Serum Hormone Characterization and Exogeneous Hormone Rescue of Bromodichloromethane-Induced Pregnancy Loss in the F344 Rat

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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®. 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®, 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.

Key Words: bromodichloromethane; disinfection by-product; pregnancy loss; progesterone; luteinizing hormone; hormonal pregnancy rescue.

Although the chlorination of public water supplies has been among the most successful of all public health interventions to decrease disease, the process generates a number of biologically active, treatment-related chemicals in the drinking water. Chlorine reacts with natural organic compounds in the source water to produce numerous halogenated by-products, one class of which is the trihalomethanes (THMs). Bromodichloromethane (BDCM), chlorodibromomethane, bromoform, and chloroform constitute the total THMs (TTHMs), regulated as a group at 80 µg/l by the U.S. EPA (U.S. EPA, 1998a) based on their carcinogenic properties. However, there is growing concern regarding the reproductive health effects associated with these compounds (Bove et al., 2002; Graves et al., 2001; Hwang et al., 2002; Levin et al., 2002).

Epidemiological studies have associated exposure to drinking water containing high TTHMs with adverse reproductive outcomes. Waller et al. (1998) reported an association between exposure to THMs in drinking water and spontaneous abortion (SAB). Increased SAB rates were associated with high TTHM level (≥75 µg/l) and daily consumption of at least five glasses of cold tap water [odds ratio (OR) = 2.0, 95% confidence interval (CI) = 1.1–3.6]. SABs in women with high personal TTHM exposure occurred on average 1 week earlier than the reference population. When results were stratified by individual THMs, only BDCM showed a significant association, (OR = 3.0, 95% CI = 1.4–6.6) in the high personal exposure group. A study in central North Carolina reported a modest, but not dose-related, association between TTHMs and SAB. However, the association was not evident when water intake was taken into account (Savitiz et al., 1995). In addition to SAB, epidemiological studies have reported other adverse reproductive outcomes associated with tap water constituents or consumption. A Canadian retrospective study of 50,000 singleton births evaluated the relationship between the level of TTHMs and specific THMs in public water supplies and the risk of
stillbirth (King et al., 2000). The strongest association was observed for BDCM exposure, where risk doubled for those exposed to a level of ≥20 µg/l compared to those exposed to a level <5 µg/l (relative risk = 1.98, 95% CI = 1.23–3.49). THMs have been linked to various other adverse reproductive outcomes, such as low birth weight, neural tube defects, uterine growth retardation, and alteration in menstrual cycle (Aschengrau et al., 1993; Bove et al., 1995; Dodds and King, 2001; Gallagher et al., 1998; Klotz and Pyrch, 1999; Kramer et al., 1992; Windham et al., 2003).

Due to its volatility, exposure to BDCM can be through dermal and inhalation routes, as well as via oral consumption. The U.S. EPA Office of Water estimates BDCM drinking water consumption at 0.8 µg/kg/day (U.S. EPA, 1998b); however, dermal and inhalation exposures are proving much more important than ingestion, making oral consumption a poor exposure index of human blood levels. In a human exposure study using tap water with an average TTHM concentration of 35 µg/l (well below the maximum contaminant level), dermal and inhalation exposures associated with a ten-minute shower or bath resulted in much higher blood levels of THMs than drinking one liter of water within 10 min (Backer et al., 2000). Specifically, in men who took showers, median blood BDCM levels started at 3.3 ng/l before showering, then rose to 19.4 ng/l 10 min after showering, and decreased to 10.3 ng/l 30 min after exposure. The median BDCM concentration in the shower water was 6 µg/l. A similar study in women of reproductive age, using water with a median BDCM level of 12 µg/l, reported median blood levels of 7.0 ng/l before showering and 46 ng/l after showering (Lynberg et al., 2001; Miles et al., 2002).

