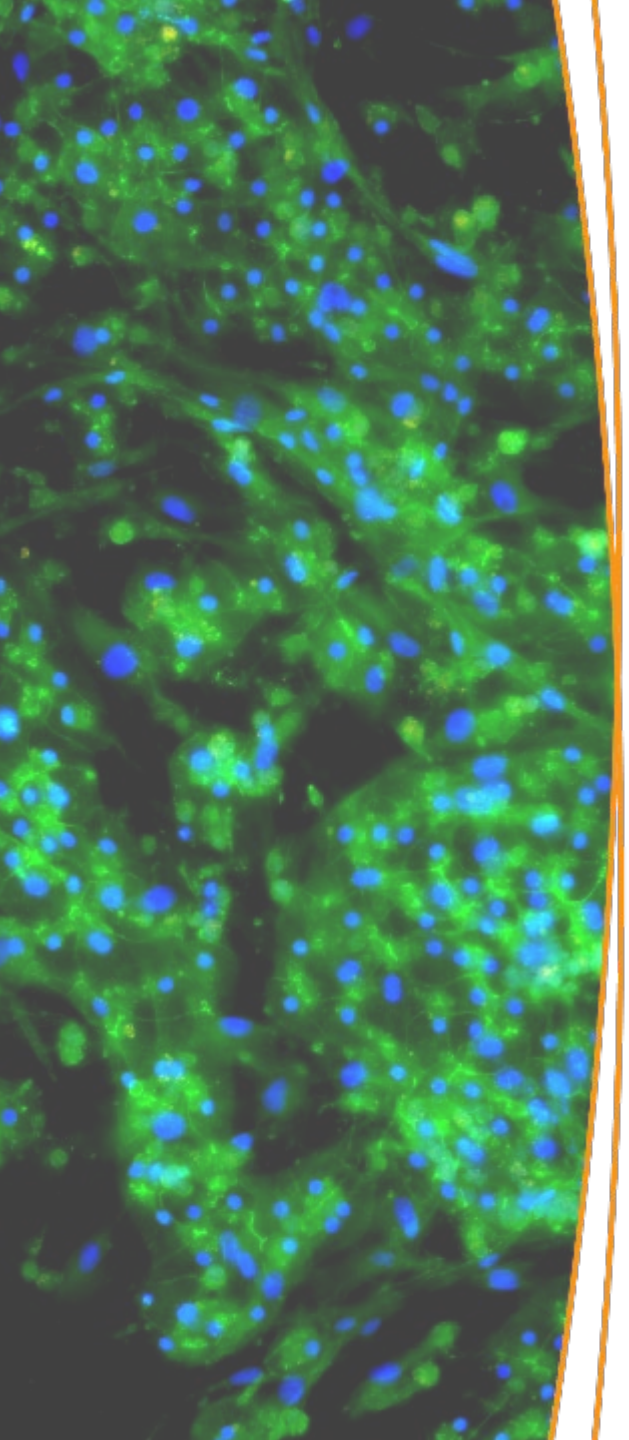

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IONTOX By LifeNet Health LifeSciences

DEVELOPING AN IN VITRO INTEGRATED ORGAN MODEL FOR PHARMACOKINETIC AND ADME PREDICTIONS

Presentation Topics

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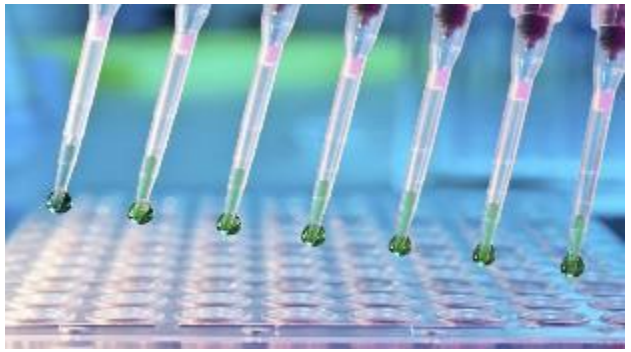
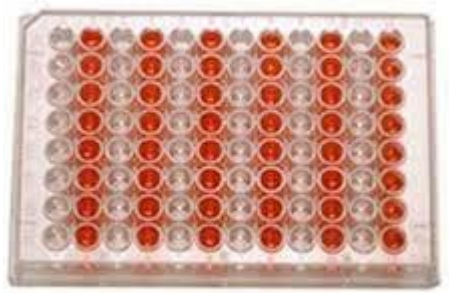
- Introduction
- Importance of both Technology & Biology
- Need for Integrated (MPS) Organ Models
- Proof-of-Concept Data Sets
- Collaborative Research Project with FDA
- Case-Study

Goal: Improve In Vitro to In Vivo Extrapolation



In Vitro Toolbox



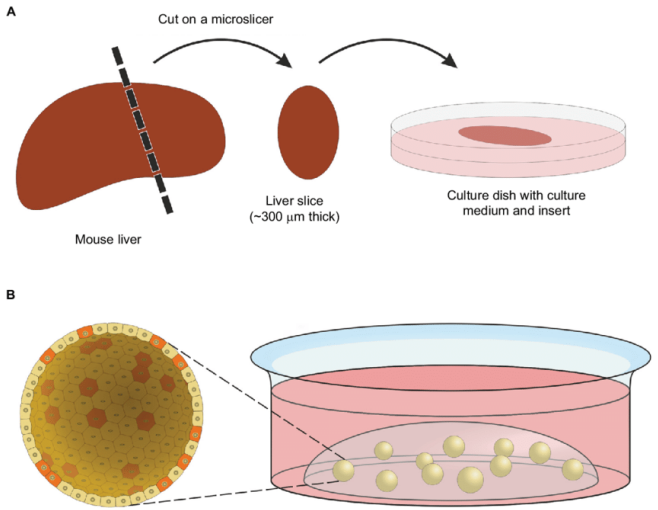
Routes of Exposure
- Intestine
- Skin
- Lung

Systemic Exposure
- Liver
- Kidney
- Heart
- Brain

Organ Integration
- Blood

- Optimization
- Relevant Endpoints (AOPs)
- New Biomarkers

- Establishing Dose Response
- Data interpretation: Models
- Validation
- Integrated Organ Platforms



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Choosing the Right In Vitro System

- Test platform selection
  - Many different technologies from which to choose
  - Every system has strengths and weaknesses
  - Select the best system to answer your primary question
- Tissue and Cell Quality
  - Need highest quality tissue or cells
  - Should mimic in vivo organs as closely as possible
- Moving toward Human relevant data
  - Predict human ADME and safety

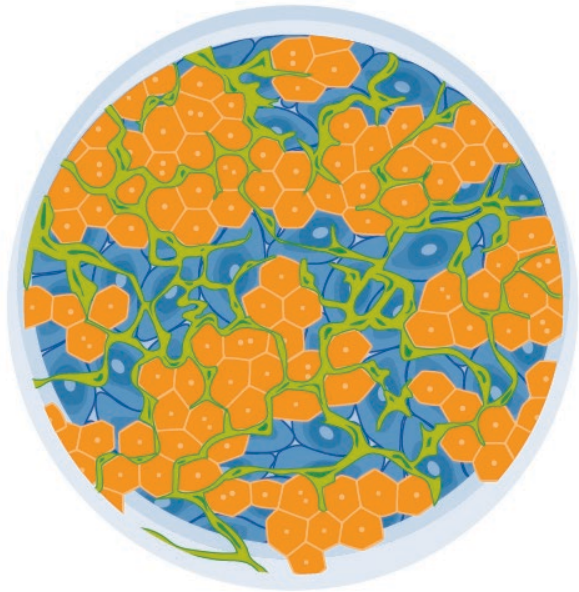
Criteria for Selecting a Cell Model

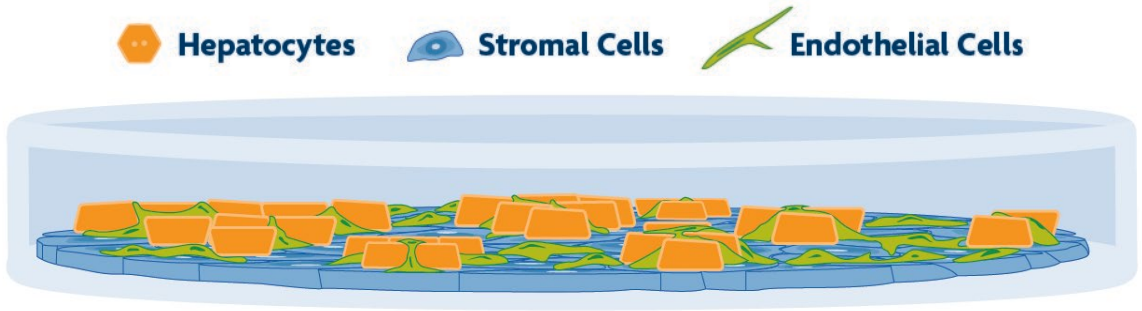
- Liver as an example
- Well characterized
  - Plateable, good morphology, longevity in culture
  - Key metabolic functions
    - CYP activity and inducibility
    - Transporter polarization/function
    - Liver metabolic function (albumin, urea)
  - Donor Information
    - Basic history
    - Genotyping
  - Large donor pools (500-1000 vials)
  - Consistent performance

All-Human Hepatic Triculture System

- All human-derived cells
  - Feeder Cells are human, not rodent
  - Hepatocytes and feeder cells are primary human cells

- Self-assembled organization
- Native cell-cell interactions
- Stable morphology & hepatic function
- Sustained metabolic activity





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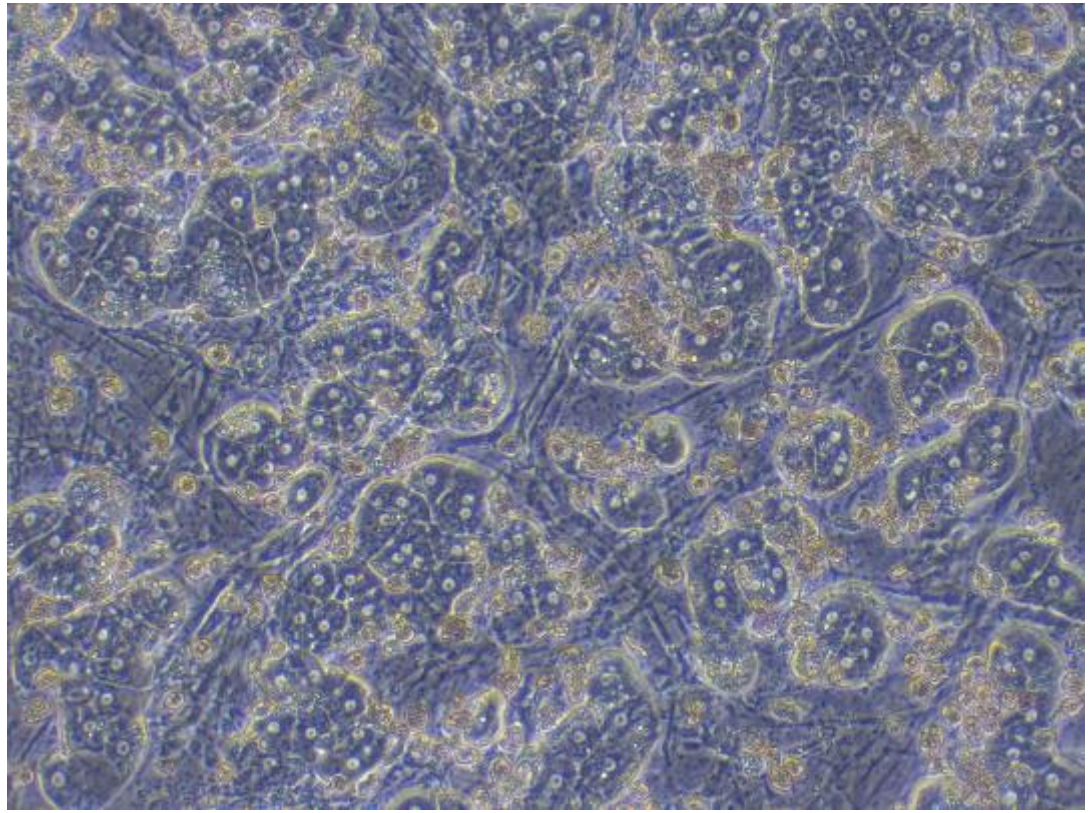
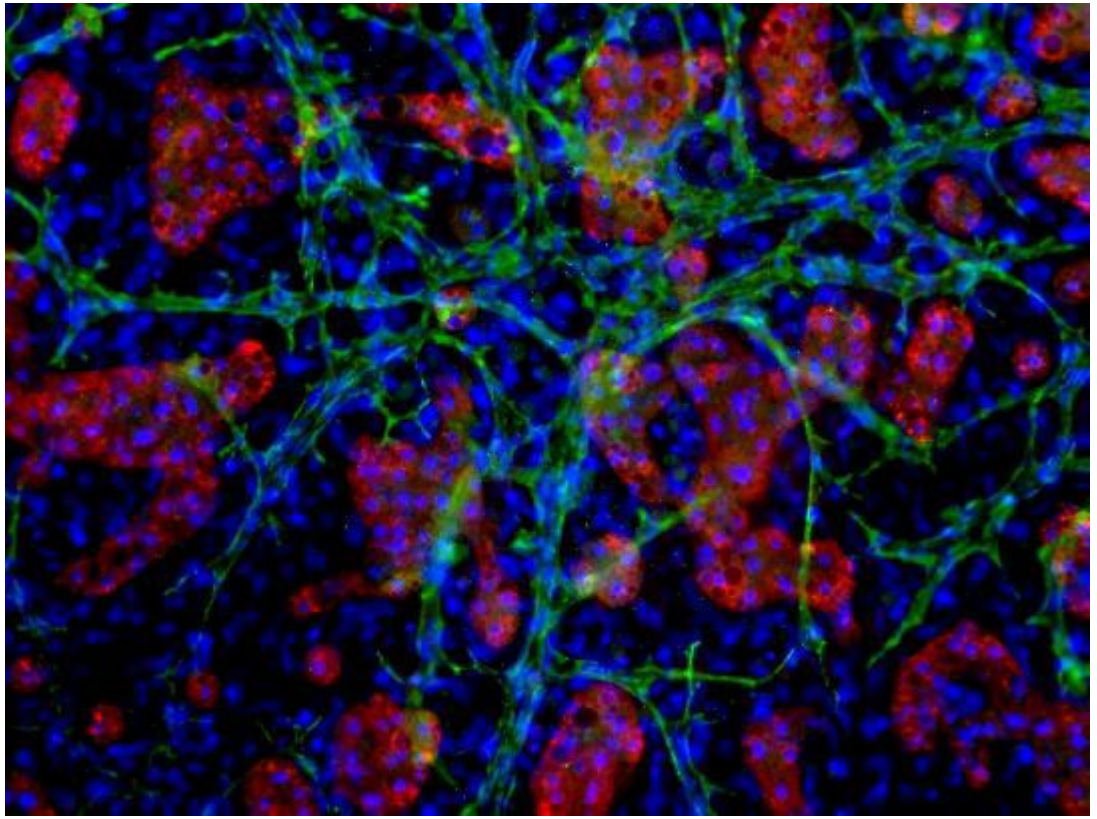
All-Human Hepatic Triculture System at 28 Days





