

## Low-Dose Pharmacokinetics and Oral Bioavailability of Dichloroacetate in Naive and GST $\zeta$ -Depleted Rats

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We studied the pharmacokinetics of dichloroacetate (DCA) in naive rats and rats depleted of glutathione *S*-transferase-zeta (GST $\zeta$ ), at doses approaching human daily exposure levels. We also compared *in vitro* metabolism of DCA by rat and human liver cytosol. Jugular vein-cannulated male Fischer-344 rats received graded doses of DCA ranging from 0.05 to 20 mg/kg (intravenously or by gavage), and we collected time-course blood samples from the cannulas. GST $\zeta$  activity was depleted by exposing rats to 0.2 g/L DCA in drinking water for 7 days before initiation of pharmacokinetic studies. Elimination of DCA by naive rats was so rapid that only 1–20 mg/kg intravenous and 5 and 20 mg/kg gavage doses provided plasma concentrations above the method detection limit of 6 ng/mL. GST $\zeta$  depletion slowed DCA elimination from plasma, allowing kinetic analysis of doses as low as 0.05 mg/kg. DCA elimination was strongly dose dependent in the naive rats, with total body clearance declining with increasing dose. In the GST $\zeta$ -depleted rats, the pharmacokinetics became linear at doses  $\leq$  1 mg/kg. Virtually all of the dose was eliminated through metabolic clearance; the rate of urinary elimination was  $<$  1 mL/hr/kg. At higher oral doses ( $\geq$  5 mg/kg in GST $\zeta$ -depleted and 20 mg/kg in naive rats), secondary peaks in the plasma concentration appeared long after the completion of the initial absorption phase. Oral bioavailability of DCA was 0–13% in naive and 14–75% in GST $\zeta$ -depleted rats. Oral bioavailability of DCA in humans through consumption of drinking water was predicted to be very low and  $<$  1%. The use of the GST $\zeta$ -depleted rat as a model for assessing the kinetics of DCA in humans is supported by the similarity in pharmacokinetic parameter estimates and rate of *in vitro* metabolism of DCA by human and GST $\zeta$ -depleted rat liver cytosol. **Key words:** animal study, dichloroacetic acid, drinking water disinfection by-products, halogenated acetic acids, human risk assessment, human *in vitro* metabolism, low-dose pharmacokinetics, oral bioavailability, rat *in vitro* metabolism, toxicology. *Environ Health Perspect* 110:757–763 (2002). [Online 13 June 2002]

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Dichloroacetate (DCA) is a drinking water disinfectant by-product commonly identified in municipal water supplies. Concentrations of DCA in finished drinking water have been reported as high as 133  $\mu$ g/L (1), although concentrations  $<$  25  $\mu$ g/L are more common (2,3). DCA is a metabolite of certain chlorinated industrial solvents and of several pharmaceuticals (4). DCA has also been used for decades as an investigational drug to treat numerous cardiovascular and metabolic disorders in humans, for example, diabetes, hypercholesterolemia, and amelioration of lactic acid during liver transplantation (4–6). Recently, DCA has been used in clinical trials to treat congenital or acquired lactic acidosis in children (5,7). Human exposure to DCA ranges from  $\sim$ 1 to 4  $\mu$ g/kg/day through consumption of drinking water and up to 50 mg/kg/day from the use of DCA as a therapeutic drug (4).

DCA is rapidly and completely absorbed from the gastrointestinal (GI) tract and is extensively metabolized both in rodents and in humans, with glyoxylate, oxalate, glycolate, and CO<sub>2</sub> being the major metabolites (8,9). Only a small percentage ( $<$  3%) of the dose is excreted as the parent compound (8–10). DCA metabolism occurs primarily in

the liver (11), mediated through a recently characterized class of glutathione *S*-transferase, GST $\zeta$  (GSTZ1–1) (12). DCA is also a mechanism-based inhibitor of GST $\zeta$ , and prolonged exposure to DCA in rodents causes both reduction in metabolism and depletion of immunoreactive GST $\zeta$  protein levels from the liver (9,13–15). Thus, in repeated dosings of DCA, the first dose is always cleared more rapidly than are subsequent doses because of the inactivation of GST $\zeta$ . This has prevented measurement of oral bioavailability of DCA using a crossover experimental design because the second dose is always eliminated more slowly regardless of the route of administration (16).

DCA has been associated with a number of toxic effects in animals exposed to high doses, including testicular abnormalities, birth defects, and liver cancer (17–20). Consumption of chlorinated water has been linked to increased risk for certain cancers in humans (21) without any specific correlation with DCA or other haloacetates. DCA presents an interesting dilemma for risk assessors because it has a history of safe use as a therapeutic, but it has created regulatory concern because of its carcinogenicity in animals. The U.S. Environmental Protection

Agency (EPA) has classified DCA as a likely human carcinogen based on the hepatocarcinogenic effects observed in rodents (22). Because of the prevalence of halogenated acetic acids in finished drinking water and their possible link to human cancer, the U.S. EPA has set standards permitting a combined total of 60  $\mu$ g/L of five common halogenated acetic acids (HAA5) in drinking water. The goal of the U.S. EPA is the virtual elimination of DCA from drinking water under stage I regulations (22).

