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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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711th Human Performance Wing
Human Effectiveness Directorate
Biosciences and Protection Division
Applied Biotechnology Branch
Wright-Patterson AFB OH 45433

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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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*Ting Li, **Irv Schultz, ***Deborah A. Keys, ****Teresa R. Sterner, *Jerry L. Campbell, *Jeffrey W. Fisher, ***** David R. Mattie

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14. ABSTRACT
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, 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 MGMGMGmg/kg/dayspectively.

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PREFACE

This research was funded in part by Department of Energy Cooperative Agreement #DE-FC09-02CH11109, a graduate assistantship from the Department of Pharmaceutical and Biomedical Sciences at the University of Georgia and a subcontract through the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) from the Air Force Research Laboratory (711 HPW/RHPB), Wright-Patterson Air Force Base (WPAFB), OH. The source of funding provided by RHPB was the Environmental Restoration Account (ERA) project, Military Site Specific Risk Management, Project Number CNBC20070001. Contract oversight for HJF (contract # FA8650-05-2-6518) was provided by Mr. Mark Hoffman of 711 HPW/RHP at WPAFB, OH. The authors would like to thank Dr. Melvin E. Andersen for his technical assistance with model development.

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*

al., 1980; Chu, 1987; Curry *et al.*, 1991; Fox *et al.*, 1996; Shangraw and Fisher, 1999; Jia *et al.*, 2006; Schultz and Shangraw, 2006). 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 (1^{st} + 2^{nd} 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 $^{13}C_1$-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.

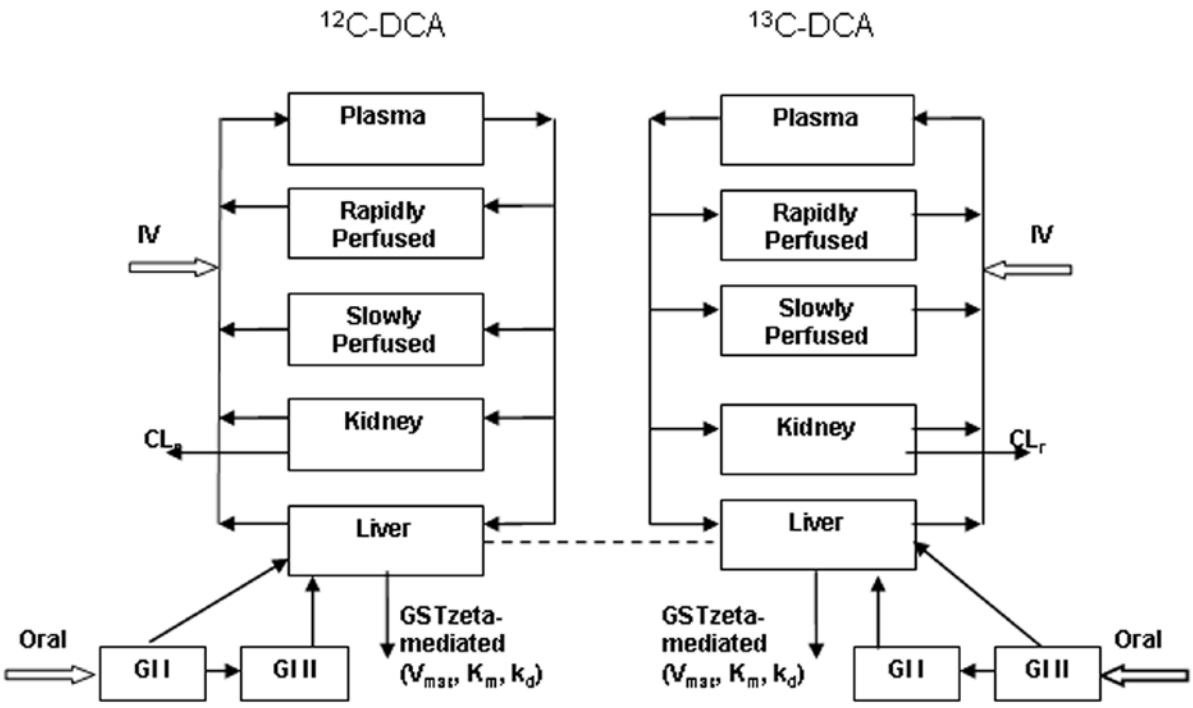


Figure 1. Schematic of DCA PBPK model for humans.
Each isotope was described separately to allow for simulation of the data reported by Schultz and Shangraw (2006). Metabolic interaction was added to the liver compartment to maintain the separate description of the isotopes.

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

$$RAM^{12} = \frac{dA_m^{12}}{dt} = \frac{V_{\max} \times Cv_l^{12}}{K_m \times (1 + Cv_l^{13} / K_m) + Cv_l^{12}} \quad (1)$$

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

$$RAM^{12} = \frac{V_{\max} \times Cv_l^{12}}{K_m + Cv_l^{13} + Cv_l^{12}} \quad (2)$$

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

$$RAM^{13} = \frac{dA_m^{13}}{dt} = \frac{V_{\max} \times Cv_l^{13}}{K_m + Cv_l^{12} + Cv_l^{13}} \quad (3)$$

where the Michaelis-Menten equation represents metabolic conversion by the GSTzeta enzyme; K_m is the Michaelis-Menten affinity constant (mg/L), V_{max} (mg/hour) is the current metabolic

capacity (inhibitable), Cv_l^{12} and Cv_l^{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.

$$\frac{dV_{\max}}{dt} = -k_d \times \left(\frac{V_{\max} \times Cv_l}{K_m + Cv_l}\right) \times \left(\frac{V_{\max} \times K_m}{K_m + Cv_l}\right) + [k_s - k_{de} \times V_{\max}] \qquad (4)$$

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}).

