



NAVAL MEDICAL RESEARCH UNIT DAYTON

In Vitro Investigation of Gulf War Illness Brain Effects: Use of a Physiologically-Based Pharmacokinetic Array Model to Inform an Electrophysiology Study

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STATEMENTS

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Animal Care and Use

The study protocol was reviewed and approved by the Wright-Patterson AFB Institutional Animal Care and Use Committee in compliance with all federal regulations governing the protection of animals and research.

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14. ABSTRACT Military deployed during the Gulf War (1990-1991) frequently exhibit signs of a neurological disorder known as Gulf War Illness (GWI). Published animal studies indicate a mixtures exposure of 1.3 mg/kg pyridostigmine bromide (PB, oral), 40 mg/kg diethyltoluamide (DEET, dermal) and 0.13 mg/kg permethrin (PER, dermal), dosed daily for 28 days, to be effective in inducing GWI-like symptoms in rats. An <i>in vitro</i> study was designed to measure the electrophysiological effects of this mixture in rat hippocampus brain slices. Physiologically-based pharmacokinetic (PBPK) modeling was utilized to predict tissue doses from exposures mirroring the <i>in vivo</i> studies. An array (vector) PBPK model was constructed to simultaneously predict brain concentrations of GWI compounds in the rat. Array models use a unified model structure to simulate single or multiple compounds. Each GWI compound was parameterized individually using kinetic data sets from literature sources. Cis- and trans-PER isomers required separate kinetic parameters. The model predicted effective brain concentration ranges of 0.1–0.5, 0.04–0.1 and 0.06–0.6 µg/mL for PB, DEET and PER (55:45 cis:trans), respectively. Directed by these predictions, slices of male Sprague-Dawley rat hippocampus incubated in 0.097, 0.13, and 0.5 µg/mL PB, DEET and PER, respectively, resulted in an increase in paired pulse facilitation and a decrease in input-output response of hippocampal neurons; these effects were not statistically significant. A 10X mixture produced statistically significant dose-dependent changes. The array PBPK model was useful in predicting tissue doses and reducing the number of animals/iterations required for successful <i>in vitro</i> testing.					
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1.0 SUMMARY

Military deployed during the Gulf War (1990-1991) frequently exhibit signs of a neurological disorder known as Gulf War Illness (GWI). Published animal studies indicate a mixture exposure of 1.3 mg/kg pyridostigmine bromide (PB, oral), 40 mg/kg diethyltoluamide (DEET, dermal) and 0.13 mg/kg permethrin (PER, dermal), dosed daily for 28 days, to be effective in inducing GWI-like symptoms in rats.

An *in vitro* study was designed to measure the electrophysiological effects of this mixture in naïve rat hippocampus brain slices. Physiologically-based pharmacokinetic (PBPK) modeling was utilized to predict tissue doses from exposures mirroring the Naval Medical Research Unit Dayton (NAMRU-D) GWI *in vivo* study, reported separately. An array PBPK model was constructed to simultaneously predict brain concentrations of GWI compounds in the rat. Array models use a unified model structure to simulate single or multiple compounds. Each GWI compound was parameterized individually using kinetic data sets from literature sources. Cis- and trans-PER isomers required separate kinetic parameters and were fit separately. The model predicted effective brain concentration ranges of 0.1 to 0.5, 0.04 to 0.1 and 0.06 to 0.6 µg/mL for PB, DEET and PER (55:45 cis:trans ratio), respectively.

Directed by these predictions, slices of male Sprague-Dawley rat hippocampus incubated in 0.097, 0.13, and 0.5 mg/L PB, DEET and PER, respectively, for more than an hour resulted in an increase in paired pulse facilitation and a decrease in input-output response of hippocampal neurons within the CA1 region; these effects were not statistically significant. A 10X mixture produced statistically significant dose-dependent changes. The array PBPK model was useful in predicting tissue doses and reducing the number of animals/iterations required for successful *in vitro* testing.

Using the optimized *in vitro* system, potential prophylactics designed to prevent long-term injuries such as GWI may be tested in the future. DEET, PB and PER are still used by military members. Therapeutic countermeasures can be based upon protective effects seen in quantitative electrophysiological studies. Once a prophylactic concentration is determined within the *in vitro* system, the PBPK model could be utilized to estimate a protective oral dose of the countermeasure under consideration.

2.0 INTRODUCTION

Gulf War Illness (GWI) is an umbrella term for a collection of chronic illnesses reported by an estimated 25 to 32 percent of the 700,000 veterans deployed to the 1991 Gulf War (Operation Desert Storm). Symptoms can include fatigue, headaches, joint pain, indigestion, insomnia, dizziness, respiratory disorders, memory problems, neuromuscular weakness, immune abnormalities, chronic inflammation and other nonspecific symptoms. To this day, many Gulf War veterans continue to experience these debilitating symptoms. Many neurotoxins were present in the Gulf War Theater. While the full etiology of these symptoms has not been identified, central nervous system (CNS) effects are prominent and mixtures of chemical exposures have become the focus of study. Psychiatric causes have been largely ruled out as Gulf War veterans have lower rates of post-traumatic stress disorder (PTSD) and other psychiatric disorders than their counterparts who served in other wars (White *et al.*, 2016).

Reports by the Research Advisory Committee on GWI implicate exposures to multiple GWI chemicals such as pyridostigmine bromide (PB), a reversible acetylcholinesterase (AChE) inhibitor used as a nerve agent prophylaxis, and pesticides like permethrin (PER) and diethyltoluamide (DEET) in the pathogenesis of GWI (Sullivan *et al.*, 2018). The molecular structure and physical properties of these chemicals are shown in Figure 1. Each of these GWI chemicals (PB, PER and DEET) can disrupt neurotransmission by acting on acetylcholinesterase, ion channels or synaptic receptors (Corbel *et al.*, 2009; Nutter *et al.*, 2013) and were used in the project herein to model GWI.

Approximately 62 percent of the Gulf War ground troops reported being exposed to pesticides (Winkenwerder, 2003), of which 44 percent reported specific pesticide sprays containing pyrethroids, commonly permethrin (Research Advisory Committee on Gulf War Veterans' Illnesses, 2008). PER is lipophilic and readily crosses the blood-brain barrier (BBB). Acute PER intoxication in humans is associated with an autoimmune clinical phenotype, including disorders like scleroderma and myasthenia gravis-like syndromes, as well as activation of CNS and peripheral adaptive immune responses. Therefore, PER may contribute to an autoimmune-type phenotype in veterans with GWI (Joshi *et al.*, 2019). Elevated autoantibodies against brain-specific proteins, such as glial fibrillary acidic protein (GFAP) and microtubule associated tau proteins in blood have been measured in veterans with GWI (Abou-Donia *et al.*, 2017). Such activation of autoimmune responses in GWI could be downstream of Gulf War PER exposures.

Pyridostigmine bromide, a prophylactic countermeasure intended to protect troops against nerve agents, was widely administered to Gulf War troops in pill form. While PB is not readily absorbed in the gut and normally does not cross the intact BBB, it may cross a compromised BBB. Thereby it increases peripheral levels of acetylcholine and may cause a disturbed homeostatic imbalance of the cholinergic system through hyperstimulation. Exposure to AChE inhibitors have been shown to induce both acute toxicity and long-term neurological deficits (Lotti and Moretto, 2005). Additionally, Abou-Donia *et al.* (2004) showed that co-exposure to PB, DEET, and PER resulted in increased ligand binding for m2-muscarinic acetylcholine receptors in the cortex and significantly increased ligand binding for nicotinic acetylcholine receptors. Thus, prolonged excessive synaptic acetylcholine levels may lead to the down-regulation or desensitization of these receptors, causing further nervous system depression post-exposure.

DEET is a very effective insect repellent and was used topically in copious amounts by Gulf War troops (White *et al.*, 2016). The compound is quickly absorbed into the skin and excessive dermal application of DEET to large areas of the body over a period of days to weeks, especially in children, has led to seizures, bradycardia, nausea, vomiting, bullous eruptions, lethargy, ataxia, encephalopathy and anaphylaxis. Neurotoxicity is the most commonly reported systemic toxic effect of DEET (Fradin, 1998).

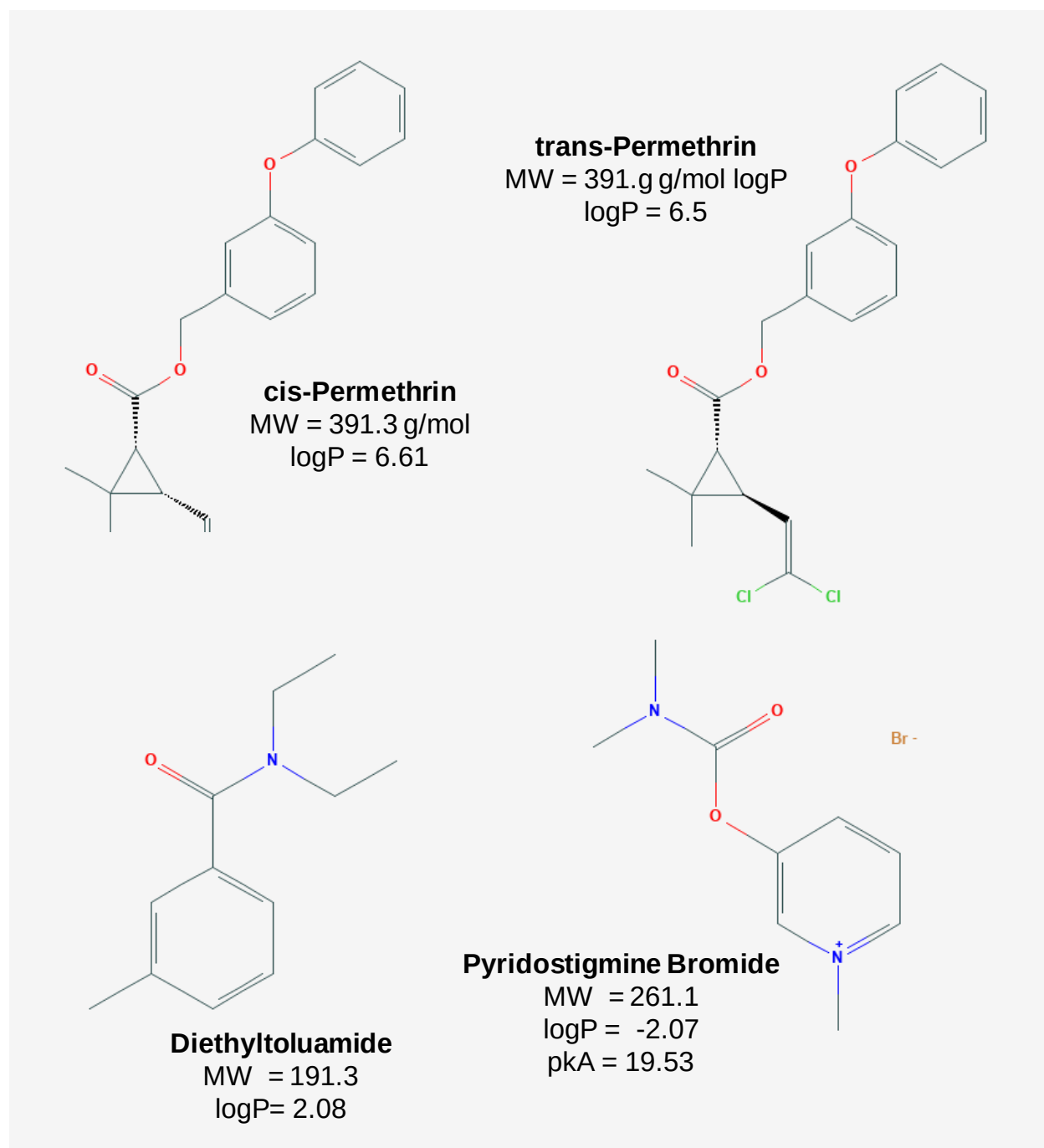


Figure 1. Chemical Structures of cis- and trans-PER, DEET and PB. Adapted from PubChem (2019a-c).

Rodent models of GWI have successfully demonstrated neurological outcomes following exposure to these 3 compounds. Abdel-Rahman *et al.* (2002) investigated the effects of a daily low dose exposure in male rats to a combination of stress and GWI chemicals (1.3 mg PB/kg orally, 40 mg DEET/kg dermally, and 0.13 mg PER/kg dermally); stress and vehicle; GWI

chemicals alone; and vehicle alone. Animals subjected to stress and chemicals for 28 days exhibited disruption of the BBB and neuronal cell death in the cingulate cortex, the dentate gyrus, the thalamus, and the hypothalamus. Other regions of the brain displayed neuronal cell death but did not exhibit indications of BBB disruption. These changes were also associated with decreased forebrain AChE activity and m2-muscarinic acetylcholine receptors. In contrast, for animals exposed to stress and vehicle or chemicals alone, the above indices were mostly comparable to that of animals exposed to vehicle alone. Thus, a combined exposure to stress and low doses of PB, DEET and PER led to significant brain injury.

Parihar *et al.* (2013) also investigated the effect of 28 days of exposure to the same low doses of PB, DEET, and PER, in the absence or presence of mild stress, on mood and cognitive function, dentate gyrus neurogenesis, and hippocampal neurons, microglia, and astrocytes. Cognitive functions and mood were assessed by spatial memory in a water maze test and behavior in a forced swim test, respectively. The chemicals alone induced depressive- and anxiety-like behaviors and spatial learning and memory dysfunction. Application of mild stress (5 minute restraint) during the chemical exposures exacerbated the extent of mood and cognitive dysfunction. Parihar and co-authors also reported reduced hippocampal volume and multiple cellular alterations, such as chronic reductions in neural stem cell activity and neurogenesis, partial loss of principal neurons, and mild inflammation comprising sporadic occurrence of activated microglia and significant hypertrophy of astrocytes.

In a follow-on to the Parihar study, Hattiangady *et al.* (2014) used a larger cohort exposed to the same GWI stressors to study the lasting effects of these compounds on rat memory and mood. Neurobehavioral assays (open field test, object location test, novel object recognition test) and mood assays (novelty suppressed feeding test, voluntary running test) were performed following the 28 days of exposure and a 3-month stressor-free waiting period. Deficits in object location memory and novel recognition memory were observed. Mood tests indicated decreased motivation and depression, compared to age-matched naïve rats.

2.1 Objective

The primary objective of this research was to utilize an *in vitro* system to assess electrophysiological effects in naïve rat hippocampus slices following exposure to GWI chemicals at concentrations comparable to levels that may occur in the rat brain during daily exposures of 1.3 mg/kg PB (oral), 40 mg/kg DEET (dermal) and 0.13 mg/kg PER (dermal). To that end, an array physiologically-based pharmacokinetic (PBPK) model was to be designed to simulate exposures from dermal or oral routes and to predict time-dependent brain concentrations to direct the perfusion of naïve brain slices. Use of the PBPK model was intended to reduce range-finding trials and thereby decrease the number of animals required to meet the primary objective.

3.0 PBPK MODEL TO PREDICT *IN VITRO* BRAIN SLICE CONCENTRATIONS

3.1 Approach

For more than 30 years, PBPK models have been a tool for quantitatively predicting maximum concentrations and concentration-time profiles in blood or plasma, organ tissues, exhaled air and urine (as the parent or metabolite). A PBPK model is a mix of algebraic and ordinary differential equations which describe the transfer of a compound or compounds from one compartment to another. Combined with physiological knowledge (e.g., organ volumes, blood flow) and chemical specific data (e.g., metabolic rates, partition coefficients), continuous simultaneous solving of these equations allows computation of concentration at any given time (Kuepfer *et al.*, 2016; Sager *et al.*, 2015).

3.1.1 Array PBPK Theory and Structure. Typical mixtures models are simply coded as parallel individual models with lines of code for each individual chemical in the mixture, for each and every tissue in each model. Interactions between the chemicals in the mixture are coded within the pertinent tissue; for example, competitive metabolism is coded in the liver (e.g., Dennison *et al.*, 2004; Martin *et al.*, 2012). Increasing the number of chemicals in the model increases the lines of code required. This practice results in very long models and many opportunities to incorporate errors into the code.

In order to streamline a mixtures model, the PBPK model in this project was written using arrays (vectors). Adapted from Sterner *et al.* (2017), this approach allows the model to have a single set of code with a unified structure. Each tissue is coded only once, regardless of the number of components in the mixture. Physiological parameters that would not change based on the chemical are still defined as scalar values, but chemical-specific parameters and physico-chemical properties (e.g., molecular weight, tissue:blood partition coefficients, metabolic constants) are defined as arrays. Once debugged, the code will work for each compound added, given appropriate parameterization, data fitting and validation.

The model structure included brain, fat, liver, rapidly perfused tissues and slowly perfused tissues (Figure 2). Three dosing routes were incorporated based on administration routes in parameter development studies: intravenous dosing straight into venous circulation, oral dosing into the slowly perfused tissues, and dermal dosing into a portion of the slowly perfused tissues mathematically extracted to represent the volume of the skin. A fourth route, inhalation, was retained from the Sterner *et al.* (2017) model; while no inhalation studies were found for the GWI compounds, DEET can be inhaled (ATSDR, 2017) and future studies could investigate inhalation exposures to this repellent or other Gulf War compounds of interest. Removal of GWI contaminants from circulation is facilitated through urinary excretion of the parent or metabolism; liver is the site of all metabolism in this model. As some compounds diffuse in and out of tissues at a slower rate than is governed by blood flow alone, diffusion limitation was added to each tissue in the model structure (Figure 2B) except the skin sub-compartment. When chemical properties are appropriate for flow limited movement, the effects of diffusion limitation can be “turned off” by setting the cell membrane permeability coefficients (PA coefficients) to a high value (10000).

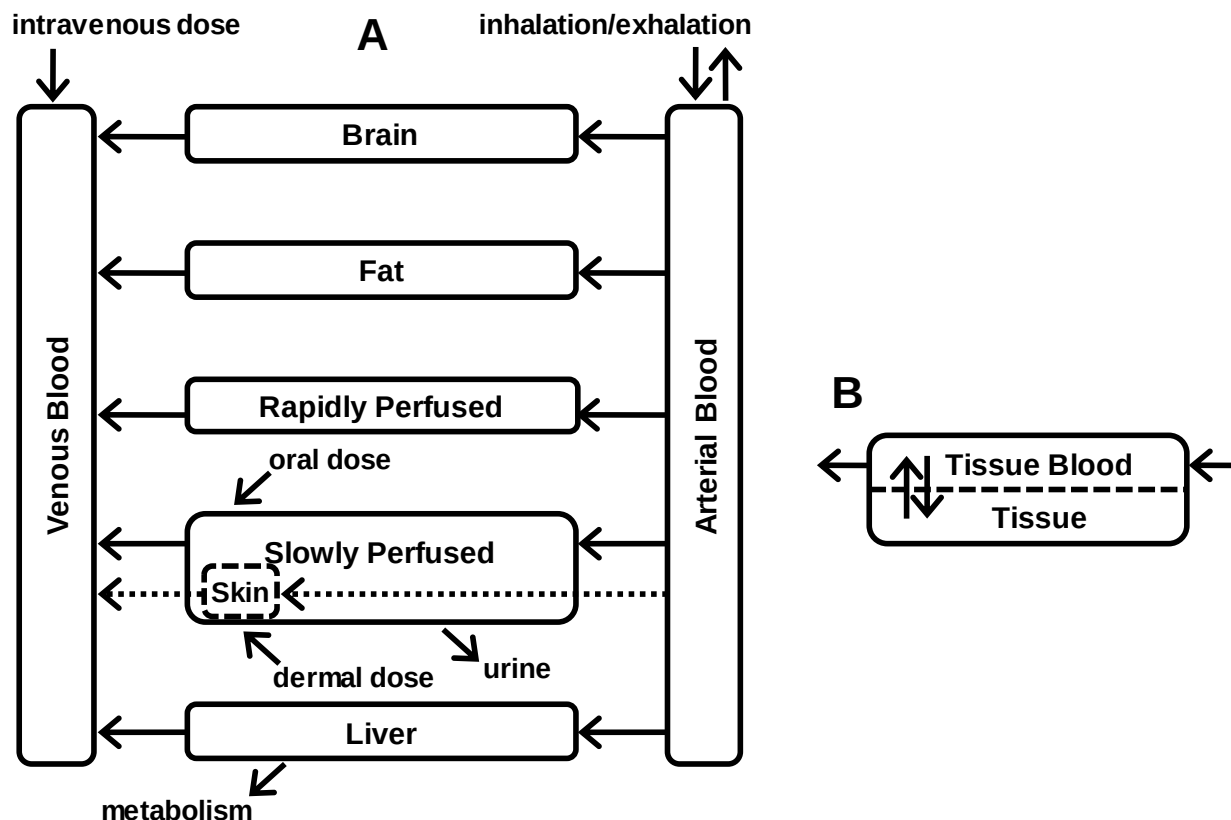


Figure 2. Array PBPK Model Structure for GWI Compounds. (A) The model structure is identical for each chemical. (B) All tissues except skin are coded for diffusion limitation.

3.1.2 Model Parameterization and Development. Although the focus of this project is pharmacokinetics of GWI compounds in male rats, the limited data for PB in rats combined with the discovery of kinetic datasets for PB in volunteers led to the array model being parameterized for both rats and humans. Physiological parameters, describing blood flows and tissue volumes for rats and humans, were based on the published version from which the array model was originally adapted (Clewett *et al.*, 2001) or obtained from experimentally measured data (Table 1). Some data were found for female rats; as male rats are the focus of the experimental design, these data were noted but not utilized at this time.

Table 1. Physiological Constants

Parameter (Constants)	Name	Rat	Human
BW	Body weight (kg)	0.22 ^a	70.0 ^a
DS	Dead space (fraction of lung not inflated)	0.15	0.15
QCC	Cardiac output (blood flow) (L/hour-kg)	14.6	12.89
Tissue Blood Flows [fraction of Cardiac Output (QCC), L/hour-kg]			
QPC	Alveolar (Pulmonary)	24.75	27.75
QBrnC	Brain	0.02	0.114
QFatC	Fat	0.07	0.052
QLivC	Liver	0.183	0.227
QRapC	Rapidly perfused	0.557	0.419
QSknC	Skin	0.058	0.058
QSlwC	Slowly perfused	0.17	0.188
Tissue Volumes [fractions of BW, kg = L]			
VAlvC	Alveolar blood	0.007	0.0079
VBrnC	Brain	0.006	0.02
VFatC	Fat	0.10	0.214
VLivC	Liver	0.034	0.026
VRapC	Rapidly perfused	0.044	0.036
VSkn	(Calculated based on surface area of application and Depth, then subtracted from VSlw; Not a constant)		
VSlwC	Slowly perfused	0.65	0.536
Tissue Blood Volumes [fraction of tissue volumes, L]^b			
VBrnBldC	Blood fraction of brain	0.03	0.04
VFatBldC	Blood fraction of fat	0.0154	0.02
VLivBldC	Blood fraction of liver	0.21	0.11
VRapBldC	Blood fraction of rapidly perfused tissues	0.2075	0.17
VSlwBldC	Blood fraction slowly perfused tissues	0.0333	0.0375
Dermal Thickness (cm)			
Depth	Skin depth	0.1	0.1

Notes: All values from Clewell *et al.* (2001), unless noted otherwise. ^aStudy specific BW values were used whenever possible. ^bSource: Sterner *et al.* (2013).

In order to streamline this report, the parameterization and fitting processes followed for each GWI compound are summarized in Appendices A through D. The appendices also provide a synopsis of previously published models for each compound and the *in vivo* studies used to develop the current model for the GWI compounds. Due to the cis- and trans-PER isomers having different physicochemical parameters, values had to be located or fit for 4 compounds instead of 3. A study in which the PER isomers were not separated at analysis is detailed in Appendix E. The array model code, written for acslX (Aegis Technologies Group, Huntsville AL), is found in Appendix F; the M files required for its execution are found in Appendices G through O.

A generalized description of how physicochemical parameters were determined follows. Tissue:blood partition coefficients (PCs), critical parameters in PBPK modeling that govern steady state chemical distribution, were obtained from published models whenever possible. However, few models have been published for GWI compounds. Prior to starting the modeling work, a mechanistic tissue composition model for the prediction of steady-state tissue:plasma

PCs was utilized for all GWI compounds. This set of algorithms uses chemical lipophilicity, pKa, phospholipid membrane binding, and the unbound plasma fraction, together with tissue fractions of water, neutral lipids, neutral and acidic phospholipids, proteins, and pH (Ruark *et al.*, 2014). Inputs included measured tissue composition data from the rat compiled by Ruark and others (2014), octanol:water partition coefficients (logP, logD) taken from ChemSpider database (www.chemspider.com), and the assumption of negligible active transport. The tissue:plasma PC values calculated were assumed to be sufficiently similar to tissue:blood PCs. These predicted PCs were used in the model iterations.

Apparent PCs were also utilized in the model. An apparent PC is the ratio of a compound in a tissue versus blood or plasma at a moment in time. The ratio changes over the time course, but can give a useful estimate of the PC. Apparent PCs, model fitting to time-course data, and prior model PCs, in general, were more useful in producing better fits (Appendices A through E) than the algorithm predicted values.

A third approach was utilized to fill in holes in each compound's PC list. If an apparent PC or previous model PC was missing for a tissue (called X), then the predicted PC for X, generated using the Ruark *et al.* (2014) algorithms, was divided by the predicted PC for rapidly perfused tissues. That ratio was then multiplied by the apparent or previous model PC for rapidly perfused tissues. The resulting PC for Tissue X was then used in the model, to good effect. Specific uses of this approach are found in Appendices A and B.

Measured values were not available for blood:air partition coefficients for GWI compounds. These values were estimated using the algorithm of Buist *et al.* (2012). Inputs include vapor pressure, octanol:water partition coefficient and molecular weight. As PB has no vapor pressure at standard temperature (25 °C), the blood:air PC is set to an arbitrary high value (200,000, Appendix B).

Time course pharmacokinetic studies for each GWI component and previous models were used to complete the metabolic, uptake and clearance parameters in rats. To account for variation associated with size, all rate constants are allometrically scaled by bodyweight to the 0.75 power ($BW^{0.75}$). This is especially important as all rat physicochemical parameters except PA coefficients were utilized for the human PB model (Appendix B).

Generally speaking, Michaelis-Menten constant (V_{MaxC}) and affinity constant (K_m) values were utilized from previously published models or fitted to *in vivo* time course data, or a combination of both. Permeability coefficients and clearance values were fitted, again utilizing *in vivo* time course data. Dermal uptake is determined by the skin:blood partition coefficient, the study specific concentration on the surface of the skin, the study specific surface area to which the dose is applied, and the skin permeability constant (KP). KP values were predicted using the modified Robinson algorithm as described in Wilschut *et al.* (1995). These values utilize the octanol:water partition coefficient and molecular weight to estimate dermal permeability.

3.2 Simulations of GWI Mixture Study

Once reasonable fits were obtained for the individual components (Appendices A through D) and both PER isomers administered and analyzed together (Appendix E), the model was ready for testing against data from a GWI simultaneous administration (mixture) study.

3.2.1 GWI Mixture Kinetics Study and Basic Assumptions. Abu-Qare and Abou-Donia (2000) published an analytical methods study with kinetic data from all three GWI compounds. SD rats of undetermined size were exposed to 13 mg PB/kg orally, 400 mg DEET/kg dermally

(440 mg/mL, 0.25 mL), and 1.3 mg PER/kg dermally (1.43 mg/mL, 0.25 mL) as a mixture. The cis:trans ratio of the PER was not stated. The authors dosed groups of rats singly or with a combination of all 3 GWI compounds. Blood, urine and metabolite concentrations were reported for rats (n = 5) euthanized at 8 hours; urine was not cumulative and therefore not useful for simulation. For modeling purposes, male SD rats were estimated to be 225 to 250 g, corresponding with another GWI study from the same laboratory (Abdel-Rahman *et al.*, 2002). No information was given on the size of the dermal exposure site; an apparently unpublished study by the same laboratory used 1 cm², which was assumed for model predictions (Abu-Qare *et al.*, 2003). The study does not indicate whether the rats were fasted or fed prior to oral dosing with PB, which profoundly affects absorption and kinetics of many drugs when taken by mouth. The related study from the same laboratory (Abdel-Rahman *et al.*, 2002) fed rats *ad libitum*; it was assumed that the Abu-Qare and Abou-Donia (2000) animals were also fed.

As discussed above and in Appendices C and D, PER exists in two isomers. The cis:trans isomer ratio of a mixture is not always defined in research articles. The 40:60 ratio is the most common for use in agricultural purposes, while the 25:75 mixture is generally used for veterinary applications (PubChem, 2019b). The PER used to impregnate uniforms during the Gulf War was a 40:60 ratio (NRC, 1994). However, researchers who don't report the isomer ratio may not confirm the authenticity of the study chemical prior to experimentation, leaving either common ratio as a possibility for use in the study. It was assumed that the Abu-Qare and Abou-Donia (2000) study used 40:60 cis:trans-PER.

DEET was shown to be excreted by rats in Abu-Qare and Abou-Donia (2000); at eight hours, 3.2 ± 0.82 µg/mL was recovered in urine. Another study had indicated that DEET as a parent compound was not excreted (Kasichayanula *et al.*, 2007). Based on this second study, the model was designed to metabolize all DEET absorbed (Appendix A). The Kasichayanula study was performed in piglets and therefore a species difference in metabolism could be the cause. However, once this disparity was discovered, the project timeline would not allow a re-parameterization of the model. The model is able to describe removal of DEET from the blood using metabolism only. While the parameter values may not be correct for application in a more physiologically appropriate model, the action of the model appears to suit the purpose of the model, which is to predict the brain concentration.

3.2.2 Mixtures Model Performance. GWI compound blood predictions using the parameters developed for kinetic studies of single compounds are shown in Figure 3. These predictions are labeled with the compound name alone without an asterisk. Following the initial predictions, the Abu-Qare and Abou-Donia (2000) mixtures exposure study allowed exploration of changes in kinetics due to simultaneous exposure of the 3 GWI compounds, in order to better simulate the data. Abu-Qare and Abou-Donia (2003) hint at differences in DEET and PER uptake and kinetics when GWI compounds are administered together. Effects listed were: decreased PER absorption, increased DEET area under the curve (AUC) for plasma (as compared with DEET administered alone), and inhibition of metabolism of both DEET and PER. No effect on PB was noted. Unfortunately, these generalities were based on a paper which can be downloaded (see Abu-Qare and Abou-Donia, 2003 reference) but doesn't appear to have been accepted for publication. Additionally, several of these listed effects could be related to absorption alone, which is dependent on vehicle, as well as compound specific parameters, and so may not translate to other studies using different methods.

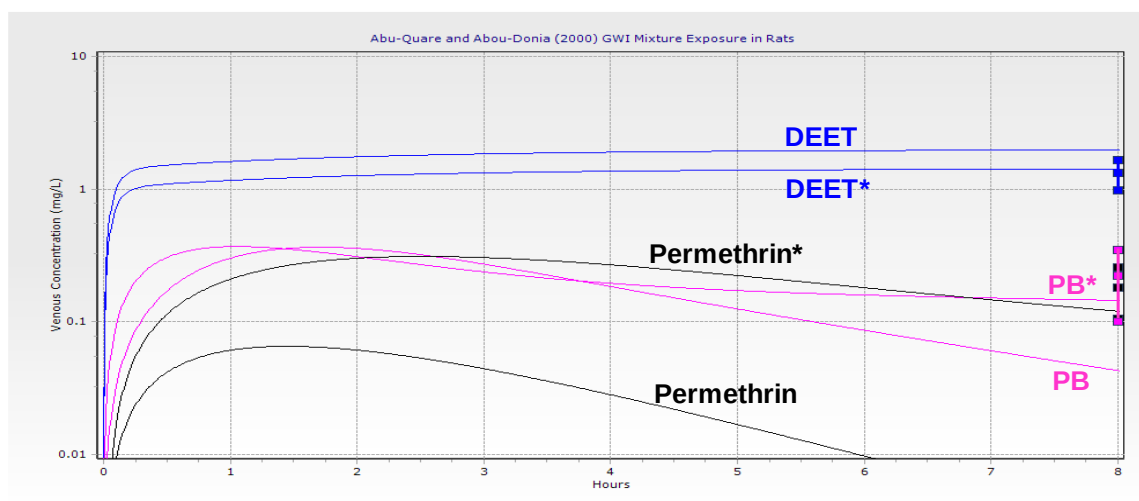


Figure 3. Predictions and Simulations of Abu-Qare and Abou-Donia (2000) GWI Mixture Blood Data. The blood concentration (mg/L) predictions and simulations are shown as lines, compared to data from the study (corresponding colored squares, representing mean and SD). Predictions and simulations are labeled for each compound. Each was first predicted using the parameters developed from kinetic studies in which the compound was administered alone (label without *). The simulations resulting from parameters optimized for this mixture exposure are labelled with *; improvements in fit indicate mixtures effects on kinetics. Male rats were exposed to 13 mg PB/kg orally, 400 mg DEET/kg dermally, and 1.3 mg PER/kg dermally.

Mixture effects were explored in the following order: DEET, PB and PER. As documented in Appendix A, larger DEET dermal doses are not completely absorbed; 62 to 82 percent absorption were reported in the kinetic time courses. The lower absorption rate was chosen for the first iteration of the model (Figure 3, DEET line) as two studies weighed in on the lower end of the range: Fediuk *et al.* (2010) reported 62 percent and Fediuk *et al.* (2011) noted 67.7 percent. A much lower absorption rate was required to fit the Abu-Qare and Abou-Donia (2000) DEET data; a 45 percent absorption rate fit the data with good success (Figure 3, DEET* line). Since both DEET and PER were applied to the skin of these rats, competition for absorption would be a natural assumption.

It should be noted that a large dose of DEET was applied and that absorption is heavily reliant on the surface area to which the dose is applied. The dose of DEET is 10 times the strength of that administered in the studies for which the model will retroactively predict brain concentrations (Abdel-Rahman *et al.*, 2002; Parihar *et al.*, 2013; Hattiangady *et al.*, 2014). A small surface area reported in the unpublished study by the same group (1 cm², Abu-Qare *et al.*, 2003) was assumed due to lack of information. Increasing the surface area parameter in keeping with the DEET kinetic studies in Appendix A (ranging from 4 to 25 cm²) greatly increases the DEET blood concentration, in opposition to the data observed (simulations not shown). The very small surface area, therefore, appears to be appropriate for this study.

Oral uptake parameters for PB initially were set to those developed for rats in the Husain *et al.* (1968) study (Appendix B). The model predicts the clearance of PB from the blood to occur too quickly (Figure 3, PB line). Since the rats in the Abu-Qare and Abou-Donia (2000) study are likely fed, the parameters were switched to those from the Coper *et al.* (1974) study. These parameters come much closer to predicting the range of blood concentrations observed by Abu-Qare and Abou-Donia (simulation not shown). Although Abu-Qare and Abou-Donia (2003) indicated that PB was unaffected by the administration of the GWI compounds together, a

significantly decreased VMaxC (2.0 decreased to 0.02 mg/(hour*kg^{0.75})) was required to retain enough PB in the blood at 8 hours (Figure 3, PB* line). The model simulation indicates that high dose combinations of GWI compounds likely has an impact on PB metabolism. This PB dose was 10 times higher than the PB dose in the studies (Abdel-Rahman *et al.*, 2002; Parihar *et al.*, 2013; Hattiangady *et al.*, 2014) for which this model is being developed.

Predicting the dermal permethrin data in this study was much more challenging. No prior PER kinetics studies were found for the dermal route. The predicted dermal uptake parameters (skin permeability constant calculated using the modified Robinson model (0.07/hour, Wilschut *et al.*, 1995) and the skin:blood PC predicted using the Ruark *et al.* (2014) algorithm (8.2 for both isomers)) were utilized along with the parameters developed in Appendices C and D for the first PER simulation (Figure 3, permethrin line). The uptake of a compound dermally is highly dependent on the surface area exposed. Assuming the very small surface area of exposure is correct, based both on another related study (1 cm², Abu-Qare *et al.*, 2003) and on the behavior of the DEET simulations, then the dermal uptake parameters or the VMaxC values (2.51 and 6.3 mg/(h*kg^{0.75}) for cis- and trans-PER, respectively, Willemin *et al.*, 2016) required adjustment to fit the Abu-Qare and Abou-Donia (2000) data. Multiple iterations adjusting the skin permeability constant and the skin:blood PC indicated that the PER remaining in the blood at 8 hours could not be simulated by their alteration (simulations not shown); these parameters were reset to their original estimated values.

The VMaxC values were then divided by 100, as was done for the PB simulation, based on the PER data provided in the study. The resulting VMaxC values (0.0251 and 0.063 mg/(hour*kg^{0.75}) for cis- and trans-PER, respectively) were sufficient to simulate the study data without altering other parameters (Figure 3, permethrin* line). As with the other GWI compounds, this study administered a dose 10 time higher than the studies on which the model will be predicting (Abdel-Rahman *et al.*, 2002; Parihar *et al.*, 2013; Hattiangady *et al.*, 2014). Again, it is likely that these high doses of GWI compounds do competitively inhibit metabolism, at least for PB and PER; DEET metabolism was apparently unaffected.

3.3 Results: Predictions for *In Vitro* Assay

Following the simulation of the mixtures study (Section 3.2), the model was utilized to predict brain tissue concentration ranges following an *in vivo* dose of 1.3 mg/kg PB (oral), 40 mg/kg DEET (dermal) and 0.13 mg/kg PER (dermal). These doses were patterned after the research of Abdel-Rahman *et al.* (2002), Parihar *et al.* (2013) and Hattiangady *et al.* (2014) and are 10 times smaller than the dose in the only kinetic mixtures study (Abu-Qare and Abou-Donia, 2000).

The projected *in vitro* study was designed in conjunction with the Naval Medical Research Unit Dayton (NAMRU-D) GWI *in vivo* study, which is in the process of being published elsewhere by Dr. Rohan. The exposure assumptions were set equivalent to the *in vivo* study for these simulations. The *in vivo* study used Sprague-Dawley rats with a maximum body weight of 225 g. PB was administered orally at 1.3 mg/kg in water; rats were fed and not fasted as the *in vivo* study was a 28 day repeated dose experiment. DEET was applied dermally at a dose of 40.0 mg/kg (45 mg/mL, 0.2 mL) over an area of approximately 3 cm². Similarly, PER was applied at 0.13 mg/kg dermally (0.13 mg/mL, 0.2 mL). The safety data sheet for the PER used indicated a 55:45 cis:trans ratio. Due to *in vivo* study endpoints, brain concentration predictions were requested for 5 minutes and 24 hours; peak concentrations and times, however, were the priority. Given the kinetic differences between single compound (Appendices A through D) and mixture kinetics (Figure 3), the parameters used to simulate the Abu-Qare and Abou-Donia

(2000) data were used to predict the blood and brain concentrations for this similar study design (Figures 4 and 5).

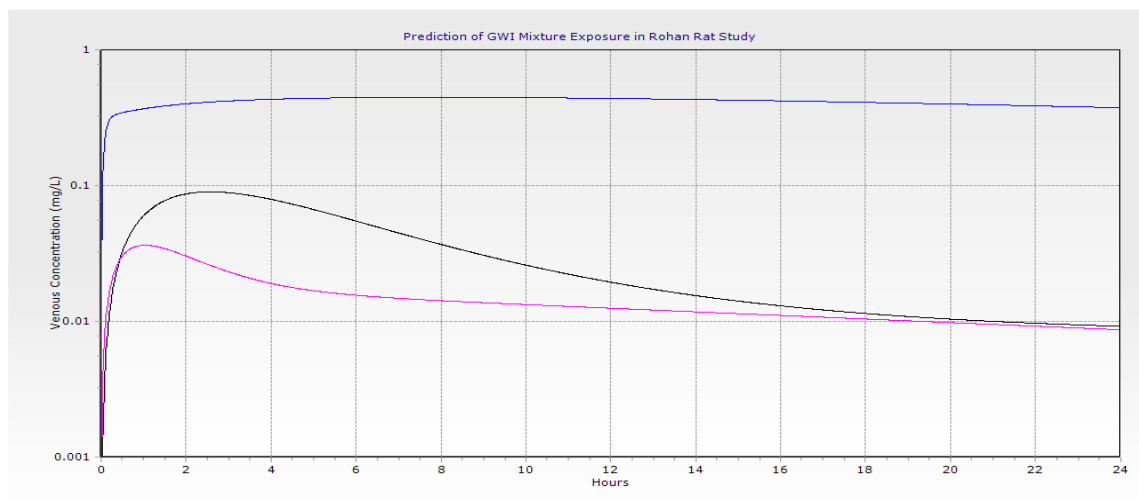


Figure 4. Predictions of *In Vivo* GWI Mixture Study Blood Concentrations. The blood concentration (mg/L) predictions are shown as a blue line for DEET, magenta line for PB and black line for permethrin. Male rats were exposed to 1.3 mg PB/kg orally, 40 mg DEET/kg dermally, and 0.13 mg PER/kg dermally.

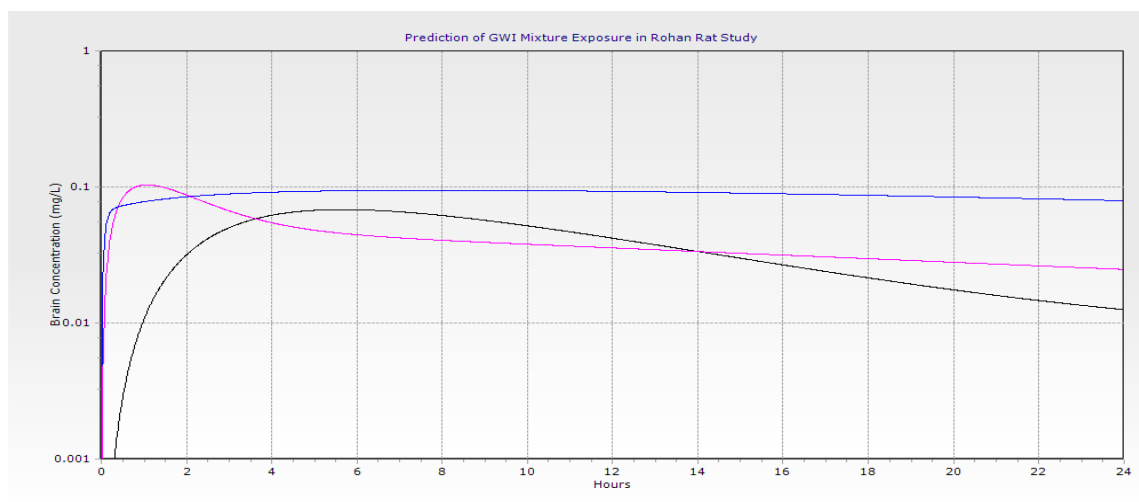


Figure 5. Predictions of *In Vivo* GWI Mixture Study Brain Concentrations. The brain concentration (mg/L) predictions are shown as a blue line for DEET, magenta line for PB and black line for permethrin. Male rats were exposed to 1.3 mg PB/kg orally, 40 mg DEET/kg dermally, and 0.13 mg PER/kg dermally.

Interstitial brain tissue concentrations were also predicted (Figure 6) by dividing the brain concentrations by the brain:blood PCs. Interstitial concentrations represent the concentrations expected to bath the synapses *in vivo*. Synaptic activities from this study are being correlated with EP and behavioral effects from the *in vivo* study. Additionally, due to the thinness of the

brain slices, it was unknown if the blood:brain barrier would be intact. Therefore, predictions of both brain tissue and interstitial concentrations were provided to reflect a relevant range of concentrations to test *in vitro*.

