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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 groundwater was associated with pediatric cancers

Supporting Information:

Supporting Information may be found in the online version of this article.

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Geospatial Distribution of Age-Adjusted Incidence of the Three Major Types of Pediatric Cancers and Waterborne Agrichemicals in Nebraska

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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 surface and 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 cancer incidence was higher than the national average in 63% of the Nebraska counties. After controlling for the counties 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 one 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 to develop 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 3,357 tons of atrazine were applied to Nebraska cornfields (Wieben, 2019). Nitrate is used as a 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 (SW) and groundwater (GW), 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

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limits (MCL) of 10 mg/L for nitrate as nitrogen and three (3) µg/L for atrazine (EPA, 2020a; Nebraska Department of Environmental Quality, 2020). Under the Safe Drinking Water Act (SDWA), the EPA has set up, for more than 90 contaminants, health-based drinking water standards and regulations. However, the 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 (DeSimone et al., 2009; EPA, 2020b).

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 pesticide 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, 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 cancers 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 cancers, leukemia, and lymphoma recorded in the Nebraska Cancer Registry between 01 January 1987 and 31 December 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 (DHHS) 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 (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 drinking water quality in a given geographic area (Nebraska Department of Environmental Quality, 2020).

2.1.3. GIS and Other Data

The Nebraska state and county boundary shapefiles were extracted from the United States Census Bureau (2019). The 2010 U.S 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 (SVI) 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):

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

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 an analysis over an extended study period using a single census year. Second, 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 cancers make the event rarer and annual case counts very small. Finally, we divided our study period to a 10-year time series (1987–1996, 1997–2006, and 2007–2016) to match the three decennial censuses (1990, 2000, and 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 a population of 200 or lower.

Table 1
Classification of Mean Atrazine and Nitrate Concentrations in Categories

	Atrazine (μg/L)		Nitrate (mg/L)	
Categories	SW[a]	GW[b]	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

[a]SW = surface water. [b]GW = groundwater.

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.

2.3. Negative Binomial Regression Analysis

2.3.1. 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:

$$\ln(\text{counts}) = \text{b0} + \text{b1} * \text{atrazine GW} + \text{b2} * \text{nitrate GW} + \text{b3} * \text{RPL\_theme1} + \text{offset}(\ln(\text{population}))$$

2.3.2. 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 do not know the duration of exposure or the latency period between exposure and cancer diagnosis. Second, 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 (Tables S1–S4 in Supporting Information S1) 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 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

Table 2
Most Common Pediatric Cancers Types in Nebraska (1987–2016) and the United States

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

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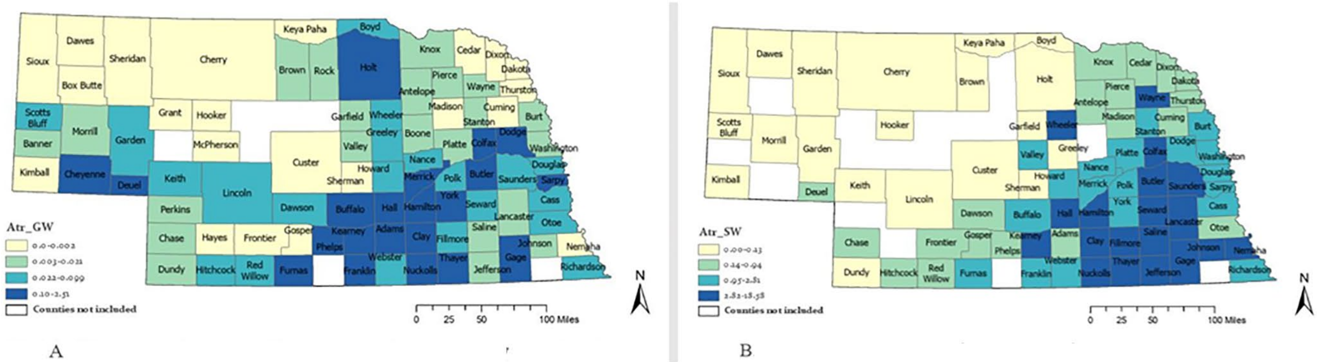

Figure 1. (a) Mean groundwater atrazine concentration per county in Nebraska from 1987 to 2016. (b) Mean surface water atrazine concentration per county in Nebraska from 1987 to 2016.

