Background for Peer Review of Chloroprene PBPK Modeling

The U.S. EPA is organizing an external peer-review of a chloroprene physiologically based pharmacokinetic (PBPK) model developed by {Ramboll, 2020, 5467423@@author-year} for possible use in updating the 2010 Integrated Risk Information System (IRIS) Toxicological Review of Chloroprene {U.S. EPA, 2010, 625433}. Specifically, the PBPK model is being considered for animal-human extrapolation for inhalation exposures based on model-predicted internal doses. The Ramboll (2020) model, which was developed specifically for the estimation of internal doses associated with the most sensitive endpoint observed in rodents from inhalation exposure, lung tumors, has been submitted to the EPA as significant new science to be considered to determine whether an update to the 2010 IRIS Toxicological Review of Chloroprene is warranted. The focus and ultimate objective of this peer review is to assist in EPA's determination of whether the model is of sufficient scientific quality and reliability to support consideration in an IRIS human health assessment.

The Ramboll (2020) model represents revisions to a published peer-reviewed model (Yang et al. 2012) in response to U.S. EPA comments and questions starting in 2018.

- *June 26, 2017* - U.S. EPA conducted a quality review of an earlier published version of this model {Yang, 2012, 3854472} as part of evaluating a Request for Correction[1] submitted by Denka.
- *January 25, 2018* - The U.S. EPA responded to the Request for Correction[2], identifying a number of technical and scientific evaluation issues with the Yang et al. (2012) published model and requesting the underlying code to fully evaluate and consider in the estimation of the IUR for chloroprene.
- *April 6, 2018* - In response to these issues identified by the U.S. EPA, Ramboll developed a workplan that outlined an approach for addressing the limitations and uncertainties raised by U.S. EPA that have prevented the use of the published Yang et al. (2012) model for the development of the IUR for chloroprene and provided the model code(s) needed to allow for full review of the published model.
- *June 13, 2018* - U.S. EPA offered further comments on the chloroprene PBPK model, as well as comments on an approach for quality assurance and review of this model that included consideration of U.S. EPA's Quality Assurance Process Plan (QAPP) in model documentation. In response to these comments, further revisions were made to the model and documentation developed.
- *July 19, 2018*[3] - U.S. EPA and Ramboll agree that EPA would review the draft model code and documentation and suggest additional improvements to Ramboll[4].

[1] Requests for Correction are the mechanism by which the public can seek and obtain, where appropriate, correction of information disseminated by the Agency that does not comply with EPA or Office of Management and Budget (OMB) Information Quality Guidelines (https://www.epa.gov/quality/frequent-questions-about-epas-quality-system#what-qg).

[2] https://www.epa.gov/sites/production/files/2018-01/documents/epa_repsonse_to_mr._holdren_jan_25_2018_complete.pdf

[3] https://cfpub.epa.gov/ncea/iris2/events.cfm#stakeholderMeetings, select "Show All Meetings Requested by Specific Members of the Public," then select "Jul 19, 2018: Stakeholder Meeting: Chloroprene Request for Correction/Request for Reconsideration (Follow-up)."

[4] https://cfpub.epa.gov/ncea/iris2/event_attachment.cfm?layout=none&attach_id=544

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- *January 31, 2019* - Ramboll provided model code to the U.S. EPA. In combination with data from a previously unpublished nose-only inhalation study in mice that would provide suitable data for validating the chloroprene model's ability to predict *in vivo* kinetics using data from *in vitro* studies (also in Ramboll 2020). Ramboll received additional comments on this version of the model from U.S. EPA in multiple emails throughout 2019, including a request for an experiment to be conducted related to the incorporation of additional parameter into the PBPK model (Kgl).
- *April 14, 2019 -* A revised version of the model and documentation was provided by Ramboll for further review and comments by U.S. EPA.
- *April 23, 2020* - The current documentation of the model for peer review (Ramboll 2020) is provided to U.S. EPA and represents Ramboll's updates in response to comments received throughout 2019, as well the documentation of the Kgl experiment (Ramboll, 2020, Supplemental Materials B).

