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# Atrazine and nitrate in drinking water and the risk of preterm delivery and low birth weight in four Midwestern states☆

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ABSTRACT

*Background:* Atrazine and nitrate are common contaminants in water, and there is limited evidence that they are associated with adverse birth outcomes. The objective of this study was to examine whether atrazine and nitrate in water are associated with an increased risk of preterm delivery (PTD) and term low birth weight (LBW).

*Methods:* The study included a total of 134,258 singletons births born between January 1, 2004 and December 31, 2008 from 46 counties in four Midwestern states with public water systems that were included in the U.S. Environmental Protection Agency (EPA)'s atrazine monitoring program (AMP). Counties with a population of > 300,000 were eliminated from the analyses in order to avoid confounding by urbanicity. Monthly child's sex, race and Hispanic ethnicity specific data were obtained from the states for estimating rates of PTD ( < 37 weeks) and very preterm (VPTD, < 32 weeks), term LBW ( < 2.5 kg among infants born at term) and very low birth weight (VLBW, < 1.5 kg). The rates were linked with county specific monthly estimates of the concentration of atrazine and nitrate in finished water. Multivariable negative binomial models were fitted to examine the association between the exposures and the adverse birth outcomes. Models were fitted with varying restrictions on the percentage of private well usage in the counties in order to limit the degree of exposure misclassification.

*Results:* Estimated water concentrations of atrazine (mean=0.42 ppb) and nitrate (mean=0.95 ppm) were generally low. Neither contaminant was associated with an increased risk of term LBW. Atrazine exposure was associated with a significant increased rate of PTD when well use was restricted to 10% and the exposure was averaged over 4–6 months prior to birth (Rate Ratio for 1 ppm increase [$RR_{1\ ppm}$]=1.08, 95%CI=1.05,1.11) or over 9 months prior to birth ($RR_{1\ ppm}$=1.10, 95%CI=1.01,1.20). Atrazine exposure was also associated with an increased rate of VPTD when when well use was restricted to 10% and the exposure was averaged over 7–9 months prior to birth ($RR_{1\ ppm}$=1.19, 95%CI=1.04,1.36). Exposure to nitrate was significantly associated with an increased rate of VPTD ($RR_{1\ ppm}$=1.08, 95%CI=1.02,1.15) and VLBW ($RR_{1\ ppm}$=1.17, 95%CI=1.08,1.25) when well use was restricted to 20% and the exposure was averaged over 9 months prior to birth.

*Conclusion:* The positive and negative findings from our study need to be interpreted cautiously given its ecologic design, and limitations in the data for the exposures and other risk factors. Nonetheless, our findings do raise concerns about the potential adverse effects of these common water contaminants on human development and health, and the adequacy of current regulatory standards. Further studies of these issues are needed with individual level outcome data and more refined estimates of exposure.

## 1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is the second most commonly used herbicide in the United States (U.S.). Approximately 76.4 million pounds of atrazine are applied annually, and it is the most commonly detected pesticide in drinking

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water in the U.S. (U.S. EPA, 2015). Particularly high concentrations of atrazine have been detected in ground and surface water in Midwestern U.S. states where it is commonly used to control weeds in the production of corn and other crops (U.S.G.S., 2007). The frequency of atrazine detection in water is related to its intense usage, moderate persistence and mobility through the soil (Jayachandran et al., 1994).

Atrazine is a well recognized endocrine disruptor. It has been shown to decrease serum and testicular testosterone and leutenizing hormone levels in rats (Stoker et al., 2000). Hayes et al. (2010) has reported feminization of male frogs exposed to atrazine in water; however, these findings were not replicated in an industry-funded study (Du Preez et al., 2008). While the exact mechanism for atrazine's estrogenic effects is unknown, there is evidence that atrazine elicits estrogen activity by up regulating aromatase activity (Fan et al., 2007; Heneweer et al., 2004; Sanderson et al., 2000, 2002) and by inhibiting cAMP specific phosphodiesterases (Kucka et al., 2012).

A few epidemiologic studies have examined the potential adverse effects of atrazine on human reproduction and birth outcomes. Atrazine exposure in drinking water has been reported to be associated with an increased risk of preterm delivery (PTD, < 37 completed weeks) (Rinsky et al., 2012), and being small for gestational age (SGA) (Munger et al., 1997; Ochoa-Acuña et al., 2009). Urinary metabolites of atrazine have been reported to be associated with fetal growth restriction (Chevrier et al., 2011) and poor semen quality (Swan et al., 2003). Occupational exposure in men has been reported to be associated with an increased risk of preterm delivery (Savitz et al., 1997). A few studies have reported an increased risk of birth defects of the central nervous system (Arbuckle et al., 1988), neural tube defects (Brender et al., 2004) spina bifida, cleft palate/lip and limb defects (Brender et al., 2013) among children of women who consumed high levels of nitrate in drinking water.

Nitrate is the most commonly found contaminant in the world's aquifers (Exner et al., 2014). Nitrate fertilizers are also commonly used in the production of corn, and drinking water contamination from nitrate is a major concern in the Midwestern U.S. (Exner, Hirsh and Spalding, 2014). High concentrations of nitrate and nitrite ( > 10 mg total nitrogen/L) in drinking water have been recognized since the 1940s to cause cyanosis (“blue baby syndrome”) in children due to methemoglobinemea (Comly, 1987). There is limited epidemiologic evidence that lower levels of nitrate in drinking water are associated with an increased risk of spontaneous abortions (Aschengrau et al., 1989; Grant et al., 1996), PTD (Manassaram et al., 2006), low birth weight (LBW < 2.5 kg) (Bukowski et al., 2001), SGA (Migeot et al., 2013) and central nervous system birth defects (Dorsch et al., 1984; Arbuckle et al., 1988; Brender et al., 2004; and Croen et al., 2001).

PTD and LBW are important public health issues affecting approximately 9.6% and 8.0% of all births in 2014 in the U.S. (Hamilton et al., 2015). PTD has been associated with an increase in infant mortality, asthma, learning, motor, visual and hearing impairment (Glass et al., 2015). LBW has been linked with neonatal mortality as well as cardiovascular disease, hypertension, diabetes and other adverse health effects observed later in life (Barker, 2006; Intapad et al., 2014).

We conducted this study in order to examine the potential risk of PTD and LBW from drinking water contaminated with atrazine or nitrate and from co-exposure to both contaminants. This work was funded by the Centers for Disease Control and Prevention's Environmental Public Health Tracking Program (EPHT) (Balluz, 2014), and an additional objective was to demonstrate the potential utility of conducting environmental health research involving health and environmental data routinely collected by state and federal agencies.

## 2. Material and methods

### 2.1. Study population

The study population included 134,258 live-born singleton births from January 1, 2004 to December 31, 2008 in 46 rural counties in four Midwestern states (Ohio, Indiana, Iowa and Missouri) that have water systems included in the United States Environmental Protection Agency's (U.S.EPA) atrazine monitoring program (AMP) at any time during 2003–2008. Counties with a population greater than 300,000 were excluded from the analysis to avoid potential confounding by urban areas, which have very low potential for exposure to atrazine and high potential for exposure to urban air pollution, lead and other socioeconomic risk factors for adverse birth outcomes relative to rural areas. In order to distinguish between prematurity and fetal growth restriction, we further restricted analysis of LBW to only include full term births ( ≥ 37 weeks) resulting in a total population of 121,604 births for this outcome.

