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Maternal periconceptional exposure to drinking water disinfection by-products and neural tube defects in offspring

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Abstract

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, the Environmental Protection Agency nor the views of the North Carolina Department of Health and Human Services, Division of Public Health.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Background: Associations between maternal periconceptional exposure to disinfection by-products (DBPs) in drinking water and neural tube defects (NTDs) in offspring are inconclusive, limited in part by exposure misclassification.

Methods: Maternal interview reports of drinking water sources and consumption from the National Birth Defects Prevention Study were linked with DBP concentrations in public water system monitoring data for case children with an NTD and control children delivered during 2000–2005. DBPs analyzed were total trihalomethanes, the five most common haloacetic acids combined, and individual species. Associations were estimated for all NTDs combined and selected subtypes (spina bifida, anencephaly) with maternal periconceptional exposure to DBPs in public water systems and with average daily periconceptional ingestion of DBPs accounting for individual-level consumption and filtration information. Mixed effects logistic regression models with maternal race/ethnicity and educational attainment at delivery as fixed effects and study site as a random intercept were applied.

Results: Overall, 111 case and 649 control children were eligible for analyses. Adjusted odds ratios for maternal exposure to DBPs in public water systems ranged from 0.8–1.5 for all NTDs combined, 0.6–2.0 for spina bifida, and 0.7–1.9 for anencephaly; respective ranges for average daily maternal ingestion of DBPs were 0.7–1.1, 0.5–1.5, and 0.6–1.8. Several positive estimates (≥1.2) were observed, but all confidence intervals included the null.

Conclusions: Using community- and individual-level data from a large, US, population-based, case–control study, we observed statistically nonsignificant associations between maternal periconceptional exposure to total and individual DBP species in drinking water and NTDs and subtypes.

Keywords

anencephaly; drinking water; haloacetic acides; neural tube defects; pregnancy; spina bifida; trihalomethanes

1 | INTRODUCTION

Neural tube defects (NTDs), largely comprising anencephaly and spina bifida subtypes, are characterized by abnormal closure of the neural tube or abnormal formation of the brain and spinal cord from the neural tube during embryogenesis. Offspring affected by anencephaly die at or before birth, whereas those with spina bifida require early surgery to close the spinal lesion, and may experience life-long paralysis, bladder and bowel dysfunction, hydrocephalus, and other health complications (Alabi et al., 2018; Botto et al., 1999). The estimated prevalence of NTDs in the United States (US) following mandatory folic acid fortification of cereal grains is seven per 10,000 live births (Williams et al., 2015). NTDs have a multifactorial etiology, including both genetic and non-genetic risk factors (Agopian et al., 2013; Copp & Greene, 2010; Lupo et al., 2017).

Disinfection by-products (DBPs) are common contaminants formed during the water disinfection process; humans are exposed to DBPs through ingestion, inhalation, and dermal absorption (Richardson & Postigo, 2012). Common disinfectants, such as chlorine, used in treating public drinking water, can react with bromide and other natural organic

matter in water and produce a complex mixture of DBPs, including trihalomethanes (THMs) and haloacetic acids (HAAs) (Singer, 1994). Citing health concerns associated with DBP exposure, the US Environmental Protection Agency established primary standards for total THMs (TTHM) and the five HAAs most commonly found in drinking water (HAA5) under the Safe Drinking Water Act (US Environmental Protection Agency, 2023). The US Environmental Protection Agency defines TTHM as the sum of four species: bromoform, chloroform, bromodichloromethane, and dibromochloromethane; HAA5 includes chloroacetic acid, dichloroacetic acid, trichloroacetic acid, bromoacetic acid, and dibromoacetic acid. Maximum Contaminant Levels (MCLs) have been established and are enforced at 80 and 60 μg/L of drinking water for TTHM and HAA5, respectively (US Environmental Protection Agency, 2010).

DBPs are associated with endocrine disruption and subsequent adverse reproductive and developmental health outcomes (Gonsioroski et al., 2020). Associations reported for maternal DBP exposure and NTDs are mixed (Bove et al., 1995; Dodds et al., 1999; Dodds & King, 2001; Hwang et al., 2002, 2008; Kallen & Robert, 2000; Klotz & Pyrch, 1999; Magnus et al., 1999; Nieuwenhuijsen et al., 2008; Righi et al., 2012; Save-Soderbergh et al., 2021; Shaw et al., 2003). Animal models provide minimal support for positive associations (reviewed in Tardiff et al., 2006) and little insight into a potential mode of action (reviewed in Colman et al., 2011). Human studies are equivocal, with some studies reporting positive associations between TTHM and NTDs (Bove et al., 1995; Hwang et al., 2008; Klotz & Pyrch, 1999) or a combined group of birth defects affecting the nervous system (Save-Soderbergh et al., 2021). Two studies examined individual THMs and NTDs, with one reporting associations for total brominated THMs and bromoform near unity (Nieuwenhuijsen et al., 2008) and the other reporting positive associations for bromodichloromethane, particularly among the most highly exposed women (Dodds & King, 2001). A study that examined HAA exposure reported associations with NTDs near unity or modestly increased (Klotz & Pyrch, 1999). Other studies that examined water chlorination, rather than specific by-products, reported slightly increased associations for NTDs (Magnus et al., 1999) and spina bifida (Kallen & Robert, 2000). A meta-analysis of DBPs and birth defects reported modestly increased summary estimates for spina bifida (five studies) and anencephaly (four studies), with confidence intervals including the null, and summary estimates for NTDs (eight studies) near the null, although these latter estimates included other central nervous system defects; and most used data prior to 2000 (Nieuwenhuijsen et al., 2009).

Equivocal findings for DBPs and NTDs have been attributed, in part, to measurement bias and inconsistencies in exposure assessment approaches (Bove et al., 2002). Except for one study (Shaw et al., 2003), these approaches lacked data to examine individual-level maternal consumption estimates. Given the limitations in previous studies, we estimated maternal DBP exposure from both community-level measurements from public water systems (community and non-community systems) and individual-level ingestion by using maternal self-reports of water consumption collected for the population-based National Birth Defects Prevention Study (NBDPS). Associations between maternal periconceptional exposure to total and individual species of THMs and HAAs were examined for all NTDs combined and selected subtypes (spina bifida, anencephaly) in offspring.

2 | METHODS

2.1 | NBDPS

NBDPS was a multisite, population-based case–control study conducted in the US, to investigate genetic and environmental (broadly defined) factors for major structural birth defects. A detailed description of NBDPS methods has been published (Reefhuis et al., 2015). Briefly, case and control mothers with pregnancies ending during or after October 1997 through pregnancies with an estimated date of delivery (EDD) during or before December 2011 were identified by birth defects surveillance programs in 10 states (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, Utah). Pregnancy outcomes including live births, fetal deaths at 20 weeks or greater gestation, and elective terminations at any gestational age were collectively considered to be eligible case children. Eligible control children were live births without a diagnosis of a major birth defect, selected through random sampling from birth certificates or birth hospitals in the same surveillance catchment area as case children in each NBDPS site. Case children with major defects due to underlying monogenic or chromosomal etiologies were not eligible for NBDPS. Mothers of case or control children were excluded from NBDPS if they had participated in the study with a previous pregnancy, could not complete the interview in English or Spanish, were incarcerated, or otherwise did not have legal custody of the child at the time of recruitment. The study protocol was approved by the institutional review board at each NBDPS site.

