EXHIBIT 1

EPA March 2022 Denial Letter of RFC 21005

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 14, 2022

OFFICE OF
RESEARCH AND DEVELOPMENT

Patrick Walsh
Denka Performance Elastomer LLC
560 Highway 44
LaPlace, LA 70068

Dear Mr. Walsh,

This letter is in response to the Request for Correction (RFC) received by the U.S. Environmental Protection Agency (EPA) from Denka Performance Elastomer LLC (Denka) on July 15, 2021. The RFC request was assigned RFC 21005 for tracking purposes. In the RFC letter, Denka asked EPA to re-evaluate certain conclusions presented in the 2010 IRIS Chloroprene Toxicological Review in consideration of new scientific information concerning the cancer effects of chloroprene on humans. The materials submitted by Denka present new analyses and express views on how these products should be used in the risk assessment of chloroprene, but the Denka submission does not identify errors in the 2010 IRIS assessment. After careful consideration, EPA has concluded that the underlying information and conclusions presented in the 2010 IRIS Toxicological Review of Chloroprene and its supporting materials are consistent with EPA's Information Quality Guidelines (U.S. 2002). Hence the RFC is denied.

The RFC process is intended to provide a mechanism to correct errors where the disseminated product does not meet information quality standards. The 2010 IRIS Chloroprene Toxicological Review was subject to rigorous independent peer review and public comment in 2010. Consistent with the EPA Information Quality Guidelines, this peer review is presumptive of objectivity and "best available" science at the time it was developed. The Information Quality Guidelines commits EPA to ensure, "to the extent practicable," that:

> "The substance of the information is accurate, reliable, and unbiased. This involves the use of (i) the best available science and supporting studies conducted in accordance with sound and objective scientific practices, including, when available, peer- reviewed science and supporting studies"...." In applying these principles, "best available" usually refers to the availability at the time an assessment is made."

EPA Information Quality Guidelines recognize that scientific knowledge about chemical hazards and risk changes and may need to be updated over time. However, the RFC process is not a mechanism to commit EPA to undertake scientific updates of its risk assessment products, such as IRIS Toxicological Reviews. EPA Information Quality Guidelines recognize explicitly that a decision to launch an updated assessment depends on important programmatic factors and resource availability. Given the finite resources of the IRIS Program, IRIS assessment activities are based on the priority needs of EPA National Program and Regional Offices identified through a structured internal nomination process. Any new scientific information submitted

through the RFC process would be considered if an update was initiated based on (1) the topic is identified as a National Program or Regional Office priority need, and (2) acceptance of the nomination by the IRIS Program given available resources. Importantly, the availability of new scientific information does not necessarily mean that existing IRIS toxicity values are outdated or not based upon the best available science. For example, EPA's 2018 denial of a prior RFC submitted by Denka indicated that the new scientific information described in that RFC would not alter the conclusions of the 2010 IRIS Assessment (see January 24, 2018, EPA Response to RFC 17002 Attachment 2 "Systematic Review of Chloroprene [CASRN 126-99-8] Studies Published Since 2010 IRIS Assessment to Support Consideration of the Denka Request for Correction (RFC)").

The RFC process does not require that EPA evaluate the potential impact of new scientific information on an existing IRIS toxicity value.

However, EPA is providing a courtesy technical review in its response to this RFC (Appendix A). This courtesy review substantially exceeds EPA's commitment toward addressing an RFC and should not be interpreted as setting a precedent for any future RFC request. Within the scope of the courtesy review, open science issues were identified concerning the PBPK model predictions proposed by Denka. EPA engaged external expert peer reviewers for aspects of this courtesy review (Versar, 2021). It should be noted that, even if the PBPK model predictions provided by the Denka were accepted at face value, the findings of EPA's courtesy review do not support Denka's assertion that applying the submitted PBPK model would lead to a large decrease in estimated risk compared with the existing IRIS assessment.

Your Right to Appeal

If you are dissatisfied with the response, you may submit a Request for Reconsideration (RFR) as described in EPA's Information Quality Guidelines. The EPA requests that any such RFR be submitted within 90 days of the date of the EPA's response. If you choose to submit an RFR, please send a written request to the EPA Information Quality Guidelines Processing Staff via mail (Information Quality Guidelines Processing Staff, Mail Code 2821T, USEPA, 1200 Pennsylvania Avenue, NW, Washington, DC 20460); or electronic mail (quality@epa.gov). If you submit an RFR, please reference the case number assigned to this original Request for Correction (RFC #21005). Additional information about how to submit an RFR is listed on the EPA Information Quality Guidelines website at https://www.epa.gov/sites/default/files/2020-02/documents/epa-info-quality-guidelines_pdf_version.pdf.

Sincerely,

Maureen R. Gwinn, Ph.D.
Principal Deputy Assistant Administrator

Cc: Vaughn Noga, Chief Information Officer and Deputy Assistant Administrator for Environmental Information, Office of Mission Support

Katherine Chalfant, Director of Enterprise Quality Management Division, Office of Mission Support

Appendix A: EPA Courtesy Technical Review of New Scientific Information Presented in RFC 21005
Appendix B: References

Appendix A: EPA Courtesy Technical Review of New Scientific Information Presented in RFC 21005

A. Background on the Denka RFC Submission

In 2010, EPA disseminated the IRIS Program's peer-reviewed Chloroprene Toxicological Review. EPA's consideration of pharmacokinetic (PK) modeling in the chloroprene assessment dates to this 2010 IRIS assessment and peer review, where a model by Himmelstein et al. (2004) proposed dosimetry estimates. The 2010 IRIS assessment explained why the Himmelstein 2004 results were not sufficient for incorporation into the IRIS assessment. In 2017, Denka filed an RFC (RFC 17002) submitting results of modeling by Yang et al. (2012) which extended the Himmelstein study with some additional *in vitro* data and expanded statistical modeling. On January 24, 2018, EPA rejected the 2017 RFC submitted by Denka. EPA evaluated the Yang results as part of its RFC response, noting limitations in the work (see Attachment 2 of EPA's denial). For example, the specific computer code used in the Yang et al. (2012) model could not be obtained. EPA needed the code to be able to adequately evaluate the model quality. Since the rejection of the 2017 RFC, EPA has engaged extensively with Denka and Ramboll[2] on the scientific issues related to Denka's proposals for applications of PBPK modeling which they view as supporting lower risk estimates for chloroprene. Notably, much of the core set of *in* vitro metabolism data underpinning the original Himmelstein et al. (2004) model remains at issue with Denka.

Denka responded to EPA's rejection of the 2017 RFC by filing a RFR (RFR 17002A) on July 24, 2018, which contained an updated and, at that time, unpublished model that had not been peer-reviewed developed by Ramboll addressing the same *in vitro* data set. EPA engaged substantially with Denka in the 2018-2020 period, contributing to quality assurance of the Ramboll model and providing suggestions on how to address model deficiencies (e.g., modeling of uptake of chloroprene by the *in vitro* reaction mix) and extend the model to attempt to address the fate of reactive metabolites. Importantly, while EPA provided feedback on quality assurance, EPA does not consider these discussions to constitute a formal quality assurance review, as the discussions alone did not satisfy the QA requirements outlined in the Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models developed by the U.S. EPA's Office of Research and Development (U.S. 2020b).

With Denka and Ramboll's cooperation, EPA hosted an extensive independent panel peer review in October 2020 to evaluate the revised model and supporting *in vitro* metabolic model, with resulting parameters, model predictions, and uncertainty analyses described by Ramboll (2020), and the alternate uncertainty analysis described by U.S. EPA (2020). The external peer reviewers identified a substantial number of key ("tier 1") recommendations necessary for: strengthening the scientific basis for the PBPK model, reducing model uncertainties, and accurately evaluating such uncertainties before the model is applied for risk assessment (see Final 2020 Chloroprene PBPK and Uncertainty Analysis Peer Review Report). The tier 1 issues identified by peer reviewers are technical matters that would require resolution before application of the model would be recommended.

After further technical interactions with EPA, Denka withdrew its RFR (RFR 17002A) on March 1, 2021. Subsequently, Denka submitted the current RFC in July 2021 (RFC 21005). This RFC contains new unpublished modeling analyses of the same *in vitro* database, more extensive statistical analyses, comparison with one *in vivo* study, and introduces modeling for reactive metabolites that has not been previously reviewed. To assist in preparing a response to RFC 21005, EPA conducted a follow-on independent letter peer review of the revised 2021 PBPK model, the results of which have been made available (see Versar, 2021). However, EPA is not obligated to review unpublished works submitted under the RFC/RFR process.

B. Technical Consideration of the 2021 Denka RFC 21005

Under EPA's Information Quality Guidelines, the RFC process does not require that EPA evaluate the potential impact of new scientific information on an existing IRIS toxicity value. However, because of significant investment by both Denka and EPA in considering the new PBPK approaches (discussed above), EPA is providing a technical analysis as part of its consideration of the July 2021 RFC. In this response, the EPA is addressing the following assertions raised in Sections III and IV in the Denka RFC 21005:

Assertion 1	IUR Should Be Corrected to Reflect the 2021 Ramboll PBPK Model (Exhibit A4 in the RFC). Denka states that: "The IUR Should Be Corrected to Reflect the 2021 Ramboll PBPK Model. Overall, the application of the 2021 PBPK model is expected to result in the estimation of an IUR that is approximately two orders of magnitude below that of the 2010 IUR."
Assertion 2	Major New Follow-Up Epidemiological Study by Dr. Gary Marsh et al., Released in 2020, Shows No Increased Cancer Mortality among U.S. Chloroprene Workers (summarized in Exhibit B5 in the RFC).
Assertion 3	New Cancer Incidence Data from the Louisiana Tumor Registry Shows the Incidence of Cancers near the Denka Faculty are At or Below State-wide Averages for Cancers of Potential Concern (summarized in Exhibit B5 in the RFC).

EPA Response to Assertion 1: IUR Should be Corrected to Reflect the 2021 Ramboll PBPK Model

EPA approached this submission by asking available peer reviewers from the Fall 2020 peer review to examine the new modeling work and advise on the extent to which it resolved tier 1 identified issues and was suitable for application (see Versar, 2021). The peer reviewers noted significant improvements in the model analysis, but multiple reviewers' comments and recommendations indicate that key uncertainties remain. These uncertainties include fundamental model assumptions, e.g., that chloroprene itself is treated as inactive but may be reactive and that data from studies on a different compound can be used to infer key metabolic rates. Some reviewers raised questions regarding whether the model was sufficiently reliable for use in risk assessment or, minimally, that additional experimental data should be obtained, and further analyses conducted to more fully quantify uncertainties. For example, two reviewer comments identify ongoing uncertainty about whether 7-ethoxycoumarin activity is an

appropriate predictor of chloroprene's oxidative metabolism and the extent to which cytochrome P450s (CYPs) enzymes other than CYP2E1 might contribute to this activity. In addressing the discrepancy between model predictions and the mouse *in vivo* pharmacokinetic (PK) data, one reviewer noted that chloroprene has constitutive chemical reactivity that may result in loss of the parent compound throughout the body. The model over-predicts blood concentrations observed after inhalation exposure to mice and the reviewer commented that this over-prediction may occur because it does not account for this constitutive reactivity. This constitutive reactivity may also explain the cancer incidence in mouse and rat tissues which do not have significant CYP enzyme metabolic activity. A separate example is noted by another reviewer regarding the statistical analysis of uncertainty in the metabolic parameters, where it appears that the joint uncertainty in Kgl may not have been incorporated. Kgl is a parameter that determines the rate of chloroprene transport between the air and liquid phases in the *in vitro* metabolic system that was used to determine the metabolic parameters for the rate of chloroprene oxidation in the lung and liver of mice, rats, and humans. Because the estimated values of those parameters depend on the value of Kgl, uncertainty in Kgl has an impact on the uncertainty of the metabolic parameters and hence overall quantitative uncertainty of the PBPK model in which they are used. Some of the uncertainties may require additional experimental data to resolve (e.g., CYP 2E1-specificity and evaluation of Kgl at the mixing speed used in the *in vitro* metabolic studies).

In addition, the Ramboll PBPK model seeks to quantify the impact on cancer risk due to differences between mice or rats and humans. These metabolic data are foundational to the PBPK modeling, and if all significant uncertainties in the PBPK model were addressed, the model predictions would incorporate these metabolic differences. In this regard, as pointed out by one of the reviewers, the Ramboll analysis does not address cancer risk outside of the lung. The limits of applicability of the Ramboll model is important because the National Toxicology Program (NTP) chronic mouse and rat inhalation bioassays, upon which the inhalation unit risk (IUR) for chloropropene was based, demonstrated the occurrence of multiple tumors beyond the lung (National Toxicology, 1998). The NTP chronic bioassays reported significantly increased incidence of neoplasms in liver, lung, forestomach, Harderian gland, mammary gland, Zymbal's gland, kidney, and the circulatory system in mice and in the lung, mammary gland, thyroid, kidney, and the oral cavity in rats. These tumor incidence results are summarized in "Background Description for Chloroprene PBPK Modeling", provided for the 2020 external peer review of the PBPK model. The 2010 IRIS assessment also cited human evidence of an association between liver cancer risk and occupational exposure to chloroprene and found suggestive evidence of an association between lung cancer risk and occupational exposure in support of reaching a hazard conclusion of "likely to be carcinogenic to humans."

Ramboll's analyses assert that the risk of human lung cancer is minimal compared to mice, making the current IRIS IUR an overestimate of risk. EPA has not undertaken the technical analysis to reach a conclusion on concurrence with this assertion. But, if accepted at face value, the lung only accounts for about 40% of the total cancer incidence in mice (National Toxicology, 1998). Since the existing Ramboll model cannot be used to address risk in other tissues, the same standard inter-species scaling as used in the 2010 IRIS Toxicological Review would need to be applied to estimate cancer risk for those other tissues. Overall, the U.S. EPA concludes that even

if the current Ramboll PBPK model were accepted at face value and applied to the extent possible, the *total* estimated cancer risk would be reduced by no more than 50%. This factor of 2 difference is well within the generally accepted uncertainty for cancer risk estimation. Hence, EPA concludes that the 2010 Toxicological Review did not over-estimate the human cancer risk by multiple orders of magnitude, as contended by Denka and Ramboll.

EPA Response to Assertion 2 and Assertion 3: Major New Follow-Up Epidemiological Study by Dr. Gary Marsh, et al., Released in 2020, Shows No Increased Cancer Mortality among U.S. Chloroprene Workers; and New Cancer Incidence Data from the Louisiana Tumor Registry Shows the Incidence of Cancers near the Denka Faculty are At or Below State-wide Averages for Cancers of Potential Concern

In addition to the PBPK model discussed above, the RFC referenced a recent update Marsh et al. (2021) to a prior epidemiologic study (Marsh et al., 2007a) described as providing evidence of no increased cancer mortality among a worker cohort exposed to chloroprene. In Exhibit B of the submitted RFC (see Section 4), unpublished analysis of Louisiana Tumor Registry data conducted by Denka (and consultants) concluded there was average or below average cancer incidence near the Denka facility for lung and liver cancer. Exhibit B of the submitted RFC also provides Denka's critique of a community survey that concluded the 23-year period prevalence of all cancer (combined) in the residential area closest to the Denka facility is elevated due to environmental exposures from the Denka facility Nagra et al. (2021).

As part of considering this RFC, the published studies were evaluated using the study evaluation approach undertaken for IRIS assessments (U.S, 2020a) and general comments were provided on Ramboll's unpublished Louisiana Tumor Registry analysis. Importantly, the studies and analyses provided by Denka and Ramboll present some new Nagra et al. (2021). and updated Marsh et al. (2021) epidemiological information, but do not identify errors in the 2010 IRIS assessment. The new epidemiological evidence provided in the 2021 Denka RFC would also not alter the 2010 IRIS conclusion given the study evaluation results presented below.

The Marsh et al. (2021) study is a follow-up analysis of additional person years for a previously published occupational cohort (Marsh et al., 2007a, b) used to examined liver, breast, and respiratory cancer mortality in relation to chloroprene exposures. The results of this study are similar[1] to earlier analyses by Marsh et al. (2007) that were considered in the 2010 IRIS

[1] The two primary cancers of interest identified in the occupational cohort studies by (Marsh et al., 2007a, b) are cancers of the liver and respiratory system. For example, increased risks of respiratory system cancers (inclusive of larynx, bronchus, trachea, lung, and other respiratory cancers) were detected in 3 of 4 plants (all but Plant L in Louisville, KY) reported in the 2007 Marsh internal rate analysis. Their more recent internal rate analysis Marsh et al. (2021) still showed increased risks for 1 of 2 plants (Plant P in Pontchartrain, LA) but without explanation did not include data on the other 2 plants with elevated respiratory system cancer risk. Some of these increased risks detected again in Plant P were strong in magnitude (RRs ranging from 1.42-5.2) across different exposure metrics. Liver cancer rates also remain elevated in Plant L based on the updated Marsh et al. (2021) internal rate analysis, although there was no evidence of an exposure-response relationship (elevated RRs ranged from 1.2-2.5). A new analysis showed that breast cancer rates were also consistently elevated across most exposure categories and metrics based on the internal rate analysis --which is deemed less prone to different biases. Although these risks

Toxicological Review and by the independent peer review committee at that time. For Marsh et al. (2021), several study quality evaluation domains were considered deficient and led to an overall judgment of *low confidence* (Figure 1-1). The epidemiological analyses had not been conducted with optimal exposure, confounder, or outcome data, and several analysis decisions likely led to substantial biases that would largely be expected to bias towards the null (i.e., not finding an association). For example, the extensive amount of healthy worker effect in the standardized mortality ratio (SMR) analysis limits the interpretation and use of these data. The healthy worker effect is a type of selection bias that can impact study validity when inappropriate comparison groups, such as external citizen groups, are compared to occupational cohort studies. This arises from the fact that less healthy individuals from the general population are more likely to be unemployed compared to those in the workforce. The healthy worker effect tends to reduce the association between an exposure and the outcome because workers, as a group, are healthier than general population comparison groups. Exposure misclassification is also anticipated in the Marsh et al. (2021) study given the lack of sampling data to estimate exposures; this reduces confidence that the study can accurately characterize any true effect of exposure. The approach for exposure categorization is also unclear and seems to have been based on cancer deaths and not on an *a priori* exposure distribution targeted to contrast higher exposure groups with an unexposed or lower exposed referent. Limited information on some key potential confounders (e.g., smoking data for respiratory cancer, and alcohol use for liver and breast cancers) precluded their full consideration and likely resulted in residual confounding. Lastly, inclusion of only part of the occupational cohort (i.e., the American plants located in Louisville, KY and in Pontchartrain, LA) raises concern over selective reporting, especially since associations (including some exposure-response relationships) were reported earlier for some outcomes in the European cohorts. These limitations reduce the study sensitivity and the ability to detect an effect that may be present.

The Nagra et al. (2021) analysis is based on a field epidemiology investigation of residents of census tracts 708 and 709 in St. John Parish, LA (within a 2.5-km radius of the Denka facility) conducted by non-profit and local citizen groups. For the Nagra et al. (2021) study, major limitations resulted in several domains that were considered deficient and led to an overall confidence of *uninformative* (Figure 1-1). The study's design and conduct likely resulted in selection of bias given that respondents who were aware of their exposure status (i.e., residential proximity to the plant) may have selectively participated and differentially reported health outcomes. This stems from considerable publicity and lawsuits surrounding these community concerns, as well as community meetings. The health outcome measures were also deficient for various reasons, including self-reported outcome data without medical confirmation and use of proxies to report on the health status of other household members over a 23-year time period. In addition, the small samples not only reduced the study sensitivity, but the examination of total

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were not monotonic, the anticipated exposure misclassification and unclear exposure categorization approaches used likely precluded detection of exposure-response relationships across these outcomes.

cancer as the primary outcome precluded analyses of more targeted and etiologically-relevant cancer-specific hypotheses related to chloroprene.

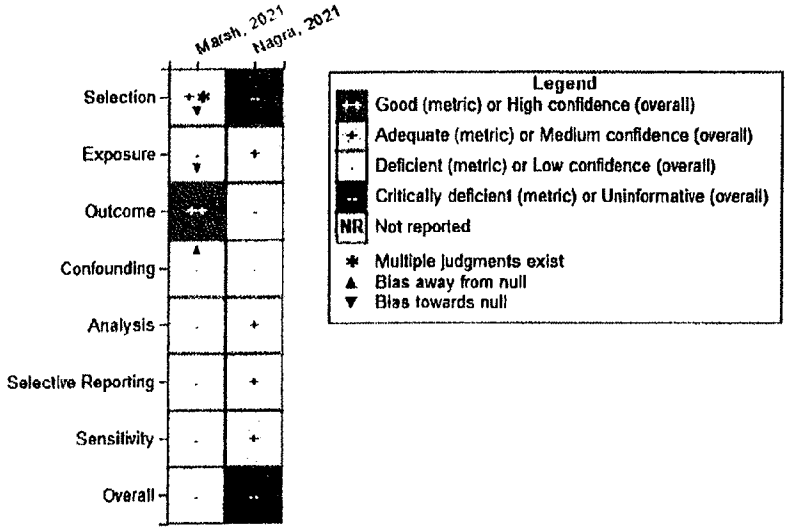

Figure 1-1. Study evaluation results for March et al. and Nagra et al. (see interactive data graphic for rating rationales).

In Exhibit B of the RFC (see Section 4), Denka conducted a tumor registry analysis to estimate cancer rates in St. John the Baptist Parish and its constituent census tracts. Denka propose that if the National Air Toxics Assessment (NATA) risk assessment was accurate, then the tumor registry analysis would identify higher cancer incidence rates in St. John the Baptist Parish than elsewhere. With respect to examining tumor registry analyses in isolation, it is important to emphasize that these data are quite limited for use in evaluating cancer risk for specific exposures, such as chloroprene. In general, and especially when epidemiologically linked with exposure data, tumor registry data are most informative when comparisons are made between local more homogenous populations. This allows for less potential for confounding and other sources of bias due to better comparability across different risk factors, demographics, and socioeconomic status. This is important as lifestyle factors and exposure to other carcinogens that different populations may be exposed to over time and location are not fully considered or controlled for when considering just tumor registry data alone. Tumor registry data may also be subject to notable differences in resources and surveillance rigor and effectiveness across healthcare systems in different regions. Many cancers are also often multifactorial in nature, and examination of tumor registry data by itself doesn't readily inform hypotheses on specific links to certain chemical exposures such as chloroprene. Thus, comparisons based on the tumor registry data alone do not further inform drawing causal inference related to specific exposures such as chloroprene. In the context of a hazard characterization, tumor registry data could be considered more descriptive and does not readily permit the examination of epidemiological associations to evaluate specific etiologic hypotheses. In addition, several limitations were noted by EPA of Denka's statewide tumor registry analysis, including that data on liver cancers are not

available in the Louisiana Tumor Registry at the parish level, which precludes examination of whether liver cancer rates are elevated in the St. John Baptist Parish compared to other relevant areas in Louisiana.

The evaluation of the epidemiological evidence, and the consideration of multiple lines of evidence to draw the conclusion that chloroprene is a likely human carcinogen, was unanimously supported by the external peer review panel for the IRIS Chloroprene Toxicological Review. In particular, the following specific points were evaluated by the peer review panel based on Charge Question 8 (Appendix A, pages A-10 to A-12) which asked: "Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (2005)", the Agency concluded that chloroprene is likely to be carcinogenic to humans by all routes of exposure. "*Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified*"? All six of the peer reviewers commented that the characterization of chloroprene as "likely to be carcinogenic to humans" was appropriate and justified based on the animal and genotoxicity data. Three reviewers commented that the animal data provided ample evidence of carcinogenesis in both sexes of two rodent species (mouse and rat) at multiple organ sites, many of which were distal to the point-of-contact. Two independent peer reviewers further suggested that the strength of the epidemiological evidence was sufficient to change the descriptor to "carcinogenic to humans." The new and updated scientific evidence provided in the 2021 Denka RFC across all the evidence streams would not alter this conclusion, given the study evaluation results presented above

Appendix B: References

Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004). Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. Toxicol Sci 79: 18-27. http://dx.doi.org/10.1093/toxsci/kfh092

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Nagra, R; Taylor, R; Hamptom, M; Lance, H. (2021). "Waiting to die": Toxic emissions and disease near the Denka Performance Elastomer neoprene facility in Louisiana's cancer alley. Environmental Justice 14: 14-32. http://dx.doi.org/10.1089/env.2020.0056

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Versar. (2021). Follow up – External peer review of a report on physiologically based pharmacokinetic (PBPK) modeling for chloroprene and a supplemental analysis of metabolite clearance. Washington, DC: U.S. Environmental Protection Agency.

Yang, Y; Himmelstein, MW; Clewell, HJ. (2012). Kinetic modeling of β-chloroprene metabolism: Probabilistic in vitro-in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans. Toxicol In Vitro 26: 1047-1055. http://dx.doi.org/10.1016/j.tiv.2012.04.004

EXHIBIT 2

EPA January 2018 Denial to DPE's RFC

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

January 25, 2018

Robert Holden
Liskow & Lewis
One Shell Square
701 Poydras Street, Suite 5000
New Orleans, LA 70139

OFFICE OF
RESEARCH AND DEVELOPMENT

Dear Mr. Holden:

This letter is in response to the Request for Correction (RFC) received by the U.S. Environmental Protection Agency (EPA) on June 26, 2017, which was assigned RFC #17002 for tracking purposes. The letter was provided on behalf of Denka Performance Elastomer LLC (DPE). In the RFC letter, DPE states that the *Toxicological Review of Chloroprene (CAS No. 126-99-8) In Support of Summary Information on the Integrated Risk Information System (IRIS)*, disseminated by EPA's Office of Research and Development (ORD) in 2010 (referred to herein as the "IRIS chloroprene assessment"), does not reflect the "best available science" or "sound and objective scientific practices" and requests correction.

Summary of the Request

The DPE RFC requests the IRIS chloroprene assessment be corrected in three ways: 1) the EPA-derived inhalation unit risk (IUR) of 5 x 10^{-4} per ug/m^3 be replaced with a value derived by Ramboll Environ of 3.2 x 10^{-6} per ug/m^3, or withdrawn; 2) the EPA cancer classification of chloroprene as a "likely" human carcinogen be classified instead as a "suggestive" human carcinogen; and 3) the EPA derived Reference Concentration (RfC) be withdrawn pending further IRIS review. The RFC letter indicates, as an alternative, that the EPA immediately withdraw the IRIS IUR and RfC values pending further review.

To support the RFC, DPE provided a document "...organized into six sections: Section I demonstrates that the 2010 IRIS Review constitutes "information" "disseminated" to the public; Section II shows that the 2010 IRIS Review is subject to heightened information quality standards because it is influential scientific information; Section III explains how the 2010 IRIS Review fails to comply with the EPA Guidelines; Section IV shows how EPA's correction of the 2010 IRIS Review would benefit DPE, which has been harmed by its errors; Section V provides DPE's contact information; and Section VI sets forth the relief that DPE is seeking."

The EPA Response to DPE Request for Correction

In the Attachments to this response, EPA addresses the assertions and topics raised in Section III of the RFC as this section is relevant to the science evaluation represented in the IRIS chloroprene assessment under EPA's *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility and Integrity of Information Disseminated by the Environmental Protection Agency (IQG).* The information and assertions in the other sections are either not in dispute or are not pertinent to the evaluation of science issues under the RFC.

Conclusion

The EPA, after careful review of the RFC submitted by DPE, has concluded that the underlying information and conclusions presented in the *Toxicological Review of Chloroprene (CAS No. 126-99-8) In Support of Summary Information on the Integrated Risk Information System (IRIS)* are consistent with the EPA's Information Quality Guidelines.

Your Right to Appeal

If you are dissatisfied with the response, you may submit a Request for Reconsideration (RFR) as described in EPA's Information Quality Guidelines. The EPA requests that any such RFR be submitted within 90 days of the date of the EPA's response. If you choose to submit a RFR, please send a written request to the EPA Information Quality Guidelines Processing Staff via mail (Information Quality Guidelines Processing Staff, Mail Code 2821T, USEPA, 1200 Pennsylvania Avenue, NW, Washington, DC 20460); or electronic mail (quality@epa.gov). If you submit a RFR, please reference the case number assigned to this original Request for Correction (RFC #17002). Additional information about how to submit an RFR is listed on the EPA Information Quality Guidelines website at http://epa.gov/quality/informationguidelines/index.html.

Sincerely,

Jennifer Orme-Zavaleta, Ph.D.
Principal Deputy Assistant Administrator for Science

Cc: Tina Bahadori, ScD ORD/NCEA Director
Stephen Fine, PhD, Acting Chief Information Officer
David Gray, EPA Region 6 Director of External Affairs
Vincia Holloman, Director of Enterprise Quality Management Division
Anne Idsal, JD, Region 6 Administrator
Kristina Thayer, ORD/NCEA IRIS Division Director
John Vandenberg, ORD/NCEA RTP Division Director

Attachment 1: U.S. EPA Response to the Denka Performance Elastomers (DPE) Request for Correction of the Toxicological Review of Chloroprene (CAS No. 126-99-8) In Support of Summary Information on the Integrated Risk Information System (IRIS)

Attachment 2: Systematic Review of Chloroprene [CASRN 126-99-80] Studies Published Since 2010 IRIS Assessment to Support Consideration of the Denka Request for Correction (RFC). January 2018. USEPA, ORD, NCEA-IRIS, Washington DC.

EXHIBIT 3

Ramboll Report – *Response to Technical Questions Regarding the Science of Chloroprene*

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

Intended for
Denka Performance Elastomer LLC

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RESPONSES TO TECHNICAL QUESTIONS REGARDING THE SCIENCE OF CHLOROPRENE

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CONTENTS

1. INTRODUCTION

The following sections provide technical responses to science issues that have been raised by the United States Environmental Protection Agency (USEPA) both in the denial letter to Denka Performance Elastomer LLC (Denka) dated March 14, 2022 (Section II of this Technical Response), as well as Appendix A of the denial letter that provides a "courtesy" technical review of new scientific information presented in Request for Correction 21005 (Section III of this Technical Response). In addition, specific responses to the recent peer review of the chloroprene PBPK model (Versar 2021) are provided (Section IV of this Technical Response).

Overall, the scientific issues surrounding the 2010 Chloroprene Toxicological Review have been discussed through continuous interactions between Denka and the USEPA via email, telephone, and face-to-face meetings over a multi-year period. These initially included identification by Denka of errors in the 2010 IRIS Chloroprene Toxicological Review, specifically related to the misinterpretation of the epidemiological evidence, the lack of consideration of the toxicological evidence related to differences in pharmacokinetics across species, and lack of consideration of evidence related to the mode of action (MOA) and the role of metabolism in the potential mutagenicity of chloroprene (Section II of this Technical Response). These interactions between USEPA and Denka also indicated that in order for USEPA to reevaluate the chloroprene IRIS assessment, new data or analyses would be needed to justify the assessment (see https://iris.epa.gov/Events/#stakeholderMeetings; further discussion in Section II of this Technical Response); these new data/analyses were provided in Denka's RFC 21005.

In the courtesy technical review provided by USEPA as part of the March 2022 denial letter, there were three main responses related to assertions stated in Denka's RFC (21005) regarding the chloroprene science. The first was regarding the estimation of an Inhalation Unit Risk (IUR) value two orders of magnitude lower than the IUR in the 2010 IRIS assessment when the PBPK model was applied, while USEPA suggested that the application of the model would only result in a factor of 2 difference. USEPA ignored an informal request to provide a clear explanation of how their estimate was derived, but it appears to have been performed using a scientifically inappropriate approach (see Section III of this Technical Response for further discussion). Application of the model to multiple tissues using methods consistent with other IRIS assessments (e.g. vinyl chloride) suggest an IUR roughly 35-fold lower than the IUR from the 2010 IRIS assessment.

The remaining two assertions in the technical review were related to the evaluation of the epidemiological evidence for cancer following exposure to chloroprene. The most significant error in both the IRIS assessment and in the current USEPA technical review is the misinterpretation of the epidemiological data presented in Marsh et al. (2007a, 2007b). USEPA incorrectly concluded that the results from these studies provide evidence of excess risk of liver and lung cancer in workers. In fact, the study results do not show evidence of increases in risk or exposure-response relationships between exposure and cancer risk in the occupational cohorts (see Section III of this Technical Response for further discussion). The cohort of the exposed Louisville workers in the Marsh et al. (2007a, 2007b) study had an SMR below 1.0. Use of the 2010 IUR estimates an excess on the order of thousands of tumors in the cohort when no excess deaths were observed. Using the PBPK model produces an IUR that estimates excess cancers on the order of 100 cases, when no excess deaths were observed. The IUR based on the PBPK model,

which is approximately 1/35th of the 2010 IUR, provides a conservative estimate considering that no excess was actually observed.

Further, USEPA misinterpreted the reasons for presentation of the Louisiana Tumor Registry (LTR) data. As discussed further in Section III of this Technical Response, we have not proposed using the LTR data to evaluate the risk associated with specific exposures or as evidence for the cancer classification of chloroprene, but rather to test the validity of USEPA's assertions regarding the magnitude of the cancer risk in the Parish surrounding the Denka facility. We found no evidence of high cancer risks in St. John the Baptist Parish where the Denka facility is located.

Finally, Section IV of this Technical Response provides responses to the Tier 1 and 2 recommendations contained in the recent peer review of the chloroprene PBPK model (Versar 2021). Overall, there were no Tier 1 or 2 recommendations that would result in significant changes to the model or that would impact the validity of the current results (see Section IV of this Technical Response for responses to the individual peer reviewer comments). Further, of those reviewers responding to questions regarding the reliability of the PBPK model for use in risk assessment, the responses indicate that the PBPK model is scientifically sound and reliable for use in a risk assessment for chloroprene.

2. RESPONSES TO DENIAL LETTER

In the denial letter provided to Denka Performance Elastomer LLC (Denka), the United States Environmental Protection Agency (USEPA) indicated that the recent Request for Consideration provided by Denka on July 15, 2021, did not identify errors in the 2010 IRIS assessment. However, the errors related to the science of chloroprene in the 2010 IRIS Chloroprene Toxicological Review have been noted in previous submissions by Denka (Request for Correction dated June 26, 2017) and include:

- Interpretation of the epidemiological evidence from the Marsh et al. studies
    - The USEPA IRIS summary of this study indicates incomplete evaluation and misinterpretation of the published results. Properly interpreted, the evidence does not demonstrate an association between occupational chloroprene exposure and human cancer incidence.
- Interpretation of the toxicological evidence related to the differences in pharmacokinetics between mice and humans
    - The available science demonstrates differences in pharmacokinetics between the mouse and the human that contribute to differences in response. These differences should be accounted for in the estimation of the IUR.
- Integration of evidence for mode of action
    - Evidence that supports an alternate MOA than that proposed by USEPA (Request for Correction dated June 26, 2021)
    - Current analysis provided as part of the peer review of the chloroprene PBPK model supporting total metabolism rather than the parent chemical as the toxic moiety.

These issues have been documented in previous submissions by Denka to the USEPA and are part of the justification leading to the peer review of the chloroprene PBPK model in 2020. These errors have also been noted by the external peer reviewers of the draft IRIS Chloroprene

Assessment in 2010 and are noted in Appendix A of the 2010 IRIS Assessment, as well as Ramboll's own peer-reviewed, published research on the subject.

Further, USEPA notes that the materials submitted by Denka present new analyses and express views on how these products should be used in a risk assessment. The information provided in the recent RFC is based on interactions and conversations with USEPA over a multi-year period (2017-2022). Meetings with USEPA during the RFR process indicated new data were necessary for IRIS to be revised. In the notes from the meeting between Denka and USEPA on June 12, 2019 (https://iris.epa.gov/Events/#stakeholderMeetings) the following are provided:

- Denka asked if USEPA would remove the 2010 IRIS assessment or its IUR value only based on the outcome of discussions.
    - USEPA stated the IRIS assessment will not be changed or removed unless science presented since the Request for Correction necessitated reassessment.
    - The process was reviewed (slide 7/USEPA): peer review model and address feedback; apply model to assessment (if appropriate); update IRIS assessment (if appropriate), building on earlier response to RFC; peer review IRIS Update (if appropriate).
- Denka asked whether USEPA's consideration of the RFR included the interpretation of the occupational epidemiology studies of workers exposed to chloroprene.
    - USEPA reiterated that those issues were addressed in the response to the Request for Correction; unless there were any new studies or results, the epidemiology would not be revisited.

3. RESPONSES TO APPENDIX A OF DENIAL LETTER

In the background section of Appendix A of the denial letter, it states that:

> "While USEPA provided feedback on quality assurance, USEPA does not consider these discussions to constitute a formal quality assurance review, as the discussions alone did not satisfy the QA requirements outlined in the Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models developed by the USEPA's Office of Research and Development."

However, in USEPA's response (dated June 13, 2018) to a PBPK workplan provided by Denka and Ramboll, USEPA indicated that:

- "The Pharmacokinetic Workgroup (PKWG) at the USEPA has developed a Quality Assurance Process Plan (QAPP) for computational modeling, focused on PBPK models, which we sent previously for your consideration. Prior to application of a PK model in its assessment work, NCEA will conduct a review according to this QAPP. Such review will be significantly facilitated if corresponding documentation is created during the modeling process. It is much easier to record this information as the modeling is being conducted than to attempt to reconstruct the information later." (Emphasis added.)
- "If sources and calculations for model parameters are not fully documented, this is likely to delay significantly USEPA's QA review of the model, hence possible use in consideration of the case for correction."

Based on the QAPP provided by USEPA, the PBPK model revised documentation considering the recommendations from the initial peer review (Versar 2020) and as requested by USEPA was developed consistent with the QAPP and provided in the recent Denka RFR 17002A.

The following sections provide technical comments on the USEPA responses to the scientific assertions regarding the chloroprene science contained in the Denka RFC 21005.

USEPA Response to Assertion 1: IUR Should Be Corrected to Reflect the 2021 Ramboll PBPK Model (Exhibit A4 in the RFC). Denka states that: "The IUR Should Be Corrected to Reflect the 2021 Ramboll PBPK Model. Overall, the application of the 2021 PBPK model is expected to result in the estimation of an IUR that is approximately two orders of magnitude below that of the 2010 IUR."

- USEPA notes that "Some reviewers raised questions regarding whether the model was sufficiently reliable for use in risk assessment or, minimally, that additional experimental data should be obtained."

In the follow-up peer review of the PBPK model (Versar 2021), there was a question (number 11) that specifically requested comments "on the capacity of the PBPK model to provide sound estimates of chloroprene inhalation dosimetry in mice, rats, and humans." Reviewers were also asked to comment on the reliability of the model predictions of the rate of chloroprene metabolism in liver and lung for use in animal-to-human extrapolation." Of the 6 recent reviewers, 5 participated in the initial peer review (Versar 2020) and only three provided comments in response to Question 11 regarding the reliability of the model for use in the risk assessment. They indicate that the model is scientifically sound and reliable for use in a risk assessment for chloroprene.

- Dr. Kenneth Portier – "Under the WHO/IPCS (2020) guidance on acceptability of predictions, Ramboll has shown that the PBPK model has the capacity to provide sound estimates of chloroprene inhalation dosimetry in mice, rats, and humans across a wide range of possible values for input and state parameters. Also, this PBPK model has been shown capable of reliably predicting rates of chloroprene metabolism in the liver and lung of animals and humans to within 2 orders of magnitude or less. Within the limitations of available data and with this accuracy acceptability target, the model should be considered a reliable tool for predicting chloroprene metabolism and for providing sound estimates of chloroprene inhalation dosimetry."
- Dr. Kan Shao – "Overall, the quality of the report has been significantly improved. Supported by deliberated sensitivity and uncertainty analyses, the results and conclusions presented by the report are scientifically sound."
- Dr. Jordan Smith – "Overall Ramboll's efforts has improved the model and increased confidence in its ability to support to chloroprene risk assessment in humans. Due to integration of many measured aspects of chloroprene pharmacokinetics (e.g. metabolism, portioning, etc.) and physiology (e.g. ventilation rates, body weights, etc.) into a model capability of extrapolating dosimetry across species, and quantitatively integrating uncertainty, this model offers an improved risk assessment tool compared to traditional standardized uncertainty factors."
- Dr. Nan-Hung Hsieh, the single new reviewer, provided a response that only demonstrated his misunderstanding of the purpose of the in vivo model validation study, a mistaken

opinion that would have been corrected by the other reviewers if he had participated in the initial review.

The remaining comments are largely based on the responses from one peer reviewer, who stated repeatedly during the initial peer review that his primary concern was that if the model were used, the risk estimates for chloroprene carcinogenicity might decrease. In our view, this reviewer's comments confused the goals of accurate quantification of risks with policy considerations relating to managing risks, which was outside the scope of the peer review. The USEPA was clear in the charge to the peer reviewers that the review was to focus only on the scientific validity of the PBPK model. This was consistent with the goals of risk assessment versus policy, as defined by the Office of Management and Budget in the Updated Principles of Risk Assessment (Dudley and Hays 2007).

The PBPK model for chloroprene was developed to support the USEPA's goal of using the best available science in order to obtain the most accurate estimate of human risk possible. In previous USEPA efforts to use the best available science, the agency has applied similar PBPK models in the risk assessments for methylene chloride and vinyl chloride, despite the fact that the use of the models resulted in substantially lower risk estimates. Moreover, as with the case of vinyl chloride (Clewell et al. 2001), a comparison of animal and epidemiological data on the carcinogenicity of chloroprene has demonstrated that the PBPK model for chloroprene provides a more accurate estimate of human risk than a default approach based on animal data (Sax et al. 2020).

- USEPA notes that remaining uncertainties in the PBPK model include, "Fundamental model assumptions, e.g. that chloroprene itself is treated as inactive but may be reactive and that data from studies on a different compound can be used to infer key metabolic rates."

The sole reviewer who suggested that chloroprene is directly reactive in tissues (Dr. Raymond Yang) was apparently unfamiliar with the extensive experimental data on chloroprene that contradicts his opinion. This evidence was thoroughly reviewed in Denka's initial RFC submission and was also reported in a peer reviewed publication (Sax et al. 2020) that unfortunately was not provided to the peer reviewers by USEPA. Briefly, the studies carried out on chloroprene overwhelmingly demonstrate that it is not genotoxic in vivo or in mammalian cells and is only genotoxic in bacterial systems when metabolism capability is added (NTP 1998; Shelby 1990; Shelby and Witt 1995; Tice 1988; Tice et al. 1988). A comprehensive review of the evidence for chloroprene mutagenicity is provided in Sax et el. (2020).

- An additional uncertainty noted by USEPA is "...whether 7-ethoxycoumarin activity is an appropriate predictor of chloroprene's oxidative metabolism and the extent to which cytochrome P450s (CYPs) enzymes other than CYP2E1 might contribute to this activity."

The use of the ratio of 7-ethoxycoumarin activity in the lung and liver as a surrogate for CYP2E1 substrates such as chloroprene has for strong scientific reasons previously been accepted by the USEPA in the current IRIS assessment for methylene chloride (USEPA 2011). Moreover, the value obtained from 7-ethoxycoumarin was further supported by an alternative approach using the CYP mRNA expression ratio. The concern raised in the initial peer review regarding the possibility that enzymes other than CYP 2E1 might contribute to the metabolism of chloroprene is unfounded. In the follow-up peer review, one of the reviewers who had previously raised the issue (Jordan Smith) indicated that he now considered the metabolism data with inhibitors from Himmelstein et al. (2001 and 2004) to serve as direct experimental evidence for the role of CYP2E1 in the metabolism of chloroprene, and his Tier 1 suggestion was that we cite that

evidence in our report to provide additional support for our modeling approach. Most importantly, Himmelstein et al. (2001) demonstrated that the metabolism of chloroprene was almost completely inhibited by 4-methyl pyrazole, a specific inhibitor of CYP 2E1.

- While the current Ramboll PBPK model focuses on cancer risk for the lung, the USEPA concludes that the model cannot be used for tissues outside the lung in which tumors were observed in the NTP (1998) bioassay.

The PBPK model for chloroprene includes metabolism in the three tissues where the metabolism of chloroprene has been characterized: liver, lung and kidney. The USEPA has suggested that the tumors in other tissues are due to circulating reactive metabolites or direct reactivity of chloroprene itself, but there USEPA has not cited, and Ramboll is not aware of, any evidence to support either of these assertions. It is more likely that tumors in other tissues result from local metabolism in the tissue, just as occurs in the liver and lung. Many other tissues that contain CYP2E1, such as the mammary glands and Harderian glands (Nishimura et al. 2003), would also be able to metabolize chloroprene. In support of this possibility, the tissues in which tumors were observed in the rodent bioassays for chloroprene are nearly identical to the tissues in which tumors were observed for the structurally similar compound vinyl chloride, which is also a CYP2E1 substrate (Clewell et al. 2001). Moreover, studies on vinyl chloride have established that the carcinogenicity of vinyl chloride is due to its metabolism, and that the metabolites produced are too reactive to circulate to other tissues (Bolt et al. 1980). This evidence is significant because the metabolites generated from chloroprene are expected to be even more reactive than those from vinyl chloride (Plugge and Jaeger 1979). In the IRIS assessment for vinyl chloride, the cancer risk assessment was based on the PBPK model-based dose metrics for liver, assuming that the mode of action for the carcinogenicity of vinyl chloride would be the same across all tissues: metabolism to reactive metabolites. The USEPA in the 2010 IRIS assessment for chloroprene also stated that, regardless of the tissue, the mode of action is expected to be the same: metabolism to reactive metabolites.

- USEPA concludes that lung cancer only accounts for about 40% of the total cancer incidence in mice and that even if the Ramboll PBPK models were applied for the lung, the total estimated cancer risk would be reduced by no more than 50% or a factor of 2.

The USEPA did not provide a clear explanation of how their estimate was derived, but it appears to have been performed by simply comparing the 2010 IUR for all tumors with an IUR based on tumors in all tissues except the lung, based on inhaled concentration in the NTP (1998) study of the female B6C3F1 mice. However, any such calculation would be based on the false assumption that the chloroprene PBPK model can only be applied to estimate risk of tumors in the lung. As the USEPA scientists who evaluated the model are aware, and as indicated by the reviewers' responses to Question 11 in the follow-up peer review, the submitted model already includes the necessary dose metrics to calculate risks for all tumors in the lung and liver. Therefore, the model submitted to USEPA can be applied to estimate dose metrics for angiosarcomas, angiomas, carcinomas and adenomas in both the lung and the liver, which account for the vast majority of the observed tumors.

Moreover, the USEPA's suggestion to use a default dose metric for tumors observed in some tissues and not in others is scientifically inappropriate, because the mode of action for chloroprene is the same in all tissues: the generation of reactive products directly in the tissue due to local metabolism of chloroprene. Therefore, the risk assessment should be conducted following the same approach used by the USEPA in their IRIS assessment for vinyl chloride,

where the agency was faced with a similar situation: the tissues in which tumors were observed in the rodent bioassays for vinyl chloride were nearly identical to the tissues in which tumors were observed for chloroprene. Based on their determination that the mode of action for vinyl chloride carcinogenicity in all tissues would be the same, the agency used a PBPK model to perform animal-to-human extrapolation based solely on liver tumor incidence (the preponderant tumors), where the scientifically appropriate PBPK dose metric could be applied. We have applied this approach with the submitted model and have determined that the risk based on all tumors in lung and liver would be roughly 35-fold lower than the IUR from the 2010 IRIS assessment. This result is similar to the difference that resulted from applying the PBPK model in the USEPA IRIS risk estimate for vinyl chloride.

USEPA's suggestion of using the default approach (based on inhaled chloroprene concentration) for the small number of tumors that were observed in tissues where quantitative metabolism data are not available is not scientifically supportable. As with many other chemicals whose carcinogenicity results from its metabolism to reactive compounds, the mouse is much more sensitive than other species (Table 1). It has been clearly demonstrated that the tumorigenicity of chloroprene across species is not consistent with inhaled concentration, but the use of total metabolism estimated by a PBPK model provides a reliable cross-species extrapolation of tumor incidence in the lung (Himmelstein et al. 1994; Clewell et al. 2019). The same result was obtained for the liver tumors from vinyl chloride (Clewell et al. 2001). Although quantitative metabolism data may not be available for some of the other tissues where tumors were observed in the mouse bioassays, the ratio of metabolism between human and mouse in these tissues would most likely be in the same range as the ratios for liver and lung, meaning that the additional risk from the small number of tumors in other tissues would not significantly impact the human risk estimate. In fact, some of the other tissues in which tumors were observed in the mouse, such as the harderian gland, zymbal gland and forestomach, are not even present in human and should not be included in the assessment.

Table 1. Exposure-Dose-Response for Rodent Lung Tumors

	Exposure Concentration (ppm)	PBPK Internal Dose	Lung Tumor Incidence	Number of Animals	Extra Risk (%)
Hamster	0	0	0	100	0
	10	0.18	0	97	0
	50	0.88	0	97	0
Wistar Rat	0	0	0	13	0
	10	0.18	0	100	0
	50	0.89	0	50	0
Fischer Rat	0	0	3	50	0
	12.8	0.22	3	49	0.3
	32	0.55	6	50	7.7
	80	1.37	9	50	14.0
B6C3F1 mouse	0	0	15	50	0
	12.8	3.46	32	50	48.3
	32	5.30	40	50	70.4
	80	7.18	46	50	89.9

USEPA Response to Assertion 2 and Assertion 3 (ORD letter to Denka): Major New Follow-Up Epidemiological Study by Dr. Gary Marsh, et al. Released in 2020, Shows No Increased Cancer Mortality among U.S. Chloroprene Workers; and New Cancer Incidence Data from the Louisiana Tumor Registry Shows the Incidence of Cancers near the Denka Faculty are At or Below State-wide Averages for Cancers of Potential Concern

- In their denial letter, the USEPA claimed that the available epidemiological data, including the update provided by Marsh et al. (2021) and observations available in the LTR, did not demonstrate errors in the 2010 IRIS risk assessment for chloroprene. The association between occupational exposure to chloroprene and human lung cancer risk was characterized as "suggestive" in the 2010 IRIS assessment.

These USEPA claims demonstrate an incorrect understanding of the weight of the epidemiological evidence. When correctly evaluated, the available epidemiological evidence does not suggest an association between occupational exposure to chloroprene and lung cancer risk.

The 2010 Toxicological Review of Chloroprene (USEPA 2010) correctly noted that the early occupational cohort studies of chloroprene exposure and cancers of liver and biliary passages (Bulbulyan et al. 1998, 1999; Leet and Selevan 1982; Li et al. 1989) and lung cancer (Bulbulyan et al. 1998; Colonna and Laydevant 2001; Li et al. 1989; Pell 1978) suffer from substantial methodological limitations. The methodological problems include lack of control for other carcinogenic chemical exposures, poorly defined or enumerated comparison groups, lack of information on potential confounders, and low statistical power due to small numbers of cases and/or short follow-up periods. More information about the methodological limitations of the early occupational cohort studies is available in published review papers (Acquavella and Leonard 2001; Bukowski 2009; Marsh and Egnot 2018; Rice and Boffetta 2001; Sax et al. 2020), in the 2010 Toxicological Review of Chloroprene, and in Appendix 1 of Denka's 2017 Request for Correction. Because of their methodological limitations, the results of these studies are unreliable and cannot be used to draw causal inferences. Furthermore, the overlapping populations in several of these studies means that they do not provide independent observations, therefore counting each one as providing an independent unit of evidence over-states the weight of evidence.

- The USEPA denial letter stated, "For Marsh et al. (2021), several study quality evaluation domains were considered deficient and led to an overall judgment of low confidence (Figure 1-1)."

These statements represent an incorrect understanding of the methods used by Marsh et al. (2021), (originally described in Marsh et al. (2007a, 2007b)) which was specifically designed to address the methodological limitations of prior studies, and also an incorrect understanding of the healthy worker effect.

Bukowski (2009) reviewed the weight of the epidemiologic evidence for the carcinogenicity of chloroprene using study quality criteria suggested by USEPA (Bukowski 2009). He noted that Marsh et al. (2007a, 2007b), in contrast to the earlier studies, was larger, characterized exposure more completely, and scored in the highest or second highest category for all USEPA criteria on methodological quality (Bukowski 2009).

Marsh et al. (2007a, 2007b) did not identify an increase in cancer risk in the employee populations compared with the relevant regional populations for all cancers (combined) or for liver or lung cancer. This was demonstrated by the standardized mortality ratios (SMRs) all

being below 1.0, indicating fewer deaths from these causes in each study population compared with the relevant populations where the plants were located. Neither of the cohorts most relevant to the USEPA risk assessment (Louisville, KY and Pontchartrain, LA), showed elevated SMRs for all cancers (combined) or for liver or lung cancers when compared with regional or national populations. SMRs were calculated for categories of both duration and amount of exposure, and there was no evidence of an exposure-response relationship associated with increasing duration or amount of exposure. The SMRs for the least exposed employees were substantially below 1.0 when compared with the general population. When used as a referent category for internal analyses, the very low risk in the lowest exposure group creates the mathematical possibility of internal relative risk ratios greater than 1.0 in the higher exposure groups, even if those higher exposure groups did not produce more cancer cases than expected (Bukowski 2009; Marsh et al. 2021; Marsh and Egnot 2018, section IV).

- The USEPA denial letter suggested that the low SMRs calculated for the occupational cohorts compared with the general population referents could be due to the healthy worker effect (HWE).

The HWE, if operating, tends to reduce the apparent association between the occupational exposure and the cause of death of interest due to the presence in the general population of both sick and healthy people. If there were a true relationship between an occupational exposure and a cause of death, and if the HWE were operating, it would cause the magnitude of the observed relationship to be closer to the null value of 1.0 than it should be, indicating no association or less association between occupational exposure and the cause of death of interest (Checkoway et al. 1989, p 78; Chowdhury et al. 2017; Thygesen et al. 2011).

The HWE is of concern when the outcome affects working-age people, when the outcome has a relatively short latent interval, and when the follow-up period is short. The HWE does not typically affect the estimation of risk of diseases that are more common in older persons, like cancer, or when follow-up is long, as it was in Marsh et al. 2021 (Burns et al. 2011; Chowdhury et al. 2017; Thygesen et al. 2011). When it is a factor, it is generally expected that the HWE will lead to approximately a 20% to 25% reduction in deaths observed among the occupational group compared with the general population, which would yield an SMR of approximately 0.75 to 0.80 if the true SMR were 1.0, i.e. if the occupational exposure was unrelated to the cause of death under study (Burns et al. 2011; Chowdhury et al. 2017).

For the HWE to explain the very low SMRs for liver and for lung cancers reported by Marsh et al. (2007a, 2007b, 2021), the chloroprene-exposed workers would have to have developed cancers and left the workforce in large numbers, and they would have had to have been lost to follow-up to be counted among the deaths in the general population rather than as deaths among cohort members (see Chowdhury et al. 2017 on the HWE as a confounder and on the healthy worker survivor effect). Had this been the case, more of the deaths from cancer would have been counted in the general population and fewer of them would have been counted in the occupational cohort. In fact, Marsh et al. (2007a) had very little loss to follow up, 0% for Pontchartrain, LA, 0.2% for Louisville, KY, and 3.5% for Grenoble, France. Marsh et al. were unable to locate records for 191/1,357 employees from Pontchartrain (12.3%) during cohort enumeration. Those individuals were considered unlikely to have been exposed to chloroprene during their employment based on their job titles and status as salaried employees (Marsh et al. 2007a), and thus would not have contributed exposure-dependent deaths to the analysis. Another 18 employees chose not to participate in the study (Marsh et al. 2007a). This very low

rate of employees lost to follow-up cannot have produced a HWE large enough to have affected the study results to the degree suggested in the USEPA denial letter.

The USEPA denial letter criticized the exposure estimation methods used by Marsh et al. and hypothesized a sufficient degree of exposure misclassification to render the estimated effect of chloroprene exposure unreliable.

As discussed in Bukowski (2009) and Sax et al. (2020), as well as the USEPA response to Public Comment 2 (page A-32) in Appendix A.3.1 of its 2010 Toxicological Review of Chloroprene (USEPA 2010), the exposure estimation methods used by Marsh et al. were more sophisticated than the methods used in any prior investigations. The earlier occupational studies characterized employees' exposure levels based on job titles, only. As described in Marsh et al. (2007b), the quantitative exposure estimates were based on work history and duration of employment in a particular job, accounting for the characteristics of the processes in use in that job, plant, and time-period.

If there were errors in the individual records used to reconstruct work history, the exposure reconstruction method used by Marsh et al. (2007b) could lead to misclassification of exposure. For such errors to have affected the study results, however, the likelihood of their occurrence would have to correlate with the likelihood of death due to cancer overall, and due to specific types of cancer. For example, the employees who eventually died from lung cancer would have had to be systematically more likely to have been misclassified as having lower exposure than employees who did not die from lung cancer, or those who survived would have been systematically more likely to be misclassified as less exposed than those who died. There is no reason to expect such systematic errors, and, in fact, exposure estimates were validated against measured exposures when measurements were available (Marsh et al. 2007b).

- The authors of the USEPA denial letter stated that they did not understand the exposure categorization methods used by Marsh et al.: "The approach for exposure categorization is also unclear and seems to have been based on cancer deaths and not on an a priori exposure distribution targeted to contrast higher exposure groups with an unexposed or lower exposed referent".

This is an inaccurate interpretation of the methods used by Marsh et al. As described in Marsh et al. (2007b), the authors defined exposure categories based on the exposures experienced by cohort members who died from any type of cancer (i.e. all types, combined). Marsh et al. used the distribution of exposures among those who died of cancer to develop the categories because the vast majority, more than 92%, of the cohort members, were exposed to chloroprene. The categorization method selected by Marsh et al. was designed to maximize the possibility of there being a sufficient number of cases and amount of person-time available for analyses of specific types of cancers to produce stable statistical results. If the referent category had comprised only the 8% of employees who were not exposed to chloroprene, the statistical results would have been even more unstable than those reported, i.e. the 95% confidence intervals around the point estimates would have been even wider than they were.

- The authors of the USEPA denial letter noted that for the Marsh et al. studies "Limited information on some key potential confounders (e.g. smoking data for respiratory cancer, and alcohol use for liver and breast cancers) precluded their full consideration and likely resulted in residual confounding."

It is unlikely that residual confounding explains the relationships reported by Marsh et al. For residual confounding to have reduced the observed relationship between chloroprene exposure and death from a given type of cancer in the SMR analyses, the confounder would have to have been more common among the employed compared with the general population, e.g. the employees would have to have used more tobacco and alcohol than the general population. For residual confounding to have reduced the relationship between chloroprene exposure and cancer deaths in the internal analyses, the confounder would have to have been more common among the less exposed cases than among the more exposed cases, e.g. the less exposed cohort members would have to have used more alcohol and tobacco than the more highly exposed cohort members. This distribution of potential confounding factors is unlikely to have occurred, as was noted in the USEPA response to comment 31 in Appendix A.2 of its 2010 Toxicological Review of Chloroprene (USEPA 2010, p A-27).

- With respect to the occupational epidemiology data, the authors of the USEPA denial letter also provided their opinion that the Marsh et al. (2021) update, which focused on two US plants and did not report updated information for the two European plants, might indicate selective reporting.

The focus in Marsh et al. 2021 on the two US plants and the exclusion of the Grenoble and Maydown plants is appropriate for evaluating the risk to the community around the Denka facility in St. John the Baptist Parish, LA, which is the subject of the USEPA risk analysis. The reason for focusing on the US plants is to mitigate the likely effects of underlying differences between employees of different nationalities, including differences in risk due to genetic and cultural/behavioral factors that are difficult to identify, measure, and quantify. In Marsh et al. 2007a, 2007b and Marsh et al. 2021, all results are presented on a plant-specific basis to avoid introducing uncontrolled confounding by these characteristics as well as confounding due to identifiable international differences in industrial processes, exposure levels to both vinyl chloride and chloroprene, and the availability of vital status data. Furthermore, the data presented in Marsh et al. (2007a, 2007b) did not show evidence of increases in risk or exposure-response relationships between exposure and cancer risk in its two European cohorts. In addition, the early studies conducted in European cohorts that did report associations have been criticized, including in the chloroprene risk assessment concluded by USEPA in 2000, for their methodological limitations. Taken together, there is no reason to believe that data from the Grenoble and Maydown plants would add meaningful information to the assessment of cancer risks in the two US cohorts that are subject to USEPA regulation.

- Our presentation of data from the Louisiana Tumor Registry (LTR) is in direct response to the USEPA assertion, based on the 2011 NATA report, that St. John the Baptist Parish has the highest cancer risk in the US.

If it were true that St. John the Baptist Parish has the highest cancer risk in the US, the cancer registry data provided by the LTR would show higher numbers of cancer cases and cancer incidence rates in St. John the Baptist Parish than elsewhere. In fact, the LTR demonstrates that cancer rates in St. John the Baptist Parish are lower than in other parts of Louisiana, including parishes that are part of the Industrial Corridor (i.e. presumed to have higher concentrations of air toxics) and parishes that are further away from the Denka facility (i.e. presumed to have lower concentrations of air toxics). The NATA risk estimate must be incorrect, based solely on the comparison between the number of cancer cases observed among residents of St. John the Baptist Parish and elsewhere.

The conclusion that the NATA risk estimate is incorrect is further supported by using the IUR together with the mean or median occupational chloroprene exposure concentrations estimated by Marsh et al. (2007a, 2007b) to calculate the expected number of excess cancers, as was incorrectly done in Appendix A (page A-17) of the Toxicological Review of Chloroprene in support of the IRIS Program (USEPA 2010). In that document, the expected number of excess cancer cases was calculated assuming the median occupational exposure concentration in the Louisville plant of the Marsh et al. (2007a, 2007b) study had been experienced as lifetime (i.e. 70 years) residential exposure (i.e. 24 hours per day, seven days per week). This exercise assumes that a proposed composite IUR of 1.4x10-4 per µg/m3, developed using the results from a chronic bioassay conducted in male mice (NTP 1998), correctly characterizes potential cancer risk for humans. Note that the final IUR recommended by USEPA, 5 x10-4 per µg/m3, was based on the female mouse and also was adjusted for other, age-specific factors. The female mouse is a more sensitive receptor than the male mouse, so the final IUR will predict more cancers than the IUR based on the male.

To estimate the upper bound on the predicted risk, the calculations shown on page A-17 of Appendix A (USEPA 2010) correctly converted the median cumulative exposure estimated for the Louisville cohort to a lifetime residential exposure estimate. Leaving aside the important questions of whether the composite cancer risk derived from the incidence of tumors observed in the male mouse is applicable to humans, and whether it validly estimates the potential risk of human liver and lung cancer specifically, USEPA (2010) incorrectly applied this upper bound predicted risk (0.13) only to the number of individuals in the Louisville plant with known cause of death. Risk estimates must account for all exposed individuals in a population, regardless of whether or not they experienced the outcome under investigation. Applying the predicted risk (upper bound) only to the individuals with a known cause of death (n=2,282) reduced the number of cancer deaths expected and resulted in a number of cancer cases (upper bound) that was similar to the number of liver and lung cancer deaths observed in the Louisville cohort, i.e. 293 vs. 283 observed the in the cohort. Had the predicted risk (upper bound) been correctly applied to the entire exposed cohort, i.e. all individuals who were at risk of developing cancer due to chloroprene exposure if chloroprene in fact causes cancer (n=5,486), the number of expected cancer deaths would have been 713 compared to 283 observed in the cohort. Of note, the number of liver and lung cancer deaths expected for the cohort based on the SMR analyses was 373. Applying the final IUR of 5 x10-4 per µg/m3 to the number of persons exposed to chloroprene in the Louisville plant (5,486) provided an estimate of 927 excess cancers versus 283 observed in the cohort, if the median exposure concentration experienced by the occupational cohort was converted to a lifetime residential exposure. If the mean occupational exposure level were used instead, which is more representative of the exposure, the number of predicted excess cancers would have been 3,891. Thus, using the best quality data available from Marsh et al. (2007a, 2007b) demonstrates a substantial disagreement between the animal and human data. Marsh et al. observed no excess cancers after exposure to chloroprene in an occupational setting.

The exercise described in Appendix A (page A-17) of USEPA (2010) attempts to demonstrate that an IUR based on rodent tumors using a default approach can be used to estimate the number of excess cancer cases expected in the occupational cohort, even though the SMR analyses completed by Marsh et al. (2007b) failed to demonstrate an excess exists. As documented in Marsh et al. (2007b), occupational exposures were highest in the past, declining from 20 ppm, or approximately 1035 µg/m3, before 1960 to less than 0.5 ppm (approximately 26 µg/m3) in 1990

(see page 303 of the publication). Thus, in spite of the highest occupational exposures being experienced longest ago, relevant to the fact that cancer develops over the course of decades, there were no excess liver or lung cancers in the cohort even with the latest update (Marsh et al. 2021): SMR values for both lung and liver cancers were <1.0. An accurate IUR should not predict a large (or any) excess in the occupational cohort. Furthermore, the occupational exposure concentrations documented in Marsh et al. (2007b) are higher than the levels expected for residents of St. John the Baptist Parish due to dispersion over distance from the source. It is implausible to suggest significant excess risks in current residents of St. John the Baptist Parish when no excess was observed at the much higher occupational exposures experienced by the cohort.

- The authors of the USEPA denial of Denka's RFC incorrectly interpreted the presentation of the LTR data as an epidemiological analysis that aimed to link an exposure with an outcome.

The only reason for providing the LTR data was to test the validity of USEPA's assertions regarding the magnitude of the cancer risk in St. John the Baptist Parish.

- The authors of the USEPA denial of Denka's RFC raised concerns that "Tumor registry data may also be subject to notable differences in resources and surveillance rigor and effectiveness across healthcare systems in different regions."

The LTR covers the entire state of Louisiana and the management and administration of the registry is the same for all parishes. Furthermore, the LTR is part of the US Centers for Disease Control and Prevention (CDC) national Surveillance, Endpoints and Epidemiology Registry (SEER) program. The LTR has not only adhered to the guidelines for managing a cancer registry set forth by the CDC, it has received awards for the quality of its data (see, for example, LSU Health New Orleans 2018). The completeness of the LTR specifically for St. John the Baptist Parish has been validated in an audit conducted by Louisiana State University (Williams et al. 2021).

- The authors of the USEPA denial letter reiterated their misunderstanding of our purpose in presenting the LTR data in their statements, "Many cancers are also often multifactorial in nature, and examination of tumor registry data by itself doesn't readily inform hypotheses on specific links to certain chemical exposures such as chloroprene. Thus, comparisons based on the tumor registry data alone do not further inform drawing causal inference related to specific exposures such as chloroprene." And "In the context of a hazard characterization, tumor registry data could be considered more descriptive and does not readily permit the examination of epidemiological associations to evaluate specific etiologic hypotheses."

We have not proposed using the tumor registry data to evaluate the risk associated with specific exposures, only to test the validity of USEPA's assertions regarding the magnitude of the cancer risk in the Parish. We found no evidence of exceptionally high cancer risks in St. John the Baptist Parish, therefore, USEPA's risk calculations have overstated the hazard.

- The authors of the USEPA denial letter misunderstood the meaning of the censored data elements in the LTR reports, evidenced by this statement: "In addition, several limitations were noted by USEPA of Denka's statewide tumor registry analysis, including that data on liver cancers are not available in the Louisiana Tumor Registry at the parish level, which precludes examination of whether liver cancer rates are elevated in the St. John Baptist Parish compared to other relevant areas in Louisiana."

The Louisiana Tumor Registry adheres to data privacy protocols that prohibit reporting data for cancers when too few cases are available for anonymized analyses. Liver cancers were among the types of cancer that occurred too infrequently in St. John the Baptist Parish to allow reporting by the Tumor Registry. This censoring therefore provides additional evidence that liver cancer rates are not elevated in St. John the Baptist Parish. Data are provided on the LTR website that compare Parish-level data for 2014-2018 to the state, overall. These data indicate that St. John the Baptist Parish has incidence in the bottom quartile for Louisiana for liver and lung cancers and for all cancers, combined.

4. SPECIFIC RESPONSES TO TIER 1/TIER 2 RECOMMENDATIONS FROM THE FOLLOW-UP PEER REVIEW REPORT (VERSAR 2021)

This section lists the Tier 1 Key Recommendations and Tier 2 Suggestions from the reviewers in the follow-up peer review report (Versar 2021) and provide Ramboll's responses.

> Tier 1: Key Recommendations – Recommendations that are necessary for strengthening the scientific basis for the PBPK model, reducing model uncertainties (especially with respect to typical expectations for a PBPK model) or accurately evaluating such uncertainties before the model is applied for risk assessment.
>
> Tier 2: Suggestions – Recommendations that are encouraged in order to strengthen confidence before the PBPK model is potentially applied in risk assessment. It is understood that other factors (e.g. timeliness) may also be considered before deciding to conduct the suggested additional research or model revisions.

The responses are organized by question, with the question from the charge to the peer reviewers noted with each comment. We have indented the peer reviewers' comments and followed them with our responses. All Tier 1 and Tier 2 comments have been addressed and resolved. We do not believe that there are here are any remaining issues that would argue against using the PBPK model in a risk assessment for chloroprene. To the contrary, the evidence described in the responses to the reviewers' comments demonstrates that the chloroprene PBPK model is based on the best available science, which is especially important when deriving an Inhalation Unit Risk involving extrapolation across species to estimate the potential human carcinogenicity of compounds, like chloroprene, whose toxicity results from reactive metabolites. Previous risk assessments for similar chemicals have demonstrated that the default cross-species extrapolation using inhaled concentration is highly inaccurate for this toxic mode of action.

Question 1 - Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

- The revised analysis for estimating the metabolic parameters is acceptable, and the joint MCMC estimation of Vmax, Km, and Kgl using male mouse liver data is informative and a correct step in the right direction. A few technical issues remain to be clarified and corrected. (Zhu – Question 1, Tier 1)
    - In Supp Mat B (page 6, last paragraph) the authors stated:

        - "Therefore, we conducted a re-analysis of the data on metabolism in the male mouse liver to simultaneously estimate Vmax, Km and Kgl ..."
    - Also in Supp Mat B (page 9 in the paragraph following equation 1) the authors stated:
        - "The flux of chloroprene between air and media (Kgl) was estimated by fixing the Km in the male mouse liver microsomal study to 1.0 μmol/L and estimating both Vmax and Kgl."
    - These two statements were inconsistent and confusing. The latter indicated the estimation of Kgl and Vmax were based on a fixed Km, not simultaneous. Clarification is needed.

Response: Unfortunately, the sentence on p. 9 of Supp Mat B was missing a few words that confused its meaning (italicized here): "The flux of chloroprene between air and media (Kgl) was estimated by fixing the Km to the value in the male mouse liver microsomal study of 1.0 μmol/L and estimating both Vmax and Kgl." This has been corrected.

- Ramboll's re-analysis reported "best" estimate of Kgl=0.22L/hr. It is unclear if it was the posterior mode. The statement (Supp Mat B p9 2nd paragraph following equation 1), "The geometric mean of Kgl was retained as a fixed value for the analysis of all the in vitro studies including the male mouse liver which was re-analyzed to estimate Vmax and Km after the Kgl was fixed" suggests that it was the mean. The footnotes of Figure B-5 also suggest the same. The posterior mode for ln(Kgl) was about -1.88 (Figure B-4), giving Kgl=exp(-1.88)=0.15. Under MCMC framework, it is crucial to use the posterior mode as the best estimate, especially when the posterior distributions are skewed, as likely the case seen for Km and Kgl (Figure B-4). It is strongly recommended that posterior modes reported and used as the estimate of the metabolism parameters. Note that only when the posterior distributions are symmetric, posterior mode and mean would be comparable. Therefore, Ramboll should examine the posterior distributions carefully.

Response: The final choice of Kgl was not based on the posterior analysis of Vmax, Km and Kgl in the male mouse liver. Due to the high correlation between Km and Kgl, and to a lesser degree between Vmax, Km and Kgl, we found the simultaneous identification of all three parameters very challenging, including having to bound some distributions from below. Based on our evaluation of the posteriors, we determined that for our purposes it was more appropriate to use the mean rather than the mode in this case. In order to evaluate the impact of this decision, we conducted a sensitivity analysis on the value assumed for Kgl. As described in the main text of the revised PBPK model documentation, the choice of 0.22 L/hr for Kgl was based on a goodness of fit analysis, which also included a value of Kgl = 0.175, which was as close to the posterior mode as it was possible to use and still be able fit the in metabolism data (Figure 1).

- In simultaneous estimation, posterior modes for Vmax and Km should be reported in conjunction with that of Kgl, in conjunction with a highest posterior density or highest credibility region/interval, the counterpart of a confidence interval in the Frequentist approach.

Response: The choice of Kgl was not based on the posterior analysis of Vmax, Km and Kgl in the male mouse liver. Due to the high correlation between Km and Kgl, and to a lesser degree between Vmax, Km and Kgl, we found that identification of parameters when

all three are included in the calibration was not reliable. Therefore, in order to evaluate the impact of the value assumed for Kgl on the estimation of metabolism parameters and dose metrics, we conducted a sensitivity analysis. As described in the revised PBPK model documentation, the choice of 0.22 L/hr for Kgl was based on a goodness of fit analysis using the data in the female liver, which indicated that the mean value of Kgl estimated from the male mouse data provided the best fit to the female mouse data (Table 2). Therefore, this value was selected for use during the in vitro calibrations in the subsequent evaluation of metabolic parameters:

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

Table 2. Goodness of fit in vitro model to the experimental data based on sum of squares error (SSE) for different values of Kgl

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

Question 2 - The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

- To address whether the uncertainty was impacted by the 'Kgl' value, the lower and upper bound of the estimated parameters (e.g. Km) should be presented and compared for different values of 'Kgl'. (Shao – Question 2, Tier 1)

Response: The issue being addressed by the Kgl analysis was not the impact of uncertainty in Kgl on the estimated metabolism parameter values, it was the impact of uncertainty in Kgl on model-predicted dose metrics. The results of this analysis were provided in Table 3 of the revised PBPK model documentation:

Table 3. Sensitivity of the dose metric predictions from the model to the value of Kgl used in the in vitro parameter estimation						
KGL value:		**0.175**	**0.22**	**0.44**	**0.88**	**1000**
Species	**Inhaled Concentration**	**Amt. Metab. Lung**	**Amt. Metab. Lung**	**Amt. Metab. Lung**	**Amt. Metab. Lung**	**Amt. Metab. Lung**
Female Mouse	**12.8 ppm**	0.85	0.85	0.85	0.86	0.86
	32 ppm	1.29	1.29	1.29	1.29	1.29
	80 ppm	1.69	1.69	1.69	1.69	1.69
Human	**1 µg/m3**	3.59E-06	3.24E-06	2.73E-06	2.54E-06	2.33E-06

- The current report contains an extensive discussion on the mass transfer coefficient Kgl, including details on estimation of Kgl. Importantly, Ramboll had the opportunity to apply the MCMC analysis on male mouse liver to other tissue/sex/species to obtain a range of Kgl estimates, therefore gaining valuable insight on the variabilities/uncertainties of Kgl. Instead Ramboll used the value Kgl=0.22 obtained from male mouse liver for all analyses on other tissue/sex/species. To a lesser extent, Ramboll could have also used values chosen within the 95% credibility interval of Kgl to investigate the propagating impact of Kgl on predicted dose metrics. Note that 95% credibility interval capture mostly uncertainties associated with sampling variations, not those associated with model and parameter. (Zhu – Question 2, Tier 1)

Response: No MCMC analysis was performed with the male mouse liver metabolism data, because the collinearity of Kgl with Vmax and Km prevented convergence. As described in the revised PBPK model documentation, the choice of 0.22 L/hr for Kgl was based on a goodness of fit analysis, and was fixed during the in vitro calibrations in the subsequent evaluation of metabolic parameters.

- In assessing the sensitivity of cancer risk quantification to the PBPK model prediction of dosimetry in general and Kgl in particular, the multi-stage Weibull dose-response model was used quantify the dose-response relationship. No justification was given to the choice of Multi-stage Weibull model as opposed to other models (e.g. Weibull model). Note also the multi-stage Weibull model is no longer supported by USEPA's BMDS software. (Zhu – Question 2, Tier 1)

Response: There are two major reasons for using the Multi-stage Weibull model in our analysis. First, there is a statistically significant difference in the survival of the dose groups of the female mice versus the survival in the control group which supports the use of a time-to-tumor model to account for the probability of survival until the tumors occur. Second, we wished to be as consistent with the USEPA methods as possible where we considered them to be appropriate. In the 2010 toxicological Review of Chloroprene (USEPA 2010), the USEPA used the multi-stage Weibull in their analyses citing the following reasons:

"Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and increased mortality with increasing exposure level, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. USEPA has generally used the multistage Weibull model, because it incorporates the time at which death-with-tumor occurred."

Although it is true that the Multi-Stage Weibull model was never directly a part of the USEPA BMDS software, it has been available on the USEPA website for many years and can presently be located at the following Web address: https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NCEA&count=10000&dirEntryId=217055&searchall=&showcriteria=2&simplesearch=0&timstype=

Question 3 - Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

- No Tier 1 or 2 recommendations

Question 4 - Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

- Address the implied recommendation of the 2020 review panel to better characterize the individuals who provided microsomal samples." (Portier – Question 4, Tier 1)

Response: The necessary information is not available to characterize the individuals from which microsomal samples were obtained, due to the age of the Lorenz et al. (1984) study. However, the USEPA has previously used this study for the same purpose in their PBPK-based IRIS assessment for vinyl chloride.

- This and Yang's (Yang et al 2012) analyses both demonstrated evidence of between-species and between-sex differences in metabolic parameters. The authors of this analysis noted the visible differences between this analysis and that of Yang et al 2012. For example, the estimates of Km in the male mouse lung and liver from this analysis were only half of those from Yang's analysis. Incorporation of a mass transport parameter Kgl made the current analysis biologically sound. However, it is plausible that Kgl could be different across tissue or sex (as evidenced by its dependence with affinity Km). The fact that the current analysis failed to obtain an acceptable estimate for the metabolism parameters in multiple species and both sexes indicating limitation in these data as well. Therefore, this analysis did not provide strong evidence that the microsomal samples or data were sufficient. (Zhu – Question 4, Tier 2)

Response: Kgl was a fixed parameter in Ramboll analyses of in vitro Vmax and Km for all tissues and genders. It is not plausible that Kgl could be different across tissue or gender: Kgl is a physicochemical parameter that represents the diffusion limitation for chloroprene uptake into the media from the air in the vial. Kgl is independent of Km, but the collinearity of Kgl and Km complicates the process of estimating values for both parameters simultaneously from the in vitro data. To address this issue, the value of Kgl=0.22 was derived in two separate ways: (1) from scaling of the mixing rate in the experimental determination of Kgl to the mixing rate in the metabolism studies, and (2) from simultaneous estimation of Kgl, Km and Vmax using the data for the male mouse liver, which had the highest rates of metabolism, and therefore served as the most informative data for the simultaneous estimation of Kgl and Km. The Kgl estimated from the male mouse liver could then be used for the estimation of Vmax and Km in all of the

other tissue data, because the mixing conditions in the vials were the same throughout the studies. There is no scientific justification for using a different value of Kgl in different tissues or genders.

Question 5 - Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

- I suggest Ramboll include metabolism data with inhibitors from Himmelstein et al. (2001 and 2004) in their report as direct experimental evidence for the role of CYP2E1 in metabolism of chloroprene. (Smith – Question 5, Tier 1)

Response: We thank the reviewer for highlighting the key evidence demonstrating that chloroprene is primarily metabolized by CYP2E1. In particular, Himmelstein et al. (2001) found that the metabolism of chloroprene in the mouse liver was almost completely inhibited by 4-methyl pyrazole, a specific inhibitor of CYP 2E1.

- Has Denka and Ramboll contacted the UK folks in Syngenta Central Toxicology Laboratory and University of New Castle where the work in the Cottrell et al. (2001) and Munter et al. (2003, 2007) papers were done? If these two groups of scientists could do the experiments, there should be other laboratories in the world with equivalent expertise and facilities to do such work. (Yang – Question 5, Tier 1)

Response: As pointed out by another reviewer, Jordan Smith, Himmelstein et al. 2001 reported that the metabolism of chloroprene in the mouse liver was almost completely inhibited by 4-methyl pyrazole, a specific inhibitor of CYP2E1. Therefore, no new studies are needed to demonstrate that chloroprene is primarily a substrate of CYP2E1.

- I recommend that Ramboll colleagues calculate Vmax and Km using enzymatic formation of 3a,b (i.e. 1-chloroethenyl oxirane) data in Table 1 [page 1296, Munter et al. (2003)]. These metabolic constants are then compared with the equivalent constants in Table 3 (page 23) of what Himmelstein et al. (2004) produced. This way we could get an idea what differences are there between two excellent groups of experimental scientists produced, using two approaches, on "total" metabolism of CP in rat, mouse, and human. This comparison will also afford us, at the very least, a ballpark idea whether there is/are major problems with the present Ramboll (2021) approach. Yes, I am aware of the fact that the Munter et al. (2003) work involved the use of acetonitrile as a solvent and, yes, I am also aware of the fact that production of 3a,b is not "total metabolism." It doesn't matter here because I am trying to avoid "major errors." (Yang – Question 5, Tier 1).

Response: As requested, we have calculated kinetic constants from Munter et al. (2003) to compare with those from Himmelstein et al. (2004). Importantly, these two studies provide different measures for a maximum velocity of metabolism (Vmax). Himmelstein et al. (2004) examined loss of CP from headspace, providing a measure of total oxidative metabolism, i.e. production of 1-CEO, 1-CEO-diol (3a,b and 4a,b from Munter) and the amount going to 2-CEO, which in liver was estimated to be 95% of total metabolism. The reported values of Vmax and Km were, respectively, 0.23 mmole/hr/mg protein and 1.03 mM.

Munter et al. (2003) added CP in 5uL acetonitrile (i.e. 3.93 mg based on a molecular weight of 41.053) with liquid phase concentrations of CP ranging from 10 to 10000 μM. The acetonitrile concentration in the absence of CP would be 95.7mM. The liquid phase concentration depends on the volumes of liquid (1 ml) and air (9mL) in the gas tight syringe and the liquid:air partition coefficient, 0.69. Based on this partition coefficient, the liquid phase concentration after equilibration will be 7.1% of the CP added in the liquid. Even at the highest CP concentration, there is close to a 10-fold excess of acetonitrile with the ratio of acetonitrile to CP increasing with decreasing [CP] and the liquid phase [CP] concentrations vastly exceed the blood [CP] in the mice exposed to 90 ppm which was 10μM.

The amounts of product formed in the assays - the sum of R- and S-1-CEO and R- and S 1-CEO diol - and the calculated liquid phase concentrations are shown in Table 4.

Table 4. Rates of formation of 1-CEO products using rat liver

CP-concentration	**Product formed**	**Adjusted rate**
(uM)	nmoles/30 min/1.5 mg protein	nmoles/hr/mg protein
0.0	0.0	0.0
0.71	1.23	1.64
7.1	7.42	9.89
71	25.29	33.72
710	31.3	40.75

The adjusted rate of production was fit to a Michaelis-Menten equation:

$V = Vmax*[CP]/(CP + Km)$.

The best fit using a single M-M equation was Vmax = 42.14 nmoles/hr/mg protein (0.042 umoles/hr/mg protein) and Km=19.38 uM (Figure 1). This Vmax needs be adjusted for the proportion of oxidation that produces 2-CEO in rat liver, estimated by Himmelstein to be 95% of the total. The adjusted Vmax for total metabolism, assuming the *in vitro* conditions in Munter et al. (2003) produce a similar split between 1-CEO and 2-CEO would be 0.042/0.05 or 0.84 μmoles/hr/mg protein. Therefore, the estimated Vmax for the two studies differs by a factor of 3.65 and the Km values differ by a factor of 18.8.

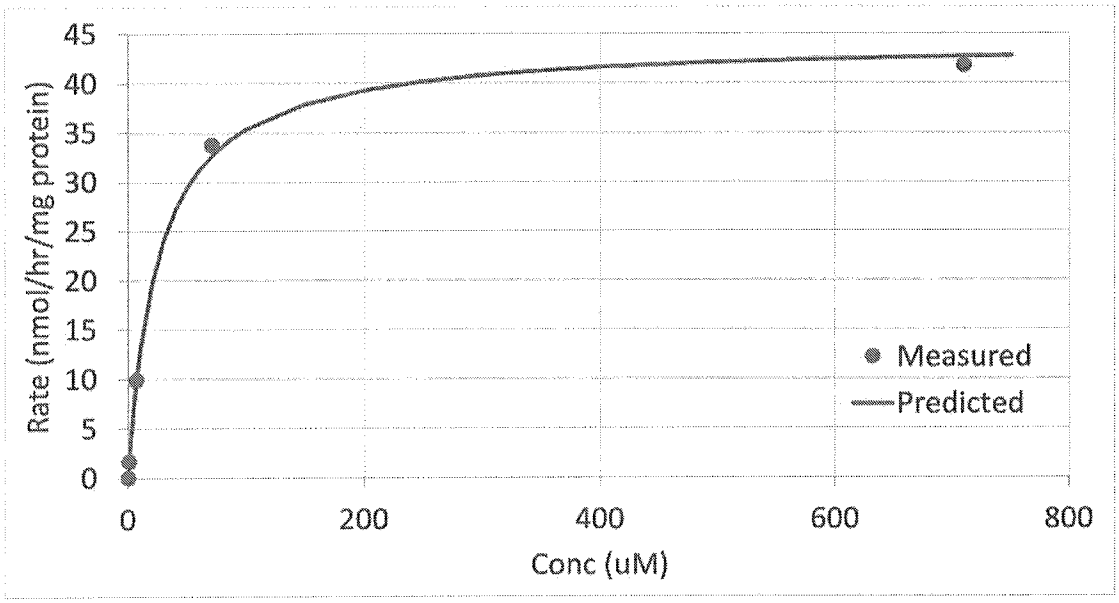

Figure 1: Michaelis-Menten Plot of data from Munter et al. (2003). Predicted values were obtained using Vmax=42.14 nmoles/hr/mg protein and Km=19.38 uM.

The large difference in Km is almost certainly due to the presence of high concentrations of a competitive low molecular weight alternative substrate, acetonitrile, in the Munter et al. (2003) study. The ratio of CP/acetonitrile varies somewhat at higher CP concentrations where the ratio of CP/acetonitrile increases. The Michaelis-Menten relationship for competitive inhibition (Andersen et al. 1987) is:

$V = Vmax*S/(S+Km\ ((KI+I)/KI)\ ) = Vmax*S/(S+(KM*(1+I/KI))$

Where I is the concentration of the inhibitor and KI is the equilibrium dissociation constant for binding of the inhibitor to the active site of the enzyme. Based on the observed Km from Munter et al. (2003), (1+I/KI) would be 18.8. Since the concentration of acetonitrile, I was 95.7 mM, the KI to give the observed inhibition would be 5.3 mM.

Thus, the difference in Km between the two studies is consistent with inhibition between acetonitrile and CP. It bears emphasis that the Km value from Himmelstein et al. (2004) is consistent with in-life Km values determined by gas uptake methods for a variety of low molecular weight chlorinated methanes, ethanes and ethylenes (Supplemental Materials B of the PBPK Model Documentation). If the Munter et al. (2003) experimental design had been intended to assess CP metabolic constants at relevant exposure levels, gas phase introduction of CP with equilibration between the gas and liquid phase would have been a preferred design. The study, however, was intended to identify metabolites not to assess kinetic constants for the high affinity pathways that dominate metabolism at concentrations relevant to the rodent bioassays and potential human exposures.

The higher estimate of Vmax in the Munter et al. (2003) study is likely to be due to the use of such high concentrations of CP. At these very high concentrations, lower affinity but higher capacity pathways, such as oxidation by CYP1A and 2B family cytochrome P450s, can also contribute substantially to total metabolism. Treatment of rats with PB (a CYP2B family inducer)

or with PCBs (a mixed CYP2B and CYP1A family inducer) increased metabolism of another chloroalkene, trichloroethylene (Clewell and Andersen 2004).

- it is not clear whether the use of relative 7-ethoxycoumarin activity in humans and lungs is a reliable way to predict the average metabolism rate. However, in the current analysis, the calculated Vmax/Km in the liver is about 4680 times (14.51/0.0031) greater than in the lung. Even in Himmelstein et al. (2004), the highest metabolism ratio between liver and lung was not over 100. (Hsieh – Question 5, Tier 2)

Response: We believe the A1 approach used by USEPA in the IRIS assessment for methylene chloride remains the best approach for estimating human lung metabolism. The analysis in Himmelstein et al 2004a only represented an upper-bound estimate of metabolism in the case of the human lung. As explained in our report, the in vitro metabolism studies conducted with chloroprene were unable to detect any metabolism in the human lung, as evidenced by the fact that the rate of change in chloroprene concentrations in the human lung metabolism vials was similar to, and in some cases less than, the rate of change of chloroprene concentrations in the control vials. Because the slow rate of metabolism in the human lung made it impossible to estimate both Vmax and Km from their in vitro data, Himmelstein et al. (2004) attempted instead to estimate a first-order rate constant for metabolism in the human lung. Unfortunately, this approach was not biologically appropriate, because the metabolism of chloroprene, regardless of tissue, results from high-affinity, low-capacity enzymes. Therefore, in our analysis we followed the same approach as in the USEPA IRIS assessment for methylene chloride, another compound where data on metabolism was not available for the human lung; that is, we assumed that the Km for the CYP 2E1 protein would be the same in liver and lung and estimated Vmax in the human lung based on the Vmax for the liver using the 7-ethoxycoumarin activity ratio between liver and lung as a surrogate for other CYP 2E1 substrates. This approach was also supported by an alternative approach based on the ratio of CYP RNA in the two tissues.

Question 6 - Please evaluate the choices of extrapolation factors and formulas used for the IVIVE calculations. Please discuss the soundness of the metabolic parameters in Table S-4 as estimates for average adult female and male mice and rats, and average adult humans (combined sexes).

- No Tier 1 or 2 recommendations

Question 7 - Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

- Clarify how the Monte Carlo assessment of uncertainty of physiological parameters was performed. (Portier – Question 7, Tier 1)

Response: The parameter distributions for the physiological parameters are based on using the set value as the mean +/- 2.5 SD with the SD calculated using a CV fraction of the mean parameter listed in Table 5. Metabolism parameters were based on the posterior chain.

Table 5. Rodent tumor sites in in bioassays for chloroprene and vinyl chloride					
Parameter	**Female Mouse**	**CV**	**Human**	**CV**	**Distribution**
BW	0.04	0.11	70	0.3	Normal
QPC	29.1	0.56	24	0.3	Normal
QCC	20.1	0.083	16.5	0.1	Normal
QLC	0.161	0.3	0.227	0.2	Normal
QFC	0.07	0.6	0.052	0.3	Normal
QSC	0.159	0.4	0.191	0.15	Normal
QKC	0.9	0.3	0.175	0.2	Normal
VLC	0.055	0.06	0.0257	0.05	Normal
VLUC	0.0073	0.3	0.0076	0.1	Normal
VFC	0.1	0.3	0.27	0.3	Normal
VRC	0.08098	0.3	0.0.533	0.1	Normal
VSC	0.384	0.3	0.4	0.3	Normal
VKC	0.0167	0.3	0.0044	0.1	Normal
PL	1.26	0.2	2.37	0.2	Log-Normal
PLU	2.38	0.2	2.94	0.2	Log-Normal
PF	17.35	0.3	28.65	0.3	Log-Normal
PS	0.59	0.2	1	0.2	Log-Normal
PR	1.76	0.2	2.67	0.2	Log-Normal
PB	7.8	0.2	4.5	0.2	Log-Normal
PK	1.76	0.2	2.67	0.2	Log-Normal

- Discuss how known/assumed correlations among partition coefficient parameters are handled in the Monte Carlo assessment of uncertainty of physiological parameters. (Portier – Question 7, Tier 1)

Response: Partition coefficients (PC) are usually sampled with independent distributions. That is, blood:air and tissue:blood PCs are assumed to have independent variability and uncertainty in the Monte Carlo analysis.

- Clearly identify the variables referred to as having the "joint posterior distribution" from which samples are drawn in the analysis to address statistical dependency among Vmax, Km and Kgl. If possible, provide a graphic to illustrate what this joint posterior distribution looks like. (Portier – Question 7, Tier 1)

Response: There is little to be gained from plotting the joint posterior distribution for a calibration that is not converged. The posterior estimate for Kgl from the male mouse liver incubation was not the basis of Kgl in the calibration to the in vitro experiments (see response to question 1 above). Given that the calibration of Vmax, Km and Kgl failed to converge, Kgl was based on the best fit to the human liver incubation in the goodness of fit evaluation shown in Table 2.

Question 8 - Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an USEPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e. tissues in which chloroprene metabolism is predicted by the model.

- From the perspective of PBPK modeling, the present Ramboll model "over-predict" the blood CP levels comparing to their experimental data [Figure 7, Ramboll (2021)]. I would recommend the Ramboll colleagues to consider CP, itself, as a part of the internal dose and incorporate an adduct-formation rate constant in the following compartments of the PBPK model: lung, slowly perfused, rapidly perfused, liver, and kidney, based on the multiple tumor sites reported in NTP TR467 (NTP 1998) as quoted above. This would certainly render CP less available in the blood stream; thus, the end results of such an incorporation into the PBPK model would have been a better fit of the simulation curves with the experimental data. Regarding "model reduction" (see discussion below under Item 2) then, the Ramboll colleagues might consider only the two-component internal dose of the parent compound CP plus "the total dose metabolized" in their PBPK modeling. In that case, the "dose metric" calculations as presented on page 27 of the Report (Ramboll 2021) would have been different. (Yang – Question 8, Tier 1)

Response: This set of comments from Dr. R. Yang raises the possibility of direct reactivity of CP with tissue macromolecules, specifically DNA. If such reactions occurred at a significant level, the dose metrics used for the risk assessment would have to include the rates/amount of direct tissue reactivity of CP as part of the dose metric. The basis for suggesting that CP would react directly was two-fold – (1) the lack of fit to the in-life blood CP concentration results in the exposures at 13.2, 32 and 90 ppm and (2) the observation of cancer in tissues other than the lungs and liver. Dr. Yang suggests evaluating the reaction of CP with calf thymus DNA, following procedures in Munter et al. (2007) where the authors were evaluating the reactivity of the 1-CEO epoxide with DNA and of reactive aldehydes derived from CP metabolism, identifying DNA adducts formed by (Z)-2-chlorbut-2-en-1-al, a reactive aldehyde (structure 7 in Munter et al. (2007). The issues raised by Dr. Yang are fully addressed in the discussion below and no further evaluation should be necessary:

Is CP expected to react with DNA? While no studies have been conducted to assess CP binding to calf thymus DNA, multiple studies have assessed the mutagenicity of CP. While there were initially conflicting results of mutagenicity in Ames assays, Westphal et al. (1994), examined the mutagenicity of both freshly prepared CP and aged CP. Freshly prepared CP showed no mutagenic response while samples aged for several days showed mutagenicity. The authors identified a group of cyclic CP dimers that were responsible for mutagenic responses. While these results do not rule out CP reactivity with DNA, they show that CP itself does not react with bacterial DNA to levels sufficient to cause mutations in an Ames assay.

Does the lack of "fit' indicate extrahepatic reactivity of CP? Dr. Yang also notes that the lack of fit to the in vivo data may be due to failure to include extra-hepatic metabolism which could increase systemic clearance (see equation (9) in Andersen, 1981) and reduce the blood concentrations of CP. Two questions to address here are a) the comment of failure to fit the in-life data and b) the nature of extra-hepatic metabolism that would be required to reduce the blood levels in the in-life study.

The curves in Clewell et al. (2019) were not generated using fitted parameters, because they were intended to demonstrate that using the previously determined default model parameters, including the metabolism parameters based on in vitro data, the model was able to provide a reasonable prediction the in vivo kinetics of CP in the female mouse. The model used experimentally measured parameters and showed that the use of these in vitro

derived constants provided an acceptable prediction of the data – within a factor of 2 – for the data sets, which themselves had significant variability. To respond to Dr. Yang's comments, we have conducted a Monte Carlo analysis of the PBPK model predictions for the in vivo results, allowing the parameters to vary using physiologically and biochemically reasonable distributions, in the same fashion as in the Monte Carlo analysis of dose metric uncertainty in Clewell et al. (2019). The resulting distributions for predicted blood concentrations (Figure 2) demonstrate that the model predictions are entirely consistent with the experimental data.

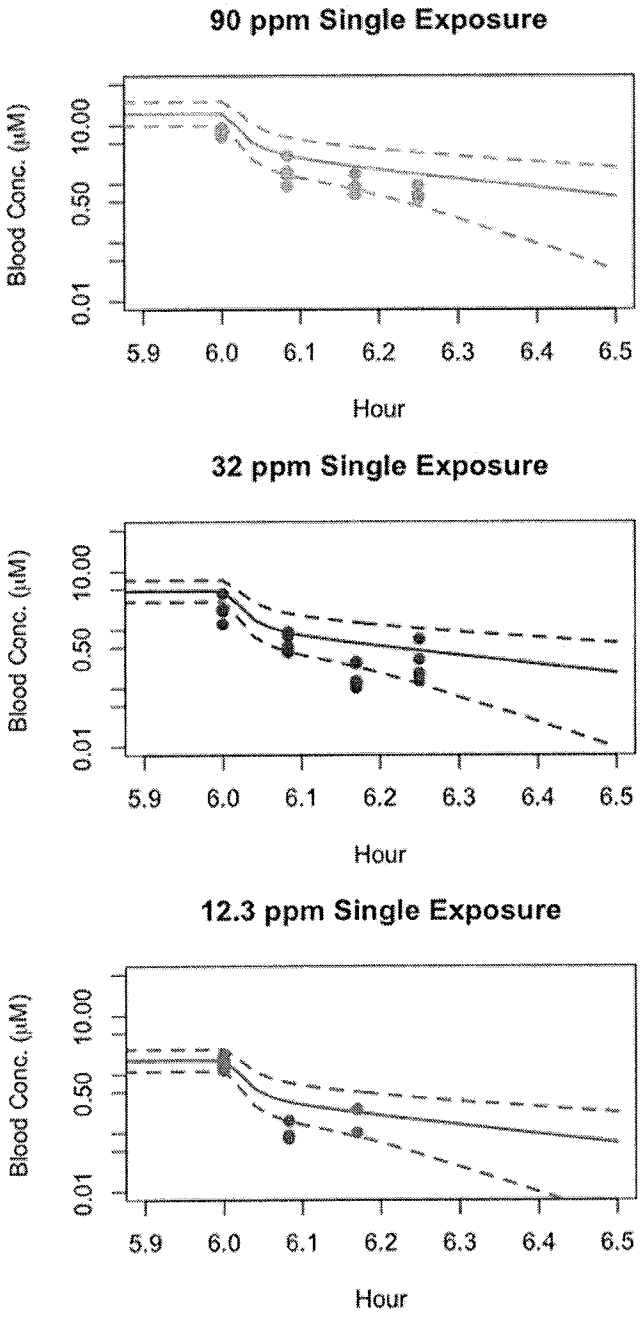

Figure 2. Monte Carlo simulation of the acute chloroprene time-course data from a single 6-hour exposure to chloroprene in female mice (12.3 ppm – red lines and circles, 30 ppm – blue lines and circles and 90 ppm – orange lines and circles). Solid lines represent the median and upper and lower dashed lines represent the 2.5 and 97.5 percentile of 5000 iterations of the model. Individual animal data are represented by the circles.

Importantly, the model predictions for the rate of clearance of CP at the end of exposure show good agreement with the experimental data. The most important parameters affecting the steady-state blood concentration during exposure are the blood:air partition coefficient and the degree of systemic extraction due to high affinity metabolism in liver. Even in the post-exposure phase, the metabolic processes required to influence the decrease in blood concentration would have to be high affinity, consistent with microsomal oxidation rather than direct reaction of CP with tissue components through lower affinity pathways or a relatively low, second order rate of reaction between CP and tissue components. Therefore, the comparison of model predictions with the mouse in vivo study does not provide any evidence of direct reactivity of CP.

Is there evidence that compounds like CP have lower affinity metabolism in tissues? In the 1970's and early 1980's several groups developed gas uptake methods to assess metabolism and associated kinetic parameters for various low-molecular weight chlorinated methanes, ethanes and ethylenes (Filser and Bolt 1979; Andersen et al. 1979). Most of the tested compounds were metabolized by high affinity, low-capacity oxidation (see Gargas et al. 1986). Several chlorinated compounds, including ethylene dichloride (D'Souza et al. 1988) and dihalomethanes (CH2Cl2 and CHBrCl), showed a second pathway related to reaction with glutathione (GSH). With the chlorinated ethylenes, there was little evidence for these secondary GSH-pathways, and when present, they were much less active than oxidative pathways. The only evidence then for alterative metabolic pathways is related to reaction, either directly or through enzyme catalyzed processes, with glutathione, and not direct reactivity with tissue macromolecules.

Does the appearance of tumors in multiple organs indicate direct CP reactivity throughout the body? CP causes tumors in multiple organs (Table 6), leading to speculation that circulating reactive metabolites such as 1-CEO, formed in lung, liver, etc. might be responsible for the carcinogenicity in these tissues. Vinyl chloride (VC), another halogenated ethylene that causes tumors in many of these same tissues, is metabolized to an epoxide intermediate that is not sufficiently stable to circulate throughout the body. For vinyl chloride and its reactive epoxide and for CP and the reactive 2-CEO metabolite, tissue exposures to these reactive products are only expected in tissues with Cyp2E1. The mapping of tumors following long-term VC or CP exposures is equivalent mapping tissues with significant levels of CYP2E1. CYP2E1 has been measured in several tissues (Nishimura et al. 2003).

Summary: Overall, we offer several conclusions regarding reactivity of CP. First, there is no evidence for direct tissue reactivity of CP at concentrations close to those used in animal testing. Second, if direct reactivity of CP were involved in altering the in-life time course, it would require a very high second-order rate constant to reduce circulating CP and there is no evidence with CP or similar compounds for these high degrees of reactivity. Lastly, there is no need to invoke CP reactivity or distribution of metabolites to remote tissues, because the presence of CYP2E1 in multiple tissues, as mapped by VC tumors and measures of content in a more limited set of tissues (Nishimura et al. 2003), indicates that tumors in these multiple tissues arise from similar modes of action to those present in lung.

Table 6. Rodent tumor sites in in bioassays for chloroprene and vinyl chloride.			
Tissue	**Tumor Type**	**Chloroprene**	**Vinyl Chloride**
Liver	Carcinoma, hemangiosarcoma	Y	Y
Lung	Carcinoma, hemangiosarcoma	Y	Y
Kidney	Renal tubule adenoma Nephroblastoma	Y	Y
Brain	Neuroblastoma		Y
Forestomach	Squamous cell carcinoma	Y	Y
Skin	Sarcoma	Y	Y
Nasal cavity	Carcinoma		Y
Bones	Osteochondroma, Leukemia		Y
Mesentery/ Mesothelium	Sarcoma Abdominal mesotheliomas	Y	Y
Harderian gland	Carcinoma	Y	
Zymbal's gland	Carcinoma	Y	Y
Mammary gland	Carcinoma	Y	Y
Other sites	Hemangiosarcoma	Y	Y

- I would recommend Ramboll/Denka colleagues at least conduct a CP DNA covalent binding study using double stranded calf thymus DNA and identify the DNA adduct of CP as described by Munter et al. (2007) on page 329, column 2. (Yang – Question 8, Tier 1)

Response: While no studies have been conducted to assess CP binding specifically to calf thymus DNA, multiple studies have assessed the direct mutagenicity of CP. Multiple studies have shown that fresh chloroprene itself (in the absence of metabolism or impurities) does not react with bacterial DNA to levels sufficient to cause mutations in an Ames assay. Further, Wadugu et al. (2010) examined the potential DNA cross-linking of 1-chloroethylene oxide (CEO) using a denaturing polyacrylamide gel electrophoresis to monitor possible formation of interstrand cross-links compared to other structurally similar DNA cross linkers including diepoxybutane (DEB) and epichlorohydrin (ECH) to better understand the cellular mechanisms associated with chloroprene toxicity. The authors determined that CEO did not form cross-links at physiological pH.

I would urge Ramboll colleagues to study the Transtrum et al. (2015) paper, if you haven't already done so, and examining carefully if any of such shortcomings mentioned in the paper, existed in your PBPK modeling and analyses. (Yang – Question 8, Tier 1)

Response: Transtrum et al. (2015) discusses uncertainty in models of complex systems where the behavior of the model is controlled by a relatively small number of parameter combinations. There is no question that PBPK models fit within this description; therefore, we have made every effort to consider this concern in every PBPK model that we have developed over the last 40 years and have written a number of publications on the topic of model reliability.

Question 9 - Given these data, please evaluate the likelihood that changes in respiration rate or metabolic induction might be factors in the observed PK relationship between exposure and

internal dose. Please comment on any other physiological or biochemical mechanisms that might be explanatory factors in the apparent discrepancy or whether experimental variability in the data may explain these differences.

- No Tier 1 or 2 recommendations

Question 10 - Given the specific considerations above, please comment on the appropriateness of the values selected for the physiological parameters in Table S-1 and partition coefficients in Table S-2, for prediction of chloroprene dosimetry.

- Discuss the importance of having an accurate estimate of PB. Address how uncertainty in the estimate of PB might or might not impact the uncertainty of estimates of other partition coefficients or critical model parameters. (Portier – Question 10)

Response: Along with ventilation rate, cardiac output and liver blood flow, the blood-air partition coefficient (PB) is one of the parameters to which model predictions of blood concentrations in the mouse validation study are highly sensitive, as shown in Figure 8 of the model documentation. However, its impact on model predictions of total metabolism dose metrics is relatively small. As shown in Figure 9 in the documentation, the most sensitive parameters for prediction of dose metrics are the metabolism parameters and volumes of the metabolizing tissues in the model. This question is also addressed in the previous response to one of Dr Yang's comments on Question 8.

I suggest that physiologies from male, female, or both independently at this stage could offer a more realistic and useful parametrization of the model. Cites ICRP 2002 as a source. (Smith – Question 10)

Response: We agree with the reviewer that formal application of the PBPK model in a risk assessment should make use of the appropriate physiological parameters for both male and female humans.

Question 11 - Please comment on the capacity of the PBPK model to provide sound estimates of chloroprene inhalation dosimetry in mice, rats, and humans. Please comment on the reliability of model predictions of the rate of chloroprene metabolism in liver and lung for use in animal-to-human extrapolation.

- No Tier 1 or 2 recommendations.
- However, one reviewer commented: "In the current analysis, the blood concentration data for female B6C3F1 mice were used to validate the model performance of the PBPK model. However, lungs and liver are the target organs that dominates the metabolism of chloroprene. It is surprising that the current analysis only collected the blood sample to conduct PBPK modeling without collect and analyze other tissue, simultaneously. The limitation of the in vivo data is a crucial factor that can reduce the reliability of model predictions and also be applied in animal-to-human extrapolation." (Hsieh – Question 11).

Response: This reviewer was added for the follow-up peer review and did not have the opportunity to benefit from the reviewers' discussions during the initial peer review. He apparently does not understand the purpose of the mouse in vivo study that was performed in response to USEPA's concern, identified during the 2010 IRIS assessment; namely, that the ability of the model to perform in vitro to in vivo extrapolation should be confirmed by an experimental study. The study did provide the data necessary to confirm this. There would be

no value in harvesting tissues from the liver and lung in this study because chloroprene is too volatile and could not be reliably measured following an in vivo exposure and its metabolites are too reactive to quantify by analytical methods.

Question 12 - Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

- No Tier 1 or 2 recommendations specifically for this question. One peer reviewer referred to his comments on question 8 (see responses to question 8, above).
- However, one reviewer commented that: "some in-vivo information (e.g. PK data) from mice and human are necessary. Since the PBPK model can only be used to make predictions. These predicted results still need to be "verified" by the real data. This critical issue should be addressed before the model is applied in risk assessment." (Hsieh – Question 12)

Response: This reviewer was added for the follow-up peer review and did not have the opportunity to benefit from the reviewers' discussions during the initial peer review. In contrast, Dr. Portier, one of the peer reviewers who participated in the initial review, provided a very positive response to this question: "I noted no Tier 1 and Tier 2 comments that had not been addressed by Ramboll. Most of the replies appear reasonable. For some of the recommendations that address issues that are outside my area of expertise or experience I was unable to assess adequacy of the response."

Question 13 - Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

- No Tier 1 or 2 recommendations

Question 14 - Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

- The list of discrepancies identified from Supplemental F should, at a minimum, be discussed (Portier – Question 14)

Response: Model parameters for a model used to simulate more than one species/sex are not set in the base model code. Simulation scripts are used to establish the parameter sets for a specific species/sex. All of the physiological parameters are shown in Exhibit A (Supp_Mat_E). Additional parameters for the epoxy submodel are reported in Table F1. Given our conclusion was that the epoxy submodel should not be used for the risk assessment, the additional files were not included in the supplement but are available upon request.

- Correct or justify the assignment of a value of 62.1 (mg/h/BW^0.75) as a reasonable value for VMAXC1 for female rat in Table F1. (Portier – Question 14)

Response: The original epoxy submodel was established for chloroprene equivalents and the in vitro metabolic rates were scaled using the MW of chloroprene. The correct 1-CEO hydrolysis rate constants for the female rat are 73.32 mg/hr/KG^0.75 for VMAXC1 and 4.34 mg/L for KM1 and for the female mouse are 12.57 mg/hr/kg^0.75 for VMAXC1 and 2.18 mg/L for KM1. The

oversight has been corrected; however, since both species were scaled the same, there essentially no difference in our simulations or conclusions regarding issues surrounding the use of 1-CEO as the dose metric for chloroprene. The confounding relationship between the clearance of 1-CEO in the female mouse and female rat livers remains.

Question 15 - *Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.*

- No Tier 1 or 2 recommendations – However, comments from each reviewer below.
  - Hsieh - Although the experiment results from Fischer female rat did not show a significant dose-response relationship in tumor incidence. The dose-response can still be observed in the mice. Also, over 0.004 of 1-CEO concentration, the tumor incidence in rats had observed higher value than low concentration. Hence, it is not a piece of strong evidence to preclude 1-CEO as a predictor for lung cancer risk.
  - Portier - This is not my area of expertise, but ... I am concerned that the 8-to-9-fold difference of female rat to female mice seen in Figure 4 is a result of the difference in estimated values of VMAXC1 assigned to rats and mice as discussed in my response to Question 14 bullet 4. This 8-to-9-fold difference in VMAXC1 could also be producing the differences observed in Figure 5-C. Before I could conclude that 1-CEO is not a reasonable predictor or chloroprene-induced lung cancer, I would need clarification of the proper value for VMAXC1 for female mice and rats as raised in Question 14 bullet 3.
  - Shao - This is outside my area of expertise, and I have no comments to add.
  - Smith - Figure F5 shows the relationships of cancer incidence as a function of 3 different predicted dose metrics: total amount of chloroprene metabolized, the concentration of total reactive products, and 1-CEO concentration in female mice and rats. Predicted 1-CEO concentration shows little if any predictive value compared to the other two predicted dose metrics evaluated across animal models. As such, I agree that these simulations provide evidence that precludes 1-CEO as a sole predictor of chloroprene-induced lung cancer risk.
  - Yang - I have no problems with using the "total amount metabolized" as a dose metric because we really don't know much, particularly quantitatively, about the metabolic processes beyond the first step oxidative transformation of CP by CYP enzymes. However, as I discussed in detail under Charge Question 8, the probability of CP, itself, being a direct-acting carcinogen cannot and should not be overlooked.
  - Zhu - This is not my specialty. It is interesting that the authors reported a consistent dose-response pattern between female rat and female mouse under both total metabolized amount and reactive products. The measurement of consistency was neither reported nor tested. It seems plausible that the between-specie difference in metabolism of chloroprene resulted in lower level of reactive products and higher concentration of 1-CEO in female rats compared with female mice, therefore leading to seemingly greater difference in the dose-response as seen in Fig F5-C where 1-CEO was the dose metric. It remains highly plausible that the dose-response could be different between mouse and rat under either the total metabolites or reactive products if we can further extend the dose-response curve for the rat to higher levels of the exposure. Furthermore, the authors' observation was limited to two species of a single sex with very limited data. I feel that the evidence is not sufficiently strong to support the

statement that the total metabolized amount and reactive products are metrics that can consistently predict lung cancer risk across species. More research is needed.

Question 16 - Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

- This is not my specialty. It is interesting that the authors reported a consistent dose-response pattern between female rat and female mouse under both total metabolized amount and reactive products. The measurement of consistency was neither reported nor tested. It seems plausible that the between-specie difference in metabolism of chloroprene resulted in lower level of reactive products and higher concentration of 1-CEO in female rats compared with female mice, therefore leading to seemingly greater difference in the dose-response as seen in Fig F5-C where 1-CEO was the dose metric. It remains highly plausible that the dose-response could be different between mouse and rat under either the total metabolites or reactive products if we can further extend the dose-response curve for the rat to higher levels of the exposure. Furthermore, the authors' observation was limited to two species of a single sex with very limited data. I feel that the evidence is not sufficiently strong to support the statement that the total metabolized amount and reactive products are metrics that can consistently predict lung cancer risk across species. More research is needed. (Zhu – Questions 15 and 16, Tier 2)

Response: The analyses shown in Figures F5-A and F5-B are far from the only data that support the use of total metabolism as the dose metric for the carcinogenicity of chloroprene, as documented in Clewell et al. (2019). However, this new comparison adds significantly to the existing evidence that the carcinogenicity of chloroprene, like that of the structurally similar compound, vinyl chloride, requires metabolism. A similar analysis (Himmelstein et al. 1994) has also demonstrated the effectiveness of the total metabolism dose metric to explain the differences in tumor dose-responses in male mice, rats and hamsters.

Question 17 - Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

- It is not recommended to use the experiment result from the lung to support the liver cancer risk assessment. They are two different organs and have different metabolic mechanisms. The additional bioassays to support this viewpoint are recommended. (Hsieh – Question 17, Tier 2).

Response: The reviewer appears to be unaware of the fact that the existing NTP bioassays provide ample data on chloroprene carcinogenicity in both lung and liver, and the necessary liver- and lung-specific metabolism data are already incorporated in the model. In addition, there are extensive data supporting the conclusion that the carcinogenic mode of action (and therefore, the most appropriate dose metric) is the same in both tissues, and the experimental data used to develop the extended model of chloroprene metabolites were obtained in studies with both lung and liver tissues (Sax et al. 2020; Clewell et al. 2019).

- Empirical dose-response based on these two studies is in itself insufficient for drawing such a conclusion. Mechanistic evidence would be useful to determine the validity of 1-CEO as a dose metric for toxicity and carcinogenicity. I do not have sufficient expertise to comment on the strength of mechanistic evidence. (Zhu – Question 17, Tier 2).

Response: We agree with the reviewer that the empirical dose-response from these studies of the evidence for the appropriate dose metric for the lung would not, in itself, be sufficient for drawing a conclusion regarding the liver. However, the experimental data used to develop the extended model of chloroprene metabolites were obtained in studies with both lung and liver tissues, and there are extensive data supporting the conclusion that the carcinogenic mode of action (and therefore, the most appropriate dose metric) is the same in both tissues (Sax et al. 2020).

Other Tier 1 or 2 Recommendations

- Lack of in-vivo data that can be used to verify the "real-world" toxicity effects in the human population. (Hsieh – General Impressions, Tier 2)

Response: The reviewer appears to be unaware that there are indeed real-world data on the potential for toxicity effects in the human population. As discussed in Section III of this document, the epidemiology study by Marsh et al. (2007a, 2007b) did not identify any increase in cancer risk in the employee populations compared with the relevant regional populations for all cancers (combined) or for liver or lung cancer. This was demonstrated by the standardized mortality ratios (SMRs) all being below 1.0, indicating fewer deaths from these causes in each study population compared with the relevant populations where the plants were located. As calculated in Sax et al. (2020), based on the IUR from the USEPA 2010 assessment, an excess of several thousand tumors would be expected in the occupational cohort, whereas no excess was observed. The predictions of the PBPK model, on the other hand were consistent with the negative result in the study, supporting its real-world relevance.

- A few issues should be addressed in the report for clarification around the BMD/BMDL analysis: (1) what is the purpose to calculate BMD and BMDL? It should be clearly stated. Based on results presented in Table 7 and associated explanation in that section, it seems that the purpose is to use the ratio of BMD/BMDL (about 3) as an indicator to justify that the estimation uncertainty is within a reasonable range. (2) How were the BMD and BMDL in Table 7 calculated? Is that a mean value over the 5,000 iterations? If so, it is more reasonable to calculate the BMD/BMDL ratio in each iteration then present the mean value of the ratio with its lower and upper bound. (3) As mentioned on Page 15, correlation analysis was performed between BMDL01s and PBPK parameters. What is purpose for this analysis? BMDL estimates are much more uncertain than BMD estimates because of the algorithms used in BMDS, so using BMD estimates in the analysis is a more reasonable choice. (Shao – Overall Impressions, Tier 2)

Response: The BMD/BMDL analysis was conducted to provide an illustration of the overall variability of PBPK model dose metric predictions resulting from variability in the PBPK model input parameters. The calculation of a BMDL (rather than a BMD) for the animal dose-response was consistent with USEPA practice for cancer risk assessments based on animal bioassay data. No BMD/BMDL rations were calculated. The 95% confidence interval for the resulting dose metric distribution spanned a range from roughly 3-fold below the mean to 3-fold above the mean, consistent with the results of previous analyses on similar PBPK models. Correlation

analysis was performed to assess the sensitivity of the predicted BMDLs to the model parameters.

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EXHIBIT 4

December 6, 2021 - Comment Report – Follow-up – *External Peer Review of a Report on Physiologically Based Pharmacokinectic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance*

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

Comment Report
Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Contract No. EP-C-17-023
Task Order 68HERH21F0287

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December 6, 2021

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I. INTRODUCTION

The U.S. EPA CPHEA is currently evaluating a Request for Reconsideration (RFR), specifically to consider use of a PBPK model in a potential IRIS reassessment of chloroprene, CAS No. 126-99-8. The 2020 report by Ramboll entitled "Incorporation of In Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene," describes new analyses and corresponding revision of a PBPK model for chloroprene, specifically using metabolic parameters derived from in vitro studies. Initial quality assurance evaluation by EPA of the previously published versions of the model (Yang et al., 2012) (for dosimetry in mice, rats and humans) identified issues which the additional data and analyses described in the report seek to address. These unpublished results have not been subjected to a formal peer review process. Such a peer review process is important in establishing the appropriateness, validity, and applicability of the revised PBPK model, in particular considering that no *in vivo* PK data are available to validate or calibrate model predictions in humans. Further, the model predicts the rate of metabolism of chloroprene to presumed toxic metabolites, but not the tissue concentrations of these metabolites.

Typically, metabolism and clearance of chemical entities in humans is assumed to be slower than in smaller mammals, with scaling by $BW^{0.75}$ used to predict the relative clearance in the absence of specific data. However in vitro data have been previously reported by the oxidative metabolite (1-chloroethenyl)oxirane (1-CEO) (Himmelstein et al., 2004). Further, while the report suggests that the toxic metabolite(s) may be completely consumed in the metabolizing tissues (liver and lung), this is contradicted by the induction of tumors in distal sites, in particular mammary tissue, which suggests that clearance by blood perfusion is a factor. Therefore a supplemental analysis (U.S. EPA, 2020) has also been developed to extrapolate the in vitro clearance of 1-CEO by the observed pathways to *in vivo*, to make the various rates comparable to each other and to clearance by blood perfusion, and to ultimately obtain relative total clearance rates in human and rodent liver and lung, and systemic distribution rates, that can be used to evaluate relative risk and whole-body dosimetry.

In October 2020, Versar, an EPA contractor, convened an independent peer review on the draft documents, Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene (Ramboll, 2020) and Supplement: Uncertainty Analysis of In Vitro Metabolic Parameters and of In Vivo Extrapolation (IVIVE) Used in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene (U.S. EPA, 2020). A final peer review report was published in December 2020.

For this peer review, Versar convened six (6) experts that previously served or were willing to serve on the panel to focus on revisions made following the original peer review and prepare written comments regarding the confidence in and applicability of the PBPK model to obtain metrics for animal-human risk extrapolation. These six (6) experts with experience and expertise in one or more of the following areas: physiologically based pharmacokinetic (PBPK) modeling, statistics with expertise in global sensitivity analysis, and metabolic rates *in vitro* were selected as peer reviewers to answer 17 charge questions and to evaluate and provide written comments on a report on physiologically based pharmacokinetic (PBPK) modeling for chloroprene (Ramboll, 2021).

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II. CHARGE TO REVIEWERS

Charge Questions:

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

A model of the *in vitro* incubation system was used to estimate the metabolic parameters from the *in vitro* data. This model is based on certain assumptions and physical parameters, such as the volume of the *in vitro* incubation vials and volumes of air and liquid media in the vials. The model of the *in vitro* system initially used for the analysis of the *in vitro* experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. *Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.*

2. *The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.*

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

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

Other information on the specific microsomal samples, preparation methods and *in vitro* experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

3. *Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.*

4. *Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.*

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

5. *Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.*

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the *Model Parameters* section of the Ramboll report, with details on scaling factors in Supplemental Material C of Ramboll and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of the Ramboll report. The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of multiple pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance.

However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism (Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on predictions of hepatic *clearance* of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE

accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

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

7. *Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.*

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. *Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.*

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

Figure 7 of Ramboll presents the extent of agreement of the model predictions with the blood concentrations in mice following inhalation exposure. It is noted that the inhalation PK data are from a single exposure (animals were not previously exposed to chloroprene) and the non-proportionality is evident by the 3-hour time-point.

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

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

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

Overall PBPK Model Soundness and Applicability

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

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

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

12. *Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.*

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. *Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.*

14. *Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.*

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. *Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.*

16. *Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?*

17. *Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.*

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III. INDIVIDUAL REVIEWER COMMENTS

Nan-Hung Hsieh, Ph.D.
California Department of Pesticide Regulation

Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Nan-Hung Hsieh, Ph.D.
September 6, 2021

I. GENERAL IMPRESSIONS

Generally, the current report provides a lot of evidence and updated information to support their study approach by incorporating in-vitro metabolism data and the PBPK model in chloroprene. Integrating the in-vitro and in-vivo data into the PBPK model also provides reliable evidence in parameter estimation and model application. This information is helpful for regulated agencies to apply in risk assessment. However, despite there are many data and comprehensive research approaches that had been applied in the current analysis, it might be challenging to use the current result as "strong" evidence in risk assessment due to the lack of in-vivo data that can be used to verify the "real-world" toxicity effects in the human population (This issue can be tier 2). This limitation has also been mentioned in the Discussion. Therefore, in my opinion, this IVIVE + PBPK approach is a powerful approach in prioritization of the chemical exposure risk. But for this case, it might still need more comprehensive information to support the risk assessment.

Aside from the above general comment, I appreciate that the current report provides a lot of supporting information (e.g., model code) that can be used to check and verify the computing result. But it will be better if the research groups can provide the comprehensive source code for review and verify the correctness of their computation process. That can improve the process in this review and also answer the question that did not mention in the main text. The comment is also mentioned below. Overall, this is well-conducted research with detailed information. Hopefully, the research group can have further improvement from this review.

