Summer 8-12-2022

Agrichemicals (Nitrate and Atrazine) In Drinking Water and Adverse Health Outcomes in Children in Nebraska

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Recommended Citation

Ouattara, Balkissa S., "Agrichemicals (Nitrate and Atrazine) In Drinking Water and Adverse Health Outcomes in Children in Nebraska" (2022). *Theses & Dissertations*. 668.
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AGRICHEMICALS (NITRATE AND ATRAZINE) IN DRINKING WATER AND ADVERSE HEALTH OUTCOMES IN CHILDREN IN NEBRASKA

by

Balkissa S Ouattara

A DISSERTATION

Presented to the Faculty of
the University of Nebraska Graduate College
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

Environmental Health, Occupational Health & Toxicology Graduate Program

Under the Supervision of Professor Eleanor G. Rogan

University of Nebraska Medical Center
Omaha, Nebraska

July 2022

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ACKNOWLEDGEMENTS

First and foremost, I acknowledge God Almighty for his unfailing grace and mercy that have followed me daily during this journey. I built my foundation on Him, the rock of ages, to be a stepping stone for others, never a stumbling block.

If I have completed this work, it is because I "stood on the shoulders of giants." Here I want to mention Dr. Rogan, whose scientific acumen, rigor, and simplicity have fascinated me during the four years I worked with her. Her guidance, advice, and support made this work possible. She has been a true mentor and leader to me. Indeed, she allowed me to be vulnerable to grow, and she was always available to teach, advise, and provide quick and constructive feedback on my work. She removed all unnecessary burdens so I could focus on all aspects of learning and research.

To my committee members, Drs. Shannon Bartelt-Hunt, Jesse Bell, Pete Iwen, and John Sparks. You dedicated your time to accompanying me on this journey. You were all available to help, and you critically assessed this work. I appreciate you and am grateful for having you on my committee.

I also want to extend my gratitude to all the colleagues in the water and health group: Jagadeesh Puvvula, Siddhi Munde, Chuyang Liu, Farzana Rahman, Drs. Azar Abadi, Karrie Weber, Alan Kolok, Joseph Naveen, and Yisong Li, I learned from each of you!

I want to acknowledge the Daugherty Water for Food Institute, the Child Health Research Institute, and the Sammy's Superheroes Foundation for financially supporting this research.

I am grateful to all COPH faculty, staff, and fellow students. A special thank you to Dr. Tibbits for her mentorship.

To Lili Bronner, I look up to you as a big sister, and I knew I could always count on you. I really appreciate you.

To Dr. Kimberly McFarland, I enjoyed all the moments we spent with you and your family– thank you!

To all my friends, thank you!

Thank you to FRENSS (French-Speaking Student Association) for allowing me to develop my leadership skills.

Finally, I dedicate this work to:

My late dad: you taught us the value of hard work and resilience by being a role model for your children. Dad, this is my second doctorate, I know you are proud of your daughter, and I wish you were among us to celebrate! Love you and miss you forever, father!

My mother: I learned with you what it means to focus on one's goals, choose the right battles, and fight until you win. You are my hero, mom!

My son, Darien. May this work be an inspiration for you. You came to school with me; you stayed in the hallway during some of my classes! I could not play with you most often because of my busy schedule. I hope you will understand that I did all this for you. You were my strongest motivation. Mom loves you and thank you for all the kisses!

Someone dear to my heart, Dr. Drissa Toure. This work is yours! You motivated and inspired me; above all, you were there every step of the way.

To my brothers and sisters, thank you for your unwavering love.

ABSTRACT

AGRICHEMICALS (NITRATE AND ATRAZINE) IN DRINKING WATER AND ADVERSE HEALTH OUTCOMES IN CHILDREN IN NEBRASKA

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University of Nebraska, 2022

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Objectives: This research was conducted to (1) determine the concentrations of atrazine, and nitrate, in Nebraska watersheds and counties; (2) calculate the incidence of pediatric cancers and prevalence of birth defects in Nebraska counties and watersheds, respectively; (3) assess the relationship between the contaminant levels and the incidence rate of pediatric cancers and birth defects prevalence.

Methods: Pediatric cancers and birth defects data were obtained from the Nebraska Department of Health and Human Services. Water quality data were collected and retrieved from the Water Quality Portal and the Nebraska Groundwater Quality Clearinghouse. Geospatial and statistical analyses were conducted at the watershed and county levels. Results: The age-adjusted incidence for pediatric brain and other central nervous system (CNS) cancers in Nebraska was 4.42 per 100,000 population between 1987 and 2016, which was higher than the national average of 3.16, and the difference was statistically significant ($p=0.004$). All the watersheds with nitrate concentrations above 10 mg/L in surface and groundwater also had pediatric CNS cancer incidence above the national average. Moreover, an association was found between atrazine concentrations > 0.0002 µg/L and nitrate concentrations > 2 mg/L and an increased incidence rate of pediatric cancers (brain and other CNS, leukemia, and lymphoma) across Nebraska counties. Furthermore, birth defect prevalence in Nebraska was 9 per 100 live births which was more than the national average of 5 per 100 live births. A positive association was observed between higher levels of nitrate in drinking water (> 6.94 mg/L) and birth defects prevalence. Similarly, watersheds with atrazine levels above 0.00 µg/L had a higher prevalence of birth defects.

Conclusions: While these findings do not indicate a causal relationship, they suggest that atrazine and nitrate may pose a risk relative to the occurrence of birth defects and pediatric cancers. They also suggest that chronic exposure to nitrate and atrazine concentrations even below the maximum contaminant levels may result in birth defects or pediatric cancers. Prospective cohort studies are recommended to support these findings so that regulations can be implemented in the form of continuous monitoring of water in private wells and improvement of agricultural practices.

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AAI	Age-adjusted incidence
ACCO	American Childhood Cancer Organization
AMD	Age-related macular degeneration
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CNS	Central Nervous System
DHHS	Nebraska Department of Health and Human Services
EPA	Environmental Protection Agency
ESRI	Environmental Systems Research Institute
GIS	Geographic Information System
GW	Groundwater
HR	Hazard Ratio
HUC	Hydrologic unit code
IARC	International Agency for Research on Cancer
IRR	Incidence rate ratio
MCL	Maximum Contaminant Level
NIH	National Institutes of Health
NOC	N-nitroso compounds
NRD	Natural Resource Districts
OR	Odd ratios
PPB	Part per billion
PPM	Part per million
SPSS	Statistical Package for the Social Sciences
SVI	Social vulnerability index
SW	Surface water
TPO	Thyroid peroxidase

TSH	Thyroid-Stimulating Hormone
UNMC	University of Nebraska Medical Center
US	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
WHO	World Health Organization

INTRODUCTION

Background

Water is a natural and essential element to the survival of all living creatures on Earth - from humans to animals and plants. Earth is often referred to as a blue planet because its surface is full of water – 71% of the Earth's surface is covered with water. Most of this water is salt water – 96.5% - not proper for human consumption (NASA, 2022). Just 3.5% of the Earth's water supplies are freshwater, which is mainly frozen (68%). The remaining freshwater is groundwater (30%) and surface water (about 2%) (Madhav et al., 2020; NASA, 2022). Freshwater is one of the most basic necessities for humankind. However, its availability, accessibility, quality, and safety have been problematic in many parts of the world, including the United States.

Water pollution can be considered as any change in water characteristics (biologic, physical, or chemical) that can negatively affect human health (Madhav et al., 2020). Many contaminants can infiltrate into the water, and their occurrence varies depending on location. Water pollution can originate from a point or nonpoint source. Point source contamination can be tracked down to a single source, such as industrial discharge in a specific water body. The nonpoint source refers to contamination from several nonspecific sources, including fertilizer runoff and pesticides from farming areas (EPA, 2022; Madhav et al., 2020). Nonpoint source pollution has been reported among the leading causes of water quality issues (EPA, 2022).

Water pollution can be the result of natural or anthropogenic factors. Geological background can contribute to natural groundwater contamination. Meanwhile, farming activities with the overuse of organic and inorganic fertilizers, the intense application of pesticides, the release of industrial waste, and seeping septic tanks all account for anthropogenic pollution of water (Madhav et al., 2020).

Drinking water is extracted from two primary sources: surface water (rivers, ponds, streams, and lakes) and groundwater (contained in an aquifer, underground rivers, and lakes). Many people in the

United States, predominantly in rural areas, rely heavily on groundwater (Nebraska Department of Environmental Quality, 2020). If the public drinking water system is regulated under the Safe Drinking Water Act with defined maximum contaminant limits established by the United States Environmental Protection Agency (EPA) for many pollutants, the reality is different for private well water (EPA, 2020b; DeSimone et al., 2009).

Nitrate and atrazine are among the chemicals with EPA-defined maximum concentrations in drinking water. The maximum contaminant limits are 10 mg/L of nitrate as N and 3 µg/L for atrazine.

What is nitrate?

Nitrate - chemical formula NO_3- - is a water-soluble molecule comprised of a central nitrogen atom bound to three oxygens. Once dissolved in water, its presence is not self-evident because nitrate is odorless, tasteless, and colorless. It is a form of inorganic nitrogen along with nitrite (NO_2) and ammonia (NH_3). Nitrates are essential for all living beings, plants, animals, and humans. They are critical for plant growth – they are plant nutrients - hence their use in industrial agriculture, especially in corn production where large quantities of fertilizers are applied (Wall, 2013).

How does nitrate get into drinking water?

Nitrate in water can originate from natural sources known as background sources. Nitrate concentrations from natural sources are usually low and safe. However, higher nitrate concentrations in water are usually associated with human-related sources such as synthetic fertilizers, manure, industrial discharges, and failing septic systems (Dubrovsky & Hamilton, 2010).

Nitrate concentrations in surface water are influenced by many factors, including the closeness to point or non-point pollution sources - farming activities and waste production (human and animals) in upstream watersheds (Dubrovsky & Hamilton, 2010; Wall, 2013). Nitrate, as previously mentioned, is soluble in water and has a negative charge allowing it to freely move through the soil profile where it can attain the groundwater (Wall, 2013). Well depth is an important factor in the occurrence of nitrate in groundwater. Higher nitrate concentrations are typically observed in shallow wells beneath agricultural lands characterized by intense use of fertilizers and manure (Burow et al., 2010). Thus, nitrate contamination is frequent in private wells that tap shallow groundwater, especially in farming areas (Dubrovsky & Hamilton, 2010).

The susceptibility of aquifers to nitrate occurrence depends on their type (higher concentration in unconfined vs. confined aquifers), the source of contamination (temporal and spatial variation in input), the groundwater age (higher nitrate concentration in younger vs. older groundwater), and the geochemical (reduced geochemical conditions vs. oxic conditions lead to denitrification) and physical (soil type, bedrock) properties of the groundwater (Burow et al., 2010; Dubrovsky & Hamilton, 2010; Lindsey, 2003). Once in groundwater, nitrate can remain for a long time – years, even decades – and its current presence in wells can be related to the previous land uses (Dubrovsky & Hamilton, 2010).

What is atrazine?

Atrazine – chemical formula $C_8H_{14}ClN_5$ - is a synthetic and systemic (moves within the plant) triazine herbicide that belongs to the chlorothiazide herbicide chemical group along with simazine and propazine. Atrazine comes in different forms - liquid, granules, and powder. Atrazine is used for broadleaf and grassy weed control, especially in the production of corn, sorghum, and sugarcane.

Atrazine is described as an endocrine-disrupting chemical that occurs in the drinking water with run-off after precipitation events or crop irrigation. Water contamination by atrazine is mainly observed in agricultural areas (Stradtman & Freeman, 2021; United States Environmental Protection Agency, 2021).

Literature review

❖ Health effects of nitrate

This research focused on nitrate in drinking water. Nitrate itself may not be harmful. However, the toxicity chain begins when nitrate is reduced to nitrite by oral bacteria. In the presence of nitrite in the blood, hemoglobin is oxidized into methemoglobin which may result in methemoglobinemia, causing tissue hypoxia manifesting as cyanosis. In addition, nitrite interacts with amides or amines to form *N*-nitroso compounds that are established carcinogens and teratogens (Mensinga et al., 2003; Ward et al., 2018). The EPA MCL for nitrate was established to prevent infant methemoglobinemia; however, subsequent adverse health outcomes were not considered (USEPA, 2021).

Nitrate and Methemoglobinemia

Methemoglobinemia, also known as “blue baby syndrome,” is a serious condition that can result in death. The condition occurs when methemoglobin levels in the blood exceed 10%. Reduced nitrate, nitrite, attaches to hemoglobin, which is oxidized, to form methemoglobin. Methemoglobin has a higher affinity for oxygen, reducing oxygen-carrying and delivery capacity to tissues by hemoglobin (Ward et al., 2018). The risk of methemoglobinemia is increased in children, especially in those less than six months old [because of their higher ability to reduce nitrate into nitrite and their reduced levels of hemoglobin reductase (Ward et al., 2005)], fed with formula or foods with high nitrate content, and in children with gastroenteric infections or taking medications with high nitrate levels (Charmandari et al., 2001; Greer & Shannon, 2005; Sanchez-Echaniz et al., 2001).

The association between methemoglobinemia and elevated nitrate level in the drinking water was first described in infants in 1945 (Comly, 1987). Subsequently, in 1998 and 1999, two cases of methemoglobinemia were reported in Wisconsin (Knobeloch et al., 2000). Both infants ingested formula made with private well water containing nitrate concentrations above 10 mg/L (22.9 and 27.4 mg/L).

More recently, research conducted in Iowa included individuals aged 1-60 years old whose private well water nitrate levels were <10 mg/ L NO_3-N. The authors observed a positive association between methemoglobin level in the blood and the quantity of nitrate consumed (Zeman et al., 2011).

More cases of methemoglobinemia were also described around the world. In a cross-sectional study, infants in Gaza (Palestine) fed with formula made using well water containing higher nitrate concentration (195 mg/L NO_3) had higher methemoglobin levels compared to infants fed with lower nitrate levels in water (119 mg/L NO_3) (Abu Naser et al., 2007). Similarly, in Morocco, infants drinking water with nitrate levels > 50 mg/L had a 22% increased risk of methemoglobin compared to their counterparts drinking water with nitrate concentrations less than 50 mg/L (Sadeq et al., 2008).

Nitrate and type 1 diabetes mellitus

Some researchers have found an association between high nitrate intake and type 1 diabetes mellitus. It has been hypothesized that some reactive nitrogen species (peroxynitrite) and nitrosamines have toxic effects on pancreatic β-cells and thus may induce type 1 diabetes mellitus (Longnecker & Daniels, 2001). Bahadoran et al. (Bahadoran et al., 2016), in their review article, include two ecological studies that found an association between nitrate concentration in drinking water and type 1 diabetes mellitus.

The first study conducted by Kostraba et al. (1992) in Colorado between 1984-1988 included children less than 18 years with type 1 diabetes mellitus reported on the diabetes mellitus registry. They found that compared to counties with drinking water nitrate in the first tertile (0.0-0.084 mg/L), counties with public and well water nitrate concentration in the third tertile (0.77-8.2 mg/L) had an increased risk of type 1 diabetes mellitus.

The second ecologic study, a population-based study conducted by Parslow et al. (1997) in Yorkshire, England, from 1978 to 1994, reported a higher incidence rate of type 1 diabetes mellitus among participants in areas with mean drinking water nitrate concentration between 14.9 and 40.0 mg/L, compared to participants with mean nitrate concentrations less than 3.2 mg/L.

On the other hand, some studies did not find any association between drinking water nitrate and type 1 diabetes mellitus (Casu et al., 2000; van Maanen et al., 2000; Virtanen et al., 1994).

Studies assessing nitrate and nitrite from food (dietary nitrate) found some association with type 1 diabetes mellitus (Benson et al., 2005; Dahlquist et al., 1990; Virtanen et al., 1994).

Nitrate and adverse pregnancy outcomes

Several studies reported a positive association between nitrate in drinking water and adverse pregnancy outcomes. The pathophysiology process is the formation of teratogens, the *N*-nitroso compounds, resulting from the reaction between nitrite and amines under specific endogenous conditions (Mensinga et al., 2003; Ward et al., 2018).

- *Spontaneous abortion*

Maternal exposure to nitrate in drinking water and subsequently to high methemoglobin levels has been investigated in early studies as a potential risk factor for spontaneous abortion. Between 1991 – 1994, four women in Lagrange County, Indiana, had a history of at least one spontaneous abortion. Genetic risk factors were assessed and were negative. However, nitrate concentrations in their drinking water were above the maximum contaminant limit of 10 mg/L. Mitigation of the contaminated wells was performed, and all four mothers conceived and delivered healthy babies afterward (CDC, 1996).

Nevertheless, a case-control study conducted in Boston, Massachusetts, found no association between spontaneous abortion and nitrate concentrations in drinking water (Aschengrau et al., 1989).

- *Preterm birth*

A study conducted in California investigated the association between drinking water nitrate and spontaneous preterm birth by using birth data from 2000 to 2011 and linked them to public water monitoring records. They found that compared to drinking water nitrate levels of less than 5 mg/L, nitrate concentration of 5 or less than 10 was associated with an increased risk of preterm birth occurring between 20 and 31 weeks (OR = 1.47; 95% CI: 1.29, 1.67) (Sherris et al., 2021)

Stayner et al., in their study, included singleton births born between 2004 -2008 in 46 counties from four midwestern states with a public water system. They assessed the relationship between drinking water nitrate and preterm birth. They found that when well use was restricted to 20 %, the risk of very preterm birth (< 32 weeks) was associated with nitrate exposure 9 months before birth (Stayner et al., 2017).

- *Birth defects*

In their review, Brender and Weyer cited a study conducted in California. The authors linked maternal periconceptional addresses with public water systems and determined nitrate levels in drinking water during the study period. They observed that women with drinking water nitrate concentrations above 10 mg/L had four-time greater odds (95% CI: 1.0-15.4) of anencephaly birth defect than their counterparts whose water supplies had nitrate concentrations at or below 10 mg/L (Brender & Weyer, 2016).

Brender and colleagues reported the compounding effect of dietary nitrate and nitrosatable drugs (such as amoxicillin, caffeine, chlorpheniramine, promethazine, and pseudoephedrine) on birth defects. They found that mothers with nitrosatable drugs and higher dietary nitrate intakes were 2.7 (95% CI: 1.4 - 5.3) times more likely to have offspring with neural tube defects than mothers not taking nitrosatable drugs or with lower nitrate intake (Brender et al., 2004).

Moreover, a case-control study in Kings County, Canada, found a positive association between nitrate and birth defects. They connected maternal residential addresses at birth to public water supply median nitrate levels and estimated drinking water concentration in 140 private wells using the kriging model of monthly concentrations. They observed a significant increase (OR = 2.44; CI:1.05–5.66) in the incidence of birth defects for drinking water with nitrate levels of 1–5.56 mg/L compared to <1 mg/L after adjusting for variables such as the infant's gender, the season of birth, the mother's age and parity and some maternal risk factors (smoking, diabetes, and thyroid disease) (Holtby et al., 2014).

Maternal daily drinking water nitrate concentration ≥ 5 mg (vs. <0.91 mg) around the conception period was associated with spina bifida, a neural tube birth defect, after adjusting for the mother's race and ethnicity, education level, and folic acid intake (OR = 2.0; 95% CI: 1.3 – 3.2). Moreover, mothers of children born with limb deficiency and cleft lip were 1.8 (95% CI: 1.1 – 3.1) times more likely to consume daily nitrate ≥ 5.42 (vs. < 1.0) mg during the preconception period (1 month) through the first trimester. Also, mothers of cleft palate children were more likely to consume higher daily nitrate levels (OR = 1.9; 95% CI: 1.2 - 3.1). The study, conducted in Texas and Iowa, linked addresses of case mothers and control groups to municipal water nitrate measurements. Bottled water nitrate concentration was measured, and total daily nitrate intake was determined from self-disclosure of daily water ingestion (Brender et al., 2013).

A study conducted in Missouri from January 2004 to December 2008 included singleton births and linked birth defects with county-specific monthly nitrate concentrations. They found that exposure to nitrate during the first trimester and more than twelve months before birth was significantly associated with an increased risk of limb deficiency (Blaisdell et al., 2019).

Several studies did not find any relationships between exposure to nitrate in drinking water and birth defects. For example, Waller and colleagues conducted a retrospective case-control study in Washington state using birth certificates and nitrate and pesticide surface water concentrations obtained from the US Geological Survey (USGS) databases (Waller et al., 2010). They observed no association between gastroschisis, an abdominal wall defect, and maternal residence closeness to water monitoring sites with surface water nitrate level above 10 mg/ L NO_3-N.

