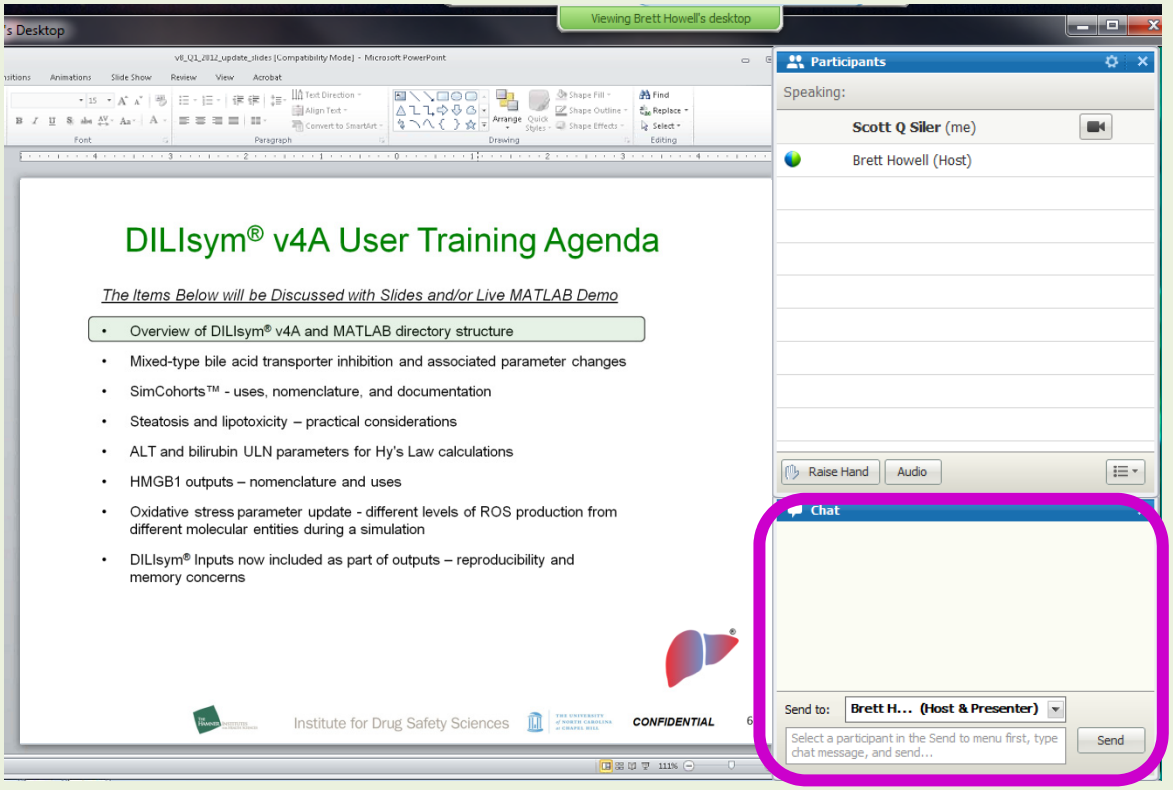
WebEx Tools for Interacting with Presenters Will Be in Use During this Meeting

- **<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® v4A User Training

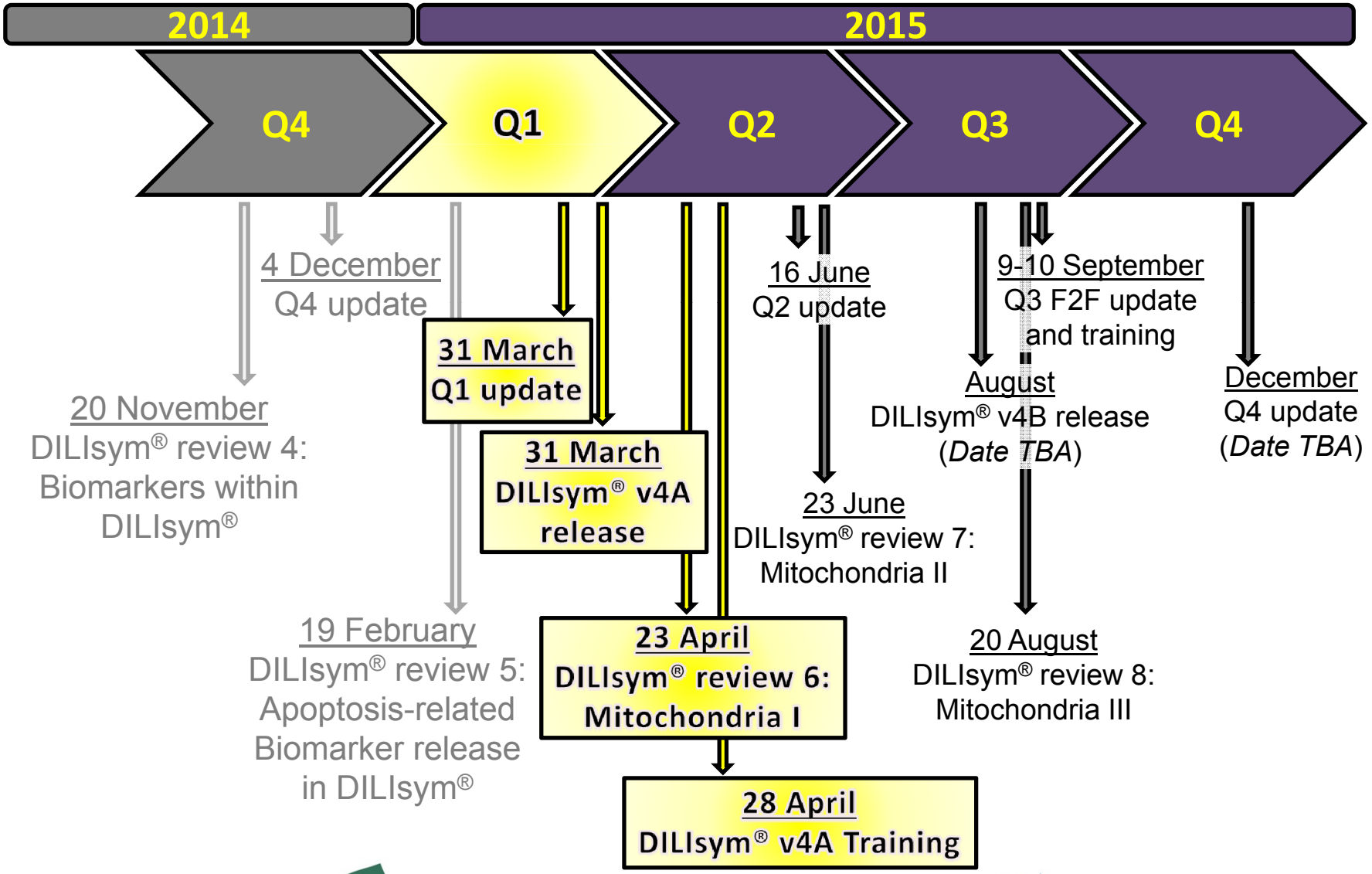
Please note: this presentation, including questions from the audience, is being recorded

April 28, 2015

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

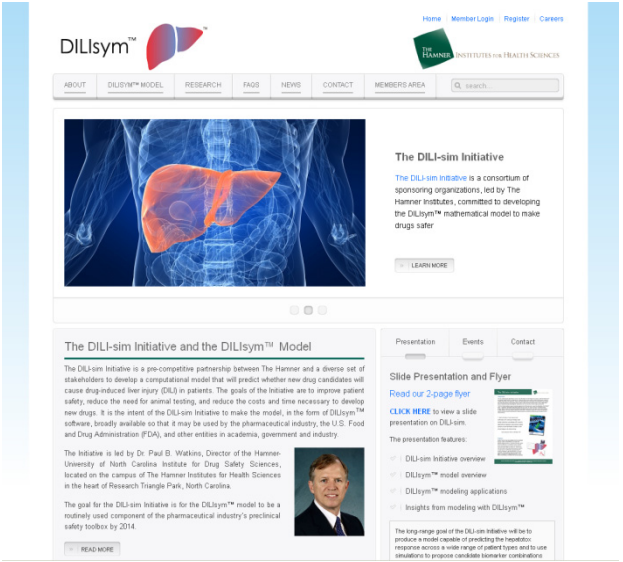
Speakers: DILIsym® Development Team

2015 DILI-sim Initiative Key Dates



The DILIsym.com Website Provides Members Access to Software, Presentations, and Training Materials

- www.DILIsym.com:
  - Stand-alone webpage, linked to the Hamner Institutes webpage, that provides information to the public
  - DILIsym® software files and documentation are available via a password protected site
    - Password protected
    - Allows for software access via download
    - See the 'MEMBERS AREA' tab
  - DILI-sim presentations and training materials are regularly uploaded for members
  - Online forum for virtual discussions through threads and post between member companies, the modeling team, and the SAB
    - Additional means for tech support, user discussions, and general questions for other members
- DILI-sim members should register for an account in the upper right-hand corner of the site to access files and forum discussions



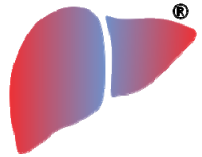
DILIsym® v4A Training Session Goals

- This training session will provide users with knowledge of updates and additions to DILIsym® as of the v4A release
- This training session should be a supplement to previously recorded training sessions
  - DILIsym® versions 1A through 3B 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® v4A User Training Agenda

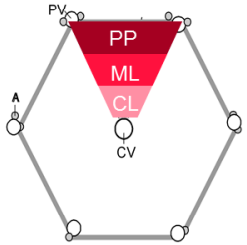
The Items Below will be Discussed with Slides and/or Live MATLAB Demo

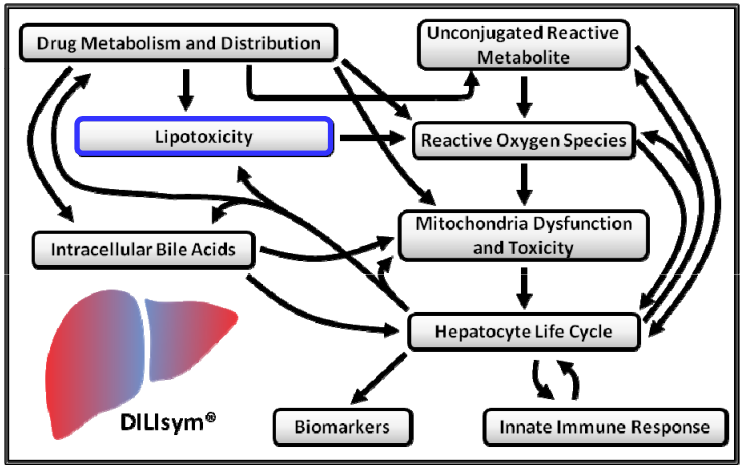
- Overview of DILIsym® v4A and MATLAB directory structure
- Mixed-type bile acid transporter inhibition and associated parameter changes
- SimCohorts™ - uses, nomenclature, and documentation
- Steatosis and lipotoxicity – practical considerations
- ALT and bilirubin ULN parameters for Hy's Law calculations
- HMGB1 outputs – nomenclature and uses
- Oxidative stress parameter update - different levels of ROS production from different molecular entities during a simulation
- DILIsym® Inputs now included as part of outputs – reproducibility and memory concerns



DILIsym® v4A 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
  - Steatosis and lipotoxicity
  - Cellular energy balance
  - Hepatocyte apoptosis and necrosis, and proliferation
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers



