DILIsym® User Training - Data Gathering For Bile Acid Transporter Inhibition

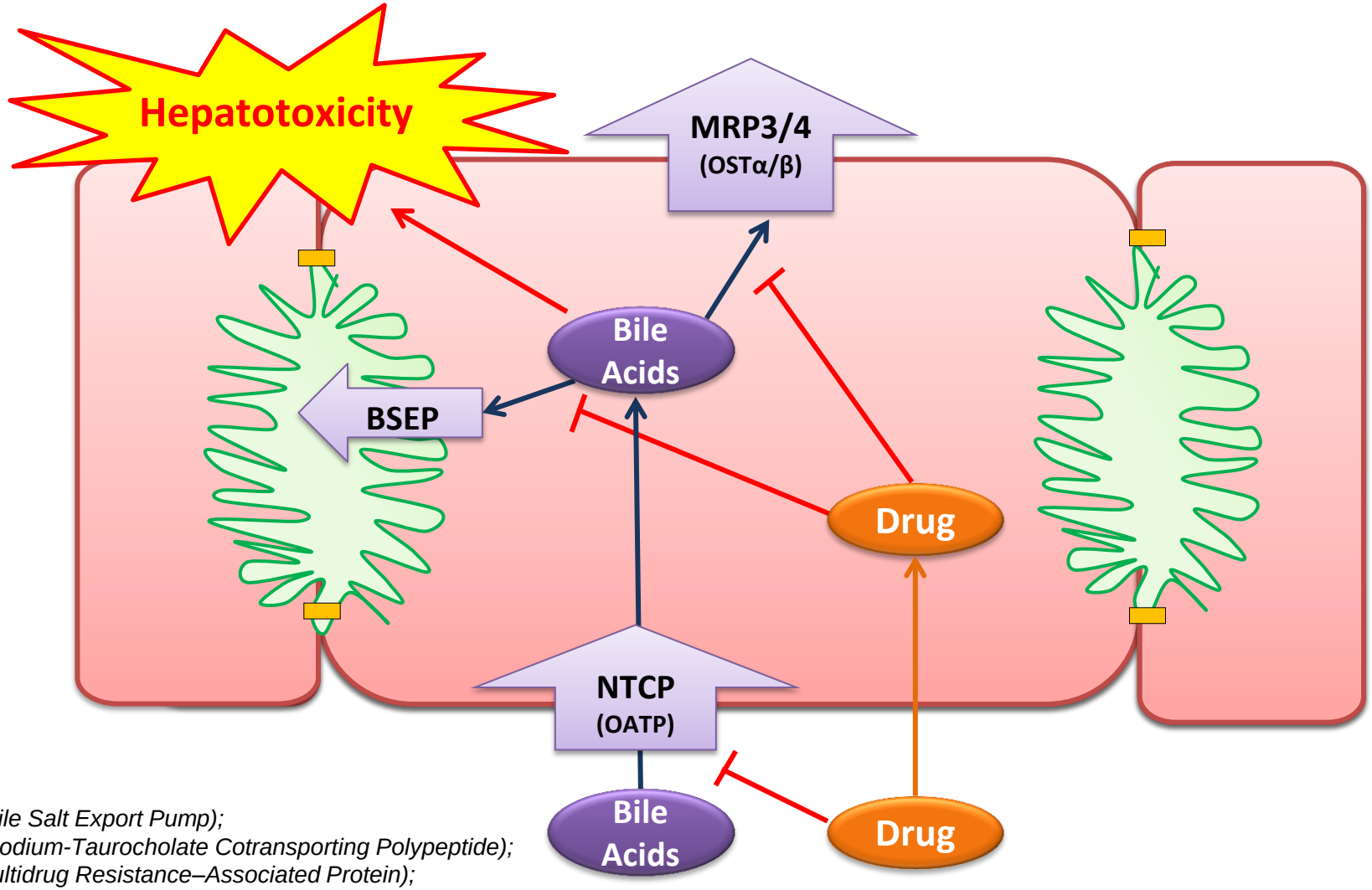
DILI-sim Team

Goal for This Training Session

Participants should understand the following general concepts:

- Methods and tips related to gathering data in the area of bile acid transporter inhibition for use within DILIsym®

Bile Acid-Mediated DILI



BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)

Gathering Data for DILIsym® Parameter Inputs: Bile Acid Transport Inhibition

- DILIsym® parameter inputs
  - Inhibition constant: K_i, IC_{50}
  - Type of inhibition: competitive, noncompetitive, uncompetitive, mixed
- *in vitro* assessment using multiple bile acid transporters is recommended