### 2.2. Outcome definitions

Data on the total number of births, PTD, very preterm delivery (VPTD), LBW and very low birth weight (VLBW) for each county were obtained from the birth registries in each state. The data were stratified by the year and month of birth, the child's sex, race and ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other/unknown). Births were classified as PTD if they were < 37 weeks of gestation and as VPTD if they were < 32 weeks of gestation. Infants were classified as LBW if they were < 2500 g and VLBW if they were < 1500 g and born full term (≥37 weeks gestation).

### 2.3. Exposure assessment

The exposure estimates for each birth were based on the monthly county-level mean atrazine (ppb) and nitrate concentrations in counties that contain one or more community water systems (CWS) that participated in the AMP at any time between 2003 and 2008. The county-level mean exposure variables were calculated as follows: 1) the average concentrations of atrazine and total nitrogen in finished water were calculated for each CWS in each calendar month, and when multiple CWSs were present in a county, 2) the monthly CWS average concentrations in each county were averaged, weighted by the population served by each CWS.

CWSs participating in the AMP are considered by the U.S. EPA as being vulnerable to atrazine contamination due to repeated measurement of high atrazine levels in routine water testing, and are required to measure the levels of atrazine approximately twice per calendar month in raw and finished water. Water quality data for nitrate and for atrazine in CWSs not part of the AMP were obtained from Safe Drinking Water Information Systems (SDWIS) maintained by each state. National Primary Drinking Water Regulations specify that CWSs using groundwater sample annually for nitrate, and that CWSs using surface water initially sample quarterly for nitrate, then annually if concentrations are below the Maximum Contaminant Level (MCL, 40 CFR § 141.62). The Regulations specify that CWSs sample for atrazine quarterly, then once every three years if concentrations are below the MCL (40 CFR § 141.61).

The Maximum Contaminant Levels (MCLs) are 10 ppm, 1 ppm and 3 μg/L for nitrate, nitrite and atrazine, respectively. The nitrate exposure estimates that were used in this study were based on measurement of nitrogen compounds (NO3-N) using a colorimetric method (EPA Method 353.2). This method actually provides a measure of nitrate and nitrite combined although nitrite is generally a minor component. Both atrazine and nitrate measurements were frequently below the limit of detection (LOD), which were 1–3 orders of magnitude below the MCL (Jones et al., 2014). Samples below the

LOD were assigned a value of the LOD/2 for this analysis. Average concentrations of atrazine and nitrate were estimated over 0–3 months, 4–6 months, and 7–9 months and 9 months prior to birth. These estimates were treated as missing if there was not a single measurement during these periods of time.

### 2.4. Statistical analysis

Negative binomial models were fitted to the data using generalized estimating equations (GEE) with an exchangeable correlation matrix, and robust standard errors to account for the clustering of the outcome data by county. The negative binomial models were generally found to provide a superior fit (i.e. lower deviance) to the data than Poisson regression models. All analyses were conducted using STATA version 13.0. (StataCorp).

Separate models were fitted for each of the four outcomes. The offset in the models was the natural log of the number of full term singleton births for LBW, and the natural log of the number of all singleton births PTD, VPTD and VLBW.

The exposure variables were modeled as continuous concentrations of atrazine or nitrate in drinking water. The monthly county-level mean atrazine and nitrate concentrations were linked to each birth by county and month of birth to tabulate the mean exposure: during the month of birth, over the 3 months prior to birth, 4–6 months prior to birth, 7–9 months prior to birth, and over the 9 months prior to birth. All models included covariates to control for the child's sex, race and ethnicity, calendar year, and season of birth (winter-January to March; spring April to June; summer- July to September and; fall- October to December), which were variables derived from the state birth records. We also included in the models county level variables that were obtained from the U.S. Census on maternal education (% attended high school, and % attended college), median income and population density (number of persons/acreage); state of birth; and data on the percentage of women smoking that was derived from the Behavioral Risk Factor Surveillance System (Dwyer-Lingren et al., 2014).

Models were fitted that included either atrazine or nitrate, and that included both contaminants. Statistical interactions between atrazine and nitrate were evaluated and tested by fitting models that included both variables and a cross-product term. The shape of the exposure-response was examined by fitting a restricted cubic spline model with three knots. Spline models offer a less biased and more efficient alternative to fitting linear or categorical models (Howe et al., 2011).

We conducted sensitivity analyses in which we restricted the data to only include counties with varying levels of private well usage ( < 20 or < 10%). Private wells are not routinely tested for either atrazine or nitrates in these states, and thus there was a large potential for misclassification of these exposures in counties that primarily use private well water. State of birth frequently had to be dropped in these models in order to achieve model convergence. Sensitivity analyses were also conducted in which data points exceeding the U.S. EPA standards for atrazine (3 ppb) and nitrates (10 ppm) were dropped.

Our study protocol was reviewed and approved by the Institutional Review Board of the University of Illinois at Chicago. It was also reviewed and approved by the states involved in the study.

## 3. Results

### 3.1. Study population

A total of 134,258 births from 46 counties were included, of which there were 13,875 (10.3%) PTD, 1882 (1.4%) VPTD, 3016 (2.2%) LBW and 1386 (1.0%) VLBW children. Characteristics of the distribution of the variables used in this study for the entire population and for the outcomes examined are presented in Table 1. Associations between the rate of these outcomes and the covariates were assessed by fitting a GEE model that only included the covariate and an intercept term. As expected, boys were significantly more likely to be PTD ($p < 0.01$) and less likely to be LBW ($p < 0.001$) than girls. Maternal race-ethnicity was strongly associated ($p < 0.001$) with all of the outcomes, which was primarily due to blacks having higher rates of PTD, VPTD, LBW and VLBW. There were strong ($p < 0.001$) differences between the states for the rates of PTD and VPTD due primarily to high rates in Missouri and Ohio. A weak association ($p=0.04$) was observed between VLBW and state due to a higher rate in Ohio. Calendar year, and season of birth were not associated with any of the outcomes. Lower percentage of high school ($p < 0.001$) and college education ($p < 0.01$), lower median income ($p < 0.05$) and higher percentage of female smokers ($p < 0.05$) were associated with higher rates of LBW. Higher percentage of households on private wells was associated with higher rates of LBW ($p < 0.01$), PTD ($p=0.001$), and to a lesser extent with VPTD births ($p=0.04$). Data on maternal age variables was only available for 2004–2006, and was thus not controlled for in the multivariable analyses. However, maternal age did not appear to be associated with the outcomes in this study except for LBW, which was just slightly higher (10.4%) among young ( < 18%) mothers ($p < 0.05$) than among all births (10.0%).

### 3.2. Exposure concentrations of atrazine and nitrate in drinking water

The mean of the monthly county-level atrazine and nitrate concentrations were 0.42 (Standard Deviation (SD)$\pm$0.40) ppm for atrazine, and 0.95 (SD$\pm$0.92) ppb for nitrate (Table 1). Only 2% of the monthly county-level estimates for atrazine and only 1.8% of the monthly estimates for nitrate exceeded the respective MCLs. Monthly county-level mean atrazine and nitrate concentrations were weakly ($r=0.06$) but statistically significantly ($p < 0.001$) correlated.

Of the linked exposure variables, exposure estimates for the 9 months prior to birth for atrazine were missing for 0.3% of births, and for nitrates were missing for 10.8% of births. Many more births did not have exposure estimates for other time periods: For example approximately 35% of births did not have nitrate exposure estimates for the 0–3 months, 4–6 and 7–9 months prior to birth.

