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Association between Aqueous Atrazine and Pediatric Cancer in Nebraska

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Abstract: Agrichemicals, chemicals used to maximize crop and animal production, can lead to water quality concerns when these chemicals run off into surface and groundwater after precipitation events. In Nebraska, one such chemical is atrazine, a suspected carcinogen. This study evaluated the association between atrazine in surface and groundwater, in relation to the incidence of pediatric cancer in Nebraska watersheds over 30 years (1 January 1987 to 31 December 2016). The watersheds were grouped into four categories based on the average atrazine concentration over the study period, using quantile classification. The associations between atrazine (ground/surface) and pediatric cancer after adjusting for social vulnerability index (SVI) variables, using pediatric population as offset, were compared using a generalized linear model (GLM) assuming a negative binomial distribution. The results from the GLM approach suggested positive associations between watersheds with higher atrazine concentration and a higher pediatric cancer incidence rate ratio. In this study, the associations were evaluated using atrazine measurements obtained from non-drinking water sources as a proxy measure. Further research is necessary to establish the causal relationship between atrazine and pediatric cancer.

Keywords: watersheds; atrazine; pediatric cancer; herbicides; water quality

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1. Introduction

According to the 2017 United States Department of Agriculture (USDA) census of agriculture, about half of Nebraska's land was used for crop production, with approximately one-quarter of the land in the state used to raise corn [1]. Atrazine, currently the second most prevalently used herbicide in the country, is routinely used in corn production [2]. Simulations have estimated that 75–89% of atrazine applied on cropland could run off through the soil, eventually infiltrating into groundwater sources, while an additional 20% may be transported from agricultural fields via surface water sources [3,4]. In Nebraska, 22% of well water samples tested for atrazine in 2018 exceeded the United States Environmental Protection Agency (US EPA) Maximum Contaminant Limit (MCL) of 3 µg/L [5].

The association between atrazine and cancer is, by no means, a resolved issue. On the one hand, a population-based study conducted in Nebraska identified an association between the interaction of atrazine with nitrate in drinking water and lymphoma among adults [6]. Similarly, an in vitro study using cell lines suggested that exposure to atrazine caused cancer [7]. In contrast, other studies suggest a null association between atrazine and cancer [8]. Despite the inconsistent findings, the International Agency for Research on Cancer (IARC) classified atrazine as unlikely to be a human carcinogen [9]. In parallel, the

US EPA reviewed the results from human-based studies and summarized that there is no aggregate risk associated with atrazine [10]. However, it is important to note that both the IARC and US EPA reports were mostly based on occupational exposure to atrazine among adults. Consequently, the association between atrazine and cancer is an unresolved issue. In addition to the studies that reported the association between atrazine exposure and pediatric cancer, there are studies that identified that atrazine is associated with fetal deaths, pre-term births, neurologic disorders, and endocrine disorders among humans [11]. Additionally, there are several pre-clinical studies that suggest that atrazine is associated with alternations in metamorphosis, reduced immune function, and abnormalities in sex hormones [12].

In Nebraska, the rate of pediatric cancer was 3.7% higher than the U.S. national average during the period 1990–2013 [13]. The objective of this study is to evaluate the potential association between aqueous atrazine and pediatric cancer. In prior work, it was determined that using watershed boundaries rather than census tracts or counties can improve our understanding of the association between water pollutants and relevant human health outcomes [14–17]. Additionally, as socioeconomic status was identified as a vulnerability factor to exacerbate the risk of cancer [18], we attempted to adjust for the social vulnerability effect on pediatric cancer. The associations were evaluated using long-term mean atrazine and pediatric cancer, accounting for social vulnerabilities on a watershed scale. Using watersheds to understand the associations between atrazine concentrations from water samples and pediatric cancer adds novelty to our study.

