UPDATING THE EPA'S 2010 IUR FOR CHLOROPRENE BASED ON PBPK MODELING RESULTS

Harvey Clewell, PhD, DABT, FATS

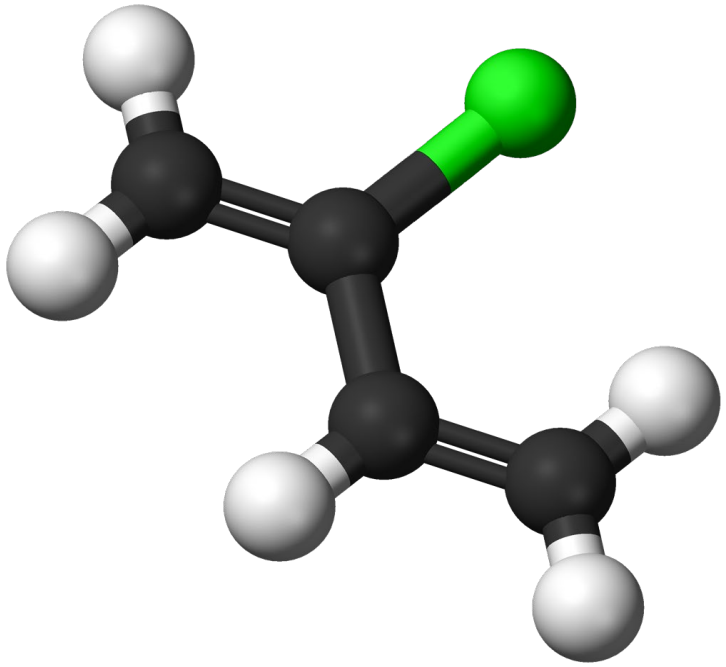
P. Robinan Gentry, PhD, DABT

Jerry Campbell, PhD

Cynthia Van Landingham, MS

Sonja Sax, ScD

EPA Meeting June 12th, 2019



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INTRODUCTION

- On behalf of Denka Performance Elastomer (DPE) and Ramboll we thank EPA for continued collaboration and dialog regarding the use of the best available science to develop a health-based standard for chloroprene
- DPE representative – Patrick Walsh
- Ramboll team –Robinan Gentry and Harvey Clewell (in person), Cynthia Van Landingham, Sonja Sax, and Jerry Campbell (on the phone)
- Supporting team – Kenneth Mundt and Mel Andersen (in person)

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OBJECTIVES

- Describe updating and validation of the PBPK model to address EPA's concerns raised in response to Denka's RFC, RFR and the PBPK Workplan
- Present results of analyses performed to evaluate the PBPK model
- Provide EPA with proposed conclusions based on model evaluation
- Discuss next steps

OVERVIEW

- Collaborations with EPA
- Evidence demonstrating the need for a PBPK correction
- Ramboll's response to EPA comments on the evaluation of the chloroprene PBPK model and the application of the revised model to estimate a chloroprene IUR
  - PBPK model overview
  - Analyses and results
- Conclusions
- Next steps

COLLABORATIONS WITH EPA 2016-2018

- **August 2016** – DPE/Ramboll first met with EPA to discuss updating the IUR
- **October 2017** - DPE/Ramboll met with EPA to discuss the submitted RFC
- **March 2018** - DPE/Ramboll submitted workplan to EPA to collaboratively develop a PBPK model for chloroprene, addressing EPA concerns raised in the RFC. Review
- **July 2018** – DPE/Ramboll met with EPA to discuss the PBPK model progress and receive feedback from EPA
- **August 2018** – DPE/Ramboll submitted PBPK model code and parameters to EPA for review/Dr. Paul Schlosser determined that "Kg" parameter was needed to validate the PBPK model
- **October/November 2018** – DPE/Ramboll developed a protocol to conduct Kg experiments together with input from Dr. Schlosser and identified TekLab as a suitable lab
- **December 2018/early January 2019 -** TekLab adapted the protocol, and the updated protocol was reviewed by EPA. Ramboll incorporated input from EPA. TekLab determined that additional equipment was needed to implement the protocol, and DPE purchased the equipment

COLLABORATIONS WITH EPA IN 2019

- **February 2019** – TekLab received all equipment and began testing the protocol to ensure reproducibility
- **March 2019** – Lab conducted the experiments; Ramboll reviewed and analyzed results, requested more data; lab collected more data
- **April 2019** – Ramboll modified PBPK model to account for Kg after discussing results with Dr. Schlosser and taking his recommendations into consideration
- **May 2019** - DPE submitted additional PBPK model documentation, and associated manuscript to EPA as an update to the RFR

EVIDENCE DEMONSTRATING THE NEED FOR A PBPK CORRECTION

- Animal studies (NTP, 1998; Trochimowicz et al., 1998) at chloroprene concentrations (10 to 80 ppm)
  - Little consistency across species in the number of tumors and in tumor locations
  - No statistically significant increases in the incidence of tumors (***including lung***) in Wistar rats and Syrian hamsters
  - Significant increases in the incidence of tumors primarily in mice and at the highest exposure levels
  - Most sensitive species/tumor site = female mouse/ the lung
- Lack of evidence of cancer in epidemiological studies of workers exposed to chloroprene
- No evidence of increased risks in the community around the Denka plant
- Differences in tumor incidence can be explained by using PBPK modeling and the calculated internal dose of metabolized chloroprene (Allen et al. 2014)
- **All lines of evidence indicate that a PBPK correction is needed to arrive at a relevant IUR for humans**

