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Incorporation of the glutathione conjugation pathway in an updated physiologically-based pharmacokinetic model for perchloroethylene in mice

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Abstract

Background—Perchloroethylene (perc) induced target organ toxicity has been associated with tissue-specific metabolic pathways. Previous physiologically-based pharmacokinetic (PBPK) modeling of perc accurately predicted oxidative metabolites but suggested the need to better characterize glutathione (GSH) conjugation as well as toxicokinetic uncertainty and variability.

Objectives—We updated the previously published “harmonized” perc PBPK model in mice to better characterize GSH conjugation metabolism as well as the uncertainty and variability of perc toxicokinetics.

Methods—The updated PBPK model includes expanded models for perc and its oxidative metabolite trichloroacetic acid (TCA), and physiologically-based sub-models for conjugative metabolites. Previously compiled mouse kinetic data in B6C3F1 and Swiss-Webster mice were augmented to include data from a recent study in male C57BL/6J mice that measured perc and metabolites in serum and multiple tissues. Hierarchical Bayesian population analysis using Markov chain Monte Carlo was conducted to characterize uncertainty and inter-strain variability in perc metabolism.

Results—The updated model fit the data as well or better than the previously published “harmonized” PBPK model. Tissue dosimetry for both oxidative and conjugative metabolites was successfully predicted across the three strains of mice, with estimated residuals errors of 2-fold for majority of data. Inter-strain variability across three strains was evident for oxidative metabolism; GSH conjugation data were only available for one strain.

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Supplementary Materials

The supplementary materials contain the model equations, a model code, and tables of model inputs and outputs and figures of convergence.

Conflict of Interest statement: The authors declare that there are no conflicts of interest.

Conclusions—This updated PBPK model fills a critical data gap in quantitative risk assessment by predicting the internal dosimetry of perc and its oxidative and GSH conjugation metabolites and lays the groundwork for future studies to better characterize toxicokinetic variability.

Graphical Abstract

Keywords

Bayesian analysis; Glutathione conjugation; Oxidative metabolism; Tetrachloroethylene; Physiologically based pharmacokinetic (PBPK) model; Risk assessment

Introduction

Perchloroethylene (tetrachloroethylene or perc) is a chemical that is widely used in commercial and industrial sectors for dry cleaning and metal degreasing. Perc is a ubiquitous environmental contaminant, found commonly in ambient and urban air, hazardous waste sites, and surface and ground water (IARC, 2014). Human exposure to perc is assumed to be mostly through inhalation and ingestion of contaminated water. Data from the National Health and Nutrition Examination Survey (NHANES) from 2005–2006 observed perc in human blood at 0.034 ± 0.79 ng/ml (mean $\pm$ S.D (CDC, 2011)). The United States Environmental Protection Agency (U.S. EPA) identified perc as one of top ten high-priority substances for risk evaluation as part of its Existing Chemicals Management Program under the Toxic Substances Control Act (TSCA), as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (U.S. EPA, 2017).

The cancer and noncancer hazards of perc have been evaluated by IARC (IARC, 2014), US EPA (USEPA, 2011a), and Office of Environmental Health Hazard Assessment (OEHHHA). Perc is classified by IARC as a probable human carcinogen [Group 2A, (IARC, 2014)], based on sufficient evidence for carcinogenesis from chronic studies in rodents and limited epidemiologic data supporting an association between perc exposure and cancer of the bladder, esophagus, kidney, and cervix. Perc exposure is also associated with noncancer toxicity in a number of tissues (USEPA, 2011b). Neurotoxicity is the most sensitive pathway

of noncancer toxicity in humans associated with perc exposure and is well correlated with parent compound concentrations at the target site (Bushnell *et al.*, 2005; USEPA, 2011a).

Investigation of the toxicokinetics of perc and its metabolites in multiple tissues is essential in understanding of perc-associated toxicity phenotypes, because delivered dose of both perc and its specific metabolites may contribute to target organ-specific toxicity (Lash and Parker, 2001; Guyton *et al.*, 2014; Cichocki *et al.*, 2016; Cichocki *et al.*, 2017b). Perc metabolism involves both cytochrome P450s (CYPs) and GSH S-transferases (GSTs) (Cichocki *et al.*, 2017b). Oxidative metabolism of perc leading to formation TCA has been presumed to be attributed to CYP2E1 substrates based on analogy to trichloroethylene (TCE), although other enzymes (including other P450s) may be involved (Lash and Parker, 2001; Cichocki *et al.*, 2016). Although the liver is the main contributor to the oxidation of perc, extrahepatic oxidative metabolism may occur, such as in the kidneys and lungs (Lash and Parker, 2001; Lash *et al.*, 2001; Cichocki *et al.*, 2016). Dichloroacetic acid (DCA) is formed locally in the kidney as a result of the GSH conjugation pathway. Toxicokinetic data on perc have been limited, making quantitative estimates of the internal dose uncertain, especially for the GSH conjugation pathway.

