Disposition of Bromodichloromethane in Humans Following Oral and Dermal Exposure

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Exposure to bromodichloromethane (BDCM), one of the most prevalent disinfection byproducts in drinking water, can occur via ingestion of water and by dermal absorption and inhalation during activities such as bathing and showering. The objectives of this research were to assess BDCM pharmacokinetics in human volunteers exposed percutaneously and orally to ^{13}C-BDCM and to evaluate factors that could affect disposition of BDCM. Among study subjects, CYP2E1 activity varied fourfold; 20% had the glutathione S-transferase theta 1-1 homozygous null genotype; and body fat ranged from 7 to 22%. Subjects were exposed to ^{13}C-BDCM in water (target concentration of 36 µg/l) via ingestion and by forearm submersion. Blood was collected for up to 24 h and analyzed for ^{13}C-BDCM by solid-phase microextraction and high-resolution GC-MS. Urine was collected before and after exposure for mutagenicity determinations in *Salmonella*. After ingestion (mean dose = 146 ng/kg), blood ^{13}C-BDCM concentrations peaked and declined rapidly, returning to levels near or below the limit of detection (LOD) within 4 h. The T_{max} for the oral exposure ranged from 5 to 30 min, and the C_{max} ranged from 0.4 to 4.1 ng/l. After the 1 h dermal exposure (estimated mean dose = 155 ng/kg), blood concentrations of ^{13}C-BDCM ranged from 39 to 170 ng/l and decreased to levels near or below the LOD by 24 h. Peak postdose urine mutagenicity levels that were at least twice that of the predose mean level occurred in 6 of 10 percutaneously exposed subjects and 3 of 8 orally exposed subjects. These results demonstrate a highly significant contribution of dermal absorption to circulating levels of BDCM and confirm the much lower oral contribution, indicating that water uses involving dermal contact can lead to much greater systemic BDCM doses than water ingestion. These data will facilitate development and validation of physiologically based pharmacokinetic models for BDCM in humans.

Key Words: bromodichloromethane; pharmacokinetics; dermal absorption; oral absorption.

Bromodichloromethane (BDCM) is a disinfection byproduct (DBP) that is commonly found in drinking water treated with chlorine or chloramine to eliminate microbial pathogens. Factors that have made BDCM one of the highest priority DBPs for research include its prevalence in disinfected drinking water (second only to chloroform among known DBPs); carcinogenic potency in experimental animals; effects on reproduction; similar target sites for adverse effects in humans and rodents; demonstrated genotoxicity; and metabolic activation to reactive intermediates (Bielmeier *et al.*, 2001; Bull *et al.*, 1995; Pegram, 1999; Ross and Pegram, 2004). A recent DBP occurrence survey of water plants distributed across the United States reported concentrations of BDCM in disinfected drinking water ranging from 1 to 50 µg/l (Weinberg *et al.*, 2002).

Epidemiological studies suggest a correlation between consumption of chlorinated water containing BDCM and cancers of the urinary bladder (odds ratios [ORs] = 1.63–3.92) and lower intestinal tracts (OR = 1.68–2.1) (Cantor *et al.*,1998; Doyle *et al.*, 1997; King and Marrett, 1996; King *et al.*, 2000b; Yang *et al.*, 1998). Currently, BDCM is listed by IARC as a class 2B, possible human carcinogen (NTP, 1987). In rodent chronic toxicity studies that used high oral doses of BDCM given in corn oil, tumors occurred in the kidneys, large intestine, and liver (NTP, 1987), likely due to a mutagenic metabolite of BDCM (DeMarini *et al.*, 1997; Pegram *et al.*, 1997; Ross and Pegram, 2004). When administered in drinking water for 13 weeks, BDCM (45 mg/kg/day) also induced preneoplastic aberrant crypt foci in the colons of rats (DeAngelo *et al.*, 2002).

In addition to carcinogenic effects, recent epidemiological studies have suggested a correlation between BDCM exposure and spontaneous abortions (OR = 1.6–3.0) (Savitz *et al.*, 2005; Waller *et al.*, 1998), stillbirths (OR = 1.98) (King *et al.*, 2000a), and neural tube defects (OR = 2.5) (Dodds and King, 2001). BDCM has been shown to induce pregnancy loss in rats given oral doses of 50–75 mg/kg body weight during gestation (Bielmeier *et al.*, 2001; Narotsky *et al.*, 1997). Very low concentrations of BDCM (0.5–20 nM) reduced the *in vitro* secretion of choriogonadotropin from human placental trophoblasts (Chen *et al.*, 2003) and prevented differentiation of mononucleated cytotrophoblasts into multinucleated trophoblasts (Chen *et al.*, 2004). These *in vitro* effects could be mechanistically relevant to both early- and later-term pregnancy loss, and they were observed at BDCM concentrations comparable to those measured in human blood (0.57 nM) after showering with ordinary tap water (Miles *et al.*, 2002). Although metabolic activation of BDCM is likely involved in its cytotoxic and carcinogenic effects, the role of metabolism is unknown in the induction of reproductive and developmental effects by BDCM.

BDCM is metabolized by three known pathways in mammals: (1) oxidation to phosgene; (2) reduction to a dichloromethyl radical (Gao *et al.*, 1996; Tomasi *et al.*, 1985); and (3) glutathione conjugation leading to formation of genotoxic intermediates. The oxidative pathway, catalyzed by CYP2E1, predominates the *in vivo* metabolism of BDCM (~70–80% of total biotransformation) and leads primarily to CO_2 production via phosgene hydrolysis (Allis and Zhao, 2002; Lilly *et al.*, 1997; Mathews *et al.*, 1990). Mutagenic intermediates are generated following glutathione transferase theta 1-1 (*GSTT1-1*)–mediated conjugation of BDCM with glutathione (DeMarini *et al.*, 1997; Pegram *et al.*, 1997; Ross and Pegram, 2003). Metabolites of this pathway covalently bind DNA, forming deoxyguanosine adducts (Ross and Pegram, 2004). *GSTT1-1* may contribute more significantly to BDCM metabolism in the kidney and colon, sites of BDCM-induced carcinomas in rats (Ross and Pegram, 2004). The known activated metabolites of BDCM are very unstable; they react with biomolecules near their site of generation to elicit toxicity and have not been detected in circulation. Given the extrahepatic effects indicated by human studies, measurement of systemic parent BDCM seems especially relevant to potential human health effects.

There are limited data on blood concentrations of BDCM in humans exposed by ingestion, showering, or bathing in water containing environmental concentrations of BDCM (Backer *et al.*, 2000; Gordon *et al.*, 2006; Lynberg *et al.*, 2001; Miles *et al.*, 2002; Nuckols *et al.*, 2005). Human blood concentrations of various trihalomethanes (THMs) including BDCM were measured recently at sites in Georgia and Texas (Lynberg *et al.*, 2001). In this study, blood BDCM levels increased approximately sixfold after showering compared to concentrations measured before water exposure activities. Backer *et al.* (2000) compared increases in human blood concentrations of BDCM after bathing, showering, or ingestion of tap water containing approximately 6 µg BDCM/l. Median blood concentrations before exposure ranged from 2.3 to 2.8 ng/l as compared to 10 min post-exposure concentrations of 17, 19.4, and 3.8 ng/l for bathing, showering, and ingestion, respectively. These findings suggest that dermal and/or inhalation exposures produce greater circulating concentrations of parent BDCM than those resulting from oral intake.

A data set suitable for a pharmacokinetic analysis of BDCM in humans from controlled exposure studies in a clinical setting has not been available. These data are critical for validation of physiologically based pharmacokinetic (PBPK) models for humans, which are needed to quantify total exposure from multiple routes and to predict target tissue concentrations of BDCM and metabolites. In addition, controlled studies have not been carried out in humans to investigate potential urinary biomarkers of exposure and mutagenicity after exposure to BDCM. The primary objective of this research was to quantify the pharmacokinetics of BDCM in humans exposed by ingestion or dermal absorption to environmentally relevant concentrations of BDCM. We examined various factors that may affect the pharmacokinetics of BDCM, including cytochrome P450 2E1 activity, *GSTT1-1* genotype, and percentage body fat. Potential markers of BDCM exposure, including pH changes in urine and urinary mutagenicity, were also measured.