Transporter	Function	Experimental System
BSEP	Biliary excretion	Membrane vesicles
MRP3, MRP4	Basolateral efflux	Membrane vesicles
NTCP	Basolateral uptake	Primary hepatocytes, transfected cell lines

- Assessment of inhibitory effects of parent and stable metabolites will provide more reliable predictions, if systemic/hepatic exposure of stable metabolites are high
  - Identification and synthesis of stable metabolites are not feasible during early stages of drug development
  - QSAR models can be used to obtain some information about transporter inhibition potencies by metabolites

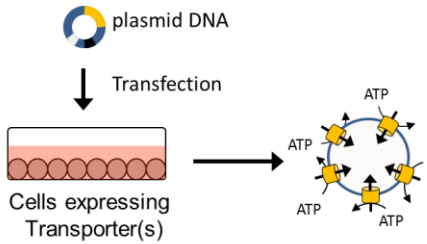
Tips for Gathering Bile Acid Transport Inhibition Data for DILIsym®

- Optimize the incubation time and the probe substrate concentration
  - Select a incubation time within linear range
  - Determine transport kinetics (i.e., K_m, V_{max}) of the probe substrate
  - For IC_{50} assays, select a probe substrate concentration below K_m
  - For K_i assays, select probe substrate concentrations spanning K_m
- Concentrations of test compounds should include predicted/observed plasma (NTCP) or liver C_{max}
  - For example, 0.33X, 1X, 3X,10X, and 30X C_{max}
- Final concentration of organic solvent should be less than 2% (v/v)
- Running positive controls is recommended to validate assay conditions

Transporter	Substrate	Positive Control (Inhibitor)
BSEP	Taurocholate (TC)	Cyclosporine A
MRP3	Estradiol 17 β-D-glucuronide ($E_2$17βD)	MK-571
MRP4	$E_2$17βD, Dehydroepiandrosterone (DHEAS)	MK-571
NTCP	Taurocholate (TC)	Taurochenodeoxycholate (TCDC)

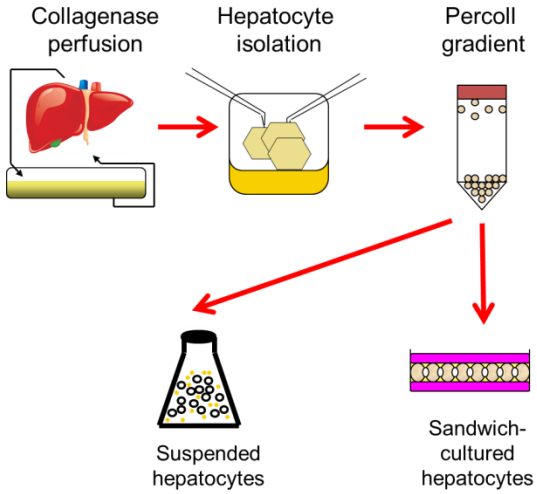
In vitro Systems to Assess Bile Acid Transport Inhibition

- Membrane vesicles
  - Used to assess efflux transporters (e.g., BSEP, MRP3, MRP4)
  - Source: liver plasma membrane, transfected cells



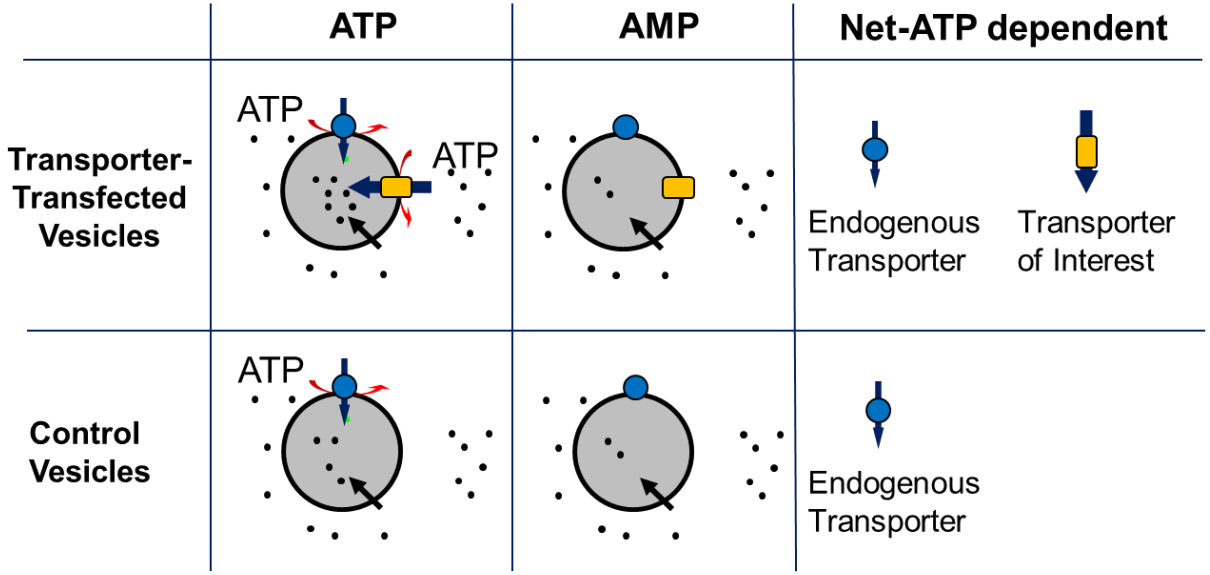
- Transfected cell lines
  - Used to assess uptake transporters (e.g., NTCP)