Similarly, Blake in his study did not observe an association between low birth weight in San Joaquin Valley, California, and elevated nitrate concentrations in drinking water at the zip code level (Blake, 2014). Moreover, Mattix et al. (2007) who studied the relationship between nitrate and birth defects in Indiana found no correlation between monthly abdominal wall defect rates and nitrate concentrations.

Nitrate and thyroid disease

Ingested nitrate ions can competitively inhibit iodide uptake, resulting in a decreased production of thyroid hormones and impairing thyroid functions (De Groef et al., 2006; Tonacchera et al., 2004). Laboratory animals (rats) exposed to different concentrations of nitrate (0, 50, 100, 250, and 500 mg/L) in their drinking water show an increased volume in the thyroid gland in all groups besides the control group (0 mg/l of nitrate). Moreover, compared to the control group, rats in the 50 mg/l nitrate group showed decreased radioiodine uptake by the thyroid, whereas an increased uptake was observed in the 250 and 500 mg/L groups (Eskiocak et al., 2005).

A study comparing school-aged children in Slovakia from an area with high nitrate concentration in drinking water (51– 274 mg/l) with their counterparts from two areas with low drinking water nitrate concentration (≤ 2 mg/L) found higher thyroid volume (on ultrasound) and other signs of thyroid disorders [(Hypoechogenicity of the thyroid on ultrasound, high thyroid-stimulating hormone (TSH) levels and positive anti-thyroid peroxidase (TPO)] among the children from the high nitrate area compared to those from low nitrate areas (Tajtáková et al., 2006).

Moreover, research conducted among the Old Order Amish in Pennsylvania found that high nitrate in private wells, above the median of 6.5 mg/L, was associated with subclinical hypothyroidism

(OR = 1.60; 95% CI: 1.11-2.32) in women, after controlling for the participants’ age and Body Mass Index (BMI). The study measured participants’ TSH and linked their address to the well tested for nitrate level only in the same location (Aschebrook-Kilfoy et al., 2012).

Additionally, a study conducted in Durango, Mexico, on 420 women measured nitrate concentrations in their drinking water and biomarkers from urine and blood samples. They observed nitrate concentration above the maximum contaminant limit of 50 mg/L with an increase in the proportion of methemoglobin and an alteration of thyroid hormones (TSH, FT3, T4T, FT4). Subclinical hypothyroidism was observed in 8.33% of the participants (Gandarilla-Esparza et al., 2021).

A study including pregnant women in two Bulgarian villages found an association between exposure to high nitrate concentration in the drinking water (93 mg/L) and an increased risk of thyroid dysfunction (OR = 5.294; 95% CI: 1.003−27.939) (Gatseva & Argirova, 2008).

Other studies investigating the relationship between nitrate exposure in drinking water and thyroid diseases did not observe an increased risk of hypothyroidism or hyperthyroidism (Ward et al., 2010).

Nitrate and age-related macular degeneration

As described above, exposure to nitrate in drinking water is associated with higher methemoglobin levels. It has also been suggested that lipid peroxidation in the retina, which can be induced by methemoglobin, can cause age-related macular degeneration (AMD).

A study conducted in Beaver Dam, Wisconsin, included a cohort of 4926 participants that had baseline eye examinations performed between 1988-1990 with follow-up exams at years 5, 10, 15, and 20. Private well drinking water was assessed for nitrate concentrations. The authors observed that compared to participants whose nitrate intake levels were low (0 - 4 ppm), the incidence of late AMD was higher for those with medium or high nitrate concentrations (Klein et al., 2013).

Nitrate and cancers

Relative to carcinogenicity, ingested nitrate is reduced to nitrite, which interacts with amines in acidic environments to form N-nitroso compounds that are established carcinogens (Mensinga et al., 2003; Ward et al., 2018). Although the EPA has not yet classified nitrates as carcinogenic (EPA, 2007), IARC, the International Agency for Research on Cancer, in its 94th volume monograph, stated that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)" (IARC, 2010).

Several studies have found a positive relationship between relatively high nitrate exposure and the risk of developing cancer, while other studies did not.

- ✓ *Thyroid cancer*

A study conducted in Iowa enrolled older females (postmenopausal) who used the same water supply for more than 10 years in a cohort and followed them from 1986 to 2004 to investigate the association between nitrate consumption in public drinking water, diet, and the risk of thyroid cancer. They found that thyroid cancer was 2.6 times higher among women whose drinking water nitrate concentrations exceeded 5 mg/L NO_3-N for five years or more than women whose drinking water supplies never exceeded 5 mg/L (Ward et al., 2010).

- ✓ *Ovarian cancer*

The same cohort of women from Iowa was followed through 2010, and different authors evaluated the risk of ovarian, bladder, and kidney cancers. Ovarian cancer risk was elevated among women with high nitrate intake (highest quartile) in public drinking water and private well water (Inoue-Choi et al., 2015).

- ✓ *Pancreatic cancer*

Looking at the cohort of postmenopausal women in Iowa from 1986 to 2011, some authors assessed the relationship between pancreatic cancer and nitrate in drinking water and food. They observed no association between drinking water average nitrate concentrations and pancreatic cancer. However, they found that higher dietary nitrite intake from processed meat was associated with pancreatic cancer (Quist et al., 2018).

- ✓ *Digestive cancers*.

From the same Iowa women cohort, Buller et al. assessed digestive system cancers diagnosed between 1986 and 2014 in relation to dietary and drinking water nitrate and nitrite concentrations. They observed no association between drinking water nitrate levels and digestive cancer risk. However, a positive association was found between nitrite ingestion from processed meat and a higher risk of stomach cancer (Buller et al., 2021).

- ✓ *Stomach cancer*

On the other hand, some studies found a correlation between nitrate levels in water and an increased risk of stomach cancer (Ledda et al., 2012)

- ✓ *Bladder cancer*

Five studies investigated the association between nitrate concentrations and bladder cancer.
Of them, Jones et al. found that long-term ingestion (≥ 4 years) of elevated nitrate in drinking water (> 5mg/L of nitrate as N) among the Iowa cohort of 34,708 postmenopausal women (1986-2010) was associated with an increased risk of bladder cancer after adjusting for covariates such as smoking status and total trihalomethane levels (HR = 1.62; 95% CI: 1.06, 2.47) (Jones et al., 2016).

A case-control study conducted among Iowa men and women newly diagnosed with bladder cancer between 1986-1989 observed no association between higher nitrate concentrations (above 5.5 mg/L) compared to those exposed to lower concentrations (Ward et al., 2003).

Additionally, a Netherlands cohort study of 120,852 men and women aged between 55–69 years did not find any association between exposure to the highest quintile of nitrate intake and the lowest quintile (Zeegers et al., 2006).

A Spanish hospital-based case-control study of bladder cancer cases from 1998 to 2001 and nitrate levels from 1940 to 2000 suggested an increased risk of bladder cancer associated with the highest and longest nitrate exposures (Espejo-Herrera et al., 2015).

A population-based case-control study conducted in Maine, New Hampshire, and Vermont among newly diagnosed bladder cancer patients aged 30-79 years old found that compared to the lowest quartile (≤ 0.21 mg/L), nitrate levels above the 95% percentile (> 2.07 mg/L) were associated with bladder cancer (Barry et al., 2020).

- ✓ *Kidney cancer*

The risk of kidney cancer was increased among women ingesting more than 5.0 mg/L NO_3-N (95% percentile) of average nitrate in public drinking water compared to those whose drinking water nitrate is in the lowest quartile (HR =2.3; 95% CI: 1.2 – 4.3). The study adjusted for trihalomethane levels (Jones et al., 2017).

- ✓ *Colorectal cancer*

Although some studies did not find any relationship between colorectal cancer and nitrate levels in drinking water (Nasseri Maleki et al., 2021), numerous studies reported an association between the two (Schullehner et al., 2018)

Indeed, a case-control study conducted by Fathmawati et al. in Indonesia demonstrated an association between prolonged exposure (more than ten years) to nitrate concentration in drinking water above 11.29 mg/L of nitrate as N with an increased risk of colorectal cancer occurrence (OR =4.31; 95% CI: 1.32–14.09) (after adjusting for smoking history, age, and family history of cancer) (Fathmawati et al., 2017).

Moreover, a case-control study conducted in Wisconsin among rural women investigated the relationship between nitrate levels in private wells (through spatial interpolation) and the risk of colorectal cancer. They found that the risk of proximal colon cancer was increased among women whose drinking water nitrate content was above 10 mg/L NO_3-N compared to nitrate levels in water < 0.5 mg/L (McElroy et al., 2008).

A study conducted in Europe between 2008-2013 included colorectal cancer cases and controls from Spain and Italy and evaluated their nitrate intake from drinking water and food. The authors observed an association between long-term exposure to drinking water nitrate concentrations below 50 mg/L of NO_3- and the risk of colorectal cancer, especially in men and participants ingesting high levels of red meat (Espejo-Herrera et al., 2016)

A systematic review and meta-analysis of drinking water nitrate and cancer risk included 48 articles addressing 13 different cancer types. Only stomach cancer was associated with median drinking water nitrate concentrations in the meta-regression analysis. The first and second stages of the meta-analysis showed that compared to the lowest odds ratios (ORs) of nitrate intake, the highest ORs are associated with brain cancer & glioma, and colon cancer (Essien et al., 2022).

Another systematic review and meta-analysis, including 15 cohort and case-control studies, concluded that nitrate intake from food was associated with an increased risk of colorectal cancer. However, there was no association between drinking water nitrate and elevated risk of colorectal cancer (Hosseini et al., 2021).

- ✓ *CNS cancers*

In Denmark, Stayner et al., in their statewide case-control study, included children no more than 15 years old with pediatric cancers: leukemia, lymphoma, and central nervous system cancers. They found that central nervous system cancers were associated with nitrate exposure (> 25 mg/L). No association was observed for leukemia and lymphoma (Stayner et al., 2021).

❖ Atrazine exposure and health effects

Birth defects

In their study using water quality data from USGS and natality data from CDC and the Indiana Department of Health, researchers from Indiana observed that rates of abdominal wall defects (gastroschisis or omphalocele) were higher in Indiana compared to those of the national averages. They also observed that increased monthly atrazine concentrations in surface water correlate with higher rates of abdominal wall defects (Mattix et al., 2007).

Similarly, a positive association was found between gastroschisis and maternal proximity to water quality monitoring sites reporting atrazine concentrations > 3 µg/L (Waller et al., 2010).

Agopian et al. (2013) observed that maternal exposure to medium-low to medium levels of atrazine during the prenatal period was associated with birth defects – male genital malformations - in Texas from 1999 to 2008.

A case-control study conducted in Texas using birth defect cases reported on the Texas Birth Defects Registry from 1999 to 2005 and randomly selected controls examined the relationship between maternal atrazine exposure in drinking water and the risk of congenital heart defects in offspring. They observed no positive association between maternal exposure to high atrazine levels and an increased risk of congenital heart defects (Kim et al., 2017).

Preterm birth

Stayner et al., in their study, included singleton births between 2004 -2008 in 46 counties from four midwestern states with a public water system. They assessed the relationship between drinking water atrazine with preterm birth. They found that when well use was restricted to 10%, exposure to atrazine 4 – 6 months or over nine months before birth was positively associated with an increased risk of preterm delivery. The risk of very preterm birth (< 32 weeks) was associated with atrazine exposure 7- 9 months prior to birth (Stayner et al., 2017).

Atrazine and Cancers

- ✓ *Pediatric cancers*

In their study, researchers from six Midwestern states linked county-level agricultural census reports with cancer incidence among children aged 0- 4 years between 2004 and 2008. They observed an association between specific crop density and the occurrence of leukemia in general (Booth et al., 2015). Similarly, Malagoli et colleagues suggested an increased risk of pediatric leukemia related to arable crop production characterized by the utilization of atrazine (Malagoli et al., 2016).

Using pediatric (children aged 0 – 14 years) cancer incidence data from 1995 to 2001 and determining the percent cropland per county, researchers found a statistically significant association between several childhood cancer types and moderate to high county agricultural activity levels (Carozza et al., 2008).

- ✓ *Ovarian and thyroid cancer*

In their study on a cohort of licensed pesticide applicators in Iowa and North Carolina, Freeman and colleagues found an increased risk (non–statistically significant) of ovarian cancer among females who ever used atrazine. They also observed some evidence for a positive association between atrazine use and thyroid cancer (Freeman et al., 2011).

- ✓ *Breast cancer*

A case-control study conducted in Wisconsin from 1987 to 2000 included females aged 20 – 79 years old with breast cancer, identified from the state cancer registry. Telephone interviews were conducted with both cases and controls, who were randomly selected from rural areas of the state. Atrazine data were collected by the Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP) as part of three separate studies of well water, and interpolation was used to estimate atrazine exposure concentrations. The study suggested no association between low-level atrazine exposure and the occurrence of breast cancer in women living in rural areas. However, the association between higher atrazine concentrations at or above the maximum contaminant limit of 3 µg/L could not be ruled out because of limited numbers in that group (Mcelroy et al., 2007).

Why this research?

The central theme of this research was inspired by several observations: (1) clinicians and pediatric oncologists have noticed that pediatric cancer is a significant concern in the state, with clusters of cases in rural areas posing the problem of access to specialized oncological care that is only available in Omaha. (2) researchers at the University of Nebraska Medical Center and the University of Nebraska – Lincoln observed that nitrate and atrazine contamination of drinking water was common, and high concentrations of these chemicals were found in monitoring wells across the state. They decided to join their efforts to conduct research to understand the health implications of high levels of these agrichemicals in drinking water (because of agricultural practices) on human health – children and adults.

An initial Nebraska legislative bill (LB 417) was introduced in 2015 to appropriate funds to the University of Nebraska Medical Center for pediatric cancer research. An annual appropriation of funds has continued and partially supported the current research (University of Nebraska Medical Center, 2019).

Study objectives

The present study was conducted to (1) determine the concentrations of the agrichemicals atrazine and nitrate in Nebraska watersheds and counties; (2) calculate the incidence of pediatric cancers and prevalence of birth defects in Nebraska counties and watersheds, respectively; (3) assess the relationship between the contaminant levels and the incidence rate of pediatric cancers or birth defects.

[1]CHAPTER 1. AGE-ADJUSTED PEDIATRIC CANCER INCIDENCE RELATED TO NITRATE CONCENTRATION MEASURED THROUGH CITIZEN SCIENCE IN NEBRASKA WATERSHEDS

Balkissa Ouattara, MD, MPH and Eleanor Rogan, PhD

Abstract

We conducted this study to calculate the mean nitrate concentration, the age-adjusted pediatric cancer incidence across Nebraska watersheds and determine the geospatial relationship between nitrate concentration and pediatric cancer. Methods: We used secondary pediatric cancer data collected in the Nebraska Department of Health and Human Services Cancer Registry from 1987-2014. We collected nitrate data from 2018 to 2019 during four sessions, through a citizen science project. We calculated the age-adjusted pediatric cancer incidence in Nebraska. Results: The mean nitrate concentrations in surface water and groundwater were 4.5 and 3.8 mg/L, respectively. Twenty percent of all groundwater measurements were above 10 mg/L. The computed age-adjusted incidence for brain and other central nervous system (CNS) cancers in Nebraska was 4.16 per 100,000 population between 1987 and 2014. This incidence was higher than the national average age-adjusted incidence for brain and other CNS cancers, reported to be 3.05 per 100,000 population between 2010 and 2014. We also found that all of the watersheds with high nitrate concentration (above 10 mg/L) in surface and groundwater also had pediatric CNS cancer incidence above the national average. Conclusions: These results suggest a possible association between groundwater nitrate concentrations and childhood CNS cancer incidence. Further study is needed to evaluate the validity of this association as compared to causation by other factors. Children living in farming and rural areas are more exposed to adverse environmental factors, such as nitrates in the water and soil, as the result of agricultural practices.

Keywords: Pediatric cancer incidence, nitrate concentration, watersheds, Nebraska

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[1] Some of the material presented in this chapter was previously published in the Int Public Health J 2021;13(4):461-469 ISSN: 1947-4989 © Nova Science Publishers, Inc. Citation : **Ouattara, B.S**., & Rogan, E. (2021). Age-adjusted pediatric cancer incidence related to nitrate concentration measured through citizen science in Nebraska watersheds. *Int Public Health J* 2021;13(4):461-469.

Introduction

Drinking water quality is a significant public health concern around the world. Surface water (rivers, lakes, reservoirs) and groundwater are the two primary sources of drinking water. Groundwater is an essential source of water for many people in North America, with 50% of the total population and 90% of the rural population using groundwater as their primary source of drinking water supply (Aschebrook-Kilfoy et al., 2012; Brender et al, 2013). Pollution resulting from anthropogenic activities impacts drinking water quality. Water contaminants include nutrients (nitrogen species and phosphorus), essential for plant and animal life, but excessive amounts pollute the water bodies (Minnesota Pollution Control Agency, 2008). Nitrate is a ubiquitous contaminant of the drinking water (Aschebrook-Kilfoy et al., 2012; Brender et al, 2013) and comes from different sources including:

- the mobilization and leaching of geologic nitrogen into groundwater through irrigation practices (Elisante & Muzuka, 2017)
- waste materials such as animal manure (beef, poultry, pork), municipal waste and septic tanks
- row crop agriculture with the excessive use of fertilizers, the inability of crops to adequately uptake nitrogen, the process of soil nitrogen mineralization and ineffective irrigation practices all account for nitrogen losses into the environment. About one half of the nitrogen fertilizer applied to enhanced crop growth diverts from its intended purpose and is lost to the water bodies (Davidson et al., 2011).

Thus, researchers conclude that anthropogenic activities (inorganic and organic fertilizer overuse in crop production and sewage) mostly account for the high concentration of nitrate in groundwater (Aschebrook-Kilfoy et al., 2012; Ward et al., 2018; Wu et al., 2019), some suggesting that nitrogen used as a fertilizer is the primary source of nitrate contamination in farming areas (Brender & Weyer, 2016).

Nitrate concentration and health outcomes

The impact of nitrate on human health was first questioned in 1945 when methemoglobinemia in infants was associated with dietary water containing nitrate above 10 mg/L (Du, Zhang, & Lin, 2007; Gilchrist, Winyard, & Benjamin, 2010; Ward et al., 2018). Since then, many studies have found a positive relationship between high nitrate levels in the drinking water and different adverse health outcomes. Nitrate itself may not be harmful. However, the toxicity chain begins when nitrate is reduced to nitrite by oral bacteria. In the presence of nitrite in the blood, hemoglobin is oxidized into methemoglobin which can result in methemoglobinemia causing tissue hypoxia manifesting as cyanosis. In addition, nitrite interacts with amides or amines to form *N*-nitroso compounds that are established carcinogens and teratogens (Mensinga, Speijers, & Meulenbelt, 2003; Ward et al., 2018).

The United States Environmental Protection Agency (EPA) set the maximum contaminant level (MCL) at 10 mg nitrate-nitrogen/liter, equivalent to 44 mg nitrate ion/liter (Gilchrist, Winyard, & Benjamin, 2010). High concentrations of nitrate in drinking water have been shown to cause various adverse health outcomes (Du, Zhang, & Lin, 2007; Gilchrist, Winyard, & Benjamin, 2010; Monti et al., 2019). For example, a study conducted among the Old Order Amish in Pennsylvania found that high nitrate in private wells, above the median of 6.5 mg/L, was associated with subclinical hypothyroidism (OR = 1.60; 95% CI: 1.11-2.32) in women, after controlling for the participants' age and Body Mass Index (BMI). The study measured participants' TSH and linked their address to the well that had been tested for nitrate level only, in the same location (Aschebrook-Kilfoy et al., 2012).

Brender and Weyer (2016) in their review cited a study conducted in California which found that women with drinking water nitrate concentration above 10mg/L had four-time greater odds (95% CI 1.0-15.4) of anencephaly birth defect than their counterparts whose water supplies had nitrate concentration below 5 mg/L.

Similarly, a study in Kings County, Canada, showed a significant increase (OR=2.44; CI:1.05–5.66) in the incidence of birth defects for drinking water with nitrate levels of 1–5.56 mg/L compared to <1 mg/L after adjusting for variables such as the infant's gender, season of birth, the mother's age and parity and some maternal risk factors (smoking, diabetes, and thyroid disease) (Holtby et al., 2014).

