The epidemiology and possible mechanisms of disinfection by-products in drinking water

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This paper summarizes the epidemiological evidence for adverse health effects associated with disinfection by-products (DBPs) in drinking water and describes the potential mechanism of action.

There appears to be good epidemiological evidence for a relationship between exposure to DBPs, as measured by trihalomethanes (THMs), in drinking water and bladder cancer, but the evidence for other cancers including colorectal cancer is inconclusive and inconsistent. There appears to be some evidence for an association between exposure to DBPs, specifically THMs, and little for gestational age/intrauterine growth retardation and, to a lesser extent, pre-term delivery, but evidence for relationships with other outcomes such as low birth weight, stillbirth, congenital anomalies and semen quality is inconclusive and inconsistent. Major limitations in exposure assessment, small sample sizes and potential biases may account for the inconclusive and inconsistent results in epidemiological studies. Moreover, most studies have focused on total THMs as the exposure metric, whereas other DBPs appear to be more toxic than the THMs, albeit generally occurring at lower levels in the water.

The mechanisms through which DBPs may cause adverse health effects including cancer and adverse reproductive effects have not been well investigated. Several mechanisms have been suggested, including genotoxicity, oxidative stress, disruption of folate metabolism, disruption of the synthesis and/or secretion of placental syncytiotrophoblast-derived chorionic gonadotropin and lowering of testosterone levels, but further work is required in this area.

Keywords: chlorination; disinfection by-products; epidemiology; cancer; reproductive health

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1. Introduction

Disinfection of drinking water has led to major improvements in public health in developed countries since its introduction in the first half of the twentieth century. It has now been more than 30 years since the discovery that by-products can be formed in small quantities as part of the chlorination process (Rook 1974). Disinfection by-products (DBPs) are formed when water is disinfected, and natural organic matter, bromide and iodide in the water react with chlorine, chlorine dioxide, chloramines and/or ozone (Bichsel & von Gunten 2000; Zhang et al. 2000). Their formation and occurrence depend on many factors including disinfectant type(s) and dose(s), type(s) of treatment, pH, temperature, contact time(s) with disinfectant(s), water source, amount and character of natural organic matter and bromide and iodide levels (Reckhow & Singer 1985; Stevens et al. 1989; Amy et al. 1991; Singer 1994). Up to 600 DBPs have been identified (Richardson 1998; Richardson et al. 2007), and these chemicals differ considerably in their physico-chemical properties (e.g. volatility). Different mixtures of by-products may exist in different locations depending on the various factors mentioned earlier, making it more difficult to ascertain the risk, if any, of health effects in relation to specific DBPs and mixtures of DBPs, as well as to compare the findings from different epidemiological studies.

Trihalomethanes (THMs) are the most commonly formed group of DBPs. These are volatile DBPs, and individuals may be exposed not only through ingestion but also through inhalation and dermal absorption during activities such as showering, bathing and swimming (Weisel & Jo 1996; Nieuwenhuijsen et al. 2000a). For non-volatile DBPs such as the haloacetic acids (HAAs), ingestion is thought to be the main route of exposure. However, dermal adsorption has also been examined for such DBPs (Kim & Weisel 1998). Recent modelling of THM uptake suggested that swimming may lead to the highest levels in the blood (Whitaker et al. 2003). Uptake of DBPs through showering, bathing and swimming was associated with an increased risk of bladder cancer in a recent Spanish epidemiology study (Villanueva et al. 2007).

In this paper, we first summarize the epidemiological evidence regarding health effects associated with exposure to DBPs, particularly for reproductive outcomes, and briefly describe the main mechanisms proposed for the action of these compounds.

2. Epidemiological studies examining health effects related to exposure to chlorination disinfection by-products

(a) Cancer

The health effects of DBPs in drinking water have been a concern since DBPs were first reported in the 1970s. According to a review by the IPCS (2000): ‘more studies have considered bladder cancer than any other cancer. The authors of the report caution against a simple interpretation of the observed associations. The epidemiological evidence for an increased relative risk for bladder cancer is not consistent—different risks are reported for smokers and non-smokers, for men and women, and for low and high water consumption. Risk may differ among
Table 1. Pooled analysis of bladder cancer and total THM exposure.

<table>
<thead>
<tr>
<th>total THM exposure level (mg)</th>
<th>OR (95% CI)</th>
<th>male</th>
<th>female</th>
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<tbody>
<tr>
<td>0–15</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>&gt;15–50</td>
<td>1.22 (1.01–1.48)</td>
<td>0.92 (0.65–1.32)</td>
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<td>&gt;50–400</td>
<td>1.28 (1.08–1.51)</td>
<td>0.94 (0.70–1.27)</td>
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<tr>
<td>&gt;400–1000</td>
<td>1.31 (1.09–1.58)</td>
<td>1.02 (0.74–1.41)</td>
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<tr>
<td>&gt;1000</td>
<td>1.50 (1.22–1.85)</td>
<td>0.92 (0.65–1.30)</td>
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</table>

THM exposure level = concentration × consumption per day × years exposed. Adapted from Villanueva et al. (2004).

Various geographic areas because the DBP mix may be different or because other water contaminants are also present. For example, as part of an improved exposure assessment (Amy et al. 2005) for two well-conducted bladder cancer epidemiology studies (King & Marrett 1996; Cantor et al. 1998), substantial differences in the mixture of DBPs were found within and between one US state and one Canadian province (e.g. amount of brominated DBPs, relative proportion of THMs to HAAs, relative proportion of di- and trihalogenated HAAs). A recent pooled analysis by Villanueva et al. (2004), which provided quantitative information on THM exposure, confirmed some of the gender differences. For men, there was an exposure–response relationship between THM intake and bladder cancer, but there was no relationship for women (table 1). For other cancers, the evidence is much weaker. Some studies have suggested an association between DBPs and colorectal cancers, whereas others have not (Wilkins & Comstock 1981; Young et al. 1981, 1987; Doyle et al. 1997, Koivusalo & Vartiainen 1997; Hildesheim et al. 1998; King et al. 2000a; Bove et al. 2007). Furthermore, there is little evidence for an association between exposure to DBPs and other cancers such as liver, kidney, brain, lung and breast cancer, lymphomas, cancer of the pancreas, but the number of studies is small (IPCS 2000). A recent report suggested an association between THMs and skin cancer, but further work needs to be conducted (Karagas et al. 2008).

(b) Reproductive outcomes

Reproductive health outcomes should be easier to study than cancer because of the shorter relevant exposure period. Among others, congenital anomalies, stillbirth, spontaneous abortion, birth weight, prematurity and semen quality have been the focus of investigation. Various thorough reviews have been conducted and have concluded that the relationship between DBP exposure and reproductive health outcomes remains unclear, mainly owing to limitations in the exposure assessment in most studies (Reif et al. 1996; IPCS 2000; Nieuwenhuijsen et al. 2000b; Gevecker Graves et al. 2001; Bove et al. 2002; Tardiff et al. 2006).

A number of studies have found statistically significant positive associations between THMs and neural tube defects (NTDs), one of the most studied groups of congenital anomalies (Bove et al. 1995; Klotz & Pyrch 1999; Dodds & King 2001), whereas other studies have not found statistically significant associations.
Table 2. Summary of epidemiological studies on chlorinated DBPs and adverse reproductive outcomes.

