

B. Re-estimation of Metabolism Parameters

The original *in vitro* concentration time-course data for metabolism of chloroprene (Himmelstein et al. 2004a, IISRP 2009b) were re-analyzed using MCMC analysis with vague priors to obtain a revised set of metabolism parameters for the model. The key differences between the alternative analysis and the original Yang et al. (2012) analysis were: (1) the incorporation of an additional parameter in the analysis of the *in vitro* metabolism data (K_{gl}) to describe the rate of transfer of chloroprene from the headspace to the media in the metabolism studies, (2) the use of updated tissue microsomal protein concentrations for scaling the *in vitro* results to *in vivo* values appropriate for the PBPK model, and (3) the adoption of a previously published approach for estimating the metabolism parameters in the human lung (Andersen et al. 1987). The details of the re-estimation of the *in vitro* metabolism parameters is described below and the results are provided in Supplemental Materials D.

Experimental Determination of Mass Transport Limitation

Schlosser et al. (1993) suggested the need to consider mass transport limitations during *in vitro* metabolism experiments conducted with volatile compounds where there is an air:liquid interface. In their studies of benzene metabolism in sealed vials (Schlosser et al. 1993), they conducted separate studies to determine the rate of approach of the system to equilibrium in order to estimate the rate of transfer of the chemical between the air and liquid phases, and then used the estimated mass transport parameter (K_{gl}) in their analysis of the metabolism of benzene. In the *in vitro* metabolism studies conducted with chloroprene (Himmelstein et al. 2004a, Yang et al. (2012)), no assessment of mass transport limitation was performed. Therefore, a new experimental study was conducted by TekLab, Inc., Collinsville IL, to estimate a K_{gl} for chloroprene following a protocol similar to Schlosser et al. (1993):

1. Add 1mL buffer solution to a 10mL crimp top vial. Crimp on top. A total of 12 vials will be needed for each series of tests.
2. Place vials in a water bath set at 37 C rotating at 60 rpm. Allow temperature to equilibrate for a minimum of 10 minutes.
3. After reaching thermal equilibrium, pierce the septa with an open needle to allow the pressure of the headspace to adjust to ambient pressure.
4. Add 0.5mL of 800ppmv chloroprene gas standard*. Immediately start a timer set for the appropriate contact time.

5. As the timer reaches zero, remove vial and insert a 1mL syringe into the vial so the needle is below the liquid level in the vial. Withdraw 0.5mL and place it into a 40mL VOA vial containing a Teflon stir bar and 4.5mL of deionized water.
6. The beginning and ending blanks are treated the same as the samples with the addition of the chloroprene.
7. A total of 10 samples are to be prepared with contact times of 5, 10, 20, 30, 45, 60, 120, 180, 240 and 360 seconds. The 5 second sample was replaced with a 600 second sample starting with replicate set R-15.
8. When all of the samples for the set are prepared in the 40mL VOA vials, they are analyzed in the VOA lab by GC/MS SW-846 Method 8260B and Method 5035. The concentration of the chloroprene is reported in ug/L.

*The vapor standard used to spike the headspace was prepared according to Denka Method PW R Gas 1805 using a chloroprene standard received from Denka. A high concentration stock was prepared and a working standard prepared from the stock by doing a 100x dilution into a second Tedlar bag. The initial vapor standards were prepared in 1L Tedlar bags. The remaining standard sets were prepared in 500mL Tedlar bags.

The original report on the study is available from the authors on request. The resulting time-courses for chloroprene concentrations in the aqueous phase of the vials were analyzed using the same approach as in Schlosser et al. (1993), resulting in an estimated value of 0.024 L/hr for Kgl, which is similar to the value previously reported for benzene (Schlosser et al. 1993). The results of the MCMC analysis are shown in Figure B-1.