II. RESPONSE TO CHARGE QUESTIONS

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

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

The model of the in vitro system initially used for the analysis of the in vitro experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

The whole data preparation and modeling process are very comprehensive. But, it might need some further information (source code or spreadsheet) to explain details (e.g., the model equation that is used to simulate Figure B-1 & B-2). It is difficult to verify the result without this information.

2. The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

I appreciate that there is a lot of information provided about the Bayesian modeling that is applied in the uncertainty analysis. However, the current analysis did not conduct the posterior predictive check and compare the predictive result with the experiment data. It is necessary to conduct the posterior predictive check and exam if the 95% confidence interval can explain the in-vitro experiment data. Under reasonable circumstances, the experiment should be located in 95% CI from the prediction. It is a common way to examine the uncertainty in Bayesian inference (This can be tier 3 issue).

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

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

Other information on the specific microsomal samples, preparation methods and in vitro experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

3. Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

The data used for the estimation of metabolic parameters are reasonable. Actually, the pool sizes are not an issue in the calibration of the model parameter. Since the modeling process is under the Bayesian approach. The most important thing is the credibility of the simulated result with the group of validation data. If the simulated results can correspond with the validation group, then we can trust the estimation of the parameter (tier 3).

4. Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

The answer is the same as the above question. I'm not worried about the sample sizes used in the parameter estimation. But I would like to ask if there are sufficient data that can be used to verify and convince that the parameter setting is adequate.

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

5. Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

It is not clear whether the use of relative 7-ethoxycoumarin activity in humans and lungs is a reliable way to predict the average metabolism rate. However, in the current analysis, the calculated Vmax/Km in the liver is about 4680 times (14.51/0.0031) greater than in the lung. Even in Himmelstein et al. (2004a), the highest metabolism ratio between liver and lung was not over 100. In my opinion, this issue can be tier 2.

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the Model Parameters section of the Ramboll report, with details on scaling factors in Supplemental Material C of Ramboll and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of the Ramboll report. The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of multiple pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance. However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism

(Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on predictions of hepatic clearance of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

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

In Table S-4, most parameters in PBPK model look reasonable and correspond with Yang et al. (2012). However, for female mice, there is no estimated kidney Vmax and Km in this analysis due to the data being insufficient. However, this value had been estimated in Yang et al. (2012). Since all metabolism parameters were estimated by MCMC. It is suggested to use the previous value as prior and update the parameter in inference (Tier 3). Theoretically, the value will not be changed due to the estimated Vmax/Km is extremely lower than liver and lung.

7. Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

Based on the description in the section of Uncertainty Analysis, the uncertainty of the metabolism and other parameters in the PBPK mode had been well address in this study. However, it seems that the MCMC simulation only quantified the uncertainty of the parameter in the modeling process. The estimated parameters in the Supplement Materials A Table S3 can only be seen as the distribution population mean. All coefficient of variations are less than 30%. The current result might not be sufficient to be used for risk estimation to represent the population in risk assessment. It is suggested to incorporate the population variability in MCMC simulation to obtain more information to quantify the variability (Tier 3).

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.

There is no critical issue found in the PBPK model code since it is a general PBPK structure that integrated the M-M metabolism in the lung liver and kidney. The only thing I want to point out here is the PBPK diagram in figure 1. According to the model code (supplement materials E), the "alveolar space" is not a dynamic compartment that is described by a differential equation.

Therefore, it causes confusion using figure 1, which has an independent compartment to describe alveolar space, as the PBPK diagram.

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

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

Changing the metabolic parameter is definitely a key factor to reflect the observed PK relationship between exposure and internal dose. Also, the chemical-specific parameters (e.g., partition coefficient, metabolic-related parameters) are more important than physiological parameters due to the higher uncertainty in IVIVE. If the current parameter setting of the PBPK model is not able to properly describe the relationship between internal and external dose from the observed data, the additional Bayesian MCMC inference with extra collected data (observed organ concentrations) might be required (Tier 3).

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

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

The PBPK model in this analysis is based on Yang et al. (2012). Some default settings of physiological parameters (QCC and QPC) are different. The reason to use the different default settings had also been provided in the Model Parameters section. Also, the partition coefficients

used in the current analysis are similar to Yang et al. (2012), which is based on the experiment in Himmelstein et al. (2004b). Therefore, there is no concern for these parameters.

Overall PBPK Model Soundness and Applicability

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

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

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

In the current analysis, the blood concentration data for female B6C3F1 mice were used to validate the model performance of the PBPK model. However, lungs and liver are the target organs that dominates the metabolism of chloroprene. It is surprising that the current analysis only collected the blood sample to conduct PBPK modeling without collect and analyze other tissue, simultaneously. The limitation of the in vivo data is a crucial factor that can reduce the reliability of model predictions and also be applied in animal-to-human extrapolation.

12. Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

The recommendation is the same as above. The most challenging part of the current result is "data". There is sufficient in-vitro information that can be used to apply in the PBPK model. However, this information can only be used in "calibration". Ultimately, we might want to exam the model performance in the "validation" part. Therefore, some in-vivo information (e.g., PK data) from mice and human are necessary. Since the PBPK model can only be used to make predictions. These predicted results still need to be "verified" by the real data. This critical issue should be addressed before the model is applied in risk assessment.

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

I'm not an expert on this question.

14. Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

The value selected in Table F1 is reasonable (with reference as supporting evidence). But, there is no evidence to support the accuracy of the model prediction. The current table only selects the central estimate of the parameter, therefore, might not be able to quantify the uncertainty in the simulation.

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.

Although the experiment results from Fischer female rat did not show a significant dose-response relationship in tumor incidence. The dose-response can still be observed in the mice. Also, over 0.004 of 1-CEO concentration, the tumor incidence in rats had observed higher value than low concentration. Hence, it is not a piece of strong evidence to preclude 1-CEO as a predictor for lung cancer risk.

16. Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for

either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

It is "likely" that the dose metrics used in Figure F5A and 5B are consistent inter-species predictors of chloroprene-induced lung cancer risk. The experiment result is significant in B6C3F1 mice. But the outcome was only observed in mice. Fischer rats did not have an obvious dose-response relationship. This might be due to the metabolite rate is much lower than mice.

17. Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

It is not recommended to use the experiment result from the lung to support the liver cancer risk assessment. They are two different organs and have different metabolic mechanisms. The additional bioassays to support this viewpoint are recommended (Tier 2).

III. SPECIFIC OBSERVATIONS

Page	Paragraph	Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)
Table B-2		The distributions of Vmax, Km, KG, and A0 mentioned here should be uniform (not log-uniform) since the value has been log-transformed.
Figure B-4	Page 7 paragraph 1	The estimated Km (0.62) after adding the transport limitation only half of the reference value (1.34) is reasonable. However, the distribution of the Km cannot cover the reference value. Also, since the Km can be set as informative prior with reference value as the central estimate, why not used normal distribution?
Table B-2		Same as above question. The prior distribution of Vmax, Km, and KG have informative reference value. Therefore, they should be set to the normal distribution with truncated at 3-4 times SD.
Figure B-4		Should be: Posterior chains (left) and distribution (right)
P17 Figure 3		The pool PK data is informative. But I would like to recommend adding the result of raw data for the individual (by the line plot) to visualize the trends.

Kenneth M. Portier, Ph.D.
Independent Consultant

Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Kenneth M. Portier, Ph.D.
September 7, 2021

I. GENERAL IMPRESSIONS

Overall, the revised documentation (referred to as Ramboll, 2021 - Exhibit A, dated July 15, 2021) of the PBPK model for chloroprene appears to accurately describe the additional research and subsequent modifications to the model and parameter estimation methodology in response to the recommendations of the external peer review conducted in October, 2020. Supplemental materials provided helped greatly to understand the work performed. The greatest issues were found with the material provided in Exhibit A – Supplemental Materials F.

II. RESPONSE TO CHARGE QUESTIONS

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

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

The model of the in vitro system initially used for the analysis of the in vitro experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

The estimated value for Kgl (0.024 L/hr) for chloroprene from the new experimental study is not very different from that reported in 2020, validating the initial estimation methodology. The value of Kgl actually used in the re-estimation of the metabolic parameters (0.22 L/hr) remains based on conjecture and not on experimental results. Despite this, the argument for using the 0.22 L/hr estimate for Kgl remains quite plausible.

The revised analysis and estimation of metabolic parameters is described in Supplemental Materials B: Re-estimation of Metabolism Parameters and summarized on page 11-12 and results on pages 17-20 of the 2021 Ramboll report. In the supplement, the MCMC estimation process for metabolism parameters is described and seems to have been implemented properly. The major difference between the 2021 and 2020 re-analysis estimates seems to be due to the

introduction of a prior distribution for A0 (initial amount in vial). The discussion of metabolism parameter re-estimation accurately summarizes and discusses the findings reported in Tables S-3 and S-4. The method of estimating metabolism parameters for the human lung (discussed on page 20) has reasonable justification and should produce appropriately conservative values.

Conclusion: The revised analysis and the estimation of metabolic parameters using the two-phase in-vitro metabolism model is clearly described and the estimation approach reasonably justified. No changes/recommendations are suggested.

2. The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

The sensitivity/uncertainty analysis methodology is discussed on pages 12-15, results presented on pages 21-28, with the Kgl sensitivity analysis results in particular presented on pages 23-25 of the 2021 Ramboll report. The Kgl sensitivity analysis results are clear and accurately characterized. The range of Kgl values used is properly justified, and the argument for retaining the Kgl value of 0.22 L/h for use in the in vitro modeling properly justified and risk conservative.

The results of the PBPK model parameter sensitivity analysis are as expected. The A1 uncertainty analysis is clearly described, and results seem reasonable from a statistical point of view. Clearly the 2021 Ramboll report presents a better assessment of the sensitivity of the model and subsequent risk estimates to parameter uncertainty than did the 2020 Ramboll report.

Conclusion: The revised analysis on the impact of uncertainty in the metabolic parameters is clearly described and the analysis reported is an improvement from that previously provided. No changes/recommendation are suggested.

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

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

while the human lung activity was measured using microsomes from 12 donors (Lorenz et al., 1984).

Other information on the specific microsomal samples, preparation methods and in vitro experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

3. Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

One 2020 Panel member recommended (Tier 2) that an estimate of the standard deviation of A1 (liver/lung activity ratio) be computed along with an approximate confidence interval and that these be used to discuss the likelihood that A1 is close to 1. The Ramboll response on pages 13, 25-27 indicated that a bootstrap sampling approach was used to derive an estimate of the distribution of A1 and to compute upper and lower 95% confidence bounds. This recommendation was fully addressed in the 2021 Ramboll report. In addition, Ramboll performed an extensive literature search for information that might further inform appropriate values for A1 and presents three additional estimates in Table 5 for human lung and liver tissues from two separate studies. Two of these three values are within the estimated 95% CI computed earlier, but the estimate using mRNA data from Bieche et al. 2007 (0.01086) is much larger than the A1 upper bound (0.00413). The reports suggests that this disparity reflects the difficulty of harvesting and preserving mRNA from tissue comparted to direct measurement of metabolism. This seemed reasonable.

One 2020 Panel member recommended (Tier 2) that Ramboll assess whether information on metabolic conversion of model substrates are available for the microsomal badges that have been used for the *in vitro* kinetic studies. Ramboll indicated that these data are not available for the Lorenz et al. (1984) study and hence could not be compared to other metabolic conversion data from well-characterized batches of human microsomes.

One 2020 Panel member questioned whether the pool size for the human (liver) microsomes used to estimate chloroprene metabolic rate *in vitro* and number of tissue samples significantly impacted the setting of bounds on the prior distributions for Vmax and Km. The Ramboll response to this concern was to point out the large range for the prior distributions used, that the prior distributions were uninformative and that the bounds on the prior distribution range did not seem to impact uncertainty/sensitivity analysis results. The Ramboll response was correct and reasonable.

Conclusion: The revised report appropriately estimates the standard deviation of A1 and computes an approximate 95% confidence interval. The report shows that the wide and uninformative priors for Vmax and Km do not adversely impact the uncertainty/sensitivity analysis results. Ramboll indicated that it could not address uncertainties in metabolic conversion of model substrates for microsomal badges from the Lorenz et al. (1984) studies due to lack of data. It seems that other metabolic conversion data from well-characterized batches of human microsomes is available, but these data are independent of the Lorenz study. While I am not an expert on this, it seems that this suggest the following recommendation.

Recommendation (Tier 3): Find or encourage research that provides quality information on metabolic conversion of model substates that better inform the *in vitro* kinetics and that also replicates or improves on the other findings of Lorenz et al. (1984) that are key to estimating relative lung:liver metabolic activity. The number of donors in this study should be sufficiently large to achieve reasonable confidence in the estimate of A1. The donor group should include adequate representation of sex and racial subpopulations.

4. Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

Although one 2020 Panelist questioned the representativeness of the individuals used to provide the microsomal samples used to characterize metabolic variation across species, sexes and tissues, no firm recommendation to address this issue was presented in the report hence Ramboll did not respond to this issue. This may have been an oversight on the part of the 2020 Panel whose recommendations on this issue centered on 1) better presentation of the available data in the final report and 2) development of new data that is better characterized and for which representativeness can be better assessed. Ramboll does not seem to have addressed either of these recommendations. The issue remains that data from only one human male is used to characterize lung cytosol levels.

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

Recommendation (Tier 1): Address the implied recommendation of the 2020 review panel to better characterize the individuals who provided microsomal samples.

Recommendation (Tier 3): Find or encourage research to provide better (mean) estimates of adult human, mouse, and rat metabolic parameters. Such studies should utilize sufficient numbers of individuals to provide acceptable confidence in the estimated mean. The mean for humans should be based on samples that have acceptable representation of sex and racial subpopulations. Rat and mouse estimates should be based on data from more than one species, preferable from those species commonly used in animal toxicity/carcinogenicity studies.

5. Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

This topic is outside my area of expertise and experience, and I have no comments/recommendations to add.

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the Model Parameters section of the Ramboll report, with details on scaling factors in Supplemental Material C of Ramboll and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of the Ramboll report. The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of multiple pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance. However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism (Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on predictions of hepatic clearance of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

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

This topic is outside my area of expertise and experience, and I have no comments/recommendations to add.

7. Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

I am not qualified to address other factors in the IVIVE application related to model structure. I will address "other factors" in the context of the physiological parameters used in the PBPK models (Table S-1) and the partition coefficients (Table S-2). The discussion of the uncertainty analysis described on pages 14-15 of the 2021 Ramboll report describes an uncertainty analysis that seems reasonable. Whether the uncertainty analysis is adequate cannot be fully assessed unless additional information is added to the report as discussed below.

The PBPK model relies on three key physiological parameters, body weight (BW), unscaled alveolar ventilation (QPC), and unscaled cardiac output (QCC) to derive all other physiological parameters. In addition, the value for QCC is often derived by dividing QPC by a constant, 1.45, the derivation of which is discussed in the 2021 Ramboll report. Given this, it is not clear how the Monte Carlo assessment of uncertainty of physiological parameters was performed. Two approaches are possible. In one, variability is assigned to BW and QPC and the remaining physiological variables are derived from the BW and QPC randomly generated values before each model run. With this approach correlations among physiological parameters are accounted for through their relationship to BW and QCC, but it is not clear whether the variation observed in, or generated for, the derived physiological variables is similar to that reported in Clewell and Jarnot (1994), the reference mentioned in the report. The other approach is to generate random values separately (independently) to each of the (11) physiological parameters before the model is run. This approach would likely not recreate the strong correlations typically seen among physiological parameters unless multivariate distributions were used to generate the random variables. Using independent distributions, "improbable" (e.g. not likely to be seen in nature) sets of values would be generated, and when entered into the model would produce "improbable" results. Regardless of the method used, the Monte Carlo runs need better description and discussion.

Recommendation (Tier 1): Clarify how the Monte Carlo assessment of uncertainty of physiological parameters was performed.

Typically, discussion of correlations among parameters occurs in the context of population (inter-individual) variability for such parameters. While it is true that correlations are a bigger issue when attempting to recreate population variability in outcomes, correlations can be observed and be important in uncertainty distributions because some of these parameters are so highly correlated leading to concerns related to estimation.

The above issue also applies to the partition coefficient parameters (PL, PLU, PF, PS, PR, PB, and PK). Since each is assigned a value independently of the others, the potential for "unlikely" sets increases since known correlations among these parameters may not have been accounted for in the analysis. This issue should also be addressed in the report.

Recommendation (Tier 1): Discuss how known/assumed correlations among partition coefficient parameters are handled in the Monte Carlo assessment of uncertainty of physiological parameters.

In the Ramboll response to a 2020 Report review panelist's question on statistical dependency among Vmax, Km and Kgl (Question 3) and the way the MCMC analysis was performed, there is mention that the Metropolis Hasting algorithm draws samples from the "joint posterior distribution." It is not clear to which variables this refers, but it is likely it refers to the metabolism parameters. There are no graphics to illustrate what this joint posterior distribution looks like. If this does refer to the joint (posterior?) distribution of Vmax and Km (conditional on the Kgl value set for the analysis). This distribution addresses the issue around covariation in the uncertainty in estimation of metabolic parameters, but not other parameters. As a result, the upper and lower bounds on metabolic parameters seem to have been adequately estimated with the MCMC analysis.

My overall assessment is that the estimates of upper and lower bounds on metabolic parameters can be used with confidence for animal-to-human risk extrapolation.

Recommendation (Tier 1): Clearly identify the variables referred to as having the "joint posterior distribution" from which samples are drawn in the analysis to address statistical dependency among Vmax, Km and Kgl. If possible, provide a graphic to illustrate what this joint posterior distribution looks like.

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.

This topic is outside my area of expertise. I have no comments/recommendations to add.

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

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

Physiological and biochemical mechanisms of action is not my area of expertise. I have no comments/recommendations to add.

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

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

This topic is outside my area of expertise and experience. But …

The fact that Ramboll has been successful in linking their estimate for cardiac output (QCC) to similar estimates used in PBPK models for similar chemicals, such as methylene chloride, lends credence to the value used in this model. Similarly, the linking of the estimate of the blood-to-air partition coefficient (PB) used in the chloroprene PBPK model to similar estimates used in other PBPK models for similar chemicals adds credence to the estimate used. Looking at the model as coded, one can understand the importance of getting an accurate estimate of QCC. This leads to deriving good estimates for other flows to tissues. It is less clear looking at the code of how getting an accurate estimate of PB supports having good estimates for the other partition coefficients since they don't seem to be linked.

Recommendation (Tier 2): Discuss the importance of having an accurate estimate of PB. Address how uncertainty in the estimate of PB might or might not impact the uncertainty of estimates of other partition coefficients or critical model parameters.

Overall PBPK Model Soundness and Applicability

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

Demonstration of the PBPK model's ability to predict in vivo PK data is shown by the level of agreement between model predictions and chloroprene venous blood concentrations in Figure 7 of the Ramboll report. For reference, where there are data, and as a rule of thumb, EPA often seeks dosimetric estimates from a model that are within a factor of two of empirical results. The results of the sensitivity analysis shown in Figure 8 for arterial concentrations indicate that these data and specific predictions are not sensitive to the estimated metabolic parameters: a relatively large range in the estimated metabolic parameters (such as the

apparent difference between male and female mouse parameters) would yield similar predictions of blood concentrations. However, as demonstrated in Figure 9, the estimation of lung dose metrics is sensitive to the estimated metabolic parameters.

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

Under the WHO/IPCS (2020) guidance on acceptability of predictions, Ramboll has shown that the PBPK model has the capacity to provide sound estimates of chloroprene inhalation dosimetry in mice, rats, and humans across a wide range of possible values for input and state parameters. Also, this PBPK model has been shown capable of reliably predicting rates of chloroprene metabolism in the liver and lung of animals and humans to within 2 orders of magnitude or less. Within the limitations of available data and with this accuracy acceptability target, the model should be considered a reliable tool for predicting chloroprene metabolism and for providing sound estimates of chloroprene inhalation dosimetry.

I have no other comments/recommendations to add.

12. Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

I noted no Tier 1 and Tier 2 comments that had not been addressed by Ramboll. Most of the replies appear reasonable. For some of the recommendations that address issues that are outside my area of expertise or experience I was unable to assess adequacy of the response.

I did note concern that a number of Tier 2 recommendations suggested the need for additional chloroprene metabolism studies to validate some key assumptions. In each case, Ramboll's reply was that *the laboratory at which the chloroprene metabolism studies were performed is no longer active and we were unable to find any commercial or academic laboratories that could perform such studies with chloroprene.* I am surprised at this response, particularly the part that no academic laboratory could be found to perform any of these studies. The real issue was likely timeliness and costs. I have no recommendation on how this issue can be addressed other than through future funding of targeted research. See, for example, my Tier 3 recommendation under Question 4 above.

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

This is not my area of expertise. I have no comments/recommendations to add.

14. Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

While I am not qualified to comment on the quality or accuracy of the parameter values selected, I did note the following discrepancies.

1) The flow rates, fractional blood flows, fractional volumes and partition coefficient parameter values shown in the model code (Appendix 1, pages 21-22) are values reported for (male and) female mice in Table S-1 and Table S-2 in Supplemental Materials A – Supplemental Tables A, page 2-4. The chloroprene tissue-specific metabolism parameter values assigned in the model code are similar but not identical to those given for female mice in Table S-4.
2) The values reported in Table F1 (page 6 of Supplemental Material F) for the 1-CEO Partition Coefficients are not the same as those reported in the model code (Appendix 1, pages 21-22). Nor are these estimates similar to those reported in Table S-2 in Supplemental Materials A – Supplemental Tables A, page 4.
3) The value for BETA (fraction of 1-CEO production available for hydrolysis/oxidative metabolism or release to blood) is set at 0.33 in Table F1, but 0.67 in the model code (page 22). On page 7 of the Supplemental Material F, the derivation of BETA is described as being *"set equal to the ratio for epoxybutene (Campbell et al. 2015) where 67% of the amount of epoxybutene produced from the metabolism of butadiene was further metabolized due to co-localization of enzymes (i.e. CYP P450 and EH) in the endoplasmic reticulum."*
4) The values for VMAXC1 (Scaled VMax for Hydrolysis Pathway:Liver – 10.65 mouse or 62.1 rat) in Table F1 are different from the values of 7.95 assigned on page 22 of the model code. These values are also different from those given in Table S-4 of Supplemental Materials A – Supplemental Tables A, page 6. While the value of VmaxC of 8.88 or 7.99 for female mouse from Table S-4 compares to the 10.65 for VMAXC1 from Table F1, the value for female rat of 62.1 in Table F1 is much larger than the VmaxC value of 9.37 or 6.36 of Table S-4. The discussion on page 7 of Supplemental Material F does not mention specifically why the female rat estimate for VMAXC1 is so much higher than that of the female mouse. I think these number are attributed to Himmelstein et al. (2004) but I am just not clear on this.
5) Similar issues are found with
    a. KM1 (0.041 in model code, 1.9 or 3.7 in Table F1),
    b. VMAXCLU1 (0.18 in model code, 0.65 or 0.85 in Table F1),
    c. KMLU1 (0.26 in model code, 4.6 or 8.0 in Table F1),
    d. VMAXC10 (7.95 in model code, 2.25 in Table F1),
    e. KM10 (0.041 in model code, 1.5 in Table F1), etc.
6) Some of these differences may be due to one value not being adjusted for BW**(3/4). Just not clear.

It is difficult to determine if the results reported in Section 3 of Supplemental Material F come from the code as reproduced in the Appendix or a modification of this code, not shown, that uses the Table F parameter values.

Recommendation (Tier 1): The discrepancies identified above should, at a minimum, be discussed and/or the code/documentation corrected/commented to reflect the values actually used.

IN ANSWERING QUESTION 15, 16 AND 17, ONE MUST ASSUME THE MODEL CODE AS PRESENTED IN THE APPENDIX IS NOT THE MODEL CODE ACTUALLY USED TO PRODUCE FIGURE 5 OF THE REPORT. I ASSUME THE MODEL CODE ACTUALLY USED WAS RUN WITH PARAMETER VALUES ASSIGNED AS INDICATED IN TABLE F1.

Assuming model code was run with Table F1 values, I still question the assignment of a value of 62.1 (mg/h/BW^0.75) as a reasonable value for VMAXC1 for female rat in Table F1, given the values for VmaxC (mg/h/kg**3/4) presented in Table S-4 for female mouse and female rat. If this value is correct it needs to be better explained. I am concerned that a decimal has been misplaced and the value should have been 6.21 which would make it closer to the 6.36 value of Table S-4.

Recommendation (Tier 1): Correct or justify the assignment of a value of 62.1 (mg/h/BW^0.75) as a reasonable value for VMAXC1 for female rat in Table F1.

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.

This is not my area of expertise, but …

I am concerned that the 8-to-9-fold difference of female rat to female mice seen in Figure 4 is a result of the difference in estimated values of VMAXC1 assigned to rats and mice as discussed in my response to Question 14 bullet 4. This 8-to-9-fold difference in VMAXC1 could also be producing the differences observed in Figure 5-C. Before I could conclude that 1-CEO is not a reasonable predictor or chloroprene-induced lung cancer, I would need clarification of the proper value for VMAXC1 for female mice and rats as raised in Question 14 bullet 3.

Recommendation (Tir 3): Address this comment as part of the last recommendation of Question 14.

16. Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and

"concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

This is not my area of expertise. See my comments for Questions 14 and 15.

17. Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

This is not my area of expertise. See my comments for Questions 14 and 15.

III. SPECIFIC OBSERVATIONS

Page	Paragraph	Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)
12	3	First reference to "R" should include the reference citation "R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/."
12	3	First reference to "ACSL" should include a reference citation.
13	4	First reference to "Crystal Ball (ver 11.1.2.3)" should include a reference citation.
13	5	Simply stating "Databases of peer-reviewed literature …" with an e.g. is inadequate these days. The details of the protocol used for the literature search and decisions made on each citation found need to be reported in an appendix. See any of the most recent TSCA chemical scoping documents for how this needs to be done.
13	4	Using uncorrelated random pairs for the lung and liver A1 parameter values in the uncertainty Monte Carlo simulation will produce "slightly broader distribution of A1 than if they were positively correlated" as stated. If the two parameters are highly correlated (say correlations greater than say +.7 or less than -.7) quite "unlikely" pairs could be generated. The correlation should be reported to help the reader judge for themselves whether your approach was reasonable.
14	4	"Crystal Ball Release 11.1.2.3.850 was used to obtain the parameter values …" Crystal Ball actually generates random values for parameters according to the distribution assigned. This needs to be clearer.
14	4	Bounds should be +- 2.5 x the standard error of the estimates when talking about uncertainty and 2.5 x standard deviation when discussing variability. When a parameter is bounded below by zero (or any other value) is the upper bound still the mean + 2.5se? Wouldn't this produce a 97.5% CI instead of the 95% CI intended?
16	Figure 2	Shifting the x axis a little to the right would allow better visualization of the 0 ppm response and range. Caption is inadequate – what do the hash marks represent?
17	Figure 3	I think the "error bars" represent mean +- one standard deviation. Be more specific in caption.

Page	Paragraph	Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)
19	Figure 5	Both graphs are titled "Human Lung". Caption does not help reader discriminate right image from left. Are these two different experiments? Two different y-axis scales?
20	2	First sentence is very long (1/2 of paragraph).
22	Figures 8,9	No Y-axis label (should say something like "Sensitivity")
23	Figure 10	No Y-axis label (should say something like "Sensitivity")
25	Figure 11	Are there units on A1. If so, add to y-axis label.

Kan Shao, Ph.D.
Indiana University

Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Kan Shao, Ph.D.
September 7, 2021

I. GENERAL IMPRESSIONS

My overall evaluation of this revised report is that, with very detailed sensitivity and uncertainty analyses regarding some important model parameters, the reliability of the PBPK model and its associated results has been significantly improved. The comprehensive and informative responses to reviewers' comments together with other supplemental materials prepared by Ramboll have substantially complement the main report regarding its presentation clarity and scientific rigor.

The Ramboll team conducted a robust sensitivity analysis to address how the value of the mass-transfer coefficient "Kgl" may impact the estimated model parameters and associated results, which was one of my critical comments on the previous version of the report. The added sensitivity analysis should be applauded for its scientific credibility. It would be even better if one or two more values of "Kgl" in the range 1~10 or 10~100 are examined and compared for model parameter estimates and other quantities listed in Tables 1 to 4. Additional uncertainty analyses on the PBPK model parameters and associated quantities justify that the soundness of the estimated results.

On the other hand, the clarity of the report still can be improved. For example, brief explanation can be added to the "Kgl Sensitivity Analysis" section on Page 23 to 24 to clarify some of the quantities compared (e.g., "Ratio to KGL = 0.022") and why.

One major change in revised report is that the target tissue dose metrics for the bioassay exposures were used in time-to-tumor modeling of the incidence of lung tumors to calculate benchmark dose and its statistical lower bound (i.e., BMDL) given BMR = 0.01. This process was repeated 5,000 times, i.e., BMD and BMDL were calculated for each iteration. A few issues should be addressed in the report for clarification: (1) what is the purpose to calculate BMD and BMDL? It should be clearly stated. Based on results presented in Table 7 and associated explanation in that section, it seems that the purpose is to use the ratio of BMD/BMDL (about 3) as an indicator to justify that the estimation uncertainty is within a reasonable range. (2) How were the BMD and BMDL in Table 7 calculated? Is that a mean value over the 5,000 iterations? If so, it is more reasonable to calculate the BMD/BMDL ratio in each iteration then present the mean value of the ratio with its lower and upper bound. (3) As mentioned on Page 15, correlation analysis was performed between BMDL01s and PBPK parameters. What is purpose for this analysis? BMDL estimates are much more uncertain than BMD estimates because of the algorithms used in BMDS, so using BMD estimates in the analysis is a more reasonable choice. (All the comments related to BMD/BMDL are Tier 2).

Overall, the quality of the report has been significantly improved. Supported by deliberated sensitivity and uncertainty analyses, the results and conclusions presented by the report are scientifically sound.

II. RESPONSE TO CHARGE QUESTIONS

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

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

The model of the in vitro system initially used for the analysis of the in vitro experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

One of my critical comments on the previous version of the Ramboll report was about the necessity of using the mass-transfer coefficient "Kgl" and its impact on the estimated parameters and risk estimates. The revised report with its supplemental materials has presented a detailed sensitivity analysis indicating that the specific value of Kgl (i.e., 0.22 L/h) has limited impact on the results. Especially, a wide range of possible values of Kgl covered the sensitivity analysis has well demonstrated the reliability and robustness of the sensitivity analysis.

2. The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

I am wondering if this charge question was accurately worded. I think that "resulting difference" is more appropriate than "resulting uncertainty". If the charge question is intended to ask about "resulting uncertainty", then the revised report didn't adequately address the uncertainty caused by the value of "Kgl". To address whether the uncertainty was impacted by the "Kgl" value, the lower and upper bound of the estimated parameters (e.g., Km) should be presented and compared for different values of "Kgl" (this will be Tier 1 if the charge question was formed accurately).

Additional explanation should be provided for clarification. For example, it is not clear how the value in the column "Ration to KGL = 0.022" was calculated or what the values are for. Actually, if it is not much work, I would like to see additional results in Tables 1 to 4 for some Kgl values between 0.88 and 1000. It is a little difficult to identify or determine if there is a clear

trend in the estimated values when limited Kgl values were examined (this could be Tier 3 suggestions).

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

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

3. Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

In my comments to the previous version of the report, I mainly used the lower and upper bound of the estimated parameters to determine if the pool size was adequate and then recommended to use sensitivity analysis to examine the impact of priors on the estimated range of the parameters. Given the response and additional explanation provided by the Ramboll, I think my previous concern has been well addressed.

4. Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

My previous comments on this charge question mainly focused on the reliability of the MCMC sampling results given the specific settings of model parameter priors. My concern was that the priors might be too specific. As clarified in Ramboll's responses to previous review comments, the priors are uninformative and with a wide range. Therefore, I believe that the adequate results generated given such uninformative priors suggest that the data and statistical modeling approach are reasonable.

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

5. Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

This is outside my area of expertise, and I have no comments to add.

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the Model Parameters section of the Ramboll report, with details on scaling factors in Supplemental Material C of Ramboll and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of the Ramboll report. The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of multiple pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance. However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism (Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on predictions of hepatic clearance of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

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

This is outside my area of expertise and I have no comments to add.

7. Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible

ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

This is outside my area of expertise, and I have no comments to add.

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.

This is outside my area of expertise and I have no comments to add.

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

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

This is outside my area of expertise, and I have no comments to add.

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

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

This is outside my area of expertise, and I have no comments to add.

Overall PBPK Model Soundness and Applicability

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

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

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

My comments on the previous version of the report mainly expressed my concern on the reliability of the PBPK model impacted by the validity of some model parameters, e.g., Kgl. In this revised report, the sensitivity analysis focusing on the "Kgl" parameter has well justified that this parameter has limited influence on other parameters of PBPK model, and hence I believe that the reliability of the PBPK model has been improved.

12. Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

My comments here are mainly based on reviewing Ramboll's responses to my previous comments and their responses to some of other reviewers' comments on statistical and modeling methods. I think that Ramboll did an excellent job providing comprehensive responses to address reviewers' comments, especially providing detailed calculation process for clarification.

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

This is outside my area of expertise, and I have no comments to add.

14. Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

This is outside my area of expertise, and I have no comments to add.

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.

This is outside my area of expertise, and I have no comments to add.

16. Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

This is outside my area of expertise, and I have no comments to add.

17. Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

This is outside my area of expertise, and I have no comments to add.

III. SPECIFIC OBSERVATIONS

Page	Paragraph	**Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)**
23,24	Tables 1 & 2	For the three bigger columns, it is better to make the column names consistent and in the same order, e.g., "Female Mouse Lung", "Female Mouse Liver", and "Human Liver"

Jordan Ned Smith, Ph.D.
Pacific Northwest National Laboratory (PNNL)

Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Jordan Ned Smith, Ph.D.
October 25, 2021

I. GENERAL IMPRESSIONS

Ramboll developed a physiologically based pharmacokinetic model (PBPK) for chloroprene. Based on the success of previous PBPK models for risk assessment of methylene chloride and vinyl chloride, Ramboll proposed this model to support chloroprene risk assessment.

In a previous review, I concluded that the chloroprene PBPK model has the potential to be useful for human risk assessment based on its many strengths over conventional risk assessment techniques. Strengths of this approach include utility for integrating pharmacokinetic knowledge and measurements (e.g. metabolism, partition coefficients, etc.) across a variety of sources and physiology values (e.g. ventilation rates, body weights, etc.) to quantitatively predict dose metrics in humans. This model was constructed by experts knowledgeable in the development and application of PBPK models. The model uses a conventional and well-accepted structure, and the most important parameters (metabolism parameters, partition coefficients, and a handful of physiological parameters) are either measured in vitro or are well-established reference values. Measured parameter values provide confidence over those predicted by algorithms or extrapolated from animal models. The model accurately simulates concentrations of chloroprene in blood of mice exposed chloroprene by nose only inhalation reasonably well. The model can be used to integrate uncertainty of sensitive parameters and translate that uncertainty to selected dose metrics of interest for risk assessment.

Previously, I also identified several areas of improvement that would increase confidence in the ability of the chloroprene model to provide accurate predictions of human dose metrics relevant for risk assessment. Three specific concerns included the assumption of CYP2E1 as the primary enzyme responsible for metabolizing chloroprene, uncertainty regarding the mass transfer coefficient Kgl and downstream implications on measured rates of in vitro metabolism, and extending the model to predict the disposition of the ultimate toxicant of chloroprene could provide better predictive capability for human risk assessment.

Ramboll addressed these primary concerns in this follow-up. Ramboll conducted sensitivity and uncertainty analyses with Kgl and quantitatively translated the Kgl uncertainty to model predictions of relevant dose metrics. Ramboll provided additional evidence to support the assumption of CYP2E1 as the primary enzyme and extrapolation based on the substrate marker activity of CYP2E1. Ramboll developed a sub model to evaluate dose metrics of reactive metabolites in lung across two animal models to support dose metric selection.

Overall Ramboll's efforts has improved the model and increased confidence in its ability to support to chloroprene risk assessment in humans.

II. RESPONSE TO CHARGE QUESTIONS

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

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

The model of the in vitro system initially used for the analysis of the in vitro experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

Equilibrium between the air and liquid phases is not instantaneous, and as such, the two-phase in vitro metabolism model seems appropriate for calculating the metabolic parameters. Since uncertainties of Kgl exist and it is not feasible to define experimentally today, the Bayesian approach used by Ramboll to estimate Kgl and uncertainty of Kgl seems appropriate and reasonable.

2. The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

This evaluation estimates the uncertainty of Kgl in the in vitro studies and translates that uncertainty to PBPK model simulations of chloroprene metabolism in the lung of humans, the proposed dose metric for risk assessment. Ramboll demonstrates that the Km for chloroprene metabolism in the lung of mice is moderately sensitive to predicting the relevant dose metric in mice (Figure 9), (~0.2-0.6 depending on the exposure concentration). Human chloroprene clearance in the lung is also sensitive (~1) for predicting the relevant dose metric. These moderate to high sensitivities demonstrate the importance of the Km for chloroprene metabolism in the lung of mice and chloroprene clearance in the human lung are important. Since lung metabolism values (Km and Cl_{int}) depend on Kgl, the estimate of Kgl uncertainty is also important. Kgl uncertainty can be used to estimate the uncertainty of the predicted dose metrics. Ramboll applies appropriate methods to translate the Kgl uncertainty from the in vitro studies to PBPK model simulations and provides the estimated uncertainty in lung metabolism of

chloroprene (Table 7). These values can be used in the risk assessment process to establish a conservative estimate of human risk in the context of Kgl uncertainty.

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

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

Other information on the specific microsomal samples, preparation methods and in vitro experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

3. Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

I have no further concerns regarding the pool size used for metabolism translation. Since these translations are based on substrate marker activities, additional measures of the appropriate substrate marker activities in samples from individuals could provide a measure of inter-individual variability of metabolism in humans. Comparison of reference substrate marker activities measured in Lorenz et al. (1984) to larger sample sizes of individuals could further inform of "representativeness" of the average human adult.

4. Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

I have not further comments.

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

5. Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

The overall concept of Ramboll's approach of using substrate marker activities to extrapolate chloroprene metabolism from liver to lung seems appropriate and preferred over extrapolation approaches based on RNA expression levels. This approach requires proper identification of a dominant enzyme and proper selection the substrate marker to represent the activity of that enzyme for extrapolating metabolism.

In my previous review, assuming CYP2E1 as the relevant enzyme for extrapolation was my top concern with Ramboll's approach, and I recommended that Ramboll experimentally determines which enzymes are responsible for chloroprene metabolism (Tier 1).

Ramboll response: As explained in the Ramboll PBPK report, both CYP2E1 and CYP2F contribute to the metabolism of chloroprene in the mouse and rat. However, in the human, the activity of CYP2F1 is extremely low, so that the metabolic clearance in both liver and lung is dominated by CYP2E1. Therefore, further experimentation is unnecessary.

In the report, Ramboll assumes that CYP2E1 is the primary enzyme responsible for metabolizing chloroprene based on surrogate data. Ramboll conducted a literature search of chloroprene surrogates to identify primary metabolizing enzymes. Among surrogates considered were trichloroethylene (TCE) and butadiene. Ramboll specifically used TCE as a surrogate for chloroprene to identify important enzymes based on "compounds with similar structures" (page 26 paragraph 2). Forkert et al. (2005) identified CYP2E1 and CYP2F as primary enzymes metabolizing TCE at relevant internal concentrations. In Exhibit A Supp Mat C, Ramboll reports CYP2A6 and CYP2E1 are responsible for metabolism of butadiene (Duescher and Elfarra 1994), a more similar compound to chloroprene than TCE. CYP2A6 is present in the lung and liver and could be involved with chloroprene metabolism. Duescher and Elfarra (1994) indicate that CYP2E1 may be driving butadiene metabolism at lower concentrations, and CYP2A6 may be driving metabolism at high concentrations. As such, CYP2E1 is probably more relevant than CYP2A6 for human exposures to butadiene, but does this translate to chloroprene? Ramboll's approach of identifying chemical similar surrogates indirectly implicates CYP2E1; however, it does not provide definitive, direct evidence for this enzyme.

In a preliminary study, Himmelstein et al. (2001) measured metabolism of chloroprene in rat liver microsomes with the with and without the addition of 4-methylpyrazole, an inhibitor of CYP2E1 and alcohol dehydrogenase. Without the inhibitor, Himmelstein et al. observed chloroprene metabolism and, with the addition of the inhibitor, did not observe metabolism (2001). Similarly in lungs of mice, Himmelstein et al. (2004) observed no oxidative chloroprene metabolism when 4-methylpyrazole was included in the incubations. These experiments provide direct evidence that CYP2E1 drives oxidation of chloroprene in liver of rats and lung of mice

and supports Ramboll's use of substrate marker activity of CYP2E1 to translate metabolism from liver to lung.

Tier 1 Key Recommendation: I suggest Ramboll include metabolism data with inhibitors from Himmelstein et al. (2001 and 2004) in their report as direct experimental evidence for the role of CYP2E1 in metabolism of chloroprene.

Ramboll uses 7-ethoxycoumarin activity, as a substrate marker for CYP2E1 activity, to extrapolate liver to lung chloroprene metabolism. In a previous review, I also recommend that a substrate marker activity is then selected based on which enzymes are identified experimentally. If CYP2E1 is indeed verified, I previously offered chlorzoxazone activity as a potentially preferred alternative to 7-ethoxycoumarin activity (Walsky 2004).

Ramboll response: Although chlorzoxazone has been used to assess CYP2E1 activity in drug evaluations, it is also metabolized by CYP3A4, CYP1A2, CYP2A6, CYP2B6, and CYP2D6 (Shimada et al. 1999), so it would not provide a specific marker for CYP2E1.

Ramboll's statement is true, however, at high substrate concentrations (≥100 μM chlorzoxazone), CYP2E1 accounts >93% of chlorzoxazone 6-hydroxylatio and increases with higher chlorzoxazone concentrations (Yamamura et al. 2015). Furthermore, 7-ethoxycoumarin O-deethylation is also associated with several CYPs other than CYP2E1 like CYP1A1, CYP1A2, CYP1B1, CYP2A6, and CYP2B6 (Shimada et al. 1999). So, specificity of 7-ethoxycoumarin O-deethylation to CYP2E1 is just as questionable as chlorzoxazone 6-hydroxylation. Inoue et al. (2000) showed chlorzoxazone 6-hydroxylation had a higher correlation to CYP2E1 levels than 7-ethoxycoumarin O-deethylation in the same human liver microsome samples (r=0.93 vs. 0.84, respectively), suggesting chlorzoxazone 6-hydroxylation may be a superior substrate marker. However, I can appreciate that 7-ethoxycoumarin O-deethylation measurements in multiple tissues of various species (Lorzen et al. 1984) can facilitate extrahepatic extrapolation of metabolism and has done so in previous modeling efforts. Similar cross-tissue and -species comparisons using chlorzoxazone 6-hydroxylation appear to be limited according to Ramboll's literature search.

Himmelstein MW, Carpenter SC, Hinderliter PM, Snow TA, Valentine R. The metabolism of beta-chloroprene: preliminary in-vitro studies using liver microsomes. Chem Biol Interact. 2001 Jun 1;135-136:267-84.

Walsky RL, Obach RS. Validated assays for human cytochrome P450 activities. Drug Metab Dispos. 2004 Jun;32(6):647-60.

Yamamura Y, Koyama N, Umehara K. Comprehensive kinetic analysis and influence of reaction components for chlorzoxazone 6-hydroxylation in human liver microsomes with CYP antibodies. Xenobiotica. 2015 Apr;45(4):353-60.

Inoue K, Yamazaki H, Shimada T. Characterization of liver microsomal 7-ethoxycoumarin O-deethylation and chlorzoxazone 6-hydroxylation activities in Japanese and Caucasian subjects genotyped for CYP2E1 gene. Arch Toxicol. 2000 Sep;74(7):372-8.

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

I have not further comments.

7. Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

Possible uncertainty and population variability of all sensitive parameters should be evaluated in the risk assessment phase. For example, amount of microsome protein per gram of tissue could be important in the IVIVE process. However, researchers have demonstrated that technician and lab-to-lab variability dominate the observed uncertainty of this parameter. Ramboll describes evaluations of this parameter in Exhibit A Supp Mat C. My previous experiences in evaluating amount of microsome protein per gram of tissue for IVIVE agree with their assessment.

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.

The model structure and dose metric selected by Ramboll is appropriate. In a previous review, I recommended (Tier 3) that Ramboll create a model to account for dosimetry of the ultimate toxicant for more accurate risk assessment predictions. Ramboll created this model and accounted for the disposition of other reactive metabolites as well.

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

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

I have no further comments.

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

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

Consistent with many PBPK models, Ramboll cites most physiological parameters from a standard source (Brown et al. 1997). Ramboll uses human physiological parameters that are mostly male as calculated in the 1975 Reference Man but includes mean male and female adipose volumes for the fat volume parameter. Previously, I suggested that male and female physiological parameters should be implemented independently to ensure that physiologies of both sexes are adequately considered (Tier 1).

Ramboll response: Concerns regarding potential sensitive human populations, including the effect of gender, is part of the application of the model for a specific risk assessment application, which USEPA will undertake if they accept the model. The physiological and metabolic structure of the PBPK model provides the necessary framework for conducting such investigations, and appropriate parameters are available in the literature (Clewell et al. 2004, Mallick et al. 2020).

I agree with Ramboll that potentially sensitive human populations could be evaluated later at the risk assessment stage. However, extrapolating to a standardized human model that contains mostly male parameters with a bend of male and female fat volume seems a bit imprecise, especially considering that fat acts as a depot for chloroprene (partition coefficient fat:blood is 28.65 in humans), although this parameter was not identified as sensitive. Using physiological parameters from male, female, or both independently at this stage could offer a more realistic and useful parametrization of the model. ICRP (2002) offers updated physiological parameters across various life-stages by sex.

Tier 2 recommendation: I suggest that physiologies from male, female, or both independently at this stage could offer a more realistic and useful parametrization of the model.

ICRP, 2002. Basic Anatomical and Physiological Data for Use in Radiological Protection Reference Values. ICRP Publication 89. Ann. ICRP. 32.

Overall PBPK Model Soundness and Applicability

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

Demonstration of the PBPK model's ability to predict in vivo PK data is shown by the level of agreement between model predictions and chloroprene venous blood concentrations in Figure 7 of the Ramboll report. For reference, where there are data, and as a rule of thumb, EPA often seeks dosimetric estimates from a model that are within a factor of two of empirical

results. The results of the sensitivity analysis shown in Figure 8 for arterial concentrations indicate that these data and specific predictions are not sensitive to the estimated metabolic parameters: a relatively large range in the estimated metabolic parameters (such as the apparent difference between male and female mouse parameters) would yield similar predictions of blood concentrations. However, as demonstrated in Figure 9, the estimation of lung dose metrics is sensitive to the estimated metabolic parameters.

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

The chloroprene PBPK model has the potential to be useful for human risk assessment. Sensitivity analyses identified metabolism parameters, partition coefficients, and a handful of physiological parameters (e.g. ventilation rates, blood flow to liver, cardiac output) as the most important parameters for determining chloroprene internal dosimetry. Most of these parameters have been measured using in vitro assays providing confidence in the parameter values. Mouse to human extrapolation of lung metabolism is based 7-ethoxycoumarin activity assuming that CYP2E1 is the primary enzyme responsible for metabolizing chloroprene. This assumption needs additional evidence (e.g. identification of enzymes involved with metabolism, assess potential of enzyme induction) to support it (see Question 5). The model predicts concentrations in blood from 13 ppm inhalation exposures to mice reasonably well, but overpredicts higher exposures (32-90 ppm) by ~2-fold. Ramboll translates uncertainty of Kgl to simulated dose metrics and calculates a ~4-fold variation in the 95% confidence interval in lung dose metrics. Which, if assumed to translate proportionally to blood, accounts for the observed ~2-fold overprediction in chloroprene concentrations in blood of mice. No chloroprene concentration data in tissues was presented. The model was used to extrapolate human exposures of 0.0003 ppm (1 $\mu g/m^3$) or 4.5 orders of magnitude lower than exposures used with mice. This magnitude of differences in exposure creates some uncertainty. Due to integration of many measured aspects of chloroprene pharmacokinetics (e.g. metabolism, portioning, etc.) and physiology (e.g. ventilation rates, body weights, etc.) into a model capability of extrapolating dosimetry across species, and quantitatively integrating uncertainty, this model offers an improved risk assessment tool compared to traditional standardized uncertainty factors.

12. Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

See my responses to Questions 5 and 10.

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

Like Dr. Yang, I also suggested modeling the disposition of the bioactivated metabolite (Tier 3). As such, I am pleased that Ramboll has undertaken this task. The biochemical processes, assumptions, and mathematical descriptions of those processes appear appropriate to my knowledge. One question that comes to mind is if the rate of GSH formation would increase with greater rates of GSH depletion as a compensatory mechanism rather than assumption of being constant regardless of GSH depletion status?

14. Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

Parameter values appear accurate and to exhibit quality. Parameters are reported or derived from published, peer-reviewed studies. One question I have is should the in vitro parameters associated with 1-CEO be re-evaluated in the context of mass transfer limitations (e.g. Kgl) similar to chloroprene metabolism parameters?

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.

Figure F5 shows the relationships of cancer incidence as a function of 3 different predicted dose metrics: total amount of chloroprene metabolized, the concentration of total reactive products, and 1-CEO concentration in female mice and rats. Predicted 1-CEO concentration shows little if any predictive value compared to the other two predicted dose metrics evaluated across animal models. As such, I agree that these simulations provide evidence that precludes 1-CEO as a sole predictor of chloroprene-induced lung cancer risk.

16. Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

There appears to be a consistent relationship between predicted dose metrics and cancer incidence across females of two species that follows a conventional sigmoidal dose response. This suggests that it is plausible that these dose metrics are "consistent inter-species predictors". Additional data from male mice or rats, other species, and/or data from species at a consistent

dose metric (e.g. overlapping x-values) would certainly increase confidence in the assertion that this relationship is real and not just coincidence.

17. Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

These results provide additional support the using the amount of chloroprene metabolized in the liver is an appropriate dose metric for risk assessment. Although somewhat limited in data, the demonstrated relationship appears predictive, and the proposed mode of action makes sense and is consistent with other similar chemicals. Further data to validate these model predictions and/or additional cancer incidence data in male mice or rats, other species, and/or data from species at a consistent dose metric (e.g. overlapping x-values) could further strengthen the evidence for using this dose metric.

III. SPECIFIC OBSERVATIONS

Page	Paragraph	Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)
		The reviewer provided no specific observations/comments.

Raymond S. H. Yang, Ph.D.
Colorado State University; Ray Yang Consulting, LLC

Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Raymond S. H. Yang, Ph.D.
September 12, 2021

I. GENERAL IMPRESSIONS

Once again, I wish to emphasize that the researchers involved in all the relevant studies to this Project, starting from the early 2000 until the present days, have all been reputable scientists from good laboratories and institutions in the fields of Toxicology and Risk Assessment. Their publications all appeared in top-notch peer-reviewed toxicology journals. The quality of their work was good and the studies were well planned and executed. Given the above, there is no reason to question the accuracy of the information presented. The clarity of presentation is good, and the conclusions, given the stated purposes and with the exceptions of the issues discussed below, are, in general, scientifically reasonable.

For this review, first of all, I wish to thank Ramboll colleagues for their responding to the 2020 peer review comments; in particular, my suggested assessment of the overall metabolism, the related simulations, and considerations of incorporating metabolic processes beyond the formation of epoxides (e.g., Exhibit A Supp Mat F and G). This revised report had somewhat strengthened their arguments, and consequently helped with their specific approach in using PBPK modeling for proposed update on chloroprene (CP) cancer risk assessment. However, while I appreciate very much their expertise on PBPK modeling and am somewhat willing to go along with their demonstrated conclusion that the metabolism beyond epoxidation might not be as important in their proposed cancer risk assessment approach, I still have reservations. Furthermore, there are clearly many remaining uncertainties with their proposed update in the latest version of the report (Ramboll, 2021). Given below in responding to some of the Charge Questions, I will present these uncertainties and the respective discussions.

With all due respect, my initial impression, in the October 2020 assessment, that the Ramboll colleagues seemed to be rather dogmatic to apply their approach which was used successfully for methylene chloride (and for vinyl chloride) risk assessment. In their insistence of using 7-ethoxycoumarin as a surrogate for estimating CP in vitro metabolic transformations, possible utilizations of CP metabolic data and/or data from a close analog, 1,3-butadiene, in the literature were dismissed. Unfortunately, this impression persisted in this peer-review. Please understand this is not a case of "my opinion is more important than yours"; rather, I am too worried about such a reactive and potent carcinogen as CP to be given relaxation on its risk assessment based on science with many uncertainties. Accordingly, under some of the Charge Questions, I have specifically recommended Tier 1 experimental and simulation work for a re-estimation of "dose metric" using new data on CP metabolism, as well as a comparison to the present Ramboll approach (Ramboll, 2021). Such a reference point will benefit Ramboll colleagues for their approach if the comparison turns out to prove the Ramboll (2021) estimated internal doses were right and us reviewers were wrong. It would also benefit the overall toxicology and risk assessment community to have a time-tested state-of-the-science approach.

II. RESPONSE TO CHARGE QUESTIONS

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

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

The model of the in vitro system initially used for the analysis of the in vitro experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

I have no further comments on this.

2. The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

I have no further comments on this.

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

The following questions address the robustness of the available metabolic data for application in the model. The questions are written with the assumption that the choice of Kgl is appropriate. Using this value of Kgl while evaluating the remaining analysis of in vitro metabolic data as described in Supplemental Material B of the Ramboll results in parameter values listed in Table S-3 of Supplemental Material A of the Ramboll report. For the chloroprene in vitro experiments, the human liver microsome samples were obtained from a pool of 15 donors while the human lung microsomes were obtained from a pool of 5 individuals (Himmelstein et al., 2004). For the 7-ethoxycoumarin in vitro experiments used to estimate the relative lung:liver metabolic activity, represented by the parameter A1, tissue

samples were not pooled; activity was measured in liver microsomes obtained from 10 donors while the human lung activity was measured using microsomes from 12 donors (Lorenz et al., 1984).

Other information on the specific microsomal samples, preparation methods and in vitro experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

3. Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

If 7-ethoxycoumarin is continued to be used as a surrogate for CP in this resubmission and beyond, an important question is: Do we have a sufficiently large N to have a decent probability distribution? If so, the Monte Carlo simulation technique advanced by Portier and Kaplan (1989) could be used to create "synthetic samples" from repeated sampling from the probability distribution to enlarge the N to a large number of samples (e.g., 1000) to obtain decent statistical analyses.

References:

Portier CJ and Kaplan NL (1989) Variability of safe dose estimates when using complicated models of the carcinogenic process a case study: methylene chloride. *Fundam. Appl. Toxicol.* 13:533-544.

4. Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

See my comments under Charge Questions 3, 5

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

5. Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

Starting from the meetings in October 2020, I have questioned the validity of using 7-ethoxycoumarin as a surrogate for CP to derive the metabolic rate constants of CP in the human being. One principal reason was the drastically different physico-chemical properties between the two chemicals. My position has not changed.

Throughout the deliberations in the October 2020 meetings to the discussions in the Ramboll (2021) Report, Supplemental Material C and the specific section on "IVIVE for first order

metabolic clearance in rat and human lung" included, the root of the problem appeared to be lack of human metabolism data, particularly human lung enzymatic transformation data. Because of this lack of human data, many metabolic parameters of CP in the PBPK modeling had to be based on assumptions which created more uncertainties persisted to this day. Thus, it seems to me that the best thing to do is to fill in the gaps of the missing human metabolic data not only to minimize the uncertainties, but to strengthen the sample size issues as well. Yes, I understood that the technical challenges to conduct such studies with CP are substantial and that the du Pont Haskell Laboratory where Dr. Himmelstein conducted his studies for Himmelstein et al. (2004) paper is no longer available. Has Denka and Ramboll contacted the UK folks in Syngenta Central Toxicology Laboratory and University of New Castle where the work in the Cottrell et al. (2001) and Munter et al. (2003; 2007) papers were done? If these two groups of scientists could do the experiments, there should be other laboratories in the world with equivalent expertise and facilities to do such work (Tier 1 recommendation). As I stated before, such a reference point will benefit Ramboll colleagues for their approach if the comparison turns out to prove the Ramboll (2021) estimated internal doses were right and us reviewers were wrong. It would also benefit the overall toxicology and risk assessment community to have a time-tested state-of-the-science approach.

In the meantime, I would also like to propose the following simple exercise to specifically compare the differences on Vmax and Km between using actual CP metabolism data from Munter et al. (2003) which were from direct enzymatic and instrumental analyses vs. what Himmelstein et al. (2004) found out which were from PBPK modeling optimized gas uptake enzymatic studies. Specifically, I recommend (Tier 1 recommendation) that Ramboll colleagues calculate Vmax and Km using enzymatic formation of 3a,b (i.e., 1-chloroethenyl oxirane) data in Table 1 [page 1296, Munter et al. (2003)]. These metabolic constants are then compared with the equivalent constants in Table 3 (page 23) of what Himmelstein et al. (2004) produced. This way we could get an idea what differences are there between two excellent groups of experimental scientists produced, using two approaches, on "total" metabolism of CP in rat, mouse, and human. This comparison will also afford us, at the very least, a ballpark idea whether there is/are major problems with the present Ramboll (2021) approach. Yes, I am aware of the fact that the Munter et al. (2003) work involved the use of acetonitrile as a solvent and, yes, I am also aware of the fact that production of 3a,b is not "total metabolism." It doesn't matter here because I am trying to avoid "major errors."

One additional comment related to the reading of the section on "IVIVE for first order metabolic clearance in rat and human lung" in Supplemental Material C. I found the discussion difficult to understand. For instance, CYP2E1 is a "high affinity, low capacity" enzyme (Andersen et al., 1987); thus, its Km should be very small. When the discussion got to the range of substrate concentrations below Km (no page number, opening page on this section, 2nd paragraph), we should be dealing with first order process, not Michalis-Menten kinetics. Therefore, there will be no Vmax and Km anymore, only a first order rate constant.

References:

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

Cottrell L, Golding BT, Munter T, Watson WP (2001) In vitro metabolism of chloroprene: Species differences, epoxide stereochemistry and a de-chlorination pathway. *Chem. Res. Toxicol.* 14:1552-1562.

Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004). Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. *Toxicol Sci* 79: 18-27. http://dx.doi.org/10.1093/toxsci/kfh092

Munter T, Cottrell L, Golding BT, Watson WP (2003) Detoxication pathways involving glutathione and epoxide hydrolase in the in vitro metabolism of chloroprene. *Chem. Res. Toxicol.* 16:1287-1297.

Munter T, Cottrell L, Ghai R, Golding BT, Watson WP (2007) The metabolism and molecular toxicology of chloroprene, *Chem Biol Int* 166:323-331.

Ramboll (2021) *Incorporation Of In Vitro Metabolism Data In A Physiologically Based Pharmacokinetic (PBPK) Model For Chloroprene- Revised Documentation In Response To USEPA Peer Review.*

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the Model Parameters section of the Ramboll report, with details on scaling factors in Supplemental Material C of Ramboll and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of the Ramboll report. The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of multiple pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance. However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism (Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on predictions of hepatic clearance of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

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

See my comments under Charge Question 5

7. Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE

calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

I have the following two concerns:

1) In page 24, under the section of "*Oxidative Metabolism of CD*" in Himmelstein et al. (2004), "...a maximum difference of about two-fold greater in the mouse than the human..." was stated, why are the "dose metrics" (i.e., internal dose calculated) in Table 6 on page 27 in the Ramboll (2021) to be approximately 46X or 30X different between the mouse and human at 80 ppm for mouse vs. 100 ppm for human or 12.8 ppm for mouse vs. 10 ppm for human, respectively? Yes, I know that Himmelstein et al. was talking about liver microsomes when they made the statement but isn't liver the principal organ of metabolism and, therefore, responsible for a major share of the "total metabolism"?
2) In their *"Conclusions"* on page 26 of Himmelstein et al. (2004), the statement "...the most dramatic of which was a faster rate of CD metabolism in the mouse lung compared with the other species..." Is this one principal reason why mouse lung tumors were chosen to do risk assessment in comparison to human to emphasize the vast difference between the two species?

References:

Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004). Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. *Toxicol Sci* 79: 18-27. http://dx.doi.org/10.1093/toxsci/kfh092

Ramboll (2021) *Incorporation Of In Vitro Metabolism Data In A Physiologically Based Pharmacokinetic (PBPK) Model For Chloroprene- Revised Documentation In Response To USEPA Peer Review.*

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.

The lead scientists working on this project from Ramboll are top-notch people; they are also the pioneers in the PBPK modeling field. Thus, there is no reason for me to question their PBPK model structure, code, and other simulation details on their PBPK modeling. This comment here is primarily to focus on the probable involvement of the parent compound, CP, in the carcinogenesis process, given the multiple tumor sites observed in experimental animal studies. Comments are also provided on possible impact on the PBPK modeling, the estimation of "dose metric," as well as the influence on the risk assessment of CP when the parent compound, CP, a direct-acting carcinogen is also part of the internal dose. Related to this, some specific concerns regarding complex, non-linear models and on Bayesian population modeling and Markov Chain Monte Carlo (MCMC) simulation are provided.

First, uncertainties related to the wide-spread carcinogenicity of CP in experimental animals:

NTP Technical Report (TR) 467 (NTP, 1998) reported that, Fischer 344 rats, when exposed to CP at 0, 12.8, 32, or 80 ppm via inhalation for two years, produced oral cavity squamous cell papillomas and carcinomas; thyroid gland follicular cell adenomas and carcinomas; alveolar and bronchiolar adenomas and carcinomas; mammary gland fibroadenomas; renal tubule adenomas and carcinomas; and urinary bladder transitional epithelium papillomas and carcinomas. Similar studies in B6C3F1 Mice (NTP, 1998) produced alveolar and bronchiolar adenomas and carcinomas; harderian gland adenomas and carcinomas; mammary gland adenoacanthomas and carcinomas; hepatocellular adenomas and carcinomas; skin and mesentery sarcomas; forestomach squamous cell papillomas; Zymbal's gland carcinomas; renal tubule adenomas and others. That multiple tumors occurred in these animals away from the lung, the portal of entry of CP, have the following toxicokinetic implications:

a) In NTP TR 467 (NTP, 1998), Dr. Ronald Melnick, the lead Study Scientist, had provided rather detailed discussion related to the potential of CP, itself, being a direct-acting carcinogen. Regarding the contradictory mutagenic activities of CP reported in the literature, Dr. Melnick indicated "...Clearly, in vivo and in vitro genotoxicity data were not predictive of the potent multisite carcinogenic effects of chloroprene. These results reveal the in-adequacy of relying on oversimplified operational classification systems, such as genotoxic versus non-genotoxic, in regard to cancer risk rather than focusing on increasing the understanding of causal relationships between exposure and cancer outcome..." [page 97, column 1, NTP TR467 (NTP, 1998)]. Concerning the then unclear metabolic transformations of CP and the possible roles of suspected reactive metabolites, Dr. Melnick stated "...These postulated oxidative intermediates of chloroprene metabolism may be protein and/or DNA reactive and may account for the cytotoxicity and carcinogenic effects of this compound. Differences in stability, distribution, and reactivity of these various intermediates may account for differences in dose-related carcinogenic effects of chloroprene and 1,3-butadiene. Further studies are needed to understand the processes involved in chloroprene carcinogenesis..." [page 97, column 2, NTP TR467 (NTP, 1998)]. The latter point regarding "stability, distribution, and reactivity" would certainly be true for the parent compound, CP, as well. It should be noted that throughout our discussions in the two-day virtual meeting organized by Versar on October 5 and 6, 2020, Dr. Clewell, the lead scientist of Ramboll had repeatedly emphasized the technical difficulties of conducting experimental work in vivo and in vitro on CP because of its reactivity. Such difficulties were also published as a cautionary note in the methodological section of the papers by Cottrell et al. (2001) and Munter et al. (2003). Therefore, the contradictory mutagenicity results reported in the literature might indeed reflect the experimental difficulties those investigators encountered rather than representing the true mutagenicity potentials in those experimental systems.

   CP, although highly reactive, is apparently stable enough to circulate in the body of experimental animals for a significant period of time (e.g., at least up to 6 hrs) as shown in Figures 3 and 7 of the Ramboll Report (Ramboll, 2021); these data were from Ramboll's own previously unpublished study. This being the case then CP, itself, could very well be an important adduct-forming reactive species for the chemical carcinogenesis. Given the above discussion by Dr. Melnick in the NTP TR 467 (NTP, 1998), the tumor formation at multiple sites in experimental animals, and the common scientific sense regarding CP's "stability,

distribution, and reactivity," it is highly likely that CP is a direct-acting carcinogen. Thus, the formation of further reactive species through metabolism of CP is not necessarily the only process leading to carcinogenesis as suggested by the Ramboll colleagues (Ramboll, 2021).

b) In addition to the evidence discussed above for the formation of multiple tumor sites away from the portal of entry, other potential indicators supporting CP's own role in adduct-formation leading to carcinogenesis might be:

(i) From the perspective of PBPK modeling, the present Ramboll model "over-predict" the blood CP levels comparing to their experimental data [Figure 7, Ramboll (2021)]. I would recommend the Ramboll colleagues to consider CP, itself, as a part of the internal dose and incorporate an adduct-formation rate constant in the following compartments of the PBPK model: lung, slowly perfused, rapidly perfused, liver, and kidney, based on the multiple tumor sites reported in NTP TR467 (NTP, 1998) as quoted above. This would certainly render CP less available in the blood stream; thus, the end results of such an incorporation into the PBPK model would have been a better fit of the simulation curves with the experimental data. Regarding "model reduction" (see discussion below under Item 2) then, the Ramboll colleagues might consider only the two-component internal dose of the parent compound CP plus "the total dose metabolized" in their PBPK modeling. In that case, the "dose metric" calculations as presented on page 27 of the Report (Ramboll, 2021) would have been different (Tier 1 recommendation). Indeed, DNA adduct formation may be identified by using post-labeling and other techniques (Randerath et al. 1985; Stiborova et al., 1998; Munter et al. 2007; Balbo et al. 2014). I would recommend (Tier 1) Ramboll/Denka colleagues at least conduct a CP DNA covalent binding study using double stranded calf thymus DNA and identify the DNA adduct of CP as described by Munter et al. (2007) on page 329, column 2.

(ii) The incorporation of vinyl chloride along with methylene chloride in their discussion in this updated report (Ramboll, 2021) was a good touch; this, however, brings in the issue of chemical reactivity, distribution, and stability in biological system in relation to their respective potencies of carcinogenicities for chemicals similar to CP such as vinyl chloride, 1,3-butadiene. If one looks at the dose levels of these chemicals in chronic and carcinogenicity studies (EPA, 2011; IARC, 2018; NTP, 1984; 1998) and their respective tumor incidences in the various tissues, one would likely reach the conclusion that CP, 1,3-butadiene are in the same class of potent carcinogens; vinyl chloride, because of the formation of a rare tumor, angiosarcoma, is also in this potent carcinogen category. Along this line of discussion, methylene chloride, not a direct-acting carcinogen, is a totally different and a milder beast.

Second, uncertainties related to complex and non-linear models:

PBPK models are complex, non-linear models with many parameters. While Ramboll colleagues are highly respected scientists and their effort to thoroughly assess the metabolism of CP (e.g., Exhibit A Supp Mat F and others) is highly appreciated, this process did introduce more parameters (Table F1) into the already complex PBPK model; many of these parameters are without experimental data. Thus, the inherent uncertainty multiplies, probably exponentially. My nervousness on this stems from the reading of a recent perspective paper (Transtrum et al., 2015). In particular, the exchanges between Fermi and Dyson as well as the statement quoted on Von Neumann in the opening paragraph on "Parameter Indeterminacy and Sloppiness" really

worries me because here we are talking about "genius" level people who were participants on the "Manhattan Project." Further, in their discussion on "Model Reduction," specifically on the case of Markov Chain Monte Carlo (MCMC) sampling of a Bayesian posterior" the issue of "Evaporated parameter" also worried me a lot. Many people, myself included, are impressed by Bayesian approach of PBPK modeling utilizing MCMC analyses because we are mostly ignorant in the finer details of such technologies. However, when we use such technologies to justify the relaxation of risk assessment on a very reactive chemical carcinogen such as CP which might have very significantly negative impact on people, particularly those without money, lawyers, we must be very, very careful. Since the Ramboll Report (Ramboll, 2021) repeatedly emphasized using "the best available science," therefore, I would urge Ramboll colleagues to study the Transtrum et al. (2015) paper, if you haven't already done so, and examining carefully if any of such shortcomings mentioned in the paper, existed in your PBPK modeling and analyses. (Tier 1 recommendation)

References:

Balbo S, Turesky RJ, Villalta PW (2014) DNA adductomics, *Chem Res Toxicol* 27:356-366.

Cottrell L, Golding BT, Munter T, Watson WP (2001) In vitro metabolism of chloroprene: Species differences, epoxide stereochemistry and a de-chlorination pathway. *Chem. Res. Toxicol.* 14:1552-1562.

EPA (2011) *Toxicological Review of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2). In Support of Summary Information on the Integrated Risk Information System (IRIS) November 2011.*

IARC (2018) *IARC Monograph on Vinyl Chloride.*

Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004). Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. *Toxicol Sci* 79: 18-27. http://dx.doi.org/10.1093/toxsci/kfh092

Munter T, Cottrell L, Golding BT, Watson WP (2003) Detoxication pathways involving glutathione and epoxide hydrolase in the in vitro metabolism of chloroprene. *Chem. Res. Toxicol.* 16:1287-1297.

Munter T, Cottrell L, Ghai R, Golding BT, Watson WP (2007) The metabolism and molecular toxicology of chloroprene, *Chem Biol Int* 166:323-331.

NTP (1984) *NTP Technical Report on the Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS NO. 106-99-0) In B6C3F1 Mice (Inhalation Studies). TR288.*

NTP (1998) *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR467*

Ramboll (2021) *Incorporation Of In Vitro Metabolism Data In A Physiologically Based Pharmacokinetic (PBPK) Model For Chloroprene- Revised Documentation In Response To USEPA Peer Review.*

Randerath K, Randerath E, Agrawal HP, Gupta RC, Schurdak ME, Reddy MV (1985) Postlabeling methods for carcinogen-DNA adduct analysis, *Environ Health Perspect* 62:57-65.

Stiborova M, Freib E, Bielerb CA, Schmeiserb HH (1998) ^{32}P-Postlabelling: a sensitive technique for the detection of DNA adducts, *Chem Listy* 92:661-668.

Transtrum MK, Machta BB, Brown KS, Daniels BC, Myers CR, and Sethna JP (2015) Perspectives: Sloppiness and emergent theories in physics, biology, and beyond. J. Chem. Phys. 143:1-13.

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

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

See my comments under Charge Question 8

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

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

No further comments other than those under Charge Question 8

Overall PBPK Model Soundness and Applicability

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

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

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

See comments under Charge Question 8

12. Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

I wish that the Ramboll colleagues were a little more receptive to my suggested utilization of the data from the UK scientists [i.e., Cottrell et al. (2001) and Munter et al. (2003)]. Even though the use of acetonitrile as a solvent is a concern, the exercise I outlined under Charge Question 5 might be helpful. In many ways, we, the reviewers, are just trying to help; we are not your adversaries.

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

Once again, I appreciated greatly the effort of Ramboll colleagues provided detailed discussions on the various enzymatic transformation, as well as the roles of GSH in the overall metabolism; it was educational for me. I also appreciated very much the added model extension and the related PBPK modeling. See my other comments under earlier Charge Questions.

14. Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

The quality and accuracy of Ramboll colleagues' work is unquestionably top-notch. See my other comments under earlier Charge Questions.

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.

I have no problems with using the "total amount metabolized" as a dose metric because we really don't know much, particularly quantitatively, about the metabolic processes beyond the first step oxidative transformation of CP by CYP enzymes. However, as I discussed in detail under Charge Question 8, the probability of CP, itself, being a direct-acting carcinogen cannot and should not be overlooked.

16. Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

See my comments under Charge Questions 7 and 8

17. Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

Because of the "highlight" by Himmelstein et al. (2004) "...the most dramatic of which was a faster rate of CD metabolism in the mouse lung compared with the other species..." (see more detailed discussion under Charge Question 7), I would be worried about placing too much stock in the mouse lung data to refute anything.

III. SPECIFIC OBSERVATIONS

Page	Paragraph	Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)
		The reviewer provided no specific observations/comments.

Yiliang Zhu, Ph.D.
University of New Mexico School of Medicine

Follow up - External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene and a Supplemental Analysis of Metabolite Clearance

Yiliang Zhu, Ph.D.
September 13, 2021

I. GENERAL IMPRESSIONS

The revised report (Exhibit A – Revised Chloroprene PBPK Model Documentation following EPA Peer Review_07122021 and supplement materials) provides adequate details on the revision and re-evaluation of the PBPK model for chloroprene, including MCMC analysis for PBPK parameter uncertainties. The improvements over the 2020 report are visible. There remains issues on clarity and completeness of the presentation. In particular, a lack of technical details and appropriate citations hampered the overall quality of the presentation. The authors appeared to have assumed readers all have intimate knowledge of kinetics and PBPK models. Throughout the report, the Michealis-Menten kinetics was repeated assumed without any description of or reference to the mathematical form. Similarly, a mass transportation kinetics (with transfer coefficient Kgl) was introduced to describe the impact of air:liquid interface on volatile compounds such as chloroprene; but the kinetic form was not given in the main report or supplemental material. The reader needs to go back to the 2020 version of the report (Supplement: Uncertainty Analysis of In Vitro Metabolic Parameters and of In Vitro to In Vivo Extrapolation (IVIVE) Used in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene), EPA_HQ-ORD-2020_0181-0003) to find appropriate description or references. Such deficiency is not uncommon throughout this report. If this report is intended for researchers and stakeholders who are not PBPK experts only, further polish of the report seems necessary to make it more self-contained with more adequate background and technical details.

It is unclear why Supp Mat B was included in the review dossier. The inclusion of Supp Mat B may have raised impression of conflict of interest. Denka Performance Elastomer LLC is at the center of the environmental health concern of communities near Denka's Louisiana facility. EPA's charge questions do not concern epidemiological evidence.

II. RESPONSE TO CHARGE QUESTIONS

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

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

The model of the in vitro system initially used for the analysis of the in vitro experiments to estimate the corresponding metabolic parameters (Yang et al., 2012; Himmelstein et al., 2004) assumed that the chloroprene in the air and liquid (incubation medium) phases was always at equilibrium, i.e., concentration in the medium was set equal to the concentration in the air times the equilibrium partition coefficient (CM = CA*P). At EPA's suggestion, the model was changed to explicitly describe separate air and liquid media compartments, with a mass-transfer coefficient (Kgl) limiting the rate of distribution between them, as described by Kreuzer et al. (1991) and others, and the authors selected a specific value (0.22 L/h) as the best

estimate. Ramboll also performed a Bayesian analysis which incorporates uncertainty in the value of Kgl, together with the metabolic parameters being estimated.

1. Please evaluate the quality of the revised analysis and estimation of metabolic parameters using the two-phase in-vitro metabolism model.

(**Tier 1**): The revised analysis for estimating the metabolic parameters is acceptable, and the joint MCMC estimation of Vmax, Km, and Kgl using male mouse liver data is informative and a correct step in the right direction. A few technical issues remain to be clarified and corrected.

In Supp Mat B (page 6, last paragraph) the authors stated:

"Therefore, we conducted a re-analysis of the data on metabolism in the male mouse liver to simultaneously estimate Vmax, Km and Kgl …"

Also in Supp Mat B (page 9 in the paragraph following equation 1) the authors stated:

"The flux of chloroprene between air and media (Kgl) was estimated by fixing the Km in the male mouse liver microsomal study to 1.0 μmol/L and estimating both Vmax and Kgl."

These two statements were inconsistent and confusing. The latter indicated the estimation of Kgl and Vmax were based on a fixed Km, not simultaneous. Clarification is needed.

Ramboll's re-analysis reported "best" estimate of Kgl=0.22L/hr. It is unclear if it was the posterior mode. The statement (Supp Mat B p9 2nd paragraph following equation 1)

"The geometric mean of Kgl was retained as a fixed value for the analysis of all the *in vitro* studies including the male mouse liver which was re-analyzed to estimate Vmax and Km after the Kgl was fixed"

Suggested that it was mean. The footnotes of Figure B-5 also suggested the same. The posterior mode for ln(Kgl) was about -1.88 (Figure B-4), giving Kgl=exp(-1.88)=0.15. Under MCMC framework, it is crucial to use posterior mode as the best estimate especially when the posterior distributions are skewed, as likely the case seen for Km and Kgl (Figure B-4). It is strongly recommended that posterior modes reported and used as the estimate of the metabolism parameters. Note that only when the posterior distributions are symmetric, posterior mode and mean would be comparable. Therefore, Ramboll should examine the posterior distributions carefully.

In simultaneous estimation, posterior modes for Vmax and Km should be reported in conjunction with that of Kgl, in conjunction with a highest posterior density or highest credibility region/interval, the counterpart of a confidence interval in the Frequentist approach.

2. The Ramboll report demonstrates that estimates of the metabolic parameter Km depend on the value of Kgl but evaluated the impact of the resulting uncertainty in the metabolic parameters on predicted dosimetry in mice and humans, in particular estimates of human lung cancer risk. The revised analysis investigates a wide range of values for the mass transfer

coefficient, Kgl. Please discuss whether this evaluation adequately addresses uncertainties regarding the parameter Kgl.

(**Tier 1**): The current report contains an extensive discussion on the mass transfer coefficient Kgl, including details on estimation of Kgl. Importantly, Ramboll had the opportunity to apply the MCMC analysis on male mouse liver to other tissue/sex/species to obtain a range of Kgl estimates, therefore gaining valuable insight on the variabilities/uncertainties of Kgl. Instead Ramboll used the value Kgl=0.22 obtained from male mouse liver for all analyses on other tissue/sex/species. To a lesser extent, Ramboll could have also used values chosen within the 95% credibility interval of Kgl to investigate the propagating impact of Kgl on predicted dose metrics. Note that 95% credibility interval capture mostly uncertainties associated with sampling variations, not those associated with model and parameter.

In assessing the sensitivity of cancer risk quantification to the PBPK model prediction of dosimetry in general and Kgl in particular, the multi-stage Weibull dose-response model was used quantify the dose-response relationship. No justification was given to the choice of Multi-stage Weibull model as opposed to other models (e.g. Weibull model). Note also the multi-stage Weibull model is no longer supported by EPA's BMDS software.

The remaining questions are repeated (with minor edits) from the original charge. The reviewers are asked to primarily evaluate Ramboll's responses and changes made to address the original review comments.

Estimation of Metabolic Parameters from *In Vitro* Metabolism Experiments

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

Other information on the specific microsomal samples, preparation methods and in vitro experiments are in Lorenz et al. (1984), Himmelstein et al. (2004) and Yang et al. (2012).

3. Please comment on the pool sizes for the human microsomes used to estimate chloroprene metabolic rates in vitro, and the number of tissue samples (donors) evaluated for 7-ethoxycoumarin activity, for the estimation of average metabolic activity for human adults.

(**Tier 3**): Now that Ramboll has done extensive analyses to estimate the metabolic rates in vitro, it is feasible to conduct a statistical power analysis to demonstrate the precision (not necessarily accuracy) of the estimates on the basis of the underlying models. Note however, a statistical

power analysis is not designed to evaluate the biological representativeness of the human microsomes and tissue samples used in this study.

4. Discuss the appropriateness of the data used and the statistical modeling approach for evaluating average (or mean) adult human, mouse, and rat metabolic parameters. Please comment on whether sufficient microsomal samples (incubations) were analyzed to represent the average values and to characterize metabolic variation across species, sexes, and tissues.

(**Tier 2**): This and Yang's (Yang et al 2012) analyses both demonstrated evidence of between-species and between-sex differences in metabolic parameters. The authors of this analysis noted the visible differences between this analysis and that of Yang et al 2012. For example, the estimates of Km in the male mouse lung and liver from this analysis were only half of those from Yang's analysis. Incorporation of a mass transport parameter Kgl made the current analysis biologically sound. However, it is plausible that Kgl could be different across tissue or sex (as evidenced by its dependence with affinity Km). The fact that the current analysis failed to obtain an acceptable estimate for the metabolism parameters in multiple species and both sexes indicating limitation in these data as well. Therefore this analysis did not provide strong evidence that the microsomal samples or data were sufficient.

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

5. Please comment on the use of the relative 7-ethoxycoumarin activity in human lung vs. liver tissue to predict the average rate of chloroprene oxidative metabolism in the human lung.

Beyond my experience/knowledge areas.

IVIVE Calculations for Chloroprene

IVIVE extrapolation is summarized in the Model Parameters section of the Ramboll report, with details on scaling factors in Supplemental Material C of Ramboll and results in Table S-4 of Supplemental Material A. (Calculations are provided in an Excel workbook, Supplemental Material D of the Ramboll report. The U.S. EPA performed a quality-assurance evaluation of the workbook to assure the calculations are as described in the report text and tables.) Wood et al. (2017) evaluated the ability of IVIVE to predict clearance for oral dosing of multiple pharmaceutical compounds with data in rats and humans and reported a systematic bias towards under-prediction with increasing clearance. However, the Wood et al. (2017) results may not be relevant to chloroprene because of differences in the route of exposure, chemical properties, metabolizing enzymes, and rate-determining processes for the set of compounds analyzed. In particular, Wood et al. (2017) evaluated IVIVE for oral dosing of drugs, but not for the inhalation of volatile compounds like chloroprene. While, IVIVE for oral exposure to drugs may be more difficult and is subject to additional sources of uncertainty compared to inhalation of volatile compounds due to variability in intestinal absorption and metabolism (Yoon et al., 2012; Liao et al., 2007), analysis of Wood et al. (2017) specifically focuses on

predictions of hepatic clearance of drugs, for which metabolism in the liver is a significant component. Thus, the analysis of Wood et al. (2017) may be considered relevant to chloroprene since it addresses the ability to predict metabolic clearance via IVIVE, not oral absorption. The U.S. EPA is not aware of a systematic evaluation of IVIVE accuracy like that of Wood et al. (2017) but focused on volatile organic (chlorinated) compounds like chloroprene for the inhalation route.

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

Beyond my experience/knowledge areas.

7. Please assess whether the analysis adequately addresses the overall quantitative uncertainty due to other factors in the IVIVE application. Please identify any factors in the IVIVE calculation or parameters in the PBPK model for which variability or uncertainty have not been adequately considered. State any concerns about predictions of the rate of chloroprene metabolism in liver and lung which should be addressed. Please discuss whether the possible ranges for metabolic parameters (upper and lower bounds) have been sufficiently estimated such that they can be used with confidence for animal-to-human risk extrapolation.

Beyond my expertise areas.

PBPK Model Structure, Physiological Parameters, and Partition Coefficients

8. Please discuss the appropriateness of the PBPK model structure presented by Ramboll for estimation inhalation dosimetry in an EPA Toxicological Review of chloroprene. Please focus on the model structure for the liver and lung, i.e., tissues in which chloroprene metabolism is predicted by the model.

(**Tier 3**): The overall model structure appears to be sound. Ramboll conducted an extensive literature review to support the use of various physiological parameters and partition coefficient. It will be helpful to conduct uncertainty/sensitivity analysis by perturbing these physiological parameters and partition coefficients to see the extent these parameters will affect metabolism parameters, dosimetry, and risk, down the stream in a cascading fashion.

Arterial blood concentrations in B6C3F1 mice after inhalation exposures to chloroprene are shown in Figure 3 of the Ramboll report. It is noted that when chloroprene exposure was increased 2.5- fold from 13 to 32 ppm, the mean arterial concentration increased less than 1.5-fold. Further, the mean arterial concentrations from 90 ppm exposure, which is seven (7) times higher than 13 ppm, are only about 4 times higher than those measured at 13 ppm. These data might indicate that some process not included in the PBPK model may have reduced chloroprene uptake or somehow increased metabolic efficiency at 90 and 32 ppm relative to 13 ppm. A factor to be considered is the high variability with large standard deviations for many of the data points, as illustrated in Figure 3 of the Ramboll report. The PBPK model structure implies that blood levels should increase in proportion to exposure while blood concentrations remain below the level of metabolic saturation and should increase

at a faster rate above saturation, unless there is some other exposure-related change in model parameters. However, the plethysmography data evaluated do not show a clear or significant dose-response in the Ramboll report.
Figure 7 of Ramboll presents the extent of agreement of the model predictions with the blood concentrations in mice following inhalation exposure. It is noted that the inhalation PK data are from a single exposure (animals were not previously exposed to chloroprene) and the non-proportionality is evident by the 3-hour time-point.

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

Beyond my expertise areas.

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

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

Beyond my expertise areas.

Overall PBPK Model Soundness and Applicability

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

Demonstration of the PBPK model's ability to predict in vivo PK data is shown by the level of agreement between model predictions and chloroprene venous blood concentrations in Figure 7 of the Ramboll report. For reference, where there are data, and as a rule of thumb, EPA often seeks dosimetric estimates from a model that are within a factor of two of empirical results. The results of the sensitivity analysis shown in Figure 8 for arterial concentrations

indicate that these data and specific predictions are not sensitive to the estimated metabolic parameters: a relatively large range in the estimated metabolic parameters (such as the apparent difference between male and female mouse parameters) would yield similar predictions of blood concentrations. However, as demonstrated in Figure 9, the estimation of lung dose metrics is sensitive to the estimated metabolic parameters.

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

None.

12. Please review the Tier 1 and Tier 2 comments from the initial review and note any which you believe have not been adequately addressed by the revised analysis. If the comment has not been adequately addressed, please provide specific suggestions as to how this can be resolved.

No comments

In response to comments from Dr. Yang during the initial review, Ramboll has introduced a new analysis (sub-model) of the fate of chloroprene's metabolic products, Supplemental Material F. Since this material has not been previously reviewed, the reviewers are asked to give it careful consideration as appropriate to your areas of expertise.

13. Please comment on how well the biochemical processes and assumptions presented in Supplemental Material F represent the likely fate of chloroprene's reactive metabolites.

Beyond my expertise areas.

14. Please comment on the quality and accuracy of the parameter values selected in Supplemental Material F, Table F1, based on details provided in the corresponding text and supporting references.

Beyond my expertise areas.

In Supplemental Material F, Ramboll concludes that the concentration of the less reactive metabolite, 1-CEO, is not an appropriate dose-metric for cross-species extrapolation, given the lack of concordance of female mouse and female rat dose-response relationships, shown in Figure F5-C. The authors also conclude that either the total amount of chloroprene metabolized (predicted by the primary PBPK model) or the concentration of reactive products (predicted by the new sub-model) provide a consistent prediction of cancer dose-response based on results depicted in Figures F5-A and F5-B, respectively.

15. Please comment on whether the results shown in Figure F5-C preclude the possibility that 1-CEO tissue concentration is a reasonable predictor of chloroprene-induced lung cancer risk.

(**Tier 2**): See my comments on Q16

16. Please comment on whether the results shown in Figures F5-A or F5-B demonstrate that the corresponding dose metrics are consistent inter-species predictors of chloroprene-induced lung cancer risk. That is, given chloroprene exposures which produce the same value for either of the proposed dose metrics ("total amount metabolized per gram lung" and "concentration of reactive products") in female mice as female rats, can one infer that the same tumor incidence would occur in those species?

(**Tier 2**): This is not my specialty. It is interesting that the authors reported a consistent dose-response pattern between female rat and female mouse under both total metabolized amount and reactive products. The measurement of consistency was neither reported nor tested. It seems plausible that the between-specie difference in metabolism of chloroprene resulted in lower level of reactive products and higher concentration of 1-CEO in female rats compared with female mice, therefore leading to seemingly greater difference in the dose-response as seen in Fig F5-C where 1-CEO was the dose metric. It remains highly plausible that the dose-response could be different between mouse and rat under either the total metabolites or reactive products if we can further extend the dose-response curve for the rat to higher levels of the exposure. Furthermore, the authors' observation was limited to two species of a single sex with very limited data. I feel that the evidence is not sufficiently strong to support the statement that the total metabolized amount and reactive products are metrics that can consistently predict lung cancer risk across species. More research is needed.

17. Please comment on whether the results for the lung shown in Figure F5-A can be used to refute or support the use of the corresponding dose metrics for estimation of liver cancer risk.

(**Tier 2**): See my comments on Q16. Empirical dose-response based on these two studies is in itself insufficient for drawing such a conclusion. Mechanistic evidences would be useful to determine the validity of 1-CEO as a dose metric for toxicity and carcinogenicity. I do not have sufficient expertise to comment on the strength of mechanistic evidence.

EXHIBIT 5

January 26, 2010 – Final Reviewer Comments – *External Peer Review Meeting on the Toxicological Review of Chloroprene*

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

III. SPECIFIC OBSERVATIONS

Page	Paragraph	Comment or Question on the draft report PBPK modeling for chloroprene (Ramboll, 2021)
Fig B6-B8	Supp Mat B	Indicate mode in the posterior density plots;
Fig B9-B10	Supp Mat B	Credibility region or highest posterior density region is a more appropriate name than "confidence ellipse plot".
p-9	Supp Mat G	The correct relationship should be: "log_10(kgl)= - 0.85 ~ -1 (kgl=0.14=0.10)"
Supp Mat B p4	Last paragraph	"Collinearity" is an incorrect term here; "dependence" is appropriate.
Sup Mat B	Figure B-4	The trace plot and posterior density plot showed up to 30000 chain iterations. It is unclear if a burn-in period was excluded from the plots. Including an adequate burn-in period is necessary
Sup Mat B	Fig B-3 and Fig B-4	Log10 was the scale in Fig B-3 and ln in Fig B-4. Use the same scale would facilitate visual inspection and comparison. The scale of log was also used in this report (Supp Mat).
		When a software was used, citation (include version) should be given. For example, PBPK simulation software acslX is off shelf since Nov 2015.

FINAL

REVIEWER COMMENTS

External Peer Review Meeting on the *Toxicological Review of Chloroprene* (CAS No. 126-99-8)

Prepared for:
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Dale Hattis, Ph.D.
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John B. Morris, Ph.D.
Avima M. Ruder, Ph.D.
Richard B. Schlesinger, Ph.D.

January 26, 2010

TABLE OF CONTENTS

I. INTRODUCTION

The Integrated Risk Information System (IRIS) is an EPA database containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances. IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose (RfD) for noncancer health effects resulting from oral exposure, a reference concentration (RfC) for noncancer health effects resulting from inhalation exposure, and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program developed a Toxicological Review of Chloroprene, an assessment which has not previously appeared in IRIS. Chloroprene was nominated for IRIS assessment in 1999. The draft document contains a chronic inhalation reference concentration (RfC) and a cancer inhalation unit risk.

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II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of chloroprene 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). Currently an IRIS assessment of chloroprene does not exist on the database.

The draft health assessment includes a chronic reference concentration (RfC) and a carcinogenicity assessment. Below are a set of charge questions that address scientific issues in the assessment of chloroprene. Please provide detailed explanations for responses to the charge questions.

General Charge Questions:

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

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chloroprene.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Chloroprene

1. An RfD was not derived for chloroprene. Has the scientific justification for not deriving an RfD been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

1. A chronic RfC for chloroprene has been derived from an inhalation toxicity study (NTP, 1998) investigating non-cancer effects in multiple organ systems. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. An increase in the incidence of degenerative nasal lesions in male rats, characterized by olfactory epithelial atrophy and/or necrosis with increasing severity, was selected as the critical effect. Please comment on the scientific justification for combining the incidence of atrophy and necrosis and for selecting this endpoint as the critical effect. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. Benchmark dose (BMD) modeling was used to define the point of departure (POD) for the derivation of the RfC. The POD was based on increased incidence of degenerative nasal lesions in male rats at a benchmark response (BMR) of 10% extra risk. Has the BMD approach been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., 10% extra risk of degenerative nasal lesions of less than moderate severity) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

(C) Carcinogenicity of Chloroprene

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that chloroprene is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

2. A two-year inhalation cancer bioassay in B6C3F1 mice (NTP, 1998) was selected as the basis for derivation of an inhalation unit risk (IUR). Please comment on whether the selection of this study for quantification is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the basis for quantification.

3. A mutagenic mode of carcinogenic action is proposed for chloroprene. Please comment on whether the weight of evidence supports this conclusion. Please comment on whether this determination is scientifically justified. Please comment on data available for chloroprene that may support an alternative mode(s) of action.

4. Data on hemangiomas/hemangiosarcomas (in all organs) and tumors of the lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin and mesentery, mammary gland and liver in B6C3F1 mice were used to estimate the inhalation unit risk. Please comment on the scientific justification and transparency of this analysis. Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the inhalation unit risk and discuss whether such approaches are preferred to EPA's approach.

5. Lung tumors have been alternatively treated as systemic or portal-of-entry effects in the modeling of cancer endpoints. Please comment on the scientific justification for this modeling approach. Please comment on whether the rationale for this decision has been transparently and objectively described. Please comment on data available

for chloroprene that may support an alternative method for modeling the observed lung tumors in mice.

6. An oral slope factor (OSF) for cancer was not derived for chloroprene. Is the determination that the available data for chloroprene do not support derivation of an OSF scientifically justified?

III. GENERAL IMPRESSIONS

Herman J. Gibb

In general, the document lays out its arguments well. The discussion of the epidemiology, however, should be more transparent and perhaps could be better organized (studies of a facility where cohorts overlap or could overlap discussed together). Elaboration on the transparency is provided in my response to Question C1. The epidemiologic studies should be evaluated more rigorously.

Dale Hattis

Overall, the judgments made in the draft IRIS document for chloroprene are sound. However the modeling of the cancer risk can be improved by taking into account the existing evidence for partial saturation of metabolic activation of chloroprene in the dose range studied in the NTP cancer bioassay. Using a simple Michaelis Menten dose response equation to model this approach to saturation indicates that low dose cancer risks in both the male and female mouse bioassays are likely to be 2-3 fold greater than the risks indicated by application of a straight linear dose response model, as was done using the Weibull equation in the current cancer slope factor analysis. For the final assessment it would be desirable either to incorporate the Michaelis-Menten saturating form into the Weibull model or (less desirably) to multiply the Weibull model result by a factor derived from the Michaelis-Menten analysis of the lifetime tumor incidence information. The former approach is preferable because it will simultaneously take into account the time-to-tumor information and the apparent saturation of activating metabolism indicated by the incidence data.

Ronald L. Melnick

The draft document is a well-written, comprehensive review and assessment of published studies on the health effects of chloroprene in humans and in experimental animals. The information is clearly presented and the conclusions are generally scientifically justified and consistent with EPA policy. One exception is the rationale for the selection of 10% extra risk for the benchmark response. Specific areas for improvement of this review are described below in my response to the "chemical-specific charge questions."

John B. Morris

From my perspective as an inhalation toxicologist with expertise in rodent studies, the Toxicological Review of Chloroprene provides an in depth review of the toxicological literature on this compound. In many ways it is quite clear and thorough. The available database appears to be presented accurately and objectively. The overall conclusion, that chloroprene is an animal carcinogen whose mechanism(s) may include genotoxicity and mutagenesis, appears well founded. In some aspects, the document is confusing and perhaps lacks transparency. For example, information is provided in the summary and synthesis sections that have not been discussed previously. There are some apparent

contradictions in interpretive approaches, for example the potential for systemic blood delivery for the pulmonary but not nasal effects. The importance of some findings has gone unrecognized. For example, the extraordinarily high pulmonary metabolism rates in the mouse calls into question the relevance of this species with respect to pulmonary injury. Overall, the fundamental conclusions appear sound; however, the document could be significantly improved with respect to clarity and interpretive issues.

It is interesting that there are no charge questions relating to the toxicokinetics of chloroprene. Since the mode of action includes activation to an epoxide as the first step, the toxicokinetics becomes an issue of great importance. The toxicokinetic section describes the available information, but could provide much more information. Moreover, the toxicokinetic data is not adequately synthesized in the overall mode of action relative to potential species differences and extrapolation to man. PBPK modeling would be a highly appropriate way to incorporate kinetic data into the risk assessment. The published model of Himmelstein may provide a useful structure. Because it includes both nasal and tracheobronchial airway compartments the styrene model of Sarangapani may be a superior approach.

Avima M. Ruder

I can only validate accuracy for the section I compared to the original papers, that on human epidemiology. There are some key relevant references that were not cited and some points that should have been discussed (latency, age at diagnosis, etc.) that were not touched on (see 2.1).

The conclusions about the human hazard potential do not evaluate the role of genetic polymorphism in genes coding for glutathione *S*-transferases, epoxide hydrolase, and other metabolic enzymes in clearing epoxide metabolites from the body. Approximately half the human population is clears those metabolites at a much slower rate [Musak, et al. 2008], presumably making them more vulnerable to exposure. The conclusion also should point out that the noncancer effects (page 6-1, lines 24-33) were observed at levels lower than the current Permissible Exposure Limit.

The statements of conclusions in section 6 are less clear than those in section 4.7. It is appropriate to include all relevant caveats about the conclusions, and all the details of the studies that support those conclusions, but the conclusions themselves should be succinctly stated.

Richard B. Schlesinger

The background information that is provided to support the selection of the key studies is clearly and accurately presented. However, the derivation of some of the quantitative factors, as noted in subsequent comments in this document, could be made more transparent. In general, the overall conclusions appear to be sound.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions:

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

Herman J. Gibb

In general, the Toxicological Review is logical, clear and concise. A more rigorous and transparent evaluation of the epidemiologic studies and an objective evaluation of how the epidemiologic studies integrate with the rest of the data should be performed, however. The descriptor of "likely to be carcinogenic to humans" is justified based on the animal and genotoxicity information, but the document overstates the human evidence.

Dale Hattis

Generally, yes. I have some reservations and suggestions for incremental improvement, as will be apparent below. But the overall evaluation in the proposed IRIS document is sound.

Ronald L. Melnick

While the Toxicological Review is clear and comprehensive, it is not obvious why a particular dose response model was selected for the determination of the benchmark dose for noncancer hazards, if more than one model provided an adequate fit to the data. The rationale for the selection of 10% extra risk for the benchmark response for non-cancer effects is not adequately justified.

Based on the animal data, mechanistic findings, and "the reasonably consistent" evidence of increased risk of liver cancer mortality "among workers exposed to chloroprene in different cohorts in different continents," it is not clear why consideration was not given to the conclusion that chloroprene is "carcinogenic to humans."

John B. Morris

In many ways, the toxicological review is logical and clear; however, the document could be significantly improved in this regard. See my specific comments (below) for more detail on this concern.

Avima M. Ruder

The review is logical but less clear and concise than it could be. In the section on human carcinogenicity, the discussion should have been consolidated by population and recommendations for additional analyses (by age at onset/death, with lags) and substudies (nested case-control) should have been included. Such analyses should be done as very

early age at cancer onset/death has been associated with occupational exposure [Kreuzer, et al. 1999; Ward, et al. 1988] and lagged analyses focus on exposure in time periods that are most relevant for the development of solid tumors [Villeneuve and Steenland 2010]. All the studies on the Louisville plant should have been discussed together. The original study includes ages at death from lung cancer for 16 workers, including four who died in their forties [Pell 1978], but no analysis of whether the ages at onset were earlier than expected (in another chloroprene cohort, earlier ages at onset among exposed workers were reported [Li, et al. 1989]). The NIOSH walk-through survey of the plant, which was not referenced in the Toxicological Review, provides useful details on plant history, processes, and personnel, noting that "there is a complete pre-employment physical" plus periodic re-examinations (presumably those who did not meet some standard of health were excluded from employment; no details were presented on how the periodic re-examinations impacted continued employment [Jones, et al. 1975]. The NIOSH re-analysis of DuPont demographic data included recommendations for improving the epidemiologic studies by including all plant employees from 1942 on [Leet and Selevan 1982]. Blood draws from 846 of the workers employed in 1977 were compared for biochemical and hematological markers, with no significant differences in age-adjusted analyses [Gooch and Hawn 1981] and workers and plant sites were monitored for exposure, and workers interviewed [McGlothlin, et al. 1984](neither referenced in the Toxicological Review).

One of the more recent University of Pittsburgh papers (not referenced in the Toxicological Review), presents SMRs for the Louisville cohort using the DuPont worker mortality database; these are significantly elevated for all causes of death, all cancers, respiratory cancers, and liver cancer [Leonard, et al. 2007]. Kentucky cancer mortality is significantly higher than U.S. national cancer mortality [U.S. Cancer Statistics Working Group 2009], and the incidence of lung cancer in both Jefferson county and all of Kentucky is almost 50% higher than the U.S. rate [Kentucky Institute of Medicine 2007], so comparisons of a working population to the population at large will show a pronounced healthy worker effect. Presumably an employment-based database would control for the healthy worker effect to some extent. The most recent studies are more comprehensive but could have included additional analyses by age at diagnosis/death, lagged analyses, comparisons with the DuPont employee mortality database, and inclusion of the pre-1949 PYAR [Marsh, et al. 2007a; Marsh, et al. 2007b]. Some discrepancies should be explored; for example, Jones stated that approximately 8000 hourly and 1000 salaried (one-third foremen) employees had been employed to the time of the 1975 visit and over 1000 workers were employed in 1975; the Marsh analysis includes 5507 employees 1949-2000 [Jones, et al. 1975; Marsh, et al. 2007a].

Some discrepancies between the report of a 1985 NIOSH walk-through of the Pontchartrain, Louisiana, plant (neoprene production from 1968, 1264 workers to 1985) and the recent epidemiologic studies (chloroprene from 1969, 1258 workers to 2000) also need to be resolved [Fajen and Ungers 1985; Marsh, et al. 2007a; Marsh, et al. 2007b].

The studies of the plant in Grenoble, Isère, France, should also have been assessed together [Colonna and Laydevant 2001; Marsh, et al. 2007a; Marsh, et al. 2007b].

As to possible human health hazards other than cancer, the two medical studies at the Louisville plant [Gooch and Hawn 1981; McGlothlin, et al. 1984] and the recent study of chromosomal aberrations [Musak, et al. 2008] should be included. Apparently there are no studies of possible human reproductive effects more recent than Sanotskii's in 1976.

Richard B. Schlesinger

In general, the Review is well written and the toxicology of chloroprene is well synthesized.

General Charge Questions:

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chloroprene.

Herman J. Gibb

The NIOSH reports by Fajen and Ungers (1985) and by McGlothin et al (1984) should be included as background on the Pontchartrain and Louisville plants, respectively. Copies were provided to the peer reviewers by Avima Ruder subsequent to the peer review meeting on January 6, 2010 and are attached. Dr. Ruder also described references of Jones et al. (1975), Gooch and Hawn (1981), and Leonard et al. (2007) in her comments. Jones et al. (1975) and Gooch and Hawn (1981) describe conditions and the population at the Louisville plant and should be added as background information on that facility. The Leonard et al. paper apparently presents mortality analyses of the Louisville cohort using a Dupont worker mortality database. These papers should be reviewed to determine what insights they may offer to the mortality analyses by Pell (1978), Leet and Selevan (1982) and Marsh et al. (2007a, 2007b).

I am not aware of any additional original studies or reports that should be considered. The following reviews by Acquavella and Leonard (2001) and Bukowski (2009) should at least be given consideration although they need not necessarily be referenced. The review by Acquavella and Leonard (2001) appeared in the same journal as the review by Rice and Boffetta (2001) which is cited in the current Toxicological Review.

Acquavella JF, Leonard RC. 2001. A review of the epidemiology of 1,3-butadiene and chloroprene. Chemico-Biological Interactions 135–136 (2001) 43-52.

Bukowski JA. 2009. Epidemiologic evidence for chloroprene carcinogenicity: review of study quality and its application to risk assessment. Risk Analysis 29(9):1203-16.

Dale Hattis

Probably the most significant omission is an analysis by Dr. DeWoskin of EPA of the potential to use a PBPK model for estimation of human vs. mouse and rat delivered doses in modeling cancer dose response relationships for chloroprene. Its omission from the list of references is surprising. The abstract of this paper I retrieved from a MEDLINE search is:

PBPK models in risk assessment--A focus on chloroprene.

DeWoskin RS.

Chem Biol Interact. 2007 Mar 20;166(1-3):352-9. Epub 2007 Feb 8.

US EPA/NCEA (National Center for Environmental Assessment), Mail Drop B243-01, Research Triangle Park, NC 27711, USA. dewoskin.rob@epa.gov

Mathematical models are increasingly being used to simulate events in the exposure-response continuum, and to support quantitative predictions of risks to human health. Physiologically based pharmacokinetic (PBPK) models address that portion of the continuum from an external chemical exposure to an internal dose at a target site. Essential data needed to develop a PBPK model include values of key physiological parameters (e.g., tissue volumes, blood flow rates) and chemical specific parameters (rate of chemical absorption, distribution, metabolism, and elimination) for the species of interest. PBPK models are commonly used to: (1) predict concentrations of an internal dose over time at a target site following external exposure via different routes and/or durations; (2) predict human internal concentration at a target site based on animal data by accounting for toxicokinetic and physiological differences; and (3) estimate variability in the internal dose within a human population resulting from differences in individual pharmacokinetics. Himmelstein et al. [M.W. Himmelstein, S.C. Carpenter, P.M. Hinderliter, Kinetic modeling of beta-chloroprene metabolism. I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans, Toxicol. Sci. 79 (1) (2004) 18-27; M.W. Himmelstein, S.C. Carpenter, M.V. Evans, P.M. Hinderliter, E.M. Kenyon, Kinetic modeling of beta-chloroprene metabolism. II. The application of physiologically based modeling for cancer dose response analysis, Toxicol. Sci. 79 (1) (2004) 28-37] developed a PBPK model for chloroprene (2-chloro-1,3-butadiene; CD) that simulates chloroprene disposition in rats, mice, hamsters, or humans following an inhalation exposure. **Values for the CD-PBPK model metabolic parameters were obtained from in vitro studies, and model simulations compared to data from in vivo gas uptake studies in rats, hamsters, and mice. The model estimate for total amount of metabolite in lung correlated better with rodent tumor incidence than did the external dose.** Based on this PBPK model analytical approach, Himmelstein et al. [M.W. Himmelstein, S.C. Carpenter, M.V. Evans, P.M. Hinderliter, E.M. Kenyon, Kinetic modeling of beta-chloroprene metabolism. II. The application of physiologically based modeling for cancer dose response analysis, Toxicol. Sci. 79 (1) (2004) 28-37; M.W. Himmelstein, R. Leonard, R. Valentine, Kinetic modeling of beta-chloroprene metabolism: default and physiologically-based modeling approaches for cancer dose response, in: IISRP Symposium on Evaluation of Butadiene & Chloroprene Health Effects, September 21, 2005, TBD--reference in this proceedings issue of Chemical-Biological Interactions] propose that observed species differences in the lung tumor dose-response result from differences in CD metabolic rates. The CD-PBPK model has not yet been submitted to EPA for use in developing the IRIS assessment for chloroprene, but is sufficiently developed to be considered. The process that EPA uses to evaluate PBPK models is discussed, as well as potential applications for the CD-PBPK model in an IRIS assessment.

In reading the document, I don't recall coming across an explanation for why the implications of this model for cancer risk were not explored. It seems to me that the high dose saturation effects that are apparent in the tumor data could be explained in part by even a basic application of this kind of model. Explaining the high dose saturation of the

metabolic activation would, I think, (1) avoid the need to eliminate the high dose for some data sets and (2) lead to an increase in the estimate of the linear coefficients for the cancer dose response model. The PBPK model may well be considered not sufficiently tested against human data for un-caveated application to human risk projection, but I think its implications should at least be explored for sensitivity analyses.

Ronald L. Melnick

No additional studies were found that would significantly impact the overall assessment.

John B. Morris

I am aware of no additional toxicity studies relative to chloroprene. The mouse bronchiolar airway lesions are reminiscent of those induced by naphthalene and styrene. In this regard, comparisons to these compounds might provide some useful perspectives.

Avima M. Ruder

Two recent studies of genetic damage in workers exposed to chloroprene are relevant to this review.

Heuser VD, de Andrade VM, da Silva J, Erdtmann B. 2005. Comparison of genetic damage in Brazilian footwear-workers exposed to solvent-based or water-based adhesive. Genet Tox Environ Mutat/Mutat Res 583(1):85-94.

This study compared Comet assay results for unexposed workers, workers using water-based adhesives, and workers using solvent-based adhesives containing polychloroprene (and, presumably, some chloroprene as a contaminant), with a significantly higher damage index among the solvent-based adhesive users than either the unexposed or workers using water-based adhesives.

It was not entirely clear from the article whether the solvent-based adhesive group used adhesives (and other compounds), as stated on page 90, or produced the polychloroprene (page 91). In either case, there are a number of additional exposures which might have been associated with the chromosome damage. Other than the chromosome results no health effects were reported.

Musak L, Soucek P, Vodickova L, Naccarati A, Halasova E, Polakova V, Slyskova J, Susova S, Buchancova J, Smerhovsky Z and others. 2008. Chromosomal aberrations in tire plant workers and interaction with polymorphisms of biotransformation and DNA repair genes. Mutat Res 641(1-2):36-42.

This study compared lymphocyte chromosome aberrations among smoking and nonsmoking tire workers (exposed to butadiene) and controls. In addition, participants were genotyped for polymorphisms in genes encoding metabolic enzymes. "Chromosomal aberrations were higher in subjects with GSTT1-null (2.4 ± 1.7%) than in

those with GSTT1-plus genotype (1.8 ± 1.4%; F = 7.2, P = 0.008)." In light of the papers on diene (butadiene, chloroprene, isoprene) metabolism that indicate that the detoxification of a mutagenic metabolite goes through the GST pathway [Himmelstein, et al. 2004a; Himmelstein, et al. 2004b; Munter, et al. 2007; Munter, et al. 2003], this result is significant. It means that the fifty percent of the human population that is GST-null may be at higher risk from exposure; any exposure-associated carcinogenicity could be higher in this susceptible subpopulation.

Other studies to consider:

Fajen JM, Ungers LJ. 1985. DuPont de Nemours and company, Pontchartrain Works, LaPlace, LA, IWS-147-31. LA, LaPlace: NIOSH, Cincinnati, OH. 1-18 p.

Jones JH, Young RJ, Selevan S. 1975. du Pont de Nemours and Company, Inc., Louisville, Kentucky, IWS-87-10. KY, Louisville: NIOSH, Cincinnati, OH. 1-9 p.

McGlothlin JD, Meyer C, Leet TL. 1984. E.I. DuPont De Nemours And Company, Louisville, KY, HETA-79-027-1459. KY, Louisville: NIOSH, Cincinnati, OH. 1-28 p.

These NIOSH site visits provide concise histories of processes and chemicals at the plants, as well as descriptions of records and medical monitoring (Fajen and Jones reports) and a Health Hazard Evaluation (McGlothlin).

Leonard RC, Kreckmann KH, Lineker GA, Marsh G, Buchanich J, Youk A. 2007. Comparison of standardized mortality ratios (SMRs) obtained from use of reference populations based on a company-wide registry cohort to SMRs calculated against local and national rates. Chem Biol Interact 166(1-3):317-22.

This study calculated SMRs for the Louisville and Pontchartrain chloroprene plants using the DuPont employee database as a reference population, rather than the U.S. national or local population. For the Louisville plant, "...the SMRs based on the total U.S. DuPont worker mortality rates for all causes of death (1.13), all cancers (1.11), and respiratory cancers (1.37) are statistically significantly increased. The SMR for liver cancer (1.27), although elevated, is not statistically significant."

Richard B. Schlesinger

There are none that I am aware of.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Chloroprene

1. An RfD was not derived for chloroprene. Has the scientific justification for not deriving an RfD been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.

Herman J. Gibb

The scientific rationale for not deriving an RfD has been clearly described.

Dale Hattis

Yes. But such a derivation would be possible if the PBPK model (or some suitable range of models derived from sensitivity analyses) were used.

The principal study selected for analysis is fine.

Ronald L. Melnick

Yes, the lack of an adequate multiple-dose oral toxicity study on chloroprene that could be used for a dose-response analysis and the lack of information on the disposition of chloroprene after inhalation or oral exposure that would enable a reliable route-to-route extrapolation justify not deriving an RfD for this chemical. Because of a likely large first-pass liver effect after oral exposure, the systemic distribution of parent compound and reactive metabolites could be very different after oral or inhalation exposures.

John B. Morris

An oral RfD was not derived for chloroprene. The current database is clearly described. The rationale for the decision to not derive an oral RfD is clearly and concisely described. The scientific justification is appropriate and the decision is well founded.

Avima M. Ruder

As the document states, there are no human data on oral exposure and only one lifetime animal study, so clearly the justification for not deriving an RfD exists.

Richard B. Schlesinger

The decision not to derive an RfD is clearly justified in the document as based upon the lack of appropriate datasets for oral exposure.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

1. A chronic RfC for chloroprene has been derived from an inhalation toxicity study (NTP, 1998) investigating non-cancer effects in multiple organ systems. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Herman J. Gibb

The selection of this study is justified. The document states that the Trochimowicz et al. study was not chosen as the principal study "primarily due to the lack of observed effects at similar exposure levels as the NTP (1998) study"(page 4-39, lines 19-20; page 5-2, lines 26-29). That doesn't seem as strong an argument as the high mortality in the low dose animals which were suffocated by the ventilation system (page 5-2, lines 13-16, 29-31).

Dale Hattis

The principal study selected for analysis is fine.

Ronald L. Melnick

The selection of the NTP chronic inhalation toxicity study as the principal study for the derivation of an RfC for chloroprene is scientifically justified. This was a well designed and conducted study, which identified several non-cancer effects in multiple organs of rats and mice exposed to a wide range of concentrations of chloroprene. A major strength of this study is the multiple histopathological reviews of lesions identified in rats and mice. The study clearly demonstrates the toxicity of chloroprene in multiple species and the data are suitable for dose-response analyses.

John B. Morris

The selection of the NTP inhalation study as the principal study is scientifically justified. It was well conducted and subject to peer review.

Avima M. Ruder

The data files for two human studies conducted at the Louisville plant [Gooch and Hawn 1981; McGlothlin, et al. 1984] might have some information on subchronic effects. Gooch and Hawn did biochemical and hematological assays on blood specimens from workers characterized by their duration of chloroprene exposure. McGlothlin and colleagues conducted medical interviews with workers who had been monitored for chloroprene exposure (personal zone air samples). The report does not present any tabular data on health effects. However, the lack of quantitative exposure data for Gooch

and Hawn and of quantitative medical data for McGlothlin et al. rule out their use as a principal study. Selection of the NTP study is justified.

Richard B. Schlesinger

This study is clearly the best one to use for derivation of the RfC. It has a range of exposure concentrations and examined two species and multiple organ systems. The other chronic bioassay of Trochimowicz et al. has a number of problems associated with it that in my mind preclude its use as the key study.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

2. An increase in the incidence of degenerative nasal lesions in male rats, characterized by olfactory epithelial atrophy and/or necrosis with increasing severity, was selected as the critical effect. Please comment on the scientific justification for combining the incidence of atrophy and necrosis and for selecting this endpoint as the critical effect. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Herman J. Gibb

It seems reasonable to combine the incidence of epithelial atrophy and necrosis. The rationale for choosing degenerative nasal lesions over epithelial hyperplasia or splenic hematopoietic proliferation (page 5-10, lines 4-10) is reasonable.

Dale Hattis

I think there is no problem with the selection of these endpoints for RfC derivation.

Ronald L. Melnick

Combining the incidences of the degenerative nasal lesions, atrophy and necrosis, seems reasonable, but does not make much difference on the overall determination – the incidence of atrophy alone in the control and three dose groups of male rats was 6, 24, 94, and 98%, while the combined incidence of atrophy and necrosis was 6, 26, 96, and 98%; and the derived human equivalent POD values were essentially the same (1.1 mg/m^3 for atrophy and 1.0 mg/m^3 for the combined lesions, respectively).

Nasal degeneration is the appropriate effect for determination of the POD, because this was the most sensitive endpoint producing the lowest human equivalent POD. The document notes that candidate endpoints considered for the critical effect were those that were statistically increased in the lowest exposure concentration group. This limitation should not be imposed because it could result in exclusion of sensitive endpoints depending on the nature of the dose-response relationship. Other endpoints that should also be considered are renal tubule hyperplasia in male rats (single and step section data) and renal tubule hyperplasia in male mice. RfCs should also be derived and presented in Figure 5-1 for other endpoints, including olfactory effects in female rats, male mice, and female mice, and renal tubule hyperplasia in male rats, female rats, and male mice.

John B. Morris

Nasal degenerative lesions in the rat were selected as the critical response because the POD-HEC derived from these data was the most protective. Several concerns could be raised relative to this recommendation. First, the rationale for combining lesions and the precise way in which the data were combined is poorly described. In my view, the concept that necrosis may precede atrophy is quite straightforward. Numerous agents

induce nasal olfactory necrosis and atrophy (esters, styrene, and naphthalene to name a few); critical evaluation of this database will provide insights into the typical progression of lesions. The concept that atrophy precedes necrosis, however, is bewildering to me. I am not aware of a nasal toxicant in which it has been shown that atrophy results in subsequent necrosis. Such an example should be provided to support this concept. In the absence of such information, it is not reasonable, in my view, to assert that atrophy causes necrosis. I, therefore, do not concur with combining the lesions. I note that the difference in POD-HEC between combined and uncombined data is quite small; why invoke a poorly substantiated approach when it results in little difference? My other concerns focus on POD issues and are provided below. In my view, the POD should not be based on nasal lesions, making the issue of combination of lesions moot.

Avima M. Ruder

Combining the effects of atrophy and necrosis appears justified. Table 5-1 does not provide the p-values for trend in dose response for various endpoints. However, it appears that the trend might be stronger for the atrophy or necrosis, with percentages affected ranging from 6 to 98% with increasing doses, than for hematopoietic cell proliferation in the spleens of female mice, with percentages affected ranging from 26 to 78% with increasing doses.

Richard B. Schlesinger

A portal of entry effect was used as the critical effect, which is appropriate for this chemical. The justification provided for combining these two degenerative changes as the overall effect of interest is appropriate, even though it would be assumed that necrosis would precede atrophy. While it appears that the chloroprene while non reactive is metabolized in the upper respiratory tract to a reactive epoxide, there needs to be some explanation as to why the nasal changes themselves were selected over effects in the bronchial tree or alveolar region that were observed at the 12 ppm exposure level as well. An explanation does appear on page 5-7 following results of modeling, but there should have been some indication earlier on as to why the upper respiratory rather than the lower respiratory tract endpoint was selected in the first place.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

3. Benchmark dose (BMD) modeling was used to define the point of departure (POD) for the derivation of the RfC. The POD was based on increased incidence of degenerative nasal lesions in male rats at a benchmark response (BMR) of 10% extra risk. Has the BMD approach been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., 10% extra risk of degenerative nasal lesions of less than moderate severity) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Herman J. Gibb

The BMD approach is preferred to other approaches for the given data. The arguments made by one of the peer reviewers, Dr. Morris, to reconsider the calculation of the RfC with regard to blood borne delivery versus airborne delivery are reasonable, and I would recommend that the Agency evaluate both approaches prior to performing dosimetric adjustment. If atrophy/necrosis is eventually selected as the endpoint, a BMR of 10% extra risk is reasonable given the arguments on page 5-4 of the document.

Dale Hattis

The saturation of metabolism to the active metabolites could be clarified with the use of the PBPK model mentioned earlier. This could facilitate dose response modeling and perhaps lead to a somewhat lower point of departure for application of uncertainty factors.

At the peer review meeting an issue arose as to whether the 10% benchmark response level was appropriate in the light of the severity of the nasal lesions in some of the animals. If counts are available on the numbers of animals showing different levels of severity in relation to dose than this would seem to be a good case for the use of the EPA's categorical regression software. With that system it would be possible to take the severity information into account and estimate a somewhat lower BMDs and BMDLs corresponding to a 10% extra risk of mildly adverse effects.

In addition, EPA might consider a modifying the benchmark dose estimation to take into account the approach to saturation of metabolic activation derived from the cancer dose response information (see below).

Finally I agree with some of the other reviewers that the RfC should be derived using the procedures for a category 3 rather than a category 1 vapor.

Ronald L. Melnick

BMD modeling is the preferred approach to derive the POD because it uses all of the dose response data and is less impacted by the group size. Some discussion is needed on why a particular dose response model was selected for the determination of the POD in situations where more than one model provided an adequate fit to the data. If it is EPA's policy to select the model that yielded the lowest AIC value, then that rationale should be explicitly noted. The characterization of chloroprene as a Category 1 gas and the application of a dosimetric adjustment factor for portal-of-entry effects have not been adequately justified.

The NTP study that was used to derive the RfC did not achieve a NOAEL, and the severity of the nasal lesions was greater than minimal in the lowest exposure concentration group. In fact, several male rats in the low exposure group (12.8 ppm) were graded with moderate severity for olfactory atrophy and necrosis. The benchmark response of 10% extra risk is not a NOAEL and the estimated BMD_{10} used to derive the RfC is approximately 60% of the lowest concentration used in the chronic toxicity study of chloroprene. Because the NTP study included 50 animals per group, a BMR of 2% or 5% extra risk would likely provide a reliable estimate for the derivation of the POD without substantially increasing statistical uncertainty at the POD. Thus, I strongly recommend BMD modeling and derivation of the POD from the 2% or 5% extra risk response; if that is not done then an additional uncertainty factor of 3 to 10X would need to be applied to the human equivalent POD.

John B. Morris

I do not concur with the approach used to derive the POD-HEC. Multiple POD-HEC values were derived for differing lesions and the most sensitive was then selected. I note that the POD values (prior to DAF correction) for all the lesions are virtually identical, spanning 2.1-8.3 mg/m3 range. The only reason the POD-HEC is lower for the nasal lesions is that the DAF is so low. Thus, the selection of the nasal lesions as the most sensitive response is simply an artifact of the DAF (RGDR) calculation and not based on the primary experimental observations.

My concerns relative to the RGDR are described below. Essentially they are: 1) the RGDR calculation is theoretically flawed and discordant with the inhalation dosimetry database, and 2) there is no basis to conclude that airborne rather than blood-borne chloroprene induces nasal olfactory lesions. The absence to consider blood-borne delivery is particularly confusing in light of the fact that the possibility of blood-borne delivery relative to pulmonary lesions received much attention. Why this was ignored for the nose is perplexing. The distribution of lesions (olfactory, but no respiratory mucosal damage) could certainly be reflective of a critical role for blood borne delivery and/or in situ metabolic activation. The absence of nasal respiratory injury suggests the parent compound and/or direct reactivity of the parent compound are not likely involved. Commonly a strong anterior/posterior gradient in respiratory mucosal injury is seen for vapors which are directly reactive. This is not the case for chloroprene, in fact, no respiratory mucosal lesions were seen. Were blood borne delivery considered I believe

the RDGR would be 1. In my view, the assumption that chloroprene is a category 1 gas is also flawed (see below). Given that numerous compounds produce nasal olfactory injury following parenteral administration, the observation of nasal olfactory injury cannot be used in support of a category 1 assignment. The partition coefficient of chloroprene is quite small (10) from a nasal dosimetric view. It is difficult, if not impossible, to envision a scenario in which nasal backpressure does not influence dosimetry and/or that nasal deposited chloroprene does not penetrate to the depth of the blood. In my view, chloroprene is a category 3 gas.