Box plots of the distribution of monthly county-level mean atrazine and nitrate concentrations by month are presented in Fig. 1. There was a distinct seasonal pattern for atrazine, with the highest concentrations occurring in the early summer months (i.e. June and July), and the lowest concentrations in winter and early spring. This is similar to the seasonal pattern that has been reported in other studies in the Midwestern U.S. (Ochoa-Acuña et al., 2009; Rinsky et al., 2012; Winchester et al., 2009) In contrast, the highest nitrate concentrations were observed in the winter and early spring and the lowest concentrations in the summer and fall months. The mean monthly county-level concentrations did not vary much by state for atrazine (range 0.41–0.49 ppb), but did vary substantially for nitrate (range 0.30–1.27 ppm), with the highest concentrations in Ohio.

### 3.3. Associations between drinking water atrazine, nitrate and birth outcomes

No evidence of an association was observed in the crude analysis for any of the outcomes and either nitrates or atrazine mean exposure occurring over 9 months prior to birth (Table 1). The results from fitting multivariable negative binomial models for each outcome are presented in Tables 2–5 and described below.

#### 3.3.1. PTD

PTD was not associated with exposure to atrazine or nitrates in models that did not restrict by well use (Table 2). Nitrates exposure was not found to be associated with PTD in any of the models fitted. A significant association was observed between atrazine exposure averaged over 9 months prior to birth (Rate Ratio for 1 ppb ($RR_{1ppb}$)=1.10,

**Table 1**
Summary statistics for key variables used in the analysis (number(percent) or mean±standard deviation) stratified by the total number of singleton births and outcomes.

| Variable | All Births (n=134,258) | PTD (n=13,875) | VPTD (n=1882) | LBW (n=3016) | VLBW (n=1386) |
|---|---|---|---|---|---|
| Child's Sex | | | | | |
| Male | 69,084 (51.5) | 7401 (53.3)** | 1023 (54.4)* | 1237 (41.0)*** | 715 (51.6) |
| Female | 65,174 (48.5) | 6474 (46.7) | 859 (45.6) | 1779 (59.0) | 671 (48.4) |
| Maternal Race-Ethnicity | | | | | |
| White | 120,964 (90.1) | 12,295 (88.6)*** | 1583 (84.1)*** | 2628 (87.1)*** | 1158 (83.5)*** |
| Black | 4619 (3.4) | 681 (4.9) | 168 (8.9) | 207 (6.9) | 126 (9.1) |
| Hispanic | 5696 (4.2) | 563 (4.1) | 91 (4.8) | 109 (3.6) | 72 (5.2) |
| Other | 2979 (2.2) | 336 (2.4) | 40 (2.1) | 72 (2.4) | 30 (2.2) |
| Season of Birth | | | | | |
| January–March | 32,675 (24.3) | 3507 (25.3) | 451 (24.0) | 713 (23.6) | 326 (23.5) |
| April–June | 33,526 (25.0) | 3490 (25.2) | 479 (25.5) | 744 (24.7) | 358 (25.8) |
| July–September | 35,548 (26.5) | 3521 (25.4) | 484 (25.7) | 800 (26.5) | 367 (26.5) |
| October–December | 32,509 (24.2) | 3357 (24.2) | 468 (25.0) | 759 (25.2) | 335 (24.2) |
| State | | | | | |
| Iowa | 5161 (3.8) | 412 (3.0)*** | 44 (2.3)*** | 106 (3.5) | 48 (3.5)* |
| Indiana | 15,732 (11.7) | 1363 (9.8) | 176 (9.4) | 343 (11.4) | 150 (10.8) |
| Missouri | 35,190 (26.0) | 4275 (31.0) | 419 (22.3) | 685 (22.7) | 274 (19.8) |
| Ohio | 78,175 (58.0) | 7825 (56.4) | 1243 (66.0) | 1882 (62.4) | 914 (65.9) |
| Year of birth | | | | | |
| 2004 | 26,510 (19.7) | 2746 (19.8)* | 341 (18.1) | 608 (20.2) | 261 (18.8) |
| 2005 | 26,871 (20.0) | 2929 (21.1) | 377 (20.0) | 587 (19.5) | 298 (21.5) |
| 2006 | 27,131 (20.2) | 2840 (20.5) | 419 (22.3) | 632 (21.0) | 293 (21.1) |
| 2007 | 26,973 (20.0) | 2696 (19.4) | 374 (19.9) | 589 (19.5) | 266 (19.2) |
| 2008 | 26,773 (19.9) | 2664 (19.2) | 371 (19.7) | 600 (19.9) | 268 (19.3) |
| Atrazine (ppb)[a] | 0.42±0.40 | 0.42±0.40 | 0.40±0.39 | 0.42±0.42 | 0.40±0.40 |
| Total Nitrates (ppm)[a] | 0.95±0.92 | 0.95±0.95 | 1.02±0.95 | 0.95±0.92 | 1.07±0.97 |
| High School (%) | 87.3±4.20 | 87.4±4.16 | 87.1±4.12 | 86.8±4.25*** | 87.1±4.22 |
| College (%) | 21.4±9.67 | 21.5±9.60 | 21.1±9.89 | 20.6±9.43** | 21.3±10.21 |
| Median Income | 53,116± 12,552 | 53,054± 12,498 | 52,777± 12,906 | 52,006± 12,186* | 53,172±13,392 |
| Population Density | 8897±6223 | 9013±6388 | 8749±5742 | 8655±5910 | 8591±5577 |
| Maternal Age (% < 18 yrs)[b] | 10.0±3.40 | 10.0±3.4 | 10.4±3.6 | 10.4±3.4* | 10.3±3.7 |
| Maternal Age (% > 40 yrs)[b] | 1.6±0.7 | 1.6±0.7 | 1.5±0.7 | 1.6±0.7 | 1.6±0.7 |
| Women Smokers (%) | 20.1±3.5 | 20.2±3.4 | 20.2±3.6 | 20.3± 3.5* | 20.1±3.7 |
| Private Wells (%) | 18.2± 16.0 | 17.4±16.1*** | 19.7±16.1* | 19.3±15.8** | 19.6±15.9* |

Abbreviations: LBW, low birth weight; VLBW, very low birth weight; PTD, preterm; VPTD, very preterm; n, number of births or cases; ppb, parts per billion; ppm, parts per million; %, percentage; SD, standard deviation, *p < 0.05, **p < 0.01, ***p < 0.001 from testing covariate in a GEE model with no other covariates.

[a] Concentrations averaged over 9 months prior to birth.

[b] Only available for 2004–2006

95%CI=1.01, 1.20; p=0.03), and 4–6 months prior to birth ($RR_{1ppb}$=1.08, 95%CI=1.05, 1.11;p < 0.001) when the analysis was restricted to counties with < 10% well use. The association with atrazine exposure over 9 months prior to birth was weakened and non-significant ($RR_{1ppb}$=1.02 95%CI=0.90, 1.16; p > 0.05), and the model for atrazine exposure 4–6 months prior to birth failed to converge in the models that included both nitrates and atrazine (i.e. dual exposure models). The exposure-response for atrazine was found to be highly significant (p=0.007) and linear (p for non-linearity=0.39) in the restricted cubic spline models using the atrazine exposure over 9 months prior to birth and a < 10% well restriction (Fig. 2).