Previous pharmacokinetic studies of DCA have focused on therapeutic (i.e., milligram per kilogram) doses. Also, the effects of GST $\zeta$  depletion on DCA disposition have not been studied in detail. Therefore, we designed this study to determine the pharmacokinetics and oral bioavailability of DCA in cohorts of naive and GST $\zeta$ -depleted rats using a range of doses down to 50  $\mu$ g/kg. We also compared the *in vitro* metabolism of DCA in human liver cytosol with cytosol obtained from naive and GST $\zeta$ -depleted rats. We made this comparison to aid in determining the appropriateness of using the GST $\zeta$ -depleted rat model for understanding low-dose pharmacokinetics of haloacetates in humans.

### Materials and Methods

**Chemicals.** We purchased DCA ( $>$  99% pure as free acid) from Fluka Chemical Corp. (Milwaukee, WI). Reagent-grade methyl-*tert*-butyl ether (MTBE) was purchased from Fisher Scientific (Pittsburgh, PA). We prepared diazomethane from

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*N*-methyl-*N*-nitrosoguanidine following Aldrich Technical Information Bulletin AL 121 (23). All other chemicals were of the purest grade available and were obtained from standard sources. All dosing solutions were prepared in 0.9% (w/v) NaCl and pH adjusted to 7.0 with NaOH.

**Animals and treatment.** The Institutional Animal Care and Use Committee of Battelle, Pacific Northwest National Laboratory approved the animal care and experimental protocols, and animal care and treatment was conducted in accordance with their established guidelines.

For pharmacokinetic experiments, we purchased 8- to 10-week-old male Fischer 344 rats ( $185 \pm 29$  g body weight, mean  $\pm$  SD;  $n = 41$  naive rats) fitted with a jugular vein cannula from Taconic Laboratories (Germantown, NY). We also purchased 6 noncannulated rats from Charles River Laboratories (Raleigh, NC) for the preparation of liver cytosol. We housed jugular vein-cannulated rats individually, whereas we housed three noncannulated rats per cage. Each cage contained wood-chip bedding and stainless steel wire tops, and rats were housed under standard conditions (22°C, 40–60% relative humidity, 12-hr light/dark cycle). We allowed rats a minimum of 48 hr for recovery from transport before use in experiments.

Initially, rats were provided with deionized water and Purina rat chow (St Louis, MO) *ad libitum*. We used deionized water throughout the experiments to avoid unwanted exposure to haloacetates, which can be present in drinking water sources and may cause some inactivation of GST $\zeta$ . Animals were fasted overnight before the administration of DCA. We dosed naive animals intravenously (iv) or by gavage (4–6/dose group) with 1, 5, or 20 mg/kg DCA and housed them in polycarbonate metabolism cages. After the initial dosing experiments, we then provided the same individual rats with 0.2 g/L DCA in their drinking water for 7 days to deplete/inactivate GST $\zeta$  activity (henceforth GST $\zeta$  depleted). We also pretreated one group of three noncannulated rats for 7 days with 0.2 g/L DCA in their drinking water to deplete GST $\zeta$ . We then switched the GST $\zeta$ -depleted animals to non-DCA-fortified water overnight (16 hr) to allow residual DCA to be cleared from the body. This treatment protocol was previously shown to reduce GST $\zeta$  activity by > 90% in rat liver cytosol (24), and the experimental results presented in this study further confirm this finding. We then dosed GST $\zeta$ -depleted rats (4–6 per dose group) iv with 0.05, 0.25, 1, 5, or 20 mg/kg or gavaged them with 0.25, 1, 5, or 20 mg/kg DCA. We gavaged two additional GST $\zeta$ -depleted rats with 100 mg/kg DCA to

estimate oral bioavailability at this highest dose using earlier reported iv data of Gonzalez-Leon et al. (9). We administered dosing solutions at a volume of 1 mL/kg.

**Sample collection and analysis.** We collected serial blood samples (0.075–0.125 mL) from individual rats through the jugular vein cannula using a 1-mL syringe coated with sodium heparin. After each blood sample, we flushed the cannula with ~0.2 mL of heparinized saline (40 U/mL heparin). We obtained plasma by centrifugation, mixed it with 0.2 mL of ice-cold 0.1 M sodium acetate buffer (pH 5.2), and stored the plasma at –20°C until analysis. We determined actual plasma volumes gravimetrically using tared vials and assuming plasma density of 1.0. A typical blood sampling schedule after iv dosing was 0, 3, 6, 10, 15, 20, 25, and 30 min and variously thereafter up to 24 hr, depending on the dose and pretreatment. For orally dosed animals, we added an additional 1-min sample. Sampling continued until plasma concentrations were expected to have declined below the method detection limit (MDL) for DCA. We calculated the MDL as described by Glaser et al. (25) using nine replicate plasma samples. The MDL for DCA in naive and GST $\zeta$ -depleted rat plasma was 6 and 10 ng/mL, respectively. The MDL for plasma removed from GST $\zeta$  rats was slightly higher because of elevated background levels. We collected urine from each rat for 24 hr, mixed an aliquot with sodium acetate buffer, and stored the samples at –20°C until analysis.

