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Physiologically based pharmacokinetic modeling of dibromoacetic acid in F344 rats

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Abstract

A novel physiologically based pharmacokinetic (PBPK) model structure, which includes submodels for the common metabolites (glyoxylate (GXA) and oxalate (OXA)) that may be involved in the toxicity or carcinogenicity of dibromoacetic acid (DBA), has been developed. Particular attention is paid to the representation of hepatic metabolism, which is the primary elimination mechanism. DBA-induced suicide inhibition is modeled by irreversible covalent binding of the intermediate metabolite α -halocarboxymethylglutathione (α H1) to the glutathione-S-transferase zeta (GSTzeta) enzyme. We also present data illustrating the presence of a secondary non-GSTzeta metabolic pathway for DBA, but not dichloroacetic acid (DCA), that produces GXA. The model is calibrated with plasma and urine concentration data from DBA exposures in female F344 rats through intravenous (IV), oral gavage, and drinking water routes. Sensitivity analysis is performed to confirm identifiability of estimated parameters. Finally, model validation is performed with data sets not used during calibration. Given the structural similarity of dihaloacetates (DHAs), we hypothesize that the PBPK model presented here has the capacity to describe the kinetics of any member or mixture of members of this class in any species with the alteration of chemical- and species-specific parameters.

Keywords

Dibromoacetic acid; Glutathione-S-transferase zeta; F344 rats; PBPK

Introduction

DHAs occur as water disinfection byproducts commonly present in public water supplies ensuring widespread human exposure. Such byproducts are formed when brominated and/or chlorinated disinfecting agents react with naturally occurring organic materials. If bromide is

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Conflict of Interest statement:

The authors declare that there are no conflicts of interest.

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present in the source water, it may be oxidized to the hypobromous acid-hypobromite ion (HOBr-OBr^-), which can react with natural organic matter to form brominated organic compounds, e.g., bromochloroacetic acid (BCA) or DBA. Haloacetic acids are second to trihalomethanes as the most commonly detected disinfection byproducts in surface drinking water supplies in the United States (Liang and Singer, 2003).

Following oral exposure, brominated and chlorinated DHAs are readily absorbed, reaching maximum peak blood concentrations in F344 rats within one hour after single gavage administration (Schultz et al., 1999). Biotransformation accounts for nearly all elimination of DHAs in rodents. Extensive research has been performed with another member of the DHA class, DCA. Approximately 2% of orally administered DCA has been recovered in feces indicating nearly complete absorption (Lin et al., 1993). In vitro experiments with DCA reveal that the majority of degradation occurs in hepatic cytosol (Lipscomb et al., 1995) requiring the presence of glutathione (GSH) to form GXA (James et al., 1997). GSTzeta catalyzes the biotransformation of DCA and DBA to GXA (Tong et al., 1998a), a mutagen in *Salmonella* (Sayato et al., 1987). Multiple studies have noted that animal pretreatment with DCA reduces the rate of metabolism of other DHAs (Gonzalez-Leon et al., 1997; James et al., 1997; Saghir and Schultz, 2002). Elimination of DCA in children or adults is also reduced as a result of prior exposure to therapeutic doses of this agent (Curry et al., 1991; Stacpoole et al., 1998). The reduction of metabolism is due to the irreversible inactivation of GSTzeta by DHAs, including both DCA and DBA (Anderson et al., 1999). Over time GSTzeta resynthesis may restore enzyme activity to pre-exposure levels, but continued exposure to DHAs may result in sustained low enzyme availability. In vitro experiments with GSTzeta knockout (KO) mouse liver samples presented in this paper confirm previous studies of BCA stereoisomers (+)-BCA and (-)-BCA in male F344 rats (Schultz and Sylvester, 2001) that a secondary non-GSTzeta-mediated metabolic pathway also produces GXA from the brominated DHAs, BCA and DBA. However, DCA is only metabolized via GSTzeta.

Further, GXA may be biotransformed to OXA or other compounds such as glycine, glycolate, and carbon dioxide. Given that approximately 25–45% of single oral gavage doses of DCA are eliminated as exhaled CO_2 alone, and under the premise that GXA is a possible source of toxicity, incorporating the kinetics of GXA is an important component in the PBPK model (Lin et al., 1993; Xu et al., 1995).

Human exposure to DCA may also occur as a metabolite of trichloroethylene (TCE), a commonly used industrial solvent, or from its therapeutic use to treat lactic acidosis. Several published TCE PBPK models describe the metabolite DCA through the inclusion of submodels (Abbas and Fisher, 1997; Clewell et al., 2000; Covington et al., 2004; Greenberg et al., 1999). Treatment of DCA kinetics is simplified within these models by tracking DCA presence only in the liver compartment (Clewell et al., 2000; Covington et al., 2004), or allowing only first-order metabolism (Abbas and Fisher, 1997; Covington et al., 2004; Greenberg et al., 1999).

Barton et al.'s (1999) DCA PBPK model was developed for mice and includes Michaelis-Menten metabolism of DCA and, while acknowledging the existence of suicide inhibition of GSTzeta, does not model it directly. A DCA PBPK model for both rats and mice was described by Keys et al. (2004). Their model incorporates the suicide inhibition and regeneration of GSTzeta via a bound intermediate inactivating the enzyme in a first order process as well as natural degradation and resynthesis of the enzyme. Additionally, a secondary non-GSTzeta metabolic pathway was included. However, as detailed below, recent data are inconsistent with the assumption that this secondary pathway is present for DCA. Also, this model lacks the GI compartment necessary for evaluation of oral exposures and omits modeling of urinary excretion, which should be included for exposure to higher dose levels where GSTzeta depletion forces the utilization of alternate elimination mechanisms. In 2008, Li and colleagues

published a human DCA PBPK model that is capable of modeling both IV and drinking water exposures. GSTzeta was modeled with the incorporation of degradation and resynthesis as well as suicide inhibition via a reactive metabolite reacting with free enzyme in a second order process (Li et al., 2008). However, earlier studies had indicated that the irreversible suicide inhibition is a result of covalent binding of the metabolite α H1 to GSTzeta during the catalytic process, rather than reacting with free enzyme (Anderson et al., 1999; Tzeng et al., 2000).

In this paper we present a PBPK model structure appropriate for application to the family of DHAs, which goes beyond the scope of any previous DHA models by including submodels for the common metabolites (GXA and OXA) that may be involved in the toxicity or carcinogenicity of these disinfection byproducts. Particular attention is paid to the representation of metabolism. DBA-induced suicide inhibition is modeled by irreversible covalent binding of the intermediate metabolite α H1 to GSTzeta. We also present data illustrating the presence of a secondary non-GSTzeta metabolic pathway for DBA that produces GXA. The model is calibrated with plasma and urine concentration data from DBA exposures in female F344 rats through IV, oral gavage, and drinking water routes. Sensitivity analysis is performed to confirm identifiability of estimated parameters. Finally, model validation is performed with data sets not used during calibration.