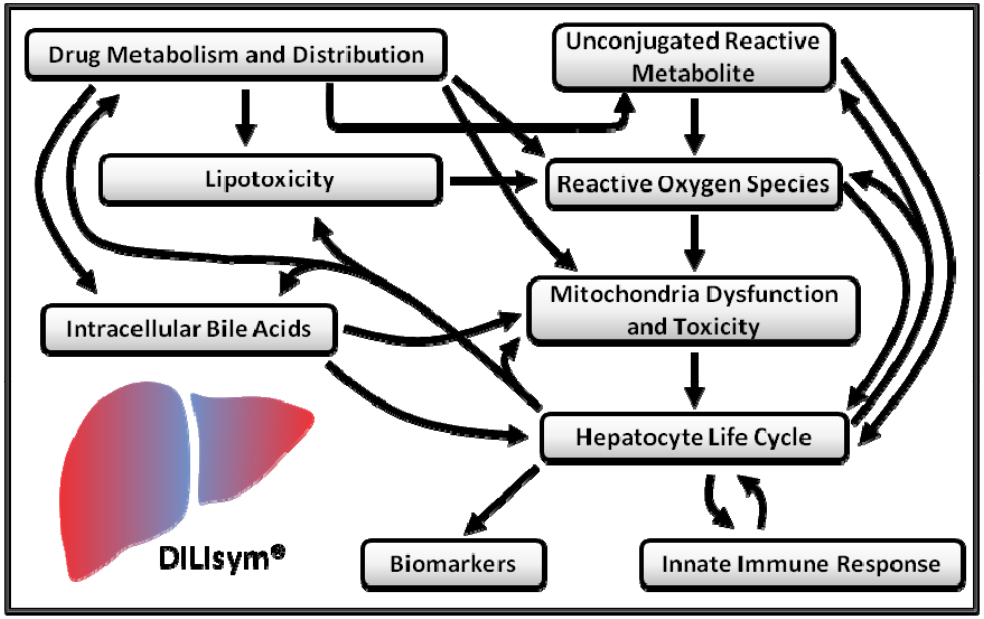


- **Compartment-based modeling**
  - >500 state variables
  - 'Form to function' connection
  - Ordinary differential equations
  - Code or GUI functionality

- **Hepatotoxicity exemplars**
  - Reactive metabolite mediated
    - Acetaminophen
    - Methapyrilene
    - Furosemide
    - Aflatoxin B1
    - Carbon tetrachloride
  - Mitochondrial dysfunction
    - Etomoxir
    - Buprenorphine
    - Tolcapone
    - Entacapone
    - CP-724714
  - Bile acid transporter inhibition
    - Glibenclamide
    - CP-724714
    - Bosentan
    - Telmisartan
    - Tolcapone
    - Troglitazone
    - Pioglitazone
    - AMG009
  - Single, multiple dose protocols
  - Single, combination drug protocols

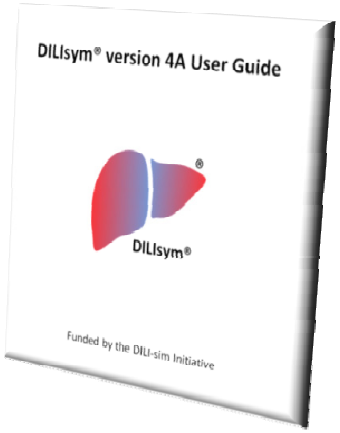
Highlights of DILIsym® v4A

- Capability for mixed-type bile acid transporter inhibition was added and input parameters were simplified
- Steatosis and lipotoxicity from saturated fatty acids was added
- Secondary necrosis sub-model was re-designed
  - More mechanistic and fundamentally based than previous version
  - Different redox states of HMGB1 now included
- MATLAB 2014b-based graphics bugs were fixed
  - DILIsym® now only compatible with MATLAB 2014b and 2015a
- Additional SimPops™, capturing impact of variability in key pathways
  - SimPops™ combining oxidative stress, mitochondrial dysfunction, caspase activation, and bile acid variability in humans and rats



- SimCohorts™ were introduced, which are subsets of existing SimPops™ selected randomly or based on sensitivity to DILI
  - Many cohort sizes included for all species
  - Greatly reduces simulation time for screening
- AMG009 compound files and data added
- Entacapone compound files and data added

Expanded Capabilities and Features of DILIsym® v4A



- Added capability for mixed-type bile acid transporter inhibition
  - Removed individual Ki's for each bile acid species and lumped them by transporter
  - Competitive and non-competitive inhibition now utilize different equation structures
- Steatosis and lipotoxicity from saturated fatty acids was added
  - De novo lipogenesis (DNL) provides pathway for carbohydrate to be converted to fatty acids
  - Added ChREBP effect
  - Fatty acid accumulation leads to more saturated fatty acids, ROS, and hepatocyte death
- MATLAB graphics bugs from 2014b changes were fixed
  - MATLAB drastically altered their graphics package as of 2014b
  - DILIsym® v4A not fully compatible with MATLAB 2014a or prior versions
- New parameters for ROS mechanism allows for up to three different levels of ROS production from different molecular entities during a simulation (as opposed to one before)
- Added ALT and bilirubin ULN parameters for Hy's Law calculations and altered Hy's Law criteria in Output Table to be consistent with FDA guidance (rather than fold change used before)
- Added all DILIsym® inputs needed for a simulation as outputs of the simulation, which allows for reproducibility (not the default for SimPops™ due to memory issues, however)
- Algebraic expressions were modified to have separate expressions for human total bile acids (including LCA sulfate) and rat total bile acids (excluding LCA sulfate) in blood and liver
- All bile acid transport inhibitors for humans (bosentan, telmisartan, troglitazone, pioglitazone, AMG009, glibenclamide, CP-724714, tolcapone, and entacapone) are now defaulted to the mechanistic bile acid toxicity model
- All bile acid transport inhibitors for rats (glibenclamide, bosentan, troglitazone, AMG009, and CP-724714) are now defaulted to the less mechanistic, ATP driven, direct bile acid toxicity model
- Expanded Zotero reference database (contact us for real-time access)
- Various bug fixes
  - License expiration issue resolved

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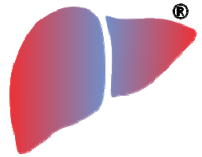
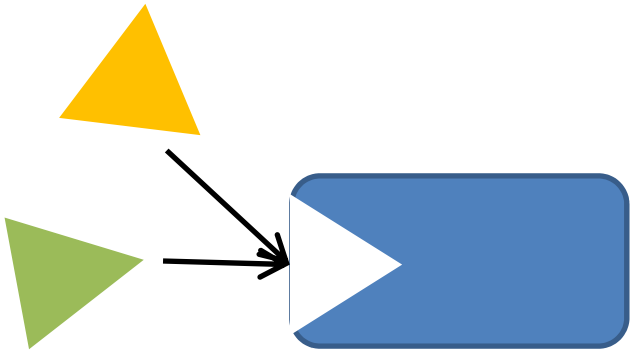
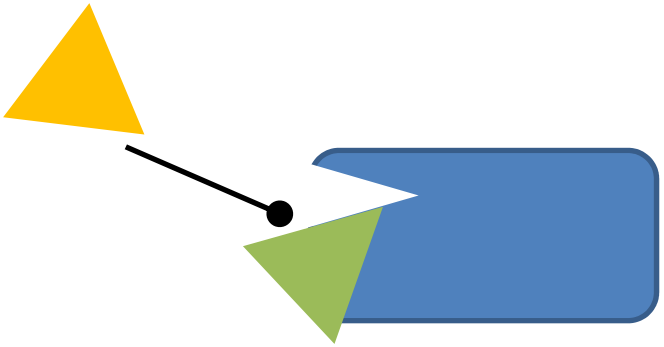


Competitive and Noncompetitive Inhibition



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}

DILIsym® v4A Includes Ability to Represent Mixed Transporter Inhibition

- Some bile acid transporter inhibitors are best described by "mixed" inhibition equation
  - Represents inhibition that is not completely noncompetitive but has some noncompetitive nature
  - Mixed inhibition governed by α parameter
  - Changes in α represent changes in the mechanism of inhibition; higher α is representative of more competitive-like inhibition while lower α is representative of more noncompetitive-like inhibition
- Mixed/noncompetitive inhibition represented together in DILIsym® v4A
  - Noncompetitive inhibition can be looked at as a special case of mixed inhibition where $\alpha = 1$
- α can be calculated from results of the normal K_i assay
  - No extra experimental effort necessary

Competitive Inhibition

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

Noncompetitive Inhibition

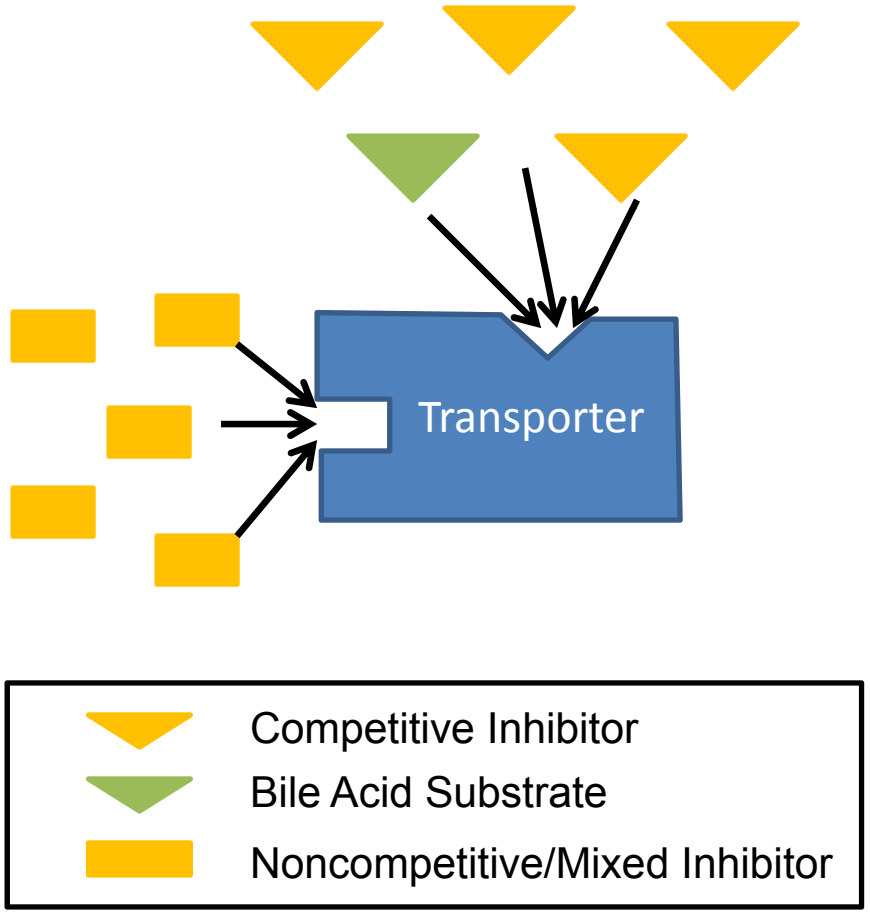
$$\frac{d[BA]}{dt} = \frac{\frac{V_{max}}{(1+\frac{[I]}{K_i})}[BA]}{K_m+[BA]}$$