The International Agency for Research on Cancer (IARC) in its 94th volume monograph stated that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)" (IARC, 2010). Several studies have found a positive relationship between high nitrate exposure and the risk of developing cancer. One example is a case-control study conducted by Fathmawati et al. (2017) in Indonesia, where they demonstrated an association between prolonged exposure (more than ten years) to nitrate concentration in drinking water above 11.29 mg/L of nitrate as N with an increased risk of colorectal cancer occurrence (OR =4.31;95%CI: 1.32–14.09) (after adjusting for smoking history, age, and family history of cancer).

Moreover, a study in Iowa found that long-term ingestion (≥ 4 years) of elevated nitrate in drinking water (> 5mg/L of nitrate as N) was associated with an increased risk of bladder cancer among postmenopausal women after adjusting for covariates such as smoking status and total trihalomethane levels (HR = 1.62; 95% CI: 1.06, 2.47) (Jones et al., 2016). Another study done in Iowa and controlled for confounders such as trihalomethane levels demonstrated that high nitrate levels (> 5 mg/L of nitrate as N) in public water supplies were associated with increased risk of renal cancer (HR=2.3, 95% CI:1.2–4.3) (Jones et al., 2017). At this time, however, the EPA has not classified nitrate as carcinogenic (EPA, 2007).

Rationale of the study

Industrial agriculture, including the widespread use of nitrogen-containing fertilizers, is conventional across Nebraska. As discussed above, fertilizer overuse is one of the primary sources of nitrate in water. Furthermore, the state does not regulate private wells; so, it is up to the well owners to assure the quality of their own drinking water. The occurrence of relatively high nitrate concentration in drinking water has been associated with adverse health outcomes in several previous studies. Additionally, childhood malignancy is a public health concern in Nebraska. The incidence of pediatric cancer in Nebraska has been above the national average for many years and is among the five highest in the United States (Farazi et al., 2018).

We hypothesize that there is an association between pediatric cancer incidence and nitrate concentration across watersheds in Nebraska. Our specific aims are to (1) calculate the average nitrate concentration per watershed, (2) determine the pediatric cancer age-adjusted incidence per watershed, and

(3) assess the spatial relationship between the average nitrate concentration and the pediatric cancer age-adjusted incidence per watershed.

Methods

We obtained longitudinal pediatric cancer data (1987 - 2014) from the Nebraska Department of Health and Human Services Cancer Registry. We collected nitrate data from 2018 to 2019 during four sessions of a citizen science project, a collaboration between the University of Nebraska-Lincoln, the University of Nebraska Medical Center (UNMC), and GC Resolve, a community partner (GR Resolve, 2019).

GC Resolve targets grassroots community development through education and mobilization to support communities and gives them the necessary tools to resolve issues that impact their daily lives. GC Resolve attended several agriculture-related conferences where they recruited volunteers to be citizen scientists. The media (TV, radio channels, newsletters) was also used in the recruitment process. Potential volunteers were invited to register on the Nebraska Watershed Network website, where they provided their mailing address and watched an educational video on testing procedures. Afterward, a testing kit (rapid test strips for nitrate), including a self-explanatory testing brochure, was sent to them to test their well water and/or local waterway. Test results were mailed back or directly logged on the Nebraska Watershed Network website. For our testing procedure, citizen scientists were instructed to collect water from their tap to test their well or dip a sample bottle into a surface water source (river, lake, or pond) to obtain a grab sample. A grab sample was chosen because it required very little equipment and there is a flexibility regarding sampling location selection. The next step was to submerge the colorimetric test strip in the water for one second; then hold it horizontally for 60 seconds. Finally, read and record nitrate concentration based on the color observed at precisely 60 seconds. For each measurement above 10 mg/L, we conducted follow up laboratory testing for confirmation. In 99% of the cases, the reading performed by the citizen scientist was proven accurate, and we only reported the correct results. Figure 1 shows a test strip being read.

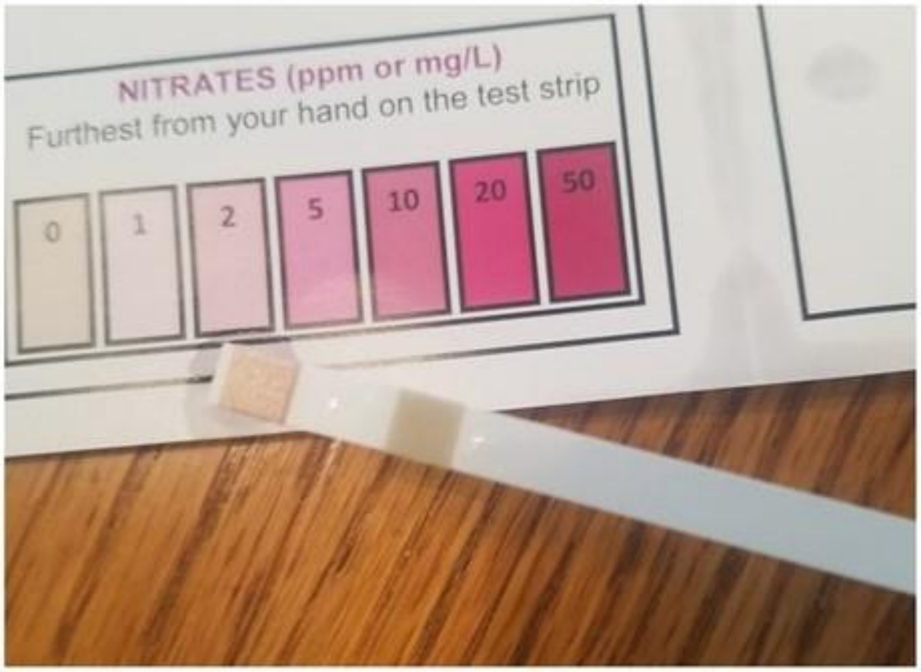

Figure 1. Nitrate test reading

Our outcome variable was the age-adjusted incidence of pediatric cancer and nitrate average concentration was the independent variable. We calculated the crude incidence of the three major pediatric cancer types for each watershed in the state and then age-adjusted to the 2010 US standard population to obtain the age-adjusted incidence for the state and for each watershed. A watershed is "a landscape that contributes surface water to a single location, such as a point on a stream or river, or a single wetland, lake, or other waterbody" (Flotemersch, 2016). The following formulas were used to calculate the crude and age-adjusted incidences (National Cancer Institute, 2021):

Crude incidence = [New cases watershed / (Population at risk watershed * Time of analysis)] *100000

Age-adjusted incidence = ∑crude incidence watershed *Age distribution of standard population age group

The age distribution of the standard population is obtained by dividing the population in any specific age group by the total US 2010 standard population. Age was categorized in four groups: 0-4, 5-9, 10-14, and 15-19 years.

We used the software SPSS (Statistical Package for the Social Sciences) version 26.0 to conduct descriptive statistics and ArcGIS Pro to analyze the spatial distribution of pediatric cancer incidence and nitrate concentration.

Results

Table 1 displays the nitrate data characteristics. Groundwater mean nitrate concentration was below the EPA maximum contaminant limit of 10 mg/L. However, of all nitrate measurements, 20% in the groundwater and 28% in the surface water were above 10 mg/L.

Table 1. Nitrate data characteristics

Water type	Sample size (n)	Minimum Concentration (mg/L)	Maximum Concentration (mg/L)	Mean Concentration (mg/L)	Standard deviation	Percent concentration above 10 mg/L (%)
Groundwater	469	0.0	20.0	3.8	5.3	20
Surface water	535	0.0	50.0	4.5	5.7	28

Three types of pediatric cancers were more prevalent in Nebraska during our study period, from 1987 to 2014. Brain and other central nervous system tumors represented 26% of all cases. It was followed by leukemia (25%) and lymphoma (16%). For children aged 0-19 years in the United States, the predominant types of pediatric cancers in 2014 were leukemia (26%), followed by tumors of the brain and central nervous system (CNS) (18%), and lymphoma (14%) (American Childhood Cancer Organization, 2014).

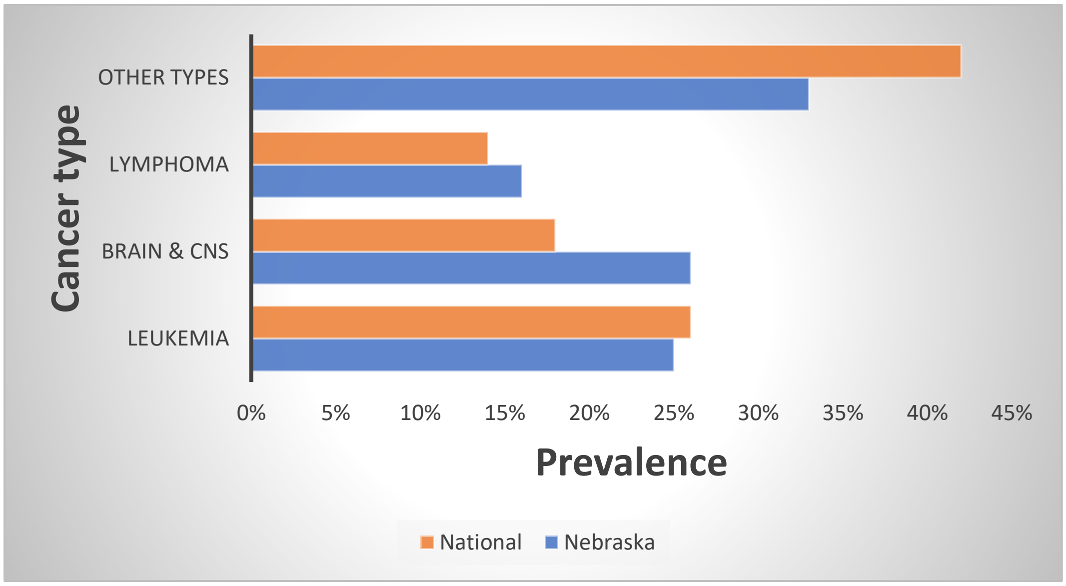

Figure 2. Prevalence of pediatric cancer types in Nebraska and the US

The computed age-adjusted incidence for brain and other CNS cancers in Nebraska was 4.16 per 100,000 population between 1987 and 2014. This incidence was higher than the national average age-adjusted incidence for brain and other CNS cancers, reported to be 3.05 per 100,000 population between 2010 and 2014 (U.S. Cancer Statistics Working Group, 2018). Leukemia and lymphoma had a lower incidence in Nebraska than the national average.

Table 2. Age-adjusted pediatric cancers incidence in Nebraska and the US

Cancer type	Nebraska age-adjusted incidence (per 100,000)	National age-adjusted incidence (per 100,000)	One-sample T Test (Two-sided p value)
Brain and other CNS	4.16	3.05	0.004
Leukemia	4.08	4.38	0.844
Lymphoma	2.82	2.94	0.848

We focused the geospatial analysis on the age-adjusted incidence for brain and other CNS tumors because the state average was higher than the national average. We represented pediatric brain and other CNS cancers incidences on a map of Nebraska with watersheds delineated. The four colors on the map represent brain and other CNS tumors incidences in quartiles. The watersheds outlined in black are watersheds with average groundwater nitrate concentration above 10 mg/L.

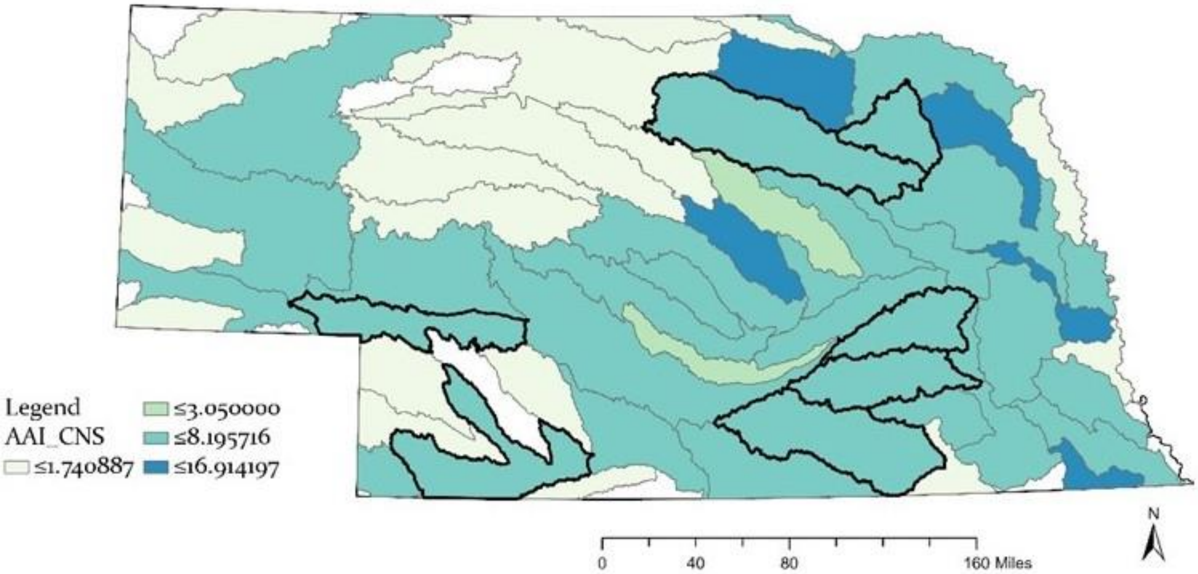

Figure 3. Age-adjusted pediatric CNS cancers vs. groundwater nitrate concentration. Watersheds outlined in black have nitrate concentration above 10 mg/L

In Figure 4 below, the watersheds outlined in black are watersheds with average nitrate concentration in surface water above 10 mg/L.

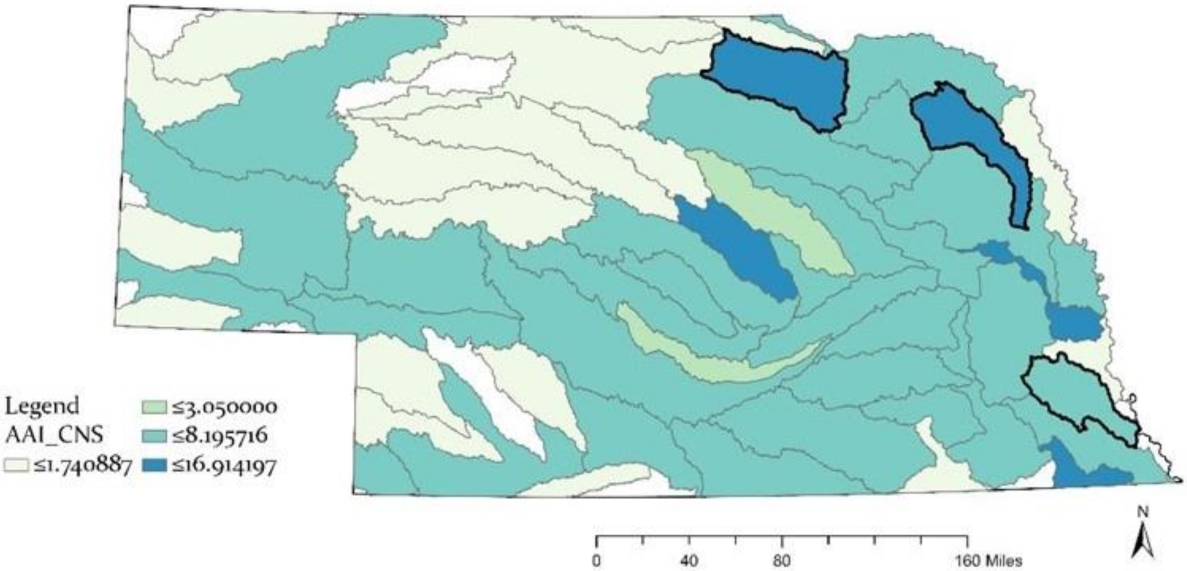

Figure 4. Age-adjusted pediatric CNS cancers vs. surface water nitrate concentration. Watersheds outlined in black have nitrate concentration above 10 mg/L

Discussion

We examined the relationship between water nitrate concentration and pediatric cancer incidence and found that all of the watersheds in rural Nebraska with high nitrate concentration (above 10 mg/L) in surface and groundwater are located in areas that have a high pediatric cancer incidence in the third and fourth quartile, above the national average, suggesting a spatial association between the nitrate concentration in water and the rate of pediatric cancers across Nebraska watersheds. Although explorative, these findings increase concern regarding the adverse effects of nitrate on human health, particularly children. As previously mentioned, agricultural activities with the widespread use of nitrogen fertilizers, in addition to livestock manure, are the primary sources of nitrate in drinking water in farming areas (Aschebrook-Kilfoy et al., 2012, Brender & Weyer, 2016; Wu et al., 2019;).

Our results suggest that agricultural activities across the state are contributing to high nitrate concentration in water and may be compromising the pediatric population's health regarding brain and other CNS tumors. Many studies have found an association between relatively high nitrate concentration in the drinking water (respectively, above 5 mg/L and 11.29 mg/L) and bladder, kidney, or colorectal cancer (EPA, 2007; Jones et al., 2016), using statistical models that controlled for confounders. We conducted a geospatial analysis to see the distribution of cancers compared to nitrate concentration across watersheds. Although our research cannot conclude that exposure to high nitrate concentration causes pediatric cancer, we did identify hot spots of cancer across the state that are associated with high nitrate concentration in water. Further research may focus on measuring nitrate concentration and other contaminants in the watersheds with a high incidence of pediatric cancers, to further assess a causal relationship between cancer incidence and nitrate concentration and other contaminants.

Strengths and limitations

Although we only measured nitrate concentration and did not account for other water contaminants, this research, through the geospatial analysis, suggested an association between nitrate concentration in drinking water and the colocation of childhood brain and other CNS cancers in Nebraska. Further limitations to this study include the use of one-time grab samples for the measurement of nitrate concentration. We also were unable to control for many possible confounding factors, including socioeconomic factors, additional chemical and environmental exposures, and familial history; therefore, further study is needed before any definitive conclusion can be reached about this relationship. Our novel methodology, however, as shown by Corley and al (2018), is beneficial in using watersheds instead of census entities when conducting a geospatial analysis of adverse health outcomes relative to agrichemicals. The authors stated that, with rainfall, agrichemical contaminants (including nitrate) travel to local waterways that flow downstream within specific watersheds (Corley et al., 2018).

Environmental health disparities

Children living in farming and rural areas are more exposed to adverse environmental factors, such as nitrates in the water and soil, as the result of agricultural practices (figure 5). Exposure to agrochemicals (including nitrate) can result in adverse health outcomes in children, as discussed above.

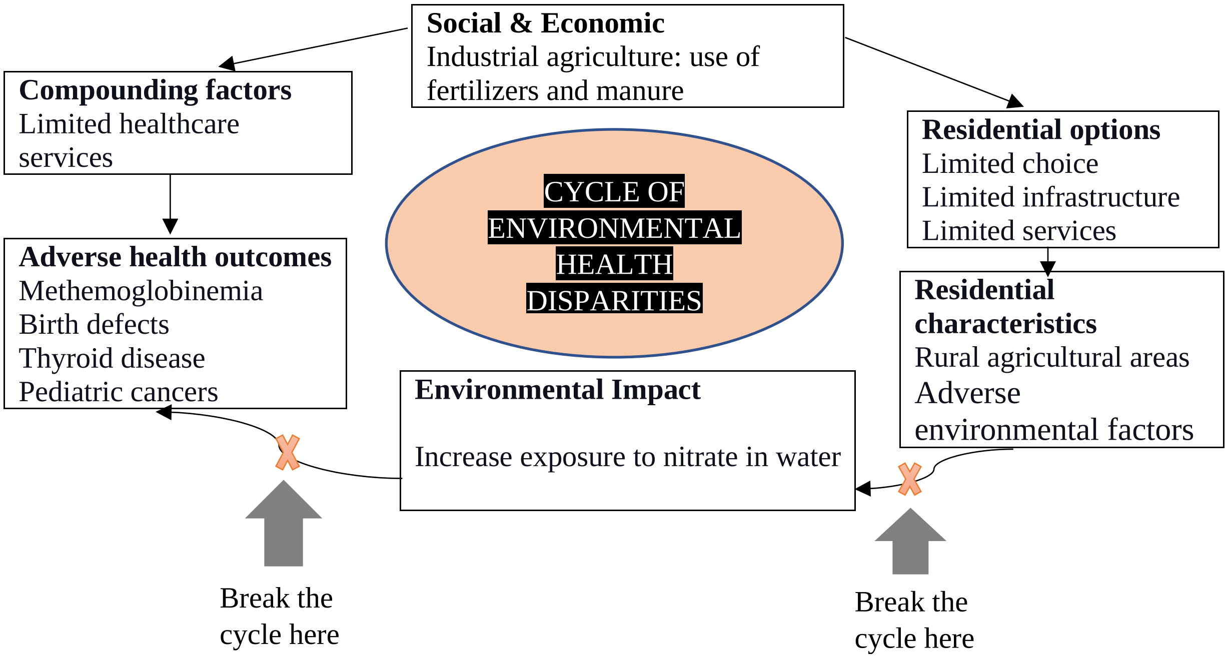

Figure 5. Cycle of children environmental health disparities

Conclusions

We found that nitrate concentration and pediatric cancer incidence are both high in specific watersheds in Nebraska. We also discovered that all waterways with nitrate concentration above the drinking water standard corresponded to watersheds with a high pediatric brain and other CNS tumor incidence above the national average. These results suggest a potential association between nitrate levels in drinking water and pediatric cancer incidence in Nebraska, but further investigation is needed to confirm this preliminary finding and assess causation.

