Intended for
Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date
July 15, 2021

INCORPORATION OF IN VITRO METABOLISM DATA IN A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR CHLOROPRENE- REVISED DOCUMENTATION IN RESPONSE TO USEPA PEER REVIEW

RAMBOLL Bright ideas. Sustainable change.

ABSTRACT

A physiologically based pharmacokinetic (PBPK) model for chloroprene in the mouse, rat and human has been developed that relies solely on *in vitro* studies for the estimation of model parameters describing tissue metabolism and partitioning. The PBPK model accurately predicts *in vivo* pharmacokinetic data from a 6-hr, nose-only chloroprene inhalation study conducted with female B6C3F1 mice, the most sensitive species/gender for lung tumors in the 2-year bioassays conducted with chloroprene. This PBPK model has been developed to support an inhalation cancer risk assessment for chloroprene using *in vitro* data on the metabolism of chloroprene to reactive metabolites in the lung target tissue of mice and humans. The approach for calculating target tissue (lung) dose metrics was based on the PBPK modeling performed in support of the inhalation cancer risk assessment for methylene chloride and represents the best available science for determining the impact of species differences in metabolism of chloroprene. The original documentation of the PBPK model that was submitted to EPA has now been revised (Ramboll 2021 – current document) in response to the recommendations from a USEPA-convened independent peer review panel advising on the suitability of the model for use in updating the IRIS assessment of chloroprene toxicity in humans.

EXECUTIVE SUMMARY

- This document describes the development of a physiologically based pharmacokinetic (PBPK) model for chloroprene in the mouse, rat and human. The intended application of the model is to estimate target tissue dose metrics (total metabolism of chloroprene to reactive epoxides in the lungs) to support an inhalation risk assessment for lung cancer.
- The chloroprene model structure and dose metric selection are based on previous PBPK models for methylene chloride and vinyl chloride that were used in cancer risk assessments by the USEPA. As with methylene chloride and vinyl chloride, the observed carcinogenicity of chloroprene in the mouse is believed to be due to the generation of a reactive metabolite in the target tissue. The chief difference from previous models is that the tissue metabolism parameters for chloroprene were based on the published results of *in vitro* studies using microsomes, rather than inferring the parameters indirectly by fitting the model to *in vivo* pharmacokinetic data.
- To assess the validity of the PBPK model, results from a previously unpublished study conducted with female B6C3F1 mice, the most sensitive species/gender for lung tumors in the 2-year bioassay conducted with chloroprene, were considered (Clewell et al. 2019). The mouse PBPK model accurately predicted the *in vivo* pharmacokinetic data from a 6-hr, nose-only chloroprene exposure.
- It was not possible to confidently estimate metabolism parameters for the model in the human lung due to the low rate of metabolism observed in this tissue. Therefore, an alternative approach, previously used in the USEPA risk assessments for methylene chloride, was applied. In the case of methylene chloride, there were no data available to estimate human lung metabolism to the reactive metabolite that was considered to be responsible for carcinogenicity. Therefore, the lung metabolism parameters were based on the parameters for liver metabolism together with the ratio of liver and lung activity for a standard substrate in an *in vitro* assay. More recent evidence for lung and liver expression of the isozymes that metabolize chloroprene supports the *in vitro* activity ratio. Applying this approach to chloroprene provides a conservative (health-protective) estimate of human lung metabolism compared to the values that could be inferred from the highly uncertain *in vitro* data for chloroprene metabolism in human lung microsomes.
- The PBPK model was used to predict dose metrics – amounts of chloroprene metabolized in the lung per gram of lung tissue per day – in female mice and humans. The ratios of the human lung metabolism dose metrics to the lung metabolism dose metrics in the female mouse are roughly two orders of magnitude lower than those calculated on the basis of inhaled chloroprene concentration.
- In response to an external peer review panel conducted for the USEPA by an independent contractor in October, 2020, additional sensitivity/uncertainty analyses and literature searches were conducted to provide additional support for the data relied upon in estimating many of the parameters in the model. Also, at the request of one peer reviewer, an extended version of the model was developed (Ramboll 2021 – current document) to describe the downstream metabolism of chloroprene in order to compare model predictions using alternative dose metrics. The results of this extension of the model demonstrate that the use of a dose metric based on total metabolism is consistent with the cross-species relationship of the toxicity and carcinogenicity of chloroprene, but

one based on epoxide area under the curve (AUC) is not. These results support the use of total metabolism as the most appropriate dose metric for the carcinogenicity of chloroprene.

- Conclusions: The revised chloroprene PBPK model is based on the best available science, including a new test animal *in vivo* validation study, an updated literature review and a new Markov-Chain Monte Carlo analysis to assess parameter uncertainty. Inclusion of the best available science is especially important when deriving an Inhalation Unit Risk based on species extrapolation for the potential carcinogenicity of a reactive metabolite, since previous risk assessments for similar chemicals have demonstrated that the default cross-species extrapolation using inhaled concentration is highly inaccurate for this mode of action.

1. INTRODUCTION

Chloroprene (CAS# 126-99-8) is a highly volatile chlorinated analog of 1,3-butadiene that is used in the manufacture of polychloroprene rubber (Neoprene). A cancer risk assessment for chloroprene conducted by the USEPA (2010) calculated an inhalation unit risk (IUR) of 5x10-4 per µg/m3 based on tumor incidence data from female mice exposed to chloroprene for 2 years (NTP 1998; Melnick et al. 1999). The USEPA (2010) assessment used a default cross-species extrapolation approach which involved linear extrapolation to the low concentration region based on chloroprene exposure concentration, despite strong evidence of quantitative differences in chloroprene metabolism in mice and humans that would have a significant impact on the calculated risk (Himmelstein et al. 2004a, 2004b). The metabolism of chloroprene results in the formation of reactive metabolites that are considered to be responsible for its carcinogenicity in rodents (USEPA 2010).

To determine the potential impact of species-specific differences in the production of these epoxides, a physiologically based pharmacokinetic (PBPK) model was originally developed in a collaborative research effort between DuPont Haskell Laboratory and the USEPA National Health and Environmental Effects Research Laboratory (NHEERL) and submitted for the USEPA (2010) IRIS Assessment. *In vitro* measurements of partition coefficients and metabolism parameters for chloroprene in mice, rats, hamsters and humans (Himmelstein et al. 2004a) were used in the PBPK model (Himmelstein et al. 2004b) to predict species-specific dose metrics for the production of epoxides in the lung, the most sensitive tissue in the mouse bioassay. The dose metric chosen for this comparison is consistent with the dose metrics used in previous PBPK-based risk assessments for methylene chloride and vinyl chloride, which are also metabolized to reactive metabolites that are considered to be responsible for the observed carcinogenicity in rodents. Closed-chamber exposures of mice, rats and hamsters were used to validate the PBPK model's ability to predict the pharmacokinetic behavior of chloroprene *in vivo*. The USEPA (2010), however, did not make use of the PBPK model from Himmelstein et al. (2004b) in their risk assessment, citing the lack of blood or tissue time course concentration data for model validation. In addition, USEPA indicated that they did not consider the comparisons of model predictions with the closed-chamber studies to be adequate because the data were limited to chloroprene vapor uptake from the closed chambers.

After the time of the USEPA (2010) evaluation, subsequent studies (IISRP 2009b) provided additional data for refining the PBPK model of Himmelstein et al. (2004b). To supplement the data in Himmelstein et al. (2004a) on liver and lung metabolism in male mouse, male rat, and pooled human cells, subsequent studies (IISRP 2009b) measured liver and lung metabolism in female mouse and female rat, as well as kidney metabolism in male and female mouse, male and female rat, and pooled human cells. The totality of the data from the Himmelstein et al. (2004a) and IISRP (2009b) *in vitro* metabolism studies was then used to refine the metabolism parameter estimates for the chloroprene PBPK model using Markov-chain Monte Carlo (MCMC) analysis. A comparison of lung dose metric estimates in mouse, rat and human was then performed using the updated metabolism parameters (Yang et al. 2012). These dose metrics were subsequently used in a study comparing genomic responses to chloroprene in the mouse and rat lung (Thomas et al. 2013) and a study comparing human risk estimates derived from mouse bioassay and human epidemiological data (Allen et al. 2014).

The objectives of the present study were to: 1) characterize the *in vivo* pharmacokinetics of chloroprene via analysis of whole blood concentrations in female B6C3F1 mice during and

following a single 6-hour nose-only inhalation exposure, and 2) determine respiratory parameters (breathing frequency and tidal volume) during chloroprene exposure. We also demonstrate the ability of the refined chloroprene PBPK model to reproduce new *in vivo* validation data and calculate PBPK dose metrics that can be used to support an inhalation cancer risk assessment that properly considers species differences in pharmacokinetics and metabolism.

In October 2020, an independent peer review of the chloroprene PBPK model was conducted by the USEPA specifically to consider use of this model in a potential IRIS reassessment of chloroprene (USEPA 2020a – external peer review report). In the charge to the peer reviewers (USEPA 2020b), USEPA requested that peer reviewers prioritize their comments to indicate their relative importance as follows:

- Tier 1: Key Recommendations – Recommendations that are necessary for strengthening the scientific basis for the PBPK model, reducing model uncertainties (especially with respect to typical expectations for a PBPK model) or accurately evaluating such uncertainties before the model is applied for risk assessment.
- Tier 2: Suggestions – Recommendations that are encouraged in order to strengthen confidence before the PBPK model is potentially applied in risk assessment. It is understood that other factors (e.g. timeliness) may also be considered before deciding to conduct the suggested additional research or model revisions.
- Tier 3: Future Work – Recommendations for useful and informative scientific exploration that may inform future evaluations of key science issues arising from any aspect of the modeling and analysis presented. These recommendations are likely outside the immediate scope and/or needs of the current PBPK model review.

The current documentation of the model provides updates in response to the peer reviewers' Tier 1 and Tier 2 comments as EPA requested of DPE by USEPA on December 15, 2020. A listing of all of the Tier 1 and Tier 2 comments from the peer reviewers by topic is provided in Supplemental Materials G, along with Ramboll's responses to each of the comments, describing how the comments were addressed in the current documentation of the model.

2. MATERIALS AND METHODS

Nose-only Exposure Study

Test Substance and Atmosphere Generation

The test substance, β-Chloroprene (CAS# 126-99-8) containing polymerization inhibitors, was supplied by the sponsor as a clear liquid. Exposure atmospheres were generated by metering saturated chloroprene vapor from a stainless-steel pressure vessel reservoir (McMaster Carr, Atlanta, GA) into the nose-only exposure chamber air supply. The concentrated chloroprene vapor was metered through a mass flow controller (MKS Instruments Inc, Andover, MA) and mixed with HEPA-filtered air approximately six feet upstream of the nose-only inlet. Chloroprene vapor was introduced counter-current to the dilution air to facilitate mixing of the vapors with the dilution air. Chloroprene concentrations were monitored on-line using a gas chromatography system with flame ionization detector (GC-FID). Calibration of the GC-FID for chloroprene analysis was conducted through the analysis of a series of calibration standards produced by introducing pure chloroprene into Tedlar® bags containing known volumes of nitrogen gas (nitrogen was metered into the bag using a calibrated flow meter).

Test Animals and Housing

Female B6C3F1 were purchased from Charles Rivers Laboratories, Inc (Raleigh, NC) at 8 weeks of age and acclimated to their surroundings for approximately two weeks prior to use. Following acclimation animals were assigned to a dosing group by randomization of body weights using Provantis NT 2000, assigned unique identification numbers, cage cards, and housed (1/cage) in polycarbonate cages with standard cellulose bedding. Animals were housed in a humidity and temperature controlled, HEPA-filtered, mass air-displacement room provided by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited animal facility at The Hamner Institutes. This room was maintained on a 12-hour light-dark cycle at approximately 64oC-79oF with a relative humidity of approximately 30-70%. Rodent diet NIH-07 (Zeigler Brothers, Gardners, PA) and reverse osmosis water was provided ad libitum except during exposures. Food and water were withheld from all animals during the chloroprene exposures. Prior to the start of the chloroprene exposure, animals were weighed, and their weights were recorded.

The Hamner Institutes for Health Sciences was fully accredited by the AAALAC during the time the study was performed. Currently acceptable practices of good animal husbandry were followed per the National Research Council Guide for the Care and Use of Laboratory Animals and were in compliance with all appropriate parts of the Animal Welfare Act. In addition, the study design and protocol were approved by The Hamner Institutes' Institutional Animal Care and Use Committee (IACUC) prior to the initiation of the study.

Inhalation Exposures

Inhalation exposures were conducted at 13, 32, and 90 ppm for 6 hours. Blood was collected by cardiac puncture at a total of 6 time-points, 0.5, 3, and 6 hours during exposure and 5, 10, and 15 minutes post-exposure. To support collection of whole blood during the exposures, nose only towers were fitted with specially designed nose only exposure tubes. These exposure tubes were manufactured from 50 mL polypropylene bulb irrigation syringes (Sherwood Medical, St. Louis, MO). Three elongated holes (0.625" x 1.125") were drilled into the wall of the syringe to allow access to the thorax of the mouse during chloroprene exposure. A second irrigation syringe was

cut to form a sleeve around the first syringe to provide an airtight barrier during the exposures. This sleeve was pulled back during the exposure to allow for the injection of pentobarbital (100 mg/kg) while the animal continued to inhale chloroprene. Blood was removed directly from the mouse via arterial-side cardiac puncture while the mouse was still housed in the syringe and breathing chloroprene.

Plethysmography

A total of 16 mice (4 per exposure group including air controls) were used for the purpose of collecting tidal volume and breathing frequency. Data were acquired using modified nose only Buxco plethysmograph tubes for pulmonary function monitoring. Data from control mice were collected prior to the first chloroprene exposure. Plethysmography data from both control and exposed mice were collected for 2-3 hours.

Blood Sampling

Whole blood was collected at 0.5, 3, and 6 hours during exposure and 5, 10, and 15 minutes post-exposure. Whole blood collection during chloroprene exposures (0.5, 3, and 6-hour time points) were done using the specially designed nose only exposure tubes described above.

Blood Analysis

Quantification of chloroprene in whole blood was conducted by headspace sampling with analysis by gas chromatography mass spectrometry (GC/MS). The sampling method to be used, headspace analysis, as well as the GC/MS method were based on the previously published method for the analysis of 1,3-butadiene in whole blood from mice and rats (Himmelstein et al. 1994).

Briefly, 200 µL of whole blood, obtained by cardiac puncture, was transferred into pre-labeled, capped, and weighed airtight headspace vials (1.5 mL autosampler vial). Sample vials were weighed to obtain an accurate estimate of sample size and allowed to equilibrate at room temperature for 2 hours. Once equilibration was complete, samples were analyzed using an Agilent 5973 mass spectrometer coupled to an Agilent 6890 gas chromatograph. The mass spectrum was run in electron impact mode with selective ion monitoring (instrumental conditions are listed below).

