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

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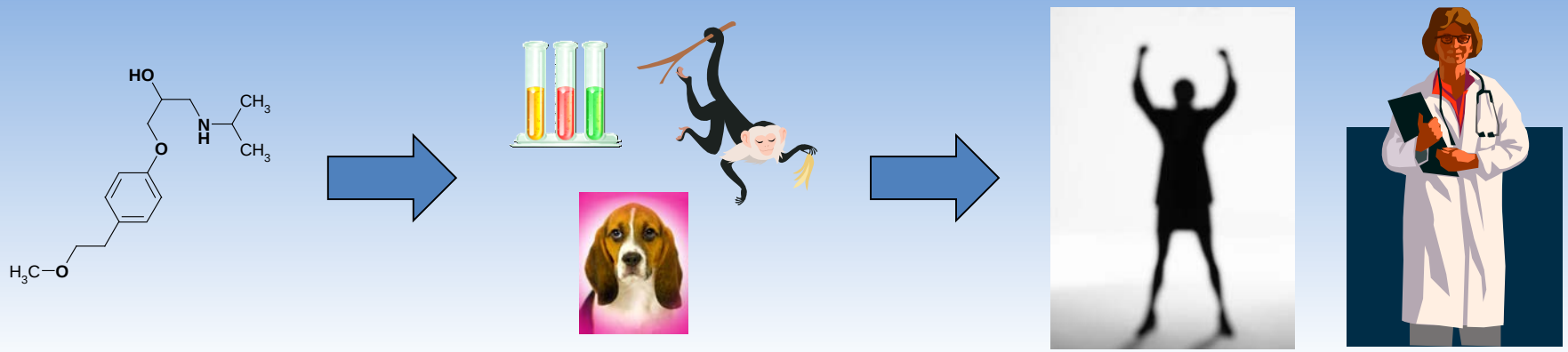
Outline

- 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

Simulations Plus Software Products



ADMET Predictor™ → GastroPlus™ → Population PK/PD Modeling & Simulations → Regulatory Submission

Cognigen

MedChem Studio™
MedChem Designer™

DDDPlus™
MembranePlus™

Consulting Services and Collaborations

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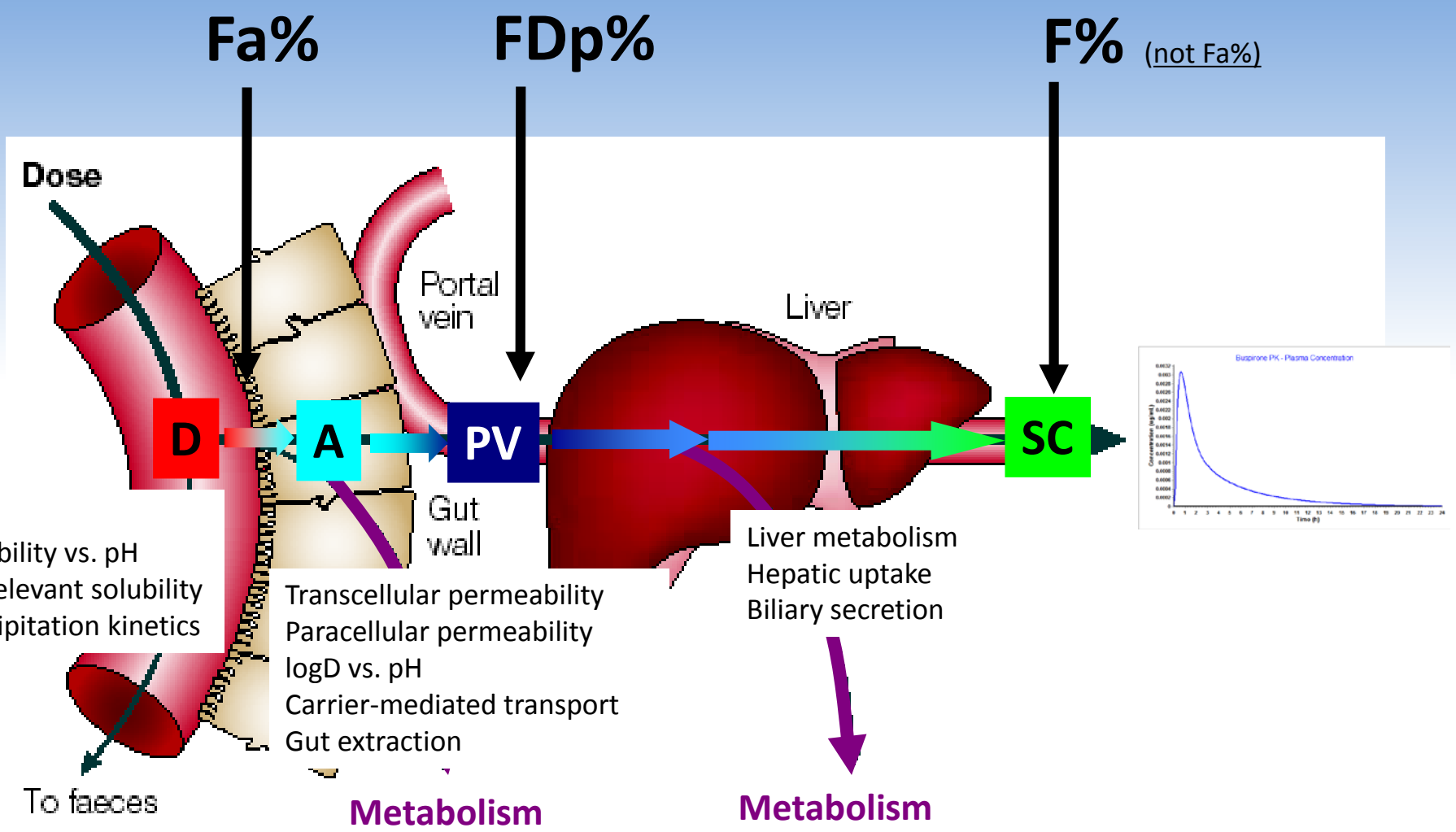
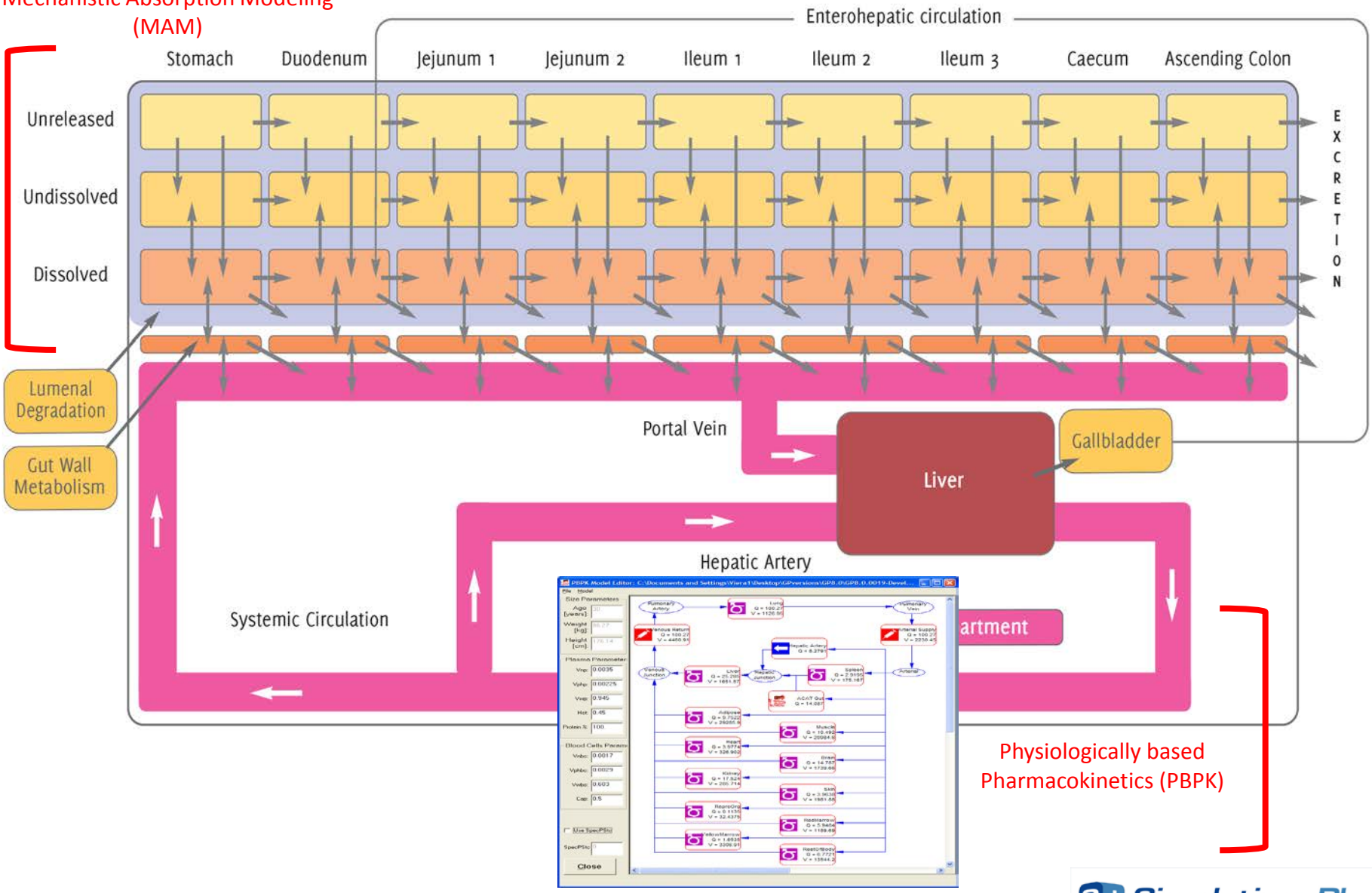
What's happening *in vivo*?



* Modified from van de Waterbeemd, H, and Gifford, E. *ADMET In Silico Modelling: Towards Prediction Paradise?* Nat. Rev. Drug Disc. 2003, 2:192-204

Advanced Compartmental Absorption and Transit Model (ACAT™)

Mechanistic Absorption Modeling (MAM)



Physiologically based Pharmacokinetics (PBPK)

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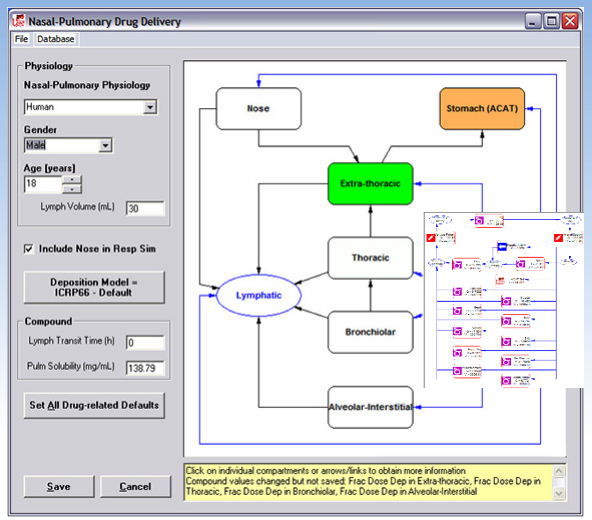
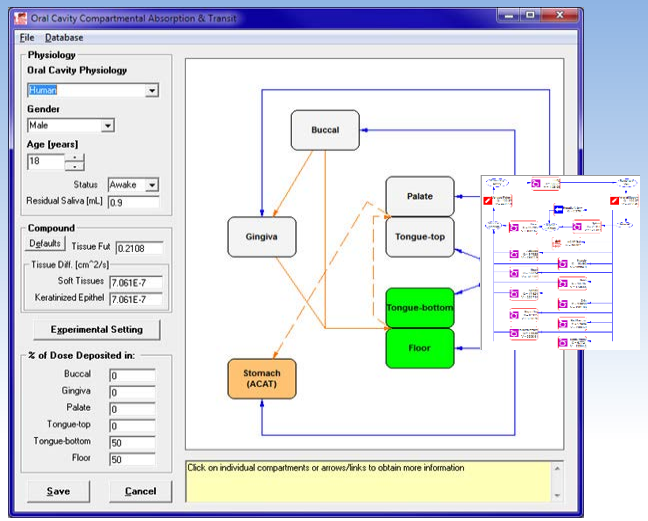
Alternative Dosage Routes Mechanistic Models

