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Congenital Anomalies, Labor/Delivery Complications, Maternal Risk Factors and Their Relationship with Perfluorooctanoic Acid (PFOA)-Contaminated Public Drinking Water

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Abstract

Background—We have previously examined the associations between perfluorooctanoic acid (PFOA) exposure, birth weight and gestational age in individuals exposed to PFOA-contaminated residential drinking water from the Little Hocking Water Association (LHWA). In this investigation, we expand the scope of our analysis to examine the associations between PFOA, congenital anomalies, labor and delivery complications and maternal risk factors.

Objectives—To compare the incidence of congenital anomalies, labor and delivery complications and maternal risk factors in neonates and their mothers residing in zip codes with public water service provided completely, partially or not at all by the LHWA.

Methods—Logistic regression analyses were performed on singleton neonatal birth outcome data supplied by the Ohio Department of Health to examine the associations between LHWA water service category and the outcomes of interest. When possible, models were adjusted for maternal age, preterm birth, neonatal sex, race, maternal education, alcohol use, tobacco use and diabetic status.

Results—Increased PFOA exposure, as assessed by water service category, was not associated with an overall increase in the likelihood of congenital anomalies or any specific diagnosis (Adjusted OR: 1.4, 95%CI: 0.34–3.3). The overall likelihood of labor and delivery complications was significantly lower among mothers with water service provided by the LHWA, as compared to mothers not serviced by the LHWA (Adjusted OR: 0.65, 95% CI: 0.46–0.92). A significant increase in the likelihood of anemia (Crude OR: 11, 95%CI: 1.8–64) and dysfunctional labor (Crude OR: 5.3 95%CI: 1.2–24) was noted for mothers residing within zip codes serviced by the LHWA, but the number of reported cases was very small.

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Conclusion—At the levels measured in the LHWA, we conclude that PFOA is not associated with increased incidence of congenital anomalies and most labor and delivery complications and maternal risk factors. Additional research is required to assess the observed associations between PFOA, anemia and dysfunctional labor.

Keywords

Perflurooctanoic Acid; PFOA; Congenital Anomalies; Pregnancy; Labor and Delivery; Complications; Maternal Risk Factors; Little Hocking; LHWA

INTRODUCTION

Perfluorooctanoic acid (PFOA) and its salts are fully fluorinated eight carbon (C8) organic compounds that have been utilized for decades in a variety of industrial and commercial applications such as the production of tetrafluoroethylene and the manufacturing of protective coatings for carpets, apparel, housewares, and fire-fighting foams [1]. PFOA is highly water soluble and resists biologic, environmental and photochemical degradation [2]. Global bio-monitoring studies have indicated that PFOA is ubiquitously found in ground water and wildlife [3–4]. In the general U.S. population, median serum PFOA values have been measured at 4–5 ng/mL with no significant gender differences [5] and a mean serum half-life in the range of 2–4 years [6–8].

Rodent studies have shown that chronic PFOA exposure is associated with developmental toxicity [9–10]. Studies conducted across two generations of rats revealed decreased weight gain in the offspring of dams who were orally dosed 30 mg/kg of PFOA per day during gestation [11]. A statistically significant increase in mortality was observed in both male and female pups in addition to reduced body weight after weaning and throughout the remainder of the study. Both sexes experienced delays in reaching sexual maturity. Similarly, Lau confirmed dose-dependent fetal toxicity of PFOA in mice and observed early pregnancy loss, delayed fetal growth and development, compromised postnatal survival, and sex-specific alterations in pubertal maturation [12]. Complete resorption of litters was observed in dams receiving 40 mg/kg of PFOA per day during gestation. Weight gain in dams that carried pregnancy to term was significantly lower at 20 mg/kg while post-natal survival was compromised and growth deficits were observed at dosages greater than or equal to 5 mg/kg. Fetal weight was also significantly reduced at oral dosages as low as 20 mg/kg. Steady-state serum PFOA concentrations were reached in dams in 7 days and measured approximately 75, 120, 185 and 275 μg/mL at term (18 days) in the 5, 10, 20 and 40 mg/kg dosage groups, respectively. Subsequent to these initial observations, more recent investigations have highlighted neurobehavioral deficits resulting from fetal PFOA exposure in addition to altered inflammatory responses, reduced lymphoid organ weights and altered antibody synthesis [13].

