DILIsym®

DILIsym User Training –

DILIsym v6A Updates Overview

August 2017

DILIsym Development Team

CONFIDENTIAL

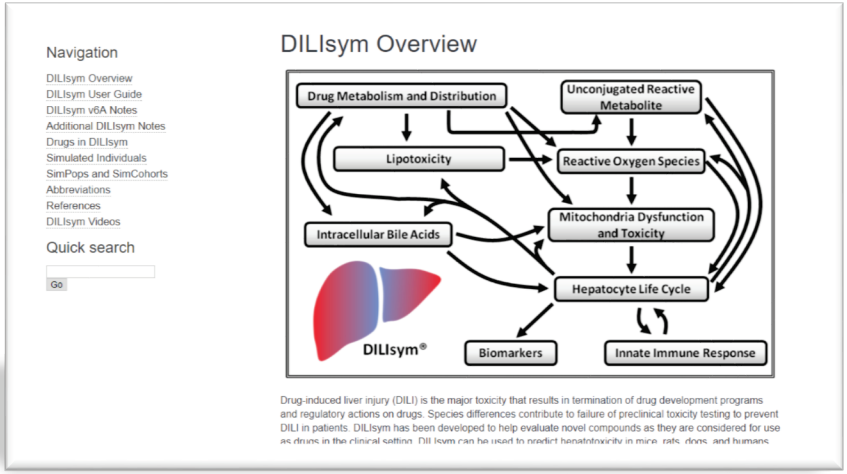
Goal for This Training Session

Participants should understand the following general concepts:

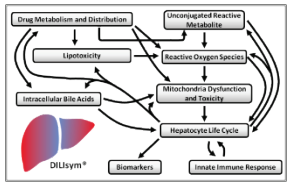
- The most notable updates included in DILIsym v6A as compared to v5A
- Some practical considerations for utilizing DILIsym v6A as compared to v5A

Highlights of DILIsym v6A (Released Aug. 2, 2017)

- Several new or updated validation compounds included with varying clinical presentations
  - Ambrisentan and sitaxsentan added as clean/toxic pair in humans
  - Metformin and phenformin added as clean/toxic pair in humans for lactic acidosis; also provide polypharmacy evaluation capabilities (mild ETC inhibitors)
  - Nelfinavir added as mechanistic bilirubin negative control validation compound
  - TAK875 parameter values updated with new data
  - PBPK representations for all v5A compounds in all species were re-optimized due to substantial PBPK sub-model updates
- DILIsym documentation resources updated and full site embedded in local software copy instead of PDF documents

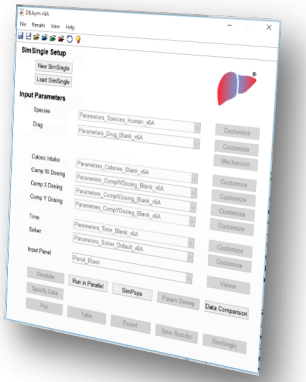


- New biomarker SimPops (v6A-1) added focused on variability in ALT parameters *(Howell 2014, Longo 2017)*
- TNF mediated cell survival / regeneration added to make TNF pleiotropic
- Capability added to input population level PK information to drive simulations (adds ability to input directly from GastroPlus)
- Simulation efficiency improved through a number of changes
- Substantial PBPK sub-model improvements made

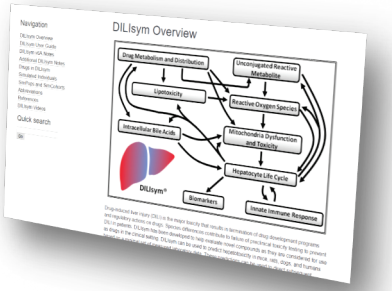


Expanded Capabilities and Features of DILIsym v6A

- PBPK representation updates:
  - Perfusion- and permeability-limited distribution are represented for all tissues
  - Additional transporter-mediated processes represented for the liver tissue
  - Only unbound, non-ionized drugs undergo passive diffusion
- New graphical user interface (GUI) tools added:
  - Batch simulation capability added to SimPops feature
  - Individual extractor feature added for SimPops feature
  - SimPops specified data profiles capability
  - Specified data efficiency improved with constant versus time dependent option
  - Time reports added to help with simulation planning



