Assessing bottled water nitrate concentrations to evaluate total drinking water nitrate exposure and risk of birth defects

Peter J. Weyer,
Center for Health Environmental Effects of Contamination, University of Iowa, Iowa City, Iowa, USA

Jean D. Brender,
Department of Epidemiology and Biostatistics, School of Rural Public Health, Texas A&M Health Science Center, Texas A&M University, College Station, Texas, USA

Paul A. Romitti,
Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, Iowa, USA

Jiji R. Kantamneni,
Center for Health Environmental Effects of Contamination, University of Iowa, Iowa City, Iowa, USA

David Crawford,
Center for Health Environmental Effects of Contamination, University of Iowa, Iowa City, Iowa, USA

Joseph R. Sharkey,
Department of Health Promotion and Community Health Sciences, School of Rural Public Health, Texas A&M Health Science Center, College Station, Texas, USA

Mayura Shinde,
Department of Epidemiology and Biostatistics, School of Rural Public Health, Texas A&M Health Science Center, Texas A&M University, College Station, Texas, USA

Scott A. Horel,
Department of Epidemiology and Biostatistics, School of Rural Public Health, Texas A&M Health Science Center, Texas A&M University, College Station, Texas, USA

Ann M. Vuong, and
Department of Epidemiology and Biostatistics, School of Rural Public Health, Texas A&M Health Science Center, Texas A&M University, College Station, Texas, USA

Peter H. Langlois
Texas Department of State Health Services, Austin, Texas, USA

corresponding author: peter-weyer@uiowa.edu.

ETHICS STATEMENT

Free and informed consent of the participants or their legal representatives was obtained and the study protocol was approved by the Human Subjects Office at the University of Iowa, Iowa City, IA, USA, Institutional Review Board ID # 200702800 (4/10/2007); met the criteria for exemption by the Office of Research Compliance at Texas A&M University, College Station, TX, Institutional Review Board ID # 2006-0682 (12/14/2006); and was approved by the Texas Department of State Health Services, Austin, TX, Institutional Review Board ID # 06-093 (2/15/2007).

Abstract

Previous epidemiologic studies of maternal exposure to drinking water nitrate did not account for bottled water consumption. The objective of this National Birth Defects Prevention Study (NBDPS) (USA) analysis was to assess the impact of bottled water use on the relation between maternal exposure to drinking water nitrate and selected birth defects in infants born during 1997–2005. Prenatal residences of 1,410 mothers reporting exclusive bottled water use were geocoded and mapped; 326 bottled water samples were collected and analyzed using Environmental Protection Agency Method 300.0. Median bottled water nitrate concentrations were assigned by community; mothers' overall intake of nitrate in mg/day from drinking water was calculated. Odds ratios for neural tube defects, limb deficiencies, oral cleft defects, and heart defects were estimated using mixed-effects models for logistic regression. Odds ratios (95% CIs) for the highest exposure group in offspring of mothers reporting exclusive use of bottled water were: neural tube defects [1.42 (0.51, 3.99)], limb deficiencies [1.86 (0.51, 6.80)], oral clefts [1.43 (0.61, 3.31)], and heart defects [2.13, (0.87, 5.17)]. Bottled water nitrate had no appreciable impact on risk for birth defects in the NBDPS.

Keywords

birth defects; bottled water; nitrate

BACKGROUND

Nitrate is a common contaminant in groundwater sources in the Midwest and other agricultural areas of the United States (Nolan *et al.* 1998). Agricultural land-use practices contribute to seasonal nitrate pollution of surface waters in these same areas (Mueller & Helsel 2013). Public water supplies use groundwater and surface water sources to provide drinking water to local and regional populations. The US Environmental Protection Agency (EPA) drinking water standard for nitrate in public water supplies is $10 \, \text{mg/L} \, \text{NO}_3^- \, \text{N}$ (nitrate-nitrogen), or $45 \, \text{mg/L} \, \text{NO}_3^-$ (total nitrate) (hence-forth, 'nitrate' refers to total nitrate). The nitrate drinking water standard was established in response to concerns related to the risk for methemoglobinemia in infants less than 6 months of age who are fed infant formula mixed with water containing elevated nitrate. Private drinking water wells are not regulated by EPA, and may also be at risk of nitrate contamination.

