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DILIsym User Training – Physiologically-based Pharmacokinetic (PBPK) Modeling in DILIsym

DILIsym Development Team

Goal for The DILIsym PBPK Sub-model Session

Participants should understand the following general concepts:

- How to use the DILIsym PBPK sub-model, including the most recent updates

DILIsym Training on PBPK Structure

- Overview of the PBPK sub-model changes in DILIsym v6A
- Parameterizing the PBPK sub-model for DILIsym v6A

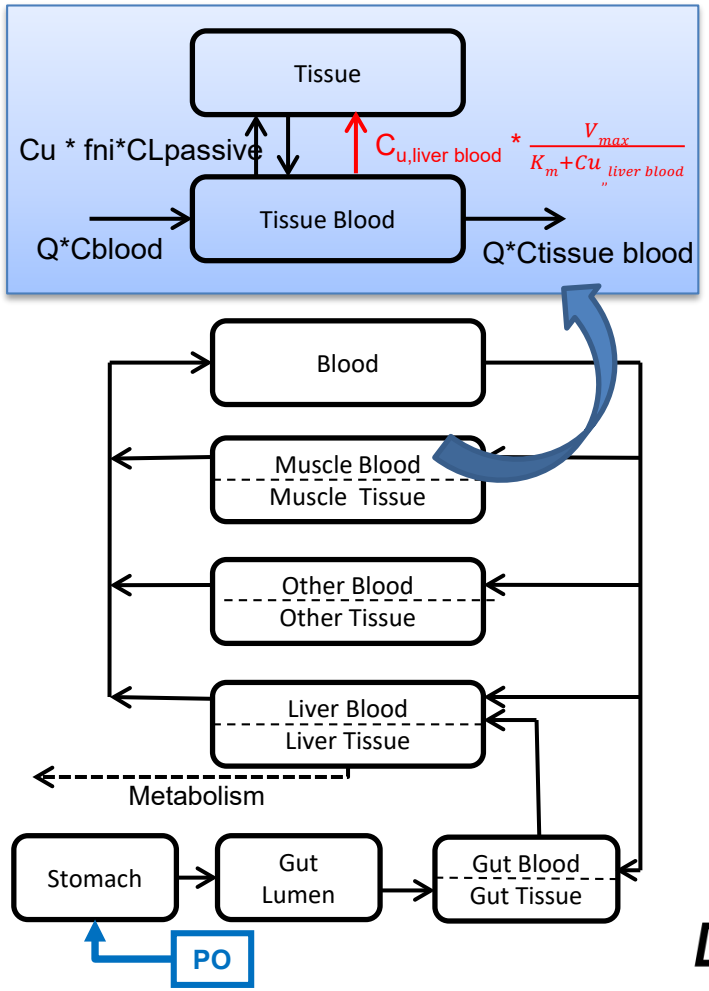
v6A PBPK Update Summary

Pathway	v5A	v6A
Passive diffusion	Only unbound compound undergoes passive diffusion	Unbound, unionized compound undergoes passive diffusion
Hepatic uptake transport	Hepatic uptake only represented for Comp W and X	Transporter-mediated hepatic uptake added for stable metabolites and Compound Y
Hepatic efflux transport	No basolateral hepatic efflux	Transporter-mediated basolateral efflux added for parent compounds, stable metabolites and Compound Y
Tissue permeability	Permeability-limited distribution represented only for the liver	Permeability-limited models added for all extra-hepatic tissues

Perfusion- and Permeability-limited Distribution Represented for All Tissues Using a Two-compartment Tissue Model

fni: fraction non-ionized

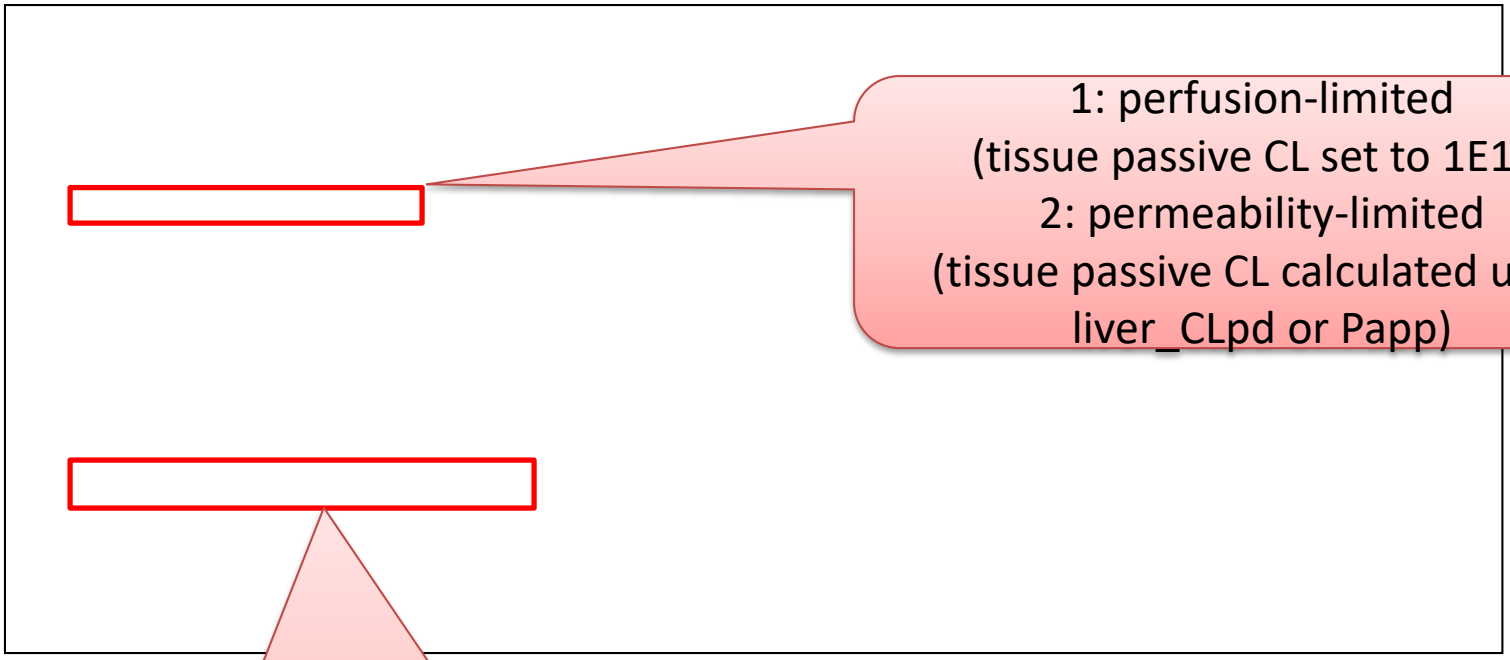
Active uptake transporter represented only in the liver



- Perfusion-limited distribution if CLpassive >> Q (default)
  - Instant mixing of tissue and tissue blood; reach equilibrium quickly
  - Extent of tissue distribution will be determined by fu_P and fu_T (calculated using tissue:blood ratio), pKa, compound type (acid/base)
  - User input: fu_P, B:P, Tissue:Blood partition coefficients, compound type (acid/base), pKa

- For permeability-limited distribution, CLpassive of each compound can be optimized or calculated from *in vitro* permeability data
  - Only unbound, non-ionized drugs can undergo passive diffusion; frac non-ionized calculated by DILIsym using compound type (acid/base) and pKa values
  - CLpassive calculated by DILIsym using *in vitro* permeability and the tissue surface area
  - User input: compound type (acid/base), pKa, *in vitro* permeability, transporter Km/Vmax if a substrate of hepatic uptake transporters

