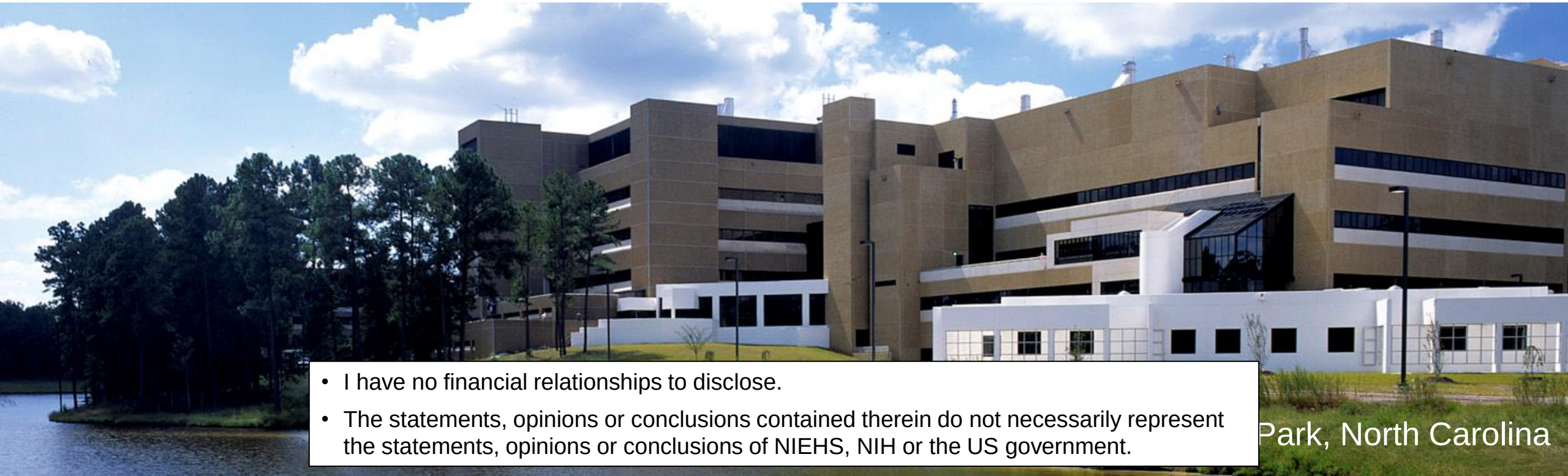
National Institute of Environmental Health Sciences
Division of Translational Toxicology

Development & Application of Microphysiological Systems (MPS) for Translational Toxicology Research

Stephen S. Ferguson, PhD
Group Leader: Predictive Toxicology & Screening

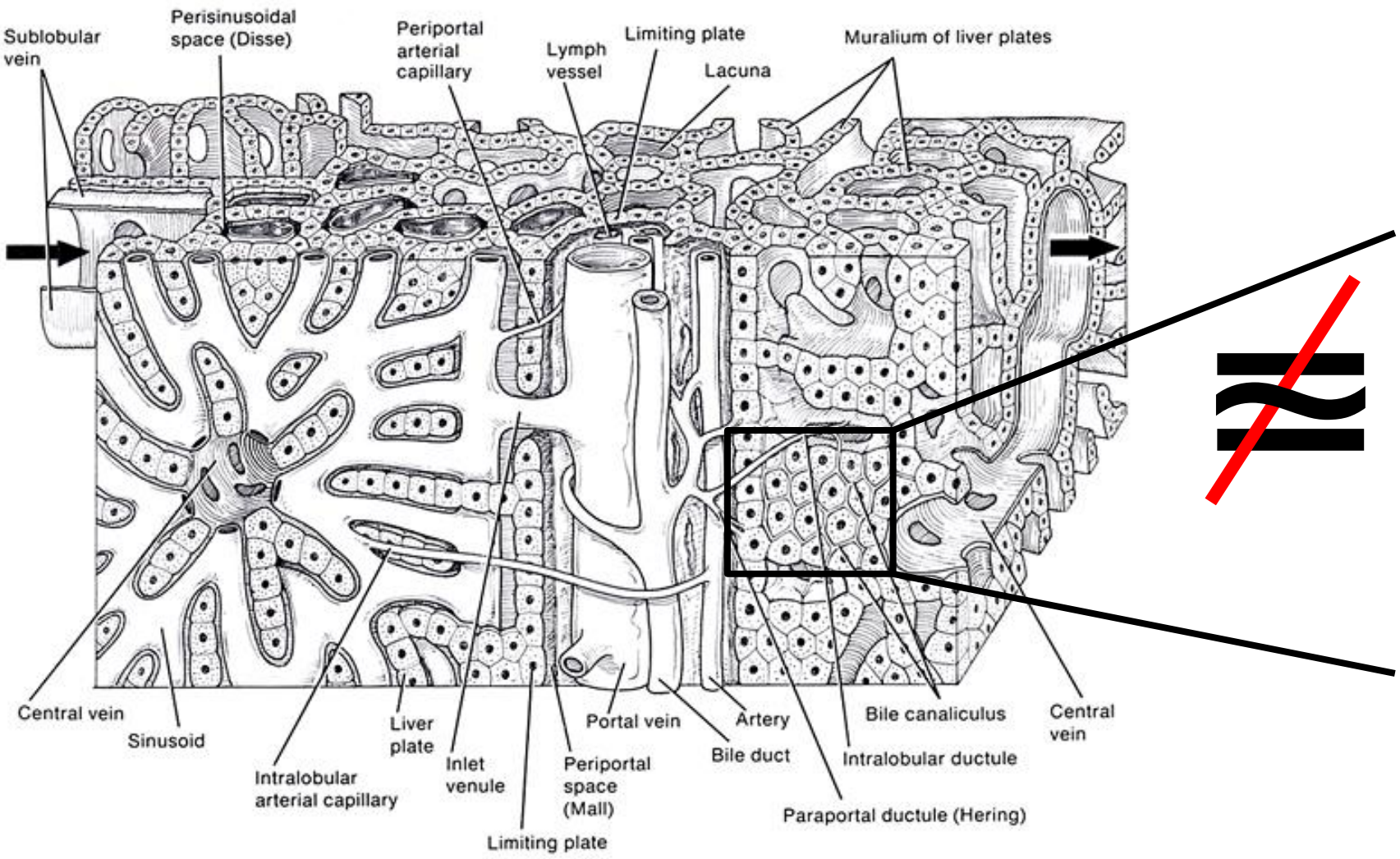


- I have no financial relationships to disclose.
- The statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH or the US government.

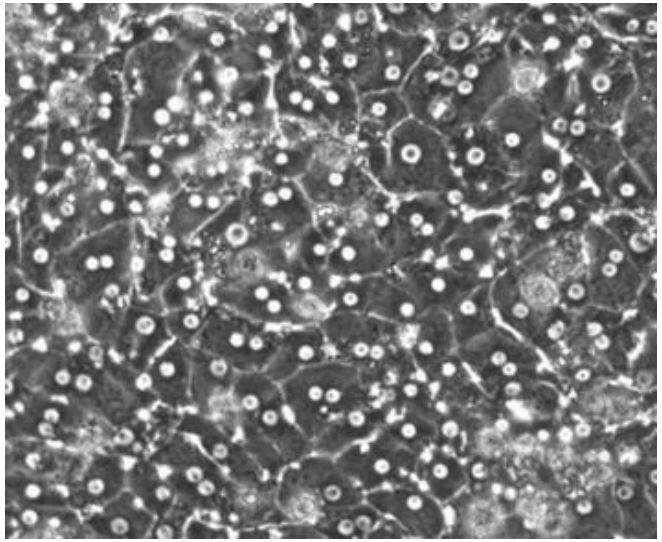
Park, North Carolina

Early In Vitro Models of Human Liver

Physiological architecture of liver

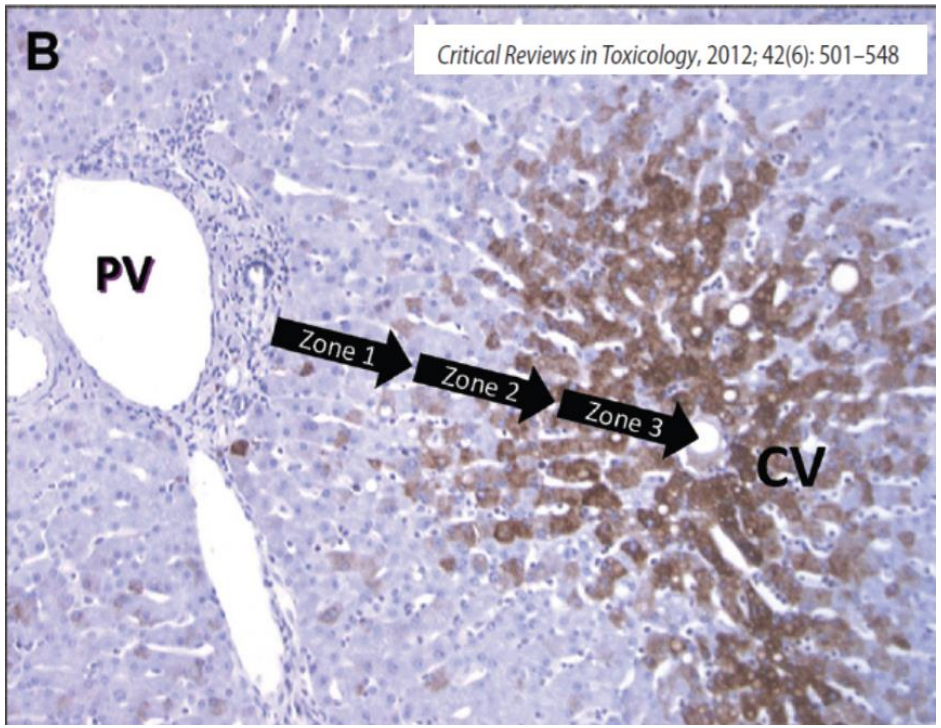


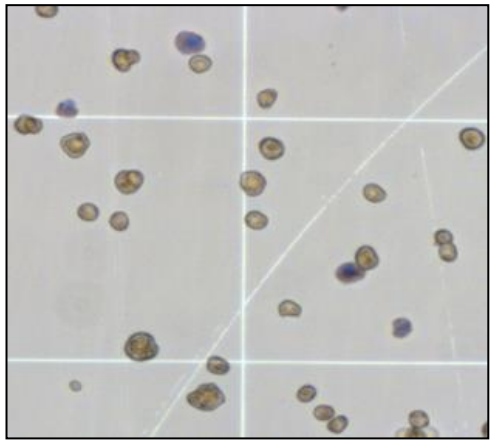
Primary human liver cells (sandwich culture)

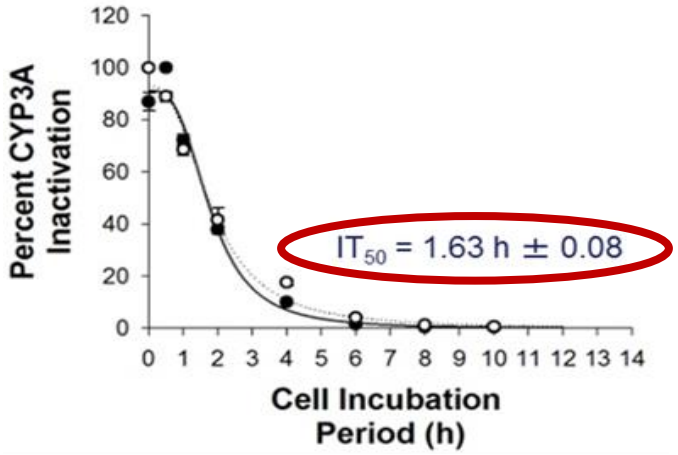


Tissues and Organs: a text of scanning electron microscopy, Kessel, RG and Kardon, RH, 1979.

Primary Liver Cells Rapidly De-differentiate Ex Vivo



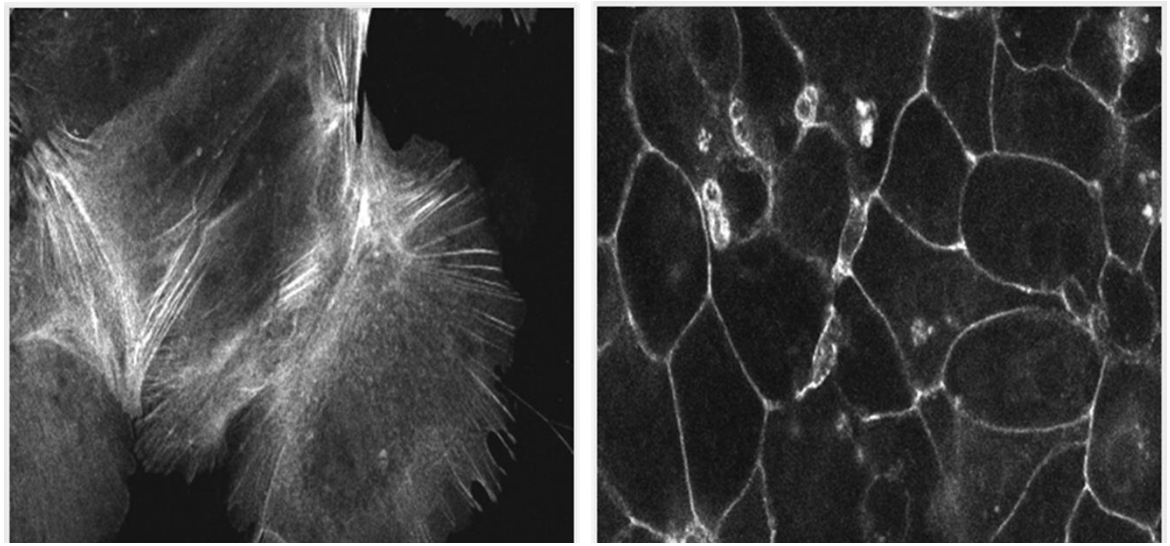




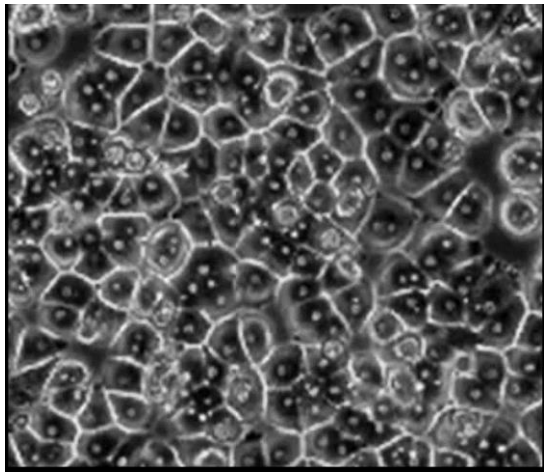
Smith et al. J. Pharm. Sci. 2012. v.101(10):3898.