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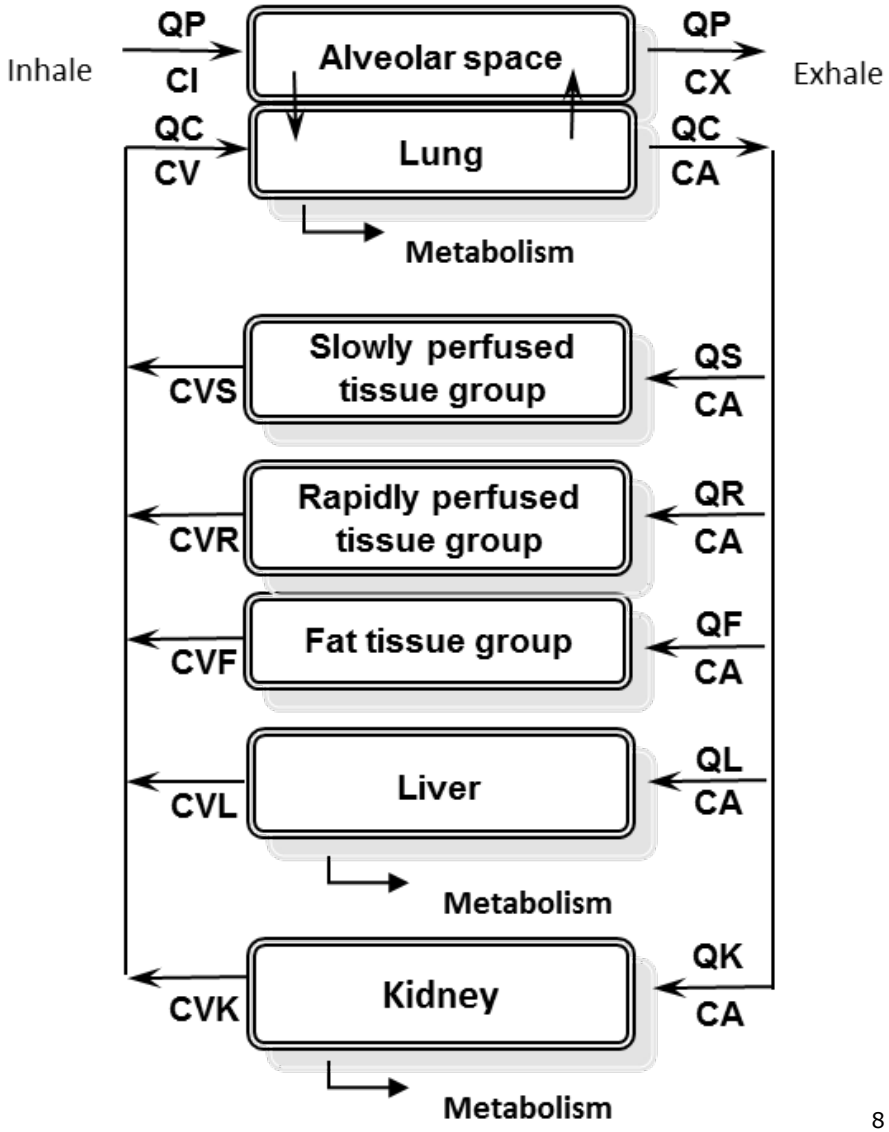
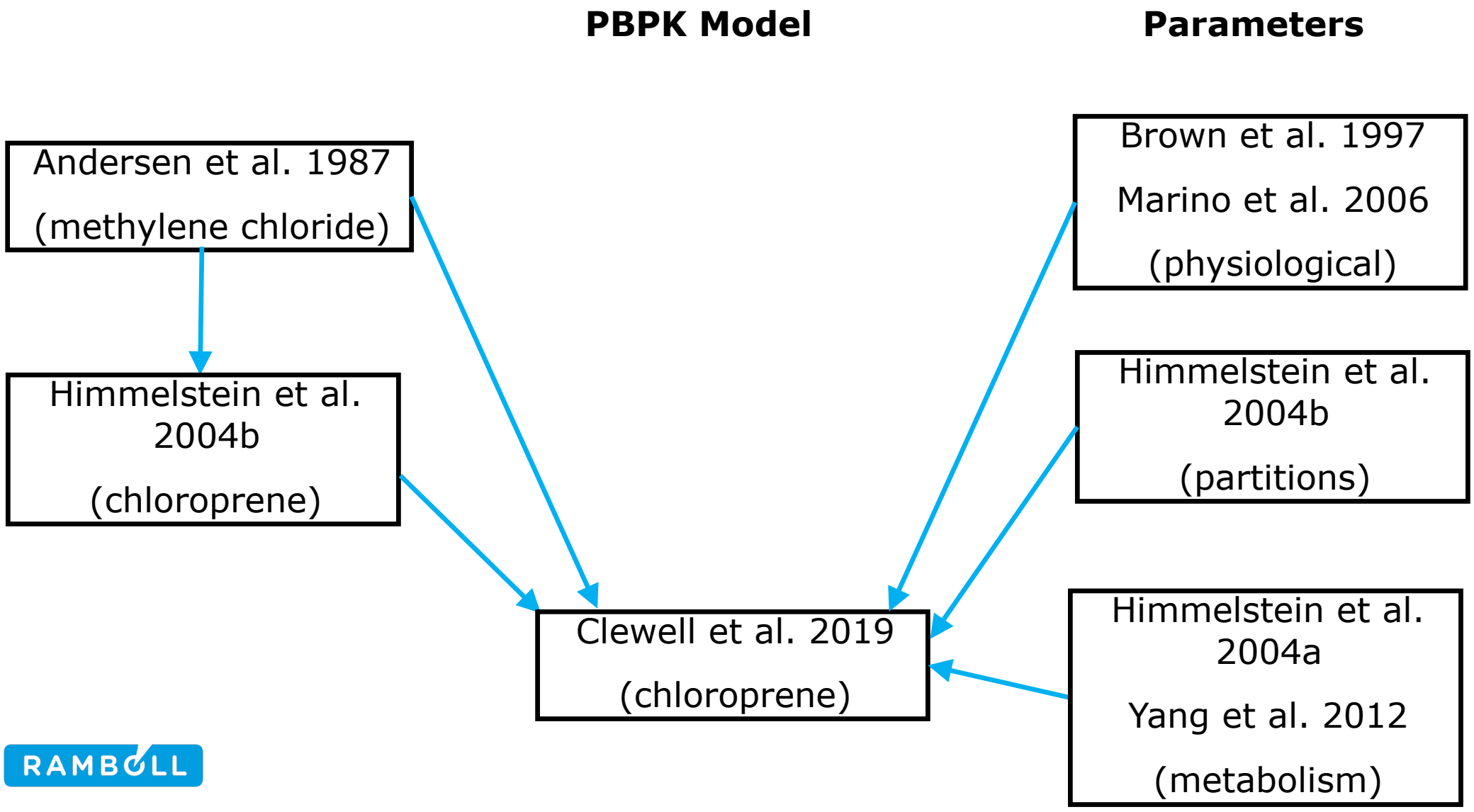
UPDATED CHLOROPRENE PBPK MODEL

- Structure based on PBPK model of methylene chloride (Andersen et al. 1987)
- Parameters obtained from the literature
  - Physiological parameters: Brown et al. (1997)
  - Partition coefficients: Himmelstein et al. (2004b)
  - Metabolism parameters: Himmelstein et al. (2004a) and Yang et al. (2012)
- Code: R programming language
- R-scripts for running mouse validation study and dose metrics in mouse, rat and human
- Documentation provided for all parameters



