Adverse Birth Outcomes and Contamination of Drinking Water by Arsenic, Atrazine, and Nitrate-Nitrite

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THESIS

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LIST OF ABBREVIATIONS

AMP	Atrazine Monitoring Program
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulation
CWS	Community Water System
DACT	Diaminochlorotriazine
DEA	Deethylatrazine
DIA	Deisopropyl-atrazine
EPHTN	Environmental Public Health Tracking Network
GEE	Generalized estimating equations
GSD	Geometric standard deviation
HPA	Hypothalamic-pituitary-adrenal
IARC	International Agency for Research on Cancer
LCL	Lower confidence limit
LOD	Limit of detection
MCL	Maximum Contaminant Level
MI	Multiple imputation
MNAR	Missing not at random
NN	Nitrate-nitrite
NRC	National Research Council
ODH	Ohio Department of Health
OH	Ohio

LIST OF ABBREVIATIONS (continued)

OR	Odds ratio
PTB	Preterm birth
PWS	Public Water System
RR	Risk ratio
SDWA	Safe Drinking Water Act
SDWIS	Safe Drinking Water Information System
SES	Socioeconomic status
SGA	Small for gestational age
Term LBW	Term low birth weight
VLBW	Very low birth weight
VPTB	Very preterm birth
UCL	Upper confidence limit
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organization
WIC	Women, Infants, and Children

SUMMARY

The goals of this research were to examine the relationships between three water contaminants; arsenic, atrazine, and nitrate-nitrite (NN); and selected birth outcomes including small for gestational age (SGA), low birth weight among term infants (term LBW), very low birth weight (VLBW), preterm birth (PTB), and very preterm birth (VPTB). We assessed these associations using data from the Safe Drinking Water Information System (SDWIS) and Atrazine Monitoring Program (AMP) as well as birth certificate data from the state of Ohio.

Our findings are consistent with the epidemiologic literature from geographic areas of high arsenic exposure that suggest arsenic exposure in drinking water adversely affects birth weight and gestation. In models with the least potential for exposure misclassification (e.g., low use of private drinking water wells), we found that the risk of VLBW and preterm birth increased with increasing levels of annual mean arsenic concentrations in drinking water. Furthermore, our findings were unchanged when we restricted our analyses to only those infants with exposure below the current permissible level of arsenic in drinking water.

The epidemiologic literature concerning NN exposure and birthweight or gestation is sparse, therefore our findings represent a valuable contribution to the field. Within rural counties in which <20 or <10% of the population derived drinking water from private wells, we observed a significant increase in the odds of VLBW births with increasing mean gestational NN concentrations. We also observed evidence of increased odds of PTB with increasing mean gestational NN exposure.

We examined the relationship between atrazine and selected birth outcomes in communities receiving public drinking water from community water systems (CWSs) that were enrolled in the United States Environmental Protection Agency's (USEPA) Atrazine Monitoring

Program (AMP). We found a significant increase in the odds of term LBW births with increasing atrazine exposure in drinking water over the entire gestational period of the pregnancy, as well as within the first and second trimesters. This suggests that exposure to atrazine in drinking water in early and mid-pregnancy may be most critical for its toxic effects on the fetus. Our results are the first to show an association between exposure to atrazine in drinking water and term LBW births, but they are somewhat consistent with previous epidemiologic research which has shown an inverse relationship between atrazine exposure and birth weight. We observed no association between exposure to atrazine in drinking water and preterm or very preterm births.

The associations observed in these analyses were present at exposure levels that are below current Maximum Contaminant Levels (MCLs) for each contaminant examined. Our findings suggest that additional epidemiologic research should examine the effects of arsenic, atrazine, and NN on term LBW, VLBW, and PTB in areas of relatively low contaminant exposure. Ideally, future research would employ biomarkers of exposure or individual measurements of exposure rather than relying on the ecologic exposure measures presented in these analyses. Despite the limitations in the exposure ascertainment, our findings suggest that linking environmental monitoring data with health outcomes data such as vital statistics databases has great utility in identifying potential associations to explore with more refined exposure and outcome ascertainment.

Future epidemiologic investigations of these contaminants should aim to improve methods to reduce exposure misclassification, perhaps through the use of biomarkers. Additional avenues for future research include assessing exposure to a mixture of contaminants, employing

multiple imputation (MI) methods to impute missing covariate data, and examining birth defects and childhood cancers as additional outcomes.

I. OVERVIEW AND SPECIFIC AIMS

Drinking water sources can be contaminated by naturally occurring and man-made contaminants that may pose serious threats to public health. Consequently, the United States Congress enacted the Safe Drinking Water Act in 1974 which authorized the Environmental Protection Agency (USEPA) to set legal limits for water contaminants to protect human health. The USEPA has published Maximum Contaminant Levels (MCLs) for many water contaminants in response to documented health effects at high exposure levels, however, concern is growing about health effects observed at levels below the MCL for many contaminants, including arsenic, atrazine, and nitrate-nitrite.

Increasing concern exists about whether prenatal exposure to arsenic, atrazine, and nitrate-nitrite in drinking water is associated with adverse reproductive and developmental outcomes. Epidemiologic evidence suggests that prenatal exposure to arsenic in drinking water may be associated with reduced birth weight and head circumference, infant death, neonatal, postneonatal, and fetal mortality, spontaneous abortions, and stillbirths (Hopenhayn et al. 2003; Yang et al. 2003; Rahman et al. 2007; Rahman et al. 2009; Vahter 2009). Nitrate causes methemoglobinemia at high levels of exposure, but at low levels, has been associated with intra-uterine growth restriction (IUGR), preterm births, multiple birth defects, and spontaneous abortions (Knobloch et al. 2000; Brender et al. 2013; USEPA 2014). Prenatal exposure to atrazine via drinking water has been associated with decreased birth weight, head circumference, and preterm births (Munger et al. 1997; Ochoa-Acuña et al. 2009; Chevrier et al. 2011; Rinksy et al. 2012). However, Villanueva et al. (2005) observed a non-significant increase in preterm births, but did not observe an association between atrazine exposure and low birth weight or small for gestational age in a study performed in Brittany, France. The lack of statistical

significance in this study, however, may be attributable to its relatively small sample size (137 PTB cases), and to the low (geometric mean = 0.03 ppm) and narrow range of atrazine concentrations.

The concentrations of arsenic, atrazine, and nitrate-nitrite in drinking water vary by water source. Surface water sources are more vulnerable to contamination by atrazine and nitrate-nitrite due to run-off and leeching from agricultural inputs, while arsenic contamination is greater in groundwater sources (Nolan and Hitt, 2006; Burrow et al. 2010; National Research Council (NRC) 2001). Furthermore, both surface water and groundwater sources in areas of intense agricultural land use are more vulnerable to contamination by atrazine and nitrate-nitrite than those in urban or undeveloped areas (Dubrovsky et al. 2010). An estimated 86% of the U.S. population relies on public water sources for their household water use, including drinking water (Kenny et al. 2005). Nearly two-thirds of water used for public supply in the U.S. is from a surface water source (Kenny et al. 2005). While contaminant concentrations are often higher in surface water, contamination of groundwater sources of drinking water is of particular concern because contamination is difficult to reverse in groundwater sources (Gilliom et al. 2006).

The specific aims of the research presented in this dissertation were to determine if county- and community-level estimates of exposure to arsenic, atrazine, nitrate-nitrite in drinking water are associated with selected birth outcomes including small for gestational age (SGA), term low birth weight (term LBW), very low birth weight (VLBW), preterm birth (PTB), and very preterm birth (VPTB).

The analyses presented herein align with the goals of Environmental Public Health Tracking Network (EPHTN), a program at the Centers for Disease Control and Prevention (CDC), which aims to integrate and analyze data from environmental hazard monitoring and

human health effects surveillance data (CDC 2006). The research used water quality data from the Safe Drinking Water Information System (SDWIS) and the USEPA Atrazine Monitoring Program (AMP) as well as birth certificate data from the state of Ohio.

II. BACKGROUND

A. Atrazine

Atrazine is the second most widely used herbicide in the United States, primarily applied to corn and sorghum crops (USEPA 2015). Much of the concern about atrazine arises from its persistence in soil and its transport to ground water, making it the most commonly detected pesticide in surface and groundwater in the United States (Gilliom et al. 2006). Due to its persistence, atrazine was banned by the European Union in 2001, but the chemical is still heavily used in the U.S. and more than 70 countries worldwide (Pathak and Dikshit 2012).

Atrazine and its metabolites (DEA, DIA, and DACT) share a common toxic effect and mechanism with other triazine herbicides (simazine and propazine): they are suspected of being endocrine disruptors (USEPA 2007; Quignot et al. 2012; Vandenburg et al. 2012). While some aspects of the toxic mechanisms are unclear, these chemicals disrupt the hypothalamic-pituitary-gonadal axis by inhibiting luteinizing hormone production, increasing aromatase production, and disrupting ovarian function (USEPA 2007; Cooper et al. 1996; Cooper et al. 2000; Zorrilla et al. 2010; Victor-Costa et al. 2010). Aromatase is a key enzyme that promotes bioconversion of androgens into estrogens. Low, ecologically relevant doses of atrazine have been shown to decrease testosterone levels, reduce spermatogenesis, and alter gonad development in amphibians, leading sometimes to complete chemical feminization of male frogs (Hayes et al. 2002; Hayes et al. 2010).

Studies in both male and female rats have shown that atrazine targets neuroendocrine regulation and reproductive development. Atrazine exposure in juvenile male rats induces delayed puberty, decreased testosterone and increased estradiol levels, reduced sperm counts, and altered testis architecture (Stoker et al. 2000; Friedmann, 2002; Belloni et al. 2011; Victor-

Costa et al. 2010; Jin et al. 2013). When exposed to atrazine, female rats experienced delayed puberty, lengthened estrous cycles, decreased number of menstrual cycles, and a significant decrease in serum prolactin (Wetzel et al. 1994; Stoker et al. 1999; Zorrilla et al. 2010). Second generation effects of prenatal atrazine exposure have been observed in rats as well, owing to a reduction in mammary gland growth in first generation female rats that yields improper suckling and significantly reduced body weight in second generation pups (Rayner et al. 2005).

The endocrine-disrupting effects of atrazine's major metabolites have also been documented. Atrazine is metabolized into diaminochlorotriazine (DACT), deethylatrazine (DEA), and deisopropyl-atrazine (DIA) in the environment and *in vivo* (Laws et al. 2009). Female offspring of rats, exposed late in gestation at low levels to a mixture of these metabolites experienced delayed mammary gland development (Enoch et al. 2007). Postnatal exposure to DIA, DEA, and DACT delayed the onset of puberty in male rats, and exposure to DACT delayed puberty in female rats (Laws et al. 2003, Stoker et al. 2002). DIA and DEA, but not DACT, were observed to increase serum corticosterone and adrenocorticotropic hormone in rats, although the mechanism of action, either through activation of the hypothalamic-pituitary-adrenal (HPA) axis or a direct effect on the pituitary gland, was unknown (Laws et al. 2009). Females exposed to DACT alone showed suppression of the luteinizing hormone surge, indicating a decrease in pituitary and/or hypothalamic function (McMullin et al. 2004).

Epidemiologic evidence of an effect of prenatal exposure to atrazine on reproductive and developmental outcomes is growing, but is limited by ecologic exposure assessment. In an ecologic study of atrazine and birth defects, Winchester et al. (2009) observed a temporal association between atrazine application and birth defects in the U.S. In case-control studies, Agopian et al. observed an increased odds of male genital malformations (2013a), choanal

atresia and stenosis (2013b), and gastroschisis (2013c) with increased atrazine exposure levels based on USGS estimates of county-level atrazine use for the maternal residences. Munger et al. (1997) reported that Iowa counties in which the public water supply had elevated levels of atrazine, metolachlor, and cyanzine had nearly twice the odds of intrauterine growth retardation (IUGR) compared to non-contaminated adjacent counties. Ochoa-Acuña et al. (2009) reported that exposure to atrazine in drinking water from community water systems (CWS) during the third trimester was associated with a 17-19% increase in the prevalence of small for gestational age (SGA) births in Indiana, but not with preterm births in a retrospective cohort study. Rinsky et al. (2012), observed a significantly increased odds of preterm births in Kentucky counties with the highest versus the lowest atrazine levels in drinking water between 2004 and 2006. However, Villanueva et al. (2005) failed to find an association between atrazine in water and low birth weight (LBW) or SGA in a study in an agricultural region of Brittany, France. In another study in the same area of France, Chevrier et al. (2011) reported that atrazine biomarkers in maternal urine were associated with lower birth weight, length, and head circumference. With the exception of the one prospective cohort study in France (Chevrier et al. 2011), all previous epidemiological studies of atrazine and birth outcomes have relied on ecologic exposure estimates obtained retrospectively through environmental monitoring data.

B. Nitrates

Nitrate (NO_3^-) is the most commonly found contaminant in the world's aquifers and a major water contaminant in the U.S. (Exner et al. 2014). Major environmental inputs of nitrate come from fertilizers, livestock manure, and human sewage (USEPA 2014). Nitrate is extremely water soluble and partitions quickly into groundwater. Further, it does not evaporate and can accumulate in groundwater over many years. Once consumed, nitrate is converted into nitrite. In

the stomach, nitrite reacts with nitrosatable compounds (e.g., amine- and amide-containing drugs) to form *N*-nitroso compounds, which have been found to be teratogens in animal models (Brender et al. 2013).

Nitrate levels in drinking water are highly variable, depending on surrounding land use, underlying geology, climate, and drinking water source (Nolan and Hitt 2006; Burrow et al. 2010). Shallow wells are more likely to exceed the USEPA MCL for nitrate of 10 mg/L than deep groundwater wells. Additionally, those who use private wells for drinking water are more likely to have higher nitrate levels in their drinking water than those served by community water systems, and surface water is more likely to have higher nitrate concentrations than groundwater, especially in areas of agricultural land use (Manassaram et al. 2006; Burrow et al. 2010). Water sources in areas of intense agricultural land use have higher median values of nitrate than those of forested and undeveloped land use (Nolan and Hitt, 2006; Burrow et al. 2010).

The acute health effects from exposure to high levels of nitrate are well understood in humans. The primary route of exposure is through ingestion of foods high in nitrate and nitrite, such as vegetables and cured meats, and through contaminated drinking water, which generally accounts for less than 14% of daily nitrate intake (World Health Organization (WHO), 2011a). Nitrite oxidizes iron atoms in hemoglobin such that it can no longer bind to oxygen, leading to a condition named methemoglobinemia, commonly referred to as “blue baby syndrome” (USEPA 2014). In infants exposed to high levels of nitrate, this can be a fatal condition (Knobeloch et al. 2000).

Reproductive effects from long term exposure to low levels of nitrate are less well understood. One study has examined the relationship between nitrate exposure and intra-uterine growth restriction (IUGR) or preterm birth. Bukowski et al. (2001) found a positive dose

response relationship between nitrates and both IUGR and preterm birth in a case control study of women on Prince Edward Island, Canada.

Most studies of reproductive outcomes have focused on birth defects and congenital anomalies. In a case control study of birth defects and nitrates, prenatal nitrate intake from drinking water was associated with increased odds of neural tube defects (NTDs), oral cleft defects, and limb deficiencies (Brender et al. 2013). Several studies suggest that exposure to nitrates in drinking water may increase the odds of cardiac defects (Cedergren et al. 2002), anencephaly (Croen et al. 2001), and spontaneous abortions (Manassaram et al. 2006). The proposed mechanism of effect is that nitrates increase endogenous *N*-nitroso compounds which are teratogenic (Croen et al. 2001; Brender et al. 2013).

C. <u>Arsenic</u>

Arsenic is the 20th most common element in the Earth's crust and is a naturally occurring water contaminant in many regions of the world (International Agency for Research on Cancer (IARC) 2004). The primary route of human exposure to arsenic is through contaminated drinking water, with additional contributions from contaminated food and air (Vahter 2009; WHO 2011b). Once ingested, inorganic arsenic is reduced from its pentavalent (As+5, arsenate) to trivalent (As+3, arsenite) form and then methylated into two major metabolites, methylarsonic acid (MMA) and dimethylarsinic acid (DMA), both of which are less toxic are more readily excreted through urine than inorganic arsenic (IARC 2004; Vahter 2002). Currently, the maximum contaminant level (MCL) for arsenic in drinking water set by the WHO and the USEPA is 10 µg/L.

IARC has classified arsenic in drinking water as a Group 1 (known) carcinogen to humans (IARC 2004). Chronic exposure to arsenic in drinking water has been associated with

cancers of the skin, bladder, kidney, liver, and lung as well as with diabetes, hypertension, and non-malignant skin conditions (NRC 1999; Yoshida et al. 2004). The effect of arsenic exposure on fetal development is less well understood. Arsenic and its metabolites readily cross the placental barrier, with cord blood arsenic levels nearly as high as maternal blood levels (Concha et al. 1998a; Hall et al. 2007). There is evidence that the primary form of arsenic transferred to the fetus is DMA, a result of increased methylation during pregnancy (Concha et al. 1998a). The mechanisms by which arsenic is toxic for fetal growth and development likely result from direct toxic effects on enzyme production and activity which alter DNA repair enzymes, oxidative stress, hormone interactions, and the one-carbon metabolism pathway (Vahter 2009). Arsenic is not transferred through breast milk (Concha et al. 1998b). Therefore, reproductive effects are likely an effect of prenatal exposure, unless infants are fed primarily formula, mixed with contaminated water.

Epidemiologic evidence of an effect of prenatal exposure to arsenic on reproductive outcomes is growing, although research on specific end points remains limited and contradictory. Studies from regions with elevated arsenic levels in drinking water suggest that arsenic exposure during pregnancy is associated with reduced birth weight. In a cross-sectional study of pregnant women in Taiwan, Yang et al. (2003) observed a significantly lower mean birth weight among women residing in areas of high drinking water arsenic (range, 0.15ppb – 3.59ppm), compared to those in low arsenic areas (<0.15ppb). Similarly, in a prospective cohort study comparing birth outcomes between two Chilean cities, one with high arsenic exposure and one with low exposure, increased arsenic levels were associated with a 57g reduction in birth weight (Hopenhayn et al. 2003). In a cohort of pregnant Bangladeshi women, a significant dose-response relationship between arsenic and birth weight, head circumference, and chest

circumference was observed only for women with lower arsenic exposure (≤100 μg/L in urine) (Rahman et al. 2009). The reduction in birth weight observed in this study observed with increased arsenic is similar to that observed with cigarette smoking during pregnancy.

In addition to birth size, various fetal and infant mortality measures have been found to be associated with arsenic levels in drinking water. In a large cohort of pregnant women in Bangladesh, risk of infant death, defined as death of a child before 12 months of age, was significantly elevated for those in the 4^{th} and 5^{th} quintiles of drinking water arsenic exposure (RR_4 = 1.29, 95% CI 1.08 – 1.53; RR_5 = 1.19, 95% CI 1.00 – 1.41) (Rahman et al. 2007). In a cross-sectional study of birth outcomes in Bangladesh, women who lived in an arsenic-affected village (mean drinking water arsenic 240 μg/L, range 200 – 1371 μg/L) had significantly increased rates of spontaneous abortions, stillbirths, and preterm births compared to those in a "nonexposed" village (≤20 μg/L in drinking water) (Ahmad et al. 2001). In West Bengal, women with high arsenic exposure (≥200 μg/L) had significantly increased odds of a stillbirth (OR = 6.07, 95% CI 1.54 – 24.0), but no increased odds of infant mortality or spontaneous abortion (von Ehrenstein et al. 2006). In a case-control study of spontaneous abortions among women in Boston, increased odds of spontaneous abortion was observed with increasing arsenic exposure, however the association was not significant at higher exposure levels (Aschengrau et al. 1989). Comparing rates of infant mortality outcomes in a highly exposed (≥50 μg/L) city in Chile, to a low exposed city (≤20 μg/L) found significantly elevated rates of late fetal mortality (RR = 1.7, 95% CI 1.0 – 1.9), neonatal mortality (RR = 1.53, 95% CI 1.4-1.7), and postneonatal mortality (RR = 1.26, 95% CI 1.2-1.3) (Hopenhayn-Rich et al. 2000). Increased rates of spontaneous abortions were observed among women working in the smelting processes containing arsenic compared to other female employees at a smelter plant in northern Sweden (Nordström et al.

1979). In a cross-sectional study of birth outcomes in Bangladesh, Milton et al. (2005) found an increased odds of spontaneous abortions (OR = 2.5, 95% CI 1.5-4.3) and stillbirth (OR = 2.5, 95% CI 1.3-4.9) among women exposed to drinking water arsenic > 50 µg/L. Kwok et al. (2006) failed to observe an association between arsenic levels in the drinking water of pregnant women and either stillbirth or birth weight in a large cohort in Bangladesh, but did find evidence of an association with birth defects.