Establishing cell-cell interactions essential for hepatocellular functionality

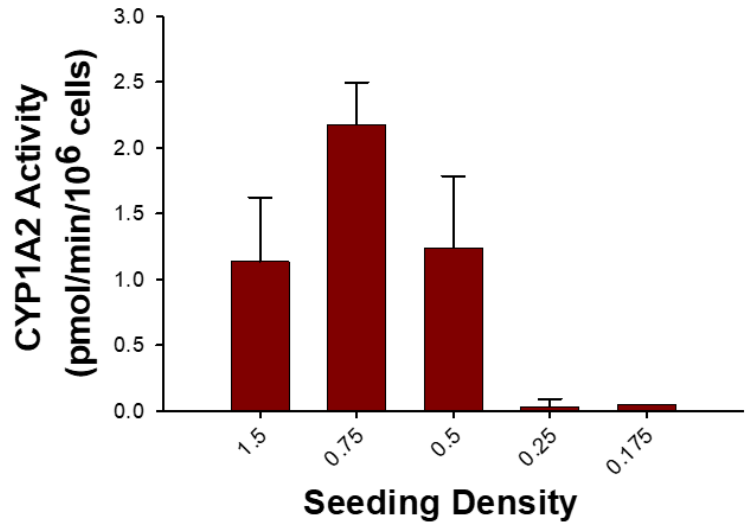
Hamilton et al., Cell Tissue Res, 2001; 306: 85-99.

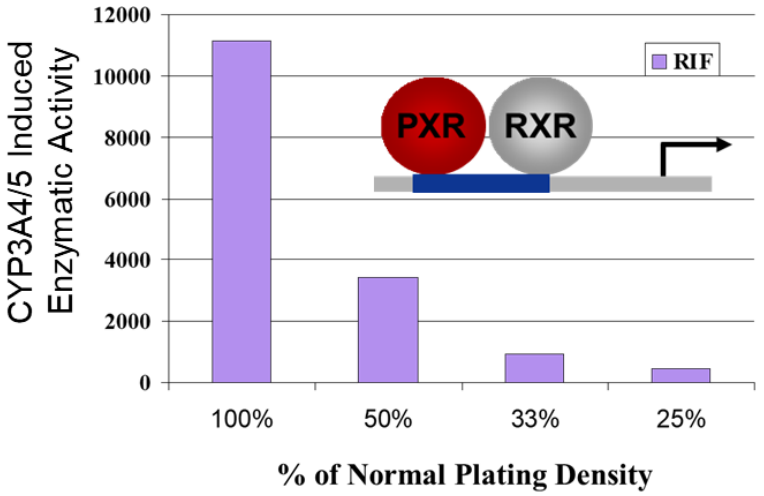


Low-density High-density



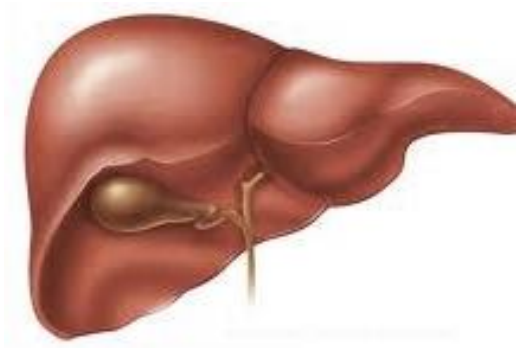
Ed LeCluyse
LifeNet Health

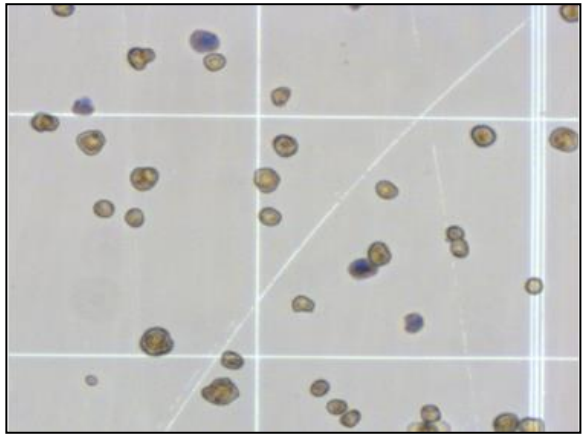


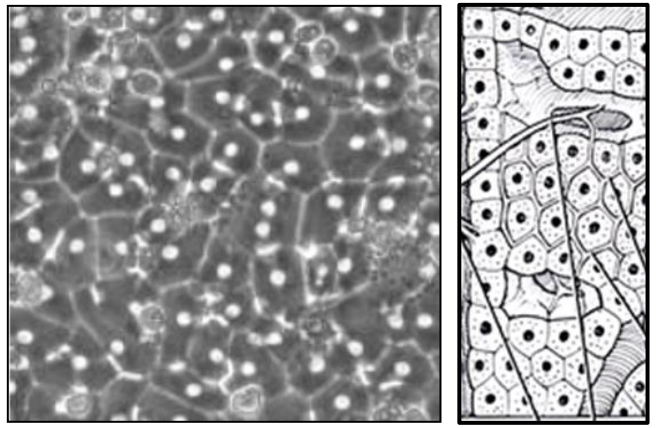


LeCluyse et al., 2010. Chapter 8: Cytochrome P450 Induction. Enzyme Inhibition in Drug Discovery and Development: The Good and the Bad. John Wiley & Sons.

Regulatory Decisions with Organotypic Human Liver Tissue Models







Drug Metabolism &
Transport Assays

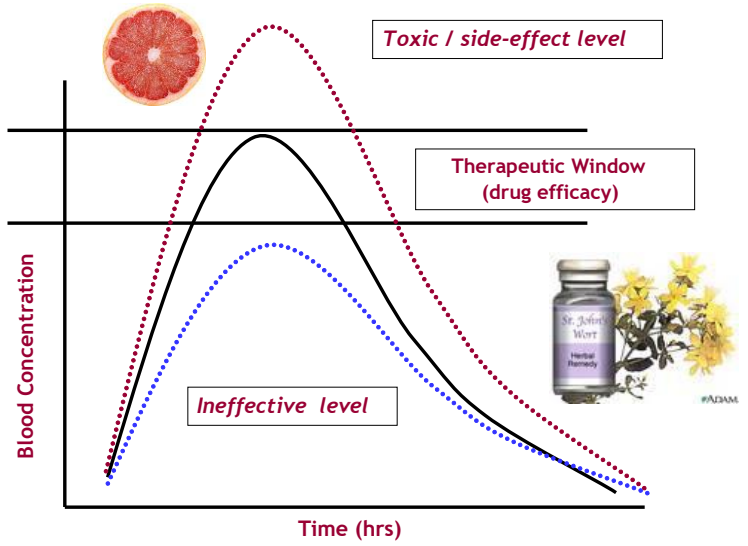
2-5 Day
Exposures

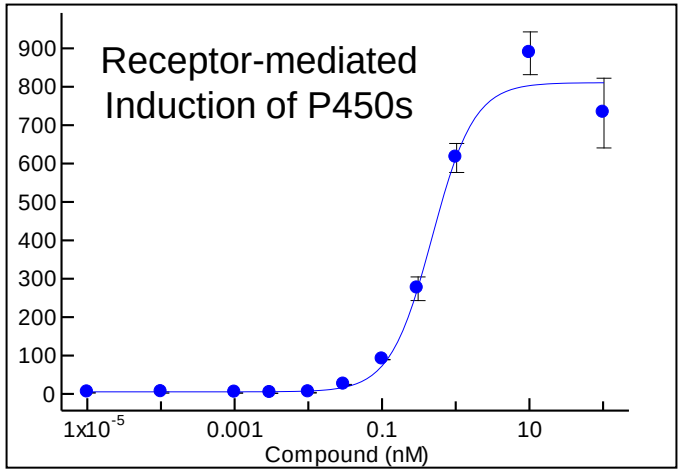
Drug Labelling

negative

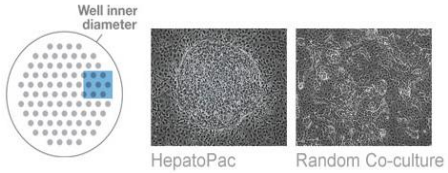
positive

Clinical trials





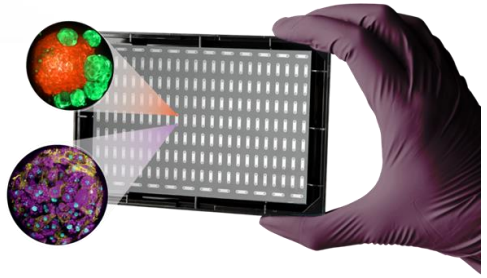
Array of MPS Platforms

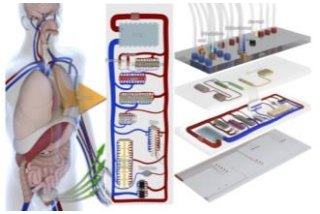


Micropatterned co-cultures

AkuraFlow™

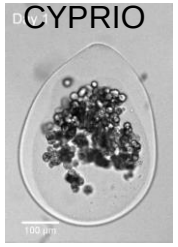
AkuraTwin™



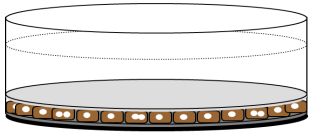


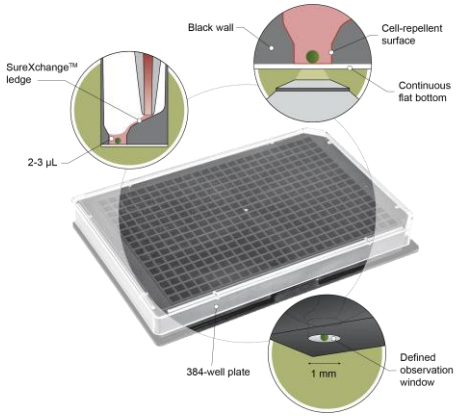
TissUSE

CYPRIO



Sandwich cultures

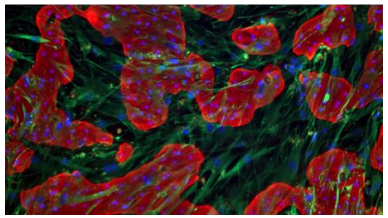




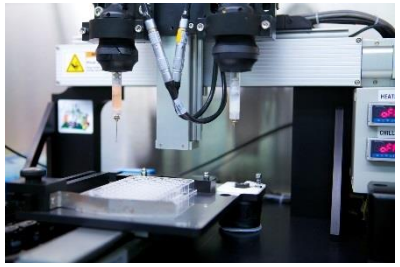
InSphero Akura™

ULA microplate

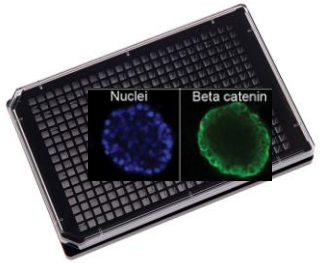
TruVivo®



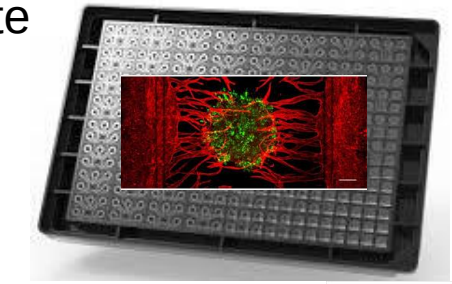
Emulate



3D Bioprinting

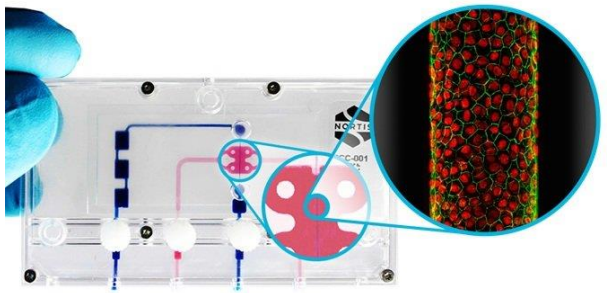


Hydrogel-based 3D models



Mimetas

Elplasia



Nortis ParVivo™

Throughput

TEX-VAL Liver Models: 2023 -2025

- Onboarding TissUse and Micro-Patterned Co-Culture (MPCC)
- TissUse liver “ring trial” (6 pharma companies and TEX-VAL)
- Comparison of perfused and static spheroids
- Comparison of hepatocytes from different species to HepaRG and iPSC-derived hepatocytes
- Gut-liver models (TissUse and Transwell-based)

Slide from Ivan Rusyn

Perfused and static spheroids

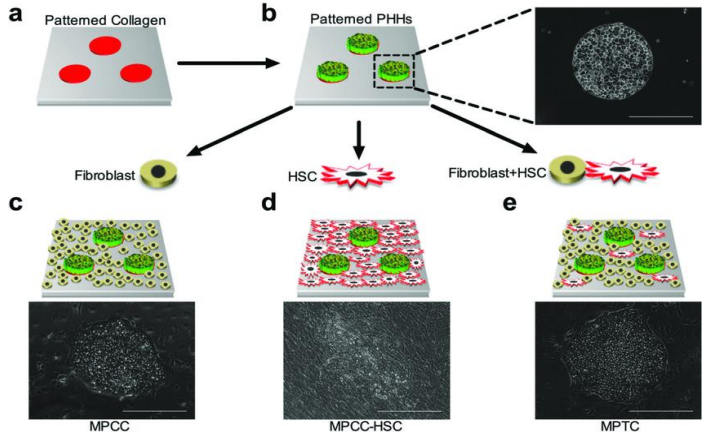
3D-TissUse (94 microcavity/chip) 100,000 cells/chip (6 Chips)