- Primary hepatocytes
  - Suspended hepatocytes
    - Used to assess hepatic uptake
    - Can determine Na^+-dependent and Na^+-independent transport
  - Sandwich-cultured hepatocytes
    - Used to assess hepatic uptake and efflux simultaneously
    - Can evaluate overall effects of drugs on bile acid disposition
    - Difficult to estimate kinetic parameters for each transporter



Membrane Vesicles Are Used to Assess Drug Effects on Efflux Transporters

- Inside-out membrane vesicles are prepared from transfected cell lines overexpressing a transporter of interest
- Perform the studies in the presence and absence of ATP to subtract the compounds taken up by passive diffusion
- Perform the studies in transporter- and control vesicles to subtract the transport by endogenous transporters



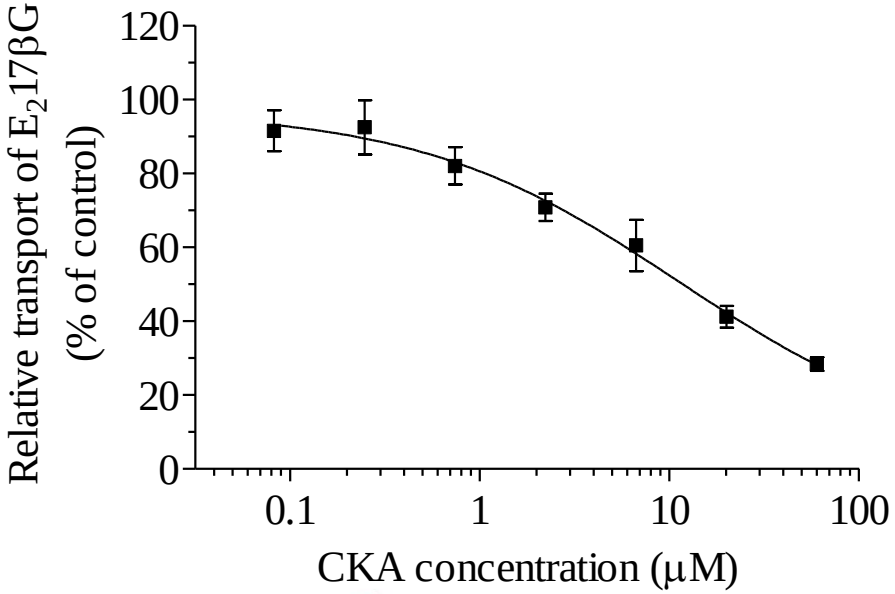
Experimental Conditions for Use of Vesicles to Assess CKA IC_{50} for Human MRP3

- Vesicles: HEK293-MRP3
  - Control vesicles not used because $E_2$17βB transport in control vesicles was negligible
- Substrate: 10 µM $E_2$17βG
- Inhibitors
  - Vehicle control: 1% (v/v) DMSO
  - CKA: 0.08, 0.25, 0.74, 2.22, 6.67, 20, and 60 µM
  - Positive control: 300 µM sulfasalazine
- Incubation conditions: 10 min, 37°C, 2 mM glutathione
- Assay groups

Assay group	Number of wells	
	ATP	AMP
CKA	3 per concentration	3 per concentration
Vehicle control	3	3
Positive control	3	3

