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Physiologically Based Pharmacokinetic (PBPK) Modeling in Drug  
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# **Challenges in IVIVE Scaling of Microsomal Data for Compounds in Preclinical Development**

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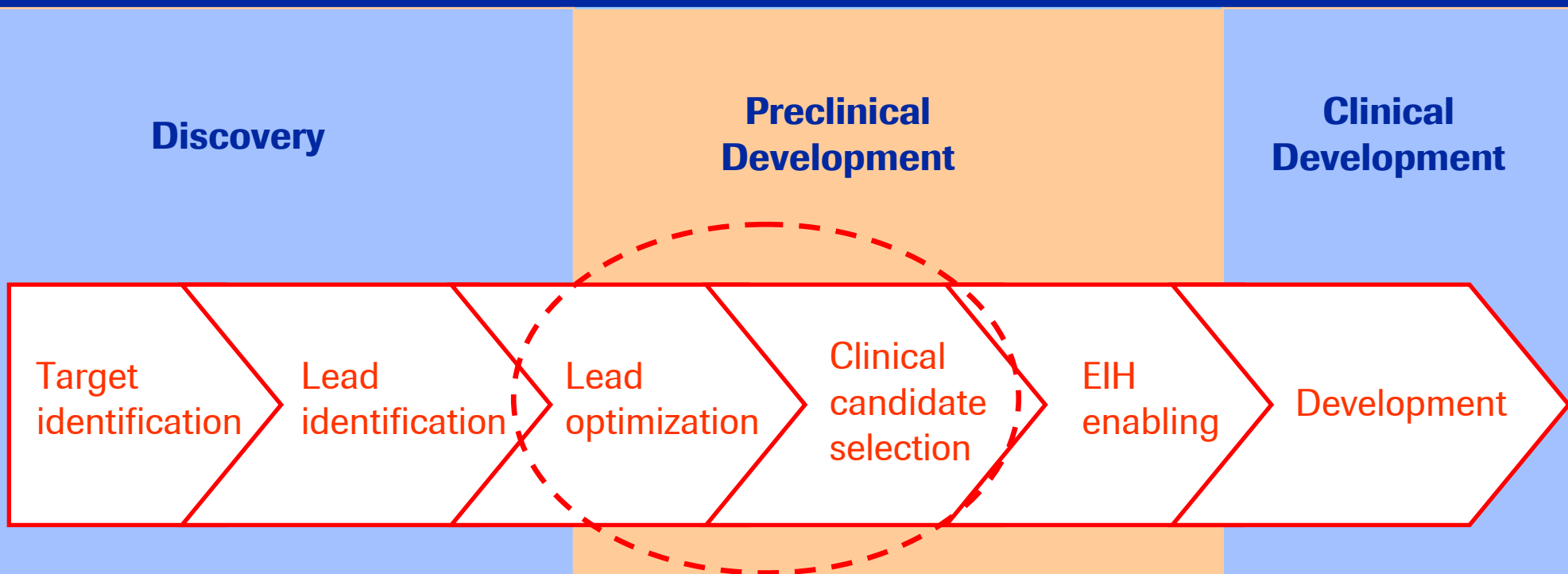
*Roche Palo Alto*

*6 April, 2009*

# Introduction

- When scaling microsome (Mx) data, failure to include pertinent binding data can result in the significant underestimation or overestimation of CL.
  - **This concern is particularly important for low CL compounds.**
- Saturable metabolism can impact in vivo and in vitro data, complicating the interpretation of in vitro data.
- For accurate scaling of Mx data, consider measuring  $f_{u_{Mx}}$  for bases with  $\log P > 2.4$ , neutrals with  $\log P > 2$ , and acids with  $\log P > 4.7$  (for 1 mg/ml Mx protein).
- **Before using Mx data to estimate human metabolic CL, make sure the data is of sufficient quality to meet your needs.**

# Overview of the drug development process



Many types of data are generated to study a compound's ADME properties. How can the data generated early in the process be used to make accurate predictions about human PK?

What about Mx data routinely generated in early screening?

# With careful interpretation, Mx data can be useful for predicting human PK.

- Metabolism (clearance, Michaelis-Menten kinetics)
- Hepatic availability,  $F_H$
- Metabolism from specific CYP450s
- Human PK extrapolation and efficacious dose/regimen estimate
- DDI clinical trial simulations

***What are the limitations of using the Mx screening assay for human PK predictions?***

# This talk will address five issues that result in inaccurate scaling of Mx data.

- ① Assay sensitivity
- ② Saturable metabolism
- ③ Partitioning into red blood cells
- ④ Plasma protein binding
- ⑤ Microsomal binding

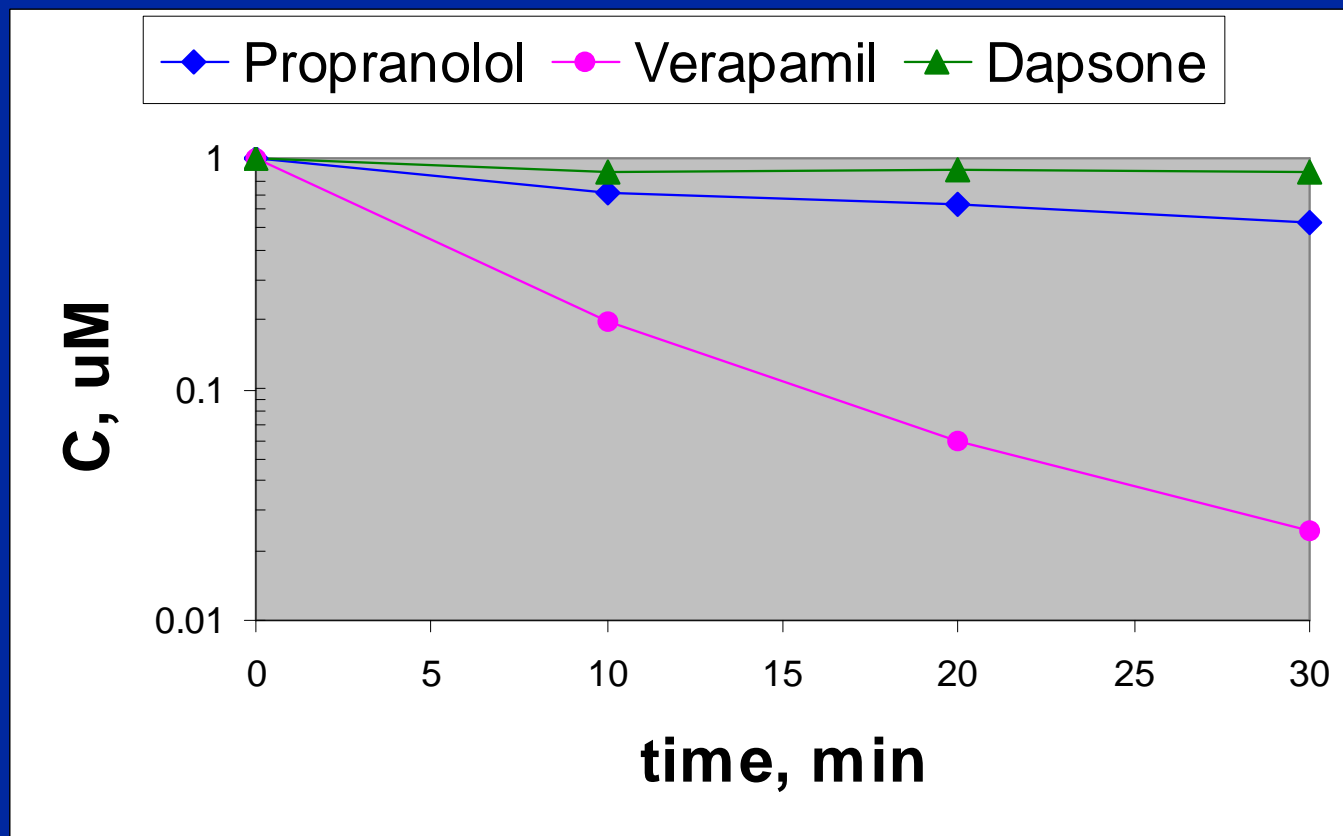
In the end, examples will be used to tie these issues together and put them into the context of the drug development process.

