High Throughput Physiologically Based Toxicokinetic Models for ToxCast Chemicals

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Abstract

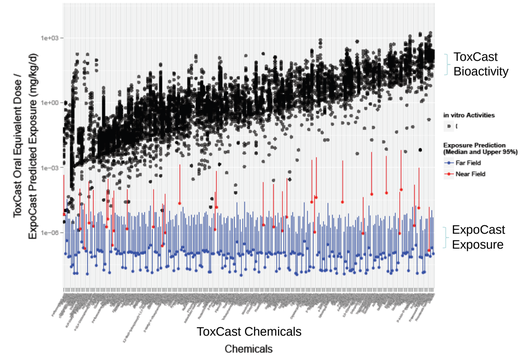
Physiologically based toxicokinetic (PBTK) models aid in predicting exposure doses needed to create tissue concentrations equivalent to those identified as bioactive by ToxCast. We have implemented four empirical and physiologically-based toxicokinetic (TK) models within a new R software package, vLiverPBPK. For the thousands of chemicals without *in vivo* TK data, all four TK models were designed to be parameterized with high-throughput (HT) *in vitro* TK experiments and structure-based physico-chemical property predictions. The models make two general types of predictions: steady-state serum concentration resulting from repeated exposures for use in reverse toxicokinetic (RTK) studies, and prediction of TK time course metrics such as C_{max} and time-integrated plasma concentration (Area Under the Curve or AUC) for evaluating model prediction by comparison to *in vivo* data. In predicting the concentrations of a chemical over time, the HTTK models primarily use *in vitro* data for both the fraction of chemical unbound to plasma and the hepatic clearance, as well as structure-derived physicochemical properties for the calculation of partition coefficients and ratios of blood flows and tissue volumes to body weight for the models with multiple compartments. We have performed simulation studies using the more sophisticated high-throughput (PBTK) model to evaluate key assumptions in the simpler three-compartment, steady-state model used in previous RTK studies and have found that although the majority of chemicals reach steady state within seven weeks, some never reach steady state within a typical human lifespan. We were also able to predict average steady state concentrations resulting from discrete dosing with predictions based on the infusion dosing assumption used in previous RTK studies; many of the chemicals that quickly reached lower steady state concentrations reached maximum concentrations of more than double the average steady state concentration. The package can currently make predictions for 350 chemicals, including 75 pharmaceuticals and 275 ToxCast chemicals, and we will continue adding chemicals as more data comes available. *This abstract does not necessarily reflect US EPA policy.*

Introduction: Bridging ToxCast and ExpoCast

• There are thousands of chemicals in our environment to which we are regularly exposed, many of which with little information for prioritizations

• ToxCast[3] *in vitro* assays (*e.g.*) generate bioactivity data that provide tools for comparing chemicals with minimal information to known toxicants

• ExpoCast[8] allows high throughput exposure predictions for comparison with bioactivity data (**point and vertical bar** in **figure at right** indicates median and upper 95% interval) **Red** indicates chemicals with some near-field (*e.g.* indoor, consumer use) sources of exposure while **blue** indicates chemicals with far field sources only.



• Each **black circle** in the **figure above** corresponds to the dose needed to cause 50% activity in an *in vitro* assay Different chemicals have different numbers of active assays, *e.g.,* if the assay dose-response was best described by a flat line (no response) then no circle is plotted.

•The ratio of oral equivalent dose for activity to predicted exposures (activity:exposure ratio, AER)[9] allows prioritization of limited testing resources for chemicals of higher concern

• *In vitro* measurements of TK determinants have allowed ToxCast activities to be translated into human[9] and rat[10] oral equivalent doses needed to reach steady state

• Although we have characterized the uncertainty in exposure predictions, there is a great need for characterizing the uncertainty of *in vitro* predictions of toxicokinetics (HTTK)

References

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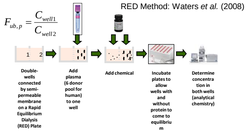
PBTK Models Parameterized *In Vitro*

- We have curated sufficient HTTK data to predict human steady-state serum concentrations (C_{SS}, in units of mg/L) equivalent to the activation concentrations observed *in vitro* for 350 chemicals[1,4,7,9,10]:
  - 75 pharmaceuticals,
  - 275 ToxCast chemicals
  - 41 NHANES chemicals
- In Wetmore *et al.* (2012) population variability was simulated via Monte Carlo method using SimCYP2 the EPA/NCCT vLiverPBPK package replaces SimCYP with distributions that better reflect *in vitro* measurement

Plasma Protein Binding (Fraction Unbound in Plasma)

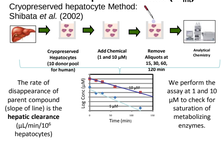
$$F_{ub,p} = \frac{C_{well1}}{C_{well2}}$$

RED Method: Waters *et al.* (2008)



Intrinsic Hepatic Clearance (Cl_{int})

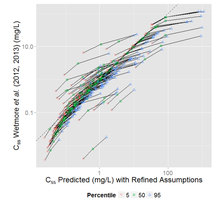
Cryopreserved hepatocyte Method: Shibata *et al.* (2002)



The rate of disappearance of parent compound (slope of line) is the **hepatic clearance** (μL/min/10^6 hepatocytes)

We perform the assay at 1 and 10 μM to check for saturation of metabolizing enzymes.

