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

The objective of this peer review is to provide advice on the applicability of the chloroprene PBPK model developed by Ramboll for possible use in a human health risk assessment for inhalation exposure to chloroprene, as well as input on the applicability of an uncertainty analysis proposed by U.S. EPA.

Scope of Review

This review is focused on the chloroprene PBPK model and supporting in vitro metabolic model, with resulting parameters, model predictions and uncertainty analyses, described by Ramboll (2020), and the alternate uncertainty analysis described by U.S. EPA (2020). In particular, the Ramboll (2020) model predictions of the rate of chloroprene oxidative metabolism in the liver, lung, and kidney of mice, rats, and humans (to the extent included in the model) and predictions of the concentration of chloroprene in all tissues, are being evaluated to determine if they are of sufficient scientific quality and quantitative confidence for use in an IRIS Toxicological Review (see "Background for the Peer Review" document). The approach for estimating uncertainty in the parameters of the in vitro system model and subsequently in the chloroprene PBPK model predictions described by U.S. EPA (2020) is likewise being evaluated as a method to quantify model uncertainty in an IRIS Toxicological Review.

Report Structure

We request that all advice given is prioritized to indicate its 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.

Charge Questions

Estimation of Mass Transfer Resistance in the *In Vitro* Metabolism Experiments

A model of the *in vitro* incubation system was used to estimate the metabolic parameters from the *in vitro* data. This model is based on certain assumptions and physical parameters, such as the volume of the *in vitro* incubation vials and volumes of air and liquid media in the vials.

The model of the *in vitro* system initially used for the analysis of the *in vitro* experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the

chloroprene in the air and liquid (incubation medium) phases was at equilibrium at all times after the start of the experiment; i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others.

1. *Please evaluate the validity and uncertainties of these two approaches to estimation of the kinetics in the vitro system and therefore in the estimation of metabolic parameters:*
   a) treating the air and liquid phases as always being at equilibrium (original model); or
   b) treating the air and liquid phases as distinct compartments with the rate of transfer limited and determined by a mass-transfer constant (Kgl).

Experiments were conducted to determine the Kgl for the *in vitro* system, however the value of Kgl obtained from those experiments is not consistent with some of the observed metabolic data (Ramboll (2020) Supplemental Material B), and Kgl would need to be at least 8 times higher to obtain results consistent with those data and to obtain a Km consistent with metabolic parameters reported for other VOCs. This inconsistency may exist because the experiments conducted to estimate Kgl used an incubator mixing speed of 60 rpm while the experiments of Himmelstein et al. (2004) and Yang et al. (2012) used 500 rpm. Also, the experiments to measure Kgl were performed without microsomal protein and the report hypothesizes that the presence of microsomal protein (1–3 mg/mL) in the metabolic experiments could increase mass transfer. It is noted that the mean value of the partition coefficient, P, estimated from the Kgl data in the absence of microsomal protein was 0.48 (Ramboll (2020) Supplemental Material B) while that reported by Himmelstein et al. (2001) for chloroprene equilibration with media containing heat-inactive protein was 0.69, 44% higher. To be clear, simulations of the metabolically active experiments used to estimate the metabolic parameters used P = 0.69, so have accounted for the difference in the equilibrium partition coefficient, but are still not consistent with the highest activity data when using the value of Kgl obtained from the 60 rpm data.

2. *Please comment on the likelihood that either the presence of microsomal protein (1–3 mg/mL) or that the higher mixing speed used in the metabolic experiments (500 rpm) vs. the mass transfer experiments (60 rpm) would increase the rate of chloroprene mass transfer between the air and liquid phases in the in vitro system by a factor of 8 or greater, relative to the rate observed in the mass-transfer experiments.*

An analysis provided in Supplemental Material B of Ramboll (2020) demonstrates that estimates of the metabolic parameter Km depend strongly on the value of Kgl. Two approaches were used to estimate the value of Kgl:

a) the measured Kgl was increased by (500/60), the ratio of mixing speeds in the metabolic experiments vs. Kgl experiments, yielding Kgl = 0.2 L/h; and
b) a Bayesian analysis used to estimate Kgl from the metabolic data yielded a mean Kgl = 0.22 L/h.