## ① Assay sensitivity

*Is it appropriate to use high-throughput Mx screening data for human PK predictions for low-clearance compounds?*

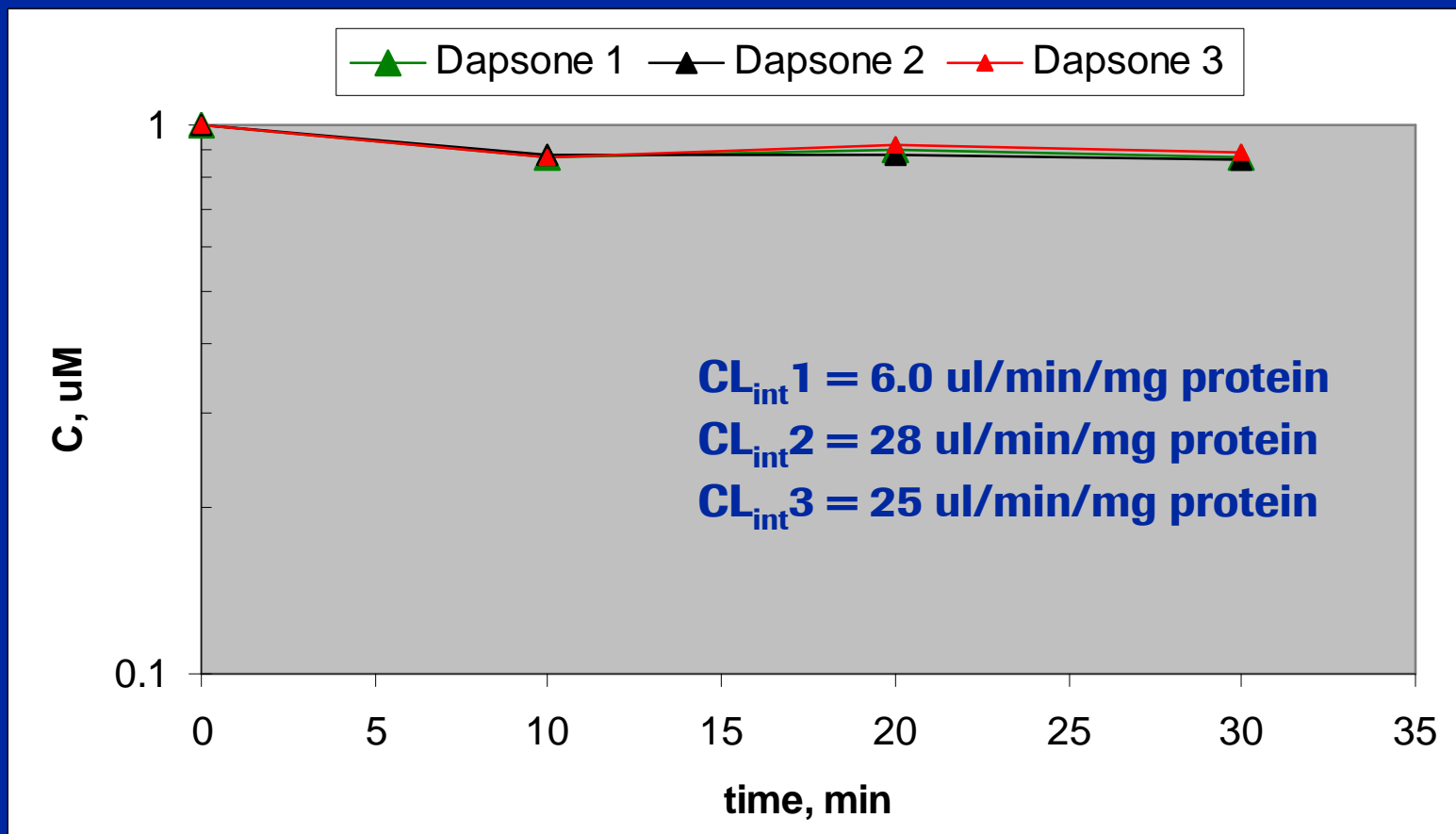
$$\text{CL}_{\text{blood}} = \frac{Q_L \times f_{u_B} \times \text{CL}_{\text{int,u}}'}{Q_L + f_{u_B} \times \text{CL}_{\text{int,u}}'}$$

High-throughput Mx assays are typically set up to estimate  $CL_{int}$  for compounds that are readily metabolized.



*Data from an automated high-throughput human Mx assay*

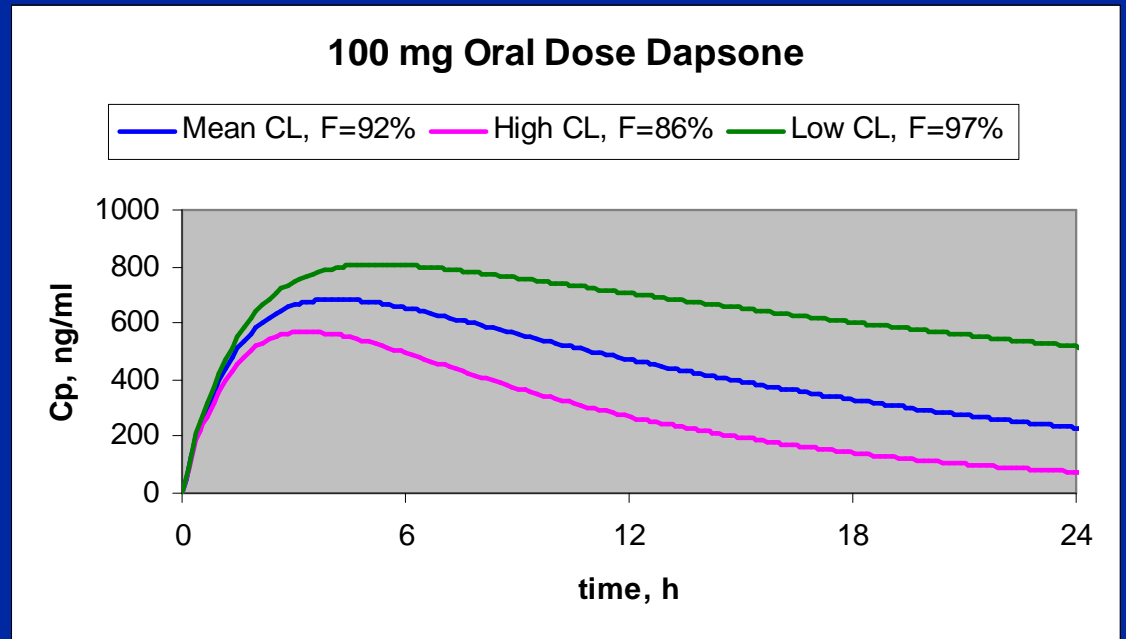
For metabolically stable compounds, the signal-to-noise ratio can cause large differences in slope and estimated  $CL_{int}$



**For metabolically stable compounds,  $CL_{int}$  from the Mx assay is less sensitive and less reproducible (reliable).**

	Dapsone	Propranolol	Verapamil
Average $CL_{int}$ ( $\mu\text{L}/\text{min}/\text{mg}$ )	14.4	41.5	358.1
SD	6.87	5.52	22.9
No. of incubations	8	10	10

**Example: Dapsone**  
**Could the difference in estimated CL impact the human PK projection and the decision to move forward? Yes.**



	$CL_{int}$ , ul/min/kg	$CL_{blood}$ , ml/min/kg	$F_H$	$Cp_{24h}$ , ng/ml	$AUC_{0-24h}$ , ng·h/ml
Mean	14.4	1.5	92	230	10800
High	28	2.7	86	520	15700
Low	6.0	0.65	97	75	6910

## A custom-designed assay can improve sensitivity for low-clearance compounds.

- The screening Mx assay is designed to screen out compounds with low metabolic stability.
- The sensitivity of the assay can be improved with a custom design.
  - use a higher concentration of microsomal protein
  - run the assay for 60 min instead of 30
  - improve the analytical method
  - measure metabolite appearance instead of drug disappearance

### When do you need a custom design?

When the screening assay does not result in two time points outside the region of bioanalytical variability.