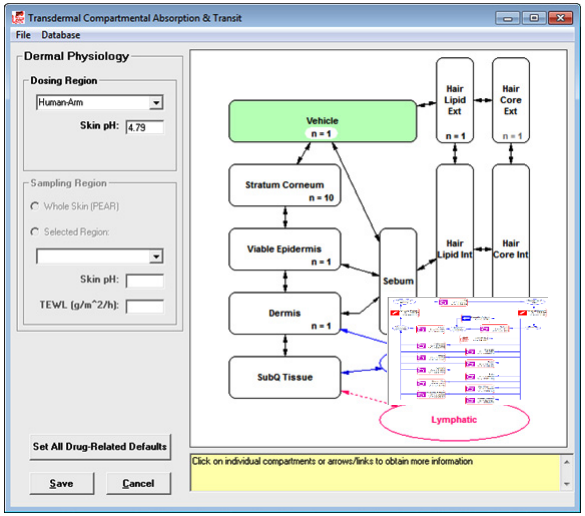
Pulmonary



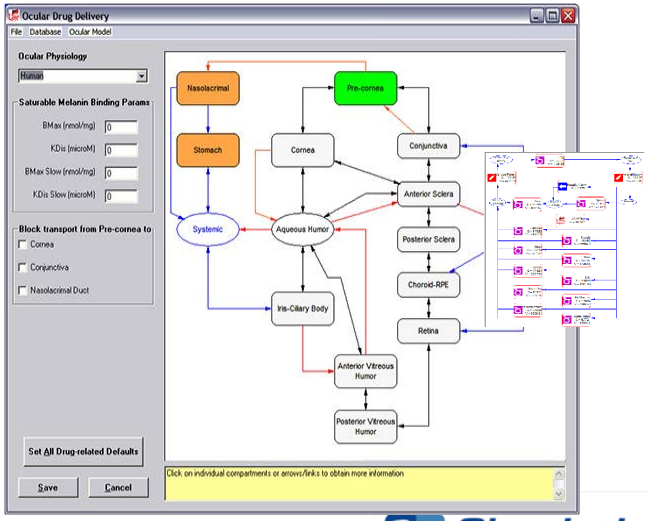
Oral Cavity



Dermal



Ocular



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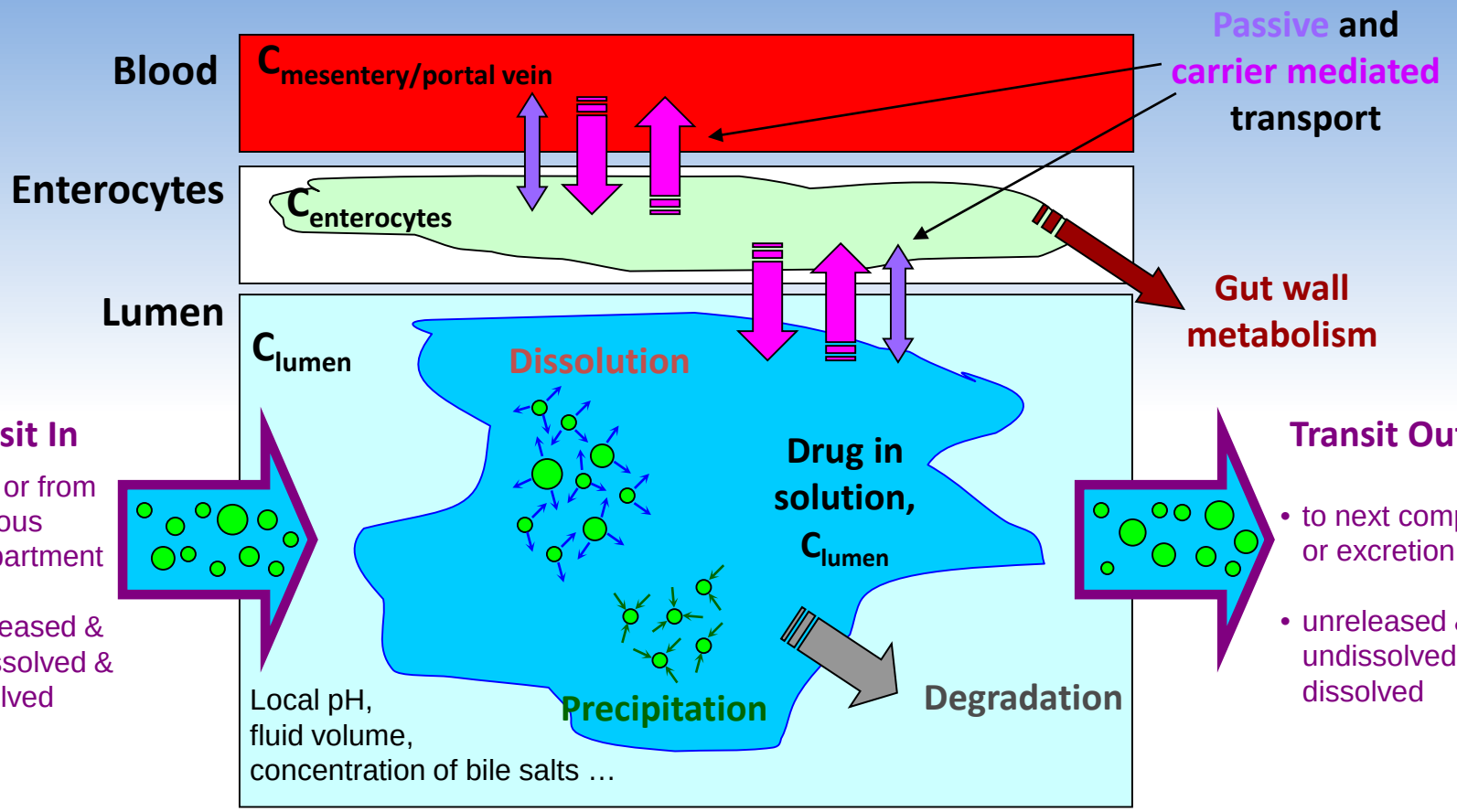
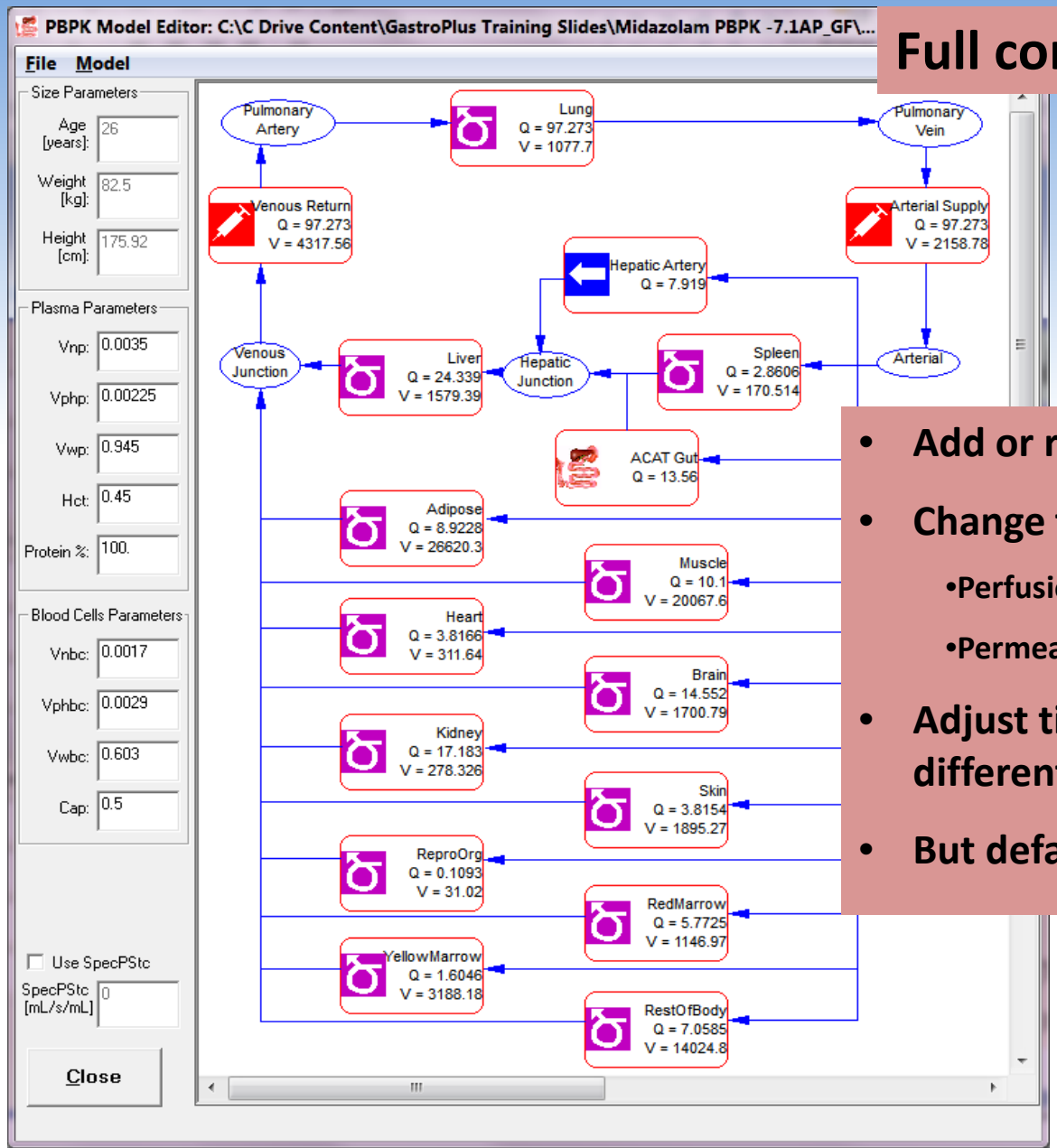
Processes Involved in Oral Absorption



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**

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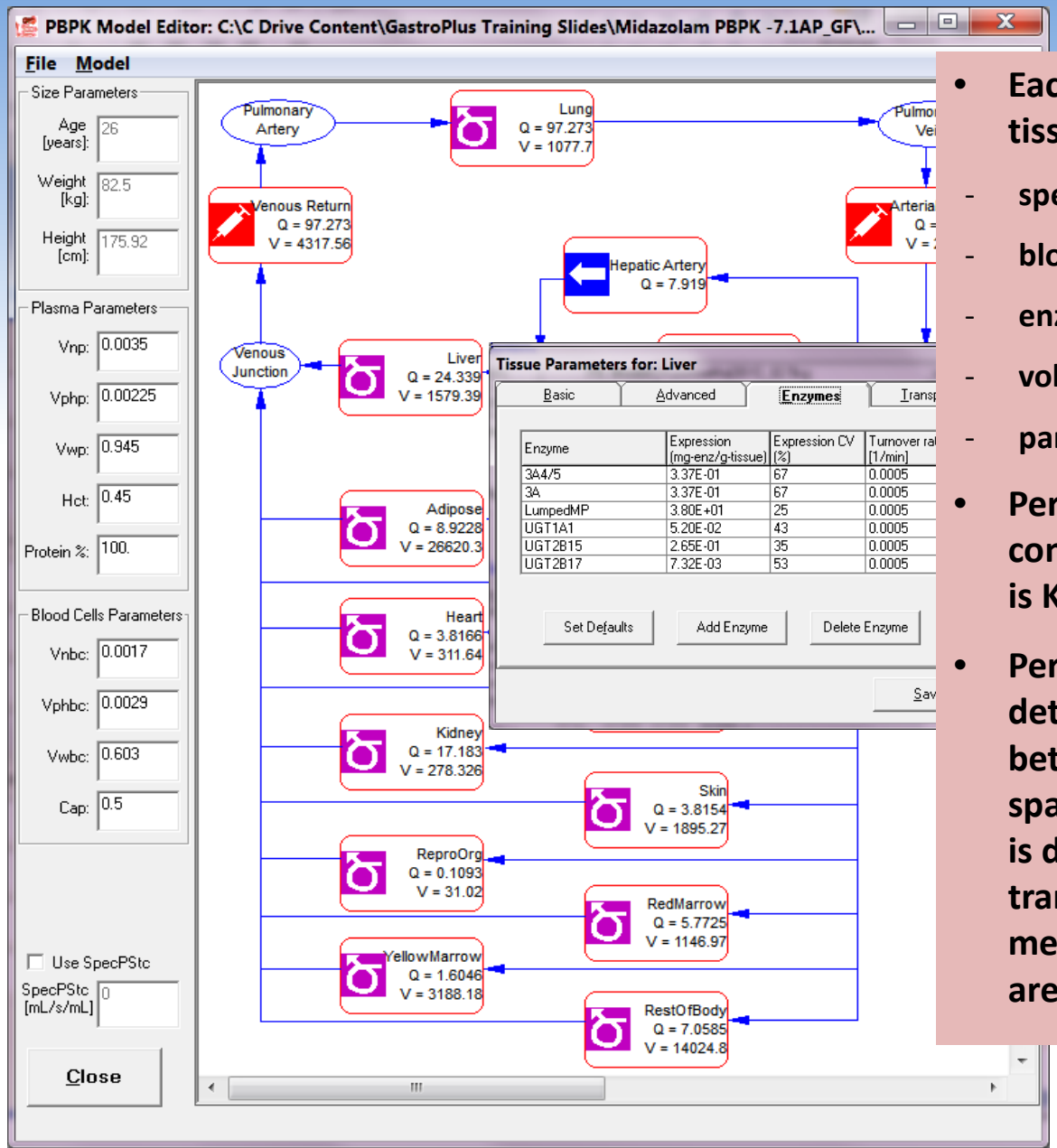
What's Defined in a PBPK Model?