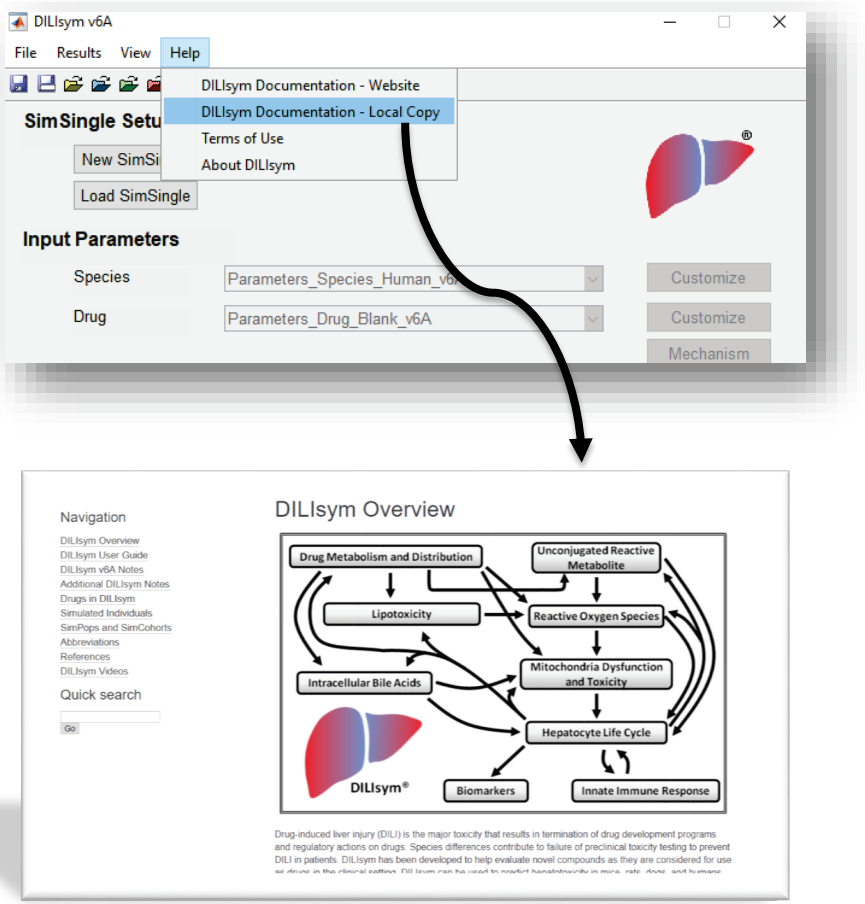
- Mitochondrial electron transport chain (ETC) inhibition mechanism updated to include new fourth parameterization spot (ETC inhibition 4) with saturable capability
- Oxidative stress (ROS) mechanism updated to include two new parameterization spots with saturable capability for ROS induction
- New human SimCohorts added for v6A-1 (ALT) SimPops
- Simulation efficiency updates:
  - Specified data addition of constant option
  - Unit conversions for PBPK, GSH and bile acid sub-models
  - Multi-dose code updated to reduce solver stiffness
- Expanded Zotero reference database (contact us for real-time access)
- Various bug fixes and enhancements to improve performance, speed, and user-friendliness



MATLAB 2015a is Recommended for DILIsym v6A Simulations as of August 2017

- MATLAB made internal changes as of MATLAB 2015b that cause variables declared within a parent function and accessed from a nested function to use much more memory and therefore take much longer to access
  - Related to Just in Time (JIT) compiler feature
- MathWorks has made progress in this area for the Fall 2017 MATLAB release – this will be tested by DILIsym Services
- In the meantime, the DILIsym Services team recommends using DILIsym v6A on **MATLAB 2015a** until further notice
- DILIsym v6A will run properly on MATLAB 2017a, but simulation time may be increased (speed decreased) in some cases

DILIsym Documentation Resources Have Migrated to www.DILIsymHelp.com

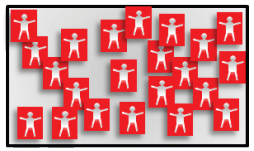


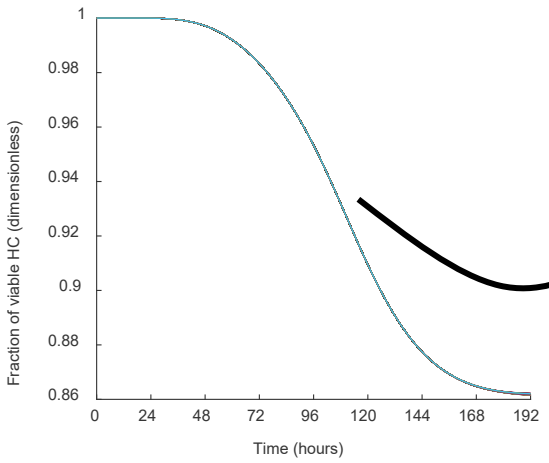
- DILIsym v6A documentation links offer hyperlink to web or local copy
- Local copy of documentation will not be capable of updates but live site will be updated in real time
  - Training and update videos also only available on live site
- All resources available in one place
  - User guide
  - DILIsym design notes
  - Drug notes
  - Detailed SimPops and SimCohorts information
  - References
  - All related videos (training and consortium meetings)

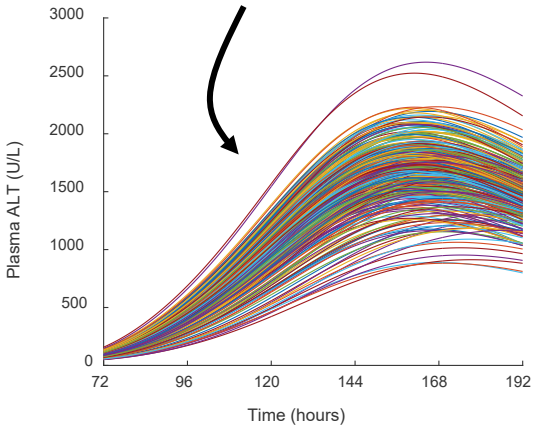
Biomarker Variability Represented in ALT (v6A_1) SimPops