Switched Added for Tissue Distribution Model



1: perfusion-limited
(tissue passive CL set to 1E10)
2: permeability-limited
(tissue passive CL calculated using liver_CLpd or Papp)

Used when permeability-limited model is selected:
1: liver_CLpd is used to calculate tissue passive CL
2: Papp is used to calculate tissue passive CL

Default values :
Liver_CL_pd: 1 mL/hr/kg^0.75
Perm_app: 1E-06 cm/sec

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Passive Diffusion CL Calculated by DILIsym from *In Vitro* Permeability or Liver Passive Diffusion CL

Variable	Name	Unit	Default Value
Comp_X_liver_CL_pd	Compound X liver passive clearance	mL/hr/kg^0.75	1
Comp_X_perm_app	Compound X apparent passive permeability	cm/sec	1e-6

- Perm_app: obtained from distributional permeability assays
- Default: 1e-06 cm/sec

- Liver_CL_pd: obtained from mechanistic modeling using SCH data, parameter optimization, or HC uptake study
- Easier to compare contribution of passive vs. active hepatic CL

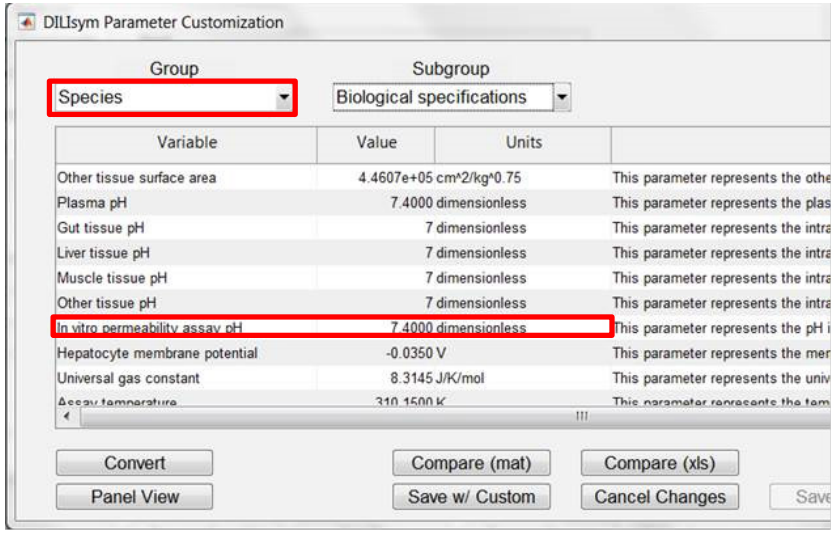
Perm_app: input parameter
(cm/sec)
P_neutral = 2*Perm_app/frac_nonionized
(cm/sec)
CL_passive_L = P_neutral*liver SA *3600
(mL/hr/kg^0.75)
CL_passive_M = P_neutral*muscle SA *3600
(mL/hr/kg^0.75)

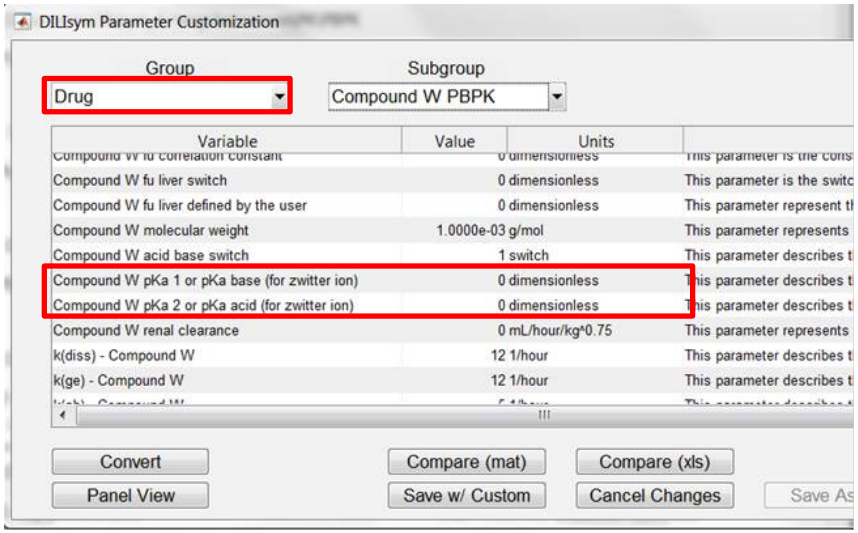
Liver_CL_pd: input parameter
(mL/hr/kg^0.75)
CL_passive_L = CL_passive_L_app/frac_nonionized
(mL/hr/kg^0.75)
CL_passive_M = CL_passive_L * muscle SA/ liver SA
(mL/hr/kg^0.75)

Compound Ionization is Determined by Compound pKa and System pH

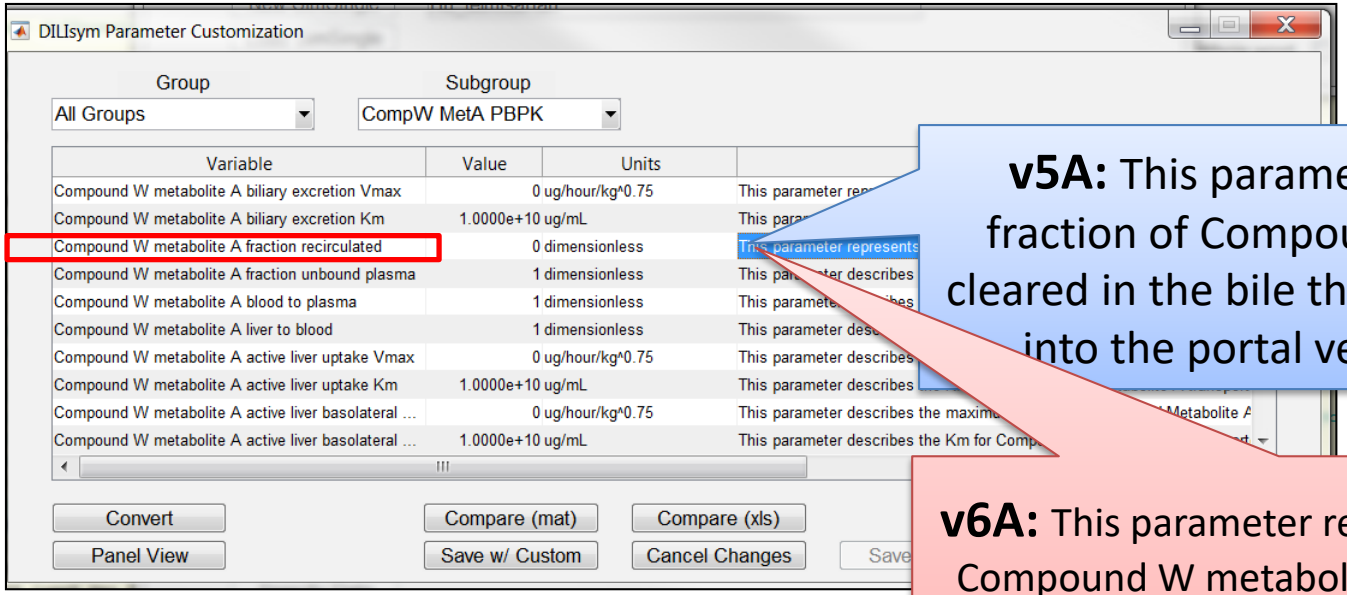
- The default assay pH for in vitro permeability is 7.4 - can be changed if in vitro experiment is run under different assay conditions

- The pKa of Comp W/X/Y and stable metabolites now need to be entered as parameters