The majority of data on arsenic and birth outcomes are from populations with very high exposure, such as Bangladesh, West Bengal, China, and Argentina. Less is known about reproductive health effects at low exposures such as those found in the Midwestern United States.

D. Birth Outcomes

Preterm birth (PTB) is defined as an infant delivered at less than 37 weeks gestation and very preterm birth (VPTB) is defined as an infant delivered before 32 weeks gestation (Wilcox, 2010). The risk of PTB in the United States is approximately 13%, with large disparities between races. In 2006, the rate of PTB was 11.7% for non-Hispanic whites, 18.5% for non-Hispanic blacks, and 12.3% for Hispanics (Martin et al. 2013). Risk factors for PTB include infections, medical procedures (e.g., early delivery for pre-eclamptic pregnancies), assisted reproductive technology, previous preterm delivery, maternal smoking during pregnancy, maternal thinness, diabetes, and illicit drug use, and stress (Behrman and Butler ed. 2007; Wilcox 2010). Limited evidence suggests certain environmental exposures are associated with PTB including lead, DDT, air pollution, PCBs, and diethylstilbestrol (DES) (Maisonet et al. 2004; Behrman and Butler Ed. 2007; Wilcox 2010). The critical window for PTB may be either proximal or early in pregnancy, and may vary by exposure.

Large racial disparities exist among rates of low birthweight (LBW) in the U.S. as well. In 2006, the rate of LBW was 7.3% for non-Hispanic whites, 14.0% for non-Hispanic blacks, and 7.0% for Hispanics. Rates of LBW are lower among term births (≥37 weeks gestation), with an overall rate of 3.1% among all races in 2012 (Martin et al. 2013). In addition to race/ethnicity, LBW is associated with socio-economic class, maternal smoking, maternal height and weight, maternal weight gain during pregnancy, parity, plurality, infant sex, and previous low birthweight delivery (Kramer, 1987; Eskenazi et al. 1995; Horta et al. 1997; Wilcox 2010). There are several environmental exposures that are associated with LBW, including lead, DDT/DDE, environmental tobacco smoke, air pollution, polycyclic aromatic hydrocarbons (PAHs), pesticides, and nitrates in drinking water (Martinez et al. 1994; Eskenazi et al. 1995, Wang et al. 1997; Dejmek et al. 1999; Perera et al. 1999; Bobak, 2000; Bukowski et al. 2001; Perera et al. 2005; Salmasi et al. 2010). Small for gestational age (SGA) is related to birthweight in that it is defined as the smallest 10% of infants born at each gestational age within a population. LBW and SGA share many risk factors including maternal smoking, thinness, nulliparity, and cocaine use, but risk of SGA is highly increased if either of the parents were born SGA (Jaquet et al. 2005; McCowan et al. 2009).

The public health implications of these adverse birth outcomes is not trivial. Neonatal mortality increases as the length of gestation decreases, resulting in a decreased survival rate among preterm and very preterm infants. The risk of neonatal mortality is also highest among the smallest and largest infants, as measured by birth weight. This same pattern of increased risk is seen later in life as well, with a reversed “J” shape association between birthweight and cardiovascular disease and all-cause mortality (Wilcox 2010).

III. ARSENIC IN DRINKING WATER AND ADVERSE BIRTH OUTCOMES IN OHIO

A. Introduction

Arsenic is the 20th most common element in the Earth's crust and is a naturally occurring water contaminant in many regions of the world (IARC 2004). The primary route of human exposure to arsenic is through contaminated drinking water, with additional contributions from contaminated food and air (Vahter 2009; WHO 2011). While arsenic in drinking water has been classified as a Group 1 (known) carcinogen to humans (IARC 2004) for bladder, lung, and skin cancers; the effect of chronic arsenic exposure through drinking water on fetal development is less well understood. The association is biologically plausible because arsenic and its metabolites readily cross the placental barrier, and arsenic levels in cord blood are nearly as high as in maternal blood (Concha et al. 1998; Hall et al. 2007). Currently, the maximum contaminant level (MCL) in drinking water set by the WHO and the USEPA is 10 µg/L, based on risk of cancers, cardiovascular diseases, and neurologic effects (40 CFR 141.62).

A growing body of epidemiologic research from regions with elevated arsenic levels in drinking water suggests that arsenic exposure during pregnancy is associated with reduced birth weight. In a cross-sectional study of pregnant women in Taiwan, women residing in areas of high drinking water arsenic (range, 0.15 ppb – 3.59 ppm) delivered infants with significantly lower mean birth weight than women in low arsenic areas (< 0.15 ppb) (Yang et al. 2003). Similarly, a prospective cohort study comparing birth outcomes between two Chilean cities, one with a mean arsenic concentration in drinking water of 42 µg/L and one with an average arsenic concentration <1 µg/L, found increased arsenic levels were associated with a 57 g reduction in birth weight (Hopenhayn et al. 2003). In a cohort of pregnant Bangladeshi women, a significant dose-response relationship between arsenic and birth weight, head circumference, and chest

circumference was observed only for women with lower arsenic exposure (< 100 µg/L in urine) compared to those with higher arsenic exposures (≥100 µg/L) (Rahman et al. 2009). The specific exposure window during pregnancy in which the fetus is most susceptible to the effects of arsenic is unknown, but one study of pregnant women in Bangladesh found a significant decrease in birthweight among women with higher arsenic exposure early in pregnancy (Huyck et al. 2007). Kwok et al. (2006), however, found no association between drinking water arsenic and birthweight among term infants born to women residing in three regions of Bangladesh with a range of exposures to arsenic through drinking water. In a prospective study of 122 pregnant women in Romania, Bloom et al. (2015) found a significant negative relationship between arsenic in drinking water and birthweight only among smokers.

Epidemiologic evidence of an association between drinking water arsenic and preterm birth is inconsistent (Bloom et al. 2014). In a cross-sectional study of birth outcomes in Bangladesh, women who lived in an arsenic-affected village (mean arsenic in drinking water 240 µg/L, range 200–1371 µg/L) had significantly increased rates of spontaneous abortions, stillbirths, and preterm births compared to those in a "non-exposed" village (≤ 20 µg/L in drinking water) (Ahmad et al. 2001). Yang et al. (2003) found a non-significant increase in the odds of preterm birth among women in a high drinking water arsenic region compared to those in a low arsenic region. A study of adverse birth outcomes in Inner Mongolia did not detect an association of drinking water arsenic levels >50 µg/L with preterm birth was observed (Myers et al. 2010). In a sample of Chinese male infants, gestational age was significantly inversely related to arsenic in maternal blood (Xu et al. 2011). Preterm birth and reduced birthweight were spatially associated with higher levels of groundwater arsenic (Shi et al. 2015).

The majority of data on arsenic and birth outcomes are from populations with very high exposure, such as Bangladesh, West Bengal, China, and Argentina. Less is known about reproductive health effects at low exposures (Quansah et al. 2015), such as those found in the Midwestern United States. The objective of this research was to examine the association between arsenic in drinking water and several birth outcomes in the state of Ohio where arsenic levels are relatively low.

B. Methods

1. Study Population

This study used birth certificate data from all births occurring in the state of Ohio between 2006 and 2008. There were 428,804 singleton births of Ohio residents during this time period. Individual-level, de-identified birth certificate data for children born in Ohio was provided by the Ohio Department of Health (ODH).

2. Birth Outcomes

The primary outcomes of interest in the study were small for gestational age (SGA), term low birth weight (term LBW), very low birth weight (VLBW), preterm birth (PTB), and very preterm birth (VPTB). SGA was defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population (Wilcox 2010). SGA status was calculated using sex- and gestational age-specific national birth weight references developed by Duryea et al. (2014). Term LBW was defined as an infant weighing <2,500g at time of delivery among term infants (≥37 weeks gestation). An infant was considered VLBW if it weighed <1,500g at time of delivery, regardless of gestational age. Preterm and very preterm births were defined as infants delivered prior to 37 and 32 weeks gestation, respectively. Gestational age was based on the reported last normal menstrual period (LMP). If the LMP was unknown or impossible, a clinical

estimate of gestation was used. All birth outcomes were either reported directly on or were calculated from variables reported on the birth certificates.

3. Exposure Assessment

The USEPA defines the legal limits of water contaminants and water testing schedules, as mandated in the Safe Drinking Water Act. The MCL for arsenic in drinking water is 10 μg/L (40 CFR 141.62). Public drinking water systems are required to monitor for arsenic every three years when using groundwater and annually when using surface water sources (40 CFR 141.23).

A total of 2,968 arsenic measurements from 975 community water systems (CWS) in Ohio from 2006 through 2008 were obtained from the Ohio EPA Safe Drinking Water Information System (SDWIS). An annual measure of arsenic in drinking water was calculated for each of the 88 counties in Ohio as follows. First all measurements in each community water system (CWS) providing drinking water in a county in a year were averaged, giving a CWS-year mean. Second, the CWS-year means in each county were averaged, weighted by the population served. The resulting county-year mean was used as the exposure measure. The median number of CWSs in each county was 11, with a range from 3 to 54. The exposure measure assumed that each CWS serves only residents in the county in which the CWS office is located (Jones et al. 2014a). Monthly mean values of arsenic were additionally calculated, but between 47 and 55% of counties were missing a monthly estimate due to the infrequent sampling requirements under current regulations. Furthermore, the monthly estimates did not show significant variation by month, therefore annual estimates were used in analysis. The limits of detection (LODs) varied, but were typically 0.5 μg/L, and measurements below the LOD were equated with LOD/2. County-level population percentages of those using private well water were obtained from the Unites States Geological Survey (USGS 2015).

The arsenic exposure measures were linked with birth outcomes by the county and year(s) of gestation. If an infant's entire gestation fell within one calendar year, the county-year arsenic measure was assigned to the birth. If an infant's gestation spanned two calendar years, an average of the two annual estimates of arsenic was assigned to that birth, weighted by months in each calendar year.

4. Covariates

The covariates examined in this study included infant sex, maternal age at birth, mother's race/ethnicity, maternal educational attainment, marital status, prenatal care status, socioeconomic status, parity, cigarette use, and maternal pre-pregnancy body mass index (BMI). Maternal age was categorized as 10-19, 20-29, 30-39, and ≥ 40 years of age. Maternal race/ethnicity was defined as non-Hispanic white, non-Hispanic black, Hispanic, and other/unknown. Maternal educational attainment was categorized as less than a high school degree, high school degree, some college, and college degree or higher. Marital status was dichotomized as married or not married. The Kotelchuck index was used to define adequate prenatal care (PNC), based on the month of entry into PNC and total number of PNC visits (Kotelchuck 1994). Maternal smoking was dichotomized as smoker versus non-smoker. The cigarette use data was non-specific to the window of time including pregnancy. Whether or not the mother was enrolled in the Women, Infant, and Children (WIC) supplemental nutrition program was used as a proxy for low socioeconomic status (SES). Maternal pre-pregnancy BMI was categorized according to the CDC definitions of underweight, normal, overweight, and obese (CDC 2015). Parity was categorized as having had 0, 1, 2, or ≥ 3 previous live births.

5. Data Analysis

We explored initial bivariate relationships between arsenic concentrations and each outcome using t-tests. Additionally, we examined initial bivariate relationships between annual concentrations of arsenic and each covariate as well as each covariate and each outcome using t-tests for continuous variables, Rao-Scott Chi-Square tests for dichotomous variables, and ANOVA test for covariates with >2 categories. Potential confounders were considered as those variables that were associated with both the exposure measures and outcome measures and were not in the causal pathway. Maternal age, maternal race/ethnicity, and infant sex were included in modeling based on *a priori* knowledge.

We developed generalized estimating equation (GEE) logistic regression models, with an exchangeable working correlation structure and robust standard errors, to estimate the association between arsenic in drinking water and dichotomous birth outcomes, including SGA, term LBW, VLBW, PTB, and VPTB while accounting for clustering at the county level. Arsenic exposure was treated as a continuous variable in the models. Confounding was indicated if the estimate for the exposure variable changed by more than 10% with the addition of the potential confounder into the model. Between 9 and 10% of observations were not used due to missing data on covariates, exposure, or outcome status. All analyses were performed using SAS®, Version 9.4 (SAS Institute Inc., Cary, NC, USA). Multiplicative interaction was assessed for smoking status through the use of a product term in the modeling process.

Several sensitivity analyses were performed to better understand the relationship between arsenic and the birth outcomes of interest. The study lacks estimates of arsenic in private well water, therefore sensitivity analyses were performed which restrict the arsenic analyses *a priori* to counties with <20% (N=31) and <10% (N=20) of county residents using private well water to

explore the exposure-outcome relationship in those counties with the least exposure misclassification. The mean percentage of private well use in Ohio was 31% (range 0 – 81%). Twenty-six percent of live singleton births in Ohio were missing either the month of entry into PNC or the number of PNC visits attended. In order to better understand the role of this important covariate, we examined the association between arsenic and each outcome in models that did not include PNC as a covariate, and again including PNC as a covariate, dropping the missing observations.

C. Results

1. Descriptive Statistics

County-year mean arsenic concentrations ranged from 0.50 – 12.2 μg/L during 2006-2008 (Table I). County-year mean concentrations were missing in 5%, 10%, and 7% of counties in 2006, 2007, and 2008 respectively, such that <4% of births in each year did not have an exposure measure.

There were 428,804 live singleton births in Ohio between 2006 and 2008, of which 51% were male (Table II). The majority of the births were born to mothers who were non-Hispanic white (76%), between 20 and 29 years old (56%), had completed more than a high-school degree (54%), were married (58%), and parous (59%). Only 38% of mothers reported intermediate or adequate prenatal care, but 26% had an unknown level of prenatal care. There was a high prevalence of obesity (25%), WIC use (42%), and reported cigarette use (26%) in this population. Among live singleton births, 10.6% were SGA, 1.2% were very low birth weight, 10.9% were preterm, and 1.8% very preterm. Among singleton term births, 2.9% were term LBW. With the exception of PNC, there were low levels of missing data on covariates. Less than 1% of births were missing data on maternal age (N = 1536), education (N = 3460), and cigarette

use (N = 44). Between 2 and 3% of births were missing data on parity (N = 11,173), pre-pregnancy BMI (14,374), and WIC status (12,309).

TABLE I
SUMMARY OF MEAN ANNUAL ARSENIC CONCENTRATIONS REPORTED IN FINISHED PUBLIC DRINKING WATER SAMPLES FOR ALL OHIO COUNTIES (N=88) BETWEEN 200 AND 2008

Year	Mean ± SD (µg/L)	Median concentration (µg/L)	Minimum concentration (µg/L)	Maximum concentration (µg/L)	Percent Missing[a] (%)
2006	2.04 ± 1.42	1.51	0.74	10.51	5
2007	2.33 ± 2.08	1.50	0.74	12.17	10
2008	1.84 ± 0.98	1.50	0.50	6.28	7

[a] Percentage of counties missing an annual mean arsenic concentration.
Data source: Ohio EPA Safe Drinking Water Information System

In bivariate analyses, nearly all covariates were significantly associated with each of the five outcomes examined which is consistent with our expectations from prior research (Table II). A slightly higher proportion of female infants were SGA and term LBW compared to male infants. Conversely, a higher proportion of male infants were preterm or very preterm than female infants. Non-Hispanic black mothers had the highest proportion of all adverse outcomes. With the exception of SGA, all adverse outcomes were more likely among the youngest and oldest maternal age categories. All outcomes were inversely related to maternal education. The proportions of mothers with any adverse outcome was significantly higher among smokers compared to non-smokers, among WIC users compared to non-WIC users, and among unmarried mothers compared to married mothers. Nulliparous mothers as well as mothers who had ≥3 previous births demonstrated elevated proportions of term LBW, VLBW, preterm, and very

preterm births compared to mothers with 1-2 previous births. SGA was most common among nulliparous women. Women receiving intermediate or adequate prenatal care had the lowest proportion of adverse outcomes, with the exception of SGA. VLBW, preterm, and very preterm births exhibited a U-shaped relationship with maternal BMI, with the highest prevalence among underweight and obese women. Due to missing data on covariates and exposure estimates, approximately 10% of observations were dropped from fully covariate-adjusted models.

2. Regression Analyses

a. SGA

We found no evidence of an association between annual measures of arsenic and SGA in either crude or fully covariate-adjust models (AOR 1.00, 95% CI 0.98, 1.01) (Table III). Similarly, we observed no association between arsenic and the odds of SGA when we restricted the analysis to those residing in counties with <20 and <10% private well use.

b. Term LBW and VLBW

Similar to the SGA analyses, we observed no association between county-year measures of arsenic in drinking water and term LBW in crude or adjusted models (AOR_A 0.99, 95% CI 0.98, 1.01) (Table III). We did observe a significant increase in the odds of term LBW with increasing arsenic in crude models restricted to those counties with <20 and <10% private well use, but these associations were attenuated after controlling for covariates ($AOR_{<20}$ 1.07, 95% CI 1.00, 1.14; $AOR_{<10}$ 1.06, 95% CI 0.98, 1.15).

We found no association between arsenic in drinking water and VLBW in models of all counties in Ohio (AOR 0.99, 95% CI 0.96, 1.03) (Table III). In crude models, restricted to those counties with <20 or <10 % private well use, we observed a non-significant increase of 8% and 13% in odds of VLBW respectively per 1µg/L increase in arsenic. The odds of VLBW births

TABLE III

DISTRIBUTION OF DEMOGRAPHIC AND ECONOMIC COVARIATES ACROSS THE SAMPLE POPULATION AND BY OUTCOME FOR ALL LIVE SINGLETON BIRTHS IN OHIO 2006-2008 (N=428,804)

		Term				
		SGA	LBW	VLBW	PTB	VPTB
Variable	**N (%)**	**%**	**%**	**%**	**%**	**%**
Gender						
Male	209,617 (51)	**10.5**	**2.3**	1.2	**11.3**	**1.8**
Female	219,187 (49)	**10.8**	**3.4**	1.2	**10.4**	**1.7**
Race/Ethnicity						
Non-Hispanic white	327,343 (76)	**9.2**	**2.4**	**0.9**	**9.8**	**1.4**
Non-Hispanic black	69,325 (16)	**16.8**	**5.2**	**2.7**	**16.0**	**3.5**
Hispanic	19,631 (5)	**1.6**	**2.7**	**1.1**	**11.9**	**1.8**
Other	12,505 (3)	**13.9**	**3.7**	**1.3**	**10.7**	**1.8**
Maternal Age at Birth						
10 − 19	47,544 (11)	**15.5**	**4.2**	**1.8**	**13.5**	**2.7**
20 − 29	239,250 (56)	**11.2**	**2.9**	**1.2**	**10.6**	**1.7**
30 − 39	132,021 (31)	**8.0**	**2.2**	**1.1**	**10.2**	**1.5**
40+	8,453 (2)	**9.2**	**3.5**	**1.8**	**13.7**	**2.5**
Maternal Education						
< High School	14,851 (4)	**11.3**	**2.9**	**1.1**	**10.6**	**1.8**
High School	178,980 (42)	**13.7**	**3.9**	**1.5**	**12.8**	**2.2**
Some College/Degree	195,754 (46)	**8.4**	**2.1**	**1.0**	**9.5**	**1.4**
> College Degree	35,759 (8)	**6.9**	**1.6**	**0.8**	**7.8**	**1.1**
Maternal Smoking						
Yes	113,552 (26)	**15.5**	**4.7**	**1.5**	**12.5**	**2.2**
No	315,208 (74)	**8.9**	**2.2**	**1.2**	**10.3**	**1.6**
Prenatal Care						
Inadequate	58,850 (14)	**14.4**	**4.5**	**1.5**	**11.9**	**2.0**
Intermediate	40,854 (10)	**11.1**	**2.2**	**0.3**	**3.9**	**0.3**
Adequate	120,563 (28)	**10.0**	**1.9**	**0.3**	**3.3**	**0.4**
Adequate Plus	94,803 (22)	**8.7**	**3.3**	**1.8**	**20.8**	**2.9**
Unknown	113,734 (26)	**10.8**	**3.0**	**2.0**	**12.7**	**2.7**
WIC use						
Yes	175,604 (42)	**13.6**	**3.8**	**1.3**	**12.1**	**1.9**
No	240,891 (58)	**8.4**	**2.1**	**1.1**	**9.8**	**1.6**
Prepregnancy BMI						
Underweight	18,811 (4)	**18.7**	**6.2**	**1.6**	**14.2**	**2.2**
Normal	197,071 (48)	**11.1**	**2.9**	**1.0**	**10.3**	**1.5**
Overweight	94,621 (23)	**9.4**	**2.3**	**1.1**	**10.3**	**1.6**
Obese	103,927 (25)	**9.2**	**2.5**	**1.6**	**11.5**	**2.1**

TABLE IIII (continued)
DISTRIBUTION OF DEMOGRAPHIC AND ECONOMIC COVARIATES ACROSS THE SAMPLE POPULATION AND BY OUTCOME FOR ALL LIVE SINGLETON BIRTHS IN OHIO 2006-2008 (N=428,804)

Variable	N (%)	SGA %	Term LBW %	VLBW %	PTB %	VPTB %
Parity						
0	170,155 (41)	**12.9**	**3.3**	**1.5**	**10.9**	**2.0**
1	131,510 (31)	**8.8**	**2.2**	**0.9**	**9.6**	**1.4**
2	68,891 (17)	**9.0**	**2.5**	**1.0**	**10.9**	**1.6**
≥3	47,075 (11)	**9.8**	**3.1**	**1.2**	**13.2**	**2.0**
Marital Status						
Married	247,516 (58)	**7.9**	**2.0**	**0.9**	**9.0**	**1.2**
Unmarried[a]	181,288 (42)	**14.4**	**4.1**	**1.8**	**13.5**	**2.5**

[a] Not married category includes mothers who responded single, widowed, or divorced.
Bold indicates a significant difference in proportions (χ^2 p-value <.05).

increased significantly with increasing arsenic concentrations in drinking water in counties with <10% of the populations using private well water in adjusted models ($AOR_{<10}$ 1.14, 95% CI 1.04, 1.24).

c. PTB and VPTB

While the odds of preterm birth did not significantly increase with increasing arsenic levels across the entire state (AOR 0.99, 95% CI 0.98, 1.01), we observed a significant 8−10% increase in the odds of PTB per 1µg/L increase in arsenic in drinking water for counties with <20% and <10% private well use ($AOR_{<20}$ 1.08, 95% CI 1.02, 1.14; $AOR_{<10}$ 1.10, 95% CI 1.06, 1.15) (Table IV).

