

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

BY MARK J. NIEUWENHUISEN^{1,2,3,4,*}, JAMES GRELLIER⁴,
RACHEL SMITH⁴, NINA ISZATT⁴, JAMES BENNETT⁴, NICKY BEST⁴
AND MIREILLE TOLEDANO⁴

¹Centre for Research in Environmental Epidemiology (CREAL), Parc de Recerca Biomèdica de Barcelona—PRBB (Office 183.05), C. Doctor Aiguader, 88, 08003 Barcelona, Spain

²Municipal Institute of Medical Research (IMIM-Hospital del Mar), Barcelona, Spain

³CIBER Epidemiología y Salud Pública (CIBERESP), Spain

⁴Imperial College London, UK

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

*Author for correspondence (mnieuwenhuijsen@creal.cat).

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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.

total THM exposure level (mg) ^a	OR (95% CI)	
	male	female
0–15	1.00	1.00
>15–50	1.22 (1.01–1.48)	0.92 (0.65–1.32)
>50–400	1.28 (1.08–1.51)	0.94 (0.70–1.27)
>400–1000	1.31 (1.09–1.58)	1.02 (0.74–1.41)
>1000	1.50 (1.22–1.85)	0.92 (0.65–1.30)

^aTHM 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.

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Aschengrau <i>et al.</i> (1989)	MA, USA, sample population: 1677	286 spontaneous abortion	surface versus ground water; chlorinated versus chloraminated water	smoking habits, contraceptive use, medical and obstetrical history, metals	surface versus ground water: 2.2 (1.3–3.6)
Kramer <i>et al.</i> (1992)	IA, USA, 151 towns with a single water source, 1989–1990, sample population: 4028	688 (total): 159 LBW, 342 pre-term delivery, 187 IUGR/SGA	based on maternal residential address and one municipal water survey to estimate individual THM levels (two or three exposure categories)	maternal age, parity, marital status, education, smoking, prenatal care	no versus medium ($1\text{--}9 \mu\text{g l}^{-1}$) versus high ($\geq 10 \mu\text{g l}^{-1}$); 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)
Aschengrau <i>et al.</i> (1993)	MA, USA, two hospitals, 1977–1980, sample population: 2348	1171 (total): 1039 major congenital anomalies, urinary tract defects, respiratory tract defects: 77 stillbirths: 55 neonatal deaths	based on maternal residential address to ascertain type of water supply, chlorination versus chloramination, and ground/mixed water versus surface water.	maternal age, pregnancy history, alcohol, ethnicity, hospital payment, other water contaminants	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)

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Bove <i>et al.</i> (1995)	NJ, USA, 75 towns with a public water supply, 1985–1988, sample population: 81 602	29 268 (total); <i>live</i> <i>births</i> : 1853 LBW, 905 very LBW, 4082 SGA, 7167 pre-term, 594 foetal deaths; <i>all births: defects</i> : 669 total, 118 central nervous system defects, 83 oral cleft, 56 NTD, 108 major cardiac	based on maternal residential address and municipal water surveys to estimate monthly TTHM levels (five or six exposure categories)	maternal age, ethnicity, infant's sex, primipara, prenatal care, education, previous stillbirth or miscarriage, other contaminants	TTHM levels >100 versus $\leq 20 \mu\text{g l}^{-1}$ LBW: 1.4 (50% CI 1.2–1.7) IUGR/SGA: 1.5 (90% CI 1.2–1.9) TTHM levels >80 versus $\leq 20 \mu\text{g l}^{-1}$ 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 >100 versus $\leq 20 \mu\text{g l}^{-1}$: oral cleft defects: 3.2 (90% CI 1.2–7.3)
Savitz <i>et al.</i> (1995)	NC, USA, six hospitals, 1988–1991, sample population: 1003	548 (total): 126 spontaneous abortion, 244 pre-term, 178 LBW	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 \times amount, and (v) TTHM dose (level \times amount)	maternal age, ethnicity, hospital, education, marital status, poverty level, smoking, alcohol consumption, employment, nausea	40.8–59.9 versus 81.1–168.8 $\mu\text{g l}^{-1}$ TTHM: spontaneous abortion: 1.2 (0.6–2.4) 40.8–63.3 versus 82.8–168.8 $\mu\text{g l}^{-1}$ TTHM: LBW: 1.3 (0.8–2.1) per 50 $\mu\text{g l}^{-1}$ TTHM increment change: spontaneous abortion: 1.7 (1.1–2.7)

