

Open-source Workflows for In Vitro to In Vivo Extrapolation

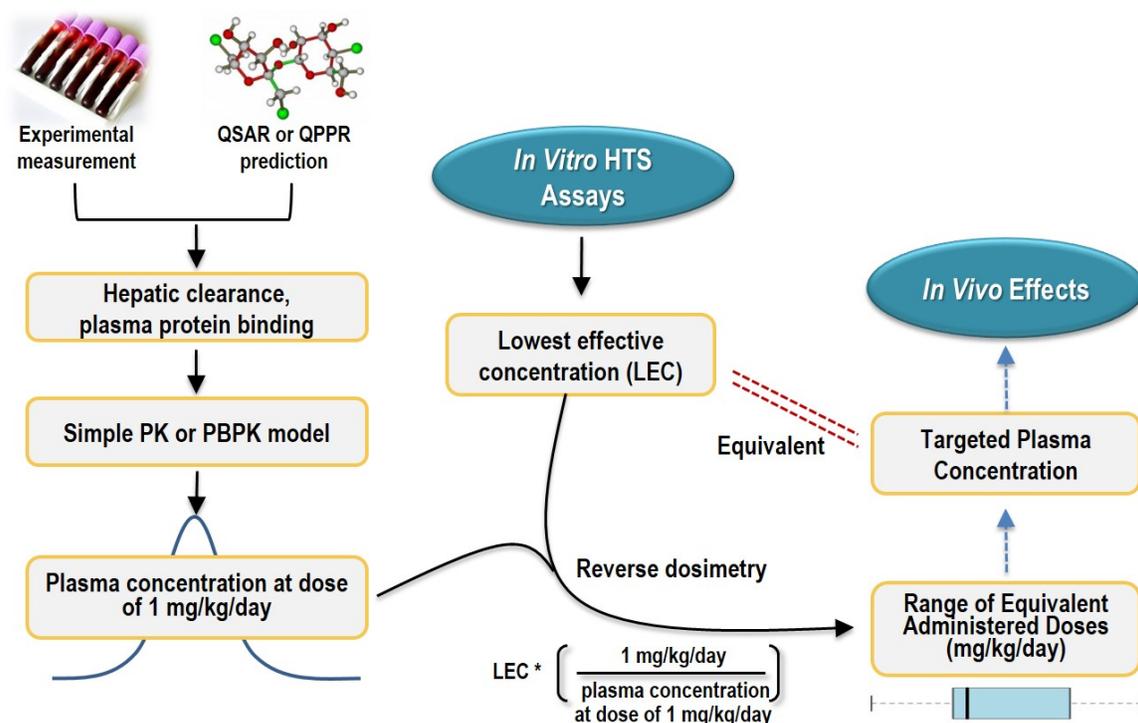
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Introduction

- In vitro to in vivo extrapolation (IVIVE) links in vitro assay results to in vivo effects. IVIVE estimates the daily in vivo equivalent administered dose (EAD) expected to produce a plasma or tissue concentration equivalent to an in vitro effective concentration (**Figure 1**).
- Pharmacokinetic (PK) and physiologically based PK (PBPK) models can link in vitro concentrations to in vivo doses, but many such models rely on proprietary software, which makes use of these models in regulatory and other applications difficult.
- Here we present workflows for IVIVE analysis using open-source PK models, and translate activity concentrations from high-throughput screening (HTS) assays into predicted doses for estrogenic activity and developmental toxicity as examples of the workflows' utility.
- To further extend the work, we adapted a published PBPK model for bisphenol A (BPA) written in a proprietary software to the open-source programming language R, for inclusion in future versions of the workflows.