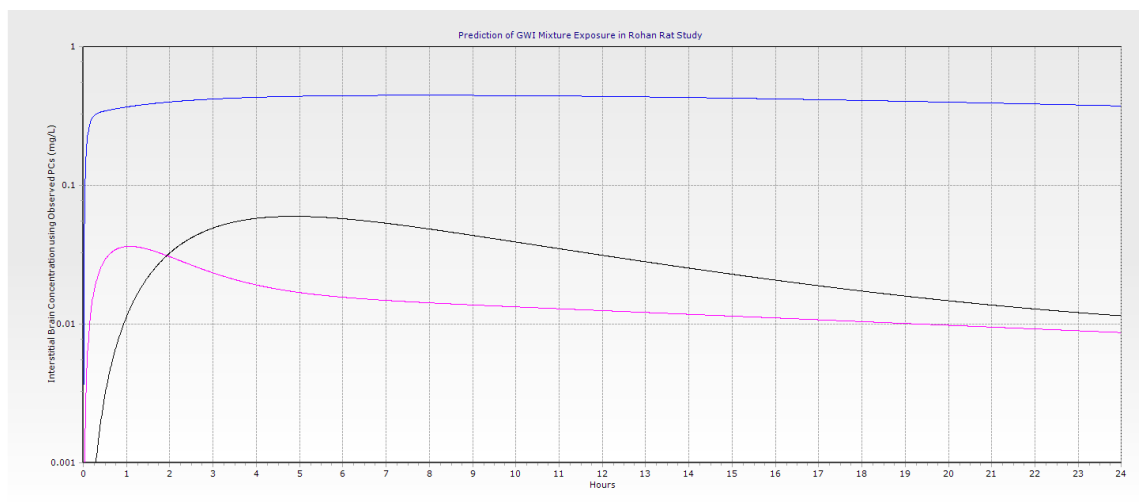


Figure 6. Predictions of *In vivo* GWI Mixture Study Interstitial Brain Concentrations. The interstitial brain concentration (mg/L) predictions are shown as a blue line for DEET, magenta line for PB and black line for permethrin. Male rats were exposed to 1.3 mg PB/kg orally, 40 mg DEET/kg dermally, and 0.13 mg PER/kg dermally.

In addition to the graphs, predictions of blood, brain and interstitial brain concentrations at 5 minutes and 24 hours are shown in Table 2, as well as peak values and times. The model predicts implicit numbers at a given time based on specific assumptions. However, the purpose of the model is to provide ballpark concentrations expected in the tissues, not a definite number. Therefore, an additional column was added with recommended concentration ranges for use in the *in vitro* study. For DEET and PB, the ranges encompass the predicted brain tissue and brain interstitial fluid (synapse region) concentrations. For PER, the confidence in the dermal uptake is less certain, given the lack of dermal kinetic data; therefore the range begins at the predicted interstitial concentration and increases by an order of magnitude.

Table 2. Predicted Values at Key Time Points and Peak Concentration for GWI Compounds in Target Tissues

GWI Compound	Tissue	5 Minute Conc. (0.08 hour)	24 Hour Conc.	Peak Time (hour)	Peak Conc.	Recommended Brain Conc. Ranges for In Vitro Study
DEET	Venous	0.21	0.37	7.86	0.45	0.1 – 0.5
	Brain	0.041	0.079	7.87	0.095	
	Brain Interstitial	0.19	0.37	7.87	0.46	
PB	Venous	0.0077	0.0087	1.03	0.036	0.04 – 0.1
	Brain	0.013	0.025	1.08	0.10	
	Brain Interstitial	0.0044	0.0087	1.08	0.036	
Total Permethrin	Venous	0.0035	0.0092	2.57	0.090	0.06 - 0.6
	Brain	0.00042	0.013	5.67	0.068	
	Brain Interstitial	0.000051	0.011	4.87	0.060	

Note: Tissue levels in mg/L

3.4 Discussion

The purpose of developing the array PBPK model of GWI chemicals, was to be able to predict brain concentrations achieved during previously published GWI studies in rats (Abdel-Rahman *et al.*, 2002; Parihar *et al.*, 2013; Hattiangady *et al.*, 2014), as well as the NAMRU-D GWI *in vivo* study being prepared for publication by Dr. Rohan. The array (vector) PBPK model successfully estimated multiple tissue concentrations simultaneously, including the brain concentrations, which were then used to inform the conditions for the *in vitro* electrophysiology study using naïve rat brain slices, described in Section 4.

The model code includes four routes of exposure (IV, inhalation, oral and dermal), two of which are pertinent to modeling exposures to GWI compounds (oral and dermal). Intravenous was preserved from the original model structure (Clewell *et al.*, 2001) to accommodate available kinetic data and is typically felt to translate well to other routes of extrapolation (Kuepfer *et al.*, 2016). While no published inhalation data were available for any of the compounds, this exposure route was retained in the model code for future use in assessing these or other volatile compounds that may have been encountered during the Gulf War. Dermal dosing was modeled as in Clewell *et al.* (2001). This pathway is critical as DEET was applied liberally by the Gulf War troops. Unfortunately, no single compound kinetic data describing dermal dosing of PER or repeated dosing for DEET were located.

Availability of data to estimate PCs for the compounds was limited. Therefore, both apparent PCs from published *in vivo* kinetic studies and QSAR-predicted values based on Ruark *et al.* (2014) were considered for each compound. Details about the estimation of PCs are provided in Appendices A through D. Whenever possible, predictions of tissues concentration were calibrated against published data. Clearance of the compounds from the body were modeled through either metabolism and/or urinary and fecal clearance. Metabolic and clearance constants were derived or fitted to data from the published literature when available. The ability to include competitive metabolism of the compounds was included in the model code and can be parameterized in the future, as there is some evidence of this between GWI compounds (Abu-Qare *et al.*, 2003). In addition, the PBPK approach used here can allow metabolites to be

modeled as separate compounds in the array if metabolites were found to be pharmacologically active.

4.0 *IN VITRO* STUDY

Based on the NAMRU-D GWI *in vivo* animal study, which will be published separately by Dr. Rohan, exposures to GWI chemicals induced lasting detectable changes in hippocampal neuronal function. Rats were exposed to 1.3 mg/kg PB (orally), 40 mg/kg DEET (dermally) and 0.13 mg/kg PER (dermally) for 28-days. Hippocampal slices from exposed rats showed a reduction in the input-output (I/O) function, increase in paired pulse facilitation (PPF) and reduction in the frequency of spontaneous activity of neurons within the Schaffer collateral-CA1 region of the hippocampus, compared to slices from unexposed rats. These alterations were observed both the next day and 3 months following the last exposure.

The Schaffer collateral-CA1 region of the hippocampus is essential for various cognitive processes, especially learning and memory, and previously has been reported to be affected by GWI compound exposures (Hattiangady *et al.*, 2014; Parihar *et al.*, 2013). Herein, it was assessed whether *in vitro* application of GWI chemicals directly to hippocampal slices could induce similar electrophysiological effects in the Schaffer collateral-CA1 synapse as those observed with *in vivo* exposures.

4.1 Methods

Electrophysiological recordings of rat hippocampal slices that were directly exposed to GWI chemicals were performed. Electrophysiology was conducted using a microelectrode array system (Alphamed MED64 Quad II system, Automate, Berkeley CA) consisting of 16 microelectrodes (50x50 μm , 150 μm apart) arranged in a 4x4 array. The Quad II system can deliver electrical currents of varying intensities to evoke synaptic responses in addition to recording spontaneous activity.

4.1.1 Hippocampal Slice Preparation. Male Sprague-Dawley rats (n = 6) weighing 200 to 225 g were anesthetized and euthanized in accordance with current American Veterinary Medical Association (AVMA) guidelines (AVMA, 2013).

Brain slices were prepared as previously described (Rohan *et al.*, 2015). Brains were rapidly removed following euthanasia and were kept viable with ice cold artificial cerebrospinal fluid (ACSF) that was continuously oxygenated (95:5 percent $\text{O}_2:\text{CO}_2$). ACSF was prepared fresh daily and consisted of (in mM): 124 NaCl, 3 KCl, 1.25 KH_2PO_4 , 10 D-Glucose, 1 MgSO_4 , 36 NaHCO_3 , and 2 CaCl_2 (pH ~7.4). Cerebellum and approximately 0.5 cm of frontal cortex were removed and the remaining brain was sectioned at 350 μm thickness using a vibratome (VT1000S Leica Microsystems, Buffalo Grove IL) in the transverse plane, at 20 to 30°, lateral from the horizontal axis. Brain slices were maintained in warmed oxygenated ACSF and allowed to recover for at least 60 minutes prior to recording.

After the recovery period, half of the brain slices were placed in another chamber filled with warmed oxygenated ACSF containing either the 1X or the 10X GWI chemical mixture (see Section 4.1.2). Slices were allowed to incubate in ACSF containing the GWI mixture for at least 1 hour prior to recording. Recordings were made using a microelectrode array system (Quad II, Automate, Berkeley CA). One hippocampal slice was placed onto the pre-coated microelectrode array probe, using small weights to anchor the edges. For recordings of the Schaffer collateral-CA1 synapses, the slice was positioned such that neurons from the CA1 and surrounding region

were recorded (Figure 7A). The probe containing the brain slice was then positioned in the Quad II system, per the manufacturer's instructions. A perfusion cap was used to deliver fresh oxygen to the slices.

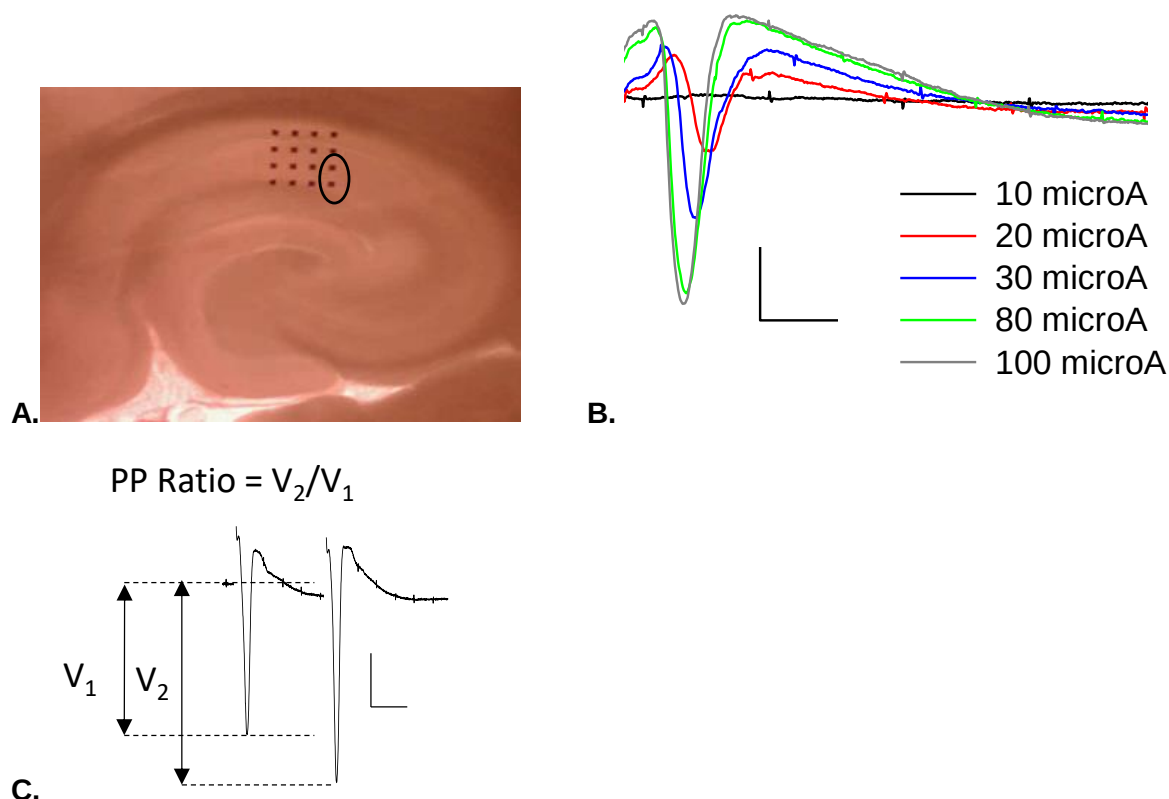


Figure 7. Measuring Evoked Responses using a Microelectrode Array System. (A) Positioning of hippocampal slices on the microelectrode array probes to measure evoked field responses from Schaffer collateral-CA1 synapses. Stimulating currents of various intensities are delivered through one of the circled probes. (B) Typical field responses from 10 to 120 μ A stimulations. Amplitudes of these responses are plotted against stimulation intensity to assess the I/O function of CA1 neurons (scale bar: 0.5 mA, 5 milliseconds). (C) Typical field responses from a paired pulse stimulation protocol in which two consecutive stimuli of equal intensity 40 milliseconds apart are delivered to assess PPF of CA1 neurons. PPF was quantified by measuring the paired pulse ratio (PP ratio) calculated by dividing the amplitude of the second response by the amplitude of the first response (scale bar: 0.25 mA, 5 milliseconds).

4.1.2 GWI Chemicals. Stock concentrations of GWI chemicals were made: 973 mg/ml DEET in water, 1.0 mg/ml PER in ethanol and 1.3 mg/ml PB in water. These stock concentrations were further diluted with ACSF to generate the working concentrations. Brain slices were incubated in two distinct working concentrations of GWI chemicals. The first was chosen to represent a value within the PBPK model-predicted concentration ranges in brain tissue; termed 1X, the mixture was 0.097 μ g/ml DEET, 0.5 μ g/ml PER, and 0.13 μ g/ml PB. The second concentration represents approximately 10X the first concentrations: 0.973 μ g/ml DEET, 1 μ g/ml PER, and 1.3 μ g/ml PB. The PER concentration was only doubled in the 10X mixture due to solubility issues.

4.1.3 Electrophysiology Measurements: All electrophysiology data were obtained using the AlphaMed Quad II systems, as previously described (Rohan *et al.*, 2015). Probes were initially pretreated with 70 percent ethanol for 5 minutes and then 0.1 percent polyethylenimine (PEI) in 25-mM borate buffer for at least 24 hours prior to use. Data acquisition and stimulation protocols were performed using Mobius software (Automate, Berkeley CA). Evoked field potentials mainly consisting of inward deflections were recorded from neurons within the CA1 region of the hippocampus at a sampling frequency of 20 kHz using the Quad II multichannel amplifier, digitized and graphically displayed using Mobius software.

Input-output data were obtained by delivering multiple biphasic stimuli at various intensities, ranging from 10 to 120 μ A. Typical I/O response is shown in Figure 7B. Paired pulse facilitation data were obtained by delivering sets of two consecutive stimuli of equal intensity that were 40 milliseconds apart. Degrees of PPF were quantified by calculating the PP ratio in which the response amplitude from the second stimulating pulse was divided by the response amplitude from the first pulse (Figure 7C). Spontaneous spiking activity of neurons within the CA1 region were recorded for 10 minutes and frequency of firing was calculated using the Mobius software. Spontaneous spiking activity data were processed through low pass (5000 Hz) and high pass (100 Hz) filters during the recordings. A threshold value of 5 times the size of the standard deviation of the baseline (typically 15 to 20 μ V) was used as the spike threshold.

4.1.4 Data Analysis. For I/O and PPF data, recordings from multiple microelectrodes within the CA1 region of a hippocampal slice were averaged together. One slice typically generated 10 to 15 recordings from spatially distinct sites within the CA1 region. Data from 9 to 12 slices were presented as mean and standard error of the mean (SEM); data were statistically compared using an unpaired, two-tailed t-test. Statistical analyses of I/O and PPF data were conducted using the averaged value per slice as the sample datum. Frequency of spontaneous firing activity was calculated by counting the spikes with amplitudes 5 times greater than the size of the standard deviation (typically 15 to 20 μ V) over the 10-minute recording period. Statistical analysis of the frequencies of spontaneous activity were completed using each recording from a microelectrode as the individual sample datum. A calculated P of less than 0.05 is considered significantly different. All quantitation and statistical analysis as well as graphs were generated using Microsoft Excel® and SigmaPlot® v.13.

4.2 Results

4.2.1 Incubation with 1X GWI Chemicals. Slices were incubated with 1X the model-predicted brain concentrations of DEET (0.097 μ g/ml), PER (0.5 μ g/ml) and PB (0.13 μ g/ml) for at least 1 hour. While a trend for differences between treated and control slices was shown, there were no statistically significant changes in the input-output function, PPF or frequency of spontaneous activity of CA1 neurons of hippocampal slices incubated with 1X GWI chemicals and control (Figure 8A through C).

4.2.2 Incubation with 10X GWI Chemicals. Slices were incubated with 10X model-predicted brain concentrations of DEET (0.97 μ g/ml), PER (1 μ g/ml) and pyridostigmine bromide (1.3 μ g/ml) for at least 1 hour. Reductions in the input-output function were not statistically significant (Figure 8D). However, there was a statistically significant increase in PPF of brain slices

incubated with 10X GWI chemicals compared to control slices at varying stimulation intensities of 10, 30, 40, 70, and 90 μ A (Figure 8E). When averaged across all stimulation intensities (10 to 120 μ A), brain slices incubated in ACSF only (control) had an average PP ratio of 1.21 ± 0.03 whereas brain slices incubated in ACSF + 10X GWI chemicals had an average PP ratio of 1.42 ± 0.04 . There was also a statistically significant reduction in the frequency of spontaneous activity of CA1 neurons when incubated in ACSF + 10X GWI chemicals (Figure 8F). The average frequency for control slices was 1.17 ± 0.2 Hz, whereas the average frequency for treated slices was 0.64 ± 0.1 Hz.

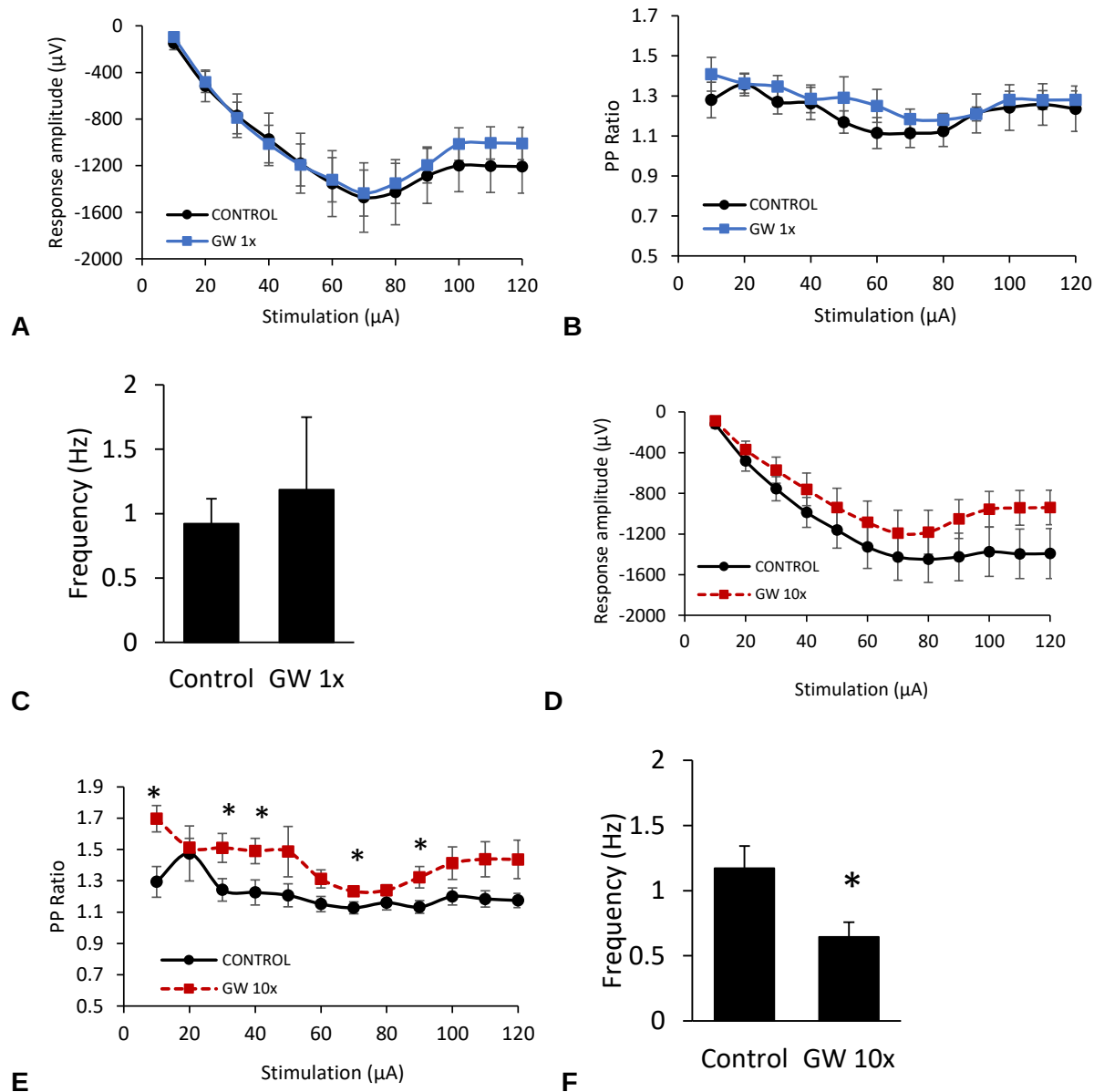


Figure 8. *In Vitro* Incubation of GWI Chemicals Directly to Acutely Dissected Hippocampal Slices.

(A-C) Slices were incubated in either ACSF (CONTROL, black) or in 0.097 μ g/ml DEET, 0.5 μ g/ml PER, 0.13 μ g/ml PB (GWI 1X, blue) for >1 hour. (A) There was no statistically significant change in I/O function of CA1 synapses as measured by amplitudes of field responses to varying stimulus intensities (n=9 slices, 10 to 15 recordings per slice). (B) There was no statistically significant change in PPF of CA1 neurons (n=9 slices, 10 to 15 recordings per slice). (C) There was no statistically significant change in spontaneous activity of hippocampal neurons (Schaffer collateral – CA1) (n=41 to 59 recordings). (D-F) Slices were incubated in either ACSF (CONTROL, black) or in 0.973 μ g/ml DEET, 1 μ g/ml PER and 1.3 μ g/ml PB (GWI 10X, red) for >1 hour. (D) There was no statistically significant change in the I/O function of hippocampal synapses (n= 10 to 12 slices, 10 to 15 recordings per slice). (E) There was a statistically significant increase in PPF at stimulation intensities of 10, 30, 40, 70 and 90 μ A in slices incubated in GWI chemicals (*P=0.005, 0.04, 0.03, 0.04, 0.03 respectively, two-tailed unpaired t test, n=10 to 12 slices, 10 to 15 recordings per slice). (F) There was a statistically significant decrease in spontaneous activity of CA1 neurons (*P=0.02, 2 tailed unpaired test, n=127 to 175 recordings).

4.3 Discussion

Hippocampal slice cultures provided a useful tool for elucidating biological mechanisms. These cultures are prepared from slices of explanted brains and represent a complex multicellular *in vitro* environment. These cultures preserve the three-dimensional architecture and local environment of brain cells, including neurons, astrocytes and microglia, as well as the neuronal connectivity and the complex glial-neuronal interactions. These systems are considered excellent models to study neuroprotection from neurotoxins and are used as a starting point for drug discovery (Cho *et al.*, 2007; Noraberg *et al.*, 2005).

The *in vitro* work was conducted following the completion of the NAMRU-D GWI *in vivo* study, in which rats were exposed to GWI chemicals with and without mild stress (5 minutes restraint) for 28 consecutive days. A finding of the *in vivo* study, which will be reported separately by Dr. Rohan, was a statistically significant increase in PPF and reduction in spontaneous activity of hippocampal CA1 neurons in rat brain slices following a 28-day exposure to these GWI compounds. Determining whether GWI chemical exposures applied directly to brain slices *in vitro* can mimic the enhanced PPF and reduce spontaneous activity observed with *in vivo* exposures was ascertained herein.

Incubation of hippocampal slices with the model-predicted brain concentrations of GWI chemicals for at least 1 hour resulted in a trend toward response alterations but did not result in statistically significant changes, possibly due to insufficient GWI chemicals diffusing into the hippocampus slices over the short *in vitro* exposure duration to exert maximal effect on synaptic function. However, incubation of hippocampal slices with 10X the model-predicted brain concentrations of GWI chemicals for at least 1 hour resulted in statistically significant increases in PPF as well as reduction in the frequency of spontaneous spiking activity similar to the NAMRU-D GWI *in vivo* study.

Our data indicate that direct incubation with GWI chemicals can elicit similar electrophysiological effects to those induced by *in vivo* exposures. PPF is a form of short term synaptic plasticity and has been implicated in a number of critical cognitive processes (Matilla *et al.*, 1998; Zucker and Regehr, 2002). Enhancement in PPF, especially when accompanied by a reduction in size of evoked response (I/O function), can result from impaired neurotransmitter release (Malenka, 2002). Changes in spontaneous activity have been documented and can result from changes in network plasticity (Usami *et al.*, 2008) and/or various insults to the brain such as cerebral ischemia (Chang *et al.*, 1989).

The effects of *in vivo* GWI chemical exposures for 28 consecutive days on hippocampus function will be further discussed in detail as a separate publication being developed by Dr. Rohan. The main finding herein is that the predicted brain concentrations obtained from a mathematical PBPK model were able to mimic electrophysiological effects of 28-day *in vivo* GWI chemical exposures in an *in vitro* setting.

5.0 CONCLUSIONS AND RECOMMENDATIONS

In order to mimic the levels of neurotoxins (GWI compounds) present within the neuronal environment during the *in vivo* GWI mixture behavioral studies, without sacrificing additional animals for tissue measurements, an array PBPK model was successfully employed. The PBPK model was parameterized and calibrated against various published *in vivo* kinetic studies. The model did a good job of predicting tissue dosimetry from *in vivo* studies across multiple doses, dose routes and species, including PB levels in humans. The estimated culture media concentrations were successful in that the 10X solution of the GWI compounds resulted in a

statistical increase in PPF and a reduction in the frequency of spontaneous activity of CA1 neurons of naïve brain slices.

5.1 Recommendations for Future Efforts

One potential reason that the model predictions were somewhat low is that the model simulated single day doses, rather than repeated dosing over 28 days, which may have been closer to effective *in vitro* doses. Future GWI PBPK models could include complex dermal dosing code to replicate the effects of multiple day dosing. This code, not readily available during this time constrained project, should account for a portion of the dose remaining on the skin between applications, plus evaporation of the compounds.

Due to the novelty of the project, it was uncertain if interstitial brain concentration predictions would yield the required ACSF concentrations or if the brain tissue concentrations were a better predictor of neurologically active doses *in vitro*. Interstitial concentrations would be required in a situation where the BBB in the brain slices was disrupted. The work herein indicates that predictions of whole brain tissue concentrations are more suitable for this hippocampal slice cultures. Diffusion from the ACSF into the brain slice must still occur prior to the diffusion of the compounds from the tissue into the synapse.

Future use of the optimized *in vitro* system includes the testing of prophylactics designed to prevent long term injuries such as GWI from occurring. Military members are commonly exposed to situations where daily DEET applications and PER impregnated uniforms may be necessary. Adding PB due to threats of irreversible AChE inhibitor exposure (chemical warfare agents) is still a possibility. Pre-incubation of naïve brain slices with a prophylactic prior to *in vitro* exposure to PB, DEET and PER could show protective effects in brain tissue based on quantitative electrophysiological measures. Following *in vitro* optimization of a prophylactic, the PBPK model could be utilized to estimate protective oral doses for a rat or human.

One novel therapeutic for possible testing is Luteolin, a plant-derived compound with anti-inflammatory properties shown to promote neuron survival against microglial inflammation (Zhu *et al.*, 2011; Al Amin and Reza, 2014). Another countermeasure candidate would be the PAF receptor antagonist (BN52021 or ginkgolide K), which may combat the GWI chemical-induced oxidative stress and inflammatory response. PAF receptor plays an essential role in oxidative stress and recent work indicates that the PAF receptor antagonist BN52021 can reduce neuroinflammation elicited by lipopolysaccharides (Cho *et al.*, 2007). Similarly, ginkgolide K possesses some neuroprotective properties via its antioxidative action (Ma *et al.*, 2012) which may partially reverse or prevent the oxidative damage elicited by GWI chemical exposures.

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APPENDIX A. DEET SIMULATIONS AND PREDICTIONS

For simplification, all citations within this appendix are contained within the main report reference list.

PRIOR PBPK MODELS

Previously published or unpublished PBPK models were not found for DEET.

KINETIC STUDIES

Fediuk *et al.* (2011) published DEET intravenous (IV) kinetic time course data. Male Sprague-Dawley rats (mean body weight = 250 g, n = 6) were exposed IV to 2 mg/kg DEET or oxybenzone (sunscreen). As no information was given regarding injection duration, 0.5 minutes (0.008 hours) was assumed. Serial 150 μ L blood samples were collected from the saphenous vein at 10 time points over 24 hours. At the 2 hour time point, saline (10 mL) was injected subcutaneously to help compensate for fluid loss. At 24 hours, liver and kidney concentrations of DEET were analyzed. The authors also included dermal kinetics of DEET, oxybenzone or a mixture of the two over the same time period. For the DEET only dermal exposure, a group of 5 250 g male SD rats were exposed to 100 mg/g DEET (0.3 percent) in a mixture of Emulphor, ethanol and water (2, 3 and 5 parts, respectively, by volume). The surface area of exposure was 25 cm² (5 x 5 cm). Eleven blood collections occurred over 24 hours, under the same method as the IV study. Terminal kidney and liver concentrations were also measured. The amount of DEET that remained on the skin or in the skin (tape stripping) accounted for 32.3 percent of the dose at 24 hours; 67.7 percent of the dose was assumed to have been absorbed.

Snodgrass *et al.* (1982) studied the intravenous and dermal kinetics of DEET in rats, rabbits and dogs. Groups of male and female Sprague Dawley rats (250 to 300 g, n = 3 per sex) were lightly anesthetized using ether and injected with 2 μ Ci (28.12 μ g = 0.102 mg/kg for a 275 g rat) DEET in the femoral vein. As no information was given regarding injection duration, 0.5 minutes (0.008 hours) was assumed. The dermal groups were exposed to the same dose prepared in absolute ethanol, using a rate of application equivalent to 4 μ g/cm², which calculates to an average surface area of 7.03 cm². The application site was covered with a non-occlusive patch. The patches (one for the first 24 hours and the other for Days 2 through 7) were analyzed to determine the amount of DEET evaporated. The application site was also quantitated on Day 7. Urine and feces were collected daily for 7 days, resulting in an informative metabolite excretion curve. Tissues were collected at the 7 day mark from the dermal groups; male rats did not retain any radioactivity but female rats retained a small portion of the dose. In the male rat, a total of 43 percent of the applied DEET was excreted as metabolites in the urine and 39 percent was recovered from the patches and dermal application site. As much as 18 percent of the dose was unaccounted for in the male rat. Since fecal elimination of DEET was not quantified to give a more complete estimate of recovery and since the applied dose was very low, absorption was assumed to be 100 percent in the simulation. Physiological parameters were not altered due to the brief and light use of anesthetic.

Schoenig *et al.* (1996) published oral and dermal DEET kinetics in rats. Male and female CD rats (n = 5 per sex and time point) were exposed to single oral doses of approximately 100 or 500 mg DEET/kg body weight in corn oil. Doses were equivalent to about 20 or 100 μ Ci/rat. A third group received repeated unlabeled oral doses of approximately 100 mg DEET/kg body weight for 13 days, followed by a labeled dose on the 14th day (~20 μ Ci/rat). A fourth group of rats received a single dermal dose of 100 mg DEET/kg body weight (~20 μ Ci/rat). The dermal

dose was applied using a 2.5 x 5 cm glass chamber glued to the rat's back; the vehicle for DEET was not mentioned. The rats were approximately 9 weeks at the beginning of the study but no body weight was given. Since the doses were apparently the same between males and females and since male and female CD rat growth curves overlap at approximately 250 g at 9 weeks (Charles River Laboratories, 2019), this body weight was chosen for modeling this study. Tissues (brain, liver, kidney, muscle, fat and blood) were collected after 0.5 (males), 2 hours (females) or 7 days following the final dose. Early (0.5 or 2 hour) time point tissue and blood concentration ratios were used as apparent PCs for model development. Feces and urine were collected for 7 days. For all routes of exposure, most of the dose was excreted as metabolites in urine but a portion of the dose was collected in feces. For male rats exposed once dermally, 77.85 percent was excreted in the urine and 3.99 percent was found in the feces; these values were used to establish the portion of the dose subject to Michaelis-Menten metabolism (urine value) and the portion eliminated due to first-order metabolism (feces value). Further, since a total of 81.84 percent of the applied dose was recovered in urine and feces, only this portion of the dose was assumed to be absorbed; 6.32 percent was left on or in the skin and the remainder (nearly 12 percent) likely evaporated. While error values were given for these data, it was not stated whether they were standard deviation (SD), SEM or some other confidence interval; the values were still used to help bracket the model fit.

Fediuk *et al.* had also published additional dermal data for DEET and oxybenzone in a prior publication (2010). Male and female SD rats (268 g males, 256 g females, n = 5 per sex) were exposed to 40 mg DEET/kg over 4 cm² (2 x 2 cm) daily for 30 days. Each daily dose was spread with the application pipette and allowed to air dry unoccluded; the vehicle in this study was 70 percent ethanol. Following the final exposure, 300 µL blood samples were collected from the saphenous vein at 2, 6, 8 and 24 hours. Following euthanasia, the application site was swabbed to collect unabsorbed DEET and tape stripped to determine the amount left in the skin itself; 38 percent of the dose was recovered using these methods. Therefore, only 62 percent of the dose was assumed to be absorbed. Liver and brain tissues also were collected for analysis.

MODEL AND PARAMETER DEVELOPMENT

As described in the Approach (Section 3.1) of the main report, blood:air PCs were calculated using the algorithm of Buist *et al.* (2012). Given the relatively high predicted PC (609.0), exhalation is not a major route for elimination of this compound (Table A-1). Upper respiratory tract uptake is a parameter in the model. Although there are no inhalation kinetic studies for this compound, DEET has been inhaled and caused neurological effects (ATSDR, 2017). An arbitrary value (1.0) was utilized in the array model to signify this possibility and inhalation as a route of uptake was left in the model for potential future use.

Table A-1. Rat Physicochemical Specific Tissue:Blood Partition Coefficients and Cell Membrane Permeability Coefficients

Parameter(Constants)	DEET
Blood:Air Partition Coefficients (PC, unitless)^a	
Blood:Air PC	609.0
Tissue:Blood Partition Coefficients (PC, unitless)	
Brain (PBrn)	0.212
Fat (PFat)	14.1*1.57 ^b
Liver (PLiv)	2.28
Rapidly Perfused (PRap)	1.57
Skin (PSkn)	0.12
Slowly Perfused (PSlw)	0.54*1.57 ^b
Cell Membrane Permeability Coefficients (PA, L/(hour*kg^{0.75}))	
Brain (PABrnC)	0.1
Fat (PAFatC)	0.1
Liver (PALivC)	1.0
Rapidly Perfused (PARapC)	10.0
Slowly Perfused (PASlwC)	0.1

Notes: All values fit to data unless otherwise noted; ^aPredicted using Buist *et al.* (2012) algorithm; ^b(Ratio of predicted tissue PC:predicted kidney PC as calculated using Ruark *et al.* (2014)) * PRap.

Multiple sets of tissue:blood PCs were considered: predictions from Ruark *et al.* (2014), apparent PCs from Fediuk *et al.* (2010 and 2011, combined), apparent PCs from Schoenig *et al.* (1996), and predictions published by Knaak *et al.* (2012). Numerous iterations of model simulations for the DEET data sets indicated the best fit across the IV and oral studies resulted from the apparent PCs calculated from the Fediuk *et al.* (2010 and 2011) data (Table A-1). These data provided brain, liver and kidney (rapidly perfused tissue) apparent PCs at the 24-hour time point.

No measured values were found for fat, slowly perfused tissues (usually muscle) or skin. Fat and slowly perfused tissues PCs were calculated for the model by estimating the PCs for fat:plasma, muscle:plasma and kidney:plasma using the method of Ruark *et al.*, (2014). The ratio of estimated fat or muscle:plasma PC divided by kidney:plasma PC indicates the relative proportion in fat or muscle tissue, as compared to the kidney (rapidly perfused tissues). This ratio was multiplied times the apparent rapidly perfused tissue:blood PC to generate a model PC for each compartment (fat and slowly perfused tissues, Table A-1). The skin permeability constant was predicted using the modified Robinson equation as published in Wilschut *et al.* (1995).

Intravenous studies were simulated first, followed by the oral studies. These studies combined served as the calibration data set for the bulk of the model parameters. The IV plasma data from Fediuk *et al.* (2011) coupled with the metabolic excretion data from Snodgrass *et al.* (1982) demonstrate an acceptable loss of DEET from the blood (Table A-2, Figure A-1) and metabolism due to Michaelis-Menten kinetics (Figure A-2). Snodgrass and colleagues indicated that not all of the DEET dose is recovered in the urine. Similarly, Schoenig *et al.* (1996) demonstrated that about 4 percent of the dose was found in feces, indicating a different metabolic pathway. First-order kinetics were added to the model to allow for approximately 4

percent of the dose to be removed from the body by this pathway (simulation in Figure A-8, paired with the appropriate dermal data).

Table A-2. Rat Metabolic, Uptake and Clearance Parameters^a

Parameter	Units	DEET
Michaelis-Menten Maximum Velocity (VMaxC)	mg/(hour*kg ^{0.75})	14.0
Affinity Constant (Km)	mg/L	2.18
First Order Metabolism Constant (KFC)	/hour	0.6
Upper Respiratory Tract Uptake (KURTC)	L/hour	1.0
Urinary Clearance (CIUrC)	L/(hour*kg ^{0.75})	0.0 ^b
Absorption from Stomach (KAS)	/hour	0.2
Transfer from Stomach to Duodenum (KTSD)	/hour	6.0
Absorption from Duodenum (KAD)	1.0	1.0
Fecal Excretion (KFec)	/hour	0.25
Skin Permeability Constant (KP)	cm/hour	0.002 ^c

Notes: ^aFit to data unless noted; ^bKasichayanula *et al.* (2007); ^cPredicted using the modified Robinson algorithm as described in Wilschut *et al.* (1995)

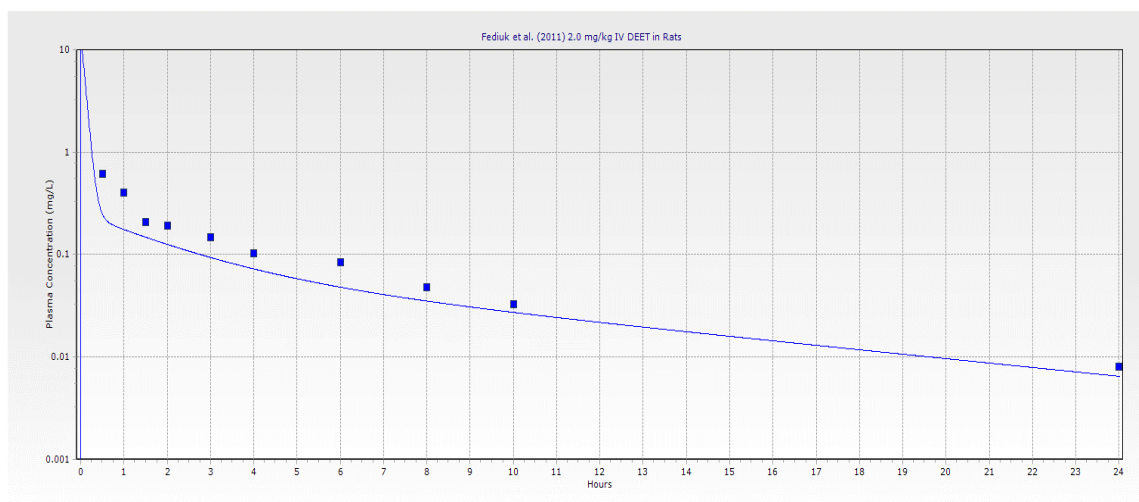


Figure A-1. Simulation of Fediuk *et al.* (2011) Intravenous DEET Plasma Data. The plasma concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 2.0 mg DEET/kg bodyweight intravenously.

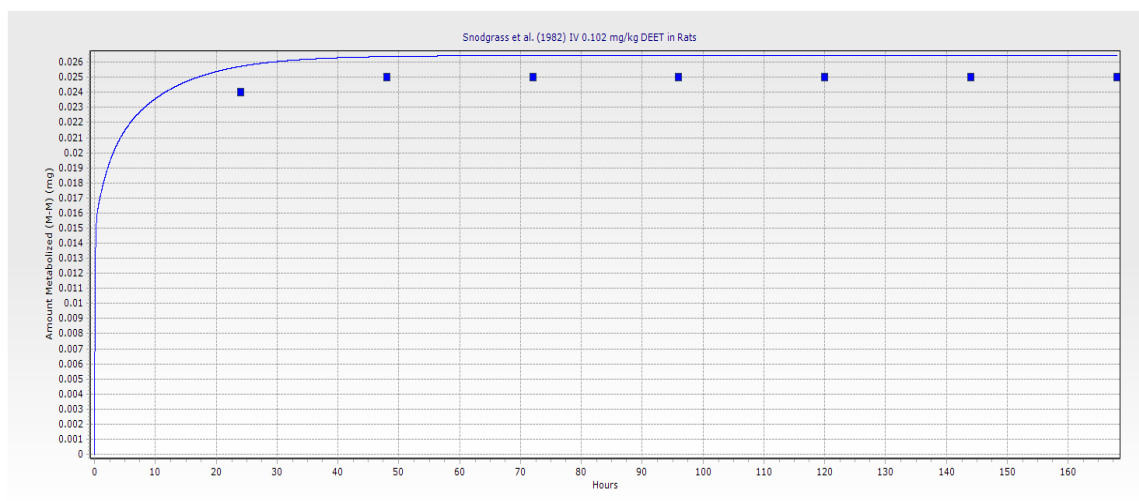


Figure A-2. Simulation of Snodgrass *et al.* (1982) Intravenous DEET Metabolism Data. The amount of Michaelis-Menten metabolite in urine (mg) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 0.102 mg DEET/kg bodyweight intravenously.

PA coefficients (Table A-2) were utilized to slow the removal of DEET from the blood and retain DEET in tissues for slow release later. Liver and rapidly perfused PA coefficients (1.0 and 10.0, respectively) were fit using the liver and kidney 24 hour coefficients from Fediuk *et al.* (2011, Figures A-3 and A-4). Fat and slowly perfused tissue PA coefficients (0.1 for both) were then estimated using the blood time course values in Figure A-1. The brain PA coefficient was initially set equal to the fat and slowly perfused coefficient (1.0).

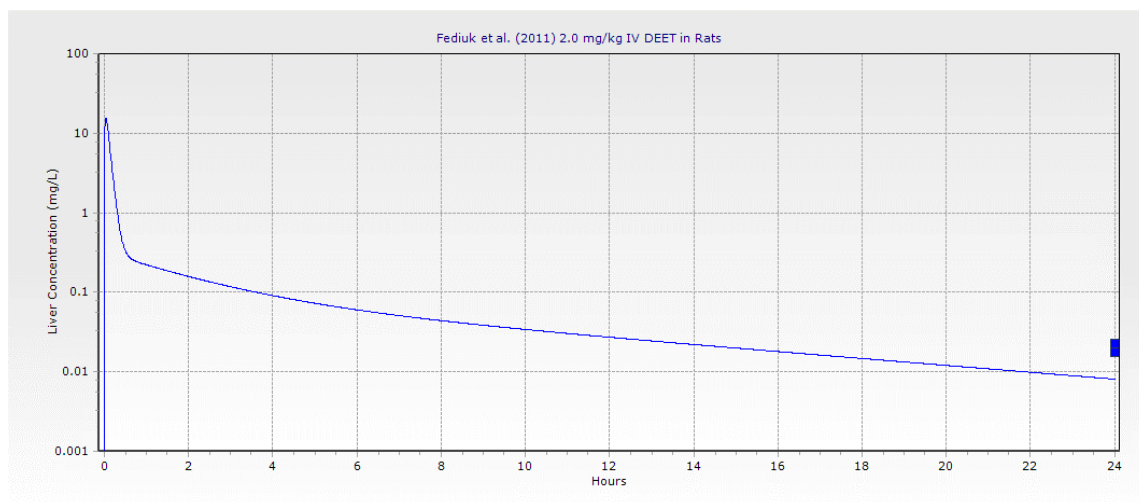


Figure A-3. Simulation of Fediuk *et al.* (2011) Intravenous DEET Liver Data. The liver concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 2.0 mg DEET/kg bodyweight intravenously.

