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DILIsym User Training - In Vitro Data Collection Considerations: Assessment of Bile Acid Transporter Inhibition and Intracellular Concentrations

DILI-sim Team

Goals for This Training Session

Participants should understand the following general concepts:

- Methods and tips related to gathering data in the area of bile acid transport inhibition for use within DILIsym
- Methods and tips related to determining intracellular concentrations in the mitochondrial toxicity and oxidative stress assays

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Gathering Data for DILIsym Parameter Inputs: Bile Acid Transport Inhibition

- DILIsym parameter inputs
  - Inhibition constant: K_i, IC_{50}
  - Type of inhibition: competitive, noncompetitive, uncompetitive, mixed
- *In vitro* assessment using multiple bile acid transporters is recommended

Transporter	Function	Experimental System
BSEP	Biliary excretion	Membrane vesicles
MRP3, MRP4	Basolateral efflux	Membrane vesicles
NTCP	Basolateral uptake	Primary hepatocytes, transfected cell lines

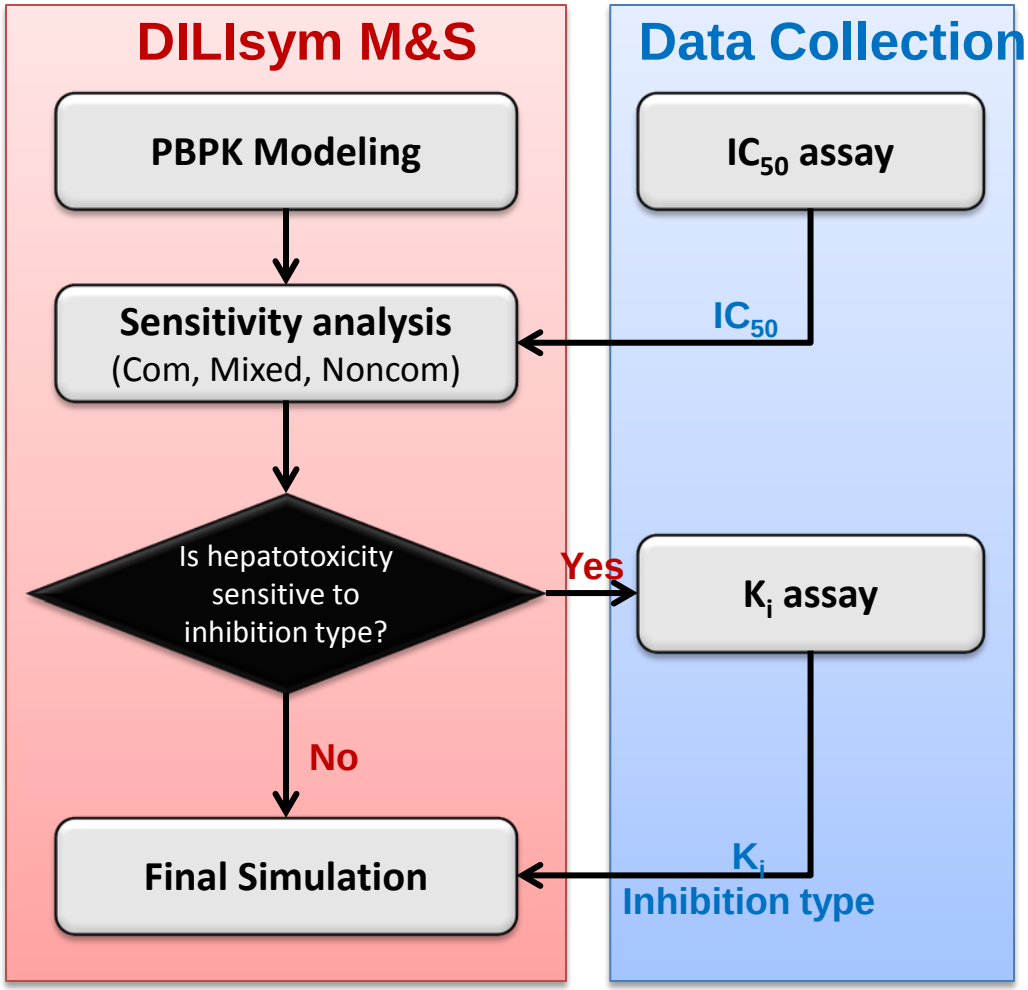
- Basolateral efflux of bile acids are represented as a single lumped pathway in the current version of DILIsym
  - Relative contribution of MRP3 and MRP4 (and potentially OSTα/β) unknown; could be updated in the future if data become available
  - Sensitivity analysis is recommended when inhibition constants for MRP3 and MRP4 are significantly different