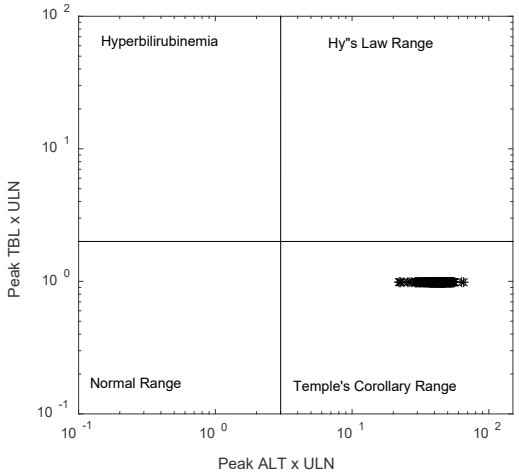
- ALT SimPops includes variability in parameters largely related to ALT levels occurring from a given level of injury
  - No mechanistic DILI parameters included
- Primary goal is to understand variability in clinical signature from given level of liver injury
- 8 parameters included; 300 simulated individuals
- SimPops v6A-1 validated indirectly with liver biopsy data after APAP overdose
- SimPops v6A-1 already used for two DILIsym publications (*Howell 2014* and *Longo 2017*)

Variables Used to Construct the ALT Biomarker (v6A-1) SimPops
Hepatocellular ALT content
ALT half-life
ALT transport rate into circulation
Hepatocyte regeneration