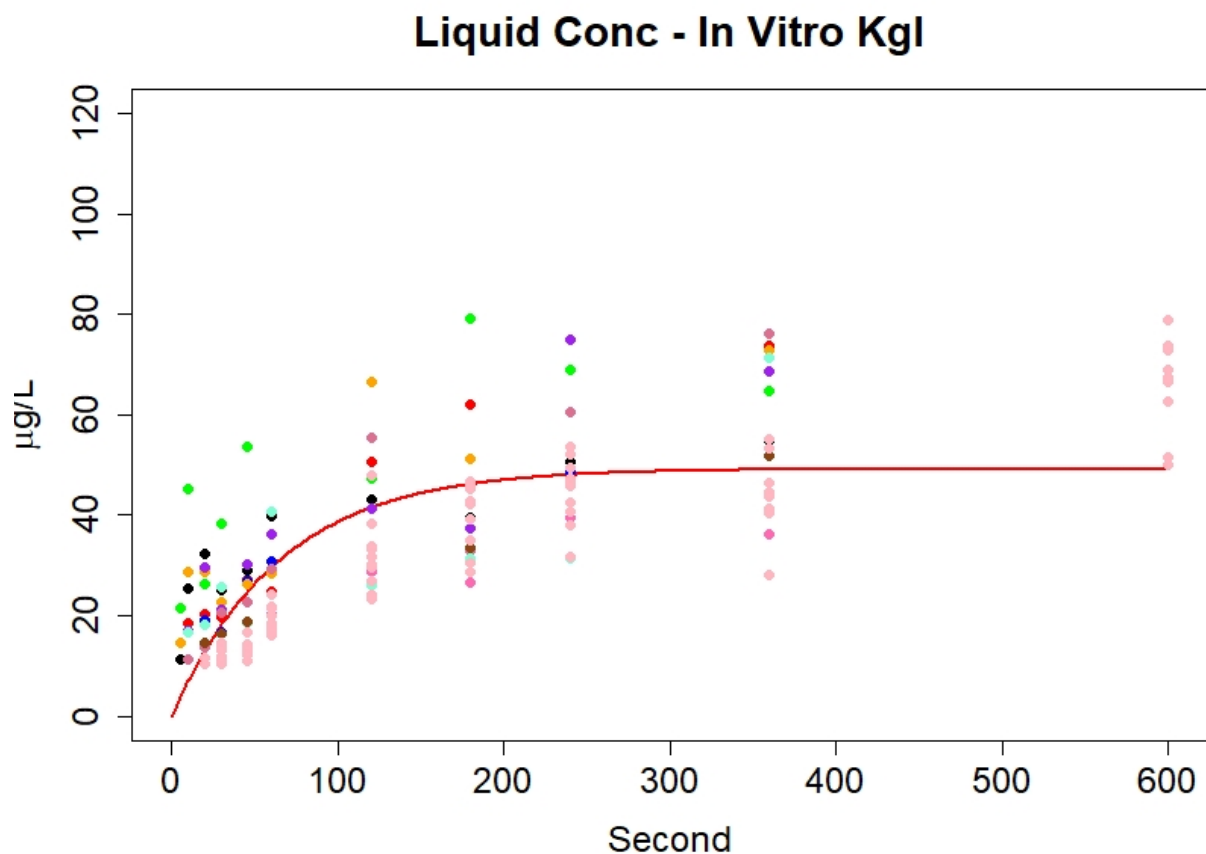


Figure B-1. Concentration of chloroprene in the aqueous phase following addition of 0.5 mL of 800 ppmV chloroprene to the air phase. The best estimates of K_{gl} and P (the liquid:air partition coefficient) were 0.024 (std. dev. = 0.0054) and 0.48 (std. dev. = 0.02).

Using this experimental value of K_{gl} , however, it was not possible to explain the high rates of liver metabolism observed at low concentrations of chloroprene; that is, the mass transport associated with $K_{gl} = 0.024$ L/hr was too slow to support the observed rates of metabolism in the media. Figure B-2 shows the closest possible fit to the experimental data using the experimental value of K_{gl} . Even if the metabolic clearance is set to an implausibly high value ($V_{max} = 1000$ $\mu\text{mol/hr}$, $K_m = 0.01$ $\mu\text{mol/L}$), it is impossible to fit the metabolism data with a K_{gl} of less than 0.11 L/hr

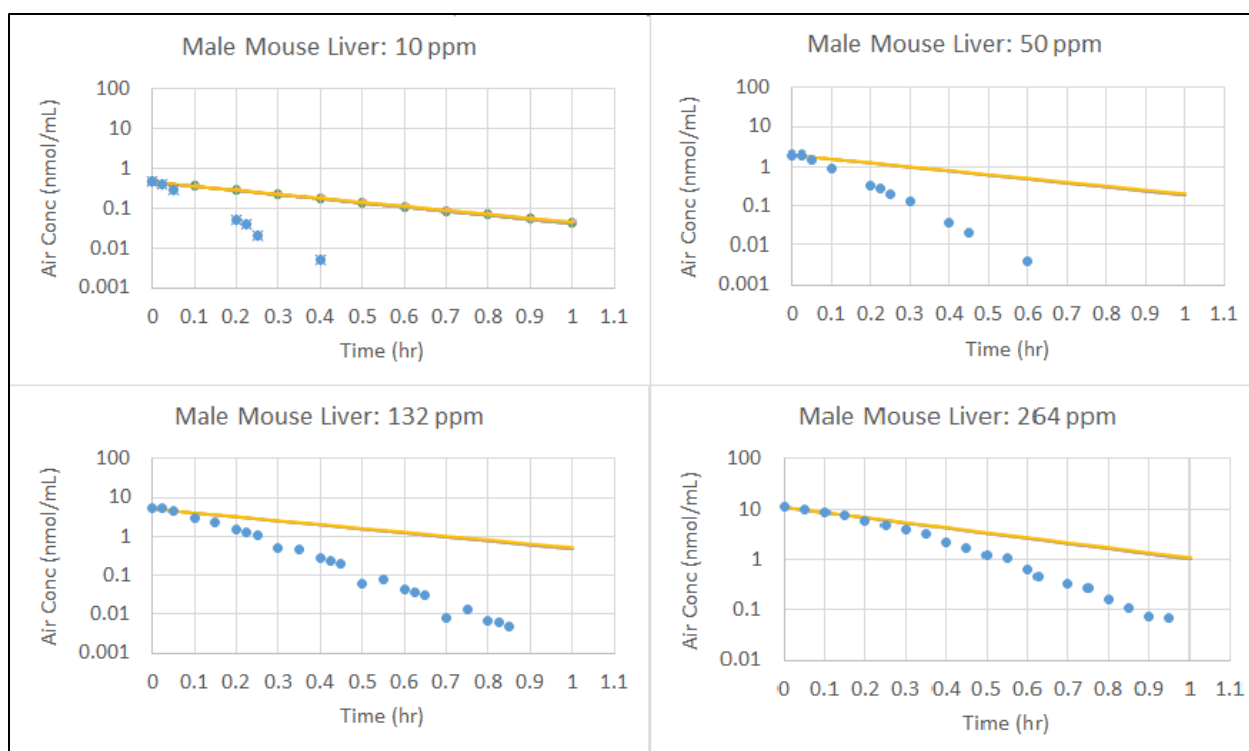


Figure B-2. Comparison of maximum metabolism rate predictions for male mouse liver (curves) and experimental data (points) using $K_{gl} = 0.024$ L/hr.

We considered it likely that the much faster uptake of chloroprene in the metabolism studies than in the K_{gl} study was due to more effective mixing during the incubations, together with non-specific surface binding of chloroprene to the microsomes, which provide a lipophilic binding component in the aqueous media. No microsomes were present in the K_{gl} experiments for chloroprene or benzene (Schlosser et al. 1993). Although the rate of shaking in the metabolism studies was not reported, we were able to determine that the Himmelstein et al. (2004a) and IISRP (2009b) studies used a Gerstel MPS2 autosampler with an agitating heater, which was set to an agitation rate of 500 rpm (Matt Himmelstein, personal communication). Based on this information, it was suggested (Paul Schlosser, personal communication) that the value of K_{gl} in the metabolism studies was likely to be higher than the value in the new experimental study by roughly the ratio of the mixing rates, that is, $K_{gl}(\text{metabolism studies}) = K_{gl}(\text{experimental study}) \times 500/60 = 0.024 \times 500/60 = 0.2$ L/hr.

To confirm this expectation, we conducted a new MCMC analysis to simultaneously estimate K_{gl} , V_{max} and K_m from the metabolism data for the male mouse (Himmelstein et al. 2004a),

the data which are most informative regarding the dose-response for the clearance of chloroprene in the vials. This analysis detected a high degree of collinearity between K_m and K_{gl} (Figure B-3), indicating that the estimates of these two parameters are not completely independent.