Bottled water from groundwater sources may also be a source of nitrate exposure. The nitrate content in groundwater depends on nitrogen input from land-use activities, aquifer vulnerability, and natural atmospheric deposition (Nolan *et al.* 1998). In the USA, bottled water use has risen substantially over the past several decades and may be underestimated given the expansion of water kiosks (stand-alone water dispensers). In the late 1970s, about 350 million gallons (1,325 M liters) of bottled water were sold yearly; by 2008, annual sales had increased to about 9 billion gallons (34 B liters). In 2008, it was estimated that an average American consumed about 30 gallons (114 liters) of bottled water annually (Gleick 2010). The US Food and Drug Administration (FDA) is responsible for the safety of bottled waters, including monitoring and inspecting bottled water products and processing plants, and requiring that companies analyze their source water and product water for contaminants

(Posnick & Henry 2002). The allowable concentration for nitrate-nitrogen in bottled water is 10, or 45 mg/L total nitrate (FDA 1998). FDA regulations require that bottled waters that exceed allowable concentrations of nitrate must have information on the label saying 'Contains Excessive Nitrate'. There is no requirement to list the actual numeric nitrate concentration on the label.

The scientific literature contains limited information on nitrate concentrations in bottled waters sold in the USA. Breuer *et al.* (1990) analyzed 39 bottled water samples purchased from supermarkets in several Iowa communities and detected nitrate in 18 samples ranging from 1 to 23 mg/L nitrate. Ikem *et al.* (2002) collected 25 brands of bottled water in an Alabama study; all brands had nitrate + nitrite content <50 mg/L, and the sum of these contaminants ranged from 0.1 to 14.6 mg/L. In a Houston study (Saleh *et al.* 2008), nitrate in bottled waters ranged from below the detection limit to 50.01 mg/L total nitrate, with spring water having the highest concentrations. In 2008, the State Hygienic Laboratory (SHL) at the University of Iowa tested 10 major brands of bottled water for nitrate and nitrite content as part of a study conducted by the Environmental Working Group; nitrate was detected in six brands ranging from 0.45 to 7.7 mg/L total nitrate (Naidenko *et al.* 2008).

Previous epidemiologic studies have reported associations between a mother's prenatal exposure to nitrate in drinking water and birth defects in their offspring (Dorsch *et al.* 1984; Croen *et al.* 2001; Brender *et al.* 2004). These studies did not account for individual consumption levels of nitrate, or the mother's use of bottled water, which includes water purchased from vending machines (dispensed water). The risk for selected birth defects associated with maternal prenatal exposure to nitrate in drinking water, the diet and nitrosatable drugs in the National Birth Defects Prevention Study (NBDPS) has been previously reported (Brender *et al.* 2013). Here, we present detailed information on the bottled water nitrate assessment conducted in two NBDPS locations on intake of drinking water nitrate and risk for birth defects.

Our main objective was to assess the impact of bottled water use on the relation between maternal exposure to nitrate from drinking water and selected birth defects in the mother's offspring. Specific aims were to: (1) examine the nitrate content of bottled water commonly sold in communities where Iowa and Texas NBDPS participants resided; (2) compare nitrate concentrations found in bottled water samples within these communities to those detected in the respective community water supplies; and (3) include bottled water nitrate concentrations in the main analysis of risk for selected birth defects.

METHODS

The NBDPS is a multi-site (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah) population-based case-control study of birth defects in the USA (Yoon *et al.* 2001). The NBDPS drinking water nitrate study included Iowa and Texas case and control mothers who completed the NBDPS telephone interview. Cases were live births, stillbirths, or elective terminations with estimated delivery dates from October 1, 1997 through December 31, 2005 and diagnosed with a neural tube defect, oral cleft defect, limb deficiency, or congenital heart defect. Controls were live births

without any major birth defects delivered during the same time period and study area randomly selected from birth certificates in Iowa and from hospital delivery records in Texas. NBDPS controls were randomly selected from two data sources. In Iowa, Massachusetts, New Jersey, North Carolina, and Utah, controls were selected from live birth certificates, while in California, New York, and Texas, controls were selected from hospital records (Cogswell *et al.* 2009). Arkansas and Georgia controls were selected from hospital records until 2001, when they switched selection to live birth certificates.

