HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Environ Res. 2019 September ; 176: 108553. doi:10.1016/j.envres.2019.108553.

Prenatal Exposure to Nitrate in Drinking Water and the Risk of Congenital Anomalies

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Abstract

Background: Nitrate is a common water contaminant that has been associated with birth defects, although the evidence is limited. The purpose of this study was to examine whether maternal consumption of nitrate through drinking water is associated with an increased risk of congenital anomalies.

Methods: The study included a total of 348,250 singletons births from the state of Missouri between January 1, 2004 and December 31, 2008. Individual-level birth defect data and maternal and child characteristics were obtained from the Missouri birth defects registry and state vital statistics records. Outcomes were linked with county-specific monthly estimates of the nitrate concentration in finished water, based on data collected for compliance with the Safe Drinking Water Standard. Poisson models were fit to examine the association between nitrate exposure and birth defects. Average nitrate exposure during the first trimester and over 12 months prior to birth were modeled as continuous variables. Sensitivity analyses included restriction of the sample to counties with < 20% and < 10% private well usage to reduce exposure misclassification as well as limiting the analyses to residents of rural counties only to account for potential confounding by urbanicity.

Results: Estimated water concentrations of nitrate were generally low and below the Environmental Protection Agency's maximum contaminant level of 10 mg/L. Nitrate exposure

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Disclaimer: The contents herein, including data analysis, interpretation or conclusions are solely the responsibility of the authors and do not represent the official views of the Missouri Department of Health and Senior Services.

Declarations of interest: none

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

was associated with a significantly increased risk of limb deficiencies (RR for 1 mg/L (RR_1) = 1.26, 95% CI = 1.05, 1.51) in models without well restriction. Nitrate was also weakly associated with an increased risk of congenital heart defects (RR_1 = 1.13, 95%CI = 0.93, 1.51) and neural tube defects (RR_1 = 1.18, 95%CI = 0.93, 1.51) in models with well restriction (< 10%).

Conclusion: The positive associations found between nitrate exposure via drinking water and congenital abnormalities are largely consistent with some previous epidemiologic studies. The results of this study should be interpreted with caution given limitations in our ability to estimate exposures and the lack information on some risk factors for congenital abnormalities. Our findings may have serious policy implications given that exposure levels in our study were well below current EPA standards for nitrate in drinking water.

Keywords

Nitrate; drinking water; congenital abnormalities

1. Introduction

1.1. Background

The Centers for Disease Control and Prevention (CDC) reports that birth defects are the leading cause of infant mortality in the United States (U.S.) (Matthews and MacDorman, 2011). Approximately 3% of babies born in the U.S. each year are affected by a birth defect and birth defects account for 20% of infant deaths nationally (Matthews et al., 2015). The economic burden of these conditions is significant, with over $2.6 billion in hospital costs for treatment and care each year (Russo et al. 2007). Birth defects can affect any area of the body, but most commonly include genetic defects such as Down syndrome, cleft lip or palate, cardiovascular anomalies, and musculoskeletal defects (CDC, 2006).

Many risk factors are associated with the development of such anomalies, including advanced maternal age, parental consanguinity, alcohol and cigarette exposure, certain infectious and chronic diseases during pregnancy, and genetics (Oliveira, 2013). There is no known cause for approximately half of all birth defects, which are thought to be the result of multiple environmental, genetic, nutritional, or infectious exposures (World Health Organization, 2015).

Nitrate is an inorganic compound found abundantly in nature. Approximately 85% of dietary nitrate comes from vegetables and the remaining 15% percent largely comes from drinking water (Lidder & Webb, 2013). The percent of nitrate ingested from drinking water will vary, based on its concentration in drinking water and individual consumption habits. Modern agricultural and livestock practices as well as other human activities have become significant contributors to surface and groundwater nitrate contamination. The use of nitrate in inorganic fertilizers and high volumes of animal waste produced by large-operation animal farms are primarily responsible for the growing levels of nitrate in groundwater systems over the past two decades (World Health Organization, 2011). Nitrate is highly water-soluble and a recent study has shown that nitrate from fertilizer will remain in the soil and continue to slowly seep into groundwater systems for more than fifty years after application (Sebilo et al., 2013). Nitrate is presently the most common aquifer contaminant worldwide and

contamination levels are rising (Spalding and Exner, 1993; Sebilo et al., 2013). The U.S. Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of 10 mg/L for nitrate measured as total oxidized nitrogen compounds (i.e. nitrate and nitrite) in drinking water and public water supplies are subject to regular testing of nitrate levels (40 CFR 141.62). This MCL was chosen to reduce the risk of methemoglobinemia in infants, which results from exposure to very high nitrate levels, but little is known about the effects of chronic nitrate exposure at levels lower than this limit (Bouchard et al., 1992). Additionally, private well systems are not required to adhere to EPA MCL limits and over 15 million U.S. households rely on such water systems (U.S. Census Bureau, 2008).

The potential reproductive effects of chronic exposure to low levels of nitrate have gained attention in recent years. Animal studies have demonstrated that maternally consumed nitrate/nitrite is capable of permeating the placenta and affecting the developing fetus (Bruning-Fann and Kaneene, 1993; Fan et al., 1987). Additionally, several epidemiologic studies have demonstrated associations between prenatal nitrate exposure and a number of different birth defects including neural tube defects, such as spina bifida and anencephaly; oral cleft malformations; limb deficiencies; and congenital heart defects (Brender et al., 2004; Croen et al., 2002; Dorsch et al., 1984). A study by Brender et al. (2004) suggests that maternal use of nitrosatable drugs, which can react with nitrite to form teratogenic N-nitroso compounds, in combination with nitrate exposure from drinking water may interact to increase the risk of neural tube defects. While these studies support an association between maternal nitrate consumption and congenital anomalies, several other studies have found no significant evidence of an association (Cedergren et al., 2002; Frecker et al., 1987; Mattix et al., 2007; Super et al., 1981). In addition to birth defects, prenatal exposure to nitrate in drinking water has been associated with other adverse birth outcomes, including intra-uterine growth restriction, preterm birth, and low birth weight (Bukowski et al., 2001; Stayner et al., 2017), as well as type 1 diabetes (Parslow et al., 1997), and pediatric brain tumors (Mueller et al., 2004; Weng et al., 2011).

1.2. Study Aims

Our study aims to address the need for further investigation of the possible relationship between prenatal exposure to nitrate and birth defects by linking birth record data from the state of Missouri with Missouri Safe Drinking Water Information System water sampling data for nitrate. The United States Geological Survey reports that Midwest states, including Missouri, are at high risk for nitrate contamination of groundwater (USGS, 1999), making this study site well situated for examining the potential association between prenatal exposure to nitrate in drinking water and the risk of birth defects.

2. Material and Methods

2.1. Study population

This study used individual-level, de-identified birth certificate data and related Birth Defects Registry data, provided by the Missouri Department of Health and Senior Services, detailing births occurring in the state of Missouri between the years of 2004 and 2008. In total, 348,250 live-born, singleton births were included in the analyses.

2.2. Birth outcomes

The Missouri Birth Defects Registry relies on birth defect data reported on birth certificates, death certificates, hospital patient abstracts, screening data, and enrollment data from state family assistance programs. Cases consist of only those defects reported within the first year of life. Occurrence of the following birth defects was examined in this study (diagnostic codes from the International Statistical Classification of Diseases and Related Health Problems, 10th Revision): spina bifida (ICD-10-CM Q05), anencephaly (ICD-10-CM Q00.0), hypoplastic left heart syndrome (ICD-10-CM Q23.4), tetralogy of Fallot (ICD-10-CM Q21.3), transposition of the great arteries (ICD-10-CM Q20.3), cleft lip and cleft palate (ICD-10-CM Q35–37), hypospadias (ICD-10-CM Q54), gastroschisis (ICD-10-CM Q79.3), upper and lower limb deficiencies (ICD-10-CM Q71–72), and Down syndrome (ICD-10-CM Q90). The presence of one or more congenital anomaly at birth was the primary outcome of interest in this study.

