Applying *in silico-in vitro-in vivo* Extrapolation (IS-IV-IVE) Techniques to Predict Exposure and Guide Risk Assessment

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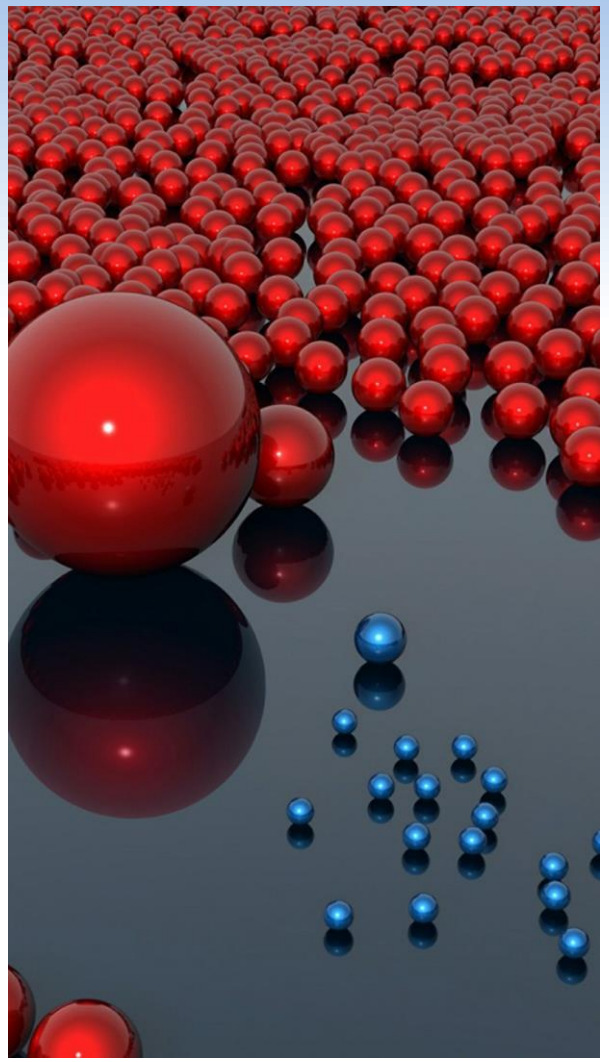
Applying IS-IV-IVE Techniques to Predict Exposure and Guide Risk Assessment

- PBPK models predict the relationship between dose and the exposure by accounting for the processes of ADMET
- IS-IV-IVE models allow
  - *in silico* and *in vitro* data to be extrapolated to estimate corresponding *in vivo* effects
- IS-IV-IVE provides
  - efficient characterization of potential chemical risk

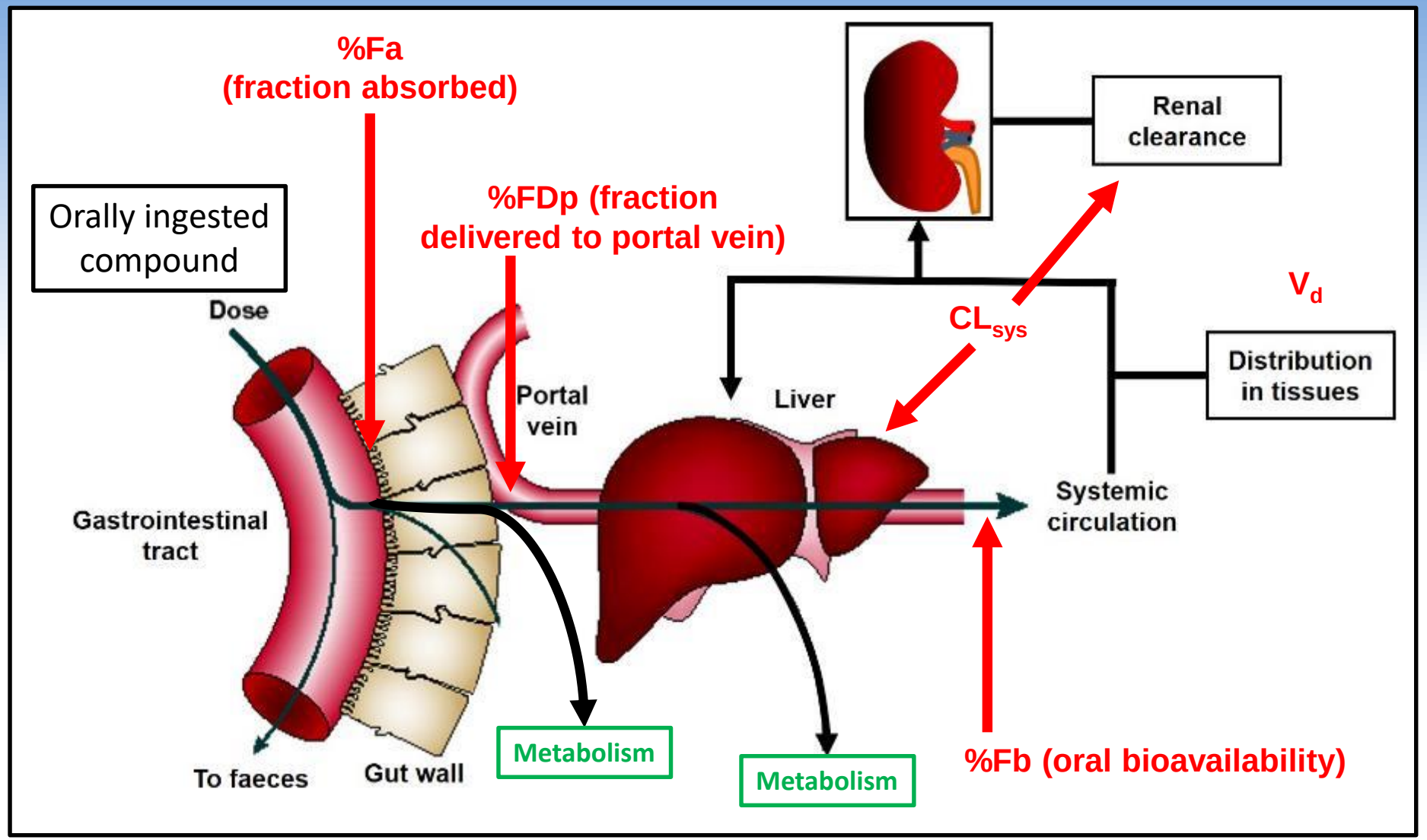


IS-IV-IVE Techniques to Predict Exposure and Guide Risk Assessment

- What's happening *in vivo*?
- HTPK and PBPK simulations – inputs and outputs
- Extended clearance classification system (ECCS) and related models
- Human and rat liver microsomal clearance models
- Cytochrome P450 models
  - Substrate/nonsubstrate models for nine (9) CYP isoforms
  - Sites of metabolism models for nine (9) CYP isoforms
  - Michaelis-Menten kinetic parameter models for five (5) CYP isoforms
- Applications – Simulations in rats and humans for agrochemical, industrial, and pharmaceutical compounds

Disclosure/Conflict of Interest
Michael Lawless is an employee of Simulations Plus, the developers of GastroPlus® and ADMET Predictor®.

What's happening *in vivo* ?



* Modified from van de Waterbeemd, H, and Gifford, E. Nat. Rev. Drug Disc. 2003, 2:192-204.

PK Simulation Inputs and Outputs

HTPK Simulation
PBPK Simulation

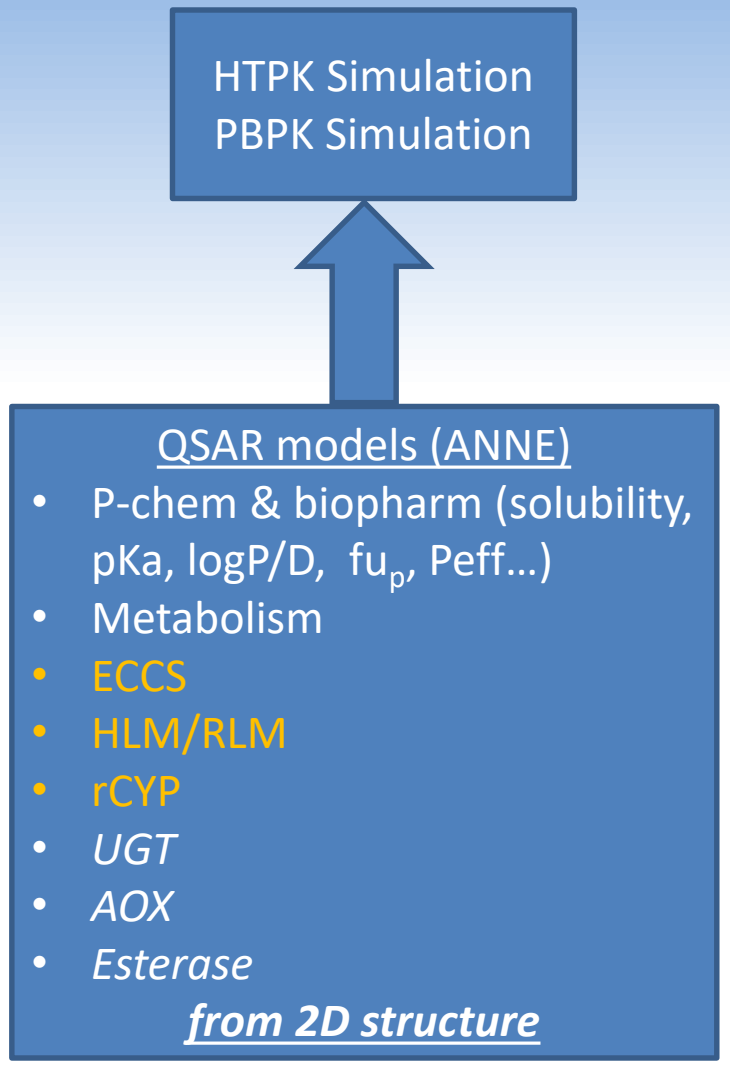
- Rat and human species
- Advanced Compartmental and Transit (ACAT™) model for GI tract
- Minimal PBPK
- IR tablet
- Passive absorption
- Linear liver clearance
- Renal clearance
- Mechanistic V_d
- **Takes less than 0.06 seconds per structure**

PK Simulation Inputs and Outputs

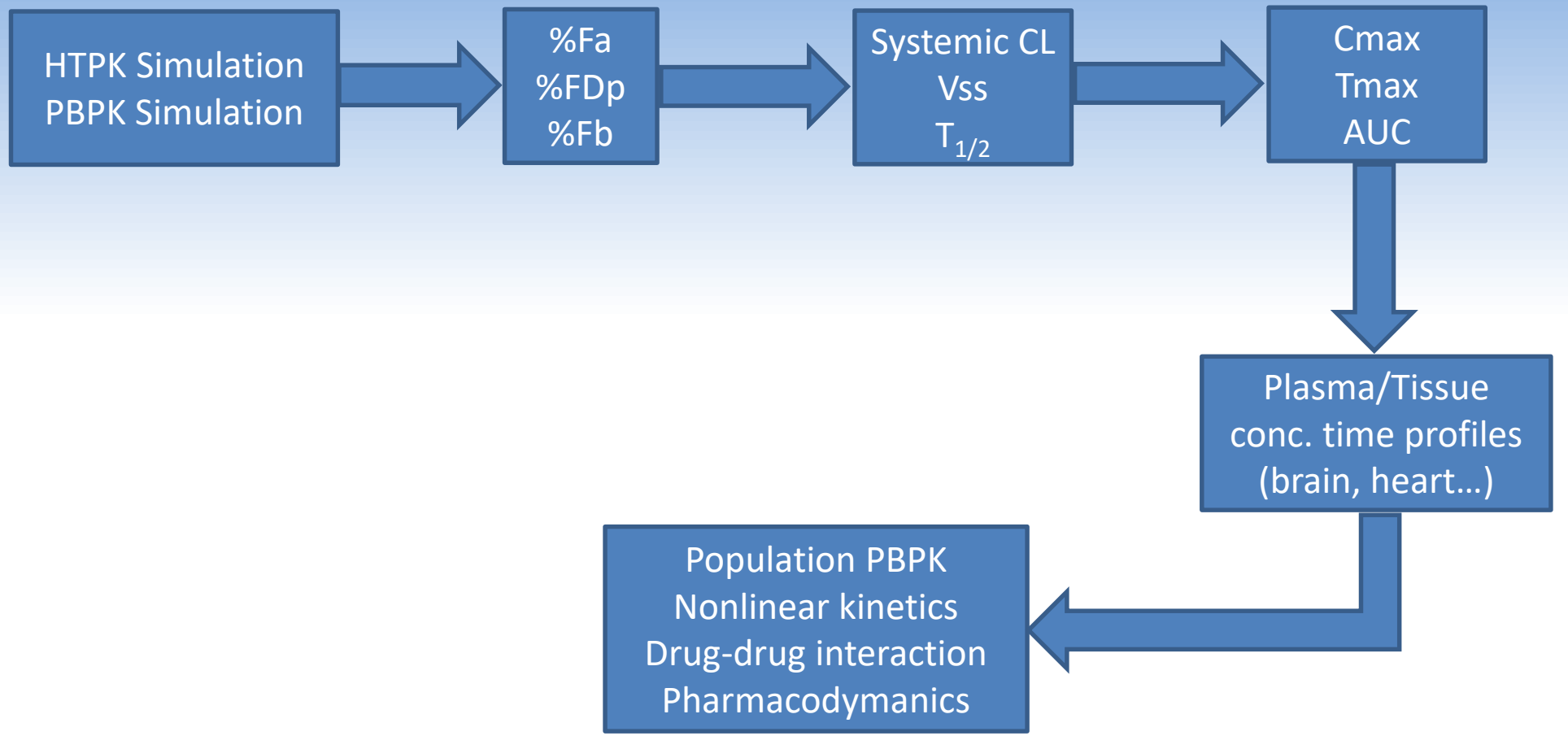
HTPK Simulation
PBPK Simulation

- Preclinical and human species
- ACAT™ model
- Full PBPK – tissue concentrations
- Multiple dosage forms (PO, IV, dermal, inhalation)
- Active and passive absorption
- Linear and non-linear liver clearance
- Renal clearance
- Mechanistic V_d