2D-96 well plate 70,000 cells/well

3D-Elplasia ULAP (79 microcavity/wells) 40,000 cells/well

3D-GravityTRAP ULAP (1 spheroid/well) 2000 cells/well

Micro-Patterned Co-Culture



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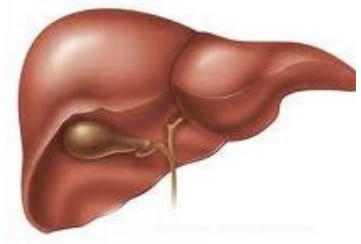
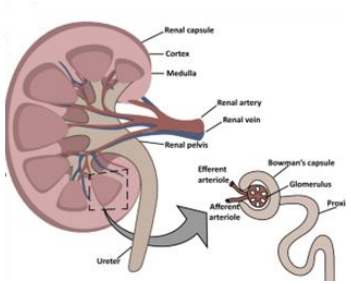
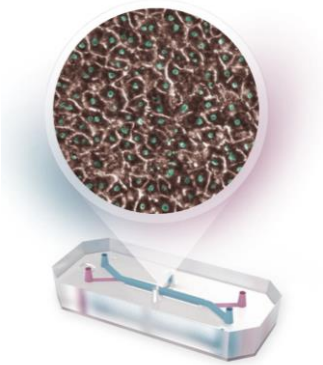
Division of Translational Toxicology

Predictive Toxicology & Screening Group

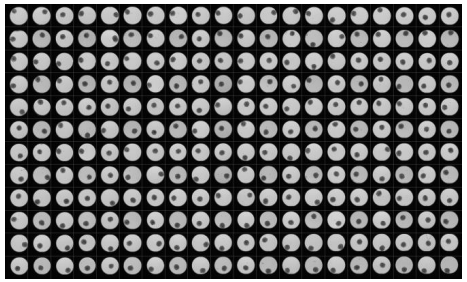
Microphysiological Systems (MPS) → **Biomarker & High-dimensional Assays** → **Computational Models & Human Translation**

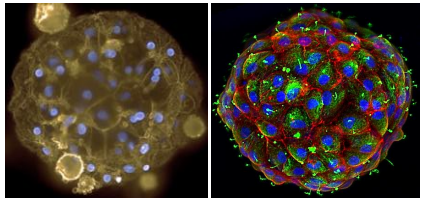


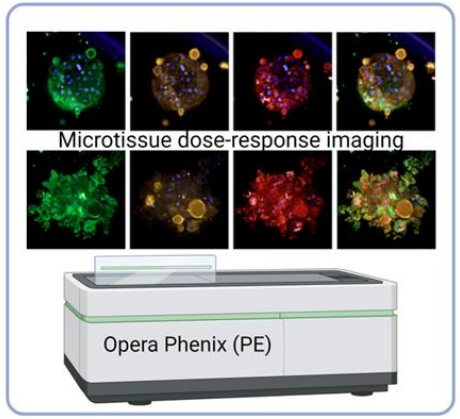


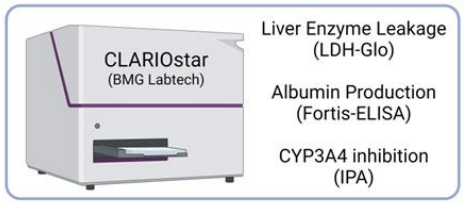


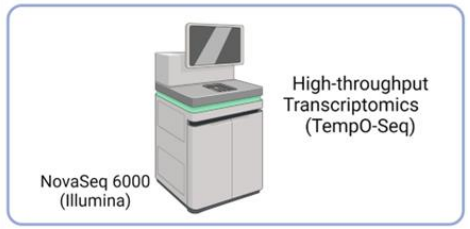
Emulate Tissue Chip Platform



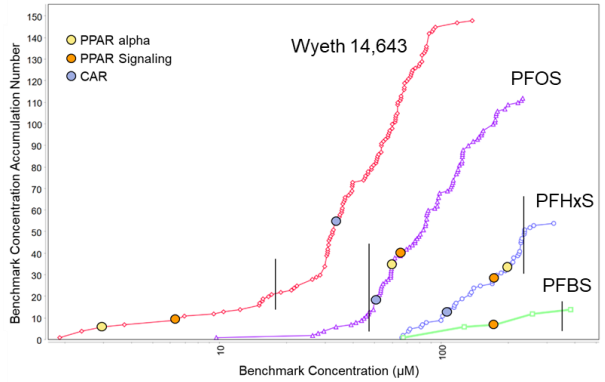


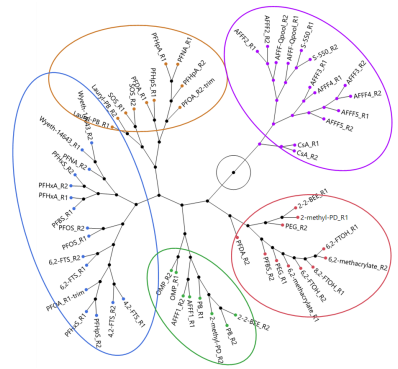






Predicting Liver Weight LOEL from Mechanistic Pathway Potencies





National Institute of Environmental Health Sciences
Division of Translational Toxicology

Extending emerging science for human translation

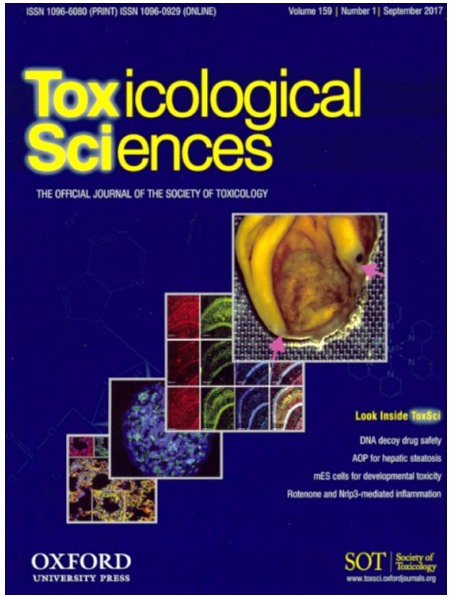
> Toxicol Sci. 2017 Sep 1;159(1):124-136. doi: 10.1093/toxsci/kfx122.

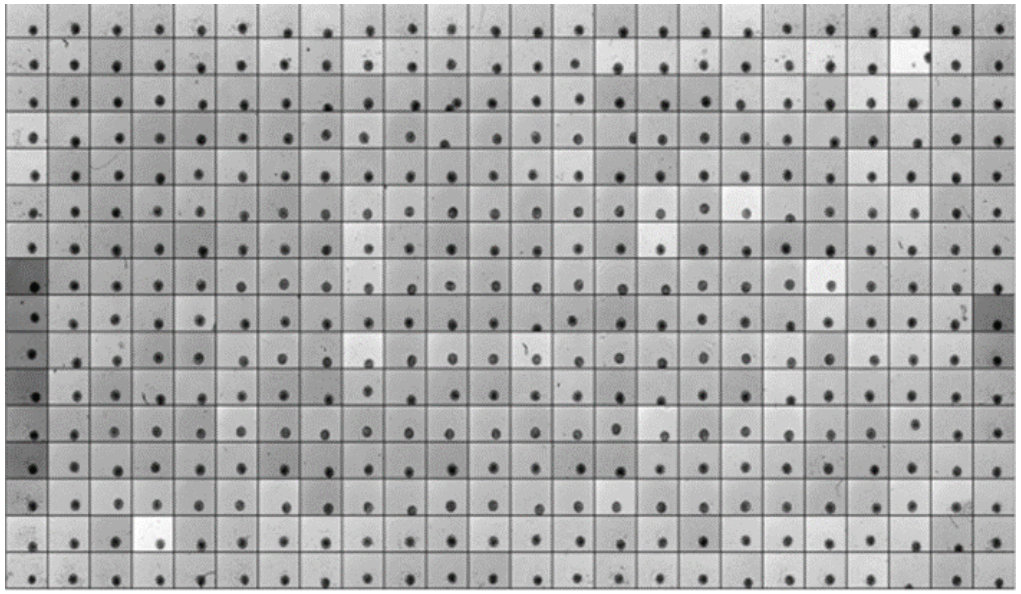
From the Cover: Three-Dimensional (3D) HepaRG Spheroid Model With Physiologically Relevant Xenobiotic Metabolism Competence and Hepatocyte Functionality for Liver Toxicity Screening

Sreenivasa C Ramaiahgari [1], Suramya Waidyanatha [1], Darlene Dixon [1], Michael J DeVito [1], Richard S Paules [1], Stephen S Ferguson [1]

Affiliations + expand

PMID: 28633424 PMCID: PMC5837526 DOI: 10.1093/toxsci/kfx122

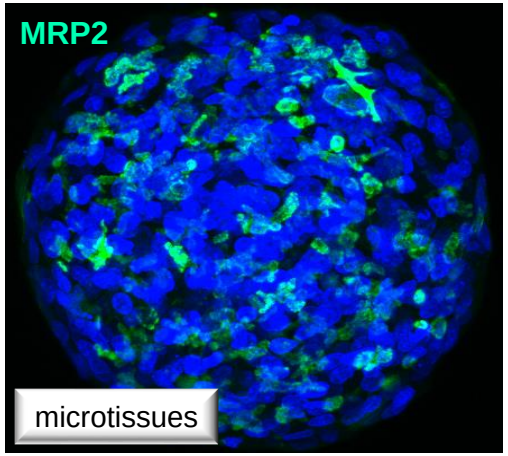


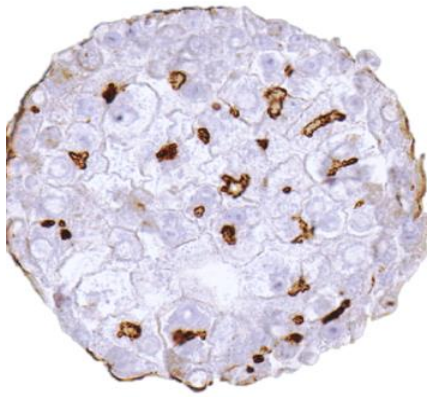


1 vial ~10 million cells = 12-25 X 384-well plates

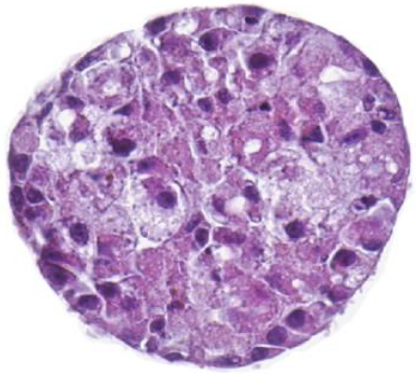
- SOT Best Paper Award by Postdoctoral Fellow
- NIEHS Paper of the Month
- NIH FARE Award

Sreeni Ramaiahgari





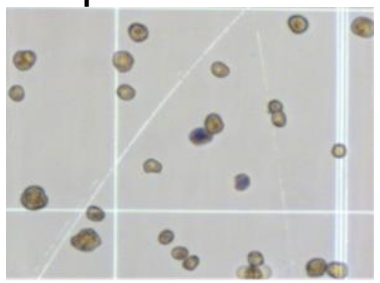
MRP2



H&E

Metabolic Competence

Suspension PHHs



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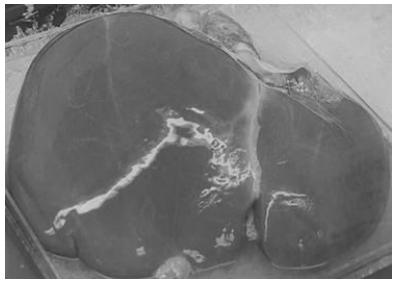
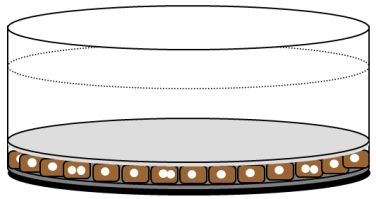
Human Liver



SC-PHHs



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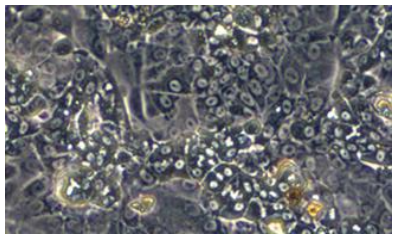
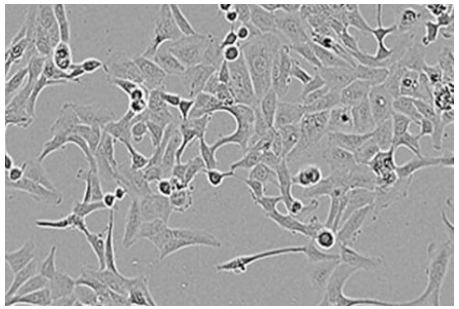
2D HepaRG

