Relation between Stillbirth and Specific Chlorination By-Products in Public Water Supplies

Will D. King,[1] Linda Dodds,[2,3] and Alexander C. Allen[2,3]

[1]Department of Community Health and Epidemiology, Queen's University, Kingston, Canada; [2]Perinatal Epidemiology Research Unit, IWK Grace Health Centre, Halifax, Nova Scotia, Canada; [3]Departments of Obstetrics and Gynecology and Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada

During water treatment, chlorine reacts with naturally occurring organic matter in surface water to produce a number of by-products. Of the by-products formed, trihalomethanes (THMs) are among the highest in concentration. We conducted a retrospective cohort study to evaluate the relationship between the level of total THM and specific THMs in public water supplies and risk for stillbirth. The cohort was assembled from a population-based perinatal database in the Canadian province of Nova Scotia and consisted of almost 50,000 singleton deliveries between 1988 and 1995. Individual exposures were assigned by linking mother's residence at the time of delivery to the levels of specific THMs monitored in public water supplies. Analysis was conducted for all stillbirths and for cause-of-death categories based on the physiologic process responsible for the fetal death. Total THMs and the specific THMs were each associated with increased stillbirth risk. The strongest association was observed for bromodichloromethane exposure, where risk doubled for those exposed to a level of ≥ 20 µg/L compared to those exposed to a level < 5 µg/L (relative risk = 1.98, 95% confidence interval, 1.23–3.49). Relative risk estimates associated with THM exposures were larger for asphyxia-related deaths than for unexplained deaths or for stillbirths overall. These findings suggest a need to consider specific chlorination by-products in relation to stillbirth risk, in particular bromodichloromethane and other by-product correlates. The finding of a stronger effect for asphyxia deaths requires confirmation and research into possible mechanisms. *Key words*: chlorination by-products, epidemiology, public water supplies, stillbirth, trihalomethanes. *Environ Health Perspect* 108:883–886 (2000). [Online 2 August 2000] *http://ehpnet1.niehs.nih.gov/docs/2000/108p883-886king/abstract.html*

In Canada, stillbirths occur at a rate of approximately 6 per 1,000 births (*1*). Despite the obvious importance of stillbirths as a health event, little is known about the risk factors that contribute to this event. In a recent study examining the relationship between chlorination by-products and several adverse birth outcomes, we reported an increase in stillbirth risk with high exposure to total trihalomethanes (THMs) (*2*). Several other studies have also implicated water chlorination by-products as a potential cause of spontaneous abortion and stillbirth (*3–5*), but this relationship requires confirmation.

During the water treatment process, natural organic substances can react with chlorine to produce a number of halogenated hydrocarbon compounds. By-products formed include THMs, halogenated acetic acids, halogenated acetonitriles, chlorinated ketones, and halogenated phenols (*6*). Quantitatively, the THMs are among the highest in concentration of the chlorination by-products and are the most consistently measured in treated water. THMs comprise four compounds: chloroform, bromodichloromethane, chlorodibromomethane, and bromoform. THM compounds as well as other by-products may occur in different concentrations in water supplies with a similar total THM level.

Five studies, including our own, have examined either stillbirth or spontaneous abortion in relation to chlorination by-products (*2–5,7*). Exposure in three of these was based on total THM level, and in another study, exposure was based on a chlorinated versus chloraminated water source. Only Waller et al. (*3*) examined risk in relation to specific THMs. They found a stronger association with bromodichloromethane than with THM compounds per se, which points to the need to examine risk in relation to specific THMs.

The etiology of stillbirths is multifactorial and likely differs depending on the specific pathophysiologic process responsible for the fetal death. Therefore, this study evaluated the relationship between specific THM compounds and stillbirth according to cause-of-death categories based on the physiologic process responsible for the fetal death.