PK Simulation Inputs and Outputs



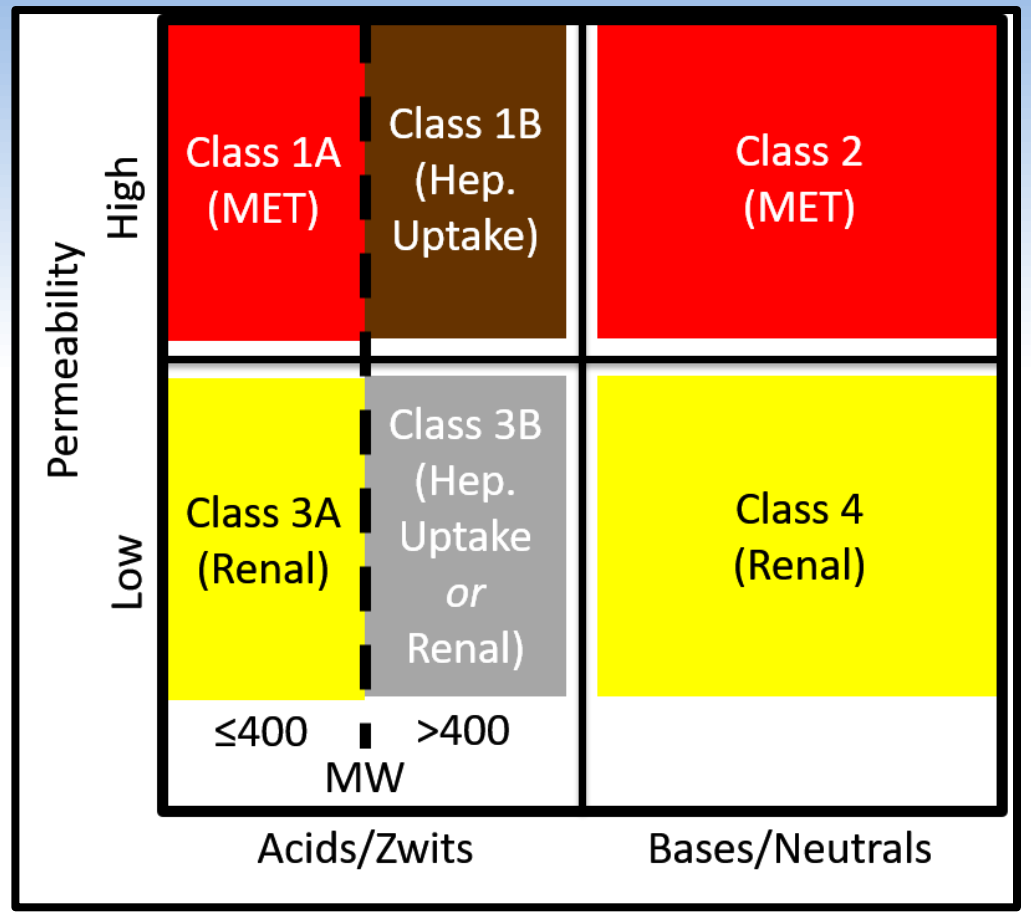
PK Simulation Inputs and Outputs



ECCS Classification System

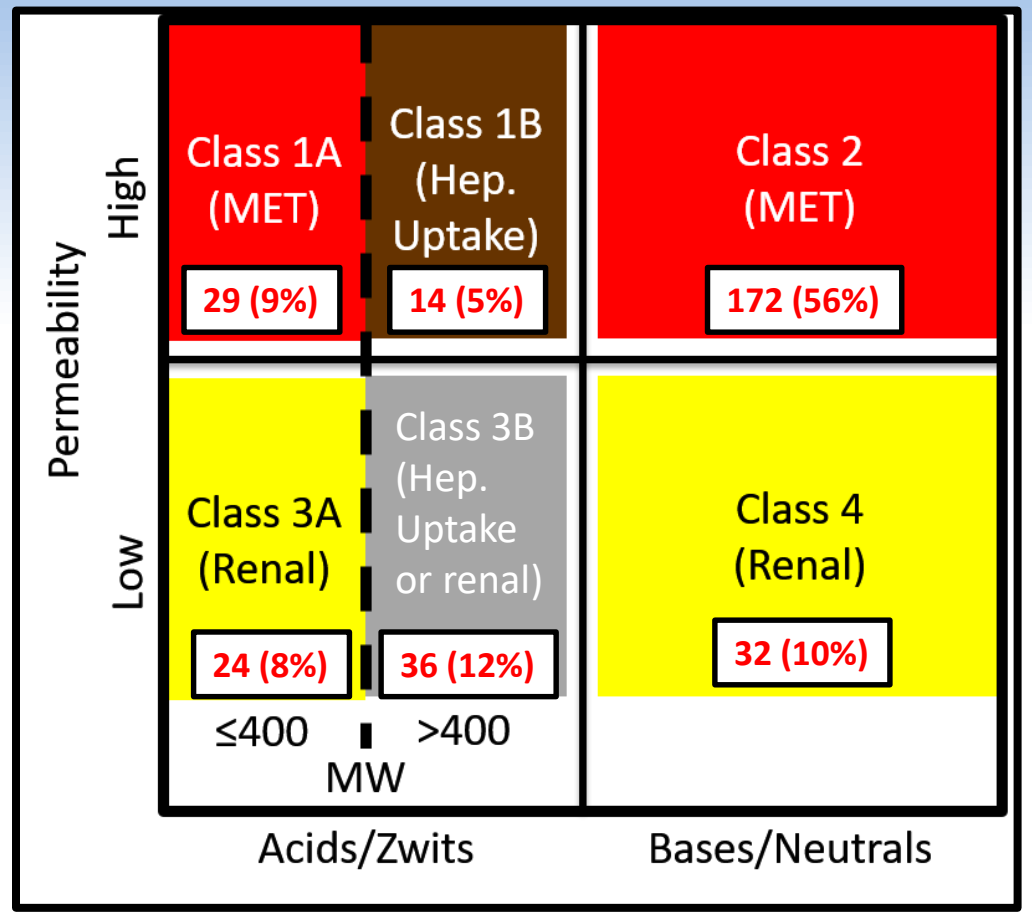
Varma MV, Steyn SJ, Allerton C, El-Kattan, AF. *Pharm. Res.* **2015**, 32(12), 3785-3802.

- Categorizes compounds according to the *rate-determining*, predominant clearance mechanism
  - Metabolism, hepatic uptake, or renal
  - Limited to small molecules, MW ≤ 700 Da
- Based on physicochemical properties and passive permeability
  - Low or high MW (cutoff is 400 Da)
  - Ionization: acids/zwits or bases/neutral
    - Used MoKa to predict pKa (at pH = 7.0)
  - Low or high MDCK permeability (cutoff is 5 x 10^{-6} cm/s)
    - Used either experimental data or predictions
- Scheme was applied to 307 compounds
  - Compounds had single clearance mechanism that accounted for >70% of systemic clearance
- Correctly categorizes ~92% of the compounds
  - Class 3B is "hep uptake OR renal". The above statistic counts these as correct if the observed value is hep uptake or renal

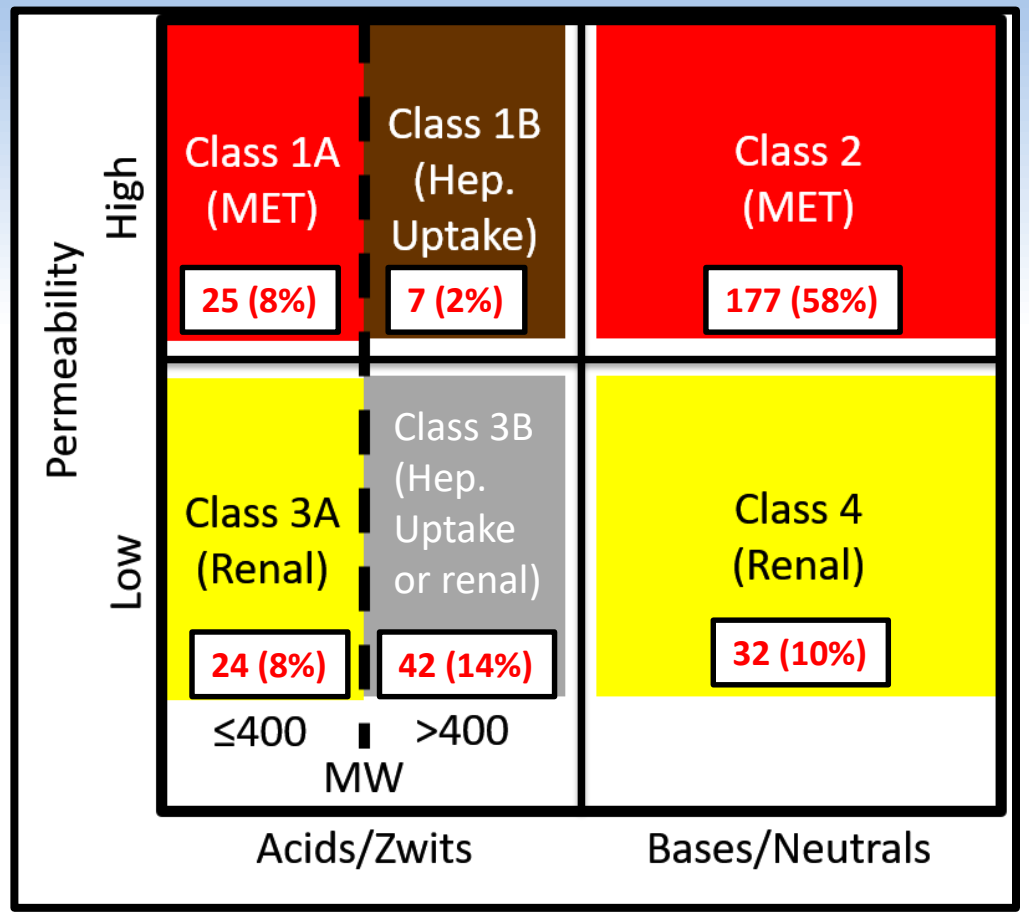


Comparison of ECCS and ECCS_Class

Varma et al. ECCS



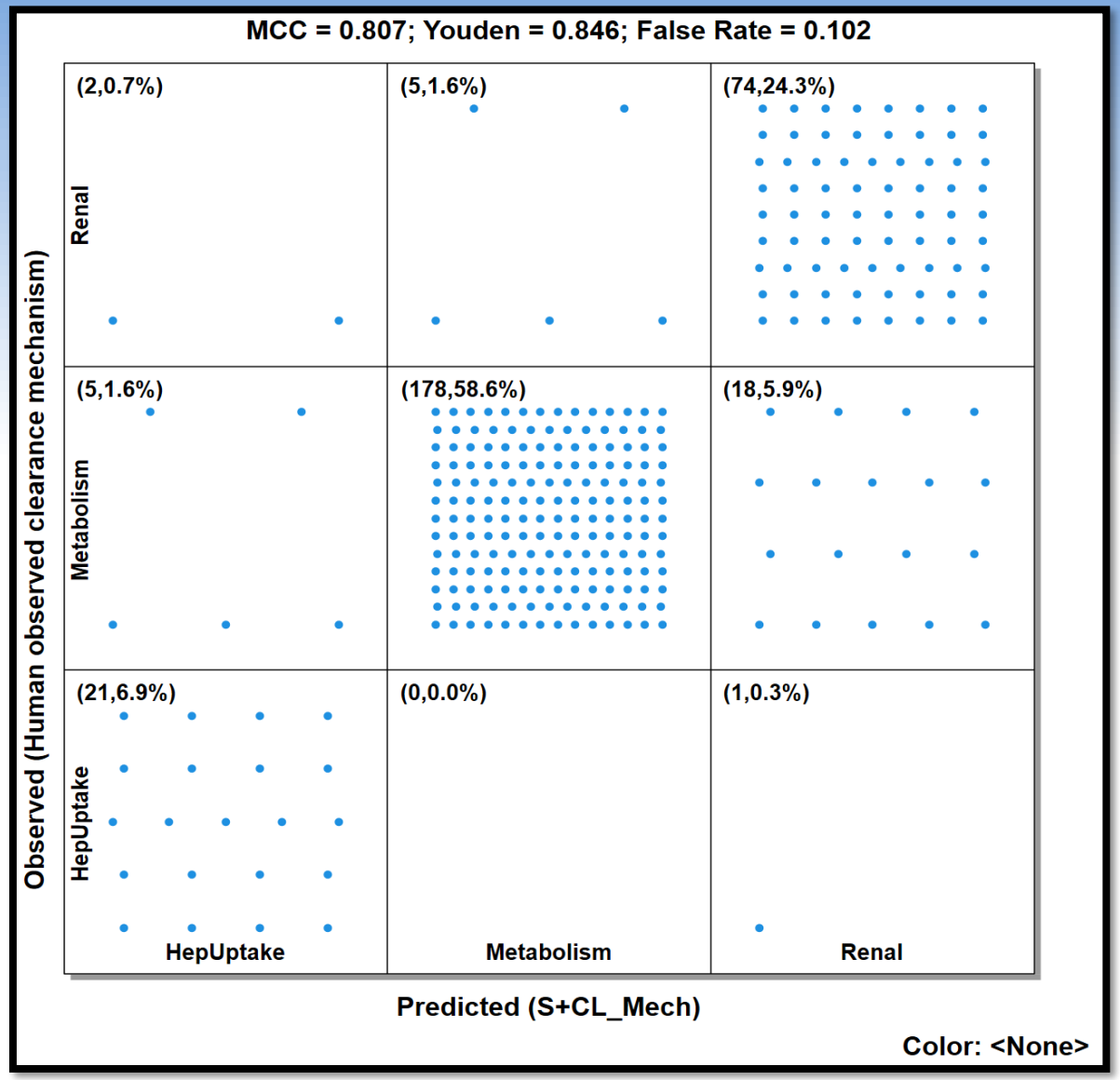
ECCS_Class
(our implementation)



