Effect of Dosing Vehicle on the Developmental Toxicity of Bromodichloromethane and Carbon Tetrachloride in Rats

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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 (CCL4) 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, CCL4 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 CCL4) compared to their aqueous-vehicle counterparts (21% for BDCM, 8% for CCL4). 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 CCL4 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.

A variety of halocarbons are of environmental concern due to their presence in drinking water supplies. Industrial halocarbons, e.g., carbon tetrachloride (CCL4), trichloroethylene, and tetrachloroethylene, are found in drinking water as a result of leakage from hazardous waste sites or industrial facilities (Deinzer et al., 1978; DHHS and U.S. EPA, 1987). CCL4 has been found in leachate from industrial landfills at concentrations ranging from <10 to 92 µg/L (Brown and Donnelly, 1988). Trihalomethanes (THMs), such as chloroform, bromoform, and bromodichloromethane (BDCM), are common drinking water contaminants as a consequence of water disinfection by chlorination (Deinzer et al., 1978). In 42 drinking water systems, BDCM levels averaged 12.7 µg/L with maximum concentrations reaching 183 µg/L (U.S. EPA, 1994). Concerns regarding the presence of these chemicals in drinking water are heightened by a recent epidemiologic study in California indicating an increased risk of spontaneous abortion among pregnant women consuming large amounts of water containing THMs, particularly BDCM (Waller, in press).

Due to the poor water solubility of halocarbons, toxicity studies with these chemicals have often used corn oil or other digestible oils as the dosing vehicle rather than an aqueous vehicle. However, since human exposure to these environmental chemicals often occurs from consumption of drinking water, several workers have investigated the effects of the vehicle on the toxicity of these agents. Withey et al. (1983) demonstrated that the vehicle can have substantial effects on the pharmacokinetics and systemic bioavailability of orally administered halocarbons in rats. They reported slower uptake, lower peak blood levels, and more complex pharmacokinetics for dichloromethane, 1,2-dichloroethane, chloroform, and trichloroethylene when administered in an oil-based vehicle as compared to an aqueous vehicle. Kim et al. (1990b) reported that the acute hepatotoxicity of CCL4 was more severe when administered by gavage in an aqueous vehicle compared to corn oil. In contrast, Larson et al. (1994) demonstrated greater hepatotoxic effects of chloroform in female B6C3F1 mice when administered by gavage in corn oil than when administered in drinking water. This finding, however, may have been due to the dose-rate differences between bolus gavage and water consumption. Lilly et al.
(1994) showed that the influence of the vehicle on halocarbon toxicity in rats may be dose-dependent; at 200 mg/kg, BDCM was more toxic to the kidney when administered in the aqueous vehicle, but at 400 mg/kg, it was more nephro- and hepatotoxic in corn oil.

In the present study we tested two volatile organic compounds, CCl₄ and BDCM, for their developmental toxicity in aqueous and oil vehicles. We have shown that both compounds, among others in this class (trichloroethylene, tetrachloroethylene, and bromoform), cause pregnancy loss, i.e., full-litter resorption (FLR), when administered orally in corn oil to Fischer-344 rats during organogenesis (Narotsky et al., 1992; Narotsky and Kavlock, 1995); no-effect levels for FLR have not been established for either BDCM or CCl₄. Wilson (1954; Wilson et al., 1969) also reported an increased incidence of FLR following oral or subcutaneous administration of CCl₄ to pregnant rats for 2–3 days. As in our laboratory, CCl₄-induced FLR appeared to be an "all-or-none" phenomenon in that resorption rates were normal in surviving litters. Schwetz et al. (1974) reported sternebral alterations and reduced fetal size in rats when CCl₄ was inhaled at maternally toxic concentrations during organogenesis. BDCM, when administered by gavage in corn oil to rats, was also nonteratogenic; no clear signs of developmental toxicity were demonstrated (Ruddick et al., 1983).

The all-or-none nature of halocarbon-induced FLR (Narotsky et al., 1992; Wilson, 1954; Wilson et al., 1969) suggests a maternal mediated mechanism. Recent data regarding the critical period of CCl₄-induced FLR (Narotsky et al., in press) and the ability of exogenous hormones to rescue the pregnancy in CCl₄-exposed FLR (Narotsky et al., 1997) suggest that the administered halocarbon is not directly embryolethal, but causes FLR by disrupting the endocrinological maintenance of pregnancy.