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.

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, 13 types of cancers 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 United States (ACCO, 2014).

3.1.2. Nitrate and Atrazine Concentration

Growing seasons groundwater (GW) and SW mean atrazine and nitrate concentrations are represented for counties included in the studies and where measurements were completed (Figures 1 and 2).

In addition to counties not included in our study, some counties were not sampled at all for SW 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 SW atrazine and nitrate, the first quartile (lowest concentration) is the most represented among the counties.

Descriptive statistics for the 10 counties that were most frequently and least frequently sampled during our study period are shown in Tables 3–6. For full list of counties please refer Tables S1-S6 in Supporting Information S1.

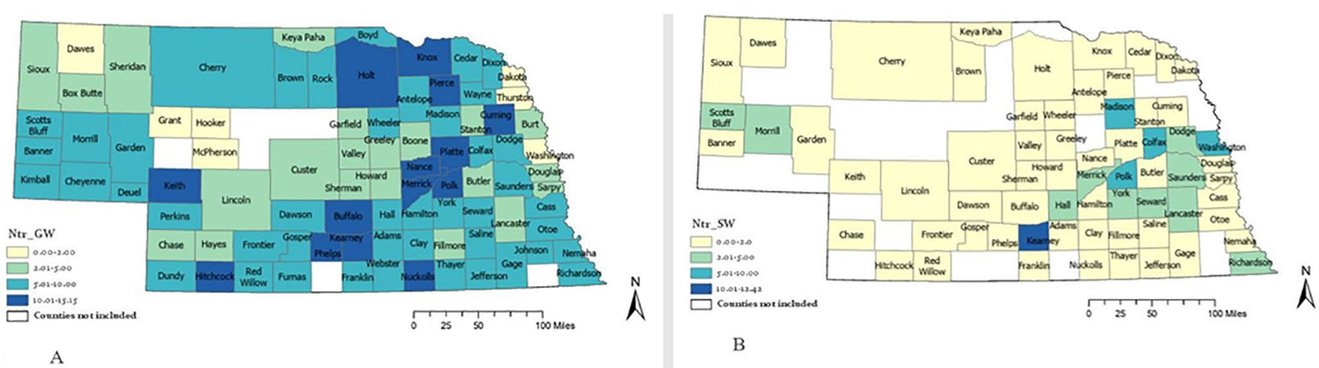

Figure 2. (a) Mean groundwater nitrate concentration per county in Nebraska from 1987 to 2016. (b) Mean groundwater nitrate concentration per county in Nebraska county, 1987–2016.

Table 3
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 to 2016. [c]Concentration in μg/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 data set into three 10-year periods. Compared to the first 10 years (1987–1996) and third 10-year period (2007–2016), CNS cancer and lymphoma crude incidences were higher in most counties during the second 10-year period (1997–2006; Figures 3a–3c).

3.2.2. Water Quality Data

We split the water quality data into three 10-year periods. Compared to the first 10 years (1987–1996) and the third 10-year period (2007–2016), atrazine in groundwater during the years 1997–2006 were more consistently measured and had higher concentrations (Figure 4a).

Table 4
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 to 2016. [c]Concentration in μg/L.

Table 5
Descriptive Statistics of Groundwater Nitrate for Selected Counties With Lower Higher and 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 to 2016. [c]Concentration in mg/L.

Regarding groundwater nitrate, measurements were less frequent but higher during the same period (1997–2006; Figure 4b).

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 7). 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 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.

