DILIsym® User Training - DILIsym® Parameters from Data: Mitochondrial Toxicity

DILI-sim Team

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Goal for This Training Session

Participants should understand the following general concepts:

- Methods to parameterize and simulate mitochondrial toxicity in DILIsym®

Entacapone and Tolcapone: Similar Mechanistic Effects but Differences in Clinical Hepatotoxicity

- Entacapone and tolcapone represent a "clean/toxic" compound pair
  - Similar structure and pharmacologic mechanism
  - No hepatotoxicity reported for patients taking entacapone
  - Clinical hepatotoxicity observed with tolcapone
- Similar mechanistic hepatotoxic effects for entacapone and tolcapone
  - Both compounds uncouple the mitochondria proton gradient
  - Modest BSEP inhibition with entacapone (IC_{50}=55.6 µM, Morgan 2013) and tolcapone (IC_{50}=36.6 µM, Morgan 2013)
- Can DILIsym® recapture the differences in hepatotoxicity observed clinically based on mechanistic information?

HUMANS

ENTACAPONE

Parameter	RCTs		EXT		Placebo	
	NDA n=406	Overall n=603	NDA n=325	Overall n=738	NDA n=296	Overall n=400
Total bilirubin	0.3%	0.2%	0	0.2%	0	0
AST	0.3%	0.3%	0	0.2%	0.7%	0.3%
ALT	0.5%	0.5%	0	0.2%	0.8%	0.6%
GGT	0	0.4%	0.3%	0.3%	0.4%	0*
Alkaline Phosphatase	0	0	0	0	0.4%	0*

FDA Comtan safety document

TOLCAPONE

Adverse Event	*Placebo*	*100 mg*	*200 mg*
PHASE III CONTROLLED TRIALS	(n=292)	(n=294)	(n=293)
High SGPT (ALAT)			
≥2x ULN			
>3x ULN	0	3 (1%)	8 (3%)
>5x ULN	0	2 (0.7%)	3 (1%)
>8x ULN	0	1 (0.3%)	1 (0.3%)
High SGOT (ASAT)			
≥2x ULN			
>3x ULN	0	4 (1%)	6 (2%)
>5x ULN	0	2 (0.7%)	3 (1%)
>8x ULN	0	0	2 (0.7%)
High alkaline phosphatase	2 (1%)	0	1 (0.3%)

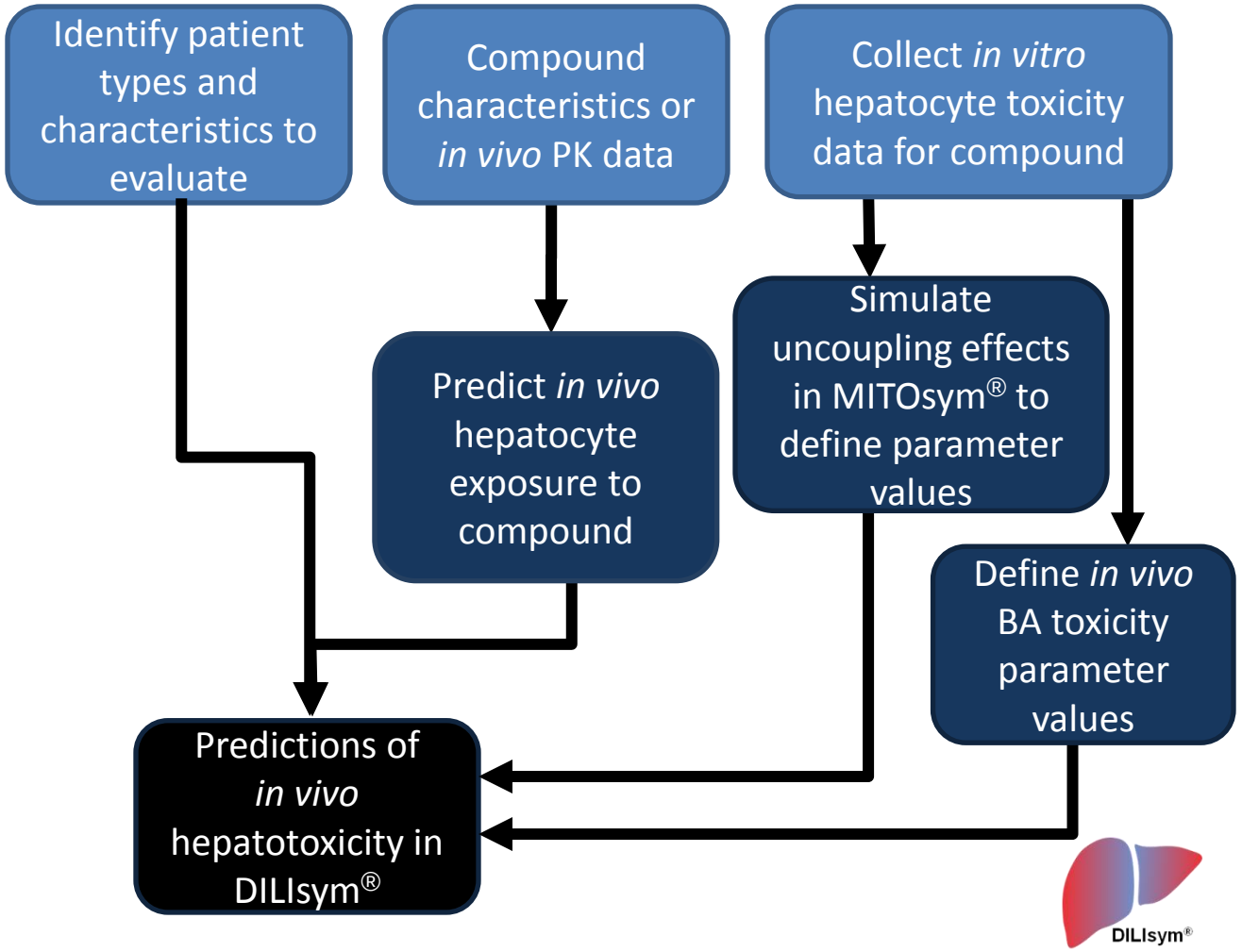
Tasmar FDA filing documents

Workflow for Modeling Entacapone and Tolcapone with MITOsym® and DILIsym®

Approach: Predict *in vivo* risk based on PK modeling and *in vitro* hepatocyte toxicity data for mitochondrial and BA toxicity mechanisms

Case study: Compare the simulated hepatotoxicity profile between tolcapone and entacapone