There was significant evidence (p < 0.001) of a statistical interaction between atrazine and nitrates exposures occurring 9 months prior to birth in the model for PTD with < 10% well use. The parameters from this model indicate that there is a protective effect of atrazine ($RR_{1ppb}$=0.87) or nitrates ($RR_{1\ ppm}$=0.89, 95%CI=0.77, 0.97) exposure alone, but an increased rate with co-exposure (RR for interaction=1.22, 95% CI=1.09,1.38).

### 3.3.2. VPTD

VPTD was significantly associated with nitrates exposure occurring 9 months prior to birth ($RR_{1ppb}$=1.08, 95%CI=1.02, 1.15; p=0.007) when well use was restricted to < 20%, and this result was virtually unchanged when atrazine was included in the model (Table 3). Atrazine exposure 7–9 months prior to birth was significantly associated with VPTD when well use was restricted to < 10% ($RR_{1ppb}$=1.19, 95% CI=1.04,1.36;p=0.01). Both atrazine ($RR_{1ppb}$=1.16, 95%CI=1.05, 1.28; p=0.003) and nitrates ($RR_{1ppb}$=1.11, 95%CI=1.02, 1.20; p=0.01) exposure 0–3 months prior to birth were significantly associated with an increased rate of VPTD in the dual exposure model.

The slope of the exposure-response for atrazine exposures occurring over 9 months prior to birth and VPTD with a 10% well use restriction was found to be significant (p=0.007) and linear (p for non-linearity=0.39) in the restricted cubic spline models using a < 20% well restriction (Fig. 2). The slope of the exposure-response for the nitrates exposure occurring over 9 months prior to birth and VPTD with a 20% well use restriction was found to be significant (p=0.02) and linear (p for non-linearity=0.97) in the restricted cubic spline models using a < 20% well restriction (Fig. 3).

### 3.3.3. LBW

There was no evidence of an exposure-response relationship between LBW and any of the atrazine or nitrates exposure variables (Table 4). There was also no evidence of an exposure-response relationship in the restricted cubic spline models for atrazine (Fig. 2) or nitrates (Fig. 3) exposure over the 9 months prior to birth.

### 3.3.4. VLBW

There was no evidence of an association between atrazine and VLBW in any of the models fitted. There was significant evidence of an

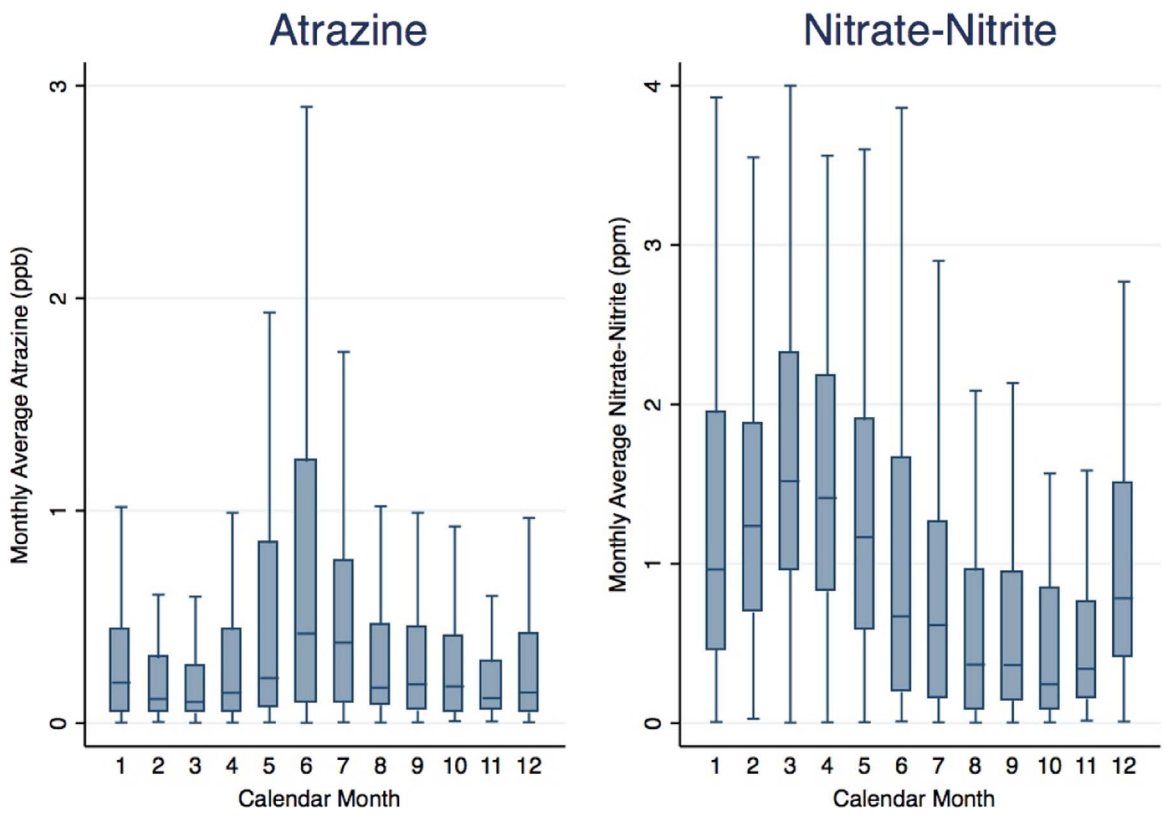


**Fig. 1.** Box and whisker plots of mean monthly county-level average concentration of atrazine (ppb) and nitrates (ppm) water concentrations by month of the year averaged over all study years.

exposure-response relationship between VLBW and exposure to nitrates over 9 months prior to birth ($RR_{1\ ppm}$=1.07, 95% CI=1.01,1.14;p=0.02). These findings were strengthened and became highly significant ($RR_{1\ ppm}$=1.17, 95%CI=1.08, 1.25; p=0.00002) when well use was restricted to < 20%. A significant exposure-response was also observed for nitrates exposures 0–3 months ($RR_{1\ ppm}$=1.18,95% CI=1.07,1.29;p=0.001) and 4–6 months prior to birth ($RR_{1\ ppm}$=1.14,95%CI=1.06,1.23;p=0.001) when well use was restricted to 20%. The magnitude of the associations between nitrates and VLBW were unchanged when atrazine was controlled for. Most models failed to converge when the analysis was further restricted to areas with < 10% well use due to the reduced sample size. The