MATERIALS AND METHODS

General approach. This was a clinical study to expose human volunteers to low concentrations of ^{13}C-BDCM in water. ^{13}C-BDCM was used in the study to quantitate concentrations above normal background levels. Because real world exposures to BDCM can occur through ingestion and dermal contact with drinking, bathing, and swimming pool water, the study included both oral and dermal exposures to ^{13}C-BDCM. Prior to exposure, the subjects were phenotyped for cytochrome P450 2E1 activity by assessing chlorzoxazone (CZ) metabolism, genotyped for *GSTT1-1*, and evaluated for percentage body fat. Concentrations of ^{13}C-BDCM were measured in blood collected during and up to 24 h after initiation of the oral and dermal exposures. The administered water concentrations of ^{13}C-BDCM were also measured. Urine was collected from subjects and used to measure pH and mutagenicity in the *Salmonella* (Ames) plate-incorporation mutagenicity assay (Maron and Ames, 1983) in a strain that expresses GSTT.

Materials. Chemicals used for clinical exposures were as follows: ^{13}C-BDCM, chemical and isotopic purity = 99% (Cambridge Isotope Laboratories, Inc., Andover, MA), CZ 250 mg tablets (Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ), and sterile water for exposures and for pre-exposure use by subjects (Baxter Healthcare Corp., Deerfield, IL). Chemicals for assays and analytical determinations included: CZ (Aldrich Chemical Co., St Louis, MO), 6-hydroxy-chlorzoxazone (Sigma RBI, St Louis, MO), β-glucuronidase from *Helix pomatia*, 110,350 units/ml β-glucuronidase and 4194 units/ml α-sulfatase (Sigma, St Louis, MO), and standard solvents for chromatography from Fisher Scientific, Raleigh, NC. *Salmonella* strain RSJ100 was kindly provided by Dr F.P. Guengerich, Department of Biochemistry and Center for Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN. RSJ100 was constructed by transfecting the base-substitution strain TA1535 (*hisG46, rfa, ΔuvrB*) with the rat glutathione *S*-transferase gene *GSTT1-1* (Thier *et al.*, 1993).

Subjects. The study was approved by Institutional Review Boards from the University of North Carolina-Chapel Hill (UNC-CH) School of Medicine and the Centers for Disease Control and Prevention (CDC). Initially a total of 22 subjects, ages 19–31, were recruited for the study, 11 for preliminary work and 11 for the actual exposures. In the actual study, data were obtained for analysis from 10 of 11 subjects (9 males and 1 female) because one subject withdrew due to difficulties collecting sequential blood samples. During recruitment, factors for exclusion from the study included: pregnancy; smoking; drug use; medical conditions such as a chronic cough, asthma, or chronic pulmonary disease; active allergy; a history of acute or chronic cardiovascular, hepatic, or renal disease; acute illness within 1 month of participating in study; current use of medication, aspirin, ibuprofen, or similar analgesics within 48 h of participation in study; other experimental exposures within the previous 2 weeks of participation; blood donation within 8 weeks of participation; and history or evidence of multiple chemical sensitivity.

Each subject was exposed by oral administration and dermal absorption (in separate experiments) to water containing ^{13}C-BDCM. A minimum of 1 week separated each exposure of the same subject. During participation in the study, subjects used sterile, distilled water for consumption and cleansing beginning 24 h prior to the start of each exposure, and they fasted beginning at 12 A.M. the night prior to exposure. Subjects were also instructed to refrain from consumption of alcohol and to avoid gasoline fumes, cigarette smoke, fried and grilled foods, hot tubs, and swimming pools for at least 48 h prior to and during participation in each exposure.

Measurement of CZ metabolism. The *in vivo* metabolism of CZ was assessed as an indicator of cytochrome P450 2E1 activity according to modified methods of Frye *et al.* (1998) and Kramer *et al.* (2003). The subject was asked to fast overnight prior to the morning of CZ administration. Following a pre-exposure venous blood draw in a heparinized vacutainer, each subject was administered a 250 mg CZ tablet orally. A second sample of venous blood (10 ml) was withdrawn in heparinized vacutainers 3 h after administration. Immediately following collection of both blood samples, the blood was centrifuged ($1000 \times g$, 10 min) to separate the plasma, which was stored at $-80°C$ until analyzed for CZ and 6-hydroxy-chlorzoxazone (CZOH) by a modified liquid chromatography-mass spectrometry (MS) method (Scott *et al.*, 1999). To free the metabolite from a glucuronide adduct, 0.25 ml plasma was incubated with 1000 U β-glucuronidase in 0.2M sodium acetate (pH 4.75; 1.0 ml final volume) at 37°C for 3 h; enzyme activity was confirmed by assay with phenolphthalein glucuronic acid as substrate. An internal standard, 2-benzoxalinone (10 μl of a 0.1 μg/μl ethanol solution), was added to the samples, which were subsequently centrifuged ($500 \times g$, 20 min, 20°C) to remove any precipitate. Samples (0.51 ml) were applied to Oasis HLB cartridges (prerinsed with methanol and water) under vacuum and washed with 5 ml distilled water. Analytes were eluted with 4 ml methanol, dried by vacuum (Speedvac, Savant Instruments, Farmingdale, NY), and redissolved in 100 μl of 1/1 Solvent A (0.01% formic acid)/Solvent B (0.01% formic acid in 95/5 acetonitrile/distilled water). The samples (25 μl) were diluted with 175 μl of solvent just before high-performance liquid chromatography-MS analyses. Samples were analyzed on a Waters Associates System (2690 HPLC Separation Module with an XTerra 3.5-μm packing C18 column (3.9 mm × 150 mm) connected to a 2487 Dual Wavelength Absorbance Detector set at 287 nm and coupled to a ZMD model MS). Separations were performed at a flow rate of 0.3 ml/min starting with 100% Solvent A for 0.2 min, increasing to 100% Solvent B (Waters gradient curve #6) over 10 min, and held for 5 min. The Micromass ZMD was operated in ESI negative mode with a capillary voltage of 2 kV, cone voltage of -10V, extractor voltage of -5V, source block temperature of 150°C, desolvation temperature of 350°C, and N_2 gas flow at approximately 500 l/h, and rf lens of 0.1. Selected ion monitoring was done at m/z 134 (IS), 168 (CZ), and 184 (CZOH). UV detection was utilized for secondary confirmation. Data were analyzed using MassLynx software, with peak areas used to calculate compound amounts based on externally injected standards (linear calibration range of 0.1–20 ng; limit of detection [LOD] of 0.1 ng) normalized to the recovery of the internal standard. The recoveries of CZ and CZOH added exogenously to plasma were 79 ± 9% and 89 ± 18%, respectively. The ratio of CZOH to CZ (CZOH/CZ) was used as an indicator of CYP2E1 activity.

Genotype of subjects for GSTT1-1. Prior to exposures, buccal cells were collected by swabbing the mouths of each subject, and DNA was isolated from the cells by standard methods using phenol/chloroform/isoamyl alcohol extraction. A multiplex PCR was performed to analyze for the presence of various genes, including *GSTT1-1* by two methods that assess *GSTT1-1* by the presence or absence of a band of different size (Bell and Pittman, 1998; Millikan *et al.*, 2000).

Body fat assessment. The body fat percentage of subjects was estimated by measuring skinfold thickness and verified in a subgroup of subjects by hydrostatic weighing. Skinfold thickness was measured at three sites according to American College of Sports Medicine standards (ACSM, 2000) and included the abdomen, chest, and thigh for males and the suprailiac, tricep, and thigh for females. The sum of the folds was converted to body fat percentage according to equations from Jackson and Pollock (1985). Hydrostatic weighing (Cohn *et al.*, 1981) was conducted at the UNC-CH Department of Exercise and Sport Science, Chapel Hill, NC.

Oral and dermal exposure to ^{13}C-BDCM. The target ^{13}C-BDCM water concentration for both oral and dermal exposures was 36 μg/l, a concentration found in U.S. EPA–compliant drinking water from three U.S. plants serving over 50,000 people (U.S. EPA, 2002). At least 1 week separated the different exposures of a given subject. The chemical purity of the neat ^{13}C-BDCM was verified by gas chromatograph-electron capture detection prior to initial use and at 2 week intervals during use over the course of the experiments; the purity was always greater than 98%. The identity of trace impurities was confirmed by full-scan gas chromatography MS and manual matching with the National Institute of Standards and Technology Standard Reference Database 1A electronic library of mass spectral data. The impurities in trace amounts included chloroethane, ^{13}C-chloroform, ^{13}C-bromotrichloromethane, toluene, ^{13}C-tetrachlorethylene, ^{13}C-dibromochloromethane, ^{13}C-dibromodichloromethane, 2-bromoethylacetate, 1,1-dibromo-2,2-dichloroethylene, and ^{13}C-1,2-dibromotetrachlorethylene.

