# BROMODICHLOROMETHANE

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of bromodichloromethane (CAS# 75-27-4). The major source of bromodichloromethane in the environment is its formation as a byproduct during chlorination of water.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. This information was used in a screen to select appropriate chemicals for presentation to the Developmental and Reproductive Toxicant Identification Committee as possible candidates for Committee consideration. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. The epidemiologic studies report on developmental and reproductive sequelae related to exposure to disinfection by-products in drinking water. Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen:

- Four epidemiologic studies of bromodichloromethane reporting increased risk of adverse developmental or reproductive outcomes were identified, all of which were analytical studies of adequate quality. Four epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified. Two related articles on bromodichloromethane were identified.

- Four animal studies of bromodichloromethane and one meeting abstract reporting reproductive or developmental toxicity were identified, as well as six animal studies that did not report reproductive or developmental toxicity. Three related studies and abstracts were identified.

# Contents

# I. Epidemiologic DART Studies

## A. Studies reporting increased risk of adverse developmental or reproductive outcomes

*** The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration.**
Wright JM, Schwartz J, Dockery DW.
Environ Health Perspect. 2004 Jun;112(8): 920-5.

Epidemiologic studies of disinfection by-products have traditionally focused on total trihalomethane (TTHM) concentration as a surrogate for maternal exposure during pregnancy. We used birth certificate data on 196,000 infants to examine the effect of third-trimester exposures on various indices of fetal development. We examined the effect of town-average concentrations of TTHM and additional exposure metrics in relation to mean birth weight, mean gestational age, small for gestational age (SGA) infancy, and preterm delivery. Trihalomethane data (TTHM, chloroform, and bromodichloromethane) from 1995-1998 were available for 109 towns in Massachusetts. Data from 1997-1998 on haloacetic acid (total haloacetic acids, dichloroacetic acid, and trichloroacetic acid), 3-chloro-4-(dichloromethyl)-5- hydroxy-2(5H) furanone (MX), and mutagenicity were available for a limited number of towns. We observed reductions in mean birth weight (12-18 g) for maternal trihalomethane exposures > the $90^{th}$ percentile compared with those < the $50^{th}$ percentile. Birth weight reductions were detected for chloroform exposures > 20 microg/L and TTHM exposures > 40 microg/L. Elevated trihalomethanes were associated with increases in gestational duration and a reduced risk of preterm delivery. We found evidence of an exposure-response effect of trihalomethanes on risk of SGA, with odds ratios (Ors) ranging from 1.09 to 1.23 for bromodichloromethane exposures > 5 microg/L. Elevated mutagenic activity was associated with SGA [OR = 1.25; 95% confidence interval (CI), 1.04 to 1.51] and mean birth weight (-27 g; 95% CI, -54 to –1). Although smaller in magnitude, our findings are consistent with previous studies reporting associations between trihalomethanes and SGA. These data also suggest a relationship between fetal development indices and mutagenic activity independent of exposure to trihalomethanes, haloacetic acids, and MX.

***Relation between trihalomethane compounds and birth defects**.
Dodds L, King WD.
Occup Environ Med. 2001 Jul;58(7):443-6.

OBJECTIVES: To evaluate the risk of birth defects relative to exposure to specific trihalomethanes in public water supplies. METHODS: A retrospective cohort study was conducted based on data from a population based perinatal database in Nova Scotia, Canada and

* Denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

from the results of routine water monitoring tests. The cohort consisted of women who had a singleton birth in Nova Scotia between 1988 and 1995 and who lived in an area with a municipal water supply. The birth defects analyzed included neural tube defects, cardiovascular defects, cleft defects, and chromosomal abnormalities. Two of the four trihalomethane compounds occur in large enough concentrations to be analyzed (chloroform and bromodichloromethane (BDCM)). RESULTS: Exposure to BDCM at concentrations of 20microg/l or over was associated with an increased risk of neural tube defects (adjusted relative risk (RR) 2.5, 95% confidence interval (95% CI) 1.2 to 5.1) whereas exposure to chloroform was not. Exposure to BDCM of 20 microg/l and over was associated with decreased risks of cardiovascular anomalies (RR 0.3, 95% CI 0.2 to 0.7). There was a suggestion of an increased risk of chromosomal abnormalities associated with exposure to chloroform, and no evidence of any association between either trihalomethane compound and cleft defects. CONCLUSIONS: In this cohort, differences were found in the RR associated with exposure to chloroform and BDCM for each of the congenital anomalies under study. These findings point to the importance of examining specific byproduct compounds relative to risk for these birth outcomes and in particular implicate BDCM and other correlated disinfection byproducts in the aetiology of neural tube defects.

*** Relation between stillbirth and specific chlorination by-products in public water supplies.**
King WD, Dodds L, Allen AC.
Environ Health Perspect. 2000 Sep;108(9):883-6.

During water treatment, chlorine reacts with naturally occurring organic matter in surface water to produce a number of by-products. Of the by-products formed, trihalomethanes (THMs) are among the highest in concentration. We conducted a retrospective cohort study to evaluate the relationship between the level of total THM and specific THMs in public water supplies and risk for stillbirth. The cohort was assembled from a population-based perinatal database in the Canadian province of Nova Scotia and consisted of almost 50,000 singleton deliveries between 1988 and 1995. Individual exposures were assigned by linking mother's residence at the time of delivery to the levels of specific THMs monitored in public water supplies. Analysis was conducted for all stillbirths and for cause-of-death categories based on the physiologic process responsible for the fetal death. Total THMs and the specific THMs were each associated with increased stillbirth risk. The strongest association was observed for bromodichloromethane exposure, where risk doubled for those exposed to a level of [greater and equal to] 20 microg/L compared to those exposed to a level < 5 microg/L (relative risk = 1. 98, 95% confidence interval, 1.23-3.49). Relative risk estimates associated with THM exposures were larger for asphyxia-related deaths than for unexplained deaths or for stillbirths overall. These findings suggest a need to consider specific chlorination by-products in relation to stillbirth risk, in particular bromodichloromethane and other by-product correlates. The finding of a stronger effect for asphyxia deaths requires confirmation and research into possible mechanisms.