Case and control mothers were asked questions (telephone interview) about personal water use beginning in 2000, including sources (private well water, unfiltered tap, filtered tap, bottled, other); presence and type of filtration; quantity of water drunk at home and at work or school on an average day; and any changes including month/year of change in source or quantity of drinking water consumed (Brender *et al.* 2013). Information on brands of bottled water consumed was not collected, due to interviewing time considerations. Prenatal (1 month pre-conception [B1] through the first trimester of pregnancy [P1-P3]) residences of 1,069 Texas mothers and 341 Iowa mothers who reported using bottled water exclusively were geocoded and mapped. We were unable to successfully geocode the prenatal residences of an additional 25 mothers who reported exclusive use of bottled water. Nearby retail grocery and convenience stores were identified using 3–5 mile buffers around maternal prenatal residences. From January through May 2010, study staff visited 42 Iowa and 32 Texas communities that mothers lived in or nearby and collected bottled water samples. Bottled waters were chosen to include various prices, brands, and types of water. In addition, dispensed waters sold by the gallon were obtained in Iowa and Texas stores, water mills, and water vending machines. These water dispensers generally filter tap water (already treated by the municipal water utility) with reverse osmosis before dispensing. All bottled and dispensed water samples were analyzed at the SHL using EPA Method 300.0 (Pfaff 1993). In samples where nitrate was below the detection limit, one-half the detection limit was assigned.

Information was not available on the brands of bottled water mothers drank, therefore, median nitrate concentrations were calculated for each community based on all bottled and dispensed water results for that community, regardless of the source of water in the bottled or dispensed water samples. If a mother lived in a community that was not included in the water collection sampling frame, results from the most proximate community in which water was collected were used. In this manner, all Texas and Iowa mothers in the study who reported exclusive use of bottled water were assigned a bottled water nitrate exposure level. Drinking water nitrate exposure levels for individuals were calculated through a multi-step process: (1) maternal residential addresses during the prenatal period (B1-P3) were geocoded; (2) geocoded addresses were linked to appropriate community water supply service areas; (3) time-appropriate drinking water nitrate data from the community supplies were linked to each mother's address during B1-P3 ('time appropriate' data were either results of water samples taken during B1-P3, or if no samples were available from that period, samples taken closest to B1 and P3 were used); (4) bottled water median nitrate concentrations were calculated for mothers reporting use of bottled water; and (5) total nitrate from drinking water per day was calculated. Total nitrate consisted of the sum of number of glasses (240 cc) of water consumed from each source of drinking water × the

respective nitrate for that source for the relevant respective time periods of pregnancy. If a woman lived in residences served by different water systems, nitrate from tap water and bottled water for those areas were weighted according to length of residence during the respective time periods of interest. For mothers who reported drinking bottled water in addition to tap water, the respective nitrate concentrations in tap and bottled water were used to calculate the overall drinking water nitrate exposure. Details of this approach and results of the overall analysis have been reported elsewhere (Brender *et al.* 2013). With respect to in-home water filtration, reverse osmosis or membrane filters and distillation are considered to be among the most effective methods to remove nitrate from water (Clifford *et al.* 1986; Seidel *et al.* 2011). If a woman reported using reverse osmosis or membrane filters to filter drinking water in the home, nitrate levels in public water supplies servicing these households were multiplied by 0.1 to account for the filtration effects from such systems; if a distillation process was used, nitrate values were multiplied by 0.01. Mean and median concentrations of nitrate in drinking water were compared with respect to source (bottled water, public water supplies) and location (Iowa, Texas, within the USA, outside the USA). Overall intake of nitrate in mg/day from drinking water was categorized into tertiles based on control mother distributions for estimates during the B1-P1 period for neural tube defects, and during the B1-P3 period for limb deficiencies, oral cleft defects, and congenital heart defects. Crude and adjusted odds ratios for these defects were estimated with mixed-effects (random-effects) models for logistic regression with the lowest tertile of nitrate intake from drinking water serving as the referent group. Variables adjusted for included maternal race/ethnicity, education, age, multivitamin/folic acid supplementation, smoking, and study center. As part of a sensitivity analysis, we compared odds ratio estimates for each defect between all mothers for which we could estimate nitrate intake from drinking water and the subset of mothers who reported only drinking community tap water.

