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Chlorination disinfection by-products in drinking water and congenital anomalies: review and meta-analyses

Subprodutos da desinfecção com cloro em água potável e anomalias congênitas: revisão e meta-análise

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Abstract ***This study aims to review epidemiologic evidence of the association between exposure to chlorination disinfection by-products (DBPs) and congenital anomalies. All epidemiologic studies that evaluated a relationship between an index of DBP exposure and risk of congenital anomalies were analyzed. For all congenital anomalies combined, the meta-analysis gave a statistically sig-nificant excess risk for high versus low exposure to water chlorination or TTHM (17%; 95% CI, 3-34) based on a small number of studies. The meta-analysis also suggested a statistically significant excess risk for ventricular septal defects (58%; 95% CI, 21-107), but based on only three studies, and there was little evidence of an exposure-response relationship. It was observed no statistically significant relationships in the other meta-analyses and little evidence for publication bias, except for urinary tract defects and cleft lip and palate. Although some individual studies have suggested an association between chlorination disinfection by-products and congenital anomalies, meta-analyses of all currently available studies demonstrate little evidence of such association.***

Key words ***Birth defects, Congenital anomalies, Disinfection by-products, Fetal development, Repro-ductive health, Trihalomethanes***

Resumo ***O objetivo deste estudo é revisar evidências epidemiológicas da associação entre a exposição a subprodutos da desinfecção com cloro (DBPs) e anomalias congênitas. Todos os estudos epidemiológicos que avaliaram a relação entre o índice de exposição a DBPs e o risco de anomalias congênitas foram analisados. Para todas as anomalias congênitas combinadas, a meta-análise resultou em um risco de excesso estatisticamente significante para alta versus baixa exposição à cloração da água ou ao TTHM (17%; 95% CI, 3-34) baseado em um pequeno número de estudos. A meta-análise também sugere um excesso de risco estatisticamente significante para defeitos septais ventriculares (58%; 95% CI, 21-107), porém com base em apenas três estudos, nos quais se encontrou pouca evidência na relação exposição-resposta. Não foram observadas relações estatisticamente significantes em outras meta-análises e pouca evidência para uma tendência de publicação, com exceção de defeitos no trato urinário e fissura labiopalatal. Apesar de alguns estudos individuais sugerirem uma associação entre subprodutos da desinfecção com cloro e anomalias congênitas, as meta-análises de todos os estudos disponíveis atualmente demonstraram pouca evidência de tal associação.***

Palavras-chave ***Defeitos congênitos, Anomalias congênitas, Subprodutos da desinfecção, Desenvolvimento fetal, Saúde reprodutiva, Trihalometano***

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Disinfection of drinking water has led to a major improvement in public health since it was first applied in the 20th century. It has now been > 30 years since it was discovered that by-products can be formed in small quantities as part of the chlorination process[1]. Chlorination disinfection by-products (DBPs) are formed when water is chlorinated and organic matter in the water reacts with chlorine. Their formation and occurrence depends on many factors, including the chlorine dose, type of treatment, pH, temperature, residence time, and bromine levels[2,3]; up to 600 DBPs have been identified[4,5]. Different mixtures of by-products may exist in different locations, depending on the various factors mentioned above, making it more difficult to assess any health effects of DBPs, particularly in epidemiologic studies.

Ingestion of water is thought to be the main route of exposure for nonvolatile DBPs such as halo acetic acids (HAAs). Exposure to volatile DBPs such as trihalomethanes (THMs) may occur through inhalation and absorption during activities such as showering, bathing, and swimming[3]. Modeling of THM uptake has suggested that swimming may lead to the highest levels of THMs in the blood[6].

Various reviews have been conducted on the epidemiology of chlorination by-products and reproductive outcomes. These have concluded that there are still many problems to overcome and that the results are inconsistent and inconclusive, except perhaps for small-for-gestational-age/intrauterine growth retardation[2,7-11]. In addition, two meta-analyses by Hwang and Jaakkola[12] and Hwang ***et al.***[13] reported evidence for an effect of exposure to chlorination by-products on the risk of neural tube defects, urinary system defects, and ventricular septal defects, but risks for other anomalies were considered hetero geneous and inconclusive. However, the meta-analyses by Hwang and Jaakkola[12] included only five studies and did not include more recent studies, and the study by Hwang ***et al.***[13] generally focused on a few sub categories of anomalies and also did not include some recent studies. In this article we review the epidemiologic evidence and provide summary risk estimates of the association between chlorination disinfection by-products and congenital anomalies; we also provide recommendations for future studies.

Methods

We searched PubMed[14] using the following key words: "chlorination", "chlorination by-products", "chlorination byproducts", "trihalomethanes", and "haloacetic acids". We searched for these key words alone and in combination with the following key words for congenital anomalies: "birth defects", "congenital anomalies", "congenital malfor-mations", "urinary", "respiratory", "cardiovascular", "neural tube defects" (NTDs), and "cleft lip/palate". Furthermore, we searched in review and meta-analysis articles on the topic[2,7-14]. We retrieved all epidemiologic studies examining the relation between chlorination by-products and congenital anomalies.

We included all epidemiologic studies that evaluated the relationship between some index of chlorination by-products (treatment, water source, DBP measurements, and both DBP measurements and personal characteristics) and any congenital anomalies. We did not score the studies for quality, given the relatively small number of studies involved.