UPDATED CHLOROPRENE PBPK MODEL

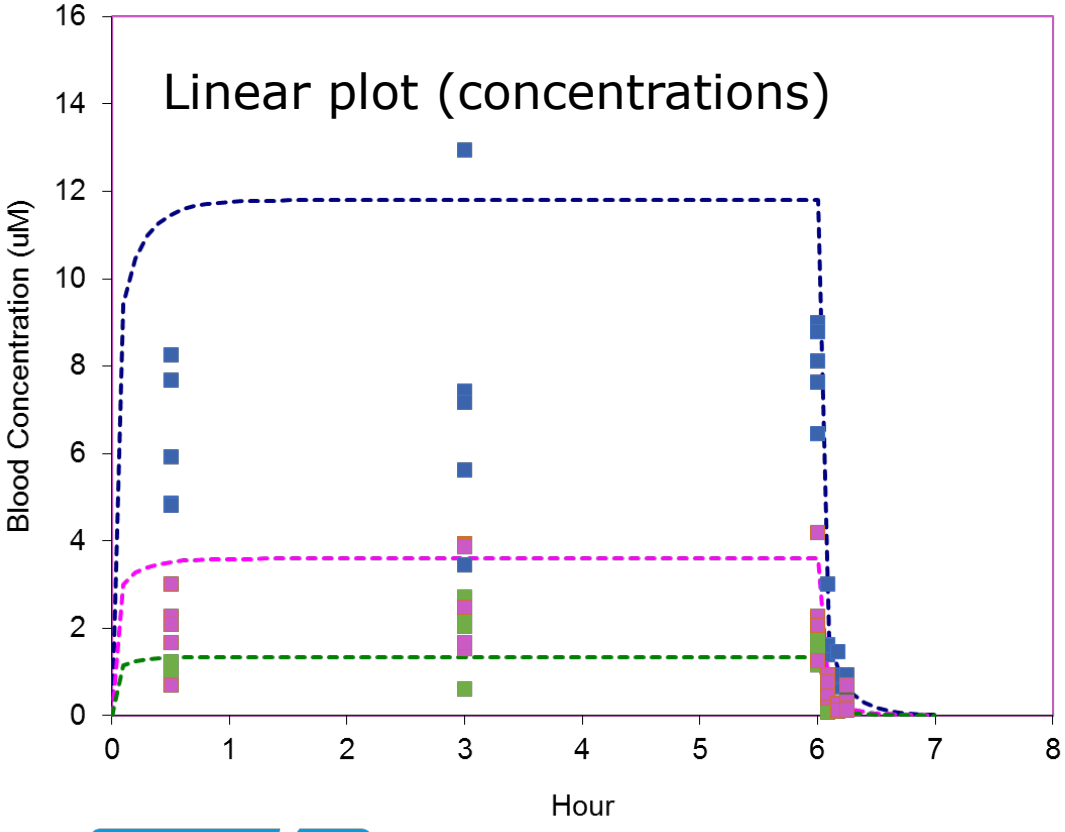


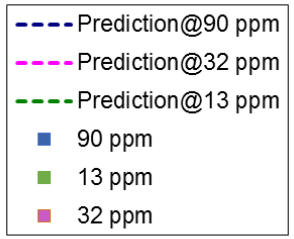
MODEL TESTING AND VALIDATION ANALYSES

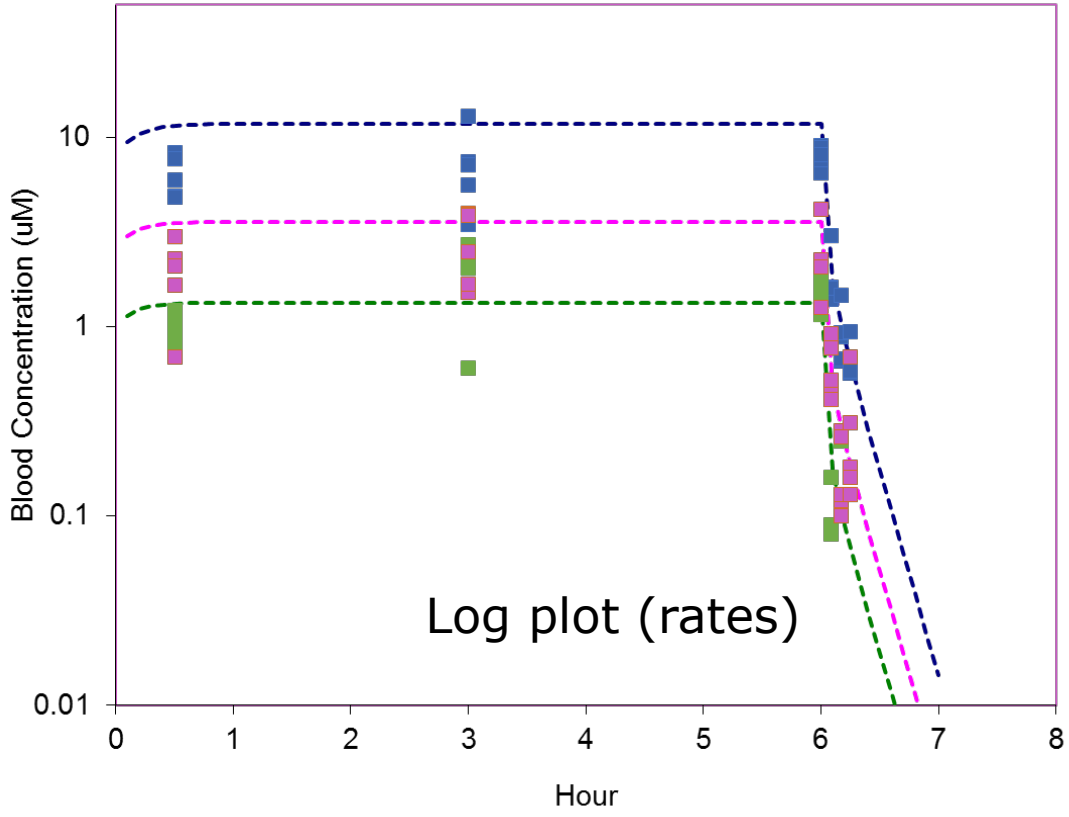
- Validation against the in vivo data
  - Ramboll tested the chloroprene PBPK model and found it was able to reproduce the blood concentrations reported in both the single and repeated exposure in vivo studies
  - Ramboll evaluated the minute ventilation data from the chloroprene single exposure study and the metabolism induction data from the repeated exposure study and determined that there was no evidence of reduced ventilation or induction of metabolism in response to chloroprene exposure
- Re-estimation of model parameters and consistency across tissues and genders
  - At the request of EPA, Ramboll investigated the impact of re-estimating the published estimates from Yang et al. (2012) using an additional estimated parameter (Kg) suggested by EPA
  - Ramboll conducted an analysis of the impact of the alternative parameter estimates on resulting dose metrics, results shown shortly
- Scale-up of in vitro data
  - Ramboll consulted with a metabolism expert, Dr. Miyoung Yoon (US FDA), on the uncertainty associated with using in vitro metabolism data, the adequacy of the in vitro data underlying the metabolic parameters and the appropriate scaling approach

VALIDATION OF THE MODEL

- 6-hour inhalation exposures of female mice to chloroprene (data from IISRP-12828-1388 2009)
- The model predictions fit the in vivo results very well (within a factor of 2 of the means of animal data) with no adjustment of parameters