The organ-specific adverse effects associated with exposure to perc are consequences of tissue-specific metabolism (Lash and Parker, 2001; Lash *et al.*, 2014; Cichocki *et al.*, 2016). For instance, carcinogenicity of perc in rat kidney has been associated with the formation of the GSH conjugate (trichlorovinyl glutathione (TCVG)) in the liver followed by conversion to the cysteine conjugate (trichlorovinyl cysteine (TCVC)) in the kidney, where it is activated by β -lyase to form a highly reactive metabolite, dichlorothioketene (Dekant *et al.*, 1986; Green *et al.*, 1990; Pähler *et al.*, 1999). Attempts to quantify interspecies variability in the extent of GSH conjugation to explain species differences in carcinogenicity, mostly based on *in vitro* data, are inconsistent (Green *et al.*, 1990; Dekant *et al.*, 1998; Lash *et al.*, 1998). Because the enzymes involved in the bioactivation pathway are also present in other tissues, there exists a potential for formation of these reactive metabolites at sites other than the kidney, such as the brain (USEPA, 2011a).

Additionally, numerous recent hazard and risk assessments pointed to a critical need for better characterization of toxicokinetics, toxicodynamics, and population variability of perc in order to improve public health protection (NRC, 2010; USEPA, 2011b; Guha *et al.*, 2012). Various toxic endpoints associated with perc and its metabolites were observed in rodents, which need to be extrapolated to humans in purpose of health risk assessment. Previous attempts at characterizing toxicokinetic variability of perc (Bois *et al.*, 1996; Gelman *et al.*, 1996; Covington *et al.*, 2007; Chiu and Ginsberg, 2011) were hindered by inadequate experimental data, particularly for GSH conjugation and for tissue dosimetry, leading to substantial uncertainty in the ultimate risk assessments.

In this study, we updated the “harmonized” perc PBPK model in mice to characterize the uncertainty and variability of perc, taking advantage of newly available internal dosimetry data on perc metabolites, particularly from GSH conjugation (Cichocki *et al.*, 2017a). Our primary goal was to expand the model to simulate the internal dosimetry of metabolites from GSH conjugation, with a secondary goal of characterizing interstrain variability. In order to

accomplish these goals, we updated the previously published “harmonized” perc PBPK model (Chiu and Ginsberg, 2011), including: (1) refinement of PBPK model structure to characterize the internal kinetics of perc GSH conjugation, using the independent physiologically based sub-models for conjugation metabolites, TCVG, TCVC, and mercapturate N-acetyl trichlorovinyl cysteine (NAcTCVC), in addition to adding a brain compartment to the parent perc and the oxidative metabolite, TCA, submodels; (2) addition of a hierarchical Bayesian population statistical model to quantify the extent of interstrain variability in perc toxicokinetics based on the available data of three different strains of mice; (3) estimation of the posterior distribution for new and key model and population parameters using updated prior distributions and Markov chain Monte Carlo (MCMC) simulations.

Methods

Perc PBPK model updates

Updated PBPK model structure—The updated model structure is depicted in Fig. 1. The major updates involve the additions of (a) a brain compartment to the parent perc and TCA submodels, (b) a kidney compartment in the TCA submodel, and (c) physiological models, incorporated blood, liver, body and kidney compartments, for conjugative metabolites, TCVG, TCVC, and NAcTCVC. The model was described by the integration of ordinary differential equations and algebraic definitions. Complete mathematical description is provided in Supplemental Materials.

PBPK model parameters and baseline values—Most PBPK model parameters and their baseline values and sources, including physiological measurements (e.g., volumes and flows) and measurements from in vitro studies (e.g., partition coefficients, binding coefficients, and metabolism and clearance rates), were previously described by Chiu and Ginsberg (2011). Model parameters, their baseline and scaling values, and their sources are summarized below, focusing on new elements of the model, with detailed descriptions in Supplemental Material, Table S-1.

Baseline values for physiological parameters: Baseline values for organ volumes and blood flows are adjusted to keep mass balance of the body after the addition of tissues and physiological submodels into a full model. As described in previous model, many of the model parameters added in this updated model are “scaled” by body weight. Parameters are further scaled to the baseline point-estimates where available, for example, to obtain the actual tissue volume in liters. Any deviation from this initial point estimate is represented by a model parameter, which is obtained by multiplying this baseline value by a separate “scaling” parameter that has a value of 1 if there is no deviation from the point estimate. These model parameters and baseline values of point estimates are specified in the structural PBPK model.

Baseline values for chemical-specific distribution parameters: Baseline values of chemical-specific distribution parameters for tissues added in the updated model, such as a brain/blood partition coefficient (PC) of perc and brain/blood and kidney/blood PCs of TCA

were taken from the same studies used previously (Gargas *et al.*, 1989; Gearhart *et al.*, 1993). Because of the lack of available *in vitro* data on the chemical-specific distribution parameters for perc conjugates, baseline values for these parameters were, in a non-informative manner, set to the assumed values equal to 1, and were later estimated using data on the internal kinetics of the conjugates. Remaining distribution parameters were reviewed and described in the previous model, and used in this model with no changes.

Baseline values for subsequent metabolism of TCVG and excretion of

conjugates: Although available data on baseline values for rates of perc GSH conjugation to produce TCVG were explicitly described in previous model, very limited data are available on subsequent metabolic pathways to yield TCVC from TCVG and its subsequent products. Available data suggest that TCVG is further processed to form the cysteine conjugate TCVC in liver and kidney. TCVC further undergoes either acetylation to its mercapturate (NAcTCVC) in both tissues or bioactivation to yield DCA by renal beta-lyase (Dekant *et al.*, 1988; Green *et al.*, 1990; Völkel *et al.*, 1998). Because the available data could not separately identify contributions from the liver and kidney, we attributed all subsequent processing after formation of TCVG to the kidney. Due to no available experimental data on baseline values, a “default” value of 1 was used as a baseline for metabolic parameters for those pathways, assuming a linear pathway. Urinary excretion was modeled only for NAcTCVC and a baseline parameter was assigned as clearance based on glomerular filtration rate from (Lin, 1995).