2.2 | Data collection

Maternal interviews were conducted by telephone in either English- or Spanish-language 6 weeks to 24 months following EDD. Mothers provided informed consent prior to participation. Data were collected on parental socio-demographics and family history of birth defects, along with maternal health history, prenatal care, residence history, and various exposures (e.g., infectious, chemical, physical, nutritional, and behavioral factors) beginning 3 months prior to conception through the EDD or end of pregnancy. Additionally, a detailed drinking water module was included for mothers with EDDs during 2000–2005 to collect data on maternal water sources, residential water treatment, drinking water consumption, and additional water use. This analysis used NBDPS data from sites funded during 2000–2005 (Arkansas, Georgia, Iowa, Massachusetts, New York, Texas) and during 2003–2005 (North Carolina, Utah) that had access to water quality data from individual public water systems.

2.3 | Outcomes

Case children eligible for this study included those diagnosed with NTDs (modified British Pediatric Association diagnostic code) defined in NBDPS as spina bifida (741.000–741.990), anencephaly (including craniorachischisis; 740.020, 740.100), and encephalocele (including cranial meningocele and encephalomyelocele; 742.000–742.090). Clinical geneticists at each NBDPS site reviewed data abstracted from medical records to confirm the NTD diagnosis and classify the child as having an isolated (i.e., NTD with no additional major, unrelated defects), multiple (i.e., NTD with one or more major, unrelated

defects), or complex (i.e., NTD with a pattern of major defects that are embryologically or pathogenically related) phenotype (Rasmussen et al., 2003).

2.4 | DBP concentration estimates

Methods used to estimate DBP exposures were described by Weyer et al. (2018). Briefly, the NBDPS interview collected detailed information on the mother's full address and residency start date (month, year) and end date (month, year). Addresses reported by all NBDPS mothers were geocoded using Centrus (Group 1 Software; Lanham, MD). Geocoding was done at the address location, street segment, or Zip centroid as data permitted at each participating site. Overall, 97% successfully matched at any level and 89% at the address level. Standardized exposure assessment approaches developed, coordinated, and conducted at the University of Iowa Center for Health Effects of Environmental Contamination were used to link geocoded addresses to public water systems across all included states. If information on the boundaries of a given public water system was lacking (e.g., cities served by multiple public water systems lacking service area boundaries within the district), the geocoded residence was linked to the public water system that served the largest number of residences in the city. Available concentrations for TTHM, HAA5, and the individual species of THMs and HAAs; sampling date; and location (distribution system/plant effluent) were obtained for each linked public water system identified in accordance with Safe Drinking Water Act regulations and guidelines (US Environmental Protection Agency, 2010). Where measurements for each of the THM and HAA species were available, but not for TTHM and HAA5, the individual species were summed and used as TTHM and HAA5 measurements. Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analyses of individual DBPs.

An inverse-time weighted mean was estimated using all sample measurement days (limiting to a maximum of 10 measurement days) for each available THM and HAA level during the period between 1 month before conception (B1) and 3 months after conception (P3) due to fluctuations in time and space in DBP concentrations throughout the system. The B1–P3 period was used to account for relevant embryologic developmental periods for all NBDPS-eligible defects and maternal pre-pregnancy behaviors that may have extended into 1 month after conception (P1) before pregnancy recognition. A higher weight was assigned to sample measurement days closest to the estimated date of conception of each case and control mother. In instances where multiple DBP sample measurements were taken in a single day at different locations served by the public water system, a mean concentration for each THM and HAA was used as the measure of the exposure for that day.

2.5 | Maternal water consumption estimates

The NBDPS interview obtained information from the mothers about whether the drinking water at the residence closest to their estimated date of conception was from a private well or public water system. Additional information collected included chemical disinfection (private wells only) and filtration of water for cooking or drinking (none, whole house filter, faucet filter, etc.), and if filtered, then the type of filter used (membrane, charcoal, etc.), along with the frequency of filter changes per year. For each reported residence, drinking water source(s) (unfiltered tap, filtered tap, bottled, other), number of 8 oz. glasses of water

consumed per day from each source, water sources used to make hot drinks and for cooking, and information on changes in drinking water consumption from B3 (3 months before conception) through the end of the pregnancy (the month of a change in amount, number of 8 oz. glasses of water consumed per day after a change in amount, and water sources used for drinking after a change in source) were obtained. Drinking water source(s) (unfiltered tap, filtered tap, drinking fountain [coded as unfiltered tap], bottled/cooler, brought from home, other), and the average number of 8 oz. glasses consumed per day from each source, were obtained to quantify exposure at each job (if employed). Where possible, responses indicating "other" water source at home or work, were recoded into one of the predefined sources. The NDBPS interview also captured water use activities including washing dishes and clothes, showering and bathing, and swimming.

Because the neural tube completes closure by day 28 after conception (Sadler, 1998), the periconceptional exposure period most relevant for NTDs includes B1–P1. Daily total water consumption during B1–P1 from each water source was estimated using a number of 8 oz. glasses of water at home, plus the number of 8 oz. glasses per each day at work (if employed). Total consumption estimates accounted for changes in estimated daily amounts of water consumption and starting or stopping work at a job if these changes occurred during B1–P1. Changes in consumption were applied to one-half of the 30-day period during which the change occurred. The number of 8 oz. glasses of water consumed per day from each source after a change in estimated daily consumption amount was not collected; thus, total drinking water consumption was distributed proportionally to the distribution of water sources before the change in consumption. The timing of a change in water sources was also not collected, so, for the purposes of this assessment, it was assumed that any changes in water sources occurred after the periconceptional period. It was also assumed that the distribution of water sources that mothers reported bringing from home to work to be proportional to the estimated distribution of water sources at home. An additional assumption was that water from a drinking fountain at work was unfiltered tap water. Consumption data for water sources used to make hot drinks and for cooking were not reported.

2.6 | Maternal DBP ingestion estimates from public water systems

Information on DBP concentrations in tap water at home and information on maternal water consumption at home and at work during the periconceptional period were combined to estimate maternal ingestion of DBPs. Private well water and bottled water were assigned 0 μg/L of DBP exposure due to minimal reported disinfection treatments. When measurements were near or below the level of detection, or reported to be 0 μg/L for DBP exposure, they were unchanged and used in the analyses as reported. For employed mothers, the water source at work was assigned to the same water district as her residence, as household estimates have been shown to be reasonable proxies for workplace estimates (Zaganjor et al., 2022). For mothers who reported a filtration system on their residential tap water source, the types or brands of filters reported were matched to the list of National Sanitation Foundation international certified drinking water treatment units to determine whether they could remove DBPs (National Science Foundation, 2020). When brand name was not reported, the effectiveness of DBP removal of the reported filter was determined based

on the description of the filtration method. Filters listed as being able to remove DBPs were assumed to reduce DBP concentrations to 10% of that measured in the public water system, whereas those unable or with undetermined capacity to remove DBPs were assumed to reduce the concentrations to 90% of that in the public water system. Because the interview did not collect information regarding the types of filters used at work, those filters were assumed to reduce the concentrations to 90% of that in the public water system. Maternal total ingestion of DBPs during the periconceptional period was estimated by multiplying the amount of tap water consumed at home and work by the concentration measured in the linked public water system, accounting for filtration information. Average daily ingestion was estimated by dividing that total by 60 days.