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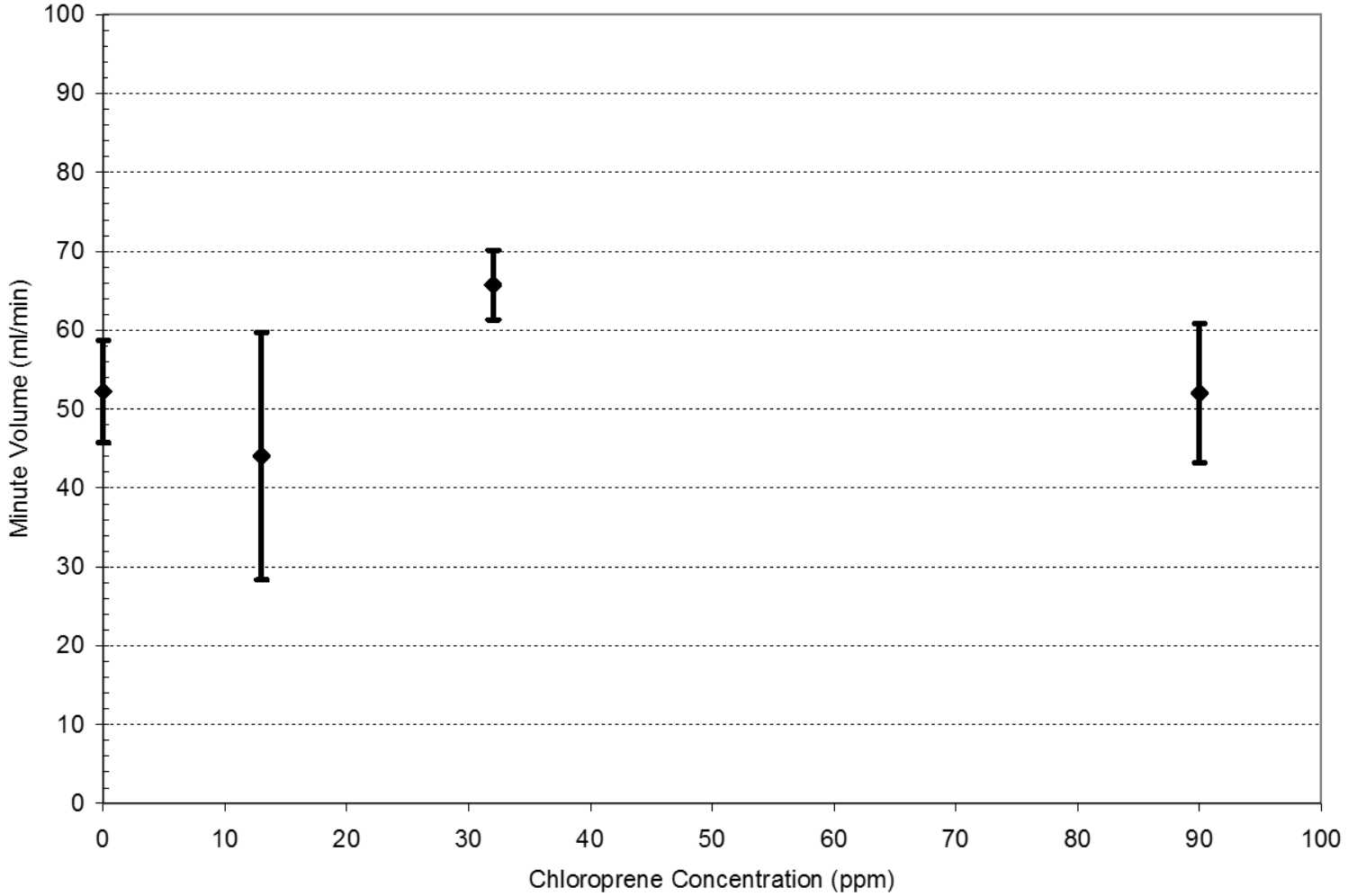
VALIDATION OF THE MODEL

Minute ventilation during 6-hour inhalation exposures of female mice to chloroprene (IISRP-12828-1388, 2009)

- Plot: measured pulmonary ventilation (ml/min) as a function of chloroprene concentration
- Results show that minute volume is not associated with chloroprene concentrations
- This suggests that respiratory depression was not an issue
- Alveolar ventilation used in PBPK model corresponds to average measured value



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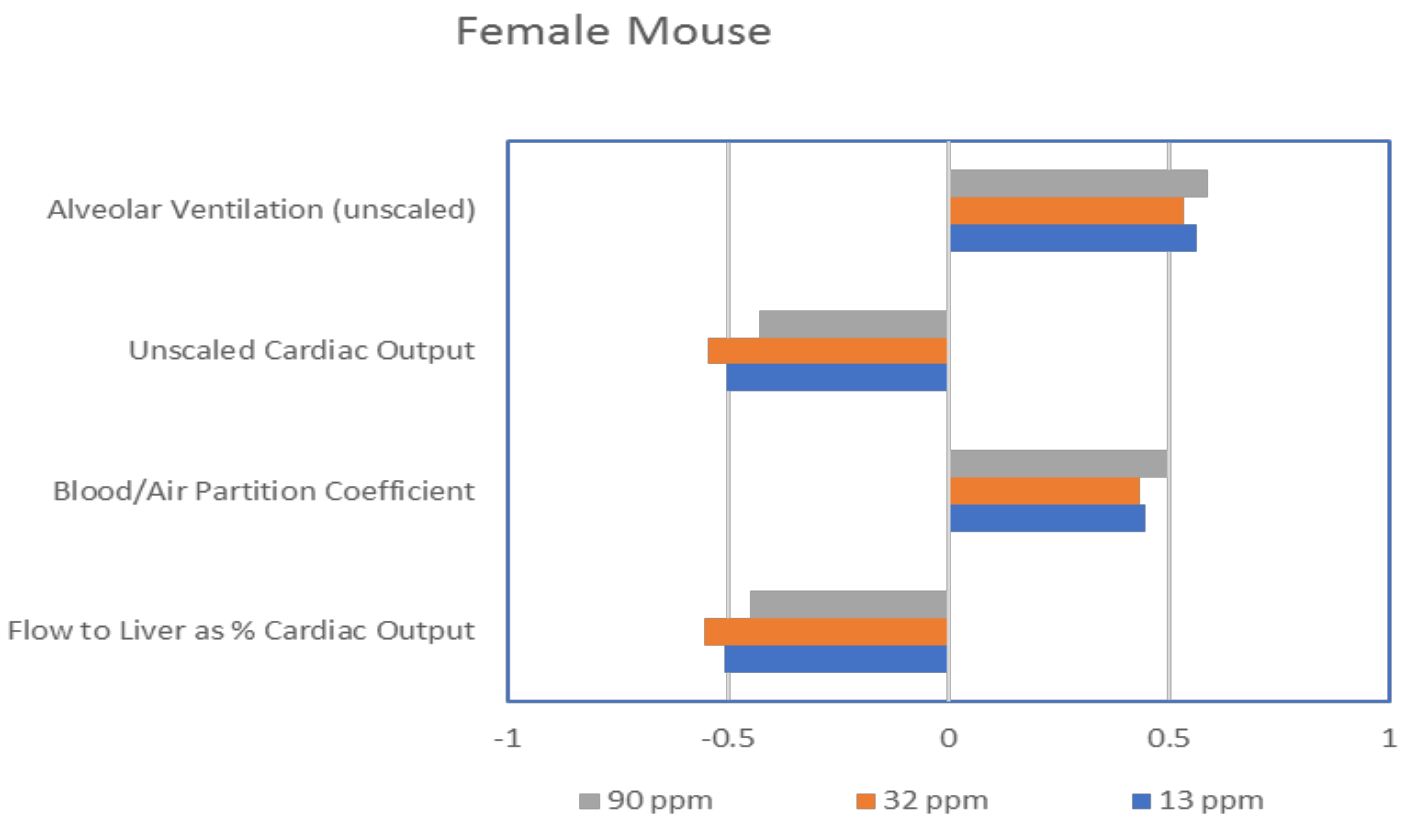
MODEL PARAMETERS: SENSITIVITY OF BLOOD CONCENTRATION (CVLC) TO CHANGES IN THE MODEL PARAMETERS