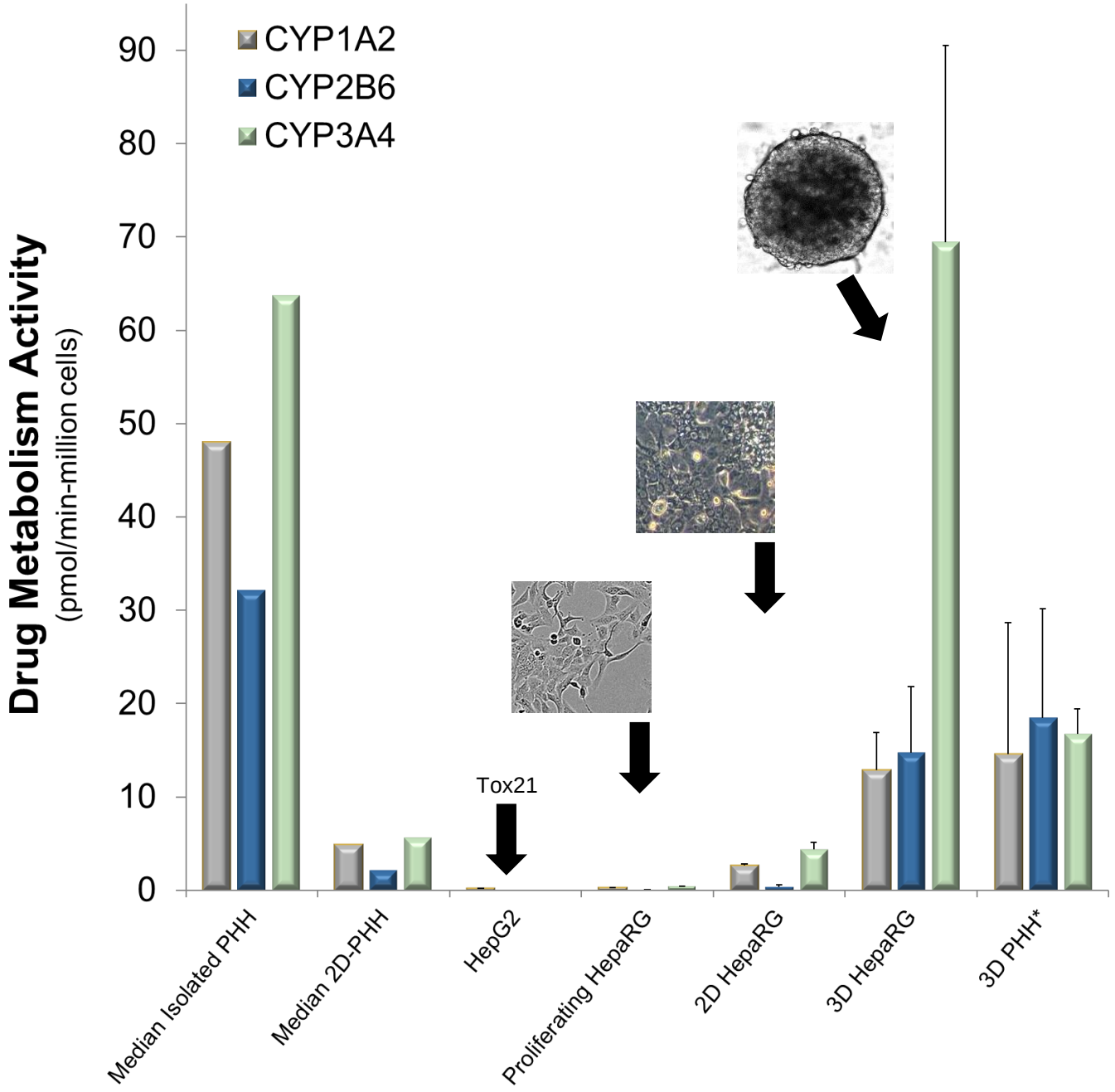


Proliferating HepaRG



iPSC-derived hepatocytes

Transformed cell lines
(e.g., HepG2)



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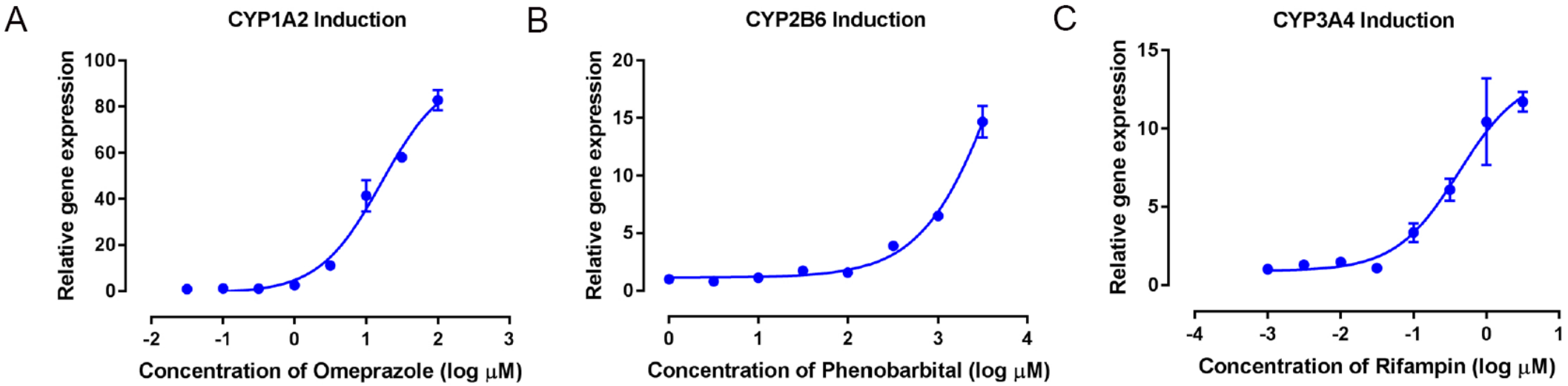
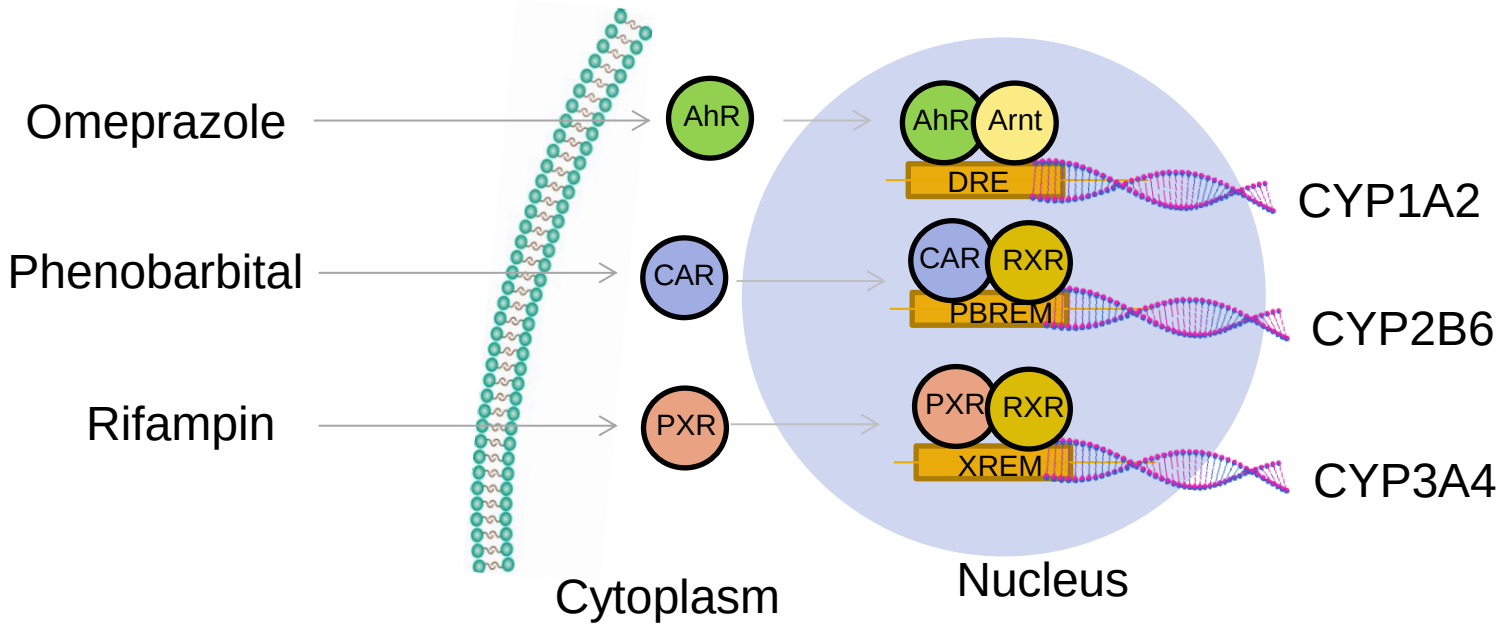
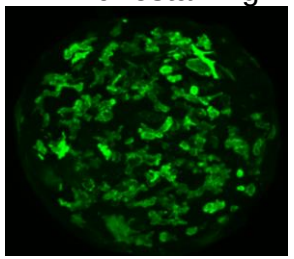
Division of the National Toxicology Program

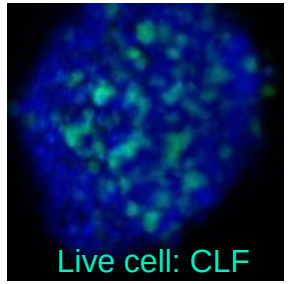
Robust Xenobiotic Metabolism & Responsiveness for Hepatic Receptor Pathways with 3D HepaRG Spheroids (AhR, CAR, & PXR)





MRP2 Immunostaining





Ramaiahgari et al., Toxicol Sci (2017) v.159 (1): 124-136

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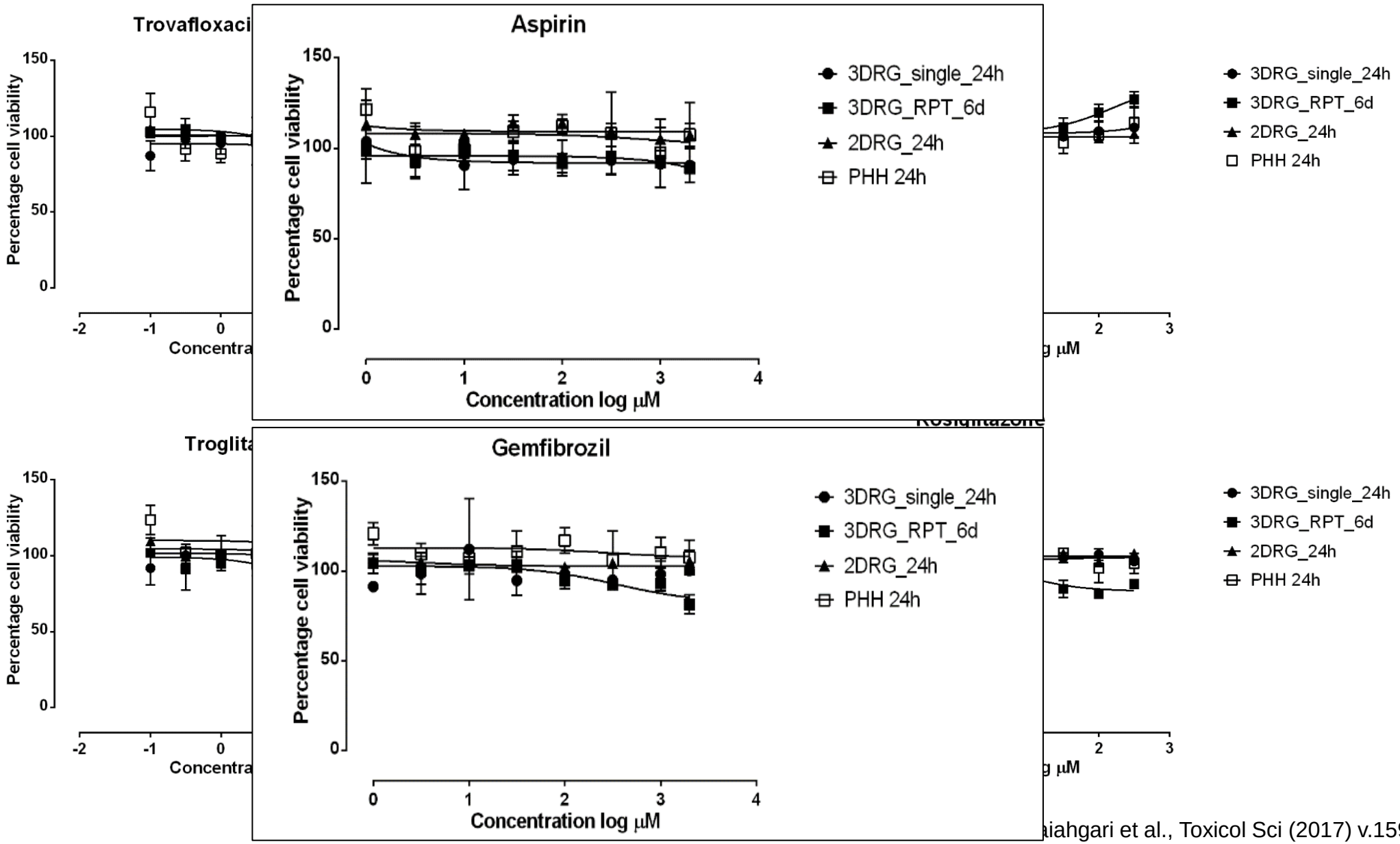
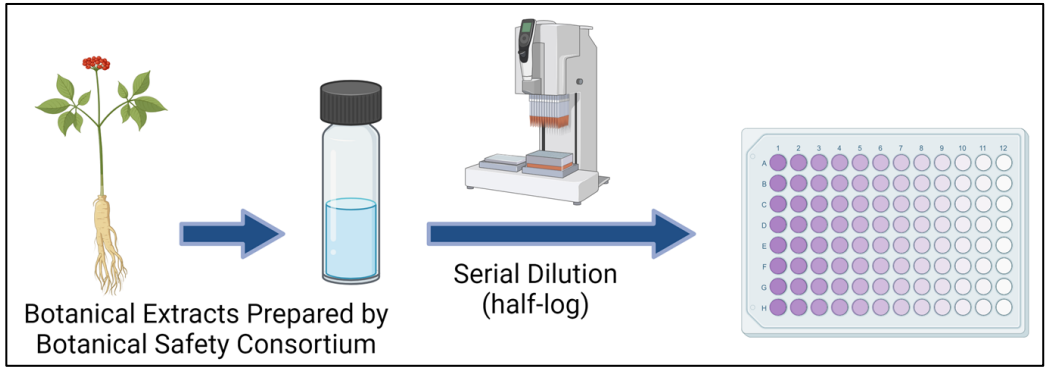
HepaRG Spheroids & ATP Depletion Model Human Liver Injury (e.g., Case Study Drug Analogues)