Methods

Data

In vitro and in vivo toxicokinetic data used for calibration and validation of model predictions were obtained from National Toxicology Program (NTP) studies. The in vivo data sets consist of plasma time-course concentrations and urine content of parent compound and the metabolites glyoxylic acid and oxalic acid (NTP, 2007). In vitro studies were carried out with liver cytosol samples obtained from both wild-type (WT) and GSTzeta KO mice. Parent compound consumption as well as GXA formation were measured after incubations at various concentrations and time points.

In vivo data—For the in vivo DBA study, plasma and urine samples were collected from female F344/N rats administered single IV injections of 10 or 110 mg DBA/kg body weight or single oral gavage doses of 10, 40, or 100 mg/kg. The IV studies captured plasma samples for two to three hours post-dose for up to nine time points. Plasma samples for the single oral gavage doses consisted of approximately 10 time points up to eight hours post-dose. DBA, GXA, and OXA urine samples up to 48 hours post-dose were collected for the two higher doses (40 and 100 mg/kg). Additionally, data were collected from two-week drinking water studies with DBA concentrations of 50, 500, or 1000 mg/L with or without a 24 hour recovery period followed by a subsequent single oral gavage challenge dose of 3.4, 34.3, or 68.6 mg/kg, respectively. Plasma samples were collected every three hours for a 24 hour period at the conclusion of the two-week drinking water studies without challenge dosing. For each of the drinking water studies with oral gavage challenge doses, plasma samples were taken at eight time points up to six hours post-dose and urine specimens collected for 96 hours subsequent to gavage challenge dosing. Some of the above studies also had associated GXA and OXA plasma concentration data. Much of these data were scattered and below the limit of quantification, hence comparisons to model predictions have been omitted. In addition, GXA plasma time course concentrations were measured from a study of single 50 mg/kg IV administration of GXA to female rats. All of these data were used during model calibration.

Furthermore, we reserved several data sets for model validation. DBA plasma samples were collected from female F344/N rats administered single IV injections of 25 mg DBA/kg body weight or single oral gavage doses of 25, 50, or 125 mg/kg. Each of these studies recorded approximately 10 time points of DBA plasma time course with 3 animals sampled for each

time point. The IV study measured from 0–1 hr post-dose and the oral gavage studies collected data up to 12 hours post-dose.

In vitro data

Preparation of Liver Cytosol: WT and GSTzeta KO mice were kindly provided by Dr. José Manuel Fernández Cañón at Universidad de León, León, Spain. The mice were euthanized by CO₂ asphyxiation and the livers perfused in-situ with ice-cold 0.9% NaCl (w/v) prior to removal. Livers from six mice of each group were pooled prior to subcellular fractionation. Preparation of the cytosolic fraction of the pooled livers was by differential centrifugation as described in Okita and Okita (1992). Freshly prepared cytosol was dialyzed for 12 hr against three changes of 100 mM phosphate buffer (pH 7.4) at 4°C using a Spectra/Por 2.1 Biotech DispoDialyzer (Spectrum, Laguna Hills, CA) with a 15,000 molecular weight cutoff. The dialyzed cytosol was stored at –70°C until use. Protein concentrations were determined using the Coomassie Protein Assay Reagent (Pierce, Rockford, IL).

DBA and DCA Metabolism in Cytosol: All cytosolic incubations were carried out at 37°C under normal atmospheric conditions. Incubations contained 1 mg/ml of dialyzed cytosolic protein and 1.0 mM GSH in a 50 mM PO₄ buffer (pH 7.4) in a total volume of 1 ml. After pre-incubating the vials for 2 min, the reaction was started by adding DBA or DCA. The initial concentration was typically 70 µM for DBA and 92 µM for DCA. The concentration-time profiles for depletion of added DHA were determined by removing 0.1 ml aliquots from the incubation at various times up to 120 minutes. The aliquots were placed in microcentrifuge tubes that contained 15 µl of 50% (v/v) H₂SO₄, which stopped the reaction. The DBA and DCA concentrations in the aliquots were then analyzed by GC-ECD after methylation with diazomethane as described previously in Schultz et al. (1999). In some experiments, the formation of GXA was measured in the remaining portion of the incubation. For this analysis, GXA was quantified using the spectrophotometric method of Vogels and Van Der Drift (1970). The residual incubation mixture was quantitatively transferred to a clean Pyrex tube and mixed with 50 µl of trifluoroacetic acid and then centrifuged (3,000 $\times$ g) for 10 minutes. The supernatant was neutralized with 1 N NaOH and then mixed with phenylhydrazine HCl for 10 minutes. Next, the mixture was acidified with concentrated HCl and then potassium ferricyanide. After 15 minutes, the absorbance of the reaction mixture was measured at 535 nm. Quantification of GXA was based on standard curves prepared with mouse WT cytosol spiked with varying amounts of GXA and processed as described above.

Model

Model structure—The DHA PBPK model is comprised of diffusion-limited compartments for aggregated tissue, kidney, and liver as well as kidney tubules and a stomach compartment receiving no blood flow but with a linear uptake to the liver as illustrated in Figure 1. Rapid venous equilibration was assumed to model blood flow. Since DHAs exhibit low binding to rat plasma proteins (Schultz et al., 1999), plasma was not modeled separately. Enterohepatic recirculation was not included in the model because data from DCA studies with bile duct cannulated F344 rats suggested DCA metabolism and distribution were unaffected by this mechanism (Schultz et al., 1999). IV, oral gavage, and drinking water administration are included as possible exposure routes. IV is described by a direct infusion into the blood and oral gavage by an infusion into the stomach compartment. Circadian rhythm (Yuan, 1993) coupled with experimental water consumption rates are used to model intake via drinking water.

Parent compound (DBA), GXA, and OXA are tracked throughout all compartments of the model. Additionally, within the liver, the model accounts for the following: free GSTzeta, DBA bound to GSTzeta, α H1 (produced from DBA) bound to GSTzeta, free α H1, and a consolidation

of other GXA metabolites (i.e., glycine, carbon dioxide, etc.). Figure 2 illustrates the interactions of these compounds within the liver. The GSTzeta-dependent pathway of the metabolic scheme is based on that presented in Tong et al. (1998a) and Wempe et al. (1999). However, simplifications have been made for modeling purposes by combining steps for which no data were available or which were assumed to happen instantaneously (e.g., conversion of α H1 to GXA).

A key feature of this model is the inclusion of suicide inhibition in the description of GSTzeta mediated metabolism. Suicide inhibition is characteristic of metabolic enzymes for the entire class of DHAs and is modeled by irreversible covalent binding of an intermediary metabolite, α H1, to GSTzeta which effectively inactivates the enzyme. The fraction of GSTzeta covalently bound to α H1 is an estimated, chemical-specific parameter. The initial amount of GSTzeta present in the animal liver is also an estimated parameter due to lack of data. Additionally, GSTzeta resynthesis and degradation are included in the model according to the reported rates for male F344 rats in Anderson et al., 1999. Although it is hypothesized that GSTzeta activity levels may be age-dependent (James et al., 1998), due to inadequate information, we are assuming all values are constant regardless of animal age.