Figure 1 A Reverse Pharmacokinetic Model for IVIVE

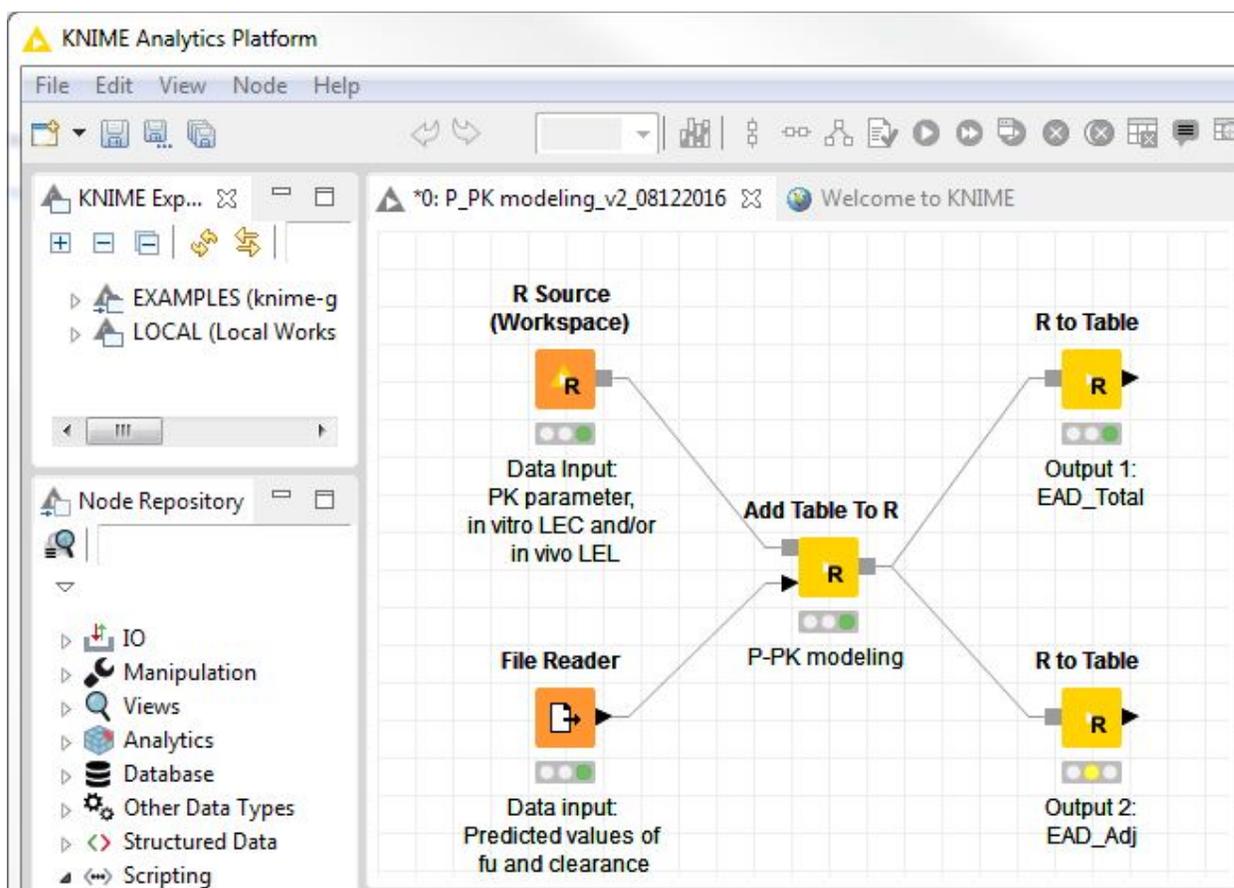


Abbreviations: HTS, high-throughput screening; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; QPPR, quantitative property-property relationship (Kirman et al. 2015); QSAR, quantitative structure-activity relationship;