- **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 $K_p * C_{plasma}$**
- **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**

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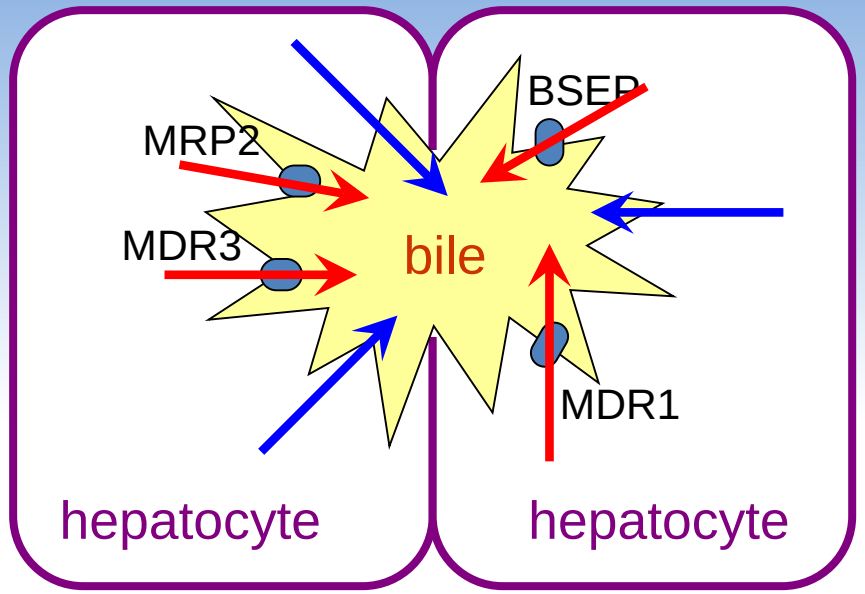
Mechanistic Liver Model



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**

$$\frac{dM_b}{dt} = \left(\frac{Activity \times V_{\max} \times C_{drug} \times Fut}{C_{drug} + K_m}\right) + \left(PStcAp \times C_{drug} \times Fut\right) + \left(M_{clear} \times F_{bcl}\right)$$

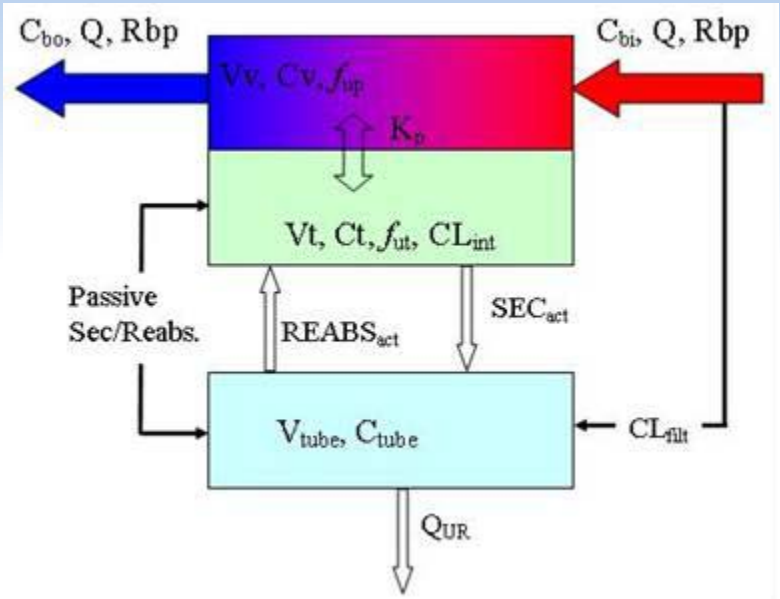
active efflux

passive diffusion

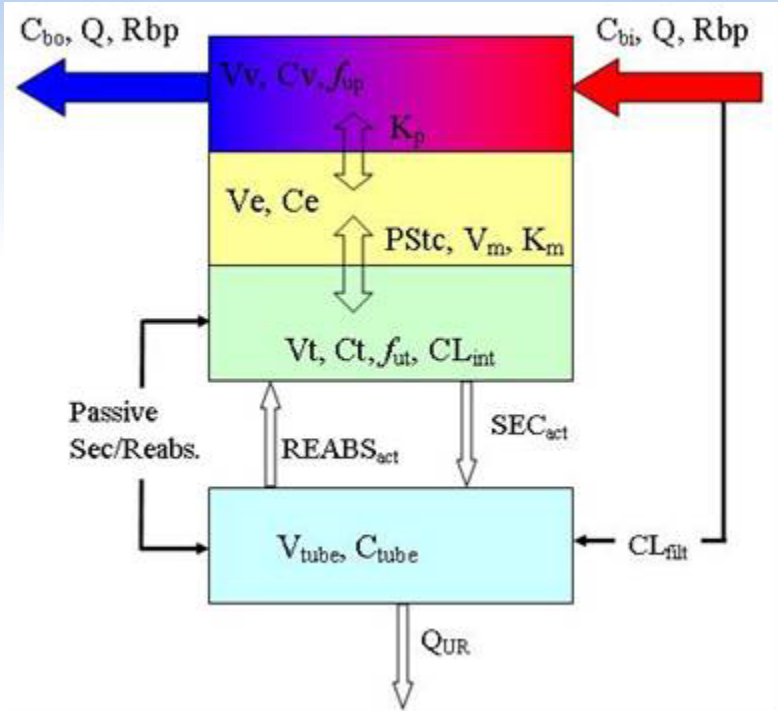
Biliary clearance fraction

Mechanistic Kidney Model

Perfusion Limited:



Permeability Limited:



CL_{filt} Estimates:

- **Fup*GFR**
- **GFR**
- **Fraction of Kidney blood flow**
- **Other**

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Distribution and Clearance

Steady State Volume of Distribution (Vdss)

$$Vd_{ss} = \sum V_t K_p * (1 - ER) + V_e (E:P) + V_P$$

$$E:P = (R_B - (1 - H_t)) / H_t$$

$$Kp = Kpu * fup$$

$$Kpu = V_{ew} + \frac{1/X_{[D],iw}}{1/X_{[D],p}} V_{iw} + \left(\frac{P \cdot V_{nlt} + (0.3 \cdot P + 0.7) \cdot V_{pht} \cdot}{1/X_{[D],p}} \right) +$$

$$(Fn + Fa) \cdot \left[\frac{1}{fup} - 1 - \left(\frac{P \cdot V_{nlp} + (0.3 \cdot P + 0.7) \cdot V_{php} \cdot}{1/X_{[D],p}} \right) \right] \cdot RAtp +$$

$$(Fc) \cdot \left(\frac{Ka \cdot [AP]_T ((1/X_{[D],IW}) - 1)}{(1/X_{[D],P})} \right)$$

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**

$$CL_{\text{int},u} = \sum_{i=1}^{nEnz} \left[\frac{V_{\max}^i}{K_m^i + C_{t,u}} \right]$$

$CL_{\text{int},u}$: Unbound intrinsic clearance

$C_{t,u}$: Unbound tissue drug concentration

Systemic Clearance:

$$CL_p = Rbp \cdot CL_b = Rbp \cdot Q \left(\frac{CL_{\text{int},u}}{CL_{\text{int},u} + Q \frac{Rbp}{fup}} \right)$$

