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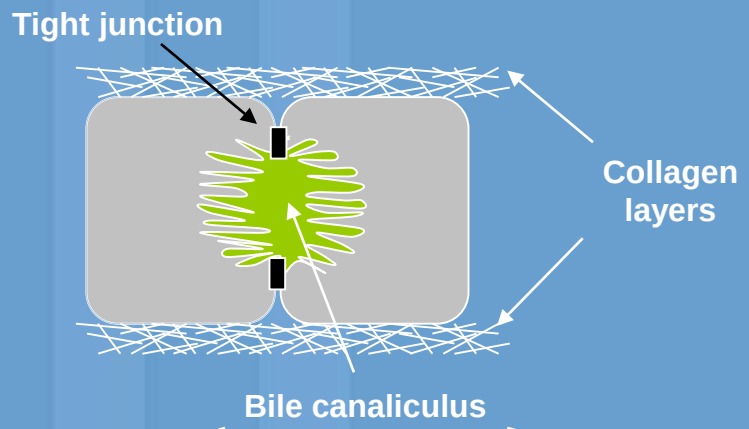
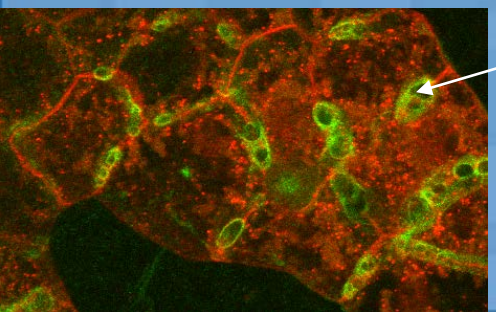
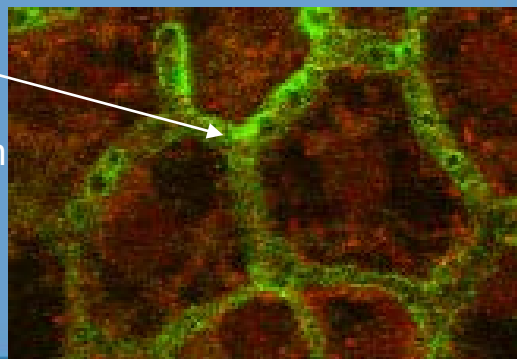
In Vitro Models for Quantitative Prediction of Hepatobiliary Clearance

Kim L. R. Brouwer, PharmD, PhD





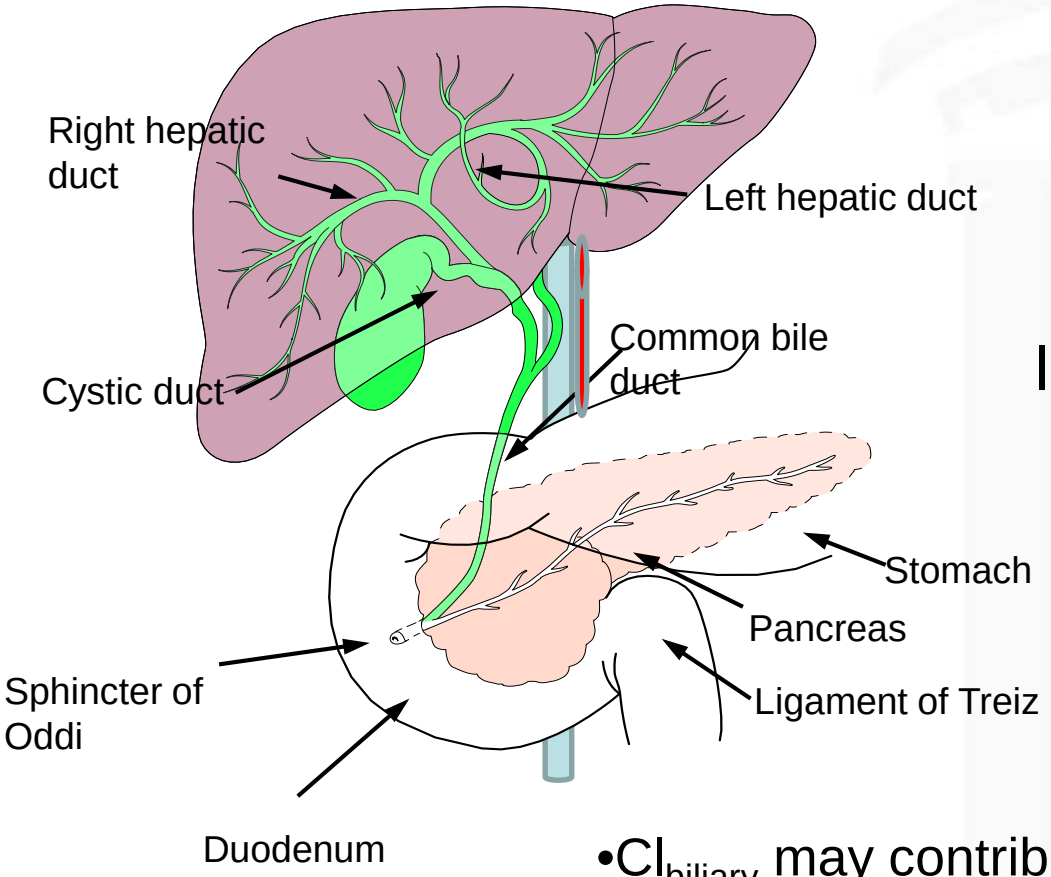
William R. Kenan, Jr., Distinguished Professor
Associate Dean for Research and Graduate Education
UNC Eshelman School of Pharmacy,
Curriculum in Toxicology,
The University of North Carolina at Chapel Hill

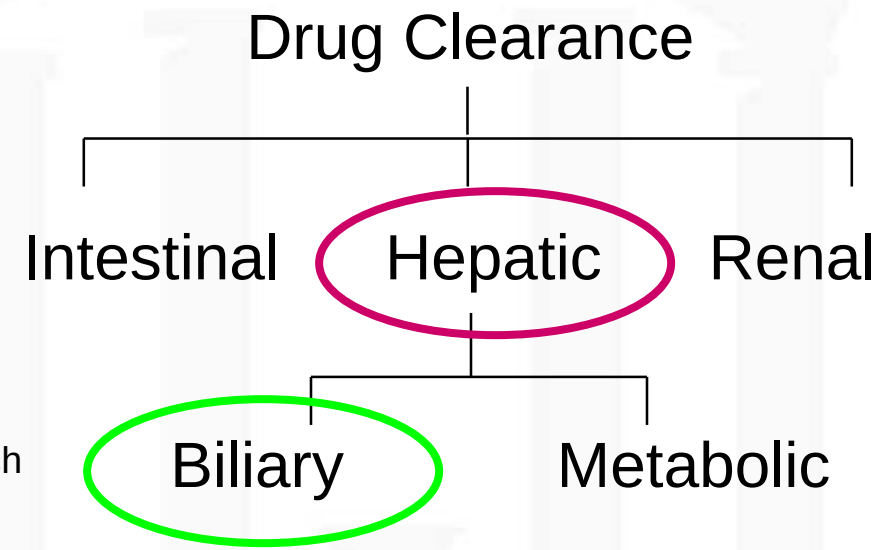


Conflict of Interest Statement

Dr. Brouwer is co-inventor of the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR®) and related technologies, which have been exclusively licensed to Qualyst Transporter Solutions.

