Intended for
Denka Performance Elastomer LLC

Document type
Report

Date
October 18, 2019

COMMENTS ON THE CHARGE TO EXTERNAL PEER REVIEWERS FOR THE EXTERNAL LETTER PEER REVIEW OF RAMBOLL (2019) AND THE USEPA (2019) REPORT

RAMBOLL Bright ideas. Sustainable change.

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ED_004105_00000033-00002

1. INTRODUCTION AND BACKGROUND

In July of 2019, scientists from Ramboll US Corporation (Ramboll), on behalf of Denka Performance Elastomers LLC (DPE), submitted a report entitled "Incorporation of In Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene" (Ramboll 2019) to the United States Environmental Protection Agency (USEPA) for consideration. The USEPA is currently evaluating this PBPK model for possible use in a human health risk assessment for the IRIS program and is coordinating an external letter peer review of the Ramboll (2019) report. Ramboll scientists involved in the development of the report have been provided with a draft of the charge to the external peer reviewers dated October 2, 2019 and provided October 10, 2019 for review and comment.

The following report provides a summary of Ramboll's comments related to the draft charge for the peer reviewers. In addition, specific questions are incorporated into the draft charge to the external peer reviewers regarding a new draft analysis developed by USEPA and included as part of the charge to the external peer reviewers entitled "Scaling of (1-chloroethenyl)oxirane (1-CEO) Metabolic Clearance at Low Concentrations from In Vitro to In Vivo and Comparison to the Expected Clearance by Blood Perfusion in the Liver and Lung of Mice, Rats, and Humans" (USEPA (2019) 1-CEO Clearance Analysis). Because the specific relevance of the USEPA (2019) 1-CEO Clearance Analysis to the peer review of the Ramboll (2019) model is not well defined in the charge, a critical review of this analysis was conducted, and any scientific issues that were identified are provided as part of the review of the draft peer review charge. The comments on the draft peer review charge are organized to provide an initial discussion of the general format and content of the charge, followed by specific comments organized by the sections of the draft charge, including comments on the USEPA (2019) 1-CEO Clearance Analysis that are presented as part of the draft charge questions to the external peer reviewers.

DPE and Ramboll recognize that USEPA has made the Quality Assurance/Quality Review (QA/QC) of the Ramboll (2019) PBPK model for chloroprene a priority, and we appreciate the diligence of USEPA's scientists in helping us to develop a model that we believe reflects sound scientific principles and methodologies. Ramboll scientists have worked diligently to respond to various concerns USEPA's scientists have raised with respect to the model and to incorporate their ideas and suggestions into our toxicological analyses where appropriate. We believe the peer review panel should now be tasked with making the final recommendations and decisions with respect to the model. In reviewing the draft peer review charge, our overarching concerns include:

- Content and format of the draft charge that is inconsistent with the USEPA Peer Review Handbook (2015) and other examples of USEPA charges for similar peer reviews;
- Presentation of USEPA opinions in the background to the draft charge questions regarding potential uncertainty in the Ramboll (2019) PBPK model as if they are resolved questions or facts and which are in some cases incorrect; and
- Inclusion of the USEPA (2019) 1-CEO Clearance Analysis in the peer review charge in the absence of clarifying how this analysis is related to the evaluation of the Ramboll (2019) PBPK model report. Further, Ramboll noted a discrepancy in the calculations of the USEPA (2019) 1-CEO Clearance Analysis that could have a significant impact on any conclusions reached from this analysis.

In its current form, the charge questions appear to be an integration of the QA/QC process and peer review process because USEPA incorporates concerns with the Ramboll (2019) PBPK model as scientific facts in the background section. We believe that this creates a serious risk of introducing bias to the peer review that could improper shape the peer reviewer responses. In our comments in the following sections on the peer review charge, we have included responses to some of the concerns raised by USEPA that we consider to be elements of the QA/QC process, and not the peer review of the Ramboll (2019) PBPK model. We believe that any concerns USEPA has with the model should be addressed through specific peer review charge questions, and that much of the background information outside of the charge questions should not be part of the charge. USEPA should revise the charge to address this concern as it will produce a higher quality and unbiased review.

2. CHARGE FORMAT AND CONTENT

Overall, the format of the draft charge questions is inconsistent with examples provided in USEPA's Peer Review Handbook (2015), as well as recent examples of peer review panel charge questions in which a PBPK model is being considered for use in a human health risk assessment (Attachment A). As noted in the USEPA (2015) Handbook, the charge is a set of ***focused questions*** that identify the scientific and technical issues on which the Agency would like feedback and invites suggestions for improving the document as a whole. The handbook further provides the essential elements of a well-prepared charge that includes:

- *"A concise overview in the introduction describing the work product, its development and its intended use."*
- *"Issues to be addressed and areas of concern or specific advice sought (in the form of charge questions)..."*

Rather than providing a concise overview of the chloroprene PBPK model report and its intended use, the Background in the peer review charge for the Ramboll (2019) report presents agency opinions as scientific conclusions related to the Ramboll (2019) PBPK model under review. This approach differs markedly from the examples contained in the USEPA (2015) handbook or in charges developed for other USEPA peer reviews. As indicated in the USEPA (2015) handbook, these types of agency concerns should be presented within the context of a neutral charge question requesting the peer reviewers' advice to eliminate any perception of trying to bias the peer review.

Examples of charges developed by USEPA that demonstrate this significant difference in format include the charge to the external peer reviewers for the IRIS Toxicological Review for Acrylonitrile (Attachment A). The scope for this charge is similar to the anticipated scope for the planned peer review for the Ramboll (2019) PBPK model. The charge for the Acrylonitrile IRIS review involves a published PBPK model (Kedderis et al. 1996; Sweeney et al. 2003) that was revised by the USEPA for application in the IRIS Review. The Acrylonitrile IRIS charge does not re-summarize the information contained in the documents under review by the panel, but rather provides a concise two-paragraph summary of the background documentation for the model, followed by concise, open-ended questions to be addressed by the external peer reviewers. Additional examples demonstrating that previous agency charges followed a significantly different format than the charge for the Ramboll (2019) PBPK model can be found in the peer review charges developed by USEPA for perchlorate, tetrachloroethylene and deltamethrin/permethrin/carbaryl (Attachment A).