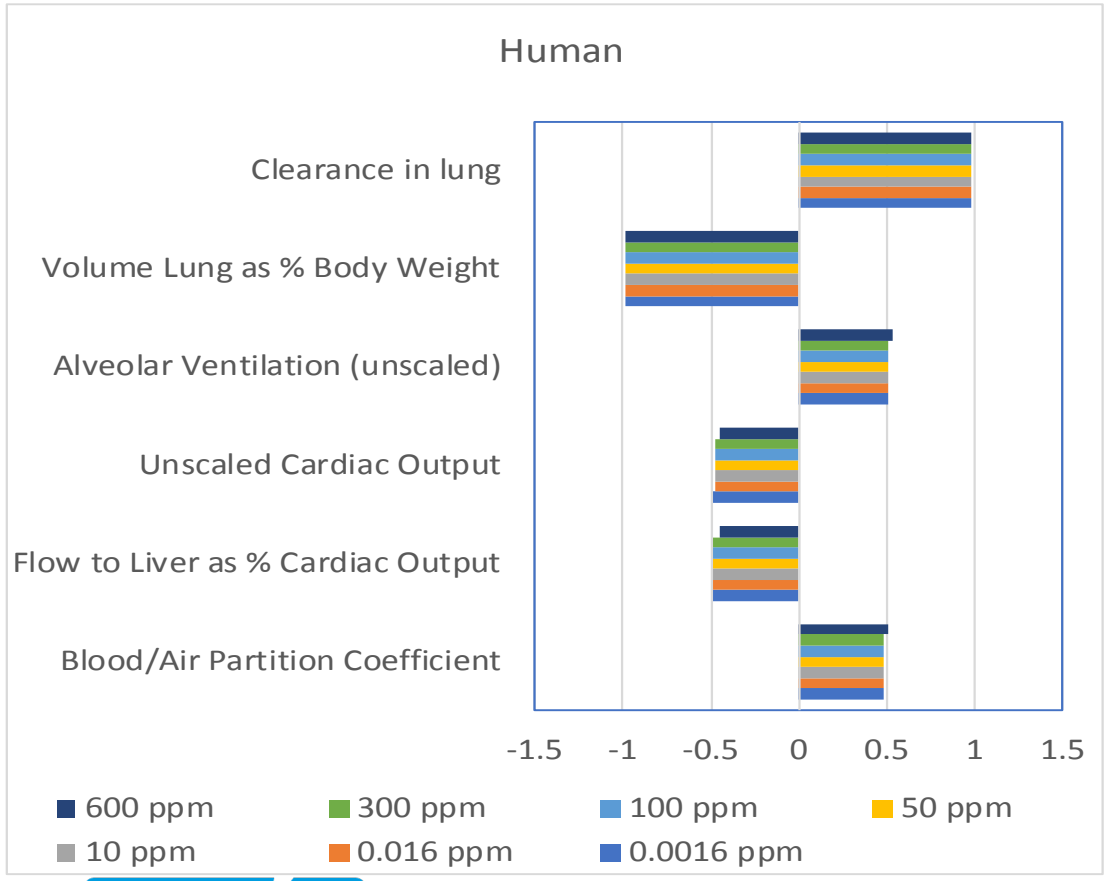


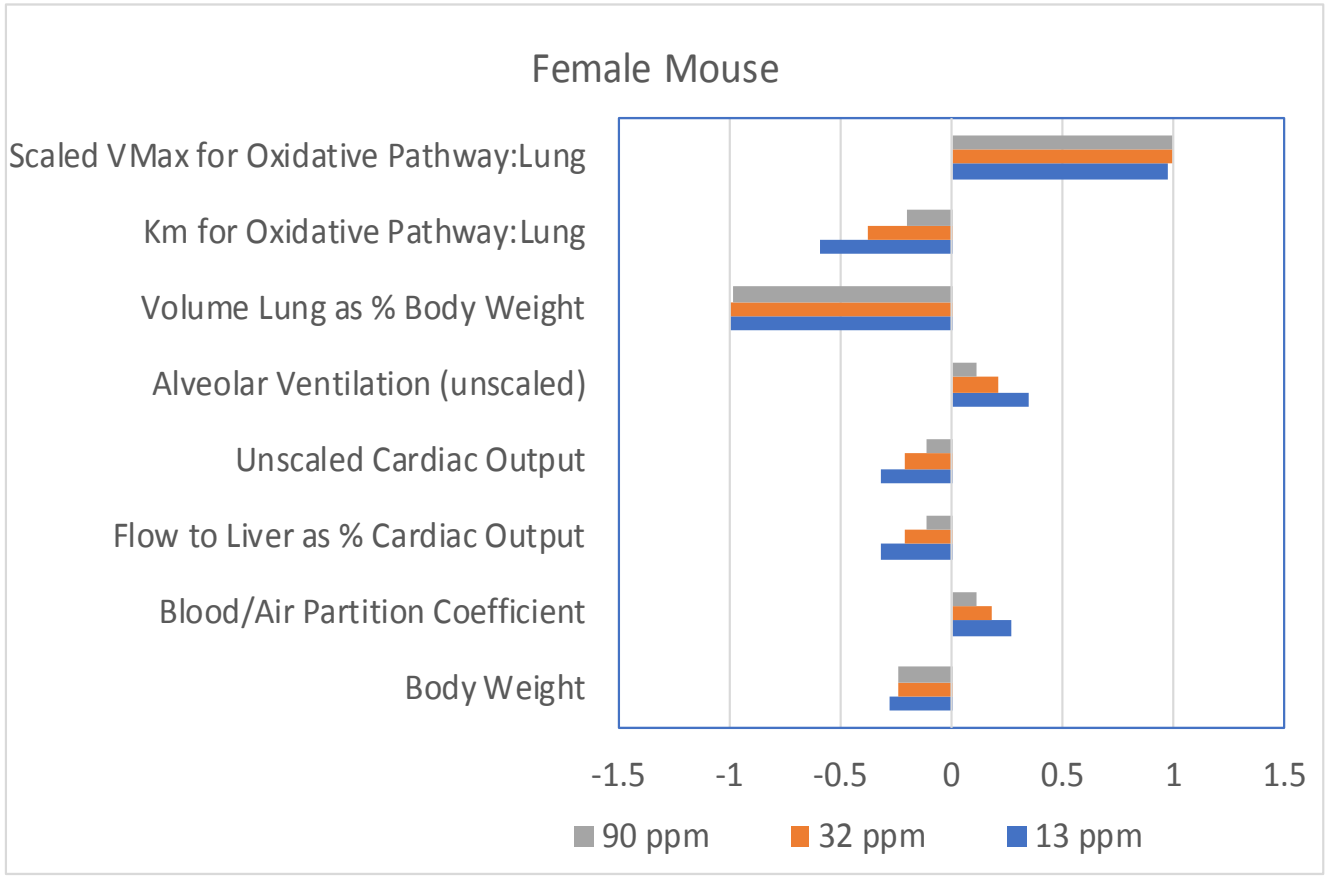
All sensitive parameters are either:

- directly measured (ventilation, blood/air partition) or
- obtained from physiological literature (cardiac output, liver blood flow)

MODEL PARAMETERS: SENSITIVITY ANALYSIS OF AMOUNT METABOLIZED IN THE LUNG DAILY PER GRAM OF TISSUE (AMPLU) TO CHANGES IN THE MODEL PARAMETERS

As expected, the lung dose metric is sensitive to the same parameters as the in vivo study, plus lung metabolism and lung volume