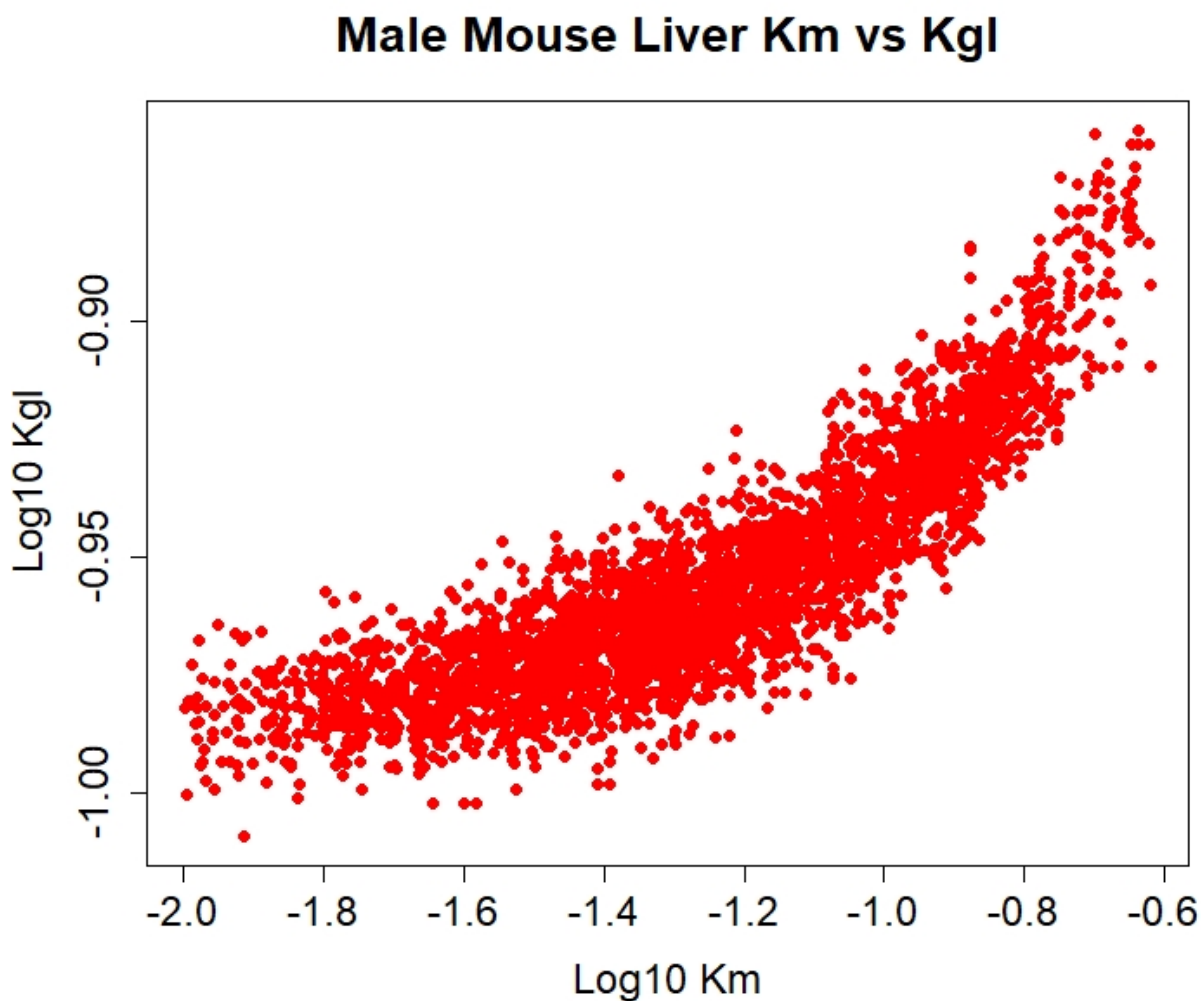


Figure B-3. Correlation plot of $\log(K_{gl})$ vs. $\log(K_m)$. Lower values of K_{gl} correlate with lower values of K_m .

Therefore, to provide a basis for a biological constraint on the K_m values estimated using this approach, we conducted a literature review of compounds with structural similarities to chloroprene, specifically, halogenated alkanes and alkenes, and found (Table B-1) that the values of K_m estimated for these compounds from a variety of *in vivo* studies in mice, rats and humans ranged from 1 to 7 $\mu\text{mol/L}$ (Andersen et al. 1987b,1991, 1994; Clewell et al. 2001; Corley et al.

1990; David et al. 2006; D'Souza et al. 1987, 1988; D'Souza and Andersen 1988; Gargas et al. 1986, 1990; Gargas and Andersen 1989; Lilly et al. 1997, 1998; Marino et al. 2006).

Compound	K _m (μmol/L)	Species
Inhibition studies:		
TCE	1.9	rat (Andersen 1987)
DCE	1.0	rat (Andersen 1987)
In vivo metabolism studies:		
MeCl ₂	5.1/6.8	human/mouse MCMC (David 2006 / Marino 2006)
DHMs	2.3-4.7	rat (Gargas et al 1986)
BDCM	3	rat (Lilly et al 1997,1998)
Closed Chamber <i>in vivo</i> studies:		
VC:	1.6	human (Clewett et al 2001)
CHCl ₃	3-4.6	rat (Corley et al 1990)
EDC	2.5	rat (DSouza et al 1987,1988)
VDC	2.5	rat (Dsouza and Andersen 1988)
Chloroethanes	3.3-5.6	rat (Gargas and Andersen 1989)
chlorinated ethylenes	1-5	rat (Gargas et al 1990)
Furan	2	rat (Kedderis et al. 1993)

Table B-1. Estimates of K_m for CYP2E1 substrates based on *in vivo* studies.

The strongest data for estimating a K_m were from studies of mutual metabolic inhibition in co-exposures to trichloroethylene and dichloroethylene (Andersen et al. 1987b), which estimated K_ms of 1.9 and 1.0 μmol/L, respectively. A K_m of 1.6 μmol/L was used by the USEPA in their risk assessment for vinyl chloride (USEPA 2000).

Therefore, we conducted a re-analysis of the data on metabolism in the male mouse liver to simultaneously estimate V_{max}, K_m and K_{gl} using uninformative priors except that the prior for K_{gl} was bounded from below at 0.11 L/hr, the minimum value that we determined could support the rate of metabolism observed in the liver, and the prior for K_m was bounded from below at a value of 0.5 μmol/L, a factor of 2 below the lowest value for a substrate of CYP2E1 from our review of the literature. There is no evidence that the posterior distributions from this analysis were clipped by the use of these lower bounds on the priors (Figure B-4).