3.4. Geospatial Analysis

The incidence of the three major types of pediatric cancers is shown on a map of Nebraska with counties delineated (Figures 5–7). 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 (yellow and green). The green color represents counties with

Table 6
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 to 2016. [c]Concentration in mg/L.

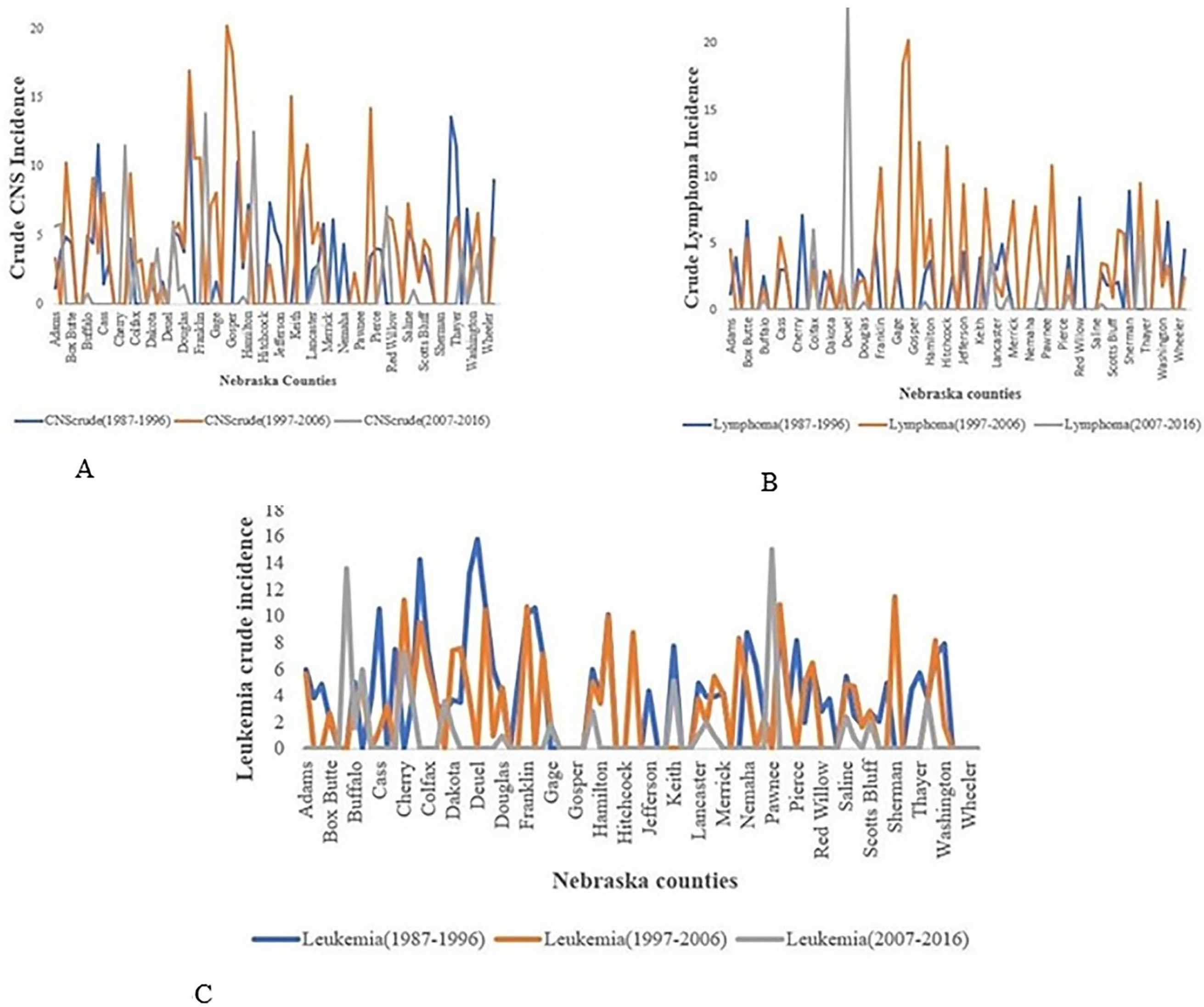

Figure 3. (a) Pediatric central nervous system crude incidence in Nebraska counties over three decades. (B) Pediatric lymphoma crude incidence in Nebraska counties over three decades. (c) Pediatric leukemia crude incidence in Nebraska counties over three decades.

incidence above the national average and the yellow counties with incidence below the national average. Counties excluded from the analysis are left blank.

