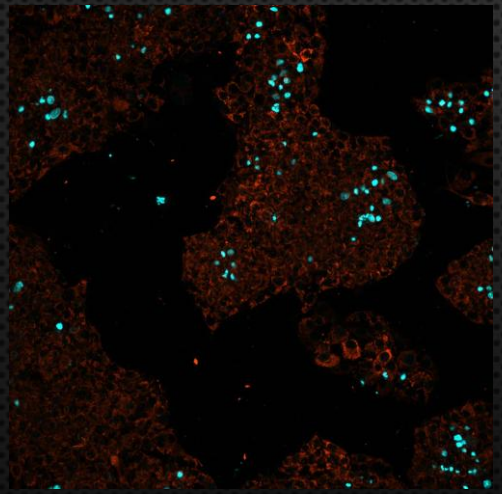
Combinatorial NAMs to Elucidate Mechanisms of Human Thyroid Hormone Disruption



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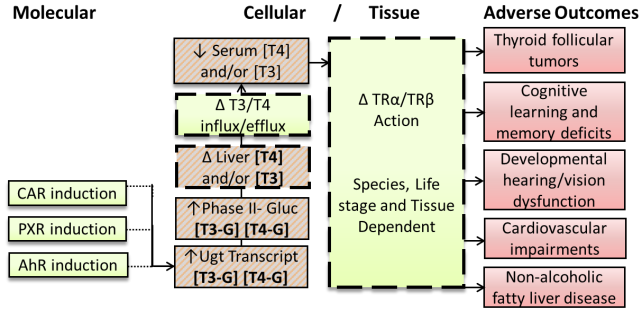
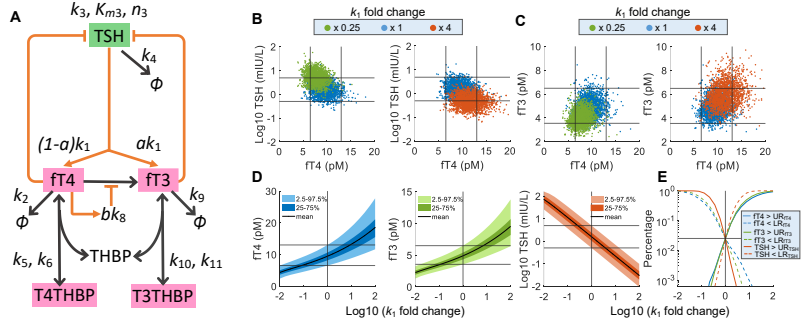
Conflict of Interest Brian Johnson owns equity in Onexio Biosystems LLC. A company that develops solutions for high-throughput toxicity testing, translational medicine and other multi-culture applications.

How can we leverage NAMs to inform how environmental contaminants cause thyroid imbalance and effects in humans

Adverse Outcome Pathway Modeling

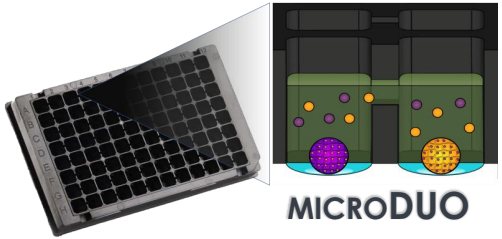


Computational PBK and IVIVE

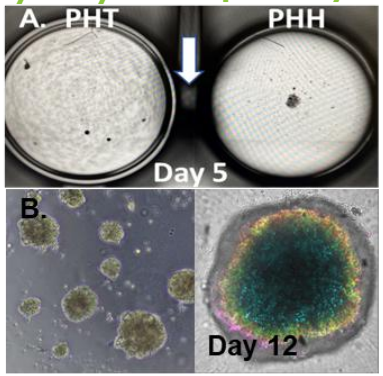


Complex in vitro

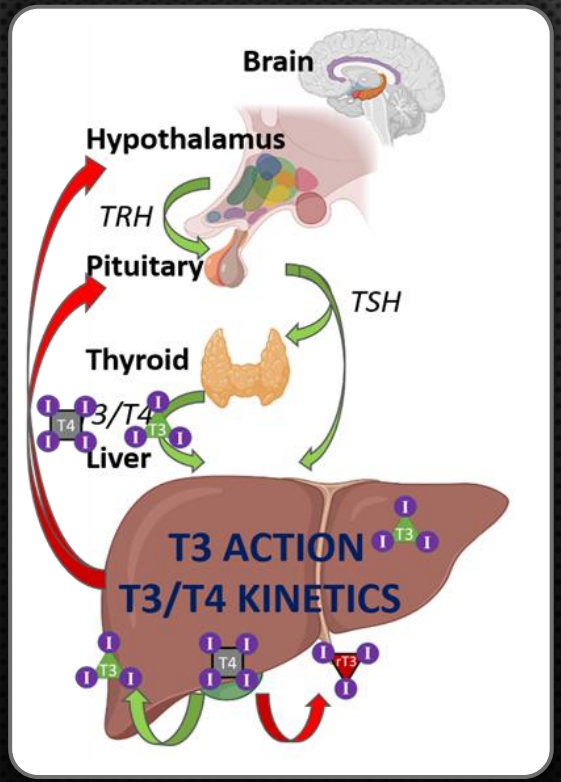
Plug and Play Coculture

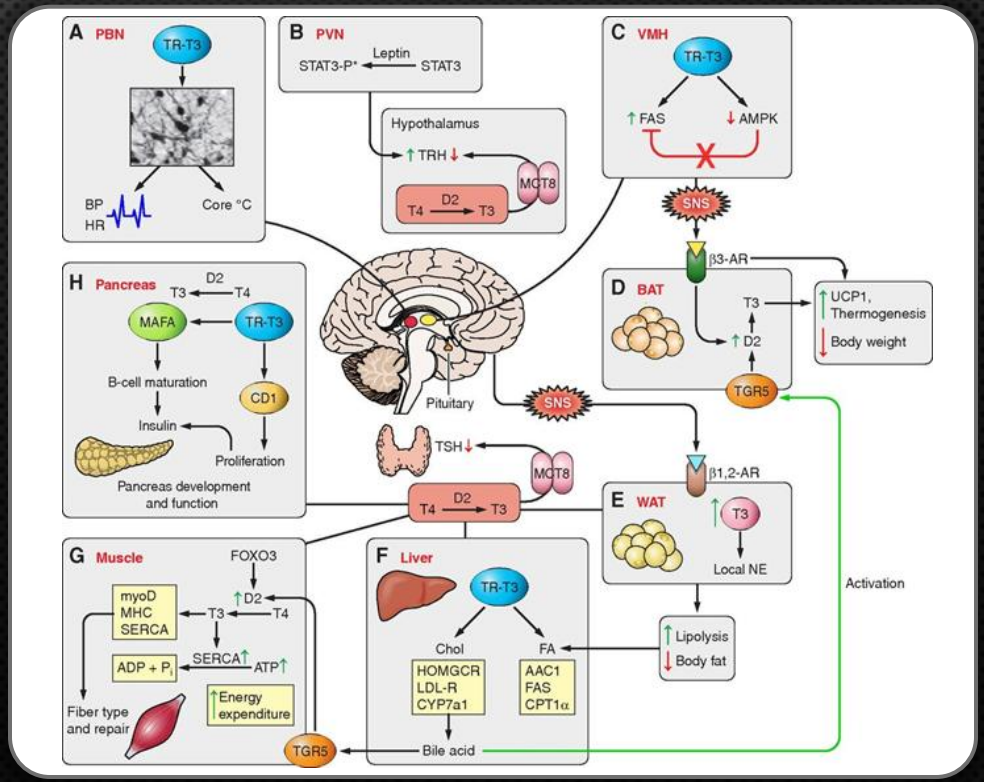


Thyrocytes Hepatocytes



THYROID IMBALANCE REPRESENTS TH KINETICS, TH ACTION DRIVES PHYSIOLOGY





Thyroid Hormone Regulation of Metabolism, Mullur R, et. al. 2014

Legacy and Emerging Superfund Contaminants Disrupt Thyroid Homeostasis Through Undetermined Mechanisms

Legacy

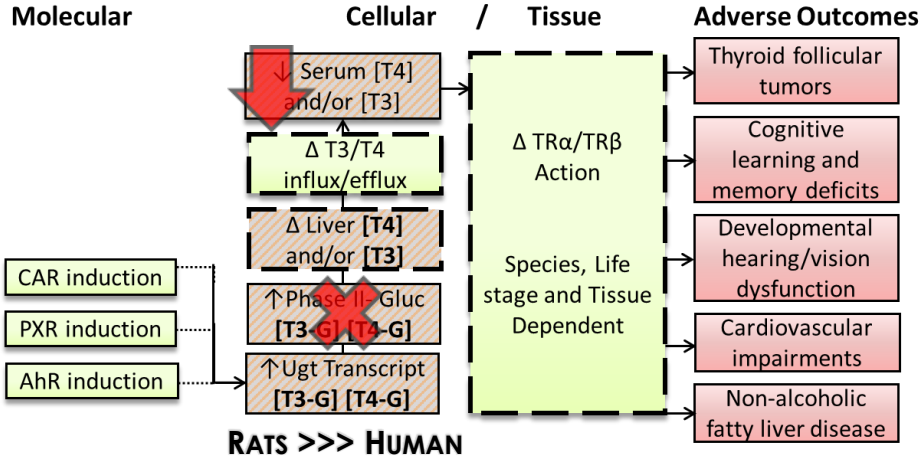
- Polychlorinated biphenyls (PCBs)
- Dioxins
- Organochlorine pesticides

Emerging

- Perfluoroalkyl substances (PFAS)
- Polybrominated diphenyl ethers (PBDEs)
- Current-use pesticides

AOP Modeling does not support current Mechanism used to make regulatory decisions (Richardson et. al., 2010; Kato et. al. 2004 and 2014)

Adverse Outcome Pathway Modeling



Published in final edited form as:
Toxicol Appl Pharmacol. 2010 October 1; 248(1): 38–44. doi:10.1016/j.taap.2010.07.010.