3. GENERAL COMMENTS BY SECTION OF THE PEER REVIEW CHARGE

3.1 Background

In the background of the draft charge, opinions of the USEPA regarding potential uncertainty in the Ramboll (2019) PBPK model are inappropriately presented as if they are resolved facts. Presenting these opinions in this way as part of the background could bias the peer reviewers during their evaluation of the Ramboll (2019) PBPK model. These opinions should be removed from the Background including:

- The agency states: "*While the U.S. EPA has previously used PBPK models in a number its risk assessments, all of these previous models have been for chemicals with sufficient in vivo pharmacokinetic (PK) data in both test animals and humans to obtain model parameters for metabolism or clearance and otherwise validate model predictions.*" This statement is misleading because it ignores the fact that (1) *in vivo* data are available for chloroprene in the animal bioassay, (2) *in vitro* data on human lung metabolism served as the basis for predicting the risk assessment dose metrics in the agency's IRIS assessment for methylene chloride and, (3) sensitivity analyses demonstrate that in the case of both methylene chloride and chloroprene, human *in vivo* data are of little value for evaluating the PBPK model. This discussion should be removed from the Background and the peer review panel should be allowed to evaluate the adequacy of the model as described in the Ramboll (2019) PBPK report.
- The agency cites conclusions from only one study (Wambaugh et al. 2015) as a basis for questioning the reliability of the Ramboll (2019) PBPK model. The results from this study, which evaluated simple *in vitro* to *in vivo* extrapolation (IVIVE) approaches for supporting high-throughput screening, are not relevant to an evaluation of the Ramboll (2019) PBPK model, which was developed with extensive chemical-specific data. The PBPK models used in Wambaugh et al. (2015) were simplified generic models intended to test the assumptions used in a rapid reverse dosimetry approach for high-throughput assays. The results provided in Figure 1 of the draft charge combine results from chemicals with a wide range of physical and chemical properties. Wambaugh et al. (2015) specifically noted that the assumptions used in the simplified models are not always appropriate. The types of compounds and exposures for which the *in vitro* data did not adequately predict the *in vivo* data are very different from chloroprene. In particular, the inhalation route of exposure, which is the relevant route of exposure for chloroprene, was not considered in Wambaugh et al. (2015). Instead, the routes considered were limited to oral, intravenous injection, and subcutaneous injection. Conducting rapid IVIVE for oral exposure, in particular, is subject to much greater uncertainty than inhalation due to the complexity of intestinal absorption and pre-systemic metabolism (Liao et al. 2007, Yoon et al. 2012). A more appropriate basis for evaluating the predictivity of the *quantitative* IVIVE (QIVIVE) conducted for the Ramboll (2019) PBPK model is provided by the Yoon et al. (2012) review, which describes approaches and gives examples of PBPK models that are more appropriate and relevant for comparison to the Ramboll (2019) PBPK model. The QIVIVE approach used in the Ramboll (2019) PBPK model should be evaluated on its own merits and not compared to rapid screening IVIVE methods, such as those used in Wambaugh et al. (2015). This discussion should be removed from the Background and assessing the predictivity of the QIVIVE approach conducted in Ramboll (2019) should be left to the peer review panel.

3.2 Context and Scope of Review

The first paragraph of the Context and Scope of Review clearly summarizes the objective of the peer review. However, it is not clear why the section entitled "In Vitro Metabolic Parameter Estimation" is needed. Much of the information that is provided in this section is a duplication of information provided in the first charge question, "Estimation of Mass Transfer Resistance in the In Vitro Metabolism Experiments." This redundant section in the Context and Scope of Review should be removed.

In the section "Relative Rate of Metabolite Clearance in Humans vs. Laboratory Animals," USEPA references the USEPA (2019) 1-CEO Clearance Analysis, but it is unclear how the USEPA (2019) 1-CEO Clearance Analysis relates to the peer review of the Ramboll (2019) PBPK model. As indicated in the charge, the primary focus of this review is the Ramboll (2019) PBPK model. While the USEPA (2019) 1-CEO Clearance Analysis is mentioned in the Context and Scope of Review, the agency does not explain how the consideration of this USEPA (2019) 1-CEO Clearance Analysis is relevant to the peer review panel evaluation of the appropriateness of the Ramboll (2019) model for use in a human health risk assessment. If the agency wants to receive comments from the panel on the USEPA (2019) 1-CEO Clearance Analysis, the discussion in the Context and Scope of Review should clearly describe what the agency believes is the relationship of an evaluation of their analysis of 1-CEO clearance to forming conclusions concerning the quality and use of the Ramboll (2019) PBPK model, which does not include a description of 1-CEO, in a risk assessment.

Footnote 1 in the Context and Scope of Review section contains what appear to be USEPA's opinions regarding adequacy of the MCMC parameter estimation process performed in the Ramboll (2019) PBPK report. This appears to represent conclusions reached by the USEPA as part of their internal QA/QC review. Importantly, the footnote does not make it clear that the statements represent USEPA's opinions. They are presented as facts that should be assumed for the peer review. This is particularly troubling because several of the opinions expressed in the footnote are incorrect, which could easily have been addressed if discussed with the model developers. In contrast to many other concerns raised by the agency during the QA/QC, where a dialog was maintained between the agency and the model developers, Ramboll has not been given an opportunity to respond to the concerns raised in the footnote. These concerns are addressed below. If there are remaining agency concerns regarding the MCMC approach in Ramboll (2019), they should be addressed by a neutral charge question to the peer-reviewers.

- Footnote 1 states:
  1. *"Further, to rigorously evaluate parameter uncertainty given these data, the likelihood function would have to be constructed in a way that reflects the repeated measurements from a single experimental incubation (Klein et al. 2012)…".*
     - In the MCMC analysis documented in the Ramboll (2019) PBPK model report, the pharmacokinetic model used to simulate the *in vitro* experiment accurately recapitulated the serial sampling. Regardless of the fact that serial sets of measurements were made on each vessel, the likelihood contributions of all of those measurements were appropriately included in the computation of overall likelihood of the parameters in the pharmacokinetic model used to simulate the time evolution of the in vitro system. Therefore, it is incorrect to suggest that the likelihood function was not constructed in a way that reflects repeated measurements from a single experimental incubation.

- In the MCMC parameter estimation process, the Ramboll (2019) PBPK model was used for deriving predictions (components of the likelihood computations) that describe the time-course of headspace concentration (as a function of various metabolic parameters and initial concentration). Specification of values for those parameters, including an initial headspace concentration and sampling times/volumes, yields predictions for the headspace concentration at all subsequent times. These predictions serve as time-varying estimates (conditional on the parameter values) of a central tendency (e.g. mean) around which actual observations may vary. The variability around the observations is another parameter that is updated as part of the MCMC procedure. The model predictions (means) and the variance term(s) in turn define the likelihood of each observation.
- Because those mean and variance estimates are for an observation at time t, and therefore the likelihood contribution for the observation at time t, they do not depend on the values of other observations. The likelihood contribution for the time t observation can be written without reference to the values of the other observations. There is one overall likelihood for each incubation, but that likelihood is the product of the within-incubation, time-specific likelihood contributions. Thus, the posterior distributions form the MCMC analysis provide reliable estimates of the uncertainty in the estimated metabolism parameters derived from the experimental data. Supplemental Materials B, provided to USEPA with the Ramboll (2019) PBPK model report, provides a full documentation of the MCMC analysis, including robust measures of convergence.

