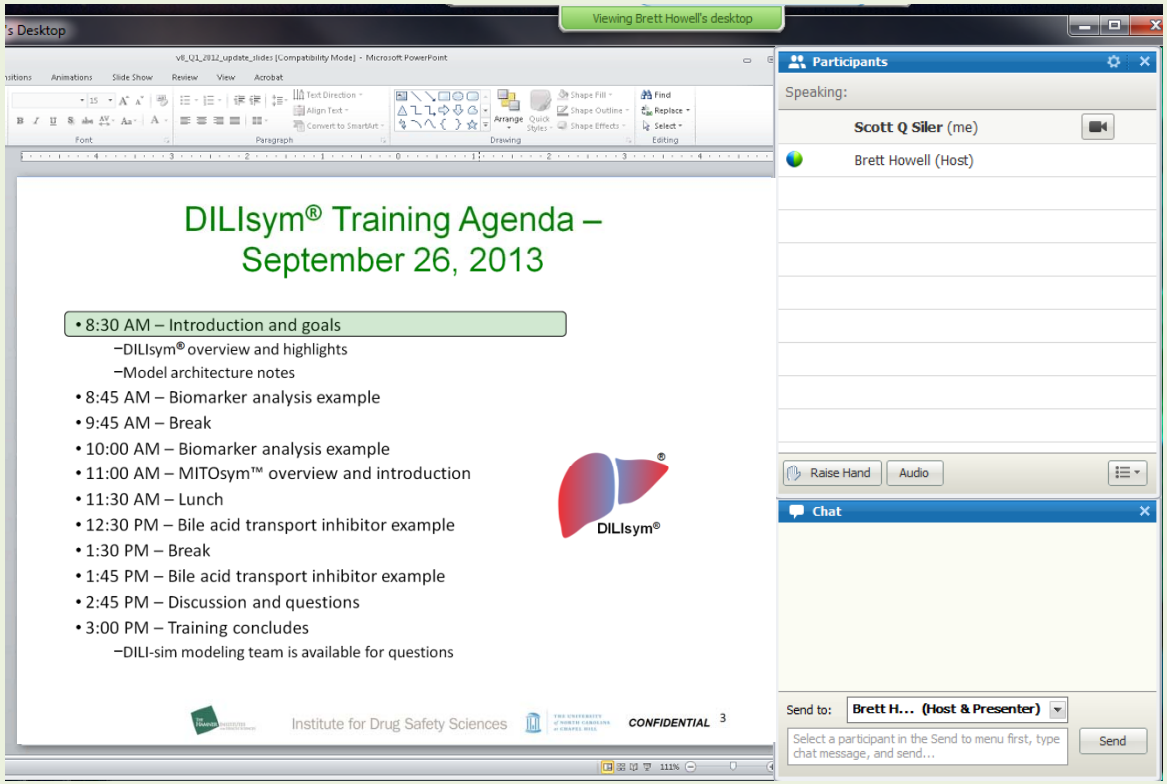
WebEx Tools will be Used to Record this Training Session

- Recording meeting for future reference and members not able to attend



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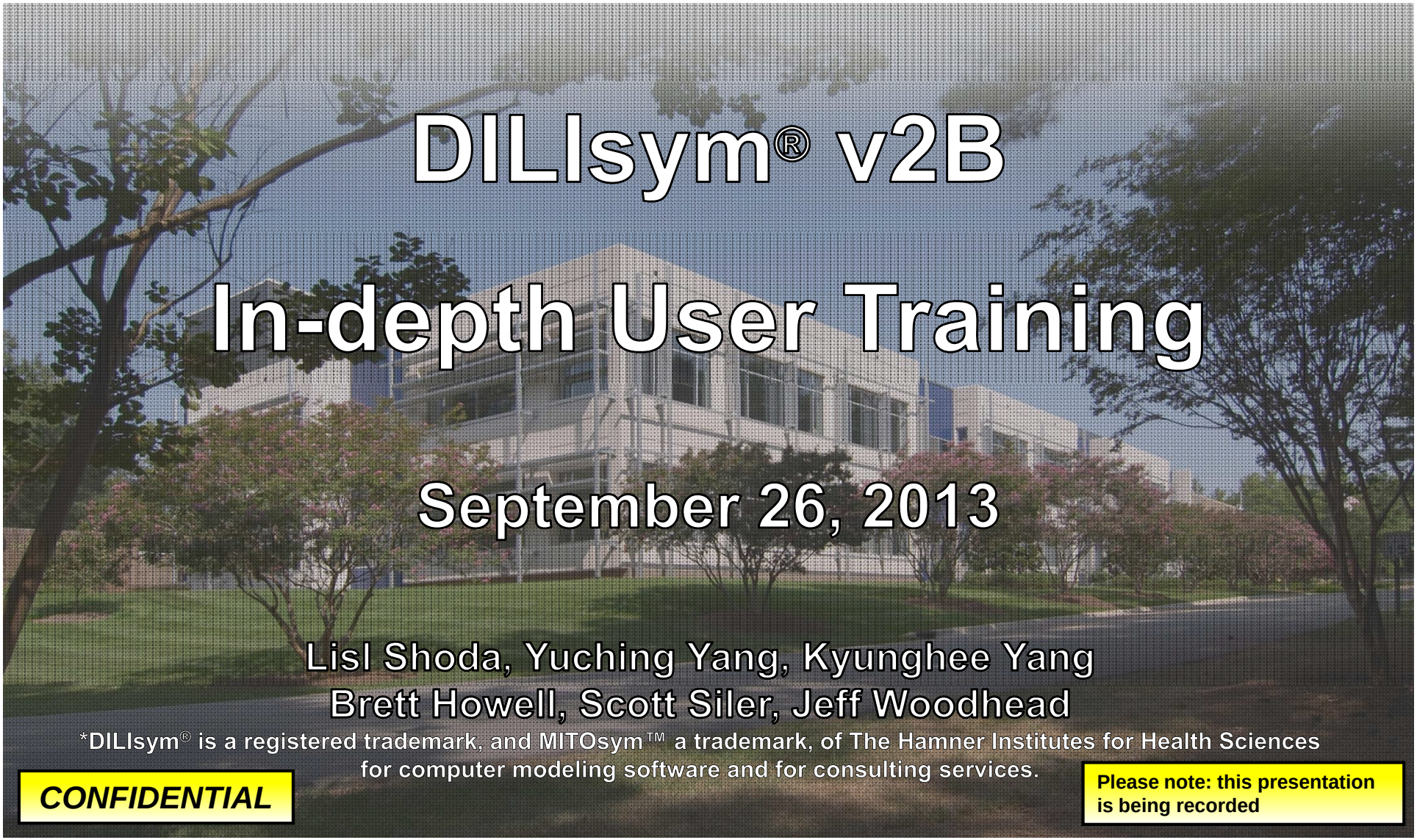

DILIsym® v2B

In-depth User Training

September 26, 2013

Lisl Shoda, Yuching Yang, Kyunghee Yang
Brett Howell, Scott Siler, Jeff Woodhead

DILIsym® Training Agenda – September 26, 2013

- 8:30 AM – Introduction and goals
  - DILIsym® overview and highlights
  - Model architecture notes
- 8:45 AM – Biomarker analysis example
- 9:45 AM – Break
- 10:00 AM – Biomarker analysis example
- 11:00 AM – MITOsym™ overview and introduction
- 11:30 AM – Lunch
- 12:30 PM – Bile acid transport inhibitor example
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- 2:45 PM – Discussion and questions
- 3:00 PM – Training concludes
  - DILI-sim modeling team is available for questions

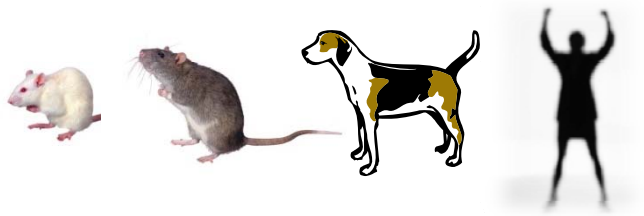
Goals for the DILIsym® v2B In-depth User Training Session

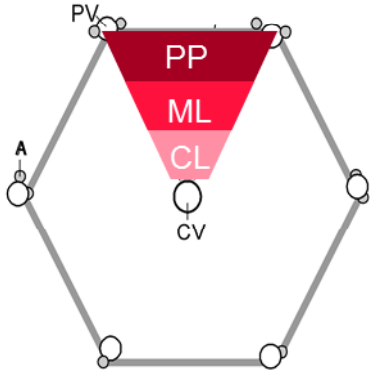
Participants should understand the following general concepts:

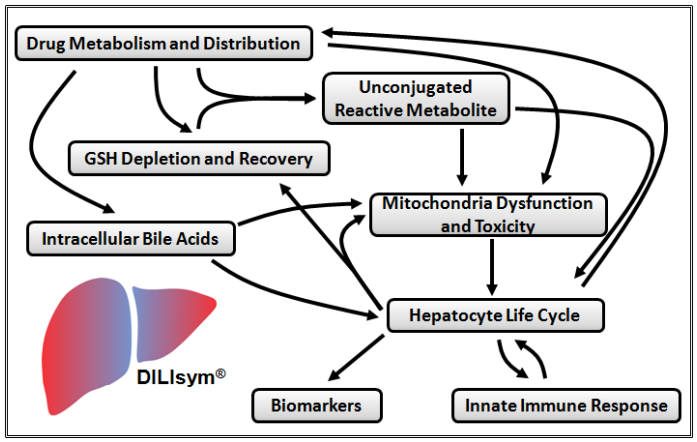
- The conceptual model architecture of DILIsym® v2B
- The concept of “translatability” as it applies to DILIsym®
- Use of DILIsym® for the retrospective interpretation of liver injury associated with clinical ALT signals
- Parameter selection for the non-mechanistic representation of hepatocyte necrosis
- Intended applications for MITOsym™ v1A, a model of mitochondrial function
- Using in vitro transporter inhibition data to parameterize DILIsym® and make predictions about the potential hepatotoxic effects of inhibitors on humans and animals

DILIsym® v2B Overview

- **Multiple species: human, rat, mouse, and dog**
  - Population variability
- **The three primary acinar zones of liver represented**
- **Essential cellular processes represented to multiple scales in interacting sub-models**
  - Pharmacokinetics
  - Dosing (IP, IV, Oral)
  - Transporter Inhibition
  - Drug metabolism
  - GSH depletion
  - Injury progression
  - Mitochondrial dysfunction, toxicity
  - Bile acid mediated toxicity
  - Cellular energy balance
  - Hepatocyte life cycle
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers

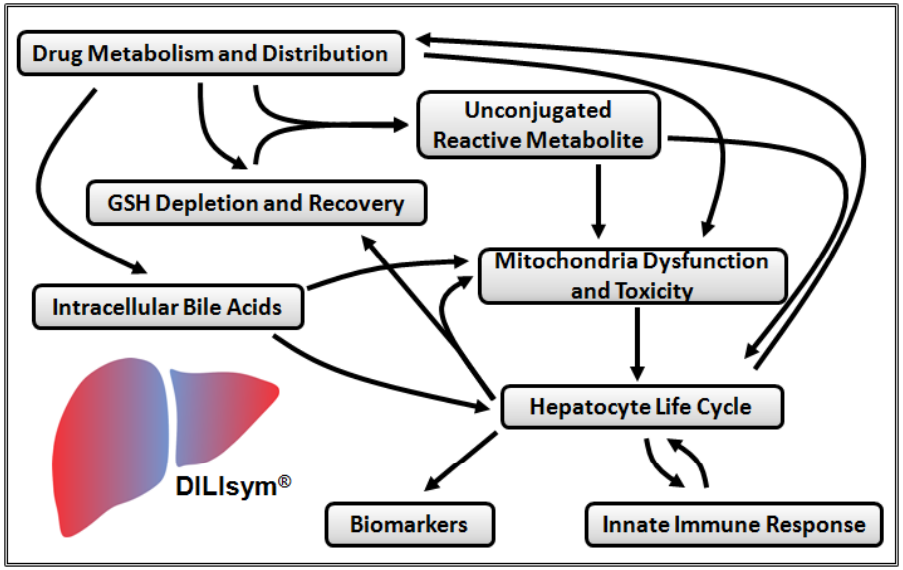






- **Hepatotoxicity exemplars**
  - Reactive metabolite mediated
    - Acetaminophen
    - Methapyrilene
    - Furosemide
    - Aflatoxin B1
  - Mitochondrial dysfunction
    - Etomoxir
    - Buprenorphine
  - Bile acid transporter inhibition
    - Glibenclamide
    - CP-724714
  - Single, multiple dose protocols
  - Single, combination drug protocols
- **Compartment-based modeling**
  - >480 state variables
  - 'Form to function' connection
  - Ordinary differential equations
  - Alternative mathematical approaches are possible
  - Simulations can be run using code or GUI developed in house

Highlights of DILIsym® v2A



- Added direct mitochondria toxicity-mediated hepatocellular necrosis
- Added bile acid-mediated toxicity hepatocellular necrosis
- Expanded representation of innate immune contributions to injury and recovery
- Expanded number of represented biomarkers of hepatocellular injury
  - Circulating (e.g., mir-122)
  - Hepatocellular (e.g., triglyceride)
- Introduced additional exemplar compounds for exposure-related toxicity
  - Etomoxir
  - Buprenorphine
  - CP-724714
- Additional SimPops™, capturing impact of variability in key pathways
- Expanded capabilities of GUI interface

Expanded Capabilities and Features of DILIsym® v2A

- *New* capability to dose up to 3 compounds at once
  - W, X, and Y; v1A included APAP, X, and NAC
  - NAC representation still available
- *New* Compound Y option includes a simple, two compartment PK model representation
- Drug and Species parameters are now split into two separate value sets
  - Easier cross-species predictions
  - Improved clarity on what parameters apply to the biology versus the intersection of the drug and the biology
- *New* Output Table feature allows for easy calculation of Max, Min, AUC, Mean, and other metrics
- *New* Parameter Sweep option allows GUI users to sweep across a range of values for a given model parameter
  - Includes all model parameters; dose sweeps and sensitivity analyses possible
- *New* 2-Parameter Sweep option (MATLAB code version only)
- *New* Load/Save options for GUI results
- *New* Override protection for standard drug and species parameter sets (GUI version only)
- Data Comparisons include many more data sets and new plot options
- Caloric intake is now included for mitochondria toxicity and bile acid homeostasis; the role of caloric intake will continue to expand
- *New* 'events' feature avoids skipping discrete events, regardless of maximum step size
  - Compound W, X, and Y doses, caloric intake (meals), and mechanistic interventions included
- Added dog optimizations and capabilities
- Streamlined code base
  - No separate algebraics file
  - ODE file and many Excel and GUI files are now automatically called
- Expanded Zotero reference database (contact us for real-time access)

DILIsym® Updates for version 2B

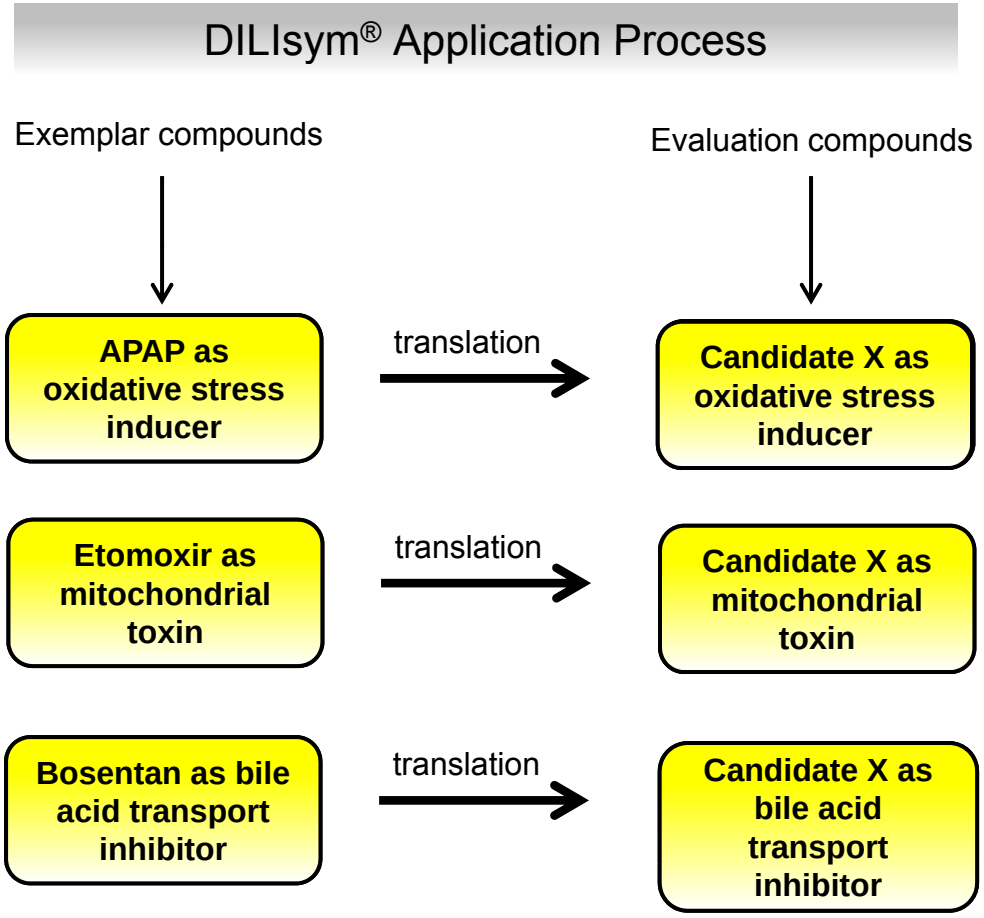
- Newly added functional model of bile acid homeostasis for the rat
- Additional SimPops™ population samples
  - Relevant to mitochondrial dysfunction and bile acid homeostasis
- Faster, more efficient simulations
- Various bug fixes and GUI improvements
  - Semi-log plotting capability
  - Log sweep capability for parameter sweeps
  - Many others

DILIsym® v2C Includes Changes for Multiple, Simultaneous Mechanisms of DILI

- Newest version of model released in September 2013
- Primary update:
  - Testing of v2B with multiple mitochondrial dysfunction mechanisms alerted DILI-sim team to changes that needed to be made for multi-hit simulations
- DILI-sim team recommends that members download and use v2C for future work, to the extent possible
- Changes do not affect simulations where any single mechanism for DILI were selected, or where one mitochondrial dysfunction mechanism or less was selected

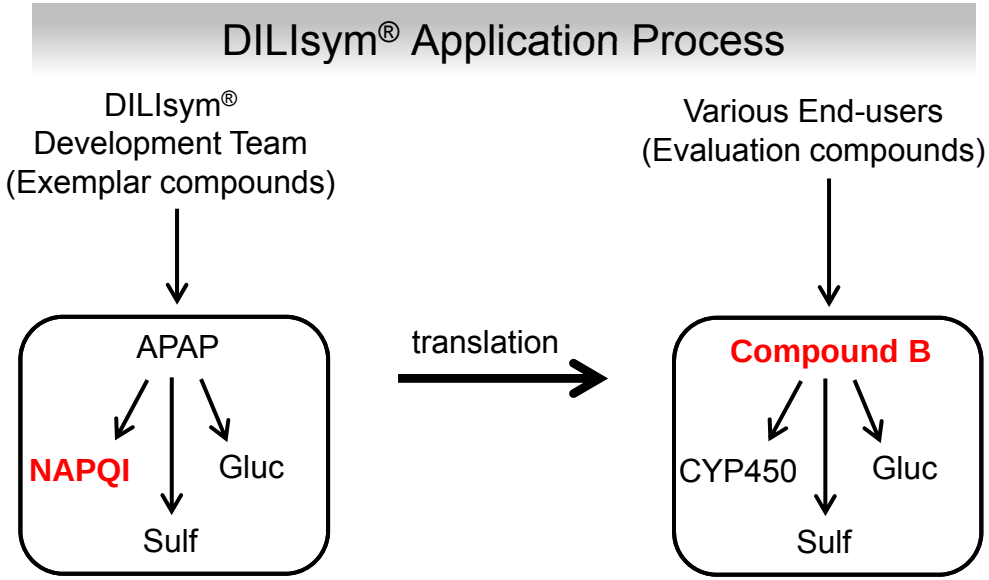
DILIsym® Architecture – Translation from Exemplar Compounds to Compounds of Interest

- The value proposition of DILIsym® lies in its ability to translate to compounds NOT used to build it
- This requires end-users with evaluation compounds to either have an idea of what mechanisms of hepatotoxicity might be in play or conduct hypothesis-based modeling
- Multiple, concurrent mechanisms of hepatotoxicity can be used and are being explored



DILIsym® Architecture – Using the Mechanism Selection Tool

- The mechanism selection tool allows the end-user to select an existing mechanism in the DILIsym® model
- Importantly, the tool also allows the mechanism to be applied anywhere in the metabolism tree
- The user can also apply multiple mechanisms to the same chemical species and different mechanisms to different levels of the tree
  - Parent and metabolite with same mechanism
  - Parent and metabolite with different mechanisms



DILIsym® Architecture – Using the Mechanism Selection Tool in the GUI

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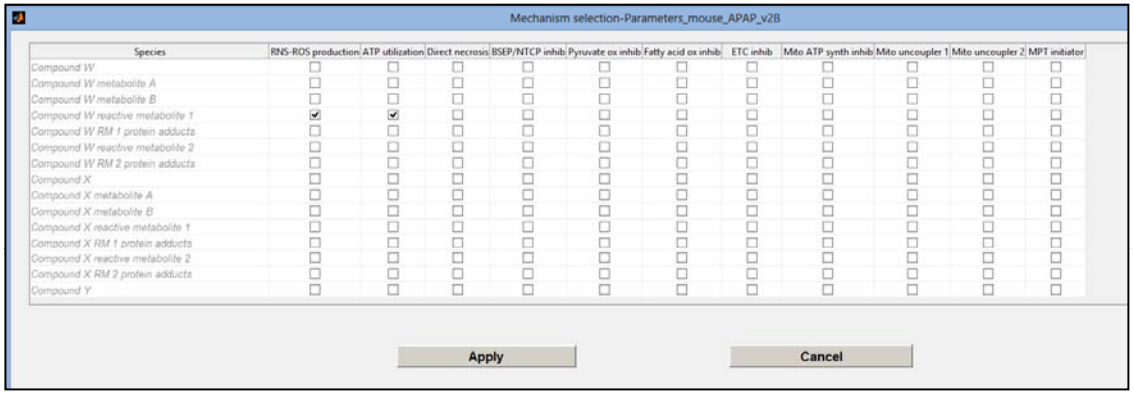

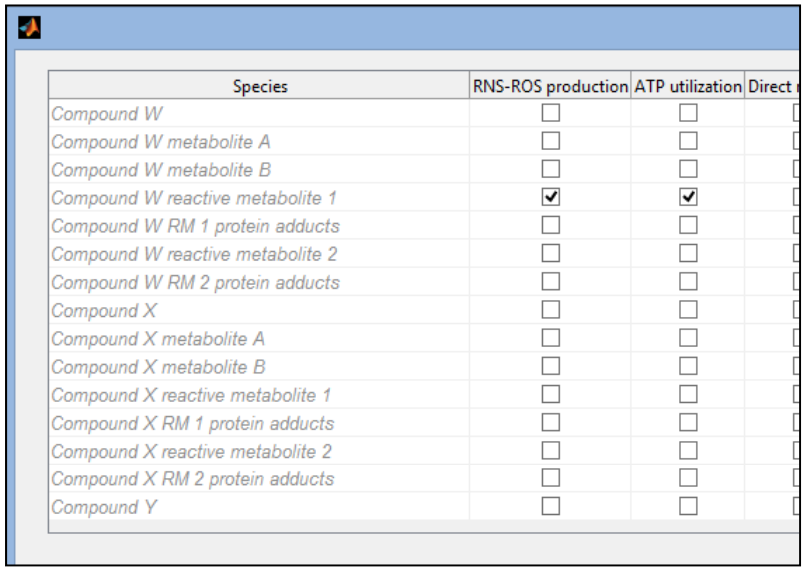

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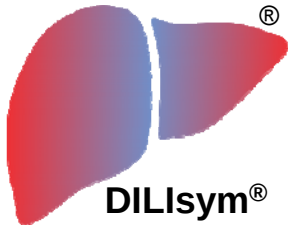
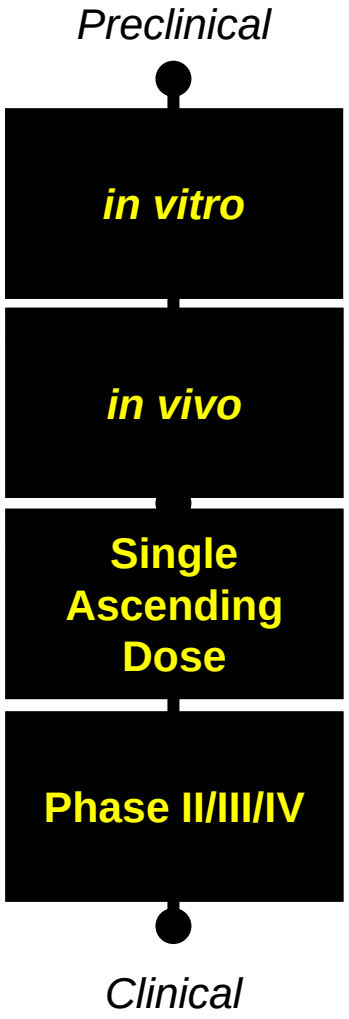


Examples of DILIsym® Applications

IVIVE

Rank compounds by risk

Preclinical biomarker study design



DILI Dose Response Estimation

Clinical biomarker analysis

Predicting variability in response

Cleveland BioLabs Project Objectives

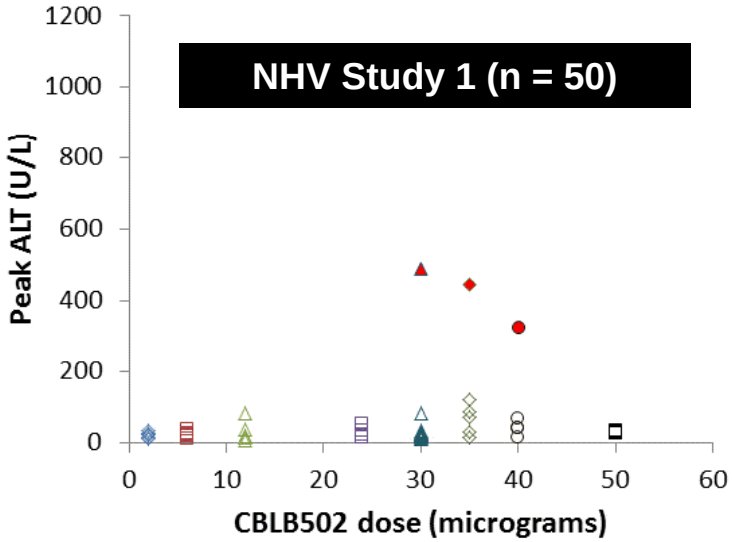
- Primary Objectives
  - Use simulations to infer hepatocellular dynamics associated with observed changes in liver biomarkers during CBLB502 clinical trials in normal, healthy volunteers (NHV)
  - Support Cleveland BioLabs in communications with regulatory agencies regarding CBLB502

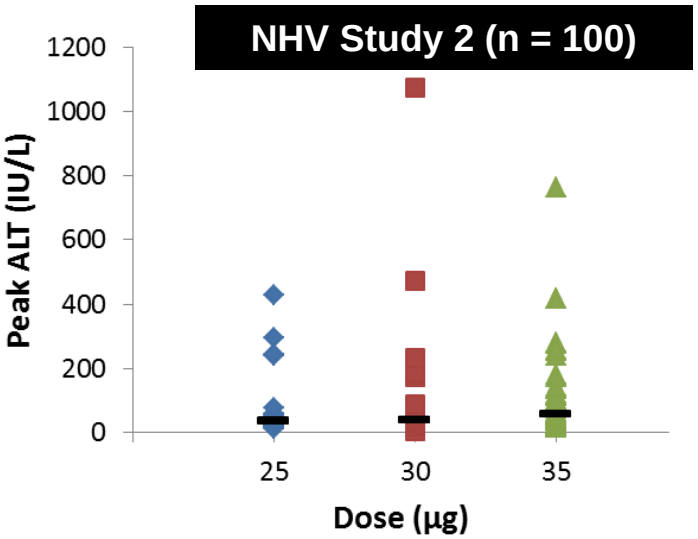
- Secondary Objectives
  - Simulate protocols of past CBLB502 clinical trials
  - Determine impact of variability in key areas of hepatocellular dynamics (i.e., necrosis, proliferation) on generation of liver biomarkers using SimPops™, individual simulated patients with variability in key areas of hepatocellular dynamics
  - Present and/or publish findings at scientific conferences or in scientific journals

Observations of CBLB502 Clinical Data Applicable to Simulations

- Initial dose-ranging trial showed that some individuals had clinically relevant ALT increases at doses ≥30 micrograms
- Second trial included more narrow dosing range (25-35 micrograms)

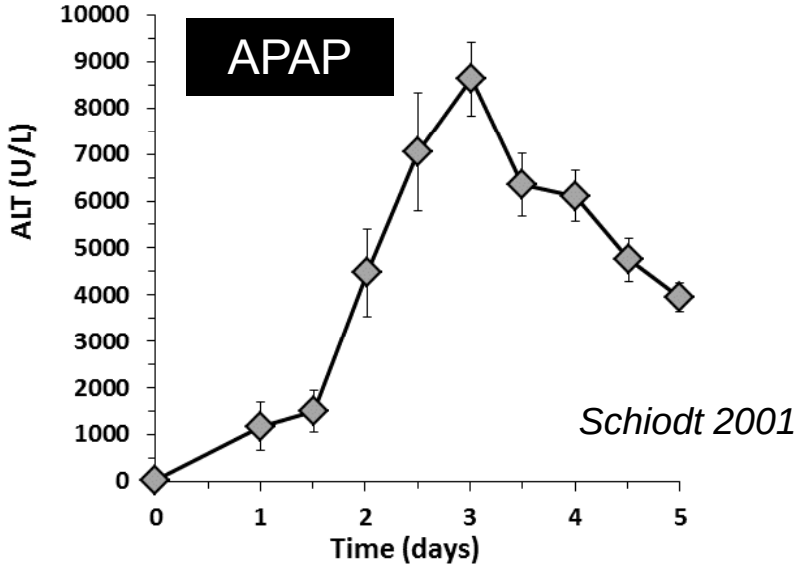
- Preponderance of NHV exhibited only minor increases in liver signals
  - 70% < 1.5x ULN for ALT
  - 65% < 1.5x ULN for AST
- Increased ALT and AST in several NHV
  - 20% > 3x ULN for ALT
  - 26% > 3x ULN for AST
- Time to peak ALT is quite rapid (8-16 h)
  - More rapid than following APAP overdose
- AST and ALT increases are coincident
  - Implies hepatic vis a vis peripheral injury
- Slight increase in bilirubin
  - No correlation with ALT or AST



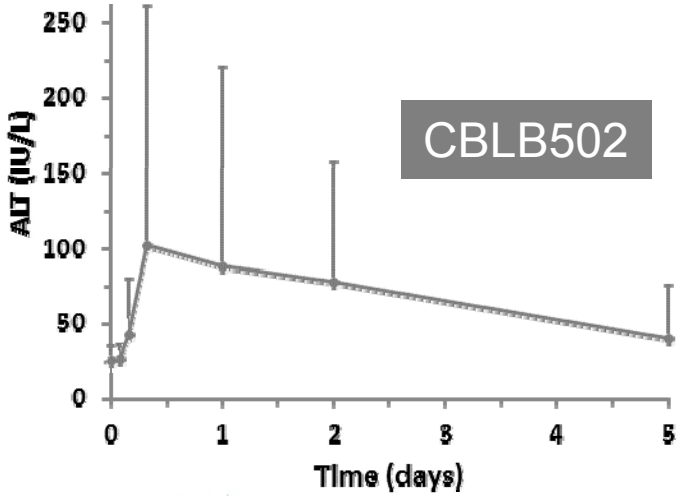


Rapid Peak ALT with CBLB502 Compared with Acetaminophen Overdose

- Peak ALT after acetaminophen (APAP) overdose reported to be 48-84 h
- Peak ALT observed after CBLB502 8-24 h after dosing
  - Mean Tmax = 14.3 h
  - Median Tmax = 8 h
- Accelerated ALT Tmax with CBLB502 treatment required adjusting existing ALT sub-model

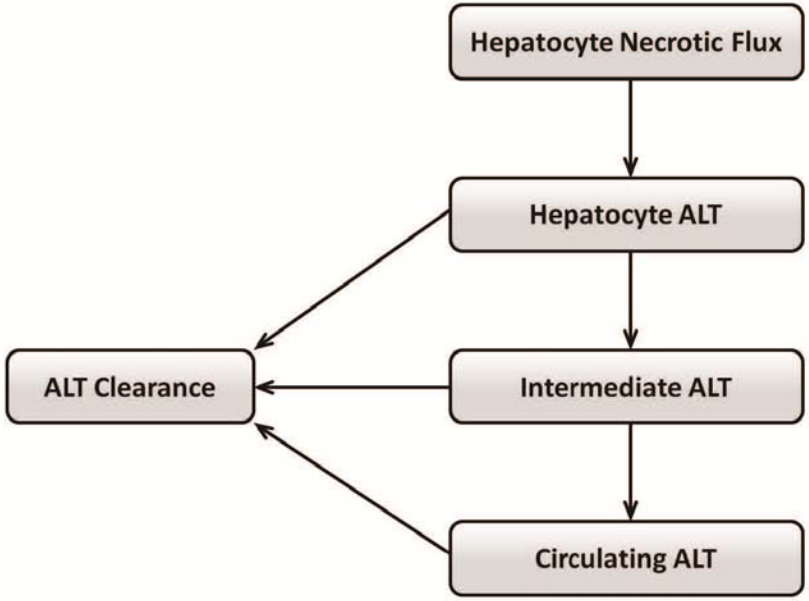


Schiodt 2001



Approach for Using Simulations to Analyze Entolimod Clinical Data

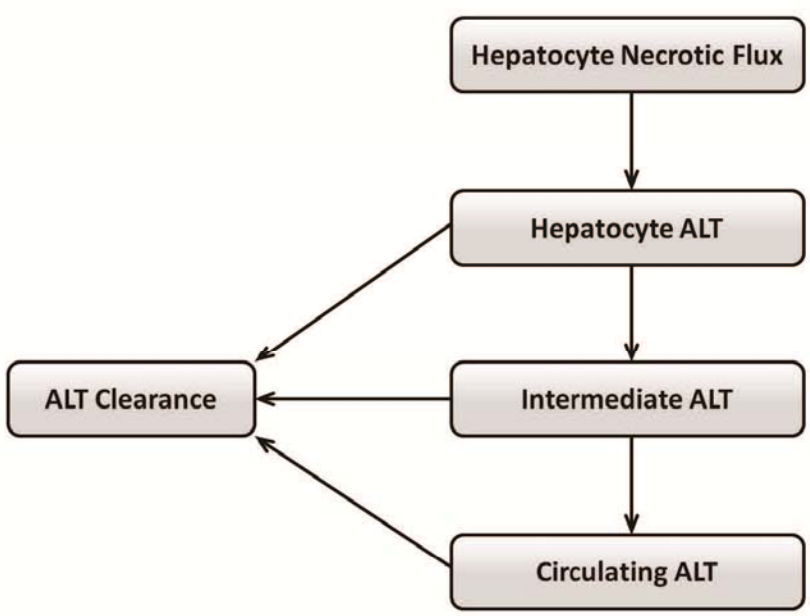
- Approach: use ALT dynamics to infer hepatocyte loss
  - ALT content per cell based on cellular measurements
    - *Boyd 1983, Remien 2012, Lindblom 2007*
  - ALT release occurs upon hepatocyte necrosis
  - ALT elimination half-life based on clinical data
    - *Nicoll 1997*



- Initial simulations in DILIsym® baseline normal healthy volunteer (NHV)

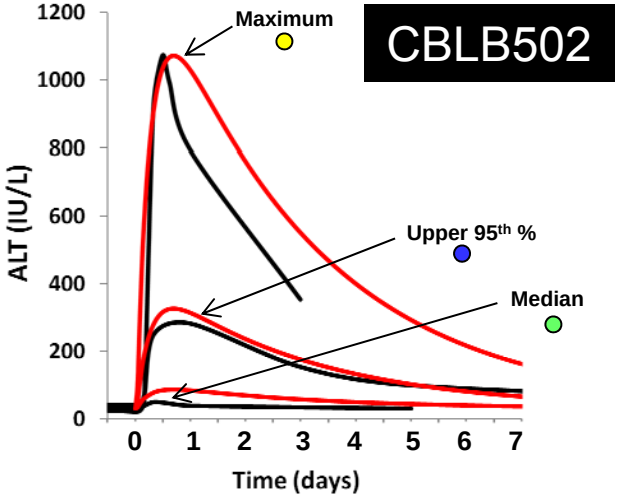
ALT Sub-Model Includes Hepatocellular Release and Clearance Dynamics

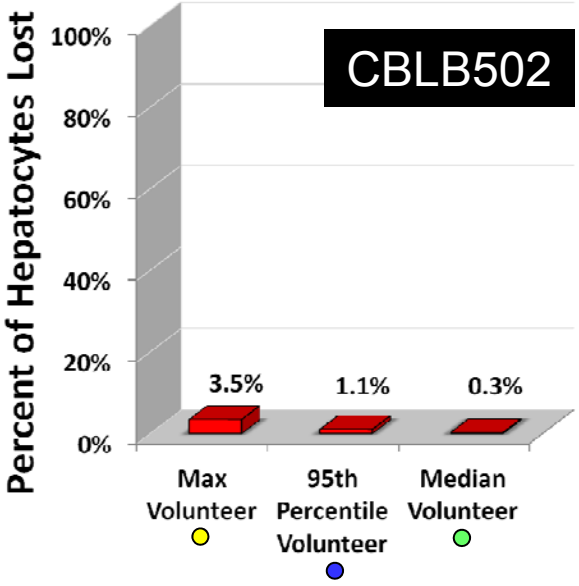
- Modeled ALT release is driven by rate of hepatocyte necrosis
- Liver, intermediate ALT pools included to provide timing of release consistent with reported clinical data
  - Primarily acute acetaminophen overdose
  - Transfer rate can be adjusted if necessary
- Model includes clearance from liver, intermediate, and plasma pools
  - Kupffer cells largely responsible for clearance from liver and intermediate ALT pools
  - Kidney largely responsible for clearance from plasma ALT pool
- AST sub-model is similarly designed