Disruption of thyroid hormone homeostasis in Ugt1a-deficient Gunn rats by microsomal enzyme inducers is not due to enhanced thyroxine glucuronidation☆

Terrilyn A. Richardson[1] and **Curtis D. Klaassen**[*]
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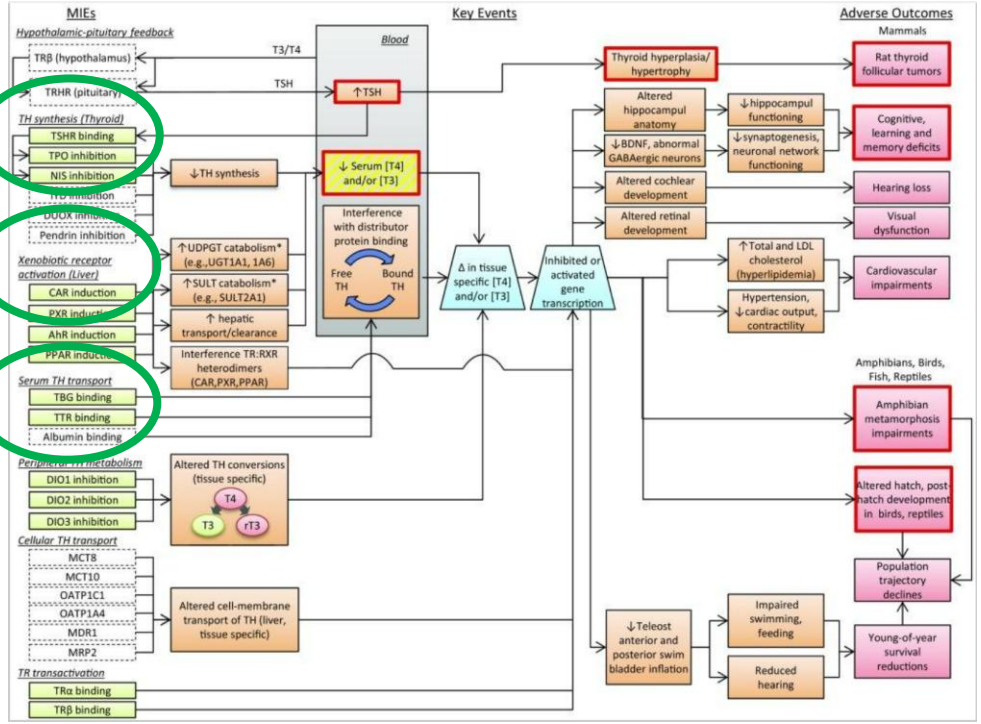
AOP IDENTIFIES CRITICAL COMPARTMENTS TO MODEL

AOP IDENTIFIES CRITICAL COMPARTMENTS TO MODEL THYROCYTES, HEPATOCYTES AND SERUM (NOYES, ET. AL, 2019)

HYPOTHESIS – THYROID HORMONE ACTION IS A SHARED KEY INTERMEDIATE EVENT LEADING TO ADVERSE OUTCOMES

ADVERSE OUTCOME PATHWAY NETWORK



(NOYES, ET. AL, 2019)

AOP IDENTIFIES ENDPOINTS TO MEASURE

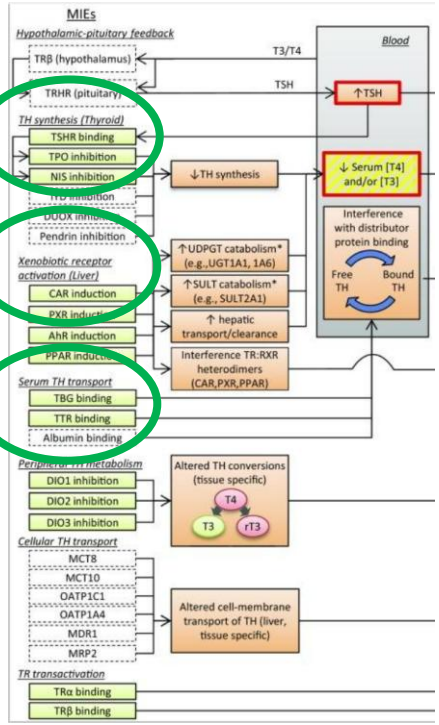
AOP IDENTIFIES CRITICAL ENDPOINTS:

TH KINETICS - HORMONE SYNTHESIS (T3/T4), METABOLISM (TH-G/TH-S), TRANSPORT (INTRA/EXTRACELLULAR) LC-MS/MS

TH ACTION – TRANSCRIPTIONAL ACTIVATION, CELLULAR ENGERGETICS (FA UPTAKE, MITOCHONDRIAL DYNAMICS, CHOLESTEROL BIOSYNTHESIS ETC.). CONFOCAL IMAGING

MANY SUPERFUND CHEMICALS ARE ALSO KNOWN TO DISRUPT CELLULAR ENERGETICS

ADVERSE OUTCOME PATHWAY NETWORK



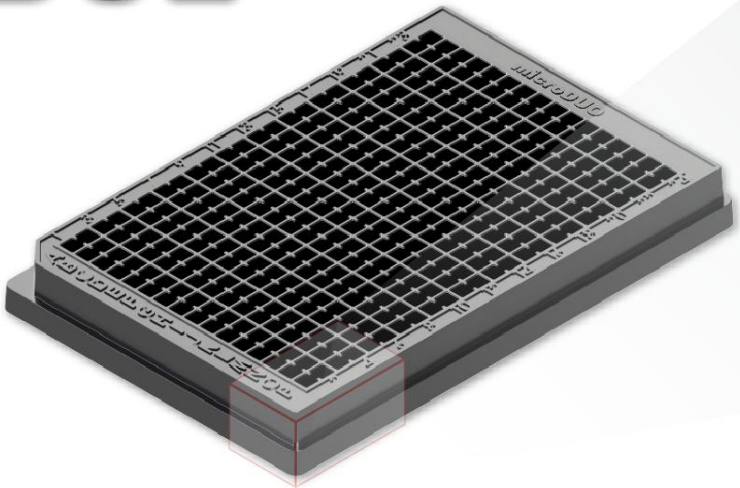
T3 : T4 Kinetics (Hepatocyte/Serum) LC-MS/MS

- ↑Phase II- Gluc **[T3-G] [T4-G]**
- ↑Phase II Sulf **[T3-S] [T4-S]**
- Δ Liver **[T4] and/or [T3]**
- ↓ Serum **f[T4]/ [T4] and/or f[T3]/ [T3]**

T3 : TRα/TRβ Action – (Hepatocyte) Confocal Imaging

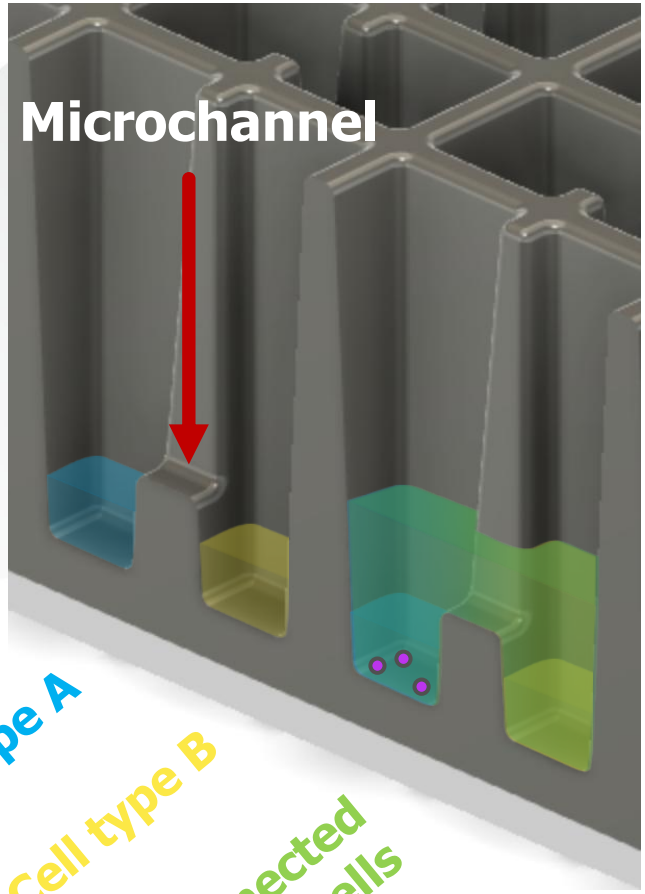
Δ Free fatty acid uptake/synthesis	**Δ Lipid droplets**	**Δ [ROS]**
Δ Fatty acids→ triglycerides	Δ Fatty acid β-oxidation	**Δ [ATP]**
Δ Cholesterol synthesis/uptake	Δ Bile acid	**Δ Mitochondrial biogenesis**

microDUO



US Patent Number 10,518,266

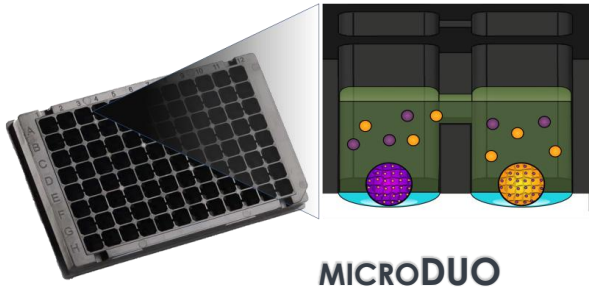
- Elegantly simple
- Fully HTS compatible
- Multi-endpoint
- 2D or 3D co-culture and multiculture