2. Materials and Methods

The atrazine water quality measurements over the study period (1 January 1987 to 31 December 2016) were obtained from three open-source databases: the Nebraska Clearinghouse (Quality-Assessed Agrichemical Contaminant Database for Nebraska Ground Water), STORage and retrieval (STORET) database by US EPA, and the United States Geological Survey (USGS). This study included 27,395 groundwater and 31,440 surface water atrazine observations. The groundwater samples were restricted to irrigation wells, and surface water samples were sampled from lakes, rivers, streams, and reservoirs. About 66% of groundwater measurements (18,072) were obtained from the clearinghouse database and 34% measurements (9323) from USGS [19,20]. About 70% of surface water measurements (21,743) obtained from USGS, and 30% (9697) from the STORET database [19,21]. The surface water data from USGS was obtained using three parameters: P39630 (atrazine, water, unfiltered, recoverable(μg/L)), P39632 (atrazine, water, filtered, recoverable (μg/L)), P99775 (atrazine, water, filtered, immunoassay, unadjusted, recoverable (μg/L)). The water samples reported by the clearinghouse were analyzed using an Enzyme-Linked Immunosorbent Assay (ELISA) method with a level of quantification (LOQ) of 0.05 μg/L [22]. The USGS samples were analyzed using Gas Chromatography with Mass spectrometry (GC/MS) with Single Ion Monitoring with a LOQ of 0.01 μg/L and Liquid chromatography with tandem mass spectrometry (LC/MS/MS) with a LOQ of 0.01 μg/L [19]. The water samples from the STORET database were based on US EPA method 507 (Gas Chromatography with a Nitrogen-Phosphorus detector) with a LOQ of 0.01 μg/L [23].

The HUC-8 (hydrologic unit code) level watershed boundary shapefiles were obtained from the USGS-National Hydrography database [24]. The water quality data was spatially standardized to HUC-8 delineations. Mean atrazine concentrations (μg/L) for both ground and surface water were estimated during the study period by watershed, then categorized into four quantiles. Since atrazine concentration was much higher in surface water relative to groundwater, different quartile classifications were used for each. The surface atrazine concentration groups could be described as negligible (0–0.03 μg/L), low (0.031–0.293 μg/L), medium (0.294–1.057 μg/L) and high (1.058–4.75 μg/L). Similarly, the groundwater atrazine concentration groups could be described as negligible (0 μg/L), low (0.001–0.007 μg/L), medium (0.0071–0.081 μg/L) and high (0.082–1.45 μg/L).

The pediatric cancer data obtained from the Nebraska Cancer Registry includes individuals diagnosed with cancer that were $\leq$19 years of age at the time of diagnosis [25].

Cancer diagnosis includes invasive and in situ tumors and does not contain benign polyps or basal/squamous cell carcinoma of the skin. The dataset contains the subject's address, primary cancer diagnosis, date of birth, and diagnosis date. The subject's cancer diagnosis was classified into 12 groups based on the International Classification of Childhood Cancer (ICCC-3). The subject's complete address was geocoded to obtain latitude and longitude using Environmental Systems Research Institute (ESRI) world geocoder [26]. Most of the study subjects were automatically matched by complete address (99.1%; n = 2538), and negligible records (0.9%; n = 21) were tied (matched with multiple locations). The tied geolocations were manually matched in a separate batch. The mean pediatric cancer count per watershed during the study period was estimated.

The 2010 decennial census dataset by census block represented as centroids were obtained from the U.S. census bureau [27]. Additionally, the 2010 social vulnerability index (SVI) variables at the census tract scale were obtained from the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (CDC/ATSDR) [28] and processed to obtain census tract centroids. The metric percentile proportion of SVI variables were used in this study. Both the pediatric population and SVI variables were then aggregated to watershed boundaries.

All the datasets (water quality, pediatric cancer, pediatric population, and SVI) were standardized to the North American Datum 1983 (NAD 1983) geographic coordinate system using ArcGIS Pro Ver. 2.3. About 32% (23/72) of the Nebraska watersheds were excluded in the analysis, as they are small subunits that lie predominantly in the neighboring states of Iowa, South Dakota, Kansas, Colorado, or Wyoming. The watersheds excluded in this study only represent 4.2% of the total Nebraska land area.

