Evaluation of Toxicokinetic Assumptions Using a 443 Chemical Library

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Abstract

With the increasing availability of high-throughput and *in vitro* data for untested chemicals, there is a need for toxicokinetic (TK) models for *in vitro* to *in vivo* extrapolation (IVIVE). Though some PBTK models have been created for individual compounds using *in vivo* data, we are now able to rapidly parameterize generic PBTK models using *in vitro* data to allow IVIVE for chemicals tested for bioactivity via high-throughput screening. However, these new models are expected to have limited accuracy due to their simplicity and generalization of assumptions. We evaluated the assumptions and performance of a generic PBTK model (R package "httk") parameterized by a library of *in vitro* TK data for 443 chemicals. We evaluate and calibrate Schmitt's method by comparing the predicted volume of distribution (V_d) and tissue partition coefficients to *in vivo* measurements. The partition coefficients are initially overpredicted, likely due to overestimation of partitioning into phospholipids in tissues and the lack of lipid partitioning in the *in vitro* measurements of the fraction unbound in plasma. Correcting for phospholipids and plasma binding improved the predictive ability (R^2 to 0.52 for partition coefficients and 0.32 for V_d). We lacked enough data to evaluate the accuracy of changing the model structure to include tissue blood volumes and/or separate compartments for richly/poorly perfused tissues, therefore we evaluated the impact of these changes on model outputs. After looking at the duration and concentration at the end of the distribution phase, elimination rate, AUC, maximum concentrations, number of days to steady state, and time elapsed for 90% of chemical eliminated, we found that the only significant change in model outputs is in the duration of the distribution phase. The richly/poorly perfused correction doubled the duration of the distribution phase while the duration for the blood volume correction was ⅔ of the original. We also determined that the effective volume of distribution is at least twice the predicted value for 153 of the chemicals, independent of the correction. Overall, comparison to *in vivo* data identified discrepancies that we reduced by refining our phospholipid partitioning and plasma binding models, which improved the accuracy of the partition coefficient predictions. However, separation of the rest of body into richly/poorly perfused compartments and consideration of blood volumes only made a significant difference in the duration of the distribution phase. *This abstract does not necessarily reflect U.S. EPA policy.*

Introduction

- Rapidly parameterized generic PBTK models allow *in vitro* to *in vivo* extrapolation (IVIVE) for chemicals tested for bioactivity via high-throughput screening.
- The models in the R package httk have been criticized in assuming negligible blood volumes and the lumping together of poorly and richly perfused tissues. The partition coefficients also showed large errors when compared to *in vivo* data.
- We evaluated these assumptions using a library of *in vitro* TK data for 443 chemicals.
- We used a simulation study to evaluate the impact of tissue lumping and negligible blood volume.

Partition Coefficient Prediction with Schmitt's Method

- To determine the ratio of the tissue and plasma concentrations at equilibrium, K_p, the partitioning into each individual component of the tissue must be determined.
- In Schmitt's method, each tissue is composed of cells and interstitium where cells are composed of neutral lipids, neutral phospholipids, acidic phospholipids, proteins, and water.
- The partition coefficients are all determined relative to water and summed together as shown in the equation below where $F_{component}$ is the fraction of the total tissue volume for each component of the tissue.

$$K_{tissue:water} = \sum_{components} F_{component} K_{component:water}$$

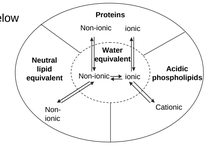


Figure from Peyret 2010

- The in vitro protein binding, f_{up}, is used as the water to plasma partition coefficient:

$$K_p = \frac{K_{tissue:water}}{K_{plasma:water}} = K_{tissue:water}\ f_{up}$$

- The volume of distribution is calculated by summing the K_ps multiplied by their volumes.

$$V_{dist} = \sum_{tissues} K_p V_{tissue} + V_{plasma}$$

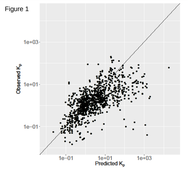
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Partition Coefficients

- Tissue:plasma partition coefficients predicted with Schmitt's method were evaluated with 1002 measured rat partition coefficients. Schmitt's method initially yielded many overpredictions by at least one order of magnitude, primarily for compounds with high logP, as seen in **Figure 1 to the right** on a log-log plot.

