

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

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Submitted to:

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Office of Research and Development
National Center for Environmental Assessment
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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
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Responses to Charge Questions

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?**

Reviewer	Comments
Byczkowski	<p>While the harmonized PBPK model developed by EPA seems to be adequately responsive to the NRC recommendations from technical standpoint, it is not clearly and transparently described in the reviewed document.</p> <p>The "<i>overall structure</i>" of updated PBPK model is presented in the Figure 3-5 (P 9). This figure is rather poorly designed - graphically and logically. The major components are not interlinked in the schematic figure, although, they are in the functional model, and the figure itself may be confusing. For example, it includes several "shortcuts" which are inappropriate in formal writing, e.g., "<i>Liver Oxidation</i>", "<i>Lung Oxidation</i>", etc. (they should be changed, for example, to "<i>Oxidation in Liver</i>", "<i>Oxidation in Lungs</i>", etc.). Also, all abbreviations not listed previously in the "<i>List of Abbreviations and Acronyms</i>" should be explained in the figure's caption (e.g., "<i>ODEs</i>" - perhaps, stands for "ordinary differential equations"?)</p> <p>Essentially, the detailed description of mechanistic workings of the "harmonized" PBPK model is missing in the reviewed document. Instead (as quoted from Section 3.5.1.2; P 8; L# 33 - 34) "...<i>The reader is referred to Chiu and Ginsberg (2011) for further details of the model structure...</i>" The publication, still "in press", contains in the "<i>Appendix A</i>" the model equations. However, this publication (<i>Chiu and Ginsberg, 2011, in press</i>) does not provide a detailed narrative description of the model. It is stating instead that the model "<i>is based on the TCE PBPK model reported by Chiu et al. (2009)</i>", thus sending the reader to yet another paper. Also, the <i>Chiu and Ginsberg (2011) in press</i> contains some problematic statements, e.g., "<i>evidence that P450s in addition to CYP2E1 are involved in perc metabolism</i>", which should be corrected before sending a final version of the paper to press (thus, CYP2E1 is a cytochrome P450, family 2, subfamily E, polypeptide 1).</p>
Emond	<p>In the tetrachloroethylene document, the U.S. Environmental Protection Agency (EPA) appeared to respond clearly and transparently to the National Research Council's (NRC's) recommendation (as mentioned in the question above. NRC also mentioned that it "<u><i>provided advice to EPA on how such a model should be developed.</i></u>" However, we did not see the advice during this review). Subsequently, the EPA made efforts to improve the earlier models published in the literature and published this model (see Chui and Ginsberg, 2011) with reviewers committee. Accordingly, this new physiologically based pharmacokinetic (PBPK) version includes the most relevant mode of action described in the previous versions and using an extensive analysis with different data sets to produce the PBPK model used in this risk assessment. This PBPK model expansion seemed to integrate a better mechanism relevant to predicting the internal dose metric in the target tissue.</p>

	<p>The PBPK model is well presented in the improvement initiative document, but there is still room for improvement. For example, the conceptual representation of the PBPK model given in Figure 3-5 (on page 9) is useful in understanding the PBPK model used in this risk assessment, but it did not provide a full understanding of the model's structure. Figure 3-5 could be expanded to also include the symbols used for the model parameters (e.g., blood flow, partition coefficient, metabolic parameters along with the appropriate arrows and volumes symbols used for the compartments). To do that, the conceptual model might be presented in two pages rather than one page as it is presented here. One page could consist of the main compartment, and the second page could contain the metabolite descriptions. In addition, the EPA should explain terms used such as the static, dynamic, and transformation or excretion compartments in the figure's legend.</p> <p>Another suggestion is to use a full line for the simulation and symbols for the experimental or epidemiological data in Figure 3-3. In Tables 3.2 to 3.5, the EPA presents predictions for the area under the curve (AUC) of tetrachloroethylene in blood and their metabolites. We can see the unit in the title but it is not obvious how we should interpret these tables, specifically, what are the differences between baseline and posterior modes? These tables could be useful if more details in the tables or in the text are presented.</p>
<p>Mumtaz</p>	<p>The US EPA has used credible science and current information to adequately respond to the recommendations of the NRC. Yes, the harmonized model has integrated important aspects of the previously published models and has used all available data from relevant species and exposure routes. The EPA has used a much wider range of experimental data than previously used, thus broadening the domain of the models application. This included the use of in vitro and in vivo data, contributions of the GSH and the P-450 metabolic pathway that allows greater use of accurate dose metrics than previously possible. The uncertainty in the assessment using this model will be within the acceptable range of variability in chemical risk assessments and should not have an impact in the decision making process. Based on the current science, further investment of resources in further evolving this model might not necessarily contribute to the improvement of the overall assessment. Computational tools such as these allow rigorous analysis of uncertainties and variation in biological data. However, these are resource intensive activities, and such tools should be refined only when the new insights justify the investment.</p>

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:
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 - 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.
 - 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.