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***Trihalomethanes in drinking water and* spontaneous abortion.**
Waller K, Swan SH, DeLorenze G, Hopkins B.
Epidemiology. 1998 Mar;9(2):134-40.

Trihalomethanes (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) are common contaminants of chlorinated drinking water. Although animal data indicate that these compounds may be reproductive toxicants, little information exists on their relation to spontaneous abortion in humans. We examined exposure to trihalomethanes and spontaneous abortion in a prospective study of 5,144 pregnant women in a prepaid health plan. Seventy-eight drinking water utilities provided concurrent trihalomethane sampling data. We calculated total trihalomethane levels by averaging all measurements taken by the subject's utility during her first trimester. We calculated exposures to individual trihalomethanes in an analogous manner. Women who drank > or = 5 glasses per day of cold tap water containing > or = 75 micrograms per liter total trihalomethanes had an adjusted odds ratio (OR) of 1.8 for spontaneous abortion [95% confidence interval (CI) = 1.1-3.0]. Of the four individual trihalomethanes, only high bromodichloromethane exposure (consumption of > or = 5 glasses per day of cold tap water containing > or = 18 micrograms per liter bromodichloromethane) was associated with spontaneous abortion both alone (adjusted OR = 2.0; 95% CI = 1.2-3.5) and after adjustment for the other trihalomethanes (adjusted OR = 3.0; 95% CI = 1.4-6.6).

## B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

**Exposure to drinking water disinfection by-products and pregnancy loss.**
Savitz, D. A.; Singer, P. C.; Herring, A. H.; Hartmann, K. E.; Weinberg, H. S.; Makarushka, C.
Am J Epidemiol. 2006; 164(11):1043-51.

Previous research has suggested that exposure to elevated levels of drinking water disinfection by-products (DBPs) may cause pregnancy loss. In 2000-2004, the authors conducted a study in three US locations of varying DBP levels and evaluated 2,409 women in early pregnancy to assess their tap water DBP concentrations, water use, other risk factors, and pregnancy outcome. Tap water concentrations were measured in the distribution system weekly or biweekly. The authors considered DBP concentration and ingested amount and, for trihalomethanes only, bathing/showering and integrated exposure that included ingestion. On the basis of 258 pregnancy losses, they did not find an increased risk of pregnancy loss in relation to trihalomethane, haloacetic acid, or total organic halide concentrations; ingested amounts; or total exposure. In contrast to a previous study, pregnancy loss was not associated with high personal trihalomethane exposure (> or =75 micro g/liter and > or =5 glasses of water/day) (odds ratio = 1.1, 95% confidence interval: 0.7, 1.7). Sporadic elevations in risk were found across DBPs, most notably for ingested total organic halide (odds ratio = 1.5, 95% confidence interval: 1.0, 2.2

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for the highest exposure quintile). These results provide some assurance that drinking water DBPs in the range commonly encountered in the United States do not affect fetal survival.

**Late pregnancy exposures to disinfection by-products and growth-related birth outcomes.**
Hinckley, A. F.; Bachand, A. M.; Reif, J. S.
Environ Health Perspect. 2005; 113(12):1808-13.

Toxicologic studies have demonstrated associations between growth-related birth outcomes and exposure to high concentrations of disinfection by-products (DBPs), including specific trihalomethane (THM) and haloacetic acid (HAA) chemical subspecies. Few prior investigations of DBPs have evaluated exposure during the third trimester of pregnancy, the time period of gestation when fetal growth may be most sensitive to environmental influences. We conducted a retrospective cohort study to examine the effects of exposure to THMs and HAAs during the third trimester and during individual weeks and months of late gestation on the risks for term low birth weight, intrauterine growth retardation, and very preterm and preterm births. The study population (n = 48,119) included all live births and fetal deaths occurring from January 1998 through March 2003 to women whose residence was served by one of three community water treatment facilities. We found evidence of associations between exposure to specific HAAs and term low birth weight as well as intrauterine growth retardation and for exposure to the five regulated HAAs (HAA5) and term low birth weight. Our findings suggest a critical window of exposure with respect to fetal development during weeks 33-40 for the effects of dibromoacetic acid and during weeks 37-40 for the effects of dichloroacetic acid. Adjustment for potential confounders did not affect the conclusions.

**The effect of trihalomethane and haloacetic acid exposure on fetal growth in a Maryland county.**
Porter, C. K.; Putnam, S. D.; Hunting, K. L.; Riddle, M. R.
Am J Epidemiol. 2005; 162(4):334-44.

**Abstract:** As water flows from treatment plants to the tap, chlorine, used to disinfect surface water meant for residential use, reacts with residual organic and inorganic matter, creating chlorine disinfection by-products. In recent years, these by-products have been scrutinized as a potential reproductive and developmental hazard. This study examined whether exposure to the four total trihalomethanes or the five haloacetic acids (two major subgroups of chlorine disinfection by-products) was related to an increased risk of intrauterine growth retardation in four regions of a Maryland county from 1998 to 2002. Maternal exposure to each by-product was evaluated for each trimester as well as over the entire pregnancy. The authors were not able to demonstrate any consistent, statistically significant effect on intrauterine growth retardation associated with any of the chlorine disinfection by-products, nor did they find any indication of a dose-response relation. However, they did find some potential for a slightly elevated risk of intrauterine growth retardation during the second and third trimesters for both total

trihalomethanes and five haloacetic acids when comparing increasing quintiles of exposure to constituents of total trihalomethanes and five haloacetic acids.

**Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England.**
Toledano MB, Nieuwenhuijsen MJ, Best N, Whitaker H, Hambly P, de Hoogh C, Fawell J, Jarup L, Elliott P.
Environ Health Perspect. 2005 Feb;113(2):225-32.