Baseline human and SimPops™



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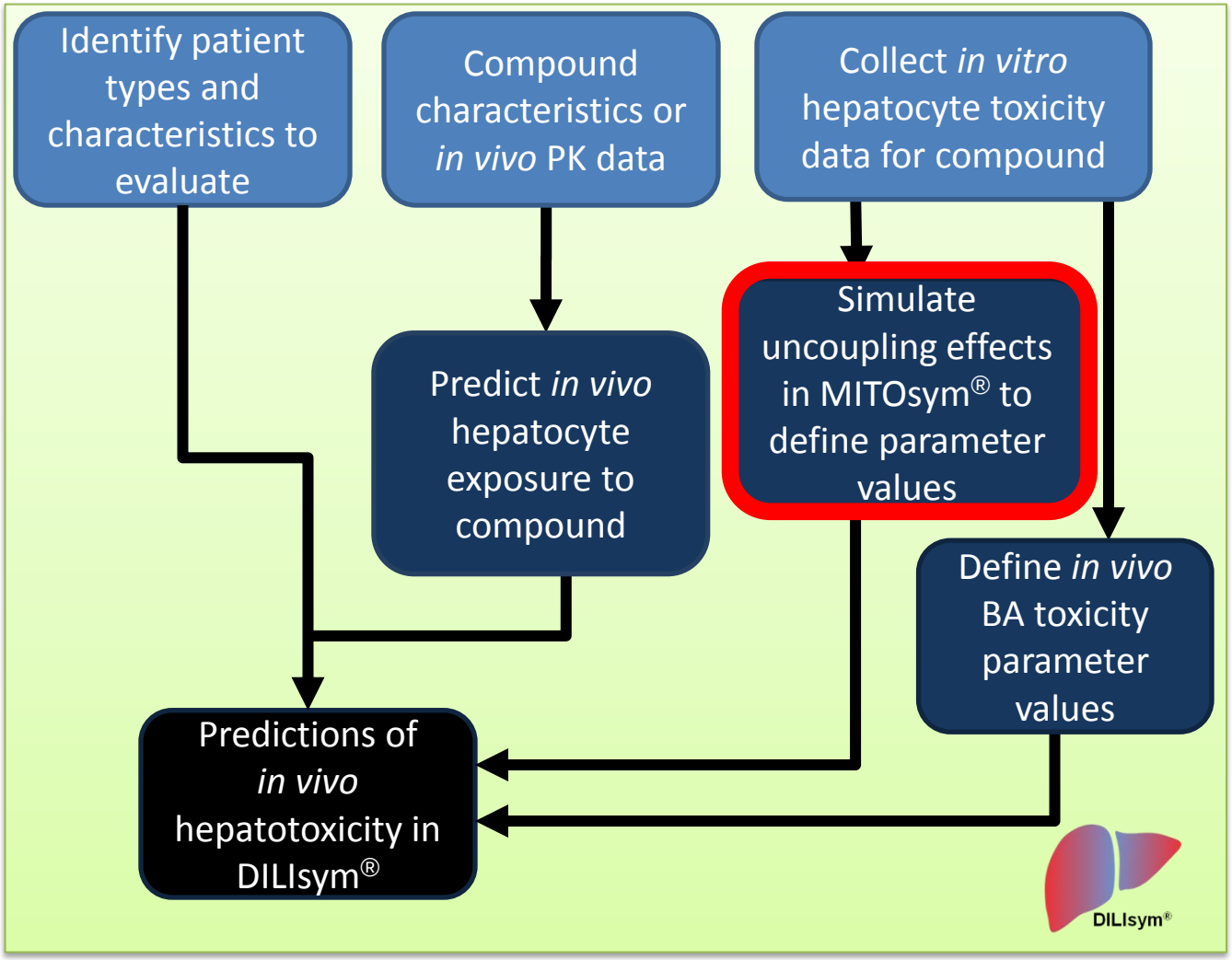
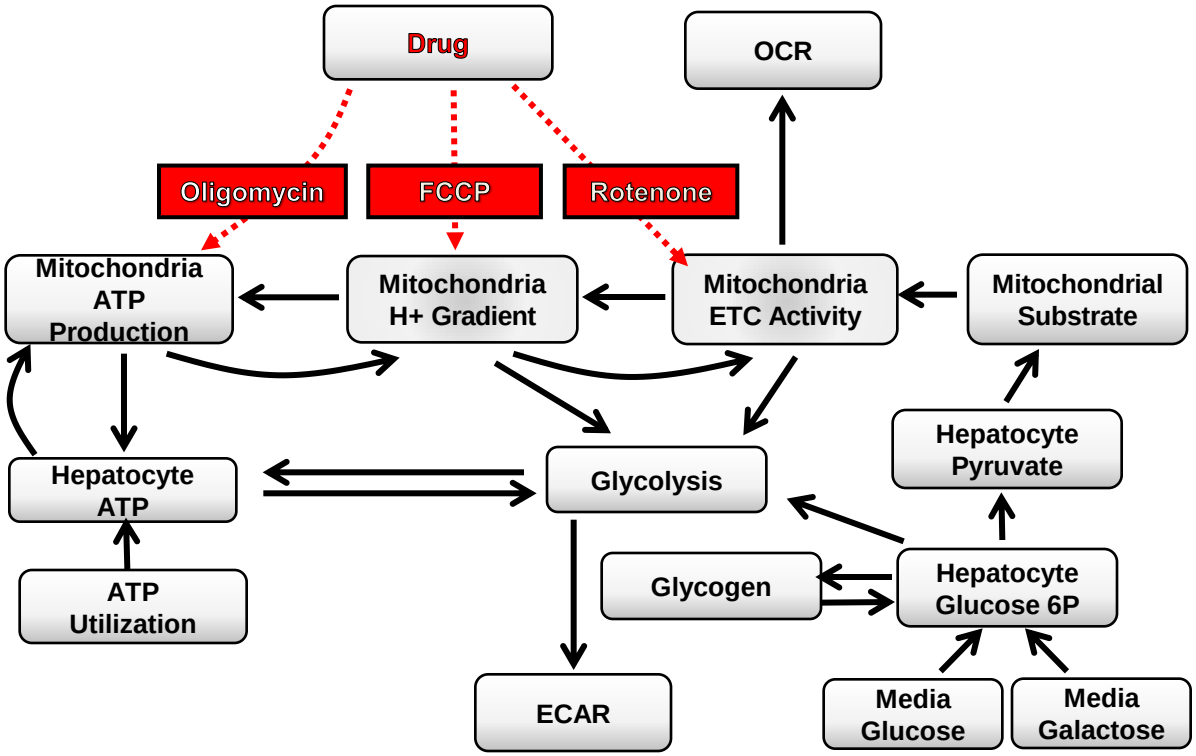


