



# **DILIsym® User Training – Bilirubin Data Collection and DILIsym® setup**

**October 2016**

**DILIsym® Development Team**

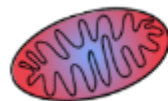
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# Goal for This Training Session

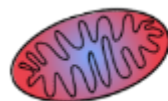
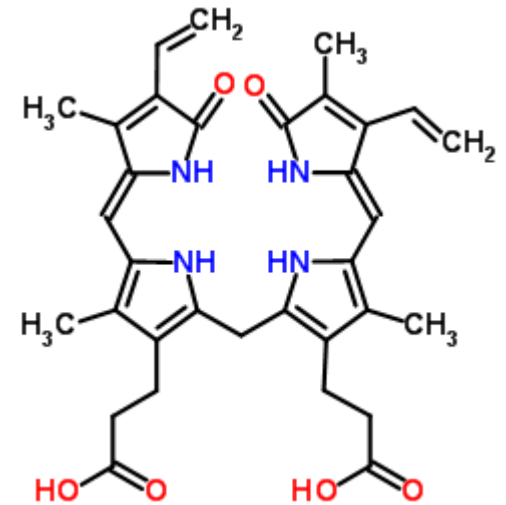
*Participants should understand the following general concepts:*

- Data collection and model setup for DILIsym<sup>®</sup> bilirubin sub-model

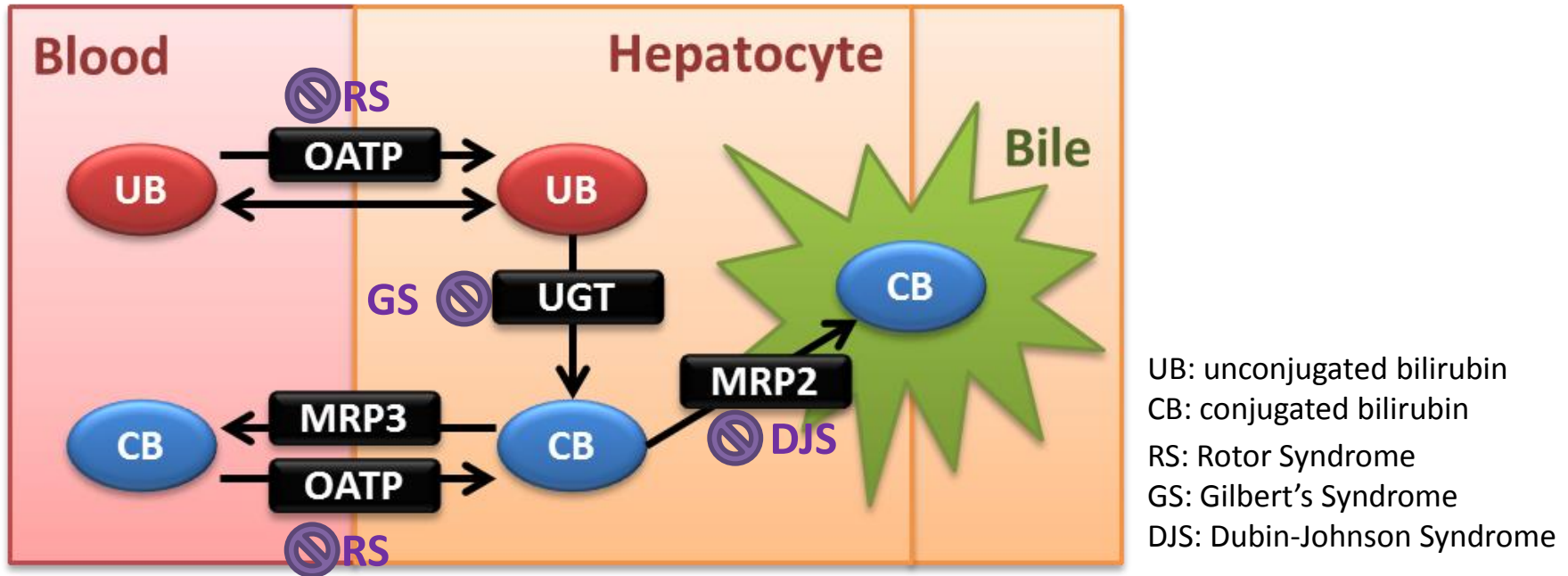


# Drug-Induced Hyperbilirubinemia

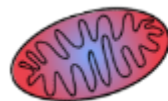
- Bilirubin, the product of heme breakdown from red blood cells, is exclusively eliminated by liver
- Circulating bilirubin is widely used as a diagnostic biomarker for liver function
- Elevations in serum bilirubin may indicate severe liver injury
- Observations of Hy's Law cases (concomitant elevations in serum ALT > 3X ULN and total bilirubin > 2X ULN) can raise concerns about irreversible liver injury that may lead to liver failure