Clarify Parameter Names and Descriptions for Recirculation



v5A: This parameter represents the fraction of Compound W metabolite A cleared in the bile that is recirculated back into the portal vein (Min:0, Max:1)

v6A: This parameter represents the fraction of Compound W metabolite A cleared in the bile that is converted to Compound W in the gut lumen and recirculated back into the portal vein as Compound W (Min:0, Max:1)

Transporter-Mediated Hepatic Basolateral Efflux Added for Comp W, X, Y

New Outputs Added for QWBA Comparison

CompW_all_liver_to_blood	**Compound W liver to blood ratio including parent and metabolites**	**dimensionless**

- Calculates liver:blood ratio for the sum of all species (parent+metabolites) in the simulation
- In the QWBA study, radioactivity is measured and reported as "ug equivalent of parent/g tissue"
  - Stable metabolite concentrations corrected for M.W. differences
  - RM and RM-adducts in molar unit converted to ug using the m.w. of the parent
  - Plasma RM-adduct concentrations converted to blood RM-adduct concentrations

DILIsym Training on PBPK Structure

- Overview of the PBPK sub-model changes in DILIsym v6A
- Parameterizing the PBPK sub-model for DILIsym v6A

The PBPK Representation in DILIsym Depends Heavily on the Development Stage of the Compound being Simulated

Early candidate screening

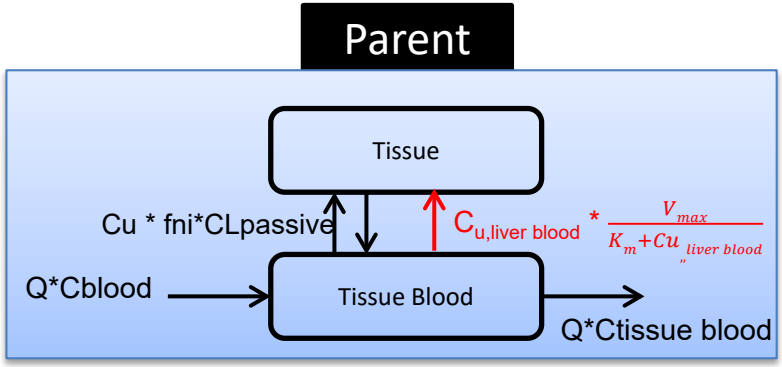
- Metabolic clearance of parent compound and coincidental appearance of specific metabolites *in vitro*
- Potential for active transport in the liver *in vitro* (rate of hepatocyte uptake); transport kinetic information if possible
- Basic molecular properties
  - Acid or base?
  - Monoprotoic or diprotic
  - pKa(s)
  - log P (oil:water and octanol:water)
  - Fraction bound to plasma or serum proteins
  - Fraction partitioned into red blood cells

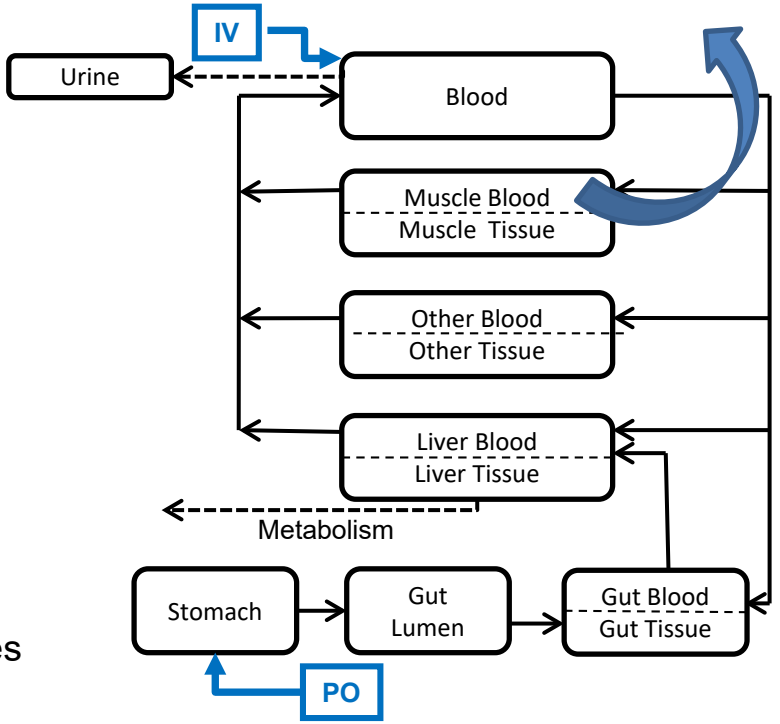
Late-stage development / OTM

- *In vivo* PK time-course and dose-response
- Mass balance tissue distribution studies in animals (*in vitro* accumulation as well)
- *In vitro* drug metabolism assays identifying the appropriate metabolizing enzymes for the drug
- Metabolic clearance of parent compound and coincidental appearance of specific metabolites
- Potential for active transport in the liver (rate of hepatocyte uptake); transport kinetic information if possible
- Basic molecular properties

DILIsym PBPK Overview – Compounds W and X

- Compound W, X, and Y PBPK models for drug combinations
- Compound W and X PBPK models feature five main compartments
  - Gut, liver, blood, muscle, other
- Parent metabolized to metabolite A or B, or reactive metabolites A or B in the liver
  - Michaelis-Menten kinetics
  - Primary metabolite models include three main compartments (liver, blood, other)
  - As of v5A, stable metabolites A and B can be generated by intestinal metabolism
- Oral and IV dosing available
  - IP dosing also included; not generally used for human
- Liver is divided into periportal (PP), midlobular (ML), and centrilobular (CL) zones to allow for zonal distribution of drug and injury
  - 5:3:1 volume distribution
  - Blood flow goes from PP→ML→CL
  - Metabolic activity can be adjusted among the zones





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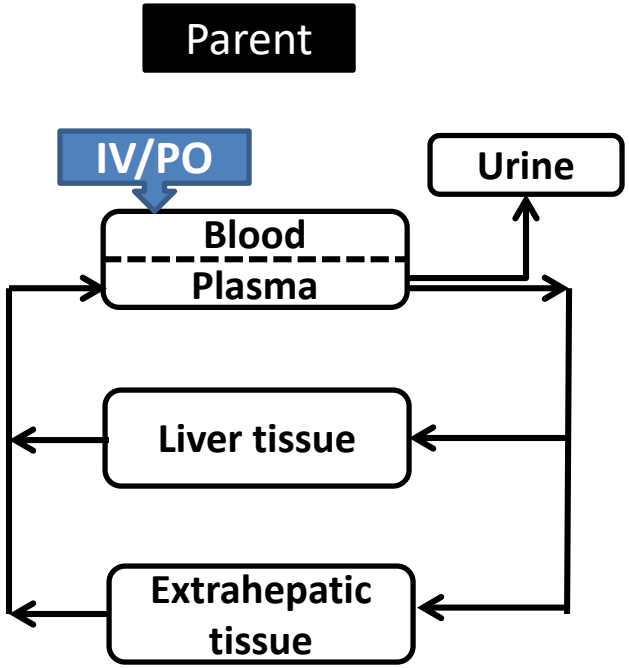
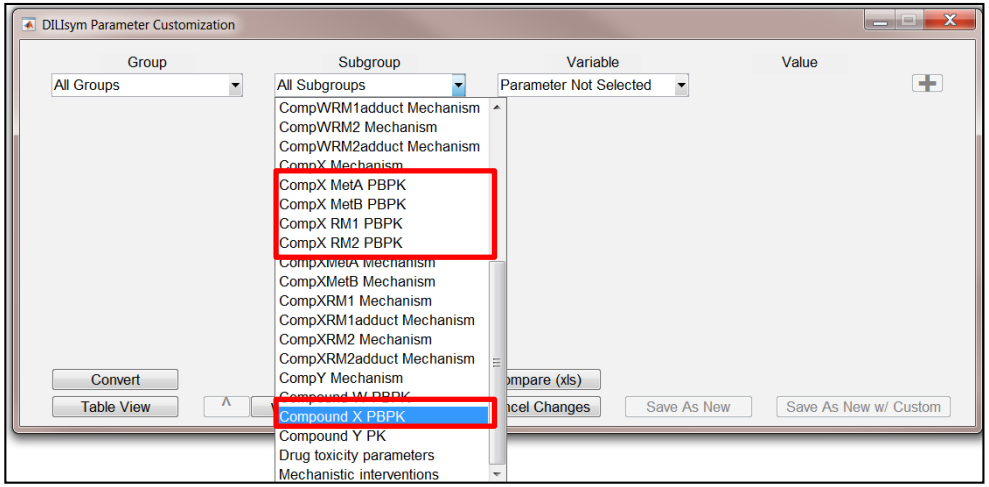
DILIsym PBPK Overview – Compound Y