For experiments measuring diurnal changes in plasma DCA levels, we provided four rats with 0.2 g/L DCA water and collected time-course blood samples up to 24 hr, after which we gave animals non-DCA water and collected the last blood samples 11 hr later. We allowed animals used in the kinetic experiments an additional 5 hr on non-DCA water (16 hr total depuration before administering DCA). This additional time was to ensure that residual DCA concentration in plasma approached background values without significant resynthesis of the GST $\zeta$  enzyme (26). We verified residual concentration after depuration by measuring DCA levels in plasma collected before dosing (time 0) and comparing them with levels in non-DCA treated rats.

We analyzed all plasma samples anticipated to contain > 100 ng DCA using a previously described method (27). Briefly, we added 0.025 mL (0.2  $\mu$ g) internal standard (dibromoacetic acid) to samples (plasma and urine), acidified them by adding 0.025 mL 50% sulfuric acid (v/v), and extracted in various volumes (0.2–1.0 mL) of MTBE depending on the dose and sampling time. We extracted samples anticipated to contain < 100 ng DCA in 0.2 mL MTBE. We then

concentrated the extracted DCA by reducing the volume of MTBE to 0.01–0.02 mL under a gentle stream of N<sub>2</sub>. We converted the free acid to the methyl ester by adding 0.01–0.02 mL ethereal diazomethane (previously diluted 1:10 with MTBE). We then analyzed samples by gas chromatography with electron-capture detection (Hewlett-Packard 5890-Series II, Avondale, PA). The additional preconcentration step increased the MDL for DCA by 50- to 75-fold when compared to our previous method. We determined stability of DCA in urine by fortifying freshly collected urine from a naive rat with DCA (10  $\mu$ g); the fortified samples were either stored at –20°C or left at room temperature for 24 hr. We then analyzed DCA as described above and compared the results. Degradation of DCA in urine was negligible; > 90% could be recovered from urinary samples left at room temperature for 24 hr.

**Kinetic analysis.** The methods we used to analyze the concentration–time profiles of DCA were similar to those used by Schultz et al. (27). Briefly, we analyzed the individual plasma profiles after both iv and oral administration by noncompartmental methods to obtain estimates of total body clearance, apparent volume of distribution at steady state ( $Cl_b$ ,  $V_{ss}$ , for iv doses only), and the mean residence time (MRT) using the standard equations for these parameters that are incorporated into the WinNonlin program (Pharsight Corp., Cary, NC). WinNonlin calculates the area under the curve ( $AUC_{0 \rightarrow \infty}$ ) by the linear trapezoidal method with the terminal portion of the curve extrapolated from time 0 to infinity by  $C_{p,t}/\beta$ , where  $C_{p,t}$  is the concentration of DCA in plasma at the last observation and  $\beta$  is the slope of the terminal phase determined by linear regression. WinNonlin calculated the elimination half-life ( $t_{1/2,\beta}$ ) as  $0.693/\beta$ . We calculated renal clearance as  $Cl_r = X_{u0 \rightarrow 24}/AUC_{0 \rightarrow 24}$ , where  $X_{u0 \rightarrow 24}$  is the total amount of DCA recovered in the urine after 24 hr. We also report the observed peak plasma concentration of DCA ( $C_{max}$ ) and the time of its occurrence ( $T_{max}$ ) after oral dosing. We calculated the oral bioavailability from the ratios of the average values for  $AUC_{0 \rightarrow \infty}$  for the oral and iv doses, and calculated the mean absorption time (MAT) as the difference between the  $MRT_{oral}$  and  $MRT_{iv}$ .

**Preparation of liver cytosol.** We prepared rat liver cytosol from male F-344 rats (8–10 weeks old;  $n = 3$  naive and 3 GST $\zeta$  depleted) by differential centrifugation as described by Okita and Okita (28). We purchased two human liver sections and a pooled S-9 fraction obtained from 10 donors from the International Institute for the Advancement of Medicine (Exton, PA). The human liver section designated Human 1 was from a

69-year-old white male (body weight 90 kg; height, 1.78 m) who died of cardiopulmonary arrest; the Human 2 liver section was from a 68-year-old white female (body weight 73 kg; height 1.63 m) who died of a brain stem infarction. The pooled S9 designated Human Pooled was prepared from liver tissue obtained from 10 white male donors of 8, 40, 40, 48, 51, 52, 53, 58, 63, and 64 years of age, who died of cardiovascular disease, brain hemorrhage, anoxia, anoxia, head injury, anoxia, stroke, head injury, anoxia, and head injury, respectively. We prepared cytosol from the liver sections as described for rats by Okita and Okita (28) and from pooled human S-9 by centrifugation at  $100,000 \times g$  for 1 hr. Liver sections had been perfused with University of Wisconsin medium and contained viable hepatocytes. We stored aliquots of cytosol at  $-70^\circ\text{C}$  until use, and determined protein concentrations as described by Bradford (29).