Calibration curves were prepared by spiking stock control whole blood with known amounts of chloroprene obtained as a certified standard solution of chloroprene in methanol. Quality control samples were prepared by spiking control rat plasma with a certified chloroprene standard. QC samples were spiked to low (near the first calibration point), medium (near the middle of the calibration curve), and high (near the highest point of the calibration curve) levels. Aliquots of the prepared QC's were placed in sealed GC vials (3 aliquots for each level, 9 total) and kept frozen at -80^{o}C until required (GC vials had a minimum of headspace prior to freezing). On the blood collection days, a low-, middle-, and high-level QC was thawed and allowed to come to room temperature for 4 hours. After this time, the QC samples were "sampled" with a syringe identical to those being used for the collection of whole blood, placed in a GC vial in a manner identical to that of the whole blood collection, and analyzed along with the samples and standards.

Additional details of the nose-only inhalation study can be found in IISRP (2009a).

Chloroprene PBPK Model

The development and documentation of the chloroprene PBPK model has been conducted in a transparent manner consistent with the WHO/IPCS (2010) guidance on PBPK modeling. The following sections describe the basis for the model structure and parameterization, as well and the methods used for sensitivity/uncertainty analysis and risk assessment application of the model.

Model Structure

The structure of the PBPK model used in this study (Figure 1) is based on the PBPK model of chloroprene described in Himmelstein et al. (2004b), as modified by Yang et al. (2012). As in previous models of volatile organic compounds (Ramsey and Andersen 1984; Andersen et al. 1987), the blood is described using a steady-state approximation and the model assumes blood-flow limited transport to tissues and venous equilibration of tissues with the blood. Metabolism is described in the liver, lung and kidney using Michaelis-Menten saturable kinetics.

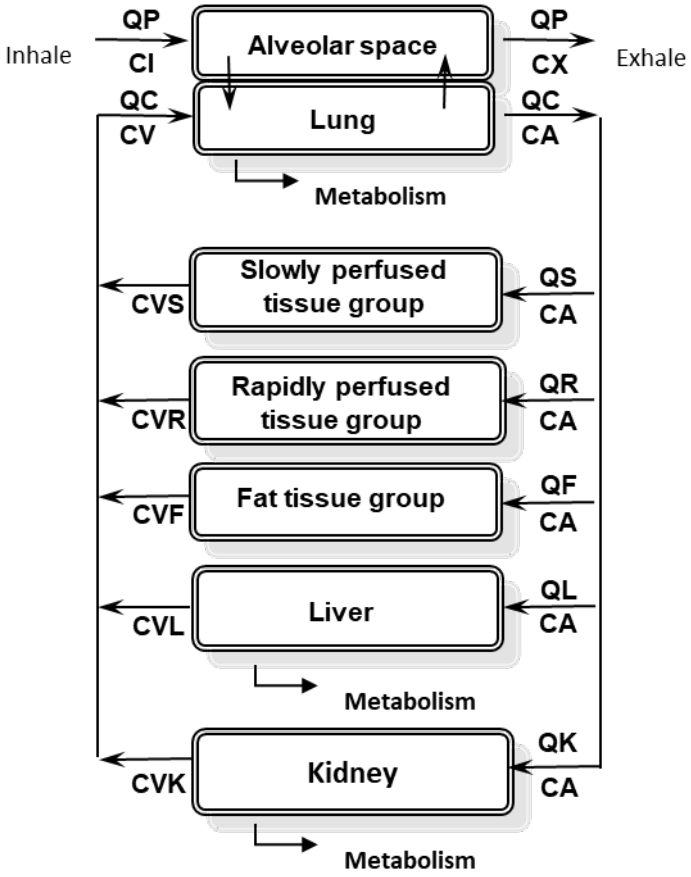

Figure 1. Chloroprene PBPK model diagram. Abbreviations: QP - alveolar ventilation; CI - inhaled concentration; CX - exhaled concentration; QC - cardiac output; CA - arterial blood concentration; CV - venous blood concentration; QS, CVS - blood flow to, and venous concentration leaving, the slowly perfused tissues (e.g. muscle); QR, CVR - blood flow to, and venous concentration leaving, the richly perfused tissues (most organs); QF, CVF - blood flow to,

and venous concentration leaving, the fat; QL, CVL - blood flow to, and venous concentration leaving, the liver; QK, CVK - blood flow to, and venous concentration leaving, the kidney.

Model Parameters

All physiological parameters in the model for mouse, rat and human (Table S-1 in Supplemental Materials A) are taken from Brown et al. (1997) except for the cardiac output in the mouse and the alveolar ventilation and cardiac output in the human. While the alveolar ventilation in the mouse is taken from Brown et al. (1997), relying on the value of cardiac output reported in Brown et al. (1997) would result in a value of 11.6 L/hr/bw3/4 for cardiac output (QCC). If used with the Brown et al. (1997) value of 29.1 L/hr/bw3/4 for alveolar ventilation (QPC), this would result in a serious mismatch between ventilation and perfusion (V/Q ratio >> 1). Andersen et al. (1987), the developers of the PBPK model for methylene chloride that was used in the USEPA (2011) IRIS assessment, suggested that it would be more biologically realistic to assume that the V/Q ratio was close to 1 at rest, and stated that their previous experience with PBPK modeling of data on clearance of chemicals in the mouse under flow-limited metabolism conditions supported the use of a higher value for QCC. Therefore, the value of QCC in the current model was calculated by dividing the alveolar ventilation from Brown et al. (1997) by an estimate of V/Q = 1.45 for the mouse based on pharmacokinetic data for exposures to another volatile organic chemical, methylene chloride (Marino et al. 2006), which was used in the USEPA (2011) inhalation cancer risk assessment for that chemical. In the case of the human, it is more appropriate to use the default USEPA ventilation rate of 20 L/day, reflecting an average activity level, rather than a resting value (Clewell et al. 2001). Since the values for alveolar ventilation and cardiac output in Brown et al. (1997) are resting values, we used the values calculated for the PBPK model of vinyl chloride (Clewell et al. 2001), which was used in the USEPA (2000) cancer risk assessment for that chemical. The parameter values, which were calculated to be consistent with the USEPA default ventilation rate of 20 L/day, were QPC = 24.0 L/hr/ $bw^{3/4}$ and a QCC of 16.5 L/hr/ $bw^{3/4}$ (V/Q ratio of 1.45).

Apart from the physiological parameters, the model parameters are based entirely on *in vitro* data. The partition coefficients (Table S-2 in Supplemental Materials A) were calculated from the results of *in vitro* vial equilibration data reported by Himmelstein et al. (2004b), using the partition coefficients for muscle and kidney to represent the slowly and rapidly perfused tissues, respectively. To obtain the model parameters for metabolism in the liver, lung and kidney, the original *in vitro* chloroprene metabolism time-course data (Himmelstein et al. 2004a; IISRP 2009b) were re-analyzed using a MCMC analytical approach similar to the one performed in Yang et al. (2012). The key differences between the new analysis and the Yang et al. (2012) analysis were: (1) the incorporation of an additional parameter in the analysis of the *in vitro* metabolism data (Kgl) to describe the rate of transfer of chloroprene from the headspace to the media in the metabolism studies, (2) the use of updated tissue microsomal protein concentrations for scaling the *in vitro* results to *in vivo* values appropriate for the PBPK model, and (3) the adoption of a previously published approach for estimating the metabolism parameters in the human lung (Andersen et al. 1987).

Re-estimation of *in vitro* metabolism parameters: Schlosser et al. (1993) suggested that mass transport limitations should be assessed when estimating metabolism from *in vitro* experiments conducted with volatile compounds where there is an air:liquid interface. Since the potential for a mass transport limitation was not addressed in the *in vitro* metabolism studies conducted with chloroprene (Himmelstein et al. 2004a; IISRP 2009b), a new experimental study was performed

by Denka Performance Elastomer LLC at the request of USEPA to estimate a Kgl for chloroprene following a protocol based on Schlosser et al. (1993). The new experimental study, which is described in Supplemental Materials B, resulted in an estimated value of 0.024 L/hr for Kgl, similar to the value previously reported for benzene (Schlosser et al. 1993). However, this experimentally estimated value of Kgl was not consistent with the high rates of liver metabolism observed at low concentrations of chloroprene; that is, the mass transport associated with a Kgl of 0.024 L/hr was too slow to support the observed rates of metabolism in the media.

We considered it likely that the much faster uptake of chloroprene in the metabolism studies than in the Kgl study was due to more effective mixing during the incubations, together with non-specific surface binding of chloroprene to the microsomes, which provide a lipophilic binding component in the aqueous media. No microsomes were present in the Kgl experiments for chloroprene or benzene (Schlosser et al. 1993). Although the rate of shaking in the metabolism studies (Himmelstein et al. 2004a; IISRP 2009b) was not reported, we were able to determine that these studies used a Gerstel MPS2 autosampler with an agitating heater, which was set to an agitation rate of 500 rpm (Himmelstein 2019, personal communication), in comparison to the 60 rpm agitation rate used in Schlosser et al. (1993) and the present study. While the agitation rate in the Himmelstein studies was much higher than that used in the Schlosser et al. (1993) study, if the high-speed agitation had denatured the microsomal enzymes, it would be apparent in the time-course and dose-response relationships of the experimental data. In particular, the fact that the data in the liver tissues from the Himmelstein et al. studies are well described by a Michaelis-Menten metabolic description is clear evidence that the microsomal enzymes were functioning normally.

To account for this difference in agitation rates, it was suggested (Paul Schlosser 2019, personal communication) that the value of Kgl in the metabolism studies was likely to be higher than the value in the new experimental study by roughly the ratio of the mixing rates, that is, Kgl(metabolism studies) = Kgl(experimental study)×500/60 = 0.024×500/60 = 0.2 L/hr. To confirm this expectation, we conducted a new MCMC analysis to simultaneously estimate Kgl, Vmax and Km from the metabolism data for the male mouse (Himmelstein et al. 2004a), which provided the strongest information regarding the dose-response for the clearance of chloroprene in the vials. The resulting value of Kgl estimated from this analysis was 0.22 L/hr, with a 95% confidence interval of 0.19 – 0.33 L/hr, consistent with the estimated value. The estimated value was then used in the re-estimation of the metabolism parameters for all tissues (Supplemental Materials B). The results of the new *in vitro* metabolism parameter estimation are provided in Table S-3 in Supplemental Materials A.

Selection of tissue scaling parameters: Based on a review of the literature (Supplemental Materials C), an updated set of scaling parameters was chosen: 35, 40, and 40 mg protein/g liver for mice, rats, and humans, respectively, Medinsky et al. (1994) for mouse, Medinsky et al. (1994) and Houston and Galetin (2008) for rat, and Barter et al. (2007) for human. For the lung, 20 mg protein/g was used for all species (Medinsky et al. 1994 for rat and mice, Boogaard et al. 2000 for rat, mouse and human). A microsomal content of kidney of 18 mg protein/g was used for mouse and rat and 11 mg protein/g for human (Yoon et al. 2007 for mouse and rat; Scotcher et al. 2017 for human). The maximum velocity and 1st order clearance rate constants were scaled allometrically (mg/hr/$BW^{0.75}$ or L/hr/$BW^{0.75}$) using the species and sex specific time and survival weighted average BW from the control group reported in the chloroprene bioassay (NTP 1998) for mouse and rat and 70 kg for human. The *in vivo* metabolism parameters derived using these revised scaling parameters with the *in vitro* metabolism estimates in Yang et al.

(2012) and with the results of the present re-analysis are listed in Table S-4 in Supplemental Materials A and the IVIVE calculations are provided in Supplemental Materials D.

Estimation of chloroprene metabolism in the human lung: Unfortunately, we found that the extremely low rates of chloroprene metabolism observed in the human lung (Himmelstein et al. 2004a) made parameter estimation for this tissue highly uncertain. Therefore, in the application of the model to calculate dose metrics we estimated the metabolism parameter for the human lung using the approach applied in the USEPA (2011) risk assessment for methylene chloride, which relied on the PBPK model developed by Andersen et al. (1987), In that model, the Km for metabolism in the human lung was assumed to be the same as the Km in the human liver, and the Vmax in the human lung was calculated from the Vmax in the human liver using a parameter (A1) derived from the ratio of the specific activities for metabolism of 7-ethoxycoumarin, a well-studied CYP2E1 substrate, in liver and lung (Lorenz et al. 1984).

Model Simulations

The previously published version of the chloroprene PBPK model (Yang et al. 2012), which was written in the Advanced Continuous Simulation Language (ACSL), was translated into R, an open source programming language, to improve its portability. The R code for the model is included in Supplemental Materials E. The full model code, including the scripts for running the model, is provided separately.

To model the experimental data from the nose-only inhalation exposures reported here, only the alveolar ventilation and cardiac output were altered. The average ventilation rate measured in the mice during the study was used to calculate an alveolar ventilation for use in the model, assuming 2/3 of total ventilation is alveolar (Brown et al. 1997), and the cardiac output was then calculated by dividing the alveolar ventilation by the V/Q ratio from Marino et al. (2006), as described in the results.

Parameter Sensitivity/Uncertainty Analyses

Parameter sensitivity analysis: An analysis of the sensitivity of model predictions to the values of its parameters was conducted with the model under two scenarios: (1) the prediction of blood concentrations in the mouse nose-only study, and (2) the prediction of dose metrics for the mouse bioassay exposures and for the human at 1 ppm continuous exposure. The results were calculated as normalized sensitivity coefficients (fractional change in prediction divided by fractional change in parameter) for parameters with a coefficient greater than 0.1 in absolute magnitude. A positive coefficient indicates the direction of change of the prediction is the same as the direction of change of the parameter. The parameters were changed by 1%, one at a time.

Kgl sensitivity analysis: In response to peer reviewer comments, an additional analysis was conducted to determine the impact of uncertainty in the value chosen for Kgl (0.22) on estimates of the metabolism parameters, dose metrics and resulting risk estimates predicted with the model. The same MCMC approach described above for the re-estimation of the *in vitro* metabolism parameters was used, holding Kgl fixed at a range of alternative values. Metabolism parameters were estimated using Kgl values of 0.175 (the lowest value of Kgl for which the MCMC analysis was able to converge in the female mouse liver), 0.22, 0.44, 0.88, and 1000 (well mixed assumption), The goodness of fit of the *in vitro* model to the metabolism data using the

various Kgl values was assessed and the impact of the resulting *in vivo* metabolism parameters on model-predicted dose metrics and risk estimates was determined.

A1 uncertainty analysis: To address peer reviewer concerns, a multi-faceted analysis was performed to assess the potential impact of uncertainty in the estimate of A1 on risk estimates obtained with the PBPK model: (1) Estimation of the 95% confidence interval for A1 based on the data in Lorenz at al. (1984), (2) Performance of an new, in-depth literature review for data to support an alternative estimate of an A1 for chloroprene, including the use of CYP mRNA expression data.

95% Confidence interval for A1 from Lorenz et al. (1984): Data from Lorenz et al. (1984) were used to define the distributions of metabolic activity in human lung and liver in units of nmole of enzyme/min/mg protein.