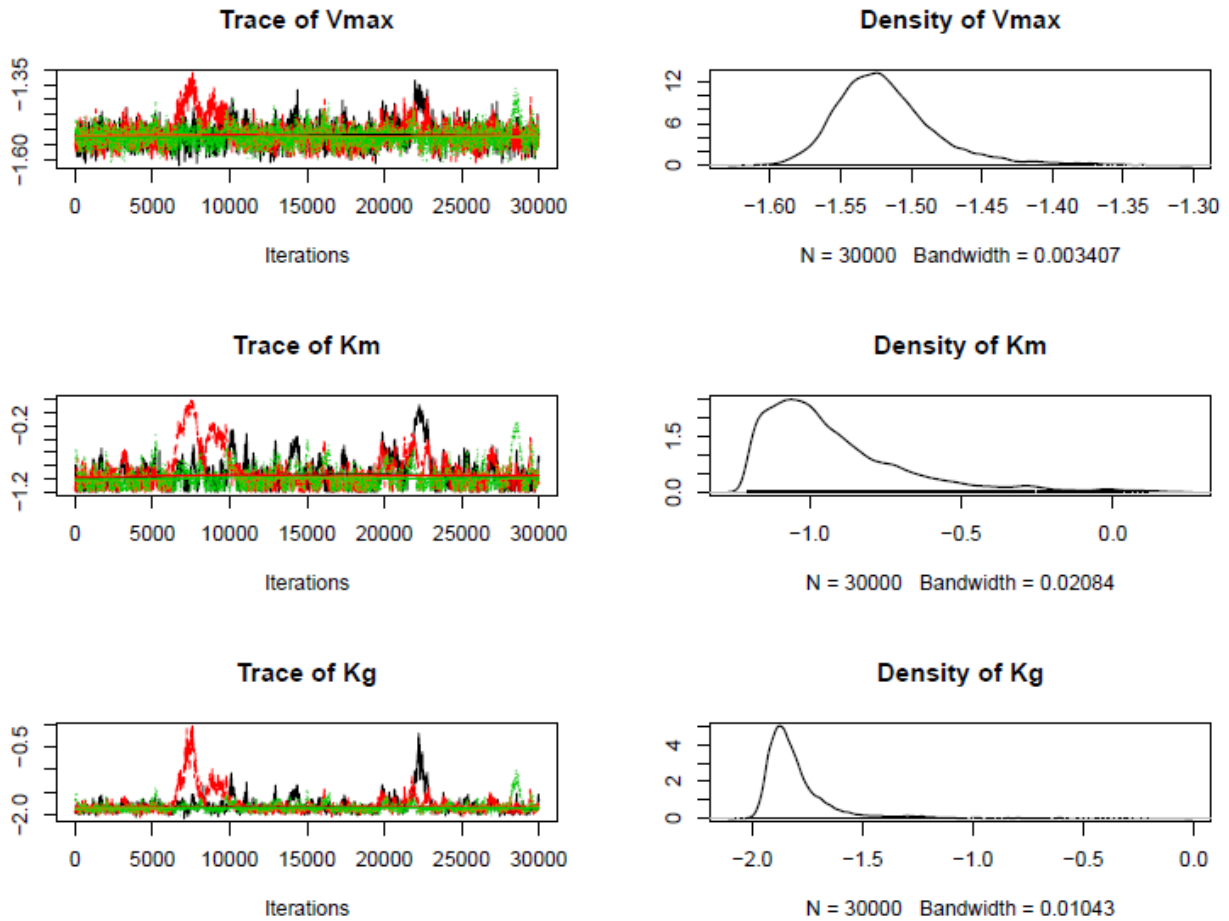


Figure B-4. Posterior chains (right) and distributions (left) of $\ln(V_{\max})$, $\ln(K_m)$, and $\ln(K_{gl})$

The resulting value of K_{gl} estimated from this analysis was 0.22 L/hr, with a 95% confidence interval of 0.19 – 0.33 L/hr, consistent with the value of 0.2 L/hr calculated from the experimental K_{gl} . Figure B-5 shows the resulting fit to the experimental data for the male mouse liver.