S+CL_Mech

- Ternary model to predict major clearance pathway (renal, metabolism, or hepatic uptake)
- There are no ambiguous categories

Set	No. of cmpds	Youden	MCC	Concordance
Training	264	0.84	0.80	0.89
Test	40	0.90	0.85	0.95



Binary Classification Models to Predict Major Clearance Mechanism

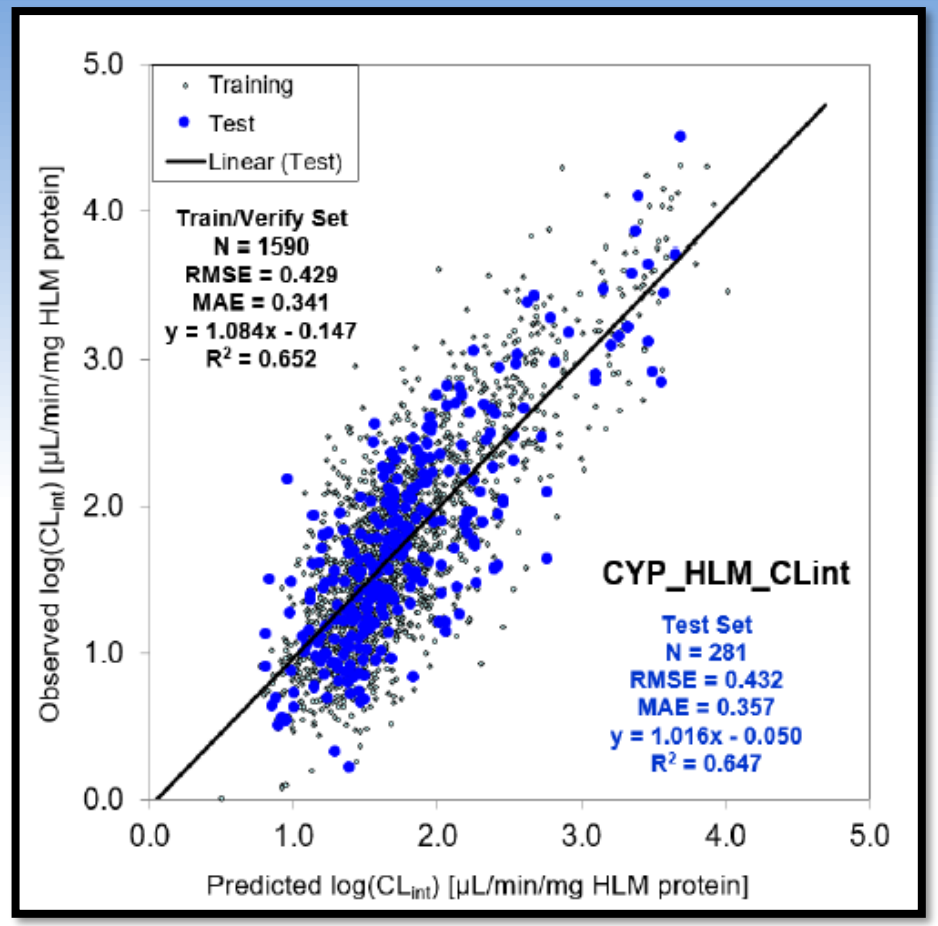
Many drugs have a mixture of clearance pathways, i.e., <70% of the drug is cleared by a single mechanism. In fact, Varma was only able to characterize 307 out of 739 drugs cleared by a predominate pathway. We constructed binary classification models with the expectation that they might be more suitable when analyzing leads that might not have a single dominate clearance mechanism.

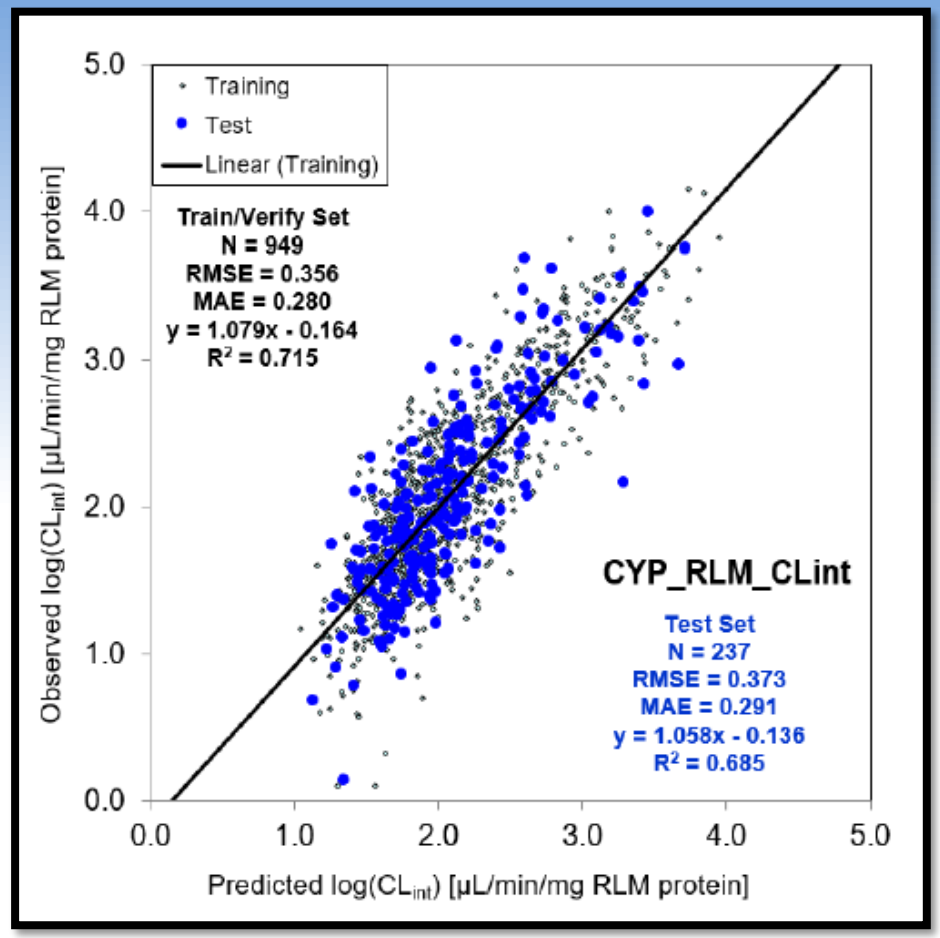
- S+CL_Metab predicts whether or not the rate-limiting clearance mechanism is metabolic
- S+CL_Renal predicts whether or not renal excretion dominates clearance
- S+CL_Uptake predicts whether or not the rate-limiting clearance mechanism is hepatic uptake

These models include *confidence estim*ates. The table below lists the statistics for each model.

Model	Set	Negatives	Positives	Total	Correct	Concordance	Sensitivity	Specificity
S+CL_Metab	**Training**	**241**	**15**	**256**	**254**	**99.2%**	**100.0%**	**99.1%**
	Test	**41**	**7**	**48**	**47**	**97.9%**	**85.7%**	**100.0%**
S+CL_Renal	**Training**	**182**	**71**	**253**	**238**	**94.0%**	**94.3%**	**93.9%**
	Test	**41**	**10**	**51**	**47**	**92.1%**	**90.0%**	**92.6%**
S+CL_Uptake	**Training**	**86**	**167**	**253**	**236**	**93.2%**	**93.4%**	**93.0%**
	Test	**17**	**34**	**51**	**47**	**92.1%**	**91.1%**	**94.1%**

HLM and RLM Models





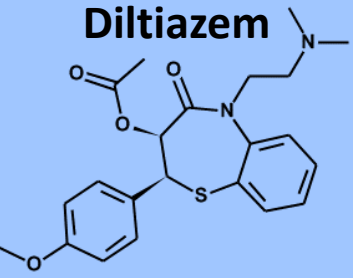
These data sets were extracted from the ChEMBL database* and contains drugs and drug leads. Original references were checked for correct units and microsomal concentrations.

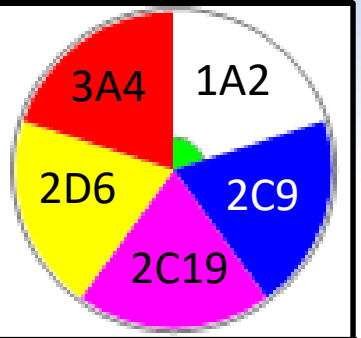
*https://www.ebi.ac.uk/chembl/

CYP Metabolism Models

CYP Substrate?