At best, the assignment of category 1 status and the exclusion of blood-borne delivery mechanisms represent a weakness of the RfC derivation. An alternate approach would be to select the POD on a parameter closely associated with the collected data rather than to pick a value subject to artifact from the RGDR approach. Were this done, a differing critical lesion would be selected – likely alveolar epithelial hyperplasia and/or hematopoietic proliferation. Given that the subsequent text includes considerable discussion of the possibility of blood borne delivery relative to pulmonary injury, the selection of an inhalation based DAF of 2.3-4.1 would need to be critically discussed and supported were lung lesions selected as the critical effect. For the cancer risk extrapolation both inhalation based and blood-borne based DAF values were used. Why not use both approaches for the non cancer endpoints as well? The lack of consistency is striking.

I am supportive of using a BMD approach as the database appears sufficiently robust to allow for this calculation. An extra risk of 10% of mild lesions is an appropriate endpoint in my view. However, if moderate grade lesions were observed at exposure concentrations approximating the calculated BMD10, it would suggest the calculated value is too permissive. As noted above, I would recommend selecting the endpoint based on the observed data and then performing a single DAF-based calculation based on those data. Such an approach would minimize artifacts due to complexities associated with selection of the most appropriate DAF.

Avima M. Ruder

I don't have the expertise in risk assessment to comment on whether the modeling and extrapolation from animal to human was appropriately conducted. However, a 10% increase in an effect appears to be a significant enough departure from good health to justify the calculation. Upon reflection, I agree with the argument made by Dr. Melnick that the proposed benchmark dose does not represent a NOAEL and that it might be better to look at a lower response level (2-5%). From the responses from EPA staff at the review meeting it appears that a 2-5% extra risk response level was considered in internal EPA discussions. I also think that the issues raised by Dr. Morris as to whether chloroprene is a category 1 gas or not need to be clarified.

Richard B. Schlesinger

The BMD approach is very well suited for the large data set of the principal study being used in this document and using chronic toxicity and carcinogenicity as endpoints. In general when using the BMD, a 10% level of acceptable risk is used. Thus, this document follows relatively standard procedures in this regard. However, based upon the data, this level may be too high and it is suggested that a lower level, perhaps 5%, be used in this case. The document could be clearer in showing the different stages in the development of the RfC. It does provide a formula on page 5-4 but does not show the use of the formula with actual numbers from the principal study. It would be helpful to the reader if such a step by step actual derivation was provided. For example, it would help to see the actual value for the PODadj (mg/m^3) that was used to derive the HEC.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s

Herman J. Gibb

The uncertainty factors seem reasonable.

Dale Hattis

I have no quarrel with the selection of uncertainty factors made in the document. The analysis seems very standard. The only area of modest controversy might be the choice of a database uncertainty factor of 3. This seems adequately justified by the absence of a two-generation reproductive study, although the negative findings for teratogenesis and dominant lethal effects could have been considered an adequate substitute.

Ronald L. Melnick

The selection of uncertainty factors of 10X for human variation, 3X for animal-to-human toxicodynamic uncertainty, and 3X for database insufficiencies are reasonable and consistent with EPA policy. However, it is not possible to know if the UFs selected for human variability and interspecies uncertainty adequately account for the extent of these variations. For example, human variability is greater than 10X for the activities of the enzymes involved in chloroprene metabolism (both activation of chloroprene and detoxification of the reactive epoxide intermediate). As noted in response #3 above, the BMD_{10} is a true effect level with several animals diagnosed with moderate lesion severity (i.e., the severity level just below marked). The EPA assumption that the BMD_{10} represents a minimal biologically significant change that was less than moderate severity is not correct. Thus, an additional uncertainty factor of 3-10X should be applied to the RfC derived from a BMD_{10}; alternatively, the POD should be derived from a BMR or 2% or 5% extra risk. An additional deficiency in the database includes lack of data on potential neurodevelopmental toxicity, or other long-term effects following perinatal exposure.

John B. Morris

The rationale for UF selection is clear and appears consistent with typical procedures. The discussion would be greatly enhanced by inclusion of discussion of the impact and uncertainty of selecting DAF factors based on airborne delivery. My concerns, in this regard, are provided above. In my view, it is important to recognize that the DAF calculation is subject to considerable uncertainty and, as such, should not be accepted as factually based. Discussion should also be included on the basis for inclusion of a database limitation uncertainty factor as a multi-generation study is available. It should be stated if this is policy-based rather than scientifically-based decision.

Avima M. Ruder

The uncertainty factors appear justified. As I commented above, there is probably considerable human variation in the metabolism of chloroprene, due to polymorphisms in the genes coding metabolic enzymes. However, as Drs. Schlesinger, Hattis, and Melnick suggested during the review (or as I understood them to suggest), it might be more appropriate to change the benchmark dose response, rather than the uncertainty factors. Their arguments should be considered.

Richard B. Schlesinger

The specific UFs chosen are well justified and appropriate for the data set used and follow standard USEPA guidelines.

(C) Carcinogenicity of Chloroprene

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgr-d.htm), the Agency concluded that chloroprene is likely to be carcinogenic to humans by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

Herman J. Gibb

The characterization is clearly justified based on the animal and genotoxicity data, but the argument for the epidemiologic data has been overstated.

The reported evidence of a liver cancer risk in the Louisville cohort studied by Marsh et al. (2007a, 2007b) summarized on page 4-18, lines 3-5 relies heavily on a purported dose response in 4 cumulative exposure categories. The document does not describe what the relative risks (and confidence limits) are in each of the four exposure categories but states that the probability of the trend is 0.09 (page 4-13, lines 13-17; page 4-71, lines 4-7)[1,2]. Furthermore, the document neglects to report what the overall SMR for liver cancer is in the Louisville cohort. Interestingly, the document concludes that there is no evidence of a dose response relationship for respiratory cancer yet describes the relative risks and confidence limits for respiratory cancer by all four cumulative exposure levels for all four facilities in the Marsh et al. study (page 4-14, Table 4-9). Why isn't the reader given that information for the liver cancer relative risks, at least for the Louisville cohort, since the document has gone to the point of suggesting that the data indicates that there is a liver cancer dose response? Furthermore, in the discussion of "biological gradient" on page 4-71, no mention is made of Table 4-11 on page 4-17 showing that two studies demonstrate evidence of a dose response for liver cancer, and two demonstrate *no* evidence of a dose response. The dose response in one of the studies (Leet and Selevan 1982) would not even exist if only deaths from liver cancer were included in the analysis since two of the three deaths from cancer of the liver and biliary passage in the high exposure category were due to gall bladder cancer. The other study in Table 4-11 that suggests a dose response is Bulbulyan (1999), but the relative risks in the high and low dose are not statistically different. The statement at the bottom of page 4-18 that there is evidence of a dose-response relationship in different cohorts in different continents (U.S., China, Russia, and Armenia) grossly misrepresents the evidence.

Known risk factors for liver cancer include Hepatitis B and C infection, aflatoxin ingestion, certain inherited metabolic diseases, cirrhosis due to alcohol abuse, obesity, and certain inherited metabolic diseases (American Cancer Society). None of these factors with the exception of alcohol consumption (page 4-69, lines 28-29) have been

[1] The document states on page 4-13, lines15-17, and page 4-13, lines 4-71, lines 5-6 the *range* for the *three* highest exposure levels was from 1.9-5.1 but doesn't state what the RR's for each of the four exposure levels are nor does it provide confidence limits on the RRs.

[2] If the p = 0.09 is calculated by the authors of the EPA document (as opposed to Marsh et al.), that should be indicated.

discussed in the review. It is interesting that in the Major Conclusions on page 6-2, lines 27-29, the document notes that "These associations (respiratory cancer) are not considered as strong as those with liver cancer due to the inability to control for confounding by smoking status, a strong indicator of lung cancer." What about the well-known risk factors for liver cancer? Were they considered in the various studies? On page 4-69, lines 28-29, the document indicates that the lack of data on alcohol consumption is a "key limitation." On lines 31-32, the document states that there is also a "high likelihood of co-exposures which may be confounders." Nonetheless, the document goes on to blithely state that "Despite this potential, there is little evidence of substantial exposure to liver carcinogens in these populations." How can such a statement be made if the study authors never considered the major risk factors?

Of particular note with respect to the Li et al. study is that the highest liver cancer rate in the world is China (as much as 10X that in the U.S.), primarily the result of Hepatitis B infection and aflatoxin ingestion. Given the considerable risk posed by these risk factors in a Chinese population and that there were only 6 liver cancer deaths in the entire cohort working in a facility where there were multiple chemical exposures, it is impossible to conclude that the study indicates an association between chloroprene and liver cancer.

The document indicates on page 4-8 that Bulbulyan et al. (1998) found 11 deaths due to cirrhosis. It is possible that these deaths could have been caused by chloroprene, but alcohol and hepatitis B/C infections are the most common causes of cirrhosis which should say something about the cohort. Liver cancer is about 50% higher in Eastern Europe than it is in North America, and alcohol consumption in Russia is reported to be almost double that of the U.S.

The analysis of the Bulbulyan (1999) study indicates that there was increasing incidence of liver cancer by duration of employment and by cumulative exposure. Presumably duration of exposure and cumulative exposure were not evaluated together in a multiple regression by the study authors (I do not have the original paper). Given that there was an increasing risk by duration of exposure, one cannot rule out that the increasing risk with cumulative exposure was not due to other exposures at the facility. Presumably, there was no analysis by intensity of exposure? If there was, what did it show?

The document should be more transparent in the presentation of the human data on liver cancer. For example:

- The liver cancer relative risks for all four exposure categories in the Louisville cohort studied by Marsh et al. should be reported.
- The SMR for liver cancer should be reported for the Louisville cohort studied by Marsh et al.
- Whether Marsh et al. (2007a, 2007b) and Leet and Selevan (1982) Louisville cohorts are independent should be addressed. If Leet and Selevan (1982) is a part of or the same as the Marsh et al. cohort (or even very similar), then use of the Leet and Selevan (1982) should not be described as providing independent results of dose

response, consistency, etc. The same is true of the Colonna and Leydavant (2011) and the Marsh et al. studies of the Pontchartrain facility.

- The confounding factors for liver cancer and whether studies addressed these risk factors should be discussed.
- The statement in the Major Conclusions on page 6-2, lines 19-20 that there was "some evidence" of liver/biliary passage cancer risk being associated with chloroprene exposure is followed by the statement on lines 22-23 that these measures of association were "strong, especially in the presence of healthy worker bias" is inconsistent.
- An association between liver cancer and chloroprene exposure being strengthened by the healthy worker effect as indicated in the Major Conclusions is not evident in the summary of the overall weight of evidence (some mention of HWE is made on page 4-69, lines 21-25 but does not indicate that the evidence is strengthened). Furthermore, a healthy worker effect for liver cancer? With such a short life expectancy following diagnosis, I would expect the healthy worker effect for liver cancer to be minimal if it even exists.
- The small number of liver cancer deaths/cases in the studies by Li et al., Bulbulyan (1998, 1999) and Leet and Selevan (1982) and the variability about such small numbers should be better described, particularly in light of the limitations of those studies with respect to calculation of the expected deaths, follow-up, etc.

As the document acknowledges on page 4-17, there is little if any evidence that chloroprene increases the risk of respiratory cancer. The limitations of the earlier studies (Li et al. 1989, Bulbulyan 1998, 1999) are significant with regard to whether or not they indicate an increased risk of liver cancer from chloroprene exposure. The largest and what appears from the document to be the best conducted study (Marsh et al., Louisville cohort) provides little if any evidence that a liver cancer risk exists. Furthermore, the document has not been transparent in its reasoning that there is a risk of liver cancer.

In summary, the descriptor of "likely to be carcinogenic to humans" is supported by the animal and genotoxicity data, but not by the human data. While the descriptor is appropriate, the document should not try to make more of the epidemiologic studies than is warranted.

Dale Hattis

Yes. The ample information on carcinogenesis in many sites in animals, the clear metabolism information to mutagenic metabolites, and the analogies to related chemical carcinogens with analogous metabolic pathways to DNA-reactive metabolites all combine to make this conclusion unequivocal. As suggested by Dr. Melnick, the final document should consider whether the available evidence warrants an upgrade of the classification to "carcinogenic to humans.

Ronald L. Melnick

Results from the NTP study demonstrating multiple organ carcinogenicity of inhaled chloroprene in both sexes of rats and mice are consistent with the EPA descriptor "likely to be carcinogenic to humans." Because the carcinogenicity of chloroprene is likely due to its epoxide metabolites, and because cytochrome P450-mediated epoxidation of chloroprene can occur in several organs including the liver, kidney, and lung, metabolism of absorbed chloroprene to a mutagenic intermediate can occur by any route of exposure. The systemic distribution of tumors in the NTP studies demonstrates that chloroprene can induce tumors beyond the sites of initial contact. Liver toxicity of chloroprene in rats after oral exposure (stomach tube) indicates the occurrence of oral absorption of this chemical. Chloroprene is absorbed by the skin (Hazardous Substances Data Bank; see page 3-1).

However, the descriptor "carcinogenic to humans" may be more appropriate based on the multiple tumor response in two species, the fact that chloroprene is activated by CYP2E1 to a DNA reactive intermediate (chloroethenyl oxirane) by rat, mouse, or human liver microsomes, the finding of a unique K-ras mutation (A→T at codon 61) in chloroprene-induced lung neoplasms in mice, and the relatively consistent evidence of an association between increased liver cancer mortality risk and occupational exposure to chloroprene. The EPA document does not adequately justify the characterization of chloroprene as "likely to be carcinogenic to humans" rather than "carcinogenic to humans," especially since many of the identified methodological limitations in the epidemiologic studies (e.g., exposure misclassifications, healthy worker effect) would result in an underestimate of risk. According to EPA's cancer risk assessment guidelines, the descriptor "carcinogenic to humans" may be applied when there is less than convincing epidemiologic evidence of a causal association between human exposure and cancer if there is strong evidence of carcinogenicity in animals, the MOA and precursor events have been identified in animals, and key precursor events in animals are anticipated to occur in humans and progress to tumors. These conditions have been demonstrated for chloroprene.

John B. Morris

I concur that the weight of evidence supports the concept that chloroprene may be carcinogenic by all routes of exposure. Multiple tumors were seen in two species in inhalation bioassays. Additionally some data suggesting increased tumor risks in humans is available. Tumors were seen in non-site of contact sites in the rodent studies. (In this regard respiratory tract as well as gastrointestinal tract tumors may be considered as site of contact because of preening activity.) Moreover, there is discussion of the possibility of a critical role blood-borne chloroprene relative to nasal and pulmonary lesions. If there is, indeed, a role for blood borne chloroprene, then the possibility of carcinogenicity after multiple routes of exposure is elevated because systemic absorption and blood-borne delivery to multiple targets is possible. (The document indicates dermal absorption may occur.) Importantly, a potential increase in liver tumors was noted in some occupationally exposed cohorts. In my view, these epidemiological data support the concept that chloroprene may represent a carcinogenic hazard to man.

Avima M. Ruder

The literature supports the likely carcinogenicity of chloroprene and the mutagenicity of its epoxide metabolites. The need for regulation of environmental (in addition to occupational) exposure to chloroprene is justified by a report on public health in the area where the Louisville DuPont plant and other industrial facilities, as well as residences, are co-located. In that report, the Agency for Toxic Substances and Disease Registry (ATSDR) stated that the volume of release of chemicals from the plants made it likely that soil and water (groundwater and the Ohio River) had been contaminated in the past; chloroprene air contamination was measured as 218 ppb or 789 $\mu g/m^3$ in 1956-7 downwind of the plants and 6 ppb or 2.68 $\mu g/m^3$ in 1988 at a monitoring station in downtown Louisville not downwind of the plants [Agency for Toxic Substances and Disease Registry 1998].

ATSDR provided a rationale for the greater vulnerability of children to toxic exposures: they are more likely to play outdoors and bring food into contaminated areas; are shorter and therefore closer to dust, soil, and contaminants; weigh less, resulting in higher doses per unit body weight; and are developing rapidly [Agency for Toxic Substances and Disease Registry 1998]. The EPA's use of age-adjustment factors seems appropriate.

Richard B. Schlesinger

While the *Guidelines for Carcinogen Risk Assessment* are being followed in the chloroprene assessment, even though there are limited to no data on exposure other than inhalation, it seems that the mode of action of the chemical is such that it may not be carcinogenic via all routes, e.g., dermal exposure. It is nonreactive chemically and relatively insoluble in water. The weight of evidence characterization is clear and justified. The animal toxicological data support the conclusion that it may likely be carcinogenic to humans. While the epidemiological evidence in this regard is equivocal, the conclusion is also supported by the fact that the MOA involves conversion to epoxides.

(C) Carcinogenicity of Chloroprene

2. A two-year inhalation cancer bioassay in B6C3F1 mice (NTP, 1998) was selected as the basis for derivation of an inhalation unit risk (IUR). Please comment on whether the selection of this study for quantification is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the basis for quantification.

Herman J. Gibb

The selection of this study is justified. The document states that the Trochimowicz et al. study was not chosen as the principal study "primarily due to the lack of observed neoplastic effects at similar exposure levels as the NTP (1998) study"(page 5-12, lines 5-8). As with the response to Question 1 for the RfC above, high mortality in the low dose animals (page 4-39, lines 19-20; page 5-2, lines 13-16, 29-31) would be a stronger argument for not choosing the Trochimowicz study than would differences in observed effects between studies. Differences in study results can occur regardless of how well the individual studies are conducted.

Dale Hattis

Choice of the two-year inhalation bioassay is beyond dispute. However, as indicated earlier, the dosimetry, in terms of active metabolite concentration AUC, could have been informed by application of a preliminary PBPK model.

Ronald L. Melnick

The selection of the NTP 2-year inhalation carcinogenicity study of chloroprene in B6C3F1 mice for derivation of an inhalation unit risk is scientifically justified. The NTP study was well designed and conducted, and identified carcinogenic effects in multiple organs of rats and mice exposed to a wide range of concentrations of chloroprene. A major strength of this study is the multiple histopathological reviews of lesions identified in rats and mice. As with the related human carcinogen, 1,3-butadiene, the carcinogenic potency of chloroprene was greater in mice than in rats.

John B. Morris

In my view, the selection of the two-year inhalation bioassay done by NTP as the critical study is appropriate. This study was well performed and peer reviewed. It is true that the Trochimowicz study provided contradictory results, but without substantive rationale the NTP study cannot be ignored. Inclusion of the mouse lung tumor data for dose-response evaluation may be scientifically problematic. As is commonly observed, the mouse metabolic activity for chloroprene is 50-fold higher (Table 3-4) than that in the human or the rat (in which lung tumors were not increased). This fact should be discussed. It is my view that the mouse lung data may overestimate the risk to humans. It is recognized that exclusion of these data may be problematic, but at a minimum a discussion of this

weakness should be provided. Because the metabolism rates in the rat appear similar to the human, the rat may offer a better species for prediction of human health risks. Certainly the document would be improved by an explicit discussion of the relevance of the mouse response considering its high metabolic capacity.

Avima M. Ruder

The text in section 5.4.4 explains the derivation of the inhalation risk but does not explain why inhalation in mice was chosen over inhalation in rats from the same study. I assume there are physiological differences which make mice a more suitable choice, but none were provided here.

Richard B. Schlesinger

The study selected for derivation of the IUR is well justified based upon the standard procedure used by USEPA in selecting the most sensitive animal model. However, they may want to consider the fact that metabolic activation rate in the rat is closer to that occurring in humans than is the situation in mice.

(C) Carcinogenicity of Chloroprene

3. A mutagenic mode of carcinogenic action is proposed for chloroprene. Please comment on whether the weight of evidence supports this conclusion. Please comment on whether this determination is scientifically justified. Please comment on data available for chloroprene that may support an alternative mode(s) of action.

Herman J. Gibb

The hypothesized epoxide metabolite mode of action is reasonable.

Dale Hattis

Yes. The ample information on carcinogenesis in many sites in animals, the clear metabolism information to mutagenic metabolites, and the analogies to related chemical carcinogens with analogous metabolic pathways to DNA-reactive metabolites all combine to make this conclusion unequivocal. I am not aware of any evidence that comparably supports any other mode of action.

Ronald L. Melnick

Based on the fact that the predominant pathway of chloroprene metabolism is via cytochrome P450-mediated oxidation to a DNA-reactive epoxide intermediate (chloroethenyl oxirane), which is mutagenic in multiple strains of Salmonella, and the finding of activating K-ras and H-ras mutations mutations in tumor tissues obtained from mice exposed to chloroprene, including unique K-ras mutations (A→T transversions in codon 61) in lung tumors, the proposed mutagenic mode of carcinogenic action is scientifically justified. This MOA is consistent with that of other epoxide-forming carcinogens, e.g., 1,3-butadiene and vinyl chloride. There is no scientific data supportive of any alternative mode of action. Recent experimental results presented to the Peer Review Panel by DuPont demonstrated the induction of changes in gene expression related to DNA damage in the lungs of mice exposed to 2.5 ppm or higher concentrations of chloroprene (Figure 8, page 79). These data also support a mutagenic mode of carcinogenic action for chloroprene.

John B. Morris

It should be stated that detailed assessment of mutagenic versus non-mutagenic modes of action is somewhat beyond my expertise. With this qualification, I concur with the proposed mutagenic mode of action of chloroprene. Chloroprene metabolite(s) are DNA reactive and mutagenic in some bacterial strains. Data presented by DuPont suggests the induction of DNA repair responses in chloroprene exposed animals. Mutations were observed in vivo in lung tumors of animals exposed to chloroprene. Were a purely cytotoxic mode of action proposed it would be important to show appropriate temporal and dose-response data supportive of this mode. I am aware of no such data. In my view there are insufficient data to exclude the possibility of a mutagenic mode of action. There

appears to be multiple lines of evidence in support of this mode of action and it, therefore, appears scientifically justified. If, however, it is concluded that a metabolite represents the ultimate toxic species, then the quantitative risk assessment should be discussed/validated in light of the large species differences in metabolism rate.

Avima M. Ruder

The metabolic pathways detailed in figure 3-1 (and in the toxicological literature from which this section is drawn) appear to justify this conclusion. The finding of increased chromosome aberrations among humans with variant metabolic enzymes that clear the epoxide metabolite more slowly [Musak, et al. 2008] also supports this conclusion.

Richard B. Schlesinger

There is much compelling evidence that chloroprene has a mutagenic mode of action due to metabolism into reactive epoxides. While this may not be the only MOA, it clearly is one of them.

(C) Carcinogenicity of Chloroprene

4. Data on hemangiomas/hemangiosarcomas (in all organs) and tumors of the lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin and mesentery, mammary gland and liver in B6C3F1 mice were used to estimate the inhalation unit risk. Please comment on the scientific justification and transparency of this analysis. Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the inhalation unit risk and discuss whether such approaches are preferred to EPA's approach.

Herman J. Gibb

The rationale for combining risks from different tumor sites is reasonable given a mutagenic mode of action. It is interesting, however, that the inhalation unit risk estimate for chloroprene is an order of magnitude higher than the inhalation unit risk estimate for butadiene which is considered a structural analog and characterized by EPA as "carcinogenic to humans". A reality check on the unit risk for chloroprene by comparing it with an upper bound on the cancer risk in the Louisville cohort studied by Marsh et al. should be performed. The Louisville cohort has the best exposure information for this purpose. From the resulting comparison, it may be necessary to adjust the unit risk estimate.

Dale Hattis

The approach is transparent and reasonable as far as it goes. However, I think it is not ideal in that it fails to make explicit use of the information that there is likely to be high dose saturation of metabolic activation.

As an alternative, at the peer review meeting I presented a series of model fits using a dose response form that incorporates an assumption of saturating metabolism on a systemic level (applicable to all tumors in the same way) but different effective background rates and potencies for the causation of tumors at low doses:

$$P(d)_i = 1 - e^{-(q0_i + \frac{Vmax_i * d}{Km + d})}$$

where:

d is the external experimental concentration in ppm

$P(d)_i$ is the fraction of animals with at least one tumor for a specific tissue (i)

$q0_i$ is a parameter estimated from data that is related to the background (control group) lifetime incidence of tumors in that tissue

V_{max} is related to the maximum tumor yield over background for the specific tissue (i)

K_m is the external dose that produces half the maximal tumor yield over all tissues (based on an assumption that metabolic activation is systemic, rather than being effective for only one tissue due to local metabolism).

This is essentially a quick and easy but approximate substitute for doing a full PBPK model, but instead uses the tumor response nonlinearity at high doses for all the tumor sites to quantify the approach toward saturation of the activating metabolism. Compared to a PBPK modeling approach, this is not informative for the issue of interspecies projection, but it does provide information about the high-dose-to-low dose projection, assuming that the saturable activating metabolism is systemic and affects the tumor frequency in all tissues in the same way. This sort of treatment is warranted by the fact that, in nearly all tissues with an appreciable tumor yield in both male and female mice, the tumor incidence over background at the highest (80 ppm) chloroprene concentration is much less than double the tumor incidence at the next highest (32 ppm) concentration (see plots below). Contrasting the results for the high-dose saturable metabolic activation model with those for a straight linear model allows us to assess how large the change in estimated low dose cancer slope might be relative to a case where there is only a term that is linear in dose:

$$P(d)_i = 1 - e^{-(q0_i + q1_i d)}$$

To maintain parallelism with the EPA analysis as much as possible, I made this comparison excluding the anomalous high-dose point for hemangiosarcomas in female mice. Because of this same anomaly, I choose to begin the discussion of the modeling and the model results with the observations in male mice.

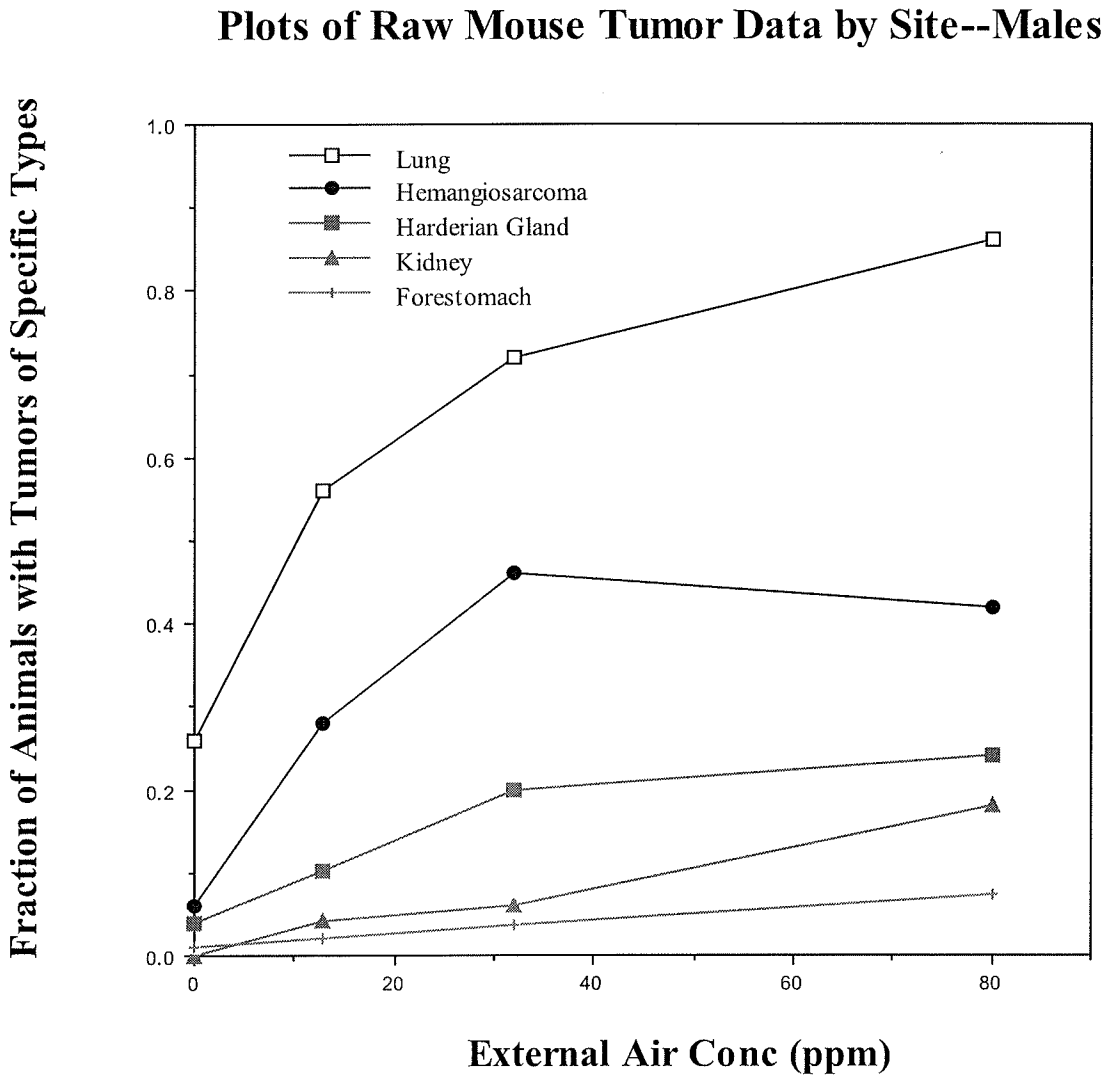
Figure 1 is a raw plot of the end of life tumor data for male mice used by EPA in its analysis (from a comment by Dr. Melnick, I understand that tumor results adjusted for mortality are also available in one of his papers; EPA should probably used those results for a more refined analysis.)

A difficulty with the raw plot the tumor data is that one might object that of course there is a flattening of the curve at higher doses and tumor incidences because no more than one tumor can be effectively detected and recorded in any specific tissue. Thus a more appropriate interpretation of the data is to say that each data point represents the fraction of animals that showed <u>at least</u> one tumor in each specific tissue studied. A more appropriate plot without the potential distortion due to multiple tumors per organ can be made by using a Poisson distribution formula

$$P_{0 \text{ tumors in an organ}} = 1 - \text{Fraction of Animals with at Least 1 tumor} = e^{-m}$$

where m = the mean number of tumor transformations per animal

Figure 1


Plots of Raw Mouse Tumor Data by Site--Males
Fraction of Animals with Tumors of Specific Types
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Lung
Hemangiosarcoma
Harderian Gland
Kidney
Forestomach
External Air Conc (ppm)

Given this, we can solve for m to find

mean number of tumor transformations per animal = - ln(1 - fraction of animals with at least 1 tumor)

Figure 2 is a plot of the male mouse tumor data using this transformations/animal parameter as the dependent variable. It can be seen that even after removing the truncation of the tumors/animal results at 1 in this way, there is still a pronounced flattening of the curves at the higher dose levels, indicating some approach to saturation. This is reminiscent of the vinyl chloride angiosarcoma case where there was saturation of metabolic activation at the higher exposure levels.

One other advantage of the transformations/animal dependent variable is that we can add up the results for the different tumor sites. Figure 3 shows a revised plot of the male tumor data showing the sum of tumor transformations/animal at all five tumor sites. It can be seen that the sum of tumor transformations at all five sites still shows a pronounced convexity as one proceeds to the highest exposure levels.

The fitting of the saturable and linear models was accomplished in Microsoft Excel workbooks designed to incorporate likelihood calculations according to the basic structure published by Haas (1994).* Copies of the final workbooks themselves will be submitted to accompany this comment. I would be pleased to explain the detailed features and operation of the modeling system if any EPA personnel would like to pursue this. Basically, each workbook consists of 3 sheets: one for optimization of the maximum likelihood estimates and two for estimation of upper and lower confidence limits on the sum of transformations/animal at all tumor sites. The optimizations were all done with the Excel solver tool, generally with multiple runs of hundreds to thousands of iterations each. Because the maximum likelihood and confidence limit estimates are done on the sum of tumor transformations per animal for all tumor sites, no Monte Carlo post-processing analysis is needed to derive confidence limits on the total tumor risk, as was needed for the separate Weibull model analyses done by/for EPA for the individual tumor sites. On the other hand, a disadvantage of this modeling system is that it only incorporated total tumor incidences observed by the end of the bioassays; not the more detailed time-to-tumor information used in the Weibull model analysis.

Figure 4 shows the overall results of this fitting for both the saturable and linear models. In the case of the saturable model, the parameters estimated are a Vmax and background (zero dose) tumor risk for each organ, and a Km (external ppm needed to achieve half of the total saturated tumor yield) common to all organs—following the hypothesis of saturable metabolism at a systemic level followed by common exposure of all organs to the activated metabolite(s). It can be seen that the saturable model fit corresponds very well with the observations of total tumors per animal (the P value is 0.51, meaning that a difference between data and model predictions as large as that observed would be expected to be produced about half the time from chance sampling-error fluctuations).

* Haas, C. N. "Dose Response Analysis Using Spreadsheets" Risk Analysis 14:1097-1100 (1994).

Figure 2

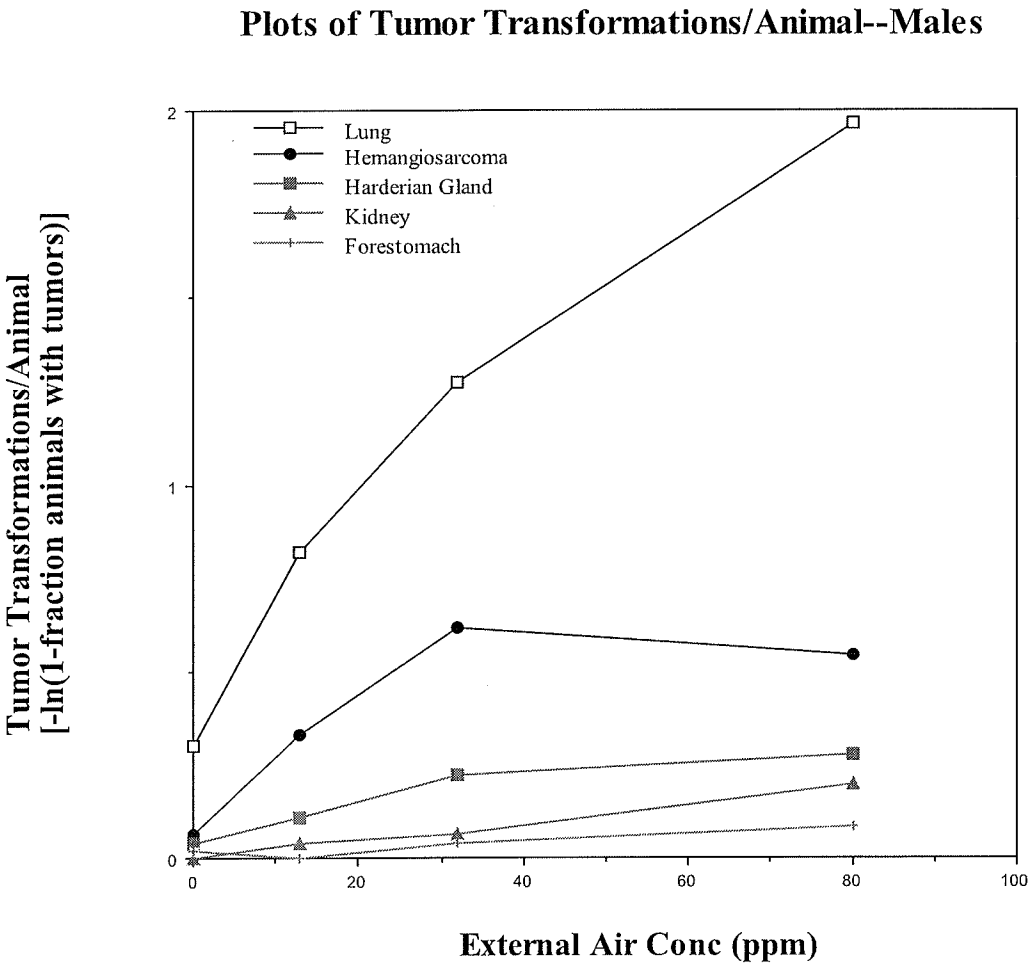

Plots of Tumor Transformations/Animal--Males
Tumor Transformations/Animal
[-ln(1-fraction animals with tumors)]
Lung
Hemangiosarcoma
Harderian Gland
Kidney
Forestomach
2
1
0
0
20
40
60
80
100
External Air Conc (ppm)

Figure 3

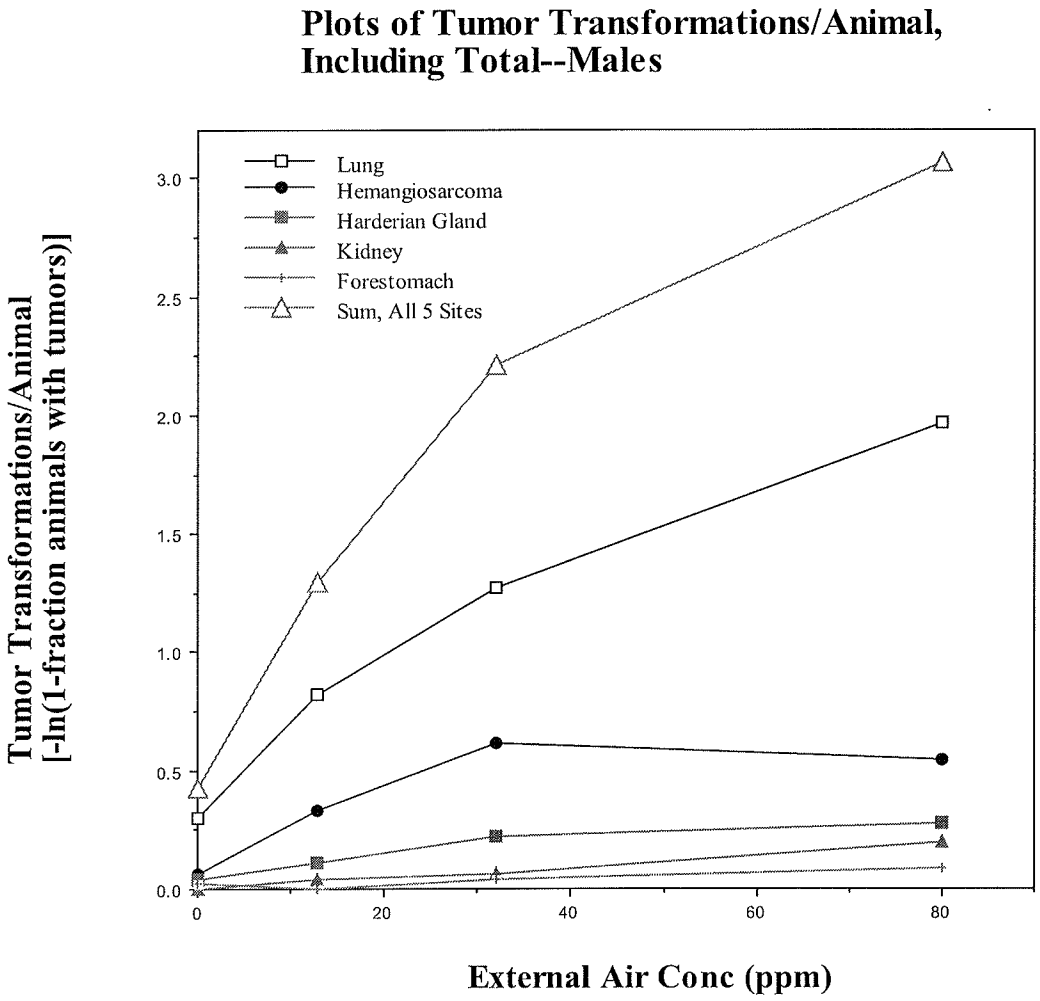
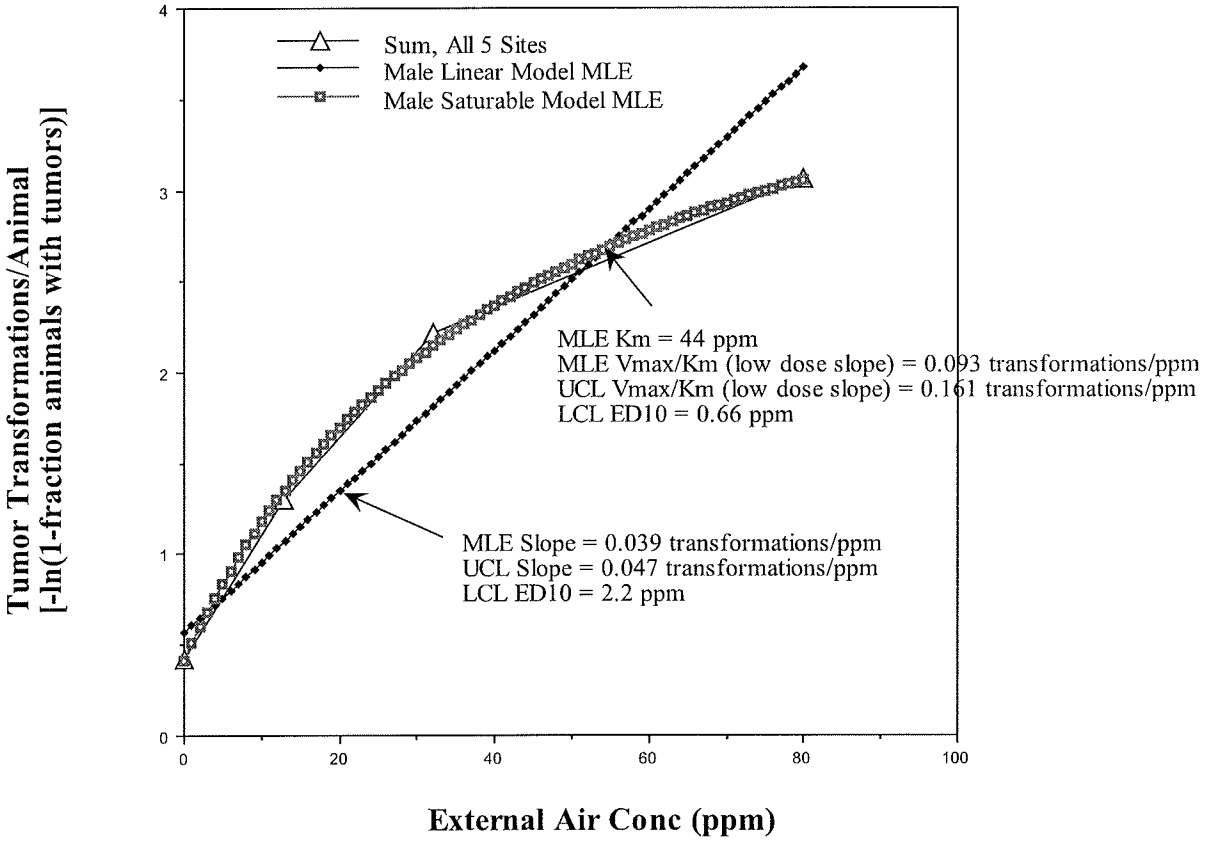

Plots of Tumor Transformations/Animal, Including Total--Males
Tumor Transformations/Animal [-ln(1-fraction animals with tumors)]
Lung
Hemangiosarcoma
Harderian Gland
Kidney
Forestomach
Sum, All 5 Sites
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
External Air Conc (ppm)

Figure 4

Comparison of Observed Tumor Transformations/Animal For All 5 Sites in Males with Maximum Likelihood Expectations for Linear and Saturable Models



The linear model fit somewhat less well at P = 0.06, although still barely within the conventional P = 0.05 criterion based on estimation of one fewer parameter (10, rather than 11, corresponding to a background rate and a transformations/ppm parameter for each tumor site).

The results in Figure 4 indicate a half saturation point (Km) of about 44 ppm, and an approximately 2-3 fold greater cancer potency at low doses for the saturable, compared to the linear model, depending on whether one makes the comparison based on MLE slopes or lower confidence limit ED10's. Thus the indication is that a simple linear formulation, as incorporated into EPA's Weibull model is likely to considerably understate the low dose potency indicated by the data for males.

Figure 5 shows a plot of the female tumor data comparable to Figure 2. The same tendency for flattening at high exposure levels is apparent. Figure 6 shows the results a similar comparison of saturable and linear model fits for the female tumor data (excluding, as did EPA, the high dose point for the hemangiosarcomas). The overall fit in this case is less successful than for the male tumor data, with a P value of about 0.02, but the saturable model still fits a great deal better than the linear model with a P value of about 9×10^{-5}. In this case the indicated Km is slightly lower (30 ppm) indicating a slightly greater effect of the indicated saturation of metabolic activation, and the saturable model again suggests a low dose cancer potency a few fold greater than expected with the linear model formulation.

In summary results lead me to five conclusions/recommendations:

- The tumor data are better fit by models incorporating systemic saturable metabolism.
- Saturable models lead to 2-3 fold increases in expected low dose risks compared to simple linear models.
- However, the current saturable models do not incorporate available time-to-tumor information.
- The best way forward would therefore be to add a saturable component to the Weibull time-to-tumor model.
- A second-best approach would be to multiply the expected ratio of saturable vs. linear model-predicted low dose risk by the existing Weibull linear model coefficient (or make a similar adjustment downward in the Weibull model estimated ED10 or LED10).

Figure 5

Plots of Tumor Transformations/Animal Excluding Hemangiosarcomas--Females

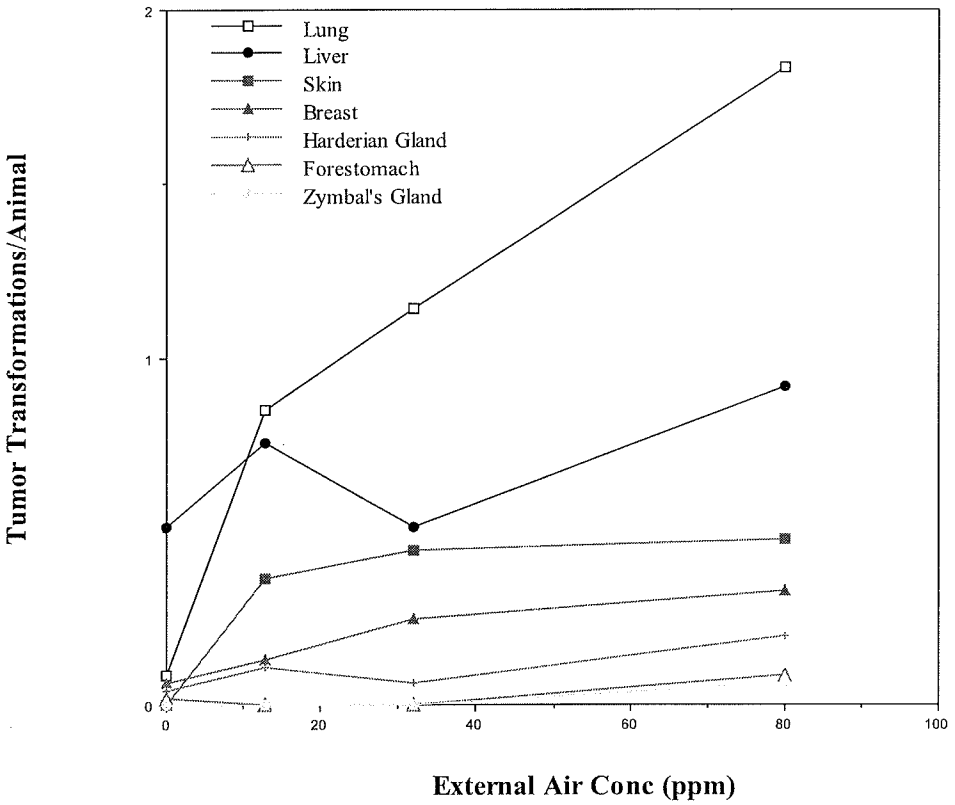
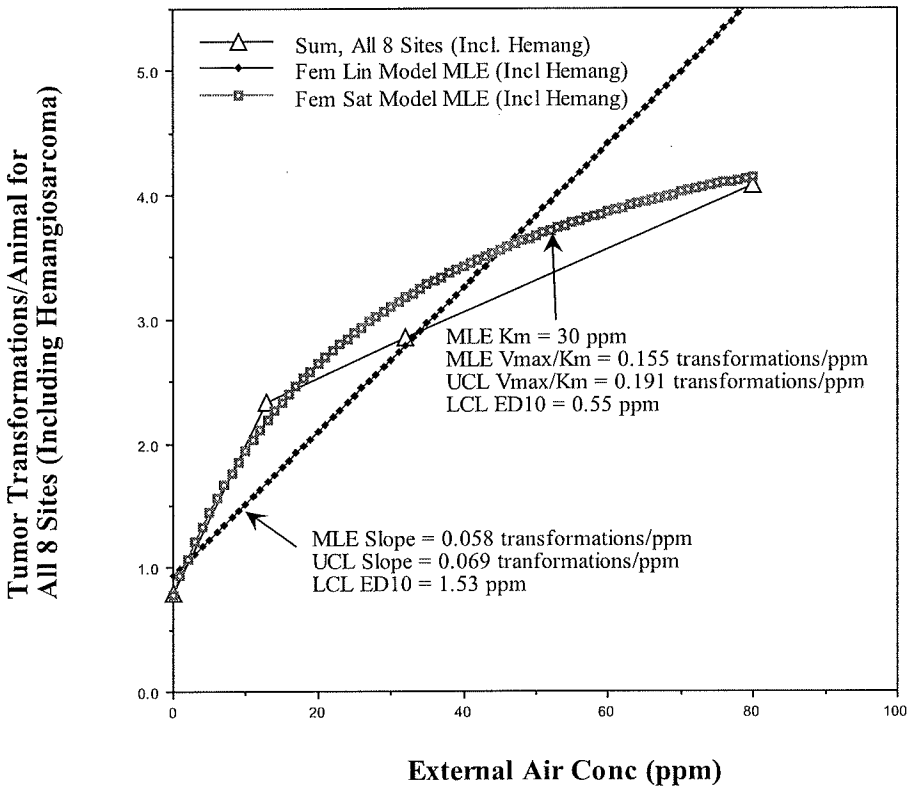


Figure 6

Comparison of Observed Tumor Transformations/Animal For All 8 Sites in Females with Maximum Likelihood Expectations for Linear and Saturable Models



Ronald L. Melnick

Yes, all of the induced tumor sites in mice should be used to estimate the inhalation cancer unit risk; an assessment based on separate modeling of each tumor type would underestimate the carcinogenic potency of chloroprene. Cancer potency estimates are increased only about 2-fold by combining all sites in the assessment compared to estimates based on only the most potent response in either male or female mice. Because of the reduced mortality of exposed mice due to induction of malignant tumors, a multistage Weibull time-to-tumor model that accounts for differences in survival among groups is most appropriate. The chloroprene document should provide discussion on why no uncertainty factor (other than early-life susceptibility) for human variability was applied to the cancer unit risk estimate. There are certainly substantial differences in human metabolism of chloroprene and its reactive epoxide metabolite and in human susceptibility to chloroprene-induced cancer.

The suggestion by Dale Hattis to apply a model that accounts for saturable metabolism of chloroprene to its epoxide intermediate should be pursued and incorporated into the estimate of the inhalation cancer unit risk. This analysis should use survival-adjusted tumor incidence values. The blood time-course data for chloroprene presented by DuPont (Figure B-1, page 99) to the Peer Review Panel clearly demonstrates saturable metabolism of chloroprene in mice at exposures between 13 and 90 ppm.

John B. Morris

The modeling approaches for the quantitative risk evaluation of chloroprene carcinogenicity were transparently described. Cancer unit risks are calculated individually for specific tumor types and an overall unit risk was calculated. Presumably the overall unit risk was calculated in concordance with accepted EPA procedures. It is beyond my expertise to comment on the generalized appropriateness of combining tumors in this way relative to overall cancer unit risk calculation. If tumors are to be combined then the human relevance of each tumor type must be considered. As noted above, in my view, some skepticism is appropriate relative to the quantitative importance of mouse bronchiolar tumors. The mode of action includes metabolic activation as the first step. The metabolic activation rates in the mouse exceed those in other species by 50-fold (Table 3-4). Clearly this is a critical observation relative to quantitative risk extrapolation. This pattern of mouse vs. human bronchiolar metabolism is certainly not unique to chloroprene. The large differences in mouse vs. human relative to pulmonary activation raise questions as to the relevance of the mouse lesions. At the very least, this issue needs to be discussed. Exclusion of the mouse lung tumors would influence the final overall unit risk estimate indicating this is not a trivial concern.

It should be noted that the epidemiological data suggests the liver at the primary target, although this may be the result of statistical issues related to the high incidence of lung tumors in humans obscuring a response. Nonetheless, a discussion of the site discordance would strengthen clarity of the text. I don't know if it is possible, but some comparison of the unit risk versus the observed tumor risks in the worker populations would seem warranted. Is it possible to estimate an upper bound risk from the human data?

Alternatively, is it possible to project human occupational risks from the unit risk factor to determine if the unit risk factors are consistent with epidemiologic observations? I recognize that only crude comparisons could be made, but a large discordance would be a cause of concern.

Avima M. Ruder

The assumption of tumor independence (p 5-20), based on the National Research Council risk assessment document, appears justified. However, the results of the animal studies should be evaluated to determine if there is a distinction (genetic, epigenetic, or other) between animals which get one tumor versus those which get more than one.

Richard B. Schlesinger

The derivation of the IUR could be made somewhat clearer in the text.

(C) Carcinogenicity of Chloroprene

5. Lung tumors have been alternatively treated as systemic or portal-of-entry effects in the modeling of cancer endpoints. Please comment on the scientific justification for this modeling approach. Please comment on whether the rationale for this decision has been transparently and objectively described. Please comment on data available for chloroprene that may support an alternative method for modeling the observed lung tumors in mice.

Herman J. Gibb

It makes sense that lung tumors could develop from a systemic as well as a portal-of-entry effect. The extent that the lung tumors occur by systemic vs. portal of entry effects may not be possible to determine, but the text should provide more elaboration for the reader so that they can better understand the approach.

Dale Hattis

The early results for the saturation modeling described in section 4 above strongly suggest that the lung tumors for both male and female mice are completely compatible with the systemic saturable metabolic activation model with a half-saturation point similar to that derived with data for other tumor locations. Therefore, I think the lung tumors should not be treated as if they depended on local metabolism and other portal-of-entry specific processes.

Ronald L. Melnick

Both treatments of the lung tumor data are appropriate because these tumors may have arisen from metabolites formed in the lung, or in other organs, particularly the liver, and subsequently distributed to the lung. No data are available to distinguish the extent of these possibilities. The EPA document did note that the induction of tumors in multiple organs after inhalation exposure to chloroprene demonstrates the systemic distribution of carcinogenic metabolites by this route of exposure.

John B. Morris

The importance of portal of entry versus systemic delivery of chloroprene is not known. A reasonable approach would be to make estimates using both approaches and then make a determination of whether or not it is of quantitative importance. Naturally, the default approach would be to select the more health protective approach. In my view, the fundamental issue in this regard is actually based on the assignment of category 1 status to chloroprene. This assignment is not appropriate (see my other comments), and at the very least needs to justified. Chloroprene should be determined to be a category 3 vapor in my view. It is a low partition coefficient vapor that does not appear to be highly reactive. Indeed, were it highly reactive it would be impossible to measure a partition coefficient. Moreover, the pattern of nasal injury (olfactory but not respiratory mucosal

damage) is inconsistent with a highly reactive vapor. Finally the modeling efforts of Himmelstein would not have been successful were chloroprene highly reactive in tissues. True it is metabolized, but the provided data do not indicate it is metabolized to such an extent that it should behave as a category 1 vapor. If category 1 vapors do not penetrate to the blood in any sufficient degree and if they should be scrubbed very efficiently in the nose, then why are distal lung tumors and non-respiratory tract tumors observed? Were chloroprene to be determined to be a category 3 vapor, then I believe the whole issue of portal of entry versus system delivery will be moot because a DAF=1 would be assumed for both cases. The regional injury pattern in the respiratory tract (olfactory and bronchiolar injury) is suggestive for a critical role of local metabolic activation. It is possible however that active metabolite is formed in and then escapes from the liver.

Avima M. Ruder

If chloroprene is indeed rapidly absorbed in mice, it makes sense that a systemic effect from the metabolite as well as a portal-of-entry effect could occur. From the text (p 5-21) I could not determine whether it is postulated that the portal-of-entry effect is from the parent compound or the metabolite; this could be made clearer.

Richard B. Schlesinger

Since it is not clear, as noted in the Document, the extent to which chloroprene induces cancer via direct contact with the lungs or via systemic contact of lungs with metabolites, the approach used is valid. However, the application of this approach is not clear from the discussion in the document.

(C) Carcinogenicity of Chloroprene

6. An oral slope factor (OSF) for cancer was not derived for chloroprene. Is the determination that the available data for chloroprene do not support derivation of an OSF scientifically justified?

Herman J. Gibb

The determination is justified. There were no data on which to base an OSF and the PBPK model developed by Himmelstein (2004) (description on page 3-7) did not seem adequate to allow route-to-route extrapolation.

Dale Hattis

Not completely. With a PBPK model formulation, an oral slope factor could be estimated.

Ronald L. Melnick

Yes, the lack of an adequate multiple-dose oral carcinogenicity study on chloroprene and the lack of information on the disposition of chloroprene, including the AUC for the DNA-reactive epoxide intermediate, after inhalation or oral exposure that might enable reliable route-to-route extrapolation justify not deriving an oral slope factor for this chemical. Because of a likely large first-pass liver effect after oral exposure, the systemic distribution of parent compound and reactive metabolites could be very different after oral versus inhalation exposures.

John B. Morris

I concur with the determination that the available data do not support derivation of an oral slope factor.

Avima M. Ruder

As there are no quantitative data on effects of oral administration (p 5-1), the determination appears justified.

Richard B. Schlesinger

The lack of oral exposure data clearly justifies not deriving an OSF.

V. SPECIFIC OBSERVATIONS

Herman J. Gibb

Page 4-1, line 8: Delete "and"

Page 4-3, line 1: Delete "also"

Page 4-3, line 8: Delete "number"

Page 4-3, lines 8-9: Delete "of these"

Page 4-3, line 14: Delete the second "were"

Page 4-5, lines 1-2: The document indicates that a limitation of Li et al. is that only three years of local area data were used to estimate the expected numbers of deaths which may not be representative with regard to the period of follow-up of the cohort. An issue not considered is the stability of the expected rates based on local data.

Page 4-5, line 5: This discussion is unclear. If the general population had a higher mortality for a given disease during the periods not examined, then there would have been a higher number of expected deaths and the SMR for that disease would have been overestimated for the period of time that was considered, not underestimated. If the mortality was lower, then the SMRs would have been overestimated. In any case, the discussion is not clear.

Page 4-6, line 18: Change "1979-1993" to "1979 to 1993".

Page 4-6, line 22: Insert "the" before "general".

Page 4-8, line 19: Change "1979-1988" to "1979 to 1988".

Page 4-9, line 12: There is an inconsistency in how the SIR is reported on line 12 and in Table 4-6. Line 12 reports as 327 with 95% CI of 147 and 727; Table 4-6 reports as 3.27 with 95% CI of 1.47 and 7.27. The epidemiology section has several examples of changing back and forth between the convention of using the convention of multiplying by 100 and the ratio. Need to make consistent.

Page 4-9, line 23: Change "suggested" to "suggest"

Page 4-9, line 23: What are "highly exposed operators"? High cumulative exposure? Intensity of exposure? Duration of exposure? It makes a difference in the interpretation.

Page 4-10, line 29: Insert "in the group employed" before "prior". Presumably the author is describing those employed prior to 1977 and not those who developed cancer prior to 1977.

Page 4-10, line 33: The document states that "all of the SIRs exceeded 100" yet Table 4-7 indicates no SIR is over 100. Again, the authors need to use a consistent convention (report as a multiple of 100 or not report as a multiple of 100).

Page 4-11, line 10: Change "cancers" to "cancer"

Page 4-11, line 15: Is there any indication of how many workers died or left the area prior to 1979? Does the author have an idea of how much impact this would have on results or is it part of a laundry list of study faults? The power of the study was low regardless of whether workers died or left.

Page 4-14, lines 16-24 and Page 4-15, lines 1-3: It is not difficult to understand why Marsh et al. would conclude that their study provided no evidence of cancer risk associated with chloroprene exposures. Table 4-9 on page 4-14 shows little evidence of a dose response. It is inappropriate to conclude as is done in lines 1-3 on page 4-15 that Marsh et al.'s explanations are "not entirely consistent with the data presented". The authors of this document have chosen one interpretation; the authors of the study have chosen another interpretation.

Page 4-15, lines 24-35: Some of the criticisms are too harsh. For example, how often are causes of death verified by histological confirmation or review of medical records? Nice if it can be done, but the vast majority of mortality studies would fall in the same boat. Incomplete enumeration of incident cases is a criticism that could be leveled at many incident studies. The statement that despite the lack of quantitative exposure information, occupational studies are still able to contribute to the overall qualitative weight of the evidence considerations (lines 31-33) states the obvious, but the statement should not be used as license to draw conclusions on studies that have serious limitations.

Page 4-16, Table 4-10: All SMRs are reported as the multiple of 100 except for Bulbulyan et al. (1998). "Sullivan" should be "Selevan". It would be more logical to have the intermediate exposure column first, followed by the high exposure column, followed by the total cohort column.

Page 4-17, Table 4-11: The relative risk is reported as a multiple of 100 for the high and intermediate exposures in the Leet and Selevan (1982) study but not for the other studies. "Sullivan" should be "Selevan." It would be more logical to have the intermediate exposure column first, followed by the high exposure column, followed by the total cohort column.

Page 4-18, lines 7-8: The limited number of cases (one in each cohort) "precluding meaningful examination" states the obvious.

Page 4-18, line 19: "these cancers"? Should this be "an increased liver cancer risk"?

Page 4-19, line 8: "No workers experienced loss of hair." This is the first place where loss of hair is mentioned. Since that is an unusual effect, it would be better to report the results of the distillation workers after the results of the polymerization workers.

Page 4-63, line 13: What is "horizontal activity"?

Page 4-66, line 30: Delete "based on available data".

Page 4-67, Table 4-38: "Sullivan" should be "Selevan"

Page 4-69, lines 6-8: "Although not statistically significant, these findings were comparable to results (RR range 2.9-7.1) detected in two other studies for high and intermediate cumulative exposures (Bulbulyan et al., 1999, 1998)." Given that there could have been considerable differences in exposure, follow-up, duration of exposure, etc. between the studies, such a statement is not justified.

Page 4-69, lines 23-26: "only Bulbulyan et al. (1999) observed a statistically significant association between chloroprene exposure and liver cancer mortality." The preceding sentence suggests that this was done by an internal analysis, but the increase in liver cancer mortality was observed from an external analysis.

Page 4-69, lines 29-30: "....although there is no direct evidence that alcohol is related to the exposure of interest (i.e., chloroprene)." There may be no "direct evidence that alcohol is related to the exposure of interest"; there is no direct evidence that is not either. More convincing that alcohol did not play a confounding role would have been clear evidence of a dose response to chloroprene since it would be unlikely that alcohol consumption would correlate with chloroprene exposure. Evidence of a dose response, however, is equivocal (see Table 4-11 on page 4-17).

Page 4-70, lines 7-10: Criticizing mortality studies for not doing a medical record review or histological examination to confirm cause of death is extreme. Almost all mortality studies could be faulted for not doing that.

Page 4-71, lines 21-24: What "current understanding" allows one to state that specificity is "one of the weaker guidelines"? Reference?

Page 6-1, line 22: Replace "th" with "the".

Dale Hattis

1. Table 3.2 should express results in fraction of total metabolites rather than relative to butanol standard. Or it could be expressed in terms of absolute rates per unit time per unit microsomal protein. Recalculate?

2. p. 3-5, lines 5-7: "Estimates for V_{max} and K_m for oxidation of chloroprene in liver microsomes ranged from 0.068–0.29 μmol/hour/mg protein and 0.53–1.33 μM, respectively."

The meaning of the ranges should be described. If these are in fact the ranges of all observations, then the number of observations should be given; also, there should be some way of describing the dependencies of the estimates of V_{max} and K_m values.

3. Presentation of metabolic data in Table 3-4 is inadequate. No error bars or statements of how many animals tested independently (or pooled?), or more crucially, how many humans and how they differ in V_{max}/K_m for various organs (obtain original papers on metabolism).

Source: Himmelstein et al. (2004a).
Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004a) Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. Toxicol Sci 79(1):18–27..

4. Table 3.5: Again, no error bars or description of the number of animals studied or experimental errors.

5. p. 3-7, lines 4-5: "The clearance of these thioethers reached a threshold at 24 hours after dosing, indicating that elimination was rapid."

Use of the word "threshold" here is unclear and ill-advised. If what is meant is that there was no further increase in thioether excretion, then that should be said explicitly.

6. Table 3-6: Why are values not provided for the major physiological parameters (body weight, cardiac output, and alveolar ventilation)?

7. Epi data discussion: The authors do qualify the discussion of the epidemiological data with the healthy worker effect. However, they do not as yet include suitable caveats for the "internal" comparisons by mentioning the distortions expected from the healthy worker survivor" effect — that longer exposed workers with higher cumulative exposures have lower mortality than shorter term workers. This must be incorporated into the analysis. Some language I have adapted from prior work (Hattis and Goble 2007) is:

> "The "healthy worker survivor" effect is a known phenomenon that produces established distortions in relationships between measured risks and measures of cumulative exposure, as shorter term workers suffer greater mortality than workers who work at exposure-producing jobs for longer periods of time (Steenland et al., 1996; Kolstad and Olsen, 1999; Garshick et al. 2004; Siebert et al. 2001; Steenland and Stayner 1991). Adjustments for this effect are at the cutting edge of current practice for the treatment of human epidemiological data, but they are vital for achieving the best possible analysis of those data. Even if the data will not support the more complex analyses [and analyses of this sort are

notoriously complex (Robins 1986; Arrighi and Hertz-Picciotto 1996; Hertz-Picciotto, personal communication)], EPA could provide at least some discussion of how large the distortions might be by citing such previous cases as the cancer risks from diesel particles (Garshick et al. 2004; 2008) and the approach that California risk assessors (and possibly others) have taken to risk analysis where the healthy worker survivor effect is even more prominent than it may be in this case. (For diesel particulates, initial estimates of the relative risk vs. cumulative dose curve even had a negative, rather than a positive slope.)"

8. The discussions of both liver and lung cancer might benefit from some attempt at integrative meta-analysis, combining the effects of multiple studies for reasonably comparable levels of exposure. This, however, likely depends on obtaining some disaggregated data from the individual investigators, and that might not be possible. Even if the combination is somewhat speculative, it might be informative to make some attempt to combine the human evidence for comparison with the projections from animal studies.

9. Chronic NTP exposures: For later modeling, the authors should report integrated average exposures that were measured, rather than the nominal target exposures. The difference may well be small, as indicated in the discussion, but the measurements should be used in preference to the target levels in the dose response modeling which appears later in the document.

10. p. 4-54, lines 16-18: "Estimates for V_{max} and K_m for oxidation of chloroprene (into (1-chloroethenyl)oxirane) in liver microsomes ranged from 0.068–0.29 μmol/hour/mg protein and 0.53–1.33 μM, respectively."

Again, what is the meaning of these ranges? Simple ranges of all best estimates for all species? 5%-95% confidence limits? What is the number of experiments based on how many different individuals in which species, particularly for humans?

Undescribed ranges of this type are absolutely useless for understanding the uncertainty and variability of the data, or for drawing inferences for subsequent steps in the risk analysis.

11. p. 4-61, lines 5-7: "A comparative report of the carcinogenicity of these compounds highlights the qualitative and quantitative concordance of their tumorigenic effects (Melnick and Sills, 2001). The female mouse lung was the most sensitive site of carcinogenicity for both chloroprene and butadiene."

It would be useful to have some quantitative comparison of cancer potency in rodents for these compounds. The full abstract is:

Comparative carcinogenicity of 1,3-butadiene, isoprene, and chloroprene in rats and mice.

Melnick RL, Sills RC.

Chem Biol Interact. 2001 Jun 1;135-136:27-42.

National Institute of Environmental Health Sciences, National Institutes of Health, PO Box 12233, Research Triangle Park, NC 27709, USA. melnickr@niehs.nih.gov

1,3-Butadiene, isoprene (2-methyl-1,3-butadiene), and chloroprene (2-chloro-1,3-butadiene) are high-production-volume chemicals used mainly in the manufacture of synthetic rubber. Inhalation studies have demonstrated multiple organ tumorigenic effects with each of these chemicals in mice and rats. Sites of tumor induction by these epoxide-forming chemicals were compared to each other and to ethylene oxide, a chemical classified by the National Toxicology Program (NTP) and by the International Agency for Research on Cancer (IARC) as carcinogenic to humans. For this group of chemicals, there are substantial species differences in sites of neoplasia; neoplasia of the mammary gland is the only common tumorigenic effect in rats and mice. Within each species, there are several common sites of tumor induction; these include the hematopoietic system, circulatory system, lung, liver, forestomach, Harderian gland, and mammary gland in mice, and the mammary gland and possibly the brain, thyroid, testis, and kidney in rats. For studies in which individual animal data were available, mortality-adjusted tumor rates were calculated, and estimates were made of the shape of the exposure-response curves and ED10 values (i.e. exposure concentrations associated with an excess risk of 10% at each tumor site). Most tumorigenic effects reported here were consistent with linear or supralinear models. For chloroprene and butadiene, the most potent response was for the induction of lung neoplasms in female mice, with ED10 values of 0.3 ppm. Based on animal cancer data, isoprene and chloroprene are listed in the NTP's Report on Carcinogens (RoC) as reasonably anticipated to be a human carcinogen. Butadiene is listed in the RoC as known to be a human carcinogen 'based on sufficient evidence of carcinogenicity from studies in humans, including epidemiological and mechanistic information', with support from experimental studies in laboratory animals. Epidemiology data for isoprene and chloroprene are not considered adequate to evaluate the potential carcinogenicity of these agents in humans.

I believe the similarity of ED10s for lung tumors is potentially helpful for the reader, however, a more comprehensive summary of potencies for other and/or all tumors would provide important background for the quantitative cancer risk analysis. Table 4-37 should be supplemented with a table giving quantification of the indicated potency for multiple- and all sites.

12. p. 4-69, lines 13-19: "One of the strengths of several of the more recent epidemiologic studies was improved exposure assessment data. These studies utilized industrial hygiene information to determine which areas or jobs were most likely to have received higher chloroprene exposures. This allowed for examination of various exposure contrasts and helped reduce the potential for exposure misclassification. As such, valid internal analyses were conducted which were less impacted by bias due to the healthy worker effect. Despite these improvements, several study limitations added to the

uncertainty in addressing the weight of evidence of the epidemiologic data."

The discussion following this paragraph should include the healthy worker survivor effect.

13. Table 5-2: DAFs greater than 1 for lung and less than 1 for nasal epithelium deserve specific discussion.

14. Page 5-20, top: Variability (uncertainty?) in slope factors follows a normal distribution? Try lognormal.

15. Cancer modeling: In view of the saturation of the generation of active metabolite, and the need to drop high doses in some cases, there should be investigation of a Michaelis Menten transformation of dose, in lieu of a full PBPK model. Demonstrate results of this for the incidence of tumors in mice (without the Weibull factor for time dependent tumor observations).

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Garshick E, Laden F, Hart JE, Rosner B, Smith TJ, Dockery DW, Speizer FE. 2004. Lung cancer in railroad workers exposed to diesel exhaust. Environ Health Perspect. 112(15):1539-43.

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worker survivor effect in a cohort of workers in the construction industry. Occup Environ Med. 2001 Dec; 58(12):774-779.

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Ronald L. Melnick

Page 3-2 to 3-5. The discussion on chloroprene metabolism is deficient in its consideration of species differences in glutathione conjugation, catalyzed by glutathione-S-transferase, in the detoxification of (chloroethenyl)oxirane.

Page 3-7 to 3-8. Discussion is needed on likely differences in chloroprene clearance among species. Factors influencing the clearance of chloroprene include fat:air partition coefficients, % of body weight as fat (mouse: 5%; rat: 7%; human 21%), metabolic elimination, etc.

Page 4-13. It seems odd that of the 652 cancer cases in the Louisville facility, only 1 case was unexposed (Table 4-8). This might suggest that a large percentage of individuals classified as exposed were essentially unexposed. The document should provide greater emphasis on the potential impact of exposure misclassifications.

Page 4-16 to 4-17. Use common units for SMR and RR values in Tables 4-10 and 4-11. On some cases the actual ratios are given, while in other cases the ratios are expressed as per cent.

Page 4-22. Contrary to the statement on lines 2-6, the data in Table 4-14 show incidences of ovarian or mammary tumors in control female rats.

Page 4-47, lines 5-7. Additional analyses are needed before dismissing the finding of increased resorptions in the 10 and 25 ppm exposure groups.

Page 4-60. Delete lines 12-15. The hypothesis that chloroprene would only produce tumors in directly exposed tissues has been disproved by the NTP studies which demonstrated the multiple organ carcinogenicity of this chemical.

Page 4-63, line a6. Severities were minimal to moderate, not minimal to mild.

Page 4-73, line 7. The document specifies a mutagenic MOA involving the reaction of epoxide metabolites formed at target sites. Until studies are conducted evaluating blood levels of epoxide intermediates it would not be appropriate to impose this target site

limitation. It is not known if epoxide formation occurs in all of the tumor target sites identified in the rodent carcinogenicity studies.

Page 5-19, Table 5-7. The unit risk value for hemangiosarcomas/hemangiomas is incorrect – it should be 2.8×10^{-5}, not 8.3×10^{-5}.

John B. Morris

Pages 3-1 - 3-6

The data on partition coefficient should be discussed more completely. It is true that it is possible to infer information on tissue distribution from such data. It is also possible to make inferences on regional respiratory tract absorption from these numbers. A vapor with a blood:air partition coefficient less than 10 is not likely to be scrubbed efficiently from the airstream in the upper airways. This is an important point because an inhalation cancer potency factor will be derived assuming category 1 status.

More detail should be provided on the metabolism kinetics for chloroprene. The information on elucidation of putative metabolites is clear and concise, but the data on kinetics is incompletely presented data and is very difficult to interpret fully. The information in Table 3-1 needs to be more fully described. Is this table cited in the text? Precisely how were these data obtained, what is the meaning of these data, particularly with respect to rodent-human extrapolations? The relative level of metabolite 1 in the humans was approximately 10-fold lower than the F344 rat and mouse. The level of metabolite in the Wistar rat and hamster was lower as well. Were these quantitative differences synthesized into a coherent explanation of species differences in response?

Similar issues could be related relative to Tables 3-4 and 3-5. The text should precisely indicate how the estimates for V_{max}/K_m for lung metabolism were obtained. The mouse – human comparison for lung metabolism is particularly important, a fact that was not adequately considered in the risk evaluation. The presented data indicate the activity in human lung is 50-fold lower than in mouse lung (Table 3-4). The liver activities in the mouse and man are much more similar. Since metabolic activation is the first step in the mode of action and lung tumors in mice drives the risk extrapolation, this comparison becomes particularly important. Exactly how was the value of 1.3 for V_{max}/K_m in the human obtained? What is the reliability of this number? Can it or can it not but used for quantitative species extrapolations? An explicit rationale for not using these data in the data synthesis sections needs to be provided. It should be noted that this type of species difference (mouse to human pulmonary metabolism) is hardly unique to chloroprene. For example, consider styrene.

Pages 4-1 - 4-18

The section on human exposures to chloroprene appears to be objectively and concisely presented. Epidemiology is not within my area of expertise. My only comment is the thought that it would be useful if as much information as possible on occupational exposure levels would be presented in the text. At least to me, information on exposure concentrations in addition to cumulative (ppm-year) would be of value. If available,

recent published reviews of the epidemiological data relative to chloroprene should be cited.

Page 4-25

Clarity would be enhanced if the table also provided information on the magnitude of injury in Table 4-16 and subsequent tables. A footnote might be adequate. Alternatively, the average injury score might be provided parenthetically in each column. The wording of the text infers there was no observed histopathological damage in the lungs of mice in the 16 day study. Clarity would be enhanced if this were explicitly stated.

Page 4-28

Clarity would be enhanced if it were explicitly stated that lesions were not observed in the nasal respiratory mucosa in the 13-week study. All lesions in Table 4-19 were in olfactory mucosa, the reader must make the inference that respiratory mucosa damage was absent. This is an important issue relative to data interpretation.

Page 4-29

Clarity might be enhanced if it is stated that preening behavior might have lead to direct gastrointestinal exposure to chloroprene. If this is not thought to be the case, then it should be explicitly stated.

Pages 4-30 - 4-43

It is noted that all nasal lesions in Table 4-16 are presented under the heading of "olfactory," implying that no nasal respiratory mucosal lesions were observed. This needs to be explicitly stated. The subsequent text is quite ambiguous in this regard. For example, in the absence of any respiratory mucosal lesions, why include speculation on the relative expression of CYP450 in olfactory versus respiratory mucosa of the rat nose? (I did a quick scan of the NTP report to confirm, at least superficially, the absence of respiratory mucosal lesions.) All subsequent descriptions of these data, e.g. chronic nasal inflammation (p5-2) should be qualified to state chronic nasal olfactory inflammation (if this is, in fact, true). Site specificity of nasal lesions is a critical aspect in the evaluation of nasal response.

Subsequent portions of the text refer to time to tumor data. Where are these data and derivation described? Should some discussion of maximum tolerated dose and whether it was exceeded be included in the text?

Clarity would be enhanced if the text provided more detail on how the survival adjusted neoplasm rates in Table 4-28 were calculated.

The description of the Trochimowicz et al. 1998 study indicates there was less chronic respiratory disease in exposed than controls. Perhaps more information should be

provided on the lesions that were present in control animals. This would seem to be a relevant issue with respect to interpretation.

Page 4-54

The text (line 20) indicates epoxide hydrolysis was faster for the human and hamster than rat or mouse. Where are these data presented?

Page 4-45

The test (lines 27-32) indicates "some activity" was observed in strains TA97A and TA98. Subsequently (p. 4-65), it is stated the epoxide mutagenicity is "positive in all strains." Are these two parts of the text concordant?

Page 4-61, Table 4-37

This table is very confusing. What was the basis for including data from the rat relative to "sites of increased incidence" of neoplasms? Listed are many sites in which statistically significant results were not enumerated in previous portions of the text. Obviously, clarity needs to be improved.

Pages 4-62 - 4-65

In general, this "synthesis" of the inhalation exposure data is not a synthesis but merely a reiteration of the results. Rather than repeat the results study by study, it might be much preferable to organize this section on the basis of target organ. It could, for example, discuss the olfactory lesion data in toto, followed by the liver, etc. On page 4-62 line 15, it is stated that chloroprene is associated with reproductive and developmental effects, yet the earlier portions of the text concluded otherwise.

Table 4-38

Table 4-38 is somewhat confusing. Why was lung cancer mortality listed under "rare tumors?" The table includes a reference to time to tumor, yet such data were not presented earlier in the text.

Page 4-72

Lines 11-12 include a listing of increased incidences of tumors, yet the basis for inclusion in this listing is unclear. Some organs are listed in which the tumor incidence was not significantly increased. The discussion of species differences (lines 27-31) should include reference to possible species differences in epoxide hydrolysis rates. Such data are presented earlier and its absence here is confusing. This section fails to include the most important species difference – the appearance of lung tumors in mice but not rats. An in situ pulmonary metabolic basis might be provided, given that the metabolic activation rate in mice appears to be 50-fold higher than the rat but that in the liver differs by only

2-fold (Table 3-4 and 3-5). This would also serve to emphasize the potential role of metabolism relative to carcinogenicity. Epoxide formation is thought to be important relative to the respiratory tract toxicity/carcinogenicity of naphthalene and styrene and the same species differences (lung tumors in mice but not in rats) is seen for these vapors. Line 32 includes a reference to Dong et al 1989; this study was not described previously.

Page 4-75

The statement that in vivo uptake of chloroprene involved the balance between epoxide formation and detoxification is confusing. Certainly the toxicity depends on the balance, but it is unlikely that uptake does. Uptake rates depend on the blood and tissue concentration of parent, downstream conversion of metabolite is not necessarily important in diffusion-based uptake. Greater clarity is needed.

Page 4-76

It is stated on lines 3-4 that there is remarkable similarities in the potency and shape of the dose response between butadiene and chloroprene. Such data are not presented in earlier portions of the text.

Page 4-77

It is stated that Melnick et al. (line 18) performed a 6 month exposure-6 month follow-up study. Where are these data presented?

Page 5-3, top

The text needs to clearly describe how the atrophy and necrotic data were combined. I am not certain there are any data indicating nasal olfactory atrophy leads to necrosis (as stated on lines 5-6). The concept that necrosis may lead to atrophy is quite straightforward however.

Page 5-5

In my view, chloroprene is not a category 1 gas (see also my comments above). Its partition coefficient is only 10, clearly backpressure in nasal tissues controls the uptake process. The presence of non-respiratory tract tumors clearly indicates it is absorbed into the bloodstream. This vapor does not possess the physical chemical characteristics required of category 1 gases; in my view, it is a category 3 gas. The text needs to rigorously support this conclusion with respect to the physical chemical characteristics of chloroprene relative to those required of category 1 gases. The presence of olfactory lesions is NOT evidence that the toxicant was delivered via the airstream. Numerous compounds produce selective olfactory injury after parenteral administration. Indeed, the presence of olfactory but not respiratory nasal mucosal injury might be considered to provide data in support of a blood-borne mechanism. Naphthalene is one example of this phenomenon. Importantly, the subsequent text describes in great detail how the lung

lesions may be due to blood-delivered rather than air-delivered chloroprene. The text needs to be consistent. Redistribution of chloroprene from fat stores during non-exposure periods is one potential mechanism for a role of blood borne chloroprene in inducing olfactory lesions.

The RfC methodology is fatally flawed with respect to RGDR calculation. The derivations of these equations are based on the faulty assumption that the mass transfer coefficient is uniform throughout the nose. Dosimetry predictions from RGDR-based evaluations are totally discordant with the data. For example, the RGDR-predictions are counter to the theoretically sound modeling and experimental data obtained for formaldehyde and vinyl acetate. The RGDR-based estimates of species differences in dosimetry are discordant with the database on acetaldehyde dosimetry in multiple laboratory animal species. While application of a flawed methodology may be consistent with EPA policy, it certainly is not consistent with the scientific state-of-the-art. Perhaps it is felt that chloroprene is truly a category 2 gas, but it is assigned category 1 status because of difficulty in implementing RGDR calculations for category 2 gases. If so, it should be explicitly stated. As noted above, its low partition coefficient and the existence of distal organ effects indicate chloroprene is likely a category 3 gas.

The mode of action is assumed to include metabolic activation to the epoxide. The RGDR of 0.28 indicates the humans will receive roughly 4-fold more toxicant (1/0.28) than the rat. Is it meant to imply that the metabolic activation rate in the human nose is 4-fold higher than the rat? Is there a single example of this being the case? The use of the RGDR needs to be discussed in light of the metabolically-based mode of action.

Page 5-8

I recognize that it may be policy to include a database limitation factor due to the lack of a two generation study, but I do not feel it is scientifically justified in this case. A multigeneration study does exist. The rationale for the selection of this uncertainty factor should include this study.

Table 5-3

Table 5-3 does not include a row in the consideration column for database limitation.

Table 5-4

This table provides time to tumor data, but such data have not been presented.

Page 5-21

Would it be possible to compare the tumor risk calculations with the human workplace experience? This might provide a useful "reality check." Even if the occupational exposure levels were only crudely known, it might be possible to determine if the estimated cancer risks were at least somewhat reflective of reality.

Page 5-25

The cross-species scaling section is deficient in that it does not include consideration of metabolism rate. The first step in the mode of action is metabolic activation to an epoxide and the toxicokinetic data indicate the mouse lung activity exceeds that in the human by 50-fold (Table 3-4). Clearly, this is highly relevant. Moreover, magnitude of species difference in metabolism is not unique, consider styrene or naphthalene. One might convincingly argue that the enormous metabolic activation rate in the mouse coupled with the low epoxide hydrolysis rate renders this species inappropriate relative to extrapolation of lung tumors. The authors of the document may not agree, but a critical discussion and rationale for using the mouse data needs to be included.

Page 6-5

The sentence on lines 18-19 is confusing. Lesions were specific to the olfactory mucosa, what is the relevance of cytochrome P450 in respiratory mucosa in this regard?

Avima M. Ruder

Page 2-1 line 12. volume produced or volume used?

Page 2-1 line 18. Is Mg a million grams? Not in List of Abbreviations.

Page 2-1 line 22. Starting material for chloroprene synthesis is butadiene *in the U.S.*

Page 2-2 line 15. Suggest rewording to: The polymerization process has been discussed…

Page 3-2 line 5. Suggest inserting "that of" between "similar to" and "vinyl chloride"

Page 3-4 Figure 3-1 and caption. Why these numbers? Why not consecutive in key/caption? Why no 2, 3, 6, etc.?

Throughout section 4, SMRs and SIRs should consistently use base 1 or base 100, not vary (cf pp 4-10 and 4-11). The adjectives low-exposure and high-exposure are not consistently hyphenated (cf p 4-2 lines 18 and 19 versus line 25, p 4.7 table 4-4 title vs. header for column 3). Deaths can be in excess but cannot be elevated (cf p 4-3 line 13). SMRs can be elevated. Deaths in and of themselves cannot be statistically significant; SMRs can be (cf p 4-3 line 13). Mortality is a rate and therefore "Mortality rate" (cf page 4-6 line 22) is redundant. Check citations! Leet and Selevan becomes Leet and Sullivan in tables 4-10 and 4-11.

Page 4-1 line 2. occupationally exposed should not be hyphenated. "during" not "from" the period …

Page 4-1 line 8. delete "and" at beginning of line

Page 4-1 line 20. Need comma after 1957. Similarly page 4-3 lines 24-25, page 4-4 line 13, etc.

Page 4-2 line 14. Change "both internal…and" to "either internal…or"

Page 4-2 lines 24-25. Needs commas after SMR and liver.

Page 4-2 line 31. Lack of adjustment (data were available) or lack of ability to adjust (data were unavailable)?

Page 4-3 line 8. A total…*was* observed

Page 4-3 line 13. Suggest rewording to: "observed cancer deaths were also in excess (SMR = 140) but the SMR was not statistically significant…"

Page 4-3 line 14. Change last phrase to "and four deaths due to lung cancer"

Page 4-3 lines 15-17. Suggest rewording to: "With five observed cancers of the urinary system (3 bladder and 2 kidney) the SMR was significantly elevated (300 compared to the DuPont population and 250 compared to the U.S.…."

Page 4-3 line 23. Suggest "accrued" instead of "worked for"

Page 4-3 line 24. Should be "*was* identified" (subject is "a cohort")

Page 4-4 line 3. Were exposures determined or estimated?

Page 4-4 lines 8-10. The sentence as written doesn't actually state that males had increased exposure. Suggest "Males had statistically significant ($p<0.005$) greater exposure to chloroprene than females based on…"

Page 4-4 line 11. Subgroup has not been defined.

Page 4-4 line 13. "their *dates* of death"

Page 4-4 line 15. Suggest "sixteen reported cancer deaths occurred among…"

Page 4-5 Table 4-2, row "researcher". All cause cell needs slash between 21 and 176.

Page 4-5 line 1. Suggest "One limitation of the Li et al. (1989) study was insufficient comparison mortality data"

Page 4-5 line 2. "years *were* not"

Page 4-5 line 4. "*time* periods"

Page 4-5 line 6. Suggest "...during the time periods with no rates available,..."

Page 4-5 line 8. "there *were* no data..."

Page 4-5 line 17. "age at death was 12.7 years *younger*"

Page 4-6 line 7. Not clear whether "lasting and making" is one or two departments

Page 4-6 line 10. Locations or departments?

Page 4-6 lines 11-12. Suggest: "year. They therefore devised a relative exposure system. Workers in the high-exposure departments were assigned..."

Page 4-6 lines 19-20. Suggest: "Thirty-seven percent of cohort members (female/male distribution was not provided) contributing 26,063 person-years..."

Page 4-6 line 22. Suggest: "Mortality of the general population of Moscow was used for comparison."

Page 4-6 line 24. Suggest "available only"

Page 4-6 line 25. "the *rate* of expected deaths"

Page 4-6 lines 29-31. Need to specify that SMRs were elevated, not just statistically significant. What are "cancer-specific SMRs for liver cancer and leukemia" as opposed to "SMRs for liver cancer and leukemia"?

Page 4-7 line 4. "low number". Is this a statistically significant decrease? Or provide expected.

Page 4-7 line 8. Delete comma after leukemia.

Page 4-4 Table 4-4 header. All cases or just high-exposure cases?

Page 4-7 lines 10-11. Suggest: "...analysis by categories of duration of employment in high-exposure jobs (1-9..."

Page 4-7 line 12. Need new paragraph starting with "The cumulative.."

Page 4-7 line 15. "Kidney cancer was increased in all categories..." Are these categories of duration of employment as in lines 10-11 or tertiles or quartiles of cumulative exposure?

Page 4-8 line 13. "Similar to *the* Li et *al.* study..."

Page 4-8 line 14. Suggest: "...values if *mortality during* these years *was* not representative..."

Page 4-8 line 20. "Death certificates were coded by using *the* ICD-9..."

Page 4-9 line 9. Suggest: "Cancer incidence data *were available for* 1979-1999..."

Page 4-9 line 10. "...were identified, with six liver.."

Page 4-9 line 13. "lung cancer *in* both the total..."

Page 4-9 line 20. "noted *in analyses* using..."

Page 4-9 lines 21-22. "...five cases in *the* highest cumulative exposure *category* of..."

Page 4-10 line 7. "adjusted for *in either* mortality..."

Page 4-10 line 12. "time" of employment—era of employment or time of first employment?

Page 4-10 line 23. "...estimated *daily* exposure..." ?

Page 4-10 lines 29-30 states that 32 cancers occurred prior to 1977. How is that possible if the registry began in 1979? Does this mean 32 cancers occurred among those exposed prior to 1977?

Page 4-10 line 32 states all SIRs exceeded 100. Table 4-7 presents SIRs using base 1. Page 4-11 Table 4-7 header 3rd column. Cases Exposed before 1977?

Page 4-11 lines 2-3. "lung cancers *occurred* in workers with >20 years of exposure..., 3 in *those with* 11-20 years...and 1 in *those with* ≤10..."

Page 4-11 line 10. "the lung *cancer* excess..."

Page 4-11 line 11. "...smoking and alcohol consumption *were*..."

Page 4-11 line 18. Suggest: "...using external regional rates and internal comparisons..."

Page 4-11 line 20. "...both chloroprene and a potential..."

Page 4-12 throughout. As done in some places, but not consistently, label data with plant initials instead of providing a string of numbers and then stating "respectively". For example, line 9, change "1.54 and 0.094 ppm, respectively" to "1.54 (L) and 0.094 (M)". Similarly in lines 11, 24, 25.

Page 4-12 lines 4-6. Suggest: "Kentucky, and Ponchartrain (P), Louisiana. The third one was the Maydown (M) plant in Northern Ireland and the fourth facility was the Enichem Elastomer plant in Grenoble (G), France."

Page 4-12 line 8. Suggest "occurred at" instead of "existed in"

Page 4-12 line 14. "cohorts" (as in line 10)

Page 4-12 line 23 states 266, 48, 12, 10 for lung cancer deaths; table 4-8 has these numbers for all respiratory cancer deaths. Were all respiratory cancer deaths lung cancers?

Page 4-12 line 26. Suggest: "deaths *than expected* from liver cancer were…"

Page 4-12 line 29. Suggest: "~~when~~ compared to expectations based on the general population. When…"

Page 4-13 line 2. "trends across quartiles of exposure were examined"

Page 4-13 line 14. "included" instead of "contained"

Page 4-13 line 23. Delete "the" at end of line

Page 4-14 line 4. "…work status *was* so highly…"

Page 4-14 line 7. "They found inverse *associations*…"

Page 4-15 lines 7-8. "cohorts had *fewer* than 1000 workers, while the remaining cohorts had *fewer* than 6000."

Page 4-17, line 8. "…Louisville, Kentucky, plant."

Page 4-18 line 16. "found in workers *who*…"

Page 4-18 line 32. "…cohorts *on* different…

Page 4-19 line 7. "…much *lower* numbers…" or "many fewer numbers"

Page 4-20 line 1. "…19-23 employed…"

(I did not read section 4.2 as closely as the preceding section; there may be errors and ambiguities I did not catch.)

Page 5-15 line 3. Delete period preceding 1st word in line

Page 5-17 line 26. "multis*t*age-Weibull…"

Page 5-21 line 21. EPA 1994A or EPA 1994B?

Page 7-3 lines 19-20. Only articles by the same author (which these are not) should be labeled 2001a and 2001b.

Page 7-5 line 33. "...*life* table analysis..."

Page B-2 Figure B-1. Abbreviations should be explained in a caption (similarly for other figures). What is the metric for the doses (horizontal axis)?

Richard B. Schlesinger

Section 4.6. The first paragraph of this section should have a subsection 4.6.1. Human Studies and the Animal Studies should be renumbered as 4.6.2.

Section 4.7. This section could be better organized. The summary in section 4.7.1 should probably be moved to the end of the entire section on carcinogenicity. The human data are discussed separately in an Evidence for Causality section, yet this is not provided for the animal studies. A true synthesis would discuss Evidence for Causality across studies in all species. This could be integrated with the discussion in Section 4.7.3.3 on Mode of Action to provide a stronger rationale for effects of chloroprene

EXHIBIT 6

Critical Review of US EPA Epidemiologic Review of Chloroprene Carcinogenicity Underlying the 2010 Toxicological review of Chloroprene and EPA's Denial of Denka Performance Elastomer LLC's Request for Correction (RFR #17002)

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

Critical Review of US EPA Epidemiologic Review of Chloroprene Carcinogenicity Underlying the 2010 Toxicological Review of Chloroprene and EPA's Denial of Denka Performance Elastomer LLC's Request for Correction (RFC #17002)

Cardno ChemRisk
Shaping the Future

Prepared by Gary Marsh, PhD, FACE
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Date 24 April 2018

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I. INTRODUCTION AND PURPOSE OF MEMO

Denka Performance Elastomer LLC (DPE) requested that Cardno ChemRisk provide a review of the epidemiological data underlying the EPA Integrated Risk Information System (IRIS) 2010 Toxicological Review of Chloroprene ("2010 Review") and the EPA January 25, 2018 denial ("the Denial") of Denka Performance Elastomer LLC's Request for Correction (# 17002). On behalf of Cardno ChemRisk, Dr. Gary Marsh and Dr. Natalie Egnot prepared this memorandum. The curriculum vitae of the authors are included as Attachments 1 and 2 to this memorandum.

Overview of Memo Contents and Conclusions

- The epidemiological literature regarding chloroprene exposure and cancer mortality reviewed by the US EPA in the 2010 Review consists of evidence from seven independent worker cohorts, four of which were included within the most comprehensive and definitive study on this topic: the 2007 University of Pittsburgh study of workers from the US, Ireland, and France who were occupationally exposed to chloroprene.

- The University of Pittsburgh study did not identify statistically significant elevations in all-cancer, lung cancer, or liver cancer deaths among workers exposed to chloroprene compared to the appropriate national or regional population rates.

- Similarly, no statistically significant evidence of a positive trend between the duration or level of chloroprene exposure and liver cancer was observed among workers in this rigorous study.

- EPA incorrectly concluded in the 2010 Review that the University of Pittsburgh study revealed evidence of a dose-response relationship between cumulative chloroprene exposure and liver cancer mortality risk. This conclusion was based on EPA's misinterpretation of certain risk values that were inflated by inordinately low liver cancer mortality rates in the baseline category used to calculate relative risks.

- EPA's assertion of a dose-response relationship for chloroprene and liver cancer starkly contrasts the University of Pittsburgh study authors' conclusion that the study provided no evidence of such an exposure-response relationship.

- Overall, the available epidemiological evidence provides no consistent or credible evidence of chloroprene carcinogenicity in humans.

II. BACKGROUND AND OVERVIEW OF MARSH ET AL. 2007 CHLOROPRENE STUDY

In the early 2000s, I, Dr. Gary Marsh, along with colleagues at the University of Pittsburgh and collaborators from the University of Illinois and University of Oklahoma, conducted the largest and most comprehensive historical cohort study of industrial workers exposed to chloroprene. The study, which specifically investigated mortality due to malignant and non-malignant causes among workers exposed to chloroprene and vinyl chloride, included 12,430 individuals employed at one of two U.S. industrial sites (Louisville, KY (n=5,507) or Pontchartrain, LA (n=1,357)) or two European sites (Maydown, North Ireland (n=4,849) or Grenoble, France (n=717)). Investigators from the University of Illinois and University of Oklahoma conducted a comprehensive retrospective exposure assessment of chloroprene, and the results of that assessment were linked to the epidemiological data from the worker cohorts in order to evaluate exposure-response relationships for lung and liver cancer. We computed standardized mortality ratios (SMRs) comparing mortality rates among the chloroprene-exposed workers to the age-sex-race-time-specific mortality rates of national and regional reference populations. We also conducted internal mortality comparisons (worker to worker comparisons) for liver and lung cancer in relation to duration and level of chloroprene exposure.

This comprehensive and definitive study (referred to as the University of Pittsburgh or UPitt) study was designed to address the major limitations of prior studies regarding the health effects of chloroprene exposure, including but not limited to small sample size, inadequate exposure assessment, and questionably appropriate reference rates of cancer mortality for the regional or national population. The results of UPitt study were reported in 2007 in a series of publications in the peer-reviewed journal, *Chemico-Biological Interactions* (Esmen et al. 2007a; Esmen et al. 2007b; Hall et al. 2007; Leonard et al. 2007; Marsh et al. 2007b, 2007a).

Ultimately, the results of the UPitt study did not identify any elevated risks of cancer, including liver and lung cancers, among the cohort of chloroprene-exposed workers. In fact, my colleagues and I identified statistically significant overall *deficits* (that is, a smaller than statistically expected number of deaths) in mortality from all-cancers among the cohorts of workers when compared to the national or corresponding regional population. Specifically, when compared to their corresponding regional populations, we consistently identified overall deficits in both liver and lung cancer mortality rates among workers in the Louisville cohort (17 deaths, SMR=0.90 95% CI=0.52-1.44, and 252 deaths, SMR=0.75 95% CI=0.66-0.85, respectively), Maydown cohort (1 death, SMR=0.24 95% CI=0.01-1.34, and 43 deaths, SMR=0.78 95% CI=0.56-1.05, respectively), and Grenoble cohort (1 death, SMR=0.56 95% CI=0.01-3.12, and 4 deaths, SMR=0.47 95% CI=0.13-1.20, respectively) (Marsh et al. 2007a). No cases of liver cancer were identified among workers in the Pontchartrain cohort; therefore, SMRs for this outcome could not be calculated for workers in this facility. However, similar to the other study sites, we observed a deficit in lung cancer mortality when comparing the Pontchartrain cohort to the regional population (10 deaths, SMR=0.55 95% CI=0.26-1.00) (Marsh et al. 2007a).

We conducted additional analyses of certain subgroups, including only those workers who had been exposed to chloroprene. Across all plants, deficits in all-cancer mortality (806 deaths, SMR=0.71 95% CI=0.66-0.76), lung cancer mortality (330 deaths, SMR=0.75 95% CI=0.67-0.84) and liver cancer mortality (17 deaths, SMR=0.71 95% CI=0.42-1.14) were observed among the exposed workers. The deficits for all-cancer and lung cancer were statistically significant. Among liver cancer cases identified within the Louisville cohort (n=17), we conducted an exposure-response analysis to evaluate possible trends in liver cancer mortality risk associated with increasing chloroprene exposure. This analysis could only be conducted within the Louisville cohort because no cases of liver cancer were identified within the entire Pontchartrain cohort, and the investigation of the Maydown and Grenoble cohorts identified only one confirmed liver cancer case at each site. The exposure-response analyses revealed no statistically significant elevations in liver cancer mortality risk among individuals at any level of chloroprene exposure, and revealed no evidence of any statistically significant trends in liver cancer mortality risk relative to three metrics of chloroprene exposure (duration of exposure, average intensity of exposure, and cumulative exposure).

III. CRITIQUE OF EPA EVALUATION AND INTERPRETATION OF STUDIES PUBLISHED PRIOR TO UPITT STUDY

In their 2010 Toxicological Review of Chloroprene, the EPA authors reviewed epidemiological studies conducted among seven worker cohorts from Armenia (Bulbulyan et al. 1999), China (Li et al. 1989), France (Colonna et al. 2001; Marsh et al. 2007b, 2007a), Ireland (Marsh et al. 2007b, 2007a), Russia (Bulbulyan et al. 1998), and the US (Marsh et al. 2007b, 2007a; Leet et al. 1982), each of which included individuals who were occupationally exposed to chloroprene. The authors of the 2010 Review made several critical errors when evaluating the studies published prior to the UPitt study. First, two of the studies considered in the 2010 Review assessed mortality among workers from the same facility that eventually constituted the Louisville cohort within the UPitt study (Leet et al. 1982; Pell 1978). The results of these studies were inappropriately considered as independent of the UPitt study within the 2010 Review even though the UPitt study included members of the prior cohorts and was specifically designed to address limitations of these studies. Second, the epidemiological studies published before the UPitt study have substantial limitations in terms of study design and analytical methods, many of which were identified in the 2010 Review's evaluation of these studies. Despite acknowledging these limitations, the authors of the 2010 Review utilized the considerably flawed epidemiological literature published prior to the UPitt study to support their conclusion that chloroprene is "*likely to be carcinogenic*" in humans (US EPA 2010b). Third, when interpreting the epidemiological evidence used to support their conclusions regarding chloroprene carcinogenicity, the authors of the 2010 Review gave many of the poorer quality studies the same weight as the more robust UPitt study.

The 2010 Review should not have treated the Leet and Selevan study as independent of the UPitt study. In 1982, Leet and Selevan reanalyzed the data collected from the DuPont Louisville facility by Pell et al. in 1978 using a modified life-table analysis, and identified a statistically significant elevation in liver/biliary cancer (4 deaths, p=0.01) among exposed workers. No statistically significant trends were identified in regard to latency or duration of chloroprene exposure. The Leet and Selevan findings were based on a crude, qualitative exposure assessment, and suffered from small sample sizes within stratified analyses. The UPitt study provided an updated and more thorough analysis of the Louisville cohort that had previously been evaluated by Leet and Selevan. The 2010 Review states that "*sufficient differences between these two studies investigating the Louisville cohort warrant independent analyses of each*" (US EPA 2010; pp.A-13). The differences in analytical approaches between these two studies do not supersede the fact that their subjects are not independent. Further, the UPitt study employed a more methodologically rigorous analytical strategy when evaluating the cohort of Louisville workers. Because these two studies included overlapping members of the same cohort and the UPitt study provided a more rigorous evaluation of these participants, it was not appropriate for the EPA to include the Leet and Selevan study in their evaluation of chloroprene carcinogenicity.

The remaining cohort studies of chloroprene and cancer mortality that the EPA considered in the 2010 Review suffer from substantial limitations such as a lack of an appropriate comparison group for effect estimate calculation, weak exposure assessment, and small sample size particularly in stratified analyses, all of which were addressed in the design of the 2007 UPitt study. For example, Li et al. published the results of a cohort mortality study of Chinese chloroprene-exposed workers in 1989 that lacked representative mortality rates to which the cohort could be compared, and conducted only a qualitative exposure assessment (Li et al. 1989). Specifically, although mortality follow-up was conducted from 1969-1983, local age- and sex-specific rates used to calculate SMRs were obtained only from 1973-1975. Similar to the Chinese study, Bulbulyan et al. utilized local liver cancer incidence and mortality rates from only two years (1992-1993) in order to calculate SMRs for liver cancer among a Russian cohort although mortality follow-up lasted from 1979-1993 (Bulbulyan et al. 1998). Internal-comparison analyses were conducted based on a qualitative assessment of chloroprene exposure, duration of high exposure, and cumulative exposure. These analyses suffered from very small sample sizes, and imprecise risk estimates. For example, the only statistically significant result among these internal comparisons was an elevated relative risk (RR) of liver cancer (RR=45) based on only one observed case of liver cancer among those with 20+ years of high chloroprene exposure resulting in a 95% confidence interval ranging from 2.2-903.

Bulbulyan et al. also conducted a mortality study among an Armenian cohort of chloroprene-exposed workers which suffered from the same limitations as the Russian study, and resulted in similarly imprecise risk estimates due to small sample size (Bulbulyan et al. 1999). More recently, Colonna and Laydevant conducted a cohort study of chloroprene exposure and cancer incidence among workers at the Isere/Grenoble facility evaluated in the UPitt study (Colonna et al. 2001). This study collected cancer incidence data from 1979-1997 and utilized cancer incidence rates from a local registry for the same time period in order to facilitate comparisons. Only one case of liver cancer was identified among the cohort and no statistically significant elevations in incidence of all-cancers, lung cancer, or liver cancer were observed within the cohort.

Ultimately, despite the substantial limitations of the studies published prior to the UPitt study, the authors of the 2010 Review gave the results of these studies equal consideration to the results of the UPitt study when forming conclusions regarding the epidemiological evidence of chloroprene carcinogenicity. The UPitt study overcame the limitations of the earlier studies by including a greater number of participants, conducting a more rigorous and comprehensive exposure assessment, and using appropriate comparison groups for the calculation of SMRs. The UPitt study included more participants than all other studies conducted on this topic combined, and included more than 350,000 person-years of follow up. Moreover, the 2007 study utilized age-sex-race-time-specific mortality rates from appropriate comparison populations (national, regional, and internal), and included detailed data on participant demographic, work history and chloroprene exposure information that was lacking in the other cohorts. Therefore, the authors of the 2010 Review should have given the conclusions of the UPitt study greater weight than the other studies published on this topic when considering the epidemiological literature. Instead, the EPA's conclusion that chloroprene is *likely carcinogenic in humans* based on the epidemiological literature is reliant on the limited and biased studies published prior to the UPitt study.

IV. EPA'S MISREPRESENTATION OF UPITT EXPOSURE-RESPONSE ANALYSIS FOR LIVER CANCER

The authors of the 2010 Review also grossly misrepresented the results of the UPitt historical cohort study in their 2010 Report. Specifically, the 2010 Review focused on a limited series of results from the UPitt study based on internal comparisons among workers at the Louisville plant, and others based on comparisons among DuPont workers nationally. Our serious concerns about how the US EPA interpreted and reported the UPitt study results are described in further detail below.

The 2010 Review suggests that the results of the UPitt study provide evidence in support of an exposure-response trend between chloroprene exposure and liver cancer. Specifically, Appendix A of the 2010 Review states, "*Although no statistically significant increase in risk of liver cancer was detected in the most recent and comprehensive cohort study involving workers at four plants (Marsh et al., 2007), the observed RR [of liver cancer] increased with increasing cumulative exposure in the plant with the highest exposure levels, indicating a dose-response trend*" (US EPA 2010b). The US EPA authors obtained these results from a limited exposure-response analysis based on a total of only 17 liver cancer deaths observed in the Louisville cohort, which was fewer liver cancer deaths than statistically expected based on regional rates. Only two liver cancer deaths were observed among the other UPitt study sites combined. The Table below shows the relevant results from the Louisville cohort in the UPitt study.

Table: Exposure-response analysis for chloroprene and liver cancer by exposure metric. Louisville plant, relative risks (RR) and standardized morality ratios (SMR). From Marsh et al. 2007b.

Metric[a]	Observed Deaths	Internal Rate Analysis			External Rate Analysis[b]	
		Non-cases[c]	RR[d] (95% CI)	p-value	Person-Years[e]	SMR (95% CI)
Duration of Exposure (years)						
<10	6	1500	1.00	Global=0.24	131,276	0.61 (0.22-1.32)
10-19	4	216	3.85 (0.76-17.09)	Trend=0.36	30,404	2.08 (0.57-5.33)
20+	7	965	1.75 (0.49-6.44)		36,239	0.99 (0.40-2.04)
Average Intensity of Exposure[f]						
<3.6216	3	714	1.00	Global=0.22	69,274	0.62 (0.13-1.80)
3.6216-8.1245	7	568	3.81 (0.77-25.76)	Trend=0.84	27,933	1.73 (0.70-3.56)
8.1246-15.99	3	388	1.84 (0.22-15.74)		28,689	0.94 (0.19-2/74)
16.0+	4	1011	1.31 (0.20-10.07)		72,023	0.59 (0.16-1.52)
Cumulative Exposure[g]						
<4.747	2	744	1.00	Global=0.17	68,918	0.43 (0.05-1.55)
4.747-55.918	3	725	1.90 (0.21-23.81)	Trend=0.09	56,737	0.59 (0.12-1.74)
55.919-164.052	7	653	5.10 (0.88-54.64)		39,840	1.62 (0.65-3.33)
164.053+	5	559	3.33 (0.48-39.26)		32,424	1.00 (0.33-2.34)

[a] Decimal places of cut points reflect precision needed for computational purposes only and not precision of exposure assessment
[b] Local county rates
[c] The number of persons in decedent's risk set used in calculation of RR
[d] Adjusted for sex
[e] Number of person-years used in calculation of SMR
[f] Ratio of cumulative exposure to duration of exposure (in ppm)
[g] Product of the number of dates in each job function and estimated average daily exposure (in ppm years)

The 2010 Review inappropriately and inaccurately suggests that the results of the exposure-response analysis of the Louisville cohort shown above indicate a "*dose-response trend*" between chloroprene exposure and liver cancer mortality (US EPA 2010b). As shown in the table, statistical tests of trend by increasing exposure metrics including duration of chloroprene exposure, average intensity of chloroprene exposure, and cumulative chloroprene exposure were performed and were consistently not-statistically significant. Moreover, none of the risk estimates based on exposure-response metrics appeared to have a monotonic, or consistent, positive relationship with liver cancer risk based on statistical tests of trend. The interpretation of these results provided in the 2010 Review is in stark contrast to the interpretation provided by the UPitt study authors: "*Although RRs for the cancer sites and exposure measures considered were elevated in many non-baseline categories due to the low baseline rates, we observed no consistent evidence that RRs were positively associated with increasing exposure in any of the study plants*" (Marsh et al. 2007b).

The not statistically significant elevation in RRs observed from the UPitt exposure-response analysis for liver cancer among the Louisville cohort can be attributed largely to the fact that the lowest exposure groups for each exposure metric, which served as the baseline category for the calculation of the RRs, had unusually low mortality rates of liver cancer. These inordinately low baseline rates are demonstrated by the large deficits in lung cancer mortality when each of the exposure groups is compared to the regional population. Specifically, the SMRs among the least exposed, or baseline groups, in terms of

duration of chloroprene exposure, average intensity of chloroprene exposure, and cumulative chloroprene exposure, were 0.61, 0.62, and 0.43, respectively. These inordinately low mortality rates in the baseline category create the impression of large excesses in risk among persons in the non-baseline categories. For example, the 5.1-fold elevation in liver cancer risk for workers in the third highest cumulative exposure category (7 deaths, RR=5.10, 95%CI=0.88-54.64) reflects the fact that persons in that exposure category had a moderate, not statistically significant 1.62 fold rate of liver cancer (SMR = 1.62, 95% CI=0.65-3.33) compared with the regional standard population, and these workers were compared with workers in the baseline category who had a 57% deficit in liver cancer mortality based on the regional comparisons (SMR = 0.43, 95% CI=0.05-1.55). Thus, an internal comparison of these two groups results in an apparent but misleading greater than five-fold excess in liver cancer mortality.

Internal comparisons are an effective method of addressing healthy worker bias, which particularly affects risks of death from non-malignant causes such as cardiovascular disease or all-cause mortality. However, as illustrated above, risk estimates obtained from internal comparisons must be interpreted with caution as they may produce misleading estimates of mortality risk if workers in the baseline exposure category used to calculate internal RRs have an inordinately low (or high) risk of mortality compared with workers in the non-baseline groups. This phenomenon was addressed in a 2007 publication regarding this study, and has been observed and discussed in other cohort studies of workers exposed to acrylonitrile and formaldehyde (Marsh et al. 2007b; Marsh et al. 2001; Marsh et al. 2014). However, this explanation of the elevated RRs obtained from the exposure-response analysis was not discussed within the 2010 Review. It is also worth mentioning that this internal comparison analysis was conducted only among liver cancer cases from the Louisville cohort (n=17), and number of deaths in each of the exposure categories ranged from only 2 to 7. The small sample size evaluated within this portion of the analysis resulted in imprecise risk estimates as shown by the wide confidence intervals. The 2010 Review thus should not have given such large weight to unremarkable and not statistically significant results obtained from a limited exposure-response analysis of liver cancer conducted in only one study site in the UPitt study.

V. EPA'S MISINTERPRETATION OF DUPONT EMPLOYEE INTERNAL COMPARISONS AND HEALTHY WORKER EFFECT IN UPITT STUDY

In 2007, Leonard et al. published the results of an internal mortality analysis comparing chloroprene-exposed workers from the Louisville and Pontchartrain facilities to regional and national samples of DuPont workers along with the series of publications regarding the UPitt study. There were no statistically significant elevations in liver cancer mortality among the Louisville workers compared to other DuPont workers regionally (SMR=1.21; p>0.05) or nationally (SMR=1.27; p>0.05) (Leonard et al. 2007). Again, because no cases of liver cancer were observed among workers in the Pontchartrain facility, risk estimates for this outcome could not be determined.

The 2010 Review highlighted statistically significant elevations in all-cancer and respiratory cancer mortality that were observed when comparing workers from the Louisville cohort to a national sample of DuPont workers. When compared to DuPont workers regionally, only SMRs for all-cause mortality and lung cancer mortality remained significantly elevated in this cohort. The increase in SMRs observed in these specific analyses was not unexpected. Some of the increase in SMRs can be attributed to regional variation, while a reduction in healthy worker bias also likely played a role, particularly in regard to the all-cause mortality outcome. However, the health worker effect is unlikely to have influenced the results related to malignant causes such as lung and liver cancers due to their relatively sudden onset, short survival time, and high case-fatality rate (Enterline 1976). Ultimately, these results provide evidence that workers may more strongly reflect their local and regional populations rather than a more widely dispersed population of workers in terms of their mortality experience.

Interestingly, the 2010 Report does not mention that exposure-response SMRs for all-cancer and lung cancer were also calculated comparing the Louisville cohort to DuPont workers regionally, and the results were, with few exceptions, not statistically significant. Instead, evidence suggesting that there was no clear consistent positive trend across the increasing exposure groups was ignored by the 2010 Review. It is also worth mentioning that 48 effect estimates were reported in the Leonard et al. paper, which should be considered within the context of the series of six epidemiological publications that reported results of

the UPitt study. These results were not adjusted based on the fact that multiple statistical comparisons were made as part of this investigation. Therefore, it is misleading for the EPA to put such weight on these few statistically significant estimates comparing the Louisville workers to DuPont workers nationally when the vast majority of results obtained from this study were consistently null.

VI. CRITIQUE OF EPA CONCLUSIONS BASED ON BODY OF EPIDEMIOLOGICAL LITERATURE

a. Application of Bradford Hill Causal Criteria

According to the 2010 Review, Bradford Hill causal criteria were utilized to assess the body of epidemiological literature as recommended by the EPA Guidelines for Carcinogenic Risk Assessment (Hill 1965; US EPA 2005). The EPA, however, did not apply the Hill criteria to the epidemiological studies of chloroprene exposure in a uniform or consistent way, rather their selective application of Hill criteria was misleading and overstated the evidence of a relationship between chloroprene and cancer mortality. A description is provided below of the selective Hill criteria with which US EPA misrepresented the epidemiological evidence or inappropriately inflated the results (strength of association, consistency, specificity, and biological gradient).

Strength of Association

When describing the strength of association between chloroprene exposure and liver cancer mortality, the 2010 Review predominately relied upon risk estimates reported from the methodologically flawed studies described within section 1.2 of this critique (Bulbulyan et al. 1998; Bulbulyan et al. 1999; Leet et al. 1982; Li et al. 1989). The authors of the 2010 Review ignored the results from the UPitt study suggesting that there was no elevated risk among chloroprene-exposed workers compared to national or regional reference populations within this section of the 2010 Review. Instead, the authors discussed the not statistically significant elevation in risk among select exposure groups when workers with higher levels of exposure were compared to those with low exposure levels as though they were statistically significant.

As discussed in Section 1.2.1 of this critique, only 17 deaths from liver cancer were observed among the Louisville cohort and were therefore included in the exposure-response analysis. In fact, some of the exposure subgroups discussed by the US EPA authors comprised only two individuals, which resulted in imprecise risk estimates and wide confidence intervals (Marsh et al. 2007b). Further, as noted above in Section 1.2.1 the elevated RRs observed in this analysis were primarily driven by the fact that the individuals in the lowest exposure or baseline groups had exceedingly large deficits in liver cancer mortality compared to what would be expected in the general regional population. Therefore, the EPA's argument that the epidemiological evidence demonstrates a strong association between chloroprene exposure and liver cancer is flawed due to its reliance on biased studies and the misinterpretation of UPitt study results.

Consistency and Specificity

The US EPA authors also incorrectly asserted that the epidemiologic evidence of a consistent and specific relationship between chloroprene exposure and liver cancer was observed among four independent epidemiological studies (Bulbulyan et al. 1998; Bulbulyan et al. 1999; Leet et al. 1982; Li et al. 1989). First, the effect estimates calculated within these limited studies vary tremendously with some of the significant estimates only identified in sub-analyses of small groups with the highest exposure (Li et al. 1989) or only among participants of one gender (Bulbulyan et al. 1998). Next, the Leet and Selevan study is not independent of the UPitt study, and therefore, should not be considered in the evaluation of the epidemiological literature as a whole. Lastly, the UPitt study included more person-years of observation than the four prior studies combined, and consistently reported no evidence of an association between chloroprene exposure and liver cancer among four worker cohorts. For these reasons, it is inaccurate for the EPA to say that there is consistent evidence of an association between chloroprene exposure and liver cancer.

Biological Gradient

When describing the epidemiological evidence of a biological gradient, or exposure-response relationship, between chloroprene exposure and liver cancer, the US EPA cites the 1999 Bulbulyan study, which conducted only a crude exposure assessment, did not account for confounding factors, and ultimately did not find a statistically significant trend of increased liver cancer risk among those with the highest chloroprene exposure (Bulbulyan et al. 1999). The US EPA authors also cited the UPitt study, claiming that elevated risks among the individuals with the highest exposures were reported. Again, contrary to the EPA's conclusions, there were no statistically significant elevations in liver cancer risk among any of the exposure groups. Furthermore, the highest risk estimates were not even among the individuals in the highest exposure groups (RR=3.85 for 10-19 years exposure vs. RR=1.75 for 20+ years exposure) indicating that a consistent trend between greater chloroprene exposure and increased liver cancer risk was not observed.

b. Failure to Address Peer Review Comments

Our observation that the 2010 Review greatly exaggerated the epidemiological evidence of an association between chloroprene exposure and liver cancer 2010 Review is echoed by the reviewer comments to the US EPA's original draft of the 2010 Review. Specifically, Dr. Herman Gibb, an epidemiologist who served on the peer review panel, stated in his comments to the EPA that the "*document overstates the human evidence*" and that the 2010 Review is not "*transparent in its reasoning that there is a risk of liver cancer*" in regards to the epidemiological data (US EPA 2010a). The epidemiologic evidence of chloroprene carcinogenicity remains overstated and in many cases misrepresented in the final version of the 2010 Review. Due to the nature of the peer review process utilized by the EPA, the US EPA authors were not required to incorporate all reviewer comments and suggestions prior to publication. Therefore, it appears as though concerns, such as Dr. Gibb's, were left unaddressed within the final 2010 Review. In particular, the EPA did not change its conclusion that the epidemiological data provides evidence of a dose-response relationship in different cohorts in different continents, which Dr. Gibb stated "*grossly misrepresents the evidence*" (US EPA 2010a).

VII. CONCLUSION

In conclusion, we maintain strongly that there is no consistent or credible epidemiological evidence of chloroprene carcinogenicity in humans. It is clear that the EPA based their conclusion on evidence from substantially flawed studies and a misinterpretation of the more rigorous UPitt study. Not only does the body of epidemiological literature not support this conclusion, but it is also not consistent with the International Agency on Cancer Research (IARC), which has classified chloroprene as "*possibly carcinogenic to humans*" (IARC 1999). In their 1999 monograph, IARC determined that there was *inadequate evidence* of the carcinogenicity of chloroprene in humans. This classification was determined even before the definitive UPitt study reported that there was definitively no evidence of a relationship between chloroprene exposure and cancer mortality across four worker cohorts.

VII. REFERENCES

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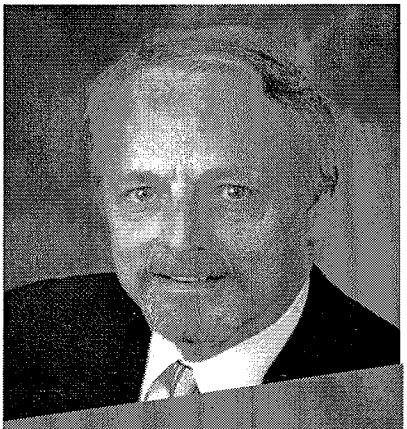
Cardno ChemRisk
Shaping the Future



Consulting Senior Science Advisor for Epidemiology

Practice Areas
> Epidemiology
> Biostatistics

Years Experience
42

Joined Cardno
2015

Education
> PhD, Biostatistics, University of Pittsburgh, 1977
> MS, Biostatistics, University of Pittsburgh, 1974
> BS, Mathematics, University of Pittsburgh, 1973

Memberships
> Fellow of the American College of Epidemiology

Gary M Marsh, PhD, FACE

Summary of Experience

Gary M. Marsh, Ph.D., F.A.C.E. is a Consulting Senior Science Advisor for Epidemiology for Cardno ChemRisk. Dr. Marsh is also a Professor of Biostatistics, Epidemiology, and Clinical and Translational Science, and the Director of the Center for Occupational Biostatistics and Epidemiology at the University of Pittsburgh, Graduate School of Public Health. He is a Fellow of the American College of Epidemiology.

Dr. Marsh directs occupational epidemiologic studies to investigate the long-term health effects of exposure to such agents as man-made mineral fibers, formaldehyde, acrylamide, acrylonitrile, arsenic, chloroprene, tungsten carbide with cobalt binder, petrochemicals, aromatic amines and pharmaceuticals. In addition, he conducts environmental epidemiologic studies of communities exposed to industrial pollutants or to hazardous waste site materials and is involved in basic methodological research related to longitudinal data analysis and quantitative risk assessment.

Dr. Marsh has more than 250 publications in the areas of biostatistics, occupational/ environmental epidemiology, quantitative risk assessment, statistical computing and health services evaluation. He is the senior author of the computer software packages, OCMAP (Occupational Cohort Mortality Analysis Program), which is used as a standard analytic tool by more than 150 domestic and 40 foreign institutions involved in occupational health research, and RACER (Rapid Assessment and Characterization of Environmental Risks). Dr. Marsh is also developer of the original Mortality and Population Data System (MPDS), a repository and retrieval system for National Center for Health Statistics (NCHS) and U.S. Census Bureau data.

Dr. Marsh is an active member of the American College of Epidemiology, the American Statistical Association, the Biometric Society, the International Society for Environmental Epidemiology, the Society for Epidemiologic Research, the Society for Occupational and Environmental Health and the International Commission on Occupational Health.