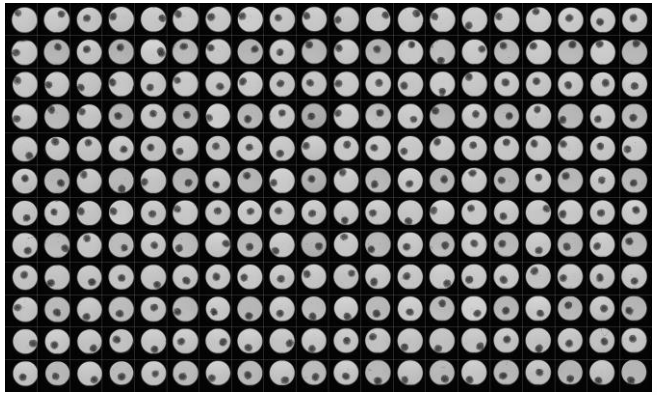
Haiahgari et al., Toxicol Sci (2017) v.159 (1): 124-136

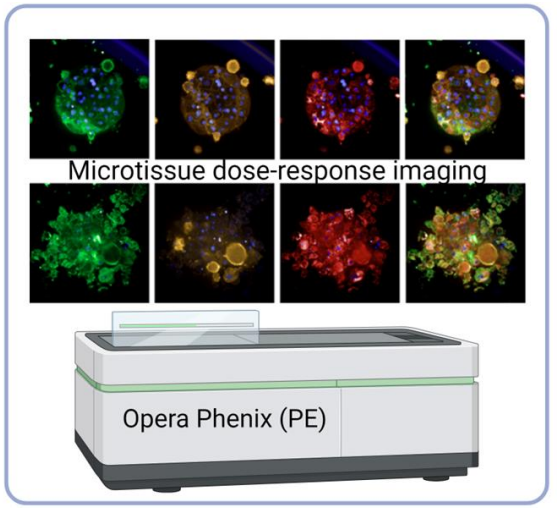
National Institute of Environmental Health Sciences
Division of Translational Toxicology

Botanical Safety Consortium Hepatotoxicity



Repeated-dose exposures (96h)





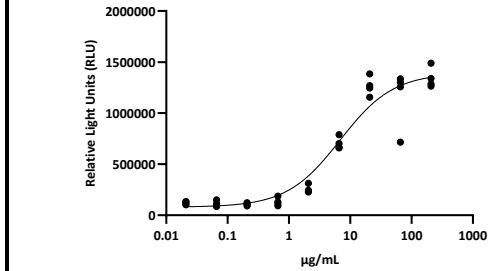
Liver Enzyme Leakage (LDH-Glo)

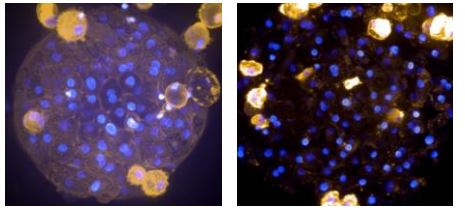
Albumin Production (Fortis-ELISA)

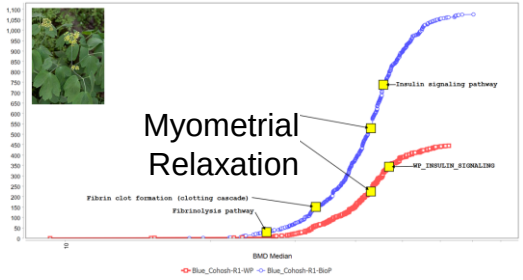
CYP3A4 inhibition (IPA)

High-throughput Transcriptomics (TempO-Seq)

BOTANICAL SAFETY CONSORTIUM



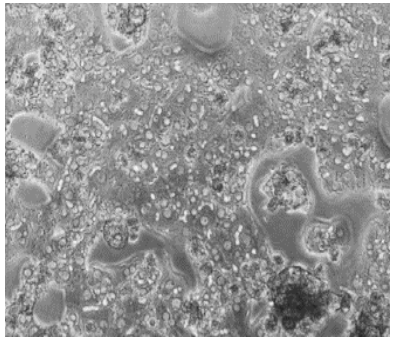




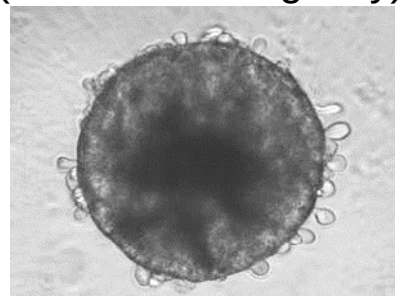
Postdoctoral Fellow
Adam Pearson, Ph.D.

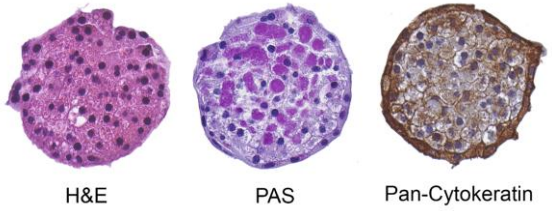
Integrated model designs for human translation

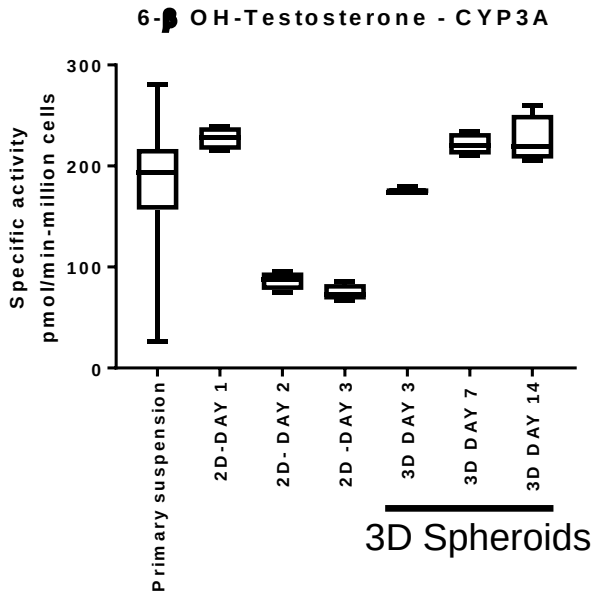
(3-5 days longevity)

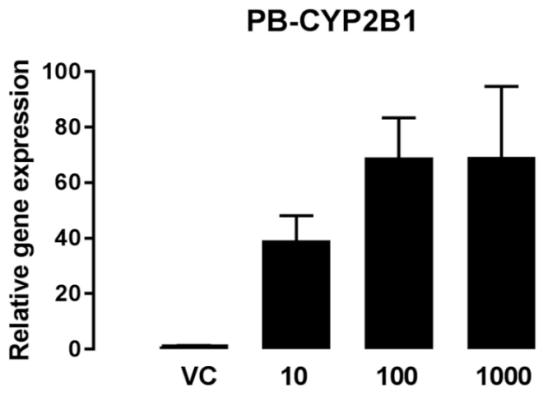


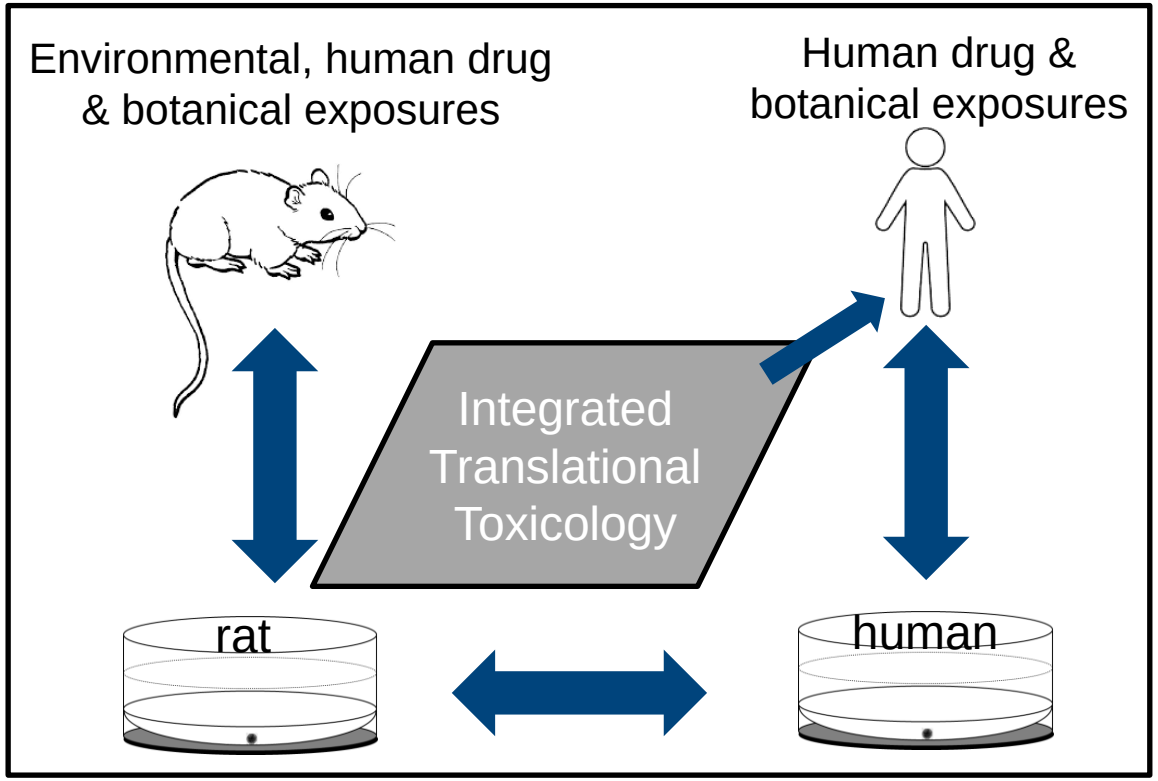
(>2 months longevity)











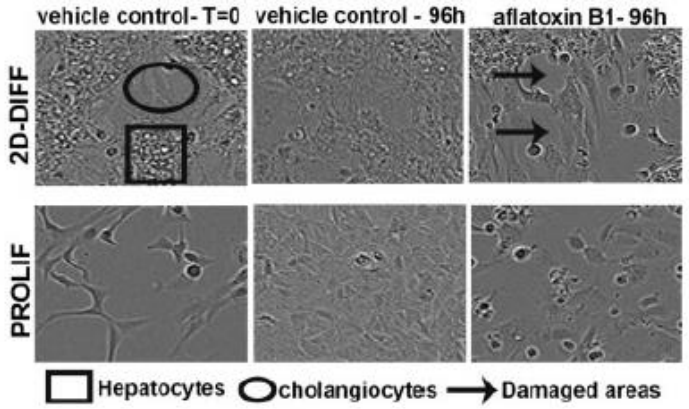
Estimating potency ranges for human liver injury with transcriptomics

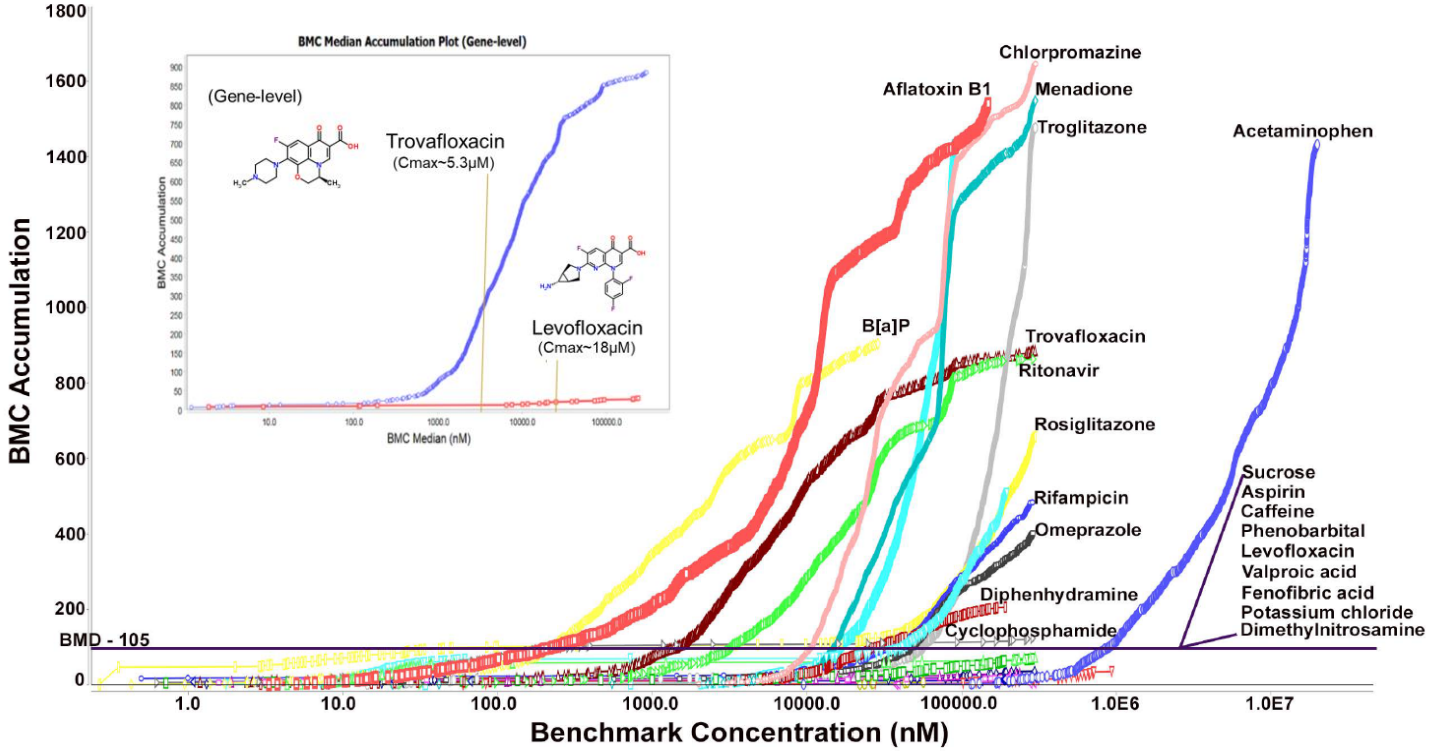
The Power of Resolution: Contextualized Understanding of Biological Responses to Liver Injury Chemicals Using High-throughput Transcriptomics and Benchmark Concentration Modeling FREE