SCALE-UP OF IN VITRO METABOLISM DATA

- EPA has raised concerns regarding the uncertainty associated with the scale-up of *in vitro* data
- The EPA Office of Pesticides has already accepted the use of PBPK models using IVIVE of microsomal metabolism data in the evaluation of early life sensitivity to pesticides, and the FDA routinely uses microsomal metabolism data to predict drug-drug interactions in vivo
- We have characterized the impact of metabolism parameter uncertainty in the chloroprene PBPK model using sensitivity analysis and a comparison of risk estimates using alternative parameter estimation approaches
- To address uncertainty in the human lung metabolism of chloroprene, which is very slow, the PBPK model has been modified to use the approach from the EPA (2011) IRIS risk assessment for methylene chloride, which was based on the PBPK model from Andersen et al. (1987), using a measure of the relative CYP abundance in human liver and lung

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TRANSPORT LIMITATION (KG) STUDY

- EPA raised a concern that a limitation (Kg) on the transfer of chloroprene from the air to the media in the vials could have affected the observed clearance rates observed in the published metabolism studies conducted by Dr. Matt Himmelstein
- To respond to this concern, a new experimental study was performed to estimate a Kg for chloroprene, following a protocol based on Schlosser et al. (1993)
- However, the experimental value of Kg obtained in this study was inconsistent with the high rates of liver metabolism observed in Dr. Himmelstein's published, peer-reviewed studies
- Therefore, we re-estimated Kg from the metabolism study data using an approach suggested by EPA, fixing Km based on published *in vivo* values for other CYP2E1 substrates

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IN VIVO EVIDENCE FOR CHLOROPRENE KM IN LIVER

2E1 substrate Kms:

Inhibition studies:		
TCE:	1.9 uM	rat (Andersen 1987)
DCE:	1 uM	rat (Andersen 1987)
In vivo metabolism studies:		
MeCl2:	5.1/6.8 uM	human/mouse MCMC (David 2006 / Marino 2006)
DHMs:	2.3-4.7 uM	rat (Gargas et al 1986)
BDCM:	3 uM	rat (Lilly et al 1997,1998)
Closed Chamber *in vivo* studies		
VC:	1.6 uM	human (Clewell et al 2001)
CHCl3:	3-4.6 uM	rat (Corley et al 1990)
EDC:	2.5 uM	rat (D'Souza et al 1987,1988)
VDC:	2.5 uM	rat (D'Souza and Andersen 1988)
chloroethanes:	3.3-5.6 uM	rat (Gargas and Andersen 1989)
chlorinated ethylenes:	1-5 uM	rat (Gargas et al 1990)
Furan	2 uM	rat (Kedderis et al. 1993)

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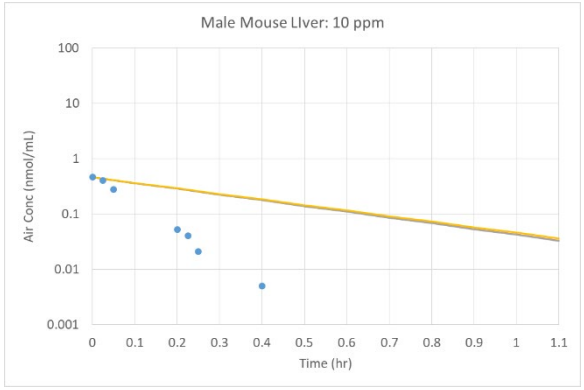
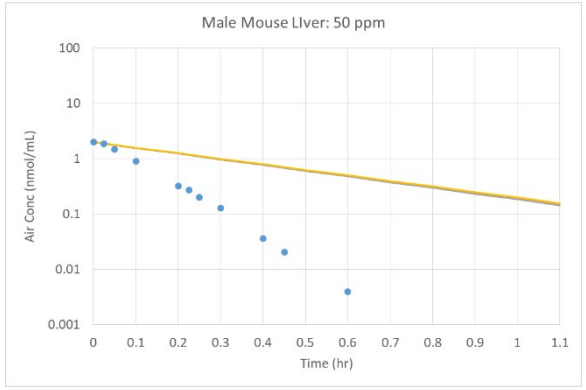
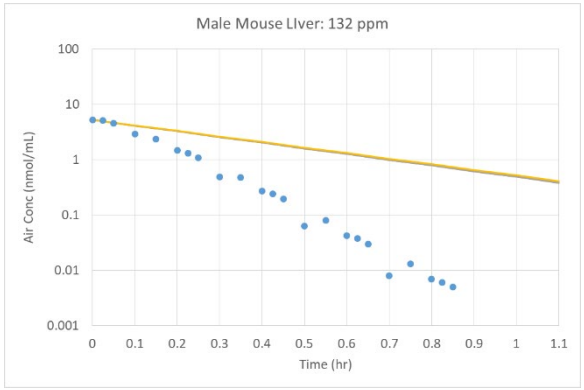
Supports Km for chloroprene no lower than 1 uM

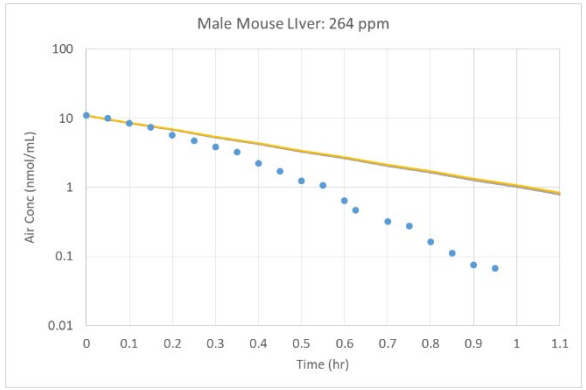
TRANSPORT LIMITATION (KG) MCMC EVALUATION

Experimental Kg = 0.020 L/hr
(95% CI. = 0.015 – 0.036)



