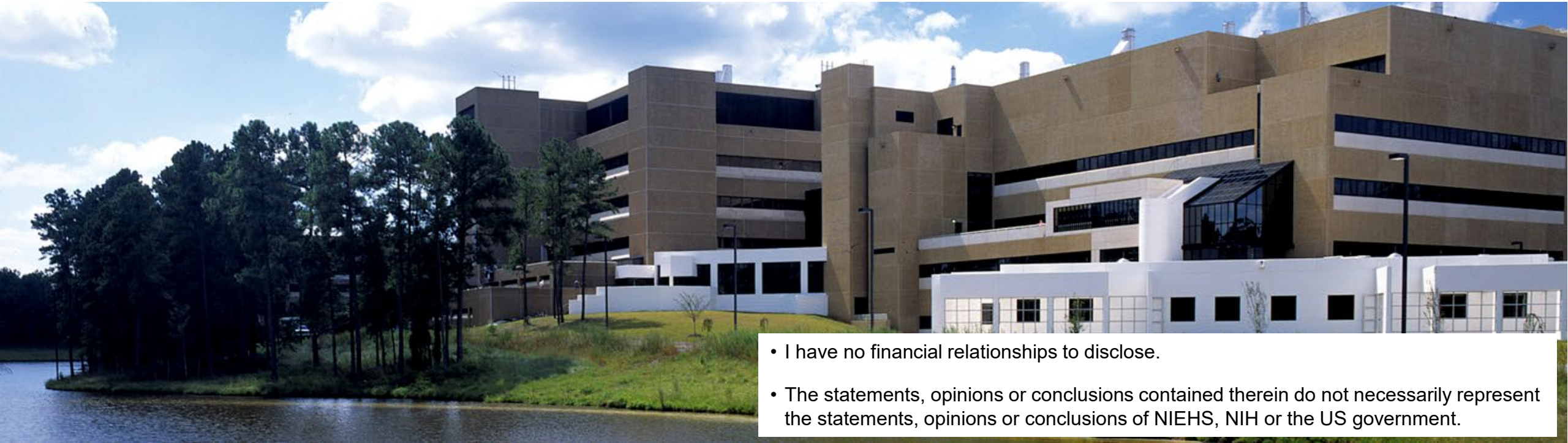
National Institute of Environmental Health Sciences
Division of Translational Toxicology

Updates from the NIEHS Division of Translational Toxicology Predictive Toxicology & Screening Group

Stephen S. Ferguson, Ph.D.
Group Leader



- 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.

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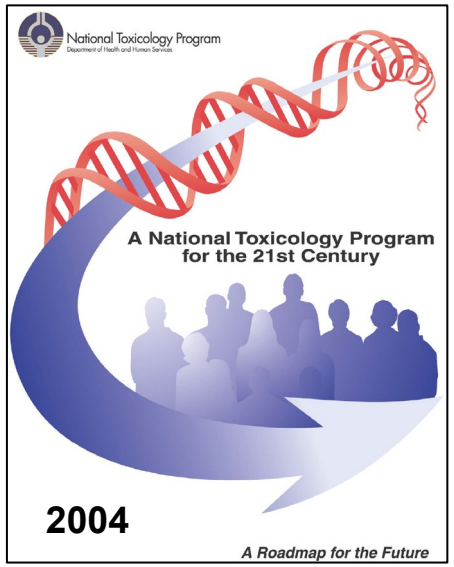
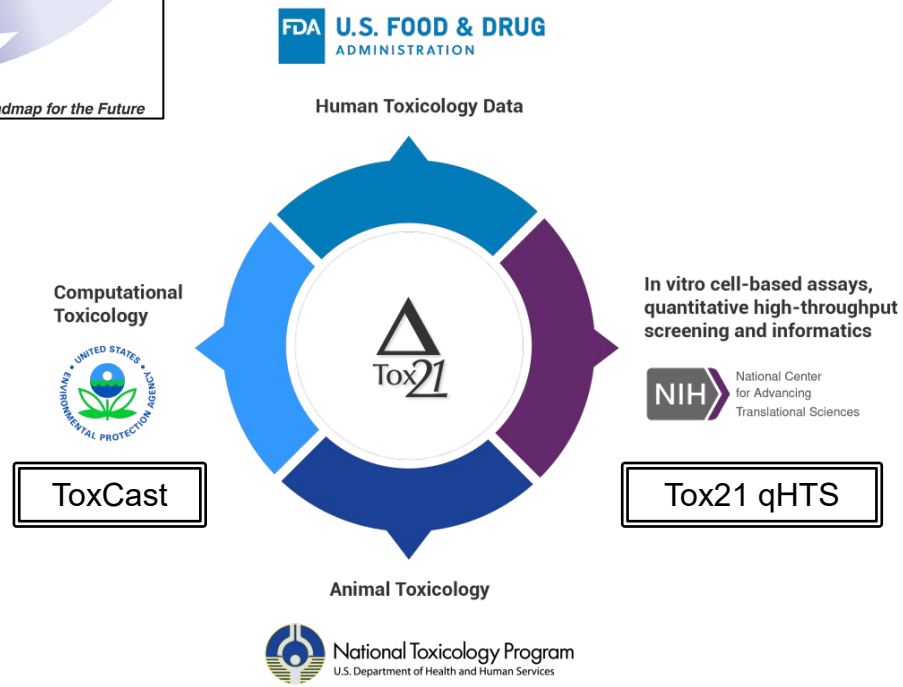
Tox21: Advancing 21st Century Tools to Evaluate & Understand Chemical Safety

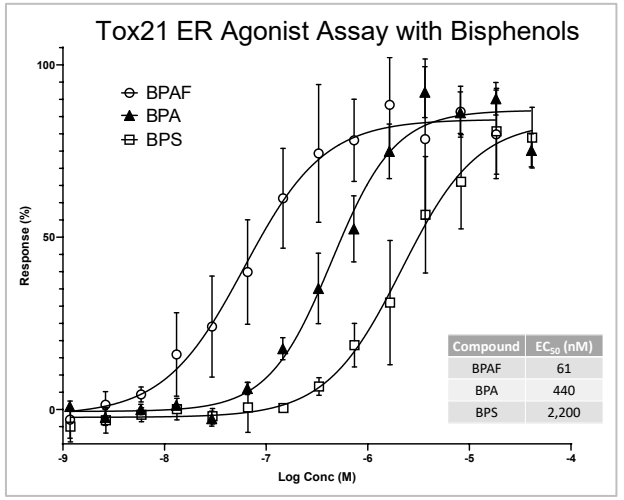
- NIEHS has been a global leader in the strategic exploration, development and application of NAMs
  - Tox21 Program, NIEHS grants, internal R&D
- In partnership with Tox21 partners (i.e., NCATS, US-EPA, US-FDA), Tox21 data for thousands of drugs/chemicals made publicly available
- Tox21 data widely used internationally (e.g., AI), yet limited adoption by regulatory agencies
  - **Why?**

