

Title: Statistical Analysis of Methylene Chloride (dichloromethane, DCM) Closed Chamber Data Using Physiologically Based Pharmacokinetic Modeling

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Objectives:

In this work we perform a statistical analysis of methylene chloride (dichloromethane, DCM) closed chamber data using a physiologically based pharmacokinetic (PBPK) model and we study the properties of the statistical methods which we employ. The data set which we analyze comes from five experiments in which 14 male B6C3F1 mice were exposed to initial concentrations of 490, 960, 2000, 3200, and 10,000 ppm of DCM in a closed chamber. For each experiment we have data which consist of observed ppm of DCM in the closed chamber over a time period of several hours following the initial exposure. These data are discussed in [1] and this reference also provides the description of an appropriate PBPK model for describing these data. We have successfully implemented this PBPK model using R code. A plot of the data along with the predictions from the PBPK model (where we set the parameters to the values used in [1]) is shown in figure 1. Some goals of the statistical analysis that we perform on this data include estimation of unknown parameters in the PBPK model along with confidence sets, testing homogeneity of parameters across different exposure levels, testing if the variation in the data is constant across different exposure levels, and studying the nature of time dependence in the data. Apart from performing the described analysis we also study the properties of the statistical methods that are employed. For example, after obtaining point estimates of unknown parameters we examine the performance of the estimators used by studying the bias, variance, and sampling distributions of these estimators.

Methods:

The basic statistical model employed in the analysis of pharmacokinetic data has the form of a nonlinear mixed effects model where the mean is the solution to a system of differential equations. These differential equations of course constitute the PBPK model. Some difficulty arises here since our mean function has no closed form expression. Hence the statistical analysis relies extensively on numerical computations. The statistical computation software package R provides a means for carrying out these numerical computations. Stochastic simulation techniques enable us to study the properties of the statistical techniques which we employ.

Results and Conclusions:

The PBPK model employed here includes many metabolic parameters for which we would like to obtain estimates. To begin the analysis we performed a graphical sensitivity analysis of the PBPK model. We found that the model was insensitive to many of these metabolic parameters with the exception of V_{max} . In figure 2 we show one example of how the PBPK model predictions change when V_{max} is varied. V_{max} is varied in figure 2 by multiplying the value of V_{max} given in [1] by a number selected at random from within the interval [0.3, 1.7]. Since the model is very sensitive to changes in the parameter V_{max} , as a starting point we can take all parameters except V_{max} to be known. As described previously, our data consist of measurements of the closed chamber DCM concentrations over time for five different initial doses of DCM (490, 960, 2000, 3200, and 10000 ppm). Below we present the least squares estimate of V_{max} obtained separately using the data from each experiment.

Initial Concentration of DCM	490	960	2000	3200	10000
V_{max} Estimate	1.240570	1.147850	1.168517	0.8896109	0.6032782

Note that the value of V_{max} given in [1] is 1.054. Looking at the estimates given above it appears that there is evidence in the data to suggest that the value of V_{max} is not constant over the five different initial concentrations. This non-homogeneity can be checked using a statistical test of significance. The statistical properties of the above estimates such as bias and variability also will be examined. As this is our current research work, more

results are forth-coming. After estimating the value of V_{max} for each experiment, we look at the residual plot to get an idea about the model fit. The residual plot for each experiment is shown in figure 3. This plot indicates that there may in fact be some time dependence in the data which needs to be modeled. We will model this time dependence using some statistical techniques which introduce correlation parameters into the statistical model.

References:

[1] Andersen, M.E., Clewell, H.J., Gargas, M.L., Smith, F.A., and Reitz, R.H. (1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicology and Applied Pharmacology*. 87, 185-205.

[2] Ramsey, J.C. and Andersen, M.E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicology and Applied Pharmacology*. 73, 159-175.

