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ADVERSE REPRODUCTIVE HEALTH EFFECTS OF EXPOSURE TO CHLORINATION DISINFECTION BY PRODUCTS

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ABSTRACT

Chlorination disinfection by-products (DBPs) are formed when water is chlorinated and the organic matter in the water reacts with chlorine to form these by-products. There have been concerns about the potential health effects of these by-products, including cancer and reproductive effects. Here we have reviewed the literature on reproductive health effects. Epidemiological studies on neural tube defects, urinary tract defects and small for gestation age/intra growth retardation have shown the most consistent statistically significant associations with an index of DBPs, but generally the risk estimates are small. The interpretation of the studies is not straight forward because they may not be directly comparable because of differences in DBP mixtures, exposure categories and actually uptake of DBPs due to differences in e.g. ingestion rates, showering, bathing, and swimming. Only few specific DBPs have been studied and THMs have often been used as a marker for other DBPs, since they are often routinely available. Sample sizes, and therefore power, have at times been low, particularly when the population was split into exposure categories. Although most studies considered some confounders, (residual) confounding by other water contaminants or other factors related to water intake, cannot always be excluded. Case ascertainment, for outcomes such as spontaneous abortion and certain congenital anomalies is far from straight forward, and for the latter at times anomalies are lumped together with different aetiology, which may be inappropriate. Furthermore, as with many reproductive epidemiological, if the putative agent affects both early pregnancy loss and later birth outcomes such as congenital anomalies, interpretation of later birth outcomes may be more difficult.

KEYWORDS: Chlorination by products, trihalomethane, reproductive health effects, cancer

INTRODUCTION

Chlorination disinfection by-products (DBPs) are formed when water is chlorinated and the organic matter in the water reacts with chlorine to form these by-products. The formation and occurrence depends on many factors, including the chlorine dose, type of treatment, pH, temperature, residence time, bromine levels (Nieuwenhuijsen *et al.,* 2000a, IPCS 2000). Up to 500 different by-products have been identified (Richardson, 1998). Different mixtures of by-product may exist in different locations depending on the various factors mentioned above, making it more difficult to assess any health effects of DBPs, particularly in epidemiological studies.

The health effects of DBPs in drinking water have been a concern since DBPs were first reported in the seventies (Rook, 1974). Early studies focused on cancer outcomes, while

the more recent studies have focused on reproductive outcomes (IPCS 2000). According to the recent review by IPCS (2000): "more studies have considered bladder cancer than any other cancer. The authors of the most recently reported results for bladder cancer risks 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 various geographic areas because the DBP mix may be different or because other water contaminants are also present. More comprehensive water quality data must be collected or simulated to improve exposure assessments for epidemiological studies." A recent pooled analysis by Villanueva *et al* (2004), that provided quantitative information, confirmed this. For men there was an exposure response related relationship between DBP intake and bladder cancer, but there was no relationship in women (Table 1).

Table 1. Pooled analysis of bladder cancer and THM*

THM		
Exposure Level	Male ORs	Females ORs
0-15 mg	1.00	1.00
>15-50 mg	1.22	0.92
>50-400 mg	1.28	0.94
>400-1000 mg	1.31	1.02
>1000 mg	1.50	0.92

* After Villanueva *et al.* 2004

Reproductive health outcomes should be easier to study from an exposure point of view, because of the shorter relevant exposure period. Amongst others, birth weight, prematurity, spontaneous abortion, congenital anomalies and still birth have been the focus of these studies. Various thorough reviews have been conducted and concluded that there are still many problems to overcome and that the results are inconsistent and inconclusive (Reif *et al.,* 1996; Nieuwenhuijsen *et al.,* 2000b; Gevecker Graves *et al.,* 2001; Bove *et al.,* 2002; IPCS 2000). Table 2 provides an overview of the main reproductive epidemiological studies, their study design, sample size, exposure indices, confounders and main positive outcomes.

A number of studies found statistically significant positive associations between THMs and neural tube defects, one of the most studied group of congenital anomalies (Bove *et al.,* 1995; Klotz and Pyrch, 1999; Dodds *et al.,* 2001), while others did not (Magnus *et al.,* 1999; Kallen *et al.,* 2000; Hwang *et al.,* 2002; Dodds *et al.,* 1999; Shaw *et al.,* 2003). Klotz and Pyrch (1999) found a statistically significant association between TTHM levels in the water and neural tube defects, but not with haloacetonitriles and haloacetates. Also, the effects were most pronounced in offspring from women that did not take supplementary vitamins, but these findings were not confirmed by the Shaw *et al.* (2003) study. Inclusion of information on ingestion, showering, bathing and swimming made little difference to the risk estimates.