We observed no association between VPTB and county-year arsenic in drinking water when using all counties in Ohio (Table IV). In models restricted to those counties with <20% and <10% private well use, we observed a non-significant increase in odds of VPTB with increasing arsenic levels ($AOR_{<20}$ 1.07, 95% CI 0.89, 1.40; $AOR_{<10}$ 1.12, 95% CI 0.96, 1.31).

TABLE III

ASSOCIATIONS[a] BETWEEN MEAN ANNUAL ARSENIC EXPOSURE (μG/L) AND SGA, TERM LBW, AND VLBW IN OHIO COUNTIES, 2006 − 2008

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[e] (95% CI)	P-value
SGA[b]	Crude		88	385,833	1.00 (0.98, 1.01)	0.755
	Adjusted		88	385,833	1.00 (0.98, 1.01)	0.551
	Crude	<20	31	241,905	1.00 (0.97, 1.03)	0.944
	Adjusted	<20	31	241,905	1.01 (0.99, 1.03)	0.577
	Crude	<10	20	216,240	1.00 (0.97, 1.04)	0.803
	Adjusted	<10	20	216,240	1.01 (0.99, 1.03)	0.224
Term LBW[c]	Crude		88	345,030	0.99 (0.96, 1.01)	0.252
	Adjusted		88	345,030	0.99 (0.98, 1.01)	0.360
	Crude	<20	31	215,394	1.08 (1.03, 1.14)	0.001
	Adjusted	<20	31	215,394	1.07 (1.00, 1.14)	0.056
	Crude	<10	20	192,236	1.09 (1.03, 1.15)	0.003
	Adjusted	<10	20	192,236	1.06 (0.98, 1.15)	0.137
VLBW[d]	Crude		88	385,833	1.01 (0.95, 1.08)	0.656
	Adjusted		88	385,833	0.99 (0.96, 1.03)	0.672
	Crude	<20	31	241,905	1.08 (0.85, 1.39)	0.523
	Adjusted	<20	31	241,905	1.10 (0.96, 1.25)	0.160
	Crude	<10	20	216,240	1.13 (0.91, 1.40)	0.253
	Adjusted	<10	20	216,240	1.14 (1.04, 1.24)	0.005

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, and parity.
[b] Small for gestational age (SGA) defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population.
[c] Term low birth weight (term LBW) defined as <2500g among term births (≥37 weeks gestation).
[d] Very low birth weight (VLBW) defined as <1500g at time of delivery.
[e] Odds ratios reflect increase in odds per 1 μg/L increase in arsenic in drinking water.

3. PNC Sensitivity Analyses

Among all live singleton births in Ohio, 17% of observations were missing data on the month of entry into PNC. Another 11% were missing data on the total number of PNC visits attended, which resulted in 26% with an unknown Kotelchuck index of PNC utilization. PNC

utilization is a confounder in the associations observed above, therefore we performed analyses of these data controlling for PNC, but dropping those individuals with a missing Kotelchuck index score.

Similar to the initial models (Table III), we observed no association between arsenic and SGA in adjusted models (Table V). We found that annual county-year measures of arsenic exposure were significantly associated with term LBW among infants with known PNC utilization in counties with <20 and <10% private well use ($AOR_{<20}$ 1.07, 95% CI 1.01, 1.13; $AOR_{<10}$ 1.06, 95% CI 1.00, 1.13). We observed no association between arsenic and VLBW among births with known PNC utilization.

Unlike in the models which did not control for PNC, we observed a significant elevation in the odds of PTB in counties with <10% private well use in models that were restricted to those births with a known PNC utilization ($AOR_{<10}$ 1.10, 95% CI 1.00, 1.21) (Table VI). Similar to the initial models of VPTB (Table IV), we observed no significant associations between VPTB and arsenic, however, the odds of VPTB were elevated in counties with <20 or <10% private well use.

D. Discussion

Previous studies from regions of endemic high arsenic concentrations in drinking water show strong associations with adverse reproductive outcomes. In this study, we aimed to examine the relationship between arsenic in drinking water and several adverse reproductive outcomes in a geographic region with relatively low arsenic concentrations, the state of Ohio in the United States. Our analyses of all live singleton births in the state showed no association between arsenic in drinking water, measured as an annual county-level average, and any of the birth outcomes assessed, but over 50% of these counties had a substantial percentage of private

well users (30-81%), thereby introducing considerable exposure misclassification. When we assessed this relationship in a subset of the population for which exposure was most accurately defined − those counties in which less than 10 or 20% of the households used private wells as their drinking water source − we found a significant increase in the odds of VLBW and PTB among live singleton births. We observed no association between arsenic and SGA, term LBW, or VPTB in this population. Furthermore, we did not observe the same interaction between arsenic exposure and smoking that was reported by Bloom et al. (2015).

TABLE IV

ASSOCIATIONS[a] BETWEEN MEAN ANNUAL ARSENIC EXPOSURE (µG/L) AND PTB AND VPTB IN OHIO COUNTIES, 2006 − 2008

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[d] (95% CI)	P-value
Preterm birth[b]	Crude		88	386,164	1.00 (0.98, 1.02)	0.921
	Adjusted		88	386,164	0.99 (0.98, 1.01)	0.455
	Crude	<20	31	242,152	1.08 (1.01, 1.15)	0.018
	Adjusted	<20	31	242,152	1.08 (1.02, 1.14)	0.005
	Crude	<10	20	216,465	1.11 (1.06, 1.16)	<.0001
	Adjusted	<10	20	216,465	1.10 (1.06, 1.15)	<.0001
Very preterm birth[c]	Crude		88	386,164	0.99 (0.94, 1.04)	0.735
	Adjusted		88	386,164	0.99 (0.96, 1.02)	0.572
	Crude	<20	31	242,152	1.06 (0.82, 1.37)	0.669
	Adjusted	<20	31	242,152	1.07 (0.89, 1.40)	0.471
	Crude	<10	20	216,465	1.11 (0.88, 1.40)	0.381
	Adjusted	<10	20	216,465	1.12 (0.96, 1.31)	0.151

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, and parity.
[b] Preterm births defined as infants delivered before 37 weeks gestation.
[c] Very preterm births defined as infants delivered before 32 weeks gestation.
[d] Odds ratios reflect increase in odds per 1 µg/L increase in arsenic in drinking water.

TABLE V

ASSOCIATIONS[a] BETWEEN MEAN ANNUAL ARSENIC EXPOSURE (μG/L) AND SGA, TERM LBW, AND VLBW IN OHIO COUNTIES, 2006 − 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[e] (95% CI)	P-value
SGA[b]	Crude		88	289,091	0.99 (0.98, 1.01)	0.363
	Adjusted		88	289,091	0.99 (0.98, 1.00)	0.189
	Crude	<20	31	172,907	1.02 (0.99, 1.04)	0.138
	Adjusted	<20	31	172,907	1.01 (0.99, 1.03)	0.213
	Crude	<10	20	155,115	1.02 (1.00, 1.04)	0.016
	Adjusted	<10	20	155,115	1.01 (0.99, 1.03)	0.162
Term LBW[c]	Crude		88	260,060	0.98 (0.96, 1.01)	0.246
	Adjusted		88	260,060	0.99 (0.97, 1.01)	0.193
	Crude	<20	31	154,503	1.09 (1.06, 1.13)	<.0001
	Adjusted	<20	31	154,503	1.07 (1.01, 1.13)	0.022
	Crude	<10	20	138,429	1.11 (1.08, 1.13)	<.0001
	Adjusted	<10	20	138,429	1.06 (1.00, 1.13)	0.047
VLBW[d]	Crude		88	289,091	1.02 (0.94, 1.10)	0.616
	Adjusted		88	289,091	0.99 (0.94, 1.05)	0.828
	Crude	<20	31	172,907	1.01 (0.72, 1.41)	0.952
	Adjusted	<20	31	172,907	1.01 (0.79, 1.29)	0.940
	Crude	<10	20	155,115	1.05 (0.78, 1.41)	0.740
	Adjusted	<10	20	155,115	1.03 (0.82, 1.30)	0.792

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, PNC utilization, and parity.

[b] Small for gestational age (SGA) defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population.

[c] Term low birth weight (term LBW) defined as <2500g among term births (≥37 weeks gestation).

[d] Very low birth weight (VLBW) defined as <1500g at time of delivery.

[e] Odds ratios reflect increase in odds per 1 μg/L increase in arsenic in drinking water.

TABLE VI

ASSOCIATIONS[a] BETWEEN MEAN ANNUAL ARSENIC EXPOSURE (μG/L) AND PTB AND VPTB IN OHIO COUNTIES, 2006 – 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[d] (95% CI)	P-value
Preterm birth[b]	Crude		88	289,303	1.00 (0.98, 1.02)	0.813
	Adjusted		88	289,303	1.00 (0.98, 1.02)	0.912
	Crude	<20	31	173,062	1.07 (1.01, 1.14)	0.026
	Adjusted	<20	31	173,062	1.09 (0.99, 1.19)	0.087
	Crude	<10	20	155,257	1.10 (1.07, 1.13)	<.0001
	Adjusted	<10	20	155,257	1.10 (1.00, 1.21)	0.056
Very preterm birth[c]	Crude		88	289,303	0.99 (0.93, 1.05)	0.675
	Adjusted		88	289,303	0.98 (0.94, 1.03)	0.445
	Crude	<20	31	173,062	1.05 (0.82, 1.36)	0.686
	Adjusted	<20	31	173,062	1.09 (0.89, 1.34)	0.400
	Crude	<10	20	155,257	1.08 (0.85, 1.37)	0.512
	Adjusted	<10	20	155,257	1.11 (0.91, 1.36)	0.306

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, PNC utilization, and parity.
[b] Preterm births defined as infants delivered before 37 weeks gestation
[c] Very preterm births defined as infants delivered before 32 weeks gestation.
[d] Odds ratios reflect increase in odds per 1 μg/L increase in arsenic in drinking water.

The mechanism by which arsenic may affect birth weight and gestational age at delivery is uncertain. Arsenic intoxication as well as chronic arsenic exposure at lower levels has been associated with anemia among pregnant women (Kyle and Pease 1965; Westhoff et al. 1975; Hopenhayn et al. 2006; Surdu et al. 2015; Taheri et al. 2015). Anemia during pregnancy has been found to be associated with low birthweight and preterm delivery (Bondevik et al. 2001). Fei et al. (2013) identified a target gene for arsenic which may lead to functional impairment of the placenta, thereby affecting fetal growth and leading to reduced birthweight. There is growing evidence that arsenic exposure increases general inflammation in chronically exposed individuals

(Wu et al. 2003) and well as inflammatory markers in newborns exposed *in utero* (Fry et al. 2007; Ahmed et al. 2011). Inflammation is associated with preterm birth, through such mechanisms as ripening of cervix and rupture of membranes (Challis et al. 2009). Other possible mechanisms by which arsenic is toxic for fetal growth and development likely result from direct toxic effects on enzyme production and activity which alter DNA repair enzymes, oxidative stress, hormone interactions, and the one-carbon metabolism pathway (Vahter 2009).

This study is one of the largest of its kind, and among the first to integrate data from environmental regulatory agencies and state vital statistics departments to examine the relationship between drinking water arsenic and adverse birth outcomes. The robust sample size of 428,804 births in Ohio from 2006-2008 allowed for greater statistical power to detect small risks than prior studies. This study also benefited from individual-level data on important covariates with very little missing data.

This study had several limitations originating from the data sources used. Both the exposure and outcome data for this study were from secondary data sources, meaning that these data were collected for purposes other than research. While meeting the regulatory requirements for arsenic testing, the counties in this study had relatively infrequent arsenic measurements from 2006 to 2008. The infrequent monitoring schedules meant that not all CWSs in each county contributed to the county-year exposure measure in all years, and some county-year exposure measures were missing. These limitations were exacerbated in more refined time scales (i.e., monthly or trimester-specific exposure measures), and prompted use of an annual measure.The exposure measure was aggregated from the CWS to the county level because we were unable to link individual births to the CWS that served as their mother's primary drinking water source during pregnancy, which introduced some exposure misclassification at the individual level. This

aggregation of exposure reduced the variability in the exposure estimates, as infants were assigned their county's (N=88) mean annual arsenic level. We used public drinking water data to calculate the exposure measures, thereby introducing exposure misclassification in those counties in which a large percentage of the population was using private well water. This misclassification is most likely to have negatively biased our results assuming that is non-differential with respect to the outcomes. We attempted to reduce exposure misclassification by restricting analyses to those counties with limited private well use. Furthermore, we had no information on drinking water consumption habits for this study population.

We used birth certificate data to ascertain all outcomes examined in this study, however, the reliability of birth certificate data varies widely by data element. Demographic variables on the birth certificate about the mother, including maternal age, race/ethnicity, and marital status are highly accurate (Querec, 1980; Schoendorf et al. 1993; Reichman and Hade, 2001; DiGiuseppe et al. 2002; Zollinger et al. 2006). There is also high agreement between birth certificates and medical records for variables including infant gender, birth weight, plurality, number of previous live births, and prenatal care received (Querec, 1980; Schoendorf et al. 1993; Green et al. 1998; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp, 2006; Zollinger et al. 2006). Gestational age, parental education, paternal demographics show moderate agreement, but maternal weight gain during pregnancy, maternal medical risk factors (e.g., chronic hypertension, previous LBW or preterm birth), tobacco and alcohol use, and number of prenatal care visits have very low reliability (Querec, 1980; Dobie et al. 1998; Reichman and Hade, 2001; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp, 2006; Zollinger et al. 2006).

Furthermore, we lacked information on whether or not the mothers of the infants in these analyses had moved at any point during their pregnancy. Rates of pregnancy mobility are estimated between 12-32% (Fell et al. 2004; Canfield et al. 2006; Miller et al. 2010; Zender et al. 2010), and vary by geography and demographic factors. We were unable to account for those women who moved during pregnancy and assumed that the residence listed on the birth certificate was the residence throughout the entire pregnancy.

E. Conclusions

To our knowledge, this study is the first of its kind to explore the association between arsenic in drinking water and multiple birth outcomes in a region of relatively low arsenic exposure; specifically, 99% of births in this study were estimated to be exposed to arsenic in drinking water below the current MCL of 10 µg/L. Our findings were similar when we deleted the 1% of the individuals with exposures above the MCL (data not shown). In models with the least potential for exposure misclassification (e.g., low use of private drinking water wells), we found that the risk of VLBW and preterm birth increased with increasing levels of arsenic in drinking water. These findings suggest that the current MCL may not be sufficiently protective against these adverse birth outcomes. Further epidemiologic research is needed to explore these associations at low levels of arsenic exposure with individual exposure measurements or biomarkers of exposure.

IV. NITRATE-NITRITE DRINKING WATER CONTAMINATION AND ADVERSE BIRTH OUTCOMES IN OHIO

A. Introduction

Nitrate (NO_3^-) is the most commonly found contaminant in the world's aquifers and a major water contaminant in the U.S. (Exner et al. 2014). Major environmental inputs of nitrate come from fertilizers, livestock manure, and human sewage (World Health Organization (WHO) 2011a; USEPA 2014). Nitrate is extremely water soluble and partitions quickly into groundwater, where it can accumulate and persist over many years. Nitrate levels in drinking water are highly variable, depending on surrounding land use, underlying geology, climate, and drinking water source (Nolan and Hitt 2006; Burrow et al. 2010). Shallow wells are more likely to exceed the USEPA MCL for nitrate of 10 mg/L than deep groundwater wells (Rowe et al. 2004). Additionally, those who use private wells for drinking water are more likely to have higher nitrate levels in their drinking water than those served by community water systems, and surface water sources are more likely to have higher nitrate concentrations than groundwater sources, especially in areas of agricultural land use (Rowe et al. 2004; Manassaram et al. 2006; Burrow et al. 2010). Water sources in areas of intense agricultural land use have higher median values of nitrate than those of forested and undeveloped land use (Nolan and Hitt 2006; Burrow et al. 2010; Dubrovsky et al. 2010).

The primary route of exposure in humans is through ingestion of foods high in nitrate and nitrite, such as vegetables and cured meats, and through contaminated drinking water, which generally accounts for less than 14% of daily nitrate intake (WHO 2011a). Once consumed, nitrate is converted into nitrite. In the stomach, nitrite reacts with nitrosatable compounds (e.g., amine- and amide-containing drugs) to form *N*-nitroso compounds, which have been found to be teratogens in animal models (Brender et al. 2013).

The acute health effects from exposure to high levels of nitrate are well understood in humans. Nitrite oxidizes iron atoms in hemoglobin such that they can no longer bind to oxygen, leading to a condition named methemoglobinemia, commonly referred to as "blue baby syndrome" (USEPA 2014). In infants exposed to high levels of nitrate, this can be a fatal condition (Knobeloch et al. 2000). The USEPA set a maximum contaminant level (MCL) of 10 mg/L nitrate in drinking water to prevent this condition among infants (40 CFR 141.62).

Reproductive effects from long term exposure to low levels of nitrate or nitrite are less well understood. Few studies have examined the relationship between nitrate-nitrite exposure and intra-uterine growth restriction (IUGR) or preterm birth. Bukowski et al. (2001) found a positive dose response relationship between nitrates and both IUGR and preterm birth in a case control study of women on Prince Edward Island, Canada. Most studies of reproductive outcomes have focused on birth defects and congenital anomalies where nitrate exposure has been associated with and increased risk of neural tube defects, oral cleft defects, limb deficiencies (Brender et al. 2013), cardiac defects (Cedergren et al. 2002), anencephaly (Croen et al. 2001), and spontaneous abortions (Manassaram et al. 2006). One proposed mechanism of effect is that nitrates increase endogenous *N*-nitroso compounds which are teratogenic (Croen et al. 2001; Brender et al. 2013). Nitrate is also an endocrine disruptor and has been reported to inhibit thyroid function in animal studies (Guillette Jr. and Edwards 2006). Subclinical hypothyroidism in pregnancy has been associated with PTD and LBW in some studies (Maraka et al. 2016).

The objective of this study was to examine the association between nitrate-nitrite (NN) concentrations in drinking water and selected adverse birth outcomes in a heavily agricultural region of the United States, the state of Ohio, between 2006 and 2008.

B. Methods

1. Study Population

This study used birth certificate data from all births occurring in counties with a population of <300,000 ("rural") in the state of Ohio between 2006 and 2008. This population restriction removed nine counties from the analysis (N=79) and all major urban centers in Ohio. There were 198,749 singleton live births in these rural Ohio counties during this time period. Individual-level, de-identified birth certificate data for children born in Ohio was provided by the Ohio Department of Health (ODH).

2. Birth Outcomes

The primary outcomes of interest in the study were small for gestational age (SGA), term low birth weight (term LBW), very low birth weight (VLBW), preterm birth (PTB), and very preterm birth (VPTB). SGA was defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population (Wilcox 2010). SGA status was calculated using sex- and gestational age-specific national birth weight references developed by Duryea et al. (2014). Term LBW was defined as an infant weighing <2,500g at time of delivery among term infants born ≥37 weeks of gestation. An infant was considered VLBW if it weighed <1,500g at time of delivery, regardless of gestational age. Preterm and very preterm births were defined as infants delivered prior to 37 and 32 weeks of gestation, respectively. Gestational age was based on the reported last normal menstrual period (LMP). If the LMP was unknown or impossible, a clinical estimate of gestation was used. All birth outcomes were either reported directly on or were calculated from variables reported on the birth certificates.