$$V_{\max} = \int_0^t \frac{dV_{\max}}{dt} + V_{\max 0} \qquad (5)$$

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:

$$PV_{\max} = \frac{V_{\max(t)}}{V_{\max 0}} \times 100\% \qquad (6)$$

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

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

Note: h=hour

Calibration of Human PBPK Model for DCA

DCA is highly hydrophilic with an octanol/water partition coefficient ($logP_{o/w}$) 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_{rc}, L/hour-kg) value was calculated by allometrically scaling the clearance rates reported in Curry *et al.* (1991). Cl_{rc} 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_{maxc0}) 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_{de}, 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 rats 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_{mb} 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

Parameters	Symbol	Value	Source
Partition coefficient			
Liver:Blood	PL	1.08	Abbas and Fisher, 1997
Kidney:Blood	PK	0.74	Abbas and Fisher, 1997
Rapidly perfused:Blood	PR	1.08	Abbas and Fisher, 1997
Slowly perfused:Blood	PS	0.11	Estimated (Schultz and Shangraw, 2006)
Metabolic and clearance parameters			
Maximal rate of GSTzeta velocity ($mg/h/kg^{0.75}$)	V_{maxc0}	50	Estimated (Curry *et al.*, 1991; Schultz and Shangraw, 2006)
Michaelic-Menten affinity constant (mg/l)	K_m	6.0	Tong *et al.*, 1998a
Inhibition constant (mg^{-1})	k_d	0.004	Estimated (Chu, 1987)
Degradation rate constant (h^{-1})	k_{de}	0.004	Estimated (Curry *et al.*, 1991)
Urinary excretion (l/h-kg)	Cl_{rc}	0.7×10^{-3}	Curry *et al.*, 1991
Oral absorption parameters following drinking water ingestion			
Transfer rate from the 1st GI compartment to the liver (h^{-1})	K_{a1}	0.01	Estimated (Schultz and Shangraw, 2006)
Transfer rate from the 1st to the 2nd GI compartment (h^{-1})	K_{a2}	7.0	Estimated (Schultz and Shangraw, 2006)
Transfer rate from the 2nd GI compartment to the liver (h^{-1})	K_{a3}	7.0	Estimated (Schultz and Shangraw, 2006)
Plasma protein binding parameters			
Maximum capacity (mg)	B_{max}	0.06	Estimated (Jia *et al.*, 2006)
Affinity constant (mg/l)	K_{mb}	0.001	Estimated (Jia *et al.*, 2006)
Dissociation constant (h^{-1})	K_{unb}	0.16	Estimated (Jia *et al.*, 2006)

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_{maxc0}, 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_{maxc0}, 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_{maxc0} 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) ($AUC_{(0\rightarrow t)}$ 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:

$$AUC_{(0\rightarrow t)}\ \text{P/M Ratio} = \frac{AUC_{(0\rightarrow t)\,predicted}}{AUC_{(0\rightarrow t)\,experimental}} \qquad (7)$$

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

$$\text{MPE\%} = \text{Median}\,(PE_1, PE_1, \ldots PE_n) \qquad (9)$$

$$\text{MAPE\%} = \text{Median}\,(|PE_1|, |PE_2|, \ldots |PE_n|) \qquad (10)$$

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

where $AUC_{(0\rightarrow t)predicted}$ and $AUC_{(0\rightarrow t)experimental}$ are the PBPK model predicted and experimental plasma AUCs until the last measured data point, respectively. $C_{measured}$ and $C_{predicted}$ are the measured and model predicted DCA plasma concentrations, $|PE_i|$ is the absolute value of PE_i and i = 1, 2….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:

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

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 $B6C3F_1$ 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 (A_{ml}, 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).

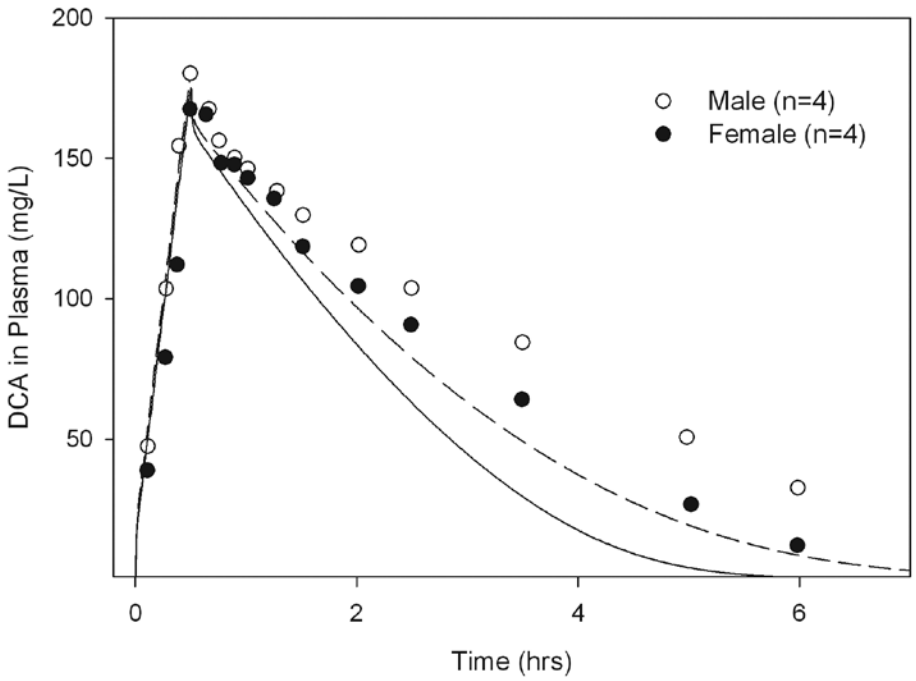


Figure 2. Model predicted (—) *vs.* observed (●) mean DCA plasma concentrations for 4 female subjects, and model predicted (----) *vs.* observed (○) mean DCA plasma concentrations for 4 male subjects following a 0.5 hour IV infusion of 50 mg/kg DCA. The measured values were originally reported by Curry *et al.* (1991).