Hwang *et al.* (2002) and Cedergren *et al.* (2002) found significant associations between chlorinated water and levels of TTHM above 10 $\mu g\ l^{-1}$ respectively and respiratory defects, but other studies did not find such an association (Magnus *et al.,* 1999; Kallen *et al.,* 2000; Bove *et al.,* 1995; Dodds *et al.,* 1999; Dodds *et al.,* 2001; Shaw *et al.,* 2003). Studies on chlorinated water and respiratory effects have been rare, but two studies found a significant positive association (Aschengrau *et al.,* 1993 and Hwang *et al.*, 2002).

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

Author (year)	**Study details (location, time, sample size)**	**Cases**	**Exposure assessment**	**Other risk factors included**	**Main positive findings OR (95% CI)**
Aschengrau *et al.,* 1989	Massachusetts., US. Sample population 1677	286 spontaneous abortion	Surface vs ground water Chlorination vs chloraminated water	Smoking habits Contraceptive use Medical and obstetrical history Metals	**Surface vs Ground water** 2.2 (1.3-3.6)
Kramer *et al.* [48]. (1992)	Iowa, US. 151 towns with a single water source. 1989-90 Sample population: 4028	588 (total) 159 low birth weight 342 pre-term delivery 187 Intrauterine growth retardation/small-for-gestational-age	Based on maternal residential address and one municipal water survey to estimate individual THM levels (2 or 3 exposure categories).	Maternal age, Parity, Marital status, Education, Smoking, Prenatal care.	No vs Medium (1-9 µg l^{-1}) vs High (=>10 µg l^{-1}): Chloroform **Low birth weight:** 1 vs 1.1 (0.7-1.6) vs 1.3 (0.8-2.2) **Intrauterine growth retardation** 1 vs 1.3 (0.9-1.8) vs 1.8 (1.1-2.9) Dichlorobromomethane **Intrauterine growth retardation** 1 vs 1.2 (0.8-1.7) vs 1.7 (0.9-2.9)
Aschengrau *et al.* [45] (1993)	Massachusetts., US. 2 hospitals. 1977-80 Sample population: 2348	1171 (total) 1039 Major congenital malformations Urinary tract defects Respiratory tract defects 77 Stillbirths 55 Neonatal deaths	Based on maternal residential address to ascertain type of water supply, chlorination vs. chloramination, and ground/mixed water vs surface water.	Maternal age, Pregnancy history, Alcohol, Ethnicity, Hospital payment, Other water contaminants.	Chlorinated vs. Chloraminated: **Stillbirth**: 2.6 (0.9-7.5) **Neonatal deaths:** 1.1 (95% CI not provided) **Congenital Malformations**: - **major malformations** 1.5 (0.7-2.1) **- respiratory defects** 3.2 (1.1-9.5) **- urinary tract defects** 4.1 (1.2-14.1)
Bove *et al.* [49] (1995)	New Jersey, US. 75 towns with a public water supply. 1985-88 Sample population: 81602	29268 (total) *Live births:* 1853 Low birth weight 905 Very low birth weight 4082 Small-for-	Based on maternal residential address and municipal water surveys to estimate monthly TTHM levels (5 or 6 exposure categories)	Maternal age Ethnicity Gender baby Primipara Prenatal care, Education, Previous still or	TTHM levels >100 µg l^{-1} vs <=20 µg l^{-1}: **Low birth weight:** 1.4 (50%CI 1.2-1.7) Intrauterine growth retardation/ **Small-for-gestational-age:** 1.5 (90% CI 1.2- 1.9)

Table 2. (continued)