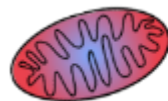
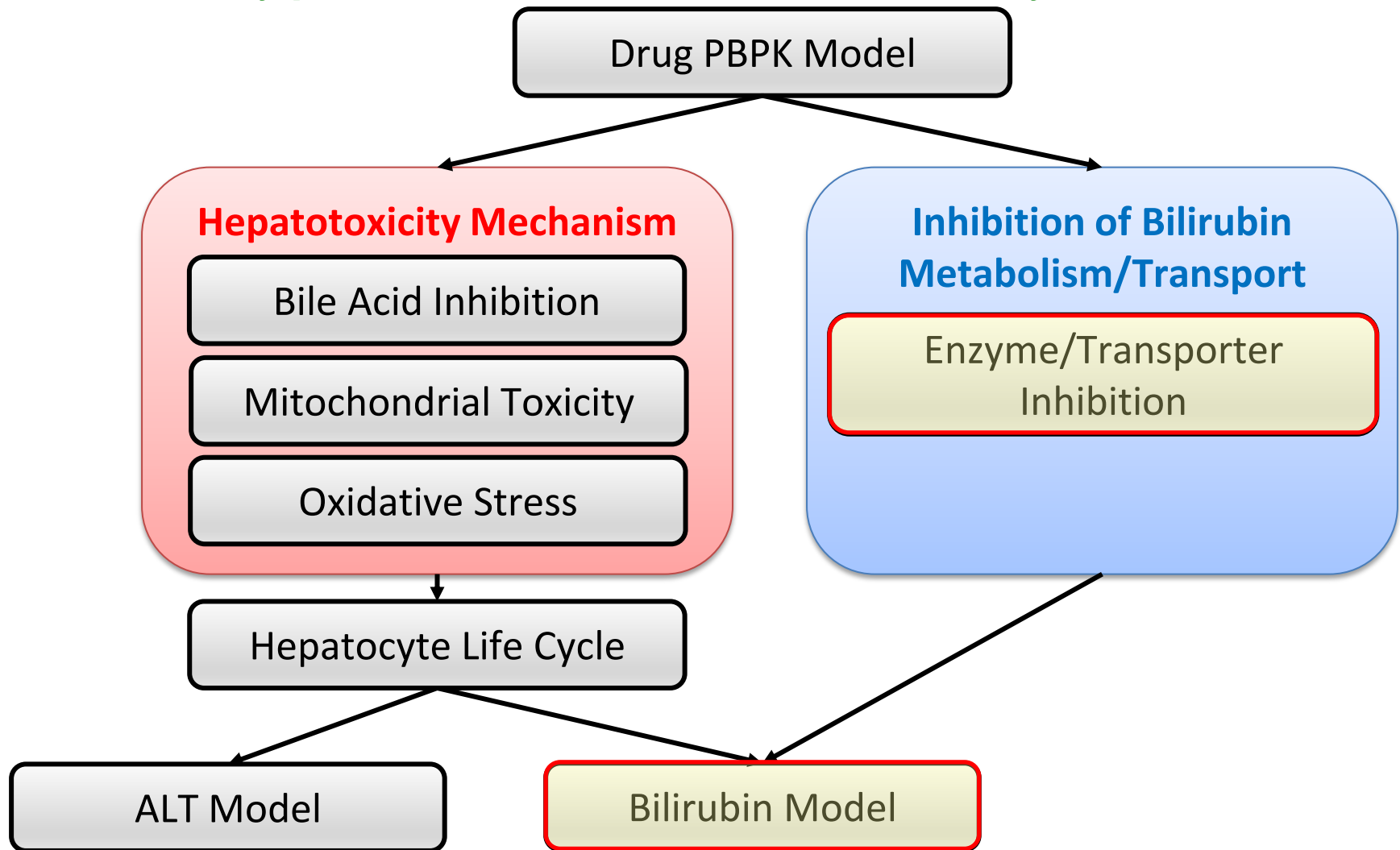
# Inhibition of Hepatic Enzyme and Transporters Can Increase Serum Bilirubin



- Multiple hepatic enzyme and transporters are involved in bilirubin elimination
- Elevated serum bilirubin observed in patients with inherited disorders of bilirubin metabolism and transport
- Drugs that interact with these enzymes/transporters can also cause hyperbilirubinemia



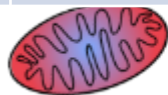
# Underlying Mechanisms of Drug-Induced Hyperbilirubinemia in DILIsym<sup>®</sup> v5A



# Gathering Data for DILIsym<sup>®</sup> Parameter Inputs: Inhibition of Bilirubin Enzyme/Transporters

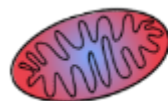
- DILIsym<sup>®</sup> parameter inputs
  - Inhibition constant:  $K_i$ ,  $IC_{50}$
  - Competitive inhibition assumed
- *In vitro* assessment using multiple bilirubin enzyme/transporters is recommended

| Enzyme/<br>Transporter | Function           | Experimental<br>System | Probe Substrate  |
|------------------------|--------------------|------------------------|--|
| OATP1B1                | Basolateral uptake | Transfected cell lines | Estradiol 17 $\beta$ -Glucuronide, estrone 3-sulfate, pitavastatin, pravastatin, rosuvastatin, atorvastatin, valsartan |
| OATP1B3                | Basolateral uptake | Transfected cell lines | Cholecystikinin octapeptide, bromosulfophthalein, estradiol 17 $\beta$ -Glucuronide, valsartan                         |
| UGT1A1                 | Metabolism         | Liver microsome        | B-Estradiol  |
| MRP2                   | Biliary excretion  | Membrane vesicles      | Leukotriene C4, estradiol 17 $\beta$ -Glucuronide, ethacrynyl glutathione, carboxy-dichlorofluorescein                 |
| MRP3                   | Basolateral efflux | Membrane vesicles      | Estradiol 17 $\beta$ -Glucuronide, carboxy-dichlorofluorescein   |

