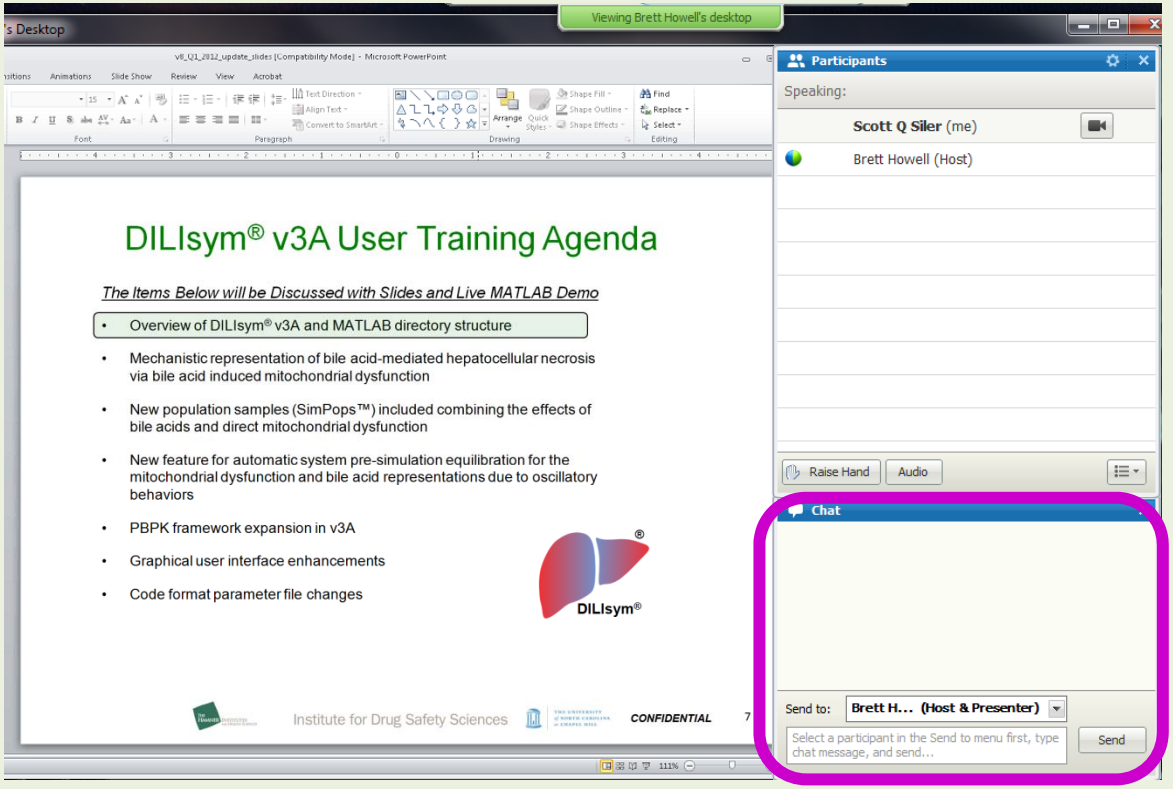
WebEx Tools for Interacting with Presenters Will Be in Use During this Meeting

- **<u>Recording meeting for future reference</u>**
- Large number of meeting participants requires system for orderly interaction
  - Will use WebEx tools
- To make a comment and/or ask a question during the presentation period:
  - Submit in text by using the Chat window (purple outline) and addressing message to 'Questions Here'
- More in-depth Q&A period planned for end of meeting



DILIsym® v3A User Training

April 17, 2014

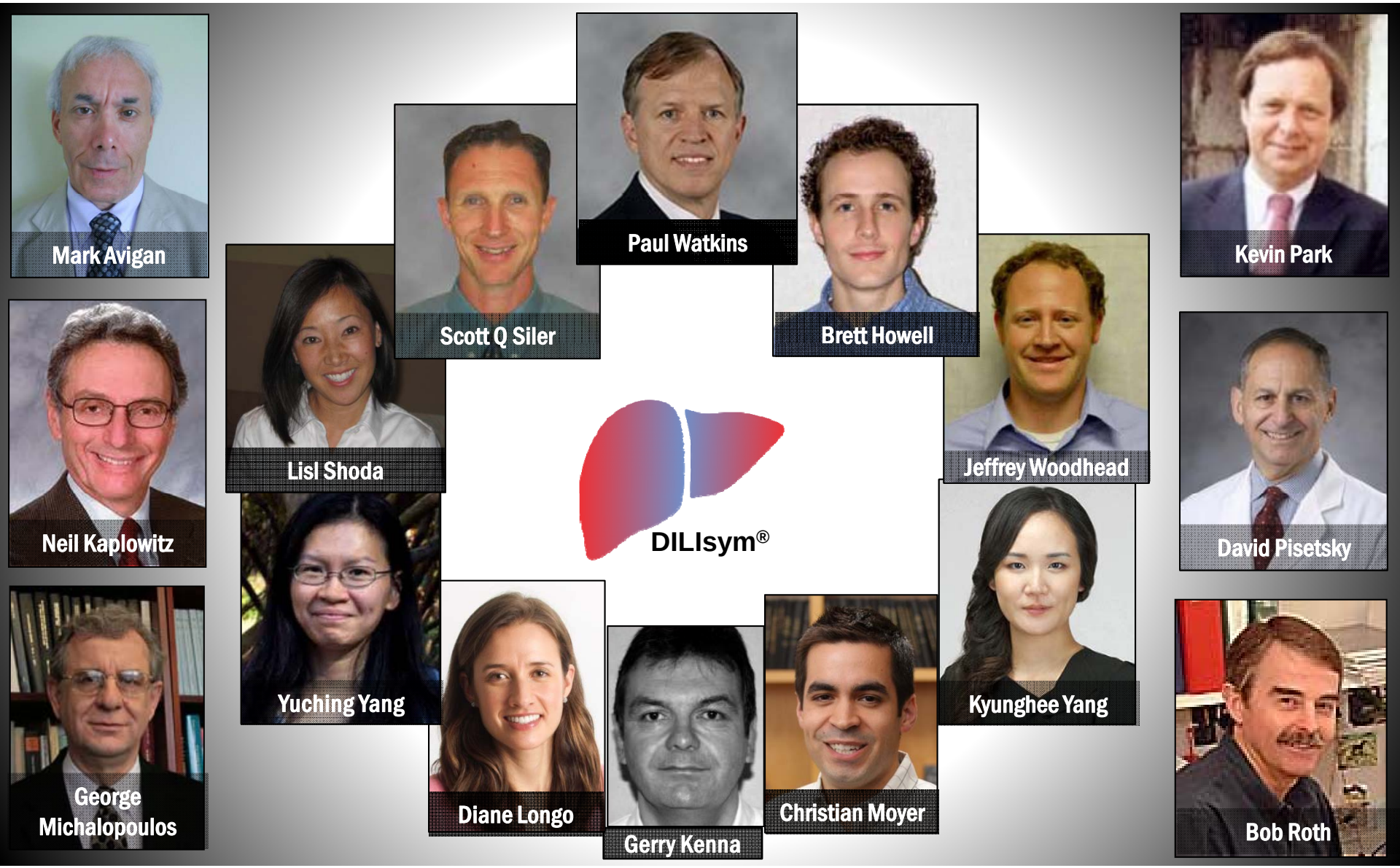
Speakers: Brett A. Howell, Scott Q. Siler