The reproductive toxicity of BDCM in animal studies appears to vary depending on strain, species, and route of administration. Klinefelter et al. (1995) reported effects on sperm motility after 52 weeks of drinking water treatment in male F344 rats. We previously reported BDCM to cause pregnancy loss (i.e., full-litter resorption) in Fischer 344 (F344) rats when treated via oral aqueous gavage on gestational days (GD) 6–10, encompassing the luteinizing hormone (LH)-dependent period, (i.e., GD 7–10) of pregnancy (Bielmeier et al., 2001; Narotsky et al., 1997b). We also reported a dramatic strain difference between F344 rats and Sprague-Dawley (SD) rats; SD rats were insensitive to BDCM-induced pregnancy loss even at much higher, maternally toxic doses (Bielmeier et al., 2001). Consistent with our finding that SD rats were relatively insensitive to BDCM, Christian et al. (2002) reported no direct adverse effects in comprehensive reproductive and developmental bioassays dosing BDCM via drinking water in the SD rat. They also found no developmental effects via drinking water in New Zealand rabbits (Christian et al., 2001a,b, 2002).

The goal of the present study was to further investigate the mode of action of BDCM-induced pregnancy loss in the F344 rat. Specifically, we sought to reexamine the maternal LH profiles during oral exposure to levels of BDCM known to cause pregnancy loss, using a more sensitive assay for LH. Moreover, we wished to assess the temporal patterns of both serum LH and progesterone decreases that occur during BDCM administration. Finally, we sought to verify the roles of LH and progesterone in BDCM-induced pregnancy loss in F344 rats by administering exogenous hormones concurrently with BDCM treatment in an attempt to prevent pregnancy loss.

MATERIALS AND METHODS

Chemicals. Bromodichloromethane (98+% stabilized with potassium carbonate) was obtained from Aldrich Chemical Co. (Milwaukee, WI). BDCM formulations were stored at 4°C in amber vials. To minimize loss due to volatilization, daily aliquots of the formulations were placed in vials with minimal headspace and sealed with Teflon-lined caps. The vehicle was aqueous 10% Alkamuls® EL-620 (ethoxylated castor oil; Rhodia, Cranbury, NJ) in distilled deionized water. Emulsions using Alkamuls EL-620 (formerly known as Emulphor EL-620) are regarded to be appropriate for the study of volatile drinking water contaminants; it has been demonstrated that such emulsions, relative to a water vehicle, do not substantially alter the pharmacokinetics of the test agent (Kim et al., 1990). BDCM dosing formulations were prepared at appropriate concentrations to provide the desired dose when administered at 1 mL/kg body weight. Pharmacokinetic data for BDCM dosed with similar formulations have been previously reported (Narotsky et al., 1997b).

Progesterone, human chorionic gonadotropin (hCG), and corn oil were obtained from Sigma Chemical Co. (St. Louis, MO). Saline was obtained from Abbott Laboratories, Inc. (North Chicago, IL). Progesterone dosing solutions were prepared in corn oil at 0 and 100 mg/kg. hCG (0.5 IU) in 0.9% saline was given at 0.2 mL/rat.

Animals and husbandry. Timed-pregnant F344 rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Animals were 60–120 days old. The day that evidence of mating (copulatory plug or vaginal sperm) was detected was designated gestational day (GD) 0. The animals were individually housed in polycarbonate cages with heat-treated wood shavings supplied as bedding. The animals were provided feed (Purina Lab Chow 5001) and tap water ad libitum and a 12:12 h light:dark cycle. Room temperature and relative humidity were maintained at 22.2 ± 1.1°C and 50 ± 10%, respectively. Dams were euthanized by cervical dislocation, pups by decapitation. All animal care, handling, and treatment procedures conformed to the NIH standards for laboratory animal research and were approved by the Institutional Animal Care and Use Committee at the National Health and Environmental Effects Research Laboratory.

Procedures. In all of the experiments, animals were distributed among treatment groups using a nonbiased randomization procedure that assured a homogeneous distribution of body weights (Narotsky et al., 1997a). The numbers of pregnant females in each group are listed in Table 1. During the morning of assigned treatment days, animals received a single BDCM dose of 0, 75, or 100 mg/kg body weight via oral gavage. Maternal body weights were determined on GD 5–15 and 20. All rats were examined throughout the experimental period for clinical signs of toxicity. Beginning on GD 20, the dams were observed periodically to determine the approximate time of parturition. The stage of parturition (complete, in progress, first pup delivered, or blood only) was recorded. Pups were individually examined and weighed on postnatal days 1 and 6. PD 1 was defined as GD 22, independent of the actual time of parturition; hence, pups were examined at the same time postcoitus. After PD 6, the dams and pups were euthanized. The number of uterine implantation sites was recorded. The uteri of females that did not deliver were stained with 2% (w/v) ammonium sulfide to enhance detection of resorption sites (Narotsky et al., 1997a).
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<th>Pregnant</th>
<th>Fully resorbed</th>
<th>With live litters</th>
<th>No. implants</th>
<th>Prenatal loss (%)</th>
<th>Postnatal loss (%)</th>
<th>No. live pups</th>
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*Statistical significance from controls, p < 0.05.
***Statistical significance from controls, p < 0.001.
Experimental Design