Baseline Human Simulations Indicate Minimal Hepatocyte Loss with CBLB502

- ALT time course data indicates consistent, early peaks
  - Variations in peak height observed
- Simulations performed in baseline NHV
  - Focused comparison of simulation results with Max, 95th percentile, and median volunteer ALT levels
- Simulations agree with ALT clinical data
  - By design via optimization
- Minimal hepatocyte loss associated with observed ALT profiles
  - Volunteer with greatest peak ALT predicted to have lost <5% hepatocytes



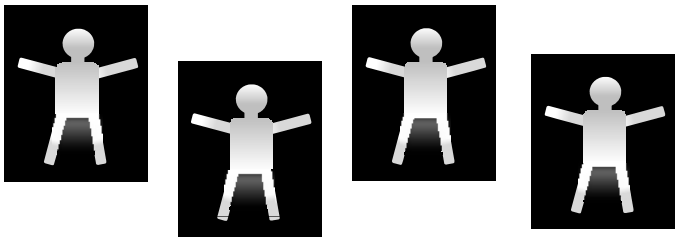


*Colored dots show correspondence between ALT profiles and hepatocyte loss predictions

Approach for Introducing Population Variability into Simulations

- Varying parameters associated with ALT dynamics in accordance with variance described in literature
  - *Remien 2012, Nicoll 1997, Portmann 1975, Prescott 1979*
- Compared simulated humans (N ≈ 300) with clinical data from Prescott 1979 and Portmann 1975
  - Indirect link between ALT and necrosis
- Simulated humans used to simulate Entolimod trial protocol

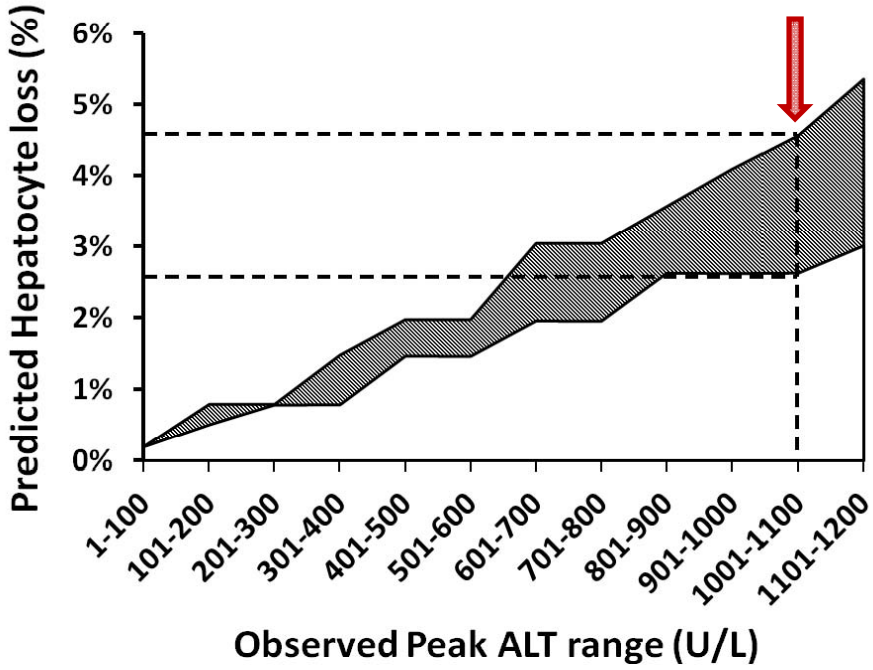
Variables Used to Construct Population Sample for Entolimod Application
Hepatocellular ALT content
ALT $t_{1/2}$
ALT transport rate
Hepatocyte proliferation rate



Variability in SimPops™ Predicts Minimal Range of Hepatocyte Loss for CBLB502 Peak ALT

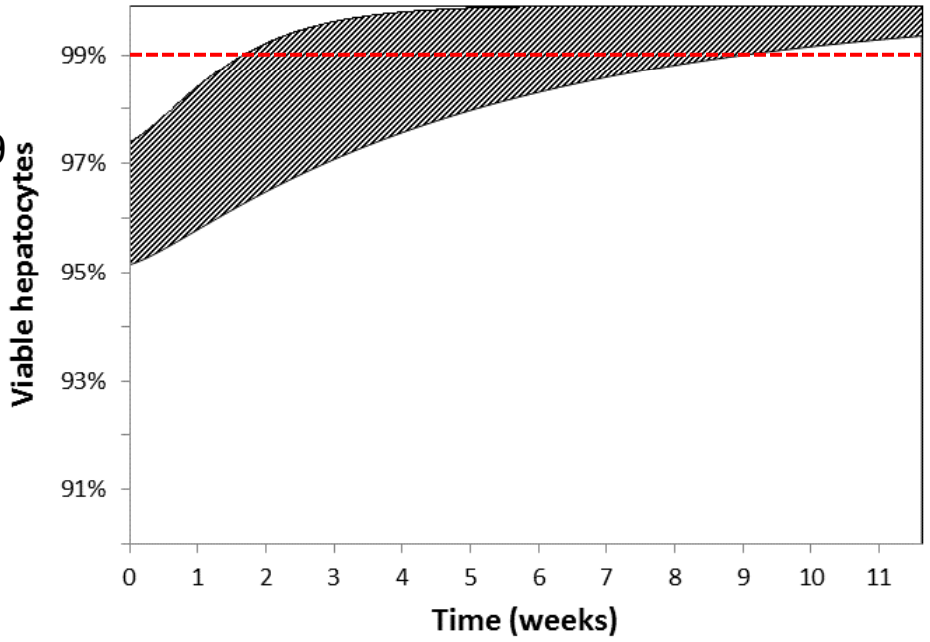
- SimPops™ generated with variability in key aspects of ALT release
- ALT 1001-1100 U/L corresponds with 2.6-4.6% hepatocyte loss
- Did not simulate hepatocyte loss-ALT variability at ALT 201-300 due to systematic simulation approach

Peak ALT	Hepatocyte loss	
Range	Lower Bound	Higher Bound
1-100	0%	0.5%
101-200	0.5%	0.8%
201-300	0.8%	0.8%
301-400	0.8%	1.5%
401-500	1.5%	2.0%
501-600	1.5%	2.0%
601-700	2.0%	3.0%
701-800	2.0%	3.0%
801-900	2.6%	3.6%
901-1000	2.6%	4.1%
1001-1100	**2.6%**	**4.6%**
1101-1200	3.0%	5.4%



Regenerative Hepatocyte Proliferation Predicted to be Complete 2-9 Weeks after CBLB502 Dosing

- SimPops™ generated with variability in hepatocyte proliferation
- Hepatocyte restoration complete within ~2-9 weeks after onset of injury (median human prediction - 3 weeks)
  - Shaded region reflects variation in degree of injury and hepatocyte proliferative response from the SimPops™
  - Viable hepatocyte restoration considered complete at 99% (dashed red line)
  - Simulation results shown for maximal ALT response to CBLB502
- Hepatocyte proliferation begins with onset of injury and persists until complete regeneration
  - Simulation results plotted from nadir of viable hepatocytes until complete restoration



Evidence from Literature to Support Safety of Minimal Hepatocyte Loss with CBLB502

- Excision of 20% of liver volume in living donors is generally considered safe (Florman 2006)
  - Living donors routinely recover fully after even greater portions (40-60%) of liver are excised for adult-to-adult donations (Florman 2006, Lee 2010)
- Heparins are widely considered to be safe despite associated increases in ALT
  - Reported ALT increases after heparins comparable to observed ALT after CBLB502
  - DILIsym® modeling team performed comparable ALT-hepatocyte loss on published clinical data (Harrill 2012, analysis on following slides)
  - Comparable, minimal hepatocyte loss predicted for heparins and CBLB502
- Clinical correlative data from literature indicate that minimal loss of hepatocytes due to injury has little to no effect on bilirubin levels and prothrombin clotting time (Portmann 1975)

Project Summary

- Analyses based on clinical data and simulation results indicate that volunteers with ALT elevations following CBLB502 administration likely incurred hepatocyte losses of ≤5%
- The vast majority of necrotic hepatocyte loss was predicted to have occurred within the first 24 h following dosing, and recovery (restoration of 99% viable hepatocytes) times ranged from 2-9 weeks
- Based on literature review, ~15% of hepatocytes can be lost to a necrotic event without an increase in bilirubin or symptoms associated with liver injury

Application Example 1: Retrospective Analysis of Observed Liver Safety Signals

Issue

- ALT (and AST) elevations were reported in a single (few) individuals from early clinical trials
- No indications of liver dysfunction were observed in the early trials
- No mechanistic data for hepatotoxicity have been identified

Pending Decision

- Does the Company continue to advance this program?
  - Assume multiple inputs and data sets, potentially including modeling and simulation

Questions to Individual(s) Responsible for Liver Safety Assessment

- Can DILIsym® be used to retrospectively interpret the observed ALT elevations?
  - What level of injury might be inferred from the reported ALT profile?
  - How much uncertainty is associated with the estimated level of liver injury?
  - What time frame of recovery would be expected for the simulated injury?

Approach for Using Simulations to Analyze Entolimod Clinical Data

- Approach: use ALT dynamics to infer hepatocyte loss
  - ALT content per cell based on cellular measurements
    - *Boyd 1983, Remien 2012, Lindblom 2007*
  - ALT release occurs upon hepatocyte necrosis
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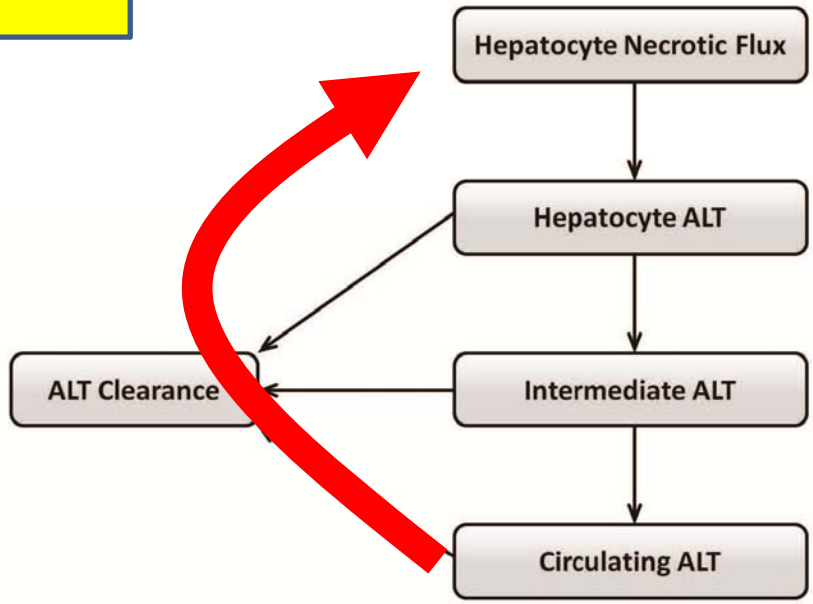
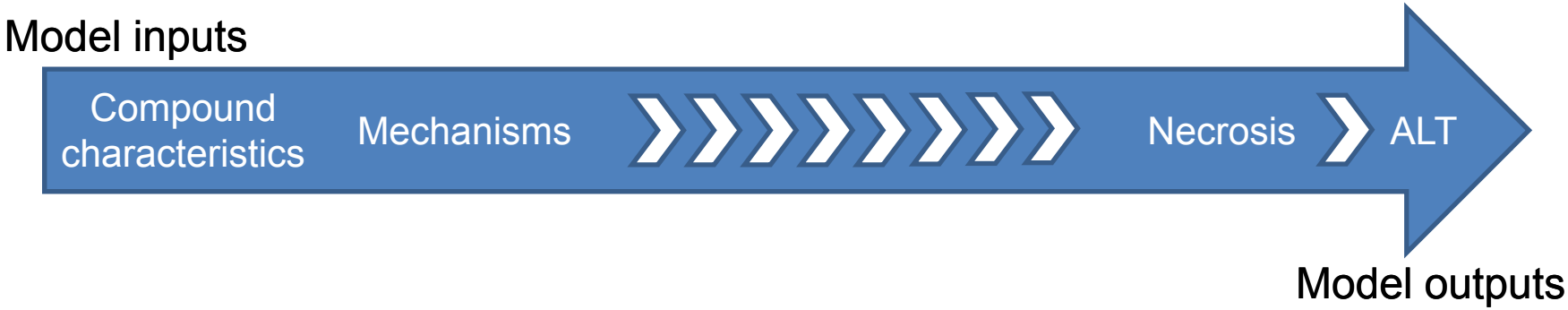


- Initial simulations in DILIsym® baseline normal healthy volunteer (NHV)

Methodological Approach for Using DILIsym® in Retrospective Analysis

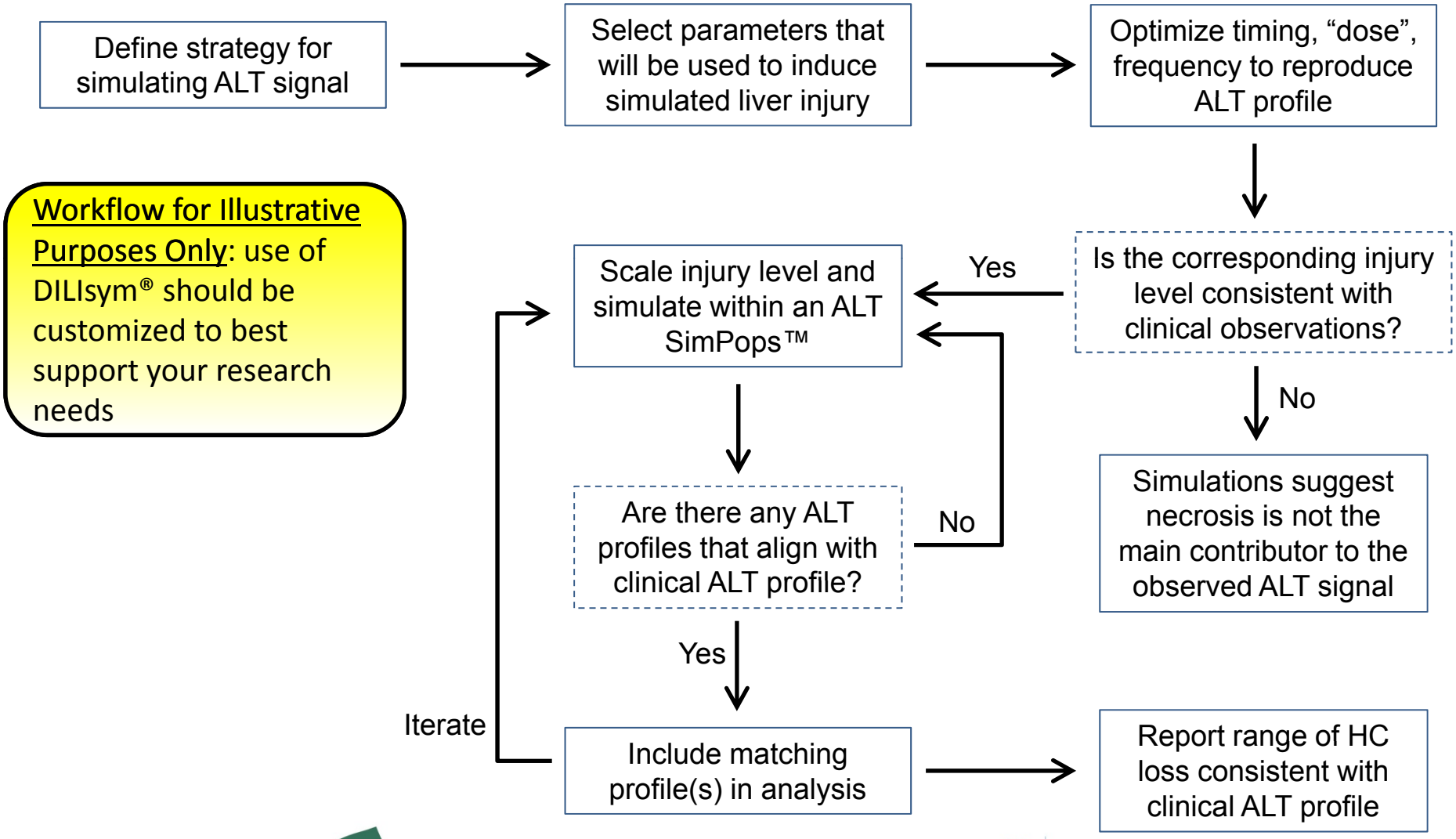
Prospective analysis: use what is known about a compound to better understand potential hepatotoxicity (e.g., degree of necrosis, ALT, bilirubin)



- *A simple retrospective analysis can be conducted <u>without</u> a detailed compound or mechanistic representation*
- *Including compound and mechanisms will result in a more robust analysis*

Retrospective analysis: use ALT to confirm when appropriate degree of necrosis has been simulated

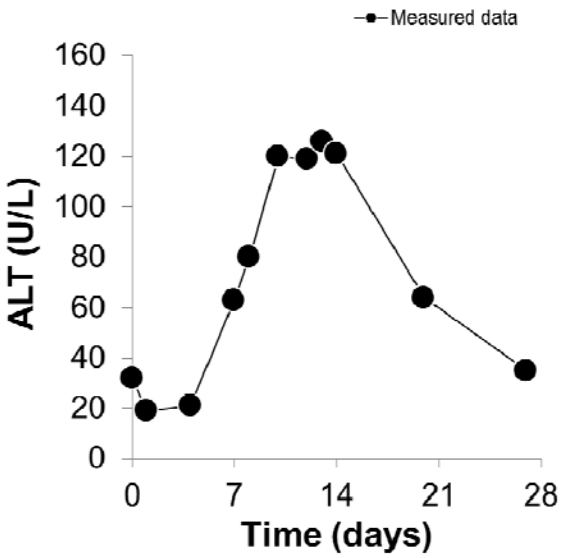
Workflow for Retrospective Analysis of Clinical ALT Signals Using DILIsym®



Workflow for Illustrative Purposes Only: use of DILIsym® should be customized to best support your research needs

How Can DILIsym® Be Used to Reproduce the Clinical ALT Signal?

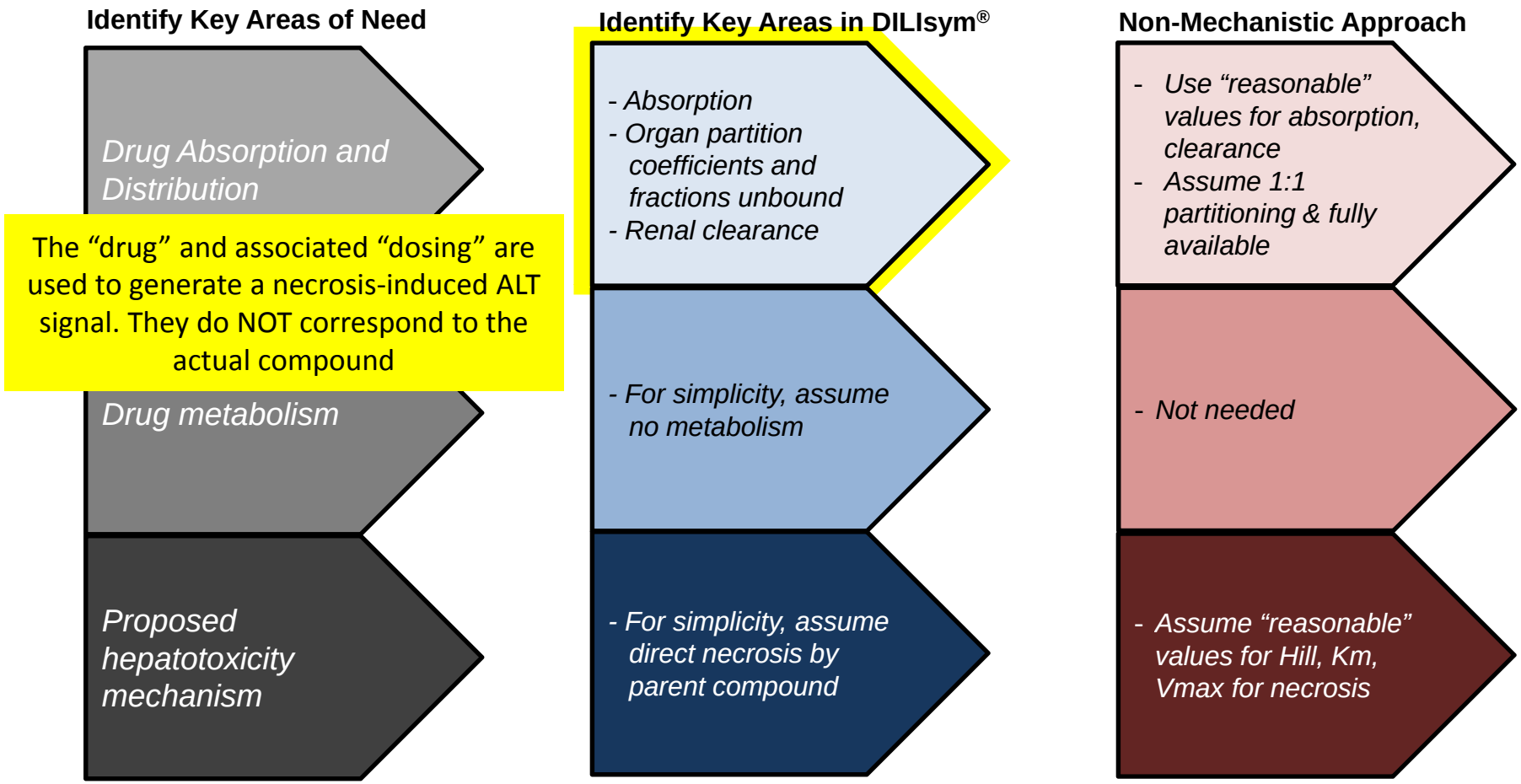
Define strategy for simulating ALT signal



Example ALT profile

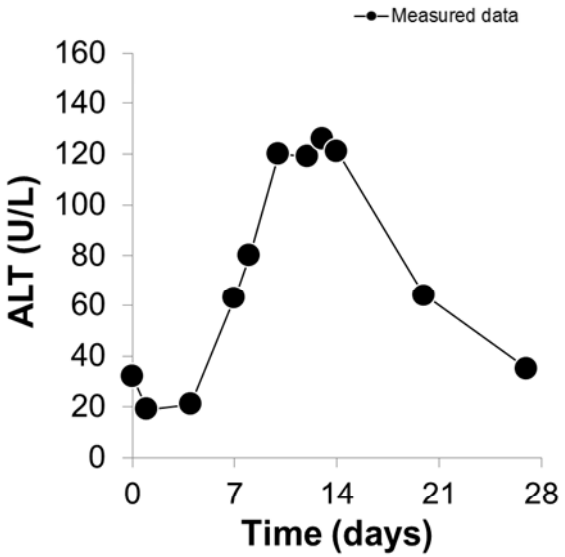
- Clinical data
  - ALT elevations observed in a single individual
  - No liver dysfunction reported
  - No mechanistic data for liver signal available
- Using the DILIsym® baseline simulated human,
  - Assume observed ALT elevations are a result of hepatocyte necrosis
  - Apply parent compound W induces direct necrosis, to “hit” the hepatocytes and generate an ALT profile similar to the experimental data

Identifying the Inputs Needed to Reproduce the ALT Profile in DILIsym® (v2B)



Identification and Selection of Injury-Inducing Parameters

Define strategy for simulating ALT signal → Select parameters that will be used to induce simulated liver injury

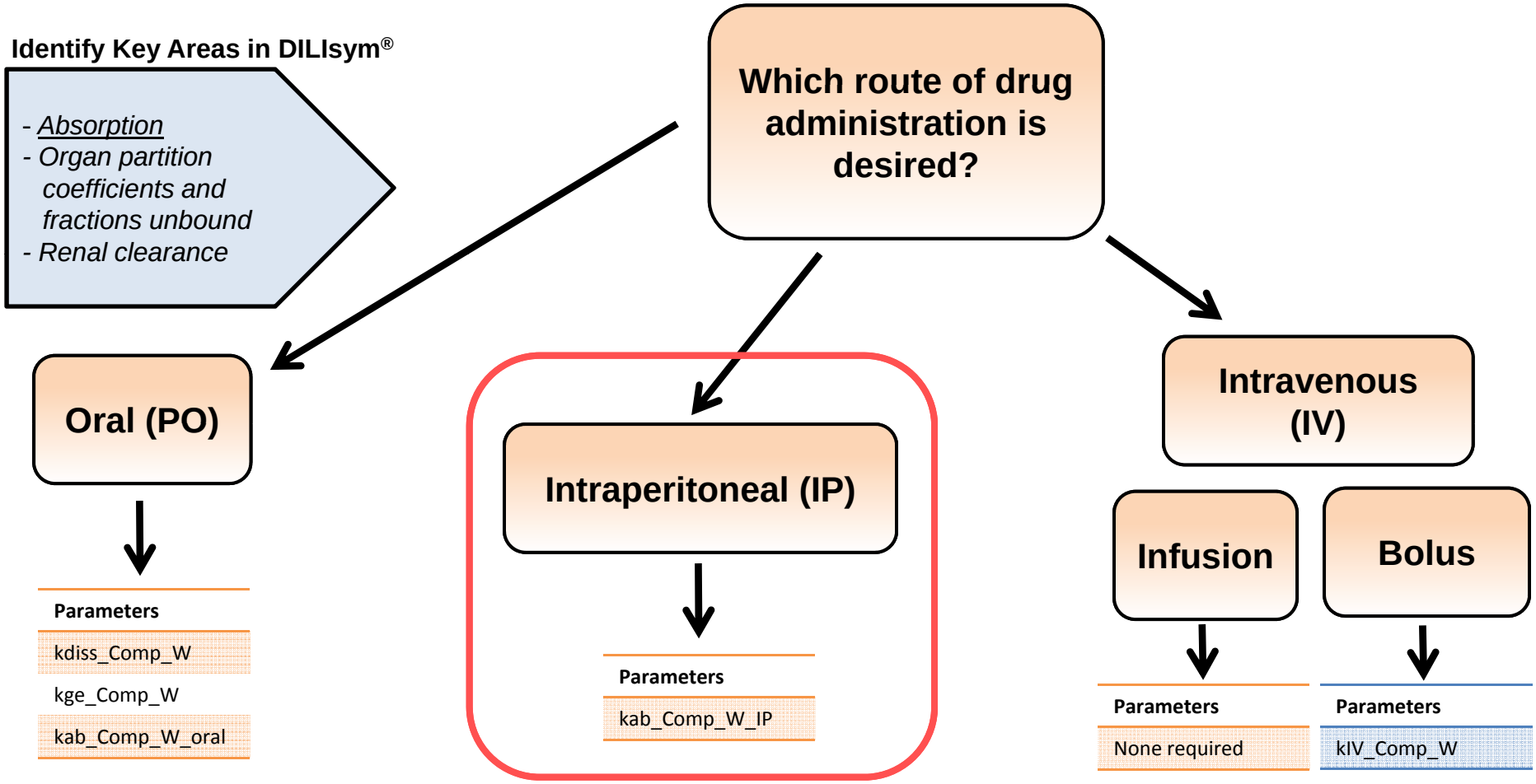


Example ALT profile

- Define simple Compound W PBPK
- Define parameters (K_m, Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

Note: Because "Compound W induces direct necrosis" is a simple stimulus & its effects will be constrained to align with the observed ALT profile, alternate parameter solutions are possible but not expected to impact the estimated liver injury

Selecting the DILIsym® Parameters to Use for Drug Delivery and Absorption



Selected for simplicity
IV would also be simple

Determining Parameter Values for Absorption

Non-Mechanistic Approach

- *Use "reasonable" values for absorption, clearance*
- *Assume 1:1 partitioning & fully available*

- *Absorption*
- *Organ partition coefficients and fractions unbound*
- *Renal clearance*

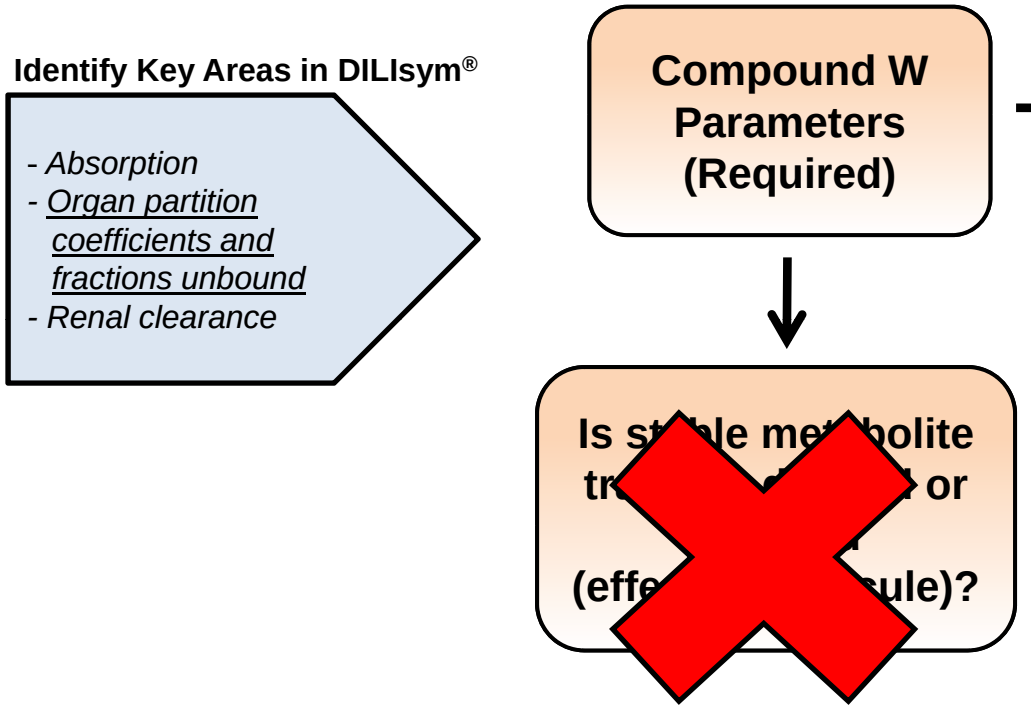
- First order dosing rate constants determine the rate of absorption
  - i.e. Concentration (mass/volume) * Rate Constant (1/hour) = Rate
- Default parameter value is ***12*** (1/hour)
- Keep default for simplicity

Parameter Syntax	Parameter Name	Given or Estimated Value
kab_Comp_W_IP	kab – compound W	12

Intraperitoneal (IP)

Parameters
kab_Comp_W_IP

Selecting the DILIsym® Parameters to Use for Drug/Tissue Partitioning and Binding



Parameters	Parameters
Comp_W_B_P	Comp_W_fu_P
Comp_W_G_B	Comp_W_fu_G
Comp_W_L_B	Comp_W_fu_L
Comp_W_M_B	Comp_W_fu_M
Comp_W_O_B	Comp_W_fu_O

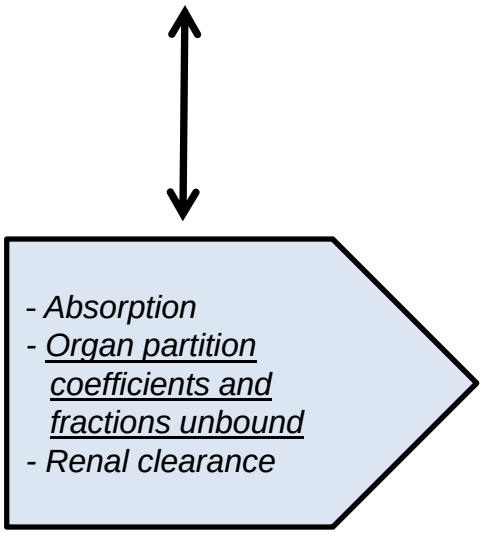
For simplicity:

- Assume complete transfer between compartments (partitioning = 1)
- Assume no binding (fu = 1)

Determining Parameter Values for Tissue Distribution and Protein Binding

Non-Mechanistic Approach

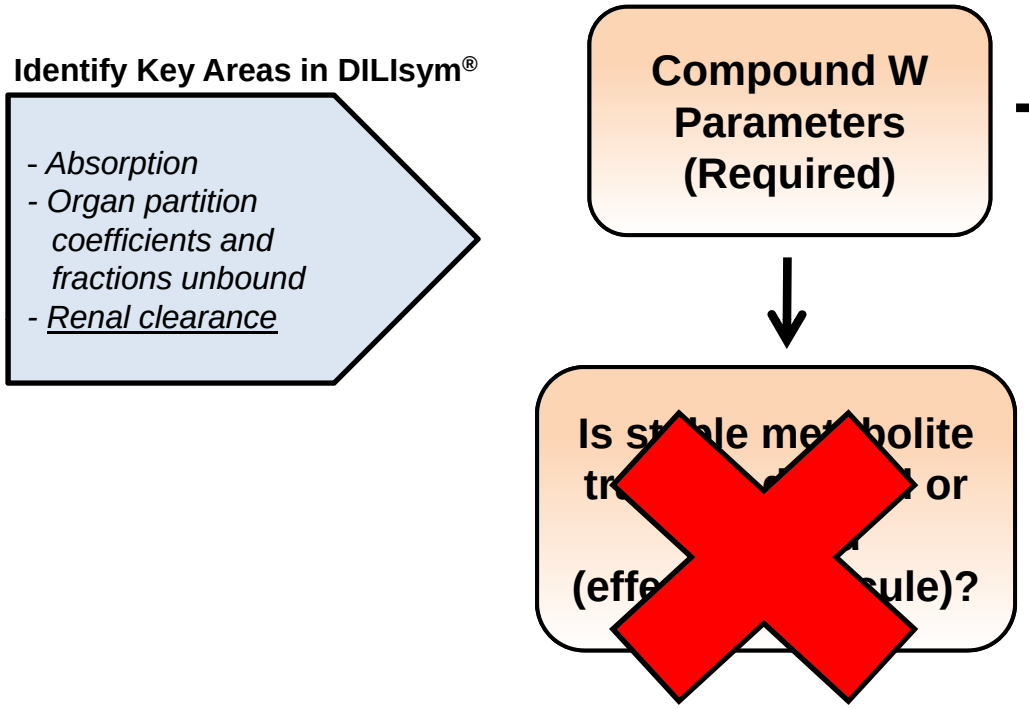
- *Use "reasonable" values for absorption, clearance*
- *Assume 1:1 partitioning & fully available*



- *Absorption*
- *Organ partition coefficients and fractions unbound*
- *Renal clearance*

Parameter Syntax	Parameter Name	Given or Estimated Value
Comp_W_B_P	Compound W blood to plasma	1
Comp_W_G_B	Compound W gut to blood	1
Comp_W_L_B	Compound W liver to blood	1
Comp_W_M_B	Compound W muscle to blood	1
Comp_W_O_B	Compound W other to blood	1
Comp_W_fu_P	Compound W fraction unbound plasma	1
Comp_W_fu_G	Compound W fraction unbound gut tissue	1
Comp_W_fu_L	Compound W fraction unbound liver	1
Comp_W_fu_M	Compound W fraction unbound muscle tissue	1
Comp_W_fu_O	Compound W fraction unbound other tissue	1

Selecting the DILIsym® Parameters to Use for Renal Clearance



Parameters
Comp_W_renal_cl

For simplicity:

- Assume renal clearance rate consistent w/other compounds
- Could use actual rate if known

Determining Parameter Values for Renal Clearance

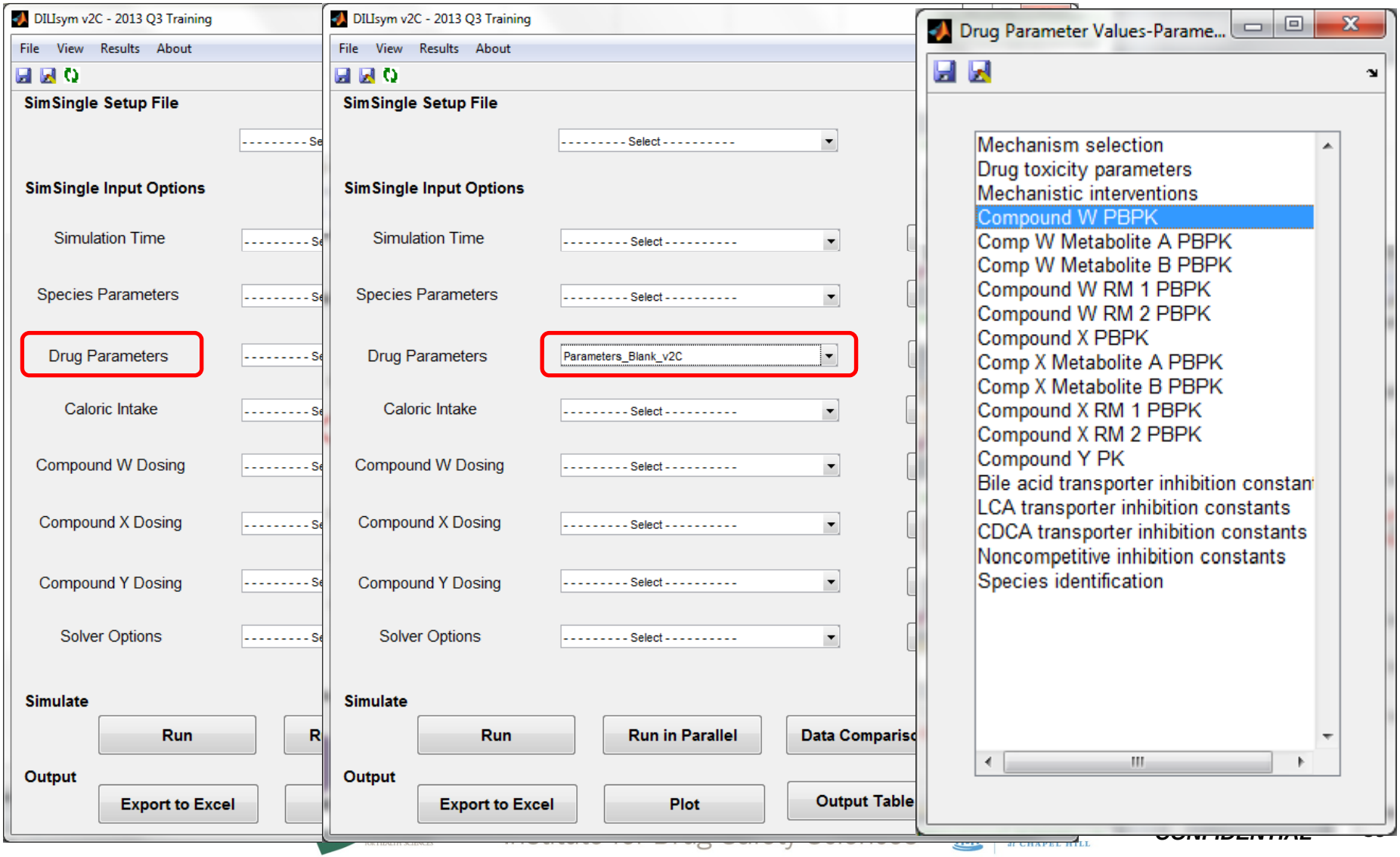
Non-Mechanistic Approach

- *Use "reasonable" values for absorption, clearance*
- *Assume 1:1 partitioning & fully available*