Data Interpretation to Assess CKA IC_{50} for Human MRP3

- ATP-dependent $E_2$17βG transport data are presented as percentages relative to vehicle control
- IC_{50} is defined as the concentration required to inhibit the transport of the probe substrate by 50%
  - IC_{50} estimated using a four-parameter logistic model
  - If IC_{50} values fall outside the concentration ranges studies, no values will be reported



$$Y = Bottom + \frac{(Top + Bottom)}{(1 + 10^{(LogIC_{50} - X) * HillSlope})}$$

CKA IC_{50} = 11.2 µM

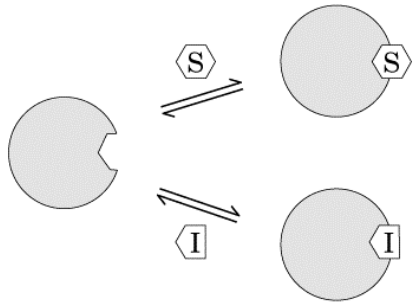
Relative transport in the presence of 300 µM sulfasalazine = 1.6 ± 1%

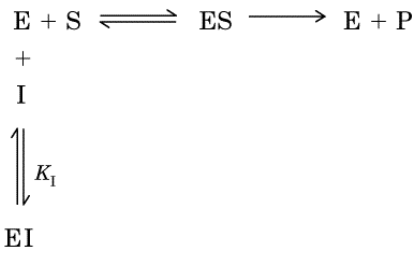
Transporter Inhibition Constants

Inhibition constant	IC_{50}	K_i
Definition	Inhibitor concentration at the half maximal activity	Affinity of the inhibitor to the probe substrate binding site
Experimental methods	Transport assays with one substrate concentration & multiple inhibitor concentrations	Transport assays with multiple substrate concentrations & multiple inhibitor concentrations
Robustness	Varies depending on the substrate concentrations IC_{50} will approach K_i, if $[S] << K_m$	A more robust parameter
Provide information on the type of inhibition?	No	Yes
Cost	$	$$$
Comment	Commonly measured	Recommended for reliable prediction of hepatotoxicity

Type of Inhibition: Reversible Inhibition

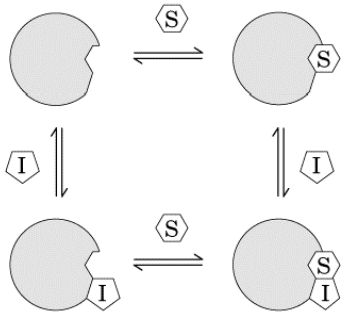
Competitive

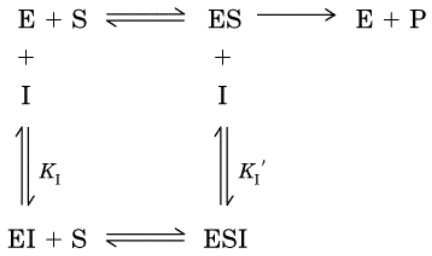




- I binds to the active or allosteric site of E and prevents binding of S
- I and S cannot bind to E at the same time
- ↔ $V_{max,app}$, ↑ $K_{m,app}$

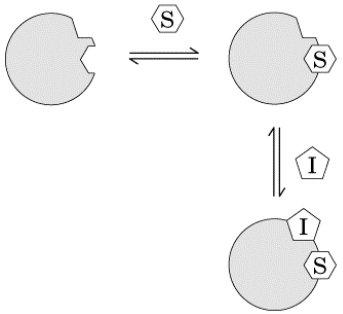
Noncom/Mixed

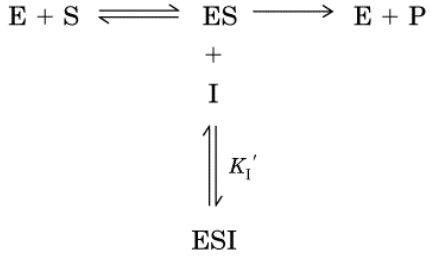




- I and S can both bind to E at any give time
- E-S-I complex cannot form P
- Mixed: I has the different affinity for E and E-S
- ↓$V_{max,app}$, ↔$K_{m,app}$

Uncompetitive





- I binds only to the E-S complex
- E-S-I complex cannot form P
- ↓$V_{max,app}$, ↓ $K_{m,app}$