Note that the naïve animals in the single IV and oral gavage studies had tap water rather than distilled water available ad libitum. Given the knowledge that DHAs may be present in municipal tap water sources at concentrations less than the current federal regulatory limit of 60 μ g/L, it is possible that the GSTzeta levels of these animals might be partially reduced compared to animals provided distilled water. However, when modeling we assume that the animals have 100% enzyme availability at the start of the study. Note that this issue is not applicable to the drinking water studies because tap water was used as the vehicle medium and compound was added until the desired dose level was reached.

In addition to GSTzeta mediated metabolism, there is evidence for an additional, unidentified metabolic degradation pathway for brominated DHAs (Schultz and Sylvester, 2001). In vitro experiments were carried out for DBA and DCA with male WT and GSTzeta KO B6C3F1 mouse liver fractions to verify the existence of this alternative pathway, understand its kinetics, and discover the end metabolite of this alternative pathway. Statistical tests were performed to validate the non-GSTzeta metabolic pathway for female F344 rats.

Mean body weight and water consumption rate measurements are study specific, and all other physiological parameters are taken from Brown et al. (1997) and Davies and Morris (1993) as described in Table 1. Abbas and Fisher (1997) determined the partition coefficients (PCs) for DCA; and due to similarities of structure, behavior, and octanol:water PCs amongst the DHAs, the PCs for DBA are assumed to be the same (Table 2). That the steady-state apparent volume of distribution approximates the total body water volume in rats, indicates that DHAs distribute uniformly outside the vascular system and do not significantly store within any single tissue (Schultz et al., 1999). The PC for the aggregated tissue compartment was derived assuming that this compartment is comprised of 80% slowly perfused tissue, using the associated PC value for muscle/blood (0.37), and 20% rapidly perfused tissue, using the associated PC value for liver/blood (1.08). PCs for both GXA and OXA were chosen as unity because of their low lipid solubility and lack of evidence found to indicate affinity of these compounds to any tissue group.

Although at low doses only minor amounts of parent compound are eliminated via urine, as GSTzeta is depleted and metabolism is reduced this elimination route gains importance. Schultz et al. (1999) suggest that since the renal clearance rate is clearly less than the urine flow rate in F344 rats, the urinary excretion of DHAs is controlled by tubular reabsorption. Therefore, we allow for saturable reabsorption from the kidney tubule space into the kidney

tissue when modeling DBA in rats. The estimated parameters, $T_{\max}$ and k_t , which describe the reabsorption are summarized in Table 3. Due to the lack of evidence to persuade otherwise, we assume reabsorption is not a factor in the urinary elimination of the metabolites GXA and OXA, and they are modeled with glomerular filtration describing distribution into kidney tissue and tubule regions and elimination via urine flow.

Data sets used for model calibration were found to include background urinary GXA and OXA concentrations, but no plasma presence, before DHA administration indicating endogenous sources separate from DHA biotransformation. In order to match model predictions to data, we used data from pre-dose urine collections over 15–18 hour time periods from the single administration oral gavage studies to female F344 rats for DBA and BCA to estimate endogenous GXA and OXA production rates (NTP, 2007; NTP, 2009). We assume that the background GXA and OXA levels present in urine remain at these estimated rates (shown in Table 1) for the duration of the experiment and incorporate them in the model predictions.

The complete system of equations comprising this model is included in the Appendix. The model was implemented in Simulink and parameter optimization as well as sensitivity analysis were performed with Matlab (Release 7.4.0 R2007a, The MathWorks, Natick, MA).

Model calibration—In all, twelve parameters were fitted to experimental data: k_{abs} , $T_{\max}$, k_t , ϵ , k_f , $\text{AGSTzeta}_{\text{liver}}(0)$, k_{bind} , k_{trans} , k_{metab} , $k_{\text{covalent_Frac}}$, $k_{\text{metabGXAtoOXA}}$, and $k_{\text{metabGXAtoOther}}$ (See Table 3 for explanation of notation). Data used for model calibration include DBA, GXA, and OXA plasma time-course concentrations and urine levels. Although no tissue measurements were available, plasma and urine measurements are adequately representative because the model is a linked system. Urine data were optimized in the log scale in an effort to normalize the values to those comparable to plasma values, creating a more even weighting amongst all data points.

Sum of squared errors between data and model predictions,

$$\epsilon^2 = \sum_{i,j} [x_i(t_j) - \hat{x}_i(t_j, \theta)]^2,$$

were computed as the minimized cost functional, where $x_i(t_j)$ are the data points for output i at time point j and $\hat{x}_i(t_j, \theta)$ are the model predictions of output i at time point j using the parameter vector θ .

Bounding criteria were imposed to mitigate against infeasible parameter values. These bounds were chosen so that: 1) the non-GSTzeta metabolic pathway was not permitted to eliminate more than 80% of the dose in single administration studies; and 2) the amount of αH1 bound to GSTzeta must be less than the amount of DBA present in the liver. Both of these were introduced to guard against unlikely scenarios for which direct data were unavailable, including in vivo measurements in GSTzeta KO animals exposed to the same dosing scenarios as well as measurements of quickly forming and transforming intermediate metabolite products. Additionally, upper and lower bounds were applied to individual parameter value estimates. Since these boundaries were administered merely to shrink the parameter space and were not necessarily motivated biologically, they were adjusted so that final optimal parameter values were not near any artificial parameter space boundary.

Given the lack of indication for potential ranges of unknown parameter values, the use of an optimizer proficient in locating global minimums was warranted. Therefore, a differential evolution (DE) optimization algorithm (Storn and Price, 1997), implemented in Matlab, was

first used to search the global parameter space restricted by the bounding criteria for seven of the unknown twelve parameters: k_{abs} , T_{max} , k_t , ε , $A_{\text{GSTzeta,liver}}(0)$, k_{bind} , and $k_{\text{covalent_Frac}}$. k_{trans} and k_{metab} were fixed as large values to signify that the associated processes happened quickly, while $k_{\text{metabGXAtoOXA}}$, $k_{\text{metabGXAtoOther}}$, and k_f were fixed to the derived values as explained in the Appendix. The output of the DE optimization was used as an initial estimate in a simplex-based routine to ensure convergence to a global minimum.

The derived values for $k_{\text{metabGXAtoOXA}}$ and $k_{\text{metabGXAtoOther}}$ (described in the Appendix), when used for model simulation with the above estimated parameter values, resulted in systematic over-prediction of OXA in urine. This indicates that the derived value of $k_{\text{metabGXAtoOXA}}$ is artificially high while the value of $k_{\text{metabGXAtoOther}}$ is artificially low. Further, statistical tests reveal that employing a calibrated value for k_f yields a superior fit to data over use of the fixed in vitro derived value. Therefore, the entire set of 12 unknown parameters was re-calibrated to the model with the simplex-based routine using the five previously fixed values as initial estimates, but allowing them to be modified. Final parameter values estimated via this scheme are summarized in Table 3.