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Kanitz <i>et al.</i> (1996)	Liguria, Italy, two hospitals, 1988–1989, sample population: 676	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).	maternal age, education, smoking, alcohol, infant's sex	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)
Waller <i>et al.</i> (1998)	CA, USA, three regions of surface, ground and mixed drinking water, 1989–1991, sample population: 5144 pregnancies	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)	maternal age, gestational age, smoking, history of pregnancy loss, race, employment	high TTHM dose (\geq 5 glasses $d^{-1} + \geq 75 \mu g l^{-1}$) versus low dose (<5 glasses $d^{-1} + < 75 \mu g l^{-1}$): spontaneous abortion: 1.8 (1.1–3.0) high BDCM dose (\geq 5 glasses $d^{-1} + \geq 18 \mu g l^{-1}$) versus low dose (<5 glasses $d^{-1} + < 18 \mu g l^{-1}$): spontaneous abortion: 3.0 (1.4–6.6).

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Waller <i>et al.</i> (2001)	see Waller <i>et al.</i> (1998)	see Waller <i>et al.</i> (1998)	see Waller <i>et al.</i> (1998)	see Waller <i>et al.</i> (1998)	re-analysis Waller <i>et al.</i> (1998) utility wide subset sample highest AOR (adjusted OR) high TTHM dose (≥ 5 glasses d $^{-1}$ + $\geq 75 \mu\text{g l}^{-1}$) versus low dose (≥ 5 glasses d $^{-1}$ + $< 75 \mu\text{g l}^{-1}$): spontaneous abortion: 5.1 (1.8–14.7) little relationship with showering
Gallagher <i>et al.</i> (1998)	CO, USA, 28 census blocks in two water districts, 1990–1993, sample population: 1244 live births	72 LBW, 29 term-LBW, 68 pre-term delivery	based on maternal residential address and municipal water surveys. Estimate of household TTHM level during last trimester based on hydraulic modelling (four exposure categories)	maternal age, smoking, marital status, parity, education, employment, prenatal care	high TTHM level ($\geq 61 \mu\text{g l}^{-1}$) versus lowest ($\leq 20 \mu\text{g l}^{-1}$): LBW: 2.1 (1.0–4.8) term LBW: 5.9 (2.0–17.0)
Dodds <i>et al.</i> (1999)	Nova Scotia, Canada, 1988–1995, sample population: 49 842 births	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	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)	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	0–49 versus > 100 $\mu\text{g l}^{-1}$ 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)

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
King <i>et al.</i> (2000b)	Nova Scotia, Canada, 1988–1995, sample population: 49 756	214 stillbirths (72 asphyxia-related stillbirths)	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)	maternal age, maternal smoking	0.49 versus >100 µg l ⁻¹ chloroform stillbirth: 1.56 (1.04–2.34) 3.15 (1.64–6.03) < 5 versus >20 µg l ⁻¹ BDCM stillbirth: 1.98 (1.23–3.49) asphyxia-related stillbirth: 1.75 (0.72–4.22)
Dodds & King (2001)	Nova Scotia, Canada, 1988–1995, Sample population: 49 842 births	77 NTDs, 430 Cardiovascular anomalies, 82 cleft defects, 96 chromosomal abnormalities	see King <i>et al.</i> (2000b)	NTDs, cardiovascular anomalies and chromosomal abnormalities: maternal age, income cleft defects: maternal age onset of prenatal care	BDCM ≥20 versus <5 µg l ⁻¹ NTDs: 2.5 (1.2–5.1)
Klotz & Pyrch (1999)	NJ, USA, 1993–1994, sample population: all births, of which 112 cases and 248 controls selected	112 NTDs	based on residential address and public water facility TTHM data, and tap water sampling for TTHMs, haloacetonitriles and HAs (three to five exposure categories)	TTHMs public monitoring data, known residence and isolated cases < 5 versus 40+ µg l ⁻¹ NTDs: 2.1 (1.1–4.0)	(Continued.)