For both exposures, spiking solutions of ^{13}C-BDCM (~396 μg/ml) were prepared gravimetrically in water. The weighed aliquot of neat ^{13}C-BDCM was added to sterile, distilled water for a total volume of 10 ml in a volumetric flask, which was sealed and vortexed for at least 30 min prior to preparation of dermal or oral dosing solutions.

For dermal exposures, the doses were prepared in 9 L of sterile water in a tank designed to expose the forearm of subjects (Prah *et al.*, 2002). Briefly the tank was constructed of glass and insulated to minimize temperature drop during exposure. The tank contained a sampling line that was positioned at approximately the midpoint of the tank along with a temperature probe. A measured volume (9 L) of sterile, distilled water was added to the tank, and the water was heated to 42°C with a thermostated recirculating water bath. Within 15 min of the start of the exposure, approximately 820 μl of the ^{13}C-BDCM spiking solution was added to the tank water, which was stirred with a glass rod and then immediately sealed to minimize loss of ^{13}C-BDCM.

Prior to and during exposure, water samples were collected into borosilicate glass vials (12 ml, screw cap, Wheaton, Millville, NJ) that were pretreated to remove volatile organics (Cardinali *et al.*, 2004). Vials contained phosphate buffer (pH 6.5) and sodium thiosulfate to quench any residual chlorine (Cardinali *et al.*, 2004). Following water sample collection, the vial was sealed with minimal headspace using a plastic open-top cap containing a Teflon-faced septum (Wheaton, Millville, NJ) and stored in the dark at 4°C.

Immediately prior to exposure, a carbon filter mask was placed over the subject's nose and mouth to prevent inhalation of volatilized ^{13}C-BDCM. To start exposure, the subject first slipped his/her arm through a Tedlar sleeve, which was attached to the top of the tank to minimize loss of BDCM from the tank into the room, and then submerged his/her arm up to the forearm. The sleeve was secured around the subject's upper arm with a Velcro band, being careful not to constrict blood flow to the arm. After 5 min, the mask was removed from the subject. At the end of the 1-h exposure, the mask was again placed over the subject's mouth and nose, and the arm was quickly removed from the tank and patted dry with a towel. The mask was removed after the dermal tank was taken out of the exposure room. Calculations based on potential loss of BDCM from the tank water to room air indicated that any exposure due to inhalation was trivial.

Sequential venous blood samples were obtained both pre-exposure and for up to 24 h following initiation of exposure. Whole blood was collected from the unexposed arm through an iv cannula in the antecubital vein into fluoride/oxalate gray-top vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) that were pretreated to remove volatile organic contaminants (Cardinali *et al.*, 1995). The time points for blood samples during and following the dermal exposure were 0, 0.083, 0.25, 0.50, 0.75, 1, 1.083, 1.25, 1.5, 2, 3, 4, 5.5, 7, and 24 h.

Urine samples were collected immediately prior to and following the dermal exposure. The urine was collected in plastic specimen cups, immediately placed on ice, and then stored at 0°C until analysis for mutagenicity and creatinine levels. Prior to storage, the pH of an aliquot of urine was measured with an Accumet model A15 pH meter with an AccuPhast microprobe combination electrode (Fisher Scientific Inc, Hampton, NH).

For oral exposures, the dose was administered in sterile, distilled water at 3.4 ml of water per kilogram body weight so that each subject drank approximately 250 ml, equivalent to an 8-oz glass of water. Approximately 45 μl of the ^{13}C-BDCM spiking solution was added to 500 ml of sterile, distilled water in a sterile glass bottle. The appropriate volume of water to administer to the subjects based on body weight was measured in a volumetric flask and then transferred to a sterile jar, which was immediately capped to prevent evaporative loss of ^{13}C-BDCM. An identical volume of the prepared water was added to a second jar that was sealed and used to determine the actual water concentration of ^{13}C-BDCM administered to the subject. To initiate the oral exposure, the subject was instructed to carefully and quickly drink all the water in the jar. The actual volume of water ingested was recorded by weighing the jar before and after administration. At the time of oral administration, a sample of water for analysis was transferred from the second jar of water with ^{13}C-BDCM to a sample collection vial as described for the dermal exposures. Subject F served as a blank control for the study and, thus, was given water that did not contain ^{13}C-BDCM (see Table 1).

Prior to and after oral administration, sequential whole-blood samples were collected and treated as described above; approximate time points were 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, and 4 h. Urine was collected prior to and up to 4 h after oral administration of ^{13}C-BDCM as described above.

Analysis of blood and water samples.   All water and blood samples were stored in the dark at 4°C until shipment on ice via overnight courier to the CDC where they were stored at 4°C until analysis. ^{13}C-BDCM in whole blood and water was analyzed by headspace solid-phase microextraction (SPME) followed by gas chromatography and MS analysis according to modifications of a previously published method (Bonin *et al.*, 2005). Blood samples from the dermal exposure regimen resulted in elevated levels of ^{13}C-BDCM relative to the oral exposure regimen; therefore, less blood (2.0 ± 0.2 g) was analyzed for dermal exposure samples compared with oral exposure samples (4.0 ± 0.2 g). Blood samples were spiked with internal standard (^{13}C-dibromochloromethane, ^{13}C-DBCM), crimp sealed (20-mm Teflon-faced silicon septum; Microliter, Suwanee, GA), and mixed to allow for equilibration of internal standard into the blood matrix. Sample headspace was subsequently extracted for 14 min with a SPME fiber (75 μm Carboxen-PDMS; Supelco, Bellefonte, PA) at 37 ± 1°C and 350 rpm agitation. Volatile components of the sample were desorbed from the SPME fiber in the injection port of a gas chromatograph (Agilent 6890, Palo Alto, CA), cryotrapped (Scientific Instruments Services, Ringoes, NJ), and chromatographically resolved (J&W DB-624, 25 m × 0.20 mm ID, 1.12-μm film) as described previously (Bonin *et al.*, 2005). Water samples (5.0 ± 0.2 g) were analyzed in a similar fashion after a 1:1000 dilution with blank water. Following chromatographic separation, the volatile sample constituents were detected with a double-focusing magnetic-sector mass spectrometer (Autospec Ultima, Micromass, Milford, MA) at 10,000 mass resolution (5% valley definition) in positive ion mode by selective ion recording method. MassLynx and QuanLynx software were used to calculate the response ratio of the analyte (^{13}C-BDCM) and internal standard (^{13}C-DBCM). Final concentrations of ^{13}C-BDCM were calculated based on seven-point calibration curves from multiple analyses of aqueous standards from 0.22 to 77.8 ng/l. Samples exceeding this linear range were diluted and reanalyzed; all reported analyte concentrations were normalized according to the sample weight. Quality control samples (bovine serum spiked with known amounts of ^{13}C-BDCM) were also analyzed along with unknown samples and evaluated using Westgard rules (Westgard *et al.*, 1981). No ^{13}C-BDCM was detected in pre-exposure blood samples or samples from the control subject; thus, background levels of ^{13}C-BDCM were effectively zero.

Mutagenicity of urine.   Urine samples collected prior to and after exposure were evaluated for mutagenicity in the *Salmonella* (Ames) plate-incorporation mutagenicity assay (Maron and Ames, 1983) in strain RSJ100 in the absence of S-9 fraction.

