Dichloroacetic acid (DCAA) is a by-product of drinking water disinfection, is a known rodent hepatocarcinogen, and is also used therapeutically to treat a variety of metabolic disorders in humans. We measured DCAA bioavailability in 16 human volunteers (eight men, eight women) after simultaneous administration of oral and iv DCAA doses. Volunteers consumed DCAA-free bottled water for 2 weeks to wash out background effects of DCAA. Subsequently, each subject consumed $^{12}\text{C}$-DCAA (2 mg/kg) dissolved in 500 ml water over a period of 3 min. Five minutes after the start of the $^{12}\text{C}$-DCAA consumption, $^{13}\text{C}$-labeled DCAA (0.3 mg/kg) was administered iv over 20 s and plasma $^{12}\text{C}/^{13}\text{C}$-DCAA concentrations measured at predetermined time points over 4 h. Volunteers subsequently consumed for 14 consecutive days DCAA 0.02 $\mu$g/kg/day dissolved in 500 ml water to simulate a low-level chronic DCAA intake. Afterward, the $^{12}\text{C}/^{13}\text{C}$-DCAA administrations were repeated. Study end points were calculation of AUC$_{0-\infty}$, apparent volume of distribution ($V_{ss}$), total body clearance ($Cl_b$), plasma elimination half-life ($t_{1/2,\text{b}}$), oral absorption rate ($K_a$), and oral bioavailability. Oral bioavailability was estimated from dose-adjusted AUC ratios and by using a compartmental pharmacokinetic model after simultaneous fitting of oral and iv DCAA concentration-time profiles. DCAA bioavailability had large interindividual variation, ranging from 27 to 100%. In the absence of prior DCAA intake, there were no significant differences ($p > 0.05$) in any pharmacokinetic parameters between male and female volunteers, although there was a trend that women absorbed DCAA more rapidly (increased $K_a$), and cleared DCAA more slowly (decreased $Cl_b$), than men. Only women were affected by previous 14-day DCAA exposure, which increased the AUC$_{0-\infty}$ for both oral and iv DCAA doses ($p < 0.04$ and $p < 0.014$, respectively) with a corresponding decrease in the $Cl_b$.

**Key Words:** haloacetic acids; chlorination; GST-zeta.

Halogenated acetic acids (HAAs) including dichloroacetic acid (DCAA) are routinely identified by-products formed during the disinfection of drinking water. Human health concerns exist over disinfection by-products in general and DCAA in particular because they have been shown to cause cancer and have adverse effects on reproduction in experimental animal models (Kaydos et al., 2004; Komulainen, 2004). Prior to the implementation of stage 1 regulations by the US EPA in 1998, concentrations of chlorinated HAAs (monochloro acetic acid, DCAA, and trichloro acetic acid [TCAA]) and specific brominated HAAs (monobromo HAA and dibromo HAA [DBAA]) were reported in excess of 100 $\mu$g/l (Arora et al., 1997; Uden and Miller, 1983). Concentrations of chlorinated HAAs in swimming pools may exceed 1000 $\mu$g/l (Loos and Barcelo, 2001) and ingestion of pool water can be an additional exposure mechanism for HAAs (Kim and Weisel, 1998). A recent nationwide survey of drinking water disinfection by-products reported concentrations of total HAAs to be less than 100 $\mu$g/l, with specific values for DCAA ranging from 1.3 to 40 $\mu$g/l (Weinberg et al., 2002). The wide variation in the concentration of specific HAAs in drinking water, both between and within sites, is caused by differences in the disinfection method used and the chemical characteristics of the source water (Pourmoghaddas et al., 1993; Williams et al., 1997). For instance, bromide-rich source water leads to increased brominated HAA concentrations.

