Quantitative Evaluation of Dichloroacetic Acid Kinetics in Human-A Physiologically-Based Pharmacokinetic Modeling Investigation

Ting Li
University of Georgia
Athens GA 30602

Irv Schultz
Battelle Pacific NW Division
1259 West Sequim Bay Road
Sequim WA 98382

Deborah A. Keys
Statistics and Modeling Supporting Informed Decisions
405 Fortson Drive
Athens GA 30606

Teresa R. Sterner
Henry M. Jackson Foundation
2729 R Street
Wright-Patterson AFB OH 45433

Jerry L. Campbell
Jeffrey W. Fisher
University of Georgia
Athens GA 30602

David R. Mattie
Biosciences and Protection Division
Applied Biotechnology Branch
Wright-Patterson AFB OH 45433

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//SIGNED//
DAVID R. MATTIE, Work Unit Manager
Applied Biotechnology Branch

//SIGNED//
MARK M. HOFFMAN, Deputy Chief
Biosciences and Protection Division
Human Effectiveness Directorate
711 Human Performance Wing
Air Force Research Laboratory

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*University of Georgia, Athens GA 30602; **Batelle Pacific NW Div, 1259 W Sequim Bay Rd, Sequim WA 98382, ***Statistics and Modeling Supporting Informed Decisions, 405 Fortson Dr, Athens GA 30606, ****HJF, 2729 R St, WPAFB OH 45433

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SUMMARY
Dichloroacetic acid (DCA) is a common disinfection byproduct in surface waters and a probable minor metabolite of trichloroethylene. DCA liver carcinogenicity has been demonstrated in rodents but epidemiological evidence in humans is not available. High doses of DCA (10-50 mg/kg) are used to treat metabolic acidosis. Biotransformation of DCA by glutathione transferase zeta (GSTzeta) in the liver is the major elimination pathway in humans. GSTzeta is inactivated by DCA, leading to slower systemic clearance and nonlinear pharmacokinetics after multiple doses.
A physiologically-based pharmacokinetic (PBPK) model was developed to quantitatively describe DCA biotransformation and kinetics in humans administered DCA by intravenous infusion and oral ingestion. GSTzeta metabolism was described using a Michaelis-Menten equation coupled with rate constants to account for normal GSTzeta synthesis, degradation and irreversible covalent binding and inhibition by the glutathione-bound-DCA intermediate. The human DCA PBPK model adequately predicted the DCA plasma kinetics over a 20,000 fold range in administered doses. Apparent inhibition of GSTzeta mediated metabolism of DCA was minimal for low doses of DCA (µg/kg/day), but was significant for therapeutic doses of DCA. Plasma protein binding of DCA was assumed to be an important factor influencing the kinetics of low doses of DCA (µg/kg/day).
Human equivalent doses (HEDs) were calculated for a 10% increase in mice hepatic liver cancer (2.1 mg/kg/day). The HEDs for the dosimetrics (area under the curve (AUC) for total and free DCA in plasma, AUC of DCA in liver and amount of DCA metabolized) per day were 0.02, 0.1, 0.1 and 1.0 mg/kg/day, respectively.
INTRODUCTION
Dichloroacetic acid (DCA) is an environmental contaminant produced primarily by disinfection of water with chlorine. The reported median concentration of DCA in surface water systems is 15 μg/L with a maximum concentration of 74 μg/L (Boorman et al., 1999). Concentrations as high as 133 μg/L may also occur (Uden and Miller, 1983). DCA is also a probable minor metabolite of the common solvent and groundwater contaminant, trichloroethylene (TCE). While DCA has been measured as a by-product of TCE exposure (Larson et al., 1992a), these data have been questioned as an artifact of sample preparation as DCA is only measurable when high levels of trichloroacetic acid (TCA) are present. Merdink et al. (1998) were unable to detect DCA in B6C3F1 mice from treatment with TCE or TCA even after pre-treatment with DCA (2 g/L in drinking water for 2 weeks). DCA is classified as a possible human carcinogen based on sufficient evidence of carcinogenicity in rats and mice (U.S. EPA, 2003). Clinically, DCA has been used to treat metabolic disorders such as lactic acidosis and diabetes mellitus due to its ability to reduce circulating glucose, lactate and pyruvate. Pharmacological doses of DCA range from 10 to 50 mg/kg/day (Barshop et al., 2004; Jia et al., 2006). Adverse effects from therapeutic use include mild liver dysfunction, transient central neuropathy, peripheral neuropathy and hypocalcemia. The clinical effects are generally reversible after withdrawal of treatments (Stacpoole et al., 1998b; U.S. EPA, 2003; Mori et al., 2004).

DCA is readily absorbed by the gastrointestinal tract as only 1% of the total dose is excreted in urine after a single oral administration of 50 mg/kg in humans. The plasma half-life of DCA in healthy humans is approximately 1 hour and increases somewhat with repeated dosing (Curry et al., 1991; Stacpoole et al., 1998a). The metabolism of DCA involves oxidative dechlorination to form glyoxylate, which is further oxidized to oxalate, carbon dioxide or incorporated into amino acids nucleophiles and other cellular molecules (Tong et al., 1998a; U.S. EPA, 2003; Anderson et al., 2004). GSTzeta is the only enzyme identified in the biotransformation of DCA (Tong et al., 1998b; Ammini et al., 2003; Lim et al., 2004). DCA has been shown to inhibit GSTzeta, through covalent modification of the enzyme, in both in vivo animal studies and in vitro studies (Anderson et al., 1999, 2002; Schultz et al., 2002). GSTzeta (GSTZ1-1), also known as maleylacetoacetate isomerase (MAAI), is an essential enzyme in phenylalanine/tyrosine catabolism pathway. Disruption of tyrosine catabolism by competitive inhibition was proposed as a possible mechanism for DCA toxicity (Cornett et al., 1999; Schultz et al., 2002; Ammini et al., 2003; Lantum et al., 2003). Four functional allelic variants of GSTZ 1-1 (GSTZ 1a-1a and GSTZ 1d-1d) have been identified in humans. These GSTZ allelic variants have different metabolic capacities and inhibition characteristics towards DCA in vitro (Tzeng et al., 2000; Blackburn et al., 2001).

The first rodent physiologically based pharmacokinetic (PBPK) models for DCA were created to track trace amounts of DCA, presumably formed as a metabolite of trichloroethylene (Abbas and Fisher, 1997; Greenberg et al., 1999; Clewell et al., 2000). In addition, Barton et al. (1999) created a mouse DCA PBPK model to understand the relationship between hepatic cancer incidence and DCA hepatic dosimetry for orally ingested DCA. The inhibitory effect of DCA on its own metabolism was not accounted for in PBPK models until Keys et al. (2004) developed rodent DCA PBPK models. The rodent PBPK models of Keys et al. (2004) were used to evaluate the impact of reduced hepatic metabolism (by suicide inhibition of GSTzeta) on DCA blood time course kinetics.

The development of a human PBPK model for DCA was possible, in part, because several pharmacokinetic studies of administered DCA have been reported (Lukas et al., 1980; Wells et...
In the present paper we report on the development of a human PBPK model for DCA formulated to account for reduced hepatic metabolism of DCA via suicide inhibition of the GSTzeta enzyme. The human equivalent doses (HEDs) for a 10% increase in mouse hepatic liver cancer after a 2 year drinking water exposure to DCA (2.1 mg/kg/day) were then estimated using the human DCA PBPK model.

MATERIALS AND METHODS

DCA Human Kinetic Studies Used for PBPK Modeling

Therapeutic DCA Dosing Studies
Curry et al. (1991) intravenously infused 4 male (average weight = 77 kg) and 4 female (average weight = 54 kg) healthy subjects over a 30 minute period with 50 mg/kg DCA. Blood samples were collected at 0.125 hour (7.5 minutes) intervals for the first hour post-dosing, at 0.25 hour (15 minutes) intervals for the second hour, at 0.5 hour (30 minutes) intervals for the third to sixth hours post-dosing, and then at 1 hour intervals until 12 hours post-dosing. These particular kinetic studies and other kinetic studies are reported in a dissertation by Chu (1987). Chu (1987) collected urine from each of these 8 subjects at various intervals up to 12 hours post-dosing and calculated the cumulative amount of DCA excreted and DCA urinary clearance rates. In other studies, Chu (1987) reported DCA plasma kinetics after a single oral dose of 50 mg/kg DCA given in gelatin capsules to 4 male subjects. The blood sampling schedule was similar to the intravenous (IV) experiments. Chu (1987) also administered 5 repeated 30 minute IV doses of DCA two hours apart to adult volunteers. Four males and one female received 25 mg/kg of DCA, and one male and three females received repeated doses of 50 mg/kg DCA. Blood samples were collected at 30 minute intervals for the first 8.5-9 hours after the first infusion and subsequently every 3-4 hours for a 24 hour period.

Three published kinetic studies with therapeutic doses of DCA were used to test the ability of the model to predict plasma kinetics. Lukas et al. (1980) intravenously administered either 10 or 20 mg/kg of DCA in saline to two adult subjects over a 20 minute period. Blood samples were drawn every hour for the first 4 hours, then every 2 hours for the next 8 hours. Shangraw and Fisher (1999) treated cirrhosis patients with 30 minute IV infusions of 35 mg/kg DCA. Five healthy men and one healthy woman were included in the control group. In these individuals, blood samples were drawn at 0, 0.25, 0.5, 1, 2, 4, 6, 10, 14, 20 and 24 hours post-dosing. In a somewhat complicated dosing schedule, Fox et al. (1996) administered two 30 minute IV infusions of DCA at 8 hour intervals to 9 subjects in each dose group. Doses were (1st + 2nd infusion): 30+15, 60+30 or 100+50 mg/kg. Blood samples were collected at several predetermined times over a 14 hour period.