Many of the reproductive toxicological effects of PFOA exposure in murines result in part from altered expression of the peroxisome proliferator- activated receptor-alpha (PPARα) signaling pathway [14–18]. The PPARα pathway plays a major role in maintaining lipid and glucose homeostasis, regulating inflammation, cell proliferation and differentiation [19–21]. In humans, however, the precise mechanisms by which PFOA and other perfluorinated compounds may disrupt fetal development are less certain. Human expression of PPARα is significantly less than that of mice and less sensitive to activation by PFOA [14,22–23]. Furthermore, PPARα-induction of gene targets related to cell or peroxisome proliferation is a mechanism not shared by human liver cells [24]. As such, PPARα-independent pathways – most of which are poorly understood – are more likely to contribute to PFOA's toxicological effects.

In humans, several studies have explored the relationship between serum PFOA levels and fetal development by examining outcomes such as birth weight, birth length, head circumference, and ponderal index [25–29]. Among these investigations, some results have been consistent (e.g. the lack of an association between PFOA and gestational age), while others have not (e.g. the negative association between PFOA and birth weight). More recently, Fei et al. have explored developmental milestones such as gross motor skills and cognitive abilities in neonates exposed to PFOA during gestation and found no significant associations [30], but suggested a potential association between population-level PFOA exposure and reduced fecundity in mothers [31].

Our own analysis of the potential adverse effects of PFOA exposure on birth weight and gestational age in residents serviced by the Little Hocking Water Association (LHWA) in Washington County, Ohio revealed no evidence of a negative association [32]. Unlike prior cohorts that lacked a distinctive source of environmental exposure, residents of the LHWA had the highest recorded serum levels of PFOA in a general population as a result of public drinking water contaminated by local industry [33]. Mean serum PFOA levels in a stratified, random sample of LHWA residents were approximately 80 times higher than those reported in the general U.S. population [5,34]. Since maternal and neonatal serum samples were not available prior to the introduction of several PFOA-reducing interventions enacted by the LHWA in September 2005, our study utilized birth records supplied by the Ohio Department of Health (ODH) in conjunction with residential public water service category as an indicator of maternal PFOA exposure and, indirectly, for fetal exposure since PFOA crosses the placenta [35–36]. This methodology was informed by analyses which demonstrated that residential drinking water service was the major determinant of elevated serum PFOA levels in the LHWA and by PFOA sampling surveys conducted by the Ohio Environmental Protection Agency (Ohio EPA) which revealed a gradient of contamination across water service categories [33]. Despite potential methodological limitations (e.g. exposure misclassification), the high levels of PFOA exposure within the LHWA were well above the range of normal biologic variation and had the distinct advantage of being less susceptible to confounding by maternal physiology, which limited prior investigations [37].

Since the publication of our original findings, the association between PFOA exposure and self-reported pregnancy outcome among LHWA residents was reassessed using direct measurement of maternal serum PFOA levels [38–39]. Although serum was sampled in 2005–2006 after the implementation of exposure-reducing interventions, differences in mean serum PFOA concentrations were still observed across water service categories with residents of the LHWA maintaining the highest levels of exposure [38,40]. Residents within Washington County who were not serviced by the LHWA had serum PFOA levels similar to those observed in the general populations of western countries while residents in regions with water service supplied in part by the LHWA (e.g. Belpre) had intermediate levels of contamination. Furthermore, no apparent association was found between PFOA exposure and low birth weight or preterm birth [39]. Preeclampsia was weakly associated with PFOA exposure as were birth defects with exposures above the 90^{th} percentile.

Given the concordance between our original findings with those that directly measured PFOA levels in the LHWA, and a continued interest in assessing the developmental impact of PFOA exposure, the current investigation expanded the scope of our prior analysis to examine the associations between PFOA exposure, congenital anomalies, labor and deliver complications and maternal risk factors in the same cohort of LHWA neonates and mothers. Unlike other analyses which were limited by quality concerns related to self-reported pregnancy outcome, this investigation utilized a dataset with medically validated outcomes provided by the ODH. The expanded ODH dataset also permitted greater ability to control for the confounding effect of parity, maternal education (as an indicator of socioeconomic

status), smoking status, diabetes and other risk factors that may affect birth outcome. In short, we assessed whether the cumulative incidence of congenital anomalies, labor and delivery complications and maternal risk factors differed among the various water service categories of the LHWA.