Table 2. (Continued.)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Magnus <i>et al.</i> (1999)	Norway, sample population: 141 077	2608 all birth defects, 62 NTDs, 250 major cardiac defects, 91 respiratory defects, 122 urinary defects, 143 oral cleft	chlorination yes versus no, colour high versus low (in chlorinated water average TTHMs = 9.4 µg l ⁻¹ and average HAAAs = 14.6 µg l ⁻¹)	maternal age, parity, geographical placement, population density, industry profile	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)
Jaakkola <i>et al.</i> (2001)	Norway, sample population: 137 145	6249 LBW, SGA, 7886 pre-term delivery	see Magnus <i>et al.</i> (1999)	see Magnus <i>et al.</i> (1999)	no chlorination low colour versus chlorination high colour pre-term delivery: 0.91 (0.84–0.99)
Källen & Robert (2000)	Sweden (1985–1994), sample population: no chlorination: 74 324 singletons; Na-hypochlorite: 24 731 singletons; chlorine dioxide: 15 429 singletons	multiple births, gestational duration, birth weight, intruterine 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	no versus sodium hypochlorite (no versus chlorine dioxide)	year of birth, maternal age, parity, maternal education, maternal smoking congenital anomalies and childhood cancer: maternal age, year of birth	no versus sodium hypochlorite LBW: 1.15 (1.05–1.26) <32 weeks' gestation: 1.22 (1.00–1.48) <37 weeks' gestation: 1.09 (1.01–1.17) <43 cm length: 1.97 (1.30–2.97) <47 cm length: 1.25 (1.10–1.43) BMI > 16 kg m ⁻² : 1.27 (1.19–1.37) <31 cm head circumference: 1.46 (1.07–1.98) spine anomalies: 3.2 (1.0–10.0)

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Yang <i>et al.</i> (2000a)	Taiwan, sample population: 18 025 first parity births: chlorinated: 10 007 non- chlorinated: 8018	LBW pre-term delivery (<37 weeks)	chlorinated (>95% population served chlorinated water) versus non-chlorinated (<5% population served chlorinated water)	maternal age, marital status, maternal education, infant's sex	chlorinated versus non-chlorinated pre-term delivery: 1.34 (1.15–1.56)
Yang <i>et al.</i> (2000b)	Taiwan, sample population: chlorinated: 24 882 non- chlorinated: 20 460	sex ratio	see Yang <i>et al.</i> (2000a)	chlorinated versus non-chlorinated sex ratio: no significant difference	
Cedergren <i>et al.</i> (2002)	Sweden, sample population: 58 669	cardiac defects	>10 versus $\leq 10 \mu\text{g l}^{-1}$ TTHM in surface water hypochlorite and chlorine dioxide versus hypochlorite in surface water ground water versus surface water	maternal age, parity, smoking, education	>10 versus $\leq 10 \mu\text{g l}^{-1}$ 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)

