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Identification of developmentally toxic drinking water disinfection byproducts and evaluation of data relevant to mode of action ☆

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ABSTRACT

Reactions between chemicals used to disinfect drinking water and compounds present in source waters produce chemical mixtures containing hundreds of disinfection byproducts (DBPs). Although the results have been somewhat inconsistent, some epidemiological studies suggest associations may exist between DBP exposures and adverse developmental outcomes. The potencies of individual DBPs in rodent and rabbit developmental bioassays suggest that no individual DBP can account for the relative risk estimates reported in the positive epidemiologic studies, leading to the hypothesis that these outcomes could result from the toxicity of DBP mixtures. As a first step in a mixtures risk assessment for DBP developmental effects, this paper identifies developmentally toxic DBPs and examines data relevant to the mode of action (MOA) for DBP developmental toxicity. We identified 24 developmentally toxic DBPs and four adverse developmental outcomes associated with human DBP exposures: spontaneous abortion, cardiovascular defects, neural tube defects, and low birth weight infancy. A plausible MOA, involving hormonal disruption of pregnancy, is delineated for spontaneous abortion, which some epidemiologic studies associate with total trihalomethane and bromodichloromethane exposures. The DBP data for the other three outcomes were inadequate to define key MOA steps.

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Introduction

Chemical disinfection of drinking water has reduced significantly the incidence of infectious waterborne disease, but reactions of disinfectants such as chlorine with natural organic matter in source waters produce chemical mixtures composed of hundreds of different disinfection byproducts (DBPs) (Krasner et al., 2001, 2006; Miltner et al., 1990; Richardson, 1998; Richardson et al., 1999, 2000a,b, 2003, 2008; Weinberg, 1999; Weinberg et al., 2002). Although inconsistent results have been reported across different epidemiological studies, some studies have reported associations between DBP exposure and increased risk of adverse developmental outcomes including term low birth weight or small for gestational age births (Gallagher et al., 1998; Hoffman et al., 2008; Infante-Rivard, 2004; Lewis et al., 2006, 2007; Wright et al., 2003, 2004), birth defects such as cardiovascular and neural tube defects (e.g., Cedergren et al., 2002; Chisholm et al., 2008; Dodds and King, 2001; Hwang et al., 2008; Klotz and Pyrch, 1998, 1999; Nieuwenhuijsen et al., 2008), spontaneous abortion (Savitz et al., 1995, 2005, 2006; Waller et al., 1998, 2001), and stillbirths (Dodds et al., 2004; Toledano et al., 2005). Because such epidemiological associations do not necessarily imply causality, developmental toxicity studies of individual DBPs have been undertaken (summarized in Klinefelter et al., 2001 and in this paper) to test the hypothesis that exposures to DBPs cause developmental effects. A number of individual DBPs cause adverse developmental effects in mammalian bioassays; however, to date, these toxicological studies do not identify any single DBP with sufficient potency to account for the relative risk estimates reported in the epidemiological literature. This observation has led some to hypothesize that exposures to mixtures of DBPs are associated with the developmental outcomes reported in epidemiological studies and that this hypothesis could be examined through toxicological experiments with DBP mixtures and components using methods for mixtures risk assessment (Simmons et al., 2002, 2004,

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2008; Teuschler and Simmons, 2003). To comply with developmental study guidelines (U.S. EPA, 1991), the DBP exposure levels employed in these bioassays are typically significantly higher than human oral DBP exposures.

Evaluations of human health risks associated with exposure to chemical mixtures rely on component or whole mixture methods (ATSDR, 2004; U.S. EPA, 1986, 2000a). For exposure to chemicals in the low dose region, where interactions are deemed unlikely to occur, two relatively simple component methods (dose addition and response addition) are used in risk assessments conducted at a screening or intermediate level (U.S. EPA, 2000a). Dose additive models are used to estimate risks posed by toxicologically similar chemicals that exhibit the same toxic mode of action (MOA) and response additive models are used to estimate risks posed by toxicologically independent chemicals that affect the same target organ or cause the same illness (e.g., cancer) but exert these effects through different toxic MOAs (Feron and Groten, 2002; U.S. EPA, 2000a). Thus, knowledge of MOA, or an assumption about the similarity or dissimilarity of MOA, is required for a component-based risk assessment. The MOA is the set of biological events (the key events) at the target tissue or target organ, and/or related tissues that influence the target organ, leading to a toxicologic outcome. MOA thus implies a general understanding of key toxicodynamic events leading to the outcome, but is not as stringent as mechanism of toxicity (Teuschler et al., 2004; U.S. EPA, 2000a, 2003, 2005). In addition to knowledge about MOA, knowledge of critical targets of toxicity can also be important determinants of other component approaches to mixtures risk assessment. Under the hazard index approach and interaction-based hazard index (U.S. EPA, 2000a), dose addition is assumed based on common target organ toxicity of the components.

Absent knowledge of MOA, information identifying the site of insult (critical tissue) for developmental toxicants (e.g., maternal tissues or fetal tissues) is valuable in grouping chemicals for risk assessment. While the MOA may include dosimetric descriptions, especially accumulation of toxic metabolites in target tissues, nearly all studied DBPs have short half-lives. Some are bioactivated to toxic metabolites while others are detoxicated; some are reduced to two-carbon molecules that are transformed via the Krebs cycle to become incorporated in normal cellular constituents (ILSI, 1999; Lipscomb et al., 2009). Some, like the haloacetonitriles, are reactive to the point that their distribution from blood and tissues may be limited by protein binding. Accordingly, in this paper, metabolism and distribution data are discussed in the text and summarized in the last column of the summary tables when potentially relevant to an MOA. While MOA descriptions for systemic toxicity other than reproductive and developmental affects have been developed for some DBPs, MOA descriptions or even analysis of sites of insult for developmental toxicity have yet to be accomplished for the majority of the DBPs.

The purpose of this review and evaluation is to identify DBPs that have been shown to cause developmental effects, and to evaluate the potential MOA(s) and/or sites of toxic insult for different developmental effects. Four outcomes of concern were targeted based on human health effects identified in the epidemiologic studies of treated drinking water and DBPs: spontaneous abortion, cardiovascular defects, neural tube defects, and low birth weight/intrauterine growth retardation. We evaluated the literature using established criteria for causality, adequacy of developmental toxicity studies, and identification of potential MOA.

Methods

We performed an extensive literature search to identify DBPs that had been examined for developmental toxicity in humans or mammalian test species and to identify MOA data relevant to developmental toxicity for these DBPs. The starting point for compound selection included the following DBP classes, individual members of these classes, and other chemicals (name, synonyms and CASRN): trihalomethanes, haloacetic acids, haloacetonitriles, halopropanones, nitrosamines, chlorine dioxide, chlorate, chlorite, chloral hydrate, chloropicrin, bromate, formaldehyde, and MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone). Literature searches included DART, PubMed, and Toxic Substances Control Act Test Submissions as well as secondary sources such as the Integrated Risk Information System (IRIS), U.S. Environmental Protection Agency (EPA) reports, Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profiles, and literature reviews and analyses (e.g., Bove et al., 2002; Graves et al., 2001; Nieuwenhuijsen et al., 2000; Tardiff et al., 2006). Searches initially were conducted in December 2006 and January 2007. Based on the results of these searches, 24 chemicals were tentatively selected for MOA analysis: the four regulated trihalomethanes (bromodichloromethane, dibromochloromethane, bromoform, chloroform), nine haloacetic acids including the five regulated haloacetic acids (monochloro-, dichloro- and trichloroacetic acid; monobromo- and dibromoacetic acid), four haloacetonitriles, a halopropanone (1,1,3,3-tetrachloropropanone), chlorine dioxide, and some other individual DBPs (chlorite, chloral hydrate, dichloromethane, bromomethane) (Table 1). Update searching on these selected chemicals has continued through July 2008 (we have updated some references that were in preparation at this time but are now published or accepted for publication, i.e., Lipscomb et al., 2009; Narotsky et al., 2011). The selection criteria included positive *in vivo* data from epidemiologic or experimental animal studies and/or MOA data for developmental effects. We also considered the availability of dose–response data, whole embryo culture data, and membership in the chemical classes (trihalomethanes and haloacetic acids) discussed in the U.S. EPA's (2006) primary drinking water regulations, if there was evidence of developmental toxicity in other members of the class.

In evaluating the data for adverse developmental outcomes, we considered established criteria for evidence of causality (Hill, 1965; U.S. EPA, 2005) and guidelines for adequacy of study design and statistical analysis in developmental toxicity studies (OECD, Organisation for Economic Co-operation and Development, 2001; U.S. EPA, 1991, 1998; U.S. FDA, U.S. Food and Drug Administration, 2000). The potential MOAs for developmental effects were evaluated according to published frameworks (Faustman et al., 2006; IPCS, International Programme on Chemical Safety, 2005; Meek et al., 2003; Seed et al., 2005; Sonich-Mullin et al., 2001; U.S. EPA, 2005). The evaluation of MOA includes identification of the potential key events, the temporal association of key events with the outcome (i.e., key events precede outcome), dose–response concordance between key events and the outcome, requirement of key events for the outcome to be expressed, consistency across studies, specificity of the association, biological plausibility and coherence, and relevance to humans.

Results and discussion

Results for the four outcomes of concern generally are discussed in the following order: the epidemiology data are reviewed first, the MOA data for that outcome (if available) are reviewed second and then supporting data are reviewed third.

Spontaneous abortion/full-litter resorption

A concern for spontaneous abortion arose because epidemiologic studies of drinking water DBPs associated this outcome with exposure to the trihalomethanes, and particularly exposure to bromodichloromethane (one of the four trihalomethanes regulated in drinking

Table 1
Selected and non-selected disinfection byproducts.

Selected	Not selected
Trihalomethanes[a]	
Bromodichloromethane	
Dibromochloromethane	
Bromoform	
Chloroform	
Haloacetic Acids[a]	
Monobromoacetic acid	
Monochloroacetic acid	
Dibromoacetic acid	
Dichloroacetic acid	
Trichloroacetic acid	
Tribromoacetic acid	
Bromochloroacetic acid	
Dibromochloroacetic acid	
Bromodichloroacetic acid	
Haloacetonitriles	
Dichloroacetonitrile	Bromoacetonitrile
Trichloroacetonitrile	
Bromochloroacetonitrile	
Dibromoacetonitrile	
Chloroacetonitrile	
Halopropanones	
1,1,3,3-Tetrachloro-propanone	1,1-Dichloropropanone
	1,3-Dichloropropanone
	1-Bromo-1-chloro-propanone
	1,1-Dibromopropanone
	1,1,1-Trichloropropanone
	1,1-Dichloro-2-bromo-propanone
	1,1,1-Trichloro-2-bromo-propanone
	1,1,1,3-Tetrachloro-propanone
	1,1-Dibromo-3,3-dichloro-propanone
	1,3-Dibromo-1,3-dichloro-propanone
	1,1,3-Tribromo-3-chloro-propanone
	1,1,3,3-Tetrabromo-propanone
	1,1,1,3,3-Pentachloro-propanone
	Hexachloropropanone
Other chemicals	
Chlorine dioxide	Chloropicrin (trichloronitromethane)
Chlorite	Dibromomethane
Chloral hydrate	MX [3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone]
Dichloromethane	
Bromomethane	

[a] The trihalomethanes and haloacetic acids that are regulated in drinking water (U.S. EPA, 2006) are shown in boldface type.

water). Savitz et al. (1995), in a case–control study in North Carolina, reported an increased risk (odds ratio [OR] = 2.8, 95% confidence interval [CI] = 1.1–2.7) in the highest sextile of total trihalomethane concentration in drinking water, but the next lower sextile had an anomalously low risk. Swan et al. (1998) examined risk of spontaneous abortion among women enrolled at ≤13 weeks of gestation in a prospective cohort study in Northern California. The authors reported an association (OR = 2.2; 95% CI = 1.2–3.9) between spontaneous abortion and consumption of six or more glasses of cold treated tap water per day compared with those who drank no cold tap water. Following further analysis of the data to include bottled water consumption, they reported that the OR for spontaneous abortion in women who consumed at least six glasses of cold, treated tap water per day and no bottled water was 4.6 (95% CI = 2.0–10.6) compared with those who drank at least six glasses of bottled water and no cold tap water. This effect, however, was observed in only one of the three geographic regions studied in California.

Waller et al. (1998) further evaluated the exposures in this study population with regard to consumption of total trihalomethanes and individual trihalomethanes in tap water. For all three regions combined, the consumption of at least five glasses of cold tap water containing at least 75 μg/L of total trihalomethanes was associated with spontaneous abortion (OR = 1.8; 95% CI = 1.1–3.0). Bromodichloromethane was the only trihalomethane associated with spontaneous abortion in each region separately and all three regions combined. The OR among subjects from all study regions combined for consumption of at least five glasses of cold tap water containing at least 18 μg/L of bromodichloromethane was 2.0 (95% CI = 1.2–3.5) and after adjustment for the other trihalomethanes was 3.0 (95% CI = 1.4–6.6).

Savitz et al. (2005, 2006) conducted an extensive prospective study of DBPs (including total and individual trihalomethanes) and pregnancy outcome in women planning pregnancy or who had completed less than 12 weeks of gestation in three southern cities in the U.S. Results for spontaneous abortion, analyzed so as to be consistent with the analysis of Waller et al. (1998), did not show an increased risk with daily consumption of at least five glasses of cold tap water containing ≥75 μg/L of total trihalomethanes (OR = 1.1; 95% CI = 0.7–1.7). However, Savitz et al. (2005) did observe an increased risk of spontaneous abortion when expectant mothers consumed at least five glasses of cold tap water containing ≥18 μg/L per day of bromodichloromethane (OR = 1.58; 95% CI = 1.02–2.47) (not adjusted for the other trihalomethanes). Using the upper quartile of bromodichloromethane concentration from their own study and consumption of at least five glasses of cold tap water, the result was virtually the same (OR = 1.6, 95% CI = 1.0–2.4) (Savitz et al., 2005, 2006). Despite largely null results from the Savitz et al. (2005,2006) study (further discussed in the following paragraph), the results for bromodichlormethane provide some evidence of an association with increased incidence of spontaneous abortion in both the Waller et al. (1998) and the Savitz et al. (2005,2006) studies.