METHODS

Study Design

This cross-sectional study received approval from the University of Pennsylvania Institutional Review Board as well as the Institutional Review Board of the Ohio Department of Health. This study was determined to be exempt from the Health Insurance Portability and Accountability Act (HIPAA). There was no requirement for informed consent due to the nature of the de-identified archival data that were analyzed. This study was also endorsed by the Decatur Community Advisory Committee, a joint partnership between residents of the Little Hocking and surrounding communities, local healthcare providers, members of the Ohio EPA and the University of Pennsylvania School of Medicine. De-identified, record-level, archival data were obtained from the Ohio Department of Health (ODH) Center for Vital Health Statistics for all births occurring in Washington County, Ohio from January 1, 2003 until September 1, 2005. This period was selected as it was prior to the implementation of several interventions designed to reduce PFOA exposure in the LHWA. January 1, 2003 was chosen as the study start date since it marked both the beginning of the first full year in which PFOA levels were measured by the Ohio EPA in the LHWA and the establishment of the relationship between the University of Pennsylvania and the Little Hocking community through a grant from the Environmental Justice Program of the NIEHS.

The dataset provided by the ODH was an update of the dataset originally obtained for our investigation on birth weight and gestational age. Data which appear in the dataset are medically validated and not self-reported. This validation applies, in general, to the diagnosis of congenital anomalies, maternal risk factors and labor/delivery complications. Maternal risk factor data are typically extracted from antepartum medical records which are transferred to the birthing hospital by the patient's obstetrician or perinatologist upon presentation and are appended to the hospital birth record. Labor and delivery complications and congenital anomalies are also recorded in the birth record at or near the time of delivery by medical personnel. Certificates of birth are transferred directly to the ODH via electronic submission for all births occurring in the state of Ohio. The ODH collects data in accordance with the U.S. Standard Certificate of Live Birth and follows the specifications for coding and key entry provided by the National Center for Health Statistics. After receipt, the ODH examines certificates for completeness and correctness. For each record the following variables were included: birth weight, gestational age (based on last menstrual period), plurality, parity, neonatal sex, race, maternal age, maternal education, paternal education, maternal tobacco use, maternal alcohol use, maternal risk factors (anemia, cardiovascular disease, pulmonary disease, diabetes, genital herpes, hyraminos, hemoglobinopathy, chronic and pregnancy-related hypertension, eclampsia, incompetent cervix, previous preterm or small for gestational age, previous infant >4000g, renal disease, Rh Sensitization, uterine bleeding), labor and delivery complications (febrile, meconium, membrane rupture, abruption placenta, placenta previa, excessive bleeding, seizure, precipitous labor, prolonged labor, dysfunctional labor, breech, cephalopelvic disproportion, cord prolapsed, anesthetic complications, fetal distress), congenital anomalies (anencephalus, spina bifida, hydrocephalus, microcephalus, CNS anomaly, heart malformation, circulatory anomaly, rectral atresia, trachea-esophageal fistula, omphalocele, gastrointestinal anomaly, renal agenesis, urogenital anomaly, cleft lip, polydactyly, club foot, diaphragmatic hernia,

musculoskeletal anomaly, Down's Syndrome, chromosomal anomaly) and zip code of the mother's residence.

Public Water Service Categories

Water service categories and the methodology utilized for their creation are identical to our prior study on birth weight and gestational age. As noted, residents of Washington County are serviced by several local public water facilities: the LHWA, Belpre Water, Marietta Water and Warren Water. On the basis of public water supply, PFOA sampling of water distributed by these facilities and serum sampling conducted between 2005–2006, the zip codes in Washington County were divided into three categories. The first category obtained public water service exclusively from the LHWA. This category ("LHWA Only") comprised the zip codes of 45724, 45742 and 45784. The second category ("Partial LHWA") included zip codes with water service in part from the LHWA, the Belpre Water System, and others. This category comprised zip codes 45712, 45713, 45714, 45729 and 45787. The third category comprised the zip codes in Washington County entirely outside the service area of the LHWA or Belpre Water System ("No LHWA"). Zip codes in this category were: 43787, 45711, 45715, 4523, 45734, 45744, 45745, 45746, 45750, 45767, 45768, 45773, 45786, 45788, and 45789. The major suppliers of public drinking water to zip codes in this category were Marietta Water and Warren Water.

