High Throughput Pharmacokinetics for Environmental Chemicals

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Abstract

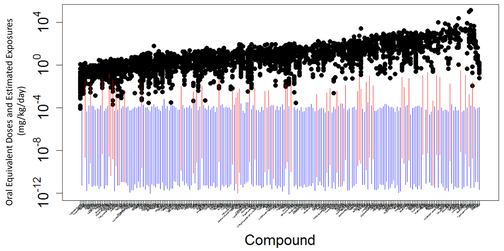
Pharmacokinetic (PK) models are critical to determine whether chemical exposures produce potentially hazardous tissue concentrations. For bioactivity identified *in vitro* (*e.g.* ToxCast) – hazardous or not – PK models can forecast exposure thresholds, below which no significant bioactivity is expected. Successful methods have been developed for pharmaceutical compounds to determine PK from limited *in vitro* measurements and chemical structure-derived property predictions. These high throughput (HT) PK methods provide a more rapid and less resource–intensive alternative to traditional PK model development. Unfortunately, predictions from HTPK approaches have demonstrated mixed success for environmental chemicals when compared to predictions made by PK models developed with extensive *in vivo* data. Here we tested assumptions of previous HTPK approaches using a simple physiologically-based PK (PBPK) model and in vitro data for 232 chemicals in human and 39 chemicals in rat. We then analyzed the discrepancy between the predictions of HTPK and in vivo literature PK data for 44, mostly pharmaceutical, chemicals, using the method of best subsets to identify those properties that correlate with poor predictive ability (*e.g., in vitro* HTPK data, physico-chemical descriptors, chemical structure, and predicted transporter affinities). We propose a framework for PK triage in stages: First, *in vitro* measurements and *in silico* predictions determine whether the simplest HTPK approaches are likely to be sufficient. Then, identify and collect any additional, targeted *in vitro* data that is needed. Finally, identify those chemicals most likely to require traditional, *in vivo* PK methods. This methodology allows prioritization of PK resources and characterizes the confidence in HTPK model predictions for potentially thousands of environmental chemicals that currently have no PK data. *This abstract does not necessarily reflect EPA policy.*

Introduction: ToxCast + ExpoCast

• There are thousands of chemicals in our environment to which we are regularly exposed

• Relatively inexpensive *in vitro* assays (*e.g.* ToxCast[4]) provide tools for comparing chemicals with minimal information to known toxicants

• Additional *in* vitro measurements of PK determinants have allowed ToxCast (Bio)Activities to be translated into human[10] and rat[11] oral equivalent doses (each **black circle** in the **figure at the right** corresponds to the dose needed to cause 50% activity in an in vitro assay – if the assay was not well-described by a Hill function, *i.e.*, systematic concentration-response, no circle is plotted).



• The ratio of oral equivalent dose for activity to predicted exposures (activity:exposure ratio, AER) allows prioritization of targeted testing resources

• High throughput exposure predictions from ExpoCast[9] project are made with a 95% confidence interval (**red** or **blue** **vertical bar** in **figure above**), the upper extent of which is often below any bioactivity (AER >> 1). **Red** indicates chemicals with some near-field (e.g. indoor, consumer use) sources of exposure while **blue** indicates chemicals with far field sources only.

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

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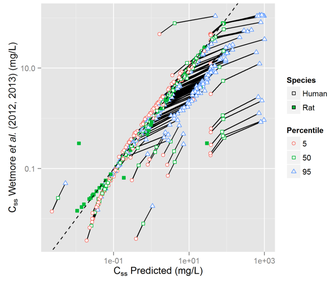
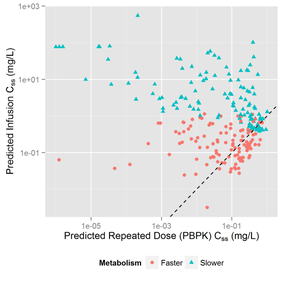
High Throughput Pharmacokinetics (HTPK)

- Relatively high throughput *in vitro* PK data allows prediction of the constant infusion dose (mg/kg BW/day) needed to produce steady-state serum concentrations (C_{ss}, in units of mg/L) equivalent to the activation concentrations observed *in vitro*[10,11]
- From literature[2,5,8,10,11] we have sufficient data to predict human C_{ss} for 308 chemicals, Including:
  - 36 pharmaceuticals,
  - 257 ToxCast chemicals
  - 278 Tox21 chemicals
  - 30 NHANES chemicals
- In Wetmore *et al.* (2012) SimCYP[3] was used to predict C_{ss} using HTPK data. We reproduce these results using the vLiverPBPK package developed by EPA/NCCT for R.
- In the Wetmore et al. papers[10,11] the rapid equilibrium dialysis (RED) assay for measuring protein binding fails in some cases because the amount of free chemical is below the limit of detection. For those chemicals a default value of 0.5% free was used.
- In the **figure at the right** we have replaced the default value with random draws from a uniform distribution from 0 to 1%.