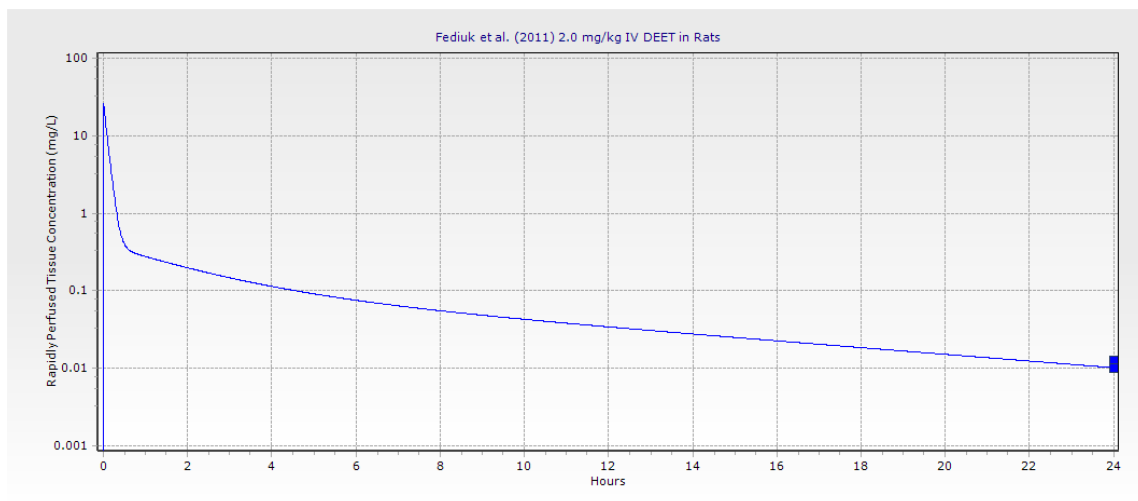


Figure A-4. Simulation of Fediuk *et al.* (2011) Intravenous DEET Kidney Data. The rapidly perfused tissue concentration (mg/L) simulation is shown as a blue line, compared to kidney data from the study (blue squares, representing mean and SD). Male rats were exposed to 2.0 mg DEET/kg bodyweight intravenously.

Oral uptake parameters (Table A-2) were set using the 100 mg DEET/kg bodyweight data of Schoenig *et al.* (1996, Figure A-5). The metabolism data from this study was then predicted using the same parameters as set above (Figure A-6). Metabolism of single and repeated oral doses of DEET at the lower dose (100 mg/kg) are predicted fairly well (blue prediction line versus the blue and magenta data points). Under-prediction of the metabolic efficiency of the 500 mg/kg dose (green prediction line versus green data points) indicates yet another route of metabolism that kicks in at a higher dose. Since this project is concerned with predicting brain tissue concentrations at a lower dose of DEET (40 mg/kg) in the NAMRU-D *in vivo* study, under-predicting metabolism at a dose more than 10 times higher was not of concern for the purpose of the model.

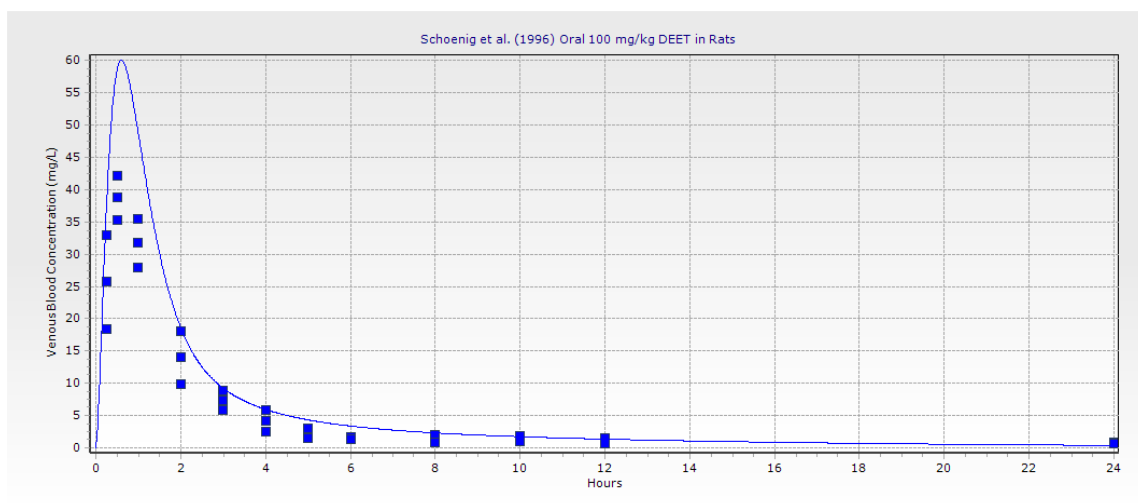


Figure A-5. Simulation of Schoenig *et al.* (1996) Oral DEET Blood Data. The blood concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and undefined error bounds). Male rats were exposed to 100 mg DEET/kg bodyweight orally.

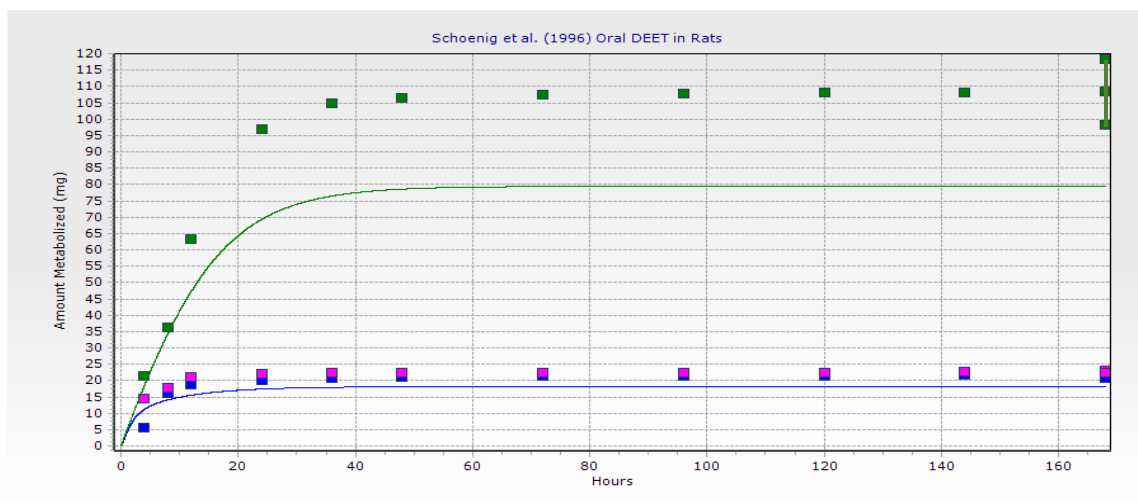


Figure A-6. Simulation of Schoenig *et al.* (1996) Oral DEET Metabolism Data. The amount of Michaelis-Menten metabolite in urine (mg) simulation is shown as a blue or green line, compared to data from the study (blue squares = single dose 100 mg/kg group, magenta squares = repeated dose 100 mg/kg group, green squares = single dose 500 mg/kg group). Multiple squares at 168 hours (7 days) represent the total metabolites measured, including final rinses of metabolism cages, and the associated undefined error bounds.

Next, the skin:blood PC (Table A-1) was fit using the blood and metabolite time course data of Schoenig *et al.* (1996, Figures A-7 and A-8). The same PC was utilized to predict blood levels for the dermal data of Snodgrass *et al.* (1982, Figure A-9) and Fediuk *et al.* (2010, Figure A-10; 2011, Figure A-11), with mixed results. The data of Snodgrass *et al.* (1982) are reasonably well predicted. However, the Fediuk *et al.* data from both studies are either under-predicted (Figure A-10) or seriously over-predicted (Figure A-11). These 2011 data are so over-predicted that a

unit reporting issue is suspected; unit conversions from the article have been checked multiple times. Dermal absorption in the current model structure will not produce such a peak as demonstrated in Figure A-10. Further, these data could not be predicted by a consistent structure or set of parameters as the data of Snodgrass *et al.* (1982) or Schoenig *et al.* (1996). It is possible that study specific differences in method have altered the uptake of DEET. The 2010 study utilized a 70 percent ethanol vehicle, while the 2011 study used a combination of Emulphor, ethanol and water, as described above. Snodgrass utilized a stronger solution of ethanol, which could result in less uptake and more evaporation; however, there are no clear indicators of the reasons for these discrepancies. Therefore, the data shown in Figures A-10 and A-11 were set aside until the model is revisited in the future.

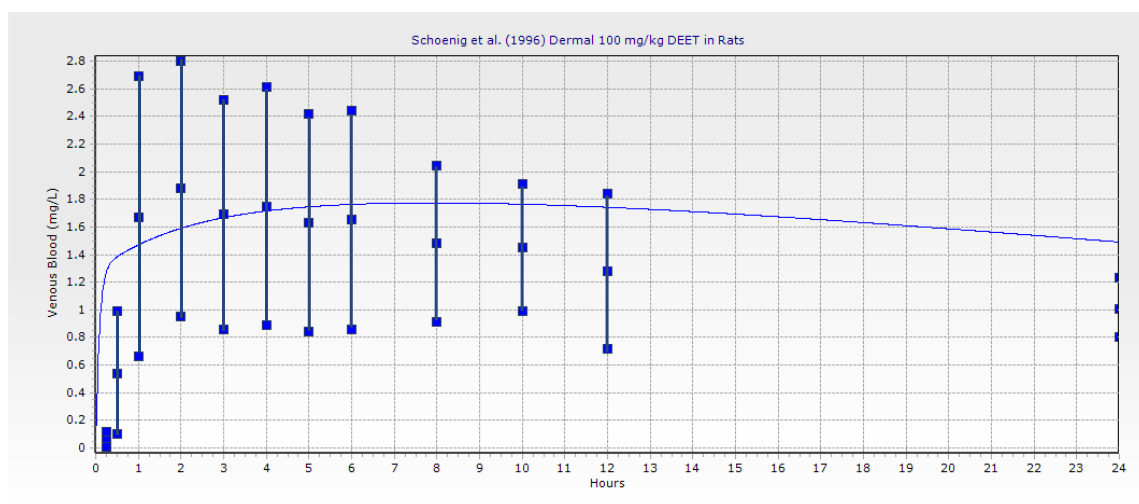


Figure A-7. Simulation of Schoenig *et al.* (1996) Dermal DEET Blood Data. The blood concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and undefined error bounds). Male rats were exposed to 100 mg DEET/kg bodyweight dermally.

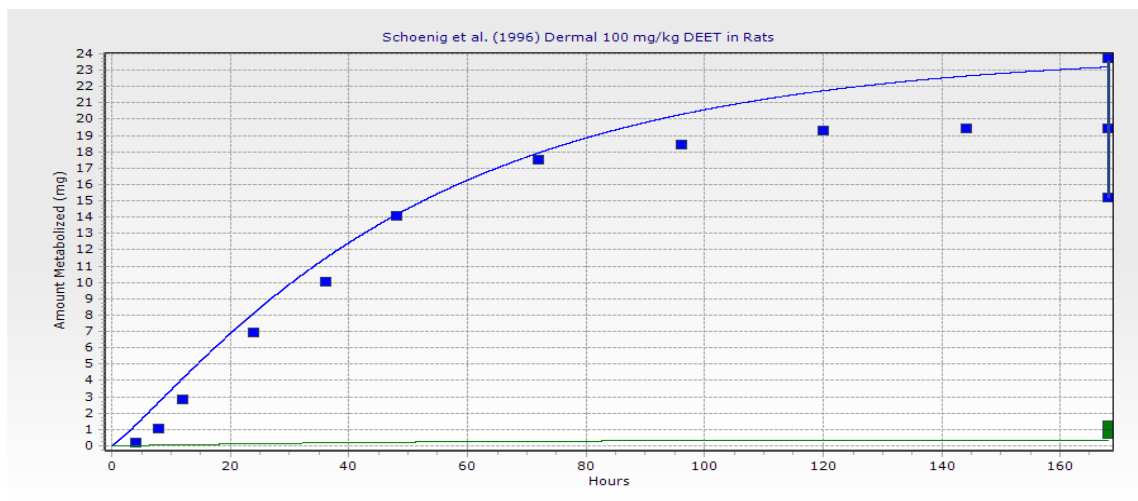


Figure A-8. Simulation of Schoenig *et al.* (1996) Dermal DEET Metabolism Data. The amount of Michaelis-Menten metabolite in urine (mg) simulation is shown as a blue line, compared to data from the study (blue squares). First order metabolite concentration in feces (mg/L) simulation is shown as a green line, compared to data from the study (green squares). Multiple squares at 168 hours (7 days) represent the total metabolites measured, including final rinses of metabolism cages, and the associated undefined error bounds. Male rats were exposed to 100 mg DEET/kg bodyweight dermally.

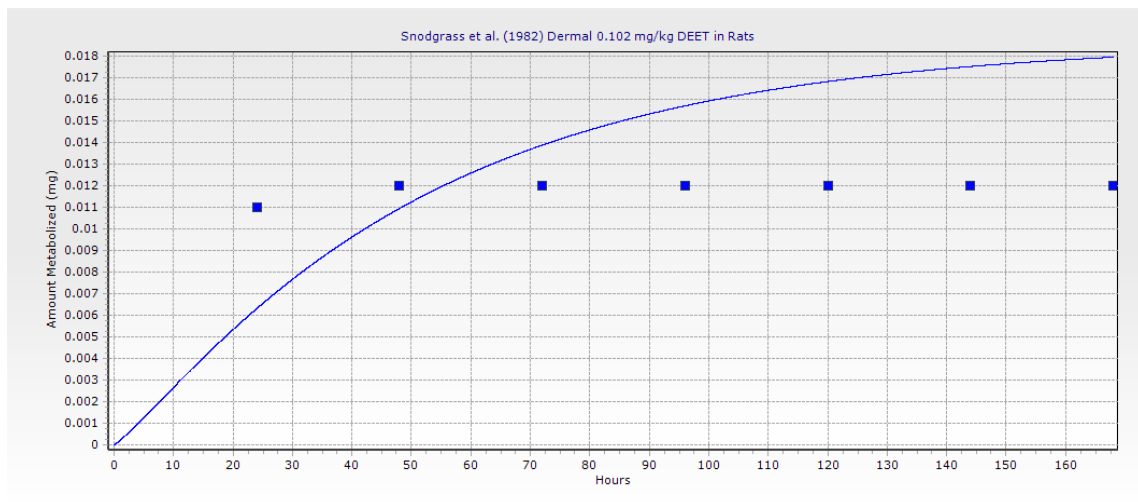


Figure A-9. Prediction of Snodgrass *et al.* (1982) Dermal DEET Metabolism. The amount of Michaelis-Menten metabolite in urine (mg) prediction is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 0.102 mg DEET/kg bodyweight dermally.

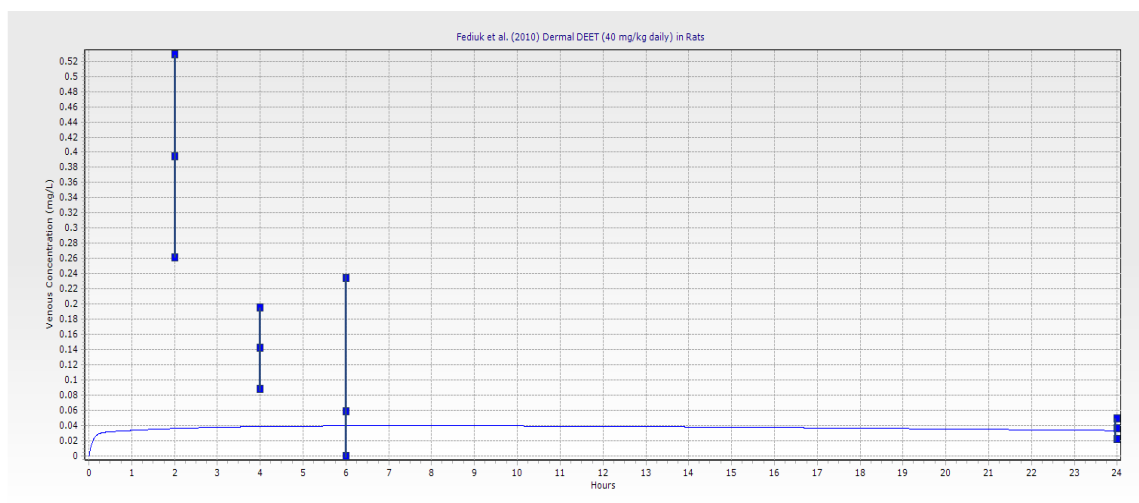


Figure A-10. Prediction of Fediuk *et al.* (2010) Dermal DEET Blood Concentrations. The blood concentration in urine (mg/L) prediction is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 40 mg DEET/kg bodyweight dermally.

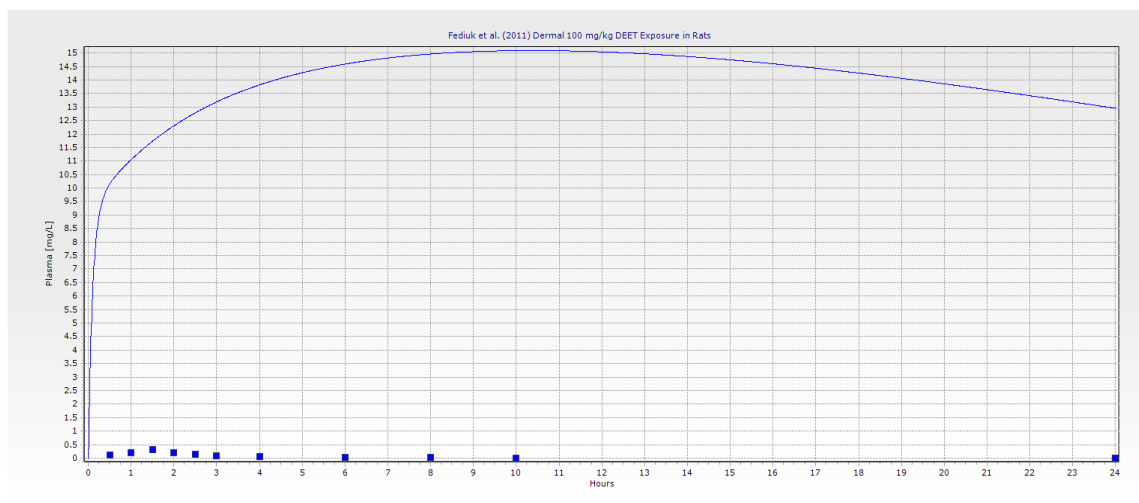


Figure A-11. Prediction of Fediuk *et al.* (2011) Dermal DEET Plasma Concentrations. The plasma concentration in urine (mg/L) prediction is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 100 mg DEET/kg bodyweight dermally.

Fediuk *et al.* (2010) had the only brain concentration data for DEET, plus some liver data. Fediuk *et al.* (2011) had liver and kidney 24-hour values. Simulations were run to determine if the liver, slowly perfused tissues, and brain would be predicted by the model, even if the blood levels were not. The 24-hour liver concentrations predicted fell within the 1 standard deviation range of the 2010 and 2011 data (Figures A-12 and A-13). The predicted rapidly perfused concentrations were only slightly over-estimated as compared to the kidney data from the 2011 study (Figure A-14). Finally, the model brain predictions were well fit to the 2010 24-hour brain data (Figure A-15). While the overall outcome is good, it should be noted that the reason the

model can fit these concentrations is because these data were used to develop the PCs utilized to “predict” them. Still the fits do indicate that the model is working as intended.

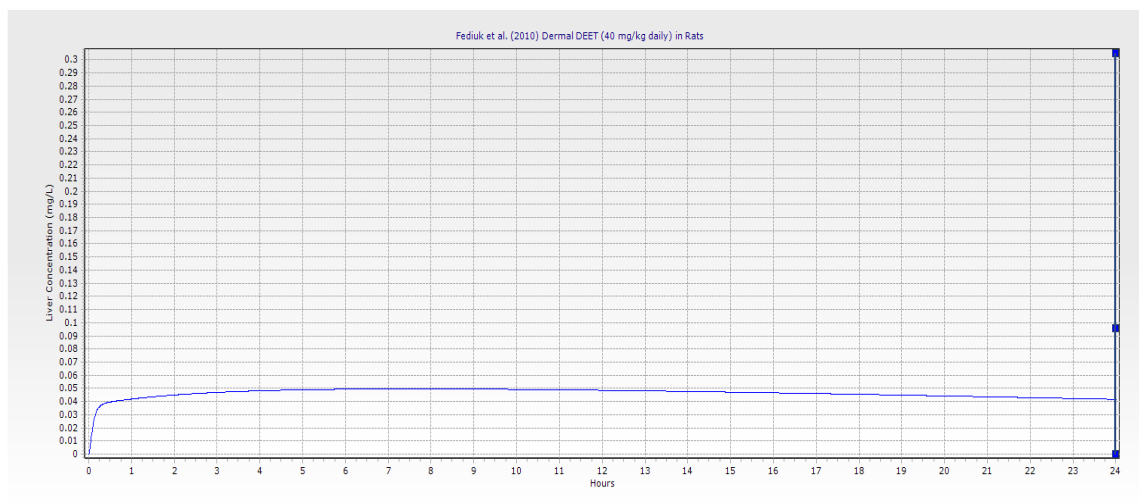


Figure A-12. Prediction of Fediuk *et al.* (2010) Dermal DEET Liver Concentrations. The liver concentration (mg/L) prediction is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 40 mg DEET/kg bodyweight dermally.

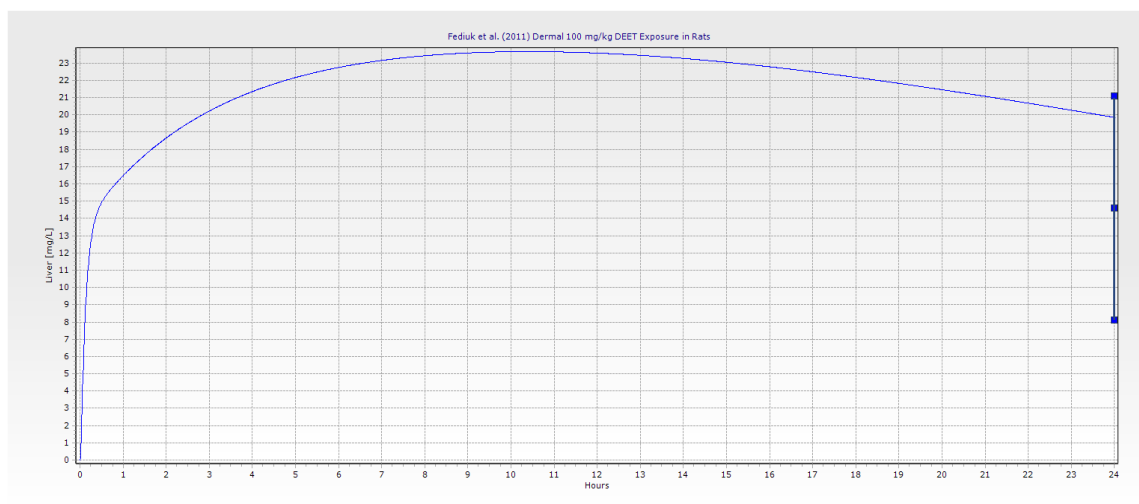


Figure A-13. Prediction of Fediuk *et al.* (2011) Dermal DEET Liver Concentrations. The liver concentration (mg/L) prediction is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 100 mg DEET/kg bodyweight dermally.

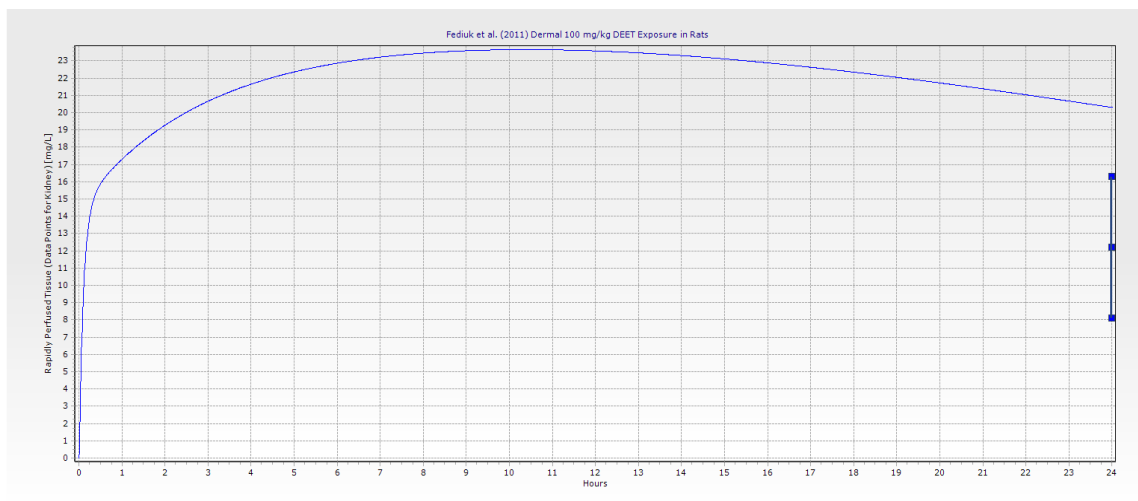


Figure A-14. Prediction of Fediuk *et al.* (2011) Dermal DEET Rapidly Perfused Tissue Concentrations. The rapidly perfused tissue concentration (mg/L) prediction is shown as a blue line, compared to kidney data from the study (blue squares, representing mean and SD). Male rats were exposed to 100 mg DEET/kg bodyweight dermally.

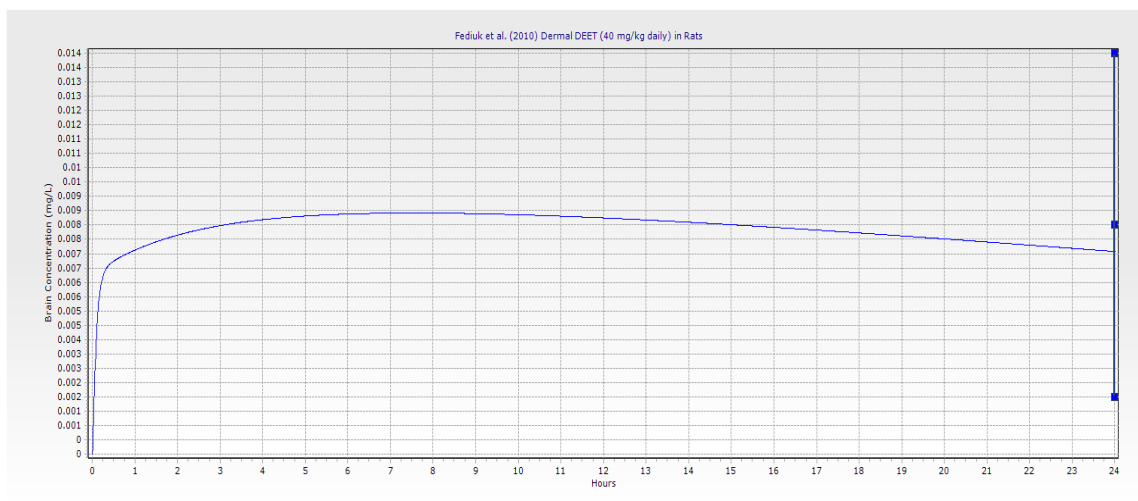


Figure A-15. Prediction of Fediuk *et al.* (2010) Dermal DEET Brain Concentrations. The brain concentration (mg/L) prediction is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 40 mg DEET/kg bodyweight dermally.

APPENDIX B. PYRIDOSTIGMINE BROMIDE SIMULATIONS AND PREDICTIONS

For simplification, all citations within this appendix are contained within the main report reference list.

PRIOR PBPK MODELS

One unpublished PBPK model was found for PB. Watson (2001) authored a thesis detailing a PBPK model for PB and the effects of stress. Her model compartments were a diffusion limited brain combined with flow limited tissues: fat, liver, rapidly perfused tissues and slowly perfused tissues. Partition coefficients for liver and rapidly perfused tissues (8 and 15, respectively) were sourced from Golomb (1999), which in turn were derived from Yamamoto *et al.* (1995). The fat and brain values that fit the model best were the same (0.002). The slowly perfused value utilized was identical to the liver value (8).

KINETIC STUDIES

Rat Studies

Yamamoto *et al.* (1995) administered 0.5, 1 or 2 μmol PB per kg bodyweight (0.13, 0.26 or 0.52 mg/kg) by the IV route to male Wistar rats (250 to 330 g) to track pharmacokinetics of the drug and determine urinary and bile excretion. No information was found on the duration of the injection; 0.5 minutes (0.008 hours) was assumed. Rats were lightly anesthetized with ether and cannulas were inserted in the femoral artery, femoral vein, bile duct and bladder. Rats were allowed 2 hours for recovery following this surgery. Blood was sampled at 6 time points following IV administration; bile and urine were sampled over 6 hours following injection. Urinary and biliary clearance was observed to be 59.5 and 0.23 percent at 6 hours post-dosing, respectively.

Coper *et al.* (1974) dosed female Wistar rats (180 to 220 g) with PB by the oral and intraperitoneal (IP) routes to determine bioavailability and calculate lethal doses for 50 percent of the population (LD_{50}). LD_{50} values were calculated to be 3 mg/kg (IP) and 115 mg/kg (oral). The IP exposure was not tracked in the model; modeling an IP study requires multiple assumptions that don't translate well for route-to-route extrapolation (Steuperaert *et al.*, 2017). The oral dose (50 mg/kg) was administered presumably via gavage and kinetics observed over 4 hours. Rats were fed *ad libitum*.

Tan *et al.* (2012) conducted an oral kinetics study with male Sprague Dawley rats (30 ± 20 g) to compare the oral administration of PB and a pyridostigmine bromide phospholipid complex (PBPLC). Both sets of rats ($n=6$) received the equivalent of 1.5 mg/kg PB. Drug kinetics were tracked for 24 hours. Although not implicitly stated for the kinetics study, these rats were likely fasted overnight, as were others described in this article.

Pyridostigmine Iodide Rat Studies. Due to the limited number of kinetic studies for PB, studies using pyridostigmine iodide (PI) were assessed. PI has a molecular weight of 308.119 g/mol. Barber *et al.* (1975) administered 0.1 or 0.63 μmol PI per kg bodyweight (0.031 or 0.194 mg/kg) intravenously to Wistar rats (250 to 350 g). The rats were assumed to be male. No information was found about the duration of the injection; 0.5 minutes (0.008 hours) was assumed. The entire exposure was performed under ethyl carbamate anesthesia with respiration supported as needed by intubation and a pump. Rats were cannulated in the jugular

vein and portal vein, to examine the first pass metabolic effect over a period of 4 hours. Jugular vein data were used in the model.

Husain *et al.* (1968) exposed male rats (150 to 160 g) of an unidentified strain to 0.5 mg (approximately 3.23 mg/kg) radiolabeled PB orally. Rats were allowed food and water *ad libitum* prior to and during the study. Rats were given 2 doses of warm tap water (5 mL/100 g BW) an hour apart prior to dosing, with the second dosing 30 minutes before administration of PB in 0.5 mL of water. All doses were given via gavage. For one subset of rats (n=5), urine and feces were collected separately and analyzed at intervals for 24 hours post PB dosing. For another subset, blood and liver were collected terminally at 1, 2, 3 and 5 hours following the PB dose (n = 3 rats/time point).

Human Studies

Two human kinetic studies were found for PB. Marino *et al.* (1998) conducted a long term tolerance test and kinetics evaluation in humans. Volunteers (30 men and 30 women) received 30 mg PB every 8 hours for 21 days. The volunteers stayed at an in-patient facility for 25 days total. Each person received doses at 0800, 1600 and 0000 (midnight). Fasting was required overnight, 2 hours following the 0800 dose, 1 hour before and after the 1600 dose, and 1 hour prior to the midnight dose. Volunteers were on average 32 years old (19 to 44 years); men weighed 77 kg (50 to 105 kg) and women weighed 68 kg (43 to 91 kg). Doses (mg/kg) were calculated with average weights for each sex but other physiological parameters were not altered (e.g., fat volume). Model results were compared with blood samples taken after the first dose.

In addition to their rat study, Coper *et al.* (1974) performed a kinetic study using 4 patients. Sex, age and weight of the subjects were not stated; 70 kg was assumed. Each patient was dosed with 120 mg of the drug mestinon (pyridostigmine bromide) orally in tablet form. Plasma levels were monitored for 4 hours.

MODEL AND PARAMETER DEVELOPMENT

PB does not normally cross the BBB. However, the combination of stress and GWI chemicals have been shown to result in BBB disruption (Abdel-Rahman *et al.*, 2002) and subsequent memory deficits (Hattiangady *et al.*, 2014; Parihar *et al.*, 2013). Therefore the model assumes that a fully functioning BBB is not in place. Instead, it was assumed that the penetration of PB into the brain would be governed by the brain:blood PC and diffusion limitations described by PA coefficients.

Rat Model

PB has an arbitrary high blood:air PC set in the model (200000, Table B-1) due to not having a vapor pressure value at 25 °C (PubChem, 2019a). PB cannot, therefore, be exhaled as a route of elimination. Inhalation as a route of exposure was left in the model for potential future use for DEET or other volatiles associated with GWI. Upper respiratory tract uptake was set at an arbitrary low value (0.001) to signify no uptake by this route for PB.

Table B-1. Rat Physicochemical Specific Tissue:Blood Partition Coefficients and Cell Membrane Coefficients

Parameter (Constants)	PB Fasted Rat	PB Fed Rat	PB Human
Blood:Air Partition Coefficients (PC, unitless)^a			
Blood:Air PC	200000.0	*	*
Tissue:Blood Partition Coefficients (PC, unitless)			
Brain (PBrn)	0.19*15.0 ^b	*	*
Fat (PFat)	0.21 ^c	*	*
Liver (PLiv)	8.0 ^d	*	*
Rapidly Perfused (PRap)	15.0 ^d	*	*
Slowly Perfused (PSlw)	0.5 ^d	*	*
Cell Membrane Permeability Coefficients (PA, L/(hour*kg^{0.75}))			
Brain (PABrnC)	0.1	10000	5.0
Fat (PAFatC)	0.1	10000	5.0
Liver (PALivC)	0.1	10000	5.0
Rapidly Perfused (PARapC)	0.1	10000	5.0
Slowly Perfused (PASlwC)	5.0	10000	5.0

Notes: All values fit to data unless otherwise noted; *Value is the same as for fasted rat; ^aVery large value as PB is not volatile (no vapor pressure at standard temperature); ^b[Ratio of predicted tissue PC:predicted kidney PC as calculated using Ruark *et al.* (2014)] * PRap; ^cWatson (2001); ^dYamamoto *et al.* (1995)

Multiple sets of tissue:blood PCs were considered in the model: predictions from Ruark *et al.* (2014), PCs used in the 2001 model by Watson, and apparent PCs from Yamamoto *et al.* (1995). The best fit across the studies resulted from a combination of these: the fat:blood PC from the Watson (2001) model and apparent PCs from Yamamoto *et al.* (1995) for liver, rapidly perfused and slowly perfused tissues (Table B-1). Since no measured or apparent values were found for brain tissue, this gap was filled using the estimated PC (Ruark *et al.*, 2014) for brain divided by the estimated PC for kidney. This ratio was then multiplied by the apparent value for rapidly perfused tissues.

Intravenous studies were simulated first, followed by the oral studies. The IV plasma data from Yamamoto were considered to be more relevant, as the only other IV study (Barber *et al.*, 1979) used anesthesia throughout the time course. Therefore, model fitting was started with the VMaxC and Km values from the Watson (2001) model. As model parameterization progressed, the Watson Km was retained and the VMaxC was fit iteratively (Table B-2). A urinary clearance value (0.25 L/(hour*kg^{0.75})) was initially fit to the Yamamoto plasma and urinary excretion data (Figures B-1 and B-2). This clearance did not fit oral studies and may have been suppressed as a lingering effect from anesthetic (Burchardi and Kaczmarczyk, 1994). Therefore, the Yamamoto model simulations were coded to retain the fit urinary clearance value for these specific data; all other physicochemical parameters were as stated in Tables B-1 and B-2.

Table B-2. Rat Metabolic, Uptake and Clearance Parameters^a

Parameter	Units	PB Fasted Rat	PB Fed Rat	PB Human
Michaelis-Menten Maximum Velocity (V _{MaxC})	mg/(hour*kg ^{0.75})	2.0	*	*
Affinity Constant (K _m)	mg/L	1.5 ^b	*	*
First Order Metabolism Constant (K _{FC})	/hour	0.0	*	*
Upper Respiratory Tract Uptake (K _{URTC})	L/hour	0.001	*	*
Urinary Clearance (C _{lUrC})	L/(hour*kg ^{0.75})	1.365	*	*
Absorption from Stomach (K _{AS})	/hour	0.1	*	*
Transfer from Stomach to Duodenum (K _{TSD})	/hour	1.5	0.75	0.5
Absorption from Duodenum (K _{AD})	/hour	0.3	0.03	1.0
Fecal Excretion (K _{Fec})	/hour	0.00038	*	*

Notes: *Value is the same as for fasted rat; ^aFit to data unless noted otherwise; ^bWatson (2001)

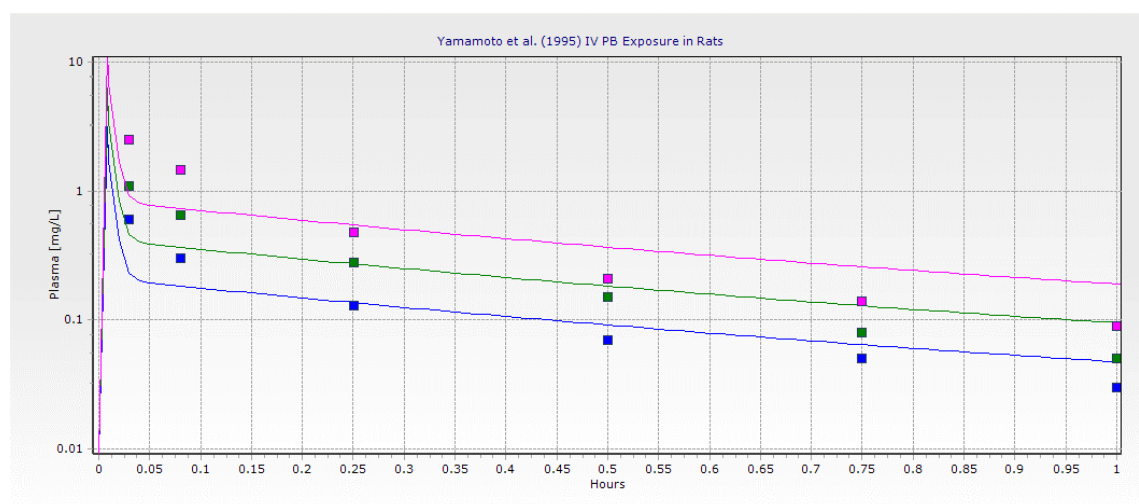


Figure B-1. Simulation of Yamamoto *et al.* (1995) Intravenous PB Plasma Data. The plasma concentration (mg/L) simulation is shown as a blue, green or magenta line, compared to data from the study (corresponding colored squares). Male rats were exposed to 0.5, 1 or 2 μ mol PB per kg bodyweight (0.13, 0.26 or 0.352 mg/kg) intravenously.

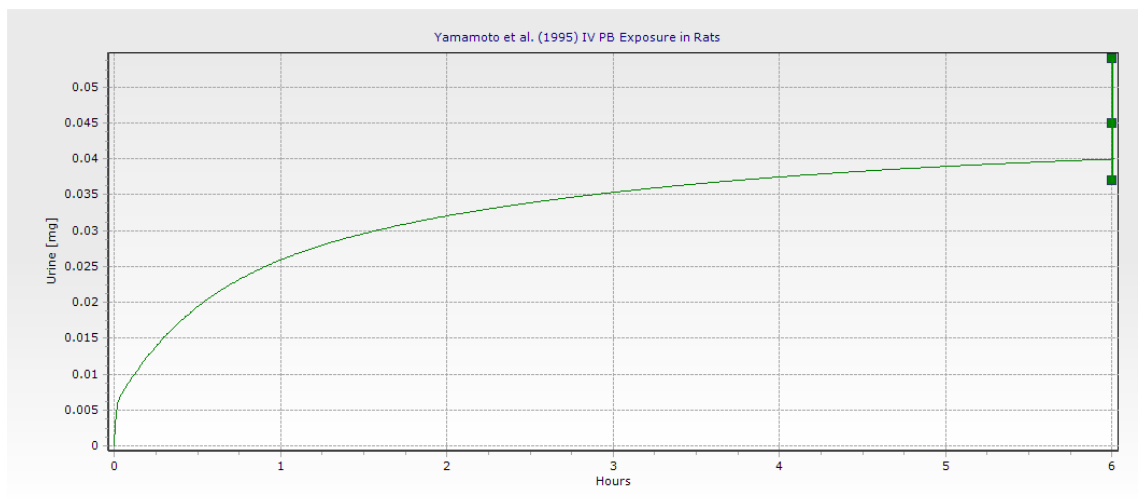


Figure B-2. Simulation of Yamamoto *et al.* (1995) Intravenous PB Urinary Excretion Data. The amount of PB in urine (mg) simulation is shown as a green line, compared to data from the study (green squares, representing mean and SEM). Male rats were exposed to 1 μmol PB per kg bodyweight (0.26 mg/kg) intravenously.

The Barber *et al.* (1975) data were completely different from the Yamamoto data. Barber and coworkers utilized pyridostigmine iodide in their study. While salts can have different kinetics primarily due to specific dissociation rates (Wiedmann and Naqwi, 2016), that shouldn't have any effect on an intravenous study in which the salts are purchased as a liquid. The dataset kinetic differences are more likely an effect of the rats being anesthetized throughout the Barber study; anesthesia is known to affect several aspects of kinetics (Burchardi and Kaczmarczyk, 1994). The data required not only an altered urinary clearance similar to that of the Yamamoto study ($0.3 \text{ L}/(\text{hour} \cdot \text{kg}^{0.75})$), but also a greatly decreased V_{MaxC} ($0.05 \text{ mg}/(\text{hour} \cdot \text{kg}^{0.75})$) and K_m (0.15 mg/L), as compared to the values reported in Table B-2. Due to these differences, the Barber data were not utilized for physicochemical parameter fitting of the compound. The simulations shown in Figures B-3 and B-4 were accomplished with the study specific parameters stated above.

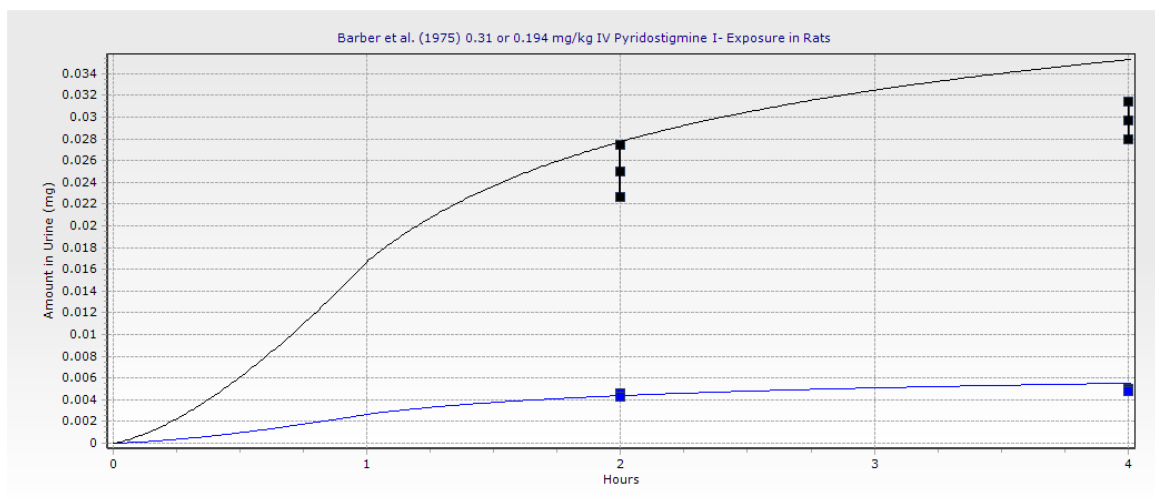


Figure B-3. Simulation of Barber *et al.* (1975) Intravenous PI Urinary Excretion Data. The amount of PI in urine (mg) simulations are shown as blue and black lines, compared to data from the study (corresponding colored squares, representing mean and SEM). Male rats were exposed to 0.1 or 0.63 μmol PI per kg bodyweight (0.031 or 0.194 mg/kg) intravenously.