Significant Experience

University of Pittsburgh

Graduate School of Public Health

> Professor of Epidemiology (2010-present)
> Professor of Clinical and Translational Science (2010-present)
> Director and Founder, Center for Occupational Biostatistics and Epidemiology (2008-present)
> Interim Chairman, Department of Biostatistics (2007, 2009-2010)
> Professor of Biostatistics (1991-present)
> Associate Professor of Biostatistics (1984-1991)
> Assistant Professor of Biostatistics (1978-1984)
> Research Associate (1977-1978)

Center for Clinical and Translational Science

> Professor of Clinical and Translational Science (2010-present)

Center for Environmental Epidemiology

> Assistant Director (1983-1985)

School of Health Related Professions

> Adjunct Assistant Professor of Health Related Professions (1981-1983)

University of Minnesota School of Public Health

> Faculty, Graduate Summer Session in Epidemiology (1984)

Wesley Institute, Bethel Park, Pennsylvania

> Mathematics Instructor (1974-1975)

Consulting Experience

Litigation Support

Dr. Marsh has provided litigation support as both a testifying and consulting expert to in-house and outside counsel on a variety of matters including:

> Railyard work and brain cancer
> Cosmetic talc and mesothelioma
> Non-occupational asbestos exposure and mesothelioma
> Asphalt adhesive and reactive airway dysfunction syndrome (RADS)
> Fiberglass and idiopathic pulmonary fibrosis
> Ethylene oxide and breast and lympho-hematopoietic tissue cancer
> Diesel exhaust and lung cancer
> Railyard work and hemaphagocytic lympohistiocytosis (HLH)
> Firefighters and kidney cancer
> Formaldehyde in hair straightening products and lung cancer
> Electric power plant occupational exposures
> Coal preparation workers and exposure to acrylamide
> FEMA trailer residents and risks from formaldehyde exposure
> Asbestos related diseases among workers and the community near an Italian manufacturing facility
> Evaluation of possible association between PFOA exposure and adverse health outcomes
> Worker exposure to amorphous silica
> Risk of mesothelioma for brake workers
> Evaluation of occupational exposures to hydroquinone and various cancer outcomes
> Evaluation of occupational exposure and adverse effects from carbonless copy paper
> Evaluation of risk of CML in workers exposed to benzene
> Evaluation of ATSDR health studies in Libby, MT

- Evaluation of community health issues associated with waste contamination near McCullom Lake, IL
- Health effects in workers exposed to ortho-toluidine
- Risk of respiratory health effects from exposure to fibrous glass in school buses
- Risks associated with occupational exposure to formaldehyde
- Advise on cases involving latex gloves and allergies
- Evaluation of case involving anophthalmia/microphthalmia in a child with potential exposure to Benomyl

Consulting Projects in Epidemiology and Biostatistics, and Advisory Positions

Dr. Marsh has been retained by numerous private sector clients to assist and advise in the evaluation of health effects associated with a wide variety of chemical, radiological and other exposures. He also has assisted managed care organizations with evaluations of health care delivery systems. Specific examples include:

- City of St. Louis, St. Louis, MO (2015-present)
  - Expert witness on case involving city firefighter
- Monsanto, St. Louis, MO (2015)
  - Member of expert scientific panel to review and critique epidemiological studies of persons exposed to glyphosate
- American Chemistry Council, Washington, DC (2015)
  - Review of epidemiological studies of persons exposed to ethylene oxide
- inXsol, Phoenix, AZ (2012-2013)
  - Statistical evaluation of smartphone application for measuring airborne chemical exposures
- Confidential Chemical Company, PA (2011-2012)
  - Advised on epidemiological study of brain cancer among workers at a chemical manufacturing facility
- Arnold & Porter, LLP, Washington, DC (2011)
  - Advised on response to NAS report on health effects of formaldehyde
- Hollingsworth, LLP, Washington, DC (2011)
  - Presentation and discussion of formaldehyde epidemiology
- ENVIRON International Corporation, Boston, MA (2010-2012)
  - Member of advisory board to evaluate manuscript reviewing association between formaldehyde exposure and lympho-hematopoietic malignancies
  - Member of advisory board to prepare comments on EPA's draft toxicological review of chloroprene
- Confidential Specialty Chemical Company, OH (2010-2011)
  - Advised on epidemiological evidence for association between formaldehyde exposure and cancer
  - Prepare presentations for NAS meeting on formaldehyde
- Confidential Heavy Duty Vehicle Manufacturer, IL (2010-2011)
  - Advised on epidemiological evidence for association between diesel exhaust exposure and lung cancer

- Review and critique of epidemiological studies of metalworking fluids exposure and cancer outcomes

> Confidential Biotechnology Company, MO (2009-2010)
- Designed probability sample to evaluate usage patterns of Botox

> North American Insulation Manufacturers Association, Alexandria, VA (2009)
- Wrote updated review of health effects associate with exposure to man-made vitreous fibers
- Presentation at NTP meeting on health effects of man-made vitreous fibers

> International Truck and Engine Corporation, Chicago, IL (2007-2011)
- Advised on epidemiological evidence for association between diesel exhaust exposure and lung cancer

> Geyer Pathology Services, LLC, Pittsburgh, PA (2007)
- Developed sampling design for selecting lung tissue for analysis

> Burdock Group Consultants, Vero Beach, FL (2006)
- Member of expert panel to review safety status of aspartame as a non-nutrative sweetener

> Energy Networks Association, London, UK (2006)
- Member of expert panel to review epidemiological literature on health effects of EMF exposure

> CEFIC AISBL European Chemical Industry Council, Brussels, Belgium (2006)
- Reanalysis of data from NCI cohort study of formaldehyde workers

> Gateway Health Plan, Pittsburgh, PA (2005-2009, 2013-2014)
- Design and analysis of health care delivery evaluations

> Confidential Construction Equipment Manufacturer, IL (2005-2010)
- Design and analysis of epidemiology study to evaluate association between welding exposures and Parkinson's Disease

> Confidential Chemical Manufacturer, PA (2005-2007)
- Advised on community studies to evaluate potential health effects of chromium exposure
- Design and analysis of epidemiological study to evaluate suspected link between working in paint production plant and testicular cancer

> FormaCare -European Chemical Industry Council (CEFIC), Brussels, Belgium (2005-2007)
- Performed various re-analyses of data from the NCI cohort study of formaldehyde exposed workers

> Formaldehyde Council Inc., Washington, DC (2004-2010)
- Advised on various studies evaluating health effects from formaldehyde exposure

> Pressley Ridge Child Care Services, Pittsburgh, PA (2004-2006)
- Designed probability sample to evaluate effectiveness of child care services

> Semi-Conductor Industry Association, Washington, DC (2003-2010)
- Member of expert scientific panel to advise on design, analysis and operational aspects of industry-wide study of semi-conductor workers

> Academy for Educational Development, Washington, DC (2003-2008)

- Advised on design and analysis of educational effectiveness studies

> Confidential Petroleum Refining Company, IL (2003)
- Design and analysis of a refinery cohort study to evaluate cancer mortality risks

> Formaldehyde Council Inc., Washington, DC (2002-2009)
- Performed various re-analyses of data from the NCI cohort study of formaldehyde exposed workers

> W.R. Grace Company, Leesburg, VA (2002)
- Advised on Libby, MT zonolite health issue

> NIOSH, Cincinnati, OH. (2001-2007)
Follow-up Investigations of Suspected Health Effects of Exposure to Effluents from a Copper Smelter, Copperhill, TN (2001-2007)

> Confidential Pharmaceutical Manufacturer, NJ (2001-2005)
- Design and analysis of epidemiological studies of pharmaceutical production workers

> Coordinated Care Network, Monroeville, PA (2001-2002)
- Statistical evaluation of coordinated care program for persons without traditional health insurance

> Confidential Aerospace Company, CT (2001-2002)
- Advised on feasibility of conducting large-scale cohort study of jet engine manufacturing workers

> The Acrylonitrile Group, Washington, DC (2001)
- Advised on plans for AN scientific conference

> Dow Chemical Co., Midland, MI (2000-2013)
- Statistical analysis of Dow benzene cohort data
- Member of scientific advisory board for epidemiological research program

> Confidential Metal Mining Company, UT (2000-2001)
- Advised on epidemiological studies of copper and zinc smelter workers
- Review and critique of protocol to evaluate association between smelter emissions and multiple sclerosis

> The Sapphire Group, Inc., Beachwood, OH (2000)
- Third-party review and critique of ethylene oxide risk assessment draft

> New Jersey Department of Health and Senior Services, Trenton, NJ (1999-2003)
- Advised on community studies to evaluate potential health effects of residing near Toms River, NJ chemical site
- Advised on design and evaluation of mail survey of chemical exposures

> University of Texas, Houston/Baylor Medical College, Houston, TX (1999-2003)
- Member, Research Advisory Committee-advised on proposed bladder cancer screening and medical surveillance program

> Confidential Chemical Manufacturing Company, MO (1999)
- Review and critique of mortality surveillance program

> Confidential Petrochemical Company, IL (1999-2002)
- Advised design and analysis of epidemiological studies of workers exposed to acrylonitrile and nitrogen products

- Orthopedic & Reconstructive Center, Oklahoma City, OK (1999-2001)
  - Advised on study protocol to evaluate treatments for carpal tunnel syndrome
- Confidential Chemical Company, PA (1999)
  - Review and critique of studies evaluating association between plasticizers and childhood asthma
- TERRA Inc., Tallahassee, FL (1998-2003)
  - Advised on various studies evaluating health effects of chemical exposures
- Confidential Chemical Company, NJ (1998)
  - Advised on possible cancer cluster study related to company. workers
- Confidential Specialty Chemical Company, NY (1998)
  - Review and provided written critique of UAB, Tom's River Plant cohort study
- The Acrylonitrile (AN) Group, Washington, DC (1997-2005)
  - Performed reanalyses of data from NCI cohort study of acrylonitrile-exposed workers
- Dow Chemical Company & Dow Corning Corporation, Midland, MI (1999)
  - Presented seminar on application of Occupational Cohort Mortality Analysis Program (OCMAP) developed by G. Marsh
- Consultant, Chemical Industry Institute of Toxicology, Research Triangle Park, NC (1998-2002)
  - Advised on reanalyses of cohort studies of formaldehyde exposed workers
- National Academy of Sciences, Institute of Medicine, Medical Follow-Up Agency, Washington, DC (1998-2002)
  - Advised on statistical analysis of large scale cohort studies
- Health Canada, Ottawa, CA (1998)
  - Participant in workshop on health effects of formaldehyde exposure
- Highmark Blue Cross Blue Shield, Pittsburgh, PA (1997-1998)
  - Design and analysis of health care delivery evaluations
- American Industrial Health Council, Washington, DC (1997)
  - Reviewed and critiqued epidemiological studies of chemical production workers
- Confidential Chemical Manufacturing Company, NJ (1996-2012)
  - Design and development of Company Mortality Registry
  - Design and analysis of cohort study of formaldehyde exposed workers
  - Design and analysis of cohort study of kidney cancer among workers exposed to acrylonitrile
  - Design and analysis of cohort study of workers exposed to acrylamide
  - Design and analysis of proportional mortality study of aerospace materials workers
  - Development of vital status tracing protocol for non-US workers
- Confidential Building Products Manufacturer, PA (1996-2007)
  - Developed mortality surveillance program with periodic proportional mortality analyses
  - Statistical analysis of mesothelioma deaths
- Electric Power Research Institute, Palo Alto, CA (1996-2006)
  - Advised on design, analysis and operational aspects of large cohort study of electrical power workers

- Member of advisory board to develop manuscript reviewing health effects studies of persons exposed to electromagnetic fields

> Chemical Manufacturers Association, Washington, DC (1996-2001)
- Reviewed health studies of chemical production workers

> Confidential Petrochemical Company, OH (1996-2001)
- Design and analysis of historical cohort study of workers exposed to acrylonitrile

> Showa Denko America, New York, NY (1996-1997)
- Member, Research Advisory Committee-advised on health studies of persons afflicted with eosinophilia myalgia syndrome (EMS)

> Confidential Petrochemical Company, PA (1996)
- Review and critique of Beaver Valley expanded mortality study
- Reviewed health studies of refinery workers

> International Center for Health Services Research, Verona, PA (1996)
- Designed sampling plan for hospital imaging services study

> Group Health Plan, St. Louis, MO (1995-1996)
- Design and analysis of health care delivery evaluations

> Ecology and Environment, Buffalo, NY (1994-2003)
- Advised on design of health survey in Kuwait

> Consultant, HealthAmerica, Pittsburgh, PA (1990-1995)
- Design and analysis of health care delivery evaluations

Research Experience

University of Pittsburgh

Graduate School of Public Health

Since the early 1980s, Dr. Marsh has directed an academic research program focused on occupational/environmental biostatistics and epidemiology, and health services evaluation. He has received research funding from a number and variety of sources, including federal and state government, foundations, trade organizations and corporations. Specific examples include:

> Cytec Aerospace Materials, Inc. (2015-present)
- Historical cohort study of aerospace adhesive materials

> Eli Lilly and Company (2015-present)
- Update of cohort mortality study of pharmaceutical production workers

> Research Foundation for Health and Environmental Effects (2013-2015)
- Additional reevaluation of the National Cancer Institute Formaldehyde Cohort Data
- Commentary on methodological and interpretational issues in the National Cancer Institute Formaldehyde Worker Cohort Study

> Eli Lilly and Company (2013-2014)

- Feasibility study of historical cohort study of pharmaceutical production workers at the Cosmopolis, Brazil site

> INEOS Nitriles, Inc. (2012-2015)
- Historical cohort study of workers exposed to acrylonitrile and nitrogen products

> International Institute of Synthetic Rubber Producers (2011-2013)
- Use of human exposure and epidemiology data in a physiologically based kinetic modeling risk assessment for chloroprene

> The Acrylonitrile Group (2011-2013)
- Statistical methods for adjusting risk estimates for potential confounding by smoking
- Analysis of pooled data from the NCI and DuPont acrylonitrile worker cohort studies

> Mining Awareness Resource Group (2011-2012)
- Evaluation of uncertainty factors in NCI-NIOSH diesel exhaust in miners study exposure assessment and their impact on risk estimates and exposure-response relationships

> North American Insulation Manufacturers Association (2010)
- Literature review of health effects from exposure to man-made vitreous fibers

> Pennsylvania Department of Health/International Tungsten Industry Association (2007-2017)
- International historical cohort and case-control studies of workers exposed to tungsten carbide with cobalt binder

> Pratt & Whitney (2002-2013)
- Historical cohort mortality and incidence studies of jet engine manufacturing workers

> International Institute of Synthetic Rubber Producers (2000-2005)
- Historical cohort study of workers exposed to chloroprene

> Owens Corning (2000-present)
- Mortality surveillance and epidemiological support program

> Highmark Blue Cross Blue Shield (1999-2014)
- A program of biostatistical support for the quality improvement department

> Solutia, Inc. (1999-2002)
- A collaborative program of biostatistical and epidemiological support

> Pennsylvania Department of Health (1998-1999)
- Evaluation of the community health project

> The Acrylonitrile Group (1997-2004)
- A program of epidemiological and biostatistical support

> Eli Lilly and Company (1996-2009)
- Historical cohort and nested case-control studies of pharmaceutical production workers

- Health America of Pittsburgh (1994-1996)
  - A program of biostatistical support for research and clinical audit activities
- Agency for Toxic Substances and Disease Control/Arizona State Health Department (1991-1995)
  - A population based case-control study of lung cancer in Arizona smelter towns
- DuPont Company (1991-1994)
  - Enhancement, modification and update of an occupational and ecological Mortality and Population Data System
- Chemical Manufacturers Association (1991-1992)
  - Identifying and responding to human disease clusters: a practical guidance document
- DuPont Company (1991-1992)
  - A Model Program for Assessing Health Risks among Communities Near Hazardous Waste Sites
- The Formaldehyde Institute (1989-1991)
  - A reanalysis of the national cancer institute study on mortality among industrial workers exposed to formaldehyde
- The U.S. Environmental Protection Agency (1989-1991)
  - A mortality update and case-control study of workers exposed to arsenic in a copper smelter
- Chemical Manufacturers Association (1989)
  - A review and critique of ecologic analyses as an epidemiologic research method
  - Development of decision and quality control criteria for conduct of pilot and epidemiology studies by ATSDR and SARA Section 110
- American Cyanamid Company/Cytec Industries, Inc. (1987-2007)
  - Historical cohort and case-control studies of workers exposed to formaldehyde
  - Historical cohort study of workers exposed to acylamide
- Pennsylvania Department of Health/NIOSH (1986-2006)
  - Bladder cancer screening program for former workers of the Drake-Kilsdonk chemical plant exposed to beta-naphthalmine
- North American Insulation Manufacturers' Association (1985-1999)
  - Historical cohort and nested case-control studies of fiberglass and rock wool production workers
- Shell Oil Company (1983-1987)
  - Historical cohort study of refinery workers
- Smelter Environmental Research Association (1981-1986)
  - Factors associated with mortality among copper and zinc smelter workers
- Development of Occupational Cohort Mortality Analysis Program (OCMAP) (1980-present)
- Development of Mortality and Population Data System (MPDS) (1980-present)

- Monsanto Company (1980-84)
  - Historical cohort study of workers in plastics producing plant
- U.S. National Cancer Institute (1980-1982)
  - Cancer in arsenic exposed populations

Service Activities

University of Pittsburgh

Biostatistics Department

- Member, PhD Admissions Committee (2015-present)
- Member, Pittsburgh Cancer Institute (1990-present)
- Member, PhD Student Admissions Committee (2015-present)
- Faculty Associate, Center for Social & Urban Research (2000-present)
- Member, Faculty Search Committee, Department of Epidemiology (2014-2015)
- Member, Curriculum Committee (2010-2016)
- Founder & Director, Center for Occupational Biostatistics and Epidemiology (2008-present)
- Interim Chairman, Department of Biostatistics (2007, 2009-2010)
- Member, Dean's Cabinet (2007, 2009-2010)
- Chair, Committee to Evaluate Departmental Biostatistics Consulting Practicum (2006-2007)
- Chair, Committee to Evaluate Master's Comprehensive Examination (2004-2005)
- Member, Health Sciences Library Advisory Committee (1997-2003)
- Member, Faculty Advancement, Promotion and Tenure Committee (1999-2001)
- Chair, Ad Hoc Search and Appointment Committees for Associate Professor and Director Occupational Medicine, Department of Environmental & Occupational Health (1996)
- Member, Budget Policies Committee (1995-1998)
- Member Fact-finding Committee for the Performance Review of Dean Mattison (1995-1996)
- Member, International Committee to Review Graduate Program of the Civil & Environmental Engineering Department (1995)

Graduate School of Public Health

- Member, Faculty Search Committee, Department of Epidemiology (2014-2015)
- Departmental Representative, Faculty Advancement, Promotion, Tenure Committee (2012-2016)
- Departmental Chair Representative, Planning and Budget Policy Committee (2009-2010)
- Member GSPH Council (2007, 2009-2010)
- Member, Committee to Evaluate MMPH Program (2005-2006)
- Member, Committee to Develop MPH Comprehensive Examination (2000-2001)
- Member, Search Committee for Dean (1999-2000)

- Member, Search Committee for Chair of EOH Department (1999-2000)
- Member, Faculty Advancement Committee (1999)
- Member, Recruitment Committee (1997)
- President, Faculty Senate (1992-1994)
- Chair, Faculty Senate Executive Committee (1992-1994)
- Member, Strategic Planning Committee (1992-1994)
- Representative, Accreditation Committee (1992-1993)

United States and International Government

- Invited Charter Member, U.S. Environmental Protection Agency, Science Advisory Board, Asbestos Panel, Washington, DC (2008)
- Invited Member, Butadiene Risk Assessment Expert Panel, Sciences International Inc., Alexandria, VA (2006)
- Invited Member, Electromagnetic Field (EMF) Risk Assessment Expert Panel, Energy Networks Association, Edinburgh, Scotland (2006)
- Invited Member, Expert Panel to Assess Health Effects of Artificial Sweetener, Burdock Group, Washington, DC (2006)
- Member, NIOSH Scientific Advisory Panel, Proposed NIOSH Study of Health Effects of Exposure to Electromagnetic Fields (EMF), Cincinnati, OH, May 4, 2001 (2001-2003)
- Member, CDC Scientific Advisory Panel to Review Protocol for Study of Long-Term Health Effects Following Administration of Anthrax Vaccine, Atlanta, GA, May 14-15 (2002)
- Invited Member, International Agency for Research on Cancer (IARC), Working Group to Re-evaluate the Carcinogenicity of Man-Made Vitreous Fibers, Lyon, France, October 9-16 (2001)
- Invited Peer Reviewer, External Peer Review Workshop on Hazard Assessment and Dose-Response Characterization for the Carcinogenicity of Formaldehyde by Route of Inhalation. Health Canada and the U.S. Environmental Protection Agency, Ottawa, Canada, March-December (1998)
- Invited Member, Site Visit Team, Veterans Health Administration, Office of Public Health and Environmental Hazards, Environmental Epidemiology Service, March 1997, Washington, DC (1997)
- Invited Member, Committee to Review the Health Consequences of Military Service During the Persian Gulf War, National Academy of Sciences, Institute of Medicine, Medical Follow Up Agency (1994-1996)
- Guest Editor, "The First International Conference on the Safety of Water Disinfection: Balancing Chemical and Microbial Risks". International Life Sciences Institute, Health and Environmental Sciences Institute (1992-1993)
- Reviewer, "Draft Health Assessment on Inorganic Arsenic", Health and Welfare Canada, May (1992)
- Invited Participant, Workshop on Environmental Epidemiology, National Research Council, National Academy of Sciences, Washington, DC, June (1992)
- Invited Participant, Advisory Committee on ATSDR Sponsored Project, "Community Health Effects of a Hazardous Waste Incinerator", The University of South Carolina, Columbia Campus (1991-1992)

Professional Honors/Awards

- Invited Member, Study Section on Safety and Occupational Health, Centers for Disease Control/National Institute for Occupational Safety and Health (1989-1992)
- Invited Member, National Scientific Advisory Committee, CDC, Center for Environmental Health, Atlanta, GA (1987-1991)

- B.S., Cum Laude (1973)
- Adolf G. Kammer Merit in Authorship Award - Best Publication in Field of Occupational Health, American Occupational Medical Association (1981)
- Delta Omega, Public Health Honorary Society (1985)
- Tenure, University of Pittsburgh, Department of Biostatistics (1986)
- Outstanding Teacher Award, Graduate School of Public Health (1994)
- Biographical Entry in *Who's Who in Science and Engineering* (1997)
- Fellowship, American College of Epidemiology (1997)
- 50 at 50 Award, Graduate School of Public Health (selected as one of 50 outstanding contributors in field of public health in 50 year history of school) (1999)
- Biographical Entry in *Who's Who in Medicine and Healthcare* (2002)
- Biographical Entry in *2000 Outstanding Scientists of the 21st Century* (2003)
- Biographical Entry in *Who's Who in America* (2004)
- Biographical Entry in *Who's Who in American Education* (2005)
- University of Pittsburgh Innovator Award for work on OCMAP software package (2006, 2008, 2009, 2013)
- Albert Nelson Marquis Lifetime Achievement Award, Marquis *Who's Who*

Membership and Service to Professional Societies

- American Statistical Association (1974-present)
  - Secretary, Vice President, President – Pittsburgh Chapter (1979-1982)
  - National Council Representative (1981-1982)
- Biometric Society (1974-Present)
- Society for Occupational and Environmental Health (1978-present)
  - National Governing Council (1986-1989)
- Society for Epidemiological Research (1979-present)
- Pennsylvania Public Health Association (1986-1995)
  - Member, Board of Directors (1989-1992)
- International Society for Environmental Epidemiology (1988-present)
- International Commission on Occupational Health (1996-present)
- American College of Epidemiology (1997-present)
  - Fellowship (1997)
- British Occupational Hygiene Society (2001-2010)

Journal Articles

- Liu, Y., G.M. Marsh, and V.L. Roggli. 2018. Asbestos fiber concentrations in the lungs of brake repair workers: An updated analysis using several regression methods to handle non-detectable measurements. J Occup Env Med. Advance online publication, March 30, 2018. doi: 10.1097/JOM.0000000000001320.
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> Marsh, G.M. and R. Day. 1988. Decision making and quality control criteria for conduct of pilot and epidemiology studies by ATSDR under SARA section 110. Technical Report submitted to the Chemical Manufacturers Association, July.

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> "Nasopharyngeal Cancer and Formaldehyde Exposure: What We Know from the Epidemiology", American Chemistry Council Conference on Health Effects from Formaldehyde Exposure, Chapel Hill, North Carolina, October 10-11, 2017.

> "The Mineralogy and Mesothelioma Epidemiology of Cosmetic Talc", The Monticello Conference, Charlottesville, VA, October17-19, 2017.

> "Mini-Symposium on the Epidemiology of Tungsten Carbide with Cobalt Binder", Coordinator and Presenter, EPICOH 2017 Conference, Edinburgh, Scotland, August 28-31, 2017.

> "Preliminary Results from the International Historical Cohort Study of Workers in the Hard-Metal Industry", Cobalt Development Institute Scientific Meeting, Kristiansand, Norway, April 19, 2016.

> "An international historical cohort study of workers in the hard-metal industry." 2014. EPICOH Conference, Chicago, IL, June 23-26.

> "Evaluating health effects of exposure to acrylonitrile: A comprehensive research program at the University of Pittsburgh." 2013. EPICOH Conference, Utrecht, Netherlands, June 18-21.

> "An international historical cohort study of workers in the hard-metal industry." 2013. EPICOH Conference, Utrecht, The Netherlands, June 18-21.

> "Formaldehyde and nasopharyngeal cancer- What do we know from the epidemiology studies?" 2012. Formaldehyde International Science Conference, Madrid, Spain, April 18-19.

> "Training workshop: Rapid assessment and characterization of environmental risk (RACER)." 2010. CDC National Environmental Public Health Tracking Conference, New Orleans, LA, April 24-26.

> "Does formaldehyde cause leukemia? Opposing viewpoint." 2010. University of Pittsburgh, Department of Epidemiology Tipping Point Seminar Series, December 2.

> "Rapid assessment and characterization of environmental risk (RACER)." 2009. CDC National Environmental Public Health Tracking Conference, Washington, D.C., February 24-26.

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> "An epidemiological study of mortality among a cohort of jet engine manufacturing workers." 2008. Society for Neuro-Oncology Annual Meeting, Las Vegas, NV, November 19-22.

> "A new software tool: Rapid assessment and characterization of environmental risks (RACER)." 2007. Presented at the Environmental Summit, sponsored by the GSPH Department of Epidemiology, Pittsburgh, PA, April 18.

> "Formaldehyde and human cancer risk understanding the nasopharyngeal cancer excess in Plant 1 of the NCI study." 2007. Formaldehyde International Science Conference, Barcelona, Spain, September 20-21.

- "EMF-ELF exposure potentials among magnetic particle inspections workers." 2006. International Workshop to Evaluate Future Needs of Occupational ELF Epidemiology, Edinburgh, Scotland, September 14-15.
- "Sampling, statistics and Iraq: Review and critique of two surveys conducted to estimate deaths in Iraq since 2003." 2006. GSPH Doctoral Student Association Invited Seminar, Pittsburgh, PA, December 10.
- "Mortality patterns among workers exposed to chloroprene and other substances." 2005. British Occupational Hygiene Society Annual Meeting, Manchester, England, April 19.
- "Mortality among industrial workers exposed to chloroprene and other substances: I. Methods and issues." 2005. International Symposium on Butadiene and Chloroprene, Charleston, SC, September 20.
- "Mortality among industrial workers exposed to chloroprene and other substances: II. Results." 2005. International Symposium on Butadiene and Chloroprene, Charleston, SC, September 20.
- "Overview of formaldehyde epidemiology." 2005. Toxicology Forum Annual Meeting, Brussels, Belgium, November 8.
- "Pharyngeal cancer mortality among workers exposed to formaldehyde." 2004. Toxicology Forum, Washington, DC, February 2.
- "Mortality patterns among pharmaceutical production workers at one U.S. site." 2004. British Occupational Hygiene Society Annual Meeting, Stratford England, April 20.
- "Bladder cancer among chemical workers exposed to nitrogen products and other substances." 2003. British Occupational Hygiene Society Annual Meeting, London, England, April 8.
- "Does fiber glass pose a respiratory cancer risk in man? Findings from the Latest update of the U.S. cohort study of man-made vitreous fiber workers." 2001. Ninth Inhaled Particles Conference, British Occupational Hygiene Society, Robinson College, Cambridge, UK, September 2-6.
- "Census 2000: Scientifically and politically correct?" 2000. Symposium Panel Member and Discussant, University of Pittsburgh, Graduate School of Public Health, May 5.
- "Industrial inorganic fibres: Assessing and controlling the risk to public health." 2000. Presented at the 26th International Congress on Occupational Health (ICOH): Mini-Symposium on Fibres-State of the Art, Singapore, August 28.
- "Historical cohort study of U.S. fiber glass production workers. I. Initial findings of 1992 Follow-Up." 2000. Presented at the 26th International Congress on Occupational Health (ICOH), Singapore, August 29.
- "Staying healthy in an unhealthy world: Occupational and environmental health." 2000. Presented at the "Mini-Medical School" Seminar Series of the University of Pittsburgh, School of Medicine, December 5.
- "A program of biostatistical support for the research and quality improvement activities of Highmark Blue Cross Blue Shield." 1999. Presented at the Annual Meeting of the Pennsylvania Public Health Association, Pittsburgh, PA, October 25.
- "The role of epidemiology in an integrated workplace surveillance program." 1999. Presented at the Eli Lilly & Co. Annual Health Fair, Indianapolis, IN, September 19.

- "Mortality surveillance program for the United States man-made mineral fiber workers cohort: Mortality patterns among rock/slagwool workers 1989 Update." 1995. Presented at the Symposium on the Health Effects of Fibrous Materials (excluding asbestos), Sydney, Australia, October 31.
- "A population-based case-control study of lung cancer mortality in four Arizona smelter towns." 1995. Presented at the ATSDR International Congress on Hazardous Waste: Impact on Human and Ecological Health, Atlanta, GA, June 6.
- "OCMAP-PLUS: A new occupational cohort mortality analysis program for multifactor work history and exposure-based analysis." 1994. Presented at the IARC Conference on Retrospective Assessment of Occupational Exposures in Epidemiology, Lyon, France, April 13-15.
- "An expert computer system to accompany the model standardized risk assessment protocol for use with hazardous waste sites." 1993. Presented at the ATSDR International Congress on the Health Effects of Hazardous Wastes, Atlanta, GA, May 3.
- "Drake Chemical Company superfund site. Notification and medical surveillance of workers at high risk of developing bladder cancer." 1993. Presented at the ATSDR International Congress on the Health Effects of Hazardous Wastes, Atlanta, GA,
- "A preliminary evaluation of new fiber and co-exposure data for the U.S. man-made vitreous fiber worker cohort." 1993. Presented at the 10th International Congress on Occupational Health (ICOH), Nice, France, September 25.
- "The University of Pittsburgh studies of man-made mineral fiber workers." 1992 Presented at the 1992 Toxicology Forum, Washington, DC, February 18.
- "Additional analysis of the National Cancer Institute study on mortality among industrial workers exposed to Formaldehyde." 1991. Presented at the American Industrial Hygiene Conference and Exposition. Salt Lake City, UT, May 23.
- "The impact of exposure misclassification and confounding on the mortality experience of U.S. man-made vitreous fiber workers." 1991. Presented at the 8th International Congress on Occupational Health (ICOH)- Epidemiology in Occupational Health, Paris, France, September 12.
- "Long-term mortality studies of man-made mineral fiber exposure." 1991. Presented at the ICOH/Congrex Symposium on Health Aspects of MMMF. Rotterdam, Netherlands, September 13.
- "Evaluating health risks in a multi-exposure environment: The case of formaldehyde." 1992. Presented at the Annual Meeting of the American Occupational Health Conference, Washington, DC, May 8.

- American Industrial Hygiene Association Journal
- American Journal of Epidemiology
- American Journal of Public Health
- Annals of Epidemiology
- Archives of Environmental Health
- Cancer Causes and Control
- Chemico-Biological Interactions
- Critical Reviews in Toxicology

Cardno ChemRisk
Shaping the Future

> Epidemiology
> Journal National Cancer Institute
> Journal of Exposure Analysis and Environmental
> Journal of Occupational and Environmental Hygiene
> Journal of Occupational and Environmental Medicine
> Lancet
> Occupational and Environmental Medicine (U.K.)
> Open Epidemiology Journal
> Regulatory Toxicology and Pharmacology
> Risk Analysis

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> Associate Editor, Open Access Epidemiology (2013-present)
> Associate Editor, Epidemiology Research International (2009-present)
> Associate Editor, Journal of Environmental and Public Health (2008-2017)
> Associate Editor, Cancer Informatics (1995-present)

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Current Position:
Health Scientist I

Practice Areas:
> Epidemiology
> Global Health
> Microbiology
> Program Evaluation
> Public Health Policy

Years Experience:
1

Joined Cardno:
2017

Education:
> DrPH, Epidemiology, University of Pittsburgh Graduate School of Public Health, 2014-2017
> MPH, Infectious Diseases and Microbiology, University of Pittsburgh Graduate School of Public Health, 2012-2014
> BS, Biology, Pennsylvania State University, 2008-2012

www.cardnochemrisk.com
www.cardno.com

Natalie Suder Egnot, DrPH

Summary of Experience

Dr. Natalie Suder Egnot is a Health Scientist with Cardno ChemRisk in the Pittsburgh, PA office. She completed her undergraduate studies at the Pennsylvania State University, and obtained both a Master's of Public Health in Infectious Diseases and Microbiology and a Doctor of Public Health in Epidemiology from the University of Pittsburgh Graduate School of Public Health. Her master's thesis research examined the association between herpesvirus coinfection and non-Hodgkin's lymphoma among men living with HIV. Dr. Egnot's dissertation work utilized novel statistical methods and imaging techniques in order to evaluate the role of inflammation in the development and progression of atherosclerotic cardiovascular disease. During her time at the University of Pittsburgh, Dr. Egnot also obtained certificates in Program Evaluation and Global Health.

Significant Projects

Epidemiology & Biostatistics

Performed statistical analysis modeling a variety of health outcomes using techniques such as multivariable regression, linear mixed effects modeling, mediation analysis, principle component analysis, and structural equation modeling. Frequently utilized statistical analysis software including SAS, SPSS, and STATA.

Conducted systematic reviews of epidemiologic literature and has contributed regularly to published manuscripts.

Litigation Support

Reviewed and summarized case materials related to occupational and para-occupational exposure to asbestos. Reviewed and interpreted epidemiologic literature related to asbestos exposure in preparation of expert reports and testimony.

Program Evaluation

Led evaluation of non-profit fellowship program in order to measure the impact of participation in the program among current and past fellows utilizing both quantitative and qualitative analysis methods. Detailed evaluation results in a final report for the fellowship program administrators and presented findings to fellowship program's board of directors.

Public Health Policy

Systematically reviewed and interpreted literature regarding strategies aimed at reducing drug overdose mortality among individuals who were recently incarcerated. Discussed existing policies with local stakeholders and developed actionable recommendations. Synthesized findings and recommendations into a white paper that was presented to local policymakers.

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> National Heart, Lung and, Blood Institute T32 Pre-Doctoral Trainee (2014-2017)
> Environmental Fellow, The Pittsburgh Albert Schweitzer Fellows Program (2013-2014)

> American Heart Association, 2014- Present

Peer-Reviewed Publications

> Hsu, S, DE Rifkin, MH Criqui, NC Suder, P Garimella, C Ginsberg, AM Marasco, BJ McQuaide, EJ Barinas-Mitchell, MA Allison, CL Wassel and JH Ix. 2017. Journal of Vascular Surgery. In Press.
> Wassel, CL, AM Ellis, NC Suder, E Barinas-Mitchell, DE Rifkin, NI Forbang, JO Denenberg, AM Marasco, BJ McQuaide, NS Jenny, MA Allison, JH Ix and MH Criqui. 2017. Femoral Artery Atherosclerosis is Associated with Physical Function Across the Spectrum of the Ankle-Brachial Index: The San Diego Population Study. Journal of the American Heart Association. July 20;6(7).
> Wukich, DK, KM Raspovic and NC Suder. 2017. "Patients with Diabetic Foot Disease Fear Major Lower-Extremity Amputation More than Death" Foot and Ankle Specialist. Feb 1.
> Wukich, DK, TL Sambenedetto, NM Mota, NC Suder and BL Rosario. 2016. "Correlation of SF-26 and SF-12 Component Scores in Patients With Diabetic Foot Disease" Journal of Foot and Ankle Surgery. Jul-Aug; 55(4): 693-6.
> Wukich, DK, KM Raspovic and NC Suder NC. 2016. "Prevalence of Peripheral Arterial Disease in Patients with Diabetic Charcot Neuroarthropathy" Journal of Foot and Ankle Surgery. Jul-Aug; 55(4):727-31.
> Sadoskas, D, NC Suder and DK Wukich. 2016. "Perioperative Glycemic Control and the Effect of Surgical Site Infections in Diabetic Patients Undergoing Foot and Ankle Surgery" Foot and Ankle Specialist. Feb; 9(1): 24-30
> Wukich, DK, W Shen, KM Raspovic, NC Suder, DT Baril and E Avgerinos. 2015. "Noninvasive Arterial Testing in Patients With Diabetes: A Guide for Foot and Ankle Surgeons" Foot and Ankle International. Dec; 36(12): 1391-9.
> Wukich, DK, BR Mallory, NC Suder and BL Rosario. 2015. "Tibiotalocalcaneal Arthrodesis Using Retrograde Intramedullary Nail Fixation: Comparison of Patents With and Without Diabetes Mellitus" Journal of Foot and Ankle Surgery. Sep-Oct; 54(5): 876-82.
> Wukich, DK, JW Dikis, SJ Monaco, K Strannigan, NC Suder and BL Rosario. 2015. "Topically Applied Vancomycin Powder Reduces the Rate of Surgical Site Infection in Diabetic Patients Undergoing Foot and Ankle Surgery" Foot and Ankle International. Sep; 36(9): 1017-24.
> Suder, NC and DK Wukich. 2012. "Prevalence of Neuropathy in Patients Undergoing Foot or Ankle Surgery" Foot and Ankle Specialist. Apr; 5(2):97-101.

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Oral Presentations

> "Associations of Biomarkers of Inflammation and Coagulation with Plaque in the Femoral Artery" American Heart Association EPI/Lifestyle Conference Trainee Session. Portland, Oregon. March 2017.
> "Recommendations for Reducing Morbidity and Mortality due to Heroin Overdose in Allegheny County" Presented to members of the Allegheny County Health Department on behalf of Health Policy and Management 2133: Law in Public Health Practice

Lectures

> "Introduction to Mentored Grant Writing" Epidemiology 2152: Student Workshop in Cardiovascular Disease Epidemiology, University of Pittsburgh Graduate School of Public Health
> "Social Determinants of Health" Health Policy: 90-861, Carnegie Mellon University Heinz College
> "Overview of Grant Writing" Epidemiology 2182: Design and Conduct of Clinical Trials, University of Pittsburgh Graduate School of Public Health

Poster Presentations

> Suder, N, E Barinas-Mitchell, M Allison, M Criqui, J Ix, N Jenny and C Wassel. "Associations of Biomarkers of Inflammation and Coagulation with Plaque in the Femoral Artery" American Heart Association EPI/Lifestyle Conference. Portland, Oregon. March 2017
> Suder, N, E Barinas-Mitchell, M Allison, M Criqui, J Ix, N Jenny and C Wassel. "Higher Levels of C-reactive Protein and Interleukin 6 are Associated with Femoral Artery Plaque Burden, but not Plaque Characteristics: The San Diego Population Study" Presented at the American Heart Association Fellows Research Day 2017
> Creppage, K, N Suder and L Torso. "A Legal Analysis of Laws Governing the Use of Naloxone (Narcan) in the United States and Pennsylvania" Presented at the Graduate School of Public Health Dean's Day 2016.
> Creppage, K, N Suder and L Torso. "Interventions to Reduce the Risk of Opioid Overdose upon Release from Jail or Prison: A Review of the Literature" Presented at the 2016 Health Disparities Poster Competition, University of Pittsburgh.
> Suder, N. "Knowledge Empowers: Communicable Disease Prevention in Abused and Neglected Children" Presented at IDM Research Day 2013, University of Pittsburgh Graduate School of Public Health. Poster based on Albert Schweitzer Fellowship and public health practicum experience.
> Suder, N. "Communicable Disease Prevention in Abused and Neglected Children Living in Beaver County, Pennsylvania" Presented at the Annual Infectious Disease Public Health Forum 2013, University of Pittsburgh Graduate School of Public Health. Poster based on proposed public health practicum.

EXHIBIT 7

DPE's RFC dated July 15, 2021 with Attachments

Request for Reconsideration
RFC #21005 (Chloroprene)
Submitted on behalf of Denka Performance Elastomer LLC

Denka Performance Elastomer LLC
560 Highway 44
LaPlace, LA 70068

July 15, 2021

Via Electronic Mail (quality@epa.gov)

Re: Request for Correction - Toxicological Review of Chloroprene (CAS No. 126-99-8) In Support of Summary Information on the Integrated Risk Information System (IRIS)

Dear Sir or Madam:

On behalf of Denka Performance Elastomer LLC (DPE), I submit this Request for Correction (RFC) under the Information Quality Act and the U.S. Environmental Protection Agency's (EPA or "the Agency") Information Quality Guidelines (IQG or "the Guidelines").[1] Through the submission of this RFC, DPE asks EPA to re-evaluate certain conclusions set forth in the "Toxicological Review of Chloroprene (CAS No. 126-99-8) In Support of Summary Information on the Integrated Risk Information System" in consideration of new scientific information concerning the cancer effects of chloroprene on humans, as discussed in this RFC and accompanying materials.

I. INTRODUCTION

In September 2010, the EPA released the "Toxicological Review of Chloroprene (CAS No. 126-99-8) In Support of Summary Information on the Integrated Risk Information System (IRIS)"[2] ("2010 Review") for the Integrated Risk Information System (IRIS). In the 2010 Review, EPA calculated a human cancer Inhalation Unit Risk (IUR) of 5 x 10^{-4} per $\mu g/m^3$ cancer risk for 70 years of exposure based on data from the female B6C3F1 mouse. In 2010, in the absence of a sufficiently rigorous Physiologically-Based Pharmacokinetic (PBPK) model to estimate human toxicological response based on the mouse data,[3] EPA defaulted to the assumption that it would use the female B6C3F1 mouse IUR as a proxy to estimate human risk from chloroprene inhalation. Without the use of a PBPK model to account for significant metabolic differences between humans and the female B6C3F1 mouse, the IUR developed by EPA overstated the risks associated with

[1] Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of Information Disseminated by the Environmental Protection Agency, EPA/260R-02-008 October 2002. Available at https://www.epa.gov/sites/production/files/2020-02/documents/epa-info-quality-guidelines_pdf_version.pdf.

[2] EPA/635/R-09/010F (September 2010).

[3] See 2010 Review, pp. 21 and 132.

human exposure to chloroprene. These risk estimates have caused unwarranted concern among members of the community surrounding DPE's facility, despite substantial epidemiological evidence indicating that EPA's risk estimate is unrealistically conservative.

Despite DPE's belief that the facility's chloroprene emissions do not pose a risk to the community, at a cost of $35 million, and in just over a year of work, DPE reduced its chloroprene emissions by 85 percent under a voluntary agreement with Louisiana Department of Environmental Quality (LDEQ) and because of DPE's commitment to excellence in environmental stewardship. Even so, we recognize that there continue to be concerns about the cancer risk posed by chloroprene, and DPE is committed to addressing those concerns based on the best available scientific information.

This RFC presents what DPE and its scientific consultants believe is the best and most up-to-date science for re-evaluating the 2010 IUR, including important new scientific information developed since 2010. Specifically, in support of this RFC, DPE is submitting the following:

1. A new PBPK model for chloroprene which underwent review by an EPA external peer review panel in 2020 and has been updated in response to comments from the peer review panel in 2021 (Exhibit A[4]); and
2. Updated epidemiological data and studies published since 2010 (summarized in Exhibit B[5]).

The above information supports the conclusion that chloroprene is less carcinogenic to humans than to the female B6C3F1 mouse. Based on discussions with EPA's Office of Research and Development, this RFC does not address all risk factors required for the determination of the IUR; however, considering the PBPK model results alone, it appears that the 2010 IUR may overstate human risk by more than 2 orders of magnitude.

DPE believes that the 2010 Review can be appropriately revised with a narrowly focused update to Section 6.2.4 ("Cancer/Inhalation," consisting of one paragraph of text on the IUR estimation on pages 147 and 148) and supplements to sections 3.5 ("Physiologically Based Toxicokinetic Models"), 4.1 ("Studies in Humans – Epidemiology, Case Reports, Clinical Controls"), and Section 5.4 ("Cancer Assessment"). We appreciate the opportunity to present this information to EPA, and we look forward to answering any questions you may have about this information.

A. How This RFC Differs from DPE's 2017 RFC

This is DPE's second RFC requesting the correction of the chloroprene IUR. On June 26, 2017, DPE filed RFC # 17002. EPA denied RFC # 17002 on January 25, 2018, primarily on the basis that DPE had not presented sufficient new information developed since 2010 to justify the

[4] Report by Ramboll entitled "Incorporation of in Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene- Revised Documentation in Response to USEPA Peer Review," and dated July 15, 2021.

[5] Report by Ramboll entitled "Epidemiological Basis for Supporting a Correction of the Chloroprene Inhalation Unit Risk (IUR): Update," dated July 15, 2021.

request. In particular, EPA concluded that the Yang, *et al.* (2012) PBPK model used to support the 2017 RFC[6] lacked quality assurance and quality control review and that EPA did not have sufficient documentation to verify the PBPK model. DPE filed a timely Request for Reconsideration (RFR # 17002A) on that decision but withdrew the RFR on March 1, 2021, in order to submit a new RFC supported with new information, including the new Ramboll 2021 PBPK model.

DPE believes that this new RFC provides the new information EPA suggested would be necessary in the denial of RFC # 17002. The new information is set forth in two separate reports prepared by scientists at Ramboll: Exhibit A, entitled "Incorporation of In Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene" and Exhibit B, entitled "Epidemiological Basis Supporting a Correction of the Chloroprene Inhalation Unit Risk (IUR)".

Exhibit A documents the new PBPK model's results and methodology, and also includes Ramboll's additional documentation and analyses undertaken to respond to comments from the peer review of the 2020 PBPK model report that was overseen by EPA. It also contains a thorough response to each of the peer reviewers' Tier 1 and Tier 2 comments, as requested by EPA on December 15, 2020.

Exhibit B provides follow-up epidemiological data on U.S. workers in Neoprene production facilities, including DPE's Louisiana facility through 2017, as published in 2021 by Dr. Gary Marsh, *et al.*, which shows no increased cancer mortality among any worker cohort exposed to chloroprene. Exhibit B also summarizes robust cancer incidence data available from the Louisiana Tumor Registry which shows only average and below average cancer incidence near the DPE facility for lung and liver cancers, the cancers of concern for chloroprene from the epidemiological studies as set out in the 2010 Review.[7]

B. This RFC Satisfies EPA's Information Quality Guidelines

Section 8.23 of EPA's Information Quality Guidelines, provide the criteria for EPA to grant an RFC. We believe that this RFC satisfies these criteria for the following reasons:

8.2 What should be Included in a Request for Correction of Information?

Persons requesting a correction of information should include the following information in their Request for Correction (RFC):

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[6] Yang, Y.; Himmelstein, MW; Clewell, HJ. (2012). Kinetic modeling of ß-chloroprene metabolism: Probabilistic in vitro – in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rates and humans. *Toxicol In Vitro* 26: 1047-1055, available at http://dx.doi.org/10.1016/j.tiv.2012.04.004.

[7] A scientific review of the IRIS study by Ramboll scientists identified serious flaws in the 2010 Review. This review has been published in the peer-reviewed scientific journal *Risk Analysis.* Sax SN, Gentry PR, Van Landingham C, Clewell HJ, Mundt KA. 2020. Extended Analysis and Evidence Integration of Chloroprene as a Human Carcinogen. *Risk Analysis.* 40(2):294-318 , available at https://onlinelibrary.wiley.com/doi/epdf/10.1111/risa.13397

- Name and contact information for the individual or organization submitting a complaint; identification of an individual to serve as a contact.
- A description of the information the person believes does not comply with EPA or OMB guidelines, including specific citations to the information and to the EPA or OMB guidelines, if applicable.
- An explanation of how the information does not comply with EPA or OMB guidelines and a recommendation of corrective action. EPA considers that the complainant has the burden of demonstrating that the information does not comply with EPA or OMB guidelines and that a particular corrective action would be appropriate.
- An explanation of how the alleged error affects or how a correction would benefit the requestor.

This RFC will go into more detail below, but this RFC meets the above criteria as follows:

First, this RFC is submitted on behalf of Denka Performance Elastomer LLC (DPE), located at 560 Highway 44, LaPlace, Louisiana 70068. The undersigned may be contacted by U.S. mail, by email at Patrick-walsh@denka-pe.com, and by telephone at 504-536-7573.

Second, the following sections of the 2010 Review contain information that does not comply with EPA and OMB Guidelines and that need to be revised or supplemented to reflect the best available scientific information:

- 6.2.4 ("Cancer/Inhalation," consisting of one paragraph of text on the IUR estimation on pages 147 and 148);
- 3.5 ("Physiologically Based Toxicokinetic Models");
- 4.1 ("Studies in Humans – Epidemiology, Case Reports, Clinical Controls"); and
- 5.4 ("Cancer Assessment").

Third, Section 4.8 of the Guidelines commits EPA "to work to ensure that our many policies and procedures are appropriately implemented, synthesized, and revised as needed"; Section 5.1 commit EPA to using "quality" scientific information; and Section 4.2 commits EPA to using peer reviewed information, where possible, and preferably externally peer reviewed information of special importance. For influential scientific risk assessment information like the 2010 Review, the EPA Guidelines (at page 22) require EPA to ensure that:

(A) The substance of the information is *accurate, reliable and unbiased.* This involves the use of:

(i) *the best available science and supporting studies conducted in accordance with sound and objective scientific practices*, including, when available, peer reviewed science and supporting studies; and

(ii) data collected by *accepted methods* or *best available methods* (if the reliability of the method and the nature of the decision justifies the use of the data).

These criteria and others support this RFC, which requests that EPA update the 2010 IUR, which was based on a default assumption in the absence of a peer reviewed PBPK model, with the new Ramboll PBPK model, which was externally peer reviewed in October 2020 and which now contains supporting documentation to address the peer review panel's suggestions.

Fourth, granting this RFC and applying the best available science to the estimation of the chloroprene IUR will benefit DPE, the only maker of Neoprene in the United States and the only major permitted emission source of chloroprene in the United States, because it will correct that misimpression that DPE's facility poses a cancer risk to people in the nearby community. It will also benefit federal and state agencies that administer air pollution laws because it will allow them to make decisions based on the best available science. This is explained in more detail in the Background section below.

II. Background

DPE's Neoprene production facility is located in the Pontchartrain Works facility near LaPlace, Louisiana, in St. John the Baptist Parish. The plant was originally built and operated by E. I. du Pont de Nemours and Company (DuPont) in the 1960s. Since then, a portion of the facility has produced Neoprene by synthesizing and polymerizing chloroprene. Neoprene is a synthetic rubber used to make medical and military equipment, clothing, and consumer products like cell phone cases.

On November 1, 2015, DPE acquired the Neoprene production facility from DuPont and established its headquarters in St. John the Baptist Parish. DPE directly employs about 230 people and is the second-largest private employer in the Parish.

In December 2015, just weeks after DPE acquired the facility, EPA released the 2011 National Air Toxics Assessment (NATA). Among other things, 2011 NATA involved a nationwide review of chemical plant emissions and a screening for potential air pollution-related health risks nationwide. The NATA used air dispersion models, 2011 emissions,stack parameters, and meteorology, along with available IRIS health risk values. Using the 2010 IUR for chloroprene, the 2011 NATA estimated on a screening level basis that the DPE plant produced the greatest offsite cancer risk of any chemical plant in the United States.

Because of the concerns arising from the 2011 NATA, DPE invested more than $35 million to substantially reduce emissions from the facility within 2 years after acquiring it from DuPont, even though DPE believed that these concerns were (and are) unwarranted. On January 6, 2017, DPE and LDEQ, in cooperation with EPA, entered into a voluntary Administrative Order on Consent (AOC), under which DPE committed to reduce chloroprene emissions by 85 percent compared to the faculty's 2014 emissions. The AOC involved:

- Interim measures (additional condensers) to reduce emissions in 2017;
- The construction and installation of a Regenerative Thermal Oxidizer (RTO) to control emissions from the Neoprene process area (started up in December 2017);
- The construction of and installation of the Monomer Emission Reduction Project (MERP) to route emissions from the Monomer process area to the facility's Halogen Acid Production Furnace (started up in December, 2017); and

- Wastewater and other controls in the Poly Building to reduce wall fan emissions.

The total cost of these projects was more than $35 million, and LDEQ acknowledged that DPE successfully met the 85 percent emission reduction target.[8] Since that time, DPE has continued to take measures to further reduce emissions to the extent feasible.

At the same time, DPE is concerned that members of the community around the facility continue to be unduly alarmed by the 2011 NATA, its 2014 update, and the IUR included in the 2010 Review. As a result, DPE has spent more than 3 years working with independent scientific experts and with EPA to assist in the development of a PBPK model in order to provide a better scientific basis for assessing the cancer risk posed by chloroprene. As discussed in Section III below, the PBPK model shows that the current IUR substantially overstates the risk of chloroprene. Further, as summarized in Section IV and Exhibit B of this RFC, recent epidemiology studies of Neoprene workers and community cancer data from the state-run Louisiana Tumor Registry (LTR) suggest that chloroprene emissions from the DPE facility do not pose increased cancer risk.

III. The Development, Methodology, and Results of the 2021 Ramboll PBPK Model

EPA's own assessment of the uncertainties of its 2010 cancer risk assessment is informative on the determination of the best available science today. Because EPA noted the need for a PBPK model in 2010 and recognized the uncertainties in its cancer risk assessment, those recognized limitations in the 2010 Review strongly suggest that the 2021 PBPK model effectively closes the self-identified gap in EPA's risk assessment in the 2010 Review.

A. In 2010, EPA Recognized the Need for a PBPK Model

PBPK models provide the best scientific approach for the quantitative adjustment for differences in pharmacokinetics among rodents and humans, which can potentially inform differences in response across species.[9] By basing the IUR on toxicological response in the female mouse, EPA "assumed that humans are as sensitive as the most sensitive rodent sex/species tested," the female B6C3F1 mouse, even though **"true correspondence is unknown."**[10] The 2010 Review explained this as follows:

> The calculated composite unit risk is based on the most sensitive endpoint (risk of any tumor type) in the most sensitive species and

[8] See letter from Lourdes Iturralde, LDEQ Assistant Secretary, to Patrick Walsh, DPE SHE Manager, dated May 20, 2020: https://edms.deq.louisiana.gov/app/doc/view?doc=12184387

[9] The U.S. EPA has long considered application of adequately validated PBPK models to be "accepted as a scientifically sound approach to estimating the internal dose of a chemical at a target site and as a means to evaluate and describe the uncertainty in risk assessments." U.S. EPA. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data In Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-05/043F, 2006, available at https://cfpub.epa.gov/si/si_public_record_Report.cfm?Lab=NCEA&dirEntryID=157668.

[10] 2010 Review, p. 139 (emphasis added).

> sex (female mouse). There is no information on chloroprene to indicate that the observed rodent tumors are not relevant to humans. **Further, no data exist to guide quantitative adjustment for differences in sensitivity among rodents and humans.**[11]

In the 2010 Review, EPA assessed the uncertainties in in the cancer risk assessment in Section 65.4.7, and noted that a PBPK model would reduce this uncertainty:

> Another source of uncertainty comes from the interspecies extrapolation of risk from mouse to human. The two rodent species for which bioassay data were available— mouse and rat—vary in their carcinogenic responses to chloroprene, in terms of both site specificity and magnitude of response (Section 4). **Ideally, a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation;** however, current PBPK models are inadequate for this purpose (Section 3)...

The 2021 Ramboll PBPK model has been developed with a carefully performed analysis of pharmacokinetic differences between mice and humans, and the calculation methodologies have been carefully evaluated with sensitivity and uncertainty analyses, and when appropriate, parameter selection based on the most conservative choice. The multiple uncertainties of unknown significance identified in the 2010 Review compared with the 2021 PBPK model clearly show that the "best available science" standard under the EPA Guidelines supports the replacement of the 2010 default assumption with the application of the 2021 PBPK model into the dose-response assessment.

B. The Development of the Ramboll PBPK Model

In the 2010 Review, EPA declined to use the Himmelstein (2004) PBPK model, concluding that that model was "inadequate for application for calculation of internal dose metrics or interspecies dosimetry extrapolations."[12] Instead, and in the absence of a PBPK model it could use in 2010, EPA adopted the default assumption that humans were as sensitive and had comparable pharmacokinetics of chloroprene as the female B6C3F1 mouse strain, the most sensitive species and sex in the 1998 National Toxicity Program (NTP) studies of chloroprene. When DPE submitted its original RFC in 2017 (RFC 17002), it suggested that EPA use the Yang, *et al.* (2012) PBPK model, but in Attachments 1 and 2 to EPA's January 25, 2018 denial of the RFC, the Agency raised a number of concerns about the Yang, *et al.* model.

In April 2018, Ramboll submitted a work plan to EPA for the development of a new PBPK model to address the concerns that EPA noted in the denial of RFC17002. Since that time, EPA has conducted an extensive quality assurance review of the PBPK model development and of the final model. At EPA's request, Ramboll conducted an experiment to provide data to inform a

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[11] 2010 Review, p. 141 (emphasis added).

[12] 2010 Review, p. 21.

chloroprene mass-transport parameter (Kgl), a parameter also included in the model based on comments from EPA. The 2019 version of the PBPK model and its documentation was peer reviewed and published in *Inhalation Toxicology.*[13]

EPA continued the QA/QC work with Ramboll and retained Versar, a third-party contractor, to oversee an external peer review of the 2020 Ramboll PBPK model. The peer review panel, which met on October 5-6, 2020, included nine experts on toxicology, PBPK models, and statistics. The peer review panel comments were set out in its Post Meeting Peer Review Report dated December 17, 2020.[14]

The Post Meeting Peer Review Report identifies approximately[15] 50 Tier 1 comments (issues necessary to address) and Tier 2 comments (issues suggested to be addressed). As part of this RFC, we are including "Ramboll's Response to External Peer Review Tier 1 and Tier 2 Comments/Suggestions, " which addresses all of these comments (see Exhibit A, Supplemental Materials G) and provides a summary of important new calculations, sensitivity analyses, and parameter analyses that have been conducted as part of the revisions to the model. Exhibit A, Supplemental Materials F,[16] provides the detail on the development of the sub model for the PBPK model to estimate epoxide and other metabolite concentrations. This was completed in response to a Tier 1 comment from a peer reviewer and is also summarized in Supplemental Materials G. We believe that all the Tier 1 and Tier 2 peer review comments have been resolved in the 2021 model and associated documentation.

In response to the peer review comments, Ramboll performed additional sensitivity/uncertainty analyses and literature searches. Also, at the request of the peer reviewers, Ramboll developed an extended version of the model to describe the downstream metabolism of chloroprene in order to compare model predictions using alternative dose metrics. The results of this extension of the model demonstrate that the use of a dose metric based on total metabolism is consistent with the cross-species relationship of the toxicity and carcinogenicity of chloroprene, but one based on epoxide area under the curve (AUC) is not. These results support the use of total metabolism as the most appropriate dose metric for the carcinogenicity of chloroprene.

C. The IUR Should Be Corrected to Reflect the 2021 Ramboll PBPK Model

Overall, the application of the 2021 PBPK model is expected to result in the estimation of an IUR that is approximately two orders of magnitude below that of the 2010 IUR. ORD has

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[13] Clewell HJ 3rd, Campbell JL, Van Landingham C, Franzen A, Yoon M, Dodd DE, Andersen ME, Gentry PR. 2020. Incorporation of in vitro metabolism data and physiologically based pharmacokinetic modelling in a risk assessment for chloroprene. *Inhalation Toxicology.* 31(13-14):468-483.

[14] Post-Meeting Peer Review Summary Report, External Peer Review of a Report on Physiologically–based Pharmacokinetic (PBPK) Model for Chloroprene (Ramboll, 2020) and Supplemental Analysis of Metabolic Clearance (U.S. EPA, 2020, December 17, 2020, U.S. EPA and Versar, Inc).

[15] Some comments have multiple parts, and some comments are redundant of others.

[16] Exhibit A, Supplemental Materials F, is entitled "Reactive Metabolite Modeling."

requested that DPE limit its request for review to the adoption of the PBPK model, and that DPE not address all risk assessment factors that IRIS may consider in re-evaluating the IUR. However, the PBPK results strongly support the revision of the IUR to include the application of the PBPK model rather than the default assumption that humans and mice metabolize chloroprene in the same manner, and therefore, would respond similarly.

IV. New Epidemiological and Cancer Incidence Data Support the Findings of the PBPK Model and this RFC

A. The 2010 Review

As explained in Ramboll's report entitled "Epidemiological Basis for Supporting a Correction of the Chloroprene Inhalation Unit Risk (IUR): Update" (Exhibit B), the epidemiological evidence does not support a causal relationship between chloroprene and cancer in humans. Dr. Herman J. Gibb, one of the two epidemiologists on the peer review panel that reviewed the 2009 draft version of the 2010 Review, agreed with this conclusion and found that the epidemiology data reviewed by IRIS provided "little if any evidence" that chloroprene exposure increases the risk of either respiratory or liver cancer, the two cancers EPA indicated were associated with chloroprene exposure. At that time, Dr. Gibb wrote:

> As the document acknowledges on page 4-17, there is little if any evidence that chloroprene increases the risk of respiratory cancer. The limitations of the earlier studies (Li et al. 1989, Bulbulyan 1998, 1999) are significant with regard to whether or not they indicate an increased risk of liver cancer from chloroprene exposure. The largest and what appears from the document to be the best conducted study (Marsh et al., Louisville cohort) provides little if any evidence that a liver cancer risk exists. Furthermore, the document has not been transparent in its reasoning that there is a risk of liver cancer.
>
> In summary, the descriptor of "likely to be carcinogenic to humans" is supported by the animal and genotoxicity data, **but not by the human data. While the descriptor is appropriate, the document should not try to make more of the epidemiologic studies than is warranted.**[17]

In its review of the epidemiological evidence on chloroprene exposure, the 2010 Review analyzed a study by Dr. Gary Marsh, *et al.*, the results of which were reported in 2007 in a series of publications[18] (referred to collectively as "Marsh 2007") in the peer-reviewed journal, *Chemico-*

[17] Final Reviewer Comments, External Peer Review Meeting on the Toxicological Review of Chloroprene (CAS No. 126-99-8), January 26, 2010, p. 27 (emphasis added).

[18] Marsh GM, Youk AO, Buchanich JM, Cunningham M, Esmen NA, Hall TA, Phillips ML. 2007a. Mortality patterns among industrial workers exposed to chloroprene and other substances. I. General mortality patterns. Chemico-Biological Interactions.;166(1-3):285-300; Marsh GM, Youk AO, Buchanich JM, Cunningham M, Esmen

Biological Interactions. Marsh 2007 was the most comprehensive and methodologically robust [study available] based on the size of the cohort, amount of follow-up time, and completeness of exposure assessment, among other strengths. [19] The study involved 12,430 workers exposed to chloroprene at various industrial sites, including two US sites: the DuPont Louisville, KY, site (5,507 individuals) and the DuPont (now DPE) Pontchartrain, LA, site (1,357 individuals). Despite finding no evidence of elevated mortality risks from lung, liver, or other cancers between workers in either cohort and the corresponding regional populations, EPA considered Marsh 2007 as supportive of a causal association between chloroprene exposures and elevated mortality from liver cancer. This conclusion was based entirely on comparisons between exposure groups within the Louisville cohort, despite the very low liver cancer mortality rates among the Louisville employees and the fact that no instances of liver cancer mortality were observed in the Pontchartrain cohort. Again, when compared to liver cancer mortality rates in corresponding regional populations, there were no excess liver mortalities among workers in the Louisville or the Pontchartrain cohort.[20]

B. A Major New Follow-Up Epidemiological Study by Dr. Gary Marsh, *et al.*, Released in 2020, Shows No Increased Cancer Mortality among U.S. Chloroprene Workers

The conclusions of Dr. Gibb and Dr. Marsh were further confirmed by a new follow-up epidemiological study by Dr. Gary Marsh, *et al.*, published in February 2021 in the *Journal of Occupational and Environmental Medicine* and entitled "Mortality Patterns Among Industrial Workers Exposed to Chloroprene and Other Substances: Extended Follow-Up" ("Marsh 2021").[21] The express purpose of the new study was "[t]o update the U.S. portion of a historical cohort mortality study of workers with potential exposure to chloroprene (CD) and vinyl chloride (VC) with focus on lung and liver cancer."[22] The subjects of the study were workers from the former DuPont Neoprene facility in Louisville, Kentucky (Plant L), and the Pontchartrain Works Neoprene facility in Laplace, Louisiana (Plant P) (the former DuPont, now the DPE Neoprene facility). The follow up period was from 2001-2017, and added 47,299 and 19,942 person-years of observation and 1399 and 214 new deaths to the Louisville and Pontchartrain Works cohorts, respectively.[23] This resulted in improved statistical precision.

NA, Hall TA, Phillips ML. 2007b. Mortality patterns among industrial workers exposed to chloroprene and other substances. II. Mortality in relation to exposure. *Chemico-Biological Interactions.* 166(1-3):301-16.

[19] Bukowski JA. 2009. Epidemiologic evidence for chloroprene carcinogenicity: Review of study quality and its application to risk assessment. *Risk Analysis*, 29(9):1203–1216.

[20] Exhibit B, p. 4.

[21] Marsh GM, Kruchten A, Buchanich JM. Mortality Patterns Among Industrial Workers Exposed to Chloroprene and Other Substances: Extended Follow-Up. J Occup Environ Med. 2021 Feb 1;63(2):126-138.

[23] Exhibit B, p. 5.

[23] Exhibit B, p. 5.

In their follow up study, Marsh, *et al.,* again performed both external and internal mortality comparisons. The external comparisons revealed statistically significant *deficits* in deaths at both plants, and internal comparisons revealed **no consistent evidence of exposure-response relationships**. Marsh 2021 concluded that "the risk of death from all cancers or from the sites of a priori interest (lung and liver cancer) **is unrelated to exposure to [chloroprene]** at levels experienced by workers in the two U.S. sites."[24]

EPA should reevaluate the 2010 IUR to consider the significant findings of Marsh 2021. As Dr. Gibb commented in 2009:

> A reality check on the unit risk for chloroprene by comparing it with an upper bound on the cancer risk in the Louisville cohort studied by Marsh et al. should be performed. The Louisville cohort has the best exposure information for this purpose. From the resulting comparison, it may be necessary to adjust the unit risk estimate.[25]

This comment is even more relevant today with the additional information from the 2021 Marsh study.

C. New Cancer Incidence Data from the Louisiana Tumor Registry Shows the Incidence of Cancers near the DPE Faculty Are at or Below State-wide Averages for Cancers of Potential Concern

The PBPK model's findings are corroborated by the health data compiled and published by the Louisiana Tumor Registry (LTR), an independent and rigorous source of cancer incidence data which has been recognized consistently as one of the leading cancer registries in the nation.[26] The LTR has compiled annual reports on cancer incidence for decades. Data that were compiled after the 2010 Review were recently published and have been compiled and released in various reports. These data show that St. John Parish, the Parish in which the DPE facility is located,

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[24] Marsh 2021, p. 135 (emphasis added).

[25] Final Reviewer Comments, External Peer Review Meeting on the Toxicological Review of Chloroprene (CAS No. 126-99-8), January 26, 2010, p. 34. See also Sax SN, Gentry PR, Van Landingham C, Clewell HJ, Mundt KA. 2020. Extended Analysis and Evidence Integration of Chloroprene as a Human Carcinogen. *Risk Analysis.* 40(2):294-318.

[26] See FN 10. The National Cancer Institute, the Centers for Disease Control and Prevention, and the North American Association of Central Cancer Regis-tries have consistently recognized and validated the Louisiana Tumor Registry's high-quality da-ta. The LTR was one of nine NCI-SEER registries awarded 1st Place for data quality in 2020, the 11th consecutive year that the Louisiana Tumor Registry was recognized for the high quality, completeness and timeliness of its data. The LTR also earned Gold Certification again in 2020 by the North American Association of Central Cancer Registries (NAACCR). This designation recognizes registries that have achieved the highest NAACCR standard for complete, accurate, and timely data to calculate standard incidence statistics. LTR has earned this designation every year since 1997. The LTR was also given the Registry of Distinction Award from the Centers for Disease Control and Prevention's National Program of Cancer Registries this year. See "Cancer Reporting in St. John Parish Findings Vali-date Louisiana Tumor Registry Data," March 1, 2021. https://www.lsuhsc.edu/newsroom/Cancer%20Reporting%20in%20St.%20John%20Parish%20Findings%20Validate%20Louisiana%20Tumor%20Registry%20Data.html.

experiences average, or even below-average rates of cancer incidence.[27] As Ramboll summarized in Exhibit B, "LTR data at neither the Parish nor the census tract level indicate elevated rates of the cancers potentially associated with chloroprene exposure in St. John the Baptist Parish compared to Louisiana."[28]

V. CONCLUSION

DPE believes that the Ramboll 2021PBPK model and the PBPK report responding to peer review comments provide a strong basis for updating the IUR included in the 2010 Review, which should make it better aligned with the epidemiological evidence.

Because of the large toxicokinetic differences between mice[29] and humans—differences which EPA did not account for in its calculation of the IUR—the IUR dramatically overstates the human cancer risks associated with chloroprene exposure. The PBPK model does not address all risk factors that affect the determination of the IUR, but it provides the foundation for a full IRIS revision of the 2010 IUR. DPE respectfully requests that EPA grant this RFC and initiate a formal revision of the 2010 chloroprene IUR.

Respectfully submitted,

Patrick A. Walsh, CIH
Safety, Health, and Environmental Manager
Denka Performance Elastomer LLC

[27] Exhibit B, pp. 7-12.

[28] Exhibit B, p. 7.

[29] For simplicity, all reference to "mice" or "the mouse" herein refer to the B6C3F1 mouse strain.

LIST OF EXHIBITS

Exhibit A: Report by Ramboll entitled "Incorporation of In Vitro Metabolism Data in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene- Revised Documentation in Response to USEPA Peer Review," and dated July 15, 2021.

Attachments to Exhibit A:

- Supplemental Materials A: Supplemental Tables
- Supplemental Materials B: Re-estimation of Metabolism Parameters
- Supplemental Materials C: IVIVE Literature Review
- Supplemental Materials D: Metabolism Parameter Calculations
- Supplemental Materials E: Model Files
- Supplemental Materials F: Reactive Metabolite Modeling
- Supplemental Materials G: Responses to Peer Reviewer Comments

Exhibit B: Report by Ramboll entitled "Epidemiological Basis for Supporting a Correction of the Chloroprene Inhalation Unit Risk (IUR): Update," dated July 15, 2021.

Intended for

Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

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

ABSTRACT

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

EXECUTIVE SUMMARY

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

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

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

1. INTRODUCTION

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

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

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

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

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

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

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

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

2. MATERIALS AND METHODS

Nose-only Exposure Study

Test Substance and Atmosphere Generation

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

Test Animals and Housing

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

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

Inhalation Exposures

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

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

Plethysmography

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

Blood Sampling

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

Blood Analysis

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

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

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

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

Chloroprene PBPK Model

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

Model Structure

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

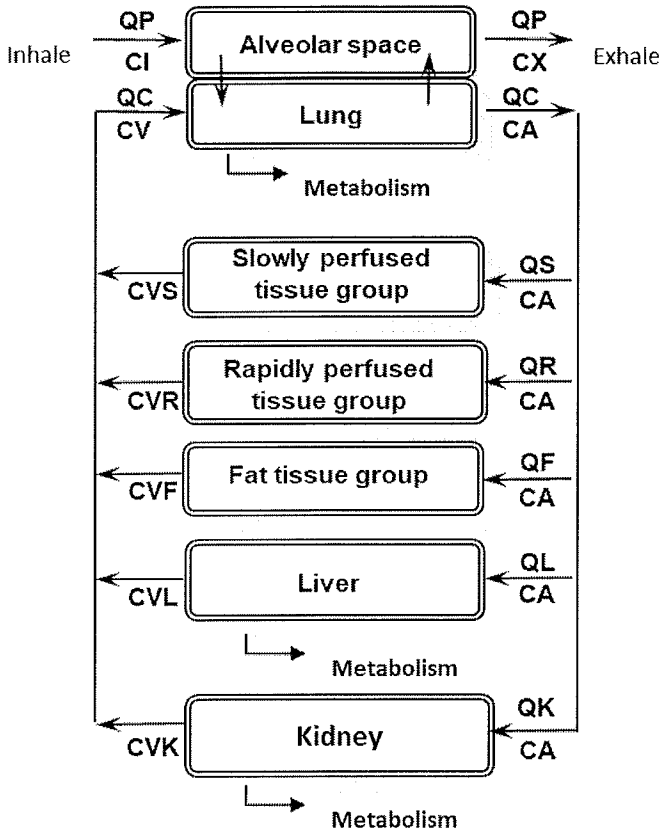

Figure 1. Chloroprene PBPK model diagram. Abbreviations: QP - alveolar ventilation; CI - inhaled concentration; CX - exhaled concentration; QC - cardiac output; CA - arterial blood concentration; CV - venous blood concentration; QS, CVS - blood flow to, and venous concentration leaving, the slowly perfused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

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

Model Parameters

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

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

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

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

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

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

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

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

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

Model Simulations

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

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

Parameter Sensitivity/Uncertainty Analyses

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

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

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

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

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

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

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

Dose metric calculations

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

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

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

Uncertainty Analysis

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

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

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

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

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

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

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

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

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

3. RESULTS

Chloroprene Exposure Atmospheres

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

Plethysmography

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

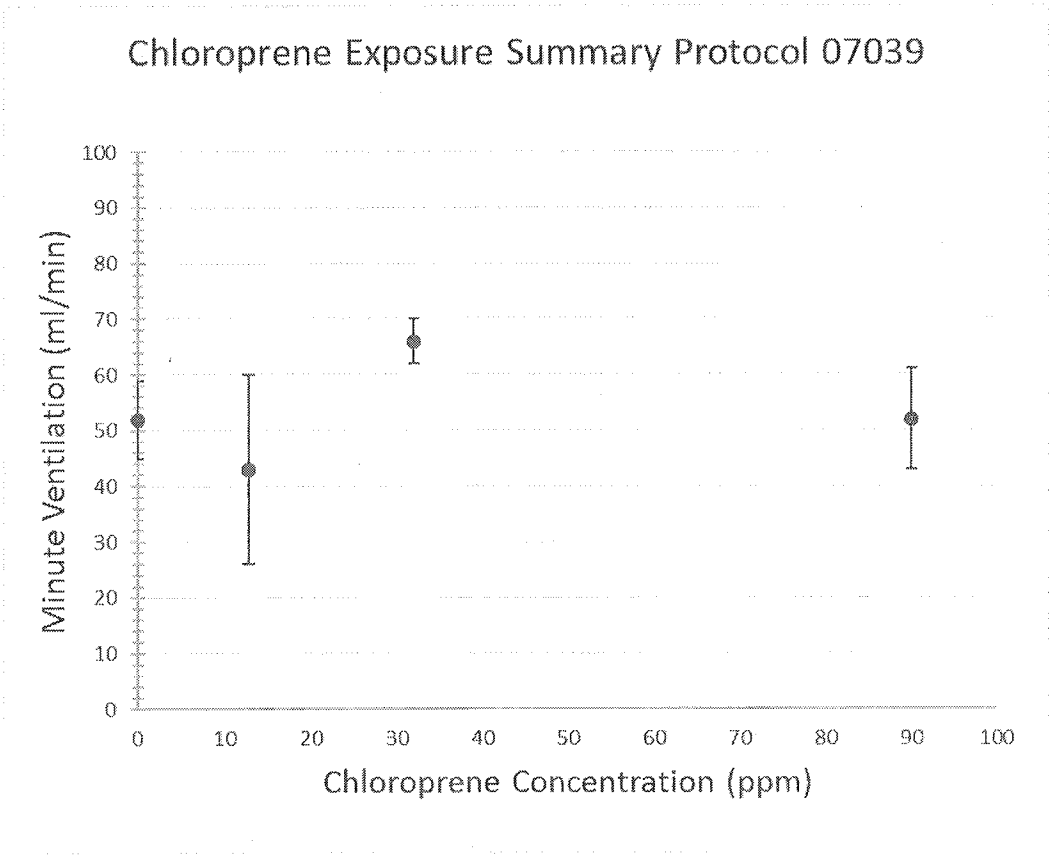

Figure 2. Measured minute ventilation during exposures.

Arterial Blood Chloroprene Concentrations

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

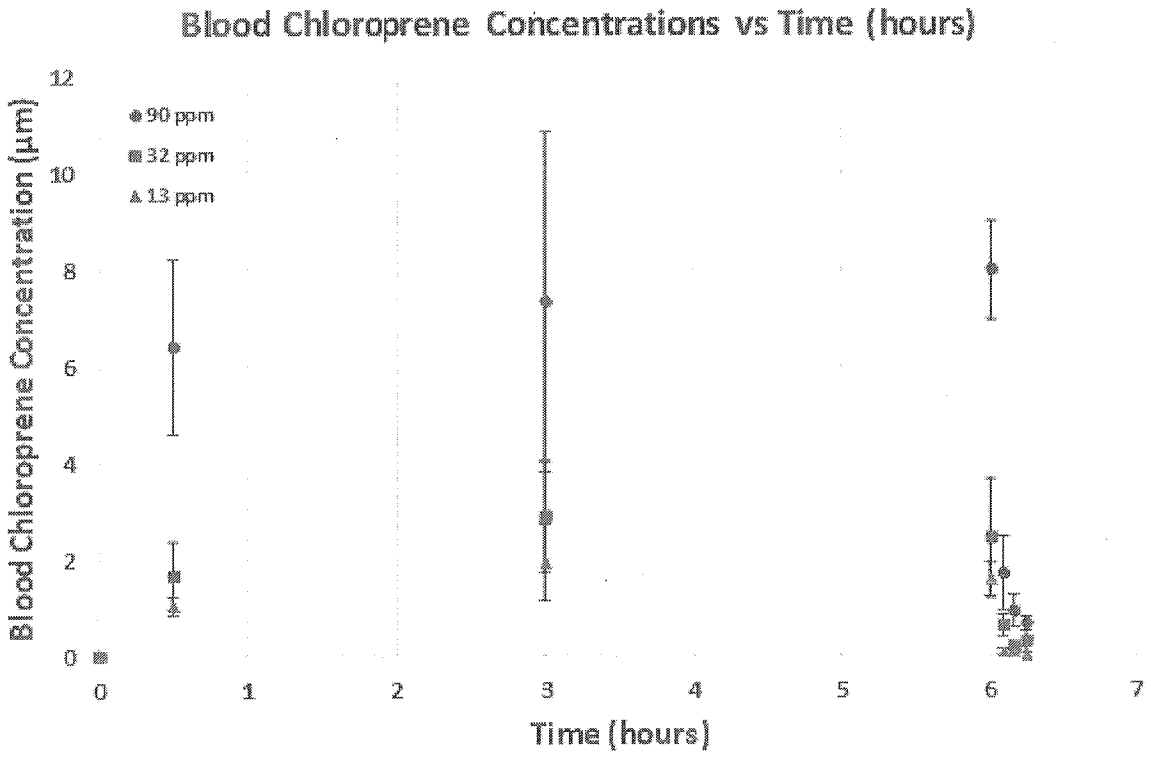

Figure 3. Arterial blood chloroprene concentrations during and following a single nose-only exposure of female B6C3F1 mice to chloroprene at 13, 32 or 90 ppm for 6 hours. Average blood chloroprene concentrations (symbols) and standard deviations (error bars) are shown for each treatment group.

Re-estimation of Metabolism Parameters

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

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

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

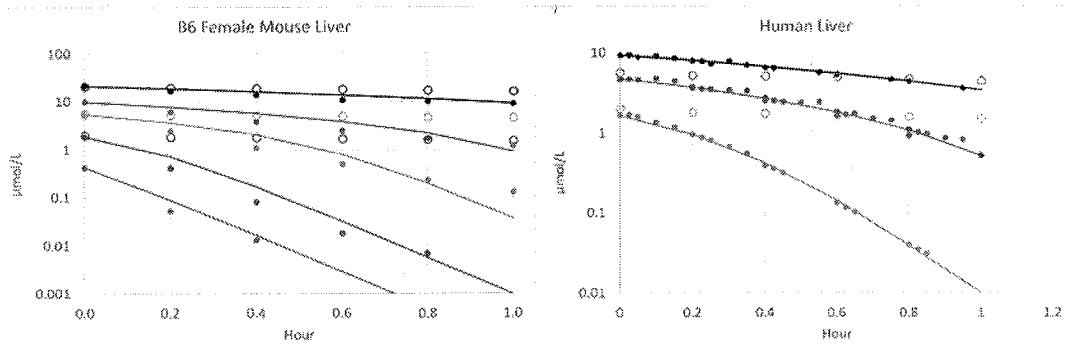

Figure 4. Predicted (curves) and measured (solid symbols) concentrations of chloroprene during *in vitro* metabolism experiments in the mouse (left) or human (right) liver. Open circles are data from control vials that lacked metabolic activity.

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

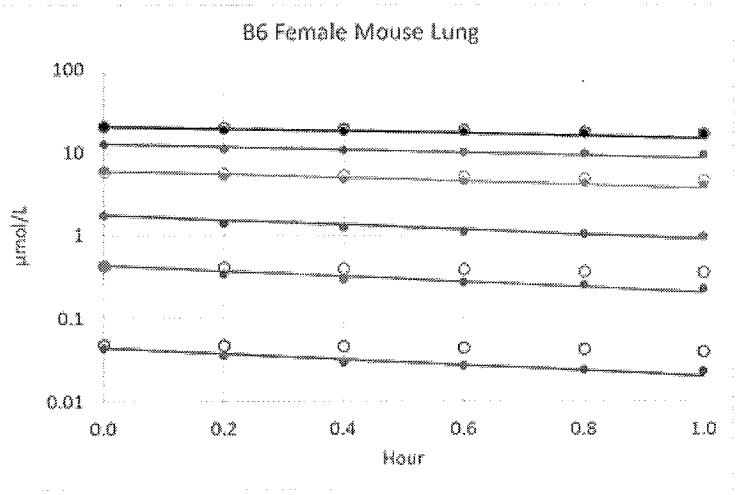

Figure 5. Predicted (curves) and measured (solid symbols) concentrations of chloroprene during *in vitro* metabolism experiments in the mouse lung. Open circles are data from control vials that lacked metabolic activity.

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

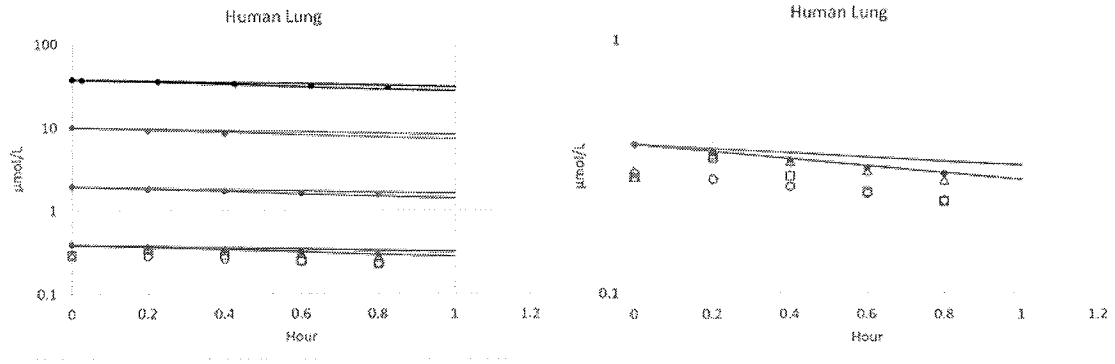

Figure 6. Measured concentrations of chloroprene during *in vitro* metabolism experiments in the human lung. (Solid symbols: metabolism vials; open symbols: control vials). Curves are model predictions assuming no metabolism is occurring.

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

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

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

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

PBPK Modeling of the Nose-Only Inhalation Study

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

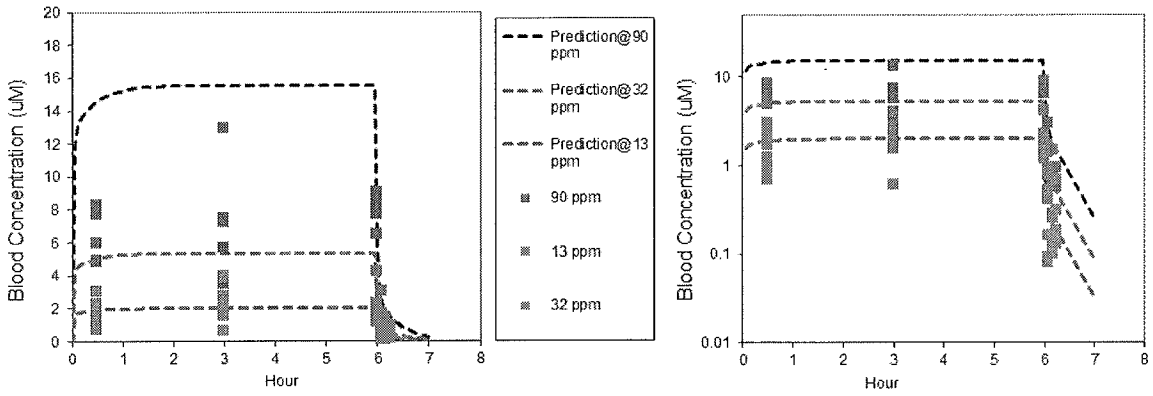

Figure 7. PBPK model predicted (dotted lines) and measured (symbols) blood concentrations during and following 6-hr exposures of B6C3F1 mice to chloroprene at 12.3 (green), 32 (fuchsia) or 90 (blue) ppm. The same data and model predictions are shown using a linear y axis (left) and a logarithmic y axis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

PBPK Model Parameter Sensitivity

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

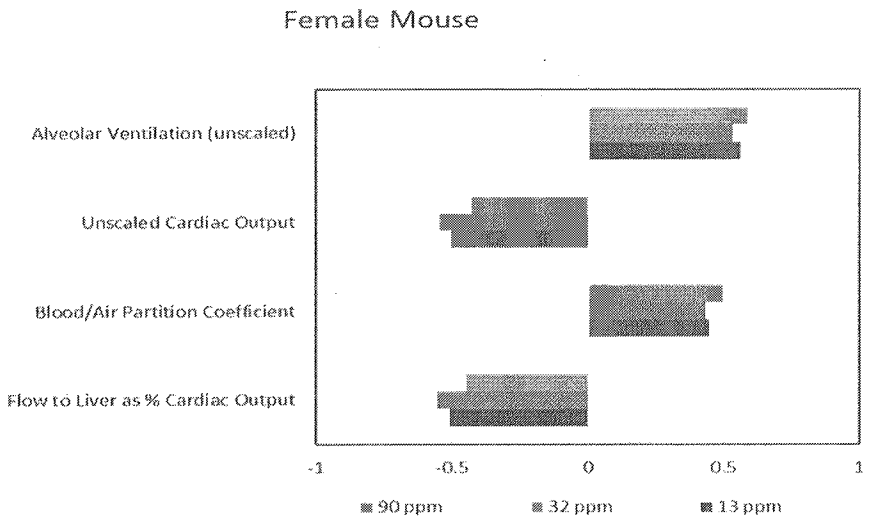

Figure 8. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of arterial blood concentrations in the nose-only study.

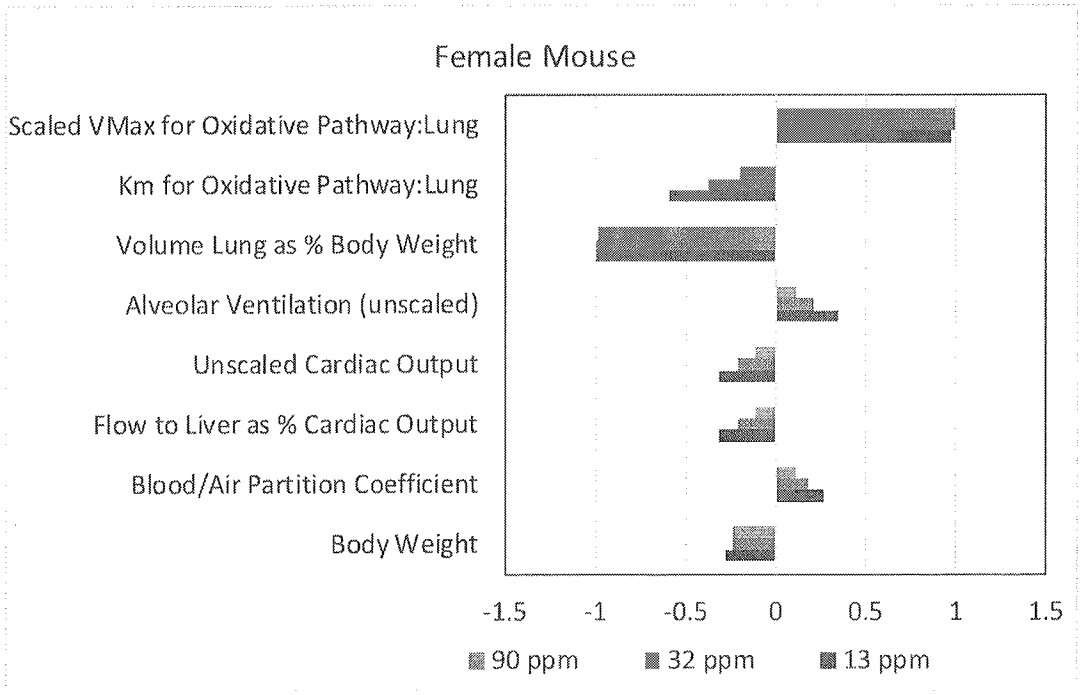

Figure 9. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of lung dose metrics in the female mouse for exposures in the 2-year bioassay.

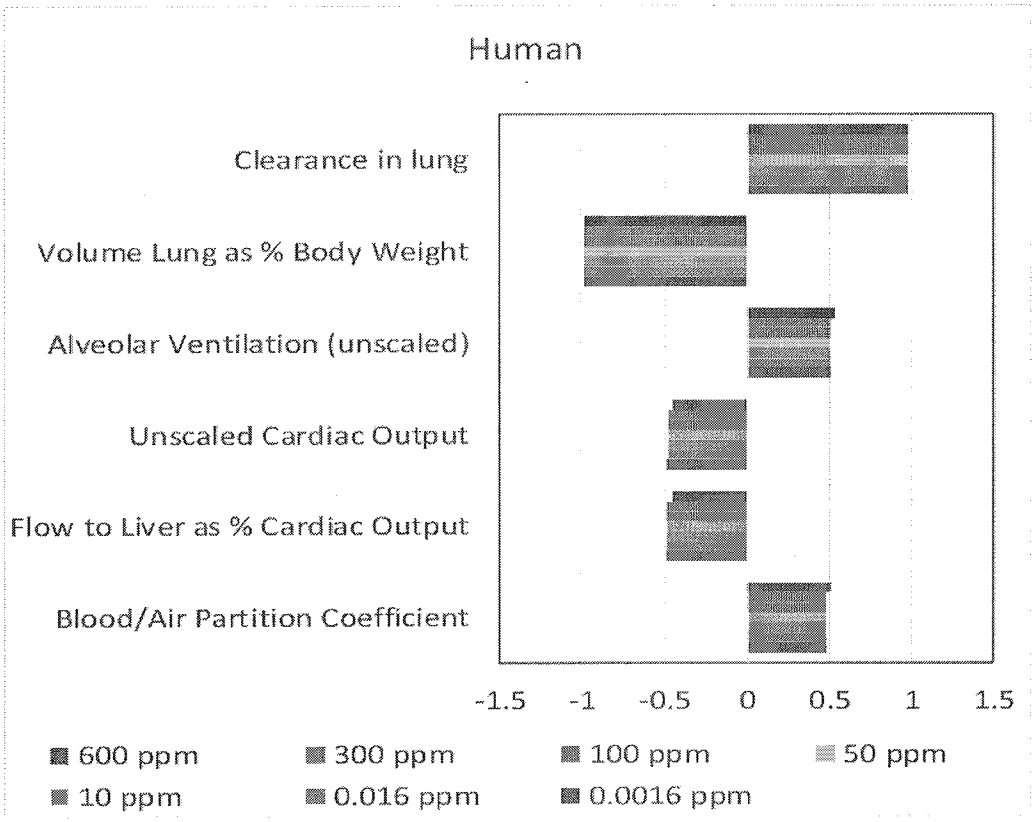

Figure 10. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of lung dose metrics in the human for continuous exposure at 1 ppm.

Kgl Sensitivity Analysis

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

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

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

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

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

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

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

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

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

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

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

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

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

A1 uncertainty analysis

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

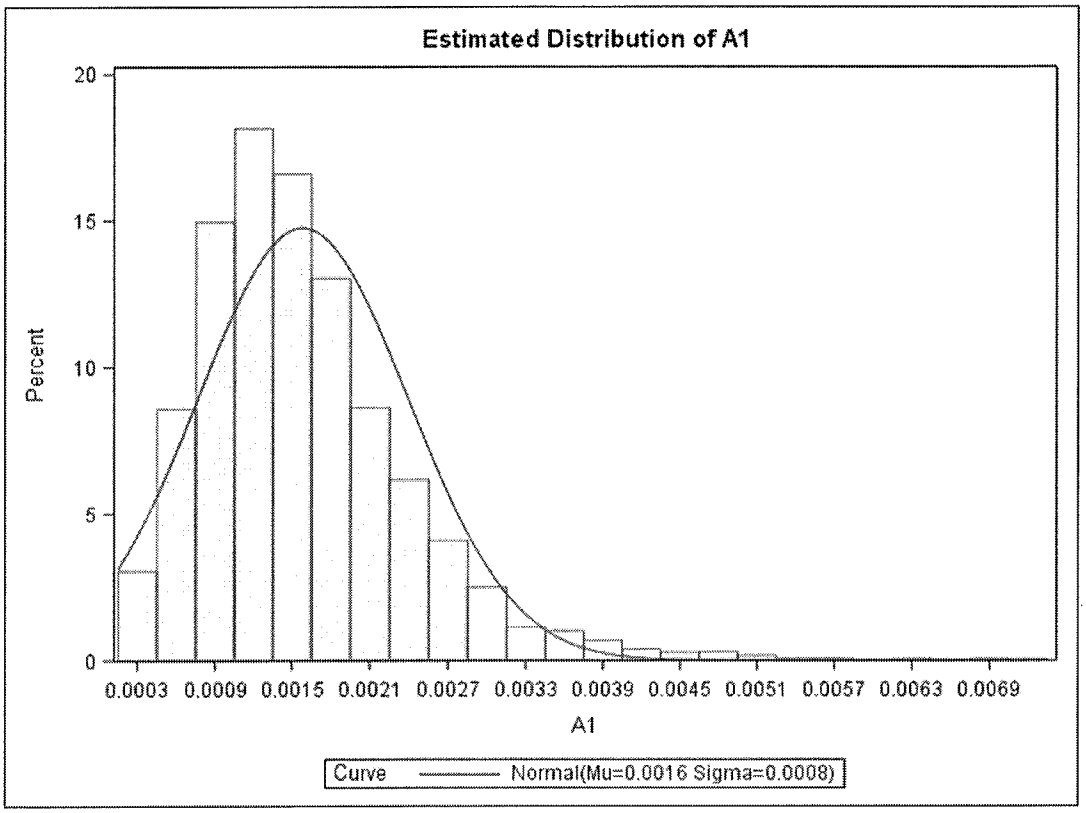

Figure 11. Distribution of A1 estimated from Lorenz et al. (1984)

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

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

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

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

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

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

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

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

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

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

PBPK-Based Dose Metrics for Chloroprene Lung Carcinogenicity

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

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

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

PBPK Model Uncertainty Analysis

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

Means and 90% Confidence Intervals from Monte Carlo Analysis				
Exposure	**Concentration**	**Mean**	**5th percentile**	**95th percentile**
Female mouse bioassay	12.8 ppm	1.11	0.52	1.99
	32 ppm	1.70	0.84	3.07
	80 ppm	2.21	1.12	4.02
Female Mouse BMDL		0.012	0.0034	0.028
Human continuous	1 $\mu g/m^3$	$4.2x10^{-6}$	$9.7x10^{-7}$	$1.1x10^{-5}$

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

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

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

4. DISCUSSION

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

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

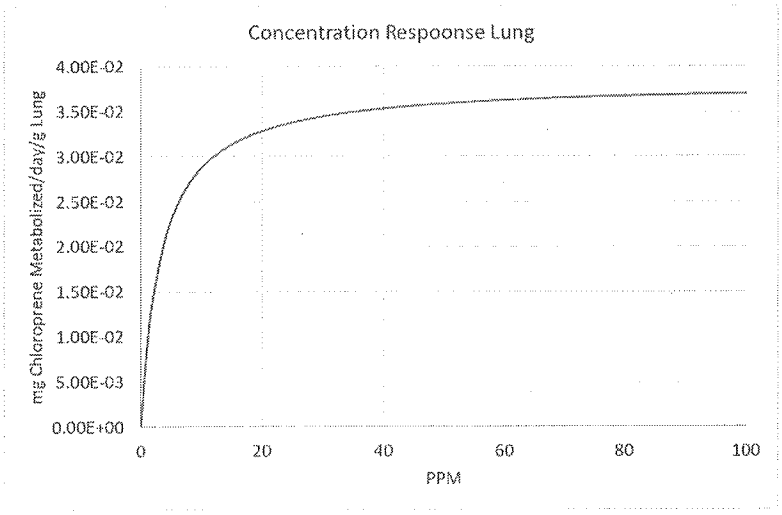

Figure 12. Inhaled concentration dependence of lung metabolism in the human for continuous exposures to chloroprene predicted with the PBPK model.

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

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

Selection of Dose Metric

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

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

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

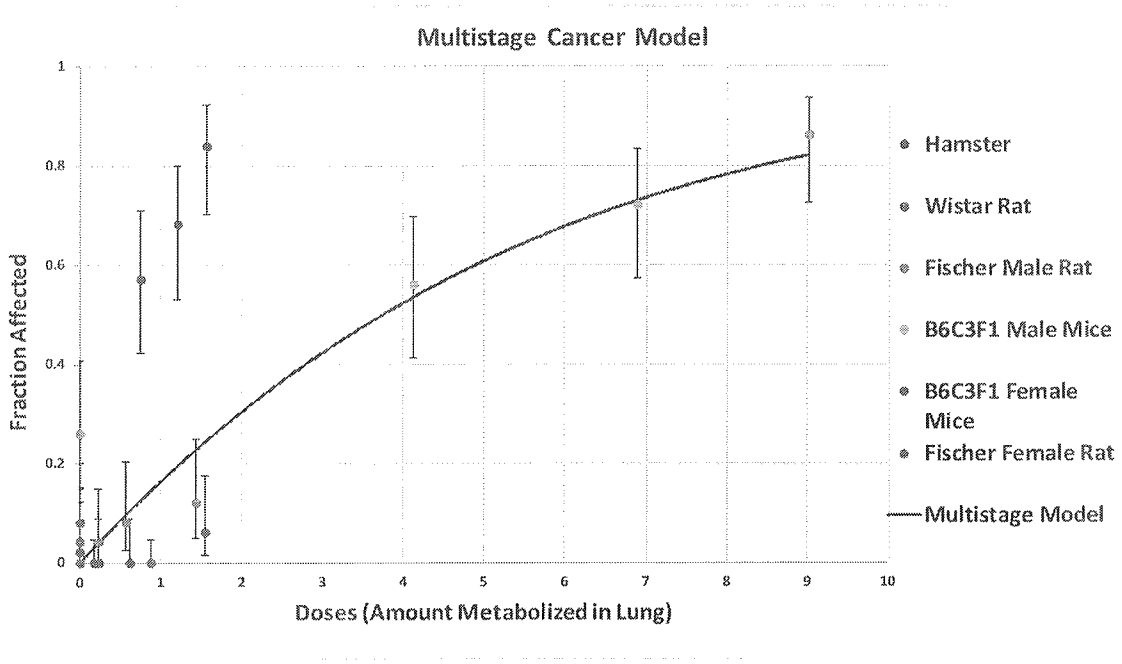

Figure 13. Comparison of Dose-Response for Lung Tumors in Chloroprene Bioassays in Rodents.

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

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

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

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

Use of *In Vitro* Metabolism Data

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5. CONCLUSION

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

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Supplemental Materials

A. Supplemental Tables

B. Re-estimation of Metabolism Parameters

C. IVIVE Literature Review

D. Metabolism Parameter Calculations

E. Model Files

F. Reactive Metabolite Modeling

G. Responses to Peer Reviewer Comments

Intended for

Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

SUPPLEMENTAL MATERIALS A
SUPPLEMENTAL TABLES

Table S-1: Physiological Parameters for PBPK Model										
Parameter	**Abbreviation**	**Units**	**Mice**		**Source**	**Rats**		**Source**	**Humans**	**Source**
			Male	**Female**		**Male**	**Female**			
Body Weight	BW	kg	0.04	0.04	NTP (1998) time and survival weighted average BW control animals	0.407	0.256	NTP (1998) time and survival weighted average BW control animals	70	USEPA 1988
Alveolar Ventilation	QPC	$L/h/kg^{0.75}$	29.1	29.1	Brown et al. 1997 (Table 31)	22.4	22.4	Brown et al. 1997 (Table 31)	24.0	Clewell et al. 2001 (Table 1)
Cardiac Output	QCC	$L/h/kg^{0.75}$	20.1	20.1	Marino et al. 2006 (QPC/QCC = 1.45)	18.7	18.7	Brown et al. 1997 (Table 22)	16.5	Clewell et al. 2001 (Table 1)
FRACTIONAL BLOOD FLOWS TO TISSUES										
Flow to Liver as fraction Cardiac Output	QLC	unitless	0.161	0.161	Brown et al. 1997 (Table 23)	0.183	0.183	Brown et al. 1997 (Table 23)	0.227	Brown et al. 1997 (Table 23)
Flow to Fat as fraction Cardiac Output	QFC	unitless	0.07	0.07	Brown et al. 1997 (Table 23; Same as rat value)	0.07	0.07	Brown et al. 1997 (Table 23)	0.052	Brown et al. 1997 (Table 23)
Flow to Slow as fraction Cardiac Output	QSC	unitless	0.159	0.159	Brown et al. 1997 (Table 23); Same as that reported for muscle	0.278	0.278	Brown et al. 1997 (Table 23); Same as that reported for muscle	0.191	Brown et al. 1997 (Table 23); Same as that reported for muscle
Flow to Kidney as fraction Cardiac Output	QKC	unitless	0.09	0.09	Brown et al. 1997 (Table 23)	0.14	0.14	Brown et al. 1997 (Table 23)	0.175	Brown et al. 1997 (Table 23)
FRACTIONAL VOLUMES OF TISSUES										
Volume Liver as fraction Body Weight	VLC	unitless	0.0549	0.0549	Brown et al. 1997 (Table 4)	0.0366	0.0366	Brown et al. 1997 (Table 5)	0.0257	Brown et al. 1997 (Table 7)

Table S-1: Physiological Parameters for PBPK Model										
Parameter	**Abbreviation**	**Units**	**Mice**		**Source**	**Rats**		**Source**	**Humans**	**Source**
			Male	**Female**		**Male**	**Female**			
Volume Lung as fraction Body Weight	VLUC	unitless	0.0073	0.0073	Brown et al. 1997 (Table 4)	0.005	0.005	Brown et al. 1997 (Table 5)	0.0076	Brown et al. 1997 (Table 7)
Volume Fat as fraction Body Weight	VFC	unitless	0.1	0.1	Brown et al. 1997 (Table 10)	0.1	0.1	Brown et al. 1997 (Table 13)	0.27	Brown et al. 1997 (Table14); Average of total male and female
Volume Rapid Perfused as fraction Body Weight	VRC	unitless	0.08098	0.08098	Brown et al. 1997 (Table 4); Sum of adrenals, brain, stomach, small intestine, large intestine, heart, lungs, pancreas, spleen and thyroid	0.04644	0.04644	Brown et al. 1997 (Table 5); Sum of adrenals, brain, stomach, small intestine, large intestine, heart, lungs, pancreas, spleen and thyroid	0.0533	Brown et al. 1997 (Table 7); Sum of adrenals, brain, stomach, small intestine, large intestine, heart, lungs, pancreas, spleen and thyroid
Volume Slow Perfused as fraction Body Weight	VSC	unitless	0.384	0.384	Brown et al. 1997 (Table 4); Same as that reported for muscle	0.4	0.4	Brown et al. 1997 (Table 5); Same as that reported for muscle	0.4	Brown et al. 1997 (Table 7); Same as that reported for muscle
Volume Kidney as fraction Body Weight	VKC	unitless	0.0167	0.0167	Brown et al. 1997 (Table 4)	0.0073	0.0073	Brown et al. 1997 (Table 5)	0.0044	Brown et al. 1997 (Table 7)

Table S-2: Partition Coefficients for PBPK Model			
	Mice	**Rats**	**Humans**
Blood:Air	7.83	7.35	4.54
Lung:Blood	2.38	1.85	2.94
Liver:Blood	1.26	1.58	2.37
Fat:Blood	17.35	16.99	28.65
Muscle:Blood [a]	0.59	0.60	1.00
Kidney:Blood [b]	1.76	2.29	2.67

[a] used for slowly perfused tissues

[b] used for rapidly perfused tissues

Table S-3: *In Vitro* Metabolism Parameters			
Sex & Species	**Parameter**	**Source**	
		Yang et al. 2012 MCMC Mean[a]	**This Analysis Mean (95% Confidence Interval)**
Female mouse	Vmax, liver (µmol/h/mg protein)	0.13 (0.11 – 0.15)	0.105 (0.087 - 0.127)
	Vmax, lung (µmol/h/mg protein)	0.03 (0.010 – 0.050)	0.022 (0.015 – 0.031)
	Vmax, kidney (µmol/h/mg protein) [b]	0.004 (-0.016 – 0.024)	ND
	Km, liver (µmol/L)	0.88 (0.606 – 1.154)	0.448 (0.302 – 0.652)
	Km, lung (µmol/L)	2.82 (-0.140 – 5.780)	2.369 (1.555 – 3.549)
	Km, kidney (µmol/L) [b]	176.11 (-1632.71 – 1984.94)	ND
Male mouse	Vmax, liver (µmol/h/mg protein) [c]	0.26 (0.240 – 0.280)	0.212 (0.161 – 0.257)
	Vmax, lung (µmol/h/mg protein) [c]	0.14 (0.120 – 0.160)	0.085 (0.069 – 0.105)
	Vmax, kidney (µmol/h/mg protein)	0.01 (0.008 – 0.012)	0.012 (0.0088 – 0.015)
	Km, liver (µmol/L) [c]	1.34 (1.183 – 1.497)	0.689 (0.472 – 0.903)
	Km, lung (µmol/L) [c]	2.22 (1.946 – 2.494)	1.194 (0.943 – 1.523)
	Km, kidney (µmol/L)	0.77 (0.594 – 0.946)	0.647 (0.469 – 0.902)
Female rat	Vmax, liver (µmol/h/mg protein)	0.09 (0.070 – 0.110)	0.069 (0.056 – 0.085)
	Vmax, lung (µmol/h/mg protein) [b]	NC	0.00408 (0.00152 – 0.00618)
	Vmax, kidney (µmol/h/mg protein)	0.003 (0.0024 – 0.0036)	0.0018 (0.0013 – 0.0026)
	Km, liver (µmol/L)	0.56 (0.501 – 0.619)	0.718 (0.544 – 0.933)
	Km, lung (µmol/L) [b]	NC	2.369 (fixed to female mouse Km)
	Km, kidney (µmol/L)	0.60 (0.443 – 0.757)	0.449 (0.298 – 0.687)
Male rat	Vmax, liver (µmol/h/mg protein) [c]	0.10 (0.094 – 0.106)	0.072 (0.066 – 0.078)
	Vmax, lung (µmol/h/mg protein) [b]	NC	ND
	Vmax, kidney (µmol/h/mg protein)	0.003 (0.0024 – 0.0036)	0.0019 (0.0014 – 0.0026)
	Km, liver (µmol/L) [c]	0.56 (0.501 – 0.619)	0.417 (0.367 – 0.477)
	Km, lung (µmol/L) [b]	NC	ND
	Km, kidney (µmol/L)	0.76 (0.544 – 0.976)	0.619 (0.437 – 0.885)
Humans	Vmax, liver (µmol/h/mg protein) [c]	0.05 (0.048 – 0.052)	0.055 (0.052 – 0.059)
	Vmax, lung (µmol/h/mg protein) [b]	NC	ND
	Vmax, kidney (µmol/h/mg protein) [b]	NM	NM
	Km, liver (µmol/L) [c]	0.45 (0.430 – 0.470)	0.349 (0.312 – 0.394)
	Km, lung (µmol/L) [b]	NC	ND
	Km, kidney (µmol/L) [b]	NM	NM

[a]95% CI calculated as mean +/- SD*1.96 reported in Yang et al. 2012

[b]ND: not determinable; NM: not measured; NC: not comparable

[c]The initial amount of chloroprene was estimated for each concentration as the time-course data were constructed from multiple vials

Table S-4: Metabolism Parameters for PBPK Model			
Sex & Species	**Parameter**	**Source**	
		Yang et al. 2012	**This Analysis (Table S-3)**
Female Mouse	VmaxC, liver (mg/h/kg**3/4)	8.88	7.99
	VmaxC, lung (mg/h/kg**3/4)	0.11	0.12
	VmaxC, kidney (mg/h/kg**3/4) [a]	0.03	ND
	Km, liver (mg /L)	0.08	0.040
	Km, lung (mg /L)	0.25	0.21
	KM, kidney (mg /L) [a]	9.59	ND
Male Mouse	VmaxC, liver (mg/h/kg**3/4) [b]	18.54	16.09
	VmaxC, lung (mg/h/kg**3/4) [b]	0.6	0.49
	VmaxC, kidney (mg/h/kg**3/4)	0.078	0.14
	Km, liver (mg /L) [b]	0.12	0.061
	Km, lung (mg /L) [b]	0.2	0.11
	KM, kidney (mg /L)	0.068	0.057
Female Rat	VmaxC, liver (mg/h/kg**3/4)	9.37	6.36
	VmaxC, lung (mg/h/kg**3/4) [a]	NC	0.03
	VmaxC, kidney (mg/h/kg**3/4)	0.018	0.015
	Km, liver (mg /L)	0.09	0.064
	Km, lung (mg /L) [a]	NC	0.21
	KM, kidney (mg /L)	0.053	0.040
Male Rat	VmaxC, liver (mg/h/kg**3/4) [b]	9.48	7.42
	VmaxC, lung (mg/h/kg**3/4) [a]	NC	ND
	VmaxC, kidney (mg/h/kg**3/4)	0.018	0.018
	Km, liver (mg /L) [b]	0.05	0.037
	Km, lung (mg /L) [a]	NC	ND
	KM, kidney (mg /L)	0.067	0.055
Humans	VmaxC, liver (mg/h/kg**3/4) [b]	20.4	14.51
	VmaxC, lung (mg/h/kg**3/4) [a]	NC	0.0031
	VmaxC, kidney (mg/h/kg**3/4) [a]	NM	NM
	Km, liver (mg /L) [b]	0.04	0.031
	Km, lung (mg /L) [a]	NC	0.031
	KM, kidney (mg /L) [a]	NM	NM

[a] ND: not determinable; NM: not measured

[b] The initial amount of chloroprene was estimated for each concentration as the time-course data were constructed from multiple vials

Table S-5: Plethysmography Data			
Pulmonary Function Data Protocol 07039 Summary			**Exposure #1**
Exposure	**Frequency (BPM)**	**Tidal Volume (ml)**	**Minute Ventilation (ml/min)**
0ppm	Average	Average	Average
Animal 1	193.5	0.247	48.4
Animal 2	168.2	0.284	47.2
Animal 3	251.9	0.212	51.6
Animal 4	201.7	0.311	61.6
Average	203.8	0.264	52.2
Stdev	35.1	0.043	6.5
Pulmonary Function Data Protocol 07039 Summary			**Exposure #2**
Exposure	**Frequency (BPM)**	**Tidal Volume (ml)**	**Minute Ventilation (ml/min)**
13 ppm	Average	Average	Average
Animal 20	221.9	0.261	57.6
Animal 21	221.0	0.264	57.6
Animal 22	140.9	0.224	32.0
Animal 23	131.2	0.218	28.9
Average	178.7	0.242	44.0
Stdev	49.4	0.024	15.7
Pulmonary Function Data Protocol 07039 Summary			**Exposure #3**
Exposure	**Frequency (BPM)**	**Tidal Volume (ml)**	**Minute Ventilation (ml/min)**
32 ppm	Average	Average	Average
Animal 54	253.3	0.269	66.1
Animal 55	225.0	0.269	59.4
Animal 56	249.8	0.282	69.5
Animal 57	235.2	0.293	67.9
Average	240.8	0.278	65.7
Stdev	13.1	0.012	4.4
Pulmonary Function Data Protocol 07039 Summary			**Exposure #4**
Exposure	**Frequency (BPM)**	**Tidal Volume (ml)**	**Minute Ventilation (ml/min)**
90 ppm	Average	Average	Average
Animal 88	216.3	0.276	59.4
Animal 89	178.4	0.242	43.2
Animal 90	217.9	0.277	59.8
Animal 91	166.2	0.278	45.8
Average	194.7	0.268	52.0
Stdev	26.4	0.017	8.8

Table S-6: Arterial Blood Concentrations			
13 ppm Exposure			
Time point	**Average [CD]**	**Std Dev. CD**	**RSD%**
Control	0	0	0%
0.5 hours	1.03	0.18	17%
3 hours	1.93	0.80	41%
6 hours	1.58	0.35	22%
5 min post exposure	0.66	0.07	101%
10 min post exposure	0.70	0.11	157%
15 min post exposure	0	0	0%
32 ppm Exposure			
Time point	**Average [CD]**	**Std Dev. CD**	**RSD%**
Control	0	0	0%
0.5 hours	1.68	0.70	42%
3 hours	2.90	1.15	40%
6 hours	2.44	1.24	51%
5 min post exposure	0.61	0.22	35%
10 min post exposure	0.18	0.09	48%
15 min post exposure	0.30	0.23	78%
90 ppm Exposure			
Time point	**Average [CD]**	**Std Dev. CD**	**RSD%**
Control	0	0	0%
0.5 hours	6.41	1.83	29%
3 hours	7.33	3.52	48%
6 hours	8.00	1.02	13%
5 min post exposure	1.71	0.78	46%
10 min post exposure	0.92	0.33	35%
15 min post exposure	0.68	0.15	23%

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Intended for

Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

SUPPLEMENTAL MATERIALS B
RE-ESTIMATION OF METABOLISM PARAMETERS

The original *in vitro* concentration time-course data for metabolism of chloroprene (Himmelstein et al. 2004a; IISRP 2009b) were re-analyzed using MCMC analysis with vague priors to obtain a revised set of metabolism parameters for the model. The key differences between the alternative analysis and the original Yang et al. (2012) analysis were: (1) the incorporation of an additional parameter in the analysis of the *in vitro* metabolism data (Kgl) to describe the rate of transfer of chloroprene from the headspace to the media in the metabolism studies, (2) the estimation of the initial amount of chloroprene in the vial for incubations where the time-course data included more than one vial (i.e. male mouse liver and lung, male rat liver and mixed human liver), (3) the use of updated tissue microsomal protein concentrations for scaling the *in vitro* results to *in vivo* values appropriate for the PBPK model, and (4) the adoption of a previously published approach for estimating the metabolism parameters in the human lung (Andersen et al. 1987a). The details of the re-estimation of the *in vitro* metabolism parameters is described below and the results are provided in Supplemental Materials D.

Experimental Determination of Mass Transport Limitation

Schlosser et al. (1993) suggested the need to consider mass transport limitations during *in vitro metabolism* experiments conducted with volatile compounds where there is an air:liquid interface. In their studies of benzene metabolism in sealed vials (Schlosser et al. 1993), they conducted separate studies to determine the rate of approach of the system to equilibrium in order to estimate the rate of transfer of the chemical between the air and liquid phases, and then used the estimated mass transport parameter (Kgl) in their analysis of the metabolism of benzene. In the *in vitro* metabolism studies conducted with chloroprene (Himmelstein et al. 2004a; Yang et al. 2012), no assessment of mass transport limitation was performed. Therefore, a new experimental study was conducted by TekLab, Inc, Collinsville IL, to estimate a Kgl for chloroprene following a protocol similar to Schlosser et al. (1993):

1. Add 1mL buffer solution to a 10mL crimp top vial. Crimp on top. A total of 12 vials will be needed for each series of tests.
2. Place vials in a water bath set at 37 C rotating at 60 rpm. Allow temperature to equilibrate for a minimum of 10 minutes.
3. After reaching thermal equilibrium, pierce the septa with an open needle to allow the pressure of the headspace to adjust to ambient pressure.
4. Add 0.5mL of 800ppmv chloroprene gas standard[1]. Immediately start a timer set for the appropriate contact time.
5. As the timer reaches zero, remove vial and insert a 1mL syringe into the vial so the needle is below the liquid level in the vial. Withdraw 0.5mL and place it into a 40mL VOA vial containing a Teflon stir bar and 4.5mL of deionized water.
6. The beginning and ending blanks are treated the same as the samples with the addition of the chloroprene.
7. A total of 10 samples are to be prepared with contact times of 5, 10, 20, 30, 45, 60, 120, 180, 240 and 360 seconds. The 5 second sample was replaced with a 600 second sample starting with replicate set R-15.

[1] The vapor standard used to spike the headspace was prepared according to Denka Method PWR Gas 1805 using a chloroprene standard received from Denka. A high concentration stock was prepared, and a working standard prepared from the stock by doing a 100x dilution into a second Tedlar bag. The initial vapor standards were prepared in 1L Tedlar bags. The remaining standard sets were prepared in 500mL Tedlar bags.

8. When all of the samples for the set are prepared in the 40mL VOA vials, they are analyzed in the VOA lab by GC\MS SW-846 Method 8260B and Method 5035. The concentration of the chloroprene is reported in µg/L.

The original report on the study is available from the authors on request. The resulting time-courses for chloroprene concentrations in the aqueous phase of the vials were analyzed using the same approach as in Schlosser et al. (1993), resulting in an estimated value of 0.024 L/hr for Kgl, which is similar to the value previously reported for benzene (Schlosser et al. 1993). The results of the MCMC analysis are shown in Figure B-1.

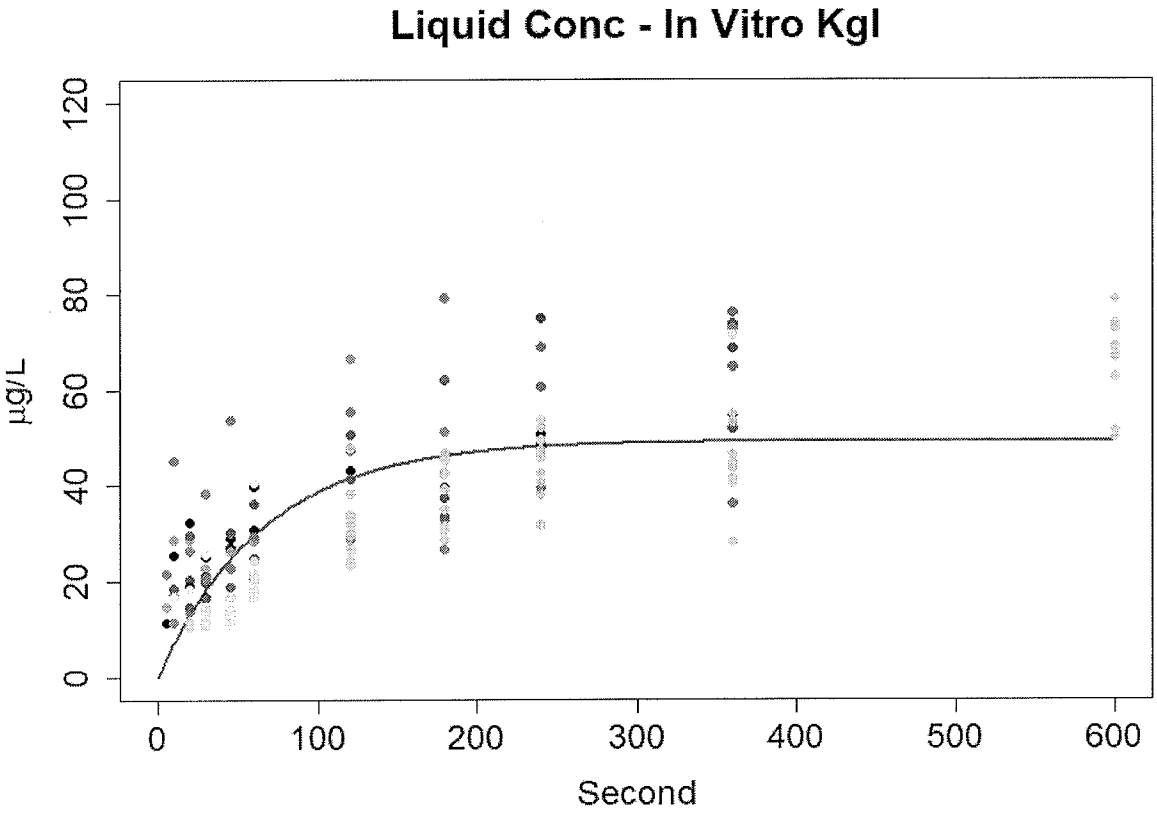

Figure B-1. Concentration of chloroprene in the aqueous phase following addition of 0.5 mL of 800 ppmV chloroprene to the air phase. The best estimates of Kgl and P (the liquid:air partition coefficient) were 0.024 (std. dev. = 0.0054) and 0.48 (std. dev. = 0.02).