We investigated the association between total trihalomethanes (TTHMs) and risk of stillbirth and low and very low birth weight in three water regions in England, 1992-1998; associations with individual trihalomethanes (THMs) were also examined. Modeled estimates of quarterly TTHM concentrations in water zones, categorized as low (< 30 microg/L), medium (30-59 microg/L), or high (greater than or equal to 60 microg/L), were linked to approximately 1 million routine birth and stillbirth records using maternal residence at time of birth. In one region, where there was a positive socioeconomic deprivation gradient across exposure categories, there was also a positive, significant association of TTHM with risk of stillbirth and low and very low birth weight. Overall summary estimates across the three regions using a random-effects model to allow for between-region heterogeneity in exposure effects showed small excess risks in areas with high TTHM concentrations for stillbirths [odds ratio (OR) = 1.11; 95% confidence interval (CI), 1.00-1.23), low birth weight (OR = 1.09; 95% CI, 0.93-1.27), and very low birth weight (OR = 1.05; 95% CI, 0.82-1.34). Among the individual THMs, chloroform showed a similar pattern of risk as TTHM, but no association was found with concentrations of bromodichloromethane or total brominated THMs. Our findings overall suggest a significant association of stillbirths with maternal residence in areas with high TTHM exposure. Further work is needed looking at cause-specific stillbirths and effects of other disinfection by-products and to help differentiate between alternative (noncausal) explanations and those that may derive from the water supply. Key words: chemical, disinfection, infant low birth weight, pregnancy outcome, stillbirth, trihalomethanes, water pollution, water purification.

## C. Related articles

**Bromodichloromethane inhibits human placental trophoblast differentiation.**
Chen J, Thirkill TL, Lohstroh PN, Bielmeier SR, Narotsky MG, Best DS, Harrison RA, Natarajan K, Pegram RA, Overstreet JW, Lasley BL, Douglas GC.
Toxicol Sci. 2004 Mar;78(1):166-74. Epub 2003 Dec 22.

Epidemiological data suggest an association between exposures to bromodichloromethane (BDCM), a trihalomethane found in drinking water as a result of drinking water disinfection, and an increased risk of spontaneous abortion. We previously hypothesized that BDCM targets the placenta and showed that the secretion of chorionic gonadotrophin (CG) was reduced in primary cultures of human term syncytiotrophoblasts exposed to BDCM. In the present study we extend

this observation by evaluating the effects of BDCM on the morphological differentiation of mononucleated cytotrophoblast cells to multinucleated syncytiotrophoblast-like colonies. Addition of BDCM to cytotrophoblast cultures inhibited the subsequent formation of multinucleated colonies in a dose-dependent manner, as determined by immunocytochemical staining for desmosomes and nuclei. The effect was seen at BDCM concentrations between 0.02 and 2 mM and was confirmed by quantitative image analysis. Secretion of bioactive and immunoreactive chorionic gonadotropin was also significantly inhibited in a dose-dependent manner under these culture conditions, and cellular levels of CG were also reduced. Trophoblast viability was not compromised by exposure to BDCM. We conclude that BDCM disrupts syncytiotrophoblast formation and inhibits CG secretion in vitro. Although other tissue targets are not ruled out, these data substantiate the idea that BDCM targets the placenta and could have implications for understanding the adverse pregnancy outcomes associated with BDCM exposure in humans.

**Effect of bromodichloromethane on chorionic gonadotrophin secretion by human placental trophoblast cultures.**
Chen J, Douglas GC, Thirkill TL, Lohstroh PN, Bielmeier SR, Narotsky MG, Best DS, Harrison RA, Natarajan K, Pegram RA, Overstreet JW, Lasley BL.
Toxicol Sci. 2003 Nov;76(1):75-82. Epub 2003 Sep 11.

Bromodichloromethane (BDCM) is a trihalomethane found in drinking water as a by-product of disinfection processes. BDCM is hepatotoxic and nephrotoxic in rodents and has been reported to cause strain-specific full-litter resorption in F344 rats during the luteinizing hormone-dependent phase of pregnancy. In humans, epidemiological studies suggest an association between exposure to BDCM in drinking water and increased risk of spontaneous abortion. To begin to address the mechanism(s) of BDCM-induced spontaneous abortion, we hypothesized that BDCM targets the placenta. Primary cultures of human term trophoblast cells were used as an in vitro model to test this hypothesis. Trophoblasts were allowed to differentiate into multinucleated syncytiotrophoblast-like colonies, after which they were incubated for 24 h with different concentrations of BDCM (20 nM to 2 mM). Culture media were collected and assayed for immunoreactive and bioactive chorionic gonadotropin (CG). Cultures exposed to BDCM showed a dose-dependent decrease in the secretion of immunoreactive CG as well as bioactive CG. The lowest effective BDCM concentration was 20 nM, approximately 35-times higher than the maximum concentration reported in human blood (0.57 nM). Trophoblast morphology and viability were similar in controls and cultures exposed to BDCM. We conclude that BDCM perturbs CG secretion by differentiated trophoblasts in vitro. This suggests that the placenta is a likely target of BDCM toxicity in the human and that this could be related to the adverse pregnancy outcomes associated with BDCM.

## II. Animal DART Studies

### A. Studies reporting developmental or reproductive toxicity

**Serum hormone characterization and exogeneous hormone rescue of bromodichloromethane-induced pregnancy loss in the F344 rat.**
Bielmeier SR, Best DS and Narotsky MG
Toxicol Sci. 2004;77:101-8.

