Applying a High-Throughput PBTK Model for IVIVE

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Abstract

Motivation:

- High throughput screening (HTS) assays used in the ToxCast and Tox21 projects provide *in vitro* bioactivity concentrations that may inform *in vivo* toxicity; accurate dosimetry information may be beneficial.
- Toxicokinetics (TK) accounts for absorption, distribution, and metabolism of chemicals in the body.
- Evaluating the application of TK for *in vitro-in vivo* extrapolation (IVIVE) may enable the use of data from HTS assays to inform regulatory decisions.

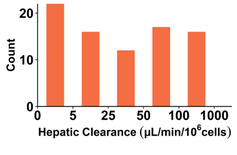
Methods:

- New data for rat-specific high-throughput toxicokinetic (HTTK) parameters of **intrinsic clearance (Cl_{int})** and **fraction unbound in plasma (f_{up})** were measured *in vitro.*
- A high-throughput, physiologically based toxicokinetic (HT-PBTK) model was used to determine predicted *in vivo* concentrations from *in vivo* doses associated with toxicological endpoints in rat.
- Regression analysis compared the strength of the correlations of:
  a) in vitro toxicity data vs unadjusted *in vivo* **dose**
  b) in vitro toxicity data vs predicted *in vivo* **concentration** calculated using the **HT-PBTK** model

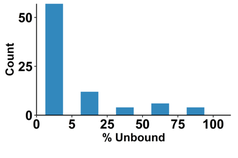
Conclusions:

- **HT-PBTK strengthens the correlation between *in vitro* and *in vivo* toxicity data.**
- The effect of assumptions in application of HT-PBTK (clearance, *in vivo* concentration selection, *in vitro* assay well models) on performance of the model demonstrated that assuming **nonrestrictive clearance improved performance** of the application of the HT-PBTK model for IVIVE.

In vitro TK parameters



- Chemicals at 1 μM and 10 μM incubated for 240 mins with primary rat hepatocytes (Wetmore et al. 2013)



- The rat based f_{up} was measured using rapid equilibrium dialysis (Wetmore et al. 2013)

References:
Armitage, J. M. *et al. Environ. Sci. Technol.* 2014, 48, 9770-9779
Fischer, F. C. *et al. Chem. Res. in Toxicol.* 2017, 30, 1197-1208
Martin, *et al. Environ. Health Perspect.* 2009, 117, 392-399
Pearce, R. G. *et al. J. Statistical Software*. 2017, 79(4)
Wetmore, B. A. *et al. Toxicological Sciences*. 2013, 132(2), 327-346

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Analysis Workflow

***In vivo* dose** – rat data, two separate analyses (effect level and TPOD)

Effect level
- **ToxRefDB** v1.3 (Martin et al. 2009)
- lowest doses where a particular effect was observed (*e.g.* body weight gain, nonneoplastic liver observations, etc.)
- Subchronic, chronic, and developmental studies

85 chemicals with ToxRef and HTTK data

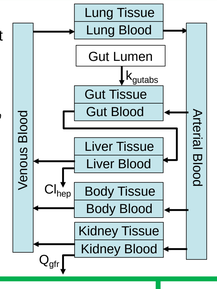
ToxVal point of departure (TPOD)
- minimum LOEL or LOAEL for a given chemical from the ToxVal database (https://comptox.epa.gov/dashboard/)
- minimum taken across acute, subchronic, chronic, and developmental studies

101 chemicals with ToxVal and HTTK data

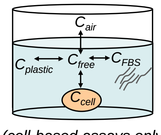
PBTK modeling result

PBTK model
part of the *httk* R package (Pearce et al. 2017)
- **Hepatic clearance**
  - restrictive – dependent on f_{up}
  - nonrestrictive – independent of f_{up}

Predicted *in vivo* concentration
- **Phase**
  - Total or free (unbound)
- **Location**
  - venous plasma or tissue
- **Value**
  - C_{mean} (i.e. AUC/time) or C_{max}



- **Assay well models** for predicting free concentration *in vitro*
  - Armitage *et al.* (2014) or Fischer *et al.* (2017)



(cell-based assays only)

Randomized result

- Perform modeling parameterized by permuted vector of chemicals
- Repeat 10x (subsequent analysis performed separately for each randomized result, medians reported)