The association between atrazine and pediatric cancer was evaluated by comparing the long-term mean atrazine and pediatric cancer, accounting for pediatric population density and social vulnerabilities. Incidence rate ratios (IRR) and 95% confidence intervals were generated by comparing pediatric cancer incidence among watersheds categorized as low, medium, and high with negligible atrazine watersheds as a reference, using a generalized linear model (GLM) assuming negative binomial distribution using R version 4.1, R library MASS version 7.3 [29]. Using the GLM framework, we evaluated the associations among three scenarios unadjusted model, adjusted_1, and adjusted_2 model. The unadjusted model does not control for covariates, using atrazine concentration as the independent variable and pediatric cancer count per watershed as the dependent variable. Both the adjusted_1 and adjusted_2 models, were an extension to the unadjusted model. The adjusted_1 model measured the association between atrazine and pediatric cancer by controlling for covariates. The covariates included in the adjusted_1 model were selected by performing sensitivity analysis using the GLM framework (Table S1). The covariates: unemployment, single parent, minority, and English language barrier were found to be positively associated with pediatric cancer and included in adjusted_1 model. The adjusted_2 model contains atrazine concentration as the independent variable; pediatric cancer count as the dependent variable and the overall SVI score as a covariate. We included the pediatric population as an offset term in all three statistical models.

3. Results

3.1. Surface and Groundwater Atrazine Concentrations

Within each watershed the atrazine ground and surface water sampling was similar between the growing season (April–October), and the non-growing season (November–March). The annual median number of groundwater samples that were collected per watershed during the growing season was 7 (interquartile range (IQR) 2–23), and in the non-growing season, it was 6 (IQR 2–18). Consistently, the annual median number of surface water samples that were collected per watershed were 9 (IQR 4–31) during the growing season, and 6 (IQR 3–15) during the non-growing season. During the growing season, 4.2% of the surface water samples, and 4.6% of groundwater exceeded the US EPA maximum contaminant limit for atrazine (3 μg/L).

Over the course of 30 years (1 January 1987–31 December 2017), the Mud, Lower Platte, Logan, and Lower North Loup watersheds had the highest mean atrazine water concentrations. We observed frequent spikes in the Lower Platte watershed surface water atrazine concentrations above the US EPA maximum contaminant limit during the growing season. The water-quality observations for Lower Platte and Lower North Loup revealed greater atrazine measurement frequency across the study period, whereas the Mud and Logan watersheds measurements were inconsistent (Table 1).

Table 1. Watersheds with higher age-adjusted pediatric cancer incidence and atrazine concentrations.

HUC-8 Watershed	Corresponding Counties	Atrazine Measuremnts		Atrazine Concentration		Incidence [a]
		Surface	Ground	Surface	Ground	
Mud	Custer, Sherman	89	33	1.857	0	36.29
Lower Platte	Cass, Sarpy, Dodge	1172	1106	0.044	0.789	35.14
Logan	Wayne, Cedar, Thurston, Cuming, Burt	148	220	0.001	0.360	34.64
Lower North Loup	Valley, Greeley, Howard, Loup, Sherman, Custer	430	83	0.001	0.354	30.57

Note: The ground and surface water atrazine were measured in µg/L. [a] Age-adjusted pediatric cancer incidence. Atrazine measurements—Total number of atrazine water quality measurements during the study period.

3.2. Pediatric Cancer

There were 2559 children diagnosed with pediatric cancer during the study period with a median age of 8 years (IQR 7–8.9 years). The mean age-adjusted pediatric cancer incidence in Nebraska is 17.21 per 100,000 children. Since the year 2000, age-adjusted pediatric cancer incidence is on an increasing trend. The annual mean age-adjusted pediatric cancer incidence in Nebraska was higher than the national average during 2011 (18.82, 18.04), 2012 (19.79, 17.80), 2015 (19.30, 19.03), and 2016 (20.79, 18.60).