TABLE 1
Subject Demographics, Administered Doses, Percentage Body Fat, Cytochrome P450 2E1 Activity, and GSTT Genotype

Subject ID	Gender	Age	BW (kg)	Oral dose (ng/kg)	Estimated dermal dose (ng/kg)[a]	Percentage Body fat (%)[b]	CZ-OH/CZ[c]	GSST1-1 genotype[d]
A	Male	23	74.3	105	96.1	14	ND[e]	+
B	Male	21	73.8	81.0	125	8.0	0.91	+
C	Male	26	64.8	127	264	8.6	0.31	+
D	Male	20	80.1	179	168	7.0 (10)	0.37	+
E	Male	31	63.9	132	151	(22)	0.22	—
F	Male	27	72.8	0[f]	87.4	11	0.38	+
G	Female	27	66.0	130	142	20 (19)	0.24	—
H	Male	29	90.9	120	321	ND	0.36	+
I	Male	19	68.3	257	100	9.4	0.62	+
J	Male	20	86.5	178	95.5	13	0.31	+

[a]Dermal dose was estimated from the water concentration, the length of exposure (1 h), and the skin permeability of BDCM (0.18 cm/h) reported by Xu *et al.* (2002). The surface areas for the current study were calculated from each subject's weight and height with the algorithm from EPA's Exposure Factor Handbook (U.S. EPA, 1997). The arm and forearm were assumed to be 5.5% of total surface area as reported by the EPA handbook.

[b]Skinfold measurements are listed with the hydrostatic weighing comparisons given in parentheses.

[c]Ratio of the metabolite 6-hydroxychlorzoxazone (CZ-OH) to CZ at 3 h after administration was the measure of cytochrome P4502E1 activity.

[d]+ indicates detection of both primers (480 and 120 bp products) whereas − indicates absence of both primers.

[e]Not determined.

[f]Subject F was used as a negative quality control for blood to ensure that the samples were not contaminated during collection, storage, transport, or analysis. Therefore, the subject drank sterile water without ^{13}C-BDCM. All bloods from subject F were blank.

Urinary organics were extracted as described previously (Peters *et al.*, 2003). Briefly, 40 ml of urine were passed through C18 resin, and the organics were eluted by methanol and then solvent exchanged into dimethyl sulfoxide (DMSO) at 150× for bioassay. Extracts were evaluated once in single plates per dose at 0.75, 1.5, 3, 7.5, and 15 ml equivalents per plate. If a dose-related increase in revertants per plate was obtained, a sample was considered mutagenic. The mutagenic potencies of positive samples, expressed as revertants per ml-equivalent, were calculated from the slope of the regression over the linear portion of the dose-response curves. These potencies were then expressed as revertants per mg creatinine based on urine creatinine measurements, which were determined by automated analysis with a Konelab 30 Clinical Chemistry Analyzer (Thermo Clinical Labsystems, Espoo, Finland) and a kit with standards purchased from Diagnostic Chemicals, Ltd. (Charlottetown, Prince Edward Island, Canada). Controls consisted of DMSO (100 µl per plate), C18 resin blanks prepared by passing 40 ml of distilled deionized water instead of urine through the columns (15 ml equivalent per plate), and sodium azide at 3 µg per plate (positive control). Control values (revertants per plate) were 9–14 (DMSO), 5–10 (resin blanks), and 377–595 (sodium azide).

Pharmacokinetic analysis of data. Following oral exposure to ^{13}C-BDCM in water, blood ^{13}C-BDCM concentration versus time data were used for calculating pharmacokinetic parameter estimates for each subject by noncompartmental methods with the pharmacokinetic software package WinNonlin (Pharsight Corporation, Cary, NC).

Following dermal exposure to ^{13}C-BDCM in the immersion tank, the concentration versus time data for each subject clearly indicated as many as two or more distinct decay phases in the terminal portions of the curves. In order to define the disposition rates associated with each phase, compartmental modeling with a nonlinear least squares fitting routine (Prism, GraphPad Software, Inc., San Diego, CA) was used to calculate pharmacokinetic parameter estimates following dermal exposure. Monoexponential and biexponential curves were fit with $1/y^2$ weighting functions to obtain the best-fit curves for each data set. Final weighting was based on a visual inspection of the curve fit to the data, analysis of residual plots, and the variability in the derived curve and pharmacokinetic parameter estimates. In addition, a statistical *F*-test was used for selection of the best-fit compartmental curve (Boxenbaum *et al.*, 1974). The equations for calculating the various pharmacokinetic parameters are described in detail by Gibaldi and Perrier (1982).

For calculation of area under the blood concentration versus time curve (AUC) by either compartmental or noncompartmental analysis, blood ^{13}C-BDCM concentrations that were less than the LOD of the bioanalytical assay (0.25 ng/l) were assigned a value of zero.

Statistics. Analysis of the urine mutagenicity data was done by grouping the samples according to the time of collection following initiation of exposure. Groups for the oral exposures were pre-exposure, 0–0.99, 1–1.99, 2–2.99, 3–3.99, and 4–4.99 h. Because of the 1-h exposure duration, a 0-0.99 group could not be included for the dermal exposures. Therefore, the dermal exposure groups were pre-exposure, 1–1.99, 2–2.99, 3–3.99, 4–4.99, 5–5.99, 6–8, and 24 h. The revertants/mg creatinine for each group were analyzed by a one-way ANOVA at $p < 0.05$. A *post hoc* test was done only if there were significant differences among groups. In addition, the times to peak levels of revertants/mg creatinine were compared between exposure routes with a *t*-test and Mann-Whitney rank sums test.

For each reported pharmacokinetic parameter (except for half-life), following each route of administration, the mean, SD, and coefficient of variation were calculated for each individual subject. For half-life estimates, the harmonic mean was calculated from the individual subject data.

The pharmacokinetic parameters and the peak levels of mutagenic activity were compared with CZOH/CZ, dose, and percentage body fat to determine if there were significant correlations. A correlation coefficient (R^2) was calculated, and the slope of the regression line was analyzed by an *F* test ($p < 0.05$) to determine if it was significantly different from zero. All statistical comparisons and plots were generated with Graphpad Prism version 4.

RESULTS

The measured physiological and biochemical parameters varied among the study subjects (Table 1). Of the 10 subjects used in the study, 2 were *GSTT1-1* homozygous null, which is consistent with reported frequencies for this gene-deletion polymorphism (Nelson *et al.*, 1995). The CZOH/CZ ratios of the subjects varied approximately fourfold. The percentage body fat of the subjects ranged from 7 to 20% based on skinfold thickness. The skinfold method yielded similar results in subjects in which the body fat was also verified by hydrostatic weighing, which is considered a more definitive assessment method. The range among the subjects was 10–22% as determined by hydrostatic weighing.

The actual water concentrations of ^{13}C-BDCM (mean ± SD) were 38 ± 20 and 60 ± 32 µg/l, for oral and dermal exposure, respectively. The actual doses administered for each subject are listed in Table 1. The dermal dose was estimated according to the following equation:

$$\text{Estimated dermal dose} = \frac{K_p \times \text{SA}_{\text{exposed}} \times C_{\text{water}} \times \text{Time}_{\text{exposure}}}{\text{body weight}}.$$

The surface area exposed (SA$_{\text{exposed}}$) was estimated according to U.S. EPA's Exposure Assessment Handbook (U.S. EPA, 1997) by calculating the total body surface area from the subject's weight and height and a fraction of 0.055 to account for the forearm and hand. The human skin permeability (K_p) of 0.18 cm/h for BDCM as determined by Xu *et al.* (2002) was used for all dermal absorption estimates. The mean water concentration was calculated over the period of exposure (C_{water}), and the exposure time (Time$_{\text{exposure}}$) was 1 h. Although measures were taken to reduce variability in the actual water concentrations, the variability was greater than expected. This could have been due to limited solubility of BDCM in the water used to prepare the spiking solutions, although the solubility of BDCM in water is reported to be 4500 mg/l, which is 10 times greater than the prepared spiking solution. The oral and estimated dermal doses (mean ± SD) were 146 ± 52 and 155 ± 78 ng/kg, respectively.

Mean blood ^{13}C-BDCM concentrations following oral administration are plotted in Figure 1. Absorption of ^{13}C-BDCM into the systemic circulation was rapid, with mean maximum concentrations (C_{max}) appearing in blood by 11 min (T_{max}) following administration (Table 2). At this time, the mean C_{max} of all subjects was 2.60 ng/l. Following C_{max}, blood ^{13}C-BDCM concentrations declined rapidly, with a mean half-life of 47 min. For many of the subjects, blood ^{13}C-BDCM was below the quantitation limits of the bioanalytical assay by 3 h after administration. Mean systemic exposure (AUC$_0^\infty$) was very low at 149 ng min/l (or 0.0152nM h).