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Hwang <i>et al.</i> (2002)	Norway, sample population: 285 631	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	chlorination (yes/no) and level of water colour (mg Pt l^{-1} : <10, 10–19.9, ≥20)	maternal age, parity, socioeconomic status: centrality and population density	chlorination (yes) and level of water colour: <10 versus ≥20 mg Pt l ⁻¹ all birth defect: 1.18 (1.02–1.36) ventricular septal defect: 1.81 (1.05–3.09)
Nieuwenhuijsen <i>et al.</i> (2002)	England, sample population: 11 462		amount of swimming (h)	maternal age, maternal education, smoking, alcohol use, drugs use, gestational age, ethnicity, infant's sex	0–60 versus >80 µg l ⁻¹ TTHM birth weight: -32 g (-47 to -18)
Wright <i>et al.</i> (2003)	MA, USA, sample population: 56 513	birth weight, LBW, SGA, gestational age, pre-term delivery	0–60, >60–80, ≥80 µg l ⁻¹ TTHM or per 20 µg l ⁻¹ TTHM increase	gestational age, infant's sex, maternal age, maternal education, maternal race, smoking, prenatal care, parity, median household income, previous infant ≥4000g, previous pre-term delivery, maternal medical history, SGA; did not include infant sex and gestational age	SGA: 1.14 (1.02–1.26) gestational age (weeks): 0.08 (0.01–0.14) per 20 µg l ⁻¹ TTHM increase birth weight: -2.8 g (-5.5 to -0.2) gestational age: 0.02 (0.01–0.03)

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Shaw <i>et al.</i> (2003)	CA, USA, study 1: 538 NTD cases and 539 controls study 2: NTDs 207 NTD cases, conotruncal heart defect cases and 409 orofacial cleft cases and 481 controls	study 1: NTDs (anencephaly and spina bifida) study 2: NTDs (anencephaly and spina bifida), conotruncal heart defects, orofacial clefts cases; ≥ 50 versus $<50 \mu\text{g l}^{-1}$ and <5 glasses; ≥ 50 versus $<50 \mu\text{g l}^{-1}$ and >5 glasses	study 1 and 2: continuous TTHMs and categorical: 0, 1–24, 25–49, 50–74 and $\geq 75 \mu\text{g l}^{-1}$ TTHMs also study 1: ≥ 50 versus $<50 \mu\text{g l}^{-1}$ and <5 glasses; ≥ 50 versus $<50 \mu\text{g l}^{-1}$ and >5 glasses	ethnicity, education, body mass index, use of vitamins, methylenetetra- hydrofolate reductase (MTHFR) genotype	study 1: NTDs NTD risk inversely related to TTHM exposure but only occasionally significant for one category

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Aggazzotti <i>et al.</i> (2004)	Italy, case-control study in nine Italian cities, 1999–2000	239 term SGA, 343 pre-term	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 $>10\mu\text{g l}^{-1}$, being a tap water consumer, and high inhalation exposure	Education, smoking, sex of child, type of drinking water, tap water-based beverages intake	overall exposure to high levels of DBPs in drinking water (THMs $\geq 30\mu\text{g l}^{-1}$, chlorites $\geq 200\mu\text{g l}^{-1}$ or chlorates $\geq 200\mu\text{g l}^{-1}$) produced an OR of 1.38 (0.92–2.07) for risk of term SGA
Dodds <i>et al.</i> (2004)	Nova Scotia and Eastern Ontario, 112 stillbirth and 398 live birth controls	stillbirth	various indices: 0, 1–49, 50–79 and $>80\mu\text{g l}^{-1}$ for total THMs and chloroform and 0, 1–4, 5–9 and $>9\mu\text{g l}^{-1}$ for BDCM quintiles for total exposure (ingestion/showering/ bathing) for TTHM, chloroform and BDCM concentration and duration	age, province, household income	stillbirth TTHM >80 versus $0\mu\text{g l}^{-1}$: 2.2 (1.1–4.4) TTHM highest versus lowest quintile: 2.4 (1.2–4.6) drinking 5+ drinks per day and TTHM $50 + \mu\text{g l}^{-1}$ versus <1 drink and THM = 0 : 4.0 (1.4–11) chloroform and BDCM generally follow TTHM trend