2.3 Covariates

Missouri birth certificate and birth defects registry data include information on the following covariates: maternal age, race/ethnicity, pre-pregnancy body mass index (BMI), years of maternal education, maternal cigarette and alcohol use, adequacy of prenatal care (inadequate prenatal care being defined as live births with fewer than five prenatal care visits for pregnancies less than 37 weeks gestation, fewer than eight visits for pregnancies 37 or more weeks, or prenatal care beginning after the first four months of pregnancy), child sex and year of birth, and participation in federal assistance programs, including Medicaid, WIC, and food stamp programs. Mothers were considered to be of low socioeconomic status if they participated in one or more of these federal assistance programs.

2.4. Exposure assessment

The U.S. federal MCL for nitrate in drinking water is 10 mg/L (40 CFR 141.62). Public water systems using groundwater sources are monitored annually for nitrate levels and those using surface water sources are monitored quarterly. If surface water sources are consistently below the MCL, monitoring can be reduced to annually (40 CFR 141.62).

Water sampling data were provided by the Missouri EPA Safe Drinking Water Information System (SDWIS). Average monthly concentrations of nitrate in drinking water (mg/L) were calculated from the finished water measurements taken from each Missouri community water system (CWS) during the years of 2004 through 2008. CWS-level monthly nitrate means were first calculated from all water samples from within a CWS in a given month. Monthly means on a county level were then calculated from the aforementioned CWS monthly-means, weighted by the size of the population served by each CWS. The resulting county-level monthly means were then used to calculate the average individual nitrate exposure occurring during the first trimester of gestation, based on the knowledge that organogenesis and limb development generally occur within the first 12 weeks of fetal development (Mueller, 1989). This estimate was treated as missing if there was no nitrate measurement available during the relevant three-month period. A first trimester nitrate exposure estimate was only available for 36% of the study cohort due to the relatively infrequent water sampling requirements. Accordingly, an additional measure of average

nitrate exposure occurring over the 12-month period prior to birth was calculated in order to minimize the missing data and thus conserve the sample size. This estimate was available for 93% of births, was highly correlated with the first trimester estimate ($r^2 = 0.84$), and was thus treated as the primary measure of exposure. This measure of exposure assumes that each CWS serves residents in the county wherein the CWS office is located and that water is not supplied from CWS outside of the county (Jones et al., 2014).

This study was reviewed and approved by the Institutional Review Board of the University of Illinois at Chicago as well as the Missouri Department of Health and Senior Services. The data used in this research was acquired from the Missouri Department of Health and Senior Services (DHSS).

2.5. Statistical methods

Poisson regression models with robust standard errors were used to estimate the association between maternal exposure to nitrate in drinking water and the presence of birth defects. In an effort to preserve statistical power, birth defects were collapsed into categories when appropriate for analysis (i.e. anencephaly and spina bifida were analyzed together as neural tube defects, hypoplastic left heart syndrome, tetraology of Fallot, and transposition of the great arteries were analyzed together as congenital heart defects, cleft lip and cleft palate were analyzed together as oral cleft defects, and upper and lower limb deficiencies were analyzed together as limb deficiencies). The monthly county-level average nitrate concentrations were linked to each birth by county and month of birth in order to estimate mean exposure during the 12 months prior to birth and during the first trimester of pregnancy. The mean nitrate exposure variables were modeled as a continuous variable (mg/L).

Models were assessed for overdispersion by fitting negative binomial models and testing the statistical significance of the dispersion parameter. There was no evidence found to suggest overdispersion and thus Poisson regression models were used. Models controlled for maternal age, race, ethnicity, and socioeconomic status based on *a priori* knowledge that these are risk factors for congenital malformations. Other covariates (i.e., BMI, education, adequacy of prenatal care, tobacco use, and alcohol use) were assessed for confounding using a 10% change in the rate ratio estimate method. Effect modification was evaluated by testing the improvement of model fit from the addition of interaction terms for each covariate into a main effects model. Restricted cubic spline models with three knots were fit for those outcomes showing evidence of a relationship with nitrate exposure in order to assess the linearity and shape of the exposure-response relationship. The knots were chosen based on percentiles as suggested by Harrell et al. (1988).

Well water estimates of nitrate concentrations were not available for this study and it was not known whether the mothers received their water from private wells or public water systems. A sensitivity analysis was conducted by restricting the analytic sample first to counties with less than 20% private well usage and then to counties with less than 10% private well usage, in order to better understand any impact of potential exposure misclassification. Another sensitivity analysis was conducted wherein the study sample was limited to residents of rural counties in order to account for potential confounding by urban areas, which have different

demographics and lifestyle than rural counties and possibly exposure to other environmental contaminants that may be risk factors for birth defects such as air pollution (Ritz et al., 2002). Urban was defined as any county with a population density greater than 150 persons per square mile or containing at least part of the central city of a Census-defined Metropolitan Statistical Area, as suggested by the Missouri DHSS in their 2015 Office of Primary Care and Rural Health Biennial Report (Missouri Department of Health and Human Services, 2015). One hundred and one of 115 Missouri counties were considered rural under this definition. Finally, a sensitivity analysis utilizing the first trimester exposure estimate was also conducted in order to assess whether the risk of birth defects was more strongly associated with exposures during this critical period of fetal development than what was observed using the 12 months pre-birth estimate of exposure in the primary analyses.

All analyses were conducted using Stata 14 (StataCorp, College Station, TX, US).

3. Results

3.1. Descriptive analyses

An estimate of mean nitrate exposure occurring over the 12 months prior to birth was available for 348,250 (92.9%) of the 375,000 live, singleton births in the state of Missouri between 2004 and 2008. Among these births were 149 with spina bifida (0.04%), 47 with anencephaly (0.01%), 127 (0.04%) with hypoplastic left heart, 341(0.10%) with Fallot/transposition of the great arteries, 415 (0.12%) with cleft lip with or without cleft palate, 279 (0.08%) with only cleft palate, 142 (0.04%) with limb deficiencies, 1,609 (0.46%) with hypospadias, 273 (0.08%) with gastroschisis, and 500 (0.14%) with Down syndrome. The average maternal age of the study sample was 26.5 ± 5.8 years, 78.7% of birth mothers identified as non-Hispanic white and 51.2% of births were male. As shown in Table 1, levels of nitrate exposure were generally comparable between mothers of infants with and without congenital defects, with the exception of mothers of children with limb deficiencies, who were significantly more likely to have higher levels of nitrate exposure.

There were differences in maternal characteristics between the full cohort and those births included in the rural only analysis. Mothers residing in rural counties were more likely to be non-Hispanic white and under the age of 34. They were also more likely to smoke, to have fewer years of education, and to be of low socioeconomic status (Table 7, Appendix). Characteristics of mothers with a 12 month pre-birth nitrate exposure estimate available, but no first trimester exposure estimate, versus those with both a 12 month pre-birth and first trimester exposure estimate were not appreciably different (Table 8, Appendix).

A summary of nitrate concentrations by year can be found in Table 2. The maximum mean concentration was 5.81 mg/L in 2004, 5.8 mg/L in 2005, 6.02 mg/L in 2006, 5.96 mg/L in 2007, and 6.36 mg/L in 2008. The average monthly concentrations ranged from 0.03 to 6.36 mg/L. All estimated average exposures were below the EPA's MCL of 10 mg/L.