We obtained summary risk estimates for general water chlorination by conducting meta-analyses when three or more studies featured the same congenital anomalies. In these analyses we calculated risk estimates for high versus low exposure, using the exposure groups as defined in the original studies [e.g., high vs. low total trihalo methane (TTHM)] concentration, chlorination vs. non chlorination, or chlorination vs. chloramina tion]. When three or more studies had measurements of individual THMs or brominated fractions, we conducted meta-analyses, again comparing the highest versus the lowest exposure groups of the original studies. We tested for hetero geneity in risk estimates using the Q test[15]. When the result of the Q test was statistically significant ($p < 0.05$), implying hetero geneity between the studies, we used random effects analyses, using the method of DerSimonian and Laird[16]. When the Q test was not statistically significant, we used fixed effects analyses, using the Mantel-Haenszel method[17]. The summary estimates were weighted by the inverse variance of each study, taking into account whether a fixed or random effects model was used. We used STATA statistical software (StataCorp, College Station, TX, USA).

Where five or more studies, each with the same outcome and exposure classification (applicable only to TTHM) were split into three or more exposure categories, we conducted a meta-analysis to obtain exposure-response risk estimates

per 10 mg/L TTHM. All the included studies provided data on exposure in a categorical way. The midpoint of each given exposure category was used in the estimation of exposure-response slopes for each of the studies. If the highest exposure category was open-ended (i.e., greater than some concentration), a value of half the width of the previous exposure category was added to the cut point of highest exposure category, using the method described by Key et al.[18]. In the absence of data to the contrary, we assumed that the natural logarithm of the measure of effect varied linearly with concentration of TTHM in drinking water. Exposure-response slopes for those exposed to TTHM in drinking water were calculated using linear regression of log-transformed risk estimates. We performed the meta-analyses using R software[19] and scripts adapted from those of Key ***et al.***[18]. A random effects model was used for the meta-analysis of exposure-response slopes of individual studies. Regression slopes of exposure-response slopes derived from individual studies were plotted together with the summary slope produced from their meta-analysis.

We also produced forest and funnel plots[20]. Forest plots show the odds ratio (OR) with its confidence interval (CI) for each study included in the meta-analyses and the estimation of the summary OR. The sizes of the square markers of OR in the plot represent the relative weight each study contributed to the regression.

Funnel plots show, for every study, the effect estimate (OR and relative risk) of the study against some precision measure (SE in this case) to examine possible bias due to publication. We also conducted a weighted Egger test, a linear regression in which the response is the estimated effect and the explanatory variable is a precision term (1/SE). A large deviation from zero of the slope term suggests publication bias[21].

Results

The extracted and evaluated studies are shown in Table 1. A third of these studies were con-ducted in the United States[22-26], followed by Sweden[27,28], England and Wales[29,30], Canada[31,32], Norway[33,34], Australia[35], and Taiwan[13]. Five were case–control studies[22,24-26,29] (includes two studies), whereas the rest were cross-sectional, population-based studies, generally using registry data, which limited the opportunity to adjust for potential confounders. Most included live births, fetal deaths, and terminations. A number of the studies used only treatment method (chlorination vs. non-chlorination, chlorination vs. chloramination) as the index of exposure[22,28,33,34], whereas the other studies used a measure of DBP exposure level, mostly THMs[23-27,29,30-32,35]. Generally, studies used TTHM levels, but some studies also evaluated individual THMs or the brominated fraction[26,29,30,32]. The levels of brominated THM were high in Australia and made up most of the TTHM there[35]. Klotz and Pyrch[24] also had a measure of halo acetonitrile (HAN) and HAA exposure, and Luben ***et al.***[25] a measure of HAA exposure. Levels of DBPs in Scandinavia[27,28,33,34,36] and Taiwan[13] tended to be lower than in the other countries, with median levels around 10 µg/L TTHM, with approximately 20% > 20 µg/L. The Canadian[31,32] and Australian[35] studies examined the highest TTHM levels (> 100 µg/L). Three studies examined personal behavioral activities such as ingestion, showering, and bathing[24,25,29]. The largest study was conducted in England and Wales and included > 2.5 million births and > 20,000 cases of congenital anomalies[30]. Dodds ***et al.***[31] and Dodds and King[32] used the same subjects but examined the effect of TTHM and individual THMs, respectively. The study by Hwang ***et al.***[33] included 3 years of subjects from Magnus at al.[34], and therefore only the former was included in any of the meta-analyses. The report by Shaw et al.[26] contained two case-control studies (study 1 and study 2), and they have been treated as separate studies.

A number of studies found statistically significant positive associations between water chlorination (THM levels or chlorinated water) and any congenital anomaly[23,33,35], whereas others did not[13,22,34]. There was evidence of heterogeneity between studies ($p = 0.041$). The meta-analysis, using a random effects model, produced a statistically significant summary effect measure of 17% excess risk (95% CI, 2–34) [(Table 2, Figure 1; see also Supplemental Material 1, Table 1, available online (doi:10.1289/ehp. 0900677.S1 via http://dx.doi.org/)].

Neural tube defects were one of the most commonly studied groups of congenital anomalies, and three studies found statistically significant positive associations between THM levels or chlorinated water and neural tube defects[23,24,32], whereas others did not[26,28,30,31,33-35] [Table 2; see also Supplemental Material 1, Tables 2a-2d (doi: 10.1289/ehp.0900677.S1)]. There appeared to be little difference between the findings of using TTHM levels or brominated species as the exposure index. The meta-analyses for high versus low

exposure for water chlorination (THM levels or chlorinated water) and bromodichloromethane (BDCM) showed some excess of risk, but it was not statistically significant. We found no evidence for an exposure-response relationship with TTHM levels (OR = 1.00; 95% CI, 0.94- 1.07 per 10 µg/L TTHM) [see Supplemental Material 3, Figures 1-3 (doi: 10.1289/ehp. 0900677.S1)].

Table 1. Summary of epidemiologic studies on chlorinated disinfection by-products and adverse reproductive outcomes.