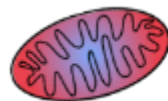
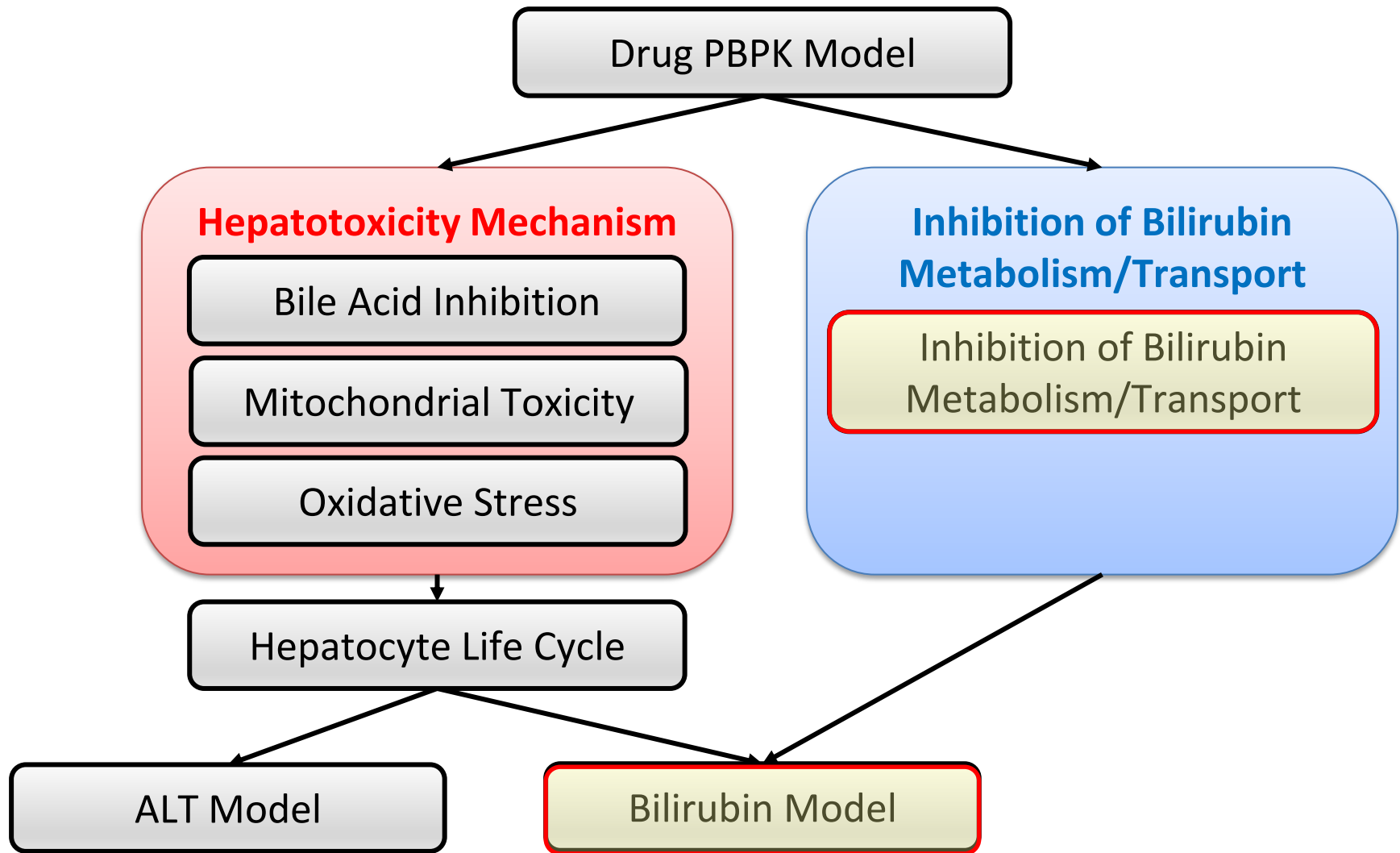


# Modeling Compounds that Inhibit Bilirubin and Bile Acid Transport: A Case Study with CKA

- Introduction
  - CKA induced dose-dependent hepatotoxicity and bilirubin increase in rats
  - Just modest increases in serum ALT, AST, and GLDH observed in humans administered CKA
  - *In vitro* assays indicate that CKA inhibits bilirubin and bile acid transporters, induces oxidative stress, and inhibits mitochondrial ETC function
- Modeling CKA-mediated hyperbilirubinemia that involves liver injury and bilirubin transporter inhibition
  - Translate bilirubin transporter inhibition data to DILIsym<sup>®</sup> parameters
- Simulate CKA-mediated hyperbilirubinemia using DILIsym<sup>®</sup>
  - Simulate CKA-mediated hyperbilirubinemia in baseline rat and rat SimPops<sup>™</sup>



# Modeling CKA-Mediated Hyperbilirubinemia

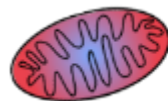




# Translate Bilirubin Transport Inhibition Data to DILIsym<sup>®</sup> Parameters for CKA

- CKA inhibits OATP1B1, MRP2, and MRP3 with IC<sub>50</sub> values of 0.84, 68.5, and 11.2 µM, respectively
- CKA effects on UGT1A1 unknown
- Unit of bilirubin enzyme/transporter inhibition constant is µM

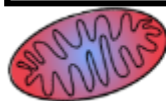
| DILIsym <sup>®</sup> Parameter        | DILIsym <sup>®</sup> Parameter Input |
|---------------------------------------|--------------------------------------|
| Compound X OATP inhibition constant   | 0.84 µM                              |
| Compound X MRP2 inhibition constant   | 68.5 µM                              |
| Compound X MRP3 inhibition constant   | 11.2 µM                              |
| Compound X UGT1A1 inhibition constant | 1e10 µM                              |



# Define CKA-Mediated Bilirubin Transport Inhibition Data in DILIsym®

The image shows two overlapping windows from the DILIsym v5A software. The background window is the 'SimSingle Setup' dialog, which has a 'Mechanism' button highlighted with a red box. A red arrow points from this button to the foreground window, which is the 'DILIsym Parameter Customization' dialog. In this foreground window, the 'Molecule' dropdown is set to 'CompX' (highlighted with a red box). The 'Mechanisms' list on the right contains various options, with 'InhibBilirubin' highlighted in blue and enclosed in a red box. At the top right of the foreground window, there is a small green plus icon in a red box. At the bottom of the foreground window, there are buttons for 'Table View', 'Cancel Changes', 'Save As New', and 'Save As New w/ Custom'.