3. Exposure Assessment

The USEPA defines the legal limits of water contaminants and water testing schedules, as mandated in the Safe Drinking Water Act. The MCL for nitrate-nitrite in drinking water is 10 mg/L (40 CFR 141.62). Nitrates are monitored annually in public water systems using groundwater sources, and quarterly for those using surface water sources. If surface water sources are consistently below the MCL, monitoring can be reduced to annually (40 CFR 141.62; Jones et al. 2014a).

We calculated average monthly concentrations of NN in drinking water (mg/L) from a total of 4,065 NN measurements in finished water from 952 community water system (CWS) facilities in Ohio from 2005 through 2008. Water sampling data was provided by the Ohio EPA Safe Drinking Water Information System (SDWIS). An initial CWS-level monthly mean was calculated from all water samples within a CWS in a particular month. County-level monthly means were then calculated from these CWS means, weighted by the population served by each CWS. The resulting county-level monthly mean was used to calculate the average exposure across all months of gestation for each birth. Gestational length in weeks was provided in the ODH birth certificate data, based on a reported or clinically estimates date of conception. Gestational length in months was calculated from this measure and gestational averages of NN exposure were matched to each unique window of gestation based on county, month, and year of birth. For example, an infant born in October of 2006 with a 9 month gestation would receive a mean NN exposure measure that averaged NN exposure in the month of birth and prior 8 months.

The exposure measure assumed that each CWS serves only residents in the county in which the CWS office is located (Jones et al. 2014a). While monthly mean values of NN were

calculated, they were not used in the analyses because between 24 and 26% of counties were missing a monthly estimate due to the infrequent sampling requirements under current regulations. The limits of detection (LODs) varied, but were typically 0.5 μg/L, and these measurements were equated with LOD/2. County-level population percentages of those using private well water were obtained from the Unites States Geological Survey (USGS 2015). The gestational NN measures were linked with birth outcomes by the county, year, and month of birth.

4. Covariates

The covariates examined in this study included infant sex, maternal age at birth, mother's race/ethnicity, maternal educational attainment, marital status, prenatal care status, socioeconomic status, parity, cigarette use, and maternal pre-pregnancy body mass index (BMI). Maternal age was categorized as 10-19, 20-29, 30-39, and ≥ 40 years of age. Maternal race/ethnicity was defined as non-Hispanic white, non-Hispanic black, Hispanic, and other/unknown. Maternal educational attainment was categorized as less than a high school degree, high school degree, some college, and college degree or higher. Marital status was dichotomized as married or not married. The Kotelchuck index was used to define adequate prenatal care, based on the month of entry into prenatal care and total number of prenatal care visits (Kotelchuck 1994). Maternal smoking was dichotomized as smoker versus non-smoker. The cigarette use data was non-specific to the window of time including pregnancy. Whether or not the mother was enrolled in the Women, Infant, and Children (WIC) supplemental nutrition program was used as a proxy for low SES. Maternal pre-pregnancy BMI was categorized according to the National Heart, Lung, and Blood Institute (NHLBI) definitions of underweight,

normal, overweight, and obese (NHLBI 1998). Parity was categorized as having had 0, 1, 2, or ≥ 3 previous live births.

5. Data Analysis

We explored initial bivariate relationships between NN concentrations and each outcome using t-tests. Additionally, we examined initial bivariate relationships between gestational concentrations of NN and each covariate as well as each covariate and each outcome using t-tests for continuous variables, Rao-Scott Chi-Square tests for dichotomous variables, and ANOVA test for covariates with >2 categories. Potential confounders were considered as those variables that were associated with both the exposure measures and outcome measures and were not in the causal pathway. Maternal age, maternal race/ethnicity, and year of birth were included in modeling based on *a priori* knowledge.

We employed generalized estimating equation (GEE) logistic regression models, with an exchangeable working correlation structure and robust standard errors, to estimate the association between NN in drinking water and dichotomous birth outcomes, including SGA, term LBW, VLBW, PTB, and VPTB while accounting for clustering at the county level. Confounding was indicated if the estimate for the exposure variable changed by more than 10% with the addition of the potential confounder into the model. Between 9 and 10% of observations were not used due to missing data on covariates, exposure, or outcome status. All analyses were performed using SAS®, Version 9.4 (SAS Institute Inc., Cary, NC, USA).

The study lacks estimates of NN in private well water, therefore we performed several sensitivity analyses to reduce exposure misclassification by restricting the analyses *a priori* to include only those counties in which <20 (N= 24) and <10 (N=13) of the population used private wells. The mean percentage of private well use in rural Ohio counties was 34% (range 0 – 81%).

Furthermore, 20% of live singleton births in Ohio were missing either the month of entry into PNC or the number of PNC visits attended. In order to better understand the role of this important covariate, we examined the association between NN and each outcome in models that did not include PNC as a covariate, and again including PNC as a covariate, dropping the missing observations.

C. Results

1. Descriptive Statistics

The geometric annual mean gestational NN exposure for births occurring between 2006 and 2008 in Ohio ranged from 0.43 – 0.53 mg/L (Table VII). Approximately 1% of births in this time period were missing gestational NN exposure measures. The maximum mean concentration was 7.7 mg/L in 2006 and 7.5 mg/L in 2007 and 2008.

TABLE VII

SUMMARY OF MEAN GESTATIONAL NITRATE-NITRITE CONCENTRATIONS FOR BIRTHS (N = 198,749) OCCURRING IN RURAL COUNTIES IN OHIO, 2006 − 2008

Year	Geometric Mean ± GSD (mg/L)	Median concentration (mg/L)	Minimum concentration (mg/L)	Maximum concentration (mg/L)	Percent Missing[a] (%)
2006	0.53 ± 3.79	0.69	0.02	7.70	1
2007	0.43 ± 3.70	0.57	0.02	7.50	1
2008	0.45 ± 3.85	0.60	0.05	7.30	1

[a] Percentage of births missing a gestational mean NN concentration.

There were 198,749 live singleton births in rural counties in Ohio between 2006 and 2008, of which 51% were male (Table VIII). The majority of the births were born to mothers who were non-Hispanic white (91%), between 20 and 29 years old (58%), had completed more than a high-school degree (53%), were married (63%), and parous (61%). Only 41% of mothers reported intermediate or adequate prenatal care utilization, but 20% had an unknown or missing level of prenatal care utilization. There was a high prevalence of obesity (31%), WIC use (41%), and reported cigarette use (30%) in this population. Among live singleton births, 9.8% were SGA, 1.0% were very low birth weight, 10.1% were preterm, and 1.5% very preterm. Among singleton term births, 2.3% were term LBW. With the exception of PNC utilization, missing data was low for the covariates examined in this analysis. Less than 1% of birth were missing information on maternal age (N = 1,516), education (N = 984), and smoking status (N = 14). Less than 3% were missing data on WIC status (N = 5,599), pre-pregnancy BMI (N = 5,430), and parity (N = 2,592).

The distribution of outcomes across the covariates examined in this study are consistent with known maternal and child health reproductive risk factors (Table VIII). A slightly higher proportion of female infants were SGA and term LBW compared to male infants. Conversely, a higher proportion of male infants were preterm or very preterm than female infants. Non-Hispanic black mothers had the highest proportion of all adverse outcomes. With the exception of SGA, all adverse outcomes were more likely among the youngest and oldest maternal age categories. All outcomes were inversely related to maternal education. Proportions of all adverse outcomes were higher among mothers who smoked, were enrolled in WIC, and were unmarried. Nulliparous mothers as well as mothers who had ≥3 previous births demonstrated elevated proportions of term LBW, VLBW, preterm, and very preterm births compared to mothers with 1-

2 previous births. SGA was most common among nulliparous women. Women receiving intermediate or adequate prenatal care had the lowest proportion of adverse outcomes, with the exception of SGA. VLBW, preterm, and very preterm births exhibited a U-shaped relationship with maternal BMI, with the highest prevalence among underweight and obese women. Due to missing data on covariates and exposure estimates, approximately 6% of observations were dropped from fully covariate-adjusted models.

2. Regression Analyses

a. SGA

We found no evidence of an association between mean gestational NN exposure and SGA in either crude or fully covariate-adjusted models without well percentage restriction (AOR 1.00, 95% CI 0.98, 1.02) (Table IX). No association between SGA and NN was also evident from models restricting the analysis to those counties with <20 or <10% private well use.

b. Term LBW and VLBW

We observed no association between mean gestational NN exposure in drinking water and term LBW (AOR_A 0.99, 95% CI 0.94, 1.04) among all rural counties in Ohio from 2006 to 2008 (Table IX).

The odds of VLBW were significantly elevated with increasing gestational NN exposure in all models, including those that restricted based on county-level private well use, (AOR 1.07, 95% CI 1.01, 1.14; AOR_{A20} 1.13, 95% CI 1.04, 1.22; AOR_{A10} 1.14, 95% CI 1.05, 1.23; Table IX).

c. PTB and VPTB

Mean gestational NN exposure was significantly associated with PTB in models without private well use restrictions (AOR 1.03, 95% CI 1.00, 1.07; Table X). We found no association

TABLE VIII

DISTRIBUTION OF DEMOGRAPHIC AND ECONOMIC COVARIATES ACROSS THE POPULATION OF LIVE SINGLETON BIRTHS (N=198,749) AND BY OUTCOME IN RURAL OHIO COUNTIES 2006 − 2008

Variable	N (%)	SGA %	Term LBW %	VLBW %	PTB %	VPTB %
Gender						
Male	102,050 (51)	**9.6**	**2.0**	1.0	**10.6**	**1.5**
Female	96,699 (49)	**10.0**	**3.1**	1.0	**9.6**	**1.4**
Race/Ethnicity						
Non-Hispanic white	181,039 (91)	**9.4**	**2.4**	**0.9**	**9.9**	**1.4**
Non-Hispanic black	8,509 (4)	**17.7**	**5.6**	**2.3**	**14.8**	**3.1**
Hispanic	5,961 (3)	**9.8**	**2.3**	**0.8**	**10.5**	**1.4**
Other	3,240 (2)	**13.2**	**3.3**	**0.9**	**10.2**	**1.5**
Maternal Age at Birth						
10 − 19	20,792 (11)	**14.1**	**3.7**	**1.4**	**12.4**	**2.1**
20 − 29	114,657 (58)	**10.4**	**2.6**	**0.9**	**9.7**	**1.4**
30 − 39	58,196 (30)	**7.3**	**1.9**	**0.9**	**9.7**	**1.3**
40+	3,588 (2)	**8.3**	**3.0**	**1.5**	**12.7**	**2.0**
Maternal Education						
High School or Less	93,826 (47)	**12.4**	**3.4**	**1.2**	**11.4**	**1.8**
Some College/Degree	103,939 (53)	**7.5**	**1.8**	**0.8**	**8.8**	**1.1**
Maternal Smoking						
Yes	60,430 (30)	**14.9**	**4.4**	**1.2**	**11.9**	**1.8**
No	138,305 (70)	**7.6**	**1.8**	**0.9**	**9.3**	**1.3**
Prenatal Care						
Inadequate	25,294 (13)	**12.9**	**3.7**	**0.9**	**9.9**	**1.5**
Intermediate/Adequate	81,150 (41)	**9.9**	**1.9**	**0.3**	**3.2**	**0.3**
Adequate Plus	52,009 (26)	**8.4**	**3.0**	**1.3**	**18.9**	**2.3**
Unknown	40,296 (20)	**9.7**	**2.7**	**2.0**	**12.8**	**2.8**
WIC use						
Yes	80,071 (41)	**12.7**	**3.5**	**1.0**	**11.3**	**1.7**
No	113,079 (59)	**7.7**	**1.8**	**0.9**	**9.0**	**1.3**
Prepregnancy BMI						
Underweight	9,152 (5)	**18.2**	**6.4**	**1.4**	**14.0**	**2.0**
Normal	91,822 (48)	**10.4**	**2.6**	**0.8**	**9.8**	**1.3**
Overweight	44,052 (23)	**8.5**	**2.0**	**0.8**	**9.2**	**1.3**
Obese	61,713 (31)	**8.2**	**2.1**	**1.2**	**10.2**	**1.6**
Parity						
0	77,179 (39)	**12.2**	**3.1**	**1.3**	**10.5**	**1.7**
1	63,508 (32)	**8.3**	**2.1**	**0.7**	**9.0**	**1.2**
2	33,498 (17)	**8.5**	**2.2**	**0.7**	**10.1**	**1.3**
≥3	21,972 (11)	**8.2**	**2.3**	**0.7**	**11.2**	**1.5**
Marital Status						
Married	125569 (63)	**7.7**	**1.9**	**0.8**	**8.9**	**1.1**
Unmarried[a]	73,180 (37)	**13.5**	**3.7**	**1.3**	**12.2**	**2.0**

[a] Not married category includes mothers who responded single, widowed, or divorced.

Bold indicates a significant difference in proportions (χ^2 p-value <.05)

between mean gestational NN concentrations and PTB in models restricted to counties with <20 and <10% private well use. We observed a significant 6 − 9% increase in the odds of VPTB in all adjusted models.

TABLE IX

ASSOCIATIONS[a] BETWEEN MEAN GESTATIONAL NITRATE-NITRITE EXPOSURE (MG/L) AND SGA, TERM LBW, AND VLBW IN RURAL[b] OHIO COUNTIES, 2006 − 2008

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[f] (95% CI)	P-value
SGA[c]	Crude		79	186,776	1.01 (0.99, 1.04)	0.413
	Adjusted		79	186,776	1.00 (0.98, 1.02)	0.966
	Crude	<20	24	64,680	1.01 (0.97, 1.06)	0.573
	Adjusted	<20	24	64,680	1.01 (0.98, 1.05)	0.485
	Crude	<10	13	37,268	0.99 (0.97, 1.01)	0.415
	Adjusted	<10	13	37,268	1.00 (0.97, 1.02)	0.846
Term LBW[d]	Crude		79	168,522	0.98 (0.91, 1.05)	0.534
	Adjusted		79	168,522	0.99 (0.94, 1.04)	0.622
	Crude	<20	24	58,105	0.95 (0.81, 1.11)	0.529
	Adjusted	<20	24	58,105	0.98 (0.89, 1.08)	0.651
	Crude	<10	13	33,357	0.90 (0.80, 1.01)	0.064
	Adjusted	<10	13	33,357	0.95 (0.87, 1.04)	0.251
VLBW[e]	Crude		79	186,776	1.08 (1.00, 1.16)	0.039
	Adjusted		79	186,776	1.07 (1.01, 1.14)	0.023
	Crude	<20	24	64,680	1.15 (1.03, 1.27)	0.011
	Adjusted	<20	24	64,680	1.13 (1.04, 1.22)	0.003
	Crude	<10	13	37,268	1.17 (1.03, 1.32)	0.016
	Adjusted	<10	13	37,268	1.14 (1.05, 1.23)	0.001

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, and parity.
[b] Rural defined as counties with <300,000 population.
[c] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population.
[d] Term low birth weight (term LBW) defined as <2500g among term births (≥37 weeks gestation).
[e] Very low birth weight (VLBW) defined as <1500g at time of delivery.
[f] Odds ratios reflect increase in odds per 1 mg/L increase in nitrate-nitrite in drinking water.

TABLE X

ASSOCIATIONS[a] BETWEEN MEAN GESTATIONAL ANNUAL NITRATE-NITRITE EXPOSURE (MG/L) AND PTB AND VPTB IN RURAL[b] OHIO COUNTIES, 2006 − 2008

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[e] (95% CI)	P-value
Preterm birth[c]	Crude		79	186,911	1.04 (1.00, 1.08)	0.043
	Adjusted		79	186,911	1.03 (1.00, 1.07)	0.041
	Crude	<20	24	64,739	1.01 (0.96, 1.06)	0.759
	Adjusted	<20	24	64,739	1.01 (0.96, 1.07)	0.589
	Crude	<10	13	37,301	1.03 (0.98, 1.08)	0.261
	Adjusted	<10	13	37,301	1.04 (0.99, 1.09)	0.154
Very preterm birth[d]	Crude		79	186,911	1.06 (1.02, 1.12)	0.009
	Adjusted		79	186,911	1.06 (1.01, 1.10)	0.013
	Crude	<20	24	64,739	1.08 (1.01, 1.15)	0.022
	Adjusted	<20	24	64,739	1.08 (1.02, 1.14)	0.010
	Crude	<10	13	37,301	1.09 (1.02, 1.17)	0.014
	Adjusted	<10	13	37,301	1.09 (1.03, 1.16)	0.002

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, and parity.
[b] Rural defined as counties with <300,000 population.
[c] Preterm births defined as infants delivered before 37 weeks gestation.
[d] Very preterm births defined as infants delivered before 32 weeks gestation.
[e] Odds ratios reflect increase in odds per 1 mg/L increase in nitrate-nitrite in drinking water.

3. PNC Sensitivity Analyses

We performed additional analyses of the NN-birth outcomes relationships that included a variable for PNC utilization, the Kotelchuck index. Twenty percent of the births in rural counties in Ohio from 2006 to 2008 had an unknown PNC status due to missing data on either the month of entry into PNC or the total number of PNC visits the mother attended. We compared the models discussed above to models that additionally included the Kotelchuck index, dropping those observations with missing data Restricting the models to those with known PNC utilization, we found similar results to those from the initial models (Tables IX and X). We

observed a non-significant inverse relationship between mean gestation NN concentrations and term LBW across all covariate-adjusted models when dropping those observations with unknown PNC data. The odds of VLBW births were elevated with increased NN in models including all counties, those with <20, and those with <10% private well use, but none were statistically significant. We observed no association between mean gestational NN concentrations and either PTB or VPTB in models restricted to births with known PNC utilization. Further restrictions by the percent of the county population using private wells did not alter these findings (Table XII).

D. Discussion

This study was aimed at examining the relationship between gestational exposure to NN from drinking water and adverse birth outcomes in one state in the Midwest with intensive agricultural land use practices. Within rural counties, we found no evidence of an association between NN in drinking water, measured as an average over the entire gestational length of pregnancy, and any of the birth outcomes in this population of Ohio births. Over 50% of Ohio counties have a substantial percentage of private well users (30-81%) which introduced considerable exposure misclassification. To overcome this, we restricted analyses to those counties with decreasing percentages of well use. Among those rural counties with <20% and <10% private well use, we observed a significant increase in the odds of VLBW births with increasing mean gestational NN concentrations. This study was restricted to rural counties only and therefore the study findings are limited in their generalizability to more developed, urban areas of the state.

Previous epidemiologic studies of this exposure's effects on the birth outcomes addressed in this study are sparse and have been conflicting, but there is some evidence that NN exposure during pregnancy is associated with IUGR and PTB (Bukowski et al. 2001). Our results do not

TABLE XI

ASSOCIATIONS[A] BETWEEN MEAN ANNUAL NITRATE-NITRITE EXPOSURE (MG/L) AND SGA, TERM LBW, AND VLBW IN RURAL[b] OHIO COUNTIES, 2006 – 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[e] (95% CI)	P-value
SGA[c]	Crude		79	150,928	1.01 (0.99, 1.04)	0.253
	Adjusted		79	150,928	1.00 (0.98, 1.02)	0.652
	Crude	<20	24	50,469	1.02 (0.98, 1.05)	0.356
	Adjusted	<20	24	50,469	1.01 (0.98, 1.03)	0.672
	Crude	<10	13	31,261	1.00 (0.97, 1.02)	0.777
	Adjusted	<10	13	31,261	1.00 (0.97, 1.03)	0.842
Term LBW[d]	Crude		79	137,038	0.99 (0.94, 1.05)	0.762
	Adjusted		79	137,038	0.99 (0.96, 1.03)	0.769
	Crude	<20	24	45,460	0.97 (0.87, 1.08)	0.533
	Adjusted	<20	24	45,460	0.98 (0.91, 1.05)	0.528
	Crude	<10	13	28,095	0.93 (0.85, 1.01)	0.083
	Adjusted	<10	13	28,095	0.96 (0.90, 1.02)	0.212
VLBW[e]	Crude		79	150,928	1.07 (0.98, 1.18)	0.148
	Adjusted		79	150,928	1.02 (0.94, 1.11)	0.593
	Crude	<20	24	50,469	1.13 (0.99, 1.29)	0.064
	Adjusted	<20	24	50,469	1.04 (0.92, 1.18)	0.509
	Crude	<10	13	31,261	1.13 (0.96, 1.33)	0.144
	Adjusted	<10	13	31,261	1.00 (0.86, 1.16)	0.972

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, parity, and PNC utilization.
[b] Rural defined as counties with <300,000 population.
[c] Small for gestational age (SGA) defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population.
[d] Term low birth weight (term LBW) defined as <2500g among term births (≥37 weeks gestation).
[e] Very low birth weight (VLBW) defined as <1500g at time of delivery.
[f] Odds ratios reflect increase in odds per 1 mg/L increase in nitrate-nitrite in drinking water.