Using this experimental value of Kgl, however, it was not possible to explain the high rates of liver metabolism observed at low concentrations of chloroprene; that is, the mass transport associated with Kgl = 0.024 L/hr was too slow to support the observed rates of metabolism in the media. Figure B-2 shows the closest possible fit to the experimental data using the experimental value of Kgl. Even if the metabolic clearance is set to an implausibly high value (Vmax = 1000 µmol/hr, Km = 0.01 µmol/L), it is impossible to fit the metabolism data with a Kgl of less than 0.11 L/hr

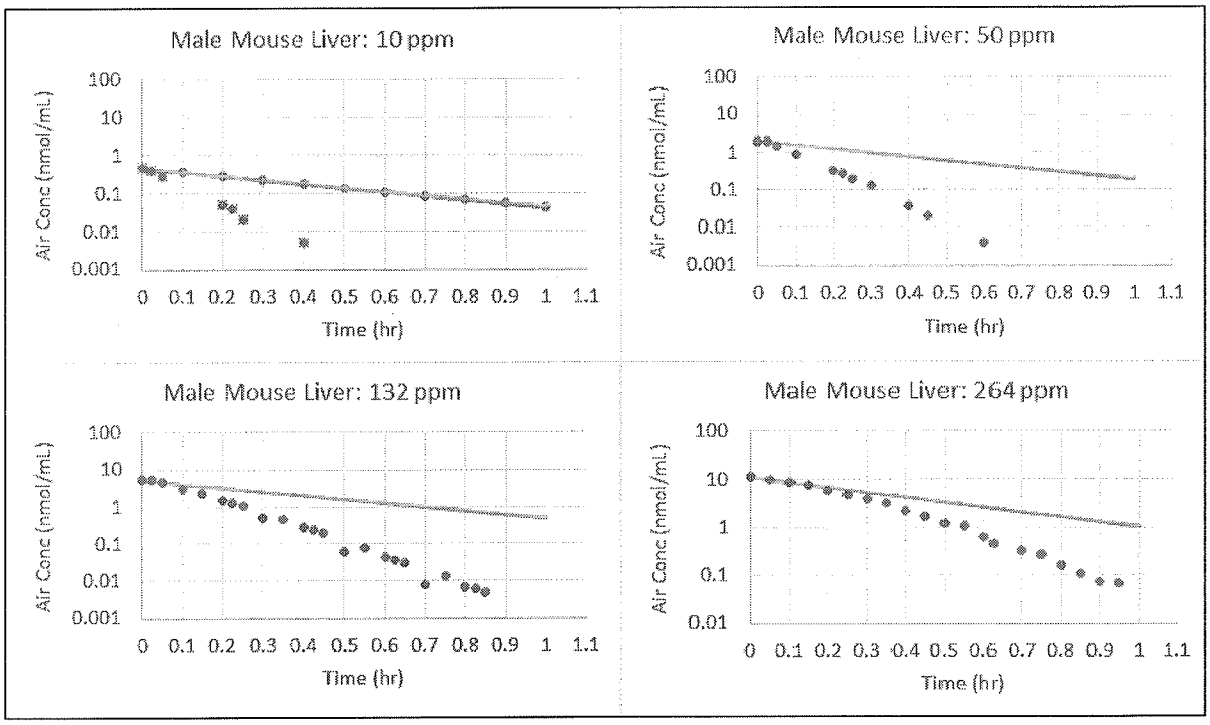

Figure B-2. Comparison of maximum metabolism rate predictions for male mouse liver (curves) and experimental data (points) using Kgl = 0.024 L/hr.

We considered it likely that the much faster uptake of chloroprene in the metabolism studies than in the Kgl study was due to more effective mixing during the incubations, together with non-specific surface binding of chloroprene to the microsomes, which provide a lipophilic binding component in the aqueous media. No microsomes were present in the Kgl experiments for chloroprene or benzene (Schlosser et al. 1993). Although the rate of shaking in the metabolism studies was not reported, we were able to determine that the Himmelstein et al. (2004a) and IISRP (2009b) studies used a Gerstel MPS2 autosampler with an agitating heater, which was set to an agitation rate of 500 rpm (Matt Himmelstein, personal communication). Based on this information, it was suggested (Paul Schlosser, personal communication) that the value of Kgl in the metabolism studies was likely to be higher than the value in the new experimental study by roughly the ratio of the mixing rates, that is, Kgl(metabolism studies) = Kgl(experimental study) × 500/60 = 0.024×500/60 = 0.2 L/hr.

To confirm this expectation, we conducted a new MCMC analysis to simultaneously estimate Kgl, Vmax and Km from the metabolism data for the male mouse (Himmelstein et al. 2004a), the data which are most informative regarding the dose-response for the clearance of chloroprene in the vials. This analysis detected a high degree of collinearity between Km and Kgl (Figure B-3), indicating that the estimates of these two parameters are not completely independent.

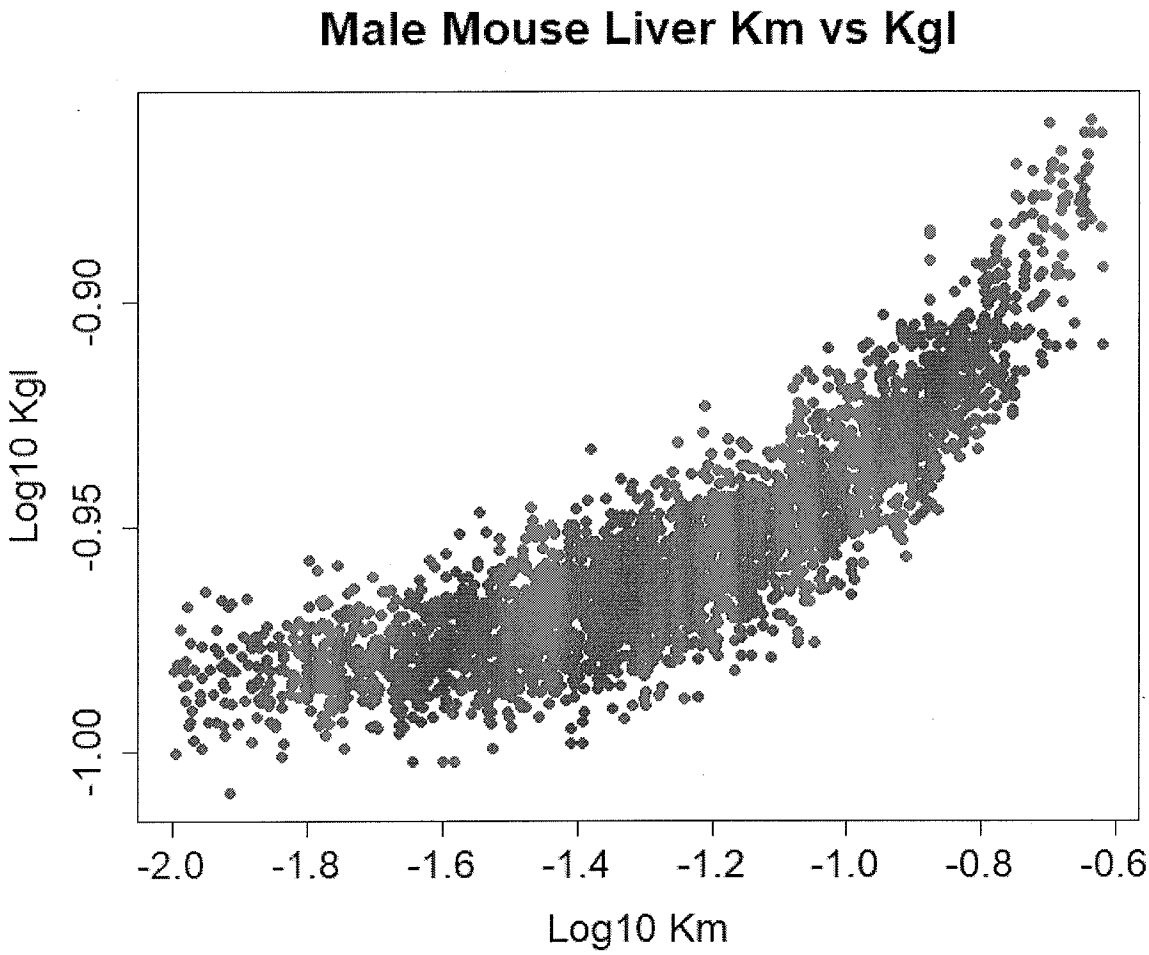

Figure B-3. Correlation plot of log(Kgl) vs. log (Km). Lower values of Kgl correlate with lower values of Km.

Therefore, to provide a basis for a biological constraint on the Km values estimated using this approach, we conducted a literature review of compounds with structural similarities to chloroprene, specifically, halogenated alkanes and alkenes, and found (Table B-1) that the values of Km estimated for these compounds from a variety of *in vivo* studies in mice, rats and humans ranged from 1 to 7 µmol/L (Andersen et al. 1987b, 1991, 1994; Clewell et al. 2001; Corley et al. 1990; David et al. 2006; D'Souza and Andersen 1988; D'Souza et al. 1987, 1988; Gargas and Andersen 1989; Gargas et al. 1986, 1990; Lilly et al. 1997, 1998; Marino et al. 2006).

Table B-1. Estimates of Km for CYP2E1 substrates based on *in vivo* studies.		
Compound	**Km (μmol/L)**	**Species**
Inhibition studies:		
TCE	1.9	rat (Andersen et al. 1987b)
DCE	1.0	rat (Andersen et al. 1987b)
In vivo metabolism studies:		
MeCl2	5.1/6.8	human/mouse MCMC (David et al. 2006; Marino et al. 2006)
DHMs	2.3-4.7	rat (Gargas et al. 1986)
BDCM	3	rat (Lilly et al. 1997, 1998)
Closed Chamber *in vivo* studies:		
VC:	1.6	human (Clewell et al. 2001)
CHCl3	3-4.6	rat (Corley et al. 1990)
EDC	2.5	rat (D'Souza et al. 1987, 1988)
VDC	2.5	rat (D'Souza and Andersen 1988)
Chloroethanes	3.3-5.6	rat (Gargas and Andersen 1989)
chlorinated ethylenes	1-5	rat (Gargas et al. 1990)
Furan	2	rat (Kedderis et al. 1993)

The strongest data for estimating a Km were from studies of mutual metabolic inhibition in co-exposures to trichloroethylene and dichloroethylene (Andersen et al. 1987b), which estimated Kms of 1.9 and 1.0 μmol/L, respectively. A Km of 1.6 μmol/L was used by the USEPA in their risk assessment for vinyl chloride (USEPA 2000).

Therefore, we conducted a re-analysis of the data on metabolism in the male mouse liver to simultaneously estimate Vmax, Km and Kgl using uninformative priors except that the prior for Kgl was bounded from below at 0.11 L/hr, the minimum value that we determined could support the rate of metabolism observed in the liver, and the prior for Km was bounded from below at a value of 0.5 μmol/L, a factor of 2 below the lowest value for a substrate of CYP2E1 from our review of the literature. There is no evidence that the posterior distributions from this analysis were clipped by the use of these lower bounds on the priors (Figure B-4).

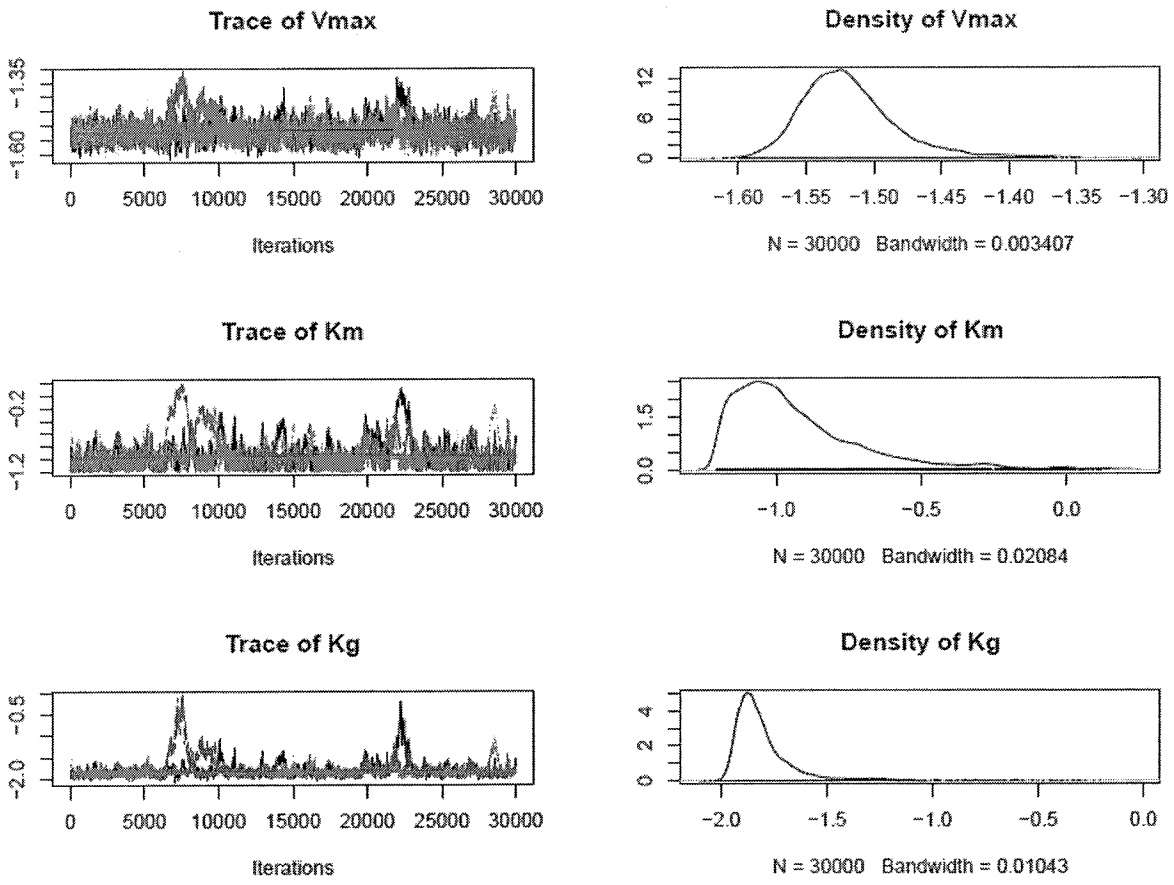

Figure B-4. Posterior chains (right) and distributions (left) of ln(Vmax), ln(Km), and ln(Kgl)

The resulting value of Kgl estimated from this analysis was 0.22 L/hr, with a 95% confidence interval of 0.19 – 0.33 L/hr, consistent with the value of 0.2 L/hr calculated from the experimental Kgl. Figure B-5 shows the resulting fit to the experimental data for the male mouse liver.

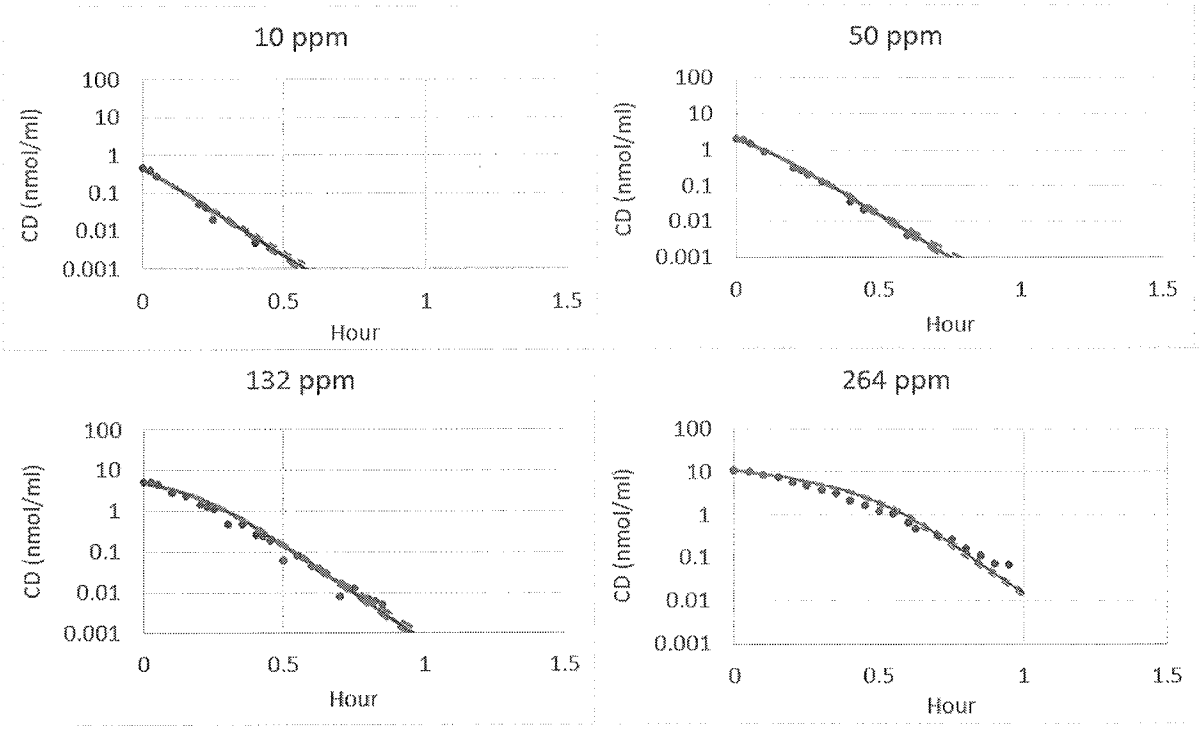

Figure B-5. Comparison of metabolism rate predictions for male mouse liver (curves) and experimental data (points) using 1000 iterations of a posterior chain from the *in vitro* metabolism MCMC (Geometric Means: Km = 0.62 µmol/L, Vmax = 0.23 µmol/hr/mg protein and Kgl = 0.22 L/hr). Solid lines represent mean and dashed lines represent the 95% confidence interval.

The value of Vmax estimated for the male mouse liver in this re-analysis (0.23 µmol/hr/mg protein) is close to the value reported in Yang et al. (2012) of 0.26 µmol/hr/mg protein; however, the Km (0.62 µmol/L) is roughly half of the Yang et al. (2012) value (1.34 µmol/L), which was derived assuming no transport limitation.

Re-estimation of *In Vitro* Metabolism Parameters

The estimated value of Kgl (0.22 L/hr) from the analysis described above was used in a re-analysis of the metabolism data for all tissues. The details of the MCMC analysis are provided below.

Yang et al. (2012) reported a two-level hierarchical Bayesian model to estimate the gender-variability of the *in vitro* metabolic parameters. In the re-analysis of the *in vitro* data presented here, the primary interest was on point estimates of metabolic constants for each species and sex (mixed gender for human) in the presence of our predicted flux of chloroprene between air and media in the *in vitro* system. 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 analysis. Parameters for both the saturable (i.e. Vmax and Km) metabolism of chloroprene are given in Table B-2.and are broad log-uniform distributions.

Table B-2. Prior Distributions Used in MCMC Analysis	
Parameter	**Distribution**
Log-likelihood Standard Deviation	Lognormal(1,1)
Vmax	Log-Uniform (-10,5)
Km	Log-Uniform(-10,5)
KG	Log-Uniform(-2.996,0)
Initial amount in vial (A0)[a]	Log-Uniform(-10,0)
[a] A0 estimated for each incubation concentration independently for each start concentration for incubations comprised of multiple vials – male mouse liver and lung, male rat liver and mixed human liver	

The likelihood contribution for any single data point is defined as follows. Suppose that μ represents the prediction of the model for a given set of parameter values (i.e. of Vmax, Km, etc.). For an observation, x, the log-likelihood of that observation is based on the assumption that that observation is log-normally distributed with median μ and a log-scale standard deviation σ. That is,

$$ln[L(x \mid \mu,\sigma)] = -ln(x) - \frac{ln(\sigma^2)}{2} - \frac{(ln(x) - ln(\mu))^2}{2\sigma^2} \quad \text{Eq. 1}$$

where L(x | μ,σ) denotes the likelihood of x given the parameters μ and σ. A broad prior for σ (lognormal with mean = 1, standard deviation = 1, truncated at 0.1 and 100) was used to avoid over-constraining the posterior parameter distributions for the metabolic parameters of primary interest.

The flux of chloroprene between air and media (Kgl) was estimated by fixing the Km in the male mouse liver microsomal study to 1.0 μmol/L and estimating both Vmax and Kgl. Initial testing of the model showed that the male mouse liver had the strongest data upon which to base the Kgl (i.e. steepest slope as low start concentrations). In the estimation of Kgl, the broad distributions reported above for metabolic parameters were retained. The geometric mean of Kgl was retained as a fixed value for the analysis of all the *in vitro* studies including the male mouse liver which was re-analyzed to estimate Vmax and Km after the Kgl was fixed. For Vmax and Km analysis, 20000 iterations of the model were run with the first 10000 discarded for the posterior analysis.

For all analysis 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 using the gelman.diag[2] routine included in the R package CODA to verify that the point estimates for the potential scale reduction factor (PSRF) were all less than 1.1. The results of the potential scale reduction factor analysis (exported from R) and the posterior distributions for the parameters and the likelihood for all MCMC analyses are listed in the supplemental Excel workbook (Supplemental Materials D) under the species-specific worksheets[3].

In three cases, female mouse kidney, male rat lung and mixed human lung, the single level MCMC failed to converge with saturable metabolism. Initial analysis with single level MCMC and a first order rate constant similar to that reported by Yang et al. (2012) showed that a lower bound on the metabolism could not be identified. It was determined that the analysis could not differentiate between metabolism and background loss based on the incubation data (not shown). In an attempt to assess the viability of estimating rate constants from these datasets, a two-level MCMC was conducted on the mixed human lung. To address uncertainty in RLOSS, both RLOSS and KF were included at the

[2] This R routine follows the proposed general approach defined in Gelman and Rubin (1992).
[3] See cells k2 to w7 on the Mouse and Rat Liver, lung and kidney sheets, and cells k2 to m7 on the human lung and liver sheets.

population level. The prior distribution of RLOSS was set to the distribution of all of the RLOSS incubations reported in Yang et al. (2012) and Himmelstein et al. (2004a) and considered to be log normally distributed with $\mu = -7.242$ and $\sigma = 0.484$). KF was given a uniform prior on the natural log with limits of -60 and 10). The prior description for parameter variability were lognormal (0.3,5). Subsequent analysis of the incubation time-course data presented by Himmelstein et al. (2004a) indicated that there is no difference between the low concentration incubation data and the control (i.e. without NADPH+) time-course data. As such, the loss attributed to metabolism in the mixed human lung and female mouse kidney was considered not determinable based on the available data (See main report text for discussion).

For the female rat lung, the single level MCMC failed to converge with saturable metabolism. In order to support the dose metric comparisons in Supplemental Materials F, an approximate estimation of female rat lung Vmax was undertaken by fixing the Km to the posterior median value estimated from the female mouse lung incubations (2.369 μM).

Posterior Distributions

The posterior chains and distributions for the female mouse liver, female mouse lung and mixed human liver are given in Figures B-6 to B-8. In all cases, the posterior distribution of the ln(parameter) represents the uncertainty in the parameter estimate given the data and not interindividual variability. The final PSRFs calculated for the female mouse and mixed human are given in Table B-3. In all cases the PSRFs were <1.2. Confidence ellipse plots for *in vitro* assay posterior chains for female mouse (liver and lung) and mixed human (liver) microsomal metabolism assays are shown in Figures B-9 and B-10. The plots show the 95% (red line) and 99% (green line) confidence ellipses over the plot of the log posterior parameters for the last 10,000 iterations of the posterior chain. As would be expected given the saturable enzymatic metabolic pathway, there is a strong positive correlation between Vmax and Km in the *in vitro* system for all three assays.

Table B-3. Potential scale reduction factors for female mouse and mixed human posterior chains.

Sex/species	Tissue	Parameter	Point Estimate	Upper CI
Female Mouse	Liver	Likelihood	1	1
		Vmax	1	1
		Km	1	1
	Lung	Likelihood	1	1
		Vmax	1.01	1.05
		Km	1.01	1.04
	Kidney	Likelihood	1	1
Mixed Human	Liver	Likelihood	1	1
		A0low	1.01	1.02
		A0mid	1.02	1.05
		A0high	1	1.01
		Vmax	1	1
		Km	1	1

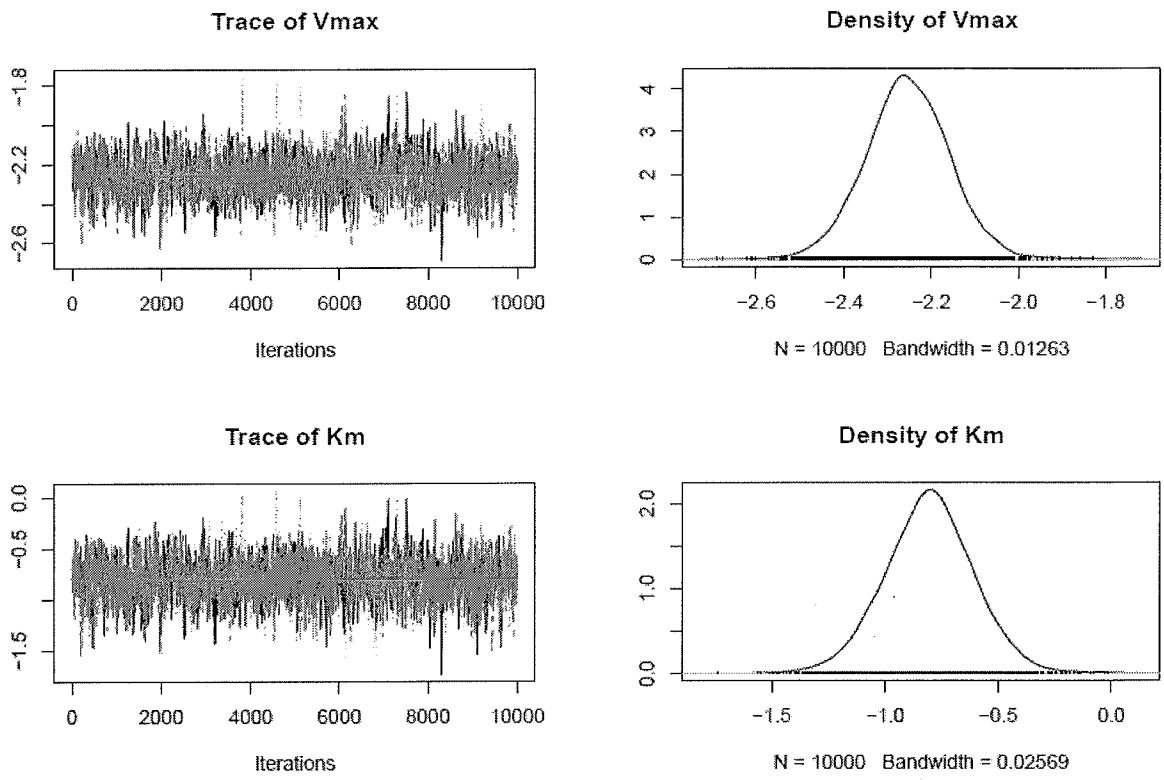

Figure B-6. Posterior chains (left) and distributions (right) for ln(Vmax) (top) and ln(Km) (bottom) in female mouse liver. Kgl was fixed at 0.22 L/hr for this analysis.

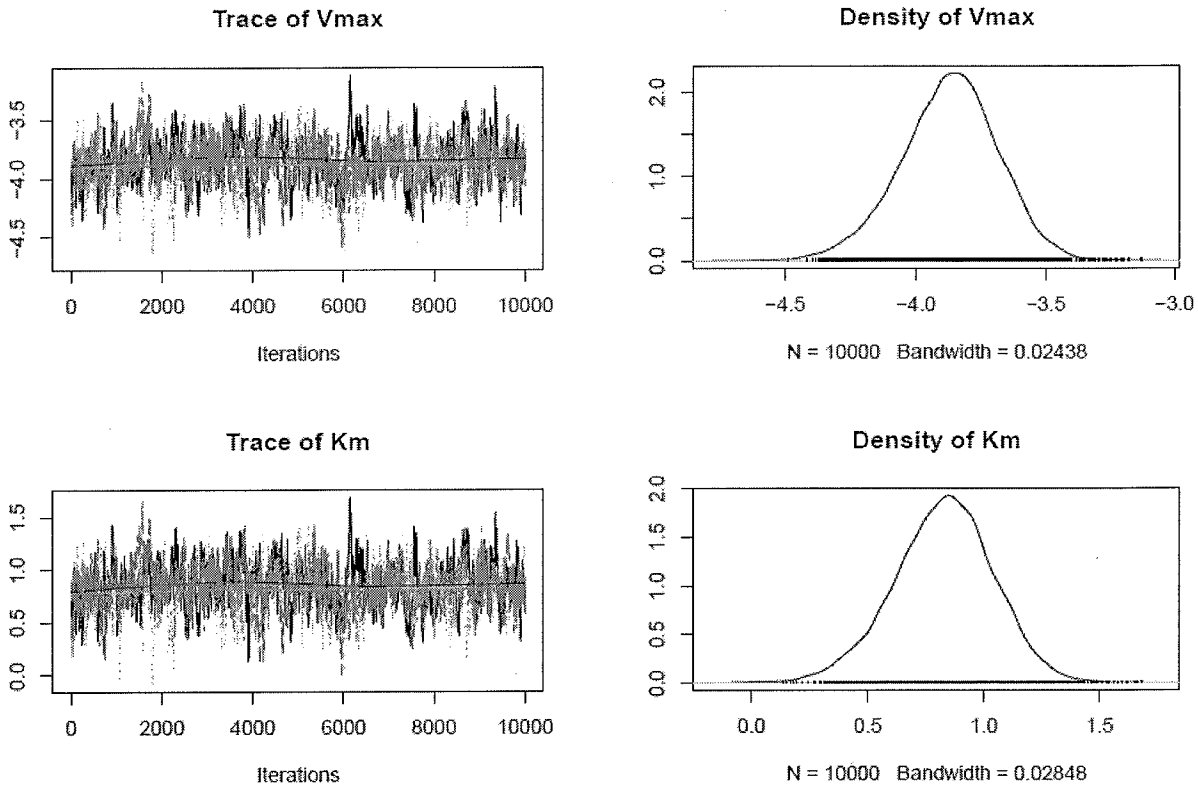

Figure B-7. Posterior chains (left) and distributions (right) for ln(Vmax) (top) and ln(Km) (bottom) in female mouse lung. Kgl was fixed at 0.22 L/hr for this analysis.

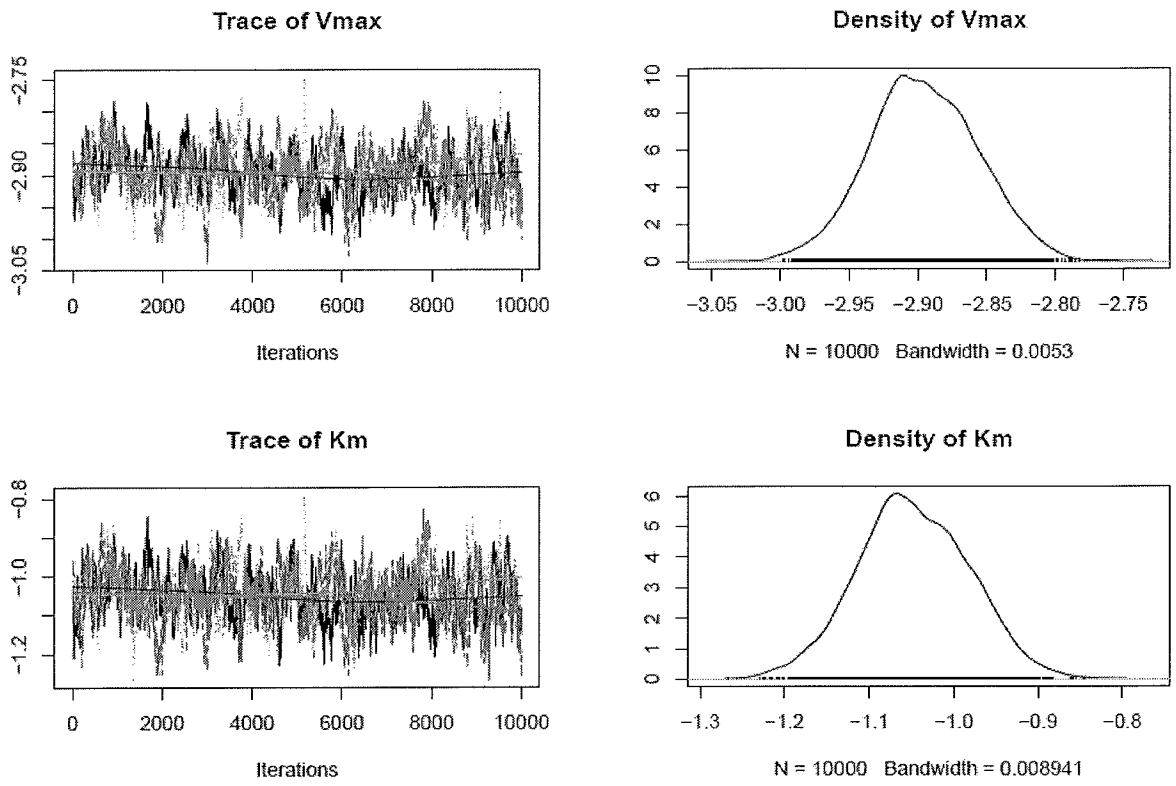

Figure B-8. Posterior chains (left) and distributions (right) for ln(Vmax) (top) and ln(Km) (bottom) in human liver. Kgl was fixed at 0.22 L/hr for this analysis.

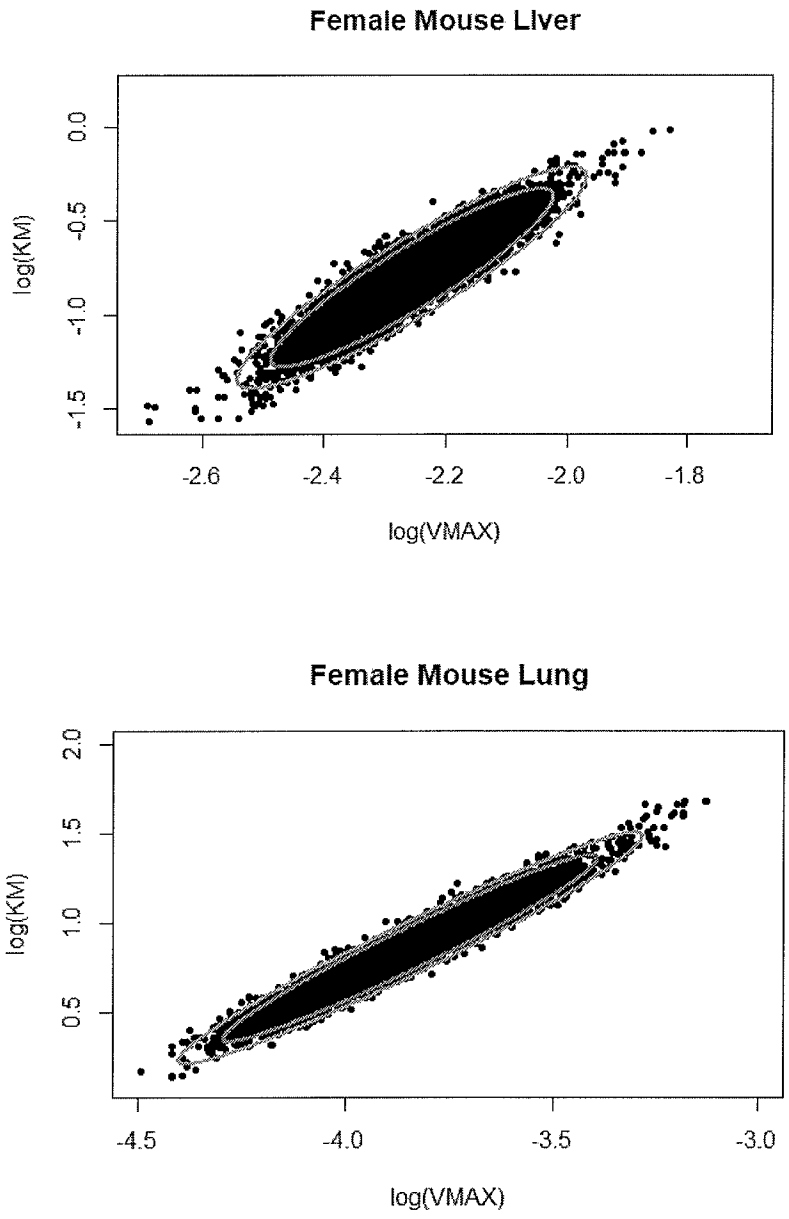

Figure B-9 Confidence (red is 95% and green is 99%) ellipse plot of Vmax vs. Km from the *in vitro* posterior chain for the female mouse microsomal metabolism assay (top panel is liver; bottom panel is lung).

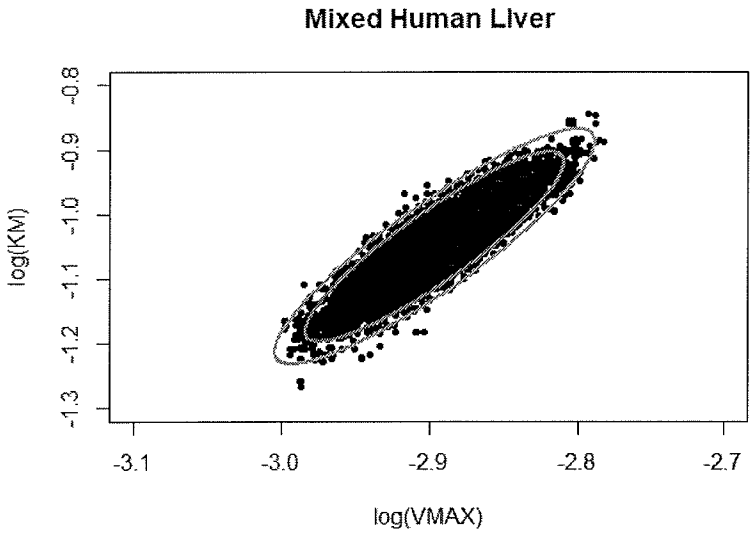

Figure B-10. Confidence (red is 95% and green is 99%) ellipse plot of Vmax vs. Km from the *in vitro* posterior chain for the mixed human liver microsomal metabolism assay.

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Intended for

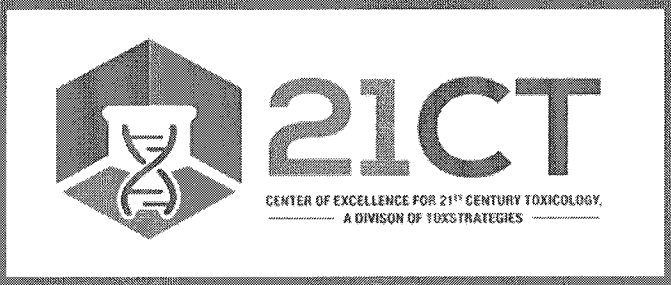
Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

SUPPLEMENTAL MATERIALS C
IVIVE LITERATURE REVIEW



In vitro to in vivo extrapolation for parameterization of the multi-species PBPK model for chloroprene

APRIL 30, 2019

In vitro to in vivo extrapolation for parameterization of the multi-species PBPK model for chloroprene

APRIL 30, 2019

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Executive Summary

The published multi-species PBPK models for chloroprene were developed using in vitro to in vivo extrapolation (IVIVE)-based parameterization. In vitro metabolism data collected in species-specific liver, lung, and kidney microsomal incubation studies (Himmelstein et al., 2004a; Yang et al., 2012) were biologically scaled to provide in vivo metabolism parameters in the PBPK model for chloroprene in liver, kidneys, and lungs of mouse, rat and human (Himmelstein et al., 2004b; Yang et al., 2012). In this study, the IVIVE used to parameterize the published model for chloroprene in these three species was reviewed to provide recommendations for any updates to the previously published IVIVE-based parameterization to support the application of the chloroprene PBPK model for risk assessment.

Background

In vitro to in vivo extrapolation for parmaeteirzation involves a series of biological scaling steps from in vitro measured metabolic constants, in most cases Vmax and intrinsic clearance (Clint), to corresponding in vivo parameters to use in PBPK models. For Km, consideration of free, i.e., available for metabolism, concentration between in vitro vs. in vivo is important for meaningful IVIVE. In the conversion of Vmax or Clint from in vitro to in vivo, it is critical to select appropriate scaling factors, which are dependent on the in vitro experimental system, the source of tissue, and the species of interest (see the Table 2 below copied from Yoon et al., 2012). For example, an in vitro Vmax of a compound determined in hepatic microsomal incubations, which is expressed in a unit of μmol/min/mg microsomal protein, can be converted to an in vivo liver Vmax that is in a unit of μmol/min/kg $BW^{0.75}$ for use in a PBPK model. In this IVIVE example, a scaling factor referred as mg microsomal protein per gram liver (MPPGL) is used along with liver weight and body weight of the species of concern. Characteristics of the tissue and the donor for the tissue used for in vitro system preparation determine which physiological scaling factors, i.e., liver weight and body weight, to be used for IVIVE. For example, microsomes prepared from an average adult rat(s) can be used to generate in vitro metabolic constants that can be extrapolated to in vivo metabolism parameters for the average adult rat.

Table 2. Summary of QIVIVE calculations for different *in vitro* systems.

System	Typical units for enzyme content in the system	Scaling factors to whole body
Expressed enzyme	pmol/min/pmol enzyme	(ISEF × CYPabundance or RAF) × MPPGL × LW
Microsomes[a]	nmol/min/mg protein	MPPGL × LW
Hepatocytes	nmol/min/10^6 hepatocytes	HPGL × LW
Liver	nmol/min/g liver	LW
Whole body	nmol/min/whole liver	

LW, liver weight; MPPGL, microsomal protein per gram liver (mg microsomal protein/g liver); HPGL, hepatocellularity per gram liver (number of hepatocytes/g liver).

[a]This scaling based on the protein amount in the subcellular fraction also applies for the cytosol and S9 fraction.

IVIVE for parameterization of the chloroprene PBPK model

It is critical to use appropriate scaling factors for accurate IVIVE for confidence in PBPK model outcomes. However, these scaling factors are not routinely measured or reported in publications and it is often hard to track down the sources for those scaling factors used in IVIVE studies (Barter et al., 2007). As such, the scaling factors used in the published chloroprene model were reviewed for their appropriateness for use in parametrization of metabolism in each tissue for mouse, rat and human as well as the sources of the selected values.

Scaling factors for the average adult of each species

In vitro enzyme kinetic parameters reported in Himmelstein et al. (2004a) and Yang et al., (2012) are considered as representatives of average adult values for each species. Therefore, extrapolation of these in vitro parameters was performed to obtain corresponding in vivo metabolism parameters in the PBPK model for the average adult of mouse, rat or human. Table 1 lists the species and gender specific scaling factors and physiological parameters recommended for use in chloroprene model along with their references. Body weight (BW) values for mouse, rat or human average adults (i.e., parameters for standard organism) are adopted from Brown et al., (1997). Fractional tissue weight values are also from Brown et al. (1997). For mouse and rat, gender specific BW values were used, but fractional tissue weight of the three tissues selected here was assumed to be the same between male and females.

Scaling of metabolic capacity

The capacity of metabolism. i.e., Vmax, is extrapolated from in vitro to in vivo based on the difference in scale between in vitro and in vivo systems. As microsomes was used to generate an in vitro Vmax for chloroprene in a given tissue, the following equation (Eq. 1) is used to scale the in vitro Vmax up to a corresponding in vivo Vmax for whole body.

$$\textit{In vivo Vmax } (mg/h/kg\ BW^{0.75}) = \textit{in vitro Vmax } (\mu mol/h/mg\ \textit{microsomal protein\_tissue}) \times \textit{MPPGL } (mg\ \textit{microsomal protein\_tissue}/g\ \textit{tissue}) \times \textit{BW } (kg) \times \textit{VtissueC} \times 1000\ (g/kg) \div BW^{0.75} \times \textit{MW } (\mu g/umol) \div 1000\ (\mu g/mg) \qquad \textit{(Eq. 1)}$$

, where MPPGL represents milligram microsomal protein content per gram of tissue, MW molecular weight, and VtissueC fractional weight of the given tissue to BW.

Selection of scaling factors for liver, lung and kidney of each species

Microsomes were used to determine in vitro enzyme kinetic parameters for chloroprene (Himmelstein et al., 2004a). Therefore, the biological scaling factor to use is the mg microsomal protein content per gram tissue (MPPGL, MPPGLU, or MPPGK for liver, lung, and kidney, respectively). In the original Himmelstein model, the values for the MPPG_tissue scaling factors for each species are 35, 49, and 56.9 mg microsomal protein/g liver for mouse, rats, and humans, respectively, whereas for lung microsomes, 23 mg

microsomal protein/g tissue was used for all animal species (Himmelstein et al., 2004b). This report reviews the appropriateness of the scaling factors selected for use in the original model.

Barter et al. (2007 and 2008) provided a comprehensive review of the liver microsomal content (MPPGL) for human and rat. Based on their meta-analysis and consensus report of the human data (Barter et al., 2007), 40 mg/g liver is recommended for human adults for chloroprene IVIVE-PBPK modeling. Lab to lab differences in microsomal preparation techniques and tissue sources are considered as the main factors for the variability in reported MPPGL values (Barter et al., 2007; Medinsky et al., 1994). Inter-species difference in microsomal protein per gram tissue appears to small in general, and is much smaller than the variability within species resulting from the experimental factors (Houston and Galetin, 2008; Barter et al., 2007; Csanády et al., 1992; Litterst et al., 1975). A MPPGL value of 35 mg/g liver was reported by Medinsky et al. (1994) for both rat and mouse. For rat, another value was available recommended by Houston and Galetin at 45 mg/g liver. We recommend to use the average of the two, 40 mg/g liver for rat for the chloroprene IVIVE-PBPK modeling. The values for mouse in Litterst et al. or Csanády et al. studies were not recommended for IVIVE directly as their results appear not corrected for experimental loss of microsomal proteins during preparation. It is important to use the scaling factors that were corrected for loss and thus, close to their in vivo values to reduce the uncertainty in IVIVE to the extent possible. Although we recommend these MPPGL values (Table 1), it should be noted that they fall in a similar range with those used by Himmelstein et al. (2004b).

For kidney microsomal content, additional caution was made. Kidney cortex is frequently used rather than using the whole tissue to prepare kidney microsomes because cytochrome P450 enzyme expression is known to be much higher in cortex compared to the medullar region. However, such details are often not reported in publications. In general, MPPGK based on kidney cortex microsomes is about two times higher than that based on whole kidney tissue microsomes (Scotcher et al., 2017). As the chloroprene model describes kidney metabolism in the whole tissue, MPPGK based on whole kidney tissue needs to be used. One half of the reported cortex-based MPPGK reported by Scotcher et al. (2017) is recommended for use as the MPPGK for whole kidney microsomes data-based extrapolation for chloroprene IVIVE in humans. For rat, 18 mg/g kidney determined by Yoon et al. (2007) is recommended as the study reported a rat specific MPPGK value for whole kidney.

It was challenging to find MPPGLU (mg lung microsomal protein per gram tissue) values, in particular for humans. Himmelstein et al. estimated MPPGLU as 23 mg per g tissue based on a few available studies and the assumed microsomal recovery. This value is in line with other studies reporting MPPGLU values about 30 - 50% of the MPPGL within species for rat and mouse (Litterst et al., 1975; Yoon et al., 2006 and 2007). It was challenging to find any data for human MPPGLU. While Boogard et al. (2000) reported the lung microsomal content in three different species, their microsomal protein recovery seems to be much lower than other studies for both liver and lung (Litterst et al., 1975 and Yoon et al., 2006 and 2007) raising a concern for the correction for protein recovery. The measured microsomal protein content in lung in this study however, appears to be similar

among the three species. As they were all determined under the same experimental condition, it would be reasonable to use the same value for all three species. Medinsky et al. (1994) reported 20 mg/g lung for mouse. As such, it is considered reasonable to keep the 20 mg per g tissue as MPPGLU based on the mouse value reported by Medinsky et al. (1994).

Table 1. Average adult parameters recommended for in vitro to in vivo extrapolation

Parameter	B6C3F1 Mouse (Female)	B6C3F1 Mouse (Male)	F344 Rat (Female)	F344 Rat (Male)	Average Human	Reference
BW (kg)	0.035	0.04	0.33	0.45	70	Brown et al, 1997 (page 415 in text)
Liver fractional weight (VLC)	0.0549	0.0549	0.0366	0.0366	0.0257	Brown et al, 1997 (Tables 4, 5, & 7)
Lung fractional weight (VLUC)	0.00730	0.00730	0.005	0.005	0.0076	Brown et al, 1997 (Tables 4, 5, & 7)
Kidney fractional weight (VKC)	0.01670	0.01670	0.0073	0.0073	0.0044	Brown et al, 1997 (Tables 4, 5, & 7)
Liver mg microsomal protein per g liver (MPPGL)	35	35	40[a]	40[a]	40	Medinsky et al., 1994 for mouse; Medinsky et al., 1994 and Houston and Galetin, 2008 for rat; Barter et al., 2007 for human
Lung mg microsomal protein per g lung (MPPGLU)	20	20	20	20	20	Medinsky et al., 1994 and Boogard et al., 2000 for all species
Kidney mg microsomal protein per g kidney (MPPGK)[b]	18	18	18	18	11	Yoon et al., 2007 for mouse and rat; Scotcher et al., 2017 for human

[a] Average of the two values reported in Medinsky et al., 1994 and Houston and Galetin, 2008.

[b] Values from these references are rounded.

IVIVE for first order metabolic clearance in rat and human lung

Yang et al. (2012) reported both Vmax and Km for chloroprene metabolism in all three tissue microsomes for mouse. However, for rat and human, only intrinsic clearance (as Vmax/Km) was reported for lung as the available in vitro data was only informative to estimate Clint in rat and human, but not informative to estimate Vmax and Km separately. The slopes of chloroprene disappearance in lung microsomes showed linear kinetics for the range of substrate concentrations used in the in vitro lung metabolism studies (Himmelstein et al., 2004a). Among those concentrations, four out of the five concentrations used in lung microsomal incubation were overlapped with the ones used for liver microsomal incubation studies. This could be suggesting a higher Km and/or much smaller Vmax for chloroprene oxidation in lung microsomes than that in liver microsomes. Whichever the case was, the measured first order clearance was similar to the level of non-biological loss observed during incubation indicating that the metabolic clearance in the lung of rat or human would be expected to be significantly lower than mouse. To be conservative, the observed first order loss in lung microsomes for rat and human was considered as metabolic clearance and was extrapolated to in vivo as first order clearance in the lung (KFLUC) in the published model. Using this first order clearance presents an issue however, when applying the model to a dose range in which the tissue concentration becomes higher than the Km in the lung. In such high dose conditions, the relative risk estimate for the lung vs. liver would shift as the current lung description cannot capture the saturable nature of chloroprene metabolism leading to an overestimation of lung metabolism and therefore risk estimate, at higher exposure conditions.

To avoid an overestimation of lung metabolism at high dose, it is recommended to estimate or infer a Km for lung metabolism in rat and human. Then estimate a Vmax from the observed first order clearance in vitro using the relationship of Clint = Vmax/Km, which holds true at low substrate concentrations, e.g., below Km. It is likely that the relative contribution of individual cytochrome (CYP) P450 enzymes toward chloroprene metabolism is tissue-dependent as the expression level of each CYP enzyme is tissue-dependent. In addition, affinity of the metabolism, i.e., Km, is different among different CYP enzymes contributing to tissue-dependent changes in relative contribution of CYP enzymes to a compound metabolism. For example, butadiene, a structural analog of chloroprene, is a substrate for at least two CYP enzymes including CYP 2El and CYP 2A6 (Csanady et al., 1992; Duescher and Elfarra, 1994). These CYPs have different affinities to butadiene as suggested by the different Km values observed for each tissue (Csanady et al., 1992). The lung Km values appear to be similar or higher in general than those for liver in all three species, this study results suggest. For mouse and rat, lung values were about 2 fold higher than the liver values. This is consistent with the in vitro observation by Himmelstein et al. (2004a), which implies a higher Km in lung than liver microsomes in rat and mice, i.e., at the overlapping substrate concentrations, no saturation was observed in lung unlike the liver. It is also consistent with the mouse results showing the lung Km being greater than that of the liver by a 1.5 – 5.3 fold, depending on the gender of the animals. Therefore, it would be reasonable to use a 2-fold higher Km for lung than the liver in each species for mouse and rat.

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Human Liver

VVIAL	0.0119573
VMED	0.001
VINJ	0.0004
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Male Mouse Liver

VVIAL	0.0119573
VMED	0.001
VINJ	0.0003858
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Female Mouse Liver

VVIAL	0.01165
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Human Lung

VVIAL	0.0119573
VMED	0.001
VINJ	0.0004
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Male Mouse Lung

VVIAL	0.0119573
VMED	0.001
VINJ	0.0003858
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Female Mouse Lung

VVIAL	0.01165
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Male Mouse Kidney

VVIAL	0.01163
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	2

Female Mouse Kidney

VVIAL	0.01163
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	2

Male Rat Kidney

VVIAL	0.01163
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	3

Male Rat Liver

VVIAL	0.0119573
VMED	0.001
VINJ	0.0003858
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Female Rat Liver

VVIAL	0.01165
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	1

Male Rat Lung

		Yang Rounded no reason given
VVIAL	0.0119573	**0.012**
VMED	0.001	**0.001**
VINJ	0.0003858	**0.00039**
VAIR	VVIAL-VMED	
P1	0.69	**0.69**
PROT	1	**1**

Female Rat Lung

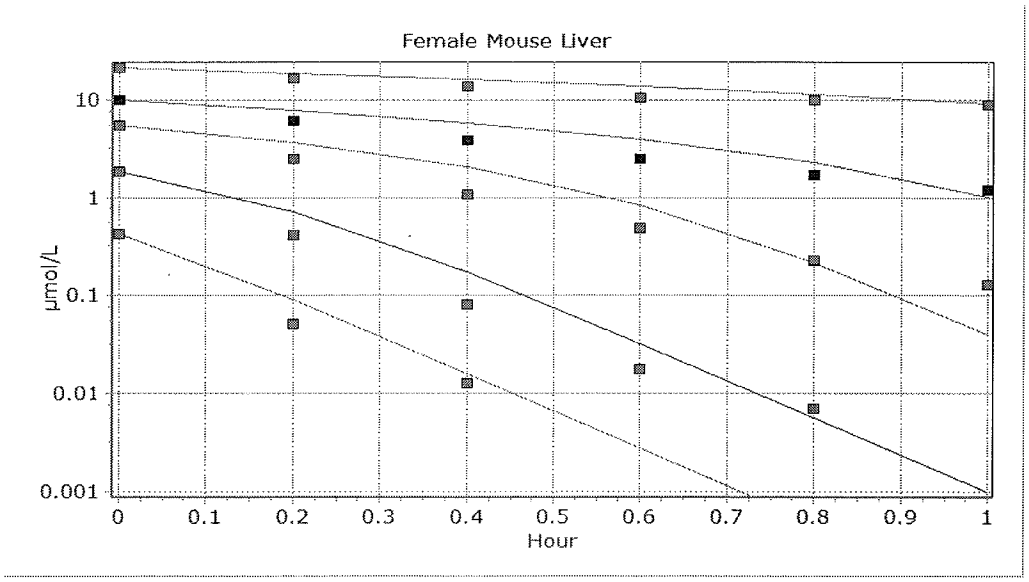
VVIAL	0.01165
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	1

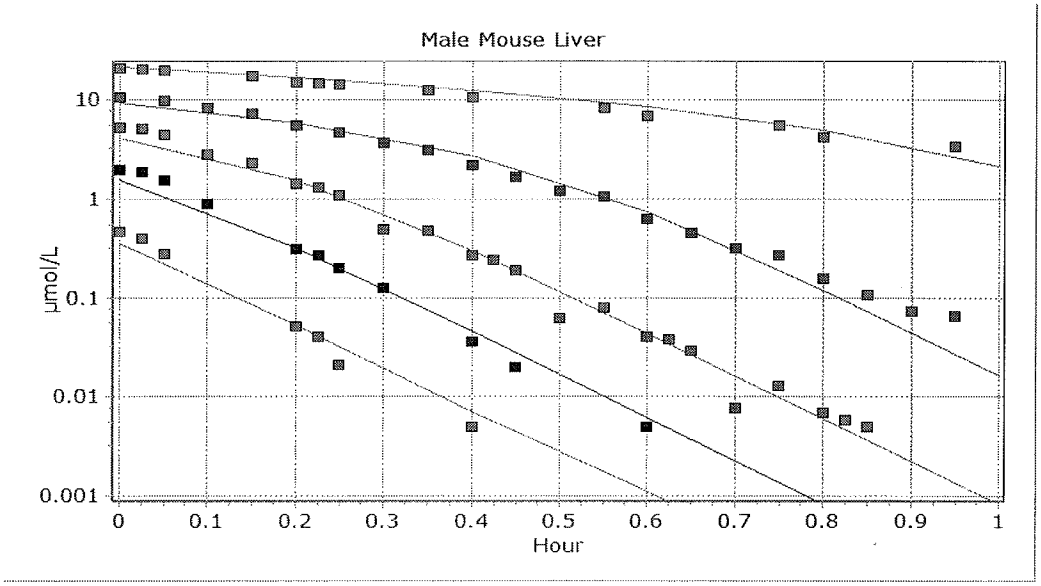
Female Rat Kidney

VVIAL	0.01163
VMED	0.001
VINJ	0.0002
VAIR	VVIAL-VMED
P1	0.69
PROT	3

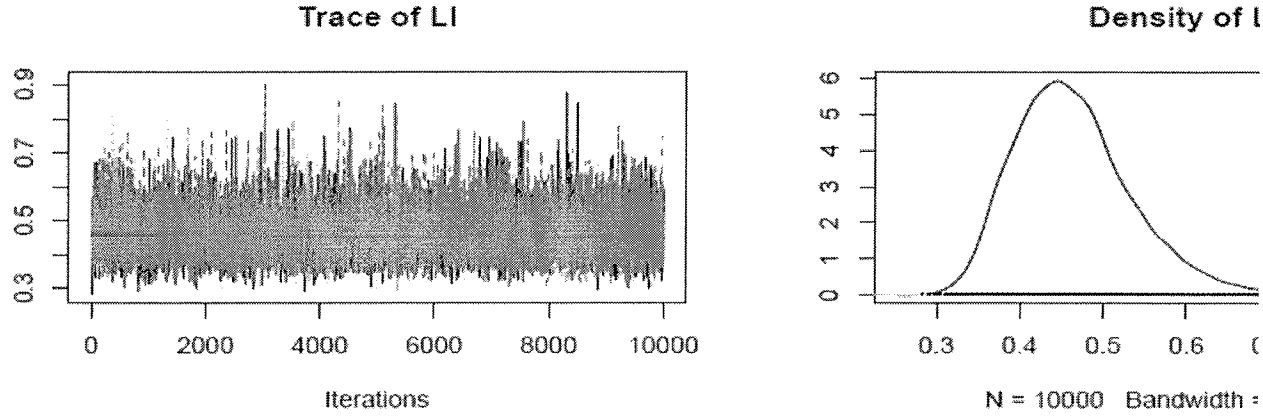
	Female				
			Median	95% CI	
Log	Vmax	µmol/hr/mg protein	-2.25	-2.45	-2.07
	Km	µmol/L	-0.80	-1.20	-0.43
	Male				
			Median	95% CI	
	A0_1	(umole)	-1.4011	-1.5533	-1.2748
	A0_2	(umole)	-2.1662	-2.3413	-2.0441
	A0_3	(umole)	-3.0364	-3.158	-2.9356
	A0_4	(umole)	-4.0041	-4.1409	-3.8725
	A0_5	(umole)	-5.4951	-5.6864	-5.2974
	Vmax	µmol/hr/mg protein	-1.5534	-1.8267	-1.3606
	Km	µmol/L	-0.3732	-0.7498	-0.1023

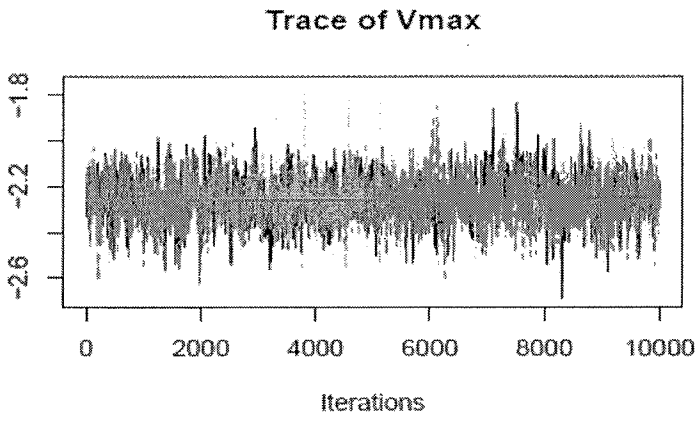
	Female				
			Median	95% CI	
EXP	Vmax	µmol/hr/mg protein	0.105	0.087	0.127
	Km	µmol/L	0.448	0.302	0.652
	Male				
			Median	95% CI	
	A0_1	(umole)	0.25	0.21	0.28
	A0_2	(umole)	0.11	0.10	0.13
	A0_3	(umole)	0.048	0.043	0.053
	A0_4	(umole)	0.018	0.016	0.021
	A0_5	(umole)	0.0041	0.0034	0.0050
	Vmax	µmol/hr/mg protein	0.212	0.161	0.257
	Km	µmol/L	0.689	0.472	0.903





Female Mouse

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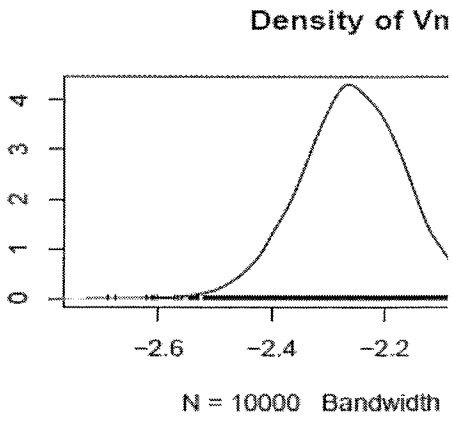
−2.6
0
2000
4000
6000
8000
10000
Iterations


Density of Vn
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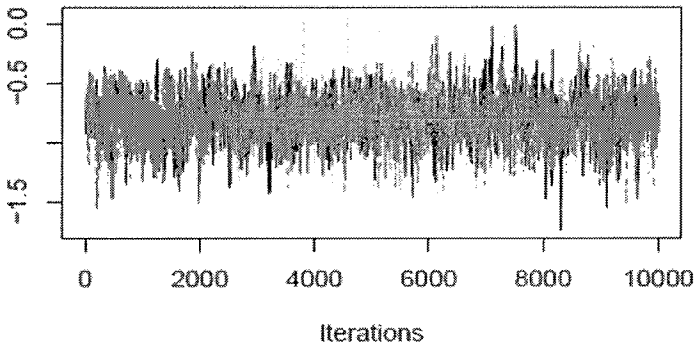
1
2
3
4
−2.6
−2.4
−2.2
N = 10000 Bandwidth


Trace of Km
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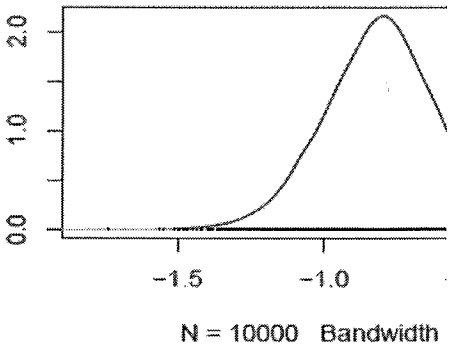
0
2000
4000
6000
8000
10000
Iterations


Density of K
0.0
1.0
2.0
−1.5
−1.0
N = 10000 Bandwidth

Female

```
> gelman.dia autoburnin
Potential scale reduction

Point est.
LI 1 1
Vmax 1 1
Km 1 1

Multivariate psrf

1
>
> summary(x1)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

Mean SD
LI 0.463 0.0714
Vmax -2.254 0.09479
Km -0.804 0.19359

2 Quantiles for

2.50% 25%
LI 0.3484 0.4115
Vmax -2.4452 -2.3159
Km -1.197 -0.9271

> summary(x2)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

Mean SD
LI 0.4648 0.07197
Vmax -2.255 0.0946
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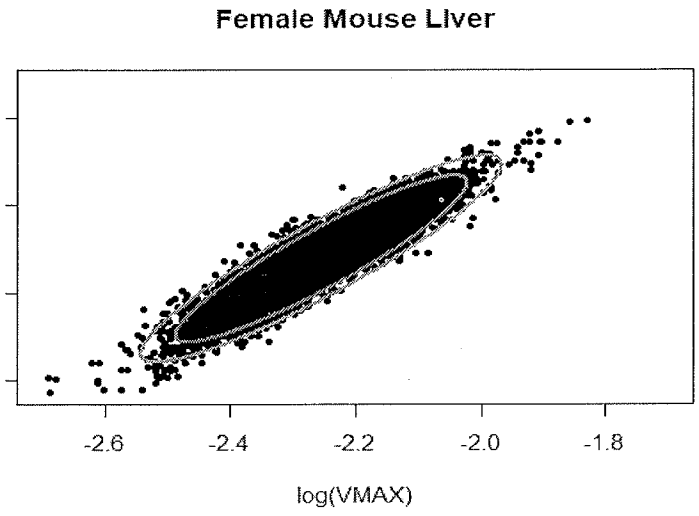
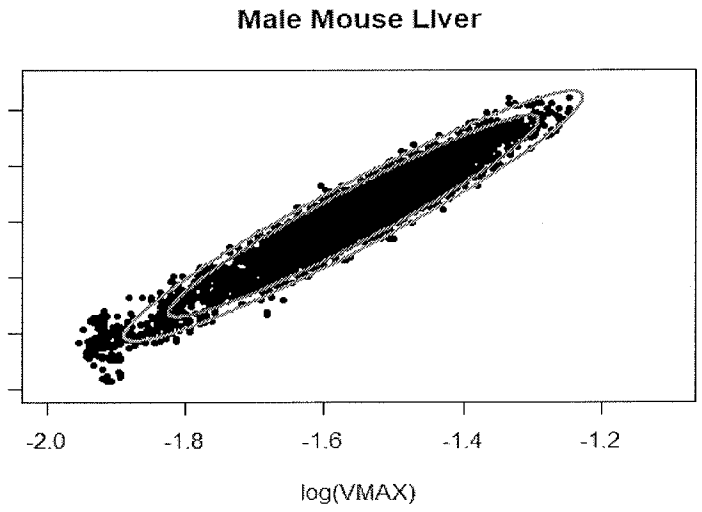




Km	-0.8064	0.19593

2 Quantiles for

	2.50%	25%
LI	0.3498	0.4155
Vmax	-2.4456	-2.3141
Km	-1.2	-0.9323

> summary(x3)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

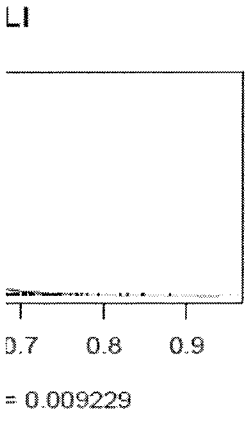
1 Empirical mean
plus standard

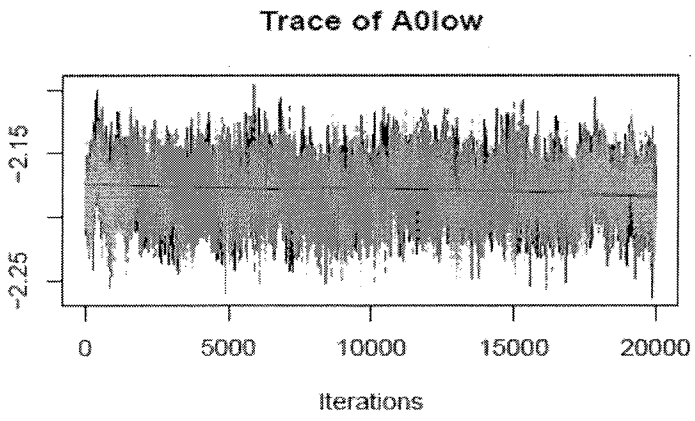
	Mean	SD
LI	0.4654	0.07343
Vmax	-2.2523	0.10187
Km	-0.8004	0.20872

2 Quantiles for

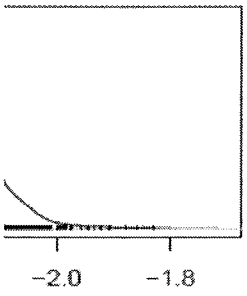
	2.50%	25%
LI	0.351	0.4128
Vmax	-2.443	-2.3192
Km	-1.196	-0.9347

Male Mouse



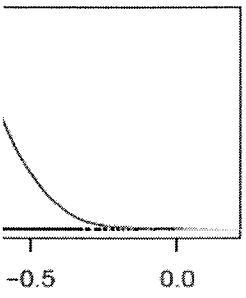


nax

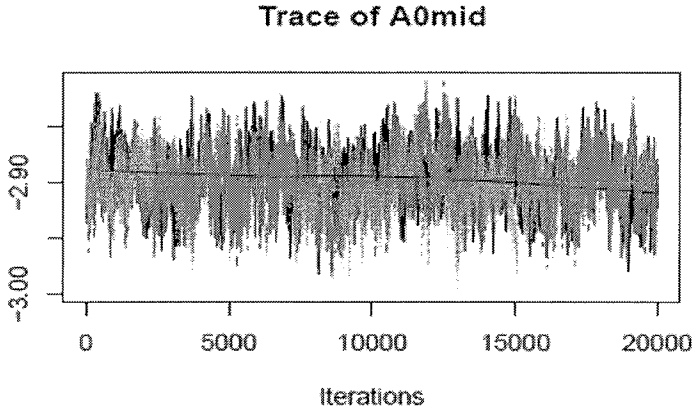


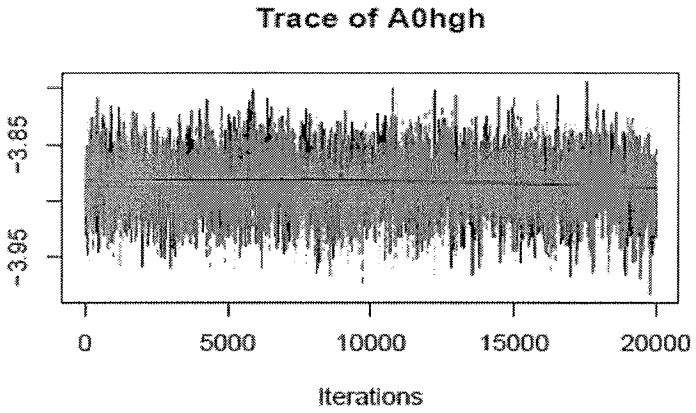
= 0.01263

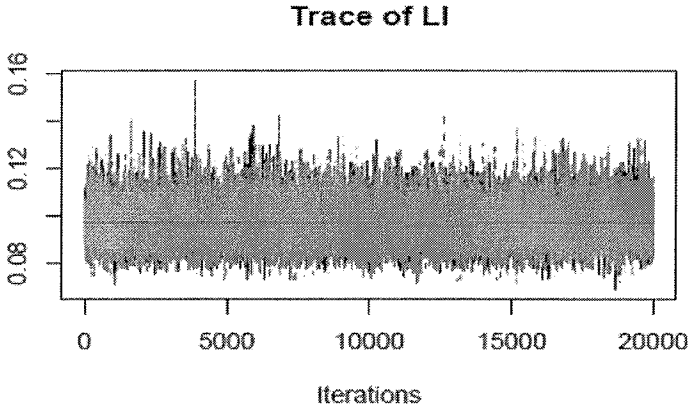
im

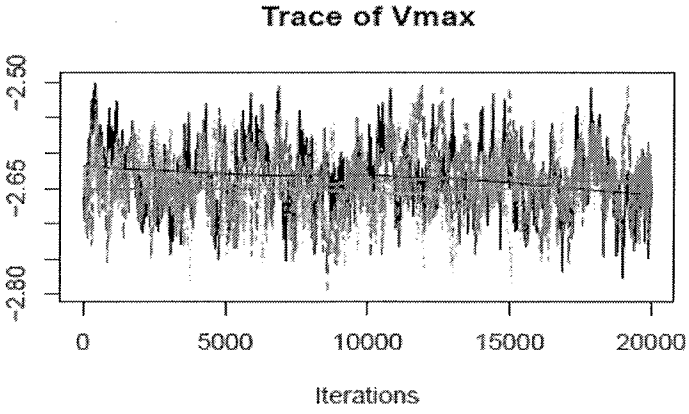


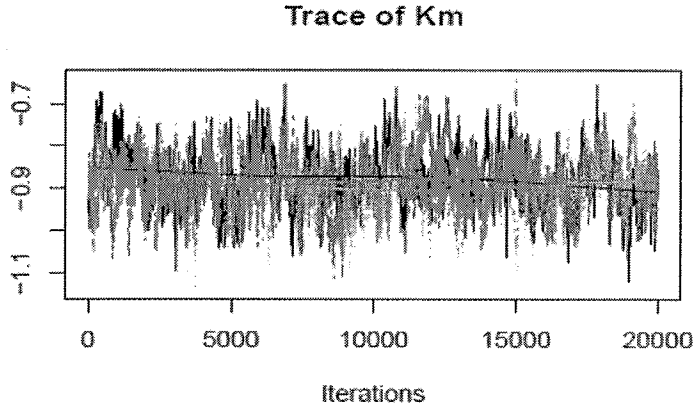
= 0.02569










Trace of Km
−0.7
−0.9
−1.1
0
5000
10000
15000
20000
Iterations

=FALSE)

factors:

Male Mouse Liver with A0 (initial mass i

KGL **0.22**

Upper C.I.

> Potential scale

Point

A0_1	1.02
A0_2	1.02
A0_3	1.01
A0_4	1
A0_5	1
LI	1
Vmax	1.03
Km	1.03

1

= 1

chain = 10000

Multivariate psrf

1.02

and standard deviation for each variable,

error of the mean:

> summary(x

Naive SE Time-serie SE

0.000714	0.001377	
0.000948	0.004866	4.2%
0.001936	0.009858	24.1%

Iterations =

Thinning interval

Number of

Sample size

each variable:

1 Empirical plus

50%	75%	97.50%
0.4552	0.5051	0.6204
-2.2542	-2.1914	-2.0651
-0.8026	-0.6756	-0.4282

Mean

A0_1	-1.4031
A0_2	-2.1699
A0_3	-3.0399
A0_4	-4.0048
A0_5	-5.4939
LI	0.2548
Vmax	-1.5577
Km	-0.379

1

= 1

chain = 10000

2 Quantiles

and standard deviation for each variable,

error of the mean:

2.50%

A0_1	-1.5533
A0_2	-2.3413
A0_3	-3.158
A0_4	-4.1409

Naive SE Time-serie SE

0.00072	0.001336
0.000946	0.005313

0.001959 0.010827

each variable:

50%	75%	97.50%
0.4559	0.5039	0.6313
-2.2558	-2.1937	-2.0711
-0.8038	-0.6782	-0.4217

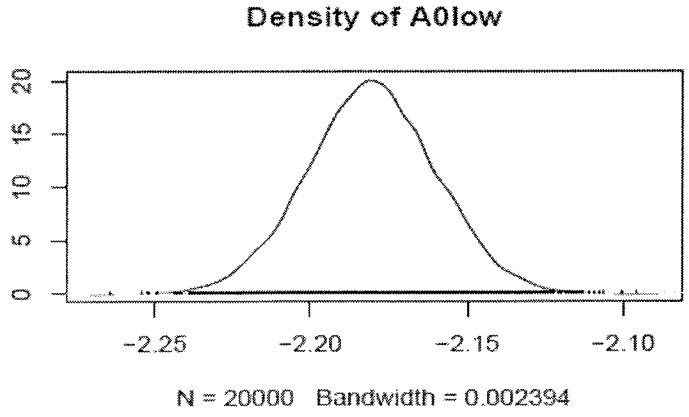
1
= 1
chain = 10000

and standard deviation for each variable, error of the mean:

Naive SE	Time-series SE
0.000734	0.001407
0.001019	0.005648
0.002087	0.01159

each variable:

50%	75%	97.50%
0.4572	0.5053	0.6375
-2.2538	-2.1886	-2.0488
-0.801	-0.6742	-0.3819



A0_5	-5.6864
LI	0.2158
Vmax	-1.8267
Km	-0.7498

> summary(x

Iterations = 1
Thinning interval = 1
Number of chains = 1
Sample size per chain = 10000

1 Empirical mean and standard deviation for each variable, plus standard error of the mean:

	Mean
A0_1	-1.4204
A0_2	-2.1879
A0_3	-3.0509
A0_4	-4.0066
A0_5	-5.4876
LI	0.2544
Vmax	-1.5867
Km	-0.4147

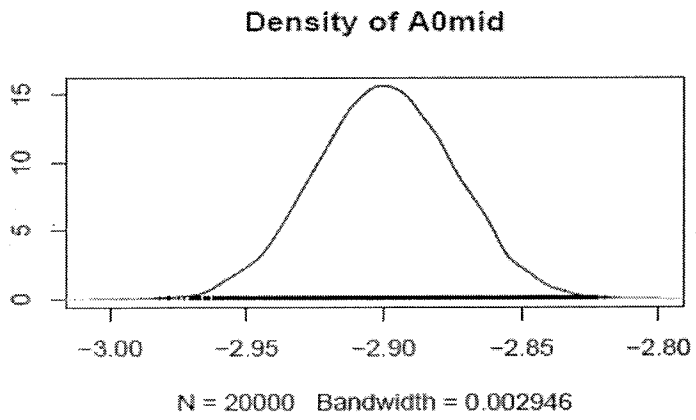
2 Quantiles for each variable:

	2.50%
A0_1	-1.5481
A0_2	-2.3234
A0_3	-3.1609
A0_4	-4.1363
A0_5	-5.6823
LI	0.215
Vmax	-1.7887
Km	-0.6893

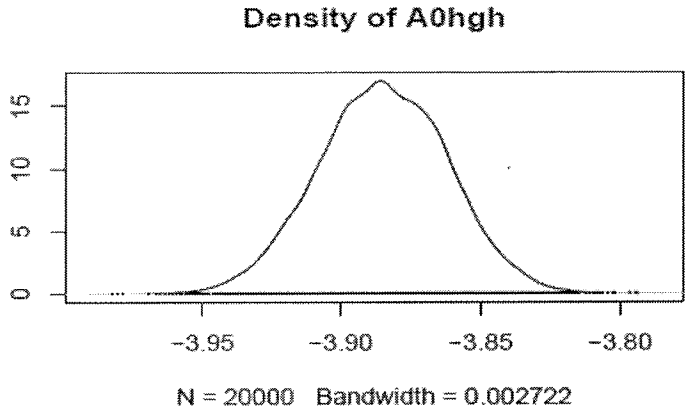
> summary(x

Iterations = 1
Thinning interval = 1
Number of chains = 1
Sample size per chain = 10000

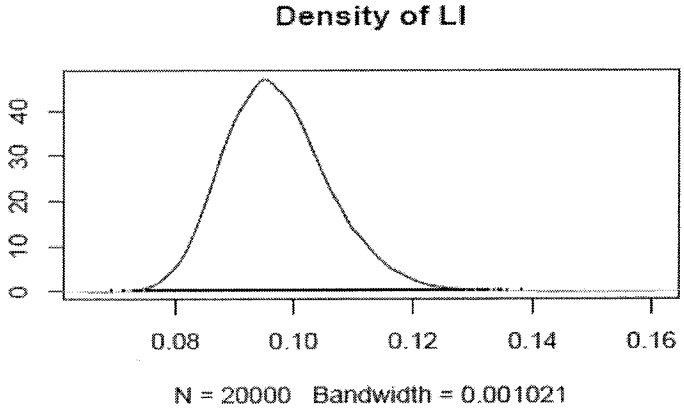
1 Empirical mean and standard deviation for each variable, plus

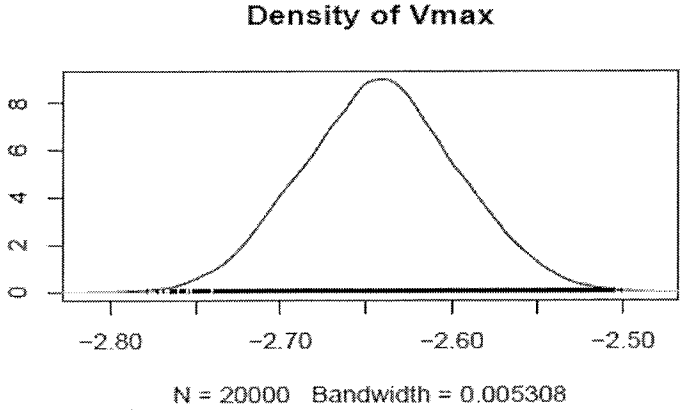


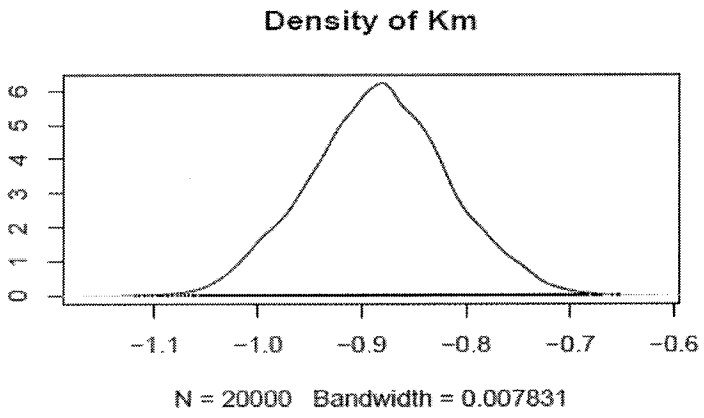
	Mean
A0_1	-1.4013
A0_2	-2.1674
A0_3	-3.0409
A0_4	-4.0073
A0_5	-5.495
LI	0.254
Vmax	-1.5519
Km	-0.3675



	2 Quantiles
	2.50%
A0_1	-1.5239
A0_2	-2.2914
A0_3	-3.1469
A0_4	-4.1416
A0_5	-5.6897
LI	0.2145
Vmax	-1.7311
Km	-0.6231






Density of Km
0 1 2 3 4 5 6
−1.1
−1.0
−0.9
−0.8
−0.7
−0.6
N = 20000 Bandwidth = 0.007831

in vial) estiamted as part of MCMC
L/hr

autoburnin=FALSE)
reduction factors:

est.	Upper	C.I.
1.07		
1.07		
1.03		
1		
1		
1		
1.09		
1.08		

1)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie: SE
0.06879	0.000688	0.010741
0.07131	0.000713	0.011831
0.05659	0.000566	0.004849
0.06784	0.000678	0.001928
0.09902	0.00099	0.002642
0.02239	0.000224	0.000514
0.10895	0.00109	0.022275
0.14721	0.001472	0.024213

for each variable:

25%	50%	75%	97.50%
-1.4453	-1.4011	-1.357	-1.2748
-2.2097	-2.1662	-2.1227	-2.0441
-3.0749	-3.0364	-3.0014	-2.9356
-4.0501	-4.0041	-3.9599	-3.8725

-5.5615	-5.4951	-5.4269	-5.2974
0.2391	0.2533	0.2684	0.3033
-1.6164	-1.5534	-1.4872	-1.3606
-0.4606	-0.3732	-0.2838	-0.1023

2)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie: SE
0.06697	0.00067	0.011224
0.07104	0.00071	0.010608
0.05793	0.000579	0.004601
0.06586	0.000659	0.001944
0.09841	0.000984	0.002364
0.02243	0.000224	0.000398
0.10464	0.001046	0.02036
0.13689	0.001369	0.021276

for each variable:

25%	50%	75%	97.50%
-1.4658	-1.4225	-1.3765	-1.2819
-2.237	-2.1894	-2.1402	-2.0409
-3.0916	-3.0503	-3.0113	-2.934
-4.0501	-4.0063	-3.9618	-3.8782
-5.5527	-5.4872	-5.4208	-5.2985
0.2386	0.2528	0.2693	0.3014
-1.656	-1.5906	-1.5222	-1.3627
-0.4984	-0.4177	-0.3338	-0.126

3)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

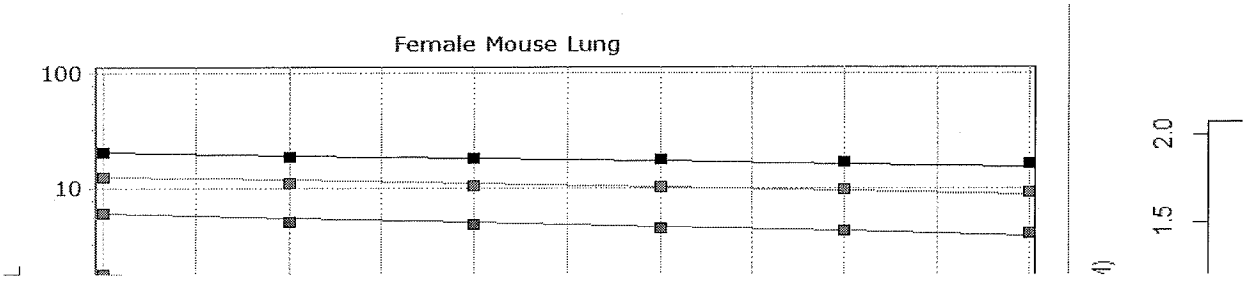
SD	Naive	SE	Time-series SE
0.06095	0.00061	0.00767	
0.06221	0.000622	0.008688	
0.05392	0.000539	0.004055	
0.06639	0.000664	0.001816	
0.10034	0.001003	0.002478	
0.02246	0.000225	0.00041	
0.09211	0.000921	0.015206	
0.12389	0.001239	0.016889	

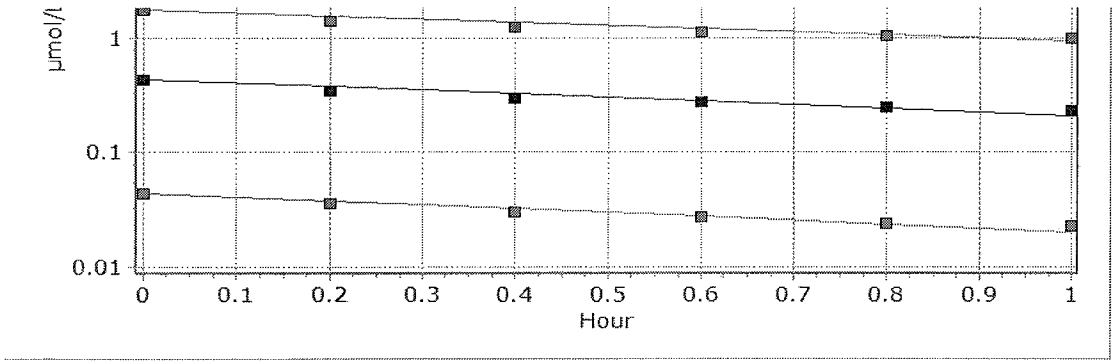
for each variable:

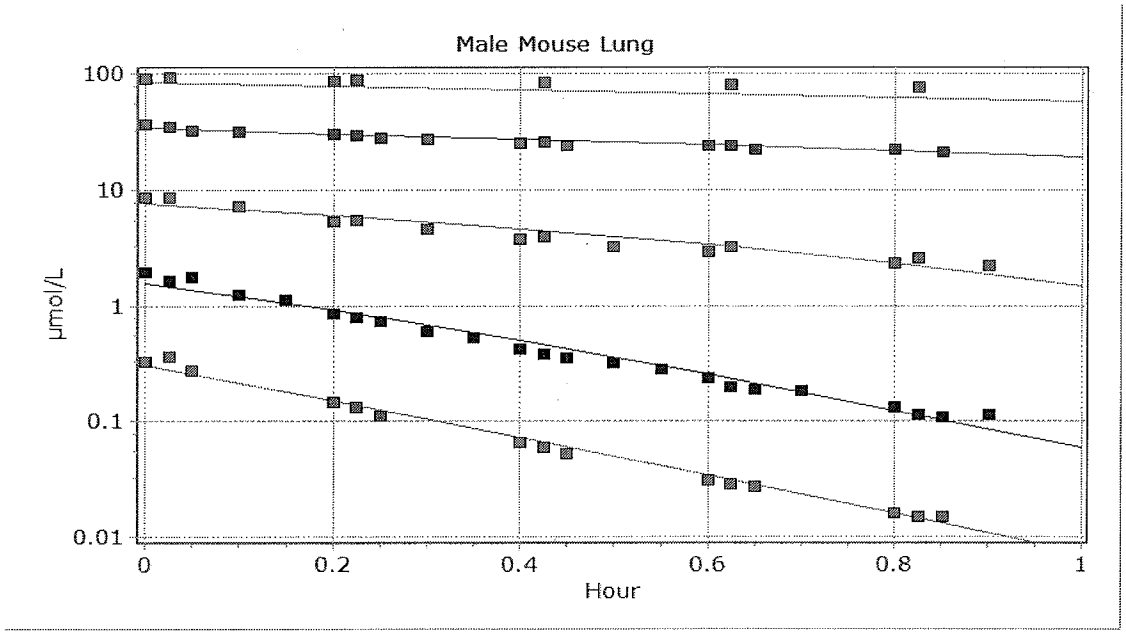
25%	50%	75%	97.50%
-1.4418	-1.3988	-1.3587	-1.2885
-2.209	-2.1661	-2.1237	-2.0542
-3.0767	-3.0413	-3.0052	-2.9345
-4.0508	-4.0072	-3.9617	-3.8783
-5.5619	-5.4949	-5.4279	-5.2964
0.2382	0.2528	0.2678	0.3014
-1.6151	-1.5468	-1.4864	-1.3898
-0.4485	-0.3621	-0.2771	-0.1494

	Female				
			Median	**95% CI**	
Log	**Vmax**	**µmol/hr/mg protein**	-3.84	-4.20	-3.48
	Km	**µmol/L**	0.863	0.441	1.27
	Male				
			Median	**95% CI**	
	A0_1	**umol/L**	-0.012	-0.054	-0.00036
	A0_2	**umol/L**	-0.94	-1.00	-0.88
	A0_3	**umol/L**	-2.43	-2.52	-2.35
	A0_4	**umol/L**	-4.03	-4.10	-3.96
	A0_5	**umol/L**	-5.64	-5.74	-5.55
	Vmax	**µmol/hr/mg protein**	-2.47	-2.67	-2.25
	Km	**µmol/L**	0.177	-0.059	0.420

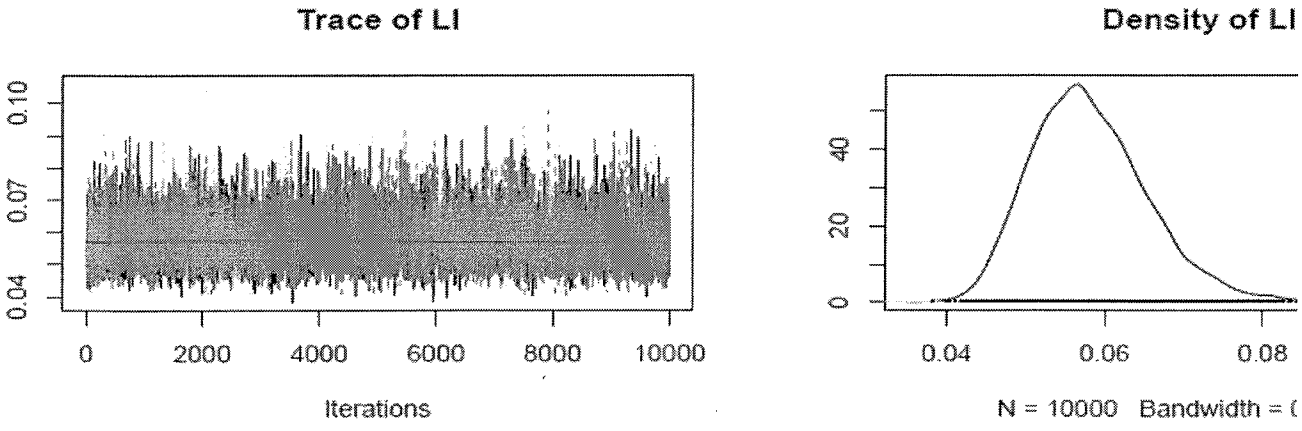
	Female				
			Median	**95% CI**	
EXP	**Vmax**	**µmol/hr/mg protein**	0.022	0.015	0.031
	Km	**µmol/L**	2.369	1.555	3.549
	Male				
			Median	**95% CI**	
	A0_1	**umol/L**	0.988	0.947	1.000
	A0_2	**umol/L**	0.391	0.367	0.414
	A0_3	**umol/L**	0.088	0.081	0.095
	A0_4	**umol/L**	0.018	0.017	0.019
	A0_5	**umol/L**	0.0035	0.0032	0.0039
	Vmax	**µmol/hr/mg protein**	0.085	0.069	0.105
	Km	**µmol/L**	1.194	0.943	1.523

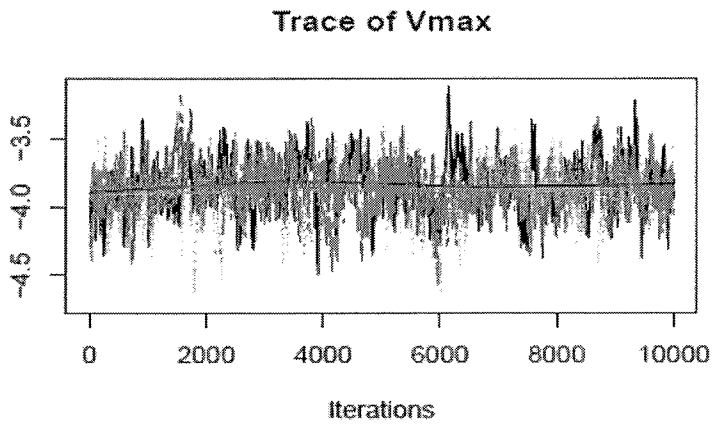


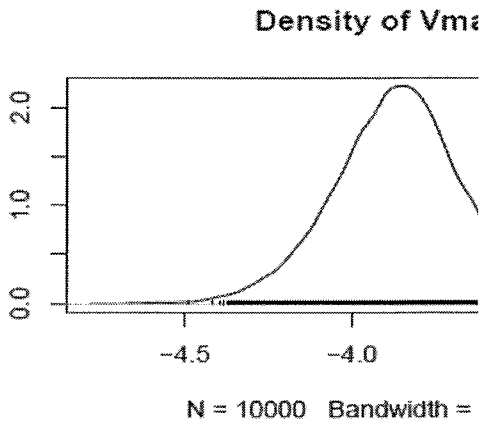


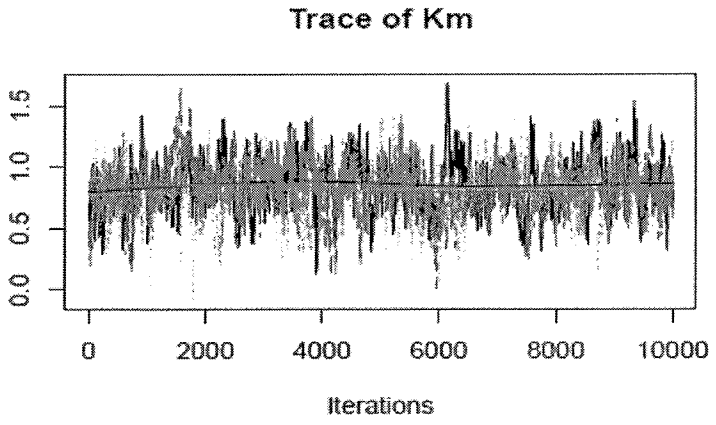


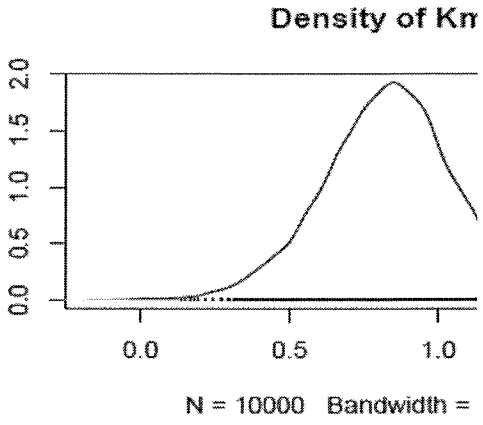
Female Mouse




Trace of Vmax
−4.5
−4.0
−3.5
0
2000
4000
6000
8000
10000
Iterations


Density of Vma
0.0
1.0
2.0
−4.5
−4.0
N = 10000 Bandwidth =


Trace of Km
0.0
0.5
1.0
1.5
0
2000
4000
6000
8000
10000
Iterations


Density of Km
0.0
0.5
1.0
1.5
2.0
0.0
0.5
1.0
N = 10000 Bandwidth =

Female > gelman.dia autoburnin

Potential scale reduction

	Point	est.
LI	1	1
Vmax	1.01	1.05
Km	1.01	1.04

Multivariate psrf

1.01

>

> summary(x1)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

	Mean	SD
LI	0.0579	0.007529
Vmax	-3.842	0.184502
Km	0.8581	0.213718

2 Quantiles for

	2.50%	25%
LI	0.04563	0.0525
Vmax	-4.20138	-3.9696
Km	0.44118	0.7097

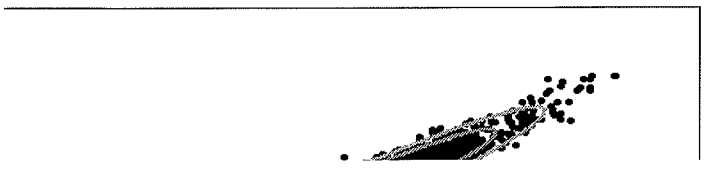
> summary(x2)

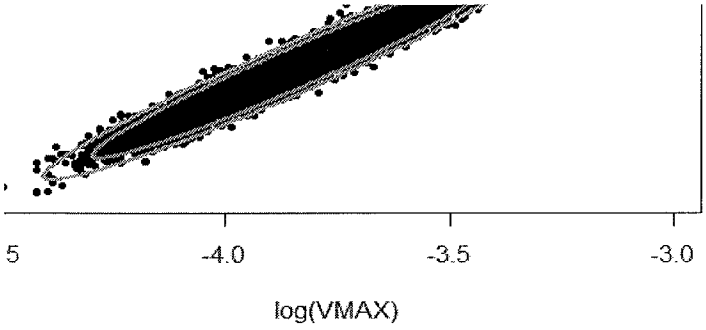
Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

	Mean	SD
LI	0.05808	0.007425

Female Mouse Lung





Vmax	-3.87608	0.185042
Km	0.81964	0.215382

2 Quantiles for

	2.50%	25%
LI	0.0455	0.0528
Vmax	-4.2715	-3.9843
Km	0.3694	0.6858

> summary(x3)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

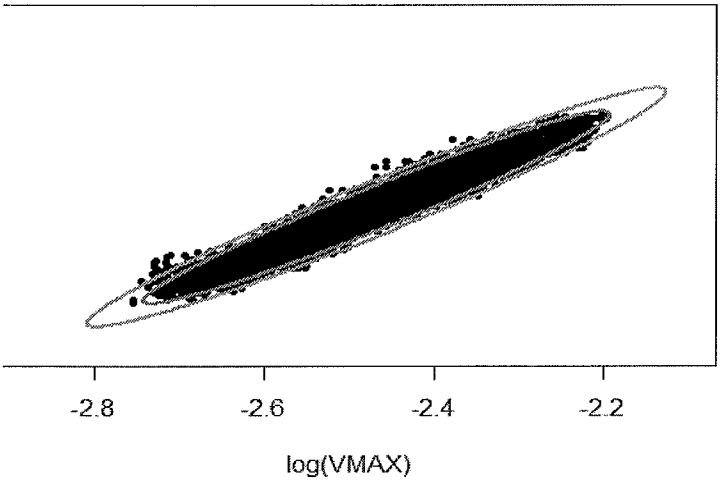
1 Empirical mean
plus standard

	Mean	SD
LI	0.05831	0.00742
Vmax	-3.89248	0.19034
Km	0.80088	0.21932

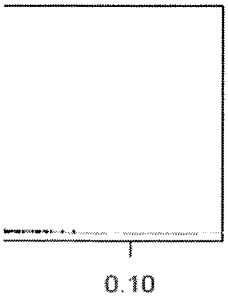
2 Quantiles for

	2.50%	25%
LI	0.04604	0.053
Vmax	-4.30746	-4.01
Km	0.32312	0.6655

Male Mouse Lung

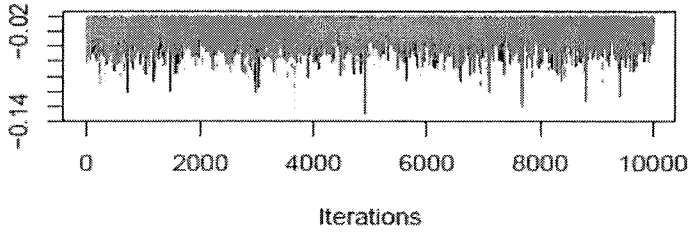


Male Mouse



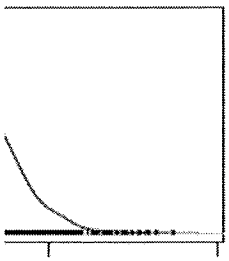
0.000988

Trace of A0_1



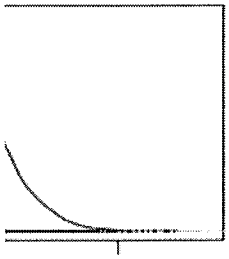
Trace of A0_2

ax

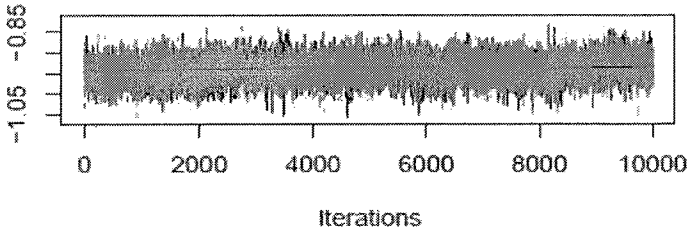


0.02438

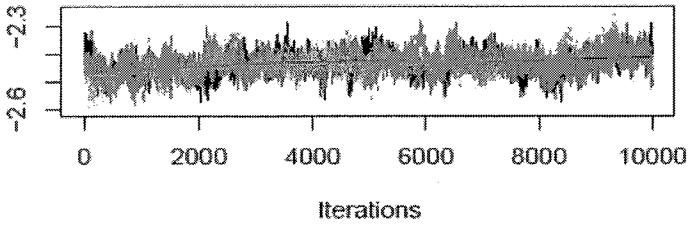
n



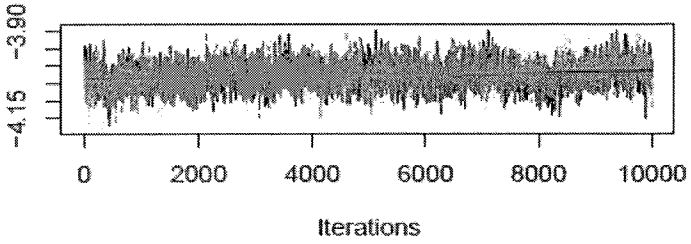
0.02848



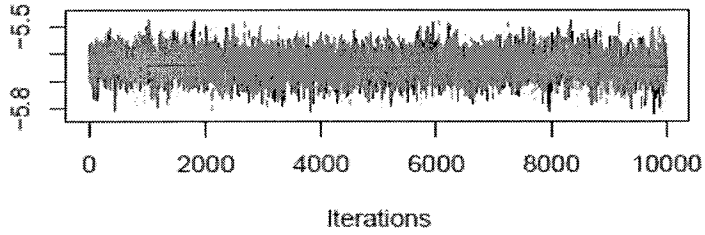
Trace of A0_3



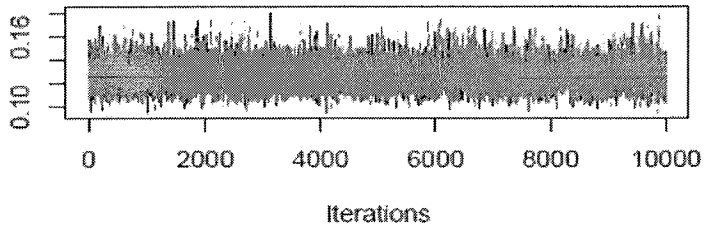
Trace of A0_4



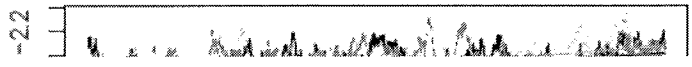
Trace of A0_5

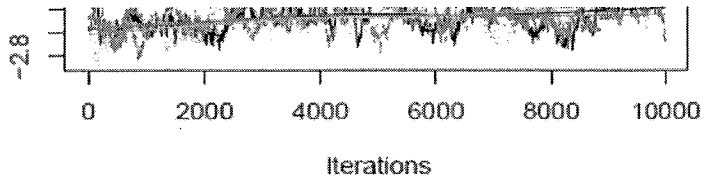


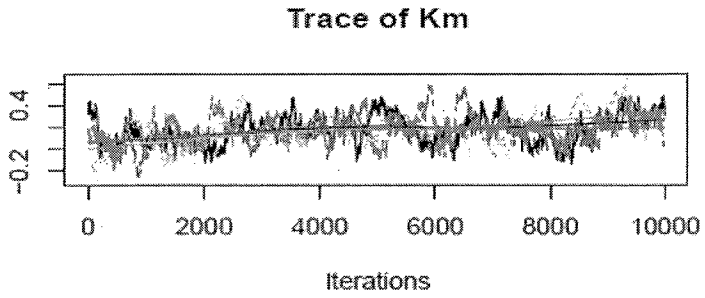
Trace of LI



Trace of Vmax




−2.8
0
2000
4000
6000
8000
10000
Iterations


Trace of Km
−0.2
0.4
0
2000
4000
6000
8000
10000
Iterations

Male Mouse MCMC included

=FALSE)
factors:

Male gelman.diag(x,
Potential scale

Upper C.I.

Point
A0_1 1
A0_2 1
A0_3 1
A0_4 1
A0_5 1
LI 1
Vmax 1
Km 1

Multivariat psrf

1
= 1
chain = 10000

and standard deviation for each variable,
error of the mean:

Naive SE Time-series SE
7.53E-05 0.00013
1.85E-03 0.015841 4.8%
2.14E-03 0.018576 24.9%

each variable:

50% 75% 97.50%
0.05717 0.0624 0.07491
-3.83749 -3.7181 -3.47993
0.86264 1.0014 1.2666

1
= 1
chain = 10000

and standard deviation for each variable,
error of the mean:

Naive SE Time-series SE
7.43E-05 0.000133

1
>
> summary(x1)

Iterations =
Thinning interval
Number of
Sample size

1 Empirical
plus

Mean
A0_1 -0.01585
A0_2 -0.9402
A0_3 -2.43407
A0_4 -4.02598
A0_5 -5.64395
LI 0.12674
Vmax -2.46803
Km 0.17784

2 Quantiles

2.50%
A0_1 -0.05397
A0_2 -1.00139
A0_3 -2.51684
A0_4 -4.09702
A0_5 -5.73964
LI 0.10695

1.85E-03	0.016448
2.15E-03	0.018973

each variable:

50%	75%	97.50%
0.05733	0.06266	0.07436
-3.87417	-3.76142	-3.51551
0.82193	0.95446	1.24341

1

= 1

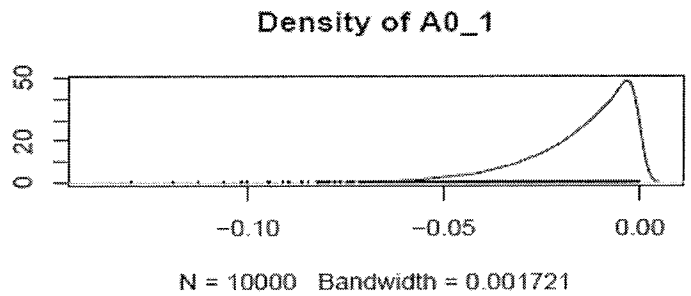
chain = 10000

and standard deviation for each variable, error of the mean:

Naive SE	Time-serie SE
7.42E-05	0.000142
0.001903	0.017027
0.002193	0.019934

each variable:

50%	75%	97.50%
0.05758	0.06264	0.07496
-3.87513	-3.76253	-3.56032
0.82077	0.95062	1.18926



Density of A0_2

Vmax	-2.67426
Km	-0.05884

> summary(x2)

Iterations =
Thinning interval
Number of
Sample size

1 Empirical plus

Mean

A0_1	-0.01522
A0_2	-0.94095
A0_3	-2.43469
A0_4	-4.02509
A0_5	-5.63906
LI	0.127
Vmax	-2.4745
Km	0.16786

2 Quantiles

	2.50%
A0_1	-0.05123
A0_2	-1.00219
A0_3	-2.51828
A0_4	-4.09717
A0_5	-5.73487
LI	0.10737
Vmax	-2.69554
Km	-0.0816

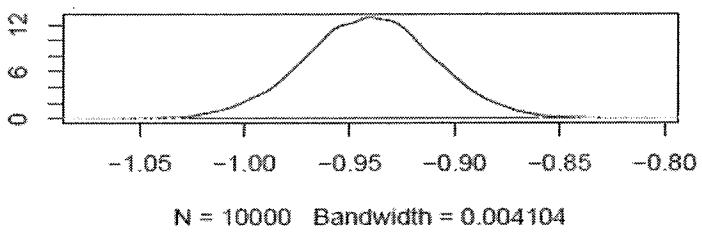
> summary(x3)

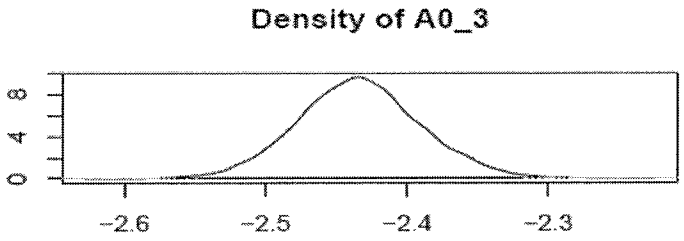
Iterations =
Thinning interval
Number of
Sample size

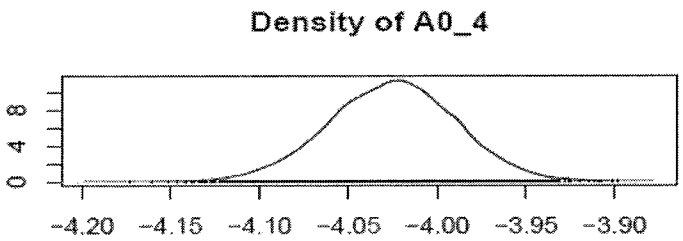
1 Empirical plus

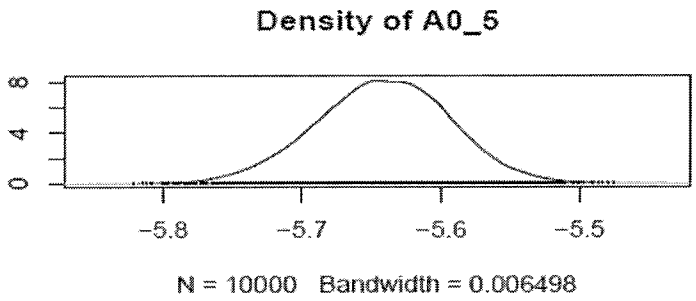
Mean

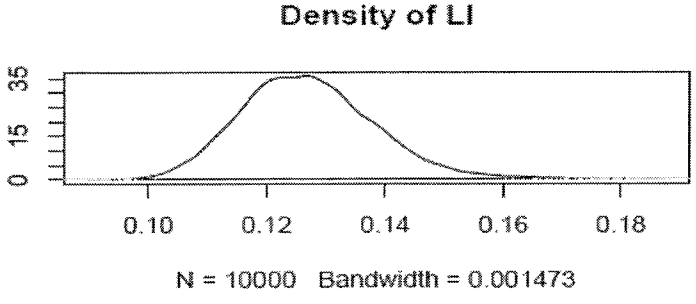
A0_1	-0.01499

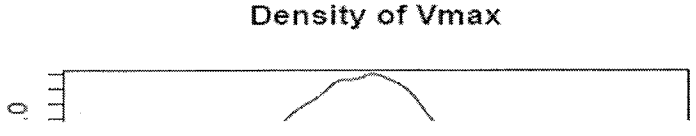








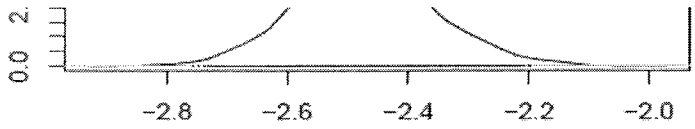




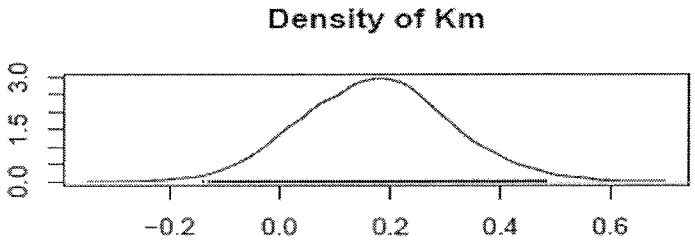
A0_2	-0.94057
A0_3	-2.43281
A0_4	-4.02546
A0_5	-5.64303
LI	0.12721
Vmax	-2.46624
Km	0.17945

2 Quantiles

	2.50%
A0_1	-0.05018
A0_2	-1.00114
A0_3	-2.52399
A0_4	-4.09933
A0_5	-5.74106
LI	0.10714
Vmax	-2.72462
Km	-0.12116



N = 10000 Bandwidth = 0.01562



N = 10000 Bandwidth = 0.01838

estimation of the initial vial amount (A10)
autoburnin=FALSE)
reduction factors:

est.	Upper	C.I.
1		
1		
1		
1		
1.01		
1		
1.01		
1.01		

1:10000 variable,
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive	SE	Time-serie: SE
0.01458	0.000146	0.000313	
0.03027	0.000303	0.001181	
0.04258	0.000426	0.005305	
0.03531	0.000353	0.00164	
0.04792	0.000479	0.001522	
0.01109	0.000111	0.000201	
0.1123	0.001123	0.018325	
0.12972	0.001297	0.020528	

for each variable:

25%	50%	75%	97.50%	
-0.02206	-0.01157	-0.00509	-0.00036	
-0.95979	-0.93976	-0.91986	-0.88184	variable,
-2.46395	-2.43489	-2.40409	-2.35246	
-4.04967	-4.02556	-4.00281	-3.95775	
-5.67606	-5.64298	-5.61096	-5.55037	
0.11919	0.12593	0.13359	0.150152	

-2.55349	-2.4688	-2.38657	-2.25465
0.07594	0.17745	0.2748	0.420482

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie SE
0.01385	0.000139	0.000277
0.03085	0.000309	0.00116
0.04286	0.000429	0.006218
0.03551	0.000355	0.001707
0.04804	0.00048	0.001574
0.01123	0.000112	0.00021
0.1139	0.001139	0.020117
0.1316	0.001316	0.021484

variable,

for each variable:

25%	50%	75%	97.50%
-0.02201	-0.01121	-0.0047	-0.00045
-0.96156	-0.94071	-0.92062	-0.88009
-2.46179	-2.43581	-2.40895	-2.34367
-4.04922	-4.02426	-4.00135	-3.9564
-5.67024	-5.63913	-5.60632	-5.54661
0.11903	0.12645	0.133848	0.150945
-2.54648	-2.47714	-2.41026	-2.22794
0.08096	0.16372	0.24467	0.455063

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie SE
0.01376	0.000138	0.00027

0.03139	0.000314	0.00149
0.04676	0.000468	0.007817
0.03717	0.000372	0.001836
0.04845	0.000485	0.001539
0.0113	0.000113	0.000207
0.1282	0.001282	0.024058
0.14681	0.001468	0.031408

for each variable:

25%	50%	75%	97.50%
-0.02121	-0.011	-0.0045	-0.00042
-0.96147	-0.9409	-0.92011	-0.87724
-2.46298	-2.433	-2.40444	-2.33627
-4.04973	-4.0252	-4.00002	-3.95299
-5.67499	-5.6421	-5.60978	-5.55109
0.1193	0.1265	0.133989	0.151696
-2.54743	-2.4616	-2.38878	-2.18845
0.08798	0.1842	0.269131	0.490299

Female

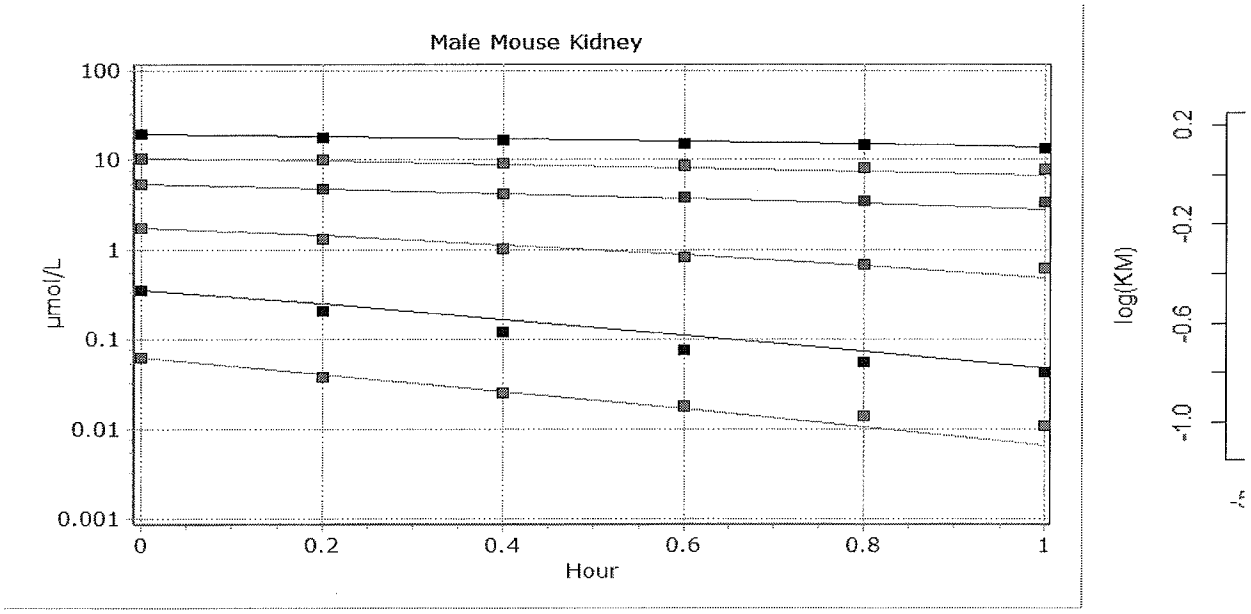
not determinable - data were insufficient to estimate in vitro metabolic parame

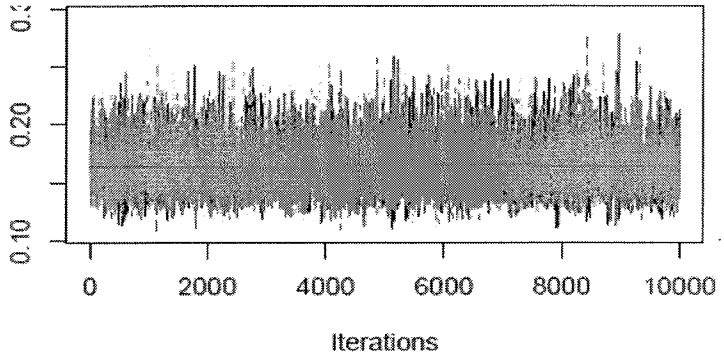
			Median	95% CI	
Log	**Vmax**	**µmol/hr/mg protein**	-4.46	-4.74	-4.18
	Km	**µmol/L**	-0.44	-0.76	-0.10

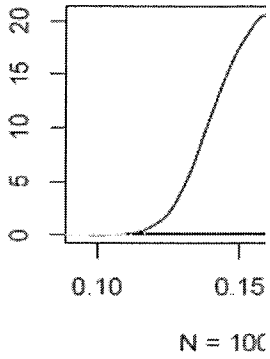
Male

			Median	95% CI	
Exp	**Vmax**	**µmol/hr/mg protein**	0.012	0.009	0.015
	Km	**µmol/L**	0.647	0.469	0.902

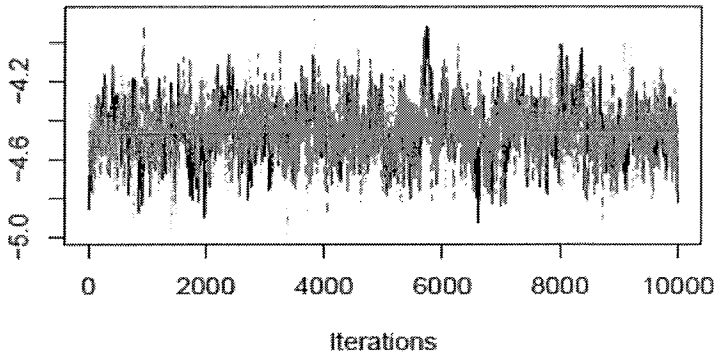
ND - Female Mouse Kidney Figure - Indeterminate - data were insufficient to estimate in vitro metaboli

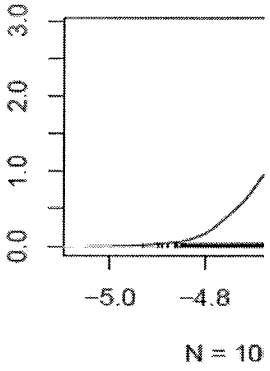




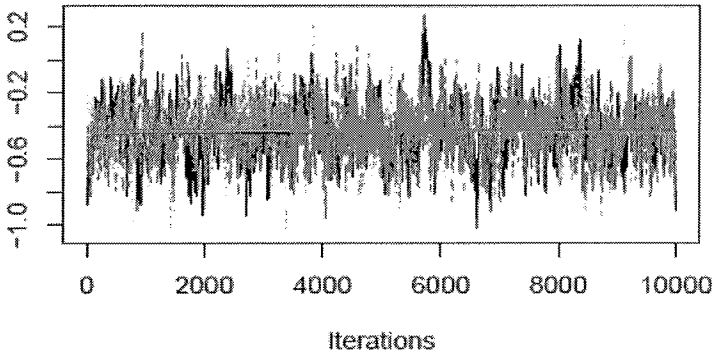


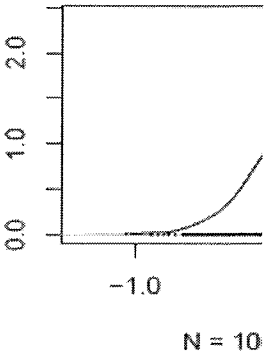
Trace of Vmax





Trace of Km



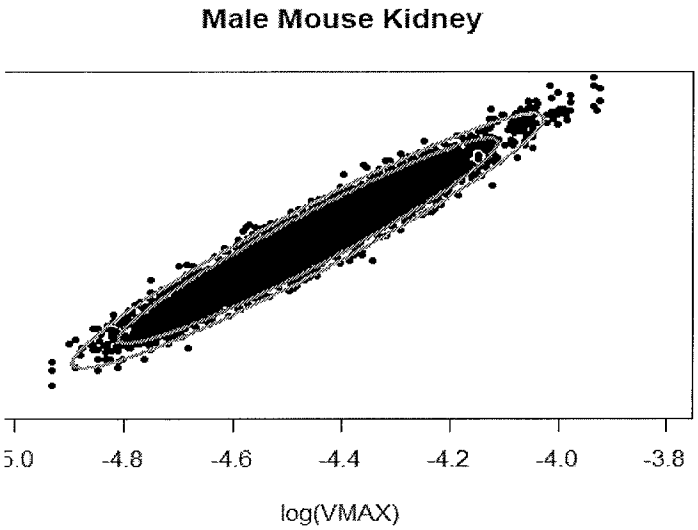


Female

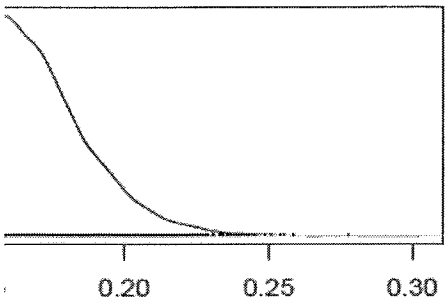
ND

ters.

ic parameters.

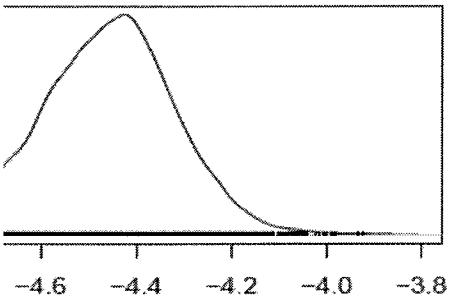


Density of LI



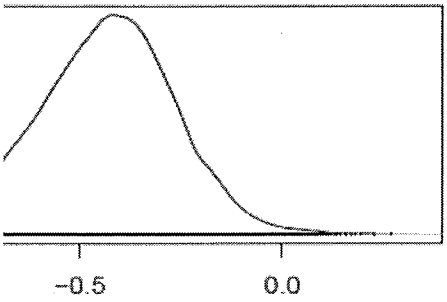
)00 Bandwidth = 0.002725

)ensity of Vmax



000 Bandwidth = 0.01896

Density of Km



000 Bandwidth = 0.02261

Male