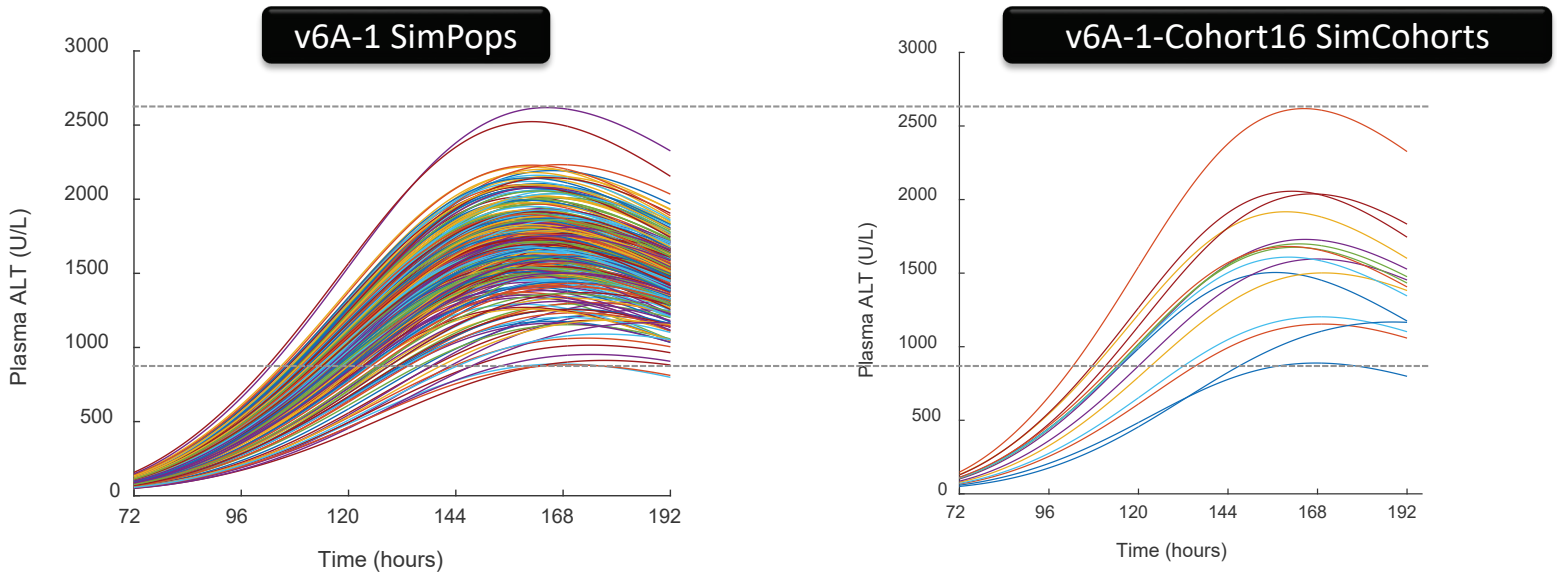






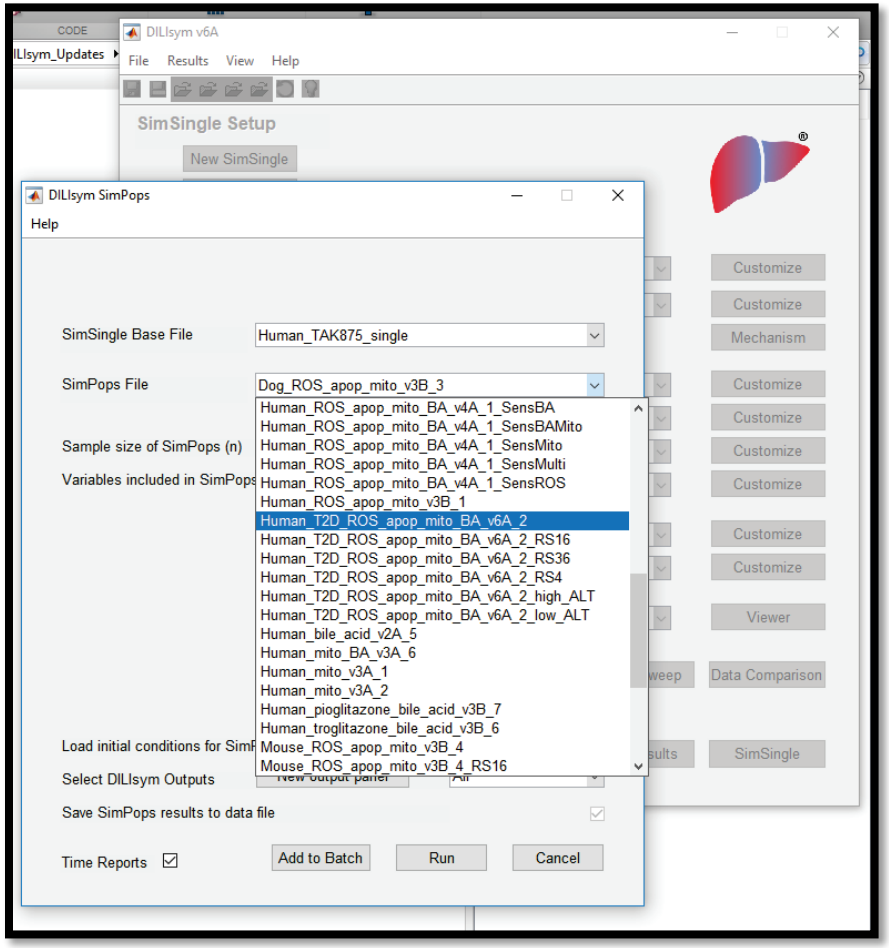
SimCohorts *v6A-1-Cohort16* from v6A-1 ALT SimPops Provides Complete Coverage of ALT Range with Fewer Simulations

- 16 individuals extracted from larger v6A-1 SimPops of 300
- Strategically chosen to provide excellent sample of full v6A-1 ALT SimPops variability
- Useful for quicker projects or screening



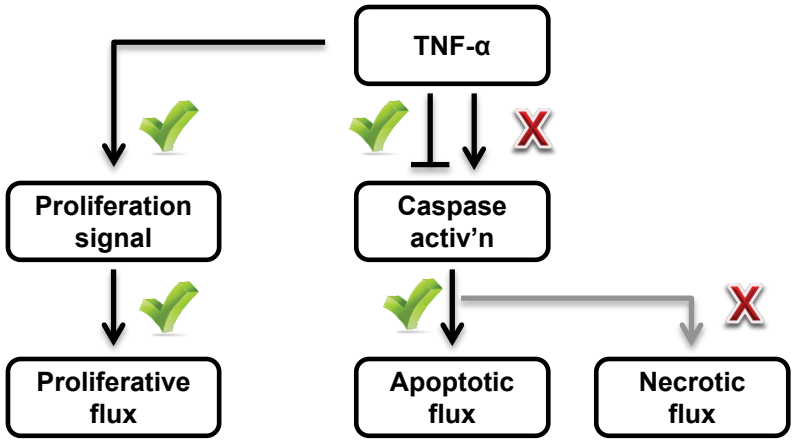
Type 2 Diabetes SimPops Previously Included in DILIsym was Renumbered to v6A-2 SimPops

- Changes made to a mitochondrial dysfunction sub-model human species parameter to improve stability led to minor SimPops parameter value adjustments for the T2D SimPops (previously numbered v4B-1)
- Renumbered the updated SimPops to be clear that some changes occurred
  - T2D SimCohorts were also all renumbered
- Changes were minor and outcomes should be similar with new (v6A-2) and older (v4B-1) version of T2D SimPops

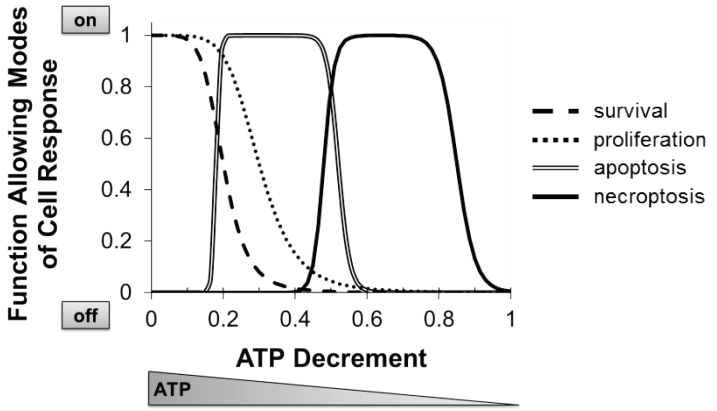


DILIsym v6A Includes Dynamic TNF-α Mediated Hepatocyte Response Based on Cell Status Checkpoints

- Modeled TNF-α ATP checkpoints
  - Healthy cells do not die in response to TNF-α
    - ATP sufficient: survival or proliferation
  - Compromised cells (*e.g.*, ActD or D-gal) undergo apoptosis
    - Partial ATP depletion: apoptosis or necroptosis
  - Can cells manage the program energetic requirements?
    - Insufficient ATP diverts cells from apoptosis to necroptosis