Serum hormone collection. Blood samples for hormone analysis were collected once daily on GD 6–11 from the lateral tail vein of control and 75 mg/kg/day-treated animals. To further characterize the timing of changes in hormone levels between GD 9 and 10, additional experiments were conducted using the same treatment regimen, but with more frequent blood collections. To minimize repeated blood collection on a single animal, this study was divided into two experiments, A and B. In Experiment A, tail blood samples were collected on GD 9 once before dosing, and then 1.5, 3, 4.5, and 6 h after treatment, and then again on GD 10 before dosing. Experiment B continued the timing of collection from Experiment A, starting approximately 8 h after the GD-9 dose and four additional times at 4-hour intervals. All serum samples were analyzed for progesterone and LH.

Hormone replacement to rescue pregnancy. To evaluate the ability of exogenous hormones to prevent BDCM-induced pregnancy loss, progesterone or human chorionic gonadotropin (hCG) were dosed concurrently with BDCM. The BDCM dose was increased to 100 mg/kg/day to ensure high response in positive controls. Exogenous hormone dosing regimens were based on results from published literature and pilot studies. In one dose-setting study, tail blood was collected on GD 6–11 to determine effects on serum progesterone and LH. Progesterone, dissolved in corn oil, was dosed at 10 mg/kg twice daily on GD 6–10 at 1 ml/kg via subcutaneous injection. In a separate experiment, hCG in saline was dosed subcutaneously on GD 8–10 at 0.5 IU/rat at 0.2 ml/rat. Control groups were dosed with vehicles via the same routes as experimental groups: Alkamuls® via oral gavage, with corn oil or saline subcutaneously injected.

Serum Collection. When scheduled, blood collection was conducted in the morning, before BDCM dosing. Blood was collected from the lateral tail vein using a 19-gauge heparinized butterfly needle, while the animal was restrained. Approximately 300 μl of whole blood was collected per sampling in Becton Dickinson Microtainer serum separator tubes, chilled on ice, and then spun at 4°C for 30 min at 1185 × g in a Beckman GS-6R centrifuge. The serum was then transferred to siliconized microcentrifuge tubes (A. Daigger and Company, Inc.) and stored frozen at −80°C.

Hormone Assays. Serum progesterone and estradiol levels were quantified utilizing a direct solid-phase enzyme-linked immunosorbent assay (ELISA). Progesterone and estradiol ELISA kits (DRG Diagnostics, Germany) were used as per provided instructions. The serum samples were thawed and agitated before introduction to the ELISA kit. Progesterone samples were diluted 1:8 with Dulbecco’s phosphate-buffered saline (lot #1013794; Gibco BRL, Life Technologies). Samples were assayed in duplicate. For quality control, three standards (CON6, lot #015; Diagnostics Products Corp.) were also analyzed. Absorbance was measured at 450 nm using a Molecular Devices ThermoMax (Columbia, MD) microplate reader spectrophotometer. The intraassay coefficients of variation involving multiple assays for progesterone ranged from 0.27 to 7.6%; the interassay coefficients of variation ranged from 0.14 to 3.0%.