Since studies have shown that the primary source of nitrate in water in farming areas is related to agricultural activities and we know that Nebraska is mainly an agricultural state, our finding suggests that children living in these areas may be harmfully exposed to agricultural chemicals.

To break the cycle of childhood exposure to high nitrate levels in drinking water, we recommend an agrarian reform in the US, optimizing the efficient use and reducing overuse of nitrate fertilizers. It may also be prudent to reassess feedlot practices, which may also be contributing to excessive groundwater nitrate. Farmers should be educated regarding the adverse health outcomes of high nitrate ingestion in children to increase their awareness. Periodic testing of private wells for contaminants is advisable, with recommendation that a treatment system (e.g., reverse osmosis, ion exchange, or distillation, as appropriate for the nature of the contaminants found) be installed to improve water quality or an alternate source of drinking water be found in case of poor drinking water quality.

Acknowledgements

We wish to thank Shannon Bartelt-Hunt, PhD, Jodi Sangster, PhD, Paul Hunt, and Break the Cycle Faculty for their assistance during this work process.

[2]CHAPTER 2. GEOSPATIAL DISTRIBUTION OF AGE-ADJUSTED INCIDENCE OF THE THREE MAJOR TYPES OF PEDIATRIC CANCERS AND WATERBORNE AGRICHEMICALS IN NEBRASKA

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Key Points: The incidence of pediatric brain and other central nervous system cancers was higher than the national average in many counties in Nebraska; atrazine concentration in groundwater was associated with pediatric cancers; nitrate concentration in water was associated with pediatric cancers

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[2] The material presented in this chapter was previously published in the GeoHealth Journal.

Citation: Ouattara, B. S., Puvvula, J., Abadi, A., Munde, S., Kolok, A. S., Bartelt-Hunt, S., Jesse, E. Bell., Wichman, C. S., & Rogan, E. (2022). Geospatial Distribution of Age-Adjusted Incidence of the Three Major Types of Pediatric Cancers and Waterborne Agrichemicals in Nebraska. GeoHealth, 6(2), e2021GH000419. https://doi.org/10.1029/2021GH000419

Abstract

This study was conducted to examine, at the county level, the relationship between pediatric cancer incidence rate and atrazine and nitrate mean concentrations in groundwater. A negative binomial regression analysis was performed to investigate the association between central nervous system (CNS) tumors, leukemia, lymphoma, and atrazine and nitrate mean concentrations in groundwater. The age-adjusted brain and other CNS cancers incidence was higher than the national average in 63% of the Nebraska counties. After controlling for the county socio-economic status and nitrate concentrations in groundwater, counties with groundwater atrazine concentrations above 0.0002 µg/L had a higher incidence rate for pediatric cancers (brain and other CNS, leukemia, and lymphoma) compared to counties with groundwater atrazine concentrations in the reference group (0.0000-0.0002 µg/L). Additionally, compared to counties with groundwater nitrate concentrations between 0 and 2 mg/L (reference group), counties with groundwater nitrate concentrations between 2.1 and 5 mg/L (group 2) had a higher incidence rate for pediatric brain and other CNS cancers (IRR = 8.39; 95% CI: 8.24-8.54), leukemia (IRR = 7.35; 95% CI: 7.22-7.48), and lymphoma (IRR = 5.59; CI: 5.48-5.69) after adjusting for atrazine groundwater concentration and the county socio-economic status. While these findings do not indicate a causal relationship, because other contaminants or cancer risk factors have not been accounted for, they suggest that atrazine and nitrate may pose a risk relative to the genesis of pediatric brain and CNS cancers, leukemia, and lymphoma.

Plain Language Summary

The rate of pediatric cancers in Nebraska is currently among the five highest in the United States. Ninety-two percent (92%) of Nebraska state’s total land area is used for agriculture (farming and ranching). It is challenging to establish childhood cancer causes because only 1 in 20 cases are related to heredity. Statistical tools were used to investigate the relationship between the exposure to nitrate and atrazine in surface and groundwater and childhood cancers in Nebraska. Nebraska counties where atrazine or nitrate levels were elevated reported more childhood cancers than counties with lower levels of these chemicals.

These results suggest that different agricultural activities across the state might present a risk for developing certain pediatric cancers.

1. Introduction

Nebraska is primarily an agricultural state, with 92% of the total land area used for farming and ranching (Nebraska Department of Agriculture and USDA NASS, Nebraska Field Office, 2020). The widespread use of agrichemicals such as atrazine and nitrate is common across many Midwestern states, including Nebraska. Atrazine, a triazine herbicide, is the second most used pesticide in Nebraska for leafy weed control (Wieben, 2019), and in 2017, more than 3357 tons of atrazine were applied to Nebraska cornfields (Wieben, 2019). Nitrate is used as fertilizer to enhance crop growth. In 2018, about 742,000 tons of nitrogen-containing fertilizers were applied to Nebraska cornfields (Nebraska Department of Agriculture, N.D.; USDA ERS, 2019).

These agrichemicals can contaminate surface water and groundwater, the latter being an essential drinking water source for more than 85% of all Nebraskans (Nebraska Department of Environmental Quality, 2020). Indeed, in Nebraska, surface and groundwater concentrations of nitrate and atrazine have been found in some locations to exceed the United States (US) Environmental Protection Agency (EPA) maximum contaminant levels (MCL) of 10 mg/L for nitrate as nitrogen and three (3) µg/L for atrazine (Nebraska Department of Environmental Quality, 2020; EPA, 2020a). Under the Safe Drinking Water Act, the EPA has set up, for more than 90 contaminants, health-based drinking water standards and regulations. However, the Safe Drinking Water Act (SDWA) does not cover private wells that serve fewer than 25 people (EPA, 2020b). It then falls under the responsibility of domestic well owners to ensure the quality and safety of their drinking water (EPA, 2020b; DeSimone et al., 2009).

A study that assessed the water quality of 2100 domestic wells in 48 states located in major aquifers of the United States reported that approximately one in five wells contained at least one contaminant with a concentration above the EPA maximum contaminant limit. The contaminants found at elevated concentrations included metals, radionuclides, and nitrate (DeSimone et al., 2009).

While research involving atrazine has been controversial, some studies have identified an association between atrazine exposure and adverse health outcomes in humans. For example, maternal atrazine exposure was associated with birth defects such as male genital malformations and gastroschisis (Agopian et al., 2013; Waller et al., 2010). Moreover, atrazine exposure has been associated with cancers such as pediatric leukemia and reproductive cancers (Booth et al., 2015; Carozza et al., 2008; Fan et al., 2007; Freeman et al., 2011; Malagoli et al., 2016). Other studies found no evidence of association between atrazine exposure and cancers. For instance, research among licensed pesticides applicators found no increase in overall cancer risk among atrazine users when comparing the highest and lowest exposure categories (Rusiecki et al., 2004) Because of these inconclusive results, the International Agency for Research on Cancer (IARC) lists atrazine in Group 3: not classifiable about its carcinogenicity to humans due to conflicting experimental results. Likewise, the EPA has concluded that human and animal evidence was not sufficient to consider atrazine carcinogenic (Boffetta et al., 2013).

In contrast to atrazine, the association between relatively high nitrate concentration in water and adverse health impacts is well established. For example, in 1945, infant methemoglobinemia was associated with elevated nitrate concentrations in drinking water (Du et al., 2007; Monti et al., 2019). Since then, relatively high nitrate concentrations in drinking water have been associated with many adverse health outcomes, including hypothyroidism (Aschebrook-Kilfoy et al., 2012), congenital anomalies (Brender & Weyer, 2016; Holtby et al., 2014), and malignant tumors such as colorectal, bladder and kidney cancer (Fathmawati et al., 2017; Jones et al., 2016; Jones et al., 2017). Relative to carcinogenicity, ingested nitrate is reduced to nitrite, which interacts with amides or amines to form N-nitroso compounds that are established carcinogens and teratogens (Mensinga et al., 2003; Ward et al., 2018). Although the EPA has not yet classified nitrates as carcinogenic (EPA, 2007), IARC (2010), in its

94th volume monograph, stated that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)."

The incidence of pediatric cancer has been high in Nebraska (Corley et al., 2018) and above the national average (National Cancer Institute, 2017). Since only about 5% of all childhood cancers are hereditary, this suggests that other external factors such as environmental exposure to carcinogenic chemicals may play a prominent role in their etiology (NIH, 2020; Robin & Farmer, 2017). This study was conducted to examine, at the county level, the relationship between pediatric cancer incidence rate and atrazine and nitrate mean concentrations in surface and groundwater; the authors hypothesized that relatively higher concentrations of nitrate and atrazine in surface and groundwater in Nebraska are positively associated with higher pediatric cancer incidence rate.

2. Materials and Methods

2.1 Case definition, study population, and data sources

2.1.1 Pediatric cancer data

Cases were defined as all children aged 0-19 years of age and diagnosed with brain and other CNS (central nervous system) cancers, leukemia, and lymphoma recorded in the Nebraska Cancer Registry between January 01, 1987, and December 31, 2016. Based on the case definition, the at-risk population encompasses all the children (0-19 years of age) who lived in Nebraska from 1987 to 2016. Pediatric cancer data were obtained from the Nebraska Department of Health and Human Services Cancer Registry.

2.1.2 Water quality data

Atrazine and nitrate data came from monitoring well sampling and were retrieved from the water quality portal (National Water Quality Monitoring Council, 2021) and Nebraska Quality-Assessed Agrichemical Contaminant Database also known as the Nebraska Groundwater Quality Clearinghouse (University of Nebraska-Lincoln, 2020). We retrieved water quality data corresponding to our study period, 1987-2016. Monitoring wells' locations and sampling frequency are decided based on findings of high contaminant levels during random field testing. Many entities including the state's 23 Natural Resource Districts performed groundwater monitoring in Nebraska to address contaminants like nitrate and atrazine in their jurisdiction (Nebraska Department of Environmental Quality, 2020).

Monitoring wells share similar geographical and geological (same aquifer for example) characteristics with drinking water wells, and they are constructed for the purposes of water quantity and quality data collection and serve as a proxy for the drinking water quality in a given geographic area (Nebraska Department of Environmental Quality, 2020).

2.1.3 Geographic Information System (GIS) and other data

The Nebraska state and county boundary shapefiles were extracted from the United States Census Bureau (2019). The 2010 US decennial census data and Nebraska county populations were obtained from the National Historical Geographic Information System database (Manson et al., 2021). The Centers for Disease Control and Prevention (CDC)'s social vulnerability index was available for download from the Centers for Diseases Control and Prevention (ATSDR, 2016).

2.2 Data analysis

2.2.1 Age-adjusted Incidence

The age-adjusted incidence for each Nebraska county and the state as a whole were determined by first calculating the crude incidence according to the equation (National Cancer Institute, 2021):

$$\text{Crude incidence} = [\text{New cases}_{\text{county}} / (\text{Population at risk}_{\text{county}} * \text{Time of analysis})] * 100000$$

The crude incidence was then used to determine the age-adjusted incidence according to the equation (National Cancer Institute, 2021):

$$\text{Age-adjusted incidence} = \sum \text{crude incidence}_{\text{county}} * \text{Age distribution of standard population}_{\text{Age group}}.$$

The ages were categorized into four groups: 0-4, 5-9, 10-14, and 15-19 years.

The age distribution of the standard population was obtained by dividing the population in any specific age group by the total U.S. 2010 standard population.

We used the U.S 2010 census population to calculate the age-adjusted incidence and as the offset variable in the negative binomial regression analysis for three reasons. First, we referred to published literature (Corley et al., 2018; Thorpe & Shirmohammadi, 2005) that conducted analysis over an extended study period using a single census year. Secondly, childhood cancers although a leading cause of death in children are rare, and our approach to look at incidence at the county level (there are 93 counties in Nebraska) and to investigate subtypes of pediatric cancer make the event rarer and annual case counts very small. Finally, we divided our study period to a ten year-time series (1987-1996, 1997-2006, 2007-2016) to match the three-decennial censuses (1990, 2000, 2010) that occurred during our study period. We found the same trend (decrease in crude incidence for all three cancer types) in both counties with rapid population growth and counties with less or no growth.

Counties with a total pediatric (0-19 years old) population of 200 or fewer people were excluded from the analysis. Out of the 93 Nebraska counties, we included 83 counties in the study. Ten counties (Arthur, Banner, Blaine, Grant, Hooker, Keya Pawa, Logan, Loup, McPherson, and Thomas) were excluded. The cutoff of 200 was based on our exploratory data analysis findings showing extreme incidence rates for the counties with population of 200 or lower.

Finally, using ArcGIS Pro version 2.4 (ESRI, 2019), the spatial distribution of the age-adjusted incidence of pediatric brain and other CNS cancers, leukemia, and lymphoma in Nebraska was compared to the national average.

2.2.2 Negative binomial regression analysis

Dependent (outcome) variables

Both univariable and multivariable negative binomial regression analyses were conducted in SPSS (Statistical Package for the Social Sciences) (IBM Corp, 2019), to identify predictors of the three most common pediatric cancer counts (brain and other CNS cancers, leukemia, and lymphoma) with offset for the county level pediatric population size. We built separate models with our three outcome variables, count of brain and other CNS cancers, count of leukemia, and count of lymphoma. Our full model was set as followed:

ln(counts) = b0 + b1*atrazine GW + b2*nitrate GW + b3*RPL_theme1 + offset(ln(population))

Independent variables

The independent variables were the growing season mean nitrate and atrazine concentrations in groundwater during our study period. We chose to use the mean concentration instead of annual measurements according to the limitations of our data. First, we don't know the duration of exposure or the latency period between exposure and cancer diagnosis. Secondly, annual atrazine and nitrate measurements were not available for many counties. About 30 % (25) of the counties included in the study have a yearly sampling frequency of less than one (Supplemental Table S1-S4) for surface and groundwater. Finally, our preliminary findings showed that nitrate or atrazine concentrations have not dramatically changed during the study period (the mean estimate changed by less than 5%). We assumed that all the children diagnosed with cancer during the study period had similar exposure regarding the contaminant levels. The growing season (months of May to October) was emphasized, as atrazine and nitrate are applied during the growing season, and their concentration in the water is expected to be higher with higher chances of exposure. We also used a covariate in the analysis: the CDC social vulnerability index (SVI) for each county to account for the county socioeconomic status (RPL_Theme1). The CDC SVI assesses the relative social vulnerability of each county using different factors that may impact the health of the population (ATSDR, 2016) Because socioeconomic status impacts cancer incidence in general (Clegg et al., 2009; Garcia-Gil et al., 2014), we opted to control for the county socioeconomic status in our analysis. The atrazine and nitrate concentrations were classified into four groups (Table 1) using the quantile classification in ArcGIS to determine automatic groups for atrazine concentrations in surface and groundwater. For nitrate concentrations in surface and groundwater, the function "manual intervals" in ArcGIS was used to set specific concentration ranges

(ESRI, 2020). We selected 2 mg as the first cutoff for the nitrate groups because studies documented that nitrate concentrations above 2 mg/L are likely related to anthropogenic activities such as the use of fertilizers (Nolan & Stoner, 2000; Rhoades et al.,2013). The 10 mg/L cutoff referred to the EPA maximum contaminant limit.

Table 3. Classification of mean atrazine and nitrate concentrations in categories

	Atrazine (μg/L)		**Nitrate (mg/L)**	
Categories	**SW[1]**	**GW[2]**	**SW**	**GW**
Group 1 (reference)	0.0000 - 0.1313	0.0000 - 0.0002	0.0000-2.0000	0.0000-2.0000
Group 2	0.1314 - 0.9473	0.0003 - 0.0213	2.1000 -5.0000	2.1000 -5.0000
Group 3	0.9474 - 2.8187	0.0214 - 0.0995	5.0100-10.0000	5.0100-10.0000
Group 4	2.8188 - 18.5750	0.0996 - 2.5118	10.0100-12.4200	10.0100-15.1500

[1]SW= surface water [2]GW=groundwater

3. Results

3.1 Descriptive Statistics

3.1.1 Pediatric cancers

Among the 2559 pediatric cancer cases reported in the Nebraska cancer registry from 1987 to 2016, thirteen (13) types of cancer were identified. Brain and other CNS cancers were the most represented with 26% (665/2559) of all cases, followed by leukemia, 24.4% (625/2559), and lymphoma, 16% (405/2559). Table 2 compares the most predominant pediatric cancer types in Nebraska and the U.S. (ACCO, 2014).

Table 4. Most common pediatric cancer types in Nebraska (1987-2016) and the US

Cancer type	Frequency in Nebraska (%)	Frequency in the U.S. (%)
Brain and other CNS	26	18
Leukemia	24	26
Lymphoma	16	14
Other types	34	42

3.1.2 Nitrate and atrazine concentration

Growing seasons groundwater (GW) and surface water (SW) mean atrazine and nitrate concentrations are represented for counties included in the studies and where measurements were completed (Figures 6 and 7).

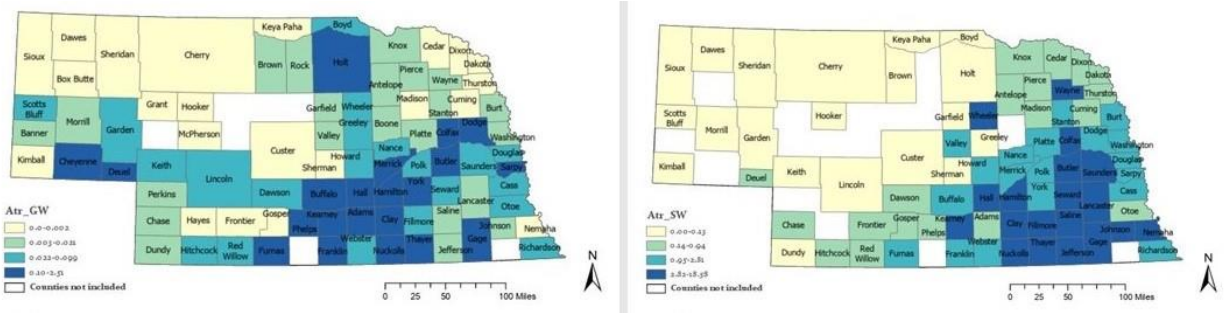

Fig 6A. Mean groundwater atrazine concentration per county in Nebraska from 1987-2016

Fig 6B. Mean surface water atrazine concentration per county in Nebraska from 1987-2016

Figure 6. Mean atrazine concentration per county in Nebraska from 1987-2016

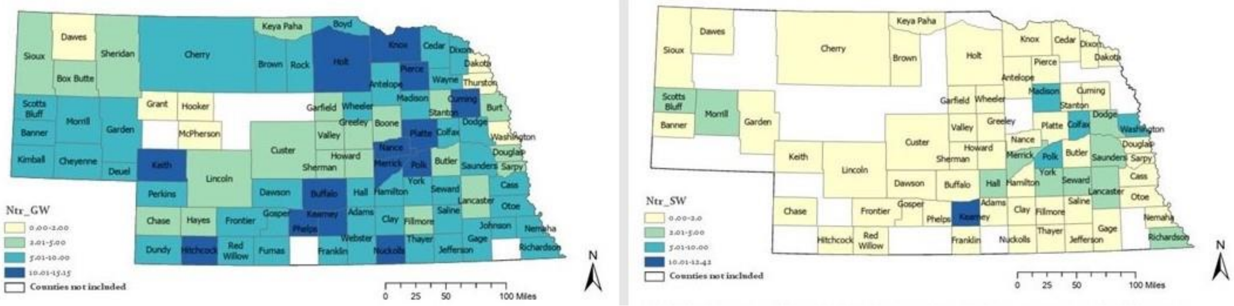

Fig 7A. Mean groundwater nitrate concentration per county in Nebraska from 1987-2016

Fig 7B. Mean surface water nitrate concentration per county in Nebraska from 1987-2016

Figure 7. Mean nitrate concentration per county in Nebraska from 1987-2016

In addition to counties not included in our study, some counties were not sampled at all for surface water atrazine and nitrate during our study period. All quartiles of mean groundwater atrazine and nitrate concentration are significantly represented across the counties included in the study. In contrast, for surface water atrazine and nitrate, the first quartile (lowest concentration) is the most represented among the counties.