Results of PFOA sampling conducted by the Ohio EPA were available for several of the public water facilities in Washington County as were serum sampling results from 2005–2006(Table 1). Water sampling results indicated substantial PFOA contamination in the LHWA and, to a lesser extent, Belpre Water. The Ohio EPA did not sample other public water systems where, in its opinion, there was no prospect of PFOA contamination. In the cases of zip codes completely and partially serviced by the LHWA, data reflect mean levels of multiple samples taken during 2002–2005. For zip codes not serviced by the LHWA in which PFOA contamination did not occur, sampling took place in 2007. A small number of residents in Washington County also use private wells for their residential water. Surveys conducted by the Ohio EPA indicated that PFOA levels in water from private wells were on the order of 12.4 ppb ± 6.9 (mean ±SD). Detectable levels in private wells followed the general pattern of the distribution observed in public water supplies with the highest levels of PFOA found in the zip codes comprising the Little Hocking water service area.

Classification by public water service was also informed by our previous investigation of a stratfied random sample of residents from the LHWA service area and by a subsequent serum analysis performed by the C8 Health Project between 2005–2006. Our findings demonstrated that residential drinking water service was the major determinant of serum PFOA levels with a smaller contribution arising from the consumption of locally-grown fruits and vegetables. Air exposure played no discernable role. The median serum PFOA level within this random sample was 354 ppb. Home use of a carbon-based water filter reduced PFOA levels by about one quarter, but 70% of residents had serum levels in excess of 200 ppb. The only significant occupational contribution was from work in production areas at a fluoropolymer manufacturing facility, which employed mostly males [33]. Similarly, in 2009, the C8 Health Project reported on serum PFOA measurements taken from a volunteer sample of residents serviced by the LHWA and Belpre water systems. Median values were 132.5 ppb and 27.1 ppb, respectively [40]. The observed gradient in serum PFOA measurements mirrors the gradient in environmental exposure (Table 1).

Study Population

We examined the incidence of congenital anomalies, labor and delivery complications and maternal risk factors in 1548 live born neonates which constituted 99.5% of the records used

in our prior investigation on birth weight and gestational age. Neonates were included in this study if they were born to mothers residing in Washington County, Ohio between January 1, 2003 and September 1, 2005, had complete records for the requested covariates and if they were singleton births. Of the confirmed 1589 singleton births in the dataset, 41 (2.6%) lacked information on one or more of the requested covariates and were censored from further analysis. 1 of 41 (2.4%) of these censored neonates was diagnosed with a congenital anomaly, 17 of 41 (41.6%) were born to mothers who experienced a labor and delivery complication and 19 of 41 (46.3%) were born to mothers diagnosed with a risk factor. Of the 19 mothers diagnosed with a risk factor, 9 (47.3%) experienced a complication during labor and delivery. The incidence of congenital anomalies, labor and delivery complications and maternal risk factors among censored observations were not significantly different from the incidence rates calculated for neonates included in the study ($p=0.76$, $p=0.83$ $p=0.30$, respectively).

Statistical Analysis

Data are presented as frequencies and percents. To compare birth outcomes across the exposure groups (e.g. water service categories) logistic regression analysis was performed to calculate odds ratios and 95% confidence intervals across the levels of each covariate. Unadjusted or “crude” odds ratios are provided for individual diagnoses. Multiple logistic regression models were used to compare the odds ratios of aggregate outcome for the overall incidence of congenital anomalies, labor and delivery complications and maternal risk factors between each water service category after adjusting for race, parity, preterm birth, maternal age, maternal education (as an indicator of socioeconomic status), diabetic status, and tobacco and alcohol use during pregnancy. To address the phenomenon of separation in the data set, a bias reduction method was utilized to generate finite parameter estimates [41–42]. An exact binomial test was utilized to assess differences in cumulative incident rates between water service categories and national averages. Maternal age was stratified into categories that have known associations with gestational outcome and which are commonly used in the literature [43–47]. With alpha set at 0.05, this investigation had an 80% power of detecting a true odds ratio of approximately 2.7 when comparing the likelihood of congenital anomalies between LHWA Only and No LHWA water service categories. Similarly, this study had an 80% power of detecting true odds ratios of 1.6 when comparing either the likelihood of labor and delivery complications or maternal risk factors across strata. All statistical analyses were performed using STATA 9.2 (StataCorp., College Station, TX).