Reliable information on HAA disposition in humans is limited to DCAA, which has been used as an experimental therapeutic agent for more than 20 years (Stacpoole et al., 1998b). The principal mechanism for elimination of DCAA is biotransformation, which primarily occurs in the liver. The importance of the liver in whole-body clearance of DCAA by humans is evidenced by a marked decrease in plasma clearance in patients with cirrhosis (Shangraw and Fisher, 1999) and the complete cessation of DCAA clearance during the anhepatic phase of liver transplantation (Shangraw and Fisher, 1996). Pharmacokinetic studies of iv administered DCAA in humans show a dose dependency of total body clearance ($Cl_b$), where higher doses are cleared more slowly, presumably due to

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saturation of metabolism (Curry et al., 1991; Fox et al., 1996; Lukas et al., 1980; Wells et al., 1980). Repeated clinical dosing of DCAA progressively reduces \( C_{lb} \) (Curry et al., 1985, 1991), and diminished \( C_{lb} \) consequent to repetitive dosing still occurs in some individuals despite a lapse of several weeks between DCAA doses (Curry et al., 1991). The variability of DCAA \( C_{lb} \) after multiple doses has prevented reliable estimation of oral bioavailability because the requirement of no change in pharmacokinetics between dosing times can never be met (Curry et al., 1991).

Rodent and in vitro human tissue studies have established that DCAA is metabolized by the cytosolic enzyme glutathione-s-transferase-zeta (GSTZ1-1; Tong et al., 1998). The initial metabolite formed by the GST-zeta pathway during DCAA and other di-HAA metabolism is glyoxylate (Tong et al., 1998). An important feature of the GST-zeta pathway is the suicide inactivation by DCAA, which causes not only a direct reduction in GST-zeta activity but also a marked and protracted reduction of tissue GST-zeta protein content (Anderson et al., 1999; Gonzalez-Leon et al., 1997; Schultz et al., 2002). This can result in a “functional” GST-zeta deficiency, slowing DCAA metabolism and prolonging its biological persistence. The decrease in hepatic GST-zeta protein is not associated with transcriptional changes as GST-Z mRNA remains unchanged (Ammini et al., 2003). Thus, at least in rodents, discontinuation of DCAA exposure allows rapid (within 48 h) recovery of GST-zeta protein content and activity to baseline levels (Anderson et al., 1999; Schultz et al., 2002).

The present study was designed to measure oral DCAA bioavailability in healthy women and men and to assess whether prolonged low-level DCAA exposure (20 \( \mu \)g/kg/day) increases its bioavailability. We determined DCAA bioavailability after simultaneous administration of unlabeled oral and \( ^{13}C \)-labeled iv doses. This approach permitted calculation of oral bioavailability of DCAA in each subject without a crossover experimental design. A final aim attempted to measure the absorption of DCAA and other HAAs naturally present in a municipal drinking water supply in male and female volunteers.

**MATERIALS AND METHODS**

**Human subjects.** This protocol was approved by the Institutional Review Boards at Oregon Health & Science University (OHSU) and the Battelle Pacific Northwest National Laboratory. Written informed consent was obtained from each volunteer prior to his or her participation in this study. Eighteen healthy volunteers (age range 23–33 years) were enrolled after written informed consent was obtained. Two subjects withdrew from the study before completion and have not been included in the analysis. Demographics of the included 16 subjects (eight men, eight women) are shown in Table 1. Health status was documented by history, physical examination, and screening laboratory evaluation. Subjects abstained from smoking, consuming alcohol, or using drugs of any kind (other than common over-the-counter medications) during the study period. Pregnancy was ruled out in female subjects by urine \( \beta \)-hCG evaluation. Subjects limited their water intake, including that for cooking, to DCAA-free bottled water throughout the study.

**Chemicals and reagents.** DCAA for oral intake was obtained from TCI Chemical (Na-DCAA salt [purity > 99%], Cat. No. D1719DMF, Portland, OR). DCAA for iv use was \( ^{13}C_1 \)-labeled by custom synthesis (Isotec, St. Louis, MO) and determined to be > 99.9% chemically pure and > 99.99% isotopically pure by GC-MS. Aliquots of iv solution were tested for sterility (OSU clinical microbiology laboratory) and for pyrogenicity by NAMSA (San Francisco, CA) using the rabbit ear technique (Stucpool et al., 1983). Chlороfluro acetic acid (CFAA) was prepared by hydrolysis of the CFAA ethyl ester (Lancaster Synthesis, Windham, NH) as described by Schultz and Sylvester (2001) and was used as an internal standard in GC-MS analysis. Diazomethane was prepared as described in the Aldrich technical bulletin no. AL-132 and stored at –70°C in sealed vials until use. All other common chemicals and solvents were of the purest grade available.