Author (year)	**Study details (location, time, sample size)**	**Cases**	**Exposure assessment**	**Other risk factors included**	**Main positive findings OR (95% CI)**
		gestational-age 7167 Pre-term 594 Foetal deaths *All births: Defects:* 669 Surveillance 118 Central nervous system defects 83 Oral cleft 56 Neural Tube 108 Major cardiac		miscarriage Other contaminants	TTHM levels >80 µg l^{-1} vs <=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) **Neural tube defects:** 3.0 (90% CI 1.3-6.6) **Major cardiac defects:** 1.8 (90% CI 1.0-3.3) TTHM levels >100 µg l^{-1} vs <=20 µg l^{-1}: **Oral cleft defects:** 3.2 (90%CI 1.2-7.3)
Savitz *et al.* [52] (1995)	Carolina, US. 6 hospitals. 1988-91 Sample population: 1003	548 (Total) 126 Spontaneous Abortion 244 Pre-term 178 Low birth weight	Based on maternal residential address and quarterly municipal water surveys to estimate average TTHM levels. Analysis of: a) surface vs ground water source, b) TTHM levels, (3 exposure categories) c) Consumption during pregnancy d) water source x amount e) TTHM dose (level x amount)	Maternal age Ethnicity Hospital Education Marital status Poverty level Smoking Alcohol consumption. Employment Nausea	40.8-59.9 vs. 81.1-168.8 µg l^{-1} TTHM: **Spontaneous Abortion**: 1.2 (0.6-2.4) 40.8-63.3 vs. 82.8-168.8 µg l^{-1} TTHM: **Low birth weight:** 1.3 (0.8-2.1) Per 50 µg l^{-1} TTHM increment change: **Spontaneous Abortion**: 1.7 (1.1-2.7)
Kanitz *et al.* [46] (1996)	Liguria, Italy. 2 hospitals. 1988-1989 Sample population: 676	548 live births in 'exposed' area. 50 Pre-term 141 Caesarean section 133 Neonatal jaundice	Based on maternal residential address to ascertain type of water source (chlorine dioxide +/or hypochlorite vs. not treated).	Maternal age Education Smoking Alcohol Gender of child	Sodium hypochlorite treated (8-16 µg l^{-1} TTHMs) vs non treated water: **Neonatal jaundice** 1.1 (0.7-2.8) **Low birth weight** 6.0 (0.6-12.6)

Table 2. (continued)

Author (year)	**Study details (location, time, sample size)**	**Cases**	**Exposure assessment**	**Other risk factors included**	**Main positive findings OR (95% CI)**
		20 Low birth weight 288 Small body length 370 Small cranial circumference			**Small body length** 2.3 (1.3-4.2) **Small cranial circumference** 3.5 (2.1-8.5)
Waller *et al.* [53] (1998)	California, US. 3 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: a) THM levels (3 or 10 exposure categories) b) consumption during first trimester from interview (2 exposure categories)	Maternal age, Gestational age, Smoking, History of pregnancy loss, Ethnicity, Employment	high TTHM dose (≥5 glasses/day + ≥75 μg l^{-1}) vs. low dose (<5 glasses/day + <75 μg l^{-1}): **Spontaneous Abortion** 1.8 (1.1-3.0). high BDCM dose (≥5 glasses/day + ≥18 μg l^{-1}) vs. low dose (<5 glasses/day + <18 μg l^{-1}): **Spontaneous Abortion** 3.0 (1.4-6.6).
Waller *et al*. (2001)	See Waller *et al.* [53] (1998)	See Waller *et al.* [53] (1998)	See Waller *et al.* [53] (1998)	See Waller *et al.* [53] (1998)	Re-analysis Waller *et al.* (1998) Utility wide subset sample highest AOR high TTHM dose (≥5 glasses/day + ≥75 μg l^{-1}) vs. low dose (≥5 glasses/day + <75 μg l^{-1}): **Spontaneous Abortion** 5.1 (1.8-14.7) Little relationship with showering
Gallagher *et al.* [50] (1998)	Colorado, US. 28 census blocks in 2 water districts. 1990-1993. Sample population: 1244 live births	72 low birth weight 29 term-low birth weight 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 (4 exposure categories)	Maternal age, Smoking, Marital status, Parity, Education, Employment, Pre-natal care	high TTHM level (=> 61 μg l^{-1}) vs. lowest (<= 20 μg l^{-1}): **Low birth weight**: 2.1 (1.0-4.8) **Term low birth weight:** 5.9 (2.0-17.0)
Dodds *et al.* [51] (1999)	Nova Scotia, Canada 1988-1995 Sample	4673 Small for gestational age 2393 Low birth weight 342 Very low birth	Based on maternal residential address and TTHM levels for public water facilities (3 sampling	Maternal age Parity Maternal smoking Attendance	0-49 μg l^{-1} vs >100 μg l^{-1} TTHMs **Still birth** 1.66 (1.09-2.52) **Chromosomal abnormalities**

Table 2. (continued)