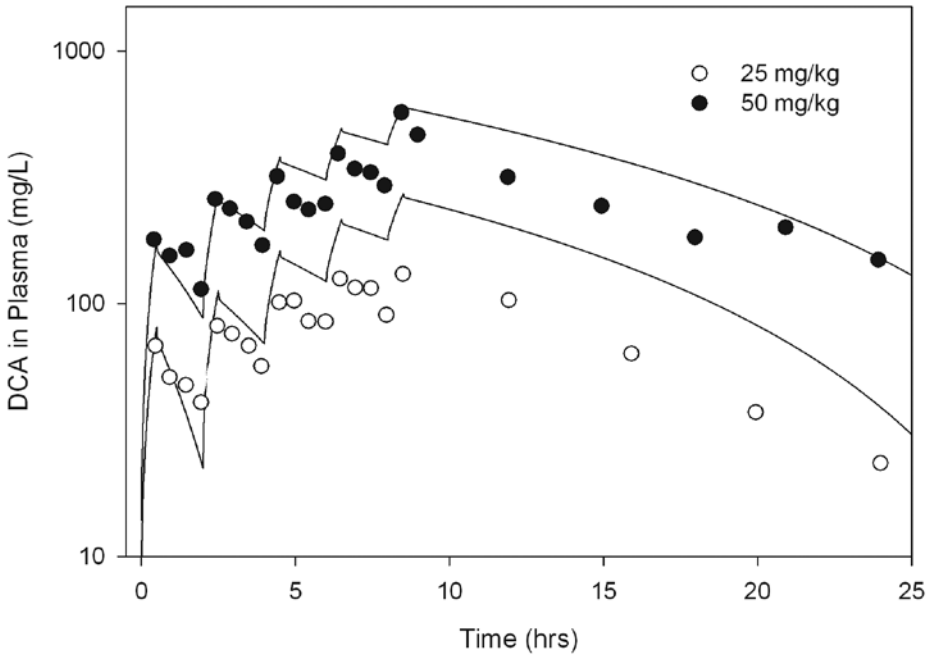


Figure 3. Model predicted and observed mean DCA plasma concentrations for 24 hours following 5-repeated IV 0.5 hour infusions at 2 hour intervals. The concentration-time course data for 2 doses are presented: 25 mg/kg (○, n=5) and 50 mg/kg (●, n=4). The measured data were originally reported by Chu (1987).

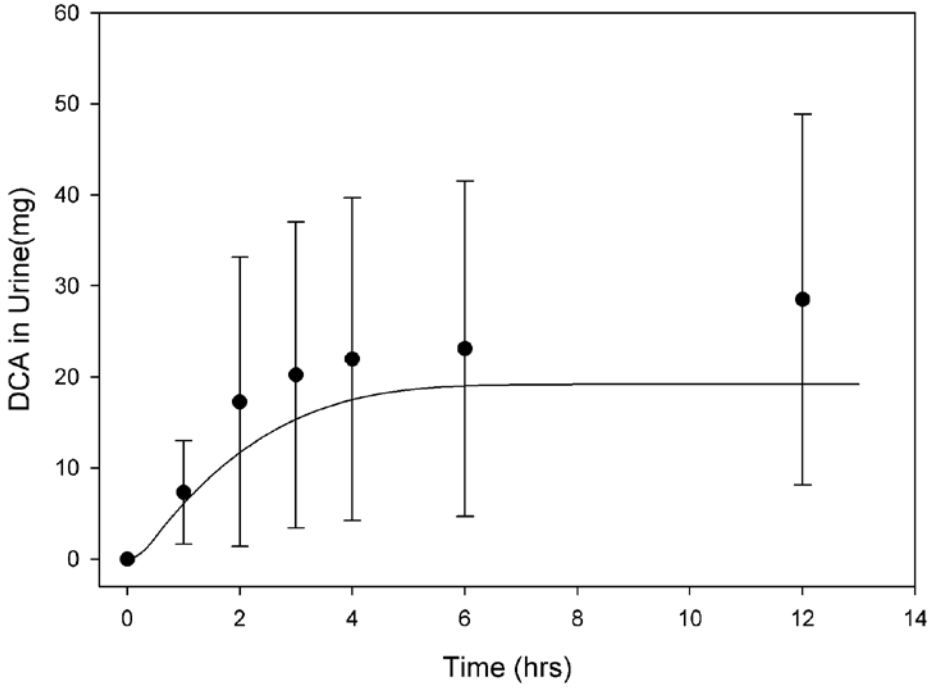


Figure 4. Model simulation (—) of the cumulative amount of DCA excreted in the urine *vs.* the average cumulative excretion measured in 8 subjects following a 0.5 hour IV infusion of 50 mg/kg DCA. Measured values were originally reported by Chu (1987). Bars represent standard deviation.

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.

