



AFRL-RH-WP-TR-2020-0059

**Investigation of Inter-Individual Genetic Variability
with Physiologically-Based Pharmacokinetic Models
and Monte Carlo Analysis**

**Tammie R. Covington, Jeffery M. Gearhart
Henry M. Jackson Foundation**

**Joseph P. Jarvis, Heather A. Pangburn, Darrin K. Ott
711 HPW/RHB**

**JULY 2020
Final Report**

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**AIR FORCE RESEARCH LABORATORY
711TH HUMAN PERFORMANCE WING,
AIRMAN SYSTEMS DIRECTORATE,
WRIGHT-PATTERSON AIR FORCE BASE, OH 45433
AIR FORCE MATERIEL COMMAND
UNITED STATES AIR FORCE**

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JOSEPH P. JARVIS, PhD
Informatics Section Lead
Biotechnology Branch

//signature//

LOGAN WILLIAMS, PhD
Airman Readiness Optimization CRA Lead
Airman Systems Directorate
711th Human Performance Wing
Air Force Research Laboratory

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REPORT DOCUMENTATION PAGE			<i>Form Approved</i> <i>OMB No. 0704-0188</i>		
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1. REPORT DATE (DD-MM-YYYY) 07-07-2020		2. REPORT TYPE Final		3. DATES COVERED (From – To) October 2017 – September 2018	
4. TITLE AND SUBTITLE Investigation of Inter-Individual Genetic Variability with Physiologically-Based Pharmacokinetic Models and Monte Carlo Analysis			5a. CONTRACT NUMBER FA8650-15-2-6608		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) *Tammie Covington, Jeffery Gearhart **Joseph Jarvis, Heather Pangburn, Darrin Ott			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER RHM Legacy		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) *Henry M. Jackson Foundation 6720A Rockridge Dr. Bethesda, MD 20817			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) ** Air Force Materiel Command Air Force Research Laboratory 711 th Human Performance Wing Airman Systems Directorate Airman Biosciences Division Performance Optimization Branch Wright-Patterson AFB, OH 45433			10. SPONSORING/MONITOR'S ACRONYM(S) 711 HPW/RHBC		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) AFRL-RH-WP-TR-2020-0059		
12. DISTRIBUTION / AVAILABILITY STATEMENT DISTRIBUTION A. Approved for public release.					
13. SUPPLEMENTARY NOTES MSC/PA-2020-0208 88ABW-2020-2709, cleared 3 September 2020					
14. ABSTRACT Genetic variability continues to be a component of interest in assessing the expected impact of chemical exposures on human health. Until the advent of modern “omics” rapid assessment technologies, broad assessment of genetic variation on individualized outcomes was limited to single gene variants. This work enlists genetic information from the DMET™ Plus Array which contains comprehensive and accurate genotyping of specific polymorphisms involved in drug metabolism from the Coriell Personalized Medicine Collaborative. The expected impact of genetic variation in metabolic and transporter processes was assessed in relation to chemicals commonly encountered in the United States Air Force environment. Ten variants in Cytochrome P450, Family 2, Subfamily E, Polypeptide 1 (CYP2E1) were chosen for initial consideration given its in processing volatile organic chemicals. Measured CYP2E1 variation and its expected impact were then incorporated into analyses via a physiologically-based pharmacokinetic (PBPK) model to assess the likely influence on blood time course of isopropanol, acetone and toluene. The PBPK model simulations show that the predictions are influenced by incorporating information on genetic variants and would, therefore, impact predicted exposure estimates. Application of these genome-informed insights will allow a refined estimate of expected exposure response and potentially the prediction of personalized health outcomes.					
15. SUBJECT TERMS genetic variability, physiologically based pharmacokinetic model, metabolism, isopropanol, acetone, toluene, polymorphisms, pilots					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joseph Jarvis
U	U	U	SAR	52	19b. TELEPHONE NUMBER (include area code) N/A

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ACKNOWLEDGMENTS

This project was funded by Research Studies and Analysis Council (RSAAC) 18-048 Investigation of Inter-Individual Genetic Variability with Physiologically-Based Pharmacokinetic Models and Monte Carlo Analysis.

When the work was conducted, Tammie R. Covington and Jeffery M. Gearhart were with FHO, Heather A. Pangburn was with FHO and RHDJ and Darrin K. Ott was with FHO. Joseph P. Jarvis was affiliated with Coriell Institute for Medical Research in Camden, New Jersey.

1.0 SUMMARY

Genetic variability continues to be a component of interest in assessing the expected impact of chemical exposures on human health. Until the advent of modern “omics” rapid assessment technologies, broad assessment of genetic variation on individualized outcomes was limited to single gene variants. This work enlists genetic information from the DMET™ Plus Array which contains comprehensive and accurate genotyping of specific polymorphisms involved in drug metabolism measured on approximately 2000 personnel in the Air Force Medical Service Patient-Centered Precision Care Program as implemented by the Coriell Personalized Medicine Collaborative (CPMC). The expected impact of genetic variation in metabolic and transporter processes was assessed in relation to chemicals commonly encountered in the United States Air Force (USAF) environment. Specifically, 10 putatively functional variants in Cytochrome P450, Family 2, Subfamily E, Polypeptide 1 (CYP2E1) were chosen for initial consideration given the role of this locus in processing volatile organic chemicals (VOCs) such as isopropanol, acetone and toluene. Measured CYP2E1 variation and its expected impact were then incorporated into analyses via a physiologically-based pharmacokinetic (PBPK) model to assess the likely influence of genetic variants on blood time course of isopropanol, acetone and toluene. The PBPK model simulations show that the predictions are influenced by incorporating information on genetic variants and would, therefore, impact predicted exposure estimates. Application of these genome-informed insights will allow a refined estimate of expected exposure response and potentially the prediction of personalized health outcomes.

2.0 INTRODUCTION

2.1 Background

Physiologically-based pharmacokinetic (PBPK) models are mathematical descriptions of the absorption, distribution, metabolism and excretion of chemicals in the body and allow for the incorporation of species-specific physiology, chemical-specific characteristics, and the chemical exposures of concern. They typically consist of a series of ordinary differential equations that describe the pharmacokinetics in blood and various tissues. Use of PBPK models allows for the determination of better estimates of the actual chemical dose delivered to a target tissue resulting in a particular response as opposed to estimating a response based on external dose alone [3, 4]. PBPK models alone, however, provide only deterministic estimates of the internal dose that don't account for even basic individual variations such as body weight or breathing rates. Integrating Monte Carlo analysis with PBPK models allows for the investigation of the impact of individual variations on different exposure scenarios.

The process of combining Monte Carlo analysis with PBPK models has been previously used to investigate the composite effect of physiological variability [5, 6, 7, 8]. These studies used PBPK models and Monte Carlo analysis to investigate the impact of inter-individual variability on PBPK model predictions and the resulting calculations of regulatory safety values for perchloroethylene and methyl mercury. In a similar vein, the previous effort based on High-Performance Aircraft Respiratory Study (HPARS) was expanded to include Monte Carlo analysis to explore the potential variation in estimated pilot exposures due to individual variations in basic physiology [1]. Employing probabilistic methods enabled the use of various distributions to describe each model parameter, and allowed for the prediction of a distribution for exposure concentrations rather than single point estimates. While the distributions from this analysis begin to inform on the potential impact of inter-individual variability, they do not incorporate any information on potential genetic variations that might result in increased susceptibility for a sub-set of the population.

Over the last several decades, a great deal of research has focused on genetic variation in loci that process and transport pharmaceuticals within the body. This work in pharmacogenomics (PGx), as the field is known, has resulted in multiple commercial products geared toward the personalization of treatments that show great promise for minimizing the side effects of therapy, maximizing treatment efficacy, and improving efficiency in healthcare spending. It has also generated a variety of publicly available resources and curated knowledgebases (*e.g.*, PharmGKB) that may be leveraged by work in other areas. For example, the very same loci that process a “voluntary” exposure to a pharmaceutical compound are involved in the body's response to an “involuntary” chemical exposure in the workplace (*e.g.*, volatile organic compounds (VOCs)). Thus, the rich information, tools, and techniques that have been developed for evaluating PGx genes and variants represent untapped sources of insights that may be deployed in efforts to understand war-fighter risk of negative outcomes following chemical exposures in the United States Air Force (USAF) environment.

There are published studies (*e.g.*, carbon tetrachloride and dichloromethane) demonstrating how genetic polymorphism data may be incorporated into Monte Carlo analysis [9, 10, 11]. In particular, the dichloromethane studies incorporated evaluation of the variability in the

polymorphism affecting metabolism (glutathione-S-transferase theta 1 (GST-T1)) (Table 1) into a Monte Carlo analysis to investigate the predicted variability in tissue dose. The published studies demonstrate that the use of Monte Carlo analysis with PBPK models can be a useful means for examining the potential inter-individual variability due to genetic polymorphisms.

Table 1. Population Distributions of GST-T1 Genotypes from David *et al.* (2006)^a

Population	Genotype Frequency		
	+/+	+/-	-/-
Caucasian	0.31	0.49	0.19
African American	0.28	0.50	0.22
Hispanic	0.47	0.43	0.10
Asian American	0.05	0.33	0.62
US Average	0.32	0.48	0.20

^a“+” and “-” refer to the wild-type and null alleles, respectively

2.2 Previous Work

The 711th Human Performance Wing F-22 Physiologic Analysis Team conducted a HPARS to potentially identify the etiology of the reported coughing/respiratory symptoms and mitigate one of the most common health-related complaints of F-22 flight. One unknown component of the proposed induction of coughing/respiratory symptoms that was addressed by HPARS was the collection and analysis of potential atmospheric chemicals in the cockpit air. United States Air Force School of Aerospace Medicine (USAFSAM) Bioenvironmental Engineering performed two types of air sampling: diffusive monitoring of the cockpit and exhaled breath before and after the flight. This study found a positive statistical association between respiratory effects or cough and a short list of known potentially toxic chemicals. Due to the rapid absorption of these volatile organic chemicals into the body via inhalation and often rapid clearance from the blood stream and organs shortly after cessation of exposure, an existing published PBPK model was utilized to predict the concentration range of probable inhalation exposures that could account for the post-flight exhaled breath concentrations.

The work presented here builds upon previous work [1] conducted using the existing published PBPK model of Clewell *et al.* [2], describing the pharmacokinetics from exposure to isopropanol and its metabolite, acetone, in conjunction with the HPARS data. The published model has flow-limited compartments for brain, fat, liver, skin and the remaining rapidly and slowly perfused tissues, plus first-order urinary excretion from blood. Due to the hydrophilic nature of isopropanol and acetone, Clewell *et al.* [2] assumed that some absorption in the upper respiratory tract could occur during inhalation, with subsequent desorption during exhalation. Their description of this cyclic phenomenon treats inhalation and exhalation as simultaneous and parallel processes and incorporates the reservoir effect of the mucus layer of the upper respiratory tract on exhaled air concentrations. The structure of the acetone sub-model is the same as that used for isopropanol with the exception of the absorption and desorption of acetone with breathing and urinary excretion from blood. The model provides the capability for

simulating exposure via intravenous injection, intraperitoneal administration, oral gavage, inhalation, and dermal application.

For the purposes of this work, the breathing portion of the metabolite sub-model was modified to include the absorption and desorption of acetone with breathing and to allow for simultaneous inhalation exposure to isopropanol and acetone; additional routes of exposure were not added to the metabolite sub-model as this work was only concerned with inhalation exposure. The metabolite sub-model was also modified to add urinary excretion from the blood compartment. Lastly, the complete model was modified to run in minutes instead of hours for ease in conducting the dose reconstructions. The modified model structure was then used to simulate inhalation exposure to isopropanol, acetone, toluene and cyclohexane by changing the chemical-specific parameters of the model.

For the dose reconstructions, the modified model was run to simulate exposure of various lengths starting at various times during a one hour flight. These simulations also included a 20 minute period following the flight in order to duplicate the actual delay between the end of the flight and the collection of exhaled breath samples. For each combination of length and start of exposure, the model was run and the predicted exhaled air concentration was compared to the measured exhaled breath concentrations from actual high-performance aircraft (HPA) pilots from HPARS. The simulated air concentrations were adjusted until the predicted exhaled air concentration at 80 minutes (one hour flight plus 20 minutes) matched the measured exhaled breath concentration. The estimated doses for all combinations were compiled along with the corresponding maximum blood and brain concentrations for all chemicals.

These reconstructions allowed for the determination of possible exposure distributions across a range of exposure lengths and times, or scenarios, that might be experienced by HPA pilots during flight, but did not account for differences in pharmacokinetics due to genetics that might result in increased susceptibility for a sub-set of the population.

2.3 Objectives

The long term goal of this effort is to produce pilot breathing air exposure guidelines that reduce the likelihood of coughing/respiratory symptoms or other reported symptomology. Incorporation of Monte Carlo analysis with PBPK modeling and information on relevant genetic polymorphisms allows for the investigation of the impact of inter-individual variation on toxic risk. Use of these probabilistic methods will be more informative than the deterministic methods as it enables accounting for inter-individual variability in a given population through the use of various distributions to describe each parameter, and allows for the prediction of distributions of potential exposure concentrations rather than single point estimates (Figure 1).

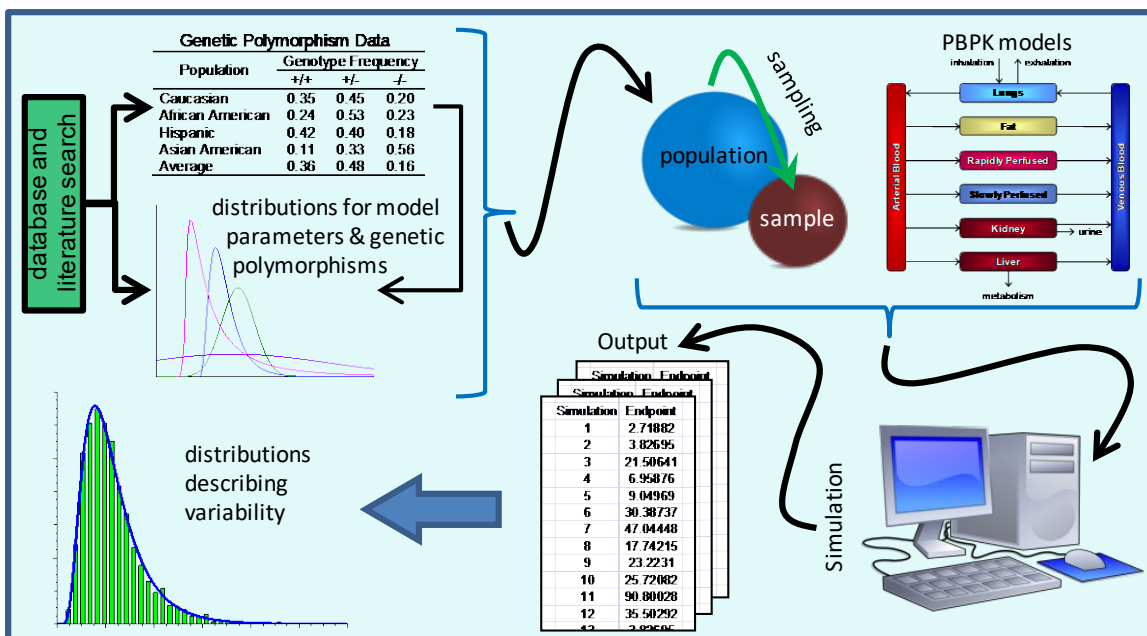


Figure 1. Work Flow Schematic

3.0 METHODS

3.1 CYP2E1 Genetic Variation in an Air Force Population

An inception study was conducted focused on 10 single nucleotide polymorphism (SNP) variants in Cytochrome P450, Family 2, Subfamily E, Polypeptide 1 (CYP2E1) that were genotyped using the Affymetrix DMET™ Plus gene chip in approximately 2200 appropriately consented USAF participants in the Coriell Personalized Medicine Collaborative (CPMC) Clinical Utility Study [12]. The CYP2E1 gene was chosen for initial consideration given its role in processing VOCs such as isopropanol, cyclohexane and aliphatic hydrocarbons. Of the 10 variants assayed, nine were bi-allelic. That is, they are known to show only two potential allelic alternatives in the human populations that have been sampled to date (*e.g.*, A or T). One variant is known to show three potential allelic alternatives (G, C, or A) though neither the “A” nor “C” allele was observed with sufficient confidence in the USAF population. One of the bi-allelic SNPs was similarly monomorphic (*i.e.*, showed no variation in the dataset with only the expected “reference” value present).