MITOsym® Model Includes Essential Components of Hepatocyte Bioenergetics

- Includes mitochondria ETC activity, proton gradient and ATP production
- Includes respiration (OCR) as a primary model output
  - Also includes ATP, $\Delta\Psi m$, ECAR
- MITOsym® simulates and recapitulates the reported dynamic changes exemplar drugs in HepG2, primary human and rat hepatocytes
- MITOsym® model is designed to provide inputs into the DILIsym® model to predict *in vivo* hepatotoxicity based on *in vitro* data

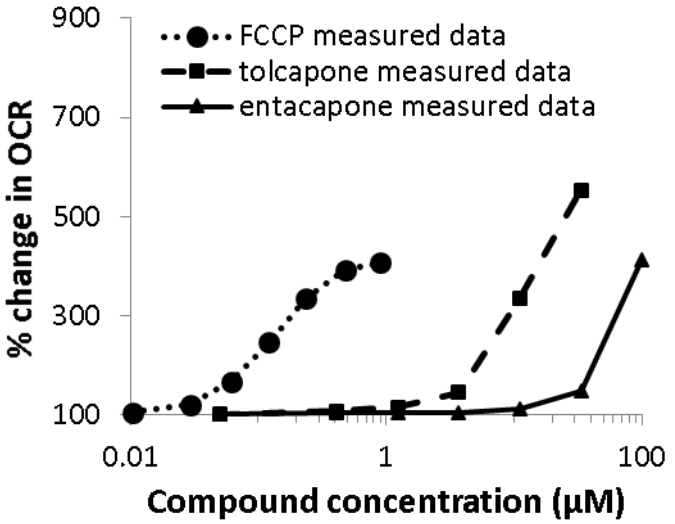


Simulating Uncoupling Effects in MITOsym® to Define Mitochondrial Toxicity Parameter Values

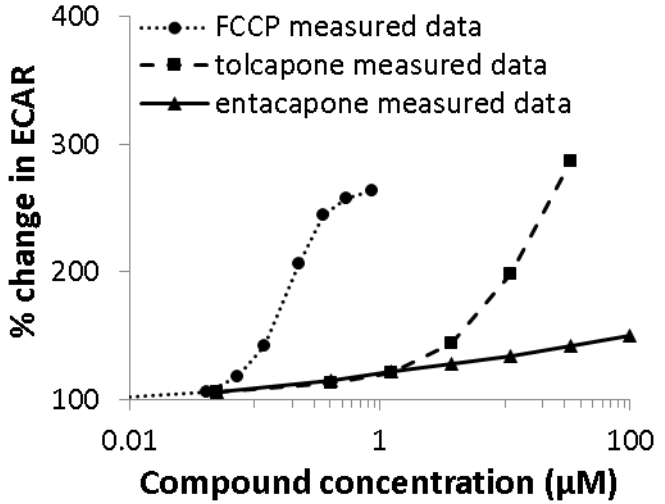
Objective:

- Use MITOsym® model to simulate changes in OCR and ECAR caused by uncoupling
- Determine uncoupling parameter values for entacapone and tolcapone by comparing simulated dose response curves to HepG2 measured data (Nadanaciva 2012)
  - FCCP is a MITOsym® exemplar compound with a strong uncoupling effect
  - Use HepG2 FCCP SimSingle available in MITOsym® as a starting point