**Experimental design.** The study consisted of two parts that were collectively designed to measure DCAA pharmacokinetic parameters and systemic bioavailability before and after a 14-day exposure of drinking water to 20 \( \mu \)g/kg/day DCAA. The first part of the study was performed after an initial 14-day period when the subjects consumed only HAA-free bottled water in lieu of municipal tap water for drinking and cooking. This intervention was to remove trace HAAs from the subjects and to ensure that GST-zeta activity and DCAA \( C_{lb} \) were at a true basal level for each subject. On the morning of day 15, subjects were admitted to the OHSU General Clinical Research Center for pharmacokinetics evaluation. After placement of bilateral iv cannulas and baseline blood sampling, subjects consumed 2 mg/kg of unlabelled DCAA dissolved in 500 ml of bottled water over a period of 2–3 min. Five minutes after initiating consumption of the bottled water, 0.30 mg/kg of \( ^{13}C_1 \)-labeled DCAA in 20 ml isotonic saline was injected iv over a period of 20 s. Time delay for administration of the iv dose was designed to optimize superimposition of the oral and iv plasma profiles, improving the accuracy of oral bioavailability estimates (Rubin et al., 1987). The dose of \( ^{13}C_1 \)-DCAA was selected to produce a molar ratio of \( ^{13}C / ^{12}C \) in plasma of approximately 0.2 to minimize error associated with GC-MS analysis of the respective \( ^{13}C / ^{12}C \)-DCAA (Bluck et al., 1996). Blood sampling (2 ml) from the contralateral arm was carried out at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, and 120 min. Blood samples were collected into tubes containing ethylenediamine tetraacetate acid, placed immediately on ice, and centrifuged to separate plasma (3000 \( \times \) g for 10 min), and the plasma was stored at –70°C until analysis. Three additional blood samples (1.0 ml) were drawn immediately prior to dosing and at 2 and 4 h after the iv DCAA administration for immediate analysis of plasma glucose and lactate concentrations (Table 1). This analysis indicated that DCAA had no effect on either plasma lactate or glucose concentrations in any subject. Subjects remained in the Clinical Research Center, did not consume food until 2 h after the start of the experiment, and were discharged home at the end of the evaluation.

The second part of the study involved the same volunteers and began the day after the initial pharmacokinetic evaluation. During this time, subjects ingested DCAA 20 \( \mu \)g/kg daily in 500 ml water for 14 days but otherwise continued to be restricted to intake of bottled water for drinking and cooking. This treatment was intended to simulate daily consumption of municipal drinking water containing approximately 1 mg total HAA per day. On the morning of day 15, 1 day after consumption of the last chronic DCAA dose, subjects returned to the OHSU Clinical Research Center for a repeat of the pharmacokinetic evaluation.

The final aim of this project was to determine oral absorption of HAAs naturally found in a U.S. municipal drinking water supply in four healthy volunteers (two women, two men) who previously consumed for 14 days only bottled water free of HAA contamination. Analysis of the drinking water identified the following HAAs: BCAA, 23 \( \mu \)g/l; DCAA, 16 \( \mu \)g/l; TCAA, 11 \( \mu \)g/l; and DBAA, 4.8 \( \mu \)g/l (mean value from two separate measurements). Also present in trace levels (< 2 \( \mu \)g/l) were BDCAA, CDBAA, and TBAA. Because of the low HAA content in the tested water, we added \(^{13}C_1 \)-DCAA at 20 \( \mu \)g/kg, which was intended to serve as a reference for comparison with measurement of the naturally occurring HAAs. Subjects consumed 1 l of this water, and blood sampling was done at 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min. HAAs were extracted from 1 ml of plasma as described below for GC-MS analysis.
except a GC-µECD was used as the primary detection method (GC-MS was used for selective detection of the \(^{13}\)C\(_1\)-DCAA). Larger sample volume and improved detector sensitivity of the µECD improved the method detection limit (MDL) to 0.2 ng/ml for DCAA, 0.3 ng/ml for DBAA, and \(\geq 0.4\) ng/ml for the other HAAs.