Descriptive statistics for the ten counties that were most frequently and least frequently sampled during our study period are shown in Table 3A-D.

Table 5. Descriptive statistics of groundwater atrazine for selected counties with lower and higher annual sampling frequency

County	Sampling frequency [a]	Total wells [b]	Minimum concentration[c]	Mean concentration[c]	Maximum concentration[c]
Nemaha	<1	2	0.00	0.00	0.00
Hooker	<1	3	0.00	0.00	0.00
Otoe	<1	7	0.00	0.05	0.10
Johnson	<1	11	0.00	0.02	0.09
Webster	<1	12	0.00	0.07	0.20
Saunders	13	125	0.00	0.10	1.64
Cass	14	69	0.00	0.05	0.50
Lancaster	26	184	0.00	0.02	0.45
York	27	66	0.00	0.73	2.08
Buffalo	223	109	0.00	1.02	3.03

[a] annual average sampling frequency
[b] Number of monitoring wells sampled from 1987-2016
[c] Concentration in µg/L

Table 6. Descriptive statistics of surface water atrazine for selected counties with lower and higher annual sampling frequency

County	Sampling frequency [a]	Total wells [b]	Minimum concentration[c]	Mean concentration[c]	Maximum concentration[c]
Clay	<1	1	3.30	3.85	4.40
Custer	<1	1	0.00	0.06	0.11
Hamilton	<1	2	5.15	18.58	32.00
Franklin	<1	1	0.54	1.14	1.36
Phelps	<1	2	0.16	0.23	0.31
Richardson	21	18	0.00	1.69	37.60
Dodge	25	27	0.01	1.80	30.80
Sarpy	29	18	0.03	1.80	26.00
Lancaster	36	44	0.00	4.69	224.00
Douglas	54	51	0.01	1.07	18.42

[a] annual average sampling frequency
[b] Number of monitoring wells sampled from 1987-2016
[c] Concentration in μg/L

Table 7. Descriptive statistics of groundwater nitrate for selected counties with lower and higher annual sampling frequency

County	Sampling frequency [a]	Total wells [b]	Minimum concentration[c]	Mean concentration[c]	Maximum concentration[c]
Hooker	1	11	0.00	0.58	1.94
Valley	3	71	0.00	3.51	9.30
Hayes	4	72	1.65	3.14	5.34
Otoe	6	83	0.00	7.04	27.80
Frontier	6	119	3.00	5.00	8.24
Platte	175	912	0.00	12.66	36.20
Box Butte	183	1133	1.65	3.38	10.18
Antelope	195	386	2.00	9.45	16.52
Buffalo	232	411	0.00	11.91	40.00
Holt	280	789	1.07	12.73	31.75

[a] annual average sampling frequency
[b] Number of monitoring wells sampled from 1987-2016
[c] Concentration in mg/L

Table 8. Descriptive statistics of surface water nitrate for selected counties with lower and annual sampling frequency

County	Sampling frequency [a]	Total wells [b]	Minimum concentration[c]	Mean concentration[c]	Maximum concentration[c]
Cedar	<1	1	0.12	0.12	0.12
Nemaha	<1	1	1.23	1.23	1.23
Dawes	<1	2	0.04	0.22	0.41
Madison	<1	1	0.00	9.50	18.99
Otoe	<1	2	0.09	0.11	0.13
Stanton	<1	1	0.00	0.00	0.00
Lancaster	19	27	0.07	2.34	8.52
Dodge	23	6	0.16	4.48	9.15
Sarpy	32	7	0.00	1.06	3.98
Douglas	52	31	0.00	1.71	5.79

[a] annual average sampling frequency
[b] Number of monitoring wells sampled from 1987-2016
[c] Concentration in mg/L

In general, the number of wells available in a county was related to the sampling frequency.

For groundwater atrazine the average concentration in all counties was below the EPA MCL. In contrast, 16% of all groundwater nitrate measurements were above the MCL of 10 mg/L.

3.2 Longitudinal Analysis

3.2.1 Pediatric cancer data

We divided the health dataset into three ten-year periods. Compared to the first ten years (1987-1996) and third ten-year period (2007-2016), central nervous system cancer and lymphoma crude incidences were higher in most counties during the second 10-year period (1997-2006) (Figure 8A-C)

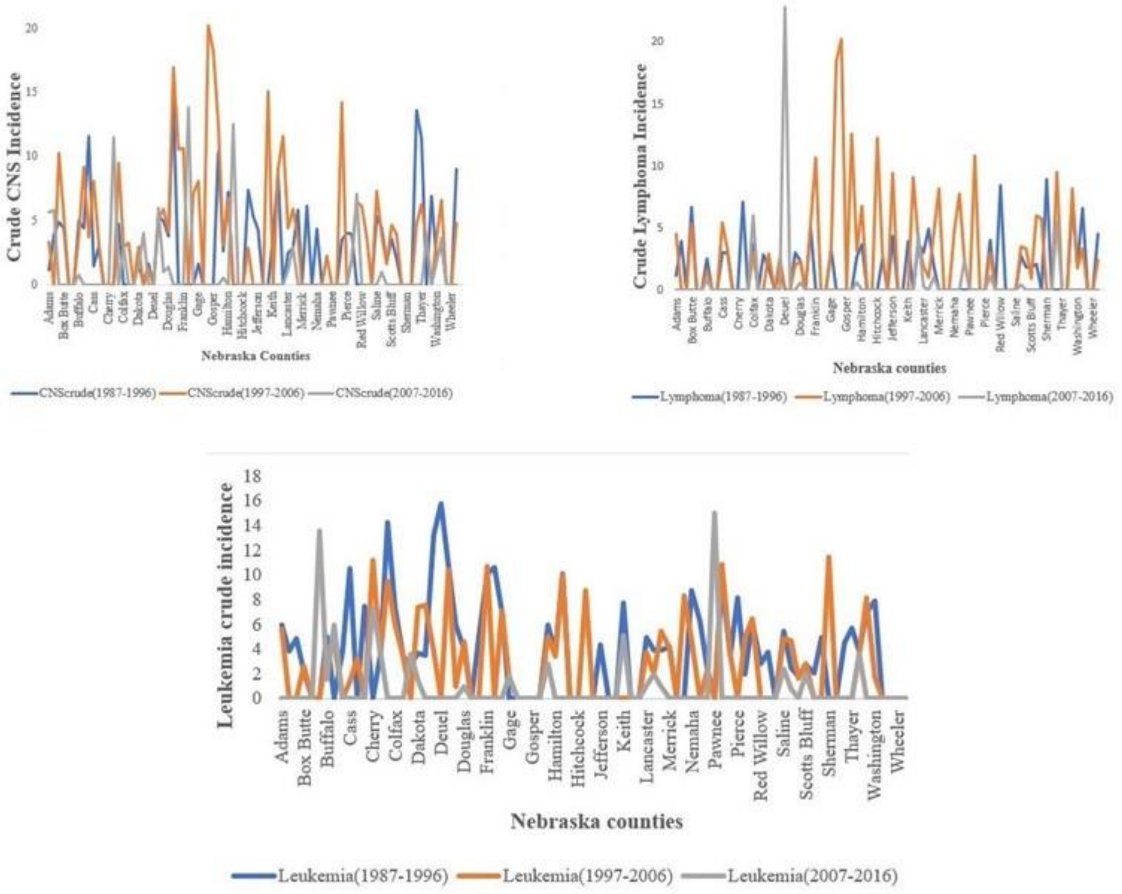

Fig 8A. Pediatric CNS crude incidence in Nebraska counties over three decades

Fig 8B. Pediatric lymphoma crude incidence in Nebraska counties over three decades

Fig 8C. Pediatric leukemia crude incidence in Nebraska counties over three decades

Figure 8. Pediatric cancers (CNS, lymphoma, and leukemia) crude incidence in Nebraska counties over three decades

3.2.2 Water quality data

We split the water quality data into three ten-year periods. Compared to the first ten years (1987-1996) and the third ten-year period (2007-2016), atrazine in the groundwater during the years 1997-2006 were more consistently measured and had higher concentrations (Figure 9A)

Regarding groundwater nitrate, measurements were less frequent but higher during the same period (1997-2006) (Figure 9B).

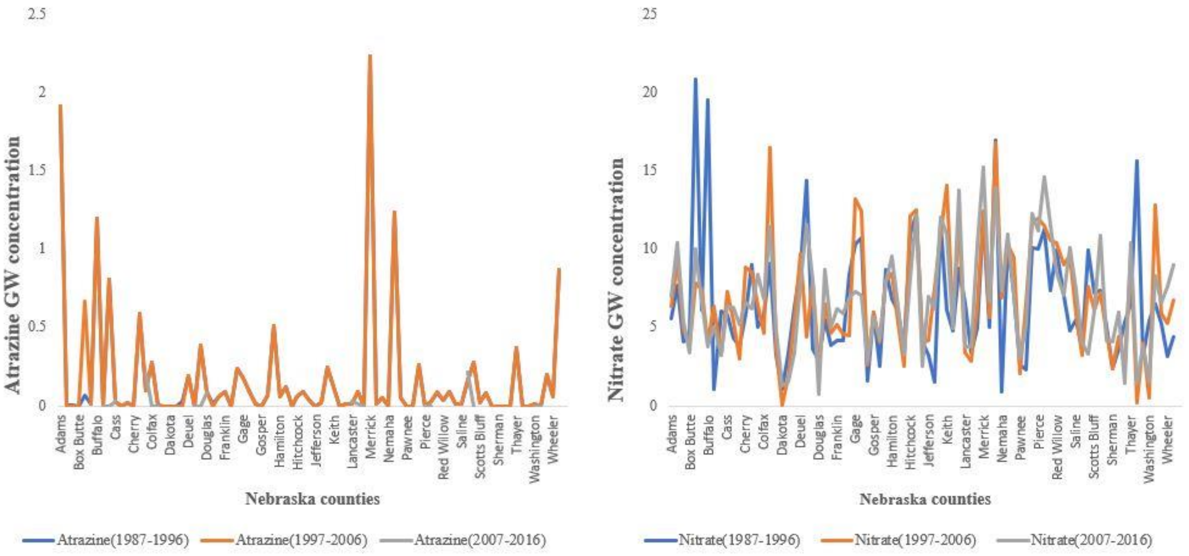

Fig 9A. Longitudinal analysis of groundwater atrazine in Nebraska counties

Fig 9B. Longitudinal analysis of groundwater nitrate in Nebraska counties

Figure 9. Longitudinal analysis of groundwater atrazine and nitrate in Nebraska counties

3.3 Age-adjusted incidence of pediatric cancers in Nebraska and the national average

The age-adjusted incidence for pediatric brain and other CNS cancers in Nebraska was 4.42 per 100,000 population between 1987 and 2016 (Table 9). This incidence was higher than the national average age-adjusted incidence for pediatric brain and other CNS cancers reported to be 3.16 per 100,000 population in average between 1999 and 2016 (U.S. Cancer Statistics Working Group, 2020). The difference between Nebraska age-adjusted brain and other CNS cancers incidence and the national average incidence was statistically significant (p=0.004). The incidence of leukemia (3.67 per 100,000 persons) and lymphoma (2.72 per 100,000 persons) was lower in Nebraska than the national average.

Table 9. Age-adjusted incidence of pediatric cancers in Nebraska and the U.S.

Cancer type	Nebraska age-adjusted incidence (per 100,000)	National age-adjusted incidence (per 100,000)	One-sample T test Two-sided p value
Brain and other CNS cancers	4.42	3.16	0.009
Leukemia	3.67	4.66	0.032
Lymphoma	2.72	2.85	0.86

3.4 Geospatial analysis

The incidence of the three major types of pediatric cancer is shown on a map of Nebraska with counties delineated (Figures 10 - 12). For each cancer type we represented side by side two maps, one with four colors on the map representing pediatric cancer incidence in quartiles with the first quartile incidence below the national average. The second map in each panel has only two colors (green and dark blue). The green color represents counties with incidence above the national average and the dark blue counties with incidence below the national average. Counties excluded from the analysis are left blank.

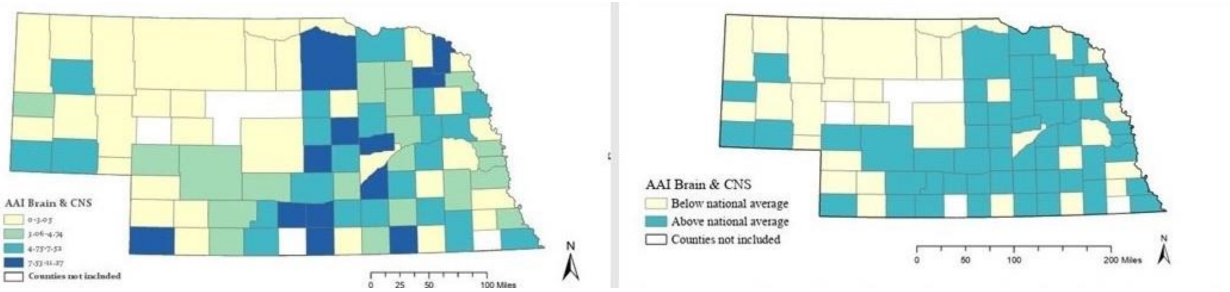

Fig 10A. Age-adjusted incidence (AAI) of pediatric brain and other CNS cancers per county in Nebraska from 1987-2016

Fig 10B. Age-adjusted incidence (AAI) of pediatric brain and other CNS cancers in Nebraska counties compared to the national average

Figure 10. Age-adjusted incidence (AAI) of pediatric brain and other CNS cancers per county in Nebraska from 1987-2016 compared to the national average

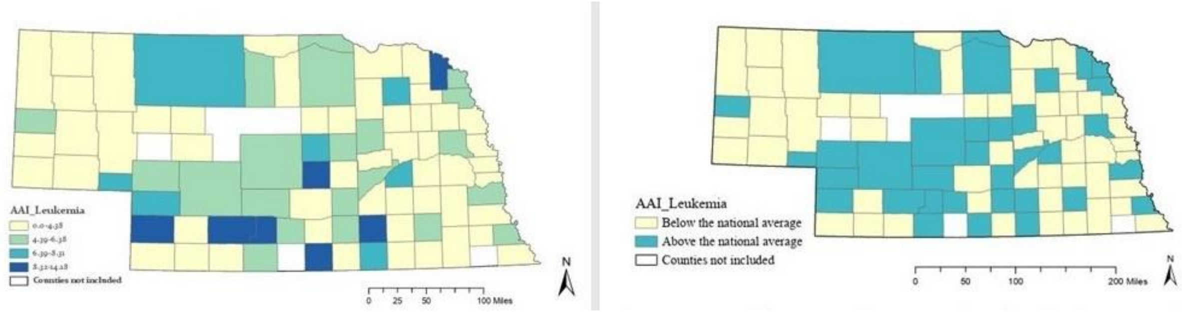

Fig 11A. Age-adjusted incidence (AAI) of pediatric leukemia per county in Nebraska from 1987-2016

Fig 11B. Age-adjusted incidence (AAI) of pediatric leukemia in Nebraska counties compared to the national average

Figure 11. Age-adjusted incidence (AAI) of pediatric leukemia per county in Nebraska from 1987-2016 compared to the national average

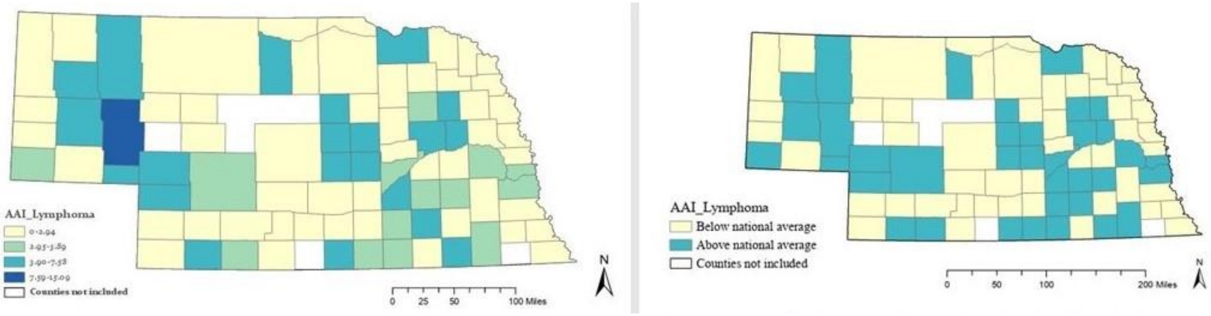

Fig 12A. Age-adjusted incidence (AAI) of pediatric lymphoma per county in Nebraska from 1987-2016.

Fig 12B. Age-adjusted incidence (AAI) of pediatric lymphoma in Nebraska counties compared to the national average

Figure 12. Age-adjusted incidence (AAI) of pediatric lymphoma per county in Nebraska from 1987-2016 compared to the national average

Relative to the national average, the age-adjusted incidence of pediatric brain and other CNS cancers (Figure 5A) is higher in 63% (54/86) of the Nebraska counties. In 41% (35/86) and 43% (38/86) of Nebraska counties; the incidence of pediatric cancers is higher than the national average, respectively, for leukemia (Figure 6A) and lymphoma (Figure 7A).

3.5 Analysis of the relation between pediatric cancers (brain and other CNS, leukemia, and lymphoma) and agrichemicals in groundwater (nitrate and atrazine)

As mentioned above, the contaminants concentrations in our dataset are a proxy for their levels in the drinking water. Thereby we focused the regression analysis on the groundwater concentrations of atrazine and nitrate. Table 10 represents the results of the negative binomial multivariable analysis.

Table 10. Multivariable analysis of the associations between pediatric cancers (Brain and other CNS, leukemia, and lymphoma) and agrichemical concentrations in groundwater (atrazine and nitrate)

Variables	**Brain and other CNS** **Full model**[a] IRR_a (95% CI)	**Leukemia** **Full model**[a] IRR_a (95% CI)	**Lymphoma** **Full model**[a] IRR_a (95% CI)
Atrazine GW[b]			
Group 1	Reference	Reference	Reference
Group 2	6.46 (6.35-6.57)	3.90 (3.83-3.96)	7.81 (7.67-7.95)
Group 3	11.20 (11.01-11.39)	6.28 (6.18-6.38)	12.76 (12.53-13.00)
Group 4	13.50 (13.26-13.74)	6.22 (6.12-6.32)	12.79 (12.55-13.04)
Nitrate GW[b]			
Group 1	Reference	Reference	Reference
Group 2	8.39 (8.24-8.54)	7.35 (7.22-7.48)	5.59 (5.48-5.69)
Group 3	0.48 (0.47-0.49)	0.51 (0.50-0.52)	0.39 (0.38-0.40)
Group 4	0.83 (0.81-0.85)	0.66 (0.65-0.68)	0.62 (0.60-0.63)
Socioeconomic status (RPL_Theme1)	4.88 (4.82-4.93)	6.34 (6.27-6.41)	3.02 (2.98-3.05)

a. Negative binomial multivariable analysis performed. IRR_a = Incidence rate ratio adjusted

b. Atrazine or nitrate concentration in groundwater.

3.5.1 Brain and other CNS cancers

Compared to counties with groundwater atrazine concentration in group 1 (reference group), counties with groundwater atrazine concentration in groups 2, 3, and 4 have brain and other CNS cancers incidence rate ratio of 6.46 (95% CI: 6.35-6.57), 11.20 (95% CI: 11.01-11.39) and 13.50 (95% CI: 13.26-13.74) respectively.

Regarding nitrate, keeping all other variables (atrazine concentration in groundwater and RPL_theme1) constant, counties with groundwater concentration in group 2 have a higher incidence rate of pediatric brain and other CNS cancers than counties with groundwater nitrate concentration in the reference group 1 (IRR=8.39; 95% CI: 8.24-8.54).

3.5.2 Leukemia

After adjusting for all other variables (nitrate concentration in groundwater and RPL_theme1) in the model, counties with groundwater atrazine concentration in groups 2, 3, and 4 have leukemia incidence rates of 3.90 (95% CI: 3.83-3.96), 6.28 (95% CI: 6.18-6.38), and 6.22 (95% CI: 6.12-6.32) times higher than leukemia incidence rate for counties with groundwater atrazine concentration in the reference group (group1), respectively. Regarding nitrate, keeping all other variables (atrazine concentration in groundwater and RPL_theme1) constant, counties with groundwater nitrate concentration in group 2 have a higher incidence rate of leukemia than counties with groundwater nitrate concentration in group 1 (IRR=7.35; 95% CI: 7.22-7.48).