While EPA has given feedback to Ramboll on the revised model and has conducted quality evaluation of previous versions of the model, it has not conducted a full quality assurance review of the current model version nor formally evaluated its suitability for use in updating the 2010 IRIS assessment. While, the current version of the model has been published and has been through peer-review as part of the publication process (Clewell et al. 2019), such journal review and publication are not considered sufficient evidence of model quality and applicability for use in an IRIS assessment {McLanahan, 2012, 1015422}.

In order to determine if the chloroprene PBPK model is suitable for use in an IRIS Toxicological Review, the toxicological endpoints and set of target tissues need to be recognized. A primary concern for chloroprene is its carcinogenic potential. In particular, the 2010 IRIS assessment concluded chloroprene was "likely to be carcinogenic to humans" primarily based on: (1) statistically significant and dose-related information from an {NTP, 1998, 42076@@author-year} chronic inhalation bioassay demonstrating the early appearance of tumors, development of malignant tumors, and the occurrence of multiple tumors within and across animal species; (2) evidence of an association between liver cancer risk and occupational exposure to chloroprene; (3) suggestive evidence of an association between lung cancer risk and occupational exposure; (4) the proposed mutagenic mode of action; and (5) structural similarities between chloroprene and known human carcinogens, butadiene and vinyl chloride. The NTP chloroprene mouse and rat inhalation bioassays reported significantly increased incidence of neoplasms in liver, lung, forestomach, Harderian gland, mammary gland, Zymbal's gland, kidney and the circulatory system in mice and in the lung, mammary gland, thyroid, kidney, and the oral cavity in rats {NTP, 1998, 42076}. The 2010 IRIS assessment utilized multi-tumor modeling in derivation of the inhalation unit risk (IUR).

As noted in Ramboll (2020), 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 most sensitive endpoint in rodents. However, the U.S. EPA also seeks to evaluate the potential for use of the model to extrapolate inhalation cancer risk for other target tissues. Therefore, the suitability of the chloroprene PBPK model for use in a human health assessment depends in part on whether it can generate an appropriate set of internal dose metrics, which are expected to be predictive of the endpoint(s) being evaluated, noted in the previous paragraph. To assist with that evaluation, the tissue-specific extra risk (ER) for neoplasm for 80 ppm chloroprene exposure vs. controls observed by {NTP, 1998, 42076@@author-year}, for male and female rats and mice, are sorted from

highest to lowest ER in the following table, along with two dose metrics predicted by the Ramboll PBPK model: the metabolic dose is the average rate of chloroprene metabolism per gram of tissue and the average tissue concentration is the average concentration of chloroprene in the corresponding tissue compartments. Evaluation of metabolic dose is important given that cancer risk is due to the formation of oxidative metabolites (U.S. EPA, 2010). More specifically, {Ramboll, 2020, 5467423@@author-year} hypothesize that it is the oxidative metabolites generated in the target tissue that should be considered as a dose metric; i.e., metabolites formed in tissues with higher metabolism, including the liver, do not contribute to risk of neoplasm in other target tissues. Work conducted by Himmelstein et al. (2004b in the Ramboll 2020 report) and summarized in Ramboll (2020) (Selection of Dose Metric; Figure 12) demonstrates that using amount metabolized in the tissue partially harmonizes the dose responses for lung tumors across multiple species. However, it is also noted the dose-response for female mice shown in this figure is considerably steeper than that of male mice. Hence the amount metabolized in lung is not a perfect predictor of lung tumor response, even within the same species. Tissue concentrations are listed as an alternative metric that the U.S. EPA is considering for use where the model does not predict a metabolic dose. A subsequent review will evaluate the biological appropriateness of using PBPK-model predicted tissue concentrations to estimate the cancer risk in tissues for which the rate of metabolism is not quantified. The current review is to determine if those model predictions are of sufficient quality for such use.