Contact: bhowell@thehamner.org, silerconsulting@comcast.net

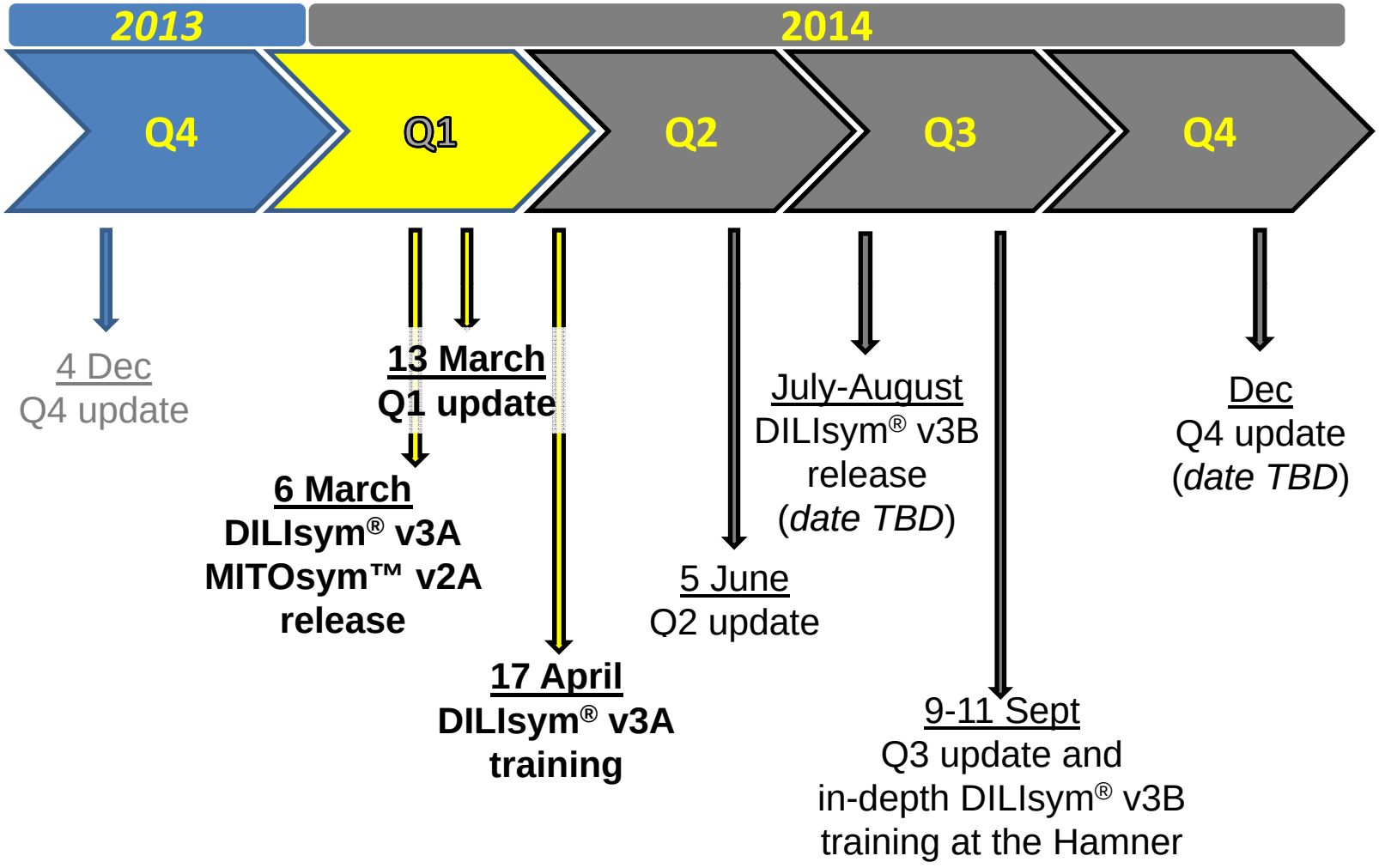
Please note: this presentation, including questions from the audience, is being recorded

Please send questions to the DILIsym® team through the individual listed as “Questions Here” (chat) so we can read them aloud and answer them, time permitting