Baseline and scaling values for remaining parameters were taken from the previous models (Table S-1). All the parameters without informative baseline values need to be estimated by Markov Chain Monte Carlo sampling using a Bayesian approach.

Parameter selection for sampling—Sensitivity analyses reported by the (USEPA, 2011a) showed that the PBPK model calibration was not sensitive to many parameters. Therefore, most of the physiological parameters and partition coefficients from previous model, were fixed at their baseline and scaling (sampled) values. Values fixed for scaling parameters used in previous perc or TCE models, and assigned as previously reported posterior means (Chiu *et al.*, 2009; Chiu and Ginsberg, 2011; Chiu *et al.*, 2014) (see Table S-1). All new parameters were selected for estimation via sampling, in addition to the ventilation–perfusion ratio, absorption and all perc metabolism parameters as were the cases in previous model (Table A-1).

In vivo toxicokinetic data—Fig. 2 depicts the *in vivo* data used in our analysis. In sum, data from three different mouse strains, including hybrid, B6C3F1, outbred, NIH Swiss Webster (SW) (albino), and the most widely used as a general purpose inbred strain, C57BL/6J, were available to characterize inter-strain variability. In vivo data for B6C3F1 and SW strains were reviewed thoroughly and used in the previous perc PBPK model (Chiu and Ginsberg, 2011). Studies in B6C3F1 mice included inhalation, closed chamber and oil gavage administration at a range of exposure levels, and reported perc and its oxidative metabolite, TCA, in blood (Gargas, 1988; Odum *et al.*, 1988; Gearhart *et al.*, 1993; Reitz *et al.*, 1996). Only data using aqueous gavage of perc was available for the SW strain, reporting concentrations of perc in blood, liver and kidney, and its oxidative metabolite, TCA, in blood

and liver (Philip *et al.*, 2007). The most recently published data (Cichocki *et al.*, 2017a) reported TCVG, TCVC and NAcTCVC in serum and liver in male C57BL/6J mice, in addition to perc and TCA, after aqueous gavage of perc. Measurements of perc and its metabolites are also made in kidney of these same animals, reported in Supplementary Materials.

Bayesian approach to calibration of PBPK model parameters

A hierarchical Bayesian population approach was used to estimate model parameters and characterize the uncertainty and inter-strain variability. Detailed methods were described previously by (Chiu *et al.*, 2009; Chiu *et al.*, 2014), with the statistical model for inter-strain variability shown in Fig 3.

Prior distributions for model and population parameters—Parameter prior distributions are summarized below, and described in detail in Supplemental Material (Table S-2).

Uncertainty distributions for the population mean of the PBPK model parameters involve informative (for parameters with independent data) and non-informative (for parameters without independent data) prior distributions. Informative prior distributions for the population mean were centered on the baseline values from the previously published multi-strain mouse PBPK model of TCE (Chiu *et al.*, 2014). The population means of new parameters of tissue/blood PCs of perc, TCA and all conjugates for all newly added tissues, were assigned a truncated normal prior distribution. Prior uncertainty distributions for parameters of PCs for perc and TCA were informative, also centered on the values of posterior means from (Chiu *et al.*, 2014). For all other parameters without independent data (e.g., conjugate PCs, absorption, metabolism/ clearance of perc metabolites), diffuse, log-uniform priors were set with upper and lower bounds separated by at least 10^4 so as to minimize potential bias.

The uncertainties in the population variability for parameters for which there were no direct data were assigned by default a coefficient of uncertainty (CU) in the population variance of 2. Generally, for parameters for which there were no direct data, a central estimate for the population coefficient of variation (CV) of 0.5 was assumed. These are similar to values used in the previous Bayesian analyses (Chiu *et al.*, 2014).

The likelihood of the data given the predictions was assumed to be lognormal, which requires specification of the variance of the “residual error” estimates (i.e., the assumed error distribution between data and model predictions). All the studies were assumed to have the same residual error. The log-uniform prior distribution was used for the variance of each of the corresponding residual error. The bounds on the log-uniform distribution were 0.01 and 3.3 for all measurements, corresponding to geometric standard deviations bounded by 1.11 and 6.15 as used in previous models (Chiu *et al.*, 2009; Chiu *et al.*, 2014).

Non-detects of perc, TCVG, TCVC and NAcTCVC in blood and liver (Cichocki *et al.*, 2017a) and kidney (Supplementary Materials) were also included in the analysis, as it was found that these data were needed to place constraints on the clearance rate of the chemicals

from blood and tissues. The limits of detection (LODs) reported in (Luo *et al.*, 2017) are provided in Supplemental Material, Table S-3. It was assumed, as is standard in analytical chemistry, that the detection limit represents a response from a blank sample at 3 standard deviations. Because detector responses near the detection limit are generally normally distributed, in the ideal case, the likelihood for observing a non-detection given a model-predicted value of y_p is equal to $P(ND|y_p) = \Phi(3*[1-y_p/LD])$, where $\Phi(y)$ is the cumulative standard normal distribution as previously described (Chiu *et al.*, 2009). However, this approach does not account for model fit uncertainty, which is a part of the lognormal likelihood used for the rest of the data. Therefore, a student's t distribution with degree of freedom of 5 was used to provide more “robust” estimate of the uncertainty around detection limits (Chiu *et al.*, 2017a).