Preclinical Data



DILI-sim Initiative



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# Define CKA-Mediated Bilirubin Transport Inhibition Data in DILIsym<sup>®</sup>

| DILIsym <sup>®</sup> Parameter        | DILIsym <sup>®</sup> Parameter Input |
|---------------------------------------|--------------------------------------|
| Compound X OATP inhibition constant   | 0.84 $\mu\text{M}$                   |
| Compound X MRP2 inhibition constant   | 68.5 $\mu\text{M}$                   |
| Compound X MRP3 inhibition constant   | 11.2 $\mu\text{M}$                   |
| Compound X UGT1A1 inhibition constant | 1e10 $\mu\text{M}$                   |

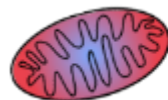
DILIsym Parameter Customization

Molecule: All Molecules (dropdown)  
Mechanisms: All Mechanisms (dropdown)

CompX (selected)  
nhBilirubin (selected)

Compound X OATP inhibition constant: 0.84 uM  
Compound X MRP2 inhibition constant: 68.5 uM  
Compound X MRP3 inhibition constant: 11.2 uM  
Compound X UGT1A1 inhibition constant: 10000000 uM

Table View | ^ | v | Save w/ Custom | Cancel Changes | Save As New | Save As New w/ Custom



# Simulating CKA-Mediated Hyperbilirubinemia in Rat SimSingle™

RAT

CKA 500mg/kg Single Dose

DILIsym v5A

File Results View Help

**SimSingle Setup**

New SimSingle Rat\_CKA\_500mpk

Load SimSingle

**Input Parameters**

Species Parameters\_Species\_Rat\_v5A Customize

Drug Parameters\_Drug\_Rat\_CKA\_v5A Customize Mechanism

Caloric Intake Parameters\_Calories\_Rat\_v5A Customize

Comp W Dosing Parameters\_CompWDosing\_Blank\_v5A Customize

Comp X Dosing CKA\_rat\_dosing\_500mgperkg Customized

Comp Y Dosing Parameters\_CompYDosing\_Blank\_v5A Customize

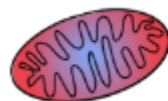
Time Parameters\_Time\_72hr\_Default\_v5A Customize

Solver Parameters\_Solver\_Default\_v5A Customize

Input Panel Panel\_Blank Viewer

Simulate Run in Parallel SimPops Param Sweep Data Comparison

Specify Data Plot Table Export Save Results SimSingle



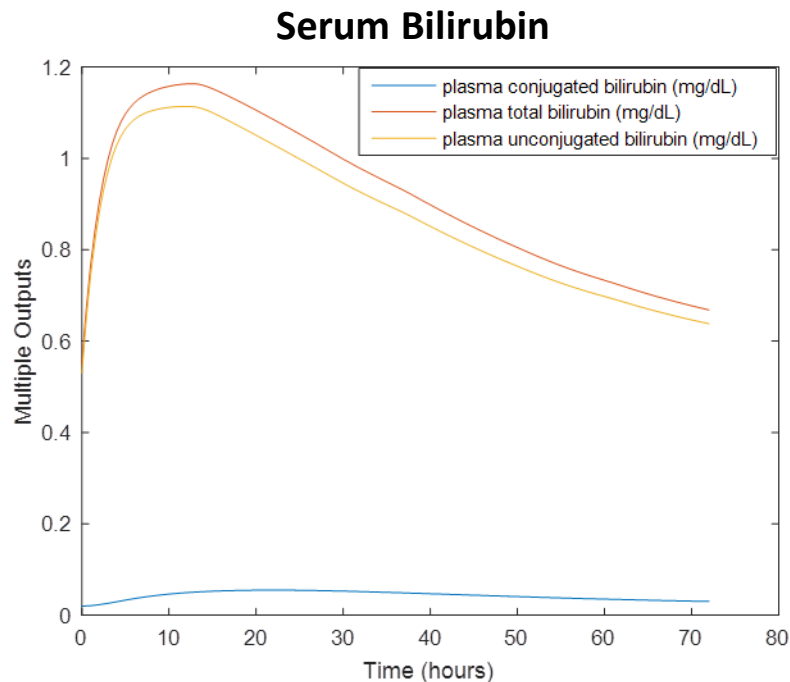
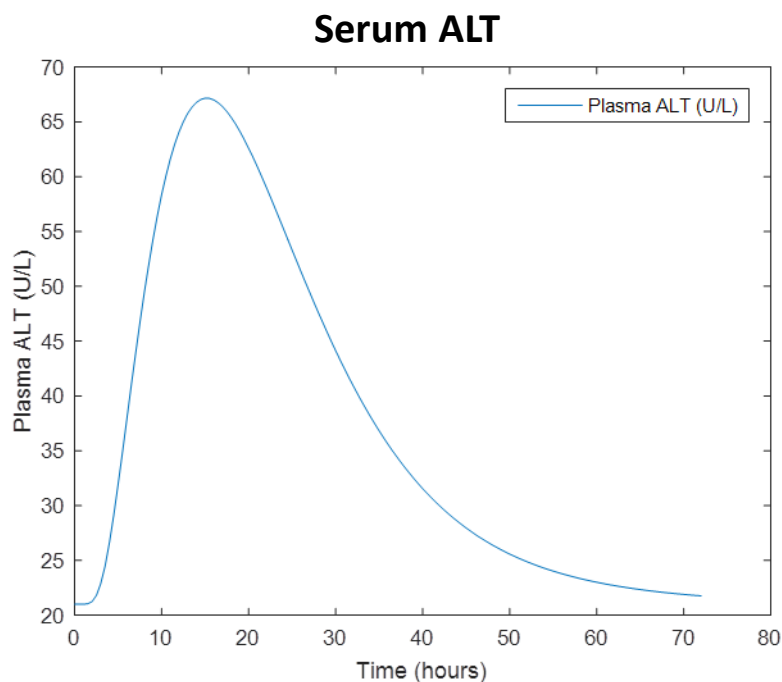
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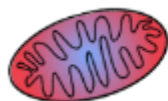
# Simulating CKA-Mediated Hyperbilirubinemia in Rat SimSingle™