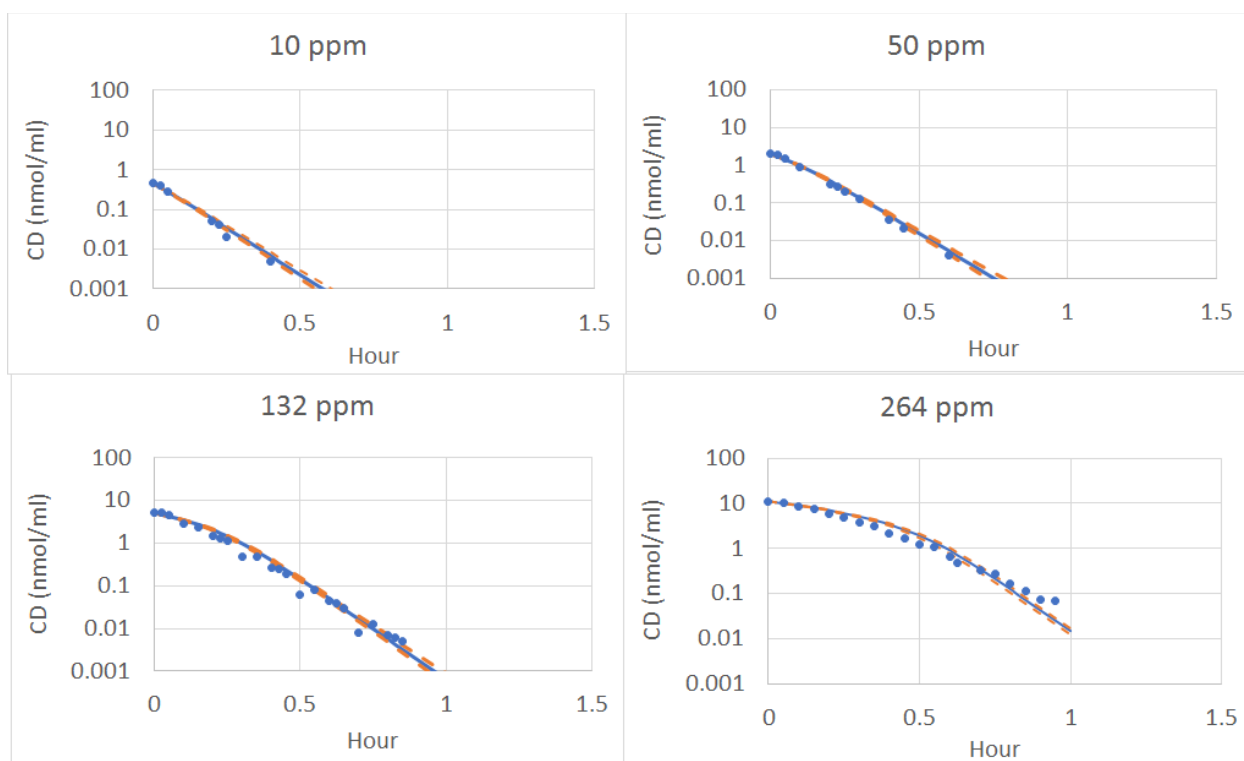


Figure B-5. Comparison of metabolism rate predictions for male mouse liver (curves) and experimental data (points) using 1000 iterations of a posterior chain from the in vitro metabolism MCMC (Geometric Means: $K_m = 0.62 \mu\text{mol/L}$, $V_{\text{max}} = 0.23 \mu\text{mol/hr/mg protein}$ and $K_{\text{gl}} = 0.22 \text{ L/hr}$). Solid lines represent mean and dashed lines represent the 95% confidence interval.

The value of V_{max} estimated for the male mouse liver in this re-analysis ($0.23 \mu\text{mol/hr/mg protein}$) is close to the value reported in Yang et al. (2012) of $0.26 \mu\text{mol/hr/mg protein}$; however, the K_m ($0.62 \mu\text{mol/L}$) is roughly half of the Yang et al. (2012) value ($1.34 \mu\text{mol/L}$), which was derived assuming no transport limitation.

Re-estimation of *In Vitro* Metabolism Parameters

The estimated value of K_{gl} (0.22 L/hr) from the analysis described above was used in a re-analysis of the metabolism data for all tissues. The details of the MCMC analysis are provided below.

Yang et al. (2012) reported a two-level hierarchical Bayesian model to estimate the gender-variability of the in vitro metabolic parameters. In the re-analysis of the in vitro data presented here, the primary interest was on point estimates of metabolic constants for each species and sex (mixed gender for human) in the presence of our predicted flux of chloroprene between air and

media in the in vitro system. Given that the primary interest was in defining uncertainty in the parameter estimates and the in vitro data are not sufficient to estimate population variability, a single level analysis was used in lieu of the hierarchical analysis reported in Yang et al. (2012). The single level analysis retained the broad prior distributions used in the Yang analysis. Parameters for both the saturable (i.e., Vmax and Km) and the 1st order (KF, where Km was unidentifiable) metabolism of chloroprene are given in Table B-2. and are broad log-uniform distributions.

Parameter	Distribution
Log-likelihood Standard Deviation	Lognormal(1,1,)
Vmax	Log-Uniform (-7,2)
Km	Log-Uniform(-10,5)
KF	Log-Uniform(-60,0)
KG	Log-Uniform(-2.996,0)

Table B-2. Prior Distributions Used in MCMC Analysis

The likelihood contribution for any single data point is defined as follows. Suppose that μ represents the prediction of the model for a given set of parameter values (i.e., of Vmax, Km, KF, etc.). For an observation, x , the log-likelihood of that observation is based on the assumption that that observation is log-normally distributed with median μ and a log-scale standard deviation σ . That is,

$$\ln[L(x | \mu, \sigma)] = -\ln(x) - \frac{\ln(\sigma^2)}{2} - \frac{(\ln(x) - \ln(\mu))^2}{2\sigma^2} \quad \text{Eq. 1}$$

where $L(x | \mu, \sigma)$ denotes the likelihood of x given the parameters μ and σ . A broad prior for σ (lognormal with mean = 1, standard deviation = 1, truncated at 0.1 and 100) was used to avoid over-constraining the posterior parameter distributions for the metabolic parameters of primary interest.

The flux of chloroprene between air and media (Kgl) was estimated by fixing the Km in the male mouse liver microsomal study to 1.0 $\mu\text{mol/L}$ and estimating both Vmax and Kgl. Initial testing of the model showed that the male mouse liver had the strongest data upon which to base the Kgl (i.e., steepest slope as low start concentrations). In the estimation of Kgl, the broad distributions

reported above for metabolic parameters were retained. The geometric mean of K_{gl} was retained as a fixed value for the analysis of all the in vitro studies including the male mouse liver which was re-analyzed to estimate V_{max} and K_m after the K_{gl} was fixed. In one case, mixed human lung, it was noted that the lower truncation limit for KF was skewing the posterior distribution due to the broad uncertainty of the data. Therefore, for the mixed human lung simulation, the lower truncation was reduced to -60. In one additional instance, female mouse kidney, the K_m was not identifiable from the in vitro study and the analysis was used to estimate a 1st order metabolic rate constant. The posterior distribution indicates that this is not the case (data not shown) and the upper 95th percentile of this posterior distribution had been represented as the mean in Yang et al. (2012). For V_{max} and K_m analysis, 20000 iterations of the model were run with the first 10000 discarded for the posterior analysis and for the KF analysis, 20000 iterations of the model were run with the final 10000 retained for posterior analysis.

For all analysis of the in vitro data with MCMC, three chains were run independently with different start values to test stability of model convergence. The truncated chains were assessed for convergence both visually (line and density plots) and using the `gelman.diag`¹ routine included in the R package CODA to verify that the point estimates for the potential scale reduction factor (PSRF) were all less than 1.1. The results of the potential scale reduction factor analysis (exported from R) and the posterior distributions for the parameters and the likelihood are listed in the supplemental Excel workbook (Supplemental Materials D) under the species-specific worksheets².

Posterior Distributions

The posterior chains and distributions for the female mouse liver, female mouse lung, mixed human liver and mixed human lung are given in Figures B-6 to B-9. In all cases, the posterior distribution of the $\ln(\text{parameter})$ represents the uncertainty in the parameter estimate given the data and not interindividual variability. The final PSRFs calculated for the female mouse and mixed human are given in Table B-3. In all cases the PSRFs were <1.1 . Confidence ellipse plots for in vitro assay posterior chains for female mouse (liver and lung) and mixed human (liver)

¹ This R routine follows the proposed general approach defined in Gelman and Rubin (1992).