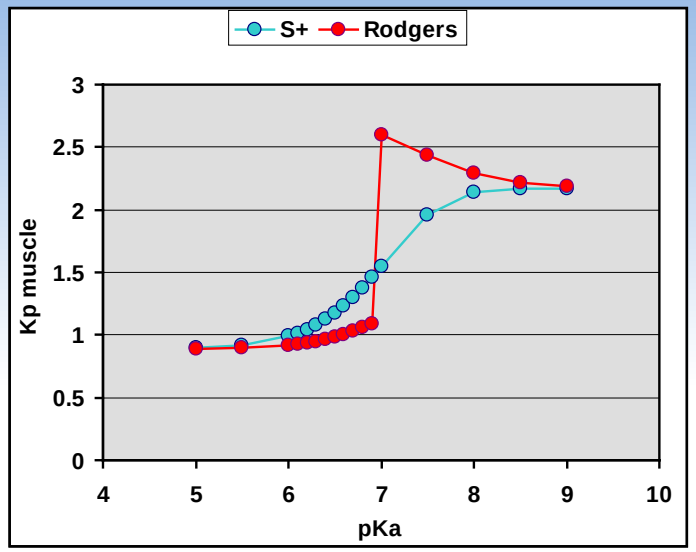
CL_p, CL_b : plasma, blood clearance

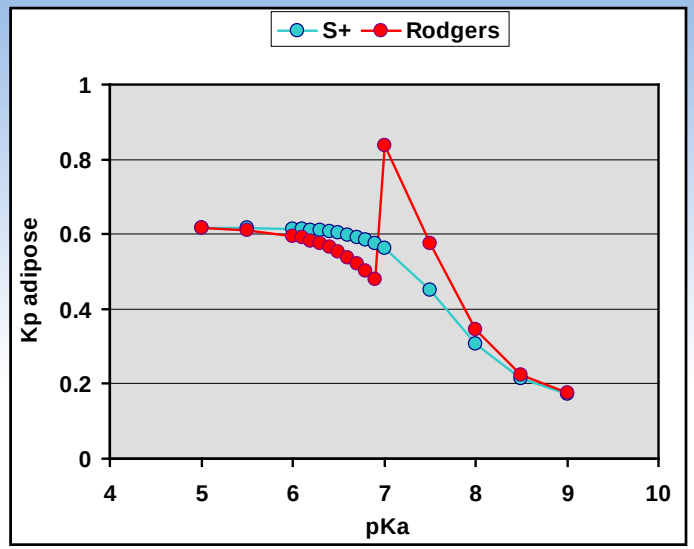
Q : Tissue blood flow

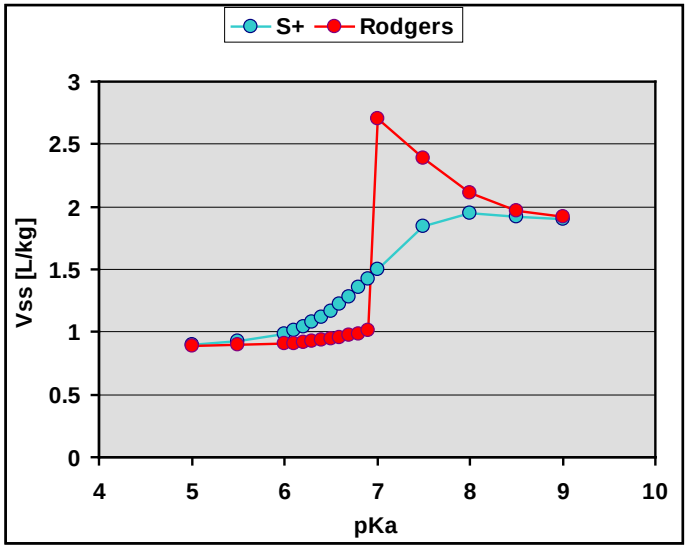
Rbp : Blood/plasma concentration ratio

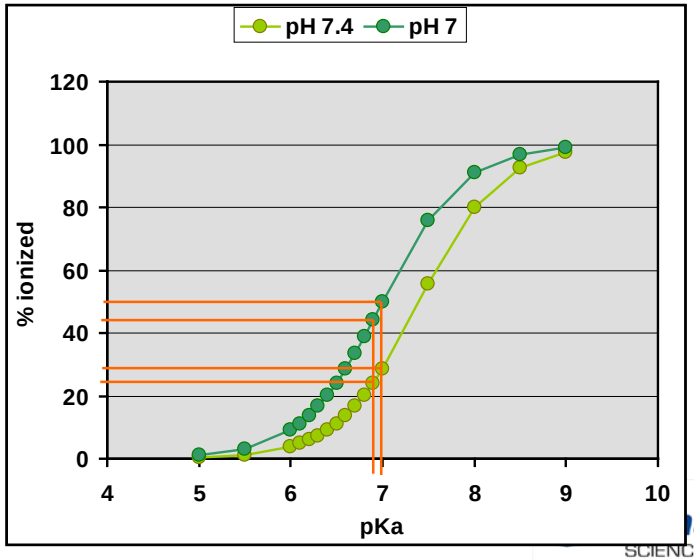
fup : fraction unbound in plasma

Predicting Kp:
Rodgers vs. Lukacova

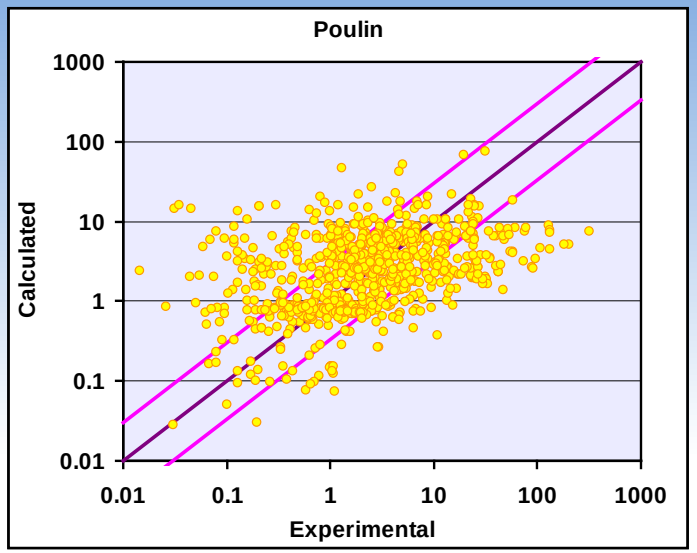


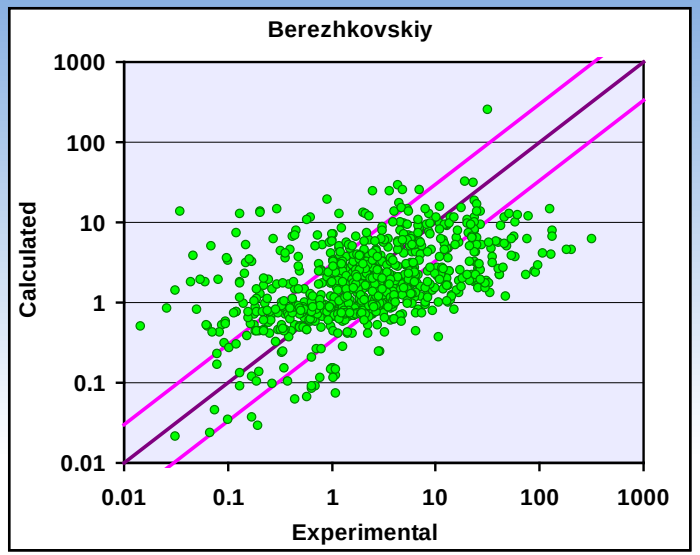


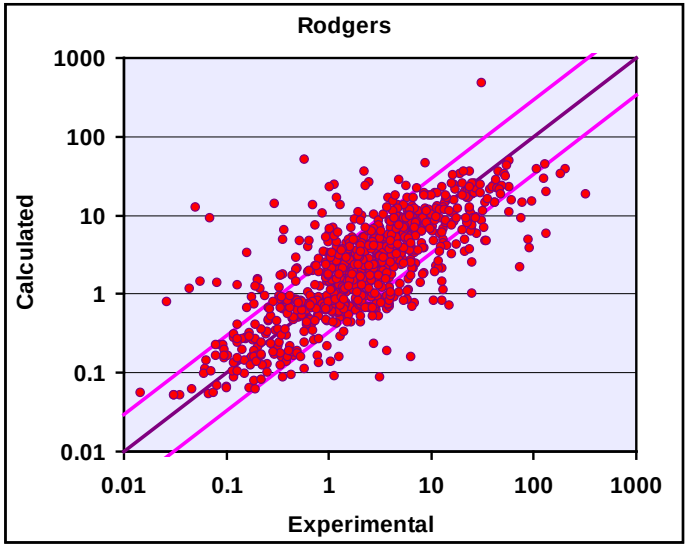


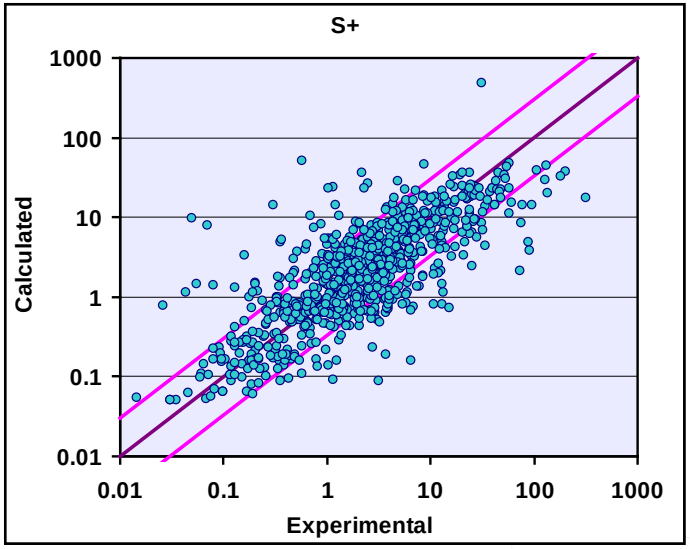


Predicting Kps









Predicting Kps

Role of Fraction Unbound in Plasma in Calculations of Tissue:Plasma Partition Coefficients

Lukacova, V.
(1) Simulations Plus, Inc. Lanc

Abstract:

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

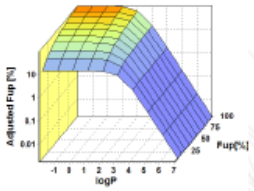
Results: While the method for prediction of Kps as published by Rodgers & Rowland provides good overall predictions for compounds with low to moderate lipophilicity, it tends to grossly overpredict Kps for highly lipophilic compounds. The corrected Fup in the Kp predictions resulted in significant improvements in predicted Kps and subsequent estimates of volume of distribution.

Conclusions: Recognizing the possible limitations of experimental techniques in capturing all the aspects of drug binding to plasma components helps to develop an approach that provides better estimates of tissue/plasma partition coefficients and subsequently better estimates of volume of distribution. This approach to predictions of drug exposure using only *in vitro* and *in silico* data will be applicable using different methods of Kp predictions for different classes of compounds.

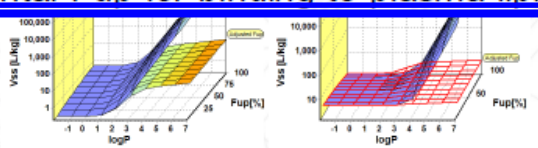
Assuming that:
(1) experimental F_{up} (by equilibrium dialysis) is a measure of drug binding only to protein
(2) logP can be used as an estimate for the drug partitioning to plasma lipids, the "corrected" fraction unbound in plasma can be calculated as:

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

where V_{lipid} is the volume fraction of total neutral lipid and phospholipid in plasma, V_{water} is the volume fraction of water in plasma, $logP_{o/w}$ is octanol/water partition coefficient, $F_{up,exp}$ is the experimentally measured value of fraction unbound in plasma, and f_{up} is the adjusted fraction unbound in plasma which will be used in Kp calculations.



Dependency of adjusted fup on logP and experimental fup



Dependency of volume of distribution (Vss) on Fup and logP using the "experimental" F_{up} directly in Kp calculations and with adjusting the Fup for binding to plasma lipids. F_{up} [%] on the Y-axis shows the "experimental" F_{up} in all graphs. The V_{ss} values were calculated for model compounds (neutral with blood-to-plasma-ratio = 1 on the left and strong base with pKa = 8.5 and blood-to-plasma-ratio = 1 on the right) using adult male physiology.
For neutral compound, the V_{ss} is increasing with increasing experimental F_{up} and increasing logP, with logP having larger impact. Adjusting F_{up} for possible binding to plasma lipids results in lower V_{ss} values reaching plateau and not increasing significantly with further increase in logP.
However, the F_{up} adjustment does not automatically result in lower V_{ss} for all compounds. For a strong base (base pKa = 8.5 and blood-to-plasma-ratio = 1), V_{ss} is increasing with increasing logP but shows much less uniform dependency on F_{up}. V_{ss} decreases with increasing F_{up} for compounds with low lipophilicity but increases with increasing F_{up} for highly lipophilic compounds. Adjustment of F_{up} for binding to plasma lipids again results in plateau in V_{ss} for highly lipophilic compounds, but for moderately lipophilic compounds, the F_{up} adjustment may result in increase in predicted Kps and subsequently V_{ss}.

Kps. Azithromycin represents compounds where the F_{up} adjustment results in increases of calculated Kps.

	Mofarotene*		Glycyrrhetinic Acid*		Azithromycin		
	Fold error of Kp prediction		Fold error of Kp prediction		Kp		
	with Exp Fup	with Adjusted Fup	with Exp Fup	with Adjusted Fup	Experimental [Rapoport 1992]	Calc with Exp Fup	Calc with Adjusted Fup
Adipose	>1000	4	>10000	>10			
Brain	500	2	>10000	5			
Gut	500	2	>5000	5			
Heart	100	3	>1000	3			
Kidney	>100	3	>1000	2	317.5	2.58	26.48
Liver	>100	3	>1000	2	442.5	3.05	24.03
Lung	>100	>5	>1000	2	205	2.19	20.59
Muscle	>100	2	>1000	2			
Repro.Org	>500	3					
Skin	>100	3	>1000	3			
Spleen	100	>5	>1000	3	1897.5	2.80	16.78

* Experimental Kp values are from unpublished Roche measurements

References:

Rodgers T., Rowland M.; J Pharm Sci 2007, 96: 3151-3152
Rodgers T., Rowland M.; J Pharm Sci 2007, 96: 3153-3154
Shepard, R. M. Falkner, F. C.; J Antimicrob Chemother 1990, 25 (suppl. A): 49-60

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Predicting Kps
Adjusted Fup

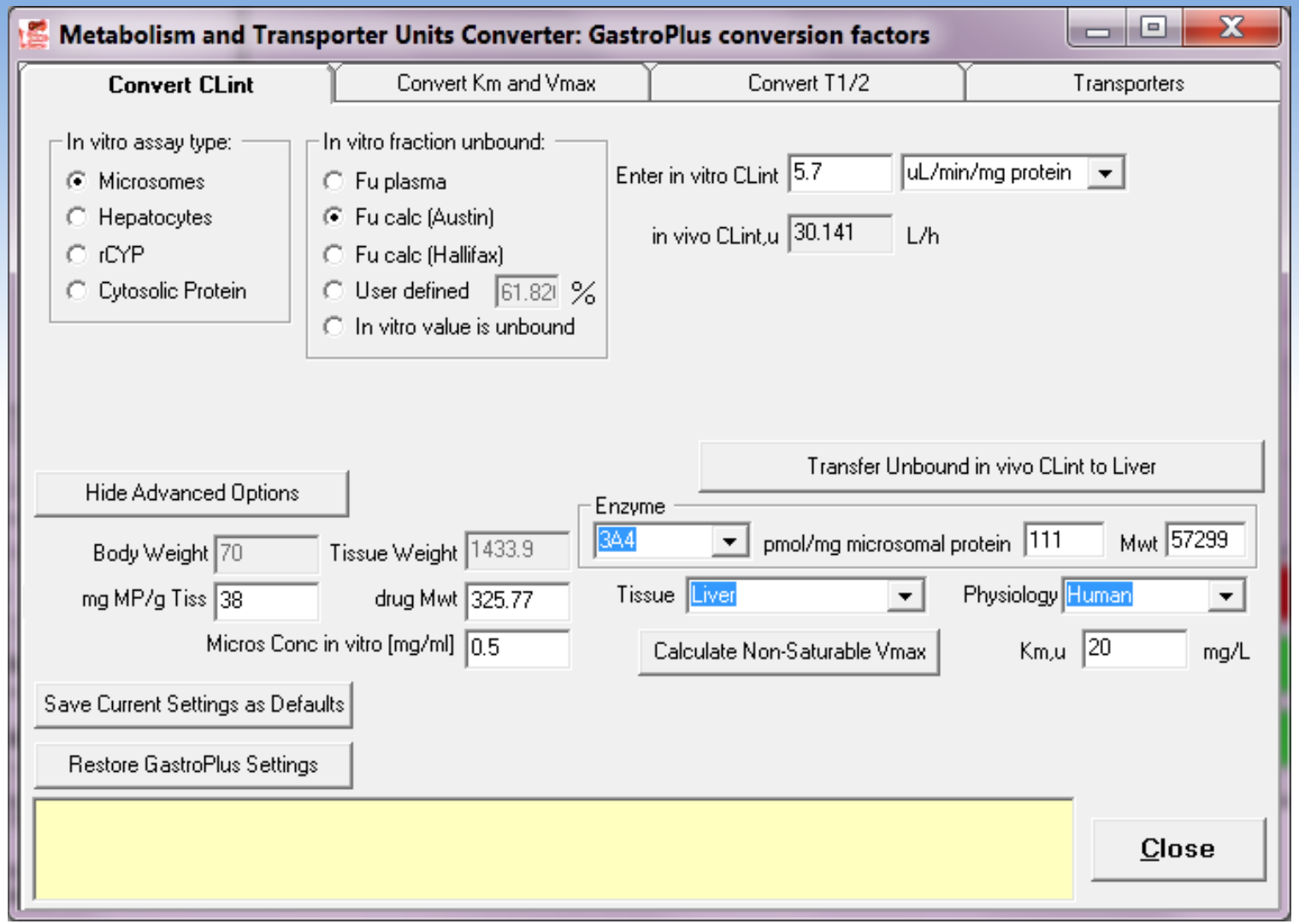
- Highly lipophilic drugs can exhibit significant binding to plasma lipids
- Binding to plasma lipids may not be captured by standard equilibrium dialysis measurement of Fup

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

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



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Obtaining Necessary Physicochemical/CYP Metabolism Properties from Chemical Structure

Structure-Based Predictions

Quantitative Structure Activity Relationships (QSAR)

Physiologically-Based Pharmacokinetics (PBPK)