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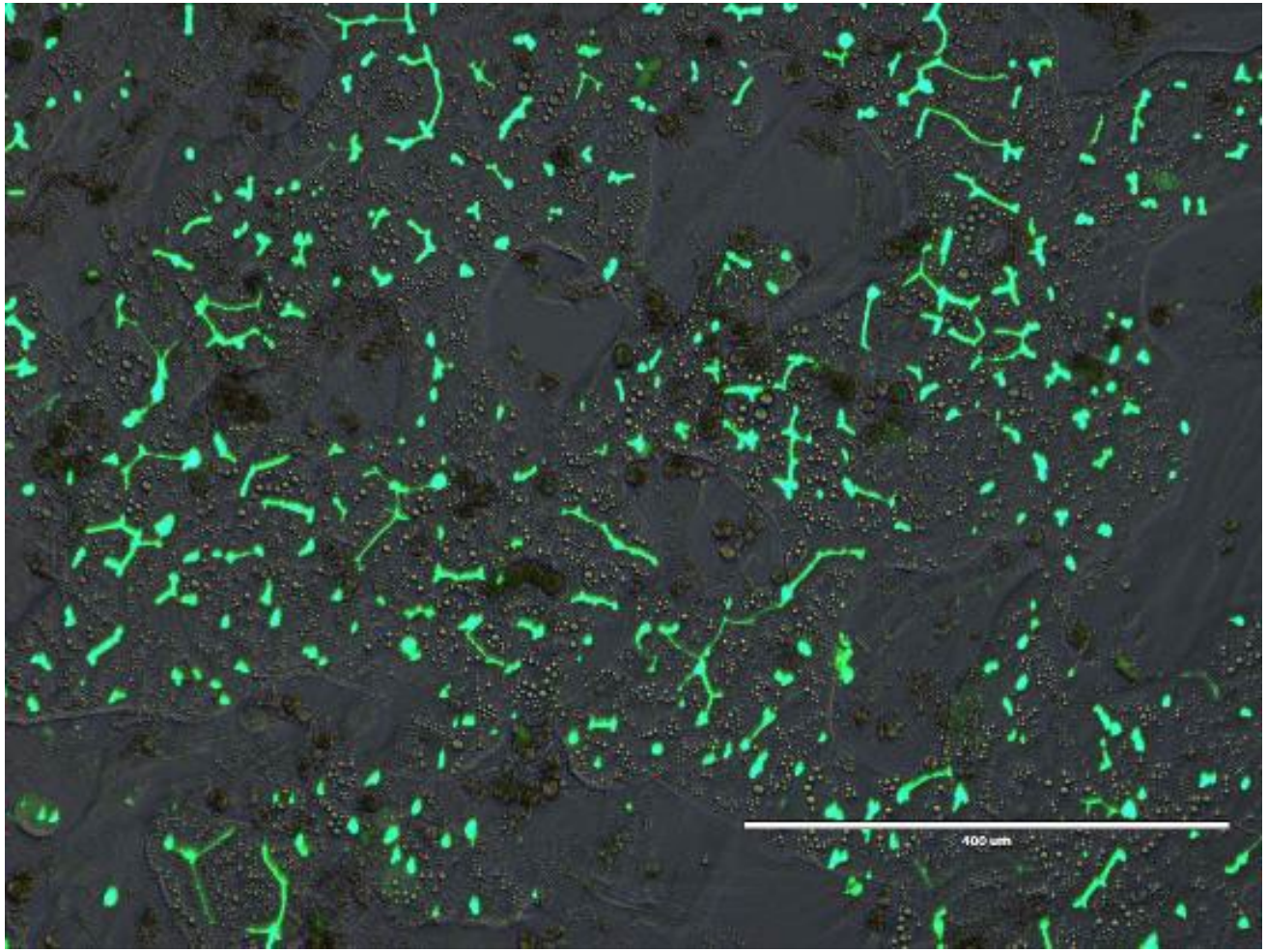
All-Human Hepatic Triculture System Tight Junction Formation and Functional Bile Canaliculi



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All-Human Hepatic Triculture System

Partners Boehringer Ingelheim CORTEVA™

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Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

The morphology, functionality, and longevity of a novel all human hepatic cell-based tri-culture system

Jessica R. Weaver [a], Justin J. Odanga [a], Kristina K. Wolf [b], Stephanie Piekos [c], Mercedes Biven [d], Mitchell Taub [c], Jessica LaRocca [d], Cody Thomas [c], Alexander Byer-Alcorace [c], Jingsong Chen [a], Jung Bok Lee [a,*], Edward L. LeCluyse [b,*]

[a] *Institute of Regenerative Medicine, LifeNet Health, 1864 Concert Drive, Virginia Beach, VA 23453, United States of America*
[b] *Research and Development, LifeSciences, LifeNet Health, 6 Davis Drive, Research Triangle Park, NC 27709, United States of America*
[c] *Non-Clinical DMPK, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, CT 06877, United States of America*
[d] *Corteva AgriScience, 9330 Zionsville Road, Indianapolis, IN 46268, United States of America*

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Characterization of Clearance Mechanisms in an All-Human Cell Based Tri-Culture System

Stephanie Piekos[1], Jessica R. Weaver[2], Cody Thomas[1], Alexander Byer-Alcorace[1], Justin J. Odanga[2], Kristina K. Wolf[3], Jingsong Chen[2], Jung Bok Lee[2], Edward L. LeCluyse[3], and Mitchell E. Taub[1]
[1]Non-clinical DMPK, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT
[2]Institute of Regenerative Medicine, LifeNet Health, Virginia Beach, VA
[3]Research and Development, LifeSciences Division, LifeNet Health, Research Triangle Park, NC

ASSESSING THE *IN VITRO IN VIVO* CORRELATION OF SMALL MOLECULE METABOLISM IN THREE LONG-TERM PRIMARY HUMAN HEPATOCYTE CULTURE MODELS

Hlaing H. Maw, Ting Wang, Klairynne Raymond, Alexander Byer-Alcorace, Stephanie Piekos, Tom S. Chan, and Mitchell E. Taub
Boehringer Ingelheim Pharmaceuticals Inc., Drug Metabolism and Pharmacokinetics Department, Ridgefield, CT, USA

CHARACTERIZATION OF MORPHOLOGY, LONGEVITY AND FUNCTIONALITY IN AN ALL-HUMAN CELL BASED TRI-CULTURE SYSTEM

Jessica R. Weaver[1], Justin J. Odanga[1], Kristina K. Wolf[2], Tammy Stone[2], Stephanie Piekos[3], Mitchell Taub[3], Cody Thomas[3], Alexander Byer-Alcorace[3], Jingsong Chen[1], Jung Bok Lee[1], and Edward L. LeCluyse[2]
[1]Institute of Regenerative Medicine, LifeNet Health; [2]Research & Development, LifeSciences, LifeNet Health; [3]Non-Clinical DMPK, Boehringer Ingelheim Pharmaceuticals, Inc

AN IN VITRO TRI-CULTURE SYSTEM TO ASSESS COMPOUND-INDUCED HEPATIC CLEARANCE OF THYROXINE IN HUMANS

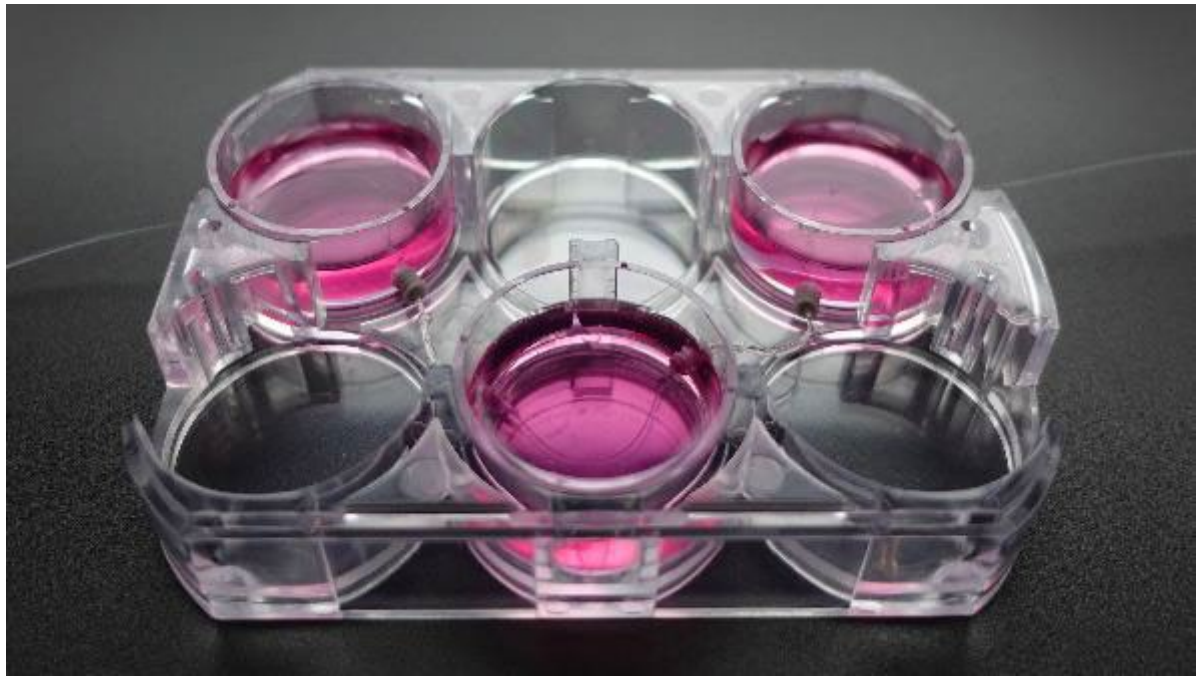
Kristina K. Wolf[1], Tammy Stone[1], Mercedes Biven[2], Margaret McIntyre[1], Bethany Hannas[2], Jessica LaRocca[2], Edward L. LeCluyse[1]
[1]LifeNet Health LifeSciences, Research Triangle Park, NC; [2]Corteva Agriscience, Indianapolis, IN

Why Do We Need Integrated Organ Models?

- To study relevant routes of exposure and subsequent organ delivery
- To study the effects of multiple organs on the test chemical
- To evaluate movement across multiple biological barriers
- Develop pharmacokinetic data and estimate key parameters (e.g., AUC)
- Understand repeated dosing in a dynamic model
- IVIVE – Provide in vitro prediction of chemical behavior in humans
- Provide Human Risk Assessment Data

Key Platform Properties

- Adaptability
- Able to incorporate many tissue or cell models
- Plastics must have low non-specific binding
- Simulated blood flow
- Isolated organ compartments (communication via blood)
- Fluid volumes and tissue mass that allows multiple time point sampling



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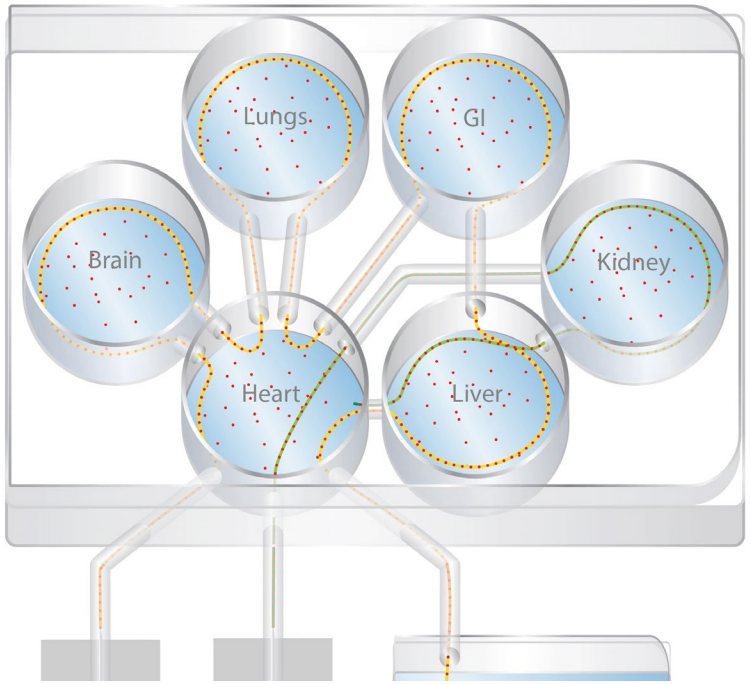
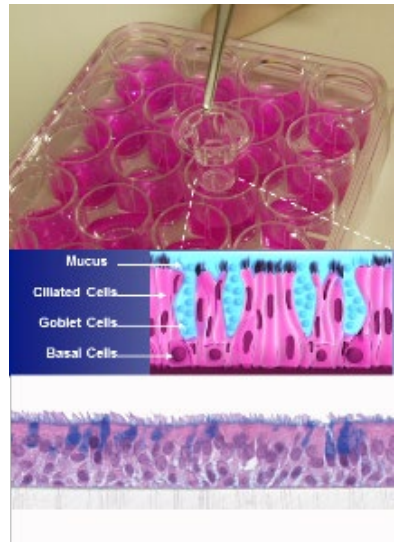
Human Dynamic Multiple Organ Plate





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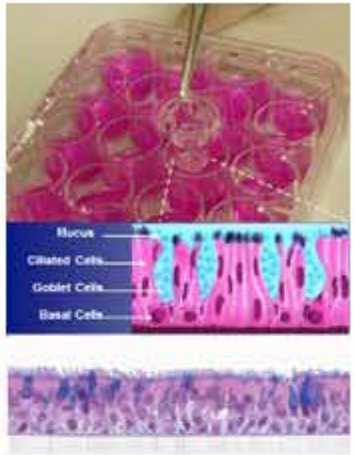
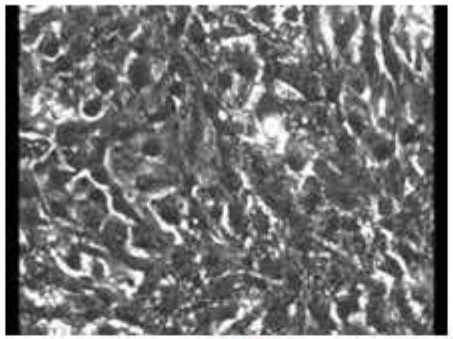
Human Dynamic Multiple Organ Plate

