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QST Applications, Use of Data and Species Differences

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Scientist II

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DILISYM OVERVIEW

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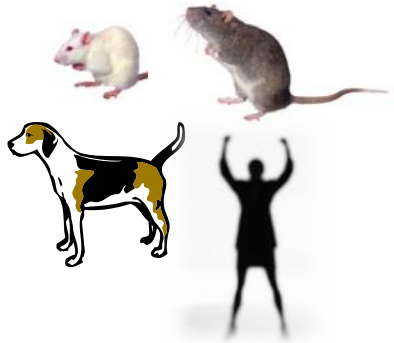
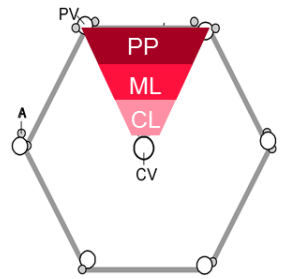
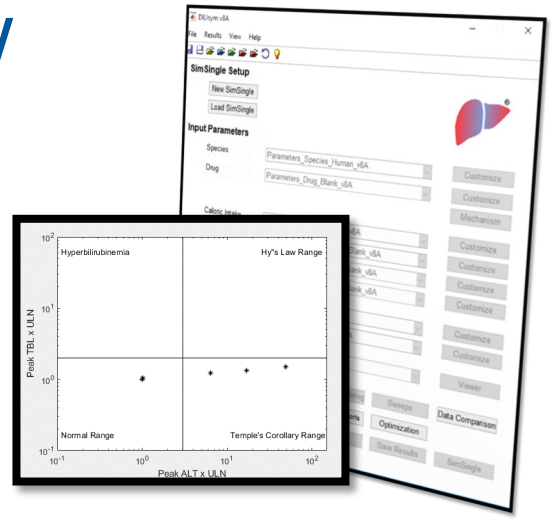
Disclaimer: DILIsym Services are developed and provided as an educational tool based on assessment of the current scientific and clinical information, and accepted approaches for drug safety and efficacy. The resultant data, suggestions, and conclusions ("Guidelines") should not be considered inclusive of all proper approaches or methods, and they cannot guarantee any specific outcome, nor establish a standard of care. These Guidelines are not intended to dictate the treatment of any particular patient. Patient care and treatment decisions should always be based on the independent medical judgment of health care providers, given each patient's individual clinical circumstances.

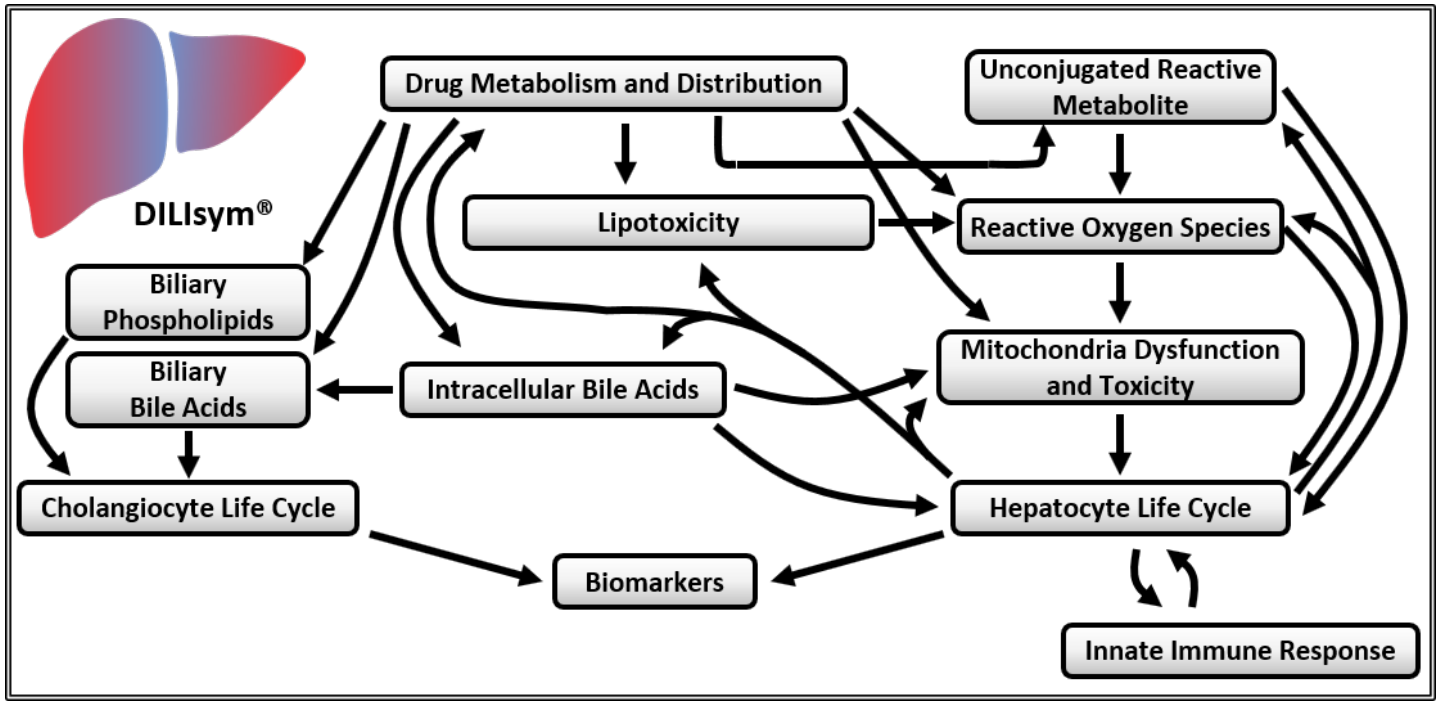
DILIsym Software Overview

- **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**
- **Over 70 detailed representations of optimization or validation compounds with 80% success**
- **Single and combination drug therapies**