Sensitivity analysis—The DHA PBPK model presented herein consists of 29 state variables (x_i), 6 with available data. It includes 38 parameters (θ_j), 26 of which were evaluated from the literature and 12 of which are estimated. Sensitivities of model state variables to parameters were computed as

$$\frac{\partial x_i(t)}{\partial \theta_j} = \frac{x_i(\theta_j + \Delta\theta_j, t) - x_i(\theta_j - \Delta\theta_j, t)}{2\Delta\theta_j},$$

where $\Delta\theta_j$ was taken to be 1% of the value of θ_j . Further, to allow for direct comparison amongst sensitivities of differing state variables and parameters, the sensitivities ($s_{i,j}$) were normalized as:

$$s_{i,j}(t) = \frac{\partial x_i(t)}{\partial \theta_j} \cdot \frac{\theta_j}{x_i(\theta_j, t)}.$$

Results

In vitro experiments

To better understand the impact of non-GSTzeta mediated hepatic metabolism of DHAs and to incorporate this secondary metabolic pathway into the PBPK model, in vitro experiments were carried out as detailed in the Methods section. From the data shown in Figure 3a, it is clear that DCA is metabolized only by GSTzeta, since the GSTzeta KO mice showed virtually no metabolism. Thus, no secondary pathway exists for this DHA. Figure 3b shows that a non-GSTzeta mediated metabolic pathway is present for DBA because this DHA is metabolized by liver cytosolic fractions prepared from GSTzeta KO mice. The lack of metabolism shown in the incubations without GSH indicates the dependence of both the GSTzeta and secondary metabolic pathways on the presence of GSH. The negative control incubation performed on ice indicates that all hepatic metabolism is most likely enzymatic. Finally, as shown in Figure 3c by comparing the amounts of DBA consumed with that of GXA formed in both the WT and KO scenarios, the resultant metabolite from this linear, non-GSTzeta path is also GXA. We

acknowledge that the means for the GSTzeta KO case differ between the DBA consumption and GXA formation amounts, but notice that the error bars indicate that the DBA consumption data has a degree of scatter. If the mean of the DBA consumption was larger than GXA formation, the possibility of a metabolite other than GXA is plausible. However, because the reverse is true we are confident in concluding that the resultant metabolite from the non-GSTzeta-mediated metabolic path is GXA.

Although the PBPK model described herein is for female rats and the in vitro experiments were for male mice, statistical tests reveal that this additional pathway is necessary to adequately fit the data and thus is also present in the female rat case. In particular, we fit the full model, with the 12 unknown parameters, to the in vivo female rat data as well as a reduced model where 11 parameters were fit and k_f was set to 0, indicating the secondary non-GSTzeta metabolic pathway was not present. Both scenarios were calibrated with DBA, GXA, and OXA plasma time-course concentrations and urine levels as described in the Methods section. This yields an F-test statistic value of 108.87. Since we have $n=557$ data points, when comparing to $F^{-1}(0.95, 12-11, 557-12)=3.86$ we reject with 95% probability the hypothesis that the reduced model with no secondary metabolic pathway fits the data as well as the full model including the path. We continued on to perform an additional statistical test to determine whether the value for k_f should be fitted to the in vivo data or derived from in vitro data as described in the Appendix. Considering the same full model of 12 fitted parameters as above along with a reduced model of 11 fitted parameters setting $k_f = 26.6 \text{ hr}^{-1}$, we arrive at an F-test statistic value of 38.66. So we reject with 95% probability the hypothesis that the reduced model with a non-GSTzeta mediated metabolic rate derived from in vitro male mouse data fits the female rat in vivo data as well as the full model.

Model predictions compared to data

Model predictions of DBA blood concentration after single IV administration are shown in Figure 4. Two doses (10 mg/kg and 110 mg/kg) were used during model calibration; the additional 25 mg/kg study is used for validation. Clearance of DBA from blood is slightly under-predicted for all doses, most notably the lowest dose.

Figures 5 and 6 depict model predictions for single administration oral gavage studies. Data in Figure 5 was used during model calibration while Figure 6 is shown for model validation. Blood time course predictions match data fairly well for the 6 presented doses, although the peak DBA concentration tends to be slightly overpredicted with the low dose data and slightly underpredicted with the high dose from the calibration data set (Figure 5a). The blood time course predictions from the validation set shown in Figure 6a provide a better fit to the experimental data and characterize especially well the decay over time. We also present, in Figures 5b and 6b, the associated GSTzeta activity as percent of control so as to understand the impact the different doses have on enzyme availability. With higher doses the enzyme activity is reduced to nearly zero, although as time elapses the enzyme begins to recover through resynthesis. To visualize the impact of non-GSTzeta metabolism we depict the associated graphs of percent of dose which is eliminated via this pathway in Figures 5c and 6c. Clearly, as enzyme activity is depleted, the secondary pathway gains importance as a clearance mechanism.

Similar model predictions for DBA blood time course concentrations, GSTzeta activity, and involvement of non-GSTzeta metabolism for the 2-week drinking water studies with gavage challenge are shown in Figure 7. These studies were comprised of a 13 day drinking water exposure followed by a 24 hour recovery period (beginning at $T=312 \text{ hr}$) and a subsequent oral gavage challenge administered at $T=336 \text{ hr}$ which was equivalent to approximately one day of drinking water exposure. There is good agreement with both the peak and clearance of the

DBA blood time course for all three doses presented. The varying degrees of enzyme inactivation and the role of the non-GSTzeta pathway are apparently nonlinear with dose.

Urine concentration data for DBA, GXA, and OXA were available from two single oral gavage studies and the drinking water with gavage challenge studies. All available urine data was used for model calibration purposes. Table 4 provides summary of the data along with model predicted values. The predictions for cumulative DBA urinary elimination were all within two standard deviations of the mean of the data. Predictions for cumulative GXA and OXA urinary elimination post-gavage dose were also mostly within two standard deviations of the mean of the data. The only exceptions are the GXA urine prediction for the 100 mg/kg single oral gavage study and the OXA urine prediction for the 40 mg/kg oral gavage study which were both slightly underpredicted. All the GXA and OXA predictions and data represented in Table 4 contain background levels, as described in the Methods section above, in addition to amounts attributed to derivation from administered DBA. Overall, given the similar nonlinearities by dose found in both the experimental data and the predicted values, the model characterizes the impact of this elimination pathway especially as it becomes more important in GSTzeta depleted scenarios.

Sensitivity analysis

Figure 8 illustrates the results of the sensitivity analysis. These subfigures show with shading the degree of sensitivity an output has to changes in parameter values and how these sensitivities change over time. The arrows along the x-axes in all subfigures indicate the particular time points where we have associated data measurements. Note that all sensitivities are presented as absolute values to illustrate only the magnitude of sensitivity and that they are normalized for easy comparison. Although some values were larger than the maximum of the scale (2), we chose to scale it in this manner to better visualize all nonzero sensitivities. As a representative case, we present the sensitivities corresponding to the 100 mg/kg single gavage administration. The sensitivities corresponding to other exposures are similar, although in general we acknowledge that the sensitivity of a model prediction to a change in a parameter value depends on time, dose, exposure route, and parameter value.