3.2. Poisson Regression Analyses

The results from the final models that included maternal age, race, ethnicity, and socioeconomic status are presented in Table 3. Further adjustment by maternal cigarette and

alcohol use did not have any appreciable effect on the results and these covariates were ultimately omitted from the final models in light of substantial missingness. As seen in Table 3, the results of the adjusted models do not greatly differ from those of the crude models.

The risk for limb deficiencies was found to significantly increase with estimated nitrate exposure (Rate Ratio for 1 mg/L (RR_1) = 1.26, 95% CI 1.05, 1.51). The rate ratio estimates changed slightly in models with private well use restrictions (< 20% well restriction RR_1 = 1.24, 95% CI 1.03, 1.48; < 10% well restriction RR_1 = 1.31, 95% CI 1.02, 1.68) (Table 3). Separate models for upper and lower limb deficiencies demonstrated a statistically significant relationship with both upper limb deficiencies (RR_1 = 1.31, 95% CI 1.06,1.62; < 20% well restriction RR_1=1.28, 95% CI 1.03, 1.59; < 10% well restriction RR_1 = 1.36, 95% CI 1.01, 1.84), and lower limb deficiencies in all adjusted models (RR_1= 1.25, 95% CI 1.02, 1.53; < 20% well restriction RR_1= 1.24, 95% CI 1.0, 1.52; < 10% well restriction RR_1= 1.35, 95% CI 1.03, 1.77) (Table 4).

There was no evidence of an association between nitrate exposure and the development of oral cleft defects (RR_1=0.93, 95% CI 0.82, 1.06), hypospadias (RR_1=0.98, 95% CI 0.91, 1.06), Down syndrome (RR_1= 0.93, 95% CI 0.80, 1.10), or gastroschisis (RR_1= 0.94, 95% CI 0.76, 1.16). A statistically non-significant, association between nitrate exposure and neural tube defects was observed, which strengthened with restriction of private well use (RR_1=1.03, 95% CI 0.84, 1.27, < 20% well restriction RR_1=1.12, 95% CI 0.92, 1.35, < 10% well restriction RR_1=1.18, 95% CI 0.93, 1.51) (Table 3). Anencephaly and spina bifida were also modeled separately and both conditions had risk estimates similar to the combined analyses (Table 4).

A statistically non-significant association was observed between nitrate exposure and congenital heart defects, which strengthened with restriction of private well use (RR_1=1.06, 95% CI 0.92, 1.23; < 20% well restriction RR_1=1.09, 95% CI 0.94, 1.27; < 10% well restriction RR_1=1.13, 95% CI 0.93, 1.38) (Table 3). Separate models for hypoplastic left heart syndrome (HLHS) and tetralogy of Fallot/transposition of the great arteries (ToF/TGA) demonstrated a stronger relationship between nitrate exposure and HLHS than for congenital heart defects. The HLHS association was statistically significant in the < 20% well restriction model (RR_1=1.32, 95% CI 1.04, 1.67). The association between ToF/TGA was strongest, but not significant, in the < 10% private well use model (RR_1=1.13, 95% CI 0.90, 1.42) (Table 4).

The results of restricted cubic spline models did not suggest a non-linear exposure response for any of the birth defect outcomes (not shown).

3.3. Sensitivity analyses

A much stronger association ($p<0.001$) was observed between congenital heart defects and nitrate exposure in the analysis using the mean first trimester exposure estimates with well restriction of < 10% (RR_1=1.33, 95%CI 1.12, 1.58) than in the primary analysis (Table 5). There was weak evidence for an association between nitrate exposure and gastroschisis in the analyses using the mean first trimester exposure estimates, which was not seen in the primary analysis. A statistically non-significant increase in risk was shown in the first

trimester exposure models, which was strongest and borderline significant when private well use was restricted to <10% (RR_1=1.26, 95% CI 1.00, 1.58). In contrast to the primary analyses, the association between nitrate exposure and limb deficiencies did not reach statistical significance in the first trimester exposure models, but did strengthen with private well use restriction (RR_1=1.18, 95% CI 0.90, 1.54; < 20% well restriction RR_1=1.28, 95% CI 0.95, 1.73; <10% well restriction RR_1=1.35, 95% CI 0.97, 1.88) (Table 5).

Models using data from only rural counties demonstrated a statistically significant (p=0.02) association for congenital heart defects (RR_1=1.38, 95% CI 1.05, 1.82) and for limb deficiencies (RR_1=1.48, 95% CI=1.08, 2.05) in the <10% well restriction models (Table 6). Mean nitrate exposure was not associated with neural tube defects, oral cleft defects, hypospadias, gastroschisis, or Down syndrome in any of the models that only included data from rural counties (Table 6).

4. Discussion

In this study, we examined the relationship between prenatal exposure to nitrate through maternal consumption of drinking water and the occurrence of birth defects using existing surveillance data from the state of Missouri. We found evidence in our study suggesting that the risk of limb deficiencies increases with exposure to nitrate in drinking water in models with and without restriction of the analyses to counties with a low percentage of private well use. These associations were observed for both upper and lower limb defects. This finding is consistent with a 2013 study by Brender et al., who used a detailed assessment of drinking water nitrate exposure, which utilized water sampling data from sources verified to supply the address of mothers included in the study, estimated nitrate levels from bottled water, and self-report data on daily water consumption habits. Expectant mothers that consumed on average more than 5.0 mg per day of nitrate through drinking water had nearly twice the odds of giving birth to a child with a limb deficiency compared to mothers that consumed less than 1.0 mg per day (RR=1.79, 95% CI 1.05, 3.08) in the Brender study. Dorsch et al. (1984) reported that women who consumed groundwater during pregnancy were nearly three times more likely to have a child with a musculoskeletal defect compared to those that drank only rainwater (RR=2.9, 95% CI 1.2, 8.0). While the authors did not report findings for nitrate concentrations, the local groundwater supply for the study cohort was known to derive from a lake contaminated with nitrate from a nearby limestone aquifer.

We found limited evidence for an association between nitrate exposure and congenital heart defects in our study. Although there was little evidence of an association in the primary analyses using the 12 month average estimates, there was highly significant evidence (p=0.001) of a positive association in models that used the first trimester estimates of nitrate exposure and well restriction <10 % (RR_1=1.33, 95%CI 1.12, 1.58). These findings are somewhat supported by the study of Brender et al. (2013) who reported a weak and statistically non-significant association between nitrate in drinking water and several different congenital heart defects. In their study, mothers that consumed 5.42 mg/day or more of nitrate via drinking water had 1.13 times the odds of having a child with a conotruncal heart defect (95% CI 0.77, 1.65), 1.48 times the odds of having a child with a right ventricular outflow tract obstruction (95% CI 0.95, 2.32), and 1.23 times the odds of

having a child with a left ventricular outflow tract obstruction (95% CI 0.81, 1.88) compared to those who consumed less than 1.00 mg/day of nitrate. Cedergren et al. (2002) similarly found a weak association between nitrate and cardiac defects, wherein mothers that consumed water with nitrate levels at or above 2 mg/L had 1.18 (95% CI 0.97, 1.44) times the odds of having a child with a cardiac defect compared to those that consumed water with nitrate levels below 2 mg/L. Although the existing evidence for a relationship between nitrate exposure and cardiac defects is not strong, the limited available data are largely consistent, warranting further investigation.