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Assess Compound X and Compound Y

CASE STUDY 1

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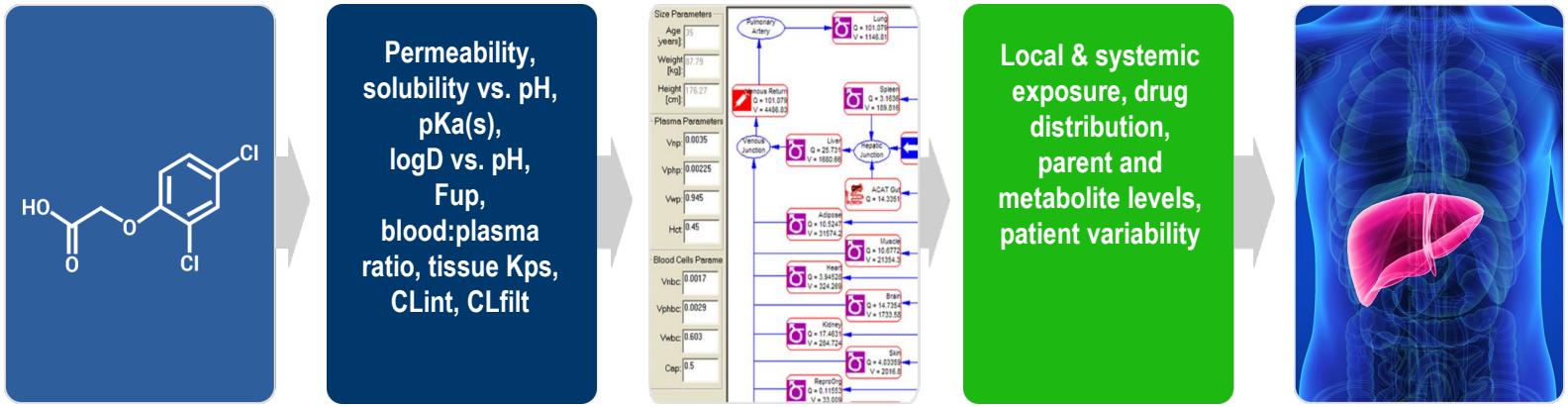
Case Study 1: Assess Compound X and Compound Y

- The primary goal of this simulation work was to:
  - quantitatively and mechanistically assess the liver toxicity potential of Compound X and Compound Y combining clinical and mechanistic *in vitro* data with DILIsym and GastroPlus software simulations of previous or prospective clinical dosing paradigms.

Saying “I do” to the QSAR / PBPK / QST marriage…



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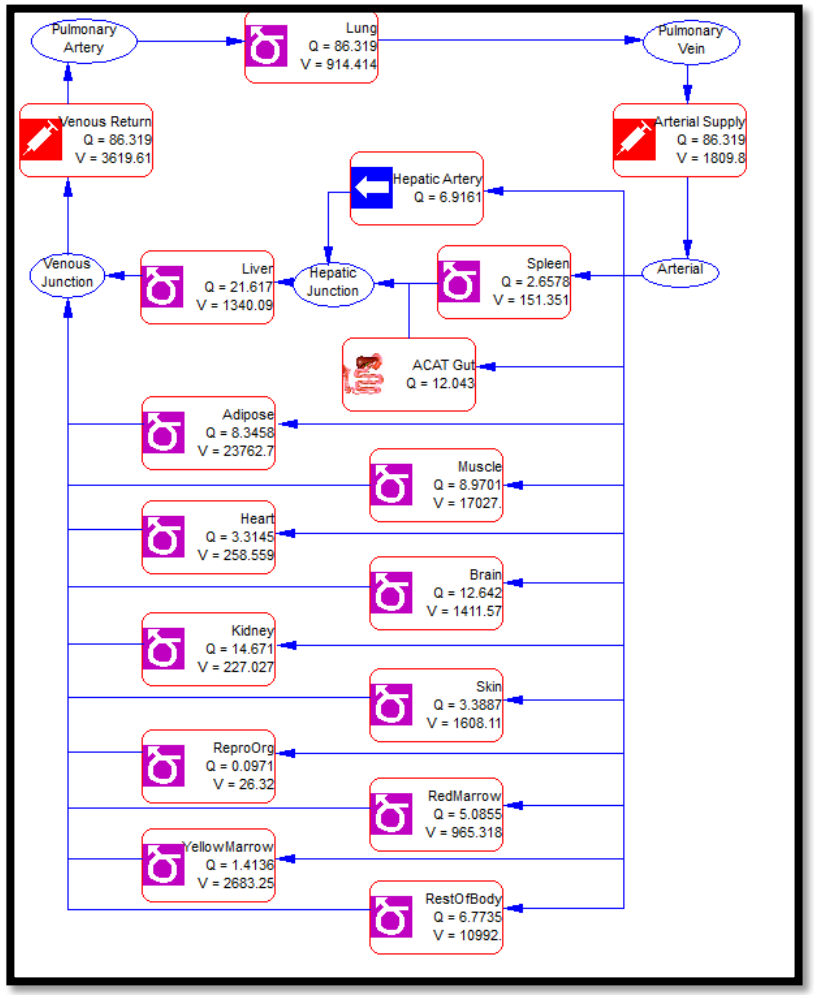
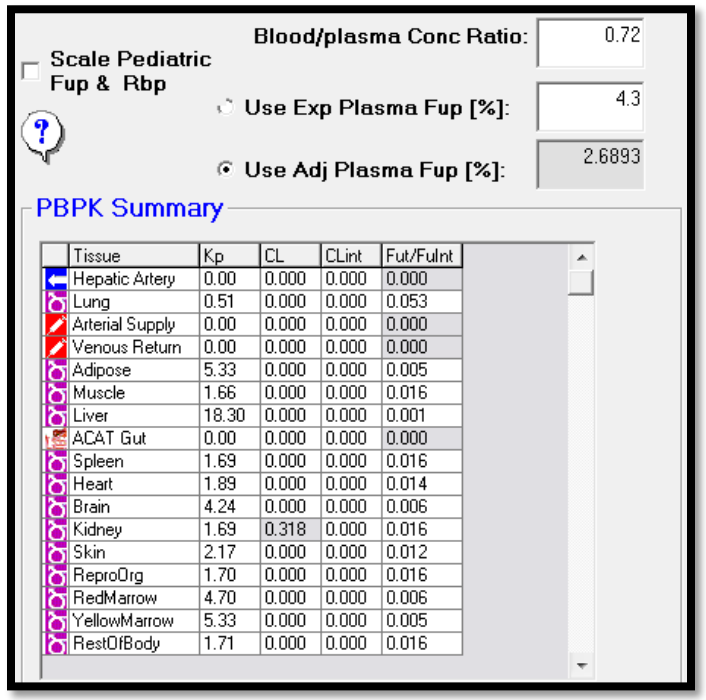
GastroPlus PBPK Model Used to Predict Liver Exposure of Compound Y and Compound X