Reference	Study details	Cases	Sample population	Exposure assessment	Other risk factors included
Aschengrau *et al.*[22]	Massachusetts, USA Two hospitals 1977–1980 1,039 major congenital malformations, urinary tract defects, respiratory tract defects; 1,177 controls	1,039 major congenital anomalies, urinary tract defects, respiratory tract defects	Ongoing population-based study of 14,130 obstetrics patients	Based on maternal residential address to ascertain type of water supply, chlorination vs. chloramination, and ground/mixed water vs. surface water	Maternal age, pregnancy history, alcohol, ethnicity, hospital payment, other water contaminants
Bove *et al.*[23]	New Jersey, USA 75 towns with a public water supply 1985–1988 Sample population: 81,602	All birth defects, 669 surveillance; 118 central nervous system defects; 83 oral cleft defects; 56 NTDs; 108 major cardiac defects	From live birth and fetal death (> 20 weeks) registries	Based on maternal residential address and municipal water surveys to estimate monthly THM levels (five or six exposure categories)	Maternal age, ethnicity, sex of baby, primipara, prenatal care, education, previous stillbirth or miscarriage, other contaminants
Dodds *et al.*[31]	Nova Scotia, Canada 1988–1995 Study population: 49,842 births	77 NTDs, 82 cleft defects, 430 major cardiac defects, 197 stillbirths, 96 chromosomal abnormalities	Live and still birth registry and fetal anomaly database	Based on maternal residential address and TTHM levels for public water facilities (three sampling locations) modeled using linear regression on the basis of observations by year, month, and facility (four exposure categories)	Maternal age, parity, maternal smoking, attendance prenatal classes, neighborhood, family income, sex of baby, pregnancy and predelivery weight Adjusted: NTDs, cardiac defects, chromosomal abnormalities, income
Dodds and King[32]	Nova Scotia, Canada 1988–1995 Study population: 49,842 births	77 NTDs, 430 cardiovascular anomalies, 85 cleft defects, 96 chromosomal abnormalities	Live and still birth registry and fetal anomaly database	Based on maternal residential address and TTHM, chloroform, and BDCM levels for public water facilities (three sampling locations) modeled using linear regression on the basis of observations by year, month, and facility (four exposure categories) (r = 0.44 for TTHM and BDCM)	Maternal age, parity, maternal smoking, attendance prenatal classes, neighborhood, family income, sex, pregnancy and predelivery weight adjusted: NTDs, cardiac defects, cardiovascular; chromosomal; maternal age and income; Cleft: maternal age

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Klotz and Pyrch[24] found a statistically significant association between TTHM levels in the water and neural tube defects, but not with levels of halo acetonitriles and halo acetates. Inclusion of personal behavioral activities made little difference in the results. In addition, the effects were most pronounced in offspring from women who did not take supplementary vitamins, but these findings were not confirmed by Shaw ***et al.***[26]. Findings of the subcategories of neural tube defects including anencephalus, spina bifida, and hydrocephalus were also mixed, and none of the studies found a statistically significant association except for Shaw ***et al.***[26] who found a statistically significant reduced risk. The meta-analyses showed some excess of risk for anencephalus and spina bifida, but it was not statistically significant.

Cedergren ***et al.***[27], Chisholm ***et al.***[35], and Hwang ***et al.***[33] found statistically significant positive associations between chlorinated water with

Table 1. Continued.

Reference	Study details	Cases	Sample population	Exposure assessment	Other risk factors included
Luben ***et al.***[25]	Arkansas, USA 1998–2002 320 cases and 614 controls, and a subset of 40 cases and 243 controls	320 and 40 hypospadias	Birth and birth defect registry from Arkansas Reproductive Health Monitoring System and National Birth Defects Prevention Study	THMs and HAAs, and personal characteristics in the subset	Maternal and paternal age, race, and education, marital status, maternal use of alcohol and tobacco, parity, prenatal care, 1- and 5-min Apgar scores, birth weight, method of delivery, any risks, procedures, or complications associated with delivery
Hwang ***et al.***[13]	Taiwan 2001–2003 396,049 births	2,148 congenital anomalies, including 43 anencephalus (ICD-9 code 740.0), 118 hydrocephalus (code 741.0), 59 ventricular septal defects (code 745.4), 19 atrial septal defects (code 745.5), 24 tetralogy of Fallot (code 745.2), 358 cleft palate or lips (code 749.0), cleft lip (code 749.1), 76 renal agenesis and dysgenesis (code 753.0), 49 obstructive urinary tract defects (code 753.2), 72 hypospadias (code 752.61), 166 chromosome anomalies (code 758)	Birth registry	High (TTHM 20 + µg/L), medium (TTHM 10–19 µg/L), low exposure (TTHM 5–9 µg/L), and 0–4 µg/L	Sex of infant, maternal age (< 20 years; 20–34 years; ≥ 35 years), plurality (singleton and multiple birth), maternal health status, population density
Iszatt ***et al.***[29]	Southeast England 1997–1998 363 hypospadias cases and 346 population controls	363 hypospadias	Population based	THMs	Income, birth weight, folate supplement use during pregnancy, maternal smoking, maternal occupational exposure to phthalates

Abbreviations: BPA, ***British Paediatric Association Classification of Disease***[37]; CDBM, chlorodibromomethane; ICD-9, ***International Classification of Diseases, 9th Revision*** (WHO[38]); ICD-10, ***International Classification of Diseases, 10th Revision*** (WHO[39]).

a higher color content, levels of TTHM > 10 µg/L, and high levels of DBPs (≥ 130 µg/L), respectively, and cardiovascular congenital anomalies, but other studies did not observe such an association[23,26,28,30-32,34] [Table 2; see Supplemental Material 1, Tables 3a–3c (doi: 10.1289/ehp.0900677.S1)]. There was some evidence for heterogeneity in study results (***p*** = 0.017). The random effects

Table 1. Continued.