We did not find consistent evidence for an association between nitrate exposure and neural tube defects in any of our analyses. The strongest finding was in our model using the 12 month average exposure and restriction of well use to <10% (RR_1=1.18, 95% CI 0.93, 1.51). An increased risk was not observed when the analysis was restricted to rural counties only, suggesting that there may be residual confounding related to urban residence. An increased risk of neural tube defects from prenatal exposure to nitrate in drinking water has been reported in several prior epidemiologic studies. A 1988 Canadian case-control study observed a twofold increase in risk for neural tube defects in association with consumption of private well water containing nitrate levels of 26 mg/L compared to a baseline nitrate level of 0.1 mg/L, although this finding was not statistically significant (Arbuckle et al., 1998). More recently, Brender et al. (2013) found that mothers with an estimated intake of 5.0 mg/day or more of nitrate from drinking water had 1.43 times the odds of a having a child with a neural tube defect compared to those mothers that consumed less than 0.91 mg/day of nitrate (95% CI 1.01, 2.04). Additionally, a 2001 case-control study observed a significantly increased risk for anencephaly in mothers with drinking water nitrate exposure above the Australian MCL of 45 mg/liter (total nitrate which is equivalent to the U.S. EPA standard 10 mg/L of nitrate-nitrogen) (OR 4.0, 95% CI 1.0, 15.4) and in mothers who consumed groundwater with nitrate levels below the MCL (OR=2.1, 95% CI 1.1, 4.1 for 5–15 mg/liter compared to < 5 mg/liter) (Croen et al., 2001).

We found weak evidence for a positive relationship between nitrate exposure and gastroschisis in the analyses using nitrate exposure during the first trimester (p=0.05 for <10 % well use). There was no evidence for an association in the models using exposure averaged over 12 months or when the analysis was restricted to rural counties. There are only two prior studies on the potential relationship between nitrate and gastroschisis. A 2007 study found a non-significant trend towards increased prevalence of abdominal wall defects with nitrate levels in Indiana (Mattix et al. 2007). A 2010 case-control study by Waller et al. did not observe a relationship between nitrate and gastroschisis.

Study Strengths and Weaknesses

This study, like all epidemiologic studies, has a number of strengths and weaknesses. Strengths of the study include the availability of individual-level data from birth certificates and a high quality birth defects registry, as well as extensive measurements of nitrate in drinking water in an area of the country with relatively high levels of nitrate contamination. The study included nearly 350,000 births, which is a large cohort, although the number of cases was still small, resulting in low statistical power for some outcomes and analyses.

A number of methodological shortcomings related to the ascertainment of exposure in our study must be recognized. Exposure estimates were calculated at the county level as individual household level estimates were not feasible with the available data. Additionally, first trimester nitrate exposure estimates were only estimable for 36% of births occurring in Missouri between 2004 and 2008 with the water quality data obtained. Federal regulations generally require public water systems be monitored for nitrate annually, which explains why data were not available in every month for each county, and why, even when a county-month value was determined, it may not have utilized data from every water system within the county. In an effort to address the substantial missingness of the first trimester nitrate exposure, we calculated an alternative estimate that consisted of mean nitrate exposure occurring over the 12 months prior to birth, which was available for nearly the entire cohort (93%). These two measures of exposure were highly correlated and there did not appear to be any meaningful difference in the distribution of the characteristics of the individuals in the analyses using 12 months or first trimester averaged exposures.

A second major source of uncertainty in our exposure estimation is related to the lack of data available on concentrations of nitrate in public wells. Our only option was to use the measurements of nitrate from public water data to estimate exposure for everyone in our study. Although analyses restricting the sample to counties with limited private well usage (<20% and <10% of the county population) were implemented to minimize the potential for exposure misclassification, the size of the cohort and statistical power of our analyses was greatly reduced by these restrictions.

A third serious limitation in our exposure assessment was the lack of information on individual water consumption habits (e.g. bottled water), and dietary sources of nitrate and nitrite. The lack of information on bottled water consumption was also an issue in most of the prior studies of nitrate in drinking water and the risk of birth defects. A 2014 study by Weyer et al., found that approximately 30% of the pregnant women included in their sample used bottled water as a primary source of drinking water and that bottled water nitrate concentrations were lower than that of the community water systems. Dietary sources of nitrate and nitrite may be a more important source of nitrate and nitrite intake than drinking water (WHO 2011).

Finally, the women included in the present study were assumed to reside in the county where they gave birth for the duration of their pregnancy, which is another source of uncertainty in our exposure assessments. A recent review of studies in the U.S. found that 9–32% of women moved during the pregnancy (Bell and Belanger 2012). Altogether these uncertainties in our exposure assessment are unlikely to have been differential with respect to disease status and thus were most likely to have weakened our ability to detect an exposure-response relationship.

The potential for misclassification of the outcomes of interest and related covariates must also be acknowledged. The state of Missouri relies on a variety of sources for their birth defects registry, including birth and death certificates, hospital patient abstracts, and enrollment data from state assistance programs. A number of studies have demonstrated that birth certificates underreport the presence of congenital anomalies (Boulet et al., 2014; Piper

et al., 1993). Furthermore, moderate-to-low agreement between birth certificates and medical records has been demonstrated for information such as gestational age, maternal medical risk factors, history of tobacco and alcohol use, and quality of prenatal care (Northam and Knapp, 2006; Zollinger et al., 2006). Missouri does not report the distribution of sources used in their registry, but does note that birth and death certificates are almost exclusively relied on for those births occurring in out-of-state hospitals, common in certain counties that lack convenient access to in-state facilities (Missouri Department of Health & Senior Services, 2017). It must also be recognized that many birth defects are not observed due to early fetal deaths, and elective terminations following prenatal diagnosis.

Finally, there are several known or suspected risk factors for birth defects that were not available and thus were not controlled for in this study. Data was lacking on use of folate supplements, paternal age, parity and other potential risk factors for birth defects. In addition, pesticides such as chlorpyrifos, atrazine, and glyphosate are also used in the production of corn and soybeans in Missouri and the Midwest. Future studies need to consider possible confounding and effect modification by these and other pesticides on the association between nitrate in drinking water and the risk of birth defects.

5. Conclusions

This study found that gestational exposure to nitrate through drinking water is associated with an increased risk for limb deficiencies. There was more limited evidence for an association with congenital heart defects, and possibly neural tube defects. These findings are largely consistent with other published studies. The public health implications of these findings may be substantial given that nitrate is a widespread water contaminant, the levels of nitrate in groundwater appear to be rising, and the levels of exposure in this study were well below the current EPA standards. This study was limited by data available for determining nitrate exposure and the lack of information on other environmental exposures and certain maternal risk factors. Further research that addresses these limitations is needed to better understand the potential effect of nitrate on birth defects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement:

The authors are grateful for the assistance by the staff of the state of Missouri Department of Health and Senior Services in obtaining the data for our study. And for the financial support received by the Center for Disease Control and Prevention’s, Environmental Public Health Tracking Network (Contract #200–2010-37442).

Funding Sources: This work was supported by a grant from the U.S. CDC Environmental Public Health Tracking Program (Contract #200–2010-37442) and National Institutes of Health/National Institute of Environmental Health Science grant P30 ES027792 Chicago Center for Health and Environment (CACHET).

This research was approved by the University of Illinois at Chicago’s Institutional Review Board.

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Highlights

- Nitrate in drinking water was associated with an increased risk of limb deficiencies.
- A weaker association was observed with heart defects, and neural tube defects.
- Our findings are reasonably consistent with previous studies.
- Our study may have policy implications since exposures were below EPA standards.

Table 1:

Maternal characteristics of the study sample (n= 348,250)* of live singleton births in the state of Missouri during 2004-2008, shown in n(%).