Plug and Play Complex in vitro Modeling – Human Hepatocytes

Complex in vitro

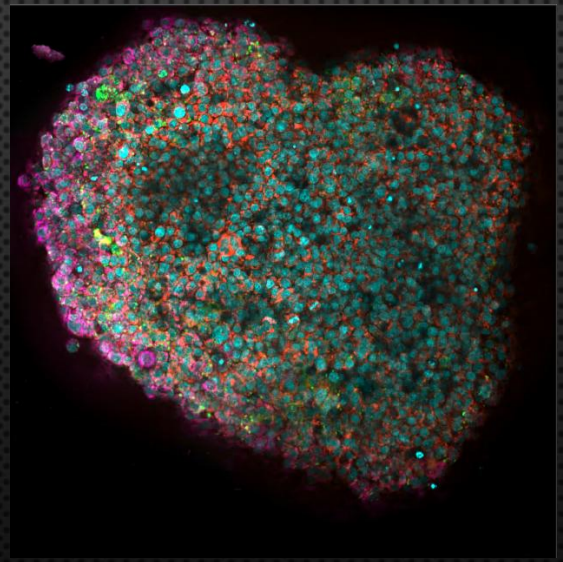
Plug and Play Coculture



microDUO

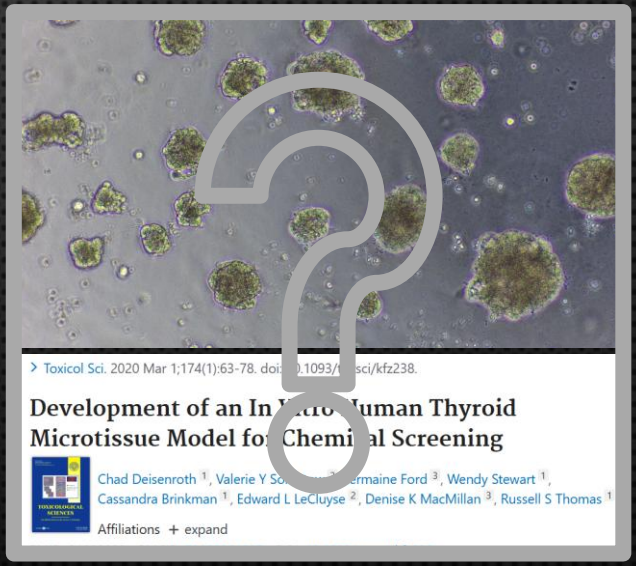
2D or 3D Compatible

Human Hepatocytes



2D or 3D Format

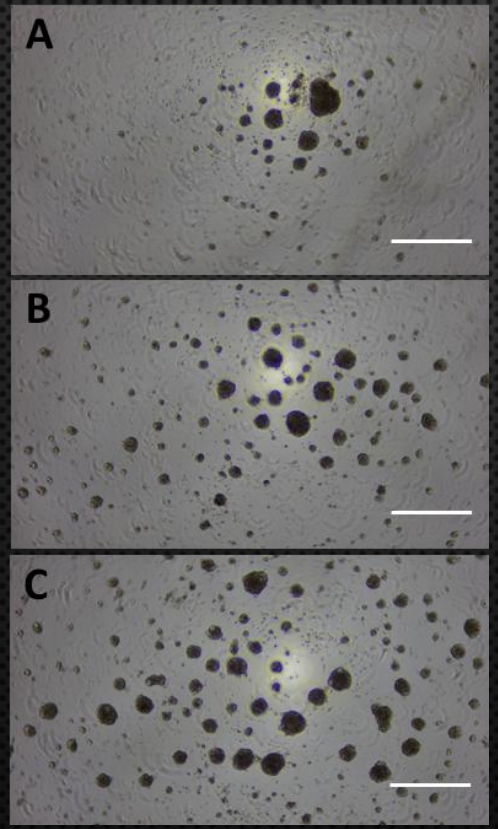
Human Thyrocyte Follicles

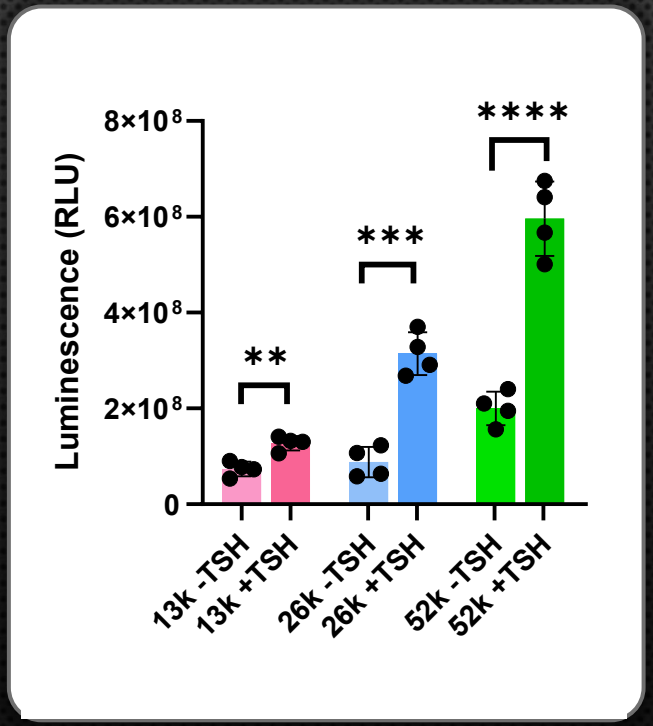


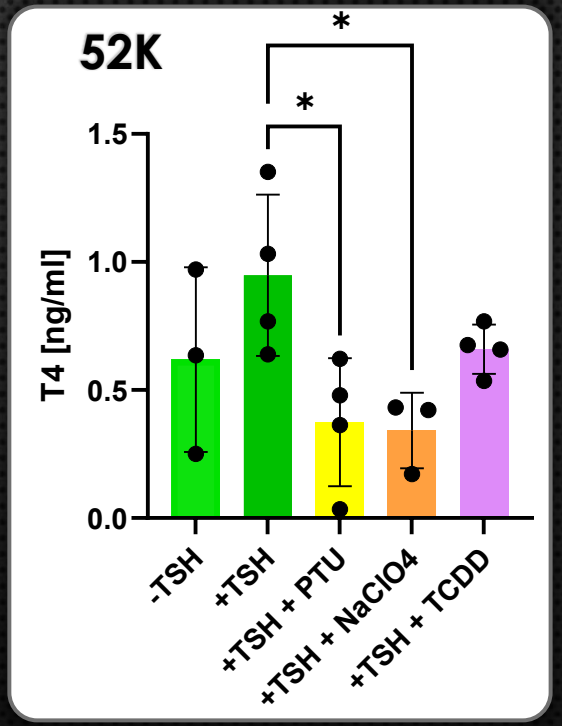
2D+ Format

Follicles respond to TSH and Chemicals

Human Thyrocyte Follicles



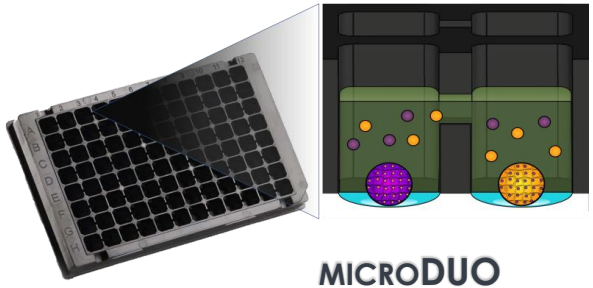




Plug and Play Complex in vitro Modeling – Human Hepatocytes

Complex in vitro

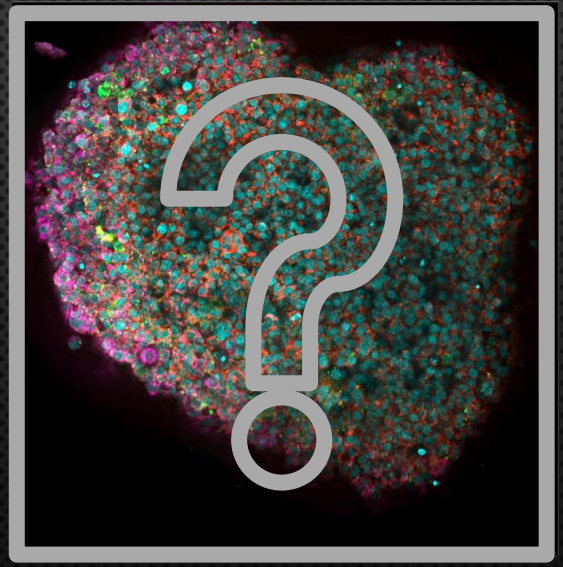
Plug and Play Coculture



microDUO

2D or 3D Compatible

Human Hepatocytes



2D or 3D Format

Human Thyrocyte Follicles

Toxicol Sci. 2020 Mar 1;174(1):63-73. doi: 10.1093/toxsci/kfz238.

