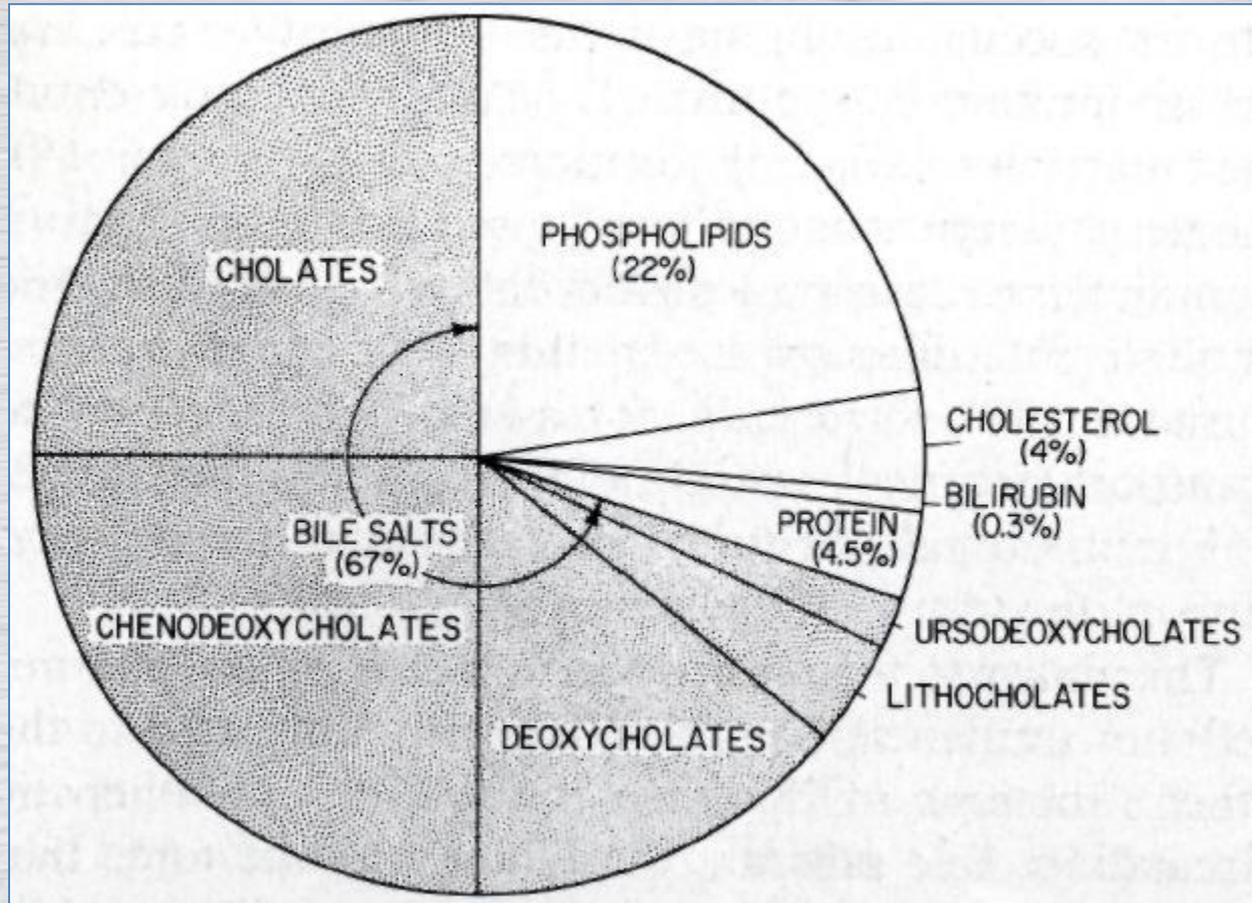


The background features a stylized illustration of a cell membrane, represented by a phospholipid bilayer. Several transporters are depicted as green and red structures embedded in the membrane. Small blue and green spheres, representing molecules, are shown moving across the membrane, with arrows indicating their direction of transport. The overall scene is set against a light, textured background.