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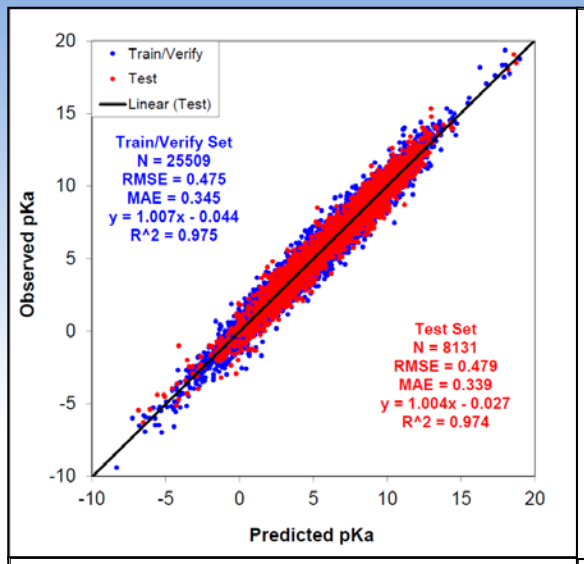
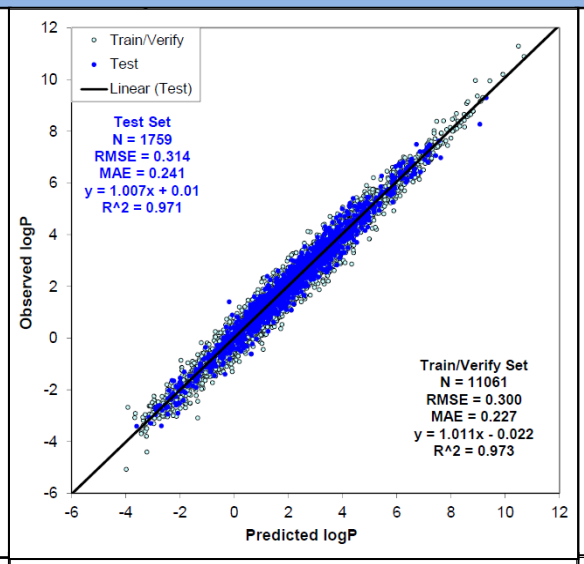
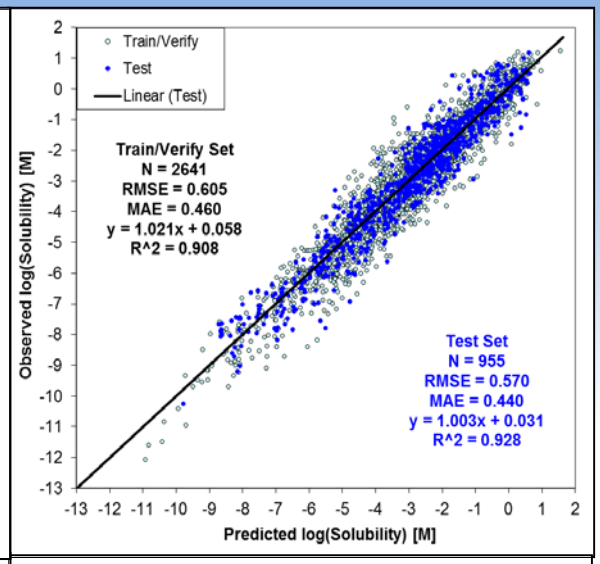
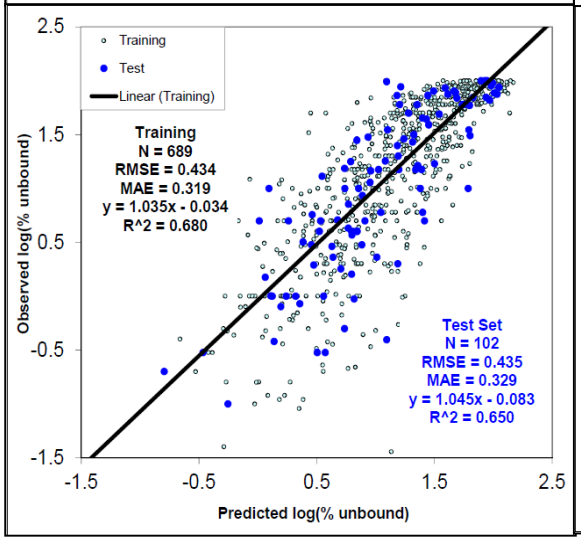
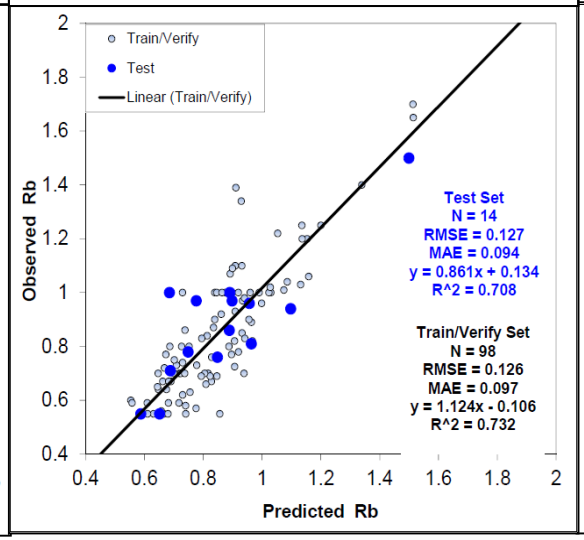
Predictive Models

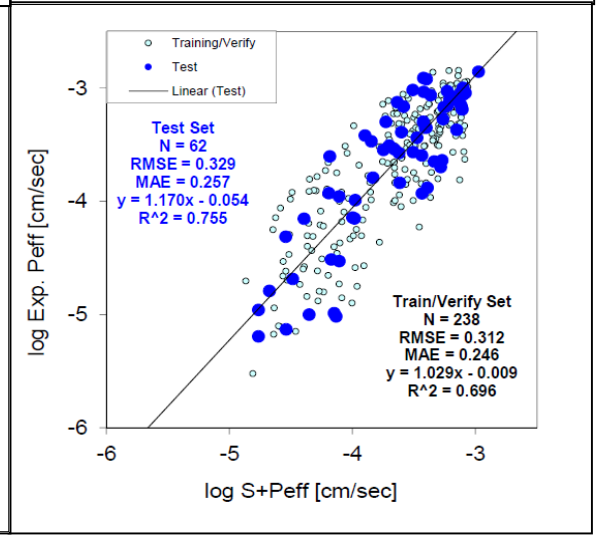












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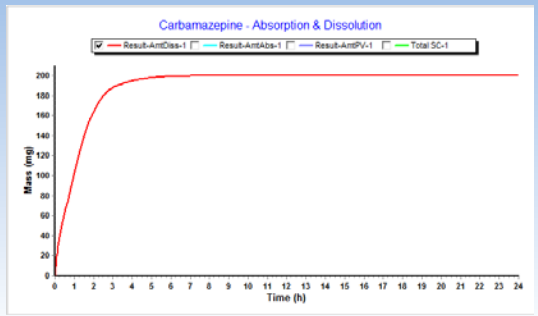
Why are pK_as so important?

PBPK Summary

	Tissue	Kp
	Hepatic Artery	0.00
	Lung	4.73
	Arterial Supply	0.00
	Venous Return	0.00
	Adipose	0.71
	Muscle	2.01
	Liver	5.60
	ACAT Gut	0.00
	Spleen	3.94
	Heart	2.84
	Brain	0.79
	Kidney	6.10
	Skin	1.74

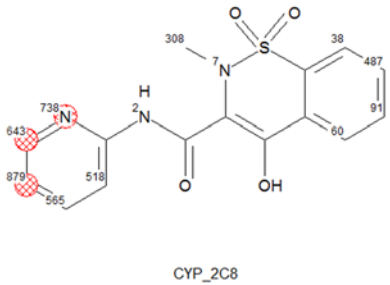
Dissolution & Precipitation



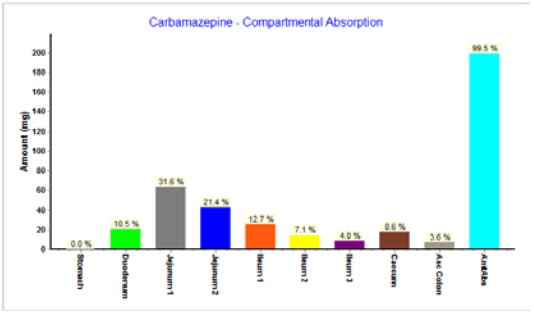
Distribution

pK_as ("ionization")

Absorption



Metabolism



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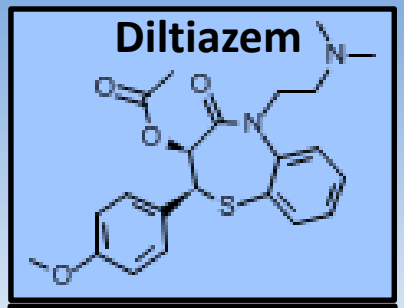
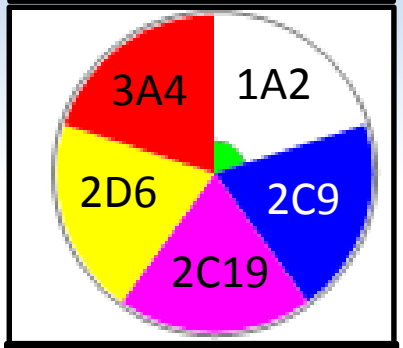
CYP Metabolism Models

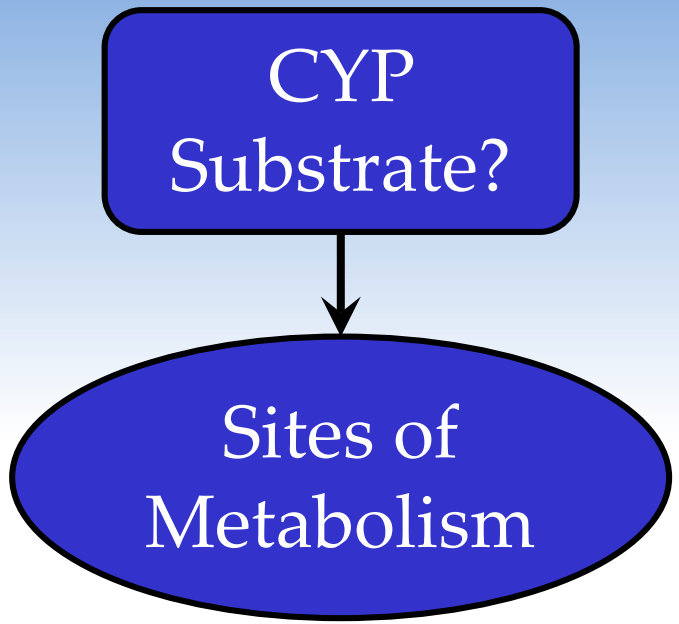
CYP Substrate?