Reference	Study details	Cases	Sample population	Exposure assessment	Other risk factors included
Shaw *et al.*[26]	California, USA Study 1: 538 NTD cases and 539 controls selected	Study 1: 538 NTDs (anencephaly and spina bifida)	Live births, fetal deaths, and terminations	Studies 1 and 2: continuous TTHM categorical: 0, 1–24, 25–49, 50–74, and ≥ 75 µg/L TTHM	Ethnicity, education, body mass index, use of vitamins
	California, USA Study 2: 265 NTD cases, 207 conotruncal heart defect cases, 109 orofacial cleft cases, 481 controls	Study 2: 265 NTDs (anencephaly and spina bifida), 207 conotruncal heart defects, 481 orofacial clefts		Also Study 1: ≥ 50 vs. < 50 µg/L and > 5 glasses ≥ 50 vs. < 50 µg/L and > 5 glasses Study 1: chloroform ≥ 13.2 vs. < 12.2 µg/L BDCM ≥ 4.2 vs. < 4.2 µg/L CDBM ≥ 1.7 vs. < 1.7 µg/L Study 2: chloroform ≥ 15.0 vs. < 15.0 µg/L BDCM ≥ 9.6 vs. < 9.6 µg/L CDBM ≥ 3.6 vs. < 3.6 µg/L MTHFR genotype	
Nieuwenhuijsen *et al.*[30]	England and Wales 2,605,226	Congenital anomalies, 1,434 respiratory (ICD-10 codes Q30–Q34), 8,809 major cardiac (Q20–Q28), 5315 urinary (Q60–Q64), 2,267 abdominal wall (Q79), 3,334 NTDs (Q00, Q01, and Q05), 3,736 cleft lip and palate (Q35–Q37)	Birth and stillbirth registries and national and local congenital anomalies registries	THMs	Maternal age, deprivation, sex
Chisholm *et al.*[35]	Perth, Australia 2000–2004 20,870 live births	1,097 congenital anomalies, 59 nervous system defects (BPA 74,000–74,299), 260 cardiovascular defects (BPA 74,500–74,299), 17 respiratory system defects (BPA 74,800–74,899), 101 gastrointestinal defects (BPA 74,900–75,199), 351 urogenital defects (BPA 75,200–75,399), 282 musculoskeletal defects (BPA 75,400–75,699), 36 congenital anomalies of integument (BPA 75,700–75,799) (***n*** = 1,097)	Western	TTH	Maternal age

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estimate of the meta-analysis for all major cardiac defects combined showed a nonstatistically significant 16% excess risk. There was no evidence of an exposure-response relationship (OR = 1.01; 95% CI, 0.95-1.08 per 10 µg/L TTHM) [see Supplemental Material 3, Figures 1-3 (doi:10.1289/ehp.0900677.S1)]. Furthermore, the brominated species did not show statistically significant associations. However, in a very large study, Nieuwenhuijsen ***et al.***[30] found a statistically significant association between bromo form levels and a subset of isolated major cardiovascular defects [2 to < 4 vs. < 2 µg/L bromoform, OR = 1.13 (95% CI, 0.99–1.29); ≥ 4 vs. < 2 µg/L bromoform, OR = 1.18 (95% CI, 1.00–1.39)]. Furthermore, ventricular septal defects showed an increased risk for high versus low exposure in all three studies examining these defects[13,3033], and a statistically significant excess risk was observed in the meta-analysis (OR = 1.59; 95% CI, 1.21–2.07) (Figure 2).

Table 1. Continued.

Reference	Study details	Cases	Sample population	Exposure assessment	Other risk factors included
Luben ***et al.***[25]	Arkansas, USA 1998–2002 320 cases and 614 controls, and a subset of 40 cases and 243 controls	320 and 40 hypospadias	Birth and birth defect registry from Arkansas Reproductive Health Monitoring System and National Birth Defects Prevention Study	THMs and HAAs, and personal characteristics in the subset	Maternal and paternal age, race, and education, marital status, maternal use of alcohol and tobacco, parity, prenatal care, 1- and 5-min Apgar scores, birth weight, method of delivery, any risks, procedures, or complications associated with delivery
Hwang ***et al.***[13]	Taiwan 2001–2003 396,049 births	2,148 congenital anomalies, including 43 anencephalus (ICD-9 code 740.0), 118 hydrocephalus (code 741.0), 59 ventricular septal defects (code 745.4), 19 atrial septal defects (code 745.5), 24 tetralogy of Fallot (code 745.2), 358 cleft palate or lips (code 749.0), cleft lip (code 749.1), 76 renal agenesis and dysgenesis (code 753.0), 49 obstructive urinary tract defects (code 753.2), 72 hypospadias (code 752.61), 166 chromosome anomalies (code 758)	Birth registry	High (TTHM 20 + µg/L), medium (TTHM 10–19 µg/L), low exposure (TTHM 5–9 µg/L), and 0–4 µg/L	Sex of infant, maternal age (< 20 years; 20–34 years; ≥ 35 years), plurality (singleton and multiple birth), maternal health status, population density
Iszatt ***et al.***[29]	Southeast England 1997–1998 363 hypospadias cases and 346 population controls	363 hypospadias	Population based	THMs	Income, birth weight, folate supplement use during pregnancy, maternal smoking, maternal occupational exposure to phthalates

Abbreviations: BPA, ***British Paediatric Association Classification of Disease***[37]; CDBM, chlorodibromomethane; ICD-9, ***International Classification of Diseases, 9th Revision*** (WHO[38]); ICD-10, ***International Classification of Diseases, 10th Revision*** (WHO[39]).

Table 2. Summary of meta-analyses of epidemiological studies on chlorinated disinfection by-products and adverse reproductive outcomes.