**DCA depletion in cytosol and determination of intrinsic metabolic clearance.** We measured the depletion of added DCA using rat and human hepatic cytosol. Incubation mixtures consisted of 1–4 mg/mL protein, 0.1 M phosphate buffer (pH 7.4), and 1.4 mM glutathione in a final incubation volume of 3 mL. We preincubated solutions for 2 min at  $37^\circ\text{C}$  in a shaking water bath and started the reaction by adding 0.025 mg DCA prepared in 0.1 M phosphate buffer (pH 7.4). We removed a 0.15-mL aliquot from each incubate at various times (0.2–60 min) and added it to a mixture of 0.25 mL 0.1 M sodium acetate and 0.05 mL of 50%  $\text{H}_2\text{SO}_4$  to stop the reactions. We added internal standard to each aliquot and extracted and analyzed DCA as described above. We plotted the loss of DCA against time and calculated AUC as described above. We calculated the intrinsic metabolic clearance ( $\text{Cl}_{\text{int}}$ ) by dividing the initial amounts of DCA (at time 0.2 min) in the incubation medium with that of the AUC values (30).

We scaled up the  $\text{Cl}_{\text{int}}$  to a whole animal/human by calculating the amount of cytosolic protein per gram of liver for rat and humans. For rats, we used measured liver and body weights. For humans, we assumed liver to be 2.5% of the body weight as reported by Davies and Morris (31). We calculated the hepatic clearance as

$$\text{Cl}_b = \frac{Q_b \times f_u \times \text{Cl}_{\text{int}}}{Q_b + (f_u \times \text{Cl}_{\text{int}})}, \quad [1]$$

where  $Q_b$  is the liver blood flow and  $f_u$  is the unbound fraction of DCA in plasma. We assumed the total cardiac output for F-344 rats was 17.38 L/hr/kg [from Hachamovitch et al. (32)]. We calculated the  $Q_b$  to be 3.13 L/hr/kg by adjusting for the percentage

blood flow (18%) to the liver (33). For humans, we assumed total cardiac output to be 312 L/hr and liver blood flow to be 22% of this value [from Astrand (34) and Williams and Leggett (35), respectively]. The calculated  $Q_b$  for a 70 kg human was 1.01 L/hr/kg. The unbound fraction for DCA in rat plasma was  $0.94 \pm 0.07$  (27) and we assumed it to be the same for humans.

**Statistics.** We assessed significant differences between pharmacokinetic parameter estimates from the different treatment groups by Student's *t*-test. We also performed analysis of variance on the individual  $\text{Cl}_b$  values to determine if they were significantly different. We considered a *p*-value of  $\leq 0.05$  to be statistically significant.

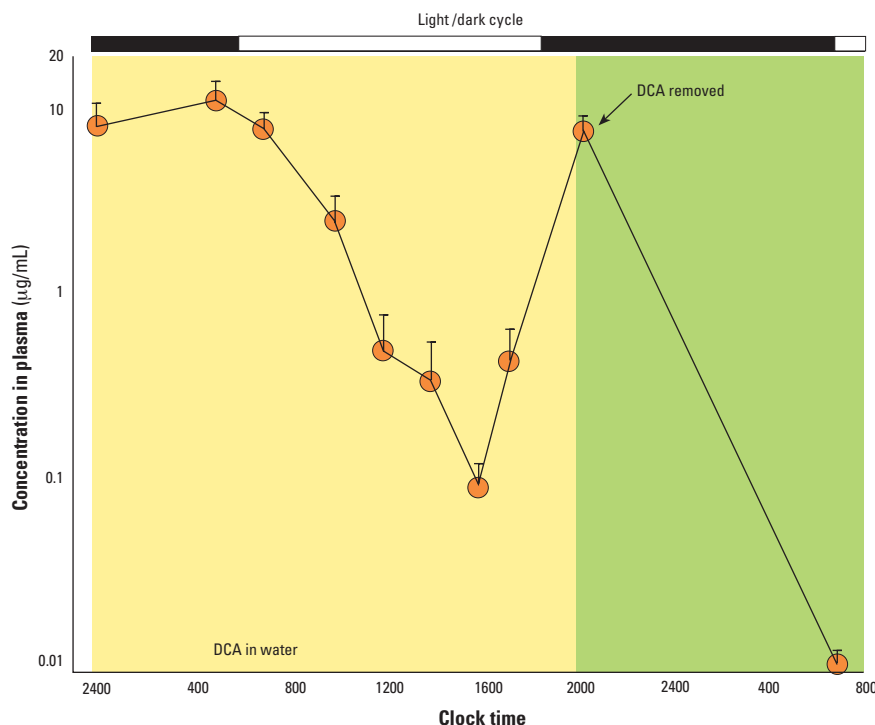
## Results

**Plasma DCA levels while receiving DCA treatment.** To study the pharmacokinetics of DCA in rats with reduced metabolism, we exposed animals to DCA (0.2 g/L) in drinking water for 7 days to effectively deplete the GST $\zeta$  enzyme. Periodically, we monitored consumption of water and plasma DCA levels to verify concentrations during the exposure and residual levels after 16 hr of washout. The average consumption of water by rats was 80 mL/kg/day, corresponding to a daily DCA dose of around 16 mg/kg. Figure 1 shows the diurnal plasma levels of DCA in rats provided with 0.2 g/L DCA-fortified water. DCA plasma levels were much higher during the dark cycle. We

observed peak plasma levels at 500 hr (1 hr before lights on), which declined thereafter. The levels of DCA in plasma started to climb again 2 hr before the lights were turned off, corresponding to the increased activity (drinking/eating). We found the minimum plasma concentration of DCA at 1600 hr. During an 11-hr depuration period after removal of DCA-fortified drinking water, plasma levels of DCA rapidly dropped to  $10 \pm 2$  ng/mL (Figure 1). We provided animals used in kinetic experiments an additional 5 hr of washout (16 hr total depuration) that allowed DCA plasma levels to fall below 10 ng/mL.