Development of an In Vitro Human Thyroid Microtissue Model for Chemical Screening

Chad Deisenroth [1], Valerie Y Soldatow [2], Jermaine Ford [3], Wendy Stewart [1], Cassandra Brinkman [1], Edward L LeCluyse [2], Denise K MacMillan [3], Russell S Thomas [1]

Affiliations + expand

PMID: 31808822 PMCID: PMC8061085 DOI: 10.1093/toxsci/kfz238

2D+ Format

ENDPOINT DEVELOPMENT

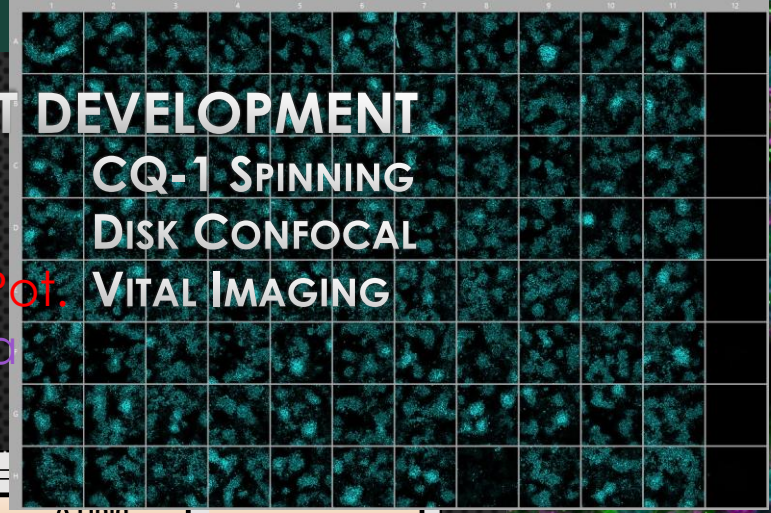
Nuclear

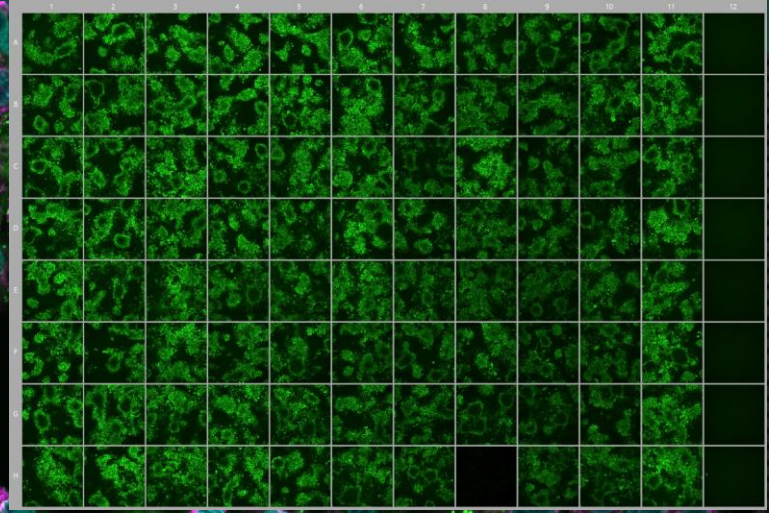
FA uptake

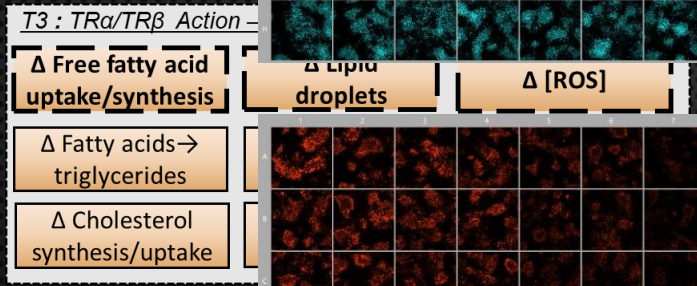
Mito. Mem. Pot.

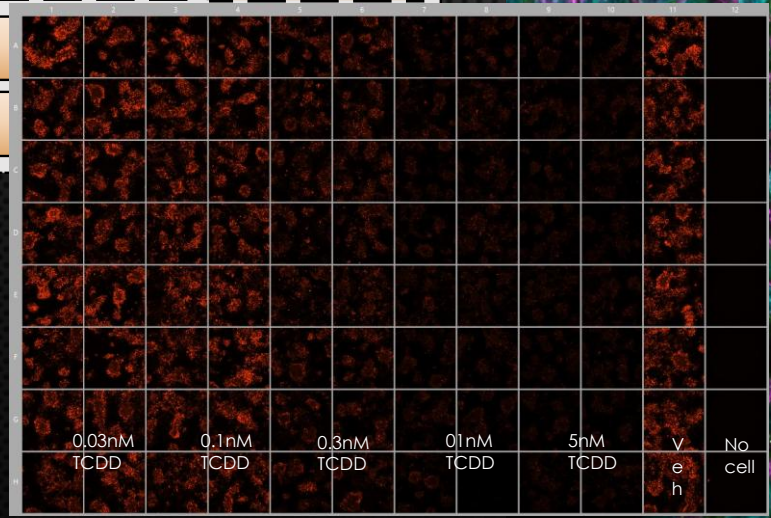
Mitochondria

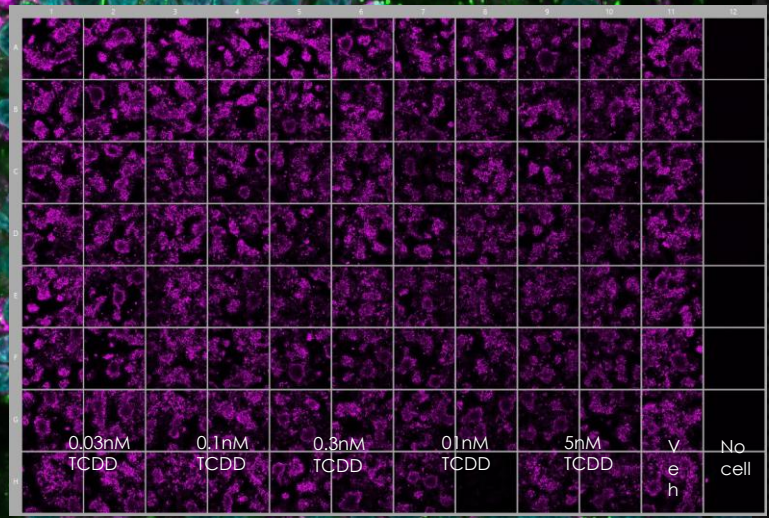
CQ-1 Spinning Disk Confocal Vital Imaging









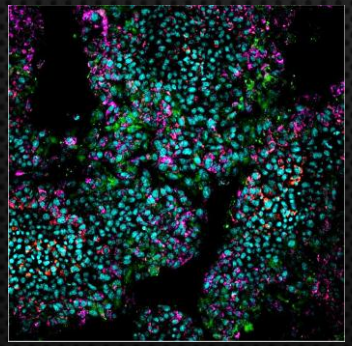


TCDD Dose-Response

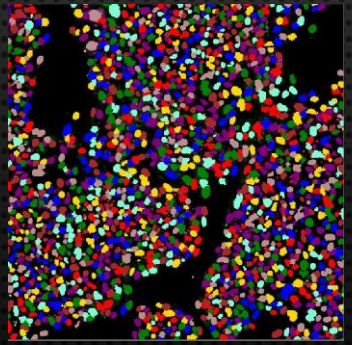
CELL SEGMENTATION AND QUANTIFICATION OF CONFOCAL IMAGES

Nucleus segmentation: Use Hoechst image to define nucleus. Apply image filter to delete noise → Set intensity threshold to identify nucleus → Set minimum point distance to segment connected nucleus.

