SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

In Vitro to *In Vivo* Extrapolation of Metabolism Data to Support Physiologically Based Modeling for Route-to-Route (RTR) Extrapolation

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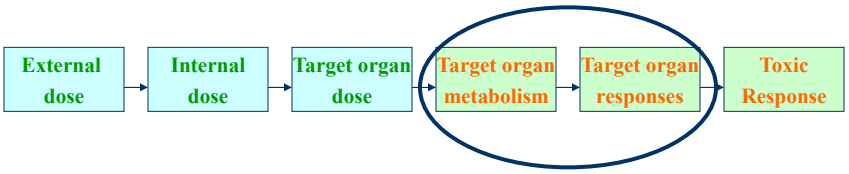
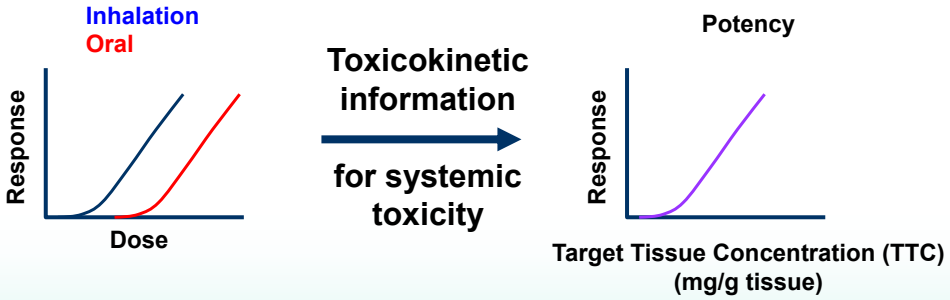
A Primary Assumption: Potency Versus Route





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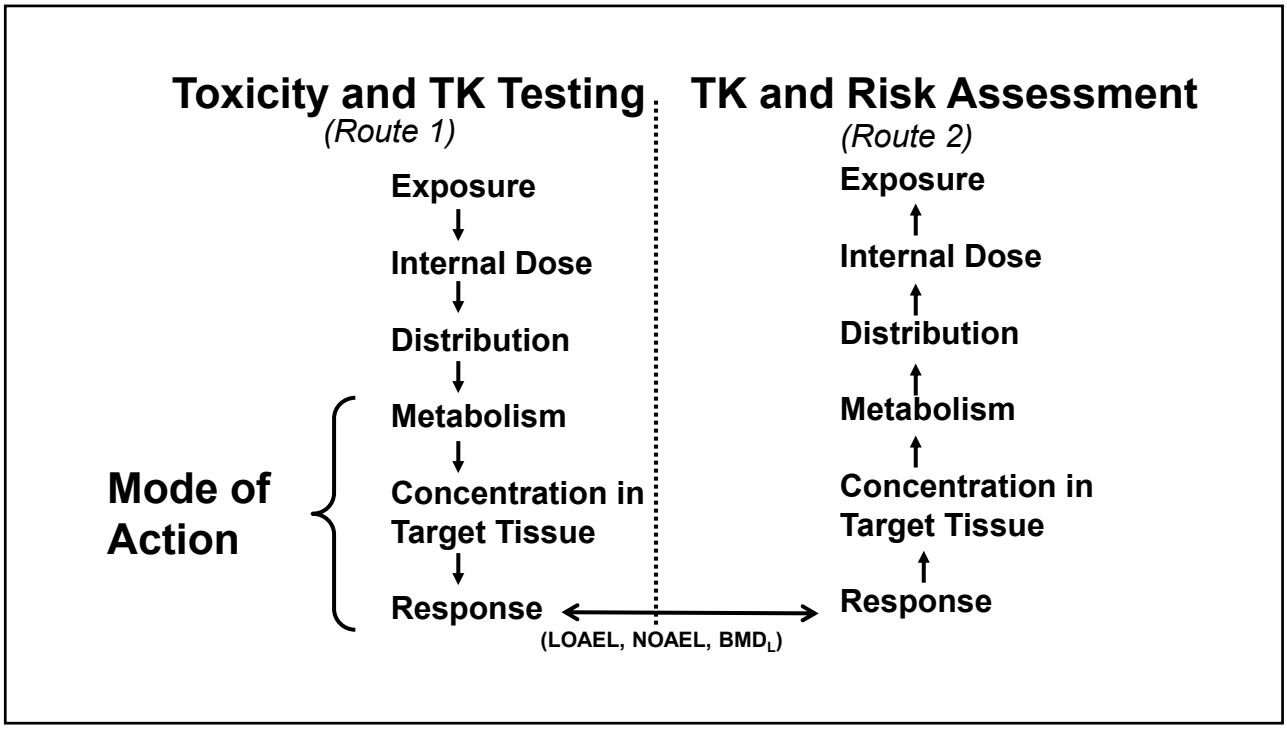
Some Basics