**Intravenous administration.** Figure 2 shows the mean ( $\pm$  SE) plasma concentration–time profiles. The decline of DCA from plasma of naive rats was so rapid that the lowest dose that could be used for kinetic analysis was 1 mg/kg, which had a plasma elimination half-life of approximately 4 min (Figure 2A, Table 1). In contrast, elimination of DCA from the plasma of GST $\zeta$ -depleted rats was much slower, allowing kinetic analysis of doses as low as 0.05 mg/kg (Figure 2B inset). Visual inspection of the plasma concentration–time profiles and the pharmacokinetic parameters presented in Table 1 for the naive rats indicate that DCA declined from plasma in a monoexponential manner. Decline of DCA from plasma of the GST $\zeta$ -depleted rats became biexponential at the higher doses (Figure 2B).

Table 1 summarizes the kinetic analysis of DCA for the naive and GST $\zeta$ -depleted



**Figure 1.** Diurnal plasma concentration profile of DCA in rats administered 0.2 g/L DCA in drinking water and its elimination upon removal of the DCA-fortified water. Each point indicates mean  $\pm$  SE ( $n = 4$ ).



rats. In general, the kinetics of DCA were similar to previous descriptions (9,27): rapid elimination by the naive rats with GST $\zeta$  depletion causing an increase in  $t_{1/2,\beta}$  and MRT and a decrease in the total body clearance ( $Cl_b$ ). DCA was essentially eliminated through metabolism by both naive and GST $\zeta$ -depleted rats. The renal clearance of DCA accounted for < 1% of the total body clearance at most doses (Table 1). The steady-state volume of distribution ( $V_{ss}$ ) did not appear to be affected by GST $\zeta$  depletion or with dose, ranging non-systematically between 223 and 618 mL/kg (Table 1). The main pharmacokinetic parameter that was affected by treatment and dose was  $Cl_b$ . In naive rats, we observed nonlinear kinetics throughout the dosing regimen (Table 1). In GST $\zeta$ -depleted rats, however, the pharmacokinetics became linear at doses < 1 mg/kg;  $Cl_b$  was not different ( $p \geq 0.4$ ) at these lower doses (Table 1).

**Oral administration.** The average plasma concentration–time profiles of DCA after gavage dosing are presented in Figure 3 and a summary of the pharmacokinetic parameters is presented in Table 2. DCA was rapidly absorbed after oral dosing and detected in plasma within 1 min after dosing (Figure 3). In naive rats, the greater capacity for metabolism limited the doses that could be used. Pilot experiments using a dose of 1 mg/kg failed to detect plasma concentrations above the MDL (6 ng/mL) because DCA was apparently completely metabolized before reaching the general circulation (Table 2). The decline in the plasma concentration of DCA after the initial peak appeared to be monoexponential at lower doses (5 mg/kg in the naive and  $\leq 1$  mg/kg in the GST $\zeta$ -depleted rats; Figure 3). At higher doses (20 mg/kg in naive rats and  $\geq 5$  mg/kg in GST $\zeta$ -depleted rats), DCA displayed complex plasma concentration–time profiles, with secondary plasma peaks appearing between 4–8 hr after dosing, long after completion of the initial absorption phase (Figure 3). This observation is consistent with a previous report of the absorption of DCA in naive rats gavaged with a 100 mg/kg dose (27). In GST $\zeta$ -depleted rats, the secondary plasma peak was less apparent at the highest gavaged dose of 20 mg/kg (Figure 3B). This observation implies that a complex dose–response relationship exists in GST $\zeta$ -depleted rats between the oral dose and appearance of the secondary plasma peaks, with both very low and high doses displaying a less pronounced secondary peak. This relationship may also apply to naive rats, although the high dose needed to obscure the secondary peak is apparently > 100 mg/kg.