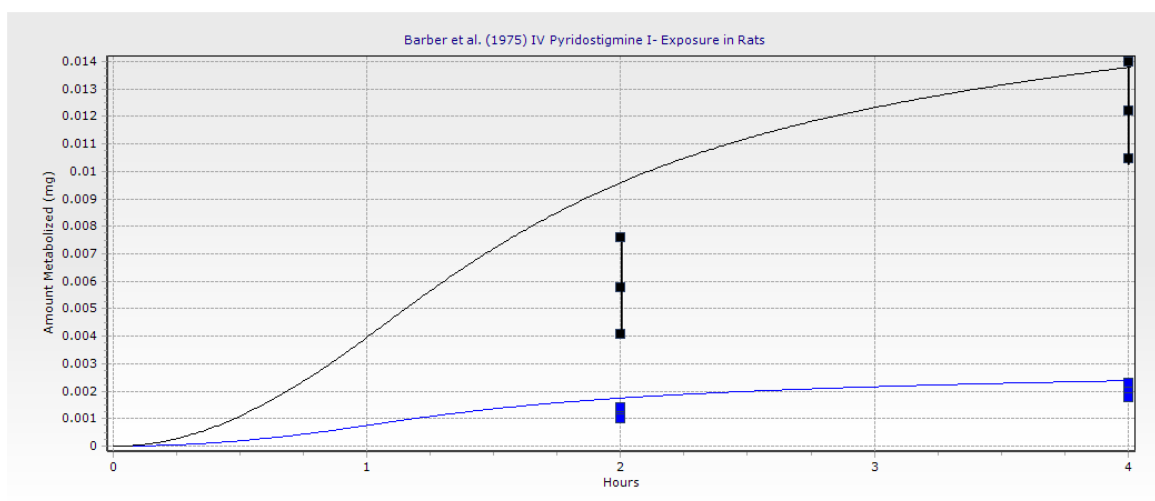


Figure B-4. Simulation of Barber *et al.* (1975) Intravenous PI Metabolite Data. The amount metabolized (mg) simulations are shown as blue and black lines, compared to data from the study (corresponding colored squares, representing mean and SEM). Male rats were exposed to 0.1 or 0.63 μmol PI per kg bodyweight (0.031 or 0.194 mg/kg) intravenously.

Following initial fitting of the IV studies, data from Husain *et al.* (1968) were considered due to their completeness, having blood, urine and liver kinetic time courses. The urinary clearance values from the IV studies were not appropriate for these oral data. Instead, a human value published by Golomb (1999) was used ($1.365 \text{ L}/(\text{hour} \cdot \text{kg}^{0.75})$), to good effect (Figures B-5 and B-6); this became the default value (Table B-2). Iterative fitting between the Husain and Yamamoto data sets yielded a consistent set of physicochemical parameters as shown in Tables B-1 and B-2; only the urinary clearance values were specially fit for Yamamoto *et al.* (1995), as discussed previously.

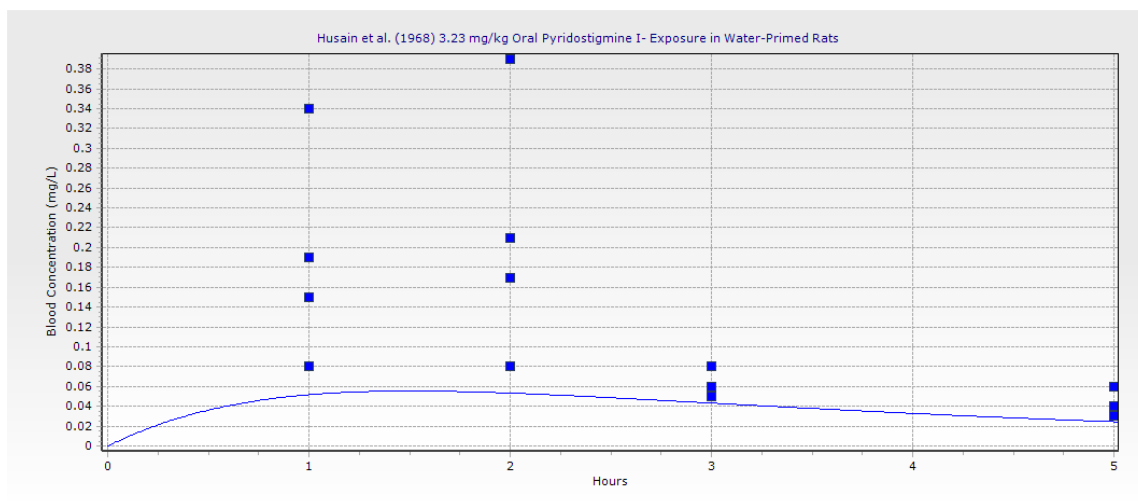


Figure B-5. Simulation of Husain *et al.* (1968) Oral PB Blood Data. The blood concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing individual concentrations from a total of 12 rats). Male rats were exposed to 0.5 mg radiolabeled PB (~3.23 mg/kg bodyweight) by oral gavage.

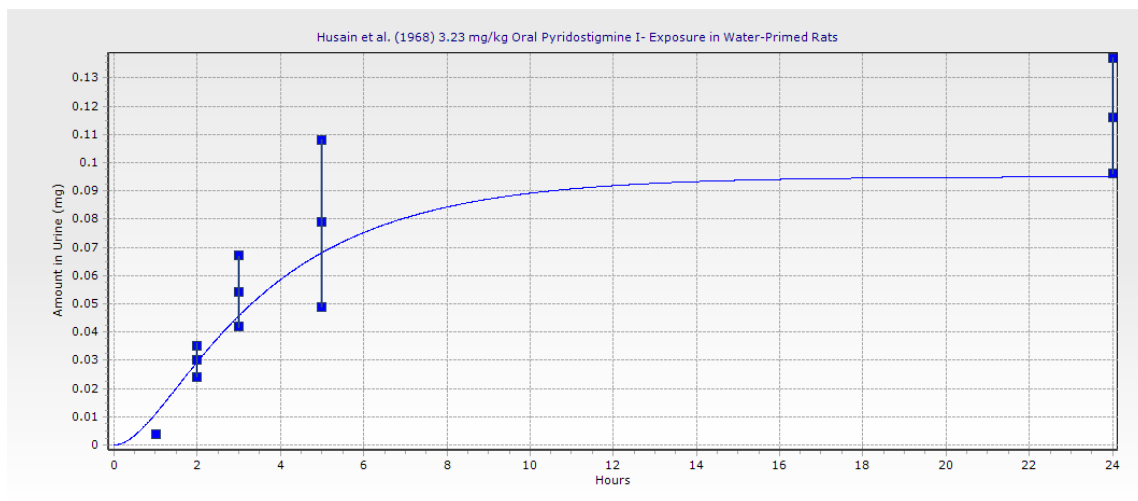


Figure B-6. Simulation of Husain *et al.* (1968) Oral PB Urine Data. The amount in urine (mg) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 0.5 mg radiolabeled PB (~3.23 mg/kg bodyweight) by oral gavage.

PB excretion (Figure B-6) was characterized well by the model and the iteratively fit parameters. The blood concentration simulation was not as successful (Figure B-5) but is still reasonable, if a bit under-predicted. The purpose for “priming” the rats with doses of water prior to the PB dose was not explained. The study also states that food was available *ad libitum* until the morning of the experiment; it is implied that food was then withheld for at least 1.5 hours prior to the PB dose. The fasting and hydration state of the GI system greatly affects absorption of drugs (McLean, 2016); the oral uptake values fit to these data became the “fasted” parameters

in Table B-2. The Husain liver data were used to set the PA coefficients across tissues (Figure B-7). Although only moderately successful, the peak concentration was well matched.

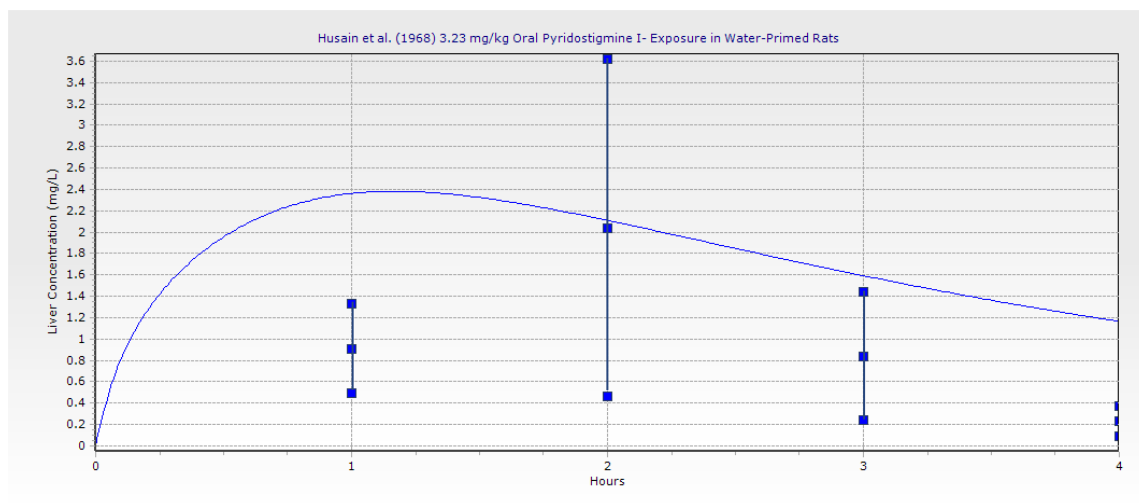


Figure B-7. Simulation of Husain *et al.* (1968) Oral PB Liver Data. The liver concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 0.5 mg radiolabeled PB (~3.23 mg/kg bodyweight) by oral gavage.

Following the Husain data, a rat oral study by Coper *et al.* (1974) was the next to model. This data set didn't fit with the PA parameters set using the Husain study. Specific oral uptake parameters had to be altered. Transfer of the compound from the stomach to the duodenum was cut in half (0.75 /hour) and the uptake of the PB from the duodenum was decreased by a factor of 10 (0.03 /hour). As the dose was significantly slowed getting to the blood stream, all PA coefficients were raised to 10000 (Figure B-8); this high value negates (turns off) the effect of diffusion limitation across all tissues. The rats in the Coper study were fed *ad libitum* and not administered water bolus doses. These oral uptake values are listed as the "fed" values in Table B-2.

For the Coper study, it is likely that feeding the rats alone was sufficient to slow the absorption of PB (McLean, 2016) and renders the diffusion limitation inconsequential, even though the physiological reason behind the need for PA coefficients still exists. Alternatively, the need for PA coefficients when administering PB intravenously or orally to fasted rats could be due to plasma binding of PB, which slows diffusion to other tissues. When fed, the absorption of PB is slowed and could essentially match the on-off rate of plasma-binding. Plasma binding was omitted from the current model due to time constraints. Inclusion of plasma binding would be an improvement for future PB modeling endeavors.

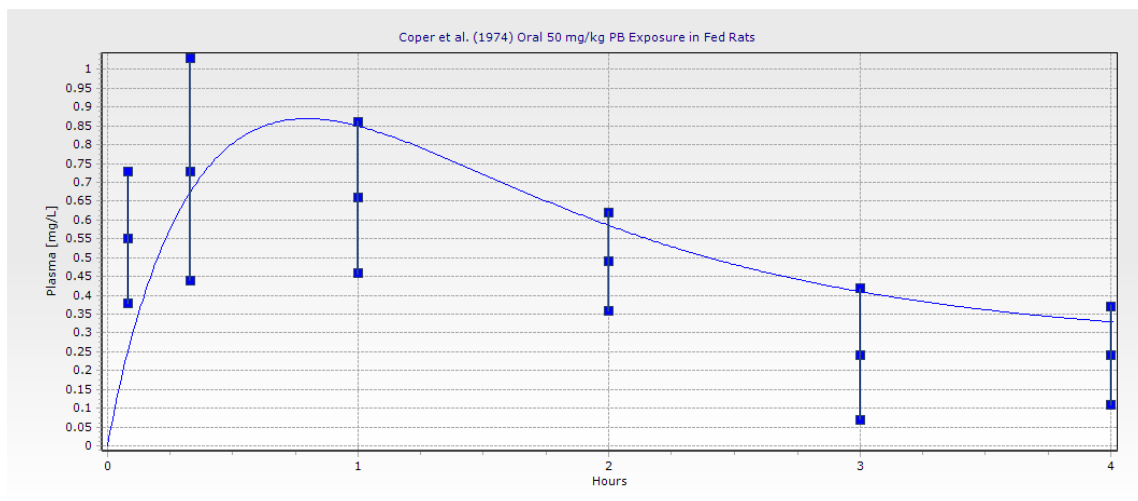


Figure B-8. Simulation of Coper *et al.* (1974) Oral PB Plasma Data. The plasma concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 50 mg PB/kg bodyweight) by oral gavage.

Finally, a plasma time course using oral study conditions by Tan *et al.* (2012) was predicted. The observed plasma data were much higher than the model predicted (Figure B-9). It is assumed that the kinetic study rats were fasted overnight prior to dosing, given that fasting was part of the treatment in another rat study covered by the same article. It seems unlikely that fasting explains the really high blood plasma levels seen in this study. Units of the published data were cross-checked several times; however a possible unit error in the publication would explain the difference between the predicted values and the observed data.

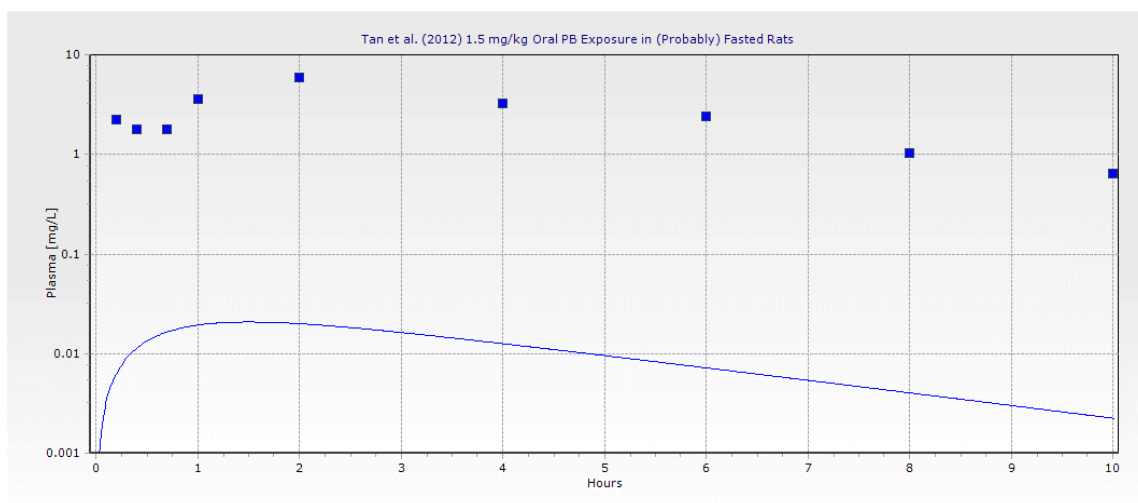


Figure B-9. Prediction of Tan *et al.* (2012) Oral PB Plasma Data. The plasma concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares, representing mean and SD). Male rats were exposed to 1.5 mg PB/kg bodyweight) by oral gavage.

The purpose of this model is to predict brain tissue levels of PB following oral exposure to GWI compounds and subsequent disruption of the BBB in the NAMRU-D *in vivo* study. Following the fitting of the few PB data sets and even fewer PI studies in rats, it was determined that the rat model was sufficient for the purpose of predicting at least some aspects of PB kinetics in rats. The variability between studies, especially in oral dosing practices, made fitting all the data impossible. Unfortunately, none of the PB or PI kinetic studies reported brain concentrations; predictions of brain levels cannot be confirmed in even a limited way.

Human Model

Two human data sets were gathered given the limited quantity data in rats for PB and PI. The rat model with human physiological parameters was utilized initially to simulate the data from Marino *et al.* (1998). The curve shape from this study could not be replicated with the oral uptake and PA coefficients from rats. The rates for transfer from the stomach to the duodenum and the uptake from the duodenum were fit to these human data (Table B-2).

All tissue PA coefficients were raised to 5.0 L/(hour*kg^{0.75}) (Table B-2). Diffusion limitation of molecules can be due to plasma binding (Kuepfer *et al.*, 2016); using PA coefficients in this unified model structure allowed for accommodation of this source of diffusion limitation without the addition of plasma binding, especially when verification through binding assays were not readily available. Drug binding in human blood and rat blood differs considerably (Colclough *et al.*, 2014); altered PA coefficients would be considered a normal physicochemical difference between species. Further investigation of plasma binding may be in order for future PB models.

With human oral uptake and uniformly raised PA coefficients, the model fits the Marino *et al.* (1998) data set well (Figure B-10). The data given are individual blood concentrations for the 30 male and 30 female volunteers; the data were digitized from the published graph and may not completely reproduce all overlapping data points. The model was used to simulate the blood concentrations for a male and a female at the average study body weight for each sex. The parameters were then used to predict plasma concentration levels from the 4 patients in the

Coper *et al.* (1974) study (Figure B-11). The model does a reasonable job of predicting blood levels for 4 patients for whom no body weight data were given.

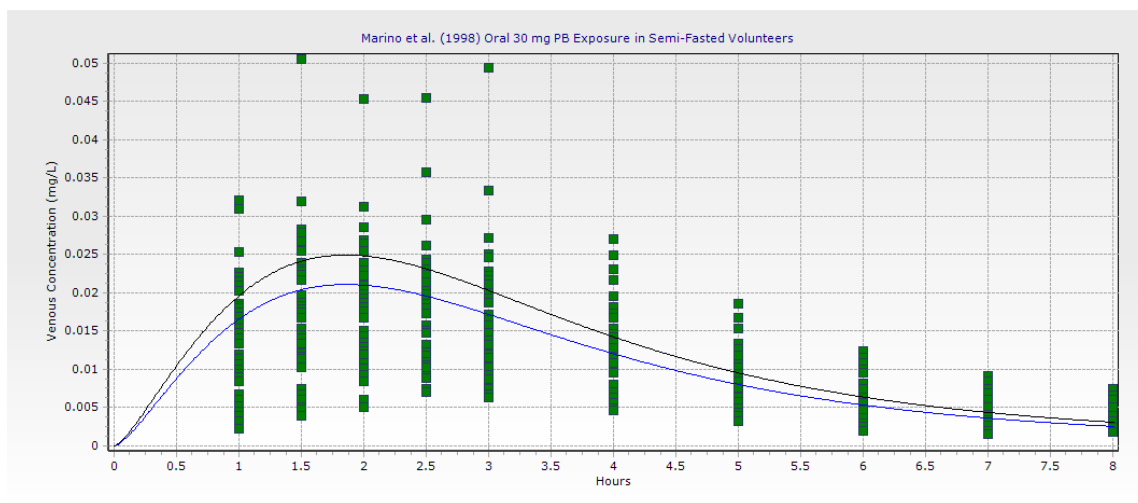


Figure B-10. Simulation of Marino *et al.* (1998) Oral PB Blood Data. The blood concentration (mg/L) simulation is shown as a blue (male) or black (female) line, compared to data from the study (green squares, representing individual volunteer concentrations). Volunteers (30 male and 30 female) were given 30 mg PB orally.

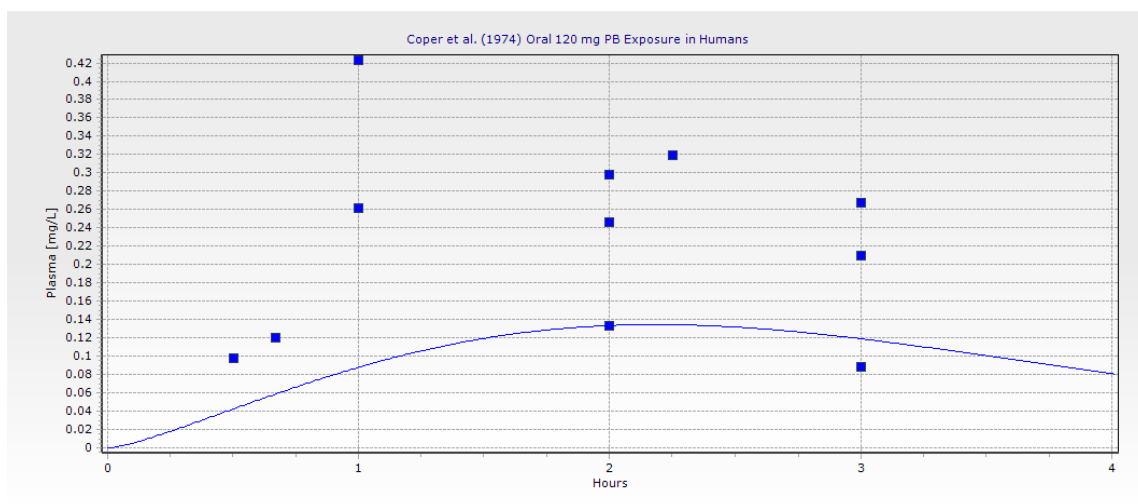


Figure B-11. Prediction of Coper *et al.* (1974) Oral PB Plasma Data. The plasma concentration (mg/L) prediction is shown as a blue line, compared to data from the study (blue squares, representing individual volunteer concentrations). Four patients were given 120 mg PB orally.

The basic model ability to simulate and predict human blood/plasma data indicate that the PBPK model structure is serviceable. The human data fitting and predictions help give confidence that the model is useful for the purpose of this project.

APPENDIX C. cis-PERMETHRIN SIMULATIONS AND PREDICTIONS

For simplification, all citations within this appendix are contained within the main report reference list.

PRIOR PBPK MODELS

Two published rat PBPK models were found for permethrin. As the purpose of this project is to predict rat brain concentrations from GWI exposures, and given the quality and quantity of models plus kinetic studies located in the literature, additional species models, including human, were not investigated.

Tornero-Velez *et al.* (2012) published an oral PBPK model for cis- and trans-PER in rats and humans based on a published deltamethrin model (Mirfazaelian *et al.*, 2006). The model contained brain, fat, liver, rapidly perfused and slowly perfused tissues. Brain, fat and slowly perfused tissues were diffusion limited. Partition coefficients for fat, liver, rapidly perfused and slowly perfused tissues originated in the deltamethrin model. The brain PC for cis-PER was fit to the data.

Willemin *et al.* (2016) published another oral PBPK model for cis- and trans-PER largely based on the Tornero-Velez model and a human model published by Wei *et al.* (2013). The model was designed for the purpose of predicting testicular concentrations and metabolite production. Tissues included brain, fat, kidney, gastrointestinal tract, liver, muscle, testes, rapidly perfused, slowly perfused and non-perfused. Brain, fat, muscle, testes and slowly perfused tissues were diffusion limited. Prior PC and PA distributions were based on the models by Tornero-Velez *et al.* (2012) and Mirfazaelian *et al.* (2006); a Bayesian framework and Markov Chain Monte Carlo (MCMC) analysis was used to calibrate these and other parameters.

KINETIC STUDIES

Tornero-Velez *et al.* (2012) exposed 70-day old male Long-Evans rats (0.295 kg) orally to 1 or 10 mg PER/kg bodyweight in 1 mL/kg corn oil. The permethrin was a 40:60 cis:trans-PER mixture. Rats were euthanized at 1, 2, 3, 4, 6, 8, 12, 24, 36, or 48 hours (n = 4/time point). Blood, brain, fat, and liver samples were collected. Blood and tissue analyses separated cis- and trans-PER, enabling the modeling of the isomers separately. Rats were fasted in this study.

The Willemin *et al.* (2016) oral study used male Sprague Dawley rats (468 g ± 21 g) and dosed cis- and trans-PER separately. Rats were dosed with 25 mg cis-PER/kg bodyweight in 2 mL/kg corn oil. Animals were not fasted in this study; however, food was withheld for 3 hours post-dosing for maximal absorption. Rats were euthanized at 0.5, 1, 1.5, 2, 4, 6, 10, 24, or 48 hours (n = 4/time point). Blood, brain, fat, liver, testes, muscle, and kidney samples were collected.

Willemin *et al.* (2016) referenced an analytical method/preliminary study paper done in their laboratory prior to their PBPK model publication. This paper, Lemestrau *et al.* (2014), contained limited kinetic data. Male SD rats of undeclared body weights were exposed to 5 or 20 mg cis-PER/kg bodyweight dissolved in 1 mL/kg corn oil. As later done in the Willemin study, rats were not fasted prior to dosing but food was withheld for 3 hours post-dosing. For modeling purposes, body weights were assumed to be equivalent to the Willemin study rats (468 g). Rats were euthanized at 2, 6, or 24 hours (n = 2/time point). Blood, liver, brain, muscle, kidneys and fat tissues were collected. Many samples were below detection limits. Parent and metabolite compounds were analyzed in feces and urine; the authors found an average of 38 percent of the cis-PER dose eliminated in the feces. cis-PER itself is not eliminated in urine.

MODEL AND PARAMETER DEVELOPMENT

Blood:air PCs were calculated using the algorithm of Buist *et al.* (2012). The very high predicted value (103000.0) indicates that PER will not be exhaled as a route of elimination. Inhalation as a route of exposure was left in the model for potential future use for DEET or other volatiles associated with GWI. Upper respiratory tract uptake was set at an arbitrary low value (0.001) to signify no uptake by this route for PER (Table C-1).

Table C-1. Rat Physicochemical Specific Tissue:Blood Partition Coefficients and Cell Membrane Permeability Coefficients

Parameter(Constants)	PERc	PERT
Blood:Air Partition Coefficients (PC, unitless)^a		
Blood:Air PC	103000.0	103000.0
Tissue:Blood Partition Coefficients (PC, unitless)		
Brain (PBrn)	1.6 ^b	0.57 ^b
Fat (PFat)	225.0 ^b	76.0 ^b
Liver (PLiv)	0.89 ^b	0.21 ^b
Rapidly Perfused (PRap)	1.1 ^b	0.21 ^b
Skin (PSkn)	8.2 ^c	8.2 ^c
Slowly Perfused (PSlw)	1.2 ^b	0.82 ^b
Cell Membrane Permeability Coefficients (PA, L/(hour*kg^{0.75}))		
Brain (PABrnC)	0.0005	0.0005
Fat (PAFatC)	0.048 ^b	0.11 ^b
Liver (PALivC)	1000.0	1000.0
Rapidly Perfused (PARapC)	1000.0	1000.0
Slowly Perfused (PASlwC)	0.31 ^b	0.48 ^b

Notes: All values fit to data unless otherwise noted; ^aPredicted using Buist *et al.* (2012) algorithm; ^bWillemin *et al.* (2016); ^cPredicted using Ruark *et al.* (2014)

Multiple sets of tissue:blood PCs were considered in the model: predictions from Ruark *et al.* (2014), PCs used in the Tornero-Velez *et al.* (2012) model, and the refined PCs developed following MCMC analysis in the Willemin *et al.* (2016) study. The Willemin *et al.* PCs were selected; however, their model did not include dermal exposure and did not have a skin PC. Therefore the skin PC for the model herein was predicted using the algorithms of Ruark *et al.* (2014). The skin permeability constant for both cis- and trans-PER was predicted using the modified Robinson equation as published in Wilschut *et al.* (1995); the equation is not equipped to differentiate between isomers.

The cis-PER PA coefficients developed in the Willemin *et al.* (2016) study using MCMC analysis were utilized for the PBPK model. The exception to this was the PA coefficient for the brain was set to ½ the Willemin coefficient as iterations of the model made this need apparent. Willemin did not find a need for diffusion limitation in the liver or rapidly perfused tissues; the PA coefficients for these compartments were set to 10000 to nullify the effect.

Willemin published an intrinsic clearance rate of 2.51 for all routes of metabolism of cis-PER. As this rate is equivalent to VMaxC/Km, the Km was set to 1 mg/L and the VMaxC to 2.51 mg/(hour*kg^{0.75}) in the model herein (Table C-2).

Table C-2. Rat Metabolic, Uptake and Clearance Parameters^a

Parameter	Units	PERc Fasted	PERc Fed	PERT Fasted	PERT Fed
Michaelis-Menten Maximum Velocity (V _{MaxC})	mg/(hour*kg ^{0.75})	2.51 ^b	*	6.3 ^b	*
Affinity Constant (K _m)	mg/L	1.0	*	1.0	*
First Order Metabolism Constant (K _{FC})	/hour	0.0	*	0.0	*
Upper Respiratory Tract Uptake (K _{URTC})	L/hour	0.001	*	0.001	*
Urinary Clearance (C _{lURC})	L/(hour*kg ^{0.75})	0.0 ^c	*	0.0 ^c	*
Absorption from Stomach (K _{AS})	/hour	0.01 ^c	*	0.01 ^c	*
Transfer from Stomach to Duodenum (K _{TSD})	/hour	0.7 ^c	0.35 ^b	0.7 ^c	0.2 ^b
Absorption from Duodenum (K _{AD})	/hour	0.9 ^c	0.52 ^b	0.9 ^c	1.3 ^b
Fecal Excretion (K _{Fec})	/hour	0.39 ^b	*	0.85 ^b	*
Skin Permeability Constant (K _P)	cm/hour	0.07 ^d	*	0.07 ^d	*

Notes: *Value is the same as for Fasted of the same isomer; ^aFit to data unless noted otherwise; ^bWillemin *et al.* (2016); ^cTornero-Velez *et al.* (2012); ^dPredicted using the modified Robinson algorithm as described in Wilschut *et al.* (1995).

Only oral kinetic studies were available in which cis-PER was analysed separately from trans-PER. Tornero-Velez *et al.* (2012) fasted the rats prior to dosing, whereas Willemin *et al.* (2016) did not. Therefore, different oral uptake values were required for each food state. The model simulations for the Tornero-Velez data used the transfer from the stomach to the duodenum and the absorption from the duodenum values published for that study (“fasted” column, Table C-2). These study specific parameters and iterative fittings were used to determine the cis-PER PA coefficient for the brain. Good fits were obtained for the data from this study (Figures C-1 through C-4).

Venous blood, brain and liver simulations suggest binding of cis-PER to plasma proteins, as the low dose simulations taper off too quickly compared to the data (Figures C-1 to C-3). Plasma binding was not included in the original models (Tornero-Velez *et al.*, 2012; Willemin *et al.*, 2016) and was omitted from the current model due to time constraints. Inclusion of plasma binding would be an improvement for the future PER modeling endeavors.

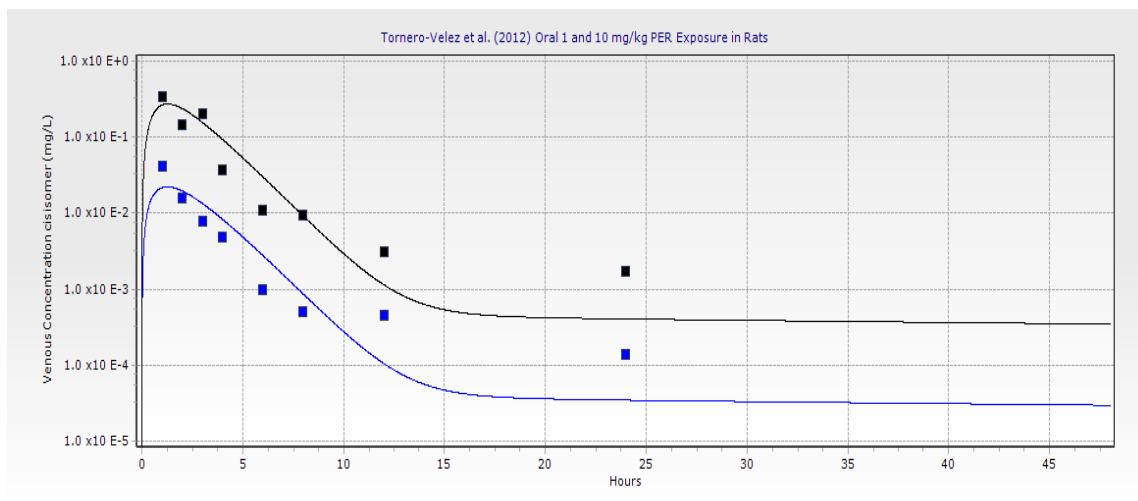


Figure C-1. Simulation of Tornero-Velez *et al.* (2012) Oral cis-PER Blood Data. The blood concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

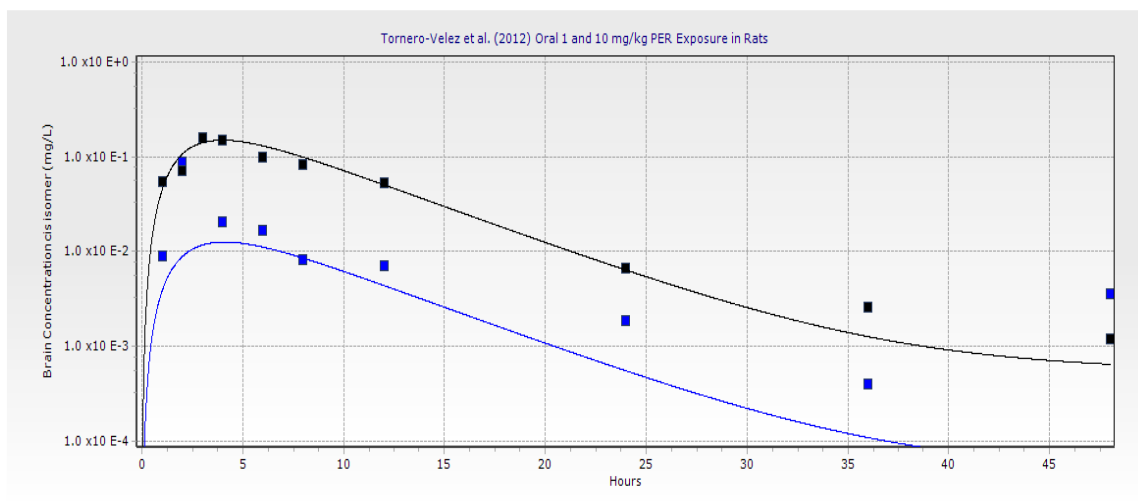


Figure C-2. Simulation of Tornero-Velez *et al.* (2012) Oral cis-PER Brain Data. The brain concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

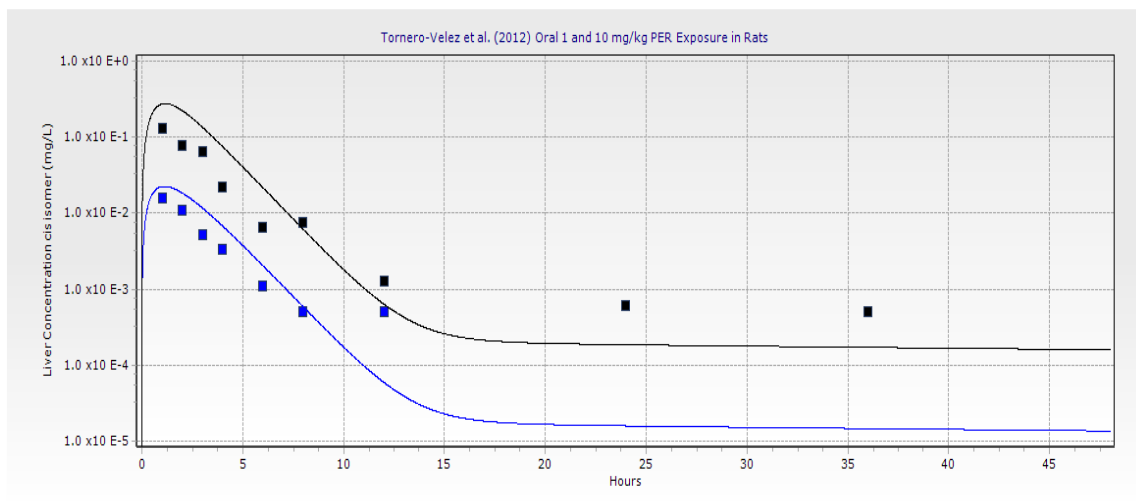


Figure C-3. Simulation of Tornero-Velez *et al.* (2012) Oral cis-PER Liver Data. The liver concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

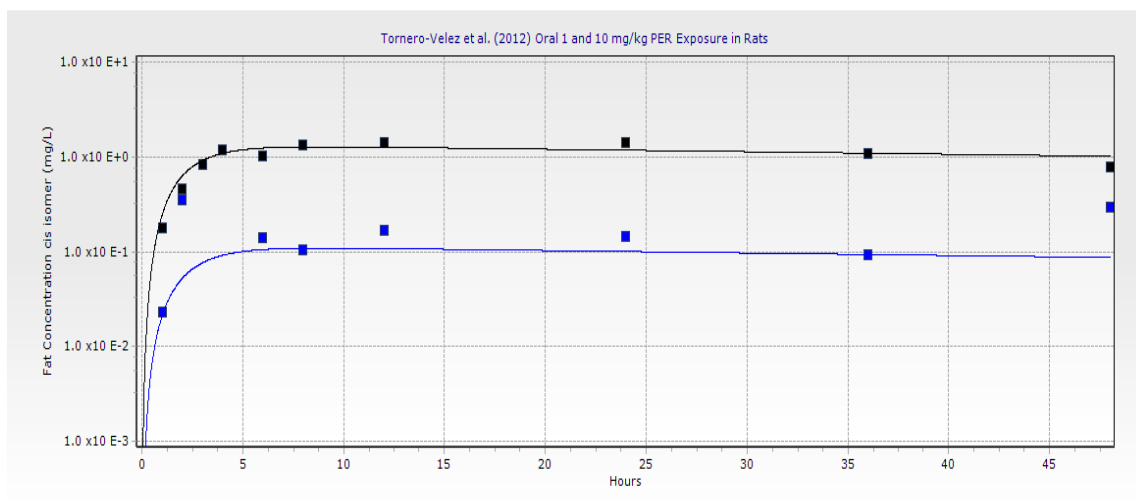


Figure C-4. Simulation of Tornero-Velez *et al.* (2012) Oral cis-PER Fat Data. The fat concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

The GWI and the Torner-Velez models use an uptake value from the stomach, which the Willemin model did not. While this value was initially set to 0 to match the Willemin *et al.* (2016) publication, the behavior of the GWI model improved with the use of the Torner-Velez model value instead. Outside of the transfer from the stomach to the duodenum and the absorption from the duodenum parameters, only body weight and exposure concentrations were altered. The model was able to produce good fits across the Willemin *et al.* data (Figures C-5 through C-8).

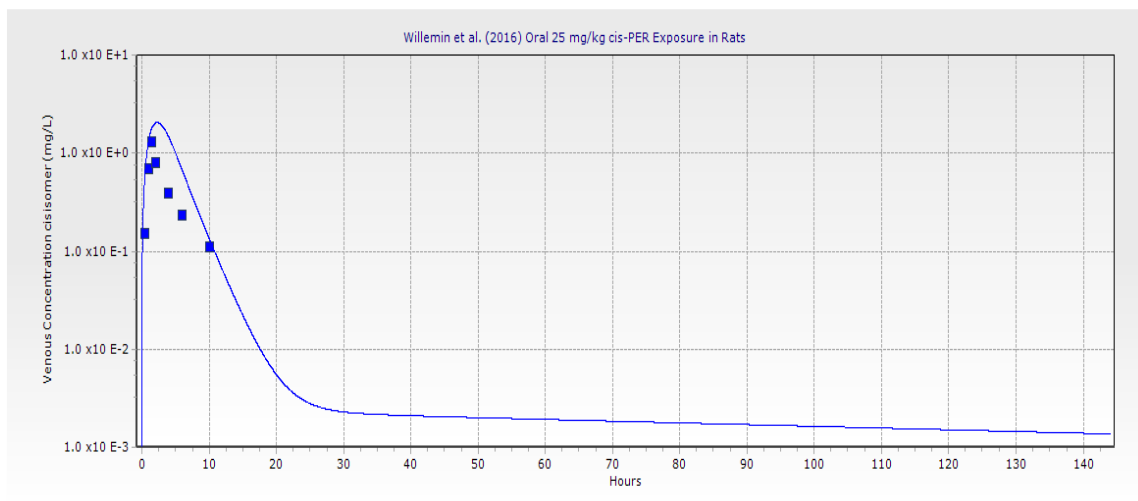


Figure C-5. Simulation of Willemijn *et al.* (2016) Oral cis-PER Blood Data. The blood concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg cis-PER/kg bodyweight by oral gavage.

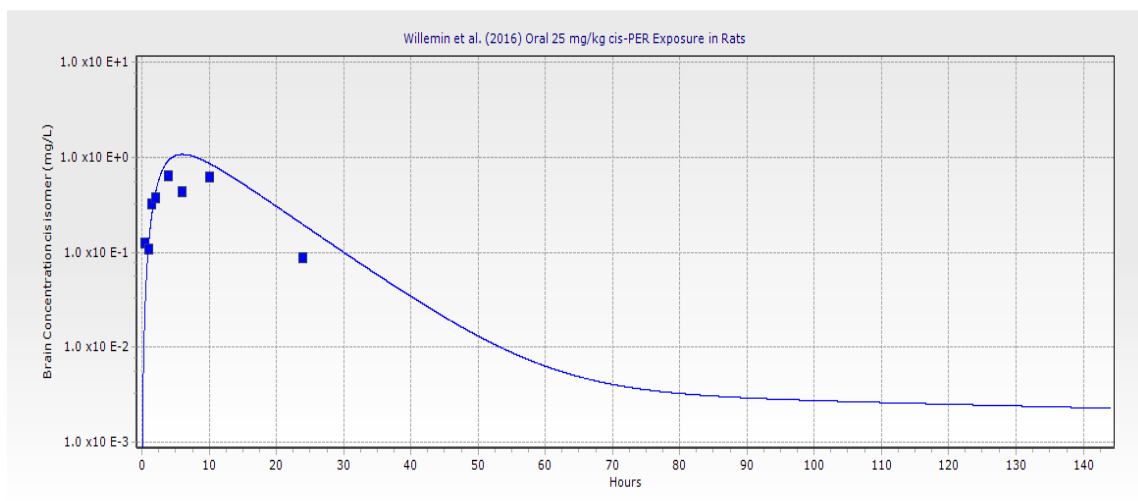


Figure C-6. Simulation of Willemijn *et al.* (2016) Oral cis-PER Brain Data. The brain concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg cis-PER/kg bodyweight by oral gavage.

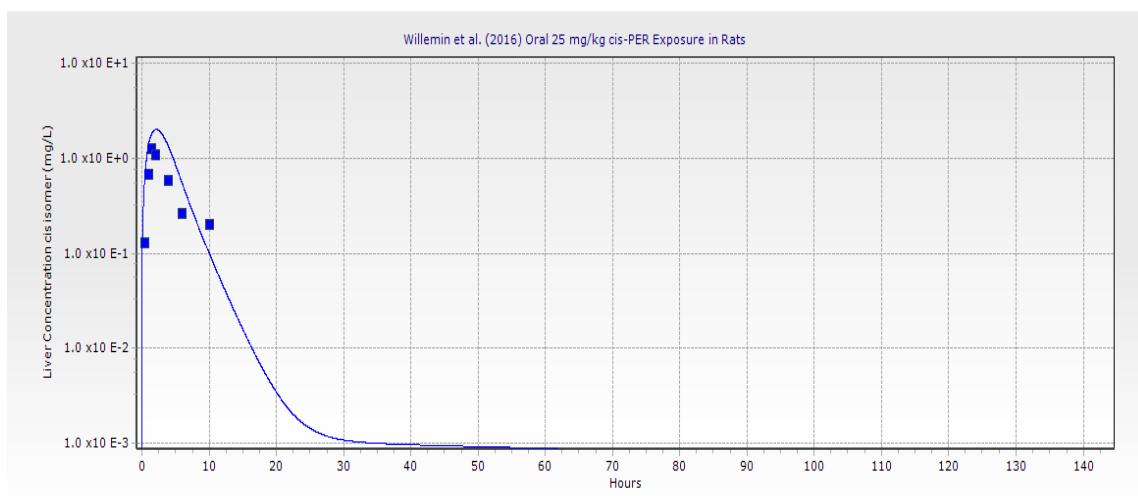


Figure C-7. Simulation of Willemijn *et al.* (2016) Oral cis-PER Liver Data. The liver concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg cis-PER/kg bodyweight by oral gavage.