Development of Open-source IVIVE Workflows

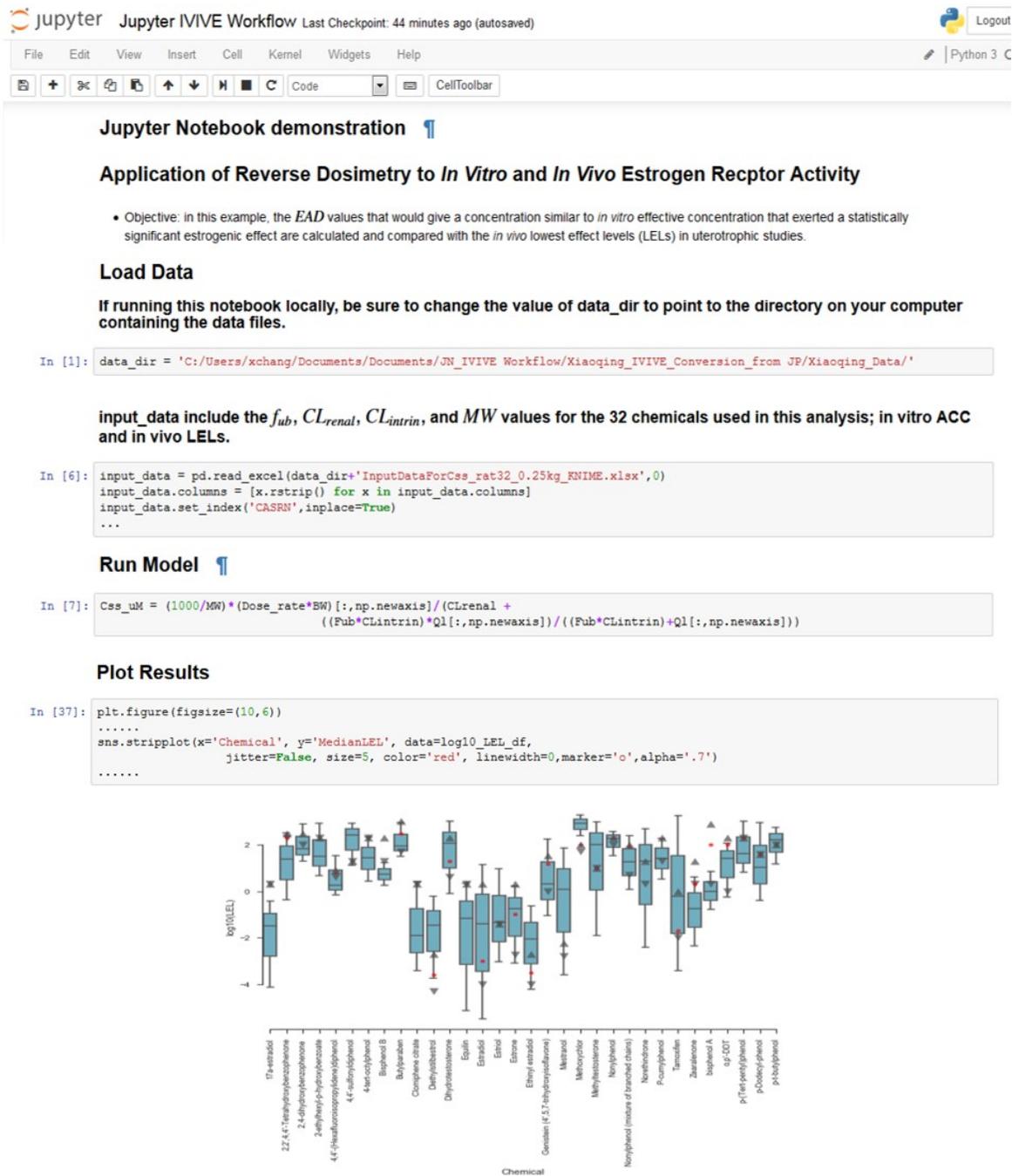
- We used two open-source platforms to develop an IVIVE workflow:
 - Konstanz Information Miner (KNIME) uses a modular data pipeline concept with a graphical user interface for data analysis and visualization (**Figure 2**).
 - Jupyter Notebook provides a web-based, interactive computing notebook environment accommodating both human-readable and executable documents in one file, giving the user more flexibility in editing the workflow to meet their own needs (**Figure 3**).
- The workflow uses a one-compartment rat population based PK (P-PK) model that incorporates Monte Carlo simulation to cover physical variability across a population of 10,000 individuals. The model predicts a daily equivalent administered dose that would lead to total (EAD_Total) or free (EAD_Adj) steady-state chemical blood concentration equivalent to the lowest effective concentration (LEC) in one or multiple in vitro assays (Wetmore et al. 2012; Chang et al. 2015; Chang et al. 2017).
- Required inputs for both IVIVE workflows include:

- In vitro data: may be expressed as LEC, half-maximal activity concentration (AC50), activity concentration at cutoff (ACC), or other concentration metric.
- PK parameters: fraction of chemical unbound to protein (f_u), intrinsic clearance, and renal clearance, which depends on f_u and glomerular filtration rate (**Figures 2 and 3**). In addition, for users that do not have their own PK parameter values, our KNIME workflow provides experimental values for 448 chemicals and values predicted from structure-based models for more than 7600 chemicals. The f_u values were predicted via a human quantitative structure-activity relationship (QSAR) model in the ADMET Predictor software package (v. 7.2) (Simulations Plus, Inc.). The intrinsic clearance values were predicted from a published quantitative property-property relationship (QPPR) model using octanol-water and water-air partition coefficients (Kirman et al. 2015).
- The output of both workflows are EAD values compared to corresponding *in vivo* lowest effective levels (LELs).

Figure 2 KNIME Workflow

The KNIME workflow uses five modules: two input modules, one modeling module containing the P-PK model, and two output modules. The user executes each module by clicking the module tab. The bar under each module tab shows the execution status. EAD_Adj, equivalent administered dose that results in free fraction of plasma concentration equivalent to in vitro LEC; EAD_Total, equivalent administered dose that results in total plasma concentration equivalent to in vitro LEC; fu, fraction of chemical unbound in the plasma; LEC, lowest effective concentration (μM); LEL, lowest effective dose level (mg/kg); PK, pharmacokinetic; P-PK, population pharmacokinetic.

Figure 3 Jupyter Notebook IVIVE Workflow



The figure shows major components of the IVIVE workflow developed using Jupyter Notebook. The workflow document consists of plain language, such as text describing objectives, data types, analysis methods, as well as equations and figures. The document also contains executable Python script (i.e., grey-shaded area).