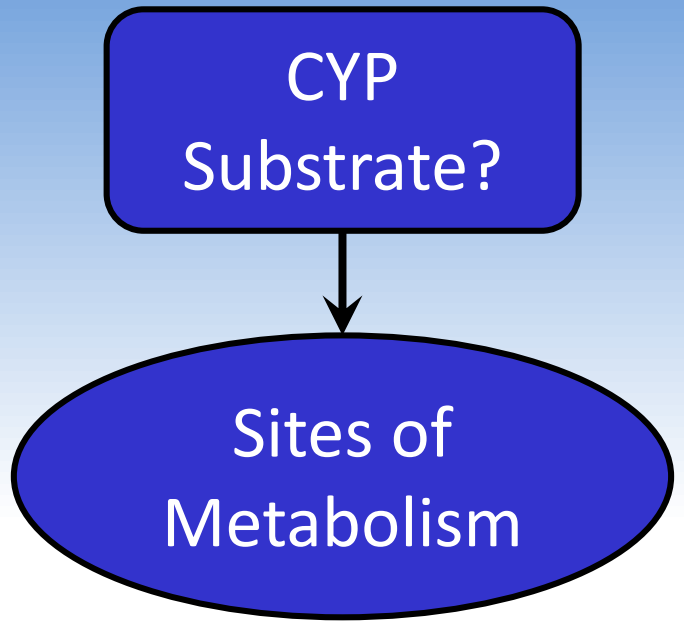
Diltiazem

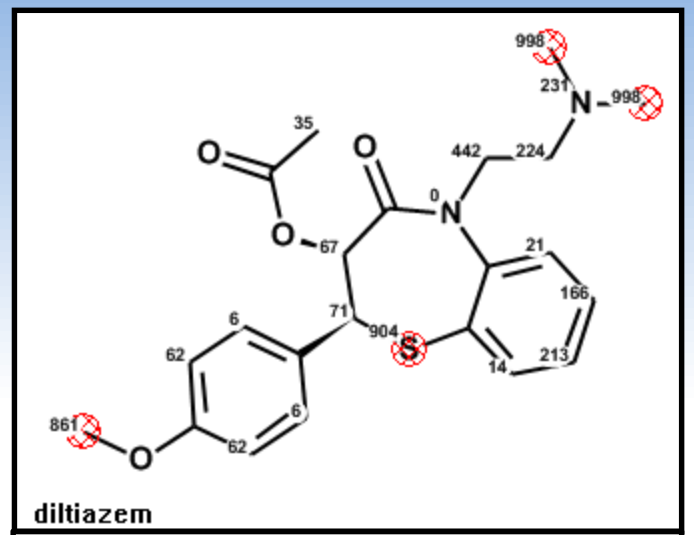




CYP Subst Star Plot: Predicted to be a substr. for all 5 CYPs except 1A2

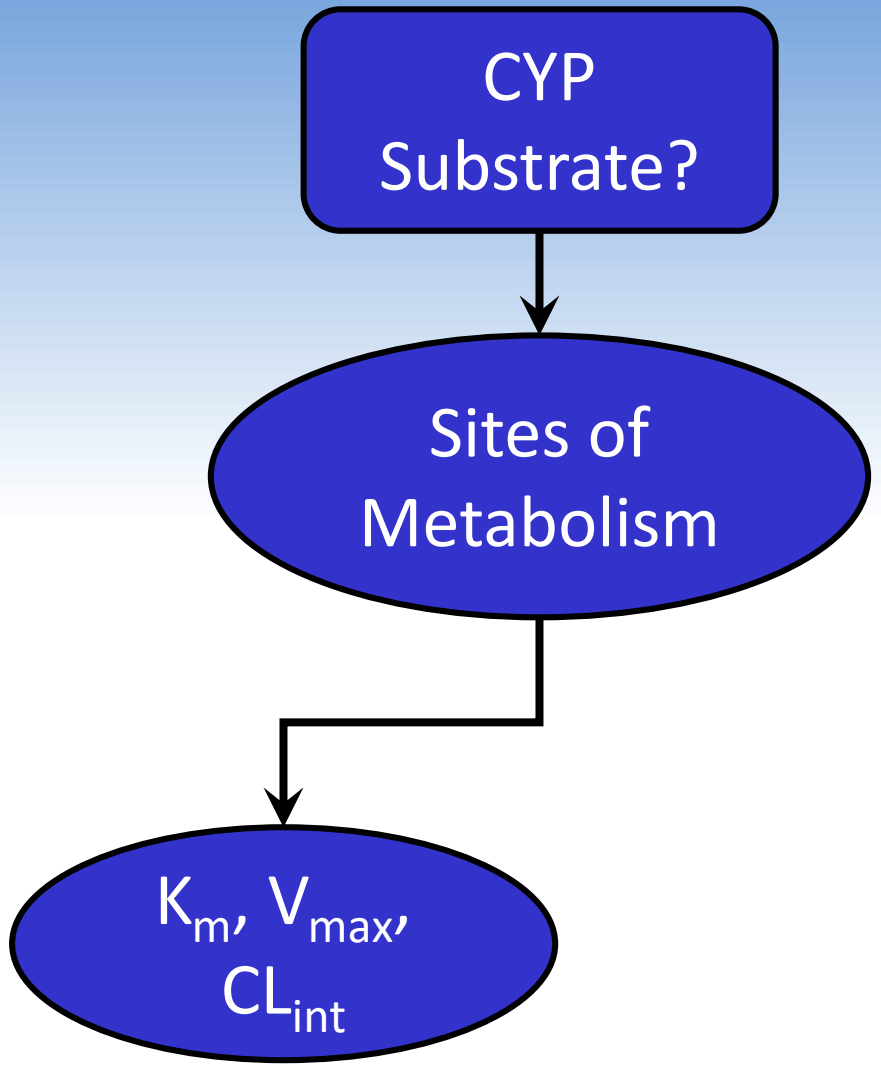
CYP Metabolism Models

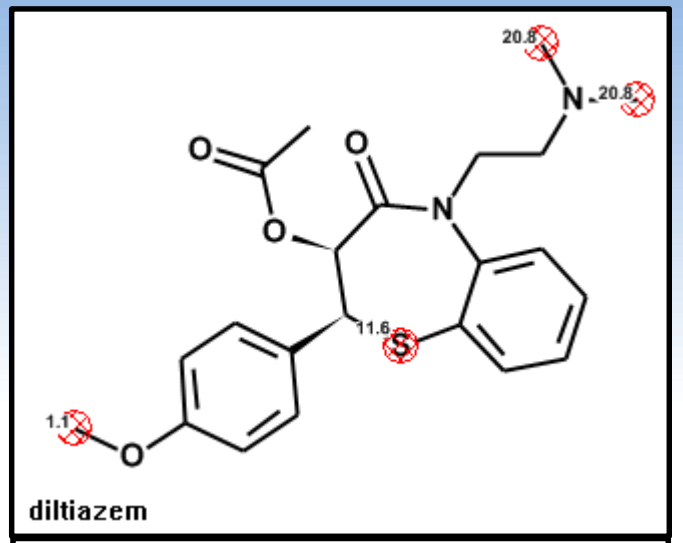




Predicted 3A4 sites of metabolism (red mesh) and scores

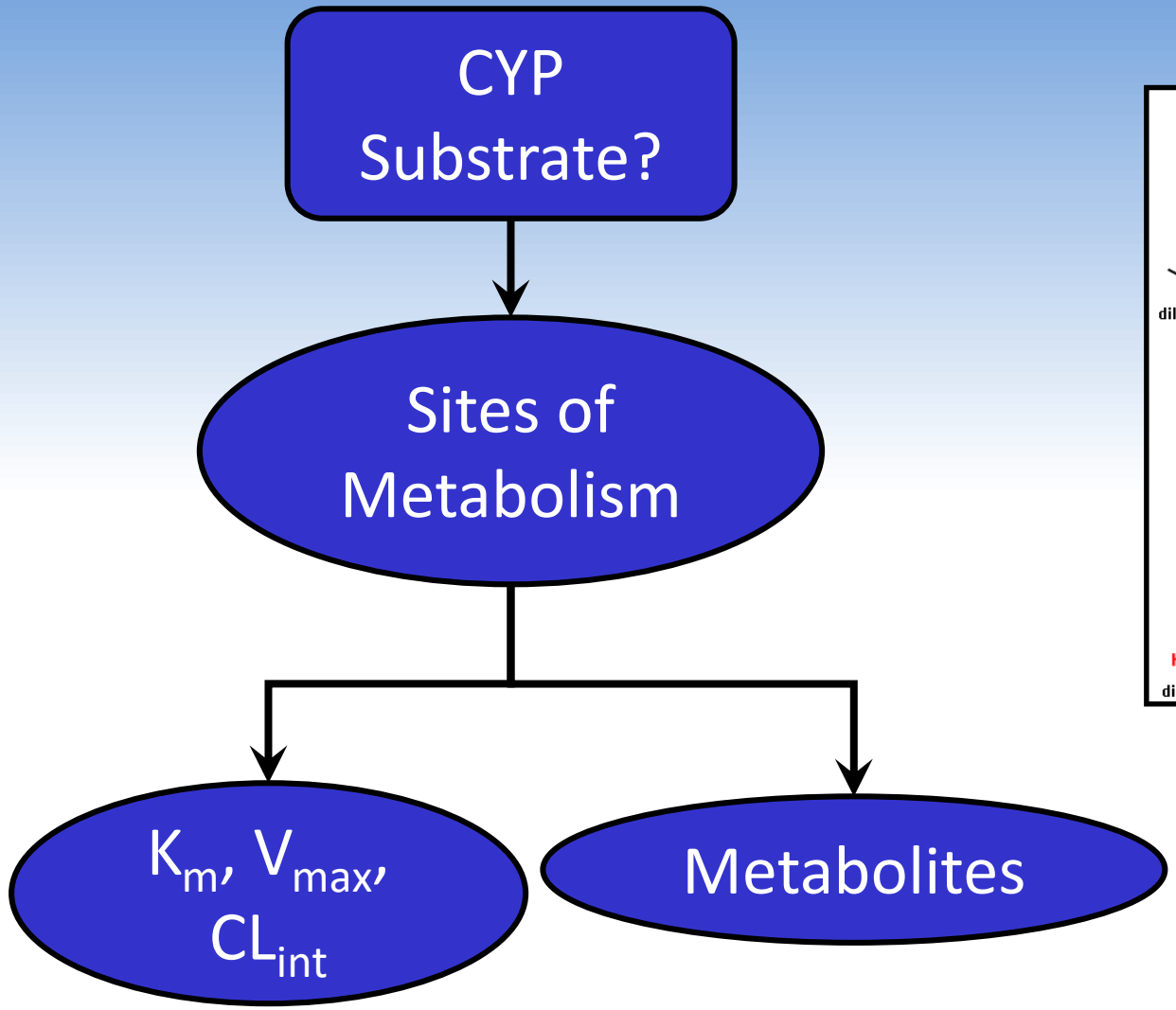
CYP Metabolism Models

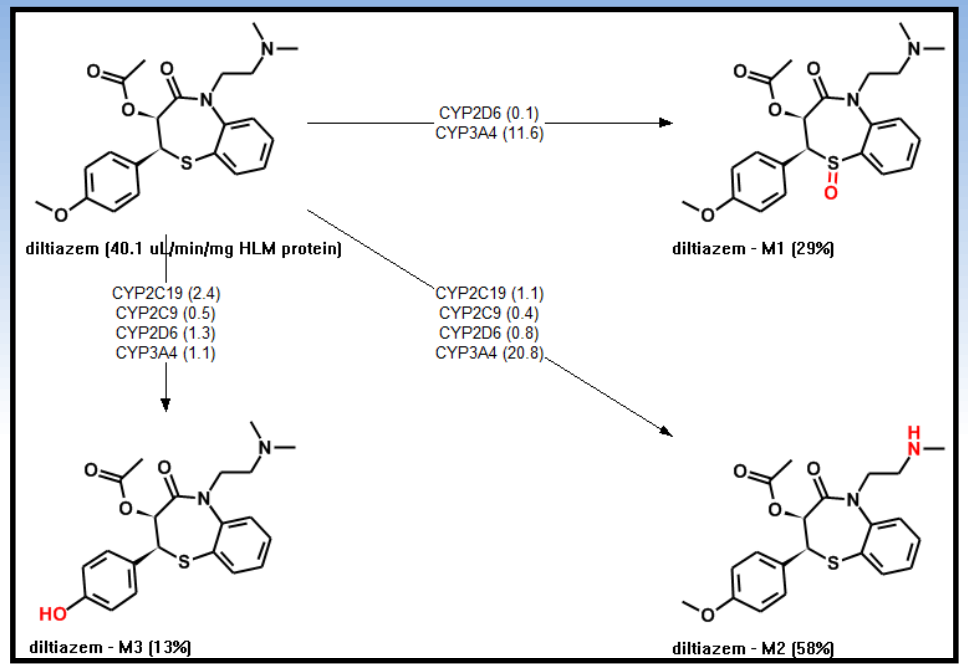


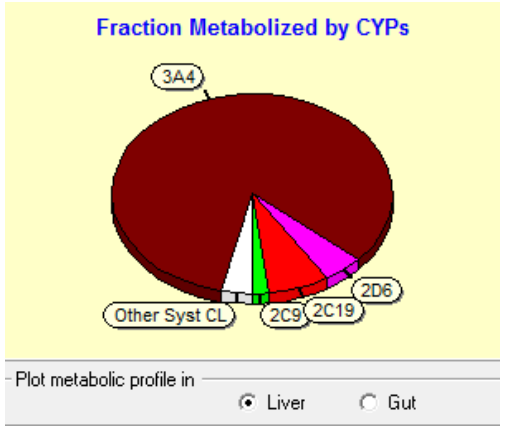


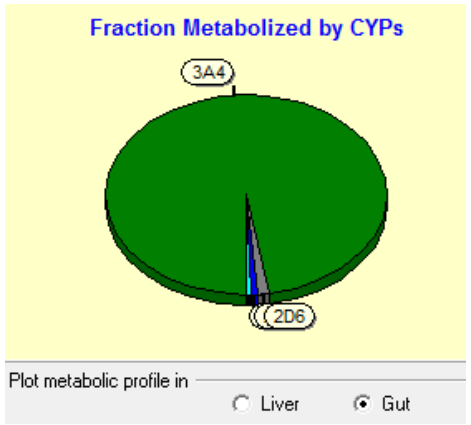
Predicted 3A4 atomic CL_{int}

CYP Metabolism Models









CYP Substrate Classification Models

Model		Negatives	Positives	Total	Correct	Concordance	Sensitivity	Specificity
CYP1A2_Substr	Trn/Ver	753	379	1132	946	83.5%	80.7%	84.9%
	Test	184	99	283	231	81.6%	80.8%	82.0%
CYP2A6_Substr	Trn/Ver	448	147	595	478	80.3%	82.3%	79.6%
	Test	119	30	149	118	79.1%	80.0%	78.9%
CYP2B6_Substr	Trn/Ver	437	192	629	507	80.6%	81.7%	80.0%
	Test	119	39	158	129	81.6%	84.6%	80.6%
CYP2C8_Substr	Trn/Ver	433	183	616	478	77.5%	77.5%	77.5%
	Test	109	46	155	120	77.4%	76.0%	77.9%
CYP2C9_Substr	Trn/Ver	794	300	1094	826	75.5%	80.0%	73.8%
	Test	196	78	274	203	74.0%	74.3%	73.9%
CYP2C19_Substr	Trn/Ver	812	259	1071	855	79.8%	80.3%	79.6%
	Test	203	65	268	216	80.5%	72.3%	83.2%
CYP2D6_Substr	Trn/Ver	814	421	1235	995	80.5%	80.2%	80.7%
	Test	149	70	219	181	82.6%	80.0%	83.8%
CYP3A4_Substr	Trn/Ver	390	892	1282	1060	82.6%	82.1%	83.8%
	Test	88	233	321	264	82.2%	82.4%	81.8%
CYP2E1_Substr	Trn/Ver	442	199	641	543	84.7%	80.9%	86.4%
	Test	102	59	161	146	90.6%	83.0%	95.0%

Data sources

- Dassault Systemes BIOVIA Metabolite Database[1]
- Drugbank database[2]
- Rendic[3]
- Sheridan[4]
- Original literature references

The data sets contain drugs, druglike and non-drug molecules like agrochemicals (chlorpyrifos, malathion, tetrachlorobenzene, butachlor, trichlorethane, methoxychlor, hexacosinoic acid) and compounds that lack both N and O; none of which are drugs. There are also compounds that have 1 oxygen and are not steroids

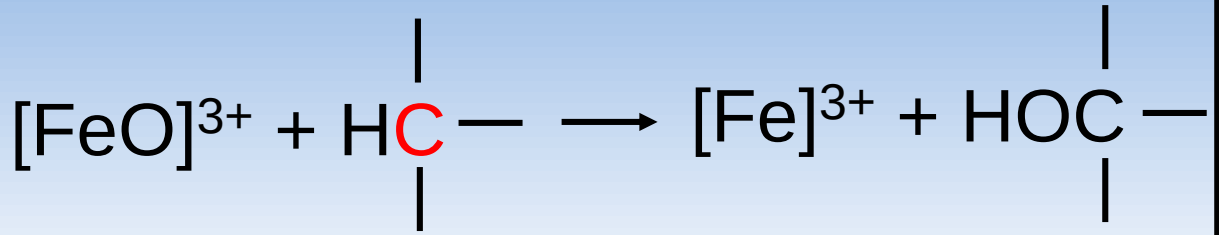
[1] http://www.3dsbiovia.com/products/collaborative-science/databases/bioactivity-databases/biovia-toxicity.html
[2] http://www.drugbank.ca
[3] *Drug Metab. Rev*. **2002**; *34*, 83-448.
[4] *J. Med. Chem.* **2007**, *50,* 3173-3184.