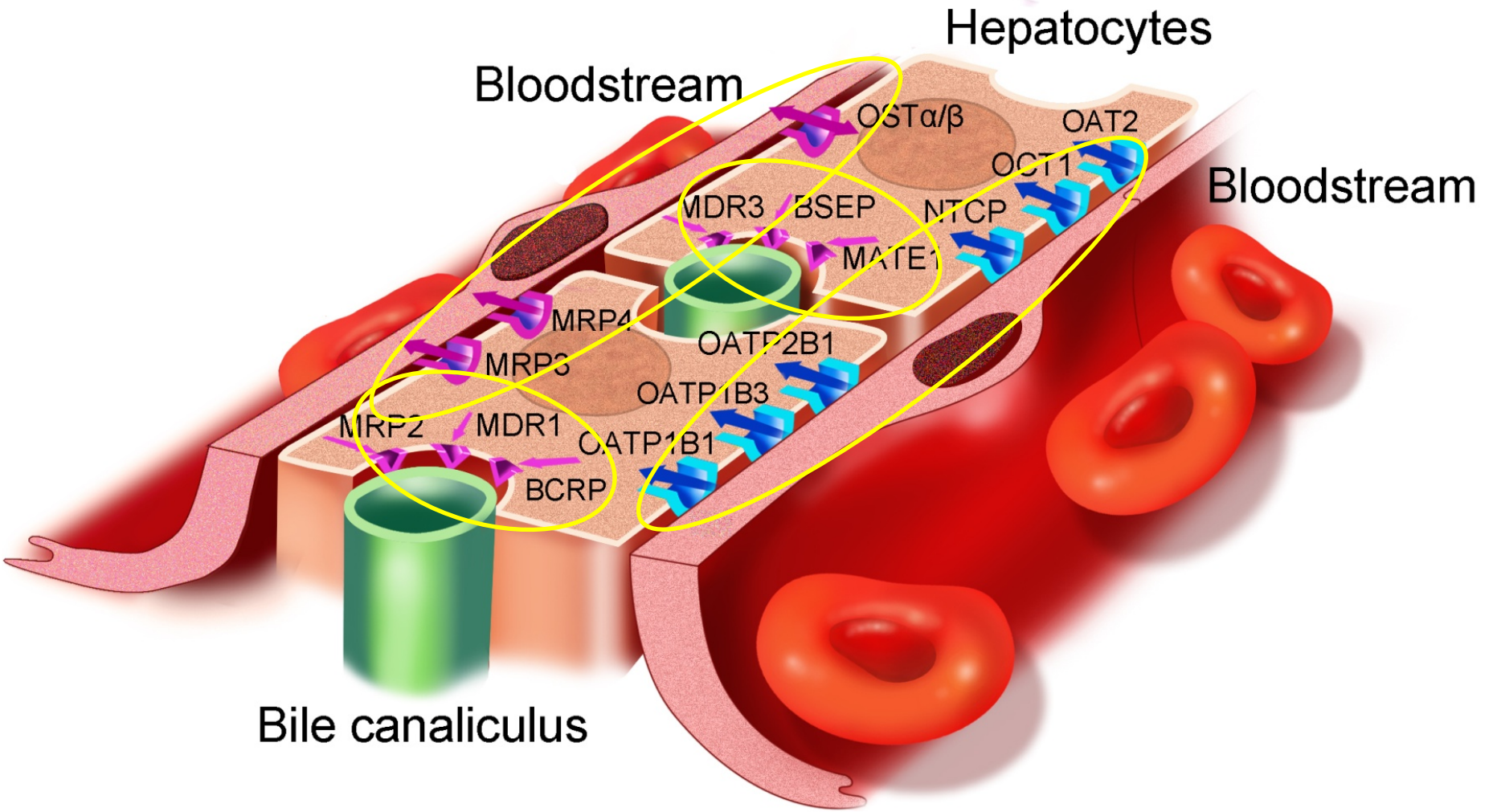
Hepatobiliary Clearance





- $Cl_{biliary}$ may contribute significantly to $Cl_{hepatic}$
- Altered $Cl_{biliary}$ due to genetics, disease states or drug interactions may affect pharmacological efficacy and/or toxicity (systemic, hepatic and/or intestinal)

Hepatic Uptake and Efflux Transporters



Köck and Brouwer, Clin Pharmacol Ther 92:599, 2012
(Adapted from Ho and Kim, Clin Pharmacol Ther, 78:260, 2005)

Selection of the appropriate model system depends on the question.
What parameter(s) do you need to predict?

- Hepatic uptake
- Hepatic clearance
- Biliary excretion (extent)
- Biliary clearance
- Hepatocyte accumulation
- Hepatotoxicity
- Hepatic transporter involvement and interactions

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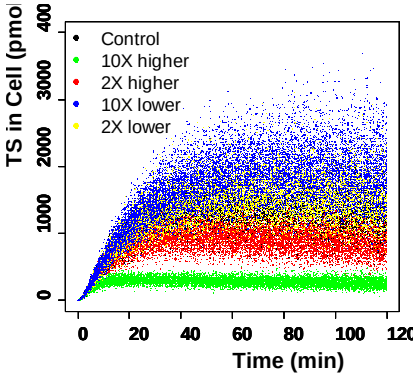
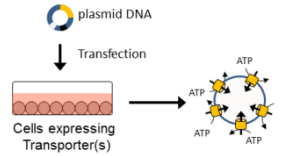
Modeling & Simulation