- Compound Y is a simpler two compartment model
- Minimal PBPK sub-model:
  - Consists of blood, liver, and extrahepatic compartments
  - Metabolite disposition is not tracked
  - Clearance options include renal, non-renal from plasma compartment, and hepatic clearance from liver compartment
- Oral and IV dosing available
  - IP dosing also included; not generally used for human
- Liver is represented with a single, well-mixed compartment
- Extrahepatic distribution is determined by volume of distribution



Selecting Proper PBPK Parameters Is Necessary to Get Maximum Value from DILIsym Simulations

- Liver concentration dynamics are important for the accurate prediction of toxicity
- DILIsym contains many PBPK parameters for the Compound W and X models
- PBPK model parameterization requires two main steps
  - Selecting appropriate metabolic scaffold
  - Parameterizing model



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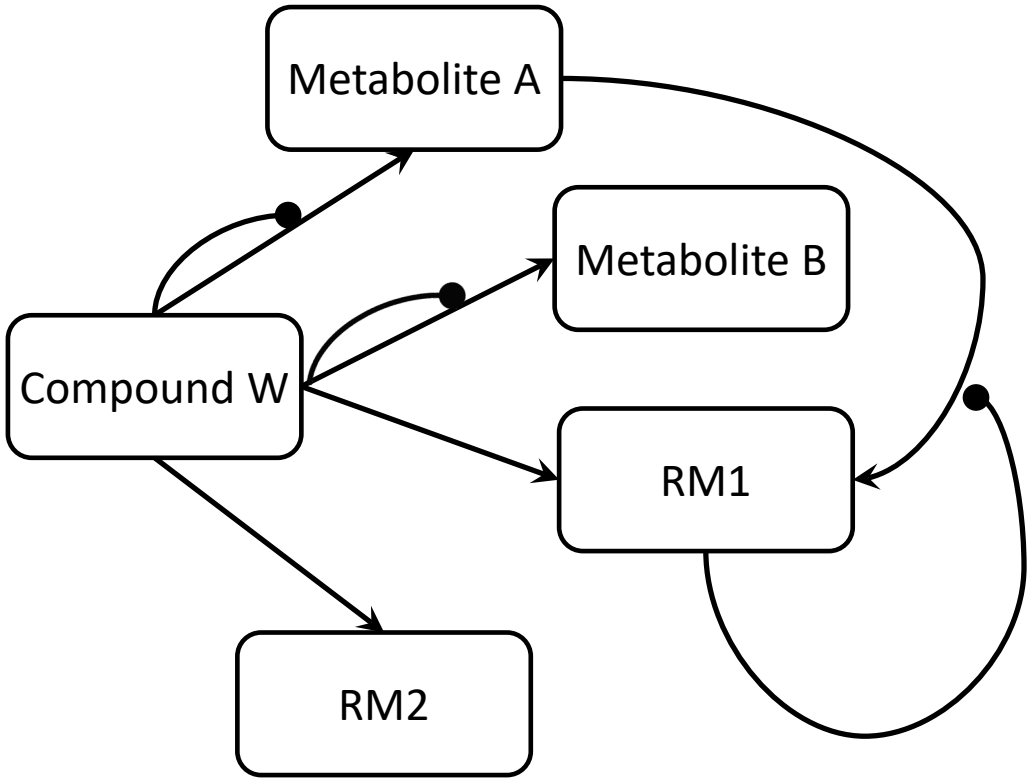
DILIsym Can Represent Up To Four Metabolites In Addition to Parent Compound



- Selecting the proper metabolism scaffold requires:
  - Knowing what data are available for each metabolite
  - Knowing what chemical species are likely to be involved in toxic mechanisms
- Example: bosentan
  - Two main metabolites, neither reactive
  - Minor metabolite involved in toxicity
  - Parent compound induces metabolism

Selecting Proper PBPK Parameters Is Necessary to Get Maximum Value from DILIsym Simulations

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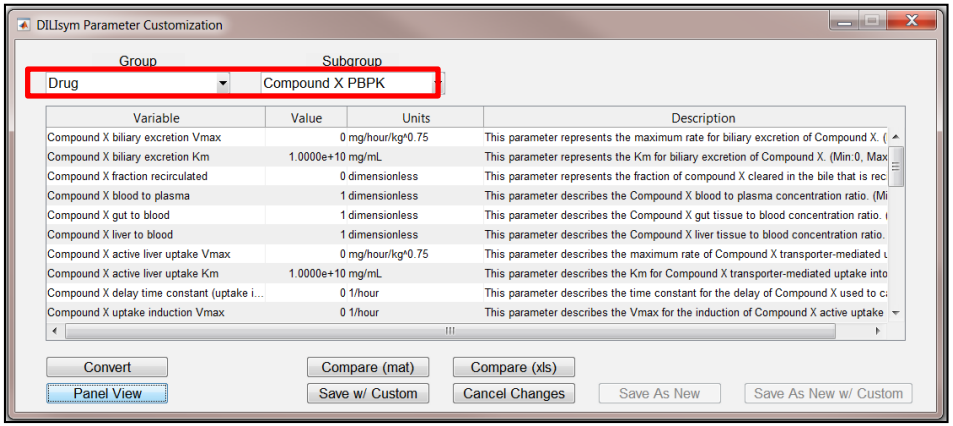
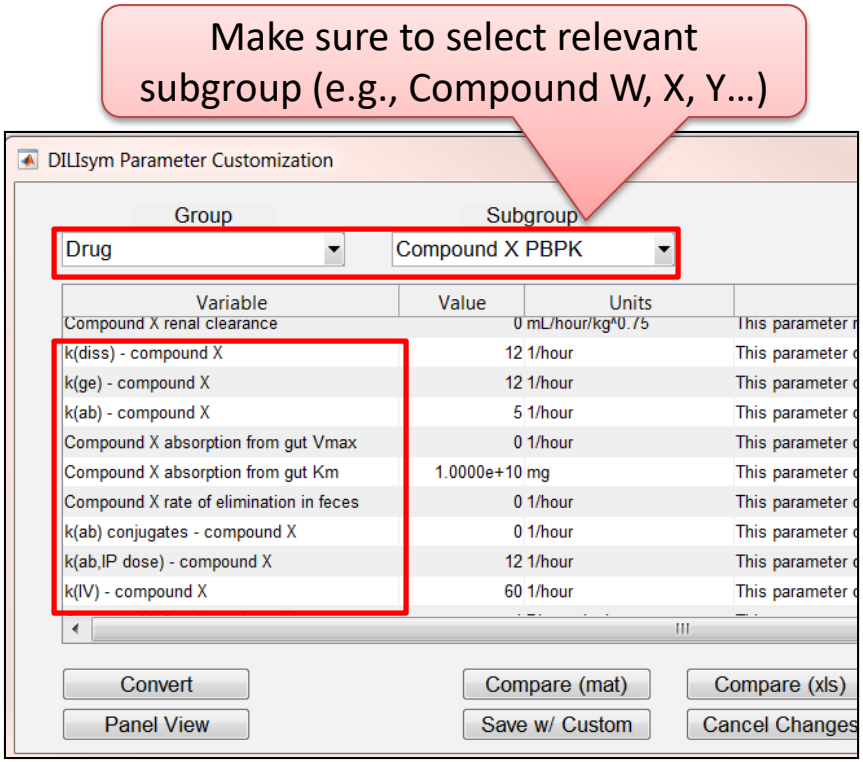