	Normal Births (n= 344,566)	Neural Tube (n= 196)	Congenital Heart (n= 442)	Oral Cleft (n= 643)	Limb Deficiencies (n= 142)	Gastroschisis (n= 273)	Hypospadias (n= 1,609)	Down Syndrome (n= 500)
Race								
White	288,158 (83.6)	**179 (91.3)**	383 (86.7)	**572 (89.0)**	127 (89.4)	219 (80.3)	1,344 (83.5)	429 (85.8)
Black	43,207 (12.5)	**14 (7.1)**	47 (10.6)	**47 (7.3)**	14 (9.9)	46 (16.8)	224 (13.9)	49 (9.8)
Other	11,920 (3.4)	-	10 (2.3)	**20 (3.1)**	-	7 (2.6)	35 (2.2)	19 (3.8)
Missing	1,281 (0.4)	-	-	-	-	-	6 (0.4)	-
Ethnicity								
Hispanic	19,449 (5.6)	15 (7.6)	**11 (2.5)**	41 (6.4)	5(3.5)	11 (4.0)	**50 (3.1)**	**40 (8.0)**
Non-Hispanic	324,745 (94.2)	181 (92.4)	**430 (97.3)**	601 (93.5)	137 (96.5)	262 (96.0)	**1,558 (96.8)**	**460 (92.0)**
Missing	372 (0.1)	0 (0.0)	-	-	0 (0.0)	0 (0.0)		**0 (0.0)**
Age Group (years)								
<18	11,876 (3.4)	-	9 (2.0)	19 (3.0)	6 (4.2)	**22 (8.1)**	58 (3.6)	**6 (1.2)**
18–34	297,112 (86.2)	168 (85.7)	376 (85.1)	546 (84.9)	125 (88.0)	**233 (85.3)**	1,388 (86.3)	**300 (60.0)**
>34	35,565 (9.7)	24 (12.2)	57 (12.9)	78 (12.1)	11 (7.7)	**18 (6.6)**	163 (10.1)	**194 (38.8)**
Missing	13 (<0.1)	0 (0.0)	0 (0.0)	0 (0.0)		**0 (0.0)**	0 (0.0)	**0 (0.0)**
BMI Pre-pregnancy								
< 18.5	16,287 (4.7)	**8 (4.1)**	**21 (4.8)**	40 (6.2)	6 (4.2)	10 (3.7)	68 (4.2)	19 (3.8)
18.5–24.9	160,560 (46.6)	**73 (37.2)**	**189 (42.8)**	279 (43.4)	68 (47.9)	150 (55.0)	702 (43.6)	229 (45.8)
25–29.9	76,963 (22.3)	**47 (24.0)**	**86 (19.5)**	138 (21.5)	30 (21.1)	52 (19.1)	381 (23.7)	112 (22.4)
>30.0	90,756 (26.3)	**68 (34.7)**	**146 (33.0)**	186 (28.9)	38 (26.8)	61 (22.3)	458 (28.5)	140 (28.0)
Missing	0 (0.0)	**0 (0.0)**	**0 (0.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Education (years)								
<12	63,773 (18.5)	**33 (16.8)**	77 (17.4)	**142 (22.1)**	28 (19.7)	**90 (33.0)**	**238 (14.8)**	**74 (14.8)**
12	106,171 (30.8)	**78 (39.8)**	146 (33.0)	**209 (32.5)**	40 (28.2)	**93 (34.1)**	**506 (31.5)**	**144 (28.8)**
13–15	79,061 (23.0)	**42 (21.4)**	100 (22.6)	**146 (22.7)**	38 (26.8)	**58 (21.3)**	**380 (23.6)**	**111 (22.2)**
>15	92,003 (26.7)	**39 (19.9)**	111 (25.1)	**140 (21.8)**	35 (24.6)	**29 (10.6)**	**469 (29.2)**	**165 (33.0)**
Missing	3,558 (1.0)	-	8(1.8)	**6 (0.9)**	-	-	**16 (1.0)**	**6 (1.2)**
Smoker								

	Normal Births (n= 344,566)	Neural Tube (n= 196)	Congenital Heart (n= 442)	Oral Cleft (n= 643)	Limb Deficiencies (n= 142)	Gastroschisis (n= 273)	Hypospadias (n= 1,609)	Down Syndrome (n= 500)
No	279,512 (81.1)	166 (84.7)	352 (79.6)	501 (77.9)	107 (75.3)	**189 (69.2)**	**1,344 (83.5)**	417 (83.4)
Yes	63,878 (18.5)	28 (14.3)	88 (19.9)	137 (21.3)	34 (23.9)	**82 (30.0)**	**255 (15.8)**	81 (16.2)
Missing	1,176 (0.3)	-	-	5 (0.7)	-	-	**10 (0.6)**	-
Alcohol during Pregnancy								
No	339,447 (98.5)	**185 4.3)**	**431 (7.5)**	636 (98.9)	139 (97.9)	269 (98.5)	1,584 (98.4)	**488 (97.6)**
Yes	2,021 (0.6)	**5 (2.6)**	**5 (1.1)**	-	-	-	12 (0.7)	**7 (1.4)**
Missing	3,098 (0.9)	**6 (3.0)**	**6 (1.3)**	5 (0.8)	-	-	13 (0.8)	**5 (1.0)**
Low SES [a]								
No	155,885 (45.2)	95 (48.4)	195 (44.1)	278 (43.2)	65 (45.8)	**56 (20.5)**	**779 (48.4)**	**277 (55.4)**
Yes	184,470 (53.5)	96 (49.0)	240 (54.3)	362 (56.2)	76 (53.5)	**213 (78.0)**	**816 (50.7)**	**217 (43.4)**
Missing	4,211 (1.2)	5 (2.6)	7 (1.6)	-	-	-	**14 (0.9)**	**6 (1.2)**
Adequate Prenatal Care [b]								
No	35,249 (10.2)	**30 (15.3)**	39 (8.8)	63 (9.8)	19 (13.4)	**33 (12.1)**	**133 (8.3)**	59 (11.8)
Yes	293,952 (85.3)	**151 (77.0)**	385 (87.1)	551 (85.7)	114(80.2)	**213 (78.0)**	**1,409 (87.6)**	422 (84.4)
Missing	15,365 (4.4)	**15 (7.7)**	18 (4.1)	29 (4.5)	9 (6.3)	**27 (9.9)**	**67 (4.2)**	19 (3.8)
Child Sex								
Male	175,548 (50.9)	102 (52.0)	263 (59.5)	354 (55.0)	76 (53.5)	152 (55.7)	**1,609 (100)**	269 (53.8)
Female	169,013 (49.1)	93 (47.4)	179 (40.5)	289 (45.0)	66 (46.4)	121 (44.3)	**0 (0.0)**	231 (46.2)
Missing	5 (≥ .1)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**0 (0.0)**	0 (0.0)
Child Birth Year								
2004	68,973 (20.0)	37 (18.9)	93 (21.04)	**115 (17.9)**	**23 (16.2)**	49 (17.9)	289 (18.0)	94 (18.8)
2005	69,596 (20.2)	48 (24.5)	95 (21.5)	**144 (22.4)**	**37 (26.1)**	51 (18.7)	346 (21.5)	108 (21.6)
2006	69,750 (20.2)	27 (13.8)	81 (18.3)	**117 (18.2)**	**31 (21.8)**	46 (16.8)	323 (20.1)	102 (20.4)
2007	70,235 (20.4)	39 (19.9)	96 (21.7)	**158 (24.6)**	**16 (11.3)**	69 (25.3)	327 (20.3)	112 (22.4)
2008	66,012 (19.2)	45 (23.0)	77 (17.4)	**109 (17.0)**	**35 (24.7)**	58 (21.3)	324 (20.1)	84 (16.8)
Nitrate Exposure (quartiles) [c]								
Ql	88,194 (25.6)	47 (24.0)	117 (26.5)	167 (26.0)	**38 (26.8)**	69 (25.3)	431 (26.7)	137 (27.4)
Q2	84,138 (24.4)	52 (26.5)	102 (23.1)	151 (23.5)	**20 (14.1)**	61 (22.3)	390 (24.2)	137 (27.4)
Q3	92,405 (26.8)	52 (26.5)	117 (26.5)	182 (28.3)	**35 (24.6)**	82 (30.0)	414 (25.7)	121 (24.2)