Truncated normal distributions were established using Crystal Ball (ver 11.1.2.3). These distributions were used to determine the distribution of A1 values where A1 was defined as the ratio of metabolic activity in lung and liver A distribution of results was determined using 5000 iterations of a Monte Carlo run using Latin Hypercube sampling with a bin size of 100. From this distribution, the 2.5^{th} and 97.5^{th} percentiles were calculated to provide a 95% confidence interval around the mean. The Crystal Ball calculations were performed assuming that the two distributions of enzymes were not correlated, which provides a slightly broader distribution of A1 than if they were positively correlated

Literature Review related to estimation of A1: A thorough review of the literature was conducted to determine whether there were additional data that could be used as an alternative to, or in support of, the A1 value for 7-ethoxycoumarin reported in Lorenz et al. (1984). The search was specifically targeted (1) to determine whether CYP isozymes other than 2E1 and 2F1 might contribute significantly to chloroprene metabolism, (2) to identify any additional studies similar to Lorenz et al. (1984) providing useable data on the *in vitro* metabolism of a CYP2E1 substrate in both human liver and human lung tissue fractions and (3) to identify any additional studies similar to Nishimura et al. (2003) providing usable data on tissue CYP isozyme mRNA expression. Initially, separate searches were performed to identify human metabolism data on compounds with similar structure or CYP affinity to chloroprene (e.g, butadiene, vinyl chloride, 1-ethoxycoumarin, chlorzoxazone, chlorinated alkenes). For the third objective, the following keyword search string was developed based on the content of the Nishimura et al. (2003) study and the comments from the peer reviewers: [Human AND (mRNA OR expression) AND (P450 OR CYP) AND (liver OR lung OR kidney)]. Databases of peer-reviewed literature (e.g. PubMed, Toxline) were searched and the abstracts of identified publications screened to identify potentially relevant studies for detailed review. The studies identified in the initial searches were then used as the basis for further searching to identify additional studies of potential interest, and this iterative process was continued exhaustively (i.e, until only previously identified studies were produced).

Dose metric calculations

Consistent with previous PBPK modeling of chloroprene (Himmelstein et al. 2004b; Yang et al. 2012), the dose metric calculated with the PBPK model is micromoles of chloroprene metabolized in the lung per gram lung per day. This dose metric was chosen because the lung is the tissue with the highest tumor incidence in the chloroprene inhalation bioassays (NTP 1998) and the

carcinogenicity of chloroprene in rodents is believed to result from its metabolism to reactive epoxides in the target tissue (Himmelstein et al. 2004a, 2004b). The dose metric selected for chloroprene is consistent with the dose metrics used in previous PBPK-based risk assessments for both vinyl chloride (Clewell et al. 2001; USEPA 2000) and methylene chloride (Andersen et al. 1987; USEPA 2011), which were also based on the production of reactive metabolites.

The PBPK model was first used to simulate the NTP (1998) bioassay exposures (12.8, 32 and 80 ppm; 6 hours/day, 5 days/week) and calculate the corresponding target tissue dose metrics (in this case, average daily production of epoxide metabolites in the lung per gram lung). The PBPK model was then used to estimate the same target tissue dose metric in a human exposed continuously to chloroprene at a concentration of 1 μg/m^3 for their lifetime. Due to the low rate of chloroprene metabolism in the human lung observed in the *in vitro* studies (Himmelstein et al. 2004a), the human lung metabolism parameters were estimated using the approach in the methylene chloride PBPK-based risk assessment (Andersen et al. 1987; USEPA 2011), where the affinity of lung metabolism was assumed to be the same as in the liver, and the relative capacity of lung to liver was based on *in vitro* data for a standard substrate, 7-ethoxycoumarin. As in the case of methylene chloride, this was done to provide a conservative (high-sided) estimate of the human dose metric, given the insufficiency of the *in vitro* chloroprene data for the human lung.

Uncertainty Analysis

Monte Carlo uncertainty analysis was conducted with the chloroprene PBPK model to estimate the uncertainty in the dose metrics resulting from the uncertainty in the estimates of the model parameters, particularly the metabolism parameters estimated from the *in vitro* studies (Himmelstein et al. 2004a, IISRP 2009b). For the purpose of evaluating uncertainty in the dose metrics, the posterior distributions for all metabolism parameters from the MCMC analysis were used, together with the uncertainty distribution of the A1 values derived from a data in Lorenz et al. (1984), as described above. Variability in the physiological and partitioning parameters was taken from Clewell and Jarnot (1994).

Crystal Ball Release 11.1.2.3.850 was used to obtain the parameter values for the mouse and human parameters used in the PBPK model. The values reported in Lorenz et al. (1984) were used to define the specified distributions for the physical parameters. Most of the parameter distributions were truncated on both the lower and upper ends of the distribution at mean ± 2.5 × std except where noted (i.e. parameters where the lower bound would be less than zero). Normal distributions were used for the body weight, tissue volumes and blood flows. Log-normal distributions were used for the partition coefficients. Five thousand iterations were performed in Crystal Ball and the data from the iterations were extracted for use as input values for the PBPK model.

The metabolism parameters were obtained by random selection without replacement from the last 5000 iterations of the Markov Chain Monte Carlo simulation, to pair with the iterations of the parameters estimated using Crystal Ball. The mouse metabolism parameters were randomized separately from the human metabolism parameters.

The target tissue dose metrics (average daily production of epoxide metabolites in the lung per gram lung) were estimated using these parameters for the mouse bioassay exposures (12.8, 32 and 80 ppm for 6 hours/day, 5 days/week) in the PBPK model. Human dose metrics were obtained using 5000 iterations of the human parameters obtained from Crystal Ball with a constant external exposure concentration of 1 μg/m3.

The target tissue dose metrics for the bioassay exposures were then used in time-to-tumor modeling of the incidence of lung alveolar/bronchiolar adenomas and carcinomas with the Multistage-Weibull model provided with the EPA BMDS software (February 25, 2010 version). The Multistage Weibull model has the following form:

$$P(d,t) = 1 - \exp[-(b0 + b1 \times d + b2 \times d2 + ... + bk \times dk) \times (t - t0)c]$$

BMDS was used to obtain a benchmark dose (BMD) and the 95% lower bound on that dose (BMDL) associated with a benchmark risk (BMR) of 0.01 for each of the 5000 iterations. The data used with the Multistage-Weibull model was the NTP (1998) female mouse combined incidence of alveolar/bronchiolar adenomas and carcinomas. For this dataset, the one animal for which the class of tumor was unknown was excluded from the analyses and the BMD and BMDL01 calculations were for incidental extra risk of 0.01 at t = 105 weeks.

In addition to the target tissue dose metrics for the mice, human dose metrics were obtained using the 5000 iterations of the human parameters obtained from Crystal Ball and a constant external exposure concentration of 1 µg/m3.

Correlation analysis was performed between the calculated BMDL01s and the PBPK model parameters used in the calculation of the dose metrics.

3. RESULTS

Chloroprene Exposure Atmospheres

Chloroprene concentrations were monitored in the nose only chambers during the 13, 32, and 90 ppm exposures, as well as in the control nose-only tower. All three target concentrations were well within 10% of their nominal levels.

Plethysmography

Figure 2 shows the measured minute volumes for the three exposure groups and controls. The data are represented as average values (circles) with standard deviation error bars. The data are provided in Table S-5 in Supplemental Materials A. There is no evidence of a concentration-related effect of short-term exposure to chloroprene on ventilation in mice. The average ventilation rate across all four exposure groups, including controls, was 56.2 mL/min. The average body weight for the mice in the study was 22g; therefore, this ventilation rate equates to a model parameter for alveolar ventilation (QPC) of 39.4 L/hr/bw3/4. The corresponding model value of QCC in this study is obtained by dividing QPC by the V/Q ratio of 1.45 for the mouse (Marino et al. 2006), yielding a value for QCC of 27.2 L/hr/bw3/4, which compares well with the QCC of 24.2 estimated for mouse exposures to methylene chloride (Marino et al. 2006).

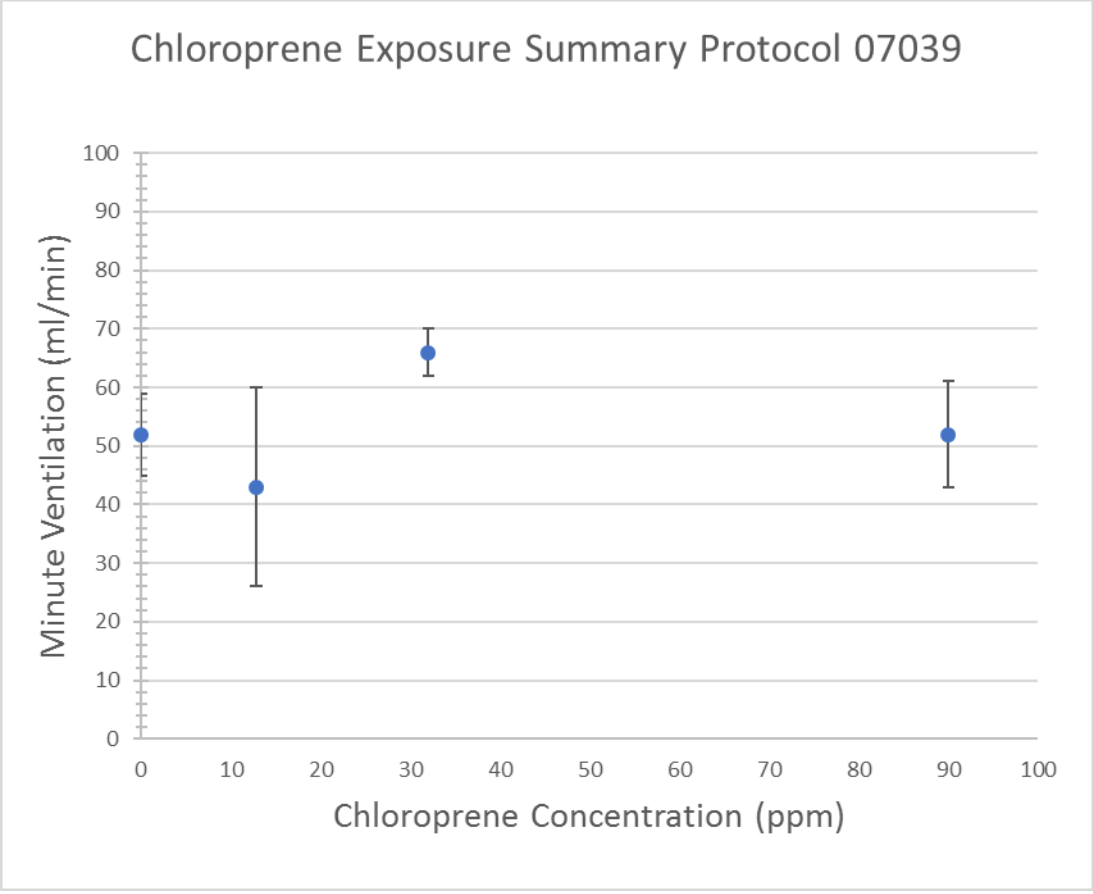

Figure 2. Measured minute ventilation during exposures.

Arterial Blood Chloroprene Concentrations

Figure 3 shows the average chloroprene (CD) blood concentrations at multiple timepoints for all three single day exposures (Data are provided in Table S-6 of Supplemental Materials A). Average blood chloroprene concentrations are represented by the symbols with standard deviations for each treatment group represented with error bars.

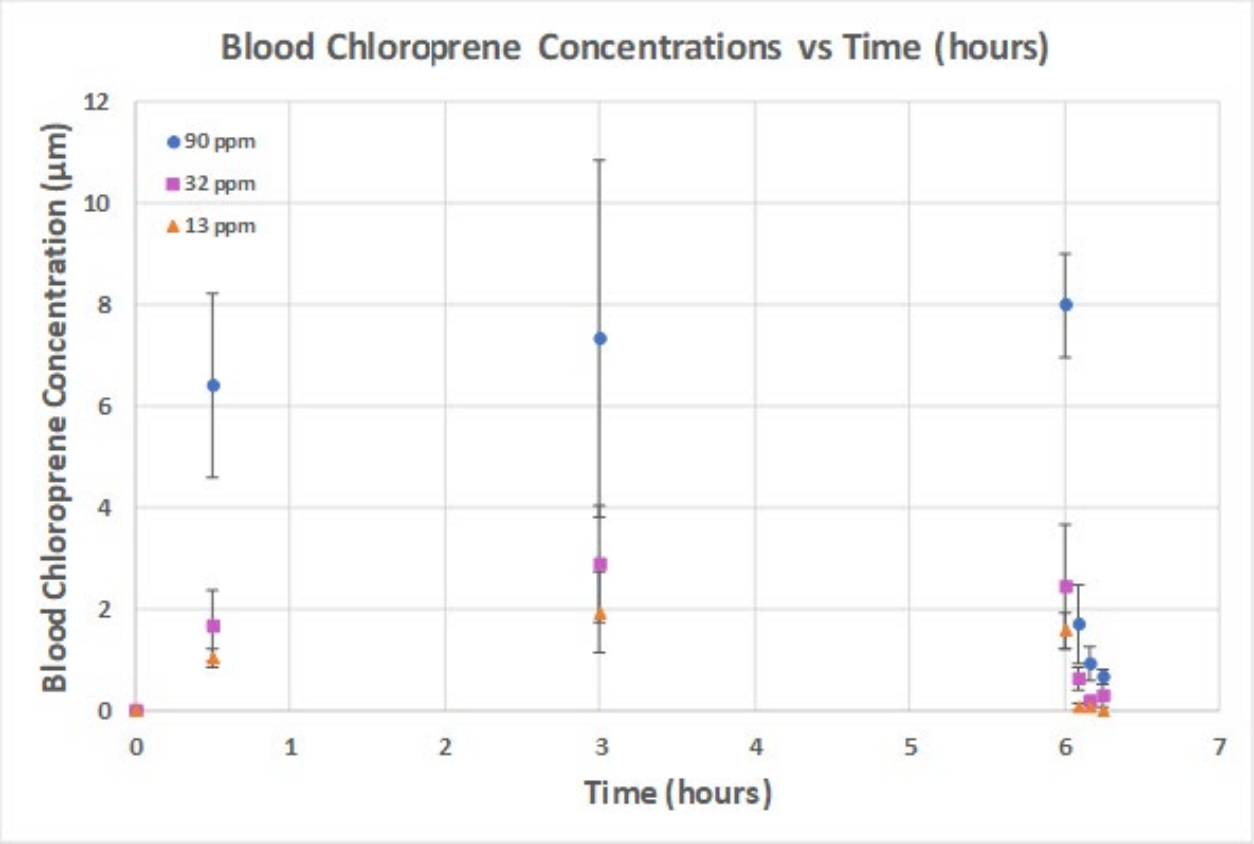

Figure 3. Arterial blood chloroprene concentrations during and following a single nose-only exposure of female B6C3F1 mice to chloroprene at 13, 32 or 90 ppm for 6 hours. Average blood chloroprene concentrations (symbols) and standard deviations (error bars) are shown for each treatment group.