Discussion

The model presented here advances DHA modeling efforts in several ways. First, although others have modeled metabolism of the DHAs, we explicitly model metabolite concentrations, which may reflect the toxicity endpoints. Furthermore, while both Keys et al. (2004) and Li et al. (2008) included GSTzeta-mediated metabolism in their models, they ignore or mischaracterize the possibility of a second metabolic pathway. Using new data presented above, we are able to model explicitly a secondary non-GSTzeta mediated metabolic pathway for bromine-substituted DHAs. The new data also illustrate that DCA is solely metabolized via GSTzeta, which counters past assumptions (Keys et al., 2004). Additionally, our model empirically describes, at a molecular binding level, the mechanisms of GSTzeta-mediated biotransformation based on scientific community consensus (Anderson et al., 1999; Tong et al., 1998a; Wempe et al., 1999). Finally, the structure of this model lends itself to application to all chemicals and mixtures of chemicals in the DHA class for various exposure patterns, while previous models focused only on DCA.

A particular challenge during model development was characterizing the mechanism of compound distribution to the tissues. Both the biologically relevant diffusion-limited compartments and the simplified flow-limited compartments were analyzed to determine which type of model best represented the available data. Chemical distribution to a flow-limited organ compartment occurs by permeation to the tissue instantly with blood flow, while distribution to a diffusion-limited compartment is a function of diffusion to and from the

separated tissue capillary space. We found that, when applied to the IV administration studies, the model incorporating the flow-limited compartments aptly characterized the DBA blood concentration while the diffusion-limited version of the model under-predicted the rate of clearance. However, the flow-limited model did not provide a reasonable fit to the data from the oral gavage studies. Specifically, the predicted decay in blood concentration curves was much too rapid as compared to the actual data; the diffusion-limited model could capture this decay. For this reason, and since our focus is characterizing DHA kinetics under the more likely exposure mechanism of oral dosing, we opted for the PBPK model to include diffusion-limited tissue compartments.

If there is significant sensitivity present for a fitted parameter where good agreement between model prediction and actual data is achieved (both for the particular timepoint and output), one has more confidence in this estimate. Large sensitivity, which is desirable, indicates that small changes of the parameter value result in large changes to model predictions. Figure 8a shows the sensitivities of DBA blood concentration predictions to the twelve fitted parameters over time. Similarly, Figures 8b, c, and d show the normalized sensitivities of cumulative DBA, GXA, and OXA urine predictions to the fitted parameters over time, respectively. Most of the fitted parameters, with the exception of k_{metab} , have significant nonzero sensitivity for at least one of the data points. DBA blood concentration is sensitive to ϵ , k_f , $A_{\text{GSTzeta,liver}}(0)$, k_{bind} , k_{trans} , $k_{\text{covalent_Frac}}$, $k_{\text{metabGXAtoOXA}}$, and k_{abs} . Further, cumulative DBA urine amounts are sensitive to T_{max} and k_t . Additionally, GXA and OXA urine amounts show sensitivity to $k_{\text{metabGXAtoOXA}}$ and $k_{\text{metabGXAtoOther}}$. The good agreement amongst model predictions and data shown in Figures 4–7 combined with the sensitivity to the fitted parameters as shown in Figure 8 provides confidence in the parameter estimates.

For completion, sensitivity analysis of model predictions to the fixed parameters was also performed (results omitted) as this can help to ascertain the importance of the fixed parameters. Of note, significant sensitivity up to a magnitude of 10.5 is present to the PC values for DBA in various tissues for which estimations and assumptions were made as outlined in the Methods section. If future advances in experimental procedures improve and measurements provide different values for fixed parameters, acknowledging the model's sensitivity to these parameters gives us a qualitative idea about the potential impact if these values were to change.

Model calibration efforts described herein call into question the method of extrapolation of in vitro derived parameter values to in vivo rate constants. The fixed parameters in this model were experimentally determined through a variety of in vivo or in vitro methods. In our first attempt, the parameters k_f , $k_{\text{metabGXAtoOXA}}$ and $k_{\text{metabGXAtoOther}}$ were fixed to values determined through common practices as outlined in the Appendix, relying on in vitro measurements. However, after several simulations we realized that these values were in fact unreasonable, so we resorted to numerically estimating these additional parameters. Future work may include further investigation into the extrapolation methods applied and the validity of other in vitro derived parameters, such as k_{de} .

DHA exposure levels comparable to those present in municipal drinking water supplies significantly decrease GSTzeta activity in rats after 8 weeks of exposure (Guo et al., 2006). Multiple authors have shown that elimination of DHAs in humans is likely similar to that in rats (James et al., 1997; Saghir and Schultz, 2002; Schultz and Shangraw, 2006; Tong et al., 1998b; Tzeng et al., 2000), so that the PBPK model presented here may be useful for risk assessment purposes. Adjustment of species-specific physiological and biochemical parameters should therefore allow application to human risk assessment.

A variety of dose metrics useful for comparison with tumor data may be derived from this model including long-term AUC predictions of DHA, GXA, and OXA for venous blood and

liver tissue as well as GSTzeta inactivation metrics. Comparing these internal dose predictions with tumor incidence data from rodent assays (Carter et al., 2003; Melnick et al., 2007; NTP, 2007; NTP, 2009), would allow stronger conclusions about the dose-response for the carcinogenicity of these chemicals. Possible hypotheses that can be examined are: 1) inactivation of GSTzeta by DHA may increase the concentrations of DHAs in the liver thus contributing to the liver alterations induced by DHAs; 2) inactivation of GSTzeta by DHA may disrupt the tyrosine catabolism pathway resulting in toxicity endpoints (Ammini et al., 2003; Cornett et al., 1999; Lantum et al., 2002); and 3) increased GXA or OXA concentrations in the liver as a result of DHA metabolism might impact a liver tumor response.

However, care should be taken when applying this model to chronic studies, as age-related body weight changes and the associated change in tissue volume fractions have not been included. Also, as noted in the Methods section, age-dependency has not been incorporated into GSTzeta related parameters although there is evidence suggesting age-related differences in DHA clearance and GSTzeta activity (Schultz et al., 2002). Application of the model to long-term studies will need to address age-related effects on enzyme activity.

Given the structural similarity of DHAs, the PBPK model presented here has the capacity to describe the kinetics of any member or mixture of members of this class with the alteration of chemical specific parameters (i.e., k_{bind} , $k_{\text{covalent_Frac}}$, etc). The PBPK model we have presented for DBA in female F344 rats is validated with data sets not used during parameter fitting procedures, implying extension to structurally similar compounds within the class is viable. The in vitro data presented from GSTzeta KO and WT mouse liver fractions confirms that a non-GSTzeta mediated metabolic pathway exists and forms the metabolite GXA for bromine-substituted DHAs. Completion of a manuscript depicting application of this model to both isomers of bromochloroacetic acid ((+)-BCA and (-)-BCA) is forthcoming and future work will also include model calibrations for DCA.