- Single oral dose of 500 mg/kg induced elevation of serum ALT and bilirubin in the baseline rat

**RAT**

*Simulation Results*



DILI-sim Initiative



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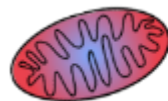
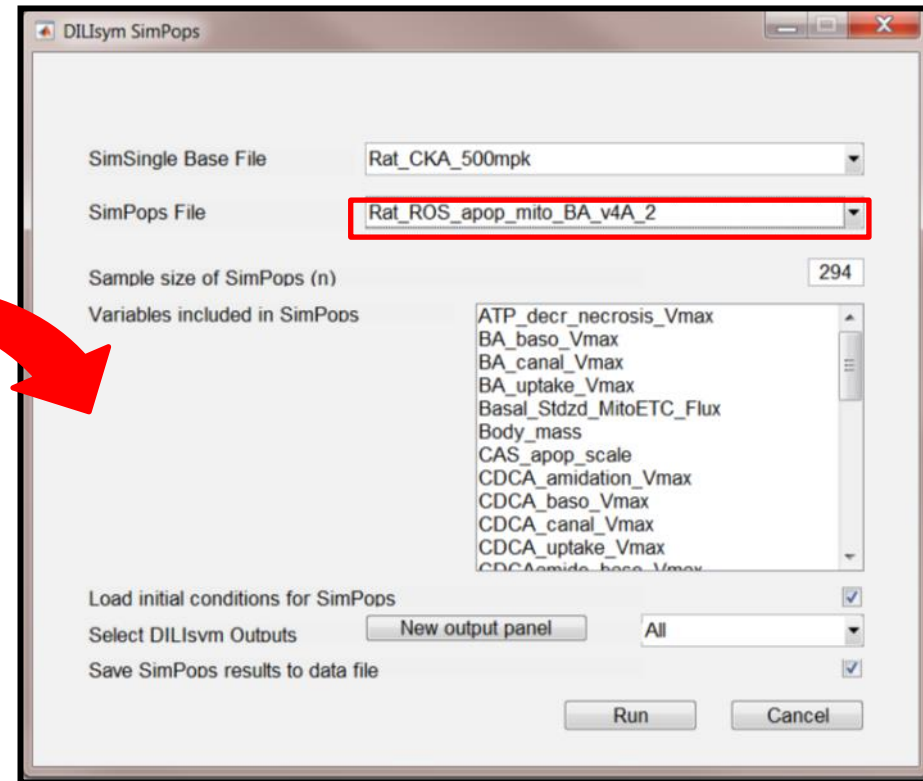
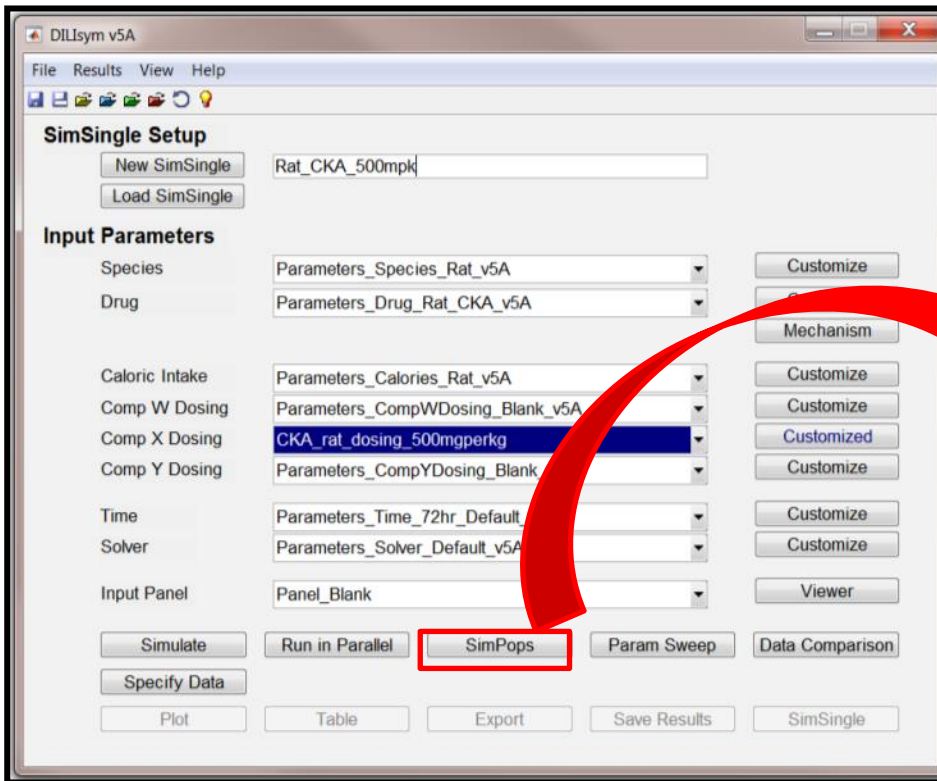
# Simulating CKA-Mediated Hyperbilirubinemia in Rat SimPops™