Mixed Inhibition

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

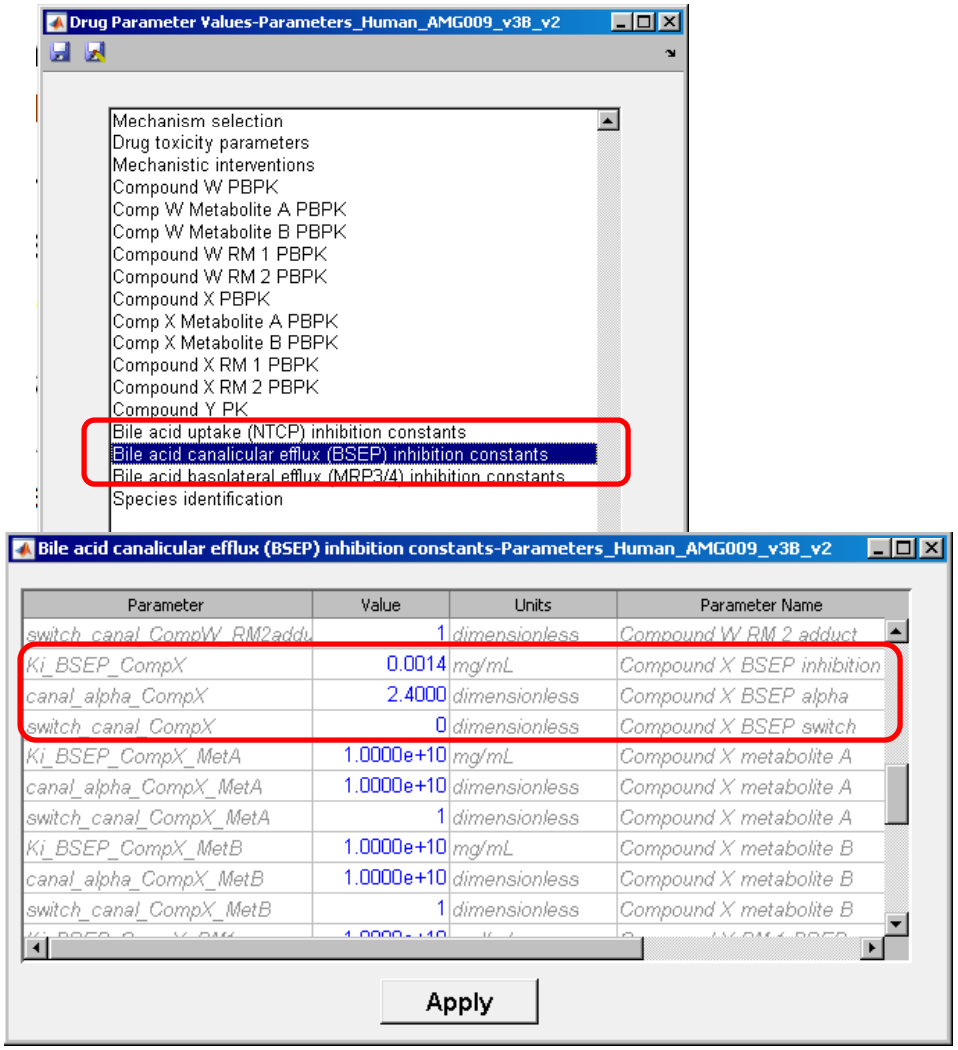
DILIsym® Model of Mixed Inhibition for v4A Contains Same Assumptions as v3B Inhibition Model

- DILIsym® assumes that transporters have one competitive inhibition binding site and one noncompetitive inhibition binding site
  - Competitive inhibitors compete with each other and with other bile acids
  - Noncompetitive inhibitors compete with each other but not with bile acids
  - Compound cannot be both competitive and mixed/noncompetitive inhibitor
- Mathematically equivalent to representation in DILIsym® v3B
  - Assumptions are the same as those made in v3B, just more explicit
  - Equations can be updated in the future if understanding of the process changes



DILIsym® v4A Includes New Input Windows for Bile Acid Transport Inhibition Constants

- Individual bile acid K_i values have been eliminated in DILIsym® v4A
  - Capability to represent inhibition differently introduced too many ease-of-use issues for not enough benefit
- Inhibition parameters grouped by transporter rather than by type
  - Uptake, basolateral, and canalicular rather than competitive and noncompetitive
- Each compound's mechanism and K_i is determined by three parameters for each transporter
  - K_i exists as before
  - Alpha is the mixed inhibition parameter – set to 1 for noncompetitive
  - Switch determines which equation is used; 0 for noncompetitive/mixed, 1 for competitive



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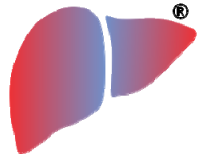
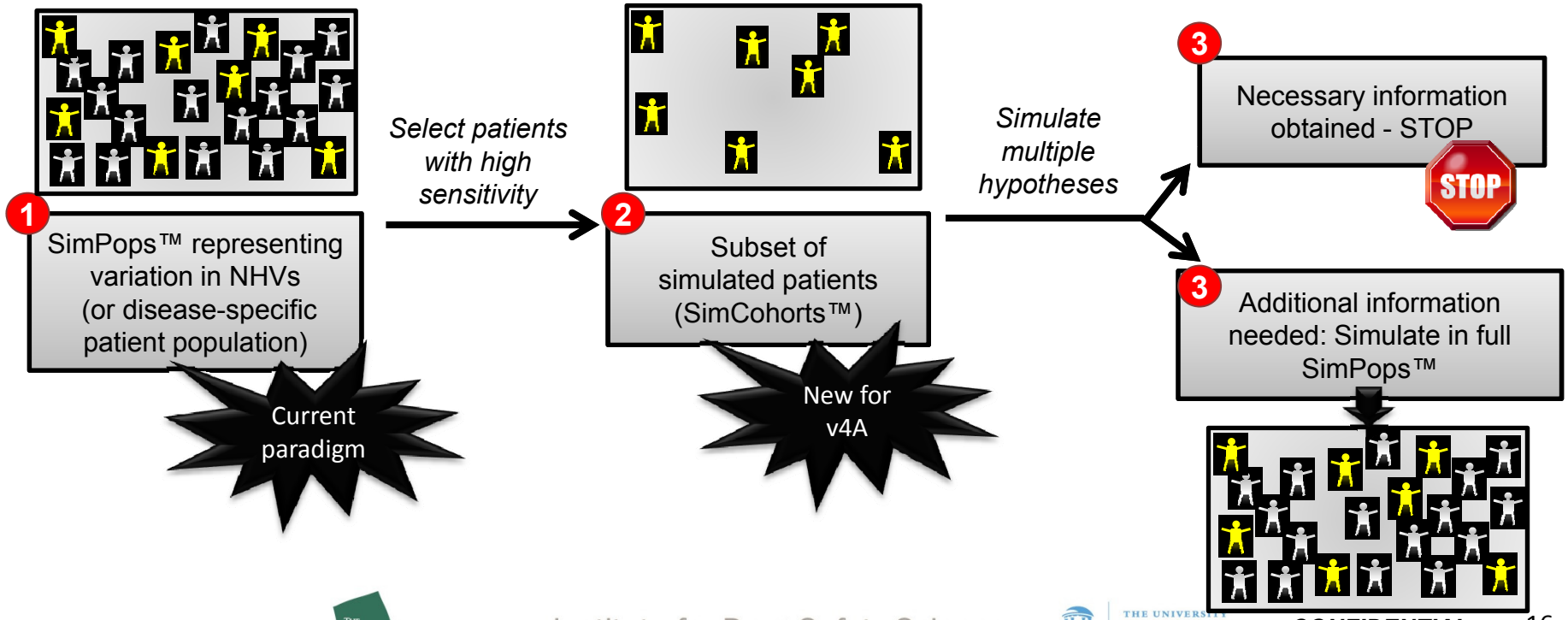


DILIsym® v4A Includes SimCohorts™

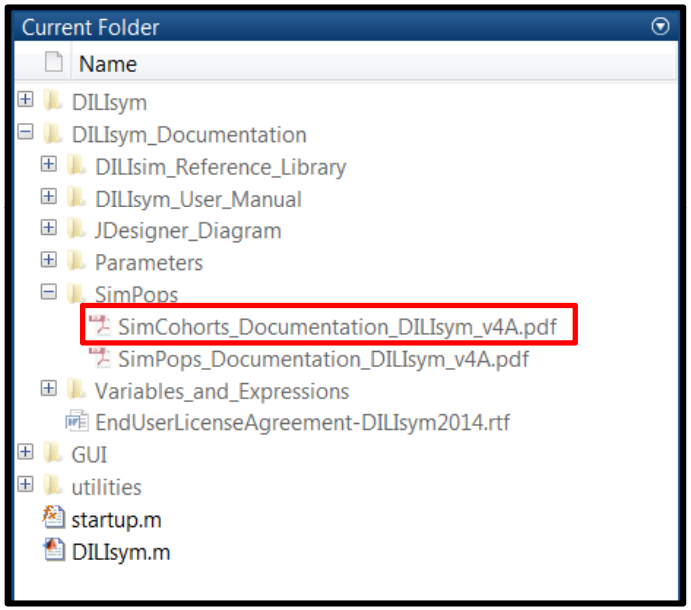
- SimCohorts™ were generated for screening purposes
  - Smaller populations consisting of subsets of simulated individuals from existing SimPops™
  - Computationally less-expensive for testing multiple hypotheses prior to full SimPops™
- Sensitive Sample SimCohorts™
  - Generated by selecting sensitive simulated individuals from larger SimPops™
- Random Sample SimCohorts™
  - Generated by randomly sampling from larger SimPops™