RESULTS

The 1548 singleton neonates included in this study comprised 773 (50%) males and 775 (50%) females. The demographics according to race were as follows: White, 1512 (97.7%); African-American, 19 (1.2%); Other, 17 (1.1%). Mean age of mothers was 26.1 ± 5.6 years (range 14–44 years). Seventy-six percent of newborns (N=1171) were born to mothers residing in zip codes without service from the LHWA (No LHWA); 13% of newborns (N=209) were born to mothers residing in zip codes partially serviced by the LHWA (Partial LHWA); and 11% of newborns (N=168) were born to mothers residing within zip codes exclusively serviced the LHWA (LHWA Only). Descriptive data for each water service category are provided (Table 2).

Congenital Anomalies

Of the 1548 records that were analyzed, 3 of 168 (1.8%) neonates born to LHWA Only mothers were diagnosed with one or more congenital anomalies (1 heart malformation, 1 circulatory malformation, 1 club foot) between January 2003 and August 2005. This

compares to 23 of 1171 (2.0%) neonates with one or more congenital anomalies reported in the No LHWA water service category (Adjusted OR: 1.1, 95%CI: 0.34–3.3) and 4 of 209 (1.9%) neonates within the Partial LHWA (Adjusted OR: 1.1, 95%CI: 0.40–3.1). After adjusting for maternal age, preterm birth, parity, sex, race, maternal education, diabetic status, alcohol and tobacco use, the likelihood of developing any congenital anomaly was not significant across water service strata ($p>0.05$). Only tobacco use was found to increase the likelihood of an anomaly (Adjusted OR: 2.6; 95%CI: 1.1–5.9). Furthermore, the incidence of congenital anomalies for each water service category was not statistically significantly different from the national incidence of 3% ($p>0.05$). Diagnosis-specific anomalies are provided along with crude odds ratios (Figure 1). Small frequency counts did not permit additional adjustment.

Labor and Delivery Complications

53 of 168 (31.5%) LHWA Only mothers developed complications associated with labor and delivery whereas 491 of 1171 (41.9%) mothers developed complications in the No LHWA water service category (Adjusted OR: 0.65, 95% CI: 0.46–0.92). 75 of 209 (35.9%) mothers had complications in the Partial LHWA (Adjusted OR: 0.79, 95%: CI: 0.57–1.1). Overall, LHWA Only births were associated with a decreased likelihood of complications ($p=0.016$) in comparison to No LHWA births. In the adjusted model, multiparous mothers were associated with a significantly decreased likelihood of developing one or more complications (Adjusted OR: 0.47, 95% CI: 0.38 – 0.60) compared to primiparous mothers. Additionally, mothers aged 40 years and older had a tendency to be associated with an increased likelihood of complications, but this association was not significant in our model (Adjusted OR: 2.0, 95% CI: 0.96 – 4.2).

Odds ratios for specific diagnoses are reported (Figure 2). LHWA Only mothers had an increased likelihood of being diagnosed with dysfunctional labor in comparison to No LHWA mothers (Crude OR: 5.3 95% CI: 1.2–24), but the number of cases was small ($N_{\text{LHWA Only}} = 3$; $N_{\text{No LHWA}} = 2$). No association between dysfunctional labor and mothers within the Partial LHWA category was observed. Both LHWA Only and Partial LHWA mothers had significantly decreased likelihoods of developing other labor and delivery complications not specifically categorized by the ODH dataset (Crude OR: 0.57, 95% CI: 0.38–0.85; Crude OR: 0.51, 95% CI: 0.35–0.74, respectively).