2.7 | Statistical analysis

Case children classified with complex defects and case and control mothers with a reported diagnosis of pregestational diabetes or periconceptional use of folate antagonist medications (aminopterin sodium, carbamazepine, cholestyramine resin, methotrexate, oxcarbazepine, pyrimethamine, sulfasalazine, triamterene, trimethoprim, phenytoin, primidone, phenobarbital, valproate sodium) were excluded. Both pregestational diabetes and the use of folate antagonist medication during the periconceptional period have been associated with an increased risk of NTDs. Mothers were eligible for inclusion if they resided at the same residence throughout B1–P1 and their DBP ingestion could be estimated.

Child characteristics evaluated included gestational age (<37, ≥37 weeks), pregnancy outcome (live birth, fetal death [≥20 weeks gestation], induced abortion), sex, family history of NTDs in a parent or sibling (yes/no), and plurality (single, multiple). Maternal characteristics evaluated were age (<20, 20–34, ≥35 years) and educational attainment at delivery (less than high school, high school graduate, post-secondary education); race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other); gravidity (1, 2, ≥3); pre-pregnancy body mass index (BMI; <18.5, 18.5–<25.0, 25.0–<30.0, ≥30.0 kg/m^2) and dietary folate intake (<600, ≥600 μg/day); periconceptional cigarette smoking (no active or passive smoking, active smoking only, passive smoking only, active and passive smoking), alcohol consumption (no drinking, binge drinking [≥4 drinks on one occasion], drinking but no binge drinking), use of folic acid-containing supplements (yes, no), and fever (yes, no); average shower duration (assessed around the time the participant became pregnant) (<15, ≥15 min); and study site (Arkansas, Georgia, Iowa, Massachusetts, New York, North Carolina, Texas, Utah). Race/ethnicity was included as a proxy for unmeasurable confounders such as access to health care, exposure to structural racism, racial inequity in unemployment and education, and residential segregation (Benmarhnia et al., 2021; Bishop-Royse et al., 2021; Wallace et al., 2017).

To evaluate the representativeness of case children and control children that were available to be included in the analytical sample, selected child and maternal characteristics and maternal exposures were compared to those of all case children and all control children, respectively, using chi-square goodness-of-fit tests (calculating exact *p*-values if expected cell counts <5). These characteristics and exposures were also compared between case

children and control children eligible for analysis using chi-square tests of independence or Fisher's exact tests (if expected cell counts <5).

Univariate analyses examined residential concentrations and average daily maternal ingestion of TTHM, HAA5, and individual species during the periconceptional period. Measures of distribution including mean, standard deviation, and quartile cut points were assessed for case and control children separately.

Associations between maternal periconceptional exposure to DBPs and NTDs were analyzed for all NTDs combined and selected NTD subtypes (spina bifida and anencephaly). Encephalocele was not examined due to insufficient sample of children eligible for analysis. Associations were examined between the outcomes and maternal exposure to TTHM, HAA5, and individual species in public tap water (measured in μg/L). Compared to ≤one-half the MCL for TTHM and HAA5 or Maximum Contaminant Level Goal (MCLG; non-enforced guideline) for individual THMs and HAAs, associations were examined at levels >one-half the MCL/MCLG to the MCL/MCLG and >the MCL/MCLG where data permitted and MCLGs were available. Associations were also examined for average daily maternal ingestion of TTHM, HAA5, and their individual species at the level of >the 50th percentile compared to ≤the 50th percentile, derived from the exposure distribution among the control mothers.

Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were estimated using mixed effects logistic regression models. Associations between DBPs and NTDs were analyzed for all NTDs combined and selected subtypes (spina bifida, anencephaly). Analyses were conducted where there were at least five exposed case mothers. Maternal race/ethnicity, educational attainment at delivery, and study site were identified as adjustment variables from a Directed Acyclic Graph (DAG). Maternal race/ethnicity and educational attainment were included in the logistic regression models as fixed effects, and study site was included as a random intercept (Figure S1). The DAG for confounding assessment was generated using the R package "DAGitty" (Textor et al., 2016). All analyses were conducted using the Statistical Analysis System (Rosenthal, #108) version 9.4 statistical software (SAS institute, Cary, NC).

3 | RESULTS

Overall, 924 NTD case and 5083 control mothers with EDDs during 2000–2005 completed the NBDPS interview (Figure 1). Excluded were 3 mothers (case = 3) whose children were classified with complex defects, 47 (case = 16; control = 31) with a reported diagnosis or an incomplete response for pregestational diabetes, 141 (case = 27; control = 114) who reported use of folate antagonist medications during B1–P1, 1175 (case = 211; control = 964) with residences in NBDPS sites that did not contribute DBP exposure data, 369 (case = 82; control = 287) who relocated during B1–P1, and 1571 (case = 233; control = 1338) with insufficient interview data to estimate exposure. Of the remaining 352 case and 2349 control mothers, 1914 (case = 262; control = 1652) reported drinking tap water provided by public water systems. Among mothers who reportedly drank public tap water, 760 (case = 111; control = 649) had their residential address geocoded and linked to public water systems

for which DBP measurements were available. These 760 mothers comprised our analytical sample (case = 111; control = 649). The 111 case children included 68 with spina bifida, 34 with anencephaly, and nine with encephalocele, of which 56, 33, and 7 presented with an isolated phenotype. The remaining case children were classified with a multiple phenotype.

Selected child and maternal characteristics and maternal exposures were compared between all control children (after exclusions for pregestational diabetes, use of periconceptional folate antagonist medications, and residence in NBDPS sites that did not contribute DBP exposure data) ($n = 3974$) and those ($n = 649$) included in the analytical sample (Table S1). These groups did not differ statistically ($p > .05$) for child characteristics but did differ ($p < .05$) for each maternal characteristic examined except periconceptional fever. Comparison of selected characteristics and exposures between NBDPS case children with all NTDs combined ($n = 667$) and those in the analytical sample ($n = 111$) showed statistical differences for pregnancy outcome, maternal age and educational attainment at delivery, race/ethnicity, study site, and periconceptional folic acid-containing supplement use. Comparisons were also made for eligible NTD case children ($n = 111$) and eligible control children ($n = 649$) included in the analytical sample (Table 1). Differences were observed for gestational age, gravidity, and study site.