RESULTS

A total of 1,435 NBDPS participants in Iowa and Texas reported consuming bottled water exclusively during their prenatal period. We were able to successfully geocode the prenatal residences of 1,410 of those women (1,069 Texas mothers, 341 Iowa mothers). Approximately 50.4% of case and control mothers combined reported community water as their usual source of drinking water. Bottled water was the next most commonly reported source at 30.5%, followed by private wells at 5.2%. Information was not available on usual home source of drinking water for 13.9% of mothers. Table 1 presents data on the mother's usual home source of drinking water by the offspring's birth defect category (from Brender *et al.* 2013).

In the bottled water survey, bottled waters were purchased from a total of 159 retail stores in Iowa and Texas. In Iowa, 129 bottled water samples were collected; in Texas, 132 bottled water samples were collected (18 additional samples exceeded laboratory holding times for nitrate analysis and were not included in the analyses). Of the total 261 samples, 10% were bottled outside the USA; 28% were from US spring sources; 16% were from US community water supply sources; and 46% were from other US sources (other/unknown/not specified). Treatment information was listed on the labels of 63% of the waters purchased, and included

reverse osmosis, ozonation, filtration, UV radiation, deionization, distillation, and 'purification' (not otherwise defined), either separately or in combination.

A total of 65 samples of dispensed waters sold by the gallon were obtained in Iowa and Texas stores, water mills, and vending machines. Twenty-one dispensed water samples were collected in Iowa, 20 from dispensing machines in retail grocery stores and 1 from a home-based water dispenser. All Iowa dispensed waters had community water supplies as source water, and all were treated with reverse osmosis. In Texas, 44 dispensed water samples were collected; 10 water dispensers had a community water supply as the source water while the other 34 water dispensers did not specify a water source. Texas dispensed waters were treated with reverse osmosis, filtration, ozone, and UV radiation, either singly or in combination.

Overall bottled water median nitrate concentrations were calculated for communities where bottled water samples were collected, and were used in the calculation of an individual's daily nitrate intake from drinking water (in mg/day). A statewide comparison of nitrate concentrations in community water supplies and in bottled water in communities where case and control mothers resided showed that median and mean bottled water nitrate concentrations were much lower than community water nitrate concentrations in both Iowa and Texas (Table 2).

Bottled water nitrate concentrations contributed minimally to the daily intake of nitrate from drinking water calculated for each individual. Median daily nitrate from drinking water for women who reported drinking bottled water exclusively was 0.75 mg/day compared with 5.57 mg/day for women who reported drinking tap water from public water supplies. Overall, nitrate concentrations in drinking water were low, contributing only 6% of the total nitrate per day in the study population (Brender *et al.* 2013). Nitrate concentrations in bottled water did not measurably affect the risk for birth defects. Among women who reported drinking bottled water exclusively, only 4–9% of the various groups were classified in the highest tertile of nitrate intake from drinking water. Crude odds ratios for defect categories for exclusive bottled water drinkers are in the same direction as odds ratios for community tap water users (Table 3; adapted from Brender *et al.* 2013). Nitrate intake from drinking water sources was categorized into tertiles for each exposure period based on the control-mothers' distributions. 95% CIs of all point estimates include 1.00 due to the small number of exposed cases. Crude odds ratios only are reported for exclusive bottled water drinkers because the small numbers of exposed cases makes it difficult to adjust for multiple potential confounders. Restriction of analyses to women reporting drinking only community tap water did not appreciably change adjusted odds ratios associated with the highest level of water intake for the various birth defect categories for all water sources combined (data not shown).