Bile salt transport profiling of hepatic transporters

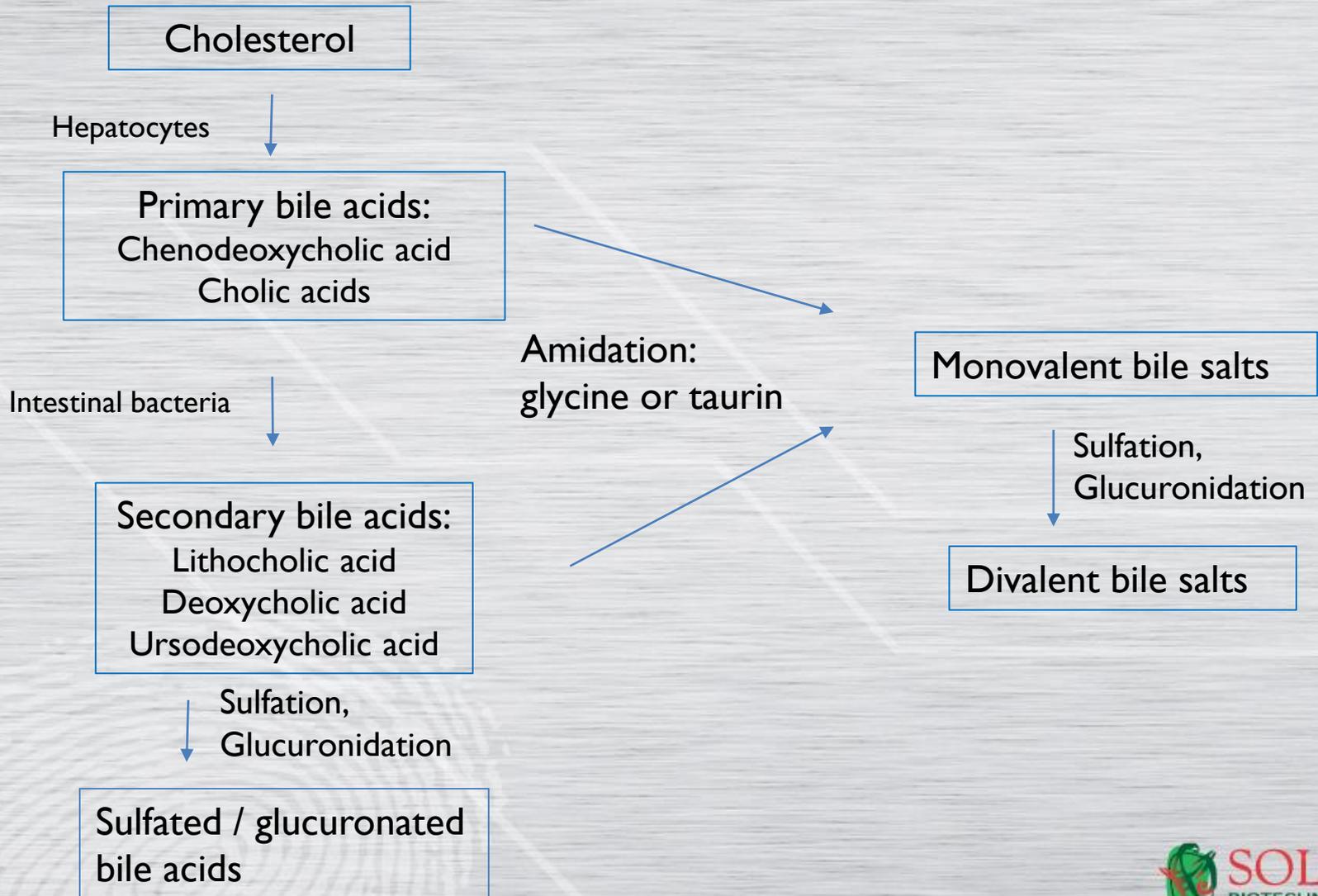
Peter Krajcsi

The human bile

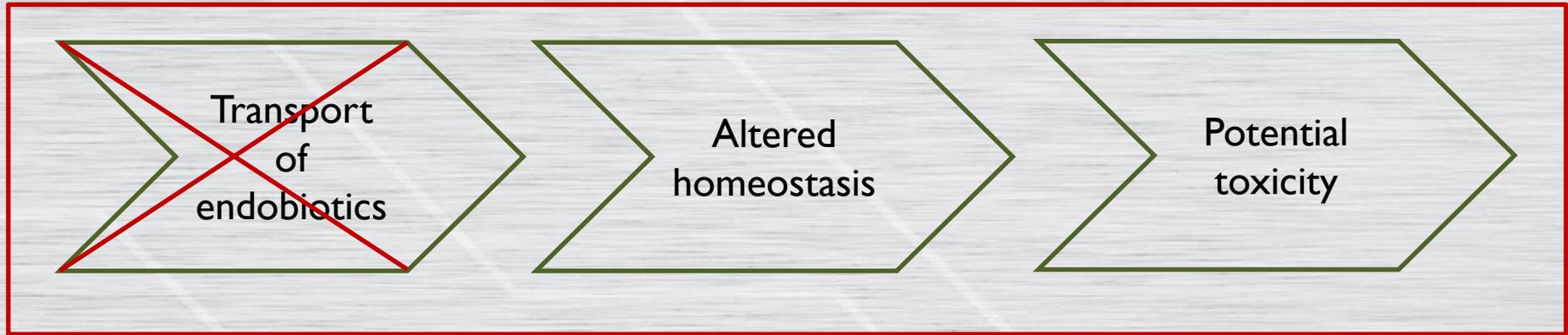


Carey and Duane: The liver, 3rd edition

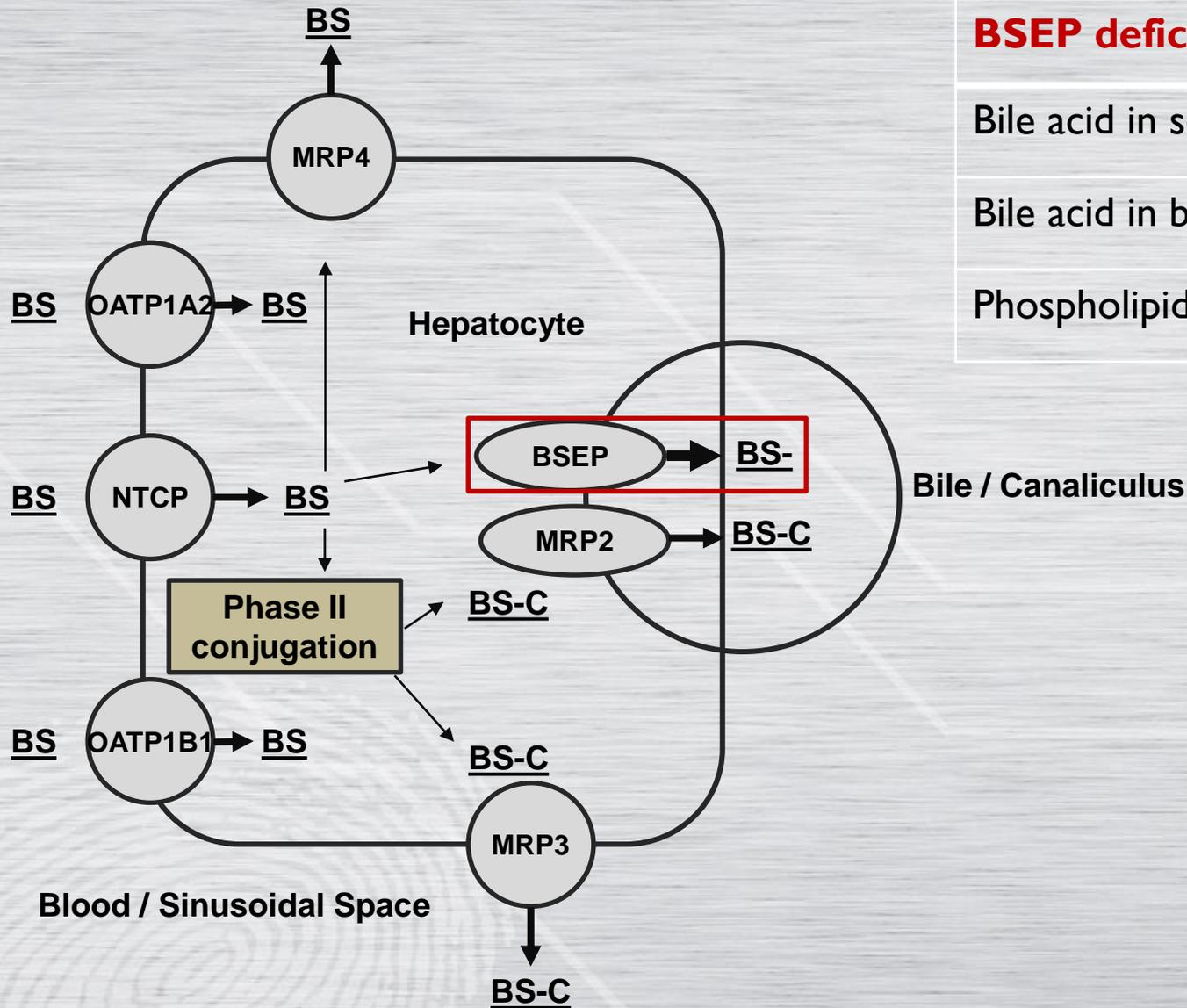
Bile acids / salts



Inhibition of transport of endobiotics



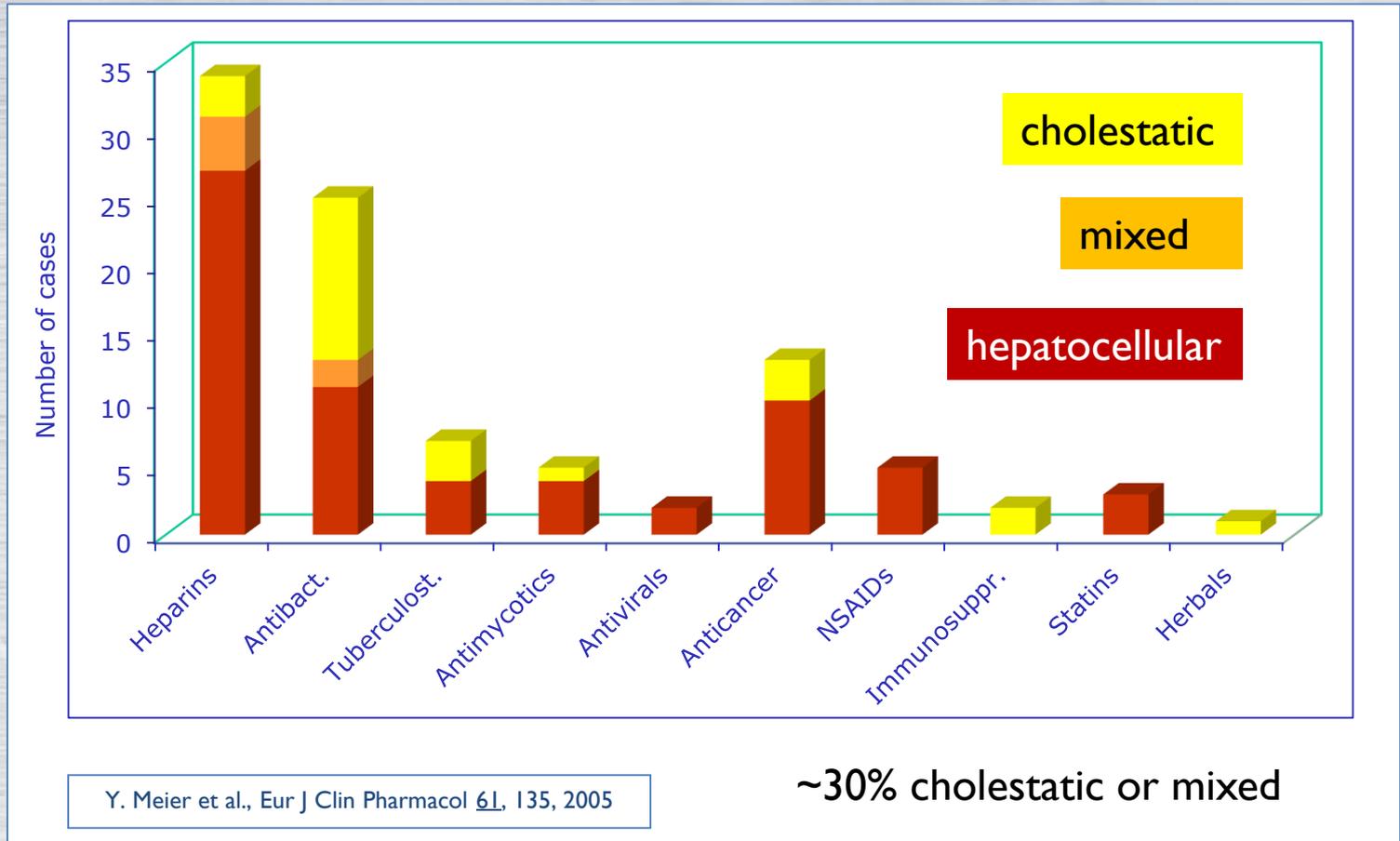
Bile salt transportin hepatocytes



BSEP deficiency / PFIC2

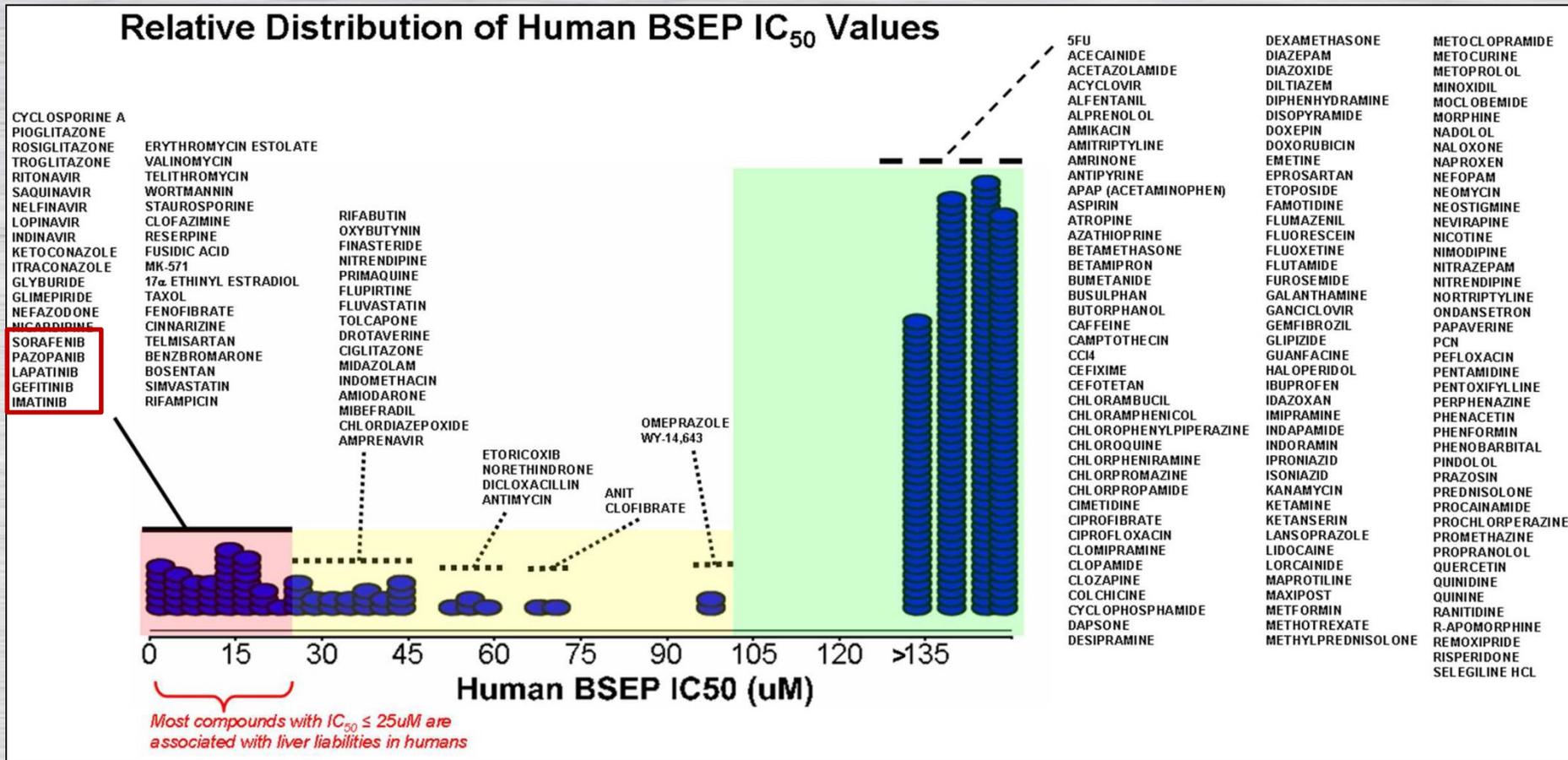
Bile acid in serum	high
Bile acid in bile	low
Phospholipid in bile	normal

Drug induced liver injury



Swedish study: almost 50% of DILI cases were cholestatic or mixed (Björnsson & Olsson 2005 Hepatology)

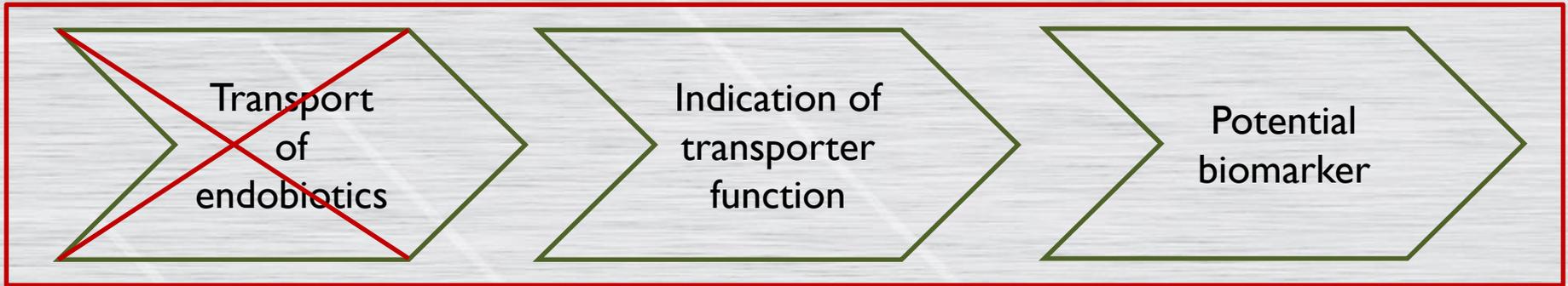
Human BSEP VT – a HT screening tool



„Of particular interest are the compounds with an IC₅₀ value of <25 uM...almost all compounds with this level of potency for BSEP interference are associated with liver injury in humans”

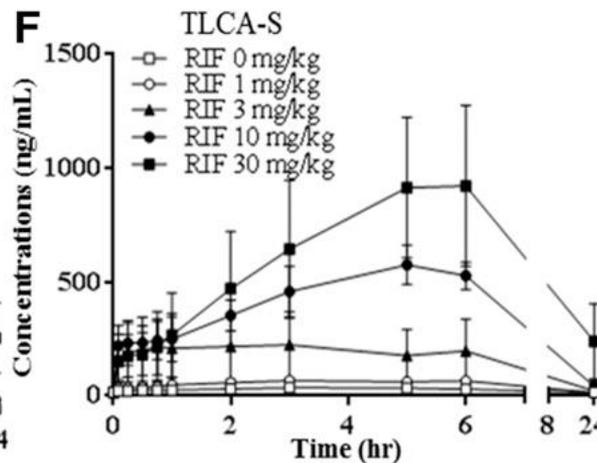
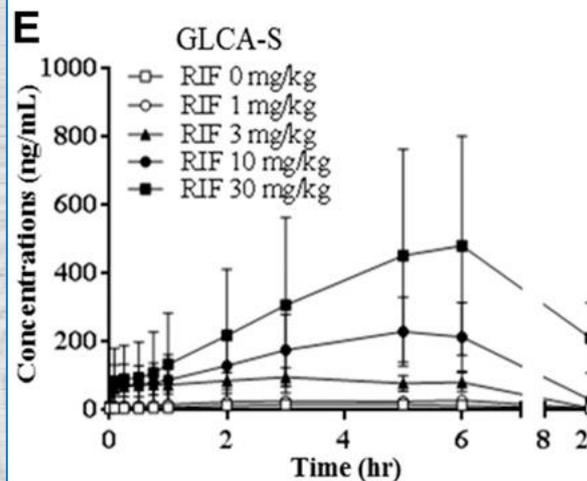
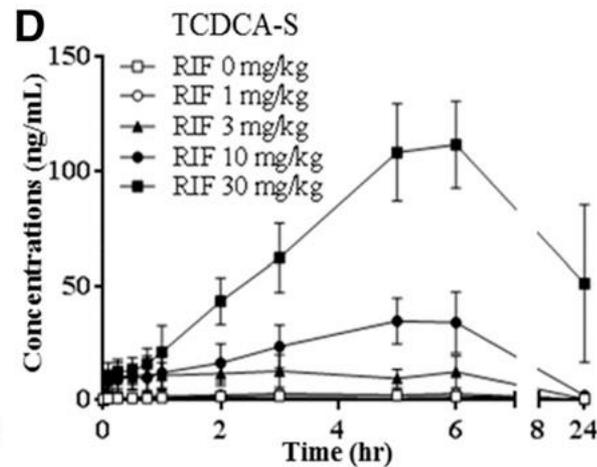
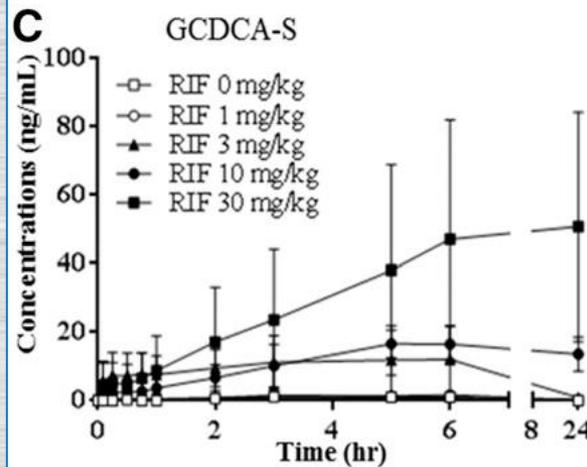
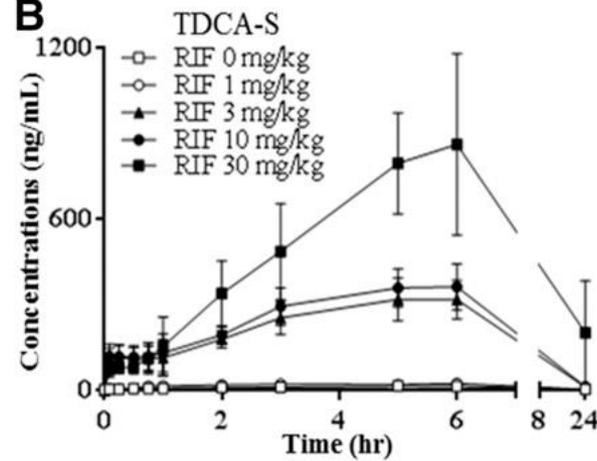
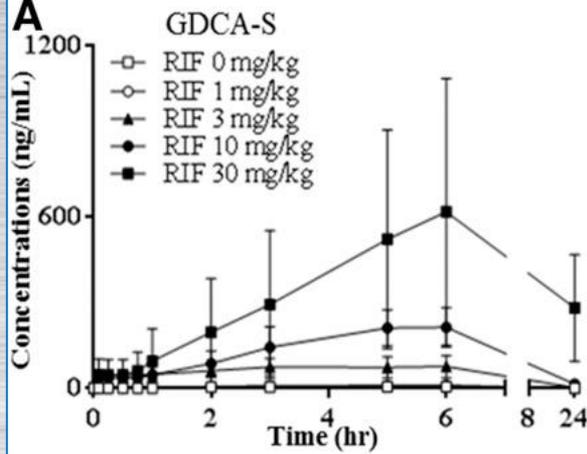
Morgan 2010 Tox Sci