Sub-therapeutic DCA Dosing Studies
Schultz and Shangraw (2006) administered an oral dose of 2 mg/kg of $^{12}$C$_1$-DCA in 0.5 L of water followed 5 minutes later by an IV dose of 0.3 mg/kg $^{12}$C$_1$-DCA to 16 healthy subjects. Blood samples were collected at 5 minute intervals for the first 0.5 hour, 10 minute intervals for the remainder of the first hour, and at 1.25, 1.50 and 2.0 hours post-dosing. These subjects were then kept on a daily oral bolus dose of 0.02 mg/kg of $^{12}$C$_1$-DCA for 14 days to mimic drinking
water contaminated with DCA. At the end of the last day of treatment (Day 15), the subjects were again given an oral bolus dose of 2 mg/kg of $^{12}$C$_1$-DCA followed by an IV dose of 0.3 mg/kg $^{13}$C$_1$-DCA.

Jia et al. (2006) administered 2.5 μg/kg 1,2-$^{13}$C-DCA daily to 20 healthy adult volunteers (8 male and 12 female) either by oral or IV administration. In one study, 15 of these volunteers (6 male and 9 female) received orally administered for either 5 or 15 consecutive days 1,2-$^{13}$C-DCA in 200 mL distilled water after an overnight fast. Blood was sampled on Days 1, 5 and 15 to determine 1,2-$^{13}$C-DCA plasma kinetics. In the 2nd study, 16 volunteers (7 male and 9 female) received daily IV doses of 1,2-$^{13}$C-DCA for up to 5 days. Intravenous 1,2-$^{13}$C-DCA (2.5 μg/kg) was infused at a constant rate in saline over 10 minutes into a forearm vein after an overnight fast. Blood was sampled on Days 1 and 5 to determine 1,2-$^{13}$C-DCA plasma kinetics. On each blood sampling day, 2 mL of venous whole blood was withdrawn 10 minutes before administration of 1,2-$^{13}$C-DCA, and then 0, 5, 10, 20, 30 minutes and 1, 2, 3, 4, 6, 8, 12 and 24 hours after administration of 1,2-$^{13}$C-DCA. Urine was also collected at 12 and 24 hours during each kinetic study. Selected volunteers participated in both the oral and IV dosing study. These 11 volunteers (5 male and 6 female) had a lapse in treatment of at least 30 days between the 2 routes of exposures.

**Human PBPK Model for DCA**

All model code was written in ASCL (acslXtreme V 2.0.1.2, AEgis Technologies Group Inc., Huntsville, AL). The model compartments include plasma, liver, kidney, slowly perfused and rapidly perfused tissues (Fig. 1). Blood flows to these compartments were described using venous equilibration equations. Intravenous dosing was described as an infusion rate directly into the mixed venous blood supply. A two-compartment modeling approach (Abbas and Fisher, 1997) was used to describe oral ingestion of DCA. The hepatic metabolism of DCA by GSTzeta was described with a Michaelis-Menten equation modified to account for suicide inhibition (Lilly et al., 1998). Minor elimination of DCA through the urine was described using a first-order rate constant. Two linked PBPK models for DCA with identical structure and model parameter values were used to describe the competitive metabolism of two isotopes of DCA after co-administration (Schultz and Shangraw, 2006). Competitive inhibition of metabolism for chemical mixtures has been successfully incorporated into PBPK models for chemicals that are metabolized by the same enzymes (Campbell and Fisher, 2007). Suicide inhibition of GSTzeta was also retained in both models where co-administration of DCA isotopes was simulated.
Figure 1. Schematic of DCA PBPK model for humans.
Each isotope was described separately to allow for simulation of the data reported by Schultz and Shangraw (2006). Metabolic interaction was added to the liver compartment to maintain the separate description of the isotopes.

The metabolism of DCA under co-exposure conditions is presented in Equations 1 and 2. The amount of each isotope (\(^{12}\text{C}\) DCA in this example) that is metabolized with respect to time (dAm/dt, mg/hour) in the liver is given by:

\[
RAM^{12} = \frac{dA_m^{12}}{dt} = \frac{V_{\text{max}} \times C_{V_i}^{12}}{K_m \times (1 + C_{V_i}^{13} / K_m) + C_{V_i}^{12}}
\]  \hspace{1cm} (1)

Assuming no isotope effect, the \(K_m\) values for each isotope are the same, thus Equation 1 for \(^{12}\text{C}\) DCA can be rearranged as:

\[
RAM^{12} = \frac{V_{\text{max}} \times C_{V_i}^{12}}{K_m + C_{V_i}^{12} + C_{V_i}^{13}}
\]  \hspace{1cm} (2)

Similarly, the rate of metabolism of \(^{13}\text{C}\) DCA is described as:

\[
RAM^{13} = \frac{dA_m^{13}}{dt} = \frac{V_{\text{max}} \times C_{V_i}^{13}}{K_m + C_{V_i}^{12} + C_{V_i}^{13}}
\]  \hspace{1cm} (3)

where the Michaelis-Menten equation represents metabolic conversion by the GSTzeta enzyme; \(K_m\) is the Michaelis-Menten affinity constant (mg/L), \(V_{\text{max}}\) (mg/hour) is the current metabolic
capacity (inhibitable), $C_{VI}^{12}$ and $C_{VI}^{13}$ are the $^{12}$C-DCA and $^{13}$C-DCA concentrations in the venous blood leaving the liver, respectively.

The proposed mechanism of DCA-mediated inactivation of GSTzeta involves the covalent binding of the glutathione-bound-DCA intermediate metabolic product with a nucleophilic site on GSTzeta. This covalent modification results in dose-dependent inactivation of GSTzeta (Anderson et al., 2002). In the present human model, the inactivation reaction rate was mathematically described as a second order process (bimolecular rate constant, $k_d$, 1/mg), which is dependent upon the intermediate reactive metabolic product(s) and free GSTzeta concentrations in the liver (Lilly et al., 1998). The rate of change in the initial value of maximal velocity of metabolism ($V_{max0}$) with respect to time ($dV_{max}/dt$, mg/h$^2$) is shown in Equation 4.

$$\frac{dV_{max}}{dt} = -k_d \times \left(\frac{V_{max} \times C_{VI}}{K_m + C_{VI}}\right) \times \left(\frac{V_{max} \times K_m}{K_m + C_{VI}}\right) + \left[k_s - k_{de} \times V_{max}\right]$$  \hspace{1cm} (4)

The resynthesis ($k_s$, mg/h$^2$) and the natural degradation ($k_{de}$, 1/hour) of GSTzeta (Equation 4) were described in a similar fashion to the Keys et al. (2004) PBPK model for rodents.

Integration of Equation 4 provided the value of $V_{max}$ as a function of inhibition of GSTzeta using starting maximal velocity in the absence of DCA ($V_{max0}$).

$$V_{max} = \int_0^t \frac{dV_{max}}{dt} + V_{max0}$$  \hspace{1cm} (5)

To maintain equilibrium of the enzyme at steady state, $k_s = k_{de} \times V_{max0}$. The percentage of reduction of the GSTzeta metabolic capacity (PV$_{max}$) was calculated as:

$$PV_{max} = \frac{V_{max(t)}}{V_{max0}} \times 100\%$$  \hspace{1cm} (6)

where $V_{max(t)}$ is the maximum metabolism rate at time t.

This PBPK model accounts for low-capacity, high-affinity binding of DCA to plasma proteins. DCA has been shown to be weakly bound to rat plasma protein (e.g., 6%, Schultz et al., 1999). Chu (1987) reported that 23% of DCA is bound in human plasma. To describe the rate of binding of DCA to plasma proteins in this model, the methods of Clewell et al. (2003) were used, where the binding (association) of DCA to plasma proteins was described using Michaelis-Menten kinetics, and the rate of dissociation (unbinding) was assumed to be a first-order process.

Physiological parameters used in the human model are summarized in Table 1. Average body weight for each pharmacokinetic study was used when reported. Gender differences in DCA kinetics were evaluated in selected studies. In the Shultz and Shangraw (2006) study, DCA kinetic data were reported for individual subjects. In this case, physiological model parameters were developed for males and females separately (Table 1) and also for both sexes combined through averaging body weight and blood flows to the liver and kidney, which are slightly different between the sexes. No appreciable gender differences were observed.