# A custom design can result in a more accurate prediction of dapsons $CL_{\text{blood}}$ \*

Protein concentration, mg/ml	0.5	2
Length of incubation, min	30	30
Average $CL_{\text{int}}$ ( $\mu\text{L}/\text{min}/\text{mg}$ )	14.4	5.90
SD	6.87	3.46
No. of incubations	8	4
$CL_{\text{blood}}$ , ml/min/kg	1.5	0.64

$CL_{\text{blood}}$  for dapsons in humans is about 0.34 – 1.7 ml/min/kg, and metabolism is the primary (but not only) mechanism of elimination.<sup>12</sup>

## ② Saturable metabolism

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*How does saturable metabolism complicate the IVIVC relationship and impact  $CL_{int,u}$ ?*

$$CL_{\text{blood}} = \frac{Q_L \times fu_B \times CL_{int,u}'}{Q_L + fu_B \times CL_{int,u}'}$$

# Saturable metabolism refers to Michaelis-Menten kinetics.

$v$  = rate of metabolism

$V_{\max}$  = maximal rate of metabolism

$S$  = substrate concentration

$K_m$  = substrate concentration when  $v$  is half of  $V_{\max}$

$$v = \frac{V_{\max} \times S}{K_m + S}$$

At low substrate concentrations:

$$v = \frac{V_{\max}}{K_m} \times S$$

(=  $CL_{\text{int}} \times S$ )

**First order kinetics**

At high substrate concentrations:

$$v \sim V_{\max}$$

**Zero order kinetics**

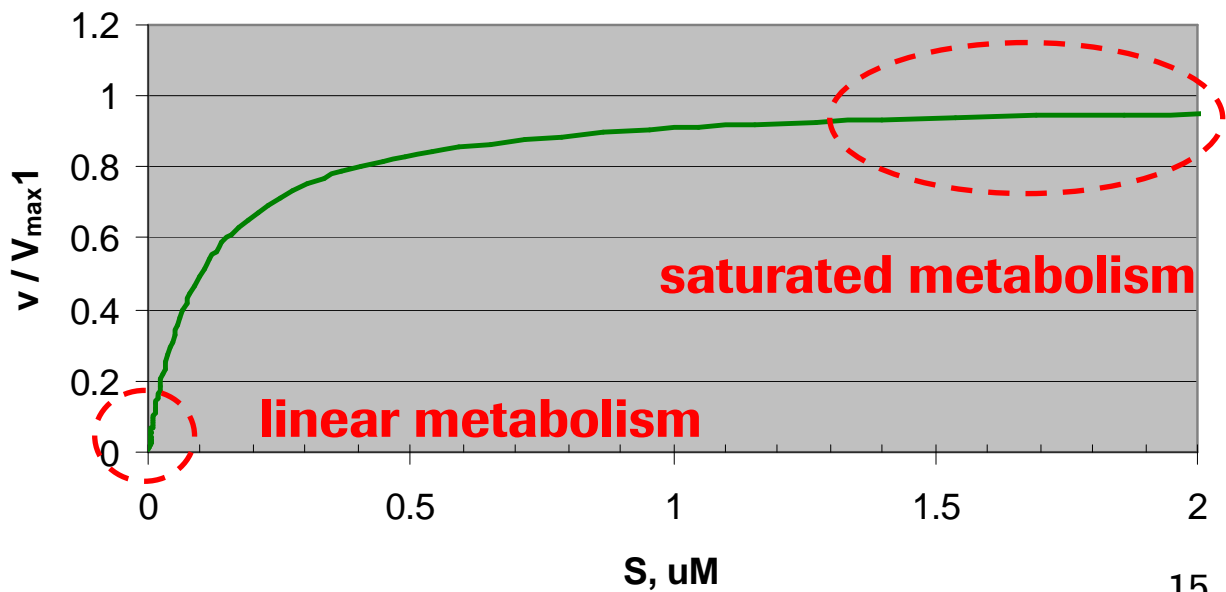
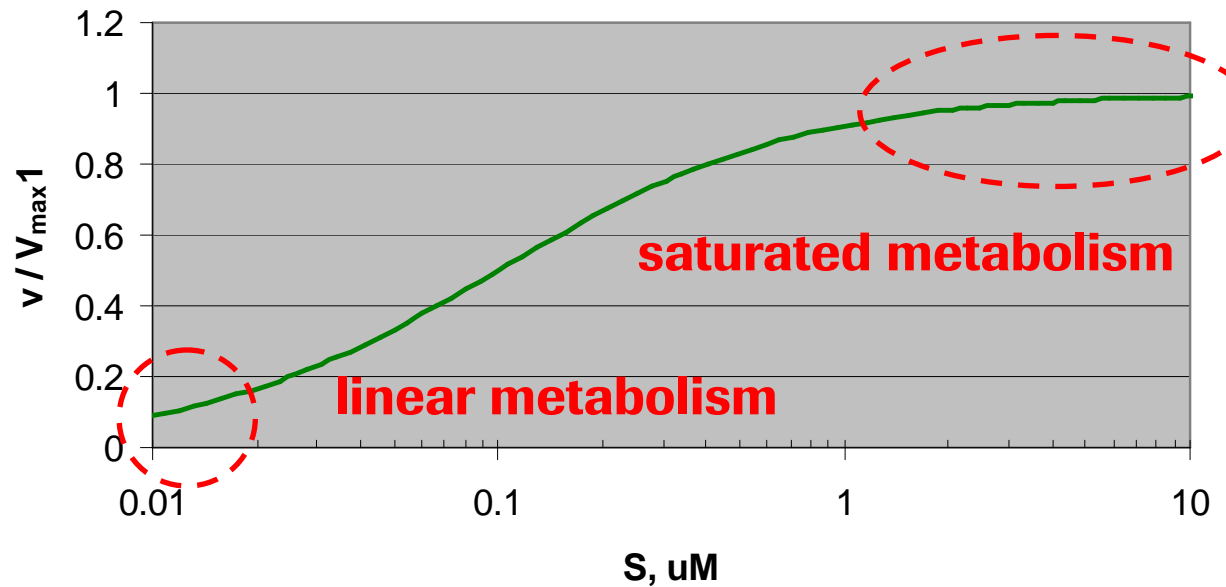
***Saturated metabolism can be an issue in vivo and/or in vitro.***

**Saturable metabolism can be one explanation for nonlinear kinetics.**

$$v = \frac{V_{\max} \times S}{K_m + S}$$

$$\frac{v}{V_{\max}} = \frac{S}{K_m + S}$$

$$K_m = 0.1 \text{ uM}$$



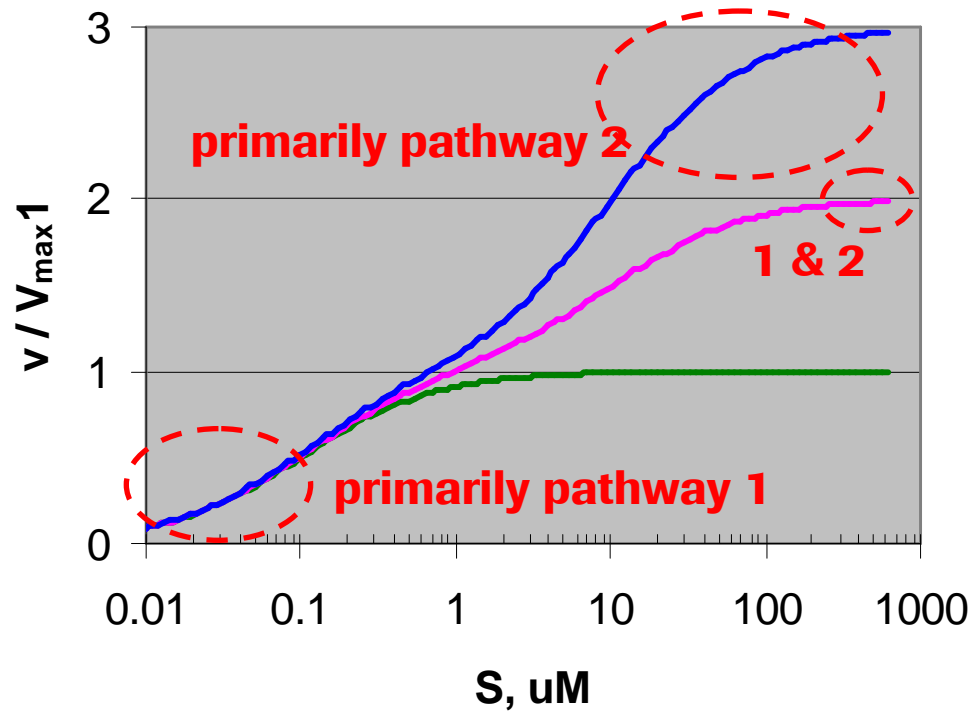
# Involvement of more than one enzyme in metabolism can result in complex nonlinear kinetics.

$$v = \frac{V_{\max 1} \times S}{K_m 1 + S} + \frac{V_{\max 2} \times S}{K_m 2 + S}$$

$$\frac{v}{V_{\max 1}} = \frac{S}{K_m 1 + S} + \frac{\frac{V_{\max 2}}{V_{\max 1}} \times S}{\frac{K_m 2}{K_m 1} + S}$$

$$K_m 1 = 0.1 \text{ } \mu\text{M}$$

$$K_m 2 = 10 \text{ } \mu\text{M}$$



- $V_{\max 2} = 0$
- $V_{\max 2}/V_{\max 1} = 1$
- $V_{\max 2}/V_{\max 1} = 2$

# Performing Mx studies at multiple concentrations can provide key information for interpretation of kinetic data.

$CL_{int}$  measured at two incubation concentrations

	0.1 uM test compound	1 uM test compound
Without quinidine *	195 $\mu\text{l}/\text{min}/\text{mg prot}$	19.2 $\mu\text{l}/\text{min}/\text{mg prot}$
With 1 uM quinidine *	18.9 $\mu\text{l}/\text{min}/\text{mg prot}$	7.0 $\mu\text{l}/\text{min}/\text{mg prot}$

\* Quinidine is a potent, specific CYP2D6 inhibitor.