Inhibition of transport of endobiotics

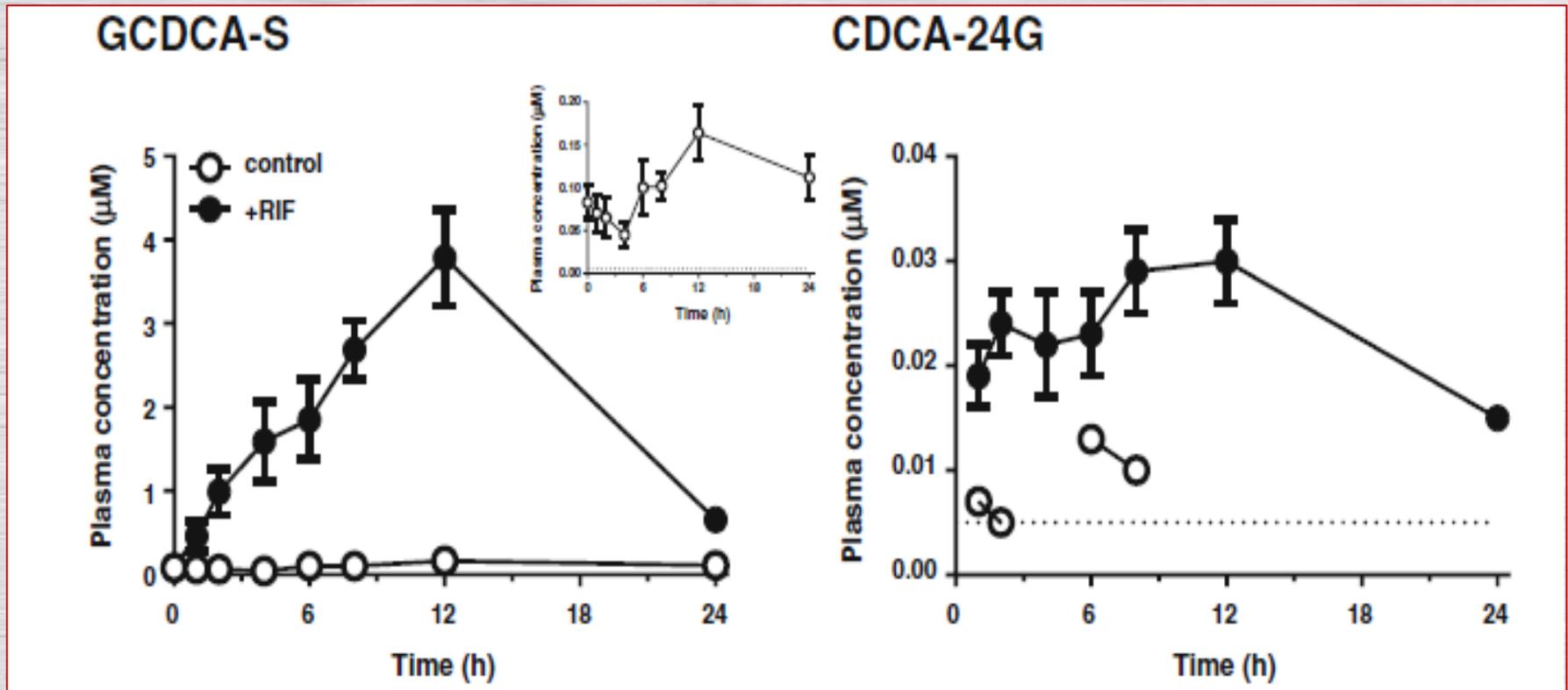


Plasma levels of sulfates in cynos

Thakare 2017 DMD

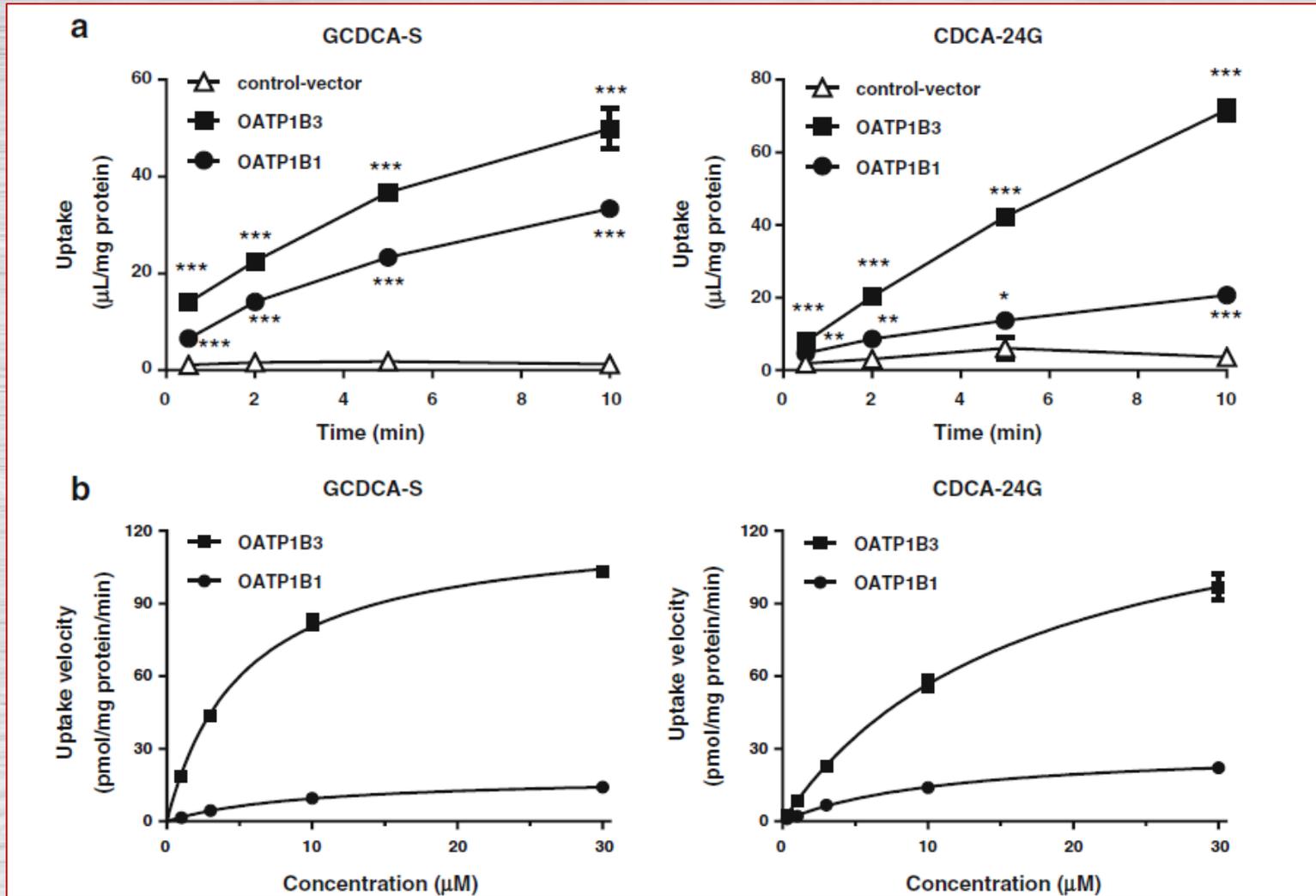


Effect of rifampicin on plasma levels of phase II conjugates of bile acids/salts

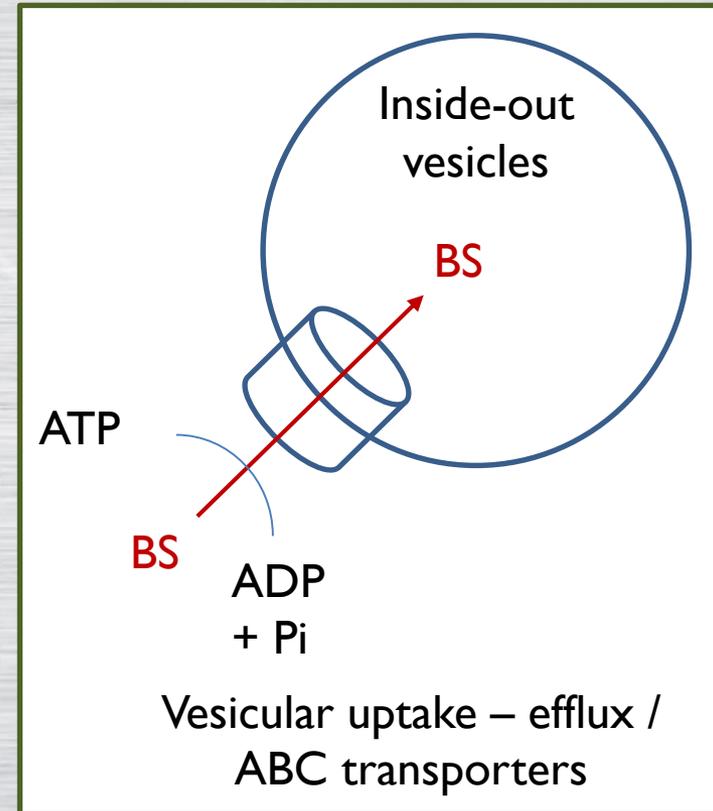
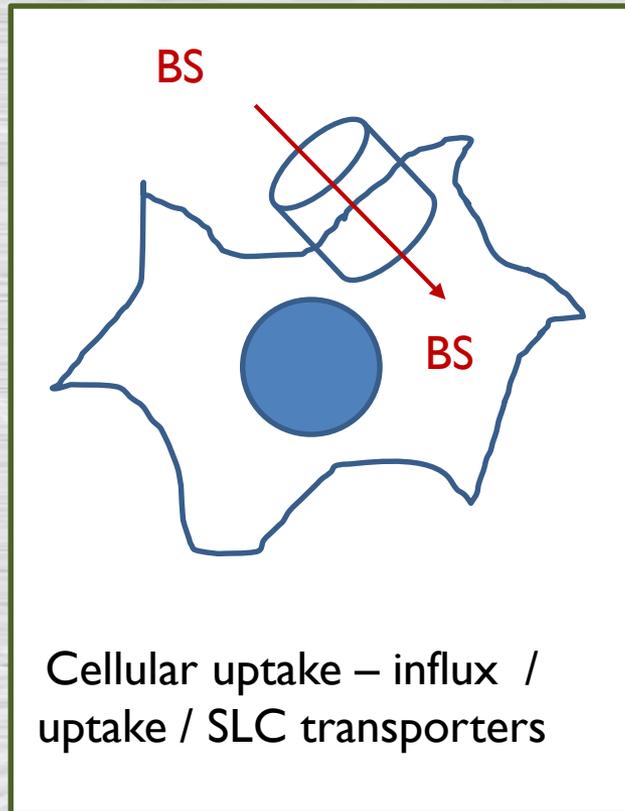


Takehara 2017 Pharm Res

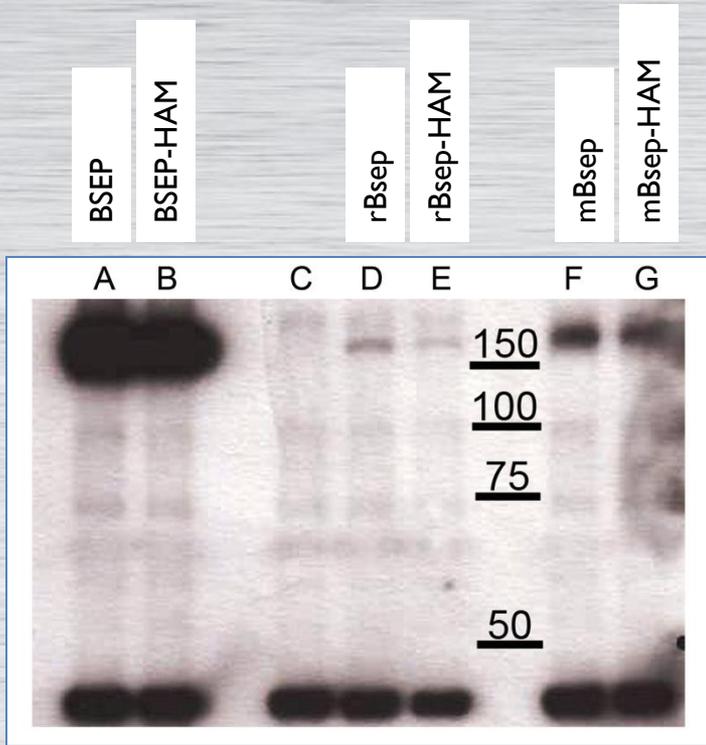
Kinetics of transport – sulfates, glucuronides



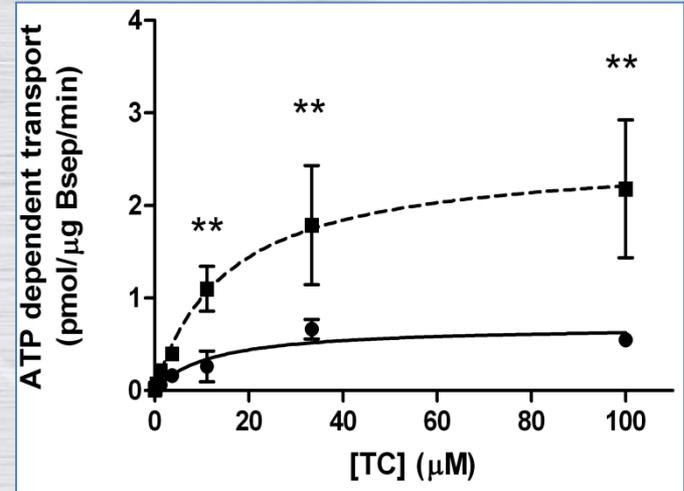
Assay systems to study bile salt - transporter interaction



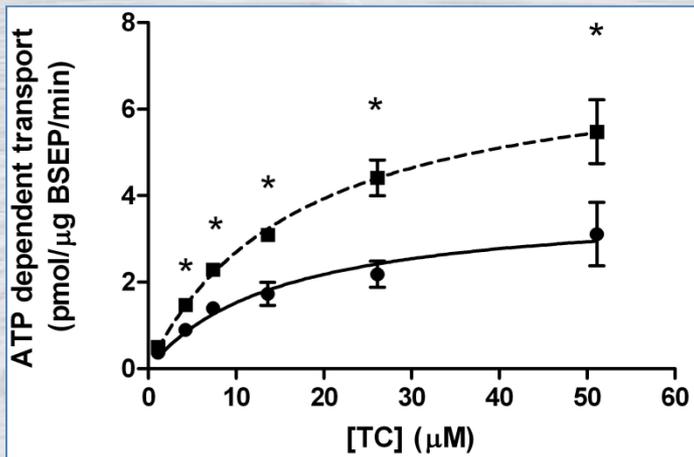
BSEP/Bsep vs BSEP/Bsep-HAM – species specificity