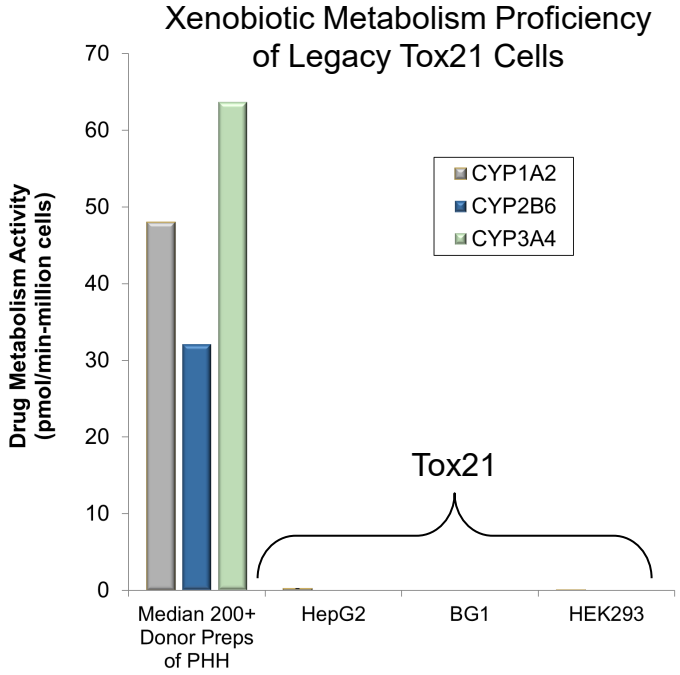




Limitations of Tox21, Evolution towards MPS

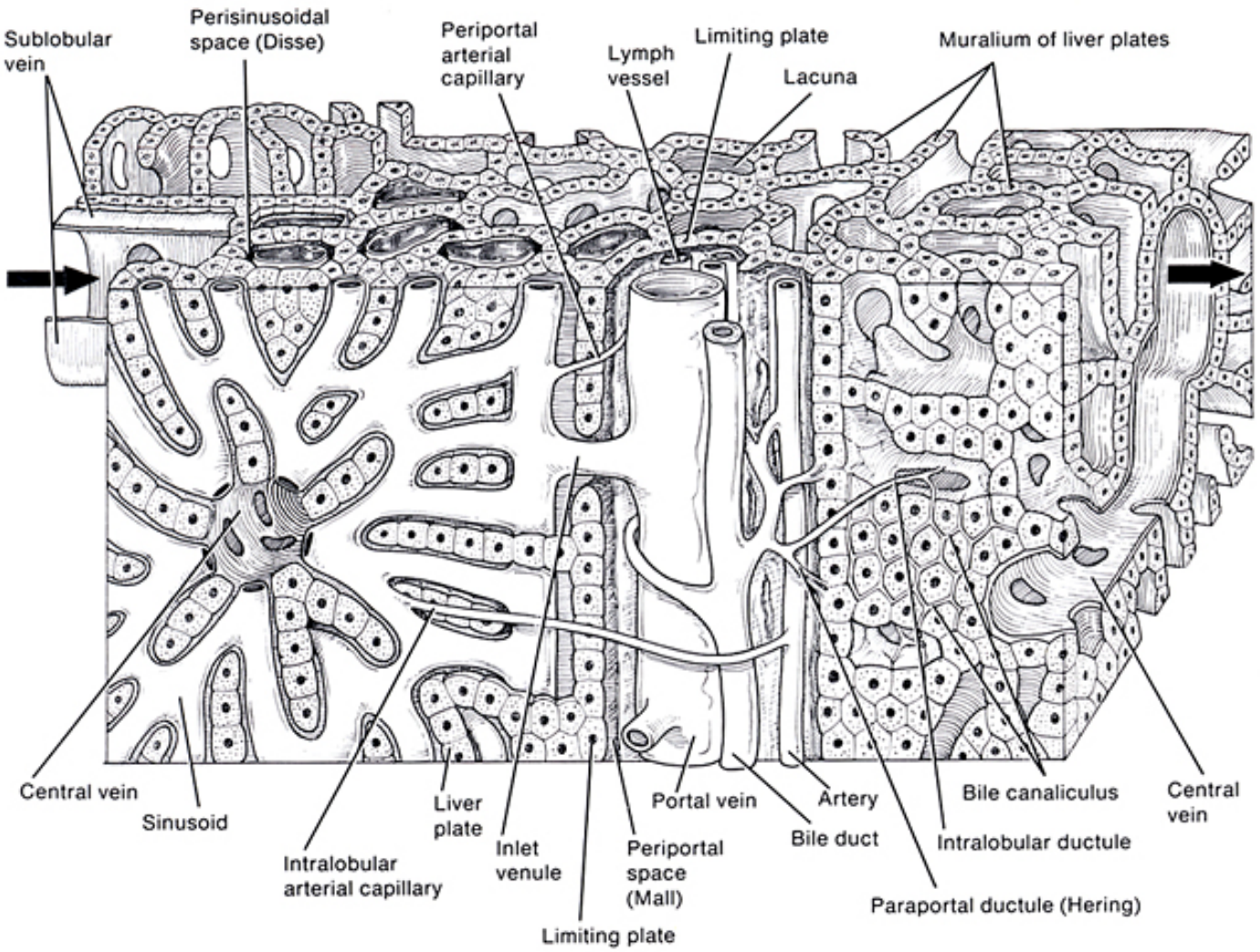
- Toxicologic Pathology not an initial focus for Tox21
  - Molecular targets & stress response pathways the focus
  - NAMs often display limited physiological relevance
- Xenobiotic Metabolism
  - Species differences not addressed without emulation of relevant metabolite profiles, adaptive pathophysiology
- Biological Coverage
  - Initial focus limited to available molecular assays for drug targets and stress response pathways with cancer cell lines
- Translation
  - Likelihood for definable human health effects
  - Intentionally focusing integrated NAMS into regulatory practice
- Uncertainties for Interindividual Susceptibility
  - Lack of NAMs that model key factors in toxicological susceptibility (e.g., sex, age, pre-existing disease)





For Tox21, in the beginning there were HepG2

Physiological architecture of liver



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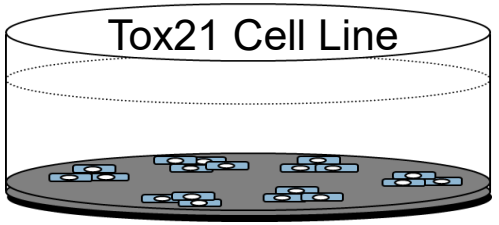
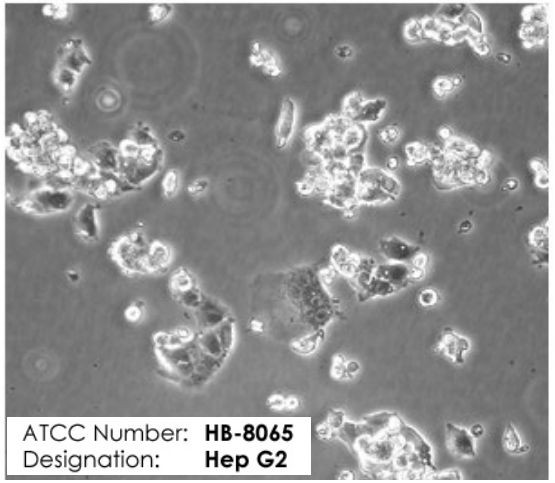
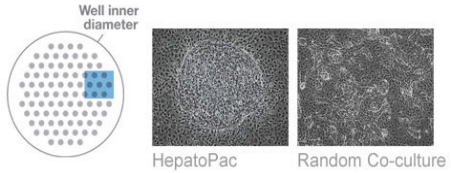




Tissues and Organs: a text of scanning electron microscopy, Kessel, RG and Kardon, RH, 1979.

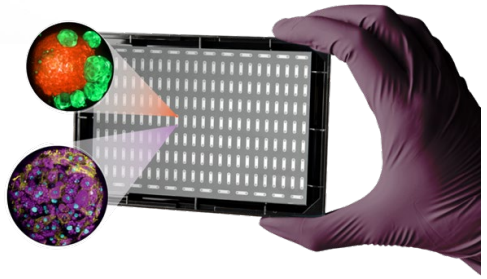
Array of MPS Platforms Have Emerged in Biomedical Research