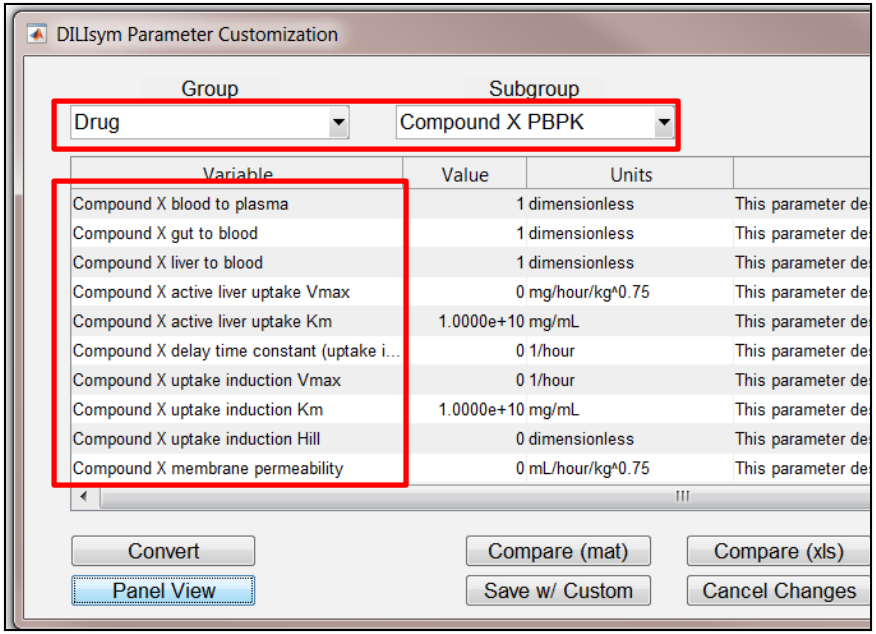
DILIsym PBPK Input Parameters Fall Into Several Main Categories

- Absorption
  - Saturable and linear models



DILIsym PBPK Input Parameters Fall Into Several Main Categories

- Absorption
  - Saturable and linear models
- Distribution
  - Linear and non-linear plasma protein binding
  - Blood to plasma partition coefficient
  - Tissue partition coefficients
  - Transporter-mediated uptake model for liver
  - Liver partition coefficient and volume of distribution for metabolites



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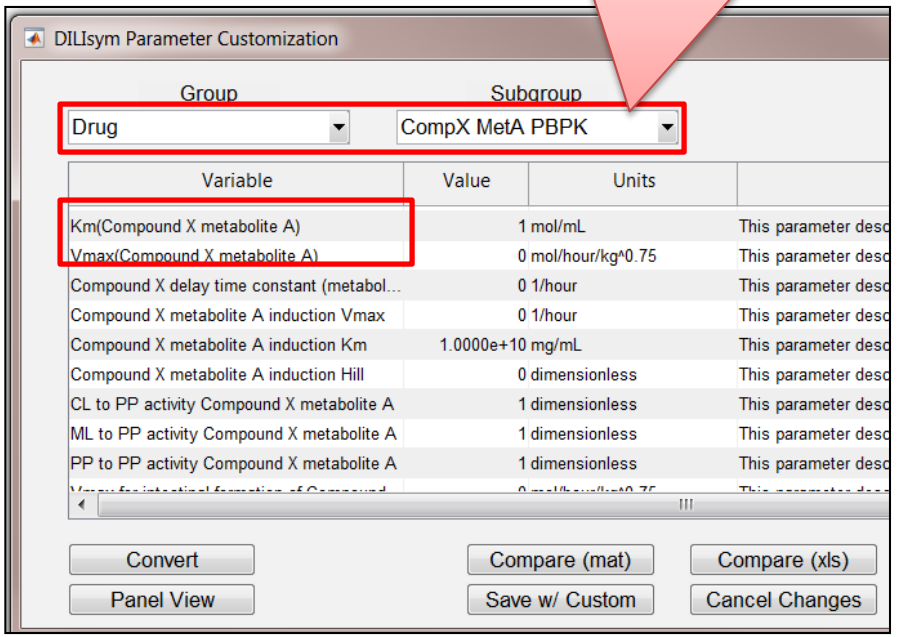
- Metabolism
  - Michaelis-Menten kinetics from parent to stable metabolites in liver and gut
  - RM reactions with GSH and protein in liver

Metabolism parameters are in the "metabolite" subgroup



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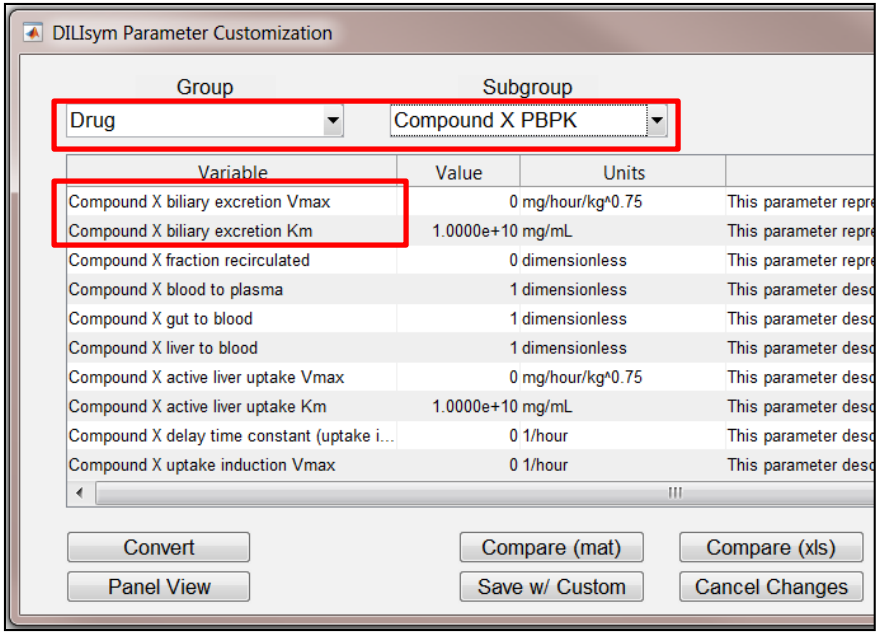
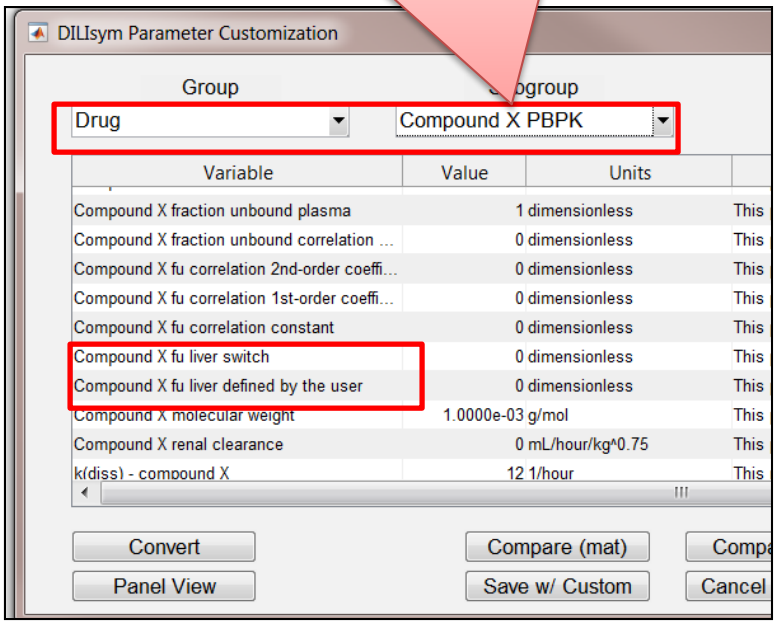
  - Transporter-mediated uptake model for liver
  - Liver partition coefficient and volume of distribution for metabolites
- Metabolism
  - Michaelis-Menten kinetics from parent to stable metabolites in liver and gut
  - RM reactions with GSH and protein in liver
- Excretion
  - Biliary excretion (K_m and V_{max}) and renal clearance of parent and main metabolites
  - Intestinal efflux
  - Clearance of protein adducts