Re-estimation of Metabolism Parameters

The mean and 95% confidence intervals for the *in vitro* metabolism parameters (Vmax and Km) resulting from the MCMC re-analysis are shown in Table S-3 of Supplemental Materials A and the scaled-up parameters for the PBPK model are listed in Table S-4. In our re-analysis of the *in vitro* metabolism data, we found that the extremely low rates of chloroprene metabolism observed *in vitro* (Himmelstein et al. 2004a; IISRP 2009b) made parameter estimation for several tissues highly uncertain: female mouse kidney, male and female rat lung, human kidney and lung. For the human lung, this uncertainty resulted from the very low rate of metabolism observed in these tissues during the *in vitro* studies conducted with chloroprene (Himmelstein et al. 2001; IISRP 2009b) compared to other sources of variability in the experiments. In particular, the data on loss of the chemical from control vials in the studies indicate that non-

metabolic losses contributed substantially to variability between assays. Unfortunately, the experimental protocol used in Himmelstein et al. (2001) and IISRP (2009b) did not include the use of internal controls to characterize non-metabolic losses in the vials in which metabolism was measured. The effect of the lack of internal controls cannot be completely overcome by *a posteriori* analysis.

Despite this experimental limitation, it was possible to reliably estimate the parameters for the capacity (Vmax) and affinity (Km) of metabolism in the majority of tissues because the enzymatic metabolism of chloroprene is known to be a saturable process (Michaelis-Menten kinetics), whereas the data from the control vials in the *in vitro* studies demonstrate that the other losses of the chemical from the vial are independent of concentration. Thus, whereas the other losses result in parallel lines on a log plot, metabolism results in downward concave curves with slopes that increase as the concentration decreases. Figure 4 shows the fit of the parameter estimates (curves) to the data (solid circles) for the *in vitro* metabolism studies in the mouse (left) and human (right) liver. It also shows the data from control vials that did not have any metabolism (open circles), which were only collected at some of the concentrations. The losses from the control vials are linear and parallel, while the rates of loss from the metabolism vials increase as the concentration decreases. Because the data spans concentrations from above to below saturation it was possible to estimate reliable values of both the capacity (Vmax) and affinity (Km) of metabolism.

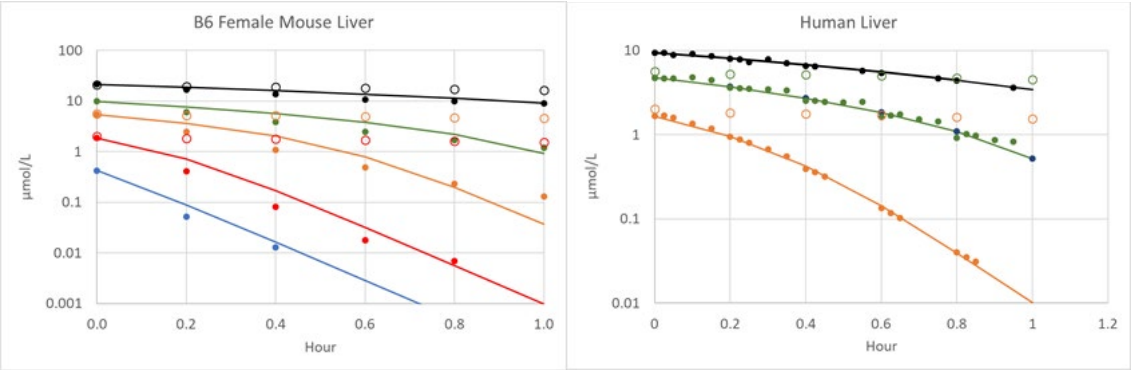

Figure 4. Predicted (curves) and measured (solid symbols) concentrations of chloroprene during *in vitro* metabolism experiments in the mouse (left) or human (right) liver. Open circles are data from control vials that lacked metabolic activity.

Metabolism in the female mouse lung (Figure 5) is much slower than in the liver, but the clearance from the metabolism vials (solid circles) is still clearly nonlinear, while the losses from the control vials (open circles) are linear. This systematic difference between the control and metabolism vials makes it possible to estimate both Vmax and Km.

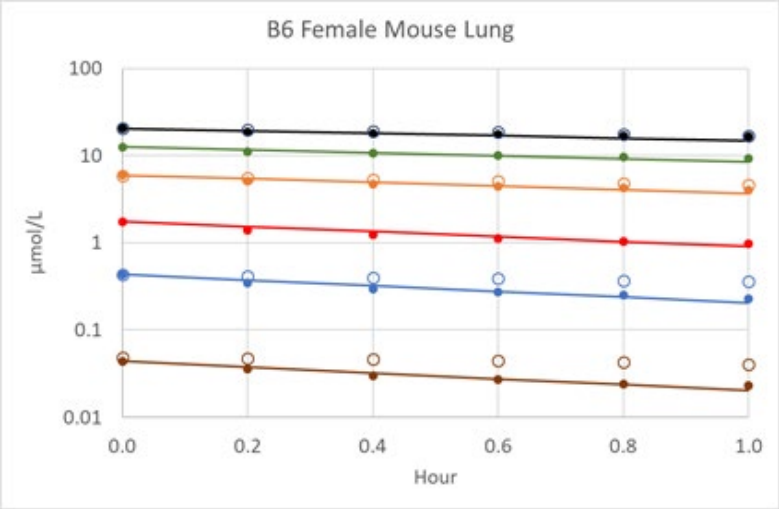

Figure 5. Predicted (curves) and measured (solid symbols) concentrations of chloroprene during *in vitro* metabolism experiments in the mouse lung. Open circles are data from control vials that lacked metabolic activity.

In the human lung, however, there is essentially no evidence of metabolism in the *in vitro* studies. Figure 6 shows the data from the metabolism vials (solid circles) along with the predictions (curves) from a model of the *in vitro* system that assumed there was no metabolism occurring. The slopes of each pair of lines represents the range of loss rates associated with taking samples from the vial headspace as well as losses associated with leakage through the vial septum after puncturing. The latter loss rates were estimated from all the control data in the *in vitro* studies (Supplemental Materials B). Controls for the human lung study were only performed at the lowest concentration (open symbols). The loss rate in the metabolism vial is within the range of loss rates in the control vials.

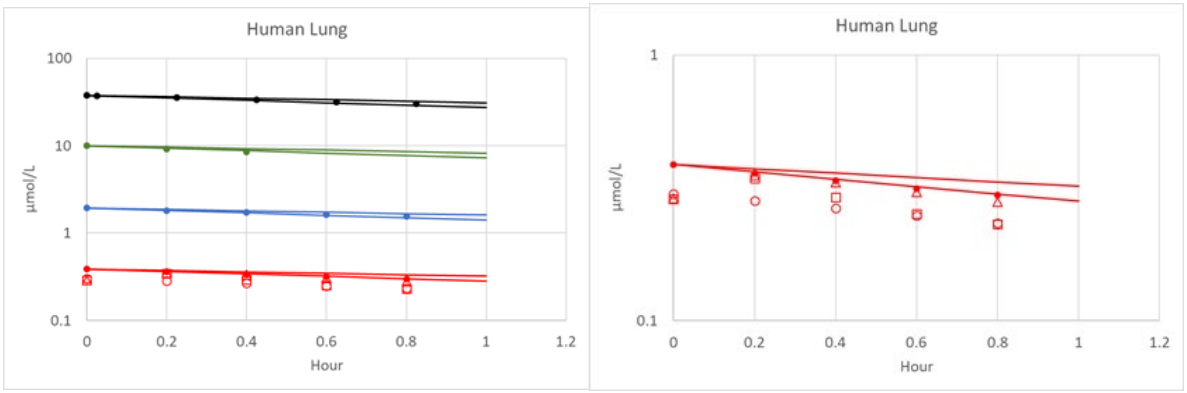

Figure 6. Measured concentrations of chloroprene during *in vitro* metabolism experiments in the human lung. (Solid symbols: metabolism vials; open symbols: control vials). Curves are model predictions assuming no metabolism is occurring.

In their analysis, Yang et al. (2012) attempted to estimate linear metabolism parameters in tissues where the MCMC analysis was unable to converge on estimates of both Vmax and Km. However, we have determined not to use that approach for two reasons: (1) estimation of a

pseudo-linear metabolic parameter would only be appropriate for concentrations well below Km, which for CYP2E1 is in the vicinity of 1 μM, but most of the data on chloroprene is at much higher concentrations, and (2) estimates of a linear metabolism component were unreliable due to confounding by other linear losses from the vials, as demonstrated by the high variability in controls.

Given the unreliability of the human lung data for chloroprene, we chose to estimate the metabolism parameters for the human lung using the same approach as the USEPA (2011) risk assessment for methylene chloride; that is, the Km for metabolism in the human lung was assumed to be the same as the Km in the human liver, and the Vmax in the human lung was calculated from the Vmax in the human liver using a parameter (A1) derived from the ratio of the specific activities for metabolism of 7-ethoxycoumarin, a well-studied CYP2E1 substrate, in liver and lung (Lorenz et al. 1984). Using the human value of A1 (0.00143), together with the estimated values of Vmax and Km in the human liver from the MCMC analysis (0.052 μmol/hr/mg protein and 0.32 μmol/L), results in a metabolic clearance in the lung of 0.24 L/hr/g microsomal protein. This human lung metabolism estimate is similar to the value of 0.32 L/hr/g microsomal protein previously estimated for chloroprene by Yang et al. (2012) and is within the confidence interval estimated by our new analysis of the *in vitro* data. In support of the applicability of A1 to chloroprene, the value of A1 in the male mouse (0.414) from Lorenz et al. (1984) is close to the ratio of the *in vitro* Vmax in the lung and liver of the male mouse in our new analysis (0.56, see Table S-3). The value of A1 is also consistent with the reported ratio of total CYP2E1 plus CYP2F1 mRNA expression in human lung and liver of 0.00059 (Nishimura et al. 2003), which is about a factor of two lower than A1.

For the tissues where metabolism was too slow to characterize (female mouse kidney, male and female rat lung, and human kidney), the model parameter for Vmax in that tissue was set to zero. Ignoring metabolism in these tissues did not perceptibly alter model predictions. In particular, it did not affect the predicted dose metrics in the female mouse lung.

PBPK Modeling of the Nose-Only Inhalation Study

The nose-only study described above was simulated with the chloroprene PBPK model using the parameters in Tables S1, S2, and S4, except for QPC and QCC, where the study-specific values derived from the plethysmography data were used. As shown in Figure 7, using only *in vitro*-derived metabolism and partitioning parameters the model predictions for blood concentrations during and after the 6-hr chloroprene exposures are in good agreement with the data collected in the study; consistent with the WHO/IPCS (2010) guidance on the use of PBPK modeling in risk assessment, model predictions are generally within roughly a factor of two of the means of the experimental data. It was not necessary to adjust any of the model parameters to provide agreement with the new data.

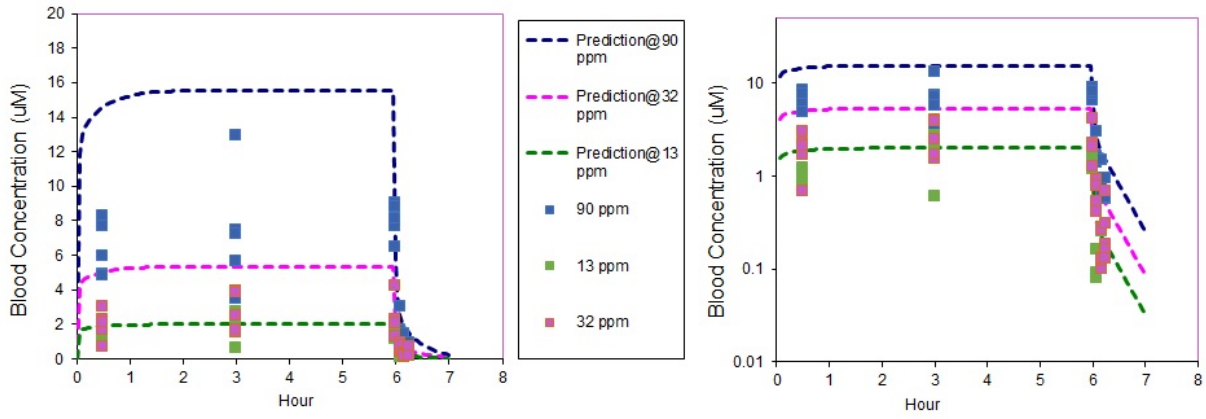

Figure 7. PBPK model predicted (dotted lines) and measured (symbols) blood concentrations during and following 6-hr exposures of B6C3F1 mice to chloroprene at 12.3 (green), 32 (fuchsia) or 90 (blue) ppm. The same data and model predictions are shown using a linear y axis (left) and a logarithmic y axis (right). The linear plot provides a better comparison for concentrations, whereas the logarithmic plot provides a clearer comparison for the post-exposure clearance.

PBPK Model Parameter Sensitivity

As shown in Figure 8, when simulating the nose-only exposures only 4 model parameters have sensitivity coefficients greater than 0.1 in absolute magnitude: alveolar ventilation, cardiac output, blood:air partition coefficient and fractional blood flow to liver. All these parameters were either directly measured or based on data from the literature, as described in the Methods, and can be considered to have low uncertainty. When predicting lung dose metrics in the female mouse (Figure 9), the sensitive parameters include the same parameters as those for the predictions of blood concentrations, with the addition of the parameters for lung metabolism and the body weight. The sensitive parameters for predictions of lung dose metrics in the human (Figure 10) are the same as those in the mouse, except that a single clearance parameter is used in the human due to the low rate of metabolism in the human lung. These analyses of the sensitivity of the model to uncertainty in its parameters suggest that performing a human *in vivo* validation study would be unlikely to provide a significant added value for model evaluation.

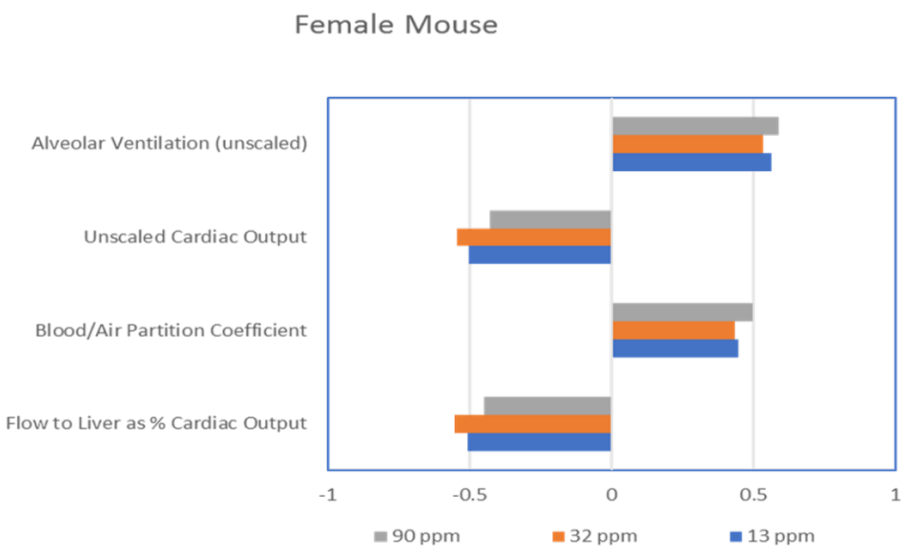

Figure 8. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of arterial blood concentrations in the nose-only study.