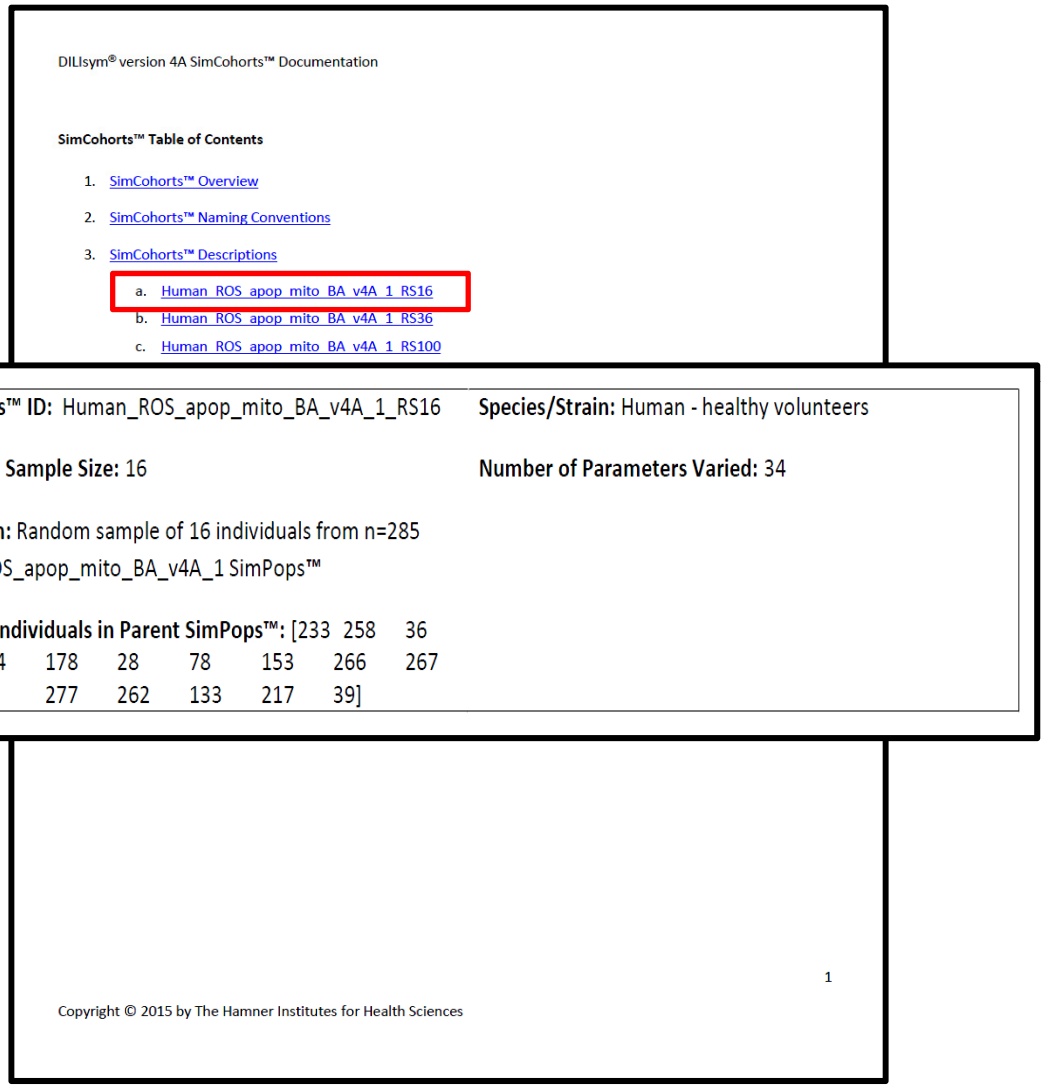


SimCohorts™ Naming Conventions

- SimCohorts™ in DILIsym® are named using the following convention:
  - "SimPops™ name" followed by either RS[XX] or Sens[Mechanism], where:
    - RS[XX] = random sample of XX, e.g. "RS16" for a random sampling of 16
    - Sens[Mechanism] = sensitivity to a particular mechanism of DILI, e.g. "SensROS" for sensitivity to RNS/ROS production
  - Examples
    - Human_ROS_apop_mito_BA_v4A_1_RS16
    - Mouse_ROS_apop_mito_v3B_4_SensMito

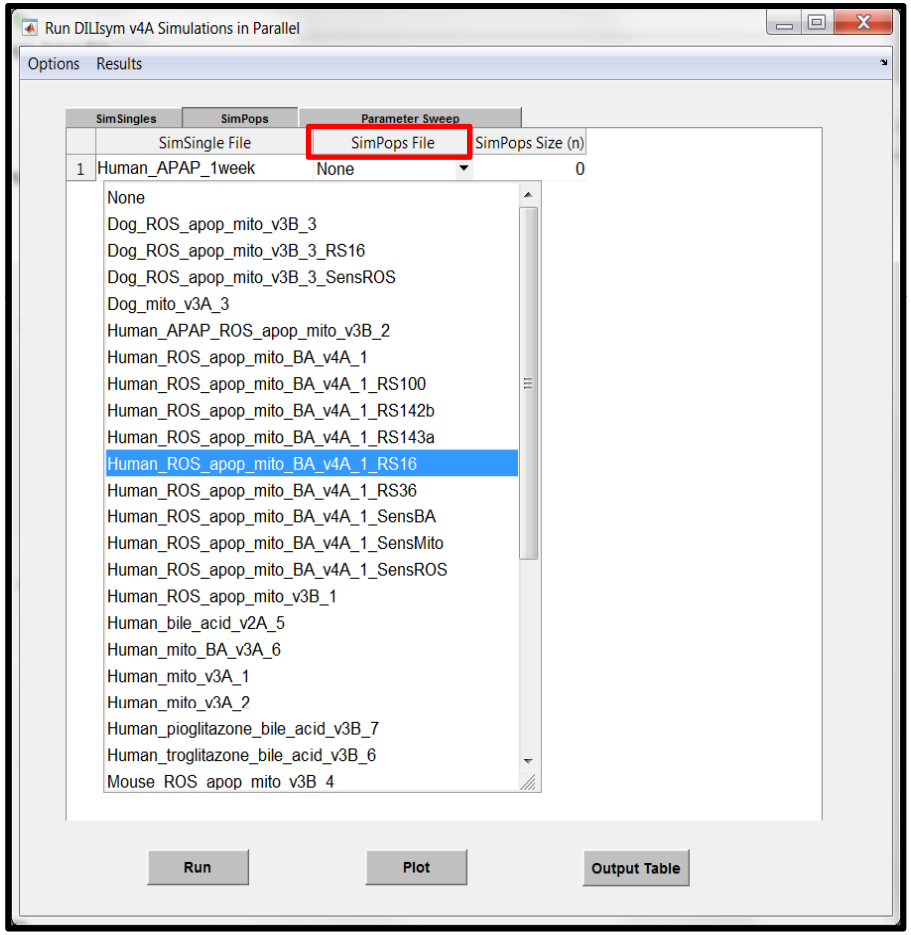
SimCohorts™ Documentation Available to Users





DILIsym® v4A GUI for Running Simulations in SimCohorts™

- Parallel Simulations window can be used to partner any existing SimSingle™ with any SimCohorts™
- All existing SimCohorts™ appear (along with all existing SimPops™) under the “SimPops File” drop-down menu
- Can plot results or use basic output table



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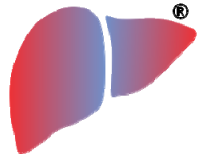
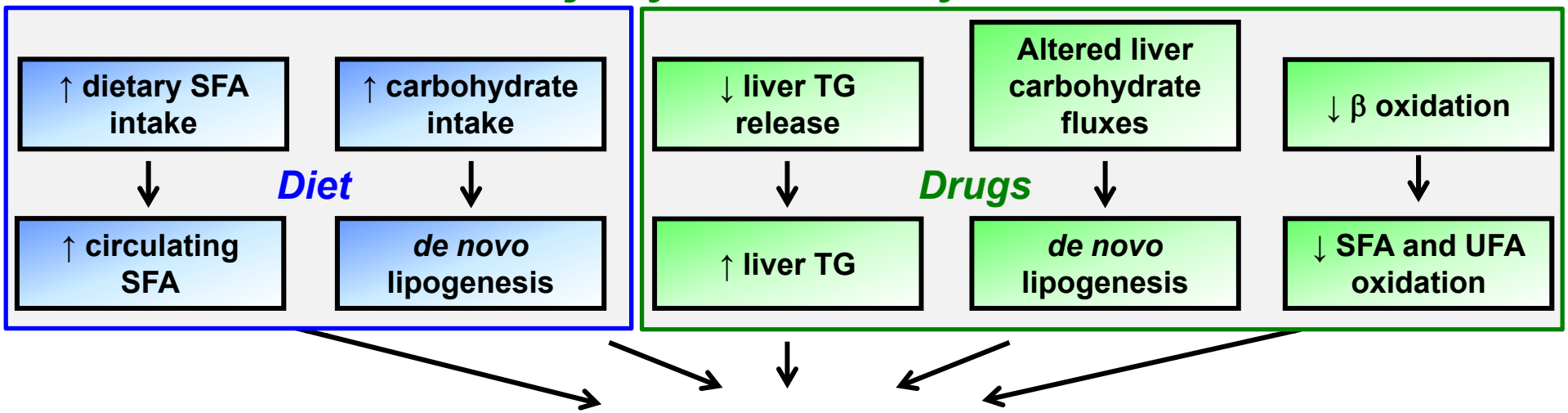
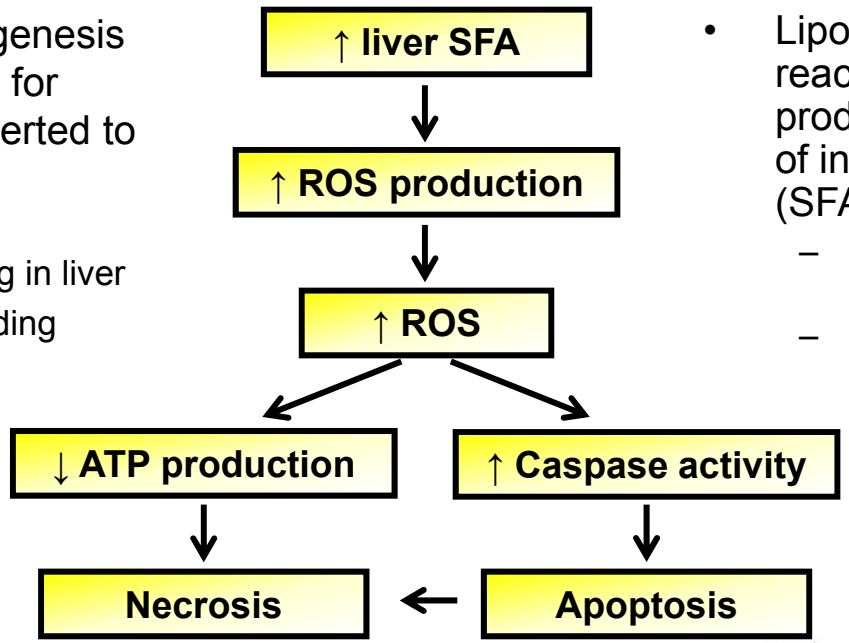


