In Vitro to *In Vivo* Extrapolation for Developmental Toxicity Potency of Selected Tox21 Chemicals

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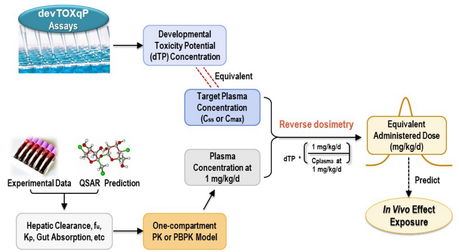
Introduction

- Selected Tox21 chemicals with the potential developmental toxicities have been tested with the devTOX *quick*Predict™ (devTOX^{qP}) assay.
- To support implementation of new approach methodologies for regulatory decision-making on developmental toxicity, the performance of the devTOX^{qP} assay for predicting the NO(A)ELs or LO(A)ELs in rat developmental toxicity studies needs to be evaluated.
- In this study, *in vitro* to *in vivo* extrapolation (IVIVE) was performed using the developmental toxicity potential (dTP) concentration from the devTOX^{qP} assay to estimate equivalent administered doses (EADs) that would result in the maximum plasma concentrations (Cmax) equivalent to the dTP concentrations **(Figure 1).** The resulting EADs were compared to rat NO(A)ELs or LO(A)ELs for developmental toxicity.
- The impacts of *in vitro* kinetics and different pharmacokinetic (PK) models on EAD estimates were also assessed.

Figure 1. Predicting *In Vivo* EAD Using *In Vitro* Activity Concentration



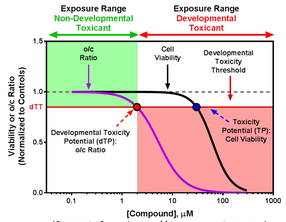
Data and Pharmacokinetics Model Inputs

In vitro assay data

- The devTOX^{qP} assay is a biomarker-based human pluripotent stem cell assay for developmental toxicity screening (Stemina Biomarker Discovery, Inc.) (Palmer et al. 2013, 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 to developmental toxicity and used for deriving the developmental toxicity potential (dTP) concentration.
  - Values of dTP concentrations are used for IVIVE include:
    - Single value.
    - Median value of replicates.
    - Maximum tested concentration (if testing results are negative).

In vivo data

- LO(A)EL, the lowest observed (adverse) effect levels in rat developmental toxicity studies.
- NO(A)EL, the no observed (adverse) effect levels in rat developmental toxicity studies



(figure is from https://www.stemina.com)

Model input parameters

- Physiochemical and ADME parameters were provided by OPERA model (v2.7) predictions (Mansouri et al. 2018):
  - The octanol-water partition coefficient (Log P_{ow}), negative $\log_{10}$ of the acid dissociation constant (pKa), fraction unbound to plasma protein (fu), intrinsic clearance
- Other parameters needed for the physiologically based pharmacokinetics (PBPK) model included:
  - Uptake rate of chemical from the gut.
  - Tissue:plasma partition coefficients of various tissues (e.g., lung, liver, gut, kidney, rest of body).

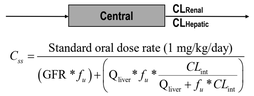
These values were obtained from the U.S. Environmental Protection Agency's httk (high-throughput toxicokinetics) R package (Pearce et al. 2017).