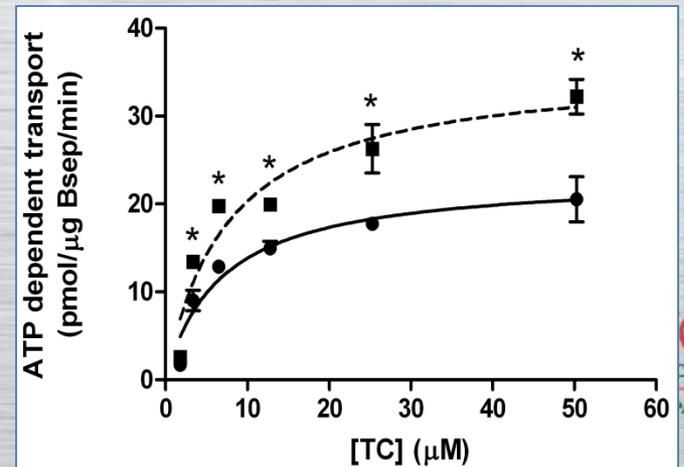
rBsep



BSEP

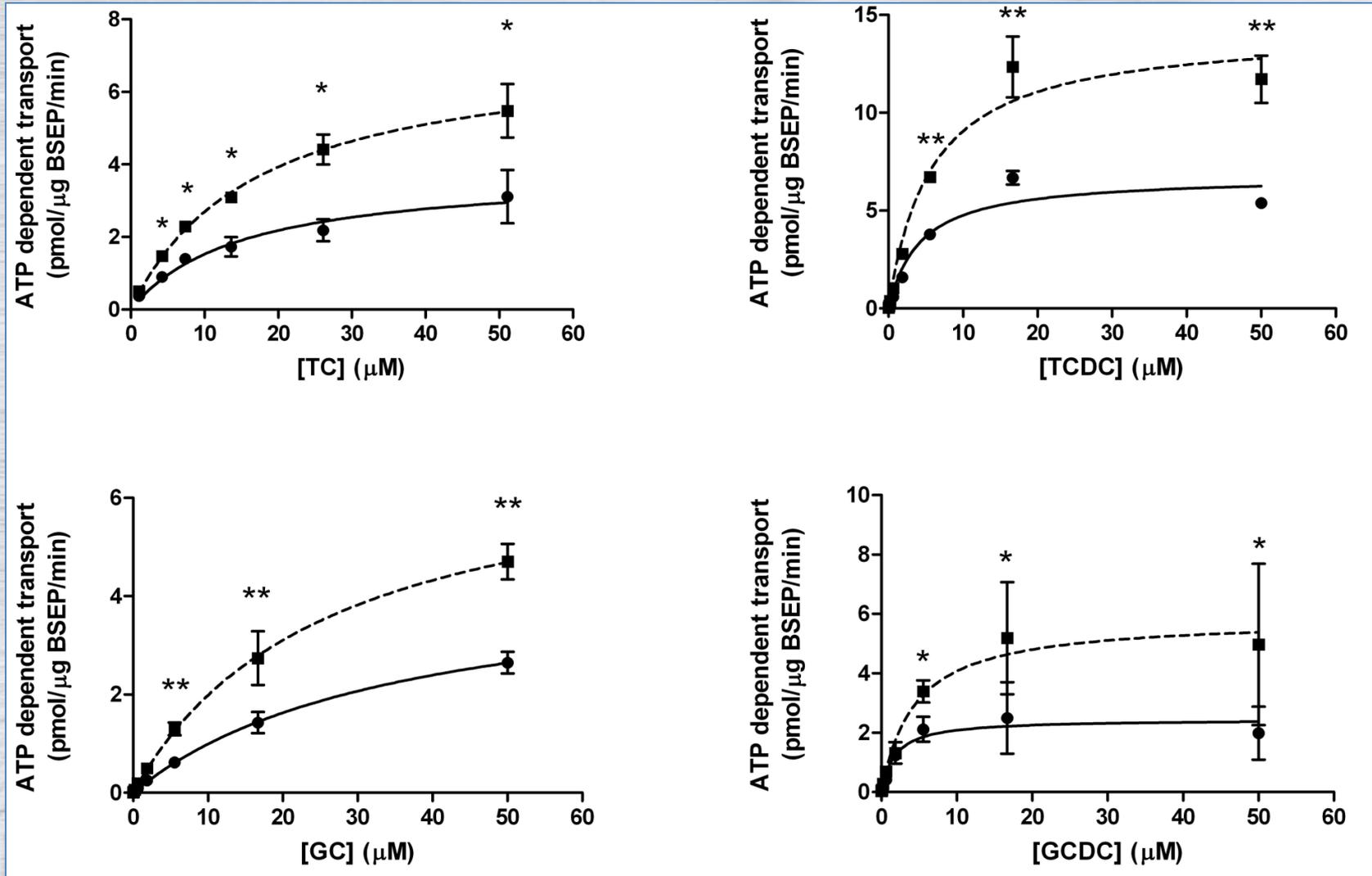


mBsep

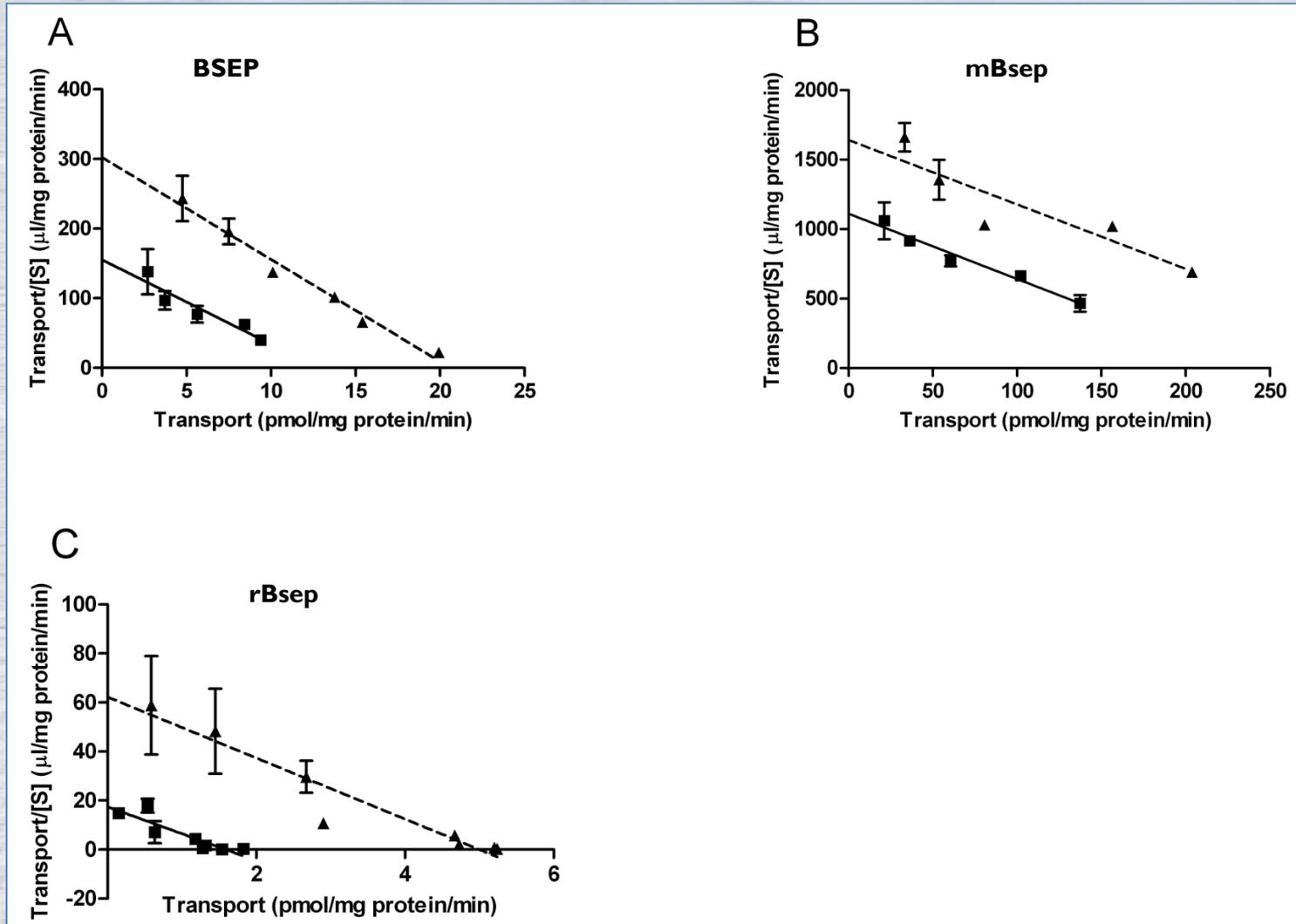


Kis 2009 DMD

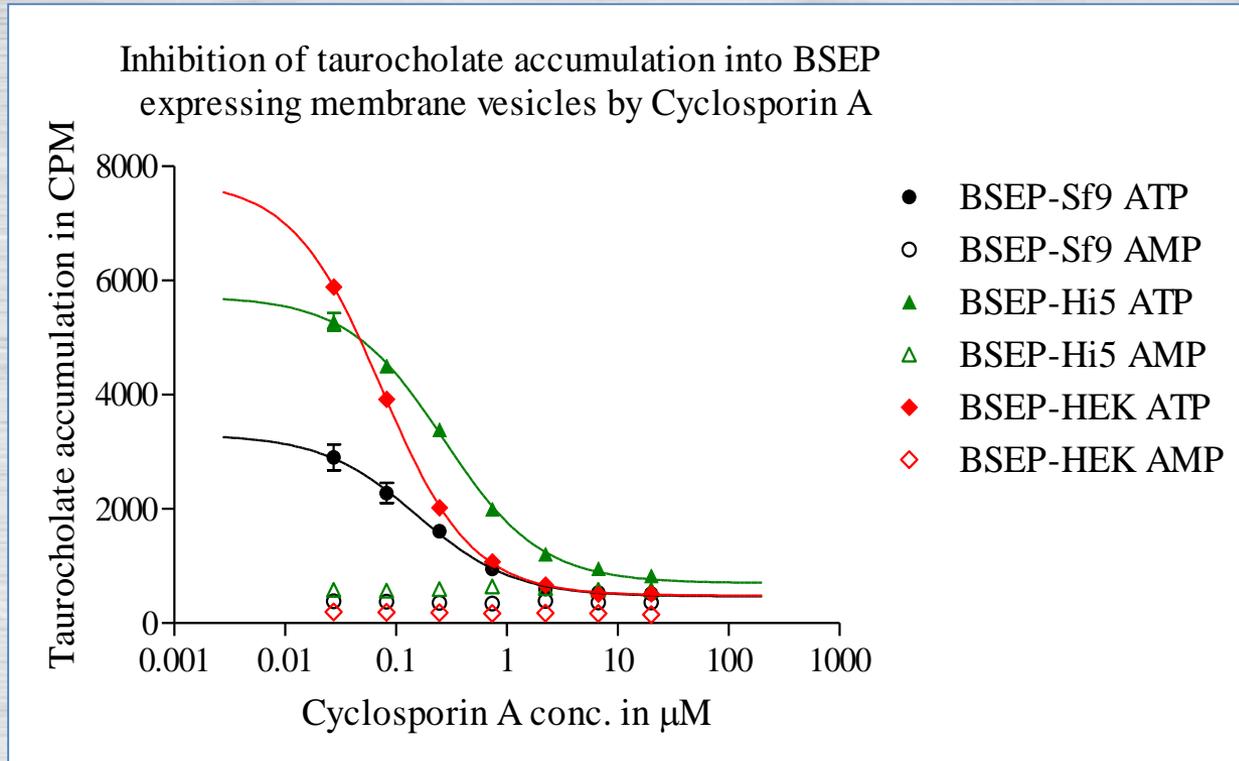
Cholesterol loading increases activity of BSEP – development of high activity membranes (HAM)



BSEP/Bsep vs BSEP/Bsep-HAM – Eadie-Hofstee plots



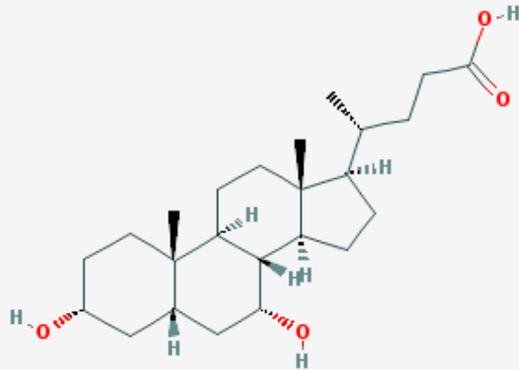
BSEP membranes – CsA inhibition



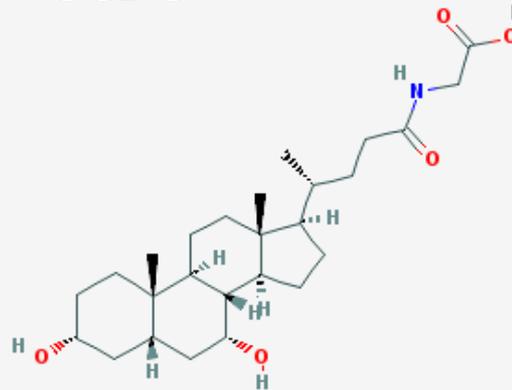
	Origin	Signal/noise (ATP+/ATP-)	IC50 for reference inhibitor (μM)
SB-BSEP-HEK293	Mammalian	40.00	0.07
SB-BSEP-Sf9	Insect	8.86	0.16
SB-BSEP-Hi5	Insect	11.64	0.27

Bile acids/salts

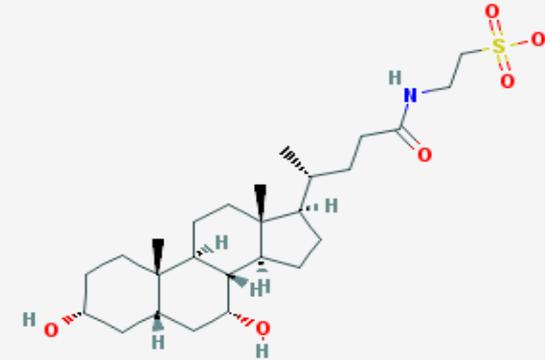
CDC



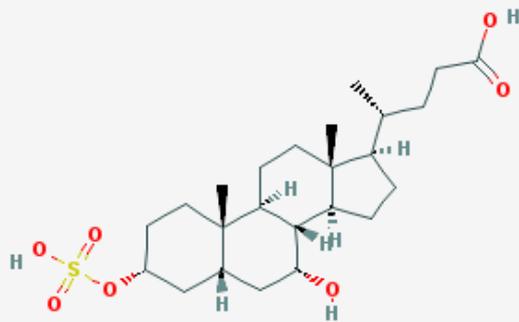
GCDC



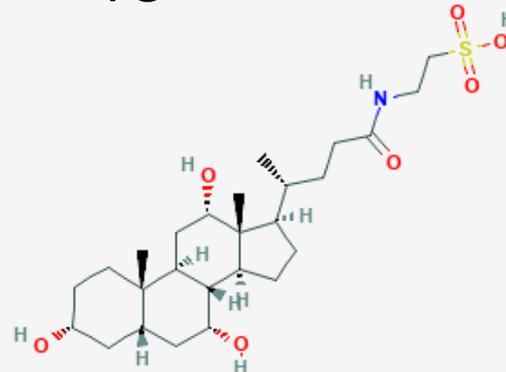
TCDC



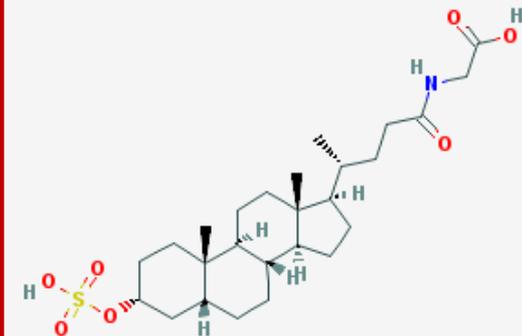
3S-CDC



TC



3S-GLC



Glycolithocholic acid-sulfate – effect on bile flow, morphology

Table 1. Effect of Albumin, Lithocholic Acid Sulfate, Taurolithocholic Acid Sulfate and Glycolithocholic Acid Sulfate Injection on Bile Flow^a

Time	Albumin (n = 6)	Lithocholic acid sulfate (n = 8)	Taurolithocholic acid sulfate (n = 8)	Glycolithocholic acid sulfate (n = 8)
Preinjection				
0-120 min	6.94 ± 0.87	7.06 ± 0.89	7.05 ± 0.80	7.10 ± 1.03
After injection				
0-15 min	7.00 ± 0.91	6.52 ± 0.91	7.30 ± 0.54	2.34 ± 0.43 ^b
15-30 min	6.56 ± 0.77	5.69 ± 0.70	7.81 ± 0.98	2.97 ± 0.63 ^b
30-60 min	6.74 ± 0.88	6.92 ± 0.84	6.75 ± 0.76	4.74 ± 0.63 ^b
60-120 min	6.26 ± 0.78	6.26 ± 0.73	6.05 ± 0.63	5.02 ± 0.50 ^b

^a Values are $\mu\text{l}/100\text{ g body wt}/\text{min}$ (means \pm SD). ^b Significantly different from corresponding control value $p < 0.05$.

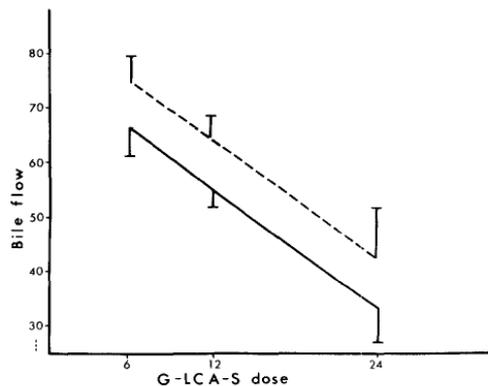
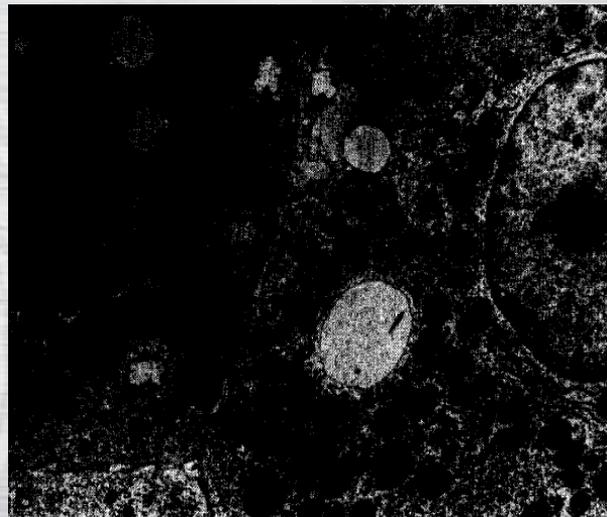


Figure 1. Percent reduction in bile flow following administration of 6, 12, and 24 moles G-LCA-S/100 g body wt. The solid line corresponds to the 0-30-min period after injection, and the broken line to the 30-60-min period after injection.

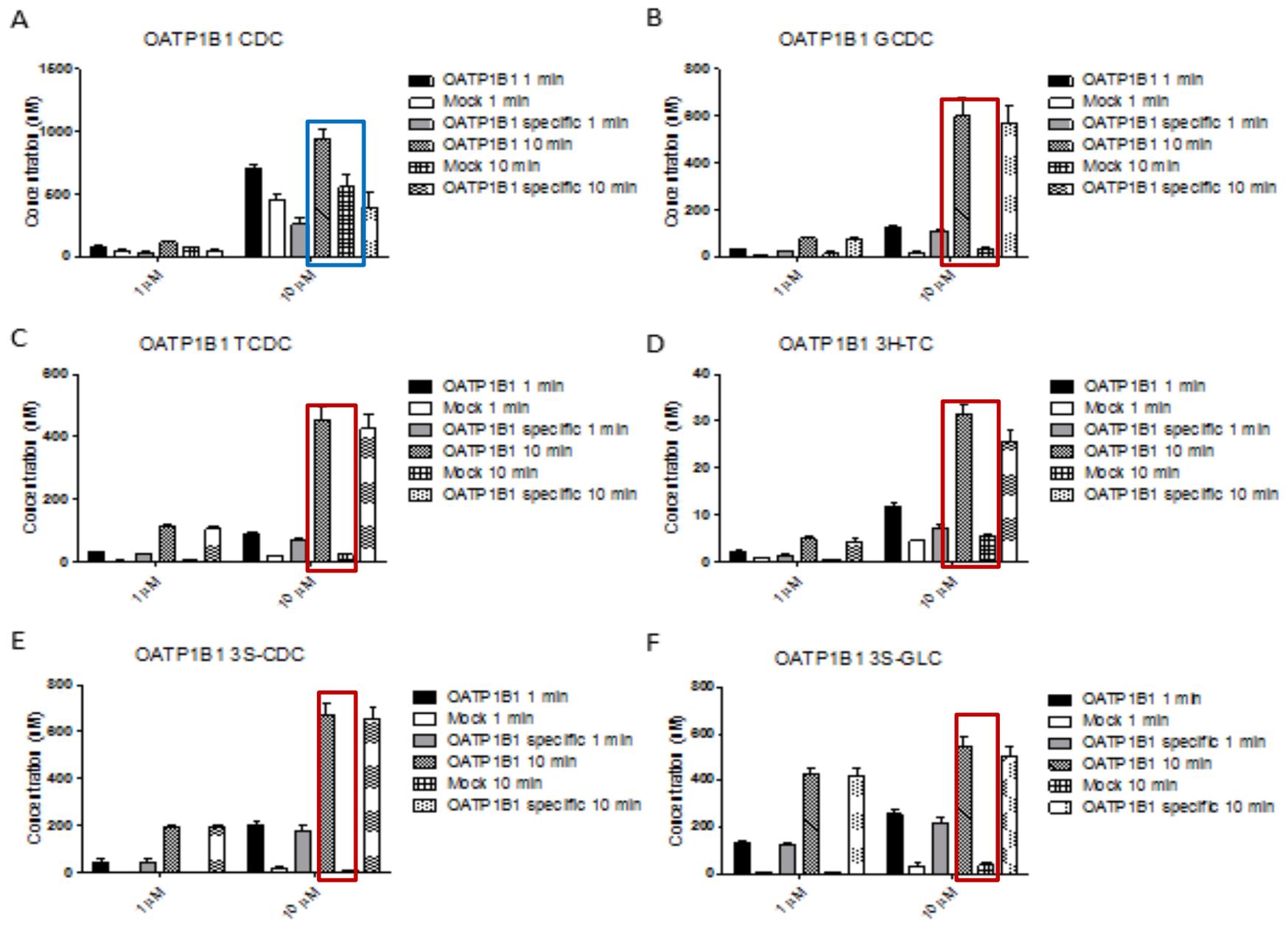


Yousef 1981 Gastroenterology

Screening strategy

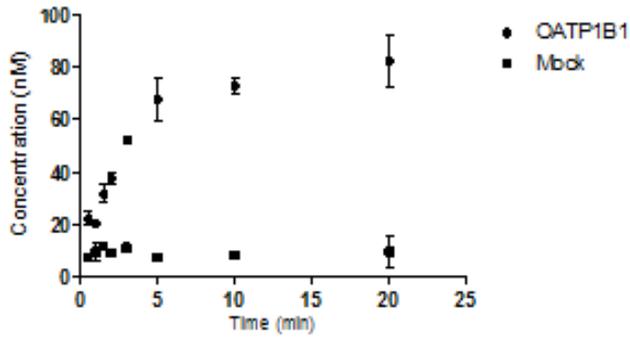
- Feasibility screen
(2 concentrations, 2 timepoints)
- Time course
- Kinetics