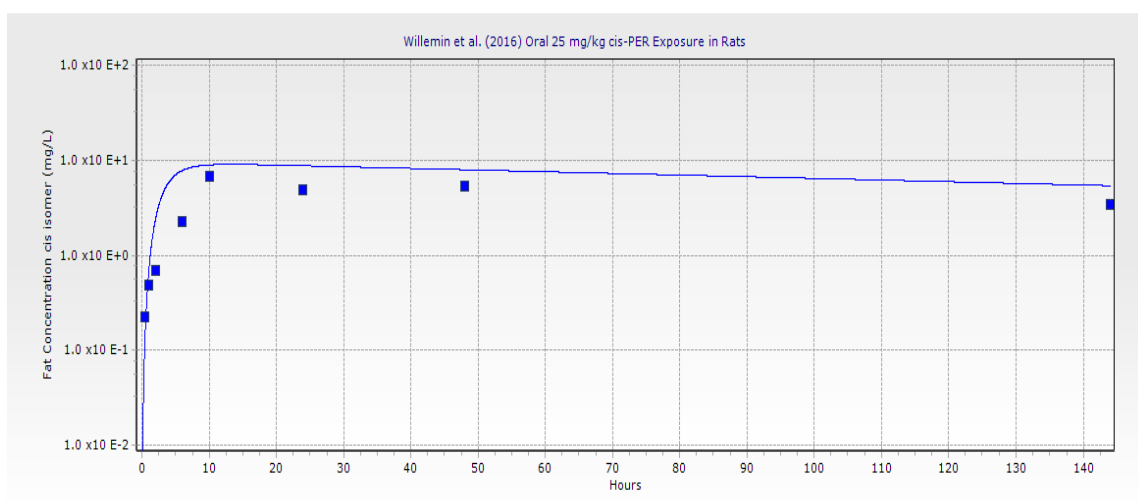


Figure C-8. Simulation of Willemijn *et al.* (2016) Oral cis-PER Fat Data. The fat concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg cis-PER/kg bodyweight by oral gavage.

To test model predictions against a different data set, the model runs were plotted against the limited pharmacokinetic data in Lestremieu *et al.* (2014). The “fed” uptake parameters were used for this study as food was not withheld until after dosing. Rat bodyweights were not reported; so those from Willemijn *et al.* (2016) were used, as the work was from the same laboratory. The predictions were good (Figures C-9 through C-14) and the model was considered fit for the purpose of the project.

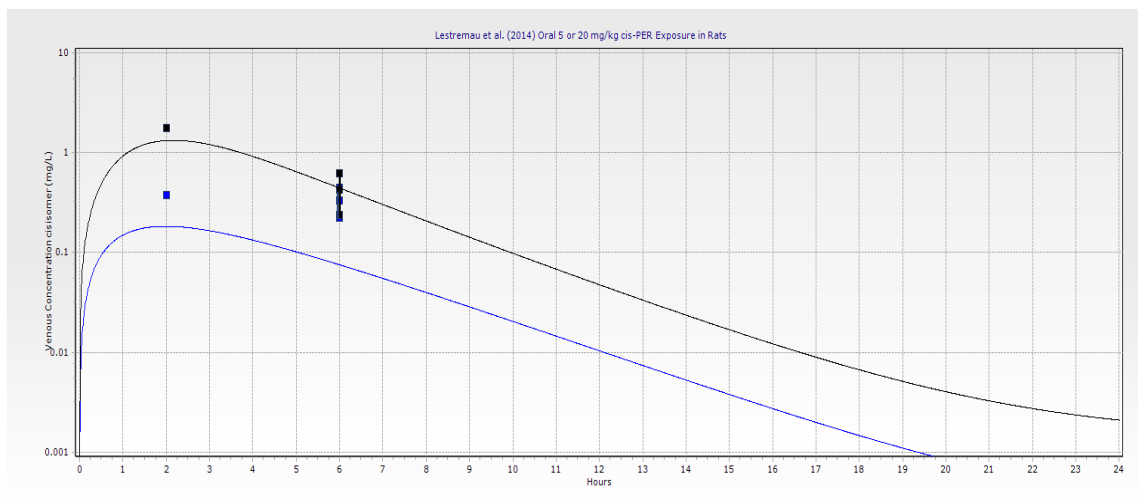


Figure C-9. Prediction of Lestremieu *et al.* (2014) Oral cis-PER Blood Data. The blood concentration (mg/L) prediction is shown as a blue or black line, compared to data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg cis-PER/kg bodyweight by oral gavage.

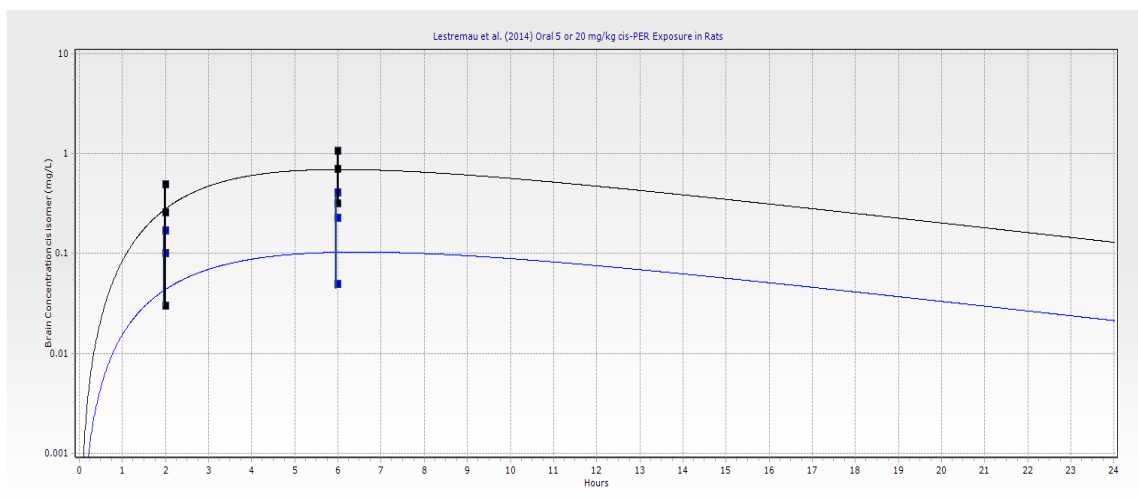


Figure C-10. Prediction of Lestremieu *et al.* (2014) Oral cis-PER Brain Data. The brain concentration (mg/L) prediction is shown as a blue or black line, compared to data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg cis-PER/kg bodyweight by oral gavage.

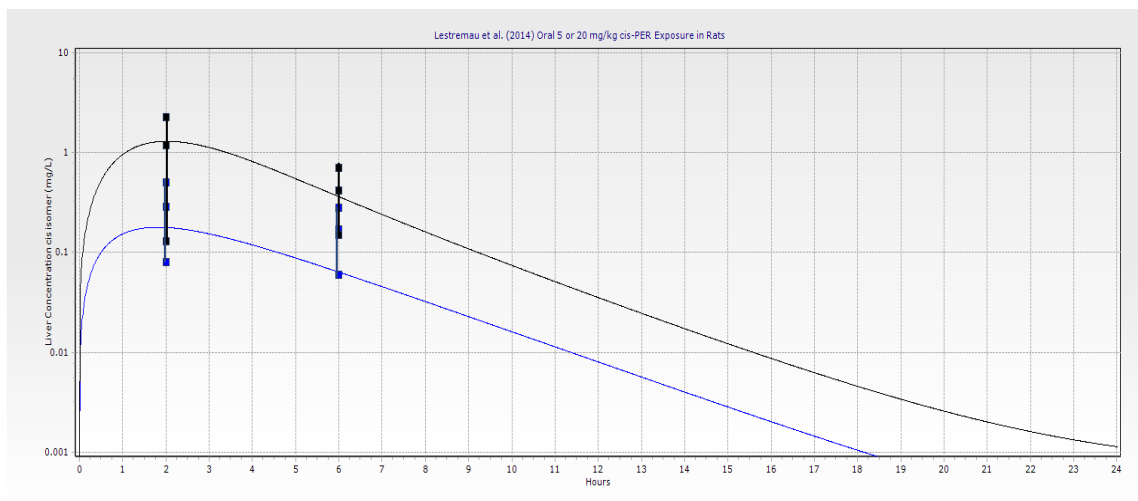


Figure C-11. Prediction of Lestremieu *et al.* (2014) Oral cis-PER Liver Data. The liver concentration (mg/L) prediction is shown as a blue or black line, compared to data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg cis-PER/kg bodyweight by oral gavage.

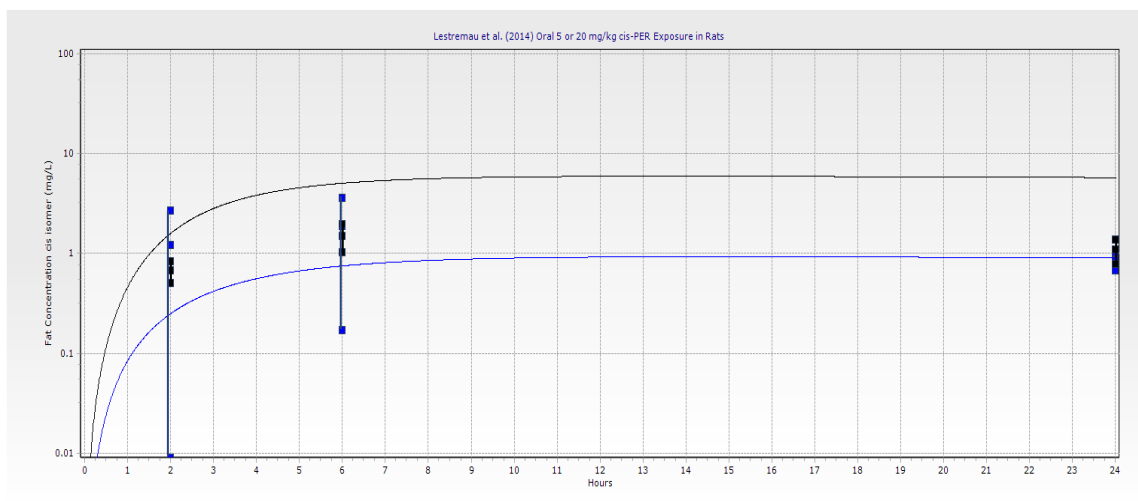


Figure C-12. Prediction of Lestremieu *et al.* (2014) Oral cis-PER Fat Data. The fat concentration (mg/L) prediction is shown as a blue or black line, compared to data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg cis-PER/kg bodyweight by oral gavage.

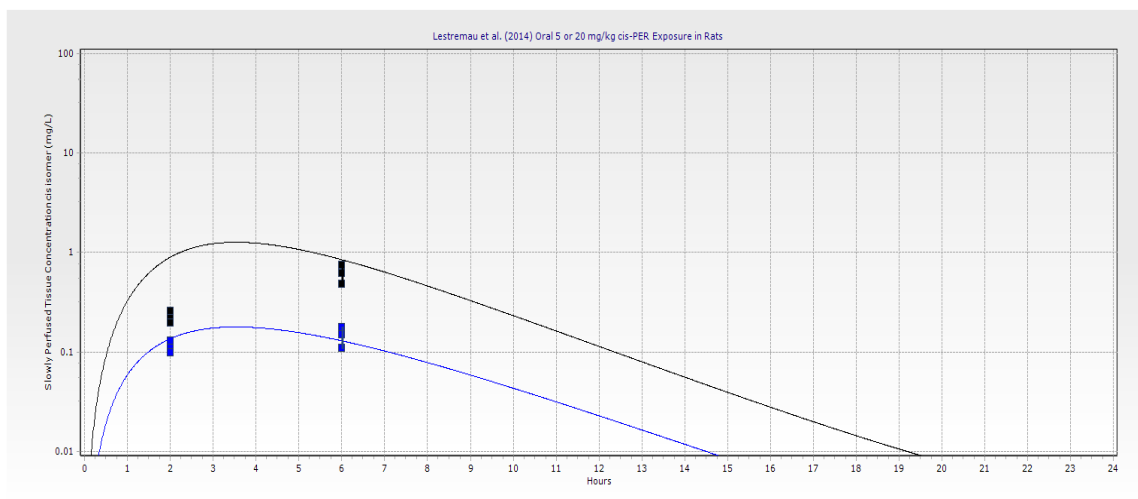


Figure C-13. Prediction of Lestremieu *et al.* (2014) Oral cis-PER Slowly Perfused Tissue Data. The slowly perfused tissue concentration (mg/L) prediction is shown as a blue or black line, compared to muscle data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg cis-PER/kg bodyweight by oral gavage.

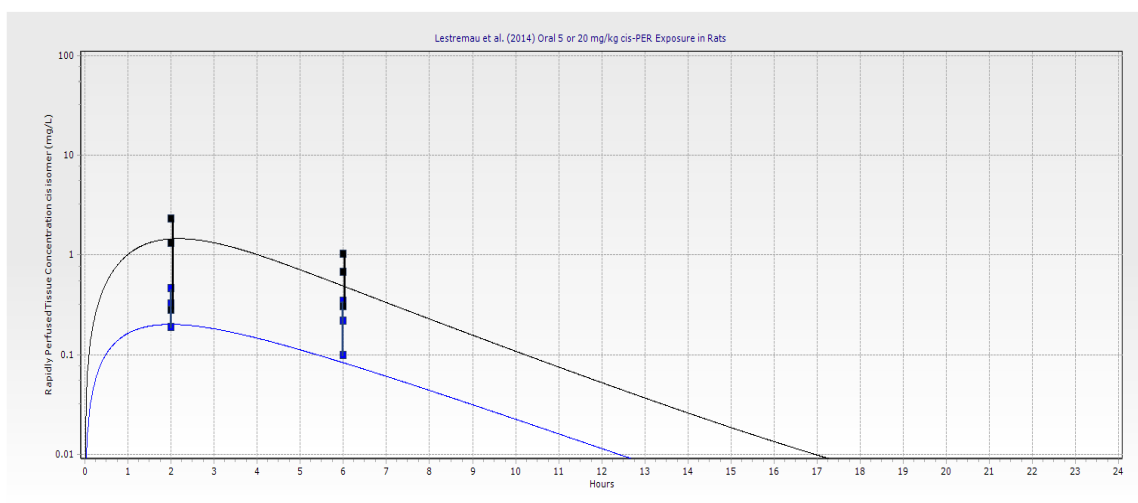


Figure C-14. Prediction of Lestremieu *et al.* (2014) Oral cis-PER Rapidly Perfused Tissue Data. The rapidly perfused tissue concentration (mg/L) prediction is shown as a blue or black line, compared to kidney data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg cis-PER/kg bodyweight by oral gavage.

APPENDIX D. trans-PERMETHRIN SIMULATIONS AND PREDICTIONS

For simplification, all citations within this appendix are contained within the main report reference list.

PRIOR PBPK MODELS

Two published PBPK models were found for permethrin. These are described in Appendix C.

KINETIC STUDIES

Descriptions of the kinetic studies are very similar to Appendix C; the narrative was repeated in order to report the differences as needed for the trans- isomer in each study. Tornero-Velez *et al.* (2012) exposed 70-day old male Long-Evans rats (0.295 kg) orally to 1 or 10 mg PER/kg bodyweight in 1 mL/kg corn oil. The permethrin was a 40:60 cis-:trans-PER mixture. Rats were euthanized at 1, 2, 3, 4, 6, 8, 12, 24, 36, or 48 hours (n = 4/time point). Blood, brain, fat, and liver samples were collected. Blood and tissue analyses separated cis- and trans-PER, enabling the modeling of the isomers separately. Rats were fasted in this study.

The Willemin *et al.* (2016) oral study used Sprague Dawley male rats (468 g $\pm$ 21 g) and dosed cis- and trans-PER separately. Rats were dosed with 25 mg trans-PER/kg bodyweight in 2 mL/kg corn oil. Animals were not fasted in this study; however, food was withheld for 3 hours post-dosing for maximal absorption. Rats were euthanized at 0.5, 1, 1.5, 2, 4, 6, 10, 24, or 48 hours (n = 4/time point). Blood, brain, fat, liver, testes, muscle, and kidney samples were collected.

Willemin *et al.* (2016) referenced an analytical method/preliminary study paper done in their laboratory prior to their PBPK model publication. This paper, Lemestrau *et al.* (2014), contained limited kinetic data. Male SD rats of undeclared body weights were exposed to 5 or 20 mg trans-PER/kg bodyweight dissolved in 1 mL/kg corn oil. As later done in the Willemin study, rats were not fasted prior to dosing but food was withheld for 3 hours post-dosing. For modeling purposes, body weights were assumed to be equivalent to the Willemin study rats (468 g). Rats were euthanized at 2, 6, or 24 hours (n = 2/time point). Blood, liver, brain, muscle, kidneys and fat tissues were collected. Many samples were below detection limits. Parent and metabolite compounds were analyzed in feces and urine; the authors found an average of 25 percent of the trans-PER dose eliminated in the feces. trans-PER itself is not eliminated in urine.

MODEL AND PARAMETER DEVELOPMENT

Much of the model development process is identical to that which was reported for cis-PER. Please see Appendix C for information on the development of the blood:air PC, upper respiratory tract uptake, tissue:blood PCs, urinary clearance, skin permeability constant, and oral uptake values (Tables C-1 and C-2).

As with cis-PER, PA coefficients from Willemin *et al.* (2016) refined using MCMC analysis were utilized for the PBPK model, with the exception of the brain PA. Based on the comparison of brain tissue cis- and trans-PER concentration in the Tornero-Velez *et al.* (2012) and Willemin *et al.* (2016) kinetic studies and on iterative fitting, the brain PA for trans-PER was set one magnitude lower than the cis-PER PA (Table C-1).

Willemin published an intrinsic clearance rate of 6.3 for all routes of metabolism of trans-PER. As this rate is equivalent to $V_{\text{Max}}C/K_m$, the K_m was set to 1 mg/L and the $V_{\text{Max}}C$ to 6.3 mg/(hour*kg^{0.75}) in the model herein. Uptake values were set to “fasted” (Table C-2).

Using the parameters as described above and iterative fittings, good simulations were obtained for the data from Tornero-Velez *et al.*, (2012, Figures D-1 through D-4). As with the cis-PER simulations, venous blood, brain and liver simulations suggest binding of trans-PER to plasma proteins, as the low dose simulations taper off too quickly compared to the data (Figures D-1 to D-3). Plasma binding was not included in the original models (Tornero-Velez *et al.*, 2012; Willemin *et al.*, 2016) and was omitted from the current model due to time constraints. Again, inclusion of plasma binding would be an improvement for the future PER modeling endeavors.

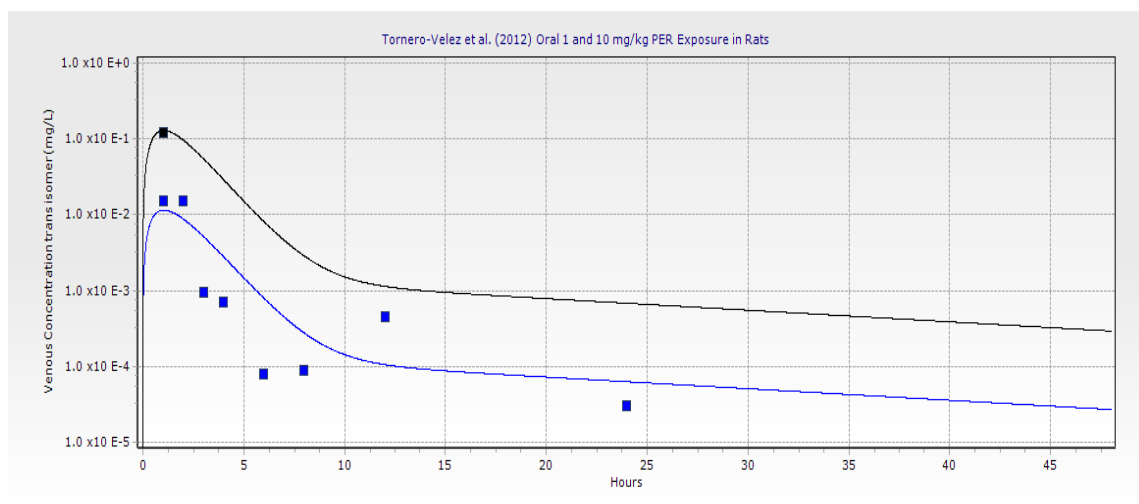


Figure D-1. Simulation of Tornero-Velez *et al.* (2012) Oral trans-PER Blood Data. The blood concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

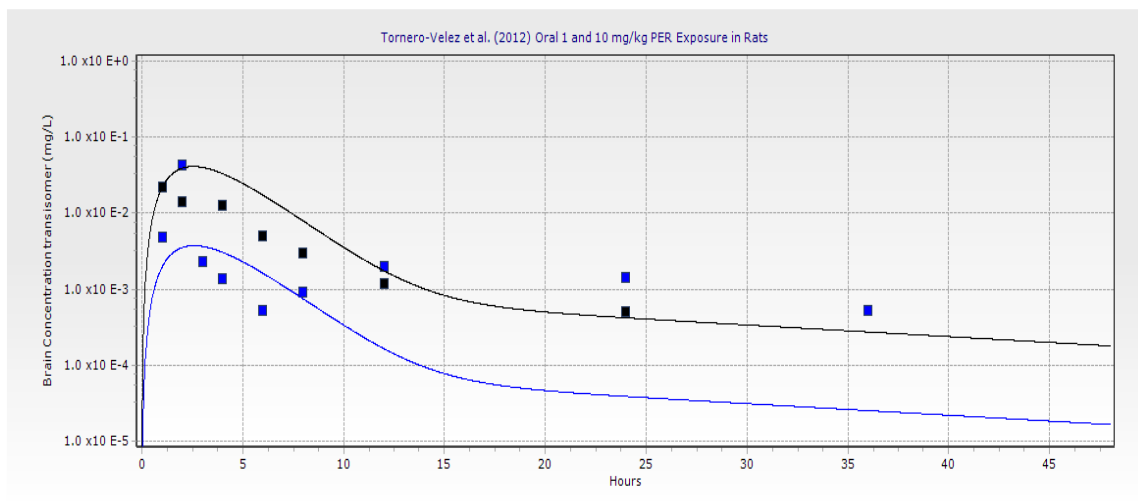


Figure D-2. Simulation of Tornero-Velez *et al.* (2012) Oral trans-PER Brain Data. The brain concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

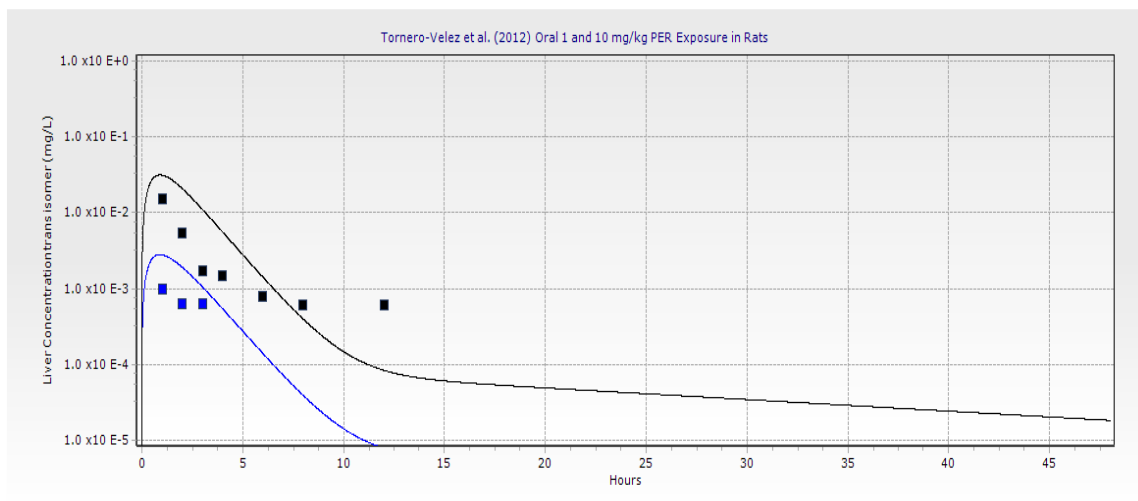


Figure D-3. Simulation of Tornero-Velez *et al.* (2012) Oral trans-PER Liver Data. The liver concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

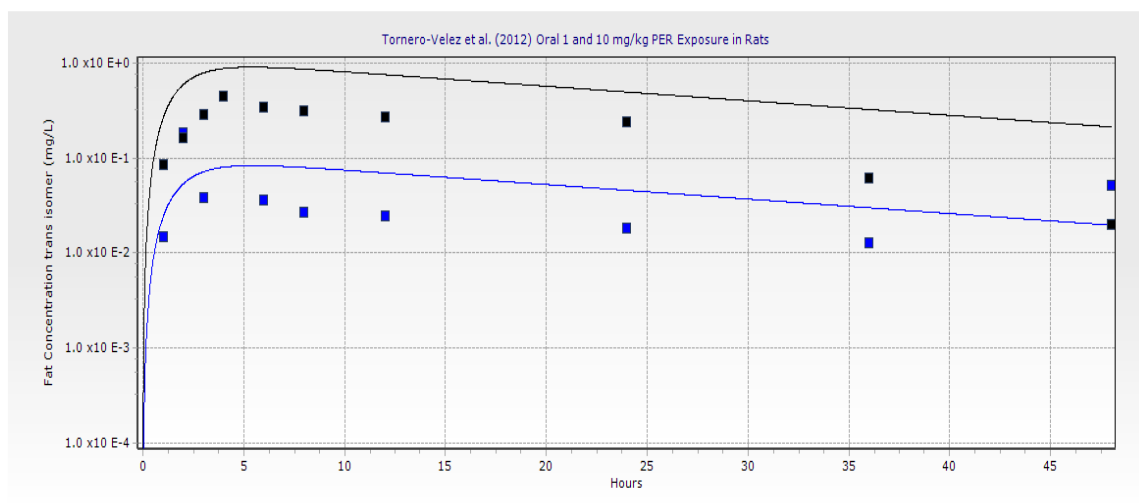


Figure D-4. Simulation of Tornero-Velez *et al.* (2012) Oral trans-PER Fat Data. The fat concentration (mg/L) simulation is shown as a blue or black line, compared to data from the study (corresponding colored squares). Male rats were exposed to 1.0 or 10.0 mg of 40:60 cis:trans-PER/kg bodyweight by oral gavage.

Next, the uptake parameters were set to “fed” values (Table C-2). As with the cis-PER model, the uptake value from the stomach matched the Tornero-Velez model value. Outside of the transfer from the stomach to the duodenum and the absorption from the duodenum parameters, only body weight and exposure concentrations were changed to study-specific values. The model was able to produce good fits across the Willemin *et al.* data (Figures C-5 through C-7). Liver data were below detection limits for trans-PER, so the liver simulation is not shown below.

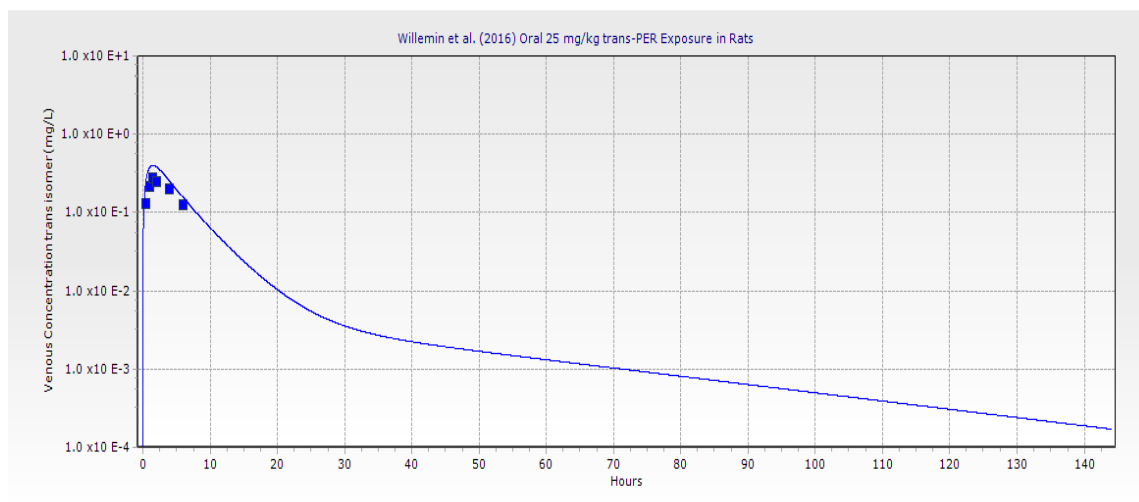


Figure D-5. Simulation of Willemin *et al.* (2016) Oral trans-PER Blood Data. The blood concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg trans-PER/kg bodyweight by oral gavage.

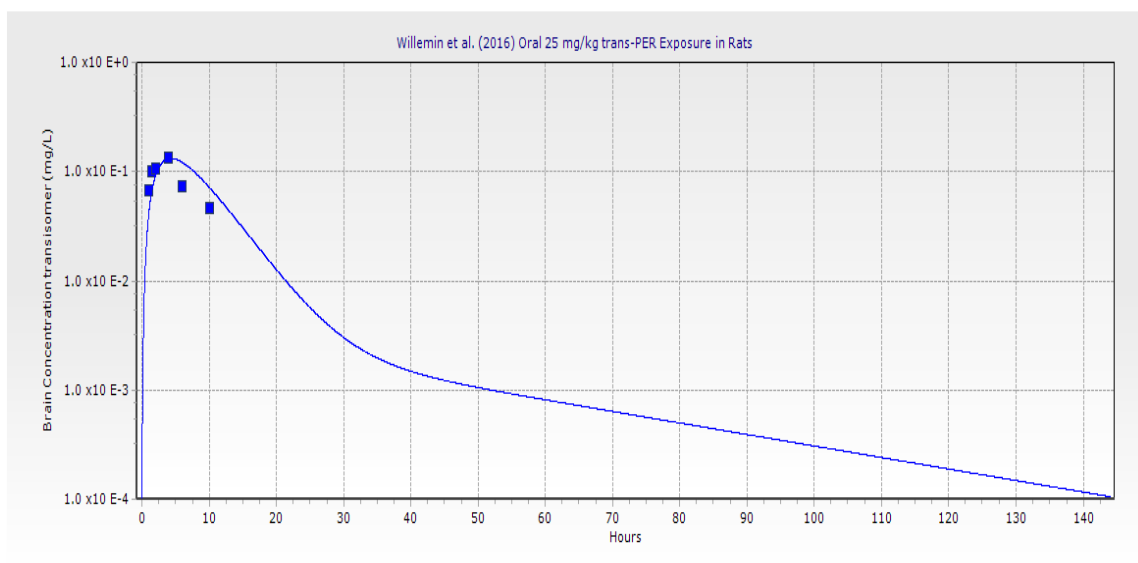


Figure D-6. Simulation of Willemijn *et al.* (2016) Oral trans-PER Brain Data. The brain concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg trans-PER/kg bodyweight by oral gavage.

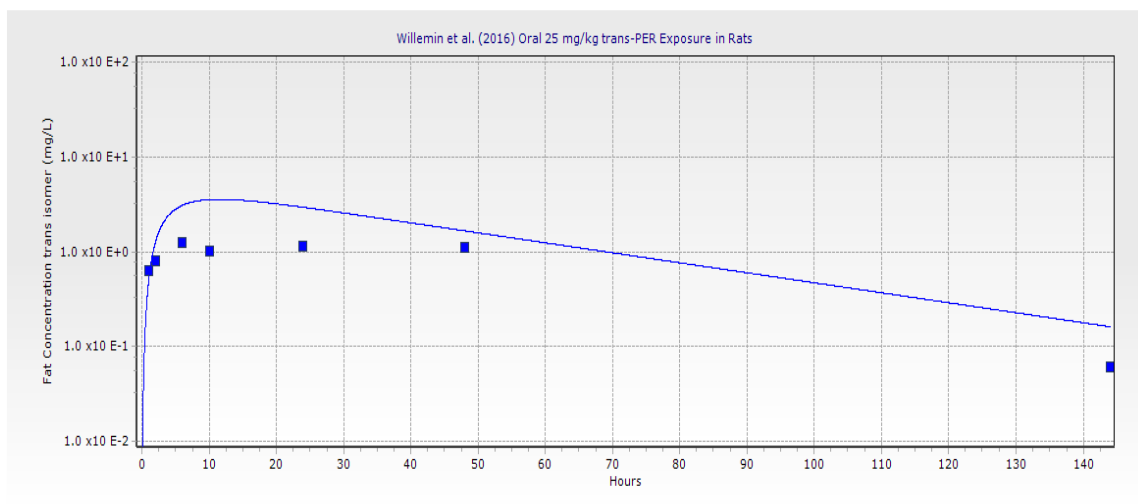


Figure D-7. Simulation of Willemijn *et al.* (2016) Oral trans-PER Fat Data. The fat concentration (mg/L) simulation is shown as a blue line, compared to data from the study (blue squares). Male rats were exposed to 25.0 mg trans-PER/kg bodyweight by oral gavage.

To test model predictions against a different data set, the model runs were plotted against the limited pharmacokinetic data in Lestremieu *et al.* (2014). The “fed” uptake parameters were kept for this study. Rat bodyweights were not reported; so those from Willemijn *et al.* (2016) were used, as the work was from the same laboratory. The predictions were reasonable for the few data points available for trans-PER from this study (Figures D-8 and D-9). Many of the tissues were non-detectable at multiple time points. Overall, the trans-PER model was considered fit for the purpose of the project.

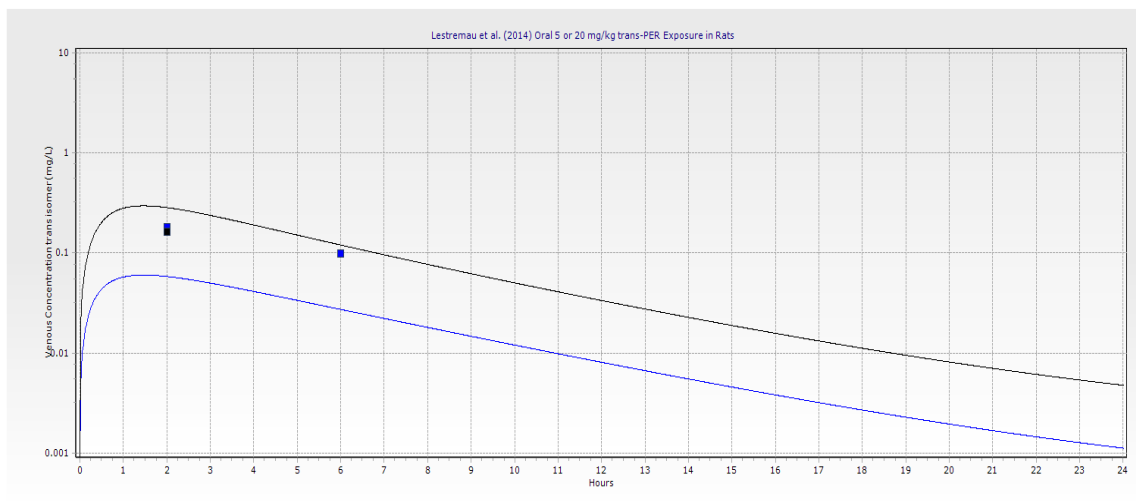


Figure D-8. Prediction of Lestremieu *et al.* (2014) Oral trans-PER Blood Data. The blood concentration (mg/L) prediction is shown as a blue or black line, compared to data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg trans-PER/kg bodyweight by oral gavage.

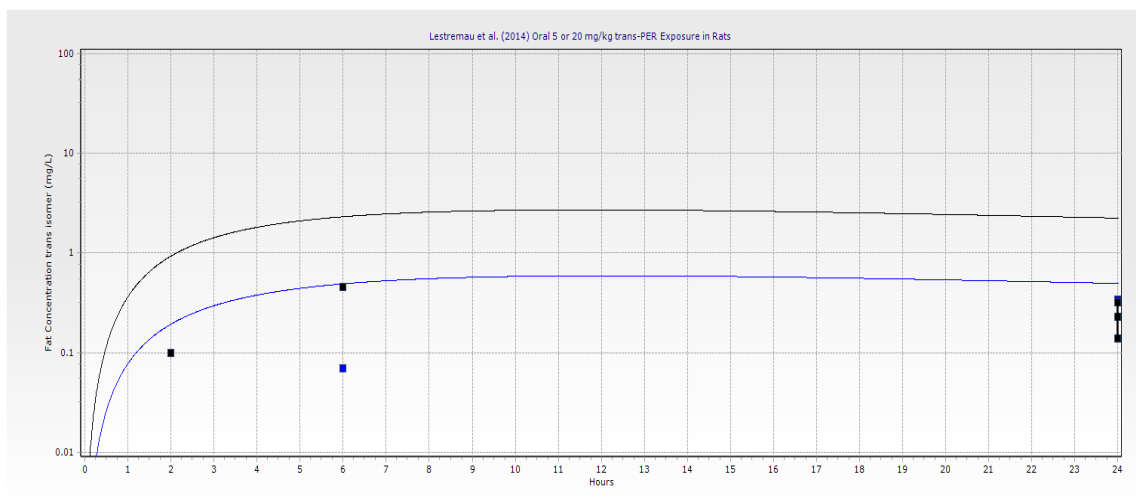


Figure D-9. Prediction of Lestremieu *et al.* (2014) Oral trans-PER Fat Data. The fat concentration (mg/L) prediction is shown as a blue or black line, compared to data from the study (corresponding colored squares, representing mean and SD). Male rats were exposed to 5.0 or 20.0 mg trans-PER/kg bodyweight by oral gavage.

APPENDIX E. MIXED PERMETHRIN SIMULATIONS

For simplification, all citations within this appendix are contained within the main report reference list.

KINETIC STUDY

A study was located in which the permethrin isomers were not separated during tissue analysis. The model is designed to simulate these data by simultaneously dosing a percentage of the dose as cis-PER and a percentage as trans-PER. These percentages are set based on the published or presumed cis:trans ratio used in the study.

Anadon *et al.* (1991) published IV and oral data sets using a PER formulation with a 25 cis-:75 trans- ratio. Male Sprague Dawley rats (180 to 200 g) were exposed intravenously to 46 mg PER/kg bodyweight in 0.1 mL glycerol formal/rat or orally to 460 mg PER/kg bodyweight in 0.5 mL corn oil/rat. No information was listed on injection times for the IV dosing group; 30 seconds or 0.008 hours was assumed for modelling. Rats were fasted prior to dosing. The rats (n = 8/time point) were euthanized at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, or 48 hours. Tissues collected include plasma, liver, sciatic nerve, and several brain regions: hypothalamus, cerebellum, frontal cortex, caudate putamen, hippocampus, and medulla oblongata. Brain concentrations were reported as sample averages for each region. The plot was digitized and the regional values averaged again to produce an overall brain value; the minimum and maximum brain concentrations were then used as bounds for the purpose of the GWI model. Metabolites were also measured.

MODEL PERFORMANCE

The purpose of modeling this data set was to determine if the overall permethrin model is adequate to describe other data sets in addition to the ones with which it was developed. Mixed PERs are representative of the exposures received by military members and most rat studies; the Willemin study (2016) is unique in dosing cis- and trans-PER separately. All others, including the Tornero-Velez study (2012), dosed with a mixture of cis- and trans-PER; few studies separate the isomers during chemical analysis.

The Anadon *et al.* (1991) data proved to be very different from the low dose data from which the model was developed. The dose administered by Anadon and co-workers was 10 to 100 times larger than the doses for which the absorption was optimized (Tornero-Velez *et al.*, 2012; Willemin *et al.*, 2016). Anadon *et al.* designed the dose in their study to be high enough to cause neurological symptoms (decreased locomotor activity and increased acoustic startle response amplitude) but not tremors and lethality.

Rats were fasted, so the uptake parameters were set to those of Tornero-Velez *et al.* (2012; fed column, Table C-2). Initial predictions indicated too much metabolism was occurring with the metabolic parameters that had worked for all 3 studies in Appendices C and D. Attempts were made to keep the intrinsic clearance values reported by Willemin *et al.* (2016); as intrinsic clearance is V_{MaxC}/K_m , the ratio was held constant but the parameters were decreased by the same divisor in each attempt. While the fits were improved in this fashion, too much metabolism was still occurring when compared to Anadon *et al.* (1991) study data.

The best fits were found by returning K_m to the original value (1.0 for both isomers; Table C-2) and dividing the original V_{MaxC} values by 5 for each isomer (0.5 and 1.26 mg/(hour*kg^{0.75}) for cis- and trans-PER, respectively) (Figures E-1 through E-3). No attempt was made to optimize the fits; efforts were merely to determine which parameters appeared to be incorrect for the data

in this study. Certainly, oral uptake parameters could be optimized for this study, as rats were given a much larger dose of PER than in the Tornero-Velez study, including a higher dose of corn oil (0.5 mL versus approximately 0.3 ml), making a specific set of uptake parameters ideal. However, the ultimate purpose of the project is to predict brain concentrations of PER from dermal doses, so focusing on the Anadon study was not appropriate.

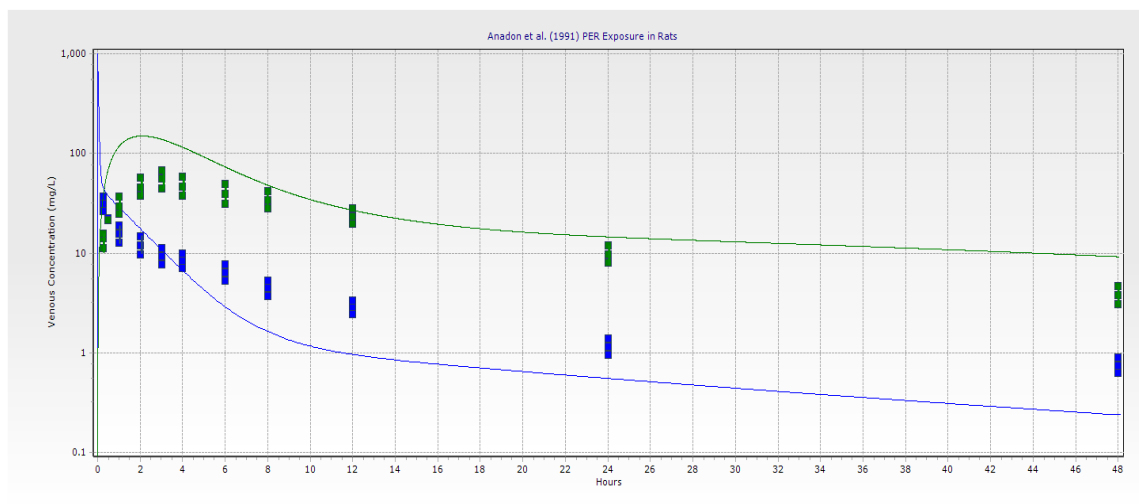


Figure E-1. Prediction of Anadon *et al.* (1991) Intravenous and Oral PER Plasma Data. The plasma concentration (mg/L) simulation is shown as a blue or green line, compared to data from the study (corresponding colored squares, representing mean and SEM). Male rats were exposed to 46 mg 25 cis-:75 trans-PER/kg intravenously or 460 mg 25 cis-:75 trans-PER/kg by oral gavage.