<table>
<thead>
<tr>
<th>author (year)</th>
<th>study details (location, time, sample size)</th>
<th>cases</th>
<th>exposure assessment</th>
<th>other risk factors included</th>
<th>main findings OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aschengrau et al. (1989)</td>
<td>MA, USA, sample population: 1677</td>
<td>286 spontaneous abortion</td>
<td>surface versus ground water; chlorinated versus chloraminated water</td>
<td>smoking habits, contraceptive use, medical and obstetrical history, metals</td>
<td>surface versus ground water: 2.2 (1.3–3.6)</td>
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<tr>
<td>Kramer et al. (1992)</td>
<td>IA, USA, 151 towns with a single water source, 1989–1990, sample population: 4028</td>
<td>688 (total): 159 LBW, 342 pre-term delivery, 187 IUGR/SGA</td>
<td>based on maternal residential address and one municipal water survey to estimate individual THM levels (two or three exposure categories)</td>
<td>maternal age, parity, marital status, education, smoking, prenatal care</td>
<td>no versus medium (1–9 μg l⁻¹) versus high (≥10 μg l⁻¹): chloroform LBW: 1 versus 1.1 (0.7–1.6) versus 1.3 (0.8–2.2) IUGR: 1 versus 1.3 (0.9–1.8) versus 1.8 (1.1–2.9) dichlorobromomethane IUGR: 1 versus 1.2 (0.8–1.7) versus 1.7 (0.9–2.9) chlorinated versus chloraminated stillbirth: 2.6 (0.9–7.5) neonatal deaths: 1.1 (95% CI not provided) congenital anomalies: major anomalies: 1.5 (0.7–2.1) respiratory defects: 3.2 (1.1–9.5) urinary tract defects 4.1 (1.2–14.1)</td>
</tr>
<tr>
<td>Aschengrau et al. (1993)</td>
<td>MA, USA, two hospitals, 1977–1980, sample population: 2348</td>
<td>1171 (total): 1039 major congenital anomalies, urinary tract defects, respiratory tract defects; 77 stillbirths; 55 neonatal deaths</td>
<td>based on maternal residential address to ascertain type of water supply, chlorination versus chloramination, and ground/mixed water versus surface water.</td>
<td>maternal age, pregnancy history, alcohol, ethnicity, hospital payment, other water contaminants</td>
<td>(Continued.)</td>
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Table 2. (Continued.)

<table>
<thead>
<tr>
<th>Study Details</th>
<th>Cases</th>
<th>Exposure Assessment</th>
<th>Other Risk Factors Included</th>
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<tr>
<td><strong>Bove et al. (1995)</strong></td>
<td>29,268 (total): live births: 1853</td>
<td>based on maternal residential address and municipal water surveys to estimate monthly TTHM levels (five or six exposure categories)</td>
<td>maternal age, ethnicity, infant's sex, primipara, prenatal care, education, previous stillbirth or miscarriage, other contaminants</td>
<td>TTHM levels &gt;100 versus ≤20 μg l⁻¹: LBW: 1.4 (50% CI 1.2–1.7) IUGR/SGA: 1.5 (90% CI 1.2–1.9) TTHM levels &gt;80 versus ≤20 μg l⁻¹ surveillance register defects: 1.6 (90% CI 1.2–2.0) CNS system defects: 2.6 (90% CI 1.5–4.3) NTDs: 3.0 (90% CI 1.3–6.6) major cardiac defects: 1.8 (90% CI 1.0–3.3) TTHM levels &gt;100 versus ≤20 μg l⁻¹: oral cleft defects: 3.2 (90% CI 1.2–7.3)</td>
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<td></td>
<td>LBW, 905 very LBW, 402 SGA, 7167 pre-term, 594 foetal deaths; all births: defects: 669 total, 118 central nervous system defects, 83 oral cleft, 56 NTD, 108 major cardiac</td>
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<td>81,602</td>
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<td><strong>Savitz et al. (1995)</strong></td>
<td>548 (total): 126 spontaneous abortion, 244 pre-term, 178 LBW</td>
<td>based on maternal residential address and quarterly municipal water surveys to estimate average TTHM levels. Analysis of (i) surface versus ground water source, (ii) TTHM levels (three exposure categories), (iii) consumption during pregnancy, (iv) water source × amount, and (v) TTHM dose (level × amount)</td>
<td>maternal age, ethnicity, hospital, education, marital status, poverty level, smoking, alcohol consumption, employment, nausea</td>
<td>40.8–59.9 versus 81.1–168.8 μg l⁻¹: TTHM: spontaneous abortion: 1.2 (0.6–2.4) 40.8–63. 3 versus 82.8–168.8 μg l⁻¹: TTHM: LBW: 1.3 (0.8–2.1) per 50 μg l⁻¹ TTHM increment change: spontaneous abortion: 1.7 (1.1–2.7)</td>
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<tr>
<td>Author et al. (year)</td>
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<td>Kanitz et al. (1996)</td>
<td>Liguria, Italy, two hospitals, 1988–1989, sample population: 676</td>
<td>548 live births in ‘exposed’ area, 50 pre-term, 141 Caesarean section, 133 neonatal jaundice, 20 LBW, 288 small body length, 370 small cranial circumference based on maternal residential address to ascertain type of water source (chlorine dioxide and/or hypochlorite versus not treated).</td>
<td>Maternal age, education, smoking, alcohol, infant’s sex</td>
<td>Sodium hypochlorite treated (8–16 μg l⁻¹ TTHMs) versus non-treated water: neonatal jaundice: 1.1 (0.7–2.8) LBW: 6.0 (0.6–12.6) small body length: 2.3 (1.3–4.2) small cranial circumference: 3.5 (2.1–8.5)</td>
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<tr>
<td>Waller et al. (1998)</td>
<td>CA, USA, three regions of surface, ground and mixed drinking water, 1989–1991, sample population: 5144 pregnancies</td>
<td>499 spontaneous abortions based on maternal residential address and quarterly municipal water surveys to estimate average TTHM and individual THM levels. Analysis based on: (i) THM levels (three or 10 exposure categories) and (ii) consumption during first trimester from interview (two exposure categories)</td>
<td>Maternal age, gestational age, smoking, history of pregnancy loss, race, employment</td>
<td>High TTHM dose (≥5 glasses d⁻¹ + ≥75 μg l⁻¹) versus low dose (&lt;5 glasses d⁻¹ + &lt;75 μg l⁻¹): spontaneous abortion: 1.8 (1.1–3.0) high BDCM dose (≥5 glasses d⁻¹ + ≥18 μg l⁻¹) versus low dose (&lt;5 glasses d⁻¹ + &lt;18 μg l⁻¹): spontaneous abortion: 3.0 (1.4–6.6).</td>
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### Table 2. (Continued.)