Methods

The cohort consisted of singleton births with known gestational age in the Canadian province of Nova Scotia between 1988 and 1995. It was assembled from a population-based perinatal database that contains mother and infant information for all live born and stillborn infants with birth weights ≥ 500 g. Data were collected prenatally, during labor, delivery, and postpartum and included demographic and risk factor information. A detailed description of this retrospective cohort study has been previously published (*2*). Information on cause of death of the stillbirths was not included in the previous study and was added to the original cohort.

Exposure information came from the Nova Scotia Department of the Environment, which provided results of monitoring for public water facilities throughout Nova Scotia during the relevant time period. Each facility measured total THM, chloroform, bromodichloromethane, bromoform, and chlorodibromomethane on an average of four samples per year. Although bromoform and chlorodibromomethane are measured in Nova Scotia, they occur at very low concentrations and were not included in this analysis. To provide estimates for each water facility for each month, we performed a least-squares regression on chlorination by-product values for year, month, and facility and predicted values were obtained.

The mother's residence at the time of delivery was assumed to be her residence during the entire pregnancy. We estimated individual exposures by linking mother's residence to the geographic area served by each water facility. The study area was restricted to those municipalities where > 90% of households were served by the public water facility, reducing the probability that subjects in a particular area did not use the public water supply. The data set was also restricted to those served by a surface water source as chlorination by-products are essentially nonexistent in groundwater sources. We averaged predicted values of by-product level for the months covering the duration of the mother's pregnancy to provide an exposure

Address correspondence to W.D. King, Department of Community Health and Epidemiology, Abramsky Hall, Queen's University, Kingston, Ontario K7L 3N6 Canada. Telephone: (613) 533-6000, ext. 74735. Fax: (613) 533 6686. E-mail: kingw@post.queensu.ca

We thank C. Woolcott and J. Pole for computing support and J. Scott and D. Briggins for their helpful discussions.

This research was supported by the Prince Edward Island Reproductive Care Program through Health Canada Green Plan funds. The perinatal data were provided by the Nova Scotia Reproductive Care Program, and the chlorination by-product data were provided by the Nova Scotia Department of the Environment.

Received 21 January 2000; accepted 17 May 2000.

measure during pregnancy. We excluded women if gestational age was unknown.

We examined risk for the by-product factors in continuous and categorical representations. Identical groupings were used for total THM and chloroform because exposures were of similar range and magnitude for these factors. The referent group for total THM and chloroform exposures were those with average pregnancy exposure of < 50 µg/L. The majority (> 95%) of women in the referent category had an average exposure in the range 25–49 µg/L. Categorical groupings for bromodichloromethane reflected the lower magnitude and narrower range of values for this by-product. To provide consistent increments of exposure level for the continuous representation of THM compounds, increments of 10 µg/L were used for total THM, chloroform, and bromodichloromethane.

In Nova Scotia, all stillbirths and neonatal deaths are reviewed by hospital perinatal committees and further reviewed by clinicians with obstetrical and neonatal expertise from the Reproductive Care Program of Nova Scotia. A primary cause of death was assigned according to guidelines prepared by the Reproductive Care Program of Nova Scotia, and these guidelines were used consistently throughout the province. During the study period, autopsies were performed in 75% of the stillbirth cases.

The categories for the underlying causes of death were adopted from methods proposed previously (*8,9*). These categories included intrauterine asphyxia, congenital anomalies, deaths related to immaturity, other specified deaths, and unexplained deaths. Asphyxia deaths included placental causes, umbilical cord causes, and labor and delivery causes. If two or more conditions were present, the condition that was most likely to have caused the fetal death was coded.

We estimated relative risks (RRs) and 95% confidence intervals (CIs) adjusted for potential confounders using Poisson regression models (*10*). The relative risk associated with exposure to each THM compound was determined for all stillbirths combined and for the cause-of-death subgroups.