The complex plasma concentration–time profile at the higher doses contributed to the

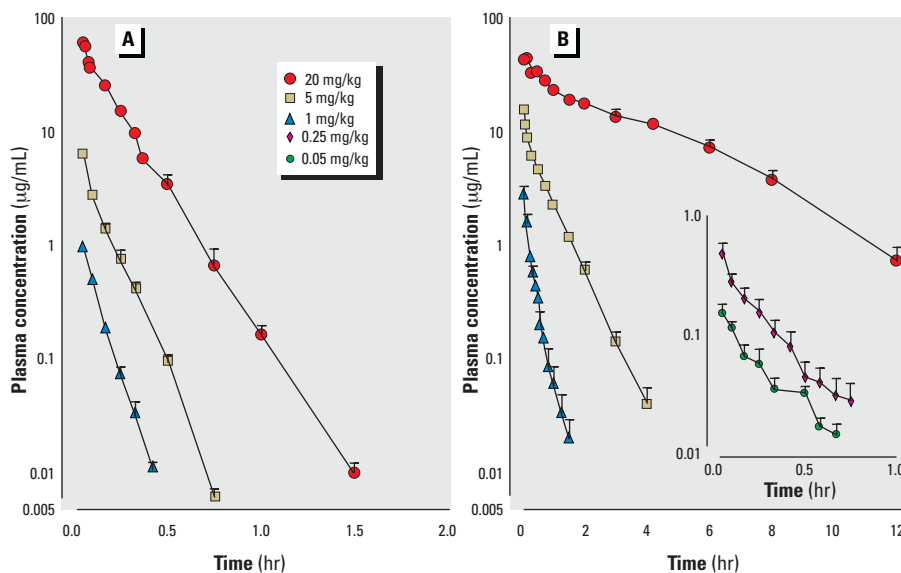
disproportionate increase in the  $AUC_{0 \rightarrow \infty}$  between the doses (Table 2). Maximum plasma concentrations were reached within 5–10 min in naive and 8–45 min in GST $\zeta$ -depleted rats (Table 2, Figure 3). In the GST $\zeta$ -depleted rats, peak plasma concentrations ( $C_{max}$ ) were 4- to 6-fold higher than in the naive rats. The higher  $C_{max}$  and longer MRT in GST $\zeta$ -depleted rats was also reflected by a 22- to 56-fold increase in the  $AUC_{0 \rightarrow \infty}$  (Table 2). The MAT in both naive and GST $\zeta$ -depleted rats was increased in a dose dependent manner.

The oral bioavailability of DCA was significantly reduced in naive rats. At doses of 5 and 20 mg/kg, bioavailability was only 10% and 13%, respectively. At a higher dose of 100 mg/kg, the oral bioavailability reached 81% (Table 2). Bioavailability at 1 mg/kg could not be calculated because of the lack

of detectable concentrations of DCA in plasma. In GST $\zeta$ -depleted rats, the oral bioavailability was 14%, 29%, 31%, and 75% at the 0.25, 1, 5, and 20 mg/kg doses, respectively, and became 100% at 100 mg/kg (Table 2).

**Correlation between dose and kinetic parameters.** Figure 4 presents the correlation between dose and percent oral bioavailability. The relationship between dose and oral bioavailability of DCA for the GST $\zeta$ -depleted rats was best defined by saturable mechanism using a hyperbolic distribution with a correlation coefficient ( $r^2$ ) of 0.90 (Figure 4). The relationship between dose and oral bioavailability for the naive rats was less clear, and Figure 4 shows only the observed data.

**Cytosolic metabolism of DCA.** The results of *in vitro* experiments using liver cytosol



**Figure 2.** The plasma concentration–time profiles of DCA after iv administration of a series of doses of 1–20 mg/kg to naive (A) and 0.05–20 mg/kg to GST $\zeta$ -depleted rats (B). Data shown are mean  $\pm$  SE ( $n = 4-6$ ). Error bars that fit within the data point are not shown. To avoid crowding of data points on the y-axis, the line was slightly shifted to the left.

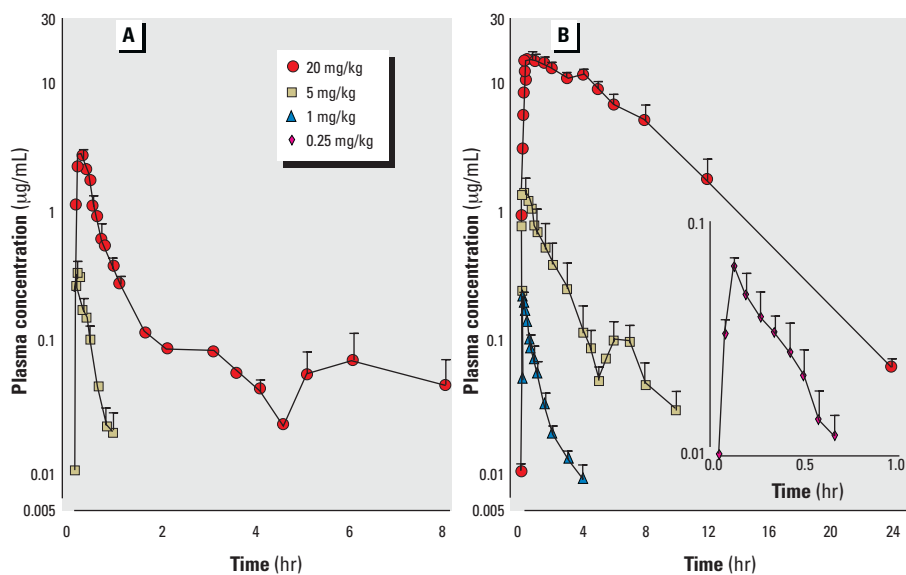
**Table 1.** Pharmacokinetic parameters of DCA after iv administration of a range of doses in naive and GST $\zeta$ -depleted adult male F-344 rats.