Extra Risk of Neoplasm in Rat and Mouse Tissues Observed by {NTP, 1998, 42076@@author-year} from 80 ppm Chloroprene Inhalation Exposure vs. Controls[a]

Tissue/neoplasm	Species/sex	Extra Risk	Metabolic dose[b]	Average tissue concentration[c]
Lung alveolar adenoma/carcinoma	Mice/female	**0.83**	**2.53**	**NA**
Lung alveolar adenoma/carcinoma	Mice/male	**0.81**	**12.9**	**NA**
Mammary gland fibroadenomas	Rats/female	**0.45**	**ND[d]**	**4.7**
Hemangioma/Hemangiosarcoma	Mice/male	**0.38**	**ND[d]**	**3.8**
Skin carcinoma	Mice/female	**0.36**	**ND[d]**	**4.3**
Liver hepatocellullar adenoma/carcinoma	Mice/female	**0.33**	**7.93**	**NA**
Oral cavity papillomas/carcinomas	Rats/male	**0.24**	**ND[d]**	**4.6**
Mammary gland carcinoma/adenocarcinoma	Mice/female	**0.23**	**ND[d]**	**4.3**
Harderian gland adenoma/carcinoma	Mice/male	**0.21**	**ND[d]**	**3.8**
Oral cavity papillomas/carcinomas	Rats/female	**0.20**	**ND[d]**	**4.7**
Kidney renal tube adenomas	Mice/male	**0.18**	**0.54**	**NA**
Harderian gland adenoma/carcinoma	Mice/female	**0.15**	**ND[d]**	**4.3**
Kidney renal tube adenomas/carcinomas	Rats/male	**0.14**	**0.26**	**NA**
Thyroid gland follicular cell adenoma/carcinoma	Rats/male	**0.10**	**ND[d]**	**4.6**
Hemangioma/Hemangiosarcoma	Mice/female	**0.09**	**ND[d]**	**4.3**
Lung alveolar adenoma/carcinoma	Rats/male	**0.08**	**0.97**	**NA**
Thyroid follicular cell adenomas/carcinomas	Rats/female	**0.08**	**ND[d]**	**4.7**
Kidney renal tube adenomas/carcinomas	Rats/female	**0.08**	**0.22**	**NA**
Forestomach squamous cell papilloma/carcinoma	Mice/female	**0.06**	**ND[d]**	**4.3**

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Forestomach squamous cell papilloma	Mice/male	**0.06**	**ND[d]**	**3.7**
Zymbal's gland carcinoma	Mice/female	**0.06**	**ND[d]**	**4.3**

[a] PBPK model simulations were run for two weeks of exposure using the NTP bioassay exposure pattern of 6 hours/day, 5 days/week, and the average dose metric during that period was calculated

[b] Average rate of chloroprene oxidative metabolism per tissue mass (µmol/g tissue/day).

[c] Average chloroprene concentration (µM); results are considered not applicable (NA) for lung, liver, and kidney since model-predicted rates of metabolism are available for those tissues; model results for richly perfused tissues were used for all others

[d] ND: metabolism in these tissues has not been determined and is not predicted by the PBPK model

The U.S. EPA has long considered application of adequately validated PBPK models to be "the optimal approach for extrapolation of dose across species" {U.S. EPA, 1994, 6488}. Use of computational models by U.S. EPA's Office of Research and Development is subject to quality guidelines and the Quality Assurance Project Plan developed for PBPK model use in IRIS Assessments includes comparison of model predictions to in vivo pharmacokinetic (PK) data {U.S. EPA, 2018, 4326432} as part of the criteria for acceptance. The PBPK model was shown to reasonably reproduce time-course blood concentration data from a chloroprene inhalation study in female mice {Ramboll, 2020, 5467423@@author-year}, but no *in vivo* PK data are available in rats or humans. The model predictions depend entirely upon the *in vitro* to *in vivo* extrapolation (IVIVE) of metabolic parameters and thus evaluation of model validity and uncertainty in model predictions depends heavily on this IVIVE application. While U.S. EPA has previously proposed or made use of *in vitro* measurements of metabolism in cross species extrapolation in a few assessments (e.g., the IRIS Toxicological Review of Acrylonitrile (External Review Draft)[5] and the Toxicological Review of Dichloromethane (Methylene Chloride)[6]), the chloroprene application provides a novel modeling scenario for the U.S. EPA IRIS Program in that it differs in its greater reliance on the *in vitro* data and in the absence of human *in vivo* pharmacokinetic (PK) data to which model predictions might be compared.

