Title: Age-adjusted pediatric cancer incidence related to nitrate concentration measured through citizen science in Nebraska watersheds.

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Abstract

Background: Nitrate is a common contaminant that gets into the drinking water from agricultural activities using fertilizers. Nebraska is an agricultural state, and the incidence of pediatric cancers in Nebraska is among the five highest of the nation. The occurrence of high nitrate concentration in water can result in many adverse health outcomes in children, including methemoglobinemia, thyroid disease, and birth defects.

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

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

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 (1,2). 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 (3). Nitrate is a ubiquitous contaminant of the drinking water (1,2). Nitrate in water comes from different sources including,

1. natural causes with the mobilization and leaching of geologic nitrogen into groundwater through irrigation practices (4);
2. waste materials such as animal manure (beef, poultry, pork), municipal waste and septic tanks;
3. 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 (5). Researchers have found that anthropogenic activities (inorganic and organic fertilizer overuse in crop production and sewage) mostly account for the high concentration of nitrate in groundwater (1,6,7). For Brender and Weyer, nitrogen used as a fertilizer is the primary source of nitrate contamination in farming areas (8).

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 (9,10,6). 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 known as cyanosis. In addition, nitrite interacts with amides or amines to form N-nitroso compounds that are established carcinogens and teratogens (11,6).

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 (9). High concentrations of nitrate in drinking water have been shown to cause various adverse health outcomes (9,10,12). 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. The participants' age and Body Mass Index (BMI) have been accounted for in the model analysis. The study measured participants' TSH and linked their address to the well that have been tested for nitrate level only in the same location (1).

Brender and Weyer, in their review, cited a study conducted in California. This research 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 (8).

Similarly, research done 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) (13).

IARC, the International Agency for Research on Cancer (14) 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)." EPA has not classified nitrate as carcinogenic at this time (15). A significant number of studies have found a positive relationship between high nitrate exposure and the risk of developing cancer. Examples of such studies include a case-control study 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) (16).

Moreover, in a study conducted in Iowa, 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) (17). Another study done in Iowa and controlling for confounders like trihalomethane levels demonstrated that high nitrate levels (> 5mg/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) (18).

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. The occurrence of relatively high nitrate concentration in drinking water has been associated with adverse health outcomes in several previous studies, although a causal relationship has not been definitively established. 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 (19). 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.

We hypothesized 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

1. Data sources

1.1. Pediatric cancer data

We obtained longitudinal pediatric cancer data (1987 -2014) from the Nebraska Department of Health and Human Services Cancer Registry.

1.2. Nitrate data

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 (20), a community partner. 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 about here]

2. Data analysis

2.1. Variables

Our outcome variable was the age-adjusted incidence of pediatric cancer. Nitrate average concentration was the independent variable.

2.2. Statistical methods

We calculated the crude incidence of the three major pediatric cancer types for each watershed and the state and then age-adjusted to the 2010 U.S. 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” (21). The following formulas were used to calculate the crude and age-adjusted incidences (22).

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

Age-adjusted incidence = $\sum_{\text{Age group}}$crude incidence watershed *Age distribution of standard population

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

1. Descriptive Statistics

1.1. Nitrate concentration

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% and 28% respectively in the groundwater and surface water were above 10 mg/L.

[Table 1 about here]

1.2. Pediatric cancer prevalence and types

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%) (23).

[Figure 2 about here]

1.3. Age-adjusted pediatric cancer incidence

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 (24). Leukemia and lymphoma had lower incidence in Nebraska compared to the national average.

[Table 2 about here]

2. Geospatial Analysis

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 about here]

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

[Figure 4 about here]

Discussion

We examined the relationship between 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 with 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 ~~raised~~ 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 (1,7,8). Our results suggest that different 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 (16,17,18), 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 ~~associated with higher~~ pediatric cancer, we did identify hot spots of cancer across the state that are associated with high nitrate concentration. Further research may focus on measuring nitrate concentration and other contaminants in the watersheds with a high incidence of pediatric cancers to assess a causal relationship between cancer incidence and nitrate concentration, thereby validating our findings.