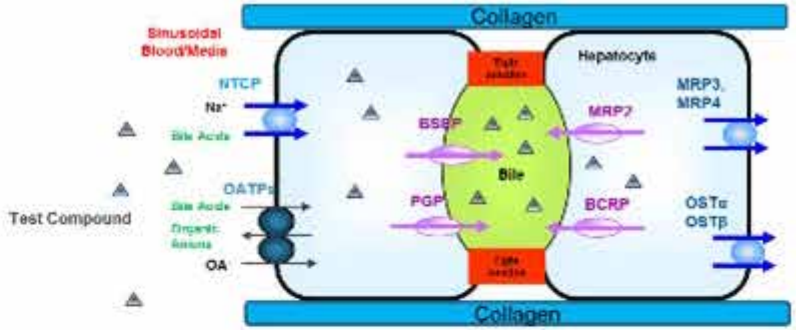
MucilAir™



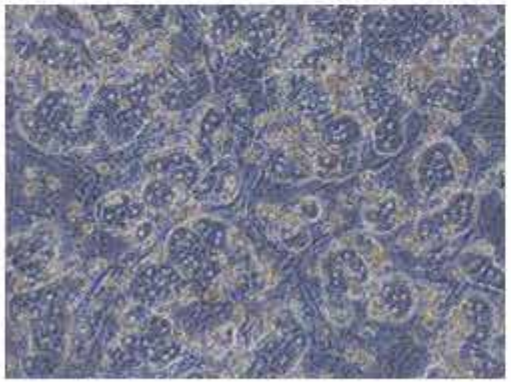
CDI iCell



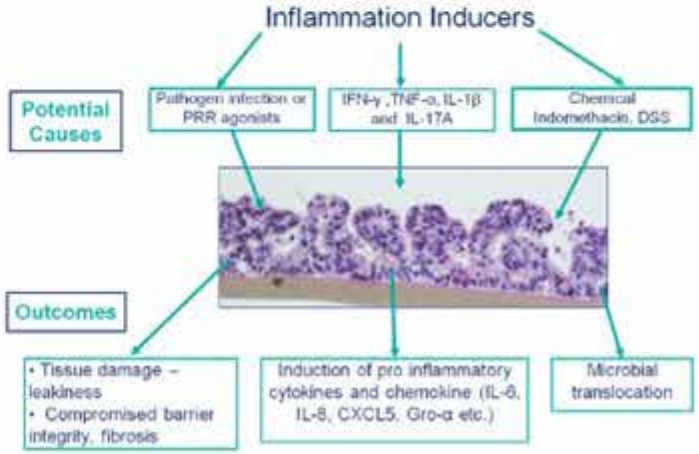
Qualyst Sandwich Cultured Hepatocytes



LifeNet Health Triculture System



MatTek Epi-Intestinal



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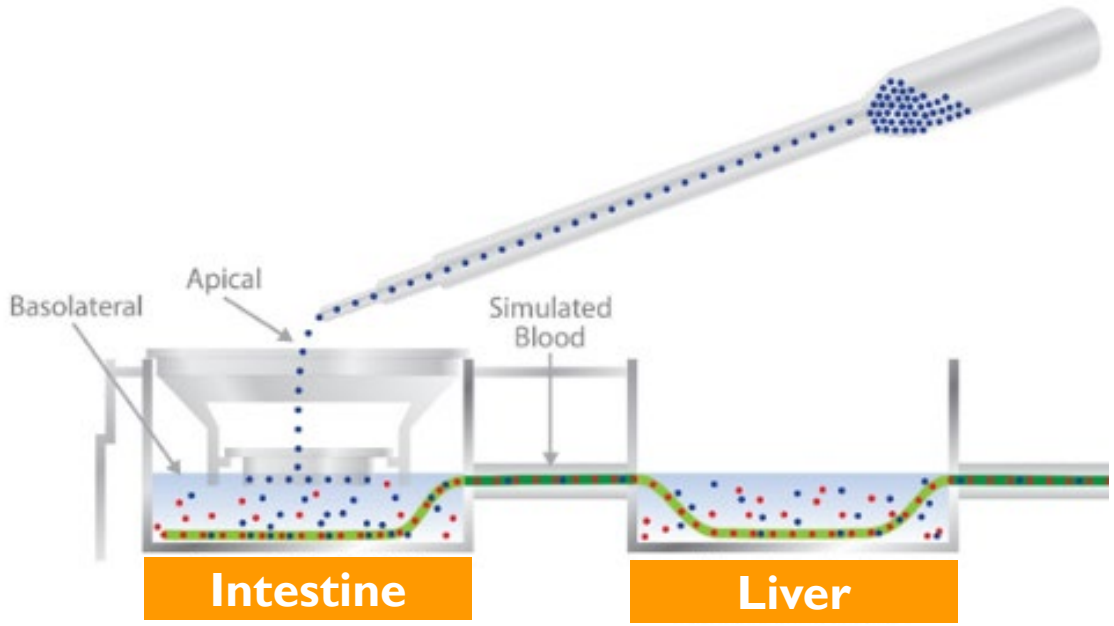
Study Process: Movement, Metabolism, and Toxicity of a Test Material

- Two organ System
- Non-specific binding
- Cytotoxicity range finder single chamber
- Confirm metabolites
- Development of LC/MS/MS methods
- Dose selection for full system



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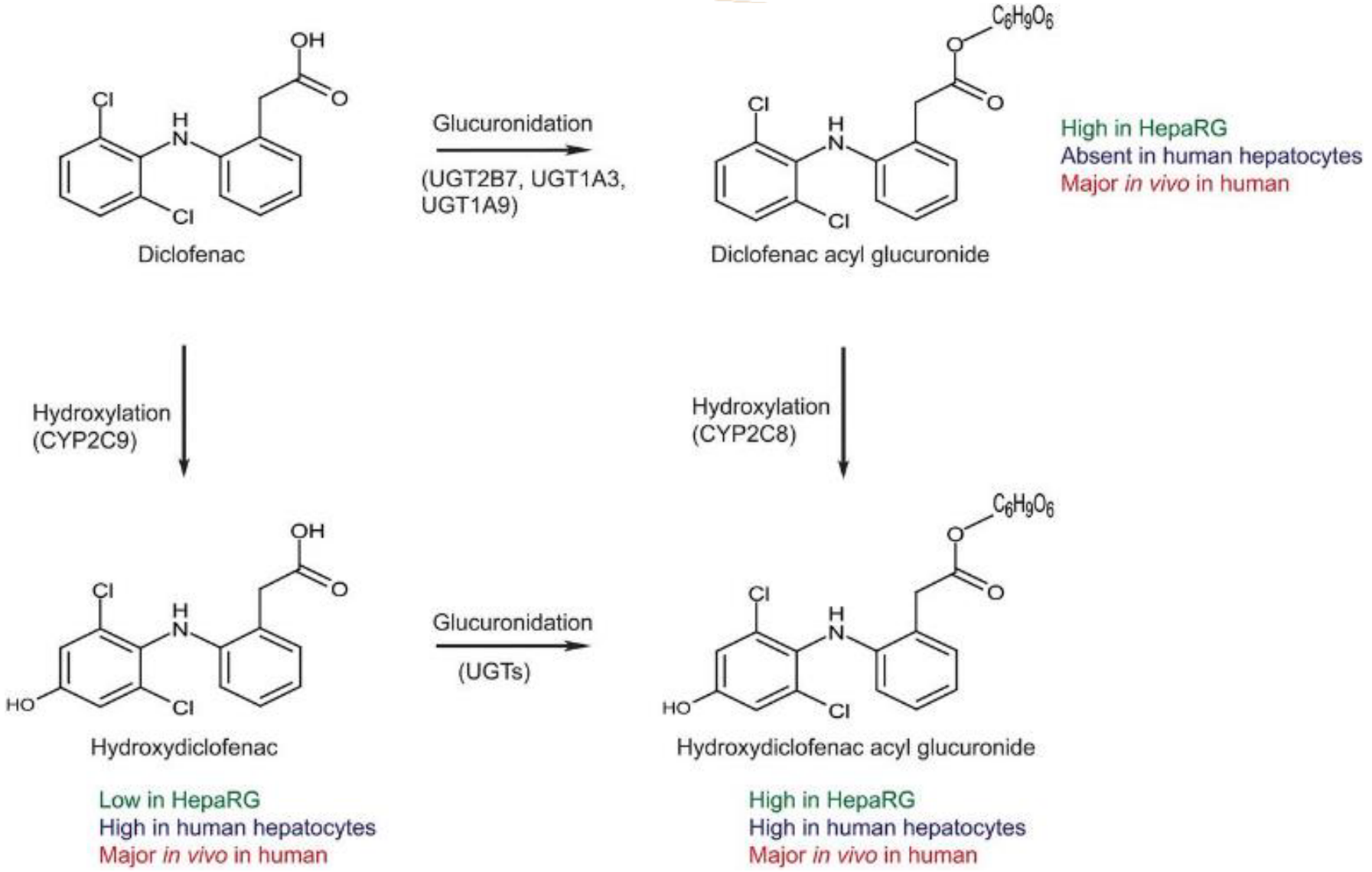
Metabolism of Diclofenac



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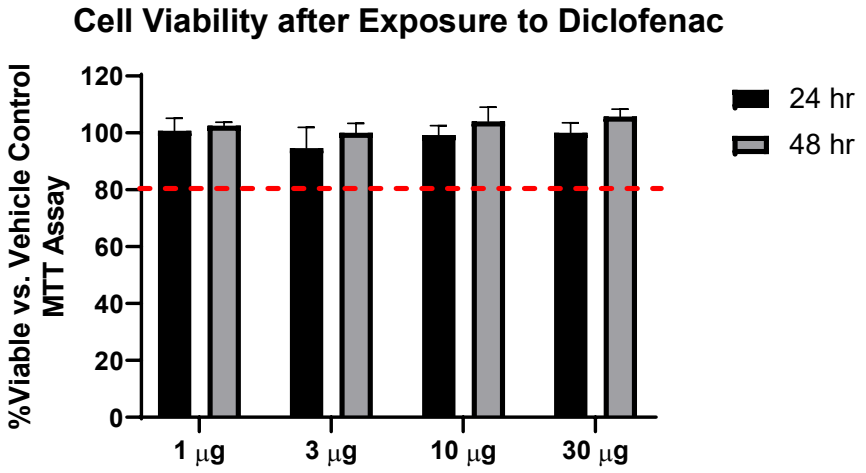
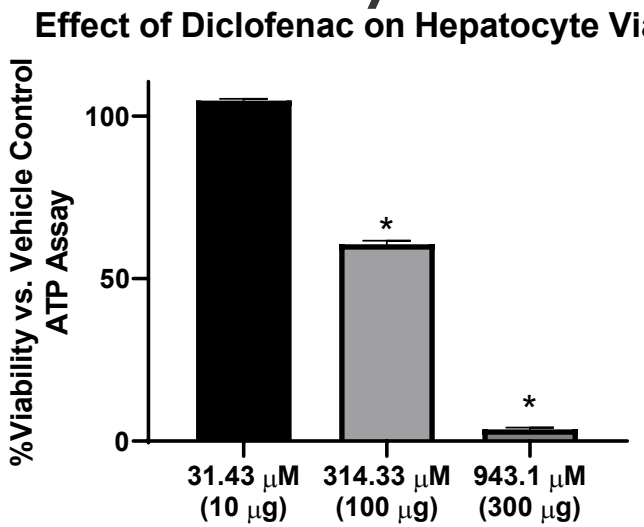
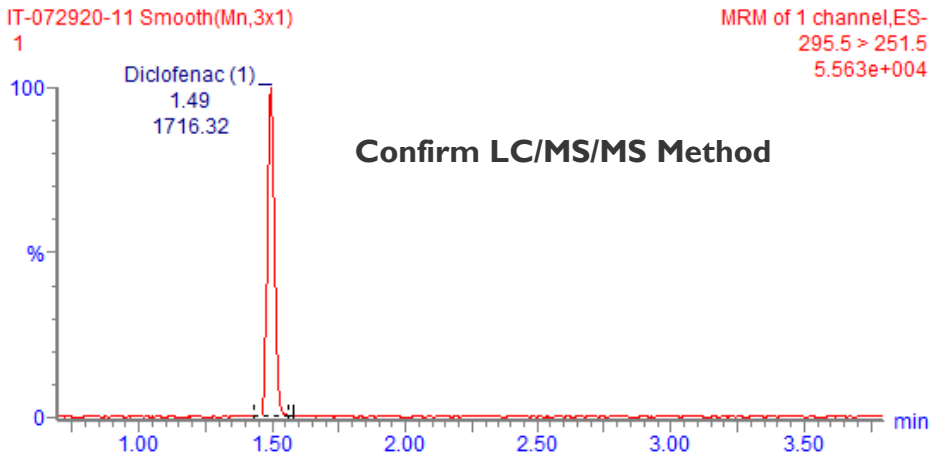
Evaluation of Cytotoxicity in Individual Organ Chambers

Skin Toxicity



Hepatocyte Toxicity





Confirm LC/MS/MS Method

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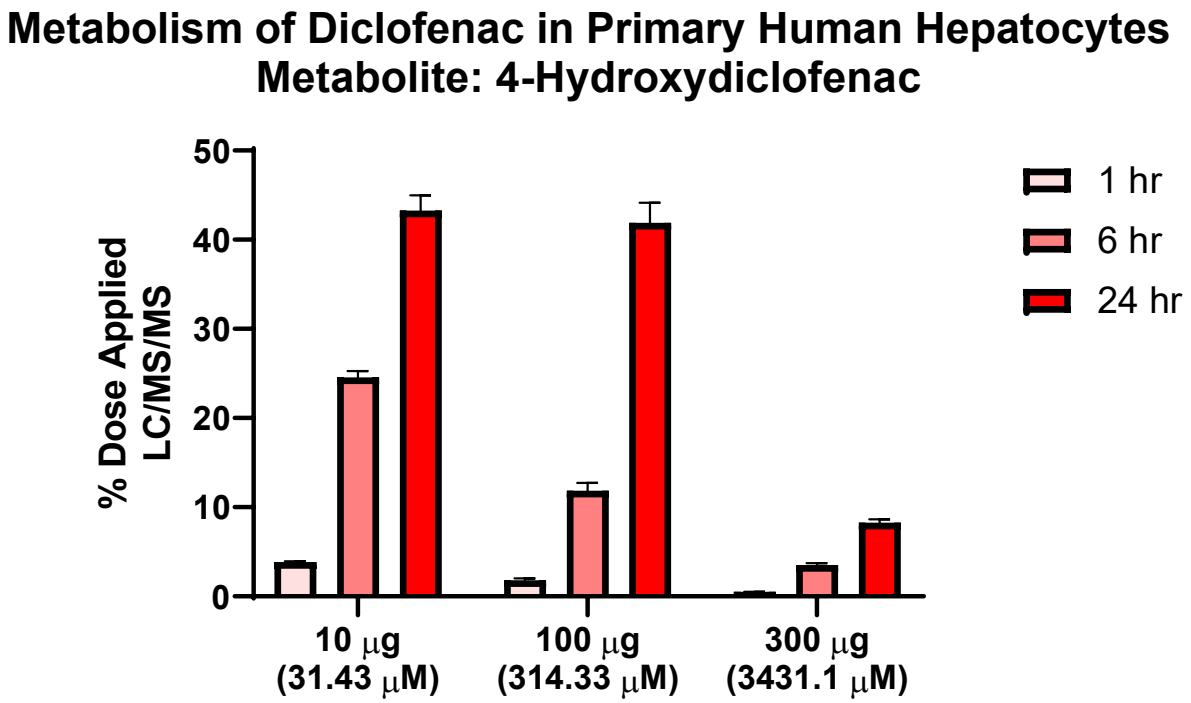
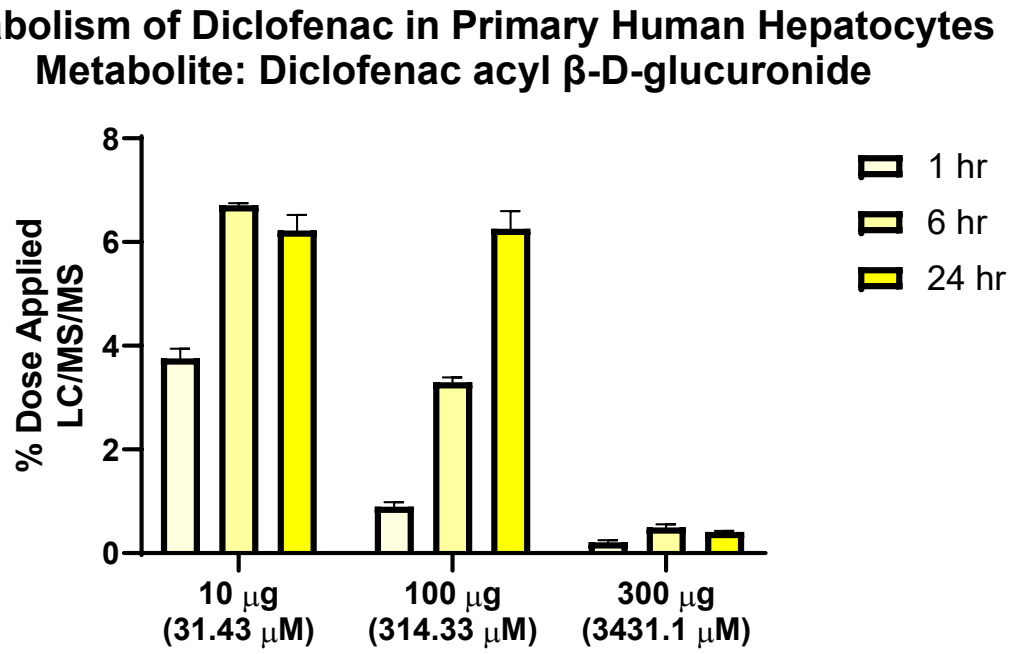
Evaluate and Optimize Metabolite Identification