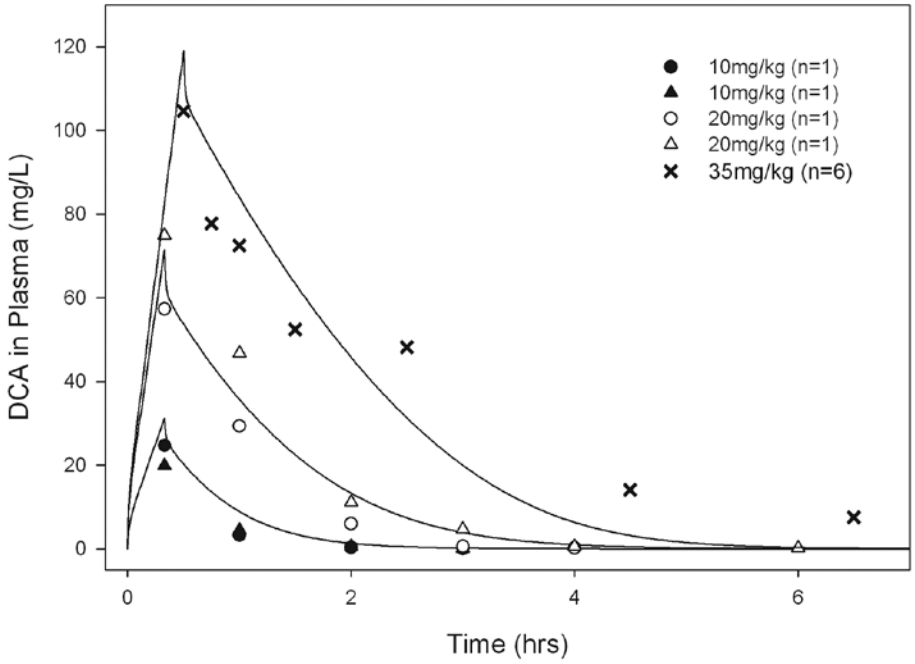


Figure 5. Model predicted (—) and observed DCA plasma concentrations of 2 subjects following 10 mg/kg (●,▲) and 2 subjects following 20 mg/kg (○, ∆) IV infusions of DCA administered over 20 minutes (originally reported by Lukas *et al.*, 1980). Also shown are the mean plasma concentrations (x, n=6) following a 0.5 hour IV infusion of 35 mg/kg DCA reported by Shangraw and Fisher (1999).

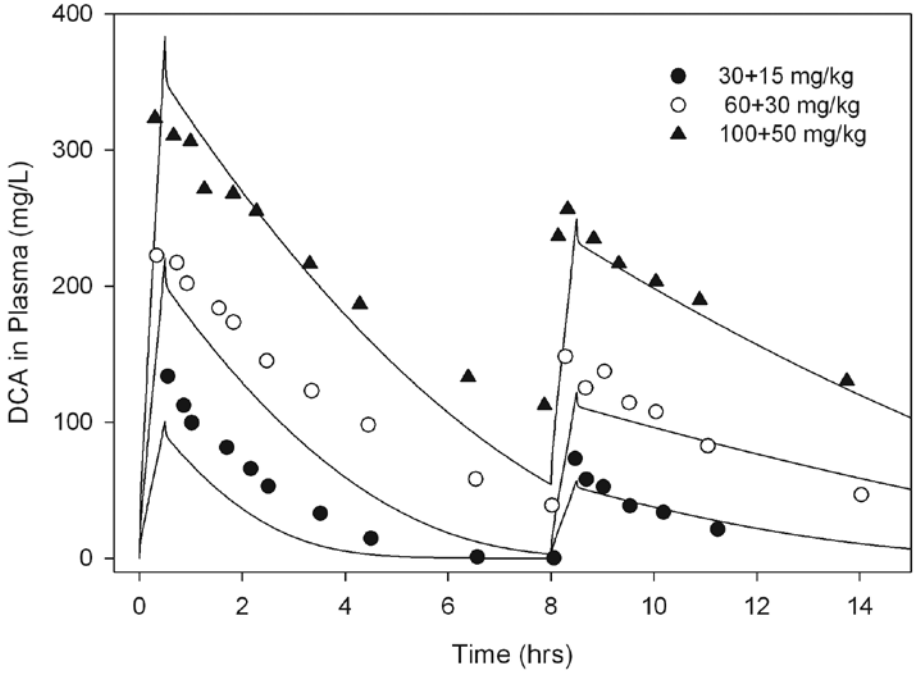


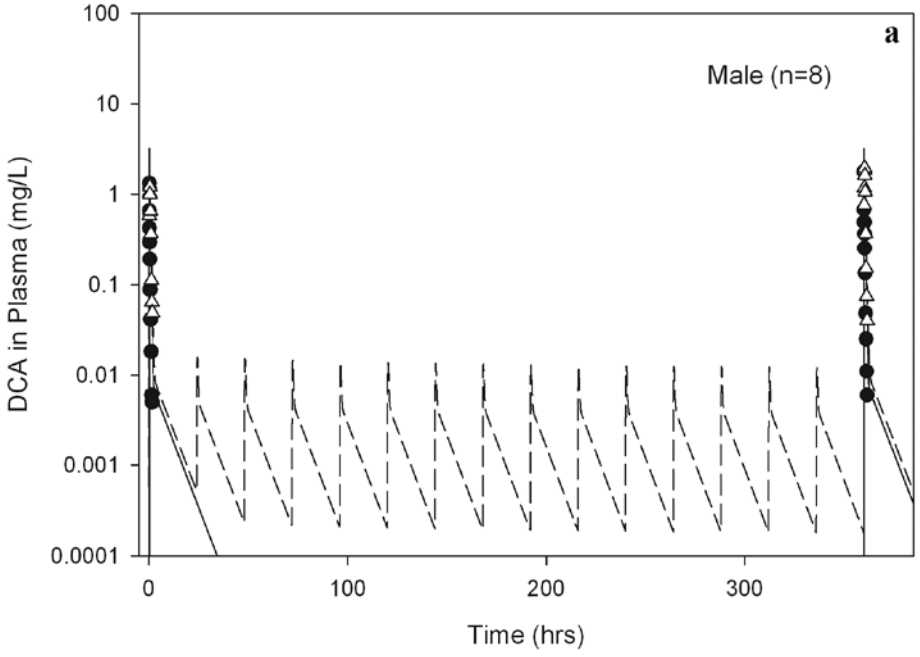
Figure 6. Model predicted (—) and observed mean DCA plasma concentrations after 2 0.5 hour IV infusions of DCA at 0 and 8 hours. The first and second administered doses of DCA were 30 + 15 mg/kg (●, n=9), 60 + 30 mg/kg (○, n=9) and 100 + 50 mg/kg (▲, n=9), respectively. Experimental data were originally reported by Fox *et al.* (1996).

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_{maxc0}, k_d) and oral absorption (K_{a2} and K_{a3}) (Table 3), the fitted values for V_{maxc0} 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_{maxc0}, 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_{maxc0}, 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_{max}) and time to reach the peak concentration, T_{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).