In vitro toxicity data

- ToxCast (ftp://newftp.epa.gov/comptox/High_Throughput_Screening_Data/Summary_Files)
- Concentration at 50% maximum activity (AC_{50}) with active hit call and no quality control flags

concentration | concentration | dose divided by MW

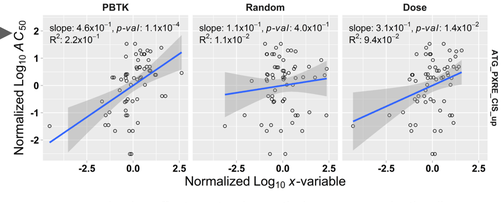
Regression Analysis

For every assay and *in vivo* effect:
- **Univariate** regressions to get relevant statistical parameters (slope, p-value, R^2, etc.)
- **Multivariate** regressions to determine most strongly correlated predictor (PBTK, Random, or Dose) based on largest coefficient

$$y_{AC50} \sim \beta_{PBTK} x_{PBTK} + \beta_{Random} x_{Random} + \beta_{Dose} x_{Dose}$$

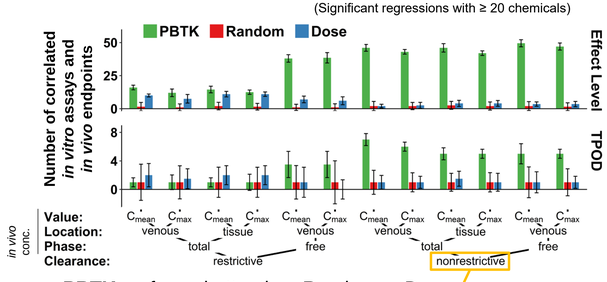
Coefficients			p-values		
PBTK	Random	Dose	PBTK	Random	Dose
0.49	-0.09	0.06	< 1.0×10^{-4}	5.0×10^{-1}	6.8×10^{-1}

Count the number of times each predictor is the most strongly correlated and the corresponding simple regression is significant (*p-value* < 0.05)

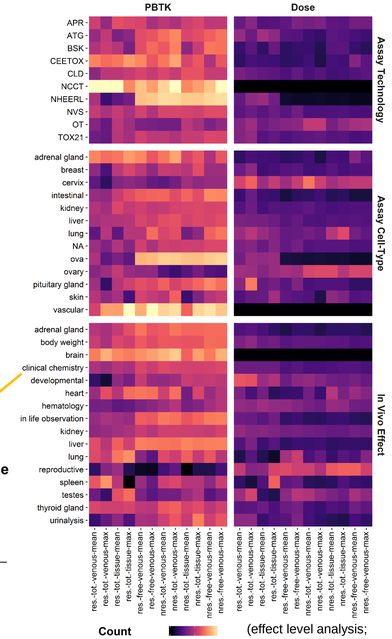


in vivo effect: systemic - pathology nonneoplastic - liver
(comparison vs a single randomized result)

Effect of HT-PBTK on correlation of *in vitro* and *in vivo* toxicity data



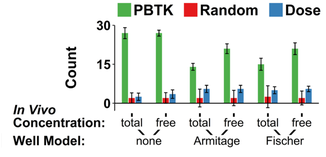
- **PBTK** performs better than Random or Dose
- Performance improves with **nonrestrictive clearance**
- Using predicted **free *in vivo* concentration** may provide some benefit, but this is less pronounced with nonrestrictive clearance
- Performance varies slightly depending on **data source** (assay technology/source, assay cell-type, *in vivo* effect)



(effect level analysis; ≥ 5 chemicals and *p-value* < 0.05)

Effect of assay well model:

- No improvement with use of *in vitro* C_{free} from assay well partitioning model
- Results may change with more detailed data of media and well composition



(effect level analysis; C_{mean}, venous plasma, nonrestrictive clearance)

Summary

- A high-throughput PBTK model was used to determine *in vivo* concentrations from external doses
- **New rat based data for Cl_{int} and f_{up} were measured** and incorporated in the model
- The application of HT-PBTK was evaluated by a regression analysis
- Results show that, although not necessarily predictive of toxicity, **HT-PBTK strengthens the correlation between *in vitro* and *in vivo* toxicity data**, particularly when assuming **nonrestrictive clearance**
- This suggests that TK with nonrestrictive clearance should be used when developing ensemble models to predict toxicity from HTS results