² See cells k2 to w7 on the Mouse and Rat Liver, lung and kidney sheets, and cells k2 to m7 on the human lung and liver sheets.

microsomal metabolism assays are shown in Figures B-10 and B-11. The plots show the 95% (red line) and 99% (green line) confidence ellipses over the plot of the log posterior parameters for the last 10,000 iterations of the posterior chain. As would be expected given the saturable enzymatic metabolic pathway, there is a strong positive correlation between Vmax and Km in the in vitro system for all three assays.

Sex/species	Tissue	Parameter	Point Estimate	Upper CI
Female Mouse	Liver	Likelihood	1	1
		Vmax	1	1
		Km	1	1
	Lung	Likelihood	1	1
		Vmax	1.02	1.06
		Km	1.02	1.06
	Kidney	Likelihood	1	1
		KFkic	1	1
Mixed Human	Liver	Likelihood	1	1
		Vmax	1	1
		Km	1	1
	Lung	Likelihood	1	1
		KF	1	1

Table B-3. Potential scale reduction factors for female mouse and mixed human posterior chains.

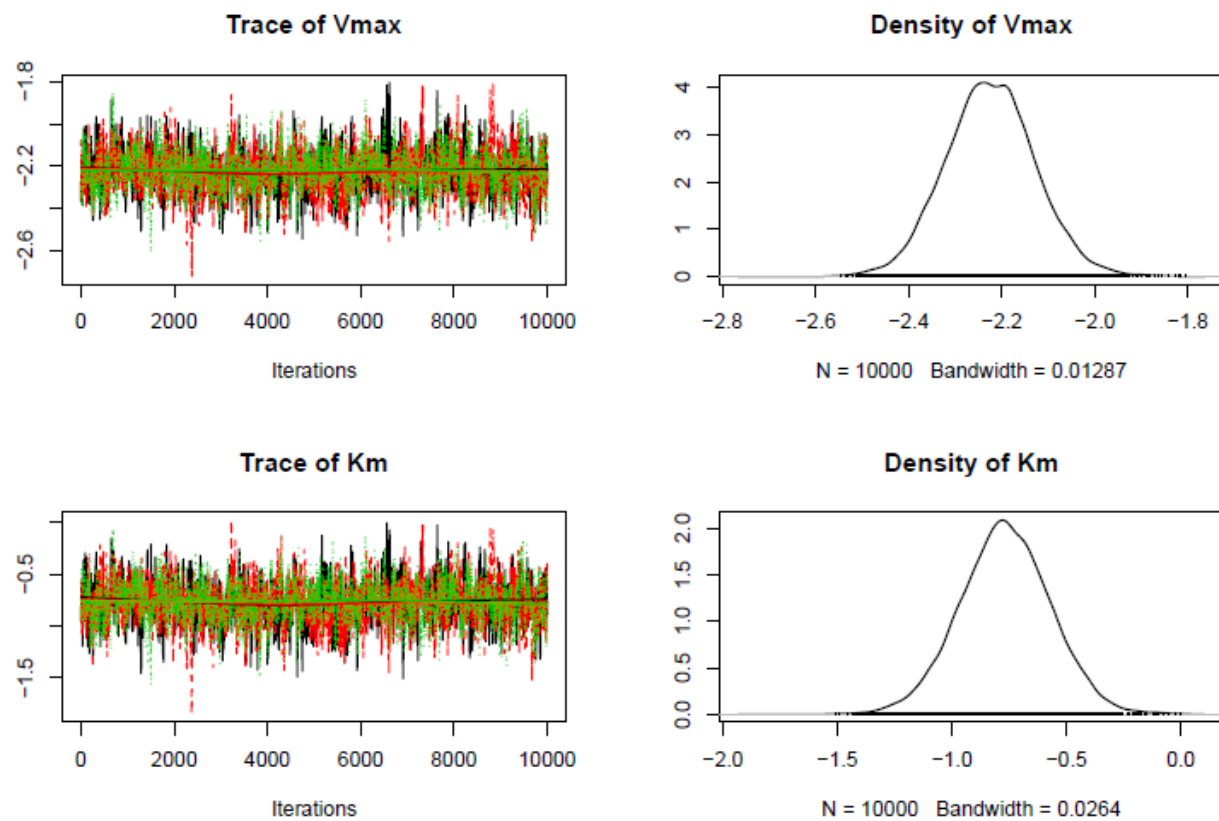


Figure B-6. Posterior chains (left) and distributions (right) for $\ln(V_{\max})$ (top) and $\ln(K_m)$ (bottom) in female mouse liver. K_{gl} was fixed at 0.45 L/hr for this analysis.