The DILIsym Team Has Begun Recommending K_i Assessments When Simulations Suggest Sensitivity to Type

Inhibition constant	IC_{50}	K_i
Definition	Inhibitor concentration at the half maximal activity	Affinity of the inhibitor to the probe substrate binding site
Experimental methods	Transport assays with one substrate concentration & multiple inhibitor concentrations	Transport assays with multiple substrate concentrations & multiple inhibitor concentrations
Robustness	Varies depending on the substrate concentrations IC_{50} will approach K_i, if $[S] << K_m$	A more robust parameter
Provide information on the type of inhibition?	No	Yes
Cost	$	$$$
Comment	Commonly measured	Recommended for reliable prediction of hepatotoxicity

DILIsym Simulations Can Inform K_i Data Collection

- A step-wise approach is recommended when collecting bile acid transporter inhibition data
  - Perform *in vitro* assays to estimate IC_{50}
  - Run simulations with competitive/mixed/noncompetitive inhibition to assess if simulated hepatotoxicity is sensitive to inhibition type
    - If sensitive, perform K_i assays to determine inhibition type
    - If not sensitive, no need to perform K_i assays
- DILIsym simulations suggested that TAK-875 hepatotoxicity is sensitive to inhibition type for BSEP and NTCP
  - Additional assays performed to determine TAK-875 K_i and inhibition type for BSEP and NTCP
  - K_i assays not run for TAK-875 glucuronide because sensitivity analysis suggested that it is a minor contributor to hepatotoxicity



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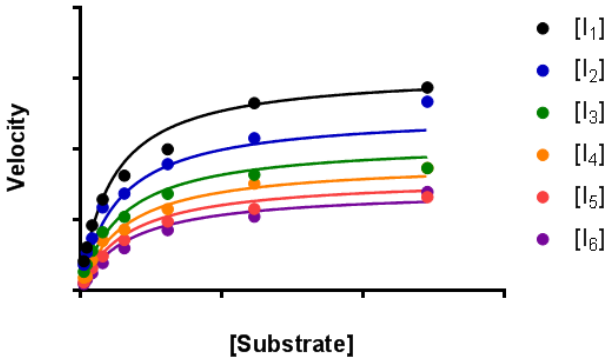
K_i Study Designs and Analysis

- Optimize the incubation time, probe substrate concentrations, and test compound (inhibitor) concentrations
  - Select an incubation time within linear range
  - Select 7 – 8 probe substrate concentrations spanning K_m
  - Select 4 – 5 inhibitor concentrations spanning IC_{50} and predicted/observed C_{max}
- Kinetic parameters (K_m, V_{max}, and K_i) and type of inhibition determined by fitting competitive, noncompetitive, uncompetitive, and mixed models to the untransformed data by nonlinear regression analysis
  - The best-fit model determined from visual inspection of the observed versus predicted data and Akaike Information Criterion (AIC)
- For more information about K_i study designs, analysis, and examples, please refer to *DILIsym Review Session 19* on the website



Competitive: $V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S}$

Mixed: $V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S \times \left(1 + \frac{I}{\alpha \times K_i}\right)}$

Noncompetitive: $V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S \times \left(1 + \frac{I}{K_i}\right)}$

Uncompetitive: $V = \frac{V_{max} \times S}{K_m + S \times \left(1 + \frac{I}{K_i}\right)}$

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Recommendations When K_i Data Cannot Be Collected

- DILIsym team recommends using mixed inhibition with α = 5 as default, if K_i data collection is not possible
  - Competitive and non-competitive inhibition types result in low and high extremes of potential bile acid accumulation, respectively
  - Mixed inhibition with α = 5 leads to a median impact on bile acid accumulation
  - K_i analysis data obtained so far suggests that mixed inhibition is more common compared to pure competitive/non-competitive inhibition
  - Simulate competitive/noncompetitive inhibition in a SimCohorts and SimPops to determine predicted ranges of response