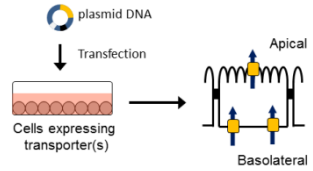
Throughput

Models

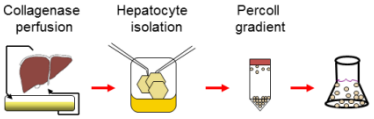
Membrane Vesicle System



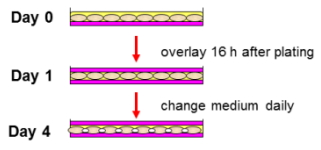
Transfected Cells



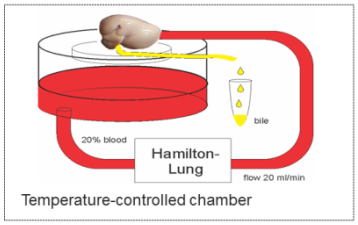
Suspended Hepatocytes



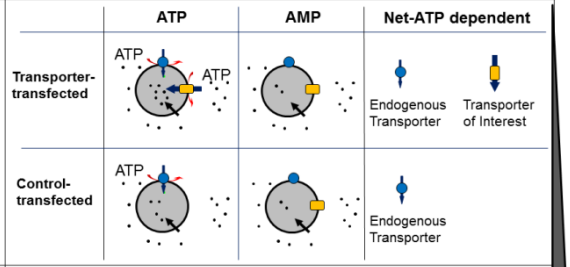
Sandwich-Cultured Hepatocytes

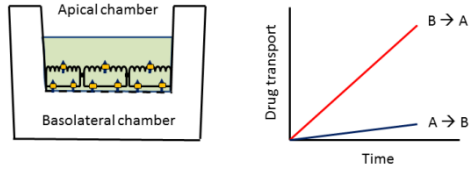


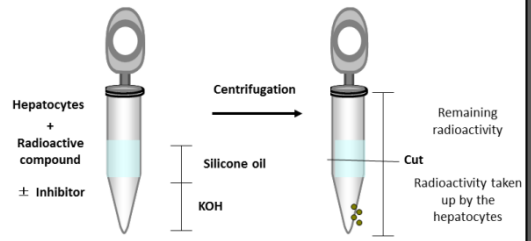
Isolated Perfused Liver

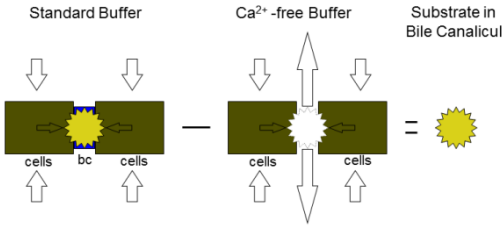


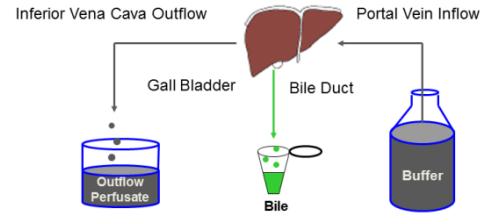
Methods



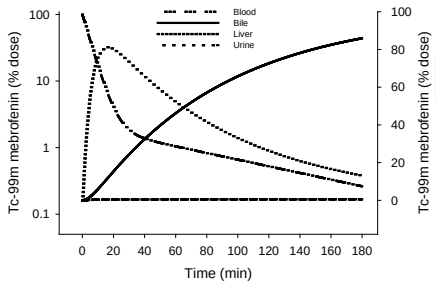




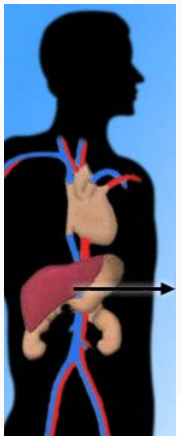




Physiologically relevant



In Vivo Imaging



Yang…Brouwer, *Transporters in Drug Development*; Chapter 9, Springer 2013

REVIEW

nature publishing group

In Vitro Methods to Support Transporter Evaluation in Drug Discovery and Development

KLR Brouwer[1], D Keppler[2], KA Hoffmaster[3], DAJ Bow[4], Y Cheng[5], Y Lai[5], JE Palm[6], B Stieger[7] and R Evers[8]; on behalf of the International Transporter Consortium

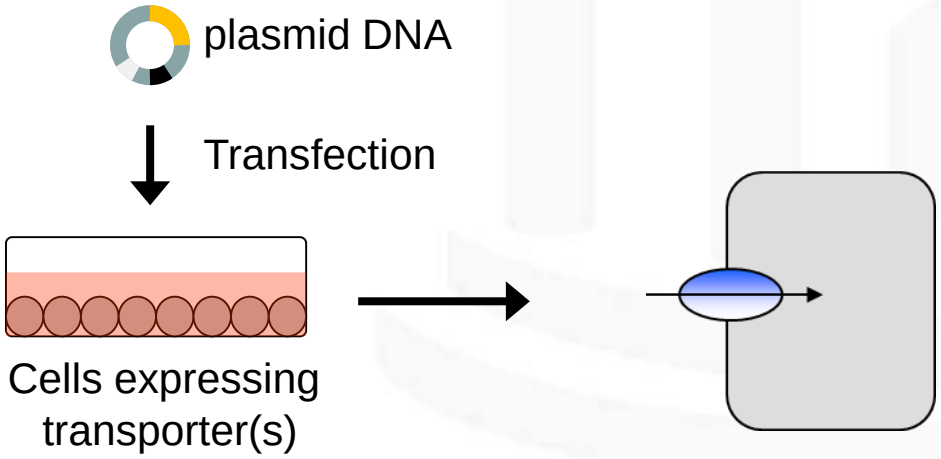
This white paper addresses current approaches and knowledge gaps concerning methods to assess the role of transport proteins in drug/metabolite disposition in humans. The discussion focuses on *in vitro* tools to address key questions in drug development, including vesicle- and cell-based systems. How these methods can be used to assess the liability of compounds for transporter-based drug–drug interactions (DDIs) *in vivo* is also explored. Existing challenges and approaches to examine the involvement of transporters in drug disposition are discussed.

- Overview of experimental systems currently employed to conduct in vitro transporter studies
- Applications, strengths and limitations of each system
- Issues concerning data interpretation
- Integration of *in vitro* transporter data to address important questions in drug discovery and development

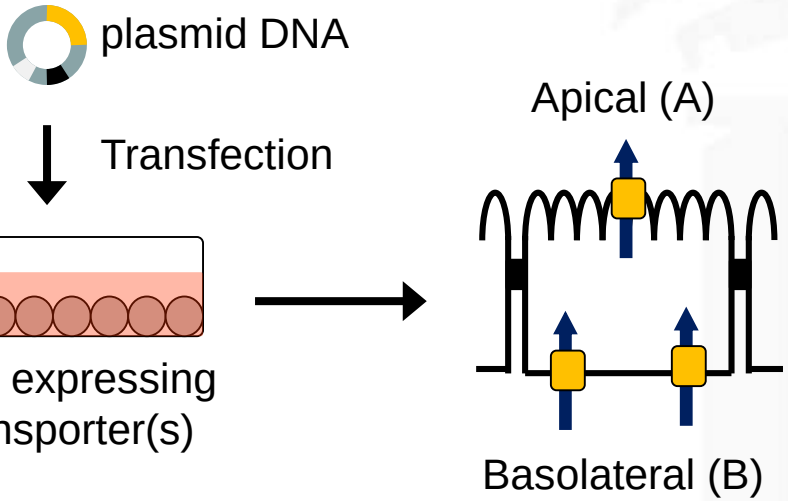
Brouwer *et al., Clin Pharmacol Ther*, **94**:95, 2013