Savitz et al. (2005, 2006) also investigated potential associations between other exposure indices for total trihalomethane or bromodichloromethane and spontaneous abortion; results were largely null. The other indices included concentration in the drinking water, ingested amount from drinking water, showering/bathing exposure, and total integrated exposure. Savitz et al. (2005, 2006) reported an association between the highest quintile for ingested amount of total organic halide and spontaneous abortion (OR = 1.5, 95% CI = 1.0–2.2). As summarized in Table 2, the previously cited and a few other epidemiologic studies largely reported null results for an association between spontaneous abortion and exposure to most individual trihalomethanes or to total haloacetic acids. Thus, although the epidemiologic results raise a concern for spontaneous abortion, the weight of evidence for this outcome as a result of DBP exposure is weak and does not include information regarding a possible MOA.

Studies in experimental animals and in human tissue *in vitro* provide insights into a potential MOA for this effect. Narotsky and Laffan (2004) have suggested that full-litter resorption (pregnancy loss) in F344 rats could serve as a model for spontaneous abortion in humans. A number of individual DBPs cause full-litter resorption in rats, as indicated in Table 2. Although some of the studies summarized in Table 2 show this effect in the presence of marked maternal and embryo/fetal toxicity, studies of the trihalomethanes in sensitive strains of rats (F344 and Wistar) have demonstrated this effect in the absence of such toxicities and are discussed below as more relevant to concerns for human health.

Bromodichloromethane has been the DBP most extensively examined for pregnancy loss and its MOA for full-litter resorption in animals is reasonably well characterized. Evidence for the MOA of bromodichloromethane-induced pregnancy loss is as follows: gavage administration of bromodichloromethane to F344 rats during a portion of the luteinizing hormone (LH)-dependent period of pregnancy, which occurs during gestation days (GD) 7–10 resulted in full-litter resorption (Bielmeier et al., 2001, 2004; Narotsky et al., 1992, 1997a). The incidences of resorption were dose-related. A single

Table 2
Summary of data on spontaneous abortion and full-litter resorption for selected disinfection byproducts (DBPs).[a]

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	MOA data
Chlorinated DW	Human, DW, epi (Swan et al., 1998)	–	–
Total trihalomethanes (THMs)	Human, DW, epi (Savitz et al., 1995) Human, DW, epi (Waller et al., 1998) F344 rat, G-aqueous emulphor (Alkamuls) (Narotsky et al., 2011)	Human, DW, epi (Savitz et al., 2005, 2006) Human, DW, epi, time to pregnancy as indicator of possible early pregnancy loss (MacLehose et al., 2008)—also negative for Brominated THM	–
Bromodichloromethane (BDCM)	Human, DW, epi (Waller et al., 1998) Human, DW, epi (Savitz et al., 2005, 2006) F344 rat, G-aqueous emulphor (Bielmeier et al., 2001, 2004) F344 rat, G-aqueous emulphor-slight but not significant increase (Narotsky et al., 1997a) F344 rat, G-corn oil (Narotsky et al., 1997a) F344 rat, G-corn oil (Narotsky et al., 1992)	Human, DW, epi, time to pregnancy as indicator of possible early pregnancy loss (MacLehose et al., 2008) SD rat G-aqueous emulphor (Bielmeier et al., 2001, 2002) SD rat, DW (Christian et al., 2001a) SD rat, rabbit, DW, fertility not affected (Christian et al., 2002a) SD rat, DW (NTP, National Toxicology Program, 1998a)	In the rat, BDCM decreases serum LH, resulting in decreased serum progesterone; F344 has less constitutive LH than SD, LH in rats is analogous to hCG in humans; hCG or progesterone prevented full-litter resorption in rats treated with BDCM (Narotsky and Laffan, 2004; Bielmeier et al., 2001, 2002, 2004, 2007) BDCM inhibited hCG-stimulated progesterone secretion by rat corpora lutea *in vitro* (Bielmeier et al., 2007) BDCM inhibited differentiation of cultured human placental trophoblast cells and decreased hCG secretion and intracellular hCG (Chen et al., 2003, 2004) Rabbit, DW, limited data suggest BDCM reaches placenta and fetus (Christian et al., 2001b) Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999)
Dibromochloromethane	–	Human, DW, epi (Savitz et al., 2005, 2006) Human, DW, epi (Waller et al., 1998) SD rat, DW (NTP, National Toxicology Program, 1996)	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999)
Bromoform	F344 rat, G-corn oil, developmental toxicity screen (Narotsky et al., 1992, 1993	Human, DW, epi (Waller et al., 1998) Human, DW, epi (Savitz et al., 2005, 2006) SD rat, G (Ruddick et al., 1983)	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999)
Chloroform	Wistar Rat, Inhal. (Baeder and Hoffmann, 1988) Also suggestive data: SD rat, Inhal., low pregnancy rate(3/20, including one fully resorbed; uteri not stained, so uncertain if full-litter resorption occurred in "nonpregnant" rats), severe maternal weight loss, fetotoxicity in the 2 surviving litters (Schwetz et al., 1974) CF-1 mouse, Inhal., low pregnancy rates, not accounted for by stain-detected resorptions: (possible very early resorption or interference with implantation), maternal liver toxicity (Murray et al., 1979)	Human, DW, epi (Waller et al., 1998) Human, DW, epi (Savitz et al., 2005, 2006) SD rat, G-corn oil, fertility not affected (Thompson et al., 1974)	At end of single 4-hour inhalation exposure on GD 17, fetal to maternal chloroform concentration ratio was 0.316; fetal concentration was not related to position in uterus (Withey and Karpinski, 1985) Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999; U.S. EPA, 2001)
Total trihalomethanes and total haloacetic acid mixture (50:50%, molar basis)	Suggestive data: F344 rat, G-aqueous emulphor (Alkamuls) (Narotsky et al., 2011)	–	Pregnancy loss seen at lower doses of THMs and HAAs than when either was tested separately, but data were not adequate to determine mode of joint toxic action (Narotsky et al., 2011)
Total haloacetic acids (HAAs)	Suggestive data: F344 rat, G-aqueous emulphor (Alkamuls) (Narotsky et al., 2011)	Human, DW, epi (Savitz et al., 2005, 2006) Human, DW, epi, time to pregnancy as indicator of possible early pregnancy loss (MacLehose et al., 2008) – also negative for brominated HAAs	–
Monobromoacetic acid	–	LE rat, G-water (Randall et al., 1991)	–
Monochloroacetic acid	–	LE rat-G-water (Smith et al., 1990) SD rat-DW (Johnson et al., 1998)	–
Dibromoacetic acid (DBA)	–	SD rat–G-water, also serum LH not affected but assay insensitive, serum progesterone not affected, serum estradiol increased (Cummings and Hedge, 1998) SD rat, DW, fertility not affected (Christian et al., 2002b)	SD rat, DW, parent compound reaches placenta, amniotic fluid and fetus (Christian et al., 2001b); was dose additive with BCA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Inhibited neural crest cell migration in cultured explants from rats and mice (Andrews et al., 2001; Hunter et al., 2001) Did not induce apoptosis in mouse embryo culture (Ward et al., 2000) Is more potent than its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dichloroacetic acid (DCA)	–	LE rat–G-water (Smith et al., 1992a) SD rat, G-water (Fisher et al., 2001)	LE rat, G, antagonistic interaction for resorption with TCA (Smith et al., 1991, 1992b) Was dose additive with DBA and/or BCA on development of rat embryo in culture (Andrews et al., 2004)

(continued on next page)

Table 2 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	MOA data
Dichloroacetic acid (DCA)			LE rat, G, at 48 hours after dosing of dams with 1- and 2-(^{14}C)-labeled DCA, ^{14}C levels were higher in embryos than in maternal plasma, and were present mainly as C2 (the dichloromethyl C), indicating a metabolite (Roth et al., 1991) DCA has multiple effects in intermediary metabolism, particularly from inhibition of the kinase that inactivates mitochondrial pyruvate dehydrogenase (Crabb et al., 1981; Smith et al., 1992a; Stacpoole, 1989) DCA induces apoptosis in mouse embryos (Ward et al., 2000) DCA did not inhibit neural crest cell migration in culture (Andrews et al., 2001; Hunter et al., 2001) Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Bromochloroacetic acid (BCA)	–	SD rat, DW (but decrease in implants and live fetuses per litter at maternotoxic dose) (NTP, National Toxicology Program, 1998b)	Was dose additive with DBA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Inhibited neural crest cell migration in cultured explants from rats and mice (Andrews et al., 2001; Hunter et al., 2001) Induces apoptosis in mouse embryos (Ward et al., 2000) Metabolites of BCA were much less potent than BCA in mouse embryo culture (Hunter and Rogers, 1999) BCA caused differential gene expression in mouse embryo culture (Karoly et al., 2004)
Tribromoacetic acid	–	SD rat, DW (NTP, National Toxicology Program, 1998c)	–
Trichloroacetic acid (TCA)	LE rat, G-water, also maternal toxicity and fetal toxicity and malformations (Smith et al., 1989a)	SD rat, G-water (Fisher et al., 2001)	LE rat, G, antagonistic interaction with DCA on resorption (Smith et al., 1991, 1992b)
Dibromochloroacetic acid	–	SD rat-DW (NTP, National Toxicology Program, 2000)	–
Bromodichloroacetic acid	–	–	–
Dichloroacetonitrile	LE rat, G-tricap, screen one dose, with replicate, GD7-15, also maternal and fetal toxicity (Smith et al., 1987) LE rat, G-tricap, also maternal mortality (non-significant), fetal toxicity and malformations (Smith et al., 1989b) Suggestive data: LE rat, G-tricap, screen one dose, GD7-15, 45% of sperm-positive rats not pregnant (possibly due to late preimplantation loss or very early resorptions) in 1 of 2 replicate dose groups (Smith et al., 1987)	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988)
Trichloroacetonitrile (TCAN)	LE rat, G-tricap, also with maternal and fetal toxicity and malformations at higher doses (Smith et al., 1988) LE rat, G-corn oil (Christ et al., 1996) LE rat, G-tricap, screen one dose, GD7-15 (Smith et al., 1987) Suggestive data: LE rat, G-tricap, replicate screen one dose, GD7-15, 45% of sperm positive rats not pregnant (possibly due to late preimplantation loss or very early resorption) (Smith et al., 1987)	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988) LE rat, G, accumulation of radioactivity in maternal liver and in fetus from two to three daily doses of ^{14}C-TCAN was higher for the trichloromethyl carbon (C2) than the cyano carbon. Accumulation in embryos was higher with tricaprylin vehicle than corn oil vehicle (Gordon et al., 1991)
Bromochloro-acetonitrile	LE rat, G-tricap, also maternal mortality and fetal toxicity and malformations (Christ et al., 1995)	LE rat, G-tricap, screen one dose, GD7-15 (Smith et al., 1987)	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988)
Dibromoacetonitrile	–	LE rat, G-tricap, screen one dose, GD7-15 (Smith et al., 1987) SD rat, DW (NTP, National Toxicology Program, 1997)	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988)
Chloroacetonitrile	–	LE rat, G-tricap, screen one dose, GD7-15 (Smith et al., 1987)	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988)
1,1,3,3,-Tetrachloropropanone	–	CF-1 mouse, rabbit, G-corn oil, fertility not affected (John et al., 1982)	–
Chlorine dioxide	–	Rat, SD, DW, but small group sizes (Suh et al., 1983) Rat, LE, but highest dose not MTD (Carlton et al., 1991)	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b) Decreased the levels of circulating thyroxine in monkeys and rats, and in developmental study

Table 2 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	MOA data
Chlorine dioxide			in rats, possibility through oxidation of dietary iodide in the GI tract, resulting in reactive species that iodinate organic matter to form potentially thyroid inhibitory compounds (Bercz et al., 1986; Orme et al., 1985)
Chlorite	SD rat, DW, GD7-14, also maternal toxicity (body weight loss and hemolysis) and greatly decreased water and food intake; only 5 rats/group (Couri et al., 1982) Suggestive data: A/J mouse, DW, fertility decreased (Moore et al., 1980)	SD rat, DW, fertility not affected (Gill et al., 2000) LE Rat, DW, fertility not affected (Carlton and Smith, 1985, Carlton et al., 1987) Rabbit, DW, fertility not affected (Harrington et al., 1995)	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b)
Chloral hydrate	–	SD rat, DW, but MTD not achieved (Johnson et al., 1998)	The metabolite trichloroethanol has sedative effects. Other metabolites are trichloroacetic acid and possibly small amounts of dichloroacetic acid (U.S. EPA, 2000a).
Dichloromethane	–	F344 Rat, G-corn oil (Narotsky and Kavlock, 1995) F344 rat, Inhal., fertility unaffected but highest exposure not MTD (Nitschke et al., 1988) SD rat, G-water, MTD not achieved, small number animals/group (IRDC, International Research and Development Corporation, 1976)	Generally, toxicity may be due to P450 metabolism to a reactive intermediate and carbon monoxide, and to direct conjugation of dichloromethane with GSH to form a bioactive product. Also, the parent compound may enter cell membranes and thereby interfere with signal transmission (ATSDR, 2000).
Bromomethane	RIV-TOX rat, also severe maternal toxicity including perforated stomach, peritonitis, and mortality (Peters et al., 1981)	Rabbit, Inhal. (Breslin et al., 1990) Wistar rat, rabbit, Inhal., fertility not affected but MTD not achieved (Sikov et al., 1981)	Generally, toxicity may be due to the alkylating (methylating) properties of bromomethane (ATSDR, 1992; Vogel and Nivard, 1994).