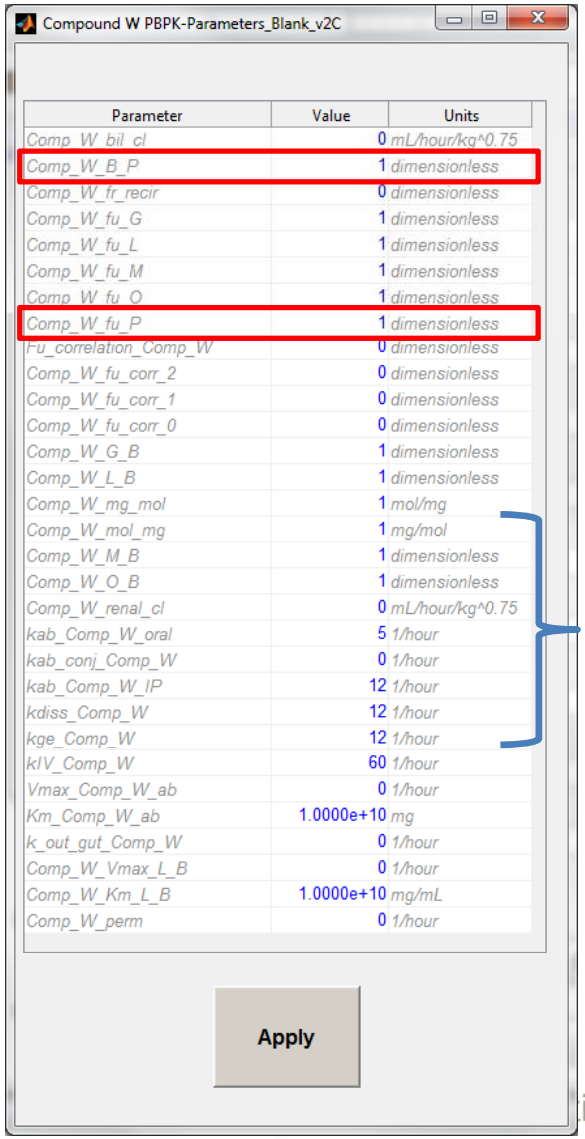
- *Absorption*
- *Organ partition coefficients and fractions unbound*
- *Renal clearance*

Parameter Syntax	Parameter Name	Given or Estimated Value
Comp_W_renal_cl	Compound W renal clearance	25

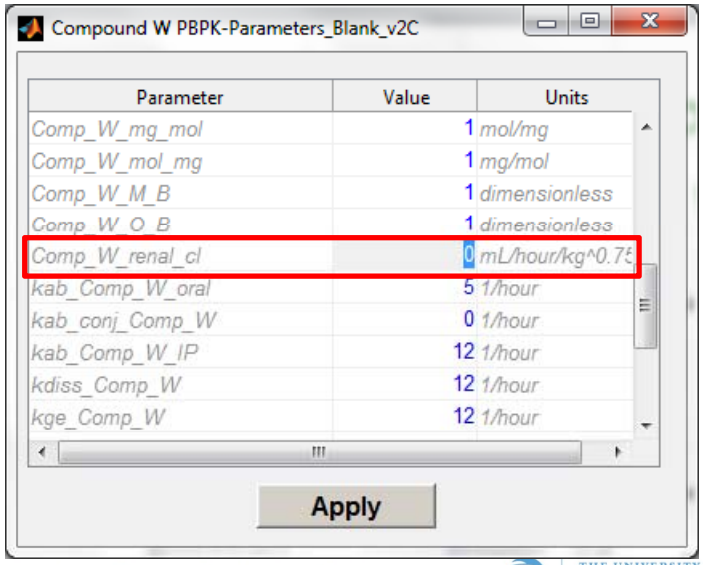
Implementing Parameter Values for Compound W PBPK (1 of 2)



Implementing Parameter Values for Compound W PBPK (2 of 2)

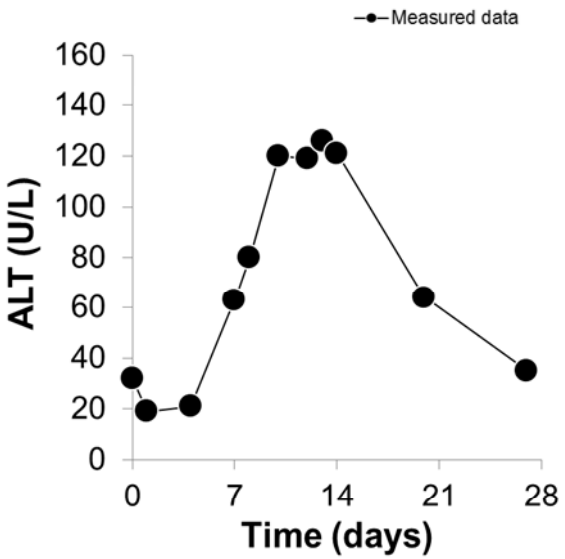


- Retain most default parameter values
  - Tissue distribution values set to 1
  - Fraction unbound set to 1
- Update renal clearance to selected value = 25
- Save parameter file by a new name, e.g.,
  - Parameters_Human_CompW_direct_necrosis



Select "Reasonable" Parameters for Compound Induction of Direct Necrosis

Define strategy for simulating ALT signal → Select parameters that will be used to induce simulated liver injury



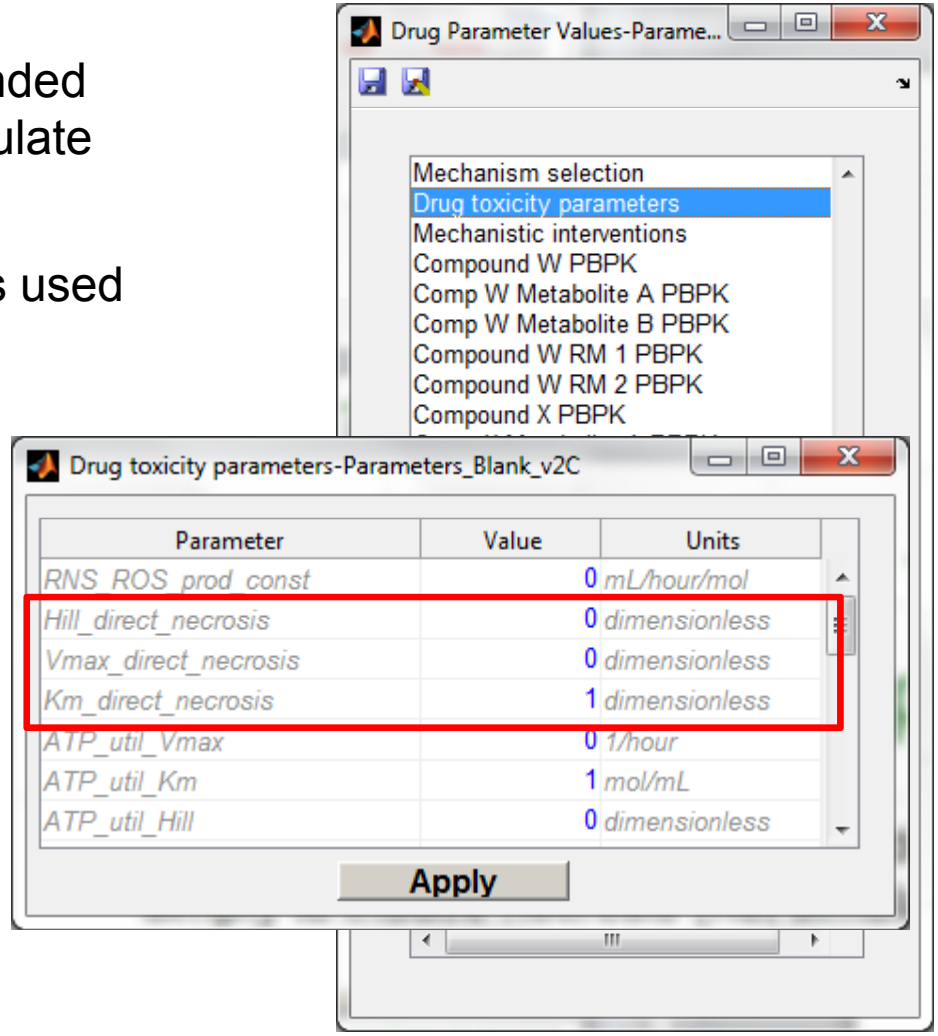
Example ALT profile

- Define simple Compound W PBPK
- Define parameters (K_m, Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

Note: Because "Compound W induces direct necrosis" is a simple stimulus & its effects will be constrained to align with the observed ALT profile, alternate parameter solutions are possible but not expected to impact the estimated liver injury

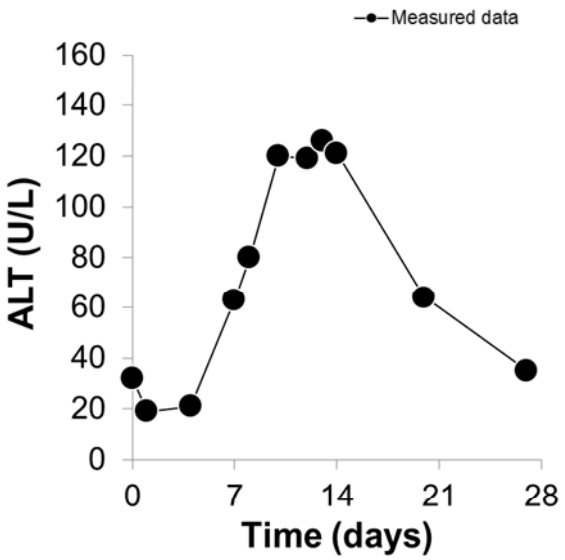
Implementing Drug Toxicity Parameters for Compound W

- Recall this is a dummy “drug” intended simply to induce necrosis (not simulate real drug)
- Insert “reasonable” values, e.g., as used in Cleveland BioLabs Project
  - Hill_direct_necrosis = 1
  - Vmax_direct_necrosis = 1
  - Km_direct_necrosis = 0.003
- Alternate values can be used at the researcher’s discretion



Specify Compound W Induces Direct Necrosis

Define strategy for simulating ALT signal → Select parameters that will be used to induce simulated liver injury



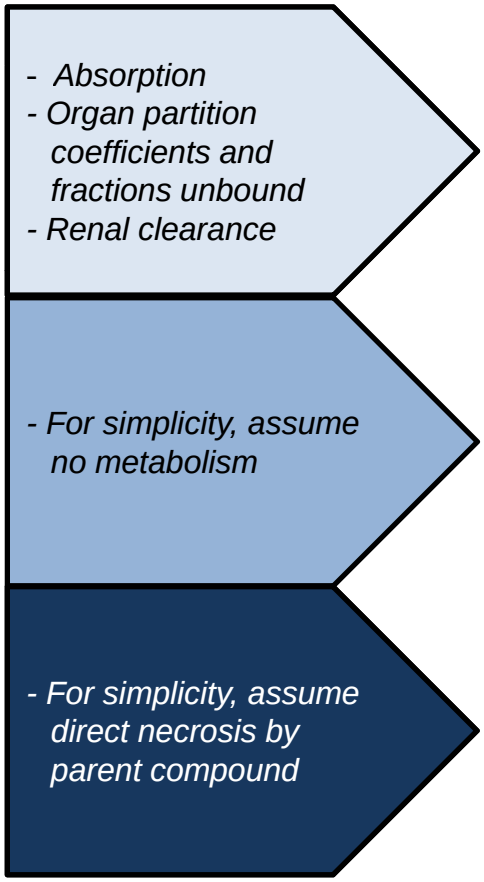
Example ALT profile

- Define simple Compound W PBPK
- Define parameters (K_m, Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

Note: Because "Compound W induces direct necrosis" is a simple stimulus & its effects will be constrained to align with the observed ALT profile, alternate parameter solutions are possible but not expected to impact the estimated liver injury

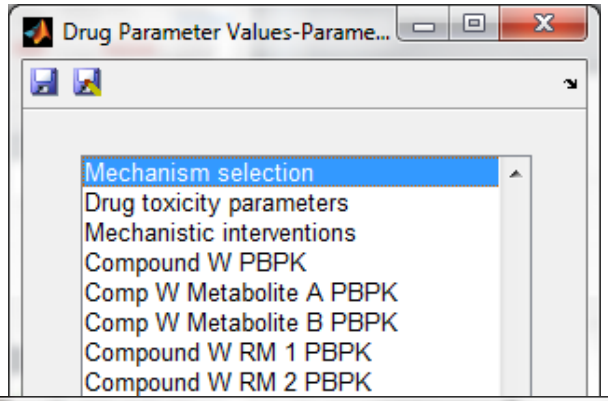
DILIsym® Hepatotoxicity Mechanism Selection for Simply Reproducing an ALT Profile

Identify Key Areas in DILIsym®

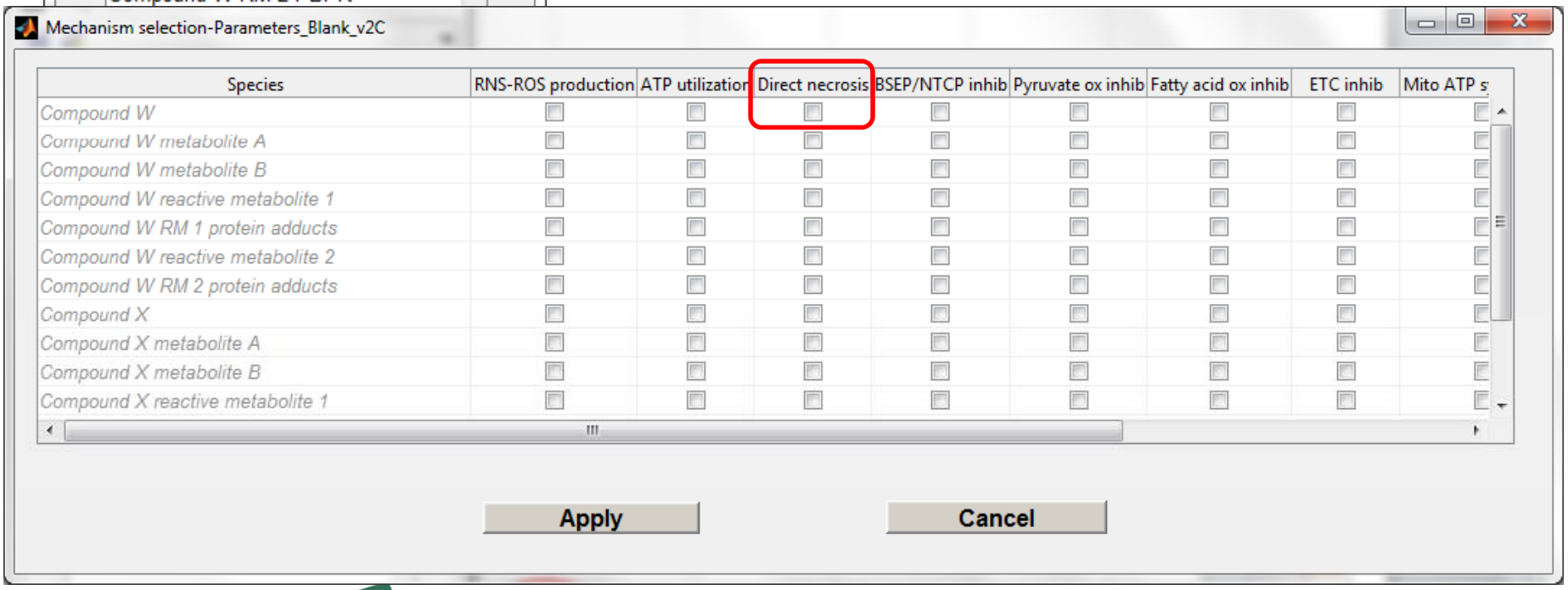


Parameter Syntax	Parameter Name	Given or Estimated Value	Units	Method of Estimation
Compound W	Mechanism for Compound W	Direct necrosis	dimensionless	Not applicable

Implementing Compound W Direct Necrosis

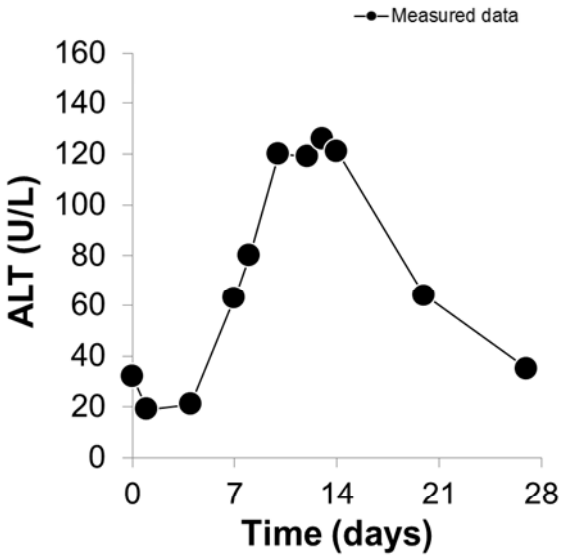


- Select “direct necrosis” for the parent compound W
- Leave all other mechanisms unchecked



Appropriate Species Selection

Define strategy for simulating ALT signal → Select parameters that will be used to induce simulated liver injury



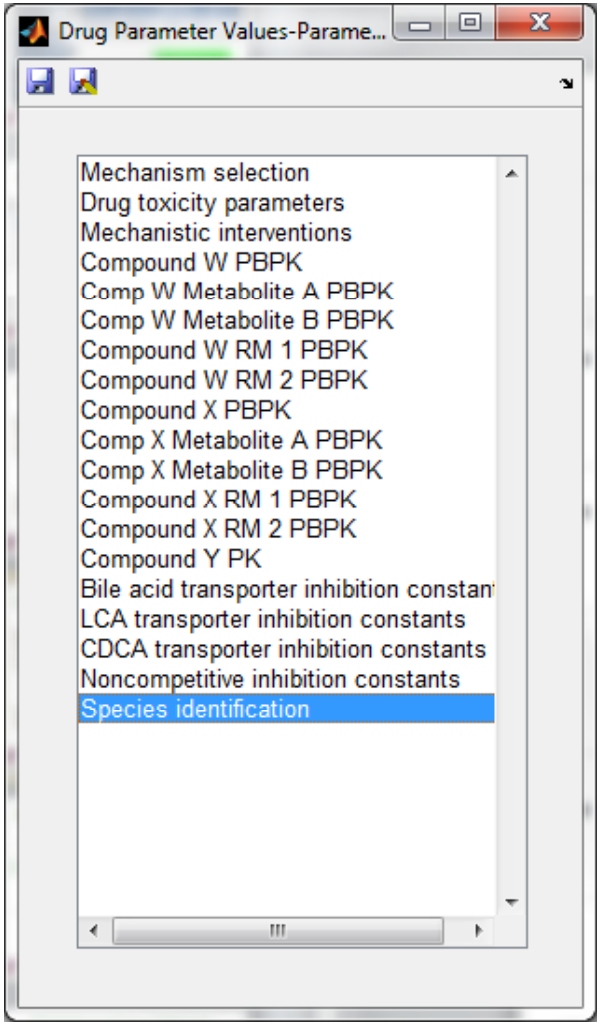
Example ALT profile

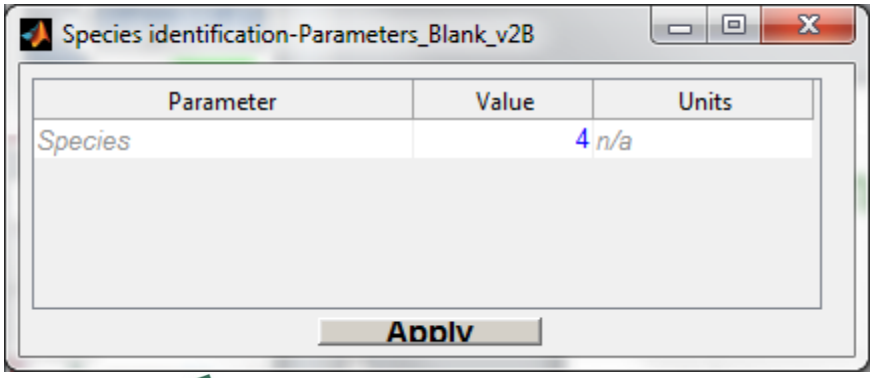
- Define simple Compound W PBPK
- Define parameters (K_m, Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

Note: Because "Compound W induces direct necrosis" is a simple stimulus & its effects will be constrained to align with the observed ALT profile, alternate parameter solutions are possible but not expected to impact the estimated liver injury

Set Species Selection for Human Simulations

- DILI simulations may be run for mice, rats, dogs, or humans
- Species is specified by number
  - ***1*** – mice
  - ***2*** – rats
  - ***3*** – dogs
  - ***4*** – humans





Optimization to Reproduce the ALT Profile

Define strategy for simulating ALT signal → Select parameters that will be used to induce simulated liver injury → Optimize timing, "dose", frequency to reproduce ALT profile

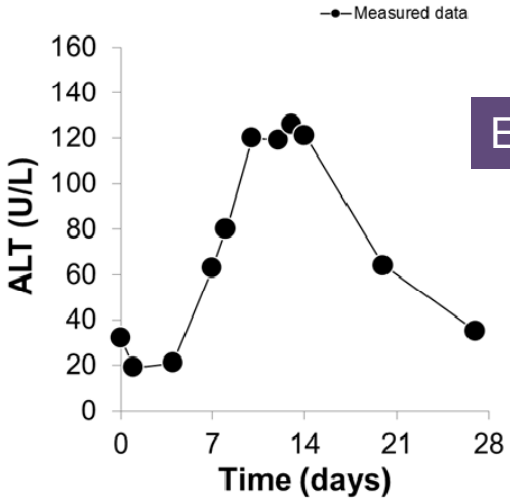
Workflow for Illustrative Purposes Only: use of DILIsym® should be customized to best support your research needs

- Optimize for a Compound W "protocol" that reproduces the ALT profile of interest
  - Time of 1st "dose" §
  - "Dose" magnitude
  - "Dose" frequency

§ *Compound W and associated "dosing" are used to induce injury. They do NOT correspond to the real compound and therefore need not reflect the real compound protocol.*

Optimization Goal: Simulate Injury that Results in the Measured ALT Profile

- Objective is to characterize:
  - Timing of ALT elevation
  - Relationship between "dose" and ALT
- Initial set-up design
  - Short duration
  - Single "dose"
- Save Compound W dose scheme & SimSingle™ under appropriate names

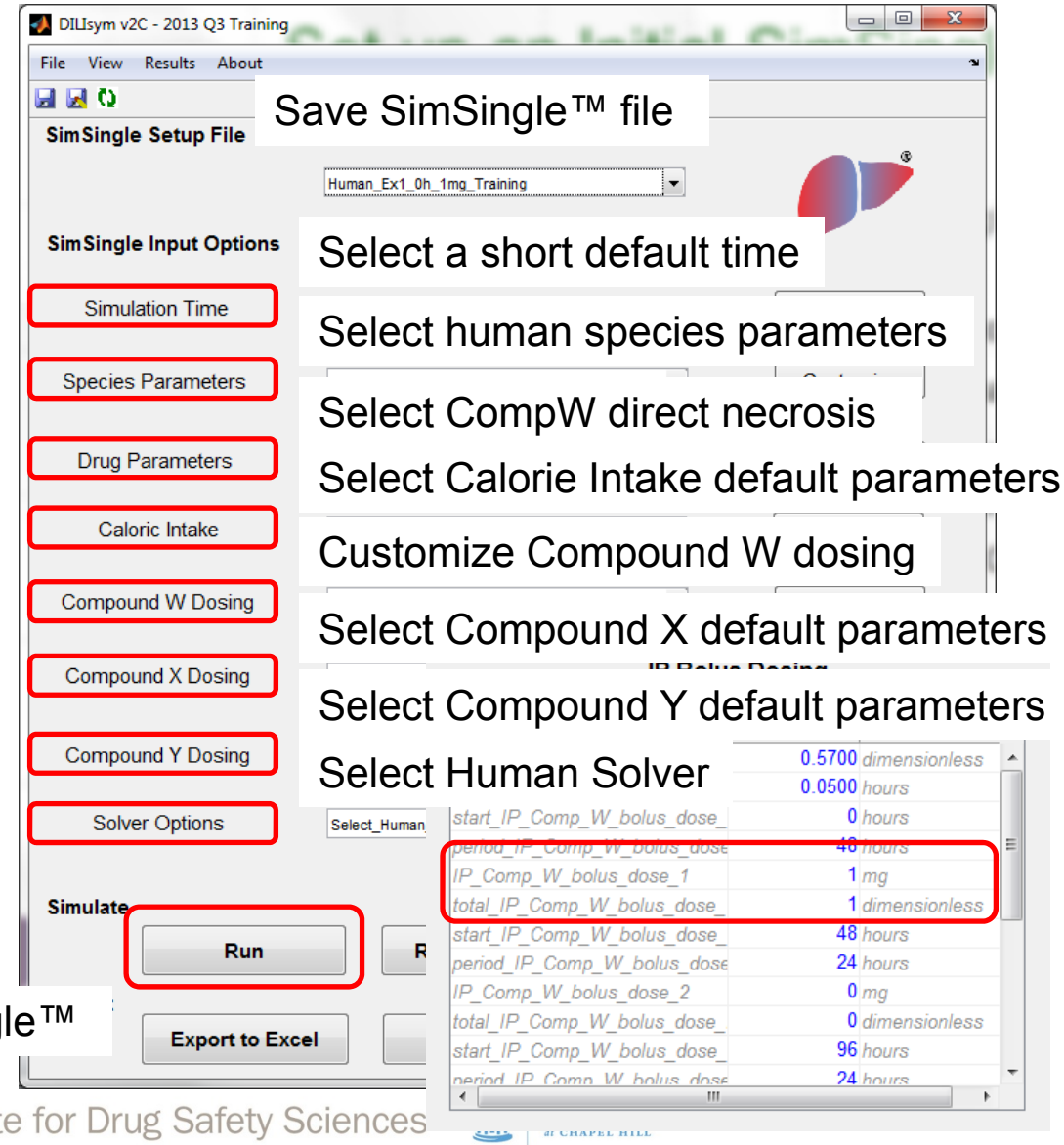


Example ALT profile

Time (days)	Time (hours)	ALT (U/L)
0	0	32
1	24	19
4	96	21
7	168	63
8	192	80
10	240	120
12	288	119
13	312	126
14	336	121
20	480	64
27	648	35

Set up an Initial SimSingle™

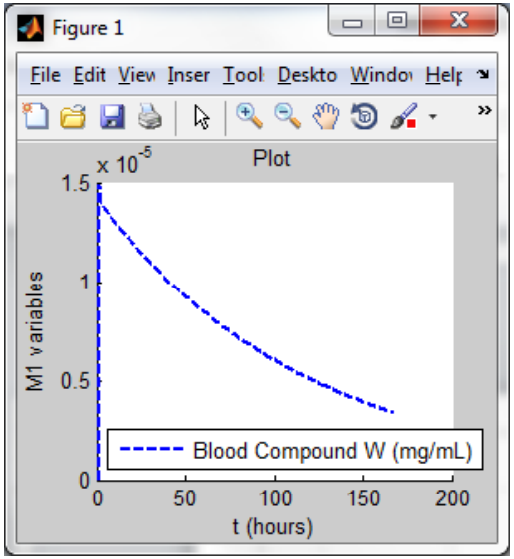
- Simulation Time
  - 1_week_Default
- Species Parameters
  - Parameters_human_specific_v2C
- Drug Parameters
  - As specified in previous section
  - Parameters_human_CompW_direct_necrosis
- Caloric Intake
  - Caloric_intake_parameters_blank_v2C
- Compound W Dosing
  - Create a new test set
  - Specify 1 mg dose, 1 total dose
- Compound X Dosing
  - Compound_X_dosing_blank_v2C
- Compound Y Dosing
  - Compound_Y_dosing_blank_v2C
- Solver Options
  - Select_Human_Sims_Solver_Options

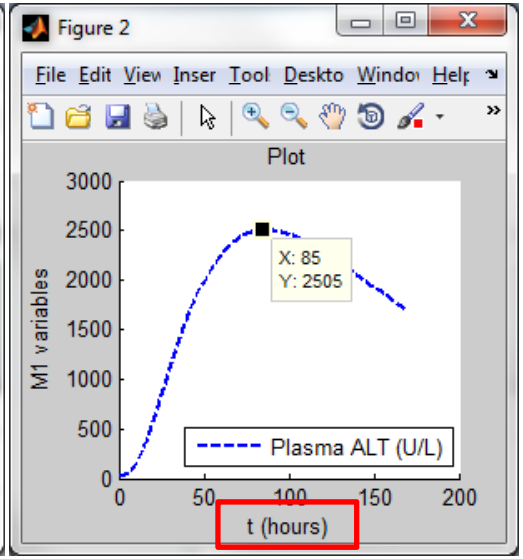


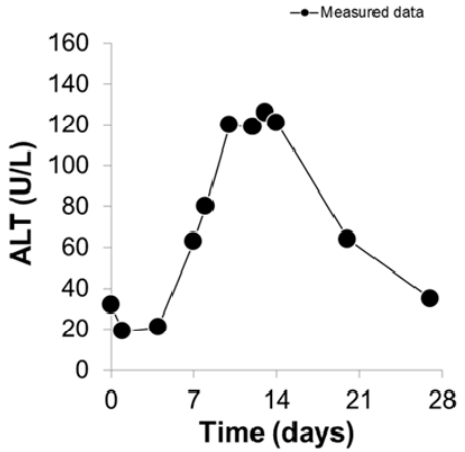
Run SimSingle™

Use Initial Results to Guide Next Steps in Optimization

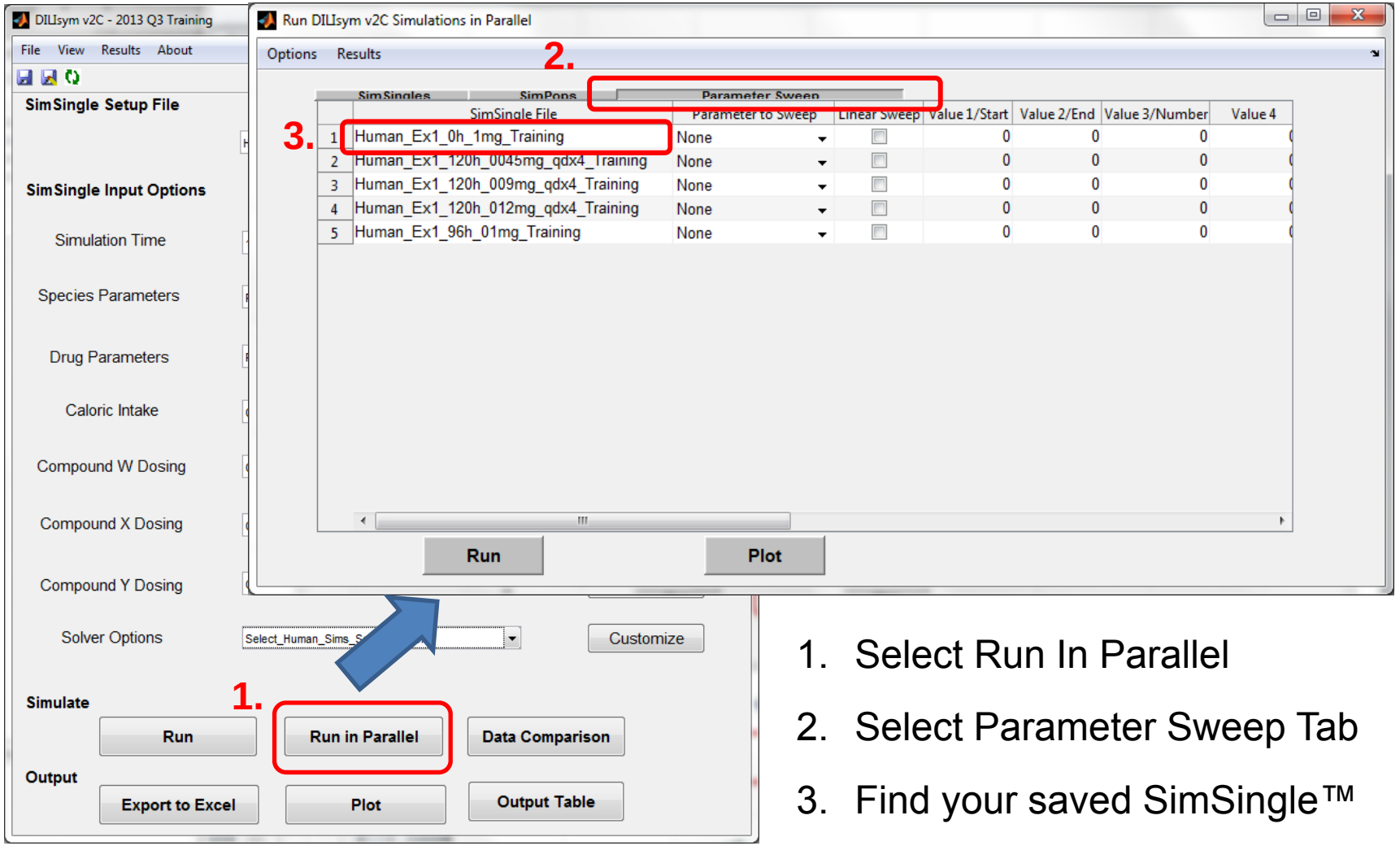
- Use **Plot** button on GUI to visualize simulation results
- Plotting blood compound W verifies that a single dose was simulated
- Plotting ALT reveals 1 mg elicits too much injury, too fast
- Use parameter sweep feature to test
  - Lower “doses”
  - Alternate start times





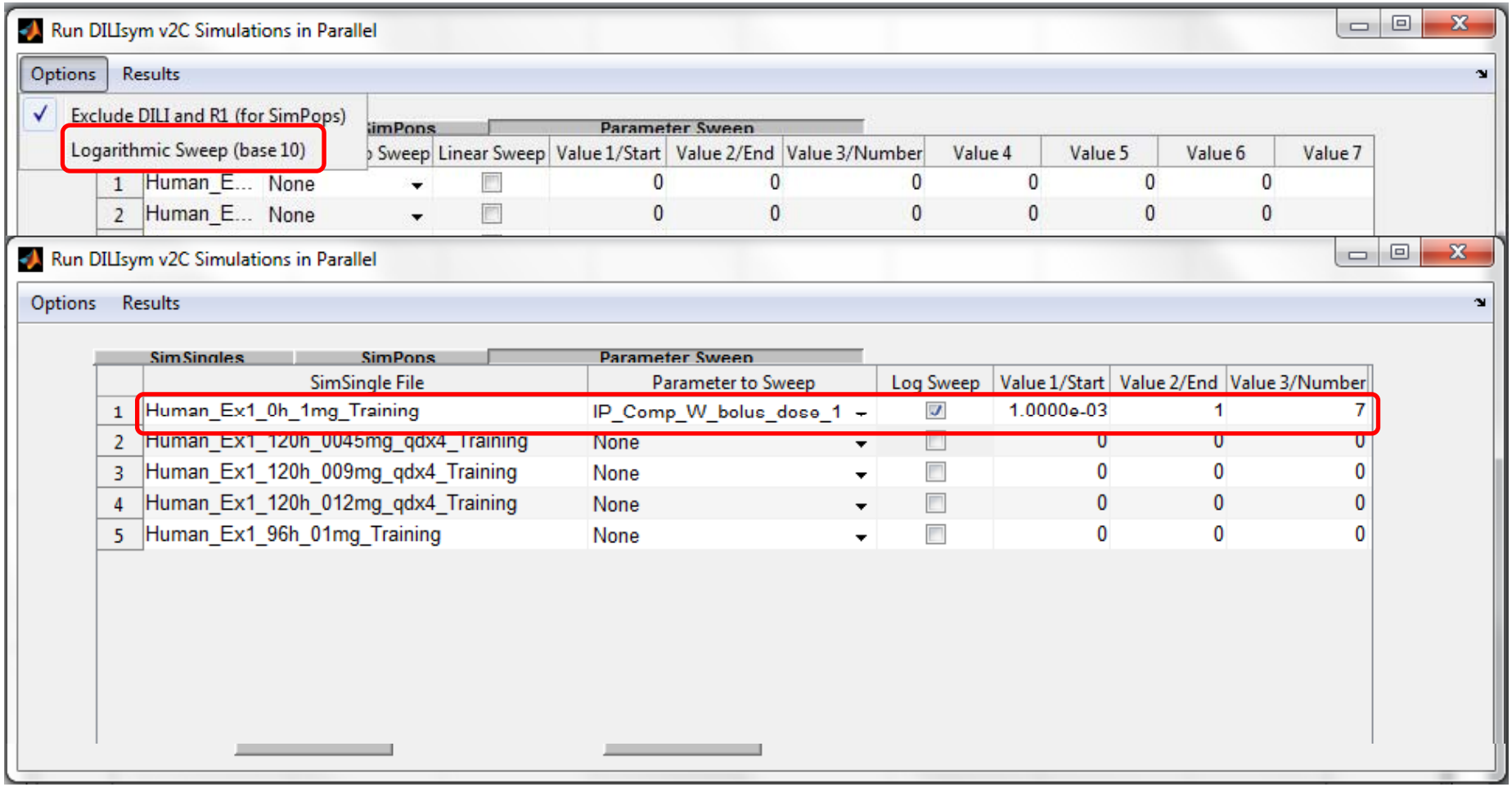


Parameter Sweep Functionality is Available Through Run In Parallel

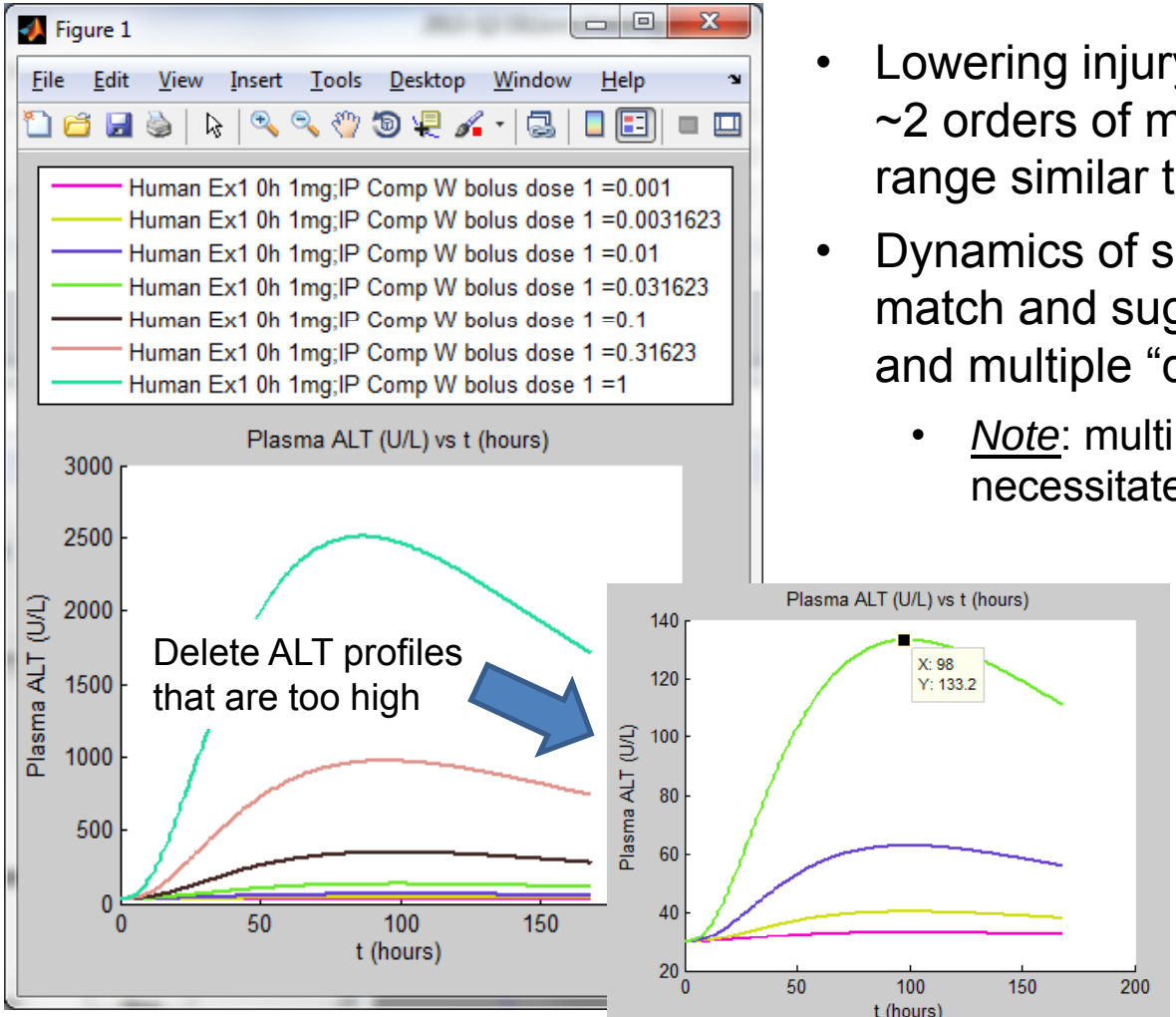


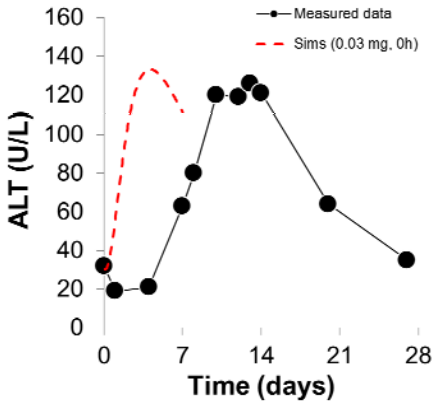
1. Select Run In Parallel
2. Select Parameter Sweep Tab
3. Find your saved SimSingle™