Additional differential equations used in the human model and a list of parameter name explanations are included in Appendix A. Appendix B contains the model code written in acslXtreme.
Table 1. Physiological model parameter values used in human PBPK model for DCA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Human</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>BW</td>
<td>~70.0</td>
<td>Subject -specific when provided</td>
</tr>
<tr>
<td>Cardiac output (l/h/kg$^{3/4}$)</td>
<td>QCC</td>
<td>16.5</td>
<td>Gender-specific when provided.</td>
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<tr>
<td></td>
<td></td>
<td>15.87 ♂</td>
<td>(Brown et al., 1997; Fisher et al., 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.73 ♀</td>
<td></td>
</tr>
<tr>
<td>Blood flows (% of cardiac output)</td>
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<td></td>
</tr>
<tr>
<td>Liver</td>
<td>QLC</td>
<td>26.5</td>
<td>Gender-specific when provided.</td>
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<tr>
<td></td>
<td></td>
<td>25.0 ♂</td>
<td>(Brown et al., 1997; Fisher et al., 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.0 ♀</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>QKC</td>
<td>19.0</td>
<td>Gender-specific when provided.</td>
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<tr>
<td></td>
<td></td>
<td>17.0 ♀</td>
<td>(Brown et al., 1997; Fisher et al., 1998)</td>
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<tr>
<td>Rapidly perfused</td>
<td>QRC</td>
<td>32.0</td>
<td>(Brown et al., 1997; Fisher et al., 1998)</td>
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<tr>
<td>Slowly perfused</td>
<td>QSC</td>
<td>24.0</td>
<td>(Brown et al., 1997; Fisher et al., 1998)</td>
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<td>Tissue Volumes (% of body weight)</td>
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</tr>
<tr>
<td>Plasma</td>
<td>VPLAC</td>
<td>4.4</td>
<td>(Merrill et al., 2005)</td>
</tr>
<tr>
<td>Liver</td>
<td>VLC</td>
<td>2.6</td>
<td>(Brown et al., 1997)</td>
</tr>
<tr>
<td>Kidney</td>
<td>VKC</td>
<td>0.44</td>
<td>(Brown et al., 1997)</td>
</tr>
<tr>
<td>Rapidly perfused</td>
<td>VRC</td>
<td>9.86</td>
<td>(Brown et al., 1997)</td>
</tr>
<tr>
<td>Slowly perfused</td>
<td>VSC</td>
<td>74.7</td>
<td>(Brown et al., 1997)</td>
</tr>
</tbody>
</table>

Note: h=hour

Calibration of Human PBPK Model for DCA

DCA is highly hydrophilic with an octanol/water partition coefficient (logP$_{ow}$) of 0.92. Chemical-specific parameters for DCA are listed in Table 2. DCA tissue/blood partition coefficient (PC) values determined for mice (Abbas and Fisher, 1997) were used for humans, except where noted. A small urinary clearance constant (Cl$_{ur}$, L/hour-kg) value was calculated by allometrically scaling the clearance rates reported in Curry et al. (1991). Cl$_{ur}$ was fixed prior to further model parameterization.

The following sequence was used to estimate metabolic parameters describing suicide inhibition of DCA metabolism. The value of the Michaelis-Menten affinity constant (K$_m$) was set to 6 mg/L, which was measured using human liver cytosol (Tong et al., 1998b). The initial value of the inhibition rate constant (k$_d$) was set to 0.001 mg$^{-1}$; as k$_d$ and V$_{maxc}$ are highly correlated, setting k$_d$ to a very small value allowed initial estimation of V$_{maxc}$ using low dose data. The degradation rate (k$_{de}$) was initially set to the value used for rodents (0.00875 hour$^{-1}$; Keys et al., 2004). It was necessary to lower the slowly perfused (PS) partition coefficient value obtained in mice (0.37, Abbas and Fisher, 1997) to 0.11 to improve the agreement between observation and model prediction following 0.3 mg/kg $^{13}$C-DCA IV bolus on Day 0 (Schultz and Shangraw, 2006). The initial estimate for the maximal rate of DCA metabolism (V$_{maxc}$) was then obtained by visual inspection using the same data set of Schultz and Shangraw (2006). This low dose of DCA was considered to have a minimal effect on GSTzeta activity. The metabolic inhibition
constant, $k_d$, was then fit to the high dose DCA plasma concentration-time course data following 5-repeated infusions of 50 mg/kg and 25 mg/kg DCA at 2 hour intervals in the study of Chu (1987). Fitting was accomplished by the maximum likelihood method with all other parameters fixed. Next, the value of $V_{maxc0}$ was again visually adjusted to fit the concentration-time course data from a 50 mg/kg single IV infusion (Curry et al., 1991).

Diminished systemic clearance of DCA after multiple or single high doses of DCA indicated that all individuals ($n=12$) needed more than 4 weeks to recover (Chu, 1987; Curry et al., 1991), suggesting a very slow rate of recovery for GSTzeta activity. The GSTzeta resynthesis rate, $k_{ds}$, was set to a value that would provide a recovery rate for GSTzeta enzymatic activity equal to 8 weeks after a single 50 mg/kg dose of DCA (2 out of 4 subjects recovered by 8 weeks). The values of the oral absorption rate constants following drinking water administration were estimated by fitting the $^{12}$C-DCA concentration-time course data after 2 mg/kg $^{12}$C-DCA oral ingestion in 500 ml of drinking water reported in the study of Schultz and Shangraw (2006). The transfer rate from the first GI compartment to the liver ($K_{a1}$) was set to 0.01 hour$^{-1}$; the values of the transfer rates from the first GI to second GI compartment ($K_{a2}$) and second GI compartment to the liver ($K_{a3}$) were estimated visually. To describe low capacity binding of DCA to plasma proteins, the parameters for protein binding ($B_{MAX}$, $K_{nb}$ and $K_{unb}$) were estimated by visually fitting the kinetic data from IV and oral administration of 2.5 μg/kg 1,2-$^{13}$C-DCA on Day 1, 5 and 15 (Jia et al., 2006). Plasma protein binding was important for describing the plasma kinetics of DCA after administration of 2.5 μg/kg of DCA.
Table 2. Chemical specific parameter values for human PBPK model for DCA

<table>
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<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td><strong>Partition coefficient</strong></td>
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<td>Liver:Blood</td>
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<td>Abbas and Fisher, 1997</td>
</tr>
<tr>
<td>Rapidly perfused:Blood</td>
<td>PR</td>
<td>1.08</td>
<td>Abbas and Fisher, 1997</td>
</tr>
<tr>
<td>Slowly perfused:Blood</td>
<td>PS</td>
<td>0.11</td>
<td>Estimated (Schultz and Shangraw, 2006)</td>
</tr>
<tr>
<td><strong>Metabolic and clearance parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal rate of GSTzeta velocity (mg/h/kg^{0.75})</td>
<td>V_{max0}</td>
<td>50</td>
<td>Estimated (Curry et al., 1991; Schultz and Shangraw, 2006)</td>
</tr>
<tr>
<td>Michaelic-Menten affinity constant (mg/l)</td>
<td>K_m</td>
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<td>Tong et al., 1998a</td>
</tr>
<tr>
<td>Inhibition constant (mg^{-1})</td>
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<td>Estimated (Chu, 1987)</td>
</tr>
<tr>
<td>Degradation rate constant (h^{-1})</td>
<td>k_{de}</td>
<td>0.004</td>
<td>Estimated (Curry et al., 1991)</td>
</tr>
<tr>
<td>Urinary excretion (l/h-kg)</td>
<td>Cl_{rc}</td>
<td>0.7×10^{-3}</td>
<td>Curry et al., 1991</td>
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<tr>
<td><strong>Oral absorption parameters following drinking water ingestion</strong></td>
<td></td>
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</tr>
<tr>
<td>Transfer rate from the 1st GI compartment to the liver (h^{-1})</td>
<td>K_{a1}</td>
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<td>Estimated (Schultz and Shangraw, 2006)</td>
</tr>
<tr>
<td>Transfer rate from the 1st to the 2nd GI compartment (h^{-1})</td>
<td>K_{a2}</td>
<td>7.0</td>
<td>Estimated (Schultz and Shangraw, 2006)</td>
</tr>
<tr>
<td>Transfer rate from the 2nd GI compartment to the liver (h^{-1})</td>
<td>K_{a3}</td>
<td>7.0</td>
<td>Estimated (Schultz and Shangraw, 2006)</td>
</tr>
<tr>
<td><strong>Plasma protein binding parameters</strong></td>
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</tr>
<tr>
<td>Maximum capacity (mg)</td>
<td>B_{max}</td>
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<td>Estimated (Jia et al., 2006)</td>
</tr>
<tr>
<td>Affinity constant (mg/l)</td>
<td>K_{mb}</td>
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<td>Estimated (Jia et al., 2006)</td>
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<tr>
<td>Dissociation constant (h^{-1})</td>
<td>K_{unb}</td>
<td>0.16</td>
<td>Estimated (Jia et al., 2006)</td>
</tr>
</tbody>
</table>

Note: h=hour

Individual metabolic capacities were estimated for the 16 subjects reported in Schultz and Shangraw (2006) to assess the inter-individual variation as inferred by differences in clearance kinetics of DCA across the study participants. Gender specific values of physiological parameters were used (Table 1) but not considered essential because gender appears to have little influence on DCA kinetics (Stacpoole et al., 1998a; Schultz and Shangraw, 2006). V_{max0}, k_d and the oral absorption parameters (K_{a2} and K_{a3}) for each subject were estimated by maximum
likelihood methods. First, the initial estimations of $V_{\text{max}0}$, $k_d$, $K_{a2}$ and $K_{a3}$ were fit simultaneously to the individual $^{12}$C-DCA and $^{13}$C-DCA concentration-time course data of each subject. Secondly, $K_{a2}$ and $K_{a3}$ were fixed at the initial estimated values and $V_{\text{max}0}$ and $k_d$ were re-optimized by fitting to $^{12}$C-DCA and $^{13}$C-DCA concentration-time course data. Finally, $K_{a2}$ and $K_{a3}$ were re-optimized by fitting to $^{12}$C-DCA concentration-time course data with all other parameters fixed.