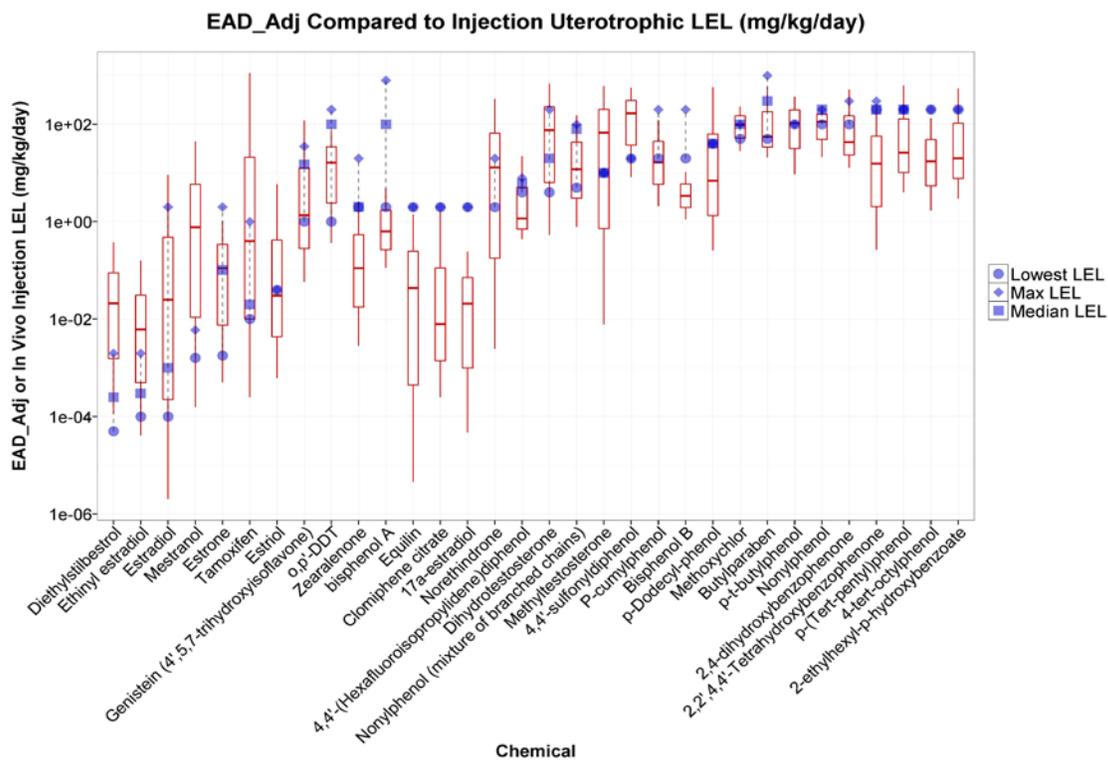
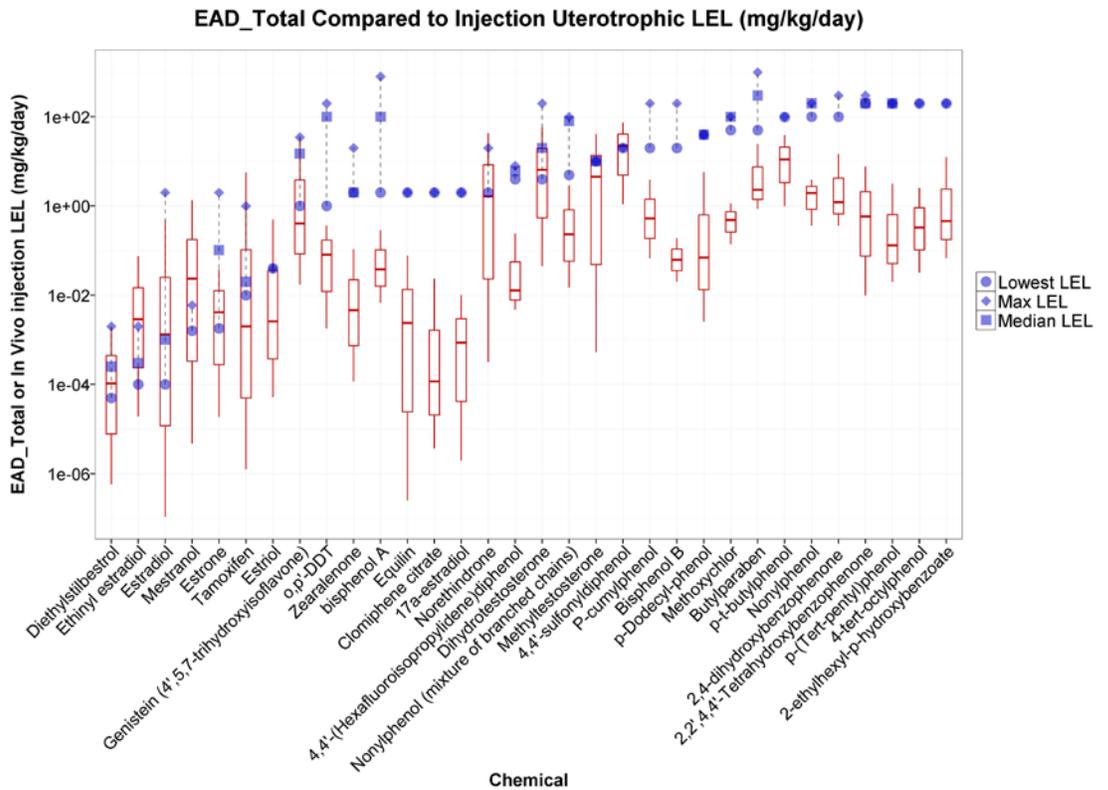
Examples Using the KNIME Workflow