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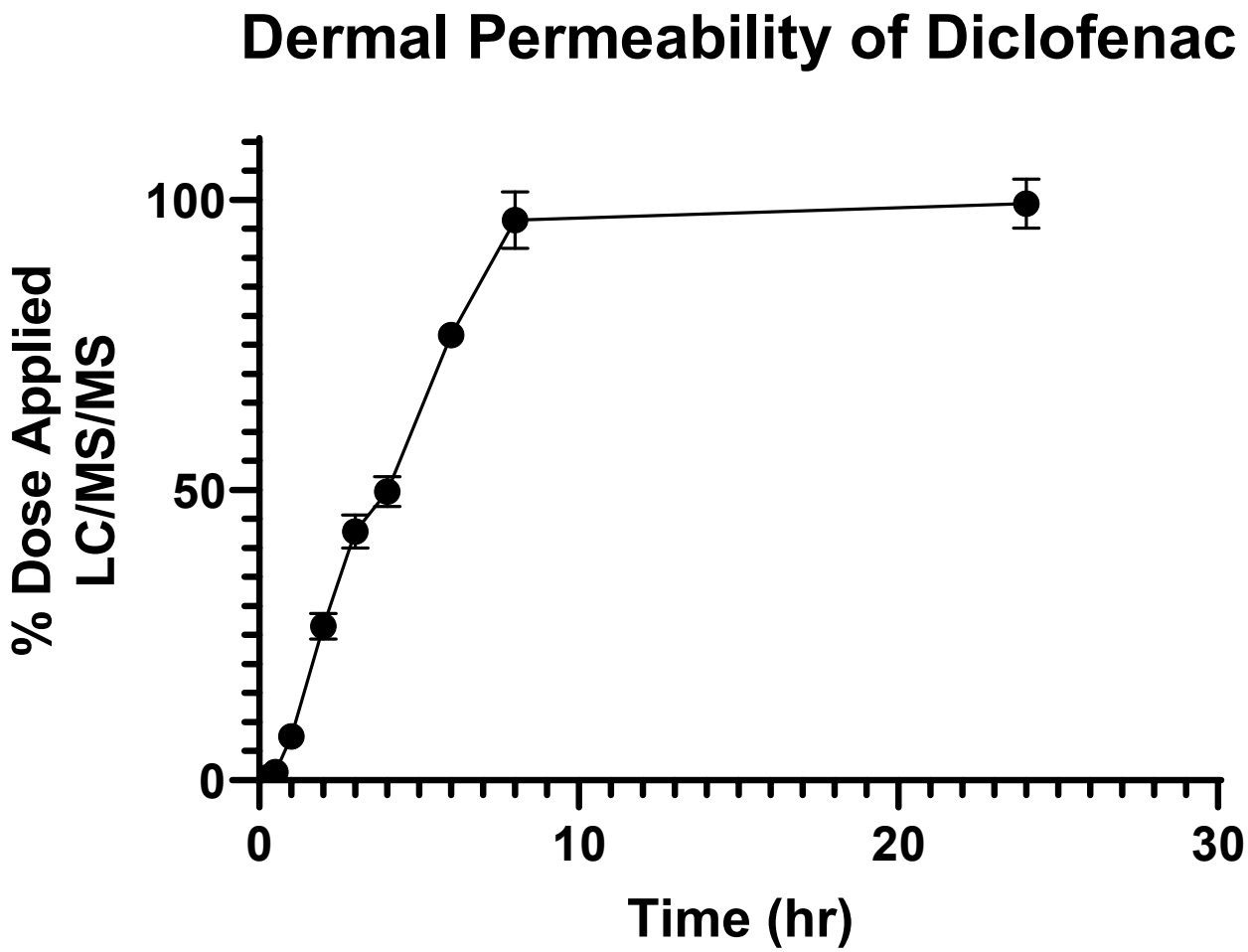
Verify that the Test Chemical is Absorbed

Initial Absorption of Diclofenac Across Skin



Dermal bioavailability between formulations can be evaluated

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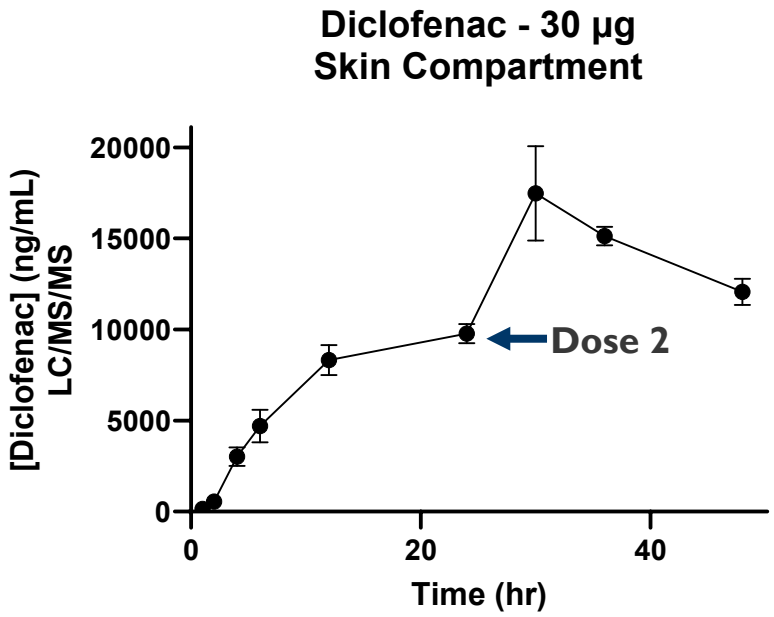
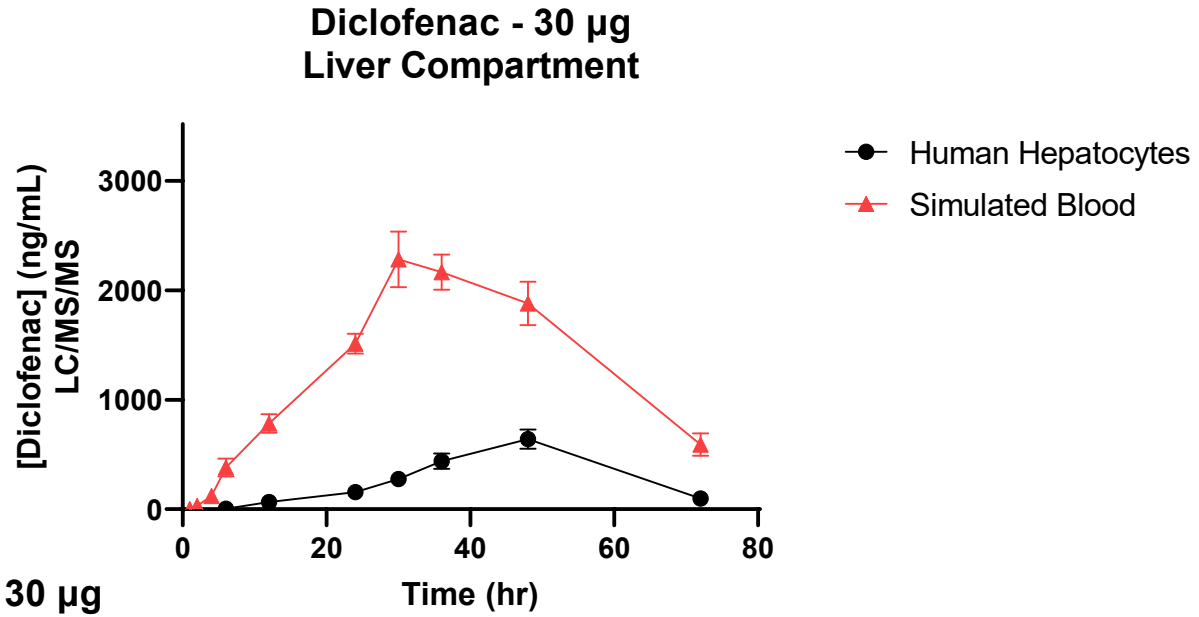
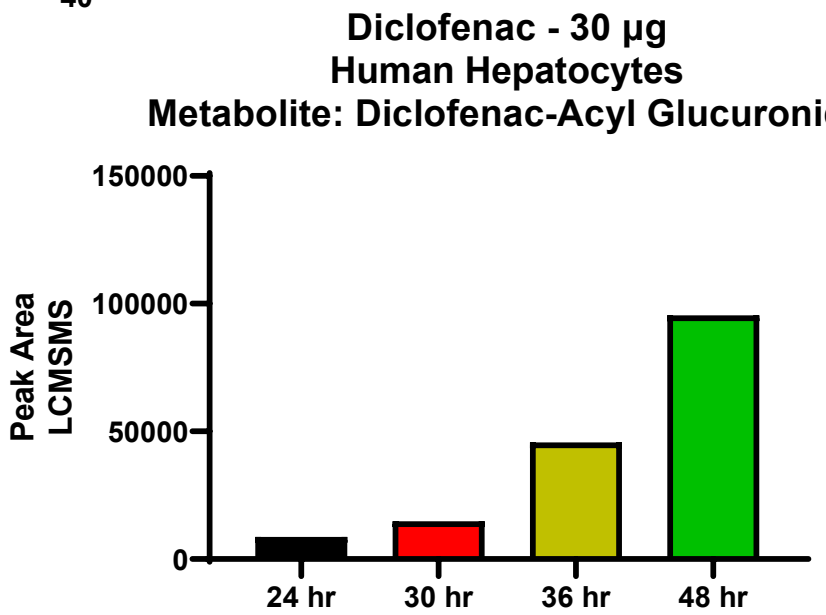
Important Pharmacokinetic Parameters







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Important Pharmacokinetic Parameters

Table 1 Plasma pharmacokinetic parameters of oral paracetamol

Parameter		Paracetamol dose[a]				*p* value[c]
		First (before[b])	Second (during[b])	Third (during[b])	Fourth (after[b])	
n		11	11	11	11	
AUC_{0-6} (μg·h/mL)[d]	Mean (SD)	31.00 (5.11)	28.51 (5.96)	25.31 (11.59)	52.38 (13.48)	<0.001
	CV%	16.5%	20.9%	45.8%	25.7%	
AUC_{0-18} (μg·h/mL)	Mean (SD)				82.50 (23.28)	
C_{max} (μg/mL)	Mean (SD)	11.6 (4.11)	7.29 (1.82)	7.25 (3.95)	13.5 (3.31)	0.188
	CV%	35.5%	25.0%	54.5%	24.6%	
C_6 (μg/mL)	Mean (SD)	2.93 (0.633)	3.71 (0.694)	4.83 (1.97)	6.83 (2.22)	<0.001
	CV%	21.6%	18.7%	40.8%	32.5%	
T_{max} (h)	Mean (SD)	1.48 (0.61)	1.64 (0.78)	3.26 (2.30)	2.84 (1.05)	0.031
	CV%	40.9%	47.5%	70.5%	37.0%	
K_{el} (/h)	Mean (SD)				0.1904 (0.0171)	
$t_{1/2}$ (h)	Mean (SD)				3.67 (0.33)	

Estimate Intestinal permeability and estimate ADME parameters

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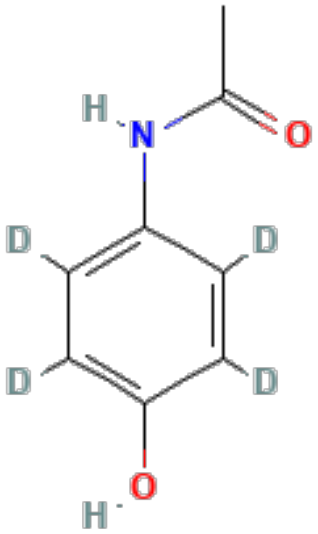
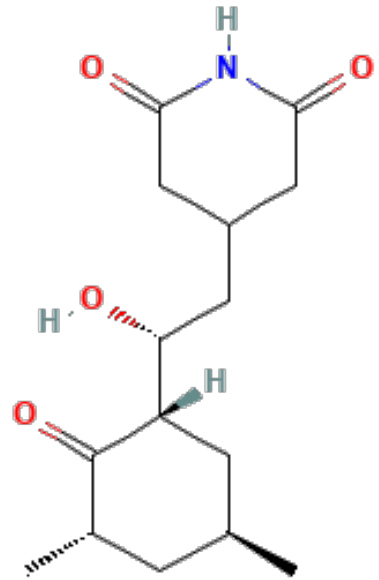
Evaluating Kinetics and Toxicity

Acetaminophen



Analgesic and Antipyretic
NSAID
MW = 155.2 cLogP = 0.50

Cycloheximide



Antifungal
Protein synthesis inhibitor
Chylomicron flow inhibitor
MW = 281.4 cLogP = 0.86

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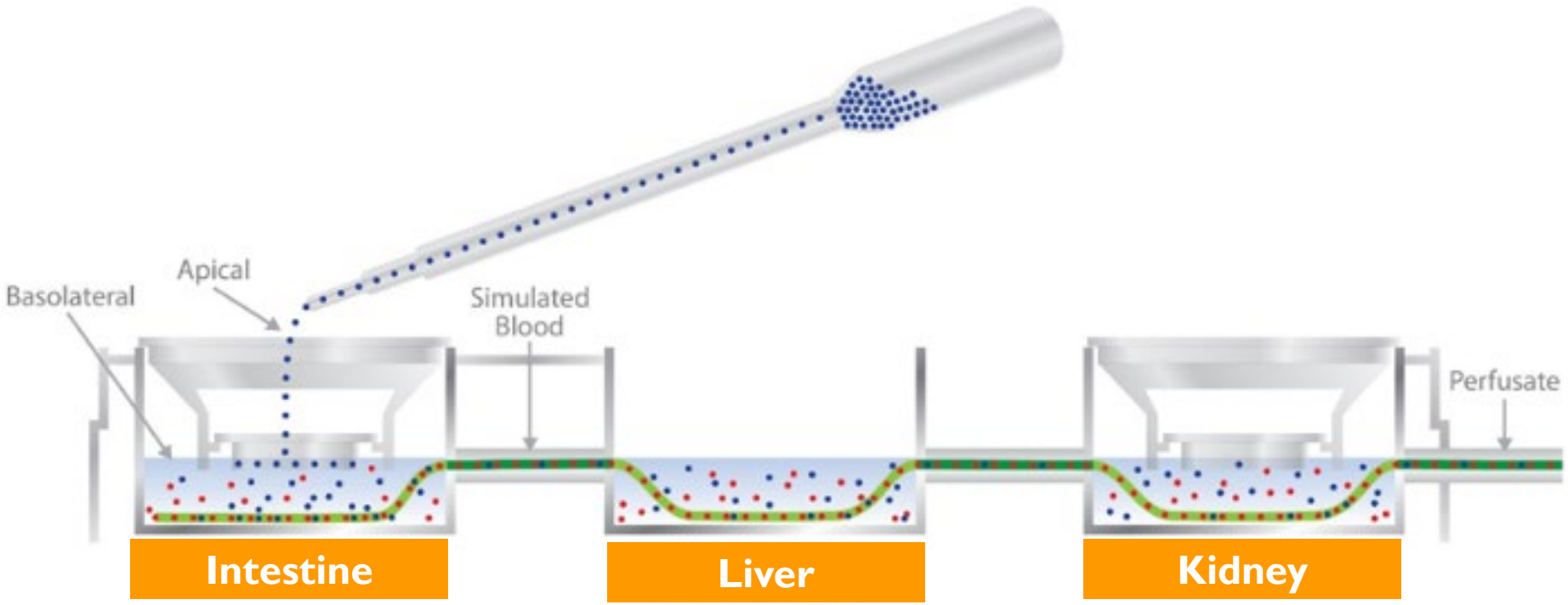
Simulated Oral Administration Three Organ Model