3.5.3 Lymphoma

The incidence rate of lymphoma in counties with groundwater atrazine concentration in groups 2, 3, and 4 were 7.81 (95% CI: 7.67-7.95), 12.76 (95% CI: 12.53-13.00), and 12.79 (95% CI: 12.55-13.04) times higher than the incidence rate for the reference group.

Regarding nitrate, keeping all other variables constant, counties with groundwater nitrate concentration in group 2 have a higher incidence rate of lymphoma than counties with groundwater nitrate concentration in group 1 (IRR=5.59; CI: 5.48-5.69).

4. Discussion

The relationship between nitrate and atrazine mean concentrations in groundwater with the three most prevalent pediatric cancer types in Nebraska was investigated through an ecological study that accounts for two critical waterborne agrichemicals used in Nebraska. We found some associations between atrazine and nitrate concentrations with pediatric cancer incidence rates.

These findings add to the growing number of studies that have observed an association between atrazine levels in water and increased cancer incidence (Booth et al., 2015; Carozza et al., 2008; Fan et al., 2007; Freeman et al., 2011; Malagoli et al., 2016). For example, Freeman et al. (2011), with a small sample size, found an increased risk of ovarian cancer among females applicators of atrazine compared to female non-applicators. Furthermore, higher incidence rates of pediatric leukemia were observed in Illinois counties with greater than the median acreage of corn (Booth et al., 2015). Atrazine is one of the most common herbicides used in corn production; thus, Booth et al (2015) in their study implied an association between atrazine use and pediatric leukemia (RR Leukemia = 2.09, 95 % CI = 1.31–3.32). Similarly, research showed an association between residence at the time of diagnosis in agriculturally intense areas and increased childhood cancer incidence. The assumption was that agriculturally intensive areas used many pesticides, including atrazine (Carozza et al., 2008). The risk of pediatric leukemia increased with arable crop production dominated by the use of atrazine, as suggested by Malagoli et al. (2016).

Furthermore, several pre-clinical studies in animal models reported that embryonic exposure to low-dose atrazine is associated with alterations in embryonic genetic expression that promote carcinogenesis (Gely-Pernot et al., 2017; Horzmann et al., 2018; Weber et al., 2013). For instance, zebrafish embryo exposure to different doses (0.3, 3, and 30 ppb) of atrazine resulted in genetic and molecular alterations which were associated with teratogenicity and carcinogenesis in these models (Weber et al., 2013).

However, many other studies did not find an association between atrazine exposure and increased cancer risk (Rusiecki et al., 2004; Sathiakumar et al., 2011). Although Rhoades et al. (2013) in their research found that the odds of developing non-Hodgkin lymphoma (NHL) were 2.9 times (CI: 1.1-7.4) higher in subjects exposed to both atrazine and nitrate in water, the study did not observe an association between NHL risk and nitrate or atrazine alone.

The present study also found that Nebraska counties with groundwater nitrate concentration in group 2 have higher incidence rates for all three major types of pediatric cancer than counties with groundwater nitrate concentration in group 1 (reference group). These findings suggest that intensive agriculture, the primary source of water contamination by nitrate, contributes to the excess rate of pediatric cancers in Nebraska. The current results will also enrich the body of evidence of a positive relationship between nitrate concentration and increased cancer risk in humans. Indeed, a significant number of studies have found an association between exposure to a relatively high nitrate concentration and the risk of developing cancer. Examples of such studies include case-control research conducted by Fathmawati et al. (2017) in Indonesia. The findings demonstrated (after adjusting for smoking history, age, and family history of cancer) an association between prolonged exposure (more than ten years) to nitrate concentration in drinking water above 11.29 mg/L of nitrate as N, with an increased risk of colorectal cancer occurrence (OR =4.31;95% CI: 1.32–14.09). Moreover, in a study conducted in Iowa, long-term ingestion (≥ 4 years) of elevated nitrate in drinking water (> 5 mg/L of nitrate as N) was associated with an increased risk of bladder cancer among postmenopausal women, after adjusting for covariates such as smoking status and total trihalomethane levels (HR = 1.62; 95% CI: 1.06, 2.47) (Jones et al., 2016). Another study conducted in Iowa and controlled for confounders like trihalomethane levels demonstrated that high nitrate levels (> 5 mg/L of nitrate as N) in public water supplies were associated with an increased risk of renal cancer (HR=2.3, 95% CI:1.2–4.3) (Jones et al., 2017).

Additionally, nitrate toxicity to laboratory animals has been reported in several studies (Alonso & Camargo, 2003; Camargo et al., 2005; Tsai & Chen, 2002). For example, long-term exposure of freshwater invertebrates to nitrate concentration of 10 mg/L resulted in adverse health outcomes in these animals (Carmargo et al. (2005).

Strengths and Limitations

This ecologic study by design has the advantage of a relatively large health dataset and controlled for the county's socioeconomic status and the two major waterborne agrichemicals used in Nebraska. However, because of the study design, individual-level exposures to drinking water were not studied and other cancer risk factors (including other water contaminants) were not accounted for. Instead, we assumed that the agrichemical concentrations in monitoring wells are the same in the drinking water, thereby captured true exposure. Additionally, the use of secondary data and the approach to investigate subtypes of pediatric cancers at the county level did not allow detailed longitudinal analysis. Moreover, the authors assumed that all the children diagnosed with cancer during the study period had similar exposure regarding nitrate and atrazine concentrations. We also assumed that the county of residence at the time of diagnosis was the county where the exposure occurred.

5. Conclusions

In this study, the authors determined the mean atrazine and nitrate concentrations and the age-adjusted pediatric cancer incidences in each county in Nebraska from 1987-2016. The age-adjusted incidence of pediatric brain and other CNS tumors was higher than the national average in 63% of the Nebraska counties. The authors also examined the relationship between atrazine concentrations, nitrate concentrations, and pediatric cancers for the three most prevalent pediatric cancers in Nebraska (brain and other CNS, leukemia, and lymphoma). An association was found between relatively higher atrazine or nitrate concentration and an increased incidence rate of pediatric cancers (brain and other CNS, leukemia, and lymphoma). Nebraska is dominated by industrial agriculture; these results do not necessarily prove a causal relationship but suggest that the use of agrichemicals such as atrazine and nitrate poses a significant threat to pediatric health regarding brain and other CNS cancers, leukemia, and lymphoma occurrence. Further research is recommended to validate these findings, such as a case-control study to measure individual-level exposure and other potential confounders.

Acknowledgments

The authors wish to extend their gratitude to Sammy's Superheroes Foundation for funding this project and the Nebraska Department of Health and Human Services for granting access to the pediatric cancer data.

Conflict of Interest

The authors declare no conflict of interest relevant to this study.

Open Research

The following datasets used for this research are publicly available. Links and parameters are provided below, and complete references are given in the Reference section:

1. The 2010 US decennial census data for Nebraska county populations were obtained from the National Historical Geographic Information System database. https://www.nhgis.org/; accessed October 07, 2020. The following parameters were used to extract the data: the geographic level was set to county, the decennial census year 2010 was selected for years, the topics included age and the datasets was set to 2010_SF1a
2. Atrazine and nitrate data were retrieved from the water quality portal (https://www.waterqualitydata.us/portal/; accessed October 7, 2020) and Quality-assessed Agrichemical Contaminant Database for Nebraska Groundwater (https://clearinghouse.nebraska.gov/well-explorer; accessed October 7, 2020). The parameters set for the water quality portal are as follow. For the location filters, the Country was set to United States of America, the State to Nebraska, all counties and all sites were selected. All data sources (USGS, STEWARDS, EPA) were selected, the date ranges were set to from 01/01/1987 to 12/31/2016; water (NWIS, STEWARDS, STORET) for selected for sample media. Nutrient and Pesticide were selected for Characteristic group. Regarding the download options we picked the

site data only and Comma-Separated. For the Nebraska Groundwater Quality Clearinghouse, the filter was set to Domestic for Well type.

3. The Nebraska state and county boundary shapefiles were extracted from the United States Census Bureau https://www2.census.gov/geo/tiger/TIGER2019/; accessed October 7, 2020. The Files named County and State were selected and downloaded separately.
4. The CDC social vulnerability index is available at https://www.atsdr.cdc.gov/placeandhealth/svi/data_documentation_download.html. For parameters chosen, the year was set to 2016, Geography to Nebraska; Geography type to Counties and File type to CVS file.

Additional data (pediatric cancer data) supporting this research is not available to the public or research community unless access is granted by the Nebraska Department of Health and Human Services (DHHS) upon completion and approval of data use agreement forms. For further information contact the Nebraska DHHS at DHHS.PublicRecords@nebraska.gov

[3]CHAPTER 3. INVESTIGATION OF A POSSIBLE RELATIONSHIP BETWEEN ANTHROPOGENIC WATER CONTAMINANTS AND BIRTH DEFECTS OCCURRENCE IN RURAL NEBRASKA

Abstract

Agrichemicals (atrazine and nitrate) are widely used in Nebraska, and relatively high concentrations have been found in drinking water in rural areas. The main objective of this research was to investigate a potential association between birth defects occurrence and nitrate and atrazine concentrations in selected Nebraska watersheds. Birth defect data from 1995 to 2014 were obtained from the Nebraska Department of Health and Human Services. Water samples were collected and analyzed between June 2021 and February 2022. Birth defect prevalence in Nebraska was 9 per 100 live births and more than 80 % of Nebraska watersheds had birth defect prevalence above the national average of 5 per 100 live births. In the negative binomial unadjusted model, a positive association was observed between higher levels of nitrate (> 6.94 mg/L) in drinking water and the prevalence of birth defects (IRR =1.44, CI: 1.40 – 1.50). Similarly, compared to watersheds with atrazine levels at 0.00 µg/L, watersheds with higher levels of atrazine had an increased prevalence of birth defects in the adjusted model (IRR = 1.62, CI: 1.56 – 1.70). This study suggested that chronic exposure to nitrate and atrazine concentrations below the maximum contaminant levels may result in birth defects. It also highlighted the relationship between anthropogenic activities (agriculture practices), water contamination, and adverse health effects on children. An additional cohort study is recommended to support these findings so that regulations can be implemented in the form of continuous monitoring of water in private wells and improvement of agricultural practices.

Keywords: birth defect, atrazine, nitrate, agrichemicals

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[3] Some of the material presented in this chapter was accepted for publication in the Water journal.
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1. Introduction

Birth defects, also known as congenital malformations, are functional or structural anomalies that can affect any body part(s). They happen during pregnancy and are detectable in utero, at birth or later during infancy (Centers for Disease Control and Prevention, 2008; World Health Organization, 2022). Birth defects annually affect approximately 3% of all babies (1 in 33 babies) born in the United States (US) (Centers for Disease Control and Prevention, 2008). Between 2011 and 2015, about 6% (2 in every 33 births) of all babies born in Nebraska had at least one birth defect (Nebraska Department of Health and Human Services, 2015).

Birth defects account for 20% of all infant deaths and represent the leading cause of infant deaths in the US (Mathews et al., 2015). Moreover, birth defects are costly. Each year, in the US, more than 2.6 billion dollars are spent in hospital costs for the care of children and adults with birth (Centers for Disease Control and Prevention, 2021; Russo & Elixhauser, 2011).

Causes for birth defects remain poorly understood. A US population-based study found that a cause was established in only 1 out of 5 birth defects cases (Feldkamp et al., 2017). Unknown etiologies were the leading cause of birth defects (79.8%), followed by genetic causes and environmental teratogens. Many researchers think that most birth defects occur due to multiple factors, including genetic, behavioral, and environmental components (Feldkamp et al., 2017). Some factors are described as associated with the occurrence of birth defects. They include prenatal smoking and substance use (alcohol, drugs), obesity, uncontrolled diabetes, infections, and advanced maternal age (Centers for Disease Control and Prevention, 2021; Harris et al., 2017).

Similarly, exposure to some anthropogenic environmental toxicants has been linked to birth defect occurrences. Among these anthropogenic contaminants are atrazine and nitrate widely used in agriculture for pest control and fertilization, respectively. Maternal atrazine exposure was associated with male genital malformations (hypospadias, cryptorchidism, and small penis), choanal atresia and stenosis in offspring (Agopian et al., 2013) and gastroschisis (Waller et al., 2010). Maternal consumption of drinking water bearing relatively high nitrate concentrations was associated with general birth defects (Holtby et al., 2014) and for specific defects such as neural tube defects (Brender & Weyer, 2016; Brender et al., 2013), and limb deficiency (Blaisdell et al., 2019).

Agricultural activities are extensively practiced in rural Nebraska (Nebraska Department of Agriculture and USDA NASS, Nebraska Field Office, 2020) with the intensive use of agrichemicals (Nebraska Department of Agriculture, N.D.; USDA ERS, 2019; Wieben, 2019). These agrichemicals can contaminate local waterways when runoffs happen, and it was elaborated that maternal exposure to nitrate, and atrazine were associated with birth defects. In rural Nebraska, drinking water is mainly supplied by private wells that are not regulated for contaminant concentrations (EPA, 2020a). In fact, under the Safe Drinking Water Act (SDWA), the United States Environmental Protection Agency (EPA) has established the maximum contaminant level (MCL) in drinking water at 10 mg/L and 3 µg/L for nitrate and atrazine, respectively (EPA, 2020b)

Thus, this research was conducted to investigate the relationship between birth defects and atrazine and nitrate concentrations in water to determine whether the reported excess risk of birth defects in Nebraska compared to the nation is related to the presence of these agrichemicals in water supplies. An innovation of this research is to conduct water quality monitoring by measuring real-time nitrate and atrazine concentrations in areas with known high incidence and low incidence of adverse health outcomes in children.

The unique approach of this research is to focus the environmental contamination and birth defect prevalence tracking based on watersheds rather than traditional geographic census entities such as census tracts, zip codes, and counties. A watershed is the land area that channels surface water from rainfall or snowmelt to a single water body, such as creeks, lakes, streams, or rivers (Flotemersch et al., 2016).

This approach considers the transport pathways of waterborne agrichemicals, which do not follow census boundaries but instead travel to local waterways that flow downstream within specific watersheds after precipitation or irrigation events (Corley et al., 2018). In terms of exposure assessment and waterborne agrichemicals, individuals living within the same watershed could have similar exposure even if they are geographically separated. In contrast, for individuals living in different watersheds, even if geographically closer to each other, could have differential environmental exposures (Corley et al., 2018).

This study also accounted for some geogenic water contaminants that are potential risk factors for birth defects. There are arsenic (Brender & Weyer, 2016; Kwok et al., 2006; Mazumdar et al., 2015; Rudnai et al., 2014) and uranium (Gholkar, 2011; Shrivastava, 2015; Yin et al., 2022).

2. Materials and Methods

2.1 Study site, population, and data sources

2.1.1 Study sites

Nebraska counts 23 natural resource districts (NRD) that are set up along hydrological boundaries allowing them to undertake natural resource management on a watershed basis (North Platte Natural Resource District, 2022). This study was conducted within two NRDs, the Lower Elkhorn NRD and the Upper Big Blue NRD. These two NRDs were selected based on prior research that indicated poor health outcomes for children which include a high incidence of pediatric cancer (Corley et al., 2018).

Groundwater samples were collected from domestic (privately-owned) wells among the rural population within the Logan, Lower Elkhorn, North Fork Elkhorn, Upper Elkhorn, Upper Big Blue, and West Fork Big Blue watersheds in coordination with the Natural Resource Districts (NRD) in the Lower Elkhorn and Upper Big Blue located in Eastern Nebraska and consent of study participants**.** Groundwater within this region is withdrawn from aquifers which can be directly connected to the watershed by gaining and losing streams. Figure 13 represents the sample collection sites.

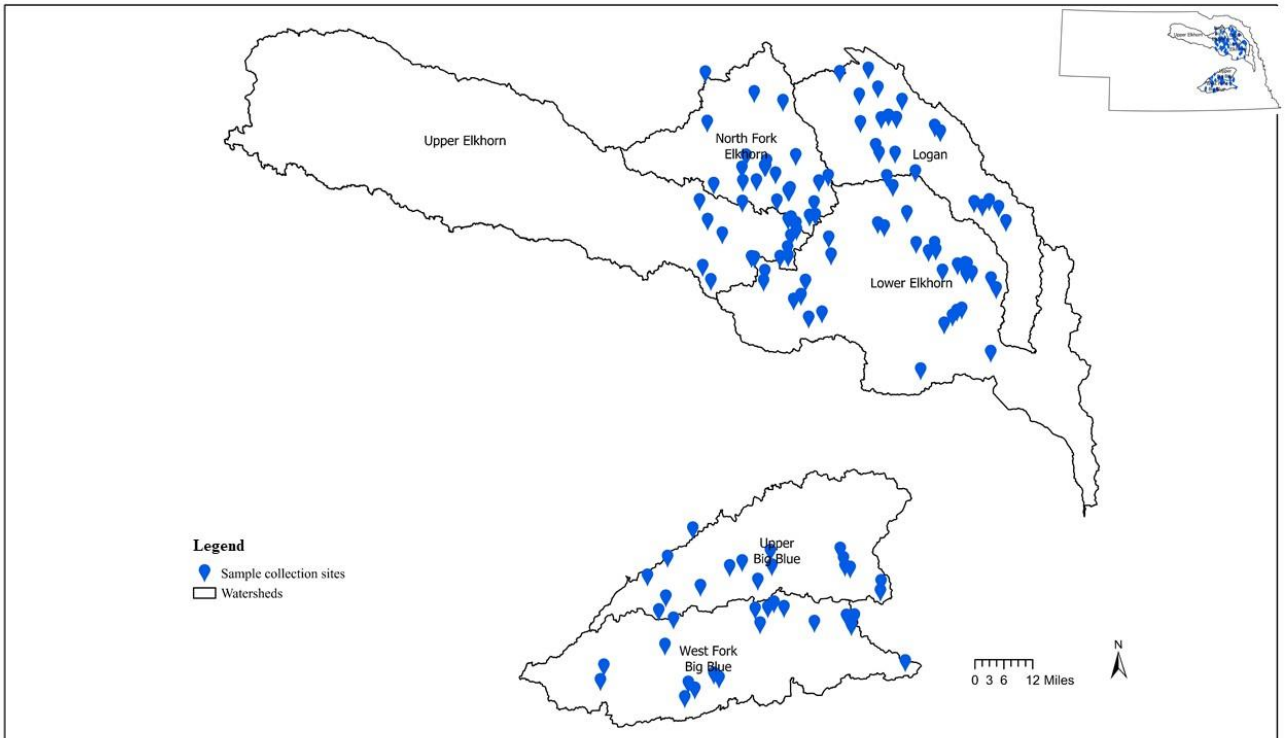

Figure 13. Sample collection sites

2.1.2 Birth defect data

Secondary birth defect data were requested through submission of data use agreement forms and obtained from the Nebraska Department of Health and Human Services (DHHS). They corresponded to all birth defects recorded in the Nebraska Birth Defect Registry from 01 January 1995 to 31 December 2014. The data from the birth defect registry included the mother's address of residence that was used to geocode to obtain geographical coordinates -longitude and latitude – for mapping purposes using the ArcGIS World Geocoding Service (Environmental Systems Research Institute (ESRI), 2021). Information on the mother's smoking and diabetes status were included in the registry.

Cases were defined as an occurrence of one or multiple birth defects in a child Thus, when a child was identified with multiple birth defects, each defect was counted as a separate case. This approach was used to calculate the prevalence of birth defects at birth. However, for the geospatial analysis, each infant with a birth defect was represented only once on the map.

The at-risk population comprised all live births in Nebraska during the study period - 01 January 1995 to 31 December 2014. Thus, corresponding live-birth data were obtained from the Nebraska DHHS. The live birth dataset included variables such as the mother's state, city, and zip code of residence. This information was used to obtain the geographical coordinates related to the mother's location for mapping purposes.

2.1.3 Water quality data

Groundwater samples were collected from domestic wells between June 2021 and February 2022 by the NRDs. Samples were collected for the analysis of anthropogenic and geogenic water quality contaminants, atrazine, nitrate, uranium, and arsenic. Prior to sample collection, if possible, the screen was removed, and the water was allowed to run for 2-3 minutes. Each collected sample was assigned a unique identification number. Samples for atrazine were collected in amber glass bottles without a preservative and immediately stored at 4 degrees° centigrade prior to analysis. Nitrate samples were immediately collected and preserved with sulfuric acid as described by EPA method 300.0. Uranium and arsenic samples were collected and preserved with nitric acid as described by 200.8.

