National Institute of Environmental Health Sciences
Division of Translational Toxicology

IVIVE to Facilitate Animal-free Risk Assessment of Potential Developmental Toxicants

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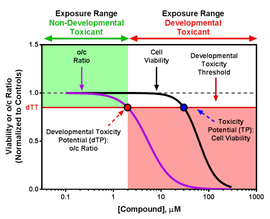
Introduction

- 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 exposures that could result in human or animal adverse effects.
- The devTOX quickPredict™ (devTOX^{qP}) assay is a human induced pluripotent stem cell (iPSC)-based assay that has been used to evaluate potential developmental toxicity of a subset 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 when available.
- EADs were also compared to predicted human exposure levels when available.
- 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 (Stemina Biomarker Discovery, Inc.; Palmer et al. 2017).
- The assay endpoint is the ornithine-to-cystine (o/c) ratio: changes in media concentrations of ornithine and cystine.
  - The o/c ratio is associated with perturbations in cell differentiation and proliferation and is used to derive the developmental toxicity potential (dTP) concentration.
- Values of dTP concentrations used for IVIVE included:
  - Single value
  - Median value of replicates
  - Maximum tested concentration (for negative results)



In vivo data from literature

- Lowest observed (adverse) effect levels (LOAELs) and no observed (adverse) effect levels (NOAELs) from rat developmental toxicity studies were obtained from literature.
  - 109 chemicals were both active in devTOX^{qP} assay and have rat LOAELs.
  - 39 additional chemicals were negative in devTOX^{qP} assay and have rat NOAELs. The maximum tested concentration was used for IVIVE to predict NOAELs for these chemicals.

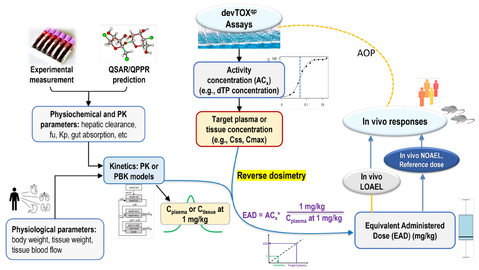
Predicted human exposure

- For another set of 109 chemicals, the population-level exposure predictions from the U.S. Environmental Protection Agency (EPA) Systematic Empirical Evaluation of Models (SEEM3) were available and obtained from the Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/).

Input parameters for PK models

- Most physicochemical and absorption, distribution, metabolism, and excretion (ADME) parameters were provided as predictions by OPERA v2.9 (Mansouri et al. 2018).
- Parameters predicted by OPERA included octanol-water partition coefficient (LogP), negative log10 of the acid dissociation constant (pKa), fraction unbound to plasma protein (fu), and intrinsic hepatic clearance (CLint).
- Other parameters needed for the physiologically based PK (PBPK) model were obtained from the EPA httk (high-throughput toxicokinetics) R package (v 2.2.2, Pearce et al. 2017).
- Parameters predicted by httk 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).

IVIVE: Putting In Vitro Activity Data into In Vivo Context



IVIVE applies PK models to estimate EADs that would result in plasma or target tissue concentrations equal to the in vitro activity concentrations (in this study, dTP concentrations) (Chang et al. 2022).

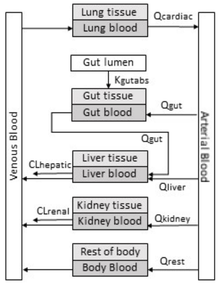
Models for In Vitro and In Vivo Kinetics

Pharmacokinetic (PK) models for simulating in vivo kinetics

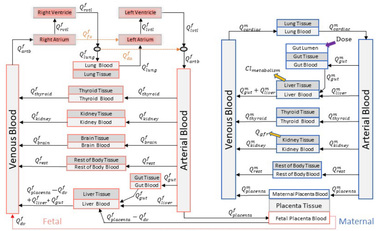
PPK: One-compartment population-based PK model

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

Httk.PBTK: physiologically based toxicokinetic model



Httk.fPBTK: pregnancy-specific PBTK model

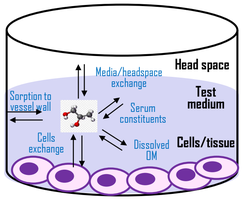


BW, body weight; CL_{Renal}, renal clearance; $CL_{Hepatic}$, hepatic clearance; CL_{int}, intrinsic clearance; Css, steady-state plasma concentration; fu, fraction unbound to plasma protein; GFR, glomerular filtration rate; Q, blood flow rate.

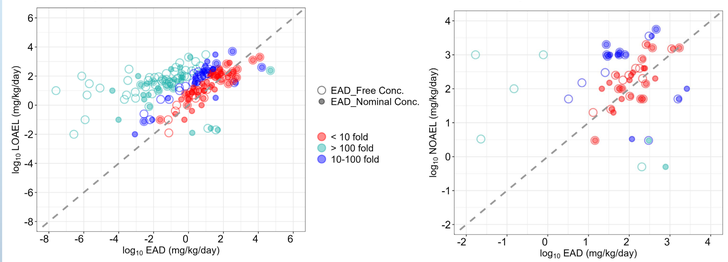
- The PPK model (upper left) estimates the upper 95th percentile Css following a given dose for a Monte Carlo-simulated population that accounts for interindividual physiological variability (Wetmore et al. 2012).
- Both PBTK models are from the EPA httk R package (v. 2.2.2; Pearce et al. 2017). The httk.PBTK model (right) estimates the dynamic plasma and tissue concentrations following a given dose. The Httk.fPBTK model (lower left) estimates the plasma and tissue concentrations in both the pregnant woman and fetus following a given dose.
- The models are used to calculate EADs that would lead to the plasma Css (for PPK model) or Cmax (for PBTK models) equal to the dTP concentration of the devTOX^{qP} assay.