```
> gelman.diag(x)
Potential scale reduction factors:

     Point est. Upper C.I.
LI            1          1
Vmax          1          1
Km            1          1

Multivariate psrf

1
> plot(x)
> summary(x)

Iterations = 1:1
Thinning interval = 1
Number of chains = 1
Sample size per chain = 1

1. Empirical mean and standard deviation for each variable,
   plus standard error of the mean:

          Mean
LI      0.1651
Vmax   -4.4603
Km     -0.4337

2. Quantiles for each variable:

       2.50%
LI    0.1303
Vmax -4.7366
Km   -0.7573

> summary(x)

Iterations = 1:1
Thinning interval = 1
Number of chains = 1
Sample size per chain = 1

1. Empirical mean and standard deviation for each variable,
   plus standard error of the mean:

          Mean
LI      0.1648
Vmax   -4.4546
```

Km	-0.4272
	2 Quantiles
	2.50%
LI	0.13
Vmax	-4.729
Km	-0.747
>	summary(x
Iterations	=
Thinning	interval
Number	of
Sample	size
	1 Empirical
	plus
	Mean
LI	0.1648
Vmax	-4.4659
Km	-0.4392
	2 Quantiles
	2.50%
LI	0.1295
Vmax	-4.7654
Km	-0.7861

autoburnin=FALSE)
reduction factors:

est.	Upper	C.I.
1		
1.01		
1		

1)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive	SE	Time-serie:	SE
2.08E-02	2.08E-04	0.000362		
1.43E-01	1.43E-03	0.010463		
0.16994	0.001699	0.012471		

for each variable:

25%	50%	75%	97.50%
1.50E-01	1.63E-01	0.1775	0.2118
-4.557	-4.4593	-4.365	-4.1838
-5.52E-01	-4.35E-01	-0.321	-0.1029

2)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive	SE	Time-serie:	SE
0.021	0.00021	0.000371		
0.1349	0.001349	0.009557		

0.1611 0.001611 0.011183

for each variable:

25%	50%	75%	97.50%
0.1501	0.1623	0.1769	0.2123
-4.543	-4.4509	-4.3685	-4.1825
-0.5326	-0.4239	-0.3237	-0.1012

3)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

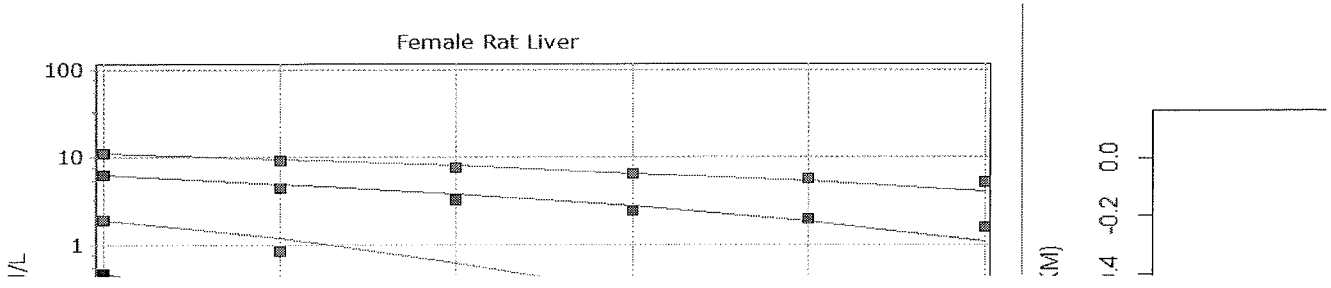
SD	Naive SE	Time-series SE
0.02117	0.000212	0.000373
0.14904	0.00149	0.012136
0.17666	0.001767	0.014207

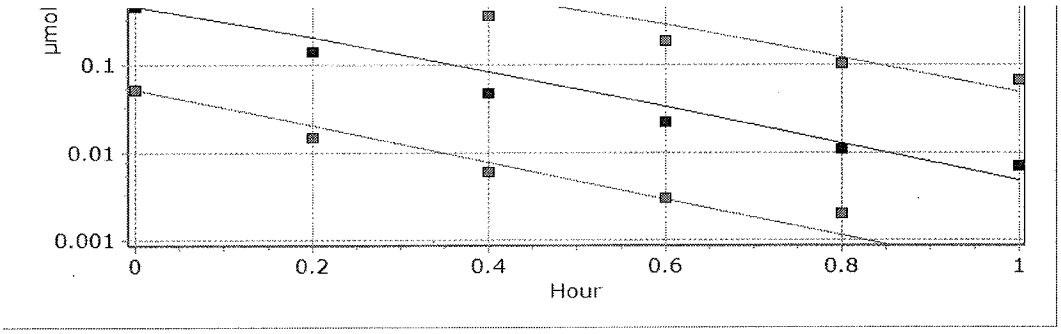
for each variable:

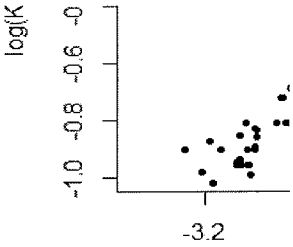
25%	50%	75%	97.50%
0.1501	0.1625	0.1772	0.2126
-4.5655	-4.4579	-4.3666	-4.1832
-0.5577	-0.4305	-0.3206	-0.1111

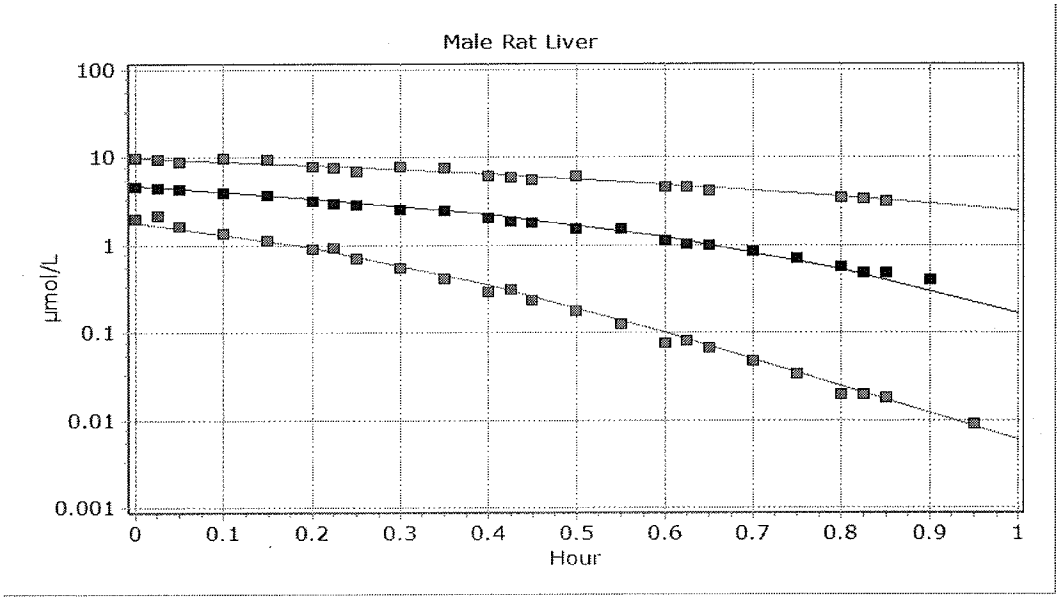
	Female				
			Median	95% CI	
Log	Vmax	µmol/hr/mg protei	-2.67	-2.89	-2.46
	Km	µmol/L	-0.33	-0.61	-0.07
	Male				
			Median	95% CI	
	A0low	µmol	-2.17842	-2.21784	-2.1387
	A0mid	µmol	-2.89622	-2.94343	-2.8474
	A0hgh	µmol	-3.88276	-3.92974	-3.8375
	Vmax	µmol/hr/mg protei	-2.63578	-2.71817	-2.5459
	Km	µmol/L	-0.87566	-1.00163	-0.7395

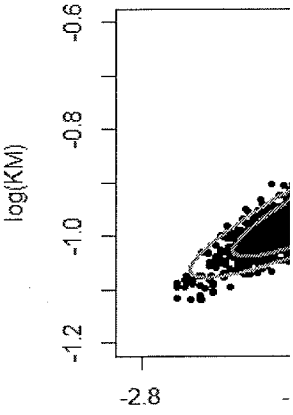
	Female				
			Median	95% CI	
EXP	Vmax	µmol/hr/mg protei	0.069	0.056	0.085
	Km	µmol/L	0.718	0.544	0.933
	Male				
			Median	95% CI	
	A0low	µmol	0.113	0.109	0.118
	A0mid	µmol	0.055	0.053	0.058
	A0hgh	µmol	0.021	0.020	0.022
	Vmax	µmol/hr/mg protei	0.072	0.066	0.078
	Km	µmol/L	0.417	0.367	0.477



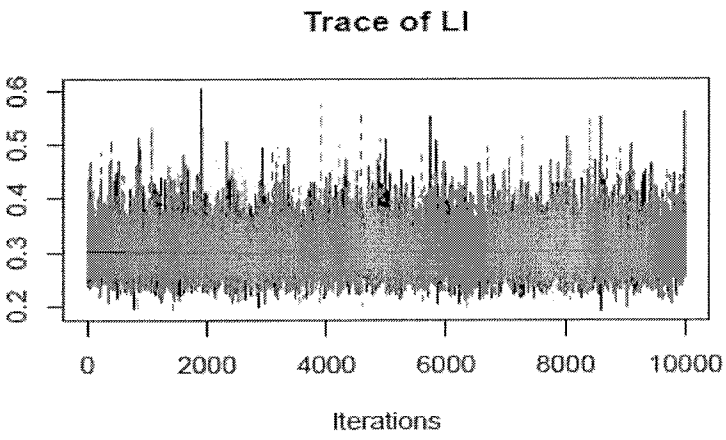

µmol
0.1
0.01
0.001
0
0.2
0.4
0.6
0.8
1
Hour

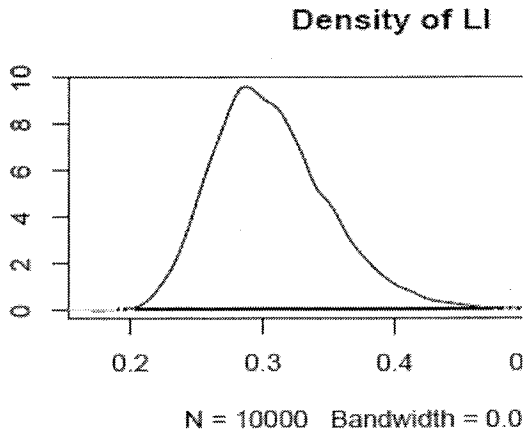

log(K
-0
-0.6
-0.8
-1.0
-3.2

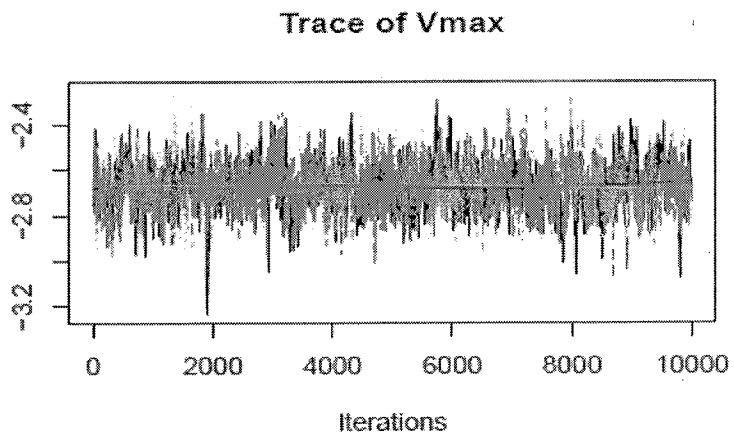

Male Rat Liver
µmol/L
100
10
1
0.1
0.01
0.001
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Hour

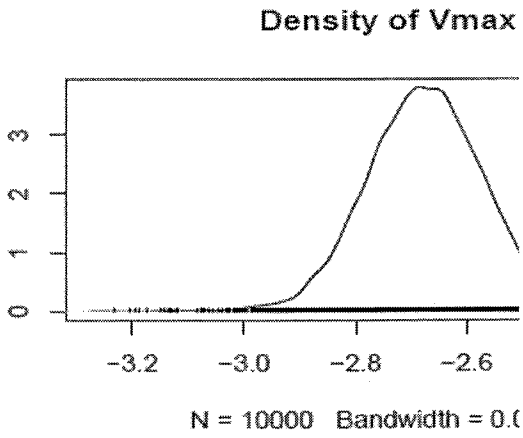

log(KM)
-0.6
-0.8
-1.0
-1.2
-2.8

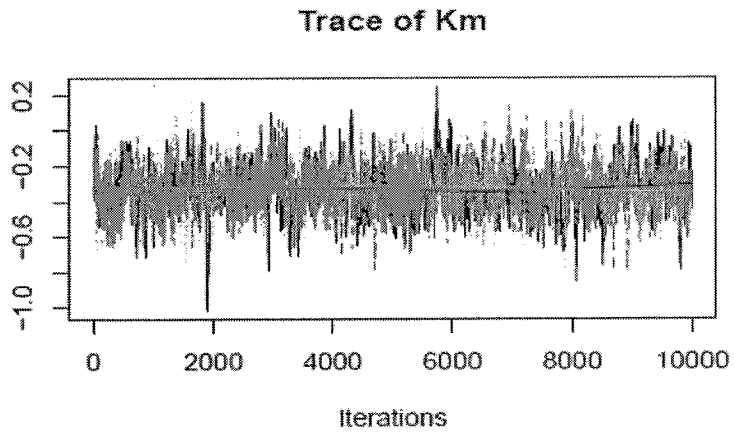
Female Rat

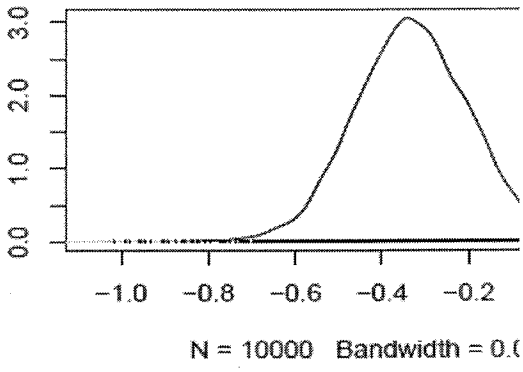

Trace of LI
0.2
0.3
0.4
0.5
0.6
0
2000
4000
6000
8000
10000
Iterations


Density of LI
0
2
4
6
8
10
0.2
0.3
0.4
0
N = 10000 Bandwidth = 0.0


Trace of Vmax
−3.2
−2.8
−2.4
0
2000
4000
6000
8000
10000
Iterations


Density of Vmax
0
1
2
3
−3.2
−3.0
−2.8
−2.6
N = 10000 Bandwidth = 0.(


Trace of Km
−1.0
−0.6
−0.2
0.2
0
2000
4000
6000
8000
10000
Iterations


Density of Km
0.0
1.0
2.0
3.0
−1.0
−0.8
−0.6
−0.4
−0.2
N = 10000 Bandwidth = 0.(

Female

```
> gelman.dia autoburnin=FALSE)
Potential scale reduction factors:

      Point est. Upper
LI            1     1
Vmax          1  1.01
Km            1  1.01

Multivariate psrf

1
>
> summary(x1)

Iterations = 1:10000
Thinning interval = 1
Number of chains = 1
Sample size per chain

1 Empirical mean and standard error

      Mean     SD  Naive
LI  0.3076 0.04605 0.000461
Vmax -2.6741 0.10833 0.001083
Km  -0.3299 0.13802  0.00138

2 Quantiles for each

      2.50%    25%    50%
LI   0.2342 0.2753 0.3028
Vmax -2.8874 -2.7434 -2.6732
Km   -0.6087 -0.418 -0.3307

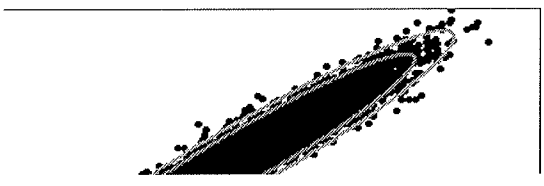
> summary(x2)

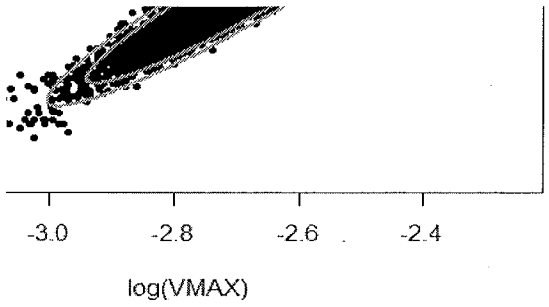
Iterations = 1:10000
Thinning interval = 1
Number of chains = 1
Sample size per chain

1 Empirical mean and standard error

      Mean     SD  Naive
LI  0.3065 0.04528 0.000453
Vmax -2.6785 0.10232 0.001023
```

Female Rat Liver





Km	-0.3362	0.13038	0.001304

2 Quantiles for each

	2.50%	25%	50%
LI	0.2331	0.2751	0.3003
Vmax	-2.8733	-2.7483	-2.6803
Km	-0.5844	-0.424	-0.3383

> summary(x3)

Iterations = 1:10000
Thinning interval = 1
Number of chains = 1
Sample size per chain

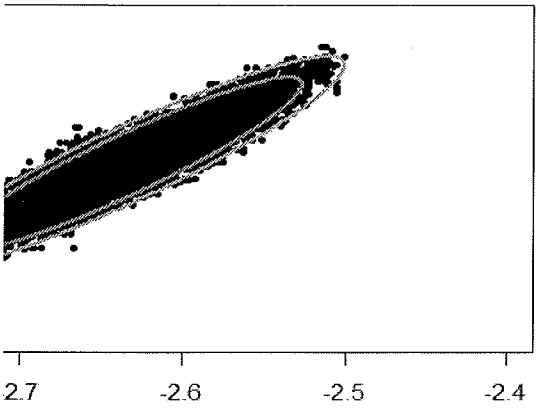
1 Empirical mean and standard error plus

	Mean	SD	Naive
LI	0.3071	0.0436	0.000436
Vmax	-2.6687	0.1051	0.001051
Km	-0.3234	0.1356	0.001356

2 Quantiles for each

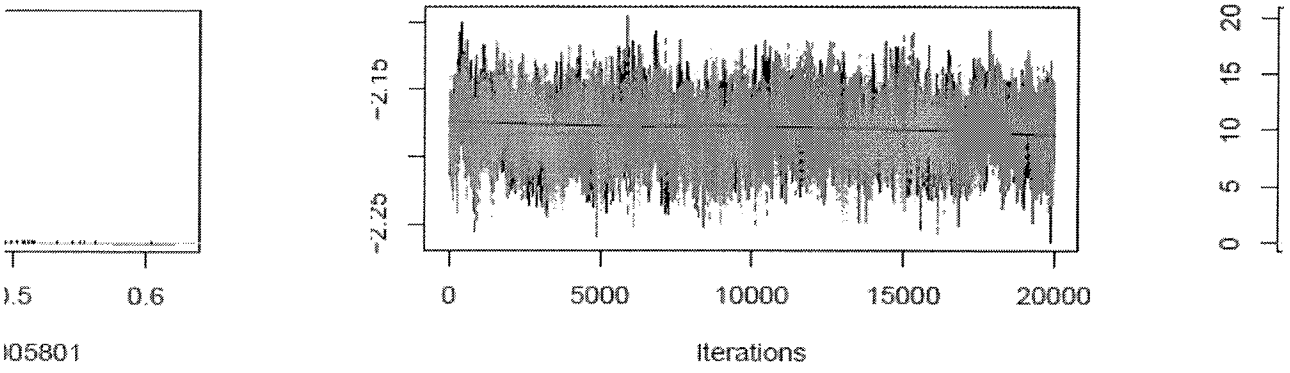
	2.50%	25%	50%
LI	0.2344	0.2754	0.3028
Vmax	-2.8753	-2.7405	-2.6674
Km	-0.5903	-0.4118	-0.3233

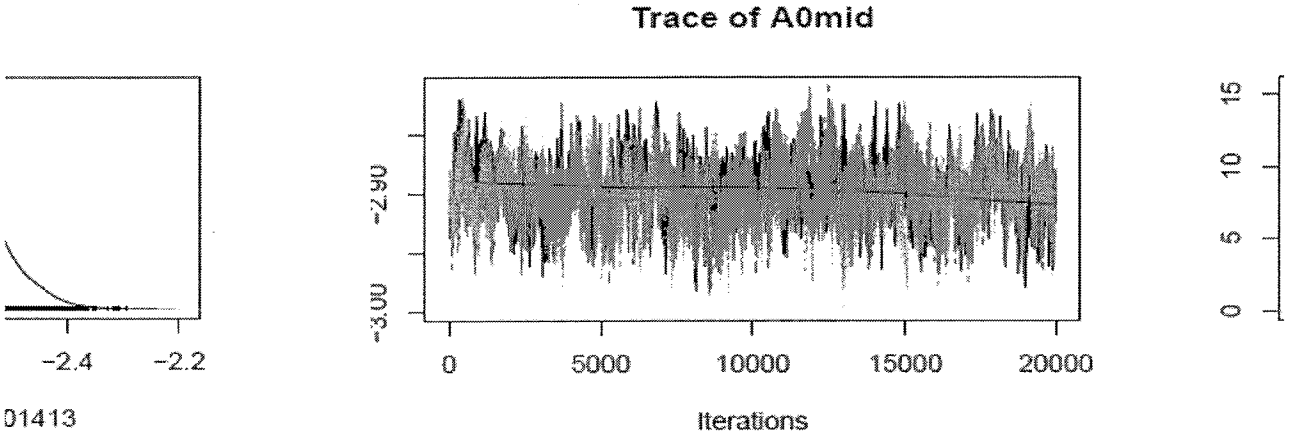
Male Rat Liver

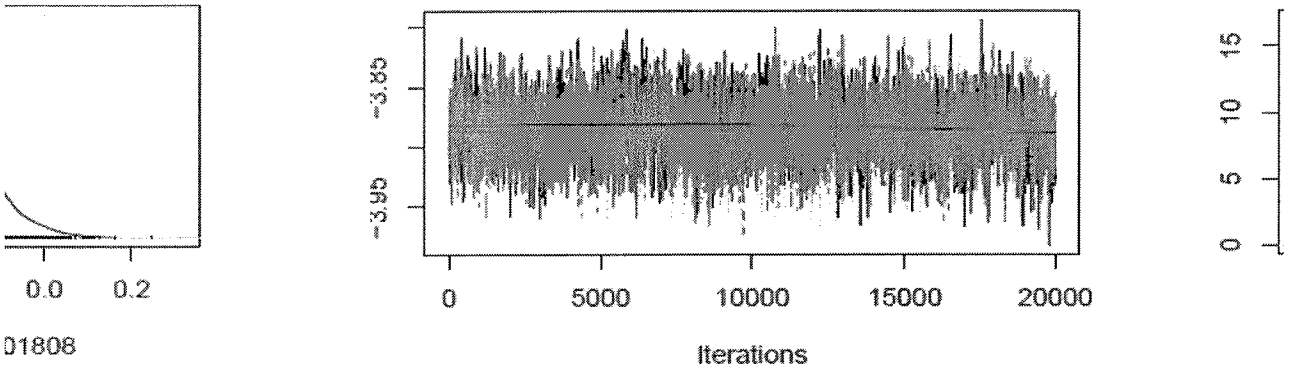


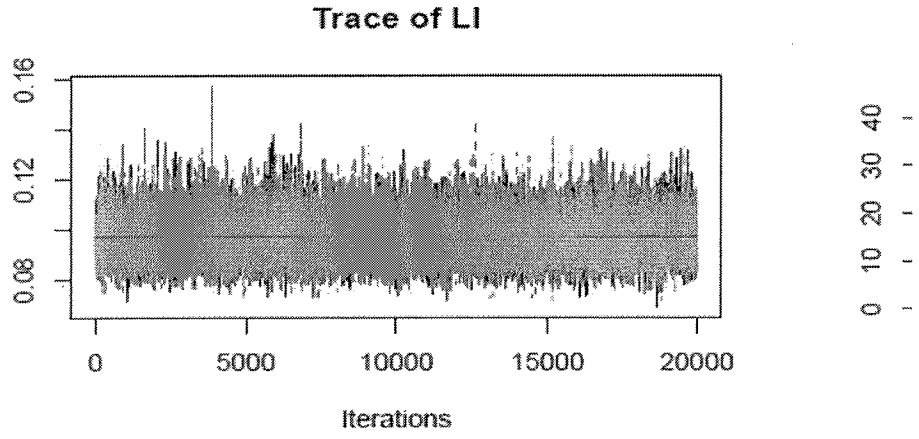
Male Rat

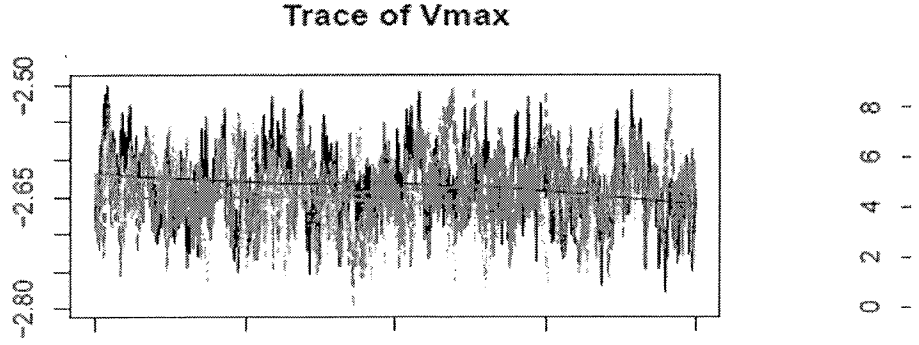
Trace of A0low

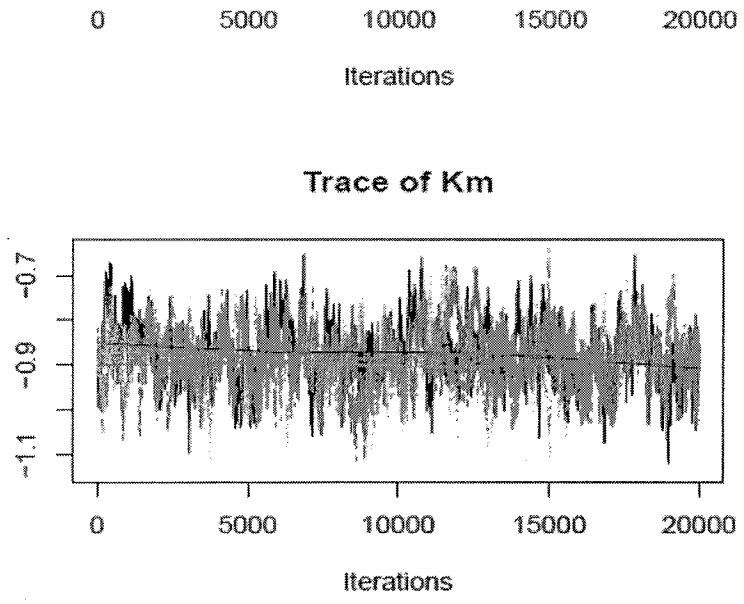



Trace of A0mid
−2.4
−2.2
01413
−3.00
−2.90
0
5000
10000
15000
20000
Iterations
0
5
10
15


Trace of A0hgh
0.0
0.2
01808
−3.95
−3.85
0
5000
10000
15000
20000
Iterations
0
5
10
15


Trace of LI
0.08
0.12
0.16
0
5000
10000
15000
20000
Iterations
0
10
20
30
40


Trace of Vmax
−2.80
−2.65
−2.50
0
2
4
6
8


0
5000
10000
15000
20000
Iterations
Trace of Km
−1.1
−0.9
−0.7
0
5000
10000
15000
20000
Iterations
0 1 2 3 4 5 6

Male

C.I.

1

= 10000

standard deviation for each variable, of the mean:

SE	Time-serie: SE
0.000901	
0.007052	
0.009067	

variable:

75%	97.50%
0.3333	0.4141
-2.6016	-2.464
-0.2365	-0.0689

1

= 10000

standard deviation for each variable, of the mean:

SE	Time-serie: SE
0.000856	
0.006857	

Potential	gelman.dia scale	autoburnin reduction
	Point	est.
A0low	1.01	1.02
A0mid	1.01	1.04
A0hgh	1.01	1.02
LI	1	1
Vmax	1.01	1.05
Km	1.01	1.04

Multivariate psrf

1.01

>

> summary(x1)

Iterations = 1:20000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean plus standard

	Mean	SD
A0low	-2.17827	0.02023
A0mid	-2.89614	0.02463
A0hgh	-3.88286	0.02364
LI	0.09722	0.00884
Vmax	-2.63501	0.04433
Km	-0.87484	0.06706

2 Quantiles for

	2.50%	25%
A0low	-2.21784	-2.19193
A0mid	-2.94343	-2.91332
A0hgh	-3.92974	-3.89875
LI	0.08166	0.09098
Vmax	-2.71817	-2.66599
Km	-1.00163	-0.92033

> summary(x2)

Iterations = 1:20000
Thinning interval =

0.008862

variable:

75%	97.50%
0.3322	0.40887
-2.6109	-2.47317
-0.2543	-0.07111

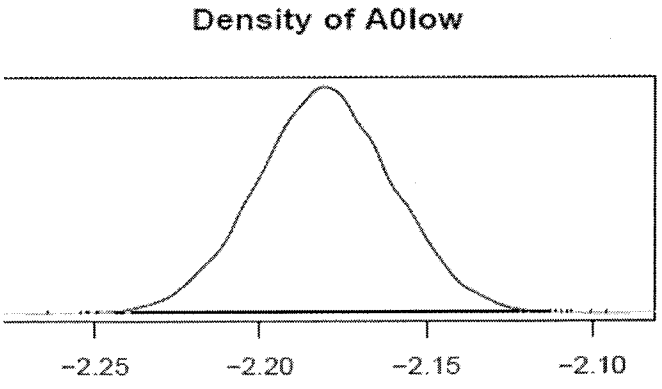
1
= 10000

standard deviation for each variable,
of the mean:

SE	Time-series SE
0.000839	
0.006884	
0.008883	

variable:

75%	97.50%
0.3334	0.40573
-2.5973	-2.4678
-0.2321	-0.06045



Number of chains
Sample size per

1 Empirical mean
plus standard

	Mean	SD
A0low	-2.18124	0.020526
A0mid	-2.90017	0.025076
A0hgh	-3.88545	0.022686
LI	0.09712	0.008951
Vmax	-2.64286	0.045271
Km	-0.88512	0.067828

2 Quantiles for

	2.50%	25%
A0low	-2.22161	-2.19472
A0mid	-2.94867	-2.91746
A0hgh	-3.9307	-3.90064
LI	0.08162	0.09081
Vmax	-2.72777	-2.67353
Km	-1.01462	-0.9304

> summary(x3)

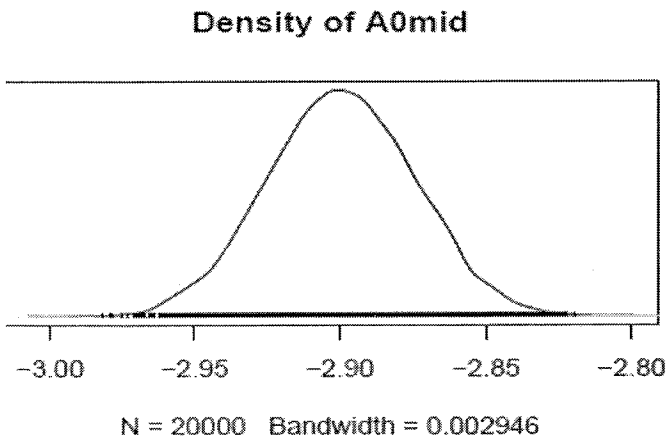
Iterations = 1:20000
Thinning interval =
Number of chains
Sample size per

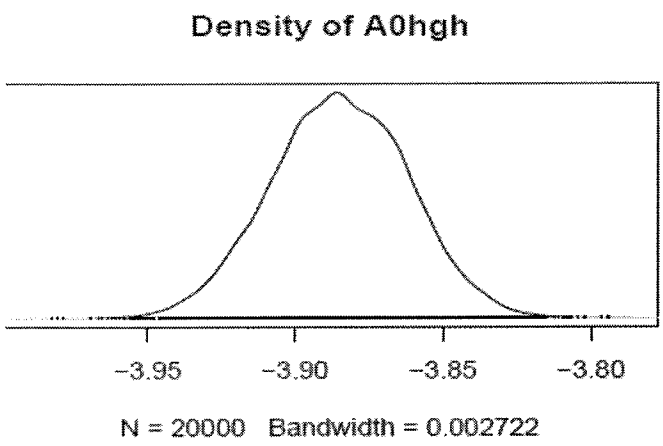
1 Empirical mean
plus standard

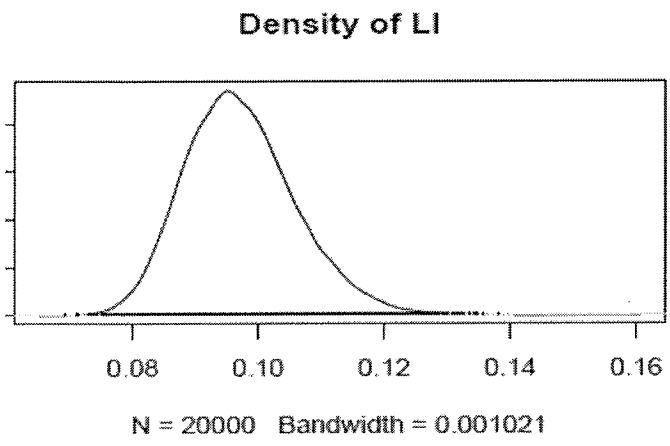
	Mean	SD
A0low	-2.18215	0.020584
A0mid	-2.9029	0.025119
A0hgh	-3.88692	0.023042
LI	0.09704	0.008725
Vmax	-2.64807	0.045076
Km	-0.89208	0.067185

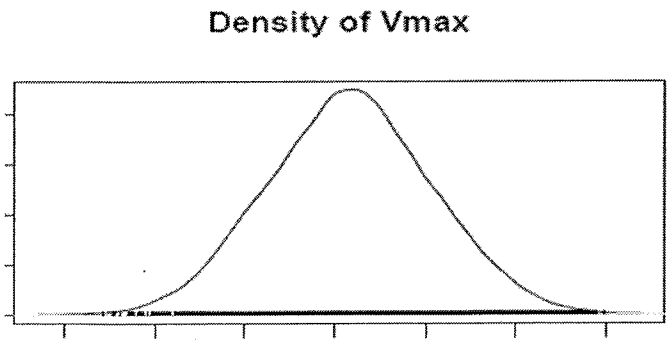
2 Quantiles for

	2.50%	25%
A0low	-2.2232	-2.19569
A0mid	-2.95412	-2.91937





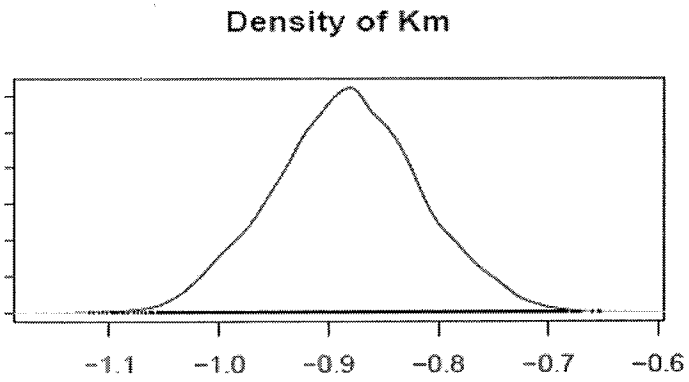




A0hgh	-3.93204	-3.90237
LI	0.08196	0.09088
Vmax	-2.74136	-2.67819
Km	-1.02787	-0.93558

−2.80 −2.70 −2.60 −2.50

N = 20000 Bandwidth = 0.005308



N = 20000 Bandwidth = 0.007831

=FALSE)
factors:

Upper C.I.

1
= 1
chain = 20000

and standard deviation for each variable,
error of the mean:

Naive SE	Time-serie: SE
1.43E-04	0.001123
1.74E-04	0.002171
1.67E-04	0.000814
6.25E-05	0.000118
3.14E-04	0.00453
4.74E-04	0.005889

each variable:

50%	75%	97.50%
-2.17842	-2.1647	-2.1387
-2.89622	-2.8789	-2.8474
-3.88276	-3.8663	-3.8375
0.09644	0.1027	0.1163
-2.63578	-2.605	-2.5459
-0.87566	-0.8312	-0.7395

1

= 1
chain = 20000

and standard deviation for each variable,
error of the mean:

Naive	SE	Time-serie:	SE
1.45E-04	0.00136		
1.77E-04	0.002124		
1.60E-04	0.000875		
6.33E-05	0.000126		
3.20E-04	0.00491		
4.80E-04	0.006864		

each variable:

50%	75%	97.50%
-2.18149	-2.1678	-2.1402
-2.90032	-2.8832	-2.8498
-3.88506	-3.8699	-3.8422
0.09656	0.1027	0.1164
-2.64321	-2.613	-2.5502
-0.88501	-0.8404	-0.7454

1
= 1
chain = 20000

and standard deviation for each variable,
error of the mean:

Naive	SE	Time-serie:	SE
1.46E-04	0.001322		
1.78E-04	0.00225		
1.63E-04	0.000857		
6.17E-05	0.000114		
3.19E-04	0.004863		
4.75E-04	0.006844		

each variable:

50%	75%	97.50%
-2.18174	-2.1682	-2.1427
-2.90262	-2.8852	-2.856

-3.88714	-3.8708	-3.8418
0.09634	0.1024	0.1161
-2.64677	-2.617	-2.563
-0.88916	-0.8467	-0.766

Female

			Median	95% CI	
Log	**Vmax**	**µmol/hr/mg protein**	-5.50	-6.49	-5.09
	Km	**µmol/L**	Km was set to the posterior median Km estimatec		

Male

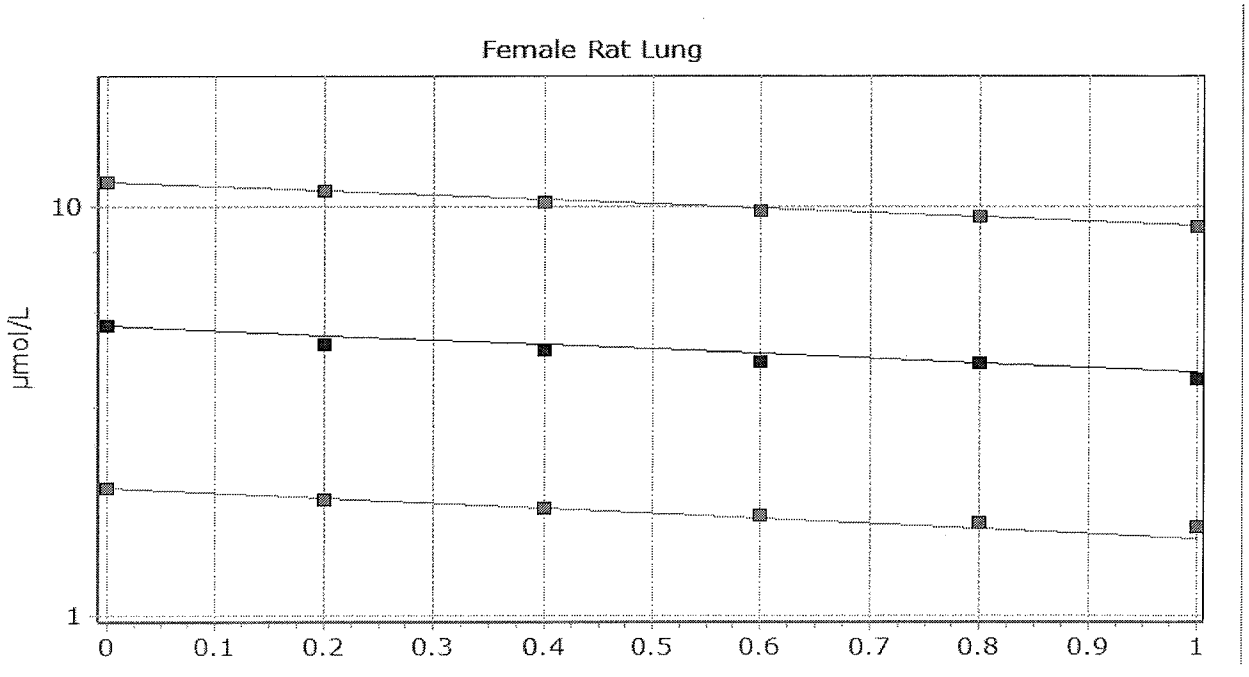
			Median	95% CI
not determinable - data were insufficient to estimate in vitro metabolic parameters				

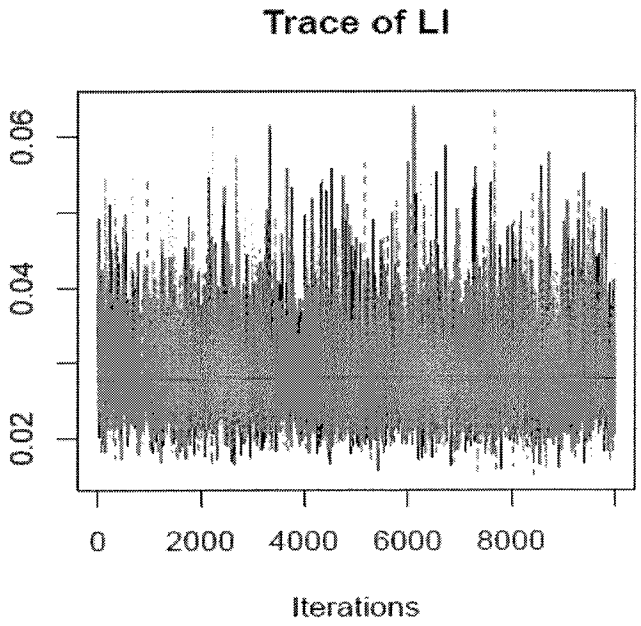
Female

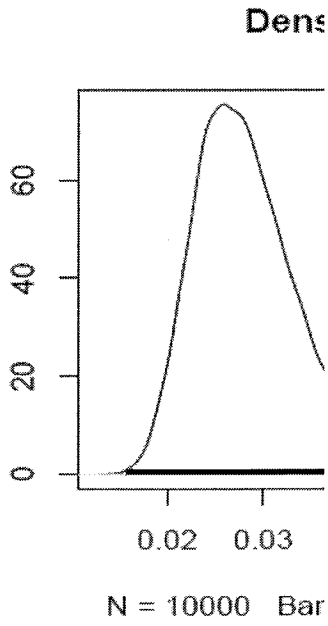
			Median	95% CI		
EXP	**Vmax**	**µmol/hr/mg protein**	0.0041	0.0015	0.0062	
	Km	**µmol/L**	2.369			Female Mou:

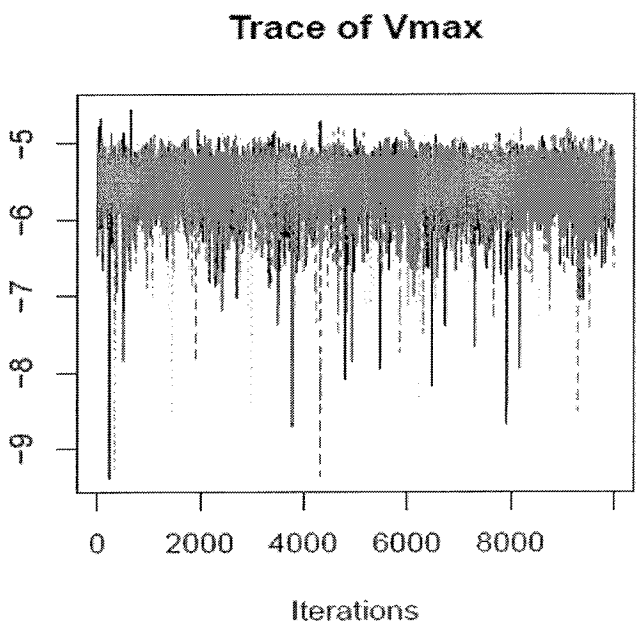
Male

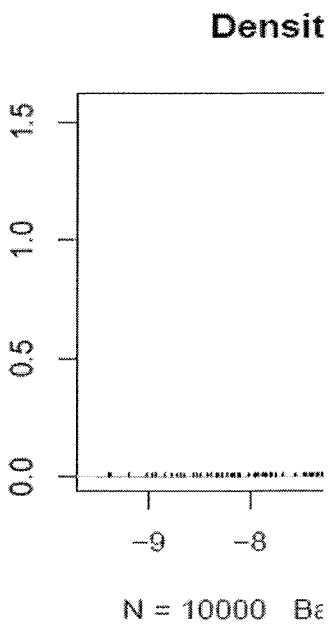
			Median	95% CI




Trace of LI
0.02
0.04
0.06
0
2000
4000
6000
8000
Iterations


Dens
0
20
40
60
0.02
0.03
N = 10000 Bar


Trace of Vmax
−9
−8
−7
−6
−5
0
2000
4000
6000
8000
Iterations


Densit
0.0
0.5
1.0
1.5
−9
−8
N = 10000 Ba

Female > gelman.dia autoburnin
Potential scale reduction

l for the female mouse lung

Point est.
LI 1 1
Vmax 1 1

Multivariate psrf

1

>
>

summary(x1)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

Mean SD
se Lung Km LI 0.02868 0.006123
Vmax -5.56714 0.378527

2 Quantiles for

2.50% 25%
LI 0.01944 0.02438
Km was fixed - no parameter correlation plot Vmax -6.48618 -5.70219

>

summary(x2)

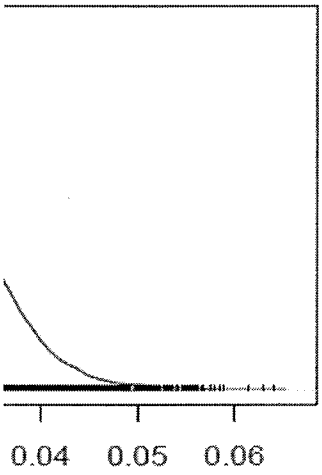
Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

Mean SD
LI 0.02865 0.005728
Vmax -5.55194 0.349512

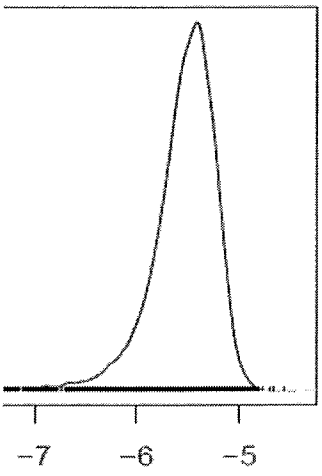
2 Quantiles for

sity of LI



ndwidth = 0.0007452

y of Vmax



andwidth = 0.03666

	2.50%	25%
LI	0.01979	0.0245
Vmax	-6.3568	-5.6959

> summary(x3)

Iterations = 1:10000
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

	Mean	SD
LI	0.02842	0.00567
Vmax	-5.5614	0.36306

2 Quantiles for

	2.50%	25%
LI	0.01947	0.02435
Vmax	-6.40378	-5.70516

> summary(x3)

Iterations = 1:19999
Thinning interval =
Number of chains
Sample size per

1 Empirical mean
plus standard

	Mean	SD
LI	0.02948	0.03966
Vmax	-5.5651	0.38375

2 Quantiles for

	2.50%	25%
LI	0.01964	0.02447
Vmax	-6.43208	-5.70606

=FALSE)
factors:

Upper C.I.

1
= 1
chain = 10000

and standard deviation for each variable,
error of the mean:

Naive SE	Time-serie: SE
6.12E-05	0.000164
3.79E-03	0.011111

each variable:

50%	75%	97.50%
0.02768	0.03187	0.04335
-5.50231	-5.3427	-5.08588

1
= 1
chain = 10000

and standard deviation for each variable,
error of the mean:

Naive SE	Time-serie: SE
5.73E-05	0.000155
3.50E-03	0.009836

each variable:

50%	75%	97.50%
0.02795	0.03191	0.04213
-5.49786	-5.32925	-5.08133

1
= 1
chain = 10000

and standard deviation for each variable,
error of the mean:

Naive SE	Time-serie: SE
5.67E-05	0.000156
0.003631	0.010678

each variable:

50%	75%	97.50%
0.02765	0.03166	0.0414
-5.50014	-5.34242	-5.0814

1
= 1
chain = 19999

and standard deviation for each variable,
error of the mean:

Naive SE	Time-serie: SE
0.000281	0.000887
0.002714	0.008891

each variable:

50%	75%	97.50%
0.02783	0.03183	0.0425
-5.50156	-5.33461	-5.075

Female

			Median	95% CI	
Log	**Vmax**	**µmol/hr/mg protein**	-6.296	-6.623	-5.95
	Km	**µmol/L**	-0.801	-1.210	-0.38

Male

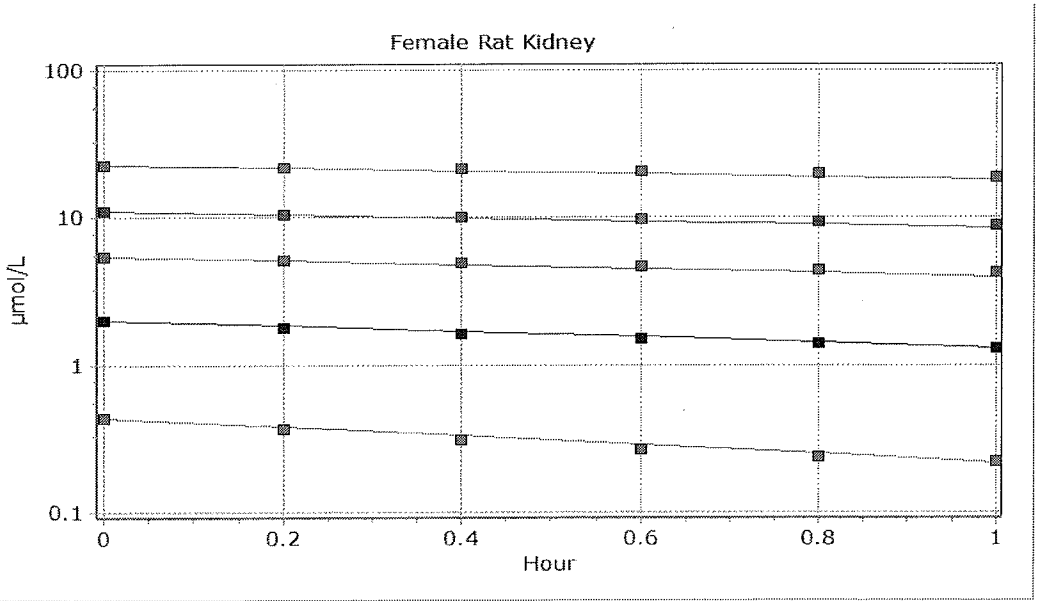
			Median	95% CI	
	Vmax	**µmol/hr/mg protein**	-6.26	-6.55	-5.97
	Km	**µmol/L**	-0.48	-0.83	-0.12

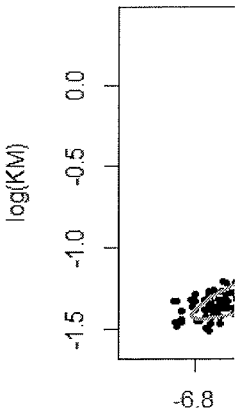
Female

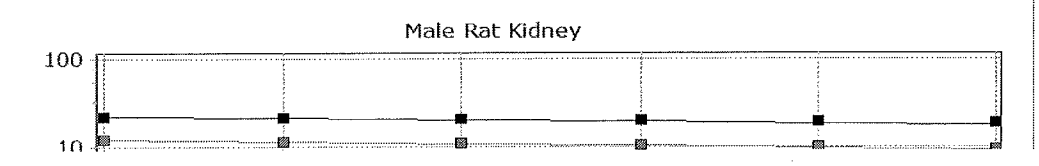
			Median	95% CI	
EXP	**Vmax**	**µmol/hr/mg protein**	0.0018	0.0013	0.0026
	Km	**µmol/L**	0.449	0.298	0.687

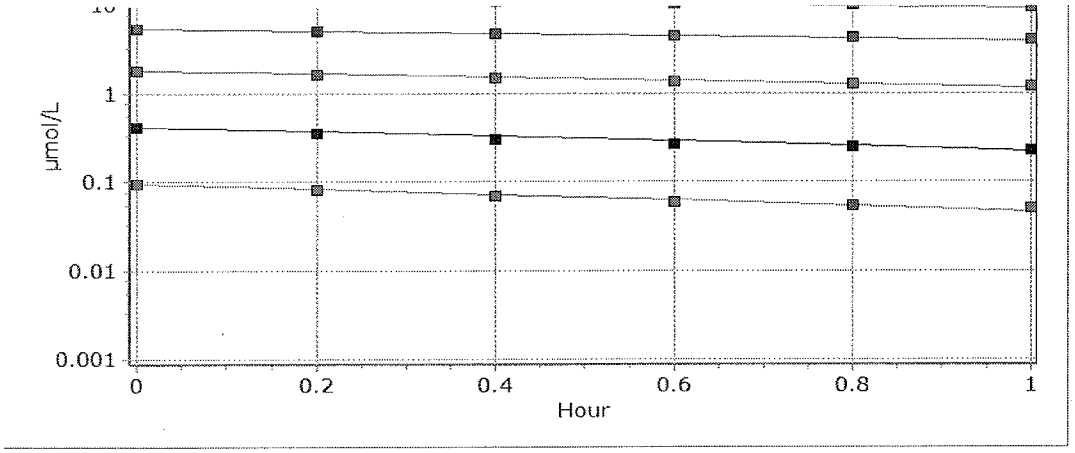
Male

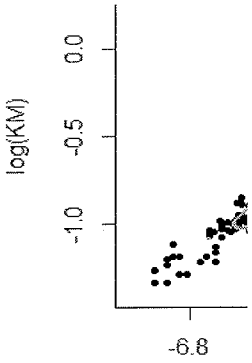
			Median	95% CI	
	Vmax	**µmol/hr/mg protein**	0.0019	0.0014	0.0026
	Km	**µmol/L**	0.619	0.437	0.885



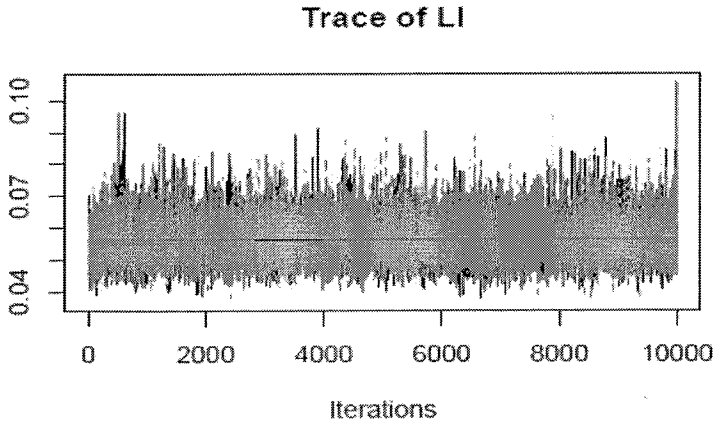


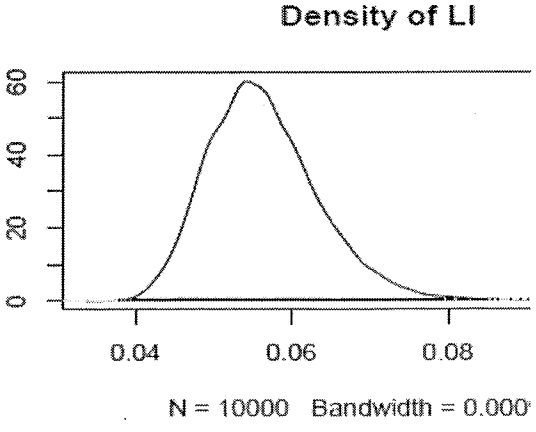


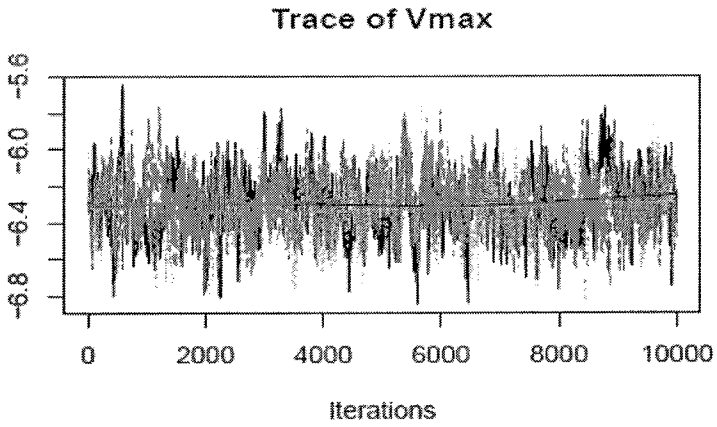

µmol/L
1
0.1
0.01
0.001
0
0.2
0.4
0.6
0.8
1
Hour

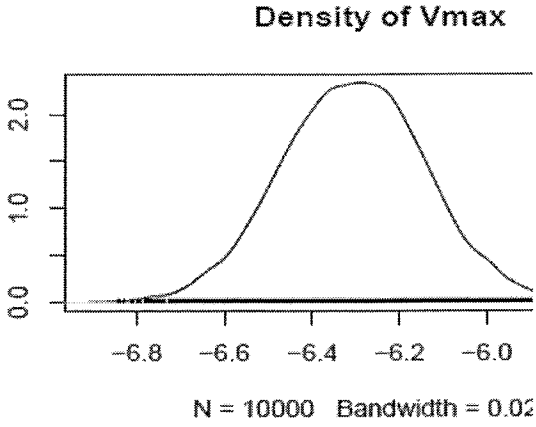

log(KM)
0.0
-0.5
-1.0
-6.8

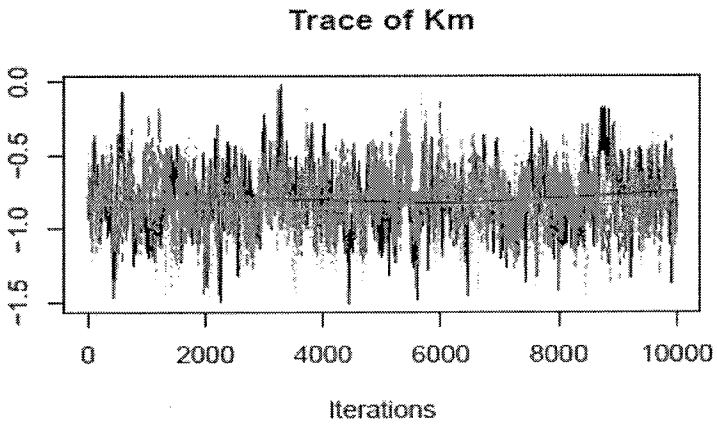
Female Rat

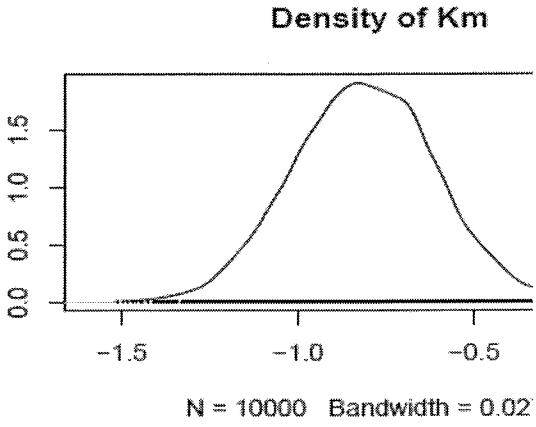

Trace of LI
0.10
0.07
0.04
0
2000
4000
6000
8000
10000
Iterations


Density of LI
60
40
20
0
0.04
0.06
0.08
N = 10000 Bandwidth = 0.000


Trace of Vmax
−5.6
−6.0
−6.4
−6.8
0
2000
4000
6000
8000
10000
Iterations


Density of Vmax
2.0
1.0
0.0
−6.8
−6.6
−6.4
−6.2
−6.0
N = 10000 Bandwidth = 0.02


Trace of Km
0.0
−0.5
−1.0
−1.5
0
2000
4000
6000
8000
10000
Iterations


Density of Km
1.5
1.0
0.5
0.0
−1.5
−1.0
−0.5
N = 10000 Bandwidth = 0.02

Female

```
> gelman.dia autoburnin=FALSE)
Potential scale reduction factors:

                Point est.  Upper
LI                      1       1
Vmax                 1.01    1.04
Km                   1.01    1.04

Multivariate psrf

1.01
>
> summary(x1)

Iterations = 1:10000
Thinning interval = 1
Number of chains = 1
Sample size per chain

1 Empirical mean and
  plus standard error

          Mean      SD  Naive
LI     0.05662 0.007231 7.23E-05
Vmax  -6.29482 0.16873 1.69E-03
Km    -0.79932 0.210893 2.11E-03

2 Quantiles for each

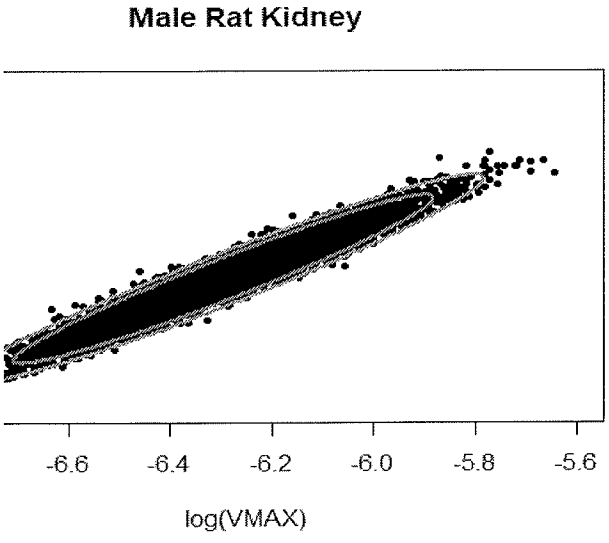
          2.50%     25%     50%
LI      0.04443  0.0517 0.05605
Vmax   -6.62274 -6.4087 -6.29574
Km     -1.20987 -0.9407 -0.80103

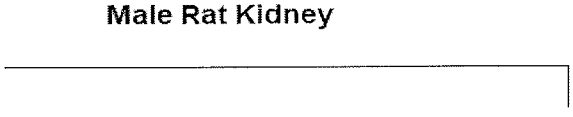
> summary(x2)

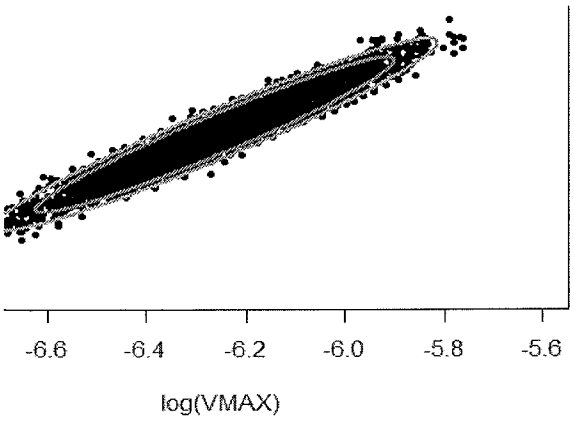
Iterations = 1:10000
Thinning interval = 1
Number of chains = 1
Sample size per chain

1 Empirical mean and
  plus standard error

          Mean      SD  Naive
LI     0.05622 0.007031 7.03E-05
Vmax  -6.28988 0.151785 1.52E-03
```







Km	-0.79265	0.187731	1.88E-03

2 Quantiles for each

	2.50%	25%	50%
LI	0.04458	0.05109	0.05554
Vmax	-6.56963	-6.39944	-6.29166
Km	-1.1409	-0.92657	-0.79655

> summary(x3)

Iterations	=	1:10000	
Thinning	interval	=	1
Number	of	chains	=
Sample	size	per	chain

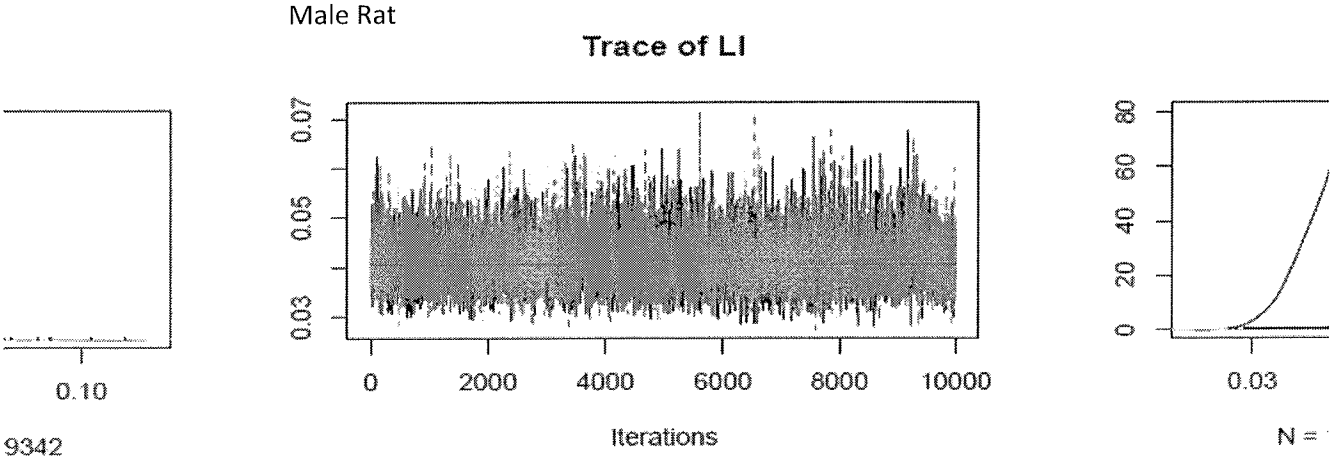
1 Empirical mean and plus standard error

	Mean	SD	Naive
LI	0.05624	0.007086	7.09E-05
Vmax	-6.32732	0.168075	1.68E-03
Km	-0.84048	0.208097	2.08E-03

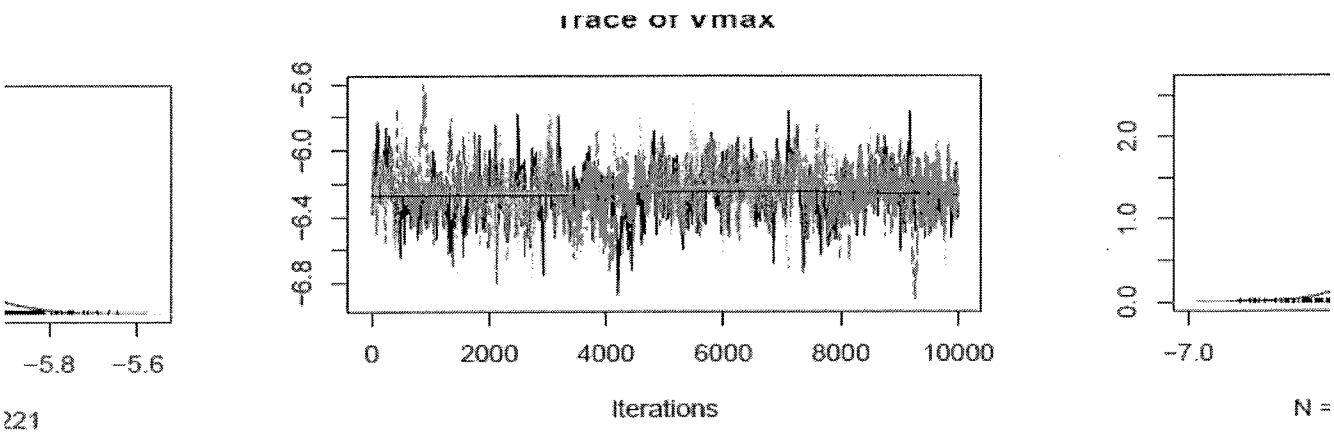
2 Quantiles for each

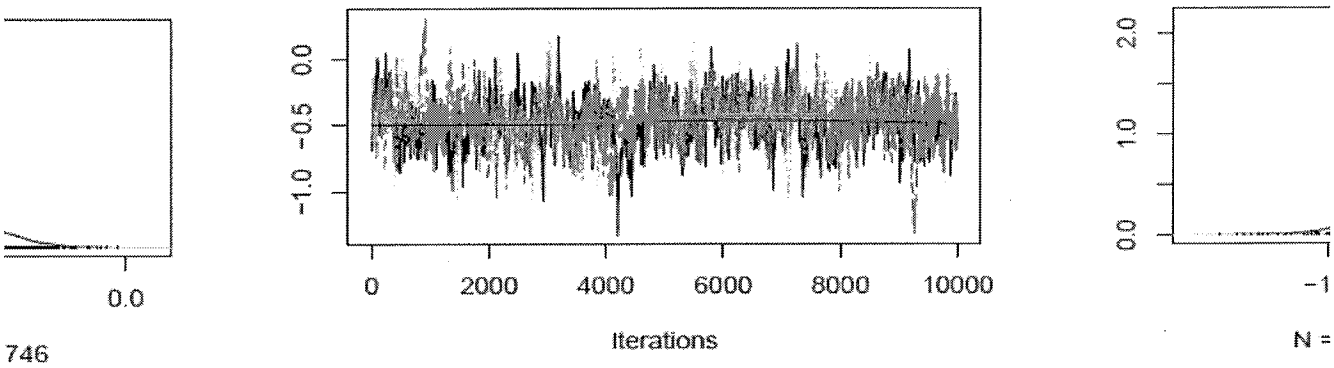
	2.50%	25%	50%
LI	0.04445	0.05121	0.05545
Vmax	-6.65409	-6.44297	-6.3222
Km	-1.24452	-0.98248	-0.83255

Male Rat



Trace of Vmax


Trace of Vmax
−6.8 −6.4 −6.0 −5.6
0 2000 4000 6000 8000 10000
Iterations
0.0 1.0 2.0
−5.8 −5.6
−7.0
221
N =


Trace of Km
−1.0 −0.5 0.0
0 2000 4000 6000 8000 10000
Iterations
0.0 1.0 2.0
0.0
−1
746
N =

Male > gelman.dia

Potential scale

C.I. Point

LI 1

Vmax 1.01

Km 1.01

Multivariate psrf

1.01

>

> summary(x

Iterations =

Thinning interval

1 Number of

= 10000 Sample size

standard deviation for each variable, 1 Empirical

of the mean: plus

SE Time-serie SE Mean

1.51E-04 LI 0.04133

1.38E-02 Vmax -6.26242

1.70E-02 Km -0.47954

variable: 2 Quantiles

75% 97.50% 2.50%

0.06083 0.07281 LI 0.03258

-6.1822 -5.95434 Vmax -6.54539

-0.66408 -0.37558 Km -0.82721

> summary(x

Iterations =

Thinning interval

1 Number of

= 10000 Sample size

standard deviation for each variable, 1 Empirical

of the mean: plus

SE Time-serie SE Mean

1.30E-04 LI 0.04152

1.15E-02 Vmax -6.23691

1.39E-02

variable:

75%	97.50%
0.0605	0.07182
-6.1871	-5.98069
-0.6641	-0.41202

1
= 10000

standard deviation of the mean: for each variable,

SE	Time-serie: SE
1.23E-04	
1.36E-02	
1.71E-02	

variable:

75%	97.50%
0.0604	0.07244
-6.211	-6.00152
-0.6972	-0.44368

Km -0.44958

2 Quantiles

	2.50%
LI	0.03273
Vmax	-6.56376
Km	-0.85279

> summary(x

Iterations =
Thinning interval
Number of
Sample size

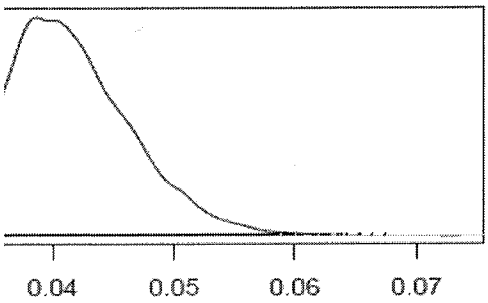
1 Empirical plus

	Mean
LI	0.04136
Vmax	-6.23539
Km	-0.44846

2 Quantiles

	2.50%
LI	0.03274
Vmax	-6.56829
Km	-0.84996

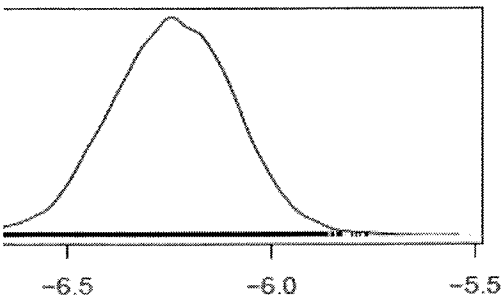
Density of LI



10000 Bandwidth = 0.0006917

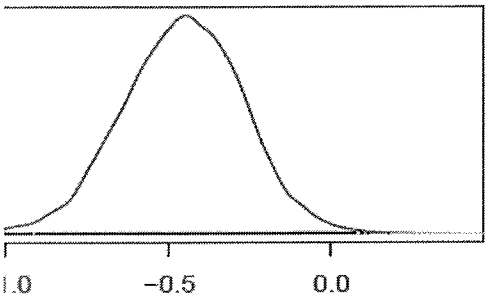
Density of Vmax

Density of Vmax



= 10000 Bandwidth = 0.02062

Density of Km



= 10000 Bandwidth = 0.02522

autoburnin=FALSE)
reduction factors:

est.	Upper	C.I.
1		
1.02		
1.02		

1)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie: SE
0.005319	5.32E-05	1.06E-04
0.14787	1.48E-03	1.25E-02
0.180938	1.81E-03	1.51E-02

for each variable:

25%	50%	75%	97.50%
0.03763	0.04073	0.04461	0.05337
-6.36184	-6.26163	-6.15961	-5.9715
-0.60111	-0.47992	-0.3559	-0.12203

2)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie: SE
0.005321	5.32E-05	1.19E-04
0.157309	1.57E-03	1.43E-02

0.19248 1.93E-03 1.73E-02

for each variable:

25%	50%	75%	97.50%
0.03785	0.04093	0.04464	0.05351
-6.33595	-6.22942	-6.13253	-5.95229
-0.57125	-0.44044	-0.32239	-0.09631

3)

1:10000
= 1
chains = 1
per chain = 10000

mean and standard deviation for each variable,
standard error of the mean:

SD	Naive SE	Time-serie: SE
0.005238	5.24E-05	9.87E-05
0.157025	1.57E-03	1.42E-02
0.190374	1.90E-03	1.74E-02

for each variable:

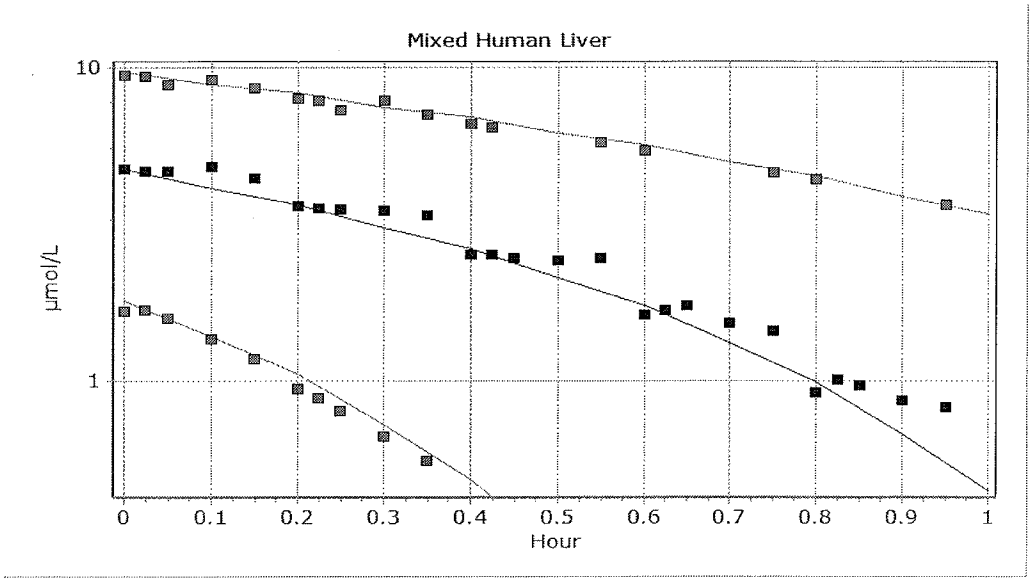
25%	50%	75%	97.50%
0.03764	0.04079	0.04445	0.05295
-6.3321	-6.2347	-6.12857	-5.9325
-0.56595	-0.44479	-0.31943	-0.07425

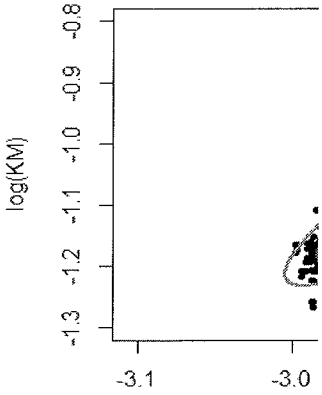
Mixed Human with estimation of the start vial amount as part of the MCMC

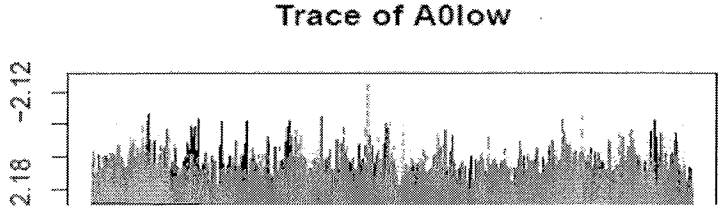
			Median	95% CI	
Log					
	A0low	**µmol**	-2.19256	-2.22353	-2.16093
	A0mid	**µmol**	-2.83933	-2.87308	-2.80278
	A0hgh	**µmol**	-3.92429	-3.96151	-3.88714
	Vmax	**µmol/hr/mg protein**	-2.89851	-2.96397	-2.82594
	Km	**µmol/L**	-1.05319	-1.1657	-0.93204

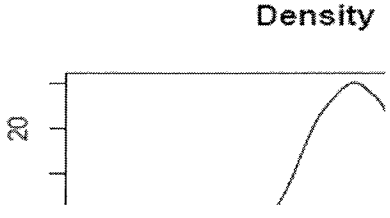
Mixed Human

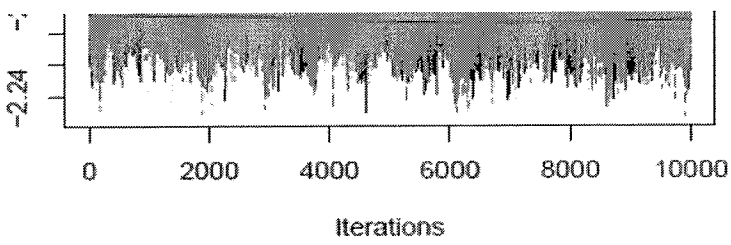
			Median	95% CI	
EXP	**A0low**	**µmol**	0.112	0.108	0.115
	A0mid	**µmol**	0.058	0.057	0.061
	A0hgh	**µmol**	0.020	0.019	0.021
	Vmax	**µmol/hr/mg protein**	0.055	0.052	0.059
	Km	**µmol/L**	0.349	0.312	0.394

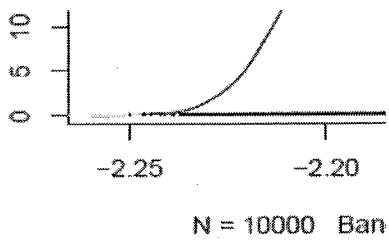






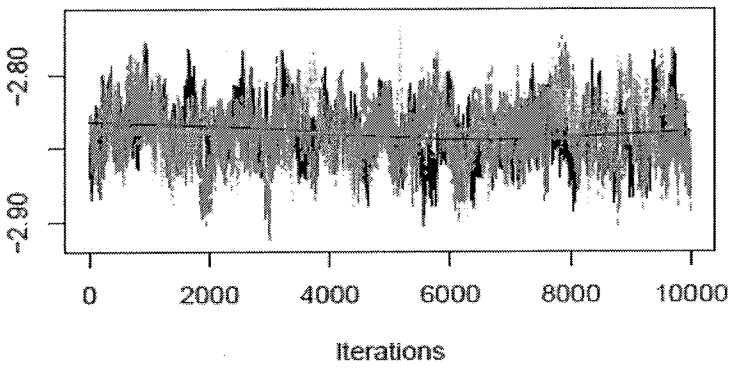


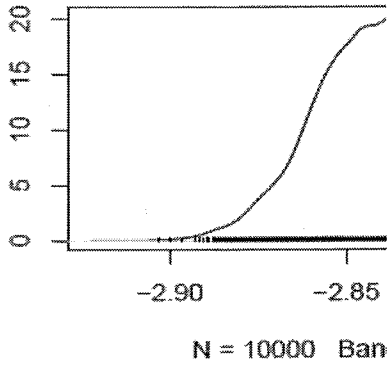




Trace of A0mid

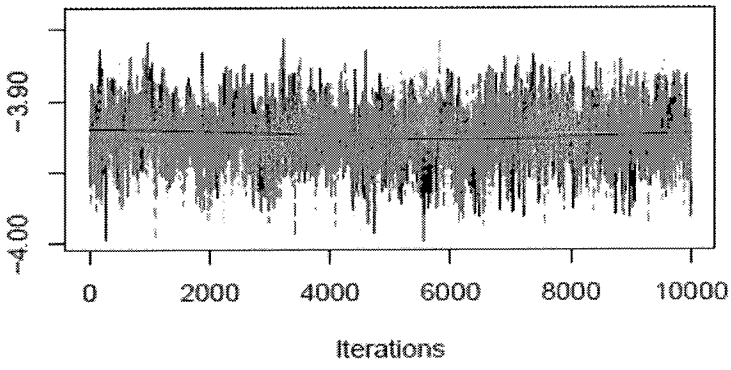
Density

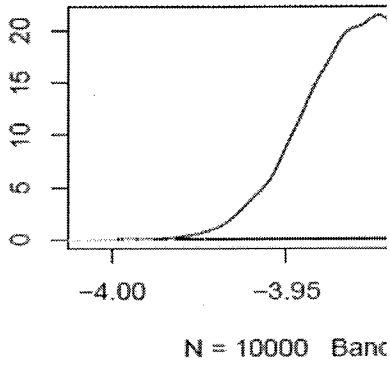




Trace of A0hgh

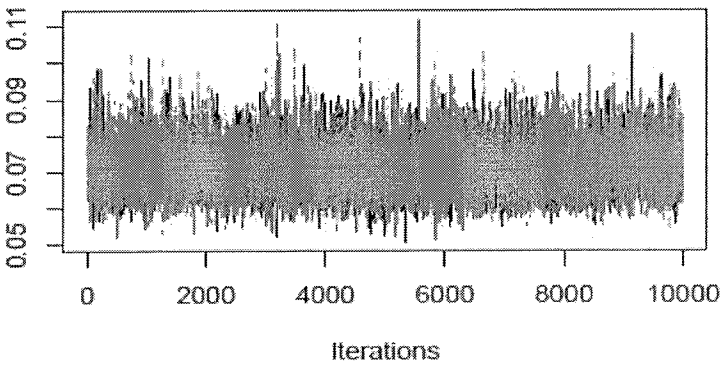
Density

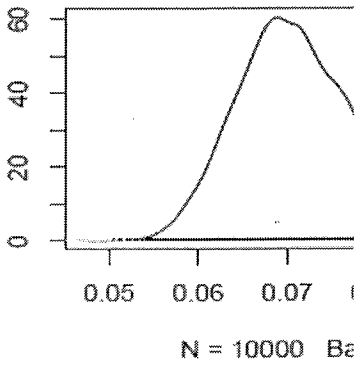




Trace of LI

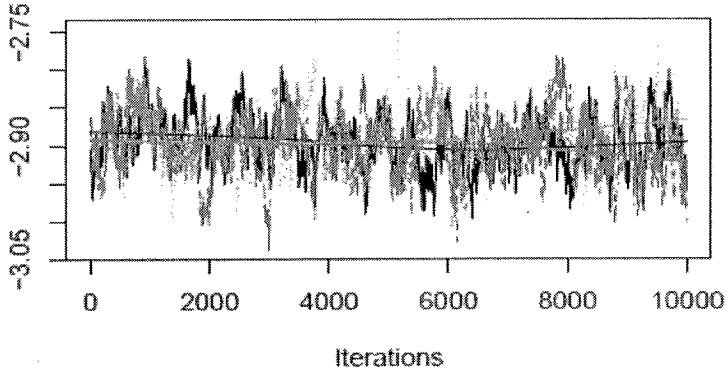
Den

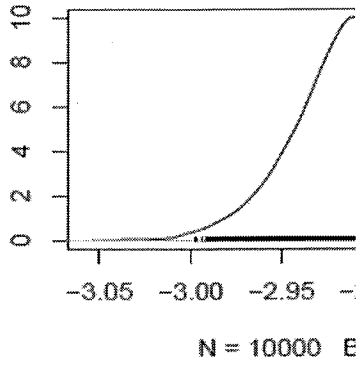




Trace of Vmax

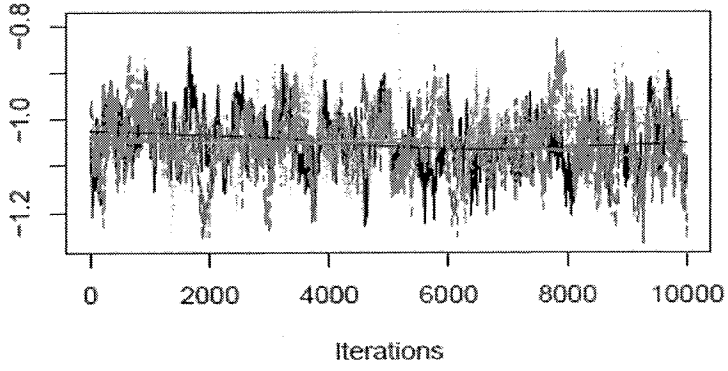
Densit

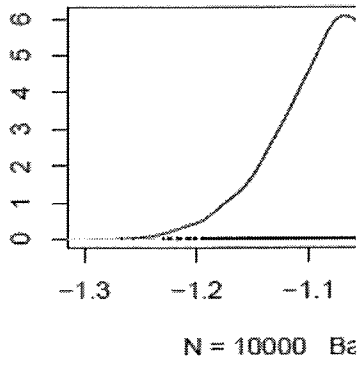




Trace of Km

Dens





```
>              gelman.dia autoburnin=FALSE)
Potential      scale      reduction  factors:

               Point      est.       Upper      C.I.
A0low          1.01       1.02
A0mid          1.02       1.05
A0hgh          1          1.01
LI             1          1
Vmax           1.02       1.06
Km             1.02       1.06

Multivariate   psrf

          1.01
>
>              summary(x1)

Iterations     =          1:10000
Thinning       interval   =          1
Number         of         chains     =          1
Sample         size       per        chain      =

             1 Empirical  mean       and        standard
               plus       standard   error      of

               Mean       SD         Naive      SE
A0low          -2.19232   0.015852   1.59E-04   0.000808
A0mid          -2.8389    0.018107   1.81E-04   0.00176
A0hgh          -3.92455   0.018913   1.89E-04   0.000857
LI             0.07132    0.006924   6.92E-05   0.000128
Vmax           -2.89635   0.035633   3.56E-04   0.004063
Km             -1.04993   0.059886   5.99E-04   0.005867

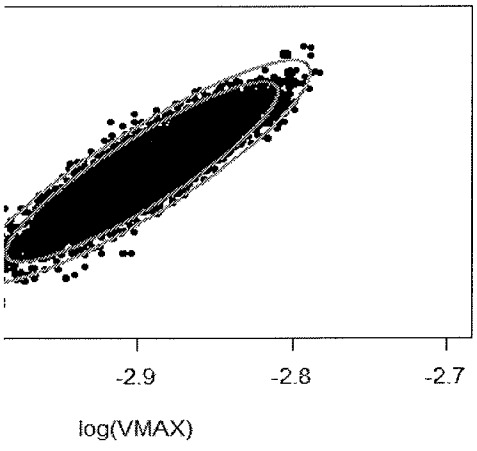
             2 Quantiles  for        each       variable:

               2.50%      25%        50%        75%
A0low          -2.22353   -2.20299   -2.19256   -2.18188
A0mid          -2.87308   -2.85165   -2.83933   -2.82698
A0hgh          -3.96151   -3.93707   -3.92429   -3.9118
LI             0.05933    0.06657    0.07076    0.07569
Vmax           -2.96397   -2.92079   -2.89851   -2.87194
Km             -1.1657    -1.0915    -1.05319   -1.00669

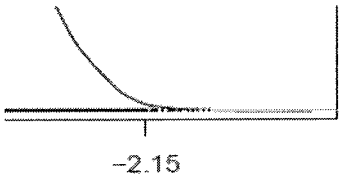
>              summary(x2)

Iterations     =          1:10000
Thinning       interval   =          1
```

Mixed Human Liver

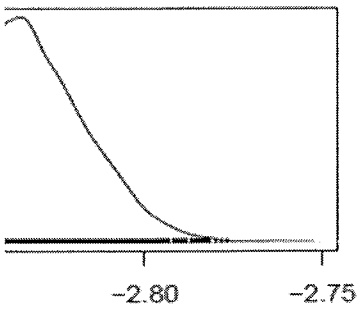


of A0low



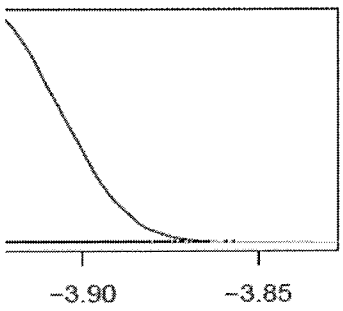
dwidth = 0.00216

of A0mid



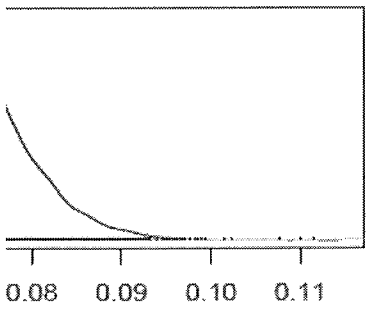
dwidth = 0.00262

of A0hgh



dwidth = 0.002513

sity of LI



andwidth = 0.000922

ty of Vmax

Number of chains = 1
Sample size per chain =

1 Empirical mean and standard
plus standard error of

	Mean	SD	Naive	SE
A0low	-2.19328	0.016581	1.66E-04	0.001185
A0mid	-2.84086	0.020773	2.08E-04	0.002357
A0hgh	-3.92683	0.018807	1.88E-04	0.000818
LI	0.07138	0.006975	6.98E-05	0.000136
Vmax	-2.90091	0.04251	4.25E-04	0.005455
Km	-1.05555	0.071698	7.17E-04	0.008742

2 Quantiles for each variable:

	2.50%	25%	50%	75%
A0low	-2.22631	-2.20397	-2.19344	-2.1823
A0mid	-2.88147	-2.85532	-2.84064	-2.8269
A0hgh	-3.96375	-3.93971	-3.92675	-3.9137
LI	0.05924	0.06657	0.07072	0.0757
Vmax	-2.98513	-2.92965	-2.90049	-2.8719
Km	-1.20086	-1.10378	-1.05501	-1.0072

> summary(x3)

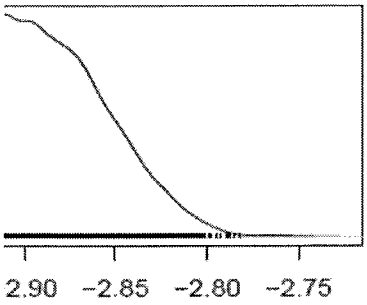
Iterations = 1:10000
Thinning interval = 1
Number of chains = 1
Sample size per chain =

1 Empirical mean and standard
plus standard error of

	Mean	SD	Naive	SE
A0low	-2.19044	0.016032	1.60E-04	0.000841
A0mid	-2.83577	0.018977	1.90E-04	0.001942
A0hgh	-3.92451	0.018085	1.81E-04	0.000794
LI	0.07119	0.006943	6.94E-05	0.000128
Vmax	-2.88915	0.038568	3.86E-04	0.00492
Km	-1.03687	0.06539	6.54E-04	0.00743

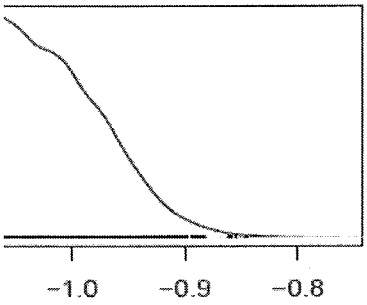
2 Quantiles for each variable:

	2.50%	25%	50%	75%
A0low	-2.2218	-2.20109	-2.19063	-2.17991
A0mid	-2.87203	-2.84939	-2.83582	-2.82276



Bandwidth = 0.0053

ity of Km



andwidth = 0.008941

A0hgh	-3.95991	-3.93657	-3.92467	-3.91274
Ll	0.05907	0.06629	0.07069	0.07557
Vmax	-2.95902	-2.91722	-2.89031	-2.86281
Km	-1.1583	-1.08281	-1.04049	-0.99046

10000

deviation for each variable,
the mean:

Time-serie: SE

1.2%
-5.7%

97.50%
-2.16093
-2.80278
-3.88714
0.08651
-2.82594
-0.93204

10000

deviation for each variable,
the mean:

Time-series SE

97.50%
-2.16052
-2.79926
-3.89062
0.08682
-2.81852
-0.91386

10000

deviation for each variable,
the mean:

Time-series SE

97.50%
-2.15882
-2.79822

-3.88842
0.08615
-2.81374
-0.90831

Mixed Human Lung Microsomes

	Mean	95% CI

not determinable - data were insufficient to estimate in vitro metabolic parameters.

MCMC Mean Parameters

			Liver			Lung	
			Mean	95% CI		Mean	95%
Kg estimated	Male Mouse	Vmax (µmol/hr/mg protein)	0.212	0.161	0.257	0.085	0.069
		Km (µmol/L)	0.689	0.472	0.903	1.194	0.943
	Female Mouse	Vmax (µmol/hr/mg protein)	0.105	0.087	0.127	0.022	0.015
		Km (µmol/L)	0.448	0.302	0.652	2.369	1.555
	Male Rat	Vmax (µmol/hr/mg protein)	0.072	0.066	0.078	ND*	
		Km (µmol/L)	0.417	0.367	0.477		
	Female Rat	Vmax (µmol/hr/mg protein)	0.069	0.056	0.085	0.00408	0.00152
		Km (µmol/L)	0.718	0.544	0.933	2.369	
	Human	Vmax (µmol/hr/mg protein)	0.055	0.052	0.059	7.88E-05	Alternative a
		Km (µmol/L)	0.349	0.312	0.394	3.49E-01	

* ND: not determinable

Female Rat Lung Km was fixed to Female Mouse Km fc

		Kidney	
CI	**Mean**	**95% CI**	
0.105	0.012	0.0088	0.015
1.523	0.647	0.469	0.902
0.031	ND*		
3.549			
	0.0019	0.0014	0.0026
	0.619	0.437	0.885
0.00618	0.0018	0.0013	0.0026
	0.449	0.298	0.687

ıpproach Not Measured

ır the MCMC

Summary In Vivo Parameters			
Species	Parameter	Liver	Lung
Male Mouse	Vmax (mg/hr/BW^0.75)	16.09	0.49
	Km (mg/L)	0.061	0.11
Female Mouse	Vmax (mg/hr/BW^0.75)	7.99	0.12
	Km (mg/L)	0.040	0.21
Male Rat	Vmax (mg/hr/BW^0.75)	7.42	ND*
	Km (mg/L)	0.037	
Female Rat	Vmax (mg/hr/BW^0.75)	6.36	0.03
	Km (mg/L)	0.064	0.21
Human	Vmax (mg/hr/BW^0.75)	14.51	0.0031
	Km (mg/L)	0.031	0.031

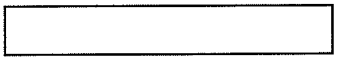
Revised Scaling

Scaling factors for average adults in each species	Parameter	BW (kg)	Liver fractional weight (VLC)
	B6C3F1 Mouse (Female)	0.04	0.0549
	B6C3F1 Mouse (Male)	0.04	0.0549
	F344 Rat (Female)	0.256	0.0366
	F344 Rat (Male)	0.407	0.0366

	Average Human	70	0.0257
	Reference	Brown et al, 1997 (page 415 in text)	Brown et al, 1997 (Tables 4, 5, & 7)

References

1. Brown et al., 1997, Toxicol. Ind. Health, July-Aug;13(4):407-84.
2. Barter et al., Drug Metab Dispos. 2008 Dec;36(12):2405-9. doi:
3. Himmelstein et al., Toxicol Sci. 2004b May;79(1):28-37. Epub 2
4. Houston and Galetin, Curr Drug Metab. 2008 Nov;9(9):940-51.
5. Scotcher et al., Drug Metab Dispos. 2017 May;45(5):556-568. (



Kidney
0.14
0.057
ND*
0.018
0.055
0.015
0.040

* ND: not determinable

Lung fractional weight (VLUC)	Kidney fractional weight (VKC)	Liver mg microsomal protein per g liver (MPPGL)	Lung mg microsomsal protein per g lung (MPPGLU)	Kidney mg microsomal protein per g kidney (MPPGK)
0.0073	0.0167	35	20	18
0.0073	0.0167	35	20	18
0.005	0.0073	40	20	18
0.005	0.0073	40	20	18

0.0076	0.0044	40	20	11
Brown et al, 1997 (Tables 4, 5, & 7)	Brown et al, 1997 (Tables 4, 5, & 7)	Medinsky et al., 1994 for mouse; Medinsky et al., 1994 and Houston and Galetin, 2008 for rat; Barter et al., 2008 2007 for human	Medinsky et al., 1994 for all species	Yoon et al., 2007 for mouse and rat; Scotcher et al., 2017 for human

. Review. PubMed PMID:9249929

: 10.1124/dmd.108.021311. Epub 2008 Sep 5. PubMed PMID: 18775982.

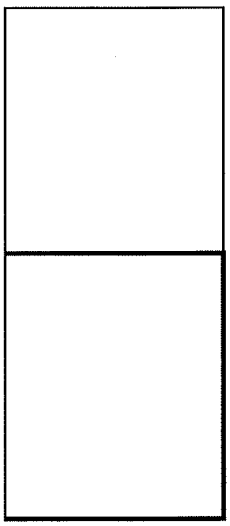
!004 Feb 19. PubMed PMID: 14976335.

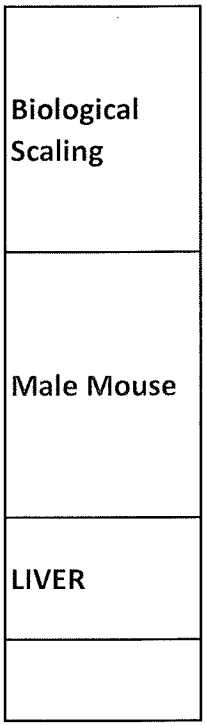
Review. PubMed PMID: 18991591.

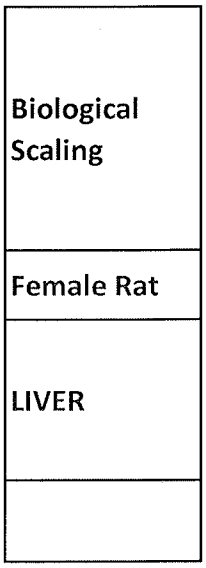
doi: 10.1124/dmd.117.075242. Epub 2017 Mar 7. PubMed PMID: 28270564; PubM

MW
88.53650
g/mol = ug/umol

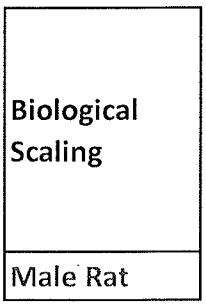
Biological Scaling
Female Mouse
LIVER

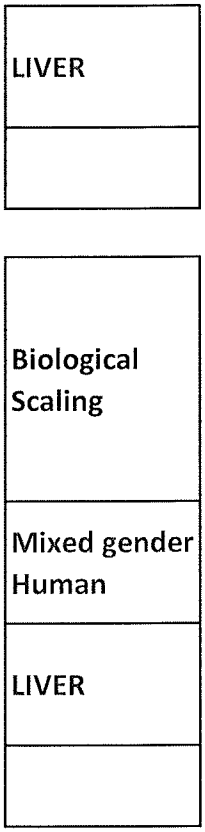






ed Central PMCID: PMC5399648.




LIVER
Biological Scaling
Mixed gender Human
LIVER

Vmaxc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
Values	0.105	35	8.07	90.19	7.99
Scaling & calculation		x MPPGL (mg MP/g liver)	x BW (kg) x VLC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
Unit	umol/h/mg MP	umol/h/g liver	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Vmaxc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
Values	0.212	35	16.26	181.77	16.09
Scaling & calculation		x MPPGL (mg MP/g liver)	x BW (kg) x VLC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
Unit	umol/h/mg MP	umol/h/g liver	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Vmaxc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
Values	0.069	40	25.87	71.89	6.36
Scaling & calculation		x MPPGL (mg MP/g liver)	x BW (kg) x VLC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
Unit	umol/h/mg MP	umol/h/g liver	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Vmaxc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
Values	0.072	40	42.70	83.80	7.42

Scaling & calculation		x MPPGL (mg MP/g liver)	x BW (kg) x VLC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
Unit	umol/h/mg MP	umol/h/g liver	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Vmaxc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
Values	0.055	40	3965.37	163.86	14.51
Scaling & calculation		x MPPGL (mg MP/g liver)	x BW (kg) x VLC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
Unit	umol/h/mg MP	umol/h/g liver	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Biological Scaling	VmaxLUc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor
Female Mouse	Values	0.022	20
LUNG	Scaling & calculation		x MPPGLU (mg MP/g lung)
	Unit	umol/h/mg MP	umol/h/g lung

Biological Scaling	VmaxLUc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor
Male Mouse	Values	0.085	20
LUNG	Scaling & calculation		x MPPGLU (mg MP/g lung)
	Unit	umol/h/mg MP	umol/h/g lung

Biological Scaling	VmaxLUc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor
Female Rat	Values	0.0041	20
LUNG	Scaling & calculation		x MPPGLU (mg MP/g lung)
	Unit	umol/h/mg MP	umol/h/g lung

Alternal Scaling

Biological Scaling	VmaxLUc (mg/h/kg BW^0.75)	In Vitro Value	x Scaling factor
Male Mouse	Values	7.88E-05	20
LUNG	Scaling & calculation		x MPPGLU (mg MP/g lung)
	Unit	umol/h/mg MP	umol/h/g lung

x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
0.126	1.41	0.12
x BW (kg) X VLUC X 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
0.495	5.53	0.49
x BW (kg) X VLUC X 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Biological Scaling	VmaxKIDc (mg/h/kg BW^0.75)	In vitro value (Yang et al. 2012, Table 3)
Male Mouse	Values	0.012
KIDNEY	Scaling & calculation	
	Unit	umol/h/mg MP

x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
0.104	0.29	0.026
x BW (kg) X VLUC X 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Biological Scaling	VmaxKIDc (mg/h/kg BW^0.75)	In Vitro Value
Female Rat	Values	0.0018
KIDNEY	Scaling & calculation	
	Unit	umol/h/mg MP

Biological Scaling	VmaxKIDc (mg/h/kg BW^0.75)	In Vitro Value
Male Rat	Values	0.0019

KIDNEY	Scaling & calculation	
	Unit	umol/h/mg MP

x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
8.38E-01	3.46E-02	3.07E-03
x BW (kg) X VLUC X 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
18	0.139	1.56	0.14
x MPPGK (mg MP/g kidney)	x BW (kg) x VKC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h/g kidney	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
18	0.062	0.17	0.015
x MPPGK (mg MP/g kidney)	x BW (kg) x VKC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h/g kidney	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

x Scaling factor	x Tissue weight	/BW^0.75	x MW = In vivo parameter (unit in PBPK model)
18	0.102	0.20	0.018

x MPPGK (mg MP/g kidney)	x BW (kg) x VKC x 1000 (g/kg)	/BW^0.75	x MW (ug/umol)/1000 (ug/mg)
umol/h/g kidney	umol/h	umol/h/kg BW^0.75	mg/h/kg BW^0.75

Uses IVIVE scaling approach from the Yoon report. (human lung Vmax calculated using approach from methyl

Dose metrics using parameters derived with MCMC

TSTOP **336 hrs** Amt. Metab. = mg/day/g tissue

Exposure: **6 hr/day 5 days/week**

Revised parameters from MCMC of In Vitro data with flux limitation included

Female Mouse Initial Parms				
	Amt. Metab.	Amt. Metab.	Amt. Metab.	**Concentration**
ppm	Liver	Lung	Kidney	**Specific** BW (kg)
12.8	1.16	0.85	-	0.040
32	2.96	1.29	-	0.040
80	7.66	1.69	-	0.036

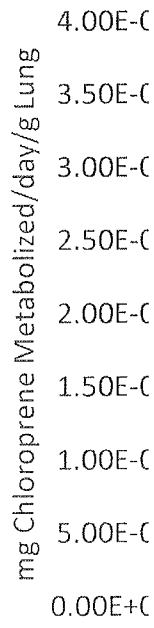
Amt. Metab. = mg/day/g tissue

Human Initial Parms			
	Amt. Metab.	Amt. Metab.	Amt. Metab.
ppm	Liver	Lung	Kidney
12.8	0.30	0.0074	-
32	0.74	0.011	-
80	1.85	0.015	-

Amt. Metab. = mg/day/g tissue

Human Continuous Exposure (24 hr/day)

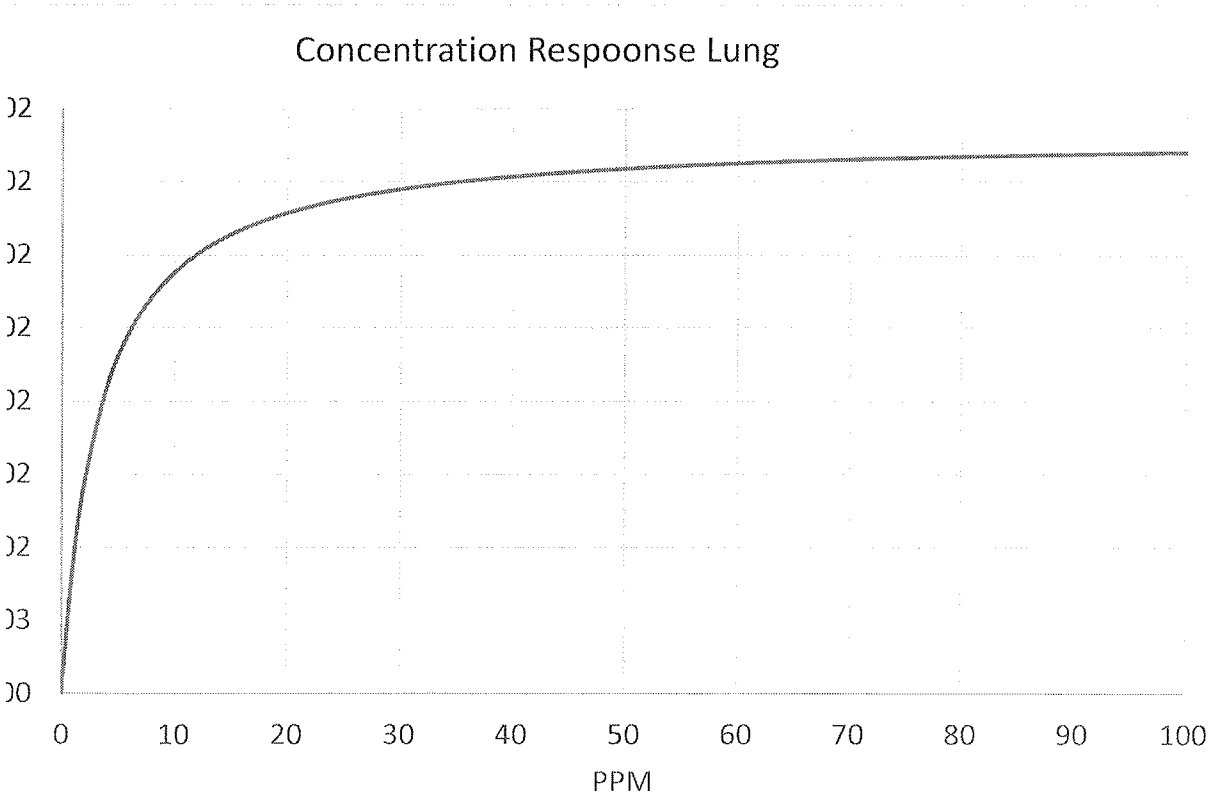
Human Parms Continuous Exposure			
	Amt. Metab.	Amt. Metab.	Amt. Metab.
PPM	Liver	Lung	Kidney
2.80E-04	**3.56E-05**	**3.24E-06**	-
1.00E-03	1.27E-04	1.16E-05	-
1.00E-02	1.27E-03	1.15E-04	-
1.00E-01	1.27E-02	1.12E-03	-
1.00E+00	1.28E-01	8.91E-03	-
2.00E+00	2.56E-01	1.45E-02	-
4.00E+00	5.13E-01	2.10E-02	-
6.00E+00	7.71E-01	2.47E-02	-
8.00E+00	1.03E+00	2.71E-02	-
1.00E+01	1.29E+00	2.88E-02	-
1.20E+01	1.54E+00	3.01E-02	-
1.40E+01	1.80E+00	3.10E-02	-
1.60E+01	2.06E+00	3.18E-02	-
1.80E+01	2.32E+00	3.24E-02	-
2.00E+01	2.58E+00	3.29E-02	-
2.20E+01	2.83E+00	3.33E-02	-
2.40E+01	3.09E+00	3.37E-02	-



2.60E+01	3.35E+00	3.40E-02	-
2.80E+01	3.61E+00	3.43E-02	-
3.00E+01	3.87E+00	3.45E-02	-
3.20E+01	4.12E+00	3.47E-02	-
3.40E+01	4.38E+00	3.49E-02	-
3.60E+01	4.64E+00	3.51E-02	-
3.80E+01	4.90E+00	3.52E-02	-
4.00E+01	5.16E+00	3.54E-02	-
4.20E+01	5.41E+00	3.55E-02	-
4.40E+01	5.67E+00	3.56E-02	-
4.60E+01	5.93E+00	3.57E-02	-
4.80E+01	6.19E+00	3.58E-02	-
5.00E+01	6.45E+00	3.59E-02	-
5.20E+01	6.70E+00	3.60E-02	-
5.40E+01	6.96E+00	3.61E-02	-
5.60E+01	7.22E+00	3.61E-02	-
5.80E+01	7.48E+00	3.62E-02	-
6.00E+01	7.73E+00	3.63E-02	-
6.20E+01	7.99E+00	3.63E-02	-
6.40E+01	8.25E+00	3.64E-02	-
6.60E+01	8.51E+00	3.64E-02	-
6.80E+01	8.76E+00	3.65E-02	-
7.00E+01	9.02E+00	3.65E-02	-
7.20E+01	9.28E+00	3.66E-02	-
7.40E+01	9.54E+00	3.66E-02	-
7.60E+01	9.80E+00	3.67E-02	-
7.80E+01	1.01E+01	3.67E-02	-
8.00E+01	1.03E+01	3.67E-02	-
8.20E+01	1.06E+01	3.68E-02	-
8.40E+01	1.08E+01	3.68E-02	-
8.60E+01	1.11E+01	3.68E-02	-
8.80E+01	1.13E+01	3.69E-02	-
9.00E+01	1.16E+01	3.69E-02	-
9.20E+01	1.19E+01	3.69E-02	-
9.40E+01	1.21E+01	3.70E-02	-
9.60E+01	1.24E+01	3.70E-02	-
9.80E+01	1.26E+01	3.70E-02	-
1.00E+02	1.29E+01	3.70E-02	-

ene chloride - scaling liver in vitro vmax to lung based on ratio of ethoxycoumarin metabolism in lung to live

: estimate of Kgl: 0.22 L/hr



Intended for

Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

SUPPLEMENTAL MATERIALS E
MODEL FILES

Contents

In Vitro Model Files

Invitro.csl (acslX model code)

PROGRAM: INVITRO.CSL

```
!MODIFIED FROM Yang et al. 2012 in vitro model
!MODEL TO SIMULATE BETA CHLOROPRENE UPTAKE
!AND METABOLISM IN A TWO-COMPARTMENT
!VIAL SYSTEM CONTAINING MICROSOMES
!Includes flux of chloroprene between air and media


VARIABLE TIME

INITIAL

CONSTANT VMAX1=0.         !MAX RATE OF MET. (uMOL/HR/mg protein)
CONSTANT KM1=0.1          !MICHAELIS CONSTANT (umol/L)
CONSTANT RLOSS = 0.001424   !Background loss rate (L/hr)

CONSTANT P1=0.69          !MEDIA/AIR PARTITION for CD

CONSTANT A10=0.           !INITIAL AMOUNT IN VIAL (uMOL)

CONSTANT VVIAL=0.01165     !VOLUME OF VIAL (L); Vial volume= 11.65 ml
CONSTANT VMED=0.001        !VOLUME OF MEDIA (L); Liquid voume
 VAIR=VVIAL-VMED                          !HEADSPACE
CONSTANT PROT = 1.0        !AMOUNT OF PROTEIN (mg)

CONSTANT TF=0             !TIME OF FIRST SAMPLE (hr); kept same
CONSTANT TI=0.2           !INTERVAL BETWEEN SAMPLES (hr)kept same
CONSTANT VINJ=0.0002       !INJECTION VOLUME (L); based on Matt email

CONSTANT KG1 = 0.11        !L/hr
 KL = KG1/0.69     !KL is set to match media:gas PC

!Initial Conditions
 TV = VAIR+VMED
 AA10 = A10*(VAIR/(VAIR+P1*VMED))                    !amount initially in vial (umol)
 CA10 = AA10/VAIR                !initial concentration in air (umol/L)
 AM10 = CA10*P1*VMED              !amount initially in media
 CM10 = AM10/VMED                          !initial concentration in media (umol/L)

 A1I=0.  !initialize injection volume loss from repeated sampling of vial
```

```
!TIMING COMMANDS
CONSTANT TSTOP=1        !LENGTH OF EXPOSURE (HOURS)
!CONSTANT POINTS=100.    !NO. OF POINTS IN PLOT

TS=TF
SCHEDULE step .AT. TF

END                !END INITIAL

DYNAMIC
CINTERVAL CINT=0.01     !COMMUNICATION INTERVAL
MAXTERVAL MAXT = 0.001
ALGORITHM IALG=2

 DERIVATIVE

 TERMT(TIME.GE.TSTOP)

!CD KINETICS (umoles/hr)
 RA1M = ((VMAX1*CM1)/(KM1+CM1))*PROT  !rate of metabolism saturable
 RRLoss = RLOSS*CA1              !rate of loss from vial
 A1M = INTEG(RA1M,0.)             !Amount metabolized saturable
 ARLOSS = INTEG(RRLoss,0.)         !Rate of loss to system
 RAG_L = KG1*CA1                 !Rate of amount leaving gas to liquid
 RAL_G = KL*CM1                  !Rate of amount leaving liquid to gas

 RAA1 = RAL_G - RAG_L - RRLoss       !Rate of change in vial air
  AA1 = integ(RAA1, AA10) - A1I
  CA1 = max(AA1/VAIR, 1.0e-7)

 RAM1 = RAG_L - RAL_G - RA1M    !Rate of change in vial media
  AM1 = integ(RAM1, AM10)
  CM1 = AM1/VMED

   A1 = CA1*VAIR+CM1*VMED             !Total amount in vial


!MASS BALANCE
 CHECK1 = A10 - (AM1+AA1+A1M+A1I+ARLOSS)

DISCRETE step
PROCEDURAL
!Routine for sample loss
   A1I= A1I+CA1*VINJ
   SCHEDULE step .AT. TS+TI
   TS=TS+TI
END                !END PROCEDURAL
END                !END DISCRETE
```

```
END             !END DERIVATIVE
END             !END DYNAMIC
END             !END PROGRAM
```

MCMC Run Scripts (m-files that run the analysis)

Female Mouse Liver

FMouse_LiverMCMC.m

```
% Simulates the MCMC for Female mouse liver

load @file=invitro.dll @format=model

prepare @clear
prepare @all


WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
CINT = 0.2 ;
MAXT = 0.01;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.01165 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR=VVIAL-VMED ;

%Simulation specific protein concentration
PROT=1.0 ;  %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69 ;        %Liquid:air PC



seedrnd(45526)

use ControlData.m

global _ca1
global _time
```

```
global data
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

B6 female mouse liver incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055
%Time  540 ppm        270 ppm        150 ppm        50 ppm 10 ppm
%Headspace Conc. (mg/ml)
B6FmiceLiver = [0      21.378 9.863   5.465   1.867   0.422
0.2     16.789 6.07    2.492  0.411   0.052
0.4     13.771 3.834   1.082  0.081   0.013
0.6     10.624 2.491   0.488  0.018   NaN
0.8     9.99   1.715   0.229  0.007   NaN
1       8.902  1.185   0.131  NaN     NaN];

b = size(B6FmiceLiver);

data = B6FmiceLiver(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = B6FmiceLiver(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);


function preds = getpreds(Vmax, Km, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
```

```
 setmdl("A10", A10);
 setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
     preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_sat.m"

chains = runmcmc();


save @file=fmouseliver1.dat @format=ascii @separator=tab chains
```

Female Mouse Lung

FMouse_LungMCMC.m

```
% Female Mouse Lung In Vitro MCMC simulation file


load @file=invitro.dll @format=model

prepare @clear
prepare @all



WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
 CINT = 0.2 ;
 MAXT = 0.01;
TF=0.0; TI=0.2; %Sample Collection start; interval

%Volumes (L) - simulation specific
VVIAL = 0.01165 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR=VVIAL-VMED ;

%Simulation specific protein concentration
PROT=1.0 ;   %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69 ;        %Liquid:air PC



seedrnd(45526)

use ControlData.m

global _ca1
global _time
global data
```

```
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

B6 female mouse lung incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055

%Time  540 ppm        270 ppm        150 ppm        50 ppm 10 ppm 1 ppm
%Headspace Conc. (mg/ml)
B6FmiceLung= [0        20.506 12.522 5.97    1.742  0.434  0.044
0.2     18.693 11.105 5.1     1.407  0.346  0.036
0.4     18.014 10.556 4.744   1.24   0.297  0.03
0.6     17.482 10.119 4.469   1.121  0.272  0.027
0.8     16.859 9.644  4.223   1.044  0.25   0.024
1       16.466 9.284  4.006   0.983  0.229  0.023];

b = size(B6FmiceLung);

data = B6FmiceLung(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = B6FmiceLung(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
  setmdl("A10", A10);
```

```
 setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_sat.m"

chains = runmcmc();


save @file=fmouselung1.dat @format=ascii @separator=tab chains
```

Female Rat Liver

FRatLiverMCMC.m

```
% Female Rat Liver In Vitro MCMC simulation file

load @file=invitro.dll @format=model

prepare @clear
prepare @all


WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
CINT = 0.2 ;
MAXT = 0.01;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.01165 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR=VVIAL-VMED ;

%Simulation specific protein concentration
PROT=1.0 ;   %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69 ;        %Liquid:air PC


use ControlData.m

seedrnd(45526)


global _ca1
global _time
global data
```

```
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

CDFS female rat liver incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055
%Female Rat Liver
%CDF Liver Summary
%Time  270 ppm        150 ppm        50 ppm 10 ppm 1 ppm
%Headspace Conc. (mg/ml)
FratFLiver=[0    11.007 6.243   1.935   0.465   0.052
0.2      9.091   4.46   0.844   0.141   0.015
0.4      7.661   3.274  0.36    0.048   0.006
0.6      6.621   2.479  0.188   0.022   0.003
0.8      5.831   1.958  0.103   0.011   0.002
1        5.202   1.607  0.066   0.007   NaN];


b = size(FratFLiver);

data = FratFLiver(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = FratFLiver(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);


function preds = getpreds(Vmax, Km, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);
```

```
 setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
 setmdl("KM1", exp(Km));
 setmdl("A10", A10);
 setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_sat.m"

chains = runmcmc();


save @file=fratliver1redo.dat @format=ascii @separator=tab chains
```

Female Rat Lung

FRatLungMCMCvmax.m

```
% Female Rat Lung In Vitro MCMC simulation file
% Km set to posterior median of the female mouse lung
% Allows estimation of the in vitro Vmax for female rat lung


load @file=invitro.dll @format=model

prepare @clear
prepare @all

WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
CINT = 0.2 ;
MAXT = 0.01;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.01165 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR=VVIAL-VMED ;

%Simulation specific protein concentration
PROT=1.0 ;  %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein

P1 = 0.69 ;        %Liquid:air PC

KM1 = 2.369 ;        %umol/L

use ControlData.m

seedrnd(45526)
```

```
global _ca1
global _time
global data
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