**GC-MS analysis of DCAA.** DCAA was extracted from 0.25 ml of acidified plasma with methyl-tetra-butyl ether (MTBE) and derivatized with diazomethane, as described previously (Schultz et al., 1999, 2002). MTBE extracts were dried under \(N_2\) to 25 \(\mu\)l and 1 \(\mu\)l was injected onto the GC. Simultaneous determination of \(^{12}\)C- and \(^{13}\)C-labeled DCAA concentrations was performed using an Agilent 5973i EI-MS in selected ion monitoring mode. The GC column was a Beta D ex 225 column (30 m, 0.25µm; Supelco, Bellefonte, PA) and the chromatographic conditions were: injection port temperature, 150°C; initial oven temperature 30°C X 3 min and ramping at 2°C/min to 60°C. DCAA-methyl ester, like other HAA methyl esters, produces a characteristic fragmentation pattern in EI-MS with the formation of two predominant ions corresponding to the C\(_1\)-methyl ester and the halogenated C\(_2\) carbon. The \(^{12}\)C\(_1\)-methyl ester of DCAA has \(m/z\) 59 and the \(^{13}\)C\(_1\)-methyl ester has \(m/z\) 60. The C\(_2\) fragment has \(m/z\) 83. Figure 1 shows the mass spectra of \(^{12}\)C- and \(^{13}\)C-DCAA methyl esters. Quantification of \(^{12}\)C- and \(^{13}\)C-DCAA in plasma used human plasma standards containing known amounts of \(^{13}\)C- \(^{12}\)C-DCAA at a \(^{13}\)C/\(^{12}\)C molar ratio of 0.2. Background \(m/z\) 60 content due to naturally occurring \(^{13}\)C-DCAA present in \(^{13}\)C-DCAA solutions was verified to be a uniform 2.3% over the DCAA concentration range measured, consistent with the relative natural incidence of \(^{13}\)C versus \(^{12}\)C. Background \(m/z\) 59 (\(^{12}\)C) levels in the \(^{13}\)C-DCAA iv dose solution were exceptionally low at < 0.01%, indicating the great isotopic purity. This allowed adjustment of measured \(m/z\) 60 levels for background by subtracting the value corresponding to \(m/z\) 59 area X 0.023. Sample results were confirmed by parallel analysis by GC-ECD (Hewlett-Packard 5890) to quantify total DCAA (\(^{13}\)C-, \(^{15}\)C-DCAA). Acceptance of the GC-MS measurements required both the GC-MS (\(^{13}\)C + \(^{15}\)C-DCAA values) and GC-ECD determined values to be within 80% agreement. Failure to achieve this level of concordance required reanalysis of samples using archived replicates for each plasma sample. MDL for DCAA was 1 ng/ml for \(^{13}\)C-DCAA and 3 ng/ml for \(^{15}\)C-DCAA.

**Pharmacokinetic analysis.** Both noncompartmental and compartmental modeling methods were used to analyze the DCAA concentration-time profiles. Noncompartmental analysis of the plasma profiles was performed by calculating the AUC\(_{0\rightarrow\infty}\) using the linear trapezoidal model with area from the last sampling time to infinity calculated from the slope of the terminal portion of the concentration-time profile (b). Steady-state volume of distribution (\(V_{ss}\)) and total body clearance (\(CL_{B}\)) were calculated from the iv pharmacokinetic profile, as described by Yamaoka et al. (1978) and Gibaldi and Perrier (1982). Plasma elimination half-life (\(t_{1/2b}\)) was calculated as \(t_{1/2b} = 0.693/b\). Bioavailability was calculated as:

\[
\text{Bioavailability} = \frac{[\text{AUC}_{0\rightarrow\infty, \text{oral}}]}{[\text{AUC}_{0\rightarrow\infty, \text{iv}}]} \times \left(\frac{\text{Dose iv}}{\text{Dose oral}}\right)
\]