	Normal Births (n= 344,566)	Neural Tube (n= 196)	Congenital Heart (n= 442)	Oral Cleft (n= 643)	Limb Deficiencies (n= 142)	Gastroschisis (n= 273)	Hypospadias (n= 1,609)	Down Syndrome (n= 500)
Q4	79,829 (23.2)	45 (23.0)	106 (24.0)	143 (22.2)	**49 (34.5)**	61 (22.3)	378 (23.4)	105 (21.0)

[a]Low socioeconomic status is defined as participation in one or more of the following public assistance programs: WIC, Medicaid, or federal food stamp programs.

[b]Adequate prenatal care is defined as live births with five or more prenatal care visits for pregnancies lasting less than 37 weeks of gestation, eight or more visits for pregnancies lasting 37 or more weeks, or prenatal care that began during the first four months of pregnancy.

[c]Q1 = 0.025 - < .082 mg/L, Q2 = .082 - < 0.18 mg/L, Q3 = 0.18 – < 0.34 mg/L, Q4 = 0.34 – 6.36 mg/L

[*]Total of summarized births is greater than 348,250 as 121 births had more than one recorded birth defect.

Dashes indicate censoring due to low cell count in order to protect subject privacy.

Bold indicates a statistically significant (i.e. p<0.05) difference in proportions in comparison to non-cases.

Table 2:

Summary of estimated drinking water nitrate exposure during 12 months prior to birth by year of birth in Missouri, 2004–2008.

Year	N (births)	Mean ± SD (mg/L)	Median concentration (mg/L)	Minimum concentration (mg/L)	Maximum concentration (mg/L)[a]
2004	69,658	0.30 ± 0. 46	0.20	0.03	5.82
2005	70,395	0.34 ± 0.50	0.24	0.03	5.80
2006	70,455	0.22 ± 0.46	0.12	0.03	6.02
2007	71,019	0.30± 0.60	0.15	0.03	5.96
2008	66,723	0.71 ± 0.99	0.33	0.03	6.36

[a]All concentrations below the EPA's MCL of 10 mg/L

Table 3:

Associations between mean nitrate exposure 12 months pre-birth (mg/L) and birth defects in Missouri, 2004–2008.

Condition	Model [a]	% Well Restriction [b]	Births (cases)	RR (95% CI) [c]	p-value
Neural Tube	Crude	n/a	348,250 (196)	0.98 (0.79, 1.22)	0.887
Defects	Adjusted	n/a	342,514 (191)	1.03 (0.84, 1.27)	0.770
	Crude	< 20	233,990 (121)	1.08 (0.88, 1.33)	0.475
	Adjusted	< 20	229,104 (117)	1.12 (0.92, 1.35)	0.264
	Crude	< 10	210,297 (97)	1.14 (0.88, 1.47)	0.335
	Adjusted	< 10	205,994 (94)	1.18 (0.93, 1.51)	0.165
Congenital Heart	Crude	n/a	348,250 (442)	1.03 (0.89, 1.19)	0.703
Defects	Adjusted	n/a	342,514 (432)	1.06 (0.92, 1.23)	0.430
	Crude	< 20	233,990 (279)	1.07 (0.92, 1.24)	0.402
	Adjusted	< 20	229,104 (272)	1.09 (0.94, 1.27)	0.267
	Crude	< 10	210,297 (245)	1.09 (0.90, 1.31)	0.366
	Adjusted	< 10	205,994 (238)	1.13 (0.93, 1.38)	0.215
Oral Cleft Defects	Crude	n/a	348,250 (643)	0.91 (0.80, 1.04)	0.178
	Adjusted	n/a	342,514 (637)	0.93 (0.82, 1.06)	0.316
	Crude	< 20	233,990 (405)	0.87 (0.74, 1.01)	0.070
	Adjusted	< 20	229,104 (402)	0.90 (0.77, 1.05)	0.168
	Crude	< 10	210,297 (364)	0.89 (0.75, 1.06)	0.202
	Adjusted	< 10	205,994 (362)	0.93 (0.79, 1.11)	0.431
Limb Deficiencies	Crude	n/a	348,250 (142)	**1.23 (1.02, 1.48)**	**0.024**
	Adjusted	n/a	342,514 (141)	**1.26 (1.05, 1.51)**	**0.011**
	Crude	< 20	233,990 (106)	**1.21 (1.01, 1.47)**	**0.044**
	Adjusted	< 20	229,104 (105)	**1.24 (1.03, 1.48)**	**0.020**
	Crude	< 10	210,297 (90)	1.25 (0.97, 1.62)	0.089
	Adjusted	< 10	205,994 (90)	**1.31 (1.02, 1.68)**	**0.032**
Gastroschisis	Crude	n/a	348,250 (273)	1.01 (0.84, 1.22)	0.908
	Adjusted	n/a	342,514 (268)	0.94 (0.76, 1.16)	0.582
	Crude	< 20	233,990 (177)	1.03 (0.84, 1.26)	0.758
	Adjusted	< 20	229,104 (173)	0.98 (0.79, 1.22)	0.879
	Crude	< 10	210,297 (161)	1.07 (0.85, 1.36)	0.542
	Adjusted	< 10	205,994 (157)	1.02 (0.80, 1.31)	0.858
Hypospadias [d]	Crude	n/a	178,295 (1,612)	0.98 (0.91, 1.06)	0.596
	Adjusted	n/a	175,329 (1,591)	0.98 (0.91, 1.06)	0.664
	Crude	< 20	119,400 (1,097)	0.98 (0.91, 1.06)	0.695
	Adjusted	< 20	116,857(1,079)	0.99 (0.91, 1.07)	0.795
	Crude	< 10	107,403 (1,010)	1.02 (0.93, 1.11)	0.675
	Adjusted	< 10	105,159 (994)	1.03 (0.94, 1.13)	0.497
Down Syndrome	Crude	n/a	348,250 (500)	0.91 (0.77, 1.07)	0.247

Condition	Model [a]	% Well Restriction [b]	Births (cases)	RR (95% CI) [c]	p-value
	Adjusted	n/a	342,514 (491)	0.93 (0.80, 1.10)	0.438
	Crude	< 20	233,990 (346)	0.92 (0.78, 1.08)	0.308
	Adjusted	< 20	229,104 (338)	0.94 (0.79, 1.12)	0.508
	Crude	< 10	210,297 (314)	0.90 (0.74, 1.09)	0.263
	Adjusted	< 10	205,994 (306)	0.91 (0.74, 1.12)	0.377

[a] All adjusted models controlled for maternal age, race, ethnicity, and socioeconomic status.

[b] Models without private well restriction included 113 Missouri counties, models with < 20% well restriction included 58 MO counties, models with < 10% well restriction included 48 MO counties.

[c] Rate Ratio for one mg/L of nitrate in water.

[d] Hypospadias models contain only male births
Bold indicates statistical significance (i.e. p<0.05)

Table 4:

Associations between mean nitrate exposure 12 months pre-birth (MG/L) and specific limb, cardiac, and neural tube defects in Missouri, 2004–2008.