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 ~~potential relationship~~ between nitrate concentration in drinking water and the occurrence of childhood brain and other CNS cancers in Nebraska. However, we 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 is, as shown by Corley and al. (25), beneficial in using watersheds instead of census entities when conducting a geospatial analysis of adverse health outcomes relative to agrichemicals. These authors stated that, with rainfall, agrichemical contaminants (including nitrate) travel to local waterways that flow downstream within specific watersheds (25). Further limitations to this study include the use of one-time grab samples for the measurement of nitrate concentration.

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. Exposure to agrochemicals (including nitrate) can result in adverse health outcomes in children, such as methemoglobinemia, thyroid disease, birth defects, and cancers.

[Figure 5 about here]

Conclusions

We found that nitrate concentration and pediatric cancer incidence are both high in specific watersheds. 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 suggested a potential relationship 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 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 to increase their awareness regarding the adverse health outcomes of high nitrate ingestion in children. 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.

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Tables

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

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)
Brain and other CNS	4.16	3.05
Leukemia	4.08	4.38
Lymphoma	2.82	2.94

Figures

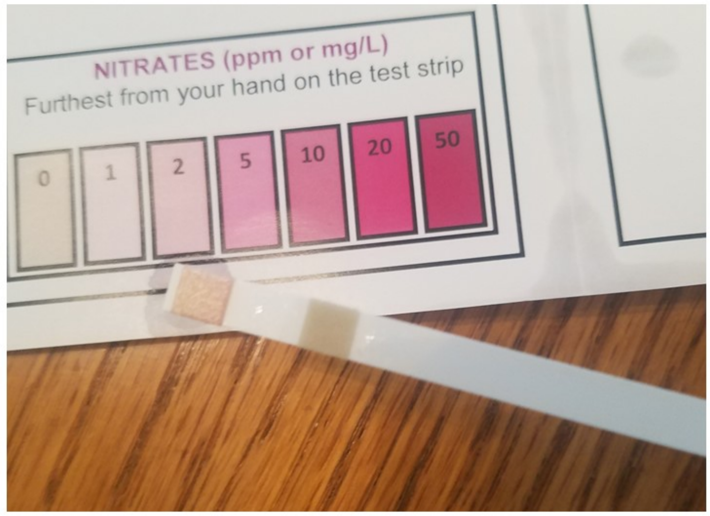

Figure 1. Nitrate test reading

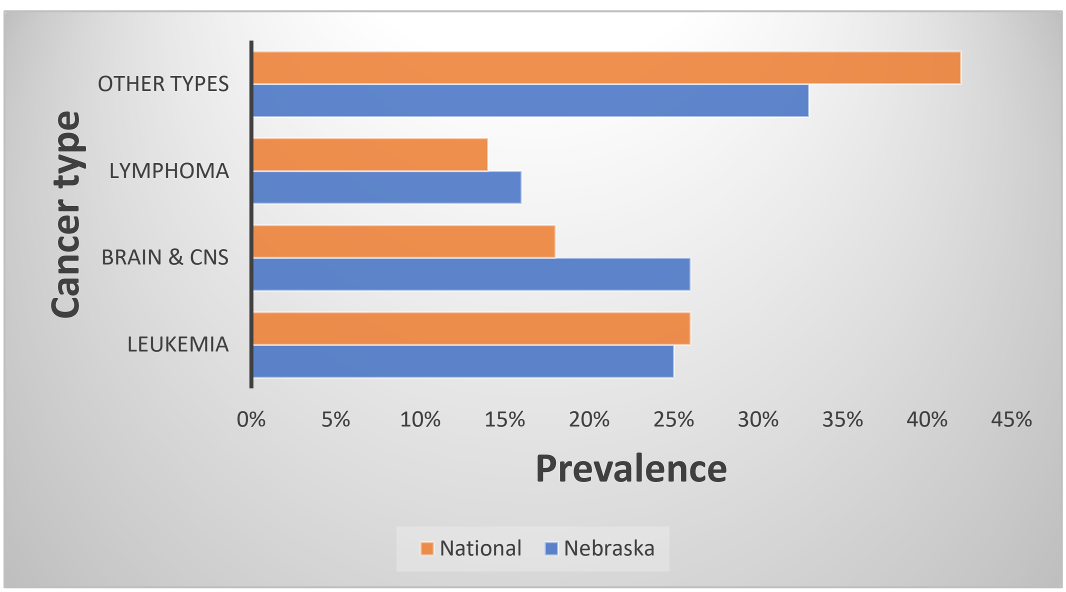

Figure 2. Prevalence of pediatric cancers types in Nebraska and the US

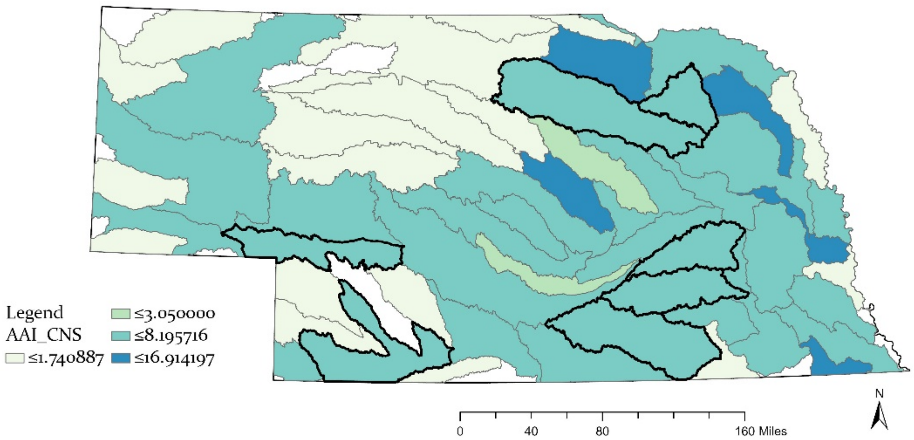

Figure 3. Age-adjusted pediatric CNS cancers vs. groundwater nitrate concentration. Watersheds outlined in black have nitrate concentration above 10mg/L

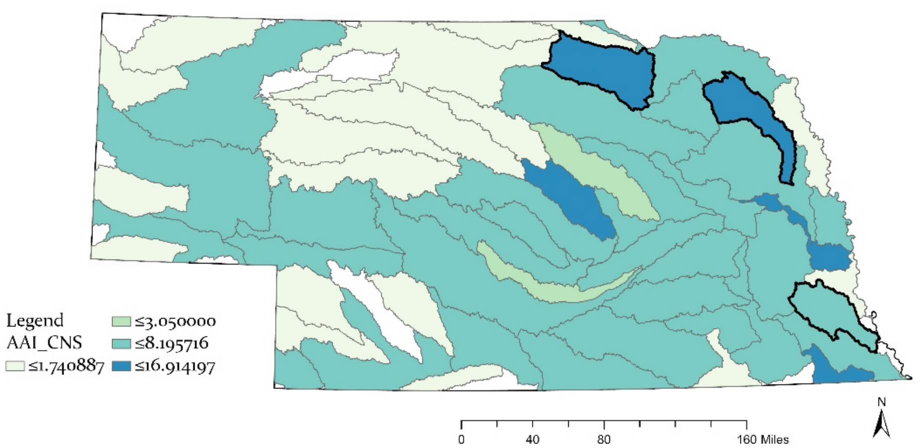

Figure 4. Age-adjusted pediatric CNS cancers vs. surface water nitrate concentration. Watersheds outlined in black have nitrate concentration above 10mg/L