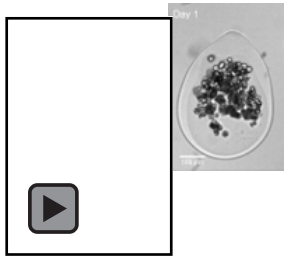


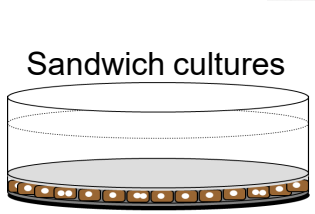
Micropatterned co-cultures

AkuraFlow™

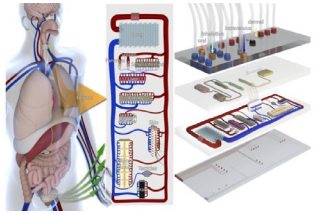
AkuraTwin™



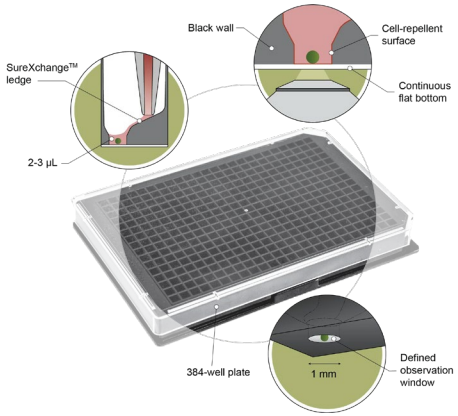




Sandwich cultures

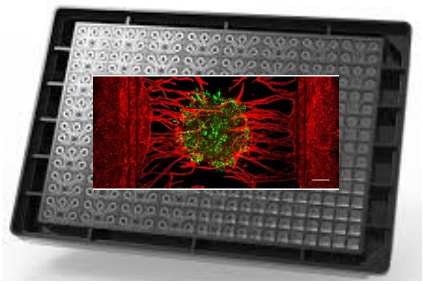


TissUSE



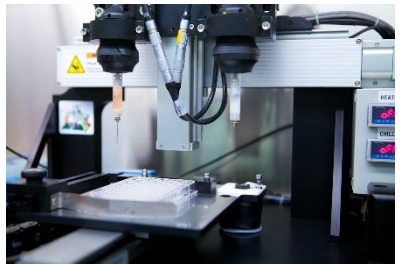
InSphero Akura™

ULA microplate



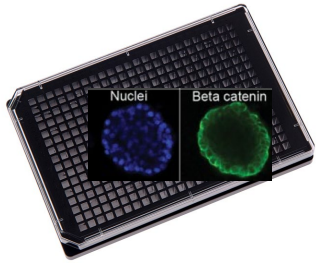
Mimetas

Emulate

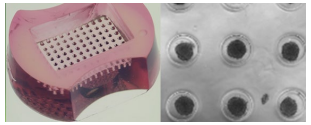


3D Bioprinting

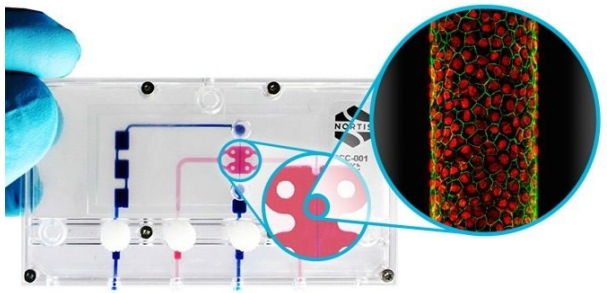
Hydrogel-base 3D models



Elplasia



Brown



Nortis ParVivo™

Throughput

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Modeling Human Liver Toxicity with 3D Microtissues

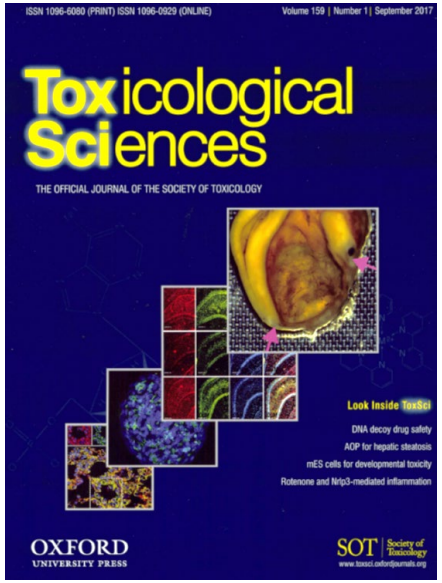
› Toxicol Sci. 2017 Sep 1;159(1):124-136. doi: 10.1093/toxsci/kfx122.

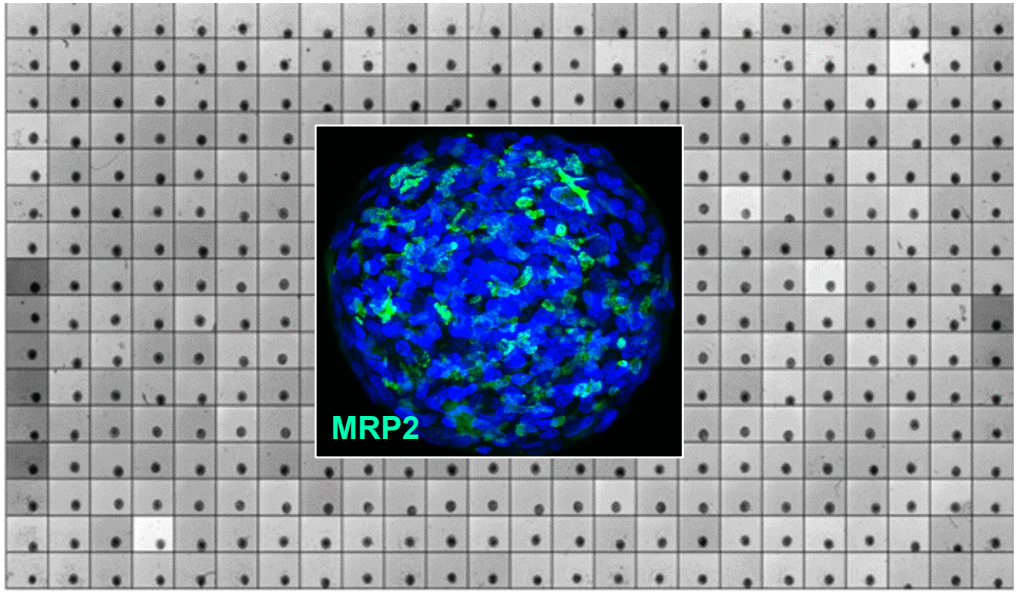
From the Cover: Three-Dimensional (3D) HepaRG Spheroid Model With Physiologically Relevant Xenobiotic Metabolism Competence and Hepatocyte Functionality for Liver Toxicity Screening

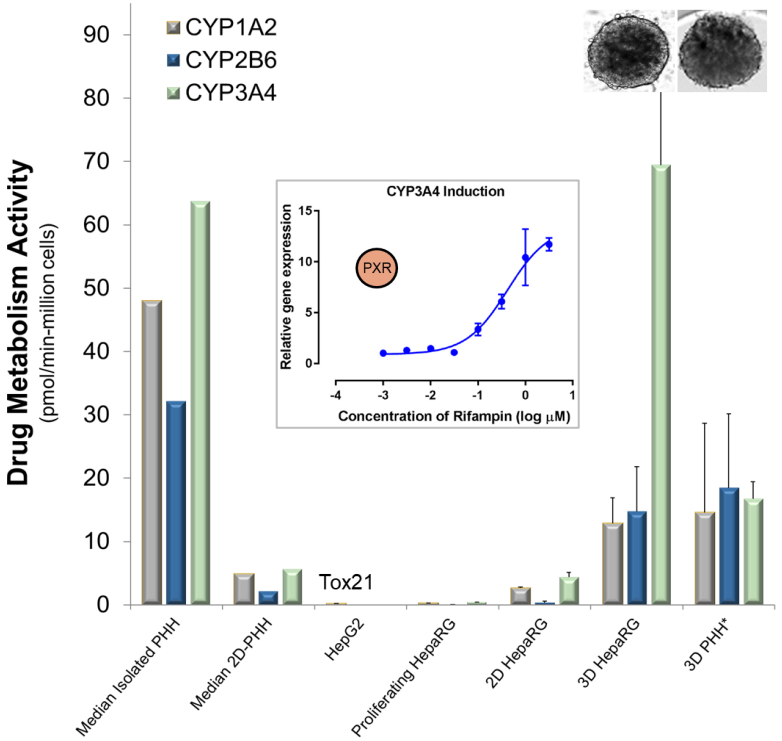
Sreenivasa C Ramaiahgari [1], Suramya Waidyanatha [1], Darlene Dixon [1], Michael J DeVito [1], Richard S Paules [1], Stephen S Ferguson [1]

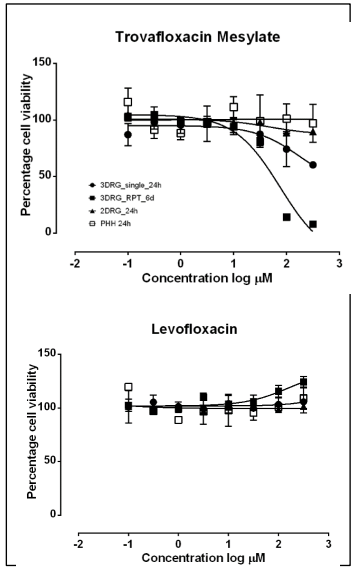
Affiliations + expand

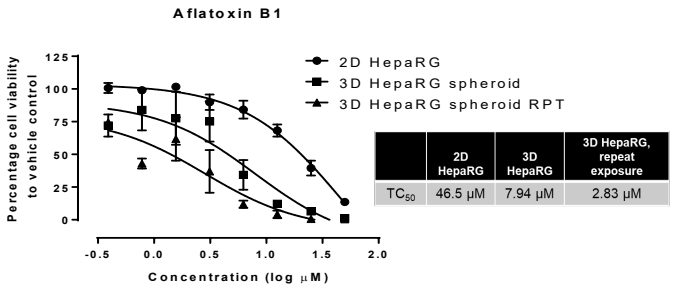
PMID: 28633424 PMCID: PMC5837526 DOI: 10.1093/toxsci/kfx122