Outcome	Exposure	Studies included	*p*-Value of test for heterogeneity	Egger test; weighted *p*-value for intercept	Summary estimate
Any congenital anomaly	High vs. low chlorination by-products	***a, b, i, l, n***	0.041	0.16	1.17 (1.02-1.34)
Nervous system defects including neural tube defects	High vs. low chlorination by-products	***b, c, e, i, j, k, l, m***	0.058	0.11	1.06 (0.89-1.26)
Nervous system defects including neural tube defects	Per 10 µg/L TTHM	***b, c, e, j, k, l, m***	0.46	0.20	1.01 (0.95-1.06)
Nervous system defects including neural tube defects	BDCM	***d, j, k***	0.005	0.12	1.15 (0.59-2.25)
Anencephalus	High vs. low chlorination by-products	***i, j, k, o***	0.45	0.17	1.48 (0.92-2.39)
Hydrocephalus	High vs. low chlorination by-products	***g, i, o***	0.18	0.54	0.92 (0.57-1.48)
Spina Bifida	High vs. low chlorination by-products	***g, i, k, l***	0.35	0.55	1.22 (0.76-1.97)
Major cardiac defects	High vs. low chlorination by-products	***b, c, g, h, i, k, l, m***	0.017	0.27	1.16 (0.98-1.37)
Major cardiac defects	Per 10 µg/L TTHM	***b, c, k, l, m***	0.49	0.39	0.99 (0.95-1.04)
Ventricular septal defects	High vs. low chlorination by-products	***i, l, o***	0.69	0.13	1.59 (1.21-2.07)
Respiratory defects	High vs. low chlorination by-products	***a, i, l, m***	0.064	0.29	1.12 (0.91-1.37)
Oral cleft or cleft palate defects	High vs. low chlorination by-products	***a, b, c, g, i, k, l, o***	0.32	0.067	0.98 (0.88-1.08)
Oral cleft or cleft palate defects	Per 10 µg/L TTHM	***b, c, k, l, o***	0.44	0.26	1.00 (0.96-1.05)
Cleft palate only	High vs. low chlorination by-products	***i, k, l, o***	0.26	0.53	1.03 (0.89-1.19)
Urinary tract defects	High vs. low chlorination by-products	***a, i, l, m***	0.012	0.002	1.33 (0.92-1.92)
Obstructive urinary defects	High vs. low chlorination by-products	***i, l, o***	0.37	0.12	1.07 (0.87-1.30)
Hypospadias	High vs. low chlorination by-products	***g, n, o, p***	0.20	0.17	1.03 (0.84-1.28)

Summary estimates are shown as OR (95%CI).
a Aschengrau ***et al.***[22]; ***b*** Bove ***et al.***[23]; ***c*** Dodds ***et al.***[31]; ***d*** Dodds and King[32]; ***e*** Klotz and Pyrch[24]; ***f*** Magnus ***et al.***[34]; ***g*** Källen and Robert[28]; ***h*** Cedergren ***et al.***[27]; ***i*** Hwang ***et al.***[33]; ***j*** Study 1, Shaw ***et al.***[26]; ***k*** Study 2, Shaw ***et al.***[26]; ***l*** Nieuwenhuijsen ***et al.***[30]; ***m*** Chisholm ***et al.***[35]; ***n*** Luben ***et al.***[25]; ***o*** Hwang ***et al.***[13]; ***p*** Iszatt ***et al.***[29].

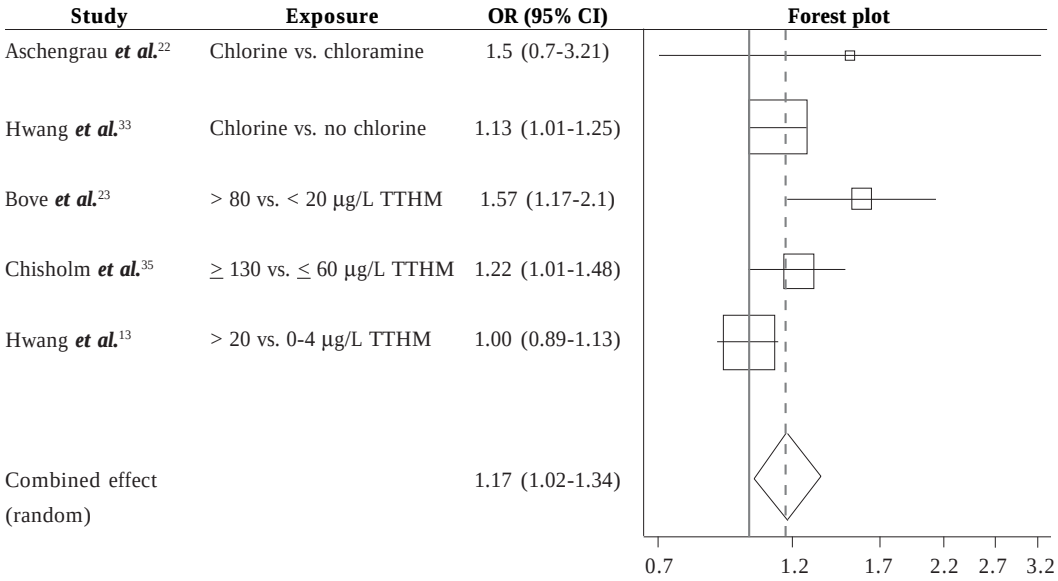

Figure 1. Study and summary risk estimates for any congenital anomalies and chlorination by-products. Test for heterogeneity: ***Q*** = 9.944 on four degrees of freedom (***p*** = 0.041). Egger test: weighted ***p***-value for intercept 0.16.