– = Data unavailable, BCA = bromochloroacetic acid, BDCM = bromodichloromethane, hCG = human chorionic gonadotropin, DCA = dichloroacetic acid, DW = drinking water, epi = epidemiological study, G = gavage, GD = gestation day, Inhal. = inhalation, LE = Long Evans, LH = luteinizing hormone, MTD = maximum tolerated dose, NS = not statistically significant, SD = Sprague–Dawley, TCA = trichloroacetic acid, TCAN = trichloroacetonitrile, tricap = tricaprylin.

[a] Animals were exposed on at least days 6–15 of gestation unless noted otherwise (where GD0 = sperm-positive or vaginal plug; GDs or embryo age adjusted to this basis). Tested at doses/exposures that included an MTD unless otherwise noted.

dose on GD 8 or 9 or repeated daily dosing on GD 6–10 or 6–15 caused the effect, whereas daily dosing on GD 11–15 did not, indicating that the effect may not occur outside a specific temporal window. In surviving litters, no changes in gestation length, no increases in malformations or resorptions, and no effects on fetal or pup weights were observed. Thus, the effect was an all-or-nothing phenomenon.

During GD 7–10 in the rat, the corpora lutea, which secrete progesterone, are dependent on LH, secreted by the pituitary, to stimulate progesterone release. The key events in the rat that led to full-litter resorption from bromodichloromethane exposure were (1) exposures to bromodichloromethane during the LH-dependent period, which resulted in (2) a decrease in serum LH, which resulted in (3) a decrease in progesterone. Bielmeier et al. (2001, 2004) observed these key events in F344 dams whose litters were fully resorbed in response to bromodichloromethane. Full-litter resorption did not occur in Sprague–Dawley rats tested at the same dose levels that caused full-litter resorption in F344 rats in the same study. Signs of maternal toxicity, including decreased body weight gain, occurred in both strains/stocks of rat in these studies, indicating that the maximum tolerated dose (MTD) was achieved.

In an intra-study comparison of the F344 and the Sprague–Dawley rats, oral bromodichloromethane decreased the serum LH levels in Sprague–Dawley rats as well as in F344 rats (Bielmeier et al., 2002). The Sprague–Dawley rats, however, had much higher constitutive levels of LH, such that even when decreased in response to bromodichloromethane exposure, their LH values were higher than in control F344 rats. Following oral bromodichloromethane exposure, the F344 rats experienced decreased serum progesterone; in contrast, the Sprague–Dawley rats did not. The comparisons between these strains/stocks indicate that a decrease in LH beyond a critical level appears to be a necessary key step resulting in decreased serum progesterone and that the decreased serum progesterone is a necessary key step for the occurrence of full-litter resorption in response to bromodichloromethane treatment. This difference across strains/stocks may explain why full-litter resorption was not seen in studies of bromodichloromethane in Sprague–Dawley rats (summarized in Table 2), including in the drinking water study by Christian et al. (2001a). *In vitro*, bromodichloromethane inhibited human chorionic gonadotropin (hCG)-stimulated progesterone secretion by rat corpora lutea, suggesting that bromodichloromethane may also affect corpora lutea responsiveness (Bielmeier et al., 2007).

Sampling for serum hormone analysis at 2- to 4-hour intervals after dosing of F344 rats with bromodichloromethane on GD 9 revealed that a decrease in serum LH occurred prior to the decrease in progesterone (Bielmeier et al., 2004), establishing the temporal sequence. The administration of either progesterone or the LH agonist, hCG, prevented full-litter resorption. These results provide further evidence that the key steps outlined in Fig. 1 are necessary for the effect. The results further suggest that bromodichloromethane may have altered LH secretion and that the consequent decreased progesterone was a key step leading to full-litter resorption.

In the pregnant rat, the pituitary gland secretes LH in response to gonadotropin-releasing-hormone (GnRH) stimulation from the hypothalamus. In the pregnant human, the placental trophoblast both secretes GnRH and expresses a receptor for this hormone. GnRH secreted by the cytotrophoblast stimulates the placental syncytiotrophoblast to secrete hCG, which in turn stimulates corpora luteal secretion of progesterone. Thus, both LH secretion in the rat and hCG secretion in the human are regulated by GnRH (as reviewed by Bielmeier et al., 2004; Chen et al., 2003, 2004). Fig. 2 provides a comparison of the rat and human hormonal maintenance of the

corpora lutea, essential for progesterone secretion and ultimately embryo survival.

Chen et al. (2003) conducted *in vitro* studies with cultures of human placental syncytiotrophoblasts and found that bromodichloromethane, at concentrations higher than found in human blood, decreased the secretion of hCG from these differentiated cells without affecting morphology or viability of the cells. Subsequently, Chen et al. (2004) reported that incubation of bromodichloromethane with undifferentiated human placental trophoblast cells inhibited hCG secretion at a bromodichloromethane concentration of 0.5 nM (0.082 μg/L), which is within the range found in human blood (0.002–0.093 μg/L, Miles et al., 2002). At higher concentrations, bromodichloromethane decreased the intracellular levels of hCG and inhibited differentiation of the trophoblasts to syncytiotrophoblast colonies. Thus, although the entire sequence of key events is not yet completely known, existing data suggest that the MOA of bromodichloromethane in the F344 rat may be qualitatively similar to that in the human and may be operative at blood levels relevant to human exposure (thus quantitatively plausible). Therefore, the developmental effects observed in the F344 rat in response to bromodichloromethane are considered relevant to humans. In addition, epidemiologic studies, described previously, report an association between exposure to the total trihalomethanes and to bromodichloroemethane in drinking water and spontaneous abortion in humans (Savitz et al., 2005, 2006; Waller et al., 1998).

Bromoform, chloroform, and a trihalomethane mixture also induced full-litter resorption in rats. Bromoform caused full-litter resorption in F344 rats treated daily by gavage on GD 6–15 (Narotsky et al., 1992, 1993). Incidences of full-litter resorption were dose-related (M. Narotsky, unpublished results). Resorption rates were not affected in the surviving litters (Narotsky et al., 1993); i.e., this was an all-or-nothing effect, similar to that seen with bromodichloromethane. Chloroform caused full-litter resorption in Wistar rats exposed by inhalation for 7 hours/day on GD 6–15 (normalized to sperm-positive = GD 0) to concentrations of chloroform that affected maternal body weights (Baeder and Hoffmann, 1988). A dose–response relationship was observed, and no effects on resorption or incidences of malformations were seen in surviving litters. These results in Wistar rats exposed to chloroform by inhalation are similar to those obtained with F344 rats treated with bromodichloromethane or bromoform by gavage as described above. Additional data suggestive of an effect of chloroform on pregnant rats (see Murray et al., 1979; Schwetz et al., 1974) are listed in Table 2. A trihalomethane mixture, using proportions similar to those found in some chlorinated tap waters, caused pregnancy loss in a dose-related manner in F344 rats following gavage administration on GD 6–20 (Narotsky et al., 2011).

While the remaining trihalomethane of interest, dibromochloromethane, did not cause developmental effects in Sprague–Dawley rats (NTP, National Toxicology Program, 1996), it has not been tested for developmental toxicity in the known sensitive strain of rat, F344, or for effects on LH or progesterone levels. Therefore, it is unclear whether dibromochloromethane would cause full-litter resorption, similar to its structural analogs. However, the non-DBP tetrahalomethane carbon tetrachloride, which is structurally related to the trihalomethanes, also causes full-litter resorption in the F344 rat (Narotsky and Kavlock, 1995; Narotsky et al., 1997a,b). Conversely, dichloromethane, a dihalomethane, did not cause full-litter resorption when tested similarly by gavage in F344 rats (Narotsky and Kavlock, 1995).

One of the nine selected haloacetic acids (eight were tested for this effect) and three of the five selected haloacetonitriles caused full-litter resorption in Long–Evans rats when administered daily by gavage on GD 6–15 or 7–15 (Table 2), but the pattern of effects was different than that seen with bromodichloromethane in the F344 rat. With bromodichloromethane, the effect appeared to be an all-or-nothing phenomenon: litters were either fully resorbed or survived with no effects (i.e., no increase in resorptions per surviving litter and no fetal effects) (Bielmeier et al., 2001, 2004; Narotsky et al., 1992, 1997a). When the haloacetic acids and haloacetonitriles (trichloroacetic acid, dichloroacetonitrile, trichloroacetonitrile, bromochloroacetonitrile) were tested in Long–Evans rats, they caused full-litter resorption with a dose-related progression of increased (per litter) resorption, malformations, and fetal weight depression in surviving litters (Christ et al., 1995, 1996; Smith et al., 1987, 1988, 1989a,b). These findings suggest that the partial and full-litter resorptions from these chemicals may be due to direct effects on the embryo or fetus rather than disruption of hormonal maintenance of pregnancy in

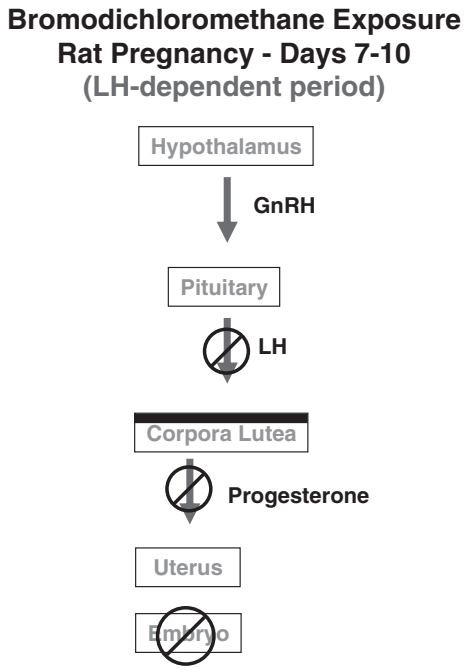

Fig. 1. Key steps in bromodichloromethane MOA for full-litter resorption.

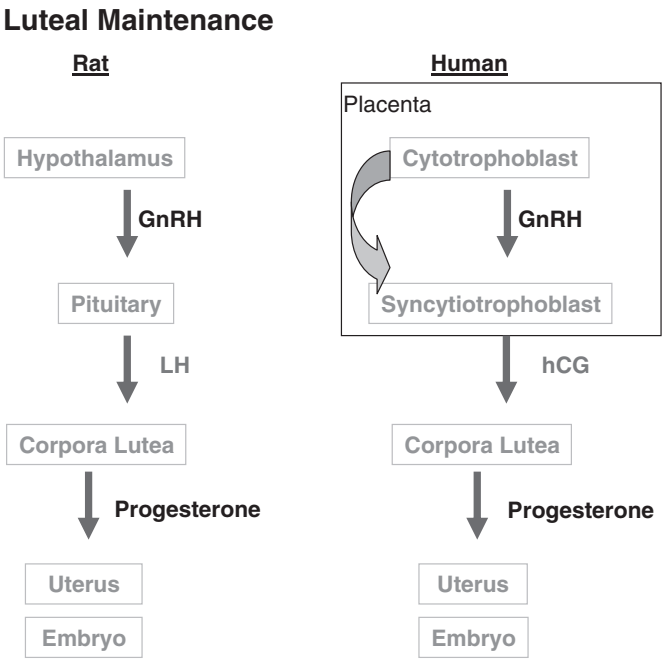

Fig. 2. Comparison of rat and human hormonal maintenance of corpora lutea.

Table 3
Summary of data on cardiovascular malformations for selected disinfection byproducts (DBPs).[a]

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
Chlorinated DW	Human, DW, epi (Hwang et al., 2002)	Human, DW, epi (Cedergren et al., 2002) Human, DW, epi (Kallen and Robert, 2000) Human, DW, epi (Magnus et al., 1999)	–	–	–
Total trihalomethanes	Human, DW, epi (Chisholm et al., 2008) Human, DW, epi (Hwang et al., 2008) Human, DW, epi (Nieuwenhuijsen et al., 2008) Human, DW, epi (Cedergren et al., 2002) Suggestive data: Human, DW also contaminated with solvents, epi (Bove et al., 1995)	Human, DW, epi (Shaw et al., 2003) Human, DW, epi (Dodds et al., 1999)	–	–	–
Bromodichloromethane (BDCM)	–	Human, DW, epi (Dodds and King, 2001) Human, DW, epi (Shaw et al., 2003) SD rat, G-corn oil (Ruddick et al., 1983) SD rat, rabbit, DW (Christian et al., 2001a)	–	–	Rabbit, DW, limited data suggest BDCM reaches the placenta and fetus (Christian et al., 2001b). Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999).
Dibromochloromethane	–	Human, DW, epi (Shaw et al., 2003) SD rat, G-corn oil (Ruddick et al., 1983) ICR Swiss mouse, DW (Borzelleca and Carchman, 1982)	–	–	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999).
Bromoform	Human, DW, epi (Nieuwenhuijsen et al., 2008)	Human, DW, epi (Shaw et al., 2003) SD rat, G-corn oil (Ruddick et al., 1983)	–	–	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999).
Chloroform	–	Human, DW, epi (Dodds and King, 2001) SD rat, G-corn oil (Ruddick et al., 1983) SD rat, rabbit, G-corn oil (Thompson et al., 1974) ICR Swiss mouse, DW, but only 6 litters/group (Borzelleca and Carchman, 1982) Wistar rat, Inhal. (Baeder and Hoffmann, 1988, 1991a,b) CF-1 mice, Inhal. (Murray et al., 1979) SD rats, Inhal. (Schwetz et al., 1974)	–	SD rat, day 10.5 (12–15 somites), diffuse cell death (except in heart), including neuroepithelium of neural tube, eye defects, but effects may be due to loss of yolk sac vascularization (Brown-Woodman et al., 1998).	At end of single 4-hour inhalation exposure on GD 17, fetal to maternal chloroform concentration ratio was 0.316; fetal concentration was not related to position in uterus (Withey and Karpinski, 1985). Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999; U.S. EPA, 2001).