The DILI-sim Team and the SAB

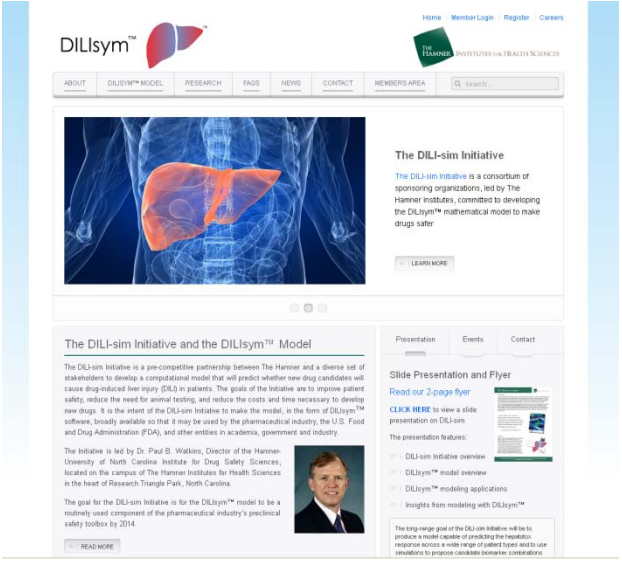


2014 DILI-sim Initiative Key Dates



The DILIsym.com Website

- www.DILIsym.com:
  - Stand-alone webpage, linked to the Hamner Institutes webpage, that provides information and file access
  - Online forum for virtual discussions through threads and post between member companies, the modeling team, and the SAB
    - Password protected
    - Allows for model access via the web
    - General scientific discussions
    - DILI-sim Initiative related discussions (operations)
    - DILIsym® model design discussions
    - DILIsym® technical/practical discussions related to use
    - Additional means for tech support for model users
  - DILIsym® files and documentation are available via a password protected site



- DILI-sim members register for an account to access files and forum discussions
  - Click ‘Register’ at the top right-hand corner of the homepage

DILIsym® v3A Training Session Goals

- This training session will provide users with knowledge of updates and additions to DILIsym® as of the v3A release
- This training session should be a supplement to previously recorded training sessions
  - DILIsym® versions 1A through 2C training sessions
  - All sessions accessible at www.DILIsym.com
- This training session is not an in-depth exploration of the application of the model, but application questions are welcome at the end of the session

DILIsym® v3A User Training Agenda

The Items Below will be Discussed with Slides and Live MATLAB Demo

- Overview of DILIsym® v3A and MATLAB directory structure
- Mechanistic representation of bile acid-mediated hepatocellular necrosis via bile acid induced mitochondrial dysfunction
- New population samples (SimPops™) included combining the effects of bile acids and direct mitochondrial dysfunction
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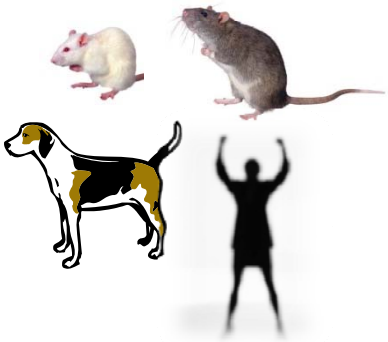
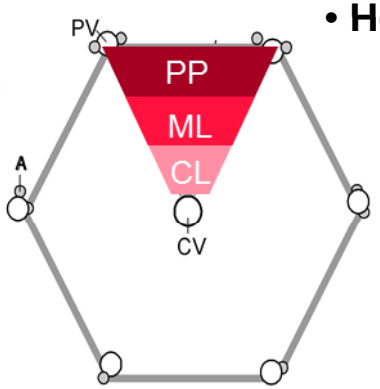
- PBPK framework expansion in v3A
- Graphical user interface enhancements
- Code format parameter file changes

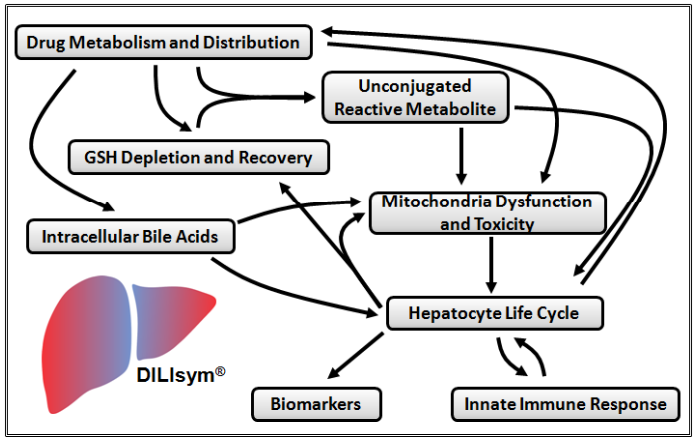
DILIsym®

DILIsym® v3A 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





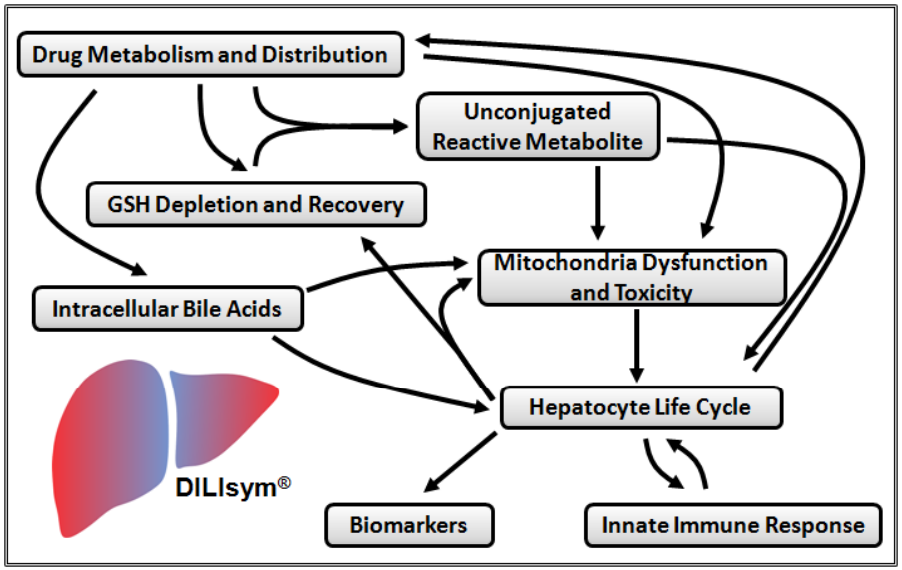


- **Compartment-based modeling**
  - >500 state variables
  - 'Form to function' connection
  - Ordinary differential equations
  - Code or GUI functionality