Since each SNP is measured independently on the DMET™ Plus Array, the precise order of allelic variants on the deoxyribonucleic acid (DNA) strand inherited from each parent (*i.e.*, the true state of the two “haplotypes” present collectively known as the “diplotype”) is not known with certainty. Rather, it must be inferred either by matching observations to data from other populations or by one of several mathematical algorithms in a process known as “phasing”. Understanding the phase of variants in each research subject is critical to interpreting their biological significance following chemical exposure. For example, if a subject is heterozygous at two SNPs for alleles that completely eliminate enzyme function, it cannot be determined without phasing if they will have one functional and one non-functional enzyme (that is impacted

by both variants) or two non-functional enzymes (each being impacted by one of the two null variants). The situation becomes even more complicated with larger numbers of variants at larger numbers of SNPs. In some cases, multiple interpretations of measured genotypes may be equally likely and the various possible phase states may or may not share physiological interpretations (*e.g.*, normal metabolism).

In order to address these complications, a custom diplotyping algorithm based on phase was developed and implemented in the statistical computing language R [13] along with available data (*e.g.*, PharmGKB, CYPalleles). This algorithm helped identify known haplotypes and resolve the independently measured genotype data into as many diplotype calls in the dataset as possible. Due to uncertainty in the *cis/trans* orientation of multiple heterozygotes, when necessary the algorithm assigns multiple possible metabolizer status categories to some combinations of variants. When there was a conflict, the lowest potential function was taken to represent the status for a given combination of variants. Thus, the output of the algorithm includes an assessment of all potential combinations of haplotypes along with their known/putative functions and inferred physiological categories (*e.g.*, normal metabolism, poor metabolism) for each individual. Specific combinations that did not fit known patterns of variation were inspected for evidence of novel haplotypes; several were identified. In all, there is evidence for as many as 21 haplotypes segregating in this population. All identified haplotypes were iteratively added to the algorithm and some level of resolution of diplotype was achieved for 2166 individuals. Eight additional individuals could not be resolved.

Little is known about the functional properties of many of the SNPs measured here with specific respect to VOCs. However, by assigning putative functional characteristics to individual haplotypes, groups of diplotypes were assigned to hypothesized metabolizer status categories (*i.e.*, normal, reduced and very low) for use in exposure modeling. Based on a review of the literature, appropriate analogous values from other work were used to assign putative function. These final physiological groupings were used in the exposure modeling.

3.2 PBPK Model Structure

The PBPK model used for this work is a modification of a previously published PBPK model [2] describing the pharmacokinetics from exposure to isopropanol and its metabolite, acetone, and is described above in Section 2. The model was further modified to remove code for oral, dermal, and intravenous dosing as this work was primarily concerned with inhalation exposure. The modified PBPK model (Figure 2) was then used, with physiological- and chemical-specific parameters from the papers describing the models for isopropanol, acetone, toluene and cyclohexane, to validate endpoints predicted with the modified isopropanol model following exposure to these chemicals. The model is executed using the Gear algorithm in acsIX (formerly available from AEGIS Technologies Group, Inc., Huntsville, AL).

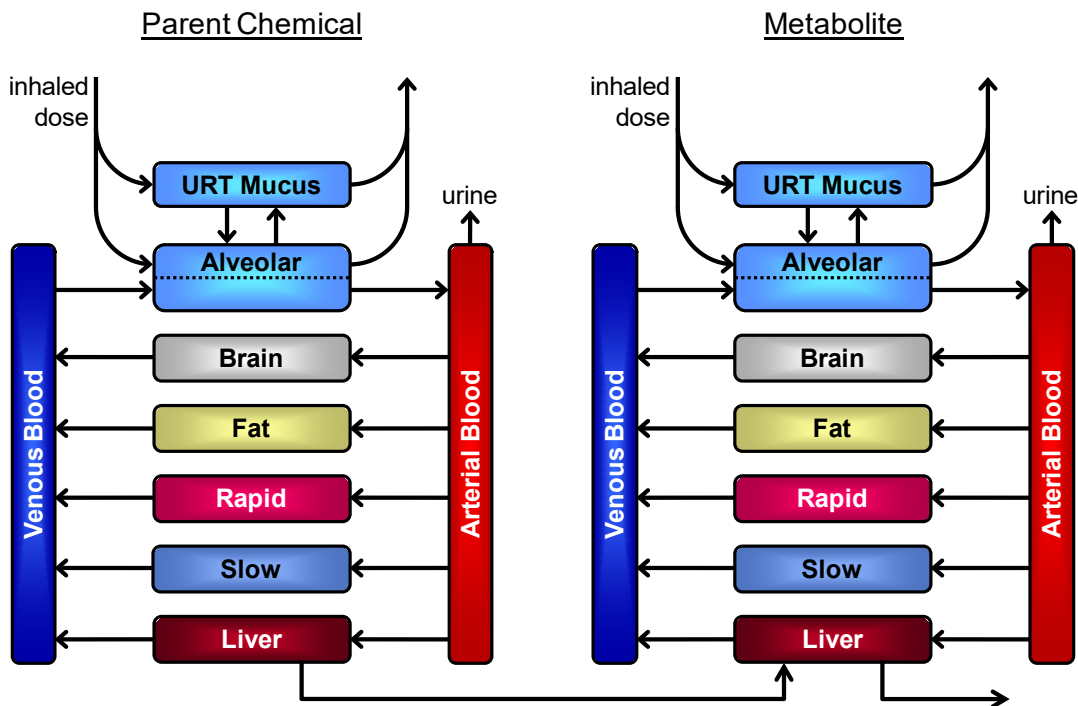


Figure 2. Schematic of Modified PBPK Model for Isopropanol and its Metabolite, Acetone. This schematic shows the model structure resulting from modifications to the Clewell *et al.* [2] model.

3.3 PBPK Model Parameters

Chemical-specific parameters were taken from papers describing the PBPK models for isopropanol, acetone, toluene and cyclohexane. The simulations use body weights taken from an Air Force (AF) biometric database [14] (pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI), physiological parameters averaged across the studies, and chemical-specific parameters. These parameters are summarized in Tables 2 and 3. The ventilation rate for the toluene model was alveolar and has been converted to a total ventilation rate in Table 2 by dividing the alveolar rate by two-thirds [15]. The exponential power used for scaling was different in the toluene model than in the isopropanol model for cardiac output, pulmonary ventilation and maximum metabolic rate; therefore, the values in Table 3 have been adjusted such that the scaled value in the simulations here would be the same as those in the toluene model for the same body weight. Parameters included in the isopropanol model (*i.e.*, clearance parameters) which are not included in the published model for toluene [16] were set to values so as to have no effect on the model predictions (*e.g.*, a value of zero for clearance parameters).

The toluene model of Tardif *et al.* [16] did not include a brain compartment but the modified isopropanol model used for the dose reconstructions does; therefore, parameter values for the brain compartment were taken from other sources: fractional brain blood flow and volume from

the isopropanol model [2] and a brain/gas partition [17] used to calculate the brain/blood partition.

Table 2. Physiological Parameters

Parameter	Isopropanol / Acetone	Toluene
Body weight (kg)	84.14 ^a	84.14 ^a
Cardiac output (L/min/kg ^{0.75})	0.2148	0.24
Pulmonary ventilation (L/min/kg ^{0.75})	0.4625	0.36
Fractional Tissue Blood Flows (fraction of cardiac output)		
Brain	0.114	0.114 ^b
Fat	0.052	0.05
Liver	0.227	0.26
Rapidly perfused compartment	0.419	0.326
Slowly perfused compartment	0.188	0.25
Fractional Tissue Volumes (fraction of body weight)		
Alveolar blood	0.0079	0.0079 ^b
Brain	0.02	0.02 ^b
Fat	0.214	0.19
Liver	0.026	0.026
Mucous	0.0001	0.0001 ^b
Rapidly perfused compartment	0.036	0.03
Slowly perfused compartment	0.536	0.62

^aAverage male body weights from an AF biometric database [14]; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI

^bParameter not included in model for this chemical – used isopropanol/acetone model value

Table 3. Chemical-Specific Parameters

Parameter	Isopropanol	Acetone	Toluene
Molecular weight (g/mole)	60.09	58.08	92.1384
Partition Coefficients (unitless)			
Blood/air	848.0	260.0	15.6
Mucus/air	848.0	260.0	-- ^a
Brain	1.33	0.69	2.33 ^b
Fat	0.32	0.44	65.4 ^c
Liver	1.16	0.58	5.36 ^c
Rapidly perfused compartment	1.25	0.69	5.36 ^c
Slowly perfused compartment	1.3	0.7	1.78 ^c
Metabolism Parameters			
Maximum reaction rate (L/min/kg ^{0.75})	5.0	0.0583	0.08
Michaelis-Menten affinity constant (mg/L)	10.0	10.0	0.55
First order rate constant (kg ^{0.75} /min)	0.0	0.0	0.0
Endogenous Acetone Parameters			
Concentration of endogenous acetone (mg/L)	0.0	0.0	-- ^a
Rate of production (mg/min/kg ^{0.75})	0.0	0.0	-- ^a
Uptake and Clearance Parameters (L/min/kg^{0.75})			
Urinary clearance	6.67×10 ⁻⁵	6.67×10 ⁻⁵	-- ^a
Upper respiratory tract uptake	0.183 ^d	0.183 ^d	-- ^a

^aParameter not used for this chemical

^bCalculated using brain/gas partition [17] and blood/air partition [16]

^cTissue/blood partitions calculated from blood/air partition and tissue/air partition [16]

^dThe value for upper respiratory tract uptake was adjusted for the dose reconstruction simulations to maintain the value as the same fraction of pulmonary ventilation as for the validation figures. The adjusted value is 0.172 L/min/kg^{0.75}.

3.4 Simulations for Metabolic Variation

For purposes of demonstrating the impact of different metabolic genotypes, it was assumed that the maximum reaction rate (V_{MaxC}) values from Table 3 would be used for “normal” metabolizers. It was determined that “reduced” and “very low” metabolizers would use one-third and one-tenth, respectively, of the “normal” value based upon the authors’ knowledge and experience with other chemicals (Table 4). Values for the Michaelis-Menten affinity constant (K_M) were not altered. For each chemical and each metabolic group, the model was executed to simulate a 1-hour inhalation exposure to the chemical at the chemical’s short-term exposure limit (STEL) and the subsequent kinetics for three hours post-exposure. The STELs are 400, 1000 and 150 ppm for isopropanol, acetone and toluene, respectively.

Monte Carlo analyses were conducted by varying only V_{MaxC} to explore the impact of variation within metabolic groups on the predicted kinetics. The analyses consisted of 10,000 iterations for each chemical for each metabolic group. The distributions for these analyses were defined using the log-space mean and standard deviation (Table 4) and were bounded at two standard

deviations. The log-space mean and standard deviation were based on a coefficient of variation (CV) of 30% to represent a moderate amount of variation.

Table 4. Adjusted VMaxC Values to Represent Genetic Polymorphisms

Chemical	Level	KM (mg/L)	VMaxC (mg/hr/kg ^{0.75})				
			Natural Space Mean	Log Space		Bounds	
				Mean	Standard Deviation	Lower	Upper
Isopropanol	Normal*	10	5.0	1.566	0.2936	2.0	8.0
	Reduced	10	1.667	0.4677	0.2936	0.6667	2.667
	Very low	10	0.5	-0.7362	0.2936	0.2	0.8
Acetone	Normal*	10	0.05833	-2.885	0.2936	0.02333	0.09333
	Reduced	10	0.01945	-3.983	0.2936	0.007778	0.03111
	Very low	10	0.005833	-5.187	0.2936	0.002333	0.009333
Toluene	Normal*	0.55	0.08	-2.569	0.2936	0.032	0.128
	Reduced	0.55	0.02667	-3.667	0.2936	0.01067	0.04267
	Very low	0.55	0.008	-4.871	0.2936	0.0032	0.0128

*Corresponds to VMaxC values of 300.0, 3.5 and 4.8 mg/hr/kg^{0.75} for isopropanol, acetone and toluene, respectively [2, 16]

For further comparison, Monte Carlo analyses were also conducted for a mixed metabolizers group. This group consisted of a mix of normal, reduced and very low metabolizers. The diplotypes were grouped by metabolic status (normal, reduced and very low – unknown metabolizers were not included) and the total frequency for each group was calculated. A portion of the simulations conducted for each individual metabolic status group, corresponding to the frequency for each group, were combined for a total of 10,000 iterations there were used for the mixed metabolizers output.

4.0 RESULTS AND DISCUSSION

4.1 Summary of Haplotypes

Haplotypes *1, *4, *5 and *7 were observed in the USAF cohort with *1 being the reference sequence for CYP2E1. The remaining haplotypes involve SNPs at the loci of rs2031920, rs2070672, rs2070673, rs2515641, rs3813867, rs6413419, rs6413420 and rs915909.

The *4 haplotype consists of an allelic variation from G to A at the rs6413419 locus. Three additional variations of the *4 haplotype, referred to as *4_Unk1, *4_Add_A and *4_Add_C in Table 5, were also observed with an allelic variation from T to A at the rs2070673 locus. *4_Unk1 has an additional allelic variation from C to T at the rs2515641 locus, and *4_Add_C has one from G to C at the rs3813867 locus. The activity levels are believed to be normal for *4 and decreased for *4_Add_C but are unknown for *4_Unk1 and *4_Add_A.

Allelic variations from G to C at locus rs3813867 and from C to T at locus rs2031920 define the *5 haplotype with an additional haplotype, referred to as *5_ *Unkl* in Table 5, having allelic variations from T to A at locus rs2070673 and from C to T at locus rs2515641. Both *5 group haplotypes exhibit a possible decrease in activity [18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28].

There were several haplotypes in the *7 group observed in the USAF cohort with all being characterized by an allelic variation from T to A at the rs2070673 locus. These haplotypes fall into one of three sub-groups: *7A, *7B and *7C. The haplotype referred to as *7A in Table 5 has no additional allelic variations, but *7A_10463 has an additional allelic variation from C to T at locus rs2515641. *7A_6498_10463 has the same variation at locus rs2515641 along with a third allelic variation from C to T at locus rs915909. The *7A haplotypes may have normal activity but the activity is unknown.

There is believed to be an increase in activity when haplotype *7A is combined with an allelic variation from G to T at locus rs6413420; this SNP defines the haplotype referred to as *7B_10463 in Table 5. The haplotype *7B is defined by the allelic variation from T to A at the rs2070673 locus and the allelic variation from G to T at locus rs6413420. These two haplotypes form the *7B sub-group and exhibit a possible increase in activity [18, 29]. It is unclear if the possible increase in activity with *7B is attributed to solely to the SNP at locus rs6413420.

The *7C sub-group of haplotypes consists of haplotypes *7C, *7C_10463 and *7C_6498_10463 and are defined by the SNP at locus rs2070673 and an additional allelic variation from A to G at locus rs2070672; all exhibit a possible decrease in activity [30]. *7C_10463 and *7C_6498_10463 have an additional allelic variation at locus rs2515641 from C to T, and *7C_6498_10463 has a third SNP from C to T at locus rs915909. The decreases for the haplotypes in sub-group *7C appear to specifically relate to the rs2070672 SNP. It should be noted that *7C_10463, which is believed to exhibit a decrease in activity, only differs from *7A_10463, which has an unknown activity level, by its SNP at locus rs2070672.

There were two additional haplotypes observed in the USAF cohort; these are labeled *CYP2E1_6498* and *CYP2E1_10463* in Table 5. Both are believed to exhibit normal activity. Synonymous with SNP I321I, *CYP2E1_6498* is defined by an allelic variation from C to T at Rs915909 and *CYP2E1_10463* (synonymous with SNP F421F) from C to T at Rs2515641.

4.2 Population Genotype and Metabolizer Status

By applying the custom algorithm, visually inspecting results to infer the presence of novel haplotypes (*i.e.*, those not present in the databases used to construct it), and applying appropriate quality control criteria, a total of 21 haplotypes were identified as segregating in the research sample. A total of 12 of these do not appear in standard public databases (*e.g.*, PharmGKB) and may represent novel combinations of variants of particular military interest.