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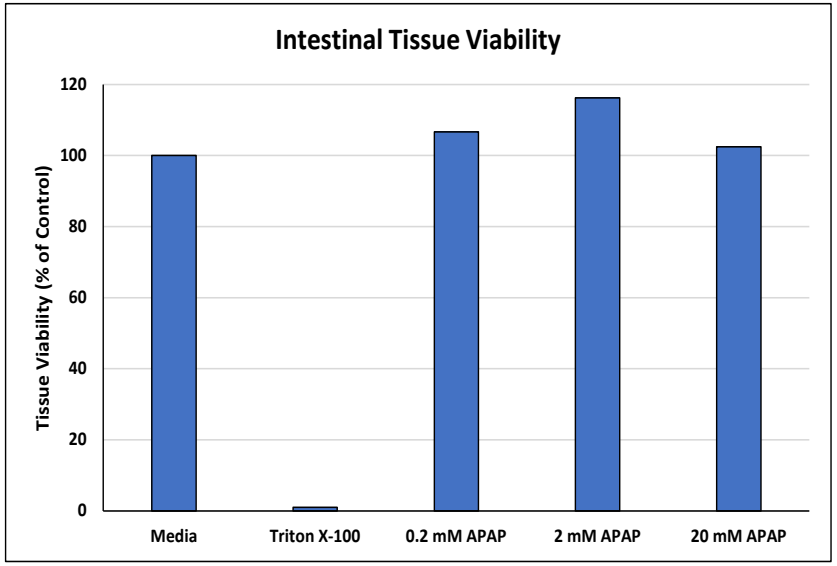
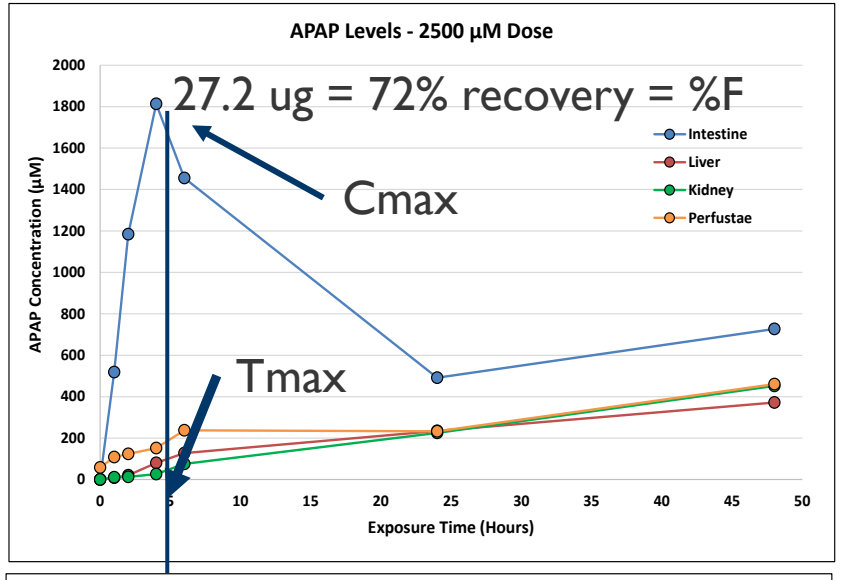
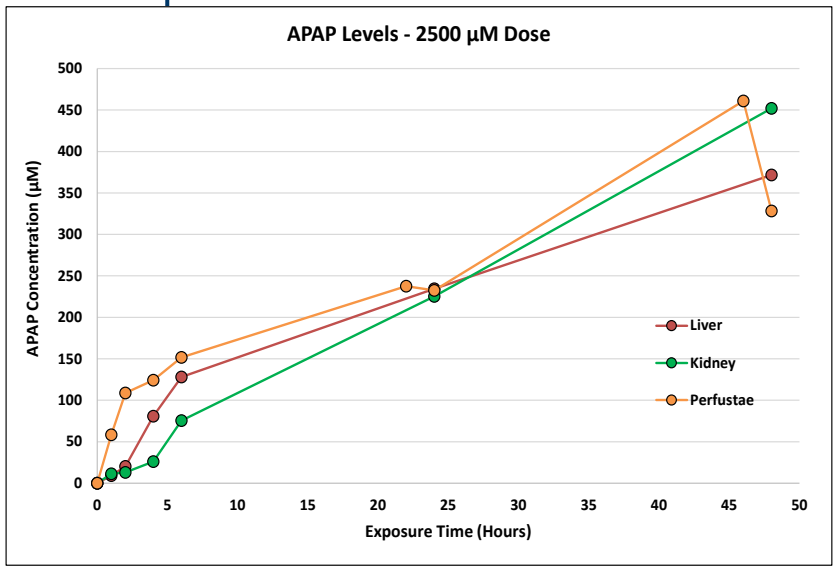
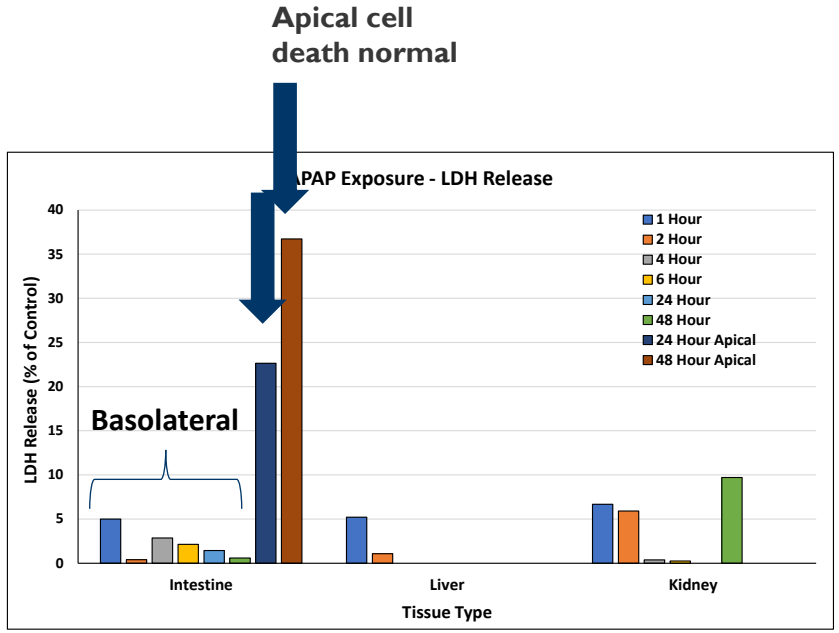
APAP – Kinetics and Toxicity

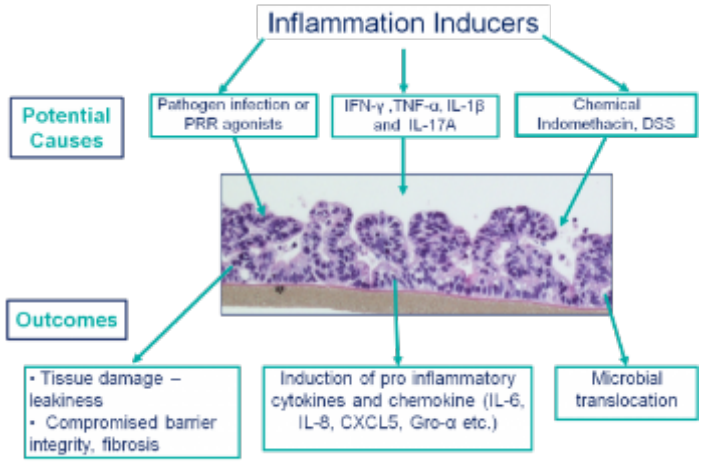
logP = 0.5











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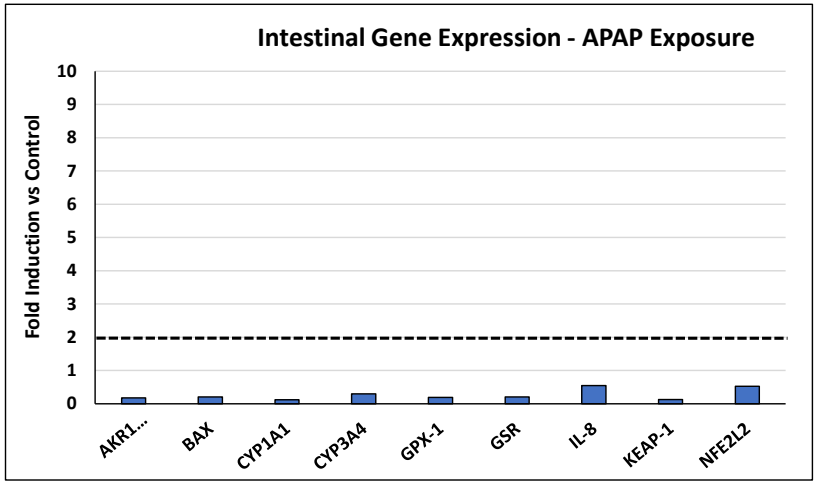
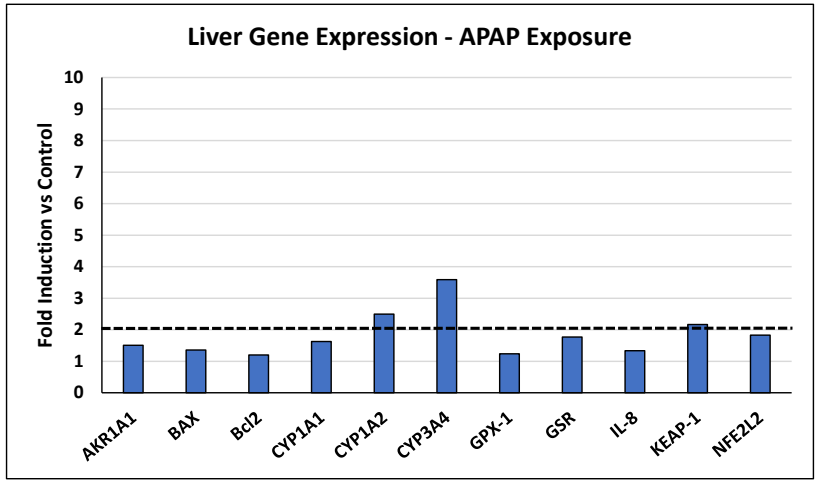
APAP – Cytotoxicity Gene Panels

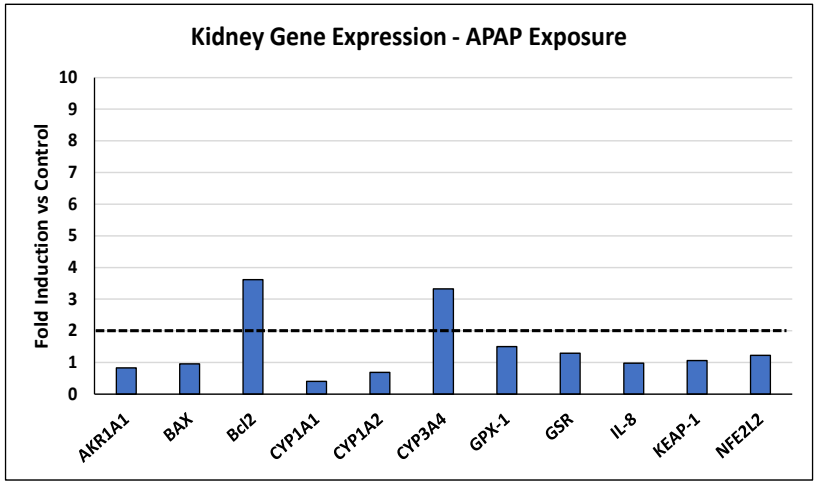
APAP	AKR1A1	BAX	Bcl2	CYP1A1	CYP1A2	CYP3A4	GPX-1	GSR	IL-8	KEAP-1	NFE2L2	TNFα
Intestine	0.174	0.203	no amp	0.116	>	0.295	0.192	0.206	0.545	0.125	0.525	>
Liver	1.509	1.358	1.196	1.630	2.495	3.589	1.235	1.771	1.334	2.167	1.831	no amp
Kidney	0.831	0.952	3.616	0.404	0.688	3.321	1.501	1.289	0.975	1.057	1.222	>

Green highlighted cells are >2-fold induction which is considered a biologically relevant induction in qPCR. “>” means the Ct value was too high.

“No amp” means there was no detectable amplification of the gene.







The black dotted line represents a 2-fold induction, which is considered a biologically relevant induction in qPCR.

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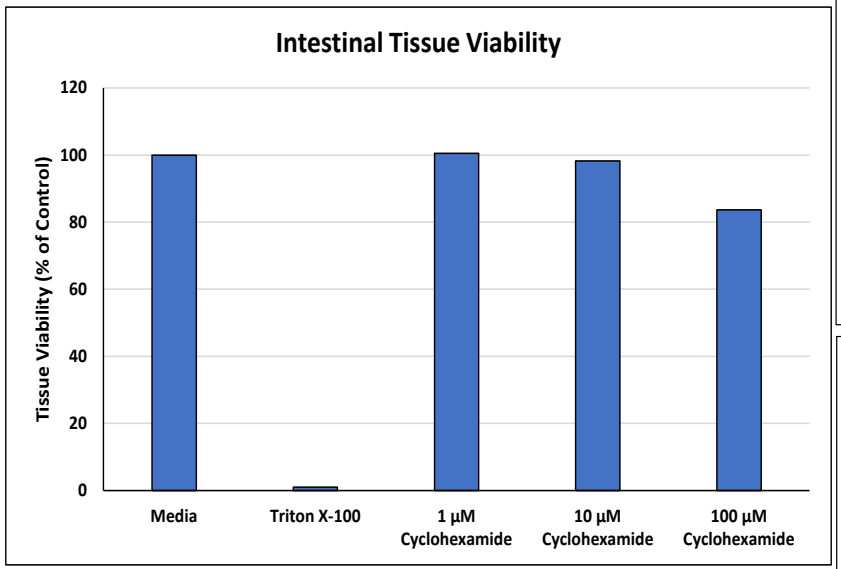
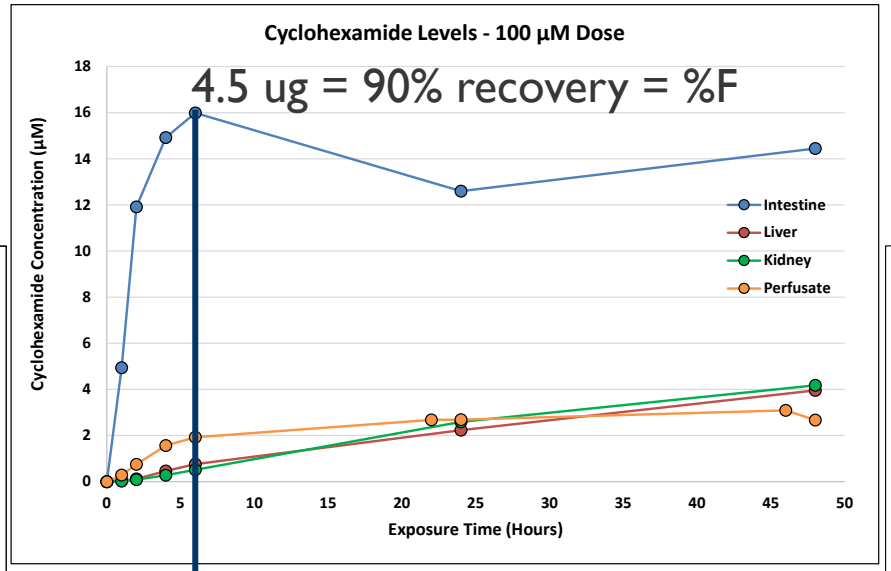
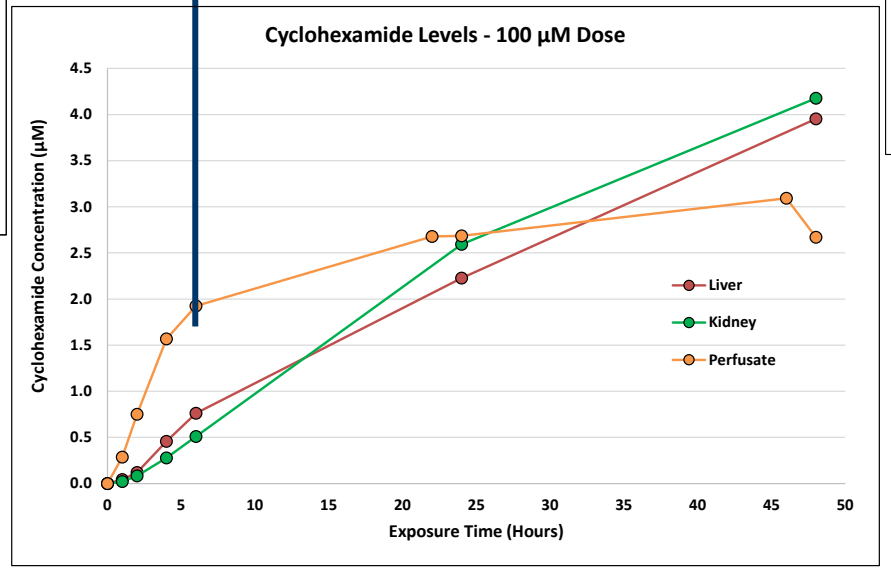
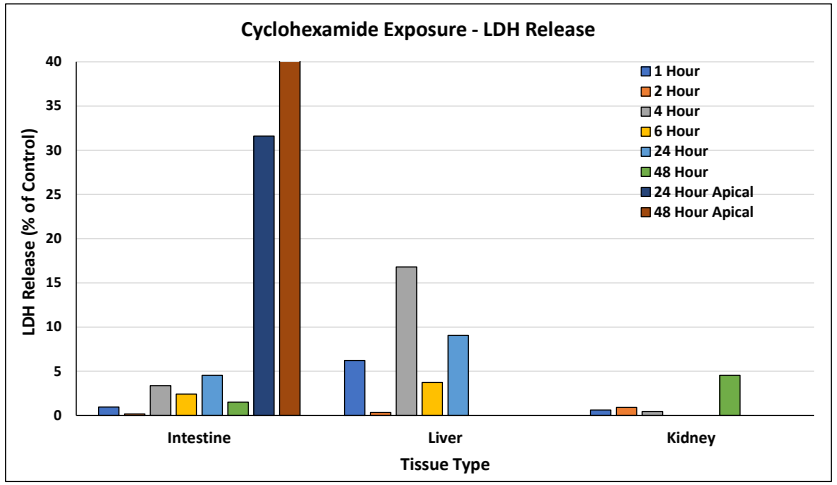
Cycloheximide – Kinetics and Toxicity