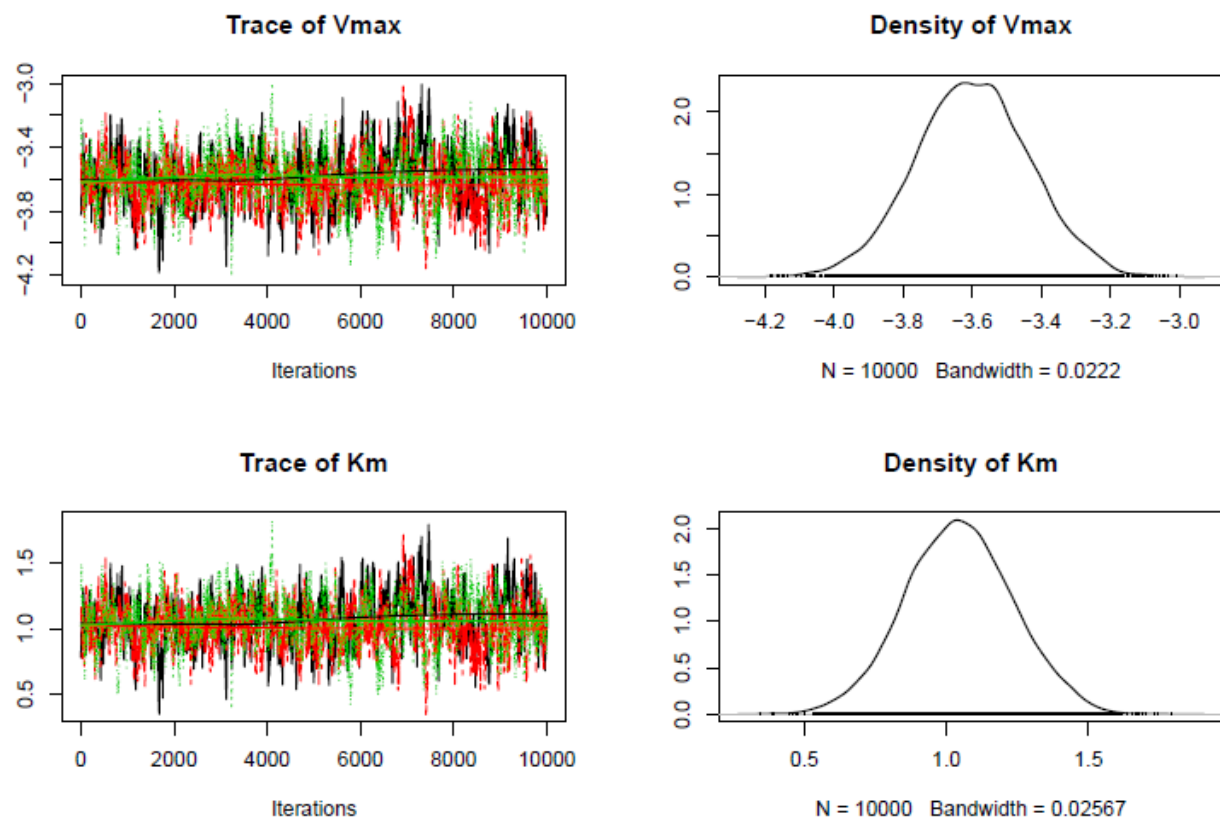


Figure B-7. Posterior chains (left) and distributions (right) for $\ln(V_{\max})$ (top) and $\ln(K_m)$ (bottom) in female mouse lung. K_{gl} was fixed at 0.45 L/hr for this analysis.

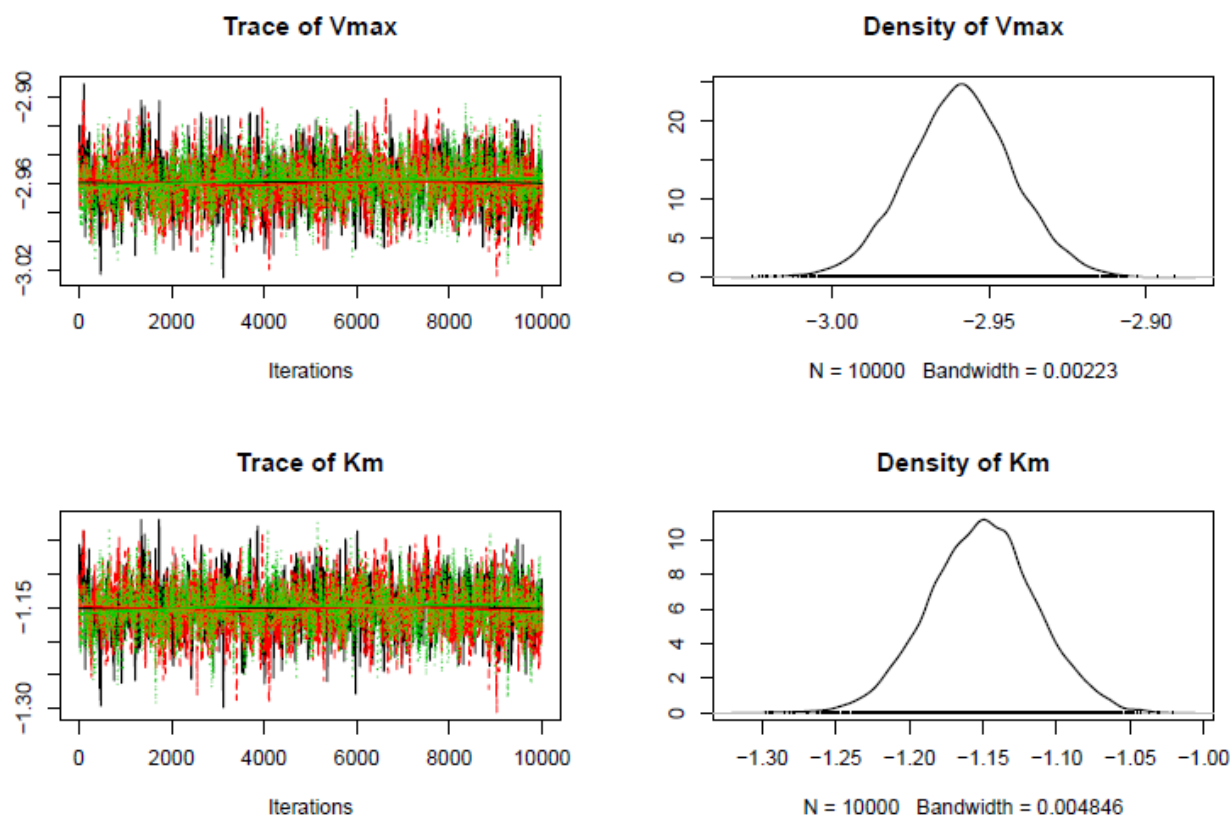


Figure B-8. Posterior chains (left) and distributions (right) for $\ln(V_{\max})$ (top) and $\ln(K_m)$ (bottom) in human liver. K_{gl} was fixed at 0.45 L/hr for this analysis.