Approach for Sites of Metabolism (SOM) Models

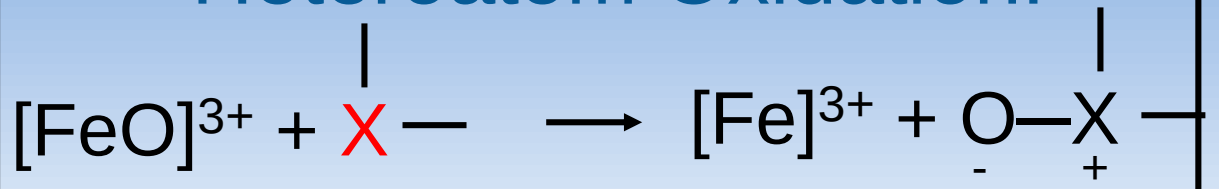
- Collect/curate data from databases, literature compilations, literature articles
  - BIOVIA Metabolite Database
  - Sheridan et al., J. Med. Chem. 50 3173 (2007)
  - *A great deal of review of literature – both old and new*
- Classify atoms of molecules as metabolized/not metabolized based on observed metabolites
- Generate atomic descriptors for each atom
  - EEM-Hückel charge model, parameterized from in-house *ab initio* database of partial charges
  - Reactivities: EEM σ atomic Fukui indices and Hückel π frontier orbital atomic densities
- Build Artificial Neural Network Ensembles (ANNEs) to predict sites of metabolism
  - Build atomic classification models for individual CYPs
    - 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4
- Each candidate atom receives a score
  - Highest scoring atoms are classified as sites

CYP 450 Typical Oxidations

- Carbon Hydroxylation:

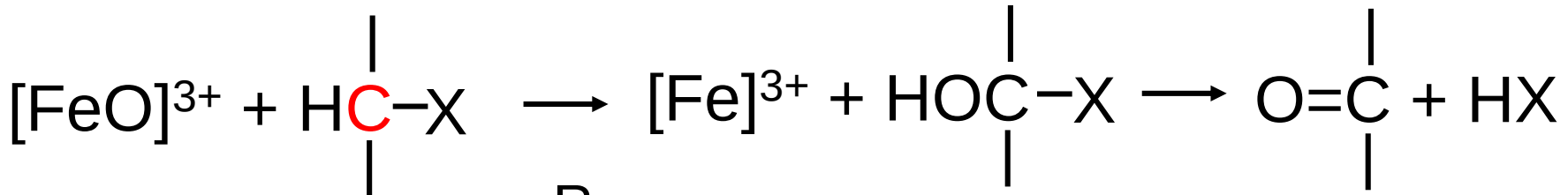


- Heteroatom Oxidation:



X = N, S

- Heteroatom Dealkylation:

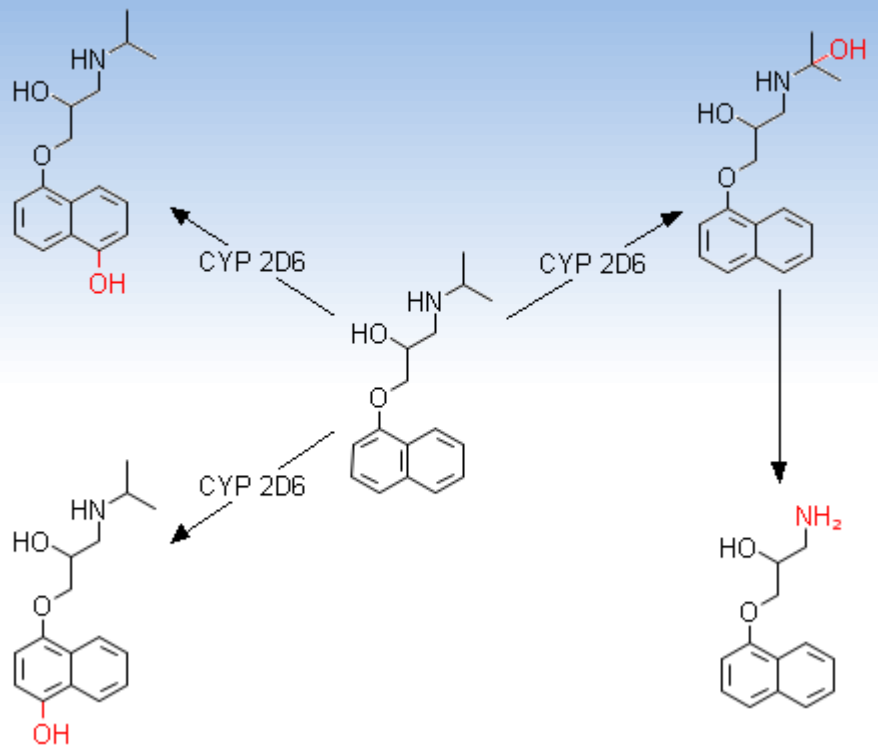


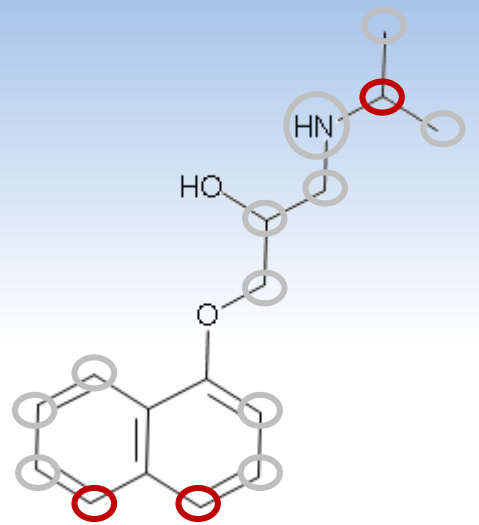
X = OR, 

, SR

Red marks site of metabolic attack

Experimental CYP2D6 SOM for Propranolol





Inputs to model

- Non-site
- Site

J. Pharmacol Exp Ther, **2000**, *294 (3)*, 1099-1105.
Drug Metab Dispos, **1994**, *22 (6)*, 909-915.

CYP Site Model Performance

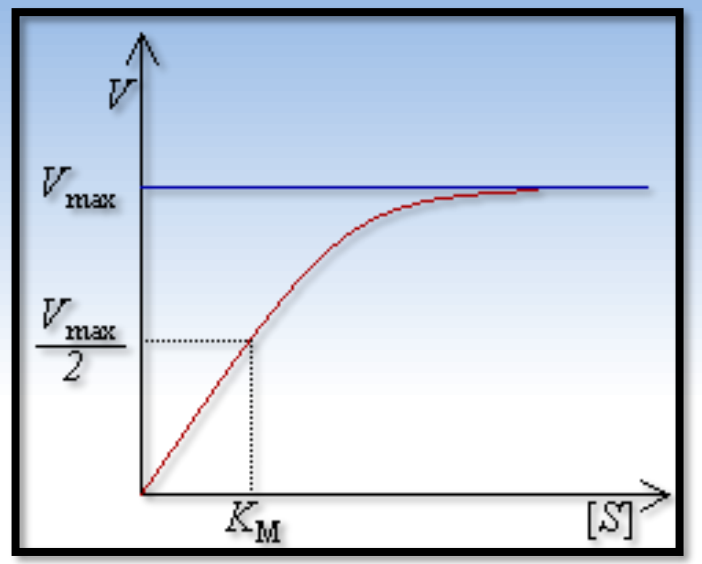
The composition of these data sets are similar to the substrate/nonsubstrate model data sets

CYP Site Model		Excluded Atoms	Included Atoms	Negatives	Positives	Concordance	Sensitivity	Specificity
1A2	Training	2374	3258	2747	511	85.9%	91.2%	84.9%
	Test	360	541	443	98	84.8%	85.7%	84.7%
2A6	Training	675	1086	920	166	87.8%	79.5%	89.2%
	Test	72	129	98	31	88.4%	80.6%	90.8%
2B6	Training	1041	1804	1559	245	90.4%	87.8%	90.8%
	Test	188	310	254	56	88.7%	87.5%	89.0%
2C8	Training	1346	2081	1842	239	89.0%	90.4%	88.8%
	Test	208	320	274	46	88.1%	84.8%	88.7%
2C9	Training	1994	3022	2651	371	88.0%	87.3%	88.1%
	Test	432	490	417	73	85.5%	80.8%	86.3%
2C19	Training	1748	2802	2400	402	90.9%	88.6%	91.3%
	Test	217	368	313	55	87.8%	80.0%	89.1%
2D6	Training	2187	4173	3646	527	90.8%	91.5%	90.7%
	Test	325	638	554	84	90.8%	88.1%	91.2%
2E1	Training	768	1154	920	234	87.7%	88.5%	87.5%
	Test	148	201	156	45	89.1%	88.9%	89.1%
3A4	Training	6401	9938	8822	1116	85.3%	87.4%	85.1%
	Test	1456	2314	2057	257	84.7%	80.2%	85.3%

Excluded Atoms were not included in the performance statistics

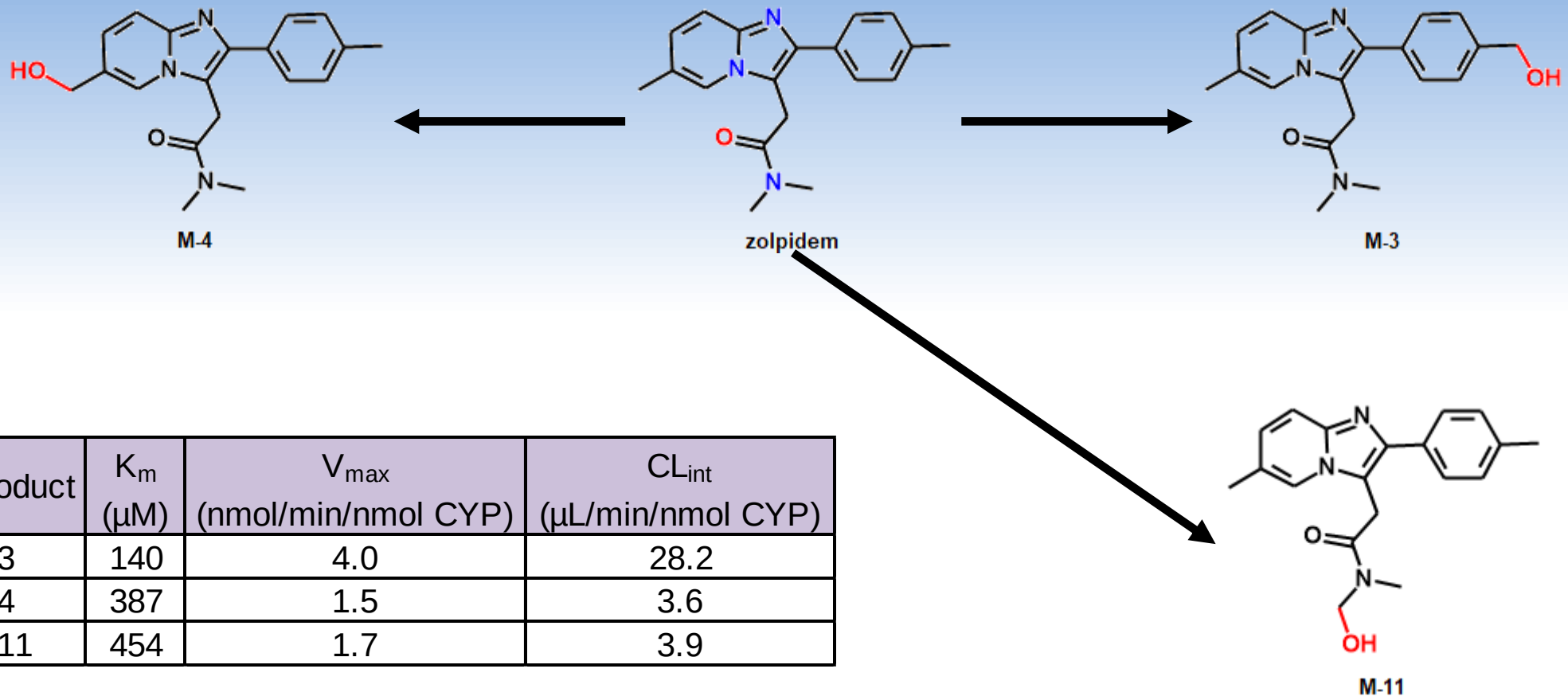
Michaelis-Menten Kinetics

- K_m : Michaelis constant
  - a measure of the affinity of the substrate for the enzyme
  - a property of ES complex not dependent on [E] and [S]
- V_{max} : Maximum metabolic velocity
  - maximum rate (velocity) at a fixed [E]
  - directly proportional to the [E]
- CL_{int}: Intrinsic clearance
  - a flow-independent measure of the tissue or organ's ability to metabolize drugs



$$CL_{int} = \frac{V_{max}}{K_m}$$

Zolpidem CYP3A4 Kinetic Parameters



Product	K_m (μM)	V_{max} (nmol/min/nmol CYP)	CL_{int} (μL/min/nmol CYP)
M-3	140	4.0	28.2
M-4	387	1.5	3.6
M-11	454	1.7	3.9

von Moltke, LL et al. *Br J Clin Pharmacol*, **1999**, 48, 89-97.

Model Building for Kinetic Parameters

- Data sources:
  - BIOVIA Metabolite Database and public domain literature
    - Drug Metabolism & Disposition
  - Careful examination of **MANY** original articles*
- K_m, V_{max}, and CL_{int} models
- CYP 1A2, 2C9, 2C19, 2D6, 3A4, and 3A4 HLM
  - 18 total models
- Use atomic and molecular descriptors
- Develop regression models for each atomic site

*M. Waldman et al. *J Comput Aided Mol Des.* **2015**, 29, 897-910.