How Lipid Accumulation Is Connected to Injury in DILIsym®

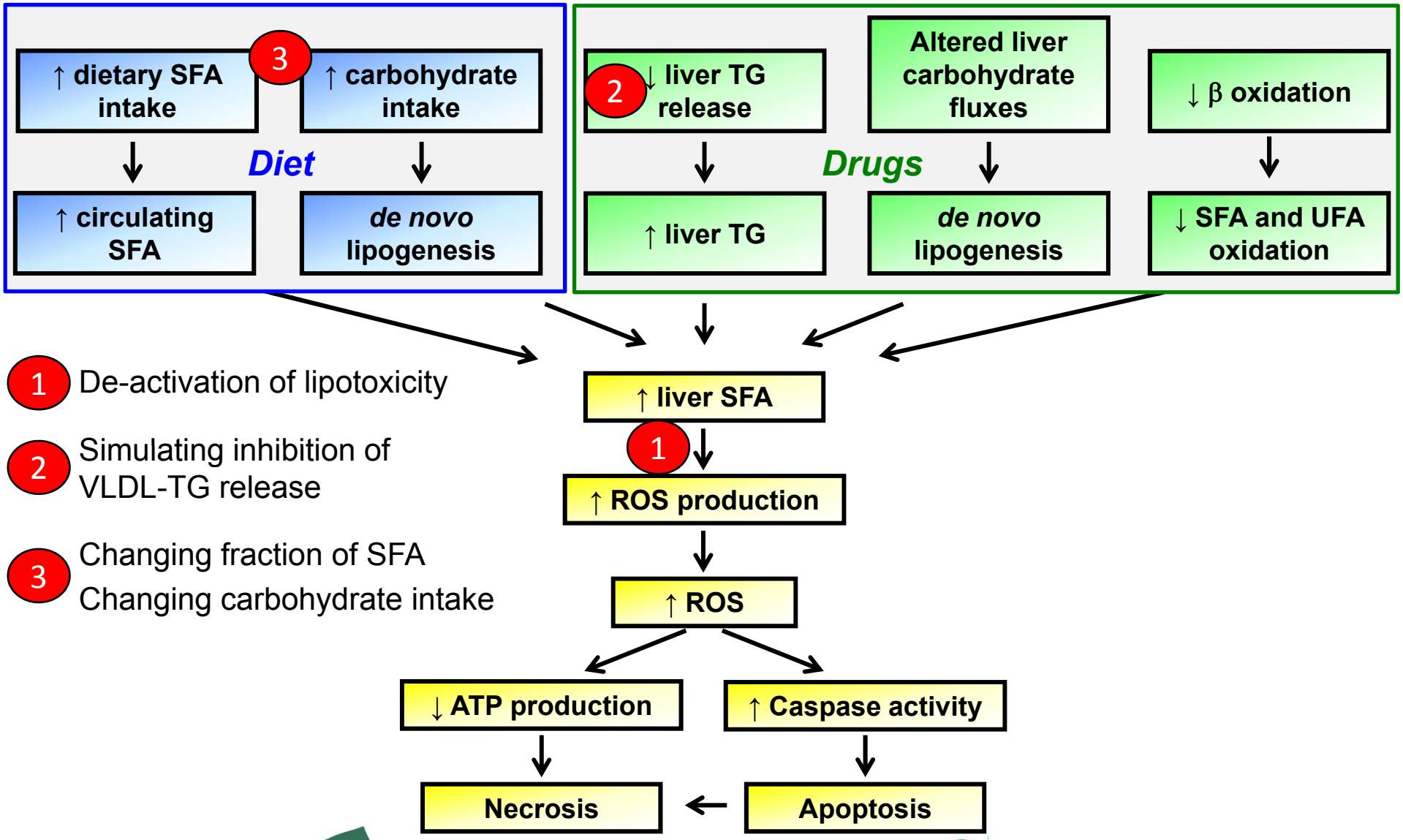


- Addition of *de novo* lipogenesis (DNL) provides pathway for carbohydrate to be converted to fatty acids
  - Drugs that manipulate carbohydrate trafficking in liver
  - Carbohydrate overfeeding



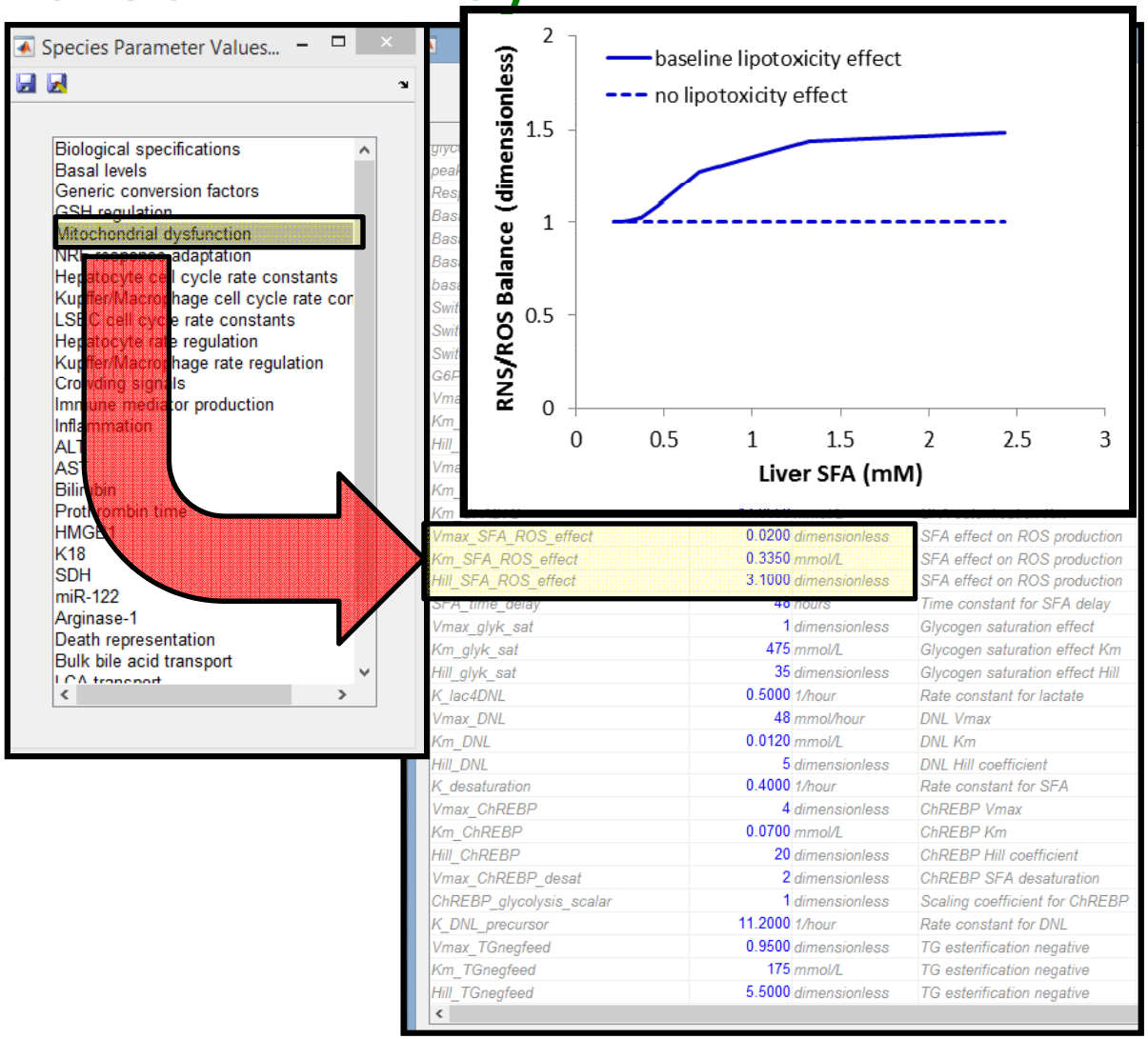
- Lipotoxicity due to increased reactive oxygen species (ROS) production as a consequence of increased saturated fatty acid (SFA) levels
  - SFA typically minority component
  - Can increase in certain situations and initiate lipotoxicity in liver

Modifying Lipotoxicity Parameters in DILIsym®



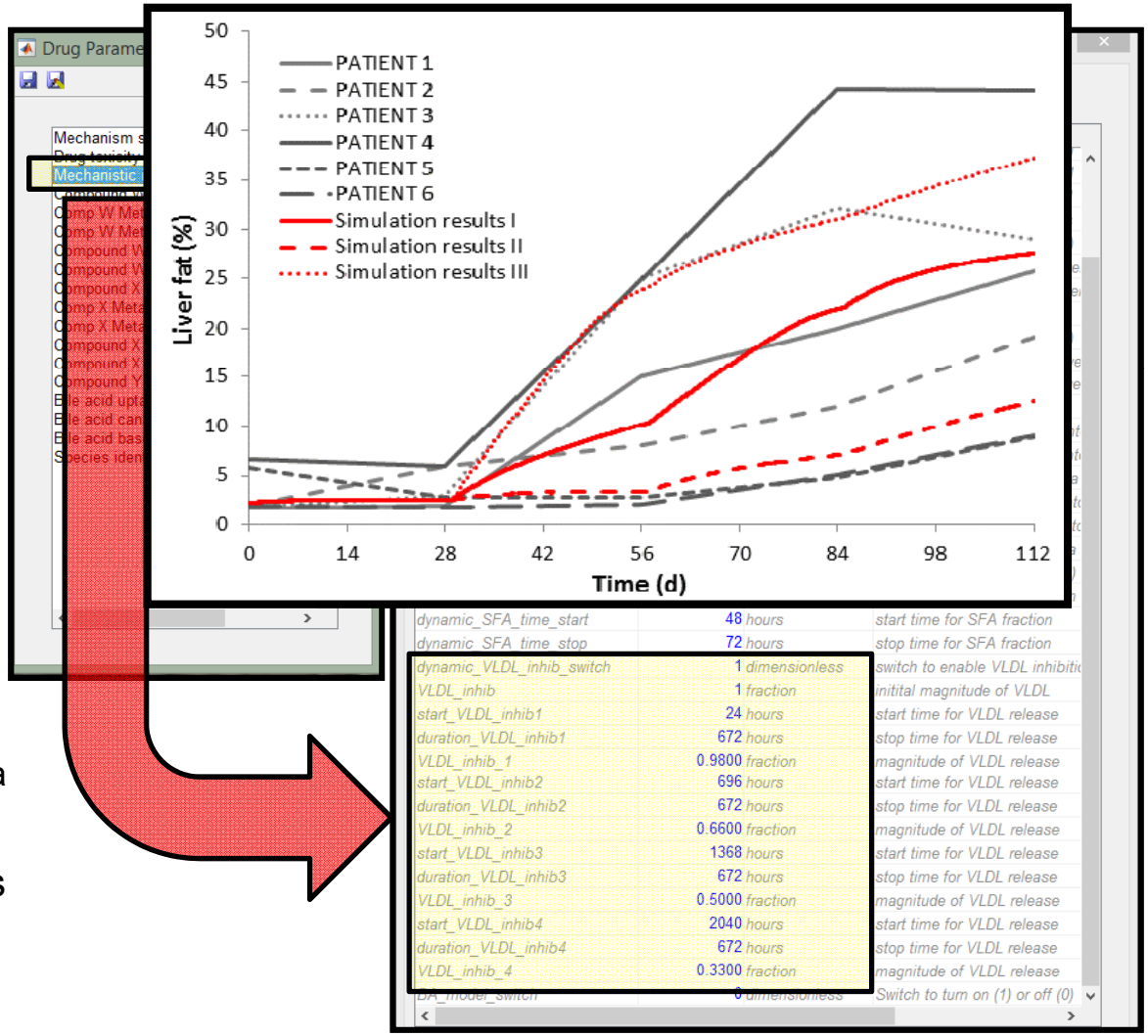
Parameters to Use to Ensure Lipotoxicity Is Activated in DILIsym®

- Lipotoxicity is active with default human parameters in DILIsym®
  - May want to perform simulations in absence of effect
  - Not active in dog, rat, mouse
- Vmax_SFA_ROS_effect = 0 to deactivate lipotoxicity effect
  - Located in 'Species Parameters'
  - Located in 'mitochondria dysfunction' parameter sub-set
  - Baseline value for humans is 0.02
  - Set to 0 for dog, rat, mouse