DISCUSSION

We found no appreciable impact on odds ratios for birth defects in offspring of mothers exposed to nitrate from bottled waters. We believe this is the first epidemiologic study of birth defects to assess nitrate concentrations in bottled/dispensed waters. The biological

plausibility of nitrate as a risk factor for adverse health outcomes, including birth defects, is discussed elsewhere (Spiegelhalder *et al.* 1976; Teramoto *et al.* 1980; Mensinga *et al.* 2003). Briefly, ingested nitrate can undergo endogenous nitrosation to form N-nitroso compounds, which are known carcinogens and teratogens. Epidemiologic studies of drinking water nitrate report mixed findings (Ward *et al.* 2005). Robust exposure assessment methods are critical in accurately assessing the contribution of drinking water nitrate and health risks. A main criticism of nitrate exposure assessment is using aggregated water quality data at the community level, which may not accurately reflect an individual's exposure at the tap (Ward *et al.* 2005). Our study used a refined drinking water exposure assessment approach that linked the mother's geocoded residential locations during B1-P3 to specific community water supply service areas. About 3.4% of mothers had more than one residence during B1-P3. While the proportion of mothers living in more than one residence during their prenatal period is quite small, not accounting for those residential changes could introduce additional error in the exposure assessment. Our approach also included community water supply nitrate data that were closest in time (sampling date) to the B1-P3 period residences. In addition, we collected and analyzed samples of bottled water in communities where mothers who reported exclusive use of bottled waters resided during B1-P3. Median nitrate concentrations for bottles and dispensed waters by community were calculated and included in the estimation of daily nitrate intake from drinking water for each mother.

Nitrate concentrations in the bottled waters we sampled were low, with medians ranging from 0.10 to 0.44 mg/L compared to community water supply medians at 3.57–6.0 mg/L (US drinking water standard is 45 mg/L total nitrate). While bottled water treatment processes varied from brand to brand, treatment information was not provided for many of the waters tested. FDA regulations do not require that contaminant concentrations be listed on the bottles, so consumers do not know what contaminants may be present in the bottled waters they purchase. In comparison, municipal water supply treatment processes are public information, and consumers receive drinking water contaminant data annually through the Consumer Confidence Reports required by EPA.

Strengths of this study include that it was the first time, to our knowledge, that representative bottled/dispensed waters were collected and analyzed for nitrate for an epidemiologic investigation of nitrate and risk for birth defects. All bottled/dispensed waters were tested at the SHL at the University of Iowa. Those nitrate concentrations were used in calculating drinking water nitrate contributions to an individual's total daily nitrate intake from water at both home and at work. A limitation was that specific brands of bottled waters consumed were not known for study participants, so median nitrate concentrations for bottled and dispensed water by community of subject's residence were used in the calculations.

Consumption of bottled water in the USA and internationally has been significant over the past several years, with 2011 per capita consumption in the USA at 29 gallons (110 liters), and in the European Union ranging from 29 to 49 gallons (110–185 liters) (Rodwan 2012). Therefore, it would be important to assess and include bottled water contaminant concentrations in epidemiologic studies of drinking water and health outcomes, particularly those with short exposure time frames of interest. Future prospective cohort studies might benefit from collecting data on the brands of bottled water purchased and consumed by

study participants, in addition to tracking personal consumption patterns of that bottled water. Those studies could consider collecting an unopened bottle of the water most commonly consumed by each study subject, which would eliminate problems with missing data for bottled waters.

CONCLUSION

Nitrate concentrations in bottled water and dispensed water were very low in this study (0.22–26.6 mg/L, 0.05–4.86 mg/L, respectively), and did not appreciably impact risk for birth defects. Bottled water has become an important source of drinking water in the USA and Europe over the past several years. Therefore, assessing possible contaminants' concentrations, such as water disinfection byproducts and synthetic organic compounds, in bottled and dispensed waters should be considered in birth defects studies, allowing researchers to construct a complete picture of the total drinking water contaminant contribution to the health risk under study.