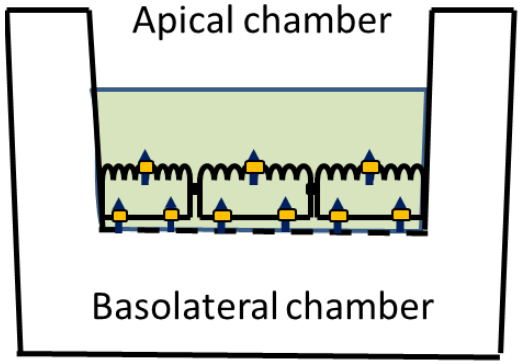
Recombinant Cell Lines Expressing Uptake Transporters

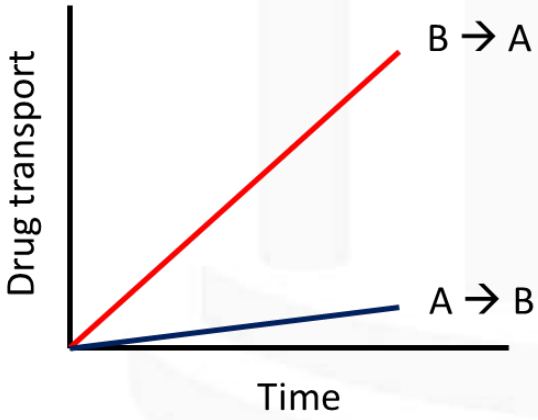
- **Applications**
  - Evaluate drug interactions with uptake transporters (i.e., OATPs, OCTs)
  - Determine substrate specificity and identify inhibitors
- **Requirements**
  - Low endogenous transport activity in cell line

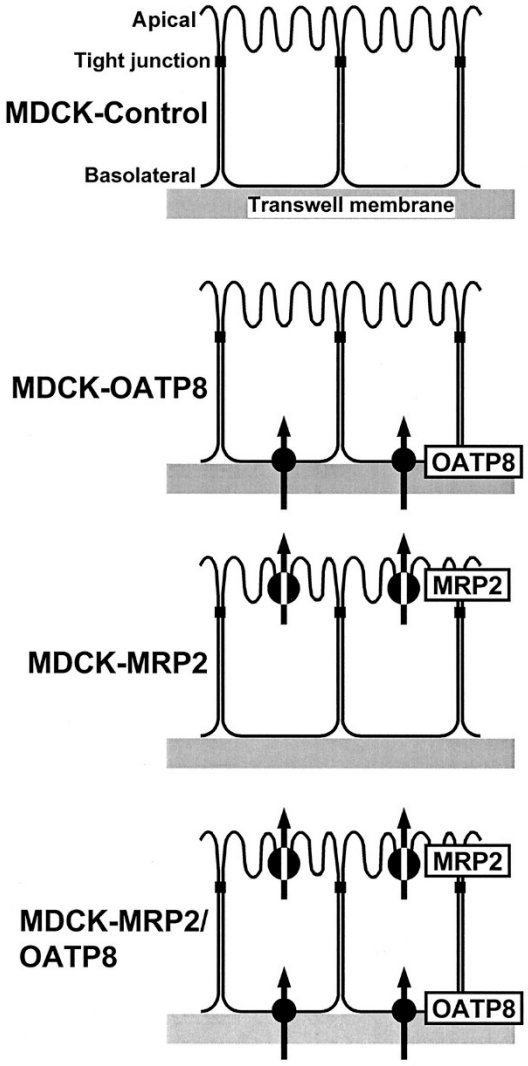


Bidirectional Transport in Recombinant Polarized Cell Monolayers









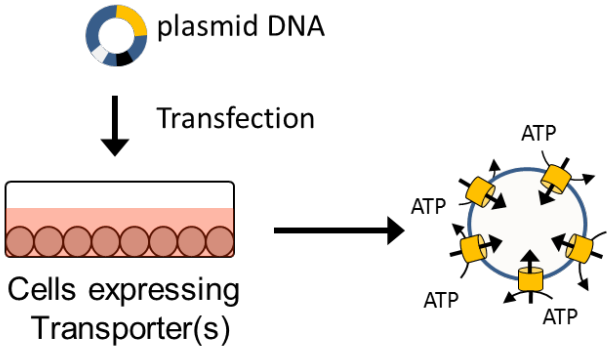
Cui *et al., Mol Pharmacol,* **60**:934, 2001

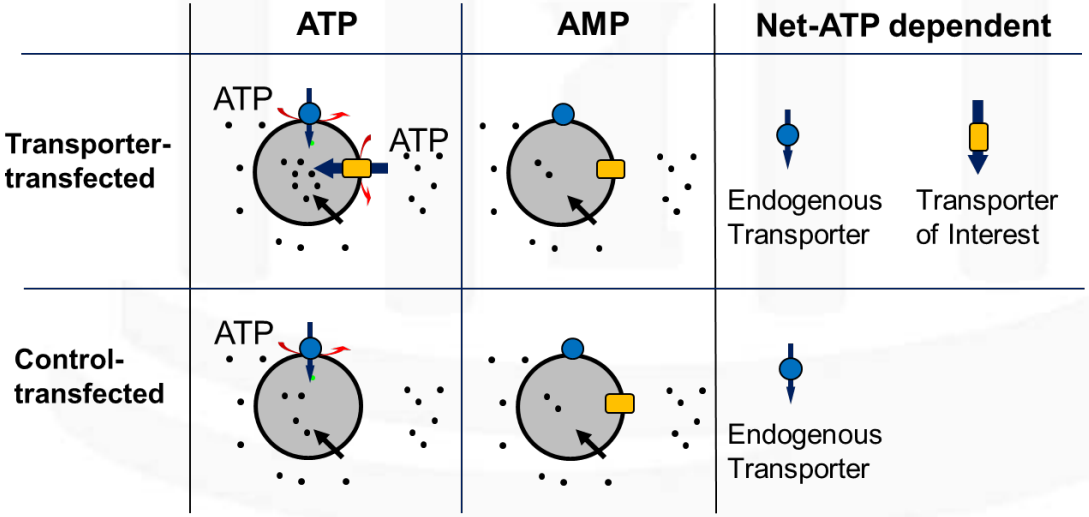
Bidirectional Transport in Recombinant Polarized Cell Monolayers

- **Strengths**
  - Transport is less influenced by nonspecific binding because only the compound crossing the cell monolayer is measured
  - Suitable to assess active transport vs. diffusion
  - Stably transfected cell lines can be passaged for multiple use or cryopreserved
- **Limitations**
  - Generation/characterization of stable recombinant cell lines is time consuming
  - Transporter expression levels vary between labs
  - Endogenous transporter activity may confound data interpretation
  - Mass balance needs to be assessed
  - Complicated kinetic studies

Membrane Vesicle-Based Transporter Assays for Efflux Transporters

- Inside-out oriented membrane vesicles prepared from:
  - Baculovirus-infected insect (Sf9 or Sf21) cells
  - Drug-selected cell lines
  - Stably or transiently transfected cell lines (HeLa, HEK293, MDCK)
  - Purified basolateral or apical plasma membranes from organ of interest