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<td>see Waller et al. (1998)</td>
<td>re-analysis Waller et al. (1998) utility wide subset sample highest AOR (adjusted OR) high TTHM dose (≥5 glasses d⁻¹ + &gt;75 μg l⁻¹) versus low dose (≥5 glasses d⁻¹ + &lt;75 μg l⁻¹); spontaneous abortion: 5.1 (1.8–14.7) little relationship with showering</td>
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<tr>
<td>Gallagher et al. (1998)</td>
<td>CO, USA, 28 census blocks in two water districts, 1990–1993, sample population: 1244 live births</td>
<td>72 LBW, 29 term-LBW, 68 pre-term delivery</td>
<td>based on maternal residential address and municipal water surveys. Estimate of household TTHM level during last trimester based on hydraulic modelling (four exposure categories)</td>
<td>maternal age, smoking, marital status, parity, education, employment, prenatal care</td>
<td>high TTHM level (≥61 μg l⁻¹) versus lowest (≤20 μg l⁻¹): LBW: 2.1 (1.0–4.8) term LBW: 5.9 (2.0–17.0)</td>
</tr>
<tr>
<td>Dodds et al. (1999)</td>
<td>Nova Scotia, Canada, 1988–1995, sample population: 49,842 births</td>
<td>4673 SGA, 2393 LBW, 342 very LBW, 2689 pre-term delivery, 77 NTD, 82 cleft defect, 430 major cardiac defects, 197 stillbirth, 96 chromosomal abnormalities</td>
<td>based on maternal residential address and TTHM levels for public water facilities (three sampling locations) modelled using linear regression on the basis of observations by year, month and facility (four exposure categories)</td>
<td>LBW, NTDs, cardiac defects: income very LBW, stillbirth: smoking maternal age, parity, maternal smoking, attendance prenatal classes, neighbourhood family income, infant’s sex, pregnancy and pre-delivery weight</td>
<td>0–49 versus &gt;100 μg l⁻¹ TTHMs stillbirth 1.66 (1.09–2.52) chromosomal abnormalities 1.38 (0.73–2.59) SGA 1.08 (0.99–1.18) NTDs 1.18 (0.67–2.10)</td>
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<tr>
<td>Author (Year)</td>
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<td>Cases</td>
<td>Exposure Assessment</td>
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<td>King et al. (2000b)</td>
<td>Nova Scotia, Canada, 1988–1995, Sample population: 49756</td>
<td>214 stillbirths (72 asphyxia-related stillbirths)</td>
<td>Based on maternal residential address and TTHM, chloroform and BDCM levels for public water facilities (three sampling locations) modelled using linear regression on the basis of observations by year, month and facility (four exposure categories) ((r = 0.44) for TTHM and BDCM)</td>
<td>Maternal age, maternal smoking</td>
<td>0–49 versus &gt;100 (\mu)g(\text{l}^{-1}), chloroform stillbirth: 1.56 (1.04–2.34), asphyxia-related stillbirth: 3.15 (1.64–6.03), &lt; 5 versus &gt;20 (\mu)g(\text{l}^{-1}), BDCM stillbirth: 1.98 (1.23–3.49), asphyxia-related stillbirth: 1.75 (0.72–4.22)</td>
</tr>
<tr>
<td>Dodds &amp; King (2001)</td>
<td>Nova Scotia, Canada, 1988–1995, Sample population: 49842 births</td>
<td>77 NTDs, 430 Cardiovascular anomalies, 82 cleft defects, 96 chromosomal abnormalities</td>
<td>See King et al. (2000b)</td>
<td>NTDs, cardiovascular anomalies and chromosomal abnormalities: maternal age, income, cleft defects: maternal age onset of prenatal care</td>
<td>BDCM ≥20 versus &lt;5 (\mu)g(\text{l}^{-1}), NTDs: 2.5 (1.2–5.1)</td>
</tr>
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<td>Klotz &amp; Pyrch (1999)</td>
<td>NJ, USA, 1993–1994, sample population: all births, of which 112 cases and 248 controls selected</td>
<td>112 NTDs</td>
<td>Based on residential address and public water facility TTHM data, and tap water sampling for TTHMs, haloacetonitriles and HAAs (three to five exposure categories)</td>
<td>TTHMs public monitoring data, known residence and isolated cases: &lt; 5 versus 40+ (\mu)g(\text{l}^{-1}), NTDs: 2.1 (1.1–4.0)</td>
<td>(Continued.)</td>
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<tr>
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<td>Magnus et al. (1999)</td>
<td>Norway, sample population: 141077</td>
<td>2608 all birth defects, 62 NTDs, 250 major cardiac defects, 91 respiratory defects, 122 urinary defects, 143 oral cleft</td>
<td>Chlorination yes versus no, colour high versus low (in chlorinated water average TTHMs = 9.4 μg l⁻¹ and average HAAs = 14.6 μg l⁻¹)</td>
<td>Maternal age, parity, geographical placement, population density, industry profile</td>
<td>No chlorination low colour versus chlorination high colour all birth defects: 1.14 (0.99–1.31) urinary tract defects: 1.99 (1.10–3.57) NTDs: 1.26 (0.61–2.62) major cardiac defects: 1.05 (0.76–1.46) respiratory tract defects: 1.07 (0.52–2.19)</td>
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<td>Jaakkola et al. (2001)</td>
<td>Norway, sample population: 137145</td>
<td>6249 LBW, SGA, 7886 pre-term delivery</td>
<td>See Magnus et al. (1999)</td>
<td>See Magnus et al. (1999)</td>
<td>No chlorination low colour versus chlorination high colour pre-term delivery: 0.91 (0.84–0.99)</td>
</tr>
<tr>
<td>Källen &amp; Robert (2000)</td>
<td>Sweden (1985–1994), sample population: no chlorination: 74 324 singletons; Na-hypochlorite: 24 731 singletons; chlorine dioxide: 15 429 singletons</td>
<td>Multiple births, gestational duration, birth weight, intrauterine growth, body length, head circumference, body mass index (BMI), infant survival up to 1 year, perinatal death, Apgar score, neonatal jaundice, congenital anomalies, including NTD, childhood cancer, hypothyroidism</td>
<td>No versus sodium hypochlorite (no versus chlorine dioxide)</td>
<td>Year of birth, maternal age, parity, maternal education, smoking, congenital anomalies and childhood cancer: maternal age, year of birth</td>
<td>No versus sodium hypochlorite LBW: 1.15 (1.05–1.26) &lt;32 weeks’ gestation: 1.22 (1.00–1.48) &lt;37 weeks’ gestation: 1.09 (1.01–1.17) &lt;43 cm length: 1.97 (1.30–2.97) &lt;47 cm length: 1.25 (1.10–1.43) BMI &gt; 16 kg m⁻²: 1.27 (1.19–1.37) &lt;31 cm head circumference: 1.46 (1.07–1.98) Spine anomalies: 3.2 (1.0–10.0)</td>
</tr>
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<tr>
<td>Yang et al. (2000a)</td>
<td>Taiwan, sample population: 18025 first parity births: chlorinated: 10007, non-chlorinated: 8018</td>
<td>LBW pre-term delivery (&lt;37 weeks)</td>
<td>chlorinated (&gt;95% population served chlorinated water) versus non-chlorinated (&lt;5% population served chlorinated water)</td>
<td>maternal age, marital status, maternal education, infant's sex</td>
<td>chlorinated versus non-chlorinated pre-term delivery: 1.34 (1.15–1.56)</td>
</tr>
<tr>
<td>Yang et al. (2000b)</td>
<td>Taiwan, sample population: chlorinated: 24882, non-chlorinated: 20460</td>
<td>sex ratio</td>
<td>see Yang et al. (2000a)</td>
<td></td>
<td>chlorinated versus non-chlorinated sex ratio: no significant difference</td>
</tr>
<tr>
<td>Cedergren et al. (2002)</td>
<td>Sweden, sample population: 58669</td>
<td>cardiac defects</td>
<td>&gt;10 versus ≤10 μg l⁻¹ TTHM in surface water hypochlorite and chlorine dioxide versus hypochlorite in surface water ground water versus surface water</td>
<td>maternal age, parity, smoking, education</td>
<td>&gt;10 versus ≤10 μg l⁻¹ TTHM cardiac defects: 1.30 (1.08–1.56) ground water versus surface water cardiac defects: 1.32 (1.10–1.58) hypochlorite and chlorine dioxide versus hypochlorite cardiac defects: 1.85 (1.42–2.39)</td>
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<tr>
<th>author (year)</th>
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<tr>
<td>Hwang et al. (2002)</td>
<td>Norway, sample population: 285 631</td>
<td>any birth defect; NTD: anencephalus, spina bifida, hydrocephalus; cardiac defects: ventricular septal defects, atrial septal defects; respiratory defects: oral cleft defects; cleft palate; cleft lip; urinary tract defect: obstructive urinary tract defect</td>
<td>chlorination (yes/no) and level of water colour (mg Pt l(^{-1}): &lt;10, 10–19.9, ≥20)</td>
<td>maternal age, parity, socioeconomic status: centrality and population density</td>
<td>chlorination (yes) and level of water colour: &lt;10 versus ≥20 mg Pt l(^{-1}) all birth defects: 1.18 (1.02–1.36) ventricular septal defect: 1.81 (1.05–3.00) chlorination (yes) and level of water colour: &lt;10 versus ≥10 mg Pt l(^{-1}) all birth defects: 1.13 (1.01–1.25) cardiac defects: 1.37 (1.00–1.89) respiratory defects: 1.89 (1.00–3.58) urinary tract defects: 1.46 (1.00–2.13)</td>
</tr>
<tr>
<td>Nieuwenhuijsen et al. (2002)</td>
<td>England, sample population: 11 462</td>
<td>birth weight</td>
<td>amount of swimming (h)</td>
<td>maternal age, maternal education, smoking, alcohol use, drugs use, gestational age, ethnicity, infant’s sex</td>
<td>no association</td>
</tr>
<tr>
<td>Wright et al. (2003)</td>
<td>MA, USA, sample population: 56 513</td>
<td>birth weight, LBW, SGA, gestational age, pre-term delivery</td>
<td>0–60, &gt;60–80, &gt;80 (\mu)g l(^{-1}) TTHM or per 20 (\mu)g l(^{-1}) TTHM increase</td>
<td>gestational age, infant’s sex, maternal age, maternal education, maternal race, smoking, prenatal care, parity, median household income, previous infant ≥4000 g, previous pre-term delivery, maternal medical history, SGA: did not include infant sex and gestational age</td>
<td>0–60 versus &gt;80 (\mu)g l(^{-1}) TTHM birth weight: −32 g (−47 to −18) SGA: 1.14 (1.02–1.26) gestational age (weeks): 0.08 (0.01–0.14) per 20 (\mu)g l(^{-1}) TTHM increase birth weight: −2.8 g (−5.5 to −0.2) gestational age: 0.02 (0.01–0.03)</td>
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Table 2. (Continued.)