The most frequently diagnosed cancer types are central nervous system (CNS) cancer, lymphoma, and leukemia. Among the children included in the study, 26% (665) were diagnosed with CNS cancer with a median age of 9 years (IQR: 4–14) at the time of diagnosis, 24% (625) were diagnosed with leukemia with a median age of 6 years (IQR: 3–12), and 16% (405) were diagnosed with lymphoma with a median age of 15 years (IQR: 9–17). Based on the median age distribution at cancer diagnosis, we observed that neuroblastoma, retinoblastoma, hepatic/renal tumors, and leukemia were diagnosed at an early age. Additionally, children living in watersheds with high surface (>0.031 µg/L)/ground (>0.001 µg/L) atrazine concentrations were diagnosed with cancer at an earlier age (median: 8.5 years) than the age of children (median: 11 years) living in watersheds with lower atrazine concentrations.

3.3. Association between Atrazine and Pediatric Cancer

During the study period, 29% (14/49) of watersheds had higher age-adjusted pediatric cancer incidence than the national average. The majority of the watersheds located towards the eastern part of Nebraska had higher age-adjusted pediatric cancer incidence than the state average. The mean ground and surface atrazine concentrations were higher in the southeastern part of Nebraska. A few watersheds with higher atrazine concentration overlapped with watersheds with high pediatric cancer incidence (Figure 1). Mud, Lower Platte, and Logan were the watersheds with the highest pediatric cancer incidence in Nebraska.