Use Log Sweep to Identify a "Dose" Range Better Aligned with Target ALT Profile



Use Parameter Sweep Results to Guide Further Optimization

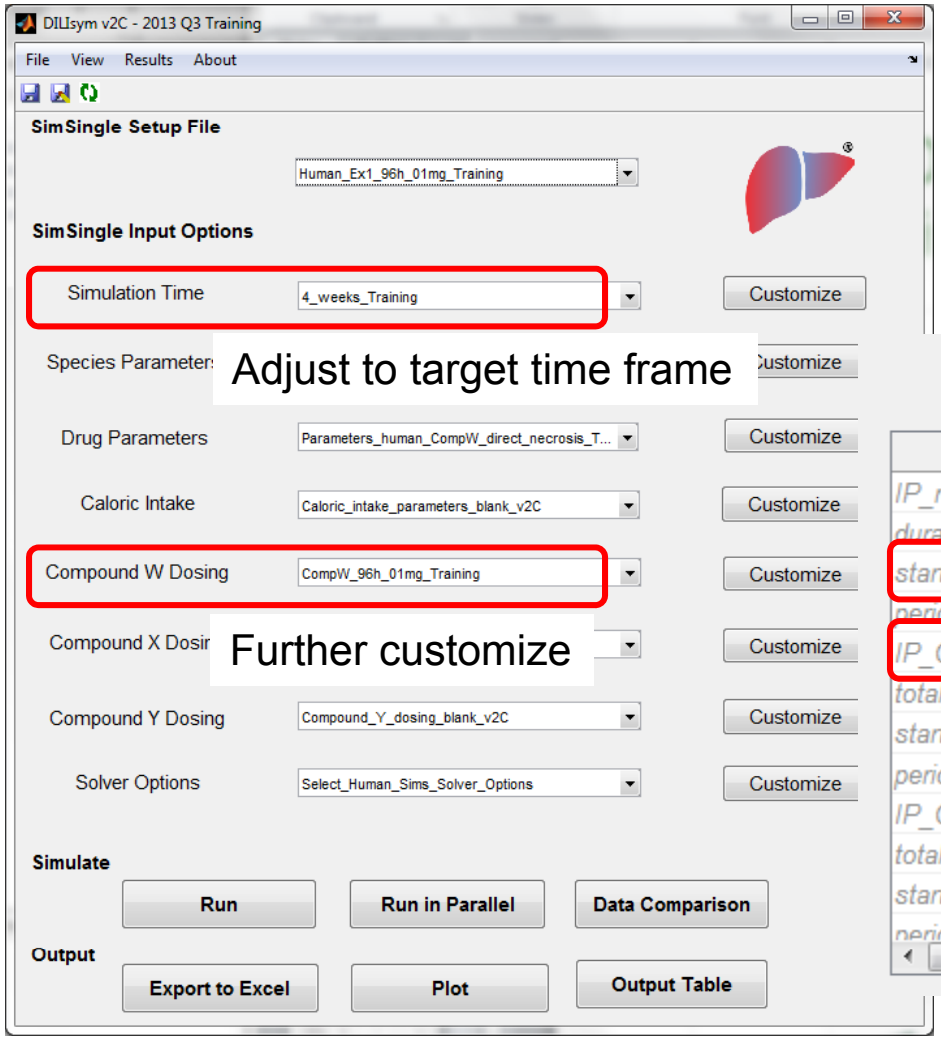




- Lowering injury-inducing "dose" range by ~2 orders of magnitude puts ALT into a range similar to data
- Dynamics of single "dose" are a poor match and suggest delayed start time and multiple "doses" should be evaluated
  - *Note*: multiple "dose" scenario will necessitate further "dose" lowering

Clinical Data and Simulation Results

Create a Derivative SimSingle™ with Closer "Dosing" to Continue Optimization

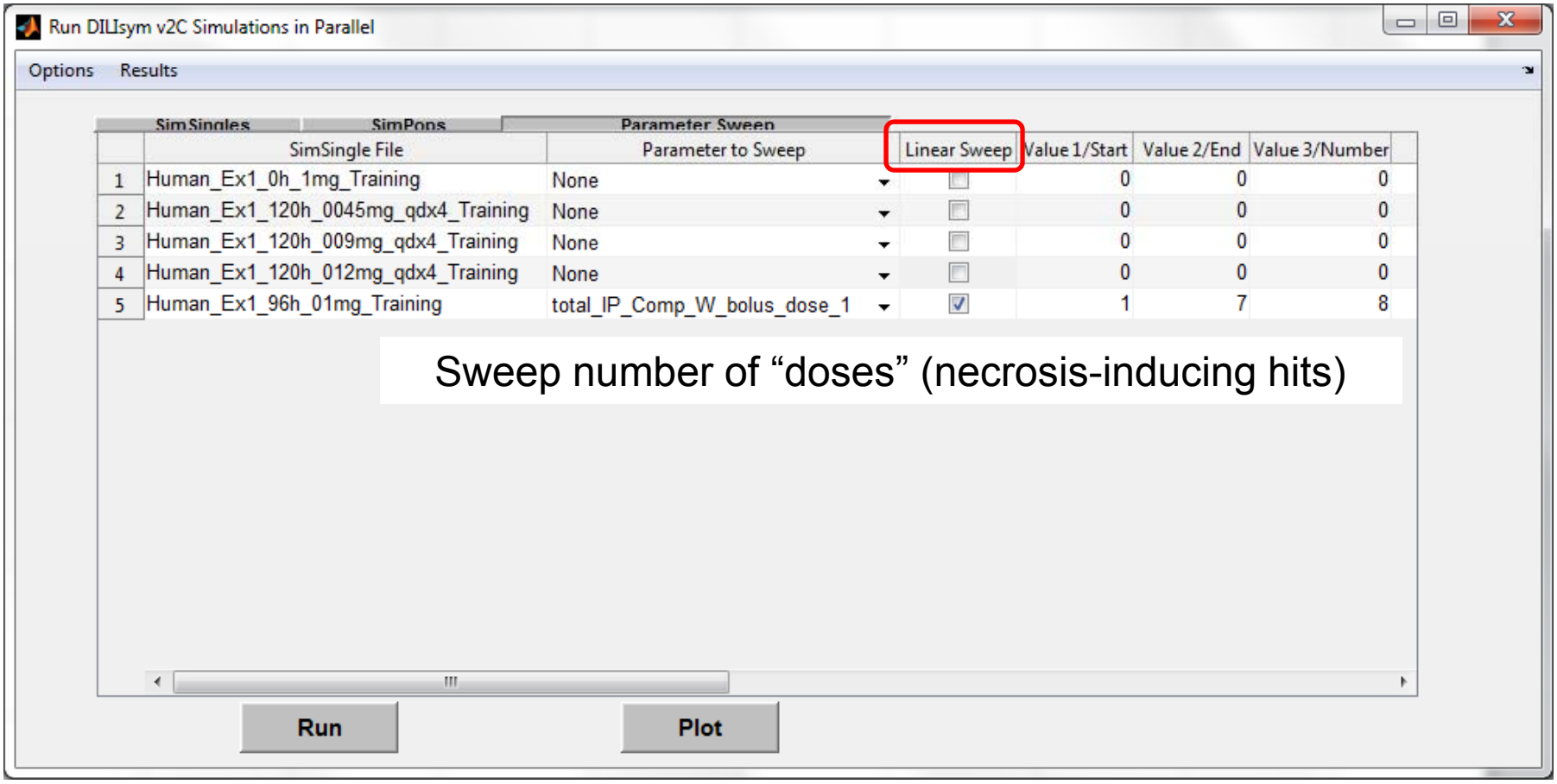


- Delay start time for 1st "dose"
- Lower "dose" to get into the reported ALT range

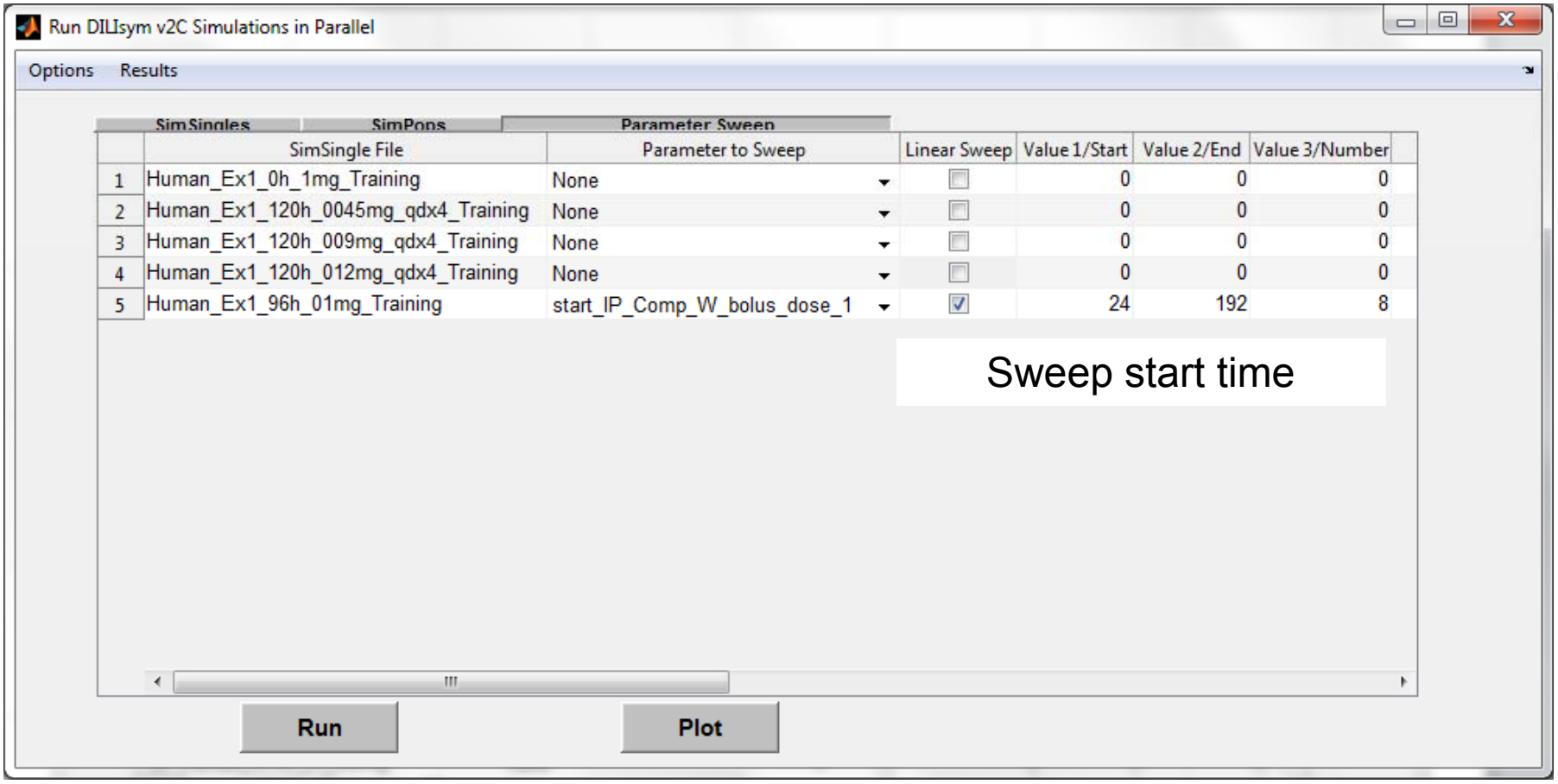
IP Bolus Dosing

Parameter	Value	Units
IP_ratio_gut_Comp_W_IP	0.5700	dimensionless
duration_IP_Comp_W_bolus	0.0500	hours
start_IP_Comp_W_bolus_dose_	96	hours
period_IP_Comp_W_bolus_dose	24	hours
IP_Comp_W_bolus_dose_1	0.0100	mg
total_IP_Comp_W_bolus_dose_	1	dimensionless
start_IP_Comp_W_bolus_dose_	48	hours
period_IP_Comp_W_bolus_dose	24	hours
IP_Comp_W_bolus_dose_2	0	mg
total_IP_Comp_W_bolus_dose_	0	dimensionless
start_IP_Comp_W_bolus_dose_	96	hours
period_IP_Comp_W_bolus_dose	24	hours

Use Linear Sweep to Identify a Frequency Range Better Aligned with Target ALT Profile



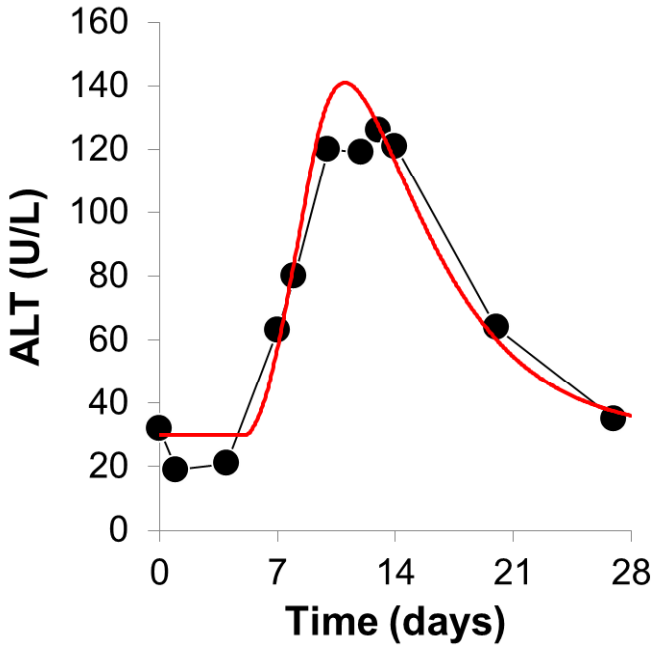
Use Linear Sweep to Identify Better Timing of 1st Dose

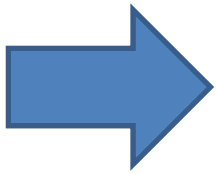


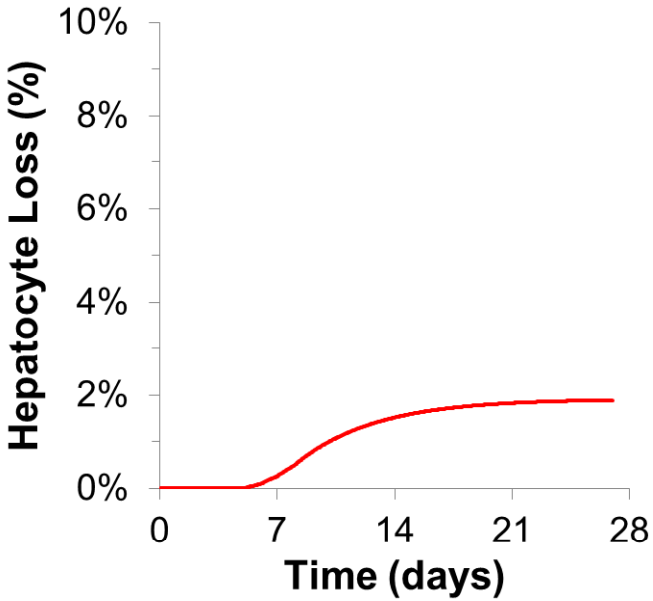
Optimization Provides an ALT Profile Similar to the Clinical Data

Time of 1st dose = 120 h
Dose number = 4
Dose frequency = daily
Dose magnitude = 0.009 mg

Reproducing the observed ALT profile in DILIsym® corresponds to ~2% hepatocyte loss

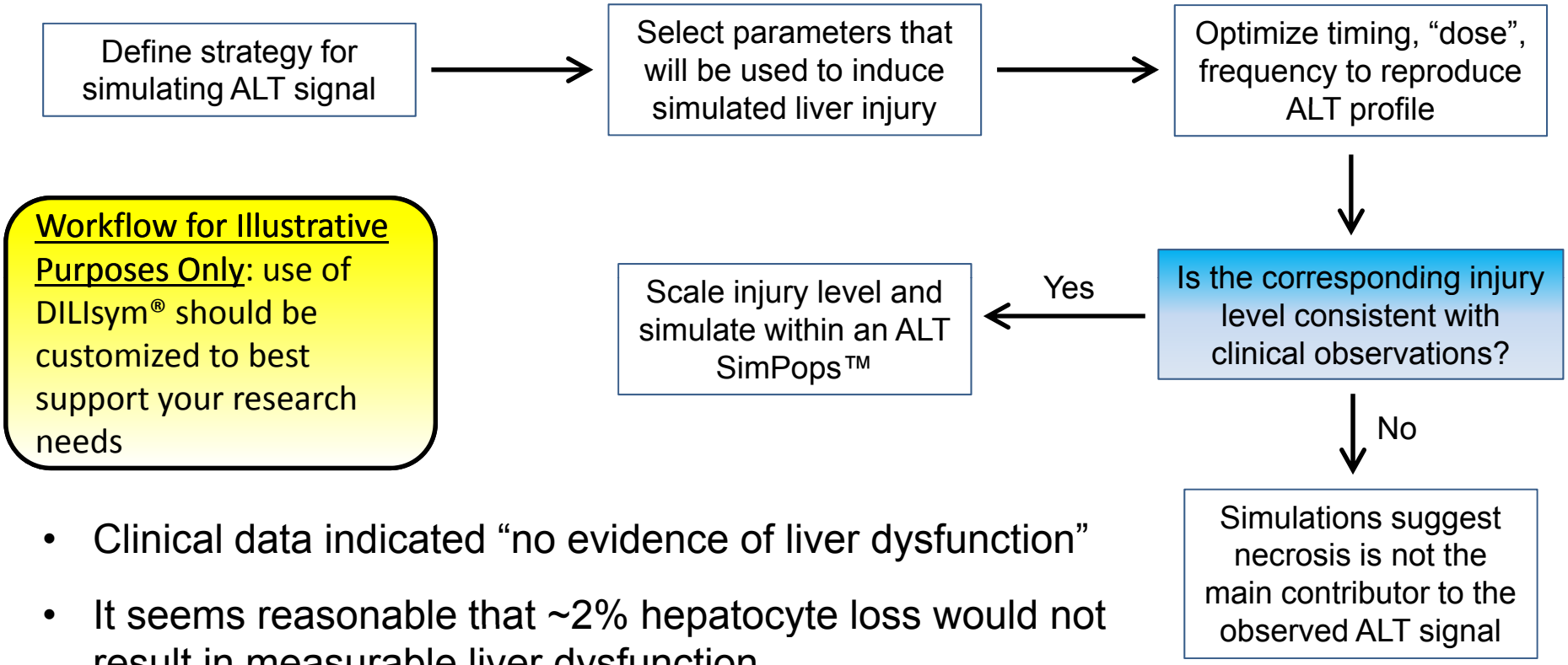






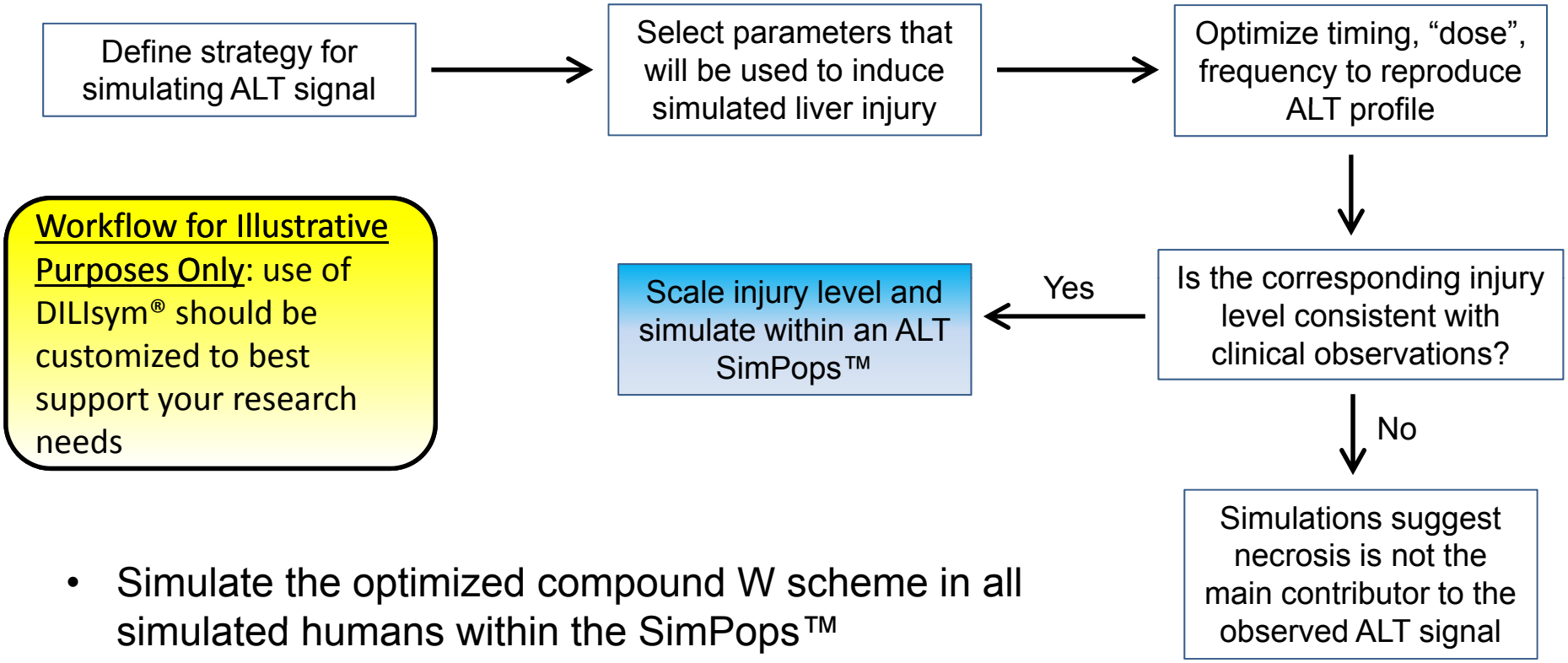
Clinical Data and Simulation Results

Injury Level Associated with Optimized ALT Profile is Evaluated



- Clinical data indicated "no evidence of liver dysfunction"
- It seems reasonable that ~2% hepatocyte loss would not result in measurable liver dysfunction
- Select "yes" direction on flow chart & continue DILIsym® retrospective analysis

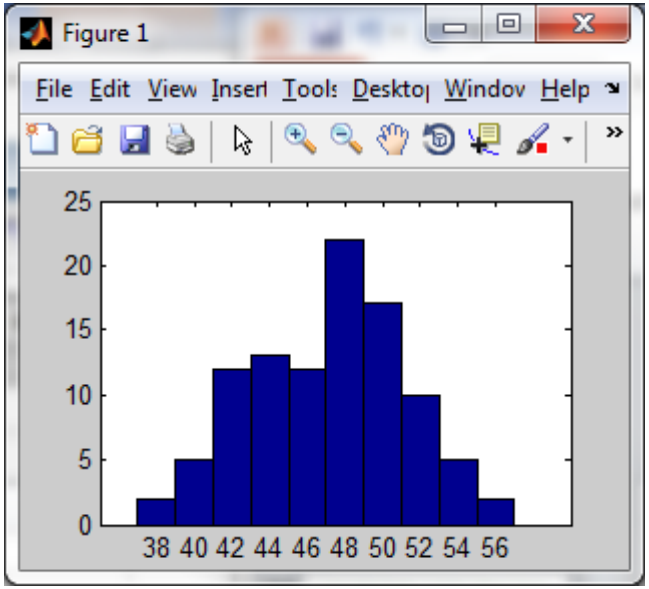
Variability in Predicted Injury Can Be Assessed Using SimPops™



- Simulate the optimized compound W scheme in all simulated humans within the SimPops™

ALT SimPops™ Include Variation in Biomarker Appearance and HC Regeneration

- SimPops™ Variables
  - HC ALT content
  - HC ALT release rate
  - ALT half-life
  - HGF production rate
  - HGF effect on regeneration
- Use normal distribution function to generate parameter combinations (alternate simulated individuals)
  - 1000 for full SimPops™
  - 100 for training SimPops™
- Screen simulated individuals against available data on liver function *vs.* biomarker

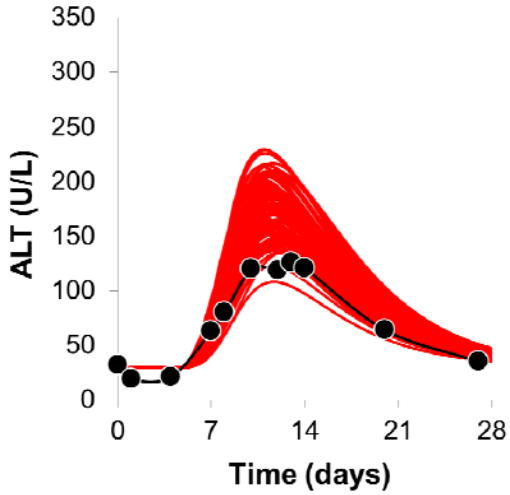


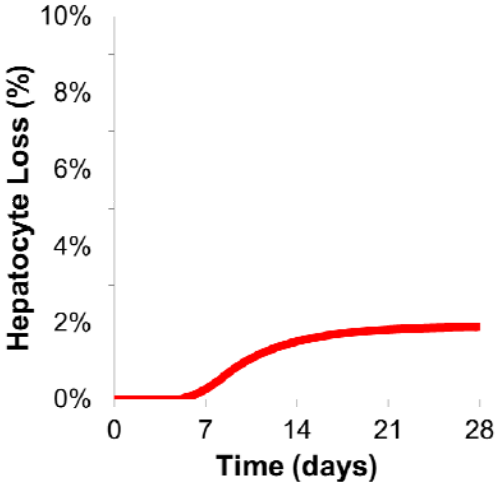
Distribution of ALT half-life across 100 simulated individuals§

§ Plasma ALT half-live of 47 ± 10h. Normal distribution function used to fill in range from 37-57h.

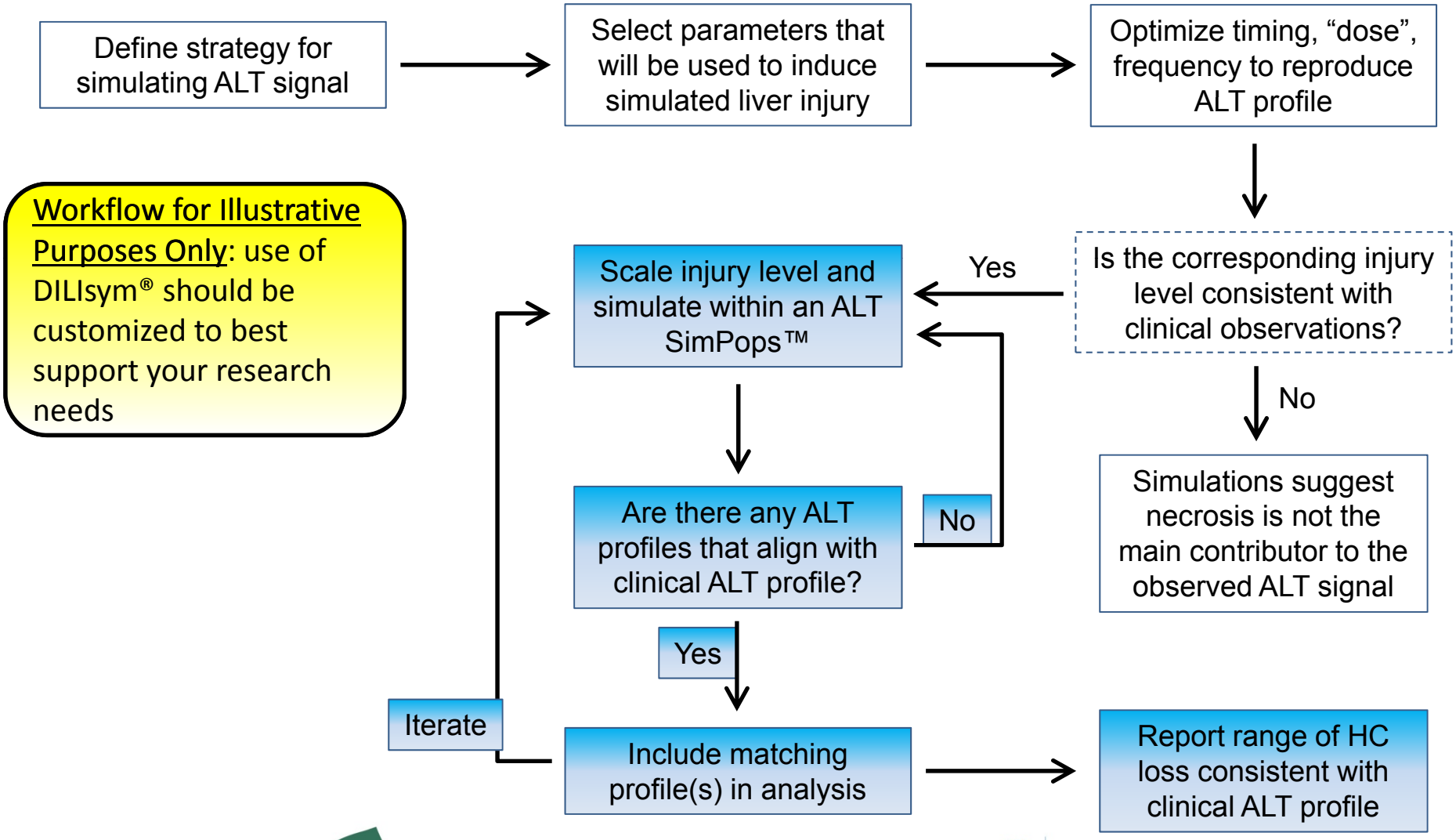
Use the Optimized Injury Profile in Training SimPops™ (n=100) to Guide Scaling

- Optimized injury profile in training SimPops™ shows peak ALT varying from 108-229 U/L
  - Max injury is 2% loss for all these profiles (lower figure)
- Evaluate max and min ALT profiles to guide "dose" scaling
  - Optimized to peak 141 U/L
  - Max 229 U/L, suggests "dose" reduction ~0.5x
  - Min 108 U/L, suggests "dose" escalation ~1.3x



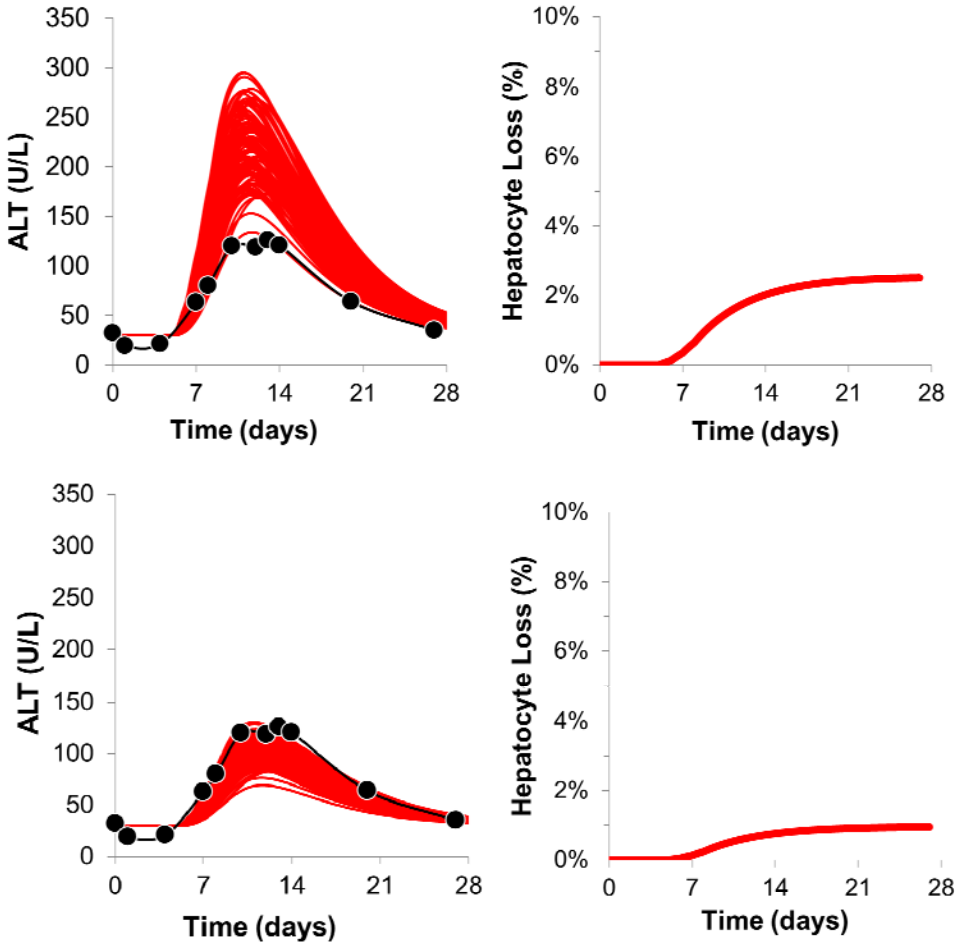


Test Different Injury Levels for Consistency with ALT Data Using SimPops™



Scale Injury to Identify the Limits of Injury that are Still Consistent with ALT Data

- Injury scaled up and down in the SimPops™
  - Injury limits identified by the ability to match the ALT profile within the SimPops™
  - Injury inducing ~3% hepatocyte loss remains consistent with the measured ALT data
  - Injury inducing ~1% hepatocyte loss remains consistent with the measured ALT data
- Analysis suggests the clinical ALT profile is consistent with 1-3% hepatocyte loss



Retrospective Analysis of Observed Liver Safety Signals

Issue

- ALT (and AST) elevations were reported in a single (few) individuals from three early clinical trials
- No indications of liver dysfunction were observed in the early trials
- No mechanistic data for hepatotoxicity have been identified

Pending Decision

- Does the Company continue to advance this program?
  - Assume multiple inputs and data sets, potentially including modeling and simulation

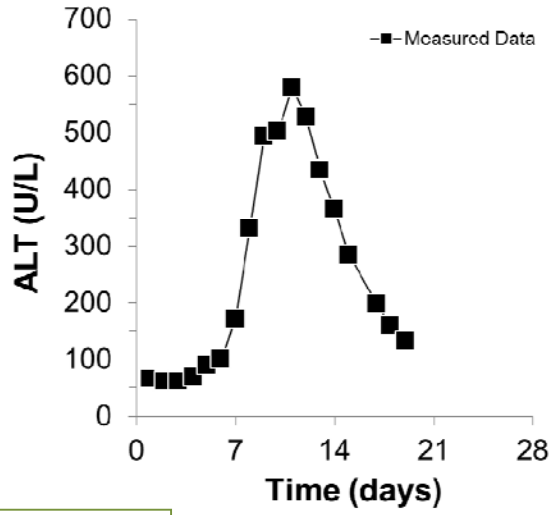
Questions to Individual(s) Responsible for Liver Safety Assessment

- Can DILIsym® be used to retrospectively interpret the observed ALT elevations?
  - What level of injury might be inferred from the reported ALT profile?
  - How much uncertainty is associated with the estimated level of liver injury?
  - What time frame of recovery would be expected for the simulated injury?