Data from a total of eight individuals could not be resolved, though the overwhelming majority of subjects (N = 2166) could be categorized. This categorization allowed the inference of the potential functional state of the two enzymes produced from the two inherited DNA sequences

based on existing data in PGx resources and the primary literature exploring CYP2E1 variation and VOC exposure specifically.

Table 5. Diplotype Frequencies

Diplotype	Metabolizer Status	N	Frequency
*1 *1	Normal	1360	62.79%
*1 CYP2E1_10463	Normal	9	0.42%
*1 *7A	Normal	4	0.18%
*1 *4_Add_A or *4 *7A	Normal	3	0.14%
*1 Unk1 or *1 *7A_10463 or *7A CYP2E1_10463	Normal	114	5.26%
*1 *4_Unk1 or *4 Unk1 or *4 *7A_10463 or *4_Add_A CYP2E1_10463	Normal	97	4.48%
*1 *7A_6498_10463 or Unk1 CYP2E1_6498 or *7A_10463 CYP2E1_6498	Normal	10	0.46%
*5 *7A	Reduced	97	4.48%
*1 *7C_10463 or *7C CYP2E1_10463	Reduced	66	3.05%
*1 *7C	Reduced	61	2.82%
*4_Add_A *5_Unk1	Reduced	8	2.32%
Unk1 *7C_10463 or *7A_10463 *7C_10463	Reduced	6	1.74%
*4_Unk1 *7C_10463	Reduced	6	1.74%
*4_Unk1 *7C or *4_Add_A *7C_10463	Reduced	5	1.45%
*1 *5_Unk1 or Unk1 *5 or *5 *7A_10463	Reduced	5	1.45%
Unk1 *7C or *7A *7C_10463 or *7A_10463 *7C_10463 CYP2E1_10463	Reduced	4	1.16%
*7C_10463 CYP2E1_10463	Reduced	4	1.16%
*1 *4_Add_C	Reduced	4	1.16%
*4_Add_C Unk1 or *4_Add_C *7A_10463	Reduced	2	0.58%
*4_Add_C *7A_6498_10463	Reduced	1	0.29%
*5_Unk1 *7A	Reduced	1	0.33%
Unk1 *7C_6498_10463 or *7A_6498_10463 *7C_10463 or *7A_10463 *7C_6498_10463	Reduced	1	0.33%
*4_Add_C CYP2E1_10463	Reduced	1	0.33%
*4_Add_A *4_Add_C	Reduced	1	0.33%
*1 *5	Reduced	1	0.33%
*7A *7C_6498_10463 or *7A_6498_10463 *7C_6498_10463	Reduced	1	0.33%
*5_Unk1 *7C	Very Low	6	2.00%
*4_Add_C *7C_10463	Very Low	5	1.67%
*7C_10463 *7C_10463	Very Low	2	0.67%
*5_Unk1 *7C_10463	Very Low	1	0.36%
*7C_6498_10463 *7C_6498_10463	Very Low	1	0.36%
*7C_10463 *7C_6498_10463	Very Low	1	0.36%
*7C *7C	Very Low	1	0.36%
*5_Unk1 *5_Unk1	Very Low	1	0.36%
*7C *7C_10463	Very Low	1	0.36%

Diplotype	Metabolizer Status	N	Frequency
*1 *7B	Unknown	8	2.85%
*7B_10463 *7B_10463	Unknown	3	1.07%
*1 *7B_10463 or *7B CYP2E1_10463	Unknown	179	63.70%
*4_Unk1 *7B_10463	Unknown	9	3.20%
Unk1 Unk1 or *7A_10463 *7A_10463 or Unk1 *7A_10463	Unknown	8	2.85%
Unk1 CYP2E1_10463 or *7A_10463 CYP2E1_10463	Unknown	7	2.49%
*4_Unk1 *7A_6498_10463	Unknown	6	2.14%
*4_Unk1 Unk1 or *4_Unk1 *7A_10463	Unknown	6	2.14%
*7B *7C_10463 or *7B_10463 *7C	Unknown	5	1.78%
*7B_10463 *7C_10463	Unknown	5	11.36%
*4_Unk1 CYP2E1_10463	Unknown	5	11.36%
*5_Unk1 *7B	Unknown	4	9.09%
Unk1 *7A or *7A *7A_10463	Unknown	4	9.09%
Unk1 *7A_6498_10463 or *7A_6498_10463 *7A_10463	Unknown	4	9.09%
*4_Unk1 *7A or *4_Add_A Unk1 or *4_Add_A *7A_10463	Unknown	3	6.82%
CYP2E1_10463 CYP2E1_10463	Unknown	2	4.55%
*4_Unk1 *4_Unk1	Unknown	2	4.55%
*4_Unk1 *7B or *4_Add_A *7B_10463	Unknown	2	4.55%
*7B_10463 CYP2E1_10463	Unknown	2	4.55%
Unk1 *7B_10463 or *7A_10463 *7B_10463	Unknown	2	4.55%
*7A_6498_10463 CYP2E1_10463	Unknown	2	4.55%
*4_Unk1 *4_Add_A	Unknown	1	2.27%
*7B *7C	Unknown	1	2.27%
*7A_6498_10463 *7A_6498_10463	Unknown	1	2.27%
*7A *7A_6498_10463	Unknown	1	25.00%
*7A *7A	Unknown	1	25.00%
*4_Add_C *7B_10463	Unknown	1	25.00%
*7A_6498_10463 *7B_10463	Unknown	1	25.00%

4.3 PBPK Model Simulations

The frequencies for each metabolizer status group are 73.73%, 12.70%, 0.88% and 12.70% for normal, reduced, very low and unknown metabolic activity. Omitting those with unknown metabolic activity and recalculating the percentages results in 84.45%, 14.54% and 1.01% for normal, reduced and very low.

Figures 4, 6 and 8 show the results of the Monte Carlo analyses for each of the three metabolic groups for isopropanol, acetone and toluene, respectively. The graphs show the mean predicted venous blood and brain concentration at each time point as well as shaded regions representing the mean \pm two standard deviations. For all three chemicals, the shaded regions are primarily separate with only slight overlap between the groups for some of the chemicals.

Figure 5, 7 and 9 show comparison of the Monte Carlo analyses for the normal metabolizers with mixed metabolizers. For the mixed metabolizers, 10,000 iterations were conducted with 84.45% of the metabolizers using a VMaxC based on the distribution for normal metabolizers, 14.54% based on the distribution for the reduced metabolizers, and 1.01% based on the distribution for the very low metabolizers. For all three chemicals and both endpoints the shaded region for the normal metabolizers is completely encompassed by the hashed region for the mixed metabolizers.

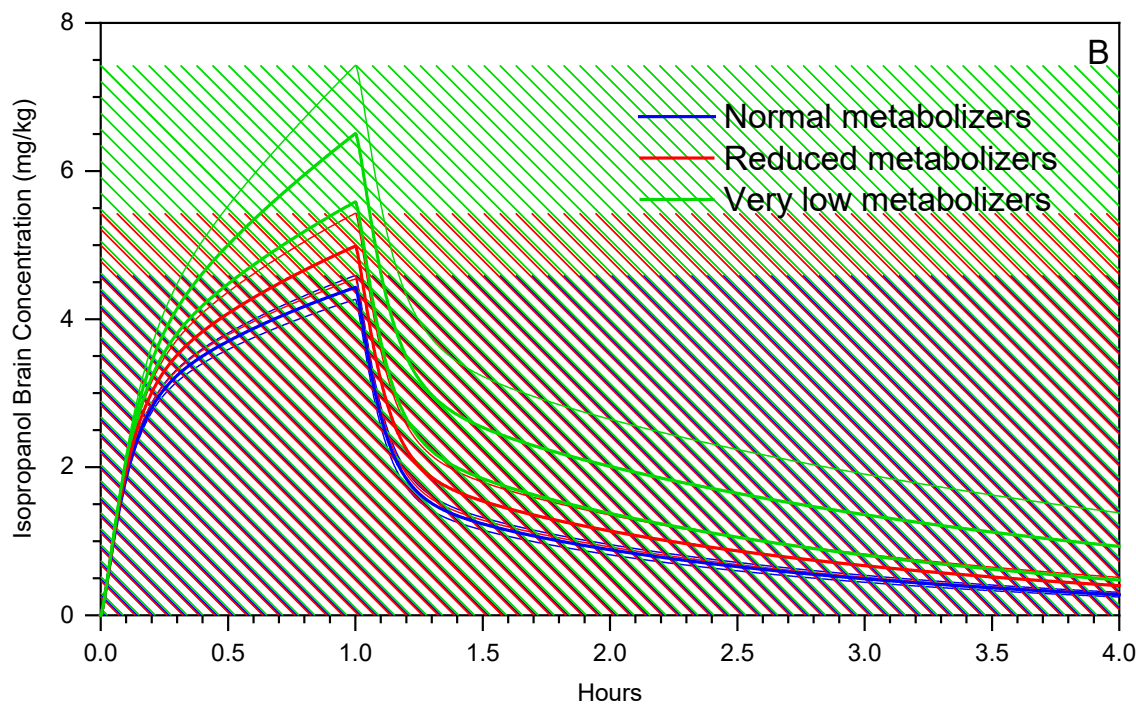
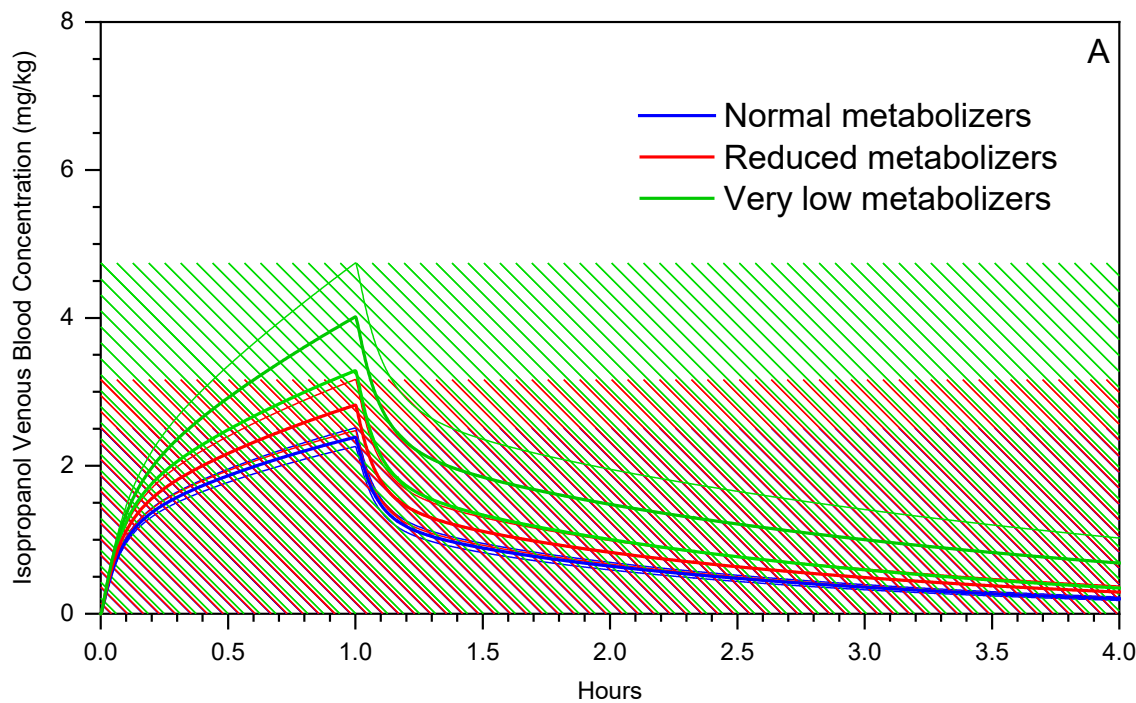


Figure 4. Isopropanol Monte Carlo Results for Normal, Reduced and Very Low Metabolizers. Simulations are for exposure to 400 ppm isopropanol for one hour. Results show predicted venous blood (A) and brain (B) concentrations for normal, reduced and very low metabolizers. Solid lines represent the means and the hashed areas represent \pm two standard deviations.

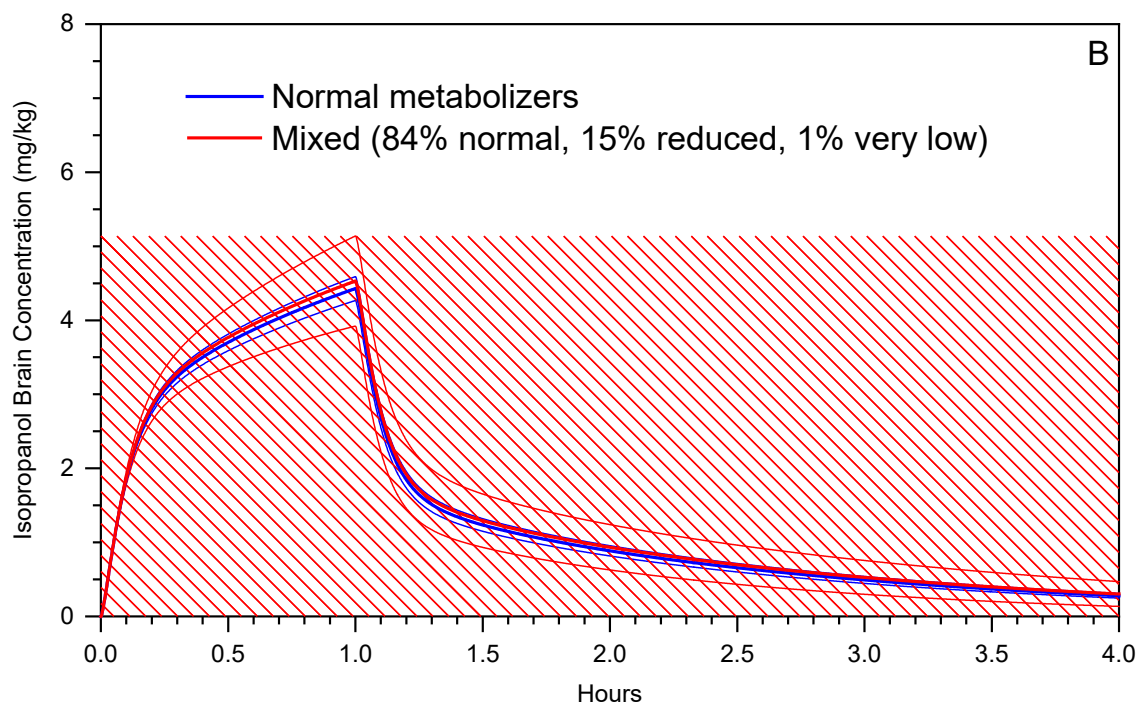
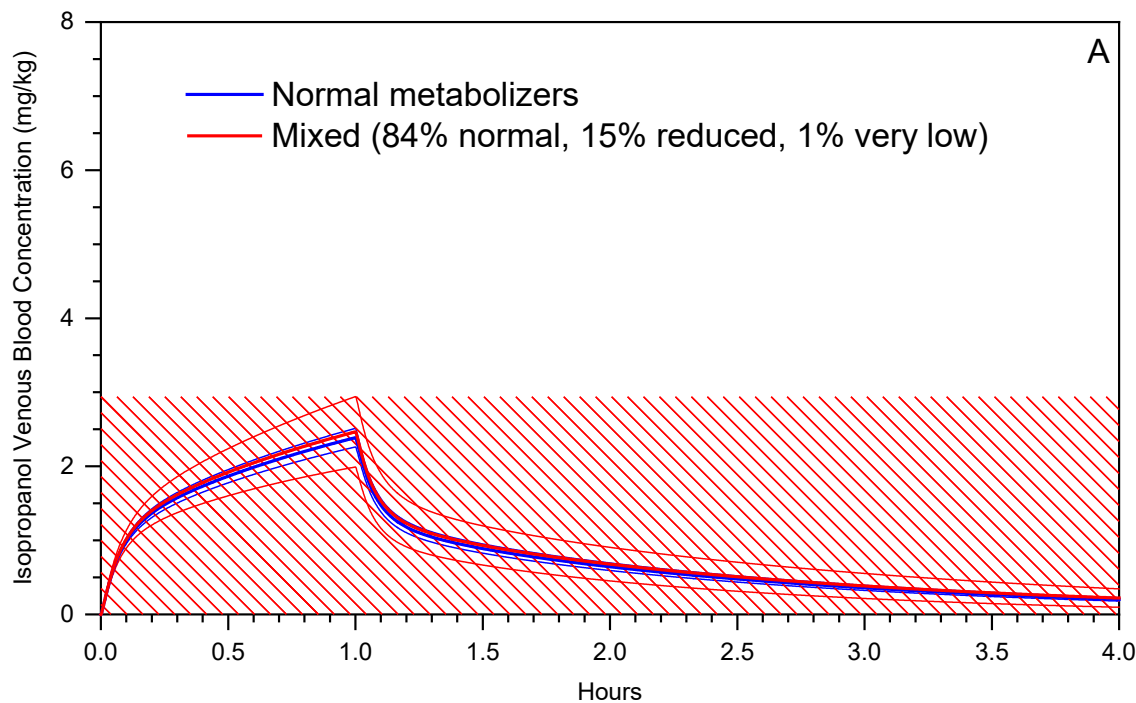


Figure 5. Isopropanol Monte Carlo Results for Normal and Mixed Metabolizers. Simulations are for exposure to 400 ppm isopropanol for one hour. Results show predicted venous blood (A) and brain (B) concentrations for normal and mixed metabolizers. Solid lines represent the means and the hashed and shaded areas represent \pm two standard deviations.