- Don't apply RTR for Portal of Entry (POE) effects:
  - Respiratory tract for inhalation toxicants
  - Some GI tissues for oral toxicants
- Must have confidence in dose-response assessment
- Requires confidence in toxicokinetic models
- This HAS been done by the US EPA
  - (e.g., Benzene, EGBE, Vinyl Chloride, Trichloroethylene)

RTR Extrapolation: Fundamentals of Application

- Dose-response data from one route must be valid
- Dose-response data from that route must be expressed in units of TTC (dose metric)
- The same model will be applied
- The same dose metric will be applied
- Dose-response data where dose is TTC will be used for predicting response via both routes



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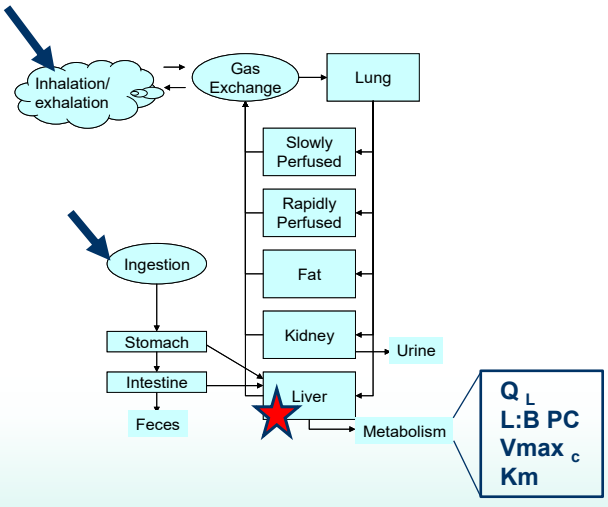
PBPK for Route-to-Route Extrapolation



- Same model structure for both routes
- Same model parameter values
- Same model equations
- Same dose metric
- Dose-response should align when dose is expressed as target tissue concentration**

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Approach

- Examine mode of action data: toxic moiety and target tissue.
- Identify enzyme responsible and quantify enzyme content.
- Characterize kinetics of metabolism: proper study design
  - tissues, preparations, cofactors and [s].
- Remember anatomy, physiology and cellular biochemistry.
- Integrate information under logical framework.

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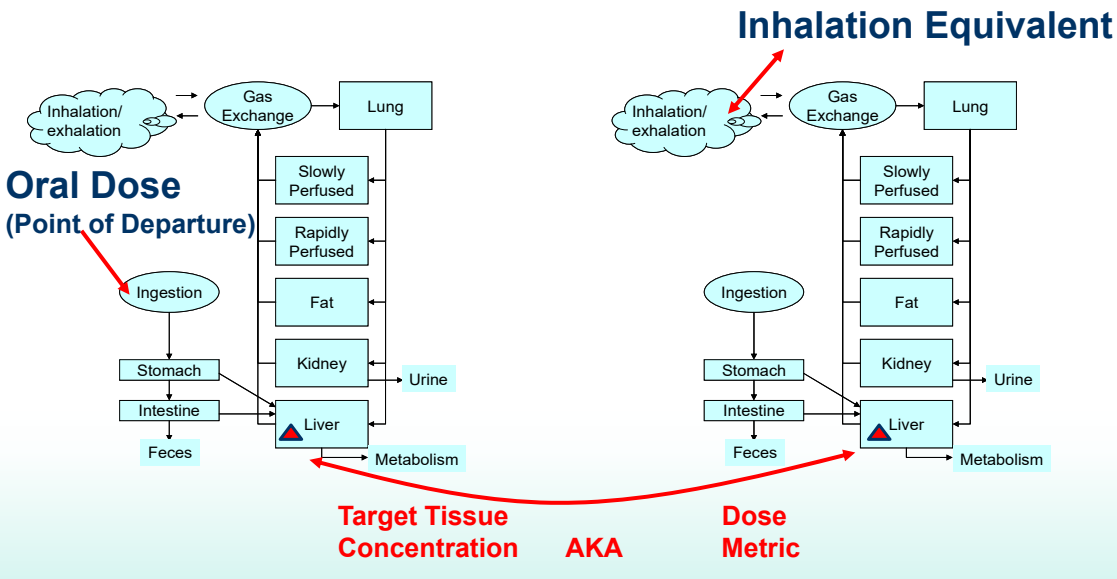
TTC-Based Route-to-Route Extrapolation