- Data on Compound Y and Compound X pharmacokinetics not available in the literature
  - *In vitro* data on liver distribution available from intracellular data collected for this project
- Structure of each compound available online
  - QSAR modeling using ADMET Predictor and GastroPlus provided the best possible estimate of Compound Y and Compound X distribution and pharmacokinetics
- Plasma time course was estimated in GastroPlus and translated into DILIsym
- Both compounds distribute significantly into the liver



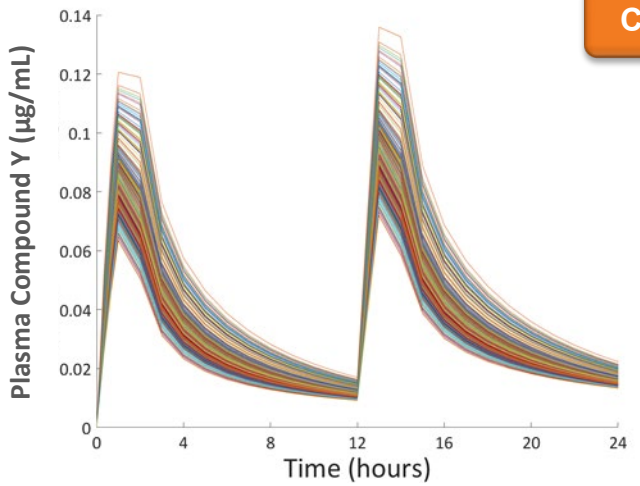
Example PBPK Representation: Compound Y at the Clinical Dose

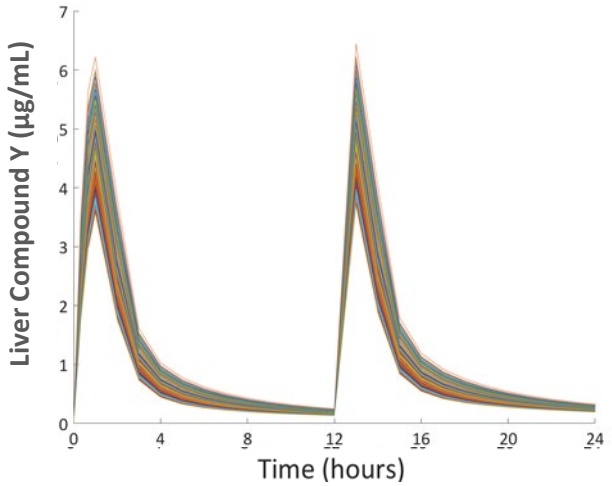
- GastroPlus predictions for liver and plasma at clinical dose shown at right
  - PBPK model specific predictions shown below
  - Dose escalation was simulated



Tissue	Kp	CL	CLint	Fut/Fulnt
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.51	0.000	0.000	0.053
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	5.33	0.000	0.000	0.005
Muscle	1.66	0.000	0.000	0.016
Liver	18.30	0.000	0.000	0.001
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	1.69	0.000	0.000	0.016
Heart	1.89	0.000	0.000	0.014
Brain	4.24	0.000	0.000	0.006
Kidney	1.69	0.318	0.000	0.016
Skin	2.17	0.000	0.000	0.012
ReproOrg	1.70	0.000	0.000	0.016
RedMarrow	4.70	0.000	0.000	0.006
YellowMarrow	5.33	0.000	0.000	0.005
RestOfBody	1.71	0.000	0.000	0.016

Compound Y





Simulation Results

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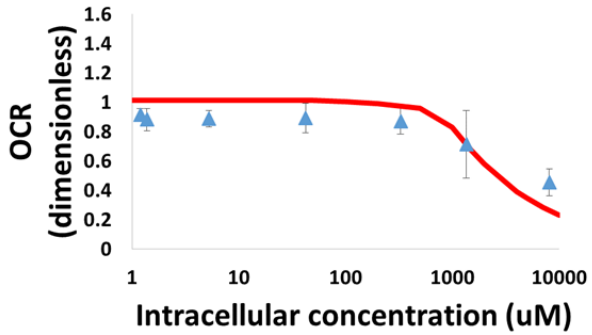
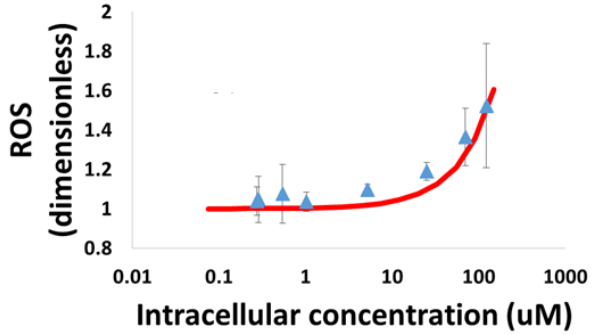
Example Toxicity Data: Compound Y *In Vitro* Data