Blood ^{13}C-BDCM concentrations increased during the 1-h dermal exposure period and were maximal at the end of exposure (Fig. 2). The mean of the subjects' blood ^{13}C-BDCM
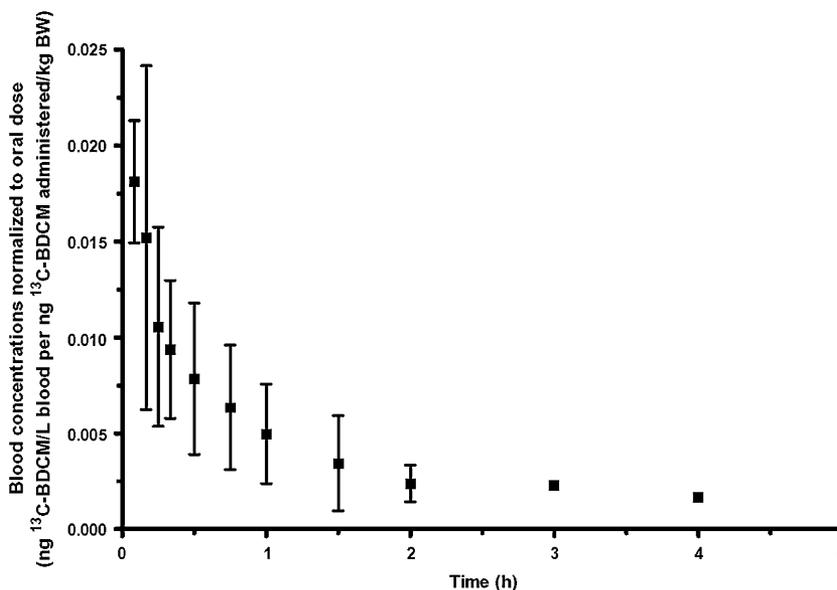
FIG. 1. Blood concentrations of ^{13}C-BDCM normalized to subject's dose after ingestion of ^{13}C-BDCM in sterile water. Each data point (blood concentration in ng/l divided by the dose in ng ^{13}C-BDCM/kg body weight) with error bars represents the mean ± SD at a given time. At 4 h, only one subject had a blood ^{13}C-BDCM concentration above the LOD (0.25 ng/l). ^{13}C-BDCM blood concentrations were not determined for subject A due to an instrument error.

concentrations at the end of the exposure was 90.5 ng/l (Table 3), and the mean maximum blood ^{13}C-BDCM concentration achieved at any time was 94.9 ng/l. Upon removing the arm from the dermal exposure tank, blood ^{13}C-BDCM concentrations generally decreased in blood in a biphasic manner and were measurable up to 24 h following initial exposure. There were two exceptions, subjects D and G, for which the decline in blood ^{13}C-BDCM concentrations

following exposure was best fit with a monoexponential equation (Table 3). For these two subjects, the blood concentrations did not decrease significantly between the 6 and 23 h postexposure time points. Overall, the mean half-life ($t_{1/2\alpha}$) of the initial elimination phase was very rapid (32.6 min), and the mean terminal elimination half-life ($t_{1/2\beta}$) was approximately nine times longer (309 min). Mean systemic exposure during the sampling time (AUC$_{0-tfinal}$) for this dose regimen was

TABLE 2
Pharmacokinetic parameters for Oral Exposures[a]

Subject ID	AUC$_{0-\alpha}$ (ng min/l)	AUC$_{0-tfinal}$ (ng min/l)	Cl/F[b] (l/min/kg)	λ_z (min^{-1})[c]	$t_{1/2\lambda z}$ (min)[d]	C_{max} (ng/l)[e]	T_{max} (min)[f]
A	ND	ND	ND	ND	ND	ND	ND
B	ND	10.5	ND	ND	ND	0.383	15.0
C	109	86.3	1170	0.0105	65.8	1.23	30.0
D	277	253	646	0.0104	66.5	3.46	15.0
E	212	142	628	0.0124	55.7	4.11	10.0
G	146	123	891	0.00865	80.2	2.06	5.00
H	52.3	47.9	2290	0.0325	21.3	1.86	5.00
I	115	103	2230	0.0134	51.8	4.02	5.00
J	133	123	1340	0.0158	43.8	3.64	5.00
Mean	149	111	1310	0.0148	46.7[g]	2.60	11.3
SD	73.9	71.9	696	0.00814		1.40	8.76
% CV	49.5	64.7	53.0	55.0		54.0	77.9

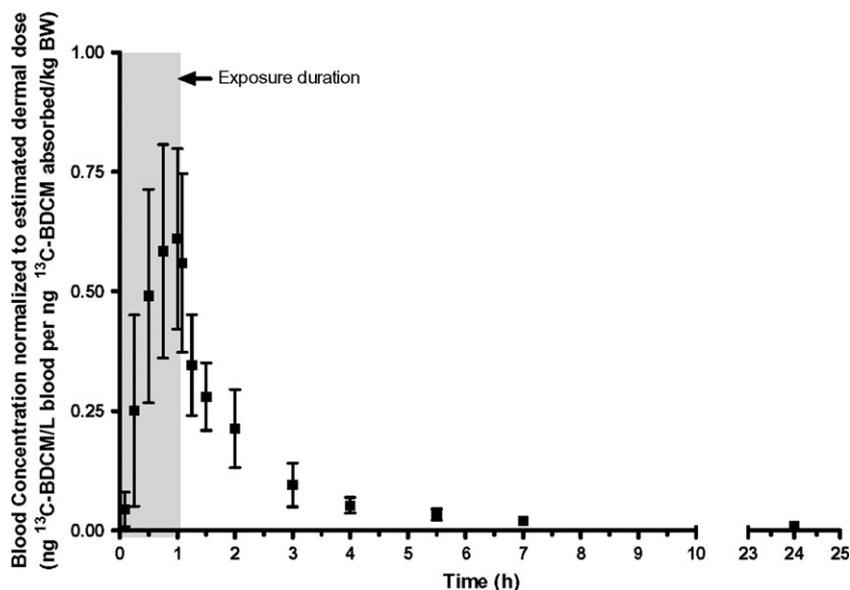[a]Noncompartmental analysis.

[b]Clearance.

[c]First-order rate constant for portion of concentration time plot described by $C_z = Ce^{-\lambda_z t}$.

[d]Half-life estimated from λ_z.

[e]Estimated maximum blood concentration.

[f]Time of C_{max}.

[g]Harmonic mean.

FIG. 2. Blood concentrations of ^{13}C-BDCM normalized to estimated absorbed dose for the dermal exposure. Each subjects submerged their left forearm for 1 h in ~40°C water containing ^{13}C-BDCM. Each data point (blood concentration in ng/l divided by the estimated dermal dose in ng ^{13}C-BDCM/kg body weight) with error bars represents the mean ± SD at a given time. At 24 h, two of the subjects had blood concentrations below the LOD (0.25 ng/l).

11,800 ng min/l (or 1.18nM h), approximately 80× greater than the oral (AUC$_0^\infty$), which is remarkable given the similarity of the oral and percutaneous doses.

The parameters estimated from the pharmacokinetic analyses of the blood concentration curves (Tables 2 and 3) were compared with administered oral dose or estimated dermal dose, CZOH/CZ, and percentage body fat for each subject. For the oral or dermal route of exposure, the C_{max} or $C_{endexposure}$ was correlated with administered oral dose or estimated dermal dose, respectively (Figs. 3A and 3B), although the correlation for the dermal exposure was stronger than for the oral exposure ($R^2 = 0.80$, $p = 0.0005$ versus $R^2 = 0.58$, $p = 0.03$, respectively). For the dermal exposure, there was a significant correlation between both AUC$_{0-1440}$ (Fig. 3C) and AUC$_{60-1440}$

TABLE 3
Pharmacokinetic Parameters for Dermal Exposures[a]