- **Hepatotoxicity exemplars**
  - Reactive metabolite mediated
    - Acetaminophen
    - Methapyrilene
    - Furosemide
    - Aflatoxin B1
    - Carbon tetrachloride
  - Mitochondrial dysfunction
    - Etomoxir
    - Buprenorphine
    - Tolcapone
    - CP-724714
  - Bile acid transporter inhibition
    - Glibenclamide
    - CP-724714
    - Bosentan
    - Telmisartan
    - Tolcapone
    - Troglitazone
  - Single, multiple dose protocols
  - Single, combination drug protocols

Highlights of DILIsym® v3A

- Added option of mechanistic representation of bile acid-mediated hepatocellular necrosis via bile acid induced mitochondrial dysfunction
- Expanded and refined the representation of innate immune contributions to injury and recovery
  - Mediator production structure altered
  - Innate immune parameter values for mouse, rat, dog, and human updated
- Introduced new structure for pre-equilibration of state variables prior to simulations
- Expanded capabilities of GUI interface
- Expanded PBPK framework



- Introduced additional exemplar compounds for exposure-related toxicity
  - Tolcapone
  - Telmisartan
  - Bosentan
  - Carbon tetrachloride
- Additional SimPops™, capturing impact of variability in key pathways

Expanded Capabilities and Changes for DILIsym® v3A

- PBPK framework expanded for additional drug metabolism schemes
  - Secondary metabolite generation now possible
  - Suicide inhibition now possible
  - Drug metabolism enzyme induction added
  - Free drug concentration representation also adjusted
- Innate immune representation expanded and enhanced
  - Mediator production structure altered
  - Innate immune parameter values for mouse, rat, dog, and human updated
- Added option of mechanistic representation of bile acid-mediated hepatocellular necrosis via bile acid induced mitochondrial dysfunction
- Added new feature for automatic system pre-simulation equilibration for the mitochondrial dysfunction and bile acid representations due to oscillatory behaviors
- New population samples (SimPops™) included combining the effects of bile acids and direct mitochondrial dysfunction
  - NASH-like patients with severely compromised mitochondrial function are also included
- New drug/chemical representations for:
  - Telmisartan
  - Carbon tetrachloride
  - Bosentan
  - Tolcapone
- Numerous graphical user interface (GUI) enhancements, including:
  - Parameter names and descriptions are now visible from within the GUI
  - New plotting feature allowing comparisons to data within GUI plot window
  - New Data comparisons feature allowing storage of population sample (SimPops) simulations, and comparisons to measured data
- Expanded Zotero reference database (contact us for real-time access)

DILIsym® v3A User Training Agenda

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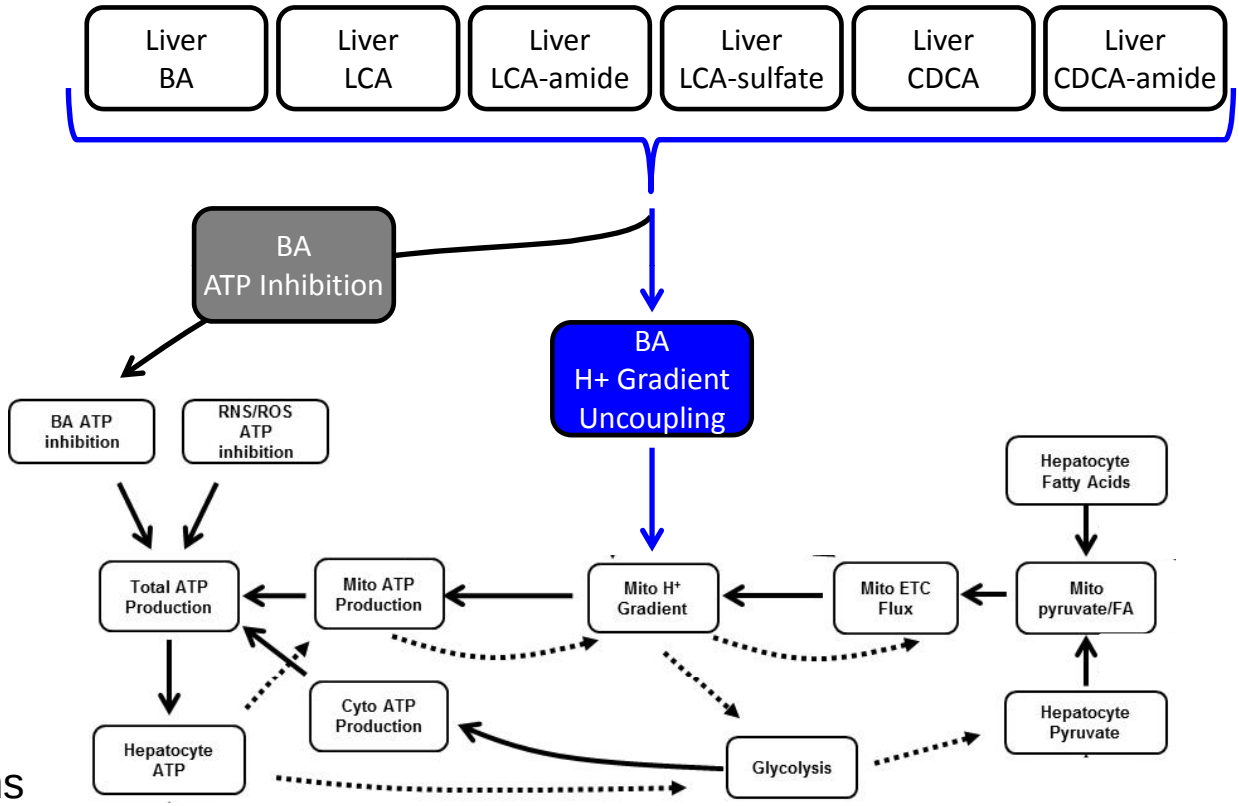
DILIsym®