Oral and iv pharmacokinetic profiles from each subject were also simultaneously fitted to a one-compartment clearance-volume model based on the two-compartment model described by Gabrielsson and Weiner (2000). Preliminary testing indicated that this model gave similar results with or without correction for a time lag for oral absorption. Therefore, a time lag parameter was not included for the final fitting of the data. The observed oral and iv profiles were then simultaneously fitted to model-based differential equations using a nonlinear least-squares regression program (WinNonlin version 2.1; Pharsight Corp. Cary, NC). For the fitting procedure, observed values were equally weighted (wt. = 1). This approach was used to estimate absorption rate (\(K_a\)), peak blood concentration (\(C_{max}\)), and oral bioavailability.

**Statistics.** Data are expressed as mean ± SD. Oral bioavailability, \(K_a\), \(C_{max}\), \(V_{ss, \text{oral}}\), \(CL_{B, \text{oral}}\), and \(t_{1/2b}\) before and after the DCAA treatments were compared by paired \(t\)-test. Gender differences in pharmacokinetic parameters were assessed by nonparametric Mann-Whitney test. Differences between groups were considered statistically significant at \(p < 0.05\).

**RESULTS**

**Baseline Pharmacokinetics and Bioavailability of DCAA**

Plasma DCAA concentration-time profiles for the volunteer groups are shown in Figures 2A and 2B. A summary of the pharmacokinetic analysis for each individual subject, using compartmental and noncompartmental approaches, is shown in Table 2. In general, the shapes of the oral and iv DCAA profiles were qualitatively similar for all subjects. Peak plasma
DCAA concentration ($C_{\text{max}}$) occurred rapidly after oral dosing, typically by 15–30 min. $C_{\text{max}}$ varied substantially between subjects, ranging from 600 to 3423 ng/ml (Table 2). Total body clearance ($Cl_b$) also varied considerably, ranging from 716 to 4300 ml/kg/h. In contrast, $V_{\text{ss}}$ and $K_{\text{a}}$ were more consistent among subjects (Table 2). Plasma DCAA concentration decreased below 10 ng/ml by 2 h in all subjects. Individual oral bioavailability estimates by dose-normalized AUC$_0$/$N$ ratios and from simultaneous fitting of the oral and iv profiles to the pharmacokinetic model yielded similar values (Table 2). By either technique, there was a wide interindividual variability in oral DCAA bioavailability, ranging from 27 to 100%.

There were no significant gender differences in any pharmacokinetic parameter ($p > 0.05$). Nevertheless, there was a trend observed for women to have a higher $C_{\text{max}}$ (30% greater than men) and AUC$_0$/$\infty$ (20% greater than men; Table 2).

**Pharmacokinetics and Bioavailability of DCAA after a 14-day Exposure to DCAA 20 $\mu$g/kg**

The volunteers who participated in the basal pharmacokinetics study continued with the 14-day exposure to low dose DCAA. Baseline plasma, obtained before the oral and iv challenges of the $^{12}$C/$^{13}$C DCAA doses, did not detect any residual DCAA. This indicates that 1 day between the last DCAA exposure and the start of the challenge sufficed for complete elimination of DCAA from plasma, within the limits of detection. The pharmacokinetic results closely resembled those in the basal state and are summarized in Table 3. There were no statistically significant differences in DCAA pharmacokinetics between men and women exposed to DCAA for 2 weeks. When compared to the basal data, women but not men were affected by the chronic DCAA exposure. After the 14-day DCAA intake, the AUC$_0$/$N$ for both oral and iv DCAA doses was respectively increased by 36 and 34% of the values measured in the basal study ($p < 0.04$ and $p < 0.014$, respectively). The $C_{\text{max}}$ was substantially lower in subject 6, but this appeared to be a unique result (Table 3). Consistent with the increased AUC, $Cl_b$ was decreased by 40% ($p < 0.014$) in women after the DCAA exposure. Although prolonged DCAA intake reduced clearance in women, it did not alter oral bioavailability for either women or men.