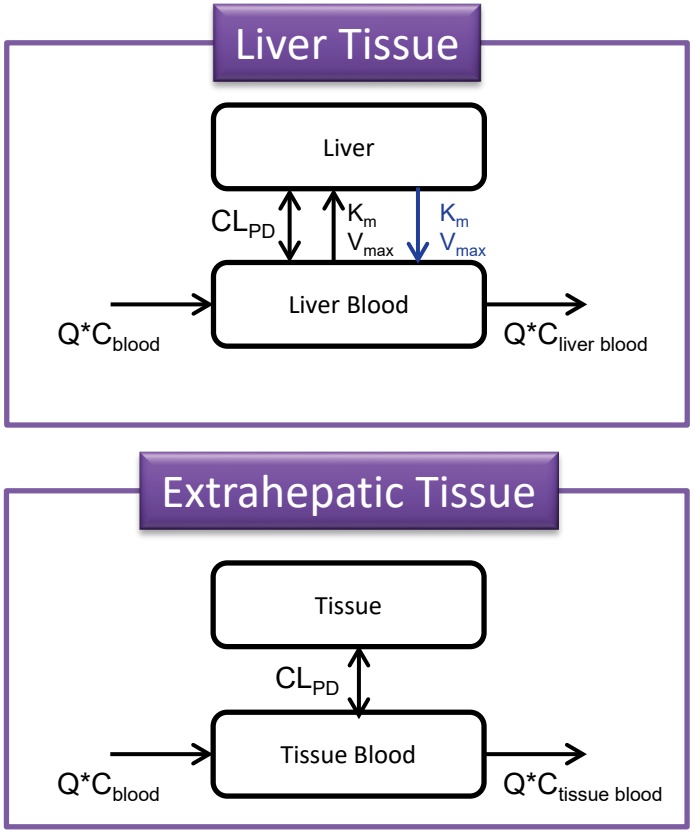


- Very low ATP results in necrosis without requirement for TNF-α
- Automatically occurs based on hepatocyte health status (no drug-specific parameters, only species parameters)
- Review recording of DILIsym Review Session 18, Innate Immunity Overview, on May 23rd, 2017 for more details



PBPK Sub-Model Updates in DILIsym v6A

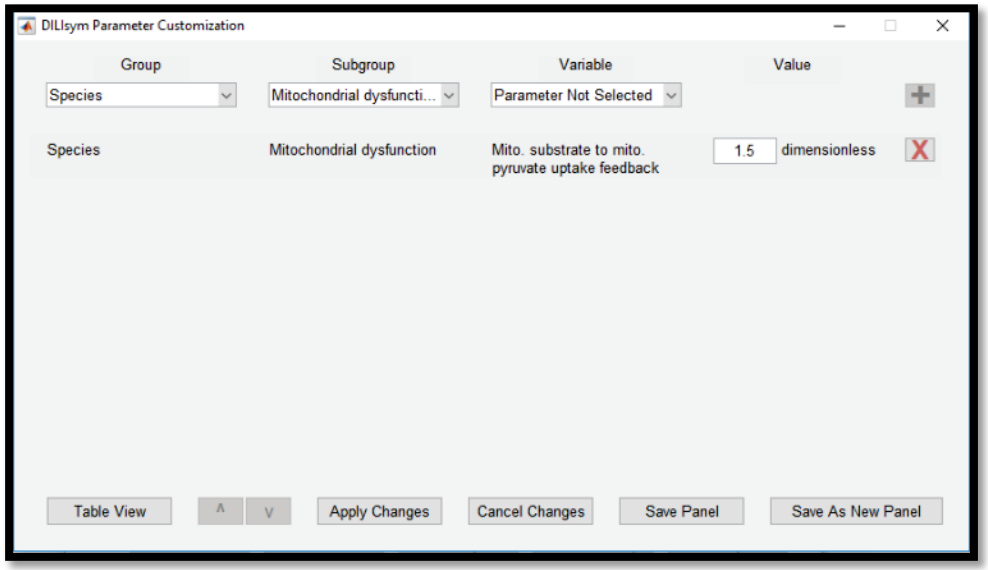
- Perfusion- and permeability-limited distribution are represented for all tissues
  - Switches added for users to select perfusion- or permeability-limited distribution
  - Transporter-mediated uptake still represented in the liver
- Additional transporter-mediated processes represented for the liver tissue
  - Active basolateral efflux added for Compound W and X
  - Transporter-mediated hepatic uptake and basolateral efflux added for stable metabolites and Compound Y
  - Electrogenic transport added for Compound W and X
- Only unbound, non-ionized drugs undergo passive diffusion
  - Fraction non-ionized calculated using pKa and pH



- Existing exemplar compounds have been revisited and updated, if necessary, to confirm that clinically/preclinically observed PK profiles and hepatotoxicity are adequately represented
  - Conversion factors temporarily included in v5A were eliminated

A Mitochondrial Dysfunction Sub-model Species Parameter Was Adjusted to Increase Equation Stability

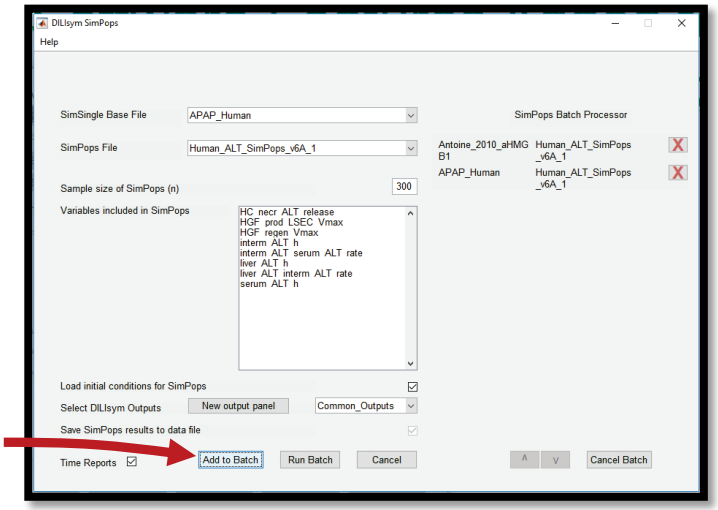
- Hill coefficient governing mitochondrial pyruvate uptake based on feedback from current mitochondria substrate levels was altered from 3 to 1.5 based on instability observed in some cases
  - Change affects humans only
  - Rat, mouse and dog parameter value stayed same