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Vinyl Chloride

- Oral to inhalation extrapolation
- Oral dose-response data available in rats
- PBPK model available for both routes in rats
  - Compartments: gas exchange lung, richly perfused, liver, poorly perfused & fat compartments
- Lifetime rat study for noncancer liver effects;
  - Point of Departure (POD) is determined

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Vinyl Chloride Risk Assessment

- Animal studies–liver effects, including tumors; other relative organ weights, testicular effects at 10X higher doses, CNS and fetotox at even higher doses
- Human epidemiology–liver, angiosarcomas, cirrhosis, degeneration (no pulmonary effects)
- Liver chosen as critical target

Adapted from US EPA Vinyl Chloride IRIS risk assessment, US EPA 2000
See summary by DeWoskin, 2007

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Vinyl Chloride

- Choice of Dose Metric
- Chronic toxicity, no split-dosing studies
- Active CYP-derived metabolite in liver
- Cumulated measure of exposure, AUC-like
- Amount of VC oxidized/unit of liver mass (AML)

- The most appropriate pharmacokinetic dose metric for a reactive metabolite is the total amount of the metabolite generated divided by the volume of the tissue into which it is produced (Andersen et al., 1987).

Andersen, M; Clewell, H; Gargas, M; et al. (1987) Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87:185-205.

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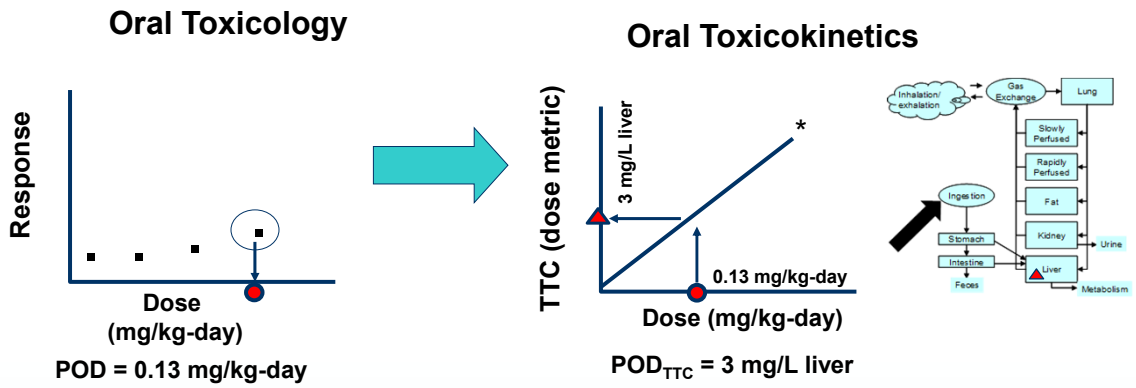
Oral Toxicity and Toxicokinetic Data