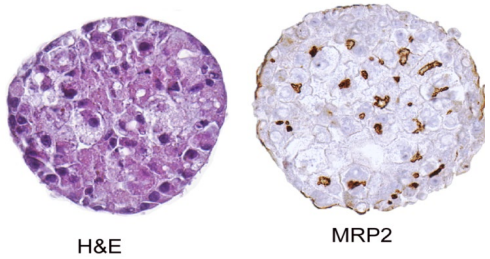




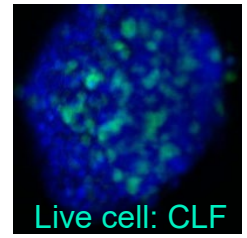




	2D HepaRG	3D HepaRG	3D HepaRG, repeat exposure
TC_{50}	46.5 μM	7.94 μM	2.83 μM



- **Human DILI Prediction**
- **Metabolically-activated hepatocellular toxicity**



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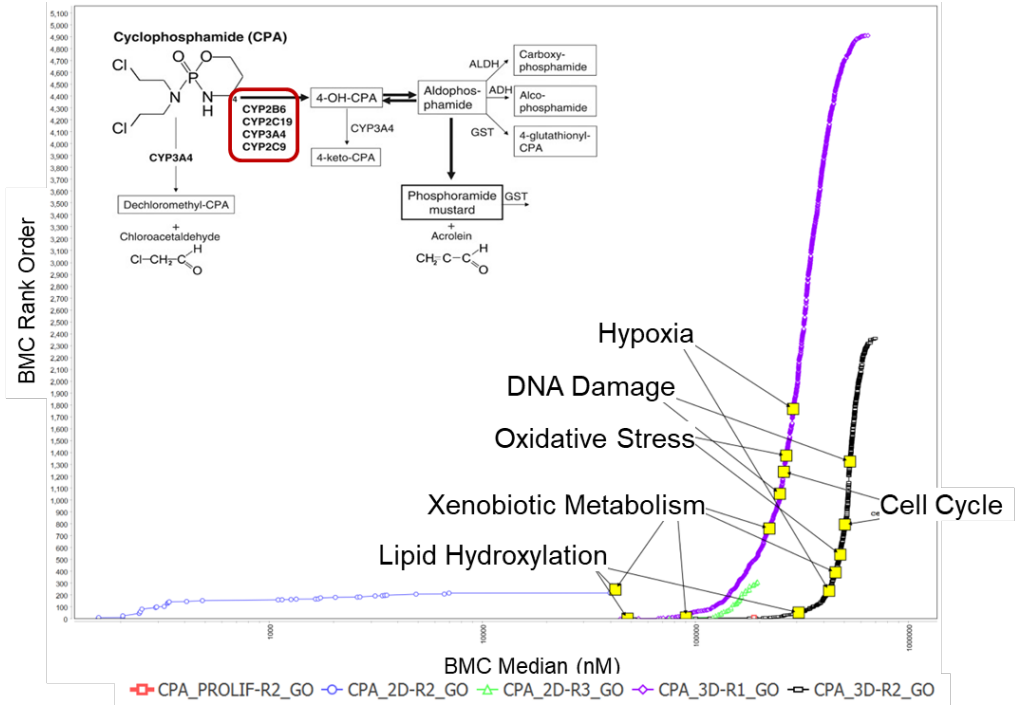
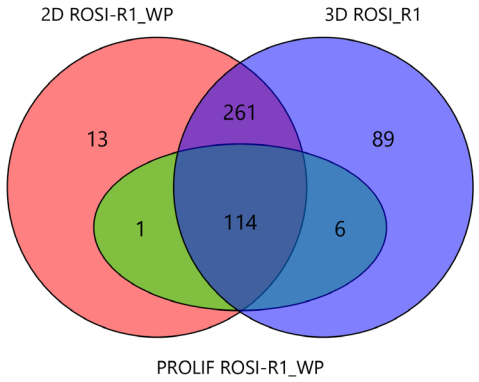
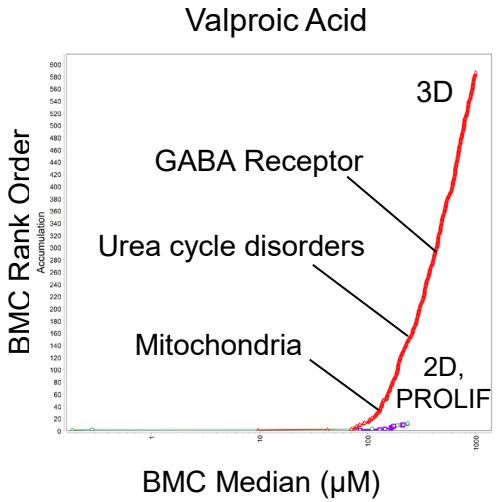
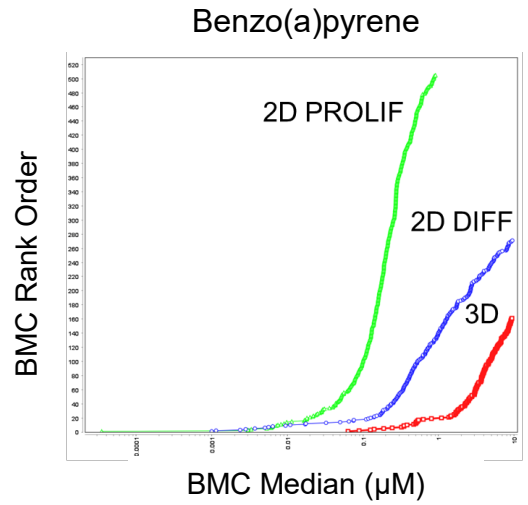
Enhanced Modeling of Drug-induced Liver Injury with 3D microtissues









- Compounds requiring metabolic activation via CYP450 enzymes respond differently in 3D vs. 2D liver tissue models
  - Cyclophosphamide
  - Valproic acid
  - Benzo(a)pyrene
  - Aflatoxin B1
- Clinically-relevant biological response pathways identified with human drug exposures in 3D that were not observed in 2D
- Enhanced transcriptomic pathway enrichment for known therapeutic targets & off-target effects with 3D liver spheroids

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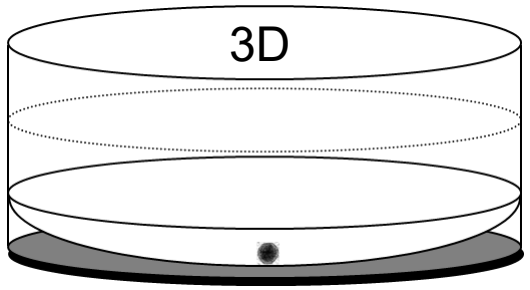
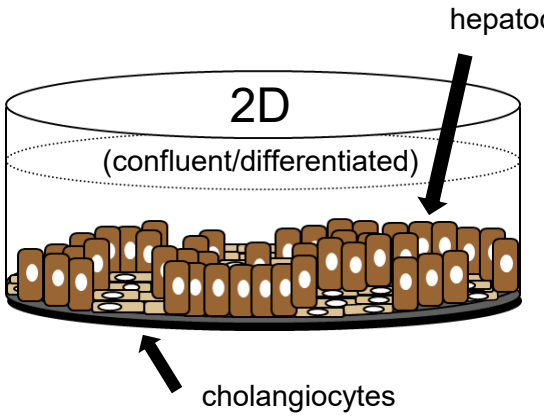
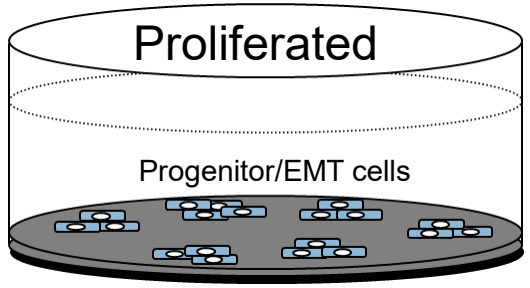
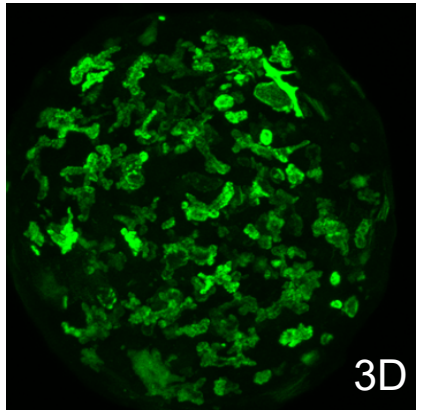
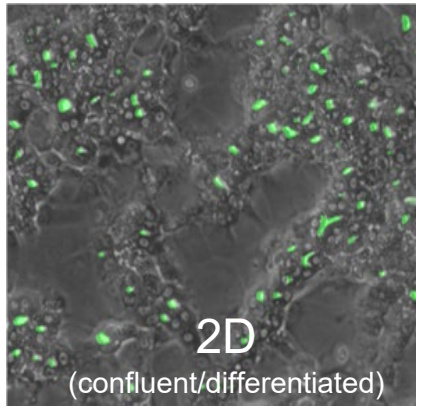
Mechanistic exploration of free-floating 3D liver microtissues