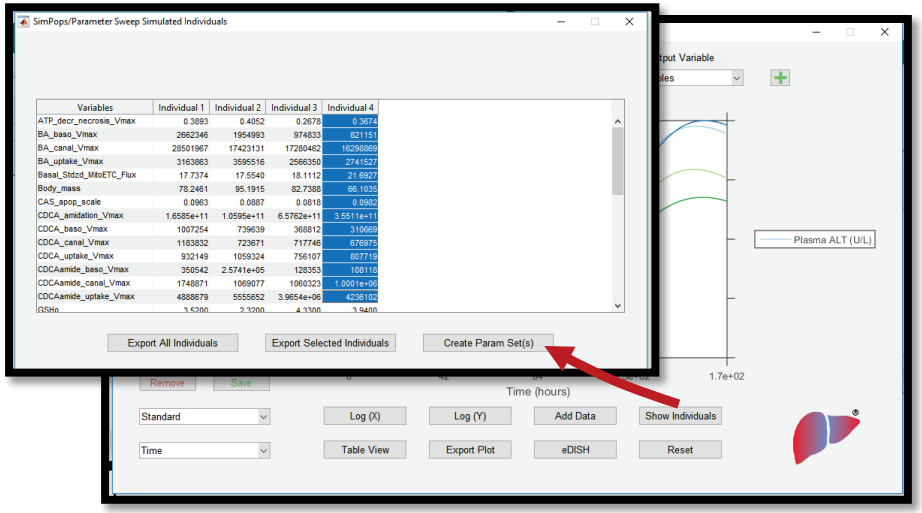


- Most simulations will not be affected
- Fasted and fed substrate utilization patterns remain consistent with literature
- A small fraction of simulations will be faster and solve without odd solver errors
- T2D SimPops name changed to reflect some necessary changes (from v4B-1 to v6A-2)

New SimPops Features Improve Simulation Workflow

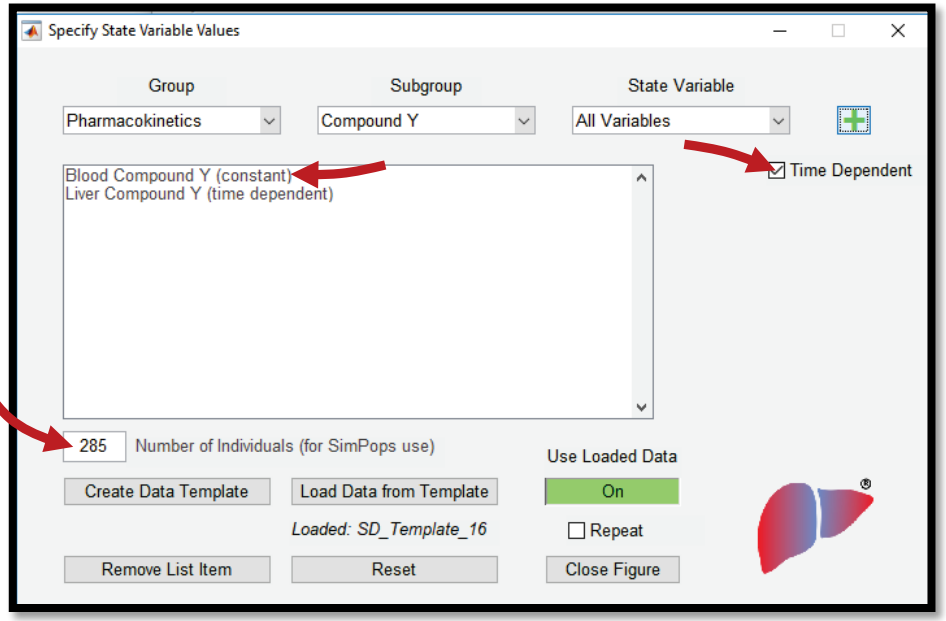
- SimPops **Batch processor** allows for setting up multiple SimPops or SimCohorts and running simulations as group
  - Simulations are saved in between each simulation
  - File names assigned automatically based on SimSingle chosen and SimPops chosen
  - Save option is mandatory
- SimPops **extraction tool** within plotting window allows for instantly selecting individuals based on outputs of interest and creating their parameter sets for SimSingles
  - “Create Param Set(s)” exports to DILIsym home screen
  - “Export” buttons create Excel files with parameter values





New Specified Data Features Allow Users to Input Population-Based PK Simulations and Improve Simulation Efficiency