We determined potentially important confounders on the basis of the confounder–outcome relationship. A backward regression approach with factors eliminated at a significance > 0.15 was used to identifiy the subset of factors with the potential to confound the relationship of interest. Factors were maternal age, parity, smoking during pregnancy, infant's sex, and neighborhood family income. With the exception of family income, this information was obtained from the perinatal database. Maternal age was represented in three categories, < 25, 25–34, and ≥ 35 years. Parity and smoking were dichotomous variables. Neighborhood family income, with adjustment for family size, was calculated based on 1991 census information for the enumeration area of the mother's residence. Enumeration area is the smallest standard geographic area for which Canadian census data are available. In 1991 the average population size of enumeration areas in Nova Scotia was 680.

Table 1. Description of stillbirths by cause-of-death category

Stillbirths	Number	Percent
Unexplained	84	39
Asphyxia	72	34
Other specified	21	10
Immaturity	20	9
Congenital anomaly	15	7
Infection	2	1
Total	214	100

Results

The cohort consisted of 49,756 singleton deliveries between 1988 and 1995, where mothers were resident in an area with municipal surface water service. Among the 214 stillbirths in the cohort, the largest subgroup included those with an unexplained cause (Table 1). Asphyxia-related causes accounted for 34% of stillbirths, with 63% of asphyxia deaths classified as abruptio placentae. The analyses to determine the relationship between total THM and individual THM compounds and cause-of-death categories were restricted to unexplained deaths and asphyxia-related deaths because the number in the other categories was too small to permit a meaningful analysis.

Table 2 presents the mean exposure value and correlation between by-product compounds. On average, 90% of the total THM value (mean 71 µg/L) was accounted for by chloroform levels (mean 64 µg/L), and exposure values for these factors were highly correlated. The mean value for bromodichloromethane was 6.9 µg/L, which correlated moderately with total THM (Pearson correlation coefficient, r = 0.44), but less so with chloroform (r = 0.26).

Table 3 presents crude and adjusted RRs for stillbirth according to the by-product exposures. Relative risks were adjusted for smoking and maternal age, and adjustment resulted in only small changes to the risk estimates. Five percent of the total cohort and 7% of those with a stillbirth are not included in this analysis due to missing information on smoking. Relative risks associated with total THM rose with increasing levels of exposure to 1.7 for exposure to an average total THM level of 100 µg/L relative to exposures < 50 µg/L. The continuous representation showed a 5% increase in risk with each 10 µg/L total THM. Results were similar according to chloroform exposure. A dose–response pattern was also observed according to levels of bromodichloromethane exposure, with a doubling of risk associated with the highest exposure category. The continuous representation displayed a 29% increase in risk with each 10 µg/L of bromodichloromethane. Because the range of exposures is much lower for bromodichloromethane, increments of 10 µg/L represent a larger proportion of the total range than for chloroform.

In an attempt to determine the independent effects of THM by-products, the continuous representations of chloroform and bromodichloromethane were entered simultaneously into a model (data not shown). Relative risk estimates in this analysis represent the effect of each by-product adjusted for the other by-product, maternal smoking, and maternal age. Chloroform was not associated with increased risk in this model (RR = 1.03 per 10 µg/L; CI, 0.98–1.07), whereas bromodichloromethane was associated with a 26% increase in risk per 10 µg/L (RR = 1.26; CI, 1.05–1.49).

The data were analyzed by the stillbirth cause-of-death classifications of unexplained and asphyxia-related deaths (Table 4). For unexplained stillbirths, the risk estimates were lower than those for all stillbirths combined, and CIs consistently overlapped the null value. In the analysis of asphyxia cases and total THM exposure, relative risks increased with increasing levels of exposure, to 5.0 for total THM ≥ 100 µg/L. The continuous representation showed a 13% increase in asphyxia stillbirth risk with each 10 µg/L total THM. Results were similar for chloroform, with an RR estimate of 3.6 for those exposed to chloroform levels ≥ 100 µg/L. Elevated relative risks were also observed for bromodichloromethane exposure. The continuous representation showed a 32% increase in risk with each 10 µg/L bromodichloromethane.