2. *"...and robust measures of MCMC convergence are needed."*
   - Robust measures of convergence were conducted in the Ramboll (2019) MCMC analysis. For all analyses of the in vitro data with MCMC, three chains were run independently with different start values to test stability of model convergence. The truncated chains were assessed for convergence both visually (line and density plots) and by verifying that the upper limit of the corrected scale reduction factor (CSRF) was less than 1.2 using the method of Gelman (gelman.diag routine included in the R package CODA).
3. *"Since the report does not provide details of the MCMC methods, including likelihood function, and convergence, the results are presumed to only provide a good estimate of population mean parameters. Parameter confidence bounds provided in the supplemental materials are not assumed to provide a robust measure of parameter uncertainty."*
   - The details of the MCMC methods, including likelihood function and convergence, are provided in Supplemental Materials B of the Ramboll (2019) report. Given that the primary interest was in defining uncertainty in the parameter estimates and the *in vitro* data are not sufficient to estimate population variability, a single level analysis was used in lieu of the hierarchical analysis reported in Yang et al. (2012). The single level analysis retained the broad prior distributions used in the Yang et al. (2012) analysis. Parameters for both the saturable (i.e. Vmax and Km) and the 1st order (KF, where Km was unidentifiable) metabolism of chloroprene were given broad log-uniform distributions (-10, 5). The likelihood function of the data given the predictions was assumed to be lognormal and a broad likelihood (lognormal, mean = 0.3, variance = 5) was used to avoid over-constraining the posterior parameters

distributions. Therefore, the posterior distributions from the MCMC analysis represent robust estimates of the uncertainty in the estimated metabolism parameters derived from the experimental data. Any concerns surrounding these scientific issues, as well as any others discussed as part of the background and scope for the charge should be provided in the form of neutral charge questions to the peer reviewers.

3.3 Charge Questions

Overall, Ramboll has limited comments related to the specific questions directed to the peer reviewers in each section of the charge. However, there are multiple introductory sections of text surrounding selected charge questions that contain errors or that introduce bias or uncertainty. These specific sections and comments by charge question section include:

- *IVIVE Calculations for Chloroprene and 1-CEO Metabolism and Estimation of 1-CEO Clearance by Blood Perfusion*
    - It is important that the peer review evaluation of the USEPA (2019) 1-CEO Clearance Analysis be clearly distinguished from the evaluation of the Ramboll (2019) PBPK model, which does not include a description of 1-CEO kinetics. Therefore, the second paragraph and question (3) in this charge, which refer to the USEPA (2019) 1-CEO Clearance Analysis, should be moved to the charge question on "Estimation of 1-CEO Clearance in Liver and Lung."
    - In reviewing the USEPA (2019) 1-CEO Clearance Analysis, which was provided to DPE with the draft USEPA Charge for the peer review, Ramboll noted a discrepancy in the calculations that could have a significant impact on the results. Specifically, the assumption in the USEPA (2019) 1-CEO Clearance Analysis that 100% of cardiac output perfuses the lung tissue is inappropriate. While total cardiac output is used in the Ramboll (2019) PBPK model to calculate the arterial concentration of chloroprene that is in turn used to calculate the rate of oxidative metabolism of chloroprene in that tissue, the metabolism of chloroprene occurs in the tracheobronchial tissues of the lung. Metabolites generated in this tissue are cleared by the arterial blood flow to the tracheobronchial region, which is only about 0.5% of total cardiac output in the mouse and 2.5% in the human (Clewell et al. 2000).
    - The reference to Wambaugh (2015) in the background for the third charge question of this section should refer to Wambaugh et al. (2015).
- *Estimation of 1-CEO Clearance in Liver and Lung* – The charge question for this section is focused on calculations performed in the USEPA (2019) 1-CEO Clearance Analysis, but it is not made clear how this question relates to the evaluation of the Ramboll (2019) PBPK model. As noted above, the calculations in the USEPA (2019) 1-CEO Clearance Analysis should be repeated using tracheobronchial blood flow instead of total blood flow for clearance of 1-CEO in the lung. It should also be noted in the charge that the USEPA (2019) 1-CEO Clearance Analysis only provides an estimate of the relative clearance across species of one of the two epoxides generated from chloroprene (1-CEO), and the results of this analysis cannot be applied to the more reactive epoxide (2-CEO), which was not measurable in the *in vitro* studies and which appears to represent the majority of the oxidation of chloroprene in the mouse (Himmelstein et al. 2004; Table 3). The rapid, non-enzymatic rearrangement of 2-CEO to reactive aldehydes leads to the expectation that its clearance would be identical across species, as was the case for methylene chloride, where the clearance of the chloromethylglutathione metabolite was non-enzymatic and rapid.

4. CONCLUSIONS/RECOMMENDATIONS

The following revisions are suggested from the review of the Draft Charge to External Peer Reviewers for the External Letter Peer Review of the Ramboll (2019) Report "Incorporation of In Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene":

- The overall form of the charge should be revised to be consistent with examples from the USEPA's Peer Review Handbook (2015), as well as recent examples of peer review panel charge questions in which a PBPK model is being considered for use in a human health risk assessment. This would involve the deletion of a significant portions of the background information and text related to each of the charge questions. Most of this information is redundant with the information already provided in the Ramboll (2019) PBPK model report and often presents what appear to be USEPA opinions as fact. Instead, USEPA should develop additional neutral charge questions to request opinions and/or conclusions from the peer review related to the scientific issues that are only discussed in the Background or Scope sections (e.g. MCMC analysis parameter estimation, IVIVE predictability). Importantly, in all cases, the questions should be objective, neutral and open-ended to ensure that the peer reviewers are free to arrive at their own conclusions and not biased by the information provided.
- References to the Wambaugh et al. (2015) paper should be removed from the Background for the charge, and references to other more relevant studies should be added for consideration, such as Yoon et al. (2012). There appears to be a charge question related to the issues; however, citation to the Wambaugh (2015) study as support for that question appears to be an error.
- Footnote 1 in the Background section of the charge presenting USEPA's conclusions regarding the MCMC parameter estimation should be deleted from the Context and Scope of Review section to remove bias, and any USEPA concerns regarding the MCMC analysis should be incorporated into additional charge questions.
- Considering the discrepancies Ramboll scientists identified in the USEPA (2019) 1-CEO Clearance Analysis, USEPA should review and revise this analysis prior to presentation to the peer reviewers. Specifically, the calculations in the USEPA (2019) 1-CEO Clearance Analysis need to be repeated using tracheobronchial blood flow instead of total cardiac output for perfusion of the lung. In addition, charge questions are needed to clarify the relevance of the review of USEPA (2019) 1-CEO Clearance Analysis to the evaluation of the Ramboll (2019) PBPK model. If the purpose for this analysis is to suggest that 1-CEO is the causative metabolite and that it should be used as the dose metric for the chloroprene IRIS risk assessment, Ramboll notes that the peer reviewers are specifically asked not to evaluate the question of whether or not 1-CEO is a causative metabolite. In addition, the draft charge indicates that the question of whether or not it is a causative agent would be evaluated in a subsequent review of specific model application. Therefore, the relevance of the USEPA (2019) 1-CEO Clearance Analysis to the current peer review of the Ramboll (2019) PBPK model should be clarified.