Compound Y

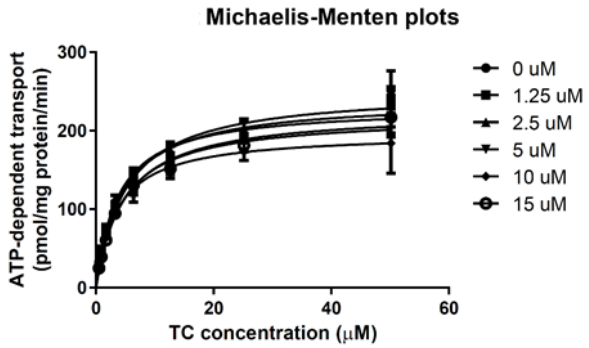
Mitochondrial toxicity



Oxidative stress



Transporter inhibition



- DILIsym collaborates with 3rd party providers to collect *in vitro* data relating compounds to mechanisms of toxicity
  - Cyprotex for mitochondrial toxicity and oxidative stress
  - Solvo for transporter inhibition
- Compound-specific toxicity parameters estimated by simulating *in vitro* data

Preclinical Data and Simulation Results

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SimPops Results Show Compound X and Compound Y to be Safe at Clinical Doses

- Simulations conducted in human simulated population (SimPops, n=285)
- Neither Compound Y nor Compound X are predicted to cause toxicity at the highest clinical dose (1X dose)
- Both Compound Y and Compound X are predicted to cause mild ALT elevations at supratherapeutic doses (5x, 10X of highest clinical dose)
  - No bilirubin elevations or Hy's Law cases occurred in simulations with Compound X
  - 2 Hy's Law cases occurred at 10x clinical dose simulations with Compound Y

HUMANS

Compound	Dosing Protocol	Simulated* ALT > 3X ULN**
Compound Y	**1X Dose, 12 weeks**	**0% (0/285)**
	2X Dose, 12 weeks	0% (0/285)
	5X Dose, 12 weeks	0.3% (1/285)
	10X Dose, 12 weeks	10.2% (29/285)
Compound X	**1X Dose, 15 days**	**0% (0/285)**
	2X Dose, 15 days	0% (0/285)
	5X Dose, 15 days	1.1% (3/285)
	10X Dose, 15 days	11.6% (33/285)

**The full v4A-1 SimPops (n=285) of normal healthy volunteers was used*

***Upper limit of normal (ULN) in DILIsym is 40 U/L*

Case Study 1: Summary

- ADMET Predictor™ and GastroPlus™ software, along with *in vitro* data, was used to construct PBPK representations to predict liver exposures for both compounds
- DILIsym parameters were successfully calculated from *in vitro* data for both compounds
- SimPops results show Compound X and Compound Y to be safe at projected clinical doses
- ALT elevations predicted within DILIsym at higher doses for both compounds
- SimPops results suggest that neither compound is likely to cause severe liver injury
- ***Phase IIb / III clinical trial results have subsequently confirmed the predictions for Compound Y***

Investigate observed species differences in DILI

CASE STUDY 2

Case Study 2: Investigating Rat *vs*. Human CKA DILI

- The primary goal of this simulation work was to:
  - investigate whether the mechanisms of toxicity represented in DILIsym can account for the observed species differences (rat DILI *vs*. human no DILI)

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Research Article

Using Quantitative Systems Toxicology to Investigate Observed Species Differences in CKA-Mediated Hepatotoxicity

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In Vitro Data Informed Mechanisms of Toxicity for Rats and Humans

- Toxicity parameter values identified for CKA interaction with all three mechanisms of toxicity
  - Most data are species-specific
- Predicted hepatotoxicity highly dependent on placing these data in the context of *in vivo* exposure

RATS

HUMANS

Table 1. Toxicity Parameters for Human and Rat

	Human	Rat
Bile acid transporter inhibition constant (μM)		
BSEP	94[a]	129.7[b]
MRP3	11.2	11.2[c]
MRP4	12.3	12.3[c]
NTCP	19.5	19.5[c]
Mitochondrial toxicity constant (mM)		
ETC inhibition constant	14.2	1.42
ROS production constant (mL/mol/h)		
ROS production constant	7278	9705

[a]Data from Astra Zeneca (unpublished).
[b]From Ulloa et al (2013).
[c]Assumed to be the same as human.

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SimPops Results Recapitulate Rat but No/Minimal Human Hepatotoxicity

- CKA simulations conducted in rat and human SimPops (n=294, n=285)
  - Different dosing protocols simulated in species-specific PBPK models
- CKA induced hepatotoxicity in simulated rats but not humans, consistent with data

RATS

HUMANS

Table 2. Summary of CKA-Mediated Hepatotoxicity in Rat and Human SimPops and Preclinical and Clinical Observations

	Species	Rat	Rat	Human	Human	Human
Simulations				Simulations[a]		
	Dose	200 mg/kg	500 mg/kg	300 mg	600 mg	900 mg
	Population size	$n = 294$	$n = 294$	$n = 285$	$n = 285$	$n = 285$
	ALT > 3× ULN (%)[b]	2.4	36.4	0	0	0
	ALT > 5× ULN (%)[b]	0	20.1	0	0	0
	ALT > 10× ULN (%)[b]	0	7.8	0	0	0
Data				Preclinical/clinical trials		
	Dose	200 mg/kg	500 mg/kg	300 mg	600 mg	900 mg
	Population size	$n = 8$	$n = 4$	$n = 5$	$n = 4$	$n = 6$
	ALT > 3× ULN (%)[b]	25	75	0	0	16.7
	ALT > 5× ULN (%)[b]	0	50	0	0	0
	ALT > 10× ULN (%)[b]	0	25	0	0	0

[a]Human simulations were run for 96 h, rat simulations for 72 h.
[b]Upper limit of normal (ULN) was 30 U/L in rat simulations and preclinical trials. ULN was 40 U/L in human simulations and clinical trials.