Compare & Contrast Two ALT Profiles from Compounds Intended for Different Indications

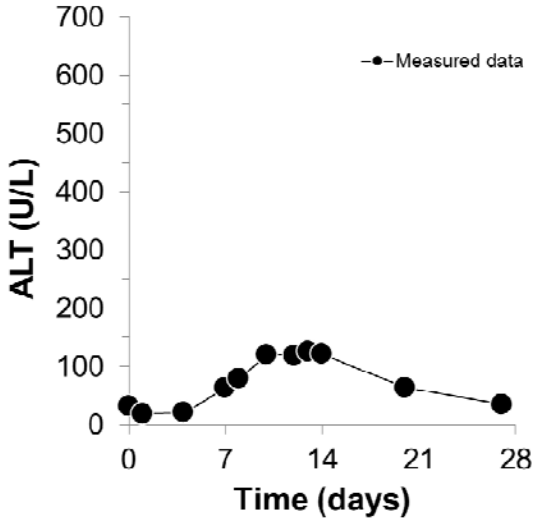
Compound 1 – Indication A

- ALT measurements shown for a single NHV
- Increase first noted at d5
- Increase >3x ULN by d8
- Max ALT ~ 600 U/L



Compound 2 – Indication B

- ALT measurements shown for a single NHV
- Increase first noted at d7
- Increase >3x ULN by d10
- Max ALT ~ 125 U/L



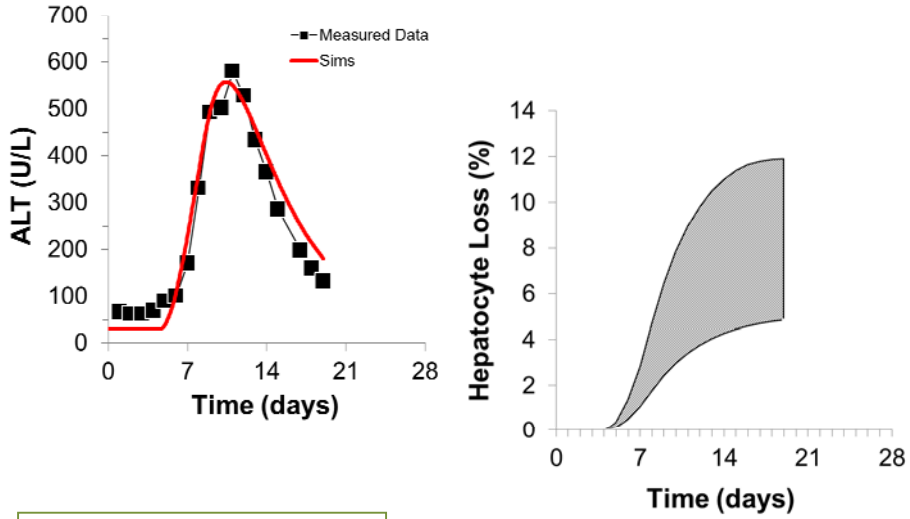
Reproducing ALT Curves Provides Estimates for Associated Hepatocyte Necrosis

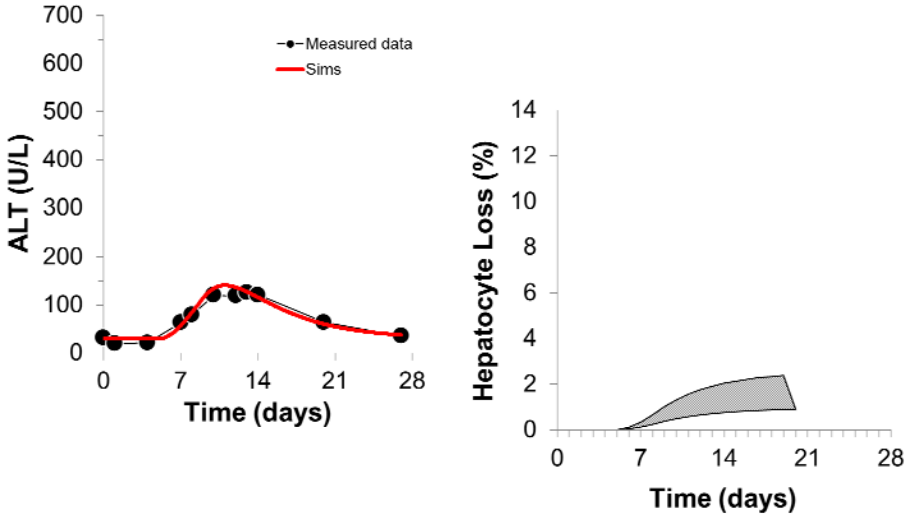
Compound 1 – Indication A

- ALT match in baseline simulated person
- Varying injury level & matching ALT profile in SimPops™ yields an estimated 4-12% range of hepatocyte loss

Compound 2 – Indication B

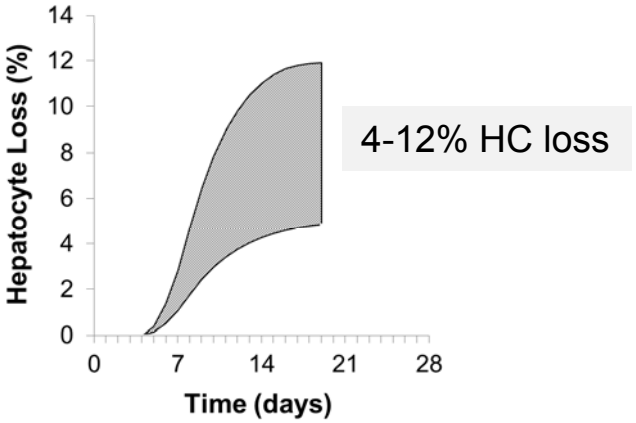
- ALT match in baseline simulated person
- Varying injury level & matching ALT profile in SimPops™ yields an estimated 1-3% range of hepatocyte loss



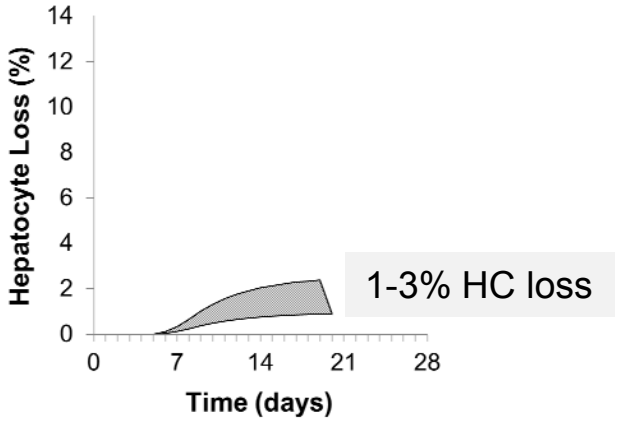


Estimated Hepatocyte Necrosis in the Context of Disease Indication

Compound 1 – Diabetes



Compound 2 – Parkinson's



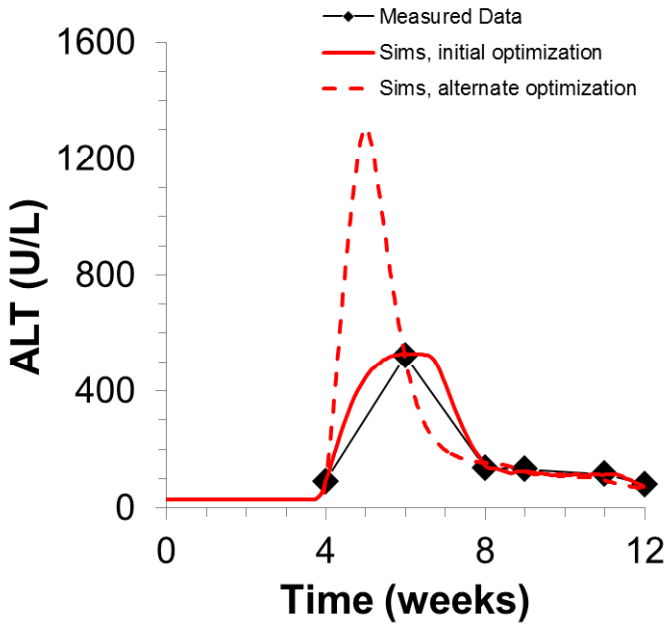
- Estimated range for hepatocyte loss may directly figure into the risk assessment
- Speed of recovery (simulation results not shown) may directly figure into the risk assessment
- Hepatocyte loss may be considered in the context of the intended indication (e.g., disease morbidity and mortality, availability and efficacy of currently approved drugs; market size)
  - Would Compound 1 estimated HC loss be considered too risky for a diabetes drug?
  - Would Compound 2 estimated HC loss be considered acceptable for a Parkinson's drug?
  - Interpretation is user and company specific – open for general discussion

Evidence from Literature to Support Interpretation of Hepatocyte Loss Simulations

- Excision of 20% of liver volume in living donors is generally considered safe (Florman 2006)
  - Living donors routinely recover fully after even greater portions (40-60%) of liver are excised for adult-to-adult donations (Florman 2006, Lee 2010)
- Heparins are widely considered to be safe despite associated increases in ALT
  - Reported ALT increases after heparins were moderate (>700 U/L peak, 1-2 week time frame)
  - DILIsym® modeling team performed comparable ALT-hepatocyte loss on published clinical data (Harrill 2012)
  - Maximal hepatocyte loss predicted for heparins of around 5% of viable hepatocytes
- Clinical correlative data from literature indicate that minimal loss of hepatocytes due to injury has little to no effect on bilirubin levels and prothrombin clotting time (Portmann 1975)

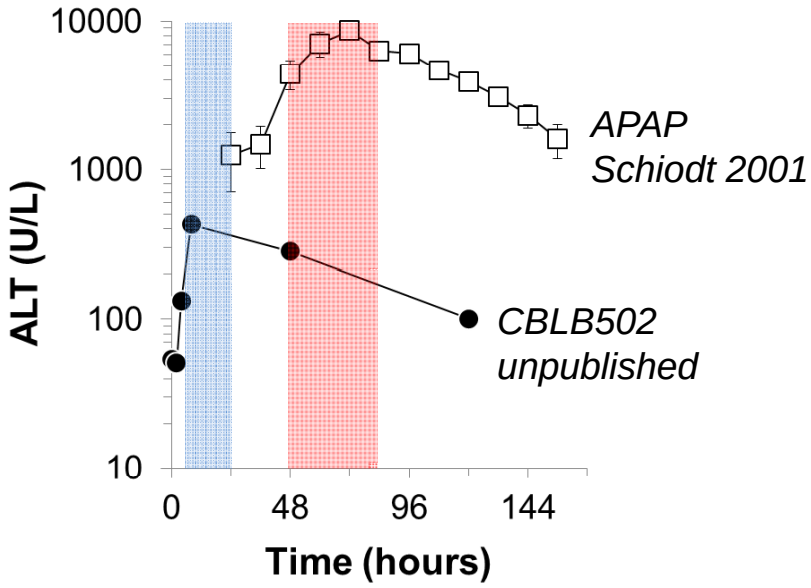
Additional Insights: Fitting Long-Term ALT Elevations Reveals Critical Uncertainties

- Long time courses (weeks to months) often include infrequent sampling
  - 1-2 week sampling intervals allow for missing the true peak
- Simulations can still provide an estimate of liver injury but with room for alternate solutions
  - Note that this can require a necrotic “event” to last for weeks to months
- Additional sources of uncertainty can have greater impact with long-term ALT elevation
  - ALT clearance rates
  - Mechanisms of injury
  - Adaptation
  - Regeneration



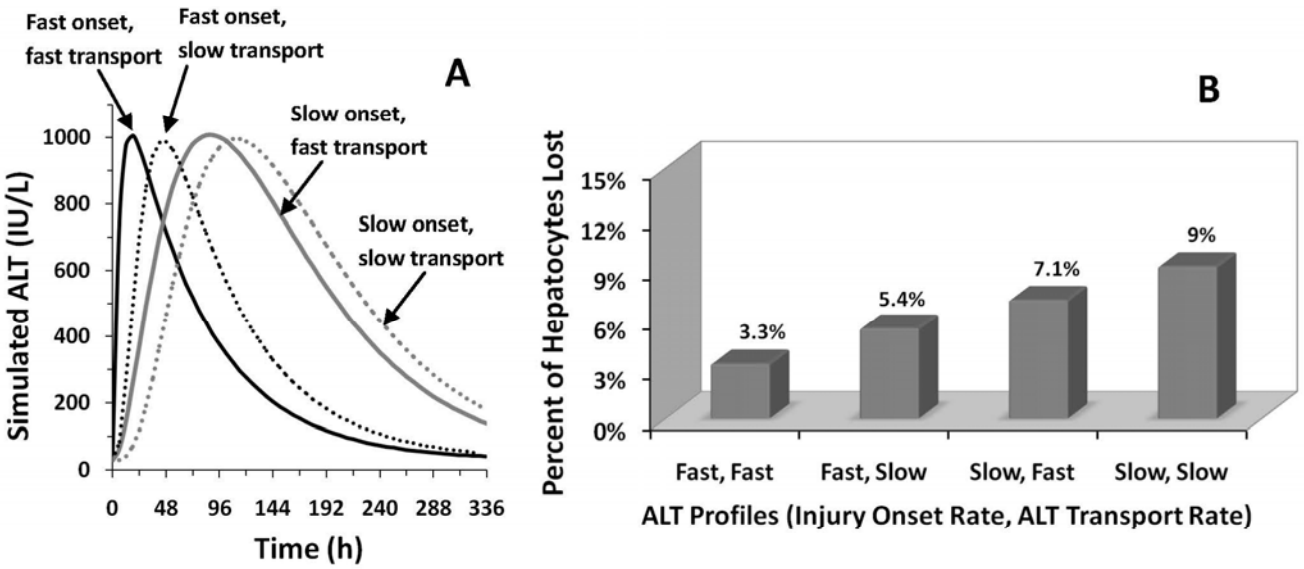
Additional Insights: CBLB502 Data Highlighted Additional Variables Affecting Measured ALT

- CBLB502 induced ALT elevations much more rapidly that the more prototypical APAP profile
  - CBLB502 peak 8-24h (shaded blue)
  - APAP peak 48-84h (shaded red)
- Dramatically different dynamics highlighted additional areas of potential variability
  - Speed of injury onset
  - Speed of ALT release from necrotic cells
- 4 possible combinations identified



	Injury onset rate	ALT transport rate
Injury onset rate	Fast, fast	Fast, slow
ALT transport rate	Slow, fast	Slow, slow

Simulating the Combinations Demonstrates the Most Conservative Assumption



- "Dose" adjusted such that all combinations achieve similar peak ALT levels
- Corresponding hepatocyte loss illustrates that slow injury onset and slow ALT release from necrotic hepatocytes is associated with the greatest level of necrosis, i.e., most conservative
- The examples described thus far have used the slow, slow parameter settings (i.e., most conservative) in the optimization of ALT profiles
  - Re-optimizing to fast, fast can be conducted and is expected to provide lower estimates of hepatocyte loss

Assumptions and Limitations

- Simulations are based on induced hepatocyte necrosis (i.e., apoptosis and extra-hepatic ALT release are not accounted for)
- Optimization examples assume the experimental data illustrate the shape of the ALT curve
  - Wide sampling intervals can potentially miss the “true” ALT peak
  - More frequent sampling increases confidence that the optimized ALT profile accurately reflects the human experience
  - Prolonged ALT elevation (weeks to months) may not be simultaneously compatible with hepatocyte necrosis and absence of clinical signs
- Alternate optimization solutions that result in the same ALT profile will give the same level of HC loss
  - Solutions resulting in a different ALT peak or AUC are expected to alter the estimated level of injury
- Optimization examples assume slow onset of injury and slow ALT release, leading to conservative estimates of hepatocyte loss
  - Speeding up either parameter and re-optimizing to the ALT data is expected to result in less simulated hepatocyte injury
- Larger SimPops™ will provide a more complete distribution of injury *vs*. ALT

Retrospective Analysis of Observed Liver Safety Signals - Summary

Participants should understand the following general concepts:

- Use of DILIsym® for the retrospective interpretation of liver injury associated with clinical ALT signals
- Parameter selection for the non-mechanistic representation of hepatocyte necrosis
- Set-up, simulation, and visualization for parameter sweeps
- Use of SimPops™ to identify a range of injury consistent with a particular ALT profile
- Key uncertainties associated with large time interval sampling
- Impact of speed of injury onset and ALT release on estimated hepatocyte injury

And for compound comparisons:

- Simulations suggest the ranges of liver injury associated with ALT signals from compound 1 and compound 2 were both less than 15%
- While no clinical measures of liver dysfunction (e.g., bilirubin) were observed, the estimated level of injury could inform the compound safety assessment

DILIsym® Training Agenda – September 26, 2013

- 8:30 AM – Introduction and goals
  - DILIsym® overview and highlights
  - Model architecture notes
- 8:45 AM – Biomarker analysis example
- 9:45 AM – Break
- 10:00 AM – Biomarker analysis example
- 11:00 AM – MITOsym™ overview and introduction
- 11:30 AM – Lunch
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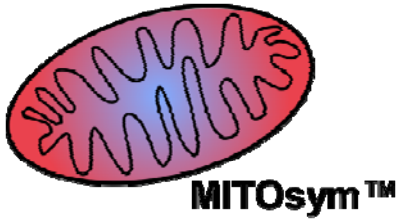
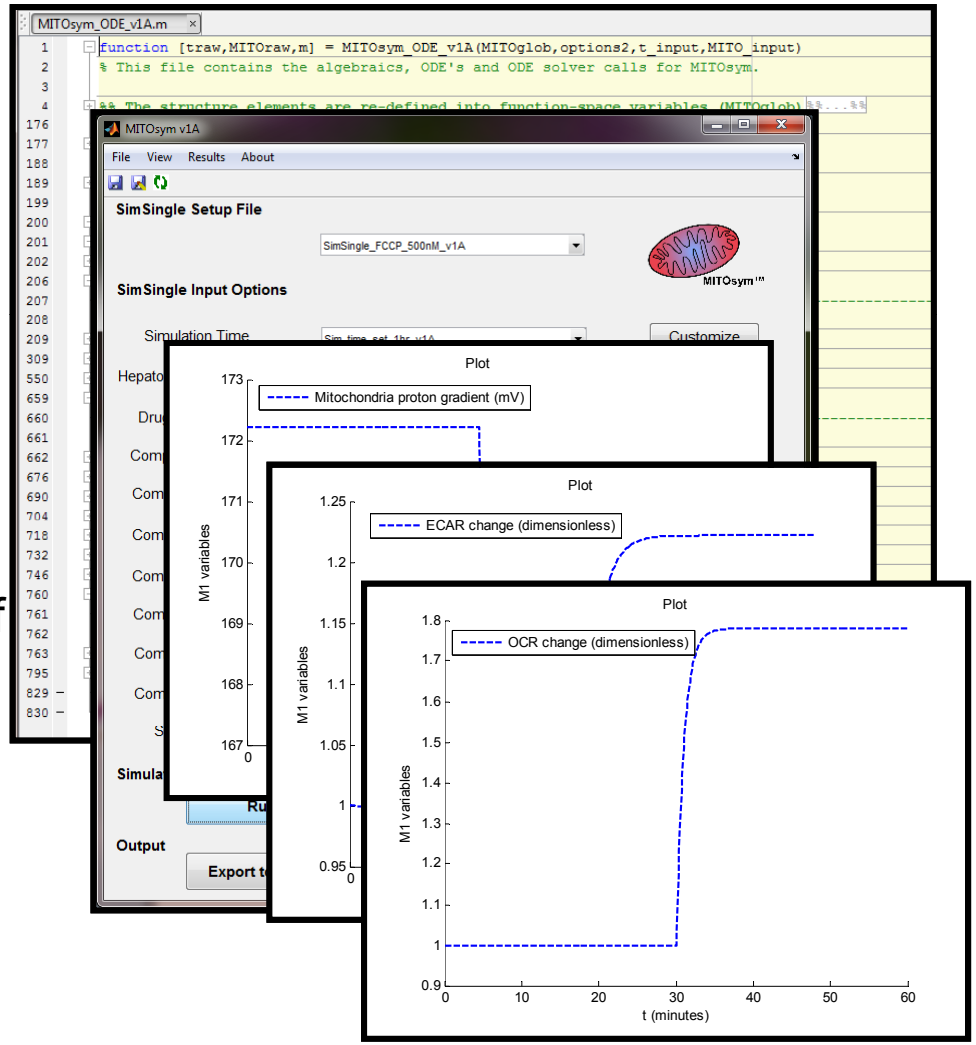
MITOsym™ Training Agenda



- Introduction
- Components of MITOsym™ model
- Optimization of MITOsym™ model
- Tolcapone as example of translation of *in vitro* data to predictions of *in vivo* toxicity

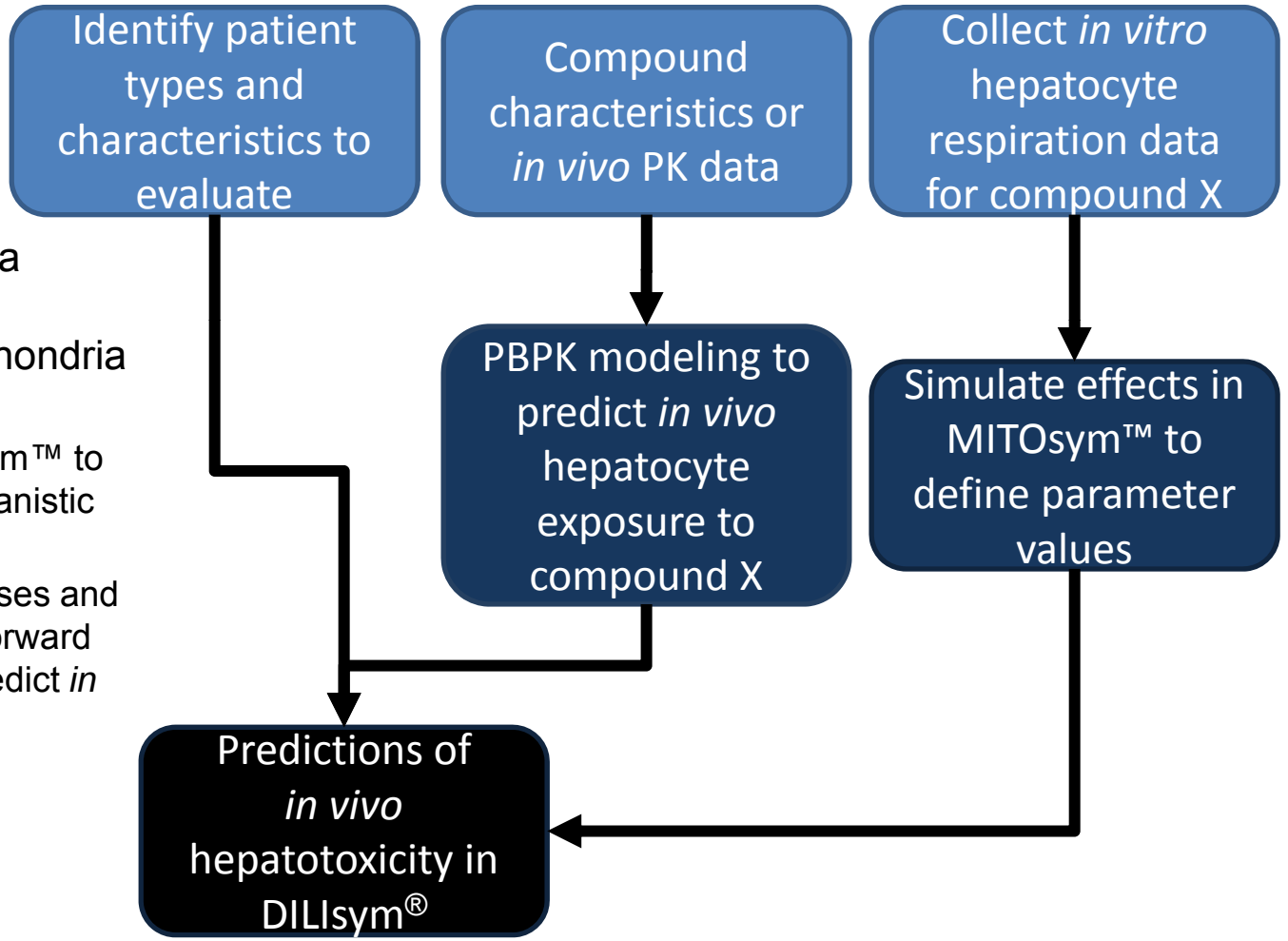
MITOsym™ Is Designed to Support IVIVE DILI Predictions and Mechanistic Data Interpretation

- MITOsym™ is a standalone model of hepatocyte bioenergetics
- MITOsym™ can be used to facilitate predictions of hepatotoxicity based on *in vitro* cellular respiration data
  - Combine with DILIsym® model
- MITOsym™ can be used to develop and explore hypotheses of the mechanisms underlying observed changes in respiration and glycolysis in hepatocytes



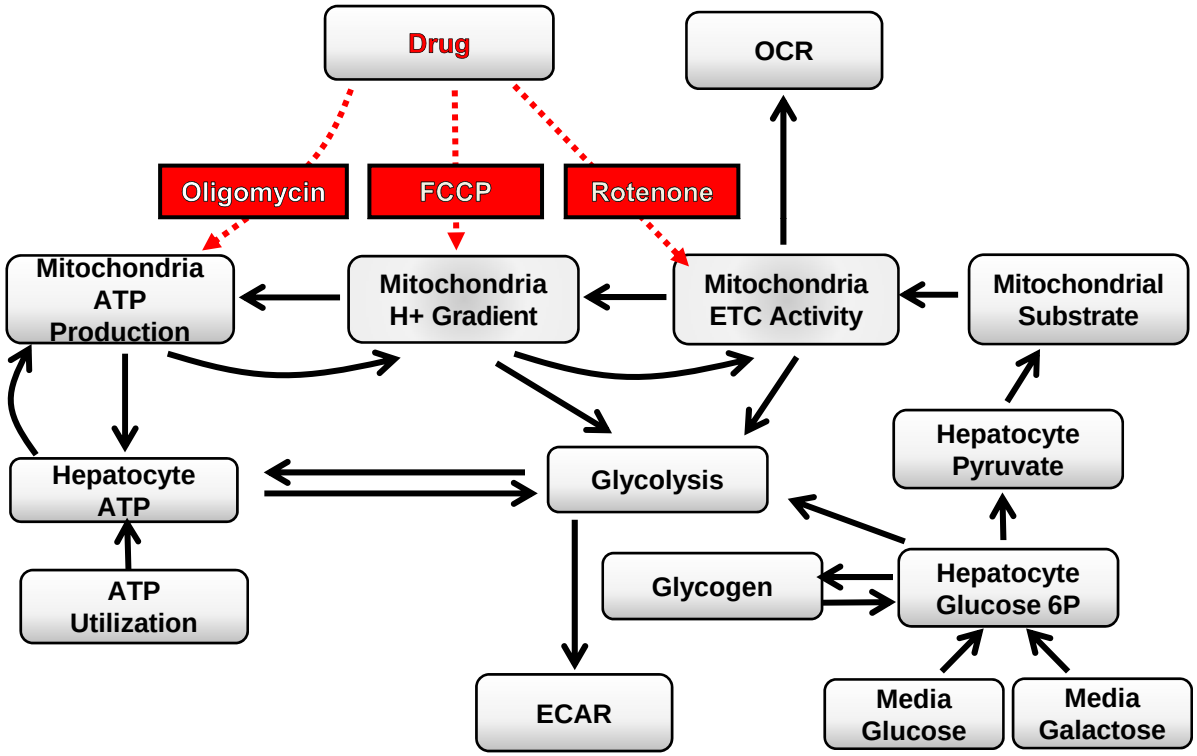
Workflow for Predicting *in vivo* Risk Based on *in vitro* Mitochondria Function Data

- *in vitro* respiration data provides insight into mechanisms of mitochondria disruption
  - Simulate in MITOsym™ to establish/test mechanistic hypotheses
  - Carry valid hypotheses and parameter values forward into DILIsym® to predict *in vivo* hepatotoxicity



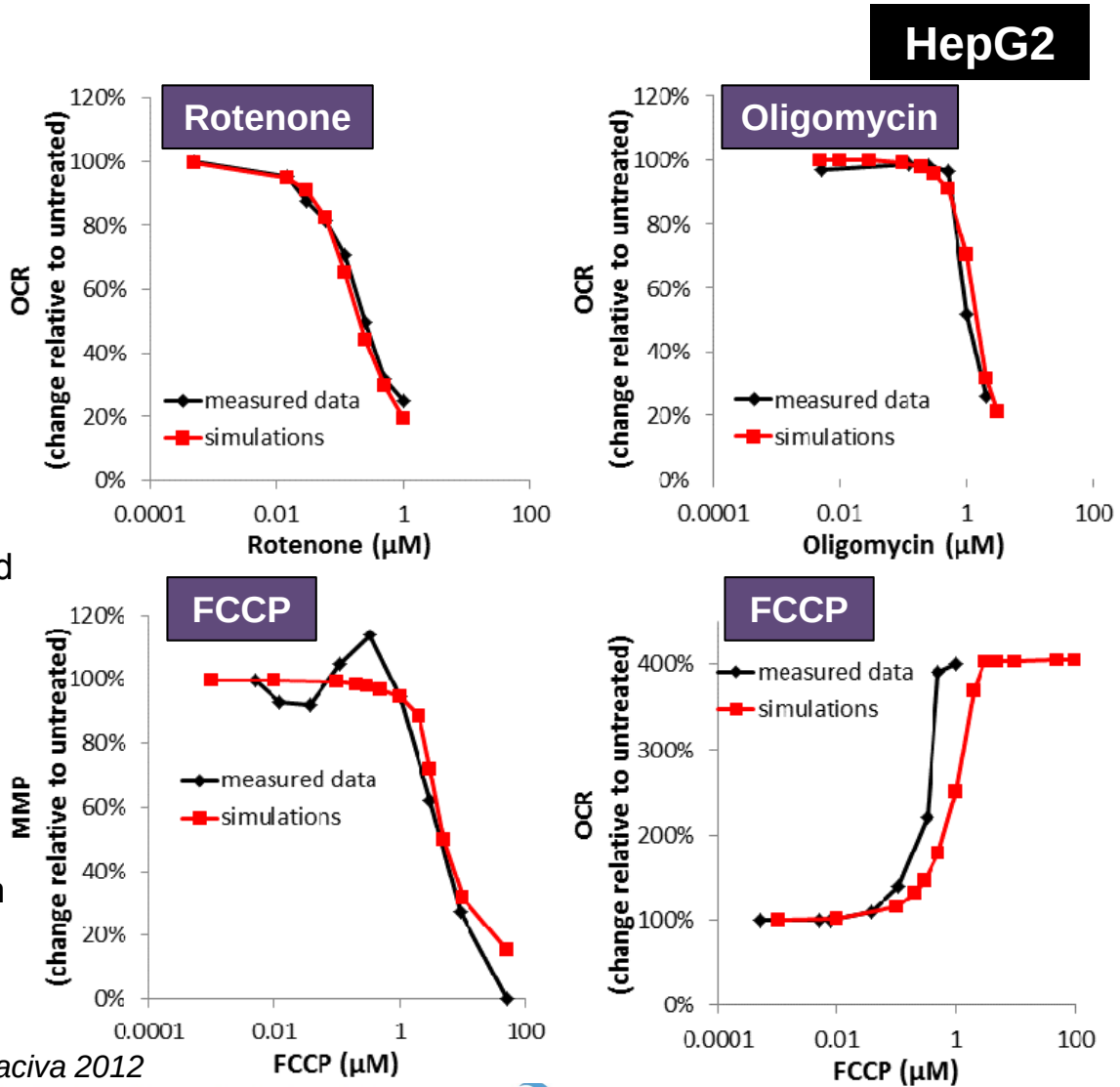
MITOsym™ Model Includes Essential Components of Hepatocyte Bioenergetics

- Includes mitochondria ETC activity, proton gradient and ATP production
  - Also includes glucose uptake, glycolysis, and ATP utilization
- Includes respiration (OCR) as a primary model output
  - Also includes ATP, $\Delta\Psi m$, ECAR
- Includes effects of exemplar drugs
- Includes adaptive, compensatory glycolysis increases with declining mitochondria function
  - Provides ATP unless galactose is primary media substrate



OCR Simulations Optimized to Align with Response to Individual Mitochondrial Effectors

- Relative changes to oxygen consumption rate (OCR)
  - Dose response vs. treatment with rotenone, oligomycin, and FCCP
  - Simulation results comparable to measured data from Nadanaciva 2012
- Reduction in mitochondria membrane potential (MMP) with increasing doses of FCCP
  - Also simulating changes in OCR and MMP with time
- Simulation results provide confidence that ETC dynamics are captured
  - Decreased respiration when ATP synthase is inhibited with oligomycin
  - Increased respiration when H^+ gradient is reduced with FCCP

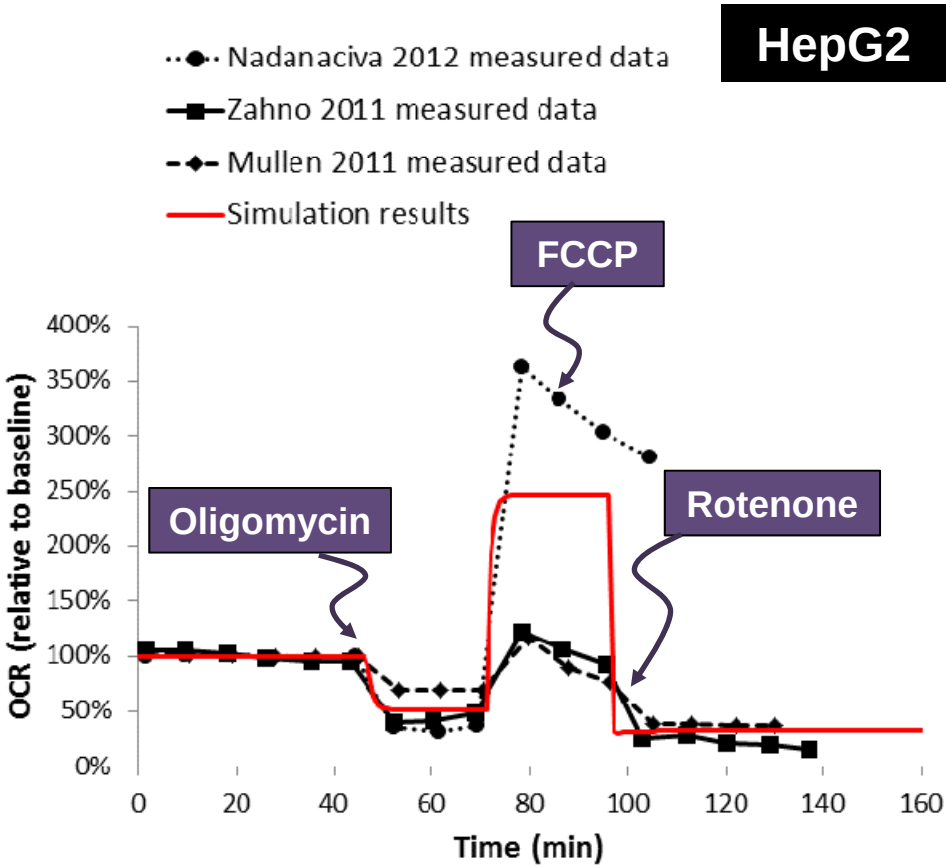


Nadanaciva 2012

Preclinical Data and Simulation Results

OCR Simulations Optimized to Align with Response to Multiple Mitochondrial Effectors

- Oxygen consumption rate (OCR) predicted to change as reported by Mullen 2011, Zahno 2011, and Nadanaciva 2012
  - Classic mitochondria disruptors used to characterize mitochondria function
  - 1 µM oligomycin, 1 µM FCCP, 1 µM rotenone added sequentially
- Simulation results provide confidence that integrated dynamics are appropriately represented
  - Decreased maximum in respiration when FCCP follows oligomycin
  - Fully suppressed respiration when rotenone follows FCCP

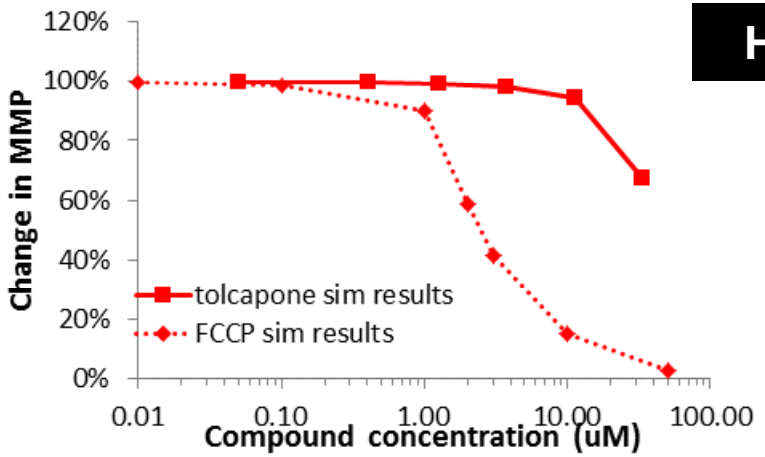


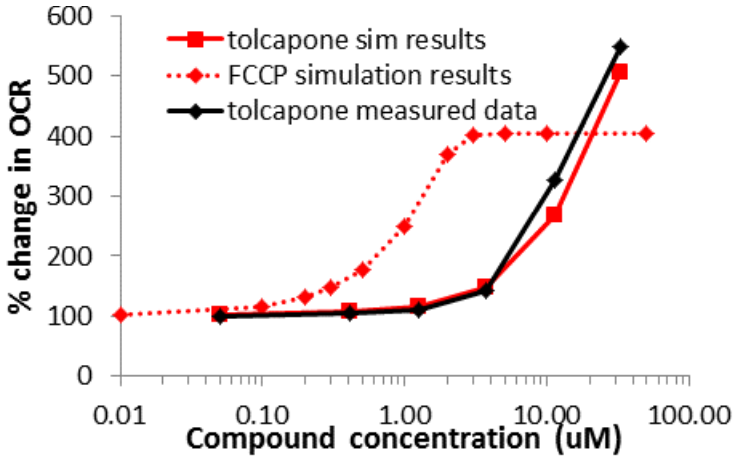
Mullen 2011, Zahno 2011, Nadanaciva 2012

in vitro Respiration Data Used to Determine Tolcapone Mitochondria Uncoupler Parameter Values with MITOsym™

- Used MITOsym™ model to simulate OCR, ECAR, and MMP response to tolcapone
  - Confirmed mechanism is uncoupling
  - Used FCCP measured data and simulations to infer tolcapone parameter values
  - Good agreement with measured OCR data (by design)
- MitoK_UC1_Km parameter value is 10x greater for tolcapone than FCCP
  - MitoK_UC1_Vmax and MitoK_UC1_Hill parameter values unchanged