TABLE XII

ASSOCIATIONS[a] BETWEEN MEAN ANNUAL NITRATE-NITRITE EXPOSURE (MG/L) AND PTB AND VPTB IN RURAL[b] OHIO COUNTIES, 2006 – 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	Well Restriction (%)	N (Counties)	N (Births)	OR[e] (95% CI)	P-value
Preterm birth[c]	Crude		79	151,017	1.03 (0.99, 1.07)	0.192
	Adjusted		79	151,017	0.99 (0.95, 1.03)	0.669
	Crude	<20	24	50,504	1.00 (0.95, 1.06)	0.911
	Adjusted	<20	24	50,504	0.99 (0.93, 1.05)	0.800
	Crude	<10	13	31,280	1.02 (0.97, 1.07)	0.515
	Adjusted	<10	13	31,280	1.01 (0.96, 1.07)	0.658
Very preterm birth[d]	Crude		79	151,017	1.05 (0.99, 1.11)	0.104
	Adjusted		79	151,017	1.00 (0.94, 1.06)	0.917
	Crude	<20	24	50,504	1.05 (0.98, 1.12)	0.204
	Adjusted	<20	24	50,504	1.00 (0.92, 1.08)	0.943
	Crude	<10	13	31,280	1.04 (0.95, 1.14)	0.423
	Adjusted	<10	13	31,280	0.98 (0.87, 1.10)	0.695

[a] Models controlled for year, infant sex, maternal race/ethnicity, maternal age, maternal education, marital status, cigarette use during pregnancy, pre-pregnancy BMI, WIC use, PNC utilization, and parity.
[b] Rural defined as counties with <300,000 population.
[c] Preterm births defined as infants delivered before 37 weeks gestation.
[d] Very preterm births defined as infants delivered before 32 weeks gestation.
[e] Odds ratios reflect increase in odds per 1 mg/L increase in nitrate-nitrite in drinking water.

confirm these findings, but are somewhat consistent with those of Blake (2014) which observed no association between nitrate contamination and LBW births in California. To our knowledge, this study is the first to report an association between NN exposure via drinking water and VLBW births. The mechanism by which NN exposure during gestation would affect birth weight remains unclear, but may be due to direct teratogenic effects of increased circulating *N*-nitroso compounds as a result of increased nitrate-nitrite intake (Brender et al. 2013).

Our failure to observe an association between NN exposure and SGA, term LBW, PTB, or VPTB may be the result of the study limitations, particularly with regard to the exposure

ascertainment. While meeting the regulatory requirements for NN testing, the counties in this study had relatively infrequent NN measurements from 2006 to 2008. The infrequent monitoring schedules mandated under the SWDA mean that not all CWSs in each county contributed to the county-month exposure measure in all months, and some county-month exposure measures were missing. These limitations were exacerbated in more refined time scales (i.e., trimester-specific exposure measures), which prompted use of gestational mean NN exposure measure. The exposure measure was aggregated from the CWS to the county level because we were unable to link individual births to the CWS that served as their mother's primary drinking water source during pregnancy, which introduced some exposure misclassification at the individual level. This aggregation of exposure reduced the variability in the exposure estimates, as infants' gestational mean was calculated from county-level monthly estimates, which did not vary across the county.

Furthermore, public drinking water data was used to calculate the exposure measures, thereby introducing exposure misclassification in those counties in which a large percentage of the population was using private well water. Nitrate levels are estimated to be higher in wells, particularly shallow wells in agricultural areas than in groundwater sources supplying public drinking water (Dubrovsky et al. 2010). The USEPA does not collect NN levels in private wells, and therefore our restrictions based on county-level estimates of private well use likely did not reduce exposure misclassification entirely. Furthermore, we had no information on drinking water consumption habits for this study population.

We used birth certificate data to ascertain all outcomes examined in this study, however, the reliability of birth certificate data varies widely by data element. Demographic variables on the birth certificate about the mother, including maternal age, race/ethnicity, and marital status are highly accurate (Querec, 1980; Schoendorf et al. 1993; Reichman and Hade, 2001;

DiGiuseppe et al. 2002; Zollinger et al. 2006). There is also high agreement between birth certificates and medical records for variables including infant gender, birth weight, plurality, number of previous live births, and prenatal care received (Querec, 1980; Schoendorf et al. 1993; Green et al. 1998; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp, 2006; Zollinger et al. 2006). Gestational age, parental education, paternal demographics show moderate agreement, but maternal weight gain during pregnancy, maternal medical risk factors (e.g., chronic hypertension, previous LBW or preterm birth), tobacco and alcohol use, and number of prenatal care visits have very low reliability (Querec, 1980; Dobie et al. 1998; Reichman and Hade, 2001; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp, 2006; Zollinger et al. 2006).

We lacked information on whether or not the mothers of the infants in these analyses had moved at any point during their pregnancy. Rates of pregnancy mobility are estimated between 12-32% (Fell et al. 2004; Canfield et al. 2006; Miller et al. 2010; Zender et al. 2010), and vary by geography and demographic factors. We were unable to account for those women who moved during pregnancy and assumed that the residence listed on the birth certificate was the residence throughout the entire pregnancy.

A further limitation of this analysis of NN and birth outcomes is the lack of information on dietary sources of nitrates. Unlike arsenic, for which drinking water is the primary route of human exposure, nitrate in drinking water generally comprises <14% of total nitrate intake (WHO, 2011a; WHO, 2011b; Brender et al. 2013).

National, as well as Ohio-specific, ground and surface water monitoring data collected by the USGS indicate that nitrate levels fluctuate seasonally and increase in areas of intense agricultural land use (Rowe et al. 2004, Dubrovsky et al. 2010). In the SDWIS data used in this

study, however, we observed no seasonal trends in NN concentrations and found no correlation between NN levels in public drinking water and the percentage of county land used for crops (rho = -0.03, P = 0.80). This may be a result of water treatment methods effectively removing NN from finished drinking water or it may reflect the failure to sample when NN levels are highest in drinking water sources. None of the county-level monthly mean estimates of NN exposure from 2006 to 2008 from these rural counties exceeded the MCL of 10 mg/L in drinking water. Our results indicate that NN levels below this threshold do not increase the odds of SGA, term LBW, PTB, or VPTB. Our results suggest that NN exposure may increase the risk of VLBW births even at levels below the current MCL.

E. Conclusions

We found that gestational exposure to NN in drinking water increased the odds of VLBW within those rural Ohio counties where private well use is not very prevalent (<20%). We did not observe significant evidence of an association between gestational NN exposure and SGA, term LBW, PTB, or VPTB. While this study benefited from the inclusion of a large number of births, it was limited by the ecologic exposure assessment. Exposure was tailored to each infant's gestational window, which is a novel exposure metric in the epidemiologic study of NN in drinking water and adverse birth outcomes. While the results of this study are largely negative, further research is needed to fully explore the relationship between NN in drinking water and adverse birth outcomes, ideally with use of individual biomarkers of exposure during pregnancy.

V. ATRAZINE CONTAMINATION OF DRINKING WATER AND ADVERSE BIRTH OUCOMES IN COMMUNITY WATER SYSTEMS ENROLLED IN THE ATRAZINE MONITORING PROGRAM IN OHIO

A. Introduction

Atrazine is the second most widely used herbicide in the United States, primarily applied to corn and sorghum crops (USEPA 2015). Much of the concern about atrazine arises from its persistence in soil and its transport to surface and groundwater drinking water sources (Jayachandran et al. 1994). Atrazine is the most commonly detected pesticide in surface water sources in the United States and is frequently detected in groundwater sources as well (Gilliom et al. 2006; USEPA 2007).

Atrazine and its metabolites (DEA, DIA, and DACT) share a common toxic effect and mechanism with other triazine herbicides (simazine and propazine), and are suspected of being endocrine disruptors (USEPA 2007; Quignot et al. 2012; Vandenburg et al. 2012). There is limited epidemiologic evidence of an effect of prenatal exposure to atrazine on adverse birth outcomes. Munger et al. (1997) reported that Iowa counties in which the public water supply had elevated levels of atrazine, metolachlor, and cyanzine had nearly twice the odds of small for gestational age (SGA) births compared to non-contaminated adjacent counties. Ochoa-Acuña et al. (2009) reported that exposure to atrazine in drinking water from community water systems (CWS) during the third trimester was associated with a 17-19% increase in the prevalence of SGA births in Indiana, but not with preterm births in a retrospective cohort study. Rinsky et al. (2012) observed significantly increased odds of preterm births in Kentucky counties with the highest ($\geq$0.1051 μg/L) versus the lowest atrazine levels ($\leq$0.0405 μg/L) in drinking water between 2004 and 2006. However, Villanueva et al. (2005) failed to find an association between atrazine in water and low birth weight (LBW) or SGA births in a study in an agricultural region

of Brittany, France. In another study in the same area of France, Chevrier et al. (2011) reported that atrazine biomarkers in maternal urine were associated with lower birth weight, length, and head circumference. With the exception of the one prospective cohort study in France (Chevrier et al. 2011), all previous epidemiological studies of atrazine and birth outcomes have relied on ecologic exposure estimates obtained retrospectively through environmental monitoring data.

The objective of this study was to examine the association between atrazine concentrations in drinking water and selected adverse birth outcomes among those communities receiving drinking water from community water systems (CWSs) that were part of USEPA's Atrazine Monitoring Program (AMP) between 2006 and 2008 in the state of Ohio.

B. Methods

1. Study Population

This study used birth certificate data from all births occurring within the 22 Ohio communities receiving drinking water from a CWS in the USEPA's AMP between 2006 and 2008. There were a total of 14,445 births in these cities which comprised 3.4% of births state-wide (N=428,804) during this time period. Individual-level, de-identified birth certificate data for children born in Ohio was provided by the Ohio Department of Health (ODH).

2. Birth Outcomes

The birth outcomes of interest in this research were small for gestational age (SGA), term low birth weight (term LBW), very low birth weight (VLBW), preterm birth (PTB), and very preterm birth (VPTB). SGA was defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population (Wilcox 2010). SGA status was calculated using sex- and gestational age-specific national birth weight references developed by Duryea et al. (2014). Term LBW was defined as an infant weighing <2,500g at time of delivery among term

infants (≥37 weeks gestation). An infant was considered VLBW if it weighed <1,500g at time of delivery, regardless of gestational age. PTB and VPTB were defined as infants delivered prior to 37 and 32 weeks gestation, respectively. Gestational age was based on the reported last normal menstrual period (LMP). If the LMP was unknown or implausible, a clinical estimate of gestation was used. All birth outcomes were either reported directly on or were calculated from variables reported on the birth certificates.

3. Exposure Assessment

The USEPA defines the legal limits for water contaminants and water testing schedules, as mandated in the Safe Drinking Water Act (SDWA). The Maximum Contaminant Level (MCL) for atrazine in drinking water is 3 µg/L (40 CFR 141.61). Public water systems are required to test for atrazine quarterly, unless atrazine concentrations are consistently below the MCL, at which point testing can be reduced to once every three years. Those water systems that have atrazine or total combined triazine (TCT) measurements exceeding 2.6 µg/L in finished water, or 12.5 µg/L in raw water, over a 90-day average are inducted into the AMP for 5 years. CWS in the AMP are required to measure atrazine, its metabolites, as well as total combined triazines weekly during the season of peak atrazine use and biweekly throughout the remainder of the year (USEPA 2006).

Drinking water measurements of atrazine in finished water from 2005 through 2008 were obtained from the USEPA's AMP public data portal (https://www.epa.gov/ingredients-used-pesticide-products/atrazine-monitoring-program-data-and-results) for all 22 AMP water systems in Ohio. Each of these water systems were enrolled in the AMP for all years of the study. We made the assumption that the service boundaries of each CWS in the AMP corresponded to the city limits in which the water system was located. To verify this assumption, we attempted to

contact an employee at each AMP water system in Ohio. We successfully reached personnel at 70% of water systems included in this study, and our assumption regarding city and water system boundaries was verified by personnel at 10 of the 15 water systems where contact was made (Ohio Atrazine Monitoring Program Community Water Systems, 2015, personal communications). Personnel at the remaining five water systems were not able to provide this information. Monthly mean estimates of atrazine in each AMP water system were calculated from the weekly and biweekly samples in the AMP data. Using the mean monthly estimates, we calculated the mean atrazine concentrations for the entire gestational period of the pregnancy ("gestational atrazine") as well as for each trimester of pregnancy. Surface water was the source for all water systems included in this analysis. Atrazine exposure measures were linked with birth records by the city code of the mother's residence, which is provided on the birth certificate, as well as the year and month of birth of the infant.

4. Covariates

The covariates examined in this study included infant sex, maternal age at birth, mother's race/ethnicity, maternal educational attainment, marital status, prenatal care status, socioeconomic status, parity, cigarette use, and maternal pre-pregnancy body mass index (BMI). Maternal age was categorized as 10-19, 20-29, 30-39, and ≥ 40 years of age. Maternal race/ethnicity was defined as non-Hispanic white, non-Hispanic black, Hispanic, and other/unknown. Maternal educational attainment was categorized as less than a high school degree, high school degree, some college, and college degree or higher. Marital status was dichotomized as married or not married. The Kotelchuck index was used to define adequate prenatal care, based on the month of entry into prenatal care and total number of prenatal care visits (Kotelchuck 1994). Maternal smoking was dichotomized as smoker versus non-smoker.

The cigarette use data was non-specific to the window of time including pregnancy. Whether or not the mother was enrolled in the Women, Infant, and Children (WIC) supplemental nutrition program was used as a proxy for low SES. Maternal pre-pregnancy BMI was categorized according to the CDC definitions of underweight, normal, overweight, and obese (CDC 2015). Parity was categorized as having had 0, 1, 2, or ≥ 3 previous live births.

5. Data Analysis

We explored initial bivariate relationships between atrazine concentrations and each outcome using t-tests. Additionally, we examined initial bivariate relationships between gestational concentrations of atrazine with each covariate as well as each covariate and each outcome using t-tests for continuous variables, Rao-Scott Chi-Square tests for dichotomous variables, and ANOVA test for covariates with >2 categories. Potential confounders were considered as those variables that were associated with both the exposure measures and outcome measures and were not in the causal pathway.

We developed generalized estimating equation (GEE) logistic regression models, with an exchangeable working correlation structure and robust standard errors, to estimate the association between atrazine in drinking water and each birth outcome − SGA, term LBW, VLBW, PTB, and VPTB − while accounting for clustering at the city level. Models of continuous and categorical (tertiles) atrazine exposure were tested. Maternal age, maternal race/ethnicity, and year of birth were included in all adjusted models based on *a priori* knowledge. We assessed confounding throughout the model building process and in an effort to maximize parsimony, we retained only those variables that had a substantial (>10%) effect on the estimate of the effect of atrazine in the models. Final adjusted models were built using the gestational atrazine exposure measure. The covariates identified as confounders in these models

were applied to models of trimester-specific atrazine exposure. Between 0.5 and 3.5% of observations were not used due to missing data on covariates, exposure, or outcome status. All analyses were performed using SAS®, Version 9.4 (SAS Institute Inc., Cary, NC, USA).

We performed a sensitivity analysis to further reduce exposure misclassification by restricting the analysis to only those water systems where we had confirmation from on-site representatives that the service boundaries of the water system corresponded to the city limits in which it was located and that >95% of the population was likely to be receiving their public water from the AMP water system in question (N = 10 water systems). Additionally, we restricted the data set to those with a gestational atrazine concentration ≤3 µg/L to evaluate the relationship between atrazine in drinking water and selected birth outcomes when exposure is below permissible levels in public drinking water.

Among births in the AMP communities, 19% were missing a PNC utilization score due to missing data on the month of entry into PNC or the number of PNC visits attended. In order to better understand the role of this important covariate, we examined the association between atrazine and each outcome in models that did not include PNC as a covariate, and again including PNC as a covariate, dropping the missing observations.

C. Results

1. Descriptive Statistics

Monthly mean atrazine concentrations in Ohio's AMP water systems ranged from 0 to 15.7 µg/L between 2006 and 2008 (Table XIII). Atrazine measurements followed a sharp seasonal pattern, peaking in the months of May and June (Figure 1). Across all years, monthly mean concentrations were missing in 2.3% of AMP water systems, with annual variation in the missing pattern between 0.5 and 5%. Overall, less than 1% of births were missing an estimate of

atrazine exposure during their entire gestation or during any of their trimester estimates of exposure.

TABLE XIII

SUMMARY OF MONTHLY MEAN ATRAZINE CONCENTRATIONS REPORTED IN FINISHED DRINKING WATER BY AMP COMMUNITY WATER SYSTEMS (N = 22) IN OHIO BETWEEN 2006 AND 2008

Year	Geometric Mean ± GSD (µg/L)	Median concentration (µg/L)	Minimum concentration (µg/L)	Maximum concentration (µg/L)	Percent Missing[a] (%)
2006	0.29 ± 3.04	0.17	0.00	7.22	1.5
2007	0.15 ± 3.43	0.16	0.03	4.23	0.4
2008	0.16 ± 4.35	0.17	0.03	15.66	4.9

[a] Percentage of community water systems missing monthly mean atrazine concentration.

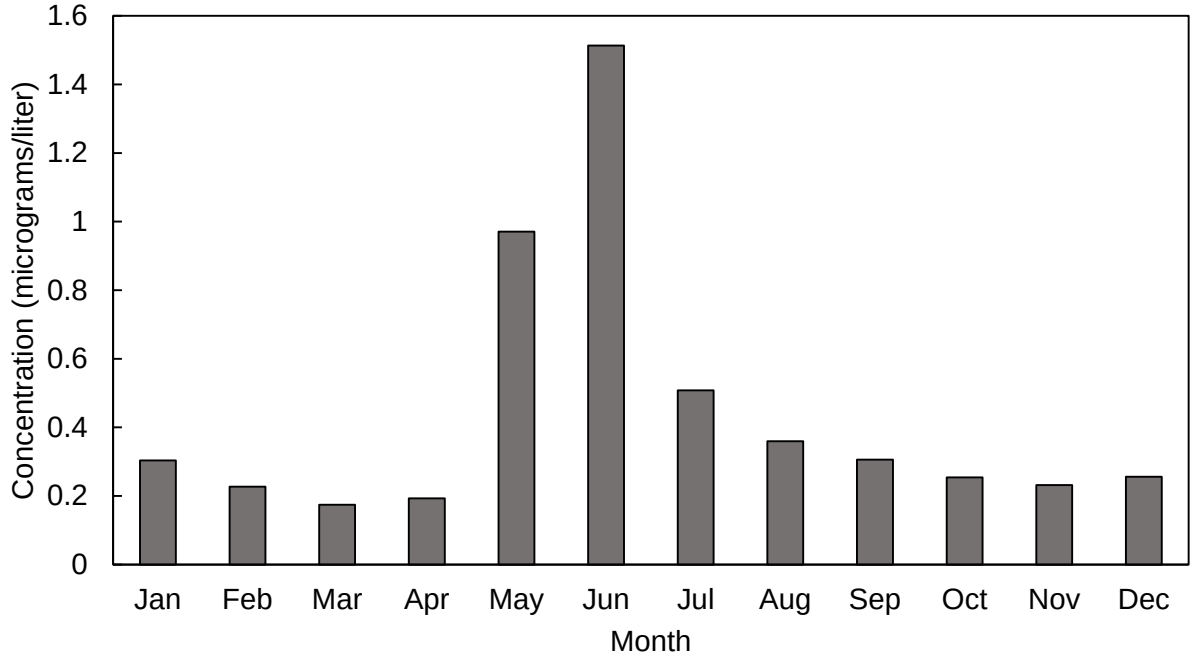

Figure 1. Seasonal variations in mean monthly atrazine concentration (µg/L) in finished water samples from 22 AMP water systems in Ohio, 2006 – 2008.

There were 14,445 live singleton births within the 22 cities which received their public drinking water supply from AMP water systems in Ohio between 2006 and 2008, of which 51% were males (Table XIV). The majority of these births were born to mothers who were non-Hispanic white (86%), between 20 and 34 years old (81%), were married (54%), and parous (59%). Half of the births during this time period were born to mothers with a high school degree or less. Only 41% of mothers reported intermediate or adequate prenatal care, but 19% had an unknown level of prenatal care. The proportion of infants born to mothers enrolled in the WIC program was higher than for the state as a whole (50% versus 42%) as was the proportion of infants born to mothers who reported smoking (35% versus 26%). There was a high prevalence of pre-pregnancy obesity (25%) among the mothers of the infants in this population. Among live singleton births, 10.3% were SGA, 1.1% were very low birth weight, 9.9% were preterm, and 1.6% very preterm. Among singleton term births, 2.4% were term LBW. Between <1% and 3% of observations were dropped in fully covariate-adjusted models due to missing data on either outcomes, covariates, or exposure estimates.