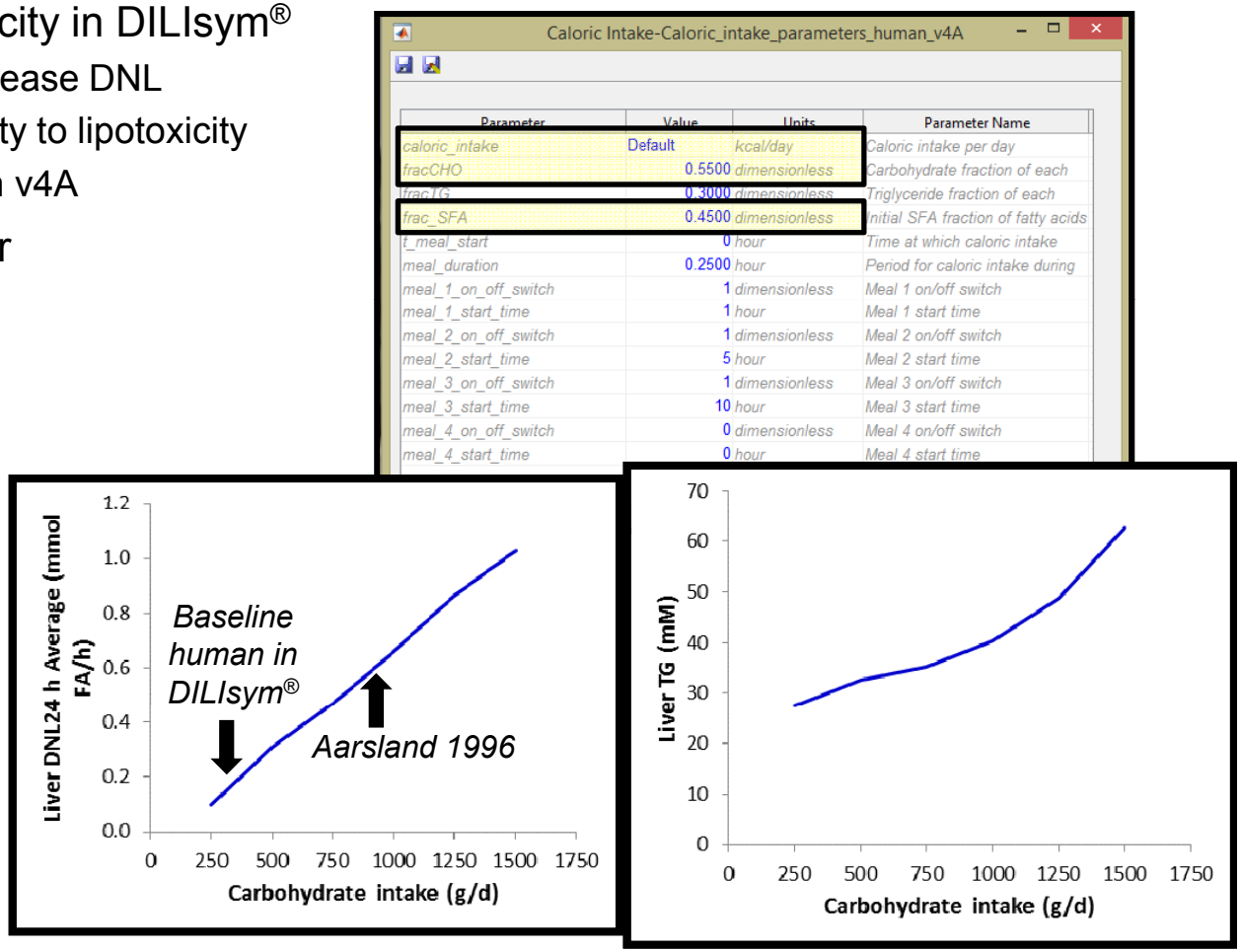
Simulating the Inhibition of VLDL-TG Release with DILIsym®

- Can simulated reductions in VLDL-TG release with DILIsym®
  - The details of the pharmacology are not represented
  - Magnitude of reduction can be set at specific time points
- Multiple parameters allow for simulating VLDL-TG inhibition
  - Located in 'Drug Parameters'
  - Located in 'mechanistic interventions' parameter sub-set
  - Dynamic_VLDL_inhib_switch = 1
  - VLDL_inhib = 1- inhibition
  - Start_VLDL_inhib1 = start time
  - Duration_VLDL_inhib1 = period of inhibition
  - Can simulate 4 inhibition levels during a single simulation
- Data Comparison experiment provides use example
  - Cuchel_2007_TG_mid
  - Cuchel 2007



Simulating Dietary Changes Relevant to Lipotoxicity in DILIsym®

- Changes to diet can impact accumulation of liver lipids and subsequent lipotoxicity in DILIsym®
  - Carbohydrate intake can increase DNL
  - SFA intake can alter sensitivity to lipotoxicity
  - DNL only active in humans in v4A
- Use 'Caloric Intake' parameter set to adjust dietary intake
  - 'caloric_intake' to adjust total calories
  - 'fracCHO' to adjust the fraction of carbohydrate
  - 'frac_SFA' to adjust the fraction of SFA
- DNL is dependent upon carbohydrate intake
  - Can cause significant increases in liver TG



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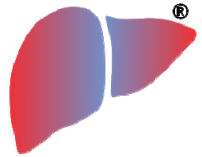
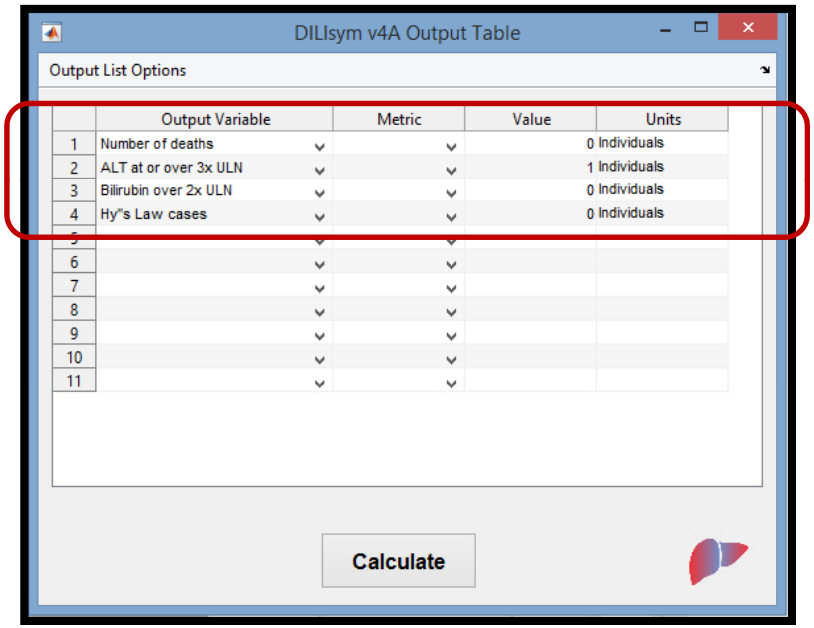


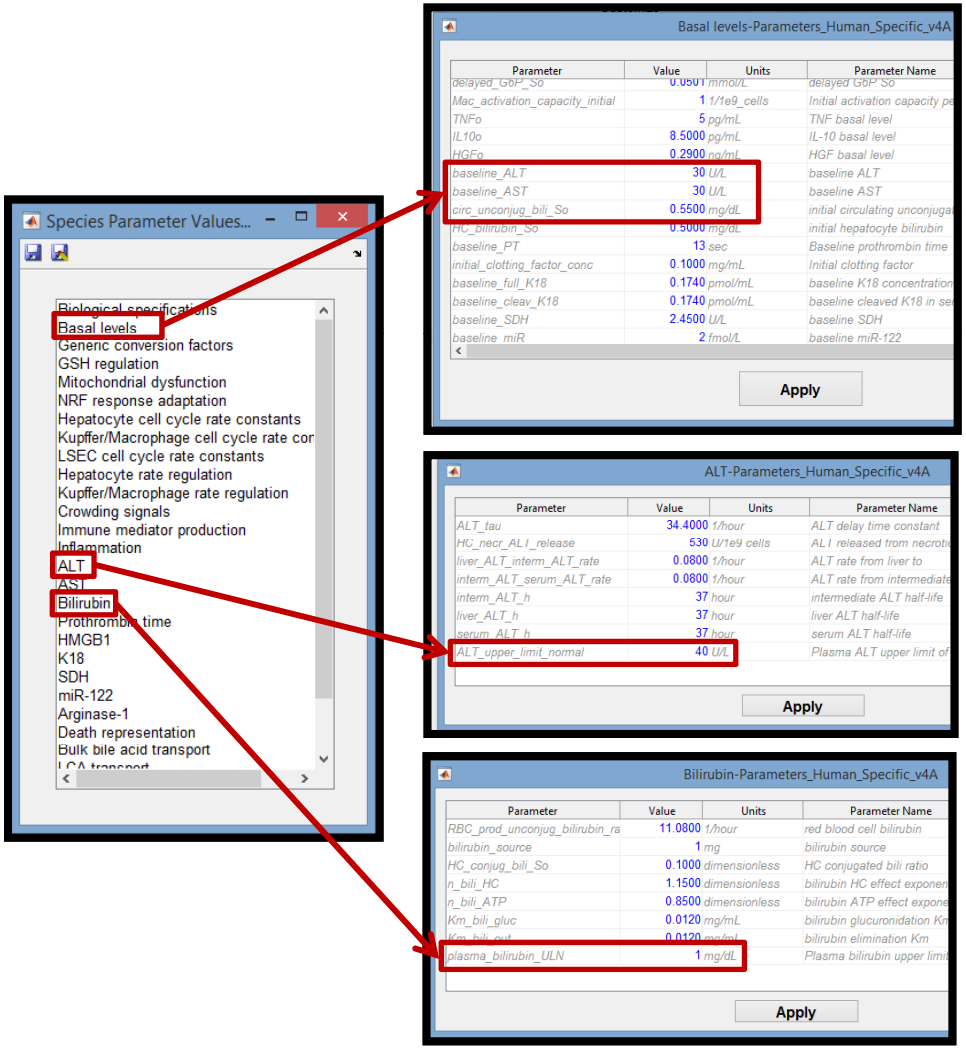
As of DILIsym® v4A, Upper Limits of Normal Have been Added for ALT and Bilirubin

- Prior to DILIsym® v4A, 3x baseline ALT and 2x baseline bilirubin were used for 'Hy's Law' designations in the output table
  - Baseline ALT is 30 U/L in baseline human
  - Baseline total bili is 0.55 mg/dL in baseline human
- Parameters for upper limit of normal (ULN) are now included
- As a result, a greater magnitude of injury is required to reach Hy's Law criteria in most simulated patients
  - ALT ULN set to 40 U/L
  - Bilirubin ULN set to 1 mg/dL
- Users may want to adjust the baseline and ULN values for their clinical study groups
- Disease groups who have higher starting ALT values will still be inadvertently declared 'Hy's Law'
  - Ongoing clinical issue that FDA is actively reviewing



Baseline and ULN Values Should be Altered in Tandem to Avoid Inconsistencies

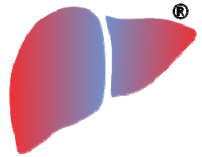
- Default baseline and ULN values for ALT and bilirubin are approximate averages across many groups, both in absolute terms and relative to one another
- If DILIsym® users opt to change the ULN values, the baseline values should also be adjusted
  - e.g. a clinical study group has an ALT ULN of 65 U/L, but a baseline of 48 U/L
  - Changing both avoids a misleading scenario where ULN is very high or low, but baseline is not corrected, and the increase in ALT to achieve Hy's Law is not in step with the spirit of Hy's Law



DILIsym® v4A User Training Agenda

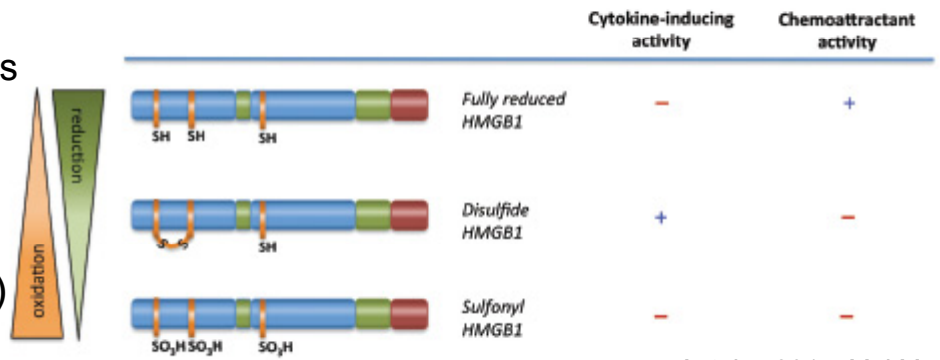
The Items Below will be Discussed with Slides and/or Live MATLAB Demo