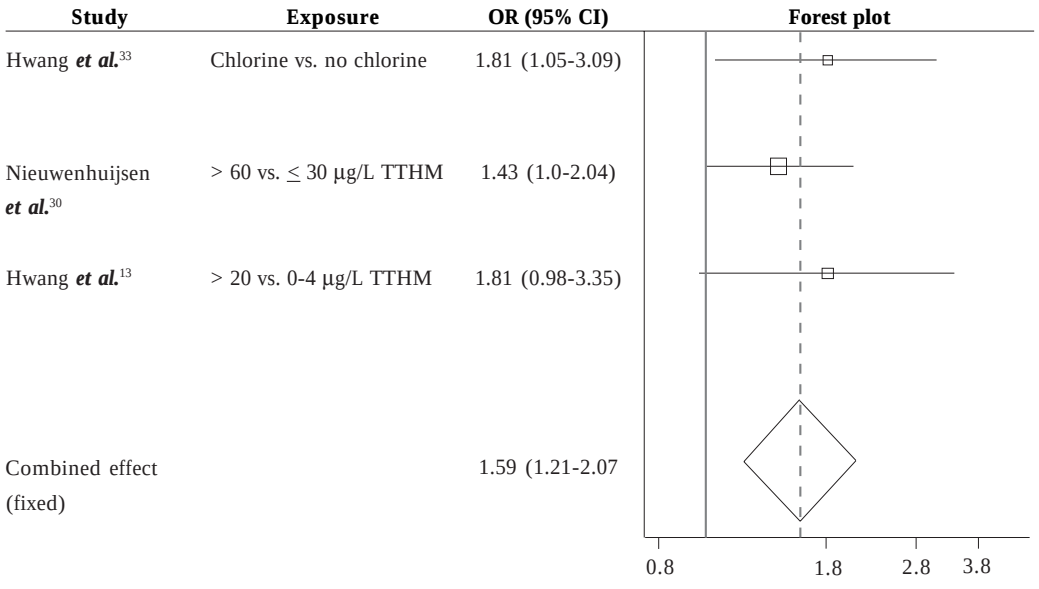

Figure 2. Study and summary risk estimates for ventricular septal defects and chlorination by-products. Test for heterogeneity: ***Q*** = 0.732 on two degrees of freedom (***p*** = 0.69). Egger test: weighted ***p***-value for intercept 0.13.

However, only Hwang ***et al.***[33] showed some indication of an exposure-response relationship. Ventricular septal defects showed little association with brominated THM levels in the study by Nieuwenhuijsen ***et al.***[30]. No statistically significant associations were observed with atrial septal defects in the two studies by Hwang et al.[13,33].

Two studies found a statistically sig-nificant positive association between chlorinated water and congenital anomalies of the respiratory system[22,33], but two other studies using TTHM levels did not[30,35] [Table 2; see also Supplemental Material 1, Table 4 (doi:10.1289/ehp.0900677.S1)]. Results of the two Swedish studies were inconsistent[33,34]. The meta-analysis showed some excess of risk, but it was not statistically significant.

Studies on oral cleft or/and cleft palate have generally shown no statistically significant associations with chlorinated water, TTHM, and brominated THMs, except for the study by Bove ***et al.***[23] [Table 2; see also Supplemental Material 1, Tables 5a,5b (doi:10.1289/ehp.0900677.S1)]. The meta-analyses showed no evidence for an association. In addition, the exposure-response relationship was not statistically significant (OR = 1.02; 95% CI, 0.97-1.06 per 10 µg/L TTHM) [see Supplemental Material 3, Figures 1-3 (doi: 10.1289/ehp.0900677.S1)].

Two studies reported statistically sig-nificant associations between chlorinated water or TTHM levels and urinary system defects[22,34], whereas one did not[30] [Table 2; see also Supplemental Material 1, Tables 6a, 6b (doi:10.1289/ehp.0900677.S1)]. Chisholm ***et al.***[35] reported almost statistically significant effects (OR = 1.40; 95% CI, 0.98-1.99), as did Hwang ***et al.***[33] (OR = 1.46; 95% CI, 1.00-2.13), and there was evidence for heterogeneity ($p = 0.012$) in study results. Nieuwenhuijsen ***et al.***[30] did not show an association with brominated THM species and found no statistically significant associations for obstructive urinary defects, although Hwang ***et al.***[13,33] did find excess risk. The meta-analyses showed some excess of risk, but it was not statistically significant.

Two studies examined the association between abdominal wall defects and chlorinated water or THM levels and did not find an association[28,30] [see Supplemental Material 1, Table 7 (doi: 10.1289/ehp. 0900677. S1)]. However, Nieuwenhuijsen ***et al.***[30] found a statistically significant association between isolated gastroschisis and bromoform levels [2–4 vs. < 2 µg/L bromoform, OR = 1.11 (95% CI, 0.85-1.45); ≥ 4 vs. < 2 µg/L bromoform, OR = 1.38 (95% CI, 1.00-1.92)].

The studies on hypospadias generally showed no statistically significant associations between chlorinated water or TTHM levels, and neither did the meta-analysis [Table 2; see also Supplemental Material 1, Table 8 (doi: 10.1289/ehp. 0900677.S1)]. However, Iszatt ***et al.***[29] reported a statistically significant excess risk between BDCM ingestion estimates and hypospadias (1st vs. 4th quartile BDCM, OR = 1.69; 95% CI, 1.04-2.73). Similarly, Luben ***et al.***[25] reported that risk of hypospadias was associated with moderate ingestion of > 0-32.5 µg/day TTHM. Luben ***et al.***[25] did not show a statistically significant association between HAA exposure levels and hypospadias but showed excess risk for TTHM uptake, which takes into account various personal behaviors and exposure routes, although this was not statistically significant. Iszatt ***et al.***[29] did not find such an association in a much larger study.