HepG2

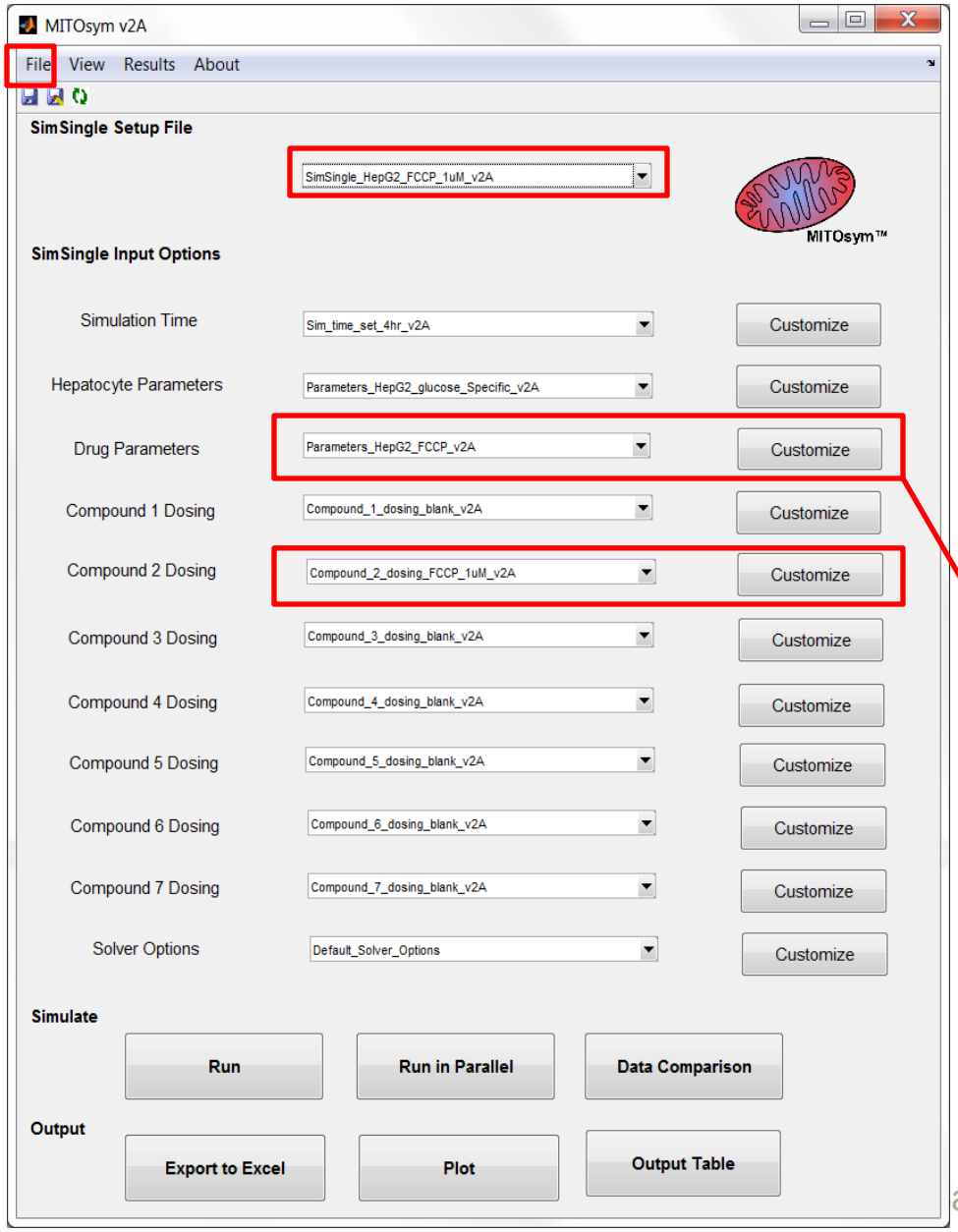


Nadanaciva 2012

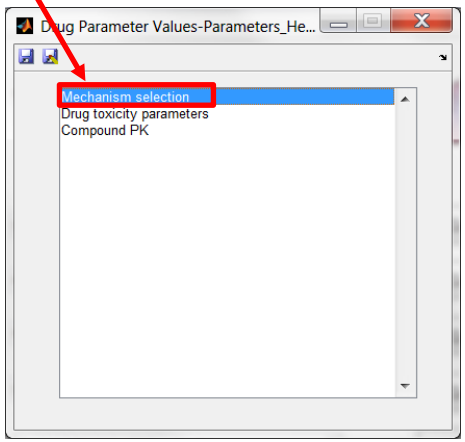


Nadanaciva 2012

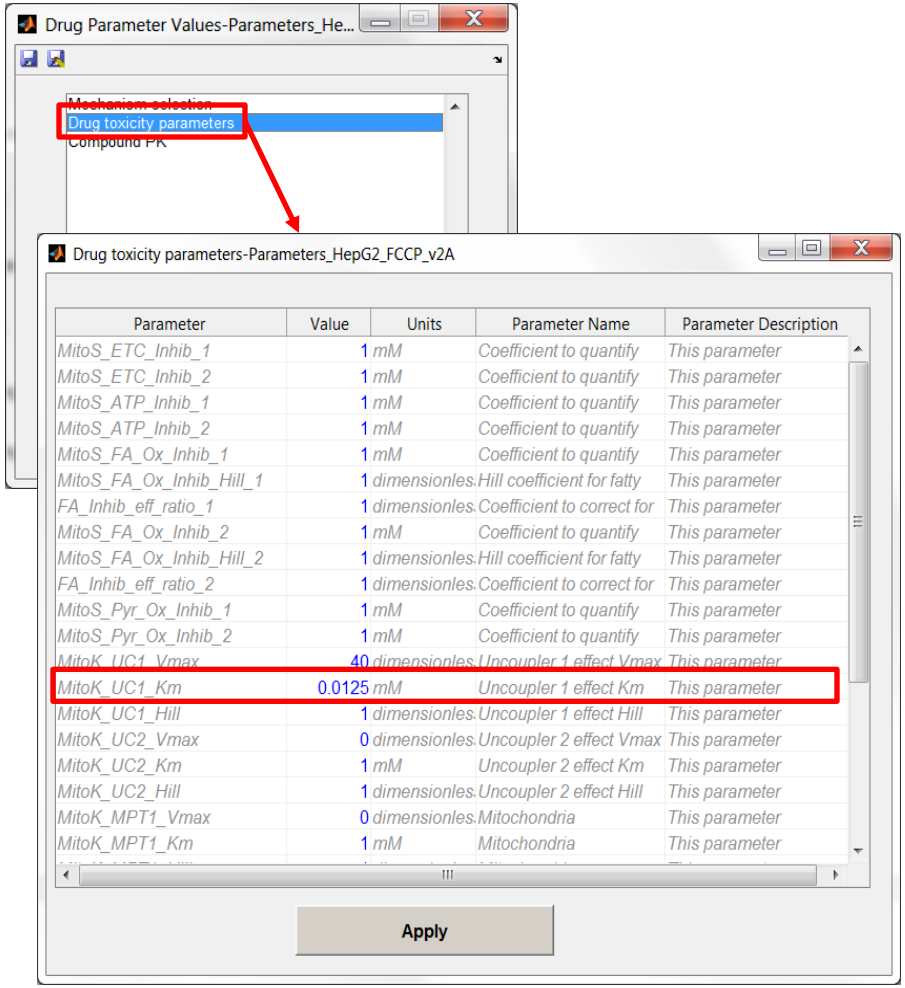
Creating Entacapone SimSingle™ in MITOsym®



1. Select HepG2 FCCP SimSingle in MITOsym®
2. Save SimSingle as: SimSingle_HepG2_Entacapone_1uM
3. View “Compound 2 Dosing” and save as: Compound_2_dosing_Entacapone
4. Select “Drug Parameters”, rename/save as: Parameters_HepG2_Entacapone
5. View “Mechanism selection”, verify “Mitochondrial uncoupler 1” is selected