Author (year)	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
	population: 49,842 births	weight 2689 pre-term delivery 77 Neural tube 82 Cleft defect 430 Major cardiac defects 197 Still birth 96 chromosomal abnormalities	locations) modelled using linear regression on the basis of observations by year, month and facility (4 exposure categories)	prenatal classes Neighbourhood family income gender Pregnancy and pre-delivery weight	1.38 (0.73-2.59) **Small for gestation age** 1.08 (0.99-1.18) **NTDs** 1.18 (0.67-2.10)
King *et al*. (2000)	Nova Scotia, Canada 1988-1995 Sample population: 49,756	214 still births (72 asphyxia related still births)	Based on maternal residential address and TTHM, chloroform, and BDCM levels for public water facilities (3 sampling locations) modelled using linear regression on the basis of observations by year, month and facility (4 exposure categories) (r=0.44 for TTHM and BDCM)	Maternal age Parity Maternal smoking Attendance prenatal classes Neighbourhood family income gender Pregnancy and pre-delivery weight	0-49 μg l^{-1} vs >100 μg l^{-1} chloroform **Still birth** 1.56 (1.04-2.34) **Asphyxia related stillbirth** 3.15 (1.64-6.03) <5 μg l^{-1} vs >20 μg l^{-1} BDCM **Still birth** 1.98 (1.23-3.49) **Asphyxia related stillbirth** 1.75 (0.72-4.22)
Dodds and King (2001)	Nova Scotia, Canada 1988-1995 Sample population: 49,842 births	77 NTDs 430 Cardiovascular anomalies 82 Cleft defects 96 Chromosomal abnormalities	Based on maternal residential address and TTHM, chloroform, and BDCM levels for public water facilities (3 sampling locations) modelled using linear regression on the basis of observations by year, month and facility (4 exposure categories) (r=0.44 for TTHM and BDCM)	Maternal age Parity Maternal smoking Attendance prenatal classes Neighbourhood family income gender Pregnancy and pre-delivery weight	BDCM ≥ 20 μg l^{-1} vs < 5 μg l^{-1} **NTDs** 2.5 (1.2-5.1)
Klotz and Pyrch [56]	New Jersey, US 1993-1994	112 Neural tube defects	Based on residential address and public water facility	Sociodemographics	TTHMs public monitoring data, Known residence&isolated cases

Table 2. (continued)

Author (year)	**Study details (location, time, sample size)**	**Cases**	**Exposure assessment**	**Other risk factors included**	**Main positive findings OR (95% CI)**
(1999)	Sample population: All births, of which 112 cases-248 controls selected		TTHM data, and tap water sampling for TTHMs, HANs and HAAs (3-5 exposure categories)	Pregnancy and medical history Parental occupational Use of vitamins	<5 μg l^{-1} vs 40+ μg l^{-1} **NTDs** 2.1 (1.1-4.0)
Magnus *et al.* [47] (1999)	Norway Sample population: 141,077	2,608 all birth defects 62 Neural tube defects 250 Major cardiac defects 91 Respiratory defects 122 Urinary defects 143 Oral cleft	Chlorination yes vs no Colour high vs low (in chlorinated water average TTHMs = 9.4 μg l^{-1}, average HAAs = 14.6 μg l^{-1})	Maternal age Parity Geographical placement Population density Industry profile	No chlorination low colour vs 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 *et al.* (2001)	Norway Sample population: 137,145	6249 Low birth weight ? Small for gestational age 7886 Pre-term delivery	Chlorination yes vs no Colour high vs low (in chlorinated water average TTHMs = 9.4 μg l^{-1}, average HAAs = 14.6 μg l^{-1})	Maternal age Parity Geographical placement Population density Industry profile	No chlorination low colour vs chlorination high colour **Pre-term delivery** 0.91 (0.84-0.99)
Kallen *et al.* (2000)	Sweden (‘85-‘94) Sample population: No chlorination: 74324 singletons Na-hypochlorite: 24,731 singletons Chlorine dioxide: 15429 singletons	Multiple births Gestational duration Birth weight Intrauterine growth Body length Head circumference Body mass index Infant survival up to 1 yr Perinatal death Apgar score	No versus sodium hypochlorite (no versus chlorine dioxide)	Year of birth Maternal age Parity Maternal education Maternal smoking Congenital malformations and childhood cancer:	No versus sodium hypochlorite **Low birth weight** 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)

Table 2. (continued)