Maternal Risk Factors

63 of 168 (37.5%) LHWA Only mothers had one or more risk factors commonly associated with potentially adverse pregnancy outcomes in comparison to 460 of 1171 (39.3%) mothers residing within the No LHWA water service area (Adjusted OR: 0.94, 95%CI: 0.67–1.3). 72 of 209 (34.4%) mothers within Partial LHWA communities had similar risk factors (Adjusted OR: 0.74 95%CI: 0.53–1.0). In the adjusted model, which was adjusted for maternal age, race, maternal education, parity, smoking and alcohol use, the cumulative likelihood of maternal risk factors across water service strata was not significantly different ($p>0.05$). Mothers aged 30 to 34 years and mothers aged 40 years and older were associated with an overall increased likelihood of developing any risk factor (Adjusted OR: 1.5, 95%CI: 1.1–2.0; Adjusted OR: 3.5, 95% CI: 1.6–7.5) as compared to mothers 25 to 29 years of age. Additionally, mothers within the LHWA had a significant increase in the likelihood of anemia compared to mothers outside the LHWA (Crude OR: 11, 95%CI: 1.8–64), but the number of reported cases was small ($N_{\text{LHWA Only}} = 3$; $N_{\text{No LHWA}} = 2$). A modest increase in the likelihood of eclampsia was also observed among No LHWA mothers, but the increase was not statistically significant (Crude OR: 7.0, 95% CI: 0.99–50). Mothers residing within the Partial LHWA also had a significantly decreased risk of small for gestational age (SGA)

compared to the No LHWA mothers (Crude OR: 0.14 95%CI: 0.02–0.99). Odds ratios and individual risk factors are reported (Figure 3).

DISCUSSION

Increased PFOA exposure, as assessed by water service category, was not associated with an increased likelihood of congenital anomalies (individually or in aggregate). Additionally, the cumulative incidence of anomalies in any of the water service categories was not significantly different from the national incidence of 3%. Large point estimates were observed for diagnose-specific crude odds ratios (e.g. club foot, Down Syndrome, etc.), but these estimates were not precise on account of small frequencies and none reached the level of statistical significance. In the fully adjusted model only smoking was found to be positively associated with a greater likelihood of anomaly development, which is consistent with published reports on the effect of tobacco use during pregnancy [48].

The overall likelihood of labor and delivery complications was significantly lower among LHWA Only mothers by 7.8% to 54.3% (95% CI), as compared to mothers within the No LHWA service category. No difference in the likelihood of complications was observed between mothers in the Partial and No LHWA strata. In the adjusted model, multiparous women had a reduced likelihood of experiencing a complication as compared to primiparous women. Differences in the cumulative incidences of complications among primiparous and multiparous women were in part the result of cephalopelvic disproportion, which is a common presentation in first time mothers. Mothers within communities partially serviced by the LHWA had a significantly decreased risk of small for gestational age (SGA) as compared to No LHWA mothers (Crude OR: 0.14 95%CI: 0.02–0.99), but no equivalent reduction was seen among LHWA Only mothers. In the adjusted model for risk factors, 30–34 year-old mothers and mothers aged 40 years and above had a significantly greater likelihood of having a risk factor as compared to mothers aged 25–29 years. This finding mirrored the tendency for mothers aged 40 and above to also have a greater likelihood of labor and delivery complications. These results were not unexpected since increased maternal age has long been established as a risk factor for many complications associated with pregnancy [49].

Two statistically significant adverse associations between water service category and outcome were observed in our analysis. Mothers residing within the LHWA Only water service area had a statistically significant increase in the likelihood of anemia as compared to LHWA Only mothers, but the number of reported cases was very small ($N_{\text{LHWA Only}}$= 2; $N_{\text{No LHWA}}$ = 3) and no relationship was observed for anemia within the Partial LHWA. Similarly, LHWA Only mothers had a significant increase in the likelihood of dysfunctional labor. Again, the number of reported cases was small ($N_{\text{LHWA Only}}$ = 3, $N_{\text{No LHWA}}$ = 4) and no similar increase was observed among mothers in the Partial LHWA.

Given the low frequency of events, broad confidence intervals, inability to adjust for confounding and the absence of a potential dose-response relationship, the veracity of these associations is not certain. With respect to anemia, our finding may represent a statistical aberration since the hematological parameters indicative of this diagnosis have not been reported in either murine or primate toxicological assessments [11–12,50]. Additionally, neither our previous serological analysis of specimens taken from LHWA residents nor any of the major occupational cohort studies of workers within the fluoropolymer manufacturing industry detected an association between PFOA exposure and reduced red blood cell count or lowered hemoglobin levels [51–54]. Nevertheless, we do not necessarily dismiss a potential biologic basis for our findings, particularly since no human investigation has examined these hematological parameters during pregnancy when the effect of PFOA

exposure may be compounded by the inherent risk of anemia resulting from plasma volume expansion and increased iron demands of the fetus [55–56]. Furthermore, PFOA exposure in the mouse is associated with reduced transferrin gene expression [57] and at least one occupational study of PFOA observed an association between PFOA exposure and altered serum iron levels [54]. In light of these findings, we conclude that additional research is required to further assess this possible association.