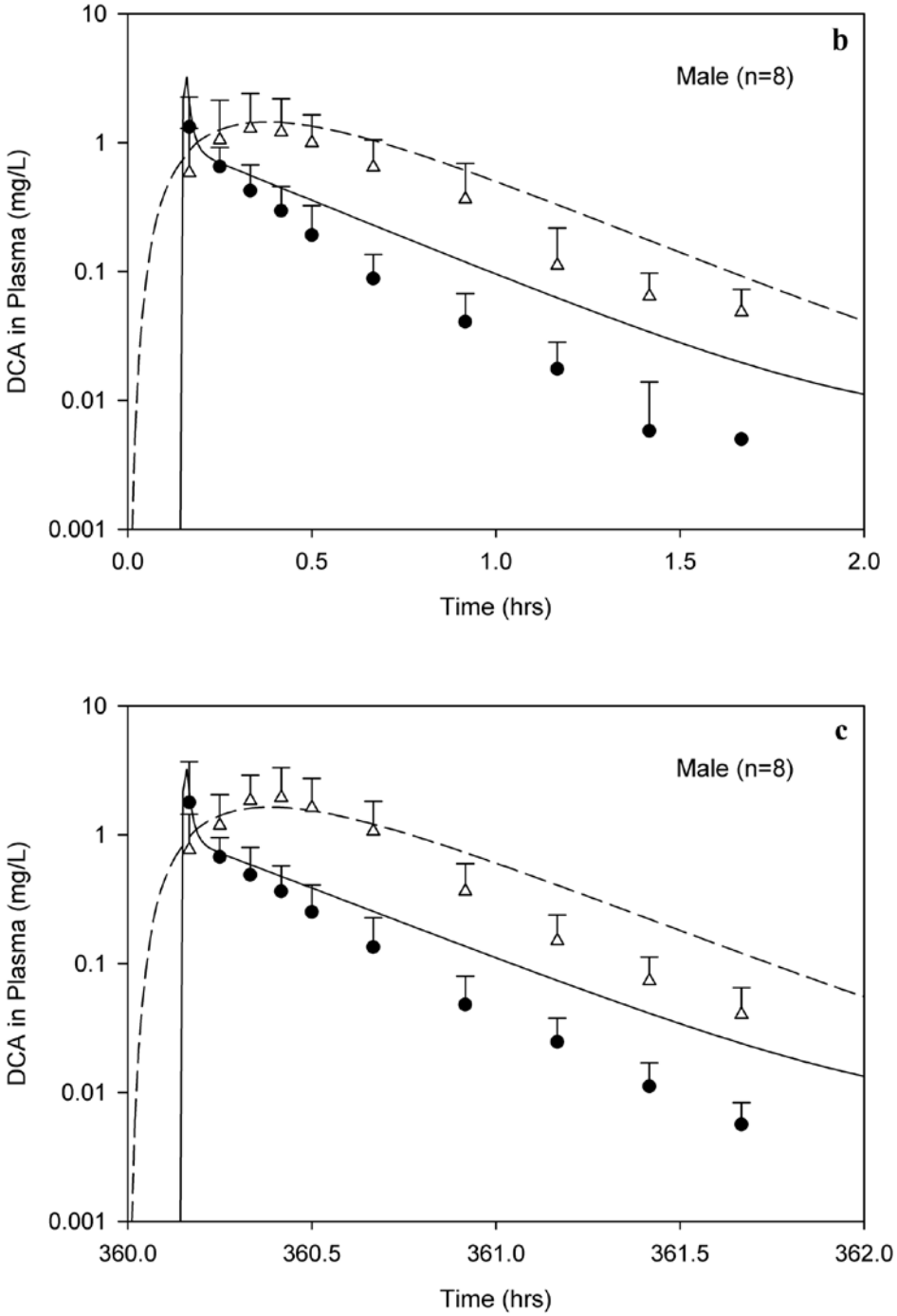


Figure 7. Model predictions of ^{12}C-DCA (----) and ^{13}C-DCA (—) *vs.* observed mean ^{12}C-DCA (Δ) and ^{13}C-DCA plasma concentrations (●) in 8 male subjects. Panel (a) includes the entire period of the study (15 days) where subjects were administered an initial oral dose of 2 mg/kg ^{12}C-DCA in 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).