- IVIVE workflow Example 1 (**Figures 3 and 4**)
 - Using the KNIME and Jupyter Notebooks workflows, IVIVE analyses were carried out on 32 chemicals known to have activity in the estrogen receptor (ER) pathway.
 - In vitro data: the ACC value was obtained for each chemical from the ToxCast October 2015 release for 16 assays that measure key events along the ER agonist pathway (Judson et al. 2015).
 - In vivo data: to cover the range of in vivo responses, for each estrogen-active chemical we calculated the lowest, median, and maximum LELs from guideline-like injection uterotrophic studies contained in a published database (Kleinstreuer et al. 2016a). These studies were considered guideline-like based on a set of minimum protocol criteria derived from test guidelines used by EPA and the Organization for Economic Co-operation and Development.
 - Results: the range of EAD_Adj estimates overlaid the range of LELs in injection uterotrophic studies for 26 of 32 chemicals. The EAD_Adj values underpredicted injection LELs for four chemicals (17alpha-estradiol, bisphenol A, bisphenol B, and zearalenone) and slightly overpredicted injection LELs for two chemicals (diethylstilbestrol, methoxychlor).
- IVIVE workflow Example 2 (**Figure 5**)
 - Using the KNIME workflow, IVIVE analyses were carried out on 35 chemicals with observed developmental toxicity potential in rats/rabbits based on in vivo prenatal guideline study data.
 - In vitro data: the ACC value was obtained for each chemical from the ToxCast October 2015 release for all the ToxCast/Tox21 assays that have a positive hit for any one of the 35 chemicals. Equivalent administered doses derived from assays relevant to the key developmental toxicity mechanisms of vascular disruption and estrogen/androgen pathway perturbation (90 assays) (Kleinstreuer et al. 2013; Tal et al. 2016; Judson et al. 2015; Kleinstreuer et al. 2016b) are plotted in red, and those from the remaining ToxCast/Tox21 assays (462 assays) are plotted in green.
 - In vivo data: we used rat/rabbit fetal LELs from EPA developmental toxicity studies retrieved from ToxRefDB (Knudsen et al. 2009).
 - Results: with the exception of three chemicals (cymoxanil, topramezone, and amitrole), the range of EAD_Total either overlapped or underpredicted the developmental LELs. All predicted doses were within two orders of magnitude of observed doses.