**Predictive Performance Analysis**

The ability of the PBPK model to predict the pharmacokinetics of DCA in humans for each data set was evaluated using descriptive measures that compare prediction with observation (Gustafson *et al.*, 2002). The predictive performance measures were: 1) ratio of the PBPK model predicted area under the concentration curve (AUC) for the total plasma concentration of DCA divided by the experimental AUC for the total plasma DCA measurements calculated by non-compartmental analysis (WinNonlin V4.1, Pharsight, Mountain View, CA) ($\text{AUC}_{(0\rightarrow t)}$ P/M Ratio, Equation 7); 2) the performance errors (PEs, Equation 8) and the median value for all the calculated PEs (MPE%, Equation 9); 3) the median value for the absolute value of all calculated PEs (MAPE%, Equation 10); and 4) the root mean squared PEs (RMSPE%). The calculations are:

$$\text{AUC}_{(0\rightarrow t)} \text{ P/M Ratio} = \frac{\text{AUC}_{(0\rightarrow t)} \text{ predicted}}{\text{AUC}_{(0\rightarrow t)} \text{ experimental}} \quad (7)$$

$$\text{Performance Error (PE)} = \frac{C_{\text{measured}} - C_{\text{predicted}}}{C_{\text{predicted}}} \times 100\% \quad (8)$$

$$\text{MPE\%} = \text{Median (PE}_1, \text{ PE}_2, \ldots, \text{ PE}_n) \quad (9)$$

$$\text{MAPE\%} = \text{Median (|PE}_1|, |\text{PE}_2|, \ldots, |\text{PE}_n|) \quad (10)$$

$$\text{RMSPE\%} = \sqrt{\frac{\sum_{i=1}^{n} PE_i^2}{n}} \quad (11)$$

where $\text{AUC}_{(0\rightarrow t)} \text{ predicted}$ and $\text{AUC}_{(0\rightarrow t)} \text{ experimental}$ are the PBPK model predicted and experimental plasma AUCs until the last measured data point, respectively. $C_{\text{measured}}$ and $C_{\text{predicted}}$ are the measured and model predicted DCA plasma concentrations, $|\text{PE}_i|$ is the absolute value of PE$_i$ and $i = 1, 2, \ldots, n$, representing the sample size in each data set. MPE% is a measure of bias and MAPE% and RMSPE% are measures of accuracy (Gustafson *et al.*, 2002).

**Sensitivity Analysis of Parameters**

A sensitivity analysis using the automated process provided in acslXtreme was carried out using AUC for DCA in plasma. Briefly, the equation used to calculate the sensitivity coefficient for each parameter is:

$$\text{Sensitivity Coefficient} = \frac{(A - B) / B}{(C - D) / D} \quad (12)$$

where A is the DCA plasma AUC predicted with a 1% increased parameter value, B is the DCA plasma AUC predicted at the starting parameter value, C is the parameter value after a 1% increase and D is the original parameter value. A large absolute value of the coefficient indicates the parameter has an important effect on the model. A positive value indicates that the model parameter and the corresponding output are positively related and a negative value indicates they
are inversely related. The calculation was performed using the forward difference algorithm with delta equal to 0.01 and normalized to both response variables and parameters. Initial calculations were checked by hand to verify the algorithm output.

**Cancer Risk Estimates**
The theoretical cancer risk for ingestion of DCA was calculated using a previously reported mouse PBPK model for DCA (Keys *et al.*, 2004) and the current human PBPK model for DCA assuming a 70 kg individual ingests 2 L of water each day. One liter is ingested over a 5 minute period every 12 hours. A point of departure (POD) for a 10% increase in cancer risk for DCA is reported to be 2.1 mg/kg/day (estimated as the 95% lower confidence limit on the benchmark dose) (U.S. EPA., 2003) based on liver carcinomas in B6C3F1 mice exposed to DCA in drinking water (DeAngelo *et al.*, 1999). The previously published DCA mouse model (Keys *et al.*, 2004) was used to estimate the dosimetrics: 1) daily AUC of free plasma DCA (mg/L/day); 2) daily AUC of total plasma DCA (mg/L/day); 3) daily liver AUC (mg/L/day); and 4) the daily amount of DCA metabolized per kg of liver (Aml, mg/kg/day) at steady state following consumption of 2.1 mg/kg/day DCA in drinking water. Assuming a 70 kg human ingests 1 L every 12 hours, the present human model was used to estimate human equivalent doses (HEDs) corresponding to the 4 dosimetrics in mice.

The rationale for using AUC for DCA in the liver and the amount of DCA metabolized in the liver is related to a lack of information pertaining to the specific mechanism of action for DCA liver carcinogenicity. DCA, reactive by-products from metabolism, or a combination of the parent and reactive metabolites may be responsible for the resulting liver cancer. These two dosimetrics are good measures of internal dose because liver is the target organ and measured liver DCA concentrations were available with which to validate. However, when extrapolating to humans, measurements of DCA in liver are not available, but measurements of DCA are available in plasma. The plasma concentration represents the dose available for delivery to the liver and, hence, is a good surrogate dosimetric for DCA liver concentration. In this case we speculate that DCA is bound to plasma proteins at low capacity and high affinity and the bound DCA is potentially not available for uptake into the liver. As there is no direct supporting experimental evidence, we calculated dose metrics for total and free DCA plasma concentrations.

**RESULTS**
The range of administered IV and oral ingestion doses of DCA described with this PBPK model was a remarkable 20,000 fold (2.5 µg/kg to 50 mg/kg) with studies conducted at several laboratories. Also noteworthy, one set of model parameter values (Tables 1 and 2) was used for the DCA PBPK model to describe this entire data set with the exception of individual subject optimized kinetic data reported by Schultz and Shangraw (2006).

Perhaps the most challenging pharmacokinetic data to describe with the present PBPK model were the high dose DCA studies conducted in the laboratory of Chu (1987). The model predicted systemic clearance of DCA over a 6 hour period was over-predicted in 4 male and 4 female subjects (Fig. 2) after a single IV infusion of 50 mg/kg (Curry *et al.*, 1991) and modestly under-predicted following 5-repeated administrations of DCA (50 and 25 mg/kg) at 2 hour intervals over a 10 hour period (Fig. 3). The cumulative urinary excretion of DCA was somewhat variable (Fig. 4) in these subjects, accounting for less than 1% of the administrated dose. The PBPK model predicted cumulative amount of DCA excreted in urine is in good
agreement with observed excretion, but is slightly below the mean cumulative amount of urine observed in these subjects (Fig. 4).

Figure 2. Model predicted (---) vs. observed (●) mean DCA plasma concentrations for 4 female subjects, and model predicted (----) vs. observed (○) mean DCA plasma concentrations for 4 male subjects following a 0.5 hour IV infusion of 50 mg/kg DCA. The measured values were originally reported by Curry et al. (1991).
Figure 3. Model predicted and observed mean DCA plasma concentrations for 24 hours following 5-repeated IV 0.5 hour infusions at 2 hour intervals. The concentration-time course data for 2 doses are presented: 25 mg/kg (○, n=5) and 50 mg/kg (●, n=4). The measured data were originally reported by Chu (1987).
Figure 4. Model simulation (—) of the cumulative amount of DCA excreted in the urine vs. the average cumulative excretion measured in 8 subjects following a 0.5 hour IV infusion of 50 mg/kg DCA. Measured values were originally reported by Chu (1987). Bars represent standard deviation.

For other high dose studies (Lukas et al., 1980), with 2 individuals each intravenously administered 10 or 20 mg/kg, good agreement was obtained between model predictions and observations (Fig. 5). In another group of 6 individuals administered 35 mg/kg of DCA (Shangraw and Fisher, 1999), the systemic clearance of DCA was slightly over-predicted (Fig. 5). For the final high dose simulation study (Fig. 6) in which groups of individuals were intravenously administered a second dose of DCA 8 hours after the first dose (Fox et al., 1996), favorable agreement was obtained between model predictions and observations.
Figure 5. Model predicted (—) and observed DCA plasma concentrations of 2 subjects following 10 mg/kg (●, ▲) and 2 subjects following 20 mg/kg (○, △) IV infusions of DCA administered over 20 minutes (originally reported by Lukas et al., 1980). Also shown are the mean plasma concentrations (x, n=6) following a 0.5 hour IV infusion of 35 mg/kg DCA reported by Shangraw and Fisher (1999).
Two independent clinical studies were used for low-dose DCA simulation studies. In the Schultz and Shangraw (2006) study, individuals were initially dosed orally with 2 mg/kg of $^{12}$C-DCA followed within minutes by an IV dose of 0.3 mg/kg of $^{13}$C-DCA. After 14 days of daily treatment with a low dose (0.02 mg/kg) of $^{12}$C-DCA in drinking water, this oral/IV dosing schedule was repeated and serial blood samples were collected. Fig. 7a depicts the entire simulation of both oral and IV dosing of men at the beginning and end of the study as well as the daily low-dose treatment. DCA plasma time course data for both the IV and oral administration are grouped tightly. In panels b and c of Fig. 7, observed and model predicted plasma concentrations are shown for a 2 hour period after oral and then IV administration of the two isotopes of DCA at the beginning and end of the treatment period. Generally speaking, good agreement was observed between model predicted DCA concentrations and measured values. The model predicted clearance of IV administered DCA in men was somewhat slower than observed for both days on which dosing occurred.

Similar trends between observed data and model prediction were found for women (Fig. 8). When individual DCA clearance kinetics were evaluated by adjusting the values for metabolism ($V_{max0}$, $k_d$) and oral absorption ($K_{a2}$ and $K_{a3}$) (Table 3), the fitted values for $V_{max0}$ had the largest range of estimated values (36.7 to 280 mg/hour/kg$^{0.75}$), while values for $k_d$ ranged from zero (no metabolic inhibition) to 0.016 mg$^{-1}$. The individual values of $K_{a2}$ and $K_{a3}$ are relatively similar and the average values are about 8.0 hour$^{-1}$. The simultaneously optimized values for $V_{max0}$, $k_d$, $K_{a2}$ and $K_{a3}$ were very close to the final values obtained after the re-optimization was
conducted separately for the metabolic parameters ($V_{\text{max}0}$, $k_d$) and the oral absorption parameters ($K_{a2}$ and $K_{a3}$). This was expected because the metabolic parameters influence the AUC of both $^{12}$C-DCA and $^{13}$C-DCA while the oral uptake parameters only influence the shape of $^{12}$C-DCA concentration-time profiles (i.e., peak concentration ($C_{\text{max}}$) and time to reach the peak concentration, $T_{\text{max}}$).