Due to the depth and breadth of Ramboll's concerns with this first draft, Ramboll and DPE request a revision of the draft charge questions and the USEPA (2019) 1-CEO Clearance Analysis to address Ramboll's concerns. We would also request a review of the revised charge and the opportunity to provide comments prior to USEPA providing the charge to the peer reviewers. Ramboll and DPE believe strongly that this will ensure an appropriately objective peer review.

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ATTACHMENT A

Charge to External Reviewers for the IRIS Toxicological Review of Acrylonitrile
June 2011

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment for acrylonitrile that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). An existing IRIS assessment for acrylonitrile includes an inhalation reference concentration (RfC), which was posted on IRIS in 1991, and an oral slope factor and inhalation unit risk (IUR), which were posted on IRIS in 1987.

The current draft health assessment includes an oral reference dose (RfD), inhalation RfC, and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in the assessment of acrylonitrile. Please provide detailed explanations for responses to the charge questions and any other significant scientific issues of concern, including recommendations for resolution. Please consider the accuracy, objectivity, and transparency of EPA's analyses and conclusions in your review.

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazards?

2. Please identify any additional studies that would be likely to make a significant impact on the conclusions of the Toxicological Review.

Chemical-Specific Charge Questions:

(A) Physiologically-based pharmacokinetic (PBPK) model for acrylonitrile

1. PBPK modeling was used for calculating the internal dosimetry in the derivation of the RfD and in the cancer assessment. A PBPK model for acrylonitrile was previously developed in rats (Kedderis et al., 1996) and extended to describe the dosimetry of both acrylonitrile and the reactive metabolite, 2-cyanoethylene oxide (CEO), in humans (Sweeney et al., 2003). While the published human version of the model (Sweeney et al., 2003) included epoxide hydrolase (EH) activity, the rat model (Kedderis et al., 1996) did not. EPA chose to also include EH in the rat PBPK model. Scientific support for this modification is based on the results of Guengerich et al. (1979) and de Waziers et al. (1990), which show the presence of EH, an enzyme that metabolized CEO, in *untreated* rat livers.

   Since the previous rat model did not include EH, Sweeney et al. assumed that the *in vivo:in vitro* ratio for EH in humans was the same as for CYP450. The approach used by EPA was to scale EH based only on enzyme content (not using an activity adjustment factor for either rats or humans) and then to re-fit the remaining metabolic parameters to the original data for rats. This parameter re-estimation in rats provided a revised value of the *in vivo:in vitro* adjustment factor for CYP450, which was then used along with human *in vitro* CYP450 data

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(i.e., the same calculation as Sweeney et al. used but with a revised adjustment factor for CYP450) to estimate the corresponding human value.

a. Please comment on whether the addition of EH to the rat model is scientifically supported and clearly described.

b. Please comment on whether the approach used for deriving the rat and human parameters is scientifically supported and clearly described.

c. Does the PBPK model, modified by EPA, adequately represent the toxicokinetics of acrylonitrile? Was the PBPK modeling appropriately conducted and clearly described? Are the model assumptions and parameters scientifically supported and clearly described? Are the uncertainties in the model structure appropriately considered and discussed, and is the sensitivity analysis in Appendix D adequately and clearly presented?

2. CEO concentration in blood (AUC expressed on a 24-hour basis) was used as the internal dose metric in PBPK modeling. For purposes of comparison, acrylonitrile concentration in blood (AUC expressed on a 24-hour basis) was also evaluated. Please comment on whether the selection of CEO concentration in blood as the internal dose metric is scientifically supported and clearly described.

(B) Oral reference dose (RfD) for acrylonitrile

1. A two-year drinking water study of acrylonitrile in F344 rats (Johannsen and Levinskas, 2002; Biodynamics, 1992) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. The incidence of forestomach lesions in male and female rats was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

3. Benchmark dose (BMD) modeling was applied to the forestomach lesion incidence data in male rats to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of forestomach lesions) scientifically supported and clearly described?

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

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II. CHARGE TO REVIEWERS

The U.S. EPA's Office of Water is in the process of developing National Primary Drinking Water Regulations for perchlorate under the Safe Drinking Water Act. In 2012, EPA submitted a white paper *Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for Perchlorate* (U.S. EPA, 2012) to the Science Advisory Board (SAB) for review. The white paper presented scientific information published since the National Research Council (NRC) released their 2005 Report "Health Implications of Perchlorate Ingestion" and described how EPA derived a range of Maximum Contaminant Level Goal (MCLG) values for life stages of concern. In their 2013 report, the SAB concluded that EPA should: 1) consider exposure to the following sensitive life stages: the fetuses of hypothyroxinemic pregnant women and infants exposed to perchlorate through either water-based formula or the breast milk of lactating women, and 2) derive the MCLG using a physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model to account for exposure differences and biological differences across life stages, including sensitive life stages. Specifically, the SAB found that the 'default algebraic approach provides limited ability to address the various exposure and biological factors affecting sensitivity to perchlorate at different life stages.' The SAB concluded that from a scientific standpoint it would be more appropriate to base the MCLG derivation on the perchlorate mode of action, using PBPK/PD modeling to relate perchlorate concentrations in drinking water to biological effects rather than the default approach.

The SAB report referenced a report by Lumen et al. (2013) on the effect of perchlorate on thyroid hormone perturbations for pregnant mothers and fetuses, as well as their work to extend a physiologically-based pharmacokinetic/pharmacodynamic iodine uptake inhibition (PBPK/PD-IUI) model with links to serum thyroid hormone levels, as a starting point for EPA's efforts.