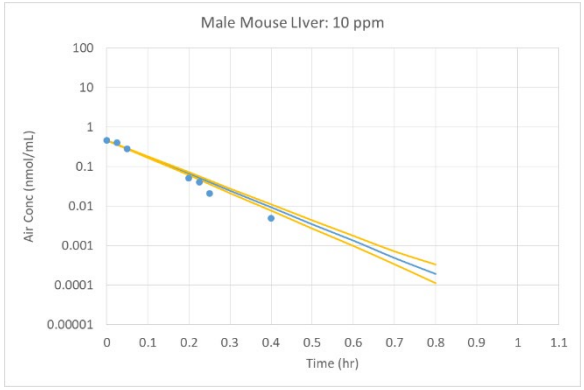


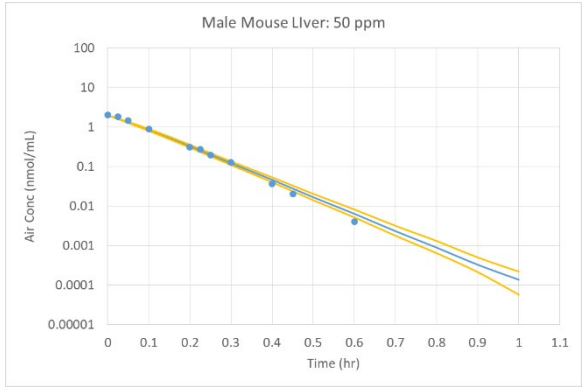


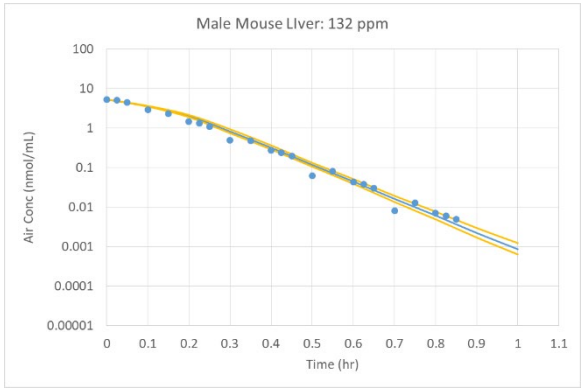


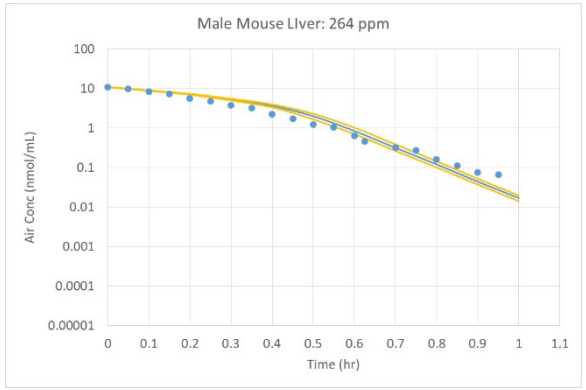
* Cannot fit metabolism data if Kg < 0.11 L/hr

Estimated Kg = 0.45 L/hr*
(95% CI. = 0.34 – 0.65)









* Estimated from male mouse liver metabolism data, with Km = 1 uM

ALTERNATIVE ESTIMATION OF TRANSPORT LIMITATION (KG) IN METABOLISM STUDIES

- Dr. Schlosser was able to contact Dr. Himmelstein and determined that the agitation in the metabolism studies was 500 rpm rather than 60 rpm
- Dr. Schlosser suggested that the Kg in the metabolism studies could be calculated from the Kg study using this information:
- Kg(metabolism study) = (500/60)*0.024 = 0.2
- This value is very close to the value of 0.45 estimated with Km =1
- We are now re-estimating the metabolism parameters using Kg = 0.2

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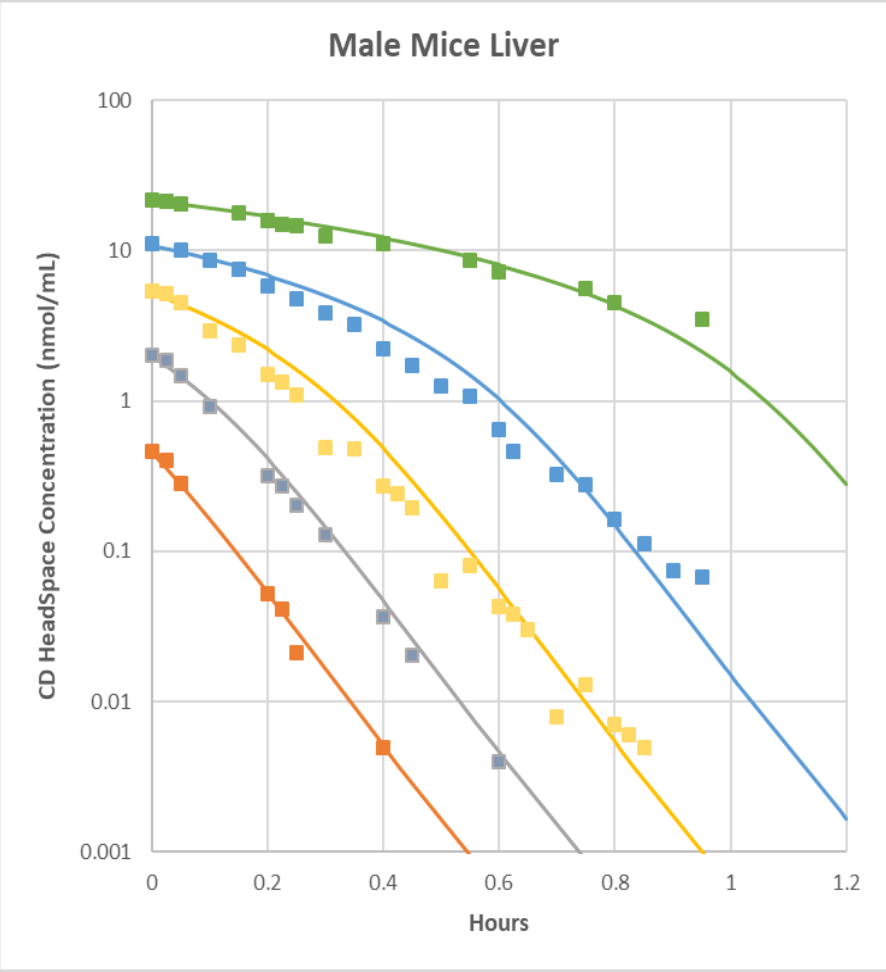
ALTERNATIVE APPROACHES FOR ESTIMATING METABOLISM

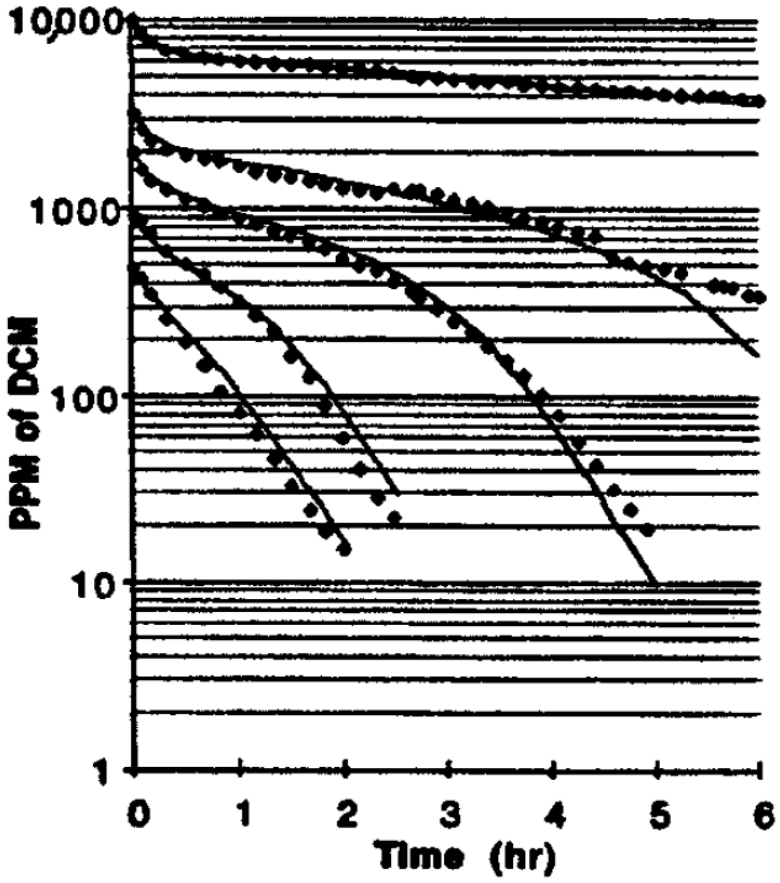
Chloroprene (2019)