Author (year)	**Study details (location, time, sample size)**	**Cases**	**Exposure assessment**	**Other risk factors included**	**Main positive findings OR (95% CI)**
		Neonatal jaundice Congenital malformations, incl NTD Childhood cancer Hypothyroidism		Maternal age Year of birth	**BMI>16 kg m^{-2}** 1.27 (1.19-1.37) **< 31 cm head circumference** 1.46 (1.07-1.98) **Spine malformation** 3.2 (1.0-10.0)
Yang *et al.* (2000a)	Taiwan Sample population 18,025 first parity births: Chlorinated: 10,007 Non-chlorinated: 8,018	Low birth weight Preterm delivery (<37 weeks)	Chlorinated (>95% pop served chlorin. water) versus non-chlorinated (<5% pop served chlorin. water)	Maternal age Marital status Maternal education Gender	Chlorinated versus non-chlorinated **Preterm delivery** 1.34 (1.15-1.56)
Yang *et al.* (2000b)	Taiwan Sample population Chlorinated: 24882 Non-chlorinated: 20460	Sex ratio	Chlorinated (>95% pop served chlorin. water) versus non-chlorinated (<5% pop served chlorin. water)		
Cedergren *et al.* (2002)	Sweden Sample population 58669	Cardiac defects	> 10 µg l^{-1} vs ≤ 10 µg l^{-1} TTHM in surface water hypochloride and chlorine dioxide vs hypochloride in surface water Ground water vs surface water	Maternal age Parity Smoking Education	> 10 µg l^{-1} vs ≤ 10 µg l^{-1} TTHM **Cardiac defects** 1.30 (1.08-1.56) Ground water vs surface water **Cardiac defects** 1.32 (1.10-1.58) Hypochloride and chlorine dioxide vs hypochloride **Cardiac defects** 1.85 (1.42-2.39)

Table 2. (continued)

Author (year)	**Study details (location, time, sample size)**	**Cases**	**Exposure assessment**	**Other risk factors included**	**Main positive findings OR (95% CI)**
Hwang *et al*., (2002)	Norway Sample population 285,631	Any birth defect Neural tube defect -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 -Population density	Chlorination (yes) and level of water colour: <10 vs ≥ 20 mg Pt l^{-1} **All birth defect** 1.18 (1.02-1.36) **Ventricular septal defect** 1.81 (1.05-3.09) Chlorination (yes) and level of water colour: <10 vs ≥ 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)
Nieuwenhuijsen *et al.* (2002)	England 11,462	Birth weight	Amount of swimming (hrs)	Maternal age Maternal education Smoking Alcohol use Drugs use Gestational age Ethnicity Infant gender	
Wright *et al.* (2003)	Massachusetts, USA 56,513	Birth weight Low birth weight Small for gestational age Gestational age Preterm delivery	0-60, >60-80, >80 µg l^{-1} TTHM or per 20 µg l^{-1} TTHM increase	Maternal age Maternal education Ethnicity Smoking Parental care Parity Infant gender	0-60, vs >80 µg l^{-1} TTHM **Birth weight** -32 g (-47 to –18) **Small for gestational age** 1.14 (1.02-1.26) **Gestational age (wks)** 0.08 (0.01-0.14)

Table 2. (continued)

Author (year)	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
					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)
Shaw *et al.* (2003)	California 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	Study 1: NTDs (anencephaly and spina bifida) Study 2: NTDs (anencephaly and spina bifida), conotruncal heart defects, orofacial clefts	Study 1 and 2: Continuous TTHMs Categorical: 0, 1-24, 25-49, 50-74 and ≥ 75 μg l^{-1} TTHMs Also study 1: ≥ 50 vs < 50 μg l^{-1} and < 5 glasses ≥ 50 vs < 50 μg l^{-1} and > 5 glasses Study 1: Chloroform ≥ 12.2 vs < 12.2 μg l^{-1} BDCM ≥ 4.2 vs < 4.2 μg l^{-1} CDBM ≥ 1.7 vs < 1.7 μg l^{-1} Study 2: Chloroform ≥ 15.0 vs < 15.0 μg l^{-1} BDCM ≥ 9.6 vs < 9.6 μg l^{-1} CDBM ≥ 3.6 vs < 3.6 μg l^{-1}	Ethnicity Education Body mass index Use of vitamins Methylenetetrahydrofolate reductase (MTHFR) genotype	Study 1: **NTDs** NTD risk inversely related to TTHM exposure but only occasionally significant for one category Chloroform ≥ 12.2 vs < 12.2 μg l^{-1} 0.50 (0.34-0.75) BDCM ≥ 4.2 vs < 4.2 μg l^{-1} 0.66 (0.45-0.97) CDBM ≥ 1.7 vs < 1.7 μg l^{-1} 0.69 (0.47-1.0) Study 2: **Multiple cleft palate/lip** Chloroform ≥ 15.0 vs < 15.0 μg l^{-1} 0.21 (0.05-0.90)
Dodds et al. (2004)	Nova Scotia and Eastern Ontario 112 stillbirth and 398 live birth controls	Still birth	Various indices: 0, 1-49, 50-79 and >80 μg l^{-1} for total THMs and chloroform and 0, 1-4, 5-9 and >9 for BDCM	Age Province Household income	**Still birth:** TTHM >80 vs 0 2.2 (1.1-4.4) TTHM highest vs lowest quintile 2.4 (1.2-4.6)