Discussion

When all stillbirths combined were considered, the level of total THM and the specific THMs in public water supplies were each

Table 2. Correlation between exposures to total THM, chloroform, and bromodichloromethane.

		Pearson correlation coefficient		
Exposure	Mean (µg/L)	Total THM	Chloroform	Bromodichloromethane
Total THM	71.3	1.00	0.98	0.44
Chloroform	64.1	0.98	1.00	0.26
Bromodichloromethane	6.9	0.44	0.26	1.00

associated with increased risk. Chloroform accounts for a large proportion of total THM, and exposure measures for these two factors were highly correlated. Accordingly, results for chloroform were similar to those reported for total THM in a previous paper (*2*). High exposure to bromodichloromethane was associated with a doubling of risk compared to low exposure. Simultaneous modeling of the two specific THMs suggested that bromodichloromethane was the stronger independent predictor of risk.

Two previous studies examined the relationship between stillbirths and water chlorination (*5*,*7*). Aschengrau et al. (*5*) reported an increased risk (odds ratio = 2.6) related to the use of chlorinated versus chloraminated surface water, whereas Bove et al. (*7*) found no association between stillbirth and total THM level.

Previous studies of spontaneous abortion are also relevant to this discussion, as they may have a similar biologic response with respect to the toxicity of chlorination by-products. Waller et al. (*3*) examined total THM and the four individual THMs. Their finding that only high bromodichloromethane exposure was associated with spontaneous abortion both alone and after adjustment for the other THMs is consistent with our results. Savitz et al. (*4*) reported increased risk of spontaneous abortion only in the highest sextile of THM concentration.

Analysis of stillbirth groups defined by cause-of-death categories was conducted with the intent of focusing on stillbirths with etiologic similarities. Two subgroups were defined with sufficient numbers to allow meaningful analysis. A stronger relationship with the THM factors was observed for asphyxia-related deaths than unexplained deaths. The observation of a stronger relationship with asphyxia-related deaths indicates that this subgroup merits specific consideration in future investigations. In this cohort, 63% of the asphyxia-related deaths were due to abruptio placentae. Research into the mechanisms responsible for abruptio placentae suggest that folate deficiency and defects within the methionine–homocysteine metabolic pathway increase the risk of placental vascular disease, including abruptio placentae (*11*). A possible biologic mechanism to explain the increased risks found for abruptio placentae and exposure to chlorination by-products may be the effect on folate or methioinine–homocysteine metabolism. Methionine biosynthesis has been shown to be inhibited by chloroform and related compounds in cell culture (*12*).

The primary methodologic limitation of this study is potential misclassification of individual exposure to THMs. Individual exposure assessment was based on a measure of specific THMs for the water distribution system serving the area of the mother's residence at the time of delivery. This measure does not take into account variations in by-product concentrations within a treatment distribution system and individual behaviors that may

Table 3. RRs and 95% CIs for the relationship between stillbirth and total THM, chloroform, and bromodichloromethane.

Exposure	No. stillbirths	Rate (%)	RR (95% CI)	Adjusted RR (95% CI)[a]
Total THM (µg/L)				
< 50	43	0.33	1.00	1.00
50–74	79	0.42	1.26 (0.87–1.83)	1.27 (0.88–1.85)
75–99	31	0.42	1.26 (0.80–2.00)	1.28 (0.81–2.03)
≥ 100	44	0.56	1.69 (1.11–2.58)	1.66 (1.09–2.54)
per 10 µg/L	–	–	1.05 (1.01–1.09)	1.05 (1.01–1.09)
Chloroform (µg/L)				
< 50	68	0.35	1.00	
50–74	63	0.42	1.19 (0.84–1.67)	1.20 (0.85–1.68)
75–99	29	0.46	1.35 (0.87–2.07)	1.35 (0.87–2.08)
≥ 100	37	0.56	1.59 (1.06–2.37)	1.56 (1.04–2.34)
per 10 µg/L	–	–	1.05 (1.00–1.09)	1.04 (1.00–1.09)
Bromodichloromethane (µg/L)				
< 5	96	0.37	1.00	
5–9	57	0.40	1.08 (0.77–1.50)	1.07 (0.77–3.19)
10–19	23	0.54	1.45 (0.91–2.28)	1.44 (0.90–2.27)
≥ 20	21	0.74	1.99 (1.24–3.20)	1.98 (1.23–3.49)
per 10 µg/L	–	–	1.30 (1.10–1.53)	1.29 (1.10–1.53)