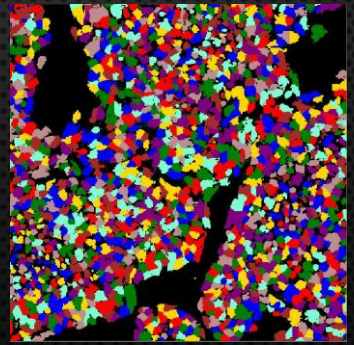
Cell body Segmentation: Use Bodipy, MitoTracker Deep Red and TMRM's images to define cell body. Apply image filter to delete noise → Merge with hoechst image to pair cell body with nucleus → Set intensity threshold to identify cell body from image → Set minimum point distance to segment connected cell body.



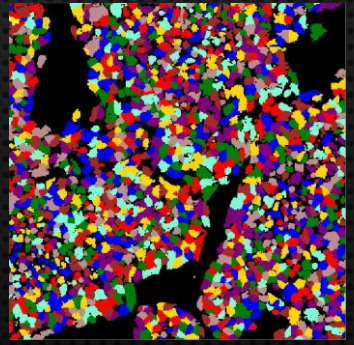
MitoTracker Deep Red-Active Mitochondria
TMRM-Mitochondrial Membrane Potential
Bodipy-Lipid Uptake
Hoechst-Nucleus



Nucleus Segmentation



Cell Body Segmentation

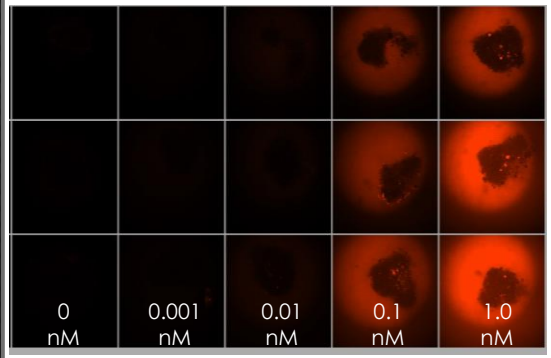


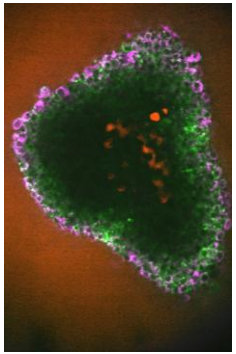
Linked Nucleus and Cell Body

Mitochondrial Membrane Potential Follows TEQ Shows Increase Sensitivity Over CYP1A1 Activity

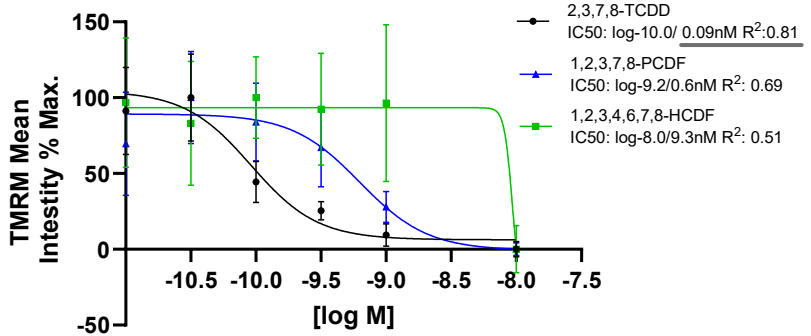
Table 1. WHO 2005 Mammalian TEFs for Dioxin and DLCs

CAS Number	Full Congener Name	Shorthand Congener Name[1]	TEF[2]
Dioxin Congeners			
1746-01-6	2,3,7,8-Tetrachloro dibenzo-p-dioxin	2,3,7,8-TCDD	1
40321-76-4	1,2,3,7,8-Pentachloro dibenzo-p-dioxin	1,2,3,7,8-PeCDD	1
39227-28-6	1,2,3,4,7,8-Hexachloro dibenzo-p-dioxin	1,2,3,4,7,8-HxCDD	0.1
57653-85-7	1,2,3,6,7,8-Hexachloro dibenzo-p-dioxin	1,2,3,6,7,8-HxCDD	0.1
19408-74-3	1,2,3,7,8,9-Hexachloro dibenzo-p-dioxin	1,2,3,7,8,9-HxCDD	0.1
35822-46-9	1,2,3,4,6,7,8-Heptachloro dibenzo-p-dioxin	1,2,3,4,6,7,8-HpCDD	0.01
3268-87-9	1,2,3,4,6,7,8,9-Octachloro dibenzo-p-dioxin	OCDD	0.0003
Furan Congeners			
51207-31-9	2,3,7,8-Tetrachloro dibenzofuran	2,3,7,8-TCDF	0.1
57117-41-6	1,2,3,7,8-Pentachloro dibenzofuran	1,2,3,7,8-PeCDF	0.03
57117-31-4	2,3,4,7,8-Pentachloro dibenzofuran	2,3,4,7,8-PeCDF	0.3
70648-26-9	1,2,3,4,7,8-Hexachloro dibenzofuran	1,2,3,4,7,8-HxCDF	0.1
57117-44-9	1,2,3,6,7,8-Hexachloro dibenzofuran	1,2,3,6,7,8-HxCDF	0.1
72918-21-9	1,2,3,7,8,9-Hexachloro dibenzofuran	1,2,3,7,8,9-HxCDF	0.1
60851-34-5	2,3,4,6,7,8-Hexachloro dibenzofuran	2,3,4,6,7,8-HxCDF	0.1
67562-39-4	1,2,3,4,6,7,8-Heptachloro dibenzofuran	1,2,3,4,6,7,8-HpCDF	0.01
55673-89-7	1,2,3,4,7,8,9-Heptachloro dibenzofuran	1,2,3,4,7,8,9-HpCDF	0.01
39001-02-0	1,2,3,4,6,7,8,9-Octachloro dibenzofuran	OCDF	0.0003

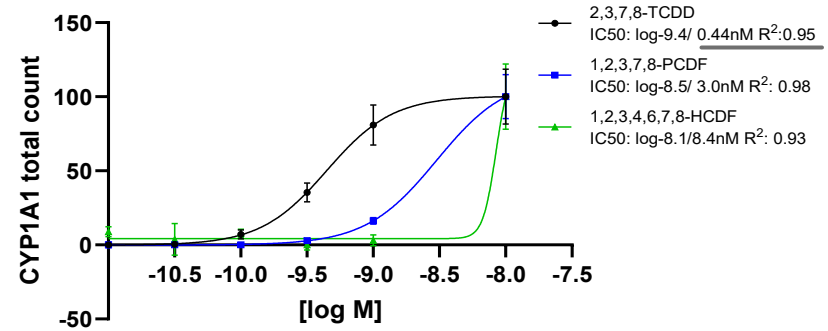




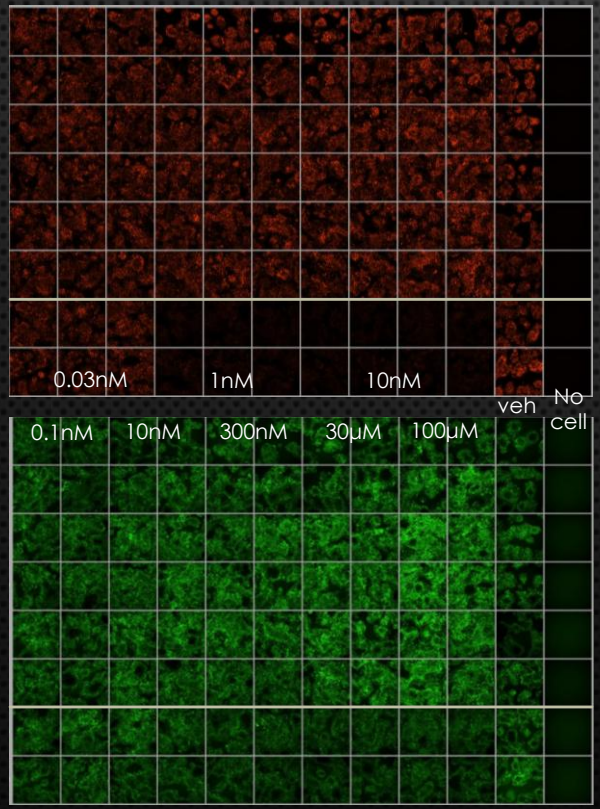
Mitochondrial Membrane Potential Dose-Response