Table 2. (continued)

Author (year)	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
			Quintiles for total exposure (ingestion/showring/bathing) for TTHM, chloroform and BDCM Concentration and duration		Drinking 5+ drinks per day and THM 50+ μg l^{-1} vs < 1 drink and THM = 0 4.0 (1.4-11) Chloroform and BDCM generally follow TTHM trend
Yang (2004)	Taiwan 182,796	Low birth weight Pre-term delivery	15 non-chlorinating municipalities (NCM) and 128 chlorinating municipalities (CM)	Maternal age Education Gestational age Birth weight Gender	**Pre-term delivery** NCM vs CM 1.37 (1.20-1.56)
Infante-Rivard (2004)	Montreal, Quebec, Canada 493 cases 472 control	Intrauterine growth restriction (10th %ile)	Regulatory data on THMs, > 90^{th} %tile vs ≤ 90^{th} %tile	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 *et al.* (2004)	Massachusetts, US 196,000 registry based	birth weight gestational age small for gestation age pre-term delivery	TTHM Ind THMs HAAs MX Mutagenicity		**SGA** >74 vs ≤ 33 μg l^{-1} TTHM 1.13 (1.07-1.20) >63 vs ≤ 26 μg l^{-1} CHCL3 1.11 (1.04-1.17) >13 vs ≤ 5 μg l^{-1} BDCM 1.15 (1.08-1.22) >2,250 vs ≤ 1250 rev/l mutagenicity 1.25 (1.04-1.51) Similar results for birth weight
King *et al.* (2005)	Nova Scotia and Eastern Ontario, Canada 112 cases 398 controls	stillbirth	HAAs	Maternal age Province Income Occupation Smoking	Stillbirth No significant results after adjustments for THMs
Toledano *et al*. (2005)	3 water regions in UK	Low birth weight Very low birth weight Still birth	THMs	Maternal age Deprivation	Stillbirth ≥60 vs < 30 μg l^{-1} TTHM 1.11 (1.00-1.23)

Similarly, for urinary tract defects, although only three studies have been conducted they all reported statistically significant associations (Aschengrau *et al.,* 1993; Magnus *et al.,* 1999, Hwang *et al* 2002). Studies on oral cleft or cleft palate have largely been negative, except for the study by Bove *et al.* (1995). In a meta-analysis Hwang *et al.* (2003) reported evidence for an effect of exposure 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 .

Only a few studies have assessed the relationship between DBPs and spontaneous abortion. The California study has attracted the most attention since they found a statistically significant association between TTHM and BDCM and spontaneous abortion (Waller *et al.,* 1998). The effects were even stronger after re-analysis (Waller *et al.,* 2001)

A number of Canadian studies and one English found statistically positive associations between DBPs and stillbirth (Dodds *et al.,* 1999, King *et al.,* 2000; Dodds *et al.,* 2004; 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 still birth (King *et al.,* 2005).

Studies on pre-term delivery have generally shown no association with DBPs, with the exception of the study by Yang *et al.* (2000). Study results on low birth weight have been more mixed, with some studies reporting statistically significant associations (Kallen *et al.,* 2000; Bove *et al.,* 1995; Gallagher *et al.,* 1998) while others did not find any statistically significant associations (Kanitz *et al.,* 1996; Jaakkola *et al.,* 2001; Kramer *et al.,* 1992; Savitz *et al.,* 1995; Dodds *et al.,* 1999; Wright *et al.,* 2003; Toledano *et al.,* 2005). Studies on small for gestational age and/or intrauterine growth retardation showed some more consistent results, and a good proportion of them have found statistically significant associations (Kramer *et al.,* 1992; Bove *et al.,* 1995; Gallagher *et al.,* 1998; Wright *et al.,* 2003; Wright *et al* 2004). Wright *et al.* (2004) found statistically significant associations with THMs and a measure of mutagenicity, but not with HAAs or MX. Infante-Rivard (2004) found that the association between THMs and intrauterine growth retardation was modified by a metabolic polymorphism, with newborns without the CYP2E1 (G1259C) variant at high risk.