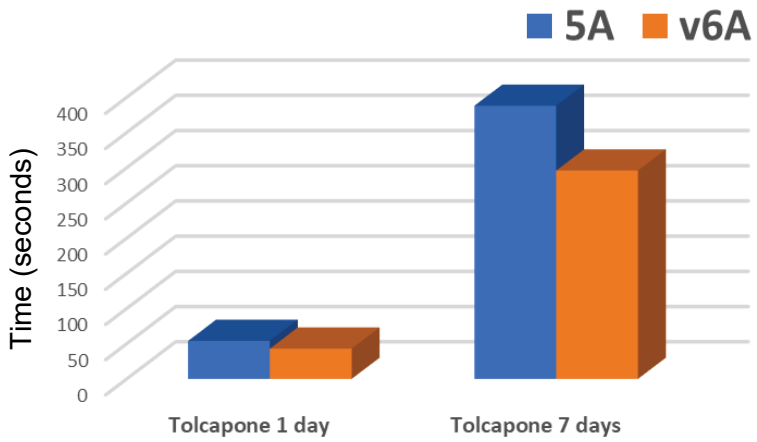
- New functionality allows users to **input more than 1 specified data profile** (up to 1000 individuals)
  - If number of data profiles put in is greater than SimPops size (N), first N profiles are used and rest are ignored
  - If number of data profiles put in is less than SimPops size (N), data profiles are repeated until full SimPops is done
- Simulation outputs from PBPK platforms can be directly imported

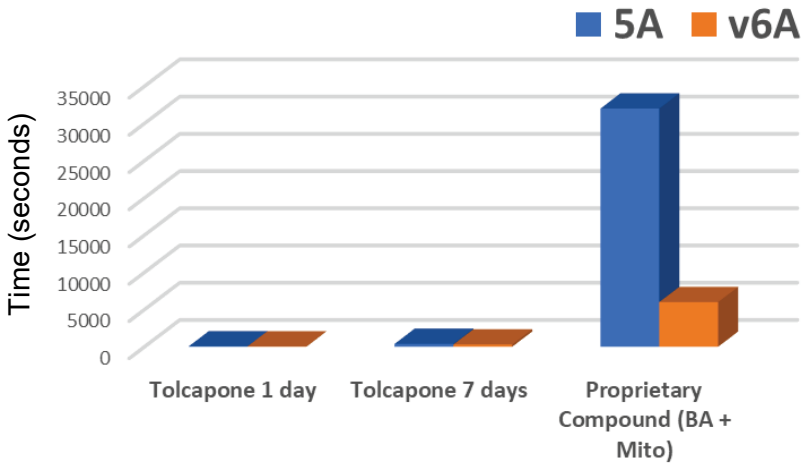


  - Feature is being added to GastroPlus to **allow easy export of population PK from GastroPlus with matched SimPops body weights and population size information automatically incorporated into a Specified Data template for direct import into DILIsym**
- Option of time dependent versus constant variable allows user to deselect "Time Dependent" when possible and greatly improve efficiency
  - Constant option is much more efficient as it avoids the need for interpolation between data points to match simulation time steps

DILIsym v6A Unit Conversions and Other Updates Will Increase Speed of Simulations

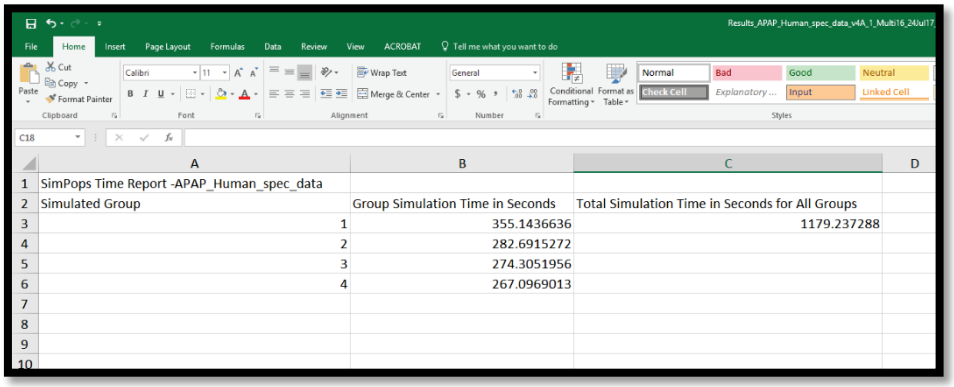
- DILIsym unit conversions were done to bring numbers closer to order 1
  - *PK: ug/mL and uM*
  - *GSH: mM*
  - *Bile acids: uM*
- Dosing code was altered so that multi-dose scenarios will run more efficiently
  - Code changes involved how discontinuities are handled in between each new dose
- Hill coefficient governing mitochondrial pyruvate uptake based on feedback from current mitochondria substrate change also speeds up some simulations



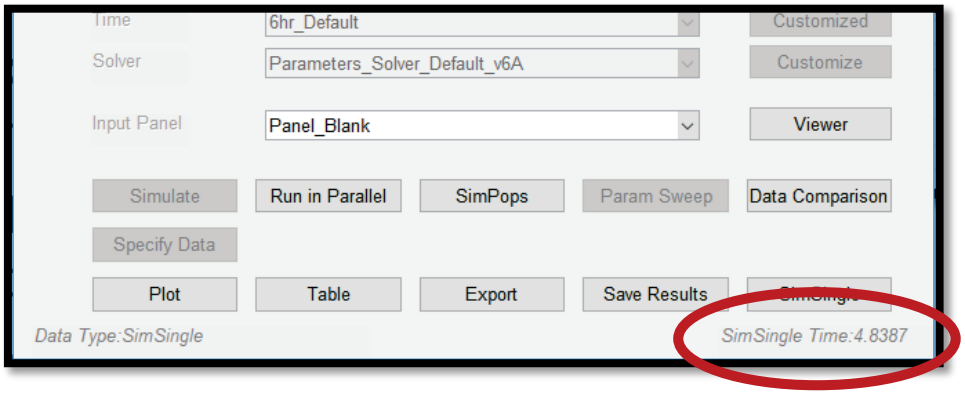


New Time Reports Feature In DILIsym v6A Provides Helpful Information for Simulation Planning