Estimation of posterior distributions—MCMC was utilized to obtain samples from the posterior distribution. Each iteration of the Markov chain generates a vector of parameters sampled from the parameter distributions and values of the posterior likelihood associated with that vector of parameters. The MCMC simulation, thereby, generates posterior parameter values at the population level, and parameter values for the strain of experiments. Posterior model predictions were made following MCMC calibration using the strain-specific posterior parameter values as well as posterior predictions for a “random strain” accounting for both interstrain variability and parameter uncertainty (Hack *et al.*, 2006). Four independent MCMC chains of up to 100,000 iterations each were run on MCSim software version 5.6.5. The MCSim model code is provided in Supplementary Materials.

Model evaluation

Evaluation of convergence—Convergence of the Markov chains to the posterior distribution was monitored using analysis of variance as described by (Gelman *et al.*, 1996). The Gelman & Rubin shrink factors (potential scale reduction factor, R), a ratio of an upper bound and a lower bound of the variance in the target distribution, and Brooks-Gelman multivariate shrink factor (MPSRF) are used to assess whether the independent MCMC chains have converged to a common distribution. As multiple, independent chains move closer together toward the same distribution, the ratio declines to unity. A convergence diagnostic “R” of 1.2 or less has been proposed as a criterion for acceptable convergence (Gelman *et al.*, 2004). A visual inspection for dependency of posterior parameter distributions using cross correlations and convergence of the chains using traces and probability density functions for each posterior parameter distributions was performed as an additional diagnostic. Figures of cross correlations, traces and probability density functions of posterior uncertainty distributions for each calibrated parameter population mean of the last-half of 100,000 iterations of four Markov chains are in Supplementary materials, Fig. S1 – S3.

Evaluation of posterior parameter distributions—Posterior distributions of the population parameters need to be checked as to whether they appear reasonable given the prior distributions. Inconsistency between the prior and posterior distributions may indicate the insufficient broad priors (i.e., overconfidence in their specification), a misspecification of

the model structure (e.g., leading to pathological parameter estimates), or an error in the data. The values of population geometric mean and standard deviation of parameter uncertainty distributions were provided in Supplementary material, Table S-4, as a qualitative evaluation.

Comparison of model predictions with data for verification of model fit—The posterior parameter distributions at both population (“random strain”) and strain-specific levels were used to generate model predictions of internal toxicokinetics of perc and its metabolites. At a population level, samples of the population parameters (means and variances) were used, and “random” strains were sampled from appropriate distributions using these population means and variances. Thus, the predictions were only conditioned on the population-level parameter distributions, representing a distribution across all the strains, and not on the specific predictions for that dataset. These strains then represent the predicted population distribution, incorporating variability in the population as well as uncertainty in the population means and variances and therefore compares predictions for the overall mouse population. In contrast to population level, because, strain-specific prediction was sampled from posterior parameter distribution of the specific strain, selectively, that prediction represents all datasets of the specific strain.

Residual error estimated for each in vivo measurements provides some quantitative measure of the degree to which there were deviations due to intra-study variability, interindividual variability, and measurement and model errors, including any difficulties fitting multiple dose levels in the same study using the same model parameters. Estimated residual error with geometric standard deviation (GSD) of more than 3-fold was assumed to indicate that the model prediction does not fit the in vivo measurement adequately.

Results

Convergence analysis

Detailed convergence analysis and comparisons are provided in Supplemental Materials, Fig. S-1–3. Initially, four chains were run to 50,000 iterations each to check convergence. As is standard, the first half of the iterations for each chain were discarded as “burn-in” iterations, i.e., iterations for which the simulation had not yet converged, and the remainders used for inferences. For the remaining iterations, the convergence diagnostic potential scale reduction factors (R) was <1.14 for all population parameters. All the initial iterations were treated as “burn-in” iterations, and each chain was subsequently run for an additional 50,000 iterations to obtain reduction in potential scale reduction factors. After these iterations, all values of R were <1.04 for all population parameters, indicating convergence. Traces of four chains were well mixed, which were hardly distinguished by visual inspection for all parameters.

Evaluation of posterior parameter distributions

Comparison of the prior and posterior distributions of population mean and geometric standard deviations of “sampled” parameters is shown in Fig. 4. Population mean of

parameter distributions were also compared with maximum likelihood estimate (MLE) estimation of the previous model.

Posterior distributions of population parameters—As shown in Fig. 4, there is a general consistency between the posterior distributions of population parameters in the current model and the corresponding priors as well as, MLE estimates from Chiu and Ginsberg (2011). In only few cases, the prior and posterior distributions did not completely overlap, but in these cases, in particular, the body/blood PCs of conjugates, the prior distributions themselves were not informed at baseline. The low PCs for these conjugates suggest a low volume of distribution, which is not necessarily unrealistic for these compounds. Prior and posterior distributions of population variability largely overlapped. Overall, the Bayesian analysis of the updated PBPK model and data exhibited no major inconsistencies in prior and posterior parameter distributions. Therefore, there were no indications based on this evaluation of prior and posterior distributions either that prior distributions were overly restrictive or that model specification errors led to pathological parameter estimates.