**Table 2**
Results from negative binomial models for PTD and the effect of continuous exposure to atrazine and Nitrates in which the exposures were modeled either separately in single exposure models or together in dual exposure models.[a]

| | Single Exposure Models | | | | Dual Exposure Models | | | |
|---|---|---|---|---|---|---|---|---|
| | Atrazine | | Nitrates | | Atrazine | | Nitrates | |
| Time Period[b] | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI |
| All (singleton births=134,258, PTD cases=13,875, counties=46) | | | | | | | | |
| 0–3 months | 0.98 | 0.94,1.01 | 1.01 | 0.98,1.04 | 0.96 | 0.89,1.04 | 1.01 | 0.98,1.04 |
| 4–6 months | 0.99 | 0.95,1.03 | 1.01 | 0.99,1.03 | 1.01 | 0.97,1.05 | 1.01 | 0.99,1.03 |
| 7–9 months | 1.00 | 0.97,1.04 | 1.01 | 0.98,1.03 | 1.04 | 0.99,1.09 | 1.01 | 0.98,1.03 |
| 9 months | 0.98 | 0.93,1.03 | 1.02 | 1.00,1.05 | 0.99 | 0.93,1.05 | 1.02 | 1.00,1.05 |
| < 20% wells (singleton births= 83,334, PTD=8849, counties=30) | | | | | | | | |
| 0–3 months | 1.01 | 0.95,1.07 | 0.98 | 0.94,1.01 | 1.01 | 0.94,1.09 | 0.97 | 0.94,1.01 |
| 4–6 months | 1.04 | 0.98,1.09 | 1.00 | 0.95,1.06 | 1.05 | 1.01,1.10 | 1.00 | 0.95,1.06 |
| 7–9 months | 1.02 | 0.96,1.08 | 1.02 | 0.96,1.08 | 1.07 | 1.02,1.12 | 1.01 | 0.96,1.07 |
| 9 months | 1.04 | 0.95,1.14 | 1.01 | 0.97,1.06 | 1.07 | 0.97,1.17 | 1.01 | 0.97,1.05 |
| < 10% wells (singleton births= 55,060, PTD cases=6113, counties=19) | | | | | | | | |
| 0–3 months | 1.05[c] | 0.99,1.12 | nc | nc | nc | nc | nc | nc |
| 4–6 months | 1.08***[c] | 1.05,1.11 | nc | nc | nc | nc | nc | nc |
| 7–9 months | 1.01[c] | 0.96,1.06 | nc | nc | nc | nc | nc | nc |
| 9 months | 1.10*[c] | 1.01,1.20 | 0.98[c] | 0.91,1.06 | 1.02[c] | 0.90,1.16 | 0.98[c] | 0.91,1.05 |

Abbreviations: RR=rate ratio for 1 ppm nitrates or one ppb atrazine, 95% CI=95% confidence interval, nc= failed to converge, *p < 0.05, **p < 0.01, ***p < 0.001.

[a] Models controlled for child's sex, race and ethnicity, state, calendar year, season of birth, maternal education, median income, population density, and smoking among women.

[b] Models were fitted using average estimates of atrazine or nitrates exposure for either 7–9 months prior to birth, 4–6 months prior to birth, 0–3 months prior to birth or over the 9 months prior to birth.

[c] State was not included in these models because of lack of model convergence.

**Table 3**
Results from negative binomial models for VPTD and the effect of continuous exposure to atrazine and nitrates in which the exposures were modeled either separately in single exposure models or together in dual exposure models.[a]

| | Single Exposure Models | | | | Dual Exposure Models | | | |
|---|---|---|---|---|---|---|---|---|
| | Atrazine | | Nitrates | | Atrazine | | Nitrates | |
| Time Period[b] | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI |
| All (singleton births=134,258, VPTD cases=1882, counties=46) | | | | | | | | |
| 0–3 months | 1.01 | 0.93,1.10 | 1.01 | 0.98,1.05 | 1.09 | 0.99,1.21 | 1.01 | 0.97,1.05 |
| 4–6 months | 0.97 | 0.91,1.03 | 1.01 | 0.96,1.06 | 0.96 | 0.86,1.08 | 0.96 | 0.86,1.08 |
| 7–9 months | 1.04 | 0.96,1.12 | 1.00 | 0.95,1.05 | 1.06 | 0.94,1.19 | 1.06 | 0.94,1.19 |
| 9 months | 1.02 | 0.91,1.14 | 1.04 | 0.99,1.09 | 1.04 | 0.91,1.20 | 1.04 | 0.99,1.09 |
| < 20% well use (singleton births=83,334, VPTD cases=1088, counties=30) | | | | | | | | |
| 0–3 months | 1.08 | 0.94,1.24 | 1.09 | 0.99,1.19 | 1.16**[c] | 1.05,1.28 | 1.11**[c] | 1.02,1.20 |
| 4–6 months | 0.95 | 0.84,1.08 | nc | nc | nc | nc | nc | nc |
| 7–9 months | 1.12 | 0.94,1.24 | nc | nc | nc | nc | nc | nc |
| 9 months | 1.11 | 0.98,1.28 | 1.08** | 1.02,1.15 | 1.18 | 0.98,1.42 | 1.08** | 1.02,1.04 |
| < 10% well use (singleton births=55,060, VPTD cases=730, counties=19) | | | | | | | | |
| 0–3 months | 1.12 | 0.97,1.29 | 1.03 | 0.93,1.13 | nc | nc | nc | nc |
| 4–6 months | 0.94 | 0.81,1.09 | nc | nc | nc | nc | nc | nc |
| 7–9 months | 1.19* | 1.04,1.36 | nc | nc | nc | nc | nc | nc |
| 9 months | 1.19 | 0.91,1.56 | 1.06 | 0.95,1.19 | 1.10 | 0.84,1.44 | 1.10* | 1.02,1.19 |

Abbreviations: RR=rate ratio for 1 ppm nitrates or one ppb atrazine, 95% CI=95% confidence interval, nc=failed to converge, *p < 0.05, **p < 0.01, ***p < 0.001.
[a] Models controlled for child's sex, race and ethnicity, state, calendar year, season of birth, maternal education, median income, population density, and smoking among women.
[b] Models were fitted using average estimates of atrazine or nitrates exposure for either 7–9 months prior to birth, 4–6 months prior to birth, 0–3 months prior to birth or over the 9 months prior to birth.
[c] State was not included in these models because of lack of model convergence.

exposure-response relationship between nitrates over 9 months prior to brith and VLBW was found to be highly significant (p=0.0001) and linear (p for non-linearity=0.8904) in the restricted cubic spline model (Fig. 3).

Our findings for all of the outcomes were unchanged when data with estimated exposures above the MCL for atrazine (3 ppb) or nitrates (10 ppm) were deleted from the analyses (results not shown).

## 4. Discussion

### 4.1. Atrazine

We observed evidence of a linear exposure-response relationship between county-level atrazine concentrations in drinking water during the prenatal period and the risk of PTD (Table 2) and VPTD (Table 3) in counties with less than 10% private well use. Our findings were particularly strong for atrazine exposures that occurred between 4 and

**Table 4**
Results from negative binomial models for LBW and the effect of continuous exposure to atrazine and nitrates in which the exposures were modeled either separately (single exposure models) or together (dual exposure models).[a]

| | Single Exposure Models | | | | Dual Exposure Models | | | |
|---|---|---|---|---|---|---|---|---|
| | Atrazine | | Nitrates | | Atrazine | | Nitrates | |
| Model[b] | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI |
| All (full term singleton births=121,604, LBW cases=3016, counties=46) | | | | | | | | |
| 0–3 months | 1.03 | 0.98,1.08 | 0.96 | 0.92,1.01 | 1.04 | 0.93,1.16 | 1.04 | 0.93,1.16 |
| 4–6 months | 1.05 | 0.99,1.10 | 0.97 | 0.91,1.02 | 1.02 | 0.93,1.11 | 0.96 | 0.91,1.03 |
| 7–9 months | 1.01 | 0.95,1.08 | 0.96 | 0.92,1.00 | nc | nc | nc | nc |
| 9 months | 1.07 | 0.98,1.16 | 0.97 | 0.92,1.02 | 1.05 | 0.95,1.15 | 1.05 | 0.95,1.15 |
| < 20% well use (full term singleton births=75,494, LBW cases=1781, counties=30) | | | | | | | | |
| 0–3 months | 1.04 | 0.91–1.20 | 0.94[c] | 0.89,1.00 | 1.05 | 0.94,1.18 | 0.95 | 0.89,1.01 |
| 4–6 months | 1.07 | 0.98,1.18 | 0.98[c] | 0.89,1.07 | 1.06 | 0.88,1.28 | 0.97 | 0.89,1.07 |
| 7–9 months | 1.00 | 0.87,1.16 | nc | nc | nc | nc | nc | nc |
| 9 months | 1.10 | 0.95,1.28 | 0.97[c] | 0.89,1.06 | 0.95 | 0.77,1.18 | 0.97 | 0.89,1.06 |
| < 10% wells use (full term singleton births=49,759, LBW cases=1157, counties=19) | | | | | | | | |
| 0–3 months | 0.95[c] | 0.77,1.16 | nc | nc | nc | nc | nc | nc |
| 4–6 months | 1.05[c] | 0.93,1.20 | nc | nc | nc | nc | nc | nc |
| 7–9 months | 0.99[c] | 0.85,1.16 | nc | nc | nc | nc | nc | nc |
| 9 months | 0.99[c] | 0.82,1.20 | 0.95[c] | 0.88,1.02[c] | 0.95 | 0.75,1.19[c] | 0.95 | 0.88,1.03[c] |