CDF female rat lung incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055

%CDF Lung Summary
%Time  150 ppm        50 ppm 10 ppm
%Headspace Conc. (mg/ml)
FratFLung=[0    11.438  5.107   2.051
0.2     10.93   4.611   1.914
0.4     10.256  4.452   1.829
0.6     9.786   4.156   1.755
0.8     9.44    4.131   1.682
1       8.88    3.774   1.641 ];


b = size(FratFLung);

data = FratFLung(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = FratFLung(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
```

```
 lossR = ControlData(tmp);

 setmdl("VMAX1", exp(Vmax)); % reset model parameter as global variables
 setmdl("A10", A10);
 setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
     preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_satfrlng.m"

chains = runmcmc();


save @file=fratlung1.dat @format=ascii @separator=tab chains
```

Female Rat Kidney

FRatLungMCMCvmax.m

```
% Female Rat Kidney In Vitro MCMC simulation file


load @file=invitro.dll @format=model

prepare @clear
prepare @all

WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
 CINT = 0.2 ;
 MAXT = 0.001;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.01163 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR = VVIAL-VMED ;

%Simulation specific protein concentration
PROT = 3.0 ;  %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69 ;        %Liquid:air PC



seedrnd(45526)

use ControlData.m

global _ca1
global _time
global data
global tindex
```

```
global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData


CDF female rat kidney incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055

%Time  540 ppm 270 ppm      150 ppm       50 ppm 10 ppm 2 ppm
%Headspace Conc. (mg/ml)
FRatKid = [0.    22.705 11.003 5.381   1.985  0.436  0.090  ;
           0.2   21.864 10.443 5.102   1.785  0.366  0.078  ;
           0.4   21.230 10.065 4.888   1.636  0.311  0.056  ;
           0.6   20.674 9.656  4.624   1.497  0.266  0.046  ;
           0.8   19.735 9.259  4.387   1.393  0.237  0.044  ;
           1.    18.879 8.792  4.216   1.297  0.222  0.043  ];


b = size(FRatKid);

data = FRatKid(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = FRatKid(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
```

```
  setmdl("A10", A10);
  setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_sat.m"

chains = runmcmc();


save @file=fratkidney1.dat @format=ascii @separator=tab chains
```

Male Mouse Liver

MmouseLiverMCMCa0.m

```
% Male Mouse Liver In Vitro MCMC simulation file
% Includes estimation of the initial amount of CP in vial
% due to multiple vials making up a single concentration


load @file=invitro.dll @format=model

prepare @clear
prepare @all

WESITG=0;
WEDITG =0;

CINT = 0.2 ;
MAXT = 0.001 ;
TSTOP = 1.0 ;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.0119573;
VMED = 0.001;
VINJ = 0.0003858 ;
VAIR = VVIAL-VMED;

%Simulation specific protein concentration
PROT=1.0 ;   %Protein mg/ml

%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;          %umol/L
P1 = 0.69;        %Liquid:air PC



seedrnd(45526)

use ControlData.m

global _ca1
global _time
global data
```

```
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

B6 male mouse liver incubation data
%Data reported in Himmelstein et al. TOXICOLOGICAL SCIENCES 79, 18–27 (2004)

% B6 male mouse liver
%[Time 529 ppm        264 ppm        132 ppm        50 ppm 10 ppm]
%Headspace Conc. (mg/ml)
B6MmiceLiver = [0      21.202  10.747  5.282   1.987   0.465
0.025   20.770  NaN    5.069   1.869   0.395
0.05    19.932  9.898  4.434   1.530   0.278
0.1     NaN     8.394  2.848   0.890   NaN
0.15    17.435  7.343  2.342   NaN     NaN
0.2     15.409  5.619  1.444   0.315   0.052
0.225   14.788  NaN    1.302   0.271   0.040
0.25    14.338  4.668  1.082   0.199   0.021
0.3     NaN     3.684  0.495   0.126   NaN
0.35    12.542  3.125  0.480   NaN     NaN
0.4     10.747  2.179  0.270   0.036   0.005
0.425   NaN     NaN    0.241   NaN     NaN
0.45    NaN     1.685  0.190   0.020   NaN
0.5     NaN     1.224  0.062   NaN     NaN
0.55    8.481   1.049  0.079   NaN     NaN
0.6     6.975   0.640  0.041   0.005   NaN
0.625   NaN     NaN    0.038   NaN     NaN
0.65    NaN     0.460  0.029   NaN     NaN
0.7     NaN     0.318  0.0077  NaN     NaN
0.75    5.561   0.270  0.013   NaN     NaN
0.8     4.299   0.159  0.0069  NaN     NaN
0.825   NaN     NaN    0.0059  NaN     NaN
0.85    NaN     0.109  0.0050  NaN     NaN
0.9     NaN     0.073  NaN     NaN     NaN
0.95    3.393   0.066  NaN     NaN     NaN];


b = size(B6MmiceLiver);

data = B6MmiceLiver(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
```

```
firstD = [1]
lastD  = [b(:,2)-1]
tindex = B6MmiceLiver(:, 1);

%AA=data(1,:)*(VAIR+P1*VMED);
%CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A0)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
  setmdl("A10", exp(A0));
  setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\mmouselivinvitroa0.m"

chains = runmcmc();

save @file=mmouseliver1a0.dat @format=ascii @separator=tab chains
```

Male Mouse Lung

MmouseLungMCMCa0.m

```
% Male Mouse Lung In Vitro MCMC simulation file
% Includes estimation of the initial amount of CP in vial
% due to multiple vials making up a single concentration


load @file=invitro.dll @format=model

prepare @clear
prepare @all



WESITG=0;
WEDITG =0;

 TSTOP = 1.0 ;
 CINT = 0.2 ;
 MAXT = 0.001;
 TF=0.0; TI=0.2; %Sample Collection start; interval

%Volumes (L) - simulation specific
VVIAL = 0.0119573;
VMED = 0.001;
VINJ = 0.0003858 ;
VAIR = VVIAL-VMED;

%Simulation specific protein concentration
PROT=1.0 ;  %Protein mg/ml

%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;          %umol/L
P1 = 0.69;         %Liquid:air PC


seedrnd(45526)

use ControlData.m

global _ca1
```

```
global _time
global data
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

B6 male mouse lung incubation data
%Data reported in Himmelstein et al. TOXICOLOGICAL SCIENCES 79, 18–27 (2004)

%time  10012.1 ppm   1000 ppm      250 ppm       50ppm  10 ppm
%Headspace Conc. (mg/ml)
B6MmiceLung = [0      90.6864       36.9563       8.5541  1.9319  0.3288
0.025  92.9729       34.7636       8.5569  1.6268  0.3629
0.05   NaN    32.7002       NaN     1.7520  0.2736
0.1    NaN    31.5351       7.1220  1.2424  NaN
0.15   NaN    NaN    NaN    1.1129  NaN
0.2    86.5465       29.6910       5.3084  0.8496  0.1464
0.225  87.6422       28.9789       5.4422  0.7895  0.1311
0.25   NaN    27.5967       NaN     0.7335  0.1104
0.3    NaN    27.2762       4.5859  0.6029  NaN
0.35   NaN    NaN    NaN    0.5335  NaN
0.4    NaN    25.3669       3.7714  0.4226  0.0652
0.425  83.6431       26.0065       3.9627  0.3831  0.0598
0.45   NaN    24.1644       NaN     0.3516  0.0523
0.5    NaN    NaN    3.1788  0.3228  NaN
0.55   NaN    NaN    NaN    0.2821  NaN
0.6    NaN    23.9130       2.9203  0.2348  0.0309
0.625  79.8247       23.9206       3.1837  0.1977  0.0290
0.65   NaN    21.9544       NaN     0.1906  0.0270
0.7    NaN    NaN    NaN    0.1861  NaN
0.8    NaN    22.2670       2.3174  0.1337  0.0163
0.825  75.2488       NaN     2.5895  0.1154  0.0148
0.85   NaN    20.9520       NaN     0.1072  0.0148
0.9    NaN    NaN    2.1820  0.1155  NaN];


b = size(B6MmiceLung);

data = B6MmiceLung(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
```

```
lastD  = [b(:,2)-1]
tindex = B6MmiceLung(:, 1);

%AA=data(1,:)*(VAIR+P1*VMED);
%CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A0)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
  setmdl("A10", exp(A0));
  setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
     preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\mmouselnginvitroa0.m"

chains = runmcmc();

save @file=mmouselung1a0.dat @format=ascii @separator=tab chains
```

Male Mouse Kidney

MMouseKidneyMCMC.m

```
% Male Mouse Kidney In Vitro MCMC simulation file


load @file=invitro.dll @format=model

prepare @clear
prepare @all



WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
 CINT = 0.2 ;
 MAXT = 0.01;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.01163 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR = VVIAL-VMED ;

%Simulation specific protein concentration
PROT = 2.0 ;  %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69 ;        %Liquid:air PC


seedrnd(45526)

use ControlData.m

global _ca1
global _time
global data
```

```
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

B6 male mouse kidney incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055

% B6C3F1 Male mouse kidney CD metabolism
% Time 540 ppm        270 ppm        150 ppm        50 ppm 10 ppm 2 ppm
% protein  = 2mg/ml
%Headspace Conc. (mg/ml)
MMiceKid = [   0.      19.674  10.591  5.341   1.764   0.358   0.063
               0.2     17.887  10.001  4.742   1.303   0.207   0.038
               0.4     16.760  9.268   4.202   1.030   0.123   0.025
               0.6     15.391  8.640   3.853   0.822   0.077   0.018
               0.8     14.566  8.100   3.505   0.692   0.055   0.014
               1.      13.509  7.809   3.327   0.617   0.043   0.011   ];


b = size(MMiceKid);

data = MMiceKid(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = MMiceKid(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);
```

```
 setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
 setmdl("KM1", exp(Km));
 setmdl("A10", A10);
 setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_sat.m"

chains = runmcmc();


save @file=mmousekidney1.dat @format=ascii @separator=tab chains
```

Male Rat Liver

MratLiverMCMCa0.m

```
% Male Rat Liver In Vitro MCMC simulation file
% Includes estimation of the initial amount of CP in vial
% due to multiple vials making up a single concentration


load @file=invitro.dll @format=model

prepare @clear
prepare @all


WESITG=0;
WEDITG =0;

CINT = 0.2 ;
MAXT = 0.001 ;
TSTOP = 1.0 ;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.0119573;
VMED = 0.001;
VINJ = 0.0003858 ;
VAIR = VVIAL-VMED;

%Simulation specific protein concentration
PROT=1.0 ;  %Protein mg/ml

%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;          %umol/L
P1 = 0.69;        %Liquid:air PC


seedrnd(45526)

use ControlData.m

global _ca1
global _time
```

```
global data
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

CDF male rat liver incubation data
%Data reported in Himmelstein et al. TOXICOLOGICAL SCIENCES 79, 18–27 (2004)


%male rat liver data
%[Time 264 ppm 132 ppm 50 ppm]
%Headspace Conc. (mg/ml)
MratMLiver=[0 9.824   4.6755  2.0125
0.025   9.454   4.503   2.18
0.05    8.939   4.318   1.634
0.1     9.767   3.918   1.354
0.15    9.603   3.708   1.113
0.2     7.856   3.217   0.893
0.225   7.581   3.007   0.931
0.25    7.02    2.885   0.706
0.3     7.925   2.559   0.545
0.35    7.679   2.478   0.419
0.4     6.097   2.0245  0.291
0.425   5.974   1.841   0.308
0.45    5.568   1.786   0.237
0.5     6.201   1.547   0.175
0.55    NaN     1.558   0.125
0.6     4.637   1.1375  0.077
0.625   4.584   1.01    0.082
0.65    4.231   0.995   0.067
0.7     NaN     0.837   0.048
0.75    NaN     0.708   0.034
0.8     3.482   0.5715  0.0195
0.825   3.428   0.483   0.02
0.85    3.18    0.489   0.018
0.9     NaN     0.397   NaN
0.95    NaN     NaN     0.009];


b = size(MratMLiver);

data = MratMLiver(:,2:b(:,2));
```

```
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = MratMLiver(:, 1);

%AA=data(1,:)*(VAIR+P1*VMED);
%CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A0)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
  setmdl("A10", exp(A0));
  setmdl("RLOSS", exp(lossR));


 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
     preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\mratlivinvitroa0.m"

chains = runmcmc();
```

save @file=mratliver1a0.dat @format=ascii @separator=tab chains

Male Rat Kidney

MRatKidneyMCMC.m

```
% Male Rat Kidney In Vitro MCMC simulation file


load @file=invitro.dll @format=model

prepare @clear
prepare @all

WESITG=0;
WEDITG =0;

TSTOP = 1.0 ;
 CINT = 0.2 ;
 MAXT = 0.01;
TF=0.0; TI=0.2; %Sample Collection start; interval


%Volumes (L) - simulation specific
VVIAL = 0.01163 ;
VMED = 0.001 ;
VINJ = 0.0002 ;
VAIR = VVIAL-VMED ;

%Simulation specific protein concentration
PROT = 3.0 ;   %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
KG1 = 0.22 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69 ;        %Liquid:air PC



seedrnd(45526)

use ControlData.m

global _ca1
global _time
global data
global tindex
```

```
global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData



CDF male rat kidney incubation data
%Data reported in Yang et al. Toxicology in Vitro 26 (2012) 1047–1055

% CDF male rat kidney CD metabolism ; (3 mg protein/mL)
% w/NADP+     Time    540 ppm          270 ppm          150 ppm          50 ppm 10 ppm 2 ppm
%Headspace Conc. (mg/ml)
MRatKid = [0.   22.510  12.125  5.405   1.810   0.418   0.096
                0.2     21.626  11.497  5.084   1.631   0.350   0.080
                0.4     21.101  11.072  4.773   1.486   0.298   0.068
                0.6     20.388  10.582  4.515   1.380   0.265   0.059
                0.8     19.658  10.032  4.305   1.284   0.247   0.054
                1.      18.978  9.587   4.058   1.206   0.228   0.051];


b = size(MRatKid);

data = MRatKid(:,2:b(:,2));
firstT = [1]
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = MRatKid(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);


function preds = getpreds(Vmax, Km, A10)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate from ControlData matrix
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);
```

```
  setmdl("VMAX1", exp(Vmax));
  setmdl("KM1", exp(Km));
  setmdl("A10", A10);
  setmdl("RLOSS", exp(lossR));



 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitromcmc_sat.m"

chains = runmcmc();


save @file=mratkidney1.dat @format=ascii @separator=tab chains
```

Mixed Human Liver

HumanLiverMCMCa0.m

```
% Human Liver In Vitro MCMC simulation file
% Includes estimation of the initial amount of CP in vial
% due to multiple vials making up a single concentration



set @format=shorte
%load @file=invitro.dll @format=model

%prepare @clear
%prepare @all


WESITG = 0;
WEDITG = 0;

CINT = 0.2 ;
MAXT = 0.001 ;
TSTOP = 1.0 ;
TF=0.0; TI=0.2; %Sample Collection start; interval

%Volumes (L) - simulation specific
VVIAL = 0.0119573;
VMED = 0.001;
VINJ = 0.0004 ;
VAIR=VVIAL-VMED;

%Simulation specific protein concentration
PROT=1.0 ;  %Protein mg/ml


%Initial values
RLOSS = 0.001424 ;  %L/hr
%KG1 = 0.44 ;       %L/hr
VMAX1 = 0.0 ;      %umol/hr/mg protein
KM1 = 1.0 ;        %umol/L
P1 = 0.69;         %Liquid:air PC



seedrnd(45526)

use ControlData.m
```

```
global _ca1
global _time
global data
global tindex


global CCC
global firstT
global lastT
global firstD
global lastD
global ControlData

Mixed human liver incubation data
%Data reported in Himmelstein et al. TOXICOLOGICAL SCIENCES 79, 18–27 (2004)

%Time  264 ppm        132 ppm        50 ppm
%Headspace Conc. (mg/ml)
humanliver = [0 9.443   4.749   1.663
0.025   9.345   4.638   1.683
0.05    8.807   4.644   1.586
0.1     9.093   4.797   1.358
0.15    8.551   4.44    1.175
0.2     7.941   3.617   0.941
0.225   7.787   3.548   0.881
0.25    7.308   3.506   0.804
0.3     7.808   3.477   0.666
0.35    7.042   3.361   0.556
0.4     6.606   2.533   0.39
0.425   6.45    2.534   0.359
0.45    NaN     2.458   0.319
0.5     NaN     2.421   NaN
0.55    5.768   2.463   NaN
0.6     5.444   1.623   0.133
0.625   NaN     1.683   0.117
0.65    NaN     1.734   0.102
0.7     NaN     1.525   NaN
0.75    4.624   1.439   NaN
0.8     4.387   0.92    0.04
0.825   NaN     1.013   0.035
0.85    NaN     0.97    0.031
0.9     NaN     0.867   NaN
0.95    3.632   0.822   NaN];


b = size(humanliver);
data = humanliver(:,2:b(:,2));
firstT = [1]
```

```
lastT  = [b(:,1)]
firstD = [1]
lastD  = [b(:,2)-1]
tindex = humanliver(:, 1);

AA=data(1,:)*(VAIR+P1*VMED);
CCC = [AA];
data = log(data);

function preds = getpreds(Vmax, Km, A0)
  global _ca1
  global _time
  global tindex
  global ControlData

  % draw back ground loss rate
  tmp = ceil(rand*500);
  lossR = ControlData(tmp);

  setmdl("VMAX1", exp(Vmax));  % reset model parameter as global variables
  setmdl("KM1", exp(Km));
  setmdl("A10", exp(A0));
  setmdl("RLOSS", exp(lossR));

 data @clear
 data("SAMPTIMES", ["T"], tindex);

 start @nocallback

 preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
   idx = find(_time == tindex(i));
   if(idx ~= [])
    preds(i) = max(0.0, _ca1(idx));
   end
end

preds = log(preds);

end

use ".\MCMCscripts\invitrohumlivsata0.m"

chains = runmcmc();

save @format=ascii @file=humanlivera0.dat @separator=tab chains
```

MCMC Control Scripts (establish priors and likelihood, called in MCMC Run Scripts)

invitromcmc_sat.m (used for female mouse liver and lung, female rat liver and kidney, male mouse and rat kidney)

```
function tchains = runmcmc(pchains = [])
  % Driver code for MCMC analysis
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds

  numParms = 3
  numChains = 3
  numIts = 50000
  funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors", "mcEvalProposal",
"mcSampleProposal"]
  updateMode = 4
  chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
  save @format=ascii @file=mcmc_results.dat chains
  tchains = chains([1:2:50000],:);
end

function mcInit()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  global OpMcmcPriorBounds
  OpMcmcPriorBounds = [...
  0.01, 100
  -10, 5
  -10, 5
```

```
    ];
    global OpMcmcAdaptive
    OpMcmcAdaptive = 1;
    global OpMcmcDelayedRejection
    OpMcmcDelayedRejection = 0;
    global OpMcmcAdaptPeriod
    OpMcmcAdaptPeriod = 30;
    global OpMcmcAdaptCovarScale
    OpMcmcAdaptCovarScale = 1;
    global OpMcmcLoggingPeriod
    OpMcmcLoggingPeriod = 50;
    global OpMcmcAdaptLowerThresh
    OpMcmcAdaptLowerThresh = 0.25;
    global OpMcmcAdaptUpperThresh
    OpMcmcAdaptUpperThresh = 0.45;
    global OpMcmcAdaptLowerThreshDR
    OpMcmcAdaptLowerThreshDR = 0.45;
    global OpMcmcAdaptUpperThreshDR
    OpMcmcAdaptUpperThreshDR = 0.65;
    global OpMcmcSigmaDecreaseFact
    OpMcmcSigmaDecreaseFact = 0.9;
    global OpMcmcSigmaIncreaseFact
    OpMcmcSigmaIncreaseFact = 1.1;
    global OpMcmcDRSigmaReduceFact
    OpMcmcDRSigmaReduceFact = 0.2;
    global OpMcmcDRSigmaReduceFactAM
    OpMcmcDRSigmaReduceFactAM = 0.1;
    global OpMcmcAdaptLowerThreshAM
    OpMcmcAdaptLowerThreshAM = 0.15;
    global OpMcmcAdaptUpperThreshAM
    OpMcmcAdaptUpperThreshAM = 0.3;
    global OpMcmcCovarScaleDecreaseFact
    OpMcmcCovarScaleDecreaseFact = 20;
    global OpMcmcCovarScaleIncreaseFact
    OpMcmcCovarScaleIncreaseFact = 20;
    global OpDemcSnookerFraction
    OpDemcSnookerFraction = 0.1;
    global OpDemcThinningFactor
    OpDemcThinningFactor = 10;
    global OpDemcB
    OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
    global data
    global firstT
    global lastT
    global firstD
```

```
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  samp = [];
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function val = mcEvalProposal(samp, prevsamp)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  val = 0;
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  LI
  Vmax
  Km
end

function names = mcSampNames()
  names = "LI";
  names = [names, "Vmax"];
  names = [names, "Km"];
  names
```

```
end

function parms = mcPackSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  parms = [];
  parms = [parms LI];
  parms = [parms Vmax];
  parms = [parms Km];
end

function mcUnpackSamples(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  idx = 1;
  LI = parms(idx); idx = idx + 1;
  Vmax = parms(idx); idx = idx + 1;
  Km = parms(idx); idx = idx + 1;
end

function parms = mcSamplePriors()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  LI = normrnd(1, 1, 0.01, 100);
```

```
  Vmax = unifrnd(-10, 5);
  Km = unifrnd(-10, 5);
  parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  val = val + normlpdf(LI, 1, 1, 0.01, 100);
  val = val + unifIpdf(Vmax, -10, 5);
  val = val + unifIpdf(Km, -10, 5);
end

function val = mcEvalLikelihoods(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  for i = firstD : lastD
    preds = getpreds(Vmax, Km, CCC(i));
    for j = firstT : lastT
      if(~isnan(data(j, i)))
        val = val + normlpdf(data(j, i), preds(j), LI);
      end
    end
  end
end
```

invitromcmc_satfrlng.m (used for female rat lung with Km fixed to female mouse Km)

```
function tchains = runmcmc(pchains = [])
  % Driver code for MCMC analysis
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global preds

  numParms = 2
  numChains = 3
  numIts = 40000
  funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors", "mcEvalProposal",
"mcSampleProposal"]
  updateMode = 4
  chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
  save @format=ascii @file=mcmc_results.dat chains
  tchains = chains([1:2:40000],:);
end

function mcInit()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global preds
  global OpMcmcPriorBounds
  OpMcmcPriorBounds = [...
  0.01, 100
  -10, 5
  ];
  global OpMcmcAdaptive
  OpMcmcAdaptive = 1;
  global OpMcmcDelayedRejection
  OpMcmcDelayedRejection = 0;
  global OpMcmcAdaptPeriod
  OpMcmcAdaptPeriod = 30;
  global OpMcmcAdaptCovarScale
```

```
    OpMcmcAdaptCovarScale = 1;
    global OpMcmcLoggingPeriod
    OpMcmcLoggingPeriod = 50;
    global OpMcmcAdaptLowerThresh
    OpMcmcAdaptLowerThresh = 0.25;
    global OpMcmcAdaptUpperThresh
    OpMcmcAdaptUpperThresh = 0.45;
    global OpMcmcAdaptLowerThreshDR
    OpMcmcAdaptLowerThreshDR = 0.45;
    global OpMcmcAdaptUpperThreshDR
    OpMcmcAdaptUpperThreshDR = 0.65;
    global OpMcmcSigmaDecreaseFact
    OpMcmcSigmaDecreaseFact = 0.9;
    global OpMcmcSigmaIncreaseFact
    OpMcmcSigmaIncreaseFact = 1.1;
    global OpMcmcDRSigmaReduceFact
    OpMcmcDRSigmaReduceFact = 0.2;
    global OpMcmcDRSigmaReduceFactAM
    OpMcmcDRSigmaReduceFactAM = 0.1;
    global OpMcmcAdaptLowerThreshAM
    OpMcmcAdaptLowerThreshAM = 0.15;
    global OpMcmcAdaptUpperThreshAM
    OpMcmcAdaptUpperThreshAM = 0.3;
    global OpMcmcCovarScaleDecreaseFact
    OpMcmcCovarScaleDecreaseFact = 20;
    global OpMcmcCovarScaleIncreaseFact
    OpMcmcCovarScaleIncreaseFact = 20;
    global OpDemcSnookerFraction
    OpDemcSnookerFraction = 0.1;
    global OpDemcThinningFactor
    OpDemcThinningFactor = 10;
    global OpDemcB
    OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global Vmax
    global preds
    samp = [];
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
```

```
end

function val = mcEvalProposal(samp, prevsamp)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global preds
  val = 0;
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global preds
  LI
  Vmax
end

function names = mcSampNames()
  names = "LI";
  names = [names, "Vmax"];
  names
end

function parms = mcPackSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global preds
  parms = [];
```

```
    parms = [parms LI];
    parms = [parms Vmax];
end

function mcUnpackSamples(parms)
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global Vmax
    global preds
    idx = 1;
    LI = parms(idx); idx = idx + 1;
    Vmax = parms(idx); idx = idx + 1;
end

function parms = mcSamplePriors()
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global Vmax
    global preds
    LI = normrnd(1, 1, 0.01, 100);
    Vmax = unifrnd(-10, 5);
    parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global Vmax
    global preds
    mcUnpackSamples(parms);
    val = 0.0;
    val = val + normlpdf(LI, 1, 1, 0.01, 100);
    val = val + uniflpdf(Vmax, -10, 5);
```

```
end

function val = mcEvalLikelihoods(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global LI
  global Vmax
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  for i = firstD : lastD
    preds = getpreds(Vmax, CCC(i));
    for j = firstT : lastT
      if(~isnan(data(j, i)))
        val = val + normlpdf(data(j, i), preds(j), LI);
      end
    end
  end
end
```

mmouselivinvitroa0.m (used for male mouse liver with initial amount in vial included)

```
function tchains = runmcmc(pchains = [])
  % Driver code for MCMC analysis
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  A0_i = zeros(5, 1);

  numParms = 8
  numChains = 3
  numIts = 50000
  funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors", "mcEvalProposal",
"mcSampleProposal"]
  updateMode = 4
  chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
  save @format=ascii @file=mcmc_results.dat chains
  tchains = chains([1:2:50000],:);
end

function mcInit()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  global OpMcmcPriorBounds
  OpMcmcPriorBounds = [...
  -10, 0
  -10, 0
  -10, 0
  -10, 0
  -10, 0
```

```
  0.01, 100
  -15, 5
  -15, 5
  ];
  global OpMcmcAdaptive
  OpMcmcAdaptive = 1;
  global OpMcmcDelayedRejection
  OpMcmcDelayedRejection = 0;
  global OpMcmcAdaptPeriod
  OpMcmcAdaptPeriod = 30;
  global OpMcmcAdaptCovarScale
  OpMcmcAdaptCovarScale = 1;
  global OpMcmcLoggingPeriod
  OpMcmcLoggingPeriod = 200;
  global OpMcmcAdaptLowerThresh
  OpMcmcAdaptLowerThresh = 0.25;
  global OpMcmcAdaptUpperThresh
  OpMcmcAdaptUpperThresh = 0.45;
  global OpMcmcAdaptLowerThreshDR
  OpMcmcAdaptLowerThreshDR = 0.45;
  global OpMcmcAdaptUpperThreshDR
  OpMcmcAdaptUpperThreshDR = 0.65;
  global OpMcmcSigmaDecreaseFact
  OpMcmcSigmaDecreaseFact = 0.9;
  global OpMcmcSigmaIncreaseFact
  OpMcmcSigmaIncreaseFact = 1.1;
  global OpMcmcDRSigmaReduceFact
  OpMcmcDRSigmaReduceFact = 0.2;
  global OpMcmcDRSigmaReduceFactAM
  OpMcmcDRSigmaReduceFactAM = 0.1;
  global OpMcmcAdaptLowerThreshAM
  OpMcmcAdaptLowerThreshAM = 0.15;
  global OpMcmcAdaptUpperThreshAM
  OpMcmcAdaptUpperThreshAM = 0.3;
  global OpMcmcCovarScaleDecreaseFact
  OpMcmcCovarScaleDecreaseFact = 20;
  global OpMcmcCovarScaleIncreaseFact
  OpMcmcCovarScaleIncreaseFact = 20;
  global OpDemcSnookerFraction
  OpDemcSnookerFraction = 0.1;
  global OpDemcThinningFactor
  OpDemcThinningFactor = 10;
  global OpDemcB
  OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
  global data
```

```
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    samp = [];
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
end

function val = mcEvalProposal(samp, prevsamp)
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    val = 0;
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    A0_i
    LI
    Vmax
    Km
```

```
end

function names = mcSampNames()
  names = "A0_i(1)";
  names = [names, "A0_i(2)"];
  names = [names, "A0_i(3)"];
  names = [names, "A0_i(4)"];
  names = [names, "A0_i(5)"];
  names = [names, "LI"];
  names = [names, "Vmax"];
  names = [names, "Km"];
  names
end

function parms = mcPackSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  parms = [];
  parms = [parms reshape(A0_i, 1, 5)];
  parms = [parms LI];
  parms = [parms Vmax];
  parms = [parms Km];
end

function mcUnpackSamples(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  idx = 1;
  A0_i = reshape(parms(idx:idx+4), 5, 1); idx = idx + 5;
  LI = parms(idx); idx = idx + 1;
```

```
  Vmax = parms(idx); idx = idx + 1;
  Km = parms(idx); idx = idx + 1;
end

function parms = mcSamplePriors()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  LI = normrnd(1, 1, 0.01, 100);
  Vmax = unifrnd(-15, 5);
  Km = unifrnd(-15, 5);
  for i = firstD : lastD
    A0_i(i) = unifrnd(-10, 0);
  end
  parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  val = val + normlpdf(LI, 1, 1, 0.01, 100);
  val = val + uniflpdf(Vmax, -15, 5);
  val = val + uniflpdf(Km, -15, 5);
  for i = firstD : lastD
    val = val + uniflpdf(A0_i(i), -10, 0);
  end
end

function val = mcEvalLikelihoods(parms)
```

```
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    mcUnpackSamples(parms);
    val = 0.0;
    for i = firstD : lastD
      preds = getpreds(Vmax, Km, A0_i(i));
      for j = firstT : lastT
        if(~isnan(data(j, i)))
          val = val + normlpdf(data(j, i), preds(j), LI);
        end
      end
    end
end
```

mmouseInginvitroa0.m (used for male mouse lung with initial amount in vial included)

```
function tchains = runmcmc(pchains = [])
  % Driver code for MCMC analysis
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  A0_i = zeros(5, 1);

  numParms = 8
  numChains = 3
  numIts = 50000
  funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors", "mcEvalProposal",
"mcSampleProposal"]
  updateMode = 4
  chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
  save @format=ascii @file=mcmc_results.dat chains
  tchains = chains([1:2:50000],:);
end

function mcInit()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  global OpMcmcPriorBounds
  OpMcmcPriorBounds = [...
  -10, 0
  -10, 0
  -10, 0
  -10, 0
  -10, 0
```

```
  0.01, 100
  -15, 5
  -15, 5
  ];
  global OpMcmcAdaptive
  OpMcmcAdaptive = 1;
  global OpMcmcDelayedRejection
  OpMcmcDelayedRejection = 0;
  global OpMcmcAdaptPeriod
  OpMcmcAdaptPeriod = 30;
  global OpMcmcAdaptCovarScale
  OpMcmcAdaptCovarScale = 1;
  global OpMcmcLoggingPeriod
  OpMcmcLoggingPeriod = 200;
  global OpMcmcAdaptLowerThresh
  OpMcmcAdaptLowerThresh = 0.25;
  global OpMcmcAdaptUpperThresh
  OpMcmcAdaptUpperThresh = 0.45;
  global OpMcmcAdaptLowerThreshDR
  OpMcmcAdaptLowerThreshDR = 0.45;
  global OpMcmcAdaptUpperThreshDR
  OpMcmcAdaptUpperThreshDR = 0.65;
  global OpMcmcSigmaDecreaseFact
  OpMcmcSigmaDecreaseFact = 0.9;
  global OpMcmcSigmaIncreaseFact
  OpMcmcSigmaIncreaseFact = 1.1;
  global OpMcmcDRSigmaReduceFact
  OpMcmcDRSigmaReduceFact = 0.2;
  global OpMcmcDRSigmaReduceFactAM
  OpMcmcDRSigmaReduceFactAM = 0.1;
  global OpMcmcAdaptLowerThreshAM
  OpMcmcAdaptLowerThreshAM = 0.15;
  global OpMcmcAdaptUpperThreshAM
  OpMcmcAdaptUpperThreshAM = 0.3;
  global OpMcmcCovarScaleDecreaseFact
  OpMcmcCovarScaleDecreaseFact = 20;
  global OpMcmcCovarScaleIncreaseFact
  OpMcmcCovarScaleIncreaseFact = 20;
  global OpDemcSnookerFraction
  OpDemcSnookerFraction = 0.1;
  global OpDemcThinningFactor
  OpDemcThinningFactor = 10;
  global OpDemcB
  OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
  global data
```

```
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  samp = [];
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function val = mcEvalProposal(samp, prevsamp)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  val = 0;
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  A0_i
  LI
  Vmax
  Km
```

```
end

function names = mcSampNames()
  names = "A0_i(1)";
  names = [names, "A0_i(2)"];
  names = [names, "A0_i(3)"];
  names = [names, "A0_i(4)"];
  names = [names, "A0_i(5)"];
  names = [names, "LI"];
  names = [names, "Vmax"];
  names = [names, "Km"];
  names
end

function parms = mcPackSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  parms = [];
  parms = [parms reshape(A0_i, 1, 5)];
  parms = [parms LI];
  parms = [parms Vmax];
  parms = [parms Km];
end

function mcUnpackSamples(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  idx = 1;
  A0_i = reshape(parms(idx:idx+4), 5, 1); idx = idx + 5;
  LI = parms(idx); idx = idx + 1;
```

```
  Vmax = parms(idx); idx = idx + 1;
  Km = parms(idx); idx = idx + 1;
end

function parms = mcSamplePriors()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  LI = normrnd(1, 1, 0.01, 100);
  Vmax = unifrnd(-15, 5);
  Km = unifrnd(-15, 5);
  for i = firstD : lastD
    A0_i(i) = unifrnd(-10, 0);
  end
  parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  val = val + normlpdf(LI, 1, 1, 0.01, 100);
  val = val + uniflpdf(Vmax, -15, 5);
  val = val + uniflpdf(Km, -15, 5);
  for i = firstD : lastD
    val = val + uniflpdf(A0_i(i), -10, 0);
  end
end

function val = mcEvalLikelihoods(parms)
```

```
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    mcUnpackSamples(parms);
    val = 0.0;
    for i = firstD : lastD
      preds = getpreds(Vmax, Km, A0_i(i));
      for j = firstT : lastT
        if(~isnan(data(j, i)))
          val = val + normlpdf(data(j, i), preds(j), LI);
        end
      end
    end
end
```

mratlivinvitroa0.m (used for male rat liver with initial amount in vial included)

```
function tchains = runmcmc(pchains = [])
  % Driver code for MCMC analysis
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  A0_i = zeros(3, 1);

  numParms = 6
  numChains = 3
  numIts = 50000
  funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors", "mcEvalProposal",
"mcSampleProposal"]
  updateMode = 4
  chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
  save @format=ascii @file=mcmc_results.dat chains
  tchains = chains([1:2:50000],:);
end

function mcInit()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  global OpMcmcPriorBounds
  OpMcmcPriorBounds = [...
  -10, 0
  -10, 0
  -10, 0
  0.01, 100
  -15, 5
```

```
  -15, 5
  ];
  global OpMcmcAdaptive
  OpMcmcAdaptive = 1;
  global OpMcmcDelayedRejection
  OpMcmcDelayedRejection = 0;
  global OpMcmcAdaptPeriod
  OpMcmcAdaptPeriod = 30;
  global OpMcmcAdaptCovarScale
  OpMcmcAdaptCovarScale = 1;
  global OpMcmcLoggingPeriod
  OpMcmcLoggingPeriod = 200;
  global OpMcmcAdaptLowerThresh
  OpMcmcAdaptLowerThresh = 0.25;
  global OpMcmcAdaptUpperThresh
  OpMcmcAdaptUpperThresh = 0.45;
  global OpMcmcAdaptLowerThreshDR
  OpMcmcAdaptLowerThreshDR = 0.45;
  global OpMcmcAdaptUpperThreshDR
  OpMcmcAdaptUpperThreshDR = 0.65;
  global OpMcmcSigmaDecreaseFact
  OpMcmcSigmaDecreaseFact = 0.9;
  global OpMcmcSigmaIncreaseFact
  OpMcmcSigmaIncreaseFact = 1.1;
  global OpMcmcDRSigmaReduceFact
  OpMcmcDRSigmaReduceFact = 0.2;
  global OpMcmcDRSigmaReduceFactAM
  OpMcmcDRSigmaReduceFactAM = 0.1;
  global OpMcmcAdaptLowerThreshAM
  OpMcmcAdaptLowerThreshAM = 0.15;
  global OpMcmcAdaptUpperThreshAM
  OpMcmcAdaptUpperThreshAM = 0.3;
  global OpMcmcCovarScaleDecreaseFact
  OpMcmcCovarScaleDecreaseFact = 20;
  global OpMcmcCovarScaleIncreaseFact
  OpMcmcCovarScaleIncreaseFact = 20;
  global OpDemcSnookerFraction
  OpDemcSnookerFraction = 0.1;
  global OpDemcThinningFactor
  OpDemcThinningFactor = 10;
  global OpDemcB
  OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
  global data
  global firstT
  global lastT
```

```
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  samp = [];
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function val = mcEvalProposal(samp, prevsamp)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  val = 0;
  % This function is a stub...
  % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  A0_i
  LI
  Vmax
  Km
end
```

```
function names = mcSampNames()
  names = "A0_i(1)";
  names = [names, "A0_i(2)"];
  names = [names, "A0_i(3)"];
  names = [names, "LI"];
  names = [names, "Vmax"];
  names = [names, "Km"];
  names
end

function parms = mcPackSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  parms = [];
  parms = [parms reshape(A0_i, 1, 3)];
  parms = [parms LI];
  parms = [parms Vmax];
  parms = [parms Km];
end

function mcUnpackSamples(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  idx = 1;
  A0_i = reshape(parms(idx:idx+2), 3, 1); idx = idx + 3;
  LI = parms(idx); idx = idx + 1;
  Vmax = parms(idx); idx = idx + 1;
  Km = parms(idx); idx = idx + 1;
end
```

```
function parms = mcSamplePriors()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  LI = normrnd(1, 1, 0.01, 100);
  Vmax = unifrnd(-15, 5);
  Km = unifrnd(-15, 5);
  for i = firstD : lastD
    A0_i(i) = unifrnd(-10, 0);
  end
  parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  val = val + normlpdf(LI, 1, 1, 0.01, 100);
  val = val + uniflpdf(Vmax, -15, 5);
  val = val + uniflpdf(Km, -15, 5);
  for i = firstD : lastD
    val = val + uniflpdf(A0_i(i), -10, 0);
  end
end

function val = mcEvalLikelihoods(parms)
  global data
  global firstT
  global lastT
  global firstD
```

```
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  for i = firstD : lastD
    preds = getpreds(Vmax, Km, A0_i(i));
    for j = firstT : lastT
      if(~isnan(data(j, i)))
        val = val + normlpdf(data(j, i), preds(j), LI);
      end
    end
  end
end
```

invitrohumlivsata0.m (used for mixed human liver with initial amount in vial included)

```
function tchains = runmcmc(pchains = [])
  % Driver code for MCMC analysis
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  A0_i = zeros(3, 1);

  numParms = 6
  numChains = 3
  numIts = 50000
  funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors", "mcEvalProposal",
"mcSampleProposal"]
  updateMode = 4
  chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
  save @format=ascii @file=mcmc_results.dat chains
  tchains = chains([1:2:50000],:);
end

function mcInit()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  global OpMcmcPriorBounds
  OpMcmcPriorBounds = [...
  -10, 0
  -10, 0
  -10, 0
  0.01, 100
  -15, 5
```

```
  -15, 5
  ];
  global OpMcmcAdaptive
  OpMcmcAdaptive = 1;
  global OpMcmcDelayedRejection
  OpMcmcDelayedRejection = 0;
  global OpMcmcAdaptPeriod
  OpMcmcAdaptPeriod = 30;
  global OpMcmcAdaptCovarScale
  OpMcmcAdaptCovarScale = 1;
  global OpMcmcLoggingPeriod
  OpMcmcLoggingPeriod = 200;
  global OpMcmcAdaptLowerThresh
  OpMcmcAdaptLowerThresh = 0.25;
  global OpMcmcAdaptUpperThresh
  OpMcmcAdaptUpperThresh = 0.45;
  global OpMcmcAdaptLowerThreshDR
  OpMcmcAdaptLowerThreshDR = 0.45;
  global OpMcmcAdaptUpperThreshDR
  OpMcmcAdaptUpperThreshDR = 0.65;
  global OpMcmcSigmaDecreaseFact
  OpMcmcSigmaDecreaseFact = 0.9;
  global OpMcmcSigmaIncreaseFact
  OpMcmcSigmaIncreaseFact = 1.1;
  global OpMcmcDRSigmaReduceFact
  OpMcmcDRSigmaReduceFact = 0.2;
  global OpMcmcDRSigmaReduceFactAM
  OpMcmcDRSigmaReduceFactAM = 0.1;
  global OpMcmcAdaptLowerThreshAM
  OpMcmcAdaptLowerThreshAM = 0.15;
  global OpMcmcAdaptUpperThreshAM
  OpMcmcAdaptUpperThreshAM = 0.3;
  global OpMcmcCovarScaleDecreaseFact
  OpMcmcCovarScaleDecreaseFact = 20;
  global OpMcmcCovarScaleIncreaseFact
  OpMcmcCovarScaleIncreaseFact = 20;
  global OpDemcSnookerFraction
  OpDemcSnookerFraction = 0.1;
  global OpDemcThinningFactor
  OpDemcThinningFactor = 10;
  global OpDemcB
  OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
  global data
  global firstT
  global lastT
```

```
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    samp = [];
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
end

function val = mcEvalProposal(samp, prevsamp)
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    val = 0;
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
    global data
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global A0_i
    global LI
    global Vmax
    global Km
    global preds
    A0_i
    LI
    Vmax
    Km
end
```

```
function names = mcSampNames()
  names = "A0_i(1)";
  names = [names, "A0_i(2)"];
  names = [names, "A0_i(3)"];
  names = [names, "LI"];
  names = [names, "Vmax"];
  names = [names, "Km"];
  names
end

function parms = mcPackSamples()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  parms = [];
  parms = [parms reshape(A0_i, 1, 3)];
  parms = [parms LI];
  parms = [parms Vmax];
  parms = [parms Km];
end

function mcUnpackSamples(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  idx = 1;
  A0_i = reshape(parms(idx:idx+2), 3, 1); idx = idx + 3;
  LI = parms(idx); idx = idx + 1;
  Vmax = parms(idx); idx = idx + 1;
  Km = parms(idx); idx = idx + 1;
end
```

```
function parms = mcSamplePriors()
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  LI = normrnd(1, 1, 0.01, 100);
  Vmax = unifrnd(-15, 5);
  Km = unifrnd(-15, 5);
  for i = firstD : lastD
    A0_i(i) = unifrnd(-10, 0);
  end
  parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
  global data
  global firstT
  global lastT
  global firstD
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  val = val + normlpdf(LI, 1, 1, 0.01, 100);
  val = val + uniflpdf(Vmax, -15, 5);
  val = val + uniflpdf(Km, -15, 5);
  for i = firstD : lastD
    val = val + uniflpdf(A0_i(i), -10, 0);
  end
end

function val = mcEvalLikelihoods(parms)
  global data
  global firstT
  global lastT
  global firstD
```

```
  global lastD
  global CCC
  global A0_i
  global LI
  global Vmax
  global Km
  global preds
  mcUnpackSamples(parms);
  val = 0.0;
  for i = firstD : lastD
    preds = getpreds(Vmax, Km, A0_i(i));
    for j = firstT : lastT
      if(~isnan(data(j, i)))
        val = val + normlpdf(data(j, i), preds(j), LI);
      end
    end
  end
end
```

In Vivo Model Files

chloroprene.model (R model code)

```
#Chloroprene PBPK Model
#Translated from the acslX model presented in Yang et al. 2012
#By Jerry Campbell 2019

States  = {
AI      ,
AX      ,
AM      ,
AMLU    ,
AMK     ,
ALU     ,
AL      ,
AK      ,
AS      ,
AR      ,
AF
};

Outputs = {
MASBAL ,
CLU     ,
CL      ,
CK      ,
CS      ,
CR      ,
CF      ,
CV  ,
CVLUM ,
ppm ,
AMP     ,
AMPLU ,
AMPK    ,
cvl ,
qcbal ,
vbal
};


Inputs = {EXPPULSE};
```

```
#BODY WEIGHT (kg)
    BW = 0.03 ;   # Body weight (kg)


#SPECIAL FLOW RATES
QPC = 29.1 ;   # Unscaled Alveolar Vent (L/h/kg^0.75)
QCC = 20.1 ;   # Unscaled Cardiac Output (L/h/kg^0.75)

#FRACTIONAL BLOOD FLOWS TO TISSUES
QLC = 0.161 ;  # Flow to Liver as % Cardiac Output (unitless)
QFC = 0.07  ;  # Flow to Fat as % Cardiac Output (unitless)
QSC = 0.159 ;  # Flow to Slow as % Cardiac Output (unitless)
QKC = 0.09  ;  # Flow to Kidney as % Cardiac Output (unitless)

#FRACTIONAL VOLUMES OF TISSUES
VLC = 0.055 ;     # Volume Liver as % Body Weight (unitless)
VLUC = 0.0073 ;   # Volume Lung as % Body Weight (unitless)
VFC = 0.1  ;      # Volume Fat as % Body Weight (unitless)
VRC = 0.08098 ;   # Volume Rapid Perfused as % Body Weight (unitless)
VSC = 0.384  ;    # Volume Slow Perfused as % Body Weight (unitless)
VKC = 0.0167 ;    # Volume Kidney as % Body Weight  (unitless)

#PARTITION COEFFICIENTS PARENT
PL = 1.26 ;   # Liver/Blood Partition Coefficient (unitless)
PLU = 2.38 ;  # Lung/Blood Partition Coefficient (unitless)
PF = 17.35 ;  # Fat/Blood Partition Coefficient (unitless)
PS = 0.59  ;  # Slow/Blood Partition Coefficient (unitless)
PR = 1.76 ;   # Rapid/Blood Partition Coefficient (unitless)
PB = 7.83 ;   # Blood/Air Partition Coefficient (unitless)
PK = 1.76 ;   # Kidney/Blood Partition Coefficient (unitless)

#KINETIC CONSTANTS
MW = 88.5  ;  # Molecular weight (g/mol)

# Metabolism in Liver
VMAXC = 7.95  ;  # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
KM = 0.041  ;  # Km for Oxidative Pathway:Liver (mg/L)

# Metabolism in Lung
VMAXCLU = 0.18;    # Scaled VMax for Oxidative Pathway:Lung (mg/h/BW^0.75)
KMLU = 0.26;       # Km for Oxidative Pathway:Lung (mg/L)

# Metabolism in Kidney
VMAXCKid = 0.0 ;   # Scaled VMax for Oxidative Pathway:Kidney (mg/h/BW^0.75)
KMKD = 1.0  ;  # Km for Oxidative Pathway :Kidney

#DOSING INFORMATION
TSTOP = 7.0 ;    # Dosing stop time
```

```
CONC = 13.0 ;  # Initial concentration (ppm)



Dynamics {

# Scaled parameters

    QC = QCC*pow(BW,0.75) ;     #Cardiac output
    QP = QPC*pow(BW,0.75) ;     #Alveolar ventilation
    QL = QLC*QC   ;          #Liver blood flow
    QF = QFC*QC   ;          #Fat blood flow
    QS = QSC*QC   ;          #Slowly-perf tissue blood flow
    QK = QKC*QC   ;          #Kidney tissue blood flow

   QRC = 1-QLC-QKC-QFC-QSC ;    #Rapily Perfused tissues
    QR = QRC*QC         ;              #Rapily-perf tissue blood flow

    VL = VLC*BW     ;        #Liver volume
   VLU = VLUC*BW    ;          #Lung volume
    VF = VFC*BW     ;        #Fat tissue volume
    VS = VSC*BW     ;        #Slowly-perfused tissue volume
    VR = VRC*BW     ;        #Richly-perfised tissue volume
    VK = VKC*BW     ;        #kidney tissue volume

  ROBC = 1 - VLC - VLUC - VFC - VSC - VRC - VKC ; #Rest of body un-perfused tissued for Monte Carlo
sims

# METABOLISM
   VMAX = VMAXC*pow(BW,0.75) ;     #Maximum rate of metabolism-Liver (mg/hr/kg-BW)
  VMAXLU = VMAXCLU*pow(BW,0.75) ;    #Maximum rate of metabolism-Lung (mg/hr/kg-BW)
  VMAXKD = VMAXCKid*pow(BW,0.75) ;   #Maximum rate of metabolism-Kidney (mg/hr/kg-BW)

# Exposure Control (mg/L)
    CIX = CONC*MW/24450 ;
    CI = CIX *EXPPULSE ;

# Tissue Venous Concentrations (mg/L)
   CVLU = ALU/(VLU*PLU) ;
    CVL = AL/(VL*PL) ;
    CVK = AK/(VK*PK) ;
    CVS = AS/(VS*PS) ;
    CVR = AR/(VR*PR) ;
    CVF = AF/(VF*PF) ;

# Concentration in Pulmonary/Arterial and venous blood Compartments (mg/L)
    CPU = (QP*CI+(QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK))/(QP/PB+QC) ;
```

```
    CX = CPU/PB ;
    CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK)/QC ;
   CPUM = CPU*1000/MW ;
    RAI = QP*CI ;
    dt(AI) = RAI ;
    RAX = QP*CX ;
    dt(AX) = RAX ;

# Amount metabolized in Liver (mg)
    RAM = VMAX*CVL/(KM+CVL) ;
    dt(AM) = RAM ;

# Amount metabolized in Lung (mg)
    RAMLU = VMAXLU*CVLU/(KMLU+CVLU) ;
    dt(AMLU) = RAMLU ;

# Amount metabolized in Kidney (mg)
    RAMK = VMAXKD*CVK/(KMKD + CVK)  ;
    dt(AMK) = RAMK ;

# Amount in Lung Compartment (mg)
    RALU = QC*(CPU-CVLU) - RAMLU ;
    dt(ALU) = RALU ;

# Amount in Liver Compartment (mg)
    RAL = QL*(CVLU-CVL) - RAM ;
    dt(AL) = RAL ;

# Amount in Kidney Compartment (mg)
    RAK = QK*(CVLU-CVK) - RAMK ;
    dt(AK) = RAK ;

# Amount in Slowly Perfused Tissues (mg)
    RAS = QS*(CVLU - CVS) ;
    dt(AS) = RAS ;

# Amount in Rapidly Perfused Tissues (mg)
    RAR = QR*(CVLU -CVR) ;
    dt(AR) = RAR ;

# Amount in Fat Compartment (mg)
    RAF = QF*(CVLU - CVF) ;
    dt(AF) = RAF ;

} # End of Dynamics

CalcOutputs {
# Mass-balance
```

```
        MASBAL = AI - AX - (AL+AM+AMLU+ALU+AK+AMK+AS+AR+AF) ;
        #Tissue Concentrations (mg/L)
         CLU = ALU/VLU ;
      CL = AL/VL ;
      CK = AK/VK ;
      CS = AS/VS ;
      CR = AR/VR ;
      CF = AF/VF ;

#Concentrations for plots
        CVLUM = CVLU*1000/MW ;  #(umol/L)
#Dose metrics
  ppm = CONC ;
  AMP = ((AM*1000/MW)/(VL*1000))/(TSTOP/24) ;
 AMPLU = ((AMLU*1000/MW)/(VLU*1000))/(TSTOP/24) ;
  AMPK = ((AMK*1000/MW)/(VK*1000))/(TSTOP/24) ;

  cvl = CVL ;

#Blood Flow balance
  qcbal = QC - QL - QF - QS - QK - QR ;

#Tissue Volume balance
   vbal = BW*(1-ROBC) - VL - VLU - VF - VS - VK - VR ;

} # End of CalcOutputs

End.
```

Mouse.R (Base model parameters for mouse)

```
#Female Mouse parameters (See Model Parameters Spreadsheet for Documentation)

parms <-c(
BW = 0.04 ,    # Body weight (kg)
QPC = 29.1 ,    # Unscaled Alveolar Vent (L/h/kg^0.75)
QCC = 20.1 ,    # Unscaled Cardiac Output (L/h/kg^0.75)

#FRACTIONAL BLOOD FLOWS TO TISSUES
QLC = 0.161 ,  # Flow to Liver as % Cardiac Output (unitless)
QFC = 0.07  ,  # Flow to Fat as % Cardiac Output (unitless)
QSC = 0.159  ,  # Flow to Slow as % Cardiac Output (unitless)
QKC = 0.09  ,  # Flow to Kidney as % Cardiac Output  (unitless)

#FRACTIONAL VOLUMES OF TISSUES
VLC = 0.055 ,  # Volume Liver as % Body Weight (unitless)
VLUC = 0.0073 , # Volume Lung as % Body Weight (unitless)
VFC = 0.1  ,   # Volume Fat as % Body Weight (unitless)
VRC = 0.08098 , # Volume Rapid Perfused as % Body Weight (unitless)
VSC = 0.384  ,  # Volume Slow Perfused as % Body Weight (unitless)
VKC = 0.0167 ,  # Volume Kidney as % Body Weight  (unitless)

#PARTITION COEFFICIENTS PARENT
PL = 1.26 ,    # Liver/Blood Partition Coefficient (unitless)
PLU = 2.38 ,   # Lung/Blood Partition Coefficient (unitless)
PF = 17.35 ,   # Fat/Blood Partition Coefficient (unitless)
PS = 0.59  ,   # Slow/Blood Partition Coefficient (unitless)
PR = 1.76 ,    # Rapid/Blood Partition Coefficient (unitless)
PB = 7.8 ,     # Blood/Air Partition Coefficient (unitless)
PK = 1.76 ,    # Kidney/Blood Partition Coefficient (unitless)

#KINETIC CONSTANTS
MW = 88.5  ,   # Molecular weight (g/mol)

#Revised Metabolism Constants based on Yoon report
# Metabolism in Liver
VMAXC = 99.0  , # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
KM = 1.0  ,    # Km for Oxidative Pathway:Liver (mg/L)

# Metabolism in Lung
VMAXCLU = 99.0 , # Scaled VMax for Oxidative Pathway:Lung (mg/h/BW^0.75)
KMLU = 1.0  ,    # Km for Oxidative Pathway:Lung (mg/L)


# Metabolism in Kidney
VMAXCKid = 00.0 , # Scaled VMax for Oxidative Pathway:Kidney (mg/h/BW^0.75)
```

KMKD = 1.0 , # Km for Oxidative Pathway :Kidney (mg/L)

#DOSING INFORMATION
TSTOP = 7.0 ,
CONC = 0.0 # Initial concentration (ppm)
)

Fmouse.R (Female mouse specific model parameters)

```
#Female Mouse parameters

source('./params/mouse.R')

#Revised Metabolism Constants based on Yoon report
#QIVIE for VMAXC and VMAXCLU were based on a 40 g mouse
#which was the rounded average female weight in the 2-year bioassay

#Female mouse average body weight (rounded to g)
parms["BW"] <- 0.040     #Weighted average of the female mouse control group NTP chloroprene
bioassay

# Metabolism in Liver
parms["VMAXC"] <- 7.99   # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
parms["KM"] <- 0.040     # Km for Oxidative Pathway:Liver (mg/L)

# Metabolism in Lung
parms["VMAXCLU"] <- 0.12  # Scaled VMax for Oxidative Pathway:Lung (mg/h/BW^0.75)
parms["KMLU"] <- 0.21     # Km for Oxidative Pathway:Lung (mg/L)

# Metabolism in Kidney
parms["VMAXCKid"] <- 00.0  # Scaled VMax for Oxidative Pathway:Kidney (mg/h/BW^0.75)
parms["KMKD"] <- 1.0      # Km for Oxidative Pathway :Kidney (mg/L)
```

Human.R (Base human model parameters)

```
#Human parameters (See Model Parameters Spreadsheet for Documentation)

parms <-c(
BW = 70.0 ,     # Body weight (kg)
QPC = 24.0 ,    # Unscaled Alveolar Vent (L/h/kg^0.75)
QCC = 16.5 ,    # Unscaled Cardiac Output (L/h/kg^0.75)

#FRACTIONAL BLOOD FLOWS TO TISSUES
QLC = 0.227 ,    # Flow to Liver as % Cardiac Output (unitless)
QFC = 0.052 ,    # Flow to Fat as % Cardiac Output (unitless)
QSC = 0.191 ,    # Flow to Slow as % Cardiac Output (unitless)
QKC = 0.175 ,    # Flow to Kidney as % Cardiac Output  (unitless)

#FRACTIONAL VOLUMES OF TISSUES
VLC = 0.0257 ,   # Volume Liver as % Body Weight (unitless)
VLUC = 0.0076 ,  # Volume Lung as % Body Weight (unitless)
VFC = 0.27 ,     # Volume Fat as % Body Weight (unitless)
VRC = 0.0533 ,   # Volume Rapid Perfused as % Body Weight (unitless)
VSC = 0.4 ,      # Volume Slow Perfused as % Body Weight (unitless)
VKC = 0.0044 ,   # Volume Kidney as % Body Weight  (unitless)

#PARTITION COEFFICIENT PARENT
PL = 2.37 ,      # Liver/Blood Partition Coefficient (unitless)
PLU = 2.94 ,     # Lung/Blood Partition Coefficient (unitless)
PF = 28.65 ,     # Fat/Blood Partition Coefficient (unitless)
PS = 1.00 ,      # Slow/Blood Partition Coefficient (unitless)
PR = 2.67 ,      # Rapid/Blood Partition Coefficient (unitless)
PB = 4.5 ,       # Blood/Air Partition Coefficient (unitless)
PK = 2.67 ,      # Kidney/Blood Partition Coefficient (unitless)

#KINETIC CONSTANTS
MW = 88.5 ,    # Molecular weight (g/mol)

#Revised Metabolism Constants based on Yoon report
# Metabolism in Liver
VMAXC = 99.0  , # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
KM = 1.0 ,    # Km for Oxidative Pathway:Liver (mg/L)

# Metabolism in Lung
VMAXCLU = 99.0 , # Scaled VMax for Oxidative Pathway:Lung (mg/h/BW^0.75)
KMLU = 1.0 ,    # Km for Oxidative Pathway:Lung (mg/L)


# Metabolism in Kidney
VMAXCKid = 0.0 , # Scaled VMax for Oxidative Pathway:Kidney (mg/h/BW^0.75)
```

KMKD = 1.0 , # Km for Oxidative Pathway :Kidney (mg/L)

#DOSING INFORMATION
TSTOP = 7.0 ,
CONC = 0.0 # Initial concentration (ppm)
)

Mhuman.R (mixed human specific model parameters)

```
#Mixed Human Parameters

source('./params/Human.R')

#Revised Metabolism Constants based on Yoon report
# Metabolism in Liver
parms["VMAXC"] <- 14.51  # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
parms["KM"] <- 0.031     # Km for Oxidative Pathway:Liver (mg/L)

# Metabolism in Lung
parms["VMAXCLU"] <- 0.0031  # Scaled VMax for Oxidative Pathway:Lung (mg/h/BW^0.75)
parms["KMLU"] <- 0.031     # Km for Oxidative Pathway:Lung (mg/L)


# Metabolism in Kidney (no renal metabolism)
parms["VMAXCKid"] <- 00.0  # Scaled VMax for Oxidative Pathway:Kidney (mg/h/BW^0.75)
parms["KMKD"] <- 1.0     # Km for Oxidative Pathway :Kidney (mg/L)
```

Fmouse_InVivo.R (simulates 15-day female mouse kinetic study with plots generated for the time-course blood data)

```
# Simulates the 15 day mouse exposure study
# Data collected during and after exposure on 1st day
# and at end of exposure on day 5 and 15 (1 day nose-only)
#VMAXC and VMAXCLU were scaled from the in vitro values to the
#rounded average BW reported in the study which was 20 g
#for all three concentrations

#set the working directory to where you downloaded the scripts
setwd(dirname(parent.frame(2)$ofile))


# load libraries needed to run scenario
library(deSolve)

# Model path and name
mName <- "chloroprene.model"

#load model inits file for the ode solver
source(paste0(mName,"_inits.R"))

#load the states files
#source(paste0(mPath,"states.R"))

#load the model dll
dyn.load(paste0(mName,.Platform$dynlib.ext))

#Scenario specific values
tstart <- 0.0
tstop <- 443.0
times <- seq(tstart, tstop , by=0.02)

# Physiolgical parameters path

#load the parameters
source('./params/Fmouse.R')
source('./states.R')

# timing variables for forcing functions
dstart <- tstart
dlength <- 6    #hours per day to expose
ddaysperwk <- 5  #days of week to expose
dexpend <- 19    #days of exposure
parms["TSTOP"] <- tstop
```

```
# Source forcing functions
# this loads the function forcing() in the namespace
source("forfunc.R")


#Scenario Specific Parameters
parms["BW"]<- 0.020  #measured in the study
parms["QPC"]<- 32.8  #measured in the study
parms["QCC"]<- parms["QPC"]/1.45  #V/Q Ratio Marino et al. 2006


parms["CONC"]<- 12.3


# Run ODE
print(system.time(
 out <- ode(Y, times, func = "derivs", parms = parms, method="vode",  atol=1.0e-10, rtol=1.0e-8,
        dllname = mName, initforc="initforc", forcings=forcings,
        initfunc = "initmod", nout = length(Outputs),
        outnames = Outputs)
))

out1 <- as.data.frame(out,stringsAsFactors = F)

#Scenario Specific Exposure
parms["BW"]<- 0.020  #measured in the study
parms["QPC"]<- 49.0  #measured in the study
parms["QCC"]<- parms["QPC"]/1.45  #V/Q Ratio Marino et al. 2006

parms["CONC"]<- 32.0



# Run ODE
print(system.time(
 out <- ode(Y, times, func = "derivs", parms = parms, method="vode",  atol=1.0e-10, rtol=1.0e-8,
        dllname = mName, initforc="initforc", forcings=forcings,
        initfunc = "initmod", nout = length(Outputs),
        outnames = Outputs)
))

out2 <- as.data.frame(out,stringsAsFactors = F)

#Scenario Specific Exposure
parms["BW"]<- 0.020  #measured in the study
parms["QPC"]<- 38.7  #measured in the study
parms["QCC"]<- parms["QPC"]/1.45  #V/Q Ratio Marino et al. 2006
```

```
parms["CONC"]<-  90.0



# Run ODE
print(system.time(
  out <- ode(Y, times, func = "derivs", parms = parms, method="vode", atol=1.0e-10, rtol=1.0e-8,
        dllname = mName, initforc="initforc", forcings=forcings,
        initfunc = "initmod", nout = length(Outputs),
        outnames = Outputs)
))

out3 <- as.data.frame(out,stringsAsFactors = F)


#unload the model dll
dyn.unload(paste0(mName,.Platform$dynlib.ext))

#displays the plots of the observed versus predicted data

## Read the dataset to be plotted
#12.8 ppm
Dataset1 <- data.frame(cbind((c(0.5, 0.5, 0.5, 0.5, 3, 3, 3, 3, 3, 6, 6, 6, 6, 6, 6.083, 6.083, 6.083, 6.17,
6.17, 102, 102, 102, 102, 438, 438, 438, 438)),
                (c(0.97, 0.82, 1.12, 1.22, 0.6, 2.7, 2.03, 2.1, 2.24, 2.08, 1.75, 1.53, 1.37, 1.16, 0.08, 0.09,
0.16, 0.1, 0.25, 0.17, 0.23, 0.2, 0.18, 0.28, 0.33, 0.24, 0.31))))
colnames(dataset1) <- c("time","cart")

#30 ppm
dataset2  <- data.frame(cbind((c(0.5, 0.5, 0.5, 0.5, 0.5, 3, 3, 3, 3, 3, 3, 6, 6, 6, 6, 6.083, 6.083, 6.083,
6.083, 6.083, 6.17, 6.17, 6.17, 6.17, 6.17, 6.25, 6.25, 6.25, 6.25, 6.25, 102, 102, 102, 102, 102, 438, 438,
438, 438, 438)),
                (c(3, 2.27, 1.66, 2.08, 0.69, 3.94, 3.9, 1.52, 2.48, 1.68, 3.87, 2.26, 1.26, 4.18, 2.06, 0.46, 0.41,
0.92, 0.52, 0.77, 0.28, 0.26, 0.1, 0.12, 0.13, 0.18, 0.31, 0.69, 0.16, 0.13, 2.32, 2.26, 1.15, 1.32, 0.88, 0.75,
2.08, 1.6, 1.12, 1.45))))
colnames(dataset2) <- c("time","cart")

#90 ppm
dataset3  <- data.frame(cbind((c(0.5, 0.5, 0.5, 0.5, 0.5, 3, 3, 3, 3, 3, 6, 6, 6, 6, 6, 6.083, 6.083, 6.083,
6.083, 6.083, 6.17, 6.17, 6.17, 6.17, 6.17, 6.25, 6.25, 6.25, 6.25, 6.25, 102, 102, 102, 102, 102, 438, 438,
438, 438, 438)),
                (c(5.92, 4.86, 4.82, 8.26, 7.69, 7.42, 12.95, 7.18, 3.46, 5.62, 9, 6.46, 7.63, 8.79, 8.12, 1.39,
3.01, 1.62, 0.92, 1.59, 0.66, 1.46, 0.67, 0.88, 0.93, 0.94, 0.63, 0.57, 0.64, 0.58, 3.73, 5.48, 4.09, 3, 6.43,
4.44, 3.64, 2.76, 3.41, 1.96))))
colnames(dataset3) <- c("time","cart")
```

```
par(mfrow=c(1,1))
plot(out1$time,out1$CV*1000/parms["MW"], type = 'l', col='red',lwd = 2,
   xlab="TIME",ylab = expression(mu*"M"), main='Mouse Study 3 Week - Day 1',
   xlim=c(0.0,7.0), ylim=c(0.0,20.0))
points(out2$CV*1000/parms["MW"]~out2$time,type = 'l',col='blue', lwd=2)
points(out3$CV*1000/parms["MW"]~out3$time,type = 'l',col='orange', lwd=2)
points(dataset1$time,dataset1$cart,type = 'p',col='red', pch=21, bg='red')
points(dataset2$time,dataset2$cart,type = 'p',col='blue', pch=21, bg='blue')
points(dataset3$time,dataset3$cart,type = 'p',col='orange', pch=21, bg='orange')


plot(out1$time,out1$CV*1000/parms["MW"], type = 'l', col='red',lwd = 2,
   xlab="TIME",ylab = expression(mu*"M"), main='Mouse Study 3 Week - Day 5',
   xlim=c(96.0,106.0), ylim=c(0.0,20.0))
points(out2$CV*1000/parms["MW"]~out2$time,type = 'l',col='blue', lwd=2)
points(out3$CV*1000/parms["MW"]~out3$time,type = 'l',col='orange', lwd=2)
points(dataset1$time,dataset1$cart,type = 'p',col='red', pch=21, bg='red')
points(dataset2$time,dataset2$cart,type = 'p',col='blue', pch=21, bg='blue')
points(dataset3$time,dataset3$cart,type = 'p',col='orange', pch=21, bg='orange')


plot(out1$time,out1$CV*1000/parms["MW"], type = 'l', col='red',lwd = 2,
   xlab="TIME",ylab = expression(mu*"M"), main='Mouse Study 3 Week - Day 19',
   xlim=c(432.0,442.0), ylim=c(0.0,20.0))
points(out2$CV*1000/parms["MW"]~out2$time,type = 'l',col='blue', lwd=2)
points(out3$CV*1000/parms["MW"]~out3$time,type = 'l',col='orange', lwd=2)
points(dataset1$time,dataset1$cart,type = 'p',col='red', pch=21, bg='red')
points(dataset2$time,dataset2$cart,type = 'p',col='blue', pch=21, bg='blue')
points(dataset3$time,dataset3$cart,type = 'p',col='orange', pch=21, bg='orange')
```

Fmouse_metric.R (simulates the bioassay study for two weeks to accumulate daily average dose metrics)

```
# Simulates female mouse for 2 weeks using mouse study protocol (6 hr/day 5 days/week)
# Uses metabolism constants from Redo of the MCMC in vitro with flux

#Set the working directory to where you downloaded the scripts
setwd(dirname(parent.frame(2)$ofile))


# Load libraries needed to run scenario
library(deSolve)

# Model path and name
mName <- "chloroprene.model"

#Load model inits file for the ode solver
source(paste0(mName,"_inits.R"))

#Load the model dll
dyn.load(paste0(mName,.Platform$dynlib.ext))

#Scenario specific values
tstart <- 0.0
tstop <- 336.0
times <- seq(tstart, tstop , by=0.01)
nend <- length(times)

#Physiolgical parameters path
#Load the parameters
source('./params/Fmouse.R')
source('./states.R')



#Timing variables for forcing functions
dstart <- tstart
dlength <- 6     #hours per day to expose
ddaysperwk <- 5  #days of week to expose
dexpend <- 12    #days of exposure
parms["TSTOP"] <- tstop

#Source forcing functions
#This loads the function forcing() in the namespace
source("forfunc.R")
```

```
#Scenario Specific Exposure
ppm <- c(12.8, 32.0, 80.0)
bw <- c(0.040, 0.040, 0.036) #wt avg bw for each exposure level rounded to g

cinh1 <- data.frame(ppm)
cinh <- lapply(cinh1, as.numeric)
outlist <- list()
ppm2 <- list()



for(i in 1:nrow(cinh1)){

 parms["CONC"] <-  ppm[i]
 parms["BW"] <-  bw[i]
 {

   out <-ode(Y, times, func = "derivs", parms = parms, method="vode", atol=1.0e-10, rtol=1.0e-8,
         dllname = mName, initforc="initforc", forcings=forcings, initfunc = "initmod", nout =
length(Outputs),
         fcontrol=list(method="linear"), outnames = Outputs)

 }
 outlist[[i]] <- out[nend,]
}
frout1 <- data.frame(outlist)
dout <- data.frame(t(frout1), row.names=paste(1:3))
rout <- cbind(dout[,c("ppm","AMP","AMPLU","AMPK")])

print("Female Mouse MCMC Redo")
print(rout)



#load the model dll
dyn.unload(paste0(mName,.Platform$dynlib.ext))
```

Human_Continuous.R (simulates continuous exposure – 7 days/week, 24 hrs/day and reports dose metrics on an average per day basis)

```
#Simulates human for 2 weeks using mouse study protocol (6 hr/day 5 days/week)
#Uses metabolism constants from Yang et al. 2012 Table 3

#Set the working directory to where you downloaded the scripts
setwd(dirname(parent.frame(2)$ofile))


#Load libraries needed to run scenario
library(deSolve)

#Model path and name
mName <- "chloroprene.model"

#Load model inits file for the ode solver
source(paste0(mName,"_inits.R"))

#Load the model dll
dyn.load(paste0(mName,.Platform$dynlib.ext))

#Scenario specific values
tstart <- 0.0
tstop <- 336
times <- seq(tstart, tstop , by=0.05)
nend <- length(times)

#Physiolgical parameters path
#Load the parameters
source('./params/Mhuman.R')   #Revised parameters from June 27 2018 update
source('./states.R')


#Timing variables for forcing functions
dstart <- tstart
dlength <- 24     #hours per day to expose
ddaysperwk <- 7  #days of week to expose
dexpend <- 100    #days of exposure
parms["TSTOP"] <- tstop

#Source forcing functions
#This loads the function forcing() in the namespace
source("forfunc.R")

#Scenario Specific Exposure
parms["CONC"]<-  12.8
```

```
#0.00028 ppm = 1.0 ug/m3
ppm <- c(0.00028, 0.001, 0.01, 0.1, 1.0, seq(2, 100, by=2))
lend <- length(ppm)
cinh1 <- data.frame(ppm)
cinh <- lapply(cinh1, as.numeric)
outlist <- list()
ppm2 <- list()



for(i in 1:nrow(cinh1)){

 parms["CONC"] <-  cinh1[i,]

 {

   out <-ode(Y, times, func = "derivs", parms = parms, method="vode",atol=1.0e-10, rtol=1.0e-8,
         dllname = mName, initforc="initforc", forcings=forcings, initfunc = "initmod", nout =
length(Outputs),
         fcontrol=list(method="linear"), outnames = Outputs)

 }
 outlist[[i]] <- out[nend,]
}
frout1 <- data.frame(outlist)
dout <- data.frame(t(frout1), row.names=paste(1:lend))
rout <- cbind(dout[,c("ppm","AMP","AMPLU","AMPK")])

#displays the output
print("Human Table 3")
print(rout)

#unload the model dll
dyn.unload(paste0(mName,.Platform$dynlib.ext))
```

Intended for

Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

SUPPLEMENTAL MATERIALS F
REACTIVE METABOLITE MODELING

Enhancing the PBPK Model for Chloroprene to include reactive oxidative metabolites responsible for the pulmonary toxicity and carcinogenicity

1. INTRODUCTION

A PBPK model was developed for chloroprene (CP; 2-chloro-1,3-butadiene) to estimate lung dose metrics and correlate these dose metrics with toxicity and carcinogenicity in the mouse (Clewell et al. 2019, 2020). The dose metric calculated with this PBPK model was total amount of chloroprene metabolized per gram of lung. Metabolism of chloroprene starts with oxidation via cytochrome P-450 enzymes producing two oxiranes – (1-chloroethenyl)oxirane (1-CEO) and (2-chloroethenyl)oxirane (2-CEO). Oxiranes produced by microsomal oxidation of ethenes, such as ethylene oxide or butadiene monoxide and butadiene diepoxide, are sufficiently stable to undergo Phase II metabolism by epoxide hydrolases (EHs) and glutathione transferases (GSTs) and to diffuse from the tissues where they are produced into the bloodstream for transport to other tissues (Johanson and Filser 1993; Kohn and Melnick 2000; Filser and Klein 2018). The goal of this present effort was to extend the PBPK model for chloroprene to include more detail on the epoxides and other reactive products formed by oxidative metabolism, and to describe the impact of production of reactive products on tissue glutathione (GSH).

In extending the PBPK model to include the production of various reactive intermediates, we examined papers on in vitro metabolism and analytical chemistry for identification of various metabolites of chloroprene (Cottrell et al. 2001; Munter et al. 2003; Himmelstein et al. 2004; Munter et al. 2007). Of the two reaction products formed by the microsomal oxidation of chloroprene, (1-chloroethenyl)oxirane (1-CEO) is expected to have a short, but measurable half-life in tissues. (2-chloroethenyl)oxirane, on the other hand, is expected to quickly rearrange to reactive aldehydes and ketones produced by both the spontaneous rearrangement of 2-CEO and the spontaneous rearrangement of the diepoxide and diepoxide diol produced from further oxidation of 1-CEO in the mouse (Himmelstein et al. 2004). This is similar to what has been observed with 1,1-DCE. The products of 2-CEO rearrangement are expected to react non-enzymatically and rapidly with GSH. The depletion of GSH by these aldehydes and ketones will affect the cellular GSH available for their detoxification. As GSH levels fall, the concentration of reactive metabolites increases, leading to exacerbation of toxic responses in the tissue because aldehydes and ketones then react with cellular constituents other than GSH. The expectations of increased tissue toxicity with GSH depletion are consistent with observations of liver and lung responses of fasted rats to CP inhalation (Jaeger et al. 1975; Plugge and Jaeger 1979) and similar responses in livers in fasted rats exposed to 1,1-DCE (McKenna et al. 1977; Andersen et al. 1980).

2. METHODS

Formation and clearance processes with 1-CEO: 1-CEO is formed by the oxidation of CP by cytochrome P450 enzymes, primarily CYP2f1 and 2e1 (Figure 1A). This oxidation step produces both 1- and 2-CEO and the relative split for the flux through both pathways was estimated separately for liver and lung microsomes (Himmelstein et al. 2004). In the subsequent equations, alpha (a) is the proportion of CYP-oxidation producing 1-CEO, and (1-a) is the

proportion producing 2-CEO (Figure 1A). In addition, the products of the first oxidation step include both the respective epoxide and diol due to the proximity of the cytochrome P450s and microsomal epoxide hydrolases within the microsomes, i.e. within the endoplasmic reticulum in the intact tissues. Thus, some diol is produced by an intracellular first-pass-like process where the proximity of the CYP enzymes and epoxide hydrolase in microsomal vesicles allows some direct conversion of the epoxide to the diol before release from the lipophilic environment of the microsome to the cytoplasm (Johanson and Filser 1993; Kohn and Melnick 2000). An estimate of the proportion of diol produced by the oxidation (b) was available from modeling with butadiene (Campbell et al. 2015). The subsequent clearance of 1-CEO occurs by three pathways in the mouse, EH/H2O hydrolysis, further microsomal oxidation of 1-CEO (Himmelstein et al. 2004) and, *in vivo*, diffusion of 1-CEO from tissue into the bloodstream. While reactions of 1-CEO with GSH, either catalyzed by GSTs or by direct non-enzymatic conjugation, are possible, there was no evidence for this pathway in human, rat, or mouse microsomal incubations (Munter et al. 2003). With human microsomes, there was no evidence of a second oxidation step consuming the 1-CEO (Himmelstein et al. 2004).

Kinetic constants for CP oxidation were estimated from in vitro studies following the loss of headspace CP from vials containing microsomal suspensions. Those for 1-CEO oxidation followed 1-CEO headspace loss using microsomal suspensions with added NADPH. 1-CEO hydrolysis was also assessed using microsomal preparations with no added NADPH. In these detailed kinetic studies of multiple pathways (Himmelstein et al. 2004), GSH conjugation was examined by evaluating loss of headspace 1-CEO with vials containing cytoplasm and 10 mM GSH – a GSH level about 5 times higher than background levels in lung (Csanady et al. 2003; Jaeger et al. 1974a). As noted in studies identifying metabolites of CP (Munter et al. 2003), there was no evidence for appreciable clearance of 1-CEO by reactions involving glutathione.

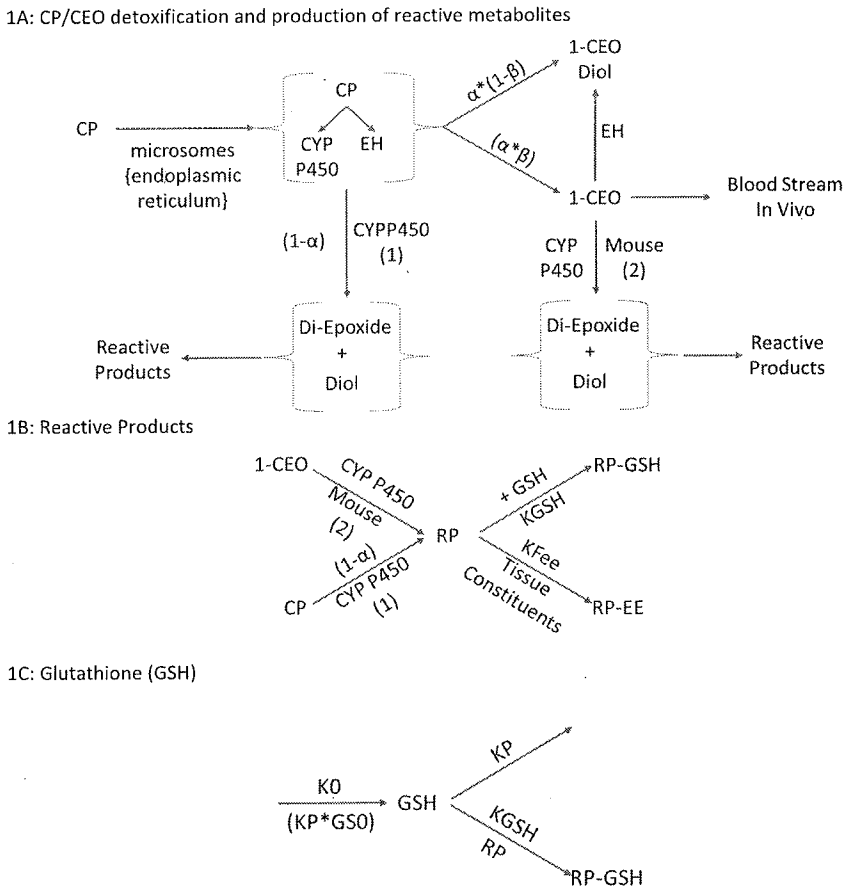


Figure F1. Schematics for the production and clearance of the three key components in the enhanced PBPK model for chloroprene (CP).

Formation and clearance processes with 2-CEO: In a fashion similar to 1-CEO, 2-CEO would be an intermediate of the oxidation of CP by CYP2e1 and 2f1 (Figure F1A). The proportion of 2-CEO formed is (1-α) times the net rate of loss of CP, where a is the proportion of the initial oxidation step that goes to 1-CEO. As with the formation of 1-CEO and 1-CEO diol, the proportion of the formation of the diol would be b times the total oxidation rate. However, neither 2-CEO nor 2-CEO diol are stable and they undergo rapid rearrangement to reactive aldehydes and ketones, all of which would react with GSH. Modeling of conjugation of reactive products with glutathione was similar to that used in PBPK models for reactive products with vinyl chloride (Clewell et al. 2001) and vinylidene chloride (D'Souza et al. 1988). In addition, the oxidative reaction of 1-CEO to a diepoxide also produces various reactive aldehydes and ketones. The net flux of all these reactive intermediates is captured in a single lumped compartment, called reactive products (RP).

Formation and tissue clearance of transient reactive products (RPs): The RP pool represents a group of reactive aldehydes and ketones that form by rearrangement of the unstable epoxide, 2-CEO, and of the diepoxide formed by oxidation of 1-CEO (Figure F2B). These products themselves are expected to be short-lived and react with other cellular components and with GSH, where the GSH conjugation pathway is favored at normal GSH concentrations. The rate

equation for RP would have the net rates of production of 2-CEO and the 1-CEO diepoxide and loss due to reaction with GSH and with other cellular constituents. The abbreviation, kfee, represent a first order rate constant for reaction of RP with "everything else". A first-order constant is used because the reaction of RP with these other tissue components is not expected to deplete the total reactant pool of these constituents to any great extent. A similar approach for modeling reactive intermediates was used with vinyl chloride (Clewell et al. 2001).

Depletion of GSH: The last process that needed to be included in the model was the production and removal of RP and the effect of higher rates of formation of RPs on GSH (Figure F1C). Higher rates of formation of RPs will cause depletion of GSH leading to increased tissue toxicity from these RPs. The rate constants for glutathione synthesis (Ko) and background loss (k1) have been approximated in various previous publications with vinyl chloride, ethylene dichloride and vinylidene chloride (D'Souza and Andersen 1988; D'Souza et al. 1988) and specifically for mouse lung in work with styrene and styrene oxide (Csanady et al. 2003). With these compounds, there is depletion of tissue glutathione, usually measured in liver, at higher exposures. While no direct measurements have been reported for GSH depletion in lungs caused by CP inhalation, the most sensitive gene ontology pathways affected by CP exposures in mice were associated with Nrf2-regulation of oxidative stress and GSH metabolism pathways (Thomas et al. 2013), an observation consistent with GSH loss during CP exposures.

Table F1. Parameters for the chloroprene metabolite model (chemical specific parameters for the physiology and chloroprene chemical specific parameters are reported in Supp Mat A).

Parameter	Description	Female Mouse	Female Rat
Chloroprene			
ALPHAL	Fraction of oxidative metabolism to 1-CEO in liver (remainder to 2-CEO)	0.02[1]	0.05[1]
ALPHALU	Fraction of oxidative metabolism to 1-CEO in lung (remainder to 2-CEO)	0.03[1]	0.15[1]
Fraction of total CP to 1-CEO privileged access			
BETA	Fraction of 1-CEO production available for hydrolysis/oxidative metabolism or release to blood	0.33[2]	0.33[2]
1-CEO			
Metabolism in Liver - Hydrolysis			
VMAXC1	Scaled VMax for Hydrolysis Pathway:Liver (mg/h/BW^0.75)	10.65[1]	62.1[1]
KM1	Km for Hydrolysis Pathway:Liver (mg/L)	1.9[1]	3.7[1]
Metabolism in Lung - Hydrolysis			
VMAXCLU1	Scaled VMax for Hydrolysis Pathway:Lung (mg/h/BW^0.75)	0.64[1]	0.85[1]
KMLU1	Km for Hydrolysis Pathway:Lung (mg/L)	4.6[1]	8.0[1]

Table F1. Parameters for the chloroprene metabolite model (chemical specific parameters for the physiology and chloroprene chemical specific parameters are reported in Supp Mat A).			
Parameter	**Description**	**Female Mouse**	**Female Rat**
Metabolism in Liver - Oxidative (Mouse pathway only)			
VMAXC1O	Scaled VMax for oxidative pathway in liver (mg/h/BW^0.75)	2.25[1]	NA
KM1O	Km for oxidative pathway in liver (mg/L)	1.5[1]	NA
LLOXACT	Lung to liver ratio for oxidative metabolism of 1-CEO (VMAXC10 scaled to lung)	0.42[4]	NA
Reactive Products			
K2LC	2nd order rate of RP reaction with GSH (L/umol/hr)	0.13[3]	0.13[3]
K2LUC	2nd order rate of RP reaction with GSH (L/umol/hr)	0.13[3]	0.13[3]
KFEEC	Conjugation rate with non-GSH (L/umol/hr)	35[3]	35[3]
LLEEACT	Lung to liver ratio for reactive products reaction with other cellular molecules (KFEEC scaled to lung)	0.14[4]	0.06[4]
GSH Parameters from ECD model			
KPC	First-order rate constant for GSH loss (/hr*kg $BW^{-0.3}$)	0.06[4]	0.06[4]
GSO	Initial GSH concentration in liver (uM)	7000[4]	5500[4]
GSOLU	Initial GSH concentration in lung (uM)	1500[4]	1200[4]
1-CEO Partition Coefficients			
PB1	Blood:Air	5.74[5]	5.74[5]
PLU1	Lung:Blood	0.69[5]	0.69[5]
PL1	Liver:Blood	1.18[5]	1.18[5]
PF1	Fat:Blood	5.15[5]	5.15[5]
PS1	Slowly Perfused:Blood	0.69[5]	0.69[5]
PR1	Rapidly Perfused:Blood	1.18[5]	1.18[5]

[1] Himmelstein et al. 2004

[2] Campbell et al. 2015

[3] Clewell et al. 2001

[4] Environ International 2004

[5] 1-CEO tissue:air and tissue:blood partitions were estimated using IndusChemFate (version 2.00, http://cefic-lri.org/toolbox/induschemfate/) and a logKow of 1.22 (KOWIN v.1.67 reported on Chemspider 2021. http://www.chemspider.com/Chemical-Structure.201536.html)

Metabolite Model Parameterization: The rate equations for the three components of the expanded model, i.e. 1-CEO (2A), RP (2B) and GSH (2C) are in Figure F2. The parameters used

in the chloroprene metabolite submodel are shown in Table F1 (IVIVE scaling of *in vitro* derived rate constants was performed in the same manner as in the parent chemical model). The fraction of total chloroprene metabolism to 1-CEO in liver (ALPHAL) and lung (ALPHALU) was reported in Himmelstein et al. (2004) for female mouse and rat. The fraction of 1-CEO that is available for distribution, hydrolysis, or oxidative metabolism (BETA) was set equal to the ratio for epoxybutene (Campbell et al. 2015) where 67% of the amount of epoxybutene produced from the metabolism of butadiene was further metabolized due to co-localization of enzymes (i.e. CYP P450 and EH) in the endoplasmic reticulum. The in vitro derived parameters for the hydrolysis and oxidative (mouse only) metabolism of 1-CEO in liver and lung were reported in Himmelstein et al. (2004). For the oxidative pathway in mouse, only the male mouse liver incubations provided levels of metabolism that allowed for estimation of the 1-CEO saturable metabolism parameters. The oxidative metabolism of 1-CEO in the lung was not measurable. As oxidative metabolism of 1-CEO could not be measured in the lung, the scaled liver maximum rate (VMAX1O) for the mouse was based on the ratio of the mixed function oxidase scaled in lung/liver (LLOXACT) previously reported for the mouse (Environ International 2004). The chemical reaction rate constants for the RP including the second order reaction with GSH in liver (K2L) and lung (K2LU), and reaction rate with other cellular molecules (KFEE) were taken from Clewell et al. (2001). For KFEELU, a scaler (LLEEACT) from liver to lung used. The partition coefficients for 1-CEO were calculated with the IndusChemFate model (ver. 2.0). Simulations with the chloroprene model were carried out in R (ver. 4.0.3). The metabolite submodel model code is included in the Appendix of this supplement.

Eq 1. 1-CEO

$$\frac{d(1CEO)}{dt} = \frac{\alpha \cdot \beta \cdot VMAX \cdot CP}{Km + CP} - \frac{VMAX1O^{*} \cdot 1CEO}{KM1O + 1CEO} - \frac{VMAX1 \cdot 1CEO}{KM1 + 1CEO} - PA_{1CEO} \cdot \frac{1CEO^{**}}{P_{1CEO}}$$

Eq 2. Reactive Product

$$\frac{d(RP)}{dt} = \frac{(1-\alpha) \cdot VMAX \cdot CP}{KM + CP} - \frac{VMAX1O^{*} \cdot 1CEO}{KM1O + 1CEO} - KGSH \cdot RP \cdot GSH - KFEE \cdot RP$$

Eq 3. Glutathione (GSH)

$$\frac{d(GSH)}{dt} = K0 - \mathrm{KP} * \mathrm{GSH} - \mathrm{KGSH} \cdot RP \cdot GSH$$

* Second microsomal oxidation step in mouse liver and lung only

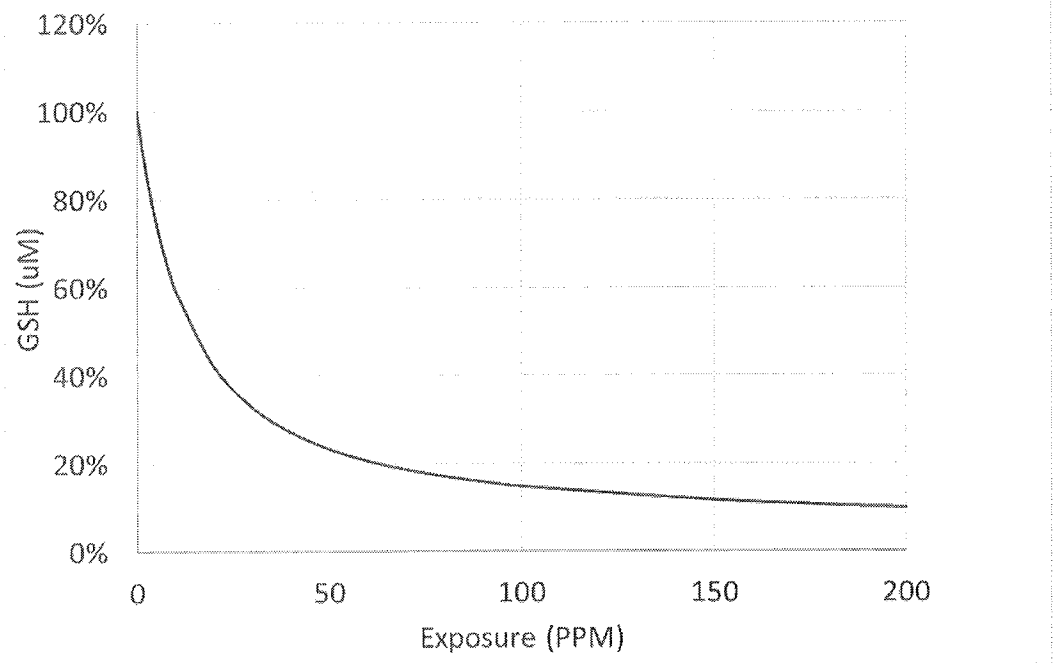
** For in vivo condition with transport out of tissue into blood stream

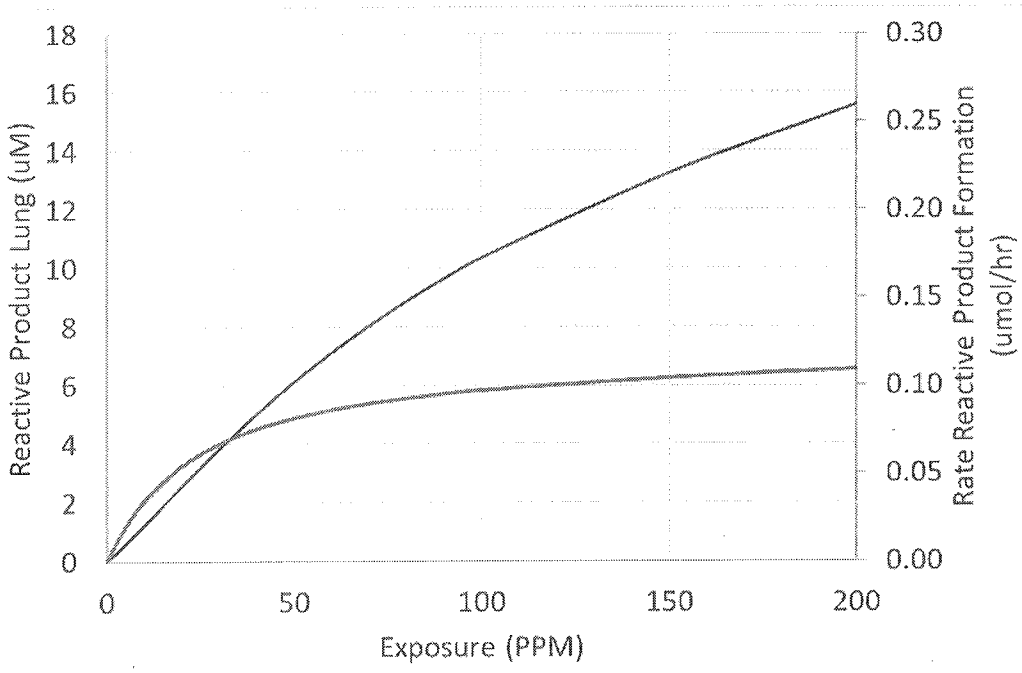
Figure F2. Rate equations for 1-CEO, reactive products (RP) and glutathione (GSH) in liver and lung.

3. RESULTS

Curves were first generated using this reactive-metabolite model to show the relationships between inhaled CP and GSH at the end of 6 hour exposures and between inhaled CP and concentrations of reactive RP and GSH at the end of 6 hours (Figure F3). The rate of metabolism versus inhaled CP follows a Michaelis-Menten form, quickly approaching a maximum rate at several 100 ppm (Figure F3 middle and bottom panel). The RPs formed by oxidation deplete GSH, with depletion to about 50% of the initial value at 15.3 ppm (Figure 3 top panel). As GSH

becomes depleted, RP cannot be cleared as efficiently and the RP concentration rise in a non-linear fashion with increasing exposure concentrations of CP.





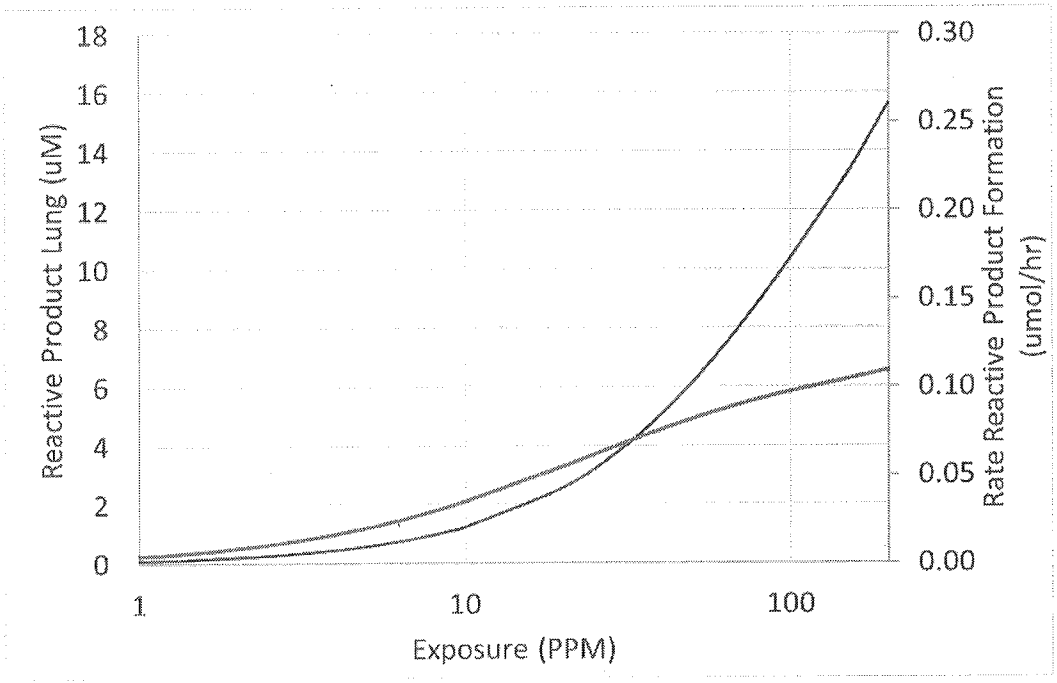


Figure F3. Predicted concentration of GSH (top panel) reactive product (middle and bottom panel, blue line) and rate of reactive product formation (middle and bottom panel, orange line) at the end of a single 6 hour exposure to chloroprene.

The extended model of chloroprene metabolism described above was exercised to evaluate three potential dose metrics for the lung toxicity and carcinogenicity of chloroprene: (1) total lung metabolism per gram lung (TMet), the dose metric used in the published PBPK models and previously submitted to the USEPA; (2) average concentration of reactive products of metabolism in the lung (PReact), and (3) average concentration of 1-CEO in the lung (1-CEO).

The first comparison performed was an evaluation of the consistency of the alternative dose metrics with the gene expression dose-response data reported in Thomas et al. (2013). In this study, female mice and rats were exposed to chloroprene by inhalation 6 hours per day, for 5 or 15 days. Mice were exposed at the bioassay concentrations, but the concentration range was extended in the rat to provide similar tissue doses based on predicted total amount of chloroprene metabolized per gram of lung tissue per day from a preliminary version of the PBPK model of Yang et al. (2012). For this comparison, two genomic responses were used: the lowest Benchmark Dose (BMD) for any gene expression change and the lowest BMD for any gene expression change related to regulation of glutathione homeostasis. A successful dose-metric for cross-species extrapolation should predict that cellular responses in the lung begin to occur at similar values of the dose metric. The results of the comparison are shown in Figure F4.

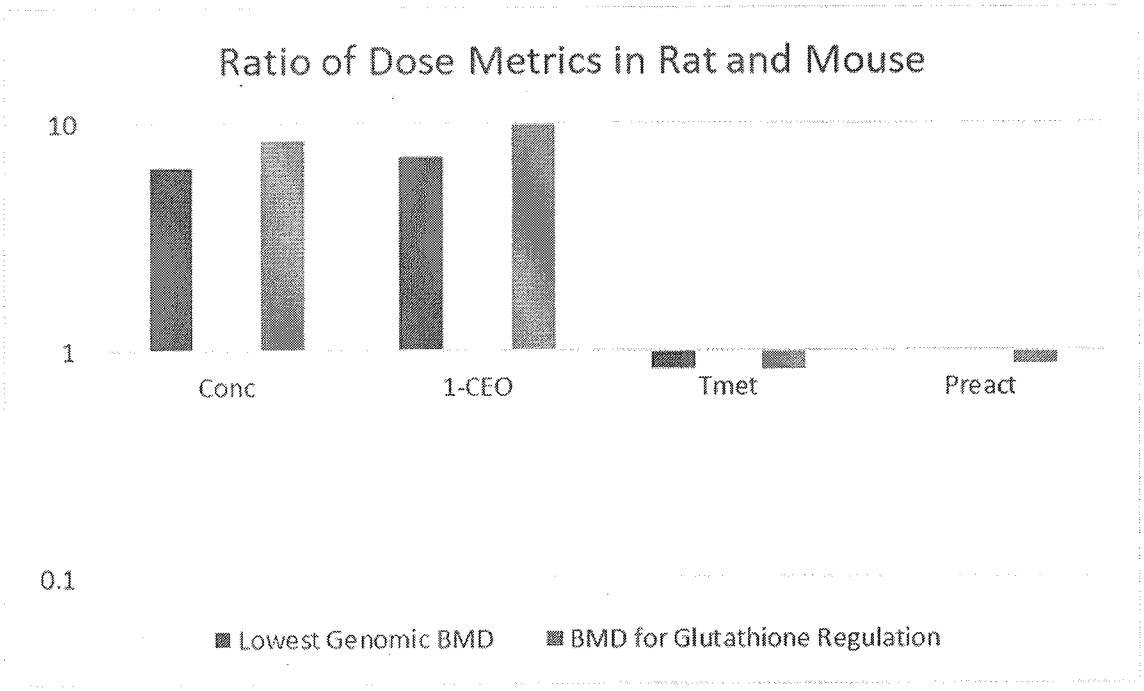
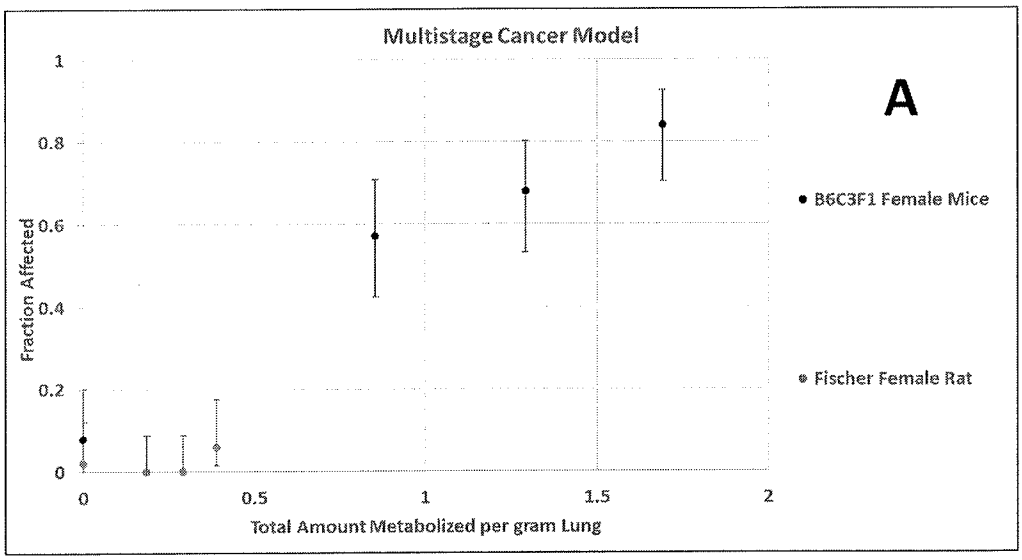


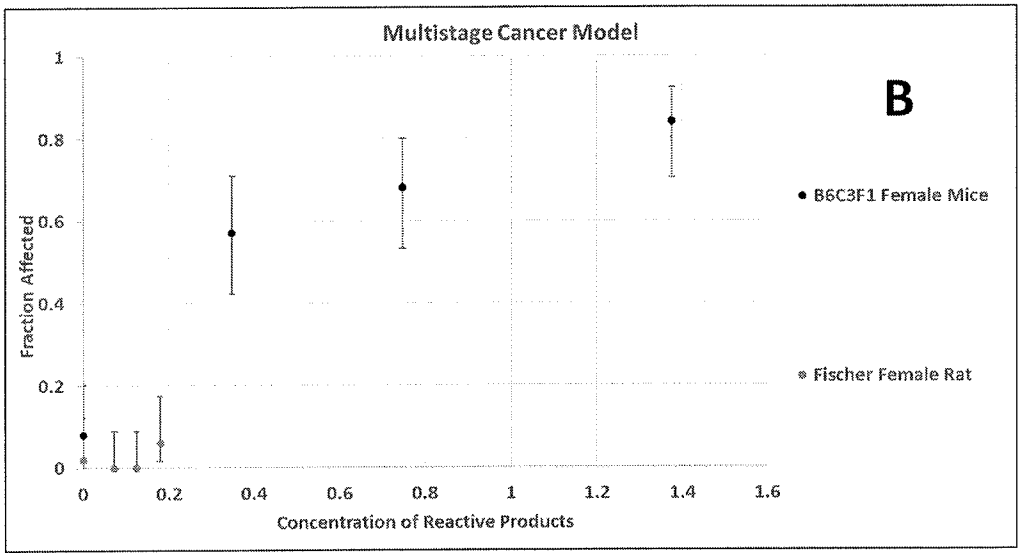
Figure F4. Cross-Species Consistency of Chloroprene Dose Metrics Based on Equivalence of Dose-Response for Gene Expression Changes (Thomas et al. 2013). An effective dose-metric for cross-species extrapolation should predict that responses in the lung occur at similar values of the dose metric, resulting in a ratio of unity. However, the lowest BMD and the lowest BMD for glutathione regulation in the rat occur at much higher inhaled concentrations (Conc) and 1-CEO concentrations (1-CEO) compared to the mouse. In contrast, they occur at similar values of total metabolism (TMet) and reactive product concentration (PReact) in the two species, supporting their appropriateness for cross-species extrapolation.

Consistent with the expectations that drove the experimental design in Thomas et al. (2013), the inhaled concentrations at which there was genomic evidence of cellular stress in the lungs of the rat were much higher than in the mouse. The predicted dose metric values for 1-CEO concentration associated with similar genomic biomarkers of cellular effects are also nearly an order of magnitude higher in the rat than in the mouse. In contrast, the model predicts similar dose metric values for both TMet and PReact in the rat and mouse, consistent with the expectation that cellular responses to chloroprene in the lung would begin to occur at similar levels of cellular stress. The consistency of these two dose metrics with the observed genomic dose-response in the female mouse and female rat, and the inconsistency of the 1-CEO or inhaled CP dose metrics, support the importance of reactive product formation in the mode of action for chloroprene.

The second comparison performed was an evaluation of the consistency of the alternative dose metrics with the tumor incidence in the bioassays for the female mouse and rat. This comparison could not be conducted in the male rat because, as described in Supplemental Materials B, the rate of metabolism in the rat lung was too low to support estimation of metabolism parameters. Figure F5(A-C) shows the predicted dose-response relationship for tumor incidence in the female mouse and female rat using the TMet, PReact and 1-CEO dose metrics. As in the previous

comparison, the TMet and Preact metrics provide a reasonable dose-response relationship with tumor incidence, whereas the 1-CEO metric does not. In fact, using the 1-CEO concentration as the dose metric would predict that the female rat should have had a higher tumor incidence than the female mouse.





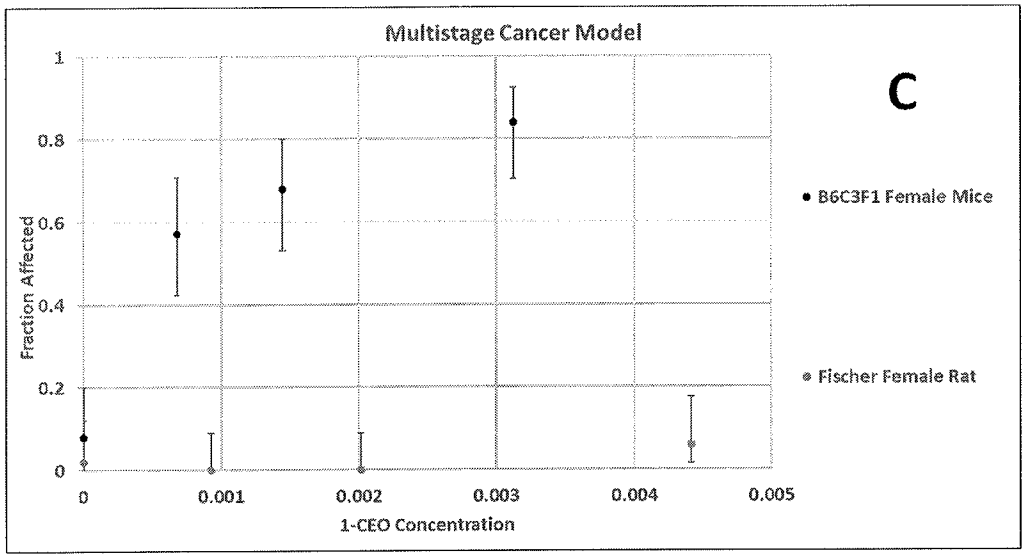


Figure F5. Benchmark dose assessment of dose metrics (A – total amount metabolized per day in lung; B – concentration of reactive products in lung; C – 1-CEO concentration in lung) predicted with the chloroprene model for the Thomas et al. 2013 concentrations.

The inconsistency of the 1-CEO dose metric with the relationships for both toxicity and carcinogenicity between the female mouse and female rat is likely due to the small proportion of total chloroprene metabolism that it represents. At the bioassay concentrations, the predicted concentrations of 1-CEO are less than 0.4% of the concentrations of reactive products in the female mouse and less than 5% in the rat.

4. DISCUSSION

Mode of Action: The toxicology and metabolism of both vinyl chloride (VC) and vinylidene chloride (VDC; 1,1-dichloroethylene) have been extremely well-characterized due to their uses as precursors for a variety of polymeric products. The research on toxicity of these compounds dates to the early 1970's. As with CP, the pathways of metabolism involve (1) CYP P450 oxidation, (2) production of reactive intermediates and (3) reaction of these reactive metabolites with glutathione and, (4), after sufficient glutathione depletion, with other cellular constituents. The reactivity toward proteins, lipids, and nucleic acids can lead to toxicity, the severity of which in turn depends on the reactivity and the dose of these metabolites. The initial studies with these two chlorinated ethylenes focused more on effects on liver rather than effects on lung, largely due to the identification of VC as a carcinogen in workers exposed to high concentrations of this monomer (Makk et al. 1974).

Cytochrome P450 mediated metabolism of VC forms an epoxide, chloroethylene oxide, which rearranges to chloroacetaldehyde. Both the epoxide and the aldehyde can react with and deplete cellular GSH (Watanabe and Gehring 1976), but even at very high inhaled concentrations, these metabolites have relatively low acute toxicity (Jaeger et al. 1974b). Both the epoxide and the aldehyde can form DNA-adducts (Green and Hathway 1978) and the tissue half-life of the epoxide will be much shorter than that of the aldehyde. Almost counter-intuitively, the carcinogenic responses to vinyl chloride arise partially due to production of metabolites that are

not themselves rapidly metabolized or overly reactive even following exposures to very high concentrations of VCM. Twenty-four hours after a 6 hr exposure to 5% VC (i.e. 50,000 ppm) there was no significant increase in alanine transaminase or sorbitol dehydrogenase in naïve rats and only a 10-to-20-fold increase in phenobarbital (PB) pretreated rats, where the PB pretreatment increases oxidative metabolism (Jaeger et al. 1974b). In the rat, the maximum metabolic rate, measured using gas uptake methods, was 40 μmoles/hr (Gargas et al. 1988) and macromolecular binding was not evident until GSH levels were decreased to 30% of basal levels (Watanabe and Gehring 1976). The cancer dose response curve was not consistent with VC as a dose metric but was consistent with the amount metabolized in the presence of significant depletion of GSH (Gehring et al. 1978; 1979), just as our extended model predicts.

Liver GSH levels are lower in fasted rats than in fed rats (Jaeger et al. 1974a). With this reduction in GSH, compounds that deplete liver GSH are more toxic to fasted than to fed rats. The LC50 of 1,1-DCE in fed rats was 15,000 ppm, but in fasted rats it was only 150 ppm. Serum enzymes increased abruptly at 100 ppm and were maximum at several hundred ppm. These responses are due to production of reactive metabolites that are cleared by GSH until the GSH becomes depleted. While the initial oxidation of 1,1-DCE produces an epoxide, this metabolite is unstable and undergoes spontaneous rearrangement producing chloroacetyl chloride, a highly reactive acid halide. These metabolites react with and deplete GSH levels. With severe GSH depletion, these metabolites react with tissue constituents leading to macromolecular binding and tissue toxicity (McKenna et al. 1977). Unlike VC, 1,1-DCE does not cause significant increases in hemangiosarcoma or any other liver tumors. However, with both VC and VDC, all metabolism goes through a single epoxide.

In early work examining the hepatic toxicity of CP in rats including the effects of fasting to restrict GSH resynthesis, Plugge and Jaeger (1979) noted that the pattern of toxicity was comparable to VDC although higher exposures of CP were required to produce equivalent increases in SAKT (Jaeger et al. 1974a; Plugge and Jaeger 1979). As noted earlier, metabolism of CP mainly produces a combination of reactive aldehydes and ketones derived from 2-(chloroethenyl)-oxirane. With only about a fraction of a*b total metabolism (3% in female mouse lung) producing 1-CEO, that itself is further oxidized to reactive products by a second oxidation in the mouse. Using kinetic constants determined for GSH synthesis and consumption from studies with styrene and styrene oxide, we showed here that the metabolism of CP in the lungs is expected to cause depletion of GSH (Figure F3 top panel) and the lung transcriptomic responses are indicative of changes in GSH metabolism as the most sensitive ontology pathway (Thomas et al. 2013). Only with sufficient GSH depletion will the reactivity with tissue components lead to extensive macromolecular binding and overt toxicity and increased tumor incidence. With VC, it was estimated that there was relatively little macromolecular binding if depletion was less than 30%. Here, our analysis showed that tumor incidence tracks with total metabolized or expected concentration of RP rather than inhaled CP or 1-CEO concentrations. All the bioassay concentrations (12.8, 30 and 80 ppm) are expected to cause much more than 30% depletion of GSH (Figure F3 top panel). Depletion of GSH to 30 % basal levels is predicted to occur at 6.8 ppm and 50% depletion at 15.3 ppm. The middle panel (Figure F3) in the plots for RP and rate of formation of RP show the non-linear relationship between RP and rate of metabolism and the increasing slope of the RP curve at low CP concentrations. Our modeling results capture the non-linear relationship between RP and total rate of metabolism. These results demonstrate the marked increase in the slope of RP as the exposure increases, and are

consistent with the body of work in the toxicology of these chlorinated compounds and on the dose response for tumors with CP.

Both 1-CEO and, (Z)-2-chlorobut-2-en-1-al, a reactive aldehyde derived from 2-CEO, formed adducts when incubated with specific nucleotides (Munter et al. 2007). While the studies are not necessarily representative of reaction conditions with native DNA in vivo, they show the ability of some 1-CEO and at least one of the reactive CP metabolites to react with bases in DNA and form adducts. Our MOA with CP does not dismiss formation of these adducts but instead highlights that there is a threshold below which macromolecular binding is small and the cancer dose response is driven by production of reactive metabolites together with increasing levels of GSH depletion. Small changes in the numbers of adducts are not expected to define the shape of the dose response curve at low doses. In fact, there is always a substantial background of various adducts with more than 40,000 altered bases per cell (Nakamura et al. 2014). At low levels of exposure, ith increases in only a small number of adducts, DNA damage response networks would still be capable of effectively maintaining the integrity of the DNA prior to cell division through non-linear feedback processes (Zhang et al. 2014; Clewell and Andersen 2016).

Overall, the dose response for lung tumors from CP is consistent with a non-linear cytotoxicity with macromolecular binding with protein, lipid and nucleic acid bases at higher exposures.

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APPENDIX 1

Chloroprene model code for the epoxy submodel

```
#Chloroprene PBPK Model
#Translated from the acslX model presented in Yang et al. 2012
#By Jerry Campbell 2019
#Added Tracheobronchial region
#Added 1-CEO, Reaction Product and GSH submodels
#By Jerry Campbell 2021

States  = {
AI ,
AX ,
AM ,
AMCP_1CE ,
AMCP_2CE ,
AMLU ,
AMLUCP_1CE ,
AMLUCP_2CE ,
AMK ,
ALU ,
AL ,
AK ,
AS ,
AR ,
AUCCR ,
AF ,
AX1 ,
AM1 ,
AMLU1 ,
AM1O ,
AMLU1O  ,
ALUE1 ,
ALU1 ,
ALE1 ,
AL1 ,
AS1 ,
AR1 ,
AF1          ,
ARPG         ,
ARPGLU       ,
ARPEE        ,
ARPEELU      ,
ALRPPRO ,
ALRP         ,
ALURPPRO ,
ALURP        ,
AGSHL        ,
AGSHLU       ,
```

```
AUCCLRP      ,
AUCCLURP     ,
AUCCEO1L  ,
AUCCEO1LU ,
AUCGSHL ,
AUCGSHLU
};

Outputs = {
CVL ,
GSHL ,
GSHLU ,
CLRP  ,
CLURP ,
VL  ,
MASBAL ,
A1CEOGEN ,
MASBAL1 ,
CLU          ,
CL           ,
CK           ,
CS           ,
CR           ,
CF           ,
CV  ,
CVLUM        ,
CVLUM1 ,
CLUE1 ,
CLU1  ,
CLE1 ,
CL1          ,
CS1          ,
CR1          ,
CF1          ,
CV1  ,
qcbal ,
vbal ,
ppm ,
AMP          ,
AMPLU        ,
AMPK         ,
AM1L ,
AM1LU ,
AM1LO ,
AM1LUO ,
ARPL  ,
ARPLU ,
```

```
ARPOTHL ,
ARPOTHLU ,
ARPGSHL ,
ARPGSHLU ,
CLRPAVG ,
CLURPAVG ,
CL1AVG  ,
CLU1AVG ,
GSHLAVG ,
GSHLUAVG
};


Inputs = {EXPPULSE}  ;


#BODY WEIGHT (kg)
BW = 0.03 ;    # Body weight (kg)


#SPECIAL FLOW RATES
QPC = 29.1 ;     # Unscaled Alveolar Vent (L/h/kg^0.75)
QCC = 20.1 ;     # Unscaled Cardiac Output (L/h/kg^0.75)

#FRACTIONAL BLOOD FLOWS TO TISSUES
QLC = 0.161 ;   # Flow to Liver as % Cardiac Output (unitless)
QFC = 0.07  ;   # Flow to Fat as % Cardiac Output (unitless)
QSC = 0.159 ;   # Flow to Slow as % Cardiac Output (unitless)
QKC = 0.09  ;   # Flow to Kidney as % Cardiac Output (unitless)


#FRACTIONAL VOLUMES OF TISSUES
VLC = 0.055 ;      # Volume Liver as % Body Weight (unitless)
VLUC = 0.0073 ;   # Volume Lung as % Body Weight (unitless)
VFC = 0.1  ;         # Volume Fat as % Body Weight (unitless)
VRC = 0.08098 ;      # Volume Rapid Perfused as % Body Weight (unitless)
VSC = 0.384  ;       # Volume Slow Perfused as % Body Weight (unitless)
VKC = 0.0167 ;       # Volume Kidney as % Body Weight  (unitless)

#PARTITION COEFFICIENTS PARENT
#Chloroprene
PL = 1.26 ;    # Liver/Blood Partition Coefficient (unitless)
PLU = 2.38 ;    # Lung/Blood Partition Coefficient (unitless)
PF = 17.35 ;    # Fat/Blood Partition Coefficient (unitless)
PS = 0.59  ;   # Slow/Blood Partition Coefficient (unitless)
PR = 1.76 ;    # Rapid/Blood Partition Coefficient (unitless)
```

```
PB = 7.83 ;    # Blood/Air Partition Coefficient (unitless)
PK = 1.76 ;    # Kidney/Blood Partition Coefficient (unitless)

#PARTITION COEFFICIENTS 1-CEO (IndusChemFate, LogKow 1.22)
PL1 = 1.26 ;     # Liver/Blood Partition Coefficient (unitless)
PLU1 = 2.38 ;    # Lung/Blood Partition Coefficient (unitless)
PF1 = 17.35 ;    # Fat/Blood Partition Coefficient (unitless)
PS1 = 0.59  ;    # Slow/Blood Partition Coefficient (unitless)
PR1 = 1.76 ;     # Rapid/Blood Partition Coefficient (unitless)
PB1 = 7.8 ;     # Blood/Air Partition Coefficient (unitless)



#KINETIC CONSTANTS
MW = 88.5  ;  # Molecular weight (g/mol)
MWCEO = 104.5 ;  # 1-CEO Molecular weight (g/mol)

#Chloroprene
#Fraction of total metabolism to 1-CEO
ALPHAL = 0.02 ;   #Fraction of liver chloroprene metabolism to 1-CEO
ALPHALU = 0.03 ;   #Fraction of lung chloroprene metabolism to 1-CEO
#Fraction of total CP to 1-CEO privaleged access
#(based on butadiene model Campbell et al. 2015; assumed same in liver and lung)
BETA = 0.67 ;

# CP Metabolism in Liver
VMAXC = 7.95  ;  # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
KM = 0.041  ;  # Km for Oxidative Pathway:Liver (mg/L)

# CP Metabolism in Lung
VMAXCLU = 0.18;     # Scaled VMax for Oxidative Pathway:Lung (mg/h/BW^0.75)
KMLU = 0.26;        # Km for Oxidative Pathway:Lung (mg/L)

# CP Metabolism in Kidney
VMAXCKid = 0.0 ;    # Scaled VMax for Oxidative Pathway:Kidney (mg/h/BW^0.75)
KMKD = 1.0  ;  # Km for Oxidative Pathway :Kidney

#1-CEO
#1-CEO Metabolism in Liver - Hydrolysis
VMAXC1 = 7.95  ;  # Scaled VMax for Hydrolysis Pathway:Liver (mg/h/BW^0.75)
KM1 = 0.041  ;  # Km for Hydrolysis Pathway:Liver (mg/L)

#1-CEO Metabolism in Lung - Hydrolysis
VMAXCLU1 = 0.18;     # Scaled VMax for Hydrolysis Pathway:Lung (mg/h/BW^0.75)
KMLU1 = 0.26;        # Km for Hydrolysis Pathway:Lung (mg/L)

#1-CEO Metabolism in Liver - Oxidative (Mouse Only!!!)
```

```
VMAXC1O = 7.95  ;   # Scaled VMax for Oxidative Pathway:Liver (mg/h/BW^0.75)
KM1O = 0.041  ;   # Km for Oxidative Pathway:Liver (mg/L)


#Reactive Products
#Liver
KGSHLC = 0.0 ;     #2nd order rate of RP reaction with GSH
K3L = 0.0 ;     #Reaction rate with cellular macromolecules
MML = 0.0 ;     #macrolecule concentration (mM)


#Lung
KGSHLUC = 0.0 ;     #2nd order rate of RP reaction with GSH (L/mmol/hr)
K3LU = 0.0 ;     #Reaction rate with cellular macromolecules (L/mmol/hr)
MMLU = 0.0 ;   #macrolecule concentration (mM)

#GSH
K0L = 0.0 ;         #Production of GSH
K1L = 0.0 ;         #Background loss of GSH

K0LU = 0.0 ;         #Production of GSH
K1LU = 0.0 ;         #Background loss of GSH

#Permeation Coefficient (fraction of blood flow)
PA1 = 1.0 ;        #Permeation Coefficient for 1-CEO in lung

#DOSING INFORMATION
TSTOP = 7.0 ;     # Dosing stop time
CONC = 13.0  ;   # Initial concentration (ppm)