* Linear relationship between dose and TTC was observed

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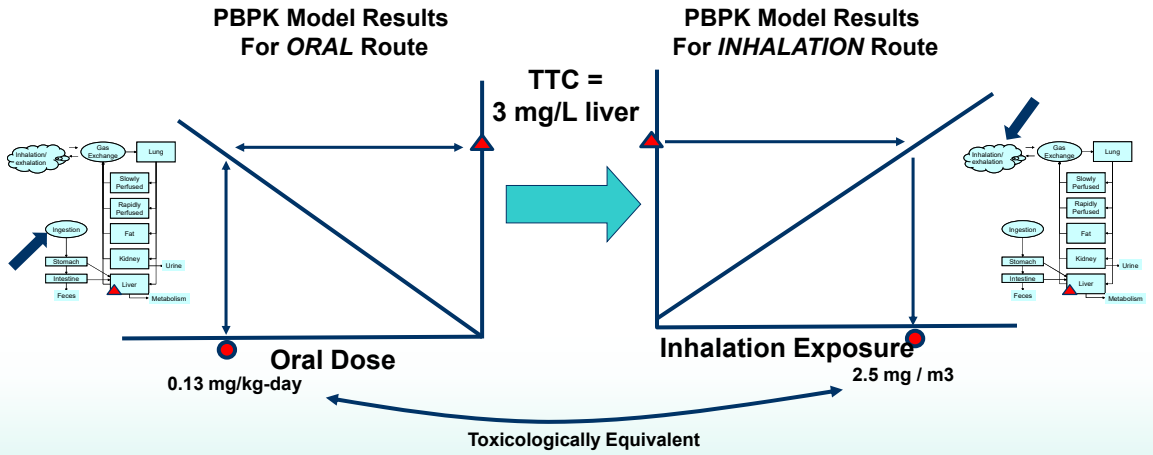
Route-to-Route Extrapolation

What inhalation exposure will produce TTC of 3 mg/L of liver?



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Metabolism

- Determines Target Tissue exposure to Toxicant
- Bioactivation or Detoxication
- Importance of Metabolic Variance among humans:
- Metabolism accounts for either 95% or 99% of the absorbed dose of a chemical
- When Detoxication, variance in risk = (100-95)/(100-99), or 5x
- When Bioactivation, variance in risk = 99/95, or about 5%

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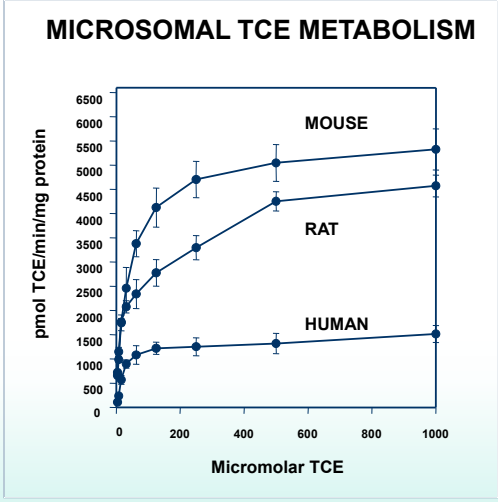
First-Order Metabolism



$$V = \frac{Vmax * [s]}{Km + [s]}$$

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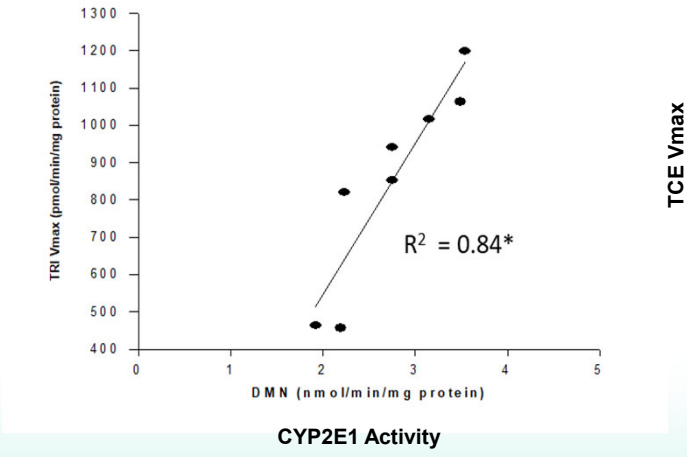
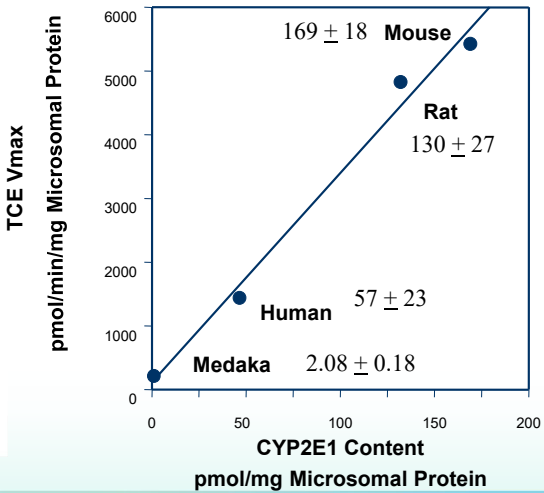
In Vitro Trichloroethylene Metabolism and CYP2E1 Content/Activity





