National Institute of Environmental Health Sciences
Division of Translational Toxicology

In Vitro to In Vivo Extrapolation to Facilitate the Animal-free Risk Assessment of Potential Developmental Toxicants

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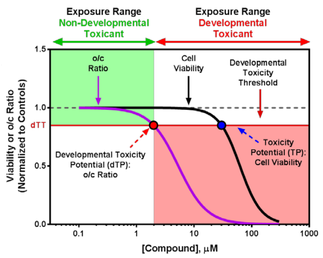
Introduction

- In vitro assays can provide insight on safe exposure levels when combined with in vitro to in vivo extrapolation (IVIVE). IVIVE uses pharmacokinetic (PK) models to relate chemical-specific in vitro activity concentrations to in vivo exposure levels that could result in corresponding human or animal adverse effects.
- The devTOX *quick*Predict™ (devTOX^{qP}) assay is a human induced pluripotent stem cell (iPSC)-based assay that has been used to evaluate potential developmental toxicity of Tox21 chemicals. The assay has been adapted to a high-throughput screening platform.
- In this study, IVIVE was performed to translate the developmental toxicity potential (dTP) concentration in the devTOX^{qP} assay to a corresponding equivalent administered dose (EAD). The resulting EADs were compared to rat oral adverse effect levels for developmental toxicity.
- The impacts of in vitro kinetics and different PK models on EAD estimates were assessed to identify the PK model providing EADs that most closely approximate in vivo effect levels.

Data and Pharmacokinetic Model Inputs

In Vitro Assay Data

- The devTOX^{qP} assay is a biomarker-based human pluripotent stem cell assay for developmental toxicity testing (Stemina Biomarker Discovery, Inc.) (Palmer et al. 2017).
  - The assay measures changes in concentrations of the amino acids ornithine and cystine following exposure, represented as ornithine to cystine (o/c) ratio.
  - The o/c ratio is associated with developmental toxicity and is used for deriving the developmental toxicity potential (dTP) concentration.
  - Values of dTP concentrations used for IVIVE included:
    - Single value
    - Median value of replicates
    - Maximum tested concentration (if testing results were negative)



  - All dTP concentrations were determined using human iPSCs (lines HYR0103 & DYR0100; ATCC).

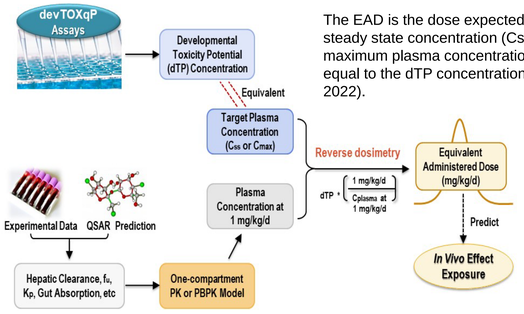
In Vivo Data from Literature

- Lowest observed (adverse) effect levels (LO(A)ELs) from rat developmental toxicity studies were obtained for 109 chemicals.
- No observed (adverse) effect levels (NO(A)ELs) from rat developmental toxicity studies were obtained for 39 chemicals.

Input Parameters for PK Models

- Most physicochemical and absorption, distribution, metabolism, and excretion (ADME) parameters were provided as predictions by the OPERA model (v 2.7, Mansouri et al. 2018).
- Parameters predicted by OPERA included octanol-water partition coefficient (LogP), negative $\log_{10}$ of the acid dissociation constant (pKa), fraction unbound to plasma protein (fu), and intrinsic clearance (Cl_{int}).
- Other parameters needed for the physiologically based pharmacokinetic (PBPK) model were obtained from the U.S. Environmental Protection Agency (EPA) httk (high-throughput toxicokinetics) R package (v 2.1.0, Pearce et al. 2017)
- Parameters predicted from the httk package included uptake rate of chemical from the gut and tissue:plasma partition coefficients of various tissues (e.g., lung, liver, gut, kidney, rest of body).

Predicting In Vivo EAD Using In Vitro Activity Concentration



The EAD is the dose expected to lead to the steady state concentration (Css) or maximum plasma concentrations (Cmax) equal to the dTP concentration (Chang et al. 2022).