In the Jia et al. (2006) clinical study, individuals were administered a very low dose of 2.5 μg/kg/day for 5 days by IV infusion or orally for 15 days. Blood was collected on specified days following treatment. These low-dose kinetic data demonstrated the requirement for plasma protein binding of DCA in the model (Fig. 9a), as the kinetic behavior could not be described without assuming plasma protein binding (Fig. 9b). In the other higher-dose DCA studies, the influence of this low-capacity, high-affinity plasma protein binding on DCA kinetics was not readily apparent. The binding of DCA to these unspecified plasma proteins was assumed to be reversible. When 2.5 μg/kg/day of DCA was administered orally, peak plasma DCA concentrations were moderately under-predicted on Days 1, 5 and 15 of treatment (Fig. 10).
Figure 7. Model predictions of $^{12}$C-DCA (-----) and $^{13}$C-DCA (—) vs. observed mean $^{12}$C-DCA ($\Delta$) and $^{13}$C-DCA plasma concentrations (●) in 8 male subjects. Panel (a) includes the entire period of the study (15 days) where subjects were administered an initial oral dose of 2 mg/kg $^{12}$C-DCA in 500 mL of water followed immediately by an IV bolus of 0.3 mg/kg of $^{13}$C-DCA. The subjects were maintained on a daily dose of 0.02 mg/kg $^{12}$C-DCA in 500 mL of water for 14 days after which time the oral/IV dosing regime was repeated on Day 15. Panel (b) shows the initial 2 hours following the first 2 mg/kg oral and 0.3 mg/kg IV doses
on Day 0 and panel (c) gives the initial 2 hours following the second 2 mg/kg oral and 0.3 mg/kg IV administration on Day 15. The data were originally reported in Schultz and Shangraw (2006).

Figure 8. Model predictions of $^{12}$C-DCA (----) and $^{13}$C-DCA (—) vs. observed mean $^{12}$C-DCA ($\triangle$) and $^{13}$C-DCA plasma concentrations (●) in 8 female subjects. Panel (a) gives the initial 2 hours following the first 2 mg/kg oral and 0.3 mg/kg IV administration on Day 0, and panel (b) gives the initial 2 hours following the second 2 mg/kg oral and 0.3 mg/kg IV administration on Day 15 (see Fig. 7 for detailed dosing explanation). Data originally reported in Schultz and Shangraw (2006).
Table 3. Optimized parameter values for metabolic constants ($V_{\text{max}0}$ and $k_d$) and oral uptake constants ($K_{a2}$ and $K_{a3}$) using individual DCA plasma pharmacokinetic data from Shultz and Shangraw (2006)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Weight (kg)</th>
<th>$V_{\text{max}0}$ (mg/h/kg$^{0.75}$)</th>
<th>$k_d$ (mg$^{-1}$)</th>
<th>$K_{a2}$ (h$^{-1}$)</th>
<th>$K_{a3}$ (h$^{-1}$)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>♂</td>
<td>90</td>
<td>113.2</td>
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<td>5.1</td>
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<td>5.3</td>
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<td>♀</td>
<td>74.5</td>
<td>118.8</td>
<td>0.011</td>
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</tr>
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<tr>
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<td>252.2</td>
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<td>0.005</td>
<td>8.4</td>
<td>8.1</td>
</tr>
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<td>SD</td>
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<td>75.7</td>
<td>0.005</td>
<td>3.1</td>
<td>3.0</td>
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</table>
Figure 9. Model predicted (---) and observed (●) $^{13}$C-DCA concentrations following a 10 minute IV infusion of 2.5 μg/kg/day for 5 days. Panel (a) shows the final model with the added low-capacity plasma protein binding and panel (b) shows the initial model without binding. The measured data originally were reported by Jia et al. (2006).
Figure 10. Model predicted (—) and observed (▲) $^{13}$C-DCA concentrations following ingestion of 2.5 μg/kg/day DCA in 200 mL of water for 15 days. Measured data originally were reported by Jia et al. (2006).

**Prediction Performance Analysis**
In addition to visual inspection, the goodness-of-fit of a model prediction to each data set was evaluated with the AUC P/M Ratio, MPE%, MAPE% and RMSPE% (Table 4). The AUC P/M Ratio values ranged from 0.4 to 2.9 for 23 pharmacokinetic data sets with a mean AUC P/M Ratio value of 1.08. A value near 1.0 indicates a good agreement between observation and prediction. The values of MPE% ranged from -50.6 to 202.2%. Thirteen of the 23 pharmacokinetic data sets had MPE% values that were negative and the remaining 10 pharmacokinetic data sets were positive. The even distribution of MPE% values around zero suggests there was little bias in the PBPK model to either under- or over- predict the experimental data systematically. MAPE% and RMSPE% values (Table 4) for the 23 pharmacokinetic data sets suggest that a few of the PBPK model predictions (Jia et al., 2006; Shangraw and Fisher, 1999) were less accurate, as indicated by large MAPE% and RMSPE% values relative to the values for the other pharmacokinetic data sets. These performance metrics appear to give more weight to the data points with smaller values because the calculations are based on relative percent error rather than absolute percent error.

**Sensitivity Assay**
Since the sensitivity analysis is both dose and time dependent, this analysis was applied using dosimetrics that reflect integrated measures over time, such as AUC (Table 5). Normalized sensitivity coefficients with absolute values that were determined to be most sensitive (>0.5) are in bold. Cardiac output (QCC) and blood flow to the liver (QLC) were found
to be sensitive for low IV doses of DCA (0.3 mg/kg and 2.5 μg/kg), while the metabolic parameters ($V_{max}$, $K_m$ and $k_d$) were the most sensitive parameters at therapeutic doses (10 to 50 mg/kg/day (Barshop et al., 2004; Jia et al., 2006)). The protein binding parameters ($B_{max}$ and $K_{unb}$) were sensitive model parameters only for the lowest DCA dose of 2.5 μg/kg/day.
Table 4. Predictive Performance of Model Predictions by $\text{AUC}_{(0→t)}$ P/M Ratio, MPE%, MAPE% and RMSPE%.

<table>
<thead>
<tr>
<th>Data Sources</th>
<th>Sampling Time (d)</th>
<th>Administration Route</th>
<th>Dose (mg/kg)</th>
<th>Gender</th>
<th>AUC$_{(0→t)}$ P/M Ratio</th>
<th>MPE%</th>
<th>MAPE%</th>
<th>RMSPE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curry et al. (1991)</td>
<td>1</td>
<td>iv</td>
<td>50</td>
<td>♂</td>
<td>0.7</td>
<td>1.8</td>
<td>3.8</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>♂</td>
<td>0.7</td>
<td>12.2</td>
<td>13.9</td>
<td>341.9</td>
</tr>
<tr>
<td>Chu (1987)</td>
<td>iv</td>
<td>25 × 5</td>
<td></td>
<td>2.9</td>
<td>-36.5</td>
<td>37.5</td>
<td>38.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>50 × 5</td>
<td></td>
<td>1.4</td>
<td>-14.1</td>
<td>20.7</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>Schultz and Shangraw</td>
<td>1.5</td>
<td>iv</td>
<td>0.3</td>
<td>♂</td>
<td>1.5</td>
<td>-54.1</td>
<td>54.1</td>
<td>55.4</td>
</tr>
<tr>
<td>(2006)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>♂</td>
<td>1.3</td>
<td>-37.1</td>
<td>37.1</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>oral</td>
<td>2</td>
<td></td>
<td>♂</td>
<td>1.2</td>
<td>-14.0</td>
<td>16.6</td>
<td>36.6</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>♂</td>
<td>1.2</td>
<td>-18.9</td>
<td>20.7</td>
<td>40.8</td>
</tr>
<tr>
<td>Jia et al. (2006)</td>
<td>1</td>
<td>iv</td>
<td>2.5 × 10$^{-3}$</td>
<td>0.8</td>
<td>13.9</td>
<td>17.6</td>
<td>56.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>iv</td>
<td>2.5 × 10$^{-3}$</td>
<td>0.4</td>
<td>157.3</td>
<td>157.3</td>
<td>11644.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>oral</td>
<td>2.5 × 10$^{-3}$</td>
<td>1.1</td>
<td>-19.5</td>
<td>44.9</td>
<td>77.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>oral</td>
<td>2.5 × 10$^{-3}$</td>
<td>0.5</td>
<td>138.0</td>
<td>138.0</td>
<td>236.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>oral</td>
<td>2.5 × 10$^{-3}$</td>
<td>0.4</td>
<td>202.2</td>
<td>202.2</td>
<td>282.8</td>
<td></td>
</tr>
<tr>
<td>Lukas et al. (1980)</td>
<td>1</td>
<td>iv</td>
<td>10</td>
<td>1.5</td>
<td>-50.6</td>
<td>50.6</td>
<td>51.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>iv</td>
<td>20</td>
<td>1.0</td>
<td>-18.0</td>
<td>30.9</td>
<td>81.9</td>
<td></td>
</tr>
<tr>
<td>Shangraw and Fisher</td>
<td>iv</td>
<td>35</td>
<td></td>
<td>0.8</td>
<td>-12.1</td>
<td>18.4</td>
<td>1985.2</td>
<td></td>
</tr>
<tr>
<td>(1996)</td>
<td>Fox et al. (1996a)</td>
<td>iv</td>
<td>30+15</td>
<td>0.7</td>
<td>47.5</td>
<td>50.2</td>
<td>165.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>60+30</td>
<td></td>
<td>0.7</td>
<td>27.2</td>
<td>27.2</td>
<td>105.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>100+50</td>
<td></td>
<td>0.9</td>
<td>4.5</td>
<td>5.8</td>
<td>38.9</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Normalized sensitivity coefficients of selected parameters in the PBPK model for DCA, based on model predictions of DCA plasma AUC in various exposure scenarios