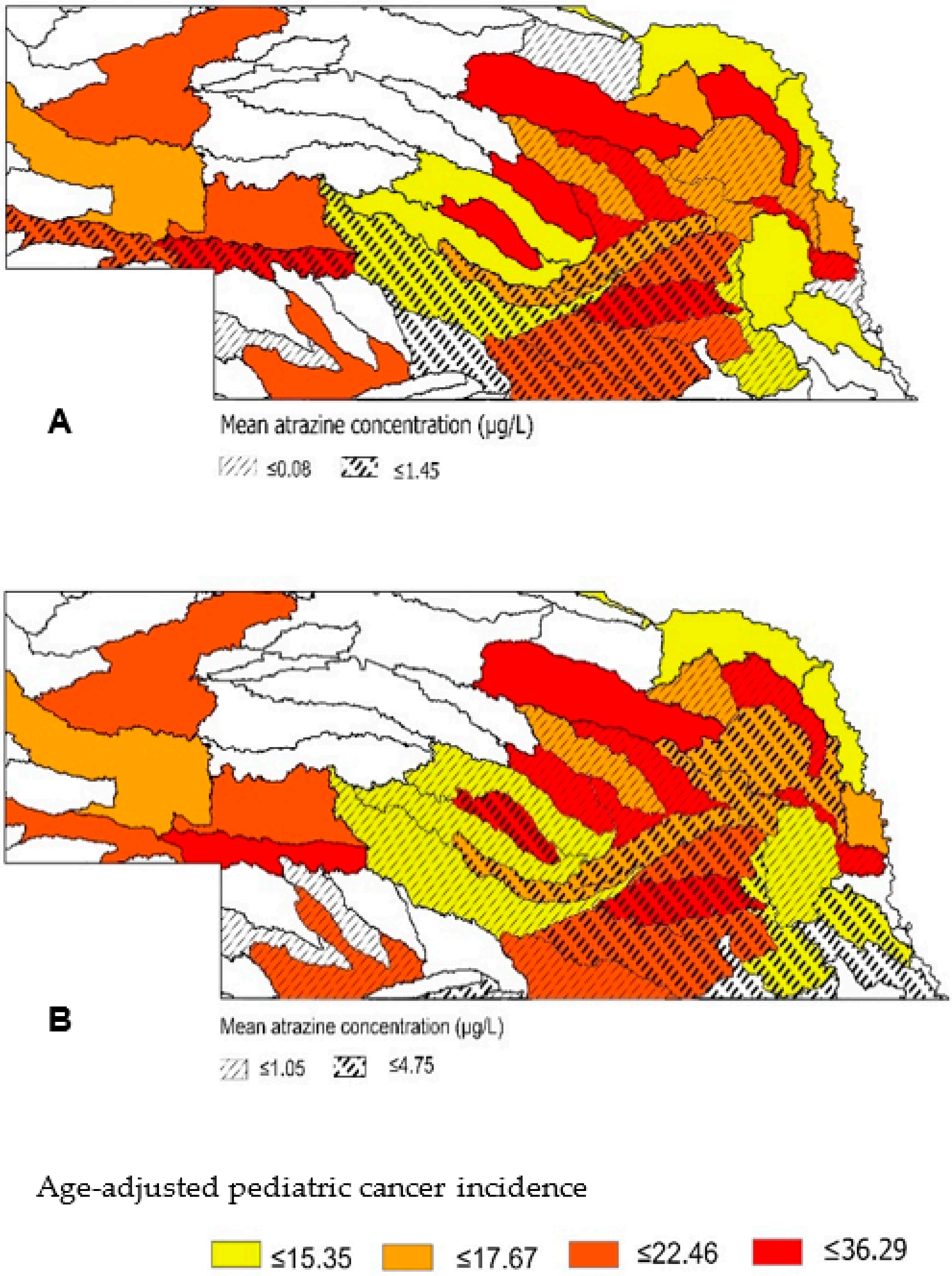

Figure 1. The spatial trend of age-adjusted pediatric cancer incidence and atrazine water concentration by watershed boundaries in Nebraska ((**A**)—Ground & (**B**)—Surface). Note: The atrazine concentration displayed on panels (**A**,**B**) contains the two highest categories (medium and high).

The associations between surface and groundwater atrazine concentrations were evaluated using a generalized linear model (negative binomial), by comparing the rate of pediatric cancer from low, medium and high atrazine watersheds to the watersheds with negligible atrazine concentration. There were positive associations between pediatric cancer and either surface or groundwater atrazine concentrations. We observed an increase in the mean surface and groundwater atrazine concentration associated with an increase in pediatric cancer incidence rate ratio (IRR) among watersheds (Figure 2). There is a positive association between atrazine and pediatric cancer was among watersheds with medium and high categories of atrazine from both ground and surface samples. The associations were consistant across the low, medium, and high atrazine groundwater watersheds using the unadjusted and adjusted_2 model. In comparison, the low groundwater category watershed was borderline associations using the adjusted_1 model, along with positive associations with medium and high categories. Among the surface water atrazine watersheds, there are positive associations among medium and high categories using all three models. In contrast to the groundwater atrazine watershed associations, the surface water atrazine watersheds has a non-significant (p-value > 0.05) association among the low concentration watersheds using unadjusted and adjusted_2 models.