DISCUSSION

Epidemiological studies on neural tube defects, urinary tract defects and small for gestation age/intra growth retardation have shown the most consistent statistically significant associations with an index of DBPs, but generally the risk estimates are small. The interpretation of the studies is not straight forward because they may not be directly comparable because of differences in DBP mixtures, exposure categories and actually uptake of DBPs due to differences in e.g. ingestion rates, showering, bathing, and swimming. Only few specific DBPs have been studied and THMs have often been used as a marker for other DBPs, since they are often routinely available. However they may not be well correlated with other DBPs and therefore not be a good marker. Sample sizes, and therefore power, have at times been low, particularly when the population was split into exposure categories. Although most studies considered some confounders, (residual) confounding by other water contaminants or other factors related to water intake, cannot always be excluded. Case ascertainment, for outcomes such as spontaneous abortion and certain congenital anomalies is far from straight forward, and for the latter at times anomalies are lumped together with different aetiology, which may be inappropriate. Furthermore, as with many reproductive epidemiological, if the putative agent affects both early pregnancy loss and later birth outcomes such as congenital anomalies, interpretation of later birth outcomes may be more difficult.

Animal evidence could shed more light on any possible relationships, but doses that have been used were often high, much higher than humans are exposed to and the DBPs are often administered in isolation rather than a mixture (Nieuwenhuijsen *et al.,* 2000b; Gevecker Graves *et al.,* 2001; IPCS 2000). THMs have generally shown no direct evidence of, but e.g. neural tube and craniodefects have been found with administration of dichloroacetic or trichloroacetic acid in rats (Smith *et al*., 1989), and cardiac malformations have been induced at high doses of dichloroacetic acid (Smith *et al.*, 1992). Andrews *et al.* (2004) found heart defects and neural tubes with HAAs. Also, Hunter *et al.* (1996) found changes in neural tube development when they exposed mouse embryos to HAAs. Several chloroacetonitrile compounds have shown an increase in malformations of cardiovascular, digestive, soft tissue and urinogenital systems (Smith *et al.,* 1989; Smith *et al*., 1988). 2-chlorophenol has also been associated with subfertility and stillbirths (Exon and Koller, 1985).

Epidemiological studies require accurate, precise and biologically relevant exposure estimates, preferably with a large range, which, as can be seen from the review, makes the exposure assessment fairly challenging. There are many different DBPs and uptake of DBPs occurs through various activities and exposure routes (Nieuwenhuijsen *et al.,* 2000a). Routinely collected THM data are often available, models are being developed, but information on personal exposure needs to be collected. Inaccurate and imprecise exposure estimates may lead to loss of power, precision and attenuation in health risk estimates, depending on the type of error model (Armstrong 1998, Nieuwenhuijsen 1997). Researchers generally have to perform some cost-benefit analysis when it comes to choosing an exposure estimate for an epidemiological study. Improving the accuracy of the exposure estimate will generally improve study power and the accuracy and/or precision of the effects of the exposure, but it is likely to come at a financial cost (Armstrong 1996). For example, personal exposure assessment such as biomonitoring of exhaled breath or urine may provide a much more accurate exposure estimate for each study subject than a group estimate such as a water zone mean estimate, at least when a sufficient number of samples are taken. However it may come at a financial cost that is also considerably higher, and may be well out of reach of the study. Relative large sample sizes may be needed for epidemiological studies of birth outcomes, for example for prospective cohort studies, given that many of the outcomes of interest are rare. Case-control studies may require fewer subjects, but the biomonitoring needs to take place after the relevant exposure period and is therefore likely to be less informative than in other study designs with a retrospective exposure assessment. Questionnaire data are also likely to be less accurate than in a prospective design. An important consideration is also whether the study sets out to find an association between a DBP and a certain outcomes, or, in addition, it also attempts to quantify the exposure/dose-response relationship, if any. In addition, one should keep in mind any policy implications, in particular risk management. DBP water zone levels can be regulated while, for example, personal activities cannot be. So what would be an efficient exposure assessment design? Would a study with a small number of subjects and a very accurate exposure estimate e.g. a biomarker in urine be as efficient and informative as a study with a large number of subjects and with a less accurate exposure estimate e.g. water zone estimate?