(continued on next page)

Table 3 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
Monobromoacetic acid	Suggestive data: (not litter-based): LE rat, G-water, aorta-ventricle communication defect, also eye defects (micro- and anophthalmia) (Randall et al., 1991)	–	CD-1 mouse, day 8 (3–6 somites), also neural tube,[b] pharyngeal arch and eye defects (Hunter et al., 1996)	–	–
Monochloroacetic acid	Suggestive data (not litter-based): LE rat, G-water, mainly levocardia (Smith et al., 1990)	SD rat, DW, MTD not achieved (Johnson et al., 1998)	CD-1 mouse, day 8 (3–6 somites), also neural tube[b] and pharyngeal arch defects (Hunter et al., 1996)	–	–
Dibromoacetic acid (DBA)	–	–	CD-1 mouse, day 8 (3–6 somites), also neural tube,[b] pharyngeal arch and eye defects (Hunter et al., 1996, 2006b) SD rat, day 9.5 (0–1 somites), also hypoplasia of prosencephalon and visceral arches and eye defects (Andrews et al., 2004)	–	SD rat, DW, parent compound reaches placenta, amniotic fluid and fetus (Christian et al., 2001b) Was dose additive with BCA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Inhibited neural crest cell migration in cultured explants from rat and mouse embryos (Andrews et al., 2001; Hunter et al., 2001) Did not induce apoptosis in mouse embryo culture (Ward et al., 2000) Is more potent than its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dichloroacetic acid (DCA)	LE rat, G-water, primarily defect between aorta and ventricle, also levocardia; eye defects without clear dose–response (Smith et al., 1992a) LE rat, G-water, higher doses on GD9-11 or 12–15, mainly interventricular septal defects (Epstein et al., 1992)	SD rat, G-water (Fisher et al., 2001)	CD-1 mouse, day 8 (3–6 somites), also neural tube,[b] pharyngeal arch and eye defects (Hunter et al., 1996, 2006b) Suggestive data: SD rat, day 9.5 (0-1 somites) (malformations stated to be similar to those from dibromoacetic acid in same study) (Andrews et al., 2004)	SD rat, day 10 (4-7 somites), hypoplasia of prosencephalon, eye defects, decreased yolk sac diameter; neural tube defects, delayed yolk sac circulation, and poor survival at higher concentration (Saillenfait et al., 1995)	LE rat, G, antagonistic interaction for cardiac malformations with TCA (Smith et al., 1991, 1992b) Was dose additive with DBA and/or BCA on development of rat embryo in culture (Andrews et al., 2004) LE rat, G, at 48 hours after dosing of dams with 1- and 2-(^{14}C)-labeled DCA, ^{14}C levels were higher in embryos than in maternal plasma, and were present mainly as C2 (the dichloromethyl C), indicating a metabolite (Roth et al., 1991). DCA has multiple effects in intermediary metabolism, particularly from inhibition of the kinase that inactivates mitochondrial pyruvate dehydrogenase (Crabb et al., 1981; Smith et al., 1992a; Stacpoole, 1989). DCA-induced malformations in cultured mouse embryos are not primarily due to reactive oxygen species (Hunter et al., 2006c). DCA induces apoptosis in mouse embryos, including in the heart (Ward et al., 2000). DCA did not inhibit neural crest cell migration in culture (Andrews et al., 2001; Hunter et al., 2001). Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)

					DCA induction of ventricular septal defects was increased by low folate maternal diets in the SD rat (Rogers et al., 2005).
Bromochloroacetic acid (BCA)	–	SD rat, DW (NTP, National Toxicology Program, 1998b)	CD-1 mouse, day 8 (3–6 somites), also pharyngeal arch and eye defects; neural tube defects but NS (Hunter et al., 2006a,b) Suggestive data: SD rat, day 9.5 (0-1 somites) (malformations stated to be similar to those from dibromoacetic acid in same study) (Andrews et al., 2004)	–	Was dose additive with DBA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Inhibited neural crest cell migration in cultured explants from rat and mouse embryos (Andrews et al., 2001; Hunter et al., 2001) Induces apoptosis in mouse embryos (Ward et al., 2000) Metabolites of BCA were much less potent than BCA in mouse embryo culture (Hunter and Rogers, 1999). BCA caused changes in gene expression in mouse embryo culture (Karoly et al., 2004), including genes networked with Akt (protein kinase B) (Hunter et al., 2005). An antagonist of an upstream regulator of Akt caused similar cardiac defects as did BCA in mouse embryo culture (Hunter et al., 2005).
Tribromoacetic acid	–	SD rat–DW, MTD not achieved (NTP, National Toxicology Program, 1998c)	CD-1 mouse, day 8 (3–6 somites), also neural tube,[b] pharyngeal arch and eye defects (Hunter et al., 1996)	–	–
Trichloroacetic acid (TCA)	SD rat, DW, ventricular and atrial septal defects (Johnson et al., 1998) LE rat, G-water, primarily levocardia, also IV septal (Smith et al., 1989a)	SD rat, G-water (Fisher et al., 2001)	CD-1 mouse, day 8 (3–6 somites), also neural tube,[b] pharyngeal arch and eye defects (Hunter et al., 1996)	SD rat, day 10 (4-7 somites), hypoplasia of prosencephalon, eye defects, decreased yolk sac diameter (Saillenfait et al., 1995)	LE rat, G, antagonistic interaction with DCA on resorption (Smith et al., 1991, 1992b) Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dibromochloroacetic acid	–	SD rat, DW, MTD not achieved (NTP, National Toxicology Program, 2000)	CD-1 mouse, day 8 (3–6 somites), yolk sac defects at same concentration as heart defects; also pharyngeal arch, eye; and neural tube defects (Hunter et al., 2006a)	–	–
Bromodichloroacetic acid	–	–	CD-1 mouse, day 8 (3–6 somites), yolk sac defects at same concentration as heart defects; also pharyngeal arch and eye defects; neural tube defects but NS (Hunter et al., 2006a)	–	–
Dichloroacetonitrile	LE Rat, G-tricap, primarily levocardia, also interventricular septal defects, also eye defects (Smith et al., 1989b)	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988)
Trichloroacetonitrile (TCAN)	LE rat, G-tricap, mainly levocardia, interventricular septal defect and common carotid trunk (Smith et al., 1988) LE rat, G-tricap, 1 dose, mainly defect between ascending aorta and right ventricle, interventricular septal defect, right-sided and ringed aortic arch (Christ et al., 1996)	LE rat, G-corn oil, increased but not significant, primarily levocardia, also eye defects (Christ et al., 1996)	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988). LE rat, G, accumulation of radioactivity in maternal liver and in fetus from two to three daily doses of ^{14}C-TCAN was higher for the trichloromethyl carbon (C2) than the cyano carbon. Accumulation in embryos was higher with tricaprylin vehicle than corn oil vehicle (Gordon et al., 1991).
Bromochloro-acetonitrile	LE rat, G-tricap ascending aorta-right ventricle defect, also interventricular septal defect and levocardia (Christ et al., 1995)	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).

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Table 3 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
Dibromoacetonitrile	–	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
Chloroacetonitrile	–	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
1,1,3,3,-Tetrachloropropanone	–	CF-1 mouse, rabbit, G-corn oil (John et al., 1982)	–	–	–
Chlorine dioxide-disinfected DW	Suggestive data: Human, DW, epi (Cedergren et al., 2002)	Human, DW, epi (Kallen and Robert, 2000)	–	–	–
Chlorine dioxide		Rat, DW, but only 6–8 dams/dose (Suh et al., 1983)	–	–	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b). Decreased the levels of circulating thyroxine in monkeys and rats, and in developmental study in rats, possibly though oxidation of dietary iodide in the GI tract, resulting in reactive species that iodinate organic matter to form potentially thyroid inhibitory compounds (Bercz et al., 1986; Orme et al., 1985).
Chlorite	–	SD rat, DW, but only 6–8 dams/dose, MTD not achieved (Suh et al., 1983) Rabbit, DW, MTD may not have been achieved (Harrington et al., 1995). SD rat, DW, also maternal toxicity (body weight loss and hemolysis) and greatly decreased water and food intake (Couri et al., 1982)	–	–	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b).
Chloral hydrate	–	SD rat, DW (Johnson et al., 1998)	SD rat, day 10 (4–7 somites), pericardial dilatation, decreased yolk sac circulation at highest exposure; more sensitive endpoints were prosencephalic hypoplasia, eye defects and decreased yolk sac diameter (Saillenfait et al., 1995).	–	The metabolite trichloroethanol has sedative effects. Other metabolites are trichloroacetic and possibly small amounts of dichloroacetic acid (U.S. EPA, 2000a).
Dichloromethane	–	LE rat, Inhal. (Hardin and Manson, 1980) SD rat, Swiss Webster mouse, Inhal. (Schwetz et al., 1975)	–	SD rat, 10.5 days (12–15 somites) craniofacial but not cardiac, but effects may be due to severe effect on yolk sac vascularization (Brown-Woodman et al., 1998).	Generally, toxicity may be due to P450 metabolism to a reactive intermediate and carbon monoxide, and to direct conjugation of dichloromethane with GSH to form a bioactive product. Also, the parent compound may enter cell membranes and thereby interfere with signal transmission (ATSDR, 2000).
Bromomethane	–	Wistar rat, Inhal. (Sikov et al., 1981)			
	Rabbit, Inhal., MTD not achieved (Sikov et al., 1981)				
	Rabbit, Inhal. (Breslin et al., 1990)		–	–	Generally, toxicity may be due to the alkylating (methylating) properties of bromomethane (ATSDR, 1992; Vogel and Nivard, 1994)

– = Data unavailable, BCA = bromochloroacetic acid, BDCM = bromodichloromethane, DCA = dichloroacetic acid, DW = drinking water, epi = epidemiological study, G = gavage, GD = gestation day, Inhal. = inhalation, LE = Long Evans, MTD = maximum tolerated dose, NS = not statistically significant, SD = Sprague–Dawley, TCA = trichloroacetic acid, TCAN = trichloroacetonitrile, tricap = tricaprylin.

[a] Animals were exposed on at least days 6–15 of gestation unless noted otherwise (where GD0 = sperm-positive or vaginal plug; GDs or embryo age adjusted to this basis). Tested at doses/exposures that included an MTD unless otherwise noted.

[b] Hunter et al. (1996) classified prosencephalic hypoplasia and neural tube closure defects together under the category neural tube defects.

the dam, as caused by bromodichloromethane. We conclude that the MOA for the haloacetic acids and haloacetonitriles that caused full-litter resorption in Long–Evans rats likely differs from that of the trihalomethanes and could be secondary to embryofetal toxicity.

A mixture of the five haloacetic acids regulated in drinking water, tested using proportions similar to those found in drinking water in F344 rats by gavage on GD 6–20, resulted in suggestive evidence that this mixture may cause pregnancy loss in this strain/stock (Narotsky et al., 2011). A non-significant incidence of full-litter resorption occurred at the mid dose level (versus no full-litter resorptions in controls or at the low dose level). A high incidence of full-litter resorption occurred in surviving dams at the high dose level, but four (of 15) died, indicating severe maternal toxicity. Based on this evidence, it would be useful to further evaluate the five haloacetic acids regulated in drinking water and the four other common haloacetic acids in the F344 rat.

The remaining two DBPs with positive results for full-litter resorption produced the effect at doses highly toxic to the dams in rat strains/stocks of lesser or unknown sensitivity (chlorite in Sprague–Dawley rats [Couri et al., 1982]; bromomethane in RIV-TOX rats [Peters et al., 1981]). Thus, the effect in these studies probably was secondary to severe maternal toxicity and does not appear at this time to be similar to the MOA of bromodichloromethane.

Cardiovascular malformations

Several retrospective epidemiologic studies have reported increased risks for cardiovascular malformations from exposure to treated drinking water. In a nationwide study of Norwegian births, Hwang et al. (2002) reported no statistically significant association between exposure of pregnant women to chlorinated water of low, medium, or high color and the occurrence of total cardiac defects in their infants. The ORs for ventricular septal defects, however, were increased following exposure to chlorinated water of medium color (OR = 1.63, 95% CI = 1.02–2.58) or high color (OR = 1.81, 95% CI = 1.05–3.09). Color has been positively correlated with the concentration of dissolved organic compounds in Norwegian waters (Hongve and Andersen, 1991). Hwang et al. (2002) expected that exposure to DBPs was more likely when water with dissolved organic compounds was chlorinated. Color, however, is a crude indicator of DBP concentrations as it also might reflect metallic ions and other compounds present in drinking water. Hwang et al. (2008), in a nationwide study of Taiwanese births, reported an increased risk of ventricular septal defects (OR = 1.81, 95% CI = 0.94–4.07) for exposure to trihalomethane drinking water concentrations ≥20 μg/L relative to those <4 μg/L. In a study of birth defects in England and Wales, Nieuwenhuijsen et al. (2008) reported excess risk for ventricular septal defects (OR = 1.43, 95% CI = 1.00–2.04) from exposure to trihalomethanes levels ≥60 μg/L (mean = 72.2 μg/L) as compared with <30 μg/L (mean = 16.4 μg/L). As summarized in Table 3, this specific cardiac defect was observed in rats administered some of the haloacetic acids and haloacetonitriles orally.

Bove et al. (1995) reported an association (OR = 1.83, 90% CI = 0.97–3.29) between major cardiac defects and total trihalomethane levels >80 μg/L (vs. ≤20 μg/L) in northern New Jersey. In addition to containing DBPs, these drinking waters were contaminated with organic solvents from landfills, industrial waste, and underground storage tanks.