Posterior distributions of strain-specific parameters—Posterior distributions of parameters were also sampled for each mouse strain in the framework of the population distributions. These strain-specific values represent the sets of parameters for each strain that produce the best agreement with all experimental data in the strain. In Fig. 4c, the posterior parameter uncertainty distributions were compared across strains. Variability of parameter distributions of oxidative metabolism across strains is evident, in particular, for the B6C3F1 strain, whose parameter values substantially differ from those for other two strains. The population variability across strains for these parameters is evident in the analysis of population geometric standard deviation, in which posterior distributions for these parameters were shifted to larger values compared to the prior distributions (Fig. 4b).

Comparison of model predictions with data

Global evaluation of model fit—A global evaluation of fits between model predictions and *in vivo* data for specific strains of mouse are shown in Fig. 5a. The GSD for estimated residual error in each *in vivo* measurement is illustrated in Fig. 5b and values were presented in Supplemental Table S-5.

Overall, model predictions for most toxicokinetic data fit *in vivo* measurements, except those for the liver concentration of TCVC, which has the largest residual error with median GSD of 5.0, and perc in kidney (median GSD=3.6), both in the C57BL/6J strain (from Cichocki et al., 2017). Model prediction for the rate of perc exhalation for data of B6C3F1 from Reitz et al. (1996) and perc concentration in liver for data of C57BL/6J strain were modest with median GSD of the residual errors of 2.9 and 2.5, respectively. All remaining *in vivo* measurements had smaller residual errors. Overall quality of our model fit was in good agreement with high goodness of fit ($R^2 = 0.95$).

Model predictions of toxicokinetic data—The representative comparisons of probabilistic population and strain-specific predictions (median and 97.5% CI) for internal

toxicokinetics of perc and its metabolites, and data from three different strains of male mice (oral gavage) are shown in Fig. 6–8. All other comparisons of model predictions of internal toxicokinetics of perc and TCA and data from inhalation studies in B6C3F1 strain of mice are shown in Supplemental Material, SI-Fig. 4–7. The current model predictions for perc and TCA were also compared with MLE results of previous model.

The current model predictions of the internal toxicokinetics of perc at both population and strain-specific levels are consistent with previous model predictions and *in vivo* data on perc and TCA in blood in male B6C3F1 mice at three different single doses of oil based oral gavage from Gearhart et al. (1993) (Fig. 6). The updated model at population level underestimated blood perc, especially at low dose (100 mg/kg) group, while it a bit overestimated blood TCA in the same dose group. However, data points are in the range of confidence intervals (CI). Model prediction fits all datapoints for blood TCA at high dose groups. In contrast to population prediction, strain-specific model prediction even better fits datapoints of all dose groups for especially perc in B6C3F1 mice. For *in vivo* data on perc and TCA in blood and tissues in male SW mice at three different single doses of aqueous based oral gavage from Philip et al. (2007), population based model predicted datapoints accurately, except for TCA in blood and liver (but in the range of CI in liver) which was a bit underestimated at low dose (Fig. 7). Rather, strain-specific model prediction fits datapoints of perc and TCA in blood and tissues at most accurately for all dose groups, in fact, it was failed with population model for TCA in blood in low dose group.

For data on perc and its oxidative metabolite, TCA, in blood and tissues in male C57BL/6J mice exposed to 300 mg/kg perc by aqueous based oral gavage from (Cichocki *et al.*, 2017a), the previous MLE predictions were in the range of uncertainty distribution of the updated model, although our model predicted higher elimination (Fig. 8a). This high elimination predicted in the updated model was consistent with datapoints of perc in blood and fat, and TCA in all compartments. Although the updated model was consistent with datapoints of kidney perc at initial timepoints, it was lower after 24 hours, though, the datapoints are still in the uncertainty range of prediction. The current model fits the datapoints for perc and TCA in other tissues and blood adequately or better than the previously published model. Strain-specific model prediction fits datapoints of TCA even more accurately.

The updated model successfully predicted the internal toxicokinetics of perc GSH conjugation products in different tissues, capturing all conjugates, including TCVG, TCVC and NAcTCVC in blood, liver and kidney with high accuracy (Fig. 8b). A very small portion of perc appears to be conjugated to TCVG in blood, while TCA appears to be accumulated in blood plasma about 9-fold higher than blood perc at one hour post dose, then the accumulation of TCA increased by 1.6-fold after 4 hours, while perc and conjugates decreased exponentially over time. Our updated model predicted this toxicokinetic profile accurately. The only discrepancy appears in liver TCVC, in which central tendency prediction underestimated the data, possibly due to regeneration of TCVC from NAcTCVC via acylase in the liver, which as not included in the model. However, the data were still well within the confidence interval of the predictions. Model prediction of disappearance of

conjugates from blood and tissues are consistent with the observations, which was characterized by chemical and compartment specific LODs.