OATP1B1 - feasibility

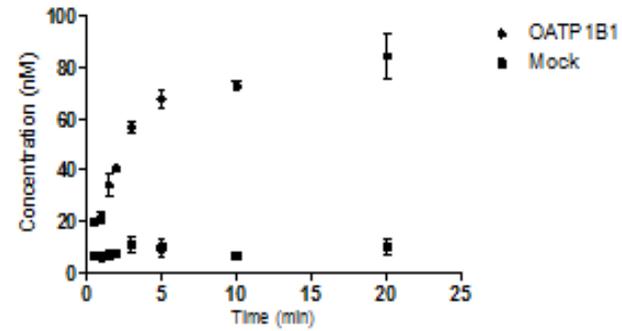


OATP1B1 – time courses

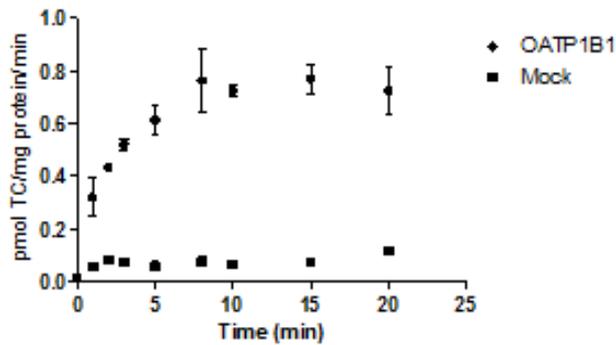
A OATP1B1 GCDC



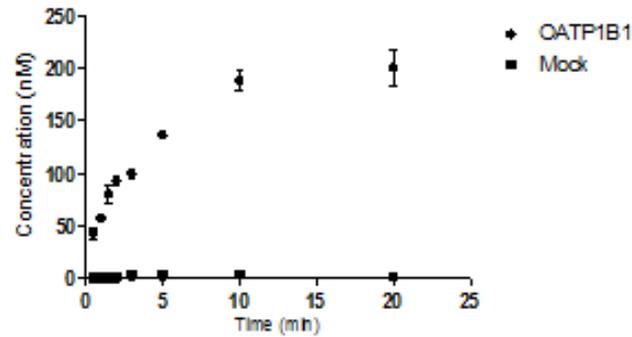
B OATP1B1 TCDC



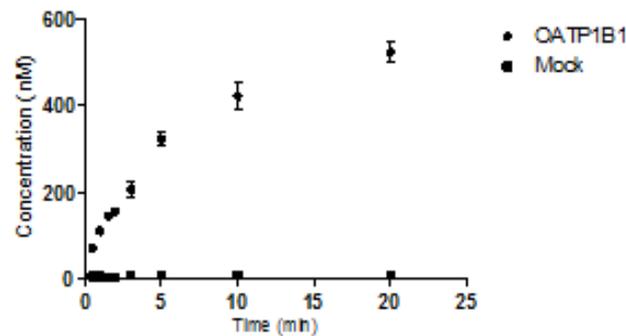
C OATP1B1 3H-TC



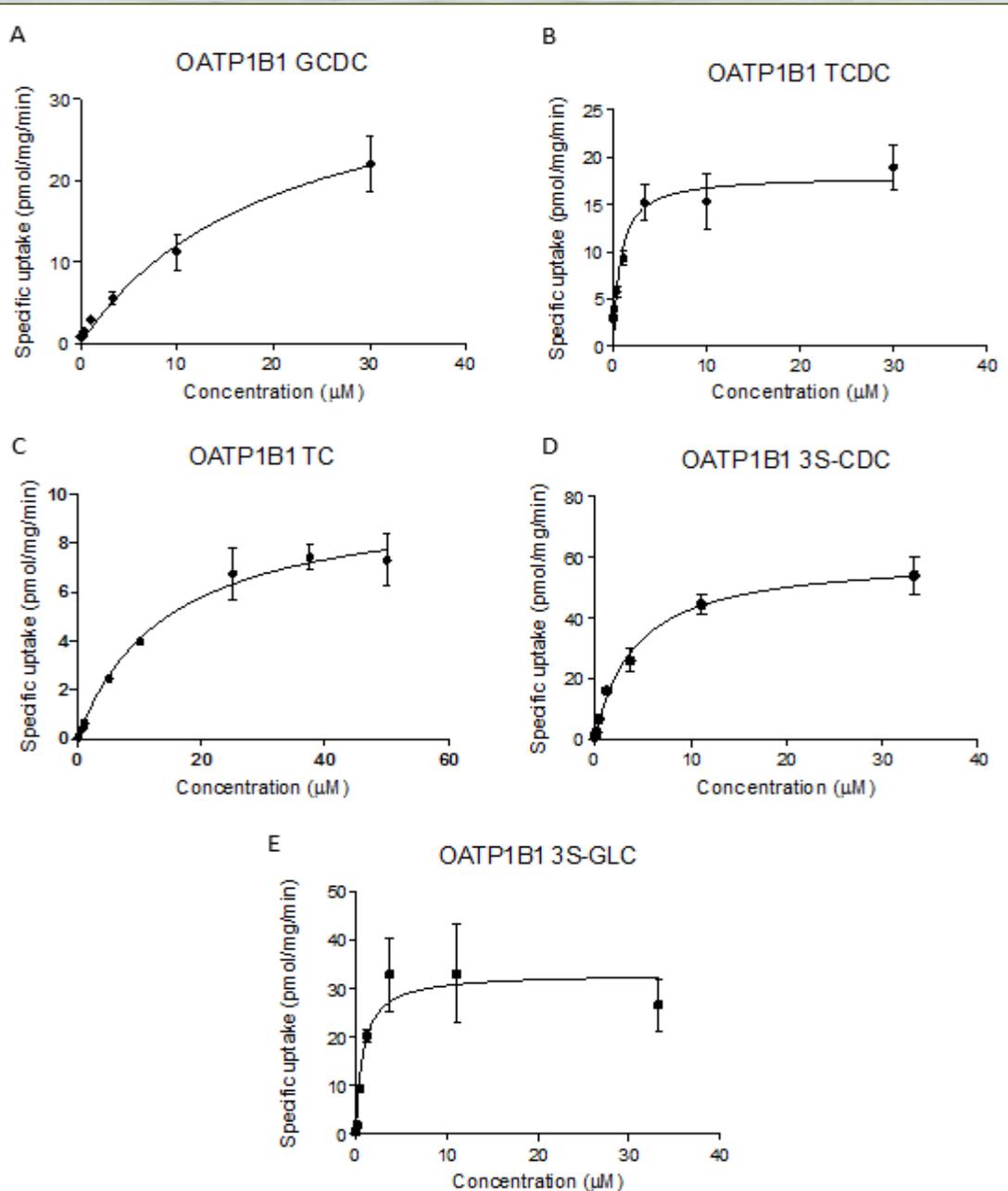
D OATP1B1 3S-CDC



E OATP1B1 3S-GLC

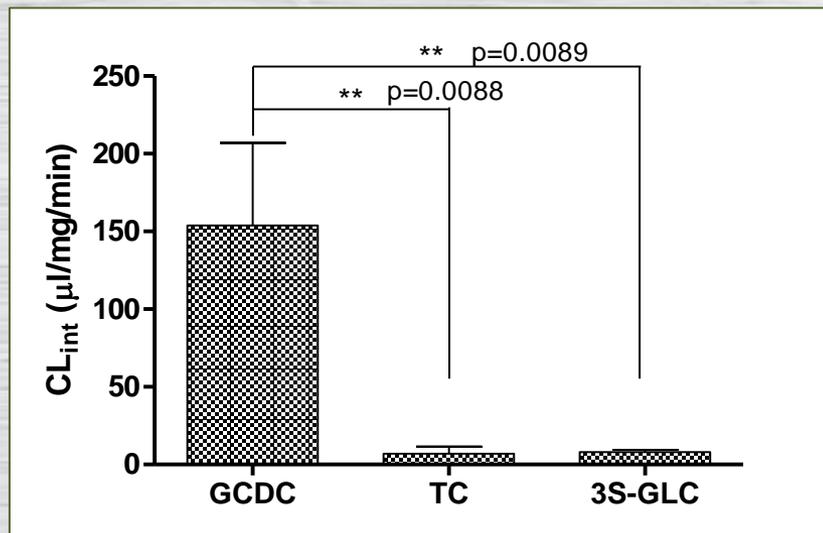


OATP1B1 - kinetics

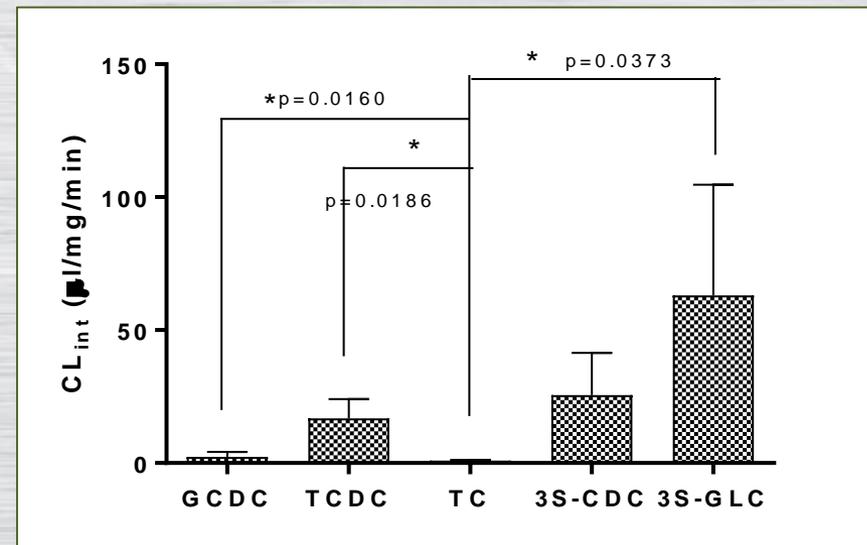


Bile salt substrate profiling – NTCP, OATP1B1

NTCP



OATP1B1



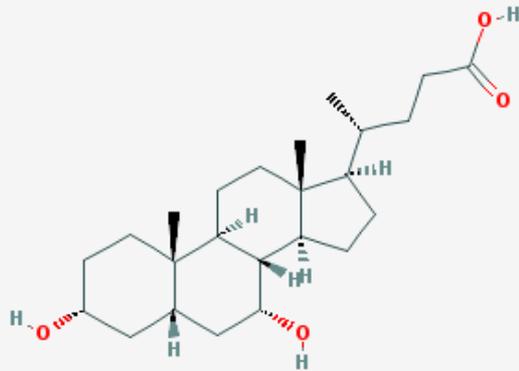
HEAT map – human transporters

Bile Salt / Transporter	NTCP	OATP1B1	Bile Salt / Transporter	BSEP	MRP2	MRP3	MRP4
CDC	Blue	Blue	CDC	Blue	Blue	Blue	Blue
TCDC	White	Red	TCDC	Red	Blue	Yellow	Yellow
GCDC	Red	Red	GCDC	Red	Blue	Red	Blue
TC	Red	Yellow	TC	Red	Blue	Yellow	Blue
3S-CDC	White	Red	3S-CDC	Red	Red	Red	Yellow
3S-GLC	Yellow	Red	3S-GLC	Red	Red	Red	Blue

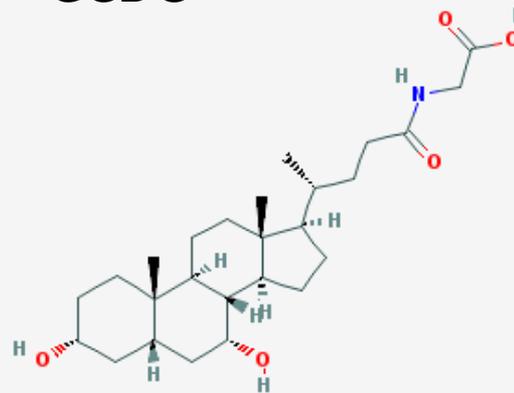
The boxes in red indicate POC folds >4, the boxes in orange indicate POC folds 4 > fold > 2.5, the boxes in yellow indicate POC folds 2.5 > fold > 1.5, and the boxes in blue indicate no transport.

Bile acids/salts

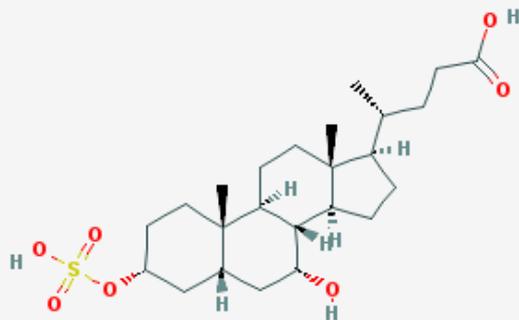
CDC



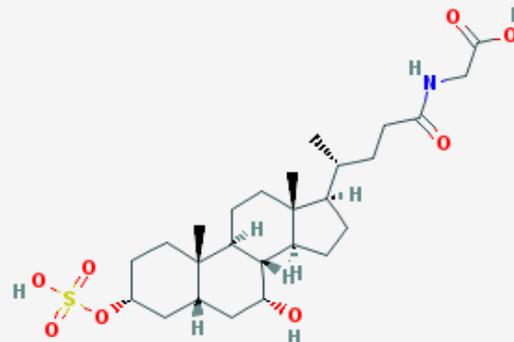
GCDC



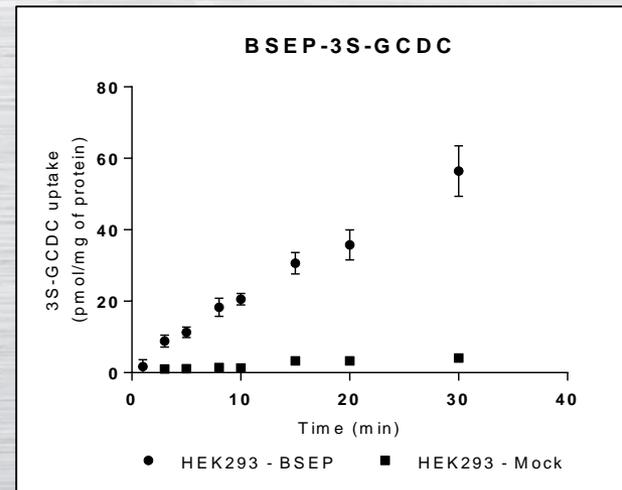
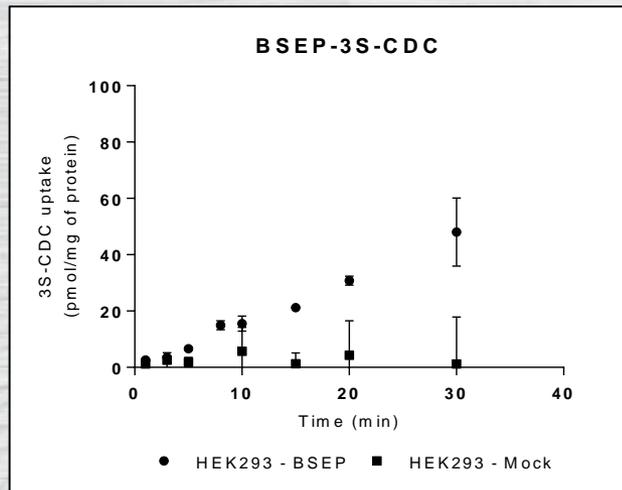
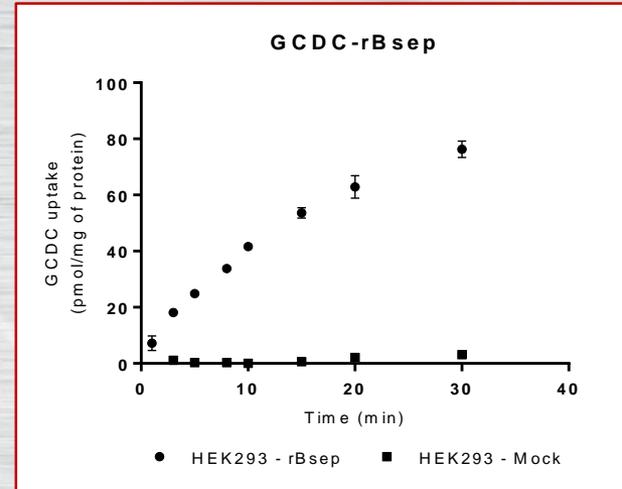
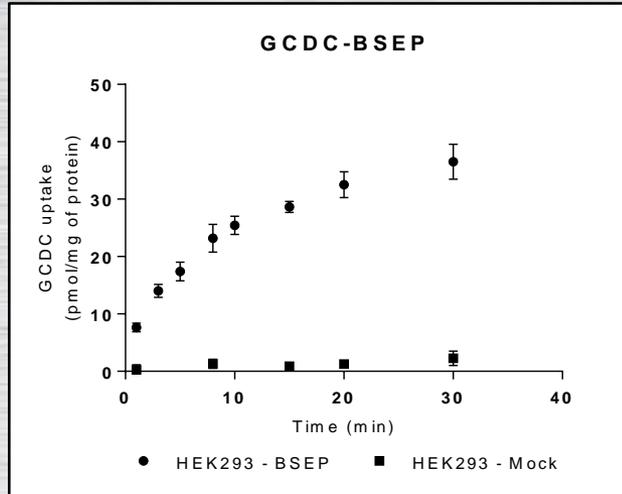
3S-CDC