E: enzyme, S: substrate, I: inhibitor, P: product

A Case Study: Use of BSEP Vesicles to Assess AMG 009 Ki and Type of Inhibition

- Vesicles: Sf9-BSEP
  - Control vesicles not used because TC transport in control vesicles was negligible
- Substrate
  - TC: 0.46, 1.4, 4.2, 12.6, 37.8 µM
- Inhibitors
  - Vehicle control: 1.3% (v/v)
  - AMG 009: 0.007, 0.02, 0.06, 0.18, 0.55, 1.64, 4.93, 14.8, 44.3, 133 µM
- Incubation conditions: 15 min, 24°C
- Assay groups

Assay group	Number of wells	
	ATP	AMP
AMG 009	3 X10 (AMG 009) X 5 (TC)	-
Vehicle control	3 X 5 (TC)	3 X 5 (TC)

Van Staden (2012) Current Protocols in Toxicology
Morgan (2013) Toxicological Sciences

A Case Study: Use of BSEP Vesicles to Assess AMG 009 Ki and Type of Inhibition

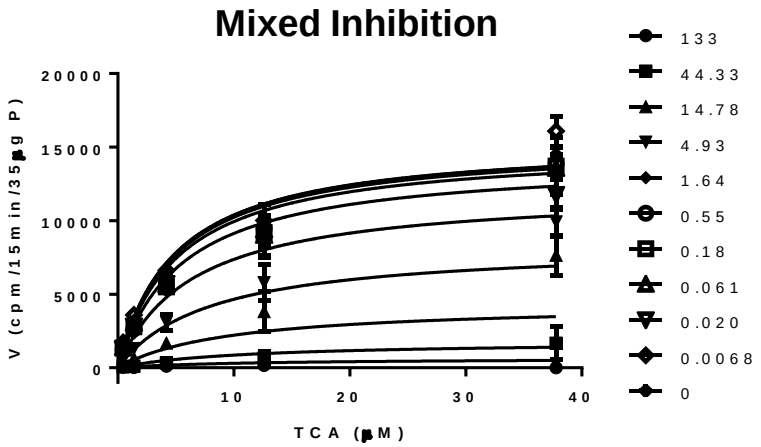
- ATP-dependent TCA transport data are presented as substrate concentration versus transport velocity for each inhibition concentration
- The kinetic parameters (K_m, V_{max}, and K_i) and type of inhibition were determined by fitting competitive, noncompetitive, uncompetitive, and mixed models to the untransformed data by nonlinear regression analysis
  - The best-fit model was assessed from visual inspection of the observed versus predicted data and Akaike Information Criterion (AIC)

Competitive: $$V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S}$$

Noncompetitive: $$V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S \times \left(1 + \frac{I}{K_i}\right)}$$

Uncompetitive: $$V = \frac{V_{max} \times S}{K_m + S \times \left(1 + \frac{I}{K_i}\right)}$$

Mixed: $$V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S \times \left(1 + \frac{I}{\alpha \times K_i}\right)}$$