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

Reviewer	Comments
Byczkowski	<p>There is very little explanation provided in the reviewed document regarding the manner of use of PBPK model. Moreover, quite limited description of the methodology of use contains some cryptic statements, e.g., "...<i>limited Bayesian analysis involving flat priors...</i>" (Section 3.5.1.2; P10; L# 22 - 23).</p> <p>It is not clear how the area under the concentration curve over time (AUC) was calculated (AUC_∞ or AUC_{day}, as it is listed in the Table 3-2). Also, how and when the uncertainty factors were applied (were all UFs applied to the external dose (POD) or some were applied to the internal dose metric, and then the quotient was back-calculated by PBPK model to find the equivalent external dose?).</p> <p>In addition, the population model, whose codes were provided to this reviewer in the "Background Material" (<i>perc.v2.1.0.mcmc.pop.model.pdf</i>), cannot be understood without a careful reading of <i>Chiu and Ginsberg (2011) in press</i> publication, including <i>Appendix A</i>.</p>

	<p>The code of population model itself is rather poorly documented. It is even not clear which version of GNU MCSim software has been used in simulations. There are no actual numerical values listed for model parameters (in the codes, the values are set to defaults). No units are listed for variables as well as for some parameters. However, most of this information is available in <i>Chiu and Ginsberg (2011) in press</i>.</p> <p>It is important to emphasize, that these critical remarks are regarding the presentation: description of PBPK methodology used and the narrative characterization of the results, but not regarding the technical soundness of the PBPK methodology or the numerical results, which seem to be adequate.</p> <p>Specifically:</p> <ol style="list-style-type: none">a. The use of the area-under-the-curve of concentration over time (AUC) of tetrachloroethylene in blood as a surrogate dose metric for chronic (long term) neurotoxicity, seems to be appropriate and the results are adequate.b. The use of the AUC of trichloroacetic acid (TCA) in the liver as a surrogate dose metric for chronic (long term) hepatotoxicity, also seems to be appropriate and the results are adequate.c. While nephrotoxicity and nephrocarcinogenesis are probably mechanistically linked to GSH conjugative metabolites of tetrachloroethylene, the quantitative experimental data for GSH conjugation metabolism are highly uncertain. Obviously the PBPK model (and any predictive model) could be only as accurate, as the data used for its calibration. Therefore, the use of AUC of tetrachloroethylene in blood as a surrogate dose metric for nephrotoxicity and nephrocarcinogenesis seems to be more appropriate than the GSH conjugation metabolites.d. Also, the use of AUC of tetrachloroethylene in blood as a surrogate dose metric for reproductive, developmental, immunological, hematological toxicity and other than kidney cancer endpoints (hemangiosarcomas, mononuclear cell leukemias, brain gliomas, and testicular interstitial cell tumors) seems to be as appropriate as it can be, due to the lack of more adequate data. <p>It is strongly suggested that in the revision of the "<i>Toxicological Review for Tetrachloroethylene</i>", the authors of the reviewed section should follow in their description and documentation of the use of PBPK model the U.S. EPA (2006) recommendations: "<i>Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment</i>." EPA/600/R-05/043F, August 2006. On line http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157668#Download, especially, the Section 3.6.3 of this guidelines.</p>
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<p>Emond</p>	<p>a. Because of the uncertainty, the unknown mechanism related the brain compartment, and in this model the brain was not described as a distinct compartment in the model; thus, using blood as a surrogate is an acceptable alternative. This approach is better than using the AUC in brain without confidence of these predictions.</p> <p>b. I agree with the way that the EPA presented its argument about TCA and proof of concept. A reason why the EPA prefers the AUC of TCA is because this metabolite is consistently the only metabolite measured. This appears to be reliable for the effect observed. In addition, the literature supports this choice as reported in Tables 4-17 to 4-19.</p> <p>c. The PBPK model developed by the EPA and published by Chui and Ginsberg (2011) can measure the GSH conjugation metabolism. However, as the EPA pointed out, a large range observed in humans tends to reduce the confidence of this mode of action and metabolite pathways. Saying that, regarding the accuracy and the transparency, it is more prudent to use the AUC in blood as a surrogate dose metric in blood.</p> <p>d. The EPA used the AUC of blood, but the Agency could also use the blood concentration. Usually a surrogate metric should meet certain criteria, including occurrence before signs of toxicity and a well-established linkage between the surrogate markers and signs of toxicity. For the tetrachloroethylene, I have not seen this evidence of a clearly linkage with blood. The fact that EPA used a blood dose metric as a surrogate to make the dose effect linkage is valuable in this case, particularly because there is a lack of available data. The EPA's rationale to use it was because the blood concentration was closer than the administrated dose, which is true. I agree with the EPA's choice for this specific situation.</p> <p>As a toxicologist, PBPK modelers learn early during their work that their models are never final. A final PBPK model today will need to be improved tomorrow or in a year or more. At this point regarding our knowledge and data that are available, I believe this model corresponds to the best model currently available. After conducting my review, it appears to me without any doubt that using a PBPK model improves the quality of the predictions for risk assessment, and I anticipate that using the current model will reduce the uncertainties that resulted from using previous PBPK models or the default approach. Because of these two statements, I believe after reviewing this document and the document provided for this review, this version of the tetrachloroethylene model is adequate for making predictions for these dose metrics.</p>
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Mumtaz	<ul style="list-style-type: none">a. The recommendation of NRC is being followed appropriately. But, using blood PERC as a surrogate for brain PERC seems to imply that all of the blood PERC goes directly to the brain (i.e., crosses the blood-brain barrier). It also implies a steady state of blood PERC with no metabolism. Those assumptions would be most unlikely and certainly could result in an unrealistically conservative RfC.b. Were the other metabolites hepatotoxic?c. No comment.d. Lack of data on what the active carcinogenic moiety(ies) was mentioned as the reason for using AUC of PERC in blood as the preferred dose metric, but what about the best metric for non-cancer effects?
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Additional Reviewer Comments