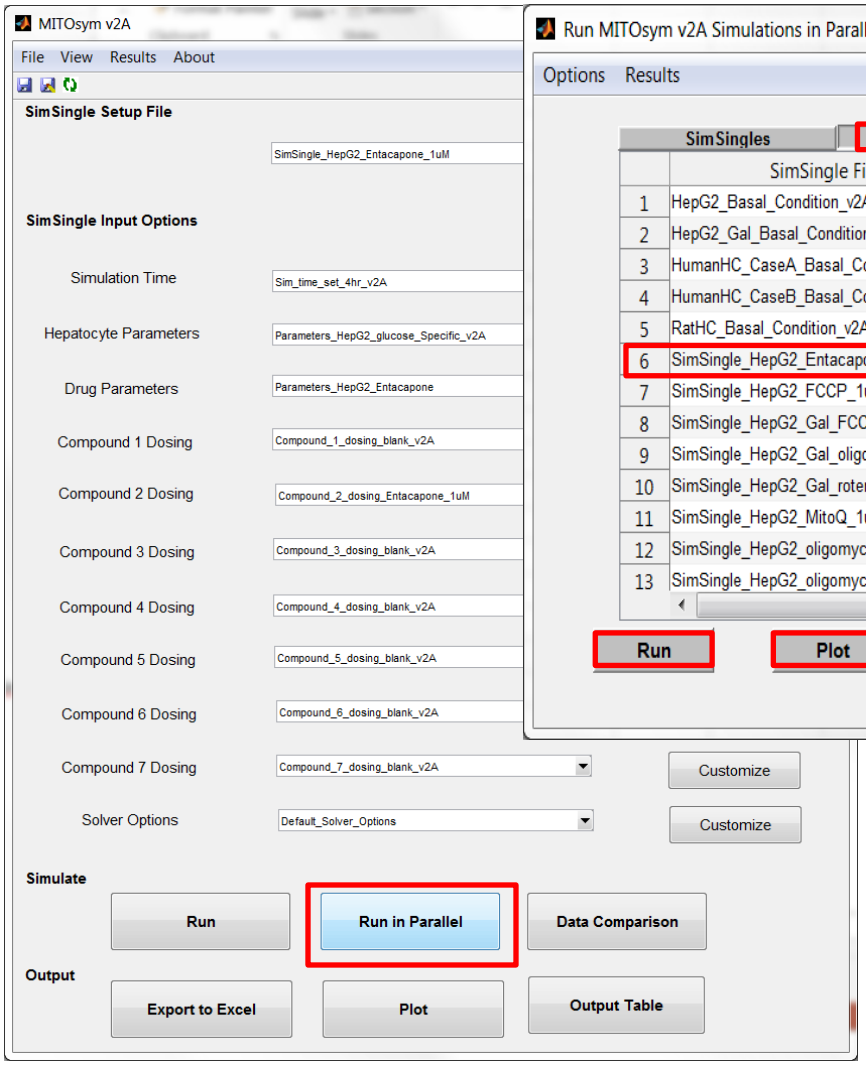


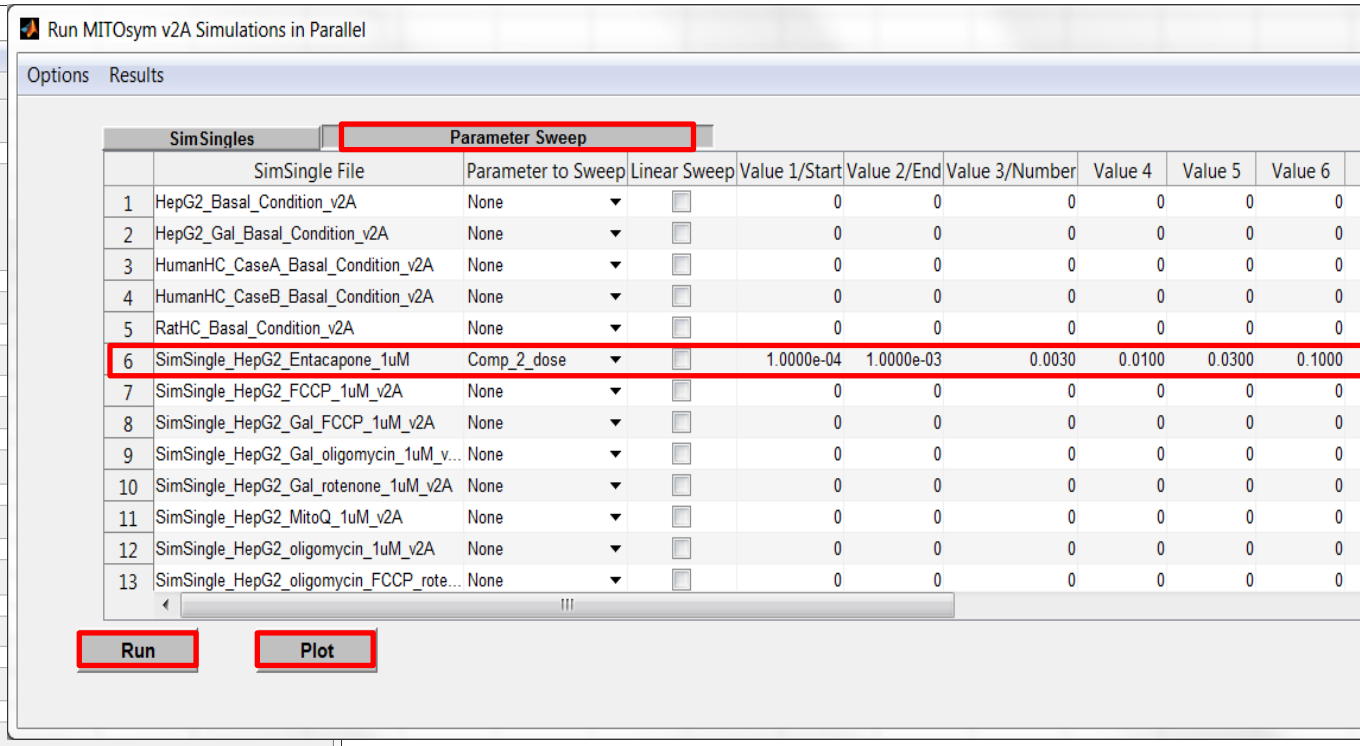
Changing the Uncoupling Drug Toxicity Parameter in MITOsym®



1. View “Drug toxicity parameters”
2. The Km for the effect of Uncoupler 1 is 0.0125 mM for FCCP
3. Based on previous simulation, Km for Tolcapone is about 5X higher than FCCP
4. Entacapone is a much weaker uncoupler than Tolcapone,
   - Try Km ~50x higher than FCCP as a first guess:
     - Change MitoK_UC1_Km to 0.5 mM
     - Apply and Save

Running a Dose Sweep in MITOsym®



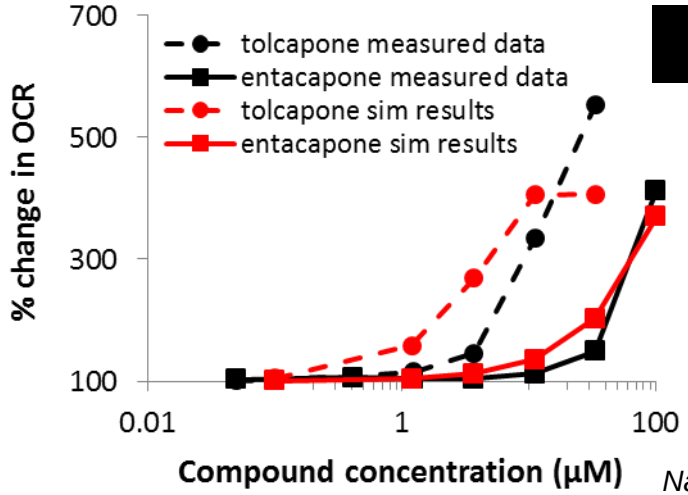


1. Run a parameter sweep for Comp_2_dose from 0.0001 to .1 mM
2. Iterate until the simulated OCR and ECAR dose-response agrees with measured data