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<thead>
<tr>
<th>author (year)</th>
<th>study details (location, time, sample size)</th>
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<th>exposure assessment</th>
<th>other risk factors included</th>
<th>main findings OR (95% CI)</th>
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<tbody>
<tr>
<td>Shaw et al. (2003)</td>
<td>CA, USA, study 1: 538 NTD cases and 539 controls study 2: 265 NTD cases, 207 conotruncal heart defect cases and 409 orofacial cleft cases and 481 controls</td>
<td>study 1: NTDs (anencephaly and spina bifida) study 2: NTDs (anencephaly and spina bifida), conotruncal heart defects, orofacial clefts</td>
<td>study 1 and 2: continuous TTHMs and categorical: 0, 1–24, 25–49, 50–74 and ≥75 μg l⁻¹ TTHMs also study 1: ≥50 versus &lt;50 μg l⁻¹ and &lt;5 glasses; ≥50 versus &lt;50 μg l⁻¹ and &gt;5 glasses</td>
<td>ethnicity, education, body mass index, use of vitamins, methylenetetrahydrofolate reductase (MTHFR) genotype</td>
<td>study 1: NTDs NTD risk inversely related to TTHM exposure but only occasionally significant for one category</td>
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<td>chloroform ≥12.2 versus &lt;12.2 μg l⁻¹: 0.50 (0.34–0.75) BDCM ≥4.2 versus &lt;4.2 μg l⁻¹: 0.66 (0.45–0.97) CDBM ≥1.7 versus &lt;1.7 μg l⁻¹: 0.69 (0.47–1.0)</td>
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<tr>
<th>Author (Year)</th>
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<th>Main Findings OR (95% CI)</th>
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<tbody>
<tr>
<td>Aggazzotti et al. (2004)</td>
<td>Italy, case–control study in nine Italian cities, 1999–2000</td>
<td>239 term SGA, 343 pre-term</td>
<td>Questionnaire on individual water habits during pregnancy, water sampling for THMs at mothers’ homes (few days after delivery), two composite third trimester exposure variables created: one based on DBP levels and tap water consumption and the other based on DBP levels and inhalation exposure, high THM exposure categorized as a combination of THM concentration &gt;10μg l(^{-1}), being a tap water consumer, and high inhalation exposure</td>
<td>Education, smoking, sex of child, type of drinking water, tap water-based beverages intake</td>
<td>Overall exposure to high levels of DBPs in drinking water (THMs ≥30 μg l(^{-1}), chlorites ≥200 μg l(^{-1}) or chlorates ≥200 μg l(^{-1})) produced an OR of 1.38 (0.92–2.07) for risk of term SGA</td>
</tr>
<tr>
<td>Dodds et al. (2004)</td>
<td>Nova Scotia and Eastern Ontario, 112 stillbirth and 398 live birth controls</td>
<td>Various indices: 0, 1–49, 50–79 and &gt;80 μg l(^{-1}) for total THMs and chloroform and 0, 1–4, 5–9 and &gt;9 μg l(^{-1}) for BDCM quintiles for total exposure (ingestion/showering/bathing) for TTHM, chloroform and BDCM concentration and duration</td>
<td>Age, province, household income</td>
<td>Stillbirth TTHM &gt;80 versus 0 μg l(^{-1}): 2.2 (1.1–4.4) TTHM highest versus lowest quintile: 2.4 (1.2–4.6) drinking 5+ drinks per day and THM 50+ μg l(^{-1}) versus &lt;1 drink and THM = 0: 4.0 (1.4–11) chloroform and BDCM generally follow TTHM trend</td>
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<tr>
<td>Author (Year)</td>
<td>Study Details (Location, Time, Sample Size)</td>
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<td>Exposure Assessment</td>
<td>Other Risk Factors Included</td>
<td>Main Findings OR (95% CI)</td>
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<tr>
<td>Yang (2004)</td>
<td>Taiwan, sample population: 182,796</td>
<td>LBW,</td>
<td>15 non-chlorinating municipalities (NCM) and 128 chlorinating municipalities (CM)</td>
<td>Maternal age, marital status, education, gestational age, infant's sex, urbanization</td>
<td>Pre-term delivery: NCM versus CM: 1.37 (1.20–1.56)</td>
</tr>
<tr>
<td>Infante-Rivard (2004)</td>
<td>Montreal, Quebec, Canada, 493 cases and 472 control (10th percentile)</td>
<td>Regulatory data on THMs, &gt;90th percentile versus ≤90th percentile</td>
<td>Gestational age, sex, race, mother's weight gain, BMI, smoking, primiparity, pre-eclampsia</td>
<td>IUGR: no association with THMs only, but with CYP2E1∗5 (G1259C) 13.2 (1.19–146.7) in newborns</td>
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<tr>
<td>Wright et al. (2004)</td>
<td>MA, USA, sample population: 196,000 registry based</td>
<td>Birth weight, gestational age, SGA, pre-term delivery</td>
<td>TTHM, individual THMs, HAAs, MX, mutagenicity</td>
<td>SGA: &gt;74 versus ≤33 μg l⁻¹ TTHM 1.13 (1.07–1.20)</td>
<td>Similar results for birth weight and SGA</td>
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<th>main findings OR (95% CI)</th>
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<tbody>
<tr>
<td>King et al. (2005)</td>
<td>Nova Scotia and Eastern Ontario, Canada, 112 cases and 398 controls</td>
<td>stillbirth</td>
<td>HAAs</td>
<td>maternal age, province, income, occupation, smoking</td>
<td>stillbirth: no significant results after adjustments for THMs</td>
</tr>
<tr>
<td>Toledano et al. (2005)</td>
<td>three water regions in UK, 920571 stillbirth and birth and 969304 births</td>
<td>stillbirth, LBW, very LBW</td>
<td>THMs</td>
<td>maternal age, deprivation</td>
<td>stillbirth: ≥60 versus &lt;30 μg l⁻¹ TTHM 1.11 (1.00–1.23)</td>
</tr>
<tr>
<td>Porter et al. (2005)</td>
<td>four regions MD, USA, sample population: 15416 births</td>
<td>IUGR</td>
<td>THMs and HAAs</td>
<td>smoking, ethnicity, prenatal care, marital status, teen birth, birth at age &gt;35</td>
<td>no association</td>
</tr>
<tr>
<td>Lewis et al. (2006)</td>
<td>MA, USA, sample population: 36259 births</td>
<td>term LBW</td>
<td>(weekly) THMs</td>
<td>previous trimester exposure, gestational age, infant’s sex, marital status, prenatal care, maternal age, maternal race/ethnicity, education, parity, smoking, prenatal care payment, per capita income, previous pre-term/SGA, conception/birth season, maternal disease</td>
<td>term LBW second trimester: all: ≥ 70 versus &lt;40 μg l⁻¹ TTHM 1.50 (1.07–2.10) per 10 μg l⁻¹ TTHM 1.08 (1.00–1.20) non-Caucasians: ≥70 versus &lt;40 μg l⁻¹ TTHM 1.60 (1.03–2.47) per 10 μg l⁻¹ TTHM 1.10 (1.00–1.22)</td>
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<tr>
<td>author (year)</td>
<td>sample details</td>
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<td>main findings OR (95% CI)</td>
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<tr>
<td>Hinckley et al. (2005)</td>
<td>AZ, USA, sample population: 48,119 births</td>
<td>LBW, IUGR, pre-term delivery</td>
<td>THMs and HAAs</td>
<td>IUGR: parity, education, smoking, Kessner index term (T) LBW: maternal age, race, education, parity, smoking, Kessner index</td>
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<td></td>
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<td>TTHM 1.09 (1.00–1.18)</td>
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<td>TLBW: ≥5 versus &lt;4 μg l(^{-1})</td>
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<td>DBAA 1.49 (1.09–2.04)</td>
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<td>IUGR: ≥8 versus &lt;6 μg l(^{-1})</td>
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<td>DCAA 1.28 (1.08–1.51)</td>
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<td>≥6 versus &lt;4 μg l(^{-1})</td>
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<td>TCAA 1.19 (1.01–1.41); weeks 37–40</td>
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<td>IUGR: ≥8 versus &lt;6 μg l(^{-1})</td>
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<td>DCAA 1.27 (1.02–1.59); weeks 33–36</td>
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<td>TLBW: ≥5 versus &lt;4 μg l(^{-1})</td>
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<td>DBAA 1.49 (1.10–2.02)</td>
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<tr>
<td>Savitz et al. (2006)</td>
<td>three US locations, 2,409 pregnancies</td>
<td>spontaneous abortion</td>
<td>THMs, HAAs, TOX</td>
<td>no association</td>
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</tr>
<tr>
<td>Lewis et al. (2007)</td>
<td>MA, USA, sample population: 37,498 births</td>
<td>pre-term birth</td>
<td>THMs</td>
<td>previous trimester exposure, gestational age, infant’s sex, marital status, prenatal care, maternal age, maternal race/ethnicity, education, parity, smoking, prenatal care payment, per capita income, previous pre-term/SGA, conception/birth season, maternal disease</td>
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<td>no association (with some exception for some groups on government pay)</td>
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Table 2. (Continued.)