Fraction Unbound in Tissue Is Calculated by DILIsym or Can Be Defined by the User

- As of DILIsym v5A, drug disposition in the PBPK sub-model is based on the unbound concentration
  - Hepatic/intestinal metabolism and transport
  - Renal and biliary excretion
- DILIsym calculates tissue fraction unbound for liver, gut, muscle, and other tissue in the static calculation (default)
  - Calculated by the user prior to v5A
- Alternatively, the user can define the unbound fraction in the liver using the “Compound (X) fu liver switch”
  - If the switch is set to 1, “Compound (X) fu liver defined by the user” will be used in the PBPK sub-model
  - This option is available from v5A

Make sure to select relevant chemical entity (e.g., Compound W/X/Y, metabolite A/B…)



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Fraction Unbound in Tissue Calculated by DILIsym v6A

- Unless the user turns on the "Compound (X) fu liver switch", DILIsym calculates tissue fraction unbound for liver, gut, muscle, and other tissue in the static calculation
- In case of passive diffusion, the unbound, non-ionized tissue concentration is equal to the unbound, non-ionized plasma concentration
  - $f_{u,tissue}$ is calculated from partition coefficients and the blood:plasma ratio

$$f_{u,tissue} = \frac{f_{u,plasma} \times f_{nonionized,plasma}}{\left(\frac{C_{tissue}}{C_{blood}}\right) \times B:P \times f_{nonionized,tissue}}$$

- In case of transporter-mediated liver uptake, the unbound liver concentration is not in equilibrium with the unbound plasma concentration
  - An empirical equation used to estimate $f_{u,liver}$ (Poulin and Theil 2000)

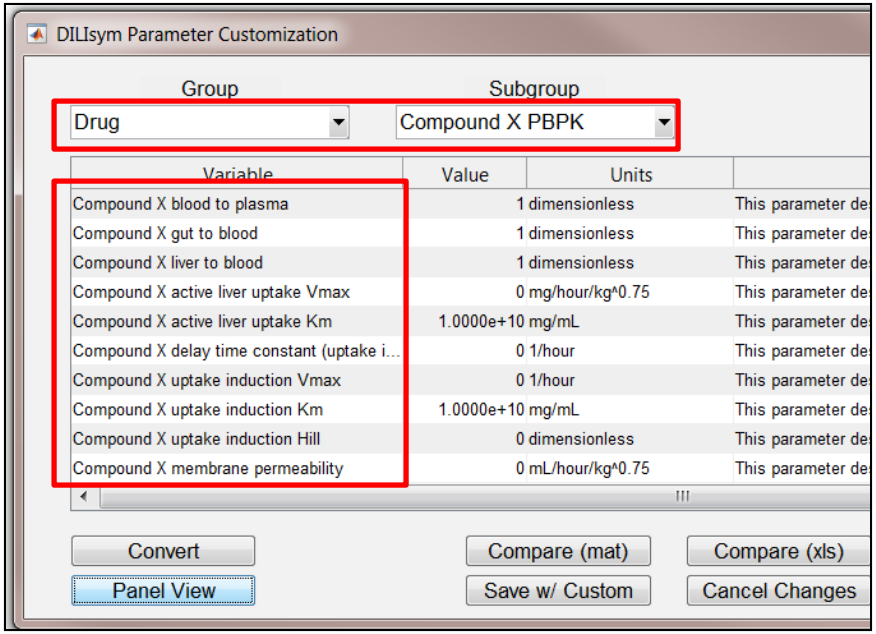
$$f_{u,cell} = \frac{1}{1 + \left(\frac{1 - f_{u,p}}{f_{u,p}} \cdot C_{m,tissue}\right)}$$

Cm,tissue: relative albumin conc in the liver compared to plasma (often assumed to be 0.5)

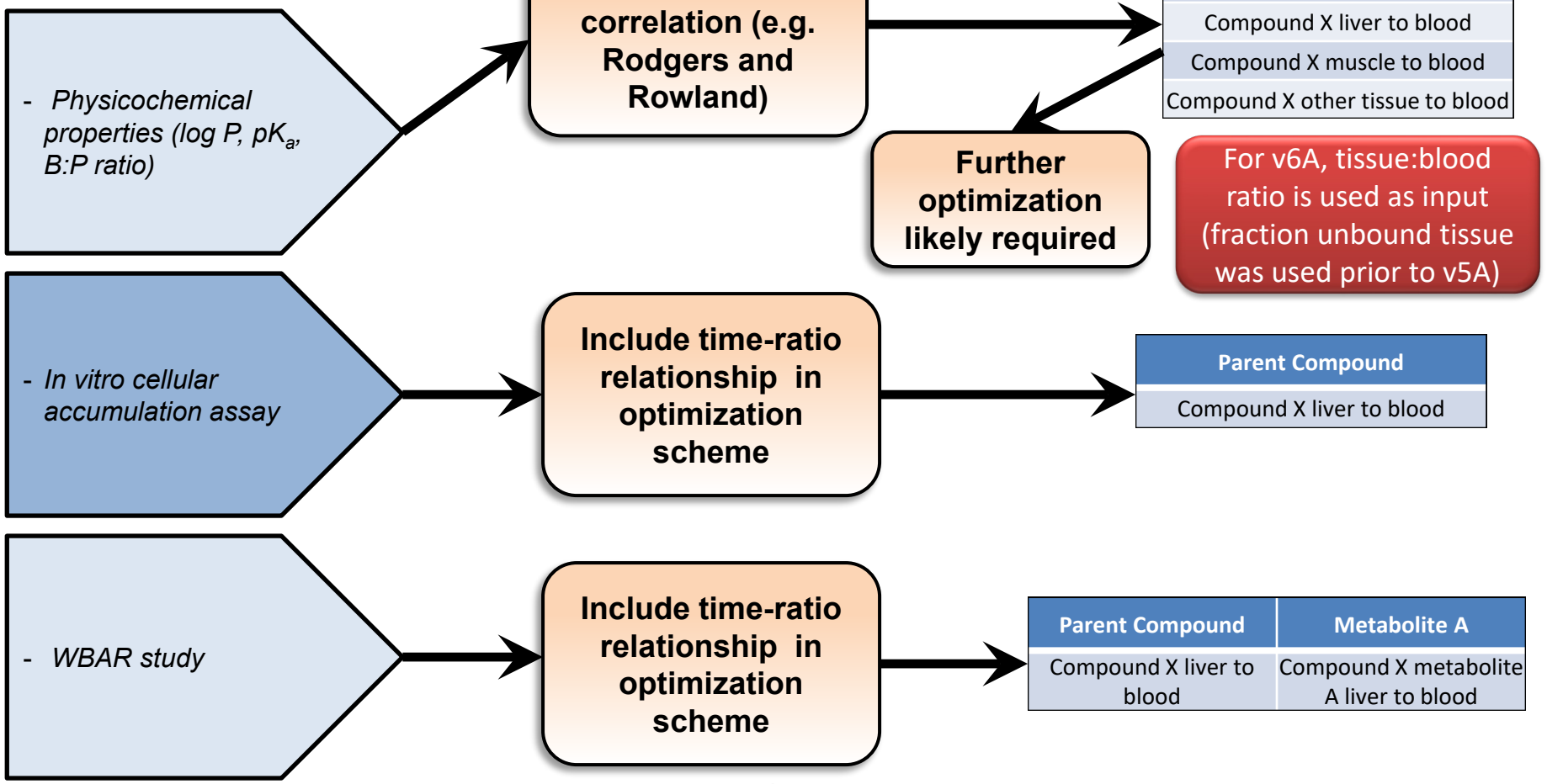
Method for Determining Distribution Parameters Depends on Data Available