Liver Bile Acids Elicit ATP Reductions in More Mechanistic Manner in DILIsym® v3A

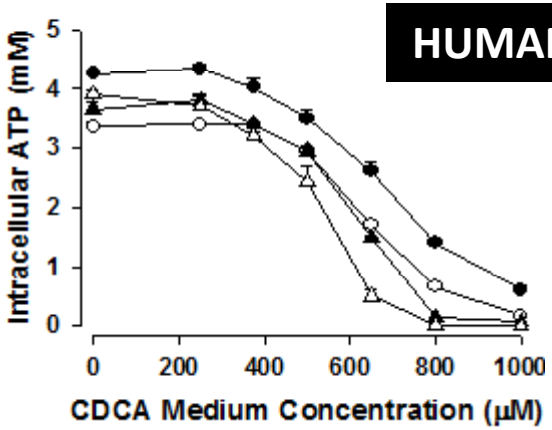
- Liver bile acids cause reductions in mitochondria proton gradient in v3A
  - BA H+ Gradient Uncoupling
- Parameter optimization based on Yang et al. hepatocyte studies (*submitted*)
  - Measured ATP and cell death following exposure to different levels of bile acids
  - Supported by DILI-sim Initiative
- Prior representation still available as an option
  - Direct effect on ATP synthesis
- BA H+ Gradient Uncoupling is better option if combined with other mitochondria mechanisms
  - Includes integration of mitochondria feedback signals
  - BA ATP Inhibition useful when it is the only mechanism



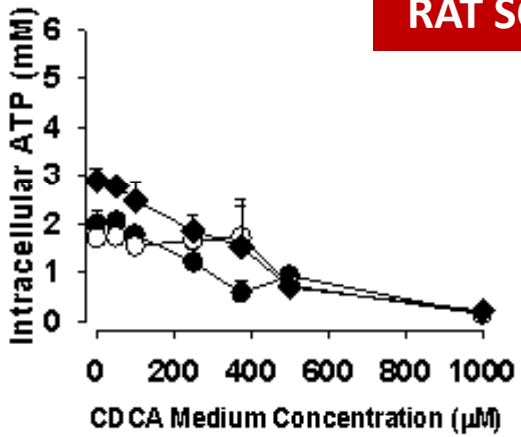
Summary of Findings from Sandwich Culture Hepatocyte Bile Acid Toxicity Studies

- Human SCH were more resistant to LCA-mediated toxicity compared to rat SCH based on the intracellular LCA concentrations
- CDCA induced medium-concentration dependent ATP loss and toxicity in rat and human SCH. Toxicity was correlated with hepatic unconjugated CDCA in rat SCH
  - Various bile acid species have been reported to initiate MPT, decrease mitochondria proton gradient, and decrease OCR[‡]
- Both BA H+ Gradient Uncoupling and BA ATP Inhibition options are optimized based on these data