Condition	Model [a]	% Well Restriction [b]	Births (Cases)	RR (95% CI) [c]	p-value
Limb Deficiencies					
Upper Limb	Crude	n/a	348,250 (100)	**1.28 (1.04, 1.59)**	**0.021**
	Adjusted	n/a	342,514 (99)	**1.31 (1.06, 1.62)**	**0.013**
	Crude	< 20	233,990 (75)	**1.26 (1.01, 1.57)**	**0.042**
	Adjusted	< 20	229,104 (74)	**1.28 (1.03, 1.59)**	**0.026**
	Crude	< 10	210,297 (63)	1.30 (0.96, 1.77)	0.091
	Adjusted	< 10	205,994 (63)	**1.36 (1.01, 1.84)**	**0.045**
Lower Limb	Crude	n/a	348,250 (65)	1.22 (0.98, 1.52)	0.072
	Adjusted	n/a	342,514 (65)	**1.25 (1.02, 1.53)**	**0.030**
	Crude	< 20	233,990 (49)	1.20 (0.96, 1.51)	0.109
	Adjusted	< 20	229,104 (49)	**1.24 (1.00, 1.52)**	**0.046**
	Crude	< 10	210,297 (42)	1.29 (0.96, 1.74)	0.093
	Adjusted	< 10	205,994 (42)	**1.35 (1.03, 1.77)**	**0.032**
Heart Defects					
HLHS	Crude	n/a	348,250 (127)	1.17 (0.89, 1.53)	0.256
	Adjusted	n/a	342,514 (124)	1.23 (0.95, 1.59)	0.123
	Crude	< 20	233,990 (82)	1.26 (0.99, 1.62)	0.058
	Adjusted	< 20	229,104 (80)	**1.32 (1.04, 1.67)**	**0.021**
	Crude	< 10	210,297 (72)	1.25 (0.89, 1.74)	0.204
	Adjusted	< 10	205,994 (70)	1.34 (0.96, 1.86)	0.087
TGA & ToF	Crude	n/a	348,250 (341)	1.01 (0.86, 1.19)	0.858
	Adjusted	n/a	342,514 (333)	1.04 (0.88, 1.22)	0.606
	Crude	< 20	233,990 (212)	1.02 (0.85, 1.24)	0.808
	Adjusted	< 20	229,104 (207)	1.04 (0.86, 1.25)	0.695
	Crude	< 10	210,297 (187)	1.10 (0.88, 1.37)	0.390
	Adjusted	< 10	205,994 (182)	1.13 (0.90, 1.42)	0.283
Neural Tube Defects					
Anencephaly	Crude	n/a	348,250 (47)	1.02 (0.71, 1.48)	0.915
	Adjusted	n/a	342,514 (47)	1.07 (0.75, 1.52)	0.705
	Crude	< 20	233,990 (33)	1.06 (0.74, 1.51)	0.745
	Adjusted	< 20	229,104 (33)	1.10 (0.78, 1.54)	0.582
	Crude	< 10	210,297 (26)	1.16 (0.74, 1.82)	0.510
	Adjusted	< 10	205,994 (26)	1.20 (0.79, 1.82)	0.394
Spina Bifida	Crude	n/a	348,250 (149)	0.97 (0.75, 1.27)	0.837
	Adjusted	n/a	342,514 (144)	1.02 (0.79, 1.31)	0.883
	Crude	< 20	233,990 (88)	1.09 (0.84, 1.39)	0.523
	Adjusted	< 20	229,104 (84)	1.12 (0.89, 1.41)	0.332

Condition	Model [a]	% Well Restriction [b]	Births (Cases)	RR (95% CI) [c]	p-value
	Crude	< 10	210,297 (71)	1.12 (0.82, 1.54)	0.460
	Adjusted	< 10	205,994 (68)	1.18 (0.88, 1.57)	0.266
Oral Cleft Defects					
Cleft Lip with or without Cleft Palate	Crude	n/a	348,250 (415)	0.86 (0.72, 1.02)	0.083
	Adjusted	n/a	342,514 (410)	0.88 (0.74, 1.05)	0.144
	Crude	< 20	233,990 (256)	**0.77 (0.62, 0.96)**	**0.021**
	Adjusted	< 20	229,104 (254)	0.82 (0.66, 1.01)	0.060
	Crude	< 10	210,297 (235)	0.81 (0.64, 1.02)	0.078
	Adjusted	< 10	205,994 (234)	0.86 (0.68, 1.08)	0.199
Cleft Palate without Cleft Lip	Crude	n/a	348,250 (279)	0.97 (0.80, 1.17)	0.760
	Adjusted	n/a	342,514 (277)	0.99 (0.82, 1.20)	0.952
	Crude	< 20	233,990 (177)	0.96 (0.78, 1.18)	0.680
	Adjusted	< 20	229,104 (175)	0.98 (0.80, 1.20)	0.825
	Crude	< 10	210,297 (150)	1.00 (0.78, 1.27)	0.972
	Adjusted	< 10	205,994 (149)	1.02 (0.80, 1.30)	0.883

Note: “HLHS” indicates hypoplastic left heart syndrome, “TGA” indicates transposition of the great arteries, and “ToF” indicates tetralogy of Fallot

[a] All adjusted models controlled for maternal age, race, ethnicity, and socioeconomic status.

[b] Models without private well restriction included 113 Missouri counties, models with < 20% well restriction included 58 MO counties, models with < 10% well restriction included 48 MO counties.

[c] Rate Ratio for one mg/L of nitrate in water
Bold indicates statistical significance (i.e. p<0.05)

Table 5:

Associations between mean first trimester nitrate exposure (mg/L) and birth defects in Missouri, 2004–2008.