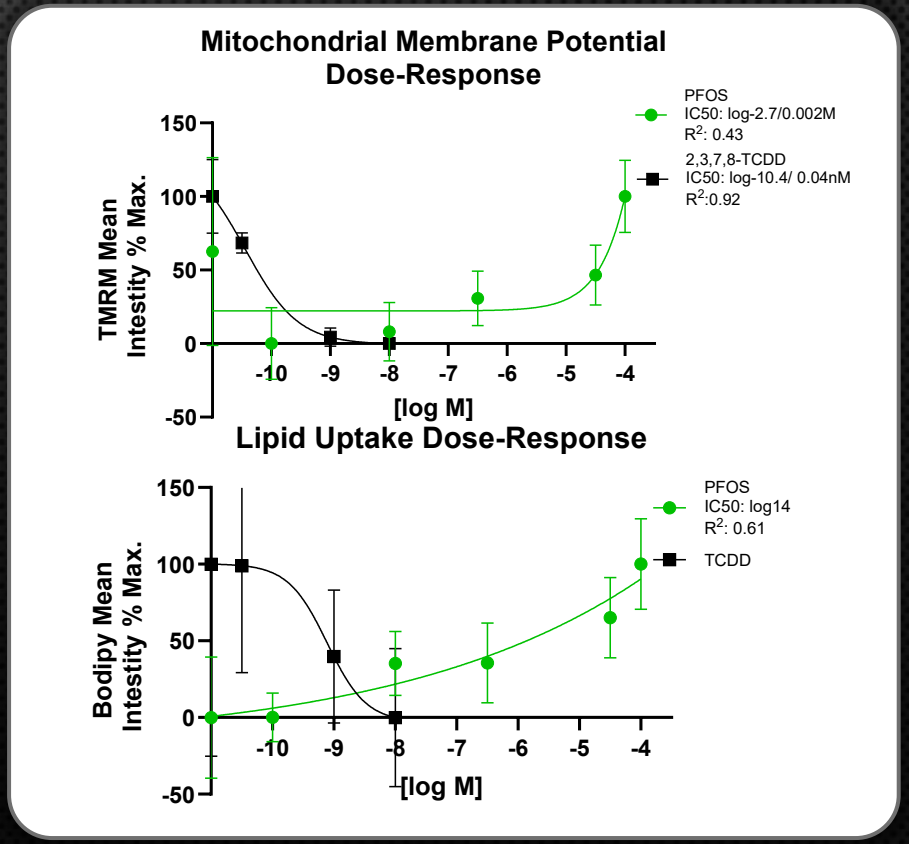


CYP1A1 Activity Dose-Response

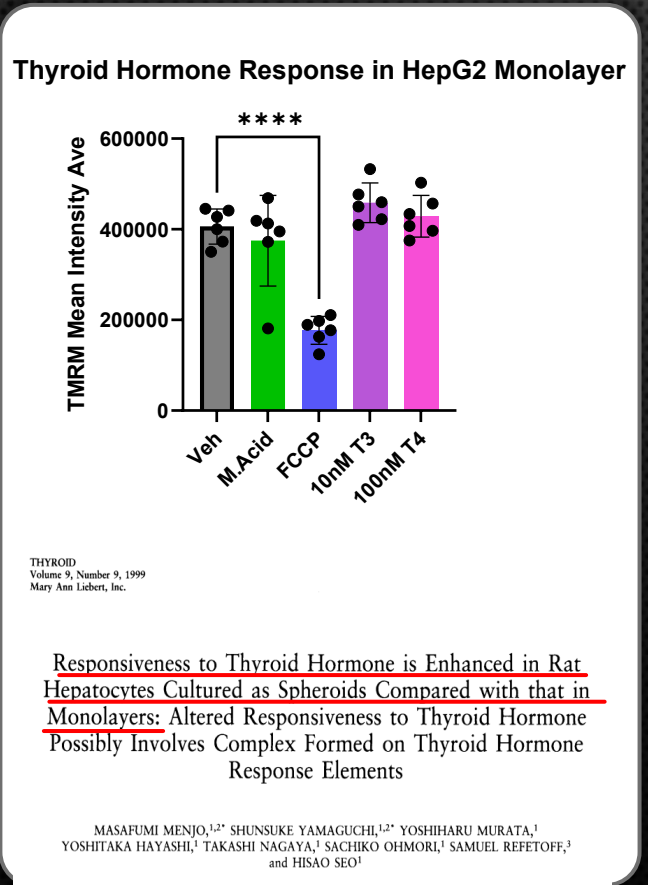


TCDD and PFOS Induce Opposite Effects





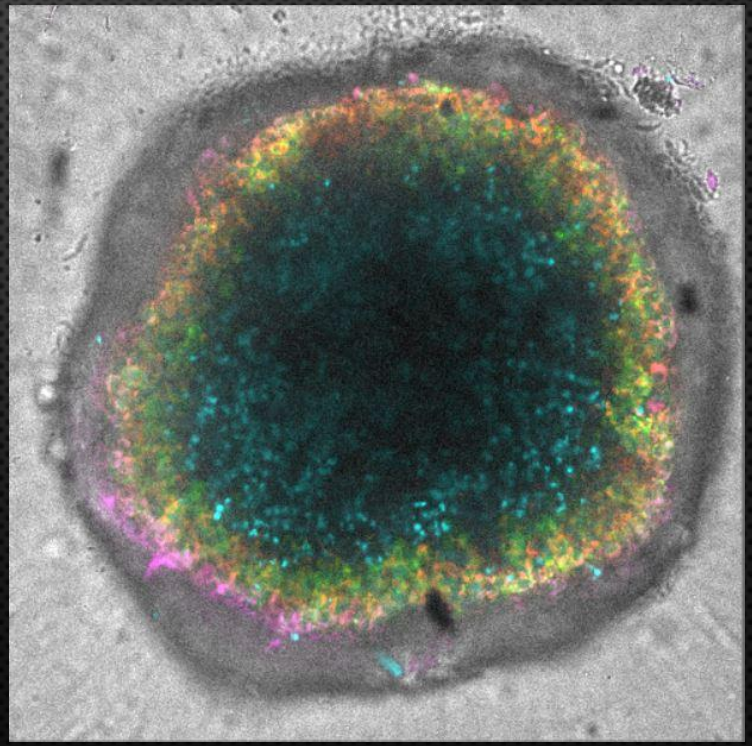
Testing a HepG2 3D Spheroid Culture Assay



THYROID
Volume 9, Number 9, 1999
Mary Ann Liebert, Inc.

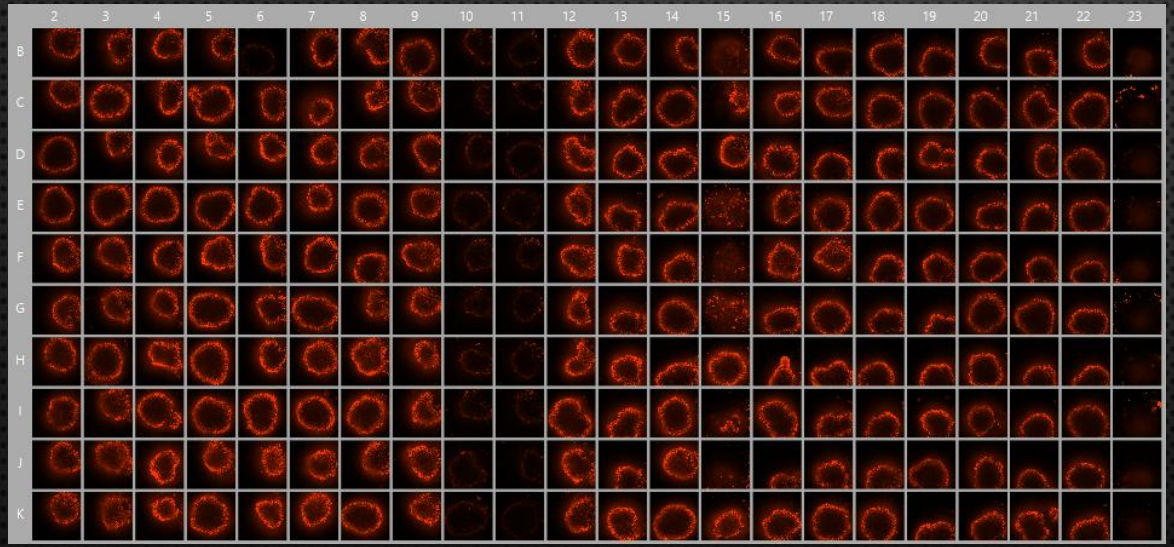
Responsiveness to Thyroid Hormone is Enhanced in Rat Hepatocytes Cultured as Spheroids Compared with that in Monolayers: Altered Responsiveness to Thyroid Hormone Possibly Involves Complex Formed on Thyroid Hormone Response Elements

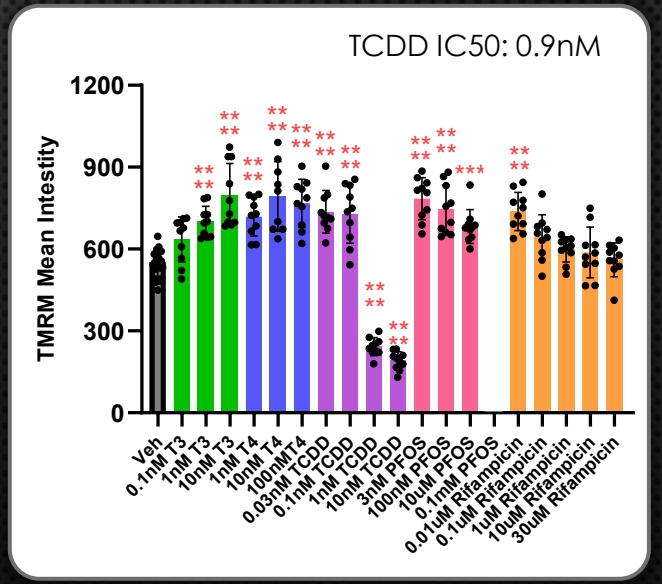
MASAFUMI MENJO,[1,2*] SHUNSUKE YAMAGUCHI,[1,2*] YOSHIHARU MURATA,[1] YOSHITAKA HAYASHI,[1] TAKASHI NAGAYA,[1] SACHIKO OHMORI,[1] SAMUEL REFETOFF,[3] and HISAO SEO[1]