- The higher clearance at 0.1 uM test compound concentration suggests saturable metabolism.
- Decreased CL in the presence of quinidine indicates that the compound is a CYP2D6 substrate, and that at low concentrations that pathway is key.

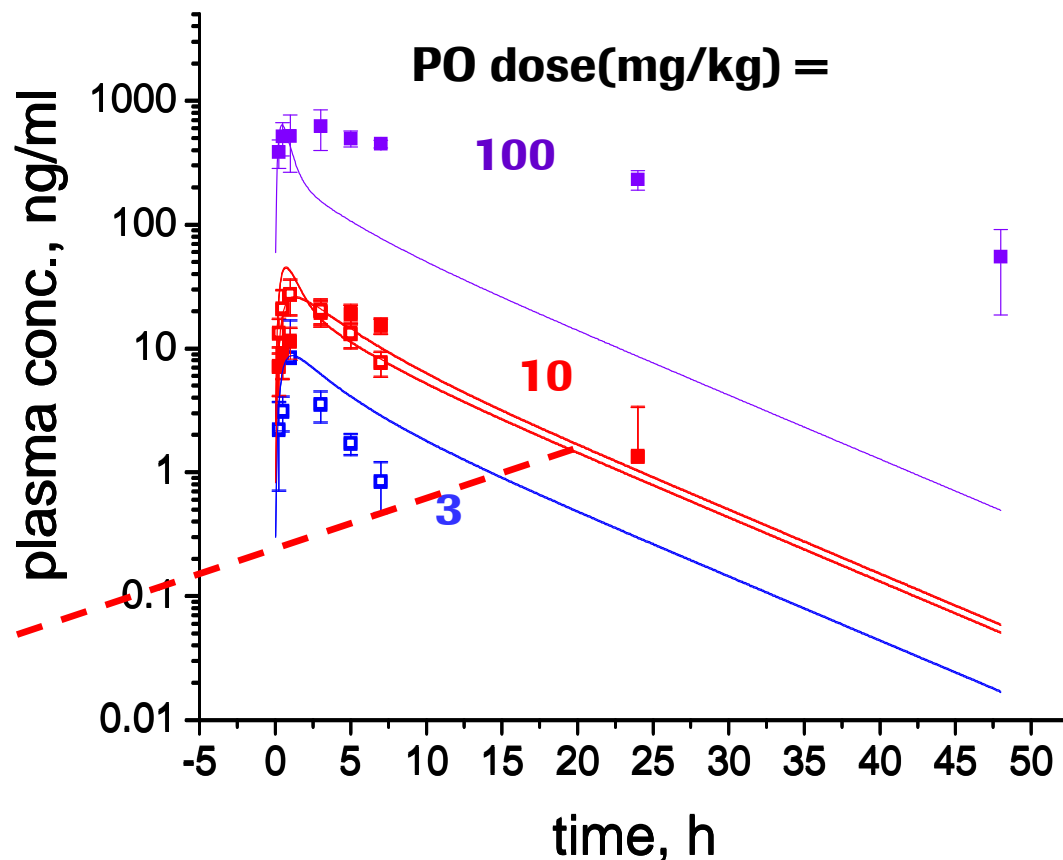
# Saturable metabolism can have a dramatic impact on in vivo PK data.

**You can have accidental IVIVC.**

If you rely on the results of single concentration experiments, you can miss important nonlinearities.

Simulated using CL estimated from 1 uM in vitro data

PO PK in the rat and PBPK model predictions



### ③ Partitioning into red blood cells

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*When is it important to measure the blood-to-plasma ratio (BPR)?*

$$CL_{\text{blood}} = \frac{Q_L \times fu_B \times CL_{\text{int,u}}'}{Q_L + fu_B \times CL_{\text{int,u}}'}$$

## The blood-to-plasma ratio (BPR) can be used to estimate $fu_B$ from $fu_p$ .

- The unbound fraction in blood ( $fu_B$ ) is not measured, but the free fraction in plasma ( $fu_p$ ) is.
- The minimum value of BPR is about 0.55 (assuming stable in RBCs).
  - If the BPR value is not measured, it is often assumed to be 1.
- The value  $fu_B$  can be estimated as  $fu_p / BPR$ .
  - *For compounds that do not enter RBCs:  $fu_B = (fu_p / BPR) \times (1 - Hc)$  where  $Hc$  is the hematocrit (Masimirembwa et al., 2003).*
- The value for  $CL_{\text{blood}}$  can be estimated as  $CL_{\text{plasma}} / BPR$  (Hinderling 1997).

# The BPR can vary from 1 and can exhibit species differences.



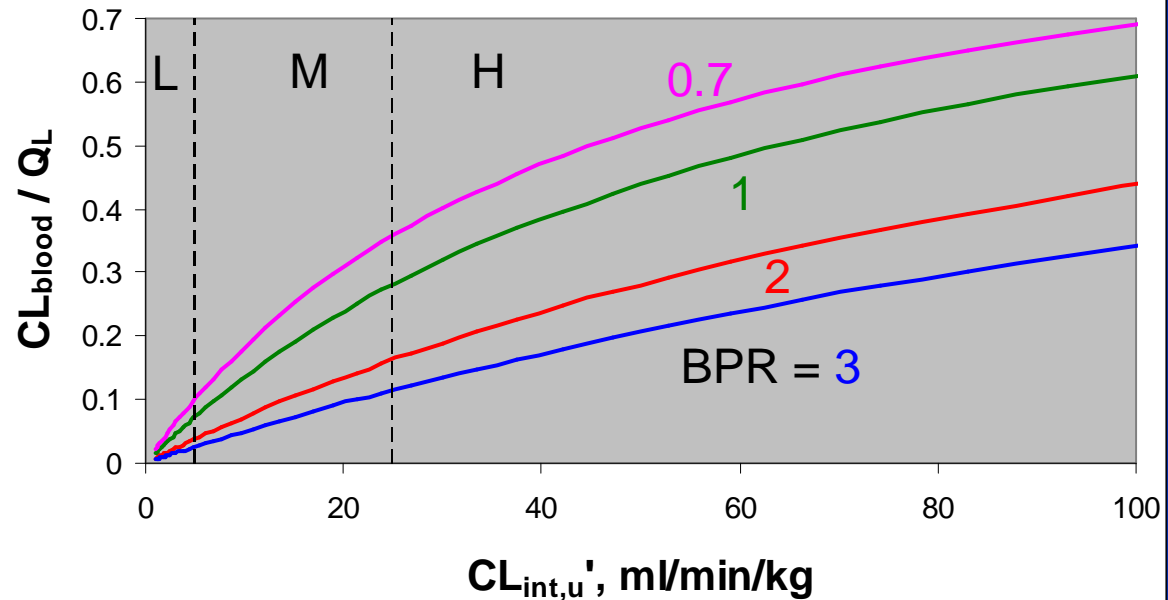
<b>Cpd.</b>	<b>MW</b>	<b>cLogP</b>	<b>Class</b>	<b>Rat BPR</b>	<b>Dog BPR</b>	<b>Monkey BPR</b>	<b>Human BPR</b>
A	556	2.56	Base	0.75	0.92	1.2	—
B <sup>a</sup>	415	5.9	Acid	0.63	0.56	0.52	0.62
C	294	3.85	Base	2.9	0.57	2.1	1.5
D	573	4.03	Neutral	26 - 1 uM 7.5 - 10 uM 8.6 - 25 uM	19 - 1 uM 9.0 - 10 uM 5.7 - 25 uM	—	18 - 1 uM 7.2 - 10 uM 4.5 - 25 uM

<sup>a</sup> Compound 12 from Jones et al. (2006). LogP was measured, not calculated.