Sreenivasa C Ramaiahgari, Scott S Auerbach, Trey O Saddler, Julie R Rice, Paul E Dunlap, Nisha S Sipes, Michael J DeVito, Ruchir R Shah, Pierre R Bushel, Bruce A Merrick, Richard S Paules, Stephen S Ferguson ✉

Toxicological Sciences, Volume 169, Issue 2, June 2019, Pages 553–566, https://doi.org/10.1093/toxsci/kfz065

- Readily distinguished drug analogues with varied clinical DILI associations
- Established novel approach to predict potencies for human liver injury (BMC105)
- Potency-ordered ‘firing sequences’ revealed
- Integration of biomarkers for clinical pathology & cell morphology imaging





Translating MPS Data to Risk Assessment & Safer Product Development Development

Environmental Science & Technology

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Article

Unraveling Human Hepatocellular Responses to PFAS and Aqueous Film-Forming Foams (AFFFs) for Molecular Hazard Prioritization and In Vivo Translation

Kevin A. Mauge-Lewis,[#] Sreenivasa C. Ramaiahgari,[#] Scott S. Auerbach, Georgia K. Roberts, Suramya Waidyanatha, Suzanne E. Fenton, Dhiral P. Phadke, Michele R. Balik-Meisner, Arpit Tandon, Deepak Mav, Brian Howard, Ruchir Shah, Barney Sparrow, Jenni Gorospe, and Stephen S. Ferguson*

Cite This: *Environ. Sci. Technol.* 2025, 59, 2423–2435

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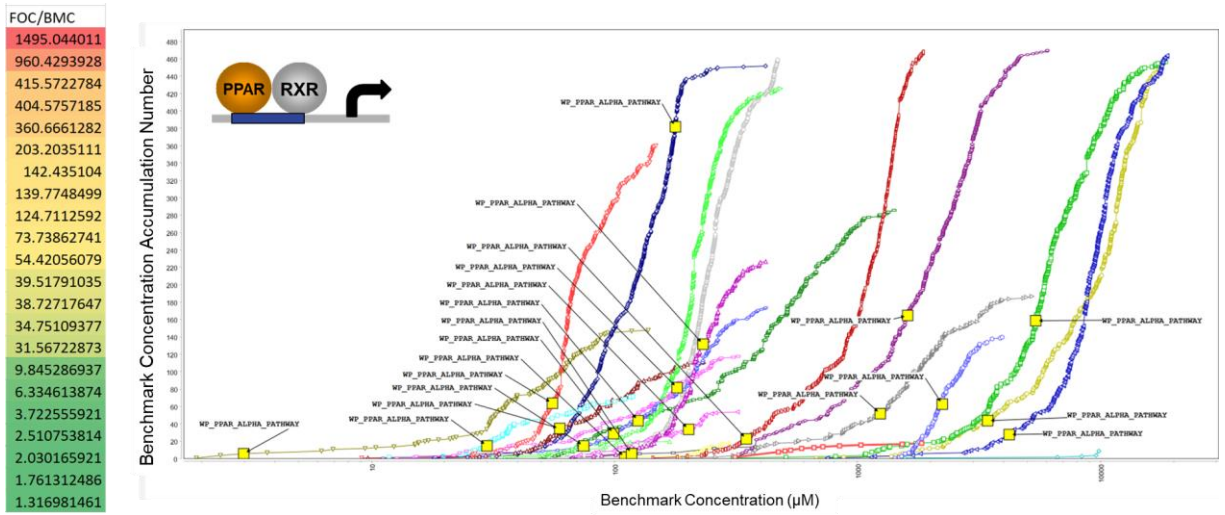
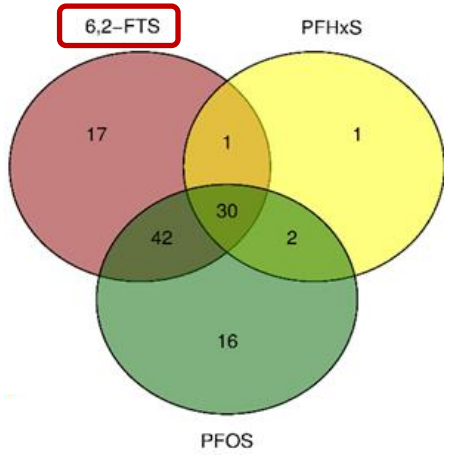
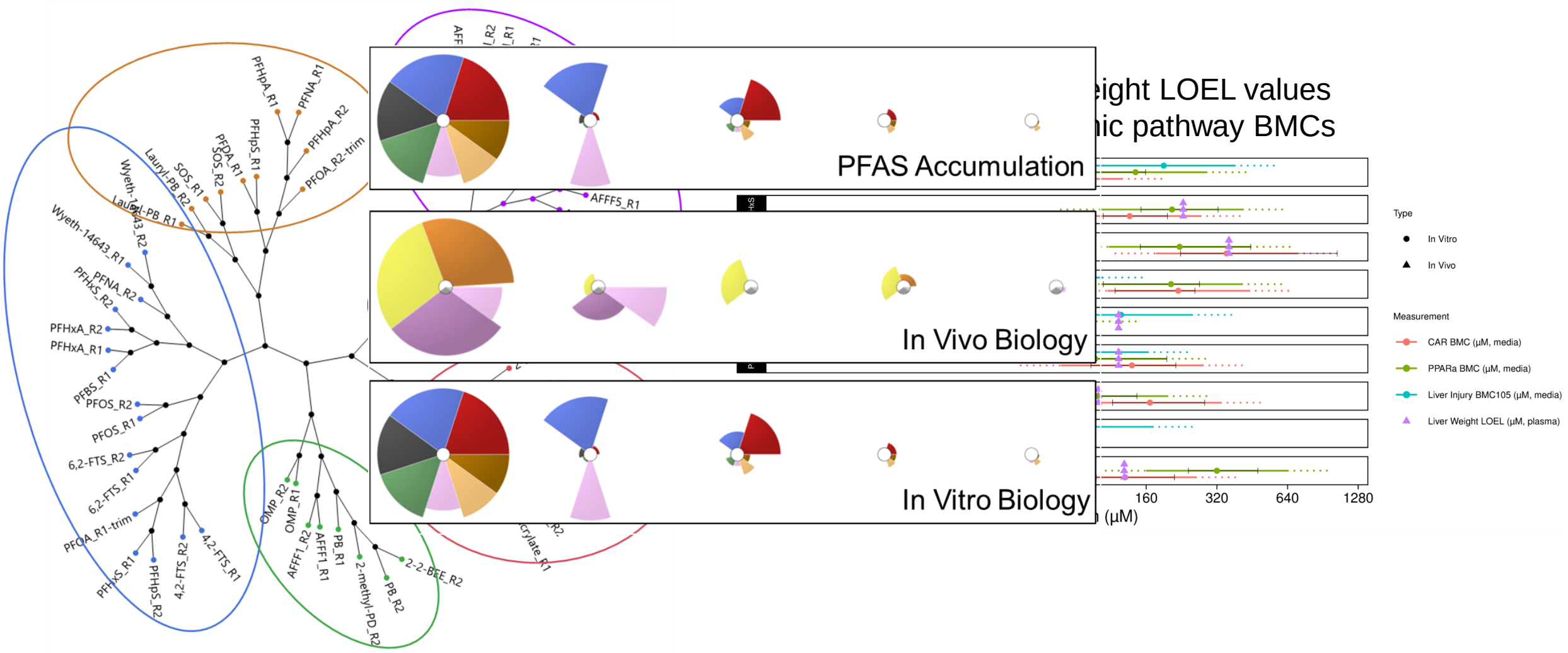




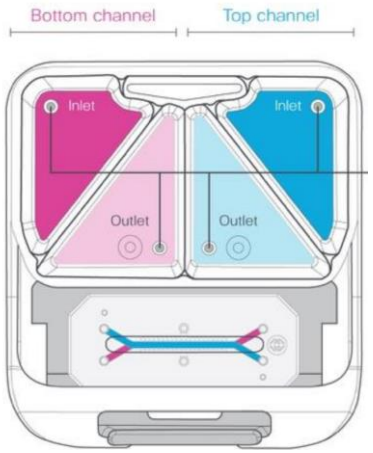
- Predicting potencies for human liver injury with PFAS and AFFF mixtures alongside human drugs
  - Machine learning image analysis
  - Clinical pathology biomarkers
  - Transcriptomics thresholding
- Identifying mechanistic pathway potencies for 30 substances (PFAS, AFFF, human drugs)
- Defining biological response similarities across PFAS and AFFF in context with hepatic receptor agonists

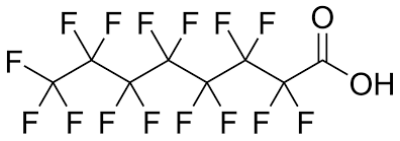
Filling Data Gaps with Mechanistic Read-across



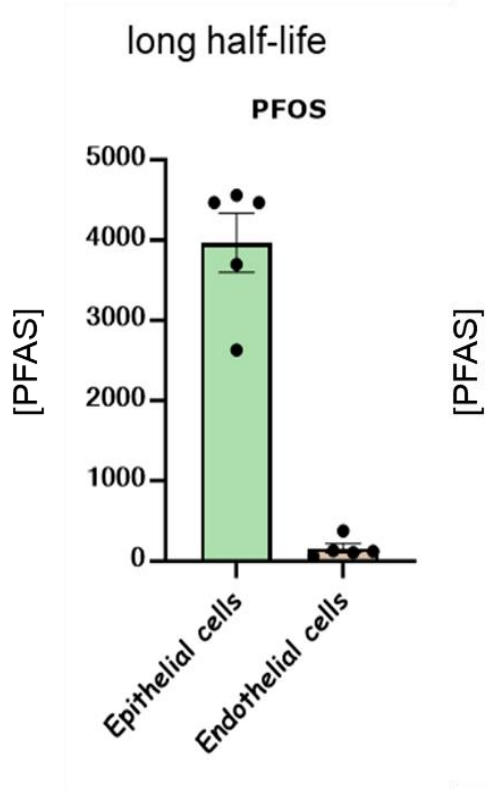
Modeling PFAS Bioaccumulation via Renal Re-uptake

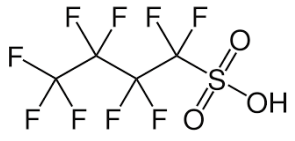
Emulate Tissue Chip Platform



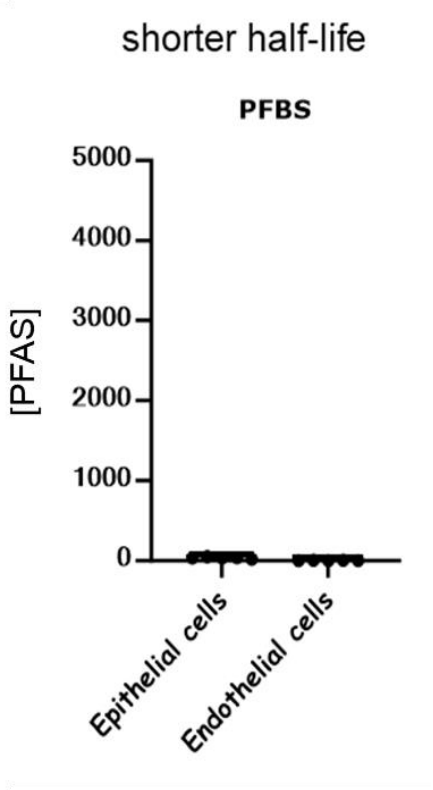


long half-life





shorter half-life



- Renal re-uptake of PFAS has been a particularly concerning from environmental exposures
- Established renal PT barrier models with the Emulate tissue chip platform
- Effective classification of long and short half-life PFAS (complex mixture) using apical, but not basolateral, exposures
- Data support the hypothesized renal re-uptake mechanism for PFOS plasma accumulation.