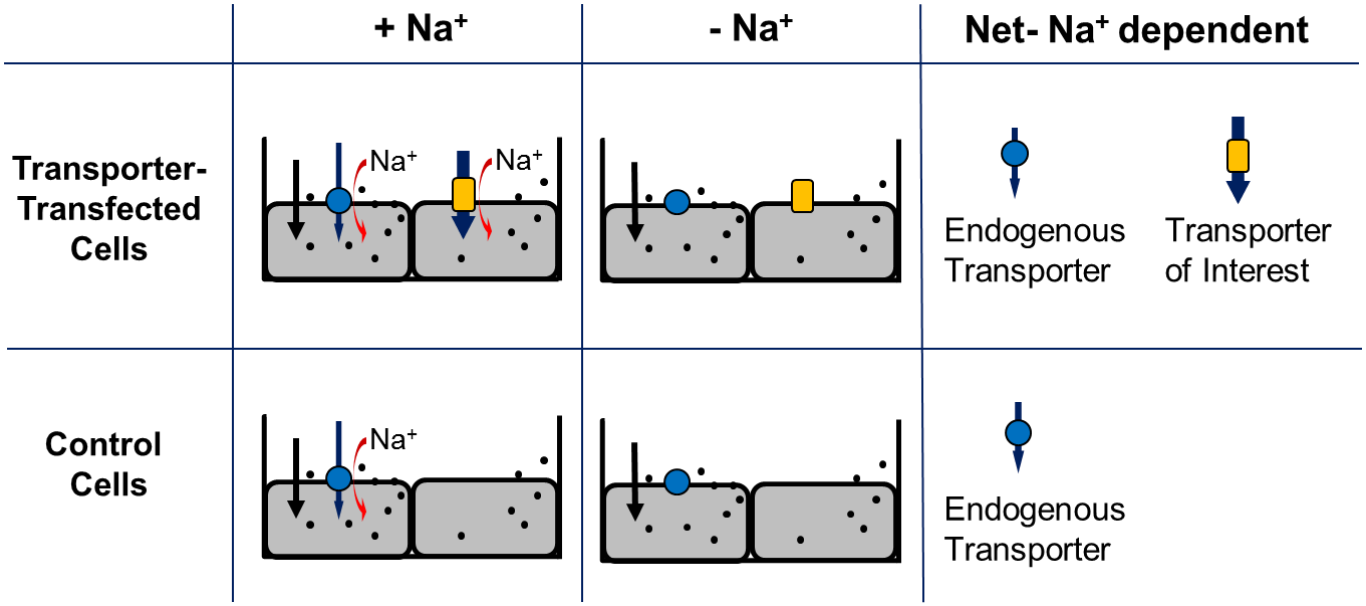


AMG 009 Ki = 2.4 µM

Type of Inhibition: mixed (α = 2.4)

Transfected Cells Are Used to Assess Drug Effects on Uptake Transporters

- Cells are stably or transiently transfected with NTCP/Ntcp
- Perform the studies in the presence and absence of Na^+ to subtract the compounds taken up by passive diffusion
- Perform the studies in transporter-transfected cells and control cells to subtract the transport by endogenous transporters



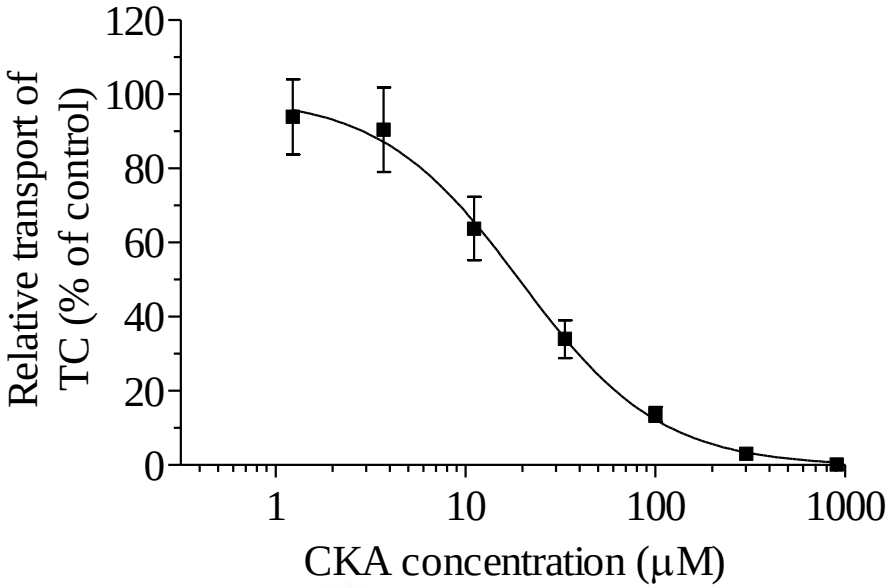
A Case Study: Use of NTCP-Transfected Cells to Assess CKA IC_{50} for Human NTCP

- Cells: CHO-NTCP, CHO-Control
- Substrate: 2 µM TC
- Inhibitors
  - Vehicle control: 1% (v/v) DMSO
  - CKA: 1.2, 3.7, 11.1, 33.3, 100, 300, 900 µM
  - Positive control: 100 µM TCDC
- Incubation conditions: 10 min, 37°C
- Assay groups

Assay group	Number of wells			
	CHO-NTCP		CHO-Control	
	+ Na^+	- Na^+	+ Na^+	- Na^+
CKA	3 per concentration	3 per concentration	3 per concentration	3 per concentration
Vehicle control	3	3	3	3
Positive control	3	3	3	3

A Case Study: Use of NTCP-Transfected Cells to Assess CKA IC_{50} for Human NTCP

- Na^+-dependent TC transport data are presented as percentages relative to vehicle control
- IC_{50} is defined as the concentration required to inhibit the transport of the probe substrate by 50%
  - IC_{50} estimated using a four-parameter logistic model
  - If IC_{50} values fall outside the concentration ranges studies, no values will be reported



$$Y = Bottom + \frac{(Top + Bottom)}{(1 + 10^{(LogIC_{50} - X) * HillSlope})}$$

CKA IC_{50} = 19.5 μM

Relative transport in the presence of 100 μM TCDC = 1.1 ± 0.2%