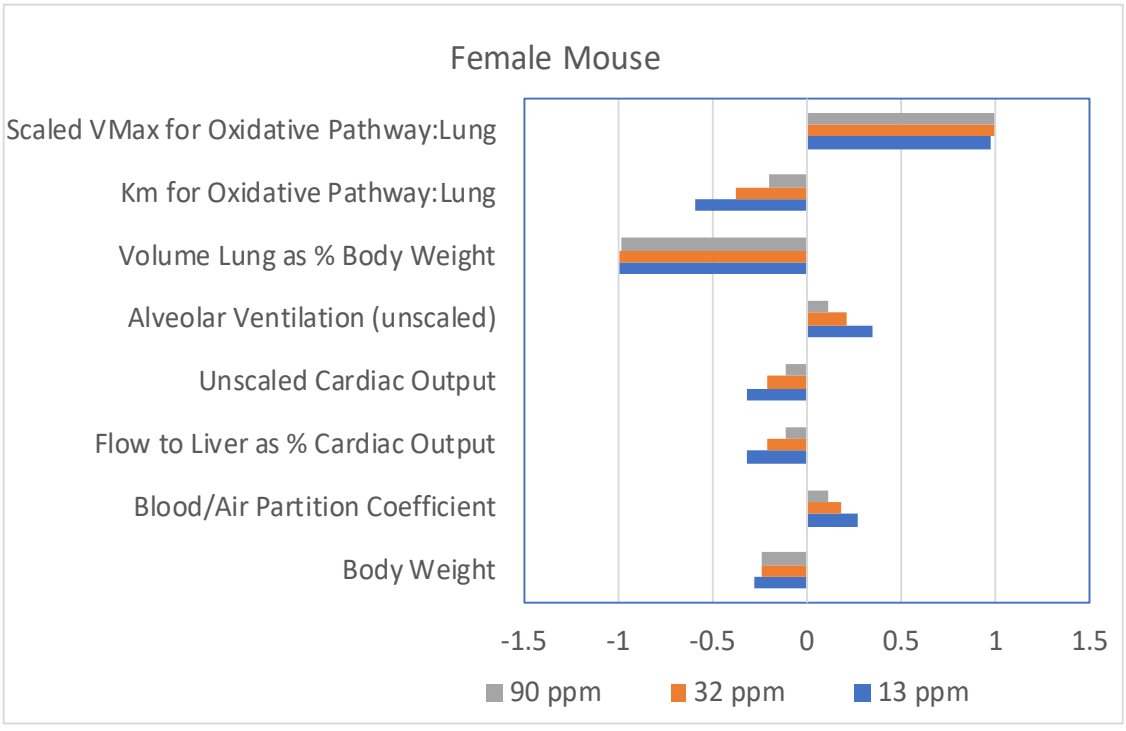

Figure 9. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of lung dose metrics in the female mouse for exposures in the 2-year bioassay.

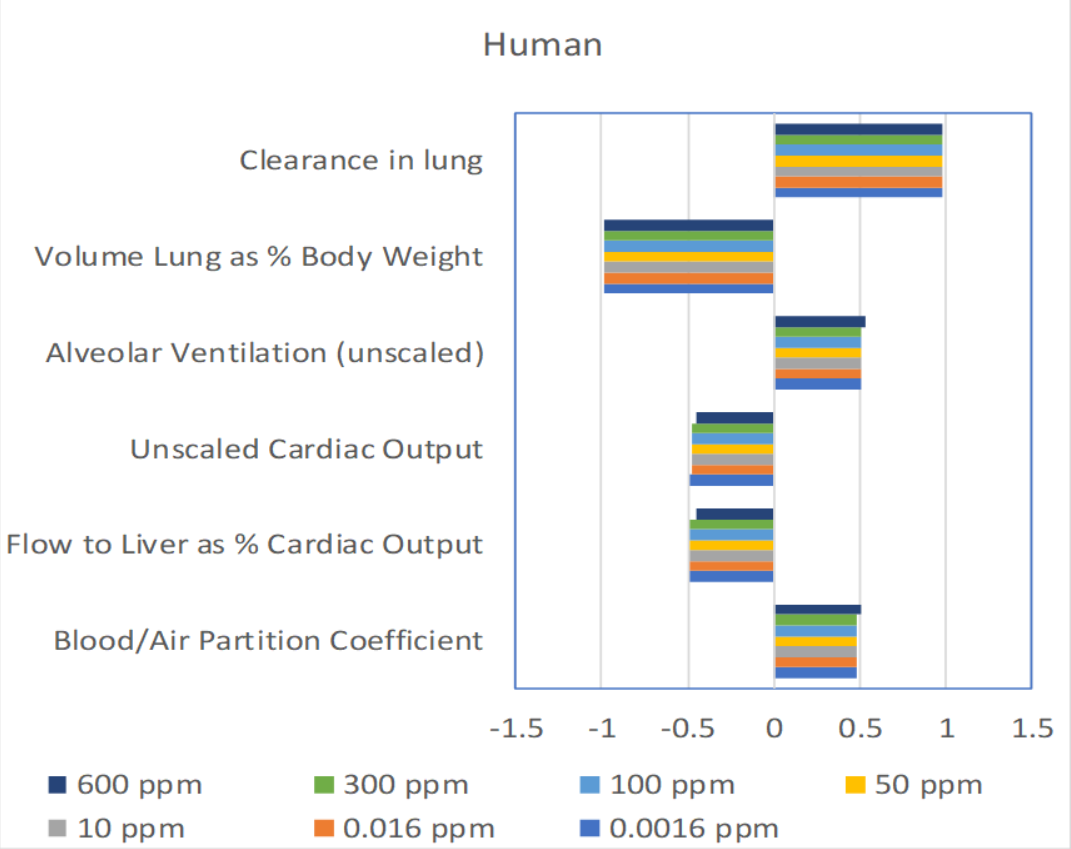

Figure 10. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of lung dose metrics in the human for continuous exposure at 1 ppm.

Kgl Sensitivity Analysis

Analysis of the goodness of fit to the experimental data based on sum of squares error (SSE) for different values of Kgl (Table 1) indicates that the best fit to data for the human liver was obtained with Kgl = 0.22. For the female mouse liver and lung, higher values of Kgl (faster mixing) provided a slightly better fit.

	Female Mouse Lung		**Female Mouse Liver**		**Human Liver**	
KGL	**Sum of Squares Error**	**Ratio to KGL = 0.022**	**Sum of Squares Error**	**Ratio to KGL = 0.022**	**Sum of Squares Error**	**Ratio to KGL = 0.022**
0.175	0.108	1.002	4.59	1.004	0.535	1.039
0.22	**0.108**	**1.000**	**4.57**	**1.000**	**0.515**	**1.000**
0.44	0.107	0.987	4.54	0.994	0.594	1.155
0.88	0.108	0.999	4.54	0.994	0.520	1.016
1000	0.108	0.998	4.54	0.993	0.580	1.126

Table 1. Goodness of fit of *in vitro* model to the experimental data based on sum of squares error (SSE) for different values of Kgl

	Female Mouse Liver		Female Mouse Lung		Human Liver	
Kgl (L/hr)	**Vmaxc**	**Km**	**Vmaxc**	**Km**	**Vmaxc**	**Km**
0.175	0.101	0.365	0.0215	2.37	0.054	0.308
0.22	**0.105**	**0.448**	**0.0215**	**2.37**	**0.055**	**0.349**
0.44	0.111	0.615	0.0213	2.37	0.057	0.431
0.88	0.113	0.691	0.0210	2.34	0.058	0.465
1000	0.115	0.771	0.0210	2.361	0.060	0.523

Table 2. Sensitivity of resulting *in vitro* metabolism parameter estimates to the values of Kgl assumed during the MCMC analysis of the *in vitro* metabolism data. The values in the human lung were obtained from the human liver values using the A1 approach.

As can be seen in Table 2, there was little impact of the choice of Kgl on the values estimated for Vmaxc: for Kgl values ranging from 0.11 to 1000 there was less than 10% variation from the values obtained with Kgl=0.22. The effect on estimates of Km (except in the mouse lung) were somewhat larger (40 – 70% variation from value for Kgl=0.22), which would be expected due to the observed collinearity of Kgl and Km in the in vitro modeling (Supplemental Materials B).

Table 3 shows the sensitivity of the dose metric predictions with the model to the value of Kgl used in the *in vitro* parameter estimation. The female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases as Kgl is increased.

KGL value:		**0.175**	**0.22**	**0.44**	**0.88**	**1000**
Species	**Inhaled Concentration**	Amt. Metab. Lung	Amt. Metab. Lung	Amt. Metab. Lung	Amt. Metab. Lung	Amt. Metab. Lung
Female Mouse	**12.8 ppm**	0.85	0.85	0.85	0.86	0.86
	32 ppm	1.29	1.29	1.29	1.29	1.29
	80 ppm	1.69	1.69	1.69	1.69	1.69
Human	**1 µg/m3**	3.59E-06	3.24E-06	2.73E-06	2.54E-06	2.33E-06

Table 3. Sensitivity of the dose metric predictions from the model to the value of Kgl used in the in vitro parameter estimation.

As shown in Table 4, human risk estimates for lung tumors based on the model-predicted dose metrics decrease as Kgl is increased.

KGL	BMDL (µmole/gram lung tissue/day)	Continuous human exposure at 1 µg/m3	IUR Per µg/m3
0.175	0.0090	3.59E-06	**4.0×10^{-6}**
0.22	0.0090	3.24E-06	**3.6×10^{-6}**
0.44	0.0090	2.73E-06	**3.0×10^{-6}**
0.88	0.0093	2.54E-06	**2.7×10^{-6}**
1000	0.0093	2.33E-06	**2.5×10^{-6}**

Table 4. Sensitivity of human risk estimates to the value of Kgl used in the in vitro parameter estimation.

Overall, the value of Kgl = 0.22 that was selected for use in the *in vitro* modeling (Supplemental Materials D) is both scientifically defensible and risk-conservative, based on (1) it was derived from a joint MCMC analysis for Kgl and Km in the male mouse, which was the most informative metabolic data (Supplemental Materials B), (2) it provides the best goodness of fit of the *in vitro* model to the experimental metabolism data in the human liver (Table 1), and (3) lower risk estimates would be obtained using higher values of Kgl. While a value of Kgl=0.175 would provide a higher risk estimate, it did not provide as good a fit to the in vitro data as Kgl = 0.22; in fact, attempting to decrease Kgl any further than 0.175 made it impossible to fit the data at all.

A1 uncertainty analysis

The results of the analysis demonstrate that there is minimal uncertainty in an estimate of A1 derived from Lorenz et al. (1984). The 95% Confidence interval for the estimate ranged from 3.59×10^{-4} to 4.13×10^{-3} (Figure 11) with a median of 1.44×10^{-3}, a mean of 1.64×10^{-3}, and standard error of the mean of only 1.37×10^{-5}. The standard deviation was 9.69×10^{-4}, resulting in a coefficient of variation of 0.69, consistent with only modest variability across samples.

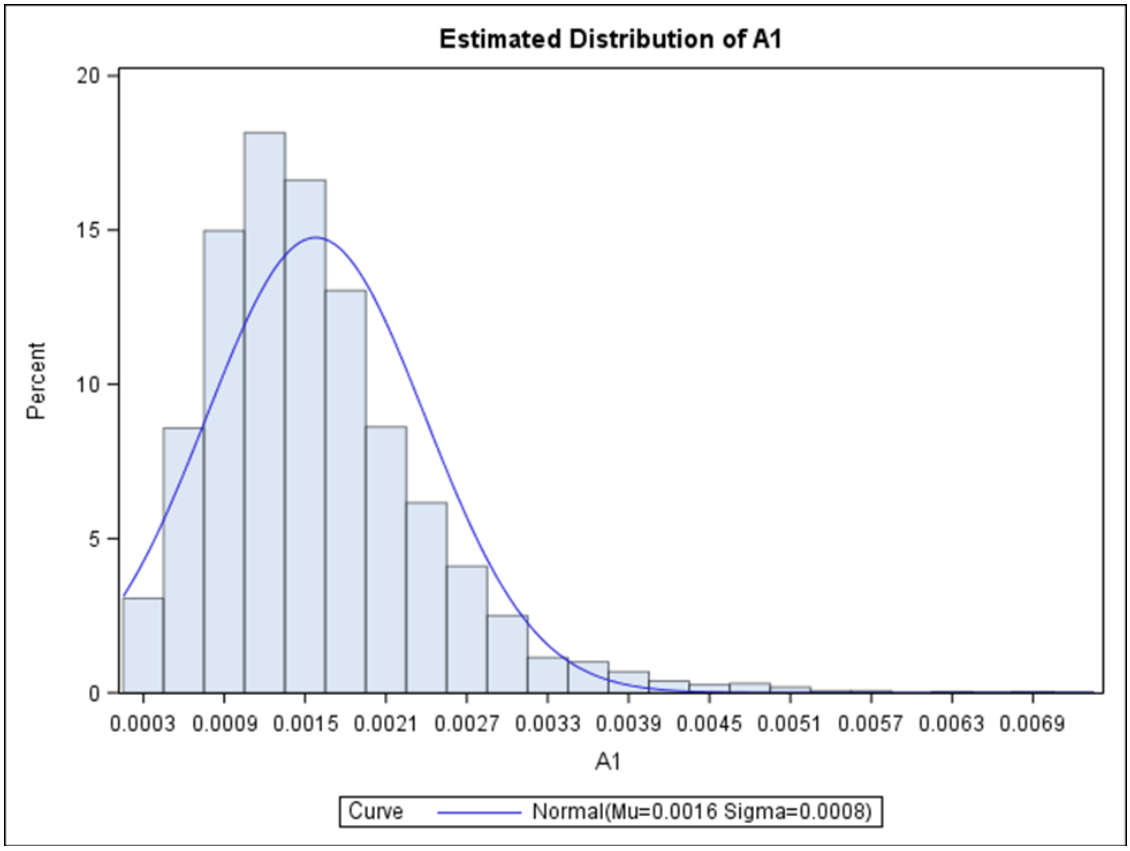

Figure 11. Distribution of A1 estimated from Lorenz et al. (1984)

Alternative values for A1: The new literature search did not identify any studies, apart from Lorenz et al. (1984), that provided *in vitro* metabolism data in both human lung and liver for a suitable analog of chloroprene based on structure or CYP affinity. Therefore, the only alternative source for an A1 value would be the use of data on mRNA expression of relevant CYPs in different

tissues. The literature search using the keyword string [Human AND (mRNA OR expression) AND (P450 OR CYP) AND (liver OR lung OR kidney)] resulted in the identification of 5,810 studies. Iterative screening of the most relevant 200 publications based on titles and abstracts identified 6 studies that were reviewed in detail (Amet et al. 1997, Bieche et al. 2007, Crawford et al. 1998, Hakkola et al. 1994, Hukkanen et al. 2002, Nishimura et al. 2003). Of these, only the Nishimura et al. (2003) and Bieche et al. (2007) studies provided data relevant to characterizing a potential value for A1.