In order to assess the developmental toxicity of BDCM and CCl₄ in different vehicles, we used an in vivo developmental toxicity screen developed by Chernoff and Kavlock (1982). In this screening protocol, the dams were exposed to the agents during organogenesis and were allowed to deliver; the offspring were examined postnatally for growth and viability. In addition, we measured blood levels of BDCM after a single dose during pregnancy to examine the pharmacokinetics of this compound in the two vehicles.

METHODS

Chemicals. Carbon tetrachloride (99.9+%) was obtained from Fisher Scientific Co. (Fair Lawn, NJ) and bromodichloromethane (98+%, stabilized with potassium carbonate) was obtained from Aldrich Chemical Co. (Milwaukee, WI). BDCM solutions and emulsions were stored at 4°C. The vehicles were corn oil (Sigma Chemical Co., St. Louis, MO) and 10% aqueous emulphor EL-620 (castor oil ethoxylated; Rhone Poulenc, Cranbury, NJ) in distilled deionized water. All dosing formulations were prepared at appropriate concentrations to provide the desired dose when administered at 1 ml formulation/kg body weight; we avoided using higher dose volumes to preclude oil-induced intestinal injury (Capasso et al., 1994). Dose volumes were based on individual gestation day-6 (GD-6) body weights and remained constant throughout the dosing period. Dosing formulations were stored in amber vials with Teflon-lined caps.

Animals and husbandry. Timed-pregnant Fischer-344 rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The day that evidence of mating (copulatory plug or vaginal sperm) was detected was designated GD 0. The animals were provided feed (Purina Lab Chow 5001) and tap water ad libitum and a 12:12 light/dark cycle. Room temperature and relative humidity were maintained at 22.2 ± 1.1°C and 50 ± 10%, respectively.

Procedures. Each chemical was tested separately, and two replicates were conducted for each chemical. For each replicate, animals were distributed among eight treatment groups (four dose levels per vehicle) using a nonbiased procedure that assured a homogeneous distribution of body weights among groups. Animals received the test agent (BDCM or CCl₄) by gavage once daily on GD 6–15 at 0, 25, 50, or 75 mg/kg or either the oil or aqueous vehicle. These dosages were selected on the basis of our previous work where corn oil was used as the vehicle. In those studies, CCl₄ caused FLR, reduced pup weights, and maternal toxicity at 112.5 and 150 mg/kg/day (Narotsky and Kavlock, 1995); and BDCM caused maternal toxicity and FLR at 50–100 mg/kg/day and reduced pup weights at 100 mg/kg/day (Narotsky et al., 1992).

Maternal body weights were determined on GD 5, 6, 8, 10, 13, 16, 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 (completed, in progress, or first pup delivered) was also recorded. Postnatal day (PD) 1 was defined as GD 22 independent of the actual time of parturition; hence, litter examinations were performed on the same day postcoitus. Pups in each litter were individually examined and weighed on PD 1 and 6. After the PD 6 examinations, the dams were killed and the number of uterine implantation sites was recorded. The uteri of females that did not deliver were stained with 10% ammonium sulfide in order to detect cases of full-litter resorption.

In a separate experiment, blood BDCM-concentration data were collected from three or four rats per vehicle per time point after receiving a single dose of 75 mg/kg on GD 6. The vehicles and dosing volume were the same as described above. Animals were killed 30 min, 90 min, 4.5 h, or 24 h postdosing. Whole blood was collected and analyzed as described by Lilly et al. (1994). Pregnancy status was confirmed at necropsy; due to the limited size of the data set, and the similar values between pregnant and nonpregnant/pseudopregnant animals, all females were included in the analyses.

Statistics. For the developmental toxicity screens with BDCM and CCl₄, dams that died or had only one implant were excluded from statistical analyses. Replicates were pooled for each agent and continuous variables were evaluated by analysis of variance (ANOVA) or covariance (Kleinbaum et al., 1988) using the general linear models (GLM) procedure on SAS (1988). Since we assumed a priori that treatment could only reduce the number of surviving progeny, one-tailed tests were used for pertinent data. Gestation lengths in each replicate were ranked according to the time and stage that parturition was observed; GLM analyses were applied to the ranked data (Conover and Iman, 1981). Pup weights were analyzed as litter means with the number of live PD 1 pups as a covariate. Similarly, the number of implants was used as a covariate in the analysis of litter size.