- Overview of DILIsym® v4A and MATLAB directory structure
- Mixed-type bile acid transporter inhibition and associated parameter changes
- SimCohorts™ - uses, nomenclature, and documentation
- Steatosis and lipotoxicity – practical considerations
- ALT and bilirubin ULN parameters for Hy's Law calculations
- HMGB1 outputs – nomenclature and uses
- Oxidative stress parameter update - different levels of ROS production from different molecular entities during a simulation
- DILIsym® Inputs now included as part of outputs – reproducibility and memory concerns

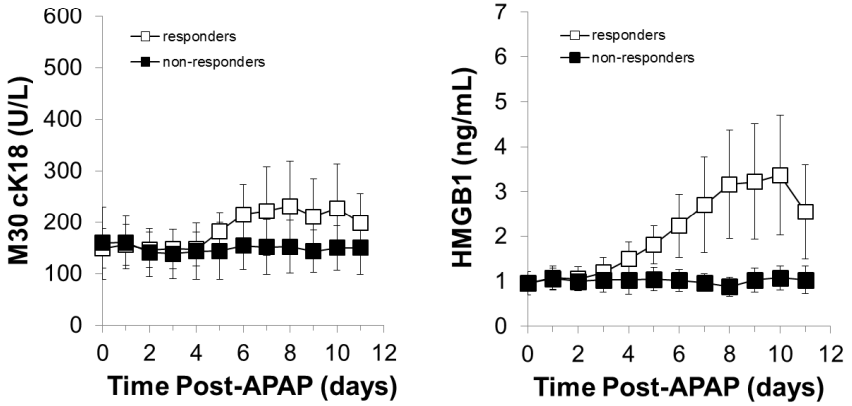


Alternate Redox Forms of HMGB1 Associate with Different Activities

- Extensive study of HMGB1 has uncovered form to function relationships, *e.g.*,
  - Caspase-dependent oxidized HMGB1 induces tolerance (Kazama 2008)
  - Disulfide HMGB1 induces cytokine release (Yang 2012)
- New nomenclature proposed (Antoine 2014)
- cK18 data indicates low dose APAP response is at least partially due to apoptosis
- HMGB1 in low dose APAP response is predominantly sulfonyl form (unpublished data), suggesting release due to apoptotic process
- DILIsym® v4A distinguishes sulfonyl HMGB1 (inactive) from reduced forms (active)



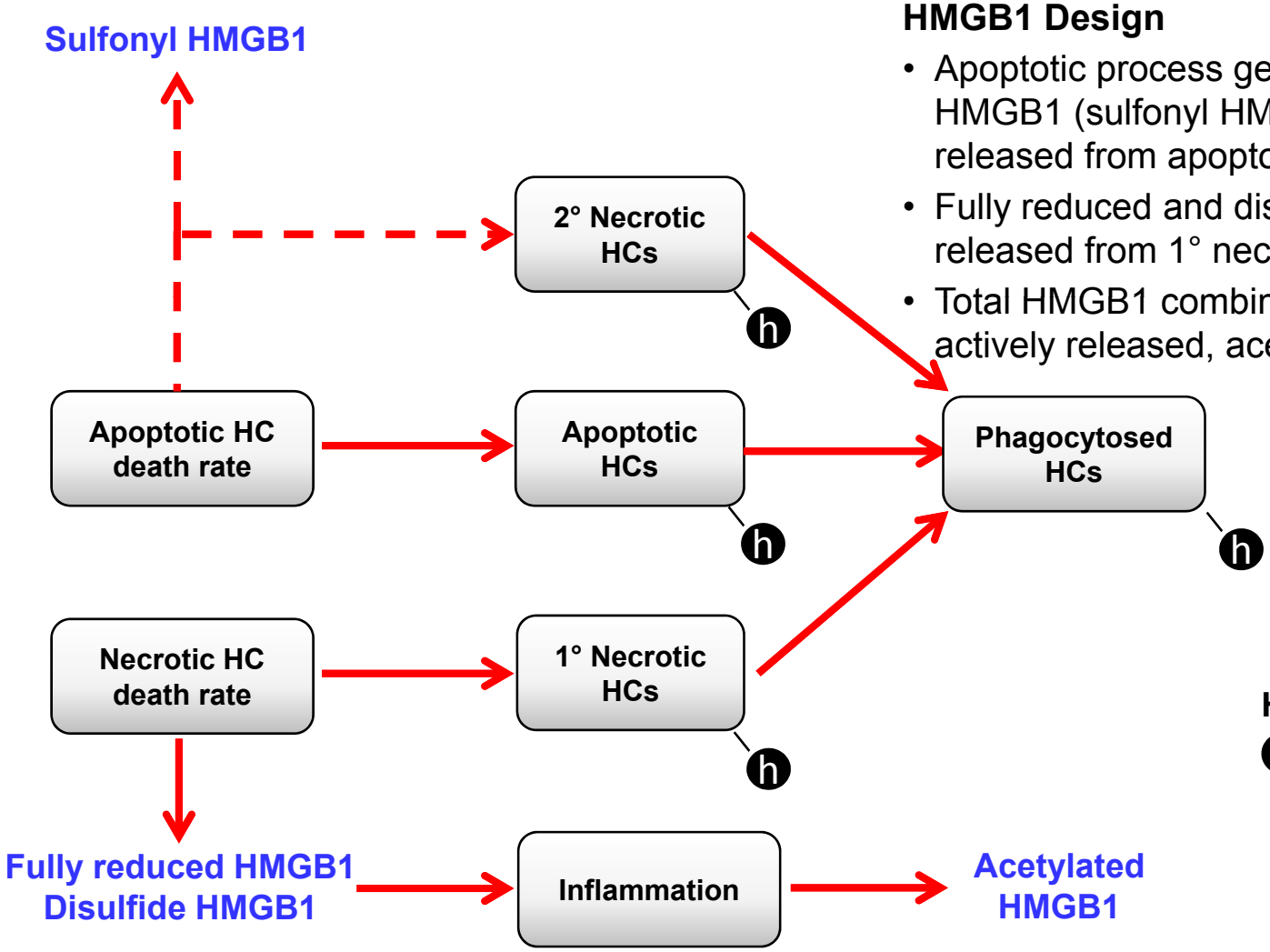
Antoine 2014, Mol Med



Thulin 2013, Watkins unpubl.

HUMANS

Updated Biomarker Representation Includes Alternate Redox Forms of HMGB1

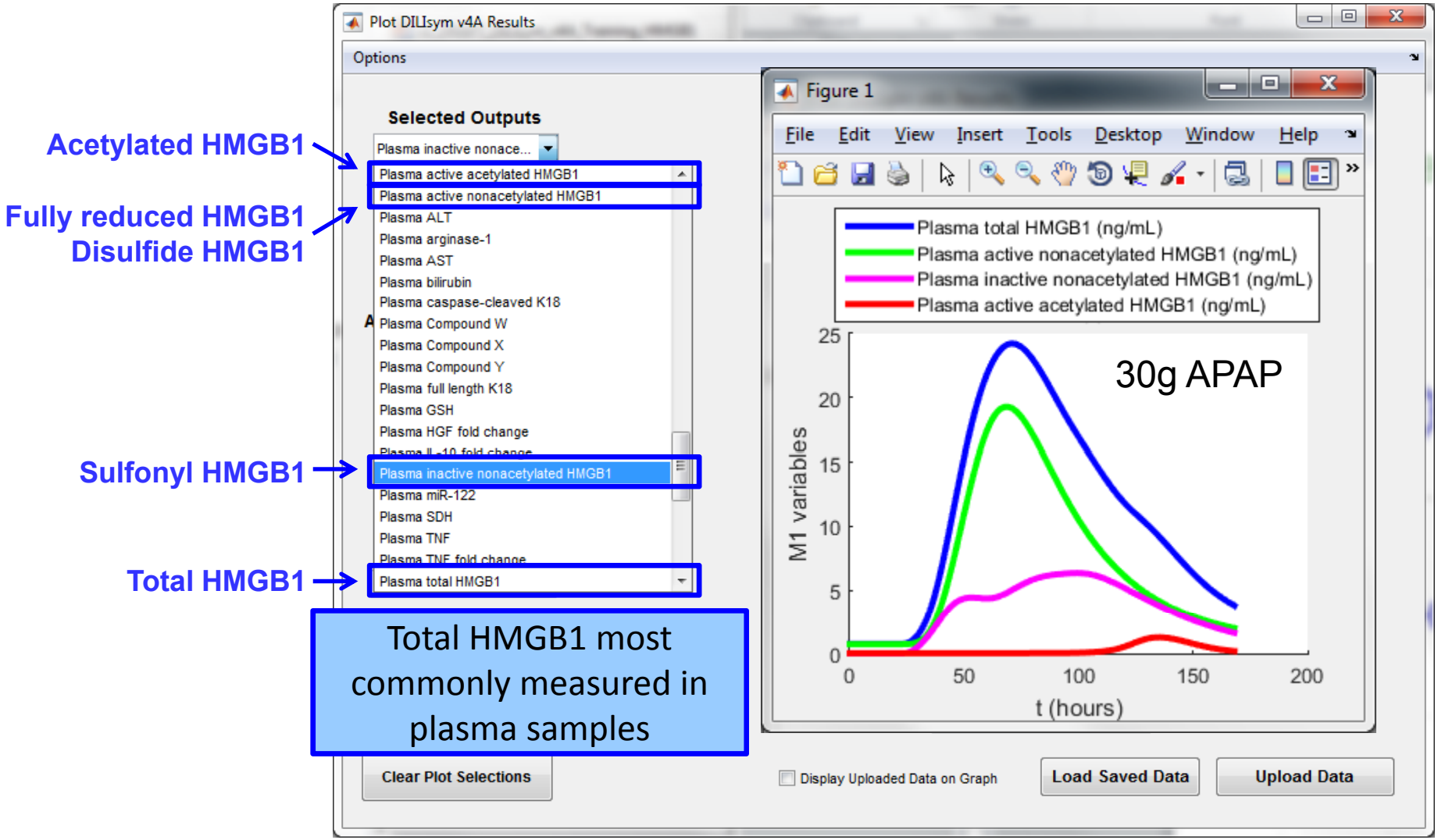


HMGB1 Design

- Apoptotic process generates fully oxidized HMGB1 (sulfonyl HMGB1), which is the form released from apoptotic or 2° necrotic HCs
- Fully reduced and disulfide HMGB1 are released from 1° necrotic HCs as a single pool
- Total HMGB1 combines cell death related and actively released, acetylated HMGB1