Based on data for compounds with similar structures, the high-affinity isozymes that primarily contribute to the metabolism of chloroprene at low (micromolar) concentrations are CYP 2E1 and 2F1 (Forkert et al. 2005). At higher (millimolar) concentrations, 2A6 may also play a role (Deuscher and Elfarra 1994). Alternative values for A1 were calculated using the tissue mRNA content data from Nishimura et al. (2003) and Bieche et al. (2007).

The equation for calculating the ratio of VmaxC in the kidney (or lung) to the liver using mRNA content data is given in Sasso et al. (2013):

$$\frac{\mathrm{VmaxC_K}}{\mathrm{VmaxC_L}} = \left(\frac{\text{pmol CF metabolized/min/pmol cortex 2E1}}{\text{pmol CF metabolized/min/pmol liver 2E1}} \times \frac{\text{pmol cortex 2E1/mg MSP}}{\text{pmol liver 2E1/mg MSP}} \right) \times \left(\frac{\text{mg MSP/g cortex}}{\text{mg MSP/g liver}} \right) \times \frac{\text{g cortex}}{\text{g liver}} \quad (2)$$

The value of A1 (lung/liver activity ratio) that is used in the PBPK model is represented by the term in the first brackets in the equation, which represents the ratio of the metabolic rate per mg microsomal protein (MSP) in the two tissues. The A1 value currently used in the model was derived from data on the ratio of the metabolic rate per mg microsomal protein (MSP) for 7-ethoxycoumarin (Lorenz et al. 1984). The Vmax in the PBPK model is obtained by multiplying A1 by the ratio of mg MSP/g in the lung and liver (11/40) and by the ratio of the lung and liver tissue weights (0.0076/0.0257).

Following the approach used in Sasso et al. (2013) it can be assumed that the first term in the equation above is unity (assuming that the metabolic efficiency of the 2E1 protein is the same across tissues). Sasso et al. (2013) measured CYP 2E1 mRNA/mg microsomal protein (MSP), but Nishimura et al. (2003) and Bieche et al. (2007) measured whole tissue CYP mRNA expression and reported the ratio of CYP mRNA content in a tissue to mRNA content of GAPDH. GAPDH is a "housekeeping protein" that is expected to be similarly expressed in all tissues and is used as a reference value for comparisons across tissues. The resulting CYP isozyme mRNA expression ratios provide a measure of the relative whole-tissue protein concentrations rather than concentration per mg MSP. Therefore, the term in the second brackets in the equation is unnecessary, and it is only necessary to multiply the CYP ratio by the ratio of the organ weights to obtain the Vmax in the lung from the Vmax in the liver. To obtain values of A1 on a per mg MSP basis, they were divided by the ratio of the tissue MSP content. The best estimates for tissue MSP content in the human (Supplemental Material C) are 40 in the liver and 20 in the lung.

Study	A1: 2E1+2F1
Nishimura et al. 2003	0.00112
Bieche et al. 2007	0.01086
Geometric average:	0.00349

Table 5. Estimated values for A1 based on CYP 21 and CYP 2F1 mRNA content data from human lung and liver tissues in two separate studies (Nishimura et al. 2003, Bieche et al. 2007.

The values of A1 derived from the two mRNA studies are shown in Table 5. The disparity between the two studies may reflect the difficulty of harvesting and preserving mRNA from tissue donors/trauma victims. While the use of mRNA data may entail greater uncertainty than direct measurement of metabolism, it is significant that the geometric average of the A1 values calculated from the two mRNA datasets (0.00349) is within the 95% confidence interval for the A1 estimated from Lorenz et al. (1984) of 0.00036 to 0.00413.

PBPK-Based Dose Metrics for Chloroprene Lung Carcinogenicity

The dose metrics for lung metabolism in the female mouse bioassay and for human continuous exposure are shown in Table 6. These estimates were obtained with the chloroprene PBPK model using the parameters in Tables S1, S2, and S4. As illustrated in Table 6, predicted dose metrics increase less than linearly above an inhaled chloroprene concentration of 1 ppm.

Exposure	Concentration	Dose metric
Female mouse bioassay	12.8 ppm	0.85
	32 ppm	1.29
	80 ppm	1.69
Human continuous	100 ppm	3.70×10^{-2}
	10 ppm	2.88×10^{-2}
	1 ppm	8.91×10^{-3}
	0.1 ppm	1.12×10^{-3}
	0.01 ppm	1.15×10^{-4}
	1 ppb	1.16×10^{-5}
	1 µg/m^3	3.24×10^{-6}

Table 6. Dose metrics for lung metabolism (average mg metabolized per gram lung per day) in the female mouse bioassay and for human continuous exposures

PBPK Model Uncertainty Analysis

Monte Carlo uncertainty analysis was performed to evaluate the impact on risk estimates associated with uncertainty in the PBPK model parameters. The input parameter distributions are provided in Table S-6 in Supplemental Materials A. The results of the analysis are presented in Table 7.

Means and 90% Confidence Intervals from Monte Carlo Analysis				
Exposure	**Concentration**	**Mean**	**5th percentile**	**95th percentile**
Female mouse bioassay	12.8 ppm	1.11	0.52	1.99
	32 ppm	1.70	0.84	3.07
	80 ppm	2.21	1.12	4.02
Female Mouse BMDL		0.012	0.0034	0.028
Human continuous	1 μg/m^3	4.2×10^{-6}	9.7×10^{-7}	1.1×10^{-5}

Table 7. Means and fifth and ninety-fifth percentiles of daily lung metabolism dose metric distributions in the mouse bioassay, with the resulting BMDLs, and for a human continuous exposure to 1 μg/m3 chloroprene using the newly estimated parameters in this study based on the in vitro assays.

The results of the Monte Carlo uncertainty analysis indicate that the variation in the predictions of the model for the animal dose metrics and the resulting BMDLs, as well as in the human dose metrics is on the order of a factor of 3 from the mean. This result is consistent with results obtained with previous similar PBPK models (Clewell and Andersen 1996).

It should be emphasized that this Monte Carlo analysis does not fully address all potential sources of human inter-individual variability, such as age and genetic polymorphisms. The intention of the Monte Carlo analysis conducted with the chloroprene PBPK model was to characterize the uncertainty in model predictions in the general population. Previous evaluations of the impact of interindividual variability in pharmacokinetics on PBPK model-based risk estimates (Clewell and Andersen 1996) have suggested that the confidence interval for inter-individual variability in human internal dose is generally consistent with the default expectation of a factor of ten; that is, the ratio of a sensitive individual (95th percentile) to an average individual is on the order of a factor of 3. More recently, a MCMC evaluation of the variability in human risk estimates with the PBPK model for methylene chloride (David et al. 2006), which included consideration of a polymorphism for the metabolism of methylene chloride, found that the upper 95th percentile risk in the US population was still within a factor of 3 of the mean risk estimate.

4. DISCUSSION

In this study, we characterized the time course blood concentrations of chloroprene in female B6C3F1 mice during and following a single 6-hour nose-only inhalation exposure over the range of concentrations used in the NTP (1998) bioassays. These data, including both arterial whole blood concentrations and respiratory parameters (breathing frequency and tidal volume) during and after these exposures provide a reliable basis for evaluating the ability of the chloroprene PBPK model to predict *in vivo* pharmacokinetics in the bioassays. We have then applied the PBPK model to calculate dose metrics to support a risk assessment that considers species differences in pharmacokinetics. The use of a PBPK model for this purpose is consistent with the conclusion of the National Academy of Science (NRC 1987) that: "relevant PBPK data can be used to reduce uncertainty in extrapolation and risk assessment." The application of the model is also consistent with recommended practice for the use of PBPK modeling in risk assessment (WHO/IPCS 2010).

It is important to note that, due to the low rates of metabolism in the *in vitro* assays for the rat and human lung, the original chloroprene model (Himmelstein et al. 2004b; Yang et al. 2012) used a linear description of metabolism in these tissues, which would only be appropriate in the concentration range below Km in the lung. Thus model-based metabolism predictions for human exposures significantly greater than 1 ppm would greatly overestimate the associated risk. Moreover, as described in the results section, estimates of linear metabolism from the *in vitro* data for chloroprene in the human lung are unreliable due to the high variability in other linear loss rates. One approach for dealing with the inability to estimate the parameters for saturable metabolism in the human lung is to use the value of Km estimated in the human liver, together with data on the ratio of metabolic activities in the liver and lung. This approach was applied by the USEPA in their risk assessment for methylene chloride using a PBPK model (Andersen et al. 1987) and in the present analysis. The impact of saturable metabolism on human dose metric predictions is shown in Figure 12. Without estimating a value for Km, the model-predicted risks above 1 ppm would continue to increase at a biologically implausible rate.

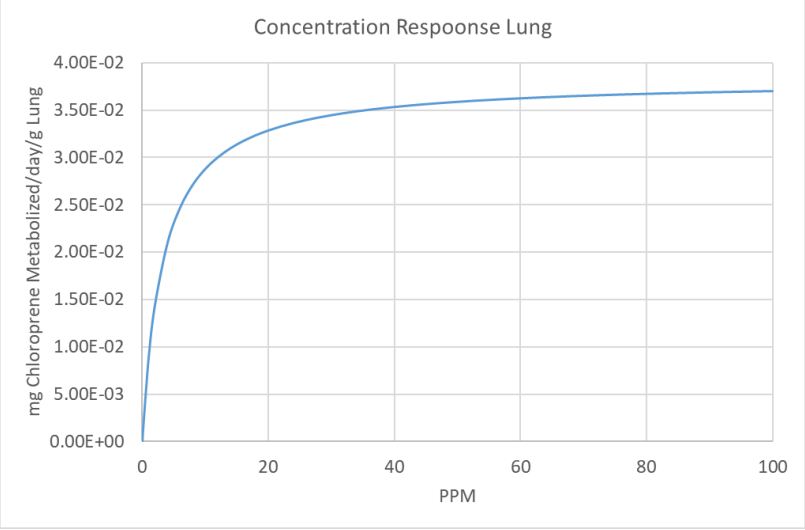

Figure 12. Inhaled concentration dependence of lung metabolism in the human for continuous exposures to chloroprene predicted with the PBPK model.

Interestingly, comparison of the Kms for chloroprene in liver and lung for male and female mice (Table S-3), which are based on the strongest data sets for estimating Kms, suggests that Km may be higher (lower affinity) in the mouse lung than in the mouse liver. This difference in apparent affinities in mouse liver and lung is consistent with differences in the relative tissue abundances of the murine CYP2E1 and CYP2F isozymes, both of which exhibit high affinities for chlorinated alkenes (Yoon et al. 2007). Whereas CYP2E1 is the predominant high affinity isozyme in the mouse liver, CYP2F is the predominant high affinity isozyme in the mouse lung (Yoon et al. 2007) and, consistent with the estimated Kms for chloroprene, the affinity of rCYP2E1 is roughly 3-fold higher (lower Km) than rCYP2F2 (Simmonds et al. 2004). However, since CYP2E1 is the predominant isozyme in both the lung and liver in the human (Nishimura et al. 2003), the estimation of human lung Km based on the human liver Km is appropriate.

It should be emphasized that the parameters in the chloroprene PBPK model represent estimates for an average mouse or human and this analysis does not address human inter-individual variability. The intention of the analysis conducted with the chloroprene PBPK model was to characterize the risk for an average individual. Previous evaluations of the impact of interindividual variability in pharmacokinetics on PBPK model-based risk estimates (Clewell and Andersen 1996) have suggested that the confidence interval for inter-individual variability in human internal dose is generally consistent with the default expectation of a factor of ten; that is, the ratio of a sensitive individual (95th percentile) to an average individual is on the order of a factor of 3. More recently, a MCMC evaluation of the variability in human risk estimates with the PBPK model for methylene chloride (David et al. 2006), which included consideration of a polymorphism for the metabolism of methylene chloride, found that the upper 95th percentile risk in the US population was within a factor of 3 of the mean risk estimate.

Selection of Dose Metric

The dose metric calculated with the PBPK model in this analysis is micromoles of chloroprene metabolized in the lung per gram lung per day (Himmelstein et al. 2004b; Yang et al. 2012). This dose metric was chosen because (1) the lung is the tissue with the highest tumor incidence in the chloroprene inhalation bioassays (NTP 1998) and (2) the carcinogenicity of chloroprene in rodents is believed to result from its metabolism to reactive epoxides in the target tissue (Himmelstein et al. 2004a, 2004b). The dose metric selected for chloroprene is consistent with the dose metrics used in previous PBPK-based risk assessments for both vinyl chloride (Clewell et al. 2001; USEPA 2000) and methylene chloride (Andersen et al. 1987; USEPA 2011), which were also based on the rate of production of reactive metabolites. The dose metric selected for the liver carcinogenicity of vinyl chloride was total mg vinyl chloride metabolized per kg liver per day, representing the production of the reactive chloroethylene epoxide. Due to the presence of chlorine in the epoxides generated from the metabolism of chloroprene, they are considered likely to have a reactivity comparable vinyl chloride (Haley 1978; Plugge and Jaeger 1979). The methylene chloride dose metric was average daily metabolism by the glutathione conjugation pathway in the lung per gram lung, which was selected based on evidence that the carcinogenicity of methylene chloride was associated with the local production of a reactive metabolite from the glutathione conjugate of methylene chloride. As with vinyl chloride and chloroprene, the assumption inherent in the dose metric was that the reactive metabolite would be completely consumed within the tissue where it was generated (Andersen et al. 1987).

Himmelstein et al. (2004b) have previously demonstrated that using the PBPK dose metric is able to harmonize the dose-responses for lung tumors in mice, rats and hamsters. However, they only had metabolism data for male animals. Figure 13 shows an update of the analysis from

Himmelstein et al. (2004b) that includes the results for the female mouse and rat. While the revised PBPK model is still able to demonstrate the consistency of the tumor incidence across male animals of different species and strains, female mice exhibit a higher tumor incidence than male mice at the same rate of lung metabolism.

