Predictive Power of PBPK Modeling and 
in silico / in vitro - in vivo Extrapolation
Using GastroPlus™ and ADMET Predictor™ Software Tools

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Lancaster, CA
Outline

• **Introduction to GastroPlus mechanistic absorption and PBPK modeling**
  – prediction of volume of distribution
  – clearance inputs
  – *in vitro* – *in vivo* extrapolation

• **In silico – in vivo extrapolation using ADMET Predictor™**
  – physicochemical property models
  – \( pK_a \): why is it so critical?
  – intrinsic clearance and metabolism models

• Validation examples

• Conclusions
What’s happening in vivo?

**Fa%**
- Dose
- Portal vein (PV)
- Liver
- SC
- To faeces
- pKₐ
- Solubility vs. pH
- Biorelevant solubility
- Precipitation kinetics

**FDp%**
- Transcellular permeability
- Paracellular permeability
- logD vs. pH
- Carrier-mediated transport
- Gut extraction
- Liver metabolism
- Hepatic uptake
- Biliary secretion

**F% (not Fa%)**

Mechanistic Absorption Modeling (MAM)

Physiologically based Pharmacokinetics (PBPK)
Alternative Dosage Routes Mechanistic Models

Pulmonary

Oral Cavity

Dermal

Ocular
Processes Involved in Oral Absorption

- **Transit In**
  - dose or from previous compartment
  - unreleased & undissolved & dissolved

- **Transit Out**
  - to next compartment or excretion
  - unreleased & undissolved & dissolved

These phenomena:
- are happening simultaneously
- are repeated in each of the compartments of the gastrointestinal tract
PBPKPlus Module

Full control over the physiology

- Add or remove tissues
- Change tissue type
  - Perfusion-limited tissue
  - Permeability-limited tissue
- Adjust tissue parameters to reflect different physiology, disease state, ...
- But default settings are used most often
What’s Defined in a PBPK Model?

- Each compartment represents a tissue:
  - specific volume
  - blood perfusion rate
  - enzyme/transporter expressions
  - volume fractions of lipids & proteins
  - partition coefficient $K_p$

- Perfusion limited tissues: concentration of chemical in the tissue is $K_p \times C_{\text{plasma}}$

- Permeability limited tissue: $K_p$ determines distribution of chemical between plasma and extracellular space, but intracellular concentration is determined by carrier-mediated transfer of chemical across cellular membrane or permeability surface area exposed to the plasma
Biliary clearance can be specified as:

- Biliary Clearance Fraction (fraction of liver clearance due to biliary excretion) – same as with Compartmental PK
- An active efflux of drug across canalicular membrane
- A passive diffusion of drug across canalicular membrane

\[
\frac{dM_b}{dt} = \left( \frac{Activity \times V_{\text{max}} \times C_{\text{drug}} \times Fut}{C_{\text{drug}} + K_m} \right) + (PStcAp \times C_{\text{drug}} \times Fut) + \left( M_{\text{clear}} \times F_{\text{bel}} \right)
\]
Mechanistic Kidney Model

Perfusion Limited:

Permeability Limited:

\[ \text{CL}_{\text{filt}} \text{ Estimates:} \]

- \( F_{up} \times \text{GFR} \)
- \( \text{GFR} \)
- Fraction of Kidney blood flow
- Other
Distribution and Clearance

Steady State Volume of Distribution (Vdss)

\[ V_{dss} = \sum V_t K_p *(1 - ER) + V_e (E : P) + V_p \]

\[ E : P = (R_B - (1 - H_t)) / H_t \]

\[ K_p = K_{pu} * f_{up} \]

\[ K_{pu} = V_{ew} + \frac{1}{X_{(D),iw}} V_{iw} + \left( \frac{P \cdot V_{nt} + (0.3 \cdot P + 0.7) \cdot V_{ph}}{1 / X_{(D),p}} \right) + \]

\[ (F_n + F_a) \cdot \left[ \frac{1}{f_{up}} - 1 - \left( \frac{P \cdot V_{nt} + (0.3 \cdot P + 0.7) \cdot V_{ph}}{1 / X_{(D),p}} \right) \right] \cdot R_{Atp} + \]

\[ (F_c) \cdot \left( \frac{K_a \cdot [AP]_T ((1 / X_{(D),iw}) - 1)}{1 / X_{(D),p}} \right) \]