Univariate analysis showing the distributions of THM and HAA concentrations in public tap water among control and case children included in the analytical sample are presented in Table 2, and correlations between these concentrations are presented in Table S2. Multivariable models were adjusted for race/ethnicity and maternal educational attainment at delivery as fixed effects; study site was included as a random intercept. Observed aOR estimates for maternal periconceptional exposure to TTHM, HAA5, and individual THM and HAA species in public tap water ranged from 0.8 to 1.5 for all NTDs combined (Table 3). Positive associations (≥1.2) were observed for exposure to chloroform levels > the MCLG and trichloroacetic acid levels that were >one-half the MCLG to the MCLG; all CIs included the null. Associations for spina bifida ranged from 0.6 to 2.0, including positive estimates observed for exposure to bromoform levels > the MCLG and trichloroacetic acid levels that were >one-half the MCLG to the MCLG. Associations for anencephaly ranged from 0.7 to 1.9, including positive estimates for exposure to TTHM levels that were >one-half the MCL to the MCL and trichloroacetic acid levels > the MCLG. All CIs for associations with spina bifida or anencephaly included the null.

Distributions of average daily THM and HAA ingestion during the periconceptional period among case and control mothers are presented in Table 4, and correlations between these estimated ingestion amounts are presented in Table S3. Compared to exposure ≤ the 50th percentile among control mothers, associations for maternal periconceptional ingestion > the 50th percentile for TTHM, HAA5, and individual THM and HAA species per day ranged from 0.7 to 1.1 for all NTDs combined with all CIs including the null (Table 5). Associations for spina bifida ranged from 0.5 to 1.5, including a positive estimate for bromoform, and those for anencephaly ranged from 0.6 to 1.8, with positive estimates for TTHM, HAA5, chloroform, dibromochloromethane, chloroacetic acid, dichloroacetic acid, trichloroacetic acid, and dibromoacetic acid. All CIs for associations with spina bifida or anencephaly included the null.

4 | DISCUSSION

We examined associations between maternal periconceptional exposure to DBPs from public tap water consumption and NTDs using community- and individual-level data from a large, US, population-based, case–control study. Several positive associations were observed for all NTDs combined, spina bifida, and anencephaly with TTHM, HAA5, and selected DBP species; however, all CIs included the null.

Previous studies examining associations between DBPs (including TTHM and HAA5, and their individual species) and NTDs (including individual subtypes) have been largely inconclusive (Bove et al., 2002; Dodds et al., 1999; Dodds & King, 2001; Hwang et al., 2002, 2008; Kallen & Robert, 2000; Klotz & Pyrch, 1999; Magnus et al., 1999; Nieuwenhuijsen et al., 2008; Righi et al., 2012; Save-Soderbergh et al., 2021; Shaw et al., 2003). Although previous US studies on DBPs and NTDs were population-based, they predated our study periods and were restricted to California and New Jersey (Bove et al., 1995; Klotz & Pyrch, 1999; Shaw et al., 2003); our study sample included data from eight other US states. Positive associations (≥1.2) in previous US studies for all DBPs reported (total or individual species) ranged from 1.2 to 5.4 with all confidence intervals containing the null. With the exclusion of the null study by Nieuwenhuijsen et al. (2008), the range of positive associations from international studies was similar (1.2–5.0) to US studies, with some studies reporting CIs that excluded the null (Dodds & King, 2001; Hwang et al., 2002; Righi et al., 2012; Save-Soderbergh et al., 2021). The range of positive associations observed in our study (1.2–1.5) for DBP concentration in public water systems and all NTDs combined was within the range for the above cited studies, and all CIs contained the null. We acknowledge, however, that our study and many other published studies are likely underpowered to detect associations that may be relatively small in magnitude for these rare outcomes.

The cutoffs used to describe DBP exposure have varied across studies, and most reported associations were between all NTDs combined and TTHM. Previous US studies reported positive associations at 20 μg/L and higher (Bove et al., 1995) and 40 μg/L and higher (Klotz & Pyrch, 1999) compared to <20 μg/L, and at 1–24 or 50–74 μg/L (Study 2, Shaw et al., 2003) compared to 0 μg/L. International studies reported positive associations at ≥100 μg/L compared to <50 μg/L (Dodds et al., 1999) and at 1 μg/L or higher compared to <1 μg/L (Righi et al., 2012) for all NTDs combined and >15 μg/L compared to exposure to non-chlorinated water for any nervous system defect (Save-Soderbergh et al., 2021). The wide range of exposure contrasts and referent population cutpoints used may preclude drawing more direct comparisons between studies resulting in a challenge when evaluating the totality of evidence, especially for studies which cannot evaluate higher levels of DBPs that may be relevant. In our study, we did not observe positive associations between all NTDs combined and TTHM as measured in public tap water even though our categories overlapped with those of the above studies. We also did not observe positive associations for all NTDs combined and TTHM as estimated by average daily ingestion; these comparisons are not directly comparable to any previous study due to our inclusion of individual-level water consumption. Few studies included or examined associations for individual species of THMs. Of those studies that included individual species and had sufficient numbers

to analyze, positive associations with all NTDs combined were reported for ≥100 μg/L of chloroform compared to 0–49 μg/L and 5–9 or ≥20 μg/L of bromodichloromethane compared to <5 μg/L (Dodds & King, 2001) and ≥1.7 μg/L compared to <1.7 μg/L chlorodibromomethane (dibromochloromethane) (Study 2, Shaw et al., 2003). Testing associations for individual THM species in public tap water in our study were limited to bromoform and chloroform due to small numbers. Our study showed a positive association below the levels previously reported for chloroform and all NTDs combined (>70 μg/L compared to ≤35 μg/L), but above the MCLG. We did not observe positive associations for individual THM species and all NTDs combined when measured by ingestion.

Two reports analyzed NTD subtypes, such as spina bifida and anencephaly, and TTHM (Hwang et al., 2008; Shaw et al., 2003). Hwang et al. (2008) reported positive associations for TTHM and anencephaly for levels at 5–9 and ≥20 μg/L compared to 0–4 μg/L; the CIs contained the null. Shaw et al. (2003) reported positive associations for stratified analyses incorporating ingestion and TTHM exposure for anencephaly (≥50 μg/L vs. <50 μg/L and ≥5 glasses/day). Compared to 0 μg/L TTHM, Shaw et al. (2003) also reported positive associations for all cutoffs at or higher than 1 μg/L for spina bifida and for cutoffs of 1–24 and 50–74 μg/L for anencephaly, with all CIs containing the null. In our analysis of TTHM as measured in the public water supply and NTD subtypes, we observed a positive association for TTHM and anencephaly at exposure levels that fell within the ranges of previous studies at levels in the range of 50%–100% of the MCL. For estimated average daily ingestion of TTHM, we observed a positive association for anencephaly at levels >the 50th percentile of exposure among control mothers (23.4 μg/day). None of the previous studies reported associations for individual species of THMs and NTD subtypes. In our study, positive associations for individual species of THMs in the public water supply were observed for any bromoform exposure (>0 μg/L) and spina bifida. We also observed positive associations accounting for ingestion at levels > the 50th percentile of exposure among control mothers for bromoform and spina bifida (>0.1 μg/day), chloroform and anencephaly (>12.3 μg/day), and dibromochloromethane and anencephaly (>1.4 μg/day).