HepG2





Nadanaciva 2012

DILIsym® and MITOsym™ Have Minor Differences in Mito Drug Parameter Values

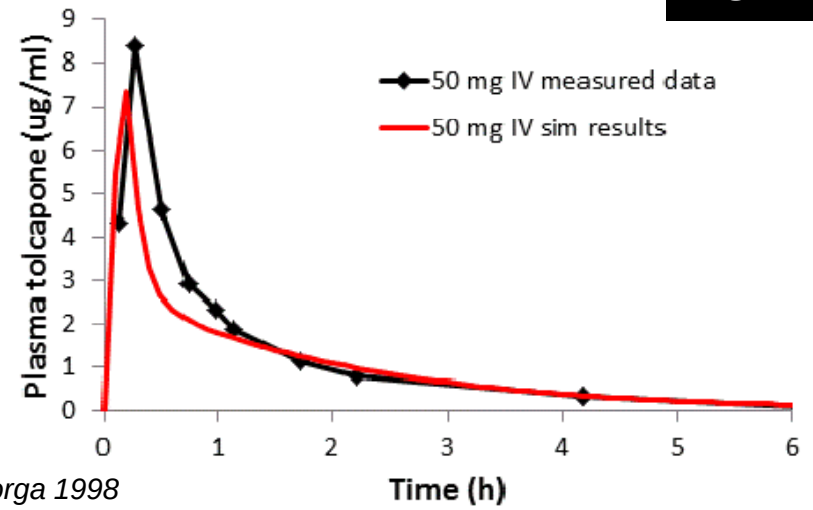
	MITOsym™				DILIsym®			
Parameter	**Rote-none**	**Oligo-mycin**	**FCCP**	**Tolca-pone**	**Rote-none**	**Oligo-mycin**	**FCCP**	**Tolca-pone**
MitoS_ETC_Inhib_1 (mM)	1.2e-05				1.2e-04			
MitoS_ATP_Inhib_1 (mM)		1.0e-03				1.0e-03		
MitoK_UC1_Vmax (unitless)			40	40			190	190
MitoK_UC1_Km (mM)			2.0e-02	2.0e-01			2.0e-03	2.0e-02
MitoK_UC1_Hill (unitless)			1	1			1	1

- Parameter values relative to the mitochondria exemplar drugs in MITOsym™ are what should be used in DILIsym®
  - e.g., MitoK_UC1_Km for tolcapone
- Minor differences between MITOsym™ and mitochondria sub-model of DILIsym®
  - Account for differences in mitochondria drug-related parameters

Good Agreement for PBPK Modeling of Tolcapone

- Used Compound Y PBPK structure in DILIsym®
  - Simpler than Compounds W and X
  - No explicit hepatic metabolism of parent compound with this paradigm
- Used data from series of tolcapone PK studies (Jorga 1998)
  - Used for parameter values and optimization
- Reasonable agreement between simulation results and measured data
  - Maintained 5-15% liver to plasma tolcapone ratio

HUMANS



Jorga 1998

Parameter	*Healthy subjects*
$t_{½}\beta$ (hr)	1.1 ± 0.2
AUC(0→∞) (hr · μg · ml^{-1})	6.2 ± 0.9
V_{ss} (L)	8.8 ± 0.7
CL (L/hr)	8.3 ± 1.3
Percentage of dose excreted in urine	0.22 ± 0.15
CL_R (L/hr)	0.02 ± 0.01
Unbound fraction (%)	0.12 ± 0.01
Albumin (gm/L)	40.8 ± 1.6
$V_{ss,unbound}$ (L)	7371.2 ± 596.7 (CI, 6872.3–7870.1)
$CL_{unbound}$ (L/hr)	6874.9 ± 1015.7 (CI, 6025.6–7724.2)

Parameters Varied in SimPops™ Used for Tolcapone Simulations

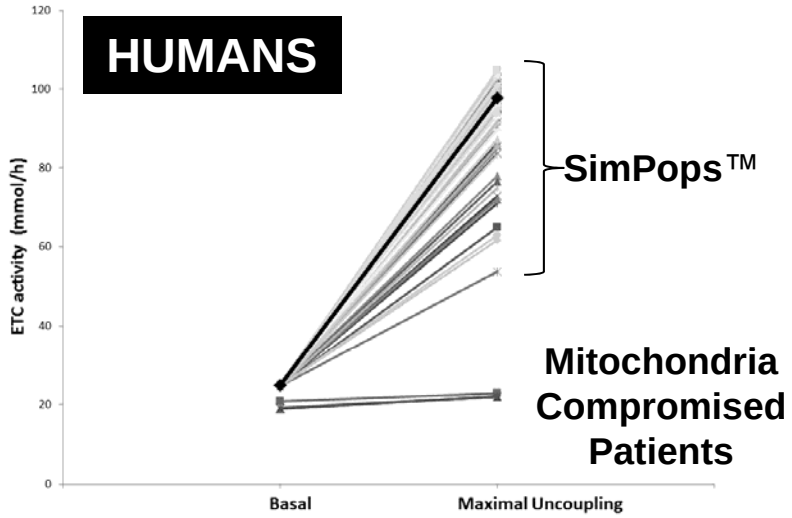
HUMANS

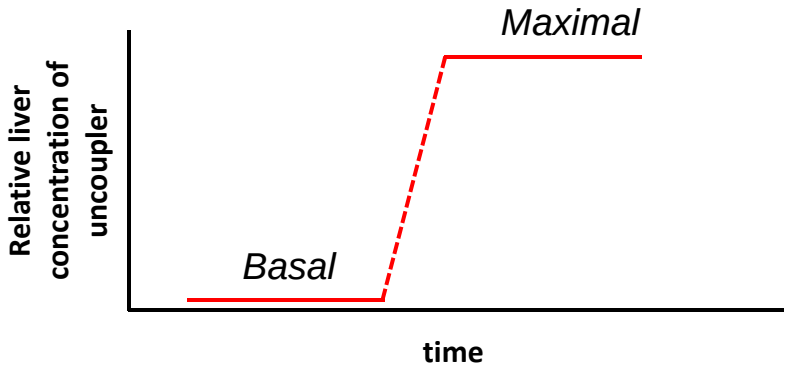
Parameter	Pathway	Baseline	Minimum	Maximum	Mitochondria compromised patients
Basal_Stdzd_MitoETC_Flux	ETC flux	25	19.26	30.77	5-25
Resp_Reserve_Scalar	Respiratory reserve	1	0.39	1.53	0.3-1.0
Basal_Glycogen_Conc	Glycogen	284	250.54	316.94	284
ATP_dec_necrosis_Vmax	ATP-dependent necrosis	0.370	0.19	0.55	0.370
Body_mass	Drug distribution	70	50.05	108.07	70
HGF_regen_Vmax	Hepatocyte regeneration	1.650	0.84	2.43	1.650

- Used SimPops™ 'Human_mito_v2B_1_exploration' in DILIsym®
  - Parameters varied based on ranges extracted from literature
  - Used a Gaussian distribution pattern, n=176
  - Currently, there aren't population response data with which to validate
- Mitochondria compromised patients included changes to mitochondria-related parameters exclusively
  - Substantial reduction in basal and adaptive ETC flux

SimPops™ Mito Characteristics Variability Described by Simulated Max Uncoupler Protocol

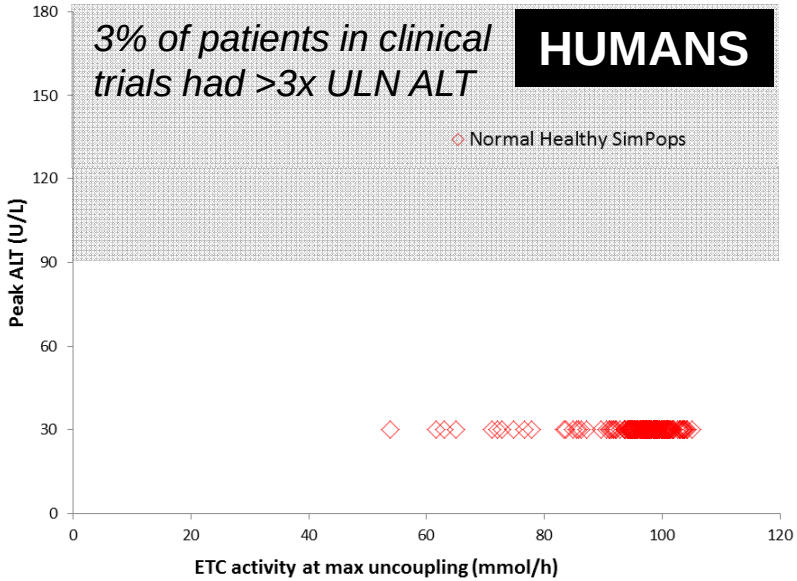
- Used simulation protocol to understand how collection of parametric changes to individual simulated patients within SimPops™ affected overall system
  - SimPops™ based on control patient mitochondria function variability in Perez-Carrera 2003
  - Mitochondria compromised patients based on NASH patient variability in Perez-Carrera 2003
- Simulated administration of a potent uncoupling agent (i.e., FCCP) to each simulated patient
  - Determined predicted ETC activity at basal and maximal response





No Injury Predicted in Normal Healthy SimPops™ with Tolcapone Treatment

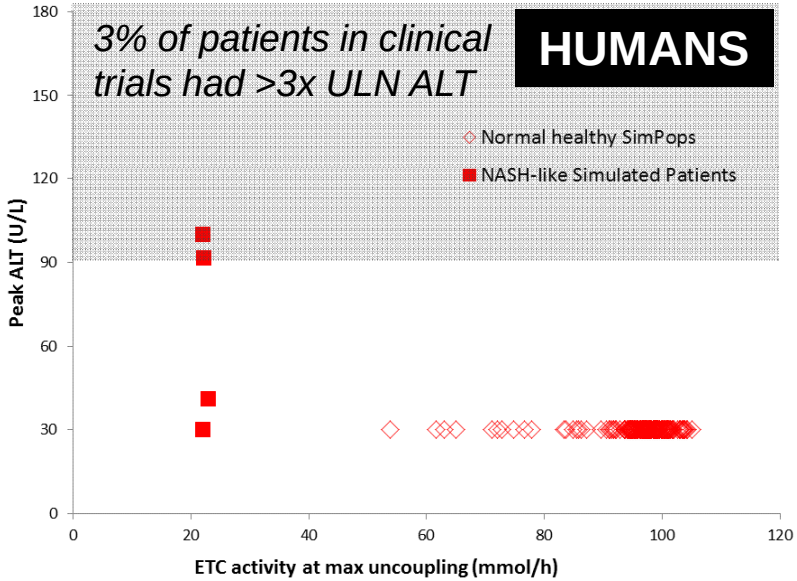
- Simulated 200 mg t.i.d. tolcapone treatment in NHV mitochondria SimPops™
- No liver injury was predicted in any of the simulated patients
  - 97% of clinical patients treated with tolcapone did not have increased ALT or AST
- ATP loss due to tolcapone uncoupling is prevented due to adaptive increase in ETC flux
- What are characteristics of patients who are sensitive to tolcapone-induced liver injury?

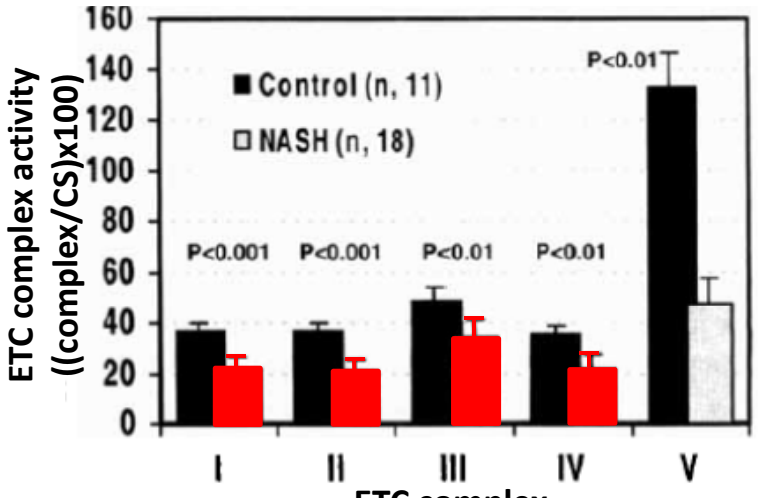


Variable	Pre-treatment	Post-treatment
ALT (U/L)	30±0	30±0
ATP decrease (%)	0.1±0.1	0.5±0.1
ETC flux increase (%)	0.7±0.2	**18±2**
simulated patients with liver signals (#)	0	0

Liver Injury Predicted when Including Patients with Severely Compromised Mito Function

- Generated simulated patients with substantial reductions in mitochondria function
  - Within observed range for NASH patients[1]
  - ETC activity at max uncoupling substantially lower in these simulated patients
- ALT increases predicted for several of the mitochondria-compromised simulated patients
  - More sensitive to effects of uncoupler
  - Unable to increase ETC flux to adequately compensate for uncoupling effect
- NASH incidence estimated to be 3-5%[2]
  - NAFLD incidence estimated to be 20%[3]
- Hypothesis: NASH-type mitochondria function patients were included in tolcapone clinical trials
  - Ability to screen and exclude these patients from treatment could reduce incidence of DILI





[1]Perez-Carrera 2003, [2]Ruhl 2004, [3]Papandreou 2007

Clinical Data and Simulation Results

DILIsym® Training Agenda – September 26, 2013

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DILIsym® Training Agenda – September 26, 2013

- 8:30 AM – Introduction and goals
  - DILIsym® overview and highlights
  - Model architecture notes
- 8:45 AM – Biomarker analysis example
- 9:45 AM – Break
- 10:00 AM – Biomarker analysis example
- 11:00 AM – MITOsym™ overview and introduction
- 11:30 AM – Lunch
- 12:30 PM – Bile acid transport inhibitor example
- 1:30 PM – Break
- 1:45 PM – Bile acid transport inhibitor example
- 2:45 PM – Discussion and questions
- 3:00 PM – Training concludes
  - DILI-sim modeling team is available for questions

Application Example 2: Potential for Bile Acid Transporter Inhibitors to Cause Clinical DILI

Issue

- Two drugs (bosentan and telmisartan) have been flagged by *in vitro* assays as BSEP inhibitors
- Clinical DILI is linked to BSEP inhibition
- Rat studies have shown no signs of liver injury

Pending Decision

- Should the Company take extra precautions for potential liver injury during clinical trials?

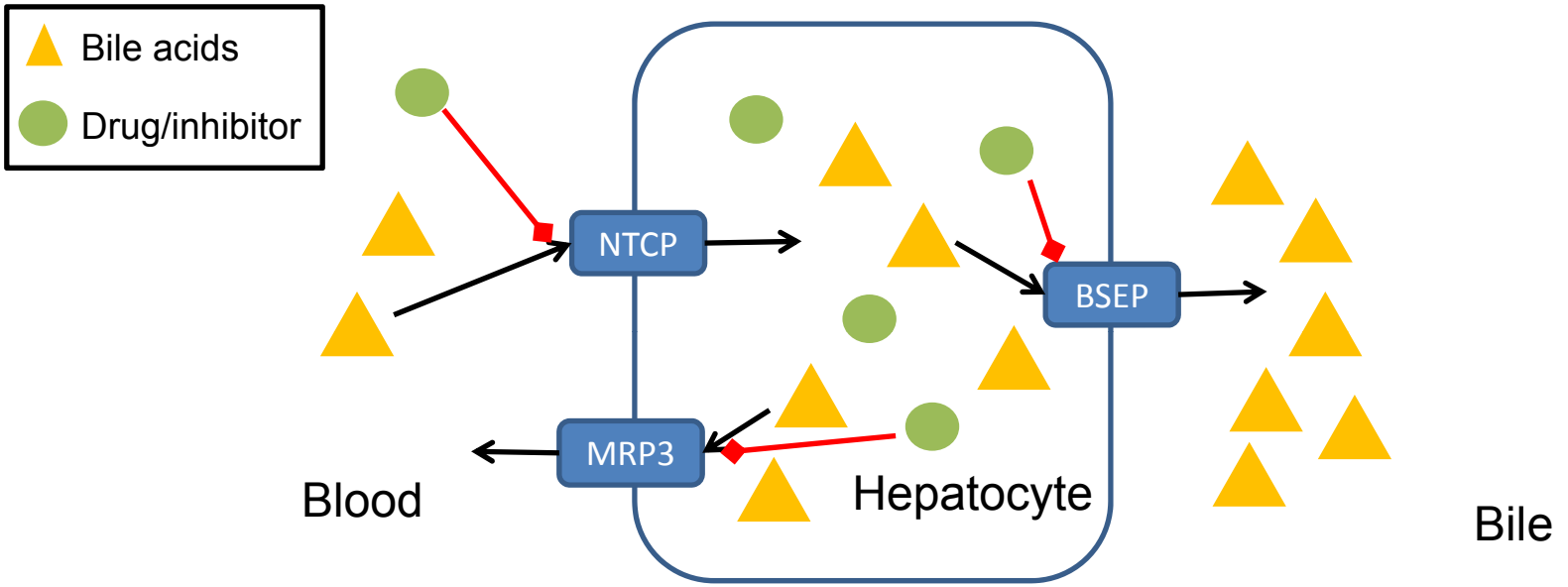
Questions to Individual(s) Responsible for Liver Safety Assessment

- Can DILIsym® be used to predict whether DILI might occur in humans?
  - Interpretation of lack of rat toxicity
  - Determination of maximum safe dose for drugs not expected to be toxic

DILIsym® Prediction of Potential Bile Acid-Induced Hepatotoxicity

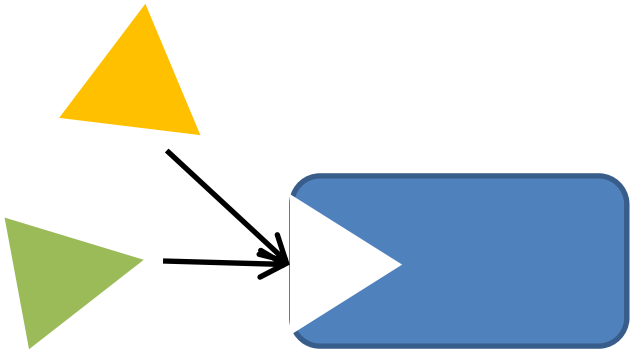
- Using the DILIsym® baseline simulated human and rat,
  - Build a model of bosentan and telmisartan including results from *in vitro* inhibition assays
    - Where mechanism of inhibition is unclear, perform simulations with both competitive and non-competitive inhibition
- Using human and rat SimPops™,
  - Simulate bosentan within the rat and human SimPops™
  - Simulate telmisartan within the human SimPops™
  - Identify the presence of human individuals with ALT elevations
  - Explore hepatocyte necrosis, ATP, and bile acid accumulation data to better understand differences between drugs and between species
  - Identify potential risk factors that would make certain individuals more sensitive to toxicity from these drugs

Bile Acid Transport Inhibition



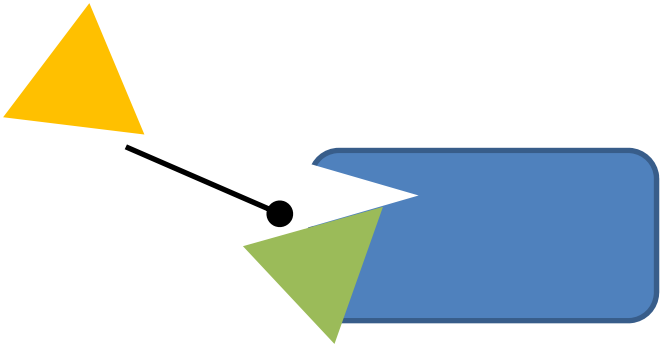
- Bile acids (▲) are taken up into hepatocytes by uptake transporters (NTCP) and transported out of cells by basolateral and canalicular efflux transporters (MRP3 and BSEP)
- Drugs (●) can inhibit any of these transport processes
- Bile acid buildup can cause toxicity in liver cells
- Our model represents bile acid transport and its inhibition by drugs mechanistically

Competitive and Noncompetitive Inhibition



Competitive Inhibition

$$\frac{d[BA]}{dt} = \frac{V_{max}[BA]}{K_m(1+\frac{[I]}{K_i})+[BA]}$$

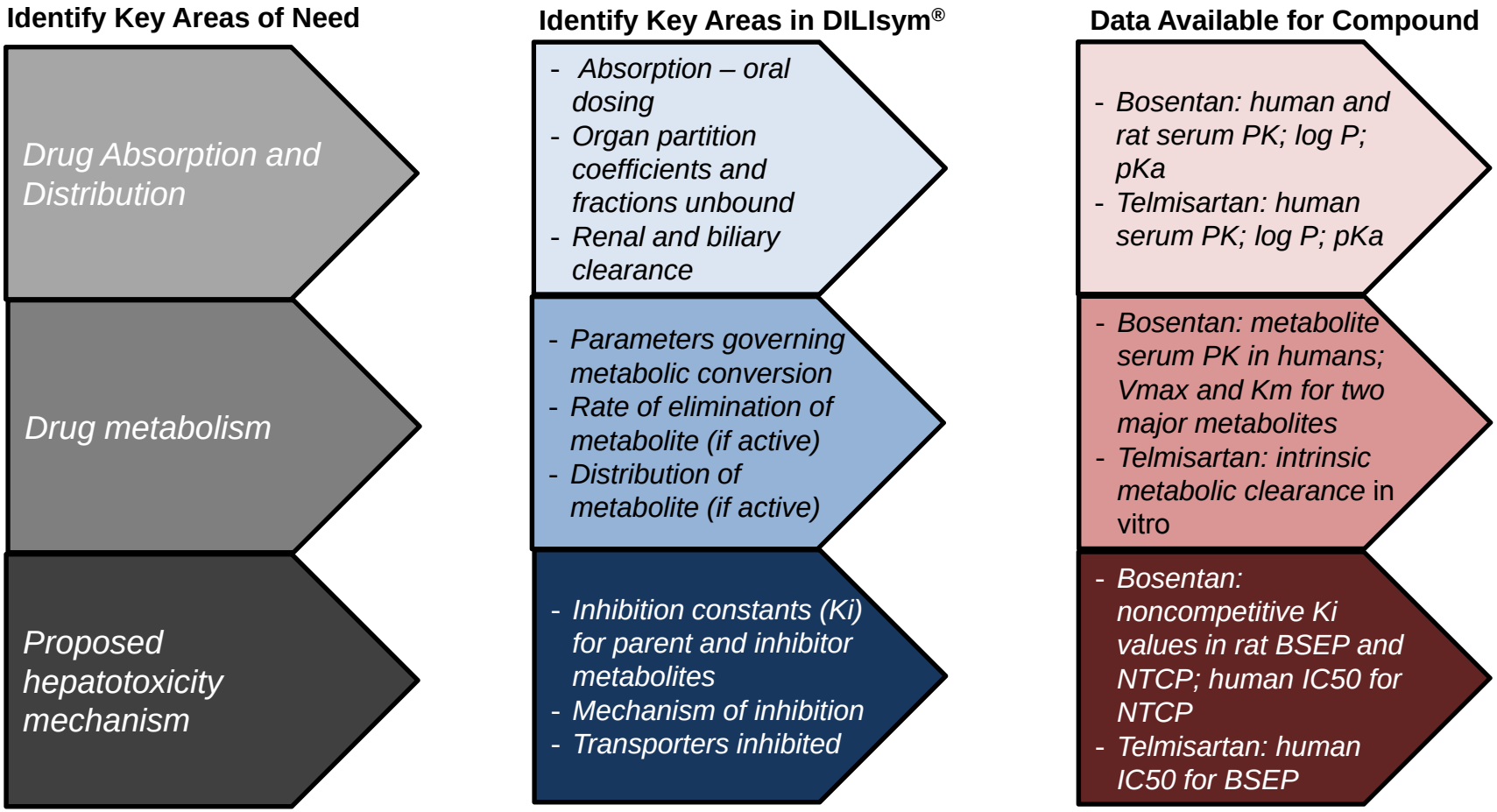


Noncompetitive Inhibition

$$\frac{d[BA]}{dt} = \frac{\frac{V_{max}}{(1+\frac{[I]}{K_i})}[BA]}{K_m+[BA]}$$

- Competitive inhibition involves drug and bile acids competing for same active site on an enzyme
  - Affects enzyme *affinity* for the bile acid, i.e. K_m
- Noncompetitive inhibition involves drug preventing bile acid from binding on the enzyme altogether
  - Affects enzyme *activity* with respect to bile acid, i.e. V_{max}

Identifying the Inputs Needed for Bile Acid Toxicity Prediction in DILIsym® v2B



Determining Parameter Values for Transporter Inhibition: Bosentan

Data Available

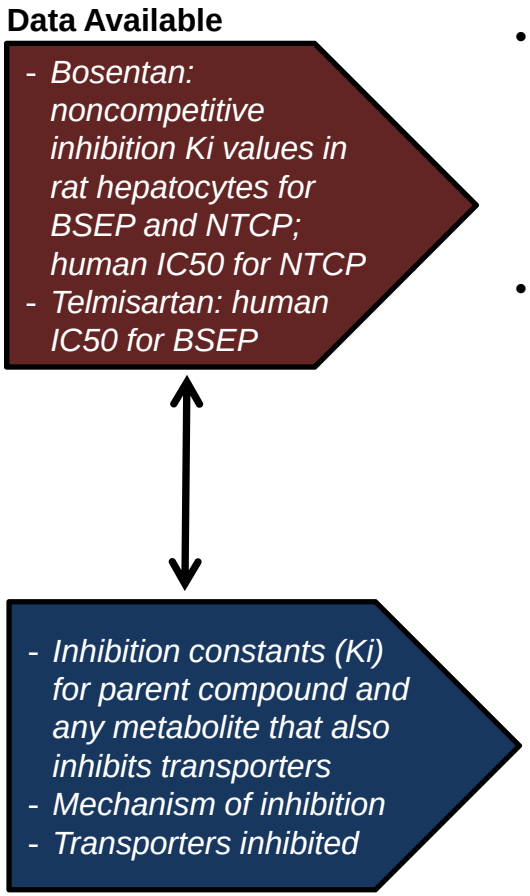
- *Bosentan: noncompetitive inhibition Ki values in rat hepatocytes for BSEP and NTCP; human IC50 for NTCP*
- *Telmisartan: human IC50 for BSEP*

- *Inhibition constants (Ki) for parent compound and any metabolite that also inhibits transporters*
- *Mechanism of inhibition*
- *Transporters inhibited*

- Value of Ki and mechanism known in rat hepatocytes for BSEP
  - Both parent and metabolite
  - Will use this value for humans too; literature has shown that bosentan has similar potency for rat and human BSEP
- Value of Ki for NTCP known in rat hepatocytes
- Ki for NTCP known in human hepatocytes

Parameter Syntax	Parameter Name	Experimental Value
Ki_noncomp_BSEP_CompX	Noncompetitive Ki for BSEP; parent Compound X	12 uM (Fattinger 2001)
Ki_noncomp_BSEP_CompX_MetA	Noncompetitive Ki for BSEP; Compound X metabolite A	8.5 uM (Fattinger 2001)
Ki_NTCP_CompX	Competitive Ki for bulk bile acid uptake; parent Compound X (human)	18 uM* (Leslie 2007)
Ki_noncomp_NTCP_CompX	Noncompetitive Ki for bile acid uptake; parent Compound X (rat)	0.28 uM (Leslie 2007)

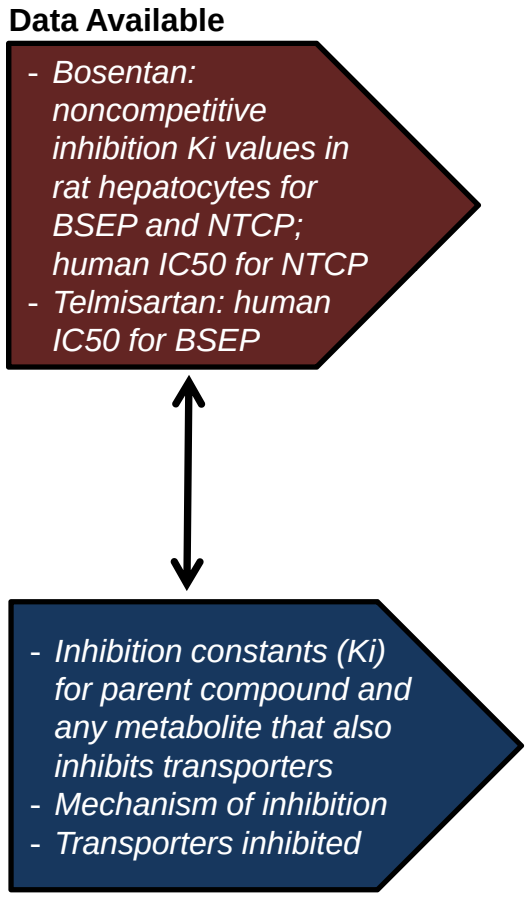
Selecting the Appropriate Mechanism



- Competitive vs. noncompetitive inhibition can be important to outcome of model
  - Can be difficult to discern from experimental data; often a blend of the two is responsible
  - Assay to differentiate competitive from noncompetitive inhibition is often not performed
- Can set the model up to run both mechanisms if mechanism is unknown/in doubt
  - In our case, that applies to telmisartan BSEP

Competitive	Noncompetitive
Ki_BSEP_CompX	Ki_noncomp_BSEP_CompX
LCA_canal_Ki_CompX	
LCAamide_canal_Ki_CompX	
LCAsulfate_canal_Ki_CompX	
CDCA_canal_Ki_CompX	
CDCAamide_canal_Ki_CompX	

Selecting the Appropriate Mechanism



- In DILIsym®, competitive inhibition is governed by individual Ki values for each bile acid species, while noncompetitive inhibition is governed by a single constant for each inhibitor
  - Constants can often be different for different bile acid species (e.g. glibenclamide)
  - However, assays are often done using only one substrate (generally TCA)
  - To represent competitive inhibition accurately, all six Ki values **must** be defined in the parameter set

Competitive	Noncompetitive
Ki_BSEP_CompX	Ki_noncomp_BSEP_CompX
LCA_canal_Ki_CompX	
LCAamide_canal_Ki_CompX	
LCAsulfate_canal_Ki_CompX	
CDCA_canal_Ki_CompX	
CDCAamide_canal_Ki_CompX	

Determining Parameter Values for Transporter Inhibition: Telmisartan

Data Available

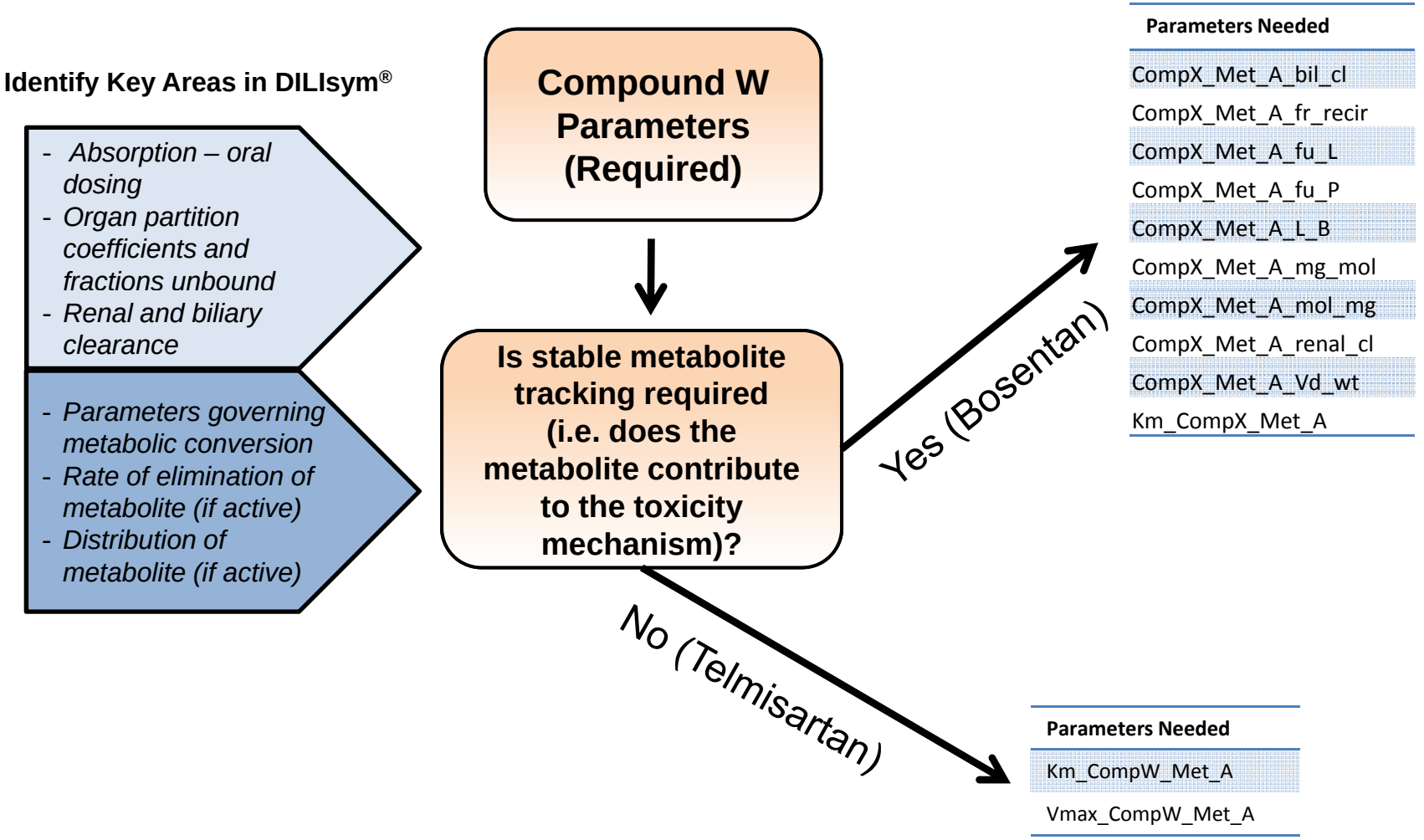
- *Bosentan: noncompetitive inhibition Ki values in rat hepatocytes for BSEP and NTCP; human IC50 for NTCP*
- *Telmisartan: human IC50 for BSEP*

- *Inhibition constants (Ki) for parent compound and any metabolite that also inhibits transporters*
- *Mechanism of inhibition*
- *Transporters inhibited*

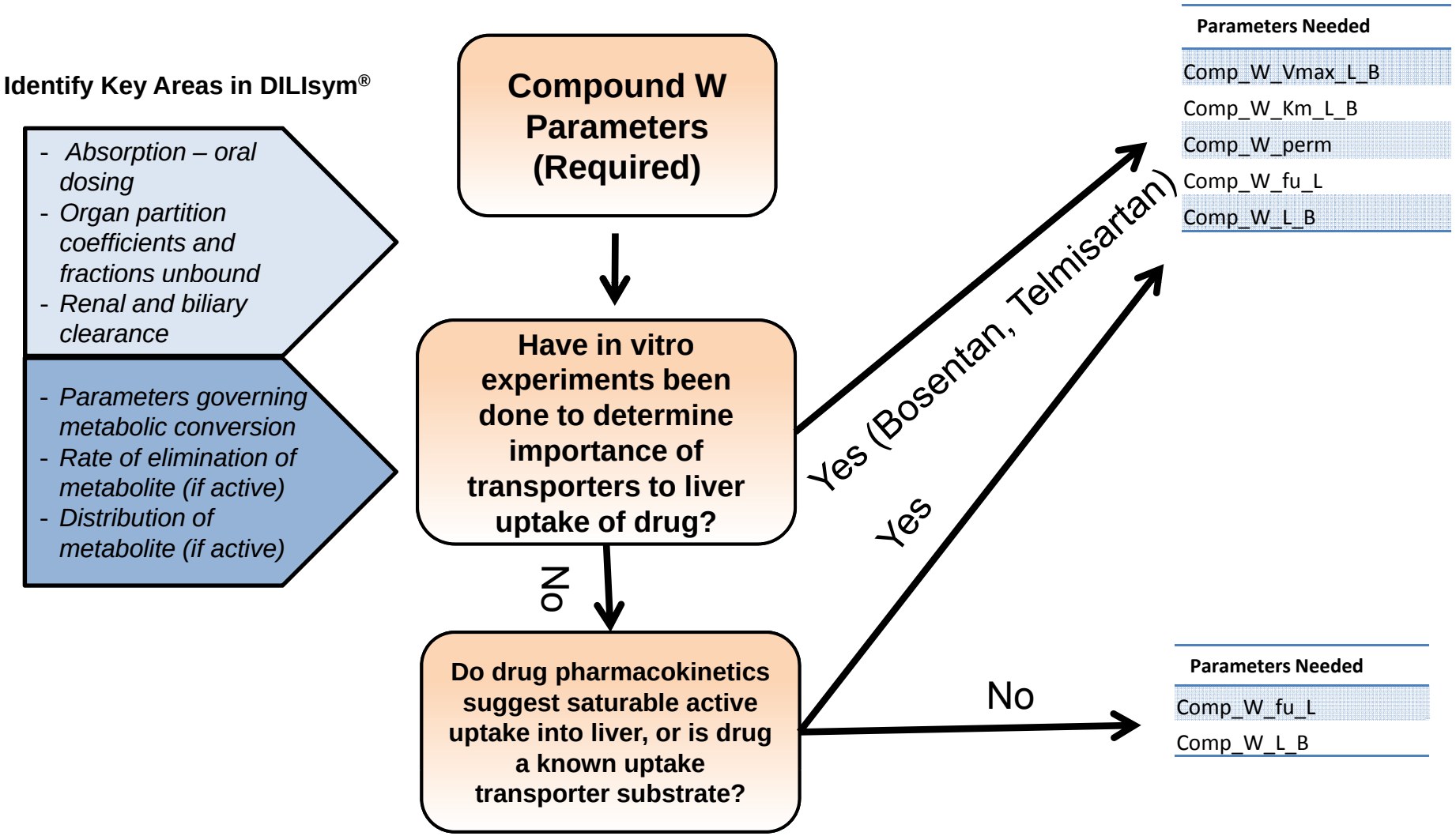
- Value of Ki unknown
- Value of IC50 for human BSEP known from *in vitro* experiment
  - Can use this value as a crude approximation of Ki
  - Will set up parameter sets representing both competitive and noncompetitive inhibition

Parameter Syntax	Parameter Name	Experimental Value
Ki_BSEP_CompW	Competitive Ki for bulk bile acids for Compound W	16.2 uM
LCA_canal_Ki_CompW	Competitive Ki for lithocholic acid for Compound W	
LCAamide_canal_Ki_CompW	Competitive Ki for lithocholic acid amide conjugates for Compound W	
LCAsulfate_canal_Ki_CompW	Competitive Ki for lithocholic acid sulfate conjugates for Compound W	
CDCA_canal_Ki_CompW	Competitive Ki for chenodeoxycholic acid for Compound W	
CDCAamide_canal_Ki_CompW	Competitive Ki for chenodeoxycholic acid amide conjugates for Compound W	