[a]RRs adjusted for smoking and maternal age.

Table 4. RRs and 95% CIs for the relationship between unexplained and asphyxia-related stillbirth and total THM, chloroform, and bromodichloromethane.

Cause of death/exposure	No. stillbirths	Rate (%)	RR (95% CI)	Adjusted RR (95% CI)[a]
Unexplained				
Total THM (µg/L)				
< 50	19	0.15	1.00	1.00
50–74	32	0.17	1.05 (0.51–2.16)	1.16 (0.66–2.04)
75–99	14	0.19	1.29 (0.65–2.57)	1.31 (0.66–2.62)
≥ 100	12	0.15	1.16 (0.66–2.04)	1.07 (0.52–2.22)
per 10 µg/L	–	–	1.00 (0.94–1.08)	1.01 (0.94–1.08)
Chloroform (µg/L)				
< 50	28	0.14	1.00	1.00
50–74	28	0.19	1.28 (0.76–2.17)	1.29 (0.77–2.19)
75–99	13	0.21	1.47 (0.76–2.83)	1.49 (0.77–2.87)
≥ 100	8	0.12	0.84 (0.38–1.83)	0.86 (0.39–1.89)
per 10 µg/L	–	–	1.00 (0.93–1.07)	1.00 (0.93–1.08)
Bromodichloromethane (µg/L)				
< 5	42	0.16	1.00	1.00
5–9	20	0.14	1.00 (0.61–1.64)	0.87 (0.51–1.49)
10–19	9	0.21	1.38 (0.69–2.74)	1.34 (0.65–2.75)
≥ 20	6	0.21	1.26 (0.54–2.96)	1.35 (0.57–3.19)
per 10 µg/L	–	–	1.13 (0.83–1.53)	1.15 (0.85–1.55)
Asphyxia				
Total THM (µg/L)				
< 50	7	0.05	1.00	1.00
50–74	27	0.14	2.65 (1.15–6.09)	2.67 (1.16–6.14)
75–99	10	0.13	2.50 (0.95–6.57)	2.49 (0.95–6.54)
≥ 100	21	0.27	4.96 (2.11–11.67)	4.57 (1.93–10.77)
per 10 µg/L	–	–	1.13 (1.06–1.19)	1.12 (1.05–1.18)
Chloroform (µg/L)				
< 50	17	0.09	1.00	1.00
50–74	18	0.12	1.36 (0.70–2.64)	1.36 (0.70–2.63)
75–99	10	0.16	1.86 (0.85–4.06)	1.82 (0.83–3.97)
≥ 100	20	0.30	3.43 (1.80–6.56)	3.15 (1.64–6.03)
per 10 µg/L	–	–	1.13 (1.06–1.21)	1.12 (1.05–1.20)
Bromodichloromethane (µg/L)				
< 5	29	0.11	1.00	1.00
5–9	18	0.13	1.13 (0.63–2.03)	1.09 (0.61–1.97)
10–19	12	0.28	2.50 (1.27–4.89)	2.32 (1.18–4.55)
≥ 20	6	0.21	1.89 (0.78–4.55)	1.75 (0.72–4.22)
per 10 µg/L	–	–	1.36 (1.04–1.78)	1.32 (1.00–1.74)

[a]RRs adjusted for smoking and maternal age.

influence exposure. The role of mobility in this study was addressed in a previous study in which we estimated that the change in municipality of residence would likely affect < 10% of study subjects (*2*). This study was limited to fetal deaths ≥ 500 g, as information on earlier fetal deaths (e.g., spontaneous abortions) is not available in the perinatal database.