Subject ID	Compartment	Weighting	AUC$_{0-1440}$ (ng min/l)	AUC$_{60-1440}$ (ng min/l)	α (min^{-1})[b]	β (min^{-1})[c]	$t_{1/2\alpha}$ (min)[d]	$t_{1/2\beta}$ (min)[e]	$C_{endexposure}$[f] (ng/l)
A	2	$1/y^2$	10800	7420	0.0217	0.00151	32.1	459	84.6
B	2	$1/y^2$	9190	7250	0.0338	0.00202	20.5	342	52.9
C	2	$1/y^2$	17700	13400	0.0203	0.00163	34.1	426	151
D	1	$1/y^2$	15300	10900	0.0120		57.7		99.0
E	2	$1/y^2$	9490	7220	0.0189	0.00149	36.7	464	62.3
F	2	$1/y^2$	4320	3220	0.0228	0.00228	30.4	304	39.4
G	1	$1/y^2$	13100	9560	0.00879		78.8		87.0
H	2	$1/y^2$	20200	12300	0.0217	0.00199	31.9	348	170
I	2	$1/y^2$	6790	4340	0.0390	0.00582	17.8	119	65.6
J	2	$1/y^2$	10900	6550	0.0140	0.00119	49.7	583	93.5
Mean			11800	8220	0.0213	0.00226	32.6[g]	309[g]	90.5
SD			4880	3300	0.00914	0.00155			41.6
% CV			41.4	40.1	42.9	68.6			45.9

[a]Compartmental analysis.
[b]First-order rate constant for initial phase of concentration time curve.
[c]First-order rate constant for terminal phase of concentration time curve.
[d]Half-life for initial phase.
[e]Half-life for terminal phase.
[f]Concentration in blood at end of 60 min dermal exposure.
[g]Harmonic mean.

(not shown) with the estimated dermal dose, $R^2 = 0.78$, $p = 0.0007$ and $R^2 = 0.73$, $p = 0.0017$, respectively. For the oral exposure, there was a weak correlation of CZOH/CZ with both apparent clearance (CL/F) ($R^2 = 0.48$, $p = 0.084$) and C_{max} normalized to dose ($R^2 = 0.45$ and $p = 0.07$) (Figs. 4A and 4B). In addition, the first order rate constant for the initial elimination phase (α) following dermal exposure was correlated with CZOH/CZ, $R^2 = 0.63$ and $p = 0.011$ (Fig. 4C). Percentage body fat was not correlated with any pharmacokinetic parameter for either route of administration.

Urine mutagenicity data sets were obtained from 10 percutaneously exposed subjects and 8 orally exposed subjects (Figs. 5A and 5B). Compared to predose values, increases in mutagenicity were observed in urine samples collected after ^{13}C-BDCM exposure from 90% (9/10) of the dermal subjects and 50% (4/8) of the oral subjects. A significant difference was noted between the times of peak urine mutagenicity for dermal and oral subjects. The mean peak time after dermal exposure was 4.5 h compared with 2.2 h after oral exposure. Peak postdose mutagenicity levels that were at least twice that of the predose mean level occurred in 60% (6/10) of the dermal subjects and 38% (3/8) of the oral subjects. Among subjects with postexposure increases in urine mutagenicity, peak mutagenicity values (revertants/mg creatinine) of the dermal subjects (9.09 ± 3.04) and oral subjects (10.08 ± 3.93) were significantly greater than their respective predose values (1.31 ± 0.42 and 1.03 ± 0.68). Urinary pH did not vary significantly over the course of sample collection for either route of exposure.

DISCUSSION

Concerns about potential adverse health effects of DBPs, especially carcinogenic, reproductive, and developmental toxicities, have motivated several investigations of household exposures to DBPs (primarily THMs) via multiple uses of tap water, including determinations of the resulting blood or exhaled breath concentrations of the most prevalent DBPs (Backer *et al.*, 2000; Lynberg *et al.*, 2001; Miles *et al.*, 2002; Nuckols *et al.*, 2005; Weisel and Jo, 1996). Although these studies have shown that oral and dermal/inhalation exposures produce significantly different postexposure blood concentrations of THMs, controlled exposures to BDCM with sequential time measurements are needed to quantify BDCM pharmacokinetics and to support development of PBPK models that describe BDCM disposition. Such models can then be further validated with results from the household studies.

Validated PBPK models can be linked with human activity and water usage models to better characterize daily internal doses of DBPs. For example, Haddad *et al.* (2006) published a PBPK model for THM exposures from showering but only had chloroform data to validate the model structure. The development of the data set presented here represents an
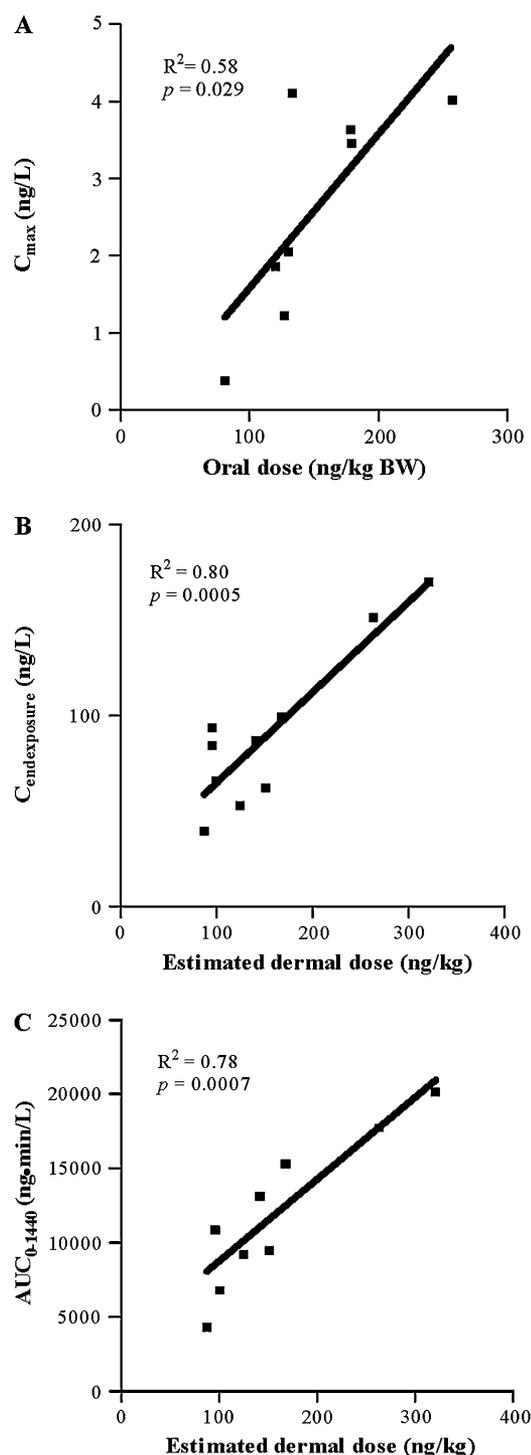

FIG. 3. Correlations of (A) C_{max} from oral exposure with administered dose; (B) $C_{endexposure}$ from dermal exposure with estimated dermal dose; and (C) AUC_{0-1440} from dermal exposure with estimated dermal dose for each subject.

important step toward eventual development of a validated human PBPK model for BDCM. In this study, the pharmacokinetics of BDCM were determined for human volunteers under controlled experimental conditions in a clinical setting.
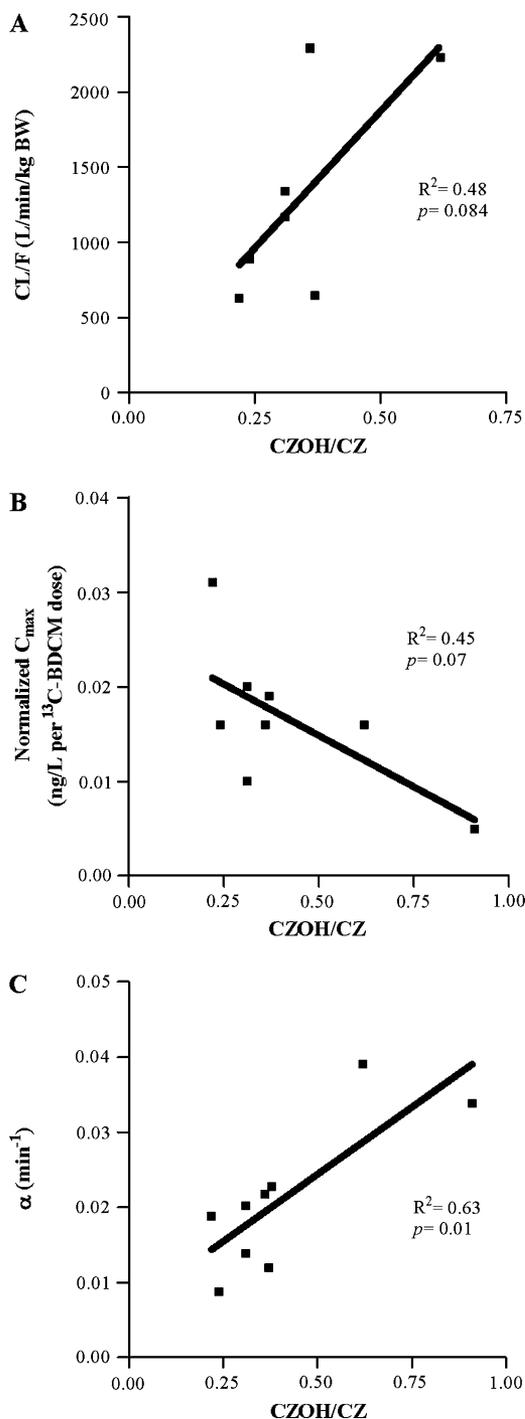
FIG. 4. Correlations of chlorzoxazone metabolic ratio (CZOH/CZ), an indicator of CYP2E1 activity, with (A) apparent clearance normalized to bioavailability from the oral exposure; (B) C_{max} normalized to dose from the oral exposure; and (C) the initial elimination phase rate constant (α) from the dermal exposure.