S+ Method (Lukacova): The binding of drug to acidic phospholipids or plasma proteins is given by actual ionization of each drug at physiological pH

• Linear Clearance
  - \( CL_{int} = \) intrinsic clearance

• Nonlinear Clearance
  - Michaelis-Menten kinetics

\[ CL_{int,u} = \sum_{i=1}^{n_{Enz}} \left[ \frac{V_{max}'}{K_m' + C_{t,u}} \right] \]

\( CL_{int,u} \): Unbound intrinsic clearance
\( C_{t,u} \): Unbound tissue drug concentration

Systemic Clearance:

\[ CL_p = R_{bp} \cdot CL_b = R_{bp} \cdot Q \left( \frac{CL_{int,u}}{CL_{int,u} + Q \frac{R_{bp}}{f_{up}}} \right) \]

\( CL_p, CL_b \): plasma, blood clearance
\( Q \): Tissue blood flow
\( R_{bp} \): Blood/plasma concentration ratio
\( f_{up} \): fraction unbound in plasma
Predicting $K_p$: Rodgers vs. Lukacova

- **$K_p$ muscle**
  - $S+$ vs. Rodgers
  - Y-axis: $K_p$ muscle
  - X-axis: pKa
  - Range: $0 \rightarrow 3$

- **$K_p$ adipose**
  - $S+$ vs. Rodgers
  - Y-axis: $K_p$ adipose
  - X-axis: pKa
  - Range: $0 \rightarrow 1$

- **$V_{ss}$ [L/kg]**
  - $S+$ vs. Rodgers
  - Y-axis: $V_{ss}$ [L/kg]
  - X-axis: pKa
  - Range: $0 \rightarrow 3$

- **% ionized**
  - pH 7.4 vs. pH 7
  - Y-axis: % ionized
  - X-axis: pKa
  - Range: $0 \rightarrow 120$
Predicting Kps

Poulin

Berezhkovskiy

Rodgers

S+
Predicting Kps

Purpose: Previous investigations have shown that the Rodgers and Rowland method [Rodgers 2007] for prediction of tissue:plasma partition coefficients (Kps) provides good prediction for compounds with low to moderate lipophilicity, but it often fails when applied to highly lipophilic compounds. The reasons for the unreasonably high Kp predictions for lipophilic compounds were investigated.

Methods: The effects on errors in predictions of experimental measurements of logP, pKa, Fup and Rbp on the accuracy of Kp prediction were evaluated. The main focus was on prediction of Kps, and the resultant volume of distribution, using the Rodgers & Rowland method for highly lipophilic compounds. The study revealed that this method tends to overpredict Kps especially for lipophilic compounds which also have fairly high measured fraction unbound in plasma (Fup). This could be due to the inability of current experimental techniques to capture the possible binding of drug to plasma lipids in Fup measurements. We have derived an equation which corrects the experimental Fup for binding to plasma lipids, assuming that the experimental Fup is

\[
\text{Fup}_{\text{corrected}} = \text{Fup}_{\text{experimental}} - \frac{\text{Fup}_{\text{lipid}}}{} 
\]

where Fup is the volume fraction of total drug lipid and phospholipid in plasma, Fup is the volume fraction of water in plasma, logP is the partition coefficient, and Fup is the adjusted fraction unbound in plasma which will be used in Kp calculations.