Chisholm et al. (2008) conducted a study of births in Perth, Australia, where trihalomethane levels are higher than is usual in other countries. The authors reported that the risk for any cardiovascular birth defect was increased (OR = 1.62, 95% CI = 1.04–2.51) for the high exposure group (total trihalomethane ≥ 130 μg/L; average = 137 μg/L) as compared with the referent group (total trihalomethane <60 μg/L; average = 54 μg/L). A main study limitation was that there was no unexposed or low exposed population to serve as the referent group, as the reference population (<60 μg/L) was comparable to intermediate and high exposure groups in other studies. The authors stated that preliminary analyses of the haloacetic acids and haloacetonitriles indicated that concentrations were very low "by international standards," so monitoring for these DBPs was discontinued.

Nieuwenhuijsen et al. (2008), in the previously mentioned study of birth defects in England and Wales, reported an increased risk for a restricted group of major cardiac defects (OR = 1.18, 95% CI = 1.00–1.39) from exposure to bromoform at concentrations ≥4 μg/L (mean = 6.7 μg/L). The referent group was exposed to <2 μg/L of bromoform in drinking water. Other epidemiologic studies of drinking water exposure to total or individual trihalomethanes in Canada and the United States (Dodds and King, 2001; Dodds et al., 1999; Shaw et al., 2003) or exposure to chlorinated water in Norway and Sweden (Kallen and Robert, 2000; Magnus et al., 1999) have not detected increased risk for cardiovascular defects.

Cedergren et al. (2002), in a study of congenital cardiac birth defects in a Swedish county, reported that chlorine dioxide-disinfected municipal drinking water was a statistically significant risk factor for the development of cardiac defects. This conclusion was based on the finding that the OR for cardiac defects from disinfection with hypochlorite was not elevated (OR = 0.85, 95% CI = 0.60–1.21), but an association was reported between cardiac defects and disinfection with both chlorine dioxide and hypochlorite (OR = 1.61; 95% CI = 1.00–2.59), as compared with no chlorination. Comparison of the combined chlorine dioxide and hypochlorite treatment with hypochlorite alone further implicated chlorine dioxide disinfection (OR = 1.85, 95% CI = 1.42–2.39). Trihalomethane concentrations >10 μg/L in drinking water were also statistically significantly associated with cardiac defects (OR = 1.30, 95% CI = 1.08–1.56) relative to concentrations ≤10 μg/L. Concentrations of DBPs (other than total trihalomethanes) or residual chlorine dioxide were not determined. This study is limited because the drinking waters contained very low trihalomethane concentrations and a narrow exposure gradient.

The epidemiologic studies which reported associations did not provide information regarding a MOA for cardiovascular birth defects and did not include an examination of DBP classes or individual DBPs other than the trihalomethanes, which were associated with cardiovascular defects. Other DBPs not examined in these studies may confound the reported associations if they are correlated with trihalomethane concentrations and are risk factors for the outcomes of interest.

Studies of the individual trihalomethanes in experimental animals, treated on at least GD 6–15 (organogenesis), have not shown increased incidences of cardiac malformations. These studies were conducted in rats, mice, and rabbits administered the chemicals by gavage, and in rats and mice exposed to chloroform by inhalation, as listed in Table 3. No cardiac developmental toxicity studies of trihalomethane mixtures similar to those in treated drinking water have been reported in experimental animals.

Some of the haloacetic acids have shown evidence of heart defects when administered to rats by gavage. Suggestive evidence was reported for monobromoacetic acid and monochloroacetic acid in Long–Evans rats, but cardiovascular malformation data were reported only as number of fetuses with each type of cardiac defect, rather than as litter-based values (Randall et al., 1991; Smith et al., 1990). Two studies of dichloroacetic acid reported fetal heart defects following gavage administration to Long–Evans rats (Epstein et al., 1992; Smith et al., 1992a). Two studies of trichloroacetic acid, one using drinking water administration to Sprague–Dawley rats (Johnson et al., 1998) and the other using gavage administration to Long–Evans rats (Smith et al., 1989a), also reported heart defects in the fetuses.

Negative results for cardiovascular malformations in the Sprague–Dawley rat also have been reported from oral exposure to monochloroacetic acid, dichloroacetic acid, bromochloroacetic acid, tribromoacetic acid, trichloroacetic acid, and dibromochloroacetic acid

(Fisher et al., 2001; Johnson et al., 1998; NTP, National Toxicology Program, 1998b,c, 2000). Except for the study by Fisher et al. (2001), these studies exposed the animals through drinking water, and some did not attain a MTD. The negative studies used lower dosage levels than did the positive studies and, in the case of drinking water studies, delivered the dose intermittently over time rather than as a single daily bolus. Thus, the difference between the positive and negative studies does not appear to be due to strain/stock of rat, since both positive and negative results were obtained for trichloroacetic acid in Sprague–Dawley rats. Rather, the difference appears to be a function of dose level.

The haloacetonitriles that have been tested adequately (see Methods) in developmental toxicity studies (i.e., dichloroacetonitrile, trichloracetonitrile, and bromochloroacetonitrile) have caused cardiovascular defects in gavage studies in Long–Evans rats (Christ et al., 1995, 1996; Smith et al., 1988, 1989b). There is some concern regarding the choice of vehicle used in the haloacetonitrile gavage studies. Tricaprylin, a triglyceride vehicle, was used due to the volatility and instability of the haloacetonitriles in water and the high concentrations required to achieve sufficient doses in a small volume. In the gavage developmental toxicity studies on the haloacetonitriles, tricaprylin controls had slight elevations in some indices of developmental toxicity relative to water controls, but these were not consistent across the studies and few were statistically significant (Christ et al., 1995, 1996; Smith et al., 1988, 1989b). A limited comparison of the developmental toxicity of trichloroacetonitrile in tricaprylin versus trichloroacetonitrile in corn oil (Christ et al., 1996) suggested that the developmental toxicity of trichloroacetonitrile, including induction of cardiac defects, was greater in tricaprylin than in corn oil. The suitability of corn oil as a vehicle has been questioned, however, because the pharmacokinetics and toxicity of chlorinated compounds have been shown to be statistically significantly different in corn oil than in aqueous vehicles (Kim et al., 1990a,b; Lilly et al., 1994; Narotsky et al., 1997a; Withey et al., 1983).

As summarized above and in Table 3, positive results for cardiovascular malformations have been observed for several of the haloacetic acids in rats *in vivo* (Epstein et al., 1992; Randall et al., 1991; Smith et al., 1990, 1992a), for all nine haloacetic acids of interest in mouse embryo culture (Hunter et al., 1996, 2006a,b), and for the three dihaloacetic acids in rat embryo culture (Andrews et al., 2004). Positive results for cardiovascular malformations also were observed for three haloacetonitriles in rats *in vivo* (Christ et al., 1995, 1996; Smith et al., 1988, 1989b), but the haloacetonitriles have not been tested in embryo culture. In both the whole embryo culture studies and the *in vivo* animal studies, the observed pattern of heart defects and craniofacial defects (including eye defects) suggests a hypothesis that the haloacetic acids and haloacetonitriles (at least trichloroacetonitrile) may exhibit a common MOA by affecting neural crest cells.

The cranial and cardiac neural crest cells migrate and differentiate into structures of the face and the musculoconnective tissues of the large arteries emerging from the heart, the membranous portion of the ventricular septum, and the septum between the pulmonary artery and the aorta. Further studies with mouse and rat embryo neural crest explants showed that some dihaloacetic acids (dibromoacetic acid and bromochloroacetic acid, but not dichloroacetic acid) affected neural crest cell development and migration at concentrations that alter development in whole embryo culture (Andrews et al., 2001; Hunter et al., 2001). Neural crest-mediated heart defects, however, occur in the outflow tracts of the heart and do not account for the full range of heart defects seen in *in vivo* studies in the rat. We suggest that a key event further upstream in development could be responsible for the full range of heart defects. Thus, key events for the cardiovascular malformations resulting from exposure to the haloacetic acids and haloacetonitriles are uncertain. For most of these DBPs, direct effects on the embryo seem likely, but for dichloroacetic acid, which produces widespread metabolic effects (see Table 3), there could be some contribution through effects on the dam.

We note that the only epidemiologic studies that reported associations of disinfected drinking water or DBPs (the trihalomethanes or bromoform) with cardiovascular malformations did not investigate potential associations with the types of DBPs (haloacetic acids or haloacetonitriles) that cause these effects in rodents. Swan and Waller (1998) suggested that trihalomethanes, which were until recently the only DBPs required to be monitored, may be proxies for other, possibly nonvolatile, etiologic agent(s) in the finished water supply. More recent U.S. studies of drinking water DBPs suggest that total haloacetic acid concentrations were correlated with reported total trihalomethane concentrations across different water systems (Savitz et al., 2005) or seasons (Porter et al., 2005); other DBP classes were not investigated. Additional studies are needed to further investigate these relationships.

Neural tube defects

The concern for an association between DBPs and neural tube defects stems from the results of some epidemiological studies. One case–control study of neural tube defects (i.e., spina bifida, anencephaly, encephalocele) examined registry data for all births from 1993 to 1994 in New Jersey in relation to public water sampling during the first trimester of pregnancy (Klotz and Pyrch (1998, 1999). The authors reported that when cases were restricted to mothers with known residence at conception and to isolated neural tube defects (i.e., other defects not present), a statistically significant association (OR = 2.1, 95% CI = 1.1–4.0) was detected between neural tube defects and the highest tertile [≥40 ppb (μg/L)] versus the lowest tertile (≤5 ppb) of total trihalomethane exposure. None of the individual trihalomethanes (also determined from public water sampling) were statistically significantly associated with neural tube defects in comparisons of the highest versus lowest exposure tertiles. In this study, trihalomethane exposure generally was determined by public water sampling at points in the distribution system representative of residences. Additional sampling and analysis of residential tap water from "index residences" were performed for the trihalomethanes 1 year after the first trimester of pregnancy; for residences served by water systems that may have included surface water sources, analyses also included the haloacetic acids and haloacetonitriles. For the residential tap water data on total trihalomethanes, and cases restricted to isolated neural tube defects, an association was detected for the highest exposure tertile relative to the lowest tertile (OR = 1.9, 95% CI = 1.0–4.0). The OR for cases restricted to mothers with known residence at conception and also to isolated neural tube defects was not reported. In the highest tertile of total trihalomethane exposure, an association with neural tube defects was seen only in the cases where the mothers did not take daily multivitamin or folate supplements during the 3 months prior to pregnancy. The authors stated in their 1999 publication that results were not markedly different when analyses controlled for volatile organic compound (VOC) contamination of the drinking water, which was generally very low (with very few cases or controls exposed to≥1 ppb of any individual VOC).

Bove et al. (1995) reported an association of neural tube defects with total trihalomethanes (OR = 2.96, 90% CI = 1.26–6.62) in infants of mothers exposed to >80 ppb in drinking water (in comparison with ≤20 ppb). This case–control study was performed in Northern New Jersey where water supplies, in addition to containing DBPs, were contaminated with organic solvents from landfills, industrial waste and underground storage tanks. VOC concentrations were low in this study as well, with few cases exposed above 1 ppb. No information regarding use of daily multivitamin or folate supplements was identified. Although it was only based on only two cases, there was some suggestive evidence of an association of neural tube defects with

exposure to carbon tetrachloride. Other epidemiologic studies reported no association of chlorinated drinking water with neural tube defects in Norway (Hwang et al., 2002; Magnus et al., 1999) and Sweden (Kallen and Robert, 2000) or for total trihalomethane exposures and risk of neural tube defects in California (Shaw et al., 2003).

One study reported an association of an individual trihalomethane, bromodichloromethane, with neural tube defects in humans. In a retrospective cohort study in Nova Scotia, Canada, Dodds and King (2001) reported an increased relative risk of 2.5 (95% CI = 1.2–5.1) for neural tube defects associated with maternal exposure to bromodichloromethane concentrations of ≥20 μg/L (as compared with exposure to <μg/L) in chlorinated drinking water during pregnancy. The study included both live and still births and also data from pregnancies terminated for prenatally diagnosed congenital anomalies. No association of bromodichloromethane (Klotz and Pyrch, 1998; Shaw et al., 2003), dibromochloromethane (Shaw et al., 2003), or chloroform (Dodds and King, 2001; Klotz and Pyrch, 1998) with neural tube defects was observed in epidemiologic studies in California and New Jersey.

The only epidemiologic data examining associations of other DBPs with neural tube defects were from Klotz and Pyrch (1998, 1999) for the haloacetic acids and haloacetonitriles, determined by residential tap water sampling and analysis 1 year after the first trimester as described previously. No statistically significant association with neural tube defects was seen for the highest tertile (≥35 ppb) versus the lowest (<3 ppb) tertile of haloacetic acid exposure (OR = 1.2, 95% CI = 0.5-2.6) or for the highest tertile (≥3.0 ppb) versus the lowest (<0.5 ppb) tertile of total haloacetonitriles (OR = 1.3, 95% CI = 0.6–2.5) in this study. Narrow exposure gradients and limited statistical power may have impacted the ability to detect an association, if one existed, between neural tube defects and other DBPs in this study.

The only MOA data directly relevant to the potential association of DBPs with neural tube defects is the observation in a case–control study that an association of exposure to total trihalomethanes with neural tube defects was seen only in the cases where the mothers did not take daily multivitamin or folate supplements during the 3 months prior to pregnancy (Klotz and Pyrch, 1998, 1999). Folic acid or folate is a coenzyme involved in single carbon transfers in the metabolism of nucleic acids and amino acids (Institute of Medicine, 1998; Padmanabhan, 2006). As discussed in these reviews, folic acid is known to be required for DNA synthesis, and its deficiency has been associated with neural tube defects. Supplementation with this vitamin lowers the risk of neural tube defects in humans. Despite these observations, the etiology of neural tube defects is poorly understood and appears highly complex. Genetic, nutritional, and environmental factors including drug and chemical exposures have been implicated. Investigations of genetic variation in enzymes involved in folic acid pathways, including the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, have not shown conclusively that these are causal factors for neural tube defects, suggesting a multifactorial etiology. Thus, a potential MOA for DBP induction of neural tube defects involves folic acid pathways, but the mechanism whereby folic acid prevents neural tube defects is not known, and the evidence associating DBPs with neural tube defects is weak. Thus, no MOA is established for DBP induction of neural tube defects.