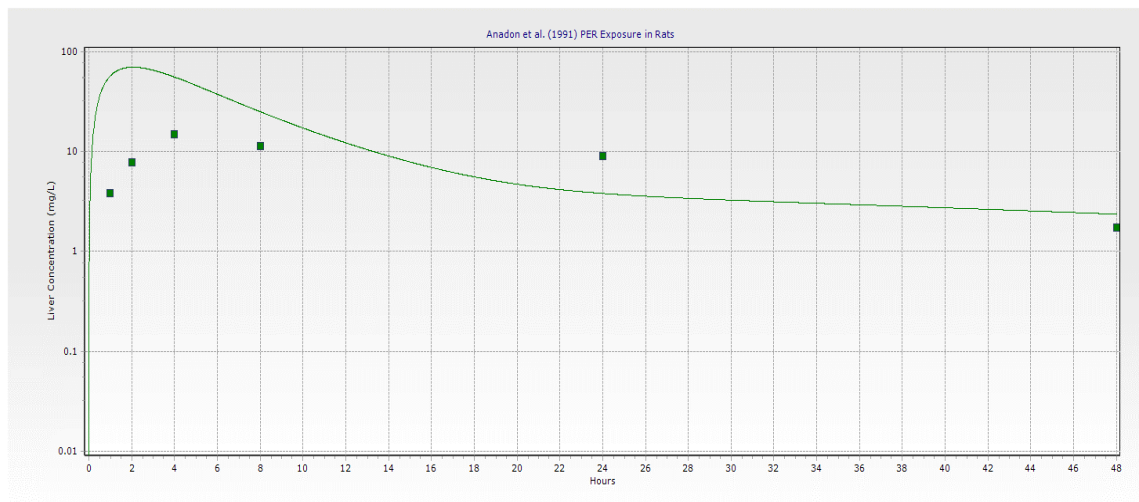


Figure E-2. Prediction of Anadon *et al.* (1991) Oral PER Liver Data. The liver concentration (mg/L) simulation is shown as a green line, compared to data from the study (green squares). Male rats were exposed to 460 mg 25 cis-:75 trans-PER/kg by oral gavage.

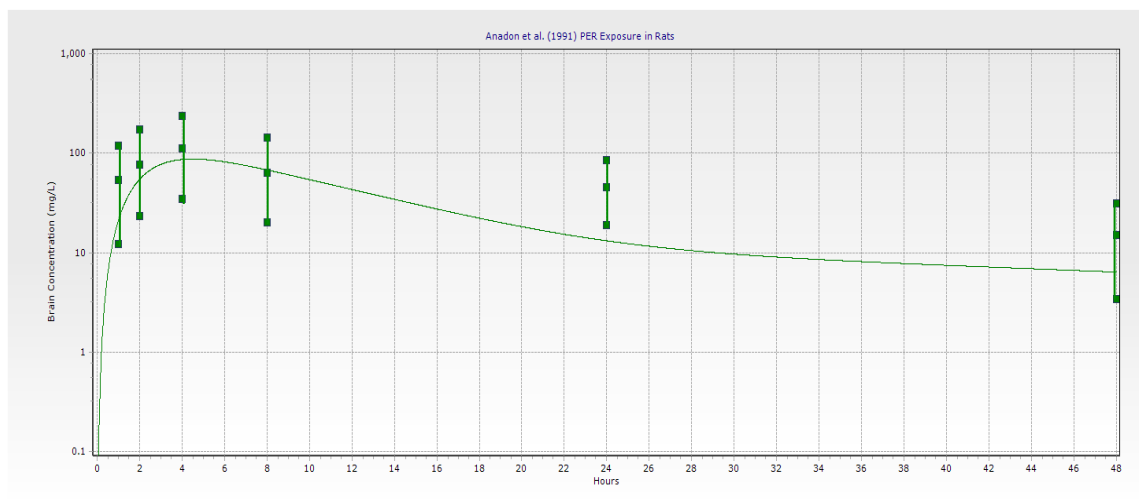


Figure E-3. Prediction of Anadon *et al.* (1991) Oral PER Brain Data. The brain concentration (mg/L) simulation is shown as a green line, compared to data from the study (green squares, representing overall mean, minimal region concentration, and maximal region concentration). Male rats were exposed to 460 mg 25 *cis*-:75 *trans*-PER/kg by oral gavage.

APPENDIX F. ARRAY MODEL CSL FILE

Note: Report formatting may introduce extraneous line breaks not in the original code.

PROGRAM: GWI_v3.CSL

! Gulf War Illness model using arrays

! Model currently set up to run for 4 chemicals: 1-DEET, 2-PB, 3-PERMc, 4-PERMt

! Will run for fewer chemicals without modifications as long as parameters are set accordingly

! Can change the model to run for more or less by only changing the "4" in the line "PARAMETER (NChem=5) "

! The fraction of PERMt and PERMc is set in study m files to suit the experiment
! e.g., IVDose(3)=0.25*46.0; IVDose(4)=0.75*46.0;

INITIAL

INTEGER NChem

PARAMETER (NChem=4)

DIMENSION MW(NChem), PB(NChem), PLq(NChem), PMuc(NChem), PBrn(NChem), PFat(NChem),
PLiv(NChem), PRap(NChem), PSlw(NChem)
DIMENSION PABrn(NChem), PAFat(NChem), PALiv(NChem), PARap(NChem), PASlw(NChem)
DIMENSION VMaxC(NChem), KM(NChem), KFC(NChem), ClUrC(NChem), kURTC(NChem)
DIMENSION kAD(NChem), kAS(NChem), kFec(NChem), kTSD(NChem)
DIMENSION Conc(NChem), IVDose(NChem), PDose(NChem), PDrink(NChem), Area(NChem),
KP(NChem), ICSurf(NChem), IVSurf(NChem)
DIMENSION QSknC2(NChem), QSkN(NChem), QSlw(NChem), VSkN(NChem), VSlw(NChem),
VSlwBld(NChem)
DIMENSION PSkNB(NChem), PSkNL(NChem)
DIMENSION VMax(NChem), KF(NChem), ClUr(NChem), kUrt(NChem)
DIMENSION IAArt(NChem), IABrn(NChem), IABrnBld(NChem), IAFat(NChem),
IAFatBld(NChem), IALiv(NChem), IALivBld(NChem)
DIMENSION IARap(NChem), IARapBld(NChem), IASlw(NChem), IASlwBld(NChem)
DIMENSION AIO(NChem), Drink(NChem), DDose(NChem), IV(NChem), TotDose(NChem)
DIMENSION IAEKh(NChem), IAMuc(NChem), IAO(NChem), IAFec(NChem), IADu(NChem),
IASt(NChem), IAMet1(NChem), IAMet2(NChem)
DIMENSION IASkN(NChem), IAABs(NChem), IAURN(NChem), IAUCCArt(NChem),
IAUCCBrn(NChem), IATotInh(NChem), IATotIV(NChem), IATotDrink(NChem)
DIMENSION DAUCCArt(NChem), DAUCCBrn(NChem), PAUCCArt(NChem), PAUCCBrn(NChem)
DIMENSION CALv(NChem), CALvPPM(NChem), CP(NChem), CEnd(NChem), CEndPPM(NChem),
PerEnd(NChem), CMix(NChem), CMixPPM(NChem), PerMix(NChem)
DIMENSION RACH(NChem), RAMuc(NChem), RAART(NChem), RAEKh(NChem), RABrn(NChem),
RABrnBld(NChem), RAFat(NChem), RAFatBld(NChem)
DIMENSION RAO(NChem), RAFec(NChem), RADu(NChem), RAST(NChem), RALiv(NChem),
RALivBld(NChem), RAMet1(NChem), RAMet2(NChem)
DIMENSION RARap(NChem), RARapBld(NChem), RAAbs(NChem), RASkN(NChem), RASlw(NChem),
RASlwBld(NChem), RAURN(NChem)
DIMENSION RATotInh(NChem)
DIMENSION CInh(NChem), CMuc(NChem), CART(NChem), CBrn(NChem), CBrnBld(NChem),
CBrnDif(NChem), CFat(NChem), CFatBld(NChem), CFatDif(NChem)
DIMENSION CLiv(NChem), CLivBld(NChem), CLivDif(NChem), CRap(NChem), CRapBld(NChem),
CRapDif(NChem), CSurf(NChem), CSkN(NChem), CSkNDif(NChem)
DIMENSION CSLw(NChem), CSLwBld(NChem), CSLwDif(NChem), CVen(NChem)
DIMENSION ACh(NChem), AMuc(NChem), AART(NChem), AUCCArt(NChem), AExh(NChem),
ABrn(NChem), ABrnBld(NChem), AUCCBrn(NChem)
DIMENSION AFat(NChem), AFatBld(NChem), AO(NChem), AFec(NChem), ADu(NChem),
AST(NChem), ALiv(NChem), ALivBld(NChem)

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    DIMENSION AMet1(NChem), AMet2(NChem), ARap(NChem), ARapBld(NChem), AAbs(NChem),
ASurf(NChem), ASkn(NChem), ASlw(NChem), ASlwBld(NChem)
    DIMENSION AUrn(NChem), ATotInh(NChem), ATotIV(NChem), ATotDrink(NChem),
TDose(NChem), AmtBody(NChem), MassBal(NChem)

    INTEGER i, j1, j2, j3, i1, i2, i3, i4, i5, i6, i7, i8, i9, i10, i11, i12, i13, i14,
i15, i16, i17, i18, i19, i20, i22
    LOGICAL CC                                ! To control whether closed or open chamber

! Total Pulmonary Ventilation Rate (L/hr for 1 kg animal)
    CONSTANT      QPC = 24.75                ! Total pulmonary ventilation (L/hr - 1 kg)

! Blood Flows (fraction of cardiac output)
    CONSTANT      QCC = 14.6                 ! Cardiac output (L/hr - 1 kg animal)
    CONSTANT      QBrnC = 0.02              ! Brain
    CONSTANT      QFatC = 0.07              ! Fat
    CONSTANT      QLivC = 0.183             ! Liver
    CONSTANT      QRapC = 0.557             ! Rapidly perfused
    CONSTANT      QSknC = 0.058            ! Skin
    CONSTANT      QSlwC = 0.17              ! Slowly perfused (includes skin)

! Tissue Volumes (fraction of body weight)
    CONSTANT      BW = 0.22                 ! Body weight (kg)
    CONSTANT      VAlvC = 0.007             ! Alveolar blood
    CONSTANT      VBrnC = 0.006            ! Brain
    CONSTANT      VFatC = 0.10              ! Fat
    CONSTANT      VLivC = 0.034            ! Liver
    CONSTANT      VMucC = 0.0001           ! Mucous
    CONSTANT      VRapC = 0.044            ! Rapidly perfused
    CONSTANT      VSlwC = 0.65             ! Slowly perfused (includes skin)
    CONSTANT      Depth = 0.10             ! Skin thickness (cm)
    CONSTANT      DS = 0.15                ! Dead space volume (fraction)

!Tissue Volumes (fraction of tissue volume)
    CONSTANT      VBrnBldC = 0.03          ! Brain
    CONSTANT      VFatBldC = 0.0154        ! Fat
    CONSTANT      VLivBldC = 0.034        ! Liver
    CONSTANT      VRapBldC = 0.2075       ! Rapidly perfused tissues
    CONSTANT      VSlwBldC = 0.0333       ! Slowly perfused tissues

! Molecular Weights (mg/mmmole)
    CONSTANT      MW = NChem*1.0

! Tissue/Blood Partition Coefficients
    CONSTANT      PB = NChem*1.0           ! Blood/air
    CONSTANT      PLq = NChem*1.0          ! Saline/air
    CONSTANT      PMuc = NChem*1.0         ! Mucous/air
    CONSTANT      PBrn = NChem*1.0        ! Brain
    CONSTANT      PFat = NChem*1.0        ! Fat
    CONSTANT      PLiv = NChem*1.0        ! Liver
    CONSTANT      PRap = NChem*1.0        ! Rapidly perfused tissue
    CONSTANT      PSlw = NChem*1.0        ! Slowly perfused tissue
    CONSTANT      PSknB = NChem*1.0       ! Skin to blood

! Tissue Permeability Coefficients
    CONSTANT      PABrn = NChem*1000.0    ! Brain diffusion limitation
    CONSTANT      PAFat = NChem*1000.0    ! Fat diffusion limitation
    CONSTANT      PALiv = NChem*1000.0    ! Liver diffusion limitation
    CONSTANT      PARap = NChem*1000.0    ! Rapidly perfused tissue diffusion limitation
    CONSTANT      PASlw = NChem*1000.0    ! Slowly perfused tissue diffusion limitation

! Metabolism Parameters
    CONSTANT      VMaxC = NChem*0.0       ! Maximum reaction rate (mg/hr)

```

```

CONSTANT      KM = NChem*1.0          ! Michaelis-Menten (mg/L)
CONSTANT      KFC = NChem*0.0        ! First order rate constant (/hr)

! Uptake and Clearance Parameters
CONSTANT      ClUrC = NChem*0.0      ! Urinary clearance (L/(hr*kg^0.75))
CONSTANT      kURTC = NChem*0.0      ! URT uptake (L/hr)
CONSTANT      kAD = NChem*0.0        ! Absorption from duodenum (/hr)
CONSTANT      kAS = NChem*0.0        ! Absorption from stomach (/hr)
CONSTANT      kFec = NChem*0.0       ! Fecal excretion (/hr)
CONSTANT      kTSD = NChem*0.0       ! Transfer - stomach to duodenum (/hr)

! Dosing Parameters
CONSTANT      Conc = NChem*0.0       ! Inhaled concentration (ppm)
CONSTANT      IVDose = NChem*0.0     ! IV dose (mg/kg)
CONSTANT      PDose = NChem*0.0      ! Oral dose (mg/kg)
CONSTANT      PDrink = NChem*0.0     ! Drinking water dose (mg/kg/day)
CONSTANT      TChng = 0.0            ! Length of inhalation exposure (hrs)
CONSTANT      DaysWk = 1.0           ! Number of exposure days per week
CONSTANT      TMax = 24.0            ! Maximum time for exposures
CONSTANT      TInf = 0.20            ! Length of IV injection (hrs)
CONSTANT      Rats = 1.0             ! Number of animals in experiment

! Chamber Parameter
CONSTANT      CC = .FALSE.           ! Default to open chamber
CONSTANT      VChC = 9.1             ! Volume of closed chamber
CONSTANT      kLCC = 0.0             ! Chamber loss

! Dermal Exposure Parameters
CONSTANT      Area = NChem*0.0       ! Exposed area (cm^2)
CONSTANT      KP = NChem*0.0         ! Permeability constant (cm/hr)
CONSTANT      ICSurf = NChem*0.0     ! Initial concentration on skin (mg/mL)
CONSTANT      IVSurf = NChem*0.0     ! Initial volume on skin surface (mL)

! Simulation Control Parameters
CONSTANT      TStop = 24.0
CINTERVAL    CINT = 0.01

! Set Chamber Volume and Loss for Open and Closed Chamber Exposures
IF (.NOT. CC) THEN
    VCh = 1.0e+20                    ! Large chamber = open chamber
    kLC = 0.0
ELSE
    VCh = VChC - (Rats * BW)         ! Calculate net chamber volume
    kLC = kLCC
ENDIF

! Scaled Pulmonary Ventilation Rate (L/hr)
QCN = QCC * (BW**0.75)
QP = QPC * (BW**0.75)
QAlv = 0.67 * QP

! Scaled Blood Flows (L/hr) and Tissue Volumes (L)
! From IPA model: (Total Area of Skin (cm^2) = 9.1 * ((BW * 1000.0)**0.666))
DO 1010 i=1, NChem
    IF (ICSurf(i) .GT. 0.0) THEN
        QSknC2(i) = QSknC * (Area(i) / (9.1 * ((BW * 1000.0)**0.666)))
        VSkN(i) = (Area(i) * Depth) / 1000.0
        !VSlw(i) = (VSlwC * BW) - VSkN(i)
        VSlw(i) = ((VSlwC * (1 - VSlwBldC) ) * BW) - VSkN(i)
    ELSE
        QSknC2(i) = 0.0
        VSkN(i) = 1.0
        !VSlw(i) = VSlwC * BW
    
```

```

      VSlw(i) = (VSlwC * (1 - VSlwBldC) ) * BW
ENDIF

QSkN(i) = QSkNC2(i) * QCN
QSlw(i) = (QSlwC * QCN) - QSkN(i)
VSlwBld(i) = VSlwBldC * VSlw(i)
1010: CONTINUE

      QBrn = QBrnC * QCN
      QFat = QFatC * QCN
      QLiv = QLivC * QCN
      QRap = QRapC * QCN
      QC = QBrn + QFat + QLiv + QRap + (QSlwC * QCN)

      VAlv = VAlvC * BW
      VMuc = VMucC * BW
      VBrn = (VBrnC * (1 - VBrnBldC) ) * BW
      VFat = (VFatC * (1 - VFatBldC) ) * BW
      VLiv = (VLivC * (1 - VLivBldC) ) * BW
      VRap = (VRapC * (1 - VRapBldC) ) * BW

      VBrnBld = VBrnBldC * VBrn
      VFatBld = VFatBldC * VFat
      VLivBld = VLivBldC * VLiv
      VRapBld = VRapBldC * VRap

DO 1030 i=1, NChem
! Calculation of Skin Partition Coefficients
      !PSkNB(i) = ((0.3 * PFat(i) * PB(i)) + (0.7 * PSlw(i) * PB(i))) / PB(i)      !
Skin/blood partition
      PSkNL(i) = ((0.3 * PFat(i) * PB(i)) + (0.7 * PSlw(i) * PB(i))) / PLq(i)      !
Skin/saline partition

! Scaled Metabolism Parameters
      VMax(i) = VMaxC(i) * (BW**0.75)
      KF(i) = KFC(i) / (BW**0.25)

! Scaled Clearance Rates
      ClUr(i) = ClUrC(i) * (BW**0.75)
      kURT(i) = (min(kURTC(i), QPC)) * (BW**0.75)

! Scaled and Initial Doses
      AI0(i) = (Conc(i) * VCh * MW(i)) / 24450.0                                ! Initial amount
in chamber
      Drink(i) = (PDrink(i) * BW) / 24.0                                         ! Drinking water
dose (mg/hr)
      DDose(i) = ICSurf(i) * IVSurf(i)                                           ! Initial
dermal dose
      IV(i) = 0.0
      TotDose(i) = 0.0

! Initialize Initial Values for Integrating State Variables
      IAArt(i) = 0.0
      IABrn(i) = 0.0
      IABrnBld(i) = 0.0
      IAFat(i) = 0.0
      IAFatBld(i) = 0.0
      IALiv(i) = 0.0
      IALivBld(i) = 0.0
      IARap(i) = 0.0
      IARapBld(i) = 0.0
      IASlw(i) = 0.0
      IASlwBld(i) = 0.0

```



```

DISCRETE Calc      ! Calculate daily average AUC
INTERVAL CalcInt = 24.0

DO 1060 j3=1, NChem
  DAUCCArt(j3) = (AUCCArt(j3) - PAUCCArt(j3)) / (CalcInt / 24.0)
  DAUCCBrn(j3) = (AUCCBrn(j3) - PAUCCBrn(j3)) / (CalcInt / 24.0)

  IF (T .GT. 0.0) THEN
    PAUCCArt(j3) = AUCCArt(j3)
    PAUCCBrn(j3) = AUCCBrn(j3)
  ENDIF
1060: CONTINUE
END      ! End of Calc

!----- Calculate Parameters Used for Plotting -----
-----
      Hours = T
      Minutes = T * 60.0
      Days = T / 24.0

DO 2010 i1=1, NChem
! Concentration in Alveolar Air (mg/L and ppm)
  CALv(i1) = CArt(i1) / PB(i1)
  CALvPPM(i1) = (CArt(i1) / PB(i1)) * (24450.0 / MW(i1))

! Concentration in Inhaled Air (ppm)
  CP(i1) = (CInh(i1) * 24450.0) / MW(i1)

! Concentration in End-Exhaled Air (mg/L, ppm and percent)
  CEnd(i1) = RAExh(i1) / QAlv
  CEndPPM(i1) = CEnd(i1) * (24450.0 / MW(i1))
  IF (Conc(i1) .GT. 0.0) PerEnd(i1) = (CEnd(i1) / ((Conc(i1) * MW(i1)) / 24450.0))
* 100.0

! Concentration in Mixed Exhaled Air (mg/L, ppm and percent)
  CMix(i1) = ((1.0 - DS) * CEnd(i1)) + (DS * CInh(i1))
  CMixPPM(i1) = CMix(i1) * (24450.0 / MW(i1))
  IF (Conc(i1) .GT. 0.0) PerMix(i1) = (CMix(i1) / ((Conc(i1) * MW(i1)) / 24450.0))
* 100.0
2010: CONTINUE

      CArtTot = 0.0
      AExhAll = 0.0
DO 2020 i2=1, NChem
! Total Concentration in Arterial Blood (mg/L)
  CArtTot = CArtTot + CArt(i2)

! Total Amount Exhaled (mg)
  AExhAll = AExhAll + AExh(i2)
2020: CONTINUE

DERIVATIVE
!-----
-----
! Amount in Inhaled Air (mg)
  ACh = INTVC(RACh, AI0)

! Concentration in Inhaled Air (mg/L) and Rate of Change in Chamber Concentration
(mg/hr)
  PROCEDURAL (CInh, RACh = QAlv, VCh, kLC, kUrt, CIZone, CMuc, CArt)
DO 3030 i3=1, NChem
  CInh(i3) = (ACh(i3) / VCh) * CIZone

```

```

      RACH(i3) = (Rats * ((-QAlv * CInh(i3)) + (QAlv * (CArt(i3) / PB(i3))) +
(kURT(i3) * ((CMuc(i3) / PMuc(i3)) - (CArt(i3) / PB(i3)))))) &
      & - (kLC * ACh(i3))
3030: CONTINUE
      END
!-----
!-----

!-----
!-----
! Amount in Mucous (mg)
      AMuc = INTVC(RAMuc, IAMuc)

! Amount in Arterial Blood (mg)
      AArt = INTVC(RAArt, IAArt)
      AUCCArt = INTVC(CArt, IAUCCArt)

! Amount in Exhaled Air (mg)
      AExh = INTVC(RAExh, IAExh)

      PROCEDURAL (CMuc, CArt = VALv, VMuc)
      DO 3040 i4=1, NChem
        CMuc(i4) = AMuc(i4) / VMuc                                ! Concentration
of in mucous (mg/L)
        CArt(i4) = AArt(i4) / VALv                                ! Concentration
of in arterial blood (mg/L)
3040: CONTINUE
      END

      PROCEDURAL (CVen = QBrn, CBrnBld, QFat, CFatBld, QLiv, CLivBld, QRap, CRapBld, QSkn,
CSknDif, QSlw, CSLwBld, IV, RAUrn, QC)
      DO 3050 i5=1, NChem
! Concentration in Mixed Venous Blood (mg/L)
        CVen(i5) = (QBrn*CBrnBld(i5) + QFat*CFatBld(i5) + QLiv*CLivBld(i5) +
QRap*CRapBld(i5) + QSkn(i5)*CSknDif(i5) + QSlw(i5)*CSlwBld(i5) &
        & + IV(i5) - RAUrn(i5)) / QC
3050: CONTINUE
      END

      PROCEDURAL (RAArt = QAlv, CInh, kURT, CInh, CMuc, PMuc, CArt, PB, QC, CVen)
      DO 3051 i15=1, NChem

! Rate of Change in Arterial Blood (mg/hr)
        RAArt(i15) = (QAlv * CInh(i15)) - (kURT(i15) * (CInh(i15) - (CMuc(i15) /
PMuc(i15)))) - (QAlv * (CArt(i15) / PB(i15))) &
        & + (QC * (CVen(i15) - CArt(i15)))
3051: CONTINUE
      END

      PROCEDURAL (RAMuc, RAExh = kUrt, CInh, CMuc, PMuc, CArt, PB, QAlv)
      DO 3052 i25=1, NChem
! Rate of Change in Mucus (mg/hr)
        RAMuc(i25) = (kUrt(i25) * (CInh(i25) - (CMuc(i25) / PMuc(i25)))) - (kUrt(i25) *
((CMuc(i25) / PMuc(i25)) - (CArt(i25) / PB(i25))))
! Rate of Change in Exhaled Air (mg/hr)
        RAExh(i25) = (QAlv * (CArt(i25) / PB(i25))) + (kUrt(i25) * ((CMuc(i25) /
PMuc(i25)) - (CArt(i25) / PB(i25))))
3052: CONTINUE
      END

```

```

!-----
!-----
! Brain Blood

      ABrnBld = INTVC(RABrnBld, IABrnBld)          ! Amount in
brain blood (mg)
      ABrn = INTVC(RABrn, IABrn)                  ! Amount in
brain tissue (mg)

      PROCEDURAL (CBrnBld, CBrn, CBrnDif, RABrnBld = ABrnBld, VBrnBld, ABrn, VBrn, CBrn,
QBrn, PBrn, PABrn, CArt)
      DO 3060 i6=1, NChem
        CBrnBld(i6) = ABrnBld(i6) / VBrnBld          ! Concentration
in brain blood (mg/L)
        CBrn(i6) = ABrn(i6) / VBrn                  ! Concentration
in brain tissue (mg/L)
        CBrnDif(i6) = CBrn(i6) / PBrn(i6)            ! Concentration
available for diffusion (mg/L)
        RABrnBld(i6) = (QBrn *(CArt(i6) - CBrnBld(i6)))+(PABrn(i6) * (CBrnDif(i6) -
CBrnBld(i6)))
                                                    ! Rate of change
in brain blood (mg/hr)

      3060: CONTINUE
      END
!-----
!-----
! Brain Tissue

      AUCCBrn = INTVC(CBrn, IAUCCBrn)              ! Area under the
curve for the concentration in brain tissue (hr*mg/L)

      PROCEDURAL (RABrn = PABrn, CBrnBld, CBrnDif)
      DO 3061 i16=1, NChem
        RABrn(i16) = PABrn(i16) * (CBrnBld(i16) - CBrnDif(i16))    ! Rate of
change in brain tissue (mg/hr)

      3061: CONTINUE
      END
!-----
!-----
! Fat Blood

      AFatBld = INTVC(RAFatBld, IAFatBld)
                                                    ! Amount in fat blood (mg)

      PROCEDURAL (CFatBld, CFatDif, RAFatBld, CFat = AFatBld, VFatBld, AFat, QFat, VFat,
PFat, PAFat, CArt)
      DO 3070 i7=1, NChem
        CFatBld(i7) = AFatBld(i7) / VFatBld          ! Concentration
in fat blood (mg/L)
        CFat(i7) = AFat(i7) / VFat                  ! Concentration
in fat tissue (mg/L)

```

```

        CFatDif(i7) = CFat(i7) / PFat(i7)                                ! Concentration
in fat available for diffusion (mg/L)
        RAFatBld(i7) = (QFat * (CArt(i7) - CFatBld(i7))) + (PAFat(i7) * (CFatDif(i7) -
CFatBld(i7)))
                                                                    ! Rate of change
in fat blood (mg/hr)
        3070: CONTINUE
        END
!-----
!-----
!-----
! Fat Tissue
        AFat = INTVC(RAFat, IAFat)                                ! Amount in fat
tissue (mg)

        PROCEDURAL (RAFat = PAFat, CFatBld, CFatDif)
        DO 3071 i17=1, NChem
                RAFat(i17) = PAFat(i17) * (CFatBld(i17) - CFatDif(i17))    ! Rate of
change in fat tissue (mg/hr)

        3071: CONTINUE
        END
!-----
!-----
!-----
! Digestive Tract
! Amount Absorbed (mg)
        AO = INTVC(RAO, IAO)

! Amount Excreted in Feces (mg)
        AFec = INTVC(RAFec, IAFec)

! Amount Duodenum (mg)
        ADu = INTVC(RADu, IADu)

! Amount Leaving the Stomach (mg)
! (ASt changed to (INTVC(RASt, IASSt)) and is replaced in other equations with
(TotDose(i) - ASt(i)) since ASt
! could not be defined as (TotDose - INTVC(RASt, 0.0)))
        ASt = INTVC(RASt, IASSt)

! Rate of Change in Amount Absorbed, Amount Leaving Stomach (mg/hr)
        PROCEDURAL (RAO, RASt = kAS, TotDose, ASt, kAD, ADu, kTSD)
        DO 3080 i8=1, NChem
                RAO(i8) = (kAS(i8) * (TotDose(i8) - ASt(i8))) + (kAD(i8) * ADu(i8))
                RASt(i8) = (kAS(i8) * (TotDose(i8) - ASt(i8))) + (kTSD(i8) * (TotDose(i8) -
ASt(i8)))

3080: CONTINUE
        END

! Rate of Change in Amount in Duodenum and Amount Excreted (mg/hr)
        PROCEDURAL (RADu, RAFec = kTSD, TotDose, ASt, kAD, ADu, kFec)
        DO 3081 i18=1, NChem
                RADu(i18) = (kTSD(i18) * (TotDose(i18) - ASt(i18))) - (kAD(i18) * ADu(i18)) -
(kFec(i18) * ADu(i18))
                RAFec(i18) = kFec(i18) * ADu(i18)

3081: CONTINUE

```

```

END
!-----
!-----
! Liver Blood
      ALivBld = INTVC(RALivBld, IALivBld)                                ! Amount in liver
blood (mg)
      PROCEDURAL (CLivBld, CLiv, CLivDif, RALivBld = ALivBld, VlivBld, ALiv, Vliv, PLiv,
QLiv, CArt, PALiv)
      DO 3090 i9=1, NChem
          CLivBld(i9) = ALivBld(i9) / VlivBld                                !
Concentration in liver blood (mg/L)
          CLiv(i9) = ALiv(i9) / Vliv                                        !
Concentration in liver tissue (mg/L)
          CLivDif(i9) = CLiv(i9) / PLiv(i9)                                !
Concentration in liver available for diffusion (mg/L)
          RALivBld(i9) = (QLiv *(CArt(i9) - CLivBld(i9)))+(PALiv(i9) * (CLivDif(i9) -
CLivBld(i9)))
                                                                    ! Rate of
change in liver blood (mg/hr)
3090: CONTINUE
      END
!-----
!-----
! Liver Tissue
      ALiv = INTVC(RALiv, IALiv)                                ! Amount in
liver tissue (mg)
      AMet1 = INTVC(RAMet1, IAMet1)                                ! Amount
metabolized, saturable (mg)
      AMet2 = INTVC(RAMet2, IAMet2)                                ! Amount
metabolized, first order (mg)

      PROCEDURAL (RAMet1, RAMet2, RALiv = VMax, CLivDif, KM, KF, Vliv, PALiv, CLivBld,
RAO, Drink)
      DO 3091 i19=1, NChem
          RAMet1(i19) = (VMax(i19) * CLivDif(i19)) / (KM(i19) + CLivDif(i19))  !
Saturable rate of metabolism in liver (mg/hr)
          RAMet2(i19) = KF(i19) * CLivDif(i19) * Vliv                                ! First-
order rate of metabolism in liver (mg/hr)
          RALiv(i19) = (PALiv(i19) * (CLivBld(i19) - CLivDif(i19))) - RAMet1(i19) -
RAMet2(i19) + RAO(i19) + Drink(i19)
                                                                    ! Rate of
change in liver tissue (mg/hr)
3091: CONTINUE
      END
!-----
!-----
! Rapidly Perfused Tissue Blood

      ARapBld = INTVC(RARapBld, IARapBld)                                ! Amount in
rapidly perfused tissue blood (mg)

      PROCEDURAL (CRapBld, CRap, CRapDif, RARapBld = ARapBld, VRapBld, ARap, VRap, PRap,
QRap, PARap, CArt)
      DO 3100 i10=1, NChem

```

```

        CRapBld(i10) = ARapBld(i10) / VRapBld          ! Concentration
in rapidly perfused tissue blood (mg/L)
        CRap(i10) = ARap(i10) / VRap                  ! Concentration
in rapidly perfused tissue (mg/L)
        CRapDif(i10) = CRap(i10) / PRap(i10)          ! Concentration
in rapidly perfused tissue available for diffusion (mg/L)
        RARapBld(i10) = (QRap * (CArt(i10) - CRapBld(i10)))+(PARap(i10) * (CRapDif(i10) -
CRapBld(i10)))
                                                    ! Rate of change
in rapidly perfused tissue blood (mg/hr)

3100: CONTINUE
      END
!-----
!-----

!-----
!-----
! Rapidly Perfused Tissue

        ARap = INTVC(RARap, IARap)                    ! Amount in
rapidly perfused tissue (mg)

      PROCEDURAL (RARap = PARap, CRapBld, CRapDif)
      DO 3101 i20=1, NChem
        RARap(i20) = PARap(i20) * (CRapBld(i20) - CRapDif(i20))    ! Rate of change
in rapidly perfused tissue (mg/hr)
3101: CONTINUE
      END
!-----
!-----

!-----
!-----
! Skin
!Amount on Skin Surface (mg)
      DermZone = RSW(T.LT.TChng, 1.0, 0.0)
      AAbs = INTVC(RAAbs, IAAbs)

! Amount in Skin (mg)
      ASkn = INTVC(RASkn, IASkn)

      PROCEDURAL (CSurf, CSkn, CSknDif, RAAbs, RASkn = ICSurf, AAbs, VSkn, ASkn, PSknB,
KP, Area, QSkn, CArt)
      DO 3110 i11=1, NChem
        CSurf(i11) = ((1000.0 * ICSurf(i11))- (AAbs(i11)/VSkn(i11))) !* DermZone
        CSkn(i11) = ASkn(i11) / VSkn(i11)                        ! Concentration
in skin (mg/L)
        CSknDif(i11) = CSkn(i11) / PSknB(i11)                  ! Concentration
available for diffusion (mg/L)

! Rate of Change in Amount Absorbed in Skin (mg/hr)
        RAAbs(i11) = ((KP(i11) * Area(i11)) / 1000.0) * CSurf(i11)

! Rate of Change in Skin (mg/hr)
        RASkn(i11) = (QSkn(i11) * (CArt(i11) - CSknDif(i11))) + RAAbs(i11)
3110: CONTINUE
      END
!-----
!-----

!-----
!-----

```

```

! Slowly Perfused Tissue Blood

      ASlwBld = INTVC(RASlwBld, IASlwBld)                ! Amount in
slowly perfused tissue blood (mg)

      PROCEDURAL (CSlwBld, CSlw, CSlwDif, RASlwBld = ASlwBld, VSlwBld, ASlw, VSlw, PSlw,
QSlw, CArt, PASlw)
      DO 3120 i12=1, NChem
        CSlwBld(i12) = ASlwBld(i12) / VSlwBld(i12)        ! Concentration
in slowly perfused tissue blood (mg/L)
        CSlw(i12) = ASlw(i12) / VSlw(i12)                ! Concentration
in slowly perfused tissue (mg/L)
        CSlwDif(i12) = CSlw(i12) / PSlw(i12)              ! Concentration
in slowly perfused tissue available for diffusion (mg/L)
        RASlwBld(i12) = (QSlw(i12) *(CArt(i12) - CSlwBld(i12)))+(PASlw(i12) *
(CSlwDif(i12) - CSlwBld(i12)))
                                                                    ! Rate of change
in slowly perfused tissue blood (mg/hr)

3120: CONTINUE
      END
!-----
!-----

!-----
!-----
! Slowly Perfused Tissue

      ASlw = INTVC(RASlw, IASlw)                ! Amount in
slowly perfused tissue (mg)

      PROCEDURAL (RASlw = PASlw, CSlwBld, CSlwDif)
      DO 3121 i22=1, NChem
        RASlw(i22) = PASlw(i22) * (CSlwBld(i22) - CSlwDif(i22))    ! Rate of change
in slowly perfused tissue (mg/hr)

3121: CONTINUE
      END
!-----
!-----

!-----
!-----
! Amount in Urine (mg)
      AUrn = INTVC(RAUrn, IAUrn)

! Rate of Urinary Excretion (mg/hr)
      PROCEDURAL (RAUrn = ClUr, CArt)
      DO 3130 i13=1, NChem
        RAUrn(i13) = ClUr(i13) * CArt(i13)

3130: CONTINUE
      END
!-----
!-----

!----- CHECK MASS BALANCE -----
!-----
! Total Amount Inhaled (mg)
      ATotInh = INTVC(RATotInh, IATotInh)

! Total Amount Injected (mg)
      ATotIV = INTVC(IV, IATotIV)

```

```

! Total Amount Drunk (mg)
  ATotDrink = INTVC(Drink, IATotDrink)

! Calculating Total Dose (mg), Total Amount in Body (mg) and Mass Balance (mg)
  PROCEDURAL (TDose, AmtBody, MassBal = QAlv, IV, TotDose, Drink, DDose, CInh,
ASurf)
    DO 3140 i14=1, NChem
      RATotInh(i14) = QAlv * CInh(i14)
      TDose(i14) = ATotInh(i14) + ATotIV(i14) + TotDose(i14) +
ATotDrink(i14) + (DDose(i14) - ASurf(i14))
      AmtBody(i14) = AMuc(i14) + AArt(i14) + ABrn(i14) + ABrnBld(i14) +
AFat(i14) + AFatBld(i14) + ALiv(i14) + ALivBld(i14) &
& + ARap(i14) +
ARapBld(i14) + ASkn(i14) + ASlw(i14) + ASlwBld(i14) + AExh(i14) &
& + AUrn(i14) + AMet1(i14)
+ AMet2(i14) + AFec(i14) + ADu(i14) + ASt(i14)
      MassBal(i14) = TDose(i14) - AmtBody(i14)
3140: CONTINUE
    END

TERMT(T.GT.TStop, 'Simulation Finished')

END          ! End of Derivative
END          ! End of Dynamic
END          ! End of Program

```

APPENDIX G. M FILES FOR ARRAY MODEL OPERATIONS

Note: Report formatting may introduce extraneous line breaks not in the original code.

Init.m

```
% Initial model settings
% Set to Execute at Load-time!!!!!!!!!!!!!!

prepare T HOURS MINUTES DAYS CP CMUC CALV CALVPPM CART AUCCART AEXH CEND CENDPPM
PEREND PERMIX CBRN AUCCBRN CFAT CLIV AMET1 AMET2
prepare CRAP CSKN CSLW CVEN AURN TDOSE AMTBODY MASSBAL AFEC ASKN AABS AST AO ICSURF
CSKN CSURF

HVDPRN=0;
WESITG=0;
WEDITG=0;
```

ResetDoses.m

```
CONC(1)=0.0; IVDOSE(1)=0.0; PDOSE(1)=0.0; PDRINK(1)=0.0;
CONC(2)=0.0; IVDOSE(2)=0.0; PDOSE(2)=0.0; PDRINK(2)=0.0;
CONC(3)=0.0; IVDOSE(3)=0.0; PDOSE(3)=0.0; PDRINK(3)=0.0;
CONC(4)=0.0; IVDOSE(4)=0.0; PDOSE(4)=0.0; PDRINK(4)=0.0;
CONC(5)=0.0; IVDOSE(5)=0.0; PDOSE(5)=0.0; PDRINK(5)=0.0;

TCHNG=0.0; DAYSWK=1.0; TMAX=24.0; TINF=1.0; RATS=1.0;

AREA(1)=0.0; SKNPERM(1)=0.0; ICSURF(1)=0.0; IVSURF(1)=0.0;
AREA(2)=0.0; SKNPERM(2)=0.0; ICSURF(2)=0.0; IVSURF(2)=0.0;
AREA(3)=0.0; SKNPERM(3)=0.0; ICSURF(3)=0.0; IVSURF(3)=0.0;
AREA(4)=0.0; SKNPERM(4)=0.0; ICSURF(4)=0.0; IVSURF(4)=0.0;
AREA(5)=0.0; SKNPERM(5)=0.0; ICSURF(5)=0.0; IVSURF(5)=0.0;

CC=0; VCHC=9.1; KLCC=0.0;
DOSEINT=1000.0; CINT=0.01;
```

APPENDIX H. ARRAY MODEL M FILES FOR PHYSIOLOGICAL PARAMETERS

Note: Report formatting may introduce extraneous line breaks not in the original code.

Human.m

```
BW=70.0; QCC=12.89; QPC=27.75;
QBRNC=0.114; QFATC=0.052; QLIVC=0.227; QRAPC=0.419; QSKNC=0.058; QSLWC=0.188;
VALVC=0.0079; VBRNC=0.02; VFATC=0.214; VLIVC=0.026; VMUCC=0.0001; VRAPC=0.036;
VSLWC=0.536;
VBRNBLDC=0.04; VFATBLDC=0.02; VLIVBLDC=0.11; VRAPBLDC=0.17; VSLWBLDC=0.0375;
DEPTH=0.1;

% Generic values from Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton
MI, Andersen ME:
% Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
% Toxicol Sci 2001, 63(2):160-172.