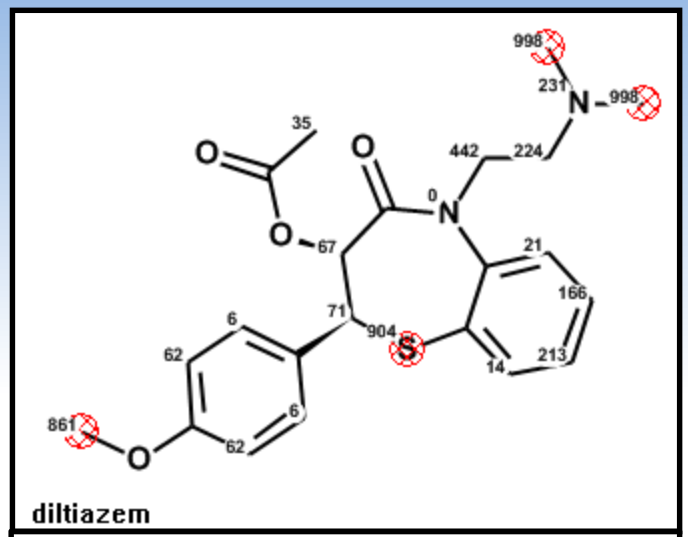




CYP Subst Star Plot: Predicted to be a substr. for all 5 CYPs except 1A2

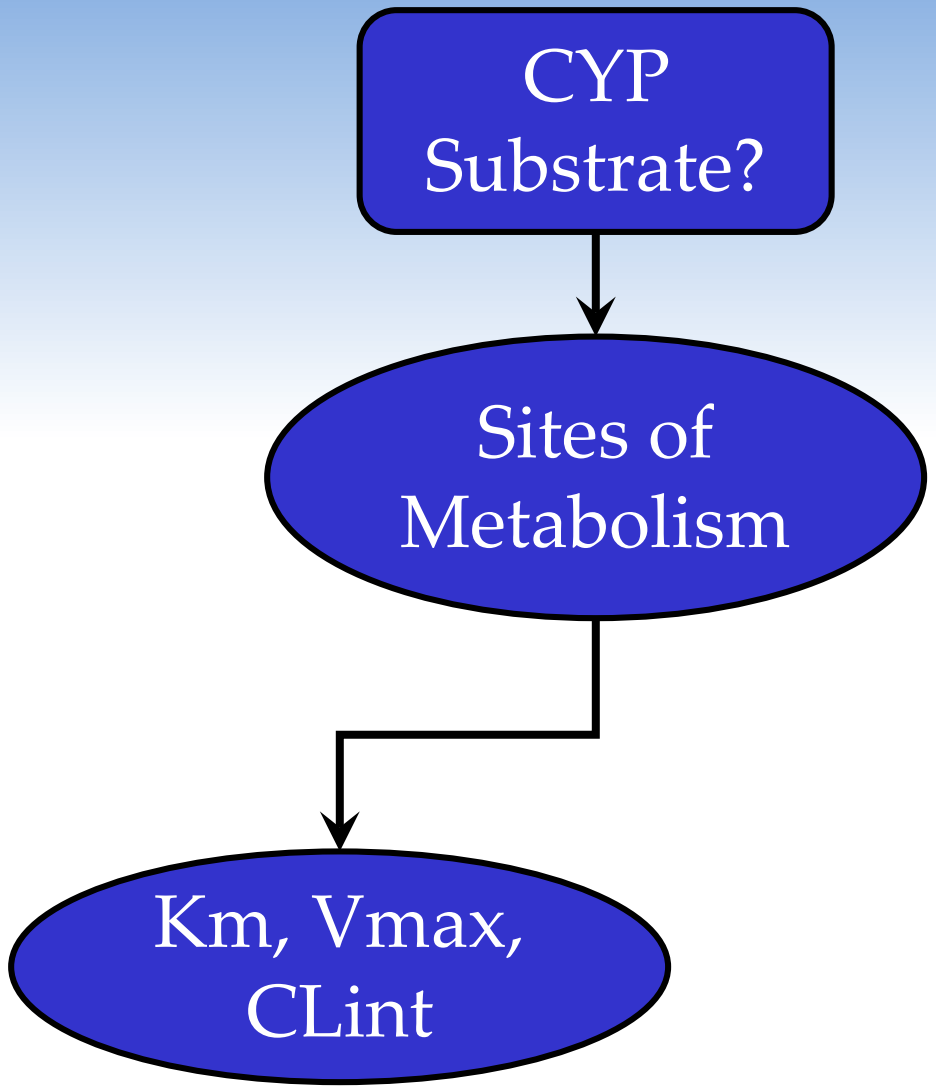
CYP Metabolism Models

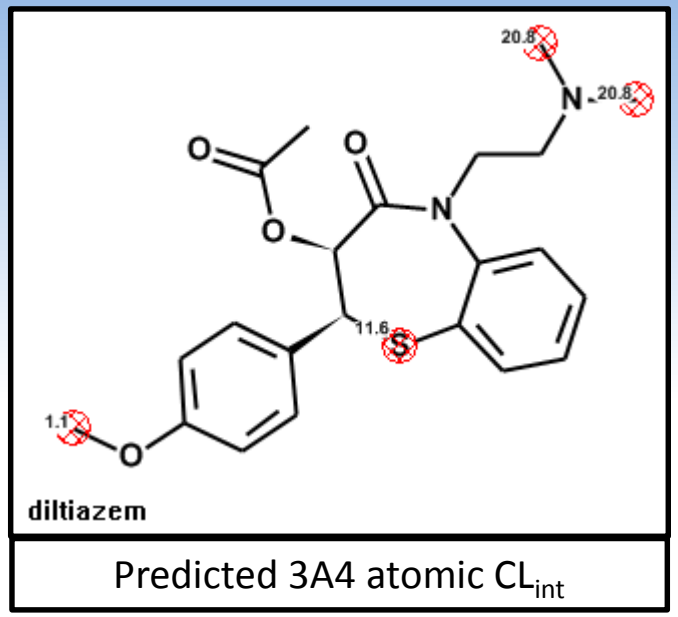




Predicted 3A4 sites of metabolism (red mesh) and scores

CYP Metabolism Models





Predicted 3A4 atomic CL_{int}

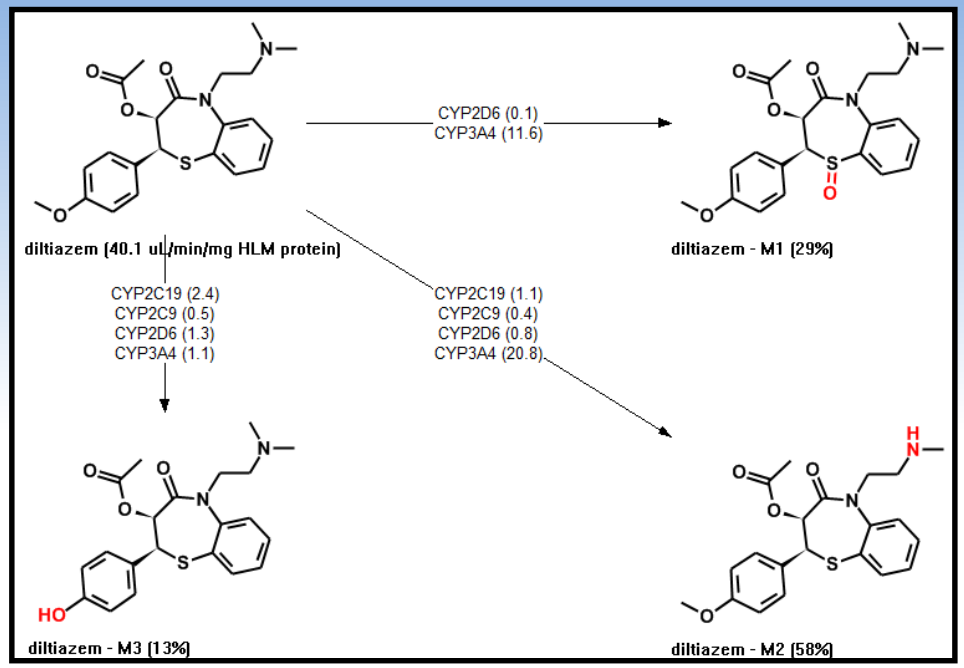
CYP Metabolism Models

CYP Substrate?

Sites of Metabolism

Km, Vmax, CLint

Metabolites



Summary of CYP Enzyme Predictions

	Inhibitor	Substrate	Km	Ki	Vmax	CLint	Sites (if substr)
CYP1A2	X	X	X		X	X	X
CYP2A6		X					X
CYP2B6		X					X
CYP2C8		X					X
CYP2C9	X	X	X		X	X	X
CYP2C19	X	X	X		X	X	X
CYP2D6	X	X	X		X	X	X
CYP2E1		X					X
CYP3A4	X	X	X		X	X	X
3A4_mid	X			X			
3A4_tes	X			X			

Validation Examples

Validation: *in vitro – in vivo* extrapolation

Chemical	$AUC_{0\text{-}inf}$ (µg-h/ml)		Ratio	Predicted F %	Reference
	In vivo	Predicted			
Erythromycin	8.43	7.48	0.9	37	Kroboth et al., *Antimocrob Agents Ch*, 21 (1982)
Acetaminophen	91.23	40.17	0.4	79	Critchley et al., *J Clin Pharm Ther*, 30 (2005)
6-Propyl-2-thiouracil	21.06	27.13	1.3	90	Kabanda et al., *J Pharm Pharmcol*, 48 (1996)
Candoxatril	0.9	5.74	6.4	58	Kaye et al., *Xenobiotica*, 27 (1997)
Flutamide	5.98	8.94	1.5	52	Anjum et al., *Br J Clin Pharmacol*, 47 (1999)
Triamcinolone	0.64	0.55	0.9	76	Hochhaus et al., *Pharmaceut Res*, 7 (1990)
Rifampicin	40.79	33.77	0.8	50	Rafiq et al., *Int J Agric Biol*, 12 (2010)
Sulfasalazine	49.76	450.8	9	56	Gu et al., *J Chromatogr B*, 879 (2011)
5,5-Diphenylhydantoin	135.56	67.25	0.5	94	Brien et al., *Europ J Clin Pharmacol*, 9 (1975)
Coumarin	0.007	0.183	25.7	64	Lamiable et al., *J Chromatogr*, 620 (1993)
Diphenhydramine hydrochloride	0.94	16.42	17.5	100	Toothaker et al., *Biopharm Drug Dispos*, 21 (2000)
Lovastatin	0.065	7.1	109	93	Kothare et al., *Int J Clin Pharm Th*, 45 (2007)
Carbaryl	0.15	0.51	3.4	37	May et al., *J Pharmacol Exp Ther*, 262 (1992)
Triabendazole	17.07	46.75	2.7	91	Bapiro et al., *Eur J Clin Pharmacol*, 61 (2005)
2,4-D	423.25	1209.5	2.9	100	Sauerhoff et al., *Toxicology*, 8 (1977)
Oxytetracycline dihydrate	14.29	97.2	6.8	50	Green et al., *Europ J Clin Pharmacol*, 10 (1976)
Picloram	0.97	166.66	171	98	Nolan et al., *Toxicol Appl Pharm*, 76 (1984)
Triclosan	1.41	0.76	0.5	96	Sandborgh-Englund et al., *J Toxicol Environ Health A*, 69 (2006)

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

PREDICTION OF ORAL BIOAVAILABILITY *in silico*

Michael Lawless, John DiBella, Michael B. Bolger, Robert D. Clark, Eva Huehn, Marvin Waldman, Jinhua Zhang, and Viera Lukacova
Simulations Plus, Inc. (www.simulations-plus.com)

Abstract

- 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 use 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_{int}) was the same as the MCP
- Overall, 58% of the molecules were predicted within 1.5-fold of their reported F%
- Scaling V_{max} by the CYP substrate model's confidence estimate resulted in fewer underpredictions

Drug	Dose [mg]	F%	MCP
fluphenazine	5	3.1	2D6
verapamil	80	22	3A4
oxycodone	15	42	3A4
irbesartan	150	70	2C9
vortioxetine	10	75	2D6
ibuprofen	400	85	2C9

Figure 1 – Examples of drugs in the data set along with their dose, F% and MCP.

Methodology

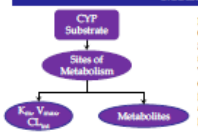


Figure 2 – CYP metabolism models for 5 CYP isoforms (1A2, 2C9, 2C19, 2D6, and 3A4). The first model predicts whether a molecule is a substrate for each CYP isoform. These predictions include confidence estimates.[5] Next, sites of metabolism are predicted for compounds that are predicted as substrates. Finally, kinetic parameters are predicted and metabolites are depicted.

QSAR Model	Description
S+Sw	aqueous solubility
S+Sp	aqueous solubility at specified pH
S+FaSSGF	solubility in simulated fasted stated gastric fluid
S+FaSSIF	solubility in simulated fasted state intestinal fluid
S+FeSSIF	solubility in simulated fed state intestinal fluid
S+logD	logD at specified pH
S+pKa	pK_a (single or multiple)
S+Peff	effective human jejunal permeability
S+PrUnbnd	percent unbound to plasma proteins
S+RBP	blood-to-plasma concentration ratio
DiffCoef	molecular diffusion coefficient in water
MET_XXX_Km	Kinetic Michaelis-Menten K_m constant (5 CYP isoforms)
MET_XXX_Vmax	Michaelis-Menten V_{max} constant (5 CYP isoforms)

Table 1 – QSAR models used in PBPK simulations.

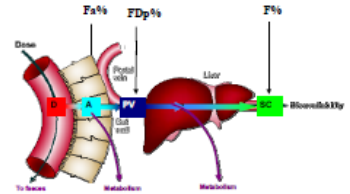
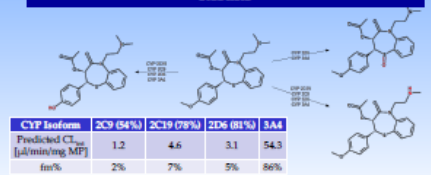


Figure 3[7] – Orally dosed drugs typically dissolve in the stomach and transit into the intestine, where they can be absorbed into the gut wall. Fa% (fraction absorbed) is the fraction of dose that is absorbed into the apical membrane of the gut epithelium. CYP enzymes metabolize some compounds in the enterocytes. FDp% is the fraction/percent of dose that makes it to the portal vein. F% is the fraction/percent of dose that enters systemic circulation. Fa%, FDp%, and F% were predicted by our GastroPlus™ PBPK simulations.

Results



CYP Isoform	2C9 (54%)	2C19 (78%)	2D6 (81%)	3A4
Predicted CL_{int} [µl/min/mg MP]	1.2	4.6	3.1	54.3
fm%	2%	7%	5%	86%

Figure 4 – Example of metabolite predictions for diltiazem. It is predicted to be a substrate of CYP 2C9, 2C19, 2D6, and 3A4 with confidence estimates shown in parenthesis. Three sites of metabolism are predicted and the metabolites are displayed. The table contains the predicted intrinsic clearances and the fraction/percent metabolized (fm%). CYP 3A4 is responsible for the majority of metabolism based on the predicted CL_{int}.

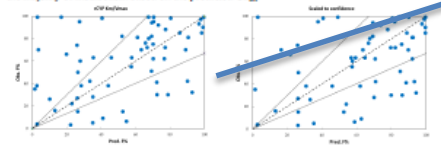


Figure 5 – Observed vs. predicted F% for 62 compounds. The dashed line is the line of unity. The dotted lines represent 1.5-fold errors. On the right-hand graph, V_{max} was scaled by the confidence estimate from the CYP substrate model (i.e., multiplied V_{max} x Confidence%/100), reducing severe underpredictions to avoid early rejection of good candidates.

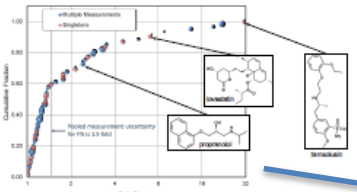


Figure 6 – Graph of cumulative fraction and fold error. F% was predicted to be within 1.5-fold of the reported value for 58% of the compounds. For 46 compounds, the reported F% either spanned a range (e.g., 80-90%) or included a standard deviation (e.g., 80 ± 15%). The area of the bubble is proportional to the expected measurement uncertainty. The F% of lovastatin is difficult to simulate due to opening and closing of the lactone ring. Tamsulosin has the highest fold error. NOTE: PBPK simulations using *in vitro* microsomal K_m and V_{max} values also resulted in large difference between reported and predicted F%. Propranolol was incorrectly predicted to be metabolized by 1A2; only including metabolism by 2D6 gives a correct F% prediction.

Conclusions

A dataset of 62 drugs along with dosage and F% was compiled. Each compound's reported MCP was CYP-mediated. Fa%, FDp%, and F% were estimated with PBPK simulations using physicochemical and CYP kinetic parameters predicted entirely from QSAR models. The CYP isoform associated with the MCP was correctly predicted in 42 of the 62 molecules. Additionally, 58% of the predicted oral bioavailability values were within 1.5-fold of the observed oral bioavailability. Scaling V_{max} by confidence estimates from our CYP substrate model reduced the number of underpredictions.

References

[1] Toshimoto K et al, *Drug Metabol. Disp.* Fast Forward. Published on August 14, 2014.
[2] Thummel KE et al., In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill; 2011.
[3] ADMET Predictor™ version 7.2, Simulations Plus, Inc., Lancaster, CA 93534 USA.
[4] GastroPlus™ version 9.0, Simulations Plus, Inc., Lancaster, CA 93534 USA.
[5] Clark RD et al., *J. Cheminform* 2014, 6:34.
[6] Hayduk W and Laudie H, *American Institute of Chemical Engineers J.* 1974, 20:611.
[7] Modified from van de Waterbeemd H and Gifford E. *ADMET In Silico Modelling: Towards Prediction Paradise?* Nat. Rev. Drug Disc. 2003, 2:192-204.

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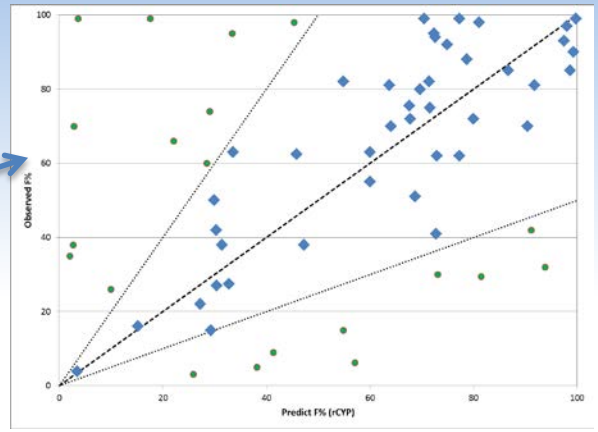
Lawless et al. (2015) ISSX Annual Meeting
Using QSAR & PBPK to predict human F%:
70% of compounds predicted within 2-fold



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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%
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  - A 35-year-old American male physiology was use 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_{int}) was the same as the MCP
- Overall, 68% of the molecules were predicted within 2-fold of their reported F%

[1] Toshimoto K et al, *Drug Metabol. Disp.* Fast Forward. Published on August 14, 2014.
[2] Thummel KE et al., In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill; 2011. Some F% values were from drug data sheet.
[3] ADMET Predictor™ version 7.2, Simulations Plus, Inc., Lancaster, CA 95354 USA.
[4] GastroPlus™ version 9.0, Simulations Plus, Inc., Lancaster, CA 95354 USA.