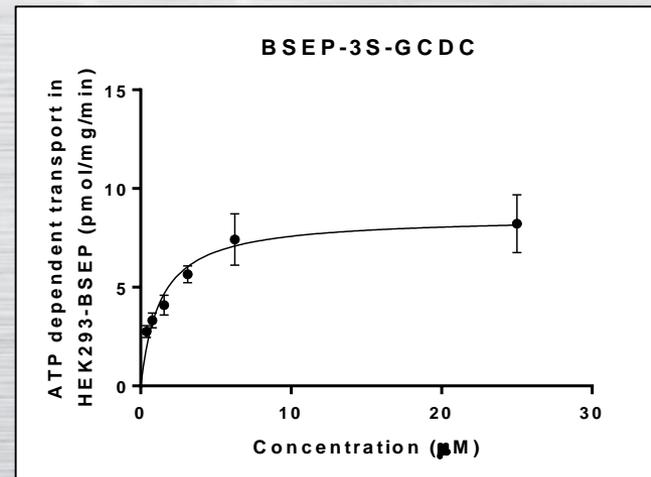
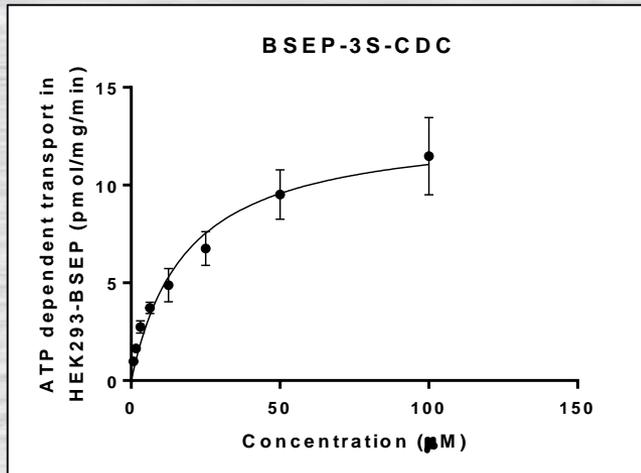
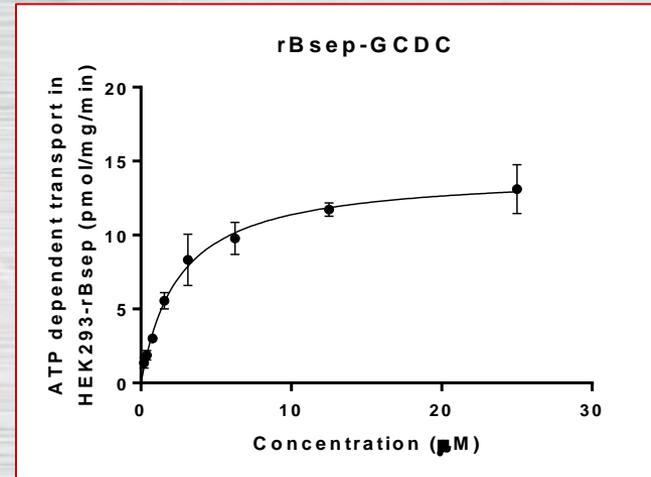
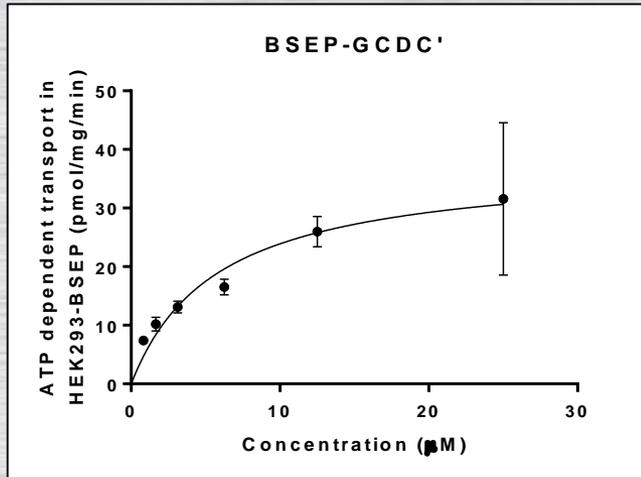
3S-GCDC



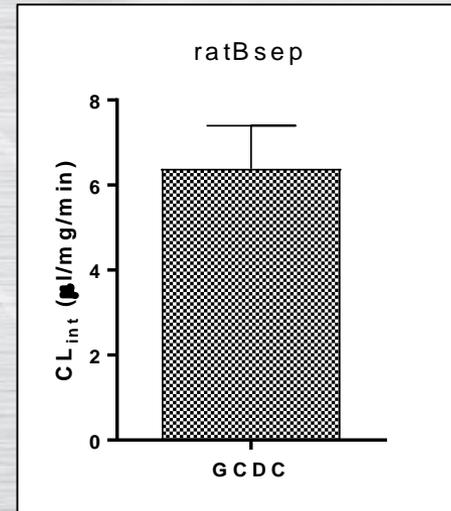
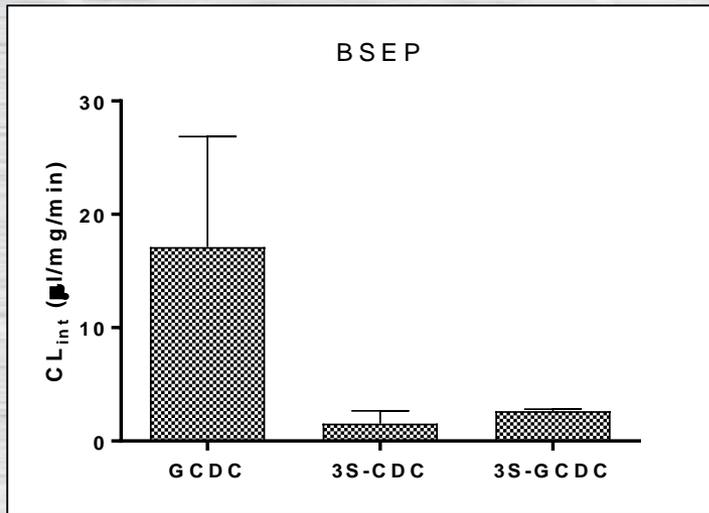
BSEP – time courses for human and rat



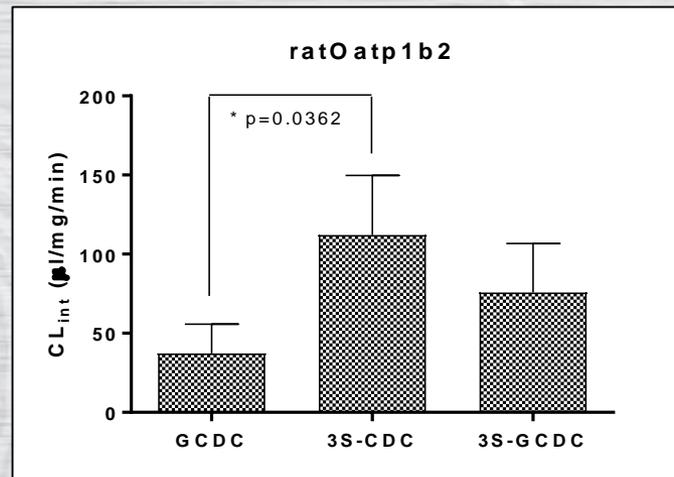
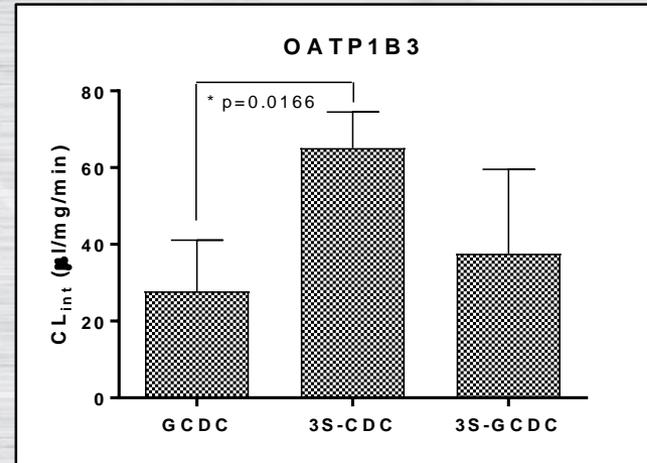
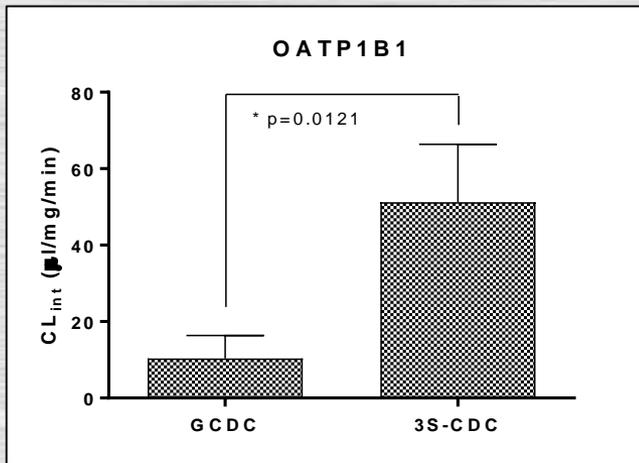
BSEP – kinetics for human and rat



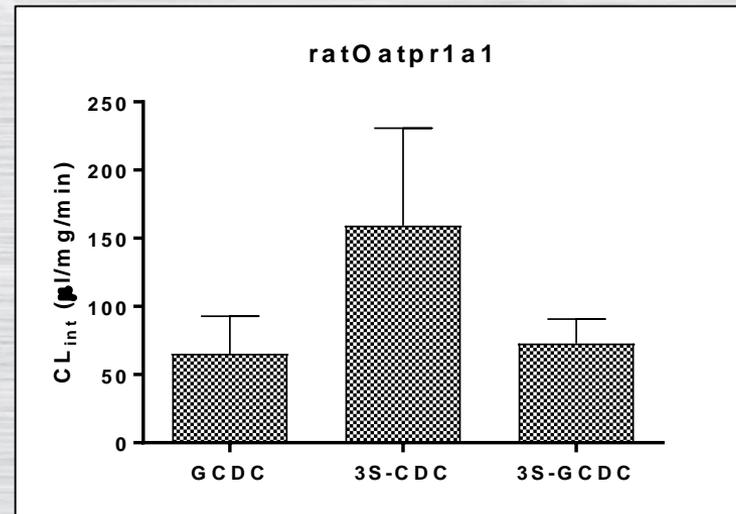
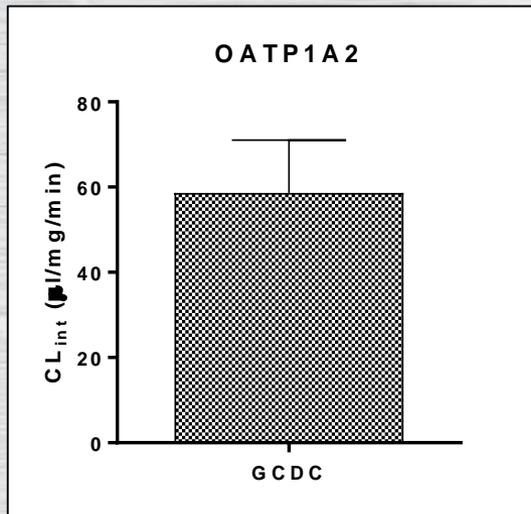
Bile salt substrate profiling – Efflux transporters



Bile salt substrate profiling – Influx transporters



Bile salt substrate profiling – Influx transporters



Heat map based on uptake ratios - human and rat transporters

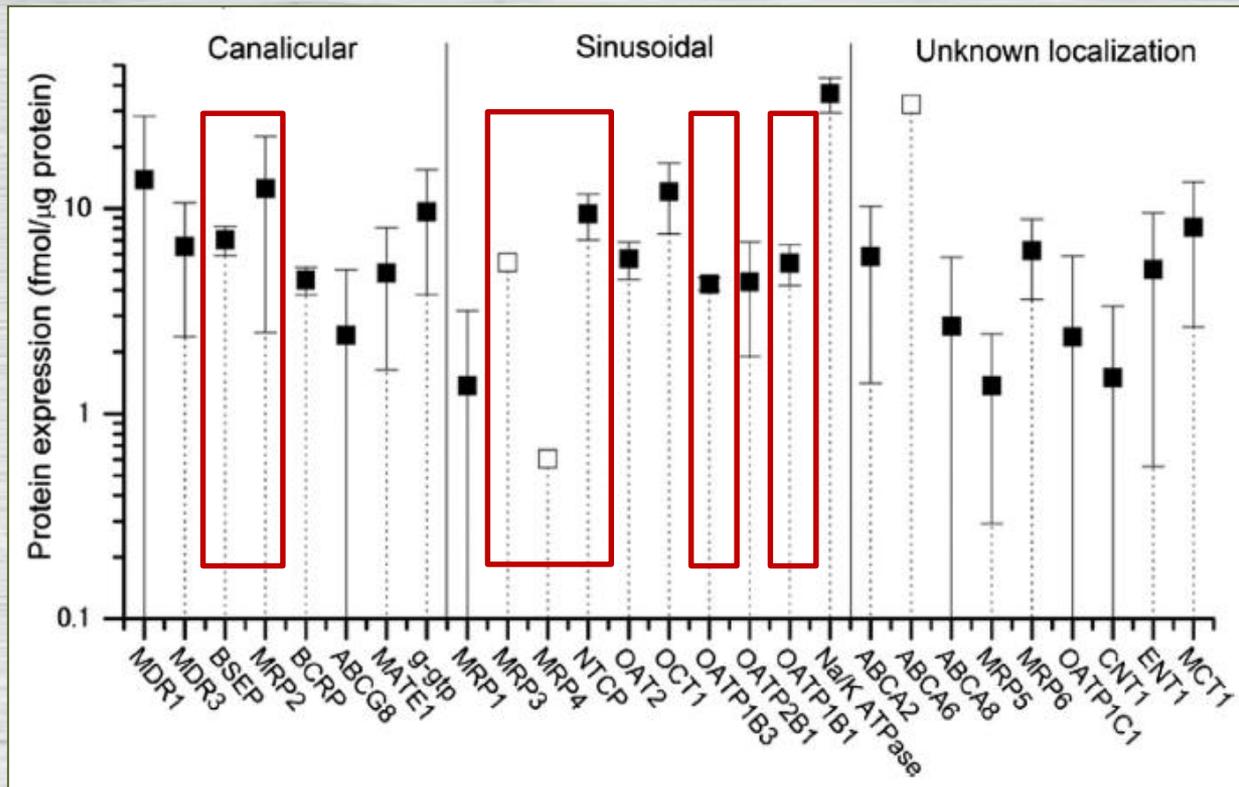
	NTCP	OATPIA2	OATPIB1	OATPIB3	BSEP
CDC	Blue	Blue	Blue	Blue	Blue
GCDC	Red	Red	Red	Red	Red
3S-CDC	Orange	Yellow	Red	Red	Orange
3S-GCDC	Blue	Blue	Yellow	Red	Yellow

	rNtcp	rOatpla1	rOatpla4	rOatplb2	rBsep
CDC	Yellow	Blue	Blue	Blue	Blue
GCDC	Red	Red	Red	Red	Red
3S-CDC	Yellow	Red	Orange	Red	Blue
3S-GCDC	Blue	Red	Blue	Red	Blue

The boxes in red indicate an average POC folds >4, the boxes in orange indicate an average POC folds 4 > fold > 2.5, the boxes in yellow indicate an average POC folds 2.5 > fold > 1.5, and the boxes in blue indicate no transport