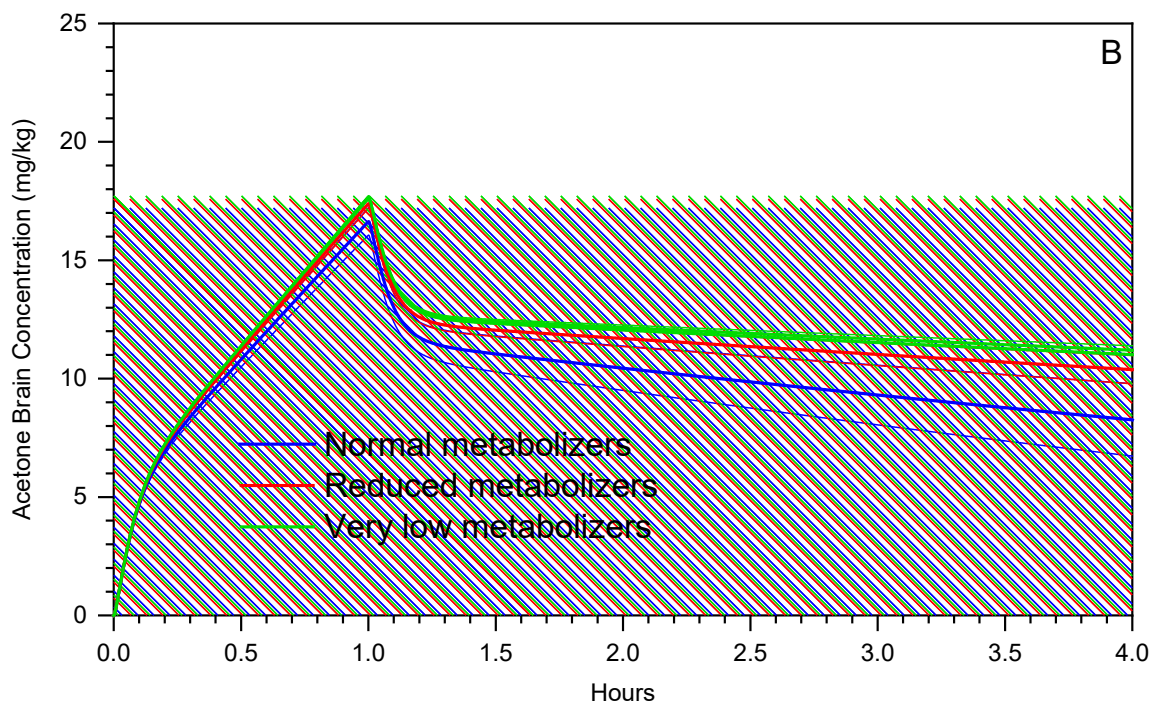
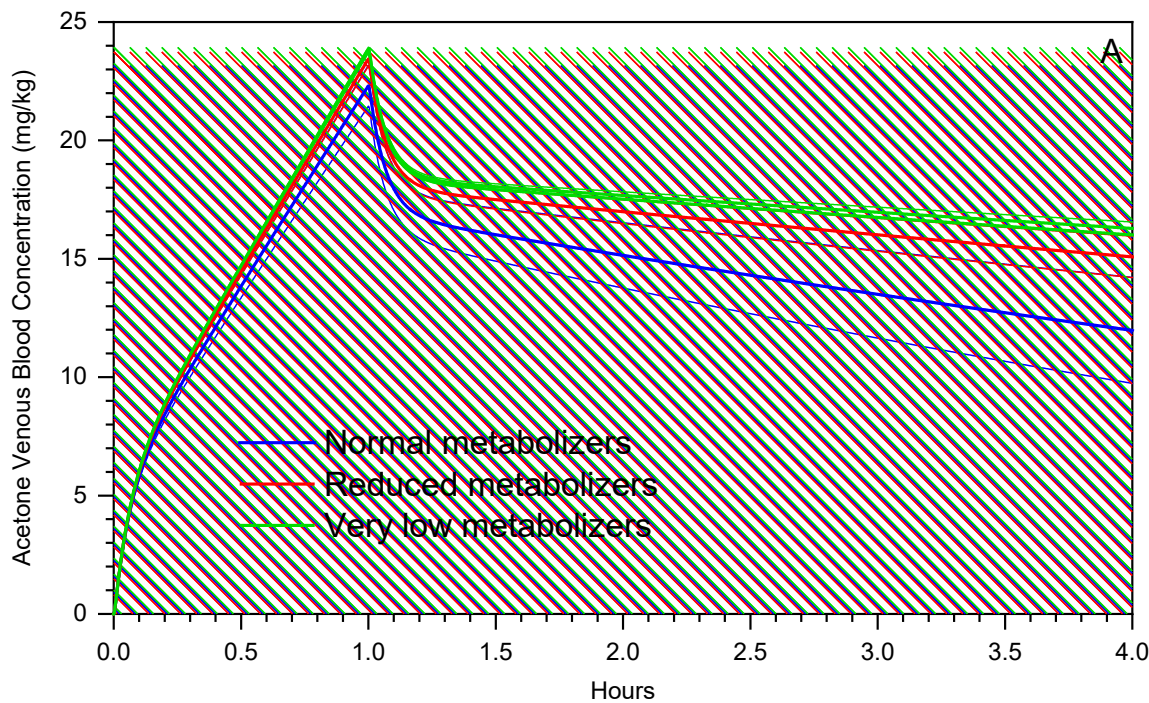


Figure 6. Acetone Monte Carlo Results for Normal, Reduced and Very Low Metabolizers. Simulations are for exposure to 1000 ppm acetone for one hour. Results show predicted venous blood (A) and brain (B) concentrations for normal, reduced and very low metabolizers. Solid lines represent the means and the hashed areas represent \pm two standard deviations.

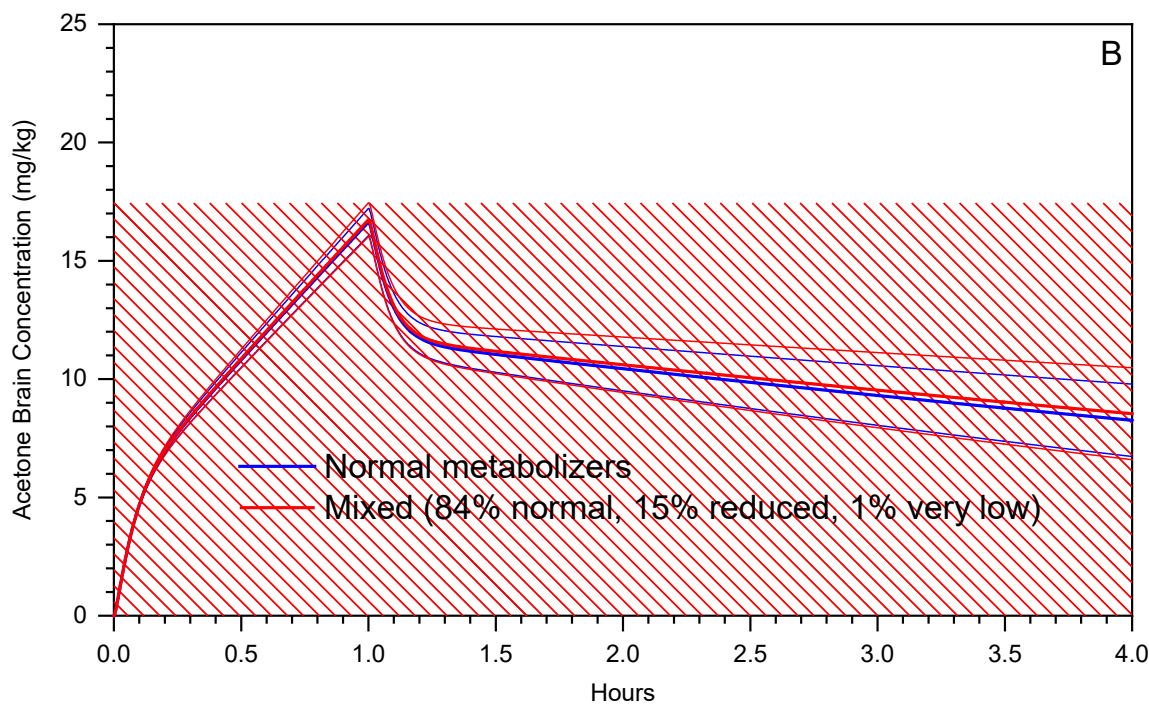
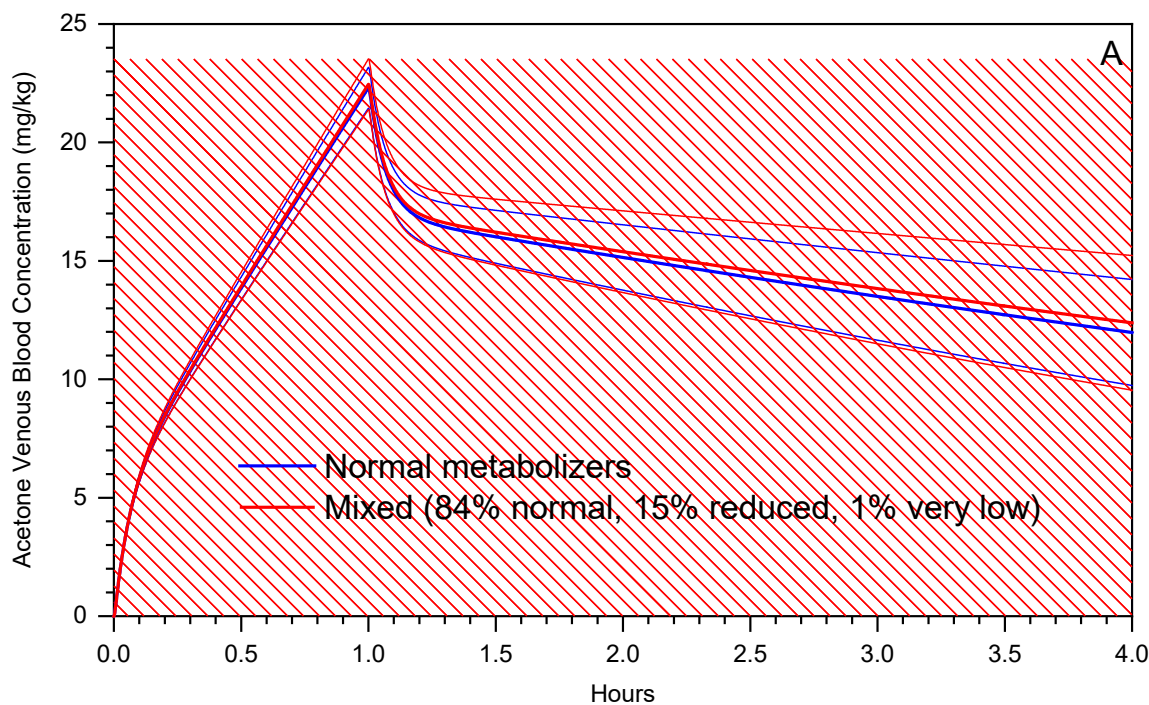


Figure 7. Acetone Monte Carlo Results for Normal and Mixed Metabolizers. Simulations are for exposure to 1000 ppm acetone for one hour. Results show predicted venous blood (A) and brain (B) concentrations for normal and mixed metabolizers. Solid lines represent the means and the hashed and shaded areas represent \pm two standard deviations.

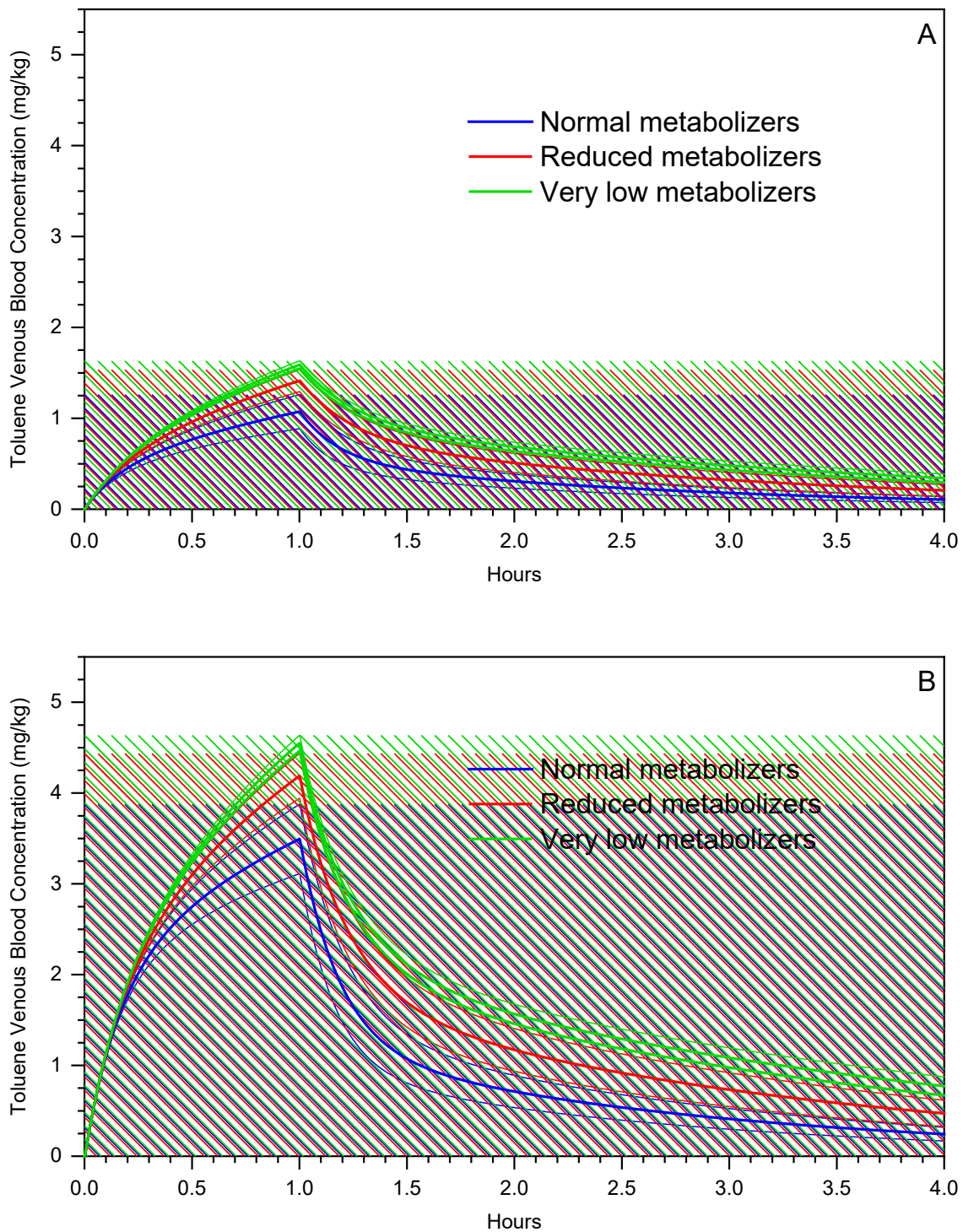


Figure 8. Toluene Monte Carlo Results for Normal, Reduced and Very Low Metabolizers. Simulations are for exposure to 150 ppm toluene for one hour. Results show predicted venous blood (A) and brain (B) concentrations for normal, reduced and very low metabolizers. Solid lines represent the means and the hashed areas represent \pm two standard deviations.