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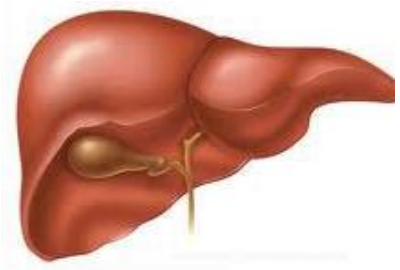
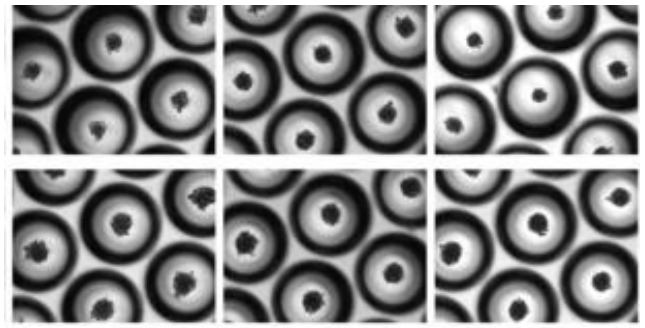
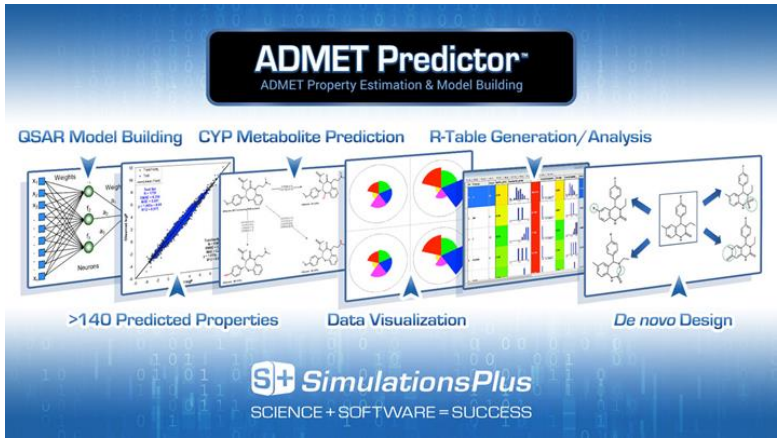
PFAS Mixtures Accumulation with MPS

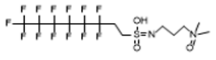


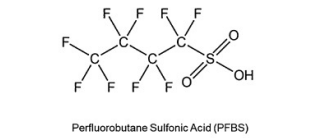
Pooled Microtissues

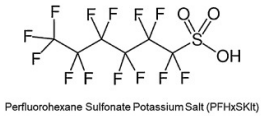


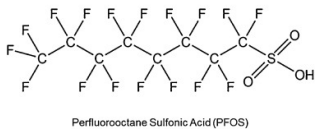


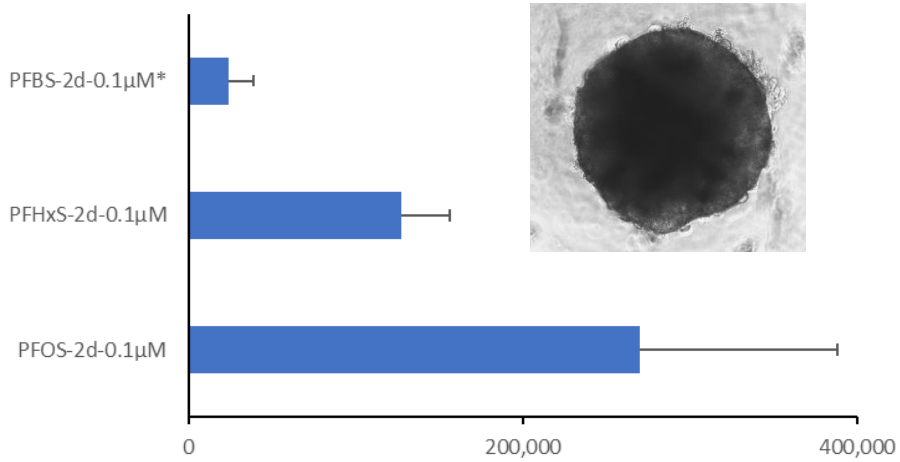
In Silico PFAS Bioaccumulation Predictions

PFAS	Structure	In Silico BCF (Fish/Water)	In Silico Hepatic $Cl_{INT,metab}$
PFOS		682	5.5
PFHxS		431	5.5
PFBS		28	3.1
4:4 FTOH		401	40
6:2-FTNO		97	19









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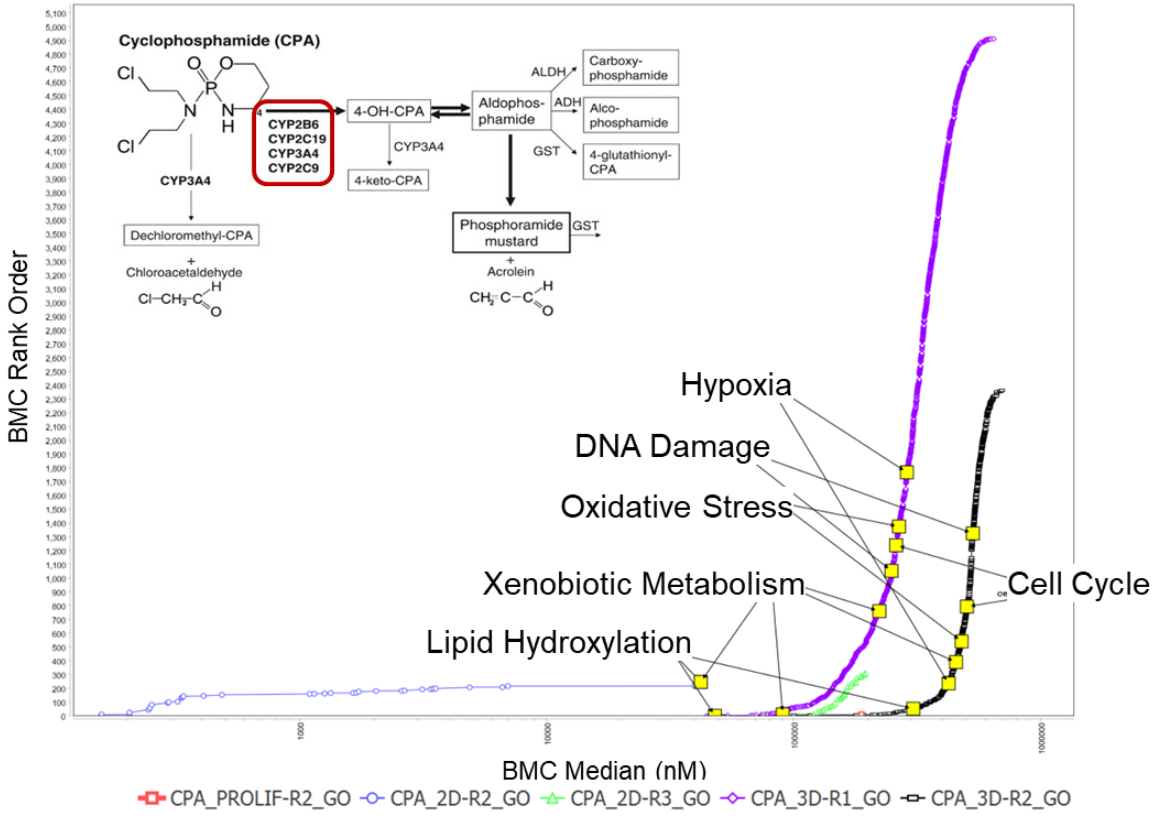
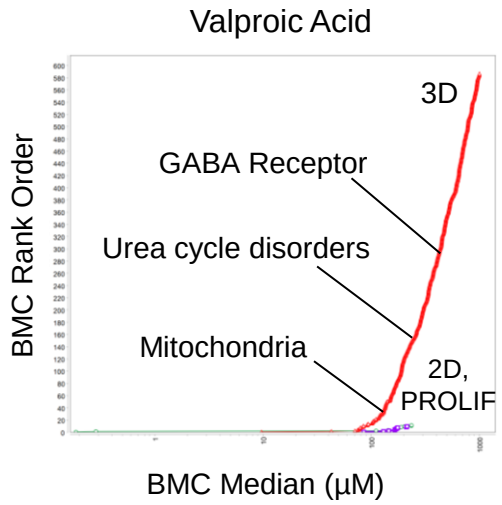
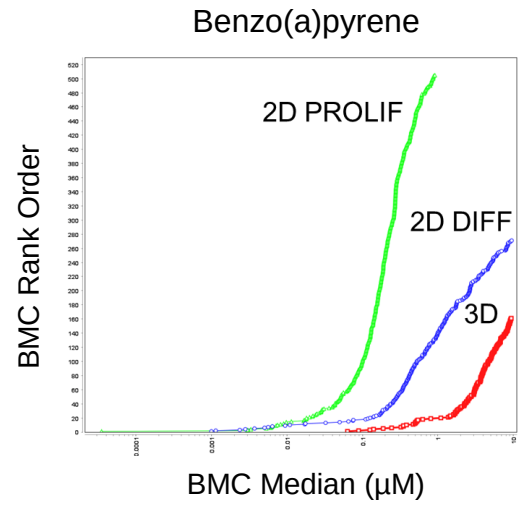
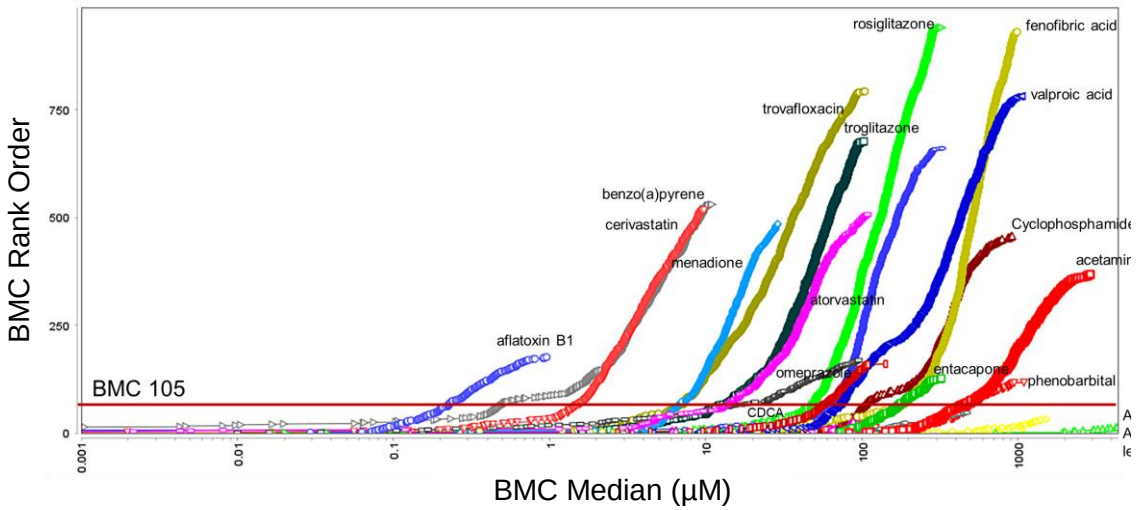
Enhanced DILI modeling with 3D microtissues





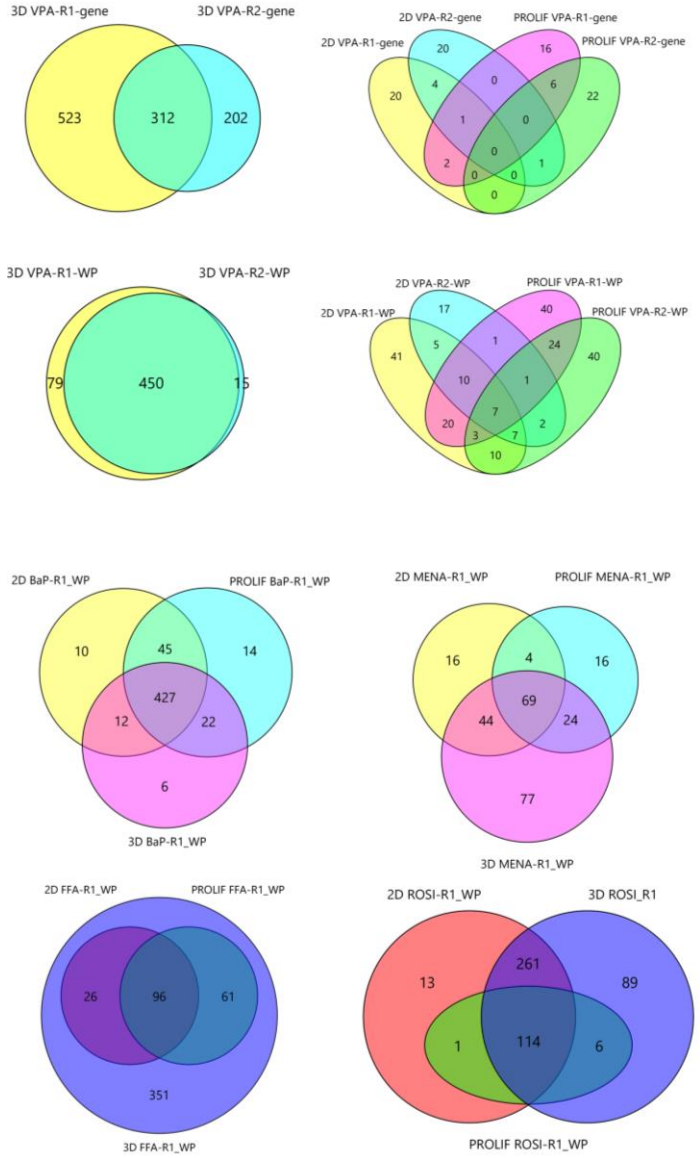




- **Valproic Acid & cyclophosphamide more sensitive in 3D**
- **Benzo(a)pyrene and aflatoxin more sensitive in 2D PROLIF mode**

National Institute of Environmental Health Sciences
Division of the National Toxicology Program

Transcriptomic Pathway Enrichment Enhanced for Known Pathways with 3D Hepatocyte Cultures