Abbreviations: RR=rate ratio for 1 ppm nitrates or one ppb atrazine, 95% CI=95% confidence interval, nc= failed to converge, *p < 0.05, **p < 0.01, ***p < 0.001.
[a] Models controlled for child's sex, race and ethnicity, state, calendar year, season of birth, maternal education, median income, population density, and smoking among women.
[b] Models were fitted using average estimates of atrazine or nitrates exposure for either 7–9 months prior to birth, 4–6 months prior to birth, 0–3 months prior to birth or over the 9 months prior to birth.
[c] State was not included in these models because of lack of model convergence.

**Table 5**
Results from negative binomial models for VLBW and the effect of continuous exposure to atrazine and nitrates in which the exposures were modeled either separately in single exposure models or together in dual exposure models.[a]

| | Single Exposure Models | | | | Dual Exposure Models | | | |
|---|---|---|---|---|---|---|---|---|
| | Atrazine | | Nitrates | | Atrazine | | Nitrates | |
| Time Period[b] | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI |
| All (singleton births=134,258, VLBW cases=1386, counties=46) | | | | | | | | |
| 0–3 months | 0.98 | 0.89,1.09 | 1.05 | 0.98,1.12 | 0.96 | 0.83,1.12 | 1.05 | 0.98,1.12 |
| 4–6 months | 1.02 | 0.93,1.11 | 1.05 | 0.99,1.11 | 0.96 | 0.83,1.10 | 1.05 | 0.99,1.11 |
| 7–9 months | 1.02 | 0.95,1.09 | 1.00 | 0.94,1.06 | 1.01 | 0.92,1.11 | 1.00 | 0.94,1.06 |
| 9 months | 1.00 | 0.88,1.15 | 1.07* | 1.01,1.14 | 1.01 | 0.86,1.18 | 1.07* | 1.01,1.14 |
| < 20% wells[c] (singleton births=83,334, VLBW cases=800, counties=30) | | | | | | | | |
| 0–3 months | 1.07 | 0.90,1.27 | 1.18*** | 1.07,1.29 | 0.96 | 0.73,1.25 | 1.18*** | 1.07,1.29 |
| 4–6 months | 1.01 | 0.85,1.21 | 1.14*** | 1.06,1.23 | 0.94 | 0.75,1.17 | 1.15*** | 1.07,1.24 |
| 7–9 months | 1.02 | 0.86,1.21 | nc | nc | nc | nc | nc | nc |
| 9 months | 1.08 | 0.85,1.37 | 1.17*** | 1.08,1.25 | 0.98 | 0.66,1.18 | 1.17*** | 1.08,1.25 |
| < 10% wells[c] (singleton births=55,060, VLBW cases=535, counties=19) | | | | | | | | |
| 0–3 months | 0.95 | 0.77,1.17 | nc | nc | nc | nc | nc | nc |
| 4–6 months | 0.95 | 0.80,1.12 | nc | nc | nc | nc | nc | nc |
| 7–9 months | 0.92 | 0.72,1.18 | nc | nc | nc | nc | nc | nc |
| 9 months | 0.88 | 0.73,1.06 | nc | nc | nc | nc | nc | nc |

Abbreviations: RR=rate ratio for 1 ppm nitrates or one ppb atrazine, 95% CI=95% confidence interval, nc=failed to converge, *p < 0.05, **p < 0.01, ***p < 0.001.

[a] Models controlled for child's sex, race and ethnicity, state, calendar year, season of birth, maternal education, median income, population density, and smoking among women.

[b] Models were fitted using average estimates of atrazine or nitrates exposure for either 7–9 months prior to birth, 4–6 months prior to birth, 0–3 months prior to birth or over the 9 months prior to birth.

[c] State was not included in these models because of lack of model convergence.

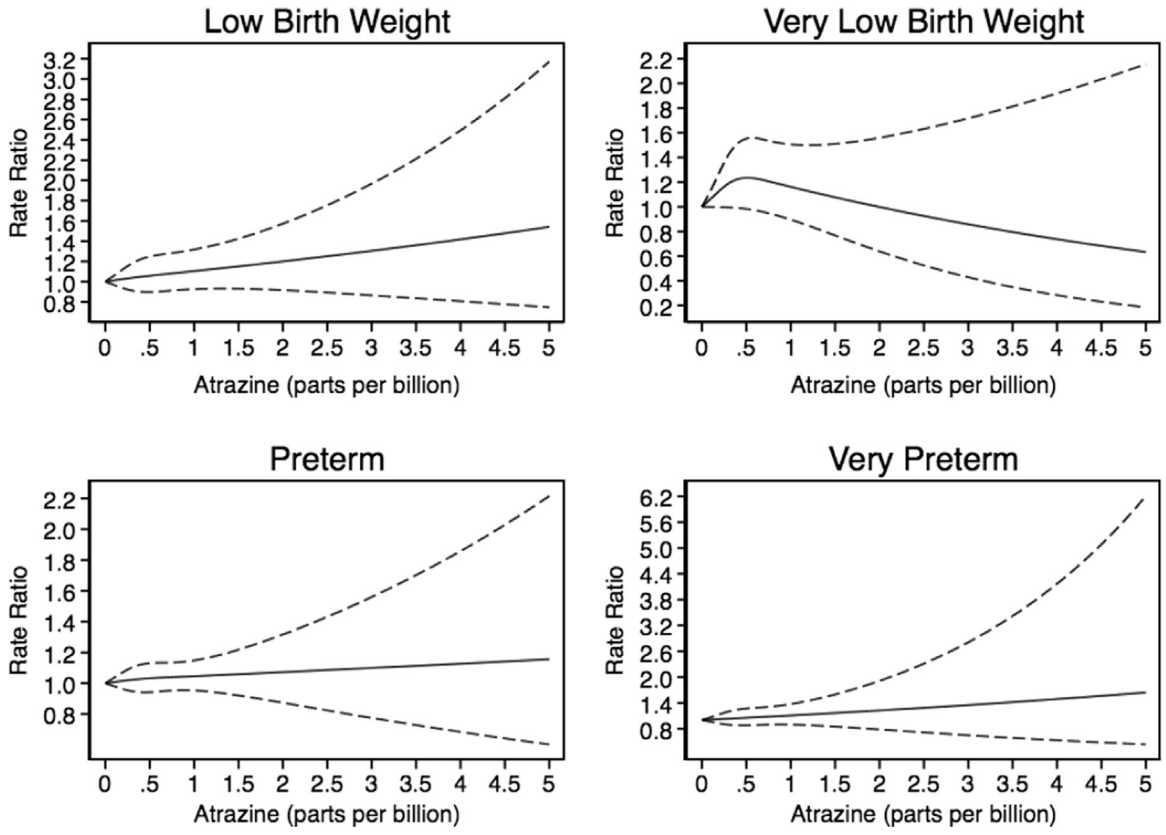


**Fig. 2.** Results from fitting restricted cubic spline (3 knots) model for atrazine exposure (ppb) averaged over 9 months prior to birth with a restriction of < 20% well usage.