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>50mg/kg iv (single) AUC$_{0-24h}$</th>
<th>50mg/kg iv (2 h interval) AUC$_{0-24h}$</th>
<th>0.3mg/kg iv (Day 0) AUC$_{0-24h}$</th>
<th>2mg/kg oral (Day 0) AUC$_{0-24h}$</th>
<th>2.5 μg/kg-d oral AUC$_{0-120h}$</th>
<th>2.5 μg/kg-d oral AUC$_{0-360h}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCC</td>
<td>-0.05</td>
<td>-0.02</td>
<td>-0.54</td>
<td>-0.02</td>
<td>-0.64</td>
<td>-0.15</td>
</tr>
<tr>
<td>QLC</td>
<td>-0.09</td>
<td>-0.03</td>
<td>-0.50</td>
<td>_</td>
<td>-0.57</td>
<td>-0.12</td>
</tr>
<tr>
<td>V$_{max0}$</td>
<td>-0.88</td>
<td>-0.53</td>
<td>-0.50</td>
<td>-1.05</td>
<td>-0.33</td>
<td>-0.72</td>
</tr>
<tr>
<td>K$_m$</td>
<td>0.47</td>
<td>0.41</td>
<td>0.43</td>
<td>0.92</td>
<td>0.33</td>
<td>0.72</td>
</tr>
<tr>
<td>k$_d$</td>
<td>0.76</td>
<td>0.22</td>
<td>0.21</td>
<td>0.42</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>k$_{de}$</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>K$_{a2}$</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.04</td>
<td>_</td>
<td>-0.09</td>
</tr>
<tr>
<td>K$_{a3}$</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.03</td>
<td>_</td>
<td>-0.08</td>
</tr>
<tr>
<td>B$_{max}$</td>
<td>_</td>
<td>_</td>
<td>0.14</td>
<td>0.06</td>
<td>0.96</td>
<td>1.01</td>
</tr>
<tr>
<td>K$_{mb}$</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>-0.07</td>
<td>-0.08</td>
</tr>
<tr>
<td>K$_{urb}$</td>
<td>_</td>
<td>_</td>
<td>-0.10</td>
<td>-0.05</td>
<td>-0.81</td>
<td>-0.90</td>
</tr>
</tbody>
</table>

Note: The coefficients less than |0.01| are not reported.

**HED for Cancer Risk Estimates**

Under steady state conditions, the estimated daily plasma and liver AUC values in mice treated with 2.1 mg/kg/day of DCA in drinking water were both 0.05 mg/L/day. The average daily amount of DCA metabolized per kg of liver (A$_{ml}$) was 38.5 mg/kg liver. In the present human model, assuming a 70 kg person ingests 2 L of water per day (1 L every 5 minutes at 12 hour increments), oral ingestion of 0.1 mg/kg/day (3.5 mg DCA/L drinking water) results in a free plasma AUC and a liver AUC equal to 0.05 mg/L/day. Oral ingestion of 0.02 mg/kg/day (0.7 mg DCA/L drinking water) results in a total plasma AUC equal to 0.05 mg/L/day, while oral ingestion of 1.0 mg/kg/day (35 mg DCA/L drinking water) results in an A$_{ml}$ equal to 38.5 mg/kg liver/day. HED values and the percent remaining GSTzeta activity (PV$_{max}$) are shown in Table 6.
Table 6. Calculated human equivalent doses (HEDs) for different dosimetrics of DCA carcinogenicity

<table>
<thead>
<tr>
<th>Dosemetrics of DCA Carcinogenicity</th>
<th>Total Plasma AUC (mg/L/d)</th>
<th>Free plasma AUC (mg/L/d)</th>
<th>Liver AUC (mg/L/d)</th>
<th>Amount of metabolized DCA (mg/kg liver/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEDs (mg/kg/day)</td>
<td>0.02</td>
<td>0.1</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>% Remaining GSTzeta (PV\text{max})</td>
<td>94.4</td>
<td>78.0</td>
<td>78.0</td>
<td>27.5</td>
</tr>
</tbody>
</table>

DISCUSSION
In the present study, PBPK modeling was used to quantitatively describe IV and oral intake of DCA in humans. The range of administered doses of DCA reported in the pharmacokinetic literature was exceptionally wide (µg/kg to mg/kg) and included both single and repeated dose administration. Collectively, these published clinical pharmacokinetic studies provide an excellent database for the development of a human DCA model, especially when coupled with information derived from existing PBPK models for DCA in laboratory animals. The historical focus of research on DCA metabolism has been on the identification of enzymes responsible for bio-transformation of DCA and the mechanisms involved in inhibition of its own metabolism. Our present human model for DCA suggests that, at low doses such as those encountered in the environment (µg/L in drinking water), DCA inhibition of its own metabolism is probably a minor contributor to the pharmacokinetic behavior of DCA, but plasma protein binding is suggested to play an important role. For higher doses of DCA, such as therapeutic doses, inhibition of its own metabolism can be substantial, resulting in slowed clearance of DCA from the body. Metabolic recovery from the DCA insult appears to be very slow and warrants further investigation.

In our view, varying degrees of success were reached in describing these diverse and sometimes variable kinetic data sets for DCA with the present human PBPK model. The model parameters were constrained to single values to represent the mean kinetic behavior for groups of individuals, thus the goal was not to fit each data set, but to determine model parameters that could adequately describe most of the data sets. When inter-individual variability was evaluated for metabolism and oral uptake of DCA, a fairly wide range of metabolic capacity values were estimated (Table 3). This variability in estimated metabolic capacity across individuals may reflect polymorphisms in GSTzeta, the primary hepatic enzyme responsible for metabolism of DCA. However, other factors such as oral uptake kinetics may be important as well.

In the present simulations, the breakdown of DCA is assumed to occur only by hepatic GSTzeta and, depending on dose, its metabolism was partially inhibited by covalent binding of a reactive intermediate metabolite. This process is described as a second order process which depends on the concentration of reactive intermediates available for binding to GSTzeta and the availability of GSTzeta in the liver (Equation 4). Experimental evidence demonstrates the dose dependent inactivation of GSTzeta by DCA (Tzeng et al., 2000) and the subsequent degradation of the GSTzeta protein (Anderson et al., 1999). With the current formulation of the human PBPK model for DCA, repeated IV administration of the lowest reported clinical dose of DCA (2.5
μg/kg/day) would result in less than 1% reduction in GSTzeta activity under steady state conditions. With combined doses of 2 mg/kg oral and 0.3 mg/kg IV (Schultz and Shangraw, 2006), the model predicted decrease in metabolic capacity is nearly 13%, which persists until the second combination dose 15 days later, due to the slow recovery of GSTzeta as well as the 0.02 mg/kg DCA administered daily in drinking water between the 2 challenges. If a person is administered 50 mg/kg/day by IV infusion until steady state is reached, only about 13% of the initial metabolic capacity is predicted to remain. Stacpoole et al. (1998a) and Larson and Bull (1992b) propose that DCA can also be dechlorinated to monochloroacetic acid; however, this alternative pathway has not been thoroughly investigated.

The mechanism for DCA induced hepatic toxicity and cancer is unknown. Possible mechanisms associated with DCA toxicity include competitive inhibition of tyrosine catabolism and the reaction of glyoxylate (the reactive metabolite of DCA) with cellular macromolecules (Anderson et al., 2004). The ability of DCA to cause hepatic cancer in humans remains equivocal. Interestingly, when DCA was used as a therapeutic, increases in hepatic cancer incidence in humans was not reported. Also, careful experimental studies are necessary to verify the DCA plasma binding constants for human plasma. Presently, the basis for our model predictions of low plasma DCA concentrations is derived from previous studies showing low-level binding in both rat (Schultz et al., 1999) and human plasma (Chu, 1987). However, in these studies, there are not enough experimental data to estimate the number of binding sites or the binding affinity. The binding parameters were estimated as described in the methods.

From a risk assessment perspective, using model-predicted DCA daily liver AUC as the dosimetric, the PBPK model-predicted human equivalent dose resulting in the same dosimetric as oral administration of 2.1 mg/kg/day DCA in mice was 0.1 mg/kg/day. Theoretically this exposure corresponds to a 10% increase in incidence in liver cancer based on 2 year rodent bioassay data (DeAngelo et al., 1999). Thus, if a 70 kg person ingested 2 L of water each day, this would equate to ingestion of 3.5 mg DCA per L of water. The current maximum contaminant level (MCL) is 60 µg/L for DCA and 4 other haloacetic acids (U.S. EPA, 2002).