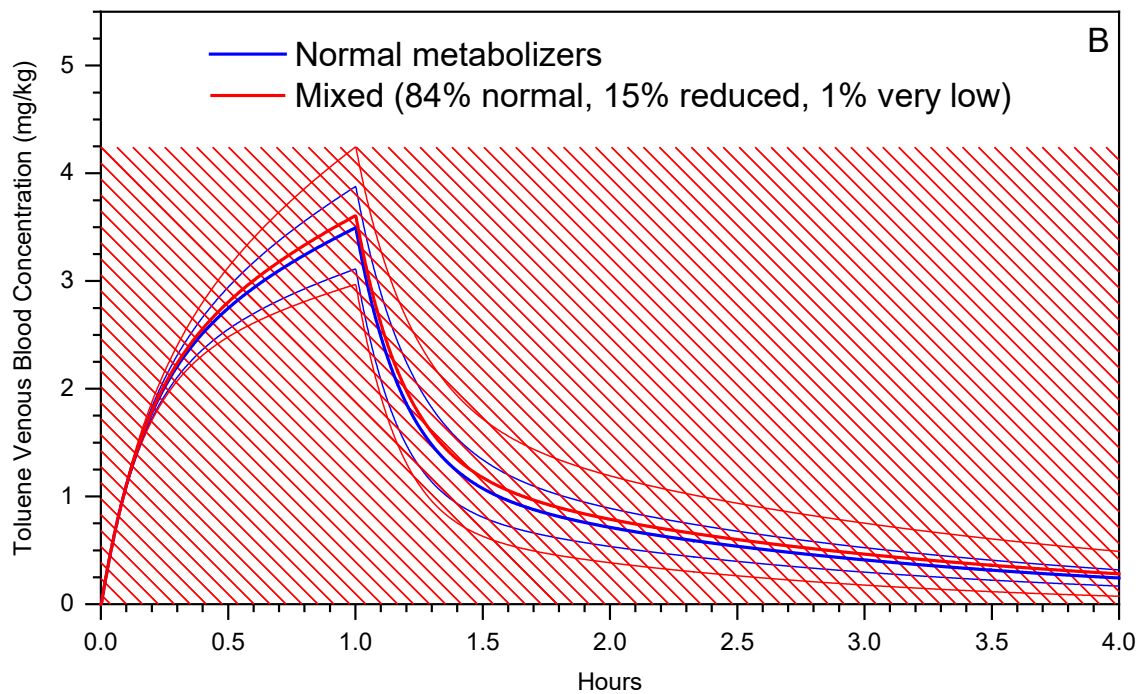
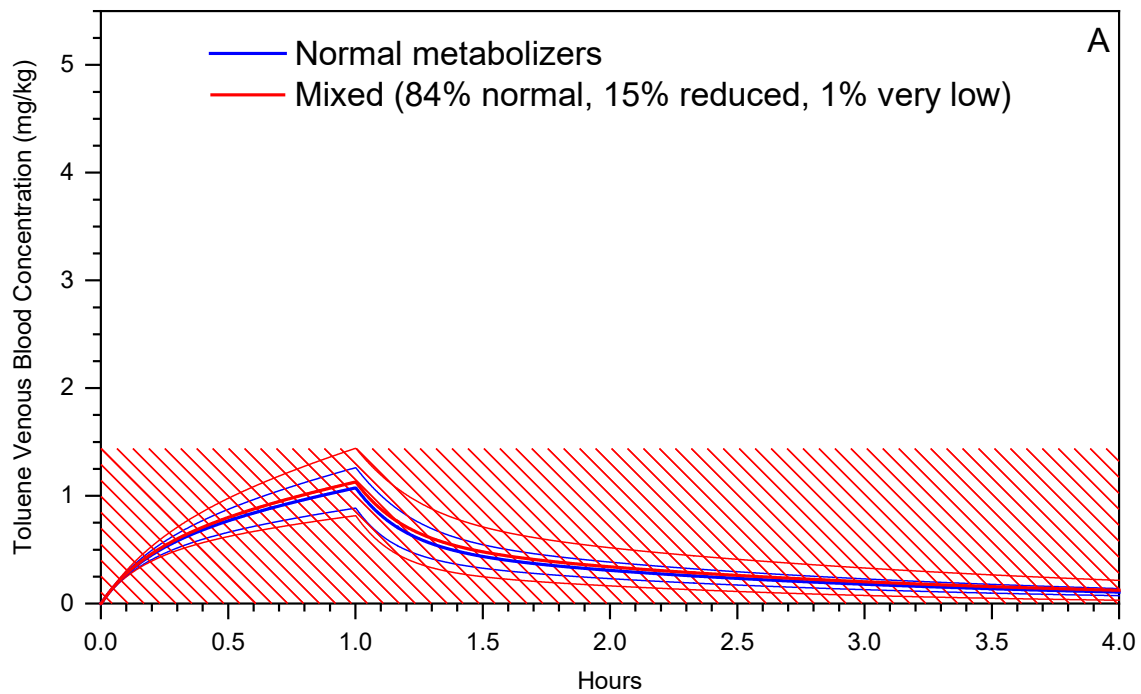


Figure 9. Toluene Monte Carlo Results for Normal and Mixed Metabolizers. Simulations are for exposure to 150 ppm toluene for one hour. Results show predicted venous blood (A) and brain (B) concentrations for normal and mixed metabolizers. Solid lines represent the means and the hashed and shaded areas represent \pm two standard deviations.

5.0 CONCLUSIONS

These results demonstrate that incorporation of information on genetic variants impacts PBPK model predictions and, therefore, would impact predicted exposure estimates. The variety of resources originally developed to support PGx research thus show potential value for improving models of environmental exposure to xenobiotics in multiple environments. Improved models, in turn, will allow a refined estimate of expected exposure response and the potential for predicting personalized health outcomes. Application of such genome-informed insights may also ultimately provide an approach to sensibly and systematically set exposure guidelines that account for key biological variation in members of the exposed population. Future work incorporating a metabolic pathway approach, for example by including Cytochrome P450, Family 1, Subfamily A, Polypeptide 2 (CYP1A2) and Cytochrome P450, Family 3, Subfamily A, Polypeptide 4 (CYP3A4) in simulations, may provide additional insights. Such increasingly comprehensive genomic profiles are likely to produce additionally comprehensive assessments of the key genetic variation involved in the inactivation of potentially toxic chemicals absorbed during deployment or normal training in military populations and, thus, account for true biological variability. Therefore, in the future, a larger chemical inventory and more diverse metabolic processes need to be addressed to expand the applicability of this analysis and correlate the end results to a broader group of individuals while delivering individualized risk.

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APPENDIX A – acsIX Model Code

```
PROGRAM: GenVar_PhaseI.CSL
! Simplified version of BrExp.csl

! THIS VERSION RUNS IN MINUTES INSTEAD OF HOURS !!!

INITIAL
! Physiological Parameters
  CONSTANT      BW = 84.14      ! Body weight (kg)
  CONSTANT      QCC = 0.2148    ! Cardiac output (L/min/kg^0.75)
  CONSTANT      QPC = 0.4625    ! Total pulmonary ventilation (L/min/kg^0.75)

! Fractional Blood Flows (fraction of cardiac output)
  CONSTANT      QBrnC = 0.114   ! Brain
  CONSTANT      QFatC = 0.052   ! Fat
  CONSTANT      QLivC = 0.227   ! Liver
  CONSTANT      QRapC = 0.419   ! Rapidly perfused
  CONSTANT      QSlwC = 0.188   ! Slowly perfused (includes skin)

! Fractional Tissue Volumes (fraction of body weight)
  CONSTANT      VAlvC = 0.0079  ! Alveolar blood
  CONSTANT      VBrnC = 0.02    ! Brain
  CONSTANT      VFatC = 0.214   ! Fat
  CONSTANT      VLivC = 0.026   ! Liver
  CONSTANT      VMucC = 0.0001  ! Mucous
  CONSTANT      VRapC = 0.036   ! Rapidly perfused
  CONSTANT      VSlwC = 0.536   ! Slowly perfused (includes skin)
  CONSTANT      VBodyC = 0.84   ! Sum of mean fractional volumes

! Inhalation Exposure Parameters
  CONSTANT      TChng = 0.0     ! Length of inhalation exposure (min)

! Dose Timing Parameters
  CONSTANT      StrtExp = 0.0    ! Time to start exposure (min)
  CONSTANT      ExpEnd = 1.0    ! Time to stop all exposures (min)
  CONSTANT      DoseInt = 0.0    ! Interval to repeat dosing (min)

! Simulation Control Parameters
  CONSTANT      TStop = 1440.0
  CINTERVAL    CINT = 0.6

! ----- PARENT CHEMICAL PARAMETERS -----
! Molecular Weights
  CONSTANT      MW = 60.09     ! Parent

! Tissue/Blood Partition Coefficients
  CONSTANT      PB = 848.0     ! Blood/air
  CONSTANT      PMuc = 848.0   ! Mucous/air
  CONSTANT      PBrn = 1.33    ! Brain
  CONSTANT      PFat = 0.32    ! Fat
  CONSTANT      PLiv = 1.16    ! Liver
  CONSTANT      PRap = 1.25    ! Rapidly perfused tissue
  CONSTANT      PSlw = 1.30    ! Slowly perfused tissue

! Metabolism Parameters
  CONSTANT      VMaxC = 5.0     ! Maximum reaction rate (mg/min/kg^0.75)
  CONSTANT      KM = 10.0      ! Michaelis-Menten (mg/L)
  CONSTANT      KFC = 0.0      ! First order rate constant (kg^0.25/min)

! Uptake and Clearance Parameters
  CONSTANT      ClUrC = 6.67e-5 ! Urinary clearance (L/min/kg^0.75)
  CONSTANT      kUrtC = 0.1833  ! URT uptake (L/min/kg^0.75)

! Endogenous Parent Production when Dosed with Parent
  CONSTANT      CEndo = 0.0     ! Concentration of metabolite (mg/L)
  CONSTANT      REndoC = 0.0    ! Rate of production of metabolite (mg/min/kg^0.75)

! Inhalation Exposure Parameters
  CONSTANT      Conc = 0.0     ! Inhaled concentration (ppm) of parent
```

```

! ----- METABOLITE CHEMICAL PARAMETERS -----
! Molecular Weight
CONSTANT    MW_Met = 58.08
           Stoch = MW_Met / MW

! Tissue/Blood Partition Coefficients
CONSTANT    PB_Met = 260.0    ! Blood/air
CONSTANT    PMuc_Met = 260.0 ! Mucous/air
CONSTANT    PBrn_Met = 0.69   ! Brain
CONSTANT    PFat_Met = 0.44   ! Fat
CONSTANT    PLiv_Met = 0.58   ! Liver
CONSTANT    PRap_Met = 0.69   ! Rapidly perfused tissue
CONSTANT    PSlw_Met = 0.70   ! Slowly perfused tissue

! Metabolism Parameters
CONSTANT    VMax_MetC = 0.05833 ! Maximum reaction rate (mg/min)
CONSTANT    KM_Met = 10.0      ! Michaelis-Menten (mg/L)
CONSTANT    KF_MetC = 0.0      ! First order rate constant (/min)

! Uptake and Clearance Parameters
CONSTANT    ClUr_MetC = 6.67e-5 ! Urinary clearance (L/min)
CONSTANT    kUrt_MetC = 0.1833 ! URT uptake (L/min) for metabolite

! Endogenous Metabolite Production when Dosed with Parent
CONSTANT    CEndo_Met = 0.5     ! Concentration of metabolite (mg/L)
CONSTANT    REndo_MetC = 0.00345 ! Rate of production of metabolite (mg/min)

! Inhalation Exposure Parameters
CONSTANT    Conc_Met = 0.0      ! Inhaled concentration (ppm) of metabolite

! ----- PARAMETER SCALING -----
! Scaled Pulmonary Ventilation Rate (L/min)
      QP = QPC * (BW**0.75)
      QAlv = 0.67 * QP

! Scaled Blood Flows (L/min)
      QCN = QCC * (BW**0.75)
      QAdjus = QBrnC + QFatC + QLivC + QRapC + QSlwC
      QBrn = (QBrnC / QAdjus) * QCN           ! Brain
      QFat = (QFatC / QAdjus) * QCN          ! Fat
      QLiv = (QLivC / QAdjus) * QCN         ! Liver
      QRap = (QRapC / QAdjus) * QCN        ! Rapidly perfused tissues
      QSlw = (QSlwC / QAdjus) * QCN        ! Slowly perfused tissues
      QC = QBrn + QFat + QLiv + QRap + QSlw

! Scaled Tissue Volumes (L)
      VTotC = VAlvC + VBrnC + VFatC + VLivC + VMucC + VRapC + VSlwC
      VAdjus = VBodyC / VTotC
      VAlv = (VAlvC * VAdjus) * BW          ! Arterial blood
      VBrn = (VBrnC * VAdjus) * BW          ! Brain
      VFat = (VFatC * VAdjus) * BW          ! Fat
      VLiv = (VLivC * VAdjus) * BW         ! Liver
      VMuc = (VMucC * VAdjus) * BW         ! Mucous
      VRap = (VRapC * VAdjus) * BW         ! Rapidly perfused tissues
      VSlw = (VSlwC * VAdjus) * BW         ! Slowly perfused tissues
      VTot = VAlv + VBrn + VFat + VLiv + VMuc + VRap + VSlw

! Scaled Metabolism Parameters
      VMax = VMaxC * (BW**0.75)
      KF = KFC / (BW**0.25)
      REndo = REndoC * (BW**0.75)
      VMax_Met = VMax_MetC * (BW**0.75)
      KF_Met = KF_MetC / (BW**0.25)
      REndo_Met = REndo_MetC * (BW**0.75)

! Scaled Clearance Rates
      ClUr = ClUrC * (BW**0.75)
      kUrt = (min((kUrtC * (BW**0.75)), QAlv))
      ClUr_Met = ClUr_MetC * (BW**0.75)
      kUrt_Met = (min((kUrt_MetC * (BW**0.75)), QAlv))

```

```

! Initial Amounts of Endogenous Parent (mg) when Dosed with Parent
  IAArt = CEndo * VALv
  IABrn = CEndo * VBrn * PBrn
  IAFat = CEndo * VFat * PFat
  IALiv = CEndo * VLiv * PLiv
  IARap = CEndo * VRap * PRap
  IASlw = CEndo * VSlw * PSlw
  InitTot = IAArt + IABrn + IAFat + IALiv + IARap + IASlw

! Initial Amounts of Endogenous Metabolite (mg) when Dosed with Parent
  IAArt_Met = CEndo_Met * VALv
  IABrn_Met = CEndo_Met * VBrn * PBrn_Met
  IAFat_Met = CEndo_Met * VFat * PFat_Met
  IALiv_Met = CEndo_Met * VLiv * PLiv_Met
  IARap_Met = CEndo_Met * VRap * PRap_Met
  IASlw_Met = CEndo_Met * VSlw * PSlw_Met
  InitTot_Met = IAArt_Met + IABrn_Met + IAFat_Met + IALiv_Met + IARap_Met + IASlw_Met

! Initialize Starting Values
  CIZone = 0.0
  CIZone_Met = 0.0
  PerEnd = 0.0
  PerEnd_Met = 0.0

  SCHEDULE DoseOn .AT. StrtExp
END      ! End of Initial

DYNAMIC
  ALGORITHM  IALG = 2      ! Gear stiff method

DISCRETE DoseOn      ! Start dosing
  SCHEDULE DoseOn .AT. T + DoseInt
  SCHEDULE DoseOff .AT. T + TChng

  IF ((T.LT.ExpEnd) .AND. (Conc.GT.0.0)) CIZone = 1.0
  IF ((T.LT.ExpEnd) .AND. (Conc_Met.GT.0.0)) CIZone_Met = 1.0
END

DISCRETE DoseOff
  CIZone = 0.0
  CIZone_Met = 0.0
END

DERIVATIVE
  Hours = T / 60.0
  Minutes = T
  Days = Hours / 24.0

! ----- PARENT PBPK MODEL -----
! Amount in Inhaled Air
  CInh = ((Conc * MW) / 24450.0) * CIZone
  CP = (CInh * 24450.0) / MW

! Amount in Mucous
  RAMuc = kUrt * (CInh - (CMuc / PMuc) + CALv - (CMuc / PMuc))
  AMuc = INTEG(RAMuc, 0.0)
  CMuc = AMuc / VMuc

! Amount Exhaled (mg)
  RAExh = ((QAlv - kUrt) * CALv) + (kUrt * (CMuc / PMuc))
  AExh = INTEG(RAExh, 0.0)

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