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Appendix

Model equations

The DHA PBPK model equations consist of the following where:

$A_{i,j}$ = Amount of compound i in tissue compartment j (nmol)

V_j = Volume in tissue compartment j (L)

$C_{i,j}$ = Concentration of compound i in tissue compartment j (nmol/L)

Q_i = Blood flow rate for tissue compartment i ; if $i=urine$, then urine flow rate (L/hr)

$P_{i,j}$ = Tissue compartment j : blood PC for compound i

The following equations represent the amount of DHA in the stomach, kidney, kidney blood, kidney tubule, urine, other aggregated tissues, other aggregated tissue blood, liver blood, and arterial blood, respectively. Note that GFR denotes glomerular filtration, in L/hour.

$$\begin{aligned}\frac{dA_{DHA, stomach}}{dt} &= -k_{abs} * A_{DHA, stomach} + drinkDoseDHA + oralDoseDHA \\ \frac{dA_{DHA, kidney}}{dt} &= \frac{\varepsilon}{1-\varepsilon} Q_{kidney} \left(\frac{A_{DHA, kidneyBlood}}{V_{kidneyBlood}} - \frac{A_{DHA, kidney}}{V_{kidney} P_{DHA, kidney}} \right) + \frac{T_{max} C_{DHA, kidTub}}{k_t + C_{DHA, kidTub}} \\ \frac{dA_{DHA, kidneyBlood}}{dt} &= (Q_{kidney} - GFR) * C_{DHA, art} - Q_{kidney} * \frac{A_{DHA, kidneyBlood}}{V_{kidneyBlood}} + \frac{\varepsilon}{1-\varepsilon} Q_{kidney} \left(\frac{A_{DHA, kidney}}{V_{kidney} P_{DHA, kidney}} - \frac{A_{DHA, kidneyBlood}}{V_{kidneyBlood}} \right) \\ \frac{dA_{DHA, kidTub}}{dt} &= GFR * C_{DHA, art} - \frac{T_{max} C_{DHA, kidTub}}{k_t + C_{DHA, kidTub}} - \frac{dA_{DHA, urine}}{dt} \\ \frac{dA_{DHA, urine}}{dt} &= Q_{urine} * \frac{A_{DHA, kidTub}}{V_{kidTub}} \\ \frac{dA_{DHA, other}}{dt} &= \frac{\varepsilon}{1-\varepsilon} Q_{other} \left(\frac{A_{DHA, otherBlood}}{V_{otherBlood}} - \frac{A_{DHA, other}}{V_{other} P_{DHA, other}} \right) \\ \frac{dA_{DHA, otherBlood}}{dt} &= Q_{other} \left(C_{DHA, art} - \frac{A_{DHA, otherBlood}}{V_{otherBlood}} \right) + \frac{\varepsilon}{1-\varepsilon} Q_{other} \left(\frac{A_{DHA, other}}{V_{other} P_{DHA, other}} - \frac{A_{DHA, otherBlood}}{V_{otherBlood}} \right) \\ \frac{dA_{DHA, liverBlood}}{dt} &= Q_{liver} \left(C_{DHA, art} - \frac{A_{DHA, liverBlood}}{V_{liverBlood}} \right) + \frac{\varepsilon}{1-\varepsilon} Q_{liver} \left(\frac{A_{DHA, liver}}{V_{liver} P_{DHA, liver}} - \frac{A_{DHA, liverBlood}}{V_{liverBlood}} \right) + k_{abs} A_{DHA, stomach} \\ C_{DHA, art} &= \frac{ivDoseDHA + \sum Q_i \frac{A_{DHA, iBlood}}{V_i}}{Q_{cardiac}}\end{aligned}$$

The following equations represent the amount of GXA in the kidney, kidney tubule, urine, other aggregated tissues, and arterial blood, respectively.

$$\begin{aligned}\frac{dA_{GXA, kidney}}{dt} &= (Q_{kidney} - GFR) * C_{GXA, art} - Q_{kidney} * \frac{A_{GXA, kidney}}{V_{kidney} P_{GXA, kidney}} \\ \frac{dA_{GXA, kidTub}}{dt} &= GFR * C_{GXA, art} - \frac{dA_{GXA, urine}}{dt} \\ \frac{dA_{GXA, urine}}{dt} &= Q_{urine} * \frac{A_{GXA, kidTub}}{V_{kidTub}} \\ \frac{dA_{GXA, other}}{dt} &= Q_{other} \left(C_{GXA, art} - \frac{A_{GXA, other}}{V_{other} P_{GXA, other}} \right) \\ C_{GXA, art} &= \frac{ivDoseGXA + \sum Q_i \frac{A_{GXA, i}}{V_i P_{GXA, i}}}{Q_{cardiac}}\end{aligned}$$

The following equations represent the amount of OXA in the kidney, kidney tubule, urine, other aggregated tissues, and arterial blood, respectively.

$$\begin{aligned}\frac{dA_{OXA, kidney}}{dt} &= (Q_{kidney} - GFR) * C_{OXA, art} - Q_{kidney} * \frac{A_{OXA, kidney}}{V_{kidney} P_{OXA, kidney}} \\ \frac{dA_{OXA, kidTub}}{dt} &= GFR * C_{OXA, art} - \frac{dA_{OXA, urine}}{dt} \\ \frac{dA_{OXA, urine}}{dt} &= Q_{urine} * \frac{A_{OXA, kidTub}}{V_{kidTub}} \\ \frac{dA_{OXA, other}}{dt} &= Q_{other} \left(C_{OXA, art} - \frac{A_{OXA, other}}{V_{other} P_{OXA, other}} \right) \\ C_{OXA, art} &= \frac{\sum Q_i \frac{A_{OXA, i}}{V_i P_{OXA, i}}}{Q_{cardiac}}\end{aligned}$$

The following equations represent, in nmol, the amounts of GSTzeta, DHA, bound DHA and GSTzeta product, bound α H1 and GSTzeta product, free α H1, inactivated GSTzeta product,

GXA, OXA, and other GXA metabolites in the liver. Note $k_s = k_{de} A_{GSTzeta,liver}(0)$ represents the rate of GSTzeta resynthesis, in nmol/hour.