- Time reports are generated for SimPops, parameter sweeps, SimSingles run in parallel and Data Comparisons
  - Reports are generated in Excel format
  - DILIsym user guide covers time reports in detail
- Time reports for SimPops batches are saved with each batch intermittently
- DILIsym home screen now also includes simulation time in bottom right corner to aid with simulation time tracking

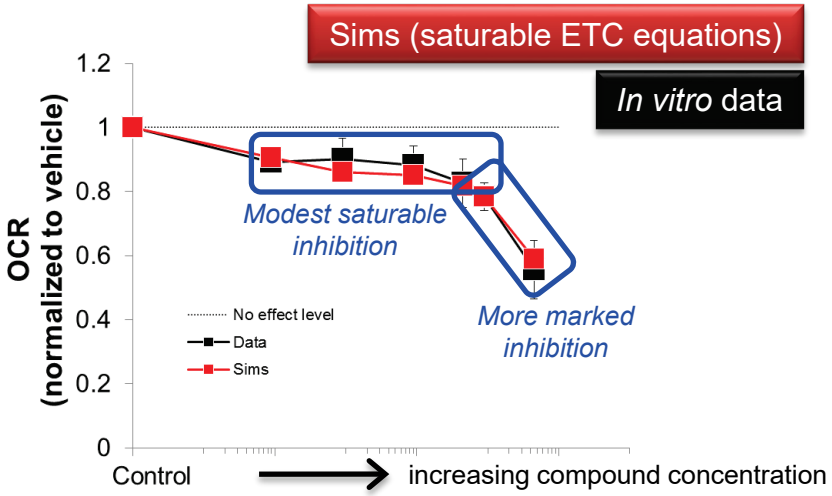


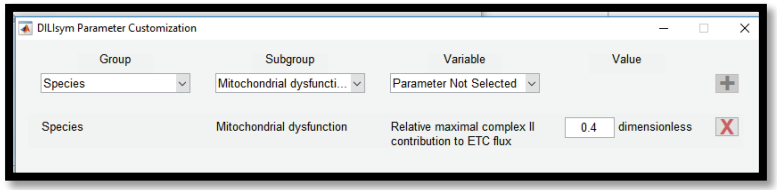
	A	B	C	D
1	SimPops Time Report -APAP_Human_spec_data			
2	Simulated Group	Group Simulation Time in Seconds	Total Simulation Time in Seconds for All Groups	
3	1	355.1436636	1179.237288	
4	2	282.6915272		
5	3	274.3051956		
6	4	267.0969013		
7				
8				
9				
10				

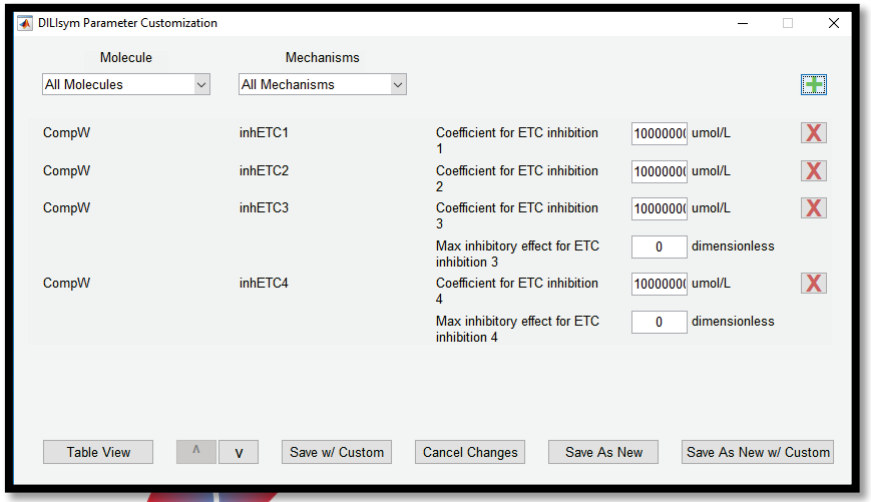


Second Set of Parameters Added for Saturable ETC Inhibition (ETC-4) with Overall Complex II Inhibition Capped

- DILIsym v5A contained ETC-3 parameters for including low level, saturable ETC inhibition
- **DILIsym v6A includes a second set of saturable ETC inhibition parameters (ETC-4)**
- This allows for representing two compounds with low level inhibition (such as metformin with other compounds)
- The overall ETC inhibition from the combination of the saturable inhibition slots, ETC-3 and ETC-4, is capped at 40% based on the reduced flux through ETC Complex II compared to Complex I
  - This can be adjusted by the user with the "Relative maximal complex II contribution to ETC flux" parameter in the Mitochondrial Dysfunction Subgroup of the Species Group of parameters







Parameter Options Added for Saturable Reactive Oxygen Species (ROS) Induction

- **DILIsym v6A includes two sets of saturable ROS/RNS induction parameters**
  - "incRNSROSproduction4 and incRNSROSproduction5"
- This allows for easier parameterization for certain measured *in vitro* ROS (oxidative stress) exposure response curves