Previously, we demonstrated that bromodichloromethane (BDCM), a drinking water disinfection by-product, causes pregnancy loss in F344 rats when given on gestational days (GD) 6-10, encompassing the luteinizing hormone (LH)-dependent period of pregnancy (GD 7-10). Pregnancy loss, i.e., full-litter resorption, was associated with reduced serum progesterone levels; however, we were unable to identify an effect on serum LH. Here, we reevaluated serum LH levels using the more sensitive technique, DELFIA(R). We further sought to better define the temporal pattern of endocrine disruption caused by BDCM during pregnancy with more frequent sampling. Lastly, we attempted to prevent BDCM-induced pregnancy loss using exogenous progesterone or human chorionic gonadotropin (hCG), an LH-agonist. BDCM, in 10% Alkamuls(R), was dosed at 75 mg/kg/day by gavage to F344 rats on GD 6-10 (plug day = GD 0). BDCM-induced pregnancy loss was associated with marked reductions in serum progesterone and LH on GD 10. The decrease in serum LH consistently preceded the decrease in progesterone. In the hormone replacement studies, BDCM and progesterone were administered on GD 6-10, hCG on GD 8-10. BDCM was delivered at 100 mg/kg/day, progesterone at 10 mg/kg twice daily, and hCG at 0.5 IU/0.2 ml/rat. Both progesterone and hCG prevented BDCM-induced pregnancy loss. Thus, BDCM-induced pregnancy loss was associated with marked GD-10 reductions in serum LH and corresponding decreases in progesterone. Furthermore, coadministration of an LH agonist prevented pregnancy loss, supporting the hypothesis that BDCM-induced pregnancy loss in the rat occurs via an LH-mediated mode of action.

**Pregnancy loss in the rat caused by bromodichloromethane.**
Bielmeier SR, Best DS, Guidici DL and Narotsky MG
Toxicol Sci. 2001;59:309-15.

Bromodichloromethane (BDCM), a trihalomethane, is a by-product of the chlorination of drinking water. In a recent epidemiological study, consumption of BDCM was associated with an increased risk of spontaneous abortion in pregnant women. We have previously shown that BDCM causes pregnancy loss, i.e., full-litter resorption (FLR), in the F344 rat. The mode of action was investigated, with three main findings. First, there was a dramatic difference in sensitivity between F344 and Sprague-Dawley (SD) rat strains. Following aqueous gavage treatment on gestational days (GD) 6-10, F344 rats had a 62% incidence of FLR at 75 mg/kg/day, whereas all SD rats maintained their litters. Second, the critical period encompassed the luteinizing hormone (LH)-dependent period of pregnancy. Rats treated on GD 6-10 at 75 mg/kg/day had a 75% incidence of FLR, but rats treated on GD 11-15 at 75 or 100 mg/kg/day

were unaffected. Third, 24 h after a single dose, all dams with FLR had markedly reduced serum progesterone levels; however, LH levels were unaffected. The high FLR rate during the LH-dependent period, the lack of response thereafter, and the reduced progesterone levels without an associated reduction in LH levels suggests that BDCM disrupts luteal responsiveness to LH.

**Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats.**
Narotsky MG, Pegram RA and Kavlock RJ
Fundam Appl Toxicol. 1997;40:30-36.
Several halocarbons have been shown to cause full-litter resorption (FLR) in Fischer-344 rats when administered orally in corn oil. Since halocarbons often occur as contaminants of drinking water, we sought to determine the influence of the vehicle, aqueous versus lipid, on the developmental toxicity of two of these agents. In separate assays, bromodichloromethane (BDCM) and carbon tetrachloride ($CCl_4$) were administered by gavage to Fischer-344 rats on gestation days (GD) 6-15 at 0, 25, 50, or 75 mg/kg/day in either corn oil or an aqueous vehicle containing 10% Emulphor EL-620. Dams were allowed to deliver and the litters were examined postnatally. Uteri of females that did not deliver were stained with 10% ammonium sulfide to detect FLR. Effects of both agents on maternal weight gain were slightly more pronounced in the aqueous vehicle at lower doses, but at the highest dose, $CCl_4$ was more maternally toxic in corn oil. Developmentally, both agents caused FLR at 50 and 75 mg/kg in both vehicles. At 75 mg/kg, dams receiving corn oil had significantly higher rates of FLR (83% for BDCM, 67% for $CCl_4$) compared to their aqueous-vehicle counterparts (21% for BDCM, 8% for $CCl_4$). Blood concentrations of BDCM following GD-6 gavage revealed a shorter elimination half-life in the aqueous dosing vehicle (2.7 h) compared to the oil vehicle (3.6 h). Benchmark doses of $CCl_4$ were similar for the oil (18.9 mg/kg) and aqueous (14.0 mg/kg) vehicles. For BDCM, the corn oil vehicle yielded a less conservative (i.e., higher) value (39.3 mg/kg) than the aqueous vehicle (11.3 mg/kg), reflecting different confidence intervals around the estimated 5%-effect dose levels.

**Preliminary screening for the potential of drinking water disinfection byproducts to alter male reproduction.**
Klinefelter GR, Suarez JD, Roberts NL and DeAngelo AB
Reprod Toxicol. 1995;9:571-8.

There is increasing epidemiologic interest in the role drinking water disinfection byproducts (DBPs) may play in adverse reproductive outcomes such as inability to conceive, spontaneous abortion, and low birth weight. Although dozens of DBPs already have been identified, only a few studies have attempted to determine whether DBPs alter male reproductive parameters such as testicular and epididymal histology, testicular and epididymal sperm numbers, and epididymal sperm morphology and motility in laboratory animals. In these studies, alterations in epididymal sperm motility seemed to be predictive of more generalized toxicity of the male reproductive system. Because there is a need to prioritize DBPs for thorough reproductive and developmental

toxicity testing, preliminary screening for the potential of DBPs to alter reproductive function seems warranted. Here, we elected to examine only cauda epididymal sperm motion parameters and testicular and epididymal histopathology. The effects of exposure to two commonly occurring DBPs, bromodichloromethane (BDCM) and chloral hydrate (CH), via drinking water were evaluated in F344 rats at an interim (52 week) necropsy during cancer bioassay studies. Exposure to 22 and 39 mg/kg BDCM and 55 and 188 mg/kg CH did not produce any systemic toxicity. Histopathologic evaluation revealed no gross lesions in the reproductive organs, and no tumors were detected in any tissues. In contrast, exposure to 39 mg/kg BDCM significantly decreased the mean straight-line, average path, and curvilinear velocities of sperm recovered from the cauda epididymidis. This BDCM exposure shifted the average path velocity distribution to a lower modal velocity range. Exposure to 188 mg/kg CH significantly decreased both the percentage of motile and progressively motile sperm. This CH exposure shifted the straight-line velocity distribution to a lower modal velocity range. These are the first reproductive toxicity data from exposure to BDCM and CH. The observed effects on sperm motion occurred in the absence of carcinogenesis. Because the effects of BDCM on sperm motility occurred at a lower exposure than that of other DBPs that compromise sperm motility, a thorough reproductive evaluation now is underway.