When a significant treatment effect was detected, Student's t test on least-squares means was used for pairwise comparisons between individual halocarbon-treated groups and their same-vehicle control and between groups receiving different vehicles at the same dosage. The incidences of FLR in groups receiving the same dosage but different vehicles were compared using Fisher's Exact Test. In addition, ANOVA was used to compare replicates and to evaluate possible vehicle–dose interactions on maternal weight gain during GD 6–8 as well as on the incidence of FLR. For the BDCM pharmacokinetics study, differences between vehicles were tested at each
time point by GLM analysis of duplicate samples averaged for each animal. No adjustments for multiple comparisons were made. The elimination half-life (t1/2) of BDCM after administration in each of the two vehicles was calculated using a two-compartment analysis of the blood concentration versus time data (Klaassen and Rozman, 1986).

Since the benchmark dose (BMD) approach has been proposed as an improvement over the no-observed-adverse-effect-level (NOAEL) approach for noncancer risk assessment (Crump, 1984; Barnes et al., 1995), the BMD for developmental toxicity was determined for each agent-vehicle combination. The BMD was defined as the lower 95% confidence limit of the administered dose predicted to cause a 5% increase in response (Allen et al., 1994). For endpoints with a zero background rate, this predicted dose is the estimated 5% effect dose (ED05). Teralog software (Howe, 1994) was used to calculate the ED05 and BMD for FLR using a generalization of a log-logistic model (Kupper et al., 1986). Intralitter correlations and litter-size variables were set to zero and, due to the all-or-none nature of the FLR endpoint, litter sizes were set to one.

RESULTS

BDCM

Maternal effects. One female, receiving 75 mg BDCM/kg in corn oil, died in the study. Clinical findings observed only in animals receiving the oil vehicle included kyphosis (75 mg/kg) and chromodacryorrhea/lacrimation (50 mg/kg). Piloerection was seen at 75 mg/kg for both vehicles and at 50 mg/kg in females receiving the aqueous vehicle. Maternal weight loss on GD 6–8 was evident at 50 and 75 mg/kg for both vehicles (Fig. 1); mean GD-6 body weights are presented in Table 1. At 25 mg/kg, a significant reduction in weight gain, compared to vehicle controls, was seen only for dams receiving the aqueous vehicle. Weight gains for the GD 6–8 period were comparable between vehicles at all dose levels. Two-way ANOVA of this endpoint did not indicate an interaction between vehicle and dose. However, there was a significant replicate effect (p < 0.001); rats from the earlier replicate tended to experience reduced weight gains or greater weight losses than from the later replicate (data not shown).

Developmental effects. All control and 25 mg/kg litters survived the experimental period; however, FLR was observed at the higher dose levels for both vehicles (Table 1; Fig. 2). The results of ED05 and benchmark dose determinations for this endpoint are presented in Table 2. For dams receiving corn oil, FLR was seen in 8 and 83% of the litters at 50 and 75 mg/kg, respectively. An additional 75 mg/kg litter was maintained to term but was delivered late (GD 23) and died by PD 6. For dams receiving the aqueous vehicle, 17 and 21% of the litters were fully resorbed at 50 and 75 mg/kg, respectively. At 75 mg/kg, BDCM caused a significantly greater incidence of FLR in corn oil (83%) than in the aqueous vehicle (21%). ANOVA of FLR incidence revealed a significant (p < 0.001) vehicle–dose interaction and no differences between replicates. In surviving litters, there were no treatment effects on gestation length, pre- or postnatal survival, pup weight (Table 1), or pup morphology.

Pharmacokinetic data. Blood concentrations of BDCM following GD-6 exposure to 75 mg/kg in oil and aqueous vehicles are presented in Fig. 3. Since the benchmark dose (BMD) approach has been proposed as an improvement over the no-observed-adverse-effect-level (NOAEL) approach for noncancer risk assessment (Crump, 1984; Barnes et al., 1995), the BMD for developmental toxicity was determined for each agent-vehicle combination. The BMD was defined as the lower 95% confidence limit of the administered dose predicted to cause a 5% increase in response (Allen et al., 1994). For endpoints with a zero background rate, this predicted dose is the estimated 5% effect dose (ED05). Teralog software (Howe, 1994) was used to calculate the ED05 and BMD for FLR using a generalization of a log-logistic model (Kupper et al., 1986). Intralitter correlations and litter-size variables were set to zero and, due to the all-or-none nature of the FLR endpoint, litter sizes were set to one.