Dependence of adjusted Fup on logP and experimental Fup

<table>
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<tr>
<th>Fup</th>
<th>logP</th>
<th>Adjusted Fup</th>
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</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>0.3</td>
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<tr>
<td>0.6</td>
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<td>0.2</td>
</tr>
<tr>
<td>0.7</td>
<td>2.0</td>
<td>0.1</td>
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</table>

References:

Lukacova – AAPS Annual Meeting 2008
Highly lipophilic drugs can exhibit significant binding to plasma lipids.

Binding to plasma lipids may not be captured by standard equilibrium dialysis measurement of Fup.

\[
f_{up} = \frac{1}{10^{\log D_{o/w}} \left( \frac{V_{lipid}}{V_{water}} \right) + 1 + \frac{1 - F_{up,exp}}{F_{up,exp}}}
\]

Assumptions:
1. \( \log D_{o/w} \) can be used as an estimate for the drug partitioning into plasma lipids.
2. Experimental \( F_{up} \) is a measure of drug binding ONLY to plasma albumin.
IVIVE in GastroPlus
Obtaining Necessary Physicochemical/CYP Metabolism Properties from Chemical Structure
Structure-Based Predictions

Quantitative Structure Activity Relationships (QSAR)

Physiologically-Based Pharmacokinetics (PBPK)

ADMET Predictor™
ADMET Property Estimation and Model Building
Predictive Models

Train/Verify Set  
N = 25559  
RMSE = 0.475  
MAE = 0.345  
y = 1.0027x - 0.044  
R^2 = 0.975

Test Set  
N = 8131  
RMSE = 0.479  
MAE = 0.339  
y = 1.0043x - 0.027  
R^2 = 0.974

Train/Verify Set  
N = 11061  
RMSE = 0.300  
MAE = 0.227  
y = 1.011x - 0.022  
R^2 = 0.973

Test Set  
N = 955  
RMSE = 0.570  
MAE = 0.440  
y = 1.003x + 0.031  
R^2 = 0.928

Train/Verify Set  
N = 669  
RMSE = 0.434  
MAE = 0.319  
y = 1.035x - 0.034  
R^2 = 0.680

Test Set  
N = 102  
RMSE = 0.435  
MAE = 0.329  
y = 1.045x - 0.083  
R^2 = 0.650

Train/Verify Set  
N = 98  
RMSE = 0.126  
MAE = 0.097  
y = 1.124x - 0.106  
R^2 = 0.732

Test Set  
N = 14  
RMSE = 0.127  
MAE = 0.094  
y = 0.891x + 0.134  
R^2 = 0.708

Train/Verify Set  
N = 238  
RMSE = 0.312  
MAE = 0.246  
y = 1.029x - 0.009  
R^2 = 0.698

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS
Why are pK_{a}s so important?

- Dissolution & Precipitation
- Distribution
- Metabolism
- Absorption

pK_{a}s ("ionization")
CYP Metabolism Models

CYP Substrate?

CYP Subst Star Plot:
Predicted to be a substr. for all 5 CYPs except 1A2
CYP Metabolism Models

CYP Substrate?

Sites of Metabolism

Predicted 3A4 sites of metabolism (red mesh) and scores

diltiazem
CYP Metabolism Models

CYP Substrate?

Sites of Metabolism

Km, Vmax, CLint

Predicted 3A4 atomic CL\text{int}
CYP Metabolism Models

CYP Substrate?