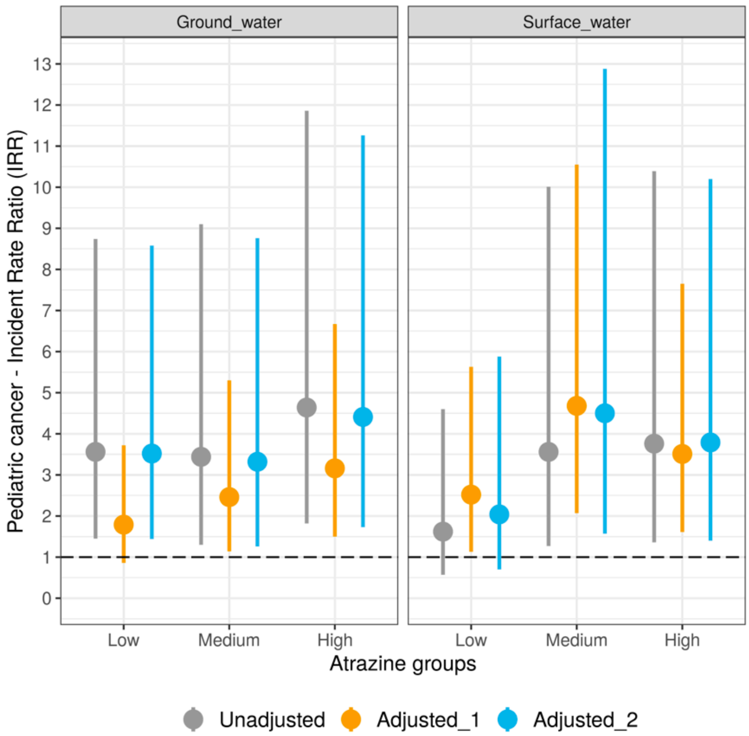

Figure 2. Association between atrazine concentration and pediatric cancer. This figure contains the incidence rate ratio (IRR) and 95% confidence interval to describe the association between atrazine and pediatric cancer. Ground and surface represent the water sample source, categorized into four quantile groups. The IRRs were estimated for Low, Medium, and High atrazine categories comparing with the negligible atrazine category (reference group). The associations were evaluated using three models unadjusted (grey), adjusted_1 (yellow), and adjusted_2 (cyan). Note: The IRR and 95% confidence interval were estimated using a generalized linear model assuming a negative binomial distribution, with population per watershed as an offset term. The IRRs with confidence interval that interest the dashed line (corresponding to "1" on the y-axis) is non-significant.

4. Discussion

The objective of this study was to evaluate the association between atrazine and pediatric cancer in Nebraska watersheds. We identified positive associations between surface/groundwater atrazine and pediatric cancer. The associations were strong among watersheds categorized in the medium and high atrazine concentration quartiles for groundwater and medium/high atrazine quartiles for surface water.

Among the three model approaches to evaluate the association between atrazine and pediatric cancer, the pediatric cancer IRRs were similar while using the unadjusted model and adjusted_2 model (controlling for overall SVI percentile proportions). At the same time, the pediatric cancer IRRs generated from the adjusted_1 model were lower than the unadjusted and adjusted_2 model for groundwater atrazine. The groundwater IRR from adjusted_1 model is 66% lower than unadjusted/adjusted_2 among the low atrazine watersheds; 34% for medium atrazine watersheds and 38% for high atrazine category. The pediatric cancer IRRs from the adjusted_1 model were similar to the unadjusted and adjusted_2 model for the surface water atrazine. As the adjusted_1 model pediatric cancer IRR estimates were generated, accounting for covariates selected based on sensitivity analysis would be ideal to understand the association between atrazine and pediatric cancer compared to the estimates from unadjusted and adjusted_2 models. We found a positive association between atrazine and pediatric cancer among medium and high surface/groundwater atrazine to generalize our findings, irrespective of the statistical model strategies. Children living in medium and high surface/groundwater atrazine watersheds are associated with 2.4–4.6 times increase in pediatric cancer rate, compared to watersheds with negligible atrazine concentrations.

During the period 2003–14, the age-adjusted pediatric cancer incidence was higher among agriculturally intensive US states [30], with Nebraska having the eighth highest incidence of age-adjusted pediatric cancer incidence among the 50 states. In Nebraska, the age-adjusted pediatric cancer incidence was higher among watersheds in Nebraska's eastern region, an area widely used for corn and soybean crop production [31]. Additionally, we observed spatial patterns where watersheds with higher age-adjusted pediatric cancer incidence have higher groundwater and higher surface water atrazine concentration. Our study's preliminary results supported our hypothesis, assuming a positive association between atrazine and pediatric cancer.

We observed positive associations between atrazine and pediatric cancer regardless of whether the water was surface water (which generally is not used in Nebraska as a drinking water source) or groundwater (which often is used as a drinking water source). The children living in watersheds categorized as medium and high atrazine than the negligible atrazine watersheds were associated with higher pediatric cancer IRR. The pediatric cancer IRR among watersheds categorized as high atrazine watershed among groundwater sources was higher than the pediatric cancer IRR relevant to the surface water sources. We hypothesized that the higher magnitude of pediatric cancer IRR associated with groundwater concentration could be due to the fact that most of the population in Nebraska is dependent on groundwater as their drinking water source.

Strengths: This study evaluated the relationship between atrazine and pediatric cancer using a watershed delineation method. Using watersheds delineations would be ideal to understand the ground and surface water contamination due to non-point source agrichemical runoff, as watersheds are geographic areas that drain water within a boundary [16]. As most of the studies evaluating the association between water quality and human health were based on geopolitical boundaries [32–37], our findings would be appropriate in attributing human health outcomes to water quality.