PK Models and In Vitro Kinetic Used in IVIVE

- **Figure 2** shows the structures of two PK models used in the IVIVE analysis.
- The open-source one-compartment population-based PK (PPK) model (**Figure 2A**):
  - Estimates the upper 95th percentile steady-state plasma concentration (Css) following a given dose for a Monte Carlo simulated population, which accounts for interindividual physical variability (Wetmore et al. 2012).
  - Calculates EADs that would lead to the total Css equal to the dTP concentration or maximum tested concentration from the devTOX^{qP} assay.
    - EAD corresponding to total chemical concentration: $EAD = In\ Vitro\ Effective\ Conc \times \frac{1}{C_{ss}} (mg/kg/day)$
- **Figure 2B** shows the *in vitro* kinetic open-source physiologically-based toxicokinetic (PBTK) model provided by the httk R package (Pearce et al. 2017).
  - This model was used to calculate EADs that result in a maximum plasma concentration (Cmax) corresponding to the *in vitro* concentration.
- **Figure 2C** shows the *in vitro* cell-based assay system. An equilibrium distribution model to describe the mass distribution of chemical in an *in vitro* assay was developed and incorporated into the httk R package (Armitage et al. 2014). Using the Armitage model, we calculated free medium concentrations based on the nominal concentration.

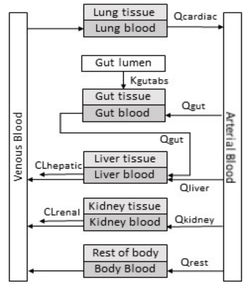
Figure 2. Structures of Kinetics Models Used in IVIVE Tool

A. PPK model

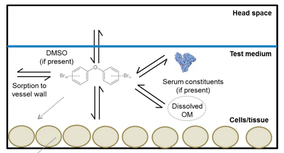


$$C_{ss} = \frac{\text{Standard oral dose rate (1 mg/kg/day)}}{(GFR * f_u) + \left(Q_{liver} * f_u * \frac{CL_{int}}{Q_{liver} + f_u * CL_{int}}\right)}$$

B. Httk.PBTK model



C. In vitro assay system



(figure is from Armitage, et al. 2014)

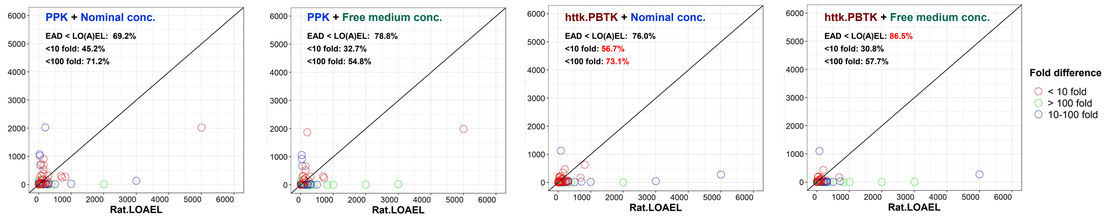
BW, body weight; CL_{Renal}, renal clearance; $CL_{Hepatic}$, hepatic clearance; CL_{int}, intrinsic clearance; fu: fraction unbound to plasma protein; GFR, glomerular filtration rate; Q, blood flow rate.

Results and Discussion

- Rat developmental toxicity NO(A)ELs were available for 39 chemicals and rat developmental toxicity LO(A)ELs were available for 104 chemicals.
- For all modeling approaches, the EADs were lower than rat NO(A)ELs for ≥ 54% of chemicals and the EADs were lower than LO(A)ELs for ≥ 70% of chemicals. These observations suggest that devTOX^{qP} assay may provide a more conservative hazard estimate for use in risk assessment
- Using free medium concentration produced lower EADs, which are more conservative, than the nominal concentration, but did not improve the overall prediction accuracy for *in vivo* effect levels, indicating a need for further characterization of conditions when this adjustment should be applied.
- Overall, the open-source, generalized httk.PBTK model using nominal dTP concentration produced the most accurate predictions for the *in vivo* effect levels. The fold difference between EAD estimates and LO(A)ELs were ≤10-fold for 57% of chemicals and ≤ 100-fold for 73% of chemicals. The fold difference between EAD estimates and NO(A)ELs was ≤ 10-fold for 64% of chemicals and ≤ 100-fold for 97% of chemicals.
- In summary, the devTOX^{qP} assay in combination with IVIVE approaches can predict rat developmentally toxic effect levels with reasonable accuracy, further supporting the utility of IVIVE in predicting *in vivo* toxic effect levels using relevant *in vitro* assays. This study also provides a good example of IVIVE application in a high-throughput context.