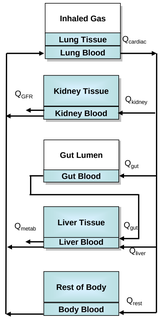
Impact of Highly Plasma Bound Chemicals



The Infusion Dose Assumption

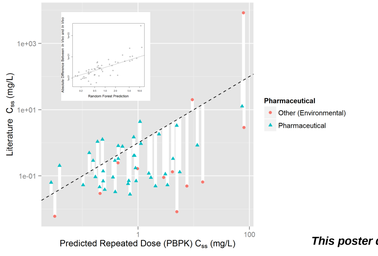


- To date we have been using a steady-state calculation assuming an infusion dose. In part, this is because we do not have an estimate of chemical partitioning to allow us estimate a volume of distribution.
- We have developed a general PBPK model (**at right)** that can be parameterized using *in vitro* hepatocyte clearance and plasma binding data, physico-chemical properties (hydrophobicity, dis/association constants from experiment or QSAR) and Schmitt's method for tissue partitioning[7]
- Perfusion-limited (free concentration in tissue equals free concentration in plasma)
- In the **figure at the left** we find that the previously used infusion model tends to over-predict C_{ss}, especially for rapidly metabolized compounds
- Eventually we would also like to know absorption rate and bioavailability to further improve these PBPK models.



- We have a small amount of *in vivo* PK data on C_{ss} from various sources, including a few environmental chemicals[10] and a large number of pharmaceuticals[6].
- This data serves as our "ground truth" for evaluation. Drawing from the literature we can compare how well the infusion model does for predicting actual human C_{ss}.
- In the **figure at the right** we can see that predictive ability is limited (R^2 ~0.23)
- Using the Random Forests method[1] we can build a statistical model for the residuals (the difference between the observation and the prediction):
  - The current residual model (**inset**) has an R^2 of ~0.56
  - We can use this predicted error for chemical-specific estimate the accuracy of HTPK.
- Note that although we are calculating "steady-state values," we do not include any metabolic induction and so these results (literature and predicted) are chronic extrapolations from acute conditions.

Random Forest Model for Errors



PK Triage for Environmental Chemicals

PK Data	PK Prediction	Confidence	Domain of Applicability	Targeted Chemicals
In vitro clearance of parent compound by hepatocytes and binding to plasma protein	Steady-State serum concentrations from Constant Infusion Exposure (empirical PK model)	If comparison with predicted exposure indicates large activity:exposure ratio (AER) then infusion Css should be sufficient (*i.e* conservative due to rapid metabolism)	Soluble non- or semi-volatile compounds, highly plasma protein bound chemicals are challenging	All chemicals amenable to *in vitro* assay, use QSAR for rest
Physico-chemical properties in conjunction with *in vitro* binding data allows prediction of partition coefficients	Steady-State from repeated dosing (generic PBPK model) & volume of distribution from sum of tissues	If comparison with predicted exposure indicates large AER then HTPBPK should be sufficient (*i.e.* conservative due to 100% absorption)		All chemicals with successful binding in vitro assay, use QSAR for rest
In vivo oral and *iv* dose PK study, serum only	Oral bioavailability, absorption rate, volume of distribution, and clearance (refined PBPK model)	If volume of distribution from HTPBPK model and in vitro estimate of clearance hold, then refined PBPK model should be excellent		Diverse members of descriptor space to sufficient to evaluate predictions from *in vitro* methodology, use QSAR for rest
QSARs for likely transporter substrates	Assumptions of perfusion-limited tissue partitioning and passive renal excretion by glomerular filtration may be questionable for chemicals that are actively transported–steady-state serum predictions only.	QSAR-defined (e.g. training set, extrapolation methodology)		All chemicals with known structures
Identification of atypical chemical structure	All assumptions must be questioned			All chemicals with known structures
CYP-specific *in vitro* data	Population variability, life-stage variability	Variation in CYP expression are well characterized, so predictions should be better, but low metabolizers will have higher tissue concentrations	Soluble non- or semi-volatile compounds	Chemicals with AER near one for general population
Transporter-specific *in vitro* data	Tissue-specific accumulation, active secretion/resorption in kidney		Soluble non- or semi-volatile compounds	Chemicals indicated by QSAR

Conclusion

•Non-detects in the in vitro protein binding assay (*i.e.* highly bound chemicals) may result in an artifactual underestimation of C_{ss} values, which in turn may overestimate the Activity:Exposure Ratio.

• As shown here, Monte Carlo approaches for PK uncertainty and variability must be able to handle censored (limit of detection) measurements

• Poor correlation between infusion dosing and PBPK models – generally infusion C_{ss} overestimates exposure, so this is a conservative assumption

• Poor correlation between C_{ss} from *in vitro* data and *in vivo* studies, **BUT** we can model the residuals using physico-chemical properties, and in *vitro* data

• Chemicals with low predicted residuals are within the domain of applicability of HTPK approaches

• Can add the predictions of transporter QSARS

• Need more environmental chemicals for statistical evaluation

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