HUMAN SCH



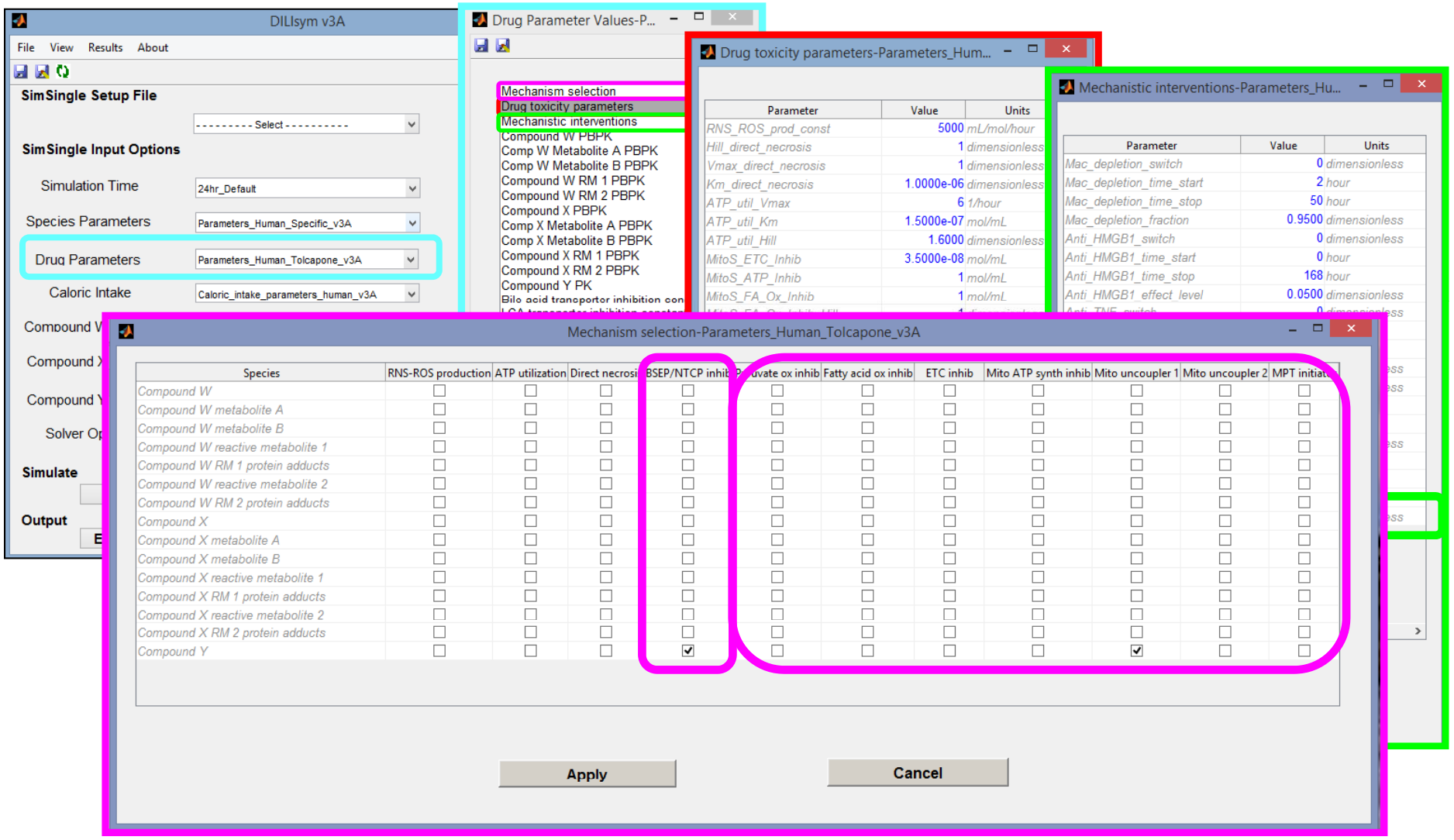
RAT SCH



Yang unpublished

‡ Denk 2012, Sokol 2005, Botla 1995, Rolo 2003, Rolo 2004, Utanohara 2005, Yerushalmi 2001, Nadanaciva unpublished

Three Parameters Required to Activate BA H+ Gradient Uncoupling



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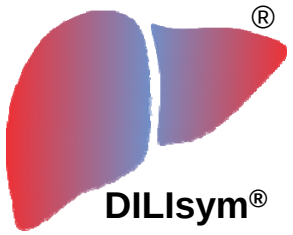
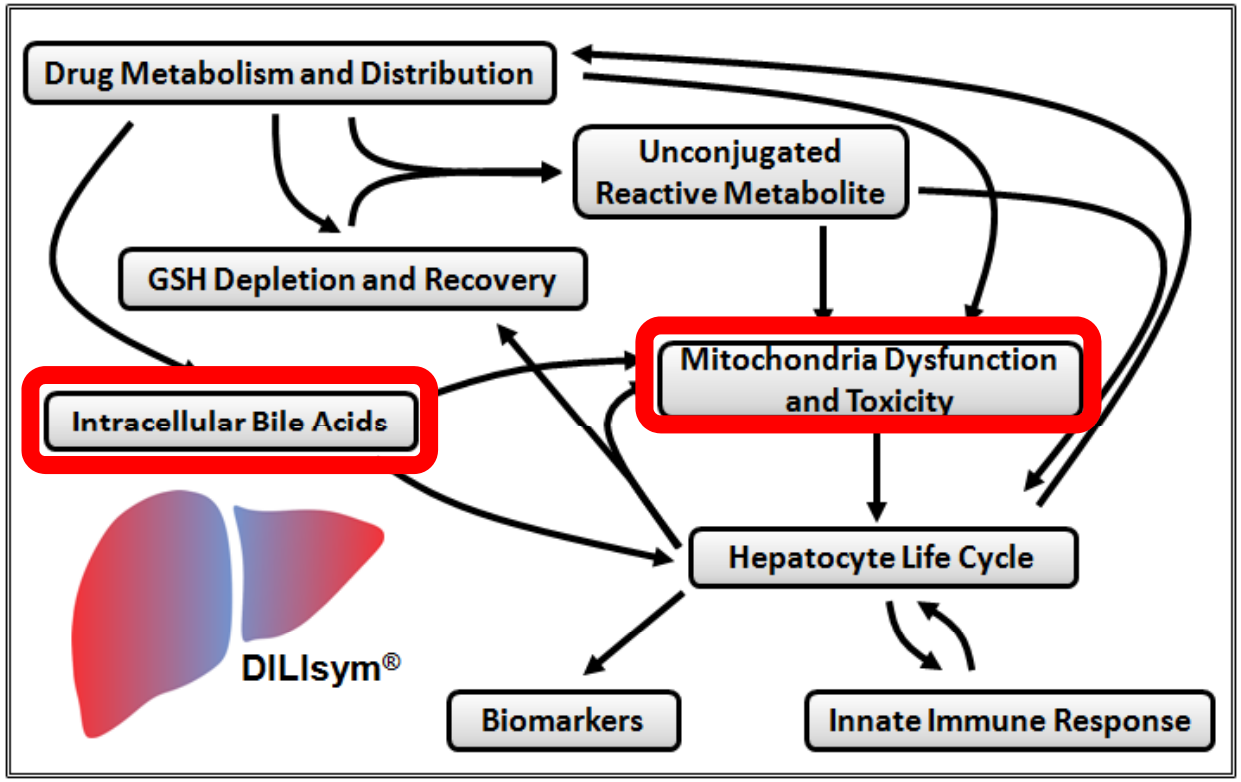


New Mitochondria and Bile Acids SimPops™ Introduced in DILIsym® v3A

SimPops™	Species/ Strain	Population Sample Size	Number of Parameters Varied	Applicable Hepatotoxicity Mechanisms
Mouse_ROS_v2A_1	Mice	400	10	Reactive oxygen species
Mouse_mito_v3A_5_exploration_only	Mice	200	5	Mitochondria
Rat_ROS_v2A_2	Rats	400	9	Reactive oxygen species
Rat_mito_v3A_4_exploration_only	Rats	200	5	Mitochondria
Rat_bile_acid_v2B_5	Rats	191	11	Bile Acids
Dog_ROS_v2A_3	Dogs	338	12	Reactive oxygen species
Dog_mito_v3A_3_exploration_only	Dogs	200	6	Mitochondria
Human_ROS_v2A_4	Humans	458	9	Reactive oxygen species
Human_bile_acid_v2A_5	Humans	331	10	Bile acids
Human_mito_v3A_1_exploration_only	Humans	150	2	Mitochondria
Human_mito_v3A_2_exploration_only	Humans	176	6	Mitochondria
Human_mito_BA_v3A_6_exploration_only	*Humans*	*229*	*15*	*Mitochondria and bile acids*

New Exploratory SimPops™ in DILIsym® v3A (v3A_6, N = 229) can be Used for Mitochondrial Dysfunction and/or Bile Acid Disruption