Structures of PK Models for IVIVE

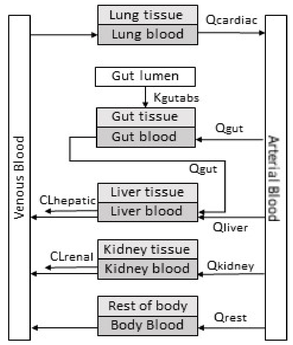
PPK: One-compartment Population-based PK Model

$$C_{ss} = \frac{\text{Standard oral dose rate (1 mg/kg/day)}}{(\text{GFR} * f_u) + \left(\frac{Q_{liver} * f_u * CL_{int}}{Q_{liver} + f_u * CL_{int}}\right)}$$

- The PPK model estimates the upper 95th percentile Css following a given dose for a Monte Carlo simulated population that accounts for interindividual physiological variability (Wetmore et al. 2012).
- The Httk.PBTK model estimates the dynamic plasma and tissue concentration following a given dose.
- Both models are used to calculate EADs that would lead to the total Css or Cmax equal to the dTP concentration of the devTOX^{qP} assay:

$$EAD = In\ Vitro\ Effective\ Conc \times \frac{1}{C_{ss}} (mg/kg/day)$$

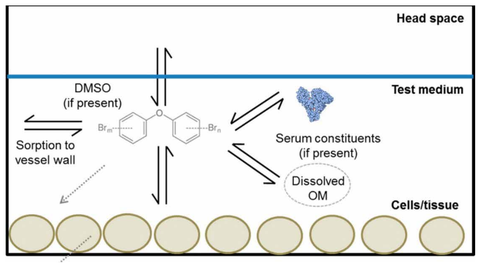
Httk.PBTK: Physiologically Based Toxicokinetic Model



From EPA httk R package (ver 2.1.0; Pearce et al. 2017)

BW, body weight; CL_{Renal}, renal clearance; $CL_{Hepatic}$, hepatic clearance; CL_{int}, intrinsic clearance; Css, steady-state plasma concentration; fu, fraction unbound to plasma protein; GFR, glomerular filtration rate; Q, blood flow rate.

In Vitro Cell-based Assay System



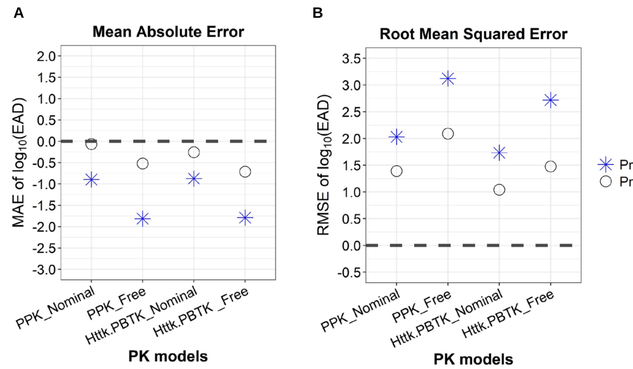
(Figure is from Armitage et al. 2014)

Equilibrium distribution model describing the mass distribution of chemical in an in vitro assay (Armitage et al. 2014). Using the Armitage model incorporated in the httk package and devTOX^{qP} assay technical specifications, we calculated free medium concentrations based on the nominal concentration. Both free and nominal concentrations were used for IVIVE.

Results and Discussion

- Across all modeling approaches, the EADs were lower than LO(A)ELs for at least 66% of chemicals and the EADs were lower than rat NO(A)ELs for at least 54% of chemicals. The PBTK model with free concentration increased this to EADs below LO(A)ELs and NO(A)ELs for 82% and 80% of chemicals, respectively. These observations suggest that the devTOX^{qP} assay may provide more conservative hazard estimates for use in risk assessment than rat toxicity studies.
- Using free medium concentration produced lower EADs in general, which are more conservative than those obtained using the nominal concentration. However, using free medium concentration did not necessarily improve the overall prediction accuracy for in vivo effect levels, indicating a need to further characterize the conditions for which this adjustment should be applied.
- Overall, the Httk.PBTK model using nominal dTP concentration produced the most accurate predictions of in vivo lowest effect levels. The difference between EAD estimates and LO(A)ELs was within 10-fold for 55% of chemicals and within 100-fold for 73% of chemicals. The difference between EAD estimates and NO(A)ELs was within 100-fold for 97% of chemicals. However, the maximum concentrations tested were used when predicting NO(A)ELs, which may not necessarily represent a true prediction.
- In summary, the devTOX^{qP} assay in combination with IVIVE approaches can predict rat developmental toxicity effect levels with reasonable accuracy, further supporting the utility of IVIVE in using relevant in vitro assay data to predict in vivo toxic effect levels.
- This study also provides a good example of how to apply IVIVE in a high-throughput context.