HC = hepatocyte
h = half life

Alternate Redox Forms of HMGB1 Available as DILIsym® Outputs



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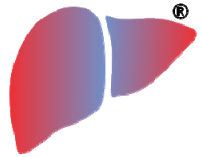
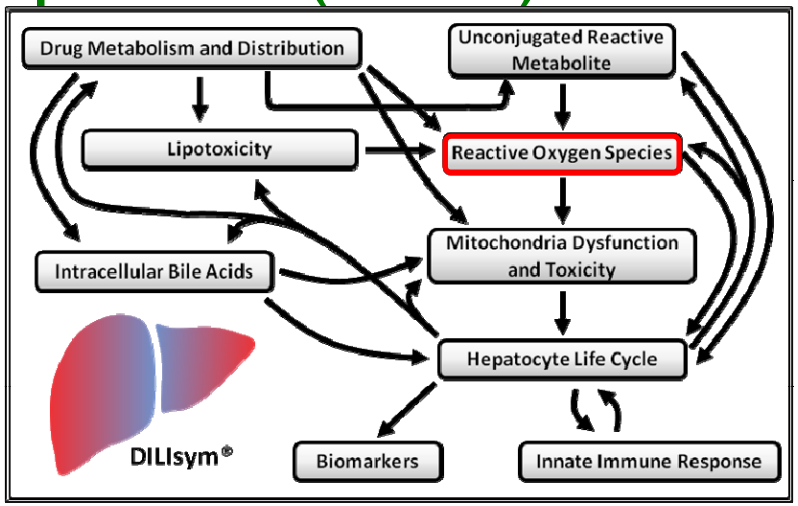


DILIsym® v4A Expands the Parameters for Reactive Oxygen Species (ROS)

- ROS is one key mechanism of hepatotoxicity
  - Reduces cellular ATP and induces apoptosis
  - Included since DILIsym® v1A
- DILIsym® v4A includes three inputs for ROS parameters
  - Expands the users ability to parameterize the ROS mechanism
  - Allows the users to simulate oxidative stress from different molecular entities independently



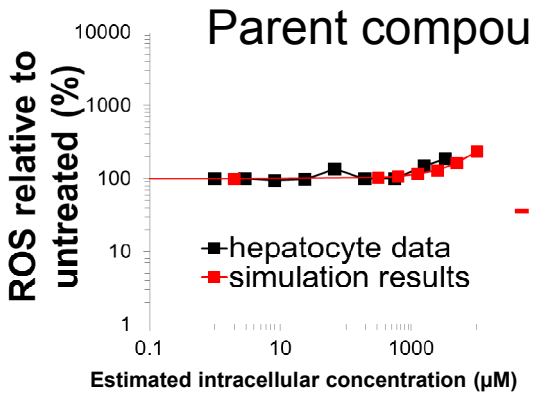
Mechanism selection-Parameters_Blank_v4A*

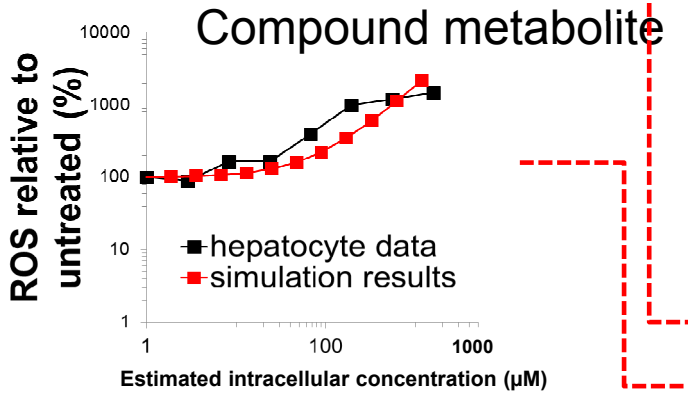
Molecular Species	RNS-ROS producer 1	RNS-ROS producer 2	RNS-ROS producer 3
Compound W	☐	☐	☐
Compound W metabolite A	☐	☐	☐
Compound W metabolite B	☐	☐	☐
Compound W reactive metabolite 1	☐	☐	☐
Compound W RM 1 protein adducts	☐	☐	☐
Compound W reactive metabolite 2	☐	☐	☐
Compound W RM 2 protein adducts	☐	☐	☐
Compound X	☐	☐	☐
Compound X metabolite A	☐	☐	☐
Compound X metabolite B	☐	☐	☐
Compound X reactive metabolite 1	☐	☐	☐

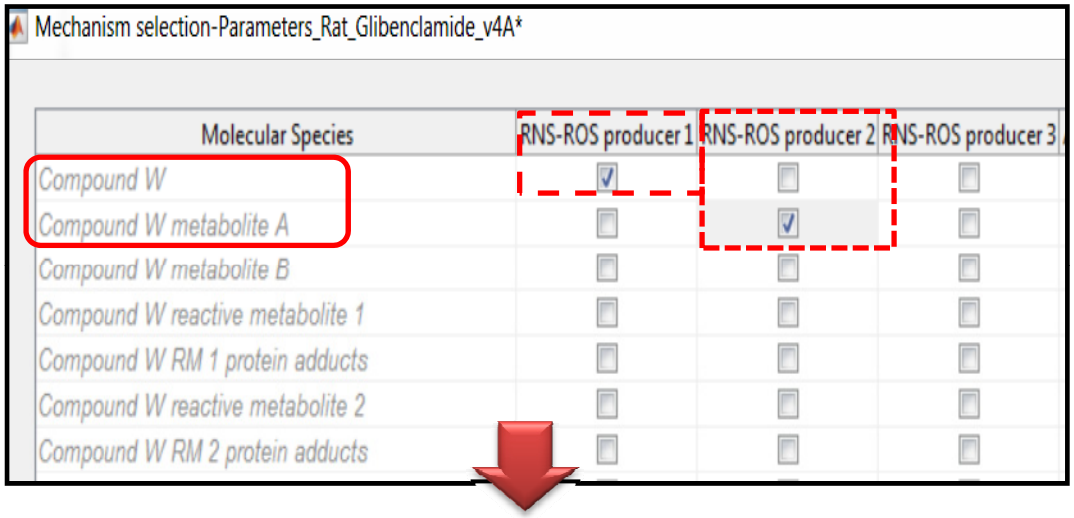
Modeling RNS-ROS Generation in DILIsym®

Collect *in vitro* data

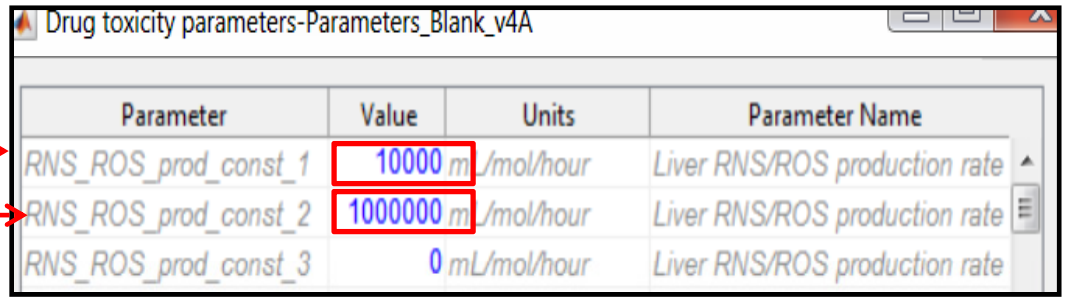
Identify toxicity mechanism







Translate in-vitro data into DILIsym® input parameters



Mock experimental data for demonstration purposes

Simulation Results

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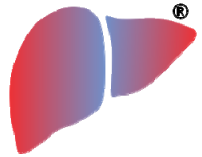
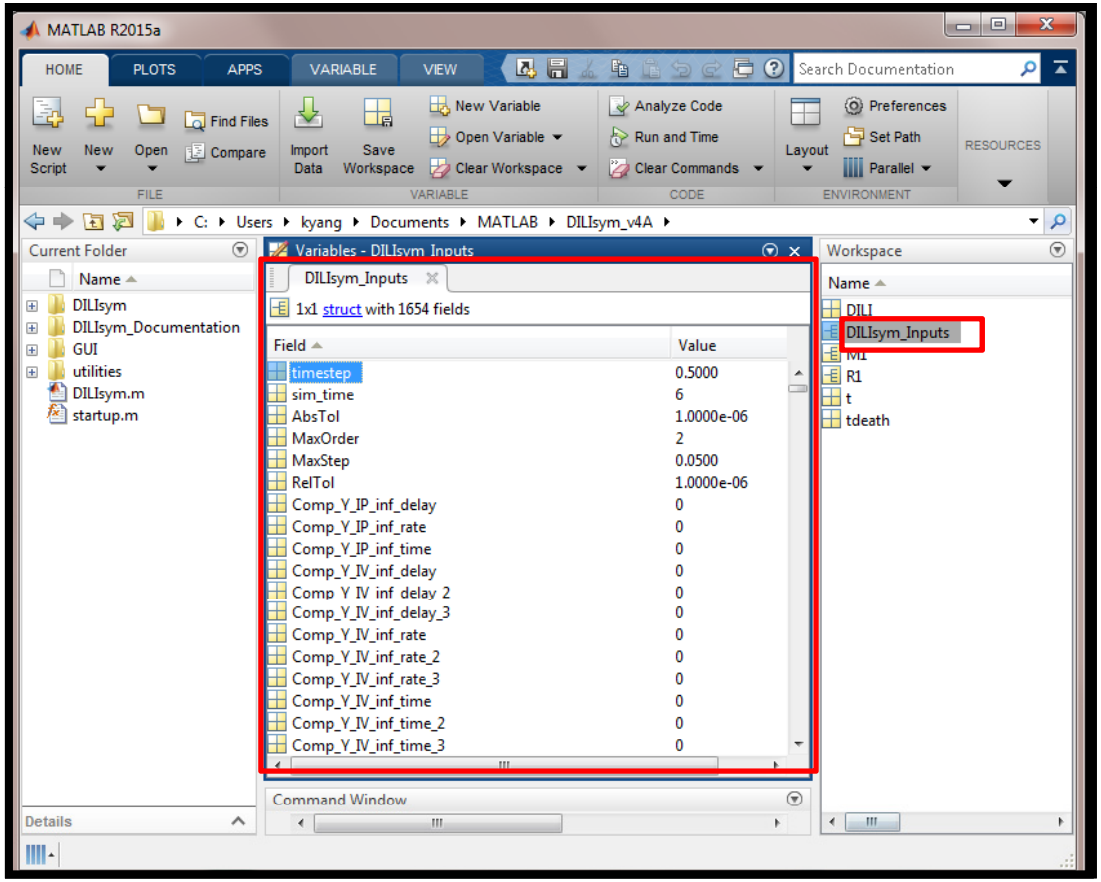
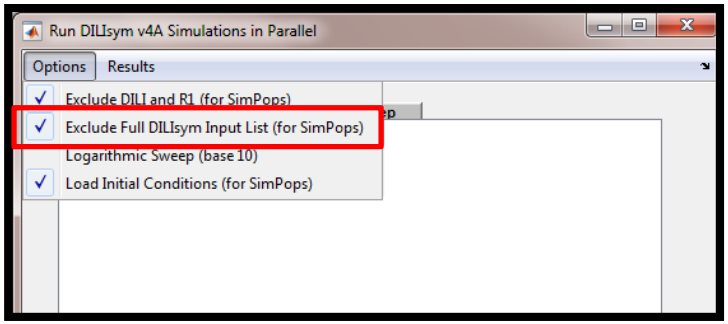


DILIsym® Inputs Now Saved with Outputs

- All the input parameters are saved as “DILIsym_Inputs”
  - Species parameters, drug parameters, and simulation setup information (e.g., simulation time, dosing information)
  - Simulation inputs improve documentation of results



- SimSingle™ and Parameter Sweep save input parameters as default
- SimPops™ does not save input parameters as default because of memory concerns
  - Uncheck “Exclude full DILIsym® input list” to save



2015 DILI-sim Initiative Key Dates