Dose (mg/kg)	No.	$AUC_{0 \rightarrow \infty}$ ( $\mu\text{g/mL/hr}$ )	$V_{ss}$ (mL/kg)	$Cl_b$ (mL/hr/kg) <sup>a</sup>	MRT (hr)	$t_{1/2}$ (hr)
Naive rats						
1	5	0.15 $\pm$ 0.01	508 $\pm$ 68.6	6,554 $\pm$ 356	0.07 $\pm$ 0.01	0.07 $\pm$ 0.001
5	6	1.24 $\pm$ 0.05	415 $\pm$ 47.2	5,265 $\pm$ 636	0.08 $\pm$ 0.01	0.08 $\pm$ 0.003
20	5	13.8 $\pm$ 0.85	223 $\pm$ 111.0	1,571 $\pm$ 97	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01
100 <sup>b,c</sup>	5	433 $\pm$ 233	618 $\pm$ 319.0	267 $\pm$ 105	2.10 $\pm$ 0.86	2.4 $\pm$ 0.15
GST $\zeta$ -depleted rats						
0.05	4	0.04 $\pm$ 0.01	277 $\pm$ 33.4	1,326 $\pm$ 342 <sup>d</sup>	0.23 $\pm$ 0.05	0.19 $\pm$ 0.05
0.25	6	0.15 $\pm$ 0.03	454 $\pm$ 57.3	1,816 $\pm$ 288 <sup>d</sup>	0.18 $\pm$ 0.03	0.17 $\pm$ 0.02
1	5	0.61 $\pm$ 0.02	261 $\pm$ 13.6	1,640 $\pm$ 57 <sup>d</sup>	0.19 $\pm$ 0.04	0.20 $\pm$ 0.05
5	4	8.21 $\pm$ 0.50	392 $\pm$ 31.4	614 $\pm$ 39	0.64 $\pm$ 0.04	0.50 $\pm$ 0.03
20	4	136.6 $\pm$ 3.4	513 $\pm$ 18.5	168 $\pm$ 22	3.45 $\pm$ 0.09	1.81 $\pm$ 0.09
100 <sup>c</sup>	6	2,410 $\pm$ 406	582 $\pm$ 146	43 $\pm$ 8	NR	10.8 $\pm$ 2.0

NR, not reported.

<sup>a</sup> $Cl_{renal}$  was < 0.7 mL/hr with the exception of 100 mg/kg (2.9  $\pm$  0.5 mL/hr for naive and 8.9  $\pm$  3.3 mL/hr for GST $\zeta$ -depleted) doses. <sup>b</sup>Data from Schultz et al. (27). <sup>c</sup>Data from Gonzalez-Leon et al. (9). <sup>d</sup>Not significantly different from each other;  $p \geq 0.4$ .

were consistent with *in vivo* kinetic analysis; that is, GSTζ depletion significantly decreases DCA metabolism/elimination. The rate of DCA metabolism by naive rat cytosol was significantly faster ( $p < 0.01$ ) than that in the GSTζ-depleted rats (Figure 5). The intrinsic metabolic clearance ( $Cl_{int}$ ) of DCA by the human liver cytosol closely resembled that in GSTζ-depleted rats (Figure 5) and was not statistically different ( $p > 0.3$ ). Table 3 presents the predicted hepatic clearance ( $Cl_h$ ) and extraction efficiency ( $E_{ss}$ ) of DCA by rats and humans. We derived these predicted values using *in vitro*  $Cl_{int}$  of DCA by the rat and human liver cytosol. The  $Cl_h$  and  $E_{ss}$  of DCA by naive rats was 3-fold higher than that by the GSTζ-depleted rats. We predict humans to have somewhat lower  $Cl_h$  and similar  $E_{ss}$  of DCA compared with GSTζ-depleted rats (Table 3).



**Figure 3.** The plasma concentration–time profiles of DCA after oral administration of 5 and 20 mg/kg to naive rats (A) and a series of doses ranging from 0.25 to 20 mg/kg to GSTζ-depleted rats (B). Data shown are mean  $\pm$  SE ( $n = 4-6$ ). Error bars that fit within the data point are not shown. To avoid crowding of data points on the y-axis, the line was slightly shifted to the left.

**Table 2.** Pharmacokinetic parameters of DCA after oral administration of a range of doses in naive and GSTζ-depleted adult male F-344 rats.<sup>a</sup>