Discussion

Reduced uncertainty in perc GSH conjugation in the mouse

There was a long debate between PBPK model-based analyses in predicting high versus low amounts of perc metabolism in humans based on the estimation of total metabolism, which mostly relied on oxidative metabolism. Chiu and Ginsberg (2011) suggested that this discrepancy may be due to uncertainty in the degree of GSH conjugation. In fact, there is some long-standing controversy surrounding the importance of the GSH conjugation pathway with regard to perc metabolism in humans. As GSH conjugation of perc was originally demonstrated only in rodents, it was thought to be not operative in humans (Green *et al.*, 1990; U.S.EPA, 2011a). More recent data clearly demonstrate the existence of this pathway in humans (Birner *et al.*, 1996; Völkel *et al.*, 1998; Schreiber *et al.*, 2002), but, with discrepancies regarding reported metabolism rates (Green *et al.*, 1990; Dekant *et al.*, 1998; Lash *et al.*, 1998; Lash and Parker, 2001). Perc clearance and metabolism are markedly slower in humans than in rodents due to the limited capacity to metabolize perc (Reitz *et al.*, 1996). However, findings concerning TCVG production could not rule out the importance of renal metabolism in susceptibility for humans as for rodents with regard to renal toxicity (Lash *et al.*, 1998). Moreover, even if the conjugative pathway is a minor route quantitatively, it is toxicologically significant because it yields relatively potent toxic metabolites (Lash and Parker, 2001).

The previous “harmonized” PBPK model-based analysis provided insight into addressing the controversy in perc toxicokinetics through reduction in uncertainty in oxidative metabolism across species (Chiu and Ginsberg, 2011). However, the previous study revealed uncertainty, which was substantially attributed to GSH conjugation after separation of total metabolism into these two pathways, as recommended by NRC (2010). There is also high uncertainty in GSH conjugation across species, in essence, characterized by its estimation based on either the limited available *in vivo* data on low portion of metabolism of perc (0–20%) by (Chiu *et al.*, 2007) and a fraction of urinary excretion of NAcTCVC by (Völkel *et al.*, 1998), or *in vitro* data on perc GSH conjugation. However, NAcTCVC only provides an indirect measure of the GSH conjugation flux due to its being a product of multiple subsequent biotransformations after perc GSH conjugation. Cichocki *et al.* (2017a) suggested that a common method (gas chromatography) to analyze perc and TCA, was not suitable for the quantitation of conjugative metabolites because of their low abundance and quick clearance from tissue (Cichocki *et al.*, 2017a). The recent development of a LC-MS/MS method has enabled investigation of toxicokinetics of a number of conjugation metabolites simultaneously, in serum and different tissues of mice (Luo *et al.*, 2017).

The updated PBPK model reported herein, therefore, reduced uncertainty in perc GSH conjugation, and provides highly informative predictions of the internal toxicokinetics of GSH conjugation in male mice through calibration and validation using *in vivo* data on consecutive products of this pathway, including TCVG, TCVC, and NAcTCVC, in blood, liver, and kidney (Cichocki *et al.*, 2017a). From a mass-balance perspective, TCVG, TCVC,

and NAcTCVC are not significant contributors to perc metabolism in the mouse, as their peak levels in liver were less than 0.05% of TCA levels and they were quickly cleared from tissues and serum. Whether this is also true in humans remains to be determined, particularly given the potential for inter-individual variability.

Starting point for characterization of inter-individual variability in perc toxicokinetics

One of the biggest challenges in risk assessment, as identified by the NRC (2009), is addressing the human toxicokinetic variability for quantitative dose extrapolation across routes of exposure, and across and within species in dose–response assessment (NRC, 2009; Zeise *et al.*, 2013). The current approaches to risk assessment are largely limited to applying default uncertainty factors to account for uncertainty associated with within-species variability (Stedeford *et al.*, 2007). There is a crucial need for the development of approaches to quantitate the impact of interindividual variability in human health risk from chemical exposures (Zeise *et al.*, 2013). Because it is unlikely that sufficient additional human toxicokinetic data will become available in the future, mouse population modeling as a surrogate to characterize and quantitate the extent of human variability may provide an avenue to address this challenge (Rusyn *et al.*, 2010; Chiu *et al.*, 2014; Chiu and Rusyn, 2018). Considerable interstrain variability in metabolism of structurally similar chemical TCE have been quantified using multistrain panels of inbred mice (Bradford *et al.*, 2011; Venkatratnam *et al.*, 2017). The data from genetically diverse mouse strains have been successfully used in population PBPK modeling for quantitative estimates of human variability in TCE metabolism for risk assessment by (Chiu *et al.*, 2014).

The metabolism of perc is complex, with multiple metabolizing tissues and interorgan transport, and, except for neurological effects, toxicity is largely attributed to metabolites rather than the parent compound. Sex- and species-dependent differences and potential contributors to interindividual variability are also identified in perc metabolism (Chiu *et al.*, 2007). Potential strain-dependent differences in metabolizing enzymes involved in perc metabolism are now known to exist among rodents. Differences in enzyme activity may lead to variations among individuals in their sensitivity to perc toxicity (USEPA, 2011a).

Our updated model utilized the available *in vivo* toxicokinetic data on perc and TCA in blood and multiple tissues in different mouse strains. Our results for the internal dosimetry of parent perc and TCA in blood/plasma across strains indicates inter-strain variability, especially between B6C3F1 and the other the two strains. With the use of Bayesian approach in analysis of model parameters, this interstrain variability is evident in the analysis of posterior distributions of strain-specific model parameters, which revealed high variability in perc oxidation.