There is limited information on understanding associations between agrichemicals and pediatric cancer, using a population-based approach [32,33]. The studies related to triazine herbicides and cancer mainly were focused on adults, using a low population size [10]. Some studies were based on agricultural crop density as a proxy for agrichemical exposure and evaluated the association with pediatric cancer [34,35]. The current study is based on a longer period (30 years) that included all children less than twenty years old, diagnosed with pediatric cancer in Nebraska, which maximized the study sample size, and the associations were based on water quality measurements which would be comparatively accurate than attributing to agricultural land use pattern.

Limitations: This study did not account for co-contaminant associations with pediatric cancer. As the use of pesticides (agricultural activities) is confined to a particular period of the year, we anticipate the concurrent presence of other agrichemicals from surface and groundwater samples that could be correlated with atrazine. Due to a low sampling frequency of other agrichemicals (compared to atrazine), we focused the analysis on a single contaminant.

As the associations were based on atrazine in water samples from non-drinking water sources, this study did not establish any causal relationships. Due to inconsistent water quality measurements across watersheds, the associations were based on the long-term average concentration of atrazine. The distribution of water sampling was not consistent among the watersheds, and it is possible that the watersheds in the highest quartile for atrazine were so only because more sampling was done during the growing season. Due to the unavailability of demographic variables (gender, race, ethnicity, and mother's medical history) that could influence cancer development, the study results could be influenced by information bias. To mitigate the information bias factor, we used covariates at a watershed scale. Using a long-term average of atrazine, pediatric cancer, and social vulnerability factors would compromise time-varying associations across the study period.

The study population contains 11 distinct cancer diagnoses; 67% of the population are diagnosed with CNS cancer (26%), leukemia (24%), and lymphoma (16%). Additionally, children diagnosed with the above cancer types are concentrated among a few watersheds (Big Papillion-Mosquito, and Salt). Due to concentrated cancer diagnosis and clustered cancer subjects among specific watersheds, this study does not contain subgroup analysis to evaluate the association between specific cancer diagnosis and atrazine due to the sample size constraints.

5. Conclusions

In summary, positive associations exist between atrazine surface water concentrations, atrazine groundwater concentrations, and the incidence of pediatric cancer in Nebraska. Children living in watersheds with higher mean groundwater atrazine concentrations are associated with an increase in pediatric cancer IRR 3.4–4.6 times higher than children living in watersheds with low mean groundwater atrazine concentrations. Similarly, children living in watersheds with higher mean surface water atrazine concentrations were associated with an increase in pediatric cancer IRR 1.6–3.7 times higher than children living in watersheds with a low mean surface water atrazine concentration. Given that the atrazine water quality data are environmental rather than exposure-based, it is truly remarkable that there is such a strong and consistent association between the two. Additionally, the results from the sensitivity analysis suggest positive associations between pediatric cancer and social vulnerabilities such as unemployment, single parent, minority status and English language barrier. Further studies to understand the relationship between atrazine and pediatric cancer based on a large-scale case–control study could establish a causal relationship between atrazine and pediatric cancer.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13192727/s1, Table S1: Sensitivity analysis of social vulnerability variables.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Nebraaska Medical Center, NE, USA (protocol code 414-16-EP and date of approval: 7 July 2016).

Informed Consent Statement: Patient consent was waived, as the patient identifiers were masked.

Data Availability Statement: The water quality data, pediatric population, and SVI data could be retrieved from the sources below mentioned. The pediatric cancer data is not publicly available. https://clearinghouse.nebraska.gov/Clearinghouse.aspx (accessed on 2 February 2018); https://waterdata.usgs.gov/nwis/sw (accessed on 2 March 2018); https://www3.epa.gov/storet/bck/dw_home.html (accessed on 10 February 2018); https://www.atsdr.cdc.gov/placeandhealth/svi/data_documentation_download.html (accessed on 15 March 2021); https://www.census.gov/data.html (accessed on 5 March 2018).

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

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