In conclusion, the present DCA PBPK model in humans is able to quantitatively describe the DCA kinetics across different populations and for two exposure scenarios. Model simulations suggest that environmental exposure to DCA in drinking water (~0.5 µg/kg/day) is expected to have very limited effects on hepatic GSTzeta activity. Future studies such as genotyping of individual subjects will allow determination of the effect that GSTZ polymorphism has on in vivo DCA pharmacokinetics.
REFERENCES


APPENDIX A: DIFFERENTIAL EQUATIONS USED IN THE PBPK MODEL

Nomenclature
QC       Cardiac output (L/h)
Cv       DCA concentration in the venous blood (mg/L)
Ca       Free DCA concentration in the arterial blood (mg/L)
RA       Rate of change in the amount of DCA (mg/h)
V        Tissue volume (L)
DIV      iv dose (mg/kg)
DO       Oral dose (mg/kg)
AO       Amount of oral absorption (mg)
V_{maxB} Maxim binding capacity rate (mg/h)
K_{mb}   Affinity constant of plasma binding (mg/L)
CL_{unb} Unbound clearance (L/h)
K_{unb}  First-order dissociate rate constant (/h)
K_{ass}  Association rate constant (L/mg/h)
B_{max}  Maximum amount of binding protein in the plasma (mg)
RAM      Rate of metabolism in the liver (mg/h)
CL_r     Urinary clearance (L/h)

Subscripts
pla      Plasma compartment
_bnd     Bound DCA in plasma compartment
_free    Free DCA in plasma compartment
_total   Total DCA concentration in plasma compartment
l        Liver
k        Kidney
s        Slowly perfused compartment
r        Rapidly perfused compartment
GI_1, GI_2 1\textsuperscript{st} and 2\textsuperscript{nd} GI compartments, respectively
The rate of change in the amount of free DCA in the plasma compartment:

\[ \frac{dA_{pla\text{free}}}{dt} = QC \times (C_v - Ca) - RA_{pla\text{bnd}} \]

\[ CV = \sum Q_i \times C_{vi} + DIV \times QC \]

\( i = \) slowly perfused, rapidly perfused, liver or kidney.

The rate of change in the amount of DCA bound in the plasma compartment:

\[ RA_{pla\text{bnd}} = \frac{dA_{pla\text{bnd}}}{dt} = \frac{V_{maxB} \times Ca}{K_{mb} + Ca} - CL_{unb} \times CA_{bnd} \]

\[ = K_{ass} \times \left( \frac{B_{max} \times K_{mb}}{K_{mb} + Ca} \right) \times Ca - K_{unb} \times V_{pla} \times CA_{bnd} = \frac{B_{max} \times K_{unb}}{K_{mb} + Ca} \times Ca - K_{unb} \times V_{pla} \times CA_{bnd} \]

where \( K_{mb} = \frac{K_{unb}}{K_{ass}} \).

The total DCA concentration in plasma compartment:

\[ CA_{\text{total}} = \frac{A_{pla\text{free}} + A_{pla\text{bnd}}}{V_{pla}} \]

The rate of change in the amount of DCA in the two GI compartments:

\[ RA_{GI1} = \frac{dA_{GI1}}{dt} = DO - K_{a1} \times A_{GI1} - K_{a2} \times A_{GI1} \]

\[ RA_{GI2} = \frac{dA_{GI2}}{dt} = K_{a2} \times A_{GI1} - K_{a3} \times A_{GI2} \]

The rate of oral absorption:

\[ RAO = \frac{dAO}{dt} = K_{a1} \times A_{GI1} + K_{a3} \times A_{GI2} \]

The rate of change in the amount of DCA in the liver compartment:

\[ RA_l = \frac{dA_l}{dt} = QL \times (Ca - C_v) - RAM + RAO \]
The rate of change in the amount of DCA in the kidney compartment:

\[ RA_k = \frac{dA_k}{dt} = QK \times (Ca - Cv_k) - CL \times Ca \]

The rate of change in the amount of DCA in the slowly perfused and rapidly perfused compartments:

\[ RA_s = \frac{dA_s}{dt} = QS \times (Ca - Cv_s) \]

\[ RA_r = \frac{dA_r}{dt} = QR \times (Ca - Cv_r) \]
APPENDIX B: HUMAN DCA PBPK MODEL CODE

PROGRAM
!Human DCA Model - December, 2007

INITIAL
!Blood Flow Rate
CONSTANT QCC = 16.5 !Cardiac output (l/hr-kg):Brown, 1997;
CONSTANT QLC = 0.227 !Fractional blood flow to liver: Brown, 1997;
CONSTANT QKC = 0.175 !Fractional blood flow to kidney: Brown, 1997;

!Tissue Volume
CONSTANT BW = 70 !Body weight (kg)
CONSTANT VLC = 0.026 !Fraction liver tissue(male):Brown, 1997;
CONSTANT VKC = 0.0044 !Fraction Kidney tissue(male): Brown, 1997
CONSTANT VPC = 0.044 !Fraction Plasma: Merrill, 2005

!Partition Coefficient for DCA : mouse values (Abbas & Fisher, 1997)
CONSTANT PL = 1.08 !Liver/blood partition coefficient: Abbas & Fisher, 1997
CONSTANT PS = 0.37 !Slowly perfused tissue/blood partition: Abbas & Fisher, 1997
CONSTANT PR = 1.08 !Richly perfused tissue/blood partition: Abbas & Fisher, 1997
CONSTANT PK = 0.74 !kidney tissue/blood partition: Abbas & Fisher, 1997

!Metabolism Parameters
CONSTANT VMAXC = 27.16 !Maximum velocity of metabolism (mg/hr-1kg)
CONSTANT KM = 6.0 !Michaelis-Menten constant (mg/l): Tong, 1998
CONSTANT KD = 0.3 !rate constant for loss of enzyme
CONSTANT KDE = 0.001 !basal enzyme destruction rate(/hr): Curry, 1991
!Proposed in rodent model (Keys, 2004). Not active in Human Model DCA Model:
!CONSTANT KFC = 0.0 !First order metabolism rate constant (/hr /kg)
!Urinary Clearance
CONSTANT Clrc = 0.0007 !1st order urinary elimin. rate constant(/hr): Curry, 1991
!Molecular Weight
CONSTANT MWDCA = 129. !Molecular weight DCA (g/mol)

!'Scaled parameters'
!Physiological
QC = QCC*BW**0.75
QL = QLC*QC
QK = QKC*QC
QR = 0.76*QC-QL-QK !Brown, 1997
QS = 0.24*QC !Brown, 1997
VL = VLC*BW !Liver volume
VK = VKC*BW !Kidney volume
VS = 0.747*BW !Brown, 1997
VR = 0.129*BW-VL-VK !Brown, 1997
VPL = VPC*BW !Plasma Volume

!Metabolism and Elimination'
VMAX = VMAXC*BW**0.75
!KF = KFC/BW**0.25 : Proposed 1st order pathway (Keys, 2004; Not active in Human Model)
Clr = Clrc*BW

!resynthesis rate (mg/hr)/hr =kde*vmax so that vmaxt=vmax at steady-state
KS = KDE*VMAX

!'Dosing'
CONSTANT PDOSE = 0.0 !first oral dose(mg/kg)
CONSTANT PDOSE14= 0.02 !dose for daily dose (mg/kg): Schultz & Shangraw (14 d); Jia (5 or 15 d).
CONSTANT TOR = 0.0 !Start time of first oral dose (hr)
CONSTANT TOR14 = 24 !start time of 14 days, 0.02 mg/kg DCA
CONSTANT IVDOSE1 = 0.0 !First IV dose(mg/kg): For Fox (1996) Data
CONSTANT IVDOSE2 = 0.0 !Second IV dose (mg/kg): For Fox (1996) Data
CONSTANT TINF = 0.5 !Length of IV infusion(hrs)
CONSTANT TIV1 = 0.0 !Start time of fist iv dosing (hr)
CONSTANT TIV2 = 8.0 !Start time of second iv dosing (hr)
CONSTANT IVINT1 = 360 !Interval of 1st iv repeat dosing (hrs)
CONSTANT IVINT2 = 10000 !Interval of 2nd iv repeat dosing (hrs)
CONSTANT FinalIV = 9 !Last IV dosing time (hr)
CONSTANT FinalOR = 337 !last oral dosing time (hr)
CONSTANT ORINT = 360 !Interval of oral dosing (hrs)
CONSTANT ORINT14 =24 !Interval of daily oral dosing (hrs)
CONSTANT TGAV = 0.05 !Length of oral gavage infusion(hrs)
CONSTANT K1 =0.001 !Oral absorption transfer rate GI1 to liver (/hr)
CONSTANT K2 = 3 !Oral absorption transfer rate GI1 to GI2 (/hr)
CONSTANT  K3= 5     !Oral absorption transfer rate GI2 to liver (/hr)

!Plasma Protein binding constants (Clewell, 2003)
CONSTANT  Vbp = 91    !Maximum Binding Capacity Rate (mg/L)
CONSTANT  CLunbp = 9   !Unbound clearance (L/hr)
CONSTANT  Kmbp = 22.6 !Binding Affinity (mg/L)

!Derived Equations for Reported Bmax and Kunb (Communication with Dr. Andersen on May 11, 2007):
Kunb = CLunbp/VPL   !Dissociated Constant (/hr)
Bmax = Vbp/Kunb    !Maximum Binding Capacity (mg)

END ! INITIAL

DYNAMIC

ALGORITHM  IALG = 2
NSTEPS    NSTP = 1
MAXTERVAL MAXT = 1.0e9
MINTERVAL MINT = 1.0e-9
CINTERVAL CINT = 0.01
DERIVATIVE

!--------------IV Multiple Doses------------------------!

!IV = Intravenous infusion rate(mg/hr)

!IV1: Repeated Dosing; IV1+ IV2: Fox (1996)- 30+15, 60+30 and 100+50
iflag1 = PULSE(TIV1,ivint1,tinf)*PULSE(0,tstop,finaliv)

iflag2 = PULSE(TIV2,ivint2,tinf)

IV1 = IVDOSE1*BW/TINF*iflag1  !'rate iv dosing(mg/hr)
AIV1 = INTEG(IV1,0.0)   ! Amount iv dosed(mg)

IV2 = IVDOSE2*BW/TINF*iflag2
AIV2 = INTEG(IV2,0.0)

!--------------oral bolus---------------------------!