Some epidemiological studies have used water zone estimates, based on routinely collected THM measurements and/or models, as exposure index (e.g. Kramer *et al.,* 1992, Bove *et al.,* 1995, Gallagher *et al.,* 1998, Dodds *et al.,* 1999). Others have used water zones estimates in combination with personal exposure characteristics (e.g. Savitz *et al.,* 1995, Waller *et al.,* 1998, Klotz and Pyrch 1999). Some questions have been raised on the interpretation of health risk estimates (e.g. odds ratios and relative risks from exposure-response relationships estimating the risk per unit exposure) when using only the mean THM estimates of the water zones as an exposure index. It was suggested

that information on personal uptake of THMs is needed to interpret, or improve the interpretation of, health risk estimates because of possible exposure misclassification (Swan and Waller, 1998).

In epidemiological studies using personal estimates, the variability in these personal activities may lead to measurement error, and therefore attenuation of health risk estimates, under the so called Classical error model (Armstrong 1998). Under the Classical error model the average of many replicate measurements would equal the true exposure and the degree of attenuation is equal to the coefficient of reliability. Attenuation is less likely to occur when a group estimate such as the mean estimates of water zones are used because the Berkson error model may apply. The Berkson error applies when an approximate exposure (proxy) is used for many subjects in the study; the true exposures vary randomly about this proxy (Armstrong, 1998). In linear, and often in log-linear models, Berkson error is generally unlikely to lead to attenuation in health risk estimates, only to less precise ones, which may reduce study power (Armstrong 1998, Armstrong 1996). The efficiency in this case is equal to the proportion of variance of personal uptake explained by water zone means i.e. the square of the correlation between personal uptake and water zone mean (Armstrong 1996). When designing and interpreting epidemiological studies these issues should be considered.

While the available evidence suggests that the risks, if any, are small, the large numbers of people exposed to chlorinated water supplies means that the population attributable risk is potentially high. The inability to eliminate the possibility that other risk factors or possible biases might explain these small excess risks, coupled with the insufficient animal data to evaluate the biological mechanisms by which these agents may exert teratogenic and other birth effects, makes interpretation of such small elevations in risks difficult. Moreover, many of these apparent associations have been seen at TTHM levels well below the established maximum standards, currently at 100 $\mu g\ l^{-1}$ in the UK and US. Reducing chlorination by products concentrations further while still using chlorine becomes increasingly difficult. It must be remembered that the public health benefits of chlorination in terms of microbiological safety far exceed the potential health risks, but alternatives to chlorination should and are being explored e.g. the use of ozone.

Further epidemiological studies are recommended for reproductive outcomes such as (low) birth weight, still birth, spontaneous abortion and birth defects e.g. heart defects, cleft lip, respiratory defects, urinary tract defects, neural tube defect and central nervous system defects, and for studies of adult male fertility based on the current available toxicological and epidemiological evidence. Such studies should use appropriate designs such as a cohort study design for more common outcomes, or case-control study design for the rarer outcomes, with sufficient sample sizes, good case ascertainment, inclusion of relevant confounders, and in-depth exposure assessment (including repeated exposure measures). In practice this may not always be practicable, partly due to the cost involved, but some of these issues could be addressed in subsets of the populations. Also cultural and water treatment differences should be considered. Large registry based studies may also be useful, but a larger effort should be focused on the exposure assessment (see below) and potential for bias and confounding.

Future epidemiological studies will remain relatively crude until exposure assessment improves. Some of the factors that need to be considered are spatial and temporal variability in individual and total THMs and other by products, correlation between different substances, large samples where feasible, the relative contribution of different exposure routes (inhalation, ingestion, dermal absorption), consumption patterns (including tap water and bottled water, hot and cold drinks, and food) and daily activities including showering, bathing and swimming. Although it is unlikely that a single study

could be carried out taking into account all these factors, future studies need to try and minimise the potential for bias from these sources, possibly by carrying out more detailed exposure characterisation among a subset of the population. This should lead to a better understanding of the distribution and determinants of uptake of chlorination by products, and the design of statistical models to predict dose estimates for epidemiological and risk assessment studies. Furthermore, more effort should be made characterise the mixture of by-products in the study area, which could be very helpful for the interpretation of previous and future epidemiological studies.

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Reproductive effects of chlorine dioxide treatment have not been discussed due to the small number of studies

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