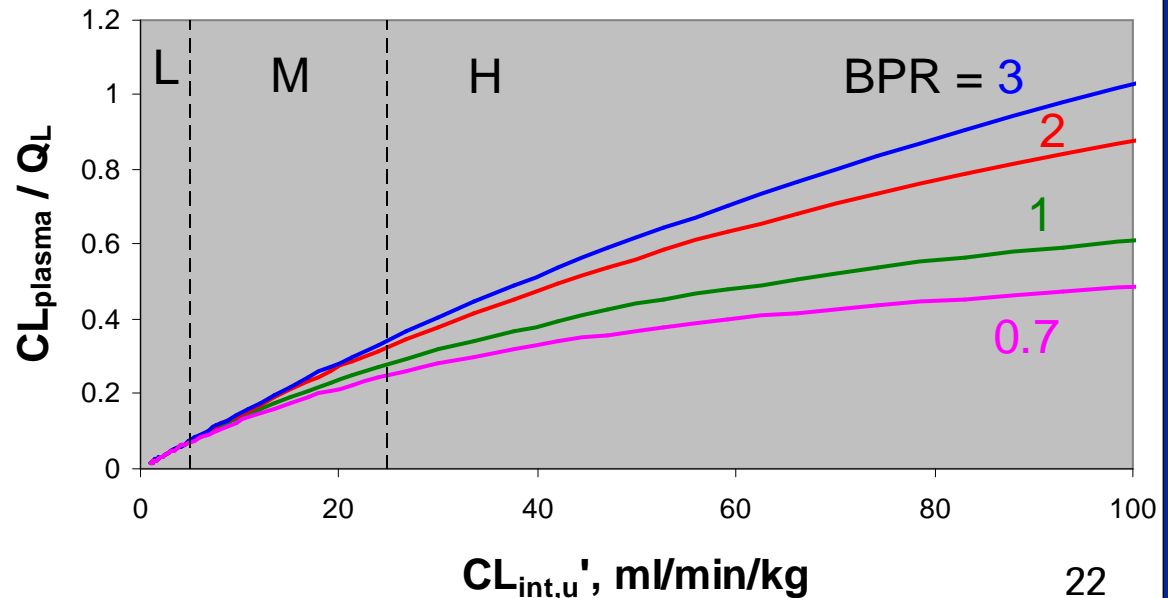
Assuming that the BPR is 1 can lead to inaccurate CL estimates.

$$CL_{\text{blood}} = \frac{Q_L \times (f_{u_p}/BPR) \times CL_{\text{int,u}'}}{Q_L + (f_{u_p}/BPR) \times CL_{\text{int,u}'}}$$

Calculations were done using the well-stirred model, assuming human liver blood flow, for  $f_{u_p} = 0.3$



For low CL compounds, CL<sub>plasma</sub> is not impacted.



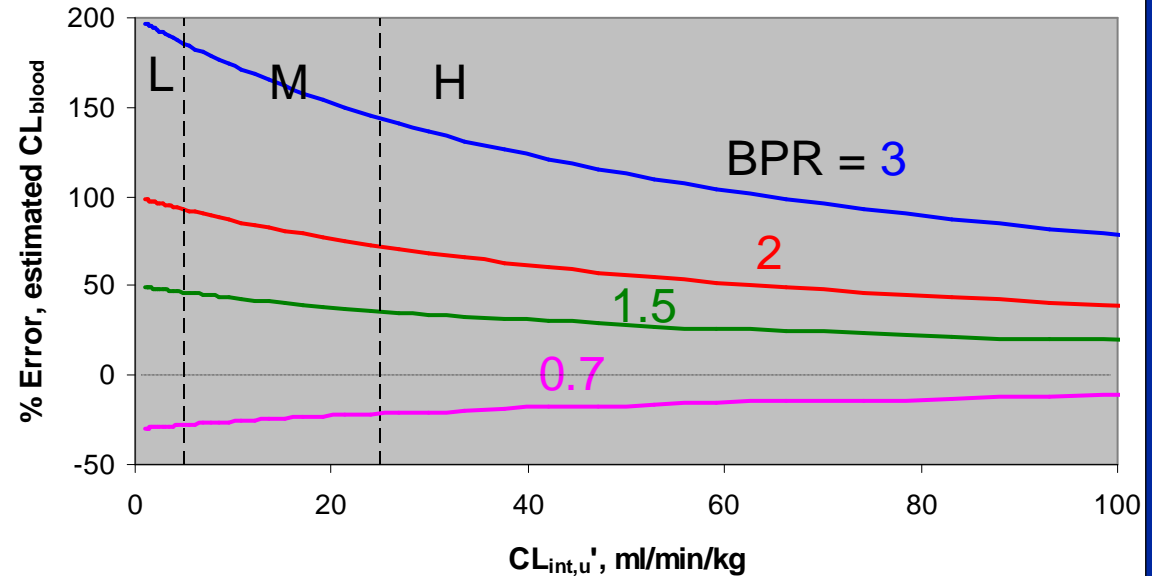
How much error is introduced into the estimated CL if it is assumed that BPR=1?

% Error =

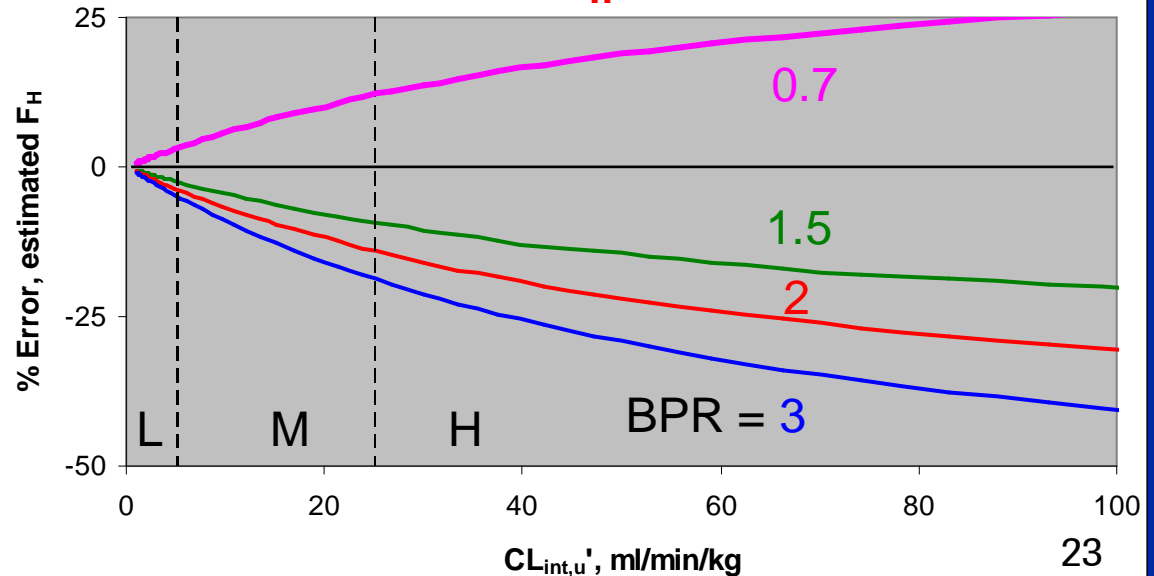
$$\frac{\text{Estimated} - \text{Actual} \times 100\%}{\text{Actual}}$$

Calculations were done using the well-stirred model, assuming human liver blood flow, for  $f_{u_p} = 0.3$

For low CL compounds, more error is introduced.



For low CL compounds, F<sub>H</sub> will be close to 100%.



## ④ Plasma protein binding

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*How much impact does plasma protein binding have on clearance?*

$$CL_{\text{blood}} = \frac{Q_L \times fu_B \times CL_{\text{int,u}}'}{Q_L + fu_B \times CL_{\text{int,u}}'}$$

# Plasma protein binding

- The well-stirred model often used for scaling CL is:

$$CL_{\text{blood}} = \frac{Q_L \times (f_{u_p}/BPR) \times CL_{\text{int,u}}'}{Q_L + (f_{u_p}/BPR) \times CL_{\text{int,u}}'}$$

- When the BPR has not been measured, but  $f_{u_p}$  data are available, the following equation is often used:

$$CL_{\text{blood}} = \frac{Q_L \times f_{u_p} \times CL_{\text{int,u}}'}{Q_L + f_{u_p} \times CL_{\text{int,u}}'}$$

- When  $f_{u_p}$  data are not available, this equation is sometimes used:

$$CL_{\text{blood}} = \frac{Q_L \times CL_{\text{int,u}}'}{Q_L + CL_{\text{int,u}}'}$$

For compounds with low binding and for compounds with flow-limited metabolism, the “simple model” may be used.