Membrane Vesicle-Based Transporter Assays

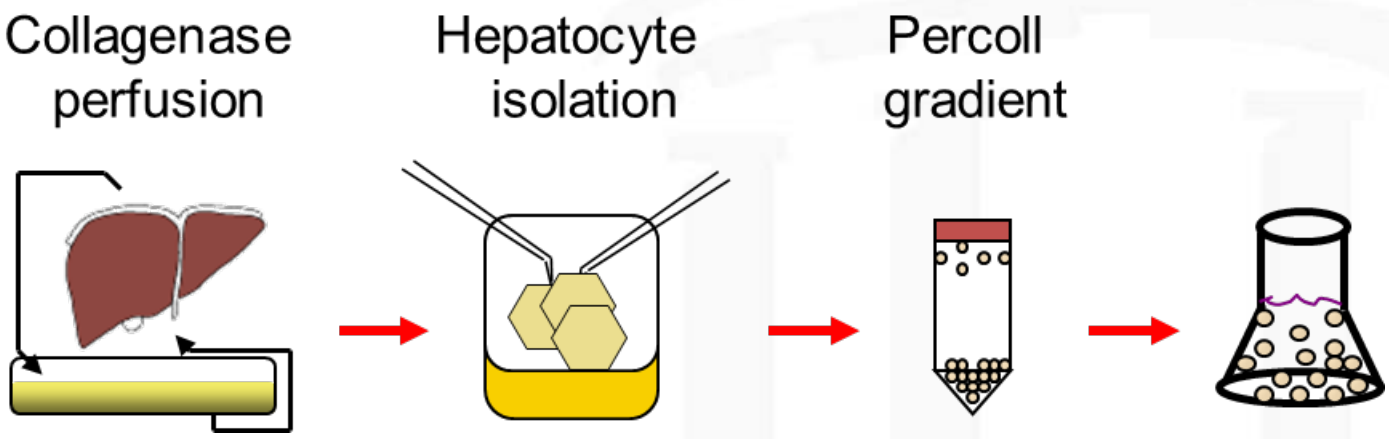
- **Strengths**
  - Good for compounds with low permeability
  - Cytotoxic compounds don't impact experimental system
  - High transporter expression levels in recombinant systems
  - Large batches can be prepared and cryopreserved
  - Able to preload with buffers and/or substances
  - Substrates in incubation buffer have direct access to active site
- **Limitations**
  - Not suitable for compounds with high permeability or high nonspecific binding
  - Hypoglycosylation in insect cells (e.g., Sf9) may alter transport
  - Endogenous transport activity in expression system may confound data interpretation
  - Transporter activity varies from batch to batch

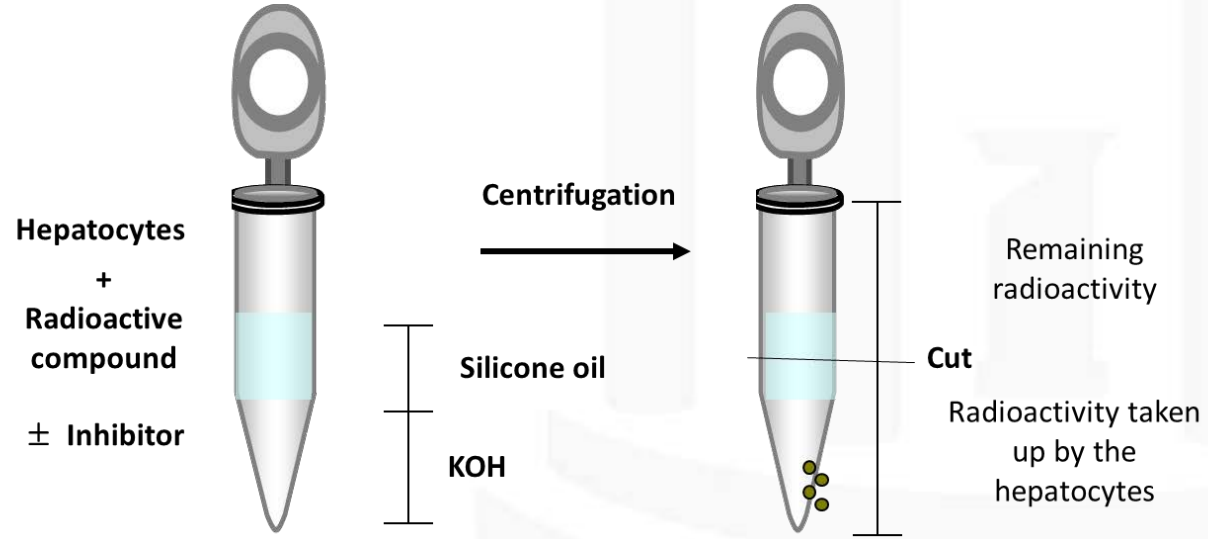
Summary

In Vitro Assays: Non-Hepatocyte Systems

- **Strengths**
  - High-throughput
  - Readily available
  - Common approach to determine whether a substrate ***can*** be transported by a specific protein
- **Limitations**
  - May not accurately predict which proteins predominantly transport substrates *in vivo* in the hepatocyte
  - Transport proteins are not expressed at physiologically relevant levels – IVIVE is challenging
  - Not a holistic system with all relevant transporters, endogenous compounds, regulatory machinery, metabolic capacity, intracellular compartments and binding proteins
  - Limited ability to assess complex drug-transport interactions (e.g., non-competitive mechanisms, metabolite interactions)