We were unable to examine other THMs (e.g., bromoform and chlorodibromomethane) because these by-products occurred in low concentrations in the water supplies examined. In this cohort, only 3% of mothers had average bromoform exposure > 1 µg/L, and only 2% had average chlorodibromomethane exposure > 1 µg/L. In contrast, 18% of the population studied by Waller et al. (*3*) were exposed to bromoform concentrations > 16 µg/L, and 16% were exposed to chlorodibromomethane of > 31 µg/L (*3*).

A relationship between total THM and stillbirth was suggested in our previous publication of total THM exposure and several adverse birth outcomes (*2*). The findings presented here focus on specific chlorination by-products and categories of stillbirth in an attempt to better understand this potentially important relationship. These results suggest a need to consider specific chlorination by-products in relation to stillbirth risk, in particular bromodichloromethane and other by-products that correlate with this THM. In addition, stronger associations and a dose–response relationship were evident with stillbirths due to asphyxia-related causes of death, a result that requires confirmation and research into possible mechanisms.

References and Notes

1. Health Statistics Division. Births and Deaths, 1995. Ottawa, Ontario, Canada:Statistics Canada, 1997.
2. Dodds L, King W, Woolcott C, Pole J. Trihalomethanes in public water supplies and adverse pregnancy outcomes. Epidemiology 10(3):233–237 (1999).
3. Waller K, Swan SH, DeLorenze G, Hopkins B. Trihalomethanes in drinking water and spontaneous abortion. Epidemiology 9:134–140 (1998).
4. Savitz DA, Andrews KW, Pastore LM. Drinking water and pregnancy outcome in central North Carolina: source, amount, and trihalomethane levels. Environ Health Perspect 103:592–596 (1995).
5. Aschengrau A, Zierler S, Cohen A. Quality of community drinking water and the occurrence of late adverse pregnancy outcomes. Arch Environ Health 48:105–113 (1993).
6. Stevens AA, Moore LA, Slocum CJ, Smith BL, Seeger DR, Ireland JC. By-products of water chlorination at ten operating utilities. In: Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol 6: Proceedings of the Sixth Conference on Water Chlorination—Environmental Impact and Health Effects (Jolley R, Condie L, Johnson JD, Katz S, Minear RA, Mattice JS, Jacobs VA, eds). Chelsea, MI:Lewis Publishers, Inc, 1990;579–604.
7. Bove FJ, Fulcomer MC, Klotz JB, Esmart J, Dufficy EM, Savrin JE. Public drinking water contamination and birth outcomes. Am J Epidemiol 141(9):850–862 (1995).
8. Cole S, Hartford RB, Bergsjo P, McCarthy B. International collaborative effort (ICE) on birth weight, plurality, perinatal, and infant mortality III: a method of grouping underlying causes of infant death to aid international comparisons. Acta Obstet Gynecol Scand 68:113–117 (1989).
9. Alberman E, Blatchley N, Botting B, Schuman J, Dunn A. Medical causes on stillbirth certificates in England and Wales: distribution and results of hierarchical classifications tested by the Office for National Statistics. Br J Obstet Gynaecol 104:1043–1049 (1997).
10. Hertz-Picciotto I. Environmental epidemiology. In: Modern Epidemiology (Rothman KJ, Greenland S, eds), 2nd ed. Philadelphia, PA:Lippencott-Raven Publishers, 1998;555–583.
11. Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. Placenta 20:519–529 (1999).
12. Alston TA. Inhibition of vitamin B12-dependent methionine biosynthesis by chloroform and carbon tetrachloride. Biochem Pharmacol 42:R25–R28 (1991).