Predicting drug bioavailability using PBPK modeling and Global Sensitivity Analysis to identify sensitive parameters

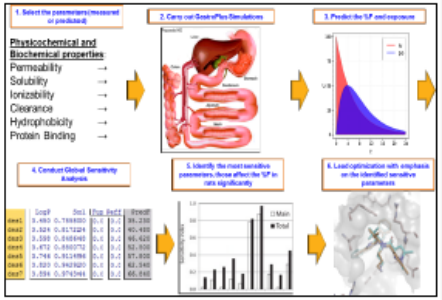
Pankaj R. Daga[1], Michael B. Bolger[2], Ian S. Haworth[3], Robert D. Clark[2], and Eric Martin[*1]

[1]Novartis Institute of Biomedical Research, Emeryville, CA 94608, United States, [2]Simulations Plus Inc., Lancaster, CA 93534, United States, [3]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90089, United States

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



Conclusions

- PBPK ADME simulations successfully adapted to lead series:
  - Predicting clearance was solved with a local QSAR for "ideal" CL_{fit}
  - In 3 cases, >80% of %F predictions within 2X all *in silico*
  - Good local QSAR for CL_{fit} 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:
    - DPP4 &HSD1 only CL_{int};
    - Kinase: CL_{int} + logD, Sw and RBP
  - Specific advice for each compound within series

Reliable results using local QSAR for fitted intrinsic clearance

DPP-4 Inhibitors (Merck)

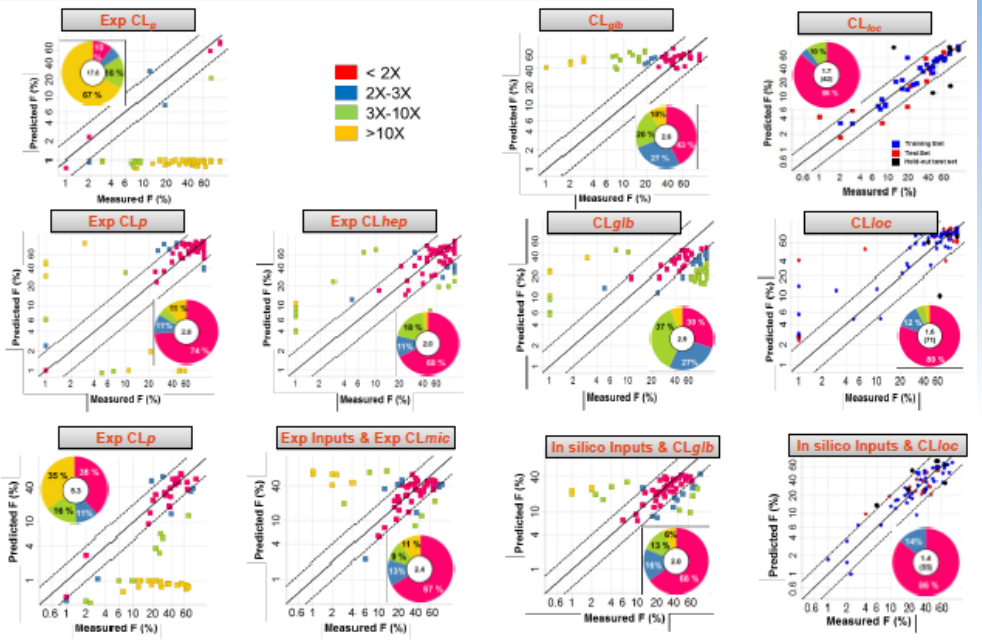
- ✓ 49 Inhibitors
- ✓ RAT *in vivo* data : %F, CLp
- ✓ Physicochem prop & *in vitro* data : --

11β-HSD1 Inhibitors (AZ)

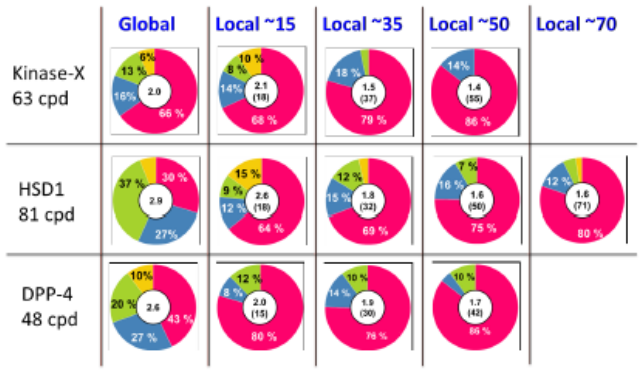
- ✓ 81 Inhibitors
- ✓ RAT *in vivo* data : %F, CLp
- ✓ Physicochem prop & *in vitro* data: $CL_{int(hep)}$

Kinase-X inhibitors (In-House)

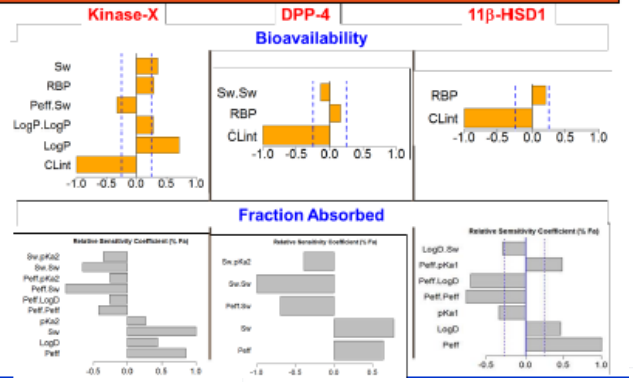
- ✓ 63 Compounds
- ✓ RAT *in vivo* data : %F, CLp, AUC, Cmax
- ✓ Physicochem prop & *in vitro* data : Sol, Perm, PPB, $CL_{int(mic)}$



Series can be modeled from as few as 15 rat studies



Sensitivity coefficients identify series-specific properties that control %F



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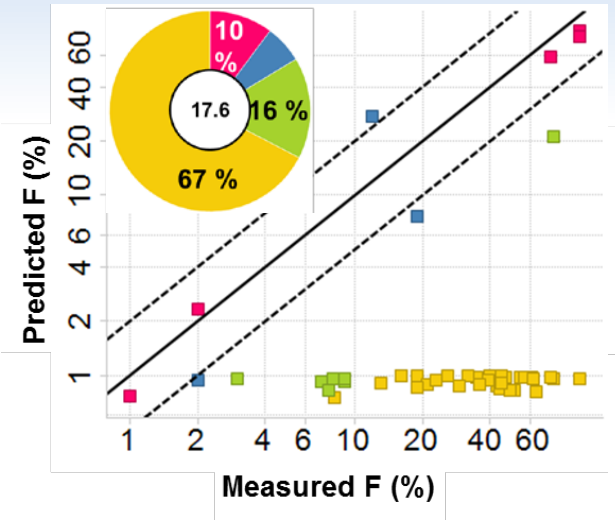
Prediction of F% Using *in silico* Physicochemical Properties and *in vitro*, Predicted or Fitted Clearance - Case Study 1

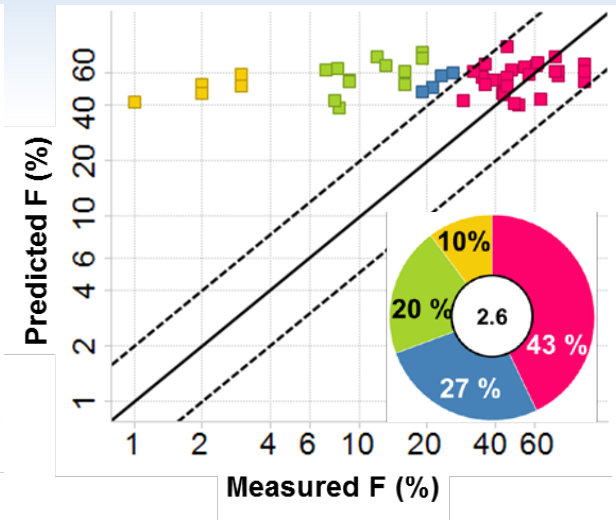
- **49 Compounds:** Single Med Chem series reported by Merck in various papers
  - RAT *in vivo* data: %F, CLp
  - Physicochemical prop & *in vitro* data: -

1 Below 2-Fold
2 Between 2-fold and 3-fold
3 Between 3- and 10-fold
4 Beyond 10-fold

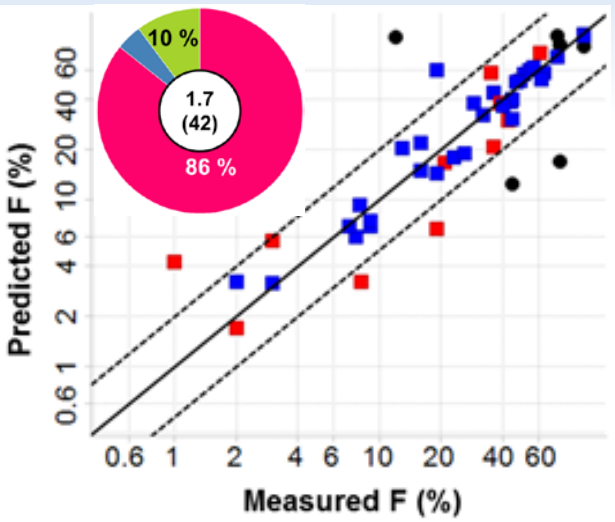
Exp CL_p



CL_{global}



CL_{local}



- **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**

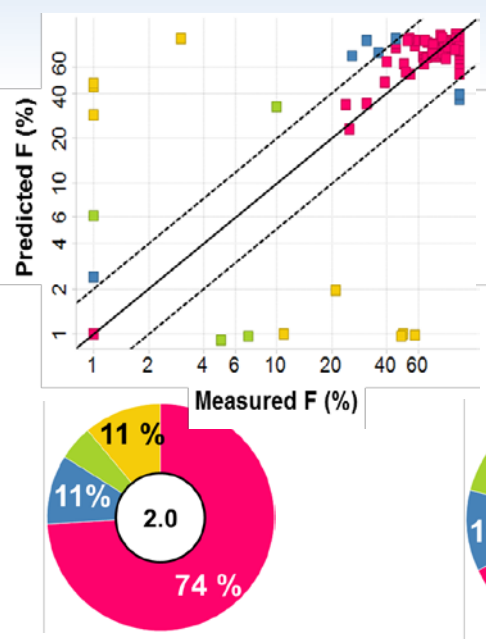
Daga et al. (2015) Gordon Research Conf.

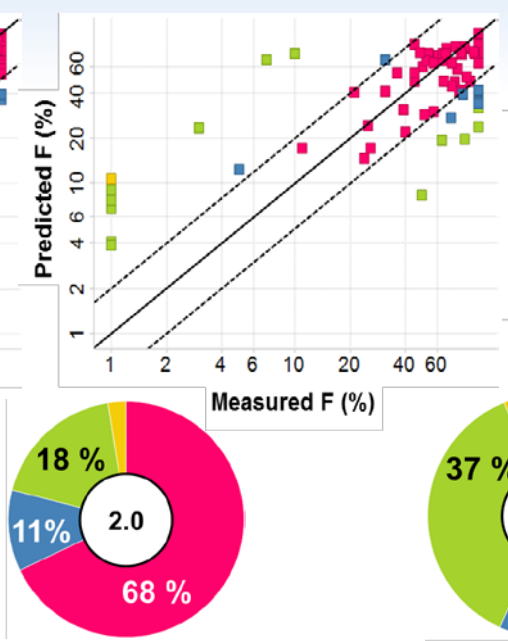
Prediction of F% Using *in silico* Physicochemical Properties and *in vitro*, Predicted or Fitted Clearance - Case Study 2

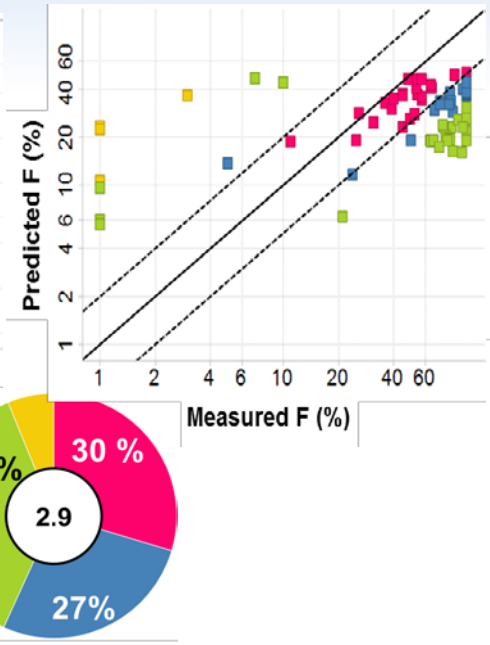
- **81 Compounds:** Single Med Chem series reported by Astra-Zeneca in 4 publications
  - RAT *in vivo* data: %F, CLp
  - *in vitro* data: CL_{int}(hep)