**RAT**

**CKA 500mg/kg Single Dose**

**SimPops™**

**Rat\_ROS\_apop\_mito\_BA\_v4A\_2**



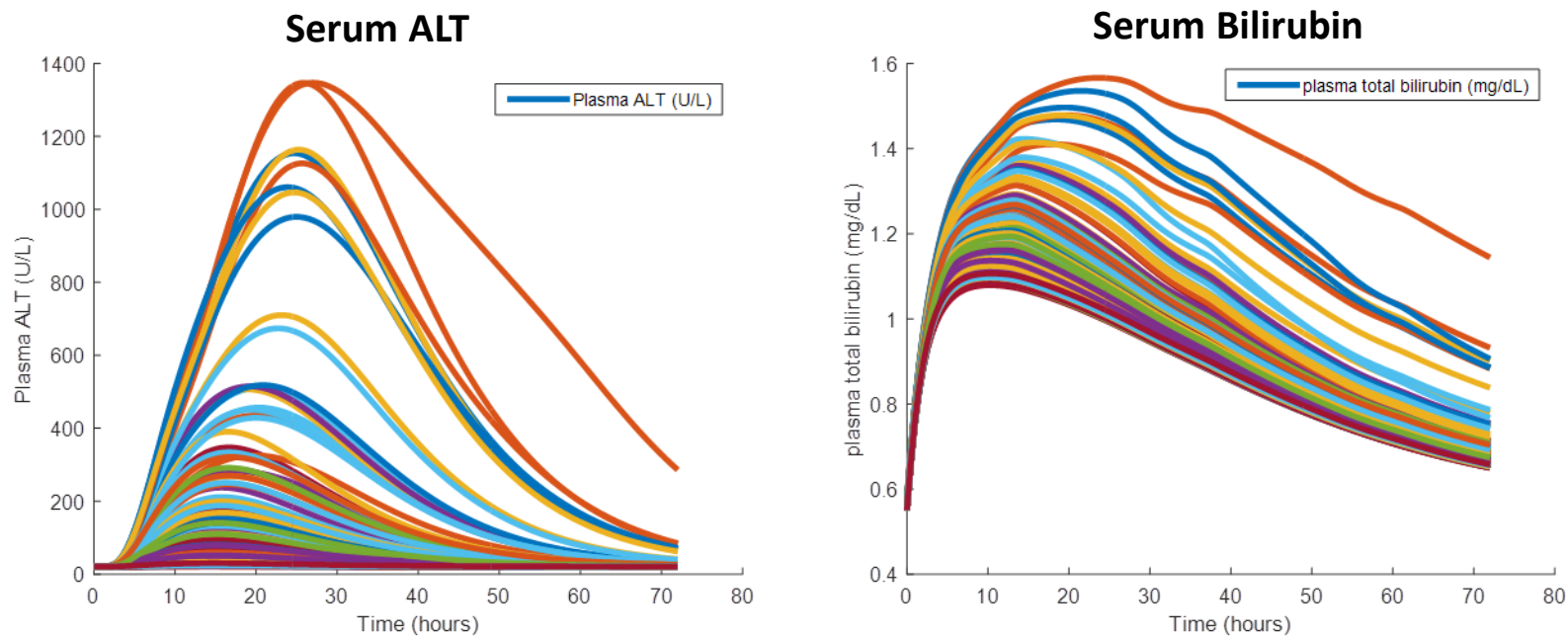
DILI-sim Initiative



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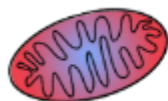
# Simulating CKA-Mediated Hyperbilirubinemia in Rat SimPops™



- Single oral dose of 500 mg/kg induced elevation of serum ALT and bilirubin in the rat SimPops™

**RAT**

*Simulation Results*



DILI-sim Initiative



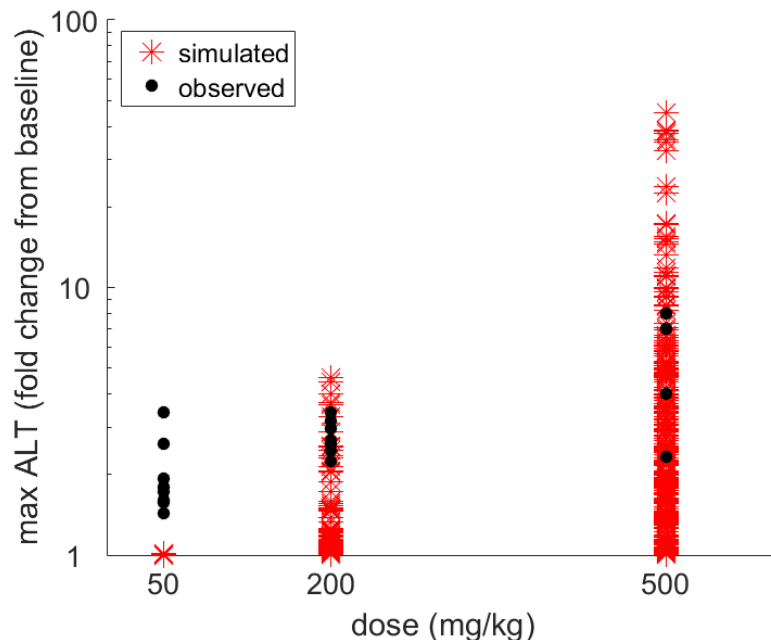
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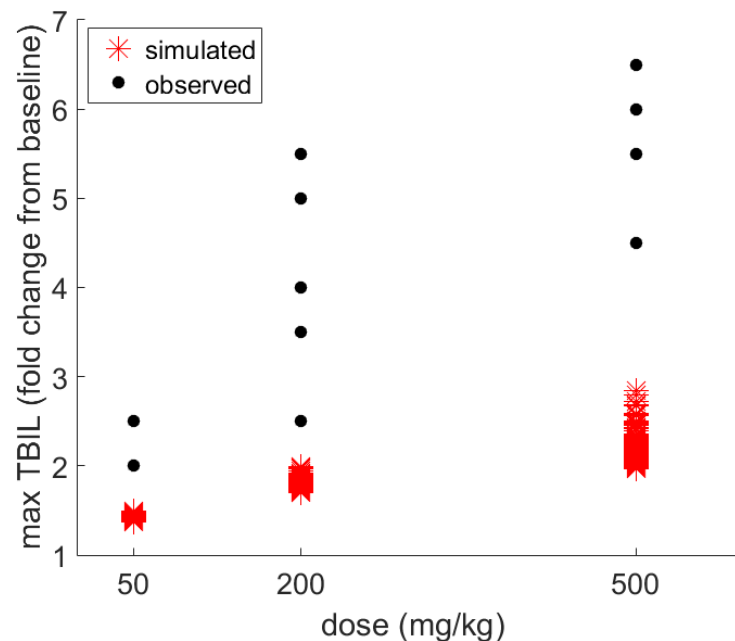
# Simulating CKA-Mediated Hyperbilirubinemia in Rat SimPops™