Condition	Model [a]	% Well Restriction [b]	Births (cases)	RR_1 (95% CI) [c]	p-value
Neural Tube Defects	Crude	n/a	134,556 (86)	1.00 (0.78, 1.29)	0.994
	Adjusted	n/a	132,475 (85)	1.02 (0.80, 1.30)	0.897
	Crude	< 20	79,478 (51)	1.12 (0.88, 1.43)	0.920
	Adjusted	< 20	77,839 (50)	1.14 (0.91, 1.42)	0.264
	Crude	< 10	69,812 (37)	1.04 (0.73, 1.50)	0.818
	Adjusted	< 10	68,422 (36)	1.07 (0.76, 1.49)	0.708
Congenital Heart Defects	Crude	n/a	134,556 (178)	**1.17 (1.00, 1.36)**	**0.037**
	Adjusted	n/a	132,475 (174)	**1.17 (1.01, 1.36)**	**0.041**
	Crude	< 20	79,478 (104)	**1.29 (1.09, 1.52)**	**0.003**
	Adjusted	< 20	77,839 (101)	**1.28 (1.09, 1.52)**	**0.003**
	Crude	< 10	69,812 (87)	**1.33 (1.12, 1.58)**	**0.001**
	Adjusted	< 10	68,422 (84)	**1.33 (1.12, 1.58)**	**0.001**
Oral Cleft Defects	Crude	n/a	134,556 (267)	0.98 (0.81, 1.19)	0.832
	Adjusted	n/a	132,475 (264)	1.00 (0.84, 1.20)	0.976
	Crude	< 20	79,478 (132)	0.88 (0.67, 1.16)	0.366
	Adjusted	< 20	77,839 (131)	0.91 (0.71, 1.17)	0.471
	Crude	< 10	69,812 (116)	0.90 (0.66, 1.22)	0.495
	Adjusted	< 10	68,422 (115)	0.93 (0.70, 1.24)	0.625
Limb Deficiencies	Crude	n/a	134,556 (46)	1.17 (0.88, 1.54)	0.285
	Adjusted	n/a	132,475 (46)	1.18 (0.90, 1.54)	0.242
	Crude	< 20	79,478 (27)	1.27 (0.92, 1.74)	0.140
	Adjusted	< 20	77,839 (27)	1.28 (0.95, 1.73)	0.105
	Crude	< 10	69,812 (22)	1.32 (0.93, 1.88)	0.115
	Adjusted	< 10	68,422 (22)	1.35 (0.97, 1.88)	0.077
Gastroschisis	Crude	n/a	134,556 (95)	1.15 (0.95, 1.39)	0.150
	Adjusted	n/a	132,475 (92)	1.12 (0.91, 1.38)	0.273
	Crude	< 20	79,478 (58)	1.20 (0.96, 1.51)	0.117
	Adjusted	< 20	77,839 (56)	1.20 (0.95, 1.51)	0.130
	Crude	< 10	69,812 (53)	1.25 (0.99, 1.57)	0.056
	Adjusted	< 10	68,422 (51)	1.26 (1.00, 1.58)	0.054
Hypospadias [d]	Crude	n/a	68,815 (615)	1.01 (0.92, 1.12)	0.771
	Adjusted	n/a	67,759 (607)	1.00 (0.90, 1.11)	0.978
	Crude	< 20	40,422 (345)	1.05 (0.94, 1.17)	0.350
	Adjusted	< 20	39,575 (338)	0.92 (0.78, 1.08)	0.309
	Crude	< 10	35,537 (313)	1.09 (0.97, 1.22)	0.132
	Adjusted	< 10	34,817 (307)	0.98 (0.84, 1.14)	0.808
Down Syndrome	Crude	n/a	134,556 (195)	1.03 (0.84, 1.26)	0.768
	Adjusted	n/a	132,475 (190)	1.04 (0.85, 1.23)	0.689

Condition	Model [a]	% Well Restriction [b]	Births (cases)	RR_1 (95% CI) [c]	p-value
	Crude	< 20	79,478 (119)	0.99 (0.78, 1.23)	0.842
	Adjusted	< 20	77,839 (115)	0.98 (0.77, 1.24)	0.852
	Crude	< 10	69,812 (109)	1.02 (0.82, 1.28)	0.839
	Adjusted	< 10	68,422 (105)	1.02 (0.81, 1.30)	0.842

[a] All adjusted models controlled for maternal age, race, ethnicity, and socioeconomic status.

[b] Models without private well restriction included 113 Missouri counties, models with < 20% well restriction included 58 MO counties, models with < 10% well restriction included 48 MO counties.

[c] Rate Ratio for one mg/L of nitrate in water.

[d] Hypospadias models contain only male births Bold indicates statistical significance (i.e. $p<0.05$)

Table 6:

Associations between mean nitrate exposure 12 months pre-birth (mg/L) and birth defects in rural counties of Missouri, 2004–2008.

Condition	Model [a]	% Well Restriction [b]	Births (cases)	RR (95% CI) [c]	p-value
Neural Tube Defects	Crude	n/a	133,081 (95)	0.78 (0.49, 1.21)	0.255
	Adjusted	n/a	131,761 (94)	0.78 (0.50, 1.22)	0.281
	Crude	< 20	52,984 (42)	0.95 (0.71, 1.27)	0.730
	Adjusted	< 20	52,247 (41)	0.97 (0.73, 1.30)	0.835
	Crude	< 10	37,945 (26)	1.03 (0.77, 1.37)	0.858
	Adjusted	< 10	37,504 (26)	1.03 (0.77, 1.39)	0.831
Congenital Heart Defects	Crude	n/a	133,081 (174)	1.07 (0.87, 1.31)	0.519
	Adjusted	n/a	131,761 (171)	1.08 (0.89, 1.32)	0.428
	Crude	< 20	52,984 (67)	1.14 (0.93, 1.40)	0.211
	Adjusted	< 20	52,247 (67)	1.14 (0.92, 1.39)	0.224
	Crude	< 10	37,945 (45)	**1.40 (1.07, 1.84)**	**0.016**
	Adjusted	< 10	37,504 (45)	**1.38 (1.05, 1.82)**	**0.019**
Oral Cleft Defects	Crude	n/a	133,081 (288)	0.88 (0.72, 1.09)	0.255
	Adjusted	n/a	131,761 (285)	0.86 (0.69, 1.08)	0.190
	Crude	< 20	52,984 (107)	0.61 (0.33, 1.12)	0.112
	Adjusted	< 20	52,247 (107)	0.62 (0.34, 1.12)	0.113
	Crude	< 10	37,945 (81)	0.44 (0.16, 1.19)	0.104
	Adjusted	< 10	37,504 (81)	0.43 (0.15, 1.18)	0.102
Limb Deficiencies	Crude	n/a	133,081 (57)	1.25 (0.98, 1.59)	0.073
	Adjusted	n/a	131,761 (57)	1.25 (0.98, 1.60)	0.070
	Crude	< 20	52,984 (33)	1.19 (0.94, 1.52)	0.148
	Adjusted	< 20	52,247 (33)	1.19 (0.93, 1.51)	0.160
	Crude Adjusted	< 10	37,945 (22)	**1.49 (1.08, 2.07)**	**0.015**
		< 10	37,504 (22)	**1.48 (1.08, 2.05)**	**0.016**
Gastroschisis	Crude	n/a	133,081 (196)	0.97 (0.71, 1.33)	0.864
	Adjusted	n/a	131,761 (191)	0.90 (0.62, 1.32)	0.602
	Crude	< 20	52,984 (121)	0.98 (0.67, 1.42)	0.909
	Adjusted	< 20	52,247 (117)	0.98 (0.67, 1.42)	0.905
	Crude	< 10	37,945 (97)	1.19 (0.71, 2.02)	0.510
	Adjusted	< 10	37,504 (94	1.21 (0.72, 2.05)	0.475
Hypospadias [d]	Crude	n/a	68,322 (566)	0.88 (0.76, 1.02)	0.094
	Adjusted	n/a	67,672 (560)	0.88 (0.76, 1.02)	0.100
	Crude	< 20	27,030 (213)	0.82 (0.65, 1.04)	0.098
	Adjusted	< 20	26,671 (210)	0.82 (0.65, 1.04)	0.101
	Crude	< 10	19,453 (166)	0.87 (0.53, 1.46)	0.607
	Adjusted	< 10	19,245 (163)	0.87 (0.53, 1.45)	0.599
Down Syndrome	Crude	n/a	133,081 (172)	0.91 (0.67, 1.22)	0.511

Condition	Model [a]	% Well Restriction [b]	Births (cases)	RR (95% CI) [c]	p-value
	Adjusted	n/a	131,761 (171)	0.93 (0.67, 1.30)	0.600
	Crude	< 20	52,984 (71)	0.93 (0.67, 1.09)	0.315
	Adjusted	< 20	52,247 (71)	0.93 (0.75, 1.10)	0.680
	Crude	< 10	37,945 (49)	0.88 (0.71, 1.10)	0.259
	Adjusted	< 10	37,504 (49)	0.88 (0.69, 1.09)	0.231

[a] All adjusted models controlled for maternal age, race, ethnicity, and socioeconomic status.

[b] Models without private well restriction included 99 Missouri counties, models with < 20% well restriction included 47 MO counties, models with < 10% well restriction included 38 MO counties.

[c] Rate Ratio for one mg/L of nitrate in water

[d] Hypospadias models contain only male births Bold indicates statistical significance (i.e. p<0.05)