3. *Given the two-compartment in vitro model structure, please comment on the two approaches for estimating Kgl and whether the value obtained is sufficiently reliable to support valid estimates of metabolic parameters and assess the uncertainties in those estimates.*

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

The following questions address the robustness of the available metabolic data for application in the model. The questions are written with the assumption that the choice of Kgl is appropriate. Using this value of Kgl while evaluating the remaining analysis of *in vitro* metabolic data as described in Supplemental Material B of Ramboll (2020) results in parameter values listed in Table S-3 of Supplemental Material A of Ramboll (2020). For the chloroprene *in vitro* experiments, the human liver microsome samples were obtained from a pool of 15 donors while the human lung microsomes were obtained from a pool of 5 individuals (Himmelstein et al., 2004). For the 7-ethoxycoumarin *in vitro* experiments used to estimate the relative lung:liver metabolic activity, represented by the parameter A1, tissue samples were not pooled; activity was measured in liver microsomes obtained from 10 donors while the human lung activity was measured using microsomes from 12 donors (Lorenz et al., 1984). Other information on the specific microsomal samples, preparation methods and *in vitro* experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

4. *Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.*
5. *Discuss the appropriateness of the data used and the statistical modeling approach with regard to representing average (or mean) adult human, mouse, and rat metabolic parameters. In particular, please comment on whether a sufficient number of microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.*
6. *Considering the experimental and computational methods, please comment on the potential order of magnitude and direction of bias of the quantitative uncertainties in the estimated in vitro metabolic rates that may be related to these factors, collectively.*

Additional discussion on the estimation of lung metabolic parameters in rats and humans is provided in Supplemental Material C of Ramboll (2020) in a section entitled "IVIVE for first order metabolic clearance in rat and human lung." However, the metabolic rate parameter values for the human lung were ultimately selected as described in the main report in a subsection entitled "Estimation of chloroprene metabolism in the human lung" because the *in vitro* chloroprene experiments with human lung microsomes showed minimal metabolism.

7. *Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.*
8. *Please comment on the possible use of a parallel approach, based on the relative activity of 7-ethoxycoumarin or another marker CYP2E1 substrate, to estimate the rate of metabolism in the rat lung and the human kidney.*

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the *Model Parameters* section of the Ramboll (2020) report, with details on scaling factors in Supplemental Material C of Ramboll (2020) and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of

Ramboll (2020). The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of a number of pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance. However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism (Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on predictions of hepatic *clearance* of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

9. *Please evaluate the choices of extrapolation factors and formulas used for the IVIVE calculations. Please discuss the soundness of the metabolic parameters in Table S-4 as estimates for average adult female and male mice and rats, and average adult humans (combined sexes).*

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

10. *Please discuss the appropriateness of the PBPK model structure presented by Ramboll (2020) for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please consider in particular the model structure for the kidney, liver, and lung; i.e., tissues in which chloroprene metabolism is predicted by the model.*

Arterial blood concentrations in B6C3F1 mice after inhalation exposures to chloroprene are shown in Figure 3 of Ramboll (2020). In particular, it is noted that when chloroprene exposure was increased 2.5-fold from 13 to 32 ppm, the mean arterial concentration increased less than 1.5-fold. Further, the mean arterial concentrations from 90 ppm exposure, which is seven (7) times higher than 13 ppm, are only about 4 times higher than those measured at 13 ppm. These data might indicate that some process not included in the PBPK model may have reduced chloroprene uptake or somehow increased metabolic efficiency at 90 and 32 ppm relative to 13 ppm. A factor to be considered is the high variability with large standard deviations for many of the data points, as illustrated in Figure 3 of Ramboll (2020). The PBPK model structure implies that blood levels should increase in proportion to exposure as long as blood concentrations remain below the level of metabolic saturation and should increase at a faster rate above saturation, unless there is some other exposure-related change in model parameters. However, the plethysmography data evaluated do not show a clear or significant dose-response Ramboll (2020). Figure 7 of Ramboll (2020) presents the extent of agreement of the model predictions with the blood concentrations in mice following inhalation exposure. It is noted that the inhalation PK data are from a single exposure (animals were not previously exposed to chloroprene) and the non-proportionality is evident by the 3-hour time-point.

11. *Given these data, please evaluate the likelihood that changes in respiration rate or metabolic induction might be factors in the observed PK relationship between exposure and internal dose. Please comment on any other physiological or biochemical mechanisms that might be explanatory factors in the apparent discrepancy or whether experimental variability in the data may explain these differences.*

In the *Model Parameters* section of the Ramboll (2020) report, the authors describe the apparent discrepancy between the rate constant for cardiac output (QCC) from Brown et al. (1997) and other data. The sensitivity of the predicted blood concentration to unscaled cardiac output is shown in Figures 5 and 6 of the report.

12. *Please comment on the analysis presented here and the proposed choice of QCC for the mouse.*

13. *Given the specific considerations above, please comment on the appropriateness of the values selected for the physiological parameters in Table S-1 and partition coefficients in Table S-2, for prediction of chloroprene dosimetry.*

Overall PBPK Model Soundness and Applicability

Model-predicted doses in model tissue compartments corresponding to tissues in which neoplasm were observed in the rat and mouse bioassay, with corresponding cancer incidence for 80 ppm chloroprene inhalation exposure, are provided in the EPA background document. In potential application to human health risk assessment, the relative risk of tumors in human liver and lung will depend on the relative rate of metabolism predicted in those tissues, compared to the mouse or rat (as well as the relative rate of clearance). Estimation of risks for tissues other than liver and lung could depend on the relative estimates of chloroprene venous blood or tissue concentration. An evaluation of the model's applicability and degree of uncertainty should consider both the absolute model predictions (i.e., does the model accurately predict the absolute rates of metabolism and blood/tissue concentrations in each species?) and also the ability to predict the relative rate of metabolism or relative concentration in human vs. rodent tissues, though some inaccuracy in the absolute values may exist. See "Background for the Peer Review" document for additional context.