For dysfunctional labor, a potential physiologic association with increased PFOA exposure is less apparent. Though recent research suggests that myometrial lactic acidosis and a decrease in oxygen saturation may lead to inefficient uterine contractions, there is nothing in the literature to suggest an association between acidosis and PFOA in animal studies [58]. Moreover, the diagnosis of dysfunctional labor applies more broadly to several etiologies encompassing cervical, fetal, uterine and iatrogenic complications. Since it is not certain which of these etiologies were implicated in the few cases (N=3) of dysfunctional labor occurring in the No LHWA water service category, it impossible to assess whether they may be associated with a common causative agent.

Lastly, we note that the C8 Science Project observed a modestly elevated risk associated with PFOA exposure and self-reported cases of preeclampsia in residents of the LHWA who submitted serum samples between 2005 and 2006 [39]. We report a similar finding with respect to the incidence of eclampsia in our study, which trended higher in LHWA Only area mothers. However, this trend did not reach the level of statistical significance and the number of reported cases was quite small ($N_{LHWA\ Only}$= 2; $N_{No\ LHWA}$ = 2).

Overall, our failure to detect an association between PFOA and the majority of birth or pregnancy outcomes is consistent with expectations based on experimental animal studies. Growth and developmental delays observed in mice were noted only at serum PFOA levels that were orders of magnitude greater than the levels observed in LHWA residents without occupational exposure [11–12]. Though highly exposed in comparison to the general U.S population, the levels of serum PFOA among residents serviced by the LHWA may not be sufficiently high to affect birth outcomes. Nonetheless, caution is warranted when extrapolating the results of murine toxicological studies to human populations particularly since the serum half-life of PFOA differs dramatically between species.

To correlate our outcomes with PFOA, we considered population PFOA exposure to be highest among zip codes exclusively serviced by the LHWA, intermediate in zip codes partially serviced by the LHWA and lowest in zip codes not serviced by the LHWA. Although the robustness of our methodology has been demonstrated by its ability to identify known predictors of birth outcome and to yield results concordant with studies that directly measured serum PFOA in the same population, it has potential limitations. In particular, the lack of individual exposure levels may introduce exposure misclassification (resulting from the consumption of drinking water outside LHWA supply areas, the use of bottled water or a home filter and the mobility of pregnant women) into our study. Mothers residing outside the LHWA service area may have consumed contaminated water at work or during critical windows of fetal development when the impact of that exposure would be most significant. Misclassification of these mothers into the No LHWA water service category would potentially bias our associations towards the null. Although mobility data are not available, the results of our prior investigations regarding PFOA exposure in the LHWA service area suggest that misclassification resulting from filtered or bottled water use may be minimized since the vast majority of LHWA residents consumed some public water (82% consumed public water exclusively) and had serum PFOA levels well above background levels for the general US population. Our previous studies also revealed that the exclusive use of bottled

water was small (3%) and that individuals who utilized a carbon water filter had only mild decreases in their serum PFOA levels (25%) [33,52].

We also must consider the possibility that the use of a data set with live born neonates may skew our conclusions regarding the association between PFOA and congenital anomalies. If exposed neonates were more likely to develop congenital defects, and if those defect resulted in spontaneous or elective termination of the pregnancy early in gestation, then the exclusion of stillborn neonates from the data set could potentially bias our associations towards the null. Nevertheless, there is no indication that stillborn rates vary within the water service categories Washington County. Furthermore, murine models pregnancy loss was only observed at the highest levels of exposures (40ppm) which were far greater than the concentrations of PFOA observed in the LHWA population [10–12].