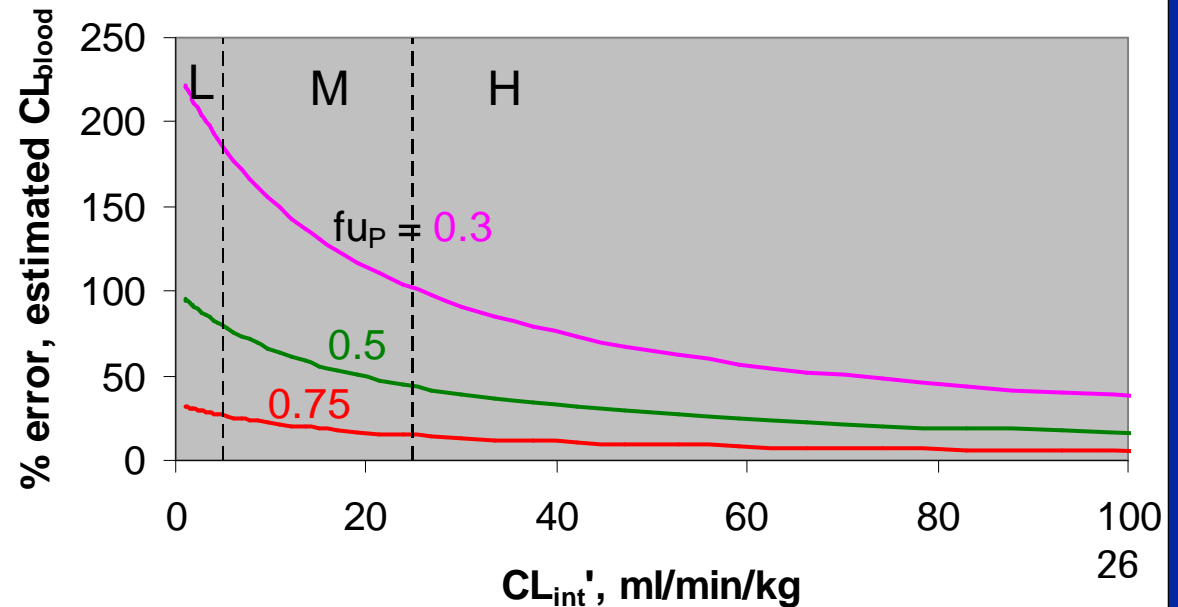
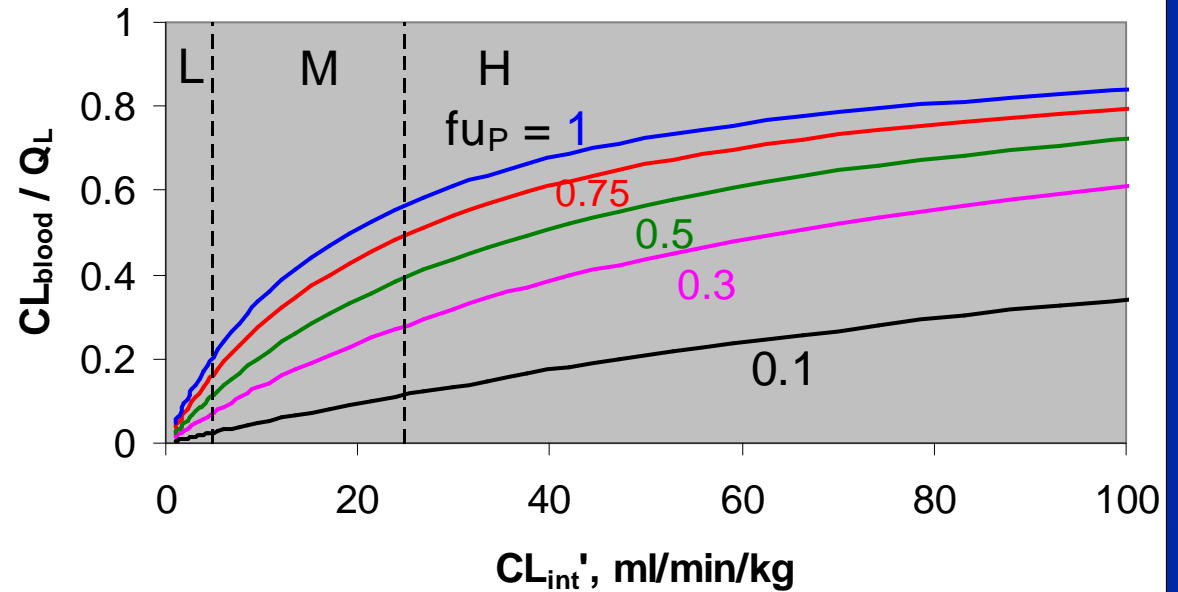
→ The extent of protein binding should be confirmed.

% Error =

$\frac{\text{Estimated} - \text{Actual} \times 100\%}{\text{Actual}}$

Actual

*Calculations were done using the well-stirred model, assuming human liver blood flow, for  $BPR = 1$*



## 5 Microsomal binding

*When will Mx binding impact your human PK prediction?*

$$\text{CL}_{\text{blood}} = \frac{Q_L \times f_{u_B} \times \text{CL}_{\text{int,u}}'}{Q_L + f_{u_B} \times \text{CL}_{\text{int,u}}'}$$

## Mx binding can reduce the concentration in the Mx incubation and the apparent $CL_{int}$ .

- The well-stirred model is used to estimate  $CL_{blood}$  from unbound intrinsic clearance.

$$CL_{blood} = \frac{Q_L \times fu_B \times CL_{int,u}'}{Q_L + fu_B \times CL_{int,u}'}$$

- $CL_{int}'$  may change depending on the protein concentration in the incubation, but  $CL_{int,u}'$  will not.

$$CL_{int,u}' = CL_{int}' / fu_{Mx}$$

- The unbound fraction in Mx,  $fu_{Mx}$ , can be measured experimentally, or estimated using correlations.

# Apparent $CL_{int}$ (and therefore scaled CL) is affected by Mx binding.

Drug	logP	Class	1 mg/ml protein		0.2 mg/ml protein		$CL_{int}$ ratio (1 mg/ml/ 0.2 mg/ml)
			$fu_{Mx}$	$CL_{int}$ , $\mu\text{l}/\text{min}/\text{mg prot}$	$fu_{Mx}$	$CL_{int}$ , $\mu\text{l}/\text{min}/\text{mg prot}$	
Desipramine	3.87	Base	0.21	10	0.66	21	0.48
Amitriptyline	4.612	Base	0.53	34	0.82	71	0.48
Midazolam	3.868	Neutral	0.65	238	0.98	319	0.75

*Data ( $fu_{Mx}$ ,  $CL_{int}$ ) are from Giuliano et al, 2005. Microsomal binding data were from equilibrium dialysis.*

$$CL_{int} = \text{Dose} / AUC_{\infty}$$

# Mx binding should be measured if it will impact estimated CL.

- Rearranging

$$CL_{\text{blood}} = \frac{Q_L \times fu_B \times CL_{\text{int}}' / fu_{Mx}}{Q_L + fu_B \times CL_{\text{int}}' / fu_{Mx}}$$

results in:

$$\frac{CL_{\text{blood}}}{Q_L} = \frac{1}{\frac{fu_{Mx} \times Q_L}{fu_B \times CL_{\text{int}}'} + 1}$$

$$R_{CL} = \frac{fu_B \times CL_{\text{int}}'}{Q_L} \quad \frac{CL_{\text{blood}}}{Q_L} = \frac{1}{\frac{fu_{Mx}}{R_{CL}} + 1}$$

$R_{CL}$  measures how efficiently the liver can remove compound relative to the rate of blood flow to the liver.