The limited availability of *in vivo* data on perc toxicokinetics led to several potential limitations of our model. Given there were only three available strains, there is substantial uncertainty in the degree of inter-strain variability. Additionally, oxidative metabolism was clearly variable across even these three strains, so median predictions of population-generated “random strain” parameter distributions for internal toxicokinetics of perc were not necessarily representative of each individual strain. In particular, median “random strain” predictions of TCA in blood at times rather underestimated datapoints for SW (in lower dose

group) and C57BL/6J strains, while accurately predicting those for B6C3F1. Notably, strain-specific predictions using strain-specific parameter distributions were more accurate. Interstrain variability in GSH conjugation was not able to be characterized in the current model due to the limited data availability, which were restricted only in one strain. Because data in all three strains were from same age of male mice, other potential factors of inter-individual variability, such as age, sex and other specific life stage differences, were not considered in this model.

The current model thus lays the groundwork for future studies to better characterize perc toxicokinetic variability. Specifically, further work is needed to expand the current population model to quantify interstrain variability in metabolism through oxidation and glutathione conjugation using a large number of strains of rodent data to understand interindividual human variability in health risk assessment (Chiu and Rusyn, 2018). Over the past decade, the utility of a mouse population-based approaches to *in vivo* toxicity testing has been demonstrated for a number of toxic agents in science and the practice of investigative pharmacology and toxicology (Rusyn *et al.*, 2010; Chiu *et al.*, 2014; Harrill and McAllister, 2017). For instance, we recently reported significant interindividual variability among strains in toxicokinetics of perc and TCA across multiple tissues using a genetically diverse mouse population of 45 Collaborative Cross (CC) mouse strains (Cichocki *et al.*, 2017b). Measurements of conjugative metabolites were also made in this same mouse population (Luo *et al.*, in preparation).

In sum, we have updated our previously published “harmonized” PBPK model for perc toxicokinetic in mice to include new data on both oxidation and GSH conjugation of perc, including internal kinetics of the parent perc and its oxidative metabolite, TCA, and full internal kinetics of conjugation metabolites, TCVG, TCVC and NAcTCVC. The updated PBPK model successfully predicted perc GSH conjugation and the internal toxicokinetics of conjugative metabolites, in addition to perc and its oxidative pathway. Our updated model, thus, fills the critical need for predictions of the internal dosimetry of perc oxidative and GSH conjugation pathways for use in dose-response analyses, especially for perc-induced health endpoints that may be associated with GSH conjugation metabolites, for which previous models were highly uncertain due to their need to indirectly estimate the GSH conjugation flux. Finally, the current Bayesian population analysis of our PBPK model suggests the potential for variability of perc and metabolite TCA toxicokinetics across different strains of mice, while also providing a foundation for incorporating multi-strain variability data on both oxidation and GSH conjugation in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CC	Collaborative Cross
CDC	Centers for Disease Control and Prevention
CU	coefficient of uncertainty
CV	coefficient of variation
CYPs	cytochrome P450s
DCA	Dichloroacetic acid
CI	confidence interval
GSD	geometric standard deviation
GSH	glutathione
GSTs	GSH S-transferases
IA	intra-arterial injection
IARC	International Agency for Research on Cancer
IV	intravenous injection
LC-MS/MS	Liquid Chromatography-tandem mass spectrometry
LODs	limits of detection
MCMC	Markov chain Monte Carlo
MLE	maximum likelihood estimate
MPSRF	Brooks-Gelman multivariate shrink factor
NAcTCVC	mercapturate N-acetyl trichlorovinyl cysteine
ND	non-detects
NHANES	National Health and Nutrition Examination Survey
NIH	National Institute of Health
NRC	National Research Council
OEHHA	Office of Environmental Health Hazard Assessment
PBPK	physiologically-based pharmacokinetic
PC	partition coefficient
Perc	perchloroethylene
PV	portal venous injection

SW	Swiss-Webster
TCA	trichloroacetic acid
TCE	trichloroethylene
TCVC	trichlorovinyl cysteine
TCVG	trichlorovinyl glutathione
TK	toxicokinetics
TSCA	Toxic Substances Control Act
U.S. EPA	The United States Environmental Protection Agency

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Highlights

- Uncertainty in perchloroethylene (perc) toxicokinetics (TK) hampers risk assessment
- An updated physiologically-based (PBPK) model was developed to address this gap
- This is the first model to predict perc glutathione conjugation in mice
- Modeling results show interstrain variability in mouse oxidative metabolism of perc
- This model reduces perc TK uncertainty and allows for characterizing TK variability

Fig. 1. Schematic presentation of the mouse PBPK model structure for perc and its metabolites after updated from Chiu and Ginsberg (2011)