- Useful for mitochondrial dysfunction simulations
- Useful for bile acid homeostasis disruption simulations
- Useful (and designed for) combinations of bile acid homeostasis disruption and mitochondrial dysfunction
- As with other mitochondrial dysfunction SimPops™, the population sample has not been validated with endpoint patient data due to lack of available data



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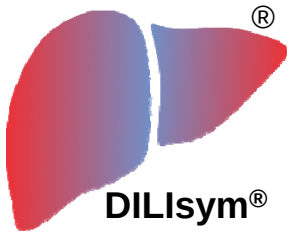
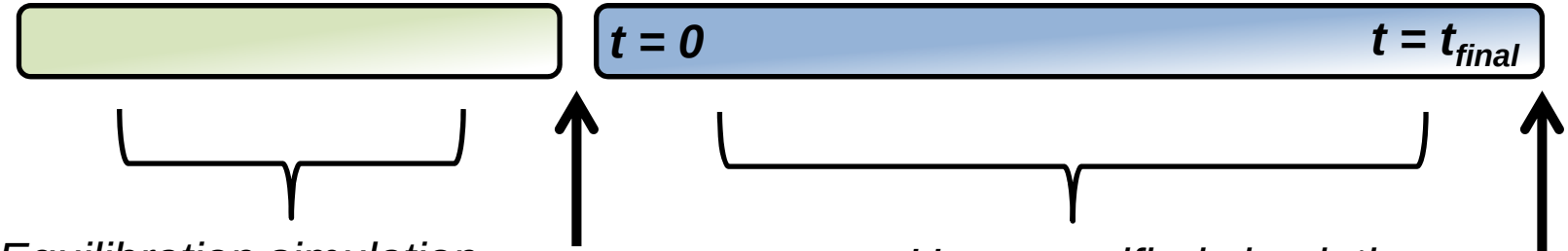


Automated Equilibration Simulations Have been Incorporated into DILIsym® as of v3A

- What are equilibration capabilities?
  - Equilibration is the process of running a simulation within DILIsym® (typically without drug) prior to running the simulation specified by the user (a 'pre-simulation')
- What is accomplished with equilibration?
  - Equilibration allows DILIsym® outputs (state variables and algebraic expressions) to reach new steady state values that are different than the initial starting values, due to the oscillatory nature of many aspects of DILIsym®, including meals, and due to the difficulty in calculating the correct steady state values for every output *a priori* with a large equation set
  - Loading stored steady state values is also an option in v3A
- What types of applications is equilibration recommended for?
  - Bile acid homeostasis simulations
  - Mitochondrial dysfunction simulations

A Chronological View of Equilibration in DILIsym®

Running an equilibration simulation



Equilibration simulation (no drug)

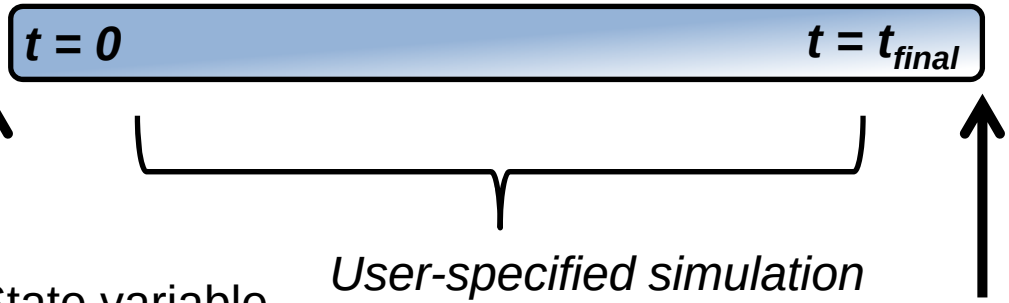
baseline_sim = 0

- Final state variable values carried forward
- User-specified simulation begins

User-specified simulation

Simulation ends

Loading equilibrated initial conditions (default)