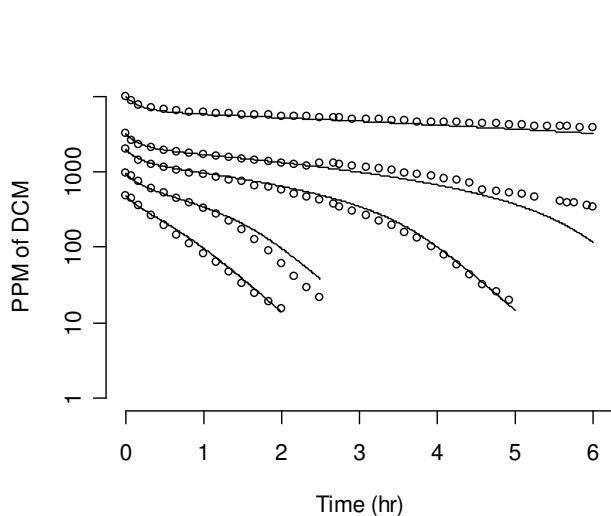


Figure 1: The points show the observed ppm of DCM in the closed chamber over time. The lines show the ppm of DCM in the closed chamber over time as predicted by the PBPK model where the parameter values used are from [1]. Note that this figure appears in [1] and we reproduced it here using our own R code.

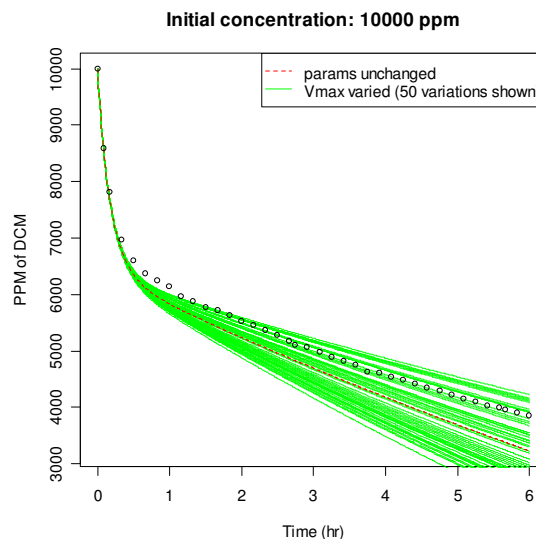


Figure 2: The red line shows the ppm of DCM in the closed chamber as predicted by the PBPK model where the parameter values used are given in [1]. The green lines also show these predicted concentrations, but V_{max} is varied from its value given in [1]. The points represent the observed data.

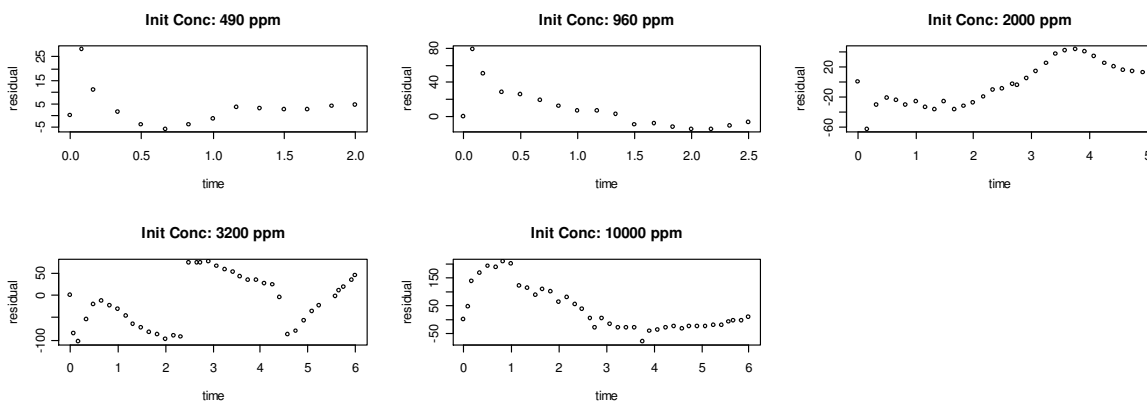


Figure 3: Plot of residuals vs. time for each experiment. The residuals are obtained as the difference between the data and the model fitted values where the model fitted values are obtained by plugging in the least squares estimate of V_{max} and setting all other parameters to the values given in [1].