```

```

! Amount in Arterial Blood (mg)
  RAArt = ((QAlv - kUrt) * CInh) - ((QAlv - kUrt) * CALv) + (kUrt * ((CMuc/PMuc) - CALv)) &
    & + (QC * (CVen - CArt)) - RAUrn
  AArt = INTEG(RAArt, IAArt)
  CArt = AArt / VALv
  CALv = CArt / PB
  CALvPPM = CALv * (24450.0 / MW)
  AUCCArt = INTEG(CArt, 0.0)

! Amount in Urine (mg)
  RAUrn = ClUr * CArt
  AUrn = INTEG(RAUrn, 0.0)

! Amount in Brain (mg)
  RABrn = QBrn * (CArt - CVBrn)
  ABrn = INTEG(RABrn, IABrn)
  CBrn = ABrn / VBrn
  CVBrn = CBrn / PBrn
  AUCCBrn = INTEG(CBrn, 0.0)

! Amount in Fat (mg)
  RAFat = QFat * (CArt - CVFat)
  AFat = INTEG(RAFat, IAFat)
  CFat = AFat / VFat
  CVFat = CFat / PFat

! Amount in Liver (mg)
  RALiv = (QLiv * (CArt - CVLiv)) + REndo - RAMet
  ALiv = INTEG(RALiv, IALiv)
  CLiv = ALiv / VLiv
  CVLiv = CLiv / PLiv

! Amount of Endogenous Metabolite Produced when Dosing with Metabolite
  AEndo = INTEG(REndo, 0.0)

! Amount Metabolised in Liver -- Saturable and 1st Order (mg)
  RAMet = ((VMax * CVLiv) / (KM + CVLiv)) + (KF * CVLiv * VLiv)
  AMet = INTEG(RAMet, 0.0)

! Amount in Rapidly Perfused Tissue (mg)
  RARap = QRap * (CArt - CVRap)
  ARap = INTEG(RARap, IARap)
  CRap = ARap / VRap
  CVRap = CRap / PRap

! Amount in Slowly Perfused Tissue (mg)
  RASlw = QSlw * (CArt - CVSlw)
  ASlw = INTEG(RASlw, IASlw)
  CSLw = ASlw / VSlw
  CVSlw = CSLw / PSlw

! Concentration in Mixed Venous Blood (mg/L)
  CVen = (QBrn*CVBrn + QFat*CVFat + QLiv*CVLiv + QRap*CVRap + QSlw*CVSlw) / QC
  AUCCVen = INTEG(CVen, 0.0)

! ----- ACETONE PBPK MODEL-----
! Amount in Inhaled Air
  CInh_Met = ((Conc_Met * MW_Met) / 24450.0) * CIZone_Met
  CP_Met = (CInh_Met * 24450.0) / MW_Met

! Amount in Mucous
  RAMuc_Met = kUrt_Met * (CInh_Met - (CMuc_Met / PMuc_Met) + CALv_Met - (CMuc_Met / PMuc_Met))
  AMuc_Met = INTEG(RAMuc_Met, 0.0)
  CMuc_Met = AMuc_Met / VMuc

! Amount Exhaled (mg)
  RAExh_Met = ((QAlv - kUrt_Met) * CALv_Met) + (kUrt_Met * (CMuc_Met / PMuc_Met))
  AExh_Met = INTEG(RAExh_Met, 0.0)

```

```

! Concentration in End-Exhaled Air (mg/L)
  CEnd_Met = RAEH_Met / QAlv
  CEndPPM_Met = CEnd_Met * (24450.0 / MW_Met)
  IF (Conc_Met.GT.0.0) PerEnd_Met = (CEnd_Met / ((Conc_Met * MW_Met) / 24450.0)) * 100.0

! Amount in Arterial Blood (mg)
  RAArt_Met = ((QAlv - kUrt_Met) * CInh_Met) - ((QAlv - kUrt_Met) * CALv_Met) &
    & + (kUrt_Met * ((CMuc_Met / PMuc_Met) - CALv_Met)) + (QC * (CVen_Met - CArt_Met)) &
    & - RAUrn_Met
  AArt_Met = INTEG(RAArt_Met, IAArt_Met)
  CArt_Met = AArt_Met / VALv
  CALv_Met = CArt_Met / PB_Met
  CALvPPM_Met = CALv_Met * (24450.0 / MW_Met)
  AUCCArt_Met = INTEG(CArt_Met, 0.0)

! Amount in Urine (mg)
  RAUrn_Met = ClUr_Met * CArt_Met
  AUrn_Met = INTEG(RAUrn_Met, 0.0)

! Amount in Brain (mg)
  RABrn_Met = QBrn * (CArt_Met - CVBrn_Met)
  ABrn_Met = INTEG(RABrn_Met, IABrn_Met)
  CBrn_Met = ABrn_Met / VBrn
  CVBrn_Met = CBrn_Met / PBrn_Met
  AUCCBrn_Met = INTEG(CBrn_Met, 0.0)

! Amount in Fat (mg)
  RAFat_Met = QFat * (CArt_Met - CVFat_Met)
  AFat_Met = INTEG(RAFat_Met, IAFat_Met)
  CFat_Met = AFat_Met / VFat
  CVFat_Met = CFat_Met / PFat_Met

! Amount in Liver (mg)
  RALiv_Met = (QLiv * (CArt_Met - CVLiv_Met)) + (Stoch * RAMet) + REndo_Met - RAMet_Met
  ALiv_Met = INTEG(RALiv_Met, IALiv_Met)
  CLiv_Met = ALiv_Met / VLiv
  CVLiv_Met = CLiv_Met / PLiv_Met

! Amount of Endogenous Metabolite Produced when Dosing with Parent
  AEndo_Met = INTEG(REndo_Met, 0.0)

! Amount Metabolised in Liver -- Saturable and 1st Order (mg)
  RAMet_Met = ((VMax_Met * CVLiv_Met) / (KM_Met + CVLiv_Met)) + (KF_Met * CVLiv_Met * VLiv)
  AMet_Met = INTEG(RAMet_Met, 0.0)

! Amount in Rapidly Perfused Tissue (mg)
  RARap_Met = QRap * (CArt_Met - CVRap_Met)
  ARap_Met = INTEG(RARap_Met, IARap_Met)
  CRap_Met = ARap_Met / VRap
  CVRap_Met = CRap_Met / PRap_Met

! Amount in Slowly Perfused Tissue (mg)
  RASlw_Met = QSlw * (CArt_Met - CVSlw_Met)
  ASlw_Met = INTEG(RASlw_Met, IASlw_Met)
  CSlw_Met = ASlw_Met / VSlw
  CVSlw_Met = CSlw_Met / PSlw_Met

! Concentration in Mixed Venous Blood (mg/L)
  CVen_Met = (QBrn*CVBrn_Met + QFat*CVFat_Met + QLiv*CVLiv_Met + QRap*CVRap_Met &
    & + QSlw*CVSlw_Met) / QC
  AUCCVen_Met = INTEG(CVen_Met, 0.0)

! ----- CHECK MASS BALANCE -----
  TDose = INTEG((QAlv*CInh), 0.0)
  Parent = AMuc + AArt + ABrn + AFat + ALiv + ARap + ASlw + AExh + AUrn + AMet - InitTot &
    & - AEndo
  Metabolite = AMuc_Met + AArt_Met + ABrn_Met + AFat_Met + ALiv_Met + ARap_Met + ASlw_Met &
    & + AExh_Met &
    & + AUrn_Met + AMet_Met - InitTot_Met - AEndo_Met
  MassBal = TDose - Parent
  MetBal = INTEG((QAlv*CInh_Met), 0.0) + (AMet * Stoch) - Metabolite

```

```
TERMT(T.GE.TStop, 'Simulation Finished')
```

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

APPENDIX B – Utility M Files for Simulations

The following M files are called within various M files utilized for this work. Some lines were too long to fit the page width and were thus reformatted to fit the page; however, these additional line breaks and space may need to be removed for the M file to run correctly.

Acetone.m

```
% Sets human acetone parameters
% kUrtC set to keep original value of kUrtC the same fraction of QPC as from the original IPA
%   paper (i.e., 11.0/27.75)

MW=58.08;
PB=260.0; PMUC=260.0; PBRN=0.69; PFAT=0.44; PLIV=0.58; PRAP=0.69; PSLW=0.7;
VMAXC=3.5/60.0; KM=10.0; KFC=0.0;
CLURC=0.004/60.0; KURTC=(11.0/27.75)*QPC;
CENDO=0.5; RENDOC=0.207/60.0;

MW_MET=1.0;
PB_MET=1.0; PB_MUC=1.0; PBRN_MET=1.0; PFAT_MET=1.0; PLIV_MET=1.0; PRAP_MET=1.0; PSLW_MET=1.0;
VMAX_METC=0.0; KM_MET=1.0; KF_METC=0.0;
CLUR_METC=0.0; KURT_METC=0.0;
CENDO_MET=0.0; RENDOC_METC=0.0;
```

IPA.m

```
% Sets human isopropanol parameters
% kUrtC set to keep original value of kUrtC the same fraction of QPC as from the original IPA
%   paper (i.e., 11.0/27.75)

MW=60.09;
PB=848.0; PMUC=848.0; PBRN=1.33; PFAT=0.32; PLIV=1.16; PRAP=1.25; PSLW=1.3;
VMAXC=300.0/60.0; KM=10.0; KFC=0.0;
CLURC=0.004/60.0; KURTC=(11.0/27.75)*QPC;
CENDO=0.0; RENDOC=0.0;

MW_MET=58.08;
PB_MET=260.0; PMUC_MET=260.0; PBRN_MET=0.69; PFAT_MET=0.44; PLIV_MET=0.58; PRAP_MET=0.69;
PSLW_MET=0.7;
VMAX_METC=3.5/60.0; KM_MET=10.0; KF_METC=0.0;
CLUR_METC=0.004/60.0; KURT_METC=(11.0/27.75)*QPC;
CENDO_MET=0.5; RENDOC_METC=0.207/60.0;
```

Toluene.m

```
% MW (molecular weight) is from NIST Chemistry Webbook
%   (http://webbook.nist.gov/cgi/cbook.cgi?Name=toluene&Units=SI)
% PBrn from Eric's toluene model (tissue/gas value of 36.4 as given in Fiserova-Bergerova et al.
%   (1984))
% Remaining parameters are from Tardif et al. (1997, 1995)

MW=92.1384;
DS=0.15;
PB=15.6; PMUC=1.0; PBRN=36.4/PB; PFAT=1021.0/PB; PLIV=83.6/PB; PRAP=83.6/PB; PSLW=27.7/PB;
VMAXC=4.8/60.0; KM=0.55; KFC=0.0;
CLURC=0.0; KURTC=0.0;
CENDO=0.0; RENDOC=0.0;

MW_MET=1.0;
DS_MET=0.5;
PB_MET=1.0; PB_MUC=1.0; PBRN_MET=1.0; PFAT_MET=1.0; PLIV_MET=1.0; PRAP_MET=1.0; PSLW_MET=1.0;
VMAX_METC=0.0; KM_MET=1.0; KF_METC=0.0;
CLUR_METC=0.0; KURT_METC=0.0;
CENDO_MET=0.0; RENDOC_METC=0.0;
```

Distrib Acetone.m

```
% Called through MC_Anal.m in MC_by_Group.m

if (Group == 'Normal ')
%   VMaxC of 0.05833 mg/min/kg^0.75 (3.5 mg/hr/kg^0.75)
    VMAXC = 0 + 1 * lognrnd(-2.8847, 0.29356, 0.023333, 0.093333);

else
    if (Group == 'Reduced')
%   VMaxC of 0.0194445 mg/min/kg^0.75 (1/3 of normal) (1.16667 mg/hr/kg^0.75)
        VMAXC = 0 + 1 * lognrnd(-3.9833, 0.29356, 0.0077776, 0.0311104);

    else
        if (Group == 'VeryLow')
%   VMaxC of 0.005833 mg/min/kg^0.75 (1/10 of normal) (0.35 mg/hr/kg^0.75)
            VMAXC = 0 + 1 * lognrnd(-5.1873, 0.29356, 0.0023333, 0.0093333);
        end
    end
end
end
```

Distrib IPA.m

```
% Called through MC_Anal.m in MC_by_Group.m

if (Group == 'Normal ')
%   VMaxC of 5.0 mg/min/kg^0.75 (300.0 mg/hr/kg^0.75)
    VMAXC = 0 + 1 * lognrnd(1.5663, 0.29356, 2.0, 8.0);

else
    if (Group == 'Reduced')
%   VMaxC of 1.666666667 mg/min/kg^0.75 (1/3 of normal) (100.0 mg/hr/kg^0.75)
        VMAXC = 0 + 1 * lognrnd(0.4678, 0.29356, 0.66668, 2.66672);

    else
        if (Group == 'VeryLow')
%   VMaxC of 0.5 mg/min/kg^0.75 (1/10 of normal) (30.0 mg/hr/kg^0.75)
            VMAXC = 0 + 1 * lognrnd(-0.7362, 0.29356, 0.2, 0.8);
        end
    end
end
end
```

Distrib Toluene.m

```
% Called through MC_Anal.m in MC_by_Group.m

if (Group == 'Normal ')
%   VMaxC of 0.08 mg/min/kg^0.75 (4.8 mg/hr/kg^0.75)
    VMAXC = 0 + 1 * lognrnd(-2.5688, 0.29356, 0.032, 0.128);

else
    if (Group == 'Reduced')
%   VMaxC of 0.026667 mg/min/kg^0.75 (1/3 of normal) (1.6 mg/hr/kg^0.75)
        VMAXC = 0 + 1 * lognrnd(-3.6674, 0.29356, 0.010667, 0.042667);

    else
        if (Group == 'VeryLow')
%   VMaxC of 0.008 mg/min/kg^0.75 (1/10 of normal) (0.48 mg/hr/kg^0.75)
            VMAXC = 0 + 1 * lognrnd(-4.8714, 0.29356, 0.0032, 0.0128);
        end
    end
end
end
```

Init.m

```
prepare T HOURS MINUTES DAYS CART CALV CALVPPM AEXH CEND CENDPPM PEREND AMET CVEN MASSBAL
prepare CART_MET CALVPPM_MET AEXH_MET CENDPPM_MET AMET_MET CVEN_MET METBAL
prepare AMUC_AART AURN ABRN AFAT ALIV ARAP ASLW CBRN CFAT CLIV CMUC CRAP CSLW
prepare AMUC_MET AART_MET AURN_MET ABRN_MET AFAT_MET ALIV_MET ARAP_MET ASLW_MET CBRN_MET CFAT_MET
CLIV_MET CMUC_MET CRAP_MET CSLW_MET
```