IVIVE Workflows Incorporating PBPK Models

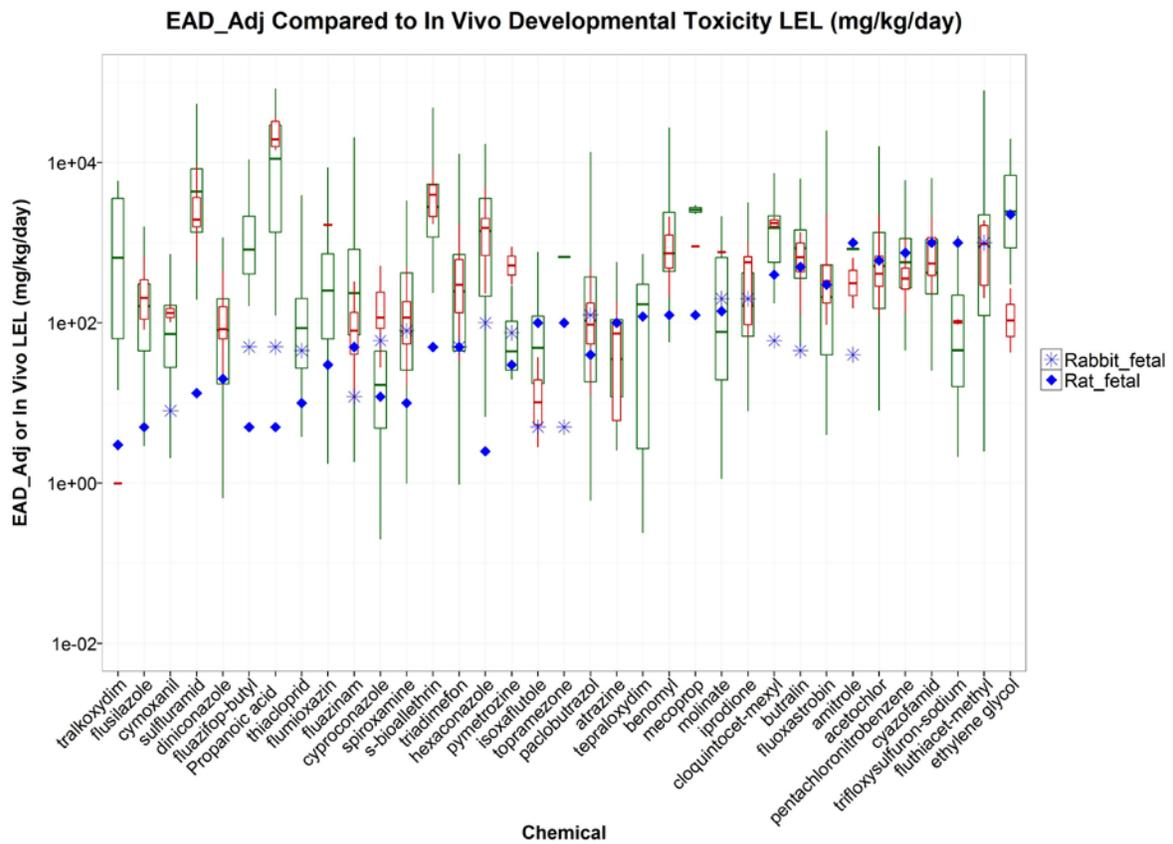
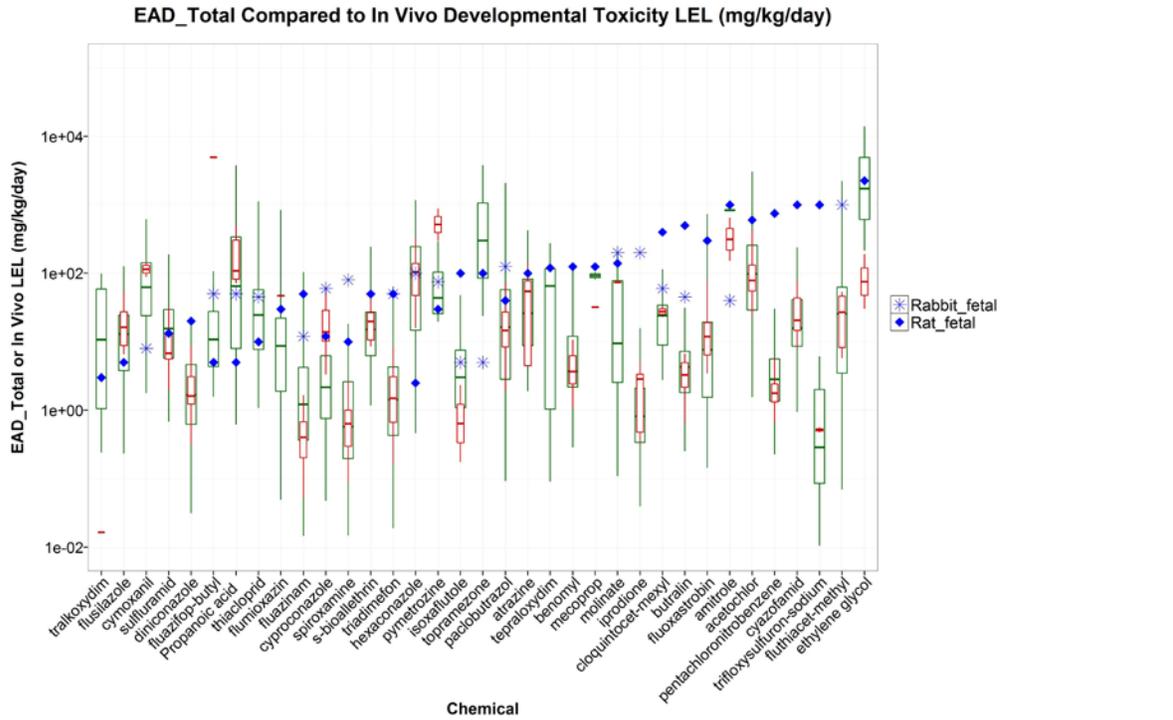
- Some applications of IVIVE require more complex representation of biology than that provided by the P-PK model described above. This more complex representation of biology can be provided by PBPK models, but these are often not available on open-source platforms.
- We used the R programming language (R Core Team 2013) to create an open-source version of a published PBPK model for bisphenol A (BPA) that incorporates glucuronidation (Yang et al. 2013), a key metabolic pathway for this chemical. This model was originally written in the proprietary software acslX, which is no longer supported.
- The open-source BPA PBPK model written in R was able to reproduce the results from the model built using the commercial software acslX in simulating concentration of both plasma BPA and BPA metabolite (**Figure 6**).
- We used the open-source PBPK model to carry out IVIVE analysis for BPA (**Figure 7**), and convert in vitro ACCs from the ToxCast/Tox21 ER pathway assays to estimate estrogenic doses in vivo. The P-PK model underpredicted LELs in uterotrophic studies for BPA, while the PBPK model gave more accurate predictions of these LELs for both injection and oral dosing (**Figure 7**).