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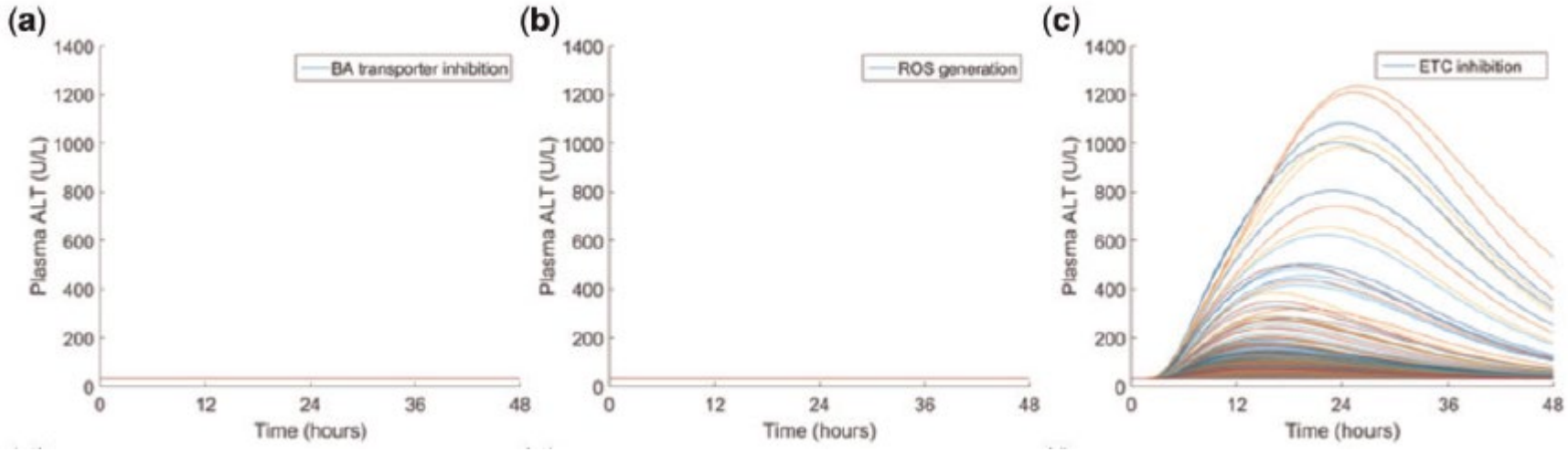
Mechanistic Investigations Reveal Main Driver of Hepatotoxicity

RATS

- Simulations conducted in rat SimPops using a single mechanism of toxicity
- Mitochondrial mechanism alone could account for CKA hepatotoxicity
- Combination of oxidative stress and transporter inhibition mechanisms absent mitochondrial mechanism were insufficient to account for CKA hepatotoxicity



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Simulation Results

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Case Study 2: Summary

- Species-specific data can be used to identify toxicity parameter values for preclinical species
- SimPops results reproduced rat but no/minimal human hepatotoxicity
- Investigative simulations implicated mitochondrial toxicity as a key driver of response
- Results support the application of QST modeling to interpret preclinical liver signals

SUMMARY

Summary

- Simulations Plus software can be used to predict chemical properties and exposure in a simulated population based on chemical structure alone
- DILIsym software can utilize exposure predictions and *in vitro* data to predict hepatotoxicity risk before compounds have been tested clinically
  - Can also provide insight into safety margins for dose selection
- DILIsym has been shown to distinguish toxicity between species for a given compound

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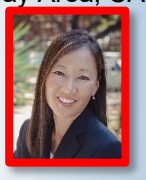

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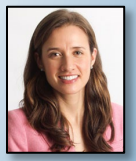

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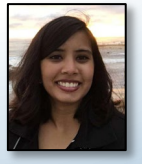

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QUESTIONS?

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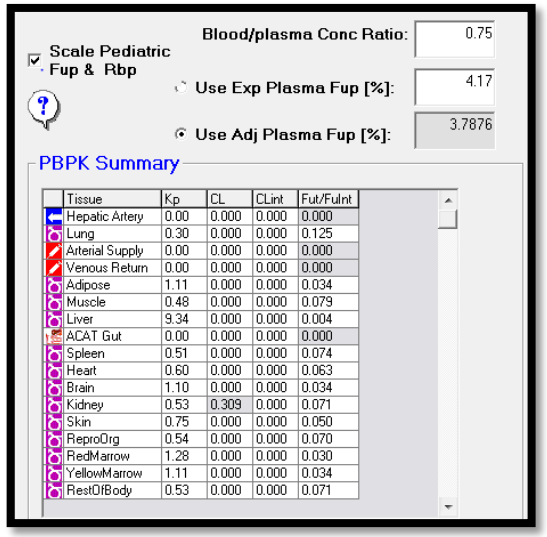
BACKUPS

Compound X PBPK Representation Calculated at Predicted Clinical Dose

- GastroPlus predictions for liver and plasma at clinical dose shown at right
  - PBPK model specific predictions shown below
  - Dose escalation and alternate protocols were also simulated