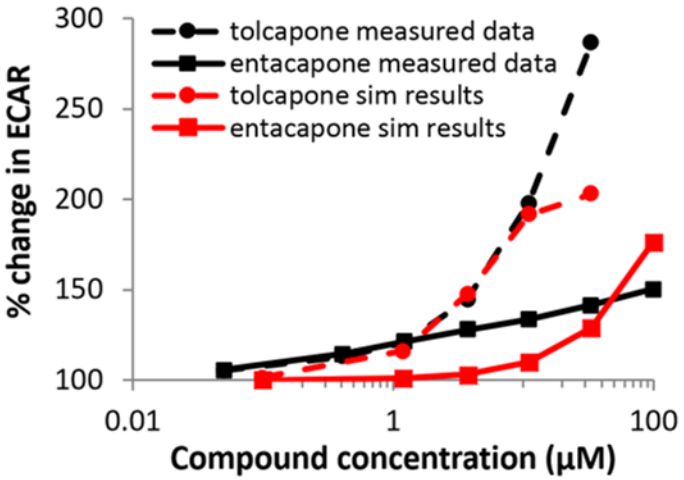
Entacapone and Tolcapone Uncoupler Parameter Values with MITOsym®

- Used MITOsym® model to simulate OCR and ECAR response to entacapone and tolcapone
  - Good agreement with measured OCR and ECAR data (by design)
- Entacapone is a weaker uncoupler than tolcapone
  - MitoK_UC1_Km parameter value is ~10x greater for entacapone than tolcapone
    - Entacapone Km 1.0
    - Tolcapone Km 0.065

HepG2



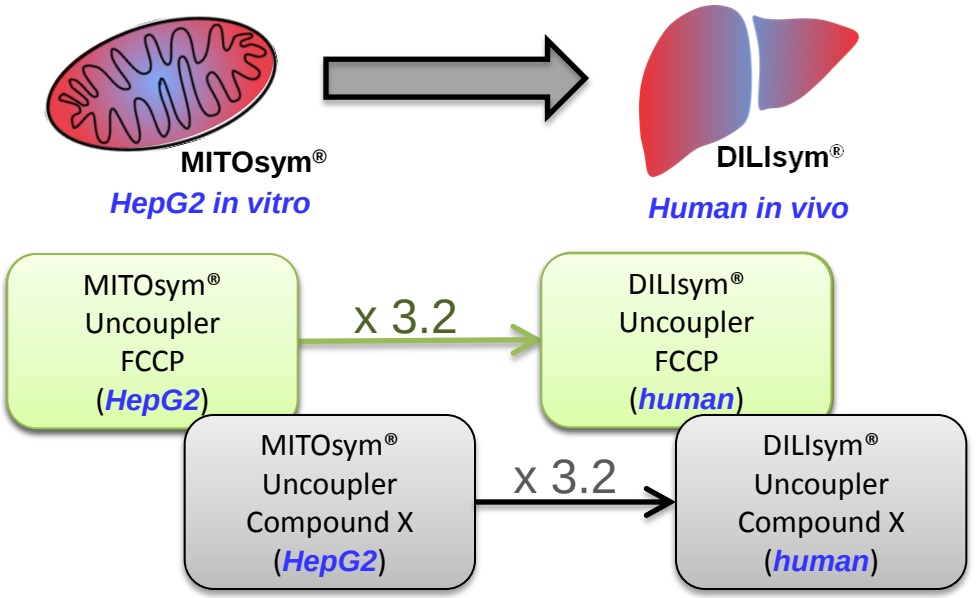
Nadanaciva 2012



Nadanaciva 2012

Exemplars Used to Translate MITOsym® Toxicity Parameters to DILIsym® Mitochondrial Toxicity Parameters

- MITOsym® exemplar compounds used to facilitate translation to DILIsym® in vivo mitochondrial toxicity parameters
- Exemplar mitochondria toxicity compounds simulated in DILIsym®
  - ETC inhibitor: rotenone
  - Uncoupler: FCCP
  - ATPase inhibitor: oligomycin
- Entacapone and tolcapone optimized Uncoupler parameter values normalized to FCCP for translation to DILIsym®
  - Conversion factor of 3.2 for uncoupling



MITOsym® to DILIsym® Mitochondrial Toxicity Parameter Value Conversion Factors

MITOsym® cell type	Mitochondria toxicity mechanism	DILIsym® species	MITOsym® to DILIsym® parameter conversion factor
HepG2	ETC inhibitor	Human	34.7
HepG2	Uncoupler	Human	3.20
HepG2	ETC inhibitor	Rat	3.47
HepG2	Uncoupler	Rat	0.40
Primary human hepatocyte	ETC inhibitor	Human	3.13
Primary human hepatocyte	Uncoupler	Human	3.20
Primary human hepatocyte	ETC inhibitor	Rat	0.31
Primary human hepatocyte	Uncoupler	Rat	0.40
Primary rat hepatocyte	ETC inhibitor	Human	3.75
Primary rat hepatocyte	Uncoupler	Human	3.20
Primary rat hepatocyte	ETC inhibitor	Rat	0.38
Primary rat hepatocyte	Uncoupler	Rat	0.40