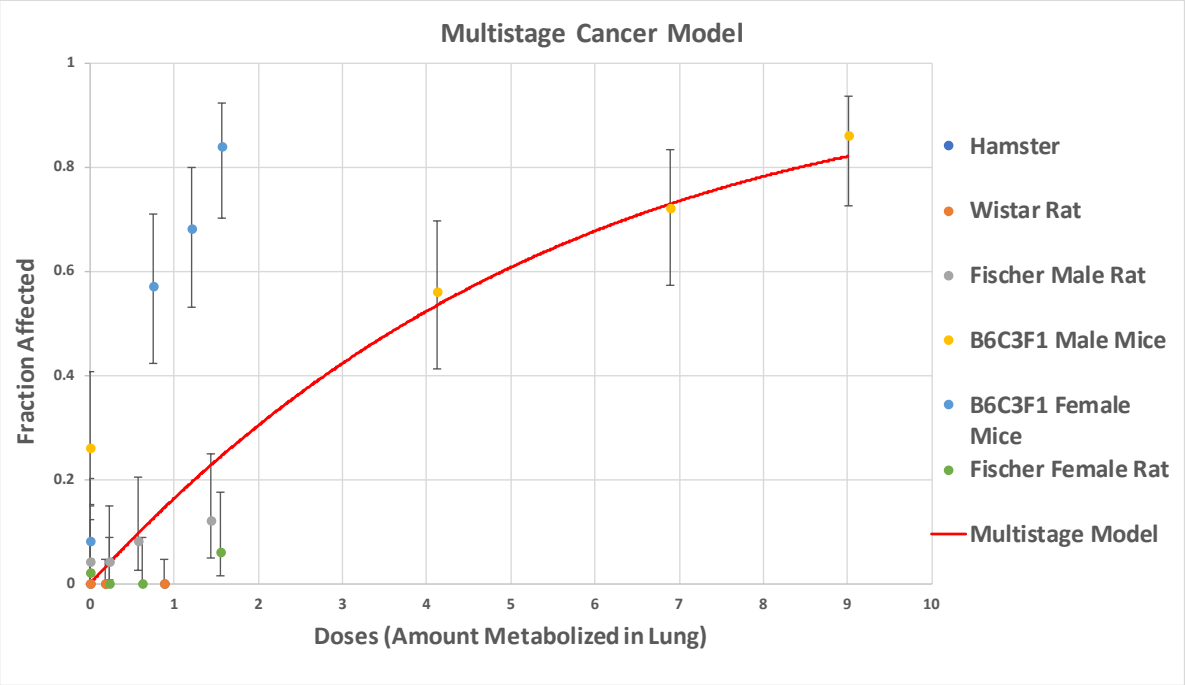

Figure 13. Comparison of Dose-Response for Lung Tumors in Chloroprene Bioassays in Rodents.

This discrepancy could indicate either of two possibilities: (1) the selected dose metric, rate of metabolism of chloroprene in the lung, is incorrect, or (2) the female mouse lung is more sensitive to the effects of chloroprene metabolites than the male mouse lung. Relatively few studies have been conducted to explore gender differences in the responses to chemical insult in the mouse lung. However, Yamada et al. (2017) provides evidence of a proliferative response of Club cells to the toxicity of permethrin in the female mouse lung that is not observed in the male mouse lung, and studies of naphthalene lung toxicity have demonstrated a greater sensitivity of the female mouse lung to both acute and repeated toxicity (Van Winkle et al. 2002, Sutherland et al. 2012). The greater susceptibility to a proliferative response to lung toxicity in the female mouse appears to result from gender differences in the tissue response to damage rather than metabolism (Laura Van Winkle, personal communication). The more sensitive response of the female mouse to oxidative stress and to a proliferative response may underlie the apparent potency difference indicated by Figure 13. Using the metabolism dose metric appropriately considers the greater sensitivity of the female mouse in a manner that is health protective, since the greater sensitivity of the female mouse results in a lower BMDL01 than would be obtained from the male mouse.

The risk assessment for vinyl chloride (USEPA 2000) demonstrated that the use of a PBPK model to estimate target tissue dose (based on total metabolism per gram liver per day) was able to produce similar human risk estimates using data from animal bioassays and human occupational exposures. As a similar test of the chloroprene PBPK model to support cross-species extrapolation, Allen et al. (2014) used a statistical maximum likelihood approach to compare risk

estimates obtained using external (air concentration) and internal (PBPK model estimated) metrics for the female mouse bioassay and human occupational exposures. The analysis concluded that if inhaled concentration was used as the dose metric, the estimates of human cancer risk using animal and human data were statistically significantly different, whereas using the PBPK metric consistent risk estimates were obtained across species. As with vinyl chloride, the use of the PBPK-based metric effectively reconciled the differences in mouse and human low-dose risk estimates.

In an independent external peer review of the PBPK model submission conducted by the USEPA, concerns were raised that the use of total metabolism as the dose metric might underestimate human risk due to potential differences in the clearance of the epoxides produced from chloroprene, and that a more appropriate dose metric might be the average concentration (area under the curve, AUC) for the epoxide concentrations. In response to this concern, a description of the downstream metabolism of chloroprene to epoxides and other reactive products was added to the model. The development of the extended PBPK model, and a comparison of dose-metric predictions, is described in Supplemental Materials F. The results of the reactive product modeling support the use of total metabolism as the most appropriate dose metric for the carcinogenicity of chloroprene and demonstrate that the use of a dose metric based on epoxide AUC is inconsistent with the cross-species relationship of the toxicity and carcinogenicity of chloroprene. The epoxide concentration dose metric was inconsistent with the relationships for both toxicity and carcinogenicity between the female mouse and female rat. The lack of evidence for a significant role of 1-CEO epoxide as a dose metric is due to the key role of tissue toxicity and glutathione depletion in determining the dose response for lung tumors. At the bioassay concentrations, the predicted portion of metabolism producing 1-CEO epoxide is 0.4% of the proportion leading to reactive products in the female mouse and less than 5% in the rat. The correlation with metabolism indicates that other reactive products dominate the mode of action for chloroprene both in determining the shape of the dose -response curve and the incidence of tumors.

Use of *In Vitro* Metabolism Data

The most notable aspect of the chloroprene PBPK model is that, apart from the physiological parameters, the parameters in the model are based on data derived solely from *in vitro* studies. The PBPK model for chloroprene is structurally similar to the PBPK model for methylene chloride (Andersen et al. 1987) and, just as in the case of the methylene chloride risk assessment, model predictions needed to support a risk assessment are critically dependent on parameters that can only be derived from *in vitro* metabolism experiments.

At the time the methylene chloride PBPK model was developed, the use of *in vitro* data to predict *in vivo* metabolism was a relatively new concept, but in the intervening years it has become common practice both for pharmaceuticals (Rostami-Hodjegan 2012) and environmental chemicals (Yoon et al. 2012). While regulatory agency acceptance of PBPK models that are not based primarily on *in vivo* data still presents a challenge (EURL ECVAM 2017), "next generation" physiologically based modeling (NG PBK, Paini et al. 2019) has gained widespread acceptance for supporting regulatory decision making. In this regard, it is important to distinguish two forms of NG PBK: high-throughput IVIVE (HT-IVIVE) and chemical-specific PBPK/QIVIVE. In the HT-IVIVE methodology, a simplified generic pharmacokinetic model is applied across chemicals regardless of the potential impact of chemical-specific properties on the processes affecting their disposition and the nature of their metabolism. The simplified generic models used in HT-IVIVE necessarily ignore many factors that could be an important determinant of steady-state blood concentrations

for a particular chemical, including incomplete absorption, pre-systemic intestinal metabolism, bypassing of hepatic pre-systemic metabolism by lymphatic uptake (in the case of lipophilic compounds), and active renal clearance or resorption. Due to the imprecision associated with this simplified generic approach (Wetmore et al. 2012; Wambaugh et al. 2015), HT-IVIVE is typically applied in screening approaches such as prioritization for further testing based on bioactivity concentrations from high-throughput testing. However, more exacting QIVIVE methods can be applied in chemical specific PBPK modeling, and there are now many examples of published NG PBK models using these techniques to provide more accurate predictions *of in vivo* kinetics (Yoon et al. 2012; Paini et al. 2019). In the development of the chloroprene PBPK model, we have followed the PBPK/QIVIVE approach described in Yoon et al. (2012) and Paini et al. (2019). Going forward it will be important to develop a consensus on standard practices for IVIVE of metabolism in PBPK modeling in order to assist agencies in their evaluations.

Comparison of current MCMC analysis with analysis in Yang et al. (2012)

In their analysis of *in vitro* data on chloroprene metabolism, Yang et al. (2012) employed both a standard frequentist approach (referred to in their analysis as a "deterministic" approach) and an approach that used a Markov Chain Monte Carlo (MCMC) method (referred to as a "probabilistic" approach) with non-informative prior distributions for all estimated parameters. The use of non-informative priors allows this Bayesian approach to be interpreted from a frequentist perspective. As stated in the Yang et al. (2012) document, the two methods were compared to demonstrate that they provided consistent estimates of metabolic parameter values. Yang et al. (2012) then relied on the MCMC-based estimates for developing dose metrics for chloroprene exposures in mouse, rat and human. Because it seeks a global optimum using a probabilistic direct search algorithm, MCMC is less likely than deterministic search algorithms to converge on a local optimum. Moreover, when used with non-informative priors, as in Yang et al. (2012), the posterior distribution represents the likelihood distribution for the parameter, and the mode of the distribution represents the maximum likelihood estimate (MLE). As pointed out in Chiu et al. (2007), the Bayesian approach, in principle, yields a more global characterization of parameter uncertainty than the local, linearized variance estimates provided by traditional optimization routines, which should be viewed as lower bound estimates of true parameter uncertainty. Because of its superior properties, we have also relied on the MCMC approach in our re-analysis of the original *in vitro* metabolism data.

The key difference between the MCMC analysis performed in this study and the original analysis (Yang et al. 2012) was that this re-analysis included an additional parameter (Kgl) for the *in vitro* experiments, representing the potential for a mass transport limitation for uptake of chloroprene from the air in the metabolism vials. Therefore, for this comparison, the PBPK model was run to obtain dose metrics with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies (Yang et al. 2012 parameters), and (2) that there was a transport limitation with Kgl = 0.22 (current re-analysis). Again, due to the high uncertainty of the human lung metabolism parameter, the approach using A1 from Andersen et al. (1987) was applied.

The results with the two parameterizations, with and without Kgl, are compared in Table 8. Using the new parameters estimated under the assumption of an air:liquid transport limitation in the *in vitro* studies, the mouse dose metrics increase by roughly 8-13% and the human dose metrics increase by roughly 20%, but the mouse/human ratios are similar, providing additional evidence of the robustness of the PBPK model.

Exposure	Concentration	Dose Metric Yang et al. 2012 parameters	Dose Metric Re-estimated parameters
Female mouse bioassay	12.8 ppm	0.75	0.85
	32 ppm	1.20	1.29
	80 ppm	1.57	1.69
Human continuous	1 μg/m^3	2.7x10^{-6}	3.24E-06

Table 8. Comparison of daily lung metabolism dose metrics in the mouse bioassay and for a human continuous exposure to 1 μg/m^3 chloroprene using either the parameters from Yang et al. (2012) or the newly estimated parameters in this study.

PBPK modeling has now been applied in risk assessments for a variety of environmental chemicals by regulatory agencies worldwide. The development of these models has typically required the use of *in vivo* experimental animal and/or human data to estimate key kinetic parameters such as uptake, metabolism and elimination. Some agencies also require the use of separate *in vivo* data to demonstrate model validity. However, it has become increasingly difficult to conduct controlled exposures of human subjects to chemicals of concern, other than for pharmaceuticals. The need for live animal studies is also being challenged, particularly in the EU, due to both ethical and practical (cost, throughput) concerns. Therefore, requirements for *in vivo* testing will increasingly limit the potential application of PBPK modeling in risk assessment, and agencies will need to consider whether *in vivo* validation data are truly necessary for assessing the fitness of a model for the specific purpose of its use in a particular risk assessment. To support these decisions, PBPK model evaluations should make greater use of uncertainty analyses to estimate the potential reduction in model uncertainty associated with the collection of additional data; that is, to determine the added value of a proposed study (Clewell et al. 2008; Keisler et al. 2013; Wilson 2015).

The original chloroprene PBPK model (Himmelstein et al. 2004b) was not used by USEPA (2010) because the agency considered it necessary to have blood or tissue time course concentration data from an *in vivo* study to adequately validate the model. The study reported here was conducted to address this requirement and we have now demonstrated that the chloroprene PBPK model accurately simulates these *in vivo* blood time course validation data.

No *in vivo* validation data for chloroprene are available in the human, and it is unlikely that such a study could be performed given the current classification of chloroprene as "likely to be a carcinogen" (USEPA 2010). However, the sensitivity analyses reported here suggest that such a study would not provide significant added value for demonstrating that the PBPK model is fit for purpose for a chloroprene risk assessment. The validity of the model instead derives from the biological validity of the physiological and biochemical underpinnings of the model structure and parameters. The key parameters for performing a risk assessment for chloroprene are those for lung metabolism, and a human *in vivo* study would not be able to provide informative data for those parameters. As shown in Figure 8, blood concentrations of chloroprene associated with inhalation are insensitive to lung metabolism, and depend only on alveolar ventilation, cardiac output, blood:air partition coefficient and fractional blood flow to liver, which serves as the primary site of metabolic clearance.

The limited value of human *in vivo* data for determining whether a PBPK model is fit for purpose in a risk assessment based on target tissue metabolism was also an issue during the

development of the PBPK model of methylene chloride (Andersen et al. 1987), where a similar dose metric was used: average daily metabolism of methylene chloride by glutathione transferase (GST) in the lung per gram lung. Although the model accurately reproduced blood and exhaled air concentration time-course data from multiple studies with human subjects, the *in vivo* data were not adequate to estimate the rates of GST metabolism in the liver and lung. Instead, it was necessary to estimate the rate of GST metabolism in the human liver by allometric scaling from animal data (Andersen et al. 1987), and to then estimate the rate of GST metabolism in the human lung using the ratio of specific activities for GST metabolism in liver and lung measured *in vitro* by Lorenz et al. (1984). This same approach was used in the chloroprene modeling documented in this report.

5. CONCLUSION

A PBPK model of chloroprene that relies solely on data from *in vitro* studies for its metabolism parameters accurately predicts the *in vivo* time course for chloroprene in the blood of female mice exposed by nose-only inhalation at the 3 concentrations used in the chloroprene 2-year cancer bioassay. This PBPK model has been used to estimate dose metrics for the metabolism of chloroprene to reactive epoxides in the lung target tissue of mice and humans to support an inhalation cancer risk assessment for chloroprene. Large differences between PBPK-based risk estimates and estimates based on inhaled concentration have been seen in previous inhalation risk assessments for chemicals where toxicity results from the production of reactive metabolites (Andersen et al. 1987; Clewell et al. 2001). The present PBPK model follows the same approach used in these previous PBPK models used in risk assessments by the USEPA and incorporates the best available science to describe the impact of species differences in metabolism on the potential cancer risk associated with chloroprene inhalation.

6. ACKNOWLEDGEMENTS

From the work at the Hamner Institutes for Health Sciences, we thank Dr. Mark A. Sochaski (analytical chemistry), Carl U. Parkinson, Jr. (inhalation generation/characterization), Paul W. Ross, Carol M. Bobbitt and Kathy A. Bragg (animal care). The authors would also like to thank Paul Schlosser, USEPA/NCEA, for his helpful comments and suggestions during the preparation of this report. This research was supported by IISRP (*in vivo* study) and Denka Performance Elastomer LLC (PBPK modeling). The authors would also like to thank Matt Himmelstein and Patrick Walsh, who served as the technical contacts for IISRP and Denka, respectively, for their helpful support and guidance during the research effort, and TekLab, Inc, Environmental Laboratory for conducting the chloroprene Kgl study.