Figure 4 IVIVE Analysis (P-PK model): ER Pathway Assays Compared to Uterotrophic LELs



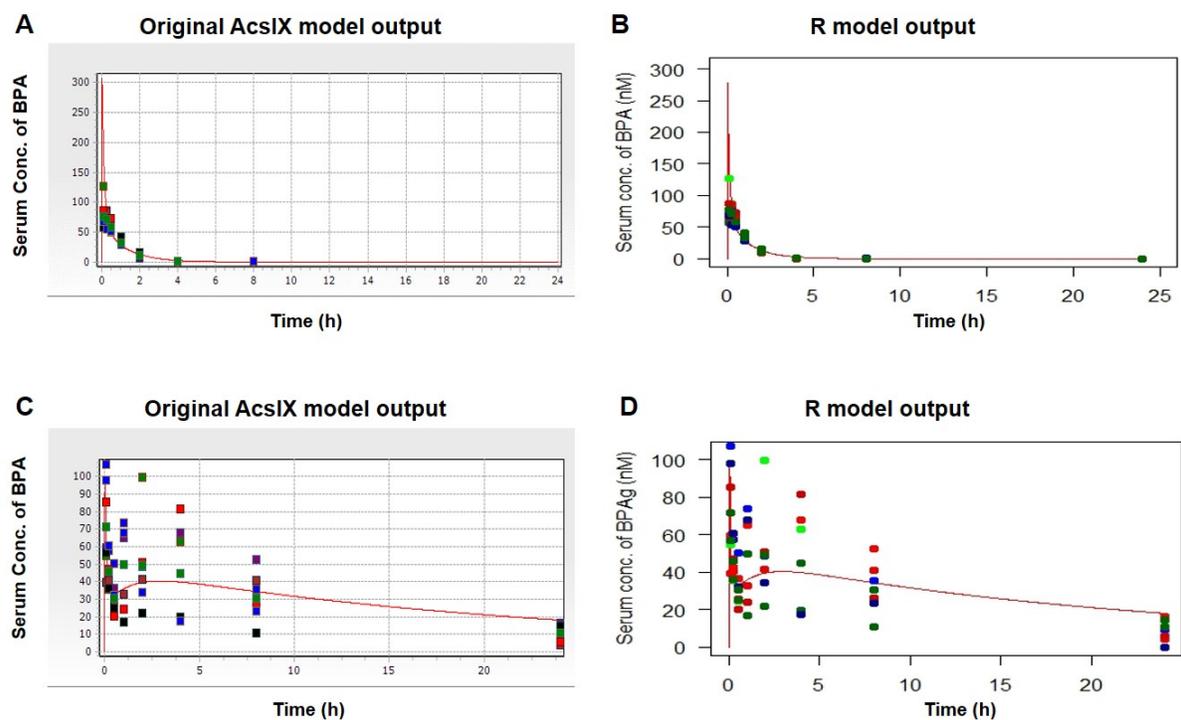
The figure compares estimated EAD_Total (A) and EAD_Adj (B) values from the KNIME workflow (mg/kg/day; red boxplots) to lowest, median, and maximum LELs (mg/kg/day; blue symbols and dashed lines) from injection uterotrophic studies. ACC, activity concentration at cutoff; EAD_Adj, daily equivalent dose that results in free chemical concentrations in the blood equivalent to corresponding ACCs; EAD_Total, daily equivalent dose that results in total chemical concentrations in the blood equivalent to corresponding ACCs; IVIVE, in vitro to in vivo extrapolation; LEL, lowest effect level; P-PK, one-compartment population pharmacokinetic.

Figure 5 IVIVE Analysis (P-PK model): ToxCast/Tox21 Assays Compared to Developmental LELs