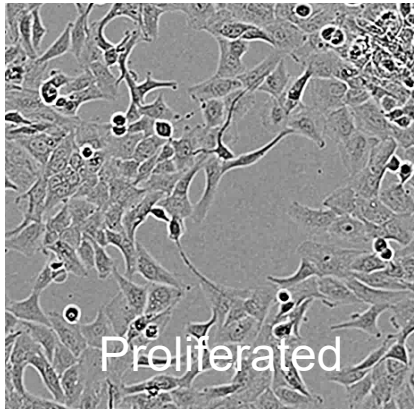


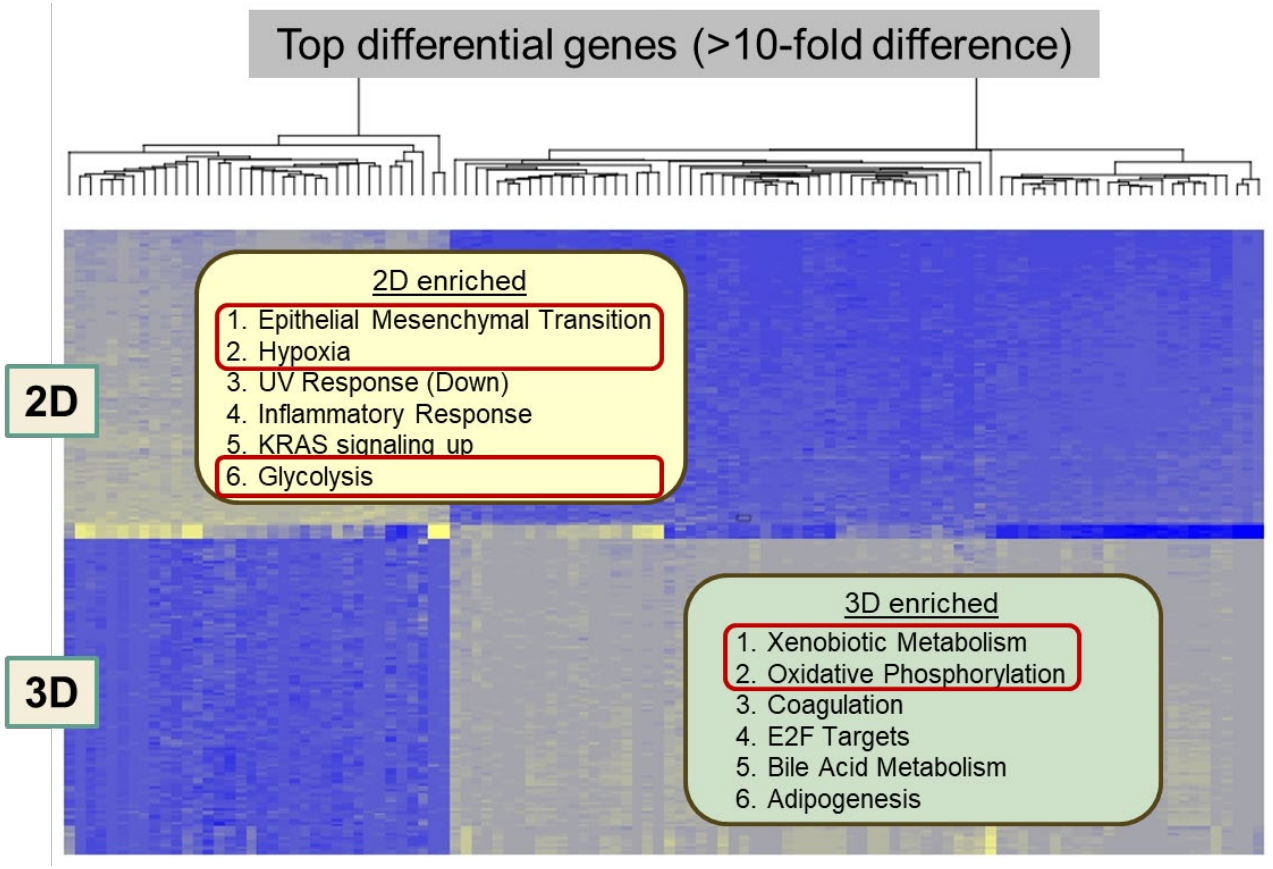












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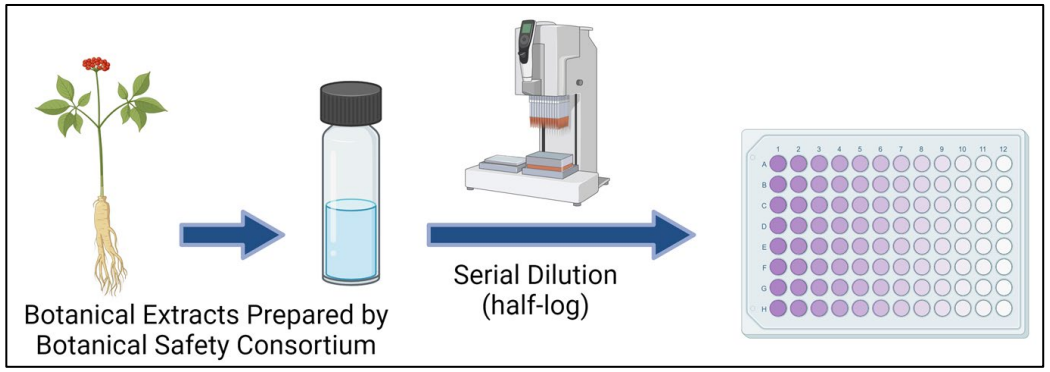
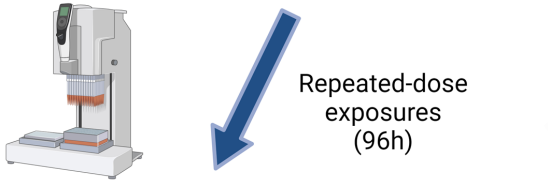
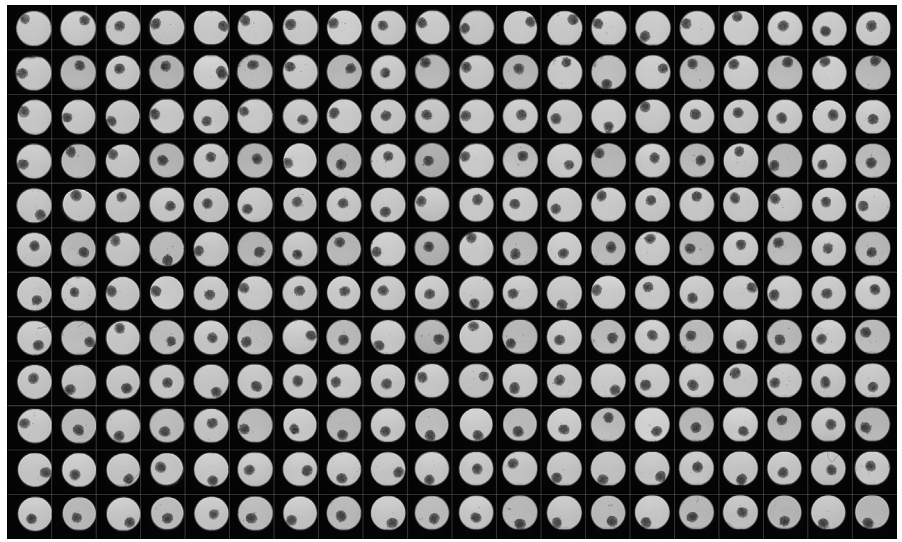
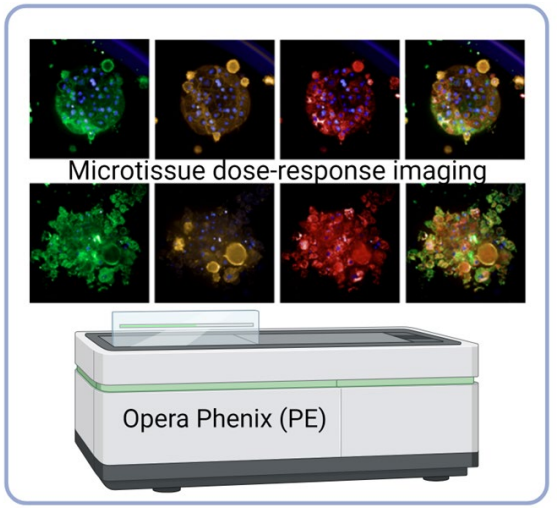
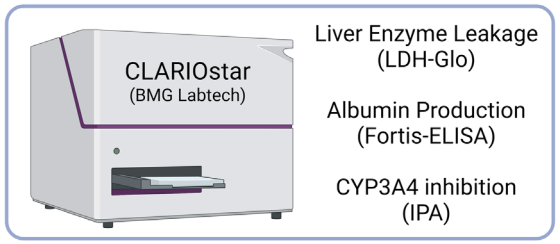
Applying Liver MPS: Botanical Safety & PFAS Mixtures