Abbreviations: IA, intra-arterial injection; IV, intravenous injection; PV, portal venous injection. A full PBPK model incorporates whole-body, flow-limited submodels of parent perc and oxidative metabolites, TCA, and GSH conjugation metabolites including, TCVG, TCVC and NAcTCVC, respectively. Perc submodel consists of gas respiratory exchange, absorption from oral gavage through gut and metabolizing, including liver, respiratory tract and kidney, and nonmetabolizing tissues, including blood, brain, fat, and the remaining were lumped to slowly and rapidly perfused tissues. TCA submodel consists of IV injection and an oral absorption, urinary excretion through kidney and liver as a metabolizing tissue and blood, plasma as a protein binding tissue, brain as a nonmetabolizing tissue, and remaining tissues were lumped to a body compartment. Based on the available supporting data, separate physiologically-based sub-models were added for conjugative metabolites, including GSH conjugate – TCVG, cysteine conjugate – TCVC, and mercapturate – NAcTCVC. Three respective conjugation submodels have the same structure, consisting of

liver, kidney, blood and remaining tissues lumped to body compartment. TCVG produced in liver and kidney is converted to TCVC in the tissues. TCVC undergoes either subsequent mercapturate to form NAcTCVC in either tissue or beta-lyase-mediated bioactivation in the kidney. NAcTCVC, the end product of conjugation in liver and kidney ultimately is excreted through urine.

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Fig. 2. *In vivo* toxicokinetic data used for analysis

Fig. 3. Hierarchical population statistical model for PBPK model parameter uncertainty and variability in mice

Square nodes denote fixed or observed quantities, circle nodes represent uncertain or unobserved quantities, and an inverted triangle denotes a nonlinear model output. Solid arrows denote a stochastic relationship represented by a conditional distribution [$A \rightarrow B$ means $B \sim P(B|A)$], dashed arrows represent a functional relationship [$B=f(A)$]. The population consists of: strain i , each of which undergo one or more experiments j with exposure parameters E_{ij} with data y_{ij} collected at times t_{ij} (see (Gelman *et al.*, 1996)). Mice within each strain are presumed to be “identical,” with the same PBPK model parameters. The PBPK model depends on measured covariates ϕ_i (e.g., body weight) and unobserved

model parameters θ_i (e.g., $V_{\max}$) drawn from a population with the mean M_θ and variance V_θ , each of which is uncertain and has a prior distribution assigned to it. The PBPK model produces outputs f_{ij} for comparison with the data y_{ij} . The difference between them (“residual error”) has variance σ^2 , with a prior distribution $\Pr_\sigma$. All strains and studies are assigned the same “residual” error variance.

Fig. 4. Densities of prior and posterior parameter uncertainty distributions of (A) population mean and (B) log-transformed standard deviations for variability, and (C) strain-specific parameter posterior distributions

In plots (A) and (B), prior distributions are colored in green and posterior distributions in pink. The distributions of parameter population mean in the current model were also compared with MLE (black points) predicted from previous model (Chiu & Ginsberg, 2011). In plot (C), pink = B6C3F1 strain, cyan = Swiss Webster strain, and purple = C57BL/6J strain.

Fig. 5. Global evaluation of model fit, compared toxicokinetic data (x-axis) and PBPK model predictions (y-axis) (A), and posterior distributions of geometric standard deviation (GSD) of estimated residual error (median (2.5%, 97.5%)) (B)

In plot (A), data points are shown for perc in blue, TCA in pink and conjugates in green, and for mouse strains, B6C3F1 (round), C57BL/6J (triangle), and SW (rectangular). The solid black diagonal line indicates where data and predictions are equal. In plot (B), posterior distributions of estimated residual error (pink) in the current model update was plotted against MLE of those in the previous model prediction (black dots).

Fig. 6.

Comparison of the current PBPK model predictions for both a population-generated “random strain” (solid black line represents the median and dashed black lines represents 2.5% and 97.5% CI) and strain-specific parameter posterior distributions (solid blue line represents the median and dashed blue lines represents 2.5% and 97.5% CI), and data for perc and TCA in blood in male B6C3F1 mice (red dots) (data from Gearhart et al., 1993) at three different single doses of oil based oral gavage, including 100 (A), 536 (B), 1072 (C) mg/kg of perc. The current model predictions were also compared with the previous model prediction (solid gray line) (Chiu & Ginsberg, 2011).

Fig. 7.

Comparison of the current PBPK model predictions for both a population-generated “random strain” (solid black line represents the median and dashed black lines represents 2.5% and 97.5% CI) and strain-specific parameter posterior distributions (solid blue line represents the median and dashed blue lines represents 2.5% and 97.5% CI), and data for perc in blood, liver and kidney and TCA in plasma and liver in male SW mice (red dots) (data from Phillip et al., 2007) at three different single doses of aqueous based oral gavage of perc, including 150 (A), 500 (B), 1000 (C) mg/kg. The current model predictions were also compared with the previous model prediction (solid gray line) (Chiu & Ginsberg, 2011).

Fig. 8.

Comparison of the current PBPK model predictions for both a population-generated “random strain” (solid black line represents the median and dashed black lines represents 2.5% and 97.5% CI) and strain-specific parameter posterior distributions (solid blue line represents the median and dashed blue lines represents 2.5% and 97.5% CI), and data for perc and its oxidative, TCA (A), and GSH-conjugation products, including TCVG, TCVC and NAcTCVC (B) in blood and tissues in male C57BL/6J mice (red dots) (data from Cichocki et al., 2017) at a single dose of 300 mg/kg aqueous based oral gavage of perc. The current model predictions for perc and TCA were also compared with the previous model

predictions (solid gray line) (Chiu & Ginsberg, 2011). A horizontal dotted red line indicates the LODs and an open reversed triangle denotes datapoints below LODs.

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