## B. Meeting abstracts reporting developmental or reproductive toxicity

**Dose additivity of atrazine and bromodichloromethane in causing pregnancy loss in F344 rats**
Narotsky MG, Best DS, Bielmeier SR and Cooper RL
Toxicologist. 2003;72:77

Atrazine (ATRZ), a widely used herbicide, and bromodichloromethane (BDCM), a disinfection by-product found in drinking water, have both been shown to cause pregnancy loss, i.e., full-litter resorption (FLR), in F344 rats. Although chemically quite different, both ATRZ and BDCM have similar modes of action; ATRZ- and BDCM-induced pregnancy loss are associated with reduced levels of luteinizing hormone (LH) and progesterone during the LH-dependent period of gestation. ATRZ and BDCM co-exist in drinking water; thus, we sought to evaluate their cumulative effect on pregnancy maintenance using a dose-additivity model. Each agent was administered alone at near-threshold doses and in combination at one-half of the near-threshold doses. If synergistic, the two sub-threshold doses would combine to cause a greater-than-threshold response. Both agents were administered by gavage (BDCM in 10% alkamuls EL-620, then ATRZ in 1% methylcellulose) on gestation days 6-10. Each agent was administered alone at 40 mg/kg/d (LOEL = 50 mg/kg); whereas the two were administered together at 20 mg/kg/d each. Dams were allowed to deliver and litters were examined on postnatal days 1 and 6. Uteri of nonparous females were stained with 10% ammonium sulfide to detect FLR. Alone or in combination, the agents were maternally toxic, causing weight loss after the first dose. As expected, 40 mg/kg of each chemical was a near-threshold dose for causing FLR; low rates (5-6%; 1 affected of 18-19 dams) were seen for each chemical alone. In combination, no FLR was seen (n = 19). Thus, in this study, ATRZ and BDCM clearly lacked synergy in their ability to

cause pregnancy loss in F344 rats. Although we did not assess the possibility of antagonism, these results are consistent with the default risk-assessment assumption of dose additivity for agents with the same mechanism.

## C. Studies reporting no developmental or reproductive toxicity

**Oral (drinking water) two-generation reproductive toxicity study of bromodichloromethane (BDCM) in rats.**
Christian MS, York RG, Hoberman AM, Fisher LC and Brown WR
Int J Toxicol. 2002;21**:**115-46

Bromodichloromethane (BDCM) was tested for reproductive toxicity in a two-generation study in CRL SD rats. Thirty rats/sex/ group/generation were continuously provided BDCM in drinking water at 0 (control carrier, reverse osmosis membrane-processed water), 50,150, and 450 ppm (0, 4.1 to 12.6, 11.6 to 40.2, and 29.5 to 109.0 mg/kg/day, respectively). Adult human intake approximates 0.8 microg/kg/day (0.0008 mg/kg/day). P and F1 rats were observed for general toxicity (viability, clinical signs, water and feed consumption, body weights, organ weights [also three weanling Fl and F2 pups/sex/litter], histopathology [10/sex, 0- and 450-ppm exposure groups]) and reproduction (mating, fertility, abortions, premature deliveries, durations of gestation, litter sizes, sex ratios, viabilities, maternal behaviors, reproductive organ weights [also three weanling Fl and F2 pups/sex/ litter], sperm parameters, and implantations. F1 rats were evaluated for age at vaginal patency or preputial separation. Ten P and F1 rats/sex from the 0- and 450-ppm exposure groups and rats at 50 and 150 ppm with reduced fertility were evaluated for histopathology (gross lesions, testes, intact epididymis, all F1 dams for number of primordial follicles). Developmental parameters in offspring included implantation and pup numbers, sexes, viabilities, body weights, gross external alterations, and reproductive parameters (Fl adults). Toxicologically important, statistically significant effects at 150 and/or 450 ppm included mortality and clinical signs associated with reduced absolute and relative water consumption, reduced body weights and weight gains, and reduced absolute and relative feed consumption (P and F1 rats). Significantly reduced body weights at 150 and 450 ppm were associated with reduced organ weights and increased organ weight ratios (% body and/or brain weight). Histopathology did not identify abnormalities. Small delays in sexual maturation (preputial separation, vaginal patency) and more Fl rats with prolonged diestrus were also attributable to severely reduced pup body weights. Mating, fertility, sperm parameters, and primordial ovarian follicular counts were unaffected. The no-observable-adverse-effect level (NOAEL) and the reproductive and developmental NOAELs for BDCM were at least 50 ppm (4.1 to 12.6 mg/kg/day), 5125 to 15,750 times the human adult exposure level, if delayed sexual maturational associated with severely reduced body weights is considered reproductive toxicity. If considered general toxicity, reproductive and developmental NOAELs for BDCM are greater than 450 ppm (29.5 to 109.0 mg/kg/day), or 36,875 to 136,250 times the human adult exposure level. Regardless, these data indicate that BDCM should not be identified as a risk to human reproductive performance or development of human conceptuses.

**Oral (drinking water) developmental toxicity studies of bromodichloromethane (BDCM) in rats and rabbits.**
Christian MS, York RG, Hoberman AM, Diener RM and Fisher LC
Int J Toxicol. 2001;20:225-37.