K_m, V_{max}, and CL_{int} Models Performance Statistics

Model	Set	No. of Cmpds	RMS	R^2
1A2 K_m	Train	85	0.38	0.86
	Test	15	0.40	0.81
1A2 V_{max}	Train	81	0.48	0.61
	Test	14	0.45	0.45
1A2 CL_{int}	Train	82	0.50	0.79
	Test	14	0.55	0.69
2C9 K_m	Train	68	0.43	0.80
	Test	12	0.40	0.87
2C9 V_{max}	Train	63	0.47	0.76
	Test	11	0.47	0.76
2C9 CL_{int}	Train	65	0.48	0.77
	Test	11	0.39	0.86
2C19 K_m	Train	67	0.32	0.86
	Test	12	0.36	0.71
2C19 V_{max}	Train	67	0.48	0.78
	Test	12	0.49	0.82
2C19 CL_{int}	Train	65	0.52	0.81
	Test	11	0.59	0.74

Model	Set	No. of Cmpds	RMS	R^2
2D6 K_m	Train	87	0.47	0.84
	Test	15	0.50	0.82
2D6 V_{max}	Train	97	0.50	0.65
	Test	17	0.49	0.55
2D6 CL_{int}	Train	86	0.59	0.59
	Test	15	0.57	0.57
3A4 K_m	Train	142	0.46	0.70
	Test	25	0.46	0.67
3A4 V_{max}	Train	146	0.59	0.59
	Test	26	0.57	0.54
3A4 CL_{int}	Train	139	0.63	0.63
	Test	24	0.63	0.63
3A4 HLM K_m	Train	117	0.47	0.83
	Test	21	0.39	0.84
3A4 HLM V_{max}	Train	105	0.44	0.68
	Test	19	0.48	0.71
3A4 HLM CL_{int}	Train	105	0.54	0.83
	Test	18	0.53	0.79

These data sets contain drugs, druglike molecules, non-drugs like naphthol and naphthalene, and agrochemicals like carbaryl, carbofuran, chlorpyrifos, diazinon, diuron, methoxychlor, and parathion.

- K_m R^2 range is 0.67-0.87
  - Test set RMSE range is 0.36-0.50 log units
- V_{max} R^2 is 0.45-0.82
  - Test set RMSE range is 0.45-0.57 log units
- CL_{int} R^2 is 0.56-0.86
  - Test set RMSE is 0.39-0.63 log units

Agrochemical development: What You Want to Know Early On!

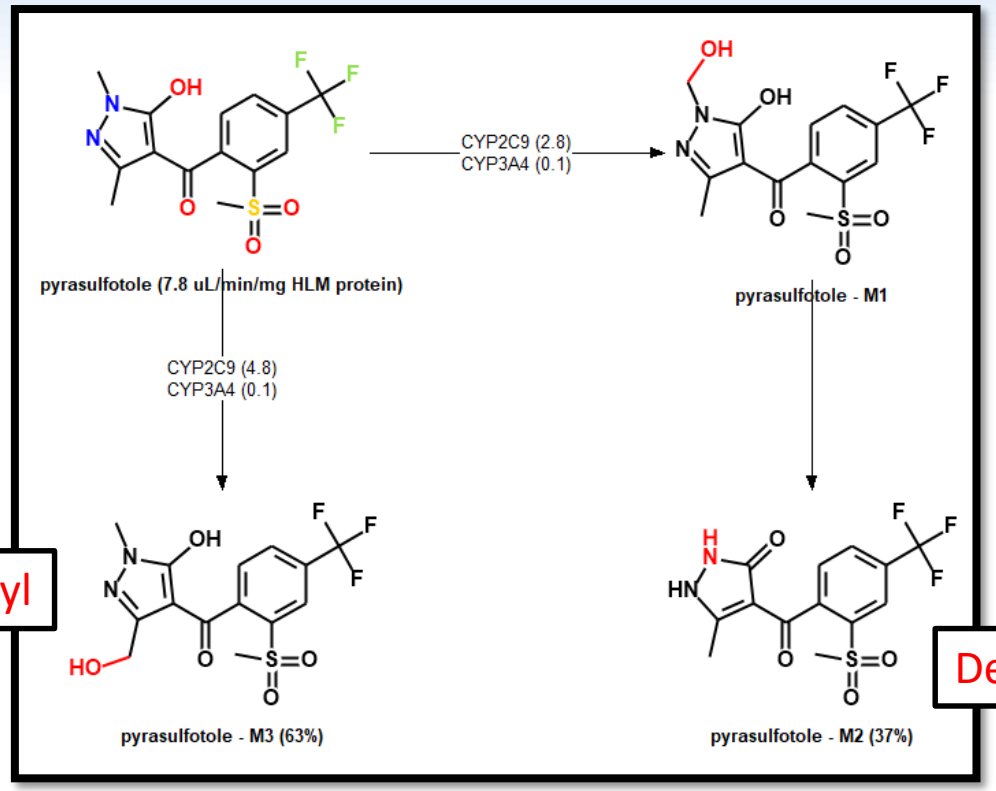
- Are the agrochemical or its residues likely to be toxic?
- How will it get metabolized *in vivo*?
  - in mammals in general
  - in humans & rats in particular
- If so, what are the likely metabolites?
- Are its metabolites likely to be toxic or not?
- How much pesticide or residue will get absorbed?
- How much of what gets absorbed will survive first-pass metabolism?

Pyrasulfotole (AE 0317309)

Paraphrasing the TOXNET record , “following both oral and intravenous administration in rat, most of the dose was excreted unchanged. Hydroxymethyl, desmethyl, and a benzoic acid fragment formed by complete removal of the pyrazole ring were observed as minor metabolites in urine and feces. Follow oral dosing, approximately 70% of the radioactivity was excreted in the urine and 30% in the feces by 48 or 52 hours.”

Rat HTPK Simulation: predictions for rat: RLM $CL_{int,u}$ = 25 µl/min/mg MP, fu_p= 5%, %Fa = 100% and %Fb = 96% for a 0.1 mg dose.

Human CYPs do not necessarily translate directly rats, however the major CYPs in the respective species (CYP3A4 and CYP3A1) belong to the same family (3A). The predictions on the right are consistent with the above TOXNET record.

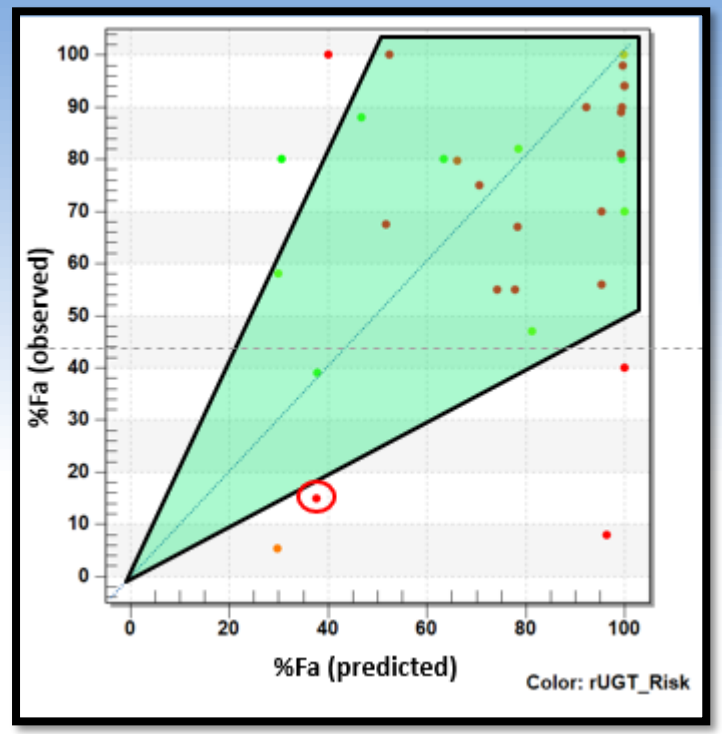


Hydroxymethyl

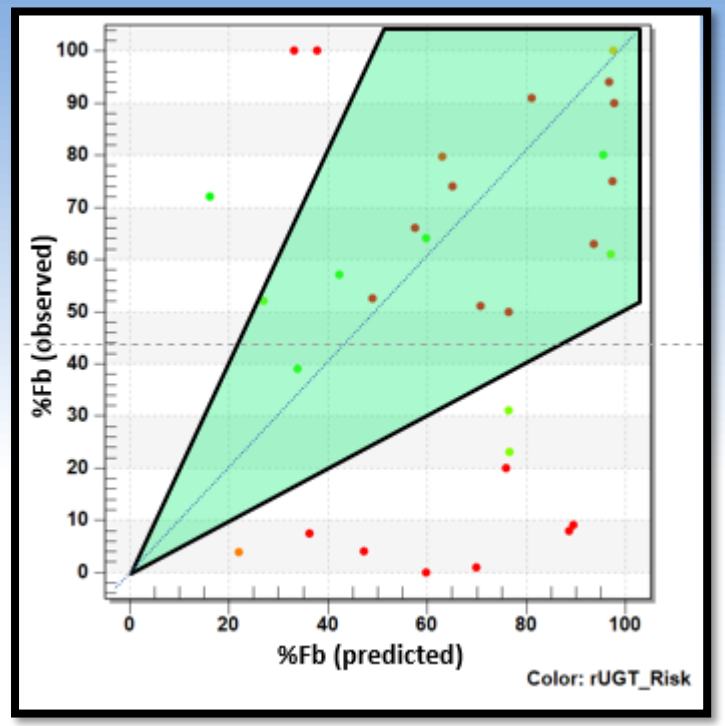
Desmethyl

RD Clark, *Pest Manag Sci.* **2018**.

Prediction of Rat %Fa and %Fb for Herbicides



81% predicted within 2-fold of the reported value and 3% within the experimental variability (±5%).



59% predicted within 2-fold of the reported value, with only 10% underestimated by more than 2-fold.

Observed values are mostly from radiolabel results extracted from EPA, EFSA, or WHO risk assessments based on radiolabel recovery. Dosages were often not reported so 2.5 mg was used. Observed %Fa comes from amount not excreted in the feces. %Fb comes from parent compound recovered in urine (it represents a lower bound).

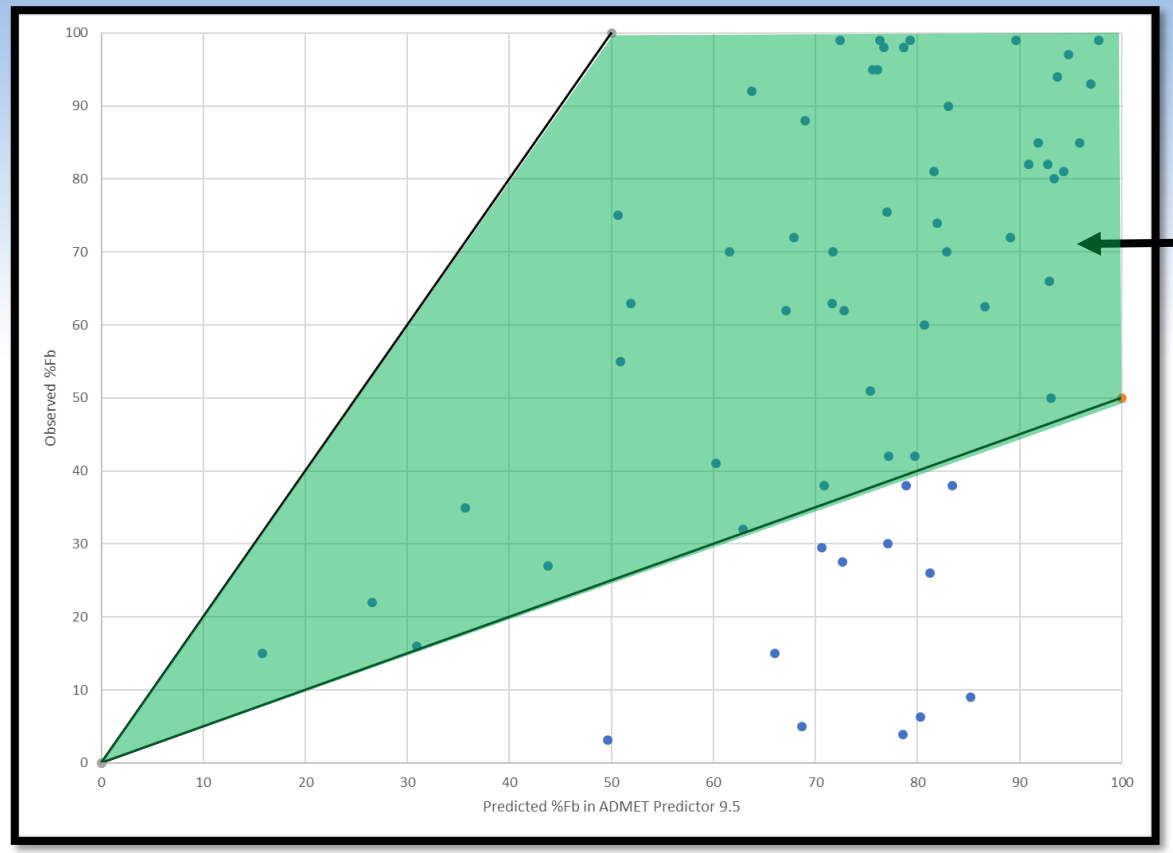
Data is from:

H Kraehmer *et al. Plant Physiology* **2014**, *166*, 1132-1148.

Y Zhang *et al., Pest Management Science* **2018**, *74*, 1979-1991.

Predicted Human %Fb from HTPK

- A database of 62 drugs including oral bioavailability (%Fb) and dose was constructed[1]
- All compounds' reported major clearance pathways (MCP) were CYP-mediated[2]



81% (50 molecules) were predicted within 2-fold of the observed value.

69% (43 molecules) were predicted within 1.5-fold of the observed value.

[1] Doses and oral biovavailabilities from drug label or "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."

[2] Toshimoto K et al, *Drug Metab. Dispos.* 42:1811-1819, November 2014.