Compound Z

Inhibition Type for BSEP/basolateral efflux	ALT > 3X ULN	Hy's Law
Noncompetitive (α=1)	15/16	15/16
Mixed (α=2)	14/16	14/16
Mixed (α=5)	9/16	9/16
Competitive (α=∞)	0/16	0/16

Human SimCohorts v4A-1-Multi16 employed in simulations

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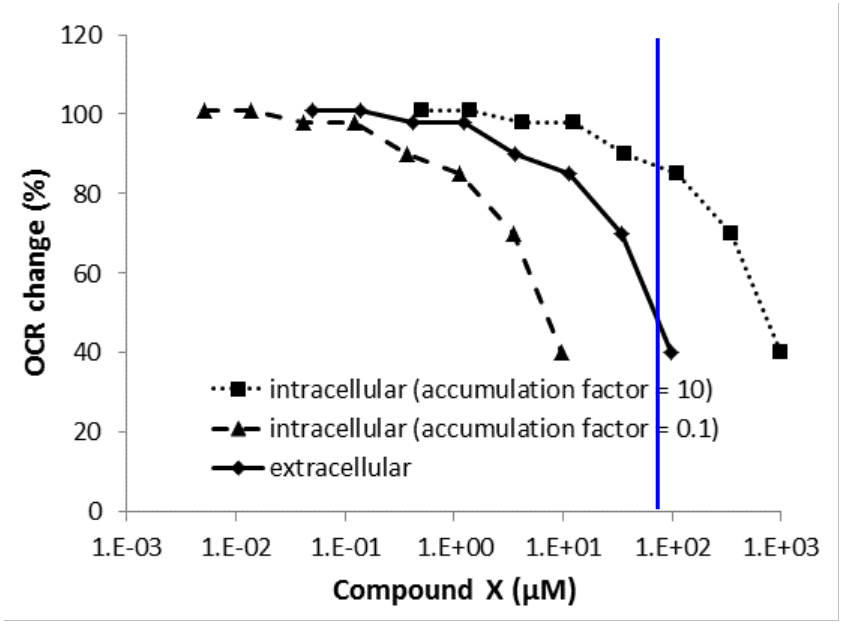
Improved DILIsym Parameter Values When Intracellular Compound Is Measured

- Most Seahorse oxygen consumption rate (OCR) or ROS/RNS data are expressed in an exposure-response relationship
  - OCR change on y-axis
  - Measured extracellular compound concentration on x-axis
- Numerous compounds have been shown to accumulate in liver
  - Potency relative to intracellular concentrations different than relative to extracellular
  - Intracellular ≠ extracellular
- Basing parameter values on extracellular concentrations introduces inaccuracy for compounds that accumulate in hepatocytes
- Recommend measuring intracellular compound concentration for cell based assays used to provide DILIsym parameter values
  - OCR, ROS production
  - For compounds that are known to have liver:blood ratio ≠ 1 (or not known)

Previously presented at Q3 2016 training session

HepG2



	extracellular	intracellular
10X accumulation	74.4 uM	744 uM
1x accumulation	74.4 uM	74.4 uM
0.1x accumulation	74.4 uM	7.44 uM