6 months prior to birth for PTD, and for 0–3 months prior to birth for VPTD, which would correspond to either a middle or late period in the pregnancy depending on how early the PTD occurred.

Our findings for an association between atrazine and PTD are biologically plausible since atrazine is known to be an endocrine disruptor (Stoker et al., 2000). A possible mechanism for this effect is that atrazine has been shown to reduce luteinizing hormone (LH) and secretion of progesterone in pregnant rats (Cooper et al., 2007). Progesterone is used as a treatment for women at high risk of PTD, and reduced progesterone synthesis has been found to increase PTD in

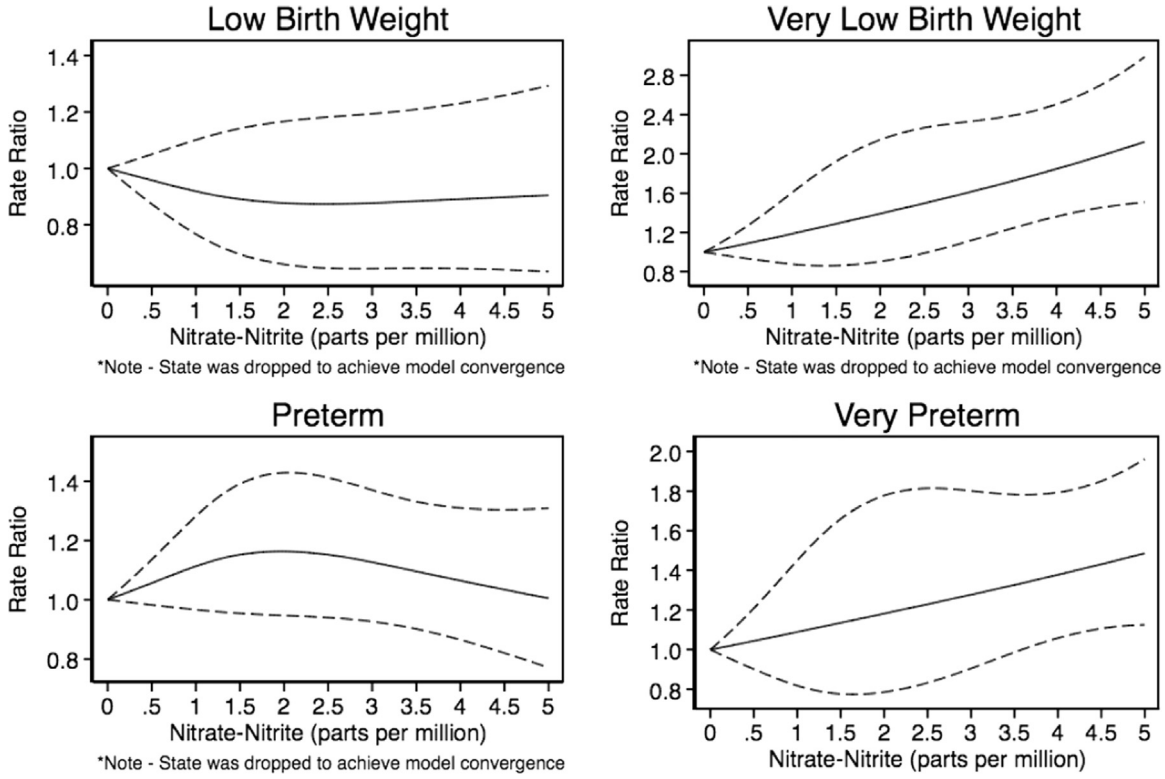


**Fig. 3.** Results from fitting restricted cubic spline (3 knots) model for nitrates exposure (ppm) averaged over 9 months prior to birth with a restriction of < 20% well usage.

experimental animals (Gawriluk and Rucker, 2015). Only a few epidemiologic studies have examined the association between and atrazine and PTD and the evidence from these studies is mixed. Our findings are consistent with Rinsky et al. (2012) who reported that PTD increased with concentrations of atrazine in public water in an ecologic study of 71,768 births in Kentucky. Villanueva et al. (2005) reported a statistically non-significant increase in the risk of PTD with atrazine in water in a cohort study of 3510 births in Brittany, France. However, the lack of statistical significance in their findings may be attributable to its relatively small size (137 PTD cases), and to the low (geometric mean=0.03 ppm) and narrow range of atrazine concentrations in this study. Munger et al. (1997) did not observe an increased rate of PTD in an ecologic study comparing a community in Iowa with relatively high concentrations of atrazine in their water supply (mean=2.2 ppm) to other counties of similar size in Iowa. This study was also quite small (492 births) and statistically underpowered for this outcome. There was no significant evidence of an association between atrazine in drinking water and PTD in a large study (24,154 births) in Indiana, which was based on individual level data, although there was borderline evidence of an exposure-response relationship when atrazine was modeled as a continuous variable (RR=1.07, 95%CI=0.99, 1.15).

We did not observe any evidence of an association between atrazine and LBW, which is regarded as a marker of fetal growth restriction (FGR). These findings are in conflict with a few studies that have reported evidence of an association between atrazine in water and increased risk of markers of FGR. The studies by Ochoa-Acuña et al. (2009) and Munger et al. (1997) (described above) both reported a significant association between atrazine in drinking water and being small for gestational age (SGA), which is another marker of FGR. Ochoa-Acuña et al. (2009) also reported that the risk of term LBW increased with atrazine exposure. Finally, Chevrier et al. (2011) in a population based cohort study of 3421 pregnant women in Brittany, France reported a significant association between having quantifiable levels of urinary biomarkers of atrazine or atrazine metabolites and an increased risk of SGA and small head circumference.

### 4.2. Nitrate

We observed significant evidence of a linear exposure-response relationship between nitrate in water and the risk of both VPTD and VLBW in several models. The results for VPTD were highly significant (p=0.007) when the analysis was restricted to < 20% well use and nitrate was averaged over 9 months prior to birth. A borderline significant (p=0.08) relationship was observed for VPTD and nitrate averaged over 0–3 months prior to birth and 20% well use restriction, which became significant (p=0.001) when atrazine was included in the model (Table 2).

Similarly, we observed a highly significant (p=0.0001) exposure-response relationship between VLBW when well use was restricted to 20% and nitrate were averaged over 9 months prior to birth, which was also evident for exposures 0–3 months (p=0.001), and 4–6 months (p=0.001) prior to birth. The similarity in the findings for VPTD and VLBW are most likely due to the fact that these outcomes are highly correlated (r=0.78, p < 0.0001).