Generally there was little evidence for publication bias, except for cleft lip and palate, specifically when comparing the exposure-response relationships ($p = 0.04$), and urinary tract defect ($p = 0.002$) [Table 2; see also Supplemental Material 2, Figures 1-15 (doi: 10.1289/ehp.0900677.S1)]. However, it should be noted that publication bias tests and funnel plots are not considered rigorous tests when the number of studies is small.

Discussion

The epidemiologic studies we reviewed showed inconsistent results for an association between drinking water chlorination by-products and risk of all congenital anomalies combined and of specific groups of anomalies. For all congenital anomalies combined, the meta-analysis gave a statistically significant excess risk for high versus low exposure to water chlorination or TTHM (17%; 95% CI, 3-34), based on a small number of studies. The meta-analysis also suggested a statistically significant excess risk for ventricular septal defects (58%; 95% CI, 21-107), but this was based on only three studies, and there was little evidence of an exposure-response relationship. We observed no statistically significant relationships in the other meta-analyses. We found little evidence for publication bias except for urinary tract defects and cleft lip and palate.

Major limiting factors in studies on chlorination by-products and congenital anomalies are crude exposure assessment (with the exception of more recent studies), small samples sizes, heterogeneity in outcomes, and, to a lesser extent, potential for bias and confounding and ability to detect susceptible groups. These factors together

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may explain some of the mixed results and possibly the lack of associations. Furthermore, combined with the small number of studies included in the meta-analyses, these factors also reduce the strength of any conclusions that can be drawn from meta-analyses. These analyses are therefore not meant to provide final conclusions on the subject, but instead evaluate the current status of this growing body of research and offer guidance for the way forward.

Use of ecologic water supply zone estimates as an exposure index may result in exposure misclassification[40]. Furthermore, ingestion has generally served as the primary exposure route of interest, in spite of the fact that uptake through showering, bathing, and swimming could be considerable, specifically for THMs; such exposure has been considered in only a few studies[24,25,29]. Combining information on individual water use with water zone estimates could provide better exposure estimates, but the individual information should be evaluated for measurement error, because within-subject variability in questionnaire data may be substantial[41] and attenuate risk estimates. Furthermore, exposure estimates have been based primarily on maternal residence at birth. This ignores any exposure that occurs outside the home (e.g., in the workplace) and also ignores the possibility that a mother has moved to another residence during her pregnancy. Therefore, exposure assessment based on maternal residence at birth may result in exposure misclassification. In addition, studies from Scandinavia[12,27,28,33] and Taiwan[13], for example, have generally shown low levels of DBPs with a small range, making risk estimation more difficult because of a higher probability of exposure misclassification, particularly where seasonal variability has not been taken into account. On the whole, epidemiologic studies have used THMs as a proxy for total DBP load, but THMs are not necessarily a good proxy measure. Only two studies have investigated other DBPs such as HAAs[12,25]. The metabolism of different DBP species varies[2], so it is insufficient to analyze DBPs as a whole or to use TTHM as a proxy. Investigation of the relation between non-THM by-products and reproductive outcomes is required to understand whether any specific DBP may cause health effects. A detailed assessment of the DBP mixture is required to provide an understanding of any observed results.

Sample sizes have often been insufficient to produce stable results, and this may have resulted in chance findings and mixed results, although there are exceptions. For example, the studies by Hwang ***et al.***[13,33] and Nieuwenhuijsen ***et al.***[30] have provided sufficiently large numbers of cases to create various exposure categories with more stable risk estimates, and were able to examine exposure-response relationships to some extent. A related limitation of the presented meta-analyses is the relatively small number of studies and therefore the need to conduct relatively simple analyses, comparing high- versus low- exposure categories and combining what could be regarded as different and/or inconsistent estimates of exposure (e.g., high vs. low TTHM concentration and chlorination vs. nonchlorination). For instance, in Table 2, for all congenital anomalies Chisholm ***et al.***[35] used cut points of < 60 μg/L TTHM for low exposure, whereas the high-exposure group used by Hwang ***et al.***[13] was > 20 μg/L TTHM. Although we assume that the meta-analysis is statistically accurate, the biological basis for comparing studies with this degree of heterogeneity in the definition of exposure is more problematic, and this should be taken into account when interpreting the results. Ideally, all the exposure categories have the same cutoffs, but in practice, this is often impossible because of the different local conditions. We also performed sensitivity analyses leaving out the studies with qualitative estimates (e.g., chlorination vs. nonchlorination), but it generally did not materially change the summary risk estimates, except for nervous system defects including NTDs [from 1.06 (95% CI, 0.89-1.26) to 1.28 (95% CI, 0.89-1.83)] and cleft palate only [from 1.03 (95% CI, 0.89-1.19) to 1.14 (95% CI, 0.80-1.62)], but they remained not statistically significant.

The analyses of TTHM exposure-response relationships combined more comparable exposure levels and were therefore probably more informative, but they could only be conducted for a few end points because of the lack of a sufficient number of studies. The question here is whether TTHM may be the putative agent or a (not so good) marker for something else. Also, as a result of the small number of cases in the studies and to increase power, congenital anomalies have often been categorized into main groups such as NTDs, major heart defects, and abdominal defects, or as all congenital anomalies combined. These anomalies, however, are generally heterogeneous with respect to both phenotype and presumed etiology, and combining them may not be appropriate. For example, Nieuwenhuijsen ***et al.***[30] showed that focusing on isolated subcategories may result in different findings. Of course, this

was possible only because of the large number of subjects in the study.