Finally, the ODH database may be subject to reporting error; however, almost all births occurred in four local community hospitals that service residents from all zip codes in Washington County. If reporting errors do exist in the data set, we have no reason to believe that they would not be randomly distributed among water service categories and bias the results in favor of a particular association. Moreover, while the fidelity of the ODH data set with respect to the reporting of congenital anomalies, maternal risk factors and labor and delivery complications has not been assessed, the ODH observed a 90% congruence between the reporting of birth weight on birth certificates and medical records and a 60% congruence for the reporting of gestational age [59]. We have no reason to believe that the reporting of the outcomes used in this investigation should be any less congruent than that of birth weight or gestational age.

In summary, our findings suggest that that PFOA is not associated with an increased incidence of congenital anomalies and most labor and delivery complications and maternal risk factors. Further investigation is required to assess the validity of the reported associations between anemia, dysfunctional labor and PFOA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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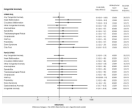

FIGURE 1.
Odds Ratios for Congenital Anomalies and Event Frequencies by Water Service Category

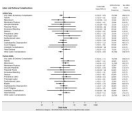

FIGURE 2.
Odds Ratios for Labor/Delivery Complications and Event Frequencies by Water Service Category

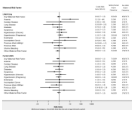

FIGURE 3.
Odds Ratios for Maternal Risk Factors and Event Frequencies by Water Service Category

TABLE 1

Mean PFOA Levels (μg/L) in Public Water Supplies for Washington County, Ohio

Public Water Facility	Mean[†] (±SD), Median (Range) PFOA (μg/L)	Dates of Sampling	PFOA Plasma Sampling[*] Median (μg/L)
LHWA	6.78 (4.2) 5.7 (1.7 – 17.1)	2002 – 2005	132.5 (0.6 – 22,412)
Belpre	0.21 (0.027) 0.22 (0.17 – 0.24)	2002 – 2005	27.1 (0.5 – 7,932)
Marietta	0.0065 (0.0074) 0.0049 (0 – 0.017)	2007	-
Warren	0.007 (0.012) 0.0 (0.0 – 0.021)	2007	-

[†]This value is the average and median of PFOA levels in each of the production wells of the indicated public water facility, as measured by the Ohio State Department of Environmental Protection

[*]From the C8 Science Project [1–2]

TABLE 2

Maternal and Neonatal Demographic Characteristics by Water Service Category

	N, %		
	LHWA Only (N=168)	**Parital LHWA (N=209)**	**No LHWA (N=1171)**
Infant Sex			
Male	73 (43.5%)	109 (52.2%)	591 (50.5%)
Female	95 (56.5%)	100 (47.8%)	580 (49.5%)
Race			
White	167 (99.4%)	202 (96.7%)	1143 (97.6%)
Black	0 (0.0%)	6 (2.9%)	13 (1.1%)
Other	1 (0.6%)	1 (0.4%)	15 (1.3%)
Maternal Education			
< High School	12 (7.1%)	28 (13.4%)	147 (12.5%)
High School Degree	63 (37.5%)	70 (33.5%)	446 (38.2%)
College Degree	86 (51.1%)	102 (48.8%)	552 (44.5%)
Advanced Degree	7 (4.2%)	9 (4.3%)	54 (4.6%)
Parity			
Primiparous	68 (40.5%)	85 (40.7%)	529 (45.2%)
Multiparous	100 (59.5%)	124 (59.3%)	542 (54.8%)
Smoking Status During Pregnancy			
Smoker	33 (19.6%)	38 (18.2%)	258 (22.0%)
Non-Smoker	135 (80.4%)	171 (81.8%)	913 (78.0%)
Alcohol Use During Pregnancy			
Yes	0 (0.0%)	1 (0.5%)	5 (0.4%)
No	168 (100%)	208 (99.5%)	1166 (99.6%)
Diabetic Status			
Diabetic	4 (2.4%)	7 (3.4%)	39 (3.3%)
Non Diabetic	164 (97.6%)	202 (96.6%)	1132 (96.7%)
	Mean ± SD		
Mean Maternal Age (Years ± SD)	26.9 ± 4.9	26.6 ± 6.3	26.0 ± 5.5
Mean Gestation (Weeks ± SD)	38.3 ± 2.0	38.1 ± 2.1	38.4 ± 2.5
Mean Birth Weight (Grams ± SD)	3276 ± 422	3278 ± 461	3264 ± 569

Differences in proportions or means are not significant ($p>0.05$) across water service categories for all characteristics.