Z-stack: slice 3

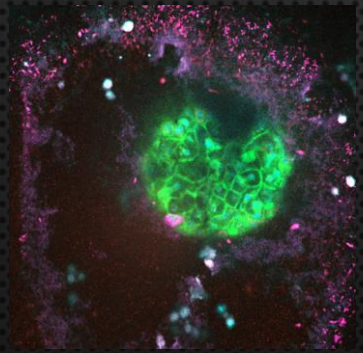
MMP IN HEPG2 3D RESPONSES TO TH,TCDD,PFOS



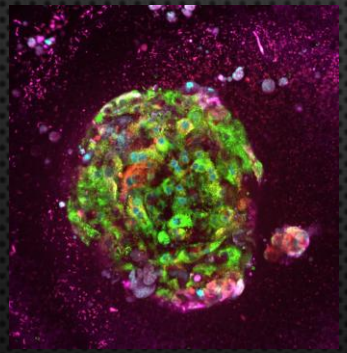


TEST THR ACTIVITY AND ENERGY METABOLISM ENDPOINTS IN OTHER POTENTIAL HEPATOCYTE CELL LINES

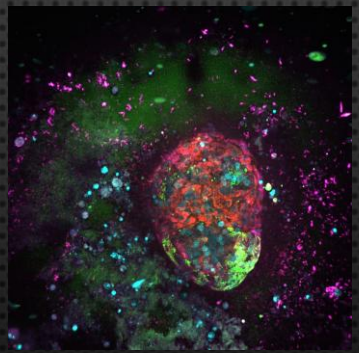
- Test THR activity and energy metabolism endpoints in other potential hepatocyte cell lines



Novabiosis

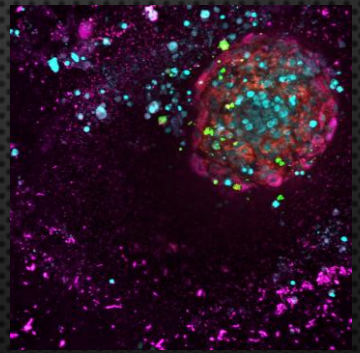


Lonza Donor 1 (D1)

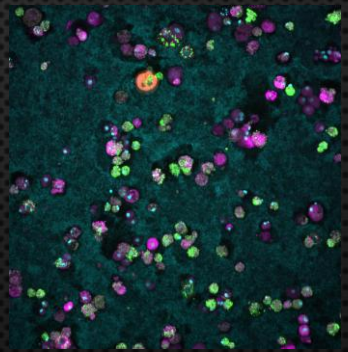


Lonza Donor 2 (D2)

Single culture spheroid
(Various Suppliers)



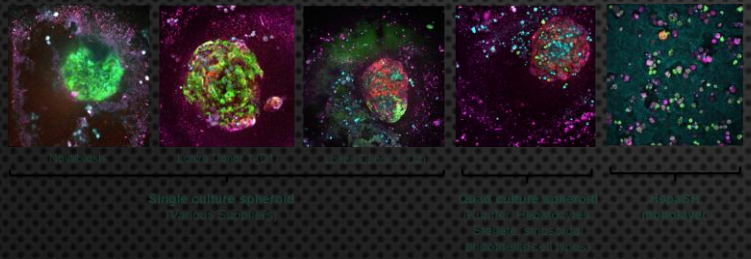
Quad culture spheroid
(Kupffer, Hepatocytes, Stellate, sinusoidal endothelial cell types)

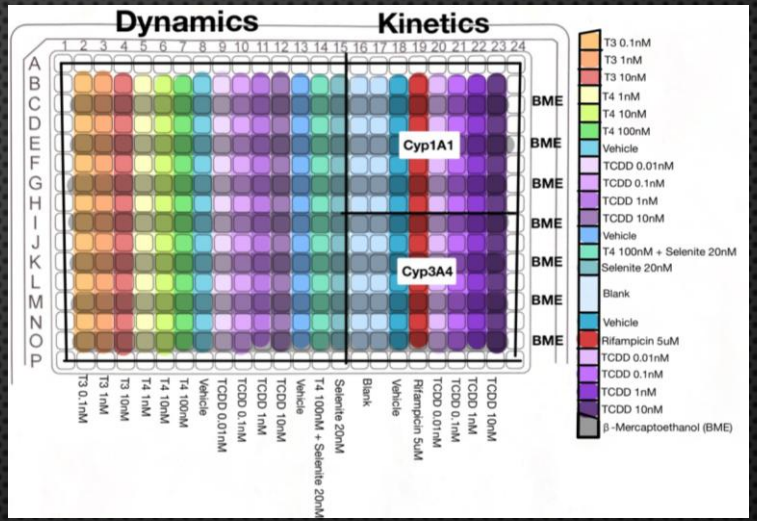


HepaSH monolayer

PHH Models

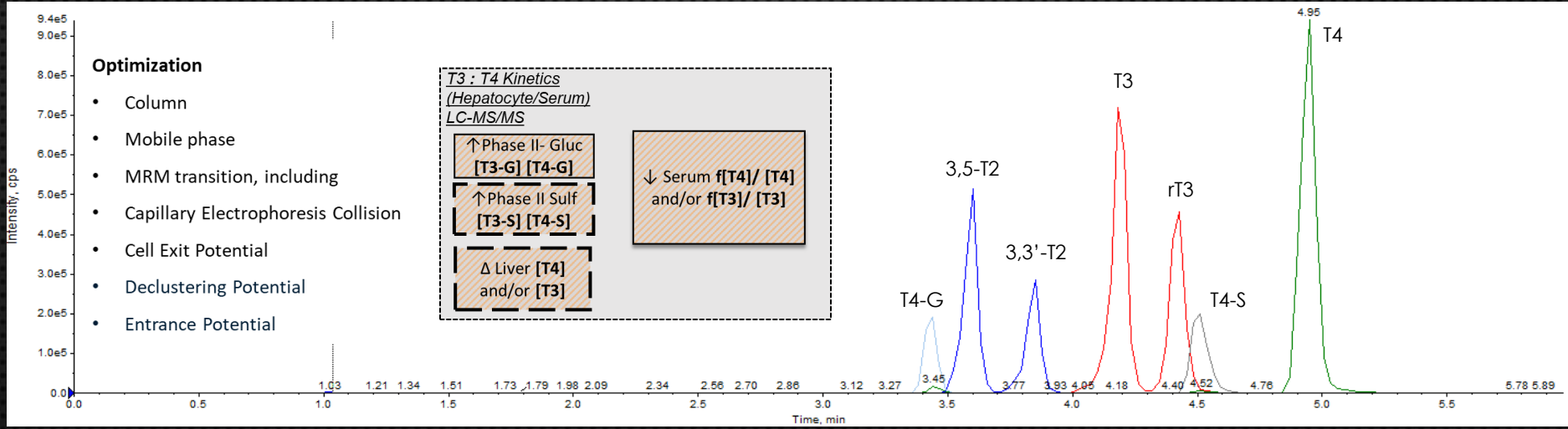
- Test THR activity and energy metabolism endpoints in other potential hepatocyte cell lines





	Lonza 1	Lonza 2	Novabiosis	Quad (+Non-Parenchymal)	Hepa SH	Huh7	HepG2
TCDD 1nM	↑Cyp1A1 (20x)	↑Cyp1A1 (22x)	↑Cyp1A1 (3x)	↑Cyp1A1 (3x)	↑Cyp1A1 (3x)	↑Cyp1A1	↑Cyp1A1 (115x)
Rifampicin 5uM	↑Cyp3A4 (5x)	↑Cyp3A4 (23x)	No Change	↑Cyp3A4 (7x)	↑Cyp3A4 (2x)	No Change	No change

LC-MS/MS Assay for TH and metabolites in 100uL media

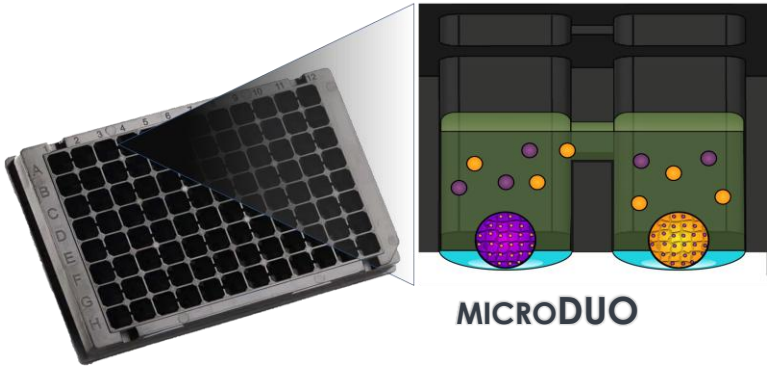


- 3,3′,5-triiodo-L-thyronine (T3), thyroxine (T4), 3,3′,5′-triiodo-L-thyronine (rT3), 3,5-diiodo-L-thyronine (3,5-T2), 3,3′-diiodo-L-thyronine (3,3′-T2), thyroxine glucuronide (T4-G) and thyroxine sulfate (T4-S)