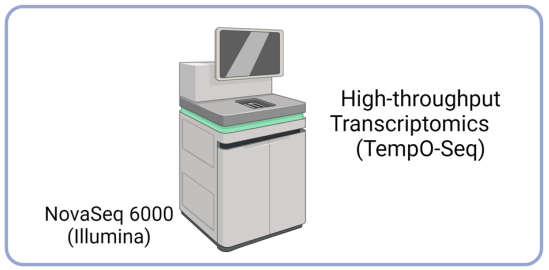




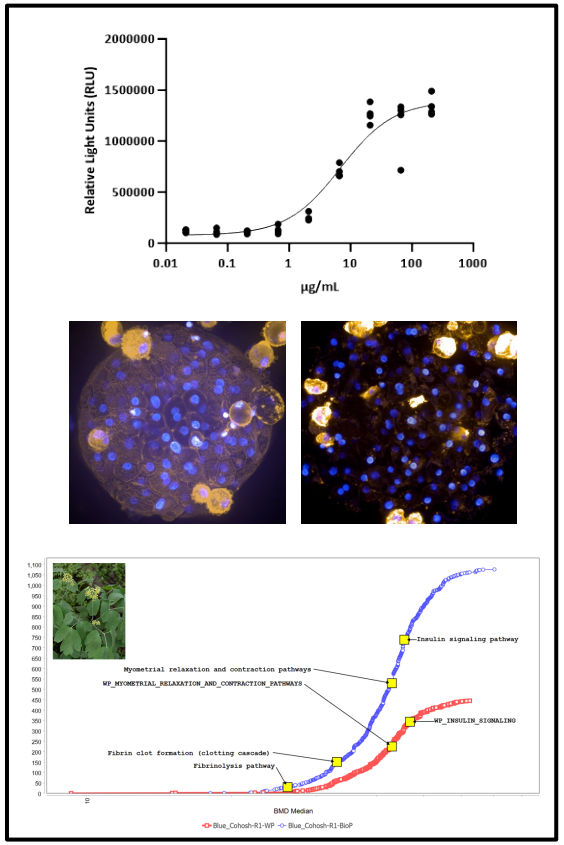








BOTANICAL SAFETY CONSORTIUM



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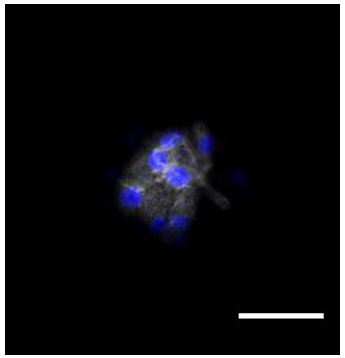
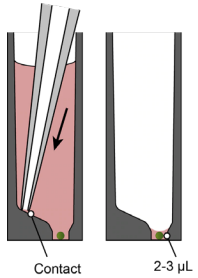
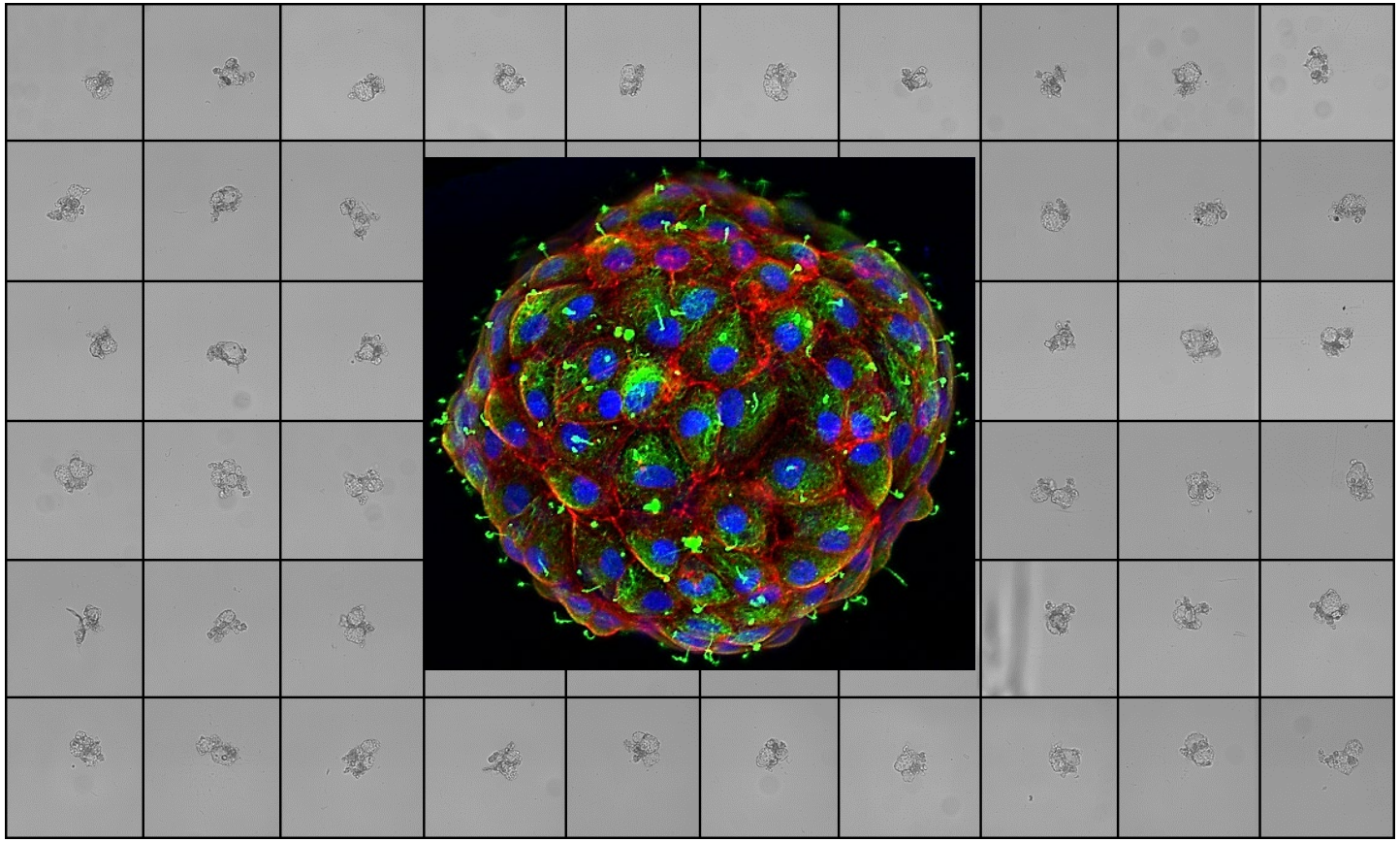
Expanding Biological Coverage: human renal proximal tubuloids