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

ResetDoses.m

```
TCHNG=0.0;
STRTEXP=0.0; EXPEND=1.0; DOSEINT=1.0;
CINT=0.5;
CONC=0.0;
CONC_MET=0.0;
```

MC by Group.m

```
% MC analysis -- 4-hr simulation with 1-hr exposure at the STEL (400 ppm for IPA, 1000 ppm for
% acetone and 150 ppm for toluene)
```

```
% Calls Start_MC_IPA, Start_MC_Acetone, Start_MC_Toluene, MC_Init.m, MC_Anal.m and MC_Sum_Mixed
```

```
% IPA -----
```

```
% Normal metabolizers
Start_MC_IPA
Group = 'Normal ';
NumMetab = round(PerNorm * NumIts);
PNumMetab = 1;
VMAXC=5.0;
MC_Init
MC_Anal
load @format=model @file=Revised_IPA_Model
```

```
% Reduced metabolizers
Start_MC_IPA
Group = 'Reduced';
PNumMetab = round(PerNorm * NumIts) + 1;
NumMetab = round(PerNorm * NumIts) + round(PerReduced * NumIts);
VMAXC=1.6667;
MC_Init
MC_Anal
load @format=model @file=Revised_IPA_Model
```

```
% Very low metabolizers
Start_MC_IPA
Group = 'VeryLow';
PNumMetab = round(PerNorm * NumIts) + round(PerReduced * NumIts) + 1;
NumMetab = round(PerNorm*NumIts) + round(PerReduced*NumIts) + round(PerVeryLow*NumIts);
VMAXC=0.5;
MC_Init
MC_Anal
load @format=model @file=Revised_IPA_Model
```

```
% Population of normal, reduced and very low metabolizers
Start_MC_IPA
MC_Sum_Mixed
load @format=model @file=Revised_IPA_Model
```

```
% Acetone -----
```

```
% Normal metabolizers
Start_MC_Acetone
Group = 'Normal ';
NumMetab = round(PerNorm * NumIts);
PNumMetab = 1;
VMAXC=0.058333;
MC_Init
MC_Anal
load @format=model @file=Revised_IPA_Model
```

```

% Reduced metabolizers
  Start_MC Acetone
  Group = 'Reduced';
  PNumMetab = round(PerNorm * NumIts) + 1;
  NumMetab = round(PerNorm * NumIts) + round(PerReduced * NumIts);
  VMAXC=0.019445;
  MC_Init
  MC_Anal
  load @format=model @file=Revised_IPA_Model

% Very low metabolizers
  Start_MC Acetone
  Group = 'VeryLow';
  PNumMetab = round(PerNorm * NumIts) + round(PerReduced * NumIts) + 1;
  NumMetab = round(PerNorm*NumIts) + round(PerReduced*NumIts) + round(PerVeryLow*NumIts);
  VMAXC=0.0058333;
  MC_Init
  MC_Anal
  load @format=model @file=Revised_IPA_Model

% Population of normal, reduced and very low metabolizers
  Start_MC Acetone
  MC_Sum_Mixed
  load @format=model @file=Revised_IPA_Model

% Toluene -----
% Normal metabolizers
  Start_MC Toluene
  Group = 'Normal ';
  NumMetab = round(PerNorm * NumIts);
  PNumMetab = 1;
  VMAXC=0.08;
  MC_Init
  MC_Anal
  load @format=model @file=Revised_IPA_Model

% Reduced metabolizers
  Start_MC Toluene
  Group = 'Reduced';
  PNumMetab = round(PerNorm * NumIts) + 1;
  NumMetab = round(PerNorm * NumIts) + round(PerReduced * NumIts);
  VMAXC=0.026667;
  MC_Init
  MC_Anal
  load @format=model @file=Revised_IPA_Model

% Very low metabolizers
  Start_MC Toluene
  Group = 'VeryLow';
  PNumMetab = round(PerNorm * NumIts) + round(PerReduced * NumIts) + 1;
  NumMetab = round(PerNorm*NumIts) + round(PerReduced*NumIts) + round(PerVeryLow*NumIts);
  VMAXC=0.008;
  MC_Init
  MC_Anal

% Population of normal, reduced and very low metabolizers
  Start_MC Toluene
  MC_Sum_Mixed
  load @format=model @file=Revised_IPA_Model

```

Start MC Acetone.m

```
% Called by MC_by_Group.m

load @format=model @file=GenVar_Phase1

% Set parameters for simulations -- WITHOUT endogenous acetone production
% BW is average from Air Force database (from e-mail from Jeff Hudson)
% Remaining parameters are from IPA published model
Init
ResetDoses
ChemName = 'Ace';
Acetone
BW=84.14; QCC=12.89/60.0; QPC=27.75/60.0; QBRNC=0.114; QFATC=0.052; QLIVC=0.227; QRAPC=0.419;
QSLWC=0.188;
VALVC=0.0079; VBRNC=0.02; VFATC=0.214; VLIVC=0.026; VMUCC=0.0001; VRAPC=0.036; VSLWC=0.536;
VBODYC=0.84;
CONC=1000.0; TCHNG=60.0; TSTOP=240.0; STRTEXP=0.0; EXPEND=60.0; DOSEINT=250.0;
CENDO=0.0; RENDOC=0.0;
CINT=0.1;
NumIts = 10000;

% Percent of individuals per metabolic group (omit those with unknown metabolic activity and
% recalculate percentages)
PerNorm = 0.8445;
PerReduced = 0.1454;
PerVeryLow = 0.0101;

cven_mixed = []; cbrn_mixed = [];
```

Start MC IPA.m

```
% Called by MC_by_Group.m

load @format=model @file=GenVar_Phase1

% Set parameters for simulations -- WITHOUT endogenous acetone production
% BW is average from Air Force database (from e-mail from Jeff Hudson)
% Remaining parameters are from IPA published model
Init
ResetDoses
ChemName = 'IPA';
IPA
BW=84.14; QCC=12.89/60.0; QPC=27.75/60.0; QBRNC=0.114; QFATC=0.052; QLIVC=0.227; QRAPC=0.419;
QSLWC=0.188;
VALVC=0.0079; VBRNC=0.02; VFATC=0.214; VLIVC=0.026; VMUCC=0.0001; VRAPC=0.036; VSLWC=0.536;
VBODYC=0.84;
CONC=400.0; TCHNG=60.0; TSTOP=240.0; STRTEXP=0.0; EXPEND=60.0; DOSEINT=250.0;
CENDO_MET=0.0; RENDO_METC=0.0;
CINT=0.1;
NumIts = 10000;

% Percent of individuals per metabolic group (omit those with unknown metabolic activity and
% recalculate percentages)
PerNorm = 0.8445;
PerReduced = 0.1454;
PerVeryLow = 0.0101;

cven_mixed = []; cbrn_mixed = [];
```

Start MC Toluene.m

```
% Called by MC_by_Group.m

load @format=model @file=GenVar_Phase1

% Set parameters for simulations -- WITHOUT endogenous acetone production
% BW is average from Air Force database (from e-mail from Jeff Hudson)
% QBrn and VBrn are values from IPA model -- Tardif model did not have a brain compartment
% Remaining parameters are from Tardif et al. (1997, 1995)
% Adjusted QCC value from (18.0/60.0) to (14.4/60.0) so that IPA model scaling by BW to the 0.75
%   gets same value as Tardiff who uses 0.7
% Adjusted QPC value from (18.0/60.0/(2/3)) to (14.4/60.0/(2/3)) so that IPA model scaling by BW
%   to the 0.75 gets same value as Tardiff who uses 0.7
Init
ResetDoses
ChemName = 'Tol';
Toluene
BW=84.14; QCC=14.4/60.0; QPC=14.4/60.0/(2/3); QBRN=0.114; QFATC=0.05; QLIVC=0.26;
QRAPC=0.44-QBRN; QSLWC=0.25;
VALVC=0.0079; VBRNC=0.02; VFATC=0.19; VLIVC=0.026; VMUCC=0.0001; VRAPC=0.05-VBRNC; VSLWC=0.62;
VBODYC=0.8940;
CONC=150.0; TCHNG=60.0; TSTOP=240.0; STRTEXP=0.0; EXPEND=60.0; DOSEINT=250.0;
CINT=0.1;
NumIts = 10000;

% Percent of individuals per metabolic group (omit those with unknown metabolic activity and
%   recalculate percentages)
PerNorm = 0.8445;
PerReduced = 0.1454;
PerVeryLow = 0.0101;

cven_mixed = []; cbrn_mixed = [];
```

MC_Init.m

```
% Run to get baseline values for MC analysis

% Called in MC_by_Group.m

% Initialize array
cven_th = []; final = []; massbal_th = [];

% Make first run
start @NoCallback
cven_th = _hours;
cven_th = addcolsj(cven_th, _cven, @Justification = 'begin');
cven_th = addcolsj(cven_th, _cbrn, @Justification = 'begin');

if (ChemName == 'IPA')
    cven_th = addcolsj(cven_th, _cven_met, @Justification = 'begin');
    cven_th = addcolsj(cven_th, _cbrn_met, @Justification = 'begin');
end

final = [final AUCCVEN];
final = [final AUCCBRN];

if (ChemName == 'IPA')
    final = [final AUCCVEN_MET];
    final = [final AUCCBRN_MET];
end

% Save mass balance to check for validity of run
massbal_th(1, 1) = max(_massbal);
massbal_th(2, 1) = min(_massbal);

SaveInitOutput

cven_th = []; final = []; massbal_th = [];
```

SaveInitOutput.m

```
% Save output to text files
% Contents of files based on file names where
% * = "ipa", "acetone" or "toluene", and
% ** = "normal", "reduced" or "verylow"
% mc_***_baseline.txt = time course output for endpoints
% mc_***_baseline_final.txt = final endpoint values
% mc_***_baseline_mb.txt = minimum and maximum mass balance values

if (ChemName == 'IPA')
    if (Group == 'Normal ')
        save cven_th @file='mc_ipa_normal_baseline.txt' @format=ascii
        save final @file='mc_ipa_normal_baseline_final.txt' @format=ascii
        save massbal_th @file='mc_ipa_normal_baseline_mb.txt' @format=ascii
    else
        if (Group == 'Reduced')
            save cven_th @file='mc_ipa_reduced_baseline.txt' @format=ascii
            save final @file='mc_ipa_reduced_baseline_final.txt' @format=ascii
            save massbal_th @file='mc_ipa_reduced_baseline_mb.txt' @format=ascii
        else
            if (Group == 'VeryLow')
                save cven_th @file='mc_ipa_verylow_baseline.txt' @format=ascii
                save final @file='mc_ipa_verylow_baseline_final.txt' @format=ascii
                save massbal_th @file='mc_ipa_verylow_baseline_mb.txt' @format=ascii
            end
        end
    end
end
else
    if (ChemName == 'Ace')
        if (Group == 'Normal ')
            save cven_th @file='mc_acetone_normal_baseline.txt' @format=ascii
            save final @file='mc_acetone_normal_baseline_final.txt' @format=ascii
            save massbal_th @file='mc_acetone_normal_baseline_mb.txt' @format=ascii
        else
            if (Group == 'Reduced')
                save cven_th @file='mc_acetone_reduced_baseline.txt' @format=ascii
                save final @file='mc_acetone_reduced_baseline_final.txt' @format=ascii
                save massbal_th @file='mc_acetone_reduced_baseline_mb.txt' @format=ascii
            else
                if (Group == 'VeryLow')
                    save cven_th @file='mc_acetone_verylow_baseline.txt' @format=ascii
                    save final @file='mc_acetone_verylow_baseline_final.txt' @format=ascii
                    save massbal_th @file='mc_acetone_verylow_baseline_mb.txt' @format=ascii
                end
            end
        end
    end
end
else
    if (ChemName == 'Tol')
        if (Group == 'Normal ')
            save cven_th @file='mc_toluene_normal_baseline.txt' @format=ascii
            save final @file='mc_toluene_normal_baseline_final.txt' @format=ascii
            save massbal_th @file='mc_toluene_normal_baseline_mb.txt' @format=ascii
        else
            if (Group == 'Reduced')
                save cven_th @file='mc_toluene_reduced_baseline.txt' @format=ascii
                save final @file='mc_toluene_reduced_baseline_final.txt' @format=ascii
                save massbal_th @file='mc_toluene_reduced_baseline_mb.txt' @format=ascii
            else
                if (Group == 'VeryLow')
                    save cven_th @file='mc_toluene_verylow_baseline.txt' @format=ascii
                    save final @file='mc_toluene_verylow_baseline_final.txt' @format=ascii
                    save massbal_th @file='mc_toluene_verylow_baseline_mb.txt' @format=ascii
                end
            end
        end
    end
end
end
end
```

MC Anal.m

```
% MC analysis for genetic variability Phase 1 work

% Called in MC_by_Group.m

final = [];
good_params = []; min_tiss_th = []; massbal_th = []; cven_th = []; cbrn_th = [];
failed_params = []; mb_maxmin_fail = []; min_tiss_fail = [];
NumFails = 0;

% Define parameters for number of iterations for Monte Carlo
NumIts2 = NumIts*2; NumSims = 0;

% Initialize random seed
seedrnd(969960349, 890917552);

% Start Monte Carlo analysis
for iter = [1 : NumIts2]
    if (ChemName == 'IPA')
        Distrib_IPA
    else
        if (ChemName == 'Ace')
            Distrib_Acetone
        else
            if (ChemName == 'Tol')
                Distrib_Toluene
            end
        end
    end
end

disp(sprintf("Starting MC Iteration #%d of %d", iter, NumIts2));
disp("-----");

start @NoCallback
mins_th = [];

if (ChemName == 'IPA')
    mins_th(:, :) = [mins_th min(_amuc) min(_aexh) min(_aart) min(_aur) min(_abrn)
                    min(_afat) min(_aliv) min(_amet) min(_arap) min(_aslw) min(_cven)
                    min(_amuc_met) min(_aexh_met) min(_aart_met) min(_aur_met)
                    min(_abrn_met) min(_afat_met) min(_aliv_met) min(_amet_met)
                    min(_arap_met) min(_aslw_met) min(_cven_met)];
else
    mins_th(:, :) = [mins_th min(_amuc) min(_aexh) min(_aart) min(_aur) min(_abrn)
                    min(_afat) min(_aliv) min(_amet) min(_arap) min(_aslw) min(_cven)];
end

% Check mass balances to make sure simulation is valid
% If simulation is valid, move on to next iteration
if (T >= TSTOP & max(_massbal) < 0.00000001 & min(_massbal) > -0.00000001 &
    min(min(mins_th)) >= 0.0)
    NumSims = NumSims + 1;
    disp(sprintf("Finished MC Simulation #%d of %d", NumSims, NumIts));
    disp("-----");

    params = [];
    min_tiss_th = addcolsj(min_tiss_th, mins_th, @Justification = 'begin');
    massbal_th = addcolsj(massbal_th, _massbal, @Justification = 'begin');
    cven_th = addcolsj(cven_th, _cven, @Justification = 'begin');
    cbrn_th = addcolsj(cbrn_th, _cbrn, @Justification = 'begin');
    params(:, :) = [params VMAXC];
    good_params = addcolsj(good_params, params, @Justification = 'begin');
    params = []; mins_th = [];

% If simulation is NOT valid, save failed parameter set, minimum tissue values and mass
% balance values
else
    NumFails = NumFails + 1;
    mins_fail = [];
    params = [];
end
```

```

    if (ChemName == 'IPA')
        mins_fail(:, :) = [mins_fail min(_amuc) min(_aexh) min(_aart) min(_aurr) min(_abrn)
                           min(_afat) min(_aliv) min(_amet) min(_arap) min(_aslw) min(_cven)
                           min(_amuc_met) min(_aexh_met) min(_aart_met) min(_aurr_met)
                           min(_abrn_met) min(_afat_met) min(_aliv_met) min(_amet_met)
                           min(_arap_met) min(_aslw_met) min(_cven_met)];
    else
        mins_fail(:, :) = [mins_fail min(_amuc) min(_aexh) min(_aart) min(_aurr) min(_abrn)
                           min(_afat) min(_aliv) min(_amet) min(_arap) min(_aslw)
                           min(_cven)];
    end

    min_tiss_fail = addcolsj(min_tiss_fail, mins_fail, @Justification = 'begin');
    params(:, :) = [params VMAXC];
    failed_params = addcolsj(failed_params, params, @Justification = 'begin');
    mb_maxmin_fail(1, NumFails) = max(max(_massbal));
    mb_maxmin_fail(2, NumFails) = min(min(_massbal));
    params = []; mins_fail = []; mins_th = [];
end

% If desired number of valid simulations have been completed, exit loop
if (NumSims == NumIts)
    break;
end
end

disp(sprintf("Ran %d simulations to get output for %d simulations", iter, NumIts));

% Save a percentage of iterations to array for mixed population calculations
for iter = [PNumMetab : NumMetab]
    i = iter;
    vmaxc_mixed(i, 1) = good_params(1, i);
end
PNumMetab = NumMetab + 1;

for iter = [PNumMetab : NumIts]
    i = iter;
    vmaxc_mixed(i, 1) = 0.0;
end

if (Group == 'Normal ')
    save vmaxc_mixed @file='mc_normal_vmaxc_mixed.txt' @format=ascii
else
    if (Group == 'Reduced')
        save vmaxc_mixed @file='mc_reduced_vmaxc_mixed.txt' @format=ascii
    else
        if (Group == 'VeryLow')
            save vmaxc_mixed @file='mc_verylow_vmaxc_mixed.txt' @format=ascii
        end
    end
end
vmaxc_mixed = [];

% Transpose matrix of time courses for endpoint to calculate statistics
[nrows, ncols] = size(cven_th);
i = 1; j = 1;
while i <= ncols
    while j <= nrows
        trans_cven_th(i, j) = cven_th(j, i);
        trans_cbrn_th(i, j) = cbrn_th(j, i);
        j = j + 1;
    end
    i = i + 1;
    j = 1;
end
cven_th = []; cbrn_th = [];