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 contaminant concentrations in our data set are a proxy for their levels in drinking water. Thereby, we focused the regression analysis on the groundwater concentrations of atrazine and nitrate. Table 8 represents the results of the negative binomial multivariable analysis.

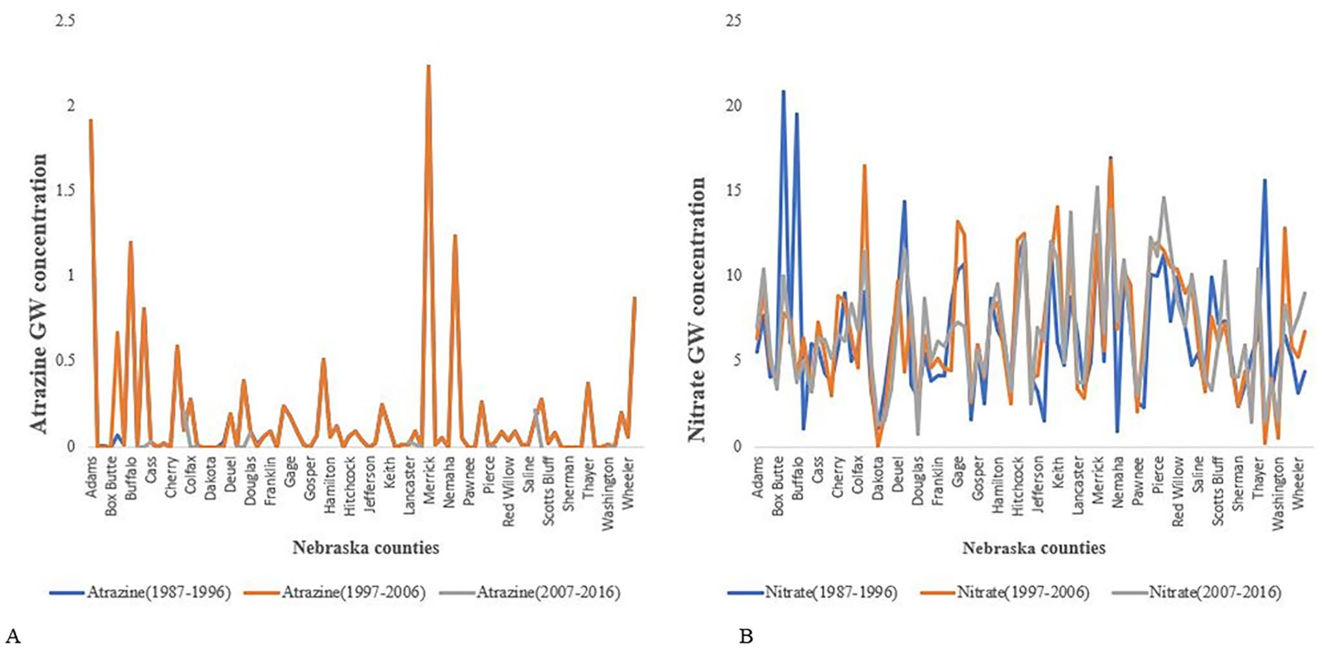

Figure 4. (a) Longitudinal analysis of groundwater atrazine in Nebraska counties. (b) Longitudinal analysis of groundwater nitrate in Nebraska counties.

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).