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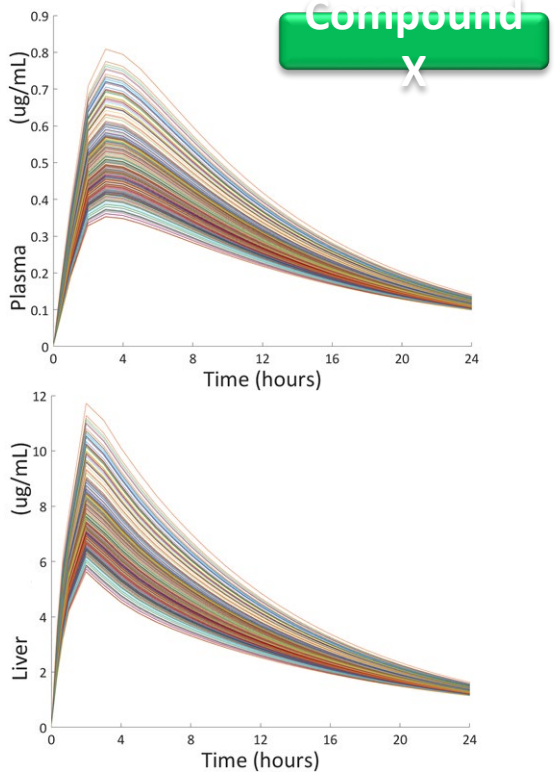
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.30	0.000	0.000	0.125
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	1.11	0.000	0.000	0.034
Muscle	0.48	0.000	0.000	0.079
Liver	9.34	0.000	0.000	0.004
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	0.51	0.000	0.000	0.074
Heart	0.60	0.000	0.000	0.063
Brain	1.10	0.000	0.000	0.034
Kidney	0.53	0.309	0.000	0.071
Skin	0.75	0.000	0.000	0.050
ReproOrg	0.54	0.000	0.000	0.070
RedMarrow	1.28	0.000	0.000	0.030
YellowMarrow	1.11	0.000	0.000	0.034
RestOfBody	0.53	0.000	0.000	0.071



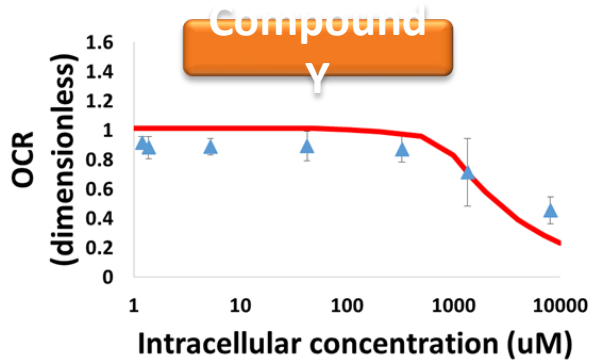
Simulation Results

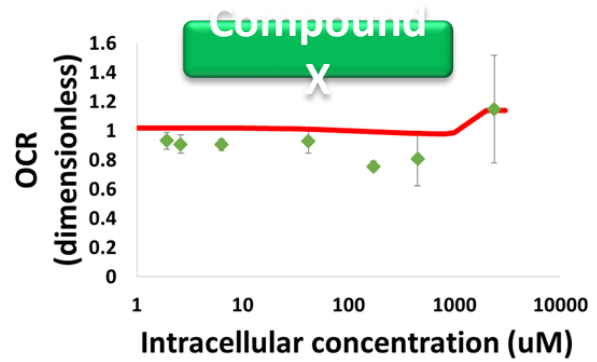
Mitochondrial Toxicity Parameters Determined for Compound Y and Compound X

- Parameter values were fit to mitochondrial data for Compound Y and Compound X
  - Electron transport chain inhibition for Compound Y
  - Both electron transport chain inhibition and uncoupling for Compound X
  - 24 hour data used
- MITOsym and D[cyprotex AN EVOTEC COMPANY]meterize both compounds

DILIsym Parameter	Compound Y Value	Compound X Value	Units
Coefficient for ETC inhibition 1	38,000	Not used	µM
Coefficient for ETC Inhibition 3	0.1	4,200	µM
Max inhibitory effect for ETC inhibition 3	0.2	0.4 (max effect)	dimensionless
Uncoupler 1 effect Km	No effect	15,000	µM
Uncoupler 1 effect Vmax	No effect	22	dimensionless
Uncoupler 1 effect Hill	No effect	4	dimensionless

Preclinical Data and Simulation Results





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Oxidative Stress Parameters Determined for Compound Y and Compound X

- Parameter values were fit to 24-hour ROS data for Compound Y and Compound X
- DILIsym representation of *in vitro* environment used to parameterize both compounds

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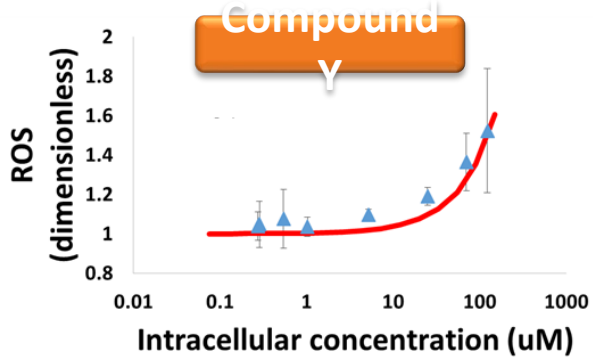
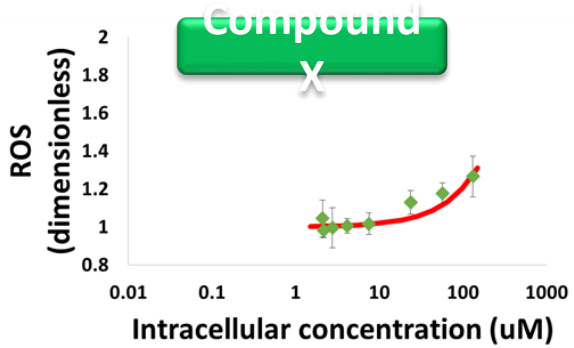
DILIsym Parameter	Compound Y Value	Compound X Value	Units
RNS/ROS production rate constant 1	3.4×10^{-4}	1.7×10^{-4}	mL/nmol/hr