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TABLE 1
Data Summary for Rats Treated by Gavage on Gestation Days 6–15 with Bromodichloromethane (BDCM) or Carbon Tetrachloride (CCL)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Vehicle</th>
<th>Dose (mg/kg)</th>
<th>No. dams</th>
<th>Maternal GD-6 body weight (g)</th>
<th>No. litters delivered</th>
<th>Day-1 live pups</th>
<th>Pup weight (g)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>BDCM</td>
<td>Corn oil</td>
<td>0</td>
<td>12</td>
<td>173.0 ± 3.3</td>
<td>12</td>
<td>8.6 ± 0.6</td>
<td>5.4 ± 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>14</td>
<td>174.9 ± 2.8</td>
<td>14</td>
<td>10.0 ± 0.5</td>
<td>5.3 ± 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>13</td>
<td>173.3 ± 2.5</td>
<td>12</td>
<td>8.6 ± 0.8</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>12</td>
<td>174.3 ± 2.1</td>
<td>2*</td>
<td>6</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>0</td>
<td>14</td>
<td>172.4 ± 3.0</td>
<td>14</td>
<td>7.9 ± 0.7</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>12</td>
<td>175.7 ± 2.8</td>
<td>12</td>
<td>8.7 ± 0.7</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
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<td>50</td>
<td>12</td>
<td>174.2 ± 2.7</td>
<td>10</td>
<td>8.0 ± 0.8</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>14</td>
<td>171.6 ± 2.6</td>
<td>11</td>
<td>8.3 ± 0.8</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td>CCl₄</td>
<td>Corn oil</td>
<td>0</td>
<td>13</td>
<td>162.2 ± 3.2</td>
<td>13</td>
<td>8.5 ± 0.8</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>13</td>
<td>160.2 ± 4.0</td>
<td>13</td>
<td>8.5 ± 0.6</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
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<td></td>
<td>50</td>
<td>12</td>
<td>162.3 ± 4.4</td>
<td>7</td>
<td>9.6 ± 0.7</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
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<td></td>
<td>75</td>
<td>12</td>
<td>160.3 ± 3.7</td>
<td>4</td>
<td>7.5 ± 1.2</td>
<td>5.4 ± 0.1</td>
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<tr>
<td></td>
<td>Aqueous</td>
<td>0</td>
<td>12</td>
<td>163.9 ± 3.7</td>
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<td>12</td>
<td>159.1 ± 3.3</td>
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<td>8.3 ± 0.6</td>
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<td>14</td>
<td>159.7 ± 3.4</td>
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<td>8.8 ± 0.9</td>
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<td>12</td>
<td>163.1 ± 4.0</td>
<td>11</td>
<td>8.4 ± 0.8</td>
<td>5.3 ± 0.1</td>
</tr>
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</table>

Note. Values are group means ± SE. Pup weights are group means of the litter means.
* Includes one litter that was delivered late and was not viable.

**Developmental effects.** All litters exposed to 0 or 25 mg/kg survived the experimental period; however, FLR was seen at the two higher doses for both vehicles (Table 1; Fig. 2). The results of the ED05 and benchmark dose determinations for this endpoint are presented in Table 2. For dams receiving corn oil, 42 and 67% of the litters were fully resorbed at 50 and 75 mg/kg, respectively. For the aqueous-vehicle groups, the respective incidences were 14 and 8%. At 75mg/kg, the incidence of FLR with the aqueous vehicle (8%) was significantly less than with corn oil (67%). ANOVA of FLR incidence revealed a significant (p < 0.01) vehicle–dose interaction, but no differences between replicates. For surviving litters, there were no effects on gestation length, pre- or postnatal survival, or pup morphology. Non-dose-related reductions in pup weights on PD 1 were noted at 25 mg/kg in corn oil compared to the vehicle control group (Table 1). Litters exposed to 25 or 50 mg/kg in corn oil had significantly reduced pup weights on PD 1 compared to their aqueous-vehicle counterparts. These nondose-related reductions in pup weight were not attributed to treatment. On PD 6, all of these pups had survived and their weights were comparable to those of controls.