The new modeling effort presented here integrates the previous EPA PBPK model (2009) for perchlorate with the model developed by Lumen et al. (2013) for pregnant mothers and fetuses (humans) and iodide models developed by Fisher et al. (2013) for lactating rats and Fisher et al. (2016) for lactating mothers and nursing infants (humans). The model has been subjected to a quality assurance (QA) review that was conducted by Oak Ridge National Laboratory (data and parameters used) and Pacific Northwest National Laboratory (model code). The Biologically Based Dose Response (BBDR) model predicts thyroid hormone perturbations for lactating mothers, breast-fed infants and bottle-fed infants resulting from exposure to perchlorate under different dietary iodine intake levels. EPA is relying on the Lumen et al. (2013) model, peer reviewed and published previously, to predict effects for pregnant mothers and fetuses. It is important to note a modification to a key parameter was made (Michaelis-Menten constant for the sodium-iodide symporter for perchlorate, Km) and the thyroid hormone predictions were adjusted for pregnant mothers and fetuses in the model report under review.

EPA is seeking comments on the scientific rigor of the model for evaluating the effects of perchlorate on thyroid hormones in lactating mothers, breast-fed infants and bottle-fed infants under different iodine intake levels. EPA is providing access to the model program code and all necessary files to facilitate use of the BBDR model by peer reviewers and the public. EPA expects that those reviewers with the appropriate software and expertise will be able to replicate

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the results described in the BBDR model report. After completing development of the BBDR model in the acslX software, the U.S. EPA created a copy of the model that can be run in the alternate software, R. The report URL with links to the code (acslX and R versions for both the pregnancy and infant/lactation models) is:
https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/3352518

Note that the model is calibrated and expected to be valid for conditions where feedback regulation due to variations in thyroid-stimulating hormone (TSH) levels is minimal, so all charge questions regarding the model are for that range.

In March 2016, the Agency invited the public to nominate scientific experts to be considered as panelists for peer review of the draft model and accompanying report. In June, EPA announced that to achieve efficiency it was expanding the scope of the peer review to include draft approaches for application of the model to inform the development of a perchlorate MCLG. EPA has since reevaluated the review process in response to concerns that a simultaneous review of approaches for application of the model to develop a perchlorate MCLG would not allow for prior consideration of peer reviewer comments specifically on the model.

Therefore, EPA seeks public comment and peer review on the modeling approaches and the ability of the model to predict outcomes that could potentially be used to inform the derivation of an MCLG. EPA intends to seek input on the application of the model for developing approaches for a perchlorate MCLG determination at a later date. Future efforts will include discussion of health endpoints and response levels that are most appropriate to base the MCLG determination.

Charge Questions

1. Model structure: Please comment on the following aspects of the Biologically Based Dose Response (BBDR) model structure and integration of the submodels for iodide/thyroid hormones and perchlorate for each of the life stages of focus (e.g., lactating mother, breast-fed infant, and bottle-fed infant):

    a. Does the developed model structure adequately and accurately describe the physiology and kinetics of iodine, thyroid hormones, and perchlorate during different life stages specified above? Please specifically note the strengths and weaknesses of the model structure for each life stage. Please also suggest approaches to address identified weaknesses in the model structure.

    b. Are the physiological and kinetic parameters for iodine, thyroid hormones, and perchlorate for these life stages supported by the published sources for those parameters or data used to identify parameters via calibration? Please identify any critical data gaps and suggest additional data or parameter (values) to these proposed model parameters to address identified gaps.

    c. While the model predicts the dose-response for fetal thyroid hormones, a reference range for the fetus was not identified. Are there additional data, parameters, or (sub)models available that might be useful for evaluating fetal effects? If not, can the

predictions for thyroid hormone changes in the pregnant mother be used as a measure of potential fetal effects?

2. Modifications to existing models: EPA has modified one parameter in the 2013 Lumen et al. model: KM_TPNG, the Michaelis-Menten constant for the sodium-iodide symporter for perchlorate based on Schlosser (2016), and a second key parameter in the 2009 USEPA/2007 Clewell et al. PBPK models: VMAXC_MKP, the maximal velocity for perchlorate transfer to breast milk from those components in the USEPA 2009 models. These two parameters affect perchlorate transport from the mammary plasma to breast milk (VMAXC_MKP) and the strength with which perchlorate inhibits iodine transport by the sodium-iodine symporter (KM_TPNG). Detailed information on the assumptions and data considered in making these changes are provided in the report appendices. Are the changes scientifically supported and reasonably estimated?

3. Model calibration and sensitivity analysis: Please address the strengths and limitations of the model calibration process and the strengths and limitations of the data used for model calibration and evaluation. Identify any additional literature, data, or peer reviewed models that EPA should consider to address identified limitations.

    a. Specifically, is the model calibration and evaluation adequately compared with what is reported in the literature for iodine and perchlorate concentrations in urine, and breast milk and thyroid hormone plasma concentrations under different iodine intake and perchlorate exposure scenarios? Please identify additional studies that would support model calibration that strengthen the proposed model.
    b. Are the influential parameters identified in the local sensitivity analysis for total thyroxine (T4) and free thyroxine (fT4) plasma concentrations consistent with known biology? Are there critical data gaps for the influential parameters, which would impact the thyroid hormone predictions? If gaps are identified, please provide approaches that would improve thyroid hormone predictions.

    c. Does the proposed model provide the appropriate level of precision and substantiated support for this approach? Please identify any additional limitations of the model's applicability?

4. Hypothyroxinemic reference levels and model predictions: The SAB (2013) recommended that the EPA focus the application of BBDR modeling on the condition of hypothyroxinemia, in which circulating levels of free thyroxine (fT4) are reduced below the normal ("reference") range, but with normal levels of thyroid stimulating hormone (TSH). While the mode of action (MOA) for perchlorate is well described, a defined set of quantitative definitions for reference ranges for hypothyroxinemia during pregnancy or lactation and in infants is not available. For interpretation of the BBDR model, EPA developed a normalization procedure to derive hypothyroxinemia reference intervals and to control for the expected differences between literature results and model predictions.

    a. Please comment on the hypothyroxinemia reference ranges derived for pregnant mothers, fetuses, lactating mothers, breast-fed infants and bottle-fed infants. Is the assumed range between the 2.5th and 10th percentile of the fT4 distribution

reasonable? Is it supported by the available data? Has the derivation of the reference ranges, including the normalization process, been appropriately described and supported in the model and the report?

b. Model predictions for zero intake of perchlorate indicate the range of iodine ingestion for which these life-stages are expected to be euthyroid vs. hypothyroxinemic. Are these predictions reasonable and have they been appropriately described and supported in the model and the report?

c. Based on the perchlorate level at which each life stage is predicted to become hypothyroxinemic (for a given iodine ingestion rate), it appears that the lactating mother is more sensitive than the breast-fed infant for PND 30-90. In part, this occurs because breast feeding reduces the iodide available for the maternal thyroid. Are these predictions reasonable and have these predictions been appropriately described and supported in the model and the report?