Additional Comments Submitted by Dr. Moiz Mumtaz

- p. 27, lines 17-18: The sentence beginning “In addition, ...” is missing something. Should the word “is” be inserted between the words “model” and “being?”
- p. 28, section 5.4.4.1: If the human data gives the lowest sRfC, why are the other sRfCs necessary?
 - ATSDR’s acute inhalation MRL for PERC is 0.2 ppm (~1.4 mg/m³) based on human exposure to 10 or 50 ppm, 4 hours/day for 4 days (Altmann et al., 1992). This does not conflict with the EPA sRfC.
 - ATSDR’s acute oral MRL is 0.05 mg/kg/day based on hyperactivity in mice (Fredriksson et al. (1993).
 - ATSDR has not derived intermediate or chronic duration MRLs (inhalation or oral) for PERC.
- p. 31, 3rd line (unnumbered) from top of page: “7 weeks” is mentioned twice (referring to Marth, 1987). Suggest re-wording to eliminate redundancy.
- p.31, numbered line 6, section 5.1.4.4: Instead of reproductive “and” developmental effects, should it actually be reproductive “or” or “and/or” effects?
- p. 33, section 5.2.2: Does the harmonized PBPK model of Chiu and Ginsberg (2011) take into account the first pass effect with oral exposure? If so, state how.
- p. 33, line 12: The use of the term “inform” is unclear.
- p. 33, line 36: What is a “continuous oral exposure”? This needs explanation.

Appendix A: Individual Reviewer Comments

COMMENTS SUBMITTED BY

Janusz Z. Byczkowski, Ph.D., DABT
Independent Consultant
Fairborn, OH

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

Comments submitted by Dr. Janusz Z. Byczkowski

1. Overall, is the harmonized PBPK model developed by EPA clearly and transparently described and adequately responsive to the NRC recommendations?

While the harmonized PBPK model developed by EPA seems to be adequately responsive to the NRC recommendations from technical standpoint, it is not clearly and transparently described in the reviewed document.

The "*overall structure*" of updated PBPK model is presented in the Figure 3-5 (P 9). This figure is rather poorly designed - graphically and logically. The major components are not interlinked in the schematic figure, although, they are in the functional model, and the figure itself may be confusing. For example, it includes several "shortcuts" which are inappropriate in formal writing, e.g., "*Liver Oxidation*", "*Lung Oxidation*", etc. (they should be changed, for example, to "Oxidation in Liver", "Oxidation in Lungs", etc.). Also, all abbreviations not listed previously in the "*List of Abbreviations and Acronyms*" should be explained in the figure's caption (e.g., "*ODEs*" - perhaps, stands for "ordinary differential equations"?)