Suspended Hepatocytes



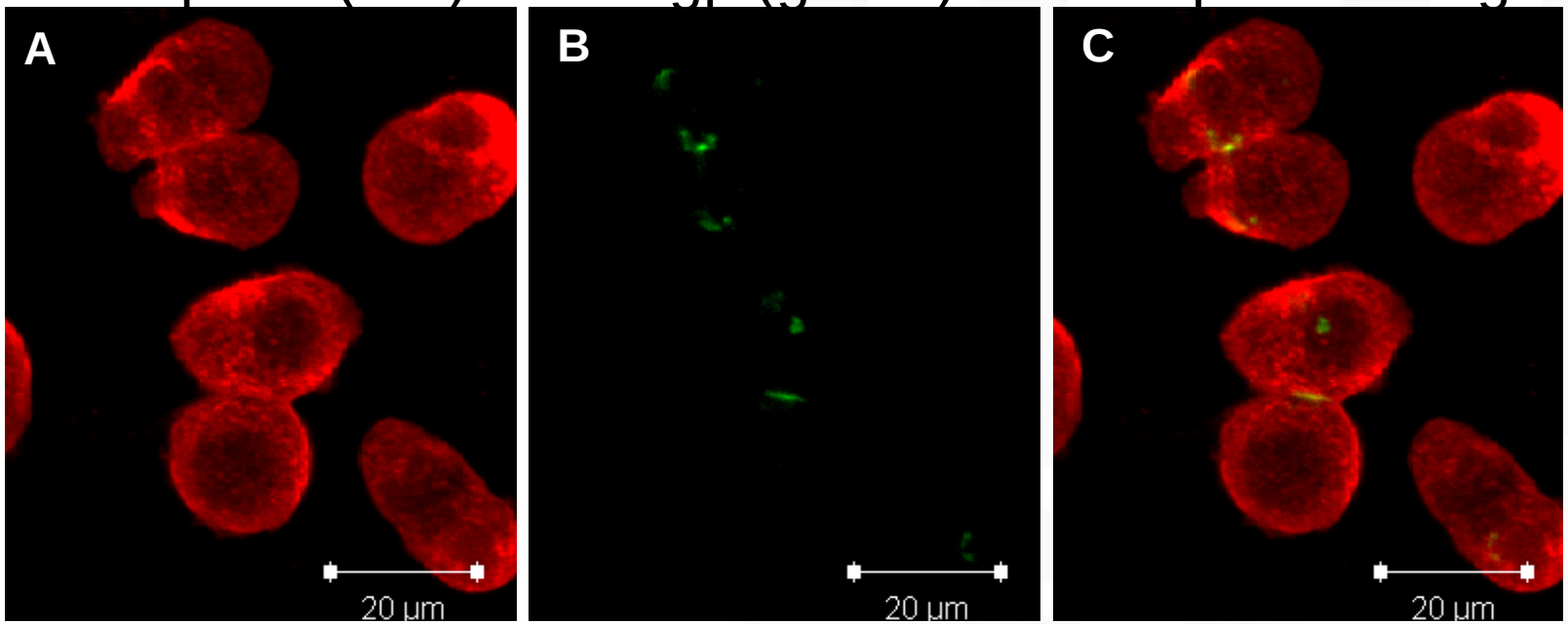


Suspended Hepatocytes

- Suitable for measuring ***initial*** hepatic uptake and transporters involved in initial substrate uptake
- ***Not*** suitable for measuring biliary clearance
  - Canalicular proteins are not properly localized

Immunohistochemical localization of P-gp (C219) and Oatp1a1 in freshly isolated rat hepatocytes. Single frames from 3D reconstruction of z-stack (1 µm slices)

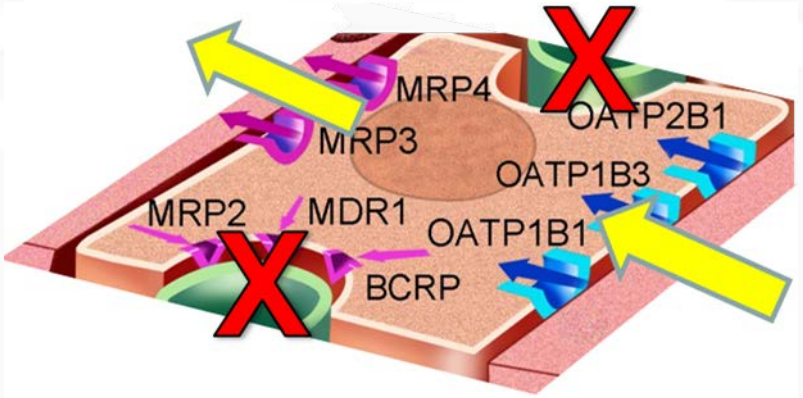
Oatp1a1 (red) P-gp (green) Composite image



Bow…Brouwer, *Drug Metab Dispos,* **36**:198, 2008

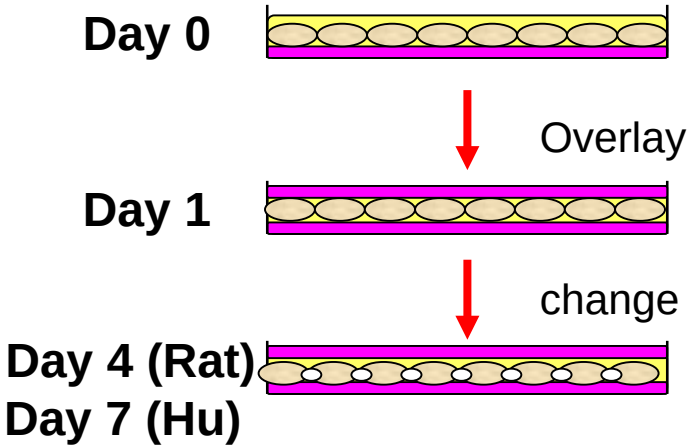
Suspended or Plated Hepatocytes

- **Strengths**
  - Cryopreserved or freshly isolated hepatocytes from the species of interest
  - Human hepatocytes can be pooled to minimize interindividual variability



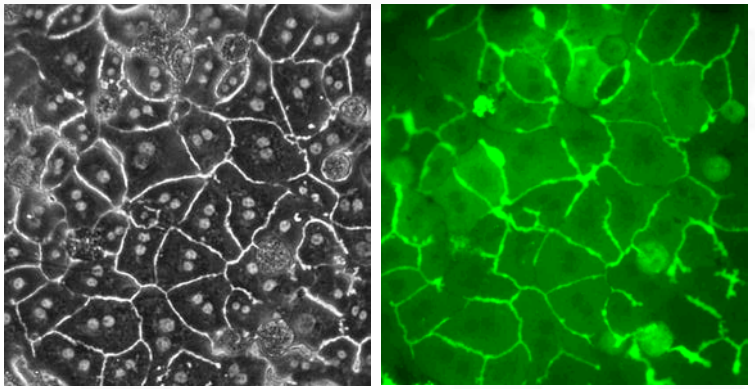
  - Expression of uptake transporters is relatively close to *in vivo*
  - Allows assessment of contribution of multiple hepatic uptake transporters simultaneously
  - Allows assessment of active uptake vs. passive diffusion
- **Limitations**
  - Loss of cell polarity
  - Minimal functional activity of canalicular efflux transporters; basolateral efflux transporters are functional
  - Rapid loss of metabolic activity in culture

Sandwich-Cultured Hepatocytes

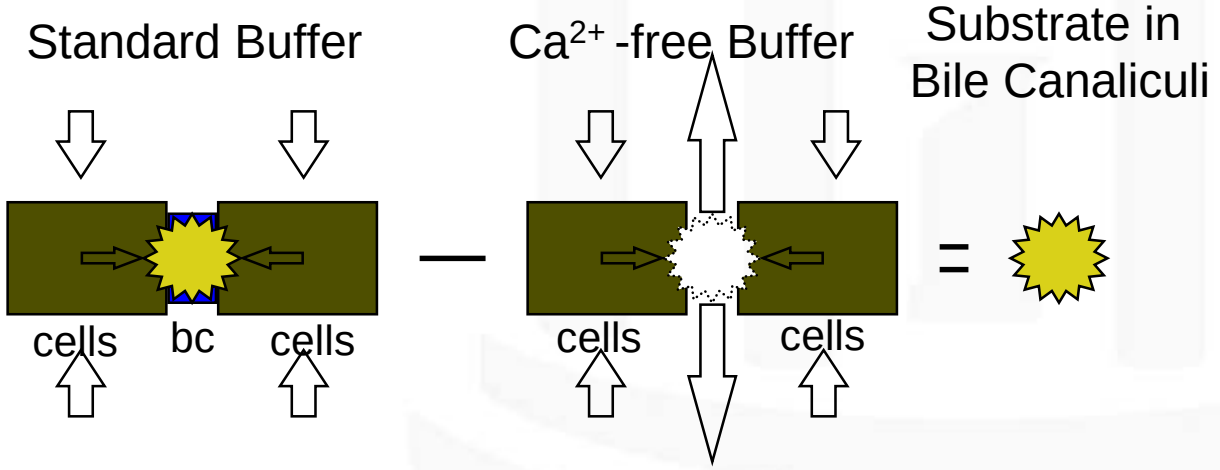


Morphology

Function:
CDF Localization



Swift *et al. Drug Metab Rev* **42**:446, 2010
CDF: 5-(6)-carboxy-2',7'-dichlorofluorescein