Selecting the DILIsym® Parameters to Use for Metabolite PBPK



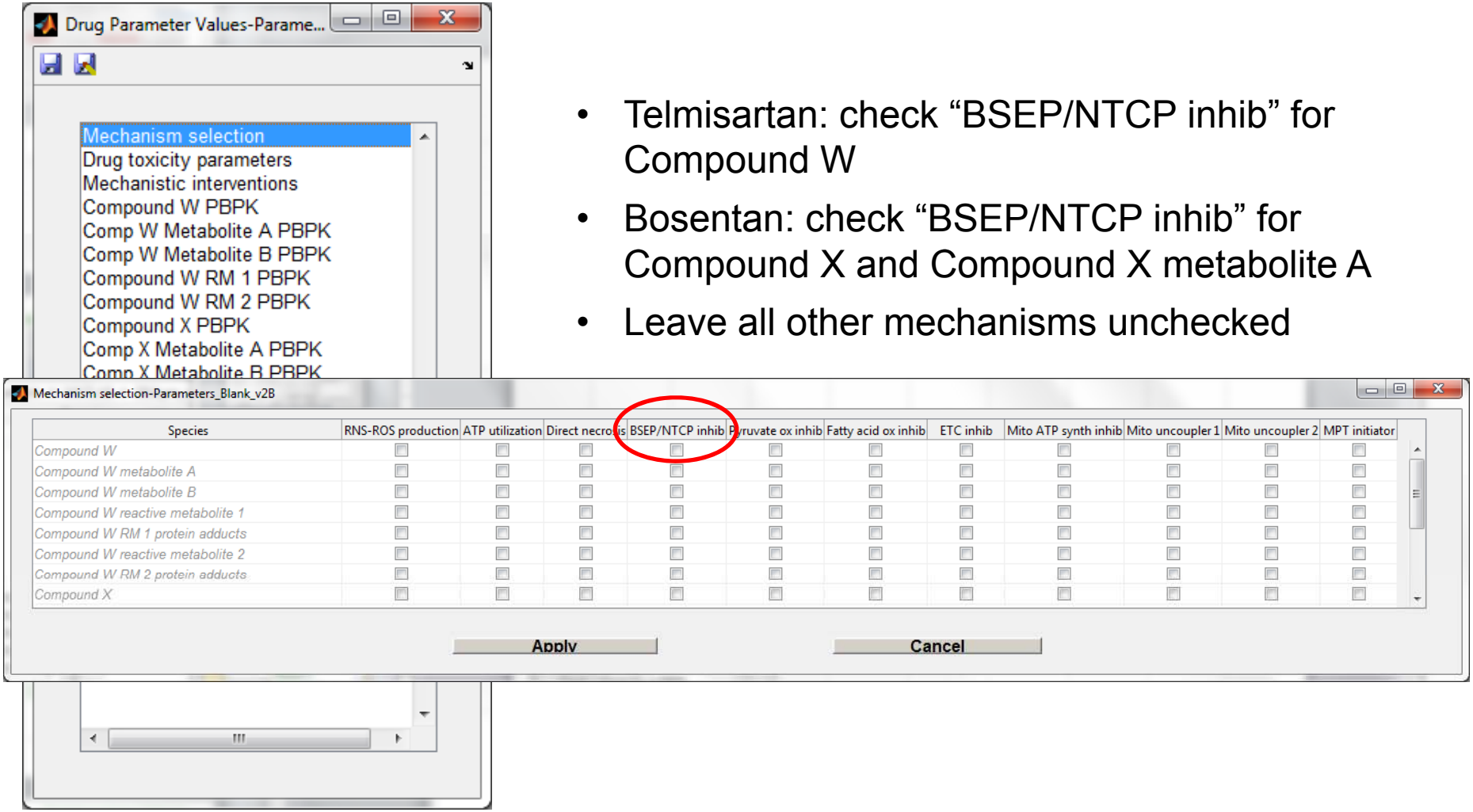
Selecting the DILIsym® Parameters to Use for Active Liver Uptake



Implementation of Bile Acid Toxicity – Creating Parameter Sets to Account for Unknown Inhibition Mechanisms

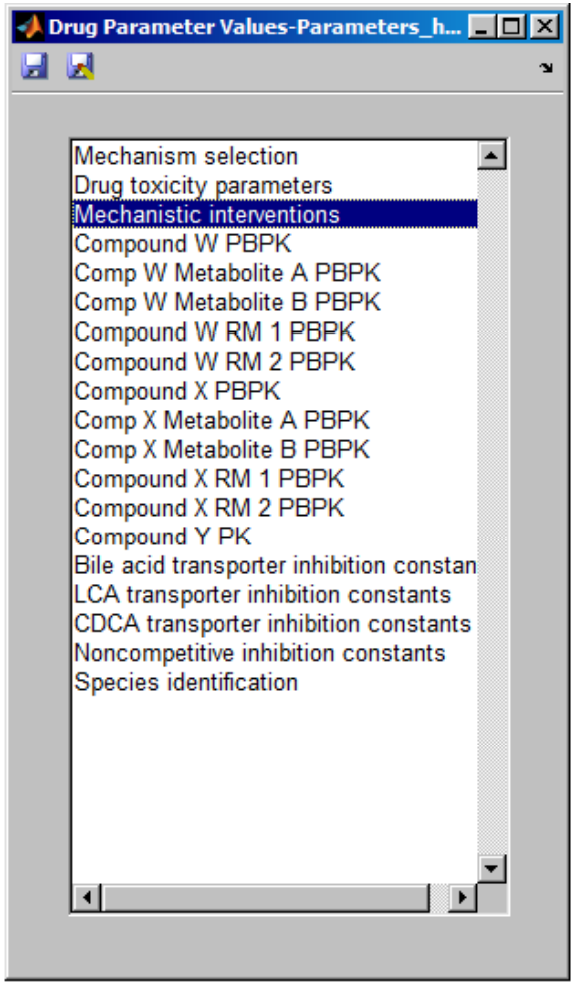
- For some transporters, the mechanism of inhibition is unknown, i.e. the drug could be either a competitive or a noncompetitive inhibitor
  - Telmisartan and bosentan for human uptake
  - We will treat bosentan as a competitive inhibitor of human uptake for this exercise
- We will need to build alternate drug parameter sets for competitive and noncompetitive inhibition and run both
- We will make four parameter sets in total today
  - Human telmisartan competitive
  - Human telmisartan noncompetitive
  - Human bosentan
  - Rat bosentan
- The PBPK portion of the input has been filled in already; we will concentrate on inputs that are special to the bile acid model

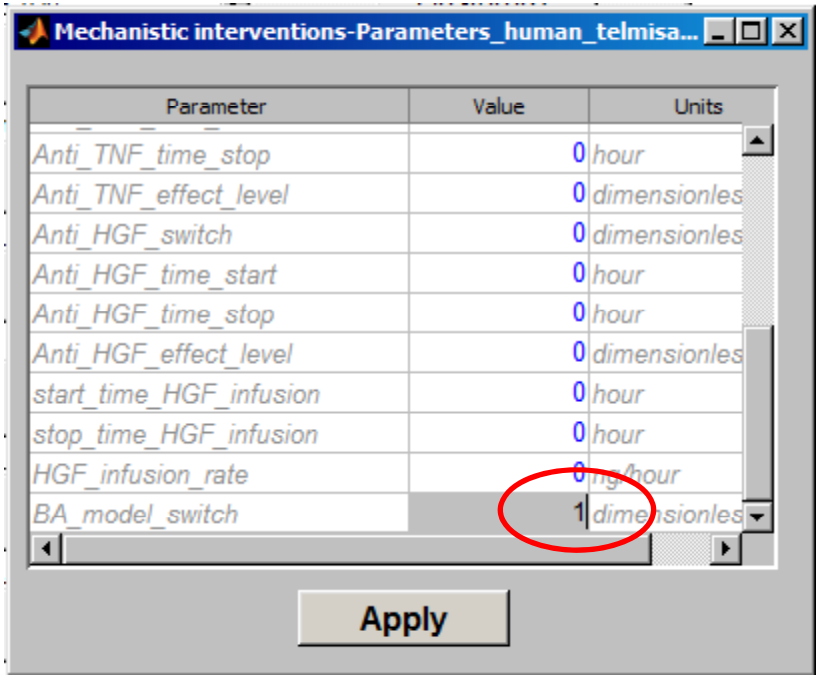
Implementing Hepatotoxicity Mechanism for Bile Acid Toxicity



- Telmisartan: check “BSEP/NTCP inhib” for Compound W
- Bosentan: check “BSEP/NTCP inhib” for Compound X and Compound X metabolite A
- Leave all other mechanisms unchecked

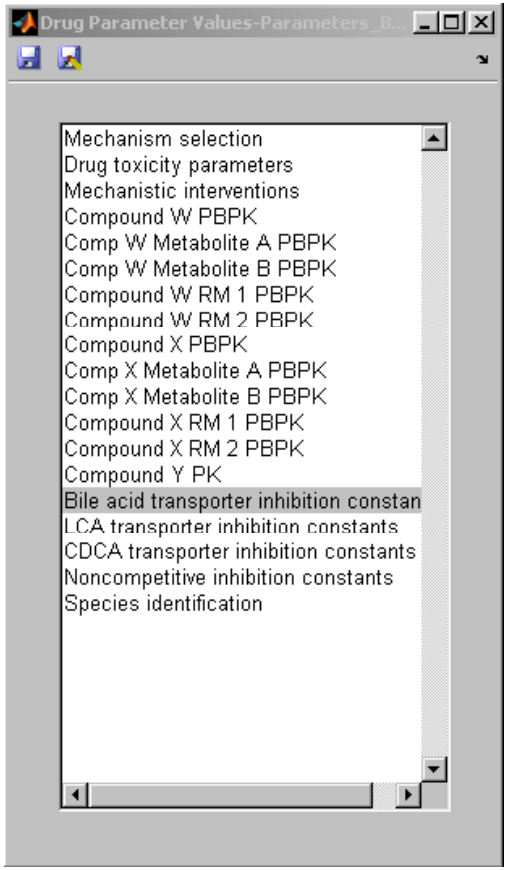
Activating the Bile Acid Model using Mechanistic Interventions

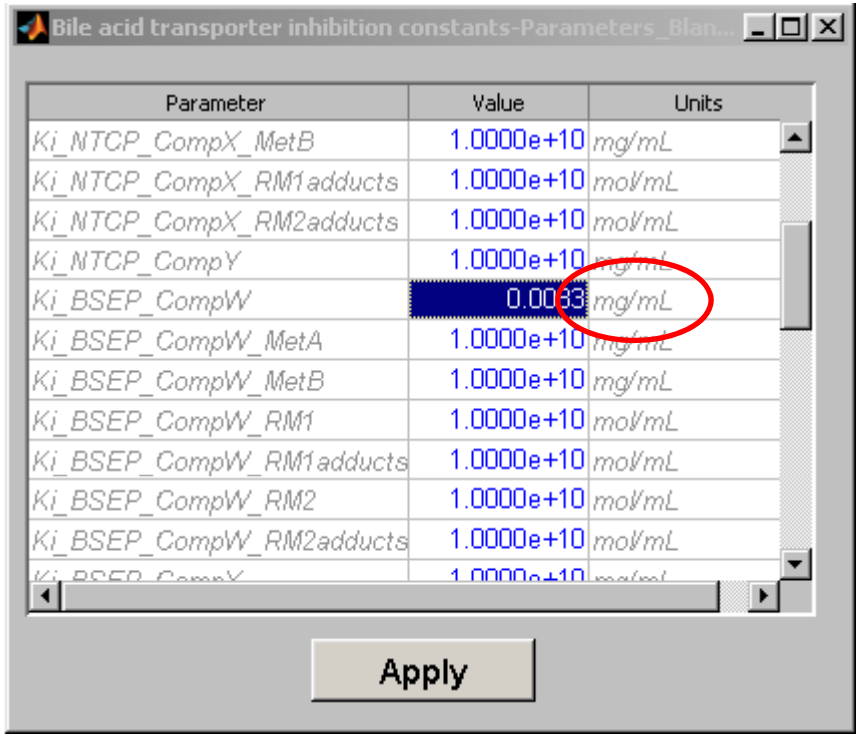




- The bile acid portion of the model is normally switched off in order to preserve computational time
- The bile acid model **must** be turned on in order to properly represent transporter inhibitors

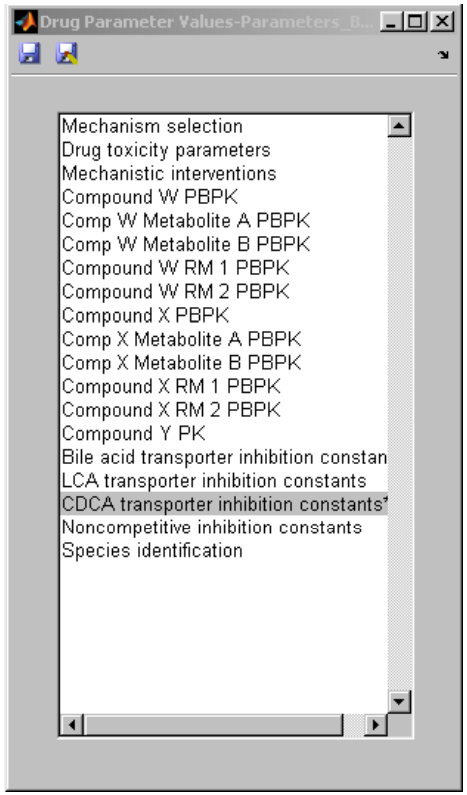
Implementation of Bile Acid Toxicity – Inhibition Constants (1 of 3)

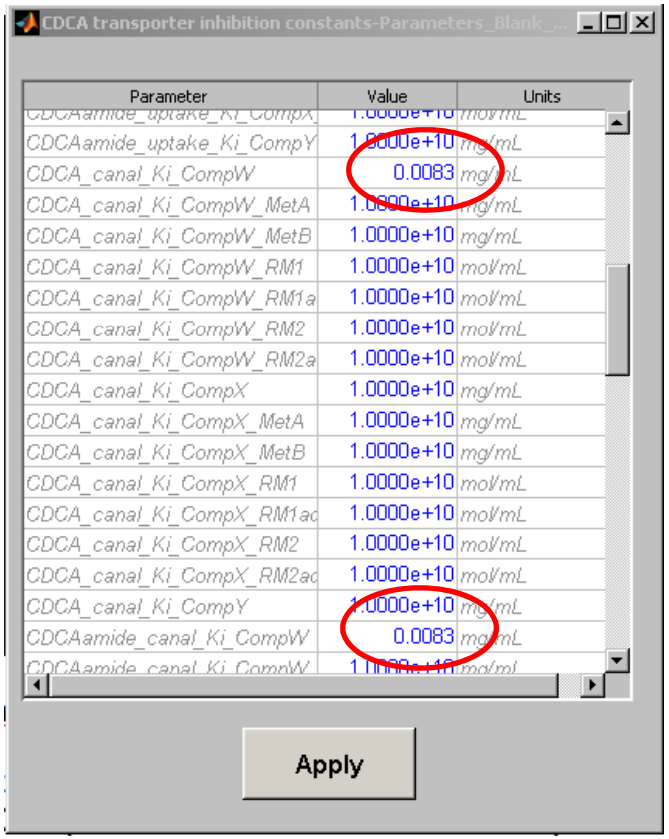




- For competitive inhibitors (telmisartan canalicular, bosentan uptake in human)
- Note units in input column
  - For telmisartan, 16.2 μM = 8.33×10^{-3} mg/mL
  - For human bosentan uptake, 19 μM = 9.67×10^{-3} mg/mL

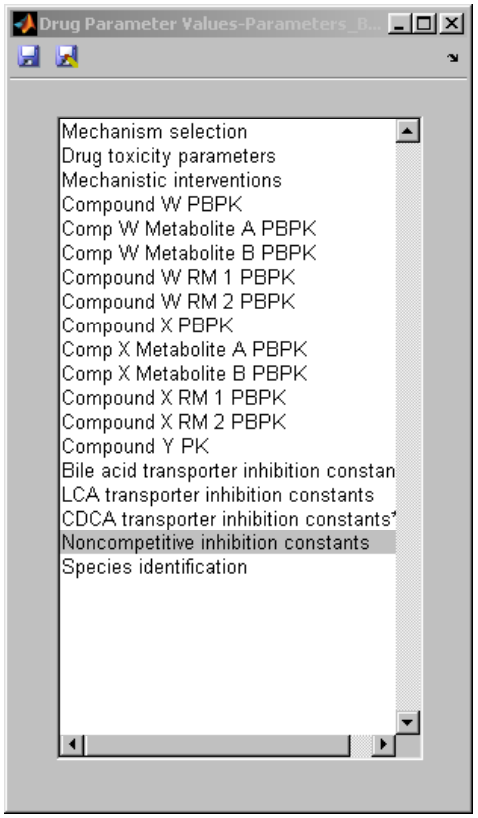
Implementation of Bile Acid Toxicity – Inhibition Constants (2 of 3)

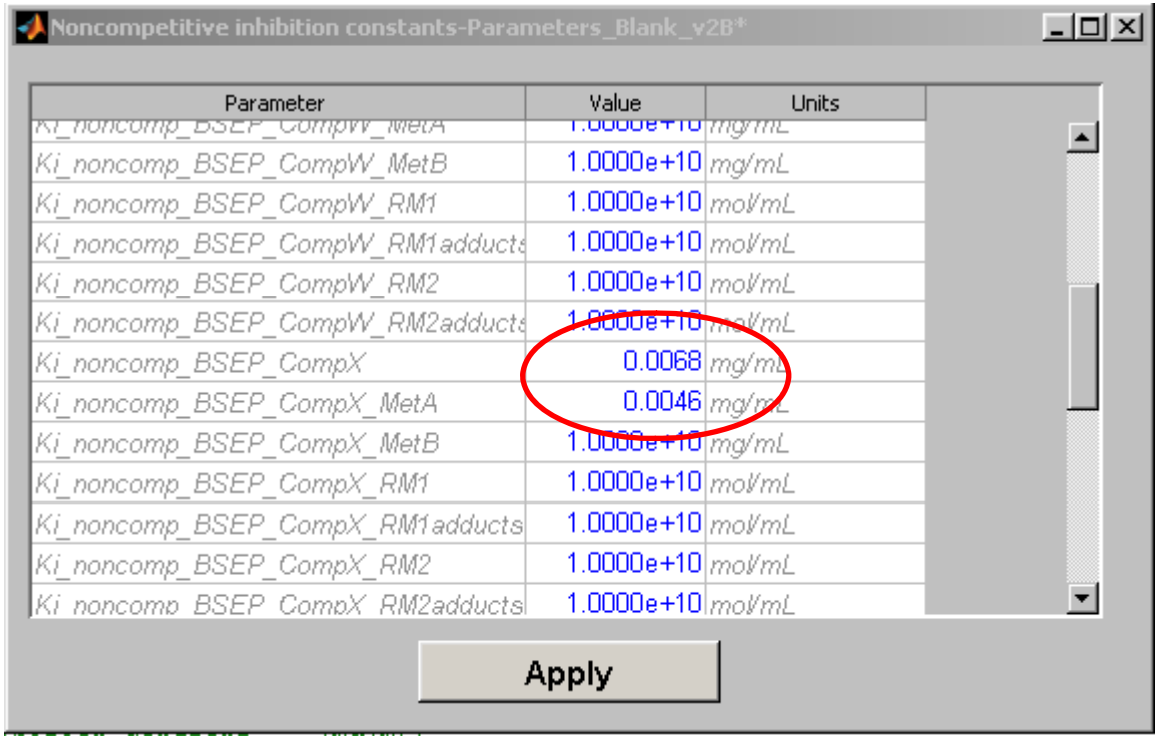




- For competitive inhibitors (telmisartan canalicular, bosentan uptake in human)
- Because we do not know the Ki values for each individual bile acid species, we must give each the same inhibition constant
- This must be done for the three LCA species as well as the two CDCA species

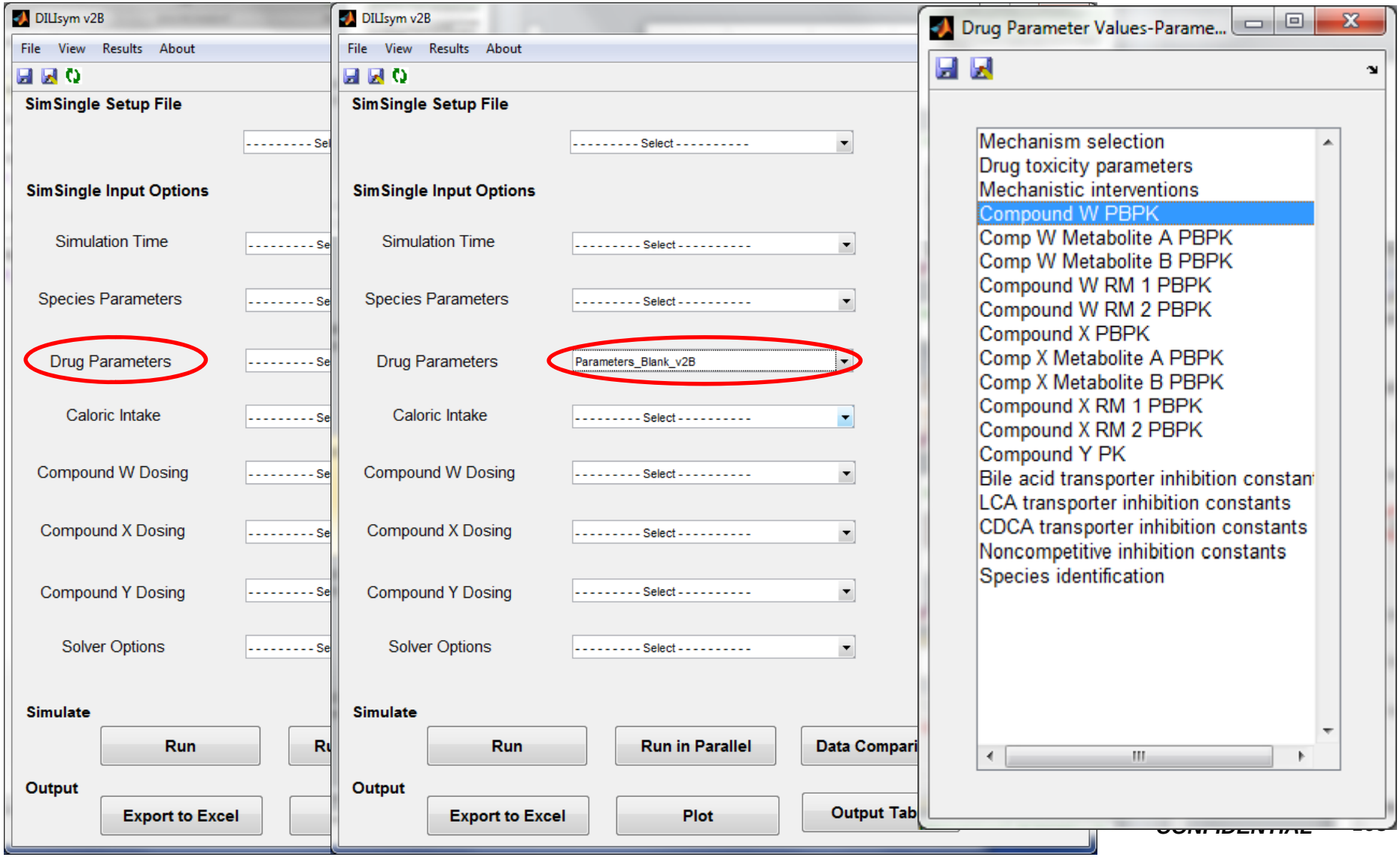
Implementation of Bile Acid Toxicity – Inhibition Constants (3 of 3)



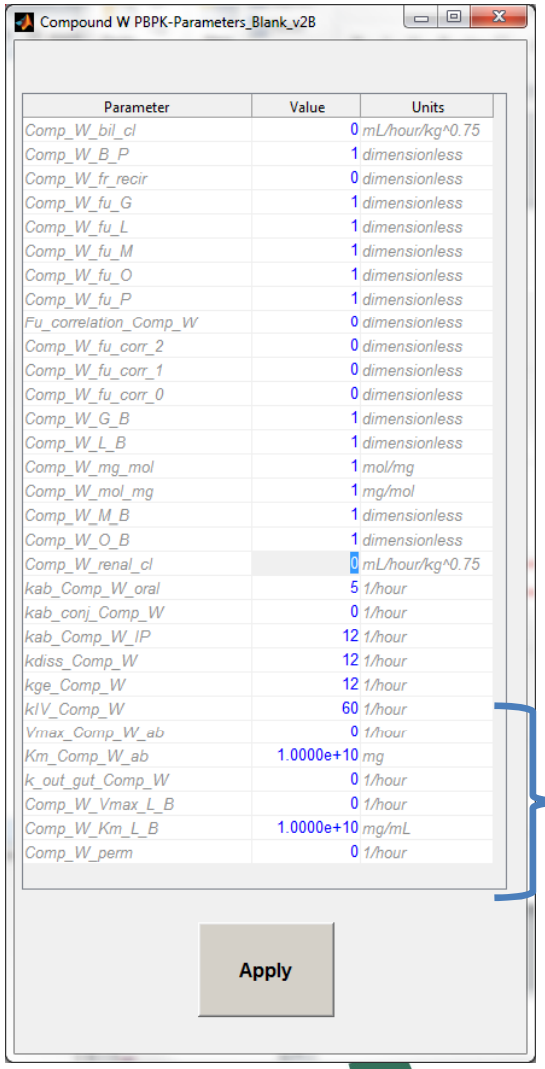


- For noncompetitive inhibitors (bosentan canalicular, bosentan uptake in rat)
  - Again, note unit conversion
  - For rat bosentan uptake, 0.28 μM = 1.51×10^{-4} mg/mL

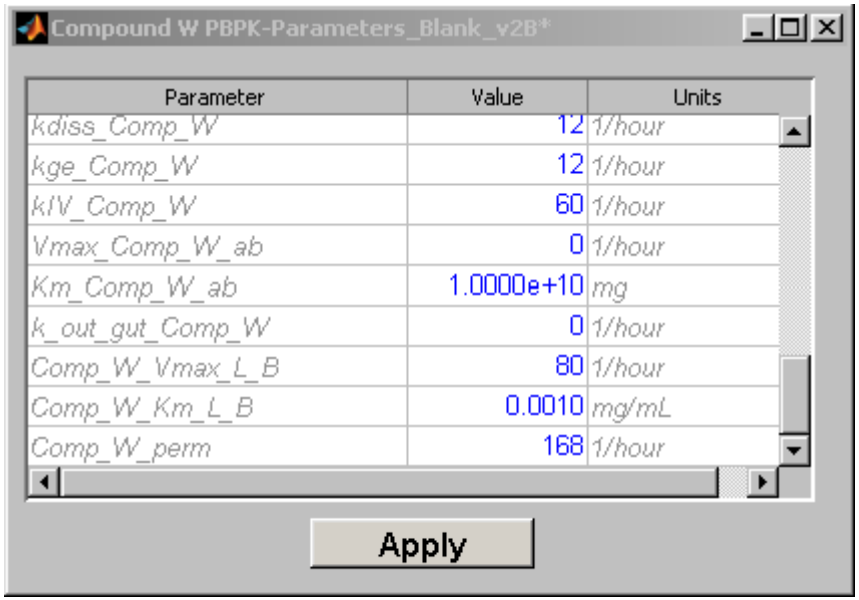
Implementing Parameter Values for Compound W/X PBPK (1 of 2)



Implementing Parameter Values for Compound W/X PBPK (2 of 2)

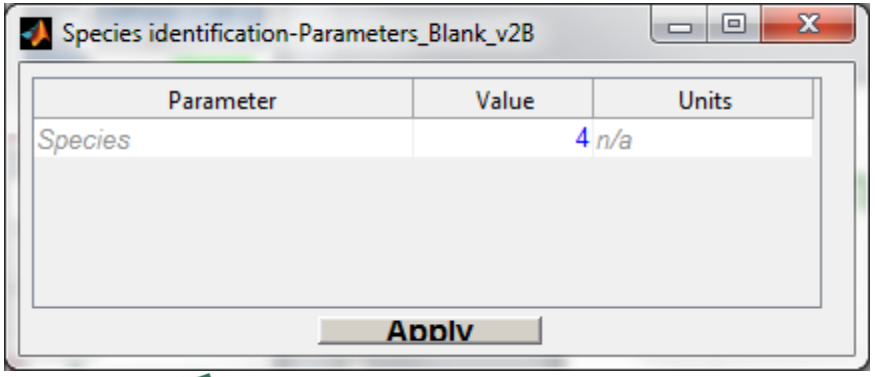


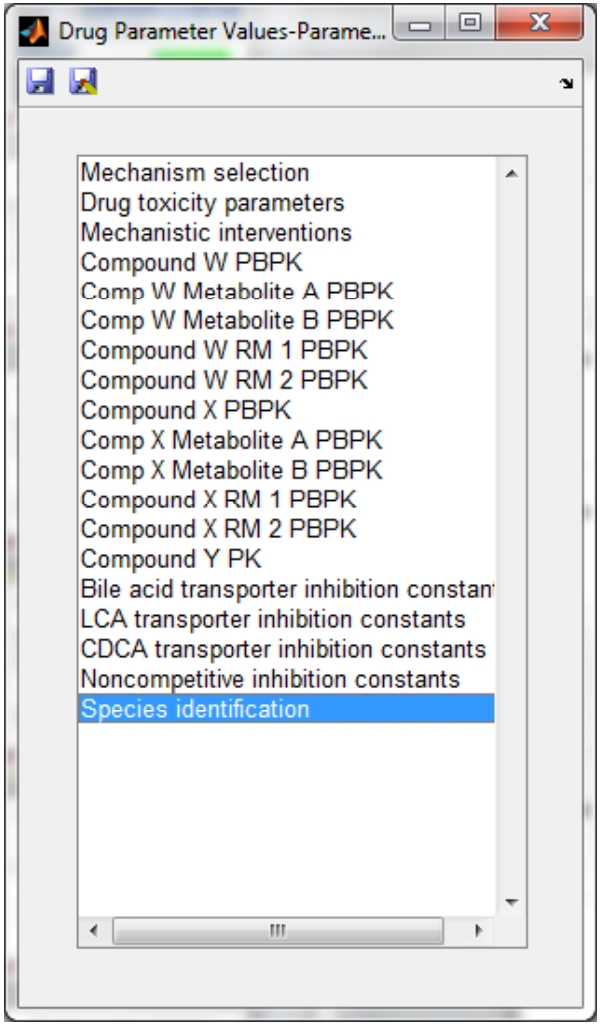
- For proprietary compounds, this would need to be filled out using data from earlier
- For bosentan and telmisartan, the values have been filled in for you



Implement Appropriate Species Selection for Simulations

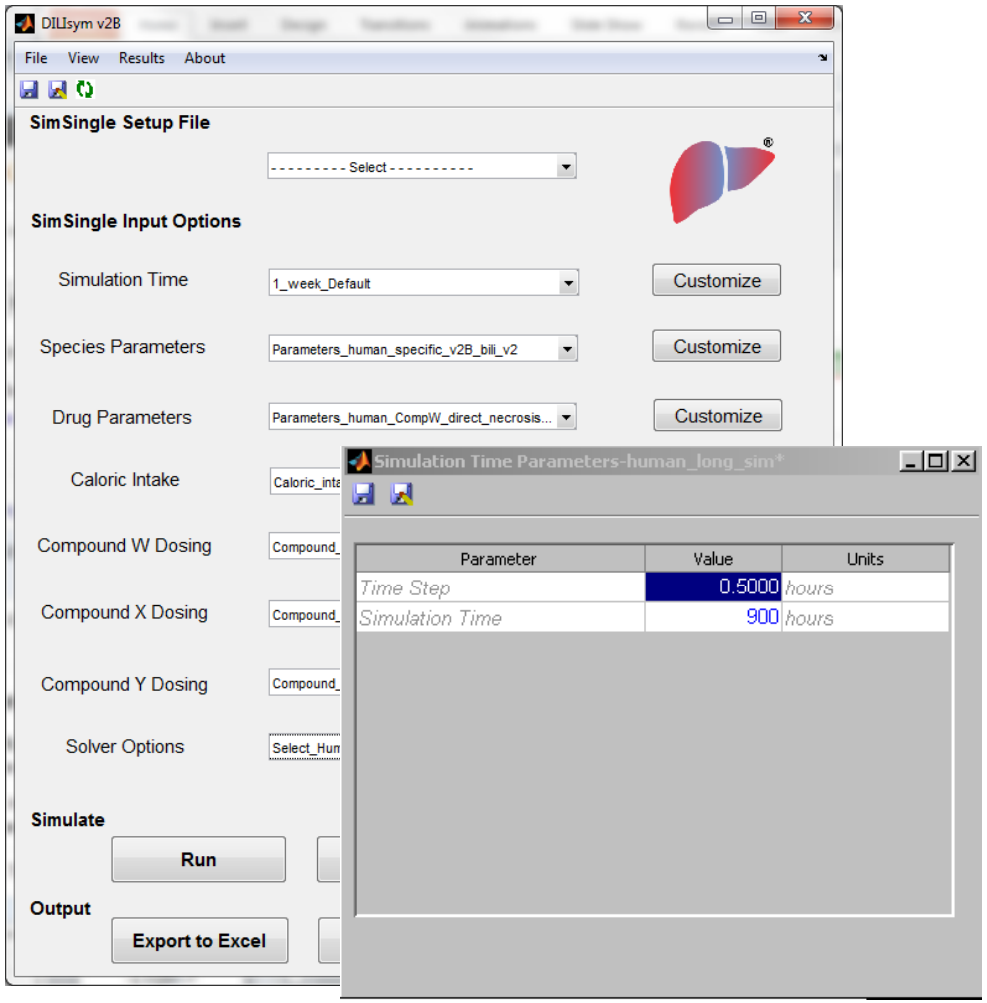
- DILI simulations may be run for mice, rats, dogs, or humans
- Species is specified by number
  - ***1*** – mice
  - ***2*** – rats
  - ***3*** – dogs
  - ***4*** – humans





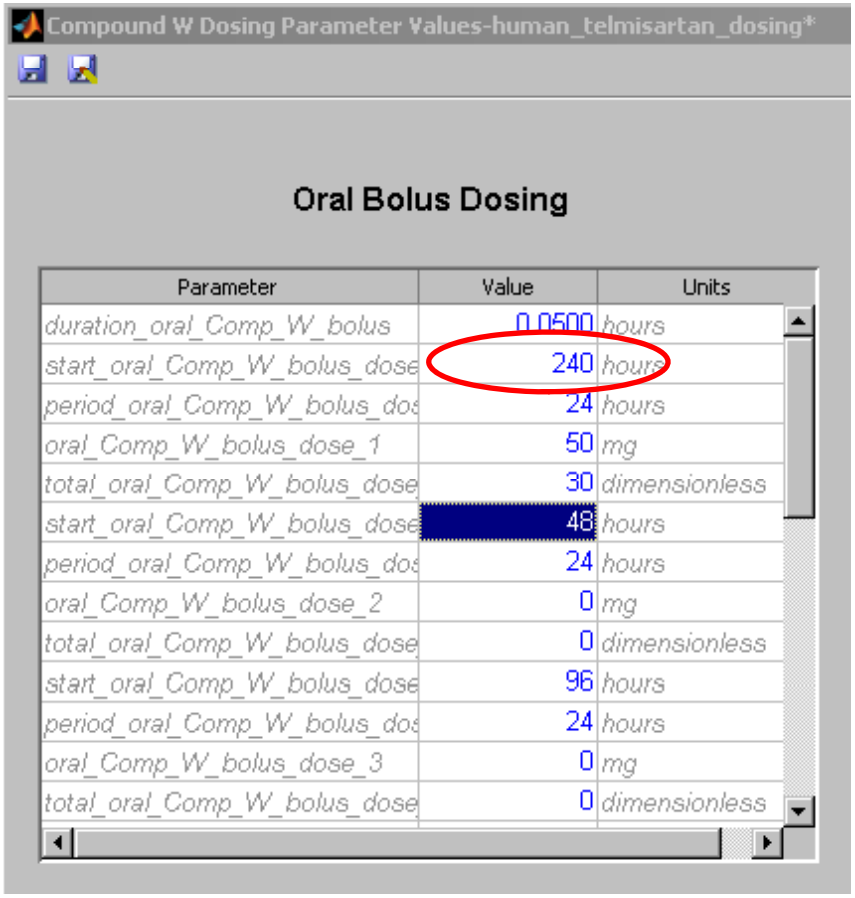
Initial SimSingle™ Set-Up

- Objective is to predict toxicity for:
  - Multiple dosing in humans
  - Standard rat protocol
- Set-up design
  - Long duration for multiple doses
- Create time files for both rat and human