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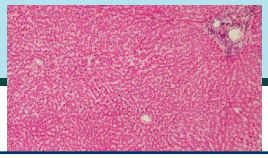
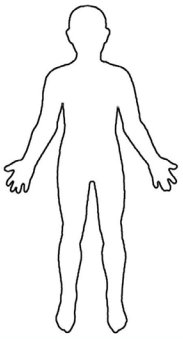
Quantitative Basis for Extrapolation

HEPATOCELLULARITY



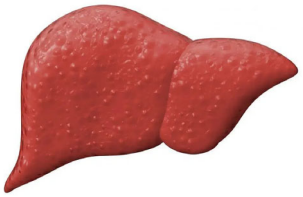
107 million cells/gram liver

BODY WEIGHT



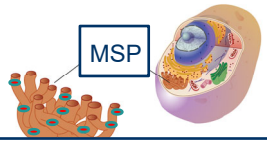
70 kg

LIVER MASS



1820 grams;
0.026 BW

MICROSOMAL PROTEIN



52.9 mg MSP/gram liver

CYP 2E1

48.9 pmol CYP2E1 / mg MSP

Risk Anal 23:1221, 2003; Br J Clin Pharmacol 56:433, 2003

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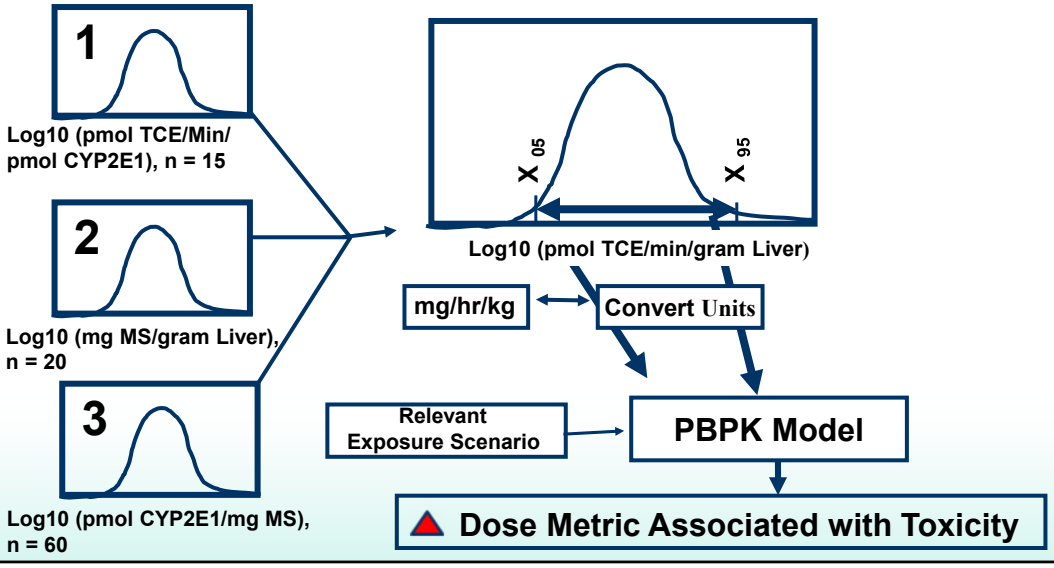
Data Integration and Interpretation