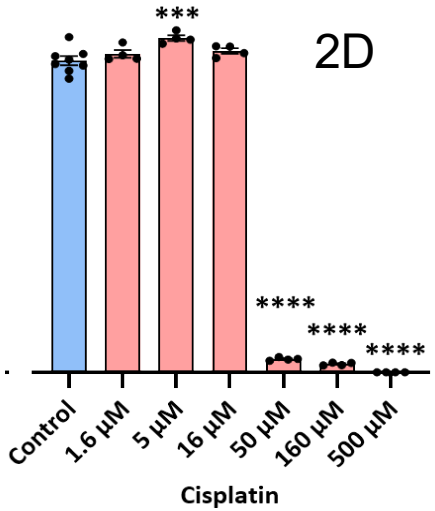
Adam Pearson

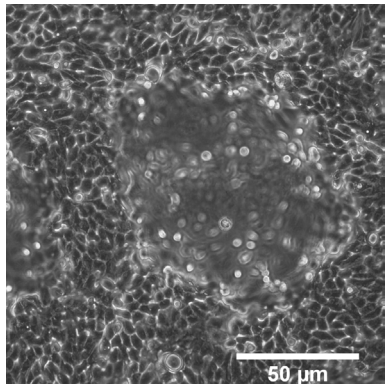


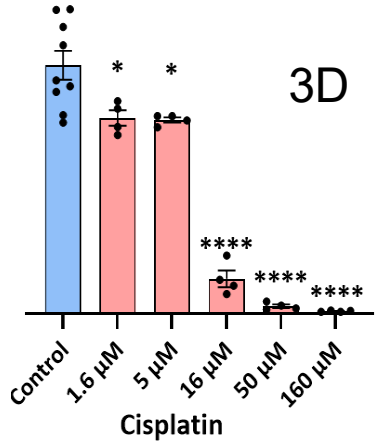
InSphero Akura Plates

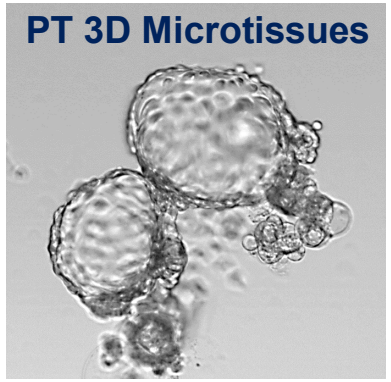












9 Commercial Liver MPS Collaborators

Axiom · BIOIVT ELEVATING SCIENCE · CN-BIO · DefiniGEN DISEASE MODEL INNOVATION · insphero · LENABIOSCIENCES · PredictCan Biotechnologies · TISSUSE Emulating Human Biology · Xellar biosystems

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Toxicological Sciences, 2023, **196(1)**, 52–70
https://doi.org/10.1093/toxsci/kfad080
Advance Access Publication Date: August 9, 2023
Research article

Analysis of reproducibility and robustness of a renal proximal tubule microphysiological system OrganoPlate 3-lane 40 for *in vitro* studies of drug transport and toxicity

Courtney Sakolish,[1] Haley L. Moyer,[1] Han-Hsuan D. Tsai,[1] Lucie C. Ford,[1] Allison N. Dickey,[2] Fred A. Wright,[2,3,4] Gang Han,[5] Piyush Bajaj,[6] Maria T. Baltazar,[7] Paul L. Carmichael,[7] Jason P. Stanko,[8] Stephen S. Ferguson,[8] Ivan Rusyn[1,*]

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https://doi.org/10.1093/toxsci/kfac061
Advance Access Publication Date: 11 June 2022
In-Depth Reviews

IN-DEPTH REVIEWS

Microphysiological Systems Evaluation: Experience of TEX-VAL Tissue Chip Testing Consortium

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An *in vitro-in silico* workflow for predicting renal clearance of PFAS

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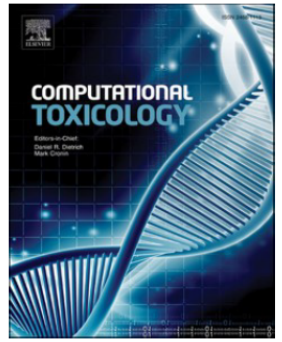

Full Length Article

Development of chemical categories for per- and polyfluoroalkyl substances (PFAS) and the proof-of-concept approach to the identification of potential candidates for tiered toxicological testing and human health assessment

G. Patlewicz [a,*], R.S. Judson [a], A.J. Williams [a], T. Butler [b], S. Barone Jr. [b], K.E. Carstens [a], J. Cowden [a], J.L. Dawson [b], S.J. Degitz [a], K. Fay [b], T.R. Henry [b,1], A. Lowit [b], S. Padilla [a], K. Paul Friedman [a], M.B. Phillips [b], D. Turk [b], J.F. Wambaugh [a], B.A. Wetmore [a], R.S. Thomas [a]

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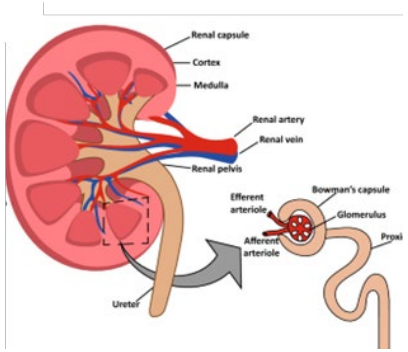
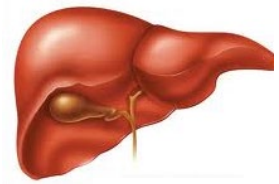
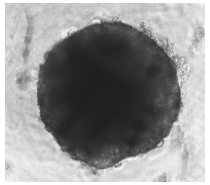
PFAS Mixtures Accumulation with MPS

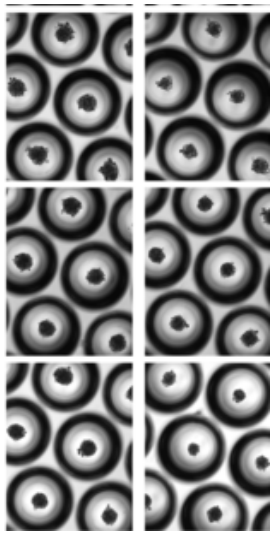
Emulate Organ-on-a-Chip Microphysiological System