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<tr>
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<th>main findings OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al. (2007)</td>
<td>Taiwan, sample population: 90,848 births</td>
<td>LBW, IUGR, pre-term delivery</td>
<td>THMs</td>
<td>maternal age, maternal education, marital status, only first birth</td>
<td>no association</td>
</tr>
<tr>
<td>Nieuwenhuijsen et al. (2008)</td>
<td>England and Wales: sample population: 2,605,226</td>
<td>congenital anomalies: respiratory, cardiovascular, urinary and NTDs and cleft lip and palate</td>
<td>THMs</td>
<td>maternal age, deprivation, gender</td>
<td>isolated ventricular septal defects: ( \geq 60 ) versus (&lt; 30 \mu g^{-1}) TTHM 1.43 (1.00–2.04) ( &lt; 4 ) versus ( &lt; 2 \mu g^{-1}) bromoform 1.13 (0.99–1.29) and ( \geq 4 ) versus ( &lt; 2 \mu g^{-1}) bromoform 1.18 (1.00–1.39) ( \geq 4 ) versus ( &lt; 2 \mu g^{-1}) bromoform 1.38 (1.00–1.92)</td>
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Table 2. (Continued.)

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<tr>
<th>Study Details</th>
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<th>Exposure Assessment</th>
<th>Other Risk Factors Included</th>
<th>Main Findings OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chisholm et al. (2008)</td>
<td>Perth, Australia 20,870 livebirths</td>
<td>Congenital anomalies nervous system defects (BPA 74000–74299); cardiovascular defects (BPA 74500–74799); respiratory system defects (BPA 74800–74899); gastro-intestinal defects (BPA 74900–75199); uro-genital defects (BPA 75200–75399); musculo-skeletal defects (BPA 75400–75699); congenital anomalies of integument (BPA 75700–75799). (n = 1097)</td>
<td>Low (TTHM &lt;60 μg l&lt;sup&gt;-1&lt;/sup&gt;), medium (TTHM &gt;60 to &lt;130 μg l&lt;sup&gt;-1&lt;/sup&gt;) and high (TTHM ≥130 μg l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Maternal age, socio-economic status, high versus low exposure any birth defect (BD): 1.22 (1.01–1.48) and cardiovascular BD: 1.62 (1.04–2.51)</td>
</tr>
<tr>
<td>Luben et al. (2008)</td>
<td>AR, USA, 320 cases and 614 controls, and a subset of 40 cases and 243 controls</td>
<td>Hypospadias</td>
<td>THMs and HAAs, and personal characteristics in the subset</td>
<td>Maternal age, no. of cigarettes smoked per day during pregnancy and maternal education, no association except for in the subset analysis: TTHM ingestion &gt;0–32.5 versus 0 μg d&lt;sup&gt;-1&lt;/sup&gt;: 2.79 (1.01–7.72)</td>
</tr>
<tr>
<td>author (year)</td>
<td>study details (location, time, sample size)</td>
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<tr>
<td>Hwang et al. (2008)</td>
<td>Taiwan, sample population: 396,049 births</td>
<td>congenital anomalies, including (ICD9) anencephalus (740.0), hydrocephalus (741.0), ventricular septal defects (745.4), atrial septal defects (745.5), tetralogy of Fallot (745.2), cleft palate (749.0), cleft lip (749.1), renal agenesis and dysgenesis (753.0), obstructive urinary tract defects (753.2), hypospadias (752.61) and chromosome anomalies (758)</td>
<td>high (TTHMs $20+ \mu g l^{-1}$), medium (TTHMs $10–19 \mu g l^{-1}$), low exposure (TTHMs $5–9 \mu g l^{-1}$) and $0–4 \mu g l^{-1}$</td>
<td>maternal age, plurality, population density</td>
</tr>
<tr>
<td>Joyce et al. (2008)</td>
<td>Perth, Australia, sample population: 16,229 women</td>
<td>pre-labour rupture of membrane</td>
<td>THMs ($&lt;110$, $110–150$, $&gt;150 \mu g l^{-1}$)</td>
<td>maternal age, smoking, socioeconomic status</td>
</tr>
<tr>
<td>Hoffman et al. (2008a)</td>
<td>three US locations, 1958 births</td>
<td>SGA, term birth weight</td>
<td>THMs, HAAs, TOX and personal characteristics</td>
<td>maternal age, race/ethnicity, income, education, employment status, marital status, pre-pregnancy BMI, parity, caffeine intake</td>
</tr>
<tr>
<td>Hoffman et al. (2008b)</td>
<td>three US locations, 2039 births</td>
<td>duration of gestation</td>
<td>THMs, HAAs, TOX and personal characteristics</td>
<td>see Hoffman et al. (2008a)</td>
</tr>
</tbody>
</table>
(Dodds et al. 1999; Magnus et al. 1999; Källen & Robert 2000; Hwang et al. 2002; Shaw et al. 2003; Nieuwenhuijsen et al. 2008) (table 2). Klotz & Pyrch (1999) found a statistically significant association between total THM (TTHM) levels in the water and NTDs, but not with levels of haloacetonitriles and HAA. Also, the effects were most pronounced in offspring from women who did not take supplementary vitamins, but these findings were not confirmed by the Shaw et al. (2003) study. Cedergren et al. (2002), Hwang et al. (2002) and Chisholm et al. (2008) found significant associations between chlorinated water, levels of TTHM above 10 μg l⁻¹ and high levels (i.e. 130 μg l⁻¹ or more) of THMs and cardiovascular congenital anomalies, respectively, but other studies did not find such an association (Bove et al. 1995; Dodds et al. 1999; Magnus et al. 1999; Källen & Robert 2000; Dodds & King 2001; Shaw et al. 2003; Nieuwenhuijsen et al. 2008).

Few studies have been published on chlorinated water and respiratory congenital anomalies, but two studies found a significant positive association (Aschengrau et al. 1993; Hwang et al. 2002), whereas two did not (Chisholm et al. 2008; Nieuwenhuijsen et al. 2008).

Similarly, for urinary tract defects, three studies reported statistically significant positive associations (Aschengrau et al. 1993; Magnus et al. 1999; Hwang et al. 2002), while one did not (Nieuwenhuijsen et al. 2008) and another one showed almost statistically significant effects (Chisholm et al. 2008; odds ratio (OR) = 1.40, 95% confidence interval (CI): 0.98–1.99).

Studies on oral cleft or cleft palate, including a meta-analysis (Hwang et al. 2008), have found no positive relationship with DBP exposure, except for the study by Bove et al. (1995).

Evidence for risk of hypospadias is also inconclusive. There was no association with THM or HAA concentrations or proxies (Källen & Robert 2000; Luben et al. 2007; Hwang et al. 2008); however, estimates of actual THM ingestion were associated with increased risk of hypospadias (Luben et al. 2007).