logP = 0.86
Delayed Intestinal Elimination



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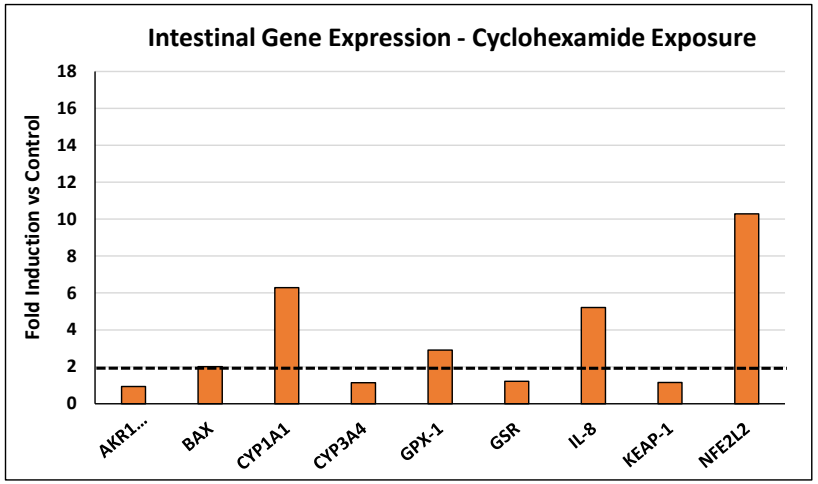
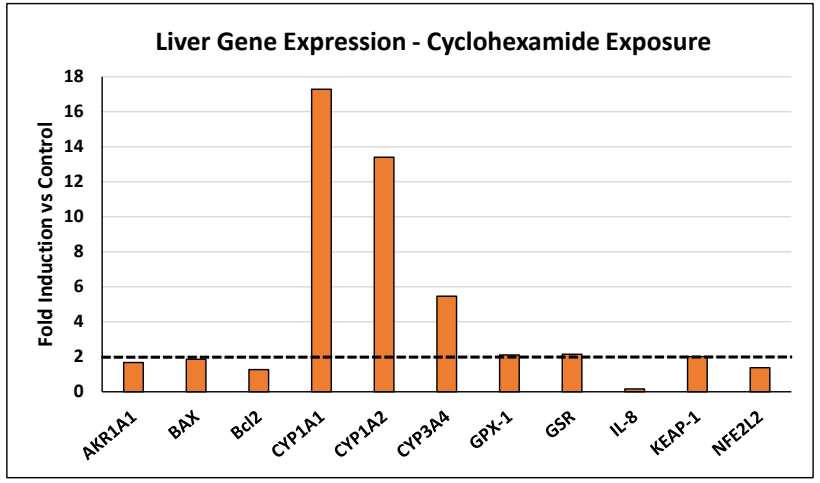
Cyclohexamide Gene Expression

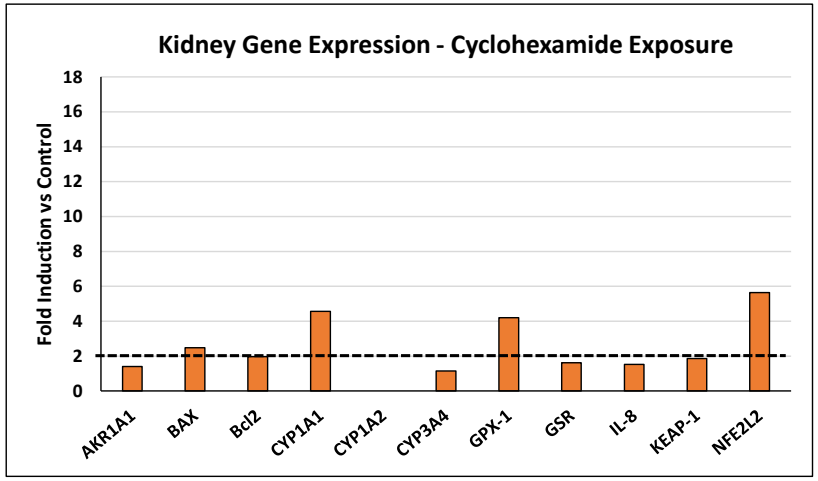
Cyclohexamide	AKR1A1	BAX	Bcl2	CYP1A1	CYP1A2	CYP3A4	GPX-1	GSR	IL-8	KEAP-1	NFE2L2	TNFα
Intestine	0.929	2.010	no amp	6.286	>	1.131	2.901	1.218	5.212	1.144	10.281	>
Liver	1.677	1.862	1.270	17.282	13.398	5.465	2.101	2.143	0.170	2.015	1.374	no amp
Kidney	1.410	2.475	1.965	4.566	no amp	1.156	4.197	1.615	1.529	1.860	5.640	>

Green highlighted cells are >2-fold induction which is considered a biologically relevant induction in qPCR. “>” means the Ct value was too high.

“No amp” means there was no detectable amplification of the gene







The black dotted line represents a 2-fold induction, which is considered a biologically relevant induction in qPCR.

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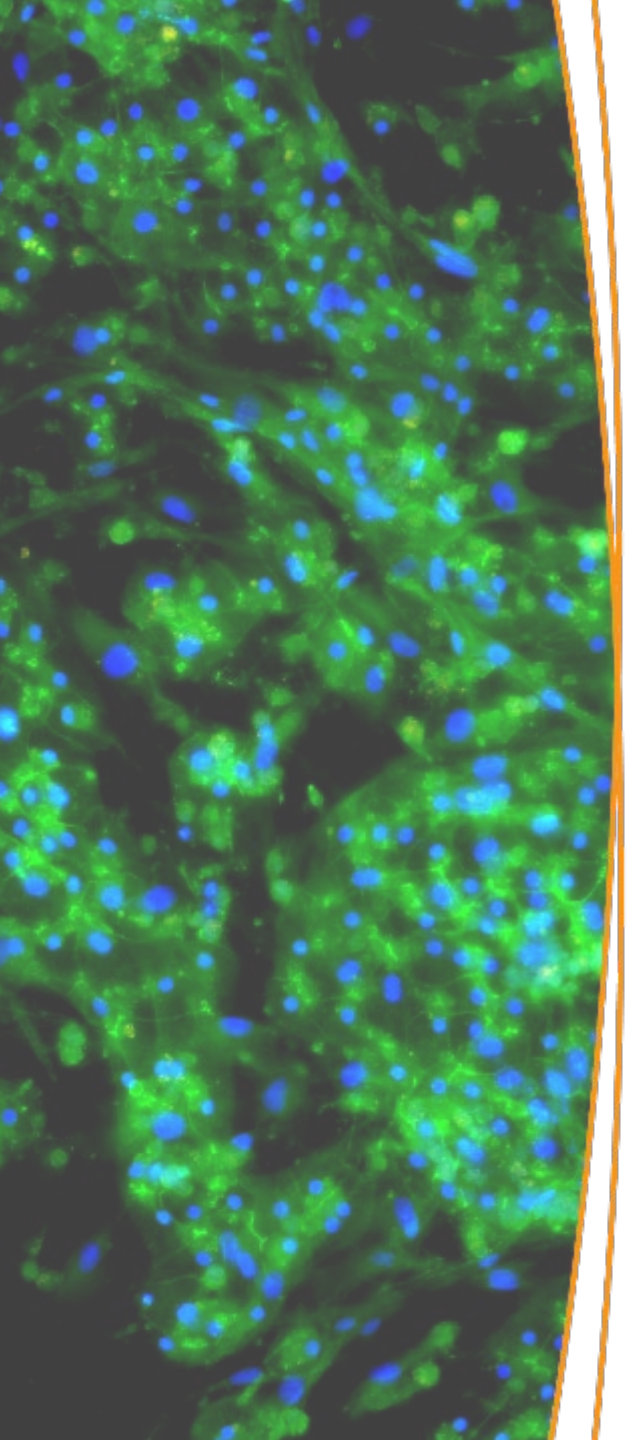

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RESEARCH COLLABORATION WITH FDA DEVELOP CASE STUDIES

SUZANNE FITZPATRICK, ROBERT SPRANDO, STEVEN HERMANSKY AND WILLIAM MATTES

Basic Goals of the Collaboration

- Evaluate the Human Dynamic Multiple Organ Plate system
- Can it provide a rapid cost-effective means of assessing human risk
  - Identifying target organs for toxicity
- Can it be used to dial in mechanisms of toxicity

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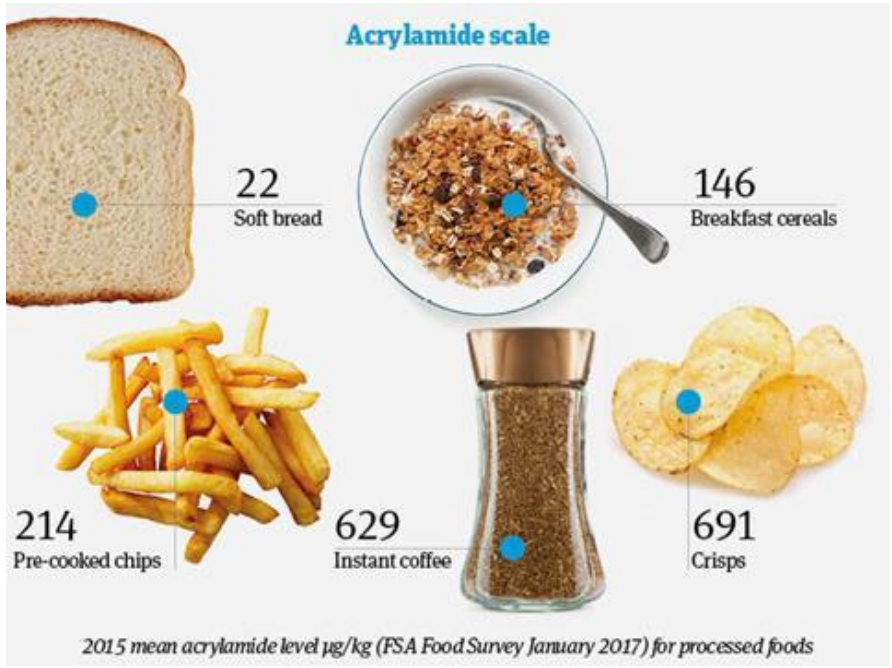
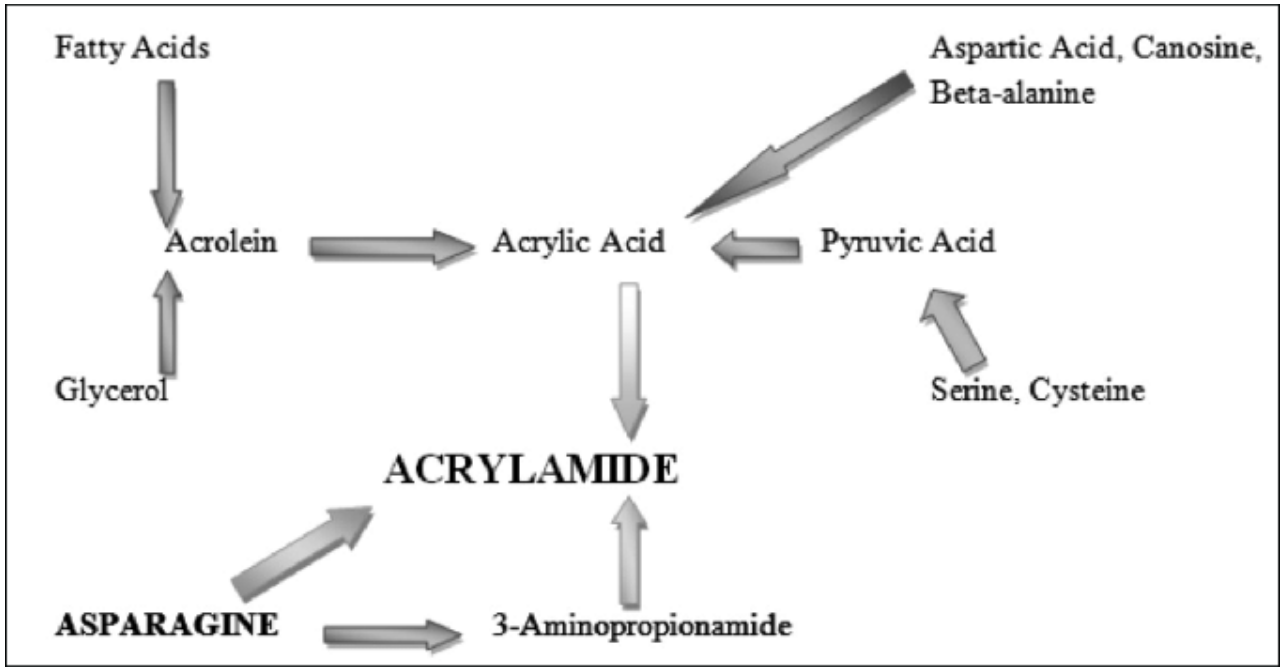
Compound Selection Based on Current FDA Issues