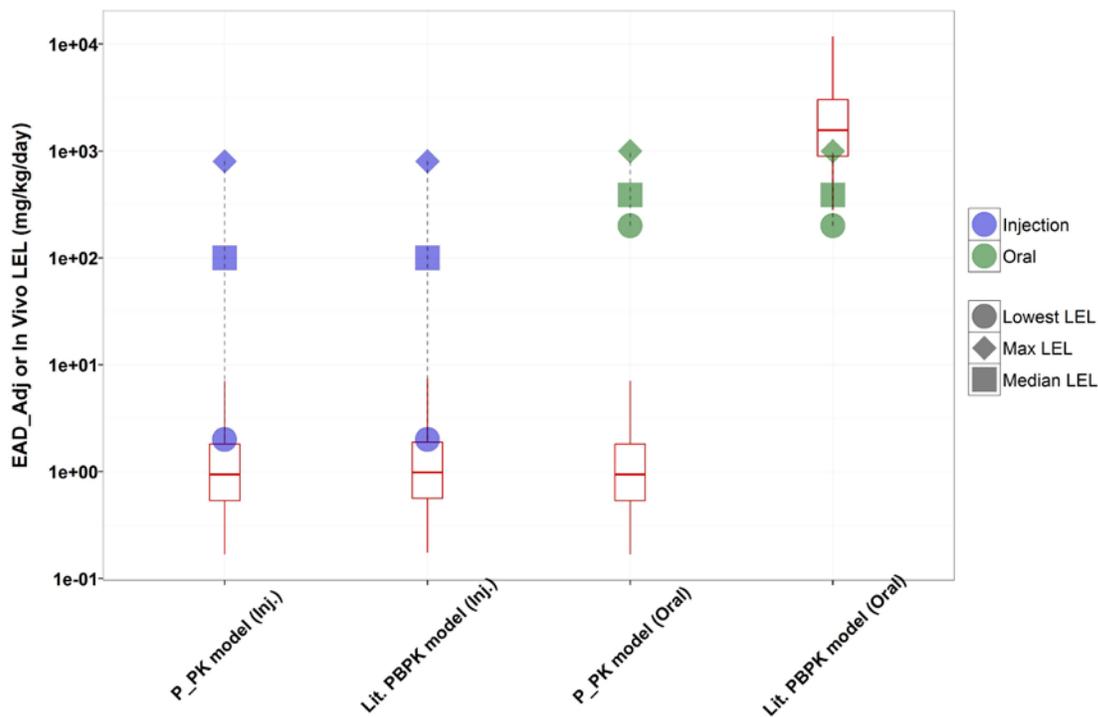
The figure compares estimated EAD_Total (A) and EAD_Adj (B) values from the KNIME workflow (mg/kg/day; boxplots) to LELs (mg/kg/day; symbols) of development and reproductive toxicity studies in ToxRefDB. EAD values derived from assays relevant to the key developmental toxicity mechanisms of vascular disruption and estrogen/androgen pathway perturbation are plotted in red; those from the remaining ToxCast/Tox21 assays are plotted in green. EAD_Adj, daily equivalent dose that results in free chemical concentrations in the blood equivalent to corresponding ACCs; EAD_Total, daily equivalent dose that results in total chemical concentrations in the blood equivalent to corresponding ACCs; IVIVE, in vitro to in vivo extrapolation; LEL, lowest effect level; P-PK, one-compartment population pharmacokinetic.

Figure 6 Simulation Result of BPA PBPK Model for BPA and BPA-g



Figures 6A and 6B compare time course simulation results of plasma concentration of BPA produced by the acsIX model and the open-source PBPK model built using R. Figures 6C and 6D provide a similar comparison for BPA-g. The symbols represent experimental measurements of serum concentration of BPA in each individual rat, while the red line represents the serum concentration of BPA or BPA-g predicted by the models. BPA, bisphenol A; BPA-g, glucuronidated bisphenol A; PBPK, physiologically based pharmacokinetic.

Figure 7 IVIVE Analysis Using PBPK Model for BPA
EAD_Adj Compared to In Vivo LEL (mg/kg/day)



The figure compares EAD_Adj values (red boxplot) estimated from the one-compartment P-PK model or the Yang et al. PBPK model to lowest, median, and maximum LELs from injection (blue symbols) or oral (green symbols) uterotrophic studies for BPA. BPA, bisphenol A, EAD_Adj, daily equivalent dose that results in free chemical concentration in the blood equivalent to corresponding activity concentrations at cutoff (ACCs); LEL, lowest effect level; P-PK, one-compartment population pharmacokinetic; PBPK, physiologically based pharmacokinetic.

Discussion and Conclusion

- Our open-source workflows provide a transparent and easy-to-use approach for IVIVE analysis.
 - The modularity and graphic interface of the KNIME platform facilitates integration with other cheminformatics workflows.
 - Jupyter Notebook provides an interactive user experience with more detailed explanations of the steps and impacts of parameters.
- The range of EAD estimates produced by these workflows correlated well with the range of in vivo LELs for the majority of chemicals tested, suggesting this IVIVE approach provides valid estimates of in vivo effective doses. The EAD_Adj appears to be more accurate when the in vitro assays and in vivo endpoints are both mapped to a specific biological pathway, but using EAD_Total and a broad suite of assays provides a more conservative estimate when predicting a complex endpoint driven by many potential mechanisms.
- In some cases, the lack of metabolism in in vitro assays included in our model could account for overpredicting the dose required to obtain the observed toxicity in vivo.
- The ER pathway assays provided more accurate estrogenic dose predictions in the uterotrophic assay when compared to the broader suite of ToxCast/Tox21 assays used to predict developmental toxicity doses. This is hardly surprising, and highlights the utility of further mechanistic characterization of complex toxicities and identification of biologically relevant assays to improve IVIVE analyses.
- Other chemical-type specific applications require more detailed biology to improve their performance; as a proof of concept, we successfully replicated the published BPA PBPK model using open-source software.
- IVIVE analysis of BPA using a published PBPK model performed better than predictions produced using a simple PK model, indicating that a specific metabolic component, likely glucuronidation, is critical for simulating pharmacokinetics of BPA.
- To improve prediction accuracy for BPA and/or BPA-like compounds, a PBPK model incorporating glucuronidation will be incorporated into the existing IVIVE workflow.

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