RAT

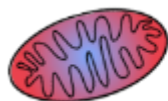
Serum ALT



Serum Bilirubin

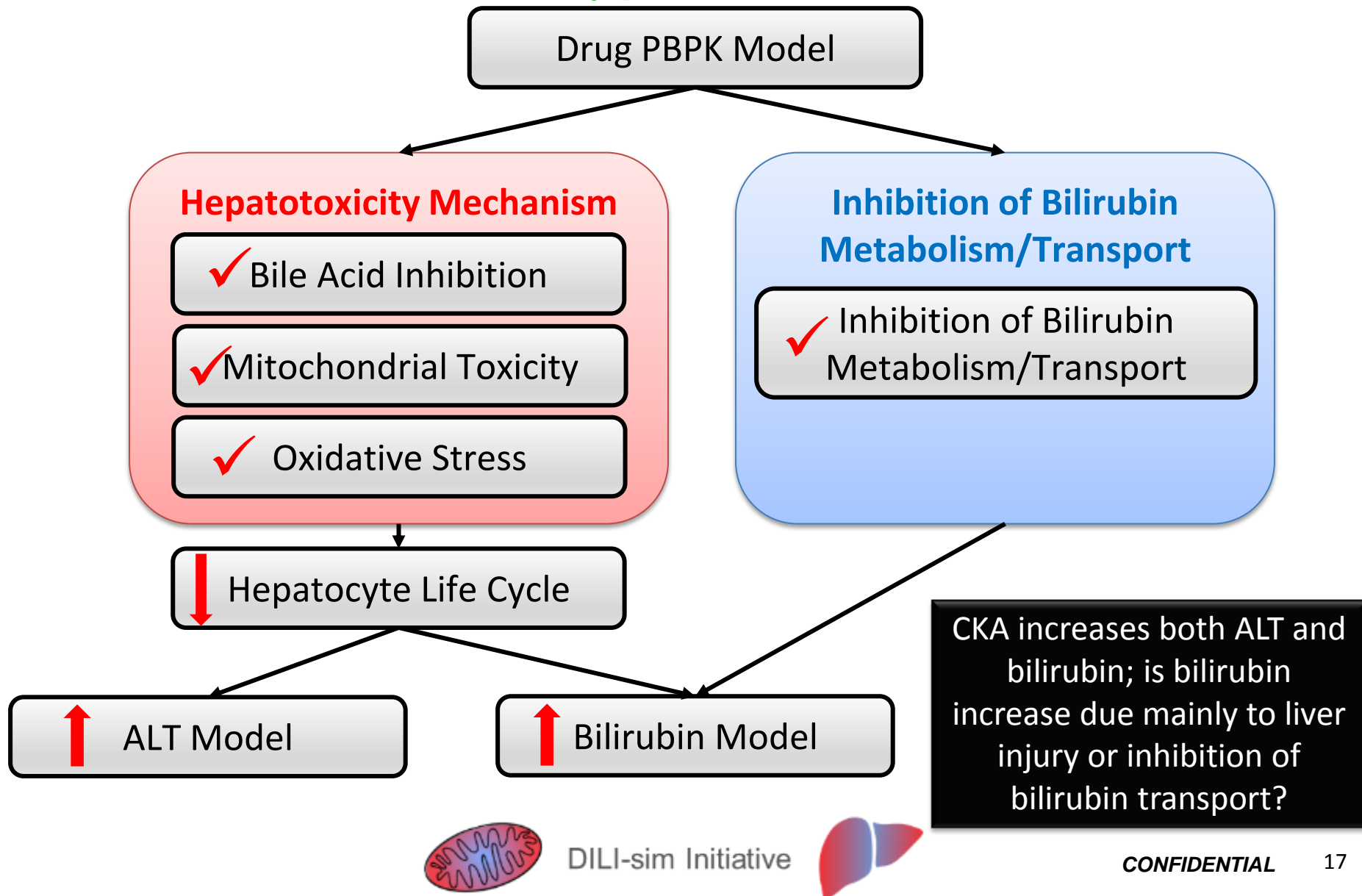


- Simulations recapitulated dose-dependent increase in serum ALT and bilirubin in rats administered CKA
- Underestimated the extent of bilirubin increase
  - Likely due to absence of metabolite effects in the current model
  - Could be due partly to lack of variability in the bilirubin model