Preclinical Data and Simulation Results

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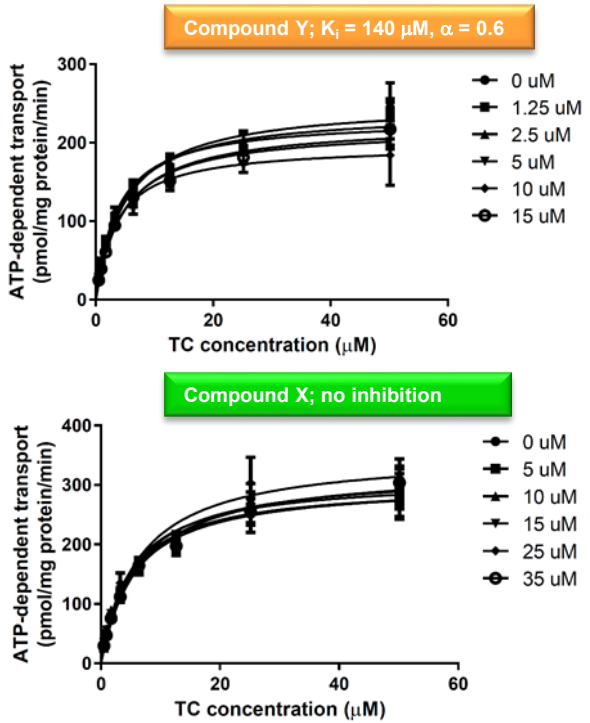
Compound Y Weakly Inhibits BSEP; Compound X Does Not

- Compound Y is a low-potency inhibitor of BSEP
  - Compound Y also inhibits MRP4 transport (not shown)
- Compound X does not inhibit BSEP
  - No changes to V_{max} or K_m of transporters observed over course of assay
  - Compound X inhibits MRP4 transport (not shown)



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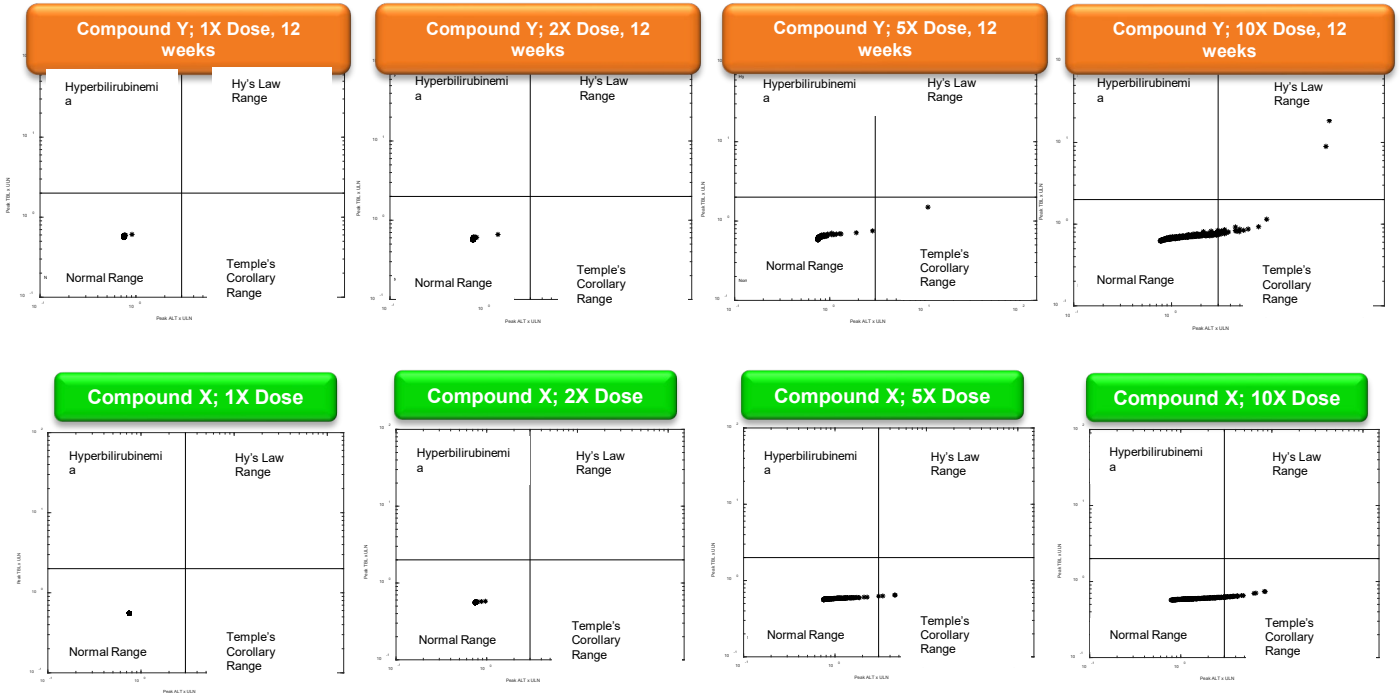
Preclinical Data

DILIsym Toxicity Parameters for Compound Y and X

Mechanism	Parameter	Unit	DILIsym Parameter Value*	
			Compound Y	Compound X
Mitochondrial Dysfunction	Coefficient for ETC inhibition 1	µM	38,000	Not used
	Coefficient for ETC Inhibition 3	µM	0.1	4,200
	Max inhibitory effect for ETC inhibition 3	dimensionless	0.2	0.4
	Uncoupler 1 effect Km	µM	No effect	15,000
	Uncoupler 1 effect Vmax	dimensionless	No effect	22
	Uncoupler 1 effect Hill	dimensionless	No effect	4
Oxidative Stress	RNS/ROS production rate constant 1	mL/nmol/hr	3.4×10^{-4}	1.7×10^{-4}
Bile Acid Transporter Inhibition	BSEP inhibition constant	µM	140	No inhibition
	BSEP inhibition alpha value	dimensionless	0.6	No inhibition
	NTCP inhibition constant	µM	No inhibition	No inhibition
	MRP4 inhibition constant	µM	40	75

*Values shown in the table for DILIsym input pa … l with exposure in DILIsym to produce simulations that have predictive and insightful v…

SimPops Results Show Lack of Severe Liver Injury for Both Compound Y and Compound X at Clinical Doses



*The full v4A-1 SimPops (n=285) of normal healthy volunteers was used
**Upper limit of normal (ULN) in DILIsym is 40 U/L for ALT and 1 mg/dL for bilirubin.