2. <u>Regression Analyses</u>

We found weak and statistically non-significant (p=0.21) evidence of a positive association between gestational averages of atrazine and SGA in either crude or fully covariate-adjust models (AOR 1.06, 95% CI 0.96, 1.17) (Table XV). In our examination of trimester-specific exposure windows, we similarly observed only a weak association between average atrazine exposure in the first trimesters and SGA (Tables XVII).

Mean gestational atrazine exposure was associated with significantly increased odds of term LBW birth in both crude and adjusted models (Table XV; AOR 1.27, 95% CI 1.10, 1.45). In our models of trimester-specific exposure windows, we observed a significant increase in odds

TABLE XIV

DISTRIBUTION OF DEMOGRAPHIC AND ECONOMIC COVARIATES ACROSS THE POPULATION OF LIVE SINGLETON BIRTHS (N=14,445) AND PREVALENCE OF OUTCOMES BY COVARIATES IN AMP COMMUNITIES IN OHIO 2006 − 2008

Variable	N (%)	SGA %	Term LBW %	VLBW %	PTB %	VPTB %
Gender						
Male	7,431 (51)	9.9	**1.9**	1.1	**10.5**	1.7
Female	7,014 (49)	10.6	**3.4**	1.1	**9.3**	1.6
Race/Ethnicity						
Non-Hispanic white	12,471 (86)	**9.7**	**2.4**	**1.0**	**9.5**	**1.5**
Non-Hispanic black	1,068 (7)	**17.8**	**6.2**	**2.4**	**13.9**	**2.9**
Hispanic	689 (5)	**8.9**	**1.8**	**1.5**	**10.3**	**2.0**
Other	217 (2)	**9.7**	**3.2**	**1.4**	**13.4**	**2.3**
Maternal Age at Birth						
<20	1811 (13)	**15.4**	**4.0**	**1.7**	**13.2**	**2.7**
20 − 34	11710 (81)	**9.7**	**2.5**	**1.0**	**9.4**	**1.4**
35+	923 (6)	**7.8**	**2.7**	**1.5**	**10.1**	**2.1**
Maternal Education						
High School or less	7203 (50)	**12.8**	**3.1**	**1.4**	**11.4**	**2.0**
Some College/Degree	7203 (50)	**7.7**	**2.2**	**0.8**	**8.4**	**1.3**
Maternal Smoking						
Yes	4,995 (35)	**7.9**	**3.9**	**1.4**	**11.5**	**2.1**
No	9,449 (65)	**14.7**	**2.0**	**0.9**	**9.1**	**1.4**
Prenatal Care						
Inadequate	1,861 (13)	**13.2**	**3.8**	**1.0**	**11.2**	**1.9**
Intermediate/Adequate	5,970 (41)	**9.7**	**1.6**	**0.3**	**3.0**	**0.4**
Adequate Plus	3,902 (27)	**9.6**	**3.8**	**1.4**	**17.5**	**2.2**
Unknown	2,712 (19)	**10.6**	**3.0**	**2.5**	**13.3**	**3.3**
WIC use						
Yes	7,064 (50)	**12.6**	**3.3**	1.2	**9.1**	1.8
No	7,108 (50)	**7.9**	**2.0**	1.0	**10.6**	1.4
Prepregnancy BMI						
Underweight	673 (8)	**17.8**	**6.8**	1.2	11.6	1.5
Normal	6,664 (47)	**11.2**	**2.8**	1.1	9.9	1.7
Overweight	3,170 (22)	**8.8**	**2.0**	1.0	9.2	1.4
Obese	3,781 (26)	**8.6**	**2.4**	1.2	10.2	1.6
Parity						
0	5,892 (41)	**12.0**	**3.2**	1.3	**10.0**	1.8
1	4,515 (32)	**8.3**	**2.3**	0.9	**9.0**	1.4
2	2,334 (16)	**9.3**	**2.0**	1.0	**9.6**	1.5
≥3	1,483 (10)	**11.2**	**2.9**	0.7	**12.8**	1.7
Marital Status						
Married	7,765 (54)	**7.1**	**1.8**	**0.8**	**8.5**	**1.0**
Unmarried[a]	6,680 (46)	**13.9**	**3.8**	**1.5**	**11.6**	**2.3**

[a] Unmarried category includes mothers who responded single, widowed, or divorced.

Bold indicates a significant difference in proportions (χ^2 p-value <.05)

of term LBW birth with increasing atrazine exposure during the first and second trimesters, but not in the third trimester (Tables XVII − XIX; Figure 2). In categorical analyses, we observed a significant increase in odds of term LBW among those in the highest tertile of mean gestational atrazine exposure compared to those in the lowest (Table XVI), although odds increased across each tertile of exposure (test for trend $p = 0.0007$).

We observed no evidence of an association between gestational or trimester averages of atrazine exposure with odds of VLBW in this population (Tables XV, XVII – XVIII). Third trimester models were not performed for the outcome VLBW because only two of the VLBW births in this population were delivered at full term.

We did not observe evidence of an association between PTB and either gestational or trimester-specific mean atrazine exposures among births in AMP communities between 2006 and 2008 in Ohio. Similarly, we found no association between atrazine exposure, either gestational or trimester-specific, and VPTB in this population (Tables XV, XVII – XVIII).

3. Sensitivity Analyses

We restricted the mean gestational and trimester-specific atrazine exposure models to include only those water systems where an on-site representative confirmed that the service boundaries corresponded to the city boundaries. There were 4,488 births within these 10 AMP water systems. We observed no association between gestational atrazine and SGA in either crude or adjusted models (Table XX). We observed elevated odds of term LBW per 1µg/L increase in atrazine in this subgroup (AOR 1.16, 95% CI 0.77, 1.74), but the association was not significant. We also observed a significant inverse relationship between atrazine and PTB. Covariate-adjusted models did not converge for VPT or VLBW, but no association between atrazine and either outcome was seen in crude models.

TABLE XV

ASSOCIATIONS[a] BETWEEN ATRAZINE CONCENTRATIONS IN DRINKING WATER AND SGA, TERM LBW, VLBW, PTB, AND VPTB IN AMP COMMUNITIES (N = 22) IN OHIO, 2006 − 2008

Outcome	Model	N	OR[g] (95% CI)	P-value
SGA[b]	Crude	13942	0.99 (0.88, 1.12)	0.915
	Adjusted	13942	1.06 (0.96, 1.17)	0.212
Term LBW[c]	Crude	12567	1.15 (1.01, 1.31)	0.030
	Adjusted	12567	1.27 (1.10, 1.45)	0.001
VLWB[d]	Crude	14089	0.90 (0.50, 1.60)	0.710
	Adjusted	14089	0.81 (0.47, 1.39)	0.444
PTB[e]	Crude	14098	1.01 (0.89, 1.14)	0.917
	Adjusted	14098	0.99 (0.88, 1.11)	0.831
VPTB[f]	Crude	14349	1.15 (0.86, 1.55)	0.346
	Adjusted	14349	1.11 (0.81, 1.51)	0.527

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, maternal pre-pregnancy BMI.
[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, marital status, and parity.
[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, and parity.
[f] Very preterm births defined as infants delivered before 32 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal marital status.
[g] Odds ratios reflect increase in odds per 1 µg/L increase in atrazine in drinking water.

TABLE XVI

ASSOCIATION[a] BETWEEN TERM LOW BIRTH WEIGHT BIRTHS AND TERTILES OF GESTATIONAL ATRAZINE EXPOSURE AMONG LIVE SINGLETON BIRTHS IN AMP COMMUNITIES (N = 22) IN OHIO, 2006 − 2008

Tertile	Exposure Range (µg/L)	OR[a] (95% CI)
1	0 − 0.1537	Ref.
2	0.1538 − 0.4622	1.11 (0.92, 1.34)
3	0.4623 − 5.9337	1.26 (1.11, 1.44)
		p for trend = 0.0007

[a] Model adjusted for maternal race/ethnicity, maternal age, birth year, infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.

TABLE XVII

ASSOCIATIONS[a] BETWEEN ATRAZINE CONCENTRATIONS IN DRINKING WATER DURING THE FIRST TRIMESTER OF PREGNANCY AND SELECTED BIRTH OUTCOMES: SGA, TERM LBW, VLBW, PTB, AND VPTB IN AMP COMMUNITIES (N = 22) IN OHIO, 2006 − 2008

Outcome	Model	N	OR[g] (95%CI)	P-value
SGA[b]	Crude	14,022	1.02 (0.95, 1.09)	0.557
	Adjusted	14,022	1.04 (0.98, 1.11)	0.186
Term LBW[c]	Crude	12,647	1.14 (1.01, 1.28)	0.029
	Adjusted	12,647	1.20 (1.08, 1.34)	0.001
VLWB[d]	Crude	14,170	1.09 (0.86, 1.37)	0.470
	Adjusted	14,170	1.07 (0.86, 1.34)	0.556
PTB[e]	Crude	14,179	1.01 (0.90, 1.13)	0.932
	Adjusted	14,179	0.99 (0.90, 1.10)	0.903
VPTB[f]	Crude	14,432	1.11 (0.81, 1.53)	0.502
	Adjusted	14,432	1.11 (0.81, 1.53)	0.512

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.

[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, and maternal pre-pregnancy BMI.

[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.

[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, and marital status.

[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, and parity.

[f] Very preterm births defined as infants delivered before 32 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal marital status.

[g] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

TABLE XVIII

ASSOCIATIONS[a] BETWEEN ATRAZINE CONCENTRATIONS IN DRINKING WATER DURING THE SECOND TRIMESTER OF PREGNANCY AND SELECTED BIRTH OUTCOMES: SGA, TERM LBW, VLBW, PTB, AND VPTB IN AMP COMMUNITIES (N = 22) IN OHIO, 2006 − 2008

Outcome	Model	N	OR[f] (95%CI)	P-value
SGA[b]	Crude	14,002	0.97 (0.93, 1.00)	0.048
	Adjusted	14,002	0.99 (0.96, 1.02)	0.478
Term LBW[c]	Crude	12,647	1.06 (0.98, 1.14)	0.119
	Adjusted	12,647	1.13 (1.07, 1.20)	<.0001
VLWB[d]	Crude	14,148	0.79 (0.55, 1.14)	0.21
	Adjusted	14,148	0.76 (0.51, 1.13)	0.175
PTB[e]	Crude	14,156	0.99 (0.95, 1.04)	0.791
	Adjusted	14,156	0.99 (0.95, 1.04)	0.749

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, and maternal pre-pregnancy BMI.
[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, and marital status.
[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, and parity.
[f] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

TABLE XIX

ASSOCIATIONS[a] BETWEEN ATRAZINE CONCENTRATIONS IN DRINKING WATER DURING THE THIRD TRIMESTER OF PREGNANCY AND SELECTED BIRTH OUTCOMES: SGA, TERM LBW, VLBW, PTB, AND VPTB IN AMP COMMUNITIES (N = 22) IN OHIO, 2006 − 2008

Outcome	Model	N	OR[d] (95%CI)	P-value
SGA[b]	Crude	12,648	0.98 (0.87, 1.10)	0.716
	Adjusted	12,648	1.00 (0.93, 1.08)	0.952
Term LBW[c]	Crude	12,647	0.97 (0.80, 1.16)	0.709
	Adjusted	12,647	1.03 (0.87, 1.22)	0.723

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, and maternal pre-pregnancy BMI.
[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

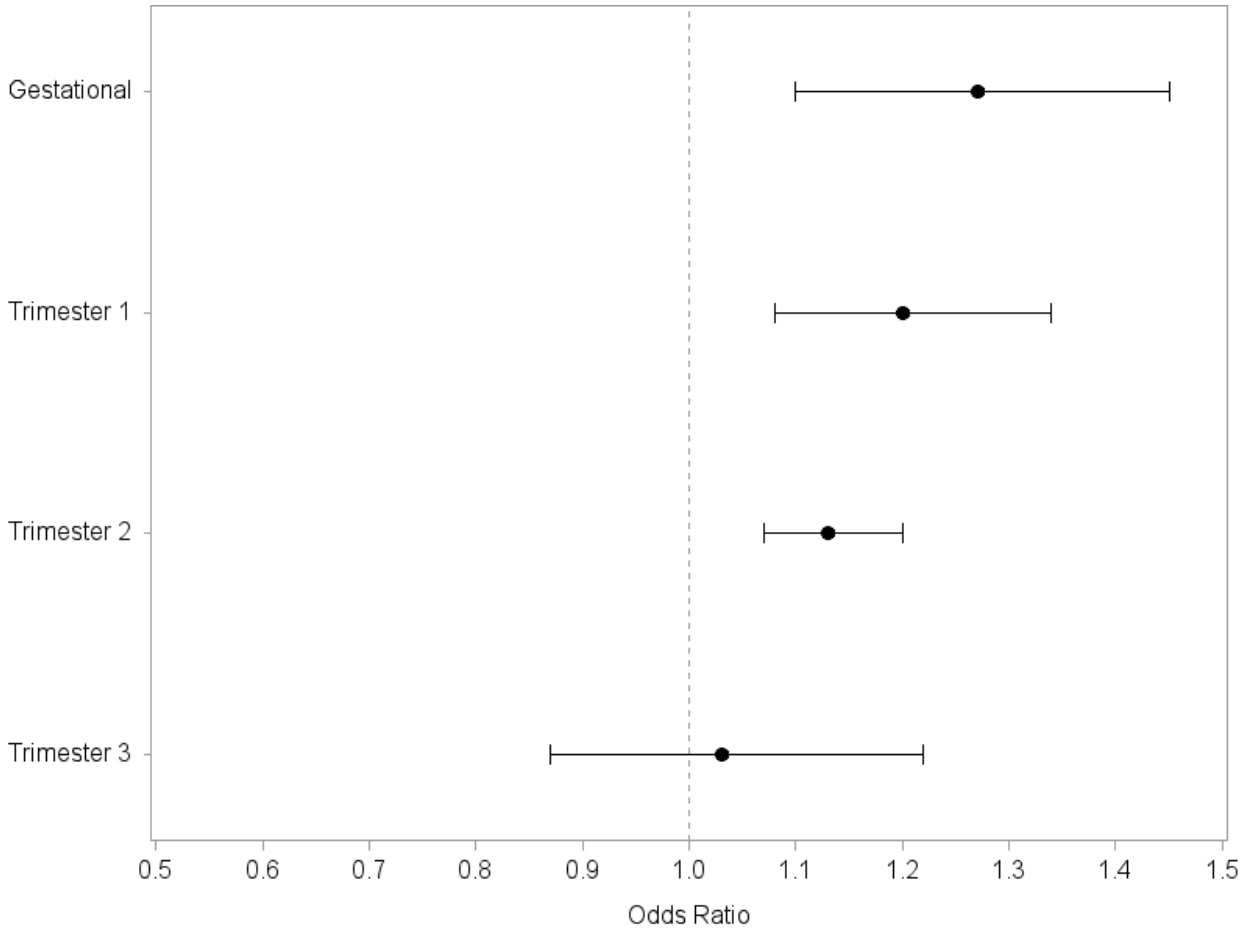

Figure 2. Association between gestational and trimester mean estimates of atrazine exposure in drinking water and term LBW in communities served by water systems enrolled in USEPA's AMP (N = 22) in Ohio, 2006 – 2008.

TABLE XX

ASSOCIATIONS[a] BETWEEN MEAN GESTATIONAL ATRAZINE CONCENTRATIONS IN DRINKING WATER AND SGA, TERM LBW, VLBW, PTB, and VPTB AMONG AMP COMMUNITIES WITH VERIFIED SERVICE BOUNDARIES (N = 10) IN OHIO, 2006 − 2008

Outcome	Model	N	OR[g] (95%CI)	P-value
SGA[b]	Crude	4,355	0.84 (0.66, 1.06)	0.148
	Adjusted	4,355	1.05 (0.86, 1.28)	0.651
Term LBW[c]	Crude	3,929	1.04 (0.62, 1.72)	0.887
	Adjusted	3,929	1.16 (0.77, 1.74)	0.485
VLWB[d]	Crude	4,435	0.50 (0.20, 1.25)	0.139
	Adjusted	4,435		-
PTB[e]	Crude	4,436	0.85 (0.74, 0.98)	0.029
	Adjusted	4,436	0.86 (0.75, 0.99)	0.038
VPTB[f]	Crude	4,453	0.83 (0.62, 1.12)	0.232
	Adjusted	4,453		-

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.

[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, maternal pre-pregnancy BMI, and prenatal care (PNC) utilization.

[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.

[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, marital status, PNC utilization, and parity.

[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, PNC utilization, and parity.

[f] Very preterm births defined as infants delivered before 32 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal marital status and PNC utilization.

[g] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

Mean exposure to atrazine in the first trimester was significantly associated with an increase in the odds of term LBW in these restricted models (AOR_{T1} 1.17, 95% CI 1.03, 1.34) (Table XXI). Atrazine exposure during the first trimester was inversely associated with the odds of PTB in crude and adjusted models. No association between atrazine exposure in the second or third trimesters and either SGA, term LBW, or PTB was observed in adjusted models. Third trimester atrazine exposure was not assessed for its relationship to PTB.

TABLE XXI

ASSOCIATIONS BETWEEN TRIMESTER-SPECIFIC ATRAZINE CONCENTRATIONS (μG/L) AND SGA, TERM LBW, AND PTB[a] AMONG AMP COMMUNITIES WITH VERIFIED SERVICE BOUNDARIES (N = 10) IN OHIO, 2006 – 2008

Trimester	Model	N (Births)	b	S.E.	P	OR	LCL	UCL
First Trimester								
SGA	Crude	4,375	-0.013	0.072	0.861	0.99	0.86	1.14
	Adjusted	4,375	0.051	0.041	0.212	1.05	0.97	1.14
Term LBW	Crude	3,961	0.119	0.084	0.157	1.13	0.96	1.33
	Adjusted	3,961	0.159	0.067	0.018	1.17	1.03	1.34
Preterm	Crude	4,468	-0.153	0.067	0.022	0.86	0.75	0.98
	Adjusted	4,468	-0.148	0.067	0.027	0.86	0.76	0.98
Second Trimester								
SGA	Crude	4,381	-0.092	0.053	0.084	0.91	0.82	1.01
	Adjusted	4,381	-0.029	0.055	0.601	0.97	0.87	1.08
Term LBW	Crude	3,961	-0.052	0.107	0.630	0.95	0.77	1.17
	Adjusted	3,961	0.009	0.099	0.929	1.01	0.83	1.22
Preterm	Crude	4,461	0.001	0.039	0.988	1.00	0.93	1.08
	Adjusted	4,461	0.031	0.037	0.399	1.03	0.96	1.11
Third Trimester								
SGA	Crude	3691	-0.138	0.039	<.001	0.87	0.81	0.94
	Adjusted	3,691	-0.054	0.045	0.226	0.95	0.87	1.03
Term LBW	Crude	3,961	-0.038	0.190	0.843	0.96	0.66	1.40
	Adjusted	3,961	0.006	0.171	0.972	1.01	0.72	1.41

[a] The number of events of VLBW and VPTB were too sparse for successful model convergence in the sensitivity analysis.

In a separate sensitivity analysis of term LBW, we restricted the data set to include only those term births with a mean gestational atrazine concentration ≤3 μg/L (N = 12,980), the current MCL set by the USEPA. Used as a continuous measure in the models, mean gestational atrazine exposure was associated with a significant increase in the odds of term LBW (AOR 1.33, 95% CI 1.08, 1.64). Odds of term LBW increased across tertiles of mean atrazine exposure ($p = 0.0017$), but only those in the highest tertile of gestational atrazine exposure were at significantly increased odds of term LBW compared to those in the lowest tertile (AOR 1.25, 95% CI 1.10, 1.43).

We performed additional sensitivity models in which we included the Kotelchuck index of PNC utilization despite the high level of missing data (19%). Results from the gestational and trimester atrazine models yielded similar results to those seen in Table XV. We observed no associations between gestational or trimester estimates of atrazine and either SGA, VLBW, PTB, or VPTB. PNC was not a confounder of the term LBW-atrazine relationship, and was not included in the models (Tables XXII – XXV, Appendix A).

D. Discussion

The aim of this study was to examine the relationship between atrazine exposure during pregnancy and selected adverse birth outcomes among communities that have been served by water systems monitored by the USEPA's Atrazine Monitoring Program. Furthermore, this research was aimed at elucidating if there is a window of exposure that is most critical for these birth outcomes. In this analysis of all live singleton births within AMP communities in Ohio between 2006 and 2008, we observed a significant increase in odds of term LBW births with increasing atrazine exposure. This association was observed within models of atrazine exposure averaged over the entire gestation of the pregnancy. Furthermore, our results suggest that

atrazine exposure within the first and second trimesters of pregnancy are most strongly associated with term LBW. We observed no significant evidence of an association between atrazine exposure via drinking water and any of the remaining birth outcomes, including SGA, VLBW, PTB, and VPTB.