Serum LH levels were quantified using the rat LH dissociation enhanced lanthanide fluorometric immunoassay (DELFIA). This assay for rat LH was designed by Haavisto et al. (1993) and is a solid-phase assay based on the sandwich technique: one antibody is used to capture the protein of interest (capture antibody), and the other is used to detect it (tracer antibody). In this case, antibody 518B7 (provided by Dr. Jan Roser, UC–Davis, CA) was biotinylated to serve as the capture antibody. The tracer antibody, clone 5303 (Medix Biochemica, Kauniainen, Finland), was labeled with europium. We created the kit formerly available commercially (from Wallace/Perkin Elmer Life Sciences, Inc., Gaithersburg, MD) using identical antibodies and reagents. Wallace/Perkin Elmer performed antibody labeling, as well as provided all other reagents: 96-well DELFIA streptavidin-coated plates, DELFIA assay buffer, wash concentrate, and enhancement solutions. The europium-labeled tracer antibody was diluted and filtered through a 22-μm syringe filter (Millipore, Inc.) prior to each use. Rat LH, provided by the National Hormone and Pituitary Program, was diluted with DELFIA Diluent I (Wallace/Perkin Elmer) to serve as standards. This DELFIA for rat LH is 10- to 50-fold more sensitive than the traditional radiolabeled immunoassay used in our earlier work (Bielmeier et al., 2001); for the DELFIA, the limit of detection with a 25 μl sample is 0.014 ng/ml. The interassay coefficient of variation was 8.9%, the intraassay coefficients of variation ranged from 3.2 to 10.1%.

Statistical analyses. Dams with only one implantation site were considered outliers and were excluded from statistical analyses; there was one such animal in these studies. Fisher’s exact test (Siegel, 1956) was used to compare the incidences of pregnancy loss between appropriate treatment groups. In order to evaluate possible relationships between maternal endpoints and subsequent litter survival, treatment groups were divided into subgroups according to litter survival status, i.e., live versus fully resorbed litters. Maternal body weights, percentage body weight changes, and hormone levels were evaluated by analysis of variance (ANOVA) (Kleinbaum et al., 1988) using the General Linear Models (GLM) procedure on SAS, Release 8 (SAS Institute Inc., Cary, NC). When a significant treatment effect was detected by ANOVA, Student’s t-test on least squares means (LSM) was used to identify individual subgroups that were significantly different from controls, as well as to compare dams with live litters versus dams with pregnancy loss within the same treatment group.

For developmental data, the litter was considered the experimental unit of analysis. Prenatal loss was defined as the number of implantation sites minus the number of live pups, divided by the total number of implantations. Postnatal loss was defined as the number of pups on PD 1 minus the number of pups on PD 6, divided by the number of pups on PD 1. The number of implantation sites, litter size, prenatal and postnatal loss, and pup weights were evaluated by GLM; LSM was used to contrast individual treatment and/or litter survival subgroups against controls. The number of live PD-1 pups was used as a covariate in the analyses of pup weights (Kleinbaum et al., 1988). Similarly, the number of implants was used as a covariate in the analyses of the numbers of live pups. A p value less than 0.05 was considered statistically significant.

RESULTS

Serum Hormone Levels

BDCM-induced pregnancy loss was associated with marked GD-10 reductions in serum progesterone and corresponding decreases in LH. The sensitivity of the DELFIA technique enabled us to demonstrate this previously undetected effect on LH. All nine control dams successfully maintained their litters, whereas 89% (eight of nine) of the BDCM-treated dams (GD 6–10 at 75 mg/kg/day) had pregnancy loss (Table 1). In controls, serum progesterone levels were >100 ng/ml throughout the study. Serum LH levels in these dams were generally 0.11–0.34 ng/ml through GD 9, but fell to mean ± SE values of 0.061 ± 0.03 ng/ml. In contrast, all BDCM-treated dams with pregnancy loss had significantly reduced progesterone levels on GD 10; all values were <40 ng/ml (Fig. 1). However, progesterone levels were comparable to controls on GD 6–9. Serum LH levels in treated animals were less than controls from GD 7 to 10, but achieved significance only on GD 7 and 10. At the later time point, five of seven dams had values below the lowest standard (0.03 ng/ml); mean ± SE values were 0.08 ± 0.03 ng/ml, versus 0.15 ± 0.02 ng/ml for controls (Fig. 2). Estradiol levels were also assayed, but frequently the values were at or below the lowest standard (13.5 pg/ml), and no differences between treatment groups were evident (data not shown).