Demonstration of the PBPK model's ability to predict *in vivo* PK data is shown by the level of agreement between model predictions and chloroprene venous blood concentrations in Figure 7 of Ramboll (2020). For reference, where there are data, and as a rule of thumb, EPA often seeks dosimetric estimates from a model that are within a factor of two of empirical results. The results of the sensitivity analysis shown in Figure 8 for arterial concentrations indicate that these data and specific predictions are not sensitive to the estimated metabolic parameters: a relatively large range in the estimated metabolic parameters (such as the apparent difference between male and female mouse parameters) would yield similar predictions of blood concentrations. However, as demonstrated in Figure 9, the estimation of lung dose metrics is sensitive to the estimated metabolic parameters.

14. *Please comment on the capacity of the PBPK model to provide sound estimates of chloroprene inhalation dosimetry in mice, rats, and humans. In particular, please comment on the reliability of model predictions of the rate of chloroprene metabolism in liver and lung for use in animal-to-*

human extrapolation. Please also comment on the reliability and uncertainty of model predictions of chloroprene concentrations in blood and other tissues from inhalation exposures. Please provide your scientific judgement about the potential order of magnitude of quantitative uncertainty in these estimates.

Proposed Uncertainty Analysis of *In Vitro* Metabolic Data and PBPK Model Predictions

The U.S. EPA seeks input on initial analyses that it has conducted, its proposed approach to evaluate quantitative uncertainty of the metabolic parameters estimated from *in vitro* data, and its proposed approach to incorporate the metabolic parameter uncertainty into an estimate of uncertainty in the PBPK model predictions U.S. EPA (2020).

15. *Please comment on the analysis and statistical assumptions for control data from Yang et al. (2012) as an approach for evaluating the underlying experiments, data, and distribution of RLOSS for use in subsequent uncertainty analyses of the metabolic data.*
16. *Considering the preliminary results for RLOSS provided, please provide any specific suggestions you may have for how the analyses methods might be improved.*

A similar analysis was conducted using data from five control incubations obtained by Himmelstein et al. (2004). Comparison of the results for RLOSS based on Yang et al. (2012) control data vs. Himmelstein et al. (2004) control data indicates that the value of RLOSS may have been lower in the Himmelstein et al. (2004) study. The two sets of experimental *in vitro* studies were conducted in the same laboratory by the same principle investigator (Matthew Himmelstein), but given the period of time between the two studies, the applicability of non-concurrent control data is a source of uncertainty.

17. *Please comment and provide any specific suggestions you have on the possible use of either:*
    a. *separate distributions of RLOSS obtained from the Yang et al. (2012) vs. Himmelstein et al. (2004) studies when analyzing the uncertainty for the different metabolic parameters obtained with data from the respective studies; or*
    b. *combining the control incubation data and analysis to obtain a distribution applicable to all metabolic data.*

U.S. EPA (2020) describes intended methods for evaluating the uncertainty in the metabolic parameters obtained from the *in vitro* data, given the distribution in RLOSS already obtained. The analysis is particularly focused on the human liver and lung data, which were obtained with pooled microsomes from 15 individuals for liver microsomes and 5 individuals for lung microsomes.

18. *Please evaluate the planned analysis as an appropriate statistical approach for evaluating the uncertainty in the metabolic parameters for the pooled tissue samples. Note any additional quantitative factors whose uncertainty you believe would not be addressed by this approach. Please provide any specific suggestions you have on how the analysis should be modified.*

U.S. EPA (2020) describes intended methods for evaluating the uncertainty in the PBPK model predictions for the rate of metabolism in liver, lung, and kidney, and in predictions of chloroprene venous blood concentrations. Since the analysis is focused on estimation of population average doses, uncertainty in human physiological parameters would be quantified as uncertainty in the *mean* values

for a healthy adult, rather than overall population variance. For model predictions based on the parameter A1 (lung:liver metabolic ratio obtained from data for 7-ethoxycoumarin) and a similar parameter for the kidney (A2), uncertainty in A1 or A2 based upon variance in tissue-specific values reported for the corresponding *in vitro* studies will be included.

19. *Please comment on whether the planned analysis for PBPK-predicted dose metrics as outlined by U.S. EPA (2020) is an appropriate approach for evaluating quantitative uncertainty in the estimated internal doses. Please provide any specific suggestions you have on how the analysis could be improved.*

References

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