Expression of transporters in human hepatocytes

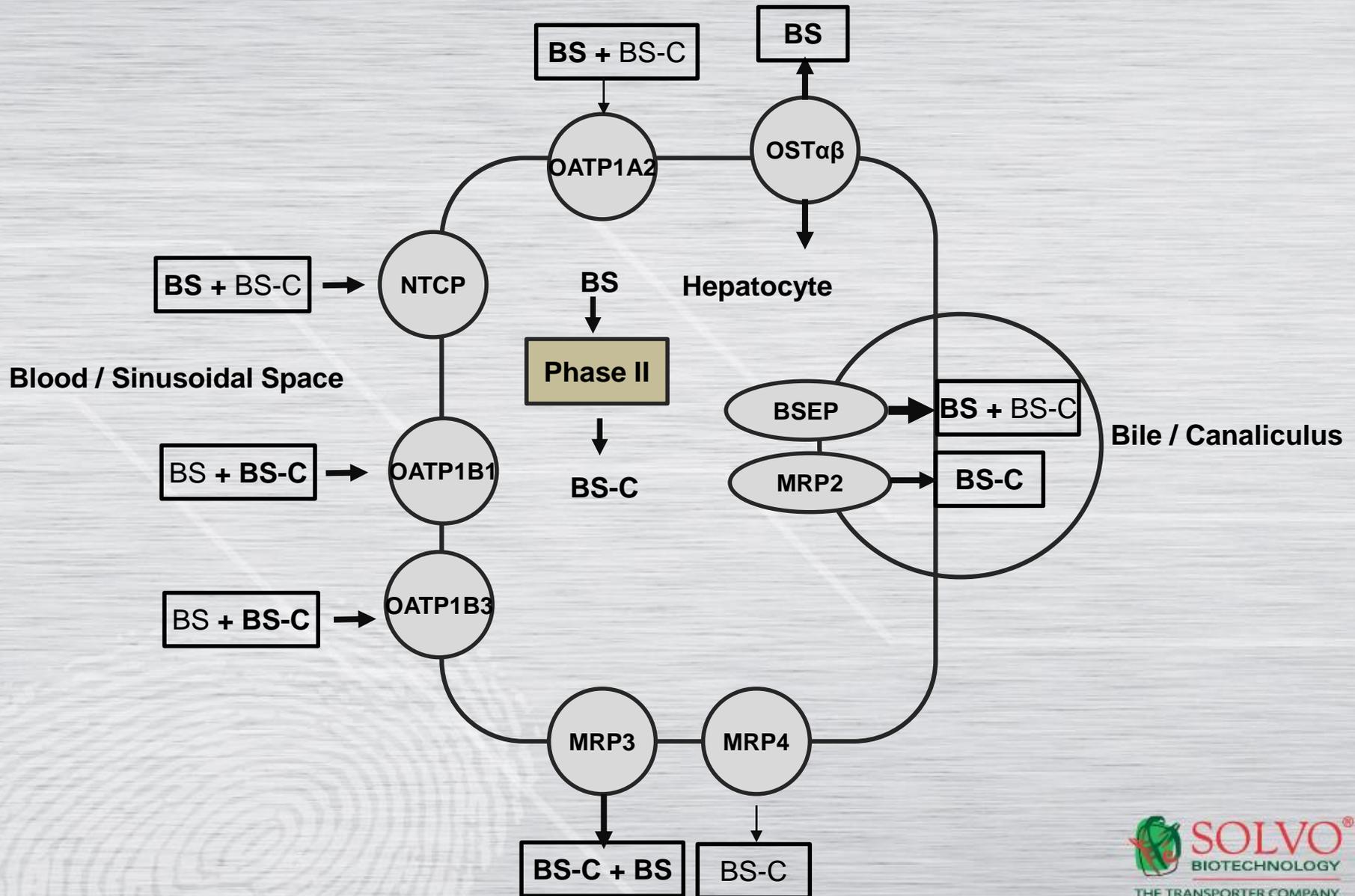
Sandwich cultured human hepatocytes



Schaefer 2012 DMD

Closed squares: $n = 2$ or 3 ; open squares $n = 1$

Human hepatic bile salt transport



Acknowledgements



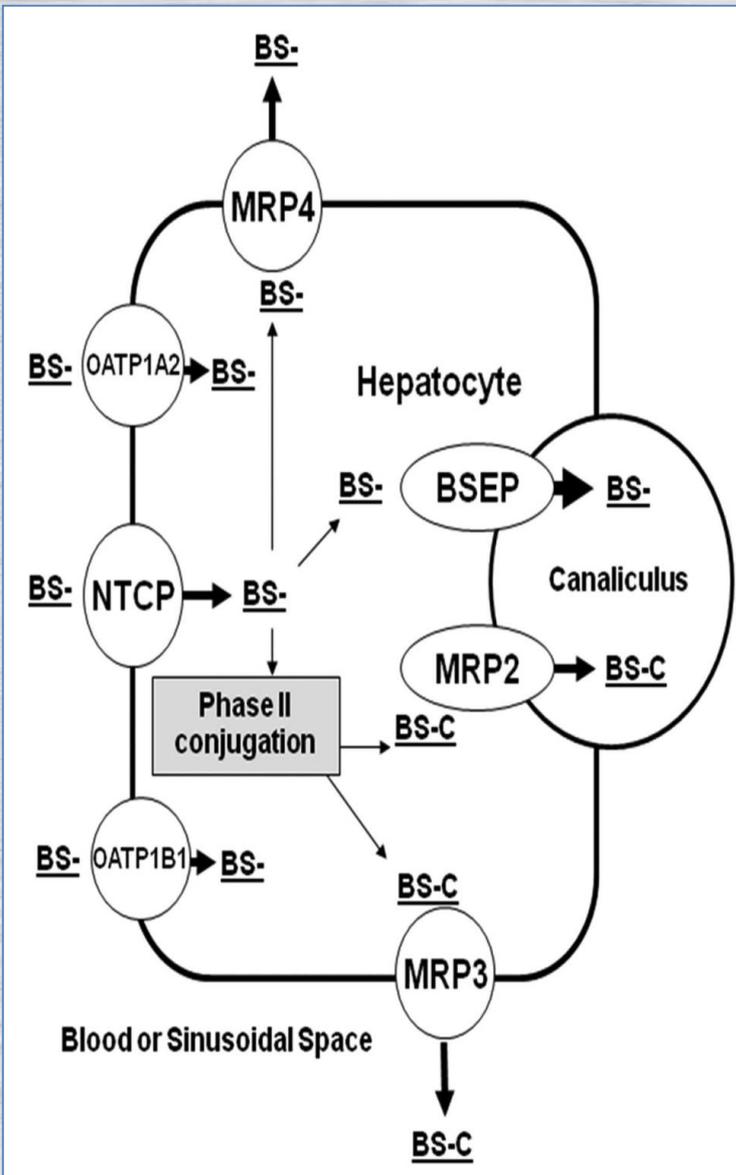
Solvo

- R&D team
 - Beata Toth
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 - Zsuzsanna Gáborik
 - Krisztina Herédi-Szabó
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- Bioanalytics group
 - Éva Molnár
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 - Kevin B Park (U Liverpool)
 - Richard W Weaver (Servier)
- Dept Med Chem, U Szeged
 - Prof Gábor Tóth & lab

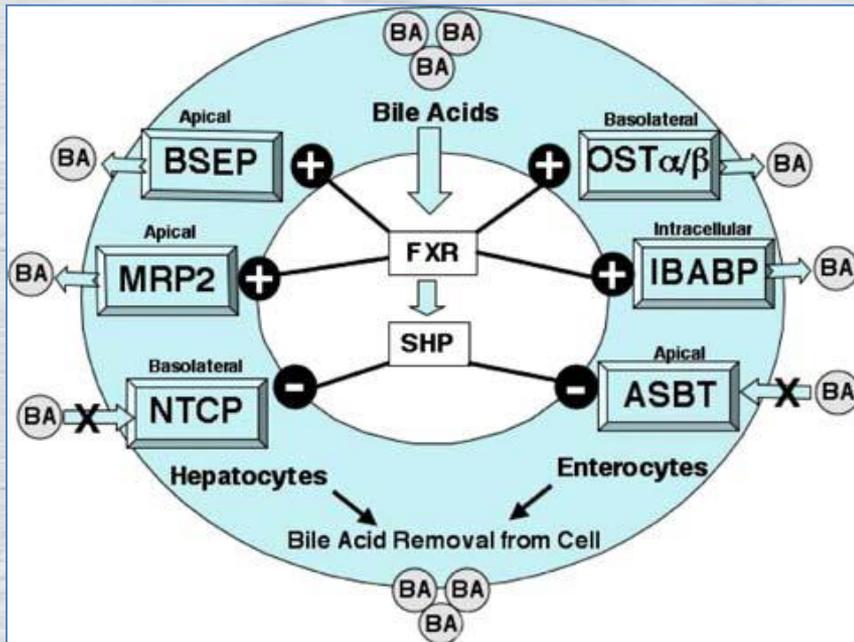
Sulfated conjugates in the focus



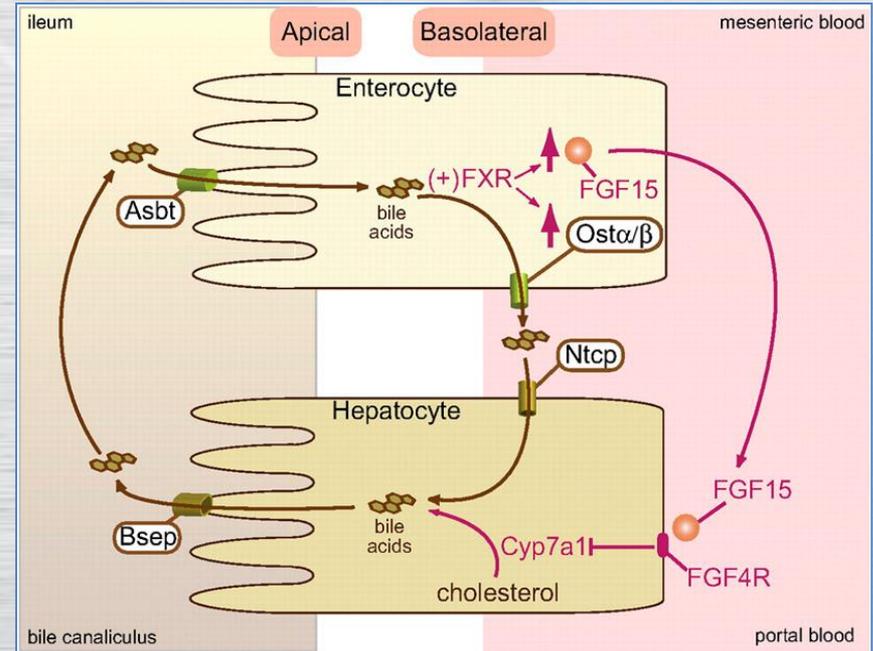
	Percentage of individual BAs: total BAs	Percentage of sulfation	Percentage of amidation ^a (G/T/U)
Serum			
LCA	13.2	93.7	73.9/21.6/4.5
UDCA	9.4	53.3	67.3/3/29.7
CDCA	30.7	28.4	66.3/20/13.7
DCA	28.2	26.0	47.7/8.7/43.6
CA	10.6	6.0	52.9/14.6/32.5

“In fact, conjugates of bile acids represented 4 of the 12 metabolites significantly associated with OATP1B1 genetic variants. These conjugates also increased following administration of CSA.”
Yee 2016 CPT