B-CLEAR® technology is covered by US Pat. No. 6,780,580 and other US and International patents, both issued and pending, and is exclusively licensed to Qualyst Transporter Solutions

Experimental Parameters:

Biliary Excretion Index (%)

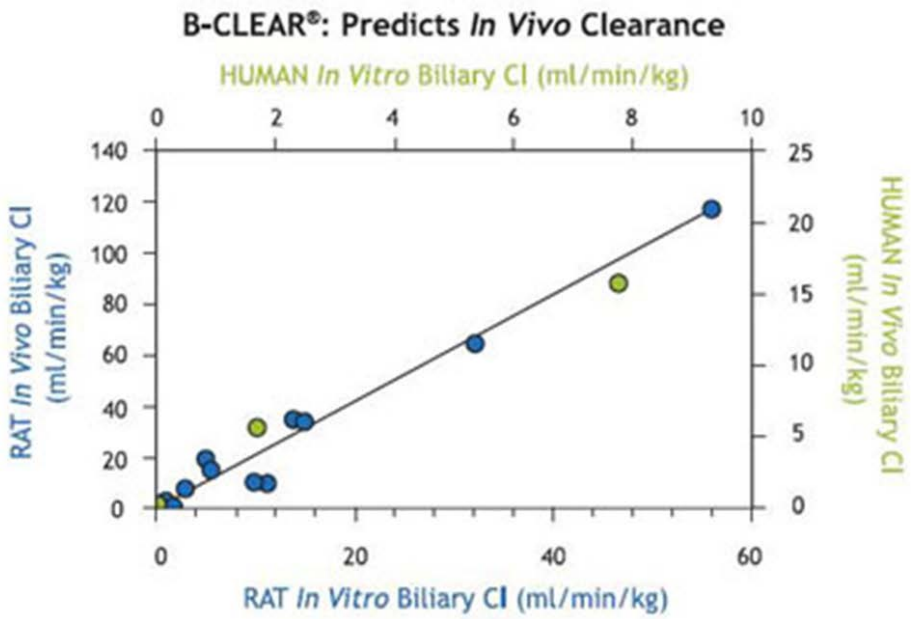
$$= \frac{\text{Total Accumulation} - \text{Cellular Accumulation}}{\text{Total Accumulation}} \times 100$$

In Vitro Biliary Clearance

$$= \frac{\text{Total Accumulation} - \text{Cellular Accumulation}}{\text{AUC}_{\text{media}}}$$

Intracellular Concentration

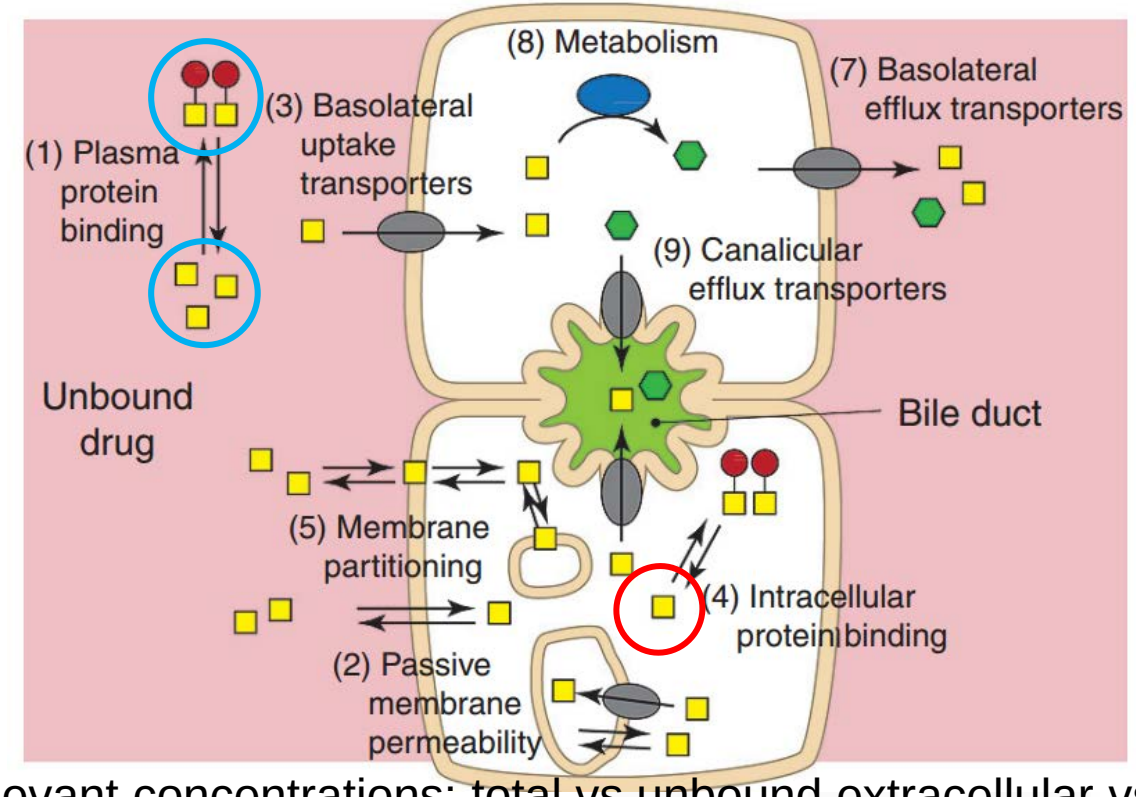
$$= \frac{\text{Cellular Accumulation}}{\text{Volume}_{\text{intracellular}}}$$



Sandwich-Cultured Hepatocytes

- **Strengths**
  - Normal cell polarity re-established
  - Biliary clearance can be measured
  - Holistic system expressing uptake and efflux transporters, metabolic enzymes and regulatory machinery
  - Cryopreserved or freshly isolated hepatocytes from the species of interest can be used
  - Suitable to identify transport inhibitors (both competitive and non-competitive) and inducers
  - Demonstrated *in vitro-in vivo* correlations between preclinical species and humans
- **Limitations**
  - Requires time in culture for proper localization of transporters
  - Less suitable for low-clearance compounds
  - Enzyme/transporter expression/activity may be modulated by culture conditions