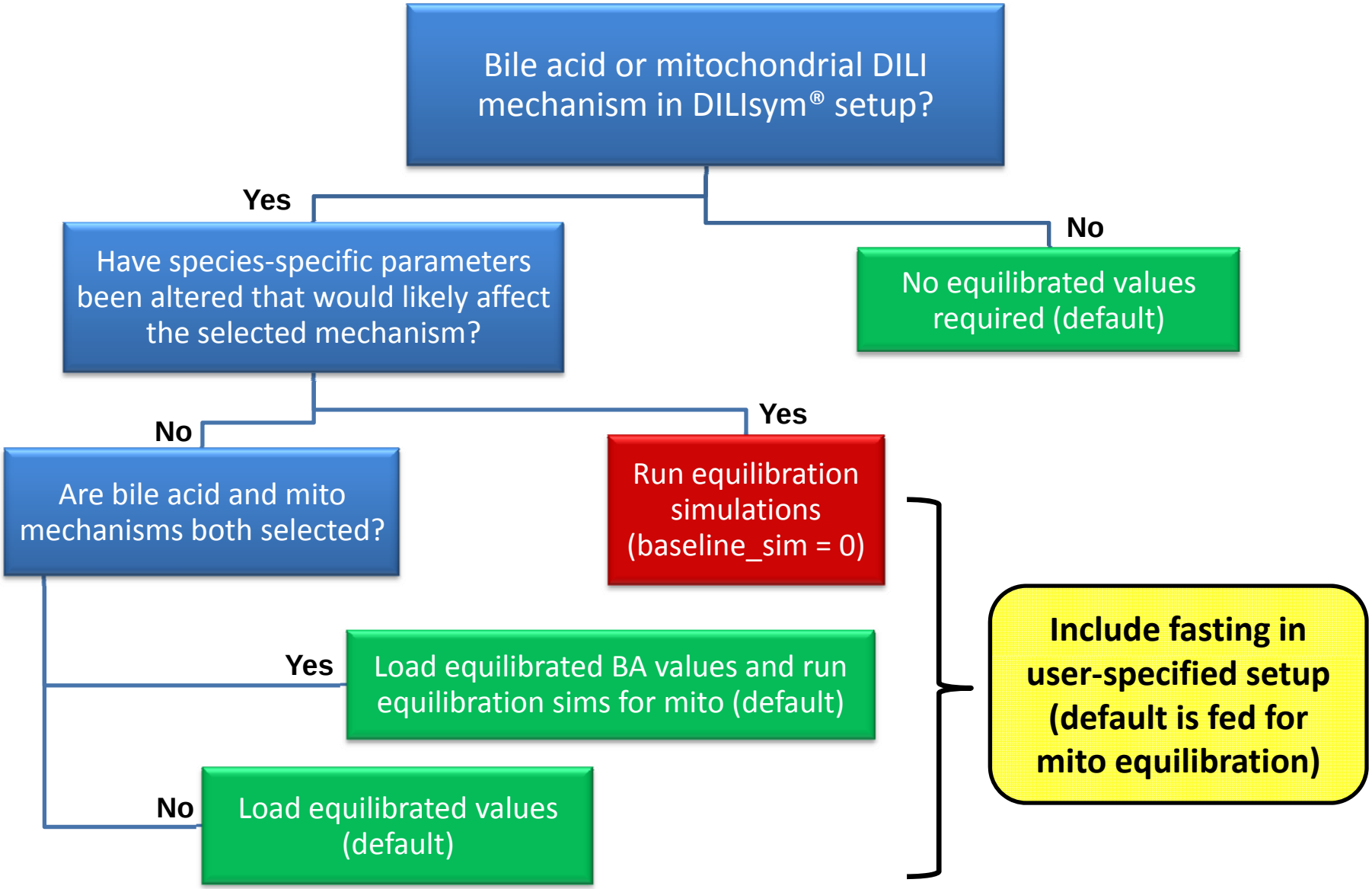
baseline_sim = 1

- State variable values loaded from previous sims

User-specified simulation

Simulation ends

DILIsym® v3A Equilibration Decision Tree



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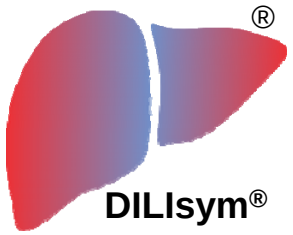
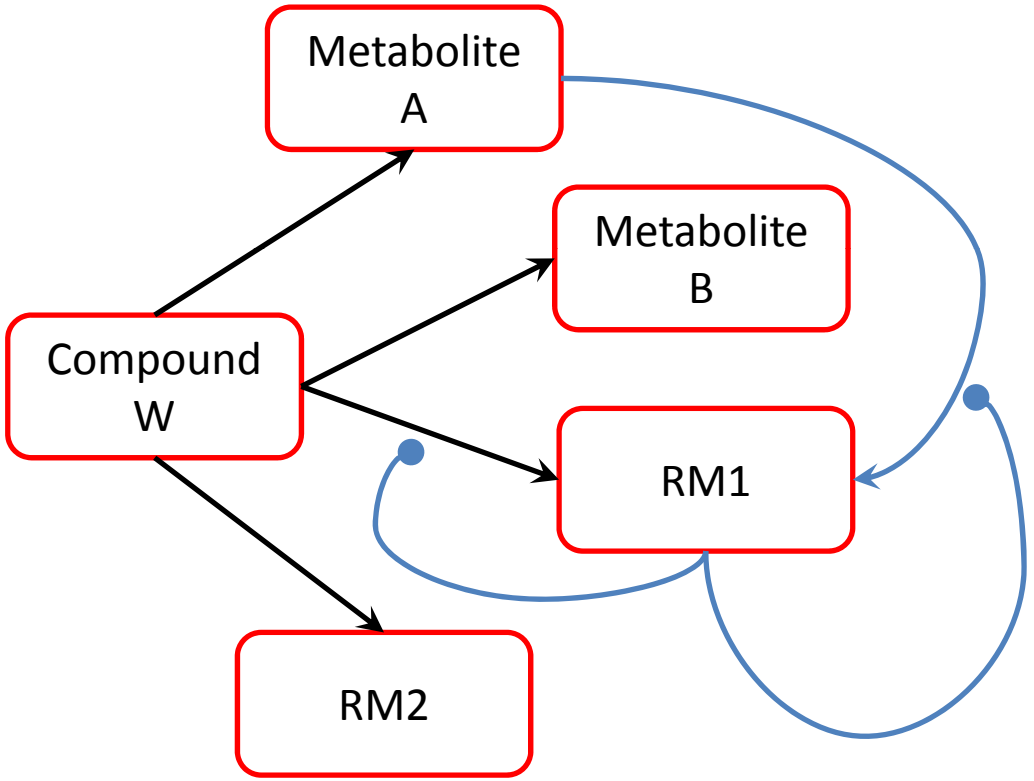


DILIsym® Metabolism Structure Changed to Accommodate Lapatinib and CCl_4



- Metabolism of Met A to RM1
- Suicide inhibition of Met A -> RM1
  - Inhibition constant model
- Suicide inhibition of parent -> RM1
  - Empirical model

Using the New Metabolism and Suicide Inhibition Parameters

- Vmax and Km defining metabolism of Metabolite A to RM1 are located in the RM1 PBPK section (applies to Compounds W and X)
  - Vmax_CompW_RM1_from_MetA (mol/h/kg$^{0.75}$)
  - Km_CompW_RM1_from_MetA (mol/mL)
- Suicide inhibition parameters for parent to RM1 reaction are located in the RM1 PBPK section
  - CompW_RM1_inhib_start_time (h)
  - CompW_RM1_inhib_stop_time (h)
  - CompW_RM1_inhib_percent (dimensionless)
- Suicide inhibition parameters for Metabolite A to RM1 reaction also located in the RM1 PBPK section
  - CompW_MetA_RM1_inhib_Vmax (mol/h/kg$^{0.75}$)
  - CompW_MetA_RM1_inhib_Ki (mol/mL)
  - k_enzyme_turnover_CompW (1/h)

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