Crl:CD(SD)IGS BR VAF/Plus (Crl SD) rats and Hra(NZW) SPF rabbits were tested for potential developmental toxicity from bromodichloromethane (BDCM) provided continuously in the drinking water during gestation (gestation days [GDs] 6 to 21 in rats and GDs 6 to 29 in rabbits). Concentrations of 0, 50, 150, 450, or 900 ppm of BDCM were used for rats; 0, 15, 150, 450, or 900 ppm were used for rabbits (in dose range-finding studies, 1350 ppm was excessively maternotoxic to both species). Investigated maternal parameters included viability, clinical signs, water and feed consumption, and body weights. Maternal gross lesions, gravid uterine weights, abnormal placentas, and numbers of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions were observed at time of Caesarean sectioning (GD 21 in rats; GD 29 in rabbits). Body weights, sex ratios, and morphological abnormalities (external, soft tissue, and skeletal) were noted in the fetuses. Mean consumed doses of BDCM were calculated to be 0, 2.2, 18.4, 45.0, or 82.0 mg/kg/day for the rats, and 0, 1.4, 13.4, 35.6, or 55.3 mg/kg/day for the rabbits (approximate human intake is 0.8 microg/kg/day [0.0008 mg/kg/day] in adults). In pregnant rats, toxicologically important, statistically significant effects included reduced absolute (g/day) and relative (g/kg/day) water consumption values at > or =50 ppm (2.2 mg/kg/day) and reduced body weight gains (also when corrected for gravid uterine weight) and absolute (g/day) and relative (g/kg/day) feed consumption values at >450 ppm (45.0 mg/kg/day). These parameters were also significantly reduced at > or =450 ppm (35.6 mg/kg/day) in pregnant rabbits (significant weight loss occurred in the rabbits at 900 ppm, i.e., 55.3 mg/kg/day). Thus, the maternal no-observable-adverse-effect level (NOAEL) for BDCM was 150 ppm, i.e., 18.4 and 13.4 mg/kg/day in rats and rabbits, respectively. No adverse effects on embryofetal viability, growth, sex ratio, gross external, soft tissue, or skeletal morphology occurred at 900 ppm in rats or rabbits. Minimal delays in the ossification of forepaw phalanges and hindpaw metatarsals and phalanges occurred in rat fetuses at 900 ppm; delays were considered marginal, reversible, and associated with severely reduced maternal weight gain. Therefore, the developmental NOAEL for rats was 450 ppm (45.0 mg/kg/day), whereas in rabbits it was 900 ppm (55.3 mg/kg/day). These NOAELs are 56,250 and 69,120 times the human adult exposure level of 0.0008 mg/kg/day, respectively. Based on the results of these studies, BDCM should not be identified as a risk to development of human conceptuses.

**Biodisposition of dibromoacetic acid (DBA) and bromodichloromethane (BDCM) administered to rats and rabbits in drinking water during range-finding reproduction and developmental toxicity studies.**
Christian MS, York RG, Hoberman AM, Diener RM, Fisher LC and Gates GA
Int J Toxicol. 2001;20:239-53.

Dibromoacetic acid (DBA) and bromodichloromethane (BDCM), by-products of chlorine disinfection of water, were provided in drinking water in range-finding

reproductive/developmental toxicity studies (rats) and a developmental toxicity study (BDCM) in rabbits. Studies included absorption and biodisposition of DBA and BDCM, including passage into placentas, amniotic fluid, fetuses (rats and rabbits), or milk (rats). The DBA and BDCM range-finding reproductive/developmental toxicity studies each included 50 Sprague-Dawley rats/sex/group. DBA (0, 125, 250, 500, or 1000 ppm) or BDCM (0, 50, 150, 450, or 1350 ppm) was provided in drinking water 14 days premating through gestation and lactation (63 to 70 days). The developmental toxicity range-finding study included 25 time-mated New Zealand white rabbits/group given 0, 50, 150, 450, or 1350 ppm BDCM in drinking water on gestation days (GDs) 6 through 29. Satellite groups (6 male, 17 female rats/group/study and 4 rabbits/group) were used for bioanalytical sampling. Rats and rabbits had exposure-related reduced water consumption caused by apparent taste aversion to DBA or BDCM, especially in the parental animals at the two highest exposure levels (500 and 1000 ppm DBA; 450 and 1350 ppm BDCM). Female rats consumed slightly higher mg/kg/day doses of DBA than male rats, especially during gestation and lactation; weanling rats consumed the highest mg/kg/day doses. DBA produced detectable and quantifiable concentrations in plasma, placentas, amniotic fluid, and milk. Plasma samples confirmed that rats drink predominately during the dark; this drinking pattern, not accumulation, produced detectable plasma concentrations for 18 to 24 hours/day. No quantifiable concentrations of BDCM occurred in plasma, placentas, amniotic fluid, or milk, suggesting that BDCM is rapidly degraded or metabolized in vivo. DBA (500 and 1000 ppm, rats) and BDCM (450 and 1350 ppm, rats and rabbits) produced secondary toxicity in the parental generation by reducing water consumption, which caused severe exposure-related apparent dehydration, reduced feed intake and weight gain. Reproductive and developmental parameters were essentially unaffected (mating possibly reduced [DBA at 1000 ppm]; exposure-related decreases in body weights of pups secondary to reduced water and feed consumption [DBA at 250, 500, and 1000 ppm; BDCM at 150, 450, and 1350 ppm]). No effects on development of rabbit fetuses occurred at BDCM concentrations as high as 1350 ppm. Results from these preliminary studies, in which DBA and BDCM were provided in the drinking water at concentrations thousands of times higher than those to which humans are exposed, suggest that neither DBA nor BDCM are reproductive/developmental risks for humans.

**Final report on the short term reproductive and developmental toxicity of bromodichloromethane (CAS #75-27-4) administered in drinking water to Sprague-Dawley rats.**
Wolfe GW et al.
R.O.W. Sciences, Inc., Gaithersburg, MD, NTIS Technical Report (NTIS/PB99-111262) (NTP/RDGT-94017) 1998 Oct.:502 pp.