Plug and Play Complex in vitro Modeling

Complex in vitro

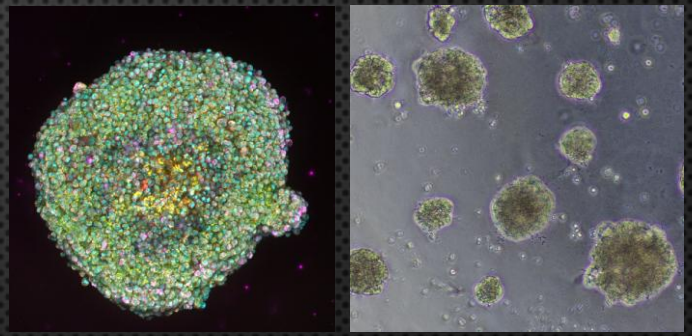
Plug and Play Coculture

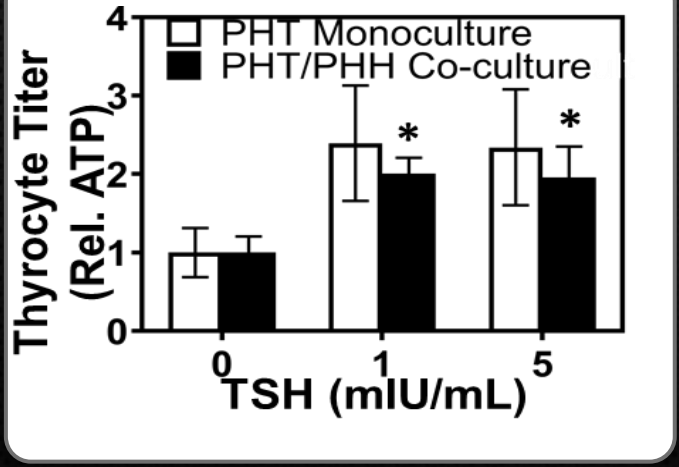


Functional integration of thyroid and liver monocultures assays microDUO.

Preserves individual model strengths while enabling cross-talk.

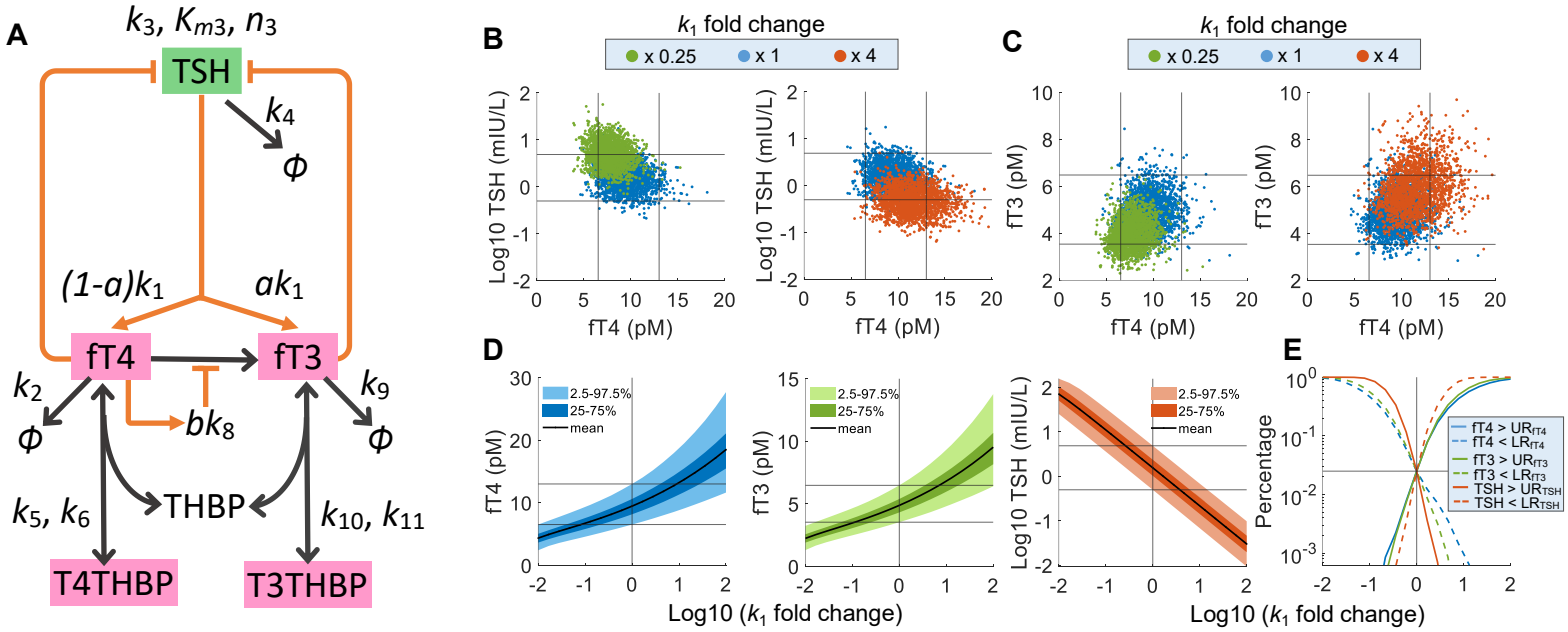
Human Hepatocytes/ Thyrocyte Follicles





IVIVE - Human population HPT model that predicts in vivo TH changes

COMPUTATIONAL PBK AND IVIVE

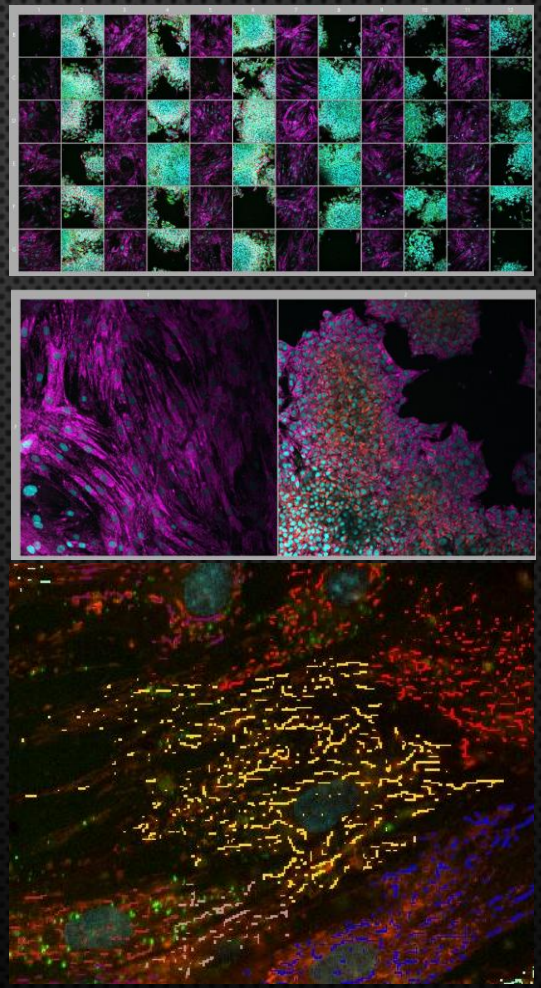


TAKE-HOMES

Combining AOPs, advanced in vitro systems, and computational modeling creates a powerful framework for predicting thyroid disruption.

Scalability and low-cost/n facilitate CIVM assay development

Integrated approach is a blueprint for future NAMs.



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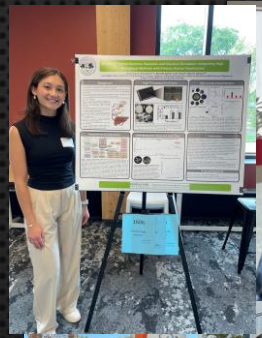
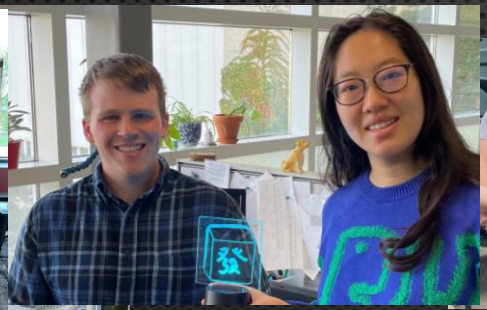


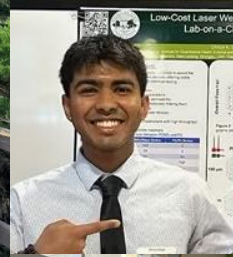


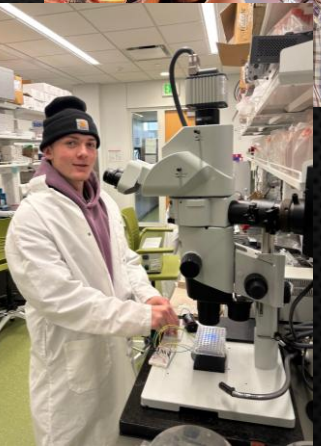


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