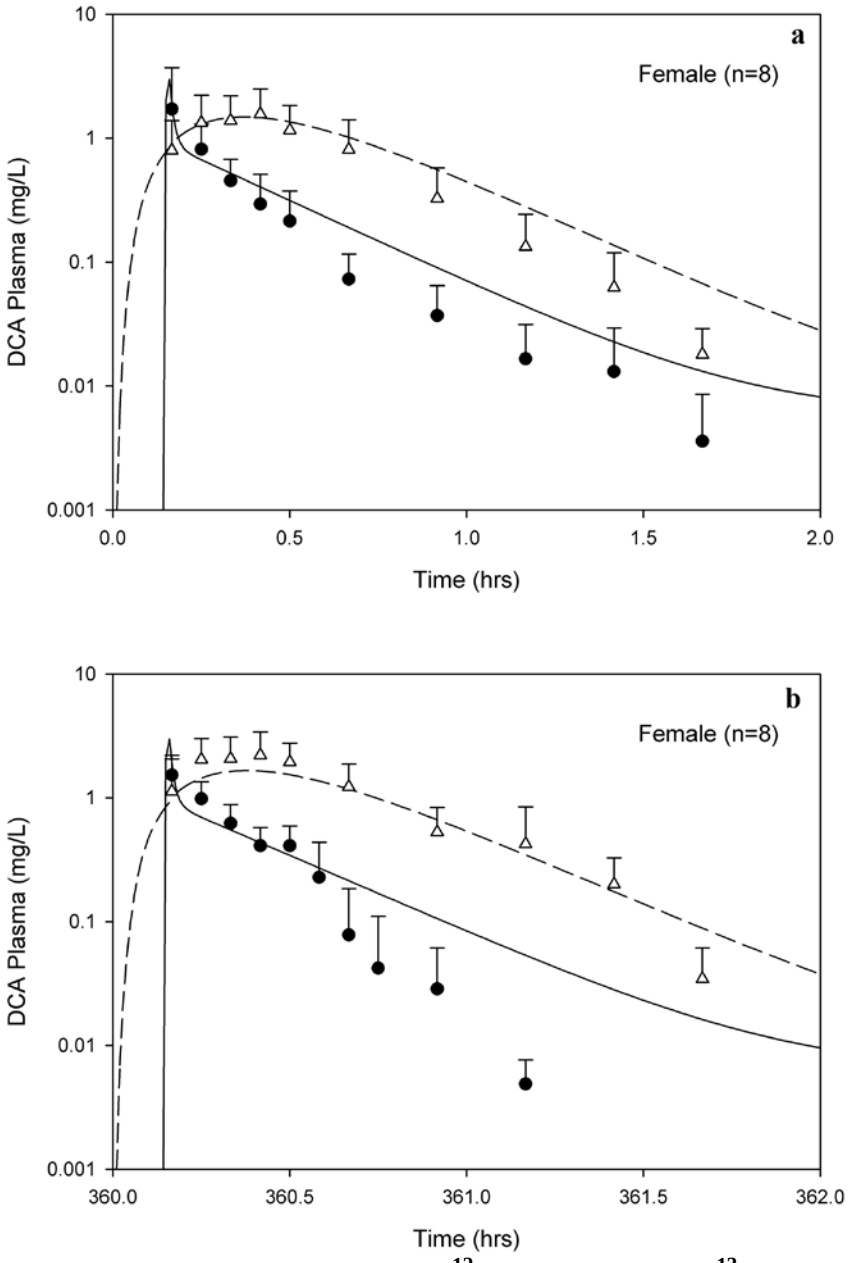


Figure 8. Model predictions of ^{12}C-DCA (----) and ^{13}C-DCA (—) *vs.* observed mean ^{12}C-DCA (Δ) and ^{13}C-DCA plasma concentrations (●) in 8 female subjects. Panel (a) gives the initial 2 hours following the first 2 mg/kg oral and 0.3 mg/kg IV administration on Day 0, and panel (b) gives the 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_{maxc0} and k_d) and oral uptake constants (Ka_2 and Ka_3) using individual DCA plasma pharmacokinetic data from Shultz and Shangraw (2006)

Subject	Gender	Weight (kg)	V_{maxc0} ($mg/h/kg^{0.75}$)	k_d (mg^{-1})	K_{a2} (h^{-1})	K_{a3} (h^{-1})
1	♂	90	113.2	0.001	5.1	5.1
2	♀	66	71.1	0.000	5.3	5.3
3	♀	74.5	118.8	0.011	9.3	9.1
5	♂	66.6	113.2	0.002	5.6	2.9
6	♀	78.3	252.2	0.016	6.9	6.9
7	♂	64.2	89.3	0.000	4.6	4.0
8	♀	59	61.4	0.005	7.9	7.9
9	♂	79	97.9	0.005	9.0	9.0
10	♀	45	225.8	0.011	7.7	12.5
11	♀	69.5	36.7	0.006	9.5	9.5
12	♂	78.6	280.0	0.010	8.8	8.9
13	♂	73.5	58.4	0.000	11.8	12.0
15	♀	67.4	60.0	0.006	17.7	5.7
16	♂	55.9	83.1	0.000	8.0	8.1
17	♀	67.8	51.1	0.000	7.7	13.3
18	♂	73.2	45.8	0.000	8.9	8.9
Mean		69.3	109.9	0.005	8.4	8.1
SD		10.6	75.7	0.005	3.1	3.0