!ODOSE = oral gavage infusion rate(mg/hr) for 1st & 2nd challenge

oflag = PULSE(TOR,orint,tgav)
ODOSE = PDOSE*BW/tgav*oflag  !'rate oral gavage dosing(mg/hr)
AO = INTEG(ODOSE,0.0)

rst = -(k1*ast)-(k2*ast)
ast = ao + integ(rst,0.0)
rug = (k2*ast)-(k3*aug)
aug = integ (rug, 0.0)
rao = k1*ast + k3*aug
aa0 = integ(rao, 0.0)

!14ODOSE = oral gavage infusion rate(mg/hr) for 14 d (Schultz)
! & Daily dosing in Jia et al. (2006) for 15 d

oflag14 = PULSE(TOR14,orint14,tgav)*PULSE(0,tstop,finalor)
ODOSE14 = PDOSE14*BW /tgav*oflag14  !'rate oral gavage dosing(mg/hr)
AO14 = INTEG(ODOSE14,0.0)

rst14 = -(k1*ast14)-(k2*ast14)
ast14 = ao14 + integ(rst14,0.0)
rug14 = (k2*ast14)-(k3*aug14)
aug14 = integ (rug14, 0.0)
rao14 = k1*ast14 + k3*aug14
aa014 = integ(rao14, 0.0)

!------VMAXT in liver-----------------!

!Isotopes (12C-DCA&13C-DCA) Competitive Metabolism in liver

CVL = (AL12+AL13)/(VL*PL)

RLME = -kd*(Vmax*CVL/(Km+CVL))*(Vmax*Km/(Km+CVL)) ! Active metabolit
& Free Enzyme Lilly, 1998
RCME = RLME+KS - (KDE*VMAXT)
VMAXT = INTEG(RCME,VMAX)

! proportion of original Vmax inhibited

VMAXP = VMAXT*100/VMAX

!------- Model for IV Dosed DCA: -----------------------------------------------!
!------- 1) 13C-DCA in Schultz & Shangraw (2006) and Jia (2006)-----!
!------- 2) 12C-DCA in therapeutic dose --------------------------------------!

! Blood 13C-DCA (No Binding)
!  Ca13 = (QL*CVL13 + QS*CVS13 + QR*CVR13 + QK*CVK13 + IV1 + IV2)/QC
! ca13 = cv13

! Plasma Compartment (with Binding)
! CA13 = Free DCA Concentration after IV (mg/L)
! CV13 = (QL*CVL13 + QS*CVS13 + QR*CVR13 + QK*CVK13 + IV1 + IV2)/QC
! RPL13 = QC*(CV13-CA13) - Rbind13
! APL13 = integ(RPL13, 0.0)
! CA13 = APL13/VPL

! Cabind13 = Bounded DCA Concentration
! Rbind13 = (Vbp*Ca13)/(Ca13+Kmbp)-CLunbp*Cabind13 !(Clewell, 2003)
! Abind13 = integ(Rbind13, 0.0)
! Cabind13 = Abind13/VPL

! Total Plasma Concentration
! Apltot13 = APL13 + Abind13
! Cat13 = (APL13+Abind13)/VPL

! AS = Amount in slowly perfused tissues (mg)'
! RAS13 = QS*(CA13-CVS13)
! AS13 = INTEG(RAS13, 0.0)
! CVS13 = AS13/(VS*PS)
! CS13 = AS13/VS

! AR = Amount in rapidly perfused tissues (mg)'
! RAR13 = QR*(CA13-CVR13)
! AR13 = INTEG(RAR13, 0.0)
! CVR13 = AR13/(VR*PR)
! CR13 = AR13/VR

! AL = Amount in liver tissue (mg)'
! RAL13 = QL*(CA13-CVL13) - RAMi
! AL13 = INTEG(RAL13, 0.0)
! CVL13 = AL13/(VL*PL)
CL13 = AL13/VL
AUCL13 = INTEG(CL13,0.0)

!Metabolism in liver
  !AM1i : GSTZ-mediated metabolism (mg)
  RAM1i = (VMAXT*CVL13)/(KM+CVL13+CVL12)
  AM1i = INTEG(RAM1i,0.0)

  !AM2i: non-specific degradation (mg)
  ! RAM2i = (KF*CVL13*VL)
  ! AM2i = INTEG(RAM2i,0)

  !AMi: total metabolism amount(mg)
  RAMi = RAM1i! + RAM2i
  AMi = AM1i !+ AM2i

!AK = Amount in kidney tissue (mg)
  RKu13 = Clr*CA13
  AKu13 = integ(RKu13,0.0)  !amount excreted in urine(mg)
  RAK13 = QK*(CA13-CVK13) - RKU13
  AK13 = INTEG(RAK13,0.0)
  CVK13 = AK13/(VK*PK)
  CK13 = AK13/VK
  AUCK13 = INTEG(CK13,0.0)

  !TMASS = mass balance (mg)
  TMASS13 = (AL13+AK13+AS13+AR13+Apltot13)+(AMi+AKu13) ! Amount Dosed + Amount out (mg)

!------------Model for oral dosed DCA (12C-DCA)-------------------------------!
!------------12C-DCA in Schultz & Shangraw (2006)----------------------------!

!Blood 12C-DCA (without Binding)
  !CV12 = (QL*CVL12 + QS*CVS12 + QR*CVR12 + QK*CVK12)/QC
  !CA12 = CV12
  !AUC12 = integ(CA12, 0.0)

! Plasma Compartment (with Binding)
  ! CA12 = Free DCA Concentration after oral (mg/L)
  CV12 = (QL*CVL12 + QS*CVS12 + QR*CVR12 + QK*CVK12)/QC

  RPL12 = QC*(CV12-CA12)- Rbind12
  APL12 = integ(RPL12, 0.0)
  CA12 = APL12/Vpl

! Bound DCA after oral bolus
\[ \text{Rbind12} = \frac{(\text{Vbp}\times\text{Ca12})}{(\text{Ca12}+\text{Kmbp})} - \text{CLunbp}\times\text{Cabind12} \]

\[ \text{Abind12} = \text{integ}(\text{Rbind12}, 0.0) \]

\[ \text{Cabind12} = \frac{\text{Abind12}}{\text{Vpl}} \]

\[ \text{AUC12free} = \text{integ}(\text{CA12}, 0.0) \]

! Total Plasma Concentration (mg/L)

\[ \text{Apltot12} = \text{APL12} + \text{Abind12} \]

\[ \text{Cat12} = \frac{(\text{APL12} + \text{Abind12})}{\text{Vpl}} \]

\[ \text{AUC12} = \text{integ}(\text{cat12}, 0.0) \]

! AS12 = Amount in slowly perfused tissues (mg)

\[ \text{RAS12} = \frac{\text{QS}\times(\text{CA12} - \text{CVS12})}{\text{VS}\times\text{PS}} \]

\[ \text{AS12} = \text{INTEG}(\text{RAS12}, 0.0) \]

\[ \text{CVS12} = \frac{\text{AS12}}{(\text{VS}\times\text{PS})} \]

\[ \text{CS12} = \frac{\text{AS12}}{\text{VS}} \]

! AR12 = Amount in rapidly perfused tissues (mg)

\[ \text{RAR12} = \frac{\text{QR}\times(\text{CA12} - \text{CVR12})}{\text{VR}\times\text{PR}} \]

\[ \text{AR12} = \text{INTEG}(\text{RAR12}, 0.0) \]

\[ \text{CVR12} = \frac{\text{AR12}}{(\text{VR}\times\text{PR})} \]

\[ \text{CR12} = \frac{\text{AR12}}{\text{VR}} \]

! AL12 = Amount in liver tissue (mg)

\[ \text{RAL12} = \frac{\text{QL}\times(\text{CA12} - \text{CVL12}) + \text{RAO} + \text{RAO14} - \text{RAMw}}{\text{VL}} \]

\[ \text{AL12} = \text{INTEG}(\text{RAL12}, 0.0) \]

\[ \text{CVL12} = \frac{\text{AL12}}{(\text{VL}\times\text{PL})} \]

\[ \text{CL12} = \frac{\text{AL12}}{\text{VL}} \]

\[ \text{AUCL12} = \text{INTEG}(\text{CL12}, 0.0) \]

! AM1w = Metabolism in liver after oral

! AM1w : GSTZ-mediated metabolism (mg)

\[ \text{RAM1w} = \frac{(\text{VMAXT}\times\text{CVL12})}{(\text{KM} + \text{CVL12} + \text{CVL13})} \]

\[ \text{AM1w} = \text{INTEG}(\text{RAM1w}, 0.0) \]

! AM2w: non-specific degradation (mg)

! \text{RAM2w} = \frac{(\text{KF}\times\text{CVL12}\times\text{VL})}{\text{PL}}

! \text{AM2w} = \text{INTEG}(\text{RAM2w}, 0.0)

! AMw: Total Metabolism Amount after Oral(mg)

\[ \text{RAMw} = \text{RAM1w} + \text{RAM2w} \]

\[ \text{AMw} = \text{AM1w} + \text{AM2w} \]

! AK12 = Amount in kidney tissue (mg)

\[ \text{RKu12} = \frac{\text{Clr}\times\text{CA12}}{\text{CLunbp}} \]

\[ \text{AKu12} = \text{integ}(\text{RKu12}, 0.0) \]

\[ \text{RAK12} = \frac{\text{QK}\times(\text{CA12} - \text{CVK12})}{\text{CLunbp}} - \frac{\text{RKU12}}{\text{CLunbp}} \]