• The workflow output is an EAD value, which can be compared to in vivo effectiveness concentrations in the blood equivalent to ER activity. Required workflow inputs include:
- Development of QSAR models to predict biochemical, biophysical, and/or ADMET Predictor models to calculate intrinsic clearance, and PK modeling.
- Open-source software may be better suited for regulatory applications due to increased transparency.

■ Figure 3. Structure of Pharmacokinetic Models

- PK parameters, including fraction of chemical unbound to protein (fu), intrinsic clearance, renal clearance for the P-PK model, and partition coefficients (PCs) between blood and tissue.
- fu and HLC QSAR models were used in combination with an open-source program for the P-PK model and to predict intrinsic clearances based on a linear quadratic property relationship (PQPR) model (Kliman et al. 2011). The fu and HLC QSAR models were used to calculate the intrinsic clearance for the P-PK model.
- Experimental data was used for each parameter, ranging from 60-180 bpm, (potentially in a proprietary software), to calculate prediction performance (P-PK model). The nonlinear parameter sets were used to calculate the QSAR models.
- Model parameters:
  - Predicted values of fu, logPC, and HLC from our open-source P-PK model were used to calculate intrinsic clearance for each chemical.
  - In vitro ACC values from HTS assays targeted to each class of ER agonists were obtained from the ToxCast October 2015 release (Judson et al. 2015).
  - In vivo LLEs from guideline animal research studies were obtained from a published curated database (Kleinstreuer et al. 2016).

- Workflow output:
  - EAD_Adj, the daily equivalent dose that results in total chemical concentrations in the blood equivalent to ER pathway ACCs.
  - EAD_Tot, the daily equivalent dose that results in total chemical concentrations in the blood equivalent to the P-PK pathway.
  - Figure 4 shows that EAD_Adj (red box) predicted in vivo uterotrophic LLEs more accurately, while EAD_Tot (green box) provided more conservative estimates of uterotrophic activity. The range of EAD_Adj estimates overlapped the range of LLEs in rodent uterotrophic studies for 26 of 32 chemicals.

Discussion and Conclusion

- Our open-source, ready-to-use workflow provides a transparent and easily accessible approach for IVIVE analysis. They can be used to study relevant toxicity potential for thousands of chemicals in vivo state, expanding the safety assessment process.
- Predictions of input parameters for PK or PRN models generated by our QSAR models correlated well with measured data, and our models generated better predictions than proprietary software for all the parameters.
- When evaluating estrogenic activity, the range of EAD estimates produced by the workflow incorporating the P-PK model correlated well with the range in vivo uterotrophic LLEs for the majority of chemicals tested. This result suggests that our models could be used to generate valid estimates of in vivo estrogenic activity from in vitro pathway assays.
- Ongoing work is focused on developing generalized PBPK models that incorporate metabolic pathways, such as glucuronidation. Such models will be included in future versions of the KNIME workflow to improve prediction accuracy for phenolic compounds, for example.

Development of Open-source IVIVE Workflows

- Figure 2 shows the scheme of an IVIVE workflow we built using Ramboll Information Miner (Knime). The workflow incorporates:
  - A one-compartment population PK (P-PK) model (model 3A) that incorporates Monte Carlo simulation to cover realistic variability across individuals (Kliman et al. 2013).
  - A generalized three-compartment physiologically based pharmacokinetic (PBPK) model (figure 4B) providing open-source software may be better suited for regulatory applications due to increased transparency.
  - To facilitate IVIVE analysis with open-source tools, we developed an IVIVE workflow incorporating transparent quantitative structure-activity relationships (QSARs) to parameterize PK models of varying complexity.

- The workflow output is an EAD value, which can be compared to in vivo effectiveness concentrations in the blood equivalent to ER activity. Required workflow inputs include:
  - Development of QSAR models to predict biochemical, biophysical, and/or ADMET Predictor models to calculate intrinsic clearance, and PK modeling.
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- Figure 3. Structure of Pharmacokinetic Models

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- Ongoing work is focused on developing generalized PBPK models that incorporate metabolic pathways, such as glucuronidation. Such models will be included in future versions of the KNIME workflow to improve prediction accuracy for phenolic compounds, for example.

Development of QSAR Models to Predict Input PK Parameters

- We built QSAR models to predict input PK parameters: fu, Henry's law constant (HLC), and PCs between plasma and gut, kidney, and liver. The fu and HLC are the proportionality factors of Henry's law, which state that the amount of dissolved gas is proportional to its partial pressures in the gas phase. HLC is used to calculate water-air partition coefficient.

- The workflow output is an EAD value, which can be compared to in vivo effectiveness concentrations in the blood equivalent to ER activity. Required workflow inputs include:
  - In vitro activity, which can be expressed as lowest effective concentration (LEC), half maximal activity concentration (AC50), activity concentration at 50% (AC50), or other concentration metric.
  - PK parameters, including fraction of chemical unbound to protein (fu), intrinsic clearance, renal clearance for the P-PK model, and partition coefficients (PCs) between blood and tissue.

- fu and HLC QSAR models were used in combination with an open-source program for the P-PK model and to predict intrinsic clearances based on a linear quadratic property relationship (PQPR) model (Kliman et al. 2011). The fu and HLC QSAR models were used to calculate the intrinsic clearance for the P-PK model.
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References

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