In Vitro Data



Methylene Chloride (1987)

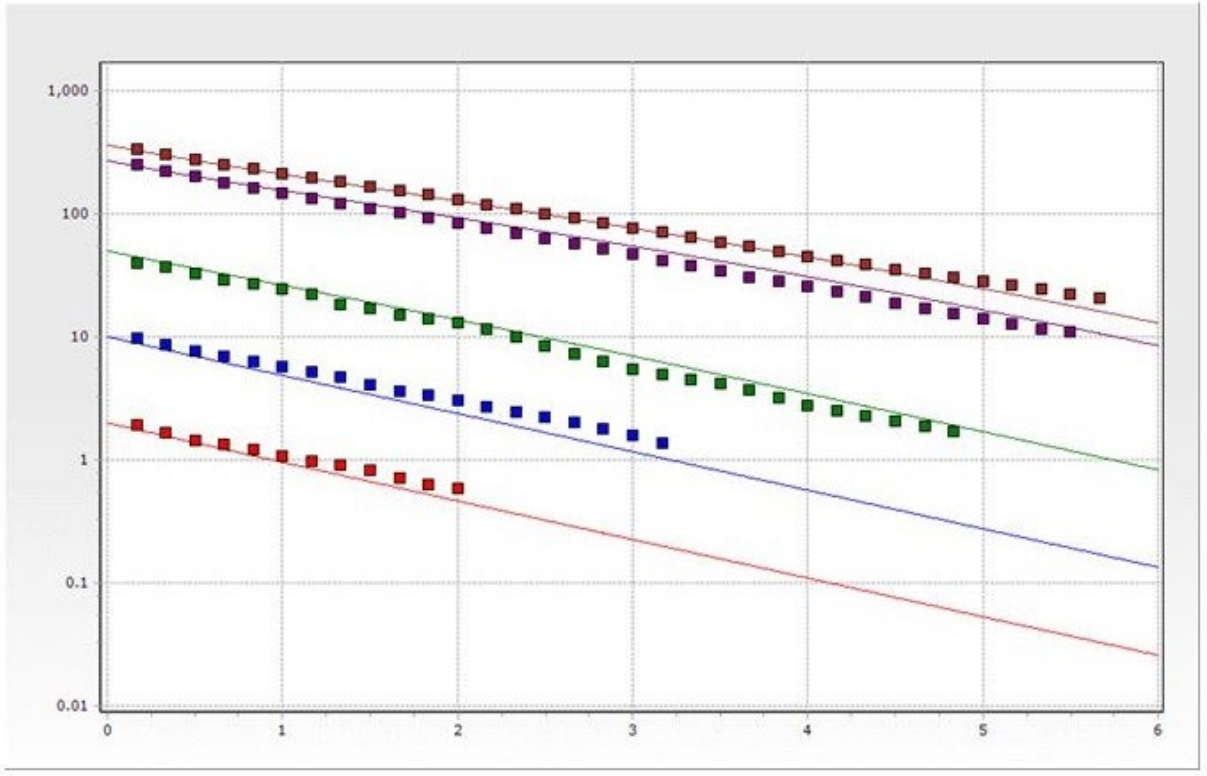
Closed Chamber Data



EVALUATION OF IN VITRO STUDIES (CONT.)

- EPA closed-chamber chloroprene inhalation data (Himmelstein et al. 2004b)
- Joint optimization of VmaxC and QPC, with Km fixed at 1 uM

  (Fixing Km allows both Vmaxc and QPC to be estimated from the data)
- Result: VmaxC = 20.1, QPC = 17.8
- Compared to IVIVE: VmaxC = 19.0



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THERE IS UNCERTAINTY IN METABOLISM PARAMETERS DERIVED FROM IN VIVO STUDIES TOO

Development of a Physiologically Based Pharmacokinetic Model of Trichloroethylene and Its Metabolites for Use in Risk Assessment

CLEWELL ET AL. 2000 EHP

Table 1. Parameter values used in the PBPK model for TCE.

Parameter	Abbreviation	Units	Mouse	Rat	Human
TCE metabolism					
Capacity	VMC	mg/hr[a]	39* (39.–60.)	12* (12.–20.)	10* (6.–10.)
Affinity	KM	mg/L	0.25	0.25* (0.25–18.)	1.5* (1.5–3.)
Fraction TCA	PO	–	0.035* (0.035–0.1)	0.02* (0.02–0.06)	0.08

METABOLISM PARAMETER SENSITIVITY ANALYSIS

Source of Parameters for Calculating Internal Dose Metric	IUR at 1 µg/m^3
EPA (2010)	5.0×10^{-4}
Using Yang et al. (2012) published parameters	3.7×10^{-6}
Re-estimated parameters assuming mass transport limitation (Kg) during *in vitro* studies	2.8×10^{-6}

Lower risk estimate using Kg compared to risk obtained with the published parameters in Yang et al. (2012) due to higher estimated lung metabolism in the female mouse when transport limitation in the *in vitro* studies is assumed

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SUMMARY

- In the Request for Correction, Ramboll relied on published data to arrive at an updated IUR
- Based on a request from EPA, Ramboll has converted the PBPK model described in Yang et al. (2012) into R
- Ramboll has provided this PBPK model for chloroprene in R to EPA so that they can verify that it accurately simulates the in vivo exposure data in the mouse
- Ramboll has sought to address EPA concerns regarding the model
- A paper documenting the model and results has been submitted to *Inhalation Toxicology* and is in review
- Ramboll has provided EPA the revised model code and all supporting documentation

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CONCLUSIONS

- PBPK modeling is the best approach for updating the IUR because of large pharmacokinetic differences demonstrated between mice and humans
- The Ramboll team appreciates the time spent on this collaboration by EPA scientists, and the detailed comments they provided, which were helpful in improving the PBPK model
- Ramboll determined that the impact of uncertainties in the PBPK model is small compared to the impact associated with ignoring important species differences in target tissue dosimetry
- A validated PBPK model has been developed and documented, and a publication documenting the model and sensitivity analysis is in review at *Inhalation Toxicology*
- The updated IUR provides a conservative risk number that will inform protective occupational and environmental exposure limits and is more than 100 times lower than the 2010 EPA IUR

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KEY POINTS

- The use of best available scientific methods as well as EPA policy dictate the need to use PBPK modeling to address pharmacokinetic differences in order to obtain the most valid risk value
- Based on our re-evaluation and testing of the PBPK model, incorporating EPA's comments we now have a validated model for chloroprene
- The validated model confirms the findings of the Himmelstein et al. (2004b) PBPK model, and the updated Ramboll IUR demonstrates that the 2010 IUR overestimates human risk from chloroprene exposure by over 100 fold

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NEXT STEPS

- What is the process and timetable for EPA's review of the revised PBPK model? Is there anything Ramboll can do to facilitate the process?
- DPE has submitted to EPA all updated materials, and we look forward to continued open communication and collaboration

THANK YOU

Robinan Gentry
rgentry@ramboll.com
Harvey Clewell
hclewell@ramboll.com
Cynthia Van Landingham
cvanlandingham@ramboll.com
Jerry Campbell
jcampbell@ramboll.com
Sonja Sax
ssax@ramboll.com
Ken Mundt
kenneth.mundt@cardno.com
Mel Andersen
andersenme@aol.com

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