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V_{MAX} Extrapolation

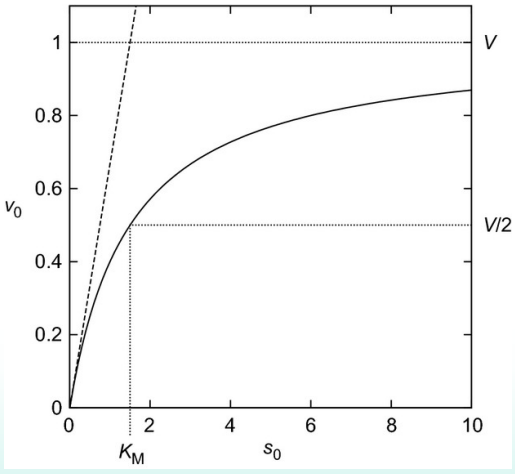
	HUMAN DATA; TCE Oxidation
***In vitro* Vmax: pmol/min/pmol CYP2E1**	**32 pmol TCE/min/pmol CYP2E1 (Set 1)**
× unit content; pmol CYP2E1/mg MSP	**× 48.9 pmol CYP2E1/mg MSP (Set 3)**
× (sub) cellular fraction; MPPGL	**× 52.9 mg MSP/gram liver (Set 2)**
× liver mass; 1820 grams	**× 1820 grams liver mass**
÷ body weight; 70 kg	**÷ 70 kg**
	= 2,152,226 pmol TCE/min/kg
Convert for time	**× 1 mmol/1,000,000,000 pmol**
Convert for mass	**= 0.00215 mmol TCE/min/kg**
= $Vmax_c$	**× 131.7 mgrams/mmol TCE**
	= 0.283 mg TCE/min/kg
	× 60 mins/hr
	= 17 mg TCE/hr/kg

Trichloroethylene data from Lipscomb et al. *Risk Anal* 23:1221, 2003

Km (affinity; [s] at one-half maximal velocity)

V at [s] << Km essentially first-order– Little impact of Km value.

***In vivo* human exposures typically result in very low blood concentrations, thereby reducing the practical impact of Km values on predicted or observed metabolic rates**



Adapted from Schnell, FEBS Journal 281:464, 2014

Km (affinity; [s] at one-half maximal velocity)

- Often expressed relative to concentration of venous blood at equilibrium with liver (CVL)
- Treat *in vitro* incubation as if it were liver tissue
- Determine Km *in vitro*
- Use Km directly in the model, or
- Extrapolate *in vitro* Km by adjusting for liver:blood PC

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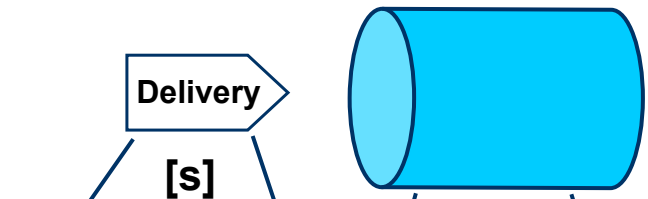
Chemical Availability	Metabolic Capacity	Amount Metabolized



Absorption into blood
Solubility in blood
Extrahepatic distribution
Blood flow to liver
Blood:Liver partitioning

Enzyme content
Enzyme activity
Affinity

Metabolite

- Rate of formation
- Average concentration
- Maximum Concentration

$$\text{Metabolic Rate} = \frac{\text{Vmax} * [s]}{\text{Km} + [s]}$$

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Summary

- PBPK models allow extrapolation across dose, species, etc.
- We can develop data *in vitro* from high quality (human) tissues
- PBPK Modeling offers a platform through which to interpret and extend *in vitro* data
- IVIVE methods abound and are expanding for metabolism data
- Including faction unbound sometimes improves prediction capability (see SimCYP)
- Methods are fairly consistent for Vmax; developing consistency for Km
- Errors in extrapolating Km may have minimal impact
- Conclusions drawn from extrapolated rate constants for flow limited substrates may not hold much weight.

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