Simulation Results

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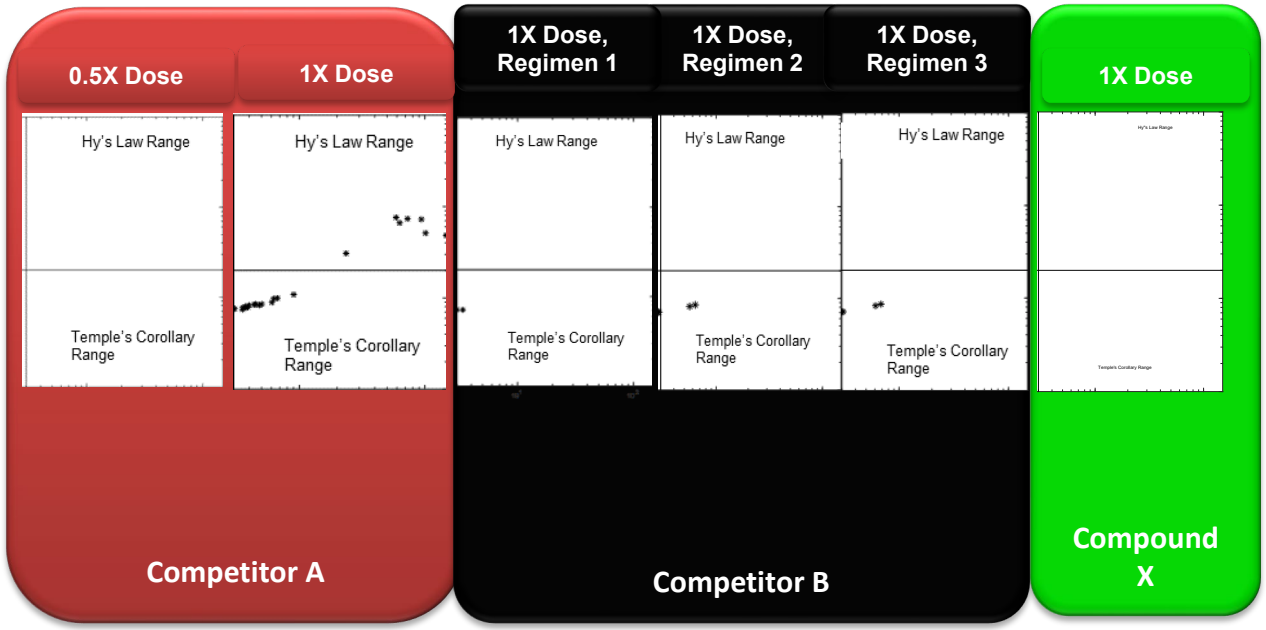
Comparison with Competitors Suggests Compound X Has a Differentiated Liver Safety Profile



Simulation Results

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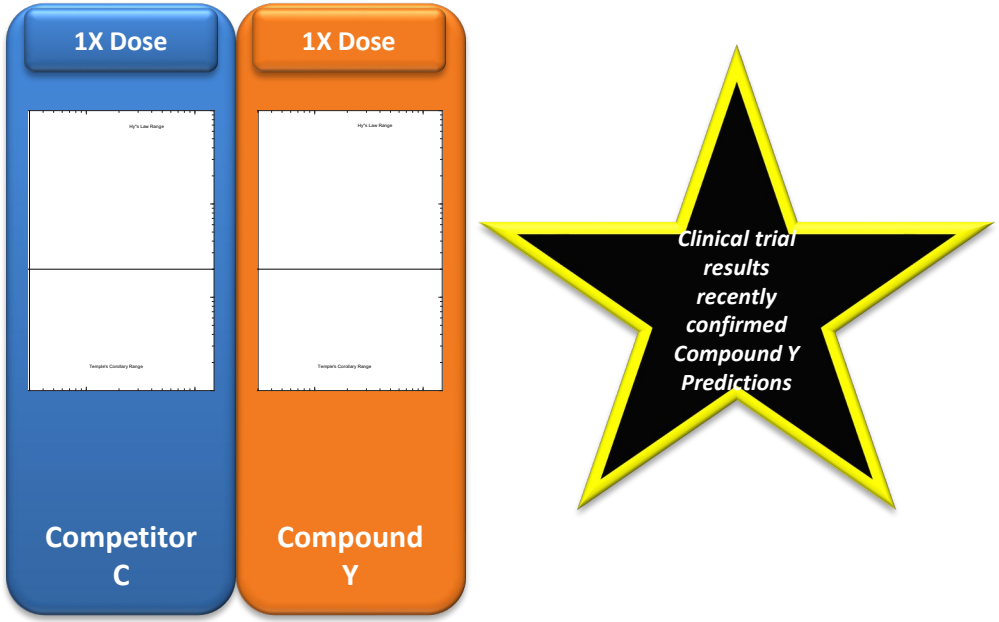
Comparison with Compound Y Competitor Suggests Comparable Liver Safety Profile



Simulation Results