In addition, none of the *in vivo* studies of developmental toxicity in laboratory mammals detected statistically significant incidences of neural tube defects as a consequence of exposure to any of the individual DBPs. These *in vivo* studies were conducted in rats, mice, or rabbits on all four of the individual trihalomethanes, on some of the individual haloacetic acids and haloacetonitriles, and on the remaining selected DBPs. All of these studies reported negative results or detected only single instances of neural tube defects. The studies are listed in Table 4. Studies that may not have been adequate to detect neural tube effects are those where the MTD was not achieved, too few pregnant dams were studied, or examinations were performed only postnatally; Table 4 highlights these limitations.

Only the *in vitro* whole embryo culture studies of mouse and rat embryos reported neural tube defects, and these defects generally occurred in the presence of yolk sac defects including reduced diameter and circulation, and/or in the presence of multiple other growth and structural defects, as noted in Table 4. The data regarding neural tube defects in the whole embryo culture studies are not reported uniformly across studies: prosencephalic hypoplasia was considered a neural tube defect in one set of data and combined with neural tube closure defects in the data tabulation (Hunter et al., 1996), and was recorded in other data series as a separate defect that may be a defect of neural tube expansion or may be simply a growth retardation reflecting overall growth. Studies reporting positive results for neural tube defects or neural tube closure defects are reported in the positive column of Table 4, whereas those reporting only prosencephalic hypoplasia (and no closure defects) in the presence of overall growth retardation are reported in the negative column of the table.

Thus, as discussed previously, although a few epidemiology studies have raised concerns regarding an association of neural tube defects with exposure to DBPs (Bove et al., 1995; Dodds and King, 2001; Klotz and Pyrch, 1998, 1999), the epidemiologic evidence associating DBPs with neural tube defects is weak. In addition, the available data are inadequate to outline key steps in an MOA for this effect and the *in vivo* animal data do not provide support for neural tube defects as an effect of exposure to any single DBP.

Low birth weight or intrauterine growth retardation

Several epidemiologic studies have reported an association with small for gestational age (i.e., intrauterine growth retardation) or low birth weight infancy and exposure to DBPs, primarily the trihalomethanes and the haloacetic acids, both as classes and individual chemicals. These studies are listed in Table 5 and the more recent U.S. studies are discussed in detail later in this section. Intrauterine growth retardation/restriction or small for gestational age is generally defined as birth weight below the 10th percentile for gestational age adjusted for certain covariates such as sex and race. Low birth weight is generally defined as birth weight <2500 g and term low birth weight is generally defined as birth weight <2500 g for infants of ≥37 weeks gestation. Small for gestational age is generally considered a better indicator of fetal growth since low birth weight is influenced by both fetal growth rate and duration of gestation.

One epidemiologic study provides some evidence for a potential MOA for fetal growth restriction outcomes. In a case–control study in Montreal, Quebec, Canada, Infante-Rivard (2004) reported that exposure to treated drinking water resulted in an increased risk of intrauterine growth restriction (retardation) associated with average total trihalomethane exposure above the 90th percentile (corresponding to 29.5 μg/L), but only in infants (cases = 45 and controls = 37) with a specific variant CYP2E1 gene [G1259C (a G-to-C substitution at position 1259 in the promoter) that defines the allele CYP2E1*5]. The adjusted OR for this effect was 13.2 (95%CI = 1.2-146.7). No association was seen in the infants with wild type CYP2E1 or when cases and controls were not subdivided on the basis of genetic polymorphisms. The authors determined that the mother's CYP2E1 gene was not associated with this outcome, and a 5,10-MTHFR gene variant (C677T) in newborns or mothers also was not associated with this outcome. CYP2E1 is the primary enzyme that metabolizes low doses of the trihalomethanes. As reviewed by Infante-Rivard (2004), the CYP2E1 gene variant G1259C, located in the regulatory region, is associated with hyperinduction, which would be expected to increase the amount of enzyme compared with individuals without the mutation, and consequently increase the

metabolic activation of trihalomethanes. This finding suggests that if there is an effect of trihalomethanes on intrauterine growth retardation, it may be related to (as yet undetermined) damage from toxic intermediates resulting from increased metabolic activation of the trihalomethanes in genetically sensitive fetuses. Christian et al. (2001b) and Withey and Karpinski (1985) have shown that dibromochloromethane, administered to pregnant rabbits orally, and chloroform, administered to pregnant rats by inhalation, reach the fetus, and therefore would be available for potential metabolic activation by fetal CYP2E1, at least in these species. Generally, toxicity of the trihalomethanes may be mediated through metabolism to reactive and toxicity intermediates (ATSDR, 2003; ILSI, 1999; U.S. EPA, 2001).

Epidemiologic studies that reported positive results for intrauterine growth retardation usually focused on DBP exposure during the third trimester or the entire pregnancy. A few epidemiologic studies have shown an association of intrauterine growth retardation with DBP exposure in the second trimester (trihalomethanes, Lewis et al., 2006 and Wright et al., 2003; monobromoacetic acid, Porter et al., 2005). These findings support the hypothesis proffered by Porter et al. (2005) that although the third trimester is the most important for fetal weight gain, insults prior to the third trimester may impact fetal growth by interfering with cell proliferation, which occurs mainly prior to the third trimester.

We did not identify additional research on these possible steps in a MOA for this adverse outcome. Thus, the data currently available for this endpoint do not adequately characterize the MOA for intrauterine growth retardation. Nevertheless, many of the more recent U.S. epidemiologic studies of this outcome support it as a developmental effect of concern for DBP exposure. These more recent U.S. studies are reviewed below.

In a study of birth weight in 36,529 singleton births in a Massachusetts population that received water from a single water utility during 1999–2001, Lewis et al. (2006) reported an association between high average total trihalomethane exposure in drinking water (≥70 μg/L) during the second trimester of pregnancy and an increased risk of term low birth weight for all races/ethnicities combined (OR = 1.50, 95% CI = 1.07, 2.10). The increase for Caucasians alone was not statistically significant (OR = 1.37, 95% CI = 0.80, 2.36), but for all minority women combined (n = 15,103) was 60% (OR = 1.60, 95% CI = 1.03, 2.47). No statistically significant increase in risk of term low birth weight was detected for first or third trimester exposures.

Wright et al. (2003) examined the effect of trimester-specific and pregnancy average total trihalomethane exposure on 56,513 singleton births in Massachusetts during 1990. They reported increased ORs for small for gestational age for second trimester (OR = 1.13, 95% CI = 1.03–1.24) and for pregnancy average (OR = 1.14, 95% CI = 1.02–1.26) total trihalomethane concentrations >80 μg/L as compared with ≤60 μg/L. Term low birth weight was not associated with total trihalomethane exposure.

Wright et al. (2004) reported on a cohort of Massachusetts women giving birth between 1995 and 1998 for whom third-trimester DBP data were available. Outcome data for 196,000 singleton births were examined. Reduced mean birth weight was associated with drinking water exposure to concentrations of total trihalomethane >40 μg/L and chloroform >20 μg/L. An increased risk of small for gestational age births was associated with concentrations of total trihalomethanes >33–74 μg/L (OR = 1.06, 95% CI = 1.02–1.10) and >74–163 μg/L (OR = 1.13, 95% CI = 1.07–1.20), chloroform >63–135 μg/L (OR = 1.11, 95% CI = 1.04–1.17), and bromodichloromethane >5–13 μg/L (OR = 1.10, 95% CI = 1.07–1.14) and >13–46 μg/L (OR = 1.15, 95% CI = 1.08–1.22). Increased risks for adverse fetal growth outcomes were not detected for the five regulated haloacetic acids as a group, dichloroacetic acid or trichloroacetic acid exposures.

Gallagher et al. (1998) investigated third trimester exposure to total trihalomethanes in Colorado and reported an association between term low birth weight and exposure to high levels (≥61 ppb) of total trihalomethanes in the maternal drinking water as compared with ≤20 ppb (OR = 5.9, 95% CI = 2.0–17.0).

Hoffman et al. (2008) reported an increased risk of delivering a small for gestational age infant in women exposed to average trihalomethane concentrations of ≥80 μg/L as compared with those exposed to <80 μg/L in tap water during the third trimester (risk ratio = 2.0, 95% CI = 1.1–3.6). [Other results from this prospective study were reported by Savitz et al. (2005, 2006) and are reviewed in the spontaneous abortion/full-litter resorption section of this paper.] Hoffman et al. (2008) did not detect an association between small for gestational age and trihalomethane exposure through showering and bathing, or between this outcome and total haloacetic acid concentration in tap water or consumption through tap water.

In a study of 15,315 births in a Maryland county, Porter et al. (2005) investigated the possible association between intrauterine growth retardation and exposure to the trihalomethanes and the five haloacetic acids ("HAA5") that have drinking water regulations (monochloro-, dichloro-, and trichloroacetic acid; monobromo- and dibromoacetic acid). The authors evaluated exposure to total trihalomethanes, HAA5, and to individual compounds for each trimester and also the entire pregnancy. No consistent statistically significant association with intrauterine growth retardation was seen for these two classes of DBPs or for the individual chemicals, and no evidence of a dose–response relationship was observed. The results for HAA5 and three of the individual haloacetic acids, however, were suggestive of potential effects. An association with intrauterine growth retardation was seen for the third trimester for the second (OR = 1.20, 95% CI = 1.01–1.68), third (OR = 1.41, 95% CI = 1.11–1.81) and fifth (OR = 1.34, 95% CI = 1.04–1.71) quintiles of total HAA5 exposure (first quintile = referent), but no dose–response was evident. Statistically significant associations with intrauterine growth retardation for second trimester exposure to monobromoacetic acid were observed for the third (OR = 1.30, 95% CI = 1.02–1.65) and fourth (OR = 1.30, 95% CI = 1.02–1.66) quintiles but not the fifth (highest) quintile of exposure. Associations with intrauterine growth retardation were reported for second trimester exposure to monochloroacetic acid for the third quintile (OR = 1.27, 95% CI = 1.00–1.61) and third trimester exposure to dichloroacetic acid for the second quintile (OR = 1.29, 95% CI = 1.02–1.64) of exposure. Statistically significant associations with intrauterine growth retardation for third trimester exposure to trichloroacetic acid were seen for the second (OR = 1.30, 95% CI = 1.01–1.65) and third (OR = 1.34, 95% CI = 1.05–1.71) quintiles and ORs were consistently ≥1.2 regardless of exposure level.

Similarly, Hinckley et al. (2005), in a study of 48,119 birth outcomes in an Arizona community, found no evidence of an association between drinking water exposure to total trihalomethanes, or to three specific trihalomethane compounds, with intrauterine growth retardation or term low birth weight; bromoform was not evaluated because drinking water concentrations of this compound were very low. The authors observed associations with exposure to specific haloacetic acids but not HAA5, as follows: (1) associations between exposure to ≥5 μg/L dibromoacetic acid (OR = 1.49, 95% CI = 1.09–2.04) or to dibromoacetic acid analyzed as a continuous variable (OR = 1.17, 95% CI = 1.03–1.32) during the third trimester with increased risk for term low birth weight; (2) associations between dibromoacetic acid exposure at ≥5 μg/L during gestation weeks 33–36 (OR = 1.49, 95% CI = 1.10–2.02) and at 3.5–5 μg/L (but not ≥5 μg/L) during gestation weeks 37–40 (OR = 1.38, 95% CI = 1.02–1.86) with an increased risk for term low birth weight; (3) an association of dibromoacetic acid analyzed as a continuous variable in both gestation weeks 33–36 (OR = 1.11, 95% CI = 1.01–1.21) and 37–40 (OR = 1.10, 95% CI = 1.01–1.20) with term low birth weight; and (4) an association of dibromoacetic acid analyzed as a continuous variable

Table 4
Summary of data on neural tube defects for selected DBPs.[a]

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
Chlorinated DW	–	Human, DW epi (Hwang et al., 2002) Human, DW, epi (Kallen and Robert, 2000) Human, DW, epi (Magnus et al., 1999)	–	–	–
Total Trihalomethanes	Human, DW, epi (Klotz and Pyrch, 1998, 1999) Human, DW also contaminated with solvents, epi (Bove et al., 1995)	Human, DW, epi (Shaw et al., 2003) Human, DW, epi (Dodds et al., 1999)	–	–	Human, DW, epi: an association with neural tube defects was seen only in the cases where the mothers did not take daily multivitamin or folate supplements during the 3 months prior to pregnancy, and not when vitamins or folate were taken (Klotz and Pyrch, 1999).
Bromodichloromethane (BDCM)	Human, DW, epi (Dodds and King, 2001)	Human, Dw, epi (Shaw et al., 2003) Human, DW, epi (Klotz and Pyrch, 1998) SD rat, G-corn oil (Ruddick et al., 1983) SD rat, rabbit, DW (Christian et al., 2001a)	–	–	Rabbit, DW, limited data suggest BDCM reaches placental and fetus (Christian et al., 2001b). Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999).
Dibromochloromethane	–	Human, DW, epi (Shaw et al., 2003) SD rat, G-corn oil (Ruddick et al., 1983) ICR Swiss mouse, DW (Borzelleca and Carchman, 1982)	–	–	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999).
Bromoform	Slightly suggestive data: CD-1 mouse, G-aqueous, exencephaly in one pup at each of two high dose groups (Narotsky et al., 2001)	SD rat, G-corn oil (Ruddick et al., 1983)	–	–	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999).
Chloroform	–	Human, DW, epi (Dodds and King, 2001) Human, DW, epi (Klotz and Pyrch, 1998) SD rat, G-corn oil (Ruddick et al., 1983) ICR Swiss mouse, DW (Borzelleca and Carchman, 1982) SD rat, rabbit, G-corn oil (Thompson et al., 1974) Wistar rat, Inhal. (Baeder and Hoffmann, 1988, 1991a,b) CF-1 mice, Inhal. (Murray et al., 1979) SD rats, Inhal. (Schwetz et al., 1974)	–	SD rat, day 10.5 (12–15 somites), diffuse cell death, including neuroepithelium of neural tube, but no effect on closure of neural tube; effects may be due to loss of yolk sac vascularization (Brown-Woodman et al., 1998).	At end of single 4-hour inhalation exposure on GD 17, fetal to maternal chloroform concentration ratio was 0.316; fetal concentration was not related to position in uterus (Withey and Karpinski, 1985). Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999; U.S. EPA, 2001).
Total haloacetic acids	–	Human, DW, epi (Klotz and Pyrch, 1999)	–	–	–
Monobromoacetic acid	–	Suggestive data: (not litter-based): LE rat, G-water (Randall et al., 1991)	CD-1 mouse, day 8 (3–6 somites), also heart, pharyngeal arch and eye defects (Hunter et al., 1996)[b]	–	–
Monochloroacetic acid	–	SD rat–DW, MTD not achieved (Johnson et al., 1998) Suggestive data (not litter-based): LE rat, G-water (Smith et al., 1990)	CD-1 mouse, day 8 (3–6 somites), also heart and pharyngeal arch defects (Hunter et al., 1996)[b]	–	–
Dibromoacetic acid (DBA)	–	–	CD-1 mouse, day 8 (3–6 somites), also heart, pharyngeal arch and eye defects (Hunter et al., 1996)[b] SD rat, day 9.5 (0-1 somites), also visceral arch hypoplasia and heart and eye defects (Andrews et al., 2004)	CD-1 mouse, day 8 (3–6 somites), prosencephalic and pharyngeal arch hypoplasia; heart and eye defects (Hunter et al., 2006b)	SD rat, DW, parent compound reaches placenta, amniotic fluid and fetus (Christian et al., 2001b) Was dose additive with BCA and/or DCA on development of rat embryo in culture (Andrews et al., 2004)