- Distribution parameters can be determined from either *in vivo* or *in vitro* data
  - Physicochemical properties
  - *In vitro* cellular uptake assays
  - Animal WBAR studies

- Input panel document provides some insight into most useful assays for best DILIsym inputs



Predicting Partition Coefficients for Use in DILIsym



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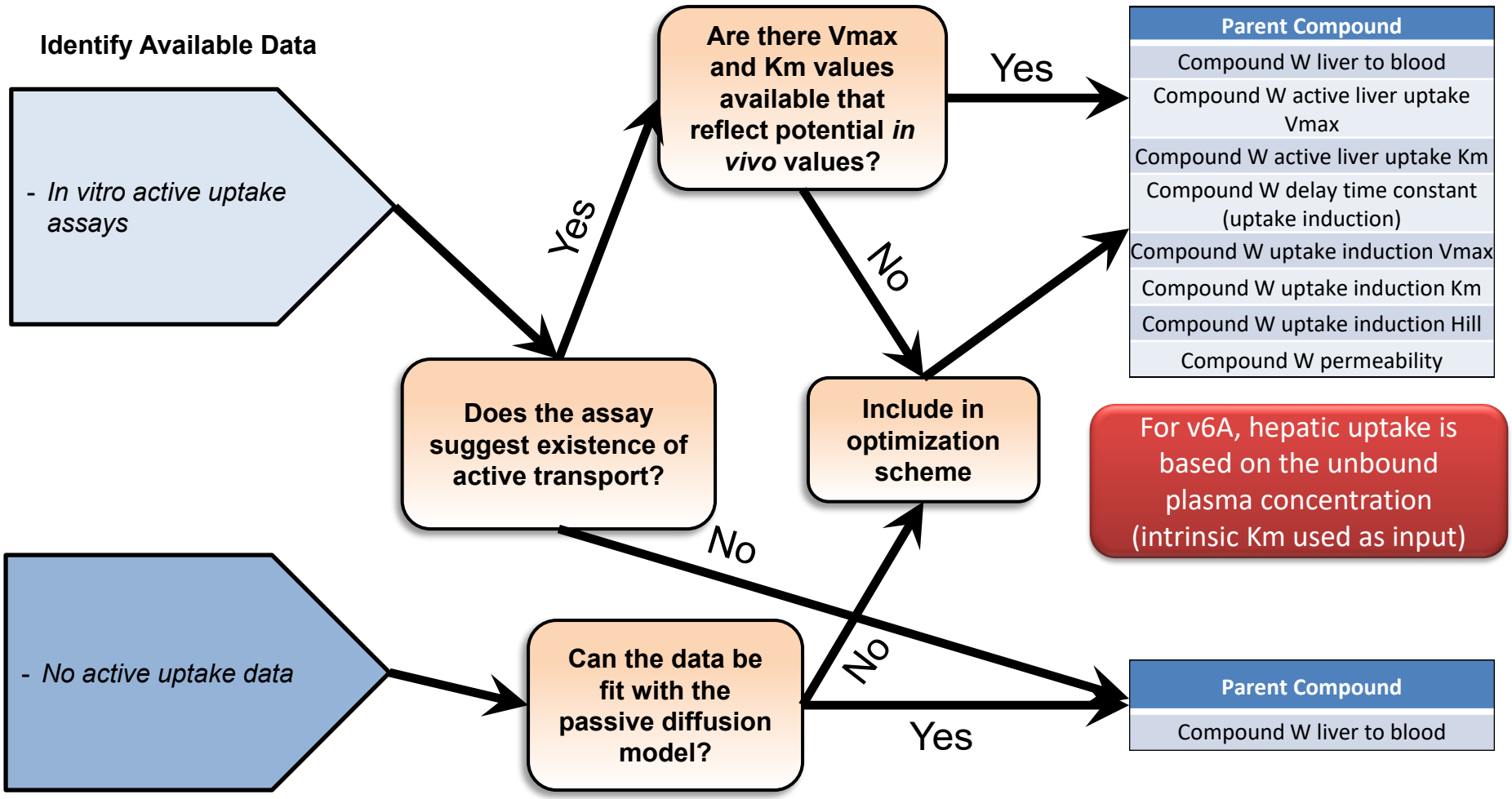
Selecting the DILIsym Parameters to Use for Active Liver Uptake