Abstract: The potential toxicity of bromodichloromethane (BDCM; CAS No. 75-27-4) was evaluated using a short-term reproductive and developmental toxicity screen. This study design was selected to identify the physiologic process (development; female reproduction; male reproduction; various somatic organs/processes) that is the most sensitive to bromodichloromethane exposure. The dose range-finding study was conducted at concentrations of 0, 100, 500, 1000, and 1500 ppm of bromodichloromethane in the drinking water for two

weeks. Based on decreases in water consumption, concentrations of 0, 100, 700, and 1300 ppm (Groups 1, 2, 3, and 4, respectively) were selected for the main study. The main study utilized two groups of male rats designated as Group A (non-BrdU treated animals, 10 per group in Groups 1-4) and Group B (BrdU treated, 5 animals in Groups 1, 2, and 3, and 8 animals in Group 4), and three groups of female rats designated as Group A (peri-conception exposure, 10 per group in Groups 1-4), Group B (gestational exposure, 13 per group in Groups 1-4), and Group C (peri-conception exposure, BrdU-treated, 5 animals in Groups 1, 2, and 3, and 8 animals in Group 4). Control animals received deionized water, the vehicle. During the treatment period, all animals except one survived to the scheduled necropsy. Body weights and feed and water consumption were decreased at many of the intervals for the 700 and 1300 ppm dose groups. Body weights were decreased by 5-13% compared to the controls at many of the intervals while feed consumption was decreased 14-47%, and water consumption was decreased by 17-86% at many of the intervals. The overall calculated mean consumption of BDCM for Groups 2-4 was 11, 53, and 88 mg/kg/day, respectively. At necropsy, clinical chemistry and hematology endpoints were unaffected by BDCM treatment except for a 43% increase in the 5'-Nucleotidase in the 1300 ppm A males, which most likely represents interference with the secretion of bile, and a 14% decrease in creatinine in the 100 ppm A males. Necropsy organ weights and organ-to-body weight ratios were comparable to the controls. Gross findings were comparable across all groups. Microscopically, cytoplasmic vacuolization of hepatocytes and individual hepatocyte necrosis were observed in tissues from the 700 and 1300 ppm A males and the 1300 ppm B males, indicative of mild liver damage. Hematopoietic cell proliferation in the spleen was observed in tissues from all dosed A males, but this is most likely an indirect change in response to stress (See Discussion). The Labeling Index (LI) for the liver and kidney from the B males were relatively comparable between treated and control groups, but the LI for the liver and kidneys from the 1300 ppm C females were significantly increased, indicating possible early stimulation of cellular proliferation. There were no treatment-related findings noted in any male and female reproductive parameters. Results of this study indicate that BDCM at doses at and above 700 ppm produced consistent decreases in body weight and food and water consumption in both sexes, but did not result in any male or female reproductive toxicity. From these data, BDCM is taste-aversive and a general toxicant in both sexes at doses at and above 700 ppm.

**A teratological assessment of four trihalomethanes in the rat.**
Ruddick JA, Villeneuve DC, Chu I and Valli VE
J Environ Sci Health B. 1983;18**:**333-49.

Four trihalomethanes were administered by gavage to Sprague-Dawley rats from day 6 to day 15 of gestation. Chloroform (Ch) was administered at levels of 100, 200 and 400 mg/kg and bromoform (Br), bromodichloromethane (BDCM) and chlorodibromomethane (CDBM) were administered at levels of 50, 100 or 200 mg/kg/day. A separate control was used for each compound. Maternal weight gain was depressed in all groups receiving Ch and at the highest dose levels of BDCM and CDBM. Ch administration caused decreased maternal hemoglobin and hematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of Ch but in no

other treatment groups. Evidence of a fetotoxic response was observed with Ch, CDBM and Br but not BDCM. No dose-related histopathological changes were observed in either mothers or fetuses as a result of treatment. None of the chemicals tested produced any teratogenic effects.

**Teratology studies in mice exposed to municipal drinking-water concentrates during organogenesis.**
Kavlock R, Chernoff N, Carver B and Kopfler F
Fd Cosmet Toxicol. 1979;17:343-347.

Organic material concentrated from the drinking-waters of five US cities selected as representative of the major sources of raw water and a sample of low-molecular-weight organohalides were administered to groups of pregnant CD-1 mice on gestation days 7-14 by oral intubation. Each of the six test materials was dissolved in dimethylsulphoxide (DMSO), and dosage levels represented 3000, 1000 and 300 times the anticipated human exposure to these materials. The dams were killed on day 18 of gestation, and the foetuses were examined for skeletal and visceral anomalies. No effects in the foetus were attributable to the administration of DMSO or to the organic materials from the municipal drinking waters. It was concluded that the organic impurities present in a wide sample of municipal drinking-waters possess very little capacity for inducing foetotoxicity in the mouse.

## D. Related articles and meeting abstracts

**Effects of defined mixtures of trihalomethanes and haloacetic acids on pregnancy maintenance and eye development in F344 rats .**
Narotsky, M. G., Best, D. S., Mcdonald, A., Myers, E. A., Hunter, E. S. 3d, and .Simmons, J. E.
Birth Defects Res A Clin Mol Teratol . 2006; 76(5):384.

Abstract: Although disinfection of drinking water is important for control of microbial contamination, it results in the formation of hundreds of disinfection by-products (DBPs). The most prevalent DBPs are trihalomethanes (THMs; chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and haloacetic acids (HAAs; chloroacetic, dichloroacetic, trichloroacetic, bromoacetic, and dibromoacetic acid). THMs and HAAs are regulated in drinking water at 80 and 60 ug/L, respectively. In rats, THMs have been shown to cause pregnancy loss (i.e., full-litter resorption, an all-or-none effect). HAAs have been shown to cause eye defects and partial-litter, as well as full-litter, resorption (i.e., not an all-or-none effect). Here, we assessed the combined toxicity of these DBPs. Rats were treated with mixtures of four THMs (THM4), five BAAs (HAA5), or nine DBPs (DBP9; THM4 + HAA5). Chemical proportions reflected those in tap water; e.g., in DBP9, molar percentages of the respective chemicals were 30.0, 11.9, 7.0, 1.0, 1.7, 22.7, 15.9, 8.2, and 1.5. Mixtures, prepared in 10% Alkamuls® EL-620, were administered daily to F344 rats by gavage on gestation days 6-20. Litters were examined on postnatal days 1 and 6. For the THM4 mixture, pregnancy loss was seen in 0/14, 0/25, 11/14 (79%), and 12/13 (92%) of the dams at 0, 307, 613, and 920 umol/kg,