The retrospective and registry-based nature of many of the studies has meant that information on lifestyle factors, such as maternal smoking and alcohol consumption, has often been lacking. These factors are not necessarily potential confounders, as it seems unlikely that levels of chlorination by-products are related to such factors, nor are they established risk factors for all congenital anomalies. However, behavioral patterns such as ingestion of tap water may be associated with these factors[41,42]. Furthermore, registry completeness is a factor that may make studies incomparable. In some places, case ascertainment occurs immediately after birth, so some malformations may not be recorded, especially malformations such as hypospadias and cardiac defects, where the less-severe forms may not immediately be recognized. In Norway and Taiwan, birth defects reported up to 7 days after birth were included in the study[13,34], whereas in California[26] and Sweden[27], cases were diagnosed up to 1 year after birth. However, we anticipate that random under reporting would weaken any observed association rather than introduce a spurious effect. A further discrepancy between studies was whether the study population had been extended beyond live births. In the European Union, terminations accounted for 18% and fetal deaths for 2% of congenital anomalies [European Surveillance of Congenital Anomalies (Eurocat)[43]]. However, whereas some study populations included terminations and stillbirths[24,30,31], others did not[13,27,35].

The strongest, albeit relatively modest, evidence for a possible association with DBPs was found for one specific type of cardiac defect: ventricular septal defect. This finding is of interest in light of studies of other environmental exposures in which associations for this particular birth defect have been observed with, for example, air pollutants[44,45]. However, ventricular septal defects are also among the anomalies that are most subject to variable diagnosis and reporting in routine anomaly registries; all three studies that reported increased risks of ventricular septal defects in relation to high chlorination exposure were based on large, regional or nationwide, congenital anomaly registries in which variable reporting is common[46] and may affect geographic comparison studies such as the DBP studies.

Only one study[26] has examined gene-environment interaction and/or the presence of susceptible groups, and this study did not find any effect. There is some evidence, however, to suggest that cytochrome P450 2E1 (CYP2E1) or glutathione ***S***-transferase T1 may play a role in the susceptibility to DBPs, and this should be further explored[47,48].

Animal and cell studies have found some effects associated with DBPs. NTDs and craniofacial defects have been found with administration of di/trichloroacetic acid in rats[49], and cardiac malformations have been induced at high doses of dichloroacetic acid[50]. Hunter ***et al.***[51] observed changes in neural tube development when mouse embryos were exposed to HAAs. Several chloroacetonitrile compounds have been shown to increase the rate of resorption, to reduce fetal body weight and survival[51], and to result in an increase in malformations of the cardiovascular, digestive, soft tissue, and urogenital systems[53,54]. However, these adverse develop mental effects have only been demonstrated at high doses in conjunction with severe feto toxicity. Furthermore, the mechanisms for possible effects are still unclear, although some have been suggested. Low levels of folate have been associated with several congenital anomalies such as neural tube defects[55]. Alston[56] found that chloroform inhibited methionine biosynthesis in cell culture. Dow and Green[57] showed that trichloroacetic acid interacts with vitamin B12, probably by a free radical mechanism, inhibiting both the methylmalonyl coenzyme A and methionine salvage pathways in rats. As a result of the latter, a secondary folate deficiency develops because of the methylfolate trap, leading to a major impairment in formate metabolism. Folate and folic acid are forms of vitamin B that are involved in the synthesis, repair, and functioning of DNA and are required for the production and maintenance of cells[58]. Folate plays an important role for cells under going rapid turnover, such as tissues in the developing fetus.

The way forward

Given the many studies conducted and the limited evidence of an association between chlorination by-products and congenital anomalies, we might ask whether there is a need to conduct more studies, and if so, what should they look at? Disinfection of drinking water is an important part of public health, and many people are exposed to chlorination by-products not only through ingestion but also through other activities such as showering and bathing. Ongoing surveillance of any possible adverse health effects is therefore war-

ranted, even though the relative risks may be small. As mentioned above, the mixture of the by-products may differ by geographic area and time, for example, because of changes in water treatment methods, and generally only indicator substances such as TTHM have been used to examine the health risks. It is important that we understand the underlying mixture of the by-products, both in existing and new studies, and where possible, we should examine any possible health risks of specific DBPs or mixtures. Studies are needed to examine the potential effects of certain mixtures, such as brominated species, in more specific locations. For example, Perth, Australia (see Chisholm ***et al.***[35]) or the Barcelona area in Spain (see Villanueva ***et al.***[59]) might be suitable places because of their high levels of brominated compounds.

Furthermore, it would be worthwhile to examine the various exposure pathways and routes other than ingestion in more detail, specifically for volatile by-products such as THMs, as the level of exposure and metabolism may be different and the measures for exposure prevention are likely to be different. This can probably only be done in case-control studies; in such studies it would be necessary to estimate exposure in the most critical (early) periods of pregnancy, which could prove difficult. Prospective exposure assessment through a cohort design would be more appropriate, but practical and financial constraints preclude such a study, as the size of the cohort would need to be extremely large to study rare congenital anomalies.

Regarding the outcomes, the focus of future studies should be on subcategories of congenital anomalies, rather than on the whole group, and should focus on anomalies for which the ascertainment is reasonably complete and consistent if registry-based designs are used. Findings for ventricular septal defects should be followed up, preferably in well-designed case-control studies. The study by Nieuwenhuijsen ***et al.***[30] showed an excess risk for bromo form and gastroschisis; these findings may be worth examining in more detail and in a different population. One of the problems in that study was the low levels of bromo form in England and Wales, and future studies should be conducted in places where bromo form levels are higher (such as Perth).

Further work is needed on the relation between potential confounders such as smoking and alcohol intake, as well as the relation with by-products in the water and personal behavioral characteristics such as tap water ingestion (instead of bottled water), showering, and bathing to examine to what extent confounding may explain findings for registry-based studies where this information is missing.

There is some suggestion that some chlorination by-products may interfere with folate metabolism; this and other potential mechanisms such as oxidative stress and genotoxicity could be examined with biomarkers in pregnant women to assess to what extent this may be possible. Furthermore, genotyping may identify susceptible populations (e.g., those with CYP2E1).

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