5. Robustness of model predictions and sensitivity at lower perchlorate dose levels: The dose-response predictions from the BBDR model predict levels of fT4 for:
   a) The pregnant mother and fetus at GW40 (Fig. 10);
   b) The formula-fed infant with fixed (Fig. 11, 7 and 30 day infants) and variable (Fig. 12, 7 through 90 day infants) iodine levels in their formula; and
   c) The breast-fed infant as a function of maternal perchlorate exposures and iodine ingestion (Fig. 13, 7 through 90 day infants).

   For each of these subgroups, please characterize the extent of uncertainty along the dose-response curve. In particular, is the level of uncertainty acceptable at lower dose levels of perchlorate (e.g., at or below 4 µg/kg/d) and higher levels of iodine intake (e.g., 150 µg/d) for each of these subgroups (left upper corner of the dose-response curves)? Should EPA assume that the model becomes more uncertain with rising perchlorate dose levels and declining iodine ingestion, i.e., as model predictions for fT4 approach and move through the range of hypothyroxinemia. (It should be noted that calibration of the iodide and thyroid hormone sub-models used data for populations with higher iodine intake and background exposure to perchlorate.)

6. Empirical functions to predict thyroid hormone levels: The pregnancy model (but not the lactation/infant models) uses empirical functions to make urinary clearance of iodine and metabolic degradation of T3 dependent on the iodine ingestion level, in order to predict observed nonlinearity in the relationship between thyroid hormone levels and iodine intake. Does this use of empirical functions result in predicted outcomes or trends that are not plausible for serum thyroid hormones, particularly fT4?

7. Characterization of model predictions: The pregnancy and infant/lactation models were developed to specifically consider the range of iodine ingestion and thyroidal uptake where TSH is expected to stay in its normal range (i.e., they do not account for HPT feedback control). Do the models represent a reasonable effort to describe the relationships between iodine intake, perchlorate exposure and thyroid function? Are they sufficiently specific to predict perchlorate-induced changes in thyroid hormones in these life-stages over the range indicated? Please characterize the robustness, precision and sensitivity of the models for the

ability to extrapolate changes in T4 associated with perchlorate exposures over the range from low exposures (less than 1 μg/kg/d) to higher levels which the model and analysis predict to be hypothyroxinemic.

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ED_004105_00000033-00020

External Peer Review of the Application on Physiologically-Based Pharmacokinetic (PBPK) Modeling in the IRIS Toxicological Review for Tetrachloroethylene

Contract No.: EP-C-07-024
Task Order 135

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August 24, 2011

Printed on Recycled Paper

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Responses to Charge Questions **1**

1. NRC (2010) recommended that EPA develop a harmonized PBPK model for tetrachloroethylene and metabolites for use in its risk assessment, and provided advice to EPA on how such a model should be developed. **Overall, is the harmonized PBPK model developed by EPA clearly and transparently described and adequately responsive to the NRC recommendations?** 3

2. EPA's dose-response assessment includes development of a chronic inhalation reference concentration (RfC) and oral reference dose (RfD) for non-cancer effects, and an inhalation unit risk and oral slope factor for carcinogenic effects. The assessment uses the following dose metric predictions from the harmonized PBPK model to conduct inter-species and/or route-to-route extrapolation for use in the dose-response assessment: 5

   a. For the critical non-cancer effect of neurotoxicity, in accordance with NRC recommendations, the area-under-the-curve (AUC) of tetrachloroethylene in blood is used as the preferred dose metric, and represents a surrogate for the AUC of tetrachloroethylene in the brain. 5

   b. For non-cancer hepatotoxicity and hepatocarcinogenesis, liver oxidative metabolism was used as the preferred dose metric, due to weight of evidence that oxidative metabolism plays a role in these endpoints for tetrachloroethylene. Results for the AUC of trichloroacetic acid (TCA) in the liver were presented as an alternative dose metric for comparison purposes. 5

   c. For non-cancer nephrotoxicity and nephrocarcinogenesis, from a toxicological perspective, glutathione (GSH) conjugation metabolism would have been the preferred dose metric due to the weight of evidence that conjugative metabolites play a role in these endpoints for tetrachloroethylene. However, due to the wide range of PBPK model predictions for GSH conjugation in humans, the surrogate dose metric of AUC of tetrachloroethylene in blood was preferred. Results for GSH conjugation were presented as an alternative dose metric for comparison purposes. 5

   d. For all other non-cancer endpoints (reproductive, developmental, immunological, hematological toxicity) and cancer endpoints (hemangiosarcomas, mononuclear cell leukemias, brain gliomas, and testicular interstitial cell tumors), the AUC of tetrachloroethylene in blood was used as the preferred dose metric, due to the lack of available data on what the active carcinogenic moiety(ies) may be for these endpoints. 5

   Is the harmonized PBPK model used appropriately for making predictions for these dose metrics, and are the results appropriately characterized? **5**

Additional Reviewer Comments **9**

Appendix A: Individual Reviewer Comments **A-1**

Janusz Z. Byczkowski A-3

Claude Emond A-7

Moiz Mumtaz A-13

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Final Report

External Peer Review of EPA's Physiologically-based Pharmacokinetic (PBPK) Model for Deltamethrin and Permethrin and PBPK-Pharmacodynamic (PBPK-PD) Model for Carbaryl

Review Panel:
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Hugh Barton, Ph.D. (Co-Chair)
Penelope Fenner-Crisp, Ph.D., DABT
Sami Haddad, Ph.D.
Zhoumeng Lin, Ph.D., DABT
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Brad Reisfeld, Ph.D.
Barry Ryan, Ph.D.
Marc-Andre Verner, Ph.D.
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Contract No. EP-C-17-023
Task Order 0007

Prepared for:
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November 9, 2018

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I. INTRODUCTION

Versar, Inc. (Versar), an independent EPA contractor, coordinated an external peer review of EPA's Physiologically-based Pharmacokinetic (PBPK) Model for Deltamethrin and Permethrin and PBPK-Pharmacodynamic (PBPK-PD) Model for Carbaryl. The purpose of this review was for ten experts to evaluate the scientific integrity of EPA's consideration of PBPK and PBPK-PD models to critically evaluate not only the structure, parameterization, and coding of these models, but also their scientific credibility for use in human health risk assessments. Versar held calls with EPA and the review panel for background information and clarification of the models. Versar also held weekly panel calls for the reviewers to discuss the challenge questions. Finally, Versar held a public meeting webinar in which the panel gave a presentation on its draft findings. This report summarizes the panel's peer review comments in response to a series of charge questions pertaining to the models.