```

```

% Calculate statistics for fixed dose, exposure length and start time for exposure
mean_cven_th = mean(trans_cven_th);
std_cven_th = std(trans_cven_th);
max_cven_th = max(trans_cven_th);
min_cven_th = min(trans_cven_th);
mean_cbrn_th = mean(trans_cbrn_th);
std_cbrn_th = std(trans_cbrn_th);
max_cbrn_th = max(trans_cbrn_th);
min_cbrn_th = min(trans_cbrn_th);
trans_cven_th = []; trans_cbrn_th = [];

% Save statistics to one array to be saved
i = 1;
while i <= nrows
    stats(i,1) = mean_cven_th(i);
    stats(i,2) = std_cven_th(i);
    stats(i,3) = mean_cven_th(i) + (2 * std_cven_th(i));
    stats(i,4) = mean_cven_th(i) - (2 * std_cven_th(i));
    stats(i,5) = max_cven_th(i);
    stats(i,6) = min_cven_th(i);
    stats(i,7) = mean_cbrn_th(i);
    stats(i,8) = std_cbrn_th(i);
    stats(i,9) = mean_cbrn_th(i) + (2 * std_cbrn_th(i));
    stats(i,10) = mean_cbrn_th(i) - (2 * std_cbrn_th(i));
    stats(i,11) = max_cbrn_th(i);
    stats(i,12) = min_cbrn_th(i);
    i = i + 1;
end
mean_cven_th = []; std_cven_th = []; max_cven_th = []; min_cven_th = [];
mean_cbrn_th = []; std_cbrn_th = []; max_cbrn_th = []; min_cbrn_th = [];

% Transpose matrix of minimum tissue values to calculate minimum across all simulations
[nrows,ncols] = size(min_tiss_th);
i = 1; j = 1;
while i <= ncols
    while j <= nrows
        trans_min_tiss_th(i, j) = min_tiss_th(j, i);
        j = j + 1;
    end
    i = i + 1;
    j = 1;
end
min_tiss_th = [];

% Find minimum for minimum tissue values across all simulations
min_th = min(trans_min_tiss_th);
trans_min_tiss_th = [];

% Save minimums to one array to be saved
i = 1;
while i <= nrows
    min_tiss(i,1) = min_th(i);
    i = i + 1;
end
min_th = [];

% Find maximum and minimum for mass balance values
mb_maxmin(1, 1) = max(max(massbal_th));
mb_maxmin(2, 1) = min(min(massbal_th));
massbal_th = [];

SaveMCOOutput

zznumbins = sqrt(250);

```

SaveMCOOutput.m

```
% Save output to text files
% Contents of files based on file names where
% * = "ipa", "acetone" or "toluene", and
% ** = "normal", "reduced" or "verylow"

% mc_*_**_good_params.txt = parameters from "good" simulations
% mc_*_**_failed_params.txt = parameters from failed simulations
% mc_*_**_mintiss_fail.txt = minimum tissue values from failed simulations
% mc_*_**_mb_fail.txt = mass balance values from failed simulations
% mc_*_**_stats.txt = statistics for endpoints
% mc_*_**_mintiss.txt = minimum tissue values
% mc_*_**_mb.txt = minimum and maximum mass balance values

if (ChemName == 'IPA')
    if (Group == 'Normal ')
        save good_params @file='mc_ipa_normal_good_params.txt' @format=ascii
        save failed_params @file='mc_ipa_normal_failed_params.txt' @format=ascii
        save min_tiss_fail @file='mc_ipa_normal_mintiss_fail.txt' @format=ascii
        save mb_maxmin_fail @file='mc_ipa_normal_mb_fail.txt' @format=ascii
        save stats @file='mc_ipa_normal_stats.txt' @format=ascii
        save min_tiss @file='mc_ipa_normal_mintiss.txt' @format=ascii
        save mb_maxmin @file='mc_ipa_normal_mb.txt' @format=ascii
    else
        if (Group == 'Reduced')
            save good_params @file='mc_ipa_reduced_good_params.txt' @format=ascii
            save failed_params @file='mc_ipa_reduced_failed_params.txt' @format=ascii
            save min_tiss_fail @file='mc_ipa_reduced_mintiss_fail.txt' @format=ascii
            save mb_maxmin_fail @file='mc_ipa_reduced_mb_fail.txt' @format=ascii
            save stats @file='mc_ipa_reduced_stats.txt' @format=ascii
            save min_tiss @file='mc_ipa_reduced_mintiss.txt' @format=ascii
            save mb_maxmin @file='mc_ipa_reduced_mb.txt' @format=ascii
        else
            if (Group == 'VeryLow')
                save good_params @file='mc_ipa_verylow_good_params.txt' @format=ascii
                save failed_params @file='mc_ipa_verylow_failed_params.txt' @format=ascii
                save min_tiss_fail @file='mc_ipa_verylow_mintiss_fail.txt' @format=ascii
                save mb_maxmin_fail @file='mc_ipa_verylow_mb_fail.txt' @format=ascii
                save stats @file='mc_ipa_verylow_stats.txt' @format=ascii
                save min_tiss @file='mc_ipa_verylow_mintiss.txt' @format=ascii
                save mb_maxmin @file='mc_ipa_verylow_mb.txt' @format=ascii
            end
        end
    end
else
    if (ChemName == 'Ace')
        if (Group == 'Normal ')
            save good_params @file='mc_acetone_normal_good_params.txt' @format=ascii
            save failed_params @file='mc_acetone_normal_failed_params.txt' @format=ascii
            save min_tiss_fail @file='mc_acetone_normal_mintiss_fail.txt' @format=ascii
            save mb_maxmin_fail @file='mc_acetone_normal_mb_fail.txt' @format=ascii
            save stats @file='mc_acetone_normal_stats.txt' @format=ascii
            save min_tiss @file='mc_acetone_normal_mintiss.txt' @format=ascii
            save mb_maxmin @file='mc_acetone_normal_mb.txt' @format=ascii
        else
            if (Group == 'Reduced')
                save good_params @file='mc_acetone_reduced_good_params.txt' @format=ascii
                save failed_params @file='mc_acetone_reduced_failed_params.txt' @format=ascii
                save min_tiss_fail @file='mc_acetone_reduced_mintiss_fail.txt' @format=ascii
                save mb_maxmin_fail @file='mc_acetone_reduced_mb_fail.txt' @format=ascii
                save stats @file='mc_acetone_reduced_stats.txt' @format=ascii
                save min_tiss @file='mc_acetone_reduced_mintiss.txt' @format=ascii
                save mb_maxmin @file='mc_acetone_reduced_mb.txt' @format=ascii
            end
        end
    end
end
```

```

else
  if (Group == 'VeryLow')
    save good_params @file='mc_acetone_verylow_good_params.txt' @format=ascii
    save failed_params @file='mc_acetone_verylow_failed_params.txt'
      @format=ascii
    save min_tiss_fail @file='mc_acetone_verylow_mintiss_fail.txt'
      @format=ascii
    save mb_maxmin_fail @file='mc_acetone_verylow_mb_fail.txt' @format=ascii
    save stats @file='mc_acetone_verylow_stats.txt' @format=ascii
    save min_tiss @file='mc_acetone_verylow_mintiss.txt' @format=ascii
    save mb_maxmin @file='mc_acetone_verylow_mb.txt' @format=ascii
  end
end
else
  if (ChemName == 'Tol')
    if (Group == 'Normal ')
      save good_params @file='mc_toluene_normal_good_params.txt' @format=ascii
      save failed_params @file='mc_toluene_normal_failed_params.txt' @format=ascii
      save min_tiss_fail @file='mc_toluene_normal_mintiss_fail.txt' @format=ascii
      save mb_maxmin_fail @file='mc_toluene_normal_mb_fail.txt' @format=ascii
      save stats @file='mc_toluene_normal_stats.txt' @format=ascii
      save min_tiss @file='mc_toluene_normal_mintiss.txt' @format=ascii
      save mb_maxmin @file='mc_toluene_normal_mb.txt' @format=ascii
    else
      if (Group == 'Reduced')
        save good_params @file='mc_toluene_reduced_good_params.txt' @format=ascii
        save failed_params @file='mc_toluene_reduced_failed_params.txt'
          @format=ascii
        save min_tiss_fail @file='mc_toluene_reduced_mintiss_fail.txt'
          @format=ascii
        save mb_maxmin_fail @file='mc_toluene_reduced_mb_fail.txt' @format=ascii
        save stats @file='mc_toluene_reduced_stats.txt' @format=ascii
        save min_tiss @file='mc_toluene_reduced_mintiss.txt' @format=ascii
        save mb_maxmin @file='mc_toluene_reduced_mb.txt' @format=ascii
      else
        if (Group == 'VeryLow')
          save good_params @file='mc_toluene_verylow_good_params.txt'
            @format=ascii
          save failed_params @file='mc_toluene_verylow_failed_params.txt'
            @format=ascii
          save min_tiss_fail @file='mc_toluene_verylow_mintiss_fail.txt'
            @format=ascii
          save mb_maxmin_fail @file='mc_toluene_verylow_mb_fail.txt'
            @format=ascii
          save stats @file='mc_toluene_verylow_stats.txt' @format=ascii
          save min_tiss @file='mc_toluene_verylow_mintiss.txt' @format=ascii
          save mb_maxmin @file='mc_toluene_verylow_mb.txt' @format=ascii
        end
      end
    end
  end
end
end
end
end

good_params = []; failed_params = []; mb_maxmin_fail = []; min_tiss_fail = []; stats = [];
min_tiss = []; mb_maxmin = [];

```

MC Sum Mixed.m

```
% MC analysis summary for genetic variability Phase 1 work

% Called in MC_Anal.m

% Parameters are from Crystal Ball results using 500 bins for LHS
load NormalParams @file=mc_normal_vmaxc_mixed.txt @format=ascii
load ReducedParams @file=mc_reduced_vmaxc_mixed.txt @format=ascii
load VeryLowParams @file=mc_verylow_vmaxc_mixed.txt @format=ascii

good_params = []; massbal_th = []; cven_th = []; cbrn_th = [];

% Start Monte Carlo analysis
for iter = [1 : NumIts]
    VMAXC = NormalParams(iter,1) + ReducedParams(iter,1) +VeryLowParams(iter,1);
    disp(sprintf("Starting Iteration #%d", iter));
    disp("-----");
    start @NoCallback
    mins_th = [];

    good_params(iter, 1) = VMAXC;
    massbal_th = addcolsj(massbal_th, _massbal, @Justification = 'begin');
    cven_th = addcolsj(cven_th, _cven, @Justification = 'begin');
    cbrn_th = addcolsj(cbrn_th, _cbrn, @Justification = 'begin');
end

disp(sprintf("Ran %d simulations to get output for %d simulations", iter, NumIts));

% Transpose matrix of time courses for endpoint to calculate statistics
[nrows,ncols] = size(cven_th);
i = 1; j = 1;
while i <= ncols
    while j <= nrows
        trans_cven_th(i, j) = cven_th(j, i);
        trans_cbrn_th(i, j) = cbrn_th(j, i);
        j = j + 1;
    end
    i = i + 1;
    j = 1;
end
cven_th = []; cbrn_th = [];

% Calculate statistics for fixed dose, exposure length and start time for exposure
mean_cven_th = mean(trans_cven_th);
std_cven_th = std(trans_cven_th);
max_cven_th = max(trans_cven_th);
min_cven_th = min(trans_cven_th);
mean_cbrn_th = mean(trans_cbrn_th);
std_cbrn_th = std(trans_cbrn_th);
max_cbrn_th = max(trans_cbrn_th);
min_cbrn_th = min(trans_cbrn_th);
trans_cven_th = []; trans_cbrn_th = [];

% Save statistics to one array to be saved
i = 1;
while i <= nrows
    stats(i,1) = mean_cven_th(i);
    stats(i,2) = std_cven_th(i);
    stats(i,3) = mean_cven_th(i) + (2 * std_cven_th(i));
    stats(i,4) = mean_cven_th(i) - (2 * std_cven_th(i));
    stats(i,5) = max_cven_th(i);
    stats(i,6) = min_cven_th(i);
    stats(i,7) = mean_cbrn_th(i);
    stats(i,8) = std_cbrn_th(i);
    stats(i,9) = mean_cbrn_th(i) + (2 * std_cbrn_th(i));
    stats(i,10) = mean_cbrn_th(i) - (2 * std_cbrn_th(i));
    stats(i,11) = max_cbrn_th(i);
    stats(i,12) = min_cbrn_th(i);
    i = i + 1;
end
```

```

mean_cven_th = []; std_cven_th = []; max_cven_th = []; min_cven_th = [];
mean_cbrn_th = []; std_cbrn_th = []; max_cbrn_th = []; min_cbrn_th = [];

% Find maximum and minimum for mass balance values
mb_maxmin(1, 1) = max(max(massbal_th));
mb_maxmin(2, 1) = min(min(massbal_th));
massbal_th = [];

% Save output to text files
% Contents of files based on file names where
% * = "ipa", "acetone" or "toluene", and

% mc_*_mixed_good_params.txt = parameters from "good" simulations
% mc_*_mixed_stats.txt = statistics for endpoints
% mc_*_mixed_mb.txt = minimum and maximum mass balance values

if (ChemName == 'IPA')
    save good_params @file='mc_ipa_mixed_vmaxc.txt' @format=ascii
    save stats @file='mc_ipa_mixed_stats.txt' @format=ascii
    save mb_maxmin @file='mc_ipa_mixed_mb.txt' @format=ascii
else
    if (ChemName == 'Ace')
        save good_params @file='mc_acetone_mixed_vmaxc.txt' @format=ascii
        save stats @file='mc_acetone_mixed_stats.txt' @format=ascii
        save mb_maxmin @file='mc_acetone_mixed_mb.txt' @format=ascii
    else
        if (ChemName == 'Tol')
            save good_params @file='mc_toluene_mixed_vmaxc.txt' @format=ascii
            save stats @file='mc_toluene_mixed_stats.txt' @format=ascii
            save mb_maxmin @file='mc_toluene_mixed_mb.txt' @format=ascii
        end
    end
end

good_params = []; stats = []; mb_maxmin = [];

zznumbins = sqrt(250);

```

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

AF	Air Force
CV	coefficient of variation
CPMC	Coriell Personalized Medicine Collaborative
CYP1A2	Cytochrome P450, Family 1, Subfamily A, Polypeptide 2
CYP2E1	Cytochrome P450, Family 2, Subfamily E, Polypeptide 1
CYP3A4	Cytochrome P450, Family 3, Subfamily A, Polypeptide 4
DNA	deoxyribonucleic acid
GST-T1	glutathione-S-transferase theta 1
HPA	high performance aircraft
HPARS	High Performance Aircraft Respiratory Study
V _{Max} C	maximum reaction rate
K _M	Michaelis-Menten affinity constant
PGx	pharmacogenomics
PBPK	physiologically-based pharmacokinetic
RSAAC	Research Studies and Analysis Council
SNP	single nucleotide polymorphism
STEL	short-term exposure limit
USAF	United States Air Force
USAFSAM	United States Air Force School of Aerospace Medicine
VOC	volatile organic chemical