**Basal Pharmacokinetics and Bioavailability of HAAs in Municipal Drinking Water**

We attempted to characterize the absorption of HAAs including DCAA, at concentrations naturally present in a U.S. municipal tap water supply in a pilot study involving two women and two men. Plasma analysis revealed the presence of DCAA and DBAA in all samples between 5 and 30 min after...
intake but at concentrations too low to permit quantitation. Because of the very low levels of total DCAA in plasma, GC-MS analysis of $^{13}$C-DCAA (intentionally added to the water at a dose of 20 µg/kg) was not performed. These findings indicated that some HAAs in drinking water are absorbed in healthy volunteers but results in very low peak plasma concentrations (e.g., < 0.1 ng/ml plasma).

### DISCUSSION

Longstanding interests in potential therapeutic applications of DCAA, and its current status as an investigational drug for treatment of congenital lactic acidosis, have encouraged several pharmacokinetic studies with human volunteers. Previous studies have focused on much larger doses of DCAA, typically 35–50 mg/kg, with doses as high as 100 mg/kg being administered to human volunteers (Curry et al., 1991; Fox et al., 1996; Henderson et al., 1997; Lukas et al., 1980; Wells et al., 1980; Shangraw and Fisher, 1996; Williams et al., 2001). The pharmacokinetic behavior of DCAA at these doses is nonlinear, with total body clearance progressively decreasing at higher doses (reviewed by Saghir and Schultz, 2002). These DCAA doses are ~30,000–100,000 times higher than those encountered in drinking water in even the most extreme cases reported in U.S. municipal water supplies.

In rats naive with respect to prior DCAA exposure, the upper limit for linear DCAA pharmacokinetics occurs at a dose of 1 mg/kg (Saghir and Schultz, 2002). The present study is consistent with a similar phenomenon existing in humans, as our $C_l$ range (730–4316 ml/h/kg) at 2 mg/kg is higher than the clearance value of 679 ml/h/kg at a dose of 10 mg/kg reported by Lukas et al. (1980). Many of the individual basal $C_l$ in the present study approach or exceed estimates of total liver blood flow in humans (e.g., 1200 ml/h/kg, Zoli et al., 1995). This is consistent with rodent studies indicating that at low doses, DCAA has a high liver extraction ratio (> 70%) and that $C_l$ can represent a major fraction (> 50%) of cardiac output (Saghir and Schultz, 2002). It is reasonable to extrapolate that DCAA pharmacokinetics may be at or near the linear range at the 2 mg/kg dose used in the present study, although we did not test the response at lower doses to confirm linearity.

Using the data from the present study to calculate pharmacokinetics at even lower doses will yield more reliable data for environmental exposure than those based on higher clinical

### TABLE 2

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>AUC$_{0-\infty}$ (ng/ml h)</th>
<th>$V_{ss}$ (ml/kg)</th>
<th>$C_l$ (ml/h/kg)</th>
<th>$t_{1/2,b}$ (h)</th>
<th>Oral dosing</th>
<th>Bioavailability$^a$</th>
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<tr>
<td></td>
<td>AUC$_{0-\infty}$ (ng/ml h)</td>
<td>$C_{max}$ (ng/ml)</td>
<td>$K_a$ (/h)</td>
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<td>% Ncomp</td>
<td>%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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Note. AUC$_{0-\infty}$, $V_{ss}$, $C_l$, and $t_{1/2,b}$ were calculated using noncompartmental methods. $C_{max}$ and $K_a$ were determined from a simultaneous nonlinear least squares fit of the oral and iv data using the pharmacokinetic model and the WinNonlin program; n.d. = not determined.

$^a$Oral bioavailability was calculated from the ratio of AUC$_{0-\infty}$ values for the oral and iv doses (with adjustment for the differences in dose) and as a fitted parameter from the pharmacokinetic model.
doses at which a “therapeutic” effect is obtained. This is important because the state-of-the-art for chemical detection is inadequate to directly characterize DCAA pharmacokinetics during environmental exposures. To overcome this limitation, our approach was to administer a dose of DCAA that permitted accurate characterization of DCAA blood levels but still was low enough to allow accurate prediction of DCAA dosing during environmental exposures.