A. Background

Federal law requires that pesticide products be registered for sale in the United States with the Environmental Protection Agency (EPA). Several statutes establish the regulations under which this process occurs. The FFDCA (Federal Food Drug and Cosmetic Act), with amendments, and the FIFRA (Federal Insecticide Fungicide and Rodenticide Act) are the primary legislation which govern this process. The process used to decide whether or not pesticide products can be legally marketed is risk-based and risk assessments for pesticides are developed using the framework outlined by the National Academy of Science criteria for risk assessment in the Federal government. EPA is also required to constantly re-evaluate all pesticide products through a continual process, which occurs every 15 years, known as Registration Review to ensure that the most up to date scientific information and methods are used for regulatory decision-making purposes.

Insecticides play a key role in modern society by enabling food, feed, and fiber production because they reduce pest pressure in production agriculture. Additionally, insecticides play critical roles in structural pest control and for public health purposes. Permethrin, deltamethrin and carbaryl are all widely used insecticides which can be used on many crops and in the other mentioned areas. The three insecticides, which are the focus of this review, are currently being evaluated in EPA's Registration Review process. In other words, the risk assessments for these three insecticides are all in the process of being updated. In typical risk assessments, animal model-derived toxicity values are coupled with pertinent exposure information to calculate risk estimates, which are then compared to an appropriate safety (uncertainty) factor. When risks are identified, EPA can opt to refine the risk assessment with additional data, tools, and techniques. In this case, physiologically-based pharmacokinetic (PBPK) or PBPK-pharmacodynamic (PBPK-PD) models have been developed to allow for more refined consideration of how animal toxicity data can be extrapolated to humans and how differing exposure patterns in humans can impact risk estimates. The focus of this review is to evaluate the individual PBPK or PBPK-PD models for these three insecticides.

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B. Peer Review Process

Versar was tasked by EPA with assembling ten scientific experts to conduct an external peer review of EPA's physiologically-based pharmacokinetic (PBPK) model for deltamethrin and permethrin and PBPK-pharmacodynamic (PBPK-PD) model for carbaryl. The peer review process provided a documented, independent, and critical review of the PBPK/PBPK-PD models, and its purpose was to identify problems, errors, or necessary improvements to the models prior to their use in further refining the risk assessments for permethrin, deltamethrin and carbaryl. In assembling these peer reviewers and coordinating the peer review, Versar was charged with evaluating the qualifications of peer review candidates, conducting a thorough conflict of interest (COI) screening process, independently selecting the ten peer reviewers, distributing review materials, managing the written peer review period, and organizing and hosting the peer review calls in developing a final peer review report.

The peer review selection process was initiated by Versar to identify candidate reviewers with demonstrated expertise in the scientific disciplines related to human health risk assessment with an emphasis on biological processes, the kinetics of environmental contaminants in human systems, and the development and use of quantitative tools to simulate such processes, especially PBPK or PBPK-PD modeling. Versar's in-depth and multi-staged evaluation of qualifications was based on each candidate's curriculum vitae (CV) and publications. In total, Versar identified over 30 potential candidate reviewers to determine their interest and availability to participate in this peer review.

In addition to the evaluation of candidates' expertise, Versar conducted a thorough COI screening of the candidate reviewers. Each candidate reviewer was required to complete a series of screening questions to help determine if they were involved with any work and/or organizations that might create a real or perceived COI. Following this screening process, Versar independently selected ten reviewers to participate in the peer review and EPA provided consent on the ten selected reviewers. In addition, Versar selected Dr. Kenneth Portier as Chair of the peer review effort and Dr. Hugh Barton as Co-Chair. The list of the ten peer reviewers who participated in this review is provided below. Following the selection process, Versar distributed to the reviewers EPA's background materials on the PBPK/PBPK-PD models along with approximately 20 charge questions (See Section II). The reviewers began work July 16, 2018 and were given approximately four months to submit a panel report of their written comments which included overall general impressions; answering approximately 20 charge questions in five areas: model representation, model coding, model evaluation, model documentation, and model application in human health risk assessment; and finding consensus where possible on specific issues. Additionally, the reviewers participated in two conference calls with EPA to supply the reviewers with background information on the models and to allow the reviewers to ask for clarification on the models and the charge questions. The first call was an initial briefing held on July 16, 2018 and the second call was a clarification call held on August 7, 2018. Finally, Versar held a public meeting webinar on October 12, 2018 (See Appendix 2 for attendees) in which the peer review panel gave a presentation on its draft findings (See Appendix 3).

Peer Reviewers:

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The reviewers were provided with the following files and supporting documents, among many others in order to run the models in R and assess the application of PBPK/PBPK-PD models to the risk assessments for permethrin, deltamethrin, and carbaryl.

File name	**Brief Description**
OPP issue paper for PBPK review	EPA's Office of Pesticide Programs' Review of PBPK Models for Carbaryl, cis-Permethrin, Deltamethrin
Carbaryl_SAP_White_Paper_Feb2018	White paper on the application of PBPK models to carbaryl risk assessment
CAPHRA_FINAL_2-2-2018	White Paper: Application of PBPK Models to Pyrethroid Risk Assessment
readme_CAPHRA_human	Instructions for the human model in R
readme_CAPHRA_rat	Instructions for the rat model in R
PyrethroidModelFile_Summary	Example of files to be submitted related to the physiologically based pharmacokinetic (PBPK) models for Deltamethrin and cis-Permethrin
Pyr_Delta_PBPKStudy Tracking	Tracked in PRISM under "Pyrethroid Working Group (PWG)" case name
CarbarylModelFile_Summary	A list of submitted documents and model files

II. CHARGE TO REVIEWERS

1. **Model Representation:** It is important that the conceptual and mathematical descriptions of the PBPK or PBPK-PD models properly represent the behaviors in critical compartments of biological systems for the insecticides being evaluated (i.e., permethrin, deltamethrin, carbaryl, and their degradates/metabolites). It is also critical that the model representations are valid and appropriate regarding the transformations from animal and other applicable data/information to adequately reflect biological processes in humans. Exposure pathways must also be adequately supported and clearly described (e.g., bolus, drinking water) as well as all assumptions made in constructing the model structure. Please address the following questions for each model under review by the panel:

   a) Please comment on whether the model structure is appropriate for describing the PK and PD properties of carbaryl, such as the assumptions of diffusion-limited compartments, the inclusion of major metabolic pathways, descriptions of oral, dermal and inhalation routes, and incorporations of cholinesterase binding in plasma and other tissues. Please comment on whether the model structure is appropriate for describing the PK properties of deltamethrin and permethrin, such as the assumptions of flow-limited and diffusion-limited compartments, the inclusion of major metabolic pathways in different tissues, and descriptions of different exposure routes.