Two studies were conducted to more precisely define the
time-course of progesterone and LH decreases. In Experiment A, hormone levels were evaluated every 2 h from dosing (0 h) to 8 h postdosing. In Experiment B, hormones were evaluated every 4 h between 8 and 24 h post GD 9 dosing. In Experiment A, 80% (8 of 10) of the animals had pregnancy loss, compared to 17% (one of six) of control animals (Table 1). The control animal with pregnancy loss maintained LH levels consistent with the other controls; progesterone levels were also comparable until a sharp decline was evident on GD 10. Unlike typical BDCM-induced resorptions, these resorption sites were not visible until after ammonium sulfide staining, perhaps indicating an earlier time of resorption (Narotsky et al. 1997a).

In Experiment B, 33% (three of nine) of treated animals had full-litter resorption, compared to 0% (zero of five) of controls (Table 1). In both experiments, a reduction in serum LH relative to controls preceded a reduction in progesterone in animals that resorbed their pregnancy. In Experiment A, serum LH values were already significantly decreased in treated animals in the initial sample on GD 9. However, serum progesterone levels were not significantly reduced until 2 h after the GD 9 dose. In Experiment B, the six animals that were treated with BDCM and maintained their litters had significantly decreased LH and progesterone levels (Fig. 3).

### Hormone Supplementation to Rescue Pregnancy

Both progesterone and hCG reduced the incidence of BDCM-induced pregnancy loss. Dams dosed with BDCM (100 mg/kg/d) on GD 6–10 and the hormone vehicles (corn oil or saline) dosed subcutaneously displayed a 70% (7 of 10) rate of pregnancy loss. Dams dosed with BDCM and progesterone twice daily had a 0% (zero of eight) incidence of pregnancy loss. Dams dosed with BDCM and hCG on GD 8–10 had an 11% (one of nine) incidence of pregnancy loss (Fig. 4).

### DISCUSSION

The DELFIA technique dramatically improved our ability to quantify LH at low levels. This allowed us to see previously undetectable, but highly important, reductions in serum LH. Information from this new method changes our hypothesis regarding the mode of action of BDCM-induced pregnancy loss. The newly detected, corresponding reduction in LH, along with the reduction in progesterone, strongly suggests that BDCM alters LH secretion rather than luteal responsiveness alone, as we previously reported.

In cases of BDCM-induced pregnancy loss, serum progesterone levels begin to decrease on the morning of GD 9 and are significantly decreased by the morning of GD 10. Given that this is before serum progesterone levels decrease during pseudopregnancy (Pang and Behrman, 1979), a situation without the conceptus, it suggests this serum progesterone decrease is a reflection of the maternal capability to maintain pregnancy, indicating the decrease in progesterone is a cause, rather than a result in BDCM-induced pregnancy loss.
The two separate experiments (A and B) with multiple collection times between GD 9 and 10, interrelated well with respect to relative serum hormone levels between treatment groups; however, the rate of pregnancy loss in the studies varied greatly. The varying response rate has occurred in the past and is not fully understood. The main goal of these experiments was to investigate the chronology of the progesterone and LH decreases. Once-a-day sampling did not clearly distinguish which hormone was affected first. Since LH stimulates the corpora lutea to secrete progesterone, one would expect a decrease in LH before a decrease in progesterone, as was seen here. In Experiment A, with collections soon after GD 9 dosing, a reduction in LH, relative to controls, was detected before a reduction in progesterone in nearly all cases. In Experiment B, with collections beginning 8 h after the GD 9 dose, serum LH was decreased relative to controls at the first collection in all treated animals, but decreases in serum progesterone were not evident in animals that resorbed their litters until 16 h after the GD 9 dose. These data support the hypothesis that the reduction in serum LH is a prerequisite to the reduction in progesterone, resulting in pregnancy loss. However, it is also clear that a significant decrease in LH is not the sole determinant in pregnancy outcome. BDCM caused decreases in LH levels in all animals; some rats maintained their pregnancies despite serum LH levels that were less than animals that lost their pregnancies. It should be noted that these time points are hours apart, analyzing a hormone that is released in an hourly pulsatile fashion during pregnancy (Gallo et al., 1985). Perhaps, reflecting this pulsatility, the standard error values are relatively large for serum LH in these studies. The severity and longevity of the disruption of LH pulsatility may be better predictors of pregnancy loss than single daily values of circulating LH.