HTPK Human Oral Simulation

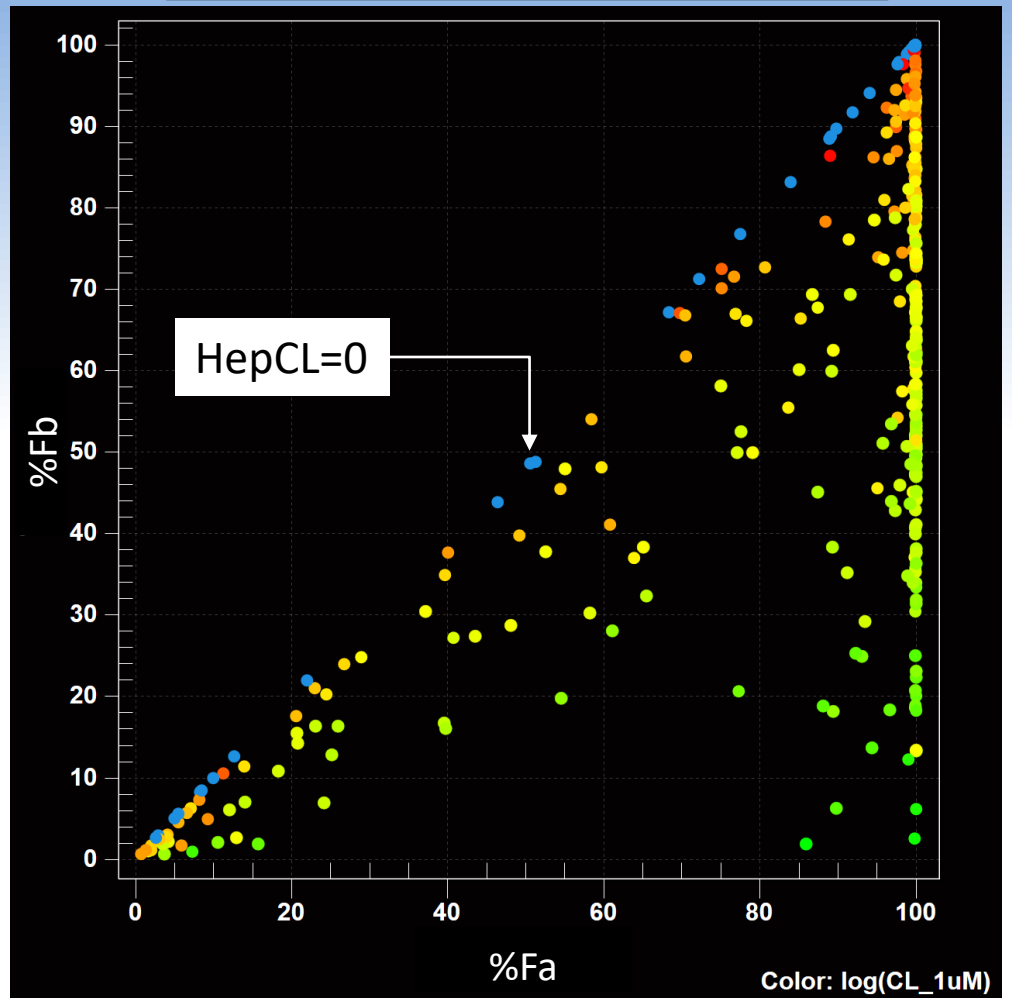
10 mg oral dose simulation using human hepatocyte clearance[1]

- 392 compounds with *in vitro* human hepatocyte CL_{int} (0 – 445 µl/min/million cells)
  - 14 compounds with out of scope aqueous solubility predictions are hidden

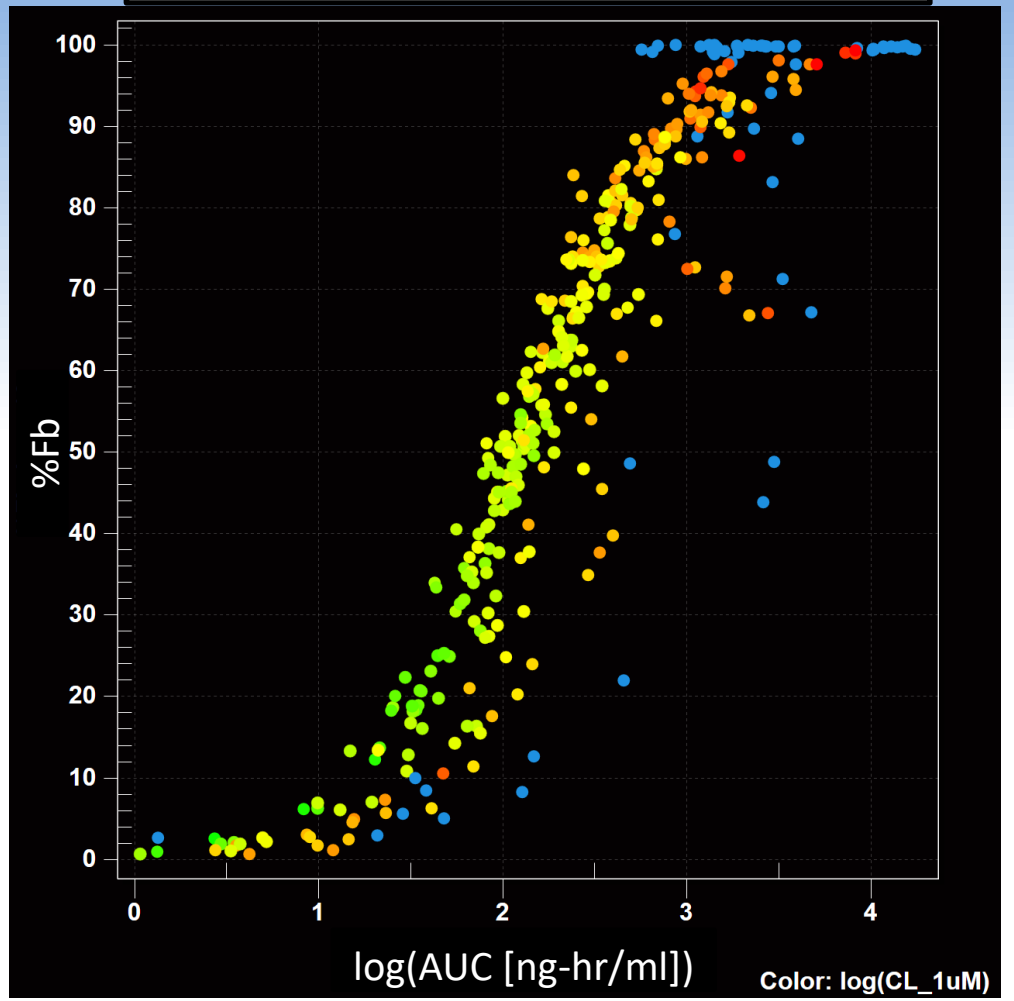
[1] Wetmore et al., *Toxicol. Sci.*, **2012**, 125(1), 157–174 and *Toxicol. Sci.*, **2015**, 148(1), 121–136.

HTPK Human Oral Simulation

%Fb v. %Fa, colored by log(HepCL)

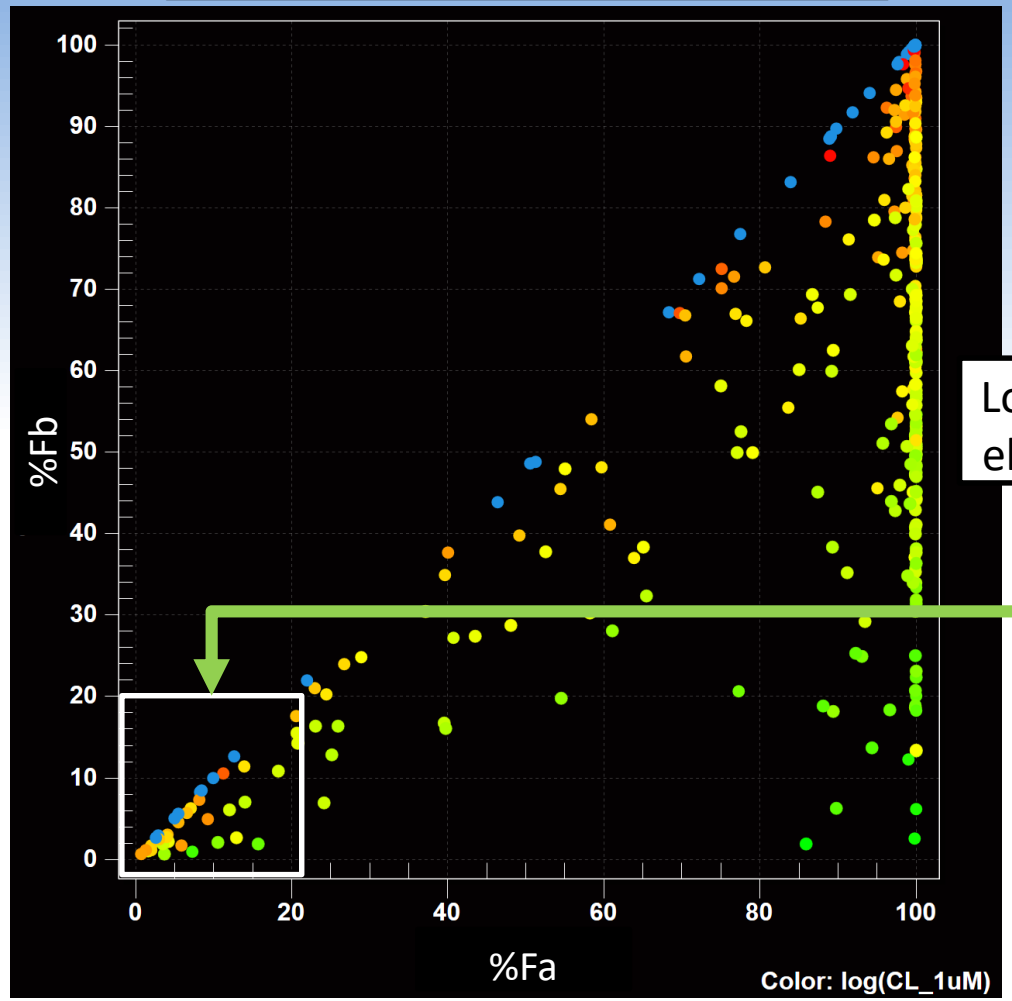


%Fb v. log(AUC), colored by log(HepCL)



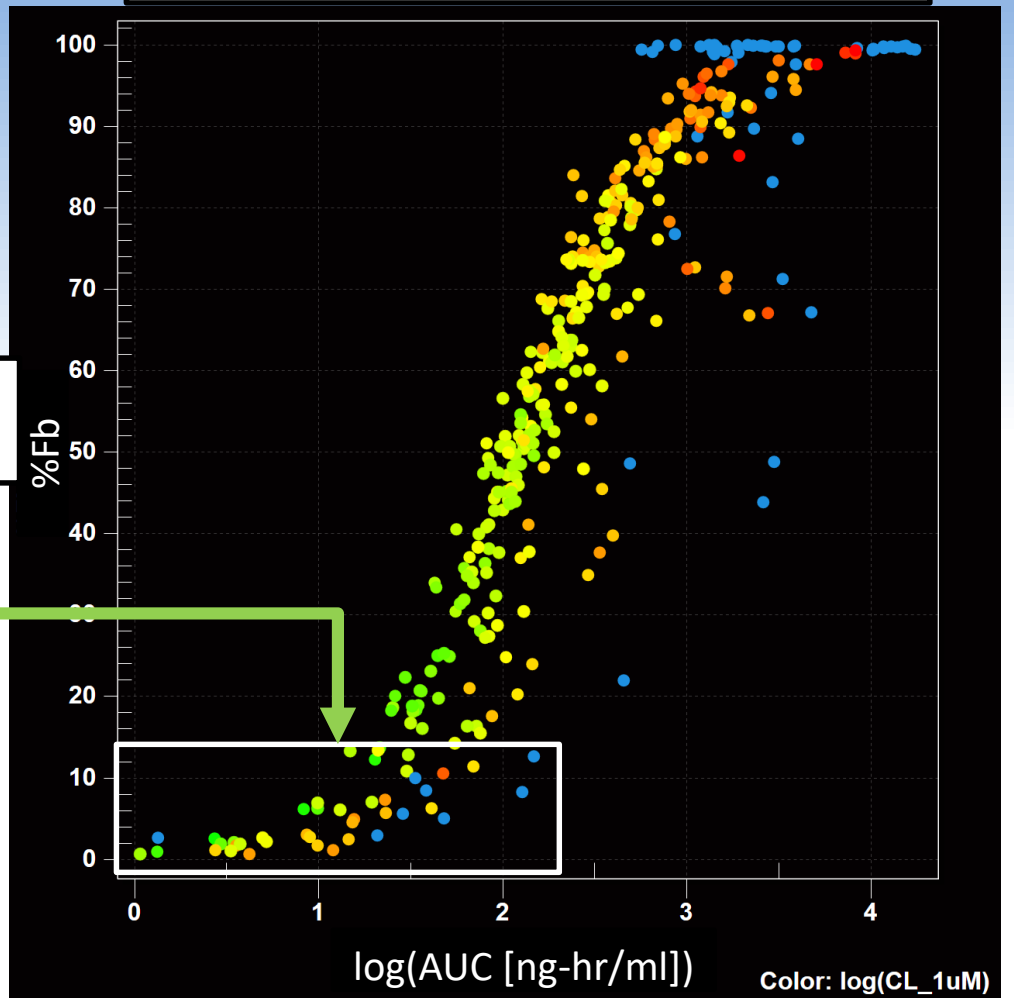
HTPK Human Oral Simulation

%Fb v. %Fa, colored by log(HepCL)