Theoretical Preclinical Data

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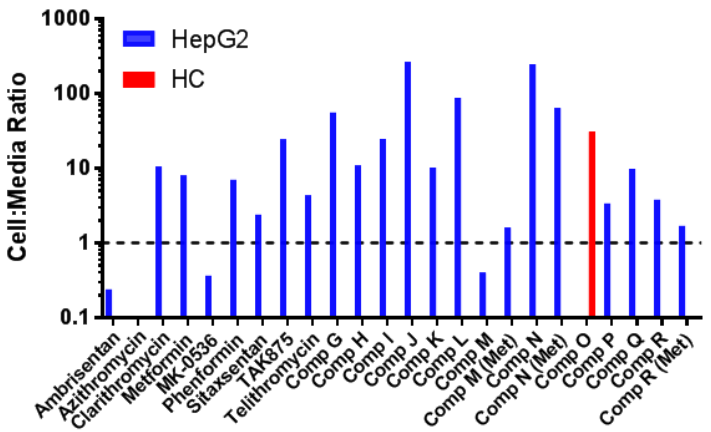
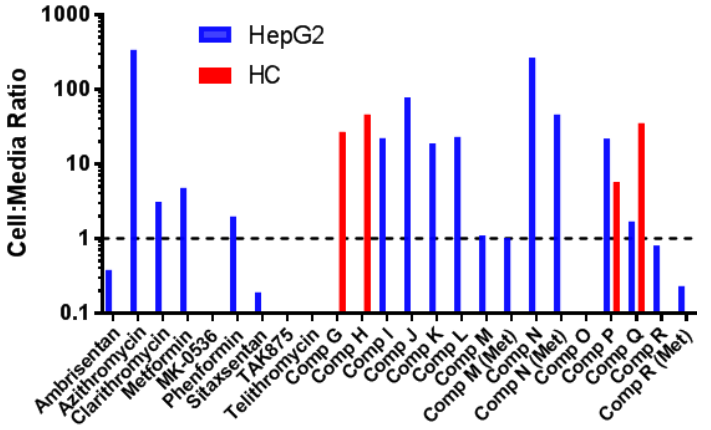
Experience from Exemplar/Proprietary Compounds Suggests the Need for Measuring Intracellular Concentration

- Intracellular concentrations have been measured for > 15 DILIsym compounds
  - Seahorse and ROS assays
  - HepG2 and primary hepatocytes
- Cell:media ratio is not equal to 1 in most cases
  - Use of nominal media concentrations to parameterize exposure-response relationship would be misleading

Seahorse

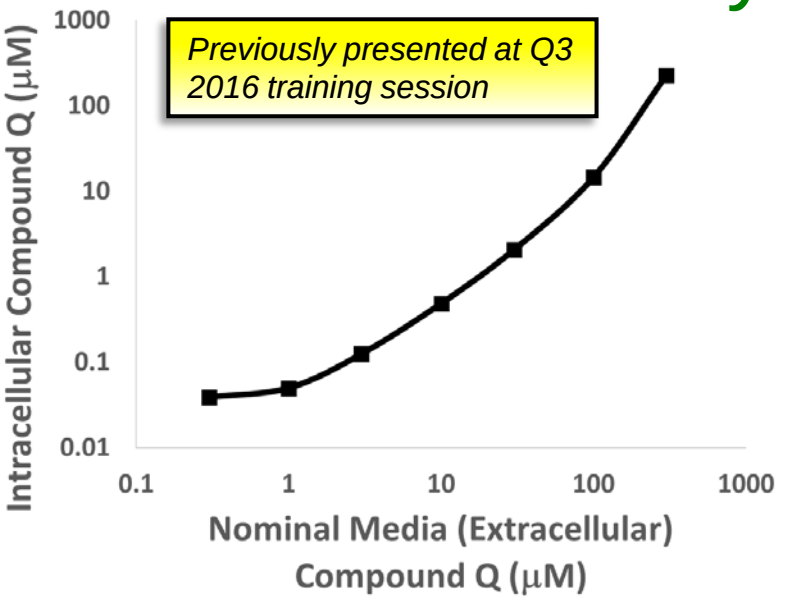


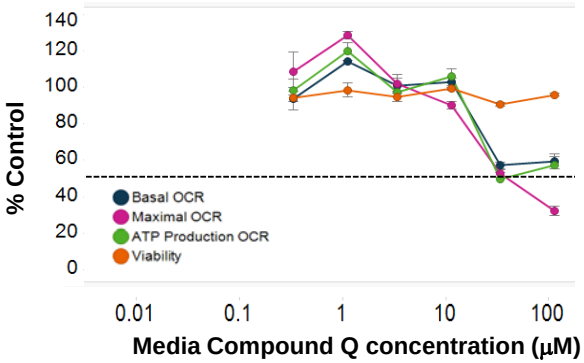
ROS

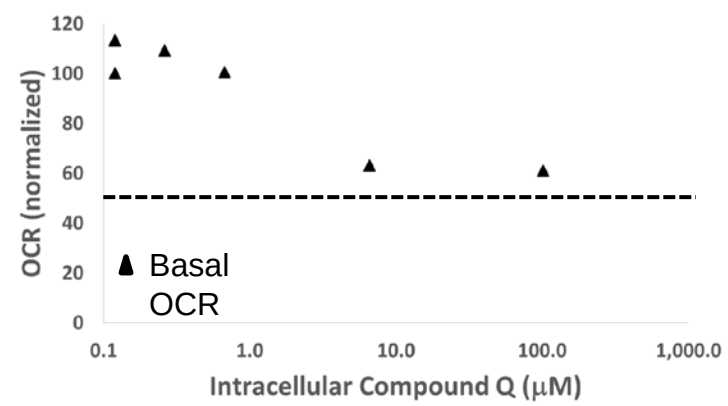


Example Using Blinded Assay Data Shows Value of Measuring Intracellular Concentration Directly