Outside of TTHM, some studies reported positive associations for measures of water quality not directly comparable to exposures quantified in our study, including A-280 s (Bove et al., 1995), haloacetonitriles (Klotz & Pyrch, 1999), specific chemical treatments such as chlorine dioxide or sodium hypochloride (Kallen & Robert, 2000), chlorate or chlorite (Righi et al., 2012), and hypochlorite or chloramine (Save-Soderbergh et al., 2021), or a combination of chlorination status and dissolved organic compounds (i.e. water color) (Hwang et al., 2002; Magnus et al., 1999). A single study reported associations between NTDs and HAAs, observing positive associations for all NTDs combined and HAA5 at 35 μg/L compared to <3 μg/L (Klotz & Pyrch, 1999). Using public tap water, we did not observe positive associations for HAA5, but we did observe associations for the individual HAA species, trichloroacetic acid. Specifically, we observed positive associations for all NTDs combined and spina bifida at levels in the range of 50%–100% (10–20 μg/L) of the MCLG (20 μg/L) each and at levels greater than the MCL for anencephaly. For exposure estimates using average daily ingestion, we observed positive associations at levels >the 50th percentile of exposure among control mothers for anencephaly and HAA5 (>13.9 μg/day),

chloroacetic acid (>0.5 μg/day), dichloroacetic acid (>8.5 μg/day), trichloroacetic acid (>5.3 μg/day), and dibromoacetic acid (>0.1 μg/day).

Our findings should be interpreted with caution. We did not include spontaneous abortions, as these outcomes are difficult to ascertain in routine birth defects surveillance, and our study was restricted to THM and HAA species that were regulated by the US Environmental Protection Agency during the study period. Also, we did not examine associations for case children with isolated and multiple phenotypes separately due to the high proportion of case children with an isolated phenotype (86.5%), nor did we examine the impact of heterogeneity of effect due to folic acid intake as was explored in two previous studies from California. These studies were conducted during 1987–1991, a time before mandatory folic acid fortification policy was implemented (Shaw et al., 2003). However, the protective effect of folic acid supplement intake on NTD risk has been shown to be neutral in the era of fortification in the US (Ahrens et al., 2011), the period examined in this study. Additionally, a large proportion of case and control mothers were not included in our analytical sample (Figure 1). We examined the influence of this attrition on the representativeness of our analytical sample, observing little difference for child characteristics but several differences for maternal characteristics and exposures (Table S1); these were either not identified as adjustment variables by our DAG or were included in adjusted models (Figure S1). Further, despite our attempt to comprehensively assess maternal DBP exposure, our estimates of periconceptional average daily ingestion lacked information on water sources used to make hot drinks and for cooking, and information collected relied on retrospective self-reports of water consumption that could have been negatively impacted by imprecise recall. However, compared to some other teratogenic or stigmatizing exposures (e.g., medications, tobacco, and alcohol use), recall related to water consumption may be less likely to lead to differential misclassification of exposure between case and control mothers. Another limitation is that there is the potential for exposure misclassification based on the nature and timing of public water system testing processes in the US and for measurement errors in DBP concentrations (Nieuwenhuijsen et al., 2000; Parvez et al., 2017), although these are not expected to result in differential misclassification. Additionally, some measurement error is anticipated in the assignment of 0 (μg/L) DBPs for bottled water users as some bottled water is known to be packaged tap water which may contain DBPs. There may be some error in the measurement of DBP concentrations near the level of detection, however since these measurements would be grouped in the lowest exposure category, the likelihood of substantial bias being introduced is relatively low. Lastly, weighted average DBP measurement data were only available for the B1–P3 period. However, due to the use of an inverse-time weighted mean, measurement values taken closest to the estimated date of conception were given the highest weight in calculating the average DBP concentration for a residence, which would coincide with the critical period for NTD risk (B1–P1).

Our study had several strengths, which improved upon the methodological limitations reported in previous studies. All case children were identified from population-based surveillance programs with clinical data abstracted from medical records reviewed by clinical geneticists, reducing the potential for misclassification. Control children were selected randomly, reducing the potential for selection bias. The study examined NTDs that occurred in live births, stillbirths, and induced abortions as well as selected NTD

subtypes (spina bifida, anencephaly). We used a detailed maternal interview module that collected individual-level information on all maternal drinking water sources, residential water filtration, drinking water consumption frequency by source, and additional water use activities, during the periconceptional period. Unlike many of the previous studies that relied only on community-level exposure to DBPs, our study also assessed individual-level exposure using geocoded maternal addresses linked to relevant public water systems, improving specificity of the exposure. Our inclusion criterion that the mother resided at the same residence throughout B1–P1 reduced the measurement error in this study. A stringent algorithm was used to estimate DBP ingestion, considering total and individual DBPs. Examining the effects of individual DBP species allowed us to quantify their effects and assess individual variations in toxicity. Time and space fluctuations of DBPs are possible, and these were integrated into the exposure assessment for each public water system. Data on several characteristics and exposures available in NBDPS assisted us in building comprehensive DAGs to identify potential confounders which were included in our multivariable analyses.