# What is the Underlying Mechanism of CKA-Mediated Hyperbilirubinemia?



# Turning Off CKA-Mediated Toxicity Mechanisms

DILIsym Parameter Customization

Molecule: All Molecules Mechanisms: All Mechanisms

|       |                      |   |                  |   |
|-------|----------------------|---|------------------|---|
| CompX | incRNSROSProduction1 | Liver RNS/ROS production rate constant 1      | 9705 mL/mol/hour | X |
| CompX | inhBAtransport       | Compound X NTCP inhibition constant           | 19.5012 uM       | X |
|       |                      | Compound X NTCP alpha constant for inhibition | 1 dimensionless  |   |
|       |                      | Compound X NTCP switch                        | 0 dimensionless  |   |
|       |                      | Compound X BSEP inhibition constant           | 129.828 uM       |   |
|       |                      | Compound X BSEP alpha constant for inhibition | 1 dimensionless  |   |

Table View ^ v Save w/ Custom Cancel Changes Save As New Save As New w/ Custom

Delete toxicity mechanisms (relevant parameters will be set to default values)

DILIsym Parameter Customization

Molecule: All Molecules Mechanisms: All Mechanisms

|       |              |                                       |                   |   |
|-------|--------------|---------------------------------------|-------------------|---|
| CompX | inhETC1      | Coefficient for ETC inhibition        | 2.5816e-01 mol/mL | X |
| CompX | inhBilirubin | Compound X OATP inhibition constant   | 0.83999 uM        | X |
|       |              | Compound X MRP2 inhibition constant   | 68.5113 uM        |   |
|       |              | Compound X MRP3 inhibition constant   | 11.1977 uM        |   |
|       |              | Compound X UGT1A1 inhibition constant | 10000000 uM       |   |

Table View ^ v Save w/ Custom Cancel Changes Save As New Save As New w/ Custom

Repeat simulations with bilirubin mechanism only

# Turning Off Inhibitory Effects of CKA on Bilirubin Metabolism and Transport

DILIsm Parameter Customization

Molecule: All Molecules Mechanisms: All Mechanisms

|       |                      |   |         |               |   |
|-------|----------------------|---|---------|---------------|---|
| CompX | incRNSROsproduction1 | Liver RNS/ROS production rate constant 1      | 9705    | mL/mol/hour   | X |
| CompX | inhBAtransport       | Compound X NTCP inhibition constant           | 19.5012 | uM            | X |
|       |                      | Compound X NTCP alpha constant for inhibition | 1       | dimensionless |   |
|       |                      | Compound X NTCP switch                        | 0       | dimensionless |   |
|       |                      | Compound X BSEP inhibition constant           | 129.828 | uM            |   |
|       |                      | Compound X BSEP alpha constant for inhibition | 1       | dimensionless |   |

Table View ^ v Save w/ Custom Cancel Changes Save As New Save As New w/ Custom

Repeat simulations with toxicity mechanisms only

DILIsm Parameter Customization

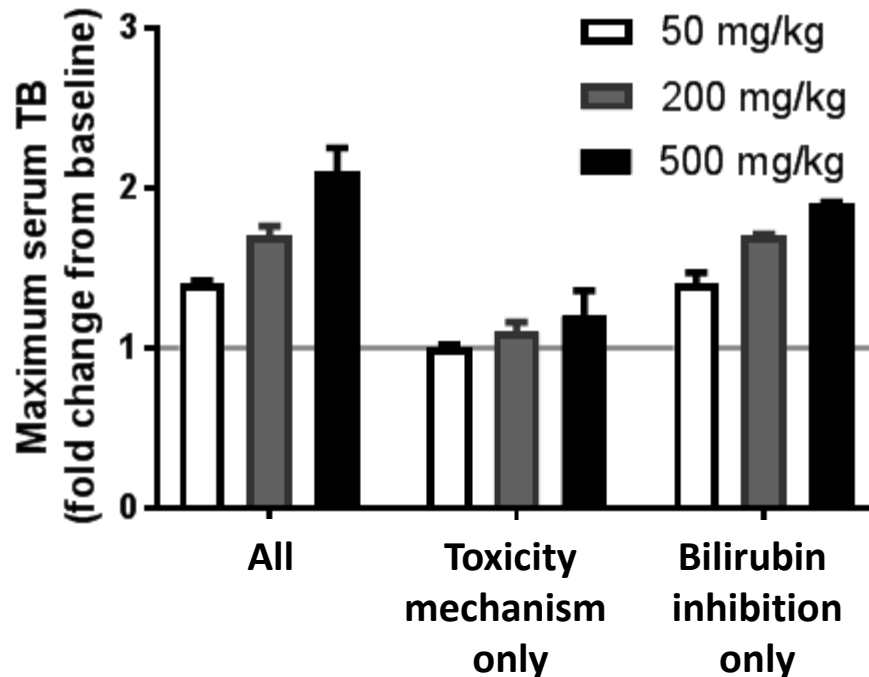
Molecule: All Molecules Mechanisms: All Mechanisms

|       |              |                                       |           |        |   |
|-------|--------------|---------------------------------------|-----------|--------|---|
| CompX | inhETC1      | Coefficient for ETC inhibition 1      | 2.5816e-0 | mol/mL | X |
| CompX | inhBilirubin | Compound X OATP inhibition constant   | 0.83999   | uM     | X |
|       |              | Compound X MRP2 inhibition constant   | 68.5113   | uM     |   |
|       |              | Compound X MRP3 inhibition constant   | 11.1977   | uM     |   |
|       |              | Compound X UGT1A1 inhibition constant | 10000000  | uM     |   |

Table View ^ v Save w/ Custom Cancel Changes Save As New Save As New w/ Custom

Delete bilirubin mechanism (relevant parameters will be set to default values)

# Simulated Serum Bilirubin with Different Mechanistic Inputs



- CKA-mediated bilirubin elevation primarily resulted from inhibition of transporters rather than liver injury