Summary of Materials for Review

Ramboll PBPK Model and Associated Analyses (Charge Questions 1 to 13)

The primary focus of this peer review is the report by {Ramboll, 2020, 5467423@@author-year}, "Incorporation of In Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene." Some aspects of the PBPK model and associated analyses are described in Supplemental Materials A through E of that report. The model code is also being provided to facilitate this review. In particular, model predictions of metabolic rates in liver, kidney and lung are included. As noted in the table above, concentrations of chloroprene in venous blood or other tissues for which metabolic rates are not determined are being considered by U.S. EPA for use as dose metrics with which one might extrapolate the risk of cancer in those tissues.

General evaluation and reliability of IVIVE (Charge Question 14)

[5] https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0206_summary.pdf
[6] https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0070tr.pdf

U.S. EPA Supplemental Uncertainty Analyses (Charge Questions 15 to 19)

Uncertainty of Metabolic Parameters Estimated from In Vitro Data

The EPA (2020) supplement "Uncertainty Analysis of In Vitro Metabolic Parameters and of In Vitro to In Vivo Extrapolation (IVIVE) Used in a Physiologically Based Pharmacokinetic Model for Chloroprene" describes initial results[7] for a statistical analysis of the control incubation data (used to evaluate chloroprene losses that occur separate from metabolism) to illustrate the type of statistical model and analysis intended to identify the uncertainty in the in vitro metabolic parameters, in particular for human liver and lung. The planned approach to estimate uncertainty in the human liver and lung metabolic parameters is then described. EPA seeks input on the proposed approaches for estimating uncertainty in the in vitro metabolic parameters.

Uncertainty in PBPK Model Predictions from IVIVE

The published chloroprene PBPK model of {Yang, 2012, 3854472@@author-year} used *in vitro* data and IVIVE calculations to estimate metabolic rates in both the liver and lung of humans. However, due to the uncertainty in the available *in vitro* data for the human lung {Ramboll, 2020, 5467423@@author-year} proposed use of *in vitro* data for 7-ethoxycoumarin, a known CYP 2E1 substrate, measured in human lung and liver by {Lorenz, 1984, 730559@@author-year} to estimate the relative metabolic activity in the human lung vs. liver, characterized by a parameter, A1 = Vmax(lung)/Vmax(liver). In particular, A1 is estimated from the lung:liver ratio of microsomal oxidation of 7-ethoxycoumarin measured *in vitro* and the ratio of estimated microsome content in lung vs. liver. The Vmax for chloroprene oxidation in the human lung is then calculated as A1*Vmax(liver), where Vmax(liver) is the maximal rate estimated from chloroprene in vitro metabolic data obtained with human liver microsomes and IVIVE calculations.

EPA had previously used the factor A1 as part of its dichloromethane assessment[8]. However, in the case of dichloromethane no *in vitro* lung metabolism data were available and Vmax(liver) was estimated by fitting the corresponding PBPK model to a set of human *in vivo* PK data. In the case of chloroprene (unlike dichloromethane), chemical-specific *in vitro* metabolism data are available for the lung and no human *in vivo* PK data are available to estimate Vmax(liver). Therefore, the EPA seeks to evaluate both the use of the A1 factor and direct IVIVE of the lung metabolism data as alternatives for estimation of human lung metabolic rates.

On the other hand, *in vitro* metabolic data are not available for the human kidney, so the EPA is also considering use of a factor corresponding to A1 for the kidney: "A2", previously estimated for human renal metabolism of chloroform based on CYP2E1 content {Sasso, 2013, 1936108}. In particular, EPA (2020) outlines an approach to evaluate the quantitative uncertainty associated with IVIVE in PBPK model predictions with kidney metabolism estimated using A2 and lung metabolism estimated with using either A1 or based on the chloroprene in vitro lung data. Since metabolism in the liver is estimated by IVIVE, and uncertainty due to use of A1 and/or A2 depends on the estimated liver metabolism, an integrated analysis is proposed to simultaneously estimate the uncertainty in IVIVE-predicted metabolism in liver, lung, and kidney, as well as venous blood and tissue concentration.

[7] Due to current telework requirements in response to COVID19, the analysis had to be suspended until access to the computer being used for this work is restored. However, the initial results and documented statistical model materials provided are sufficient for peer reviewers to evaluate and address relevant charge questions.
[8] https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?&substance_nmbr=70