5 | CONCLUSION

We observed no statistically significant associations between maternal periconceptional exposure to total and individual DBP species and NTDs and selected subtypes; however, there were a few estimates that indicated positive associations. Continued improvements in the assessment of individual-level exposure data may be warranted to build on the current knowledge. The various teratogenic mechanisms that increase the risk of NTDs and their individual subtypes through exposure to various DBPs in a dose-dependent manner could be informed by future research with larger sample sizes. Additionally, interactions between different DBPs that may create synergistic effects of increased toxicity could be explored. The above steps may help to inform and develop guidelines to better monitor and simultaneously minimize the adverse effects of DBPs on human reproduction and health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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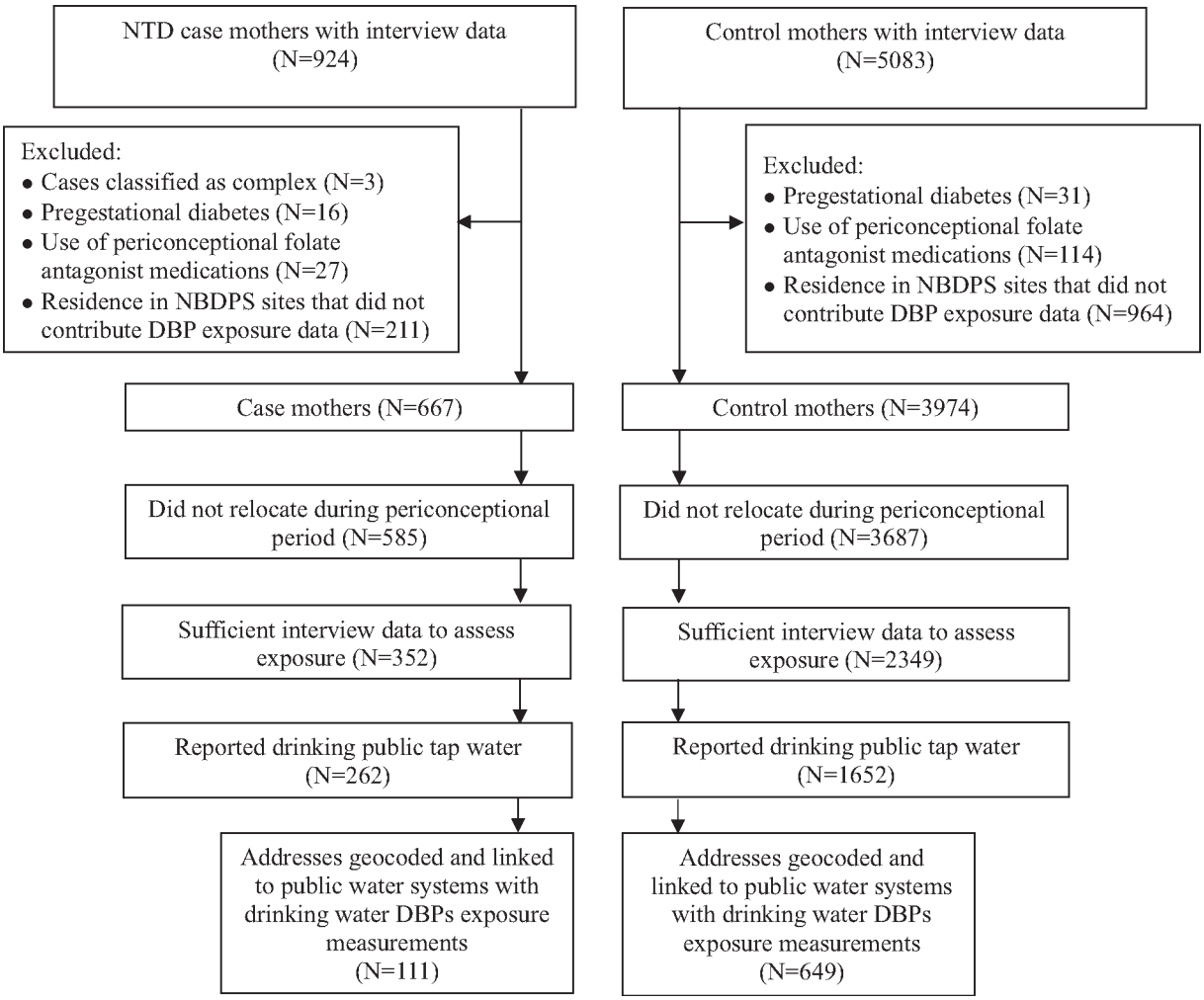

FIGURE 1.

Subject selection flow chart—National Birth Defects Prevention Study, 2000–2005. DBPs, disinfection by-products; NBDPS, National Birth Defects. Prevention Study; NTD, neural tube defects.

TABLE 1

Chi-square tests of independence for child and maternal characteristics and maternal exposures for participants included in the analytical sample, National Birth Defects Prevention Study, 2000–2005.

	Control children		Case children, all NTDs combined		
Characteristics	***N*** [a]	**%**	***N*** [a]	**%**	***p***
Total	**649**		**111**		
Child characteristics					
Pregnancy outcome					
Livebirth	649	100.0	69	62.2	NC
Fetal death (≥20 weeks gestation)	0	0.0	3	2.7	
Induced abortion	0	0.0	39	35.1	
Missing	0		0		
Sex					
Male	339	52.2	52	52.5	.96
Female	310	47.8	47	47.5	
Missing	0		12		
Gestational age (weeks)					
Preterm (<37)	70	10.8	57	51.4	<.01
Term (≥37)	579	89.2	54	48.6	
Missing	0		0		
Family history of NTDs					
First-degree relative	1	0.2	1	0.9	.27[b]
None	648	99.8	110	99.1	
Missing	0		0		
Plurality					
Single birth	622	95.8	104	93.7	.32[b]
Multiple birth	27	4.2	7	6.3	
Missing	0		0		
Maternal characteristics					
Age at delivery (years)					
<20	37	5.7	3	2.7	.23

Characteristics	Control children N [a]	Control children %	Case children, all NTDs combined N [a]	Case children, all NTDs combined %	p
20–34	508	78.3	85	76.6	
≥35	104	16.0	23	20.7	
Missing	0		0		
Educational attainment at delivery (years)					
<12	43	6.6	10	9.0	.46
12	131	20.2	18	16.2	
>12	475	73.2	83	74.8	
Missing	0		0		
Race/Ethnicity					
Non-Hispanic white	451	69.5	77	69.4	.95
Non-Hispanic black	95	14.6	15	13.5	
Hispanic	70	10.8	12	10.8	
Other	33	5.1	7	6.3	
Missing	0		0		
Gravidity					
First pregnancy	188	29.0	38	34.2	.04
Second pregnancy	226	34.8	25	22.5	
Third or later pregnancy	235	36.2	48	43.2	
Missing	0		0		
Pre-pregnancy BMI (kg/m^2)					
Underweight (<18.5)	17	2.7	6	5.7	.43
Normal (18.5-<25.0)	370	58.2	60	56.6	
Overweight (25.0-<30.0)	149	23.4	23	21.7	
Obese (≥30.0)	100	15.7	17	16.0	
Missing	13		5		
Study site					
Arkansas	124	19.1	20	18.0	.04
Georgia	109	16.8	15	13.5	
Iowa	143	22.0	25	22.5	
Massachusetts	77	11.9	4	3.6	

Characteristics	Control children N [a]	Control children %	Case children, all NTDs combined N [a]	Case children, all NTDs combined %	p
New York	13	2.0	3	2.7	
North Carolina [c]	95	14.6	22	19.8	
Texas	49	7.6	8	7.2	
Utah [c]	39	6.0	14	12.6	
Missing	0		0		
Maternal exposures					
Periconceptional cigarette smoking [d]					
No active or passive smoking	477	73.6	73	65.8	.07
Active smoking only	44	6.8	5	4.5	
Passive smoking only	83	12.8	24	21.6	
Active and passive smoking	44	6.8	9	8.1	
Missing	1		0		
Periconceptional alcohol consumption [d]					
No drinking	401	62.3	70	64.8	0.88
Binge drinking (≥4 drinks/occasion)	82	12.7	13	12.0	
Drinking but no binge drinking	161	25.0	25	23.1	
Missing	5		3		
Prepregnancy dietary folate intake (μg/day)					
<600	489	75.3	86	77.5	.63
≥600	160	24.7	25	22.5	
Missing	0		0		
Periconceptional folic acid containing supplement use [d]					
Yes	408	63.2	76	71.0	.12
No	238	36.8	31	29.0	
Missing	3		4		
Periconceptional fever [d]					
Yes	28	4.8	9	9.8	.05
No	560	95.2	83	90.2	
Missing	61		19		

Characteristics	Control children N [a]	Control children %	Case children, all NTDs combined N [a]	Case children, all NTDs combined %	p
Average shower duration [e]					
<15 min	366	57.5	53	51.0	.21
≥15 min	270	42.5	51	49.0	
Missing	13		7		

Note: Because of rounding, percentages might not total 100.

Abbreviations: BMI, body mass index; NC, not calculated; NTD, neural tube defect.

[a] Missing values not included in chi-square tests.

[b] Fisher's exact test.