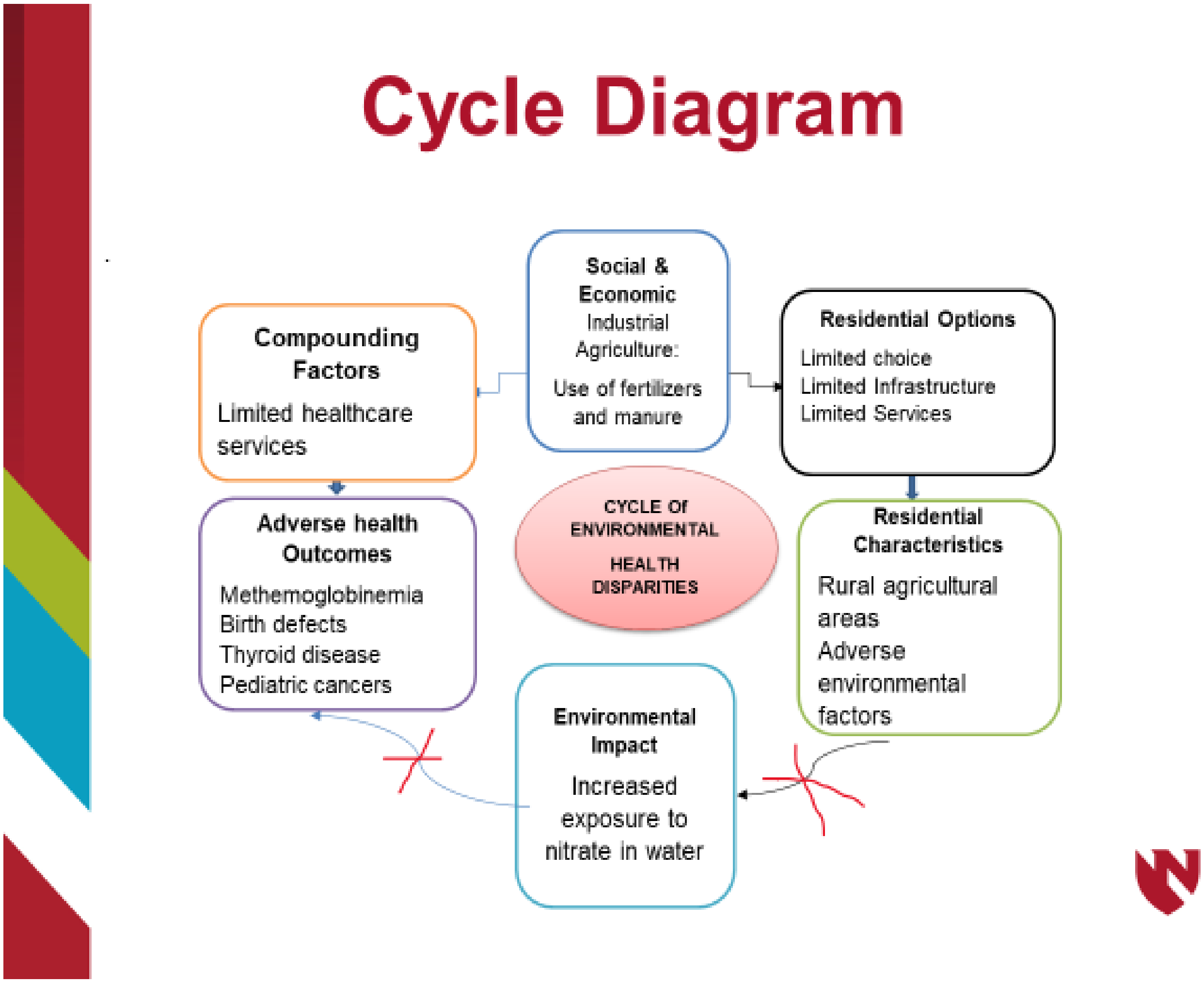

Figure 5. Cycle of children environmental health disparities.