This study is the first to show an association between exposure to atrazine in drinking water and term LBW births. Our findings are consistent with those of Chevrier et al. (2011) who found an inverse relationship between atrazine exposure, as measured through urinary biomarkers, and birth weight on a continuous scale; and with those of Ochoa-Acuña et al. (2009) who reported a significant decrease in birthweight among term infants with increasing atrazine concentrations in drinking water averaged over the entire pregnancy. Ochoa-Acuña et al. (2009), however, also observed a significant decrease in birth weight among term infants with increasing atrazine concentrations specifically in the third trimester, which we did not observe in the present study. Our findings are inconsistent with those of Villanueva et al. (2005), who found no association between atrazine exposure and low birth weight in a population of infants in Brittany, France. In this study, however, women were assigned atrazine exposure measures based on the water service district in which they lived, which may have included multiple water systems. Furthermore, the lack of positive findings may have been due to the relatively low levels of atrazine and the narrow range of exposures (<0.029 μg/L in the referent group versus >0.036 in the high exposure group).

While previous studies have shown evidence of an association between atrazine exposure and small for gestational age (Munger et al. 1997; Ochoa-Acuna et al. 2009) and preterm birth (Rinsky et al. 2012), we found no evidence of these associations in our study of singleton births occurring within communities served by AMP water systems in Ohio from 2006 to 2008.

Atrazine and its metabolites disrupt the hypothalamic-pituitary-gonadal axis by inhibiting luteinizing hormone production, increasing aromatase production, and disrupting ovarian function (USEPA 2007; Cooper et al. 1996; Cooper et al. 2000; Zorrilla et al. 2010; Victor-Costa et al. 2010). Aromatase is a key enzyme that promotes bioconversion of androgens into estrogens. Exposure to atrazine has been associated with numerous adverse neuroendocrine and reproductive effects in amphibian and rat models, including reduced testosterone levels (Hayes et al. 2002; Friedmann 2002; Stoker et al. 2002; Jin et al. 2013); altered gonadal development and structure (Hayes et al. 2010; Hayes et al. 2011; Victor-Costa et al. 2010); delayed puberty and decreased number of menstrual cycles in females (Zorilla et al. 2010); and reduced body weight among mature and newly born rats exposed during pregnancy and *in utero* to atrazine (Rayner et al. 2007). Caution is needed however when interpreting results of animal studies for humans.

Previous epidemiologic studies of atrazine and birth outcomes have been limited by ecologic exposure and outcome assessment. In the present study, birth outcomes and covariates were assessed at the individual level from birth certificates, providing more accurate outcome ascertainment and robust confounding control. Atrazine exposure was estimated at the water system level in this study, which offers substantial refinement of exposure classification from the ecologic measurements that combine observations across multiple CWSs used in some of the prior studies (Munger et al. 1997; Villanueva et al. 2005; Rinsky et al. 2012). Furthermore, the sampling frame under the USEPA's Atrazine Monitoring Program is more intensive than the frame for low-risk CWSs, which allows more robust determination of monthly atrazine concentrations and minimizes the number of months missing data in this analysis. Despite the reduction in exposure misclassification by estimating atrazine for each unique water system, we

remain unable to account for personal drinking water behaviors (e.g., use of bottled water or filters), which can substantially influence an individual's exposure.

We made an assumption that the service boundaries of the AMP water systems in this study corresponded to the geographic boundaries of the city in which each was located. For nearly half of these water systems, we received verbal confirmation from treatment plant operators and water system managers that this was in fact the case. We performed a sensitivity analysis by restricting the gestational atrazine models to only these confirmed water systems to attempt to further reduce exposure misclassification. In this sub-group analysis, we saw consistent magnitude and direction of association between atrazine and term low birth weight, but lacked sufficient numbers to detect a significant increase in odds of this rare outcome.

This study had several additional limitations originating from the data sources used. Both the exposure and outcome data for this study were secondary data, collected for purposes other than research. The frequent sampling of finished water under the AMP sampling scheme allowed our study to investigate geographically and temporally refined windows of atrazine exposure which we were unable to assess in the studies of arsenic and nitrate-nitrite in Chapters III and IV. Despite this improvement, we lacked water samples from the individual drinking water sources (e.g., household taps) of the mothers in the study. Our outcome and covariate data originated from birth certificates. The reliability of birth certificate data, however, varies widely by data element. We chose to only use those covariates that are considered to be well-reported and highly accurate on birth certificates, such as maternal age, race/ethnicity, marital status, parity, plurality, infant gender, birth weight, and gestational age (Querec 1980; Schoendorf et al. 1993; Green et al. 1998; Reichman and Hade 2001; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp 2006; Zollinger et al. 2006). We assessed several other important covariates

that have moderate to poor reporting due to their strong associations with the outcomes of interest including maternal cigarette use, number of prenatal visits, and maternal education (Querec 1980; Reichman and Hade 2001; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp 2006; Zollinger et al. 2006).

We lacked information on whether or not the mothers of the infants in these analyses had moved at any point during their pregnancy. Rates of pregnancy mobility are estimated between 12 and 32% (Fell et al. 2004; Canfield et al. 2006; Miller et al. 2010; Zender et al. 2010), and vary by geography and demographic factors. We were unable to account for those women who moved during pregnancy and assumed that the residence listed on the birth certificate was the residence throughout the entire pregnancy.

Our study was restricted to a small percentage of births (3%) in the state of Ohio for this analysis of AMP water systems. The population in these AMP communities differed from the state population in important ways. A much higher percentage of infants were born to mothers who were non-Hispanic white (86%) compared to the state as a whole (76%). Additionally, these AMP communities had a higher proportion of births from women enrolled in WIC (50% versus 42%) and who reported smoking (35% versus 26%). The small sample size relative to the state population and the demographic differences between the AMP communities and the state as a whole limit the generalizability of the study results. Ideally, future research on the association between atrazine in drinking water and adverse birth outcomes would include a representative sample of births to increase the generalizability of study findings.

Despite these limitations, the study had several notable strengths. The exposure estimates used in this study are highly geographically and temporally refined, which allowed specific exposure windows, such as trimesters, to be examined. The large number of births included in

this study allowed the examination of two rare outcome, very preterm birth and very low birth weight, which have not been reported previously. Furthermore, this study also benefited from individual-level data on important covariates with very little missing data.

E. Conclusions

We found a consistent association between atrazine concentrations in drinking water and the odds of term LBW births within communities served by water systems enrolled in USEPA's Atrazine Monitoring Program in Ohio. This is the first study to show such an association for term LBW by linking maternal residence to a specific water system. Water systems are enrolled in the AMP as a result of repeated exceedances of the 3 µg/L MCL for atrazine, but only 4% of samples from the water systems in this study exceeded the MCL. We observed an increase in the odds of term LBW births among those with average gestational atrazine below the current MCL. While further epidemiologic research is needed, these results suggest that the current MCL for atrazine may not be protective against some adverse birth outcomes such as term LBW.

VI. DISCUSSION

A. Summary of Findings

The aims of this research were to examine the relationships between three water contaminants − arsenic, atrazine, and NN − and selected birth outcomes including SGA, term LBW, VLBW, PTB, and VPTB. We assessed these associations using data from the Safe Drinking Water Information System (SDWIS) and Atrazine Monitoring Program (AMP) as well as birth certificate data from the state of Ohio.

The vast majority of previous epidemiologic research on arsenic in drinking water arises from geographic locations in which arsenic levels in drinking water sources are quite high, such as Bangladesh, West Bengal, China, and Argentina. Our research was able to evaluate the effects of arsenic in drinking water on birth outcomes within a relatively low exposure range, on which there is a paucity of literature, as Quansah et al. (2015) has noted. To our knowledge, this study is the first of its kind to explore the association between arsenic in drinking water and multiple birth outcomes in a region of relatively low arsenic exposure; specifically, 99% of births in this study were estimated to be exposed to arsenic in drinking water below the current MCL of 10 µg/L. In models with the least potential for exposure misclassification (e.g., low use of private drinking water wells), we found that the risk of VLBW and preterm birth increased with increasing levels of arsenic in drinking water.

The mechanism by which arsenic may affect birth weight and gestational age at delivery remains uncertain, although biologic plausibility exists for both of these outcomes. Chronic arsenic exposure may reduce birth weight and gestational age through its anemic effects, placental impairment, chronic inflammation, or direct toxic effects on enzyme production and activity.

Our analyses of NN and adverse birth outcomes found that the odds of VLBW and VPTB increase significantly with increasing mean gestational NN exposure. This association remained significant in models with reduced exposure misclassification (<20% and <10% private well use). Models that included a categorical variable for the utilization of PNC received by the mother of the infant yielded somewhat different results than the models that excluded this confounder. Notably, we found that the association between NN exposure and VLBW was weakened, such that while the odds remained increased, the association was no longer significant, however this may be a result of reduced sample size or selection bias in these models. Furthermore, we observed no association between mean gestational NN concentrations and either PTB or VPTB in models restricted to births with known PNC utilization.

These findings suggest that is it important to control for PNC utilization when examining the associations between NN exposure and these birth outcomes. The level of missing data in the PNC variable, however, introduces considerable bias when 20% of the observations are removed. Having a missing PNC utilization score was more common among non-Hispanic black and other race infants, as well as among infants born you younger mothers (Table XXVI, Appendix B). Given the large number of covariates provided in the birth certificate data, PNC utilization may be imputed in future studies to reduce the potential for bias due to missing data.

We found a significant increase in the odds of term LBW births with increasing atrazine exposure in drinking water in our analysis of all live singleton births within AMP communities in Ohio between 2006 and 2008. Unlike in the analyses of arsenic or NN, we were able to examine trimester exposures in the AMP data due to the frequent sampling scheme for AMP CWSs. Mean gestational atrazine exposure was associated with an increase in the odds of term LBW, as was atrazine exposure in the first and second trimesters. This indicates that exposure to

atrazine in drinking water in early and mid-pregnancy may be most critical for its toxic effects on the fetus. We observed no association between atrazine exposure via drinking water and any of the remaining birth outcomes, including SGA, VLBW, PTB, and VPTB.

Our results are the first to show an association between exposure to atrazine in drinking water and term LBW births. This finding is supported by previous epidemiologic research which has shown an inverse relationship between atrazine exposure and birth weight (Chevrier et al. 2011), but conflicts with another study which found no association between atrazine exposure and low birth weight in a population of infants in Brittany, France (Villanueva et al. 2005). While previous studies have shown evidence of an association between atrazine exposure and SGA (Munger et al. 1997; Ochoa-Acuna et al. 2009) and PTB (Rinsky et al. 2012), we found no evidence of these associations in our study.

The birth outcomes in this study – SGA, term LBW, VLBW, PTB, and VPTB – have serious public health impacts. Neonatal mortality increases as the length of gestation decreases, resulting in a decreased survival rate among preterm and very preterm infants. The risk of neonatal mortality is also highest among the smallest and largest infants, as measured by birth weight. This same pattern of increased risk is seen later in life as well, with a reversed "J" shape association between birthweight and cardiovascular disease and all-cause mortality (Wilcox 2010). Our findings suggest that the morbidity and mortality burden from these adverse birth outcomes can be lessened through reducing gestational exposures to arsenic, NN, and atrazine in drinking water.

It is important to note that the associations presented in these analyses were observed within exposure ranges that rarely exceeded the MCL for each contaminant. Specifically, only 1% of observations in Ohio had annual arsenic exposures that exceeded the arsenic MCL and

only 4% had gestational atrazine estimates that exceeded the atrazine MCL. All mean gestational concentrations were below the NN MCL. These findings, therefore, suggest that the current MCLs for arsenic, atrazine, and NN may not be sufficiently protective against term LBW, VLBW, PTB, and VPTB.

MCLs are established as a result of considering human health effects from exposure to each contaminant as well as feasibility and cost issues related to its implementation and enforcement. In the case of arsenic, the MCL was set at 10 µg/L, which is higher than EPA's maximum contaminant level goal of 0 µg/L, based on cancer and non-cancer effects, and feasibility (USEPA 2001). The non-cancer health effects considered in this determination, however, did not include the adverse birth outcomes examined in this study. Our findings suggest that exposure to arsenic at levels below the MCL may increase risk of PTB and VLBW. Few epidemiologic studies of adverse birth outcomes have examines chronic exposure to low levels of arsenic in drinking water. Our research suggests that the MCL for arsenic should be lowered from the current 10 µg/L standard.

B. Strengths and Limitations

1. Birth Outcome Data

We used birth certificate data to ascertain all outcomes and covariates examined in this study. Birth certificate data are a commonly used data source because there is one for almost every birth in the United States and they are standardized across the country. The reliability of birth certificate data, however, varies widely by data element. Demographic variables on the birth certificate about the mother, including maternal age, race/ethnicity, and marital status are highly accurate (Querec, 1980; Schoendorf et al. 1993; Reichman and Hade, 2001; DiGiuseppe et al. 2002; Zollinger et al. 2006). When comparing birth certificates to medical records,

considered the “gold standard”, there is high agreement for variables including infant gender, birth weight, Apgar score, plurality, insurance, delivery type (vaginal versus Cesarean), number of previous live births, number of previous fetal deaths, and prenatal care received, as a dichotomous variable (Querec, 1980; Schoendorf et al. 1993; Green et al. 1998; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp, 2006; Zollinger et al. 2006). Gestational age, parental education, paternal demographics show moderate agreement, but complications of labor and delivery, maternal weight gain during pregnancy, maternal medical risk factors (e.g., chronic hypertension, previous LBW or preterm birth), tobacco and alcohol use, number of prenatal care visits, and obstetric procedures have very low reliability (Querec, 1980; Dobie et al. 1998; Reichman and Hade, 2001; DiGiuseppe et al. 2002; Roohan et al. 2003; Northam and Knapp, 2006; Zollinger et al. 2006). There is evidence that suggests that reliability of birth certificate data differs be race/ethnicity, which higher reliability among white mothers compared to black mothers (Schoendorf et al. 1993). Furthermore, we used de-identified birth certificate data and could not identify births from the same mother during this time period. Without being able to exclude multiple singleton births from the same women in this study, we may have underestimated the variance in our models because these observations are no longer independent.

The majority of epidemiologic research on NN exposure in drinking water has focused on birth defects and congenital anomalies where nitrate exposure has been associated with and increased risk of neural tube defects, oral cleft defects, limb deficiencies (Brender et al. 2013), cardiac defects (Cedergren et al. 2002), anencephaly (Croen et al. 2001), and spontaneous abortions (Manassaram et al. 2006). Unfortunately, birth certificate data is particularly poor for the assessment of birth defects. The accuracy of birth defect data from birth certificates varies greatly by anomaly, with major malformations recorded with more accuracy than minor

malformations (Mackeprang et al., 1972). Several comparisons of birth certificates and birth defects registries have shown that congenital anomalies are greatly underreported on birth certificates, even for defects that are readily identifiable at the time of birth, such as anencephaly, spina bifida, gastroschisis, cleft lip/palate, clubfoot, and Down's Syndrome (Hexter et al., 1990; Snell et al., 1992; Watkins et al., 1996). Reliability of birth certificate data for multiple anomalies and anomalies not easily identifiable at birth, such as heart defects, is particularly poor (Hexter et al., 1990; Watkins et al., 1996). Consequently, we were unable to examine the relationship between NN in drinking water and birth defects in our data.

The de-identified individual-level birth certificate data provided by the ODH allowed for excellent covariate adjustment to better control for confounding in multivariable models. In previous analyses of these data, confounders and other covariates have been assessed at the ecologic level (Almberg et al. 2014). Overall, the ODH birth certificate data contained low levels (<3%) of missing data on the key covariates used in these analyses. A notable exception is the high level of missing data on the variables needed to construct the Kotelchuck index score for utilization of prenatal care. For births occurring from 2006 to 2008 in Ohio, 24% of infants lacked data on the month in which their mother entered prenatal care. Another 11% were missing data on the number of prenatal care visits they completed during their pregnancy. Together, this resulted in 26% of all singleton live births in the state of Ohio lacking a measure of PNC utilization. PNC was strongly associated with each of the outcomes analyzed and was a confounder in most multivariate models presented in these analyses. Those who were missing data on their prenatal care status were more likely to be non-Hispanic black, "Other" race/ethnicity, and young (Table XXVI, Appendix B).

2. Exposure Data

These analyses were performed within the CDC's EHPTN framework which aims to identify environmentally-related diseases by utilizing regulatory and other existing data sets on exposures and health outcomes. Among the goals of the EPHTN is providing and utilizing data on drinking water contaminants for the purposes of linking them with data on health outcomes (CDC 2006). Using drinking water contaminant data for public health research has unique challenges stemming from the discrete boundaries of water delivery infrastructure (Jones et al. 2014a). Water quality in one water system is likely to be independent from adjacent systems as water sources and treatment processes can differ at the water system level. We made several key assumptions in the development of our exposure measures, chief among them that the service boundaries of each CWS remained within the borders of the county in which the treatment plant was located. This allowed us to calculate annual, gestational, and trimester estimates of exposure for arsenic, NN, and atrazine respectively.

Exposure estimates for arsenic, atrazine, and NN were developed from regulatory sampling data under the SDWA and AMP. Individual water system measurements were aggregated to the county-level in both the arsenic and NN analyses largely as a result of the low frequency of contaminant sampling in the data set. This ecologic exposure measure introduced misclassification of exposure at the individual level. Furthermore, we were unable to link women to the CWS that supplied their residence with drinking water. Even if this linkage were possible for the entire state, the infrequent monitoring scheme for NN would result in many women missing an exposure value for their CWS during their pregnancy.

Atrazine samples in CWSs in the AMP were sufficiently frequent to estimate mean atrazine exposure at the CWS-level, which reduced the bias introduced through aggregation of

the data to the county level. Furthermore, we confirmed that nearly half of all AMP CWSs had service boundaries that matched city boundaries, allowing us to link infants to a specific water system using the city of residence reported on the birth certificate.

We made several attempts to reduce the exposure misclassification in our analyses. In the atrazine analyses, we limited our analyses of atrazine and birth outcomes to those communities served by a CWS enrolled in the AMP, which requires frequent sampling. This increased frequency of monitoring allowed us to further evaluate shorter time frames of exposure, notably trimesters, with substantially reduced levels of missing exposure values.

In the arsenic and NN analyses, we performed analyses that were restricted to those counties with a low proportion of the population using private well use (<20 and <10%). Private wells are not routinely monitored for arsenic or NN by any regulatory body, therefore we lacked estimates of their concentrations in private wells. In both of these analyses, our results changed substantially with well use restriction. Restricting the arsenic analyses by well use yielded stronger associations between arsenic exposure and term LBW, VLBW, PTB, and VPTB. The NN analyses did not show a similar consistent pattern of change in the results after restricting based on private well use estimates. NN analyses restricted to counties with <20 or <10% well use yielded moderately higher odds of VLBW and VPTB, but weaker associations with term LBW. One explanation for the increased positive associations we observed in restricted models is that the reduction in exposure misclassification strengthened our results. It is also possible, however, that the counties that were excluded had different exposure and risk profiles from those with low levels of private well use. In crude analyses of county estimates of private well use and each birth outcome, odds of each outcome decrease with increasing percentage of the population using private wells.

The arsenic and NN analyses used regulatory data collected by the USEPA on public drinking water sources. By definition, these data are collected for purposes other than research, and as such, their sampling frequency is not always ideal for epidemiologic analyses. We observed no evidence that water systems were not sampling within the required time frame (Jones et al. 2014a), however, sampling remained sparse. Furthermore, there are concerns that water systems sample for certain contaminants when they are likely to be at their lowest (e.g., pre-application or plating season). The high level of censored and missing data points at a more refined geographic and temporal scale was addressed with multiple imputation, but the data are missing not at random (MNAR) and therefore imputation methods should be used with caution (Jones et al. 2014b).

While the atrazine data was also collected for regulatory purposes, the frequency of sampling for the AMP is better suited for epidemiologic research. CWSs in the AMP are required to measure atrazine weekly during the season of peak atrazine use and biweekly throughout the remainder of the year. This frequent sampling allowed us to examine temporally refined windows of exposure (e.g., trimesters). A limitation of using the AMP data is that it collects these data from a relatively small number of CWSs, thereby dramatically reducing the sample size available for analysis.