**DISCUSSION**

FLR was the prominent developmental effect of both CCl₄ and BDCM in either vehicle. Only at the highest dose level (75 mg/kg) was the incidence of FLR significantly increased by the lipophilic vehicle. Concerning the risk assessment of these drinking water contaminants, the developmental NOAEL for both agents, regardless of vehicle, was 25 mg/kg and the lowest observed adverse effect level (LOAEL) was 50 mg/kg. For CCl₄, the BMDs were similar for the two vehicles (Table 2), whereas for BDCM, the corn oil vehicle yielded a greater BMD (implying lower risk) than the aqueous vehicle. In view of the high FLR response rate at the highest dose (75 mg/kg), the greater BMD value may
TABLE 2
Summary of ED05 Estimates and Benchmark Doses for Full-Litter Resorption

<table>
<thead>
<tr>
<th>Agent</th>
<th>Vehicle</th>
<th>NOAEL (mg/kg)</th>
<th>Response at LOAEL (%)</th>
<th>Estimated ED05 (mg/kg)</th>
<th>Benchmark dose (mg/kg)</th>
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<tbody>
<tr>
<td>BDCM</td>
<td>Corn oil</td>
<td>25</td>
<td>8</td>
<td>48.4*</td>
<td>39.3*</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>25</td>
<td>17</td>
<td>33.3</td>
<td>11.3</td>
</tr>
<tr>
<td>CCl₄</td>
<td>Corn oil</td>
<td>25</td>
<td>42</td>
<td>30.0</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>25</td>
<td>14</td>
<td>39.5</td>
<td>14.0</td>
</tr>
</tbody>
</table>

*The estimated ED05 and benchmark dose for whole-litter loss (including the death of one litter that was maintained to term) was 48.1 and 37.1 mg/kg, respectively.

seem counterintuitive. However, one of the experimental dose levels (50 mg/kg) was associated with a response rate (8%) that closely approximated 5%. This yielded a smaller confidence interval around the ED05, resulting in a less conservative BMD (Kavlock et al., 1996).

Both BDCM and CCl₄ caused similar degrees of maternal toxicity following high-dose exposure in corn oil. A conspicuous difference, however, was the lacrimator effect (chromodacryorrhea, lacrimation) observed only following BDCM treatment. Maternal effects at lower doses of both agents were slightly more pronounced in the aqueous vehicle, but, at the highest dose, CCl₄ was clearly less toxic in the aqueous vehicle. A vehicle-dose interaction on maternal weight gain during GD 6 to 8 was evident for CCl₄, but not BDCM.

These dose-dependent results for CCl₄ maternal toxicity differ from the findings of Kim et al. (1990b) where the hepatotoxicity of acute CCl₄ exposure was reduced when the agent was administered in corn oil over a wide range of doses (10–100 mg/kg). Pharmacokinetic data following oral dosing of 25 mg CCl₄/kg (Kim et al., 1990a), indicated that the corn oil vehicle resulted in slower absorption from the gastrointestinal tract, causing lower peak blood concentrations and delayed removal from the bloodstream.

For BDCM, our findings were similar to those of Lilly et al. (1994) in that they reported dose-dependent differences in vehicle effects. Although they also demonstrated increased toxicity with the oil vehicle at a high dose and reduced toxicity at a lower dose, they used acute, rather than repeated, exposures of substantially higher dosages (200 and 400 mg/kg rather than 25–75 mg/kg). Thus, corn oil resulted in greater BDCM-induced toxicity at 400 mg/kg (acute), but reduced toxicity at 200 mg/kg (acute) and 25 mg/kg (repeated exposure) compared to the aqueous vehicle. Although there are several differences in the conditions of the two studies (e.g., sex, pregnancy, dosing volume), these findings indicate that vehicle differences depend on a variety of factors including exposure duration as well as dose.