Table 7
Age-Adjusted Incidence of Pediatric Cancers in Nebraska and the United States

Cancer type	Nebraska age-adjusted incidence (per 100,000)	National age-adjusted incidence (per 100,000)
Brain and other CNS cancers	4.42	3.16
Leukemia	3.67	4.66
Lymphoma	2.72	2.65

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 female applicators of atrazine compared to female nonapplicators. 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 preclinical 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-con-

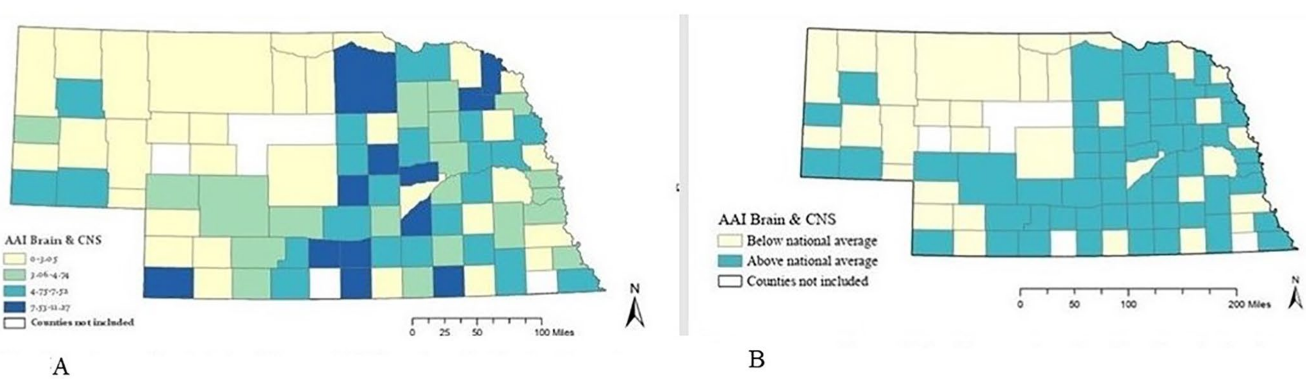

Figure 5. (a) Age-adjusted incidence (AAI) of pediatric brain and other CNS cancers per county in Nebraska from 1987 to 2016. (b) Age-adjusted incidence (AAI) of pediatric brain and other CNS cancers in Nebraska counties compared to the national average.

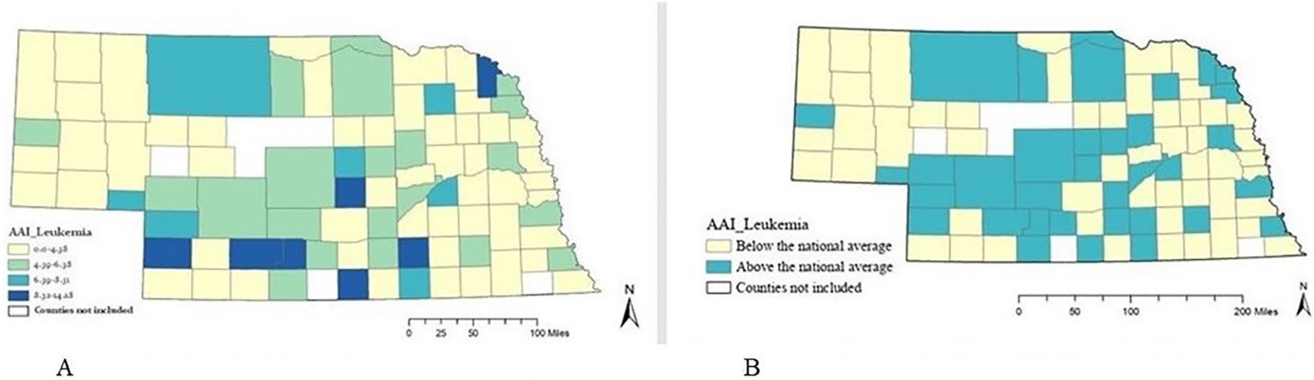

Figure 6. (a) Age-adjusted incidence (AAI) of pediatric leukemia per county in Nebraska from 1987 to 2016. (b) Age-adjusted incidence (AAI) of pediatric leukemia in Nebraska counties compared to the national average.

trol 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 10 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).