   b) Please comment on the correctness of mathematical equations, as presented in the model code, to describe the model structure.

   c) Please comment on the selection of the dose-response or time-concentration studies that are used to support the construction of the rat model. Are these studies sufficient to support the development of the PBPK-PD model to predict Acetylcholinesterase (AChE) inhibition in Red Blood Cells (RBC) and in the brain from exposure to carbaryl, or the development of the PBPK model to predict peak brain and plasma concentrations from exposure to deltamethrin and permethrin. Please comment on whether it is appropriate to construct the human model based on the structure of the rat model, for which time- concentration or dose-response data exist.

   d) Please comment on the selection and/or estimation of values and distributions for all model parameters, such as age- and gender-dependent physiological parameters and enzyme ontogeny, tissue/blood partition coefficients, metabolic constants. And for carbaryl only, please comment on the selection and/or estimation of values and distributions for pharmacodynamic parameters that determine the binding of carbaryl to cholinesterase's active site, carbamylation of the active site, and subsequent reactivation of enzyme activity.

      - Please comment on whether the model properly accounts for human variability in PK and PD by considering age- and gender-differences.

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- Please comment on the correctness of allometric scaling of parameters. Please comment on the methods used to extrapolate in vitro-derived data (e.g., metabolism constants) to an in vivo system. Please comment on the in vitro to in vivo extrapolation (IVIVE) and enzyme ontogeny approaches for estimating intrinsic clearance for humans at different ages.

- For carbaryl only, please comment on whether it is appropriate to use empirical ratios to adjust an in vitro measured bimolecular rate to fit in vivo AChE inhibition data in brain and in plasma.

- For deltamethrin and permethrin only, please comment on the justification and appropriateness of using an empirical factor to adjust the apparent Km from an *in vitro* system to an *in vivo* free- concentration based K_m. Please comment on the appropriateness of including lymphatic absorption and protein binding in the model.

2. **Model Coding:** The accuracy of coding used to reflect model equations in a programming language (in this case "R") is central to ensuring the proper implementation of any model. It is also important to ensure sources for values of model parameters are appropriately documented and that they are correctly implemented in the model code (e.g., calculation of model parameters such as converting in vitro metabolism rate to in vivo metabolism rate). In cases for which values of model parameters are estimated by fitting the model predictions with data it is also important to ensure such estimates are reasonable as such approaches are often used to calibrate models (e.g., eyeball fitting or statistical optimization). Please address the following questions for each model under review by the panel:

   a) Are the model equations correctly coded in programming language R?.

   b) Are sources for values of model parameters (with units) appropriately documented and annotated, and are these values correctly implemented in the model code?

   c) Are all additional calculations not related to model structure (e.g., calculating concentrations from predicted amounts) correct in the model code?

   d) Please run the model simulations and comment on whether these simulations provide the same outputs as those documented in the materials supplied to the panel.

3. **Model Evaluation:** Examination of the theoretical construct and coding of any model is critical in an overall review process. There are basic criteria associated with such evaluations, which are noted below. Please address the following questions for each model under review by the panel:

   a) Is the mass balance achieved?

   b) Are all units of model parameter correct?

c) Do model simulations agree with diverse sets of experimental data that have not been used for calibrating the model?

d) In cases where values of model parameters were estimated by fitting the model predictions with time concentration or dose-response data, what method was used to objectively determine the model's goodness-of-fit (e.g., eyeball fitting, statistical optimization) and was such method acceptable? Please comment of the model fit to the selected time-concentration and dose-response data. For carbaryl only, please comment on whether the PBPK-PD model is capable of simulating the general trend of published human time-concentration data, as well as time profiles of AChE inhibition in RBC. For deltamethrin and permethrin only, please comment whether the PBPK model is capable of simulating the general trend of published time-concentration data measured in post-natal day 90 (PND90) and PND15 rats, and whether the parallelogram approach is appropriate to support the development of a human life-stage PBPK model in the absence of human *in vivo* data.

e) Please comment on the conclusions from sensitivity and uncertainty analyses, and the implications from these results on the estimation of data-derived extrapolation factors (DDEFs) and derivation of scenario-specific human points of departure (PoDs) (Note that exposure scenarios are not subjected to this peer review).

4. **Model Documentation:**

a) Is the rationale for conducting the PBPK or PBPK-PD analysis clearly stated and supported by the modeling efforts?

b) Is the model code properly annotated?

5. **Model Application:** PBPK or PBPK-PD models can be (and have been) used to estimate critical inputs for the risk assessment process including DDEFs and PoDs. Model code can be developed and implemented to automate the process of estimating these values from animal data. The models under review represent pesticide uses which can occur under a variety of conditions (e.g., durations, pathways, routes). Please address the following questions for each model, as appropriate, under review by the panel:

a) Please comment on the approach used to determine the most sensitive age and gender combination that results in the highest peak AChE inhibition in RBC and in brain at a given dose of carbaryl or the highest peak brain or plasma concentration of deltamethrin/permethrin at a given dose of deltamethrin/permethrin. Please comment on the subsequent use of the most sensitive age and gender combination for a specific exposure scenario to estimate the PoD for that scenario.

b) Please comment on whether the Monte Carlo simulations, including the selection of parameters to be varied and their distributions, are appropriate for use in estimating distributions of the peak AChE inhibition levels in RBC and in brain after exposure to carbaryl, and for use in estimating DDEFs for carbaryl. Similarly, please comment on

whether the Monte Carlo simulations, including the selection of parameters to be varied and their distributions, are appropriate for use in estimating distributions of the peak deltamethrin and permethrin concentrations in plasma and in brain, and for use in estimating DDEFs for deltamethrin and permethrin.

c) Please comment on whether the carbaryl PBPK-PD model is appropriate for use to replace the default uncertainty factors for PK and PD with the DDEFs, and to derive scenario-specific PoDs. Please comment on whether the PBPK models for deltamethrin/permethrin are appropriate for use to replace the default uncertainty factors for PK with the DDEFs, and to derive scenario-specific PoDs

d) Please comment on the potential to apply a read-across approach that uses one generic model structure in conjunction with chemical-specific parameter values to construct PBPK or PBPK-PD models for other chemicals in the same class (i.e., carbaryl mode structure for other n-methyl carbamates, deltamethrin/permethrin model structure for other pyrethroids.

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