Physiological and biochemical parameters that may affect interindividual variability of BDCM pharmacokinetics were also measured in the study subjects and analyzed for correlations with observed disposition.
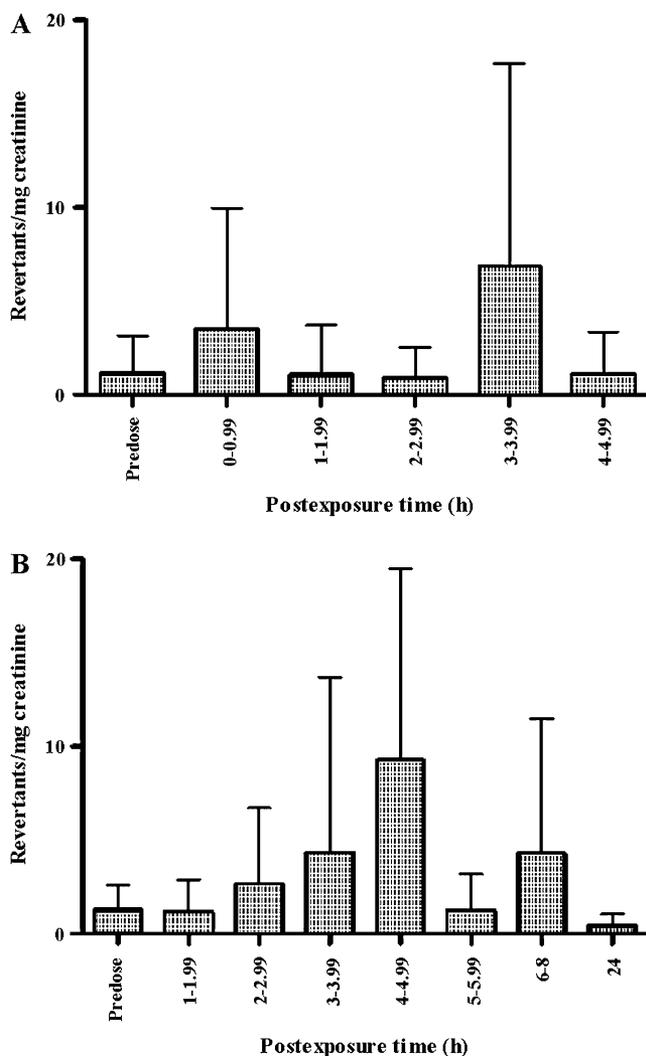
FIG. 5. Mutagenic activity of urine normalized to creatinine concentration following (A) oral and (B) dermal exposure to ^{13}C-BDCM.

In the present study, oral and dermal exposures produced dramatically different blood concentration profiles of ^{13}C-BDCM. Although the oral doses were actually slightly greater than the dermal doses for most subjects (Table 1), the maximum blood concentrations attained after dermal exposure ($C_{endexposure}$) were 25–130 times higher than the C_{max} values attained after oral exposure. In addition, blood concentrations returned to pre-exposure levels within a few hours following ingestion of ^{13}C-BDCM, whereas ^{13}C-BDCM was still detectable in the blood at 24 h after dermal exposure in the majority of subjects. These findings are also consistent with the results of previous BDCM exposure assessments in human subjects (Backer et al., 2000; Lynberg et al., 2001; Miles et al., 2002; Nuckols et al., 2005). These investigators observed increases in postactivity blood concentrations of BDCM in subjects exposed via showering or bathing but not in subjects exposed via ingestion of tap water. This difference in pharmacokinetics

between the two routes of exposure indicates that significant first-pass hepatic removal (i.e., metabolism) of ^{13}C-BDCM occurred following ingestion of water containing ^{13}C-BDCM. A significant potential for hepatic CYP2E1 metabolism of BDCM has been reported previously (Allis and Zhao, 2002; Lilly *et al.*, 1997, 1998; Zhao and Allis, 2002). As mentioned previously, no hepatic effects have been reported in association with human exposure to BDCM. However, the extrahepatic effects that have been correlated with human exposure would seem to warrant a focus on BDCM in the systemic circulation. Additional explanations for the lower circulating levels of ^{13}C-BDCM after oral exposure in this study may include lower bioavailability and/or absorption from the gut, perhaps due to gastrointestinal metabolism of BDCM. However, these would likely be minor factors because 81–97% of orally dosed BDCM was absorbed in rats (Mathews *et al.*, 1990).

After the dermal exposures, elimination of ^{13}C-BDCM was rapid; the mean half-life during the initial phase was 32.6 min, which is consistent with expected half-lives of volatile compounds in blood. Most of the subjects had a second phase of elimination, which was approximately nine times slower and would be consistent with distribution from compartments in the body such as adipose tissue. In previous studies of environmental blood concentrations of DBPs, measurable preactivity blood concentrations of BDCM were observed, which were postulated to be due to accumulation of BDCM from slower elimination after the initial rapid removal from blood (Backer *et al.*, 2000; Lynberg *et al.*, 2001). The longer terminal half-life measured in this study corroborates these previous findings and indicates that dermal exposures during water-use activities result in measurable amounts of BDCM for several hours after exposure. A similar result would also be expected after inhalation exposures to BDCM and other volatile DBPs.

Previously, researchers reporting environmental exposure results for BDCM (Backer *et al.*, 2000; Lynberg *et al.*, 2001; Miles *et al.*, 2002; Nuckols, 2005) have suggested that variability in measured blood concentrations of BDCM could be due to interindividual differences in metabolic capacity. In the current study, the estimated dermal dose was highly correlated with C_{max} and AUC, but the association between C_{max} and the oral dose was weaker. In addition, there was a weak correlation between CZOH/CZ and the oral C_{max}, but not the dermal $C_{endexposure}$. Therefore, intersubject variability in CYP2E1 activity could be partly responsible for the variability seen in measured blood concentrations after BDCM ingestion. However, other xenobiotic metabolizing enzymes, such as GSTT1-1, CYP1A2, and CYP2B, are likely involved (Allis and Zhao, 2002; Pegram *et al.*, 1997) and may thus play a role in interindividual variability in human BDCM pharmacokinetics. Without the significant hepatic first-pass metabolism observed with oral exposure, the primary determinant of maximum systemic BDCM levels attained via percutaneous absorption appears to be the water concentration of BDCM.

The blood concentrations observed in the present study correlated well with blood concentrations measured from environmental exposures to BDCM. Table 4 compares the estimated dermal dose from this study to estimated dermal doses from Backer *et al.* (2000) and Nuckols *et al.* (2005). Lynberg *et al.* (2001) and Miles *et al.* (2002) could not be included because detailed exposure information was not given. The water concentrations of BDCM in those studies were 6.2 µg/l for Backer *et al.* (2000) and 32 µg/l and 9 µg/l for the North Carolina and Texas sites in Nuckols *et al.* (2005). The dermal doses were calculated as in the current study, accounting for surface area for males and females and length of exposure. There was good correspondence between the ratios of doses compared with the ratios of measured blood concentrations at each of the postexposure time points.