#Parameters for GSH submodel
LLOXACT = 0.14 ;     #Scaler for liver to lung oxidative metabolism 1-CEO (mouse only)
LLEEACT = 0.06 ;     #Scaler for liver to lung oxidative metabolism 1-CEO (mouse only)

KFEEC = 4500.0 ;     # 1/hr/kg Conjugation rate with non-GSH
 KPC = 0.06  ;     # First-order rate constant for GSH loss
 GSO = 5500.0  ;   # Initial GSH concentration liver
GSOLU = 1200.0  ;   # Initial GSH concentration lung



Dynamics {
#########################################################################
#####################
# Scaled parameters

     QC = QCC*pow(BW,0.75)  ;       #Cardiac output
```

```
    QP = QPC*pow(BW,0.75)  ;      #Alveolar ventilation
    QL = QLC*QC    ;             #Liver blood flow
    QF = QFC*QC    ;             #Fat blood flow
    QS = QSC*QC    ;             #Slowly-perf tissue blood flow
    QK = QKC*QC    ;             #Kidney tissue blood flow

   QRC = 1 - QLC - QKC - QFC - QSC ;  #Rapily Perfused tissues
    QR = QRC*QC           ;                        #Rapily-perf tissue blood flow

    VL = VLC*BW      ;           #Liver volume
   VLU = VLUC*BW     ;            #Lung volume
    VF = VFC*BW      ;           #Fat tissue volume
    VS = VSC*BW      ;           #Slowly-perfused tissue volume
    VR = VRC*BW      ;           #Richly-perfised tissue volume
    VK = VKC*BW      ;           #kidney tissue volume

  ROBC = 1 - VLC - VLUC - VFC - VSC - VRC - VKC ;  #Rest of body un-perfused tissue for MC

# METABOLISM
   VMAX = VMAXC*pow(BW,0.75)  ;       #Maximum rate of metabolism-Liver (mg/hr)
 VMAXLU = VMAXCLU*pow(BW,0.75) ;      #Maximum rate of metabolism-Lung (mg/hr)
 VMAXKD = VMAXCKid*pow(BW,0.75)  ;    #Maximum rate of metabolism-Kidney (mg/hr)

  VMAX1 = VMAXC1*pow(BW,0.75)  ;      #Maximum rate of metabolism-Liver (mg/hr)
VMAXLU1 = VMAXCLU1*pow(BW,0.75) ;     #Maximum rate of metabolism-Lung (mg/hr)
 VMAX1O = VMAXC1O*pow(BW,0.75)  ;     #Maximum rate of metabolism-Liver (mg/hr)

 VMAXLU1O = VMAX1O*LLOXACT ;         #Liver Vmax scaled to lung (ECD model)

  KGSHL = KGSHLC;
   KFEE = KFEEC ;
 KGSHLU = KGSHLUC;
 KFEELU = KFEE*LLEEACT  ;
    KP = KPC*pow(BW,-0.3)  ;
  KOTDL = KP*GSO ;
KOLUTDL = KP*GSOLU ;



#############################################################
#####################
# Exposure Control (mg/L)
    CIX = CONC*MW/24450 ;
     CI = CIX *EXPPULSE  ;

#############################################################
#####################
```

```
# Chloroprene
# Tissue Venous Concentrations (mg/L)
    CVLU = ALU/(VLU*PLU) ;
     CVL = AL/(VL*PL) ;
     CVK = AK/(VK*PK) ;
     CVS = AS/(VS*PS) ;
     CVR = AR/(VR*PR) ;
     CVF = AF/(VF*PF) ;

#Tissue Concentration (mg/L)
# 1-CEO
# Tissue Venous Concentrations (mg/L)
     CLUE1 = ALUE1/VLU ;
     CVLU1 = ALU1/(VLU*PLU1) ;
     CLU1  = (ALUE1+ALU1)/VLU ;
     CLE1  = ALE1/VL ;
     CVL1  = AL1/(VL*PL1) ;
     CL1   = (ALE1 + AL1)/VL ;
     CVS1  = AS1/(VS*PS1) ;
     CVR1  = AR1/(VR*PR1) ;
     CVF1  = AF1/(VF*PF1) ;


#Concentration of GSH in Liver and Lung
 GSHL = AGSHL/VL ;     #GSH concentraiton in liver (mM)
 GSHLU = AGSHLU/VLU ;  #GSH concentraiton in lung (mM)



#########################################################################
######################
# Concentration in Pulmonary/Arterial and venous blood Compartments (mg/L)
     CPU = (QP*CI+(QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK))/(QP/PB+QC)  ;
      CX = CPU/PB ;
      CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK)/QC ;
    CPUM = CPU*1000/MW ;
     RAI = QP*CI ;
     dt(AI) = RAI ;
     RAX = QP*CX ;
     dt(AX) = RAX ;

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#Rate amount metabolized in liver, lung and kidney
```

```
# Amount metabolized in Liver (mg)
    RAM = VMAX*CVL/(KM+CVL) ;
    dt(AM) = RAM ;

    #Total 1 and 2-CEO from CP
    RAMCP_1CEO = RAM*ALPHAL ;    #CP metabolized to 1-CEO in Liver
    dt(AMCP_1CE) = RAMCP_1CEO ;

    RAMCP_2CEO = RAM*(1-ALPHAL) ; #CP metabolized to 2-CEO in Liver
    dt(AMCP_2CE) = RAMCP_2CEO ;


# Amount metabolized in Lung (mg)
    RAMLU = VMAXLU*CVLU/(KMLU+CVLU) ;
    dt(AMLU) = RAMLU ;

    RAMLUCP_1CEO = RAMLU*ALPHALU ; #CP metabolized to 1-CEO in Lung
    dt(AMLUCP_1CE) = RAMLUCP_1CEO ;

    RAMLUCP_2CEO = RAMLU*(1-ALPHALU) ; #CP metabolized to 2-CEO in Lung
    dt(AMLUCP_2CE) = RAMLUCP_2CEO ;

# Amount metabolized in Kidney (mg)
    RAMK = VMAXKD*CVK/(KMKD + CVK)  ;
    dt(AMK) = RAMK ;

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# Amount in Lung Compartment (mg)
    RALU = QC*(CPU-CVLU) - RAMLU  ;
    dt(ALU) = RALU ;

# Amount in Liver Compartment (mg)
    RAL = QL*(CVLU-CVL) - RAM ;
    dt(AL) = RAL ;

# Amount in Kidney Compartment (mg)
    RAK = QK*(CVLU-CVK) - RAMK ;
    dt(AK) = RAK ;

# Amount in Slowly Perfused Tissues (mg)
    RAS = QS*(CVLU-CVS) ;
    dt(AS) = RAS ;
```

```
# Amount in Rapidly Perfused Tissues (mg)
    RAR = QR*(CVLU-CVR) ;
    dt(AR) = RAR ;
    dt(AUCCR) = AR/VR ;

# Amount in Fat Compartment (mg)
    RAF = QF*(CVLU-CVF) ;
    dt(AF) = RAF ;

###############################################################
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#1-CEO submodel

###############################################################
######################
###############################################################
######################
###############################################################
######################
# Concentration 1-CEO in Pulmonary/Arterial and venous blood Compartments (mg/L)
     CV1 = (QF*CVF1 + QL*CVL1 + QS*CVS1 + (QR+QK)*CVR1)/QC ;
    CPU1 = (QC*CV1)/(QP/PB1+QC)  ;
     CX1 = CPU1/PB1 ;
    RAX1 = QP*CX1 ;
  dt(AX1) = RAX1 ;

#Rate amount 1-CEO metabolized in liver and lung
#Hydrolysis (1-CEO to diol)
# Amount metabolized in Liver (mg)
      RAM1 = VMAX1*CVL1/(KM1 + CVL1) ;
   dt(AM1) = RAM1 ;

# Amount metabolized in Lung (mg)
    RAMLU1 = VMAXLU1*CVLU1/(KMLU1 + CVLU1) ;
  dt(AMLU1) = RAMLU1 ;

#Oxidative (Mouse Only!!!)
# Amount metabolized in Liver (mg)
      RAM1O = VMAX1O*CVL1/(KM1O + CVL1) ;
   dt(AM1O) = RAM1O ;

# Amount metabolized in Lung (mg)
      RAMLU1O = VMAXLU1O*CVLU1/(KM1O + CVLU1) ;
   dt(AMLU1O) = RAMLU1O ;
```

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# 1-CEO in Lung Compartment
# Amount in Lung Epithelium (mg)
    RALUE1 = PA1*QC*(CVLU1 - CLUE1/PLU1) + RAMLUCP_1CEO*BETA*MWCEO/MW - RAMLU1
- RAMLU1O ;
    dt(ALUE1) = RALUE1 ;

# Amount in Lung Submucosa (mg)
    RALU1 = QC*(CPU1-CVLU1) + PA1*QC*(CLUE1/PLU1 - CVLU1) ;
    dt(ALU1) = RALU1 ;

# 1-CEO in Liver
# Amount in Liver Epithelium (mg)
    RALE1 = PA1*QL*(CVL1 - CLE1/PL1) + RAMCP_1CEO*BETA*MWCEO/MW - RAM1 - RAM1O ;
    dt(ALE1) = RALE1 ;

# Amount in Liver Compartment (mg)
    RAL1 = QL*(CVLU1-CVL1) + PA1*QL*(CLE1/PL1 - CVL1) ;
    dt(AL1) = RAL1 ;


# Amount in Slowly Perfused Tissues (mg)
    RAS1 = QS*(CVLU1 - CVS1) ;
    dt(AS1) = RAS1 ;

# Amount in Rapidly Perfused Tissues (mg)
    RAR1 =(QR+QK)*(CVLU1 - CVR1) ;
    dt(AR1) = RAR1 ;

# Amount in Fat Compartment (mg)
    RAF1 = QF*(CVLU1 - CVF1) ;
    dt(AF1) = RAF1 ;

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#Reactive Products (converted to umol or umol/L for GSH submodel)
```

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 CLRP = ALRP/VL ;    #(umol/L)
CLURP = ALURP/VLU ;    #(umol/L)


# ACMG = AMOUNT METABOLITE CONJUGATED WITH GLUTATHIONE (UMOLES)
    RARPG = KGSHL*GSHL*CLRP*VL ;
  dt(ARPG) = RARPG ;

   RARPGLU = KGSHLU*GSHLU*CLURP*VLU ;
 dt(ARPGLU) = RARPGLU ;

# ACMEE = AMOUNT METABOLITE CONJUGATED WITH OTHER THINGS (UMOLES)
    RARPEE = KFEE*CLRP*VL ;
  dt(ARPEE) = RARPEE ;

  RARPEELU = KFEELU*CLURP*VLU ;
dt(ARPEELU) = RARPEELU  ;


 #Reactive products in liver (umol)
dt(ALRPPRO) = (RAMCP_2CEO/MW)*1000 + (RAM1O/MWCEO)*1000 ;
    RALRP =  (RAMCP_2CEO/MW)*1000 + (RAM1O/MWCEO)*1000 - RARPG - RARPEE ;
  dt(ALRP) = RALRP ;

 #Reactive products in lung (umol)
dt(ALURPPRO) = (RAMLUCP_2CEO/MW)*1000 + (RAMLU1O/MWCEO)*1000 ;
   RALURP = (RAMLUCP_2CEO/MW)*1000 + (RAMLU1O/MWCEO)*1000 - RARPGLU - RARPEELU
;
 dt(ALURP) = RALURP ;


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#GSH

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 #GSH in liver (umol)
  RAGSHL = KOTDL*VL - KP*GSHL*VL - RARPG  ;
dt(AGSHL) = RAGSHL ;

 #GSH in LU (umol)
  RAGSHLU = KOLUTDL*VLU - KP*GSHLU*VLU - RARPGLU  ;
dt(AGSHLU) = RAGSHLU ;

#AUCs for reactive products and 1-CEO:
dt(AUCCLRP) = CLRP ;  #uM*hr
dt(AUCCLURP) = CLURP ;  #uM*hr
dt(AUCCEO1L) = CL1/MWCEO*1000 ;  #uM*hr
dt(AUCCEO1LU) = CLU1/MWCEO*1000 ;  #uM*hr

dt(AUCGSHL) = GSHL ;  #uM*hr
dt(AUCGSHLU) = GSHLU ;  #uM*hr

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} # End of Dynamics

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CalcOutputs {
# Mass-balance
            MASBAL  = AI - AX - (AL+AM+AMLU+AK+AMK+AS+AR+AF+ALU) ;
            A1CEOGEN = AM*ALPHAL*(1-BETA)*MWCEO/MW + AMLU*ALPHALU*(1-
BETA)*MWCEO/MW ;
            MASBAL1 =  A1CEOGEN - AX1 - (AM1+AMLU1+AM1O+AL1+AS1+AR1+AF1+ALU1)
;

            #Tissue Concentrations (mg/L)
      CLU = ALU/VLU ;
       CL = AL/VL ;
       CK = AK/VK ;
```

```
        CS = AS/VS ;
        CR = AR/VR ;
        CF = AF/VF ;
#Concentrations for plots
                CVLUM = CV*1000/MW ;  #(umol/L)
  CVLUM1 = CV1*1000/MWCEO ;  #(umol/L)

                #Tissue Concentrations 1-CEO (mg/L)
        CS1 = AS1/VS ;
        CR1 = AR1/VR ;
        CF1 = AF1/VF ;

#Blood Flow balance
   qcbal = QC - QL - QF - QS - QK - QR ;
#Tissue Volume balance
    vbal = BW*(1-ROBC) - VL - VLU - VF - VS - VK - VR ;

#Dose metrics are only correct when simulation time=tstop
   ppm = CONC ;

   #Total Metabolism umol/g/day
   AMP = ((AM*1000/MW)/(VL*1000))/(TSTOP/24) ;
 AMPLU = ((AMLU*1000/MW)/(VLU*1000))/(TSTOP/24) ;
  AMPK = ((AMK*1000/MW)/(VK*1000))/(TSTOP/24) ;

  #Hydrolase Metabolism of 1-CEO (umol/g/day)
  AM1L = ((AM1)/(VL*1000))/(TSTOP/24) ;
 AM1LU = ((AMLU1)/(VLU*1000))/(TSTOP/24) ;

  #Oxidative metabolims of 1-CEO umol/g/day
  AM1LO = ((AM1O)/(VL*1000))/(TSTOP/24) ;
  AM1LUO = ((AMLU1O)/(VLU*1000))/(TSTOP/24) ;

  #Total production of RP (umol/g/day)
  ARPL = ((ALRPPRO)/(VL*1000))/(TSTOP/24) ;
 ARPLU = ((ALURPPRO)/(VLU*1000))/(TSTOP/24) ;

  #Total reaction of RP with other (umol/g/day)
  ARPOTHL = ((ARPEE)/(VL*1000))/(TSTOP/24) ;
 ARPOTHLU = ((ARPEELU)/(VLU*1000))/(TSTOP/24) ;

  #Total reaction of RP with GSH (umol/g/day)
  ARPGSHL = ((ARPG)/(VL*1000))/(TSTOP/24) ;
 ARPGSHLU = ((ARPGLU)/(VLU*1000))/(TSTOP/24) ;

 #Average concentration of RP (uM)
 CLRPAVG = AUCCLRP/TSTOP ;
```

```
CLURPAVG = AUCCLURP/TSTOP ;

#Average concentration of 1-CEO (uM)
CL1AVG = AUCCEO1L/TSTOP ;
CLU1AVG = AUCCEO1LU/TSTOP ;

#Average concentration of GSH (uM)
GSHLAVG = AUCGSHL/TSTOP ;
GSHLUAVG = AUCGSHLU/TSTOP ;


} # End of CalcOutputs

End.
```

Intended for

Denka Performance Elastomer LLC, Request for Correction

Exhibit A

Date

July 15, 2021

SUPPLEMENTAL MATERIALS G
RESPONSES TO PEER REVIEWER COMMENTS

Ramboll's Response to External Peer Review Tier 1 and Tier 2 Comments/ Recommendations[1]

Ramboll scientists have reviewed the Post-Meeting Peer Review Summary Report entitled "External Peer Review of a Report on Physiologically Based Pharmacokinetic (PBPK) Modeling for Chloroprene (Ramboll 2020) and a Supplemental Analysis of Metabolite Clearance (USEPA 2020)" and dated December 8, 2020. These Supplemental Materials list the Tier 1 Key Recommendations and Tier 2 Suggestions from the reviewers and provide Ramboll's responses, along with a description of the associated revisions to the original PBPK model documentation.

> Tier 1: Key Recommendations – Recommendations that are necessary for strengthening the scientific basis for the PBPK model, reducing model uncertainties (especially with respect to typical expectations for a PBPK model) or accurately evaluating such uncertainties before the model is applied for risk assessment.
>
> Tier 2: Suggestions – Recommendations that are encouraged in order to strengthen confidence before the PBPK model is potentially applied in risk assessment. It is understood that other factors (e.g. timeliness) may also be considered before deciding to conduct the suggested additional research or model revisions.

The responses are organized by general topic, with the question from the charge to the peer reviewers noted with each comment. All Tier 1 and Tier 2 comments have been addressed and resolved. We appreciate the reviewers' comments and believe that the additional analyses we have performed at their request have increased the strength of the revised PBPK model.

COMMENTS AND RESPONSES RELATED TO KGL

Tier 1

Annie Lumen (Question 1.b.): Specifically, to evaluate the degree of under-estimation of metabolic parameters from the current estimates, in other words what is the maximum dose-metric value will the lowest possible value of Kgl (0.11 L/hr as stated in Pg. 6 of Supplementary Materials B or whatever the appropriate equivalent is) would yield in each tissue.

***Ramboll response**: Table 2 of the Ramboll chloroprene PBPK model report provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact of Kgl on risk estimates derived from the model would be negligible.*

In response to the reviewer's recommendation, we have conducted a new sensitivity analysis of the impact of Kgl values ranging from 0.055 to 1000 on the metabolism parameter estimates and the dose metrics obtained with the model. This new analysis, which has been added to the methods and results sections of the Model Documentation, demonstrates that (1) a value of Kgl = 0.22 provides the best fit to the data, (2) the impact of the choice of Kgl on the values estimated for Vmaxc is less than 10% and the effect on estimates of Km is less than a factor of

[1] The comments/recommendations are all direct quotes from the Draft "Post-meeting Peer Review Summary Report" December 8, 2020.

2, (3) the female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases about 30% as Kgl is increased from 0.175 (the lowest value for which the MCMC analysis could converge) to 1000.

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

Annie Lumen (Question 3): The authors of the report have cited the range of Km values for similar compounds as supportive reasoning for the choice of this value fixing for Chloroprene. I recommend that since this overall process is to estimate the respective metabolic parameters in each tissues/species including Km perhaps it would be useful to at least understand whether and by how much would this initial choice of fixed Km value impact the final metabolic parameter estimations – perhaps a range of values around the 1.0 µmol/L (below and above) be evaluated to see if the initialization of that value carries any considerable impact.

***Ramboll response**: A new sensitivity analysis of the Kgl value has been conducted in response to the comments from the reviewers (see response to previous comment). The analysis is documented in the methods and results sections of the revised documentation. The results of the new analysis provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate.*

Annie Lumen (Question 6): Evaluate the worst-case scenario using Kgl value of 0.11L/h instead of 0.22 L/h given the high correlation to Km

***Ramboll response**: A new sensitivity analysis of the Kgl value has been conducted in response to the comments from the reviewers (see initial response in this section). The analysis, which includes a comparison of Kgl = 0.11 vs 0.22, is documented in the methods and results sections of the revised documentation. The result of the analysis provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate. A value of Kgl=0.175 would provide a higher risk estimate, but it did not provide as good a fit to the in vitro data as Kgl = 0.22; attempting to decrease Kgl any further than 0.175 made it impossible to fit the data at all.*

Kan Shao (Question 1.b.): (1) Comparing the estimated results (including confidence intervals) of Vmax and Km before and after introducing the "Kgl" parameter, so that the impact of introduction "Kgl" can be evaluated.

***Ramboll response**: Table 2 of the Ramboll chloroprene PBPK model report provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact on risk estimates derived from the model would be negligible.*

A new sensitivity analysis of the Kgl value has been conducted in response to the comments from the reviewers. The analysis is documented in the methods and results sections of the revised documentation. The results of the analysis (see initial response in this section) provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate.

(2) Investigating the impact of the specified prior distribution, i.e. log-uniform distribution, on the "Kgl" estimation. The estimated confidence interval shown on Figure B-5 is very narrow, I am wondering if this is related to the specified uninformative prior for these parameters. Additionally, it seems to me that the resulting posterior distribution of "Kgl" is not closely related to the specified lower bound "0.11" used in the prior distribution for "Kgl". Therefore, it is worth to investigate if the "Kgl" estimate is sensitive to its specified prior distribution.

***Ramboll response**: A sensitivity analysis of the Kgl value has been conducted in response to this comment. The analysis is documented in methods and results sections of the revised documentation. The results of the analysis (see initial response in this section) provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate.*

Kan Shao (Question 3): perform a more detailed analysis (as suggested in my response to Charge Question 1) to understand the possible value range of "Kgl" with uncertainty and sensitivity.

***Ramboll response**: See initial response in this section.*

Kan Shao (Question 4): As mentioned earlier, additional analysis results should be presented to better evaluate if introducing the "Kgl" parameter is appropriate, i.e. how the estimates of Vmax and Km changed before and after including "Kgl".

***Ramboll response**: See initial response in this section.*

Raymond Yang (Question 1.b.): (1) Dr. Clewell and Team at Ramboll provide more detailed descriptions, to be included in this Report, of the incubation system as well as explaining how 500 rpm stirring was achieved in such a system.

***Ramboll response**: It has not been possible for Ramboll to obtain a more detailed description of the incubation system beyond what was provided in the publications of the work. The studies were performed more than 10 years ago, and the original investigators no longer have access to the raw data.*

(2) Dr. Schlosser and Team at the USEPA provide written description of how he and the USEPA colleagues examining the kinetic behavior of the above system and reached their conclusion that the high speed agitation at 500 rpm had not denatured the microsomal enzymes.

***Ramboll response**: If the high-speed agitation had denatured the microsomal enzymes, it would be apparent in the time-course and dose-response relationships of the experimental data. In particular, the fact that the data in the liver tissues is well described by a Michaelis-Menten metabolic description is clear evidence that the microsomal enzymes are functioning normally. This response has been added to the documentation on page 10 in the section discussing re-estimation of in vitro metabolism parameters.*

Kenneth M. Portier (Question 3): Resolve the confusion between the bounds on km provided in Table B-1 and the suggested limits in Figure B-3.

***Ramboll response**: Figure 3 shows the relationship between Kgl and Km in the range of $log_{10}(Kgl) = 0.85 - 1$ ($Kgl = 0.14 - 0.10$), to demonstrate that above a Kgl value of approximately 0.11, a lower estimate of Kgl is associated with a lower estimate of Km, whereas further reduction in Kgl below a value of 0.11 no longer effects the estimate of Km (because Kgl becomes rate-limiting). Table B-1 displays values of Km that have been estimated for compounds that, like chloroprene, are substrates for CYP2E1, demonstrating that the range of likely values of the Km for chloroprene is in the range from 1 – 7 μM. This range of Kms is well within the region in which Km and Kgl are highly correlated (above a Km of about 0.05, Figure 3). This point has been clarified in Supplemental Materials B.*

Yiliang Zhu (Question 3): (1) Given that the three kinetic parameters Vmax, Km, and Kgl are biologically and statistically dependent, the MCMC analysis must sample data from the joint posterior distribution. This requires specification of the likelihood for the parameters, a prior for each parameter, the joint posterior, and MCMC implementation strategies.

(2) Supp B failed to describe the model log(u) in the likelihood (Supp B, Eq 1) and the joint posterior distribution. The first step to implement MCMC is to specify the likelihood function where log(u) must be explicit with respect to u=(Vmax, Km, and Kgl), and the kinetic model underlying log(u) should be also specified. A log-normal likelihood is reasonable. Re-parameterization of the kinetic parameters may be useful or even necessary to utilize the fact that a normal likelihood in conjunction with appropriate prior (e.g. non-informative) implies normal posterior for the kinetic parameters.

(3) A non-informative prior for each kinetic parameter can be specified if an informative prior is not plausible. However, use of the log-normal distribution as a prior for SD is highly unusual, justifications are needed. Common priors for SD include uniform and inverse gamma (ref: Gelman A. Prior distributions for variance parameters in hierarchical models. Bayesian Analysis (2006) 1, Number 3, pp. 515–533).

(4) MCMC can be implemented most effectively in an iterative fashion as illustrated below:

a) draw posterior k samples from P(σ|data);

b) for fixed σ (e.g. the kth sample), draw k samples from the posterior distribution p (Kgl |σ;data)

c) for fixed σ and Kgl, draw k samples from posterior p(Km | Kgl, σ; data)

d) for fixed σ, Kgl, and Km, draw k samples from posterior p(Vmax | Km, Kgl, σ; data)

e) iterate between steps a)-d)

(5) The iterative approach above ensures a multivariate posterior distribution resembling what Figure B-3 depicts. Consider presentation of the MCMC results in a joint fashion when feasible.

(6) Describe the MCMC sampling process and report results in greater details to ensure transparency and reproducibility.

(7) Describe convergence criteria adopted, including graphic tools such as trace plot.

***Ramboll response:** As a point of clarification, Kgl was a fixed parameter in the Ramboll analysis of in vitro Vmax and Km. A value of Kgl=0.22 was derived in two separate ways: (1) from scaling of the mixing rate in the experimental determination of Kgl to the mixing rate in the*

metabolism studies, and (2) from simultaneous estimation of Kgl. Km and Vmax using the data for the male mouse liver, which had the highest rates of metabolism. The Kgl estimated from the male mouse liver was used for the estimation of Vmax and Km in all of the tissue data, on the assumption that the mixing conditions in the vials were the same throughout the studies. Due to the collinearity between Km and Kgl, we conducted an analysis of the data on metabolism in the male mouse liver to simultaneously estimate Vmax, Km and Kgl, using uninformative priors except that (1) the prior for Kgl was bounded from below at 0.11 L/hr, the minimum value that we had previously determined could support the observed rate of metabolism, and (2) the prior for Km was bounded from below at a value of 0.5 µmol/L, a factor of 2 below the lowest value for substrates of CYP2E1 from our review of the literature. Importantly, there was no evidence that the posterior distributions from this analysis were clipped by the use of these lower bounds on the priors. Kgl would be expected to have minimal deviation from vial to vial in the robotic system used in the experiments.

While we did investigate the interaction between Kgl and Vmax (see Supplemental Material B), this was not the basis of the final analysis presented in Ramboll (2020). We will expand on the description of the kinetic model below as it was used to estimate posterior distributions of Vmax and Km in the "Re-estimation of In Vitro Metabolism Parameters" (Supplemental Material B).

The kinetic model is a series of differential equations:

$$d(Aa)/dt = Kgl/PC * Am/Vm - Kgl * Aa/Va - Rloss* Aa/Va$$

$$d(Am)/dt = Kgl * Aa/Va - Kgl/PC * Am/Vm - (Vmax * Am/Vm)/(Km + Am/Vm)$$

where: Aa is the amount of CP in the vial headspace, Va is the volume of the vial headspace, Kgl is the mass transfer rate between air and media, PC is the air:media partition coefficient, Am is the amount of CP in media, Vm is the volume of media, Vmax is the maximal rate of metabolism, Km is the affinity constant for CP metabolism and Rloss is the background loss from the vial headspace. The observed headspace concentrations (obs) are modeled as:

$$log(obs_{t,i}) \sim Normal\ (log(u_t), \sigma^2)$$

where u = Aa/Va which is the predicted headspace concentration at sampling time (t). The differential equations are solved numerically in acslX using a stiff system algorithm. The MCMC routine within the software package implements an adaptive random walk Metropolis Hasting algorithm to draw samples from the joint posterior distribution. Parameters are sampled individually and updated with the exception that all parameters except the one being updated are fixed at their last value (acslX MC Modeler User's Guide, Version 3.1). For the analysis, observed data, Vmax and Km were log transformed. The prior for the model parameters (Vmax and Km) were given broad uniform distributions (-10, 5) and the residue standard deviation σ was given a truncated normal distribution (u=1, sd=1, lb=0.01, ub=100). Convergence was assessed using Gelman-Rubin potential scale reduction factor (PSRF) conditional on an upper threshold value of 1.1 for each parameter and σ^2. Plots for the posterior chains, density and correlation between posterior Vmax and Km for the female mouse liver and lung and the human liver are shown in Figures B-6 through B-10.

Tier 2

Annie Lumen (Question 1): I've not conducted such assays to speak from experience about the validity of treating the air and liquid phases to be always at equilibrium for these class of chemicals. If this has not been confirmed experimentally it might be good to evaluate by making this assumption that the two phases are in equilibrium if and by how much the metabolic parameters would be under-estimated by. Especially, since under-estimation of metabolic capacities could contribute to lesser health-conservative dose metrics.

***Ramboll response**: As noted above for the Tier 1 comment, the sensitivity of the model predictions to Kgl was evaluated during the development of the model and the results were shown in Table 2 of the Ramboll PBPK report, which provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact on risk estimates derived from the model would be negligible. In addition, a new sensitivity analysis of the impact of the Kgl value has been conducted in response to the comments from the reviewers. The results are presented in the methods and results sections of the revised documentation. The results of the analysis (see initial response in this section) provide evidence that the value of 0.22 L/h currently used in the model is the most scientifically defensible and conservative estimate from a risk perspective.*

Annie Lumen (Question 3): I wanted to note that for the second approach, I was a little confused when the Kgl value of 0.22 L/h estimated for male mouse liver was stated to be used in the re-analysis of metabolism data for all tissues (Pg. 8 of Supplementary Materials B) but elsewhere in the same document Kgl was said to be fixed at 0.45 L/hr in the MCMC analysis (Figure legends for Figures B-6, B-7,B-8 in Pgs. 11,12,13). During the meeting, it was clarified that 0.45 L/hr was not used in the final metabolic parameter estimations. The implications of this as it relates to Figure B-6, B-7, B-8 and estimated parameters needs to be verified.

***Ramboll response**: The Kgl value used in the final metabolic parameter estimations was 0.22. Supplemental Material B was corrected accordingly.*

Kan Shao (Question 1): I comment on this question only from a perspective of quantitative analysis. Using this simplified assumption may ignore the potential uncertainty and variability in the rate of air:liquid transfer, which should be reasonably characterized. A sensitivity analysis is suggested to justify the validity of the assumption.

***Ramboll response**: As noted above (Annie Lumen comments on Question 1): The sensitivity of the model predictions to Kgl was evaluated during the development of the model and the results were provided in Table 2 of the Ramboll PBPK report, which provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact on risk estimates derived from the model would be negligible. In addition, a new sensitivity analysis of the Kgl value has been conducted in response to the comments from the reviewers (see above). The analysis is documented in the methods and results sections of the revised documentation. The results of*

the analysis (see initial response in this section) provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate.

Jeffrey Heys (Question 1.b.): Retain the current two compartment model with separate air and liquid phases. If the experiments recommended in response to question 2 demonstrate that mass transfer is nearly instantaneous, then the model can be modified to use a single compartment and an equilibrium assumption.

***Ramboll response**: The current two-compartment model was retained.*

Kenneth M. Portier (Question 3): (1) Perform a literature search to better justify that the mass-transfer coefficient for volatile compounds is likely to be proportional to mixing speeds.

***Ramboll response**: The dependence of mass-transfer on mixing rate is a well-established principle, and the value of Kgl=0.22 was confirmed by simultaneous estimation of Kgl and Km using MCMC analysis with the data for the male mouse, which had the highest rates of metabolism. Further, the sensitivity analysis conducted in response to peer reviewer comments (see above) provides support for the value of 0.22 L/h currently used in the model as the most conservative estimate.*

(2) Perform a sensitivity analysis on the impact of placing bounds on the range of prior distributions as well as modifying the form of the prior distribution. The current assumptions are given in Table B-2. Table B-1 suggests that an informed upper bound for ln(km) is closer to -7 than to 5. What is the impact of assuming a priori that Km ~ Log-Uniform (-10, -7) instead of Log-Uniform (-10,5)? What is the impact of assuming a priori that Kgl ~ Log-Uniform (-4,0) instead of Log-Uniform (-3,0)? It is plausible that the lower bound for Kgl is below exp(-3)=0.05.

***Ramboll response:** In the new analysis conducted to estimate Kgl, the prior for Kgl was bounded from below at 0.11 L/hr, the minimum value that we had previously determined could support the observed rate of metabolism, and the prior for Km was bounded from below at a value of 0.05 µmol/L, a factor of 2 below the lowest value for substrates of CYP2E1 from our review of the literature. Importantly, there was no evidence that the posterior distributions from this analysis were clipped by the use of these bounds on the priors.*

(3) Following the approach by Lampert et al. (2005), perform a sensitivity analysis to determine how specification of the prior distribution of the standard deviation impacts the estimates of Vmax, Km and Kgl in the re-analysis.

***Ramboll response:** As noted previously, Table 2 of the Ramboll chloroprene PBPK model report provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact on risk estimates derived from the model would be negligible. However, a new sensitivity analysis of the Kgl value has been conducted in response to reviewer comments. The analysis is documented in the methods and results sections of the revised documentation. The results of the analysis (see initial response in this section) provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate.*

Jordan Smith (Question 6): I suggest a sensitivity analysis should be conducted to better identify sensitive parameters (referencing Kgl specifically).

Ramboll response: *See responses above.*

COMMENTS AND RESPONSES RELATED TO A1

Tier 1

Annie Lumen (Question 6): Consider the range values the parameter A1 can take and evaluate its impact as a part of supplemental uncertainty analysis if seen fit. For example, from Table 2 of Lorenz et al. 1984 a range of value for A1 can be derived. Of which the highest bound of A1 value is 0.0083 (0.0013/0.156) which is approximately 6-fold higher than the average value currently proposed to be used.

***Ramboll response**: The value of A1 used in the chloroprene PBPK model is the same value used by USEPA in their IRIS risk assessments for methylene chloride. A multi-faceted analysis of the uncertainty in the estimate of A1 was conducted in response to the peer reviewers' comments, including estimating a 95% confidence interval for the Lorenz et al. (1984) value and conducting a literature search to support estimation of an A1 value for chloroprene based on CYP expression. These additional analyses are documented in the methods and results sections of the revised model documentation and support the current approach for A1 used in the PBPK model.*

Annie Lumen (Question 7): (1) If CYP2E1 is shown to be the only enzyme metabolizing Chloroprene (please gather sufficient evidence from the literature or other sources to verify this), and if 7-ethoxycoumarin is a CYP2E1 specific substrate (please gather sufficient evidence from the literature or other sources to verify this), then assuming that Km and the enzyme catalytic rate are the same (please gather sufficient evidence from the literature or other sources to verify this assumption), and that the in vitro activity translates well in vivo (please gather sufficient evidence from the literature or other sources to verify this assumption), then the ratio of Vmax in human liver and lung for 7-ethoxycoumarin can be used to estimate the proportional enzyme expression levels between the two tissues.

***Ramboll response**: The value of A1 used in the chloroprene PBPK model is the same value used by USEPA in their IRIS risk assessments for methylene chloride. Prior to using this value, we reviewed the literature to determine whether there were more recent data that could be used as an alternative to, or in support of, the A1 value used previously. The only alternative we were able to find at that time was a study on tissue mRNA expression of CYPs (Nishimura et al. 2003), where the ratio of total CYP2E1 plus CYP2F1 mRNA expression in human lung and liver was consistent with the value of A1 used by USEPA for methylene chloride. In further support of the applicability to chloroprene of the human value of A1 based on Lorenz et al. (1984), the value of A1 in the male mouse (0.414) based on data from Lorenz et al. (1984) is very close to the ratio of the in vitro Vmax estimates in the lung and liver of the male mouse in our new analysis (0.56, see Table S-3). In addition, in response to comments from the reviewers, additional analyses of the uncertainty in A1 have been conducted that provide additional support for the current value used in the PBPK model (see description of A1 uncertainty analysis in the methods and results sections of the revised model documentation).*

(2) Each of the verification criteria stated above are of equal ranking in my opinion to confidently use the 7-ethoxycoumarin relative activity to predict chloroprene metabolism in human lung. Appropriate uncertainty analysis can be undertaken if some of the verification criteria are not met. This ratio can then perhaps be used to scale the Vmax for chloroprene oxidative metabolism from human liver to human lung.

***Ramboll response**: See response to previous recommendation. We believe the A1 approach used by USEPA in the IRIS assessment for methylene chloride remains the best approach for estimating human lung metabolism. As explained in our report, the* in vitro *metabolism studies conducted with chloroprene were unable to detect any metabolism in the human lung, as evidenced by the fact that the rate of change in chloroprene concentrations in the human lung metabolism vials was similar to, and in some cases less than, the rate of change of chloroprene concentrations in the control vials.*

Annie Lumen (Question 8): For rats it would be good to confirm that other enzymes such as CYP2F does not contribute to chloroprene metabolism (please gather sufficient evidence from the literature or other sources to verify this) and if found to be the case please evaluate if CYP2E1 substrate is a good choice to estimate relative activity between tissues in that specific species. My only additional comment is that if the parallel approach is agreed to be appropriate for use in estimating human lung metabolism from liver values then it seems reasonable that the same approach will be applied to other metabolically relevant tissues as well. For CYP2E1 the mRNA expression correlates very poorly to its protein expression levels and that protein expression levels are better correlated to CYP2E1 activity than mRNA expression levels (Ohtsuki et al. 2012; Sadler et al. 2016).

***Ramboll response**: CYP2F4 does contribute to the metabolism of chlorinated alkenes in the rat. In general, CYP2E1 and CYP2F have similar substrates. In deriving values of A1 from mRNA expression, we added the expression of CYP2E1 and CYP2F1. Because the CYP enzymes are subject to induction by substrate stabilization (Parkinson 1996), an individual's protein expression varies with exposure to substrates, meaning that the activity in a particular donor tissue would reflect effects from recent exposures to food and drugs. CYP mRNA expression, on the other hand, provides an assessment of the baseline enzyme activity in the individual. Therefore, mRNA expression provides a more reliable value for estimating an average value for a human population from a small number of subjects.*

Parkinson A. 1996. An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. Toxicologic Pathology, 24(1): 48-57.

Jochem Louisse (Question 8): Perform a literature study to obtain data on the in vitro conversion of CYP2E1 substrates in human liver and human lung tissue fractions (see as example above butadiene), also including other organs of interest. These data may provide insight into whether the derivation of a factor to scale the Vmax obtained with liver microsomes to a Vmax for lung microsomes (and other tissue fractions) may be feasible. If these analyses provide values for A1 that largely differ, this would indicate that this approach is not valid. If these data are limited to do such a comparative assessment, one should be cautious using the approach. In that case, the recommendation below would become a Tier 1 recommendation. In any case, in order to obtain the most reliable estimation of chloroprene oxidation in the lung, I would highly recommend to perform in vitro biotransformation studies with microsomes and determine the time- and concentration-dependent formation of metabolites (epoxide metabolites), instead of using a substrate depletion approach, to derive kinetic constants for chloroprene oxidation.

***Ramboll response**: We performed the literature search suggested by the reviewer during the development of the chloroprene PBPK model. The only data we were able to find was on tissue mRNA expression of CYPs in the human (Nishimura et al. 2003), where the ratio of total CYP2E1 plus CYP2F1 mRNA expression in human lung and liver was consistent with the value of A1 used*

by USEPA for methylene chloride. Additional literature searching conducted in response to the peer review of the model resulted in the identification of a second publication (Bieche et al. 2007) publication that provided additional data (see discussion of A1 uncertainty analysis in the methods and results sections of the revised model documentation).

In further support of the applicability to chloroprene of the A1 based on Lorenz et al. (1984), the value of A1 in the male mouse (0.414) from Lorenz et al. (1984) is very close to the ratio of the in vitro Vmax estimates in the lung and liver of the male mouse in our new analysis (0.56, see Table S-3).

Tier 2

Jochem Louisse (Question 4): Assess whether information on metabolic conversion of model substrates are available for the microsomal badges that have been used for the in vitro kinetic studies. These data should then be compared with metabolic conversion data of well-characterized batches of human microsomes (e.g. available by suppliers of these materials). This may provide some insight into whether the microsomal badges that have been used in the original studies can be considered representative for the 'average' human.

***Ramboll response**: Due to the age of the Lorenz et al. (1984) study, and the inability to access the original data, it is not possible to obtain such information.*

Kenneth M. Portier (Question 4): Provide an estimate of the standard deviation of A1, compute an approximate confidence interval and use this to discuss the likelihood that A1 is close to 1.

***Ramboll response**: The requested analysis has been performed and is documented in the methods and results sections of the revised model documentation.*

Kan Shao (Question 4): Regarding the pool sizes for the human microsomes, the estimate results presented in Supplemental Materials A and B demonstrate that the pool sizes are reasonably sufficient to generate adequate parameter estimates with confidence intervals. However, sensitivity analysis on the prior distribution is highly recommended which will be very useful to determine whether the relatively small confidence intervals are resulted from narrow priors or sufficient sample sizes.

***Ramboll response**: To be clear, the human liver microsome data are based on a single purchased pool, and are not data that were pooled after incubations with microsomes from different subjects. The pool size for the human liver microsomes was presumably based on the available pool from the vendor at the time of the study. The pooled microsomes would have undergone multiple quality control tests conducted by the vendor to ensure the pool provided reasonable levels of metabolism based on a battery of standard compounds (i.e. EROD and PROD substrates). It is unclear why the reviewer appears to believe the prior distributions may have been narrow. The prior distributions for Vmax and Km in the in vitro analysis covered 15 orders of magnitude on the log of the parameter (-10, 5). This is a highly uninformative prior chosen to allow the data to predominate in the analysis. The narrowness of the posterior confidence intervals is due to the consistency of the pooled human liver incubation data across concentrations and times. We agree that the prior, if too narrow, can influence the posterior distribution; however, that is not the case in this analysis. The reviewer could be noting the prior distribution of Kg in Table B-2 (-2.996-0). The prior for Kg was only used in initial examination of the transport limitation in the male mouse liver and a semi-informative prior for Kg was*

necessary to allow the simulation to converge, as there is a very strong correlation between Kg and Km. For the MCMC analysis of the in vitro incubation data presented in Supp Mat D, Kg was fixed at 0.22 L/hr.

Annie Lumen (Question 7): The validity of using mRNA expression ratio to support enzyme activity ratio was discussed in detail as part of the meeting. Panel members who have expertise in this area provided supporting references that suggests that this could be true for some enzymes but for CYP2E1 the mRNA expression correlates very poorly to its protein expression levels and that protein expression levels are better correlated to CYP2E1 activity than mRNA expression levels (Ohtsuki et al. 2012; Sadler et al. 2016). Based on this information my suggestion is perhaps to not rely on the mRNA expression ratios to support the choice of A1 value. And if possible, other approaches be sought or the associated uncertainties in this value be appropriately evaluated.

***Ramboll response**: Because the CYP enzymes are subject to induction by substrate stabilization (Parkinson 1996), an individual's protein expression varies with exposure to substrates, meaning that the activity in a particular donor tissue would reflect effects from recent exposures to food and drugs. CYP mRNA expression, on the other hand, provides an assessment of the baseline enzyme activity in the individual. Therefore, mRNA expression provides a more reliable value for estimating an average value for a human population from a small number of subjects.*

Parkinson A. 1996. An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. Toxicologic Pathology, 24(1): 48-57.

Annie Lumen (Question 8): In my preliminary comments I had indicated that a consensus be reached on what is the lowest limit of metabolism below which we accept that no metabolism needs to be described for that particular tissue. During the meeting this was clarified that if by using MCMC analysis Vmax and Km values were found to be identifiable from the low metabolism data then metabolism parameters were derived from that data.

***Ramboll response**: No response required.*

COMMENTS AND RESPONSES RELATED TO MODELING OF THE REACTIVE METABOLITES OF CHLOROPRENE

Raymond S.H. Yang ("Post-meeting Thoughts"):

(1) Ramboll should use the kinetic information in Cottrell et al. (2001) and Munter et al. (2003) in their chloroprene PBPK modeling. For instance, capturing as many data points as needed, digitally, from the curves in Figure 9 by software such as Getdata-Graph-Digitizer. After obtaining Vmax and Km from double reciprocal plots for chloroprene for this reaction, PBPK model simulations could be done without the issue of using surrogates. Since Cottrell et al. (2001) and Munter et al. (2003) also did comparisons of microsomal enzyme assays with or without epoxide hydrolase (EH) inhibitor, quantitative information on the detoxication of EH was buried in there somewhere to be uncovered.

(2) Ramboll scientists should effectively use the 1,3-butadiene pharmacokinetic and metabolism data as additional supporting information in the building of a robust and complete PBPK model for chloroprene. (This last sentence should be considered as a Tier 1 recommendation).

(3) Another important note is that the Cottrell et al. (2001) and Munter et al. (2003) studies are microsomal enzyme studies. They did not investigate the role of glutathione S-transferase (GST) which is a cytosolic enzyme in chloroprene metabolism. GST, being a high capacity low affinity enzyme (Andersen et al. 1987), would serve as an important detoxifying enzyme for chloroprene epoxides as indicated by similar data in 1,3-butadiene metabolism studies (Csanady et al. 1992; Kohn and Melnick 2000, 2001). Ramboll scientists should incorporate kinetic information of reactive metabolites detoxication into their PBPK modeling.)

(4) The Cottrell et al. (2001) and Munter et al. (2003) studies also reported the GSH detoxication of reactive metabolites of chloroprene as a body's chemical defense system as outlined in Scheme 2 in Cottrell et al. (2001) and Scheme 1 in Munter et al. (2003). These chemical detoxication processes, as other detoxifying enzymatic processes, would certainly impact on the "dose metric" of the Ramboll/Denka PBPK modeling approach. Ramboll scientists should incorporate such detoxication processes into their PBPK modeling.

(5) Ramboll scientists should compare the simulation results of three PBPK modeling approaches: (i) their present approach modified and refined with consideration of downstream detoxication processes; (ii) PBPK modeling using kinetic constants estimated using data from Cottrell et al. (2001) and Munter et al. (2003) with consideration of downstream detoxication processes; and (iii) PBPK modeling using kinetic constants from 1,3-butadiene data from Csanady et al. (1992) and Kohn and Melnick (2000, 2001) with consideration of downstream detoxication processes.

***Ramboll response**: The five comments from Dr. Yang raise his concerns about the adequacy of the chloroprene PBPK model for assessing appropriate dose measures for carcinogenicity of chloroprene in the mouse lung and extrapolating the dose metric from the mouse to the human. Concerns voiced were (1) the need to make more quantitative use of the Cottrell et al. (2001) and Munter et al. (2003) data on specific chloroprene metabolites to establish kinetic parameters for EH hydrolysis and GSH-conjugation of the epoxides formed by microsomal oxidation; (2) the need to account for knowledge available from butadiene modeling for parametrizing aspects of the chloroprene model; (3) the need to ensure appropriate consideration of the role of GSH conjugation, both enzymatically and non-enzymatically, in the detoxification of reactive intermediates (a concern that covers both comments 3 and 4 from Dr. Yang). Dr. Yang*

suggested comparing the results from the present model with (1) a model using quantitative reaction rates for chloroprene metabolism extracted from Cottrell et al. and Munter et al. and (2) a model using kinetic constants from the PBPK models for butadiene. In response to the concerns raised by Dr. Yang we have added a description of the downstream metabolism of chloroprene to epoxides and other reactive products. The development of the extended PBPK model, and comparison of dose-metric predictions with the current parent chemical model, is described in Supplemental Materials F. The extended model incorporates information derived from the Cottrell and Munter studies and includes a description of the role of glutathione in detoxification, including the impact of glutathione depletion and regeneration. Before summarizing the results of the reactive metabolite modeling, we will directly address two of the key concerns that were raised.

Regarding the extraction of kinetic parameters/information from Munter et al. and Cottrell et al.: There are two questions to consider about parameterizing the CP model to explicitly include oxidation, EH hydrolysis and glutathione conjugation based on these papers. The first question is whether the Cottrell and Munter studies were designed in a manner to allow confident estimation of rate constants for hydrolysis and GSH conjugation, and the second is whether this information, if available, would enhance the ability of the CP model to estimate dose metrics for highly reactive metabolites in the lung.

On the first point, we agree that the two papers provide important qualitative information about pathways of metabolism and the metabolite formed, and we took this information into account in designing our PBPK model for CP. However, for several reasons, these papers were not well-designed to extract quantitative information as suggested by Dr. Yang. First, CP was introduced into the vials by injection in 5 μL acetonitrile (described explicitly in Munter et al. but only inferred by a careful reading of Cottrell et al.) with resultant CP concentrations ranging from 0.01 to 10 mM in Munter et al. and from 1 to 40 mM in Cottrell et al. The estimated end of exposure CP concentration in blood at 80 ppm is only 0.015 mM, well below most of the concentrations examined in these two papers. A second confounder is the use of another potential low-molecular weight CYP2E1 substrate – acetonitrile – as diluent, raising concerns for competitive inhibition of CP metabolism between CP and acetonitrile. Thirdly, when GSH was added, the achieved levels were much below those that occur in tissues, which are from 2 to 6 mM.

Despite these concerns for extracting specific kinetic constants, the papers provided solid information on the pathways of metabolism to the epoxides and expected reactive metabolites – especially the reactive aldehydes and ketones. The Cottrell paper discussed incubations with and without an EH inhibitor and incubations with GSH. In incubations with active EH, neither 1-CEO or 2-CEO were found in the incubations, leading to respectively to diol from 1-CEO and various reactive aldehyde and ketone metabolites from 2-CEO. Using the EH inhibitor, it was found that the 2-CEO epoxide was still very unstable, producing metabolites that quickly reacted with GSH either in the presence or absence of cytosol, i.e. the conjugation reaction does not require soluble glutathione transferases. Cottrell also noted that the reaction of the 2-CEO breakdown product, 1-hydroxy-but-3-ene-2-one with GSH is rapid and likely an effective detoxication pathway. The takeaway from these results was that the dominant pathway – formation of 2-CEO – leads to rapid, nearly quantitative production of GSH-reactive metabolites, observations that supported a dose metric of amount metabolized per unit tissue for this arm of the pathway, which accounts for about 90% of total metabolism (i.e. α in Figure 1 is 0.9) as reported in Himmelstein et al. (2004a).

The remainder of the total oxidation (1-α) is through 1-CEO. The half-life of 1-CEO must be longer than 2-CEO since 1-CEO is found in the airspace over the microsomal incubations (Himmelstein et al. 2004a); however, 1-CEO formation is a relatively minor pathway compared to 2-CEO production. With respect to GSH conjugation of 1-CEO, Munter et al. also report that 1-CEO either does not react with GSH or the reaction is very slow (Munter et al. pages 1294-1295). Thus, there would be no need to consider glutathione transferase reactions in the overall metabolic scheme with either 2-CEO or 1-CEO.

With regard to exposure of the lung tissue to a slightly longer-lived 1-CEO, one interspecies difference could arise based on EH activities. Humans have higher EH activity than does the mouse (Himmelstein et al. 2004a). If a quantitative correction were to be included in the reactive metabolite tissue dose metric in the CP model for longer tissue exposure to 1-CEO, the expected tissue dose would decrease for human compared to mouse, i.e. the present configuration of our risk-assessment estimate of tissue dose is conservative and would produce even lower risk estimates if we included interspecies EH hydrolysis rates to reduce tissue exposure to 1-CEO.

Regarding the use of kinetic constants from the PBPK model for butadiene to describe the kinetics of the chloroprene epoxides: Chloroprene differs substantially from non-chlorinated dienes, such as BD or isoprene, due to the presence of the electron withdrawing chloride atom at the 2-position. As pointed out in Cottrell et al. the chorine atom leads the formation of chloro-aldehydes and ketones in addition to epoxides. These metabolites themselves rapidly react with glutathione and, when formed in sufficient amounts, could lead to GSH depletion and attendant tissue toxicity. These differences in epoxide reactivity have been well documented for ethenes with the studies on ethylene metabolism to ethylene oxide versus kinetic models for vinyl chloride or 1,1-dichloroethylene with metabolism to halogenated epoxides that are unstable and whose reaction products readily react with GSH. The glutathione depletion with these halogenated ethene epoxides plays important roles in their toxicity and similarly are important in the tissue effects of chloroprene noted in in older studies in rats.

The purposes in developing models for CP and BD were quite different: the CP model was designed to understand dose of locally produced reactive metabolites in lung and the BD model was intended to examine the production of epoxides in tissues, primarily liver, and the expected steady-state circulating concentration of the mutagenic mono- and diepoxide arising from subsequent distribution of the epoxides to other tissues. The Kohn and Melnick work, called a privileged-access model and referenced by Dr. Yang, was essentially an extension of an earlier model by Johanson and Filser (1993) that included first-pass clearance of the CYP produced epoxide by EH in a microsomal compartment in the liver. In both cases (privileged access and intrahepatic first-pass), the model structure was designed to enhance epoxide clearance before its appearance in the tissue and its transfer to tissue blood and the general circulation (shown by the inclusion of both EH and CYP2E1 in the reaction scheme from BD to butadiene monoepoxide (BMO) and from BMO to butadiene diepoxide (BDO). These BD epoxides are much more stable than those produced by CP and due to longer half-lives are available for downstream reactions and diffusion into blood. In addition, there was a significantly greater data base on which to parametrize the model – especially the gas uptake studies from Kreiling et al. (1987) and concentrations of circulating epoxides. The CP model depends primarily on the detailed in vitro examinations of CP metabolism from Cottrell et al., Munter et al. and Himmelstein et al.

Key differences in modeling with BD and CP: Modeling systemic exposures to stable metabolites (BMO and BDO for BD) is very different from modeling for transient tissue exposures to reactive

intermediates that do not leave tissues in significant amounts. The metabolism pathways are similar, but the consequences of forming stable versus highly reactive metabolites lead to very different demands on the PBPK model for tissue of formation dose measures –proportion escaping the liver as BMO and BDO for BD - versus total amount metabolized for CP. With BD, the goal was to understand the systemic delivery of epoxides to tissue throughout the body, including blood time course for the epoxides; with CP the goal is more restricted to understand tissue dose in a specific tissue containing CYP2E1 and how possible species differences in CYP2E1 and EH in this tissue might affect local exposures. The Himmelstein et al. paper provided the key data for parameterizing the model and the papers by Munter and Cottrell provided key data for understanding the reactive metabolites from 1-CEO and especially 2-CEO.

Results of reactive metabolite modeling: The extended model of chloroprene metabolism described above was exercised to evaluate three potential dose metrics for the lung toxicity and carcinogenicity of chloroprene: (1) total lung metabolism per gram lung (TMet), the dose metric used in the published PBPK models and previously submitted to the USEPA; (2) average concentration of reactive products of metabolism in the lung (PReact), and (3) average concentration of 1-CEO in the lung (1-CEO).

The first comparison performed was an evaluation of the consistency of the alternative dose metrics with the gene expression dose-response data reported in Thomas et al. (2013). In this study, female mice and rats were exposed to chloroprene by inhalation 6 hours per day, for 5 or 15 days. Mice were exposed at the bioassay concentrations, but the concentration range was extended in the rat to provide similar tissue doses based on predicted total amount of chloroprene metabolized per gram of lung tissue per day from a preliminary version of the PBPK model of Yang et al. (2012). For this comparison, two genomic responses were used: the lowest Benchmark Dose (BMD) for any gene expression change and the lowest BMD for any gene expression change related to regulation of glutathione homeostasis. A successful dose-metric for cross-species extrapolation should predict that cellular responses in the lung begin to occur at similar values of the dose metric.

Consistent with the expectations that drove the experimental design in Thomas et al. (2013), the inhaled concentrations at which there was genomic evidence of cellular stress in the lungs of the rat were much higher than in the mouse. The predicted dose metric values for 1-CEO concentration associated with similar genomic biomarkers of cellular effects were also nearly an order of magnitude higher in the rat than in the mouse. In contrast, the model predicts similar dose metric values for both TMet and PReact in the rat and mouse, consistent with the expectation that cellular responses to chloroprene in the lung would begin to occur at similar levels of cellular stress. The consistency of these two dose metrics with the observed genomic dose-response in the female mouse and female rat, and the inconsistency of the 1-CEO or inhaled CP dose metrics, support the dominant role of reactive product formation in the mode of action for chloroprene.

The second comparison performed was an evaluation of the consistency of the alternative dose metrics with the tumor incidence in the bioassays for the female mouse and rat. While both the TMet and Preact metrics provided a reasonable dose-response relationship with tumor incidence, the 1-CEO metric did not. In fact, using the 1-CEO concentration as the dose metric would predict that the female rat should have had a higher tumor incidence than the female mouse. The inconsistency of the 1-CEO dose metric with the relationships for both toxicity and carcinogenicity between the female mouse and female rat is likely due to the small proportion of total chloroprene metabolism that it represents. At the bioassay concentrations, the predicted concentrations of 1-

CEO are less than 0.4% of the concentrations of reactive products in the female mouse and less than 5% in the rat.

In summary, the results of the reactive product modeling support the use of total metabolism as the most appropriate dose metric for the carcinogenicity of chloroprene and demonstrate that the use of inhaled concentration or epoxide area under the curve is inconsistent with the cross-species relationship of the toxicity and carcinogenicity of chloroprene.

COMMENTS AND RESPONSES RELATED TO OTHER TOPICS

Tier 1

Jochem Louisse (Question 5): (1) Make a clear overview of the kinetic data in a Table that has been used to derive the kinetic constants for chloroprene conversion. This allows a better assessment as to whether the data, with regard to number of replicates, independent experiments, etc., can be considered as being adequate to provide robust data.

***Ramboll response**: The section of the documentation entitled "Model Parameters" outlines the sources for the kinetic data used in the modelling. Further, it points the reader to Supplemental Materials A which provides tables comparable to those suggested by the peer reviewer. Additional information is also provided in Supplemental Materials D. In addition to these data, all of the underlying data that have been relied upon and provided as part of the modeling scripts are included with citations along with the model code in Supplemental Materials E. The raw data underlying these modeling scripts are not currently available. The studies were performed more than 10 years ago, and the original investigators no longer have access to the raw data.*

(2) It is not clear to me whether all data have been obtained from one experiment or whether independent studies have been performed. It would have been of help for the analysis to present an overview of the data points that have been obtained in the Himmelstein et al. (2004) and Yang et al. (2012) papers, e.g. in a Table like this:

		Himmelstein et al. (2004), experiment 1	Himmelstein et al. (2004), experiment 2	Yang et al. (2012), experiment 1	Yang et al. (2012), experiment 2
Female mouse	Liver	concentrations technical replicates per concentration time points per replicate			
	Lung				
	Kidney				
Human	Liver				
	Lung				
	Kidney				

***Ramboll response**: As discussed in the text, all of the metabolic parameters in both the Yang et al. (2012) paper and the current analysis are based on the in vitro chloroprene metabolism time-course data reported by Himmelstein et al. (2004a) and IISRP-17520-1288 (2009). The key differences between the new analysis and the original Yang et al. (2012) analysis were: (1) the incorporation of an additional parameter in the analysis of the* in vitro *metabolism data (Kgl) to describe the rate of transfer of chloroprene from the headspace to the media in the metabolism studies, (2) the use of updated tissue microsomal protein concentrations for scaling the* in vitro *results to* in vivo *values appropriate for the PBPK model, and (3) the adoption of a previously published approach for estimating the metabolism parameters in the human lung (Andersen et*

al. 1987). The differences in these parameters in the Yang et al. (2012) paper and the current analysis are documented in Table S-3 of Supplemental Materials A.

Jochem Louisse (Question 6): (1) To facilitate assessment of the kinetic data, it would be of help to not only present the data points in graphs but to include these in accompanying tables, allowing better assessment by the reviewers.

***Ramboll response**: For Figures 2, 3 and 7, as noted in the text, the data are provided in Supplemental Materials A. In addition, as noted previously, the underlying data relied upon for the model are provided with citations along with the model code in Supplemental Materials E.*

(2) Assess whether in vitro kinetic data of optimization studies are available and include these in the table presenting all in vitro kinetic data. This allows reviewers to assess whether kinetic studies have indeed been performed at optimal conditions.

***Ramboll response**: All of the in vitro kinetic data are now listed in Supplemental Materials E, as they were received from the original investigators.*

Annie Lumen (Question 6): Based on the description in Pg. 17 of Ramboll report I calculated the metabolic clearance in the lung to be 0.24 L/h/g of protein (0.052*0.00143*1000/ 0.316) but the report indicates an estimate of metabolic clearance of 0.16 L/h/g of protein. I doubt this glitch is real and could be something that I've missed but I thought I'll bring it up since it caught my eye and I'm fine with being proved wrong.

***Ramboll response**: The reviewer is correct. The number in the report must not have been updated with the final MCMC results. This has been corrected in the current documentation. The calculated metabolic clearance should be 0.24 L/hr/kg, which is close to the upper bound estimate of metabolism from Yang et al. (2012) of 0.32 L/hr/g.*

Annie Lumen (Question 10): Approximately, 36% of mouse body weight and 24% of human body weight doesn't seem to be contributing to chemical disposition. This seems to be a rather large fraction of body weight to remain unperfused. Please verify.

***Ramboll response**: The bones contribute about 10% to body weight and the gut lumen, hair and integuments contribute another 10% to 15%. The fraction of non-perfused body weight in the chloroprene PBPK model results from the use of the tissue data in Brown et al. (1997).*

Annie Lumen (Question 14): (1) Since blood flow to the liver is identified as the primary determinant of the overall elimination of Chloroprene and no influence of tissue-specific metabolism is noted to affect blood concentrations, the predictive evaluation using blood concentration data should not be weighted to validate the extrapolative performance of IVIVE, its related PBPK parameterization, and subsequent overall predictive performance of tissue-dose metrics (parameters that influence blood concentrations have normalized sensitivity coefficients < 0.5 in influencing lung dosimetric).

***Ramboll response**: We agree with the reviewer that the in vivo study does not serve as validation of model predictions for tissue-specific metabolism. The sole purpose of the* in vivo *validation study, which was performed in response to an USEPA concern, was to demonstrate the ability of the model to predict the* in vivo *pharmacokinetics of chloroprene inhalation, since that is the exposure route of concern. The validity of the model predictions of for target tissue (e.g. lung) metabolism rests on the correctness of the IVIVE methodology. This dichotomy of the*

validation process was also the case with the PBPK model for methylene chloride that was used by the USEPA in their IRIS assessments. In that case, the lung metabolism parameters were also based on in vitro *data and the available* in vivo *data was insensitive to their values.*

(2) The average measured blood concentrations between 13ppm and 90ppm are only 3.8-fold and 5.1-fold apart at 3h and 6h respectively (Table S-6). That said, from a health-protective standpoint, an overprediction of blood concentration I suppose in better than an under-prediction. Nevertheless, this (the observed discrepancy in model predictions and observations) is a model uncertainty and needs to be evaluated as such.

***Ramboll response**: On the contrary, the model predictions for blood concentrations during and after the 6-hr chloroprene exposures are in good agreement with the data collected in the study. Consistent with the WHO/IPCS (2010) guidance on the use of PBPK modeling in risk assessment, model predictions are generally within roughly a factor of two of the means of the experimental data. It was not necessary to adjust any of the model parameters to provide agreement with the new data.*

I recommend caution be exercised when making predictions of tissue-dosimetric (rate of metabolite production) in other metabolically relevant tissues with only blood concentrations.

***Ramboll response**: It's unclear what the reviewer is trying to say, but caution is always a good thing in risk assessment. It is not possible to obtain* in vivo *validation data on the production of a reactive metabolite in a tissue. As in the case of methylene chloride and vinyl chloride, the validity of such model predictions must be based on biological plausibility and correct methodology.*

Kan Shao (Question 6): The comparison results presented in Table S-3 in the Supplemental Material A confuse me. First of all, without showing the confidence interval estimates for these parameters, it is very difficult to judge the magnitude of uncertainties in the estimated results obtained using Yang et al. (2012) approach.

***Ramboll response**: The confidence intervals for the Yang et al. analysis are available in the publication. Only the means were included in this appendix, have been added to Table S-3 in Supplemental Materials A as requested.*

Jordan Smith (Question 13): I suggest that male and female physiological parameters are implemented independently to ensure that physiologies of both sexes are adequately considered. ICRP (2002) could serve as a reference for male and female physiologies across various life-stages.

***Ramboll response**: Concerns regarding potential sensitive human populations, including the effect of gender, is part of the application of the model for a specific risk assessment application, which USEPA will undertake if they accept the model. The physiological and metabolic structure of the PBPK model provides the necessary framework for conducting such investigations, and appropriate parameters are available in the literature (Clewell et al. 2004, Mallick et al. 2020).*

Clewell HJ, Gentry PR, Covington TR, Sarangapani R, Teeguarden JG. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Toxicol. Sci. 79:381-393.

Mallick P, Moreau M, Song G, Efremenko AY, Pendse SN, Creek MR, Osimitz TG, Hines RN, Hinderliter P, Clewell HJ, Lake BG, Yoon M. 2020. Development and Application of a Life-Stage

Physiologically-Based Pharmacokinetic (PBPK) Model to the Assessment of Internal Dose of Pyrethroids in Humans. Toxicol Sci. 173(1): 86–99

Tier 2

Annie Lumen (Question 10): I had suggested a proof of concept modeling evaluation for epoxide hydrolase activity using available literature data as an added component to the current model. In the public meeting, when discussing this comment, it was mentioned that 1-CEO might only constitute 4-5% of the total metabolites produced and that other metabolites might be more reactive. Therefore, I'll leave this suggestion here as a lower tiered one for consideration if potential concern for downstream metabolite accumulation is seen to be likely.

***Ramboll response**: At the request of the peer reviewers, we have conducted a more extensive analysis of the downstream metabolism of chloroprene (see previous section on COMMENTS AND RESPONSES RELATED TO MODELING OF THE REACTIVE METABOLITES OF CHLOROPRENE). The results of the reactive product modeling support the use of total metabolism as the most appropriate dose metric for the carcinogenicity of chloroprene and demonstrate that the use of inhaled concentration or epoxide area under the curve is inconsistent with the cross-species relationship of the toxicity and carcinogenicity of chloroprene.*

Annie Lumen (Question 11): Based on the current model structure, to understand the plausible mechanistic reasoning, perhaps it might be useful to run a time-dependent sensitivity analysis (particularly between 0-3hrs) to see if any unsuspected change in parameter sensitivities are observed at early timepoints across exposure levels. This could only be useful to generate hypothesis challenging the current assumptions of chloroprene uptake (e.g. need for saturable uptake kinetics at higher exposures for example) but might not provide a solution to the discrepancy directly.

***Ramboll response**: We have performed the time-dependent sensitivity analysis suggested by the reviewer on PBPK models of similar chemicals and found that the early times are driven by ventilation and blood flow (Clewell et al. 1994).*

Clewell HJ III, Lee T, Carpenter RL. 1994. Sensitivity of physiologically based pharmacokinetic models to variation in model parameters: methylene chloride. Risk Analysis 14: 521-531.

Annie Lumen (Question 12): The logic of reducing any large discrepancy between ventilation rate and cardiac output and to have them match is reasonable. One recommendation is that since cardiac output value in a mouse model would most likely be a well-studied parameter, a secondary check from a source external to Brown et al. 1997 or the current Ramboll estimates/references could be useful as a confirmation to validate if the selected QCC is reflective of an average cardiac output for mouse models.

***Ramboll response**: The value of QCC for the mouse in the chloroprene model (QCC=30), is similar to the mouse value (QCC=28) in the PBPK model of Andersen et al. (1987) that was used by EPA in the IRIS assessment for methylene chloride, and is consistent with the physiology of ventilation and perfusion.* As discussed in *Brown et al. 1997, while the value of cardiac output used in the PBPK model of Andersen et al. (1987) for the rat is in agreement with the experimental measurements reported in Table 22, the value for the mouse is about double the reported values. The higher value of QCC in the mouse was determined by comparisons of PBPK modeling with closed chamber exposure data for a number of chemicals (Gargas et al. 1986).*

Gargas ML, Andersen ME, Clewell HJ. 1986. A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol Appl Pharmacol 86:341 352.

Annie Lumen (Question 13): I did want to note that the blood-to-air partition coefficient, a sensitive parameter determining of chloroprene blood concentration, is determined in humans based on a sample size of 3 healthy male adult volunteers (Himmelstein et al. 2004 PartII). However, the values do seem to be tight and less variable so I'm not sure if there is any room for uncertainties here but changing this parameter considerably changes the model predictions of blood concentrations (as shown by the sensitivity analysis in Pg. 19 of the Ramboll report). It might be worth verifying that there is no room for uncertainties in the blood-to-air partition coefficient.

***Ramboll response**: The relationship of the experimental chloroprene blood-air partitions across species is consistent with results for similar volatile organic compounds, such as methylene chloride, vinyl chloride and trichloroethylene (Gargas et al. 1989).*

Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol. 1989 Mar 15;98(1):87-99.

Jordan Smith (Question 11): I suggest an uncertainty analysis, such as a Monte Carlo type approach, to quantify model uncertainty. This would allow quantitative evidence to better assess if the model overpredictions are explained by data variability.

***Ramboll response**: An uncertainty analysis has now been conducted using the revised Ramboll PBPK Model, and the results are described in the PBPK Model documentation.*

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

Yiliang Zhu (Question 11): the over-estimation of blood concentrations during and following 6-h exposure of B6C3F1 mice to Chloroprene raises questions regarding its reliability of model prediction and robustness toward key kinetic parameters. A systematic approach to sensitivity analysis involving the parameters of this high dimension would be useful. Sensitivity/robustness should be one criterion for selecting the kinetic parameters for prediction purposes.

***Ramboll response**: Such a sensitivity analysis was performed during the development of the PBPK model and the results are provided in the main report (Figures 8-10).*

Kan Shao (Question 14): A few technical issues undermine the overall quality of the PBPK model, including how to justify the necessity and validity of introducing a new parameter "Kgl" to quantify the air and liquid mass-transfer, the lack of detailed analytic results prevent better evaluating the statistical approaches' ability to characterize uncertainty and variability, etc. Therefore, better addressing and more clearly explaining these issues will certainly improve the quality of the report.

***Ramboll response**: As noted previously, Table 2 of the Ramboll chloroprene PBPK model report provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport*

limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact on risk estimates derived from the model would be negligible. However, a new sensitivity analysis of the Kgl value has been conducted in response to this and other comments. The analyses are documented in the methods and results sections of the revised documentation. The results of the analysis (see initial response in the section on Kgl) provide support for the value of 0.22 L/h currently used in the model as the most scientifically defensible and conservative estimate.

COMMENTS AND RESPONSES RELATED TO THE RAMBOLL MCMC ANALYSES

Tier 2

Kan Shao (Question 5): I also would like to point out that the performance of MCMC simulation and consequently the estimation results are closely related to the modeling implementation: (1) using a single level MCMC analysis instead of a hierarchical structure essentially treated the samples from various incubation vials equally and increased the sample size, which may reduce the uncertainty/variability in the posterior sample and facilitate the convergence of posterior sample; (2) the selected prior distributions for the parameters may also have important impact on the resulting estimates. So, my suggestion is to employ additional sensitivity analysis to investigate how the various settings in the MCMC method may impact the results (**Tier 2**).

***Ramboll response**: We do not believe this sensitivity analysis would provide significant additional value, since the USEPA is conducting their own MCMC analysis.*

Kan Shao (Question 6): it is likely that the uncertainty quantified in the present analysis may be underestimated. Additionally, it is not clear why the estimated value of Km_liver and the estimated value of Km_lung of the present analysis presented in Table S-3 in the Supplemental Material A are different given they were assumed to be the same (**Tier 2**).

***Ramboll response**: The values of Km in liver and lung are for the mouse and rat. The assumption that the Km in the liver and lung were the same was made for the human. As explained in the main report, both CYP2E1 and CYP2F contribute to the metabolism of chloroprene in the mouse and rat, resulting in different estimated Km values in the two tissues. However, in the human, the activity of CYP2F1 is extremely low, so that the metabolic clearance in both liver and lung is dominated by CYP2E1, and the Km of CYP2E1 is the same.*

COMMENTS AND RESPONSES RELATED TO CONDUCTING ADDITIONAL EXPERIMENTAL STUDIES

Tier 1

Jordan Smith (Question 7): If the A1 approach is going to be used to extrapolate extrahepatic metabolism, I recommend that Ramboll experimentally determine which enzymes are responsible for chloroprene metabolism.

***Ramboll response**: As explained in the Ramboll PBPK report, both CYP2E1 and CYP2F contribute to the metabolism of chloroprene in the mouse and rat. However, in the human, the activity of CYP2F1 is extremely low, so that the metabolic clearance in both liver and lung is dominated by CYP2E1. Therefore, further experimentation is unnecessary.*

I also recommend that a substrate marker activity is then selected based on which enzymes are identified. For example, an alternative substrate marker for CYP2E1 may be chlorzoxazone activity, which is commonly used by vendors to assess CYP2E1 activity in commercially available samples.

***Ramboll response**: Although chlorzoxazone has been used to assess CYP2E1 activity in drug evaluations, it is also metabolized by CYP3A4, CYP1A2, CYP2A6, CYP2B6, and CYP2D6 (Shimada et al. 1999), so it would not provide a specific marker for CYP2E1.*

Shimada T, Tsumura F, Yamazaki H. 1999. Prediction of human liver microsomal oxidations of 7-ethoxycoumarin and chlorzoxazone with kinetic parameters of recombinant cytochrome P-450 enzymes. Drug Metabolism and Disposition, 27(11): 1274-1280.

Tier 1/2

Jochem Louisse (Question 5): Perform in vitro studies with well-characterized batches of microsomes for which data on metabolic conversion of model substrates are available (provided by vendor). Include one or more of these substrates in the studies (reference chemicals) to assess whether the system works (quality control). I am actually of the opinion that such quality controls should always be included if one generates data to be used in a regulatory setting. From that perspective, this can also be seen as a Tier 1 recommendation. [I think it is important to have at least data of two independent experiments per type of microsome (so for a certain tissue/species/sex) in order to assess the robustness of the method (the experimental setup)].

***Ramboll response**: The laboratory at which the chloroprene metabolism studies were performed is no longer active and we were unable to find any commercial or academic laboratories that could perform such studies with chloroprene. Metabolism studies with volatile compounds are difficult and time-consuming, and require strong experience, such as the experience of the investigator, Dr. Matthew Himmelstein, who performed the studies used in the Ramboll PBPK model development. Further, as noted by Dr. Paul Schlosser during the peer review, the USEPA is not looking to conduct additional experimentation related to development of this model or its parameters. However, in response to the reviewers' concerns, we have conducted a new sensitivity analysis of the impact of Kgl values ranging from 0.055 to 1000 on the metabolism parameter estimates and the dose metrics obtained with the model. This new analysis, which has been added to the methods and results sections of the Model Documentation, demonstrates that (1) a value of Kgl = 0.22 provides the best fit to the data, (2) the impact of*

the choice of Kgl on the values estimated for Vmaxc is less than 10% and the effect on estimates of Km is less than a factor of 2, (3) the female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases about 30% as Kgl is increased from 0.175 (the lowest value for which the MCMC analysis could converge) to 1000. Overall, the value of Kgl = 0.22 that was selected for use in the in vitro modeling (Supplemental Materials D) is both scientifically defensible and risk-conservative, based on (1) it was derived from a joint MCMC analysis for Kgl and Km in the male mouse, which was the most informative metabolic data (Supplemental Materials B), (2) it provides the best goodness of fit of the in vitro model to the experimental metabolism data in the human liver (Table 1 of the main report), and (3) lower risk estimates would be obtained using higher values of Kgl. While a value of Kgl=0.175 would provide a higher risk estimate, it did not provide as good a fit to the in vitro data as Kgl = 0.22; in fact, attempting to decrease Kgl any further than 0.175 made it impossible to fit the data at all.

Tier 2

Jeffrey Heys (Question 2): (1) In Csanady, Guengerich et al. (1992), they state that equilibrium experiments employed "heat-inactivated microsomes or phosphate buffer." I think that conducting air-liquid equilibrium studies with heat-inactivated microsomes would provide some insight into the magnitude of non-specific binding (it would not provide insight into specific binding).

(2): If the first recommendation is not feasible, it also seems relatively straightforward to conduct equilibrium experiments identical to those shown in supplement B at various mixing rates up to at least 500 rpms to determine the impact of mixing on mass transport.

***Ramboll response**: As noted previously, the laboratory at which the chloroprene metabolism studies were performed is no longer active and we were unable to find any commercial or academic laboratories that could perform such studies with chloroprene. Even if such a study could be performed, it would be difficult to assure that the experimental conditions in the new Kgl study were sufficiently similar to those in the original metabolism studies, so the actual value of Kgl in the metabolism studies would still be uncertain. However, in response to the reviewers' concerns, we have conducted a new sensitivity analysis of the impact of Kgl values ranging from 0.055 to 1000 on the metabolism parameter estimates and the dose metrics obtained with the model. This new analysis, which has been added to the methods and results sections of the Model Documentation, demonstrates that (1) a value of Kgl = 0.22 provides the best fit to the data, (2) the impact of the choice of Kgl on the values estimated for Vmaxc is less than 10% and the effect on estimates of Km is less than a factor of 2, (3) the female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases about 30% as Kgl is increased from 0.175 (the lowest value for which the MCMC analysis could converge) to 1000. Overall, the value of Kgl = 0.22 that was selected for use in the in vitro modeling (Supplemental Materials D) is both scientifically defensible and risk-conservative, based on (1) it was derived from a joint MCMC analysis for Kgl and Km in the male mouse, which was the most informative metabolic data (Supplemental Materials B), (2) it provides the best goodness of fit of the in vitro model to the experimental metabolism data in the human liver (Table 1 of the main report), and (3) lower risk estimates would be obtained using higher values of Kgl. While a value of Kgl=0.175 would provide a higher risk estimate, it did not provide as good a fit to the in vitro data as Kgl = 0.22; in fact, attempting to decrease Kgl any further than 0.175 made it impossible to fit the data at all.*

Jordan Smith (Question 2): I recommend that these hypotheses are tested using the *in vitro* metabolism experiment. Phase transfer experiments with inactivated microsomes at various concentrations could be used to measure Kgl at different RPMs. Properly designed experiments could address these hypotheses specifically and definitively.

***Ramboll response**: Table 2 of the Ramboll chloroprene PBPK model report provides a comparison of the dose metrics obtained with the model assuming (1) that there was no diffusion limitation on transport in the metabolism studies, and (2) that there was a transport limitation with Kgl =0.22. The resulting dose metrics only differed by roughly 30%, and the differences were similar across concentrations and in mouse and human. Therefore, the impact on risk estimates derived from the model would be negligible.*

As noted previously, the laboratory at which the chloroprene metabolism studies were performed is no longer active and we were unable to find any commercial or academic laboratories that could perform such studies with chloroprene. Even if such a study could be performed, it would be difficult to assure that the experimental conditions in the new Kgl study were sufficiently similar to those in the original metabolism studies, so the actual value of Kgl in the metabolism studies would still be uncertain. However, in response to the reviewers' concerns, we have conducted a new sensitivity analysis of the impact of Kgl values ranging from 0.055 to 1000 on the metabolism parameter estimates and the dose metrics obtained with the model. This new analysis, which has been added to the methods and results sections of the Model Documentation, demonstrates that (1) a value of Kgl = 0.22 provides the best fit to the data, (2) the impact of the choice of Kgl on the values estimated for Vmaxc is less than 10% and the effect on estimates of Km is less than a factor of 2, (3) the female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases about 30% as Kgl is increased from 0.175 (the lowest value for which the MCMC analysis could converge) to 1000 Overall, the value of Kgl = 0.22 that was selected for use in the in vitro modeling (Supplemental Materials D) is both scientifically defensible and risk-conservative, based on (1) it was derived from a joint MCMC analysis for Kgl and Km in the male mouse, which was the most informative metabolic data (Supplemental Materials B), (2) it provides the best goodness of fit of the in vitro model to the experimental metabolism data in the human liver (Table 1 of the main report), and (3) lower risk estimates would be obtained using higher values of Kgl. While a value of Kgl=0.175 would provide a higher risk estimate, it did not provide as good a fit to the in vitro data as Kgl = 0.22; in fact, attempting to decrease Kgl any further than 0.175 made it impossible to fit the data at all.

Jordan Smith (Question 3): As recommended in question 2, measuring Kgl with an experiment specifically designed to assess mixing speed and microsome concentration with inactivated microsomes would be a preferred approach.

***Ramboll response**: As noted previously, the laboratory at which the chloroprene metabolism studies were performed is no longer active and we were unable to find any commercial or academic laboratories that could perform such studies with chloroprene. Even if such a study could be performed, it would be difficult to assure that the experimental conditions in the new Kgl study were sufficiently similar to those in the original metabolism studies, so the actual value of Kgl in the metabolism studies would still be uncertain. However, in response to the reviewers' concerns, we have conducted a new sensitivity analysis of the impact of Kgl values ranging from 0.055 to 1000 on the metabolism parameter estimates and the dose metrics obtained with the model. This new analysis, which has been added to the methods and results sections of the*

Model Documentation, demonstrates that (1) a value of Kgl = 0.22 provides the best fit to the data, (2) the impact of the choice of Kgl on the values estimated for Vmaxc is less than 10% and the effect on estimates of Km is less than a factor of 2, (3) the female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases about 30% as Kgl is increased from 0.175 (the lowest value for which the MCMC analysis could converge) to 1000 Overall, the value of Kgl = 0.22 that was selected for use in the in vitro modeling (Supplemental Materials D) is both scientifically defensible and risk-conservative, based on (1) it was derived from a joint MCMC analysis for Kgl and Km in the male mouse, which was the most informative metabolic data (Supplemental Materials B), (2) it provides the best goodness of fit of the in vitro model to the experimental metabolism data in the human liver (Table 1 of the main report), and (3) lower risk estimates would be obtained using higher values of Kgl. While a value of Kgl=0.175 would provide a higher risk estimate, it did not provide as good a fit to the in vitro data as Kgl = 0.22; in fact, attempting to decrease Kgl any further than 0.175 made it impossible to fit the data at all.

Kan Shao (Question 3): Therefore, my suggestions to the Ramboll/USEPA team are either (1) conducting a lab experiment to verify the "Kgl" value...

***Ramboll response**: As noted previously, the laboratory at which the chloroprene metabolism studies were performed is no longer active and we were unable to find any commercial or academic laboratories that could perform such studies with chloroprene. Even if such a study could be performed, it would be difficult to assure that the experimental conditions in the new Kgl study were sufficiently similar to those in the original metabolism studies, so the actual value of Kgl in the metabolism studies would still be uncertain. However, in response to the reviewers' concerns, we have conducted a new sensitivity analysis of the impact of Kgl values ranging from 0.055 to 1000 on the metabolism parameter estimates and the dose metrics obtained with the model. This new analysis, which has been added to the methods and results sections of the Model Documentation, demonstrates that (1) a value of Kgl = 0.22 provides the best fit to the data, (2) the impact of the choice of Kgl on the values estimated for Vmaxc is less than 10% and the effect on estimates of Km is less than a factor of 2, (3) the female mouse dose metrics are essentially unaffected by the value of Kgl assumed, while the human dose metric decreases about 30% as Kgl is increased from 0.175 (the lowest value for which the MCMC analysis could converge) to 1000, and (4) the highest risk estimate is obtained for Kgl = 0.22. These results support the appropriateness of using a value of 0.22 for Kgl.*

Jordan Smith (Question 7): I recommend that CYP2E1 induction be evaluated in lung tissue, if it is determined that this enzyme is primarily responsible for chloroprene metabolism.

***Ramboll response**: The recommended study would be extremely difficult due to the volatility of chloroprene and the difficulty of maintaining lung cell cultures over a sufficient period of time to see induction. Moreover, as discussed in the main report, there was no evidence of metabolic induction in liver microsomes from mice exposed to 90 ppm chloroprene for several weeks.*

Jochem Louisse (Question 10): Perform plasma binding studies to provide insight into whether chloroprene plasma protein binding is limited and whether description of plasma protein binding is indeed not needed in the PBPK model, or whether description of plasma protein binding should be included in the PBPK model.

***Ramboll response**: It is well known that plasma protein binding does not impact the kinetics of volatile organic compounds like chloroprene (Yoon et al. 2012), and it was not considered in the PBPK models used by EPA for similar compounds such as methylene chloride, vinyl chloride and trichloroethylene.*

Yoon, M, Campbell, JL, Andersen, ME, and HJ Clewell. 2012. Quantitative in vitro to in vivo extrapolation of cell-based toxicity assay results. Crit Rev Toxicol. 42(8):633-652.

Intended for
Denka Performance Elastomer LLC, Request for Correction

Exhibit B

Date
July 15, 2021

EPIDEMOLOGICAL BASIS FOR SUPPORTING A CORRECTION OF THE CHLOROPRENE INHALATION UNIT RISK (IUR): UPDATE

RAMBOLL Bright ideas. Sustainable change.

CONTENTS

1. INTRODUCTION

The community living near the Denka Performance Elastomer facility in St. John the Baptist Parish, LA has been in a state of high alert after being identified by United States Environmental Protection Agency (USEPA) as having exceptionally high cancer risk, based on estimated, modeled chloroprene exposures used in the two most recent National-Scale Air Toxics Assessments (NATA). As discussed in Section 2, below, these USEPA risk conclusions for the community were based on the results of screening-level models that are designed to identify areas for more detailed, data-driven evaluation and targeted environmental interventions, and do not provide a definitive assessment of cancer risks (USEPA 2018). Section 3 summarizes the recent epidemiological evidence regarding the carcinogenicity of chloroprene, highlighting results from the most recent occupational epidemiology studies (Marsh et al. 2007a, 2007b, 2021). If there were an association between chloroprene exposure and cancer(s), it would be more easily detected in an occupational setting where exposures are higher than in the general population and can be reasonably well estimated.

In addition, we summarize the latest data from the Louisiana Tumor Registry (LTR) in Section 4. These data as a whole – the updated epidemiological evidence together with current data from the LTR – continue to support the position that the USEPA NATA model results, used to suggest high cancer rates in St. John the Baptist Parish, are not substantiated empirically. Despite this, some groups, in particular the University Network for Human Rights (UNHR), have attempted to promote the opposite conclusion, that cancer risks in St. John the Baptist Parish are higher than expected. Therefore, to ensure the body of epidemiological evidence is accounted for and correctly interpreted, we discuss in Section 5 the numerous scientific deficiencies in a report issued by the University Network for Human Rights (UNHR 2019) and a follow up publication (Nagra et al. 2021).

2. NATA SUMMARY

Residents living in the community near the Denka Performance Elastomer facility became concerned about cancer risks from the Denka facility's chloroprene emissions after the USEPA publicized the results of their NATA screening and interpreted them as showing the community to be at elevated cancer risk compared to other regions of the United States.[1] The NATA is conducted by USEPA about every three years to evaluate sources, levels, and potential risks of hazardous air pollutants (HAPs), or air toxics. The NATA analyses rely primarily on source emissions inventories as inputs into air dispersion models and are used to predict population-level exposures. Multiple complex models are involved in the screening that may not reflect local site conditions. Toxicity factors, such as inhalation unit risk values for cancer effects, are then applied to the results of the exposure models to predict risks at the population level. The multiple steps in the analysis incorporate conservative estimates at each level, usually resulting in overestimated risks.

USEPA has highlighted that NATA does not include information that applies to specific locations, because exposure data at the county and census-tract level are not usually available. Instead, the NATA applies models to estimate exposures at the county and census-tract level. According

[1] National Air Toxics Assessment | US EPA

to USEPA, the NATA assessment is designed as a comparative tool that should be used to evaluate relative variations in air concentrations, exposures, and risk among geographic areas rather than to identify or estimate risks in any given, specific location. These data can then be used to help communities design local assessments, improve emissions inventories, and find areas where the air toxics monitoring network could be expanded or improved. USEPA specifically notes that NATA should not be used to address epidemiological questions such as the relationship between cancer risk and proximity to certain sources. There is significant uncertainty in modeled risk estimates. USEPA highlights that the NATA results should be applied cautiously, because of these large uncertainties, which vary from location to location as well as from pollutant to pollutant.

In 2015 and 2018, USEPA published the NATA results based on 2011 and 2014 emissions data, respectively, for the United States, including Louisiana. The risk calculations presented in the 2015 report (USEPA 2015) combined estimated exposures based on emissions data with a cancer inhalation unit risk (IUR) value derived by USEPA's Integrated Risk Information System (IRIS), documented in the Toxicological Review of Chloroprene (USEPA 2010). An updated assessment based on 2014 emission data was published in 2018 that relied on the same IUR as the prior assessment (USEPA 2018). As we have previously shown, the IUR is highly inflated due to the lack of consideration of pharmacokinetic differences between species (e.g. Sax et al. 2020; Clewell et al. 2020). Because they relied on the inflated IUR, both NATA assessments reported that Louisiana residents experienced the highest cancer risks in the US and attributed these risks to chloroprene exposures. The epidemiological evidence cited by USEPA as supporting its IUR in its 2010 report (USEPA 2010) is discussed in Section 3.1, as it provides a basis for the epidemiological evidence published since 2010, which is discussed in Section 3.2.

3. EPIDEMIOLOGICAL EVIDENCE

3.1 Summary of Epidemiological Evidence Considered in the USEPA Toxicological Review of Chloroprene

Occupational epidemiological studies are relevant to the identification of health risks associated with specific exposures because occupational exposures tend to be substantially higher than environmental exposures experienced by community members. Therefore, if the exposure is truly related to the outcome, that relationship is more likely to be detected in the occupational vs. the community setting.

As summarized in the USEPA Toxicological Review of Chloroprene (USEPA 2010) and in Sax et al. (2020), the epidemiological literature on chloroprene exposure and cancer risk includes studies of occupational cohorts from several countries, published over approximately 30 years. Among the available occupational epidemiological studies, those by Marsh et al. (2007a, 2007b) represent the most comprehensive and methodologically robust based on the size of the cohort, amount of follow-up time, and completeness of exposure assessment, among other strengths (Bukowski 2009; Sax et al. 2020). The results from earlier studies conducted in the US (Pell 1978; Leet and Selevan 1982) were included in the update by Marsh et al. (2007a, 2007b).

The studies by Marsh et al. (2007a, 2007b) include a cohort of 12,430 workers from two US facilities in Louisville, KY (Plant L, n= 5507) and Pontchartrain, LA (Plant P, n=1357)) and two European facilities (Maydown, Northern Ireland; Plant M, n = 4849 and Grenoble, France; Plant G, n=717). Chloroprene production at these facilities dates back to 1942 (Plant L) and 1969

(Plant P). Two of the facilities (Plants L and M) used an acetylene process to make chloroprene, which also produces vinyl chloride, a known risk factor for liver cancer (IARC 2008). One of the strengths of these epidemiological studies is that the authors quantitatively estimated historical exposures for individual workers for both chloroprene and, where applicable, vinyl chloride. Mortality follow-up was conducted through 2000, therefore the study benefitted from an extensive follow-up time and ample time for the development of the two cancers of interest, lung and liver cancer. Overall, the study found no evidence of elevated mortality risks from lung, liver, or other cancers.

In contrast, studies conducted in China (Li et al. 1989), Russia (Bulbulyan et al. 1998), and Armenia (Bulbulyan et al. 1999) have not been updated and have serious limitations as described in Acquavella & Leonard (2001), Bukowski (2009), Rice & Boffetta (2001) and Sax et al. (2020). Briefly, these limitations include insufficient statistical power due to small cohort sizes, incomplete exposure assessments, poor control for confounding factors (e.g. smoking and drinking), poor documentation of cohort enumeration, and inappropriate reference rates.

In 2010, USEPA concluded that chloroprene was "likely to be carcinogenic to humans," in part because of its assessment of the epidemiological evidence (USEPA 2010). In their evaluation of this evidence, USEPA designated the evidence presented in the Marsh et al. (2007a, 2007b) studies as supporting a causal association between chloroprene exposures and elevated mortality from liver cancer. As noted in Sax et al. (2020), USEPA misinterpreted the Marsh et al. findings as providing evidence of an exposure-response relationship between chloroprene exposure and liver cancer mortality based on comparisons between exposure groups within the cohorts. In fact, the internal comparisons were misleading due to the very low liver cancer mortality rates among the employees. For example, the standardized mortality ratio (SMR, their mortality rate compared with the general population) for those in the lowest cumulative exposure category in Plant L was 0.40, or 60% lower than expected when compared with the general population in the area surrounding the plant. The SMR for the highest cumulative exposure category was 0.85, or about 15% below the expected rate compared with the general population (Marsh et al. 2007b). When these two rates are compared in the relative risk calculation, the ratio of these two low rates provides a mathematical result that is above 1.0 (specifically, 2.32), and misleadingly implies an excess risk for those in the highest cumulative exposure category. In part because of USEPA's reliance on and misinterpretation of the Marsh et al. (2007a, 2007b) results, Marsh et al. (2021) published analyses of an extended follow-up period for the US occupational cohorts, summarized below.

3.2 Summary of Epidemiological Evidence Published since the USEPA Toxicological Review of Chloroprene

A recent update by Marsh et al. (2021) includes additional follow-up of the US cohorts, from 2001 to 2017. Marsh et al. (2021) conducted an update in order to increase the person-years and total numbers of deaths observed, and thereby provide a more reliable evaluation of cancer mortality patterns in relation to chloroprene exposure in the two large US plants that were included in the 2007 studies (i.e. Plants L and P).

The updated study by Marsh et al. (2021) added 47,299 and 19,942 person-years of observation and 1399 and 214 new deaths from the Plant L and Plant P cohorts, respectively. Using the National Death Index, the authors identified 4,118 deaths and with an underlying cause of death recorded for 97.2% of them (n=4004). Exposure estimates were not updated for the re-analysis but were based on the exposure estimates as described in the earlier publications (Marsh et al.

2007a, 2007b). This is appropriate given the decades-long induction period for solid tumors: any recent exposure data added to the updated analysis would not contribute information to cancer risk calculations, because it would have occurred outside of the time period relevant for the development of cancer. Marsh et al. (2021) used the same statistical analyses reported in Marsh et al. (2007a, 2007b), including both external and internal comparisons. External comparisons included national and local cancer rates, and internal comparisons included comparisons based on exposure levels (i.e. exposure duration in years, exposure concentration in ppm, or cumulative exposure in ppm-yrs), with the lowest exposure group as the referent category.

The external comparisons showed statistically significant deficits at both plants (SMR<1.0) for all types of cancer (combined) using both national and local rates as comparisons. Compared to local cancer mortality rates, there were statistically significant deficits reported for the key outcomes of lung cancers (each plant) and liver cancers (Plant P). There were no statistically significant excess risks in either plant (Tables 1 and 2, below, and Marsh Table 4). As in the earlier reports, (Marsh et al. 2007a, 2007b) the internal analysis showed some elevated relative risks (RR) for some exposure categories at each plant, but these were arithmetical results due to comparisons between pairs of low rates. As discussed above, the internal referent categories had substantially lower mortality rates than both national and county rates, again yielding a comparison between a deficit of deaths in one group and a larger deficit of deaths in another group. Overall, the authors noted that "Although we observed elevated RRs in many exposure categories, we found no compelling evidence of a positive exposure-response relationship in either study plant" (Marsh et al. 2021).

Table 1. Observed Deaths and SMRs for Selected Causes of Death Total Plant L (Louisville, KY) Cohort

Cancers	Observed	US		Local County	
		SMR	95% CI	SMR	95%CI
All cancer	974	0.89**	0.84-0.95	0.75**	0.70-0.80
Biliary Passages & Liver Primary	31	1.06	0.72-1.51	0.95	0.65-1.35
Bronchus, Trachea, Lung	340	1.0	0.89-1.11	0.72**	0.65-0.80

From Marsh et al. (2021, Table 3); Observed deaths between 1960-2017; ** P<0.01

Table 2. Observed Deaths and SMRs for Selected Causes of Death Total Plant P (Pontchartrain, LA) Cohort

Cancers	Observed	US		Local County	
		SMR	95% CI	SMR	95%CI
All cancer	92	0.69**	0.56-0.85	0.64**	0.52-0.78
Biliary Passages & Liver Primary	1	0.2	0.01-1.10	0.16*	0.00-0.88
Bronchus, Trachea, Lung	30	0.71	0.48-1.02	0.62**	0.42-0.89

From Marsh et al. (2021, Table 4); Observed deaths between 1960-2017; * P<0.05 **P<0.01

As with the prior studies (Marsh et al. 2007a, 2007b), this follow-up study has several strengths that are discussed in detail in Bukowski (2009) and Sax et al. (2020). Briefly, they include a large cohort size, long follow-up period, comprehensive case ascertainment, a detailed exposure assessment (including of vinyl chloride) and use of appropriate local and national population

comparisons. The follow-up study adds confidence to the prior findings by the increased statistical power from added person-years and numbers of deaths. The choice to not update work histories and exposure estimates would not affect the results because 97% and 70% of workers from Plant L and Plant P, respectively, left their jobs before 2001 and because the original exposure assessment is more pertinent to the assessment of cancer risk, due to the long latency period for cancers.

Occupational epidemiology data, especially the best quality data from the occupational cohorts described by Marsh et al. (2007a, 2007b) and Marsh et al. (2021), do not support the elevated risk estimates suggested by the results of the NATA assessments.

4. LOUISIANA TUMOR REGISTRY REPORTS

The Louisiana Tumor Registry (LTR) participates in the CDC Surveillance, Epidemiology, and End Results (SEER) program, which records incident cancers in 43 US states and Washington D.C., Guam, Puerto Rico, the U.S. Virgin Islands, and Bermuda[2,3]. As part of the SEER program, LTR is held to specific standards of quality and completeness. It has received awards for meeting or exceeding these standards in every year since 2002 (Maniscalco et al. 2020). The results of a recent audit for St. John the Baptist Parish, specifically for the parts of the parish nearest to the Denka facility, have further demonstrated that the LTR is complete and accurate (Williams et al. 2021)—i.e. all cancers reported by the community members who participated in the audit were found in the LTR data.

The purpose of the LTR, and all cancer registries, is to collect information to identify locations or population subgroups that experience unusual patterns of cancer, such as higher than expected rates of specific cancer types, unusual occurrences of rare forms of cancer, or unexpected numbers of cancers in certain age ranges. This is accomplished by comparing patterns of cancer incidence between regions.[4] Therefore, the LTR periodically issues a report summarizing cancer incidence and mortality rates (all cancers and specific cancers) in Louisiana as a whole, in the seven parishes comprising the Industrial Corridor (IC), and in each individual parish. In the context of identifying cancer risks in the vicinity of the Denka facility, the LTR provides an important means of verifying the risk estimates suggested by NATA. If the NATA risk assessment were accurate, and the area around the Denka facility were at high risk of cancer, the LTR would identify higher cancer incidence rates in St. John the Baptist Parish than elsewhere. In fact, the incidence rates of cancers of concern, i.e. cancers of the lung/bronchus and liver/intrahepatic bile duct, were similar to or statistically significantly lower than the incidence of these cancers in the IC compared with Louisiana as a whole in each of the periods reported in the last three LTR reports, covering the years 2007-2011, 2011-2015, and 2013-2017 (Table 3). Among white men, the incidence of all cancers (combined) was higher in the IC than in Louisiana as a whole during 2007-2011, but rates were similar or lower for all other time intervals. For all time intervals and all other race/gender groups reported (White women, Black men, Black Women)

[2] National Program of Cancer Registries program standards, 2017-2022 (cdc.gov), accessed March 2, 2021 and Scope of Standards | SEER Training (cancer.gov), accessed March 2, 2021
[3] National Interstate Data Exchange Agreement (naaccr.org), accessed March 2, 2021
[4] How Cancer Registries Work | CDC, accessed March 3, 2021; Cancer Registries' Value for You | CDC, accessed March 3, 2021; How Cancer Registries Work | CDC, accessed March 3, 2021;Cancer Registries' Value for You | CDC, accessed March 3, 2021

the incidence was similar to or statistically significantly lower than the incidence rate for Louisiana as a whole.

Statistical comparisons between individual parishes or between parishes and the IC or the state are not included in the published LTR reports. Since 2019, however, parish-level comparisons have been available from the LTR by way of an on-line data visualizer[5]. These data indicate that, for the period 2012-2016, cancer incidence rates in St. John the Baptist parish have been below the state-wide average for all cancers (combined) and for cancers of the lung/bronchus and liver (Figures 1, 2, and 3). Therefore, the premise offered by the estimates modeled by NATA, that this parish has or its constituent census tracts have the highest rates of cancer in the U.S., is incorrect.

In 2018, the LTR began reporting cancer data for individual census tracts, in addition to providing data at the Parish and State levels.[6] These reports provide data aggregated over a 10-year period to protect the privacy of residents and to increase the statistical reliability of the estimates. Specifically, the LTR is legally restricted from reporting data for populations of less than 20,000. Reliable statistics can only be obtained if there is a sufficient number of cases (generally greater than 16 cases or more).

The latest LTR census tract-level report provided for the period from 2008-2017 (Maniscalco et al. 2021). The results from this report were consistent with the findings from the Parish-level analysis. Specifically, for St. John the Baptist Parish, none of the 11 census tracts reported a statistically significantly elevated rate of all cancers (combined) compared to the State-level rates. Similarly, for lung and bronchus cancers, none of the 9 census tracts with reported data showed statistically significant elevations compared to the State rates. There were no census tract-level data reported for St. John the Baptist Parish for liver cancers.

LTR data at neither the Parish nor the census tract level indicate elevated rates of the cancers potentially associated with chloroprene exposure in St. John the Baptist Parish compared to Louisiana.

[5] Louisiana Cancer Data Visualization - Public Health (lsuhsc.edu). Accessed June 2021

[6] Cancer Incidence in Louisiana by Census Tract - 2018 - Public Health (lsuhsc.edu) Accessed June 2021

Table 3. Average annual incidence rates by race and sex in Louisiana (LA), the Industrial Corridor, St. John the Baptist and surrounding parishes, 2007-2011, 2011-2015, and 2013-2017

	All cancer			Lung & Bronchus			Liver and Intrahepatic Bile Duct		
	2013-2017	**2011-2015**	**2007-2011**	**2013-2017**	**2011-2015**	**2007-2011**	**2013-2017**	**2011-2015**	**2007-2011**
White men									
LA state	547.2	544.8	578.1	77.9	82.4	92.3	13.4	12.5	10.8
Industrial corridor‡	559.0	551.3	**595.3§**	**66.6§**	**69.9§**	**78.8§**	11.9	**10.2§**	**7.3§**
St. John the Baptist	539.5	487.5	524.5	75.9	72.2	104.3	NR		
Ascension	573.0	581.1	595.5	81.4	82.4	95.5	NR		
Jefferson	522.4	530.4	555.7	71.0	74.0	83.3	NR		
Lafourche	580.1	569.5	547.2	74.5	78.2	84.8	NR		
Livingston	542.1	562.5	615.9	91.3	111.8	115.7	NR		
St. Charles	503.4	499.2	583.9	42.8	61.6	86.1	NR		
St. James	626.1	642.2	599.3	84.2	70.9	65.7	NR		
St. Tammany	562.4	555.4	589.3	76.3	75.5	82.3	NR		
Tangipahoa	527.8	539.0	600.8	88.0	90.3	110.5	NR		
Black men									
LA state	592.4	605.1	652.1	99.1	105.8	113.8	22.6	21.5	16.0
Industrial corridor‡	599.9	629.2	675.2	**88.0§**	98.0	104.0	25.2	23.6	17.2
St. John the Baptist	597.0	619.8	627.4	89.2	103.0	90.6	^		
Ascension	463.6	562.0	690.0	51.1	104.9	116.4	^		
Jefferson	603.8	601.1	640.5	98.1	99.1	103.5	26.8		
Lafourche	611.8	634.5	593.8	124.0	123.3	103.2	^		
Livingston	573.5	566.9	619.6	^	^	^	^		
St. Charles	524.4	547.0	586.0	75.2	102.2	96.7	^		
St. James	553.9	638.7	813.9	92.6	103.5	132.7	^		
St. Tammany	659.1	614.9	579.5	90.1	111.1	108.7	25.1		
Tangipahoa	613.3	604.4	684.0	112.9	108.8	126.8	^		
White women									
LA state	432.1	420.6	413.1	56.8	57.1	59.1	4.2	3.4	3.2
Industrial corridor‡	**418.1§**	**398.4§**	**397.6§**	**46.4§**	**43.5§**	**52.7§**	3.1	2.5	3.3
St. John the Baptist	439.7	422.8	396.9	47.7	41.5	43.9	NR		
Ascension	421.7	393.1	392.2	56.6	52.3	69.3	NR		

Table 3. Average annual incidence rates by race and sex in Louisiana (LA), the Industrial Corridor, St. John the Baptist and surrounding parishes, 2007-2011, 2011-2015, and 2013-2017

	All cancer			Lung & Bronchus			Liver and Intrahepatic Bile Duct		
	2013-2017	**2011-2015**	**2007-2011**	**2013-2017**	**2011-2015**	**2007-2011**	**2013-2017**	**2011-2015**	**2007-2011**
Jefferson	449.9	442.2	423.5	57.6	60.4	60.6	NR		
Lafourche	434.3	417.2	408.0	52.9	49.6	56.0	NR		
Livingston	417.0	405.0	414.3	62.2	65.1	56.3	NR		
St. Charles	415.7	427.9	399.3	59.9	53.9	63.1	NR		
St. James	395.1	332.4	373.2	^	^	44.8	NR		
St. Tammany	452.3	447.3	434.2	49.5	50.3	59.6	NR		
Tangipahoa	415.7	405.7	405.6	53.8	56.0	55.0	NR		
Black women									
LA state	421.9	415.4	415.4	46.7	49.0	52.7	4.9	4.5	4.4
Industrial corridor‡	422.0	416.3	418.6	**40.5**§	**41.5**§	48.3	5.0	3.9	4.6
St. John the Baptist	351.9	359.5	392.7	31.7	38.2	56.5	NR		
Ascension	370.4	389.8	429.0	30.5	32.6	54.2	NR		
Jefferson	450.4	421.8	429.1	49.1	50.9	62.1	NR		
Lafourche	377.8	352.9	395.4	^	^	70.1	NR		
Livingston	426.9	391.7	458.1	^	^	^	NR		
St. Charles	482.7	418.5	410.7	65.1	67.3	65.1	NR		
St. James	502.8	439.1	355.0	51.5	48.8	^	NR		
St. Tammany	434.8	458.7	461.6	48.5	57.9	56.7	NR		
Tangipahoa	414.2	429.2	430.3	41.8	44.8	70.8	NR		

Figure 1. Cancer Incidence (All Cancers) in Louisiana: 2012-2016

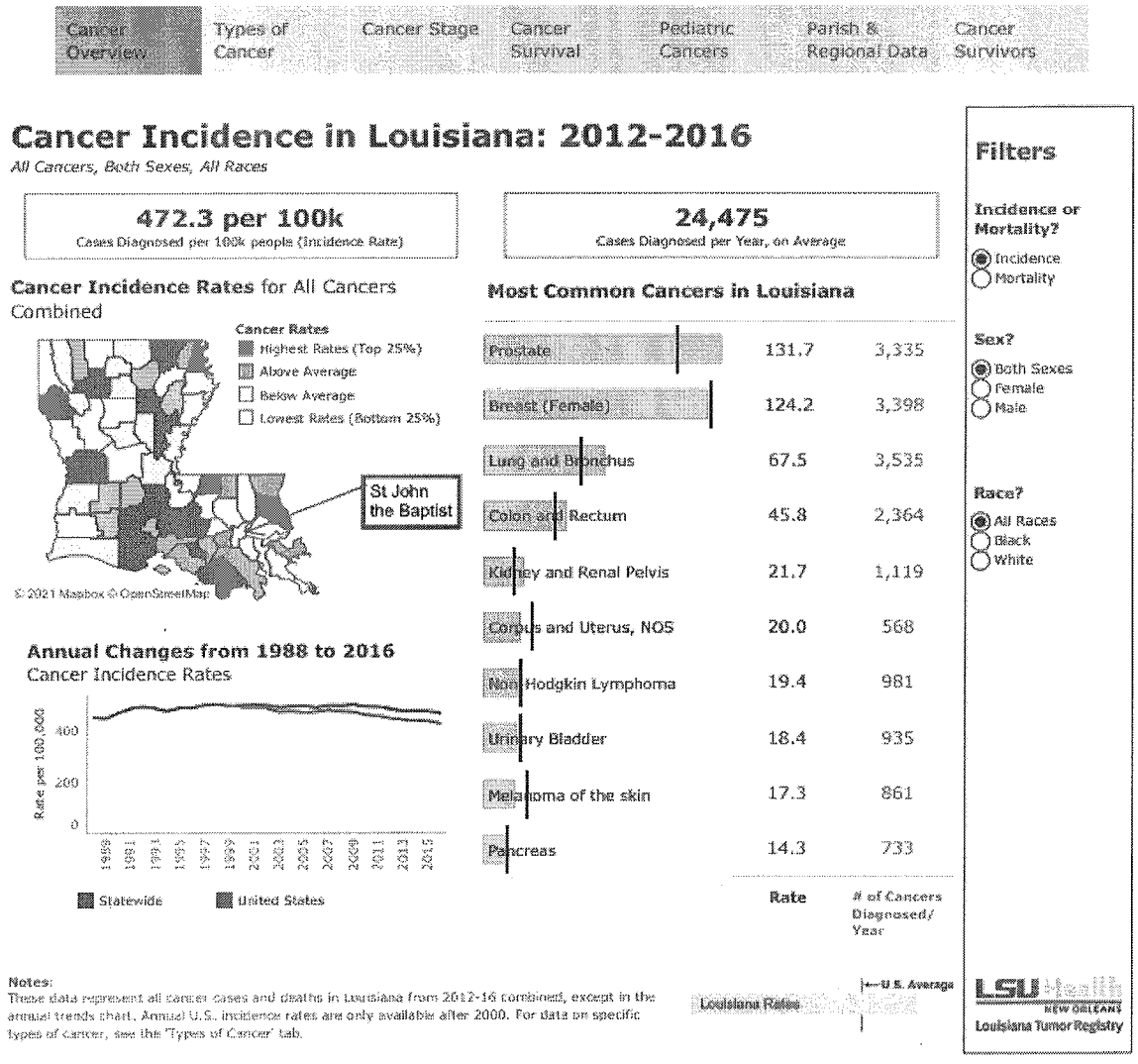
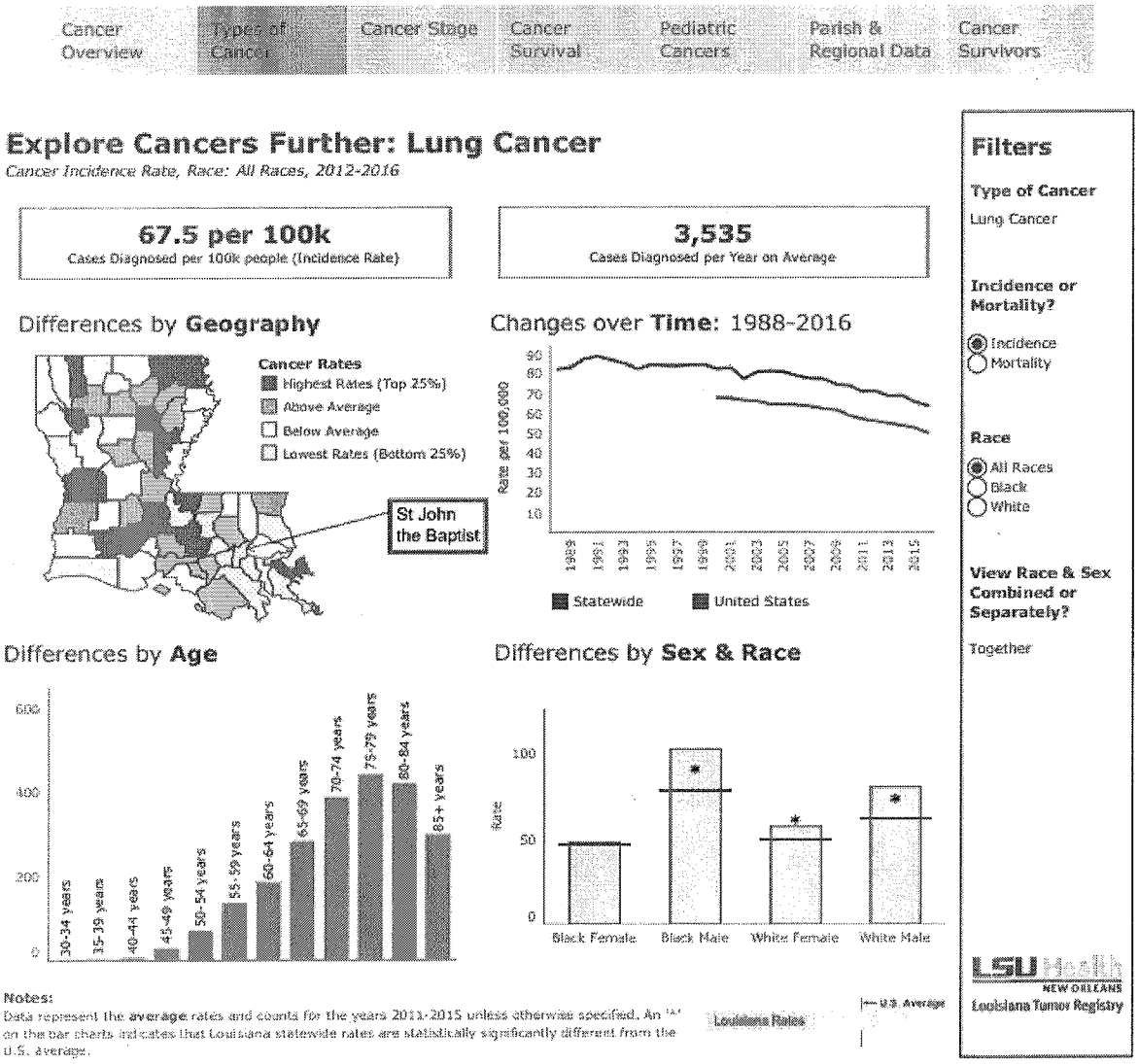
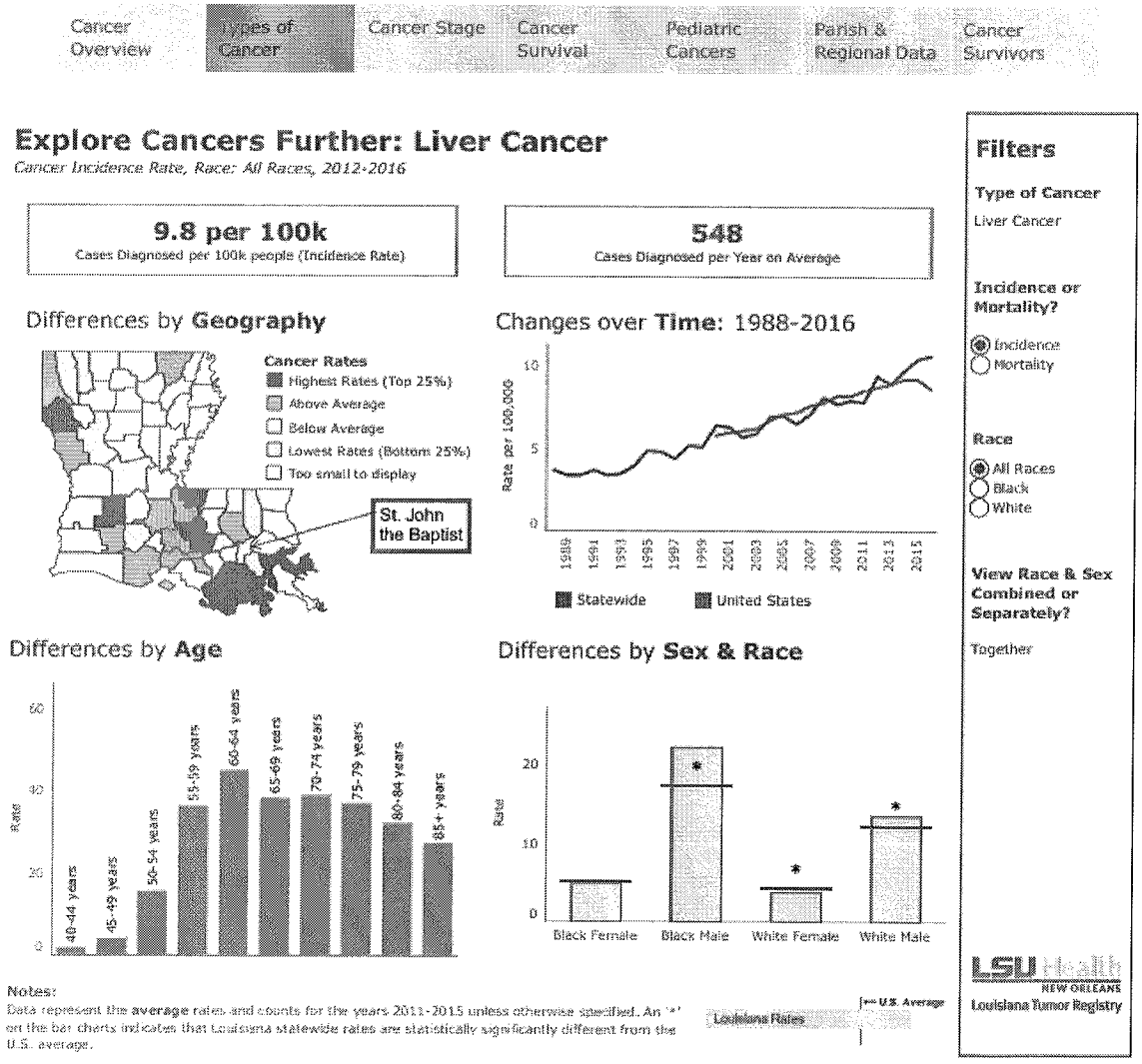


Figure 2. Lung Cancer Incidence in Louisiana: 2012-2016



Source: Louisiana Cancer Data Visualization - Public Health (lsuhsc.edu). Accessed June 2021

Figure 3. Liver Cancer Incidence in Louisiana: 2012-2016



Source: Louisiana Cancer Data Visualization - Public Health (lsuhsc.edu). Accessed June 2021

5. UNIVERSITY NETWORK FOR HUMAN RIGHTS (UNHR) 2019 REPORT AND FOLLOW-UP PUBLICATION

In 2019, the University Network of Human Rights (UNHR) self-published a report describing a community survey conducted in two residential areas surrounding the Denka facility in St. John the Baptist Parish. This report, in slightly revised form, was published in 2021 in the journal Environmental Justice (Nagra et al. 2021). As outlined in a response to the unpublished UNHR report[7], the survey and its analyses used incorrect and non-standard methods that led to an incorrect conclusion that the 23-year period prevalence of all cancer (combined) in the residential area (so-called Zone 1) closest to the Denka facility is elevated due to environmental exposures from the Denka facility. The comments that follow apply to both the original, unpublished UNHR report and to the paper published in 2021; they are collectively referenced here as Nagra et al. (2021).

Nagra et al. (2021) used residential distance from the Denka facility as a surrogate for exposure to chloroprene, without using any measured exposures and assuming that all the residences within each of the zones they identified had the same exposure. We focused our assessment of their methods on Zone 1, where they reported an apparent increase in the 23-year cancer prevalence.

Nagra et al. (2021) relied on data from a subset of households to represent the entire population of interest. This is often done in community health surveys, but properly conducted surveys attempt to corroborate the validity of data collected by interviews, and particularly data collected from proxy respondents. In the Nagra et al. (2021) study, one volunteer per included household answered survey questions, including questions about cancer diagnoses, for all household members. It is impossible to speculate about the validity of these self- and proxy-reports, but, had the authors obtained appropriate Institutional Review Board approval and informed consent from the participants, and had they recorded individually identifying information, they could have verified the reported cancer cases against either medical records or data recorded in the LTR. This was not done.

In properly conducted surveys, the included participants represent the target population. To validly estimate the 23-year cancer prevalence in Zone 1, the study should have been based on a representative sample of current and former residents covering the 23-year time-period of interest. If the population of Zone 1 had been stable over time, data for current residents might have provided a valid estimate of cancer prevalence in the target population. This was not the case, however. Almost all current residents of Zone 1 can be mapped to Block 1 of US Census tract 708, where the US Census American Community Survey (ACS) found a shift in the gender and age distributions with relatively more men and residents aged 70+ years in 2015-19 compared with 2010-14. Because older age and male gender are risk factors for cancer (see, for example, Clegg et al. 2009; Ward et al. 2004; Yin et al. 2010, and data from SEER[8]), the cancer prevalence Nagra et al. (2021) estimated based on the current population does not represent the true cancer prevalence in Zone 1 over the past 23 years, and likely overestimates that value. An analysis conducted according to standard epidemiological practice would have evaluated and

[7] https://edms.deq.louisiana.gov/app/doc/view.aspx?doc=11830771&ob=yes

[8] https://seer.cancer.gov/statfacts/html/all.html

adjusted for such changes in the distribution of cancer risk factors before applying the current data to the target population.

The survey questions used by Nagra et al. (2021, appendix A1) did not clearly indicate if respondents should only provide information for current household members or if former household members should be included; if former household members were to be included, the questions could be interpreted as applying only if the former household members had had cancer. If only those former household members who had had cancer were included, rather than a representative sample of former residents with and without cancer, then the cancer prevalence estimated for Zone 1 would again have been artificially inflated.

It is imperative that epidemiological studies be constructed to avoid bias. In the community survey conducted by Nagra et al. (2021) an unknown proportion of the volunteer participants in the survey are among the community members who are plaintiffs suing Denka and DuPont (the owner of the facility until November 1, 2015) for personal injury and damages related to alleged chloroprene exposure. If plaintiffs in the lawsuit are among the survey participants, there is increased likelihood that they may – consciously or otherwise -- over-report health conditions among themselves and their family members.

The effect of the non-representative study population on the prevalence estimate might have been reduced if Nagra et al. (2021) had excluded cancer cases that must have resulted from causes other than environmental chloroprene exposure instead of analyzing all cancers (combined). For example, Nagra et al. (2021) could have focused on lung and liver cancers, which have been proposed to be potentially associated with chloroprene exposure (USEPA 2010). In addition, under their questionable assumption that residence location is a valid surrogate for environmental exposure, Nagra et al. (2021) should have confirmed that the individuals with cancer included in their prevalence estimate had lived in Zone 1 during a time period relevant to the development of their cancers. This was not done, however.

By restricting the study to a sub-population that had recently undergone a sociodemographic shift, which resulted in an increase in cancer risk factors that were unrelated to any environmental exposures, and by analyzing all cancers (combined), thereby including types of cancer that could not—even theoretically—have been associated with environmental chloroprene exposure, Nagra et al. (2021) likely created an artificial, false association between residential proximity to the Denka plant and cancer. This major shortcoming would have been exacerbated if they included former household members only if they had had cancer and by failing to assess or account for the likely participation of plaintiffs suing Denka among the study population.

Overall, Nagra et al. (2021) incorrectly analyzed the data they collected, ignored relevant, available data, and conducted an analysis that was fundamentally flawed to reach the incorrect conclusion that there was an increase in cancer prevalence attributable to environmental exposures in the surveyed population living closest to the Denka facility.

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