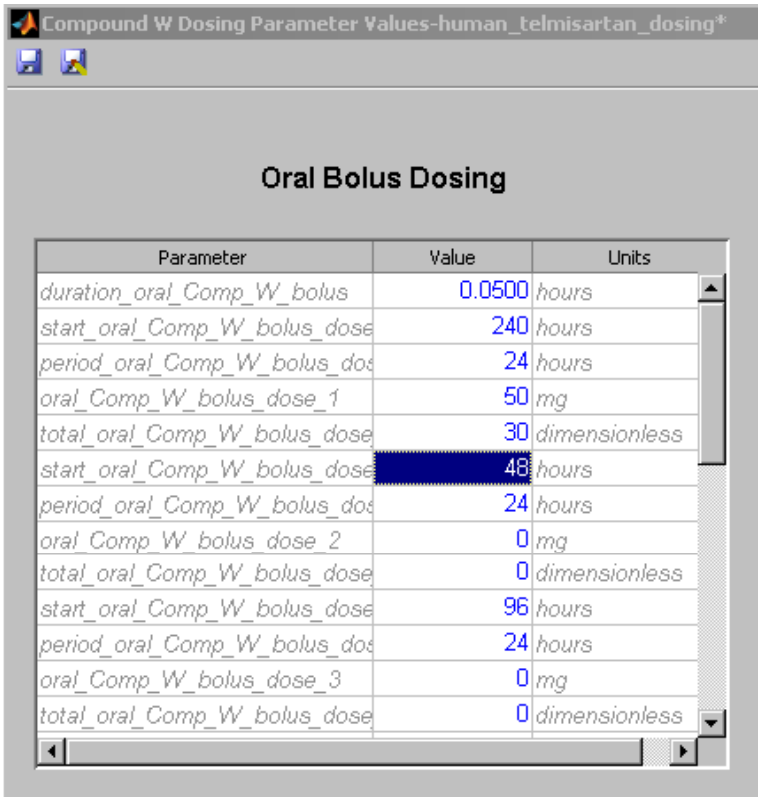


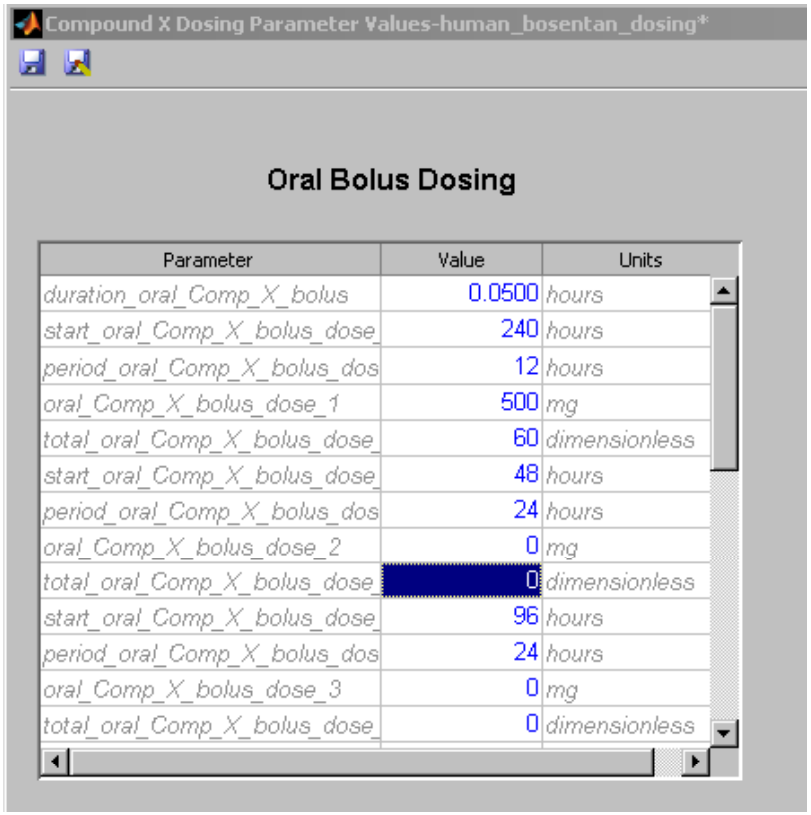
Setting Up the Bile Acid Model to Equilibrate Properly

- Bile acid model must be run for a period of time without drug dosing so bile acids can reach their initial concentrations
- Human model reaches stable bile acid concentrations in 240 hours
- Rat model requires 480 hours to reach equilibrium



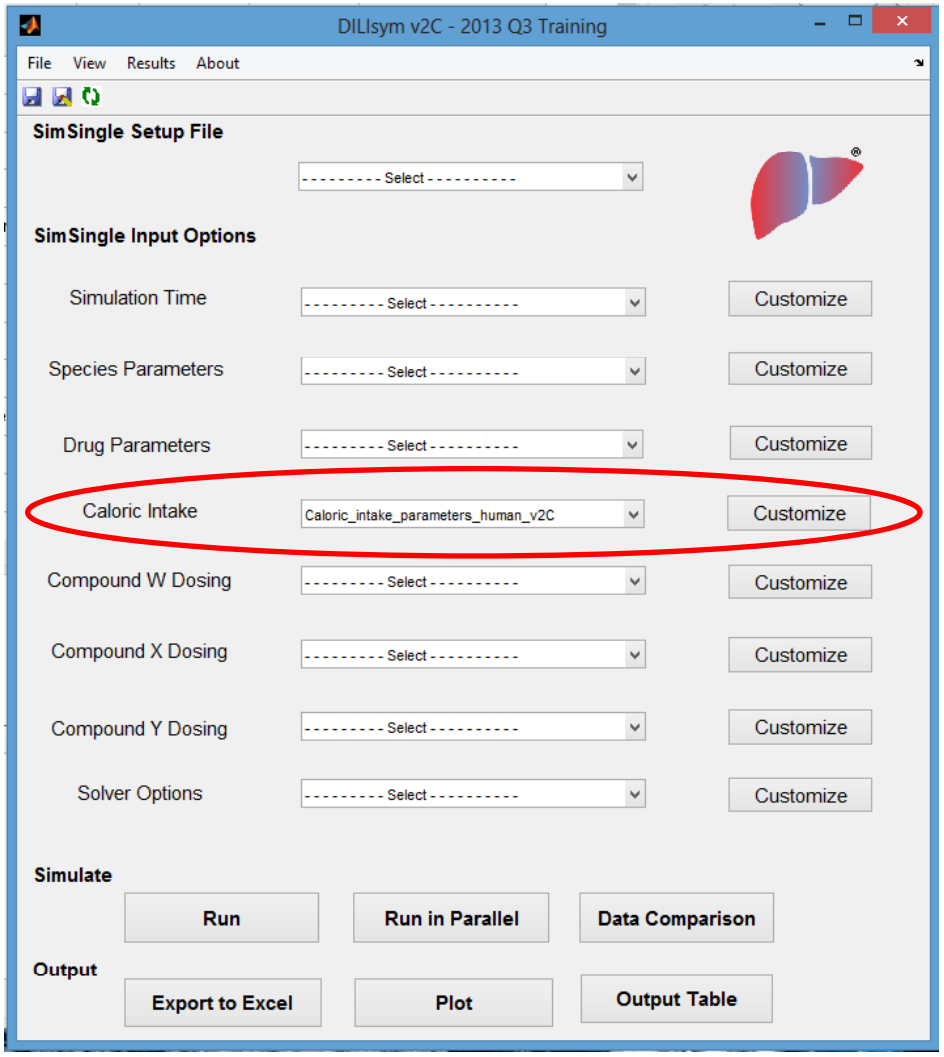
Drug Dosing for Bile Acid Simulations

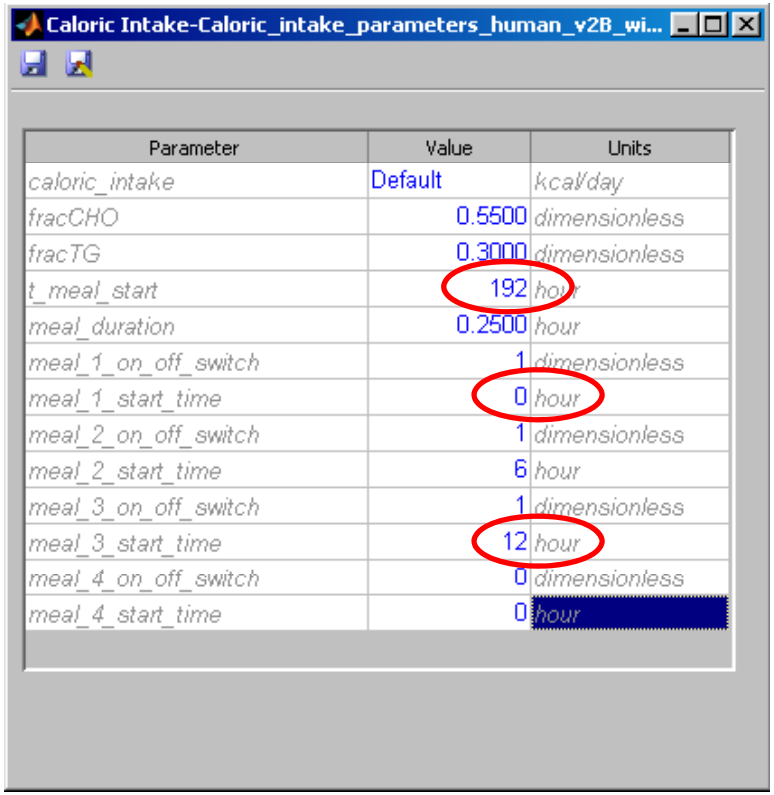




- Set up simulations to run at maximum clinical dose
  - Telmisartan: 50 mg QD
  - Bosentan: 500 mg BID

Caloric Intake for Bile Acid Simulations

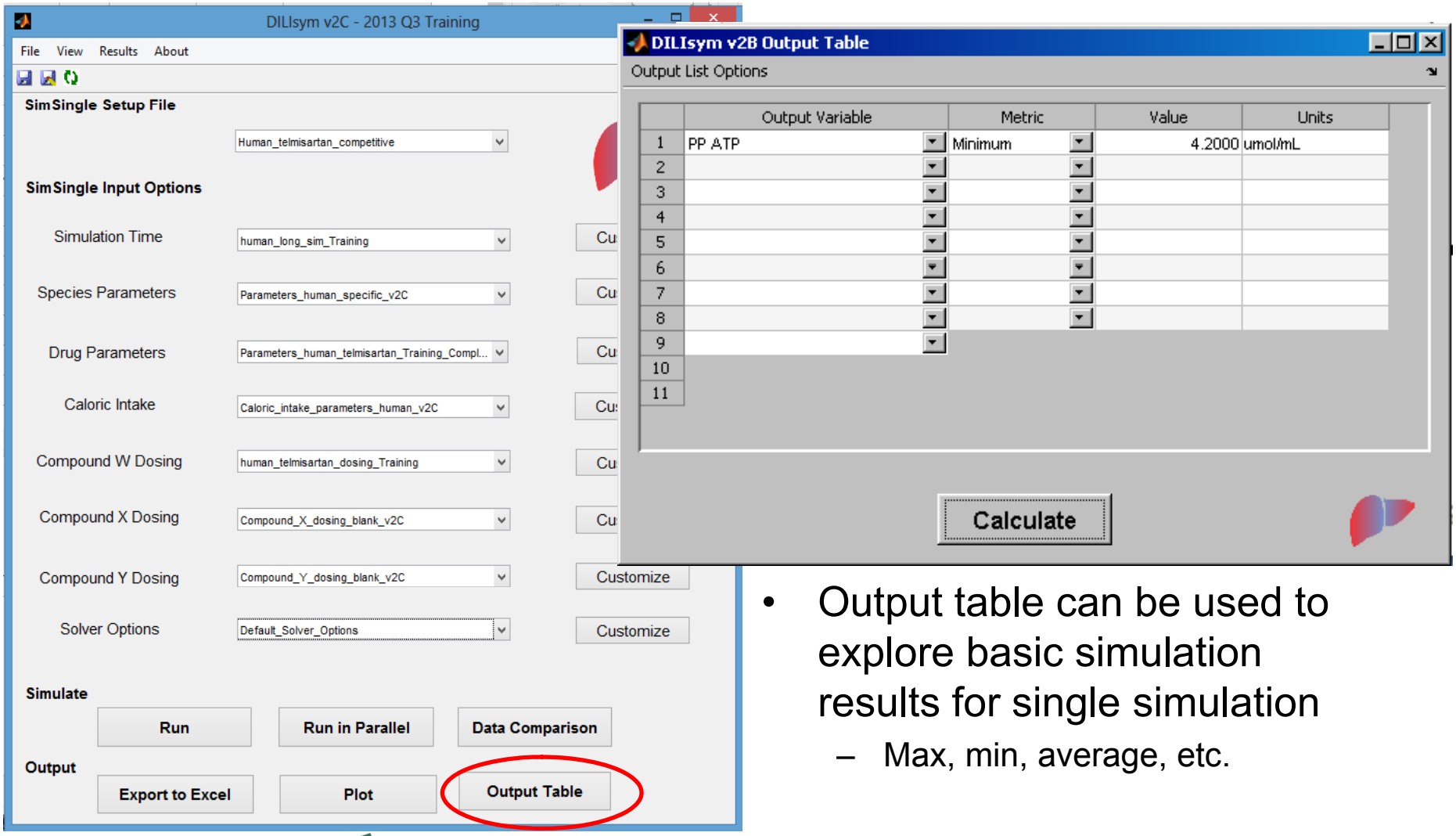




Parameter	Value	Units
caloric_intake	Default	kcal/day
fracCHO	0.5500	dimensionless
fracTG	0.3000	dimensionless
t_meal_start	192	hour
meal_duration	0.2500	hour
meal_1_on_off_switch	1	dimensionless
meal_1_start_time	0	hour
meal_2_on_off_switch	1	dimensionless
meal_2_start_time	6	hour
meal_3_on_off_switch	1	dimensionless
meal_3_start_time	12	hour
meal_4_on_off_switch	0	dimensionless
meal_4_start_time	0	hour

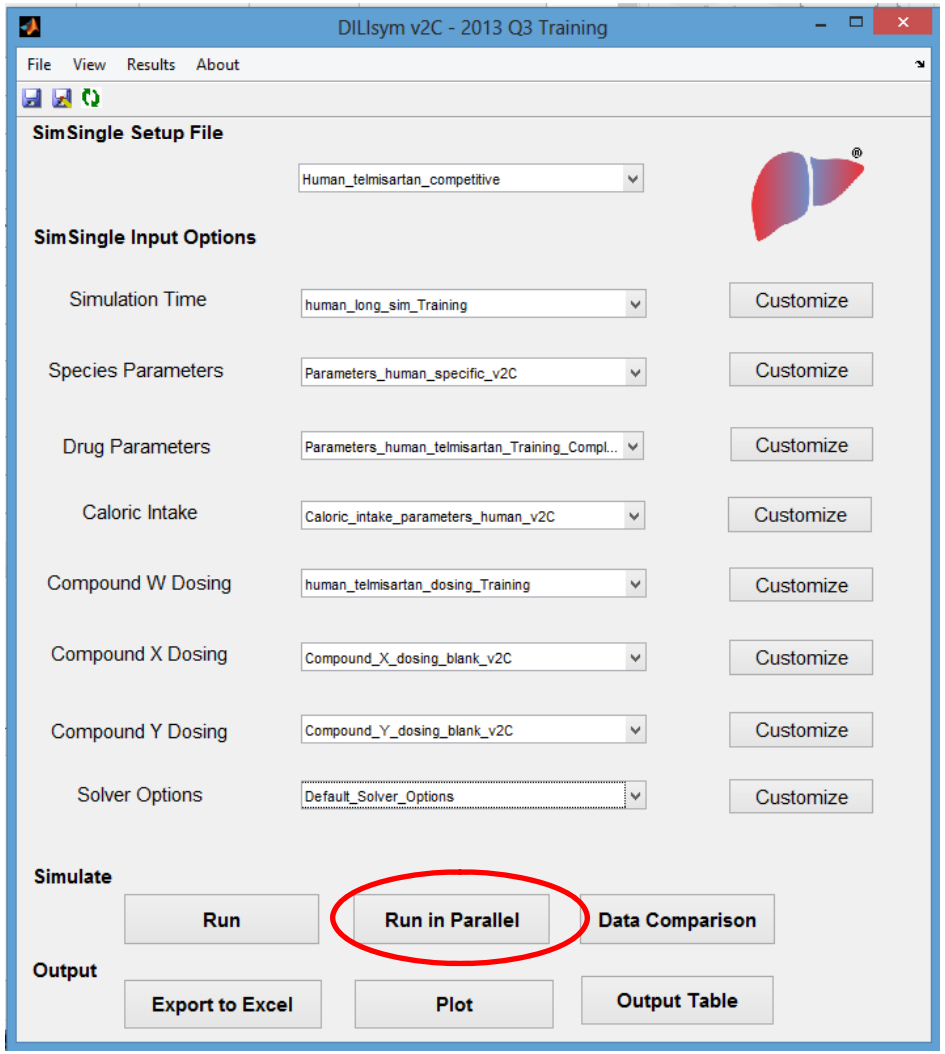
- Set up caloric intake so that meals are taken at same time as drug

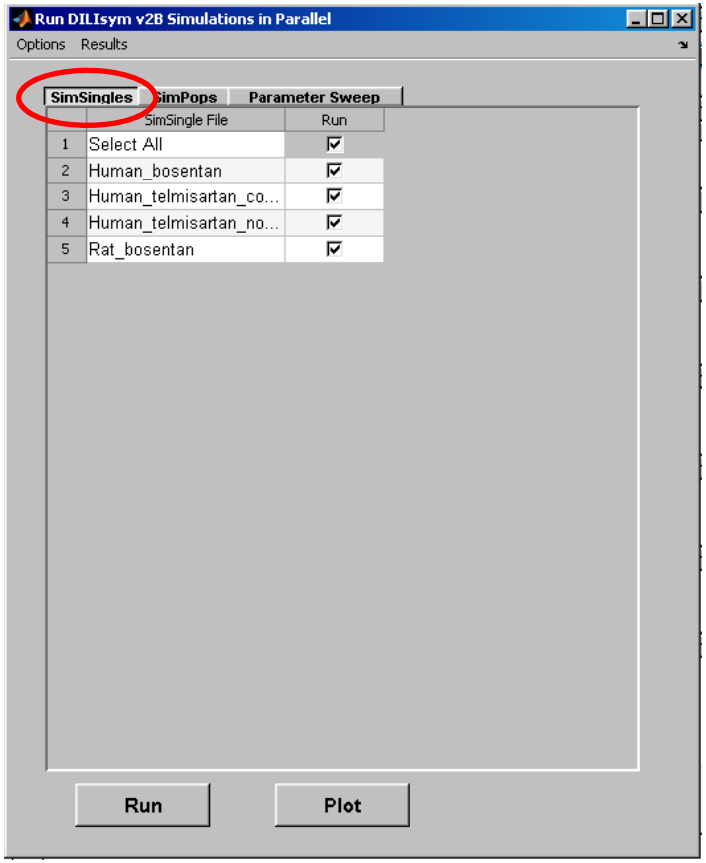
Exploring Simulation Results Using the Output Table



- Output table can be used to explore basic simulation results for single simulation
  - Max, min, average, etc.

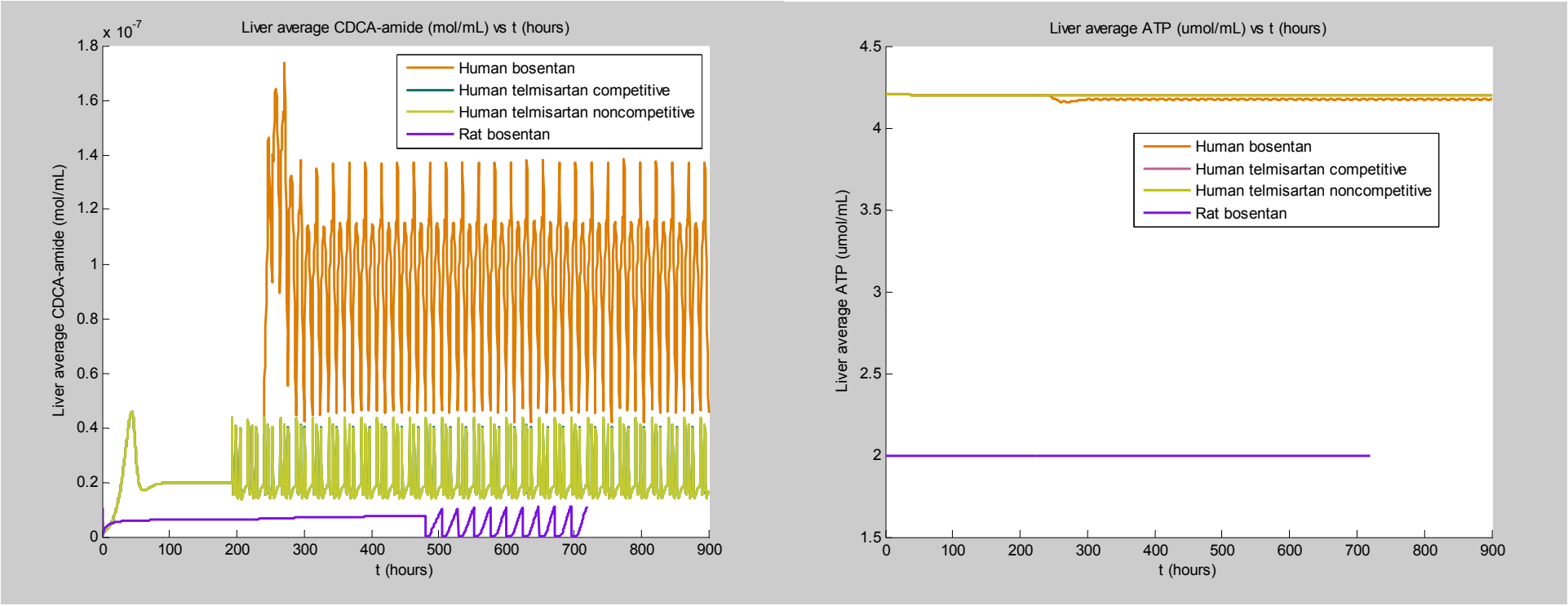
Running Different SimSingles™ in Parallel





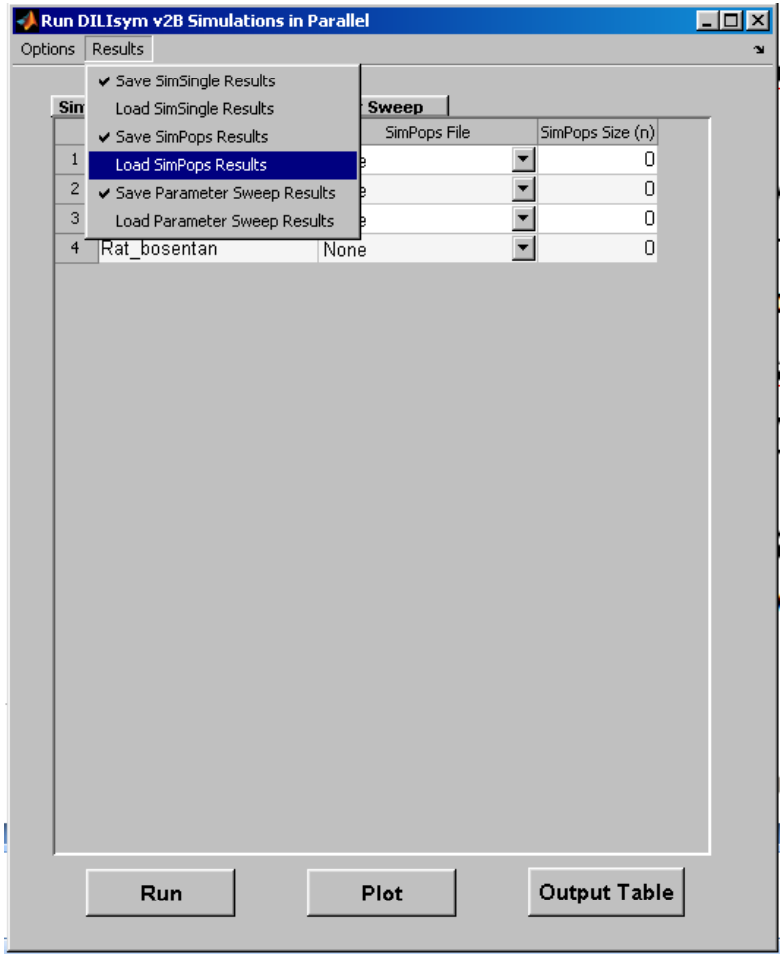
- Can compare the results of each simulation by running them in parallel together

SimSingle™ Results Summary



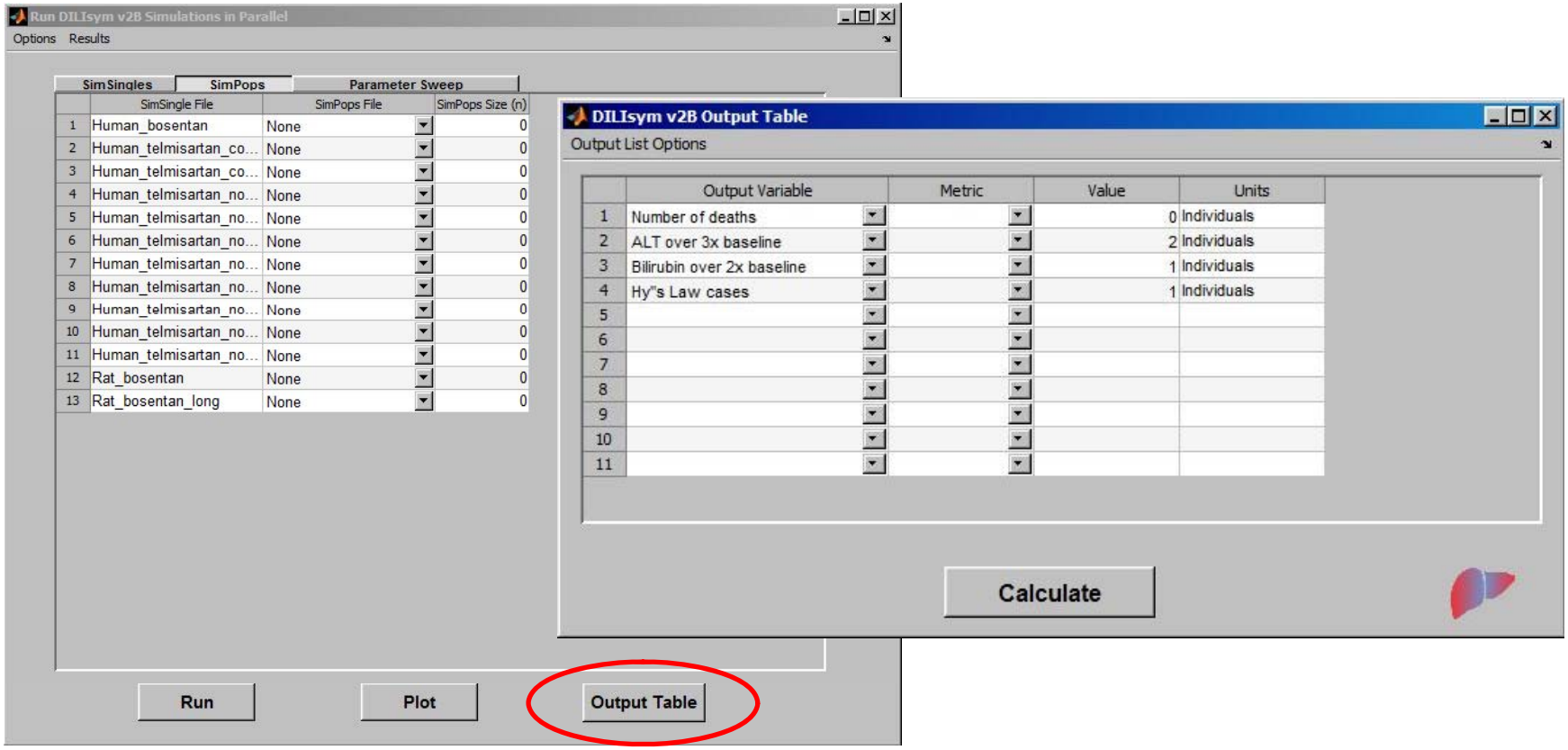
- SimSingle™ results show no toxicity in average individual
  - Average individual does not generally show bile acid-induced toxicity
  - There are some slight elevations of bile acids in the human bosentan baseline
- Will need SimPops™ to investigate if these elevations could cause problems in the general population

Load SimPops™ results



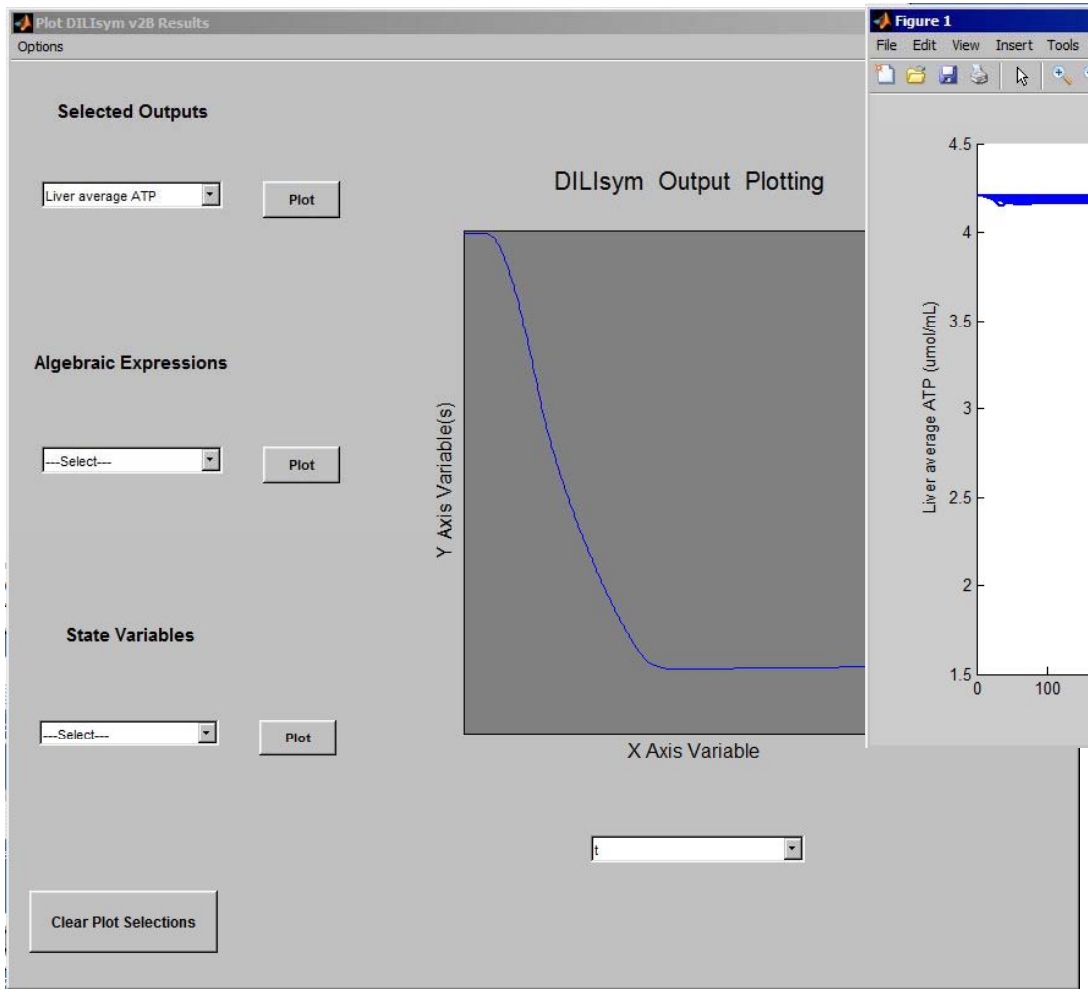
- SimPops™ for human bosentan, rat bosentan, and human telmisartan have been run
  - Time to run would be prohibitive (~2 days)
  - Files are too large to fit on thumb drives; I will show results here
- Bosentan results include enzyme induction equations that are not in v2B
  - Will be included in v3A

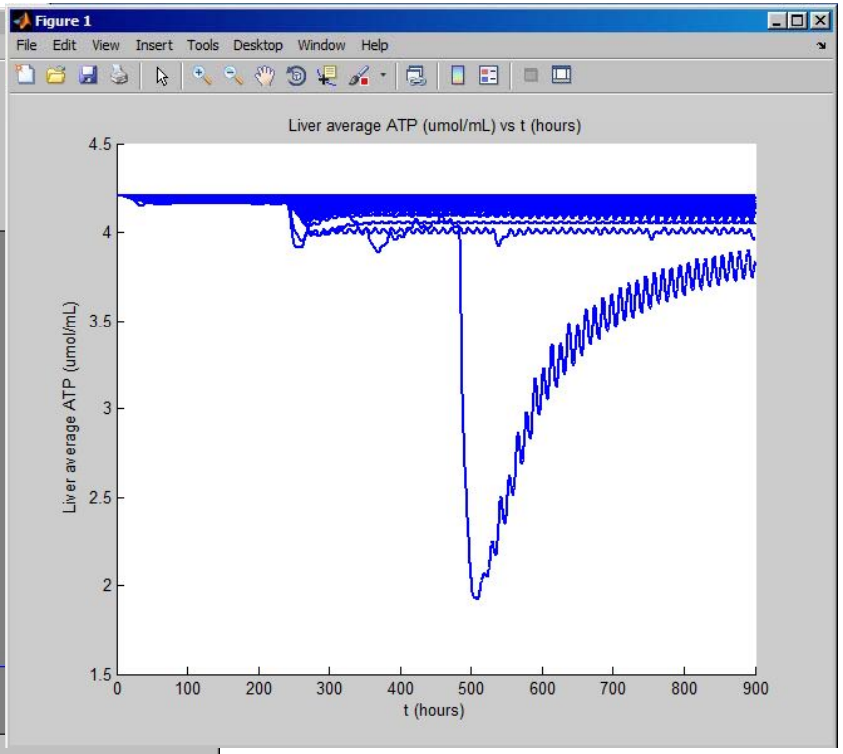
Exploring SimPops™ Using the Output Table



- Bosentan SimPops™ shows 2 individuals with elevated ALT
- Potential cause for worry; deeper investigation of the results is needed

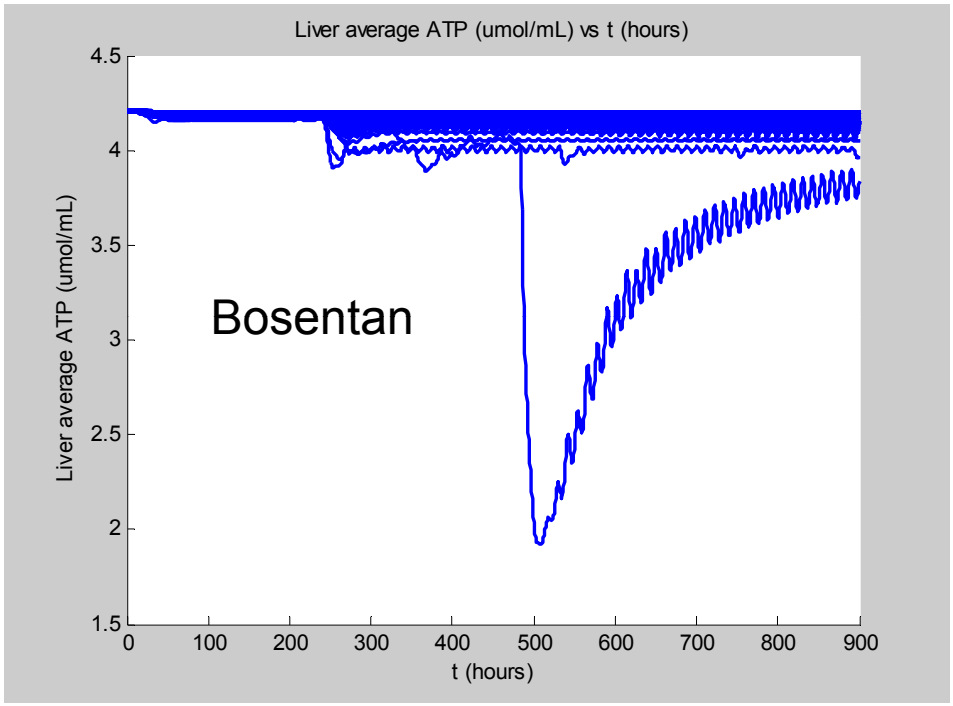
Exploring SimPops™ Using the Plotting Function (1/2)

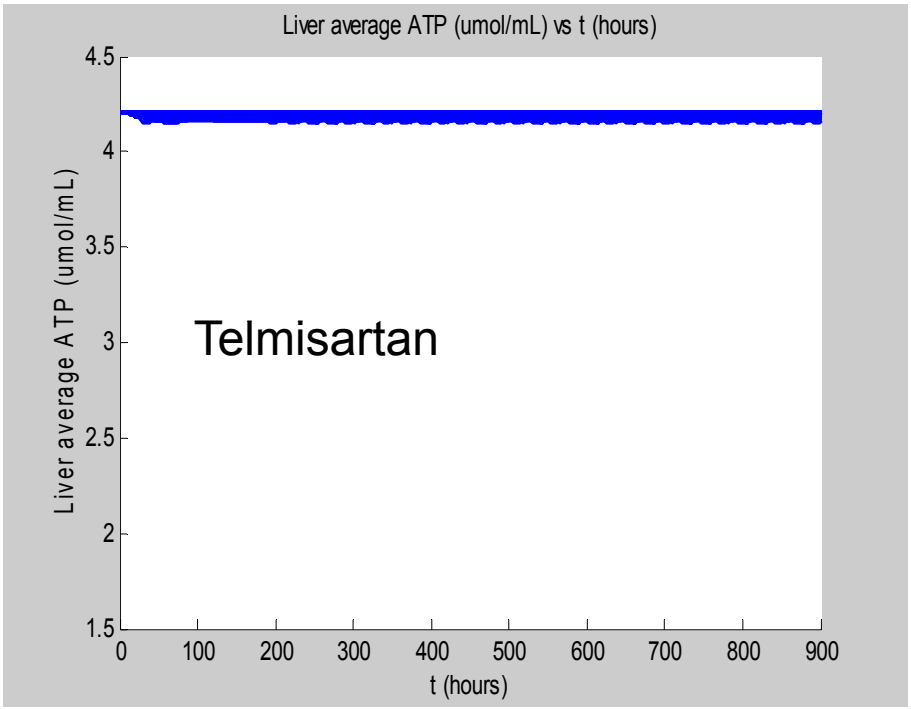




- Many individuals have small decreases in ATP with bosentan

Exploring SimPops™ Using the Plotting Function (2/2)





- ATP does not decline at all in telmisartan SimPops™

Application Example 2: Analysis of Modeling Results

Issue

- Two drugs (bosentan and telmisartan) have been flagged by *in vitro* assays as BSEP inhibitors
- Clinical DILI is linked to BSEP inhibition
- Rat studies have shown no signs of liver injury

Pending Decision

- Should the Company take extra precautions for potential liver injury during clinical trials?

Conclusions from DILIsym® Modeling

- Bile-acid induced hepatotoxicity may be an issue with bosentan in certain individuals
  - Average individual will be fine; toxicity may appear rare
- Rat models are not predictive of the hepatotoxicity that may be seen with bosentan
  - Serum bile acid measurements can be misleading in this regard
- Telmisartan is likely clear of any bile-acid induced hepatotoxicity

DILIsym® Training Agenda – September 26, 2013

- 8:30 AM – Introduction and goals
  - DILIsym® overview and highlights
  - Model architecture notes
- 8:45 AM – Biomarker analysis example
- 9:45 AM – Break
- 10:00 AM – Biomarker analysis example
- 11:00 AM – MITOsym™ overview and introduction
- 11:30 AM – Lunch
- 12:30 PM – Bile acid transport inhibitor example
- 1:30 PM – Break
- 1:45 PM – Bile acid transport inhibitor example
- 2:45 PM – Discussion and questions
- 3:00 PM – Training concludes
  - DILI-sim modeling team is available for questions