Atrazine was determined using an enzyme immunoassay method (MODERN-WATER, 2013) following the EPA SW-846 method #4670. Atrazine groundwater concentrations were determined relative to a standard curve on a photometer with a method detection limit of 0.046 ppb. Nitrate was determined in accordance with the EPA method 300.0 for drinking water on a DIONEX-ICS-300 ion chromatograph equipped with conductivity detection using a AS9-HC-column.The method detection limit was 0.48 mg/L. Uranium and arsenic were determined in accordance with EPA method 200.8 for the determination of trace elements in drinking water by inductively coupled plasma-mass spectrometry

(ICPMS) on an iCAP RQ ICPMS (model). Indium was used as an internal standard to validate recovery. The detection limits were 0.036 µg/L for arsenic and 0.030 µg/L for uranium.

2.1.4 Geographic Information System (GIS) data

The Nebraska state and watersheds (hydrologic unit code - HUC 8 level) boundary shapefiles were retrieved from the Nebraskamap.gov website (Nebraskamap.gov, 2020). The birth defect and water quality data were geocoded in ArcGIS Pro Ver. 2.4 to include geographic coordinates. The join function in ArcGIS was used to represent both the birth defect data and water quality data at the HUC- 8 watershed level. The geographic coordinate system used corresponded to the North American Datum (NAD) 1983.

2.2 Data analysis

2.2.1 Birth defects prevalence

In the current study the prevalence of birth defects was calculated. In ideal conditions, birth defect occurrence is measured by incidence rates instead of prevalence. Incidence rates quantify the occurrence of new cases in a population during a specific period. The formula to calculate the incidence of birth defect would be (National Birth Defect Prevention Network, 2016) :

$$\frac{\text{Number of new cases of birth defects in an area and period}}{\text{the number of conceptions at risk of developing birth defects in that area and period}} \times \text{multiplier}$$

However, in this formula, the number of conceptions is not known because the number of spontaneous abortions is unknown. Thus, we are not able to appropriately determine incidence of birth defects. As a result, most professionals addressing birth defect use the concept of “prevalence” for birth defect occurrence. Birth defect occurrence is measured using the formula for birth at prevalence (National Birth Defect Prevention Network, 2016). The current study used the following formula:

$$\frac{\text{Number of new cases of birth defects in each Nebraska watershed from 1995 - 2014}}{\text{the number of live births in Nebraska watershed from 1995 – 2014}} \times 10$$

Watersheds with a total live birth less than 100 infants during the study period (1995 – 2014) were not included in the analysis. Out of the 72 Nebraska watersheds at the HUC – 8 level, birth defect prevalence was calculated for fifty-two (52) watersheds. The cutoff of 100 was based on preliminary data analysis showing extremely high prevalence for watersheds with live birth count between 0 and 100 infants.

2.2.2 Analysis of the association between birth defects and water quality data

All analyses were conducted in SPSS (Statistical Package for the Social Sciences) version 28.0 (Corp, 2021). The outcome variable was the count of infants with at least one birth defect per watershed with the watershed total live births during the study period as the scale weight variable. The main independent variables were comprised of the mean nitrate and atrazine concentrations per selected watershed. Mean arsenic and uranium concentrations, and diabetes, count per selected watershed were used as covariates. With an outcome variable that is a count variable, the negative binomial regression analysis was used, and the full model was set as follows:

$$\mathbf{\ln(\text{birth defect counts}) = b0 + b1 \ast \text{mean nitrate} + b2 \ast \text{mean atrazine} + b3 \ast \text{mean uranium} + b4 * \text{mean arsenic} + b5 * \text{diabetes} + \text{offset}(\ln(\text{population}))}$$

Mean nitrate and atrazine concentrations were categorized into two groups for each contaminant. Group 1 represented the watersheds with the lowest mean nitrate concentration measured (6.94 mg/L) and non-detectable atrazine levels, 0.02 µg/L (half the detection limit). Group 2 represented all other watersheds included in the study.

Univariable analyses were conducted first between each independent variable and the outcome variable. The multivariable analysis model was built with the dependent variable and the predictors that showed a positive association in the univariable analysis.

3. Results

3.1 Descriptive Statistics

3.1.1.Birth defects

From 1995 to 2014, 24,965 children born in Nebraska were diagnosed with at least one type of birth defect reported in the Nebraska Birth Defect Registry. A total count of 45,134 birth defect cases were reported. Birth defect prevalence per watershed ranged from 2.76 to 23.79 per 100 live births (Table 10). The average birth defect prevalence for Nebraska was 9 cases per 100 live births compared to 3 – 5 cases per 100 live births for the national average prevalence; the difference was statistically significant ($p < 0.001$).

Table 11. Birth defect prevalence per watershed in Nebraska from 1995 – 2014

Watershed Name	**Prevalence (per 100 live births)**
Beaver	9.96
Big Nemaha	10.16
Big Papillion-Mosquito	10.91
Blackbird-Soldier	18.67
Cedar	6.18
Frenchman	6.84
Harlan County Reservoir	5.59
Horse	4.86
Keg-Weeping Water	9.39
Keya Paha	3.45
Lewis and Clark Lake	20.47
Little Nemaha	8.62
Logan	7.71
Loup	5.24
Lower Elkhorn	10.19
Lower Little Blue	5.44
Lower Lodgepole	2.76
Lower Middle Loup	7.76
Lower Niobrara	13.61
Lower North Loup	9.52
Lower North Platte	7.68
Lower Platte	18.57
Lower Platte-Shell	15.32
Lower South Platte	9.31
Medicine	9.07
Middle Big Blue	8.07
Middle Niobrara	5.51
Middle North Platte-Scotts Bluff	4.57
Middle Platte-Buffalo	6.55
Middle Platte-Prairie	7.88
Middle Republican	6.68
Mud	5.31
Niobrara Headwaters	3.45
North Fork Elkhorn	6.87
Ponca	23.79
Red Willow	16.02
Salt	8.74
South Fork Big Nemaha	9.74
South Loup	10.11
Stinking Water	8.56
Tarkio-Wolf	13.53
Turkey	10.13
Upper Big Blue	7.32
Upper Elkhorn	12.37
Upper Little Blue	4.62
Upper Middle Loup	3.33
Upper Niobrara	4.61
Upper North Loup	19.50
Upper Republican	5.46
Upper White	3.97
West Fork Big Blue	13.15
Wood	7.34

3.1.2 Groundwater quality data

Groundwater samples were collected from within the Lower Elkhorn, Upper Elkhorn, North Fork Elkhorn, Logan, Upper Big Blue, and West Fork Big Blue watersheds. Anthropogenic agrichemical contaminants nitrate and atrazine were detected across the watersheds at varying concentrations. Groundwater nitrate concentrations ranged from 0.56 mg/L to 97.21 mg/L among the samples collected with those highest in the Logan watershed region in Northeast Nebraska (Table 11). The mean groundwater nitrate concentration ranged from 6.94 to 21.37 mg/L. Groundwater uranium concentration ranged from 0.00 to 143.7 µg/L. Groundwater arsenic concentrations ranged from 0.38 to 19.84 µg/L (Table 11).

Table 12. Descriptive Statistics for the water quality data

		Nitrate (mg/L) MCL = 10			**Atrazine (µg/L MCL = 3**			**Uranium (µg/L) MCL = 30**			**Arsenic (µg/L) MCL =10**		
Watershed	**Samples (n)**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Logan	20	21.37	1.18	97.21	0.00	0.00	0.00	8.02	0.11	28.03	2.61	0.4	8.04
Lower Elkhorn	37	9.09	1.14	24.58	0.01	0.00	0.29	9.81	0.06	83.08	3.17	0.4	10.51
North Fork Elkhorn	21	8.38	0.56	39.67	0.04	0.00	0.76	3.92	0.00	8.32	4.73	0.47	14.08
Upper Elkhorn	17	6.94	1.36	16.47	0.09	0.00	0.63	5.99	0.04	34.24	4.84	0.11	19.84
Upper Big Blue	17	18.24	0.73	58.35	0.18	0.00	1.39	13.18	0.55	143.77	3.61	1.22	6.13
West Fork Big Blue	20	16.12	0.87	55.97	1.11	0.00	11.23	6.33	0.24	15.03	3.14	0.33	5.04

Table 12 below shows the percentage of samples per selected watershed above the MCL for each water contaminants analyzed.

Table 13. Percentage of samples with analyte concentration above the specific MCL

		Percent above MCL (%)			
Watershed	**Samples (n)**	**Nitrate**	**Atrazine**	**Uranium**	**Arsenic**
Logan	20	50 (10/20)	0.0	5 (1/20	0.0
Lower Elkhorn	37	41 (15/37)	0.0	5 (2/37)	3 (1/37)
North Fork Elkhorn	21	19 (4/21)	0.0	0.00	5 (1/21)
Upper Elkhorn	17	24 (4/17)	0.0	6 (1/17)	12 (2/17)
Upper Big Blue	17	65 (11/17)	0.0	6 (1/17)	0.0
West Fork Big Blue	20	65 (14/20)	10 (2/20)	0.0	0.0
All	132	43.9 (58/132)	1.5 (2/132)	3.8 (5/132)	3.0 (4/132)

Among the domestic wells tested in the watersheds included in the study, 19 to 65% of them exceeded the MCL of 10 mg/L for nitrate concentrations. While nitrate exceeds the MCL in over half of the domestic wells, groundwater atrazine concentration was only found to exceed the MCL (3 µg/L) in 2 of the 132 wells tested. Groundwater atrazine concentration ranged from 0.00 to 11.23 µg/L (Table 12). Among the domestic wells sampled in this study 4% of the wells exceeded the drinking water MCL for uranium (30 µg/L) with elevated levels in the Lower Elkhorn, Logan, Upper Elkhorn, and Upper Big Blue Watershed. Within the Upper Big Blue and West Fork Big Blue, groundwater arsenic concentrations remained below the MCL. However, in the Upper Elkhorn, North Fork Elkhorn, and Lower Elkhorn groundwater arsenic concentrations were measured in excess of the MCL (10 µg/L) with the highest value in the Upper Elkhorn (Table 12).

3.1.3 Correlations between variables

The table below shows the correlation matrix between all the variables included in the study. All data points corresponding to nitrate, atrazine, uranium, and arsenic concentrations measured were used to conduct the analysis. Birth defect counts, tobacco use, and diabetes status were also included in the analysis.

Table 14. Pearson Correlation Coefficient

	Nitrate	Atrazine	Uranium	Arsenic	Diabetes	Tobacco	Birth defect cases
Nitrate	1	0.02	0.03	-0.18	-0.23	-0.26	-0.24
Atrazine	0.02	1	-0.01	-0.04	-0.09	-0.03	0.10
Uranium	0.03	-0.01	1	-0.08	0.03	0.01	-0.02
Arsenic	-0.18	-0.04	-0.08	1	-0.06	-0.01	-0.01
Diabetes	-0.23	-0.09	0.03	-0.06	1	**0.96**	0.76
Tobacco	-0.03	-0.03	0.01	-0.01	**0.96**	1	0.88
Birth defect cases	-0.24	0.10	-0.02	-0.01	0.76	0.88	1

Correlation analysis revealed a high correlation between the covariates, diabetes, and tobacco (r>0.80), thus only one of these variables will be included in statistical full model.

3.2 Geospatial analysis

Figure 14 represents a map of Nebraska birth defect prevalence distribution per watershed in quartiles with the first quartile prevalence (yellow color) below the national average.

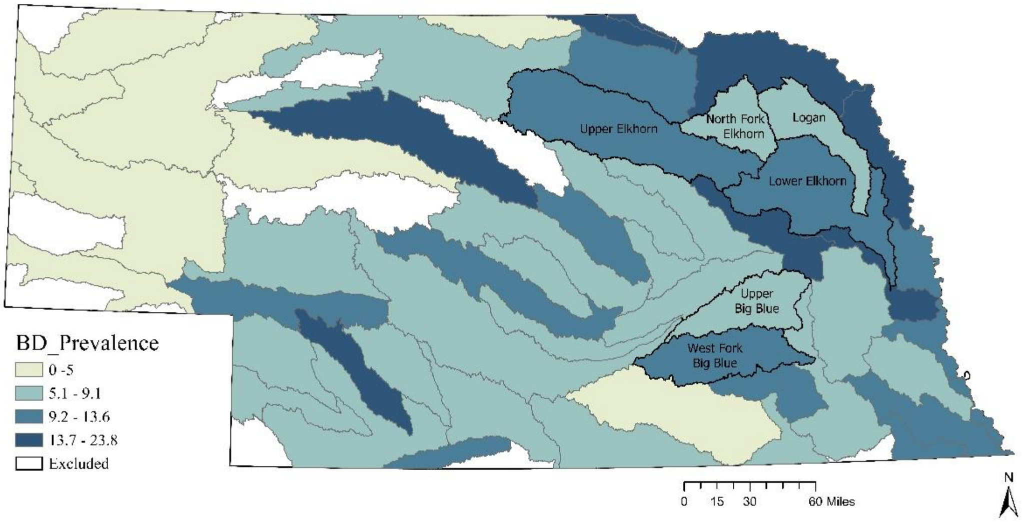

Figure 14. Birth defects prevalence in Nebraska watersheds from 1995 – 2014

More than 80% of Nebraska watersheds have birth defect prevalence above the national average (3-5 per 100 live births).

Figure 15 represents sampling locations with nitrate concentrations above 10 mg/L and birth defect (infant count) distribution per selected watershed.

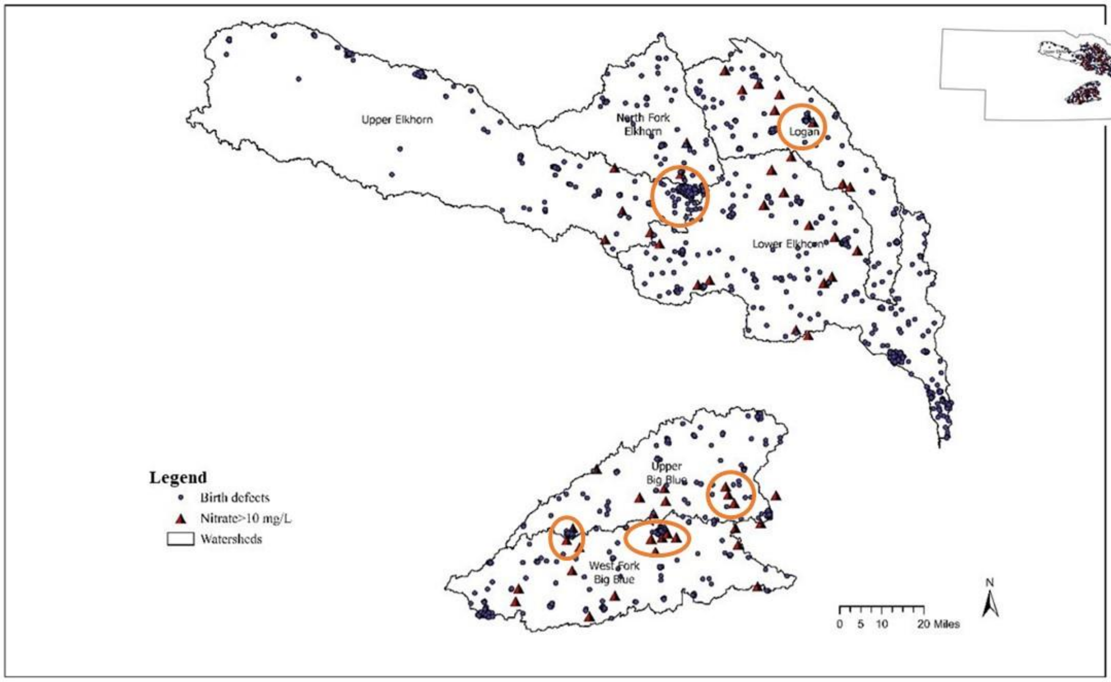

Figure 15. Geospatial representation of birth defect counts vs. nitrate concentration above 10 mg/L per selected watershed

We observed that several clusters of birth defect locations also have high nitrate concentrations as shown inside the circles on the map.

In the following map, birth defect counts in selected watersheds were plotted against all sites with detected atrazine levels and sites with atrazine concentration above the MCL (Figure 16).

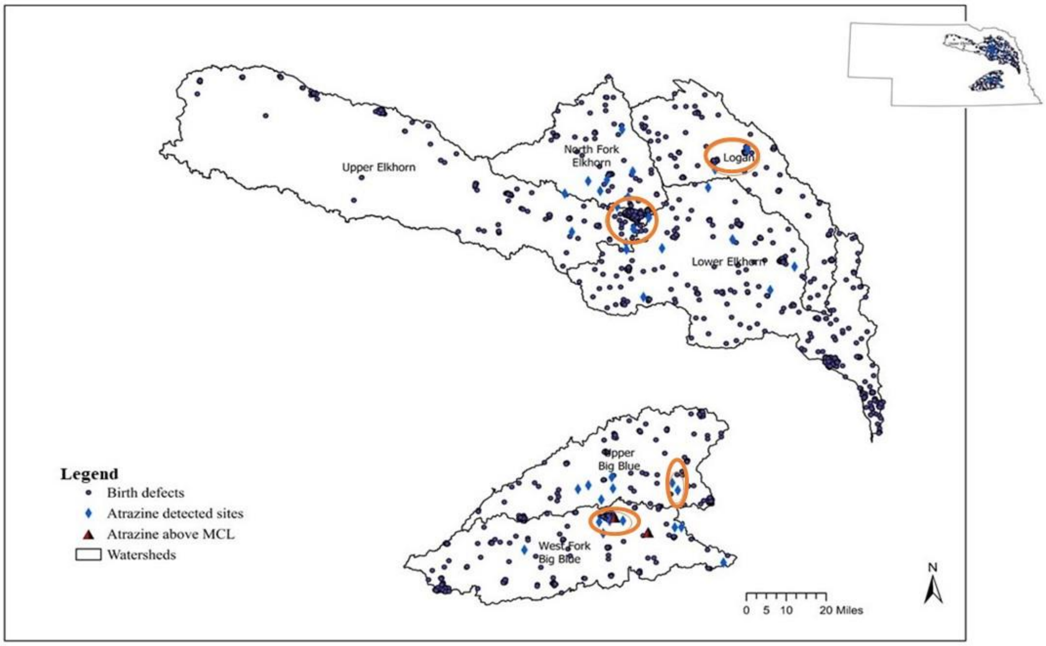

Figure 16. Geospatial representation of birth defect counts vs. atrazine concentration detected and above MCL (3 µg/L) per selected watershed

We observed that atrazine was detected in many locations with clusters cases of birth defects as indicated inside the circles on the map.

3.3 Inferential statistics

We conducted a negative binomial regression analysis to assess the association between birth defects and nitrate and atrazine. Table 14 shows the results of the univariable and multivariable negative binomial regression analysis.

Table 15. Analysis of the associations between birth defects and nitrate and atrazine concentrations in drinking water

Variables	**Birth Defects**	
	Univariable analysis IRR$_c$ (95% CI)[a]	**Multivariable analysis IRR$_a$ (95% CI)**[a]
Nitrate		
Group 1	Reference	Reference
Group 2	1.44 (1.40 – 1.50)	0.86 (0.83 – 0.89)
Atrazine		
Group 1	Reference	Reference
Group 2	2.84 (2.75 – 2.93)	1.62 (1.56 – 1.70)
Diabetes	1.03 (1.03 -1.04)	1.03 (1.02 – 1.04)
Tobacco[b]	1.01 (1.01 – 1.02)	N/A
Uranium[c]	1.03 (1.02 - 1.03)	0.96 (0.95 – 0.97)
Arsenic[c]	0.85 (0.84 - 0.86)	0.92 (0.89 – 0.94)

[a] Negative binomial regression analysis performed. The estimate of the model is IRR$_c$/IRR$_a$ = Incidence rate ratio (crude/adjusted). In this study it is referred to as birth defects prevalence.

[b] the variable tobacco was not included in the multivariable analysis, because it is collinear with the variable diabetes; only one of them should be in the model.

3.3.1 Birth defects and nitrate

While the univariable analysis showed a positive association between nitrate concentrations above 6.94 mg/L and birth defects (IRR = 1.44; CI: 1.40 – 1.50); the multivariable analysis showed a negative association between the two (IRR = 0.86; CI:0.83 – 0.89) (Table 5).

3.3.2 Birth defect and atrazine

In both the crude and adjusted models (Table 14), watersheds with atrazine concentrations in group 2 have a higher incidence rate of birth defects compared to the watersheds in group 1 (concentration not detected).