In a meta-analysis, Hwang & Jaakkola (2003) reported evidence for an effect of exposure to chlorination by-products on the risk of neural tube and urinary system defects, but results for respiratory system, major cardiac and oral cleft defects were heterogeneous and inconclusive. The exposure index used was, however, fairly crude, without levels of DBPs being taken into account. Since the meta-analysis was published in 2003, the largest study published to date by Nieuwenhuijsen et al. (2008) was conducted, which was larger than all previous studies combined and which reported no association between THMs and cleft palate/lip, abdominal wall, major cardiac, neural tube, urinary and respiratory defects, except for a restricted set of anomalies with isolated defects, which appears to be due to a more reliable means of case identification. There were excess risks in the highest exposure categories of TTHMs (i.e. 60 μg l⁻¹ or more) for ventricular septal defects and the highest exposure category of bromoform (i.e. 4 μg l⁻¹ or more) and a subset of major cardiovascular defects and gastroschisis (Nieuwenhuijsen et al. 2008). In the meta-analysis by Hwang et al. (2008), the summary OR for ventricular septal defects (OR 1.59, 95% CI: 1.21, 2.07) for high versus low exposure to DBPs was statistically significant, but the exposure categories in the individual studies were inconsistent (different levels of THMs, or chlorination as a proxy), rendering the results difficult to interpret.
Only a few studies have assessed the relationship between DBPs and spontaneous abortion. The Californian study has attracted the most attention since its authors found a statistically significant association between TTHMs (i.e. 75 μg l\(^{-1}\) or more), especially for bromodichloromethane (BDCM) (i.e. 18 μg l\(^{-1}\) or more)—together with a high consumption of water (five glasses or more per day)—and spontaneous abortion (Waller et al. 1998). The ORs were even larger after re-analysis when restricting it to subjects for whom exposure had been characterized with greater confidence (Waller et al. 2001). However, in a study trying to replicate these results with substantially improved exposure assessments—including a study site with high-bromide water—Savitz et al. (2006) found no evidence for an association between a number of DBPs and spontaneous abortion, nor did they find any such association in an earlier study (Savitz et al. 1995).

A number of Canadian studies and one English study found statistically positive associations between DBPs and stillbirth (Dodds et al. 1999, 2004; King et al. 2000b; Toledano et al. 2005). However, the case–control study by Dodds et al. (2004) did not show a monotonic relationship between THM levels and stillbirth, and they did not find an association between HAAs and stillbirth (King et al. 2005).

Studies on pre-term delivery have generally shown no statistically significant associations with DBPs (Kramer et al. 1992; Bove et al. 1995; Savitz et al. 1995; Gallagher et al. 1998; Wright et al. 2003; Aggazzotti et al. 2004; Hinckley et al. 2005; Lewis et al. 2007; Yang et al. 2007), with the exception of the study by Yang et al. (2000a) and Yang (2004), who found a statistically significant increased risk. Wright et al. (2004) and Jaakkola et al. (2001) found a statistically significant decreased risk of pre-term delivery.

Study results on (term) low birth weight (LBW) have been mixed, with some studies reporting statistically significant associations (Bove et al. 1995; Gallagher et al. 1998; Källen & Robert 2000; Lewis et al. 2006) and others not (Kramer et al. 1992; Savitz et al. 1995; Kanitz et al. 1996; Dodds et al. 1999; Yang et al. 2000a, 2007; Jaakkola et al. 2001; Wright et al. 2003; Yang 2004; Toledano et al. 2005). Hinckley et al. (2005) found no association with THMs, but did for some specific HAAs. Studies on small for gestational age (SGA) and/or intrauterine growth retardation (IUGR) showed some more consistent results, and a good proportion of them have found statistically significant associations (Kramer et al. 1992; Bove et al. 1995; Wright et al. 2003, 2004), while some did not (Dodds et al. 1999; Porter et al. 2005; Yang et al. 2007; Hoffman et al. 2008b) (table 3). Aggazzotti et al. (2004) found some effects with by-products of chlorine dioxide. Wright et al. (2004) found statistically significant associations with THMs and a measure of mutagenicity, but not with HAAs or the chlorinated furanone 3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone (MX) (table 4). Infante-Rivard (2004) found that the association between THMs and IUGR was modified by a metabolic polymorphism, with newborns with the CYP2E1 (G1259C) variant at high risk.

Two small case–control studies have investigated the relationship between DBPs and semen quality (Fenster et al. 2003; Luben et al. 2007). Halogenated acetic acids have been found to cause testicular damage in rats through disruption of spermatogenesis and motility, with the brominated analogues being the strongest toxicants (Smith et al. 1989; Toth et al. 1992; Linder et al. 1994a,b,
Table 3. Association between SGA/IUGR and DBP exposure.

<table>
<thead>
<tr>
<th>study</th>
<th>exposure</th>
<th>risk estimate</th>
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<tr>
<td>Källen &amp; Robert (2000)</td>
<td>chlorinated (average TTHM = 9.4 μg l(^{-1})) versus non-chlorinated</td>
<td>1.07 (0.96–1.19)*</td>
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<tr>
<td>Jaakkola et al. (2001)</td>
<td>chlorinated (average TTHM = 9.4 μg l(^{-1})) versus non-chlorinated</td>
<td>1.00 (0.91–1.10)**</td>
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<tr>
<td>Kramer et al. (1992)</td>
<td>0 versus ≥10 μg l(^{-1}) CHCl(_3)</td>
<td>1.8 (1.1–2.9)*</td>
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<tr>
<td>Bove et al. (1995)</td>
<td>≤20 versus &gt;100 μg l(^{-1}) TTHM</td>
<td>1.5 (1.2–1.9)*</td>
</tr>
<tr>
<td>Dodds et al. (1999)</td>
<td>0–49 versus &gt;100 μg l(^{-1}) TTHM</td>
<td>1.08 (0.99–1.18)**</td>
</tr>
<tr>
<td>Wright et al. (2003)</td>
<td>0–60 versus &gt;80 μg l(^{-1}) TTHM</td>
<td>1.14 (1.02–1.26)</td>
</tr>
<tr>
<td>Wright et al. (2004)</td>
<td>&gt;74–163 versus 0–33 μg l(^{-1}) TTHM</td>
<td>1.13 (1.07–1.20)</td>
</tr>
<tr>
<td>Aggazzotti et al. (2004)</td>
<td>THMs ≥30 μg l(^{-1}), chlorite or chloride ≥200 μg l(^{-1})</td>
<td>1.38 (0.92–2.07)</td>
</tr>
<tr>
<td>Infante-Rivard (2004)</td>
<td>&gt;90th percentile versus ≤90th percentile: 29.4 μg l(^{-1}) TTHM</td>
<td>0.97 (0.57–1.62)</td>
</tr>
<tr>
<td>Porter et al. (2005)</td>
<td>highest versus lowest quintile</td>
<td>1.17 (0.96–1.42)</td>
</tr>
<tr>
<td>Hinckley et al. (2005)</td>
<td>≥53 versus &lt;40 μg l(^{-1}) TTHM</td>
<td>1.09 (1.00–1.18)</td>
</tr>
<tr>
<td>Yang et al. (2007)</td>
<td>THM4 &gt;13.1 versus ≤4.9 μg l(^{-1})</td>
<td>0.96 (0.91–1.02)</td>
</tr>
<tr>
<td>Hoffman et al. (2008)</td>
<td>74.9–108.8 versus 2.2–4.6</td>
<td>1.3 (0.7–2.3)</td>
</tr>
</tbody>
</table>

*≤5th percentile; **≤10th percentile; ***≤2 s.d.

Table 4. Association between SGA/IUGR and various DBPs (μg l\(^{-1}\)) (except for MX, which has ng l\(^{-1}\) units) and mutagenicity (rev l\(^{-1}\)) (Wright et al. 2004).

<table>
<thead>
<tr>
<th>exposure</th>
<th>level</th>
<th>risk estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTHM</td>
<td>&gt;74–163 versus 0–33</td>
<td>1.13 (1.07–1.20)</td>
</tr>
<tr>
<td>CHCl(_3)</td>
<td>&gt;63–135 versus 0–26</td>
<td>1.11 (1.04–1.17)</td>
</tr>
<tr>
<td>BDCM</td>
<td>&gt;13–46 versus 0–5</td>
<td>1.15 (1.08–1.22)</td>
</tr>
<tr>
<td>total HAAs</td>
<td>&gt;49–58 versus 4–30</td>
<td>0.97 (0.77–1.23)</td>
</tr>
<tr>
<td>TCAA</td>
<td>&gt;27–37 versus 0–18</td>
<td>0.95 (0.76–1.19)</td>
</tr>
<tr>
<td>DCAA</td>
<td>&gt;22–24 versus 2–15</td>
<td>0.90 (0.75–1.09)</td>
</tr>
<tr>
<td>MX</td>
<td>&gt;46–80 versus 4–20</td>
<td>1.14 (0.95–1.37)</td>
</tr>
<tr>
<td>mutagenicity</td>
<td>&gt;2250 versus &lt;1250</td>
<td>1.25 (1.04–1.51)</td>
</tr>
</tbody>
</table>

The results of the two epidemiology studies were inconclusive, with inconsistent evidence across various measures of semen quality and DBP exposure. Fenster et al. (2003) found that TTHM levels were not associated with decrements in semen quality. Per cent normal morphology decreased and per cent head defects increased at higher levels of a THM ingestion metric compared with the lowest level, although there were no monotonic dose–responses, and at this level, they observed a small decrease in per cent morphologically normal sperm. BDCM exposure was inversely related to linearity (a motility parameter). Luben et al. (2007) studied the relation between exposure to classes of DBPs and sperm.
concentration and morphology, as well as DNA integrity and chromatin maturity, but found no association—or consistent pattern—of increased abnormal semen quality with elevated exposure to THMs or HAAs.