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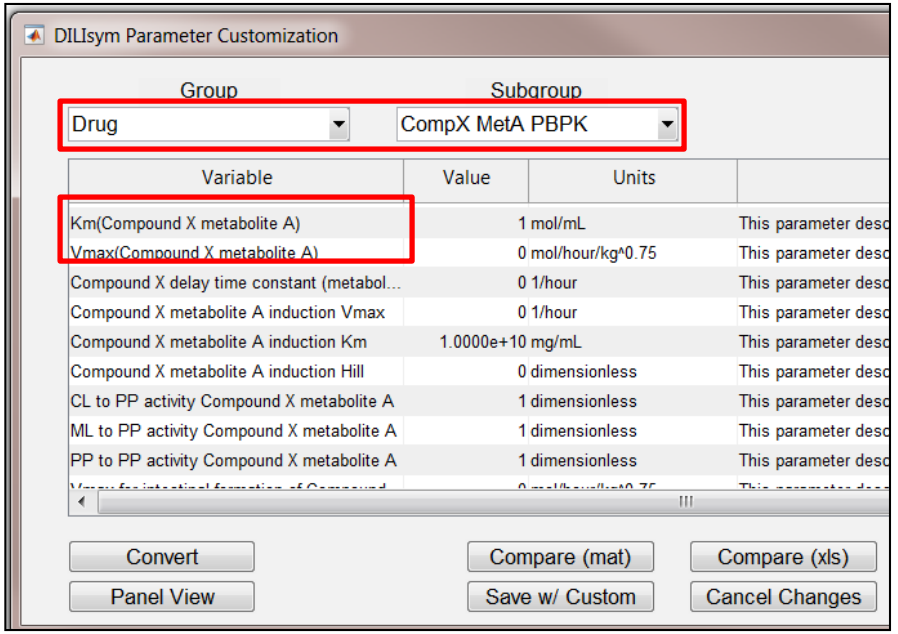
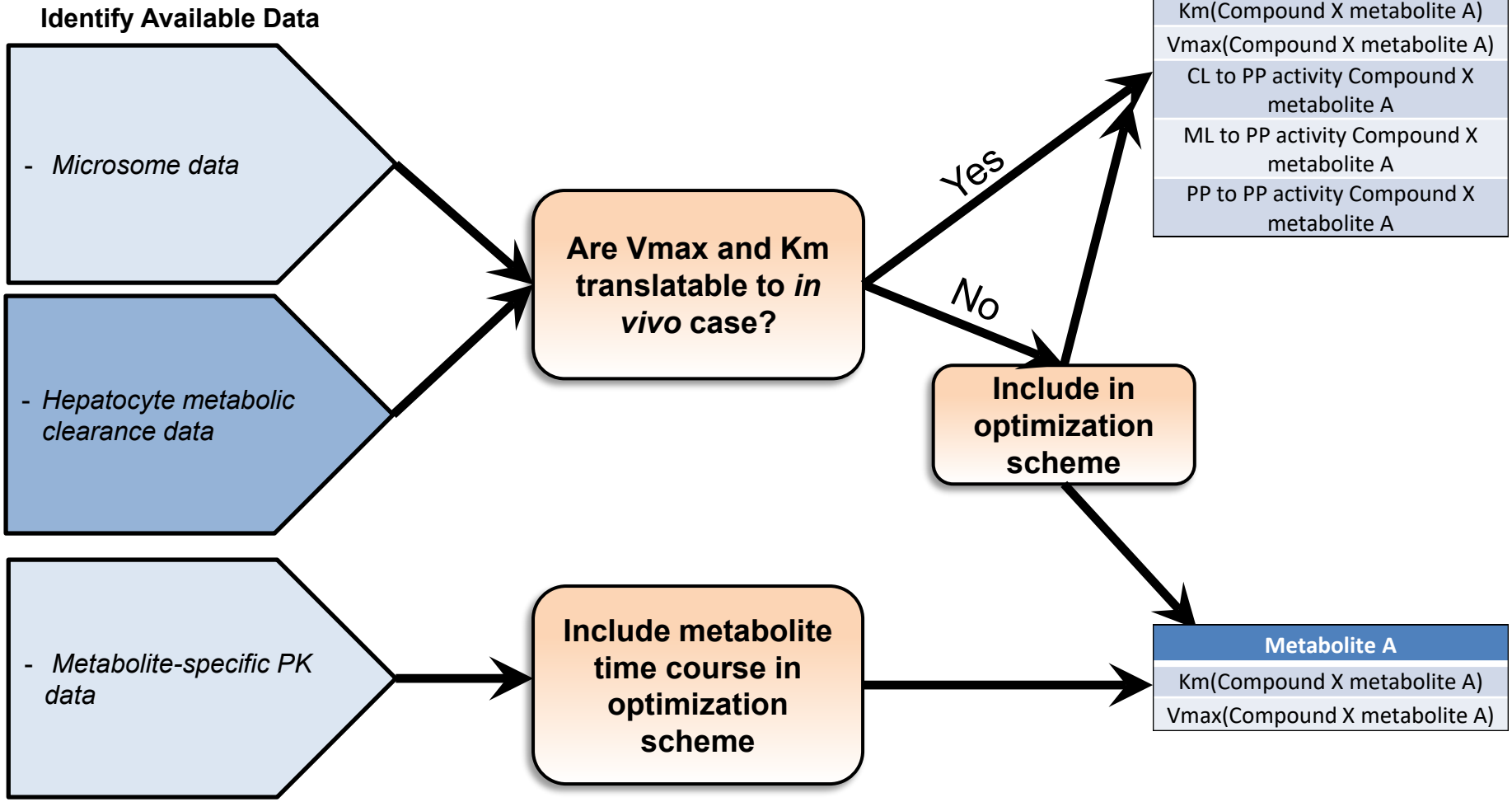
Method for Determining Metabolism Parameters Depends on Data Available

- Metabolism parameters can be determined from *in vitro* data or by fitting to PK data
  - *In vitro* microsome data
  - Hepatocyte metabolic clearance data
  - PK data **including complete metabolite time course in plasma**
- Input panel document provides some insight into most useful assays for best DILIsym inputs



Determining Metabolism Parameters for Use in DILIsym



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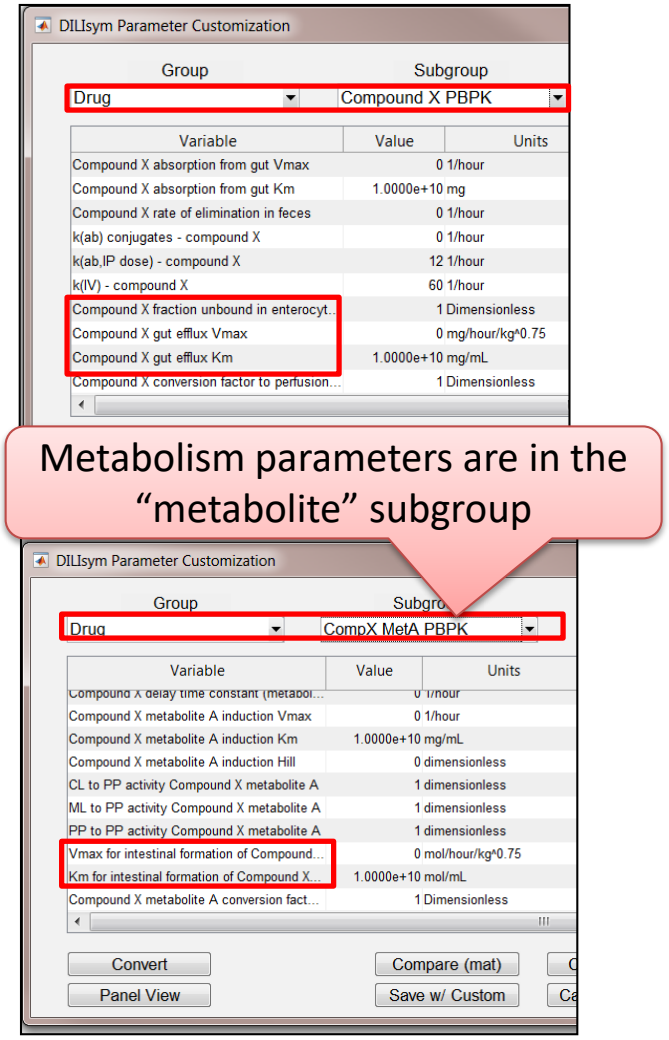
Intestinal Metabolism and Transport Represented in DILIsym v6A

- As of v5A, intestinal metabolism and transport available for Compound X and W
  - Based on the unbound gut concentration
  - The user can define “Compound X fraction unbound in enterocyte”
- Stable metabolites (metabolite A and B) can be generated by gut metabolism
  - Saturable process (K_m and V_{max})
  - Generated metabolites enter into liver tissue and are combined with liver-generated metabolites
- Efflux of parent compounds from gut tissue to intestinal lumen represented
  - Saturable process (K_m and V_{max})



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Determining DILIsym Parameter Values for Biliary Excretion

Data Available for Compound

- *Log P*
- *pKa*
- *Fraction unbound (blood)*
- *B:P ratio*

- *Absorption – IV dosing*
- *Organ partition coefficients and fractions unbound*
- *Renal clearance*
- *Biliary excretion*

- Prior to v5A, biliary excretion was represented as a linear biliary clearance
  - Based on the total liver concentration
- As of v5A, biliary excretion is represented as a saturable process
  - Michaelis Menten kinetics employed (K_m and V_{max})
  - Based on the unbound liver concentration
  - Kinetic parameters can be obtained by translating *in vitro* transport data or optimized to in vivo data (e.g., biliary recovery)

Parent Compound
Comp_W_bil_cl

→

Parent Compound
Comp_W_bil_Vmax
Comp_W_bil_Km

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