Regulation

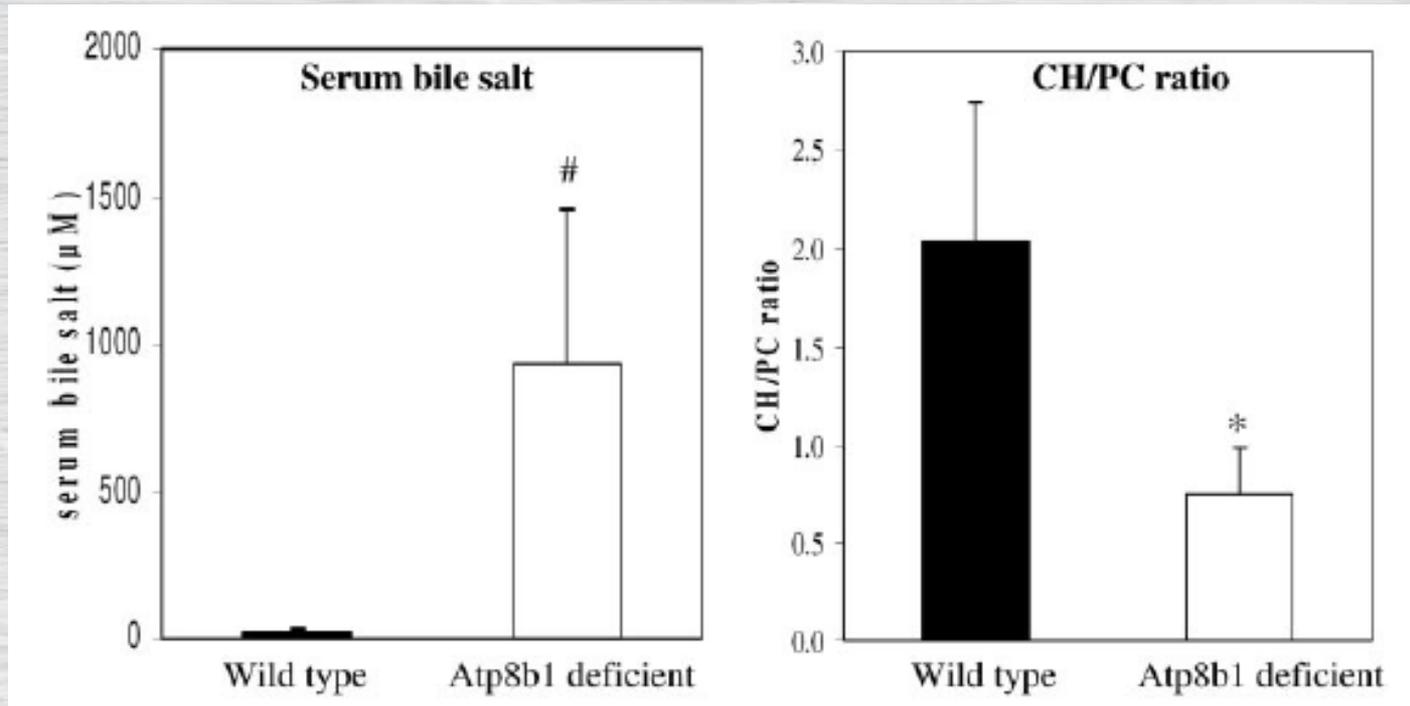


Alferai 2007 Pharm Res

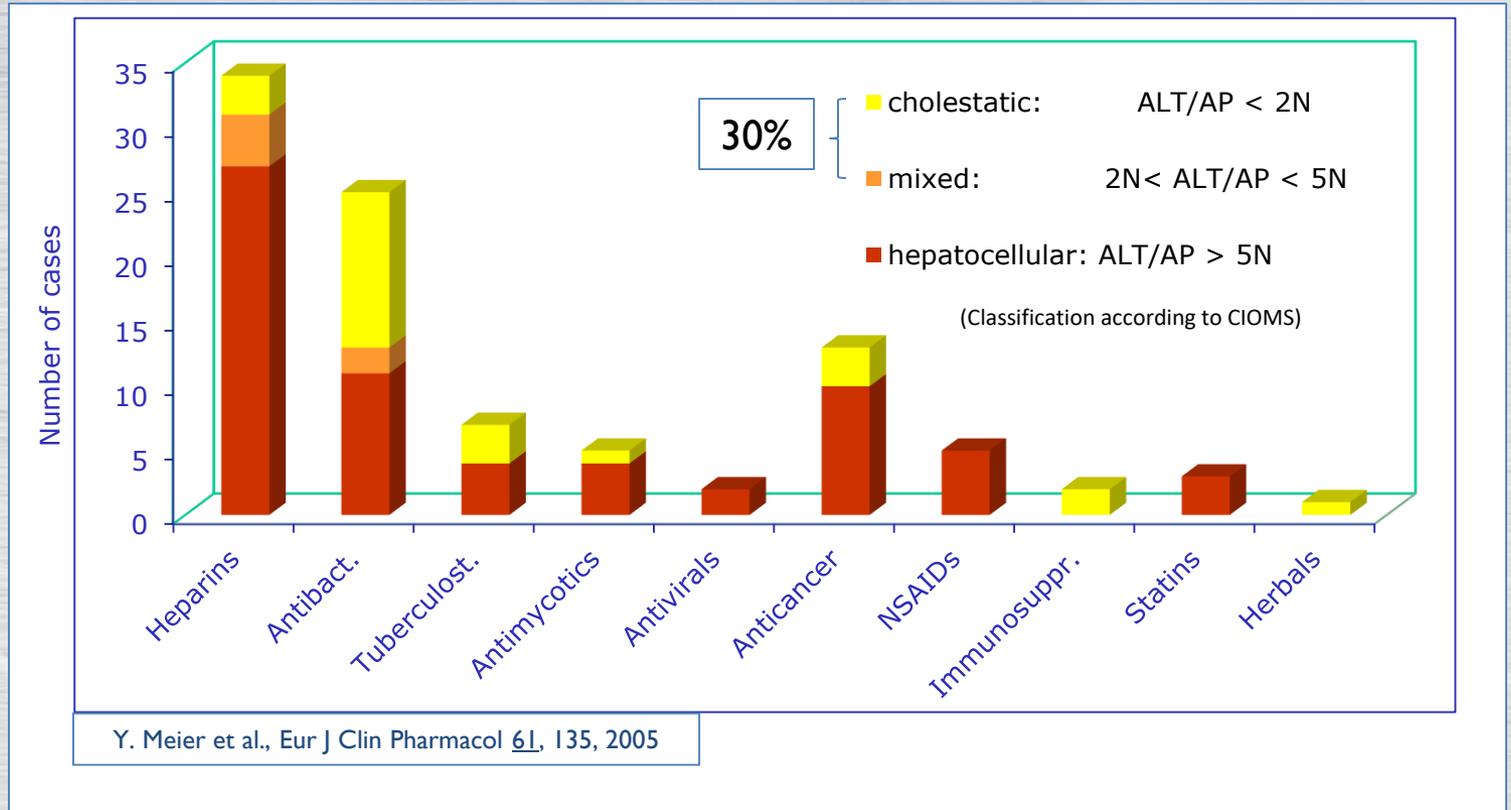


Dawson 2009 JLR

FIC1 deficiency

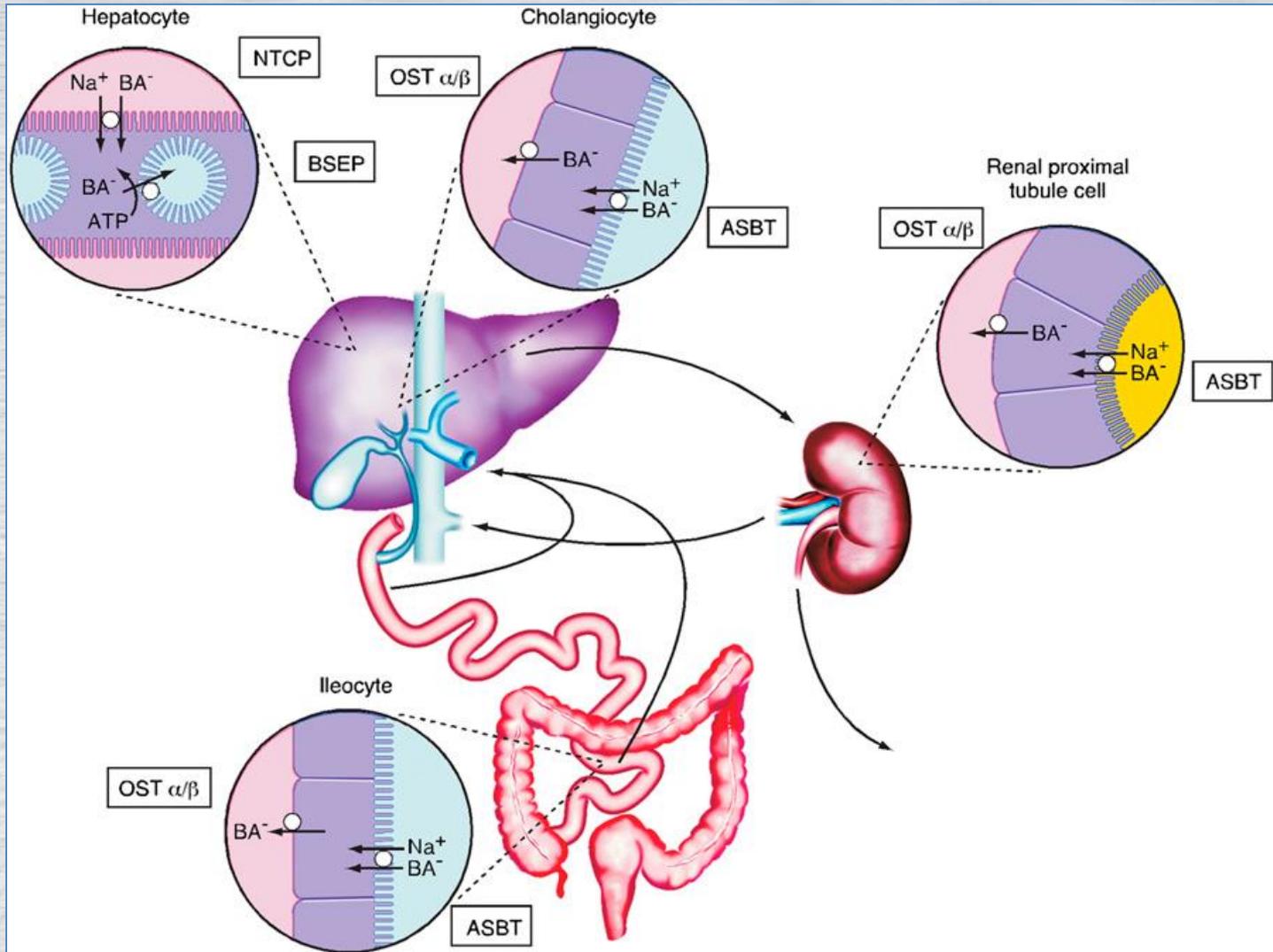


Drug induced liver injury



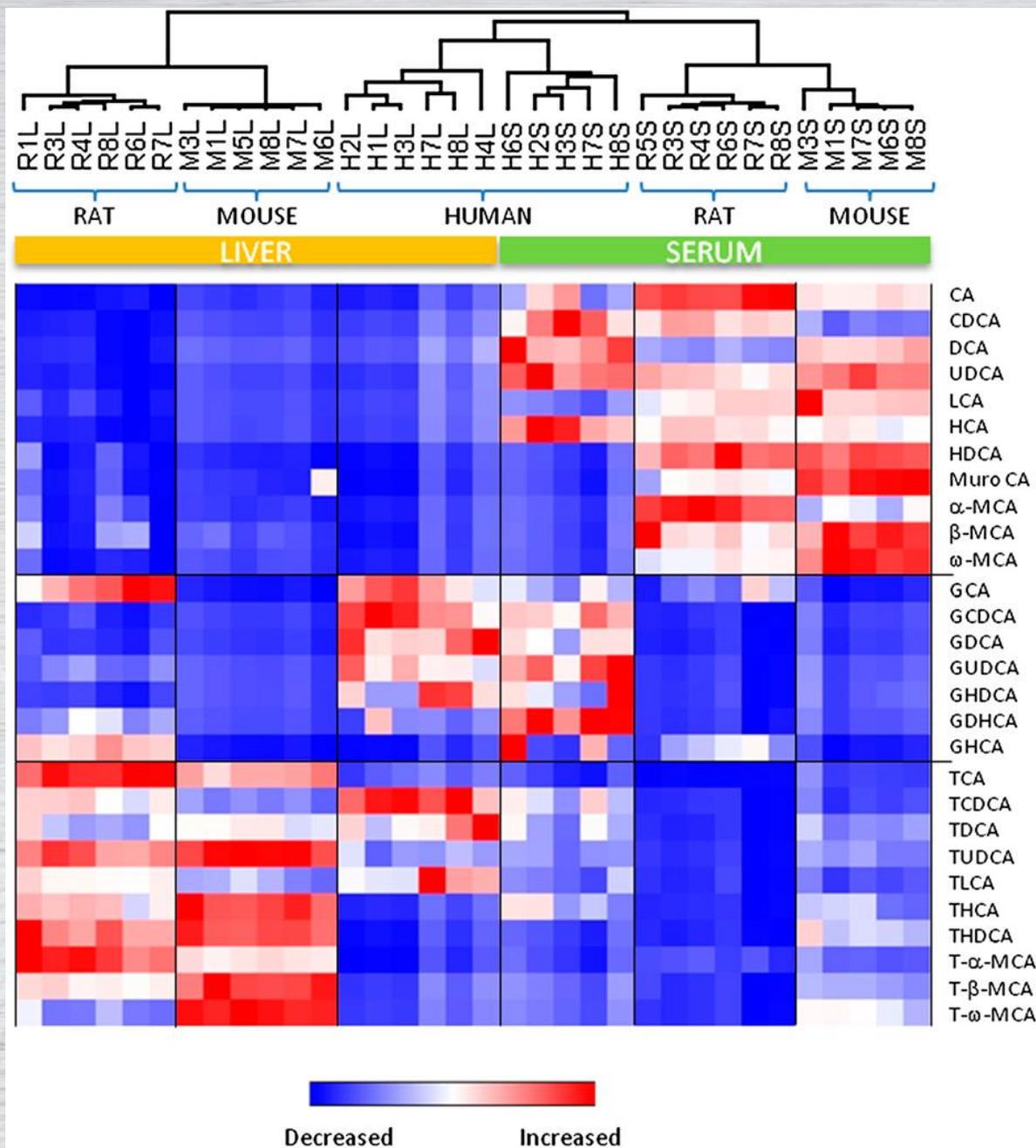
Swedish study: almost 50% of DILI cases were cholestatic or mixed (Björnsson & Olsson 2005 Hepatology)

Bile salt transport



Dawson
2009 JLR

Bas/BSs in human and rodent liver and serum

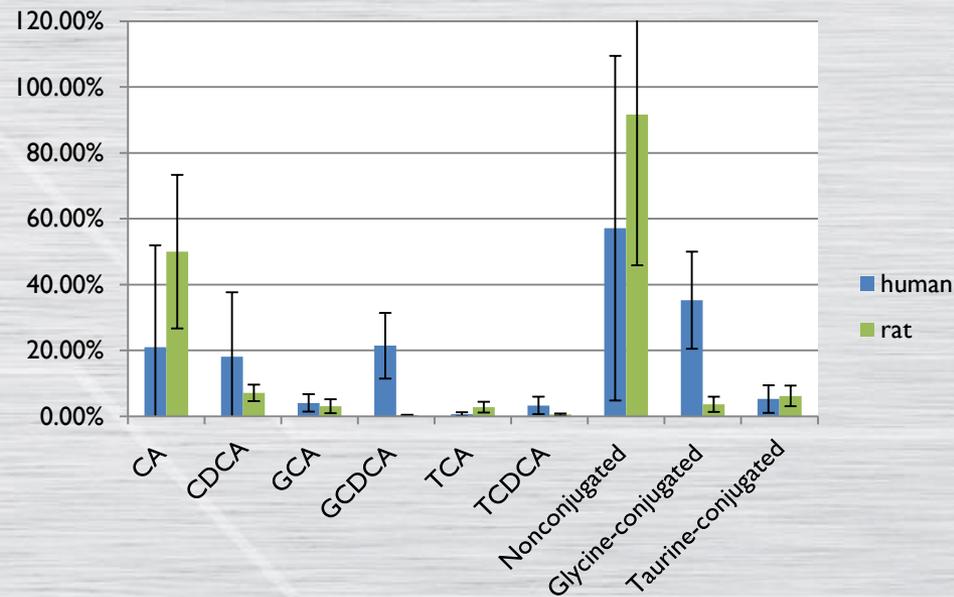
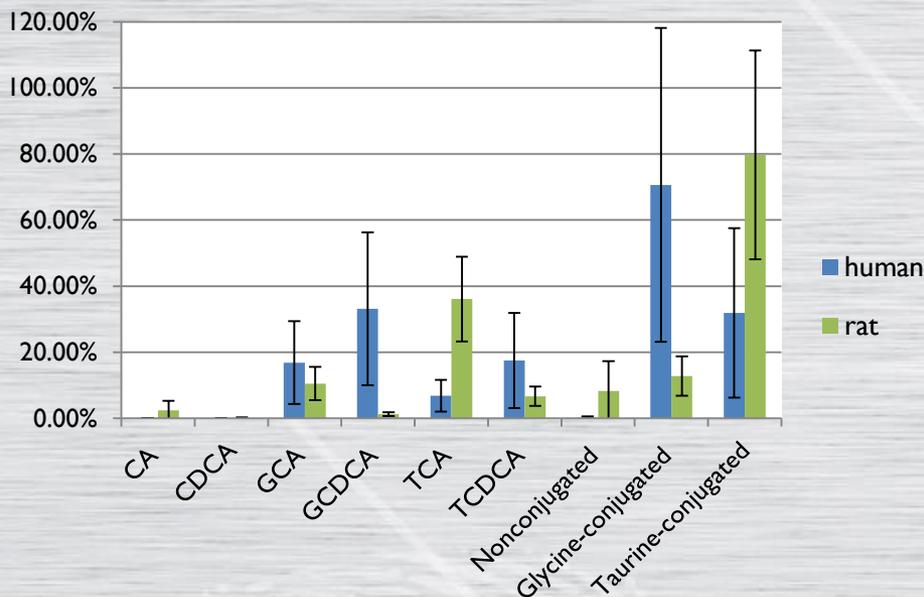


Garcia-Canaveras
2012 JLR

Composition of the BA/BS pool in human and rat liver and serum

Liver

Serum



Human

CDCA:CA=1:1
G:T=2-3.7:1

Rat

CDCA:CA=1:2
G:T=1:3-6

Heat map based on uptake ratios - human and rat transporters

	NTCP	OATPIA2	OATPIB1	OATPIB3
CDC	Blue	Blue	Blue	Blue
GCDC	Red	Red	Red	Red
3S-CDC	Orange	Yellow	Red	Red
3S-GCDC	Blue	Blue	Yellow	Red

	MRP2	MRP3	BSEP
CDC	Blue	Blue	Blue
GCDC	Blue	Yellow	Red
3S-CDC	Red	Red	Orange
3S-GCDC	Red	Red	Yellow

	rNtcp	rOatpla1	rOatpla4	rOatplb2
CDC	Yellow	Blue	Blue	Blue
GCDC	Red	Red	Red	Red
3S-CDC	Yellow	Red	Orange	Red
3S-GCDC	Blue	Red	Blue	Red

	rMrp2	rMrp3	rBsep
CDC	Blue	Blue	Blue
GCDC	Red	Yellow	Red
3S-CDC	Red	Orange	Blue
3S-GCDC	Yellow	Yellow	Yellow

The boxes in red indicate an average POC folds >4, the boxes in orange indicate an average POC folds 4>fold>2.5, the boxes in yellow indicate an average POC folds 2.5>fold>1.5, and the boxes in blue indicate no transport

Heat map based on Km - human and rat transporters

	NTCP	OATPIA2	OATPIB1	OATPIB3
CDC	Blue	Blue	Blue	Blue
GCDC	Orange	Yellow	Orange	Orange
3S-CDC	Blue	Blue	Orange	Orange
3S-GCDC	Blue	Blue	Blue	Orange

	MRP2	MRP3	BSEP
CDC	Blue	Blue	Blue
GCDC	Blue	Orange	Orange
3S-CDC	Yellow	Orange	Yellow
3S-GCDC	Yellow	Red	Orange

	rNtcp	rOatpla1	rOatpla4	rOatplb2
CDC	Blue	Blue	Blue	Blue
GCDC	Orange	Yellow	Blue	Yellow
3S-CDC	Blue	Yellow	Yellow	Yellow
3S-GCDC	Blue	Orange	Blue	Orange

	rMrp2	rMrp3	rBsep
CDC	Blue	Blue	Blue
GCDC	Blue	Orange	Orange
3S-CDC	Yellow	Orange	Blue
3S-GCDC	Yellow	Orange	Blue

The boxes in red indicate $K_m < 1 \mu M$, the boxes in orange indicate $K_m 10 > K_m > 1 \mu M$, the boxes in yellow indicate $K_m > 10 \mu M$, and the boxes in blue indicate no significant transport

Inhibition profiling of human rat and monkey OATPs/Oatps

	OATP 1A2								
OATP 1A2	8/8	OATP 1B1							
OATP 1B1	1/8	8/8	OATP 1B3						
OATP 1B3	1*/8	6/8	8/8	rOatp 1a1					
rOatp 1a1	5*/8	3/8	7*/8	8/8	rOatp 1a4				
rOatp 1a4	6*/8	2/8	2/8	4/8	8/8	rOatp 1b2			
rOatp 1b2	1/8	4/8	5/8	6/8	1/8	8/8	cyOatp 1b1		
cyOatp 1b1	1/8	7/8	5/8	3/8	2/8	5/8	8/8	cyOatp 1b3	
cyOatp 1b3	2*/8	3/7	5*/8	6*/8	2/8	7*/8	4/8	8/8	

- Atorvastatin
- CCK8
- Diclofenac
- NMQ
- Pravastatin
- Taxol
- Telmisartan
- Troglitazone

Substrate: taurocholate