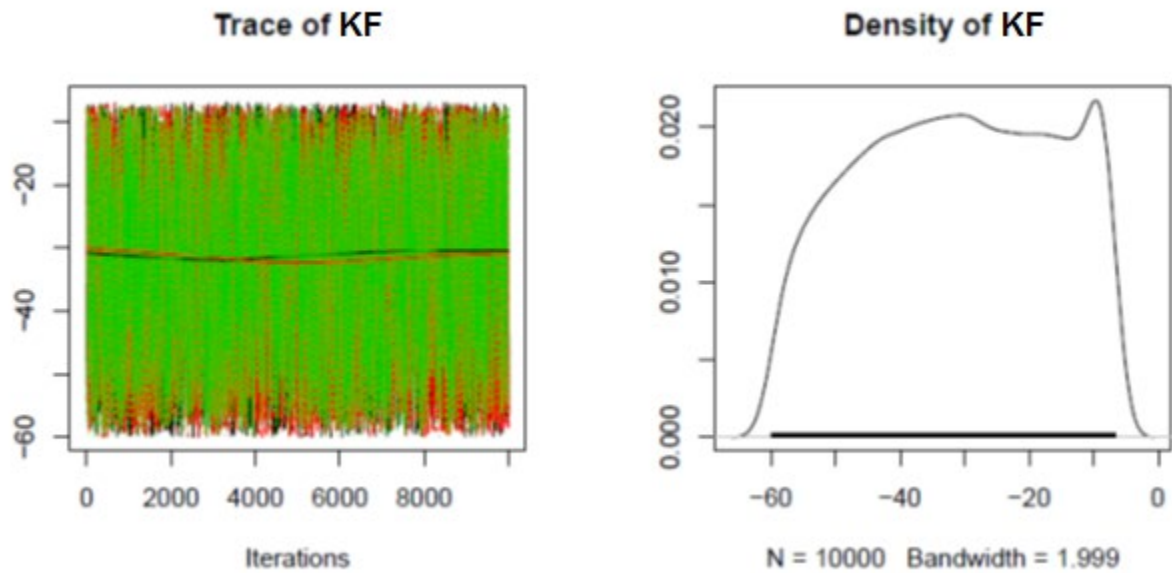


Figure B-9. Posterior chains (left) and distributions (right) for $\ln(KF)$ in mixed human lung. K_{gl} was fixed at 0.45 L/hr for this analysis.

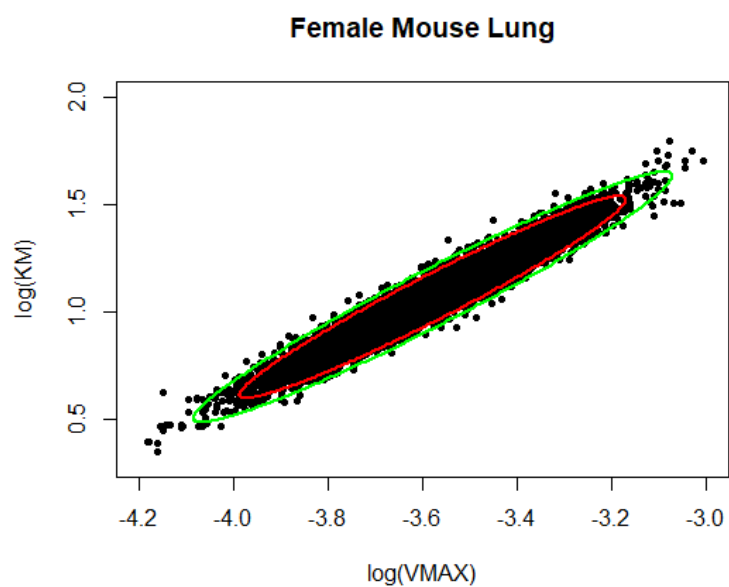
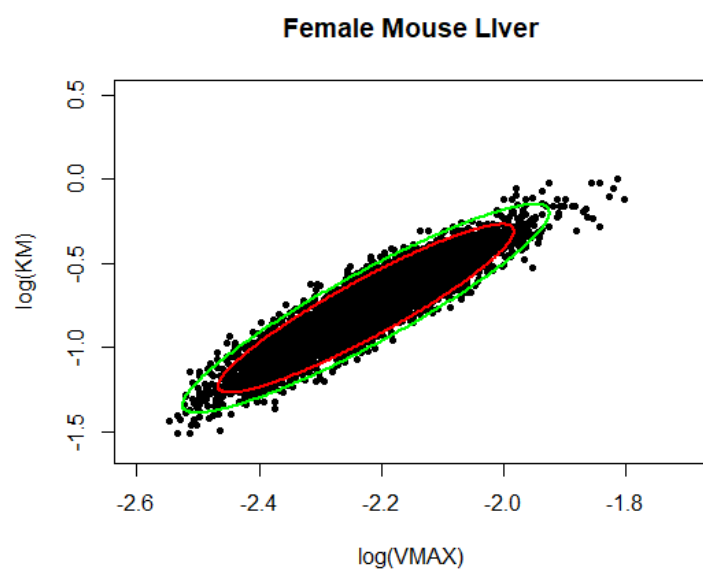


Figure B-10 Confidence (red is 95% and green is 99%) ellipse plot of V_{max} vs. K_m from the in vitro posterior chain for the female mouse microsomal metabolism assay (top panel is liver; + bottom panel is lung).

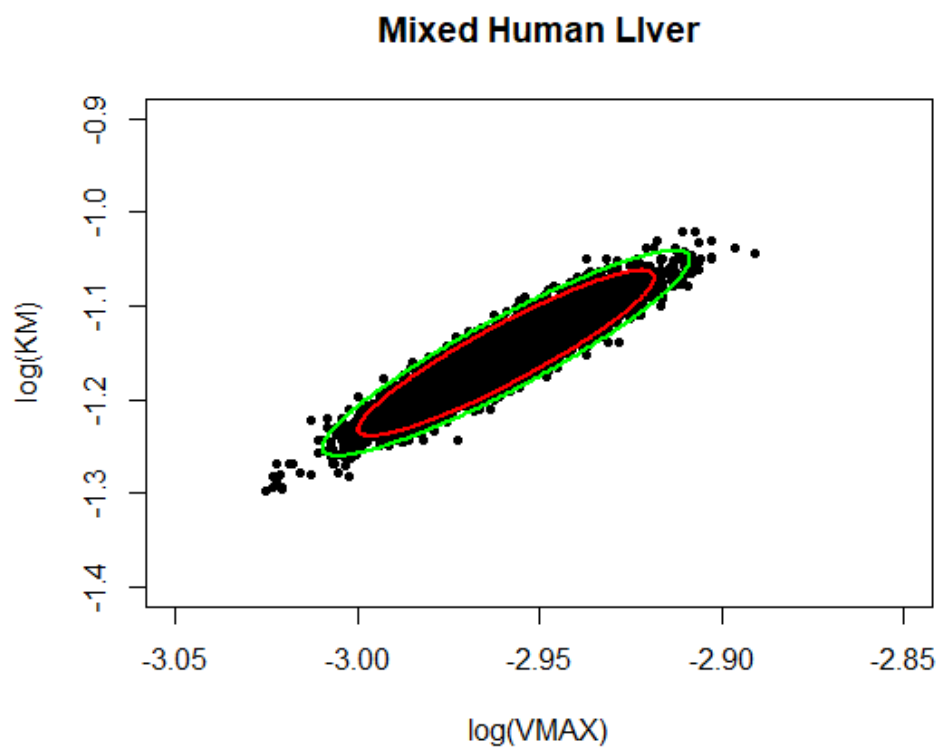


Figure B-11. Confidence (red is 95% and green is 99%) ellipse plot of Vmax vs. Km from the in vitro posterior chain for the mixed human liver microsomal metabolism assay.