$$\begin{aligned}
 \frac{dA_{GSTzeta,liver}}{dt} &= k_s - k_{de} A_{GSTzeta,liver} - k_{bind} A_{DHA,liver} A_{GSTzeta,liver} \\
 A_{GSTzeta,liver}(t) &= A_{GSTzeta,liver}(t) + (1 - k_{covalent_frac}) A_{\alpha H1BoundGSTzeta,liver}(t) \\
 \frac{dA_{DHA,liver}}{dt} &= \frac{Q_{liver}}{V_{liver}} \left(\frac{A_{DHA,liverBlood}}{V_{liverBlood}} - \frac{A_{DHA,liver}}{V_{liver} P_{DHA,liver}} \right) - k_{bind} A_{DHA,liver} A_{GSTzeta,liver} - k_f \frac{A_{DHA,liver}}{P_{DHA,liver}} \\
 \frac{dA_{DHABoundGSTzeta,liver}}{dt} &= k_{bind} A_{DHA,liver} A_{GSTzeta,liver} - k_{trans} A_{DHABoundGSTzeta,liver} \\
 \frac{dA_{\alpha H1BoundGSTzeta,liver}}{dt} &= k_{trans} A_{DHABoundGSTzeta,liver} \\
 \frac{dA_{\alpha H1,liver}}{dt} &= -k_{metab} A_{\alpha H1,liver} \\
 A_{\alpha H1,liver}(t) &= A_{\alpha H1,liver}(t) + (1 - k_{covalent_frac}) A_{\alpha H1BoundGSTzeta,liver}(t) \\
 A_{deactivated_ \alpha H1BoundGSTzeta}(t) &= A_{deactivated_ \alpha H1BoundGSTzeta}(t) + k_{covalent_frac} A_{\alpha H1BoundGSTzeta,liver}(t) \\
 \frac{dA_{GXA,liver}}{dt} &= Q_{liver} \left(C_{GXA,art} - \frac{A_{GXA,liver}}{V_{liver} P_{GXA,liver}} \right) + k_f \frac{A_{DHA,liver}}{P_{DHA,liver}} + k_{metab} A_{\alpha H1,liver} \\
 &\quad - (k_{metabGXAtoOXA} + k_{metabGXAtoOther}) A_{GXA,liver} \\
 \frac{dA_{OXA,liver}}{dt} &= Q_{liver} \left(C_{OXA,art} - \frac{A_{OXA,liver}}{V_{liver} P_{OXA,liver}} \right) + k_{metabGXAtoOXA} A_{GXA,liver} \\
 \frac{dA_{otherGXAmetab,liver}}{dt} &= k_{metabGXAtoOther} A_{GXA,liver}
 \end{aligned}$$

Deriving metabolic rates from data

In vitro data was used in conjunction with the liver portion of the PBPK model, excluding the GSTzeta enzyme interactions, to calibrate the non-GSTzeta mediated linear metabolic rate per mg cytosolic protein. Using a reference value of 108,000 mg cytosolic protein per 1 kg liver tissue in rats, this rate was linearly extrapolated to 26.6 hr^{-1} for the female F344 rat.

The elimination rate constant describing the metabolism from GXA to OXA ($k_{metabGXAtoOXA}$) was obtained as the slope of the linear regression of the log-transformed concentrations of these chemicals in female Sprague-Dawley rat liver supernatants versus time data in the terminal portion of the curve from the data presented in Sharma and Schwille (1997). We assumed a similar rate constant for F344 rats. Given the in vitro rates of conversion from GXA to OXA per mg protein over one hour, the log-transformed concentration was regressed against time based on the experimental system details. The associated slope was computed. Finally, this was scaled by mg protein present in the female F344 rat liver. Using the mean body weight of the female rats from the NTP study, liver weight fractions (Brown et al., 1997) and liver protein concentrations (Sharma and Schwille, 1997), the in vivo elimination rate was calculated at 308.7 hr^{-1} .

As indicated in Figure 2, GXA is either metabolized to OXA or a range of other products including glycine, glycolate, and carbon dioxide. We have grouped all GXA metabolites, excluding OXA, into an alternative pathway for GXA metabolism. Data for specific members of this group were unavailable for model calibration or validation, but the inclusion of this mechanism in the model is vital given the evidence that approximately 25–45% of single oral gavage doses of DCA are eliminated as exhaled CO_2 alone (Lin et al., 1993; Xu et al., 1995). To calculate the rate of transformation from GXA along this alternate pathway to this group of non-OXA metabolites ($k_{metabGXAtoOther}$), we coupled the transformation rate $k_{metabGXAtoOXA}$ calculated as detailed above along with the GXA clearance information from the GXA IV studies. Specifically, we log-transformed the GXA blood time course and found the terminal slope (for $t > 0.15 \text{ hr}$), which was -3.9851 for female rats. Then, the flow-limited model was run for these dosing scenarios including the GXA to OXA transformation rate and urinary elimination of both GXA and OXA to ascertain the terminal slope of log-transformed GXA blood-time course without including the transformation of GXA to non-OXA

metabolites. At this point, the terminal slope was -3.4157 for female rats. From here, values for $k_{\text{metabGX} \rightarrow \text{Other}}$ were iteratively substituted until the model predicted terminal slope was within 5% of the terminal slope suggested by data. Using this method, we arrive at the value 299.1 hr^{-1} .

Figure 1.

Schematic of DHA PBPK model with GXA and OXA submodels. DHA is tracked in 8 compartments (stomach, kidney tubule space, tissue and capillary space of aggregated tissue, kidney, and liver) and the metabolites GXA and OXA are tracked in 4 compartments (other aggregated tissue, kidney, kidney tubule, and liver). Administration of DHA via oral gavage and/or drinking water, as well as administration of DHA and/or GXA via IV is described. Elimination of DHA may occur through two possible biotransformation mechanisms or through urine. Elimination of GXA may occur through biotransformation or urinary elimination; the only modeled elimination route of OXA is via urine.

Figure 2.

Metabolic scheme of DHAs as implemented in the model. The model includes two possible biotransformation pathways for DHA to GXA. The first pathway is GSTzeta dependent and includes the following steps: (1) binding of DHA to GSTzeta, (2) displacement of a halide by glutathione from bound DHA:GSTzeta product to form bound α H1:GSTzeta product, (3) irreversible inactivation OR (4) release of α H1, (5) elimination of glutathione from α H1 to form GXA. The second pathway (6) is a direct transformation of DHA to GXA, as determined from in vitro data. The metabolism of GXA to OXA (7) and GXA to other metabolites such as glycine or CO₂ (8) occurs regardless of the path taken to form GXA from DHA.

Figure 3. In vitro experiments for DBA and DCA with both WT and GSTzeta KO mice liver cytosol

- a) In vitro DCA metabolism in WT (solid line, open circles) and GSTzeta KO (dashed line, open triangles) mouse cytosol, where $n=3$ for each case.
- b) In vitro DBA metabolism in WT (solid line, open circles) and GSTzeta KO (dashed line, open triangles) mouse cytosol ($n=3$ for each case). Incubations of WT on ice (dotted line, stars, $n=3$) and WT without GSH (dash-dot line, open squares, $n=1$) are included as negative controls.
- c) Comparison of DBA consumed versus GXA produced from in vitro experiments with 50 μM DBA, where $n=3$ for each case.

Figure 4. DBA single administration IV studies. Two of the three dosing scenarios shown here were used for model calibration, namely: 10 mg/kg (solid line, open circles) and 110 mg/kg (dashed line, stars). The remaining dosing scenario, 25 mg/kg (dotted line, open triangles) is shown for model validation purposes. All lines represent model predictions and markers represent observations.