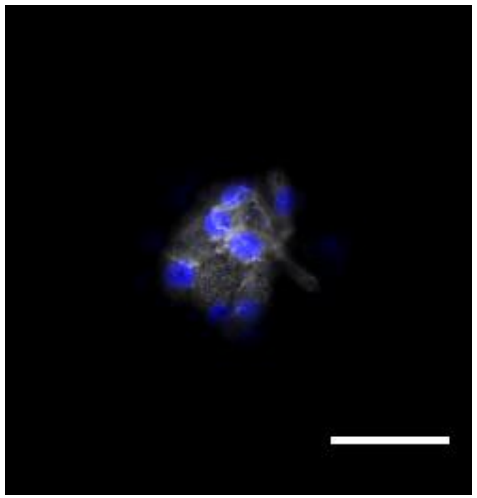


Drug	Culture Mode	WikiPathway	BMCs Identified in Pathway
Valproic Acid	3D	Non-alcoholic Fatty Liver Disease	27
Valproic Acid	2D	Non-alcoholic Fatty Liver Disease	0
Valproic Acid	PROLIF	Non-alcoholic Fatty Liver Disease	0
Valproic Acid	3D	GABA Receptor Signaling	4
Valproic Acid	2D	GABA Receptor Signaling	0
Valproic Acid	PROLIF	GABA Receptor Signaling	0
Valproic Acid	3D	PI3KAKT Signaling Pathway	73
Valproic Acid	2D	PI3KAKT Signaling Pathway	0
Valproic Acid	PROLIF	PI3KAKT Signaling Pathway	0
Afltx B1	3D	DNA Damage Response	36
Afltx B1	2D	DNA Damage Response	54
Afltx B1	PROLIF	DNA Damage Response	47
B(a)P	3D	Oxidation by Cytochrome P450	21
B(a)P	2D	Oxidation by Cytochrome P450	12
B(a)P	PROLIF	Oxidation by Cytochrome P450	13
Cyclophos	3D	Cell Cycle	51
Cyclophos	2D	Cell Cycle	4
Cyclophos	PROLIF	Cell Cycle	1
Fenofibric Acid	3D	PPAR Signaling Pathway	28
Fenofibric Acid	2D	PPAR Signaling Pathway	13
Fenofibric Acid	PROLIF	PPAR Signaling Pathway	12
Menadione	3D	Oxidative Stress	7
Menadione	2D	Oxidative Stress	9
Menadione	PROLIF	Oxidative Stress	5
Omeprazole	3D	Aryl Hydrocarbon Receptor Pathway	17
Omeprazole	2D	Aryl Hydrocarbon Receptor Pathway	12
Omeprazole	PROLIF	Aryl Hydrocarbon Receptor Pathway	6
Omeprazole	3D	Gastrin Signaling Pathway	7
Omeprazole	2D	Gastrin Signaling Pathway	5
Omeprazole	PROLIF	Gastrin Signaling Pathway	2
Phenobarbital	3D	Constitutive Androstane Receptor	19
Phenobarbital	2D	Constitutive Androstane Receptor	12
Phenobarbital	PROLIF	Constitutive Androstane Receptor	5
Rosiglitazone	3D	PPAR Signaling Pathway	28
Rosiglitazone	2D	PPAR Signaling Pathway	12
Rosiglitazone	PROLIF	PPAR Signaling Pathway	9
Troglitazone	3D	PPAR Signaling Pathway	21
Troglitazone	2D	PPAR Signaling Pathway	10
Troglitazone	PROLIF	PPAR Signaling Pathway	9

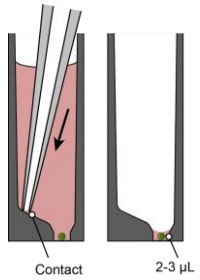
National Institute of Environmental Health Sciences
Division of Translational Toxicology

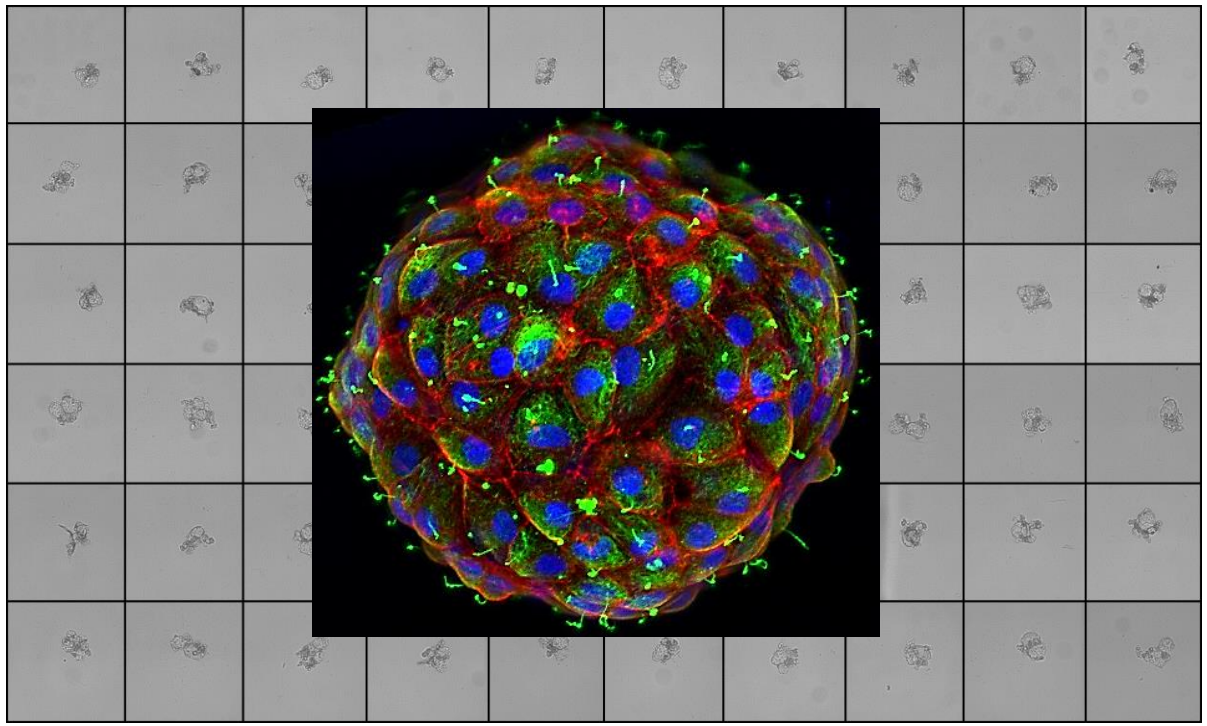
Expanding Biological Coverage: human renal proximal tubuloids

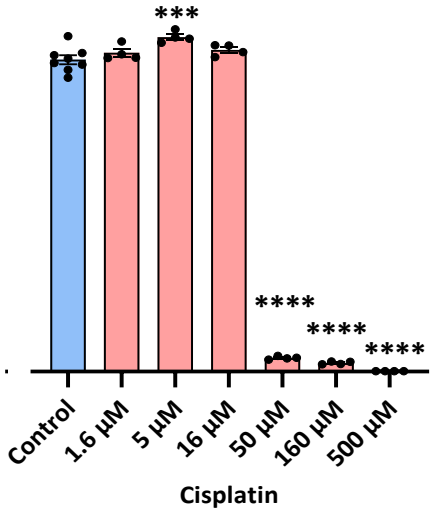
Adam Pearson

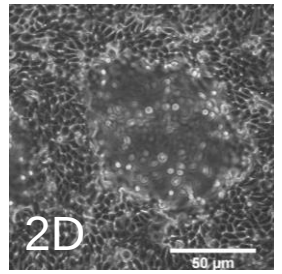


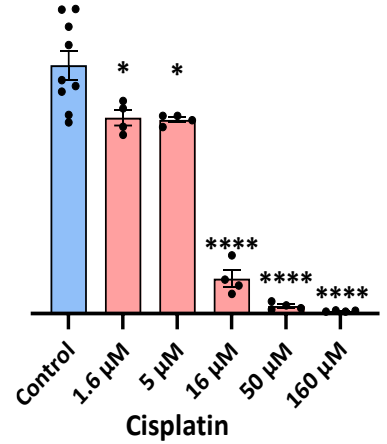
InSphero Akura™ Plates

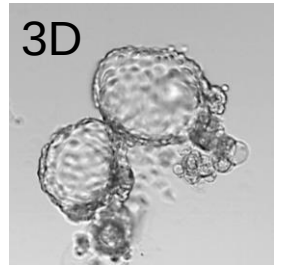






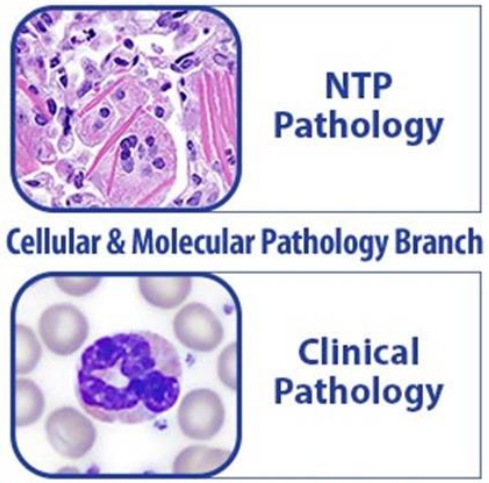


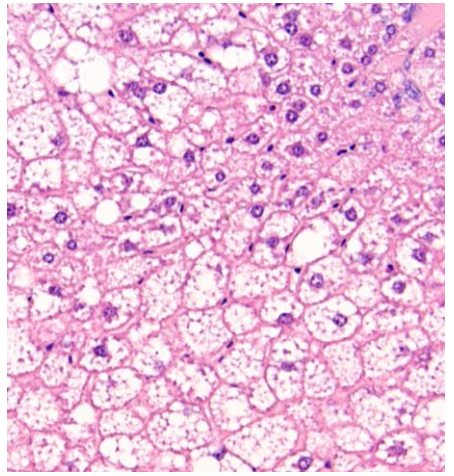


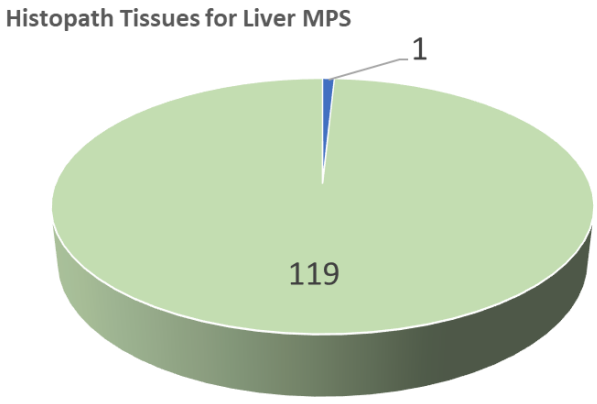


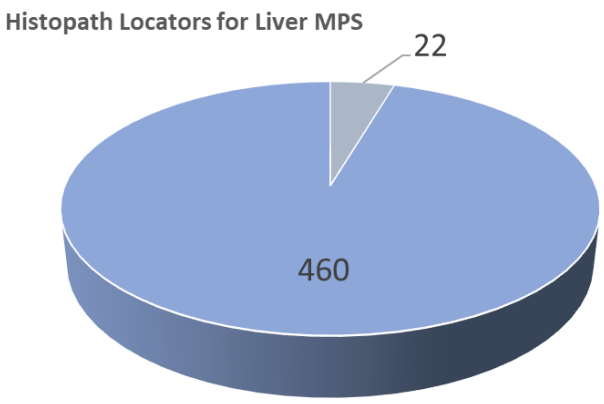
What are we trying to predict with liver MPS?

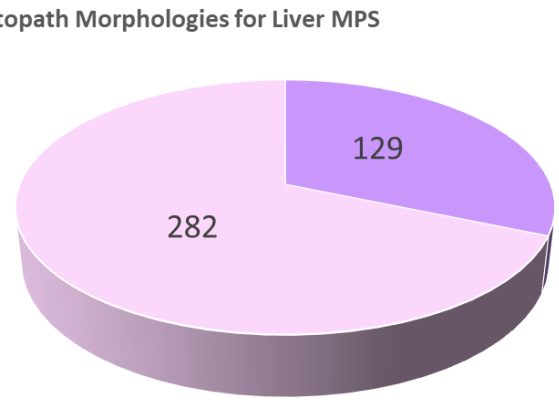
- NTP Histopathology Glossary
  - Mapping diagnostic morphologies feasible with liver MPS
    - Steatosis (micro and microvesicular)
    - Hepatomegaly & Involution (e.g., hypertrophy, hyperplasia)
    - Fibrosis (e.g, zonal-specific)
    - Cholestasis
  - Developing a ‘Rosetta Stone’ to translate liver MPS data to recognizable pathology findings
  - Calibrating MPS with human drug effectors

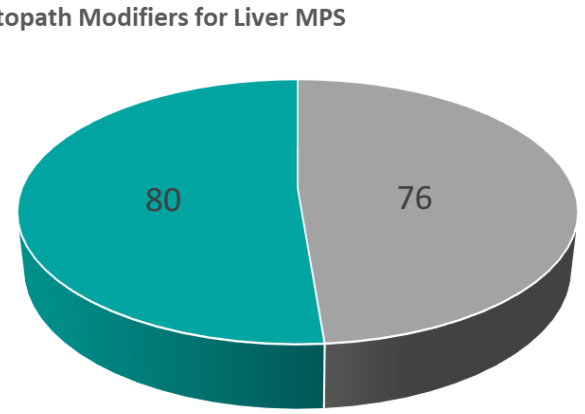






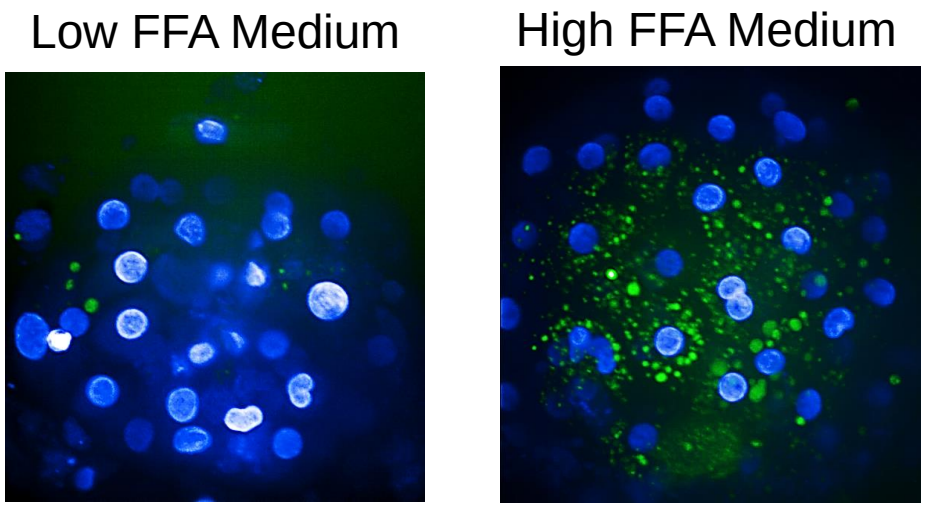






Future Directions

- Extending MPS from promising research tools into NAM-based solutions with capacity to model recognizable toxicological phenotypes
  - Regulatory decision frameworks
  - Safer product development
  - Effective therapeutics



- Addressing interindividual susceptibility
  - age: fetal, neonatal, childhood, adult, geriatric
  - Sex differences
  - Genetic
  - Pre-existing disease states (e.g., steatosis)
- Expanding tissue coverage, axes of pathophysiology (e.g., liver-thyroid & PFAS)

National Institute of Environmental Health Sciences
Division of Translational Toxicology

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