[c] Includes estimated dates of delivery during 2003–2005.

[d] Periconceptional period defined as 1 month before conception and 1 month after conception.

[e] Assessed around the time the participant became pregnant.

TABLE 2

Summary of distributions of residential trihalomethanes (THM) and haloacetic acids (HAA) concentrations in public tap water among participants included in the analytical sample, National Birth Defects Prevention Study, 2000–2005.

	Control children						Case children, all NTDs combined					
Exposure (μg/L, B1-P1)[a]	***N***	**Mean**	**SD**	**p25**	**p50**	**p75**	***N***	**Mean**	**SD**	**p25**	**p50**	**p75**
TTHM	643	43.5	35.9	21.6	38.0	55.0	111	39.9	37.1	18.1	36.3	53.3
Bromoform	472	2.5	7.0	0.0	0.5	1.4	80	2.3	4.8	0.0	0.2	2.0
Chloroform	472	28.2	30.4	8.1	22.2	36.7	80	28.3	30.9	5.9	23.1	37.3
Bromodichloromethane	471	8.7	7.3	4.7	7.3	10.6	80	9.3	10.1	4.0	7.0	12.5
Dibromochloromethane	471	4.4	6.8	1.0	2.1	5.6	80	4.3	6.1	0.9	1.9	6.0
HAA5	459	29.8	26.8	13.3	25.7	38.5	87	30.0	34.1	14.2	26.3	36.6
Chloroacetic acid	388	2.8	4.4	0.0	2.0	3.3	72	3.1	7.6	0.0	2.0	3.3
Dichloroacetic acid	388	15.8	14.2	7.6	13.0	20.7	72	16.6	16.4	9.3	14.3	17.6
Trichloroacetic acid	388	12.0	11.7	3.4	10.6	16.3	72	12.6	11.3	6.9	11.2	14.0
Bromoacetic acid	388	0.7	1.3	0.0	0.0	1.0	72	0.8	2.4	0.0	0.0	1.0
Dibromoacetic acid	388	1.6	3.1	0.0	0.6	1.7	72	1.4	4.6	0.0	0.0	1.4

Abbreviations: B1,1 month before conception; HAA5, group of five most common haloacetic acids; NTD, neural tube defect; P1,1 month after conception; p25, 25th percentile; p50, 50th percentile; p75, 75th percentile; SD, standard deviation; TTHM, total trihalomethanes.

[a]Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analysis of individual disinfection by-products.

TABLE 3

Odds ratios for neural tube defects (NTDs) and residential trihalomethane (THM) and haloacetic acid (HAA) concentrations in public tap water for participants included in the analytical sample, National Birth Defects Prevention Study, 2000–2005.

	Control children (*N* = 649)		Case children, all NTDs combined (*N* = 111)[a]			Case children, spina bifida (*N* = 68)			Case children, anencephaly (*N* = 34)		
Exposure (B1-P1)	***N***	**%**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**
TTHM[c]											
≤1/2 MCL	343	53.3	62	55.9	Reference	45	66.2	Reference	13	38.2	Reference
>1/2 MCL - MCL	238	37.0	40	36.0	0.9 (0.6, 1.4)	19	27.9	0.6 (0.3, 1.1)	17	50.0	1.9 (0.9, 4.1)
>MCL	62	9.6	9	8.1	0.8 (0.3, 1.6)	4	5.9	NC	4	11.8	NC
Missing	6		0			0			0		
Individual THMs[d,e,f]											
Bromoform[g]											
≤MCLG	218	46.2	40	50.0	Reference	19	42.2	Reference	14	51.9	Reference
>MCLG	254	53.8	40	50.0	0.9 (0.5, 1.5)	26	57.8	1.3 (0.7, 2.4)	13	48.1	0.7 (0.3, 1.9)
Missing	177		31			23			7		
Chloroform[h]											
≤1/2 MCLG	341	72.2	56	70.0	Reference	34	75.6	Reference	18	66.7	Reference
>1/2 MCLG - MCLG	100	21.2	18	22.5	1.0 (0.6, 1.9)	10	22.2	0.9 (0.4, 2.0)	5	18.5	0.9 (0.3, 2.5)
>MCLG	31	6.6	6	7.5	1.2 (0.5, 3.0)	1	2.2	NC	4	14.8	NC
Missing	177		31			23			7		
HAAS[i]											
≤1/2 MCL	266	58.0	50	57.5	Reference	32	64.0	Reference	14	50.0	Reference
>1/2 MCL - MCL	161	35.1	31	35.6	1.0 (0.6, 1.7)	17	34.0	0.9 (0.5, 1.7)	10	35.7	1.0 (0.4, 2.5)
>MCL	32	7.0	6	6.9	0.9 (0.3, 2.3)	1	2.0	NC	4	14.3	NC
Missing	190		24			18			6		
Individual HAAs4[d,j,k,l]											
Trichloroacetic acid[m]											
≤1/2 MCLG	182	46.9	28	38.9	Reference	14	35.9	Reference	12	48.0	Reference
>1/2 MCLG - MCLG	155	39.9	36	50.0	1.5 (0.9, 2.7)	23	59.0	2.0 (1.0, 4.0)	8	32.0	0.9 (0.3, 2.3)
>MCLG	51	13.1	8	11.1	1.0 (0.4, 2.4)	2	5.1	NC	5	20.0	1.6 (0.5, 5.2)

	Control children (*N* = 649)		Case children, all NTDs combined (*N* = 111)[a]			Case children, spina bifida (*N* = 68)			Case children, anencephaly (*N* = 34)		
Exposure (B1-P1)	***N***	**%**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**
Missing	261		39			29			9		

Note: Because of rounding, percentages might not total 100.

Abbreviations: aOR, adjusted odds ratio; B1, 1 month before conception; CI, confidence interval; HAA5, group of five most common haloacetic acids; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; NC, not calculated; P1, 1 month after conception; TTHM, total trihalomethanes.

[a] Nine case children had encephalocele.

[b] Adjusted for maternal race/ethnicity and maternal educational attainment at delivery; study site was included as a random intercept.

[c] MCL for TTHM is 80 μg/L.

[d] Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analysis of individual disinfection by-products.

[e] MCLG for bromodichloromethane is 0 μg/L, but there were fewer than five case children unexposed.

[f] MCLG for dibromochloromethane is 60 μg/L, but there were fewer than five control children or case children exposed to >1/2 MCLG-MCLG or >MCLG.

[g] MCLG for bromoform is 0 μg/L.

[h] MCLG for chloroform is 70 μg/L.

[i] MCL for HAAS is 60 μg/L.

[j] MCLG for chloroacetic acid is 70 μg/L, but there were fewer than 5 control children or case children exposed >1/2 MCLG-MCLG or >MCLG.

[k] MCLG for dichloroacetic acid is 0 μg/L, but there were fewer than five case children unexposed.

[l] No established MCLG for bromoacetic acid or dibromoacetic acid.

[m] MCLG for trichloroacetic acid is 20 μg/L.

TABLE 4

Summary of distributions of average daily maternal ingestion of trihalomethanes (THMs) and haloacetic acids (HAAs) among participants included in the analytical sample, National Birth Defects Prevention Study, 2000–2005.