A similar comparison (Table 5) was conducted with blood concentrations of ^{13}C-BDCM following oral exposure from the current study and with Backer *et al.* (2000). The study population from Backer *et al.* (2000) ingested 1 liter of water containing 6.2 µg/l of BDCM. Although the water concentrations were higher in the current study, the Backer *et al.* (2000) subjects drank larger volumes of water and, therefore, were exposed to similar oral doses of BDCM. Again, the ratio of doses between studies corresponded well with the ratio of the blood concentrations measured after exposure.

The correspondence of the current blood concentrations from ^{13}C-BDCM dermal and oral exposures with previously published studies measuring environmental exposures raises two points. First, a PBPK model developed based on the results of this controlled human study can be expected to accurately predict the pharmacokinetics of populations exposed environmentally to BDCM. Second, inhalation appears to play a minor role in contributing to blood concentrations of BDCM from bathing and showering exposures. Otherwise, the ratio of the blood concentrations between other studies and the current study would have been significantly larger than the ratio of the doses. This differs from chloroform as demonstrated by the Jo *et al.* (1990) study, which reported that the mean internal dose of chloroform due to dermal absorption while showering was approximately equal to that due to inhalation. Lower volatility, greater lipophilicity (Lilly *et al.*, 1997), and the greater dermal permeability of BDCM compared to chloroform (Xu *et al.*, 2002) are likely factors contributing to these differences.

Kerger *et al.* (2000) quantified the air concentrations of the THMs, chloroform, BDCM, and dibromochloromethane in showers and baths resulting from their volatilization from water. Due to its greater vapor pressure, chloroform air concentrations were approximately twice those of BDCM. Kerger estimated air concentrations of 0.6 and 1 µg/m^3 per µg/l of BDCM in water for baths and showers, respectively. Based on average alveolar ventilation rates, the amount of BDCM inhaled in the Backer *et al.* (2000) and Nuckols *et al.* (2005) studies would have been approximately 10–17% of the amount absorbed percutaneously.

Urinary mutagenicity has been used extensively to measure genotoxic exposures resulting from cigarette smoke, benzidine dyes, chemotherapeutic agents, fried meat consumption, etc.

TABLE 4
Comparison of Literature Dermal Doses and Blood Concentrations with Current Study

Study	Water concentration (μg/l)	Estimated dose[a] (ng)	Postexposure blood concentration (ng/l)			Ratio dose:current study dose[b]	Ratio blood concentration:current study concentration[c]		
			5 min	10 min	15 min		5 min	10 min	15 min
Current Study	60.4	11,500	83	62[d]	41	1.0	1.0	1.0	1.0
Backer *et al.* (2000)	6.2	2900	—	17	10	0.25	—	0.27	0.24
Nuckols *et al.* (2005)									
NC site	32	18,000[e]	93	—	—	1.6	1.1	—	—
Texas site	9	6000	36	—	—	0.5	0.43	—	—

[a]Dermal dose was estimated from the water concentration, the length of exposure, and the permeability of BDCM (0.18 cm/h) reported by Xu *et al.* (2002). The surface area for the current study was calculated from each subject's weight and height with the algorithm from EPA's Exposure Factor Handbook (U.S. EPA, 1997). The arm and forearm were assumed to be 5.5% of total surface area as reported by the EPA handbook. Mean total surface areas for males and females, 1.94 and 1.69 m^2, respectively, were used for Backer *et al.* (2000) and Nuckols *et al.* (2005). The head and neck were excluded from the exposures for the bath or shower, so a total of 88% of the body was assumed to be exposed in those studies. The dose was averaged based on the number of women and men participating in each of the study populations.

[b]The doses from each study were divided by the estimate of dose from the current study; the ratio is unitless.

[c]The mean blood concentrations from each study were divided by the mean blood concentrations from the current study; the ratio is unitless.

[d]Interpolated from 5- and 10-min mean concentrations.

[e]For the NC site the comparison of dose was made to the showering exposure. The blood concentration from the bathing exposure was low compared with the reported water concentration of 26 μg/l. Postexposure blood concentrations from the bath were 41 ng/l compared with 36 ng/l from the Texas site, whose water concentrations were 9 μg/l.

(Cerna and Pastorkova, 2002). A recent clinic-based, case-control study showed that colorectal adenoma risk was 2.4-fold higher in subjects in the highest versus the lowest quintile of urinary mutagenicity (Peters *et al.*, 2003), and colon cancer is one of the cancers associated with human exposure to chlorinated drinking water (King *et al.*, 2000b) and BDCM exposure in rats (NTP, 1987). Therefore, urinary mutagenicity was measured as a potential biomarker of BDCM exposure and genotoxicity in the present study.

Because we have shown that BDCM is activated to a mutagen in the *GSTT1-1*–transfected *Salmonella* strain RSJ100, thus providing a very sensitive mutagenicity assay for BDCM (DeMarini *et al.*, 1997; Pegram *et al.*, 1997), we used this strain to evaluate urinary mutagenicity among the subjects before and after ^{13}C-BDCM exposure. The increases in urinary mutagenicity that were observed after the exposures were likely due to the excretion of unmetabolized BDCM into the urine, which was then activated by *GSTT1-1* expressed within the RSJ100 *Salmonella* cells. Mutagenic BDCM metabolites produced via *GSTT1-1 in vivo* would probably have reacted with macromolecules at the site of their generation, thus never appearing in urine. If any metabolites of BDCM were excreted into the urine, their hydrophilicity would have likely prevented their extraction by C18/methanol for mutagenicity testing.

Due to the reasons described above, one would not necessarily expect the *GSTT1-1* genotype of the subjects to correlate with urinary mutagenicity. However, the two null

TABLE 5
Comparison of Literature Oral Doses and Blood Concentrations with Current Study

Study	Water concentration (μg/l)	Dose[a] (ng/kg)	Postexposure[b] increase in blood concentration (ng/l)	Ratio dose:current study dose[c]	Ratio blood concentration:current study concentration[d]
Current study	38.9	146	2.3	1.0	1.0
Backer *et al.* (2000)	6.2	89	1.2	0.61	0.52

[a]For the current study, the reported dose is the mean of all administered doses. For Backer *et al.* (2000) the subjects drank 1 l of water. The dose was estimated assuming an average body weight of 70 kg because the average body weight of the study subjects was not given. Nuckols *et al.* (2005) was not included in the comparison because postexposure blood concentrations were not elevated, and the subjects drank water during meals over an extended time period (30 min).

[b]The blood concentrations were for 10-min postexposure for both studies.

[c]The doses from each study were divided by the estimate of dose from the current study; the ratio is unitless.

[d]The mean blood concentrations from each study were divided by the mean blood concentrations from the current study; the ratio is unitless.

GSTT1-1 subjects also had the two lowest CYP2E1 activities; thus, one might expect to have observed lower total BDCM metabolism in these subjects and, perhaps, greater excretion of parent compound into the urine. Interestingly, these two subjects were, in fact, among those with the highest C_{max} values and greatest peak levels of urinary mutagenicity after BDCM exposure. However, the role of genotype cannot be conclusively assessed in this study due to the small number of subjects.

Although there are several exposure assessment studies that have measured tap water concentrations of BDCM and exhaled breath or blood concentrations from daily activities, this is the first controlled study to provide time-course data for BDCM absorption and disposition from human oral and dermal exposures. Our results demonstrate a highly significant contribution of percutaneous absorption to circulating levels of BDCM and confirm the much lower contribution of oral consumption, thus indicating that showering, bathing, and other water uses involving dermal contact will lead to much greater systemic BDCM doses than ingestion of water. This observation supports recent epidemiology studies showing that longer duration of showering or bathing with water containing THMs was associated with a two-fold increased risk for bladder cancer compared to the lowest duration group (Villanueva *et al.*, 2007).

The pharmacokinetic data measured in the current study will be valuable for developing and validating human PBPK models to describe disposition of BDCM. PBPK models for BDCM can be combined with exposure-assessment data, such as the activity analyses from Lynberg *et al.* (2001) and Haddad *et al.* (2006), to better predict tissue dosimetry of BDCM and its reactive metabolites resulting from multi-route exposures via disinfected drinking water. Such an analysis will help identify mechanistic links between drinking water exposures to BDCM and possible health effects, which will improve risk estimates for BDCM.

ACKNOWLEDGMENTS

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