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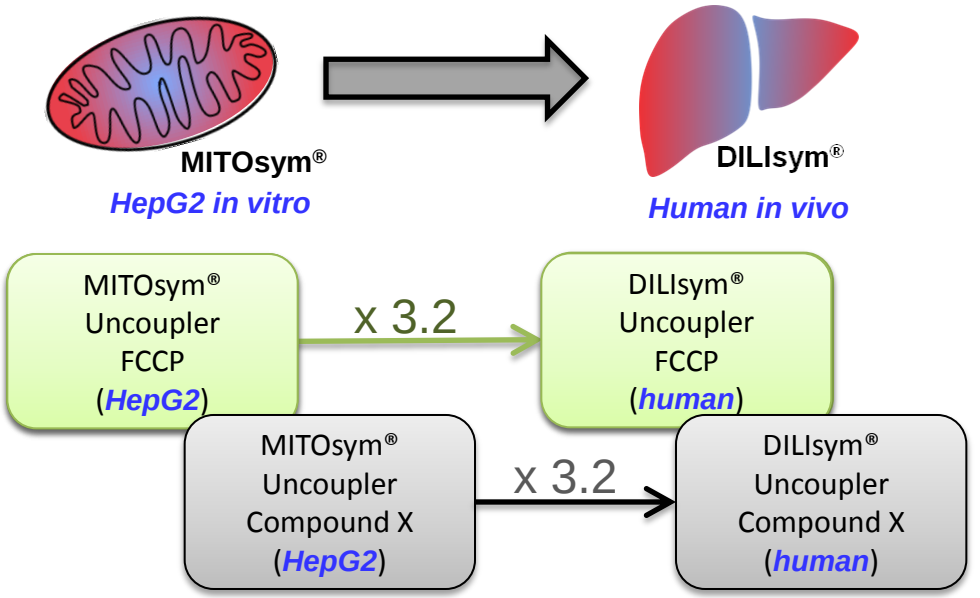
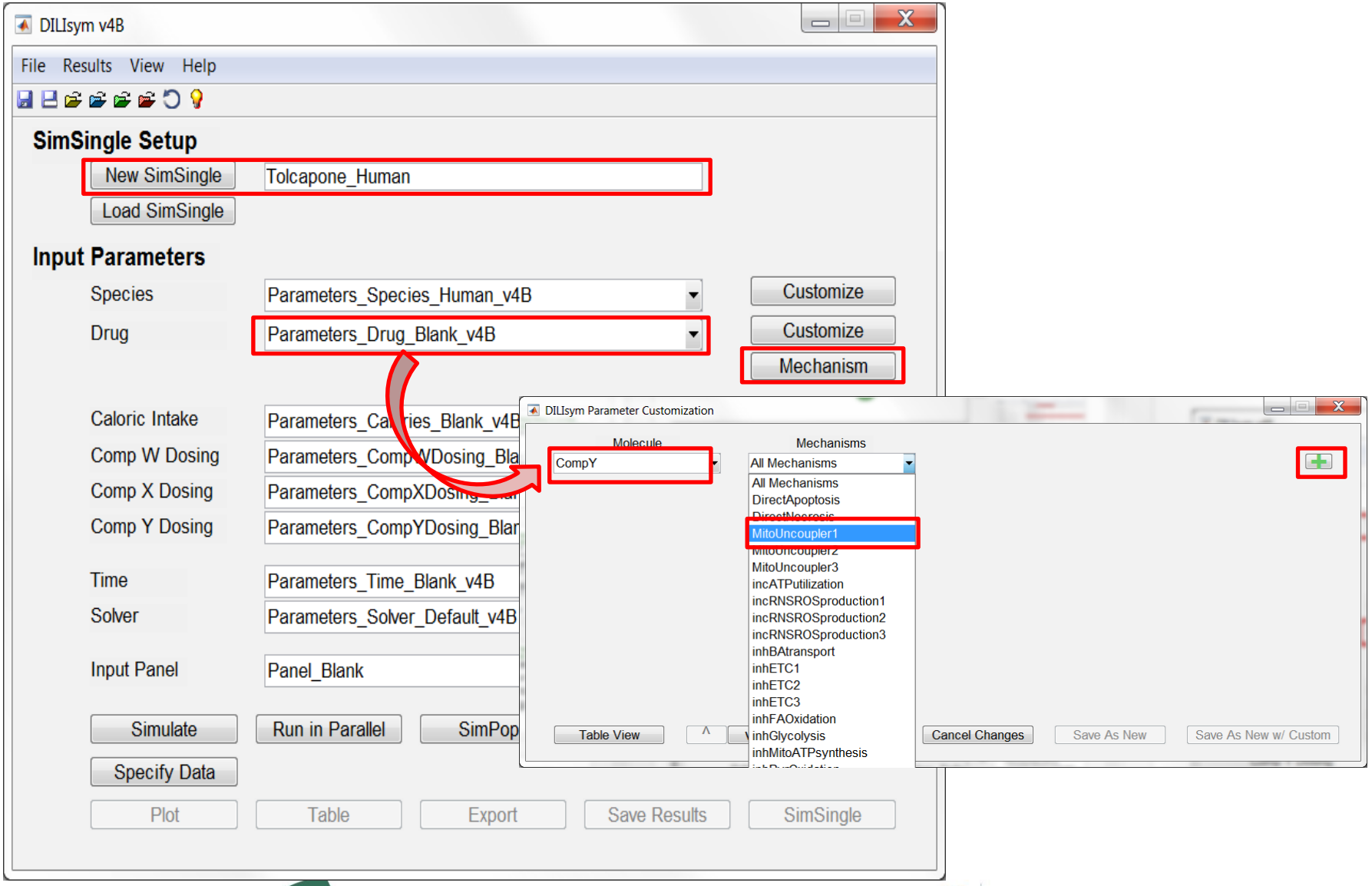
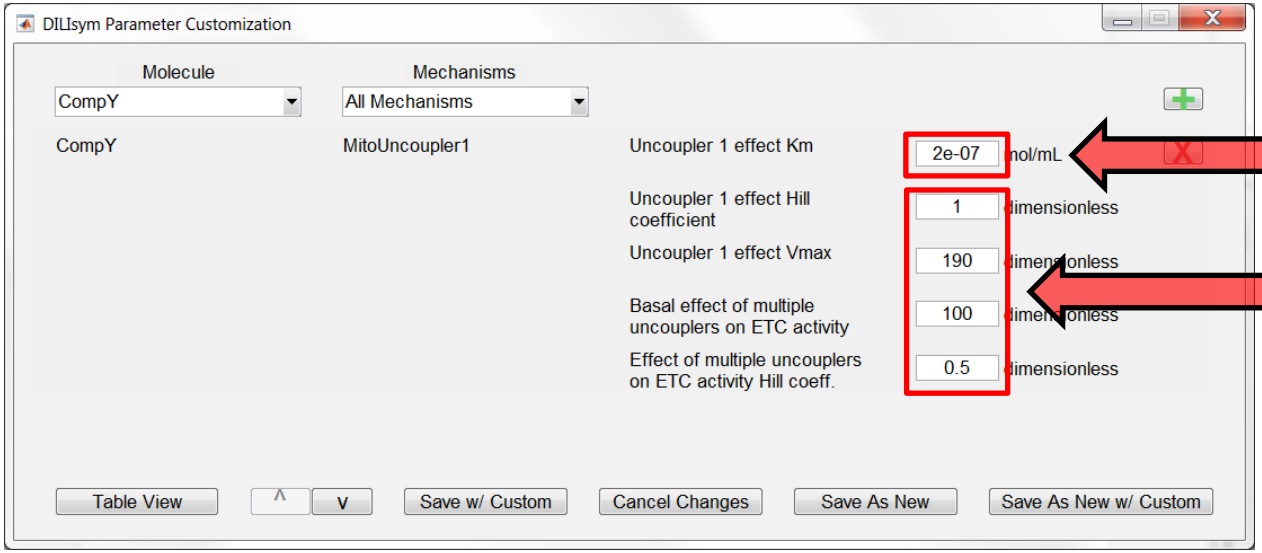