Modeling Measurement Limitations

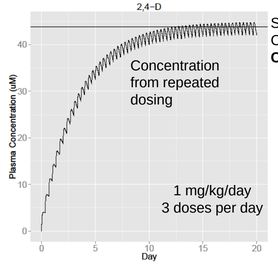


The 5%, median, and 95% quantiles for each chemical are connected by a line. The 95% quantile (highest C_{SS} for a fixed dose) is sensitive to assumptions about the protein binding assay.

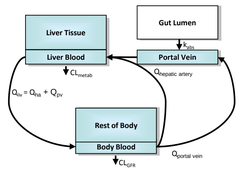
Predicting Steady State and Equivalent Dose

- Most chemicals reach a steady state concentration (C_{SS}) in a manner similar to the way it is reached in the **figure on the right**. The **horizontal line** represents the steady state reached with the constant infusion dosing assumption made in Wetmore *et al.* (2012).
- The **equation on the right** is used in the Monte Carlo sampler and Wetmore et al. (2012). The equations is equal to the steady state concentration of the liver in the three compartment model without partition coefficients,

$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

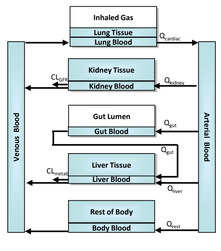


- Using HTPBTK we can simulate discrete doses to better approximate discrete dosing from proximate (near-field) sources. We can then compare the maximum concentration with the infusion dosing results at steady state, which are equivalent to the average of the discrete dosing steady states.
- The steady state concentration from 1 mg/kg/day dosing is used to calculate the dose needed to reach any steady state for that chemical using the linear dose-concentration relationship of the model.



Three Compartment Model

- The models **at the left and right** are included in the vLiverPBPK package with functions for solving for the concentration vs. time curve for each compartment , finding the steady state plasma concentration, simulating steady state and oral equivalent variability with Monte Carlo methods, calculating and listing parameters, and listing the chemicals and data within the package.



HTPBPK Model

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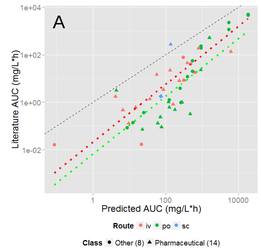
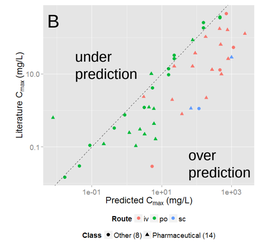
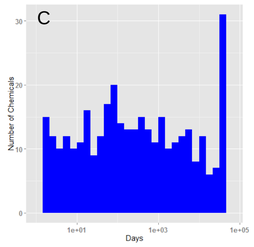
Results

The HTPBTK model predictions for the area under the plasma concentration versus time curve (AUC – shown in **A**) and the maximum concentration for a single dose (C_{max} – shown in **B**) correlate well with the *in vivo* data taken from various literature sources.

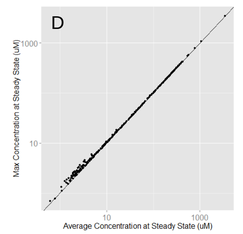


The HTPBTK model assume 100% bioavailability for oral doses and should therefore usually over predict in vivo measurements, as in **A** and **B.**





C) The number of days it takes a chemical to reach steady state ranges from 2 days to over 100 years with about half taking less than a year. The density of chemicals decreases as the time to steady state increases.



D) The maximum concentration at steady state does not vary significantly from the average concentration. For a few of the chemicals with lower steady state concentrations it can be up to a factor of 1.35 larger at three doses per day.

Conclusion

- The models within vLiverPBPK provide rapid and efficient predictions of steady state and other concentrations of interest, allowing *in vitro – in vivo* extrapolation (IVIVE) of ToxCast bioactivity results
- We find the assumptions and results from Wetmore *et al.* (2012) to be consistent with our own and reasonable for most chemicals:
  - The comparison of our HTPBTK model predictions to literature values demonstrates that we can account for a significant amount of the variance in *in vivo* concentrations between chemicals.
  - Our models predict that some chemicals with long half lives never reach steady state.
  - These models show that the steady-state concentrations predicted with discrete and infusion dosing assumptions are consistent and significantly different from the peak concentration at steady state.