% Blood content values for most tissues from published TMB-4 model
% Sterner TR, Ruark CD, Covington TR, Yu KO, Gearhart JM. 2013. A physiologically
based pharmacokinetic
% model for the oxime TMB-4: simulation of rodent and human data. Archives of
toxicology. 87(4):661-680.
```

Rat.m

```
BW=0.22; QCC=14.6; QPC=24.75;
QBRNC=0.02; QFATC=0.07; QLIVC=0.183; QRAPC=0.557; QSKNC=0.058; QSLWC=0.17;

VALVC=0.007; VBRNC=0.006; VFATC=0.10; VLIVC=0.034; VMUCC=0.0001; VRAPC=0.044;
VSLWC=0.65;
VBRNBLDC=0.03; VFATBLDC = 0.0154; VLIVBLDC = 0.21; VRAPBLDC = 0.2075; VSLWBLDC =
0.0333;
DEPTH=0.10; %Skin thickness (cm)

% Generic values from Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton
MI, Andersen ME:
% Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
% Toxicol Sci 2001, 63(2):160-172.
% Blood content values for most tissues from Brown et al. 1997
% VBrnBldC from published TMB-4 model
% Sterner TR, Ruark CD, Covington TR, Yu KO, Gearhart JM. 2013. A physiologically
based pharmacokinetic
% model for the oxime TMB-4: simulation of rodent and human data. Archives of
toxicology. 87(4):661-680.
```

APPENDIX I. ARRAY MODEL M FILES FOR PHYSICOCHEMICAL PARAMETERS

Note: Report formatting may introduce extraneous line breaks not in the original code.

RatDEET.m

```
% Sets rat DEET parameters

%----- Chemical Parameters-----
MW(1)=191.27; %https://en.wikipedia.org/wiki/DEET
DS=0.15; %Clewell et al. 2001

%-----PCs-----
PB(1)=609.0; %Predicted using Buist et al. 2012 algorithm
PLQ(1)=609.0; PMUC(1)=609.0;

% Apparent PCs from Fediuk et al. 2010 and 2011
% Other values predicted by Ruark method to be: fat = 14.1*kidney, skin = 0.92*kidney,
and slow = 0.54*kidney (below)
PBRN(1)=0.212; PFAT(1)=14.1*1.57; PLIV(1)=2.28; PRAP(1)=1.57; PSLW(1)=0.54*1.57;

% Fit PC for skin based on apparent uptake
PSKNB(1)=0.12;

%-----PAs-----
% Diffusion Limitation - not needed = 1000.0
PABRN(1)=0.1;
PAFAT(1)=0.1;
PALIV(1)=1.0;
PARAP(1)=10.0;
PASLW(1)=0.1;

%-----Metabolism-----
VMAXC(1)=14.0; KM(1)=12.8; KFC(1)=0.6;
% Km (4.39 & 12.8) = weighted average values for both major metabolites from rat
liver microsomes (Usmani et al., 2002)
%Snodgrass et al. (1982) IV data indicate that entire dose is not excreted in urine
% Added a bit of first order metabolism that would be removed fecally

%-----Clearance-----
KURTC(1)=1.0; % DEET has been inhaled and caused neurological issues. No ADME
studies have been conducted with inhalation.
% It is not considered a major route of exposure. (ATSDR Tox Profile,
2017)
CLURC(1)=0.0; % In piglets, no DEET was excreted except as a metabolite
(Kasichayanula et al. 2007)

%-----Oral Uptake-----
KAS(1)=0.2; KTSD(1)=6.0; KAD(1)=1.0; KFEC(1)=0.25;

%-----Dermal Uptake-----
KP(1)=0.002; %Calculated by Peter Robinson using Wilschut et al. 1995 = 0.0029

% Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton MI, Andersen ME:
% Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
% Toxicol Sci 2001, 63(2):160-172.
% Buist HE, de Wit-Bos L, Bouwman T, Vaes WHJ. 2012. Predicting blood:air partition
coefficients using basic
% physicochemical properties. Regul Toxicol Pharmacol 62:23-28.
```

```
% Ruark CD, Hack CE, Robinson PJ, Mahle DA, Gearhart JM. 2014. Predicting passive and
active tissue:plasma
% partition coefficients: interindividual and interspecies variability. Journal of
pharmaceutical sciences.
% 103(7):2189-2198.
% Kasichayanula S, House JD, Wang T, Gu X. 2007. Percutaneous characterization of the
insect repellent DEET
% and the sunscreen oxybenzone from topical skin application. Toxicol Appl
Pharmacol 223:187-94.
% Schoenig GP, Hartnagel RE, Osimitz TG, Llanos S. 1996. Absorption, distribution,
metabolism, and excretion
% of N,N-diethyl-m-toluamide in the rat. Drug Metab. Disposit. 24(2): 156-163.
% Snodgrass HL, Nelson DC, Weeks MH. 1982. Dermal penetration and potential for
placental transfer
% of the insect repellent, N, N-diethyl-m-toluamide. Am. Ind. Hyg. Assoc. J.
43(10): 747-753.
% Usmani KA, Rose RL, Goldstein JA, Taylor WG, Brimfield AA, Hodgson E. 2002. In vitro
human metabolism and
% interactions of repellent n,n-diethyl-m-toluamide. Drug Metab Disposit. 30(3):
289-294.
% Wilschut, A., Berge, ten, W. F., Robinson, P. J., & McKone, T. E. (1995). Estimating
skin permeation. The validation
% of five mathematical skin permeation models. Chemosphere, 30(7), 1275-1296
```

RatPB_fast.m

```
% Sets rat pyridostigmine bromide parameters for fasted animals

MW(2)=261.119; % https://pubchem.ncbi.nlm.nih.gov/compound/pyridostigmine_bromide
DS=0.15;      % IPA model

PB(2)=200000.0; PLQ(2)=200000.0; PMUC(2)=200000.0; % Not volatile
%Values below based on Watson 2001, mostly from apparent PCs published by Yamamoto
et al. 1995
PBRN(2)=0.19*15.0; % Predicted to be 19% of kidney PC
PFAT(2)=0.21; % 0.21 Watson 2001
PLIV(2)=8.0; % Yamamoto 8 (6-11, +/- SE)
PRAP(2)=15.0; % Kidney, Yamamoto 15 (10-20, +/- SE)
PSLW(2)=0.5; % Muscle from Yamamoto 0.5 (0.4-0.6, +/- SE)

%Values if we want to use PA to simulate plasma binding (80% bound in rat)
PABRN(2)=0.1; PAFAT(2)=0.1; PALIV(2)=0.1; PARAP(2)=0.1; PASLW(2)=5.0;

VMAXC(2)=2.0;
KM(2)=1.5; % 1.5 Watson 2001;

CLURC(2)=1.365; % 1.365 Golomb
% Yamamoto has really low clearance (0.25), probably due to 2
hours post-anesthesia
% Barber uses 0.35
KURTC(2)=0.001; % URT uptake is going to be low - there won't be any in the air
anyway
KAS(2)=0.1;
KTSD(2)=1.5;
KAD(2)=0.3;
KFEC(2)=0.00038; % Biliary excretion is about 0.23% at 6 hours (Yamamoto)

% Watson KM. 2001. Thesis. A Pharmacokinetic Study of the Effects of Stress and
Exercise on Chemical Exposure.
% Wright-Patterson AFB OH: Air Force Institute of Technology. AFIT/GEE/ENV/01M-
23.
```

```
% Yamamoto K, Sawada Y, Iga T. 1995. Comparative pharmacokinetics of four
cholinesterase inhibitors in rats.
% Biol. Pharm. Bull. 18(9): 1292-95.
% Golomb BA. 1999. Appendix A-Pyridostigmine Pharmacokinetic Data. In: A Review of
the Scientific Literature
% as it Pertains to Gulf War Illnesses: Volume 2: Pyridostigmine Bromide. Santa
Monica, CA: RAND Corporation.
% <https://www.rand.org/pubs/monograph\_reports/MR1018z2.html>.
```

RatPB_fed.m

```
% Sets rat pyridostigmine bromide parameters in fed animals

MW(2)=261.119; % https://pubchem.ncbi.nlm.nih.gov/compound/pyridostigmine\_bromide
DS=0.15;      % IPA model

PB(2)=200000.0; PLQ(2)=200000.0; PMUC(2)=200000.0; % Not volatile
%Values below based on Watson 2001, mostly from apparent PCs published by Yamamoto
et al. 1995
PBRN(2)=0.19*15.0; % Predicted to be 19% of kidney PC
PFAT(2)=0.21; % 0.21 Watson 2001
PLIV(2)=8.0; % Yamamoto 8 (6-11, +/- SE)
PRAP(2)=15.0; % Kidney, Yamamoto 15 (10-20, +/- SE)
PSLW(2)=0.5; % Muscle from Yamamoto 0.5 (0.4-0.6, +/- SE)

%Fed animals have different uptake parameters which negates the need for diffusion
limitation
PABRN(2)=10000.0; PAFAT(2)=10000.0; PALIV(2)=10000.0; PARAP(2)=10000.0;
PASLW(2)=10000.0;

VMAXC(2)=2.0;
KM(2)=1.5; % 1.5 Watson 2001;
KFC(2)=0.0;

CLURC(2)=1.365; % 1.365 Golomb
% Yamamoto has really low clearance (0.25), probably due to 2
hours post-anesthesia
% Barber uses 0.35
KURTC(2)=0.001; % URT uptake is going to be low - there won't be any in the air
anyway
KFEC(2)=0.00038; % Biliary excretion is about 0.23% at 6 hours (Yamamoto)

%Fed animals have different uptake parameters
KAS(2)=0.1; KTSD(2)=0.75; KAD(2)=0.03;
%KTSD was 1.5 and KAD was 0.3 with fasted animals
%McLean 2016 saw a 2 fold decrease in a hydrophilic drug absorption with fed mice

% Watson KM. 2001. Thesis. A Pharmacokinetic Study of the Effects of Stress and
Exercise on Chemical Exposure.
% Wright-Patterson AFB OH: Air Force Institute of Technology. AFIT/GEE/ENV/01M-
23.
% Yamamoto K, Sawada Y, Iga T. 1995. Comparative pharmacokinetics of four
cholinesterase inhibitors in rats.
% Biol. Pharm. Bull. 18(9): 1292-95.
% Golomb BA. 1999. Appendix A-Pyridostigmine Pharmacokinetic Data. In: A Review of
the Scientific Literature
% as it Pertains to Gulf War Illnesses: Volume 2: Pyridostigmine Bromide. Santa
Monica, CA: RAND Corporation.
% <https://www.rand.org/pubs/monograph\_reports/MR1018z2.html>.
```

HumanPB.m

```

% Sets human pyridostigmine bromide parameters

MW(2)=261.119; % https://pubchem.ncbi.nlm.nih.gov/compound/pyridostigmine_bromide
DS=0.15;      % IPA model

PB(2)=200000.0; PLQ(2)=200000.0; PMUC(2)=200000.0; % Not volatile
%Values below based on Watson 2001, mostly from PCs published by Yamamoto et al.
1995
PBRN(2)=0.19*15.0; % Predicted to be 19% of kidney PC
PFAT(2)=0.21; % 0.21 Watson 2001
PLIV(2)=8.0; % Yamamoto 8 (6-11, +/- SE)
PRAP(2)=15.0; % Kidney, Yamamoto 15 (10-20, +/- SE)
PSLW(2)=0.5; % Muscle from Yamamoto 0.5 (0.4-0.6, +/- SE)

%Values if we want to use PA to simulate plasma binding (>80% bound in humans at low
doses; ~20% at high doses)
PABRN(2)=5.0; PAFAT(2)=5.0; PALIV(2)=5.0; PARAP(2)=5.0; PASLW(2)=5.0;

VMAXC(2)=2.0;
KM(2)=1.5; % 1.5 Watson 2001;
KFC(2)=0.0;

CLURC(2)=1.365; % 1.365 Golomb
KURTC(2)=0.001; % URT uptake is going to be low - there won't be any in the air
anyway

KAD(2)=1.0; % Rat = 0.3, may be due to use of corn oil
KAS(2)=0.1; % 2.0
KFEC(2)=0.00038; % Biliary excretion is about 0.23% at 6 hours (Yamamoto)
KTSD(2)=0.5;

% Watson KM. 2001. Thesis. A Pharmacokinetic Study of the Effects of Stress and
Exercise on Chemical Exposure.
% Wright-Patterson AFB OH: Air Force Institute of Technology. AFIT/GEE/ENV/01M-
23.
% Yamamoto K, Sawada Y, Iga T. 1995. Comparative pharmacokinetics of four
cholinesterase inhibitors in rats.
% Biol. Pharm. Bull. 18(9): 1292-95.
% Golomb BA. 1999. Appendix A-Pyridostigmine Pharmacokinetic Data. In: A Review of
the Scientific Literature
% as it Pertains to Gulf War Illnesses: Volume 2: Pyridostigmine Bromide. Santa
Monica, CA: RAND Corporation.
% <https://www.rand.org/pubs/monograph_reports/MR1018z2.html>.

```

RatPERc_fast.m

```

% Sets rat cis-permethrin parameters for fasted rats

MW(3)=391.288; %https://pubchem.ncbi.nlm.nih.gov/compound
DS=0.15; % Dead space, Clewell et al. IPA model

PB(3)=103000.0; %Predicted using Buist et al., 2012
PLQ(3)=103000.0; PMUC(3)=103000.0;

%Values below from Willemin et al. 2016
PBRN(3)=1.6; PFAT(3)=225.0; PLIV(3)=0.89; PRAP(3)=1.1; PSLW(3)=1.2;
%In this reference, muscle (1.2) and slowly perfused tissues (19.0) are separate
PSKNB(3)=8.2; %Ruark predicts skin to be 8.2 or 0.98*kidney;

% Diffusion Limitation
%Tornerio-Velez et al. 2012 (1st #) and Willemin et al. 2016 (2nd #) model values in
comment for retention

```

```

PABRN(3)=0.0005;    %0.003, 0.001**
PAFAT(3)=0.048;     %0.1, 0.048
PASLW(3)=0.31;      %0.7, 0.32(muscle) 0.31(slowly perfused)
%Tornerio-Velez et al. 2012 and Willemmin et al. 2016 models did not use diffusion
limitation for these tissues (default = 1000.0)
PALIV(3)=1000.0;
PARAP(3)=1000.0;

VMAXC(3)=2.51; % Willemmin optimized number for liver metabolism = 2.4 L/h/kg (2.51
if you add all metabolism together)
KM(3)=1.0;
KFC(3)=0.0;

CLURC(3)=0.0; %Tornerio-Velez et al. 2012 model - no urinary clearance
KURTC(3)=0.001; %URT uptake is going to be low - there won't be any in the air
anyway
KAS(3)=0.01; %Stomach uptake (0.01/hr) Tornerio-Velez et al. 2012 model, Willemmin
did not use uptake from the stomach
KFEC(3)=0.39; %Fecal excretion 0.39/hr in Willemmin model

%Fasted parameters
KTSD(3)=0.7; %Stomach-intestine transfer, Tornerio-Velez et al. 2012 model (0.7/hr
KAD(3)=0.9; %Absorption from duodenum (/hr)
%Intestinal transfer (0.9/hr) Tornerio-Velez et al. 2012 model

%-----Dermal Uptake-----
KP(3)=0.07; %0.07 = Calculated by Peter Robinson using Wilschut et al. 1995

% IPA model values from Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton
MI, Andersen ME:
% Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
% Toxicol Sci 2001, 63(2):160-172.
% Tornerio-Velez R, Davis J, Scollon EJ, Starr JM, Setzer RW, Goldsmith M-R, Chang DT,
Xue J,
% Zartarian V, DeVito MJ, Hughes MF. 2012. A pharmacokinetic model of cis- and
trans-
% permethrin disposition in rats and humans with aggregate exposure application.
Toxicol.
% Sci. 130(1): 33-47.
% Willemmin M-E, Desmots S, Le Grand R, Lestremiau F, Zeman FA, Leclerc E, Moesch C,
% Brochot C. 2016. PBPK modeling of the cis- and trans-permethrin isomers and
% their major urinary metabolites in rats. Toxicol. Appl. Pharmacol 294:65-77.
% Buist HE, de Wit-Bos L, Bouwman T, Vaes WHJ. 2012. Predicting blood:air partition
coefficients using
% basic physicochemical properties. Regul Toxicol Pharmacol 62:23-28.
% Wilschut, A., Berge, ten, W. F., Robinson, P. J., & McKone, T. E. (1995). Estimating
skin permeation. The validation
% of five mathematical skin permeation models. Chemosphere, 30(7), 1275-1296
% Ruark CD, Hack CE, Robinson PJ, Mahle DA, Gearhart JM. 2014. Predicting passive and
active tissue:plasma
% partition coefficients: interindividual and interspecies variability. Journal of
pharmaceutical sciences.
% 103(7):2189-2198.

RatPERc_fed.m
% Sets rat cis-permethrin parameters for fed rats

MW(3)=391.288; %https://pubchem.ncbi.nlm.nih.gov/compound
DS=0.15; % Dead space, Clewell et al. IPA model

```

```

PB(3)=103000.0; %Predicted using Buist et al., 2012
PLQ(3)=103000.0; PMUC(3)=103000.0;
%Values below from Willemín et al. 2016
PBRN(3)=1.6; PFAT(3)=225.0; PLIV(3)=0.89; PRAP(3)=1.1; PSLW(3)=1.2;
    %In this reference, muscle (1.2) and slowly perfused tissues (19.0) are separate
PSKNB(3)=8.2;    %Ruark predicts skin to be 8.2 or 0.98*kidney;

% Diffusion Limitation
    %Torneró-Velez et al. 2012 (1st #) and Willemín et al. 2016 (2nd #) model values in
comment for retention
    PABRN(3)=0.0005;    %0.003, 0.001**
    PAFAT(3)=0.048;    %0.1, 0.048
    PASLW(3)=0.31;    %0.7, 0.32(muscle) 0.31(slowly perfused)
    %Torneró-Velez et al. 2012 and Willemín et al. 2016 models did not use diffusion
limitation for these tissues (default = 1000.0)
    PALIV(3)=1000.0;
    PARAP(3)=1000.0;

    VMAXC(3)=2.51; % Willemín optimized number for liver metabolism = 2.4 L/h/kg (2.51
if you add all metabolism together)
    KM(3)=1.0;
    KFC(3)=0.0;

    CLURC(3)=0.0;    %Torneró-Velez et al. 2012 model - no urinary clearance
    KURTC(3)=0.001; %URT uptake is going to be low - there won't be any in the air
anyway

    KAD(3)=0.52;    %Absorption from duodenum (/hr), 0.52/h in Willemín et al. 2016
    KAS(3)=0.01;    %Stomach uptake (0.01/hr) Torneró-Velez et al. 2012 model, Willemín
did not use uptake from the stomach
    KFEC(3)=0.39;    %Fecal excretion 0.39/hr in Willemín model
    KTSD(3)=0.35;    %Stomach-intestine transfer 0.35 in Willemín

%-----Dermal Uptake-----
    KP(3)=0.07;    %0.07 = Calculated by Peter Robinson using Wilschut et al. 1995

% IPA model values from Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton
MI, Andersen ME:
    % Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
    % Toxicol Sci 2001, 63(2):160-172.
% Torneró-Velez R, Davis J, Scollon EJ, Starr JM, Setzer RW, Goldsmith M-R, Chang DT,
Xue J,
    % Zartarian V, DeVito MJ, Hughes MF. 2012. A pharmacokinetic model of cis- and
trans-
    % permethrin disposition in rats and humans with aggregate exposure application.
Toxicol.
    % Sci. 130(1): 33-47.
% Willemín M-E, Desmots S, Le Grand R, Lestremau F, Zeman FA, Leclerc E, Moesch C,
    % Brochot C. 2016. PBPK modeling of the cis- and trans-permethrin isomers and
    % their major urinary metabolites in rats. Toxicol. Appl. Pharmacol 294:65-77.
% Buist HE, de Wit-Bos L, Bouwman T, Vaes WHJ. 2012. Predicting blood:air partition
coefficients using
    % basic physicochemical properties. Regul Toxicol Pharmacol 62:23-28.
% Wilschut, A., Berge, ten, W. F., Robinson, P. J., & McKone, T. E. (1995). Estimating
skin permeation. The validation
    % of five mathematical skin permeation models. Chemosphere, 30(7), 1275-1296
% Ruark CD, Hack CE, Robinson PJ, Mahle DA, Gearhart JM. 2014. Predicting passive and
active tissue:plasma
    % partition coefficients: interindividual and interspecies variability. Journal of
pharmaceutical sciences.
    % 103(7):2189-2198.

```

RatPERT_fast.m

```
% Sets rat trans-permethrin parameters in fasted animals

MW(4)=391.288; %https://pubchem.ncbi.nlm.nih.gov/compound
DS=0.15; % Dead space, Clewell et al. IPA model

PB(4)=103000.0; %Predicted using Buist et al., 2012
PLQ(4)=103000.0; PMUC(4)=103000.0;
%Values below from Willemin et al. 2016
PBRN(4)=0.57; PFAT(4)=76.0; PLIV(4)=0.21; PRAP(4)=0.21; PSLW(4)=0.82;
    %In this reference, muscle (0.82) and slowly perfused tissues (8.4) are separate
PSKNB(4)=8.2; %Ruark predicts skin to be 8.2 or 0.98*kidney;

% Diffusion Limitation
%Tornerio-Velez et al. 2012 (1st #) and Willemin et al. 2016 (2nd #) model values in
comment for retention
PABRN(4)=0.0005; %0.003, 0.0012**
PAFAT(4)=0.11; %0.1, 0.11
PASLW(4)=0.48; %0.7, 0.48(muscle) 0.065(slowly perfused)
%Tornerio-Velez et al. 2012 and Willemin et al. 2016 models did not use diffusion
limitation for these tissues (default = 1000.0)
PALIV(4)=1000.0;
PARAP(4)=1000.0;

VMAXC(4)=6.3; % Willemin optimized for liver metabolism = 5.7 L/h/kg (6.3 if you
add all metabolism together)
KM(4)=1.0;
KFC(4)=0.0;

CLURC(4)=0.0; %Tornerio-Velez et al. 2012 model - no urinary clearance
KURTC(4)=0.001; %URT uptake is going to be low - there won't be any in the air
anyway

KAS(4)=0.01; %Stomach uptake (0.01/hr) Tornerio-Velez et al. 2012 model, Willemin
did not use uptake from the stomach
KFEC(4)=0.85; %Fecal excretion 0.85/hr in Willemin model
%Fasted parameters
KTSD(4)=0.7; %Stomach-intestine transfer Tornerio-Velez et al. 2012 model (0.7/hr)
KAD(4)=0.9; %Absorption from duodenum (/hr)
    %Intestinal transfer (0.9/hr) Tornerio-Velez et al. 2012 model

%-----Dermal Uptake-----
KP(4)=0.07; %0.07 = Calculated by Peter Robinson using Wilschut et al. 1995

% IPA model values from Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton
MI, Andersen ME:
% Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
% Toxicol Sci 2001, 63(2):160-172.
% Tornerio-Velez R, Davis J, Scollon EJ, Starr JM, Setzer RW, Goldsmith M-R, Chang DT,
Xue J,
% Zartarian V, DeVito MJ, Hughes MF. 2012. A pharmacokinetic model of cis- and
trans-
% permethrin disposition in rats and humans with aggregate exposure application.
Toxicol.
% Sci. 130(1): 33-47.
% Willemin M-E, Desmots S, Le Grand R, Lestremau F, Zeman FA, Leclerc E, Moesch C,
% Brochot C. 2016. PBPK modeling of the cis- and trans-permethrin isomers and
% their major urinary metabolites in rats. Toxicol. Appl. Pharmacol 294:65-77.
```

```
% Buist HE, de Wit-Bos L, Bouwman T, Vaes WHJ. 2012. Predicting blood:air partition
coefficients using
%   basic physicochemical properties. Regul Toxicol Pharmacol 62:23-28.
% Wilschut, A., Berge, ten, W. F., Robinson, P. J., & McKone, T. E. (1995). Estimating
skin permeation. The validation
%   of five mathematical skin permeation models. Chemosphere, 30(7), 1275-1296
% Ruark CD, Hack CE, Robinson PJ, Mahle DA, Gearhart JM. 2014. Predicting passive and
active tissue:plasma
%   partition coefficients: interindividual and interspecies variability. Journal of
pharmaceutical sciences.
%   103(7):2189-2198.
```

RatPERT_fed.m

```
% Sets rat trans-permethrin parameters in fed animals

MW(4)=391.288; %https://pubchem.ncbi.nlm.nih.gov/compound
DS=0.15; % Dead space, Clewell et al. IPA model

PB(4)=103000.0; %Predicted using Buist et al., 2012
PLQ(4)=103000.0; PMUC(4)=103000.0;
%Values below from Willemin et al. 2016
PBRN(4)=0.57; PFAT(4)=76.0; PLIV(4)=0.21; PRAP(4)=0.21; PSLW(4)=0.82;
%In this reference, muscle (0.82) and slowly perfused tissues (8.4) are separate
PSKNB(4)=8.2; %Ruark predicts skin to be 8.2 or 0.98*kidney;

% Diffusion Limitation
%Tornero-Velez et al. 2012 (1st #) and Willemin et al. 2016 (2nd #) model values in
comment for retention
PABRN(4)=0.0005; %0.003, 0.0012**
PAFAT(4)=0.11; %0.1, 0.11
PASLW(4)=0.48; %0.7, 0.48(muscle) 0.065(slowly perfused)
%Tornero-Velez et al. 2012 and Willemin et al. 2016 models did not use diffusion
limitation for these tissues (default = 1000.0)
PALIV(4)=1000.0;
PARAP(4)=1000.0;

VMAXC(4)=6.3; % Willemin optimized for liver metabolism = 5.7 L/h/kg (6.3 if you
add all metabolism together)
KM(4)=1.0;
KFC(4)=0.0;

CLURC(4)=0.0; %Tornero-Velez et al. 2012 model - no urinary clearance
KURTC(4)=0.001; %URT uptake is going to be low - there won't be any in the air
anyway

KAD(4)=1.30; %Absorption from duodenum (/hr), 1.30/h in Willemin et al. 2016
KAS(4)=0.01; %Stomach uptake (0.01/hr) Tornero-Velez et al. 2012 model, Willemin
did not use uptake from the stomach
KFEC(4)=0.85; %Fecal excretion 0.85/hr in Willemin model
KTSD(4)=0.20; %Stomach-intestine transfer 0.20 in Willemin

%-----Dermal Uptake-----
KP(4)=0.07; %0.07 = Calculated by Peter Robinson using Wilschut et al. 1995

% IPA model values from Clewell HJ, III, Gentry PR, Gearhart JM, Covington TR, Banton
MI, Andersen ME:
%   Development of a physiologically based pharmacokinetic model of isopropanol and
its metabolite acetone.
%   Toxicol Sci 2001, 63(2):160-172.
% Tornero-Velez R, Davis J, Scollon EJ, Starr JM, Setzer RW, Goldsmith M-R, Chang DT,
Xue J,
```

% Zartarian V, DeVito MJ, Hughes MF. 2012. A pharmacokinetic model of cis- and trans-permethrin disposition in rats and humans with aggregate exposure application. Toxicol. Sci. 130(1): 33-47.

% Willemain M-E, Desmots S, Le Grand R, Lestremieu F, Zeman FA, Leclerc E, Moesch C, Brochet C. 2016. PBPK modeling of the cis- and trans-permethrin isomers and their major urinary metabolites in rats. Toxicol. Appl. Pharmacol 294:65-77.

% Buist HE, de Wit-Bos L, Bouwman T, Vaes WHJ. 2012. Predicting blood:air partition coefficients using basic physicochemical properties. Regul Toxicol Pharmacol 62:23-28.

% Wilschut, A., Berge, ten, W. F., Robinson, P. J., & McKone, T. E. (1995). Estimating skin permeation. The validation of five mathematical skin permeation models. Chemosphere, 30(7), 1275-1296

% Ruark CD, Hack CE, Robinson PJ, Mahle DA, Gearhart JM. 2014. Predicting passive and active tissue:plasma partition coefficients: interindividual and interspecies variability. Journal of pharmaceutical sciences. 103(7):2189-2198.

APPENDIX J. ARRAY MODEL M FILES FOR DEET SIMULATIONS AND PREDICTIONS

Note: Column formatting introduces extraneous line breaks not in the original code.

DEET_rat_Fediuk10.m

```
% Fediuk DJ, Wang T, Raizman JE,
Parkinson FE, Gu X. 2010 Tissue
deposition of the insect
% repellent DEET and the sunscreen
oxybenzone from repeated topical skin
applications in
% rats. Int. J. Toxicol. 29(6): 594-
603.
%
% Sprague-Dawley rats, n = 10 (5 M, 5
F)
% M mean BW = 268 g
% F mean BW = 256 g
% 40 mg/kg DEET daily for 30 dy
% 4 cm2 area
% 2.5 mg/mL, which means that males
received 4.3 mL and females received
4.1 mL
% 38% of dose left on skin after 31
dy
% Euthanized at 24 hr

resetdoses
rat
ratdeet

% Without repeated dermal dosing in the
code, the following is as if the last
day of dosing is the first (until
repeated dosing is added)
% Time, Conc, +SEM, -SEM (hour, mg/L,
mg/L, mg/L)
plasma = [
2.0      0.395 0.529 0.261
4.0      0.142 0.196 0.088
6.0      0.059 0.235 0.000
24.0     0.036 0.050 0.022];

% Without repeated dermal dosing in the
code, the following is as if the last
day of dosing is the first (until
repeated dosing is added)
% Time, Liver, Brain (hour, mg/L, mg/l)
% Points are median, +SD, -SD (top to
bottom)
tiss = [
24.0     0.096 0.008
24.0     0.305 0.014
24.0     0.000 0.002];

% Male rat
BW=0.268;

ICSURF(1)=2.5*0.62; IVSURF(1)=4.3;
```

```
%38% of dose remained on or in
surface layers of skin; 62% absorbed
AREA(1)=4.0;

TCHNG=24.0; TSTOP=24.0;
start @NoCallback

plotcven = plot (0, plasma(:,1),
plasma(:,2), '+b', plasma(:,1),
plasma(:,3), '+b',...
                plasma(:,1), plasma(:,4),
'+b', _t, _cven(:,1), '-b');

plotcliv = plot (0, tiss(:,1),
tiss(:,2), '+b', _t, _cliv(:,1), '-b');

plotcbrn = plot (0, tiss(:,1),
tiss(:,3), '+b', _t, _cbrn(:,1), '-b');
```

DEET_rat_Fediuk11_dml.m

```
% Fediuk DJ, Wang T, Chen Y, Parkinson
FE, Namaka MP, Simons KJ, Burczynski
FJ, Gu X. 2011.
% Tissue disposition of the insect
repellent DEET and the sunscreen
oxybenzone following
% intravenous and topical
administration in rats. Biopharm. Drug.
Dispost. 32: 369-79.
%
% Sprague-Dawley rats, n = 5-6 (male)
% M mean BW = 250 g
% Dermal: 100 mg/kg
% 5 cm2 area
% 0.25 mL vol
% 100 mg/mL
% 32.1% of dose left on skin after 24
hr + 0.2% in stratum corneum (recovered
from tape stripping)
% Euthanized at 24 hr

% Metabolism information for same doses
available in :
% Fediuk DJ, Wang T, Chen Y, Parkinson
FE, Namaka MP, Simons KJ, Burczynski
FJ, Gu X. 2012.
% Metabolic disposition of the insect
repellent DEET and the sunscreen
oxybenzone following
% intravenous and skin administration
in rats. Internat. J. Toxicol. 31: 467-
76.
```

```

% Model is not set up to simulate
metabolite concentration in liver or
plasma at this time.

resetdoses
rat
ratdeet

% time, IV, Dermal (hour, mg/L)
plasma = [
0.5      0.110
1.0      0.206
1.5      0.302
2.0      0.204
2.5      0.138
3.0      0.094
4.0      0.053
6.0      0.032
8.0      0.015
10.0     0.008
24.0     0.007];

% Time, Liver (hour, Dermal mg/l)
% Points are median, +SD, -SD (top to
bottom)
liver = [
24.0    14.6
24.0    21.1
24.0     8.1];

% Time, Kidney (hour, Dermal mg/l)
% Points are median, +SD, -SD (top to
bottom)
kidney = [
24.0    12.2
24.0    16.3
24.0     8.1];

BW=0.25;

ICSURF(1)=0.677*100.0;
% 32.3% recovered from skin or
stratum corneum; remainder 67.7%

IVSURF(1)=0.25;
AREA(1)=25.0;

TCHNG=24.0; TSTOP=24.0;
start @NoCallback

plotcven = plot (0, plasma(:,1),
plasma(:,2), '+b', _t, _cven(:,1), '-
b');
plotcliv = plot (0, liver(:,1),
liver(:,2), '+b', _t, _cliv(:,1), '-
b');
plotcrap = plot (0, kidney(:,1),
kidney(:,2), '+b', _t, _crap(:,1), '-
b');

DEET_rat_Fediuk11_iv.m

```

```

% Fediuk DJ, Wang T, Chen Y, Parkinson
FE, Namaka MP, Simons KJ, Burczynski
FJ, Gu X. 2011.
% Tissue disposition of the insect
repellent DEET and the sunscreen
oxybenzone following
% intravenous and topical
administration in rats. Biopharm. Drug.
Dispost. 32: 369-79.
%
% Sprague-Dawley rats, n = 5-6 (male)
% M mean BW = 250 g
% IV: 2 mg/kg tail vein injection
% Euthanized at 24 hr

% Metabolism information for same doses
available in :
% Fediuk DJ, Wang T, Chen Y, Parkinson
FE, Namaka MP, Simons KJ, Burczynski
FJ, Gu X. 2012.
% Metabolic disposition of the insect
repellent DEET and the sunscreen
oxybenzone following
% intravenous and skin administration
in rats. Internat. J. Toxicol. 31: 467-
76.
% Model is not set up to simulate
metabolite concentration in liver or
plasma at this time.

resetdoses
rat
ratdeet

% time, IV (hour, mg/L)
plasma = [
0.5      0.611
1.0      0.405
1.5      0.209
2.0      0.192
3.0      0.149
4.0      0.104
6.0      0.085
8.0      0.048
10.0     0.033
24.0     0.008];

% Time, Liver (hour, IV mg/L)
% Points are median, +SD, -SD (top to
bottom)
liver = [
24.0    0.0202
24.0    0.0226
24.0    0.0178];

% Time, Kidney (hour, IV mg/L)
% Points are median, +SD, -SD (top to
bottom)
kidney = [
24.0    0.0112
24.0    0.0124
24.0    0.0100];

```

```
% Male rat IV
BW=0.268;
IVDOSE(1)=2.0;
TINF=0.008; % no information on
injection time - assuming 0.5 min
TCHNG=24.0; TSTOP=24.0;
start @NoCallback

plotcven = plot (0, plasma(:,1),
plasma(:,2), '+b', _t, _cven(:,1), '-
b');

plotcliv = plot (0, liver(:,1),
liver(:,2), '+b', _t, _cliv(:,1), '-
b');

plotcrap = plot (0, kidney(:,1),
kidney(:,2), '+b', _t, _crap(:,1), '-
b');
```

DEET_rat_Schoenig_dml.m

```
% Schoenig GP, Hartnagel RE, Osimitz
TG, Llanos S. 1996. Absorption,
distribution, metabolism, and excretion
% of N,N-diethyl-M-toluamide in the
rat. Drug Metab. Disposit. 24(2): 156-
163.
```

```
% CD rats, n = 4 or 5 M, 4 or 5 F)
% ~9 weeks at start of study
% BW likely similar between sexes as
applied dose is similar (~100 mg/kg,
~20 uCi/rat)
% Assuming BW = 250 g
% Dermal Study: 100 mg/kg radiolabeled
DEET
% 2.5 x 5 cm (12.5 cm2 area)
% Volume not stated, assuming 1 mL
% ICSurf = 25 mg/mL
% Error not defined as SE or SD
```

```
% Data below are for male rats only,
unless otherwise noted
```

```
resetdoses
rat
ratdeet
```

```
% Blood
%Time, Conc, +Err, -Err (hour, mg/L,
mg/L, mg/L)
dblood = [
0.25 0.06 0.12 0.01
0.50 0.54 0.99 0.10
1.00 1.67 2.69 0.66
2.00 1.88 2.80 0.95
3.00 1.69 2.52 0.86
4.00 1.75 2.61 0.89
5.00 1.63 2.42 0.84
6.00 1.65 2.44 0.86
8.00 1.48 2.04 0.91
```

```
10.0 1.45 1.91 0.99
12.0 1.28 1.84 0.72
24.0 1.01 1.23 0.80];
```

```
% Amount metabolized, excreted in urine
%Time, AMet1, +Err, -Err (hour, mg/L,
mg/L, mg/L)
dmet = [
4.0 0.21
8.0 1.05
12.0 2.85
24.0 6.93
36.0 10.03
48.0 14.10
72.0 17.53
96.0 18.47
120.0 19.28
144.0 19.41
168.0 19.46
168.0 23.73
168.0 15.19];
```

```
% Amount metabolized, excreted in feces
%Time, AMet2, +Err, -Err (hour, mg/L,
mg/L, mg/L)
dfec = [
168.0 1.00
168.0 1.28
168.0 0.72];
```

```
% Male rat dermal dose
BW=0.250;
ICSURF(1)=25.0*0.8184;
% 81.84% of the radioactivity was
recovered in urine + feces
% 6.32% was definitely left on the
skin (93.68% possibly absorbed)

IVSURF(1)=1.0; AREA(1)=2.5*5.0;

TCHNG=168.0; TSTOP=168.0;
start @NoCallback
```

```
plotderm = plot (0, dblood(:,1),
dblood(:,2), '+b', dblood(:,1),
dblood(:,3), '+b',...
dblood(:,1), dblood(:,4),
'+b', _t, _cven(:,1), '-b');
plotmet = plot (0, dmet(:,1),
dmet(:,2), '+b', _t, _amet1(:,1), '-
b',...
dfec(:,1), dfec(:,2), '+g',
_t, _amet2(:,1), '-g');
```

DEET_rat_Schoenig.m

```
% Schoenig GP, Hartnagel RE, Osimitz
TG, Llanos S. 1996. Absorption,
distribution, metabolism, and excretion
% of N,N-diethyl-M-toluamide in the
rat. Drug Metab. Disposit. 24(2): 156-
163.
```

```

% CD rats, n = 4 or 5 M, 4 or 5 F)
% ~9 weeks at start of study
% BW likely similar between sexes as
applied dose is similar (~100 mg/kg,
~20 uCi/rat)
% Assuming BW = 250 g
% Oral Studies: 100 or 500 mg/kg
radiolabelled DEET single dose
% 18 hr fast
% Oral Repeated Study: 100 mg/kg daily
for 13 days, then 100 mg/kg
radiolabelled DEET
% Error not defined as SE or SD

% Data below are for male rats only,
unless otherwise noted

resetdoses
rat
ratdeet

% Oral dosing, low dose (100 mg/kg)
% Time, Conc, +Err, -Err (hour, mg/L,
mg/L, mg/L)
oblood = [
0.25 25.74 33.02 18.45
0.50 38.80 42.23 35.37
1.00 31.75 35.52 27.97
2.00 13.99 18.14 9.83
3.00 7.39 8.94 5.84
4.00 4.15 5.81 2.49
5.00 2.31 3.09 1.54
6.00 1.54 1.73 1.36
8.00 1.42 1.97 0.87
10.0 1.38 1.80 0.96
12.0 1.10 1.45 0.75
24.0 0.75 0.92 0.59];

% Oral dosing, low dose (100 mg/kg)
% Time, AMet, +Err, -Err (hour, mg/L,
mg/L, mg/L)
olmet = [
4.0 5.53
8.0 16.15
12.0 18.79
24.0 20.21
36.0 20.70
48.0 21.05
72.0 21.30
96.0 21.48
120.0 21.60
144.0 21.65
168.0 21.68
168.0 22.44
168.0 20.92];

% Oral dosing, repeated (14 day) low
dose (100 mg/kg)
% Time, AMet, +Err, -Err (hour, mg/L,
mg/L, mg/L)
rolmet = [
4.0 14.53
8.0 17.96
12.0 21.22
24.0 22.17
36.0 22.33
48.0 22.43
72.0 22.50
96.0 22.55
120.0 22.59
144.0 22.62
168.0 22.65
168.0 23.02
168.0 22.28];

% Oral dosing, high dose (500 mg/kg)
% Time, AMet, +Err, -Err (hour, mg/L,
mg/L, mg/L)
o5met = [
4.0 21.36
8.0 36.23
12.0 63.16
24.0 96.81
36.0 104.74
48.0 106.38
72.0 107.44
96.0 107.83
120.0 108.06
144.0 108.24
168.0 108.38
168.0 118.40
168.0 98.35];

% Male rat oral dose
BW=0.250;
TCHNG=24.0; TSTOP=168.0;
PDOSE(1)=100.0;
start @NoCallback

plotoral = plot (0, oblood(:,1),
oblood(:,2), '+b', oblood(:,1),
oblood(:,3), '+b',...
oblood(:,1), oblood(:,4),
'+b', _t, _cven(:,1), '-b');
plotmet = plot (0, olmet(:,1),
olmet(:,2), '+b', rolmet(:,1),
rolmet(:,2), '+m',...
_t, _amet1(:,1), '-b');

resetdoses
PDOSE(1)=500.0;
start @NoCallback

plot (plotmet, 1, o5met(:,1),
o5met(:,2), '+g', _t, _amet1(:,1), '-
g');

DEET_rat_Snodgrass.m

% Snodgrass HL, Nelson DC, Weeks MH.
1982. Dermal penetration and potential
for placental transfer

```

```

% of the insect repellent, N, N-
diethyl-m-toluamide. Am. Ind. Hyg.
Assoc. J. 43(10): 747-753.
%
% Sprague-Dawley rats, M & F
% 250-300 g (use 275 g)
% Dermal Study: 4 ug/cm2 rate of
administration
% 28.12 ug/rat of radiolabeled DEET
% SA = (28.12 ug)/(4 ug/cm2) = 7.03
cm2
% Area is reported to be varied but
they did report the rate, so perhaps
the volume varied,
% which caused the area to change
somewhat
% Volume is a guess (1 mL) so initial
conc = 0.02812 mg/mL

resetdoses
rat
ratdeet

% Amount metabolized (in urine)
% t, amet1 (hr, mg)
metab = [
24.0 0.011
48.0 0.012
72.0 0.012
96.0 0.012
120.0 0.012
144.0 0.012
168.0 0.012];

% Amount metabolized (in feces)
% t, amet2 (hr, mg)

% Male rat dermal dose (skin
permeability from IPA rat dermal m
file)
BW=0.275;
ICSURF(1)=0.02812; IVSURF(1)=1.0;
AREA(1)=7.03;

TCHNG=168.0; TSTOP=168.0;
start @NoCallback

plotderm = plot (0, metab(:,1),
metab(:,2), '+b', _t, _amet1(:,1), '-
b');

```

DEET_rat_Snodgrass.m

```

% Snodgrass HL, Nelson DC, Weeks MH.
1982. Dermal penetration and potential
for placental transfer
% of the insect repellent, N, N-
diethyl-m-toluamide. Am. Ind. Hyg.
Assoc. J. 43(10): 747-753.

% Sprague-Dawley rats, M & F
% 250-300 g (use 275 g)
% IV study: radiolabelled DEET single
dose
% 28.12 ug/rat
% Lightly anesthetized

% Has cumulative % metabolized, if I
can figure out how to use it (Figs 2 &
3).

resetdoses
rat
ratdeet

% Amount excreted as metabolites
% t, amet1 (hr, mg)
metab = [
24.0 0.024
48.0 0.025
72.0 0.025
96.0 0.025
120.0 0.025
144.0 0.025
168.0 0.025];

% Male rat iv dose
BW=0.275;
IVDOSE=0.102;
TINF=0.008; %0.5 minute
administration assumed

TCHNG=168.0; TSTOP=168.0;
start @NoCallback

plotmet = plot (0, metab(:,1),
metab(:,2), '+b', _t, _amet1(:,1), '-
b');

```

APPENDIX K. ARRAY MODEL M FILES FOR PB SIMULATIONS AND PREDICTIONS

Note: Column formatting introduces extraneous line breaks not in the original code.

```

PB_hmn_Coper.m
% Figure 4 data from:

% Coper H, Deyhle G, Dross K. 1974.
Studies on the absorption of
pyridostigmine:
%   The application of a
spectrophotometric method for the
determination of
%   pyridostigmine in plasma.
Z.Klin.Chem.Klin. Biochem. 273-275.
%
% 4 patients, no idea of sex or weight
%   Assuming 70 kg
% Dosed with the drug mestinon =
pyridostigmine bromide
%   120 mg oral dose in Fig 4

    resetdoses
    human
    humanpb

% Time, Plasma from 4 individuals

plasma = [
0.50  0.098
1.00  0.423
2.00  0.298
3.00  0.267
0.67  0.120
1.00  0.262
2.00  0.246
3.00  0.088
2.25  0.319
3.00  0.267
2.00  0.133
3.00  0.210];

    BW=70.0; PDOSE(2)=120.0/BW;
    TCHNG=4.0; TSTOP=4.0;
    start @NoCallback

    plotcven = plot (0, plasma(:,1),
plasma(:,2), '+b', _t, _cven(:,2), '-
b');

PB_hmn_Marino.m
% Figure 2 data from:

% Marino, M. T., B. G. Schuster, et al.
(1998). "Population pharmacokinetics
and pharmacodynamics of
%   pyridostigmine bromide for
prophylaxis against nerve agents in
humans." J Clin Pharmacol 38: 227-35.

```

1.5	0.0267	2.5	0.0214
1.5	0.0255	2.5	0.0203
1.5	0.0239	2.5	0.0198
1.5	0.0231	2.5	0.0190
1.5	0.0228	2.5	0.0185
1.5	0.0224	2.5	0.0180
1.5	0.0216	2.5	0.0173
1.5	0.0197	2.5	0.0158
1.5	0.0187	2.5	0.0151
1.5	0.0183	2.5	0.0147
1.5	0.0178	2.5	0.0132
1.5	0.0174	2.5	0.0122
1.5	0.0168	2.5	0.0118
1.5	0.0153	2.5	0.0110
1.5	0.0145	2.5	0.0106
1.5	0.0141	2.5	0.0102
1.5	0.0133	2.5	0.0092
1.5	0.0121	2.5	0.0089
1.5	0.0117	2.5	0.0075
1.5	0.0114	2.5	0.0071
1.5	0.0102	3.0	0.0494
1.5	0.0075	3.0	0.0334
1.5	0.0067	3.0	0.0271
1.5	0.0061	3.0	0.0251
1.5	0.0051	3.0	0.0246
1.5	0.0048	3.0	0.0228
1.5	0.0039	3.0	0.0222
2.0	0.0453	3.0	0.0217
2.0	0.0312	3.0	0.0211
2.0	0.0286	3.0	0.0200
2.0	0.0269	3.0	0.0193
2.0	0.0265	3.0	0.0187
2.0	0.0254	3.0	0.0171
2.0	0.0239	3.0	0.0164
2.0	0.0229	3.0	0.0157
2.0	0.0222	3.0	0.0148
2.0	0.0210	3.0	0.0138
2.0	0.0198	3.0	0.0132
2.0	0.0193	3.0	0.0120
2.0	0.0187	3.0	0.0115
2.0	0.0181	3.0	0.0103
2.0	0.0176	3.0	0.0093
2.0	0.0168	3.0	0.0087
2.0	0.0151	3.0	0.0075
2.0	0.0144	3.0	0.0067
2.0	0.0135	3.0	0.0063
2.0	0.0130	4.0	0.0270
2.0	0.0124	4.0	0.0249
2.0	0.0119	4.0	0.0230
2.0	0.0108	4.0	0.0217
2.0	0.0101	4.0	0.0196
2.0	0.0093	4.0	0.0181
2.0	0.0085	4.0	0.0173
2.0	0.0061	4.0	0.0167
2.0	0.0051	4.0	0.0154
2.5	0.0455	4.0	0.0146
2.5	0.0357	4.0	0.0142
2.5	0.0296	4.0	0.0135
2.5	0.0261	4.0	0.0128
2.5	0.0244	4.0	0.0116
2.5	0.0239	4.0	0.0108
2.5	0.0230	4.0	0.0095
2.5	0.0224	4.0	0.0080

```

4.0 0.0070
4.0 0.0064
4.0 0.0056
4.0 0.0049
4.0 0.0047
5.0 0.0186
5.0 0.0168
5.0 0.0154
5.0 0.0133
5.0 0.0126
5.0 0.0118
5.0 0.0107
5.0 0.0103
5.0 0.0094
5.0 0.0084
5.0 0.0076
5.0 0.0064
5.0 0.0058
5.0 0.0049
5.0 0.0043
5.0 0.0039
5.0 0.0033
6.0 0.0124
6.0 0.0115
6.0 0.0109
6.0 0.0100
6.0 0.0096
6.0 0.0081
6.0 0.0074
6.0 0.0064
6.0 0.0056
6.0 0.0047
6.0 0.0037
6.0 0.0030
6.0 0.0025
6.0 0.0020
7.0 0.0091
7.0 0.0084
7.0 0.0075
7.0 0.0069
7.0 0.0061
7.0 0.0050
7.0 0.0041
7.0 0.0034
7.0 0.0027
7.0 0.0020
7.0 0.0016
8.0 0.0074
8.0 0.0066
8.0 0.0060
8.0 0.0050
8.0 0.0040
8.0 0.0032
8.0 0.0024
8.0 0.0018];

% Running model for male human
BW=77.0; PDOSE(2)=30.0/BW;
TCHNG=24.0; TSTOP=8.0;
start @NoCallback

plotcven = plot (0, marinodat(:,1),
marinodat(:,2), '+g', _t, _cven(:,2),
'-b');

% Running model for female human
BW=68.0; PDOSE(2)=30.0/BW;
start @nocallback

plot (plotcven, 1, _t, _cven(:,2), '-
k');

PB_rat_Barber75.m
% Table 1 data from:

% Barber HE, Bourne GR, Calvey TN, Muir
KT. 1975 The pharmacokinetics of
pyridostigmine and
% 3-hydroxy-N-methylpyridinium in
the rat: Dose-dependent effects after
portal vein administration.
% Br. J. Pharmac. 55:335-41.