Dose (mg/kg)	No.	AUC <sub>0→∞</sub> (µg/mL/hr)	T <sub>max</sub> (hr)	C <sub>max</sub> (µg)	MRT (hr)	MAT (hr)	Bioavailability (%)
Naive rats							
1	3	BD	BD	BD	0	0	0
5	6	0.12 $\pm$ 0.01	0.09 $\pm$ 0.02	0.36 $\pm$ 0.07	0.28 $\pm$ 0.04	0.20	9.68
20	6	1.82 $\pm$ 0.10	0.17 $\pm$ 0.01	2.91 $\pm$ 0.24	1.70 $\pm$ 0.49	1.56	13.20
50 <sup>b</sup>	4	11.7 $\pm$ 1.68	0.27 $\pm$ 0.04	9.29 $\pm$ 1.87	NR	NR	ND
100 <sup>c</sup>	5	218 $\pm$ 74.5	8.0	27.4	6.70 $\pm$ 1.44	4.5	80.93
GSTζ-depleted rats							
0.25	6	0.02 $\pm$ 0.01	0.13 $\pm$ 0.01	0.08 $\pm$ 0.01	0.39 $\pm$ 0.03	0.19	14.0
1	4	0.18 $\pm$ 0.03	0.12 $\pm$ 0.02	0.26 $\pm$ 0.01	1.05 $\pm$ 0.07	0.86	29.4
5	4	2.58 $\pm$ 1.05	0.25 $\pm$ 0.03	1.66 $\pm$ 0.31	1.80 $\pm$ 0.19	1.16	31.4
20	4	103 $\pm$ 14.0	0.75 $\pm$ 0.25	17.0 $\pm$ 1.90	4.63 $\pm$ 0.69	1.18	75.0
100	2	2,730	3.32	151	12.5	ND	100

Abbreviations: BD, below detection (< MDL); MAT, mean, absorption, time; ND, not determined; NR, not reported.

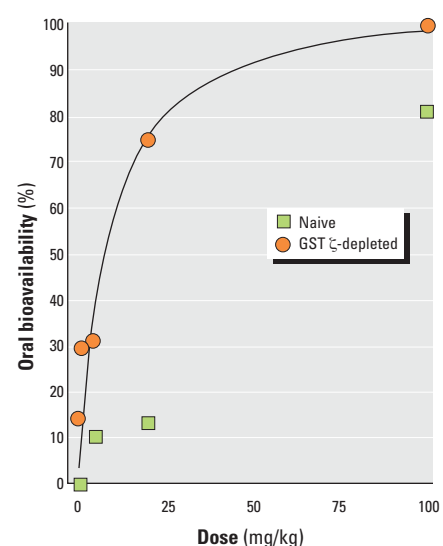
<sup>a</sup> $Cl_{renal}$  was < 0.8 mL/hr with the exception of 100 mg/kg dose, where it was 2.32 mL/hr.  $Cl_{renal}$  for 50 mg/kg has not been reported by the James et al. (10). <sup>b</sup>Data from James et al. (10). <sup>c</sup>Data from Schultz et al. (27).

## Discussion

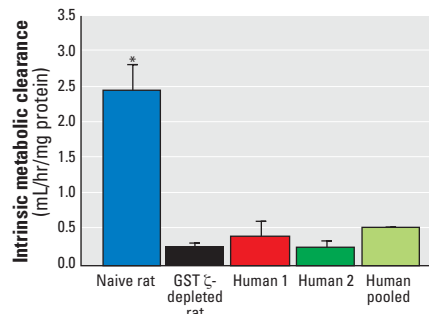
The results of this study demonstrate that the elimination of DCA in naive rats exhibits nonlinear behavior at all doses that allowed pharmacokinetic analysis. The  $Cl_b$  continued to increase at lower iv doses and exceeded 6.5 L/hr/kg at the 1 mg/kg dose. The cardiac output in F-344 rats of body size comparable to those used in this study has been reported to be 17.38 L/hr/kg (32). Therefore, the clearance of DCA in naive rats at low doses is at least 38% of cardiac output, which would exceed liver blood flow and implies extensive extrahepatic elimination of DCA occurs. In contrast, the pharmacokinetics of DCA in GSTζ-depleted rats becomes linear at doses  $\leq$  1 mg/kg (Table 1); the  $Cl_b$  ranged between 1.33 and 1.82 L/hr/kg after iv doses of 0.05–1 mg/kg. Assuming liver blood flow is 3.13 L/hr/kg (i.e., fraction of cardiac output to

the liver is 0.18) (33) and liver metabolism accounts for the bulk of DCA elimination (in GSTζ-depleted rats), DCA clearance appears to correspond to 42–58% of liver blood flow. This finding indicates that DCA is moderately extracted by liver under linear kinetics by GSTζ-depleted rats. At higher doses, metabolism becomes saturated, and liver extraction decreases.

The complex plasma concentration–time profiles of DCA observed after some oral doses (Figure 3) agreed with those in an earlier report (27). The extent of the secondary peaks appears to be reduced at lower doses and was absent after doses that only produced detectable levels of DCA until 4 hr after dosing (5 and 20 mg/kg in this study).



**Figure 4.** Correlation of dose and oral bioavailability of DCA for the naive and GSTζ-depleted rats.  $Y = 106.8x \div (8.32 + x)$ ,  $r^2 = 0.90$ . To avoid crowding of data points on the y-axis, the line was slightly shifted to the left.



**Figure 5.** Intrinsic metabolic clearance of DCA calculated from the progress metabolism experiment using liver cytosol from naive and GSTζ-depleted rats ( $n = 3$ ). Human hepatic cytosol was from two donors or pooled from 10 individuals. For rats, each bar represents the mean  $\pm$  SE ( $n = 3$ ); for humans, each bar represents the mean  $\pm$  SE of three experiments. Error bars that fit within the data point are not shown.

\* $p < 0.01$ .

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