Figure 3. Scatter Plots for Rat EADs Predicted Using dTP or Maximum Testing Concentration and Rat LO(A)ELs or NO(A)ELs

A. Compared to Rat LO(A)ELs (104 chemicals)



B. Compared to Rat NO(A)ELs (39 chemicals)

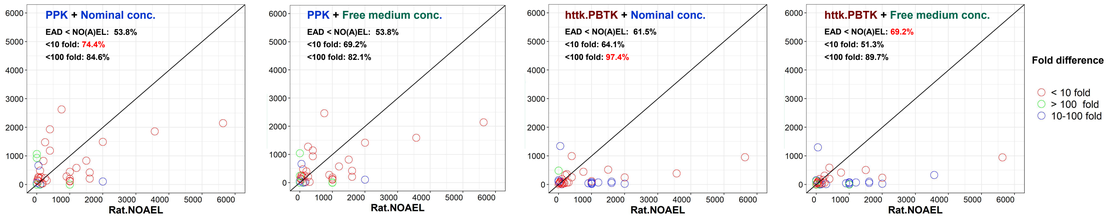


Figure 3. EAD values estimated from *in vitro* concentrations less than 3,000 µM are shown. The open circle **colors** represent different ranges of fold differences between EAD and NO(A)EL (**Fig. 3A**) or LO(A)EL (**Fig. 3B**). Font in red indicates highest % across different model approaches. Conc., concentration; EAD, equivalent administered dose corresponding to the *in vitro* concentration; LO(A)EL, the lowest observed (adverse) effect level in rat developmental toxicity studies; NO(A)EL, the no observed (adverse) effect level in rat developmental toxicity studies.

Figure 4. Performance Comparison Between PK models

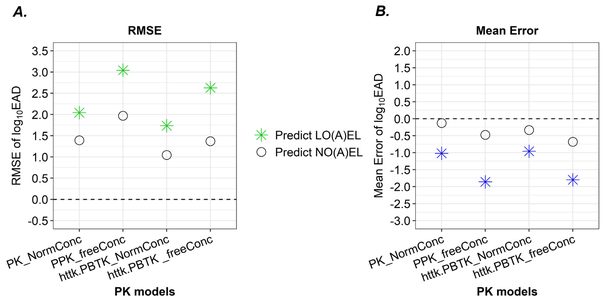


Figure 4. The overall performance of two PK models with nominal or free medium concentration across all chemicals in the set was evaluated using the root mean squared error (RMSE) (**A**) and mean error (**B**) between log10 values of EAD and LO(A)ELs or NO(A)ELs from rat developmental toxicity studies. RMSE is a standard statistical metric used to measure error between actual and predicted values. The mean error is used to inform directional bias of error, i.e., over- or under-prediction of *in vivo* effect levels overall. NormConc, nominal dTP concentration was used for IVIVE; freeConc, free medium concentration was used for IVIVE.

References

Armitage et al. 2014. Environ Sci & Tech 48(16).
Mansouri et al. 2018. J Cheminform 10(1):10.
Palmer et al. 2013. Birth Defects Res B Dev Reprod Toxicol 98(4): 343-63.
Palmer et al. 2017. Reprod Toxicol 73:350-361.
Pearce et al. 2017. J Stat Softw 79(4): 1-26.
Wetmore et al. 2012. Toxicol Sci 125:157-174.

Acknowledgements

We thank Catherine Sprankle, ILS, for editorial input. The Intramural Research Program of the National Institute of Environmental Health Sciences (NIEHS) supported this poster. Technical support was provided by ILS under NIEHS contract HHSN273201500010C.The views expressed above do not necessarily represent the official positions of any federal agency.

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