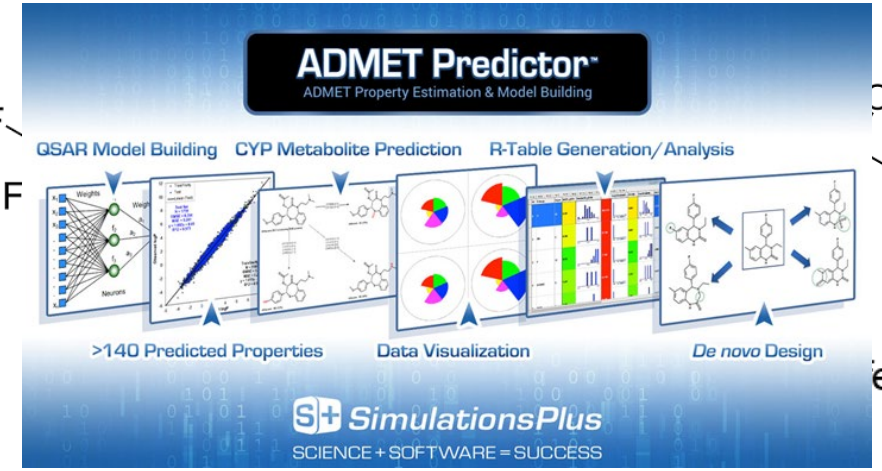


3D PHH

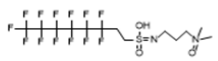




Pooled Microtissues



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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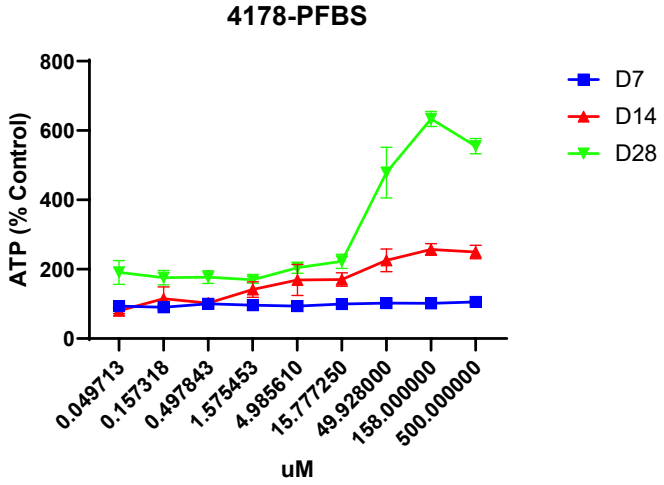
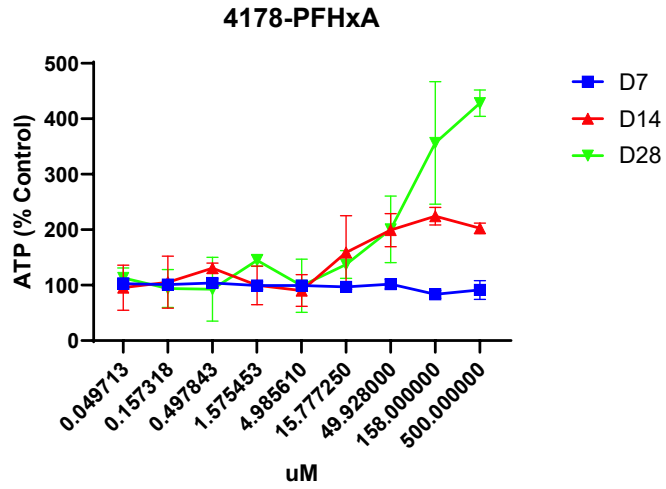
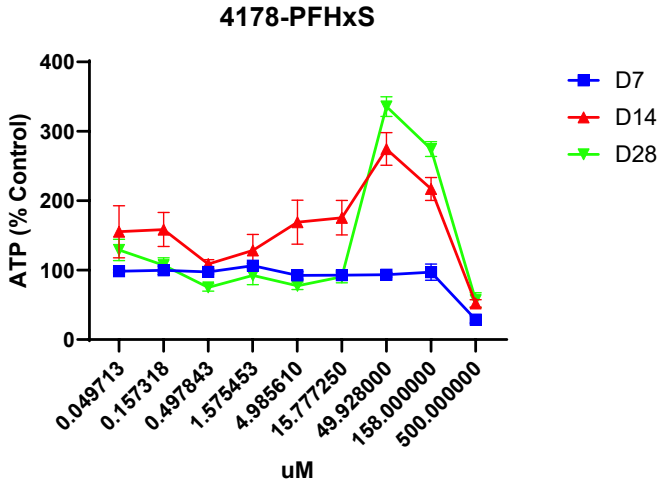
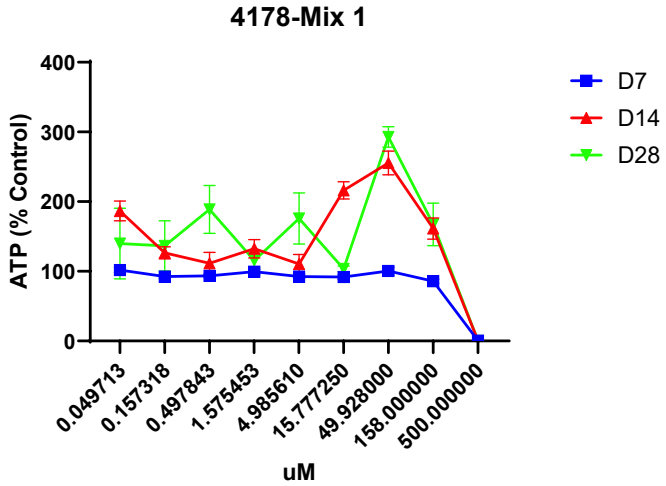
PFAS Mixtures Exposures Over 28-days in PHH Spheroids

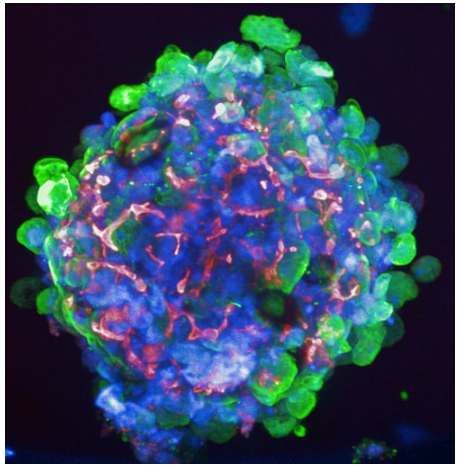


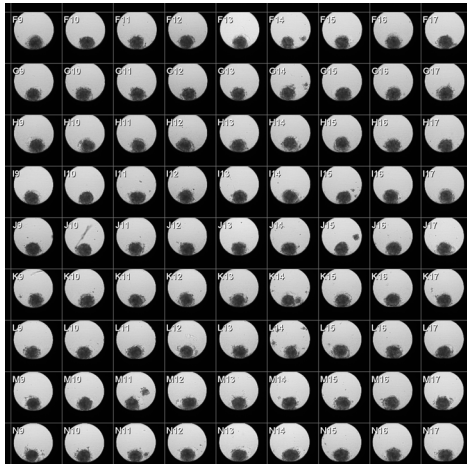






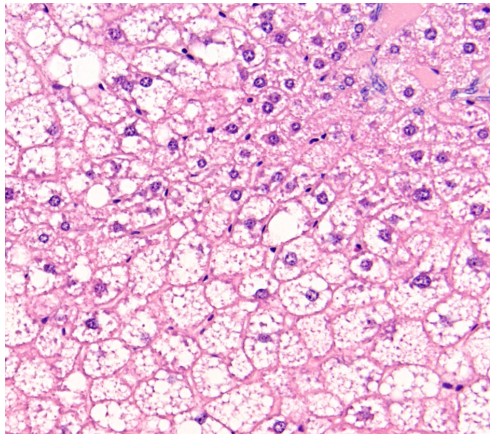
- Longer-term exposures with some PFAS led to surprisingly robust ATP accumulation
  - PFHxS, PFHxA, PFBS
- Longer-chain PFAS (e.g., PFOA, PFNA, PFOS) tended to show sharp declines in ATP with smaller increases
- Transcriptomic assays in progress





The Path Forward

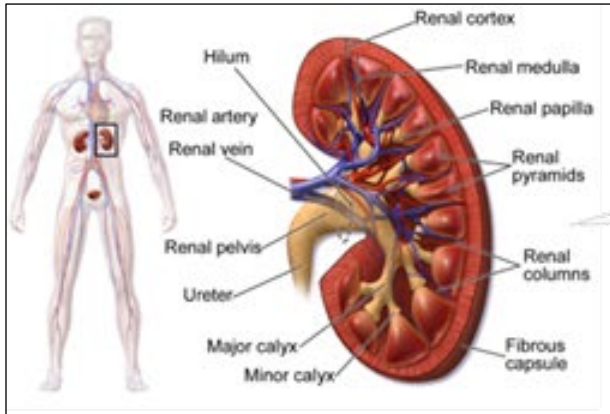
- What are we trying to predict?
  - Comprehensive safety?
  - Hazard/risk for specific health effects? Which ones?
- NTP Histopathology Glossary: Mapping morphologies to liver MPS
  - Diagnostic histology and clinical pathology equivalents within MPS
  - MPS that display recognizable phenotypes of toxicity and disease
    - Fatty liver disease, zonal-specific fibrosis, cholestasis
    - 130 out of 411 total morphologies feasible with liver MPS
    - Developing a 'Rosetta Stone' to relate imaging and assay data with liver MPS into recognizable findings for risk assessors
- Blind spots with MPS:
  - How wrong are NAM-based potency range estimates for more vulnerable individuals?
    - Sex differences
    - Age ranges (e.g., neonatal, childhood, adult, geriatric)
    - Pre-existing disease states
- Expanding tissue coverage & establishing MPS functional benchmarks with the precious gifts of donated organs & tissues
  - PFAS toxicity involves the liver-thyroid axis



The Director's Challenge
Innovation Award Program

NIH
NCATS

Rationale for Prioritization of MPS Development for Chemical Safety



Renal proximal tubule

Renal glomerulus

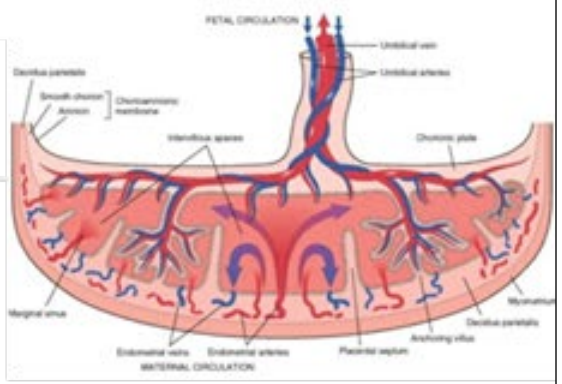
Liver

Small intestine

Placenta

Blood-brain barrier

Lung



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It takes a village to raise the bar.

Predictive Toxicology Screening

US EPA
Josh Harrill
Mike DeVito
Katie Paul-Freedman
Richard Judson
Rusty Thomas
John Wambaugh
Nisha Sipes

NICEATM

ScIome

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