Sites of Metabolism

Km, Vmax, CLint

Metabolites

diltiazem (40.1 μ/l/min/mg HLM protein)

Diltiazem - M1 (23%)

Diltiazem - M3 (13%)

Diltiazem - M2 (50%)
## Summary of CYP Enzyme Predictions

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Substrate</th>
<th>Km</th>
<th>Ki</th>
<th>Vmax</th>
<th>CLint</th>
<th>Sites (if substr)</th>
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<td>X</td>
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<td>X</td>
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<td>3A4_tes</td>
<td>X</td>
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</table>
Validation: *in vitro – in vivo* extrapolation

<table>
<thead>
<tr>
<th>Chemical</th>
<th>( \text{AUC}_{0-\text{inf}} ) (( \mu \text{g-h/ml} ))</th>
<th>Ratio</th>
<th>Predicted F %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{In vivo} )</td>
<td>Predicted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8.43</td>
<td>7.48</td>
<td>0.9</td>
<td>37</td>
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<tr>
<td>Acetaminophen</td>
<td>91.23</td>
<td>40.17</td>
<td>0.4</td>
<td>79</td>
</tr>
<tr>
<td>6-Propyl-2-thiouracil</td>
<td>21.06</td>
<td>27.13</td>
<td>1.3</td>
<td>90</td>
</tr>
<tr>
<td>Candoxatril</td>
<td>0.9</td>
<td>5.74</td>
<td>6.4</td>
<td>58</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>0.64</td>
<td>0.55</td>
<td>0.9</td>
<td>76</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>40.79</td>
<td>33.77</td>
<td>0.8</td>
<td>50</td>
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<tr>
<td>Sulfasalazine</td>
<td>49.76</td>
<td>450.8</td>
<td>9</td>
<td>56</td>
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<tr>
<td>5,5-Diphenylhydantoin</td>
<td>135.56</td>
<td>67.25</td>
<td>0.5</td>
<td>94</td>
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<tr>
<td>Coumarin</td>
<td>0.007</td>
<td>0.183</td>
<td>25.7</td>
<td>64</td>
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<tr>
<td>Diphenhydramine hydrochloride</td>
<td>0.94</td>
<td>16.42</td>
<td>17.5</td>
<td>100</td>
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<tr>
<td>Lovastatin</td>
<td>0.065</td>
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<td>109</td>
<td>93</td>
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<td>Carbaryl</td>
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<td>3.4</td>
<td>37</td>
</tr>
<tr>
<td>Triabendazole</td>
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<td>46.75</td>
<td>2.7</td>
<td>91</td>
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<tr>
<td>2,4-D</td>
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<td>1209.5</td>
<td>2.9</td>
<td>100</td>
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<td>Picloram</td>
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<td>166.66</td>
<td>171</td>
<td>98</td>
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<tr>
<td>Triclosan</td>
<td>1.41</td>
<td>0.76</td>
<td>0.5</td>
<td>96</td>
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</tbody>
</table>

Ref: Haiying Zhou et al., Using Physiologically Based Pharmacokinetic Modeling for *in vitro – in vivo* Extrapolation to Predict Chemical Exposure, Poster presented here at the IVIVE workshop.
Validation: *in silico* – *in vivo* extrapolation

Lawless et al. (2015) ISSX Annual Meeting
Using QSAR & PBPK to predict human F%: 70% of compounds predicted within 2-fold
Prediction of F%

- A database of 62 drugs including oral bioavailability (F%) and dose was constructed
  - All compounds’ reported major clearance pathways (MCP) were CYP-mediated\(^1\)
  - For 43 drugs with more than one reported value of F%, the average experimental CV\(\%\) was 29\%
- Reported F% values\(^2\) varied from 3\% (fluphenazine) to 99\% (diazepam, galantamine, glimepiride, indomethacin, and tamsulosin), with an average of 60\%
- F% was predicted by integrating quantitative structure activity relationship (QSAR) model predictions\(^3\) and physiologically based pharmacokinetic (PBPK) simulations\(^4\)
  - A 35-year-old American male physiology was used for all PBPK simulations
- All molecules were predicted to be substrates of the CYP associated with their MCP
- In 42 of the 62 molecules, the CYP isoform with highest predicted intrinsic clearance (CL\(_{\text{int}}\)) was the same as the MCP
- Overall, 68\% of the molecules were predicted within 2-fold of their reported F%