Regulatory agencies have a keen interest in water HAAs in general, and DCAA in particular, because of their association with cancer and adverse pregnancy outcomes. It is therefore important to better predict the actual human tissue exposure level of DCAA after consumption of drinking water. Rodent studies have shown that tissue concentrations of DCAA (and other HAAs) are equal to or slightly less than plasma levels (e.g., tissue:plasma ratios of 0.5–1.0; Abbas and Fisher, 1997; Saghir and Schultz, 2005). This is consistent with our findings for the apparent volume of distribution of DCAA ($V_{ss}$, Tables 2 and 3), which is equivalent to 50–75% of total body water. Thus, the plasma DCAA concentration is a reasonable surrogate for most well-perfused tissue concentrations.

We estimated DCAA plasma concentrations after consumption of 1 l of drinking water containing 15 $\mu$g DCAA (equivalent to 0.2 $\mu$g/kg for a 70-kg person) using the pharmacokinetic model. The selected DCAA concentration represents the approximate median level reported in a recent nationwide survey of disinfection by-products in U.S. drinking water supplies (Weinberg et al., 2002). DCAA plasma concentration-time profiles were predicted using average values for $K_{a,o}$ or oral bioavailability, and $V_{ss}$. Two separate simulations were performed using the highest and lowest values for $Cl_b$ determined in this study (Table 2; Fig. 3). Our simulation predicts that peak plasma concentrations will be < 0.2 ng/ml and are likely to be considerably lower in individuals with high $Cl_b$ for DCAA. The AUC0–∞ for these simulations was 0.028 and 0.173 ng/ml h for the high- and low $Cl_b$, respectively. The predicted plasma concentrations shown in Figure 3 represent the mean plasma DCAA exposure in an average individual for the specified DCAA intake. However, actual DCAA exposures may be lower if bioavailability is reduced, as has been shown in rodents when the DCAA intake is < 1 mg/kg (Saghir and Schultz, 2002). Reduced bioavailability would also explain our inability

### TABLE 3

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>iv dosing</th>
<th>Oral dosing</th>
<th>Bioavailability$^a$</th>
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</thead>
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<tr>
<td></td>
<td>$AUC_{0-\infty}$ (ng/ml hr)</td>
<td>$V_{ss}$ (ml kg$^{-1}$)</td>
<td>$Cl_b$ (ml/h/kg)</td>
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<tr>
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</tr>
<tr>
<td>SD</td>
<td>133</td>
<td>271</td>
<td>668</td>
</tr>
</tbody>
</table>

**Note:** $AUC_{0-\infty}$, $V_{ss}$, $Cl_b$, and $t_{1/2, b}$ were calculated using noncompartmental methods. $C_{max}$ and $K_a$ were determined from a simultaneous nonlinear least-squares fit of the oral and iv data using pharmacokinetic model and the WinNonlin program; n.d. = not determined.

$^a$Oral bioavailability was calculated from the ratio of the $AUC_{0-\infty}$ values for the oral and iv doses (with adjustment for the differences in dose) and as a fitted parameter from the pharmacokinetic model.

$^b$Greater ($p < 0.042$ and $p < 0.014$ for oral and iv, respectively) versus basal value.

$^c$Lower ($p < 0.014$) versus basal value.
to measure the $^{13}$C-DCAA added to the municipal tap water at a
dose of 20 g/kg. This dose would be expected to produce
a peak $^{13}$C-DCAA plasma concentration between 5 and 20 ng/ml (simulations not shown), well within our analytical detection
limits. However, our lack of detection suggests that the oral
bioavailability of DCAA is less than 10% at the dose of 20 g/kg
used in this experiment.

Based on the results of the present study, human exposures to
DCAA and related disinfection by-products (e.g., di-HAAs) is
very short lived, for which complete absorption and excretion
occurs within the first hour after consumption drinking water.
Our prediction creates a challenge for environmental monitoring
programs that seek to determine actual exposure levels of
disinfection by-products in people consuming chlorinated
drinking water. Our results indicate that sampling must occur
immediately after consumption of drinking water and that very
sensitive analytical detection methods that are capable of
accurately measuring DCAA at low picogram/milliliter levels
in plasma must be used. Even current state-of-the-art analytical
methods (e.g., Jia et al., 2003) are inadequate to quantitatively
measure plasma DCAA at the low concentrations (< 50 pg/ml)
expected from drinking water exposures.