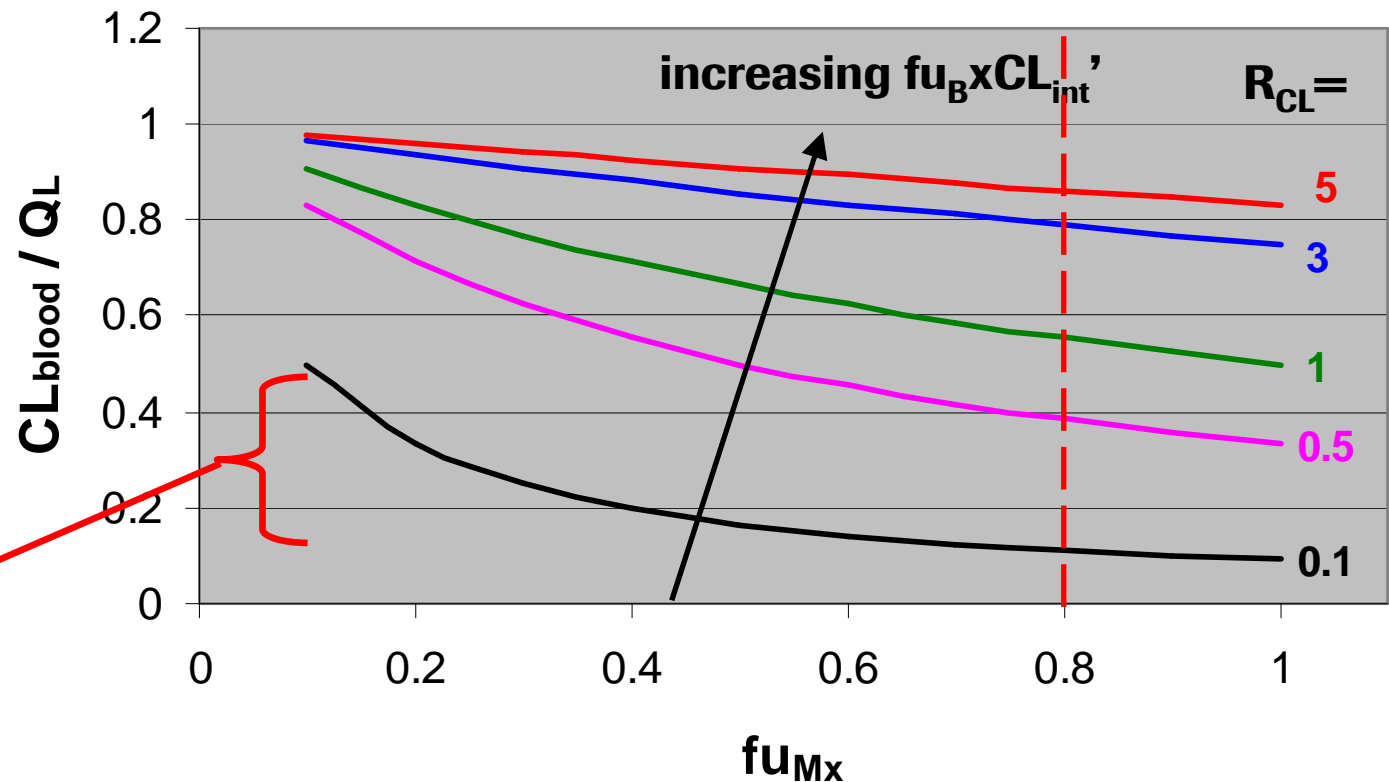
# Failure to account for Mx binding has the greatest relative impact on the accuracy of scaled CL for low CL compounds.

$$R_{CL} = \frac{fu_B \times CL_{int}'}{Q_L}$$

$R_{CL}$  is a measure of metabolic efficiency.

More than a four-fold impact

Effect of Mx binding on  $CL_{blood}$



# Correlations have been developed to relate Mx binding to logP.

- Bases  $\log K_{mic} = 0.58 \times \log P - 2.02$
- Neutrals  $\log K_{mic} = 0.46 \times \log P - 1.41$
- Acids  $\log K_{mic} = 0.20 \times \log P - 1.54$
- $f_{u_{Mx}} = 1 / (1 + K_{mic})$

From poster by Turner, Rostami-Hodjegan, Tucker, and Yeo, "Prediction of non-specific hepatic microsomal binding from readily available physicochemical properties." Other methods include that of SimCyp (Emoto et al. 2009), Hallifax and Houston (2006), and Austin et al. (2002).

- These correlations were developed for a protein concentration of 1 mg/ml, but  $f_{u_{Mx}}$  can be adjusted to values for other protein concentrations (Austin et al. 2002).

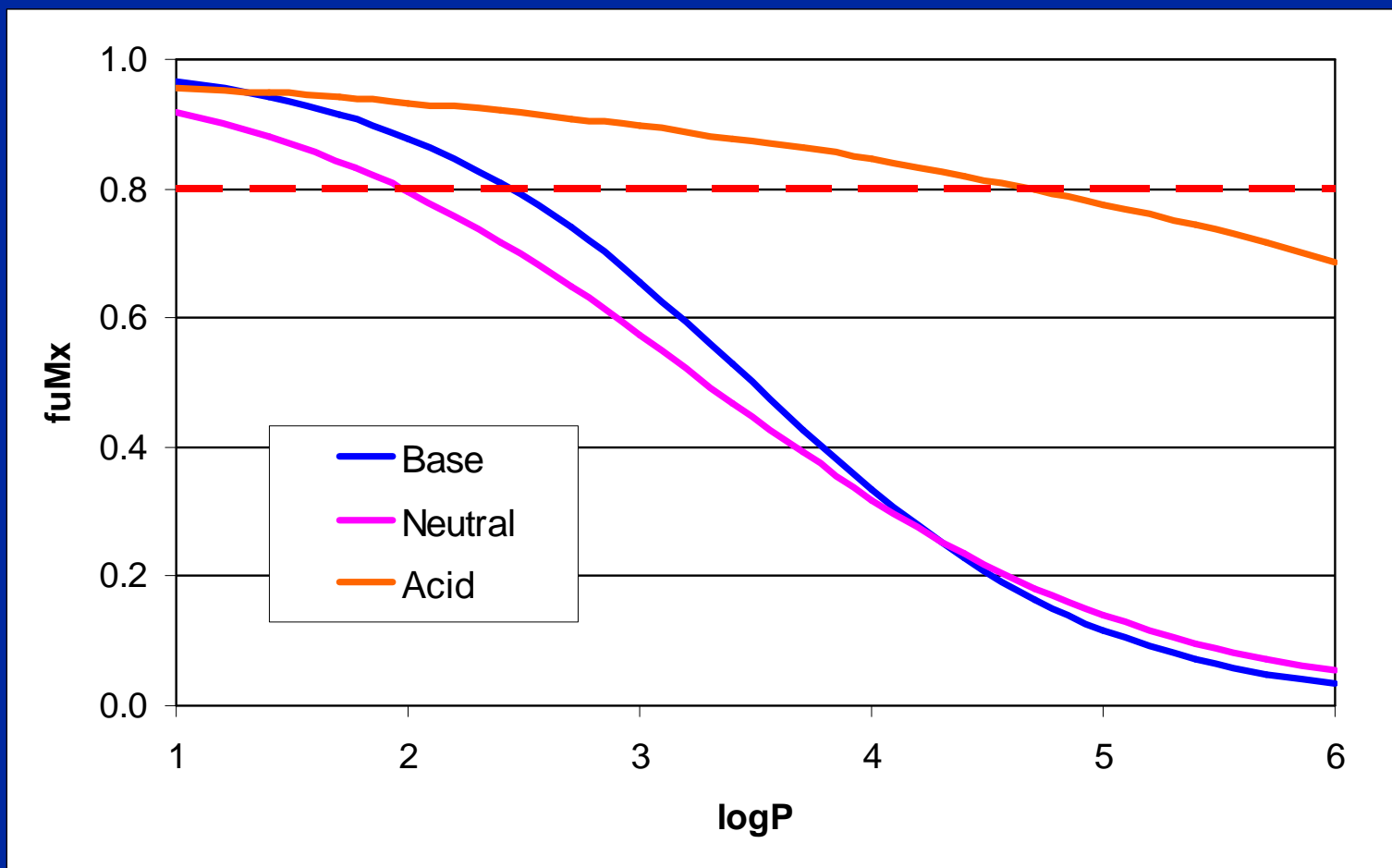
$$f_{u_{Mx2}} = \frac{1}{\left[ \frac{C_{Mx2}}{C_{Mx1}} \right] \left[ \frac{1 - f_{u_{Mx1}}}{f_{u_{Mx1}}} \right] + 1}$$

$C_{Mx1}$  = concentration of Mx protein where  $f_{u_{Mx}}$  is known (i.e.,  $f_{u_{Mx1}}$ )