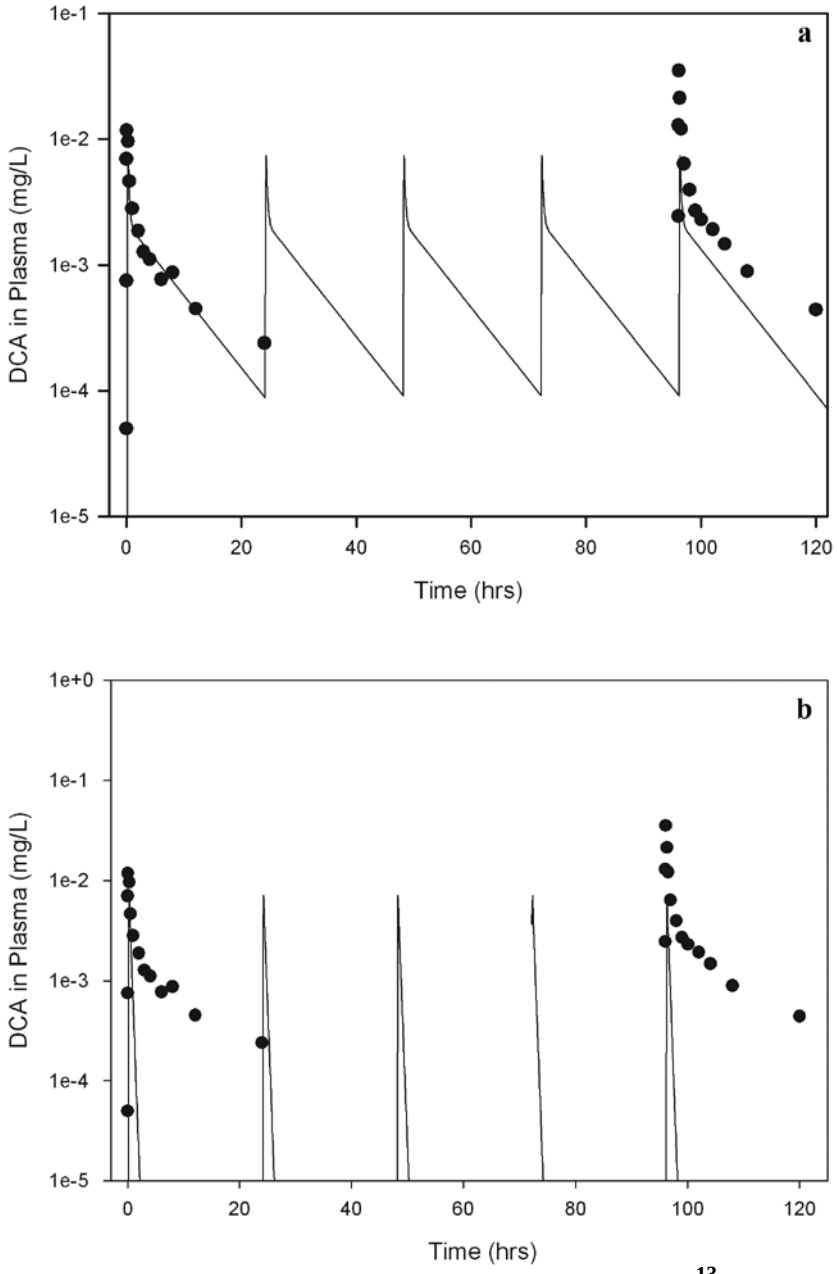


Figure 9. Model predicted (—) and observed (●) ^{13}C-DCA concentrations following a 10 minute IV infusion of 2.5 µg/kg/day for 5 days. Panel (a) shows the final model with the added low-capacity plasma protein binding and panel (b) shows the initial model without binding. The measured data originally were reported by Jia *et al.* (2006).

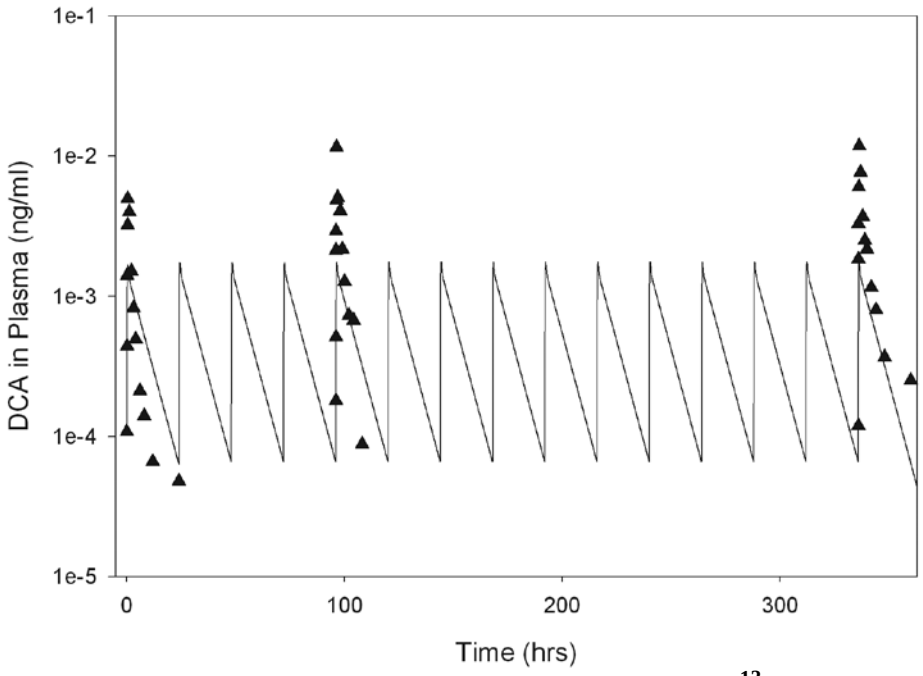


Figure 10. Model predicted (—) and observed (▲) ^{13}C-DCA concentrations following ingestion of 2.5 µg/kg/day DCA in 200 mL of water for 15 days. Measured data originally were reported by Jia *et al.* (2006).

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_{maxc0}, 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 $AUC_{(0\rightarrow t)}$ P/M Ratio, MPE%, MAPE% and RMSPE%

Data Sources	Sampling Time (d)	Administration Route	Dose (mg/kg)	Gender	$AUC_{(0\rightarrow t)}$ P/M Ratio	MPE%	MAPE%	RMSPE%
Curry *et al.* (1991)		iv	50	♂	0.7	1.8	3.8	18.4
				♀	0.7	12.2	13.9	341.9
Chu (1987)		iv	25 × 5		2.9	-36.5	37.5	38.6
		iv	50 × 5		1.4	-14.1	20.7	23.9
Schultz and Shangraw (2006)	0	iv	0.3	♂	1.5	-54.1	54.1	55.4
				♀	1.3	-37.1	37.1	44.7
		oral	2	♂	1.4	-35.6	35.6	39.7
				♀	1.2	-14.0	16.6	36.6
	15	iv	0.3	♂	1.7	-41.0	41.0	48.8
				♀	1.2	-11.0	30.1	50.6
		oral	2	♂	1.2	-18.9	20.7	40.8
				♀	0.8	19.9	25.2	30.7
Jia *et al.* (2006)	1	iv	2.5×10^{-3}		0.8	13.9	17.6	56.4
	5	iv	2.5×10^{-3}		0.4	157.3	157.3	11644.0
	1	oral	2.5×10^{-3}		1.1	-19.5	44.9	77.9
	5	oral	2.5×10^{-3}		0.5	138.0	138.0	236.0
	10	oral	2.5×10^{-3}		0.4	202.2	202.2	282.8
Lukas *et al.* (1980)		iv	10		1.5	-50.6	50.6	51.3
		iv	20		1.0	-18.0	30.9	81.9
Shangraw and Fisher (1996)		iv	35		0.8	-12.1	18.4	1985.2
Fox *et al.* (1996a)		iv	30+15		0.7	47.5	50.2	165.6
		iv	60+30		0.7	27.2	27.2	105.5
		iv	100+50		0.9	4.5	5.8	38.9