7. REFERENCES

Allen BC, Van Landingham C, Yang Y, Youk AO, Marsh GM, Esmen N, Gentry PR, Clewell HJ III, Himmelstein MW. 2014. A constrained maximum likelihood approach to evaluate the impact of dose metric on cancer risk assessment: Application to b-chloroprene. Regulatory Toxicology and Pharmacology, 70(1): 203–213.

Amet Y, Berthou F, Fournier G, Dréano Y, Bardou L, Clèdes J, Ménez JF. 1997. Cytochrome P450 4A and 2E1 expression in human kidney microsomes. Biochemical Pharmacology, 53(6): 765–771.

Andersen ME, Clewell HJ III, Gargas ML, Smith FA, Reitz RH. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicology and Applied Pharmacology, 87(2): 185-205.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health, 13(4): 407–484.

Barter ZE, Bayliss MK, Beaune PH, Boobis AR, Carlile DJ, Edwards RJ, Houston JB, Lake BG, Lipscomb JC, Pelkonen OR, Tucker GT, Rostami- Hodjegan A. 2007. Scaling factors for the extrapolation of *in vivo* metabolic drug clearance from *in vitro* data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. Current Drug Metabolism, 8(1): 33-45.

Bièche I, Narjoz C, Asselah T, Vacher S, Marcellin P, Lidereau R, Beaune P, de Waziers I. 2007. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues. Pharmacogenetics and Genomics, 17(9): 731–742.

Boogaard PJ, de Kloe KP, Bierau J, Kuiken G, Borkulo PE, Watson WP, van Sittert NJ. 2000. Metabolic inactivation of five glycidyl ethers in lung and liver of humans, rats and mice *in vitro*. Xenobiotica. 30(5): 485-502. Erratum in: Xenobiotica 2002 32(10): 935.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health, 13(4): 407–484.

Chiu WA, Barton HA, DeWoskin RS, Schlosser P, Thompson CM, Sonawane B, Lipscomb JC, Krishnan K. 2007. Evaluation of physiologically based pharmacokinetic models for use in risk assessment. Journal of Applied Toxicology, 27(3): 218-237.

Clewell HJ III, Andersen ME. 1996. Use of physiologically-based pharmacokinetic modeling to investigate individual versus population risk. Toxicology, 111(1-3): 315-329.

Clewell HJ 3rd, Jarnot BM. 1994. Incorporation of pharmacokinetics in noncancer risk assessment: example with chloropentafluorobenzene. Risk Analysis, 14(3): 265–276.

Clewell HJ 3rd, Campbell JL, Van Landingham C, Franzen A, Yoon M, Dodd DE, Andersen ME, Gentry PR. 2019. Incorporation of *in vitro* metabolism data and physiologically based pharmacokinetic modeling in a risk assessment for chloroprene. Inhalation Toxicology, 31(13-14): 468-483.

Clewell HJ III, Gentry PR, Gearhart JM, Allen BC, Andersen ME. 2001. Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. The Science of the Total Environment, 274(1-3): 37-66.

Clewell HJ III, Andersen HJ, Blaauboer BJ. 2008. On the incorporation of chemical-specific information in risk assessment. Toxicology Letters, 180(2): 100-109.

Crawford EL, Weaver DA, DeMuth JP, Jackson CM, Khuder SA, Frampton MW, Utell MJ, Thilly WG, Willey JC. 1998. Measurement of cytochrome P450 2A6 and 2E1 gene expression in primary human bronchial epithelial cells. Carcinogenesis, 19(10): 1867–1871.

David RM, Clewell HJ III, Gentry PR, Covington TR, Morgott DA, Marino DJ. 2006. Revised assessment of cancer risk to dichloromethane II: Application of probabilistic methods to cancer risk determination. Regulatory Toxicology and Pharmacology, 45(1): 55-65.

Duescher RJ, Elfarra AA. 1994. Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: evidence for major roles by cytochromes P450 2A6 and 2E1. Arch Biochem Biophys. 311(2):342-349.

EURL ECVAM. 2017. Workshop on New Generation of Physiologically-Based Kinetic Models in Risk Assessment, EUR 28794 EN, Publications Office of the European Union, Luxembourg,2017, ISBN 978-92-79-73849-4, doi 10.2760/035371, PUBSY No. JRC108231

Forkert PG, Baldwin RM, Millen B, Lash LH, Putt DA, Shultz MA, Collins KS. 2005. Pulmonary bioactivation of trichloroethylene to chloral 14hydrate: relative contributions of CYP2E1, CYP2F, and CYP2B1. Drug Metab Dispos. 33(10):1429-37.

Hakkola J, Pasanen M, Purkunen R, Saarikoski S, Pelkonen O, Mäenpää J, Rane A, Raunio H. 1994. Expression of xenobiotic-metabolizing cytochrome P450 forms in human adult and fetal liver. Biochemical pharmacology, 48(1): 59–64.

Haley T. 1978. Chloroprene (2-chloro-1,3-butadiene) —What is the evidence for its carcinogenicity? Clinical Toxicology, 13(2): 153-170.

Himmelstein MW, Turner MJ, Asgharian B, Bond JA. 1994. Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3-butadiene by inhalation. Carcinogenesis, 15(8): 1479-1486.

Himmelstein MW, Gladnick NL, Donner EM, Snyder RD, Valentine R. 2001. *In vitro* genotoxicity testing of (1-chloroethenyl)oxirane, a metabolite of beta-chloroprene. Chemico-Biological Interactions, 135-136: 703-713.

Himmelstein MW, Carpenter SC, Hinderliter PM. 2004a. Kinetic modeling of beta-chloroprene metabolism: I. *In vitro* rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. Toxicological Sciences, 79(1): 18-27.

Himmelstein MW, Carpenter SC, Evans MV, Hinderliter PM, Kenyon EM. 2004b. Kinetic modeling of beta-chloroprene metabolism: II. The application of physiologically based modeling for cancer dose response analysis. Toxicological Sciences, 79(1): 28-37.

Houston JB, Galetin A. 2008. Methods for predicting *in vivo* pharmacokinetics using data from *in vitro* assays. Current Drug Metabolism, 9(9): 940-951.

Hukkanen J, Pelkonen O, Hakkola J, Raunio H. 2002. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. Critical Reviews in Toxicology, 32(5): 391–411.

IISRP. 2009a. Chloroprene: Blood Concentration Toxicokinetics in Female Mice by Single and Repeated Inhalation Exposure. International Institute of Synthetic Rubber Producers, IISRP-12828-1388.

IISRP. 2009b. β Chloroprene: *In Vitro* Rate Constants for Metabolism in Liver, Lung, and Kidney Microsomes. International Institute of Synthetic Rubber Producers. IISRP-17520-1388. Docket ID No. EPA-HQ-ORD-2009-0217.

Keisler J, Collier Z, Chu E, Sinatra N, Linkov I. 2013. Value of Information Analysis: The State of Application. Environment, Systems, Decisions, 34(1): 3-23.

Lorenz J, Glatt HR, Fleischmann R, Ferlinz R, Oesch F. 1984. Drug metabolism in man and its relationship to that in three rodent species: monooxygenase, epoxide hydrolase, and glutathione S-transferase activities in subcellular fractions of lung and liver. Biochemical Medicine, 32(1): 43-56.

Marino DJ, Clewell HJ III, Gentry PR, Covington TR, Hack CE, David RM, Morgott DA. 2006. Revised assessment of cancer risk to dichloromethane I: Bayesian PBPK and dose-response modeling in mice. Regulatory Toxicology and Pharmacology, 45(1): 44-54.

Medinsky MA, Leavens TL, Csanády GA, Gargas ML, Bond JA. 1994. *In vivo* metabolism of butadiene by mice and rats: a comparison of physiological model predictions and experimental data. Carcinogenesis, 15(7): 1329-1340.

Melnick RL, Sills RC, Portier CJ, Roycroft JH, Chou BJ, Grumbein SL, Miller RA. 1999. Multiple organ carcinogenicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344/N rats and B6C3F1 mice and comparison of dose-response with 1,3-butadiene in mice. Carcinogenesis, 20(5): 867-878.

NRC. 1987. Pharmacokinetics in Risk Assessment. Drinking Water and Health, Volume 8. National Research Council, National Academy Press. Washington DC.

NTP. 1998. Toxicology and carcinogenesis studies of chloroprene (CAS No. 126-99-8) in F344 rats and B6C3F1 mice (inhalation studies). Public Health Service, United States Department of Health and Human Services; NTP TR-467. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Available at: http://ntp.niehs.nih.gov/ntp/htdocs/LT)rpts/tr467.pdf.

Nishimura M, Yaguti H, Yoshitsugu H, Naito S, Satoh T. 2003. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. Yakugaku Zasshi. 123(5): 369-375.

Paini A, Leonard JA, Joossens E, Bessems JGM, Desalegn A, Dorne JL, Gosling JP, Heringa MB, Klaric M, Kliment T, Kramer NI, Loizou G, Louisse J, Lumen A, Madden JC, Patterson EA, Proença S, Punt A, Setzer RW, Suciu N, Troutman J, Yoon M, Worth A, Tan YM. 2019. Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. Computational Toxicology, 9: 61-72.

Plugge H, Jaeger RJ. 1979. Acute inhalation of 2-chloro-1,3-butadiene (chloroprene): Effects on liver and lung. Toxicology and Applied Pharmacology, 50(3): 565-572.

Ramsey JC, Andersen ME. 1984. A physiological model for the inhalation pharmacokinetics of inhaled styrene monomer in rats and humans. Toxicology and Applied Pharmacology, 73(1): 159–175.

Rostami-Hodjegan A. 2012. Physiologically based pharmacokinetics joined with *in vitro-in vivo* extrapolation of ADME: a marriage under the arch of systems pharmacology. Clinical Pharmacology and Therapeutics, 92(1): 50-61.

Sasso AF, Schlosser PM, Kedderis GL, Genter MB, Snawder JE, Li Z, Rieth S, Lipscomb JC. 2013. Application of an updated physiologically based pharmacokinetic model for chloroform to evaluate CYP2E1-mediated renal toxicity in rats and mice. Toxicological Sciences, 131(2): 360–374.

Schlosser PM, Bond JA, Medinsky MA. 1993. Benzene and phenol metabolism by mouse and rat liver microsomes. Carcinogenesis, 14(12): 2477-2486.

Scotcher D, Billington S, Brown J, Jones CR, Brown CDA, Rostami-Hodjegan A, Galetin A. 2017. Microsomal and cytosolic scaling factors in dog and human kidney cortex and application for *in vitro-in vivo* extrapolation of renal metabolic clearance. Drug Metabolism and Disposition, 45(5): 556-568.

Simmonds AC, Reilly CA, Baldwin RM, Ghanayem BI, Lanza DL, Yost GS, Collins KS, Forkert PG. 2004. Bioactivation of 1,1-dichloroethylene to its epoxide by CYP2E1 and CYP2F enzymes. Drug Metabolism and Disposition, 32(9): 1032–1039.

Sutherland KM, Edwards PC, Combs TJ, Van Winkle LS. 2012. Sex differences in the development of airway epithelial tolerance to naphthalene. American Journal of Physiology, 302(1): L68-L81.

Thomas RS, Himmelstein MW, Clewell HJ III, Yang Y, Healy E, Black MB, Andersen ME. 2013. Cross-species transcriptomic analysis of mouse and rat lung exposed to chloroprene. Toxicological Sciences, 131(2): 629-640.

USEPA. 2000. Vinyl Chloride: CASRN 75-01-4. Integrated Risk Information System (IRIS): Chemical Assessment Summary. United States Environmental Protection Agency; National Center for Environmental Assessment. Available at: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/1001_summary.pdf

USEPA. 2010. Toxicological Review of Chloroprene (CAS No. 126-99-8) In support of Summary Information on the Integrated Risk Information System (IRIS). United States Environmental Protection Agency. Washington, DC. Available at: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0070tr.pdf

USEPA. 2011. Dichloromethane: CASRN 75-09-2. Integrated Risk Information System (IRIS): Chemical Assessment Summary. United States Environmental Protection Agency; National Center for Environmental Assessment. Washington, DC. Available at: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0070_summary.pdf

USEPA. 2020a. External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene (Ramboll, 2020) and a Supplemental Analysis of Metabolite Clearance (U.S. EPA, 2020). Post-Meeting Peer Review Summary Report. December 17, 2020. Prepared by Versar, Inc. for the United States Environmental Protection Agency. Washington, DC.

USEPA. 2020b. Draft Charge Questions for Peer Review of Chloroprene PBPK Modeling (July 2020).

Van Winkle LS, Gunderson AD, Shimizu JA, Baker GL, Brown CD. 2002. Gender differences in naphthalene metabolism and naphthalene-induced acute lung injury. American Journal of Physiology, 282(5): L1122-L1134.

Wambaugh JF, Wetmore BA, Pearce R, Strope C, Goldsmith R, Sluka JP, Sedykh A, Tropsha A, Bosgra S, Shah I, Judson R, Thomas RS, Setzer RW. 2015. Toxicokinetic Triage for Environmental Chemicals. Toxicological Sciences. 147(1): 55-67.

Wetmore BA, Wambaugh JF, Allen B, Ferguson SS, Sochaski MA, Setzer RW, Houck KA, Strope CL, Cantwell K, Judson RS, LeCluyse E, Clewell HJ 3rd, Thomas RS, Andersen ME. 2015. Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing. Toxicological Sciences, 148(1): 121-136.

WHO/IPCS. 2010. Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment. Harmonization Project Document No. 9. World Health Organization. International Programme on Chemical Safety, Geneva, Switzerland.

Wilson EC. 2015. A practical guide to value of information analysis. Pharmacoeconomics. 33(2): 105-121.

Yamada T, Kondo M, Miyata K, Ogata K, Kushida M, Sumida K, Kawamura S, Osimitz TG, Lake BG, Cohen SM. 2017. An evaluation of the human relevance of the lung tumors observed in female mice treated with permethrin based on mode of action. Toxicological Sciences, 157(2): 465-486.

Yang Y, Himmelstein MW, Clewell HJ III. 2012. Kinetic modeling of b-chloroprene metabolism: Probabilistic *in vitro–in vivo* extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans. Toxicology *in Vitro,* 26(6): 1047–1055.

Yoon M, Madden MC, Barton HA. 2007. Extrahepatic metabolism by CYP2E1 in PBPK modeling of lipophilic volatile organic chemicals: Impacts on metabolic parameter estimation and prediction of dose metrics. Journal of Toxicology and Environmental Health Part A, 70(18): 1527-1541.

Yoon M, Campbell JL, Andersen ME, Clewell HJ III. 2012. Quantitative *in vitro* to *in vivo* extrapolation of cell-based toxicity assay results. Critical Reviews in Toxicology, 42(8): 633-652.

Supplemental Materials

A. Supplemental Tables

B. Re-estimation of Metabolism Parameters

C. IVIVE Literature Review

D. Metabolism Parameter Calculations

E. Model Files

F. Reactive Metabolite Modeling

G. Responses to Peer Reviewer Comments