4.1. Strengths and Limitations

This ecological study by design has the advantage of a relatively large health data set and controlled for the counties 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 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

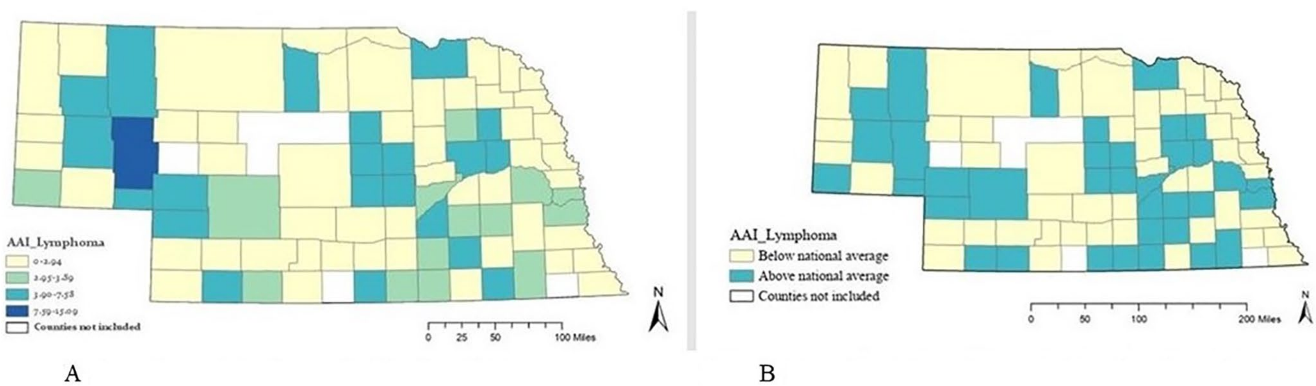

Figure 7. (a) Age-adjusted incidence (AAI) of pediatric lymphoma per county in Nebraska from 1987 to 2016. (b) Age-adjusted incidence (AAI) of pediatric lymphoma in Nebraska counties compared to the national average.

Table 8
Multivariable Analysis of the Associations Between Pediatric Cancers (Brain and Other CNS, Leukemia, and Lymphoma) and Agrichemical Concentrations in Groundwater (Atrazine and Nitrate)

	Brain and other CNS full model[a]	Leukemia full model[a]	Lymphoma full model[a]
Variables	IRR_a (95% CI)	IRR_a (95% CI)	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.

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 to 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.

Conflict of Interest

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

Data Availability Statement

The following data sets used for this research are publicly available. Links and parameters are provided below and complete references are given in the References section: (a) 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 07 October 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 data sets were set to 2010_SF1a. (b) Atrazine and nitrate data were retrieved from the water quality portal (https://www.waterqualitydata.us/portal/; accessed 7 October 2020) and Quality-assessed Agrichemical Contaminant Database for Nebraska Groundwater (https://clearinghouse.nebraska.gov/well-explorer2020;accessed 7 October 2020). The parameters set for the water quality portal are as follows: 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 from 01/01/1987 to 12/31/2016; water (NWIS, STEWARDS, and STORET) was selected for sample media. Nutrients and Pesticides were selected for the 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. (c) The Nebraska state and county boundary shapefiles were extracted from the United States Census Bureau https://www2.census.gov/geo/tiger/TIGER2019/; accessed 7 October 2020. The Files named County and State were selected and downloaded separately. (d) The CDC SVI 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 DHHS upon completion and approval of data use agreement forms. For further information contact the Nebraska DHHS at DHHS.PublicRecords@nebraska.gov.

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