Figure 5. Fitted DBA single administration oral gavage studies. The dosing scenarios shown here were used for model calibration, namely: 10 mg/kg (solid line, open circles), 40 mg/kg (dashed line, stars), and 100 mg/kg (dotted line, open triangles)

- Blood time course from single administration oral gavage studies.
- Predicted GSTzeta activity, as percent of control, for single administration oral gavage studies.
- Predicted percent of dose eliminated through secondary non-GSTzeta mediated metabolic pathway for single administration oral gavage studies.

Figure 6. Validation DBA single administration oral gavage studies. The dosing scenarios shown here were used for model validation, namely: 25 mg/kg (solid line, open circles), 50 mg/kg (dashed line, stars), and 125 mg/kg (dotted line, open triangles)

- a) Blood time course from single administration oral gavage studies.
- b) Predicted GSTzeta activity, as percent of control, for single administration oral gavage studies.
- c) Predicted percent of dose eliminated through secondary non-GSTzeta mediated metabolic pathway for single administration oral gavage studies.

Figure 7. Fitted DBA 2-week drinking water studies with single gavage challenge. The dosing scenarios presented here were used for model calibration, namely: 2-week exposure to 50 mg/L drinking water with 3.4 mg/kg single oral gavage challenge (solid line, open circles), 500 mg/L drinking water with 34.3 mg/kg single oral gavage challenge (dashed line, stars), and 1000 mg/L drinking water with 68.6 mg/kg single oral gavage challenge (dotted line, open triangles)

- a) Blood time course after single administration oral gavage challenge.
- b) Predicted GSTzeta activity, as percent of control, for drinking water studies.
- c) Predicted percent of dose eliminated through secondary non-GSTzeta mediated metabolic pathway for drinking water studies.

Figure 8. Sensitivity of model predictions to fitted model parameters for the 100 mg/kg single gavage administration dose. Arrows indicate the time points for which we have data

- Normalized sensitivity of DBA blood concentration predictions to fitted model parameters for 100 mg/kg single gavage administration dose.
- Normalized sensitivity of cumulative DBA urine predictions to fitted model parameters for 100 mg/kg single gavage administration dose.
- Normalized sensitivity of cumulative GXA urine predictions to fitted model parameters for 100 mg/kg single gavage administration dose.
- Normalized sensitivity of cumulative OXA urine predictions to fitted model parameters for 100 mg/kg single gavage administration dose.

Table 1Physiological parameters for female F344 rats.^a

Parameter (units)	Value
Body weight (kg) ^b	0.175
Cardiac output (L/hour per kg ^{0.75} body weight)	14.1
Water consumption rate (mL/day per kg ^{0.75} body weight) ^b	49.7
Tissue volumes as fraction of body weight	
Kidney tissue	0.0073
Kidney blood	0.00119
Kidney tubule	0.000073
Liver	0.0366
Liver blood	0.00769
Other aggregated tissue	0.776
Other aggregated tissue blood	0.0574
Blood flows as fraction of cardiac output	
Kidney	0.141
Liver	0.183
Other aggregated tissue	0.676
Urinary elimination	
Glomerular filtration (L/hour per kg body weight) ^c	0.314
Urine flow (L/hour per kg body weight) ^c	0.00833
GXA urine background rate (microgm/hour) ^b	6.45
OXA urine background rate (microgm/hour) ^b	19.0

^aExcept as noted, parameter values determined from Brown et al. (1997).^bComputed as means from experimental animals. N=378 (body weight), 504 (water consumption), and 11 (urine).^cParameter value taken from Davies and Morris (1993).

Table 2Partition coefficients.^a

Tissue	DBA	GXA	OXA
Kidney	0.74	1	1
Liver	1.08	1	1
Other aggregated tissue	0.513	1	1

^a All partition coefficients are expressed as tissue: blood ratios; values for DBA are the same as or derived from those provided for DCA by Abbas and Fisher (1997).

Table 3

Model parameters.

Parameter (units)	Symbol	Value	Source
Absorption rate from stomach compartment to liver (1/hr)	k_{abs}	2.27	Estimated
Maximum rate of reabsorption from kidney tubule space into kidney tissue (nmol/hr)	T_{max}	62500	Estimated
Michaelis-Menten constant associated with reabsorption from kidney tubule space into kidney tissue (nmol/L)	k_t	461000	Estimated
Permeability coefficient (unitless)	ε	0.303	Estimated
Non-GSTzeta mediated DHA metabolic rate (1/hr)	k_f	12.5	Estimated
Initial amount of GSTzeta present in liver (nmol)	$A_{\text{GSTzeta,liver}}(0)$	124	Estimated
Degradation rate of GSTzeta (1/hr)	k_{de}	0.00875	Anderson et. al, 1999
Binding rate of GSTzeta to DHA (1/nmol/hr)	k_{bind}	12.5	Estimated
Metabolism rate of DHA to α H1 (1/hr)	k_{trans}	553	Estimated
Transformation rate of α H1 to GXA (1/hr)	k_{metab}	465	Estimated
Fraction of bound GSTzeta to α H1 product which is covalently bound (unitless)	$k_{\text{covalent_Frac}}$	0.00275	Estimated
Metabolism rate of GXA to OXA (1/hr)	$k_{\text{metabGXAtoOXA}}$	59.9	Estimated
Metabolism rate of GXA to "other" metabolites (i.e. glycine, CO ₂ , etc.) (1/hr)	$k_{\text{metabGXAtoOther}}$	1530	Estimated

Model prediction and data (N=3 for all studies, presented as mean $\pm$ standard deviation) of cumulative urinary elimination (in micrograms) post-gavage dose for DBA and the metabolites GXA and OXA. Note that values for the metabolites GXA and OXA include both quantities derived from DBA and endogenous amounts as described in the Methods section.

Table 4

Study	DBA	GXA		OXA	
		Prediction	Data	Prediction	Data
40 mg/kg DBA single oral gavage ^a	11.4 $\pm$ 5.50	15.3	516 $\pm$ 61.0	437	1640 $\pm$ 130.
100 mg/kg DBA single oral gavage ^a	128 $\pm$ 22.8	104	649 $\pm$ 37.0	456	2000 $\pm$ 248.
2-week 50 mg/L DBA drinking water with 3.4 mg/kg DBA single oral gavage challenge ^b	1.09 $\pm$ 0.283	1.19	617 $\pm$ 49.9	620	1980 $\pm$ 147.
2-week 500 mg/L DBA drinking water with 34.3 mg/kg DBA single oral gavage challenge ^b	54.2 $\pm$ 19.5	42.9	627 $\pm$ 47.2	629	2160 $\pm$ 186.
2-week 1000 mg/L DBA drinking water with 68.6 mg/kg DBA single oral gavage challenge ^b	121 $\pm$ 14.5	115	942 $\pm$ 250.	639	2240 $\pm$ 305.
					2010

^a cumulative 48 hours post-gavage dose.

^b cumulative 96 hours post-gavage dose