MacLehose et al. (2008) investigated time to pregnancy in relation to DBP exposure, but found little evidence for a relationship. Joyce et al. (2008) investigated the effect of DBPs on pre-laboural rupture of membranes but found no relationship.

Very few studies have examined the gene–environment interaction and/or the presence of susceptible groups. Infante-Rivard (2004) found that newborns with a high-metabolism CYP2E1 gene variant who experienced pregnancy average exposures of more than 29.4 μg l\(^{-1}\) for TTHMs were at much higher risk (OR = 13.2, 95% CI: 1.19–146.7) of IUGR compared with those without this CYP2E1 variant, but found no indication that MTHFR C677T modified the effect of exposure to chloroform and risk of foetal growth in humans. A study investigating NTDs, isolated cleft lip palate with or without cleft palate, also found no evidence that MTHFR C677T modified the effect of TTHM exposure (Shaw et al. 2003). Lewis et al. (2006) reported an increased risk of term LBW in non-Caucasians associated with second trimester exposure to THMs greater than the increased risk found for Caucasians and non-Caucasians combined.

(c) Limitations

The major limiting factor in these studies has often been crude exposure assessment, with the exception of some of the more recent studies. The use of ecological water supply zone estimates as an exposure index may result in exposure misclassification (Whitaker et al. 2003), which likely biases the measures of effect towards the null. Furthermore, while ingestion has generally been the primary exposure route of interest, uptake through showering, bathing and swimming could be considerable, specifically for THMs owing to their volatility and dermal adsorption, but these routes have only been considered in a few studies (e.g. Savitz et al. 2006; Luben et al. 2007, 2008; Villanueva et al. 2007; Hoffman et al. 2008a,b; MacLehose et al. 2008). Combining information on individual water use with water supply zone estimates would provide more detailed exposure assessment, but the individual information should be evaluated for measurement error because within-subject variability in questionnaire data may be substantial (Forssén et al. in press) 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 may have moved her residence during her pregnancy. Exposure assessment based on maternal residence at birth may, therefore, result in exposure misclassification.

In addition, studies from countries including Scandinavia (Magnus et al. 1999; Källen & Robert 2000; Jaakkola et al. 2001; Cedergren et al. 2002; Hwang et al. 2002) and Taiwan (Yang et al. 2000a,b; 2007; Yang 2004; Hwang et al. 2008) have generally shown low levels of DBPs with a small range, making the assessment of risks more difficult owing to both a higher probability of exposure misclassification and a smaller difference in exposure between dose groups. Furthermore, Cedergren et al. (2002) (in Sweden) and Chisholm et al. (2008) (in Australia) found significant associations between levels of TTHM above 10 μg l\(^{-1}\)
and high levels greater than or equal to 130 μg l\(^{-1}\) of THMs and cardiovascular congenital anomalies, respectively, but the low exposure group in the latter study (i.e. less than 60 μg l\(^{-1}\)) represented higher levels of THM exposure than that of the cases in the former study (i.e. more than 10 μg l\(^{-1}\)), making the comparison more difficult. Moreover, other studies did not find such an association, such as in Magnus et al. (1999) (in Norway), in which the average level of TTHMs for chlorinated water was 9.4 μg l\(^{-1}\). In the latter study, exposure assessment was based on whether the mothers received unchlorinated or chlorinated water, albeit with relatively low levels of THMs (on average) in the latter group. Thus, neither group had high exposure to THMs (on average). Where seasonal variability in DBPs has not been taken into account, further errors in the exposure assessment are likely.

Particularly for reproductive epidemiological studies, the sample sizes have often been insufficient to produce robust results, especially for congenital anomalies and, to a lesser extent, for stillbirth, semen quality and other outcomes, but there are exceptions. For example, studies on SGA/IUGR by Dodds et al. (1999), Wright et al. (2003, 2004) and Hinckley et al. (2005), on congenital anomalies by Hwang et al. (2002, 2008) and Nieuwenhuijsen et al. (2008) and on stillbirth by Toledano et al. (2005) provide sufficiently large numbers of cases to create various exposure categories with more robust risk estimates, which could improve the overall assessment of risk.

The retrospective and registry-based nature of many of the reproductive studies has meant that information on potential confounders, and other risk factors for birth outcomes, such as maternal smoking and alcohol consumption, have often been lacking.

On the whole, epidemiological studies have used TTHMs as a proxy for total DBP load, but TTHMs are not necessarily a good proxy measure. Some studies have examined individual (brominated) THM species (e.g. Waller et al. 1998; King et al. 2000b; Dodds & King 2001; Shaw et al. 2003; Dodds et al. 2004; Wright et al. 2004; Nieuwenhuijsen et al. 2008). In addition, some studies have investigated other DBPs such as HAAs and/or MX (e.g. Klotz & Pyrch 1999; Wright et al. 2004; Hinckley et al. 2005; Porter et al. 2005; Savitz et al. 2006; Luben et al. 2007, 2008; Hoffman et al. 2008; MacLehose et al. 2008) and/or total organic halides (TOX) (e.g. Savitz et al. 2006; Hoffman et al. 2008). The metabolism of different DBP species varies (IPCS 2000), the toxicity of different DBP classes varies, specific DBPs in a particular class have substantially different toxicities (e.g. Hunter et al. 2006) and the relationship of THMs to that of other DBPs (e.g. HAAs and TOX) varies, so it is insufficient to use TTHMs as a proxy for DBPs as a whole. Investigation of the relation between non-THM by-products and reproductive outcomes is required in order to help elucidate the specific DBPs driving the associations observed. A detailed assessment of the DBP mixture (including speciation within different DBP classes) is necessary to explain any observed epidemiological results. Wright et al. (2004) is a good example of a study in which different DBP classes were examined, as well as specific DBPs within these classes (table 4).

Furthermore, outcomes such as spontaneous abortion, foetal growth restriction or congenital anomalies have not been defined well and/or are difficult to study. Previous epidemiological studies have used a variety of outcomes as proxies for foetal growth restriction: terms LBW, IUGR and SGA. There are
some limitations to these measures. LBW is rather crudely defined—the fixed criterion of birth weight below 2500g takes no account of population-specific birth weight distributions (Wilcox 2001). Somewhat confusingly, the terms IUGR and SGA have been used interchangeably in the literature, and criteria for IUGR/SGA diagnosis have varied, some studies using the 5th and some the 10th percentile of gestational specific weight according to a standard population growth chart as a cut-off point. These measures fail to distinguish between those babies who are constitutionally small and those who are pathologically small (i.e. growth restricted). Some small but normally grown babies will fall below the cut-off point, and some growth-restricted babies will reach a weight above the cut-off point. Therefore, a proportion of infants are misclassified, and in epidemiological studies, this may bias any association towards the null. There is evidence to show that the use of customized foetal growth charts, which take into account factors such as maternal height and ethnicity, significantly reduce the proportion of false-positive and false-negative diagnoses of foetal growth restriction, compared with using standard population growth charts (Gelbaya & Nardo 2005; Gardosi 2006), but these are poorly developed at present.

Congenital anomalies have often been analysed either as one group or in main categories, e.g. neural tube, major heart and abdominal defects, owing to the small number of cases in each study. These anomalies, however, are generally heterogeneous with respect to both phenotype and presumed aetiology. Nieuwenhuijzen et al. (2008) showed that focusing on isolated subcategories may result in different findings. Furthermore, in some countries, registration of congenital anomalies may occur up to 1 year after the birth (e.g. in Taiwan), which will improve the completeness of the registry by including cases, such as hypospadias, that are more difficult to identify at birth.