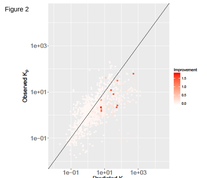
Figure 1



- Many of these points improved after using a new regression for predicting phospholipid partitioning (membrane affinity) and assuming higher protein binding than the *in vitro* measurement for un-ionized compounds to account for lipid in plasma.
- The fraction unbound in plasma, f_{up}, (concentration in unbound plasma divided by the total plasma concentration) was modified to account for the binding to lipid in plasma that is not measured *in vitro*, using the fractional volume of lipid in plasma, F_{lipid} , and the distribution coefficient, D_{ow} , calculated with logP and the ionization in plasma.

$$f_{up} = 1/((Dow - 1) * F_{lipid} + \frac{1}{f_{up\ in\ vitro}})$$

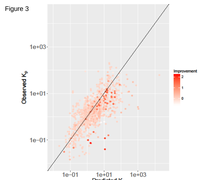
Figure 2



- Only highly lipophilic un-ionized compounds with high fub significantly improved (**Figure 2 at Right**).
- A new regression, shown below, used for predicting partitioning into phospholipids for compounds without membrane affinity data from Yun et al. 2013 on logP using Schmitt's data set (**Figure 3 at right**) yielded much better results. Most improved points were highly lipophilic and ionized.

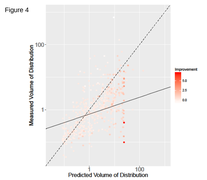
$$logMA = 1.294 + 0.304\ * logP$$

Figure 3



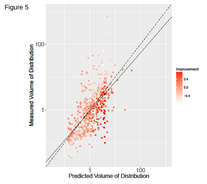
- V_{dist} was initially overpredicted when compared to 498 measured values but greatly improved when the two partition coefficient corrections were applied (**Figure 4 at right**). 94 points improved by at least half an order of magnitude.

Figure 4



- The error in K_p prediction correlated with the value. Regressions were made to predict the observed K_p based on the predicted K_p, and this correction was applied again to predicting V_{dist}.
- Only 47 compounds from the Schmitt evaluation data set overlap with the Obach V_{dist} measurements, suggesting that the improvement is not due to the compounds used in the evaluations.
- After applying the regressions to the partition coefficients used in the volume of distribution calculation, 72 V_{dist} predictions further improved by at least half an order of magnitude (**Figure 5 at right**).

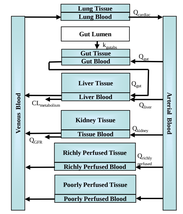
Figure 5



Tissue Lumping and Blood Volume

- The PBTK models included in httk assume a negligible blood volume in each compartment and have all extra tissues lumped together in the rest of body compartment.
- To test the affect of these assumption on model outputs, the rest-of-body compartment was separated into richly and poorly perfused compartments, determined by the blood flow to a tissue divided by its volume, as shown in the figure to the right. The volumes of the blood compartments were also set to realistic fractions of the total organ volume.

Figure 6



- The duration of the distribution phase turned out to be the only significant change in the concentration-time curve. The end of the distribution phase was determined by the maximum of the rest of body concentration curve.
- **Figure 7 at the right** shows the concentration-time curve of permethrin with the PBTK model. The distribution phase lasts 0.25 days, and if we trace the line after this time point back to the origin, we have the curve for the one compartment model.

Figure 7

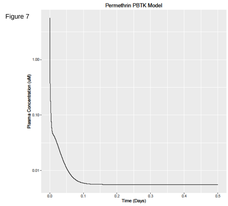
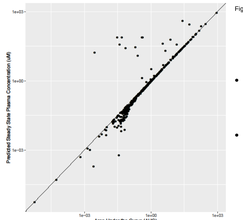
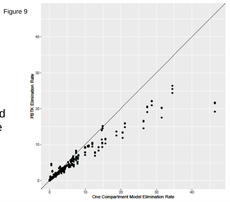


Figure 8



- In **Figure 8 at the left**, the AUC of the PBTK model is plotted against its steady state plasma concentration. A few compounds did not entirely clear the system in a reasonable time and are thus less than the true AUC.
- Now we can assume that AUC and steady state equivalence is true for the PBTK model as in a one or two compartment model.

Figure 9



- In **Figure 9 at the right**, the decay rate of the PBTK model after the distribution phase is plotted against the elimination rate of a one compartment model with clearance equal to the sum of the glomerular filtration rate multiplied by f_{ub} and the well-stirred model for liver clearance.

Conclusion

- Adding the additional complexity of realistic blood volumes and separating the rest-of-body compartment into richly and poorly perfused compartments had little affect on the model outputs. The most significant change was the duration of the distribution phase increasing by a factor of 2 with the richly/poorly perfused correction.
- The corrections to Schmitt's method in the treatment of neutral phospholipid in tissue and neutral lipid in plasma dramatically reduced the partition coefficient and volume of distribution prediction error for many high logP compounds.
- One and two compartment models both have the observed property of the PBTK model that steady state concentration equals AUC, suggesting a predictable PBTK model clearance. Fitting a PBTK model to a one compartment model yields a larger volume of distribution than expected and no distribution phase. However, a two compartment model with the predicted volume of distribution and clearance can better fit a PBTK model.

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