Compound	Mechanism	MITOsym® (mM)	DILIsym® (mM)
FCCP	Uncoupler	.0125	.040
Entacapone	Uncoupler	1.000	3.20
Tolcapone	Uncoupler	.065	0.208

Defining Drug Toxicity Parameters in DILIsym®: Mitochondrial Uncoupler Example



Drug Parameters in DILIsym®: Input MitoUncoupler1 Toxicity Parameter Values



Optimized value

Default values for DILIsym® uncouplers, need to input

Bile Acid Transport Inhibition DILIsym® Parameters for Entacapone and Tolcapone

- Bile acid transport inhibition constants (IC_{50}) for entacapone and tolcapone have been measured in Morgan 2013
  - Assumed noncompetitive BSEP and MRP inhibition
  - Used reported BSEP IC_{50} data as basis for noncompetitive BSEP Ki
  - Used reported MRP4 IC_{50} as basis for noncompetitive basolateral Ki

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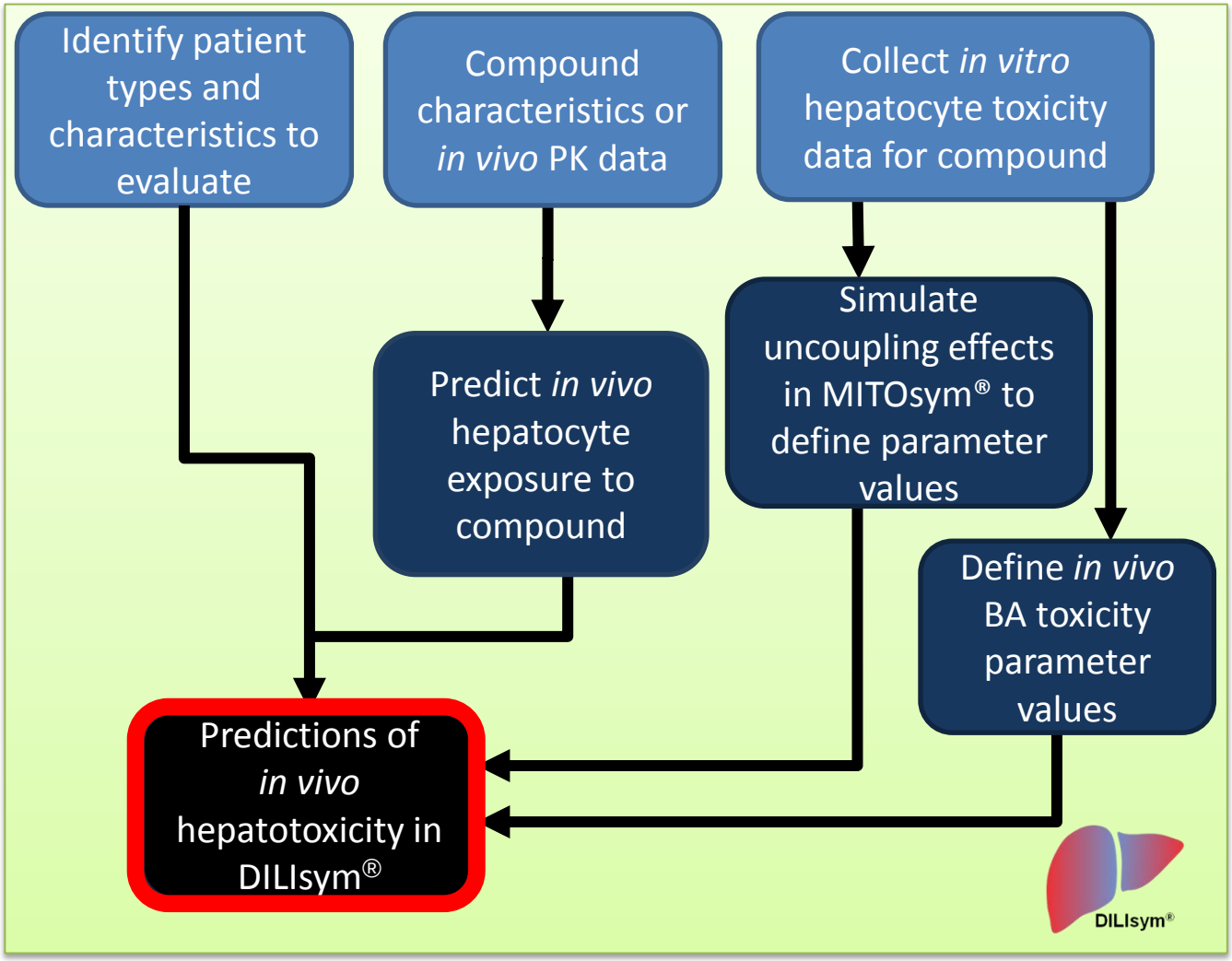

in vivo Hepatotoxicity Profiles Assessed Using Human BA-MITO SimPops™

- No ALT elevations observed in simulations at the population level following oral administration with entacapone
  - Clinical protocol (up to 8 oral doses of 200mg)
  - None of the human SimPops™ exhibited serum ALT elevations greater than 3x ULN
  - Consistent with lack of clinical hepatotoxicity reported for entacapone
- Small percentage of simulated patients treated with tolcapone with elevated ALT
  - Consistent with infrequent clinical hepatotoxicity reported for tolcapone
  - 3% of patients in clinical trials had >3x ULN ALT
  - NAFLD/NASH simulated patients most responsive to tolcapone hepatotoxic effects
- Simulation results revealed BSEP transporter inhibition contributed minimal liver toxicity

HUMANS

Simulated with Human_mito_BA_v3A_6 SimPops™, n=229	Simulated ALT >3x ULN	Clinical Data
Entacapone 200mg oral 8xday 1 week	0/229 (0%)	0/1000s (0%)
Tolcapone 200mg oral TID 1 week	6/229 (3%)	8/293 (3%)

Clinical Data and Simulation Results