Lilly et al. (1994) demonstrated that BDCM can reach peak blood concentrations (Cₘₐₓ) within 20 min after gavage in the rat, with greater Cₘₐₓ values following aqueous, rather than oil, administration. Thus, the earliest time point included in the current study, 30 min, was unlikely to yield realistic Cₘₐₓ values. Data from the later time points, however, were consistent with those from earlier work (Lilly et al., 1994) demonstrating slower BDCM elimination when administered in corn oil compared to the aqueous vehicle. In addition, the t₁/₂ of 3.6 h in the corn oil vehicle compares favorably to a previous observation in adult male Fischer-344 rats of 44.5% elimination of an oral dose of [¹⁴C]BDCM (10 mg/kg in corn oil) at 4 h after dosing (Mathews et al., 1990). In the present study, BDCM blood concentrations were examined only after a single GD-6 dose. Thus, it is possible that BDCM pharmacokinetics may be different after repeated dosing during the GD 6–15 treatment regimen used in the developmental toxicity screens. Previous results suggest that repeated dosing could lead to slightly decreased metabolism via inhibition of a minor pathway; however, five consecutive doses of 75 mg BDCM/kg/day caused no change in total cytochrome P450 levels in mature female rats of the same strain (Thornton-Manning et al., 1994).

No biotransformation data were collected in the present...
study; however, like other halogenated methanes (Hanzlik, 1981), the adult (and perhaps developmental) toxicity of BDCM may be mediated by toxic metabolites rather than the parent compound (Thornton-Manning et al., 1993; Gao et al., 1996). As suggested by Lilly et al. (1994), the lower BDCM C\textsubscript{max} in an oil vehicle may lead to a reduced degree of metabolic saturation, allowing more of the administered dose to be converted to a toxic metabolite rather than be eliminated unmetabolized via the urine or exhalation. Thus, oil, compared to aqueous, administration of saturating doses of BDCM may result in greater toxic insult due to transformation of a larger fraction of the administered dose to a toxic metabolite. In the present study, the longer elimination half-life of BDCM seen following oil administration is also consistent with this result; slower elimination would be expected to allow more complete metabolism, and hence, greater maternal toxicity.

Two epidemiologic studies have recently examined potential relationships between THMs in drinking water and spontaneous abortion. In a study conducted in California (Waller, in press), there was an increased risk of spontaneous abortion in women consuming large amounts of water containing THMs, especially BDCM. Although a North Carolina case-control study (Savitz et al., 1995) did not show increased risk with total THM exposure, this may have been due to the relatively low levels of brominated THMs in North Carolina compared to California (U.S. EPA, 1994).

The lowest dose of BDCM causing FLR in the present study was approximately 5000- to 10,000-fold greater than the doses that would be consumed by humans drinking highly contaminated water (assuming 70 kg body weight, 2–4 L water consumption, 180 \( \mu \)g BDCM/L). Thus, in view of the findings of the California epidemiologic study (Waller, in press) showing an association between BDCM-contaminated water consumption and spontaneous abortion, there may be large differences between species in effective doses for BDCM-induced pregnancy loss; this apparent contrast in sensitivity to BDCM can be reconciled only by future mechanistic investigation. For example, known interspecies variation in THM metabolism could be important. If an oxidative metabolite is causally involved in pregnancy loss, one can postulate that less BDCM would be required to elicit the response in humans, because considerably more oxidative metabolism of THMs occurs in humans than in rats (Corley et al., 1990). In addition, species differences in physiological mechanisms of pregnancy maintenance could yield different target sites of toxicity and contribute to greater human susceptibility relative to rats. For example, in humans, the corpus luteum of postimplantation pregnancy is maintained by chorionic gonadotropin (Zeleznik and Fairchild-Benyo, 1994), whereas in rats the corpora lutea are maintained by luteinizing hormone (Madhwa Raj and Moudgal, 1970) or placental lactogens (Tabarelli et al., 1982), depending on the period of gestation.

In summary, both BDCM and CCl\textsubscript{4} caused FLR at 50 and 75 mg/kg in either vehicle and may provide a valuable animal model for halocarbon-associated spontaneous abortion. At 75 mg/kg, the oil vehicle significantly increased the FLR response compared to the aqueous vehicle. At lower doses, however, maternal toxicity was slightly more pronounced when either agent was administered in the aqueous vehicle. Thus, the influence of the vehicle on the toxic effects of these drinking water contaminants is dose dependent. These findings are consistent with the pharmacokinetic data showing slower BDCM elimination following dosing with an oil vehicle. Also, the increased FLR rates in the corn oil vehicle for both agents clearly suggest no increase in developmental hazard for aqueous delivery of these volatile organic compounds.

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