Acrylamide is a chemical that can form in some foods during high-temperature cooking processes, such as frying, roasting, and baking. Acrylamide in food forms from sugars and an amino acid that are naturally present in food

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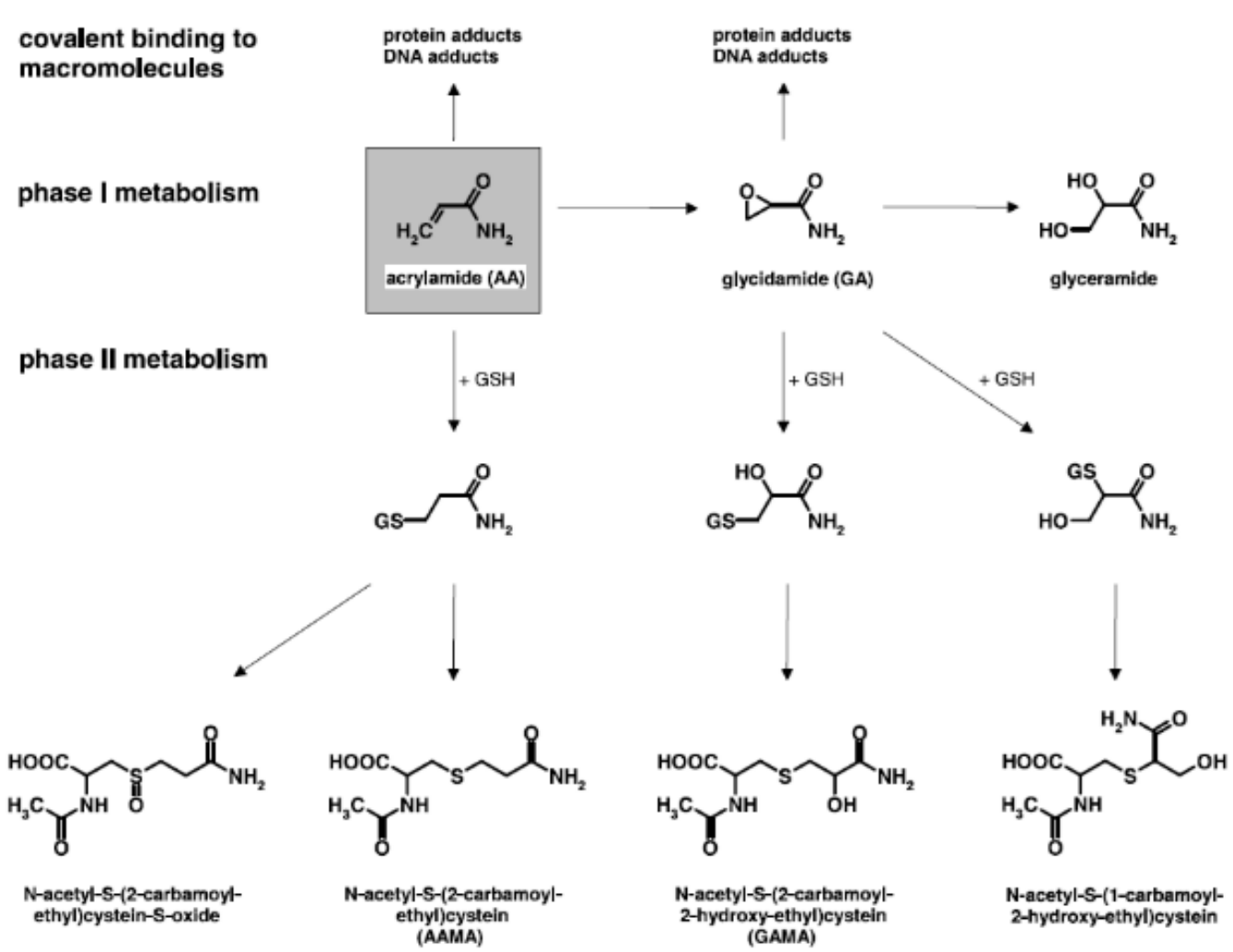
Acrylamide Metabolism



est on 03 October 2022

Figure 1. Presumed metabolic scheme of acrylamide. The scheme was partly adopted from Boettcher et al. (22), Dybing et al. (6), and Fennell et al. (24). Not all of the metabolites shown have been confirmed unequivocally in humans. In the present study, only acrylamide, glycidamide, AAMA, and GAMA have been quantified, with glycidamide concentrations being lower than the lower limit of quantification (2.5 ng/mL) in all samples.

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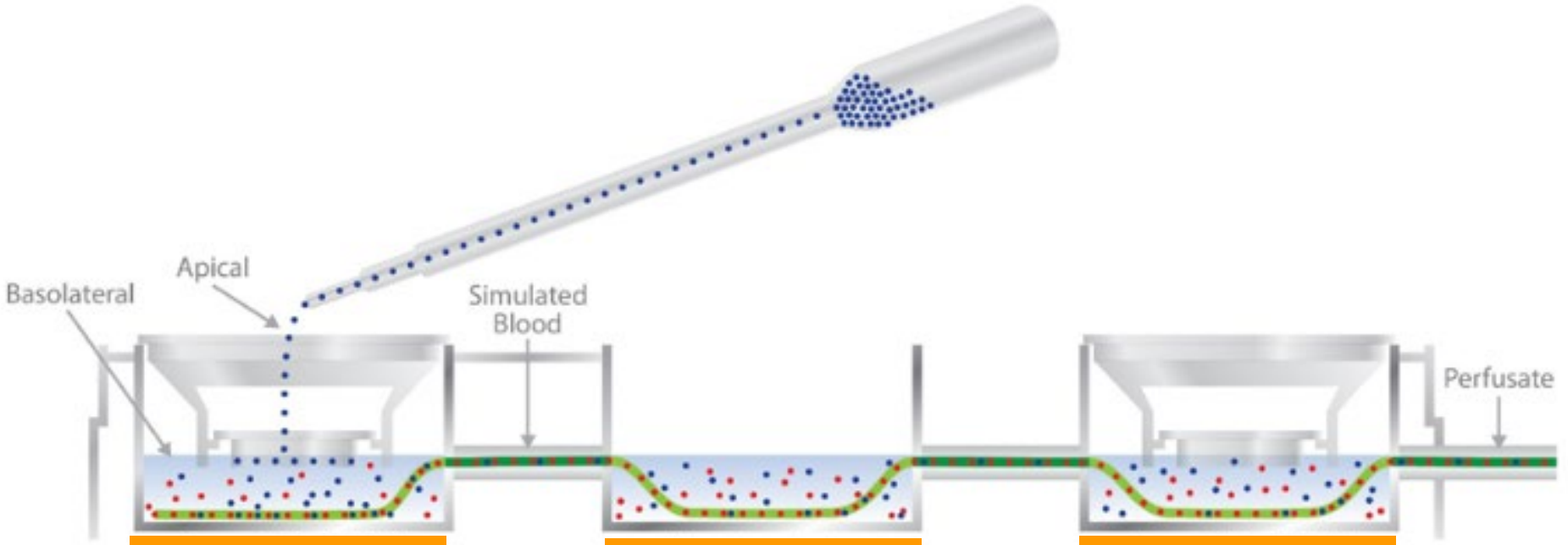
Three Organ Model



Intestine	Liver	Kidney
EpiIntestinal	LifeNet Health Human Hepatocytes	HK-2 Cells

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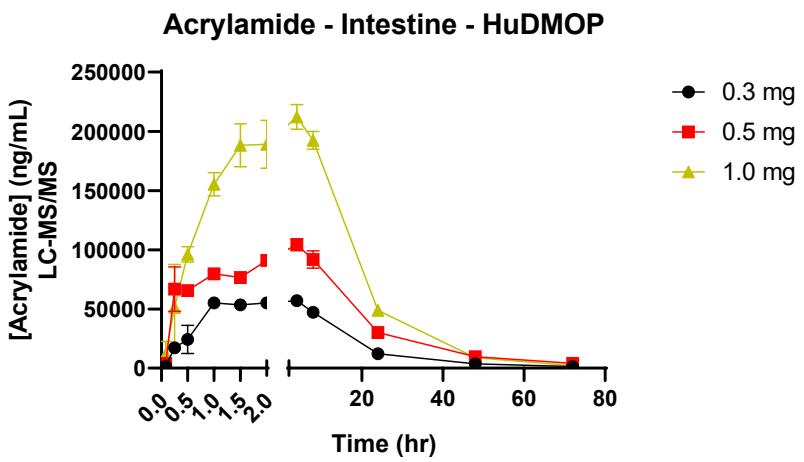
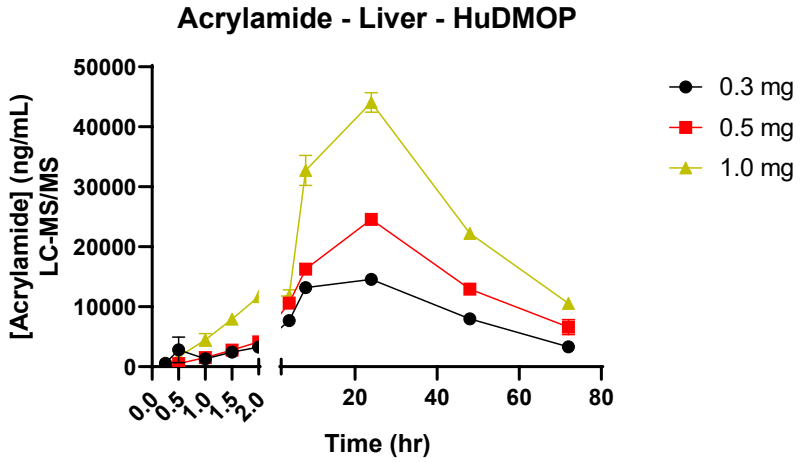
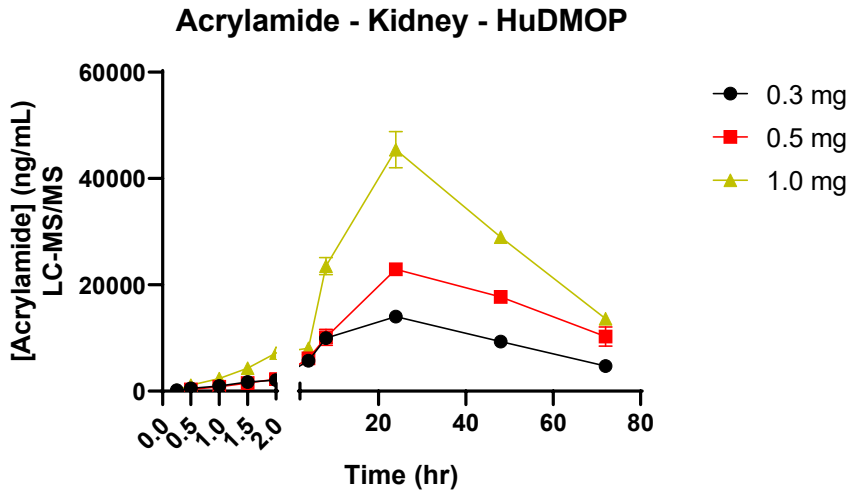
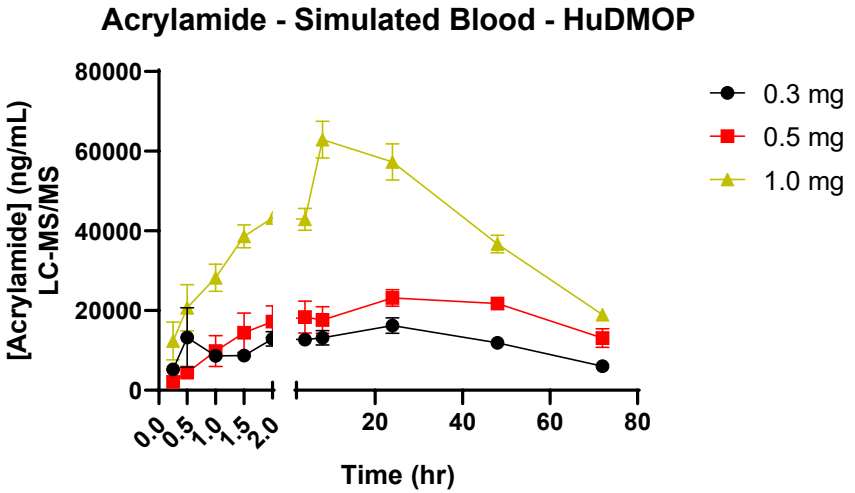
Developing Pharmacokinetic and Toxicology Data in a Single System









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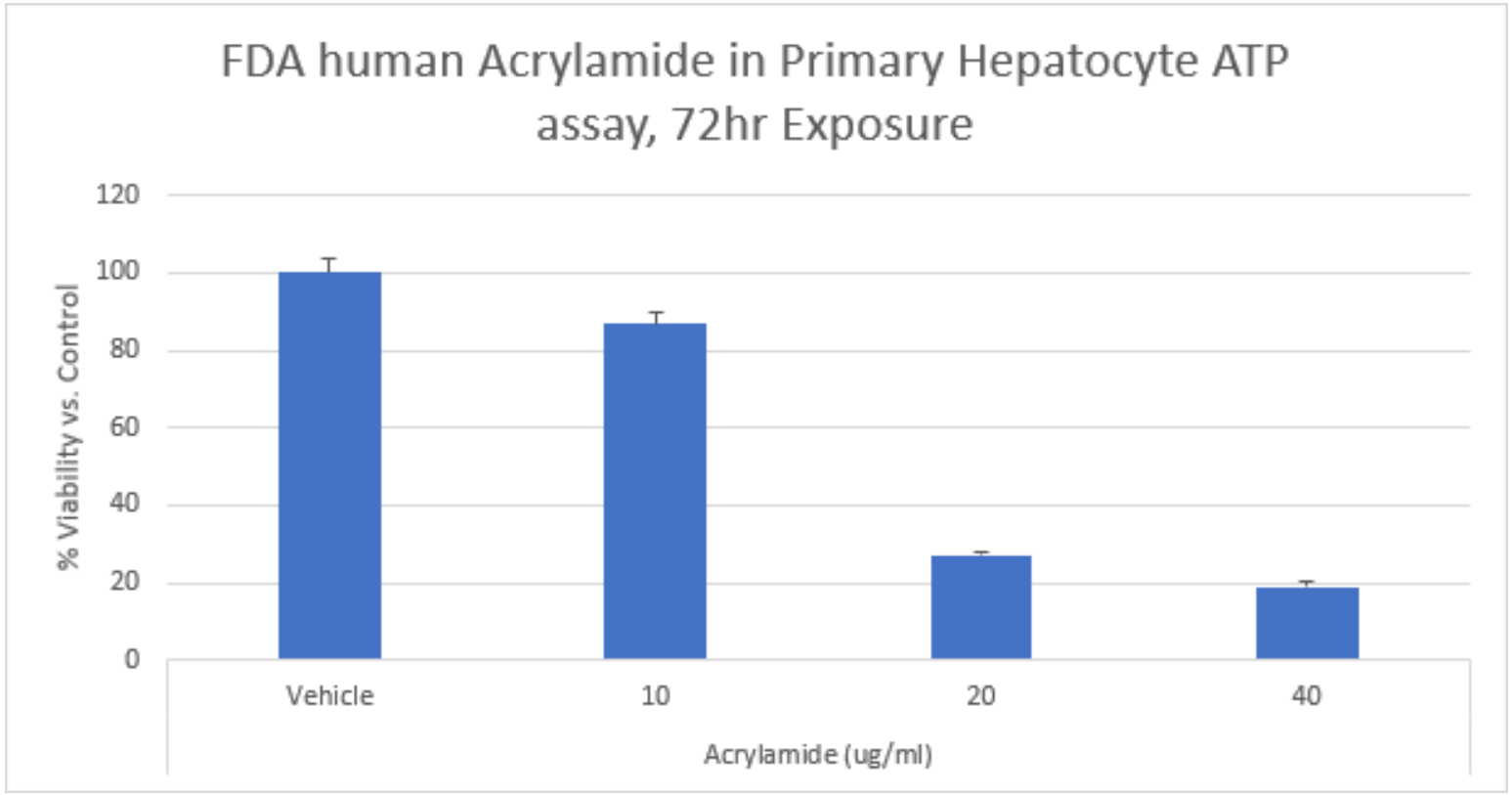
LIVER: Acrylamide Induced Loss of ATP