$C_{Mx2}$  = concentration of Mx protein where you would like to know  $f_{u_{Mx}}$  (i.e.,  $f_{u_{Mx2}}$ )

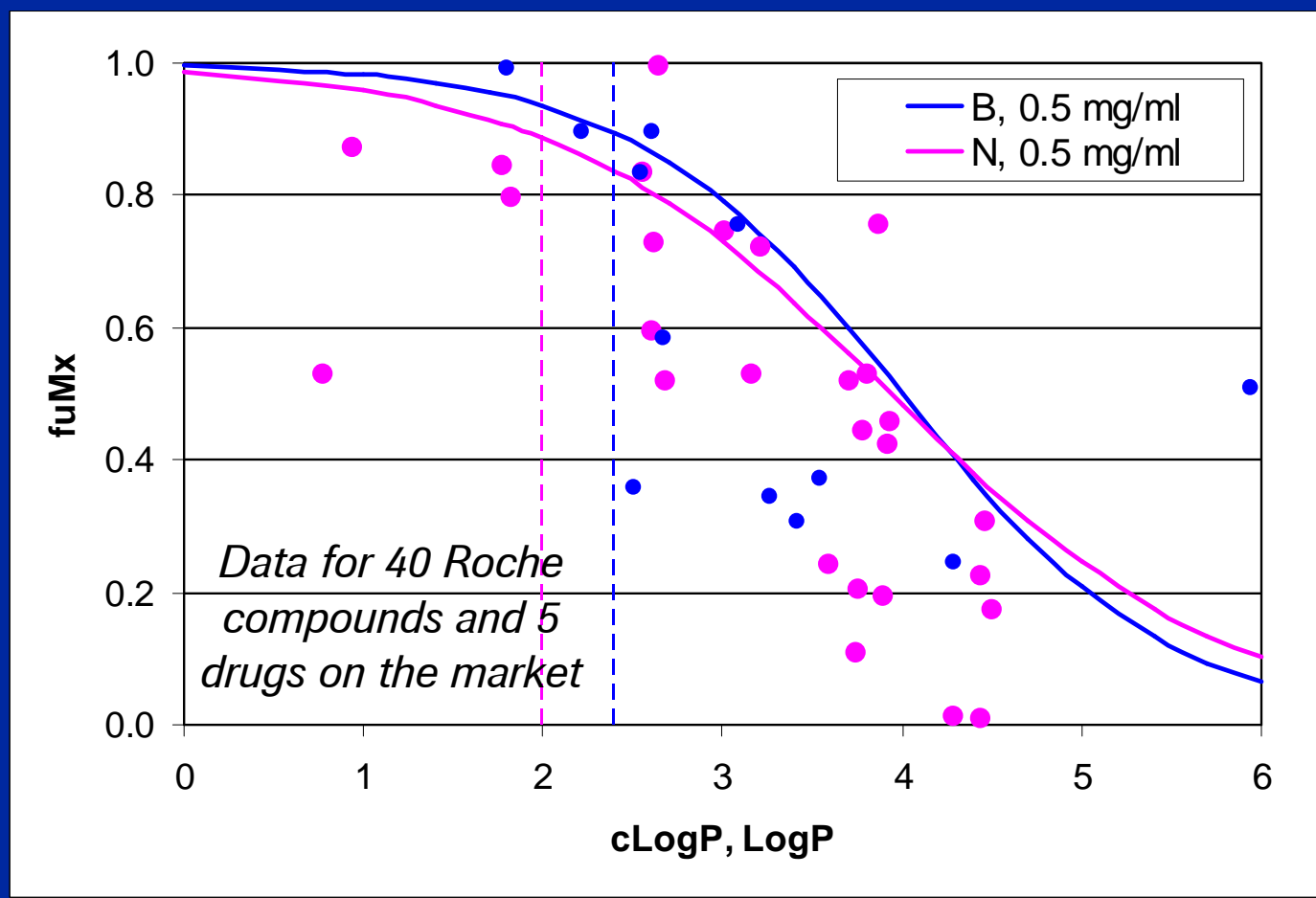
For 1 mg/ml protein, consider measuring  $fu_{Mx}$  for:

- bases with  $\log P > 2.4$
- neutrals with  $\log P > 2$
- acids with  $\log P > 4.7$



*Plot constructed using correlations for 1 mg/ml protein in Mx assay.*

# By measuring Mx binding, you can minimize one more possible contributor to error in scaled CL.



*Plot constructed using correlations for 0.5 mg/ml protein in Mx assay.*

# Mx data is scaled with many different models.

- George Box stated, “...all models are wrong, but some are useful.”
- **Some models are more wrong than others.**
  - **If a model misleads instead of informs, it should not be used.**
- The stage of preclinical development can impact model choice.
- Before using CL values scaled from Mx data, clarify:
  - the model used
  - evidence that in vitro and in vivo CL are linear
  - if the BPR was measured
  - whether scaled CL is  $CL_{\text{plasma}}$  or  $CL_{\text{blood}}$

# Example of an IVIVC analysis – as additional data becomes available, the model can be refined.



CL, ml/min/kg	Rat	Human
IV CL <sub>plasma</sub> <sup>a</sup>	13	5.1
IV CL <sub>blood</sub> <sup>a</sup>	19	7.3
CL <sub>int</sub> '	610	66
CL <sub>blood</sub> , Model 1	54 (420% of 13)	—
CL <sub>blood</sub> , Model 2	0.96 (7.3% of 13)	—
CL <sub>blood</sub> , Model 3	1.37 (7.2% of 19)	—
CL <sub>blood</sub> , Model 4	8.6 (45% of 19)	1.0

<sup>a</sup> Data from Jones et al. (2006).

## Model 1

$$CL_{blood} = \frac{Q_L \times CL_{int}'}{Q_L + CL_{int}'}$$

## Model 2 (fu<sub>p</sub>)

$$CL_{blood} = \frac{Q_L \times fu_p \times CL_{int}'}{Q_L + fu_p \times CL_{int}'}$$

## Model 3 (fu<sub>p</sub>, BPR)

$$CL_{blood} = \frac{Q_L \times (fu_p/BPR) \times CL_{int}'}{Q_L + (fu_p/BPR) \times CL_{int}'}$$

## Model 4 (fu<sub>p</sub>, BPR, fu<sub>Mx</sub>)

$$CL_{blood} = \frac{Q_L \times (fu_p/BPR) \times CL_{int,u}'}{Q_L + (fu_p/BPR) \times CL_{int,u}'}$$

# Sometimes, the model choice does not impact data analysis.



CL, ml/min/kg	Rat
IV CL <sub>plasma</sub>	30.9
IV CL <sub>plasma</sub> *	18.7
IV CL <sub>blood</sub>	24.9
CL <sub>int</sub> '	4.22
CL <sub>blood</sub> , Model 1	3.94
CL <sub>blood</sub> , Model 2	2.74
CL <sub>blood</sub> , Model 3	3.59
CL <sub>blood</sub> , Model 4	4.10 (16% of 24.9)

\* This compound is primarily eliminated by biliary excretion.

Model 1

$$CL_{blood} = \frac{Q_L \times CL_{int}'}{Q_L + CL_{int}'}$$

Model 2 (fu<sub>p</sub>)

$$CL_{blood} = \frac{Q_L \times fu_p \times CL_{int}'}{Q_L + fu_p \times CL_{int}'}$$

Model 3 (fu<sub>p</sub>, BPR)

$$CL_{blood} = \frac{Q_L \times (fu_p/BPR) \times CL_{int}'}{Q_L + (fu_p/BPR) \times CL_{int}'}$$

Model 4 (fu<sub>p</sub>, BPR, fu<sub>Mx</sub>)

$$CL_{blood} = \frac{Q_L \times (fu_p/BPR) \times CL_{int,u}'}{Q_L + (fu_p/BPR) \times CL_{int,u}'}$$

\* Urinary CL<sub>plasma</sub> subtracted.

# Conclusions

- When scaling Mx data, failure to include pertinent binding data can result in the significant underestimation or overestimation of CL.
  - **This concern is particularly important for low CL compounds.**
- Saturable metabolism can impact in vivo and in vitro data, complicating the interpretation of in vitro data.
- For accurate scaling of Mx data, consider measuring  $f_{u_{Mx}}$  for bases with  $\log P > 2.4$ , neutrals with  $\log P > 2$ , and acids with  $\log P > 4.7$  (for 1 mg/ml Mx protein).
- **Before using Mx data to estimate human metabolic CL, make sure the data is of sufficient quality to meet your needs.**

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