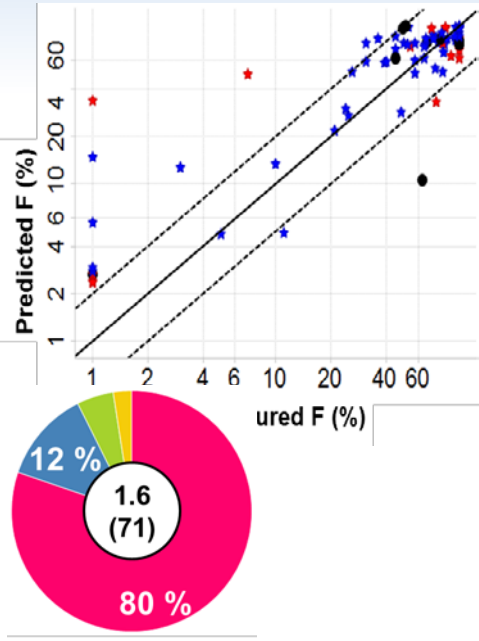
1 Below 2-Fold
2 Between 2-fold and 3-fold
3 Between 3- and 10-fold
4 Beyond 10-fold

Exp CL_p	Exp Hep CL_{int}	CL_{global}	CL_{local}









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

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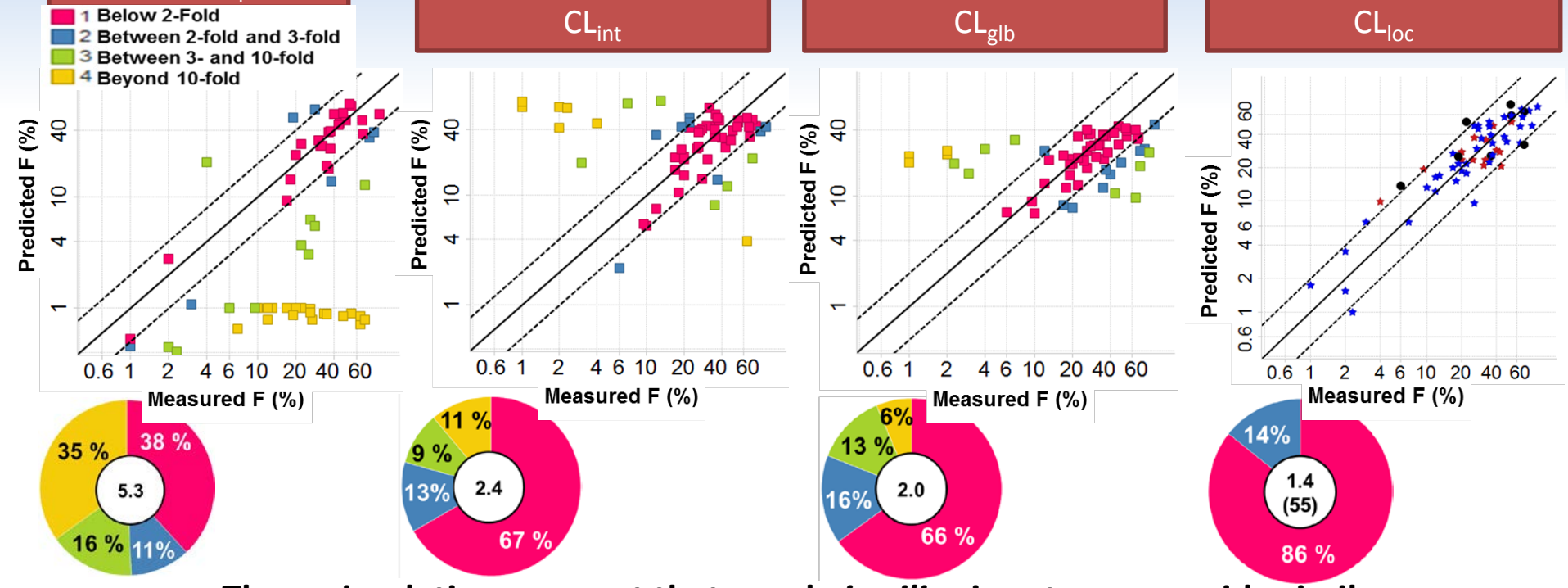
Prediction of F% Using *in silico* as well as Experimental Physicochemical Properties and *in vitro*, Predicted, or Fitted Clearance - Case Study 3

- **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})

Exp CL_p	Exp input properties & CL_{int}	in silico input prop & CL_{glb}	In silico input prop & CL_{loc}



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

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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 CL_{int} and $t_{1/2}$:

$$CL_{\text{int}} = \frac{0.693}{t_{1/2}} * \frac{\text{ml incubation}}{\text{mg microsomes}} * \frac{38\,\text{mg microsomes}}{\text{g liver}} * \frac{x\,\text{g liver}}{\text{kg b.w.}}$$

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

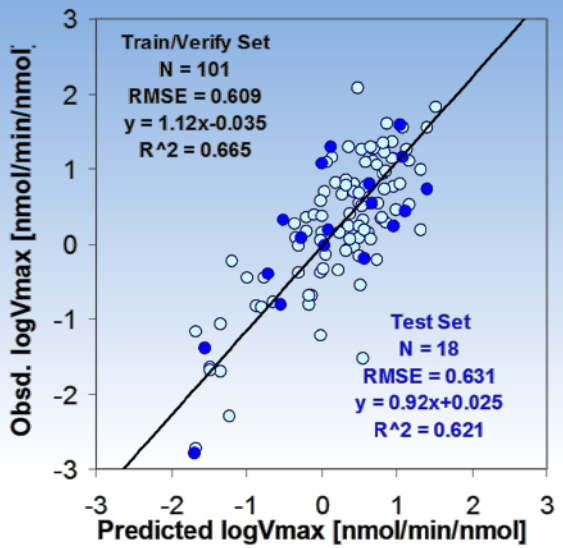
$$\frac{\text{rate}}{[\text{one million cells}]} \times \frac{[\text{millions of cells}]}{[\text{g of tissue}]} \times [\text{g of tissue}] = \frac{\text{rate}}{[\text{tissue}]}$$

rCYP

$$\frac{\text{rate}}{[\text{pmol of enzyme}]} \times \frac{[\text{pmol of enzyme}]}{[\text{mg of microsomal protein}]} \times \frac{[\text{mg of microsomal protein}]}{[\text{g of tissue}]} \times [\text{g of tissue}] = \frac{\text{rate}}{[\text{tissue}]}$$

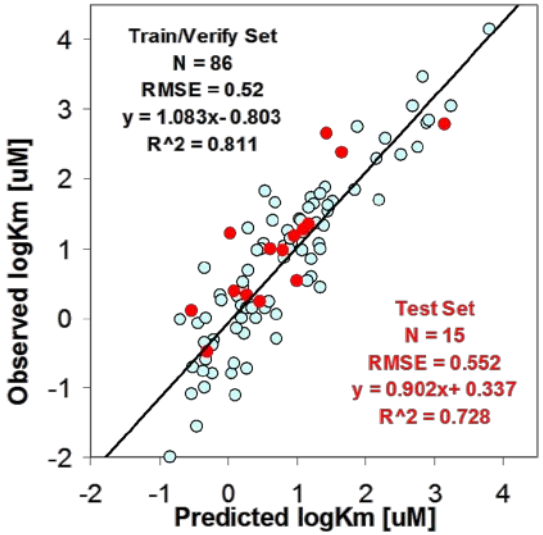
Model performance… CYP2D6

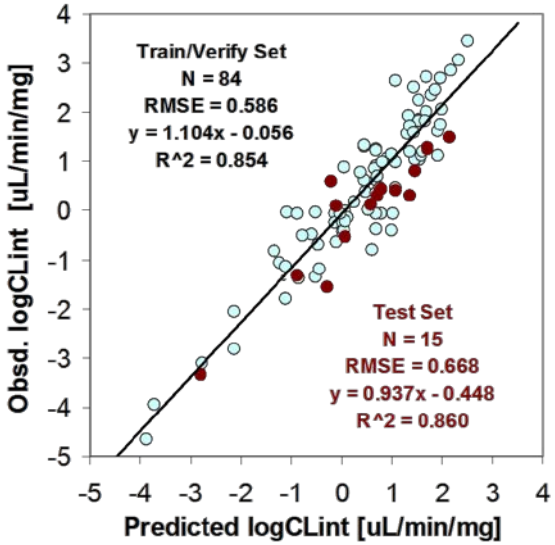
Model	Data Sets	Negatives	Positives	Total	Correct	Concordance	Sensitivity	Specificity
CYP_1A2_Substr	Training	799	410	1209	988	81.7%	79.8%	82.7%
	Test	138	75	213	170	79.8%	80.0%	79.7%
CYP_2A6_Substr	Training	479	154	633	516	81.5%	80.5%	81.8%
	Test	89	23	112	89	79.5%	78.3%	79.8%
CYP_2B6_Substr	Training	470	200	670	551	82.2%	80.5%	83.0%
	Test	87	31	118	97	82.2%	80.6%	82.8%
CYP_2C8_Substr	Training	461	199	660	510	77.3%	72.9%	79.2%
	Test	83	34	117	90	76.9%	73.5%	78.3%
CYP_2C9_Substr	Training	838	333	1171	917	78.3%	72.7%	80.5%
	Test	154	53	207	161	77.8%	71.7%	79.9%
CYP_2C19_Substr	Training	859	283	1142	934	81.8%	76.0%	83.7%
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CYP_2D6_Substr	Training	822	416	1238	1032	83.4%	81.0%	84.5%
	Test	142	76	218	174	79.8%	80.3%	79.6%
CYP_2E1_Substr	Training	472	213	685	573	83.6%	79.8%	85.4%
	Test	74	47	121	109	90.1%	80.9%	95.9%
CYP_3A4_Substr	Training	416	971	1387	1165	84.0%	85.3%	81.0%
	Test	79	166	245	202	82.4%	81.3%	84.8%



V_{max}

K_m





CL_{int}

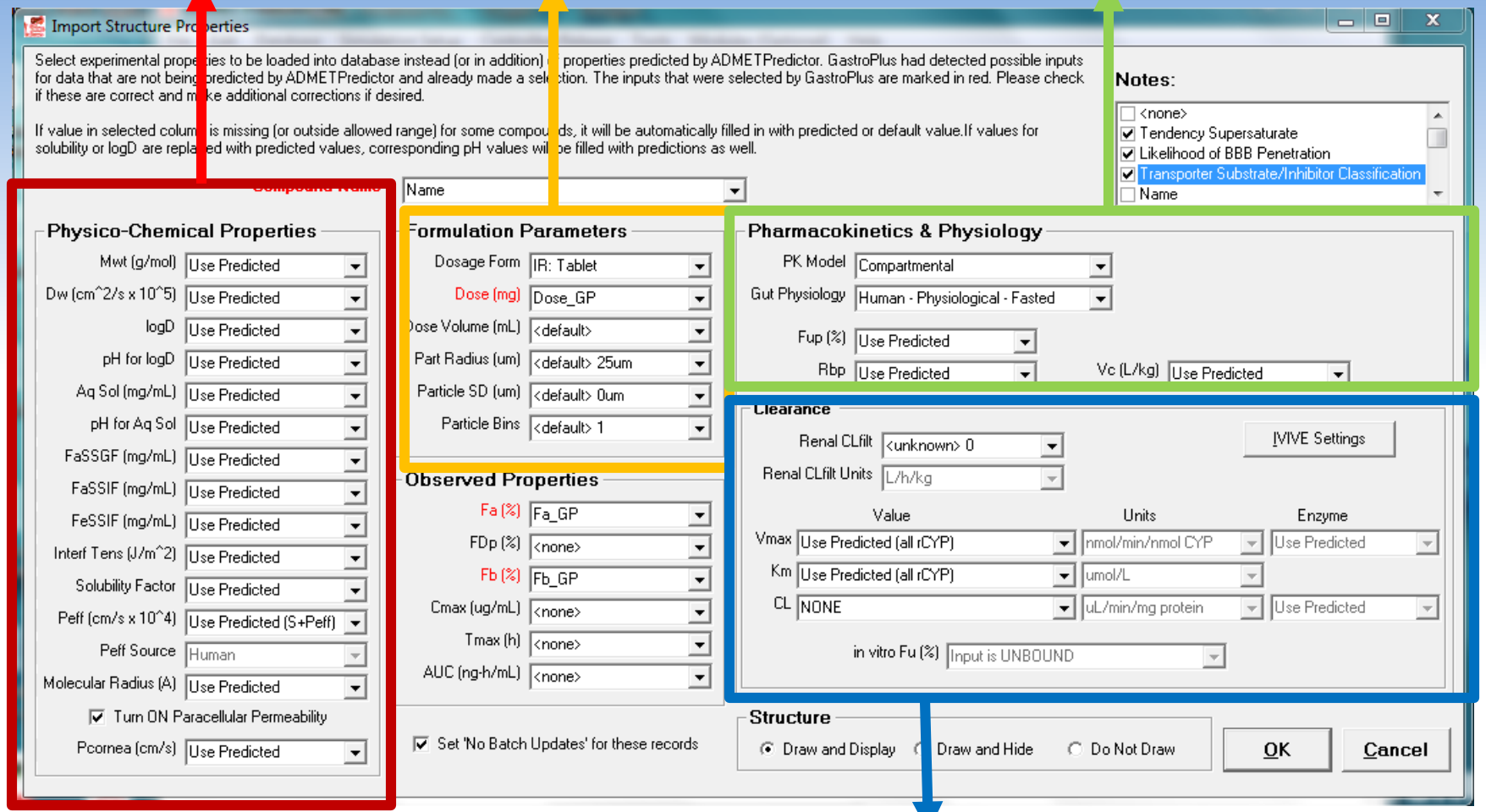
Zhang et al., ACS National Meeting (2013)

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

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)?