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1 Toshimoto K et al, *Drug Metabol. Disp.* Fast Forward. Published on August 14, 2014.
3 ADMET Predictor\(^\text{TM}\) version 7.2, Simulations Plus, Inc., Lancaster, CA 95354 USA.
4 GastroPlus\(^\text{TM}\) version 9.0, Simulations Plus, Inc., Lancaster, CA 95354 USA.
Predicting drug bioavailability using PBPK modeling and Global Sensitivity Analysis to identify sensitive parameters

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Introduction
- ADME modeling in lead optimization typically includes only QSAR/QSPR predictions of physicochemical properties or simple allometric scaling to predict species variation.
- Many physicochemical properties might be modified to improve exposure. Prioritizing is difficult.
- Physiologically-Based Pharmacokinetic (PBPK) modeling, typically applied on individual compounds for clinical trials, gives more accurate and detailed mechanistic results.
- Inputs required for PBPK modeling are the very same properties, that med chemists intend to modify to improve bioavailability.
- Predicting clearance is the challenge in modeling whole series, and that was solved with the help of local QSAR for an apparent intrinsic clearance.
- Global Sensitivity Analysis (GSA) of PBPK models for whole chemical series in lead opt. could identify the most effective properties to improve drug exposure.

Approach
- PBPK ADME simulations successfully adapted to lead series:
  - Predicting clearance was solved with a local QSAR for "ideal" C\text{lp}.
  - In 3 cases, >90% of %F predictions within 2X all in silico
  - Good local QSAR for C\text{lp} with only 15-20 in vivo %F's
- Global Sensitivity Analysis finds key properties:
  - Methods developed for GSA of chemical series
  - Unique advice for each series:
    - DPP-4 46 cpd only C\text{lng}
    - 11\beta-HSD1 C\text{lng} + logD, logP and RBP
  - Specific advice for each compound within series

Conclusions
- Series can be modeled from as few as 15 rat studies

Prediction of F% Using \textit{in silico} Physicochemical Properties and \textit{in vitro}, Predicted or Fitted Clearance - Case Study 1

- **49 Compounds**: Single Med Chem series reported by Merck in various papers
  - RAT \textit{in vivo} data: %F, CLp
  - Physicochemical prop & \textit{in vitro} data: -

• The low accuracy of the 1st approach was due to significant renal clearance that this series of compounds undergoes
• Global QSAR model built on a wide variety of compounds was not accurate enough for this series of compounds
Prediction of F% Using \textit{in silico} Physicochemical Properties and \textit{in vitro}, Predicted or Fitted Clearance - Case Study 2

- **81 Compounds**: Single Med Chem series reported by Astra-Zeneca in 4 publications

  - **RAT \textit{in vivo} data**: %F, CLp
  - **\textit{in vitro} data**: \( \text{CL}_{\text{int}}(\text{hep}) \)

  • These simulations suggest that this class of compounds undergo extensive hepatic clearance and that extrahepatic clearance mechanisms are either absent or minimal

Prediction of F% Using *in silico* as well as Experimental Physicochemical Properties and *in vitro*, Predicted, or Fitted Clearance - Case Study 3

- **61 compounds**: Single Med-Chem series with experimental data
  - Physicochemical prop & *in vitro* data: (Solubility, Caco2 permeability, Plasma Protein binding, CL_{int})
  - RAT PK data: (%F, AUC, C_{max}, T_{max}, CL_{plasma}, V_{ss})

- These simulations suggest that purely *in silico* inputs can provide similar results to the experimentally obtained values

Conclusions

• Quality of predictions produced by Mechanistic Absorption and PBPK modeling greatly depends on the input parameters and the routes of clearance that any given compound is subjected to *in vivo*.

• In general, volume of distribution is predicted well with the default GastroPlus PBPK methodology if the provided physicochemical and biopharmaceutical properties are correct. The main reasons for underprediction of Vd are: specific binding to some tissues, lysosomal trapping, and active transport (influx and efflux) into the tissue(s).