% Wistar rats, assuming male, 250-350 g
% Compared dosing in jugular vein
and portal vein, to examine first pass
effect
% Used jugular vein data; consistent
with IV dosing in model
% Dosed with pyridostigmine iodide
% Low dose = 0.1 umol/kg or 0.031
mg/kg
% High dose = 0.63 umol/kg or 0.194
mg/kg

resetdoses
rat
ratpb_fast

% Hours Low Dose AUrn (incl. +SE, -
-SE), High Dose AUrn (incl. +SE, -SE)

urine = [
2.0 0.0044 0.0250
2.0 0.0046 0.0274
2.0 0.0043 0.0227
4.0 0.0049 0.0297
4.0 0.0050 0.0314
4.0 0.0048 0.0280];

% Hours, Low Dose AMet1 (incl. +SE, -
SE), High Dose AMet1 (incl. +SE, -SE)
metab = [
2.0 0.0012 0.0058
2.0 0.0014 0.0076
2.0 0.0010 0.0041
4.0 0.0020 0.0122
4.0 0.0023 0.0140
4.0 0.0018 0.0105];

% Switch to different salt
MW(2)= 308.119;

```

```

    BW=0.3; IVDOSE(2)=0.031;
    TINF(2)=0.008; % Assuming 0.5 minute
infusion

```

```

    %These data will not fit with the
same metabolism and urine settings as
other studies

```

```

    VMAXC(2)=0.05; %0.15;
    KM(2)=0.15; %0.1;
    KFC(2)=0.0;
    CLURC(2)=0.3; %0.35;

```

```

    TCHNG=4.0; TSTOP=4.0;
start @NoCallback

```

```

    ploturn = plot (0, urine(:,1),
urine(:,2), '+b', _t, _aurn(:,2), '-
b');

```

```

    plotmet = plot (0, metab(:,1),
metab(:,2), '+b', _t, _amet1(:,2), '-
b');

```

```

IVDOSE(2)=0.194;
start @nocallback

```

```

    plot (ploturn, 1, urine(:,1),
urine(:,3), '+k', _t, _aurn(:,2), '-
k');
    plot (plotmet, 1, metab(:,1),
metab(:,3), '+k', _t, _amet1(:,2), '-
k');

```

PB_rat_Coper.m

```

% Figure 3 data from:

```

```

% Coper H, Deyhle G, Dross K. 1974.
Studies on the absorption of
pyridostigmine:
% The application of a
spectrophotometric method for the
determination of
% pyridostigmine in plasma.
Z.Klin.Chem.Klin. Biochem. 273-275.

```

```

% Wistar rats, female, 180-220 g
% Compared ip and oral dosing to
determine bioavailability and LD50
% LD50 ip = 3 mg/kg
% LD50 po = 115 mg/kg
% Dosed with the drug mestinon =
pyridostigmine bromide
% 50 mg/kg oral dose in Fig 3
% Rats were fed ad libitum

```

```

resetdoses
rat
ratpb_fed

```

```

% Time, Plasma, +SD, -SD (hour, mg/L,
mg/L, mg/L)

```

```

plasma = [
0.08 0.55 0.73 0.38
0.33 0.73 1.03 0.44
1.00 0.66 0.86 0.46
2.00 0.49 0.62 0.36
3.00 0.24 0.42 0.07
4.00 0.24 0.37 0.11];

```

```

    BW=0.2; PDOSE(2)=50.0;
    TCHNG=4.0; TSTOP=4.0;
start @NoCallback

```

```

    plotcven = plot (0, plasma(:,1),
plasma(:,2), '+b', plasma(:,1),
plasma(:,3), '+b',...
                    plasma(:,1), plasma(:,4),
'+b', _t, _cven(:,2), '-b');

```

PB_rat_Husain.m

```

% Husain MA, Roberts JB, Thomas BH,
Wilson A. 1968. The excretion and
metabolism of oral
% 14C-pyridostigmine in the rat. Br.
J. Pharmac. 34: 445-50.

```

```

% male rats, no idea of strain, 150-160
g
% 155 g median
% Dosed with pyridostigmine iodide
% 0.5 mg, radiolabeled
% 3.23 mg/kg

```

```

% Rats were primed with 2 doses of
water (5 mL/100 g BW) an hour apart,
the second 30 min before dosing with
PB.
% This study used radiolabelled 14C-
PI.

```

```

resetdoses
rat
ratpb_fast

```

```

% Time, AUrn (hour, mg) - Value sets
are Mean, +SE, -SE (top to bottom)
urine = [
1.0 0.004
2.0 0.030
3.0 0.054
5.0 0.079
24.0 0.116
2.0 0.035
3.0 0.067
5.0 0.108
24.0 0.137
2.0 0.024
3.0 0.042
5.0 0.049
24.0 0.096];

```

```

% Time, CLiv (hour, mg/L) - Value sets
are Mean, +SE, -SE (top to bottom)

```

```

liver = [
1.0 0.91
2.0 2.04
3.0 0.84
4.0 0.23
1.0 1.33
2.0 3.62
3.0 1.44
4.0 0.37
1.0 0.49
2.0 0.46
3.0 0.24
4.0 0.09];

% Time, CVen (hour, mg/L) - Values from
12 individual rats
blood = [
1.0 0.34
1.0 0.15
1.0 0.08
1.0 0.19
2.0 0.39
2.0 0.17
2.0 0.08
2.0 0.21
3.0 0.05
3.0 0.08
3.0 0.06
3.0 0.06
5.0 0.04
5.0 0.06
5.0 0.03
5.0 0.04];

% Switch to different salt
MW(2)= 308.119;

BW=0.155;
PDOSE(2)=3.23;
TCHNG=24.0; TSTOP=24.0;
start @NoCallback

ploturn = plot (0, urine(:,1),
urine(:,2), '+b', _t, _aurn(:,2), '-
b');

plotliv = plot (0, liver(:,1),
liver(:,2), '+b', _t, _cliv(:,2), '-
b');

plotbld = plot (0, blood(:,1),
blood(:,2), '+b', _t, _cven(:,2), '-
b');

PB_rat_Tan.m
% Tan Q-Y, Hu N-N, Liu G-D, Yin H-F,
Zhang L, Wang H, Lu L-Y, Zhang J-Q.
2012
% Role of a novel pyridostigmine
bromide-phospholipid nanocomplex in
improving

% oral bioavailability. Arch. Pharm.
Res. 35:499-508.

% SD rats, male, 300 +/- 20 g
% Compared oral administration of PB
and PBPLC
% 1.5 mg/kg PB equivalent of each

resetdoses
rat
ratpb_fast

% Time, Plasma PB (hour, mg/L)

plasma = [
0.2 2.26
0.4 1.80
0.7 1.80
1.0 3.57
2.0 5.86
4.0 3.26
6.0 2.41
8.0 1.04
10.0 0.65];

BW=0.3; PDOSE(2)=1.5;
TCHNG=24.0; TSTOP=10.0;
start @NoCallback

plotcven = plot(0, plasma(:,1),
plasma(:,2), '+b', _t, _cven(:,2), '-
b');

PB_rat_Yamamoto.m
% Figure 1 data from:
% Yamamoto K, Sawada Y, Iga T. 1995.
Comparative pharmacokinetics of
% four cholinesterase inhibitors in
rats. Biol. Pharm. Bull. 18(9): 1292-
95.

% Wistar rats, male, 250-330 g
% IV dosing to determine blood
kinetics & urinary/bile excretion
% Dosed with pyridostigmine bromide
% 0.5, 1, 2 umol/kg or 0.13, 0.26,
0.52 mg/kg

resetdoses
rat
ratpb_fast

% Time, Plasma for low, mid, hi doses
% (+SE, -SE not discernable from
average symbol (hour, mg/L, mg/L, mg/L)
plasma = [
0.03 0.60 1.09 2.51
0.08 0.30 0.65 1.45
0.25 0.13 0.28 0.48
0.50 0.07 0.15 0.21
0.75 0.05 0.08 0.14
1.00 0.03 0.05 0.09];

```

```

% Time, amount in urine for mid (0.26
mg/kg) dose (hour, mg)
% Values are mean, -SE, +SE
urine = [
6.0    0.045
6.0    0.037
6.0    0.054];

BW=0.29;
CLURC(2)=0.25;    % 0.25; Urinary
excretion is 59.5% at 6 hours
                % Clearance is much
slower than Husain et al. 1968;
                % In the current
study, the rats were anesthetized 2
hours prior
    IVDOSE(2)=0.13;
    TINF=0.008; %0.5 minute
administration assumed
    TCHNG=6.0; TSTOP=6.0;
    start @NoCallback

```

```

    plotcven = plot (0, plasma(:,1),
plasma(:,2), '+b', _t, _cven(:,2), '-
b');

    IVDOSE(2)=0.26;
    start @NoCallback

    plot (plotcven, 1, plasma(:,1),
plasma(:,3), '+g', _t, _cven(:,2), '-
g');
    ploturine = plot (0, urine(:,1),
urine(:,2), '+g', _t, _aurn (:,2), '-
g');

    IVDOSE(2)=0.52;
    start @NoCallback

    plot (plotcven, 1, plasma(:,1),
plasma(:,4), '+m', _t, _cven(:,2), '-
m');

```

APPENDIX L. ARRAY MODEL M FILES FOR PERc SIMULATIONS AND PREDICTIONS

Note: Column formatting introduces extraneous line breaks not in the original code.

```

PER_rat_Lestremau_cis_orl.m
% Lestremau F, Willemine ME, Chatellier
C, Desmots S, Brochot C. 2014.
Determination of
% cis-permethrin, trans-permethrin
and associated metabolites in rat blood
and
% organs by gas chromatography-ion
trap mass spectrometry. Anal Bioanal
Chem.
% 406(14):3477-87.

% RATS WERE NOT FASTED as they were for
the Tornero-Velez study
% Sprague-Dawley male rats, 468 g from
Willemine et al. study (same authors) -
NOT STATED IN CURRENT STUDY
% n=2 at each time point
% Each group received an oral dose of
PER: 5 or 20 mg/kg cis-permethrin in 2
mL/kg corn oil
% Euthanized at 2, 6, 24 hr (lots of
non-detects at 24 hr)
% Collected BLOOD, brain, fat,
liver, testes, muscle, kidney
% Analytical methods paper; Metabolites
were measured

% 5 mg/kg dose cis isomer: Time (hr)
Blood Brain Liver Fat Muscle
Kidneys (mg/L)
% (_hours, _cven, _cbrn, _cliv,
_cfat, _cslw, _crap)
cis = [
2.0 0.379 0.10 0.29 1.22 0.11
0.33
2.0 NaN 0.17 0.50 2.69 0.13
0.47
2.0 NaN 0.03 0.08 0.00 0.10
0.19
6.0 0.335 0.23 0.17 1.91 0.15
0.22
6.0 0.445 0.41 0.28 3.64 0.18
0.35
6.0 0.224 0.05 0.06 0.17 0.11
0.10
24.0 NaN NaN NaN 0.85 NaN
NaN
24.0 NaN NaN NaN 1.03 NaN
NaN
24.0 NaN NaN NaN 0.68 NaN
NaN];

% 20 mg/kg dose cis isomer: Time (hr)
Blood Brain Liver Fat Muscle
Kidneys (mg/L)

% (_hours, _cven, _cbrn, _cliv,
_cfat, _cslw, _crap)
cis20 = [
2.00 1.778 0.26 1.19 0.68 0.23
1.32
2.00 NaN 0.49 2.25 0.84 0.26
2.35
2.00 NaN 0.03 0.13 0.51 0.20
0.28
6.00 0.427 0.70 0.42 1.51 0.62
0.68
6.00 0.617 1.07 0.70 1.99 0.75
1.04
6.00 0.238 0.32 0.15 1.04 0.49
0.31
24.0 NaN NaN NaN 1.09 NaN
NaN
24.0 NaN NaN NaN 1.39 NaN
NaN
24.0 NaN NaN NaN 0.78 NaN
NaN];

ResetDoses
Rat
RatPERc_fed

BW=0.468;
PDOSE(3)=5.0;
TSTOP=24.0;
start @NoCallBack

plotbldc = plot (0, cis(:,1),
cis(:,2), '+b', _t, _cven(:,3), '-b');
plotbrnc = plot (0, cis(:,1),
cis(:,3), '+b', _t, _cbrn(:,3), '-b');
plotlivc = plot (0, cis(:,1),
cis(:,4), '+b', _t, _cliv(:,3), '-b');
plotfatc = plot (0, cis(:,1),
cis(:,5), '+b', _t, _cfat(:,3), '-b');
plotslwc = plot (0, cis(:,1),
cis(:,6), '+b', _t, _cslw(:,3), '-b');
plotrapc = plot (0, cis(:,1),
cis(:,7), '+b', _t, _crap(:,3), '-b');

PDOSE(3)=20.0;
start @NoCallBack

plot (plotbldc, 1, cis20(:,1),
cis20(:,2), '+k', _t, _cven(:,3), '-
k');
plot (plotbrnc, 1, cis20(:,1),
cis20(:,3), '+k', _t, _cbrn(:,3), '-
k');
plot (plotlivc, 1, cis20(:,1),
cis20(:,4), '+k', _t, _cliv(:,3), '-
k');

```

```

    plot (plotfatc, 1, cis20(:,1),
cis20(:,5), '+k', _t, _cfat(:,3), '-
k');
    plot (plotslwc, 1, cis20(:,1),
cis20(:,6), '+k', _t, _cslw(:,3), '-
k');
    plot (plotrapc, 1, cis20(:,1),
cis20(:,7), '+k', _t, _crap(:,3), '-
k');

```

PER_rat_TV_cis.m

```

% Contains only cis data for fitting
% Tornero-Velez R, Davis J, Scollon EJ,
% Starr JM, Setzer RW, Goldsmith M-R,
% Chang DT, Xue J,
% Zartarian V, DeVito MJ, Hughes MF.
% 2012. A pharmacokinetic model of cis-
% and trans-
% permethrin disposition in rats and
% humans with aggregate exposure
% application. Toxicol.
% Sci. 130(1): 33-47.

```

```

% Long-Evans 70-day old male rats, n =
% 4/timepoint
% Each group received an oral dose of
% PER: 1 or 10 mg/kg in 1 mL/kg corn oil
% 40:60 cis:trans
% Bodyweight = 0.295 kg (Table 1)
% Euthanized at 1, 2, 3, 4, 6, 8, 12,
% 24, 36, 48 hr
% Collected BLOOD, brain, fat, liver
% Metabolites were not measured

```

```

% 1 mg/kg dose cis isomer: Time in
% hours, Blood Brain Liver Fat (mg/L)
% (_hours, _cven, _cbrn, _cliv,
% _cfat)
cis1 = [
1.0 0.04100 0.0090 0.0160
0.0228
2.0 0.01570 0.0890 0.0109
0.3500
3.0 0.00783 NaN 0.0052 NaN
4.0 0.00479 0.0208 0.0034 NaN
6.0 0.00098 0.0167 0.0011
0.1413
8.0 0.00050 0.0082 0.0005
0.1038
12.0 0.00045 0.0071 0.0005
0.1672
24.0 0.00014 0.0019 NaN
0.1442
36.0 NaN 0.0004 NaN
0.0933
48.0 NaN 0.0036 NaN
0.2967];

```

```

% 10 mg/kg dose cis isomer: Time in
% hours, Blood Brain Liver Fat (mg/L)
% (_hours, _cven, _cbrn, _cliv,
% _cfat)

```

```

cis10 = [
1.0 0.3420 0.0557 0.1290
0.1777
2.0 0.1480 0.0714 0.0767
0.4531
3.0 0.2071 0.1600 0.0654
0.8260
4.0 0.0366 0.1505 0.0220
1.1746
6.0 0.0111 0.0984 0.0064
1.0304
8.0 0.0094 0.0827 0.0076
1.3390
12.0 0.0031 0.0533 0.0013
1.4120
24.0 0.0017 0.0068 0.0006
1.3988
36.0 NaN 0.0026 0.0005
1.0912
48.0 NaN 0.0012 NaN
0.7800];

```

```

ResetDoses
Rat
RatPERc_fast

```

```

BW=0.295;
PDOSE(3)=0.4*1.0; %Calculates dose
for cis isomer, 1 mg/kg dose
TSTOP=48.0;
start @NoCallBack

```

```

plotbldc = plot (0, cis1(:,1),
cis1(:,2), '+b', _t, _cven(:,3), '-b');
plotbrnc = plot (0, cis1(:,1),
cis1(:,3), '+b', _t, _cbrn(:,3), '-b');
plotlivc = plot (0, cis1(:,1),
cis1(:,4), '+b', _t, _cliv(:,3), '-b');
plotfatc = plot (0, cis1(:,1),
cis1(:,5), '+b', _t, _cfat(:,3), '-b');

```

```

PDOSE(3)=0.4*10.0; %Calculates dose
for cis isomer, 10 mg/kg dose
% Note: Lestremieu et al. (2014) low
% dose was comparable (12 mg/kg) and had
% about 38% fecal excretion
start @NoCallBack

```

```

plot (plotbldc, 1, cis10(:,1),
cis10(:,2), '+k', _t, _cven(:,3), '-
k');
plot (plotbrnc, 1, cis10(:,1),
cis10(:,3), '+k', _t, _cbrn(:,3), '-
k');
plot (plotlivc, 1, cis10(:,1),
cis10(:,4), '+k', _t, _cliv(:,3), '-
k');
plot (plotfatc, 1, cis10(:,1),
cis10(:,5), '+k', _t, _cfat(:,3), '-
k');

```

PER_rat_Willemin_cis.m

% Willemin M-E, Desmots S, Le Grand R,
Lestremiau F, Zeman FA, Leclerc E,
Moesch C,
% Brochot C. 2016. PBPK modeling of
the cis- and trans-permethrin isomers
and
% their major urinary metabolites in
rats. Toxicol. Appl. Pharmacol 294:65-
77.

% RATS WERE NOT FASTED as they were for
the Tornero-Velez study
% Sprague-Dawley male rats, 468 g +/-
21 g
% Each group received an oral dose of
PER: 25 mg/kg cis-permethrin in 2 mL/kg
corn oil
% Euthanized at 0.5, 1, 1.5, 2, 4, 6,
10, 24, 48 hr
% Collected BLOOD, brain, fat,
liver, testes, muscle, kidney
% Metabolites were measured

% 25 mg/kg dose cis isomer: Time (hr)
Blood Brain Liver Fat (mg/L)
% (_hours, _cven, _cbrn, _cliv,
_cfat)
cis = [

0.5	0.153	0.126	0.129	0.223
1.0	0.692	0.109	0.671	0.490
1.5	1.320	0.318	1.254	NaN
2.0	0.807	0.372	1.077	0.687
4.0	0.390	0.641	0.588	NaN
6.0	0.231	0.433	0.258	2.243
10.0	0.110	0.621	0.203	6.715
24.0	NaN	0.088	NaN	4.863
48.0	NaN	NaN	NaN	5.359
144.0	NaN	NaN	NaN	3.422];

ResetDoses
Rat
RatPERc_fed

BW=0.468;
PDOSE(3)=25.0;
TSTOP=144.0;
start @NoCallBack

plotblcdc = plot (0, cis(:,1),
cis(:,2), '+b', _t, _cven(:,3), '-b');
plotbrnc = plot (0, cis(:,1),
cis(:,3), '+b', _t, _cbrn(:,3), '-b');
plotlivc = plot (0, cis(:,1),
cis(:,4), '+b', _t, _cliv(:,3), '-b');
plotfatc = plot (0, cis(:,1),
cis(:,5), '+b', _t, _cfat(:,3), '-b');

APPENDIX M. ARRAY MODEL M FILES FOR PERT SIMULATIONS AND PREDICTIONS

Note: Column formatting introduces extraneous line breaks not in the original code.

```

PER_rat_Lestremau_trans_orl.m
% Lestremau F, Willemin ME, Chatellier
C, Desmots S, Brochot C. 2014.
Determination of
% cis-permethrin, trans-permethrin
and associated metabolites in rat blood
and
% organs by gas chromatography-ion
trap mass spectrometry. Anal Bioanal
Chem.
% 406(14):3477-87.

% RATS WERE NOT FASTED as they were for
the Tornero-Velez study
% Sprague-Dawley male rats, 468 g from
Willemin et al. study (same authors) -
NOT STATED IN CURRENT STUDY
% n=2 at each time point
% Each group received an oral dose of
PER: 5 or 20 mg/kg trans-permethrin in
2 mL/kg corn oil

% Euthanized at 2, 6, 24 hr (lots of
non-detects at 24 hr)
% Collected BLOOD, brain, fat,
liver, testes, muscle, kidney
% Analytical methods paper; Metabolites
were measured

% 5 mg/kg dose trans isomer: Time (hr)
Blood Fat (mg/L)
% (_hours, _cven, _cfat)
trans = [
2.0 0.181 NaN
6.0 0.098 0.07
24.0 NaN 0.34];

% 20 mg/kg dose trans isomer: Time (hr)
Blood Fat (mg/L)
% (_hours, _cven, _cfat)
trans20 = [
2.0 0.162 0.10
6.0 NaN 0.46
24.0 NaN 0.23
24.0 NaN 0.32
24.0 NaN 0.14];

ResetDoses
Rat
RatPERT_fed

BW=0.468;
PDOSE(4)=5.0;
TSTOP=24.0;
start @NoCallBack

plotbldc = plot (0, trans(:,1),
trans(:,2), '+b', _t, _cven(:,4), '-
b');
plotfatc = plot (0, trans(:,1),
trans(:,3), '+b', _t, _cfat(:,4), '-
b');

PDOSE(4)=20.0;
start @NoCallBack

plot (plotbldc, 1, trans20(:,1),
trans20(:,2), '+k', _t, _cven(:,4), '-
k');
plot (plotfatc, 1, trans20(:,1),
trans20(:,3), '+k', _t, _cfat(:,4), '-
k');

PER_rat_TV_trans.m
% Contains only trans data for
fitting
% Tornero-Velez R, Davis J, Scollon EJ,
Starr JM, Setzer RW, Goldsmith M-R,
Chang DT, Xue J,
% Zartarian V, DeVito MJ, Hughes MF.
2012. A pharmacokinetic model of cis-
and trans-
% permethrin disposition in rats and
humans with aggregate exposure
application. Toxicol.
% Sci. 130(1): 33-47.

% Long-Evans 70-day old male rats, n =
4/timepoint
% Each group received an oral dose of
PER: 1 or 10 mg/kg in 1 mL/kg corn oil
% 40:60 cis:trans
% Bodyweight = 0.295 kg (Table 1)

% Euthanized at 1, 2, 3, 4, 6, 8, 12,
24, 36, 48 hr
% Collected BLOOD, brain, fat, liver

% Metabolites were not measured

% 1 mg/kg dose trans isomer: Time in
hours, Blood Brain Liver Fat (mg/L)
% (_hours, _cven, _cbrn, _cliv,
_cfat)
trans1 = [
1.0 0.01500 0.00489 0.00100
0.01506
2.0 0.01550 0.04300 0.00064
0.18600
3.0 0.00096 0.00234 0.00063
0.03793

```

```

4.0    0.00070    0.00140    NaN    NaN    PDOSE(4)=0.6*10.0;    %Calculates dose
6.0    0.00008    0.00052    NaN    for trans isomer
0.03596    start @NoCallBack
8.0    0.00009    0.00093    NaN
0.02726    plot (plotbldt, 1, trans10(:,1),
12.0    0.00046    0.00201    NaN    trans10(:,2), '+k', _t, _cven(:,4), '-
0.02479    k');
24.0    0.00003    0.00144    NaN    plot (plotbrnt, 1, trans10(:,1),
0.01825    trans10(:,3), '+k', _t, _cbrn(:,4), '-
36.0    NaN    0.00052    NaN    k');
0.01271    plot (plotlivt, 1, trans10(:,1),
48.0    NaN    NaN    NaN    trans10(:,4), '+k', _t, _cliv(:,4), '-
0.05216];    k');
    plot (plotfatt, 1, trans10(:,1),
    trans10(:,5), '+k', _t, _cfat(:,4), '-
    k');

% 10 mg/kg dose trans isomer: Time in
hours, Blood Brain Liver Fat (mg/L)
%    (_hours, _cven, _cbrn, _cliv,
_cfat)
trans10 = [
1.0    0.1230    0.0220    0.0150
0.0864
2.0    NaN    0.0141    0.0054
0.1650
3.0    NaN    NaN    0.0017
0.2920
4.0    NaN    0.0129    0.0015
0.4560
6.0    NaN    0.0051    0.0008
0.3477
8.0    NaN    0.0030    0.0006
0.3143
12.0    NaN    0.0012    0.0006
0.2712
24.0    NaN    0.0005    NaN
0.2412
36.0    NaN    NaN    NaN
0.0618
48.0    NaN    NaN    NaN
0.0203];

ResetDoses
Rat
RatPERT_fast

BW=0.295;
PDOSE(4)=0.6*1.0;    %Calculates dose
for trans isomer
TSTOP=48.0;
start @NoCallBack

plotbldt = plot (0, trans1(:,1),
trans1(:,2), '+b', _t, _cven(:,4), '-
b');
plotbrnt = plot (0, trans1(:,1),
trans1(:,3), '+b', _t, _cbrn(:,4), '-
b');
plotlivt = plot (0, trans1(:,1),
trans1(:,4), '+b', _t, _cliv(:,4), '-
b');
plotfatt = plot (0, trans1(:,1),
trans1(:,5), '+b', _t, _cfat(:,4), '-
b');

PER_rat_Willemin_cis.m
% Willemin M-E, Desmots S, Le Grand R,
Lestremau F, Zeman FA, Leclerc E,
Moesch C,
% Brochot C. 2016. PBPK modeling of
the cis- and trans-permethrin isomers
and
% their major urinary metabolites in
rats. Toxicol. Appl. Pharmacol 294:65-
77.

% RATS WERE NOT FASTED as they were for
the Tornero-Velez study
% Sprague-Dawley male rats, 468 g +/-
21 g
% Each group received an oral dose of
PER: 25 mg/kg cis-permethrin in 2 mL/kg
corn oil
% Euthanized at 0.5, 1, 1.5, 2, 4, 6,
10, 24, 48 hr
% Collected BLOOD, brain, fat,
liver, testes, muscle, kidney
% Metabolites were measured

% 25 mg/kg dose cis isomer: Time (hr)
Blood Brain Liver Fat (mg/L)
%    (_hours, _cven, _cbrn, _cliv,
_cfat)
cis = [
0.5    0.153    0.126    0.129    0.223
1.0    0.692    0.109    0.671    0.490
1.5    1.320    0.318    1.254    NaN
2.0    0.807    0.372    1.077    0.687
4.0    0.390    0.641    0.588    NaN
6.0    0.231    0.433    0.258    2.243
10.0    0.110    0.621    0.203    6.715
24.0    NaN    0.088    NaN    4.863
48.0    NaN    NaN    NaN    5.359
144.0    NaN    NaN    NaN    3.422];

ResetDoses
Rat
RatPERc_fed

BW=0.468;

```

```

PDOSE(3)=25.0;
TSTOP=144.0;
start @NoCallBack

    plotbldc = plot (0, cis(:,1),
cis(:,2), '+b', _t, _cven(:,3), '-b');

    plotbrnc = plot (0, cis(:,1),
cis(:,3), '+b', _t, _cbrn(:,3), '-b');
    plotlive = plot (0, cis(:,1),
cis(:,4), '+b', _t, _cliv(:,3), '-b');
    plotfatc = plot (0, cis(:,1),
cis(:,5), '+b', _t, _cfat(:,3), '-b');

```

APPENDIX N. ARRAY MODEL M FILE FOR MIXED PERMETHRINS SIMULATIONS AND PREDICTIONS

Note: Column formatting introduces extraneous line breaks not in the original code.

```

PER_rat_Anadon.m
% Anadon A, Martinez-Larranaga MR, Diaz
MJ, Bringas P. 1991. Toxicokinetics of
permethrin in the rat.
% Toxicol.Appl.Pharmacol. 110:1-8.

% Sprague-Dawley male rats, n = 8
% This batch of permethrin had a 25
cis:75 trans ratio
% Each group received an IV dose of
PER: 46 mg/kg in 0.1 mL glycerol
formal/rat
% OR an oral dose of PER: 460 mg/kg
in 0.5 mL corn oil/rat
% Bodyweight was listed as 180-200 g
(assume 190 g)
% Euthanized at 0.25, 0.5, 1, 2, 3, 4,
6, 8, 12, 24, 48 hr
% Collected plasma, liver, sciatic
nerve and several brain regions:
% hypothalamus, cerebellum, frontal
cortex, caudate putamen, hippocampus,
medulla oblongata
% Metabolites were also measured in all
tissues listed above. This information
may be useful in the future.

% Assuming that plasma = blood
% Time in hours, PER Plasma Conc in
mg/L from IV and oral dose with + and -
SE
% (_hours, _cven IV, _cven IV+SE,
_cven IV-SE, _cven Oral, _cven+SE,
_cven-SE)
anadon1 = [
0.25 31.23 36.72 26.23 13.97 15.74
11.25
0.50 22.53 nan nan 21.56
nan nan
1.00 15.57 18.74 12.68 29.56 36.72
24.83
2.00 12.00 14.60 9.66 45.68 57.36
37.53
3.00 9.35 11.13 7.69 55.50 68.18
44.68
4.00 8.39 9.98 7.04 46.62 58.64
37.95
6.00 6.32 7.69 5.26 39.65 49.25
31.53
8.00 4.47 5.26 3.71 34.05 41.83
28.29
12.00 2.86 3.37 2.43 23.54 28.29
19.77
24.00 1.14 1.38 0.95 9.66 12.00
8.03

48.00 0.74 0.90 0.63 3.75 4.67
3.09];

% Assuming that plasma = blood
% Time, PER Plasma, Liver, Average
Brain, Max Brain, Min Brain Conc in
mg/L
% (_hours, _cliv, _cbrn, _cbrn,
_cbrn)
anadon2 = [
1.0 3.82 53.96 119.40 12.32
2.0 7.84 75.86 173.63 23.36
4.0 15.00 111.45 235.48 34.95
8.0 11.40 63.50 142.66 20.14
24.0 9.09 45.61 85.45 19.00
48.0 1.74 15.02 31.36 3.40];

resetdoses
rat
ratperc_fast
ratpert_fast

%Note that this dose is 200x larger
than the doses for which the absorption
was optimized (Willemin)
% Animals were fasted (like
Tornero-Velez) but used only 0.5 mL
corn oil (Willemin ~1 mL, Tornero-Velez
~0.3 mL)

VMAXC(3)=0.5; %Willemin (2.51);
Original Anadon fit 0.4
KM(3)=1.0; %Willemin (1.0);
Original Anadon fit 1.0
%Attempted to hold intrinsic
clearance = 6.3 - does not produce
fits. Changing VMaxC alone works
better

VMAXC(4)=1.26; %Willemin (6.3);
Original Anadon fit 1.2
KM(4)=1.0; %Willemin (1.0);
Original Anadon fit 1.0
%Attempted to hold intrinsic
clearance = 6.3 - does not produce
fits. Changing VMaxC alone works
better

IVDOSE(3)=0.25*46.0;
IVDOSE(4)=0.75*46.0;
TINF=0.008; % Assuming 30 second
injection in the absence of data
TCHNG=48.0; TSTOP=48.0;

CINT=0.1;

```

```

start @NoCallback

cventot = (_cven(:,3)+_cven(:,4));

plotcven = plot (0, anadon1(:,1),
anadon1(:,2), '+b', anadon1(:,1),
anadon1(:,3), '+b',...
                anadon1(:,1), anadon1(:,4),
'+b', _t, cventot, '-b');

resetdoses
PDOSE(3)=0.25*460.0;
PDOSE(4)=0.75*460.0;
TSTOP=48.0;
start @NoCallBack

cventot2 = (_cven(:,3)+_cven(:,4));

clivtot2 = (_cliv(:,3)+_cliv(:,4));
cbrntot2 = (_cbrn(:,3)+_cbrn(:,4));

plot (plotcven, 1, anadon1(:,1),
anadon1(:,5), '+g', anadon1(:,1),
anadon1(:,6), '+g',...
      anadon1(:,1), anadon1(:,7),
'+g', _t, cventot2, '-g');
plotliv = plot (0, anadon2(:,1),
anadon2(:,2), '+g', _t, clivtot2, '-
g');
plotbrn = plot (0, anadon2(:,1),
anadon2(:,3), '+g', anadon2(:,1),
anadon2(:,4), '+g', anadon2(:,1),
anadon2(:,5), '+g',...
               _t, cbrntot2, '-g');

```

APPENDIX O. ARRAY MODEL M FILES FOR GWI MIXTURES EXPOSURES AND PROJECT PREDICTIONS

Note: Column formatting introduces extraneous line breaks not in the original code.

Mix_rat_AQAD00.m

```
% Abu-Qare AW and Abou-Donia MB. 2000.
Simultaneous determination of
pyridostigmine bromide,
% N,N-diethylm-toluamide,
permethrin, and their metabolites in
rat plasma and urine by
% high-performance liquid
chromatography. J.Chromatogr.B.
749:171-8.

% Sprague-Dawley rats, n = 5
% (Same authors, different GWI
study, male SD rats, 225-250 g BW,
Abdel-Rahman, 2002)
% Each group received PB, PER, DEET or
3-way mixture
% 13 mg/kg PB oral
% 400 mg/kg DEET dermal (ICSurf =
440 mg/mL, 60 to 80% enters the system
per other studies)
% 1.3 mg/kg PER dermal (ICSurf =
1.43 mg/mL)
% No mention of cis:trans ratio
and ChemService Inc. doesn't know the
ratio with their current product
% As a pesticide, the mixture is
assumed to be approximately 40:60
cis:trans
% That is the ratio featured in
the PubChem Open Chemistry Database
% The acceptable range when
measured would be 30-50% cis.
% Food and Agricultural
Organization of the United Nations,
2009
% Euthanized at 8 hr; Collected blood
and cumulative urine
% No information: size of dermal
exposure site (assuming 1 cm2) based on
the 2003 study
% volume of dermal
exposure (using 0.25 mL DEET or
permethrin, 0.5 mL combined (different
study, same authors))
%(Area from Abu-Qare A.W., Abdel-Rahman
A., Abou-Donia MB. 2003.
Pharmacokinetic interactions
% between DEET (N,N-diethyl-m-
toluamide) and permethrin following
intravenous and
% dermal dose in rats. In: Abou-Donia
M.B. Toxic Interactions of Prophylactic
Drugs
```

```
% and Pesticides. Final Report. Ft
Detrick MD: U.S. Army Medical Research
and
% Materiel Command. ADA 424099.
pp.181-230. <
https://apps.dtic.mil/docs/citations/AD
A424099>.)
```

```
% Urine data were reported as
concentrations; without volumes, the
data can't be converted to an amount to
compare with AMet1
% Metabolites also measured in ng/mL:
107.3 +/- 21.5 m-toluamide, 98.4 +/-
14.8 m-toluic acid,
% 107.5 +/- 5.7 m-phenoxybenzyl
alcohol and 142.7 +/- 27.1 m-
phenoxybenzoic acid
```

```
resetdoses
rat
ratdeet
ratpb_fast
ratperc_fast
ratpert_fast
```

```
% Time, Average, -SD, +SD (_hours,
_cven DEET, _cven PER, _cven PB) in
mg/L
```

```
aqad00 = [
8.0 1.320 0.182 0.224
8.0 0.974 0.106 0.101
8.0 1.666 0.258 0.347];
```

```
BW=0.2375;
```

```
%DEET - estimating 62% absorption
ICSURF(1)=0.62*440.0; IVSURF(1)=0.25;
AREA(1)=1.0;
```

```
%PB
PDOSE(2)=13.0;
```

```
%PERc and PERT - KP estimated by
modified Robinson model from Wilschut
et al. (1995)
```

```
% PSkn estimated using Ruark et al.
2014, VMaxC from Willemijn et al. 2016
model
```

```
ICSURF(3)=1.43*0.4;
IVSURF(3)=0.25*0.4;
AREA(3)=1.0;
KP(3)=0.07;
PSKNE(3)=8.2;
```

```

VMAXC(3)=2.51;

ICSURF(4)=1.43*0.6;
IVSURF(4)=0.25*0.6;
AREA(4)=1.0;
KP(4)=0.07;
PSKNB(4)=8.2;
VMAXC(4)=6.3; %Anadon 1.26, Willemin
6.3

```

```

TCHNG=8.0; TSTOP=8.0;
start @NoCallback

cventot = (_cven(:,3)+_cven(:,4));

plotcven = plot (0, aqad00(:,1),
aqad00(:,2), '+b', _t, _cven(:,1), '-b', ...
aqad00(:,1), aqad00(:,3),
'+k', _t, cventot, '-k', ...
aqad00(:,1), aqad00(:,4),
'+m', _t, _cven(:,2), '-m');

```

```
%second run
```

```
%DEET - optimized % absorption
ICSURF(1)=0.45*440.0;
```

```
%PB - using fed parameters and
optimized VMaxC
ratpb_fed
VMAXC(2)=0.02; %Fit value 2.0
```

```
%PERC and PERT - Optimizing KP, PSkn,
VMaxC
KP(3)=0.07;
PSKNB(3)=8.2; %Ruark 8.2
VMAXC(3)=0.0251; %Anadon 0.5,
Willemin 2.51, Willemin/100
```

```

KP(4)=0.07;
PSKNB(4)=8.2; %Ruark 8.2
VMAXC(4)=0.063; %Anadon 1.26,
Willemin 6.3, Willemin/100

```

```

start @NoCallBack

cventot2 = (_cven(:,3)+_cven(:,4));

plot (plotcven, 1, _t, _cven(:,1), '-b',
_t, _cven(:,2), '-m', _t, cventot2,
'-k');

```

Mix_rat_Rohan_AQAD_param.m

```
% Prediction of Rohan tissue
concentrations using Abu-Qare and Abou-
Donia 2000 simulation parameters
```

```
% Sprague-Dawley rats
```

```

% Guessing BW is max weight in
protocol (225 g)
% Mixture dosing daily for 28 days
% 1.3 mg/kg PB oral
% 40.0 mg/kg DEET dermal (ICSurf =
45 mg/mL, 60 to 80% enters the system
per other studies)
% 0.13 mg/kg PER dermal (ICSurf =
0.13 mg/mL)
% SDS indicates 55:45 cis:trans ratio
% Need prediction at 5 min and 24 hour,
plus peak
% No concrete information on size of
dermal exposure site (assuming 3 cm2)
% Volume of exposure is 0.2 mL of
permethrin plus 0.2 mL DEET, both in
70% IPA
% Since it's a daily dose, assuming
they're fed.

```

```

resetdoses
rat
ratdeet
ratpb_fed
ratperc_fed
ratpert_fed

```

```
%ADAQ parameters
ICSURF(1)=0.45*45.0; IVSURF(1)=0.2;
AREA(1)=3.0;
```

```

PDOSE(2)=1.3;
VMAXC(2)=0.02; %Fit value 2.0

```

```

ICSURF(3)=0.13*0.55;
IVSURF(3)=0.55*0.2; AREA(3)=3.0;
VMAXC(3)=0.0251; %Anadon 0.5,
Willemin 2.51, Willemin/100

```

```

ICSURF(4)=0.13*0.45;
IVSURF(4)=0.45*0.2; AREA(4)=3.0;
VMAXC(4)=0.063; %Anadon 1.26,
Willemin 6.3, Willemin/100

```

```

TSTOP=24.0;
start @NoCallback

```

```

cventot = (_cven(:,3)+_cven(:,4));
cbrntot = (_cbrn(:,3)+_cbrn(:,4));
%calculating interstitium
concentrations in brain
% o=using observed/model PC, p=using
predicted PC
cbrninto1 = (_cbrn(:,1)/0.212);
cbrnintp1 = (_cbrn(:,1)/0.24);
cbrninto2 = (_cbrn(:,2)/2.85);
cbrnintp2 = (_cbrn(:,2)/0.016);
cbrninto3 =
((_cbrn(:,3)/1.6)+(_cbrn(:,4)/0.57));
cbrnintp3 =
((_cbrn(:,3)+_cbrn(:,4))/17.1);

```

```

    plotcven = plot (0, _t, cventot, '-
k', _t, _cven(:,2), '-m', _t,
_ cven(:,1), '-b');
    plotcbrn = plot (0, _t, cbrntot, '-
k', _t, _cbrn(:,2), '-m', _t,
_ cbrn(:,1), '-b');
    plotcbrninto = plot (0, _t,
cbrninto3, '-k', _t, cbrninto2, '-m',
...
_t, cbrninto1, '-b');
    plotcbrnintp = plot (0, _t,
cbrnintp3, '-k', _t, cbrnintp2, '-
m',...
_t, cbrnintp1, '-b');

```

LIST OF ACRONYMS

AChE	acetylcholinesterase
ACSF	artificial cerebrospinal fluid
AUC	area under the curve
AVMA	American Veterinary Medical Association
BBB	blood-brain barrier
CNS	central nervous system
DEET	diethyltoluamide
GFAP	glial fibrillary acidic protein
GWI	Gulf War Illness
I/O	input-output
IP	intraperitoneal
IV	intravenous
K _m	affinity constant
K _P	skin permeability constant
LD ₅₀	lethal doses for 50 percent of the population
MCMC	Markov Chain Monte Carlo
NAMRU-D	Naval Medical Research Unit Dayton
PB	pyridostigmine bromide
PBPK	physiologically-based pharmacokinetic
PBPLC	pyridostigmine bromide phospholipid complex
PC	partition coefficient
PEI	Polyethylenimine
PER	permethrin
PI	pyridostigmine iodide
PP ratio	paired pulse ratio
PPF	paired pulse facilitation
PTSD	post-traumatic stress disorder
RAC	Research Advisory Committee
SD	standard deviation
SEM	standard error of the mean
V _{Max} C	Michaelis-Menten constant