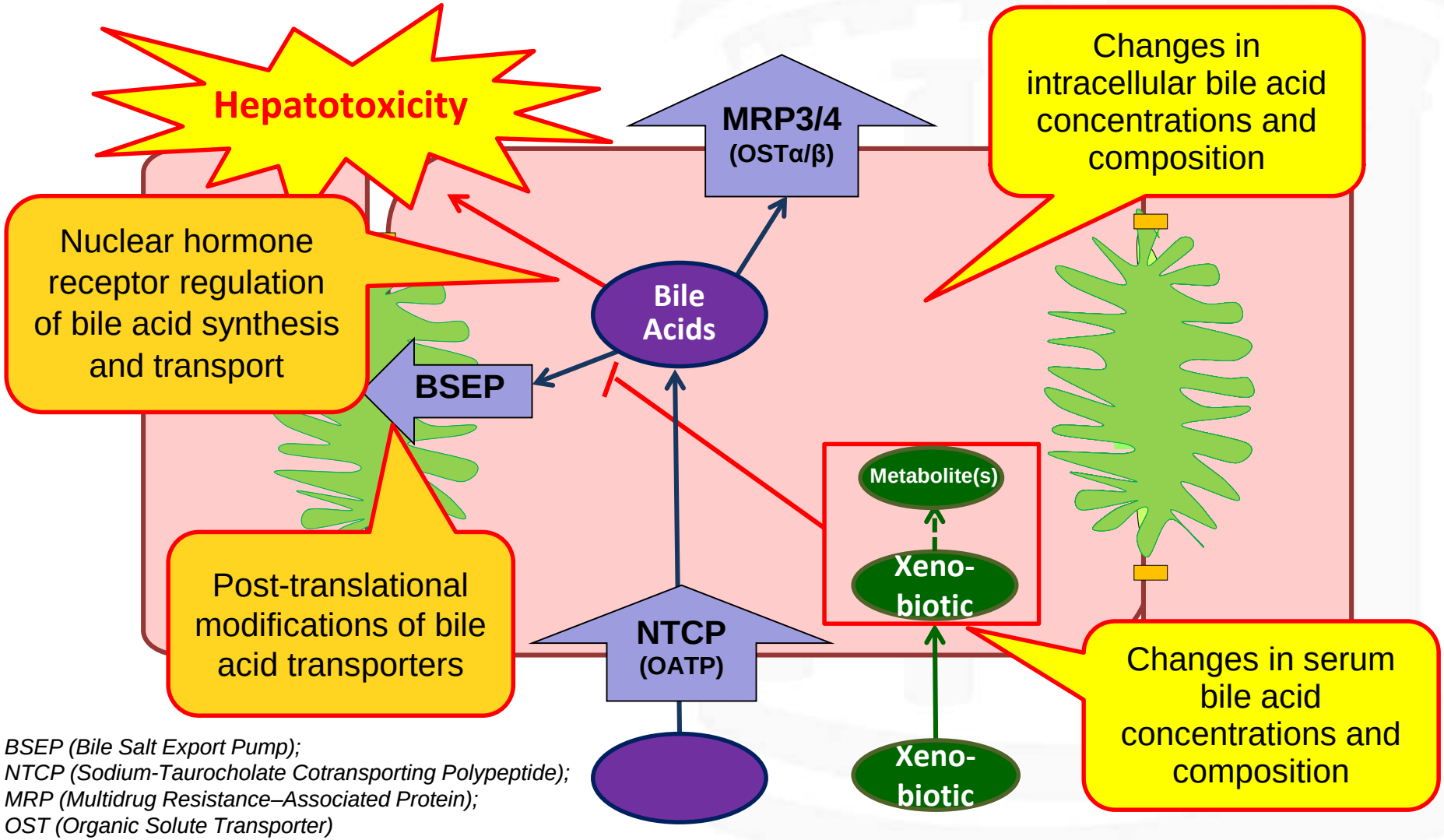
What is the Relevant Concentration for Accurate Risk Assessment?



- Relevant concentrations: total vs unbound extracellular vs intracellular?
- Should protein be included in the incubation medium?
- How do we extrapolate IC_{50} data from *in vitro* systems (e.g., vesicle-based transporter assays) to *in vivo*?

Chu…Brouwer, *Clin Pharmacol Ther*, **94**:126, 2013

Mechanisms of Hepatotoxicity: Direct and Indirect Interactions with Bile Acids

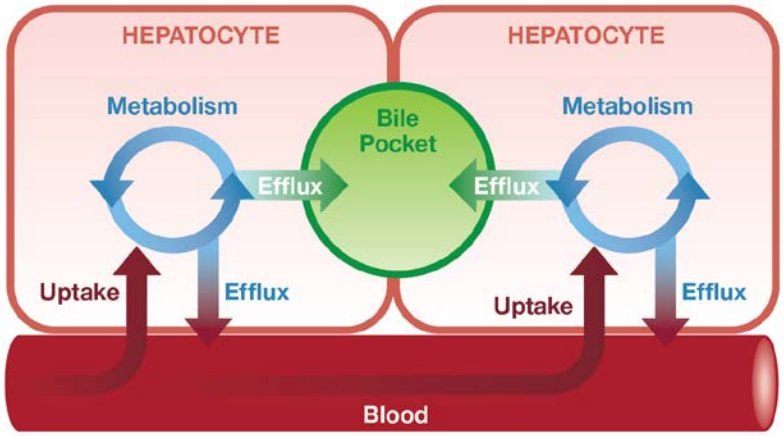


BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)

Essential Components of a Predictive Hepatic Model for Risk Assessment:

- ✓ **Uptake**
  - Sinusoidal uptake transport proteins
- ✓ **Efflux**
  - Biliary and/or basolateral efflux transport proteins
- ✓ **Metabolism**
  - Metabolic enzymes for elimination
- ✓ **Regulation**
  - Induction of transport and metabolism

The intracellular concentration determined in sandwich-cultured hepatocytes is a function of all of these processes



Generation of *in vivo* relevant intracellular concentrations using B-CLEAR® hepatocytes

Courtesy of Qualyst Transporter Solutions

Recommendations

- Numerous *in vitro* assays are available to predict hepatic uptake and the involvement of transporters in hepatobiliary clearance
- Assays must be selected based on the compound characteristics and the question(s) that need to be answered; assay limitations always must be considered
- Sandwich-cultured hepatocytes are the only *in vitro* model that can accurately predict biliary clearance for IVIVE
- Additional research is needed to characterize the assay conditions (*e.g.,* protein, bile acids, other media additives) and concentration(s) (*e.g.,* unbound, total) that are most predictive for IVIVE, cellular regulatory mechanisms that impact hepatobiliary clearance, and whether more complex models are able to improve prediction accuracy

Acknowledgments

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Kyunghee Yang

THE UNIVERSITY
of NORTH CAROLINA
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Kathleen Köck, PhD