Comparison of PK Models With or Without In Vitro Kinetic Adjustment

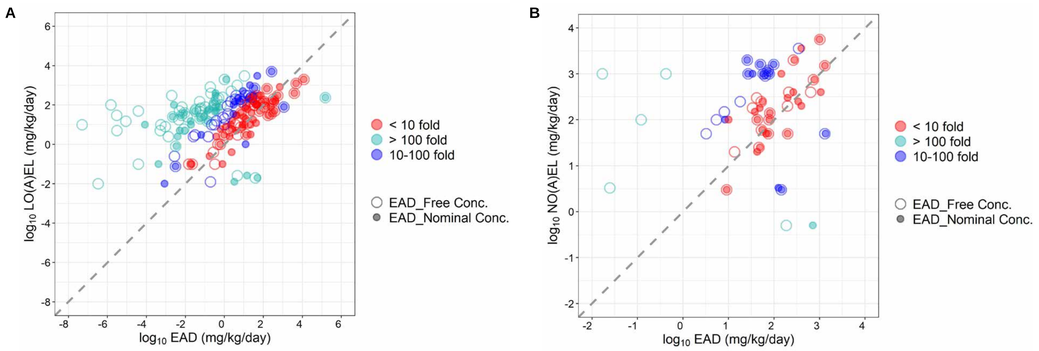


The figure presents an evaluation of overall performance of PK models across all chemicals in the set. Differences between $\log_{10}$ values of EADs and LO(A)ELs or NO(A)ELs from rat developmental toxicity studies were evaluated using root mean squared error (RMSE) and mean absolute error (MAE).

- RMSE is a standard statistical metric used to measure errors between actual and predicted values.
- MAE can inform on directional bias of error, i.e., over- or under-prediction of in vivo effect levels overall.

Free: free medium concentration used for IVIVE (calculated from nominal dTP concentration using Armitage model); Nominal: nominal dTP concentration used for IVIVE.

Correlation Between Predicted EADs and LO(A)ELs or NO(A)ELs



Plots show EAD values estimated from dTP concentrations using the Httk.PBTK model compared to in vivo LO(A)EL (plot A) or NO(A)EL (plot B) values. For both plots, the colors of the open (free) or solid (nominal) circles represent different ranges of fold differences between EAD and NO(A)EL or LO(A)EL values. Conc., concentration.

	EAD Compared to LO(A)EL: Percentage of chemicals			EAD Compared to NO(A)EL: Percentage of chemicals		
	EAD < LO(A)EL	<10-fold difference	<100-fold difference	EAD < NO(A)EL	<10-fold difference	<100-fold difference
PPK + Nominal conc.	66.1% (72/109)	43.1% (47/109)	71.6% (78/109)	53.8% (21/39)	74.4% (29/39)	84.6% (33/39)
PPK + Free medium conc.	75.2% (82/109)	30.3% (33/109)	54.1% (59/109)	59.0% (23/39)	66.7% (26/39)	82.1% (32/39)
Httk.PBTK + Nominal conc.	74.3% (81/109)	55.0% (60/109)	72.5% (79/109)	64.1% (25/39)	64.1% (25/39)	97.4% (38/39)
Httk.PBTK + Free medium conc.	81.7% (89/109)	32.1% (36/109)	55.0% (60/109)	79.5% (31/39)	48.7% (19/39)	87.2% (34/39)

The table summarizes the percentage of chemicals having EADs less than LO(A)EL or NO(A)EL values, and the percentage of chemicals with EAD less than 10- or 100- fold of LO(A)EL or NO(A)EL values. Ratios inside the parentheses indicate the number of chemicals used to calculate the ratios. The highest percentage across different model approaches is highlighted in red.

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