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Yang (2004)	Taiwan, sample population: 182 796	LBW, pre-term delivery	15 non-chlorinating municipalities (NCM) and 128 chlorinating municipalities (CM)	maternal age, marital status, education, gestational age, infant's sex, urbanization	pre-term delivery NCM versus CM: 1.37 (1.20–1.56)
Infante-Rivard (2004)	Montreal, Quebec, Canada, 493 cases and 472 control	intruterine growth restriction (10th percentile)	regulatory data on THMs, >90th percentile versus ≤90th percentile	gestational age, sex, race, mother's weight gain, BMI, smoking, primiparity, pre-eclampsia, previous IUGR	IUGR: no association with THMs only, but with CYP2E1*5 (G1259C) 13.2 (1.19–146.7) in newborns
Wright <i>et al.</i> (2004)	MA, USA, sample population: 196 000 registry based	birth weight, gestational age, SGA, pre-term delivery	TTHM, individual THMs, HAAs, MX, mutagenicity	SGA see Wright <i>et al.</i> (2003)	>74 versus ≤33 µg l ⁻¹ TTHM 1.13 (1.07–1.20) >63 versus ≤26 µg l ⁻¹ CHCl ₃ 1.11 (1.04–1.17) >13 versus ≤5 µg l ⁻¹ BDCM 1.15 (1.08–1.22) >2250 versus ≤1250 rev l ⁻¹ mutagenicity 1.25 (1.04–1.51) similar results for birth weight

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Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
King <i>et al.</i> 2005	Nova Scotia and Eastern Ontario, Canada, 112 cases and 398 controls	stillbirth	HAAs	maternal age, province, income, occupation, smoking	stillbirth: no significant results after adjustments for THMs
Toledano <i>et al.</i> (2005)	three water regions in UK, 920 571 stillbirth and birth and 969 304 births	stillbirth, LBW, very LBW	THMs	maternal age, deprivation	stillbirth: ≥ 60 versus $<30 \mu\text{g l}^{-1}$ TTHM 1.11 (1.00–1.23)
Porter <i>et al.</i> (2005)	four regions MD, USA, sample population: 15 416 births	IUGR	THMs and HAAs	smoking, ethnicity, prenatal care, marital status, teen birth, birth at age >35	no association
Lewis <i>et al.</i> (2006)	MA, USA, sample population: 36 259 births	term LBW (weekly) THMs	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	term LBW second trimester: all: ≥ 70 versus $<40 \mu\text{g l}^{-1}$ TTHM 1.50 (1.07–2.10) per $10 \mu\text{g l}^{-1}$ TTHM 1.08 (1.00–1.20) non-Caucasians: ≥ 70 versus $<40 \mu\text{g l}^{-1}$ TTHM 1.60 (1.03–2.47) per $10 \mu\text{g l}^{-1}$ TTHM 1.10 (1.00–1.22)	(Continued.)

Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Hinckley <i>et al.</i> (2005)	AZ, USA, sample population: 48119 births	LBW, IUGR, pre-term delivery	THMs and HAAs	IUGR: parity, education, smoking, Kessner index term (T)LBW: maternal age, race, education, parity, smoking, Kessner index	IUGR: ≥53 versus <40 µg l ⁻¹ TTHM 1.09 (1.00–1.18) TLBW: ≥5 versus <4 µg l ⁻¹ DBAA 1.49 (1.09–2.04) IUGR: ≥8 versus <6 µg l ⁻¹ DCAA 1.28 (1.08–1.51) and ≥6 versus <4 µg l ⁻¹ TCAA 1.19 (1.01–1.41); weeks 37–40 IUGR: ≥8 versus <6 µg l ⁻¹ DCAA 1.27 (1.02–1.59); weeks 33–36 TLBW: ≥5 versus <4 µg l ⁻¹ DBAA 1.49 (1.10–2.02) no association
Savitz <i>et al.</i> (2006)	three US locations, 2409 pregnancies	spontaneous abortion	THMs, HAAAs, TOX	maternal age, race, ethnicity, education, marital status, alcohol intake age at menarche, vitamin use + random-effects term for study site	no association (with some exception for some groups on government pay)
Lewis <i>et al.</i> (2007)	MA, USA, sample population: 37498 births	pre-term birth	THMs	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	(Continued.)

Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Yang <i>et al.</i> (2007)	Taiwan, sample population: 90 848 births	LBW, IUGR, pre-term delivery	THMs	maternal age, maternal education, marital status, only first birth	no association
Nieuwenhuijsen <i>et al.</i> (2008)	England and Wales: sample population: 2 605 226	congenital anomalies: respiratory, cardiovascular, urinary and NTDs and cleft lip and palate	THMs	maternal age, deprivation, gender	isolated ventricular septal defects: ≥ 60 versus $<30 \mu\text{g l}^{-1}$ THM 1.43 (1.00–2.04) subset isolated major cardiovascular defects: 2 to <4 versus $<2 \mu\text{g l}^{-1}$ bromoform 1.13 (0.99–1.20) and ≥ 4 versus $<2 \mu\text{g l}^{-1}$ bromoform 1.18 (1.00–1.39) isolated gastroschisis: 2 to <4 versus $<2 \mu\text{g l}^{-1}$ bromoform 1.11 (0.85–1.45) and ≥ 4 versus $<2 \mu\text{g l}^{-1}$ bromoform 1.38 (1.00–1.92)

(Continued.)

Table 2. (*Continued.*)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Chisholm <i>et al.</i> (2008)	Perth, Australia 20 870 livebirths	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) hypospadias	low (TTHM <60 µg l ⁻¹), medium (TTHM >60 to <130 µg l ⁻¹) and high (TTHM ≥130 µg l ⁻¹)	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)
Luben <i>et al.</i> (2008)	AR, USA, 320 cases and 614 controls, and a subset of 40 cases and 243 controls	THMs and HAAAs, and personal characteristics in the subset	maternal age, no. of cigarettes smoked per day during pregnancy and maternal education	no association except for in the subset analysis: TTHM ingestion >0–32.5 versus 0 µg d ⁻¹ ; 2.79 (1.01–7.72)	(Continued.)

Table 2. (Continued.)

author (year)	study details (location, time, sample size)	cases	exposure assessment	other risk factors included	main findings OR (95% CI)
Hwang <i>et al.</i> (2008)	Taiwan, sample population: 396 049 births	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)	high (TTHMs $20+\mu\text{g l}^{-1}$), medium (TTHMs $10\text{--}19\mu\text{g l}^{-1}$), low exposure (TTHMs $5\text{--}9\mu\text{g l}^{-1}$) and $0\text{--}4\mu\text{g l}^{-1}$	maternal age, plurality, population density	high versus low cleft palate: 1.56 (1.00–2.41)
Joyce <i>et al.</i> (2008)	Perth, Australia, sample population: 16 229 women	pre-labour rupture of membrane	THMs (<110 , $110\text{--}150$, $>150\mu\text{g l}^{-1}$)	maternal age, smoking, socioeconomic status	no association
Hoffman <i>et al.</i> (2008a)	three US locations, 1958 births	SGA, term birth weight	THMs, HAAAs, TOX and personal characteristics	maternal age, race/ethnicity, income, education, employment status, marital status	no association
Hoffman <i>et al.</i> (2008b)	three US locations, 2039 births	duration of gestation	THMs, HAAAs, TOX and personal characteristics	per $10\mu\text{g l}^{-1}$ TTHM increase 0.95 (0.91, 1.00) per $10\mu\text{g l}^{-1}$ HAA increase 0.89 (0.82, 0.98)	see Hoffman <i>et al.</i> (2008a)