Investigation of gene–environment interaction and/or the effects on susceptible groups has been limited (e.g. Shaw et al. 2003; Infante-Rivard 2004). Preliminary studies suggest that certain groups may be more susceptible to the influence of DBPs (Lewis et al. 2006), and thus these effects may be masked in studies that only look at the population in general.

3. Mechanisms

The mechanisms through which DBPs may cause adverse health effects, including cancer and adverse reproductive effects, are not well investigated. Several mechanisms have been suggested that involve genotoxicity, oxidative stress, disruption of folate metabolism, disruption of the synthesis and/or secretion of placental syncytiotrophoblast-derived chorionic gonadotropin and lowering of testosterone levels.

(a) Genotoxicity/mutagenicity

Richardson et al. (2007) reviewed 30 years of research on the occurrence, genotoxicity and carcinogenicity of 85 DBPs. Of these, 11, including THMs, are currently regulated by the United States Environmental Protection Agency and 74 are considered emerging DBPs owing to their moderate occurrence levels and/or toxicological properties. Sixty-eight of the 85 DBPs reviewed were
considered genotoxic, including the regulated brominated THMs, where the THMs are generally at higher levels in drinking water. In general, the brominated DBPs are more genotoxic and carcinogenic than chlorinated compounds, and iodinated DBPs are the most genotoxic (Plewa et al. 2008). Moreover, certain nitrogenous DBPs were found to be more genotoxic than the regulated carbonaceous DBPs (i.e. THMs and HAAs) (Plewa et al. 2008). Recently, Ross & Pegram (2004) reported GSTT1-1-dependent covalent binding of brominated THMs to DNA and formation of deoxyguanosine adducts \textit{in vitro}. Because there is structural similarity among the brominated THMs and evidence for common pathways of bioactivation (DeMarini et al. 1997; Pegram et al. 1997), the findings of Ross & Pegram (2004) support the idea that glutathione (GSH) conjugation of tribromomethane may lead to the formation of DNA-reactive metabolites in the liver, and perhaps even more likely in the colons, of rodents and humans.

\textbf{(b) Oxidative stress}

There is evidence that maternal oxidative stress during pregnancy may play an important role in adverse foetal development (Scholl & Stein 2001; Meek et al. 2002; Myatt & Cui 2004; Kim et al. 2005; Min et al. 2006). For example, increased concentrations of oxidative stress biomarkers (8-OH-dG and MDA) observed in the urine of pregnant women have been associated with decreased birth weight (Scholl & Stein 2001; Kim et al. 2005). In late gestation, an increase in oxidative stress is observed in pregnancies complicated by IUGR, pre-eclampsia and diabetes, and this is associated with increased trophoblast apoptosis and alterations to placental vascular reactivity (Myatt & Cui 2004). There is also evidence to suggest that exposure to DBPs can cause oxidative stress in human cells. An \textit{in vitro} study on human hepatoma (HepG2) cells reported that increasing chloroform dose resulted in decreasing GSH, which induces oxidative stress (Beddowes et al. 2003). Another \textit{in vitro} study on human HepG2 cells found that when exposed to chlorinated drinking water, MDA increased and GSH decreased in a dose-dependent manner, indicating oxidative stress (Yuan et al. 2005).

Cytochrome P-450E1 (CYP2E1) is the primary enzyme involved in the metabolism of low doses of chloroform (Meek et al. 2002), and Tomasi et al. (1985) showed that chloroform metabolism generates free radicals. Chloroform is oxidatively metabolized and decomposed to electrophilic phosgene, which is highly reactive and will bond to cell components including proteins, phospholipid polar heads and reduced GSH (Gemma et al. 2003).

Other compounds such as trichloroethanol, trichloroacetic acid and dichloroacetic acid have all been shown to induce lipid peroxidation, a biomarker of oxidative stress, presumably via a free radical mechanism (Larson & Bull 1992; Ni et al. 1996).

Polymorphisms in pro-inflammatory cytokines (i.e. tumour necrosis factor (TNF)) have been associated with pre-term births (Crider et al. 2005; Engel et al. 2005a), whereas polymorphisms in anti-inflammatory cytokines (i.e. interleukin (IL)-4) have been associated with SGA outcomes (Engel et al. 2005b). Animal studies have shown that both pro-inflammatory (TNF, IL-6 and IL-8) and anti-inflammatory (IL-10 and transforming growth factor) cytokines have been
affected by exposure to carbon tetrachloride, a haloalkane similar to chloroform, and to phosgene, a metabolite of chloroform (Sciuto et al. 2003; Weber et al. 2003).

The observed associations between various adverse birth outcomes and markers of oxidative stress, and the associations between exposure to DBPs and their metabolites and markers of oxidative stress, suggest the possibility that DBPs may act on foetal growth via the oxidative stress mechanism.

(c) Folate metabolism

One suggested mechanism by which DBPs could cause cancer and adverse birth outcomes is the interference of folate metabolism by DBPs. Folate and folic acid are the forms of the B vitamin that are involved in the synthesis, repair and functioning of DNA and required for the production and maintenance of cells (Kamen 1997). Folate plays an important role for cells that are undergoing rapid turnover such as tissues in the colon and the developing foetus. Folate is involved in the synthesis of methionine, an essential amino acid. Low levels of folate have been associated with several forms of cancer and congenital anomalies such as NTDs. Furthermore, defects in the methionine–homocysteine metabolic pathway, which can be the result of low folate levels and result in elevated homocysteine levels, may be a contributing factor for abruptio placentae (Ray & Laskin 1999). Both chloroform and TCAA have been found to inhibit the vitamin B12-dependent methionine biosynthesis pathway. Inhibition of this pathway can lead to vitamin B12 deficiency and consequently folate deficiency. Alston (1991) found that chloroform inhibited methionine biosynthesis in cell culture. Dow & Green (2000) showed that trichloroacetic acid interacts with vitamin B12, probably by a free radical mechanism, inhibiting both the methylmalonyl CoA and methionine salvage pathways in rats. As a result of the latter, a secondary folate deficiency develops owing to the ‘methyl folate trap’, leading to a major impairment in formate metabolism. Geter et al. (2005) showed that rats exposed to bromoform and fed a no-folate diet had significant increases in aberrant crypt foci (putative precursor lesions in the development of colon cancer) when compared with rats exposed to bromoform and fed a normal diet.

(d) Chorionic gonadotropin disruption

Chen et al. (2003) showed that the THM BDCM reduced the secretion of immunoreactive and bioactive chorionic gonadotropin in primary cultures of human trophoblasts and thus appears to target human placental trophoblasts. Trophoblasts are the sole source of chorionic gonadotropin during normal human pregnancy; a decrease in the amount of this bioactive hormone could have adverse effects on pregnancy outcome, including those leading to growth retardation. Chen et al. (2004) reported that BDCM directly inhibits the morphological differentiation of mononucleated placental cytotrophoblast cells to multi-nucleated syncytiotrophoblast-like colonies in vitro. Syncytiotrophoblast formation was inhibited in a dose-dependent manner and was accompanied by no loss of cell viability.

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Testosterone

Potter et al. (1996) showed that all of the THMs reduced serum testosterone in rats treated with 1.5 mmol kg\(^{-1}\) by oral gavage for 7 days. The finding that male F-344 rats treated with THMs had decreased circulating concentrations of testosterone also raises the question as to whether THMs may produce androgenic deficiency in male rats.

The various mechanisms described earlier begin to provide plausible biological pathways through which DBPs may cause adverse health effects, including cancer and adverse reproductive effects. However, they are clearly still in their infancy and further research is required to provide more definitive evidence for causal biological mechanisms.

4. Conclusion

There appears to be good epidemiological evidence for an association between chlorination by-products, as measured by THMs, in drinking water and bladder cancer, but the evidence for other cancers including colorectal cancer appears to be inconclusive and inconsistent. There appears to be some evidence for a relationship between chlorination by-products and SGA and IUGR and, to a lesser extent, pre-term delivery, but evidence for other outcomes such as LBW, stillbirth, congenital anomalies and semen quality appears to be inconclusive and inconsistent. Major limitations in exposure assessment, small sample sizes and potential biases may account for the inconclusive and inconsistent results in epidemiological studies. Moreover, most studies have focused on TTHMs as the exposure metric, whereas some emerging DBPs appear to be more toxic than the THMs. The mechanisms through which DBPs may cause adverse health effects, including cancer and adverse reproductive effects, have not been well investigated to date.

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