(continued on next page)

Table 4 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
					Inhibited neural crest cell migration in cultured explants from rat and mouse embryos (Andrews et al., 2001; Hunter et al., 2001) Did not induce apoptosis in mouse embryo culture (Ward et al., 2000) Is more potent than its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dichloroacetic acid (DCA)	Slightly suggestive data: CD-1 mouse, G-water, spina bifida aperta in one pup each at highest and 4th highest dose levels (Narotsky et al., 1996)	LE rat, G-water, eye defects (Smith et al., 1992a)	CD-1 mouse, day 8 (3–6 somites), also heart, pharyngeal arch and eye defects (Hunter et al., 1996[b], 2006b) SD rat, day 10 (4-7 somites), may be secondary to decreased yolk sac circulation, also poor survival; prosencephalic hypoplasia, eye defects, and decreased yolk sac diameter more sensitive (Saillenfait et al., 1995). Suggestive data: SD rat, day 9.5 (0-1 somites) (malformations stated to be similar to those from dibromoacetic acid in same study) (Andrews et al., 2004)	–	LE rat, G, antagonistic interaction for resorptions, cardiac and total visceral malformations with TCA (Smith et al., 1991, 1992b) Was dose additive with DBA and/or BCA on development of rat embryo in culture (Andrews et al., 2004) LE rat, G, at 48 hours after dosing of dams with 1- and 2-(^{14}C)-labeled DCA, ^{14}C levels were higher in embryos than in maternal plasma, and were present mainly as C2 (the dichloromethyl C), indicating a metabolite (Roth et al., 1991). DCA has multiple effects in intermediary metabolism, particularly from inhibition of the kinase that inactivates mitochondrial pyruvate dehydrogenase (Crabb et al., 1981; Smith et al., 1992a; Stacpoole, 1989). DCA-induced malformations in cultured mouse embryos are not primarily due to reactive oxygen species (Hunter et al., 2006c). DCA induces apoptosis in mouse embryos, including in the heart (Ward et al., 2000). DCA did not inhibit neural crest cell migration in culture (Andrews et al., 2001; Hunter et al., 2001). Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Bromochloroacetic acid (BCA)	–	SD rat, DW, but looked only at brain on PND 5 (NTP, National Toxicology Program, 1998a)	Suggestive data: CD-1 mouse, day 8 (3–6 somites), neural tube defects NS but small sample size, also prosencephalic and pharyngeal arch hypoplasia; heart and eye defects (Hunter et al., 2006a) SD rat, day 9.5 (0-1 somites) (malformations stated to be similar to those from dibromoacetic acid in same study) (Andrews et al., 2004)	CD-1 mouse, day 8 (3–6 somites), prosencephalic and pharyngeal arch hypoplasia; heart and eye defects (Hunter et al., 2006b)	Was dose additive with DBA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Inhibited neural crest cell migration in cultured explants from rat and mouse embryos (Andrews et al., 2001; Hunter et al., 2001) Induces apoptosis in mouse embryos (Ward et al., 2000) Metabolites of BCA were much less potent than BCA in mouse embryo culture (Hunter and Rogers, 1999). BCA caused changes in gene expression in mouse embryo culture (Karoly et al., 2004) including genes networked with Akt (protein kinase B) (Hunter et al., 2005).

Tribromoacetic acid	–	SD rat-DW, MTD not achieved, looked only at brain at PND 5 (NTP, National Toxicology Program, 1998c)	CD-1 mouse, day 8 (3–6 somites), also cardiac, pharyngeal arch and eye defects (Hunter et al., 1996)[b]	–	–
Trichloroacetic acid (TCA)	–	LE rat, G-water, eye defects (Smith et al., 1989a) SD rat, DW (Johnson et al., 1998) SD rat, G-water (Fisher et al., 2001)	CD-1 mouse, day 8 (3–6 somites), also cardiac, pharyngeal arch and eye defects (Hunter et al., 1996)[b]	SD rat, day 10 (4-7 somites), prosencephalic hypoplasia, eye defects, decreased yolk sac diameter (Saillenfait et al., 1995)	LE rat, G, antagonistic interaction with DCA on resorption and cardiac and total visceral malformations (Smith et al., 1991, 1992b) Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dibromochloroacetic acid	–	SD rat, DW, MTD not achieved, examined external malformations at PND 1, 3, 5 and brain malformations at PND 5 (NTP, National Toxicology Program, 2000)	CD-1 mouse, day 8 (3–6 somites), yolk sac defects at same concentration as neural tube defects; also prosencephalic hypoplasia, heart, pharyngeal arch, and eye defects (Hunter et al., 2006a)	–	–
Bromodichloroacetic acid	–	–	CD-1 mouse, day 8 (3–6 somites), yolk sac defects at same concentration as NS neural tube defects; also prosencephalic hypoplasia, and heart, pharyngeal arch and eye defects (Hunter et al., 2006a)	–	–
Total haloacetonitriles	–	Human, DW, epi (Klotz and Pyrch, 1999)	–	–	–
Dichloroacetonitrile	–	LE rat, G-tricap (Smith et al., 1989b)	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
Trichloroacetonitrile (TCAN)	–	LE rat, G-tricap (Smith et al., 1988) LE rat, G-corn oil, also G-tricap but only one dose (Christ et al., 1996)	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988). LE rat, G, accumulation of radioactivity in maternal liver and in fetus from 2–3 daily doses of ^{14}C-TCAN was higher for the trichloromethyl carbon (C2) than the cyano carbon. Accumulation in embryos was higher with tricaprylin vehicle than corn oil vehicle (Gordon et al., 1991).
Bromochloroacetonitrile	–	LE rat, G-tricap (Christ et al., 1995)	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
Dibromoacetonitrile	–	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988)
Chloroacetonitrile (CAN)	–	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
1,1,3,3,-Tetrachloropropanone	–	CF-1 mouse, rabbit, G-corn oil (John et al., 1982)	–	–	–
Chlorine dioxide-disinfected DW	–	Human, DW, epi (Kallen and Robert, 2000)	–	–	–
Chlorine dioxide	–	Rat, DW, but only 6–8 dams/dose (Suh et al., 1983) Rat, DW, but examined external malformations during lactational period (Carlton et al., 1991)	–	–	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b). Decreased the levels of circulating thyroxine in monkeys and rats, and in developmental study in rats, possibly though oxidation of dietary iodide in the gastrointestinal tract, resulting in reactive species that iodinate organic matter to form potentially thyroid inhibitory compounds (Bercz et al., 1986; Orme et al., 1985)

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Table 4 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
Chlorite	–	SD rat, DW, but only 6–8 dams/dose, MTD not achieved (Suh et al., 1983) Rabbit, DW, MTD may not have been achieved (Harrington et al., 1995). SD rat, DW, also maternal toxicity (body weight loss and hemolysis) and greatly decreased water and food intake (Couri et al., 1982) LE rat, DW, but examined external malformations postnatally (Carlton and Smith, 1985; Carlton et al., 1987)	–	–	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b).
Chloral hydrate	–	SD rat, DW (Johnson et al., 1998) D-1 mouse, DW, only five dams/dose, examined external malformations postnatally (Kallman et al., 1984)	–	SD rat, day 10 (4-7 somites), hypoplasia of prosencephalon, pericardial dilatation, eye defects, decreased yolk sac diameter (Saillenfait et al., 1995)	The metabolite trichloroethanol has sedative effects. Other metabolites are trichloroacetic and possibly small amounts of dichloroacetic acid (U.S. EPA, 2000c).
Dichloromethane	–	LE rat, Inhal. (Hardin and Manson, 1980) SD rat, Swiss Webster mouse, Inhal. (Schwetz et al., 1975) F344 rat, G-corn oil, examined postnatally for external malformations and if dead with no external malformations, then for soft tissue malformations (Narotsky and Kavlock, 1995)	–	SD rat, 10.5 days (12–15 somites) craniofacial but not cardiac, but effects may be due to severe effect on yolk sac vascularization (Brown-Woodman et al., 1998)	Generally, toxicity may be due to P450 metabolism to a reactive intermediate and carbon monoxide, and to direct conjugation of dichloromethane with GSH to form a bioactive product. Also, the parent compound may enter cell membranes and thereby interfere with signal transmission (ATSDR, 2000).
Bromomethane	–	RIV-TOX rat, G-peanut oil (Peters et al., 1981) Wistar rat, Inhal. (Sikov et al., 1981) Rabbit, Inhal., MTD not achieved (Sikov et al., 1981) Rabbit, Inhal. (Breslin et al., 1990)	–	–	Generally, toxicity may be due to the alkylating (methylating) properties of bromomethane (ATSDR, 1992; Vogel and Nivard, 1994).

– = Data unavailable, DW = drinking water (household water), epi = epidemiologic study, G = gavage, 2 gen = 2 generation reproduction study, Inhal. = inhalation, LE = Long Evans, NS = not statistically significant, SD = Sprague–Dawley, tricap = tricaprylin.

[a] Animals were exposed on at least days 6–15 of gestation unless noted otherwise (where GD 0 = sperm-positive or vaginal plug; GD or embryo age adjusted to this basis). Tested at doses/exposures that included a maximum tolerated dose (MTD) unless otherwise noted.

[b] Hunter et al. (1996) classified prosencephalic hypoplasia and neural tube closure defects together under the category neural tube defects.

Table 5
Summary of data on low birth weight/intrauterine growth retardation for selected DBPS[a].

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
Chlorinated DW	Equivocal data: Human, DW, epi (Kallen and Robert, 2000) and Human, DW, epi (Kanitz et al., 1996)	Human, DW, epi (Jaakkola et al., 2001)	–	–	–
Total trihalomethanes	Human, DW, epi (Hoffman et al., 2008) Human, DW, epi (Lewis et al., 2006) Human, DW, epi (Wright et al., 2003, 2004) Human, DW, epi (Gallagher et al., 1998) Human, DW also contaminated with solvents, epi (Bove et al., 1995) Equivocal:	Human, DW, epi (Yang et al., 2007) Human, DW, epi (Hinckley et al., 2005) Human, DW, epi (Porter et al., 2005) Human, DW, epi (Dodds et al., 1999)	–	–	Maternal exposure to total trihalomethanes resulted in an increased risk of intrauterine growth retardation only in newborns with a variant CYP2E1 gene; the mother's CYP2E1 was not associated with this outcome, and a 5,10-methylenetetrahydrofolate reductase gene variant in newborns or mothers also was not associated with this outcome (Infante-Rivard, 2004).
Bromodichloromethane (BDCM)	Human, DW, epi (Toledano et al., 2005) Human, DW, epi. (Wright et al., 2004)	Human, DW, epi (Porter et al., 2005) Human, DW, epi (Hinckley et al., 2005) Human, DW, epi (Kramer et al., 1992) SD rat, rabbit, DW (Christian et al., 2001a) SD rat, G-corn oil (Ruddick et al., 1983) SD rat, DW (NTP, National Toxicology Program, 1998b) F344 rat, G-aqueous (Bielmeier et al., 2004) F344 rat, G-corn oil or aqueous (Narotsky et al., 1997a)	– –	–	Rabbit, DW, limited data suggest BDCM reaches placenta and fetus (Christian et al., 2001b). Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999).
Dibromochloromethane	–	Human, DW, epi (Porter et al., 2005) Human, DW, epi (Hinckley et al., 2005) Human, DW, epi (Kramer et al., 1992) SD rat, G-corn oil (Ruddick et al., 1983) SD rat, DW (NTP, National Toxicology Program, 1996)	–	–	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999).
Bromoform	–	Human, DW, epi (Porter et al., 2005) Human, DW, epi (Kramer et al., 1992) SD rat, G-corn oil (Ruddick et al., 1983) CD-1 mouse, G-corn	–	–	Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ATSDR, 2003; ILSI, 1999).