Modeling kinetics for in vitro cell-based assay

The figure at right (adapted from Armitage et al. 2014) shows an equilibrium distribution model describing the mass distribution of a chemical in an in vitro assay. Using the Armitage model 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.



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



The plots above show EAD values estimated from dTP concentrations (Conc.) using the Httk.PBTK model compared to in vivo LOAELs (plot A, 109 chemicals) or NOAELs (plot B, 39 chemicals). In both plots, the colors of the open (free) or solid (nominal) circles represent different ranges of fold differences between EAD and LOAEL or NOAEL values. “Free” denotes that free medium concentration was used for IVIVE. “Nominal” denotes that nominal dTP concentration was used for IVIVE.

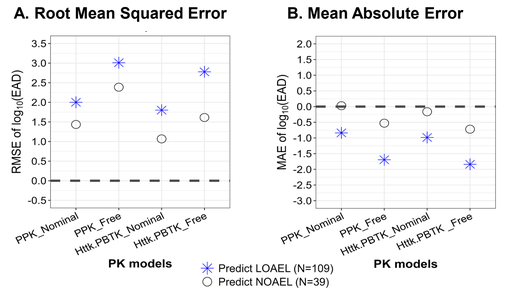
	EAD Compared to Rat LOAEL: Percentage of Chemicals			EAD Compared to Rat NOAEL: Percentage of Chemicals		
	EAD < LO(A)EL	<10-fold difference	<100-fold difference	EAD < NO(A)EL	<10-fold difference	<100-fold difference
PPK + Nominal conc.	66.1% (72/109)	44.0% (48/109)	69.7% (76/109)	48.7% (19/39)	69.2% (27/39)	84.6% (33/39)
PPK + Free medium conc.	74.3% (81/109)	34.9% (38/109)	56.0% (61/109)	53.8% (21/39)	61.5% (24/39)	79.5% (31/39)
Httk.PBTK + Nominal conc.	78.9% (86/109)	46.8% (51/109)	72.5% (79/109)	61.5% (24/39)	61.5% (24/39)	94.9% (37/39)
Httk.PBTK + Free medium conc.	84.4% (92/109)	32.1% (35/109)	53.2% (58/109)	71.8% (28/39)	51.3% (20/39)	84.6% (33/39)

The table summarizes the percentage of chemicals having EADs less than LOAEL or NOAEL values, and the percentage of chemicals with EAD less than 10- or 100- fold of LOAEL or NOAEL values. Ratios inside the parentheses indicate the number of chemicals used to calculate the ratios. The highest percentage across different model approaches for each comparison category is highlighted in red.

Results and Discussion

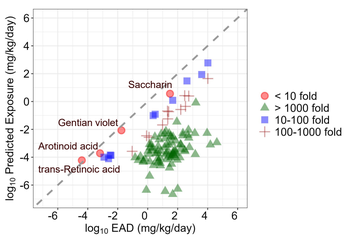
- Overall, the Httk.PBTK model using nominal dTP concentrations produced the most accurate predictions of rat LOAELs and NOAELs. The difference between EAD estimates and LOAELs was within 10-fold for ~50% of chemicals and within 100-fold for 73% of chemicals. The difference between EAD estimates and NOAELs was within 10-fold for 62% of chemicals and within 100-fold for 95% of chemicals.
- Across all modeling approaches, the EADs predicted using httk.PBTK model and free medium concentration were lower than rat LOAELs for > 80% of chemicals, suggesting using the devTOX^{qP} assay and this IVIVE method is a conservative approach for hazard estimates.
- Using free medium concentration produced lower EADs in general but did not necessarily improve the overall accuracy for predicting rat effect levels, indicating a need to further characterize the conditions for which this adjustment should be applied.
- For most chemicals, the predicted human exposure is far below the EADs estimated using the human gestational (httk.fPBTK) model, suggesting a low human exposure risk concern. However, a subset of chemicals was identified whose EAD values were within 10-fold of human exposure predictions.
- In summary, the devTOX^{qP} assay in combination with IVIVE methods can predict rat developmental toxicity effect levels with reasonable accuracy, further supporting the utility of IVIVE in using mechanistically relevant in vitro assay data to predict in vivo toxic effect levels.

Impact of In Vitro Kinetic Adjustment on IVIVE Outcome



The plots above show overall performance of PK models to predict LOAELs (109 chemicals) or NOAELs (39 chemicals) from rat developmental toxicity studies for each set of chemicals. Differences between log_{10} values of EADs and LOAELs or NOAELs 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.

Comparison of EADs to Predicted Human Exposure



The plot shows EAD values estimated using the human httk.fPBTK model compared to 95th percentile of predicted human exposure from the EPA SEEM3 model. For most chemicals, the predicted human exposure is far below the EADs.

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Acknowledgments

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