Our study also addressed the concern that repeated exposure
to DCAA and related di-HAAs found in drinking water
functionally inhibits GST-zeta activity to the extent that it
reduces $C_l$. This would have the potential effect of increasing
the persistence of HAAs and associated health risks in humans.

**FIG. 2.** Plasma concentration-time profiles in human subjects after a 2 mg/kg $^{12}$C-DCAA po (open circles) and 0.3 mg/kg $^{13}$C-DCAA iv (black squares) dose. Data are expressed as mean ± SD for (A) $n = 8$ male and (B) $n = 8$ female subjects. Solid lines are predicted values using the pharmacokinetic model. Data shown are from the initial baseline pharmacokinetic assessment.

**FIG. 3.** Model-predicted plasma DCAA concentration-time profiles in
a 70-kg human after consuming 1 l of water containing 15 µg DCAA. Values
were calculated using the pharmacokinetic model described in the text and
assuming oral absorption rate = 5.7/h, bioavailability = 0.53, and $V_{ss} = 370$ ml/
kg. Model simulation range was defined between a rapid $C_l$ (4300 ml/h/kg, lower curve on graph) and a slow $C_l$ (716 ml/h/kg, upper curve).
Previous studies in rodents have shown that reductions in hepatic GST-zeta activity (> 25%) can reduce \( C_{lb} \) and in turn prolong the presence of DCAA in plasma (Schultz et al., 2002). The present study demonstrates that prolonged DCAA exposure at the relatively low exposure rate of 20 \( \mu \)g/kg/day can induce similar changes in healthy women volunteers. It is possible that a larger sample size would have increased the power of our study sufficiently to show a statistically significant inhibition of clearance in men as well. It is worth noting the 20 \( \mu \)g/kg/day DCAA dose is still > 10 times higher compared with typical drinking water exposures of di-HAAs. Also, the observed reductions in \( C_{lb} \) (Table 3) may have been influenced by the initial 2 mg/kg DCAA dose administered as part of the basal pharmacokinetic study two weeks earlier. Keeping in mind these considerations, we predict that typical drinking water exposure exerts only a minimal effect on GST-zeta activity and \( C_{lb} \) of di-HAAs in the human population. However, additional exposure to DCAA such as from clinical use or from chlorinated swimming pools would likely cause a clinically significant reduction in \( C_{lb} \).

**Gender and Interindividual Differences**

Two interesting findings from this study are the gender differences and the large interindividual differences in DCAA clearance. Although gender differences were not statistically significant, there was a trend for women to have higher \( C_{max} \) values (Table 2; Fig. 2), which can be attributed to the modest increase in \( K_{a} \) and decrease in \( C_{lb} \) observed in women. The lack of statistical significance is due to high interindividual variation in some pharmacokinetic parameters, especially clearance. This large interindividual variation in DCAA \( C_{lb} \) has been reported since the 1970’s and has complicated the development of appropriate clinical dosing regimens (Stacpoole et al., 1998a). Because DCAA \( C_{lb} \) is primarily determined by the GST-zeta metabolism pathway, it is tempting to speculate that the differences are attributable to variations in GST-zeta activity and expression.

GST-zeta is polymorphic in the human population, with at least four characterized variants (GST-Za, b, c, and d; Blackburn et al., 2000, 2001). Experiments using recombinantly expressed hGST-Z isoforms in cell-free test systems indicate the hGSTZ1a-1a isoform has greater activity with respect to DCAA metabolism and is less susceptible to inactivation by DCAA than other isoforms (Lantum et al., 2002b; Tzeng et al., 2000). Therefore, the potential exists for individuals to express GST-Z variants with lower or higher metabolic activity toward DCAA. Individuals expressing a GST-Z variant more sensitive to di-HAA inactivation would also be expected to be more susceptible to the effects of repeated exposure to di-HAAs, particularly at lower exposure rates where differences are likely to be more pronounced. Thus, understanding the importance of specific GST-Z genotypes as it relates to DCAA pharmacokinetics in humans should be a priority in future studies.

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