	Control children						Case children, all NTDs combined					
Exposure (μg/day, B1-P1)[a]	***N***	**Mean**	**SD**	**p25**	**p50**	**p75**	***N***	**Mean**	**SD**	**p25**	**p50**	**p75**
TTHM	643	38.0	50.9	7.0	23.4	49.8	111	33.9	35.3	5.2	18.8	52.0
Bromoform	472	2.6	10.1	0.0	0.1	1.2	80	1.9	6.3	0.0	0.0	1.0
Chloroform	472	24.8	37.3	2.5	12.3	31.9	80	20.7	25.1	2.1	10.7	31.2
Bromodichloromethane	471	8.0	11.8	1.5	4.7	10.1	80	6.9	8.2	1.5	3.8	9.5
Dibromochloromethane	471	4.4	12.9	0.4	1.4	4.4	80	3.2	4.6	0.3	1.2	3.9
HAA5	459	27.3	40.3	4.2	13.9	35.9	87	23.0	24.2	5.7	11.7	33.9
Chloroacetic acid	388	2.5	5.3	0.0	0.5	2.7	72	2.1	3.0	0.0	0.6	3.0
Dichloroacetic acid	388	14.7	20.8	3.0	8.5	19.7	72	12.1	11.2	3.4	8.5	19.4
Trichloroacetic acid	388	11.2	17.9	1.4	5.3	15.2	72	10.1	11.0	2.3	5.4	14.6
Bromoacetic acid	388	0.6	1.4	0.0	0.0	0.7	72	0.8	2.5	0.0	0.0	0.3
Dibromoacetic acid	388	1.6	4.7	0.0	0.1	1.4	72	0.8	1.5	0.0	0.0	0.9

Abbreviations: B1, 1 month before conception; HAA5, group of five most common haloacetic acids; NTD, neural tube defect;P1, 1 month after conception; p25, 25th percentile; p50, 50th percentile; p75, 75th percentile; SD, standard deviation; TTHM, total trihalomethanes.

[a]Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analysis of individual disinfection by-products.

TABLE 5

Odds ratios for neural tube defects (NTDs) and average daily maternal ingestion of trihalomethanes (THMs) and haloacetic acids (HAAs) for participants included in the analytical sample, National Birth Defects Prevention Study, 2000–2005.

	Control children (*N* = 649)		Case children, all NTDs combined (*N* = 111)[a]			Case children, spina bifida (*N* = 68)			Case children, anencephaly (*N* = 34)		
Exposure percentile (B1-P1)	***N***	**%**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**
TTHM											
≤50th	322	50.1	59	53.2	Reference	41	60.3	Reference	14	41.2	Reference
>50th	321	49.9	52	46.8	0.9 (0.6, 1.3)	27	39.7	0.7 (0.4, 1.1)	20	58.8	1.4 (0.7, 3.0)
Missing	6		0			0			0		
Individual THMs[c]											
Bromoform											
≤50th	237	50.2	40	50.0	Reference	19	42.2	Reference	14	51.9	Reference
>50th	235	49.8	40	50.0	1.1 (0.7, 1.8)	26	57.8	1.5 (0.8, 2.9)	13	48.1	0.9 (0.4, 2.4)
Missing	177		31			23			7		
Chloroform											
≤50th	236	50.0	43	53.8	Reference	29	64.4	Reference	11	40.7	Reference
>50th	236	50.0	37	46.3	0.8 (0.5, 1.4)	16	35.6	0.5 (0.3, 1.0)	16	59.3	1.5 (0.6, 3.6)
Missing	177		31			23			7		
Bromodichloromethane											
≤50th	236	50.1	46	57.5	Reference	29	64.4	Reference	14	51.9	Reference
>50th	235	49.9	34	42.5	0.7 (0.4, 1.2)	16	35.6	0.5 (0.3, 1.0)	13	48.1	0.9 (0.4, 2.0)
Missing	178		31			23			7		
Dibromochloromethane											
≤50th	238	50.5	42	52.5	Reference	25	55.6	Reference	11	40.7	Reference
>50th	233	49.5	38	47.5	0.9 (0.6, 1.5)	20	44.4	0.8 (0.4, 1.5)	16	59.3	1.4 (0.6, 3.3)
Missing	178		31			23			7		
HAA5											
≤50th	230	50.1	46	52.9	Reference	30	60.0	Reference	12	42.9	Reference
>50th	229	49.9	41	47.1	0.8 (0.5, 1.4)	20	40.0	0.7 (0.4, 1.2)	16	57.1	1.3 (0.6, 2.9)
Missing	190		24			18			6		
Individual HAA[c]											

	Control children (*N* = 649)		Case children, all NTDs combined (*N* = 111)[a]			Case children, spina bifida (*N* = 68)			Case children, anencephaly (*N* = 34)		
Exposure percentile (B1-P1)	***N***	**%**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**	***N***	**%**	**aOR (95% CI)[b]**
Chloroacetic acid											
≤50th	196	50.5	35	48.6	Reference	19	48.7	Reference	10	40.0	Reference
>50th	192	49.5	37	51.4	1.1 (0.6, 1.8)	20	51.3	1.0 (0.5, 2.0)	15	60.0	1.6 (0.7, 4.1)
Missing	261		39			29			9		
Dichloroacetic add											
≤50th	194	50.0	36	50.0	Reference	23	59.0	Reference	10	40.0	Reference
>50th	194	50.0	36	50.0	1.0 (0.6, 1.7)	16	41.0	0.7 (0.4, 1.4)	15	60.0	1.6 (0.7, 3.9)
Missing	261		39			29			9		
Trichloroacetic acid											
≤50th	194	50.0	35	48.6	Reference	22	56.4	Reference	10	40.0	Reference
>50th	194	50.0	37	51.4	1.1 (0.6, 1.8)	17	43.6	0.8 (0.4, 1.5)	15	60.0	1.7 (0.7, 4.1)
Missing	261		39			29			9		
Bromoacetic acid											
≤50th	216	55.7	46	63.9	Reference	23	59.0	Reference	17	68.0	Reference
>50th	172	44.3	26	36.1	0.7 (0.4, 1.2)	16	41.0	0.8 (0.4, 1.7)	8	32.0	0.6 (0.2, 1.6)
Missing	261		39			29			9		
Dibromoacetic acid											
≤50th	194	50.0	37	51.4	Reference	22	56.4	Reference	9	36.0	Reference
>50th	194	50.0	35	48.6	0.9 (0.6, 1.6)	17	43.6	0.7 (0.4, 1.5)	16	64.0	1.8 (0.7, 4.7)
Missing	261		39			29			9		

Note: Because of rounding, percentages might not total 100.

Abbreviations: aOR, adjusted odds ratio; B1, 1 month before conception; CI, confidence interval; HAA5, group of five most common haloacetic acids; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; P1, 1 month after conception; TTHM, total trihalomethanes.

[a] Nine case children had encephalocele.

[b] Adjusted for maternal race/ethnicity and maternal educational attainment at delivery; study site was included as a random intercept.

[c] Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analysis of individual disinfection by-products.