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

Model Parameters	Normalized Sensitivity Coefficients					
	50mg/kg iv (single) $AUC_{0\rightarrow 24h}$	50mg/kg iv (2 h interval) $AUC_{0\rightarrow 24h}$	0.3mg/kg iv (Day 0) $AUC_{0\rightarrow 24h}$	2mg/kg oral (Day 0) $AUC_{0\rightarrow 24h}$	2.5 µg/kg-d iv $AUC_{0\rightarrow 120h}$	2.5 µg/kg-d oral $AUC_{0\rightarrow 360h}$
QCC	-0.05	-0.02	**-0.54**	-0.02	**-0.64**	-0.15
QLC	-0.09	-0.03	**-0.50**	_	**-0.57**	-0.12
V_{maxc0}	**-0.88**	**-0.53**	**-0.50**	**-1.05**	-0.33	**-0.72**
K_m	0.47	0.41	0.43	**0.92**	0.33	**0.72**
k_d	**0.76**	0.22	0.21	0.42	_	_
k_{de}	_	_	_	_	_	_
K_{a2}	_	_	_	0.04	_	-0.09
K_{a3}	_	_	_	0.03	_	-0.08
B_{max}	_	_	0.14	0.06	**0.96**	**1.01**
K_{mb}	_	_	_	_	-0.07	-0.08
K_{unb}	_	_	-0.10	-0.05	**-0.81**	**-0.90**

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

	Dosemetrics of DCA Carcinogenicity			
	Total Plasma AUC (mg/L/d)	Free plasma AUC (mg/L/d)	Liver AUC (mg/L/d)	Amount of metabolized DCA (mg/kg liver/d)
HEDs (mg/kg/day)	0.02	0.1	0.1	1.0
% Remaining GSTzeta (PV_{max})	94.4	78.0	78.0	27.5

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.

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APPENDIX A: DIFFERENTIAL EQUATIONS USED IN THE PBPK MODEL

Nomenclature

QC	Cardiac output (L/hour)
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/hour)
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/hour)
K_{mb}	Affinity constant of plasma binding (mg/L)
CL_{unb}	Unbound clearance (L/hour)
K_{unb}	First-order dissociate rate constant (/hour)
K_{ass}	Association rate constant (L/mg/hour)
B_{max}	Maximum amount of binding protein in the plasma (mg)
RAM	Rate of metabolism in the liver (mg/hour)
CL_r	Urinary clearance (L/hour)

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	1st and 2nd GI compartments, respectively

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

$$RApla_{\_free} = \frac{dApla_{\_free}}{dt} = QC \times (Cv - Ca) - RApla_{\_bnd}$$

$$Cv = \frac{\sum Q_i \times Cv_i + DIV}{QC}$$

i = slowly perfused, rapidly perfused, liver or kidney.

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

$$RApla_{\_bnd} = \frac{dApla_{\_bnd}}{dt} = \frac{V_{\max B} \times Ca}{K_{mb} + Ca} - CL_{unb} \times Ca_{\_bnd}$$

$$= K_{ass} \times (\frac{B_{\max} \times K_{mb}}{K_{mb} + Ca}) \times Ca - K_{unb} \times Vpla \times Ca_{\_bnd} = \frac{B_{\max} \times K_{unb}}{K_{mb} + Ca} \times Ca - K_{unb} \times Vpla \times Ca_{\_bnd}$$

where $K_{mb} = K_{unb} / K_{ass}$.

The total DCA concentration in plasma compartment:

$$Ca_{\_total} = \frac{Apla_{\_free} + Apla_{\_bnd}}{Vpla}$$

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:

$$RA_l = \frac{dA_l}{dt} = QL \times (Ca - Cv_l) - RAM + RAO$$

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

$$RA_k = \frac{dA_k}{dt} = QK \times (Ca - Cv_k) - CL_r \times Ca$$

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
 aao = 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
 aao14 = integ(rao14, 0.0)

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

!Isotopes (12C-DCA&13C-DCA) Competitive Metabolism in liver
          CVL = (AL12+AL13)/(VL*PL)
          RLME = -kd*(Vmaxt*CVL/(Km+CVL))*(Vmaxt*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)
        ! Cv13 = (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
```

```
      Rbind12 = (Vbp*Ca12)/(Ca12+Kmbp)-CLunbp*Cabind12
      Abind12 = integ(Rbind12, 0.0)
      Cabind12 = Abind12/Vpl
      AUC12free = integ(CA12, 0.0)

      ! Total Plasma Concentration (mg/L)
      Apltot12 = APL12+Abind12
      Cat12 = (APL12+Abind12)/Vpl
      AUC12 = integ (cat12, 0.0)

 !AS12 = Amount in slowly perfused tissues (mg)'
 RAS12 = QS*(CA12-CVS12)
  AS12 = INTEG(RAS12,0.0)
 CVS12 = AS12/(VS*PS)
  CS12 = AS12/VS

 !AR12 = Amount in rapidly perfused tissues (mg)'
 RAR12 = QR*(CA12-CVR12)
  AR12 = INTEG(RAR12,0.0)
 CVR12 = AR12/(VR*PR)
  CR12 = AR12/VR

 !AL12 = Amount in liver tissue (mg)'
 RAL12 = QL*(CA12-CVL12) + RAO + RAO14 - RAMw
  AL12 = INTEG(RAL12,0.0)
 CVL12 = AL12/(VL*PL)
  CL12 = AL12/VL
AUCL12 = INTEG(CL12,0.0)

 !AM1w = Metabolism in liver after oral
        !AM1w : GSTZ-mediated metabolism (mg)
         RAM1w=(VMAXT*CVL12)/(KM+CVL12+CVL13)
         AM1w = INTEG(RAM1w,0.0)

       !AM2w: non-specific degradation (mg)
       ! RAM2w=(KF*CVL12*VL)
       ! AM2w = INTEG(RAM2w,0)

       !AMw: Total Metabolism Amount after Oral(mg)
        RAMw = RAM1w !+ RAM2w
 AMw = AM1w !+AM2w

 !AK12 = Amount in kidney tissue (mg)'
       RKu12 = Clr*CA12
 AKu12 = integ(RKu12,0.0)
 RAK12 = QK*(CA12-CVK12) -RKU12
```