respectively. Pup weights were reduced at 613 and 920 umol/kg. Postnatal loss was increased at 920 umol/kg. For the HAA5 mixture, pregnancy loss was seen in 0/9, 0/15, 3/17 (18%), and 11/11 (100%) of the dams at 0, 308, 615, and 1231 umol/kg, respectively. Eye malformations (anophthalmia or microphthalmia) were seen in 0, 53%, and 79% of the live litters at 0, 308, and 615 umol/kg. Prenatal loss was unaffected in live litters. For the DBP9 mixture, pregnancy loss was seen in 0/18, 0/19, 6/17 (35%), and 7/8 (88%) of the dams at 0, 307, 615, and 1228 umol/kg, respectively. In live litters, prenatal loss was unaffected. Thus, THM4, HAA5, and DBP9 each caused pregnancy loss at > = 613 umol/kg; i.e., both HAAs and THMs contributed to DBP9-induced pregnancy loss. The presence of THMs in the full mixture, however, reduced the incidence of BAA-induced eye defects.

**Effects of Defined Mixtures of Trihalomethanes and Haloacetic Acids on Pregnancy Maintenance in F344 Rats.**
Narotsky, M. G., Best, D. S., Mcdonald, A., Myers, E. A., Hunter, E. S., and Simmons, J. E.
Birth Defects Res A Clin Mol Teratol . 2005; 73(5):358.

Abstract: Although disinfection of drinking water is vitally important for eliminating microbial contamination, it also causes formation of hundreds of disinfection by-products (DBPs). The most prevalent DBPs are trihalomethanes (THMs; chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and haloacetic acids (HAAs; chloroacetic, dichloroacetic, trichloroacetic, bromoacetic, and dibromoacetic acids). Some epidemiology studies have shown an increased risk of spontaneous abortion associated with consumption of water with high concentrations of THMs. We have previously shown that bromodichloromethane causes pregnancy loss, i.e., full-litter resorption, in F344 rats via a luteinizing hormone-mediated mechanism (low-effect level = 305 umol/kg). HAAs, however, have not been quantified in these epidemiology studies, nor have they been tested in the F344 strain for their effects during gestation. In this study, we tested mixtures of the four THMs (THM4) as well as mixtures of the THMs and HAAs (DBP9; THM4 HAA5). Chemical proportions mimicked those found in drinking water. The DBP9 mixture consisted of ~50% THM4 and ~50% HAA5. Mixtures, prepared in 10% Alkamuls® EL- 620, were administered to F344 rats by gavage on gestation days 6-20. Litters were examined on postnatal days 1 and 6. For the THM4 mixtures, pregnancy loss was seen in 0/5, 0/10, 11/14 (79%), and 12/13 (92%) of the dams at 0, 307, 613, and 920 umol/kg, respectively. Among surviving litters, pup weights were reduced at 613 and 920 umol/kg. Postnatal loss was increased at 920 umol/kg. For the DBP9 mixtures, pregnancy loss was seen in 0/18, 0/19, 6/17 (35%), and 7/8 (88%) of the dams at 0, 307, 615, and 1228 umol/kg, respectively; no effects on pup weight or postnatal mortality were observed. It is noteworthy that all dams maintained pregnancy at 307 umol THM4/kg; however, the addition of HAA5 to this mixture (i.e., 307 umol THM4/kg 308 umol HAA5/kg 615 umol DBP9/ kg) resulted in pregnancy loss. This finding suggests that either HAAs cause pregnancy loss in F344 rats, or that HAAs potentiate the THMs' disruptive effect on pregnancy. We plan to evaluate HAAs for their effects on pregnancy in the F344 rat.

**Effects of bromodichloromethane (BDCM) on ex vivo luteal function in the pregnant F344 rat.**
Bielmeier, S. R., Murr, A. S., Best, D. S., Goldman, J. M., and Narotsky, M. G.
Toxicologist. 2003; 72(S-1):26-27.

Abstract: We have reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption, in F344 rats when treated on gestation day (GD) 6-10, encompassing the luteinizing hormone (LH)-dependent period. BDCM-induced pregnancy loss was associated with reductions in serum progesterone (P) and corresponding decreases in LH on GD 10, suggesting BDCM disrupts the maternal hypothalamic-pituitary-gonadal axis. These and other data indicate that BDCM affects the hypothalamus or pituitary gland; however, an effect on luteal responsiveness to LH had not been definitively excluded. To address this data gap, we used an ex vivo approach to assess luteal function. Dams were dosed by gavage on GD 6-9 (plug day = GD 0) at 0 or 100 mg/kg/d (n = 11, 12). One hour after the GD-9 dose, rats were sacrificed, blood was collected and corpora lutea (CL) were incubated with or without hCG, an LH agonist, to stimulate P secretion. During the 24 h incubation, media were periodically sampled for hormone analysis by dissociation enhanced lanthanide flouroimmmunoassay (DELFIAÑ). Luteal responsiveness was unaffected; both groups displayed a 2.4-fold increase in P secretion in response to hCG challenge. Paradoxically, the BDCM-exposed CL showed greater than 2-fold increases in P secretion ex vivo regardless of the presence of hCG; whereas the same animals, i.e., the CL donors, had decreased serum P and LH levels in vivo. It is unclear if this 'rebound' effect reflects the removal of the CL from a possible direct inhibitory influence of BDCM, or a response to the diminished LH stimulation in vivo. Regardless, the lack of effect on luteal responsiveness is further evidence that BDCM-induced pregnancy loss in the rat is due to reduced pituitary LH secretion.