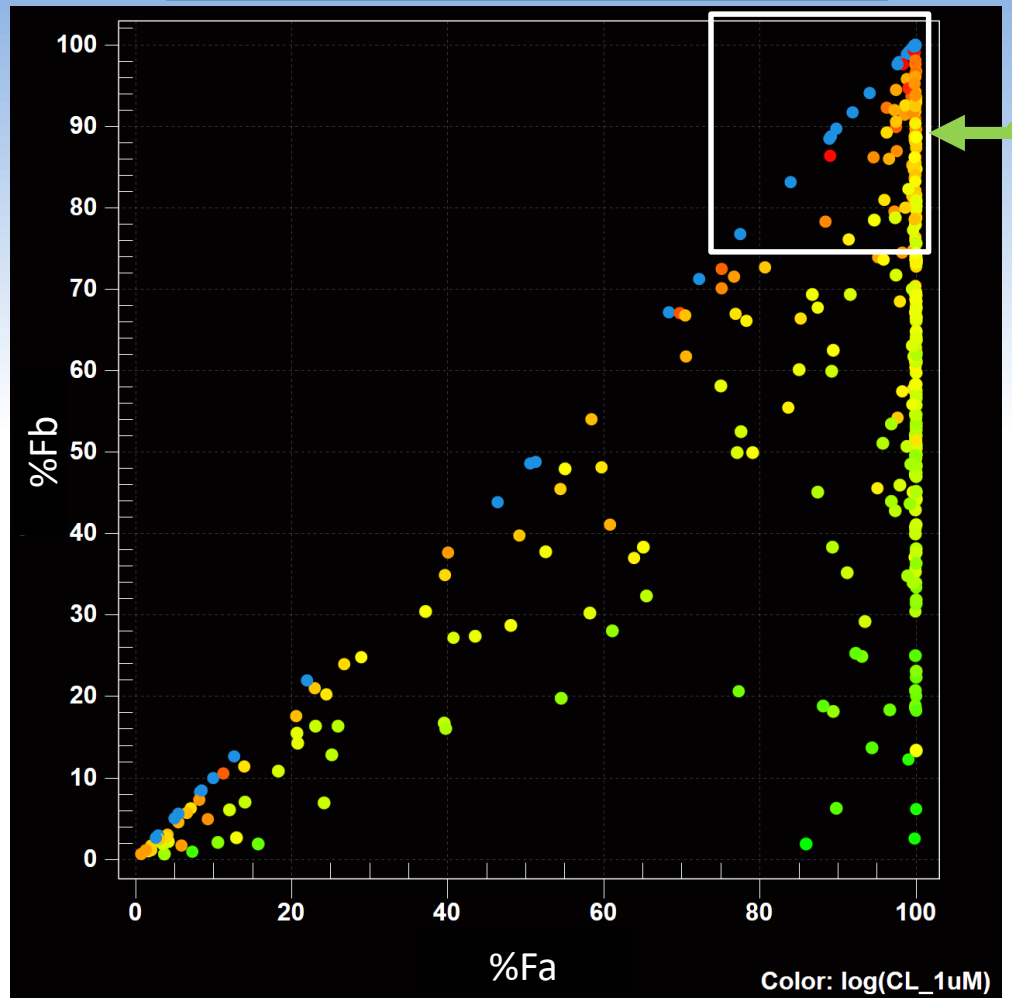
Lower risk because eliminated in feces

%Fb v. log(AUC), colored by log(HepCL)



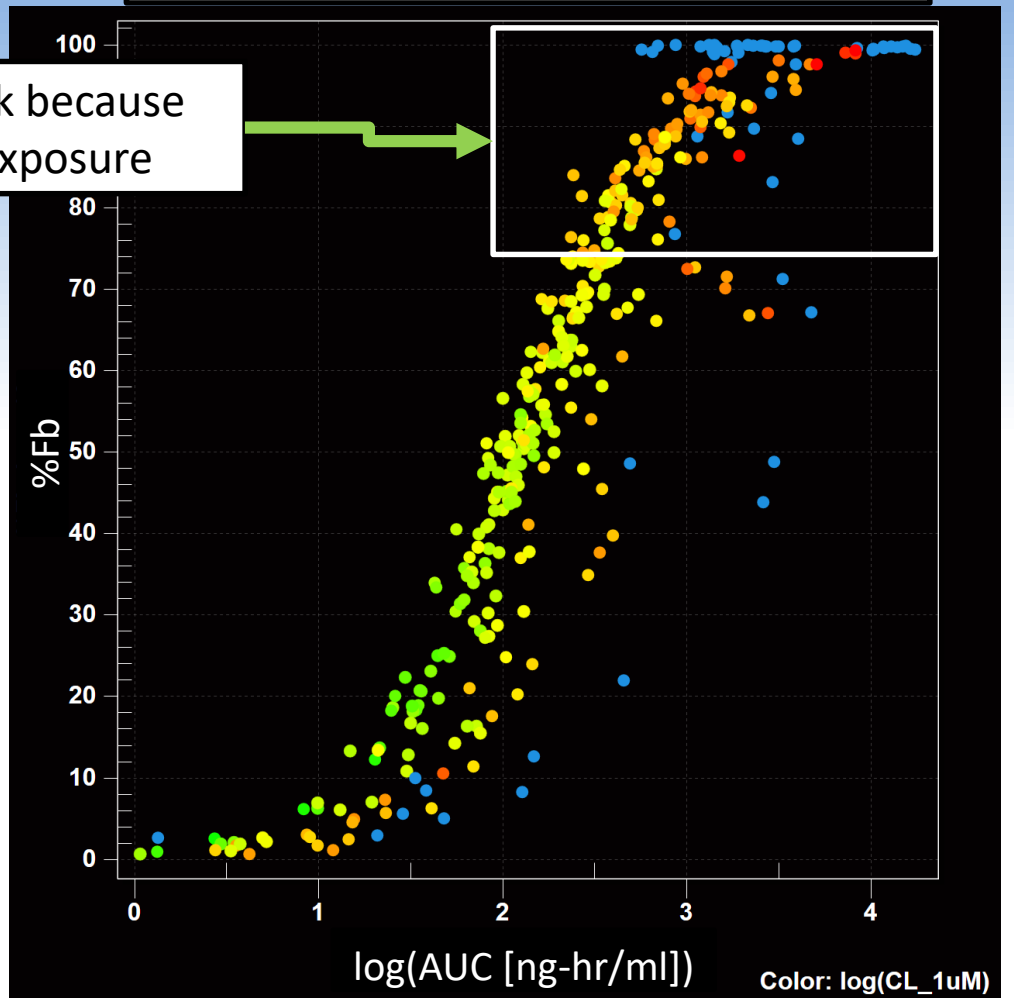
HTPK Human Oral Simulation

%Fb v. %Fa, colored by log(HepCL)



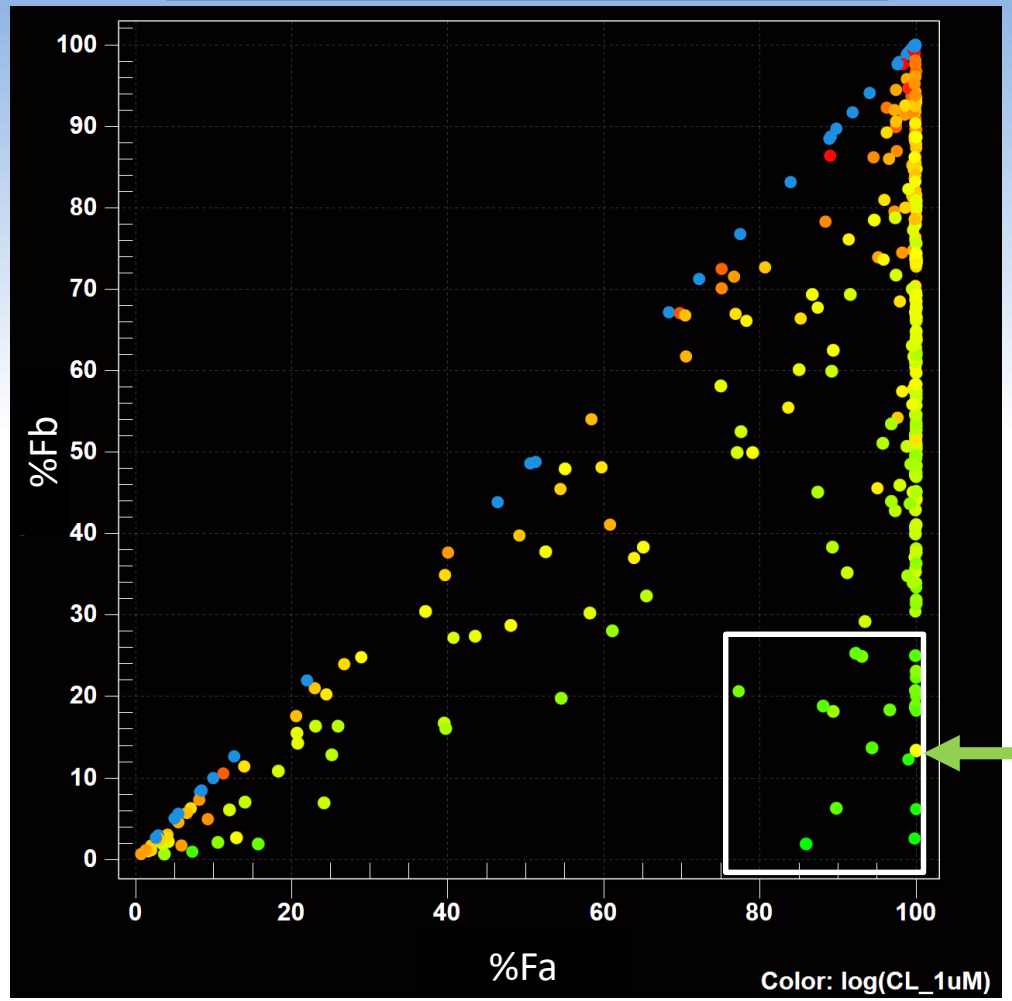
Higher risk because higher exposure

%Fb v. log(AUC), colored by log(HepCL)

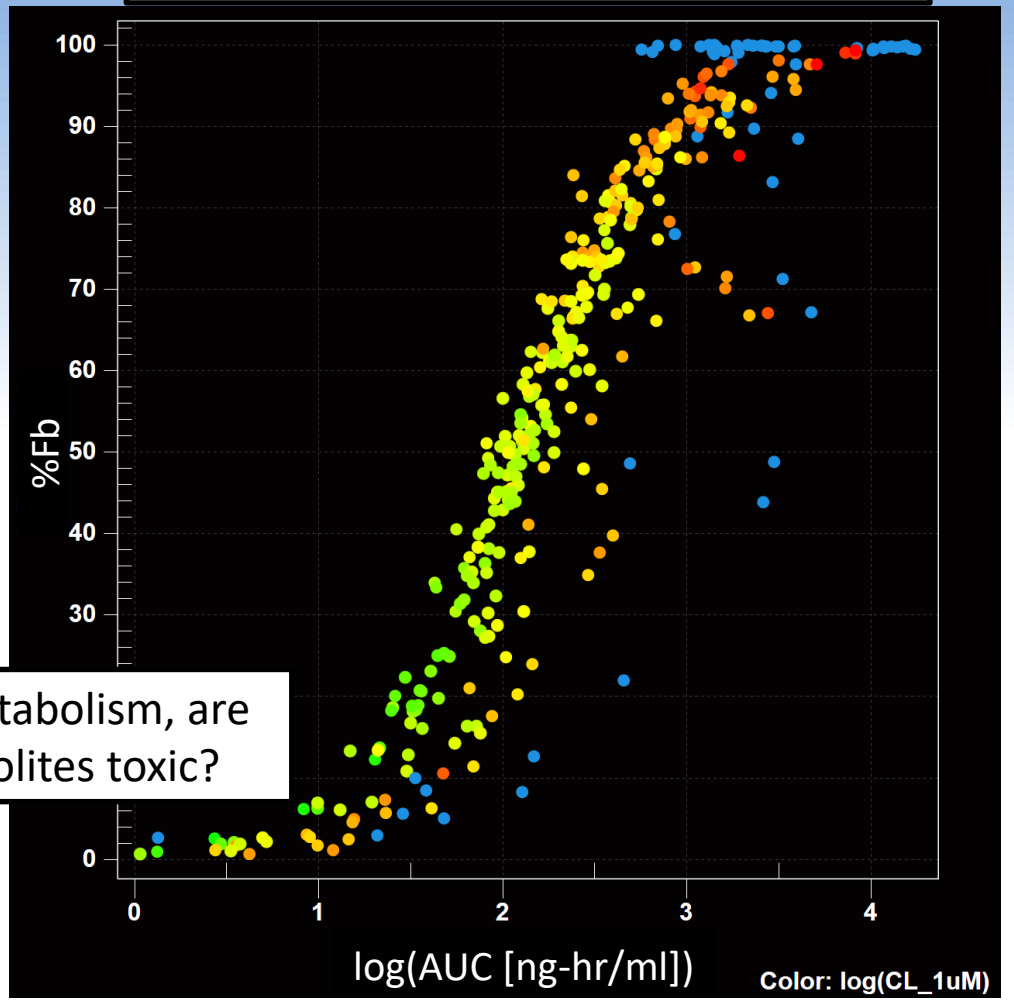


HTPK Human Oral Simulation

%Fb v. %Fa, colored by log(HepCL)



%Fb v. log(AUC), colored by log(HepCL)



High metabolism, are metabolites toxic?

IS-IV-IVE Techniques to Predict Exposure and Guide Risk Assessment

- Mechanistic PK simulations require parameters, e.g., solubility, permeability, fraction unbound to plasma, hepatic clearance, etc.
  - QSAR/QSPR models based on the 2D structure of the molecule can be used to estimate these parameters
  - *In vitro* values can also be used as they become available
- ECCS and related models can be used to predict the major clearance pathway
- HLM and RLM clearance models were developed and used to estimate liver clearance in PK simulations
- Cytochrome P450 models are based on specific CYP isoforms and used to predict metabolites and kinetic parameters
- PK examples included agrochemicals and drugs

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