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Table 5 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
		oil (NTP, National Toxicology Program, 1989)			
Chloroform	Human, DW, epi (Kramer et al., 1992) May be secondary to maternal effects in the following: SD rat, G-corn oil (Ruddick et al., 1983) SD rat, G-corn oil (Thompson et al., 1974) Wistar rat, Inhal (Baeder and Hoffmann, 1988, 1991a,b) SD rat, Inhal. (Schwetz et al., 1974) CF-1 mouse, Inhal. (Murray et al., 1979)	Human, DW, epi (Dodds and King, 2001) Human, DW, epi (Klotz and Pyrch, 1998) CD-1 (ICR) mouse (NTP, National Toxicology Program, 1988) Human, DW, epi (Hoffman et al., 2008)	SD rat, day 10.5 (12–15 somites), may be due to loss of yolk sac vascularization (Brown-Woodman et al., 1998)	–	At end of single 4-hour inhalation exposure on GD 17, fetal to maternal chloroform concentration ratio was 0.316; fetal concentration was not related to position in uterus (Withey and Karpinski, 1985). Generally, toxicity may be mediated through metabolism to reactive and toxic intermediates (ILSI, 1999; U.S. EPA, 2001).
Total haloacetic acids	Human, DW, epi (Porter et al., 2005)	Human, DW, epi (Hinckley et al., 2005)	–	–	–
Monobromoacetic acid	Human, DW, epi, suggestive (Porter et al., 2005) LE rat, G-water, may be secondary to maternal effects (Randall et al., 1991)	–	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 1996)	–	–
Monochloroacetic acid		Human, DW, epi (Porter et al., 2005) SD rat–DW, MTD not achieved, 10 dams/group (Johnson et al., 1998) LE rat, G-water (Smith et al., 1990)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 1996)	–	–
Dibromoacetic acid (DBA)	Human, DW, epi (Hinckley et al., 2005) CD-1 mouse, G-water, may be secondary to maternal effects (Narotsky et al., 1996)	Human, DW, epi (Porter et al., 2005) SD rat, DW, 2-gen (Christian et al., 2002b)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 1996) SD rat, day 9.5 (0-1 somites) (Andrews et al., 2004)	–	SD rat, DW, parent compound reaches placenta, amniotic fluid and fetus (Christian et al., 2001b). Was dose additive with BCA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Did not induce apoptosis in mouse embryo culture (Ward et al., 2000) Is more potent than its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dichloroacetic acid (DCA)	Human, DW, epi (Hinckley et al., 2005) Human, DW, epi, suggestive (Porter et al., 2005) May be secondary to maternal effects in the following: LE Rat, G-water (Smith et al., 1992a) SD rat, G-water (Fisher et al., 2001)	Human, DW, epi (Wright et al., 2004)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 1996) SD rat, day 10 (4-7 somites), possibly secondary to effects on yolk sac (Saillenfait et al., 1995) SD rat, day 9.5 (0-1 somites) (Andrews et al., 2004)	–	LE rat, G, antagonistic interaction with TCA for resorptions and cardiac and total visceral malformations (Smith et al., 1991, 1992b) Was dose additive with DBA and/or BCA on development of rat embryo in culture (Andrews et al., 2004) LE rat, G, at 48 hours after dosing of dams with 1- and 2-(^{14}C)-labeled DCA, ^{14}C levels were higher in embryos than in maternal plasma, and were present mainly as C2 (the dichloromethyl C), indicating a metabolite (Roth et al., 1991). DCA has multiple effects in intermediary metabolism, particularly from inhibition of the kinase that inactivates mitochondrial pyruvate dehydrogenase (Crabb et al., 1981; Smith et al., 1992a; Stacpoole, 1989). DCA induces apoptosis in mouse embryos (Ward et al., 2000). Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)

Bromochloroacetic acid (BCA)	–	SD rat, DW (NTP, National Toxicology Program, 1998a)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 2006a) SD rat, day 9.5 (0-1 somites) (Andrews et al., 2004)	–	Was dose additive with DBA and/or DCA on development of rat embryo in culture (Andrews et al., 2004) Induces apoptosis in mouse embryos (Ward et al., 2000) Metabolites of BCA were much less potent than BCA in mouse embryo culture (Hunter and Rogers, 1999). BCA caused changes in gene expression in mouse embryo culture (Karoly et al., 2004).
Tribromoacetic acid	–	SD rat-DW, MTD not achieved (NTP, National Toxicology Program, 1998c)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 1996) SD rat, day 10 (4-7 somites), may be secondary to yolk sac effects (Saillenfait et al., 1995)	–	–
Trichloroacetic acid (TCA)	Human, DW, epi (Hinckley et al., 2005) Human, DW, epi, suggestive (Porter et al., 2005) LE rat, G-water (Smith et al., 1989a) SD rat, G-water, may be secondary to maternal effects (Fisher et al., 2001)	Human, DW, epi (Wright et al., 2004) SD rat, DW (Johnson et al., 1998)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 1996)	–	LE rat, G, antagonistic interaction with DCA for resorptions and cardiac and total visceral malformations (Smith et al., 1991, 1992b) Has similar potency to its metabolites in mouse embryo culture (Hunter and Rogers, 1999)
Dibromochloroacetic acid	–	SD rat, DW, MTD not achieved (NTP, National Toxicology Program, 2000)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 2006a)	–	–
Bromodichloroacetic acid	–	CD-1 mouse, G-water (Narotsky et al., 2001)	CD-1 mouse, day 8 (3–6 somites) (Hunter et al., 2006a)	–	–
Total haloacetonitriles	–	–	–	–	–
Dichloroacetonitrile	LE rat, G-tricap (Smith et al., 1989b) LE rat, G-tricap, may be secondary to maternal effects (Smith et al., 1987)		–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
Trichloroacetonitrile (TCAN)	LE rat, G-tricap (Smith et al., 1988) May be secondary to maternal effects in the following: LE rat, G-tricap (Smith et al., 1987) and LE Rat, G-corn oil (Christ et al., 1996)	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988). LE rat, G, accumulation of radioactivity in maternal liver and in fetus from 2–3 daily doses of ^{14}C-TCAN was higher for the trichloromethyl carbon (C2) than the cyano carbon. Accumulation in embryos was higher with tricaprylin vehicle than corn oil vehicle (Gordon et al., 1991).
Bromochloroacetonitrile	LE rat, G-tricap (Smith et al., 1987)				
LE Rat, G-tricap (Christ et al., 1995)	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).	
Dibromoacetonitrile	LE rat, G-tricap, may be secondary to maternal effects (Smith et al., 1987)	SD rat, DW, MTD not achieved (NTP, National Toxicology Program, 1997)	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
Chloroacetonitrile	LE rat, G-tricap, may be secondary to maternal effects (Smith et al., 1987)	–	–	–	Metabolism to cyanide does not appear to account for developmental toxicity (Christ et al., 1995; Smith et al., 1988).
1,1,3,3,-Tetrachloropropanone	–	CF-1 mouse, rabbit, G-corn oil (John et al., 1982)	–	–	–
Chlorine dioxide-disinfected DW	Human, DW, epi (Kanitz et al., 1996)	Human, DW, epi (Kallen and Robert, 2000)	–	–	–
Chlorine dioxide	SD rat, DW, but maternal body weight not reported (Mobley et al., 1990)	MTD not achieved in any of the following: SD rat, DW (Orme et al., 1985)	–	–	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b).

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Table 5 (*continued*)

DBP exposure measure	*In vivo* positive studies	*In vivo* negative studies	Embryo culture positive studies	Embryo culture-negative studies	MOA data
		SD rat, DW (Taylor and Pfohl, 1985) and LE Rat, DW (Carlton et al., 1991)			Decreased the levels of circulating thyroxine in monkeys and rats, and in developmental study in rats, possibly through oxidation of dietary iodide in the gastrointestinal tract, resulting in reactive species that iodinate organic matter to form potentially thyroid inhibitory compounds (Bercz et al., 1986; Orme et al., 1985).
Chlorite	SD rat, DW (CMA, Chemical Manufacturers Association, 1996; Gill et al., 2000)	Human, DW, epi (Aggazzotti et al., 2004) MTD not achieved in any of the following: SD rat, DW, but only 6–8 dams/dose (Suh et al., 1983) SD rat, DW (Mobley et al., 1990) Rabbit, DW (Harrington et al., 1995) A/J mouse, DW, only 10 treated litters (Moore et al., 1980)	–	–	Generally, toxicity is considered due to oxidative properties (U.S. EPA, 2000b).
Chloral hydrate	–	MTD not achieved in any of the following: SD rat, DW (Johnson et al., 1998) D-1 mouse, DW, only 5 dams/dose (Kallman et al., 1984)	SD rat, day 10 (4–7 somites), may be secondary to effect on yolk sac diameter and circulation (Saillenfait et al., 1995)	–	The metabolite trichloroethanol has sedative effects. Other metabolites are trichloroacetic and possibly small amounts of dichloroacetic acid (U.S. EPA, 2000c).
Dichloromethane	LE rat, Inhal. (Hardin and Manson, 1980)	SD rat, Swiss Webster mouse, Inhal. (Schwetz et al., 1975) F344 rat, Inhal., MTD not achieved (Nitschke et al., 1988) F344 rat, G-corn oil (Narotsky and Kavlock, 1995)	SD rat, 10.5 days (12–15 somites) but may be secondary effect on yolk sac vascularization (Brown-Woodman et al., 1998)	–	Generally, toxicity may be due to P450 metabolism to a reactive intermediate and carbon monoxide, and to direct conjugation of dichloromethane with GSH to form a bioactive product. Also, the parent compound may enter cell membranes and thereby interfere with signal transmission (ATSDR, 2000).
Bromomethane	Rabbit, Inhal., may be secondary to maternal effects (Breslin et al., 1990)	RIV-TOX rat, G-peanut oil (Peters et al., 1981) Wistar rat, Inhal. (Sikov et al., 1981) Rabbit, Inhal., MTD not achieved (Sikov et al., 1981)	–	–	Generally, toxicity may be due to the alkylating (methylating) properties of bromomethane (ATSDR, 1992; Vogel and Nivard, 1994).

– = Data unavailable, DW = drinking water (household water), epi = epidemiologic study, G = gavage, 2 gen = 2 generation reproduction study, Inhal. = inhalation, LE = Long Evans, NS = not statistically significant, SD = Sprague–Dawley, tricap = tricaprylin.

[a] Animals were exposed on at least days 6–15 of gestation unless noted otherwise (where GD 0 = sperm-positive or vaginal plug; GDs or embryo age adjusted to this basis). Tested at doses/exposures that included a maximum tolerated dose (MTD) unless otherwise noted.

in gestation weeks 25–28 (OR = 1.06, 95% CI = 1.01–1.12) with intrauterine growth retardation; (5) an association between dichloroacetic acid exposure at ≥8 μ/L (OR = 1.28, 95% CI = 1.08–1.51) or to trichloroacetic acid at ≥6 μ/L (OR = 1.19, 95% CI = 1.01–1.41) during the third trimester with intrauterine growth retardation.

Thus, the trihalomethanes and the haloacetic acids, both the classes and also some of the individual compounds, have been associated with intrauterine growth retardation and low birth weight in humans. Results are fairly consistent across the epidemiologic studies especially for total trihalomethanes and intrauterine growth retardation with all but one of the studies reporting relative risks in excess of 1.1. However, some of these results are not statistically significant and most are small in magnitude. These findings likely result from limited exposure assessment data and other study limitations which can decrease statistical power inhibiting the ability to detect risk small in magnitude and assess dose–response relationships.

As summarized in Table 5, developmental and reproductive studies of the individual DBPs in experimental animals have generally reported no adverse effects on fetal or newborn body weight or have reported adverse effects that may be secondary to maternal effects, including decreased maternal body weight gain, liver lesions, and clinical signs of toxicity. The exceptions are trichloroacetic acid; dichloro-, trichloro-, and bromochloroacetonitrile; chlorite; and dichloromethane, for which the fetal body weight effects appear to be relatively sensitive as compared with maternal effects. The studies in cultured mouse and rat embryos also reported growth retardation, often in the presence of adverse effects on the yolk sac (decreased diameter and circulation), and were inconsistent in whether and how they reported effects on the yolk sac, such that interpretation of these studies is problematic.

In summary, although a number of recent epidemiology studies have raised concerns regarding an association of low birth weight or intrauterine growth retardation with exposure to DBPs, the available *in vivo* and *in vitro* data are inadequate to outline key steps in an MOA for this effect.

Conclusions

Although an understanding of the MOA for an adverse developmental outcome is needed for a component-based risk assessment, such detailed knowledge typically is rare for developmental toxicants (Faustman et al., 2006; NRC, National Research Council, 2000), and, as presented in this paper, for the DBPs. A major reason is the complexity and plasticity of development. The need for understanding at different levels of biological organization and processes including cell migration, proliferation, and differentiation also complicates the study and interpretation of data relevant to developmental MOA (Faustman et al., 2006; NRC, National Research Council, 2000).

Our review and evaluation of the literature focused on 24 developmentally toxic DBPs and four adverse developmental outcomes associated with DBP exposure in humans. A plausible MOA is delineated for one of these outcomes, spontaneous abortion, which was associated with total trihalomethane exposure and with bromodichloromethane exposure in epidemiologic studies (Savitz et al., 1995; Waller et al., 1998). The available data seem to indicate that the target tissue is maternal and that the MOA may involve a hormonal disruption of pregnancy. It is reasonably well established for one of the trihalomethanes, bromodichloromethane, based on studies in rats *in vivo* and in human and rat tissues *in vitro*. Some MOA data were available for the other three outcomes (cardiovascular defects, neural tube defects, and low birth weight or small for gestational age), but the data were inadequate for any DBP to define key steps and clearly associate key steps (define a MOA) for those outcomes. Thus, to conduct a component-based health risk assessment of the developmentally toxic DBPs, further development of risk assessment methods is needed to assess the possibility that they may act by similar or dissimilar MOAs. MOA-based risk assessments provide a better understanding of dose–response in the region of low response than do risk assessments based only on assumptions of similarity or dissimilarity of MOA.

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