4. Discussion

In this study we determined the prevalence at birth of all birth defects in Nebraska watersheds. More than four-fifth of Nebraska watersheds reported a higher birth defect prevalence (Table 10) than the national average of 5 per 100 live births (Mai et al., 2015). Moreover, the watersheds included in the study had birth defect prevalence in the second and third quartiles (Figure 14), all above the national average.

These results aligned with the Nebraska Department of Health and Human Services report of a birth defect rate of 6 cases per 100 live births between 2011 and 2015 in Nebraska (Nebraska Department of Health and Human Services, 2015). Additionally, Corley et al. (2018) in their study revealed that Nebraska shared a disproportionate burden of birth defect-related deaths compared to the nation. Indeed in 2011, the mortality rate from birth defect was 1.94 per 100,000 in Nebraska compared to 1.27 per 100,000 for the United States.

We found relatively high nitrate mean concentrations in the watersheds included in the study. The mean nitrate concentrations ranged between 6.94 and 21.37 mg/L (Table 11). Three watersheds have nitrate concentrations above the MCL, and one is less than one unit lower than the MCL. Those concentrations correspond, as mentioned above, to watersheds with birth defect incidence above the national average.

In our univariable analysis we found that watersheds with nitrate concentration above 6.94 mg/L had higher incidence rate of birth defects compared to the reference group (Table 14). In the multivariable analysis, after controlling for the covariates of arsenic and uranium concentrations and a maternal risk factor (diabetes), we found a negative association between nitrate concentration and birth defect incidence rate. This observation can be explained by the fact that when additional birth defect risk factors are combined, higher nitrate concentration resulted in miscarriages, giving the impression that the number of birth defects has decreased; instead, the higher nitrate concentration was lethal in utero.

The teratogenic potential of nitrate is supported by the formation of *N*-nitrosamines in acidic conditions when nitrite reacts with amines. It is suggested that *N*-nitrosamines can cause malformation to the embryo or fetus (Ward et al., 2018).

The findings of the current research corroborate many other studies that reported a positive association between higher nitrate concentration in drinking water and the occurrence of birth defects. Among them, Brender and Weyer (2016) described a study that observed that women with drinking water nitrate concentration above 10 mg/L had four-time greater odds (95% CI: 1.0-15.4) of a birth defect (anencephaly) than their counterparts whose water supplies had nitrate concentration at or below 10 mg/L.

Additionally, another study found a significant increase (OR = 2.44; CI:1.05–5.66) in the incidence of birth defects for drinking water with nitrate levels of 1–5.56 mg/L compared to <1 mg/L, after controlling for variables such as the infant's gender, season of birth, the mother's age and parity and some maternal risk factors (smoking, diabetes, and thyroid disease) (Holtby et al., 2014).

Moreover, maternal ingestion of drinking water with nitrate concentration at or above 5 mg/L in the periconceptual period was associated with a greater odds of a birth defect (spina bifida) compared to mothers whose drinking water had nitrate level below 0.91 mg/L, after adjusting for confounders such as the mother's age, ethnicity, education level and folic acid intake (OR = 2.0; 95% CI: 1.3 – 3.2) (Brender et al., 2013).

We also found both in the univariable and adjusted models that detectable atrazine levels (>0.00 µg/L) were associated with higher incidence rates of birth defects.

Atrazine is potentially teratogenic because of its abilities to cause oxidative stress and congenital anomalies that were observed in animal models (Adeyemi et al., 2015; Wu et al., 2007).

The correlation between atrazine in drinking water and birth defect occurrence has been reported in many other studies. Indeed, Mattix et al. (2007) in their study using water quality data from USGS and birth defect data from the CDC and the Indiana Department of Health observed that increased monthly atrazine concentrations in surface water correlated with higher rates of abdominal wall defects (Mattix et al., 2007). Similarly, a positive association was found between gastroschisis and maternal proximity to water quality monitoring sites reporting atrazine concentrations > 3 μg/L (Waller et al., 2010) Moreover, Agopian et al. (2013) observed that maternal exposure to medium-low to medium levels of atrazine during the prenatal period was associated with birth defects – male genital malformations - in Texas from 1999 – 2008.

It is necessary to recognize that some studies that investigated the relationship between birth defects and nitrate or atrazine did not find any association. They include a case-control study conducted in Washington state that observed no association between gastroschisis and mother living close to a water quality monitoring site that reported nitrate concentrations above the MCL (10 mg/L) (Waller et al., 2010). Additionally, Mattix et al. (2007), who studied the relationship between nitrate and birth defects in Indiana, found no correlation between monthly abdominal wall defect rates and higher nitrate concentrations in drinking water. Moreover, researchers in Texas conducted a case-control study, and observed no positive association between maternal exposure to high atrazine levels in drinking water and an increased risk of congenital heart defects (Kim et al., 2017).

Compared to our study, the abovementioned studies - that did not find any association between birth defects and nitrate or atrazine concentrations – focused on specific birth defect types (abdominal wall defect or congenital heart defects). It is possible that if other birth defect types were investigated, a correlation might have been found. In our research, we looked at the relationship between all birth defects and nitrate or atrazine levels in drinking water.

Study Strengths and Limitations

This study has the advantage of using a large dataset of birth defects that occurred in Nebraska from 1995 – 2014. Many risk factors for birth defects were controlled in this study: arsenic, uranium, and maternal diabetes status. However, as an ecological study, individual-level exposure was not measured. Additionally, it was assumed that current contaminant levels were similar to levels during the exposure period. This is supported by the agricultural activities and practices that have been steady or increasing for many decades in Nebraska. Moreover, our previous findings (Ouattara et al., 2022) showed that nitrate and atrazine concentrations measured in monitoring wells have not dramatically changed from 1987 - 2016 (the mean estimate changed by less than 5%).

5. Conclusions

In this study, the hypothesis supported a potential positive association between birth defects and nitrate or atrazine concentrations in drinking water in the selected watersheds. The prevalence at birth of birth defects was calculated for each Nebraska watershed from 1995 – 2014. The mean nitrate and atrazine concentrations were computed for the watersheds included in the study. The findings showed that birth defect prevalence was above the national average for the watersheds included in the study. Mean nitrate concentrations were relatively high (6.94 – 21.37 mg/L), and atrazine was detected in all but one of the watersheds included in the study. In the regression analysis, a positive association was found between higher levels of nitrate in drinking water and the incidence rate of birth defects. Similarly, compared to watersheds with non-detectable atrazine levels, watersheds with any detected level of atrazine had higher incidence rates of birth defects. This study suggests that chronic exposure to nitrate and atrazine concentrations below the maximum contaminant limits may result in birth defects. It also highlights the relationship between anthropogenic activities (agriculture practices), water contamination, and adverse health effects in children.

Recommendations to address the health effects of water contamination include (1) continuous monitoring of private well water by state jurisdictions to ensure contaminants are present at safe levels, (2) provision of adequate water filtration systems or alternate sources of water to households with high contaminant levels in drinking water, and (3) implementation of efficient agricultural practices (carbon injection, efficient irrigation techniques, cover crops) to improve soil uptake and reduce agrichemical runoff into water supplies.

Acknowledgements: A special thank you to the Lower Elkhorn and Upper Big Blue natural resource districts for their support with water sample collection.

DISCUSSION

Conceptual model

This study applied the conceptual framework based on the eDPSEEA model (*ecosystems-enriched Drivers, Pressures, State, Exposure, Effects, Actions*) (Figure 17). The model was developed to describe the complex relationship between the environment and human health (Reis et al., 2015).

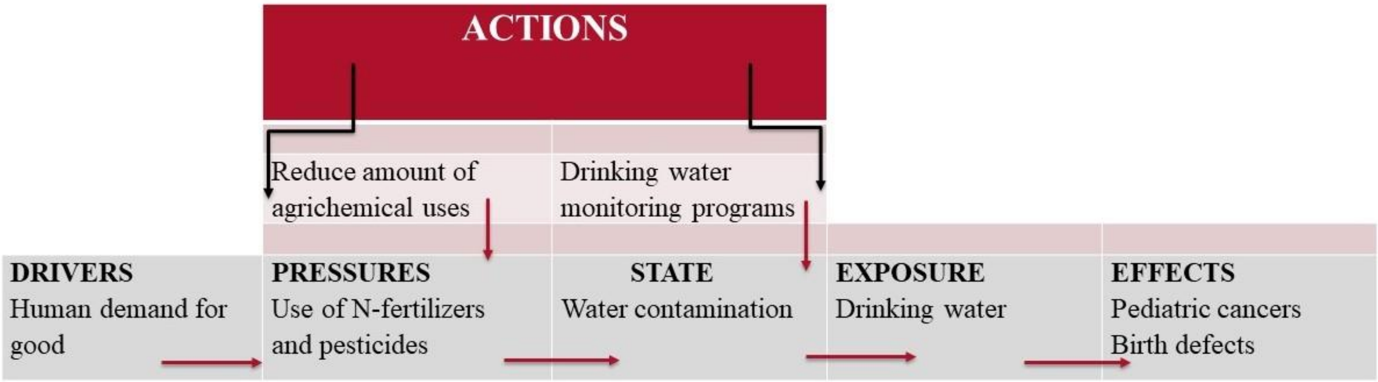

Figure 17. Conceptual Framework

The drivers in our research represent the human activities to produce goods and services. Industrial agriculture requires the use of nitrate-containing fertilizers and pesticides. In our model, the excessive use of nitrate-containing fertilizers and pesticides represents the pressures in response to the drivers. The State is the result of the "pressures." In our context, the “state” is the occurrence of nitrate and atrazine in water. Precipitation and irrigation events lead to nitrate and atrazine in water, with human exposure happening through drinking contaminated surface water or groundwater. Nitrate and atrazine health effects include methemoglobinemia, thyroid disease, congenital anomalies, and cancers. Actions could be taken to influence the drivers and the pressures to reduce or mitigate the effects of the State.

Health outcomes data sources and challenges

Different health outcomes have been investigated in this research: pediatric cancers of all types, brain and other central nervous systems cancers, leukemia, and lymphomas, and birth defects of all types.

The health data (pediatric cancer and birth defect data) were secondary longitudinal data collected throughout the years from different sources, including hospital and public health records. The data were aggregated by the state health department and maintained in specific registries. Since the data are not publicly available, the research team requested them from the Nebraska Department of Health and Human Services. Data obtention has been challenging at some point due to a Nebraska legislative act that was passed restraining data sharing by the health department. Some variables were denied to the research team, not allowing certain types of analyses to be conducted.

Water quality data

For the water quality parameters, both primary and secondary data were used. The secondary data were aggregated from different sources available to the general public – the water quality portal (National Water Quality Monitoring Council, 2021) and Nebraska Quality-Assessed Agrichemical Contaminant Database (University of Nebraska-Lincoln, 2020). Those data were mainly from monitoring wells. Private wells are not regularly tested because they are not regulated under the Safe Drinking Water Act that does not cover them, thus the scarcity of publicly available domestic well water data. For the monitoring well data, the general observation involved the inconsistency of sampling and measurement availability across counties or watersheds. Some locations were sampled regularly - monthly or annually– and have monthly or annual water quality data available. Other locations were not sampled periodically during the study, showing significant gaps in available data. It is necessary to mention that monitoring well construction and testing frequencies are not conventional but based on the levels of contaminants found during random field controls or in support of various state or federal water quality monitoring programs, and program priorities may change from year to year. These inconsistencies in water quality measurements limited the ability to conduct detailed time-series analyses, which impacted the research findings.

Primary water quality data were collected to address some of the limitations of the secondary data. Primary nitrate data collection was conducted through a citizen science project. Participants, mainly in rural Nebraska, consented to self-test (test kits and testing instructions provided) their drinking water and reported the results to the research team. The citizen science project has proven to be a cost-effective method for obtaining private well water quality data and providing recommendations to residents whose water tested high for specific contaminants. The recruitment of citizen scientists was a collaborative effort between researchers and community organizations.

Primary water data were also collected as part of a separate project in which water samples were obtained from volunteer well owners in two NRDs and tested in laboratories for different water contaminant levels. The involvement of local NRD personnel was imperative to gain residents' consent to participate in the research. These experiences suggest that future research needs to employ a community-based participatory research approach to be successful with a higher participation rate.

Similar trends were observed when comparing historical water quality data and recently collected data. Counties or watersheds with average high contaminant levels in this research have had high contaminant concentrations in the past. Thus, this study assumed that concentrations of water contaminants today provide a reasonable approximation of what they were in previous relevant decades, capturing accurate exposure for the health outcomes being assessed. This approach was partially based on the knowledge that rural families, especially farm families, do not tend to move often and use of farmland is also relatively unchanging.

Pediatric cancers and agrichemicals used in Nebraska

The calculated age-adjusted pediatric cancer incidence was higher than the national average (4.42 vs. 3.16) between 1987 and 2016 in Nebraska. These results aligned with clinical observations and previous research that reported high pediatric cancer incidence in Nebraska, among the five highest in the nation. When broken down into specific types of cancers, the three major types of pediatric cancers in Nebraska were brain and other CNS cancers, leukemia, and lymphoma, respectively. At the national level, the three predominant types of pediatric cancers were: leukemia, brain and other CNS cancers, and lymphoma, respectively. It stood out that the most prevalent type of pediatric cancer in Nebraska is brain cancer, but not leukemia as for the nation. That led to exploring pediatric cancer risk factor profiles in Nebraska compared to the nation.

One fact is that Nebraska is among the most intensive agricultural states in the USA, with extensive use of nitrate-containing fertilizers and pesticides. As described previously, the ingestion of nitrates in drinking water may result in the formation of *N*-nitroso compounds (NOC). NOCs are potent carcinogens in animals and able to cross the placenta and the blood-brain barrier. Thus, the hypothesis is that NOCs can cause brain cancers in children (Vienne-Jumeau et al., 2019).

A question that would immediately spring to mind is: do all the intensive agricultural states like Iowa – which is a neighboring state to Nebraska - have a high incidence of pediatric cancers? The quick answer is no, but there is more to know: some states do not have a well-established cancer registry, and researchers have not investigated the question of cancer risk profile in these states.

Through geospatial and statistical analyses, the current study observed positive associations between pediatric cancers - all types, brain and other CNS cancers, leukemia, and lymphoma - and nitrate or atrazine concentrations in drinking water. Although a causal relationship was not established, these results suggest that atrazine and nitrate can be added to the pediatric cancer risk profile in Nebraska.

These results also add to the scientific evidence of a positive correlation between nitrate or atrazine concentrations and pediatric cancers.

IARC and nitrate or atrazine carcinogenicity

The International Agency for Research on Cancer (IARC) is the World Health Organization (WHO) agency that promotes international collaboration in cancer research. IARC working groups conduct a comprehensive review of the scientific literature to gather evidence from all types of studies (animal models, laboratory testing, and epidemiological studies) to help classify chemicals based on their carcinogenic potential. All agents reviewed by IARC have been classified in one of the following four groups (International Agency for Research on Cancer, 2022):

Group 1 Carcinogenic to humans

Group 2A Probably carcinogenic to humans

Group 2B Possibly carcinogenic to humans

Group 3 Not classifiable as to its carcinogenicity to humans

For the chemicals involved in this study, nitrate has been classified by IARC as "Probably carcinogenic to humans," which corresponds to Group 2A (International Agency for Research on Cancer, 2010). Atrazine was classified in 1999 in group 3 because of conflicting study findings (IARC, 1999). One can argue that new evidence has emerged from recent studies, and the IARC may consider reevaluating atrazine regarding its carcinogenic potential.

EPA and nitrate or atrazine carcinogenicity

The United States Environmental Protection Agency (EPA) does not consider nitrate or atrazine as carcinogens. The US EPA conducts periodic risk assessments on chemicals and is responsible for identifying and regulating cancer-causing agents. According to the EPA, there is insufficient evidence to categorize nitrate or atrazine as carcinogenic to humans.

Risk-based vs. precautionary approach

It is necessary to emphasize that the U.S. focuses on risk-based approach regarding chemical safety and toxicity: a chemical is not regulated until sufficient, overwhelming evidence has been gathered regarding its ability to cause harm to humans. In contrast, the European Union follows precautionary-based approach: chemicals are highly regulated when sufficient information is not available regarding their safety (Klinke & Renn, 2002).

Pediatric cancer latency period and exposure window

The latency period for pediatric cancers is unknown, making it challenging to determine the exposure period using secondary cancer data. In the dataset used for this study, pediatric cancers occurred at any age between 0 and 19 years old. If environmental carcinogens were incriminated, it is problematic to determine when exposure occurred – periconceptionally through the mother? or after birth?

Chemicals MCL in drinking water and cancer

The EPA has established the MCL of nitrate at 10 mg/L of nitrate as N. This was the safe concentration to avoid methemoglobinemia in children. Methemoglobinemia was the first-ever described health problem related to acute exposure to nitrate in drinking water. In this research as well as in some previous studies, nitrate concentrations as low as 1 mg/L - below 10 mg/L - were associated with pediatric cancers. Cancer being a chronic health outcome, chronic exposure to lower concentrations of nitrate may be harmful to humans, especially children. That raises the question of the current MCL safety and thus suggests and invites the EPA to revise the maximum contaminant limit of nitrate in drinking water.

Lower levels or even just detected levels of atrazine had been found to be associated with pediatric cancers in this research and previous studies. This raises the question of whether the MCL of 3 μg/L is really safe for human consumption in drinking water. To optimize human health and prevent disease, we recommend appropriate regulation for atrazine and other pesticides to not have any allowable levels in drinking water. In other words, atrazine should not be detected in drinking water. These chemicals are not natural – they are fully artificial

Birth defects and agrichemicals

Birth defect prevalence was higher in Nebraska than the national average during the study period (1995 – 2014). Additionally, high birth defect prevalence locations also have relatively high nitrate and atrazine concentrations. Nitrate and atrazine concentrations below the MCL have been associated with birth defects in the current research and previous studies. This emphasizes the concern regarding the safety of the established MCL for nitrate, 10 mg/L and atrazine, 3 ug/L. Based on our observations, chronic exposure to lower levels than the MCL of agrichemicals may lead to birth defects. Contrary to the health outcome pediatric cancer, where the exposure period is difficult to establish, for birth defects exposure to environmental toxin occurs before or during conception.

Nebraska is a highly agricultural state and a national leader in the production of corn, soybeans, and beef cattle. Women represent 27% of farmers in Nebraska and 36% in the U.S. (United States Department of Agriculture, 2017; USDA National Agricultural Statistics Service, 2022)**.** Female farmers are younger and more likely to live on the farm than their counterpart male farmers (USDA National Agricultural Statistics Service, 2022), where they are potentially exposed to agrichemicals used in agricultural production.

The current research suggests that these agrichemicals pose a risk for birth defects in general. Specific types of birth defects were not assessed. It is possible that exposure to atrazine or nitrate was associated with certain birth defect types but not others. More than one hundred birth defect types were found in each of the selected watersheds, not allowing us to focus on any specific type.

CONCLUSION

This research investigated the risk that agrichemicals – atrazine and nitrate – pose to children's health. It has been suggested that both atrazine and nitrate represent risk factors for pediatric cancers and birth defects in Nebraska at concentrations below the maximum contaminant levels defined by EPA for the two chemicals. Nebraska is underlain by the High Plains Aquifer, which extends from the Dakotas into Texas. The methodologies employed in this study could be used by other researchers in states existing within the footprint of the High Plains Aquifer, including states with significant agricultural activity. Additionally, the findings of this research laid the foundation for future research in the field. Based on the findings of our research and previous studies and applying a precautionary approach, we can therefore make recommendations regarding environmental exposures to nitrate and atrazine in drinking water and the risk of pediatric cancers and birth defects:

1. Develop and implement statewide private well water monitoring programs.
2. While waiting for regulations to be implemented, advise pregnant women and parents of young children to regularly test their drinking water if it is supplied by a private well.
3. Teach farmers appropriate irrigation practices to reduce runoff of farm contaminants into waterways
4. Conduct studies to determine the appropriate quantity of fertilizers and pesticides to use per acre of cropland.
5. Use carbon injection technology to improve nitrogen intake and reduce its losses in the soil.
6. Use advanced statistical analyses such as the multiple imputation method to fill in water quality values where measurements were inconsistent.
7. Conduct prospective cohort studies to help establish causality. Additional studies with more rigorous methodologies are required to increase the level of evidence allowing an updated classification of atrazine and nitrate as established carcinogens and teratogens.
8. Revise the MCL for nitrate to lower levels in drinking water
9. Revise the MCL for atrazine to zero concentrations in drinking water.

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