• Plasma concentrations and F% are typically predicted within 10-fold for the majority of chemicals. Compounds that undergo only passive renal clearance and/or hepatic CYP clearance can be predicted within 2-fold – even with only *in silico* inputs. Other routes of clearance such as: biliary in liver and transporter-based (in liver or kidney) are difficult to predict and are the major reasons for underpredicting clearance when *in vitro-in vivo* extrapolation is used.
Acknowledgments

• Co-authors:
  – Haiying Zhou
  – Michael Lawless
  – Pankaj R. Daga
  – Michael B. Bolger

• Contributors:
  – Viera Lukacova
  – Robert Fraczkiewicz
  – Marvin Waldman
  – Robert D. Clark
  – Jinhua Zhang
  – John DiBella
  – Walter Woltosz
Additional Slides
Mechanisms: Clearance

Relationship between $\text{CL}_{\text{int}}$ and $t_{1/2}$:

$$\text{CL}_{\text{int}} = \frac{0.693}{t_{1/2}} \times \frac{\text{ml incubation}}{\text{mg microsomes}} \times \frac{38 \text{ mg microsomes}}{\text{x g liver}} \times \frac{\text{g liver}}{\text{kg b.w.}}.$$
IVIVE

- Predict metabolic clearance in vivo from in vitro measurements (microsomes, hepatocytes, recombinant systems)
- Convert Vmax measured in rate of metabolism per ‘unit amount of enzyme’ to rate of metabolism in the entire tissue (liver, gut, etc.)
- in vitro ‘unit amount of enzyme’ is given by the in vitro assay:
  - mg of microsomal protein (microsomal assay)
  - 1 million cells (hepatocyte assay)
  - pmol of enzyme (recombinant enzymes)

To obtain in vivo Vmax in the entire tissue:

**microsomes**  \[
\frac{\text{rate}}{[\text{mg of microsomal protein}]} \times \frac{[\text{mg of microsomal protein}]}{[\text{g of tissue}]} \times [\text{g of tissue}] = \frac{\text{rate}}{[\text{tissue}]}\]

**hepatocytes**  \[
\frac{\text{rate}}{[\text{one million cells}]} \times \frac{[\text{millions of cells}]}{[\text{g of tissue}]} \times [\text{g of tissue}] = \frac{\text{rate}}{[\text{tissue}]}\]

**rCYP**  \[
\frac{\text{rate}}{[\text{pmol of enzyme}]} \times \frac{[\text{pmol of enzyme}]}{[\text{mg of microsomal protein}]} \times \frac{[\text{mg of microsomal protein}]}{[\text{g of tissue}]} \times [\text{g of tissue}] = \frac{\text{rate}}{[\text{tissue}]}\]
Model performance... CYP2D6

<table>
<thead>
<tr>
<th>Model</th>
<th>Data Sets</th>
<th>Negatives</th>
<th>Positives</th>
<th>Total</th>
<th>Correct</th>
<th>Concordance</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>CYP_1A2_Substr</td>
<td>Training</td>
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<td>410</td>
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<td>938</td>
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<td>79.6%</td>
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<td>Test</td>
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CYP_2D6_Substr

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</table>

\[ V_{\text{max}} \]

\[ K_m \]

\[ \text{CL}_{\text{int}} \]

Zhang et al., ACS National Meeting (2013)
Define the physicochemical properties for your compounds.

Define the initial formulation conditions for your compounds.

Define the pharmacokinetic model (compartmental or PBPK) for your compounds, along with the Fu, plasma and blood:plasma concentration ratio.

Define how the clearance will be estimated for your compounds:

a. Include renal filtration clearance?

b. Use Vmax and Km for CYP enzymes OR intrinsic clearance – **not both**!

c. If Vmax and Km are selected, use HLM data to calculate 3A4 Vmax and Km, or rCYP data (rCYP data is used for all other CYPs)?