Acknowledgments

ABBREVIATIONS

B1-P3	1 month prior to conception through the first trimester of pregnancy
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
NBDPS	National Birth Defects Prevention Study
P1-P3	first trimester of pregnancy
SHL	State Hygienic Laboratory at the University of Iowa

References

Brender JD, Olive JM, Felkner M, Suarez L, Marckwardt W, Hendricks KA. Dietary nitrates and nitrites, nitrosatable drugs, and neural tube defects. Epidemiology. 2004; 15(3):330–336. [PubMed: 15097014]

Brender JD, Weyer PJ, Romitti PA, Mohanty BP, Shinde MU, Vuong AM, Sharkey JR, Dwivedi D, Hrel SA, Kantamneni J, Huber JC, Zheng Q, Werler MM, Kelley KE, Griesenbeck JS, Zhan FB, Langlois PH, Suarez L, Canfield MA. The National Birth Defects Prevention Study. Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the National Birth Defects Prevention Study. Environ Health Perspect. 2013; 121(9):1083–1089. [PubMed: 23771435]

Breuer GM, Friell LA, Moyer NP, Ronald GW. Testing of bottled waters sold in Iowa. August. 1990; 30:1990. No. 90-1. http://www.shl.uiowa.edu/publications/archive/research/bottledWater.xml.

Clifford D, Subramonian S, Sorg TJ. Removing dissolved inorganic contaminants from water. Environ Sci Technol. 1986; 20(11):1072–1080.

Cogswell ME, Bitsko RH, Anderka M, Caton AR, Feldkamp ML, Hockett Sherlock SM, Meyer RE, Ramadhani T, Robbins JM, Shaw GM, Mathews TJ, Royle M, Reefhuis J. National Birth Defects Prevention Study. Control selection and participation in an ongoing, population-based, case-control study of birth defects: the National Birth Defects Prevention Study. Am J Epidemiol. 2009; 170(8): 975–985. [PubMed: 19736223]

Croen LA, Todoroff K, Shaw GM. Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. Am J Epidemiol. 2001; 153(4):325–331. [PubMed: 11207149]

Dorsch MM, Scragg RK, McMichael AJ, Baghurst PA, Dyer KF. Congenital malformations and maternal drinking water supply in rural South Australia: a case-control study. Am J Epidemiol. 1984; 119(4):473–486. [PubMed: 6711537]

EPA Method 300.0, Inorganic Anions by Ion Chromatography, Rev 2.1 in Methods for the Determination of Inorganic Substances in Environmental Samples (EPA/600/R-93/100).

Food and Drug Administration. Beverages – Requirements for Specific Standardized Beverages. 1998 21 CFR 165.110(b).

Gleick, PH. Bottled and Sold: The Story Behind our Obsession with Bottled Water. Island Press; Washington, DC: 2010.

Ikem A, Odueyungbo S, Egiebor NO, Nyavor K. Chemical quality of bottled waters from three cities in eastern Alabama. Sci Total Environ. 2002; 285:165–175. [PubMed: 11874039]

Mensinga TT, Speijers GJ, Meulenbelt J. Health implications of exposure to environmental nitrogenous compounds. Toxicol Rev. 2003; 22(1):41–51. [PubMed: 14579546]

Mueller, DK.; Helsel, DR. Nutrients in the Nation's Waters – Too Much of a Good Thing?. U.S. Geological Survey Circular 1136. 2013. http://pubs.usgs.gov/circ/circ1136/circ1136.html

Naidenko, O.; Leiba, N.; Sharp, R.; Houlihan, J. Bottled Water Quality Investigation: 10 Major Brands, 38 Pollutants. 2008. http://www.ewg.org/book/export/html/27010

Nolan, BT.; Ruddy, BC.; Hitt, KJ.; Helsel, DR. A National Look at Nitrate Contamination of Ground Water. U.S. Geological Survey. 1998. http://water.usgs.gov/nawqa/nutrients/pubs/wcp_v39_no12/

Pfaff, JD. Method 300.0 Determination of Inorganic Anions by Ion Chromatography. Revision 2.1, August 1993. Environmental Monitoring Systems Laboratory, US Environmental Protection Agency; Cincinnati, OH: 1993. http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_300_0.pdf

Posnick, LM.; Henry, K. Food Safety Magazine. Aug-Sep. 2002 2002 Bottled water regulation and the FDA.