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- a. The use of the area-under-the-curve of concentration over time (AUC) of tetrachloroethylene in blood as a surrogate dose metric for chronic (long term) neurotoxicity, seems to be appropriate and the results are adequate.
- b. The use of the AUC of trichloroacetic acid (TCA) in the liver as a surrogate dose metric for chronic (long term) hepatotoxicity, also seems to be appropriate and the results are adequate.
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COMMENTS SUBMITTED BY

Claude Emond, Ph.D.
Associate Professor
University of Montreal
Quebec, Canada

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Comments submitted by Dr. Claude Emond

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?**

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The PBPK model is well presented in the improvement initiative document, but there is still room for improvement. For example, the conceptual representation of the PBPK model given in Figure 3-5 (on page 9) is useful in understanding the PBPK model used in this risk assessment, but it did not provide a full understanding of the model's structure. Figure 3-5 could be expanded to also include the symbols used for the model parameters (e.g., blood flow, partition coefficient, metabolic parameters along with the appropriate arrows and volumes symbols used for the compartments). To do that, the conceptual model might be presented in two pages rather than one page as it is presented here. One page could consist of the main compartment, and the second page could contain the metabolite descriptions. In addition, the EPA should explain terms used such as the static, dynamic, and transformation or excretion compartments in the figure's legend.

Another suggestion is to use a full line for the simulation and symbols for the experimental or epidemiological data in Figure 3-3. In Tables 3.2 to 3.5, the EPA presents predictions for the area under the curve (AUC) of tetrachloroethylene in blood and their metabolites. We can see the unit in the title but it is not obvious how we should interpret these tables, specifically, what are the differences between baseline and posterior modes? These tables could be useful if more details in the tables or in the text are presented.

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Because of the uncertainty, the unknown mechanism related the brain compartment, and in this model the brain was not described as a distinct compartment in the model; thus, using blood as a surrogate is an acceptable alternative. This approach is better than using the AUC in brain without confidence of these predictions.

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COMMENTS SUBMITTED BY

Moiz Mumtaz, Ph.D.

Science Advisor

Computational Toxicology and Methods Development Laboratory

Division of Toxicology and Environmental Medicine

Agency for Toxic Substances and Disease Registry

Atlanta, GA 30333

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

Comments submitted by Dr. Moiz Mumtaz

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?**

The US EPA has used credible science and current information to adequately respond to the recommendations of the NRC. Yes, the harmonized model has integrated important aspects of the previously published models and has used all available data from relevant species and exposure routes. The EPA has used a much wider range of experimental data than previously used, thus broadening the domain of the models application. This included the use of in vitro and in vivo data, contributions of the GSH and the P-450 metabolic pathway that allows greater use of accurate dose metrics than previously possible. The uncertainty in the assessment using this model will be within the acceptable range of variability in chemical risk assessments and should not have an impact in the decision making process. Based on the current science, further investment of resources in further evolving this model might not necessarily contribute to the improvement of the overall assessment. Computational tools such as these allow rigorous analysis of uncertainties and variation in biological data. However, these are resource intensive activities, and such tools should be refined only when the new insights justify the investment.

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:
 - 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.**
 - 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.**
 - 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.

- 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.**

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

2.a. The recommendation of NRC is being followed appropriately. But, using blood PERC as a surrogate for brain PERC seems to imply that all of the blood PERC goes directly to the brain (i.e., crosses the blood-brain barrier). It also implies a steady state of blood PERC with no metabolism. Those assumptions would be most unlikely and certainly could result in an unrealistically conservative RfC.

2.b. Were the other metabolites hepatotoxic?

2.c. No comment.

2.d. Lack of data on what the active carcinogenic moiety(ies) was mentioned as the reason for using AUC of PERC in blood as the preferred dose metric, but what about the best metric for non-cancer effects?

Other comments:

- p. 27, lines 17-18: The sentence beginning “In addition, ...” is missing something. Should the word “is” be inserted between the words “model” and “being?”
- p. 28, section 5.4.4.1: If the human data gives the lowest sRfC, why are the other sRfCs necessary?
 - ATSDR’s acute inhalation MRL for PERC is 0.2 ppm (~1.4 mg/m³) based on human exposure to 10 or 50 ppm, 4 hours/day for 4 days (Altmann et al., 1992). This does not conflict with the EPA sRfC.
 - ATSDR’s acute oral MRL is 0.05 mg/kg/day based on hyperactivity in mice (Fredriksson et al. (1993).
 - ATSDR has not derived intermediate or chronic duration MRLs (inhalation or oral) for PERC.
- p. 31, 3rd line (unnumbered) from top of page: “7 weeks” is mentioned twice (referring to Marth, 1987). Suggest re-wording to eliminate redundancy.
- p.31, numbered line 6, section 5.1.4.4: Instead of reproductive “and” developmental effects, should it actually be reproductive “or” or “and/or” effects?

- p. 33, section 5.2.2: Does the harmonized PBPK model of Chiu and Ginsberg (2011) take into account the first pass effect with oral exposure? If so, state how.
- p. 33, line 12: The use of the term “inform” is unclear.
- p. 33, line 36: What is a “continuous oral exposure”? This needs explanation.