- Intracellular concentration was measured in parallel to Seahorse study in the same conditions as in the preparations for the assay
  - Same nominal media (extracellular) concentrations, incubation time, and assay conditions
- Allows for **direct translation** from nominal concentration to intracellular concentration when fitting toxicity parameters
  - Can calculate effect of Compound Q on mitochondrial ETC with fewer assumptions







Preclinical Data

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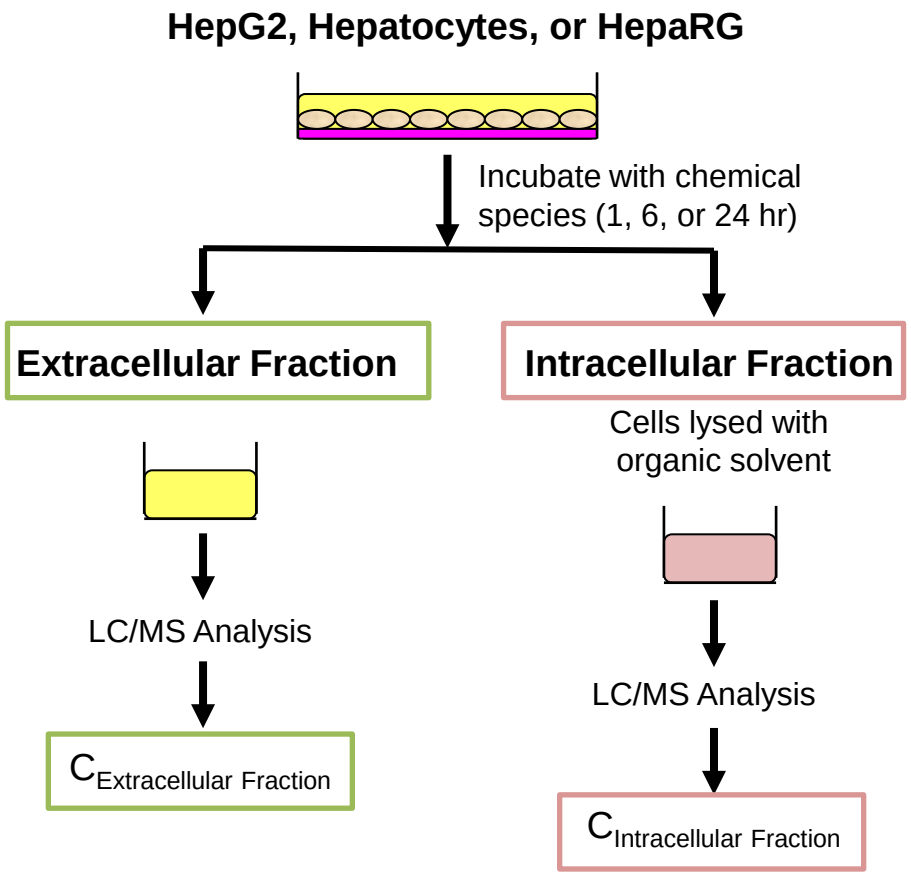
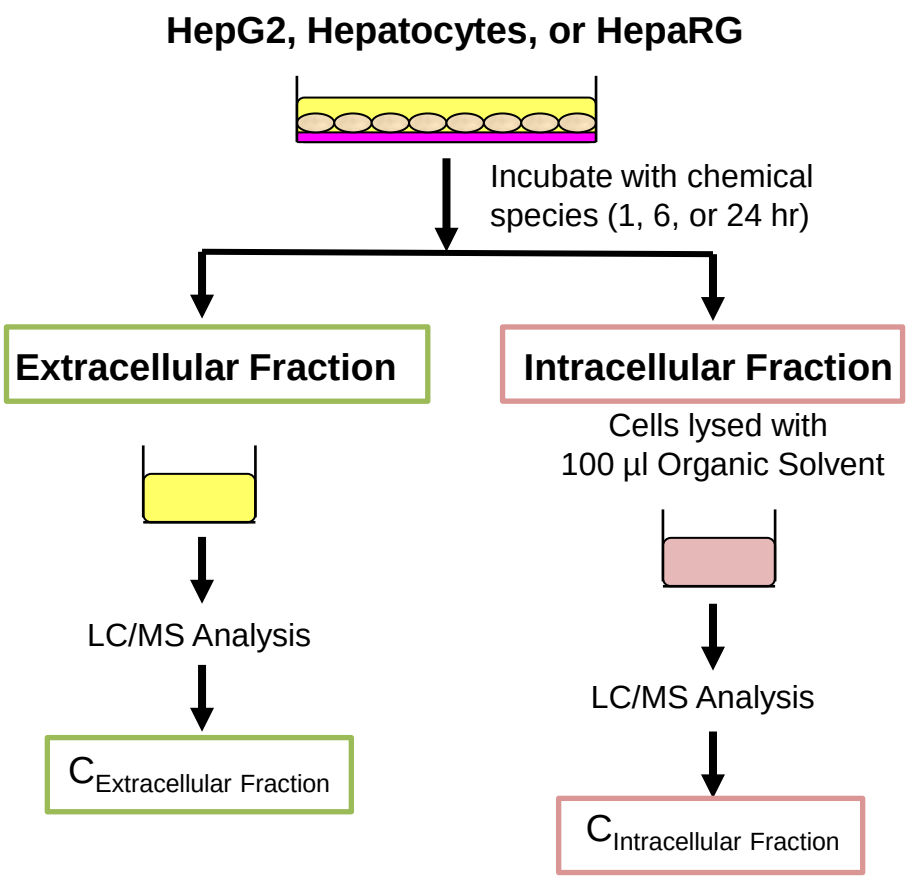
DILIsym Team Recommends LC/MS/MS Method for Determining Intracellular Concentrations