Rodwan, JG., Jr. Bottled Water 2011: The Recovery Continues – U.S. and International Development and Statistics. 2012. http://www.bottledwater.org/files/2011BWstats.pdf

Saleh MA, Abdel-Rahman FH, Woodard BB, Clark S, Wallace C, Aboaba A, Zhang W, Nance JH. Chemical, microbial and physical evaluation of commercial bottled waters in greater Houston area of Texas. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2008; 43:335–347. [PubMed: 18273738]

Seidel, C.; Gorman, C.; Darby, JL.; Jensen, VB. An Assessment of the State of Nitrate Treatment Alternatives. The American Water Works Association; Denver, CO: 2011.

Spiegelhalder B, Eisenbrand G, Preussmann R. Influence of dietary nitrate and nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. Food Cosmet Toxicol. 1976; 14:545–548. [PubMed: 1017769]

Teramoto S, Saito R, Shirasu Y. Teratogenic effects of combined administration of ethylenethiourea and nitrite in mice. Teratology. 1980; 21(1):71–78. [PubMed: 7385057]

Ward MH, deKok TM, Levallois P, Brender J, Gulis G, Nolan BT, VanDerslice J. Workgroup report: Drinking-water nitrate and health – recent findings and research needs. Environ Health Perspect. 2005; 113(11):1607–1614. [PubMed: 16263519]

Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, Costa P, Druschel C, Hobbs CA, Romitti PA, Langlois PH, Edmonds LD. The national birth defects prevention study. Public Health Rep. 2001; 116(Suppl 1):32–40.

Table 1

Frequency of controls and cases (Iowa and Texas) by defect category and drinking water source, NBDPS, 1997–2005

Usual home source of drinking water	Controls n = 1,551 No. (%)	Neural tube defects n = 317 No. (%)	Limb deficiencies n = 177 No. (%)	Oral cleft defects n = 654 No. (%)	Heart defects n = 2,011 No. (%)
Tap water, community	738 (47.6)	173 (54.6)	96 (54.2)	354 (54.1)	1,011 (50.3)
Tap water, private well	72 (4.6)	19 (6.0)	14 (7.9)	42 (6.4)	99 (4.9)
Bottled water, exclusively	455 (29.3)	77 (24.3)	40 (22.6)	178 (27.2)	685 (34.1)
Information not available	286 (18.4)	48 (15.1)	27 (15.3)	80 (12.2)	216 (10.7)

Table 2

Nitrate concentrations in bottled, dispensed, and municipal water samples by state (Iowa and Texas), NBDPS

Type/source of bottled water	Number of samples	Range mg/L total nitrate	Median mg/L total nitrate	Mean mg/L total nitrate
Iowa (*n* = 129)				
Spring (US domestic)	31	0.05 *–6.0	4.5	2.99
Community (US domestic)	21	0.05 *–1.5	0.05 *	0.12
Other/unknown (US domestic)	66	0.05 *–4.8	0.07	0.69
Spring (outside USA)	5	0.05–0.8	0.05 *	0.35
Other/unknown (outside USA)	6	0.3–1.1	0.35	0.46
Texas (*n* = 132)				
Spring (US domestic)	39	0.22 *–21.69	5.31	8.36
Community (US domestic)	23	0.22 *–7.96	0.22 *	1.52
Other/unknown (US domestic)	57	0.22 *–2.65	0.22 *	0.44
Spring (outside USA)	4	0.22 *–3.54	3.31	2.60
Other/unknown (outside USA)	9	0.22 *–3.54	0.88	1.10
Bottled water – Iowa	129	0.22 *–26.6	0.40	0.50
Bottled water – Texas	132	0.22 *–21.7	0.33	0.47
Dispensed water – Iowa	21	0.05 *–1.7	0.10	0.35
Dispensed water – Texas	44	0.22 *–4.86	0.44	0.75
Community water – Iowa	113,232	0.05 *–49.0	6.0	10.6
Community water – Texas	84,579	0.05 *–74.4	3.57	4.75

*
Sample result <detection level, used one-half of the detection level.