Hormone replacement studies offer further evidence regarding the mode of action of pregnancy loss. Progesterone and hCG each dramatically reduced the incidence of BDCM-induced pregnancy loss. The capability of exogenous progesterone to prevent BDCM-induced pregnancy loss supports the hypothesis that the mode of action is maternally mediated, rather than directly embyotoxic. The ability of hCG, a LH agonist, to prevent pregnancy loss supports the hypothesis that BDCM-induced pregnancy loss is mediated, at least in part, by an effect on LH secretion. Furthermore, these findings suggest that the corpora lutea were able to secrete progesterone in response to hCG. Alternatively, the administered hCG may have been a dose large enough to stimulate progesterone release despite reduced luteal responsiveness. Regardless, the fact that an LH agonist can prevent BDCM-induced pregnancy loss strongly implicates an LH-mediated mode of action.
Recent human data suggest that inhalation and dermal exposure model systems should be investigated. This is of concern because animal toxicological studies vary greatly based on exposure route, as well as species and strain. This phenomenon of contrasting outcomes with different modes of exposure has also been reported in the BDCM carcinogenicity bioassays. Kidney and colon tumors were produced in rats and liver tumors in mice dosed via oral gavage; whereas, exposure via drinking water only produced liver neoplasms in the rat at comparable doses (George et al., 2002; NTP, 1987). It should be noted that in the Christian et al. (2001b) range-finding study for a developmental study in rats exposed via drinking water, they were unable to detect BDCM in plasma, placentae, amniotic fluid, or milk; however, BDCM has been detected in human blood. Higher human blood levels occurred after showering or bathing, rather than drinking water (Backer et al., 2000). Due to the relative insensitivity of the analytical technique (0.11 μg BDCM limit of detection) in the Christian et al. (2001b) report, it is difficult to make direct comparisons. Because body levels of BDCM through oral uptake are much less than via inhalation or dermal absorption, the administered drinking water concentration would have to be so high it would affect palatability, making the desired internal dose unattainable via drinking water. Regardless, current human exposure data indicate that the drinking water route poorly mimics human exposure scenarios.

Drinking water studies in rodents may not be the most relevant exposure route. According to recent studies, humans receive bolus exposures during showering, bathing, or doing other household related activities with water. Blood levels dramatically decrease within an hour after exposure. Animal dosing that also results in a rapid increase of BDCM blood levels would better replicate this exposure scenario. Toxicokinetic models in male F344 rats predict that, via the gavage route, blood levels peak within 15 min and diminish very quickly (Lilly et al., 1998). Thus, this pattern, following gavage dosing, may better mimic the human internal dose profile than does the pattern following drinking water administration.

Further investigation of these phenomena may provide insight into the epidemiological association of BDCM and reproductive toxicity. We hypothesize that BDCM is disrupting hypothalamic control of pituitary LH secretion. If this is confirmed, this could be relevant to the epidemiological studies implicating BDCM in miscarriage. First, if BDCM disrupts LH secretion via GnRH signaling in the rat, this could have implications in the human as LH secretion in the rat and placental hCG secretion in humans are both regulated by GnRH (Fink, 1988). GnRH, from the cytotrophoblast, stimulates human placental trophoblast cells to secrete hCG, a hormone critical for human pregnancy maintenance in the first trimester (Currie et al., 1992). It is important to note that LH and hCG act on the same receptor to elicit the same response (Griffin and Ojeda, 1996). Recently, Chen et al. (2003) demonstrated that BDCM perturbs hCG secretion from differentiated human placental trophoblasts in vitro. Also, altered LH secretion in women could potentially hinder normal menstrual cycling and/or fertility in women. Disruption of the menstrual cycles of women has recently been associated with disinfection by-products (Windham et al., 2003).

In summary, these experiments involving the F344 rat clearly support the hypothesis that BDCM acts via an LH-mediated mode of action, disrupting LH secretion. We demonstrated that BDCM administration decreases serum LH and progesterone levels in a chronological fashion, and that exogenous progesterone and hCG can protect against BDCM-induced pregnancy loss. However, an effect on LH responsiveness of the corpora lutea may also contribute to BDCM-induced pregnancy loss in the F344 rat.

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