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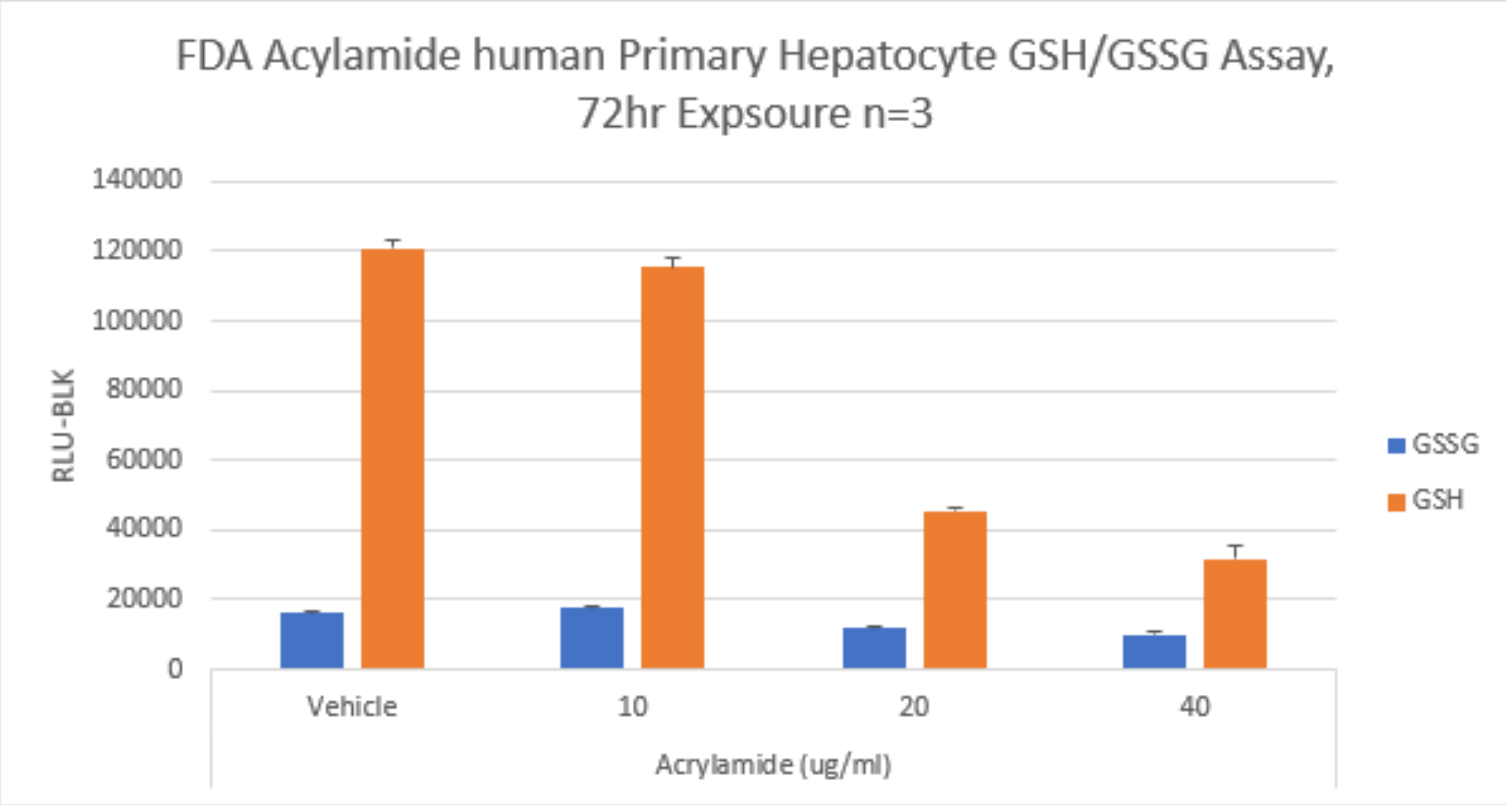
LIVER: Acrylamide Depletion of GSH



Direct loss of GSH a strong indication of a reactive molecule and potential mutagenicity

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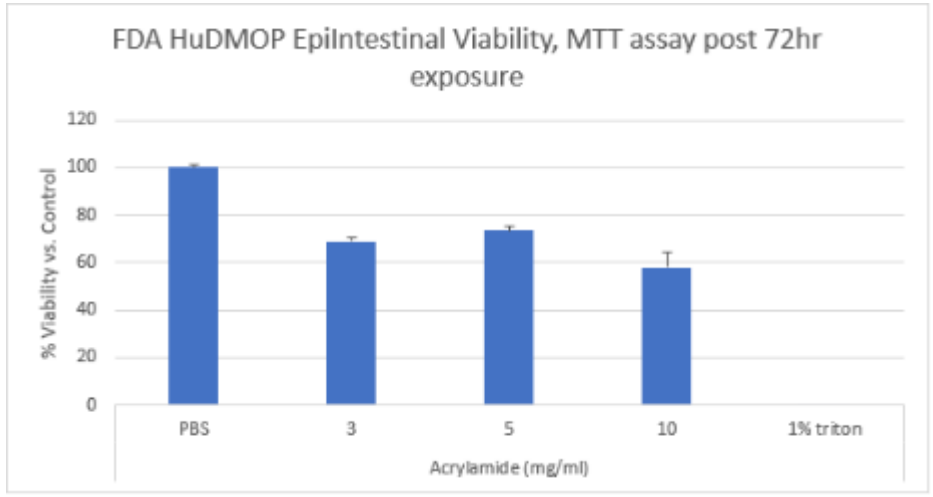
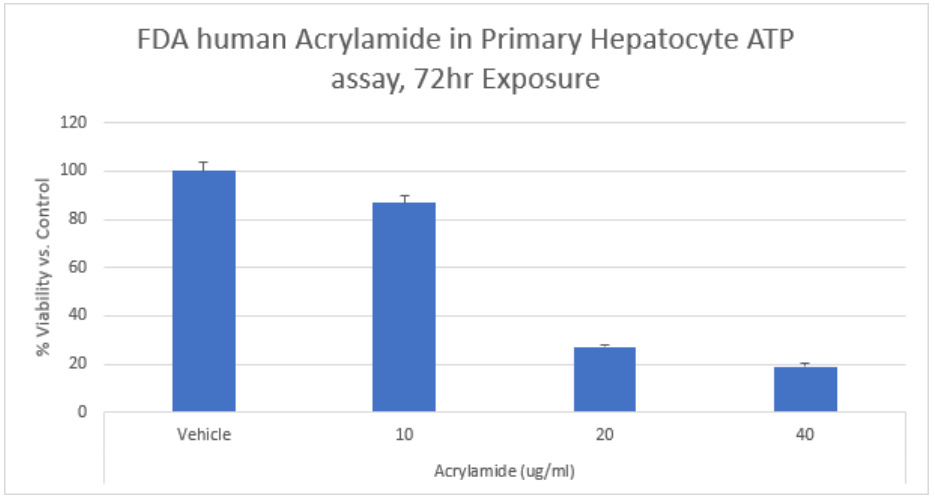
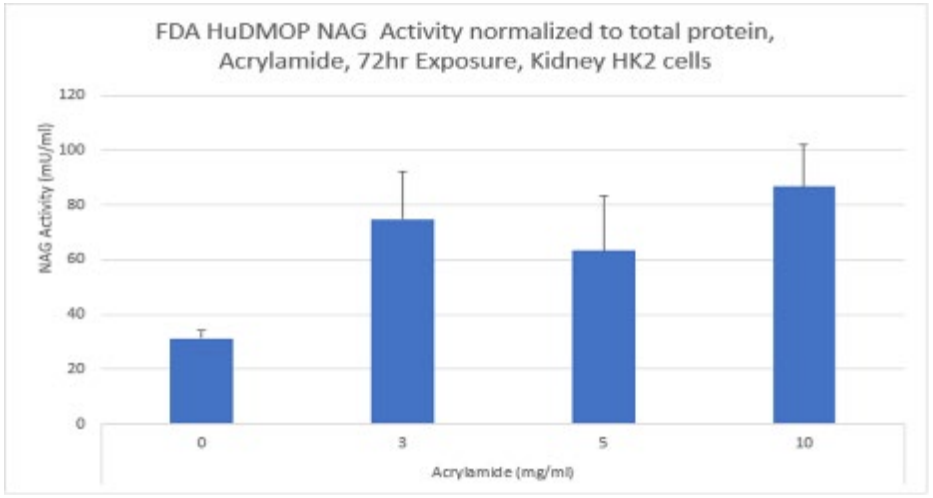
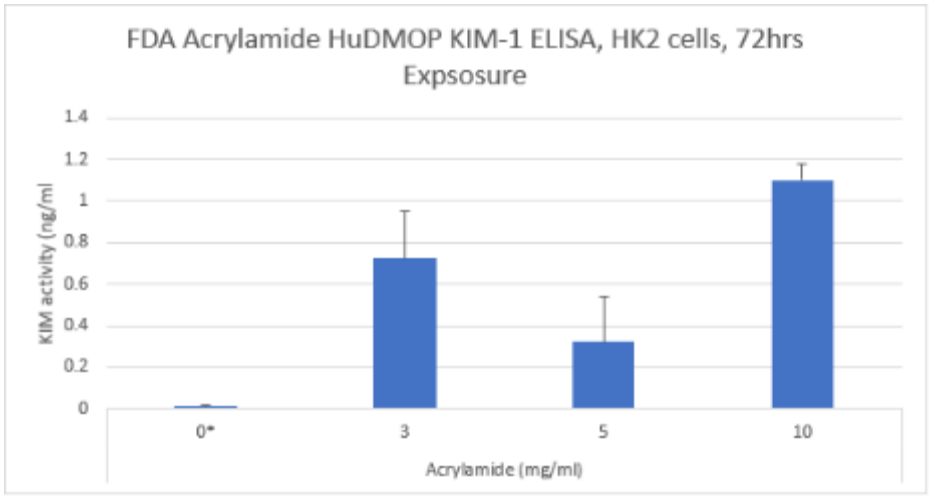
Identifying Acrylamide Organ Toxicity









The System identified Liver and Kidney as potential sites of toxicity which agrees with animal and human literature

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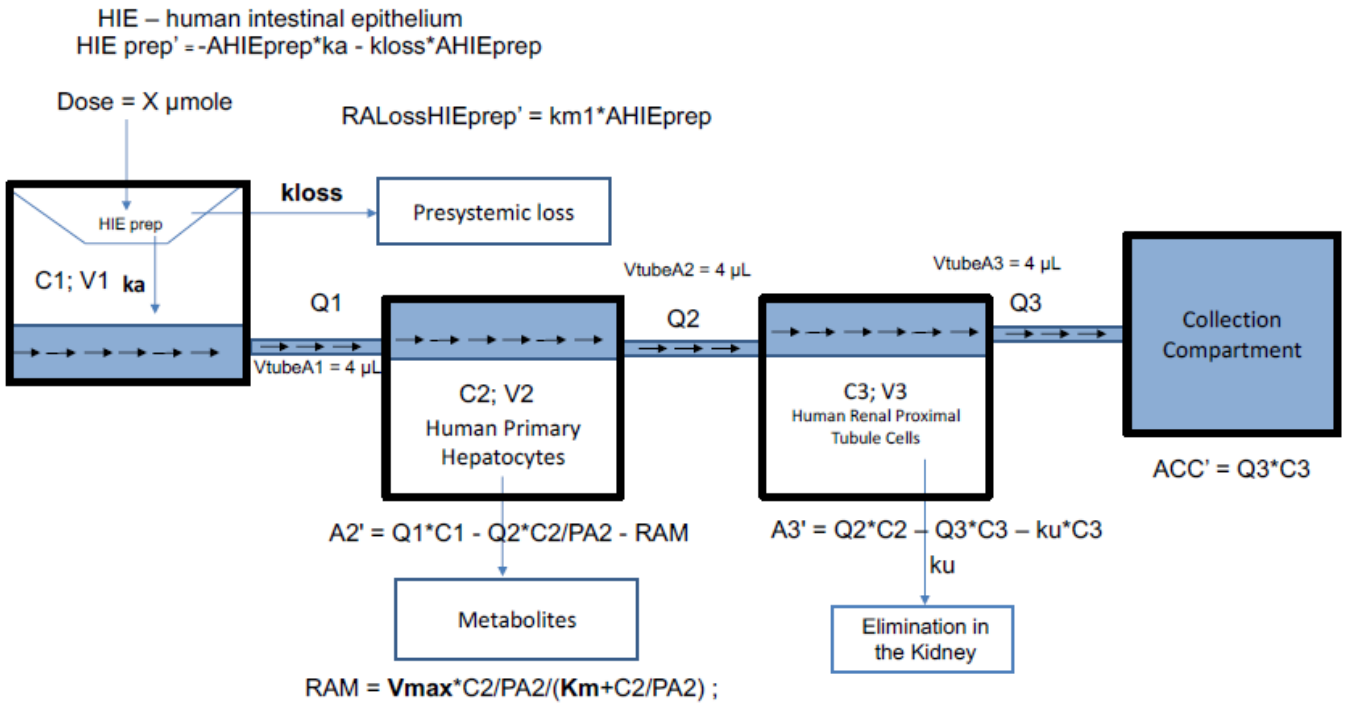
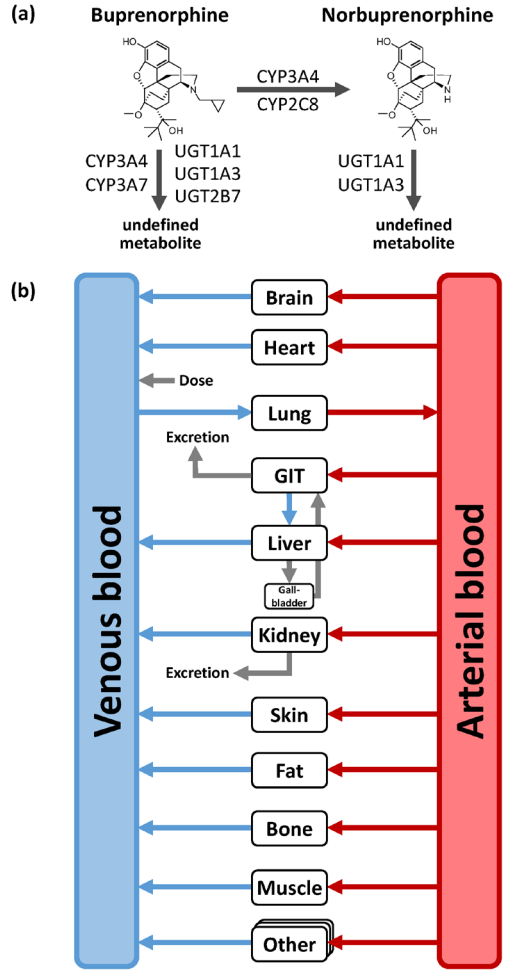
Development of PBPK Models to Enable Better IVIVE





In Summary, Human Dynamic Multiple Organ Plate

- Selection of technology and cell or tissue should match question
- An in vitro integrated organ system, combined with well characterized cell models, can provide kinetic and cytotoxicity data
- Parameterization of the system should allow PBPK models and accurate IVIVE

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