Table 3

Maternal daily nitrate intake for bottled water users only *vs.* community tap water users only and risk for selected birth defects in offspring, NBDPS, 1997–2005

Birth defect category	Daily nitrate intake from water (mg/day)[a]	Cases No. (%)	Controls No. (%)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)[b]	p-value for linear trend
Any neural tube defect[c]						
Bottled water	<0.91	40 (55.6)	239 (56.9)	1.00	–	
Bottled water	0.91–4.9	27 (37.5)	160 (38.1)	1.01 (0.60, 1.71)	–	
Bottled water	5.0	5 (6.9)	21 (5.0)	1.42 (0.51, 3.99)	–	
Community tap water	<0.91	25 (16.7)	125 (18.9)	1.00	1.00	
Community tap water	0.91–4.9	38 (25.3)	199 (30.2)	0.95 (0.55, 1.66)	0.91 (0.52, 1.60)	
Community tap water	5.0	87 (58.0)	336 (50.9)	1.29 (0.79, 2.11)	1.25 (0.76, 2.06)	0.230
Any limb deficiency[d,e]						
Bottled water	<1.0	24 (63.2)	253 (60.1)	1.00	–	
Bottled water	1.0–5.41	11 (28.9)	151 (35.9)	0.77 (0.37, 1.61)	–	
Bottled water	5.42	3 (7.9)	17 (4.0)	1.86 (0.51, 6.80)	–	
Community tap water	<1.0	4 (6.6)	114 (17.2)	1.00	1.00	
Community tap water	1.0–5.41	19 (31.2)	215 (32.2)	2.52 (0.84, 7.58)	2.43 (0.80, 7.43)	
Community tap water	5.42	38 (62.3)	335 (50.5)	3.23 (1.13, 9.26)	3.19 (1.09, 9.35)	0.030
Any oral cleft defect[e,f]						
Bottled water	<1.0	94 (59.1)	253 (60.1)	1.00	–	
Bottled water	1.0–5.41	56 (35.2)	151 (35.9)	1.00 (0.68, 1.47)	–	
Bottled water	5.42	9 (5.7)	17 (4.0)	1.43 (0.61, 3.31)	–	
Community tap water	<1.0	47 (16.9)	114 (17.2)	1.00	1.00	
Community tap water	1.0–5.41	74 (26.5)	214 (32.4)	0.84 (0.55, 1.29)	0.80 (0.52, 1.25)	
Community tap water	5.42	158 (56.6)	333 (50.4)	1.15 (0.78, 1.70)	1.15 (0.77, 1.72)	0.191
Conotruncal heart defects[e,g]						
Bottled water	<1.0	56 (62.9)	253 (60.1)	1.00	–	
Bottled water	1.0–5.41	25 (28.1)	151 (35.9)	0.75 (0.45, 1.25)	–	
Bottled water	5.42	8 (9.0)	17 (4.0)	2.13 (0.87, 5.17)	–	
Community tap water	<1.0	15 (15.8)	114 (17.2)	1.00	1.00	

Birth defect category	Daily nitrate intake from water (mg/day)[a]	Cases No. (%)	Controls No. (%)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)[b]	p-value for linear trend
Community tap water	1.0–5.41	25 (26.3)	215 (32.4)	0.88 (0.45, 1.74)	0.92 (0.46, 1.83)	
Community tap water	5.42	55 (57.9)	335 (50.5)	1.25 (0.68, 2.29)	1.29 (0.69, 2.41)	0.252

[a]For neural tube defects, water nitrate intake 1 month preconception to 1 month post-conception was estimated. For limb, oral cleft, and congenital heart defects, water nitrate intake 1 month preconception through the first trimester was estimated.

[b]Adjusted odds ratios not presented for bottled water users because of small numbers of exposed cases for several defects.

[c]Adjusted for maternal race/ethnicity, education, folic acid supplementation, and study center.

[d]Adjusted for maternal race/ethnicity, education, age, multivitamin supplementation, and study center.

[e]For community tap water drinkers, birth defects are restricted to isolated defects.

[f]Adjusted for maternal race/ethnicity, education, age, folic acid supplementation, smoking, and study center.

[g]Adjusted for maternal race/ethnicity, education, multivitamin supplementation, smoking, and study center.