Nitrate and nitrite in drinking water have been found to cause fetal growth restriction, lower birth weights and other adverse reproductive effects in animal experiments, but only at extremely high levels ( > 1000 ppm) (Fan and Steinberg, 1996). High levels of nitrate in drinking water are also a well recognized cause of methemoglobinemia ("blue baby syndrome") in children (Comly, 1987). The underlying mechanism is believed to involve an increase in methemoglobin, which is unable to carry oxygen, by the reduction of heme iron by nitrites to its ferric state. Infants less than 6 months of age are believed to be at particularly high risk of methemoglobinemia because they have low levels of methemoglobin reductase, which converts methemoglobin back to hemoglobin (Lukens, 1987). Maternal anemia has been associated with an increased risk of LBW and PTD (Haider et al., 2013). Nitrate have also been reported to to inhibit steroidogenesis and thyroid function in animal studies (Guillette, 2006), which may be alternative mechanism since subclinical hypothyroidism in pregnancy has been associated with PTD and VLBW in some studies (Maraka et al., 2016).

There is a paucity of epidemiologic data examining the potential relationship between low levels of nitrate in water and the risk of adverse reproductive outcomes. Only two epidemiologic studies have examined whether nitrate-nitrites in drinking water are associated with an increased risk of PTD and LBW, and the results from these studies are contradictory. Bukowski et al. (2001) conducted a population based case control study of term LBW (n=210 cases) and PTD (n=336 cases) on Prince Edward Island, Canada. The study relied on an ecologic exposure assessment of nitrate, which was based on extensive monitoring of both public and private water systems. Significant evidence of an exposure response relationship was reported between nitrate and both term LBW and PTD ($p < 0.001$) in this study. Super et al. (1981) in a study in Africa did not report an increased risk of either term LBW or premature birth in an area of Southwest Africa known to have relatively high levels of nitrate ( > 20 ppm), however, this was a very small study with only 486 births. Migeot et al. (2013) reported a significant association between nitrate exposure and elevated risk for SGA in a cohort study of 11,446 mother child pairs in western France. Migeot et al. (2013) also reported that the association with nitrate exposure was only evident when there were no detectable metabolites of atrazine. We did not observe any evidence of an interaction between nitrate and atrazine for term LBW in our study. However, we did observe a highly significant ($p < 0.001$) statistical interaction between exposure to nitrate and atrazine for PTD. The results from this model suggest that the risk increases with joint exposure to nitrate and atrazine, and not with either exposure alone. Atrazine is a secondary amine which can react with nitrite to produce N-nitrosoatrazine, which has been found to be over 1000 times more potent than nitrite or atrazine alone in increasing chromosomal breakage in cultured lymphocytes (Meisner et al., 1993). An interaction between prenatal exposure to secondary and tertiary amines and nitrite and the risk of PTD has recently been reported (Vuong et al., 2016).

### 4.3. Study strengths and weaknesses

An obvious strength of our study is the large number of births (n=134,258), which makes it one of the largest studies ever conducted exploring agrichemical exposures through drinking water and birth outcomes. The large size of our study made it possible for us to examine the rate of VPTD and VLBW, which to our knowledge has not been considered any other studies. Another strength of our study is the extensive data that was available on atrazine concentrations, since we focused our study on counties that had water systems that have participated in the EPA's AMP program. This extensive data allowed us to examine how variations in seasonal exposure might affect the risk of adverse birth outcomes.

The positive as well as the negative findings from this study need to be cautiously interpreted due to several major limitations in the study design. First of course is the ecologic design of the study. Both the exposures and the outcomes were only measured at the county level. Thus our study may be subject to the well known ecologic fallacy that may occur from attributing group characteristics to individuals. Although this is a clear limitation, our study was conducted with support from the CDCs EPHT program, and one of the objectives of this study and program was to demonstrate the utility of linking publically available data, which is only obtainable at a grouped level. In this respect, we believe our study should best be viewed as a useful effort in identifying hypotheses that merit further study.

There are also several substantial issues regarding the information that was used in our study for quantifying exposures to atrazine and nitrates in drinking water. First, the exposure data in this study were gathered for regulatory and not for research purposes. It is conceivable that some public water systems perform their sampling at times when they expect the concentrations to be the high, or conversely low, and thus the data may not be representative of the true exposure distribution. Second, although the data available for atrazine were extensive due to the requirements of the AMP, measurements for nitrates were performed quarterly and annually in systems that are believed to be at low risk of nitrate contamination or that use groundwater. Thus, although the percentage of missing data was low for atrazine, it was larger for nitrates (e.g. 0.3% versus 10.8% for average over 9 months prior to birth). Second, although the data available for atrazine were extensive due to the requirements of the AMP, measurements for nitrates were performed quarterly and annually in systems that are believed to be at low risk of nitrate contamination or that use groundwater. Thus, although the percentage of missing data was low for atrazine, it was larger for nitrates (e.g. 0.3% versus 10.8% for average over 9 months prior to birth). The pattern of missingness was clearly not random and varied substantially by county and states. It was higher in counties with low median income, low percentage of high school or college graduates, and in counties that were predominantly white. A high percentage of missing data might indicate better water quality if public water systems were no longer testing regularly because prior tests were low or non-detectable. Missingness was also high in counties with a large percentage of well use, which may simply be due to the lack of use of public water systems in these counties. Third, many of the atrazine and nitrates measurements were left censored because they were below the limit of detection. Fourth, not all of the CWSs in a county may have participated in the AMP program, or were not in the program for the entire study time period (2004–2008). Fifth, the lack of data on private well use is another major source of exposure misclassification in our study. In order to address this concern, we conducted analyses in which we restricted the percentage of the population that had a low percentage ( < 10 or 20%) of private wells. In fact, we only observed significant evidence of associations between the adverse outcomes and nitrates or atrazine when we applied these restrictions. Restricting the study to only include counties with a low percentage of well use resulted in a severe loss of data and statistical power. It also resulted in our not being able to control for the state of birth or co-exposure to nitrates and atrazine in some models.

We had no information on personal water use habits and it is quite possible that women who are pregnant would drink filtered and/or bottled water in areas that are known to have high levels of atrazine or nitrates in their drinking water. A recent study in the U.S. reported that approximately 30% of women in their study exclusively drank bottled water, and that nitrate levels in the water were found to be low compared to levels in community water systems (Weyer et al., 2014). Thus failure to account for bottled water consumption may have led to serious overestimation of exposures to nitrate for some women in our study.

Our study also lacked information on many important risk factors for adverse birth outcomes such as parity, parental income, maternal age, and reproductive histories. Furthermore, many of the covariates controlled for in our analyses were at the county level and relatively crude (e.g. the percent of female smokers) and were based on a county level. Thus there is a strong possibility for residual confounding by these covariates in our study. Our study lacked information on the date of conception which limited our ability to accurately estimate trimester specific exposures and other covariates such as season of conception, particularly for preterm births. Finally, we tested over 100 hypotheses in this study and thus some of our findings may be expected based upon chance alone.

## 5. Conclusions

The associations between nitrates and the risk of VPTD and VLBW, and between atrazine and PTD observed in this study may have serious public health implications. Only approximately 2% of the monthly average atrazine and nitrates exposure levels in the study exceeded the EPA's MCL (3 ppb and 10 ppm respectively). Analyses performed excluding the data that exceeded the MCLs did not alter the results. However, due to the exploratory nature and limitations of our study,

additional research is clearly warranted before any causal conclusions may be drawn. Future studies are needed with individual level data on both the outcomes and exposures, and better information on other risk factors for PTD and LBW.

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