- LC/MS/MS method measuring cellular and media concentrations can be used to estimate intracellular concentrations present during toxicity assay
  - Using same cell type and media (e.g. protein) as used for toxicity assay
- Measure concentrations in extracellular fraction and cell lysate separately
  - DILIsym team no longer recommends non-specific binding correction for determining intracellular concentrations (*Review session 16*)
  - Intracellular concentrations can be calculated by adjusting for cell lysate volume and cellular volume
  - Ratio of extracellular:intracellular can be used to estimate hepatic accumulation
  - Actual intracellular concentration can be used to correct extracellular media concentrations in toxicity assay

LC/MS/MS Data Used to Determine Intracellular Concentrations of Compounds in Cell-Based Assays



$$C_{Media} = C_{Extracellular\ Fraction}$$

$$C_{Hepatocyte} = \frac{C_{Intracellular\ Fraction} \times V_{Intracellular\ Fraction}{}^{\dagger}}{V_{Hepatocytes}{}^{\ddagger}}$$

† 100 µl (cell lysate volume)
‡ Calculated by multiplying cell count per well and cellular volume per cell (2.85 pl/cell for HepG2)

In Silico Methods Can Be Used to Estimate Intracellular Concentrations When Experimental Data Cannot Be Collected

- Literature-based correlation (Rodgers & Rowland 2005)
  - Use physiochemical properties to estimate partition coefficients using a mechanistic model
  - Assumes passive transport, no hepatic elimination (e.g., metabolism, biliary excretion), and non-saturable conditions
- PBPK Model-based estimation
  - Use a PBPK model to estimate liver concentration at steady-state
    - IV infusion protocol can be used to mimic assay environment
    - More information can be found in the 2016 Q3 training
  - Simulated K_p and $K_{p,u}$ reflect active hepatic transport and/or hepatic elimination processes such as biliary excretion and metabolism
- Predictivity of *in silico* methods still remains to be determined, although they are deemed better than use of nominal media concentrations
  - Best method is to measure intracellular concentration by LC/MS/MS due to current limited data and variability of data
  - Mechanistic models representing *in vitro* cell systems (e.g., MembranePlus™) may be used to improve prediction of intracellular concentrations

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