(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 $10 \mu\text{g l}^{-1}$ and high levels (i.e. $130 \mu\text{g l}^{-1}$ 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. $60 \mu\text{g l}^{-1}$ or more) for ventricular septal defects and the highest exposure category of bromoform (i.e. $4 \mu\text{g l}^{-1}$ 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. $75 \mu\text{g l}^{-1}$ or more), especially for bromodichloromethane (BDCM) (i.e. $18 \mu\text{g l}^{-1}$ 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.

study	exposure	risk estimate
Källén & Robert (2000)	chlorinated (average TTHM = 9.4 µg l ⁻¹) versus non-chlorinated	1.07 (0.96–1.19)*
Jaakkola <i>et al.</i> (2001)	chlorinated (average TTHM = 9.4 µg l ⁻¹) versus non-chlorinated	1.00 (0.91–1.10)**
Kramer <i>et al.</i> (1992)	0 versus ≥10 µg l ⁻¹ CHCl ₃	1.8 (1.1–2.9)*
Bove <i>et al.</i> (1995)	≤20 versus >100 µg l ⁻¹ TTHM	1.5 (1.2–1.9)*
Dodds <i>et al.</i> (1999)	0–49 versus >100 µg l ⁻¹ TTHM	1.08 (0.99–1.18)***
Wright <i>et al.</i> (2003)	0–60 versus >80 µg l ⁻¹ TTHM	1.14 (1.02–1.26)
Wright <i>et al.</i> (2004)	>74–163 versus 0–33 µg l ⁻¹ TTHM	1.13 (1.07–1.20)
Aggazzotti <i>et al.</i> (2004)	THMs ≥30 µg l ⁻¹ , chlorite or chlorate ≥200 µg l ⁻¹	1.38 (0.92–2.07)
Infante-Rivard (2004)	>90th percentile versus ≤90th percentile: 29.4 µg l ⁻¹ TTHM	0.97 (0.57–1.62)
Porter <i>et al.</i> (2005)	highest versus lowest quintile	1.17 (0.96–1.42)
Hinckley <i>et al.</i> (2005)	≥53 versus <40 µg l ⁻¹ TTHM	1.09 (1.00–1.18)
Yang <i>et al.</i> (2007)	THM4 >13.1 versus ≤4.9 µg l ⁻¹	0.96 (0.91–1.02)
Hoffman <i>et al.</i> (2008)	74.9–108.8 versus 2.2–4.6	1.3 (0.7–2.3)

*≤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).

exposure	level	risk estimate
TTHM	>74–163 versus 0–33	1.13 (1.07–1.20)
CHCl ₃	>63–135 versus 0–26	1.11 (1.04–1.17)
BDCM	>13–46 versus 0–5	1.15 (1.08–1.22)
total HAAs	>49–58 versus 4–30	0.97 (0.77–1.23)
TCAA	>27–37 versus 0–18	0.95 (0.76–1.19)
DCAA	>22–24 versus 2–15	0.90 (0.75–1.09)
MX	>46–80 versus 4–20	1.14 (0.95–1.37)
mutagenicity	>2250 versus <1250	1.25 (1.04–1.51)

1995, 1997*a,b*). 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 $29.4 \mu\text{g l}^{-1}$ for TTHMs were at much higher risk ($\text{OR} = 13.2$, 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 $10 \mu\text{g l}^{-1}$

and high levels greater than or equal to $130 \mu\text{g l}^{-1}$ of THMs and cardiovascular congenital anomalies, respectively, but the low exposure group in the latter study (i.e. less than $60 \mu\text{g l}^{-1}$) represented higher levels of THM exposure than that of the cases in the former study (i.e. more than $10 \mu\text{g l}^{-1}$), 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 $9.4 \mu\text{g l}^{-1}$. 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 2500 g 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. Nieuwenhuijsen *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 *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.

(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 *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 *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.

(e) 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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