An additional source of exposure misclassification in these analyses arises from the lack of data on personal water consumption patterns and behaviors in this population. In a sample of 2,280 pregnant women from three geographic locations in the United Sates, Forssen et al. (2007) estimated that 28% drank bottled water during their pregnancy and 19% reported drinking filtered tap water. Furthermore, water ingestion behaviors varied significantly by certain demographic factors such as race/ethnicity, maternal education, and marital status. In a smaller

sample of pregnant women (N = 71), Zender et al. (2010) found that 25% of women drank filtered or bottled water during their pregnancy. Despite this relatively high proportion of women using bottled or filtered water, Forssen et al. (2009) did find a significant increase in the ingestion of cold tap water from early to mid-pregnancy among a sample of 1,990 pregnant women, suggesting that tap water remains a significant route of exposure to water contaminants for pregnant women. Pregnant women are estimated to ingest roughly a third of their total water intake outside of the home (Kaur et al. 2004; Zender et al. 2010). The current analyses were unable to control for personal water consumption patterns.

We lacked information on whether or not the mothers of the infants in these analyses had moved at any point during their pregnancy. Rates of pregnancy mobility are estimated between 12-32% (Fell et al. 2004; Canfield et al. 2006; Miller et al. 2010; Zender et al. 2010), and vary by geography and demographic factors. We were unable to account for those women who moved during pregnancy and assumed that the residence listed on the birth certificate was the residence throughout the entire pregnancy. Miller et al. (2010) found that among a sample of 991 pregnant women in the Atlanta, Georgia region, 51% of mothers that moved during pregnancy moved within the same county. For the analyses of arsenic and NN, where exposure was at the county-level, exposure misclassification from pregnancy mobility may be reduced.

Each of the analyses assessed the relationship between one exposure and multiple birth outcomes, which does not address the fact that drinking water contains varying levels of multiple contaminants. We assumed that the results for the arsenic, NN, and atrazine analyses are independent from one another. Mean monthly estimates of arsenic were not correlated with either monthly means of NN (rho = -0.04, P = 0.16) or atrazine (rho = 0.01, P = 0.78), lending support for the assumption of independence. We could not directly compare county-level means

of NN with the AMP water system means of atrazine in our data, but when we compare monthly means of NN and atrazine from the SDWIS data, we find a significant, but weak correlation (rho = 0.22, P = <.0001).

One previous epidemiologic study of reproductive birth outcomes assessed the combined effect of atrazine and nitrates in drinking water and found that risk of SGA increased with increasing nitrate exposure in the second trimester, but only when atrazine was not present (Migeot et al. 2013). Assessing multiple exposures in this data set would be a valuable avenue for future research, although the sparsity of NN exposure data would need to be addressed.

C. Implications for Future Research

Our findings suggest that additional epidemiologic research should examine the effects of arsenic, atrazine, and NN on term LBW, VLBW, and PTB in areas of relatively low contaminant exposure. Ideally, future research would employ biomarkers of exposure or individual assessment of drinking water exposures rather than relying on the ecologic exposure measures presented in these analyses. Despite the limitations in the exposure ascertainment, our findings suggest that linking environmental monitoring data with health outcomes data such as vital statistics databases has great utility in identifying potential associations to explore with more refined exposure and outcome ascertainment.

There are fruitful avenues of research in the data employed in these analyses, chiefly assessing the effects of contaminant mixtures on these birth outcomes. The USEPA AMP data contains samples of total chlorinated triazines (TCTs), as well as atrazine's metabolites and simazine. TCTs were not consistently reported until 2007, but would be a valuable exposure measure to evaluate in the future using these data resources with the birth outcomes examined in these analyses.

An additional area for future research is to examine the relationship between mean gestational NN concentrations in drinking water and birth defects. Birth defect data is maintained by the Ohio Connections for Children with Special Needs in the Ohio Birth Defects Information System (http://www.odh.ohio.gov/odhprograms/cmh/bdefects/birthdefects1.aspx). Reporting from the entire state of Ohio began in 2007, and the registry relies on passive data collection from hospitals. Despite the reliance on passive data collection, the birth defects registry data would be substantially more reliable than the birth certificate data for ascertaining anomalies.

Initially, we employed several techniques to model the relationship between arsenic, atrazine, and NN in drinking water and each birth outcome while controlling for important covariates. Because the exposure was an ecologic measure, either at the county or water system level, we employed model designs that would control for the correlation within counties and cities. Random effects models, allowing for a random effect of county or city, failed to converge for many of the models, perhaps a result of the small number of clusters (counties/cities) relative to the overall sample size. As a result, we employed GEE models with an exchangeable working correlation structure to examine the relationships between arsenic, atrazine, and NN and each outcome. Resolving the disparity between the actual number of unique exposure estimates ($N_{arsenic} = 88$, $N_{atrazine} = 22$, $N_{NN} = 79$) and the number of births in this region would be a valuable avenue for research in this area which employs aggregate exposure estimates. An additional modeling limitation is that we did not adjust for the multiple comparisons made across these analyses. These analyses were intended to explore the relationship between arsenic, NN, and atrazine and each birth outcome by linking data from environmental hazard monitoring and human health effects surveillance data.

Multiple imputation (MI) methods could be employed to resolve the level of missingness in the exposure and covariate data, namely utilization of prenatal care. In the NN and atrazine analyses, we restricted our study population in part to reduce exposure misclassification and reduce missingness exposure data which limited the generalizability of the findings from these analyses. MI has the potential to allow greater population inclusion in all analyses, thereby increasing the generalizability and sample size, while reducing potential bias of the study findings. Our research team has investigated MI for filling in missing water quality measurements when frequent monitoring is not required (Jones et al. 2014). These methods were generally effective in filling in missing contaminant concentrations in drinking water, despite the fact that the data are missing not at random (MNAR). Use of the AMP data allowed us to examine the effects of atrazine in a population with virtually no missing exposure data, but MI would be required in an analysis of the entire state, for which only SDWIS data is available. The vast majority of reported atrazine measurements in SDWIS are below the censoring threshold.

VII. CONCLUSIONS

We found a consistent association between atrazine concentrations in drinking water and odds of term low birth weight within communities served by water systems enrolled in USEPA's Atrazine Monitoring Program. This is the first study to show such an association linking maternal residence to a specific water system. Water systems are enrolled in the AMP as a result of repeated exceedances of the 3 µg/L MCL for atrazine, but only 4% of samples from the water systems in this study exceeded the MCL. Our findings are unchanged when we remove those observations for which gestational atrazine estimates exceeded the MCL. While further epidemiologic research is needed, these results suggest that the current MCL for atrazine may not be protective against some adverse birth outcomes such as term low birth weight.

We found that gestational exposure to NN in drinking water increased the odds of VLBW within those rural Ohio counties where private well use is not very prevalent (<20%). We did not observe significant evidence of an association between gestational NN exposure and SGA, term LBW, PTB, or VPTB. While this study benefited from the inclusion of a large number of births, it was limited by the ecologic exposure assessment. Exposure was tailored to each infant's gestational window, which is a novel exposure metric in the epidemiologic study of NN in drinking water and adverse birth outcomes. While the results of this study are largely negative, further research is needed to fully explore the relationship between NN in drinking water and adverse birth outcomes, ideally with use of individual biomarkers of exposure during pregnancy.

To our knowledge, this study is the first of its kind to explore the association between arsenic in drinking water and multiple birth outcomes in a region of relatively low arsenic exposure; specifically, 99% of births in this study were estimated to be exposed to arsenic in drinking water below the current MCL of 10 µg/L. Our findings were unchanged when we

deleted the 1% of the individuals with exposures above the MCL. In models with the least potential for exposure misclassification (e.g., low use of private drinking water wells), we found that the risk of VLBW and preterm birth increased with increasing levels of arsenic in drinking water. These findings suggest that the current MCL may not be sufficiently protective against these adverse birth outcomes. Further epidemiologic research is needed to explore these associations at low levels of arsenic exposure with individual exposure measurements, preferably biomarkers of exposure.

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APPENDICES

APPENDIX A

TABLE XXII

ASSOCIATIONS[a] BETWEEN MEAN GESTATIONAL ATRAZINE CONCENTRATION AND SGA, TERM LBW, VLBW, PTB, AND VPTB IN AMP COMMUNITIES IN OHIO, 2006 − 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	N	b	S.E.	OR[g] (95% CI)	P
SGA[b]	Crude	11,224	-0.007	0.079	0.99 (0.85, 1.16)	0.927
	Adjusted	11,224	0.030	0.078	1.03 (0.88, 1.20)	0.697
Term LBW[c]	Crude	12,567	0.144	0.0662	1.15 (1.01, 1.31)	0.030
	Adjusted	12,567	0.236	0.0699	1.27 (1.10, 1.45)	0.001
VLWB[d]	Crude	11,503	0.183	0.3232	1.20 (0.64, 2.26)	0.572
	Adjusted	11,503	0.145	0.2556	1.16 (0.70, 1.91)	0.570
PTB[e]	Crude	11,508	0.024	0.007	1.02 (1.01, 1.04)	0.697
	Adjusted	11,508	-0.003	0.0599	1.00 (0.89, 1.12)	0.957
VPTB[f]	Crude	11,646	0.250	0.1365	1.28 (0.98, 1.68)	0.067
	Adjusted	11,646	0.187	0.1228	1.21 (0.95, 1.53)	0.129

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, maternal pre-pregnancy BMI, and PNC utilization.
[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, marital status, parity, and PNC utilization.
[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, parity, and PNC utilization.
[f] Very preterm births defined as infants delivered before 32 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal marital status and PNC utilization.
[g] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

APPENDIX A (continued)

TABLE XXIII

ASSOCIATIONS[a] BETWEEN MEAN ATRAZINE CONCENTRATION DURING THE FIRST TRIMESTER AND SGA, TERM LBW, VLBW, PTB, AND VPTB IN AMP COMMUNITIES IN OHIO, 2006 − 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	N	b	S.E.	OR[g] (95%CI)	P-value
SGA[b]	Crude	11407	0.022	0.040	1.02 (0.95, 1.11)	0.578
	Adjusted	11407	0.035	0.042	1.04 (0.95, 1.12)	0.398
Term LBW[c]	Crude	12647	0.130	0.059	1.14 (1.01, 1.28)	0.029
	Adjusted	12647	0.184	0.057	1.2 (1.08, 1.34)	0.001
VLWB[d]	Crude	11581	0.060	0.204	1.06 (0.71, 1.58)	0.770
	Adjusted	11581	0.102	0.186	1.11 (0.77, 1.59)	0.584
PTB[e]	Crude	11586	-0.027	0.056	0.97 (0.87, 1.09)	0.633
	Adjusted	11586	-0.016	0.058	0.98 (0.88, 1.10)	0.782
VPTB[f]	Crude	11726	0.045	0.196	1.05 (0.71, 1.54)	0.818
	Adjusted	11726	0.051	0.198	1.05 (0.71, 1.55)	0.795

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, maternal pre-pregnancy BMI, and PNC utilization.
[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, marital status, and PNC utilization.
[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, parity, and PNC utilization.
[f] Very preterm births defined as infants delivered before 32 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal marital status and PNC utilization.
[g] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

APPENDIX A (continued)

TABLE XXIV

ASSOCIATIONS[a] BETWEEN MEAN ATRAZINE CONCENTRATION DURING THE SECOND TRIMESTER AND SGA, TERM LBW, VLBW, AND PTB IN AMP COMMUNITIES IN OHIO, 2006 − 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	N	b	S.E.	OR[f] (95%CI)	P-value
SGA[b]	Crude	11397	-0.046	0.027	0.96 (0.91, 1.01)	0.090
	Adjusted	11397	-0.031	0.027	0.97 (0.92, 1.02)	0.260
Term LBW[c]	Crude	12647	0.059	0.038	1.06 (0.98, 1.14)	0.119
	Adjusted	12647	0.125	0.031	1.13 (1.07, 1.20)	<.0001
VLWB[d]	Crude	11569	-0.018	0.107	0.98 (0.80, 1.21)	0.866
	Adjusted	11569	-0.023	0.093	0.98 (0.81, 1.17)	0.801
PTB[e]	Crude	11574	0.009	0.025	1.01 (0.96, 1.06)	0.709
	Adjusted	11574	0.008	0.029	1.01 (0.95, 1.07)	0.772

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] Small for gestational age defined as the smallest 10% of infants, according to birth weight, at each gestational age in the population. In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, maternal pre-pregnancy BMI, and PNC utilization.
[c] Term low birth weight is defined as <2,500g among term births (≥37 weeks gestation). In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Very low birth weight is defined as <1,500g at time of delivery. In addition to *a priori* variables, the final model for term LBW included maternal education, marital status, and PNC utilization.
[e] Preterm births defined as infants delivered before 37 weeks gestation. In addition to *a priori* variables, the final model for PTB included maternal education, maternal smoking status, parity, and PNC utilization.
[f] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

APPENDIX A (continued)

TABLE XXV

ASSOCIATIONS[a] BETWEEN MEAN ATRAZINE CONCENTRATION DURING THE THIRD TRIMESTER AND SGA AND TERM LBW IN AMP COMMUNITIES IN OHIO, 2006 − 2008, CONTROLLING FOR PRENATAL CARE UTILIZATION

Outcome	Model	N	b	S.E.	OR[d] (95%CI)	P-value
SGA[b]	Crude	10380	-0.028	0.075	0.97 (0.84, 1.13)	0.708
	Adjusted	10380	-0.013	0.061	0.99 (0.88, 1.11)	0.830
Term LBW[c]	Crude	12647	-0.035	0.094	0.97 (0.80, 1.16)	0.709
	Adjusted	12647	0.030	0.085	1.03 (0.87, 1.22)	0.723

[a] All adjusted models included maternal race/ethnicity, maternal age, and birth year *a priori*.
[b] In addition to *a priori* variables, the final model for SGA included maternal education, WIC status, marital status, maternal pre-pregnancy BMI, and PNC utilization.
[c] In addition to *a priori* variables, the final model for term LBW included infant sex, maternal education, WIC status, marital status, maternal smoking status, and maternal pre-pregnancy BMI.
[d] Odds ratios reflect increase in odds per 1 μg/L increase in atrazine in drinking water.

APPENDIX B

TABLE XXVI

DISTRIBUTION OF COVARIATES ACROSS EACH CATEGORY OF PRENATAL CARE UTILIZATION AMONG ALL LIVE SINGLETON BIRTHS (N=428,804) IN OHIO 2006 − 2008

		Kotelchuck Index Category of Prenatal Care			
Variable	**N (%)**	**Unknown %**	**Inadequate %**	**Int/Adequate[a] %**	**Adequate + %**
Gender					
Male	209,617 (51)	**26.5**	**13.8**	**37.3**	**22.4**
Female	219,187 (49)	**26.6**	**13.7**	**38.0**	**21.8**
Race/Ethnicity					
Non-Hispanic white	327,343 (76)	**24.2**	**11.8**	**40.1**	**23.9**
Non-Hispanic black	69,325 (16)	**35.7**	**20.8**	**27.4**	**16.1**
Hispanic	19,631 (5)	**26.2**	**21.7**	**34.6**	**17.5**
Other	12,505 (3)	**36.4**	**13.1**	**34.0**	**16.5**
Maternal Age at Birth					
10 − 19	47,544 (11)	**29.2**	**20.8**	**31.1**	**18.9**
20 − 29	239,250 (56)	**26.3**	**14.6**	**37.3**	**21.8**
30 − 39	132,021 (31)	**26.3**	**9.7**	**40.6**	**23.4**
40+	8,453 (2)	**27.5**	**12.5**	**34.3**	**25.7**
Maternal Education					
High School or Less	193,831 (46)	**26.9**	**19.7**	**32.9**	**20.5**
Some College/Degree	231,513 (54)	**25.8**	**8.7**	**41.9**	**23.6**
Maternal Smoking					
Yes	113,552 (26)	**27.4**	**16.9**	**33.9**	**21.7**
No	315,208 (74)	**26.2**	**12.6**	**39.0**	**22.3**
WIC use					
Yes	175,604 (42)	**26.8**	**17.4**	**34.0**	**21.8**
No	240,891 (58)	**25.6**	**11.2**	**40.8**	**22.4**
Prepregnancy BMI					
Underweight	18,811 (4)	**27.7**	**16.3**	**34.4**	**21.6**
Normal	197,071 (48)	**26.0**	**13.4**	**39.6**	**21.0**
Overweight	94,621 (23)	**25.2**	**13.5**	**38.5**	**22.9**
Obese	103,927 (25)	**26.3**	**13.9**	**35.5**	**24.3**
Parity					
0	170,155 (41)	**24.9**	**12.9**	**39.3**	**22.9**
1	131,510 (31)	**25.3**	**11.7**	**39.8**	**23.2**
2	68,891 (17)	**25.9**	**14.0**	**37.4**	**22.7**
≥3	47,075 (11)	**26.9**	**22.8**	**31.3**	**19.1**
Marital Status					
Married	247,516 (58)	**24.5**	**9.6**	**42.0**	**23.9**
Unmarried[b]	181,288 (42)	**29.3**	**19.4**	**31.7**	**19.7**

[a] Intermediate or adequate prenatal care categories are combined.

[b] Not married category includes mothers who responded single, widowed, or divorced.

APPENDIX C

UNIVERSITY OF ILLINOIS AT CHICAGO HUMAN SUBJECTS APPROVAL FOR ALL AIMS

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

September 21, 2015

Leslie T Stayner, PhD
Epidemiology and Biostatistics
1603 West Taylor Street
Room 971 sphpi, M/C 923
Chicago, IL 60612
Phone: (312) 355-3692 / Fax: (312) 996-0064

RE: **Protocol # 2010-0907**
"A Linkage Study of Health Outcome Data in Children and Agrichemical Water
Contamination Data in the Midwest"

Dear Dr. Stayner:

Your Continuing Review was reviewed and approved by the Expedited review process on September 17, 2015. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: October 14, 2015 - October 13, 2016
Approved Subject Enrollment #: 3,980,000 (3,259,233 cases analyzed)
Additional Determinations for Research Involving Minors: The Board determined that this research satisfies 45CFR46.404', research not involving greater than minimal risk.
Performance Sites: UIC, Illinois Department of Public Health,

APPENDIX C (continued)

Wisconsin
Division of Public Health, Iowa
Department of Public Health, Indiana
State Department of Health, Michigan
Department of Community Health, Indiana
State Dept. of Health, Cancer Registry,
Ohio Department of Health, Missouri
Department of Health and Senior Services

Sponsor: None
PAF#: Not applicable
Research Protocol:

a) A Linkage of Study of Health Outcome Data in Children and Agrichemical Water; Version 6, 11/07/2014

Recruitment Material:

a) Secondary data analysis of public health data obtained under separate data transfer agreements with non-UIC entities; no recruitment materials will be used

Informed Consent:

a) Waivers of consent/permission/assent have been granted under 45 CFR 46.116(d) for the secondary analysis of public health data obtained via separate data transfer agreements with non-UIC entities

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific category:

(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
09/16/2015	Continuing Review	Expedited	09/17/2015	Approved

Please remember to:

→ Use your **research protocol number** (2010-0907) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the OPRS website under:

APPENDIX C (continued)

"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the right to seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 996-9299. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Anna Bernadska, M.A.
IRB Coordinator, IRB # 2
Office for the Protection of Research Subjects

Enclosure: None

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923

VITA

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	M.S., Public Health – Epidemiology, University of Illinois at Chicago, Chicago, IL 2012
	Ph.D., Public Health – Epidemiology, University of Illinois at Chicago, Chicago, IL 2016
TEACHING EXPERIENCE:	Teaching Assistant, Division of Health Policy and Administration, University of Illinois at Chicago, Chicago, IL, 2013
	Teaching Assistant, Division of Epidemiology and Biostatistics, University of Illinois at Chicago, Chicago, IL, 2013
HONORS:	National Institute for Occupational Safety and Health Education and Research Center Trainee, University of Illinois at Chicago, Chicago, IL, 2010 − 2015
	Delta Omega Society, University of Illinois at Chicago, School of Public Health, Chicago, IL, 2013
	Dr. Scholl Research Fellowship, Lincoln Park Zoo, Chicago, IL, 2008
PROFESSIONAL EXPERIENCE:	Guest Researcher, National Institute for Occupational Safety and Health, Surveillance Branch, Respiratory Health Division, Morgantown, WV, 2014 − present
PUBLICATIONS:	Almberg, K.S., Turyk, M.E., Jones, R.M., Anderson, R., Graber, J., Banda, E., Waller, L.A., Gibson, R., Stayner, L.T. 2014. A Study of Adverse Birth Outcomes and Agricultural Land Use Practices in Missouri. *Environmental Research, 134C,* 420−426.
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PRESENTATIONS: Almberg, K.S. "A linkage study of adverse birth outcomes with agricultural land use practices in Missouri." UIC School of Public Health, Chicago, IL; October 10, 2012.

Almberg, K.S. "Methodologic Issues in Tracking Children's Health & Agrichemical Water Contaminant Data." International Society for Environmental Epidemiology Pre-Conference Workshop Session, Columbia, SC; August 26, 2012.

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