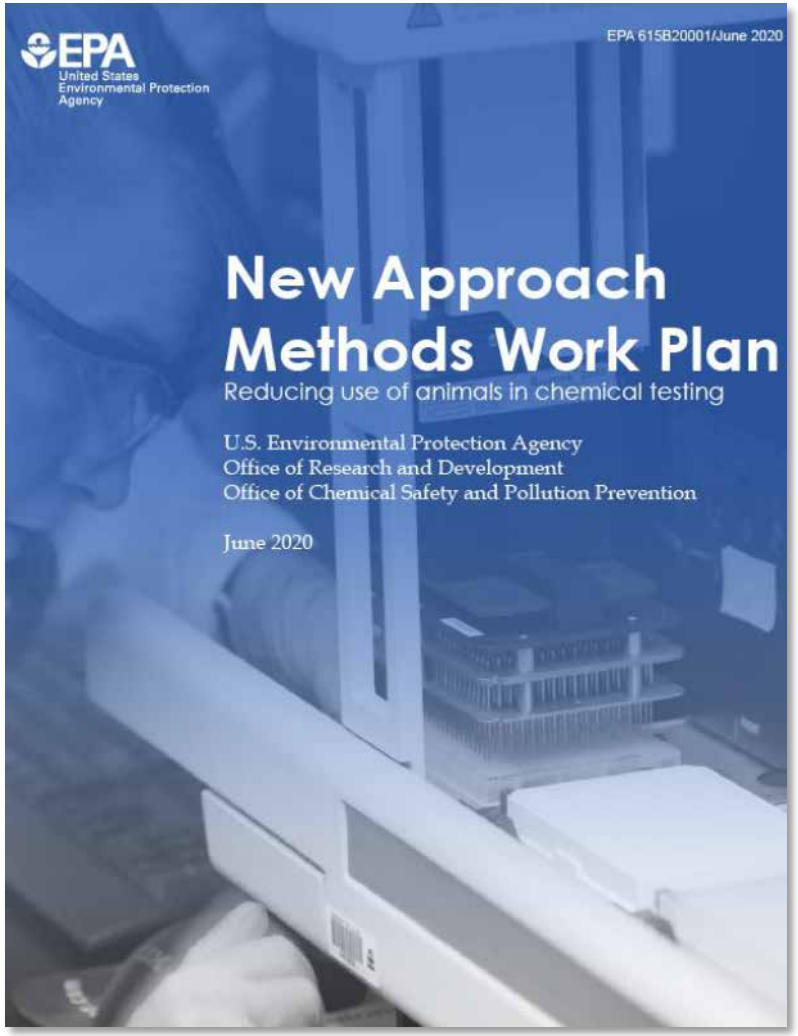
Update on Alternatives Research Activities at EPA

ICCVAM Public Forum

May 27, 2021

Rusty Thomas
Director
Center for Computational Toxicology and Exposure

The Release of the EPA NAM Work Plan Provided Clear Objectives, Strategies and Deliverables



- Five objectives for achieving the reduction goals while ensuring that Agency decisions remain fully protective of human health and the environment
  - Evaluate Regulatory Flexibility
  - Develop Baselines and Metrics
  - Establish Scientific Confidence and Demonstrate Application
  - Develop NAMs to Address Information Gaps
  - Engage and Communicate with Stakeholders
- Short- and long-term strategies EPA will use to accomplish the objectives
- Specific deliverables and timelines linked with each objective
- Recognition that the EPA NAMs Work Plan represents a snapshot in time and will evolve as EPA's knowledge and experience grows

Moving Forward with NAM Work Plan Deliverable to Set Expectations for Alternative Models

EPA Developed an Operational Blueprint to Focus and Facilitate Progress

SOT | Society of Toxicology

www.toxsci.oxfordjournals.org

FORUM

The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

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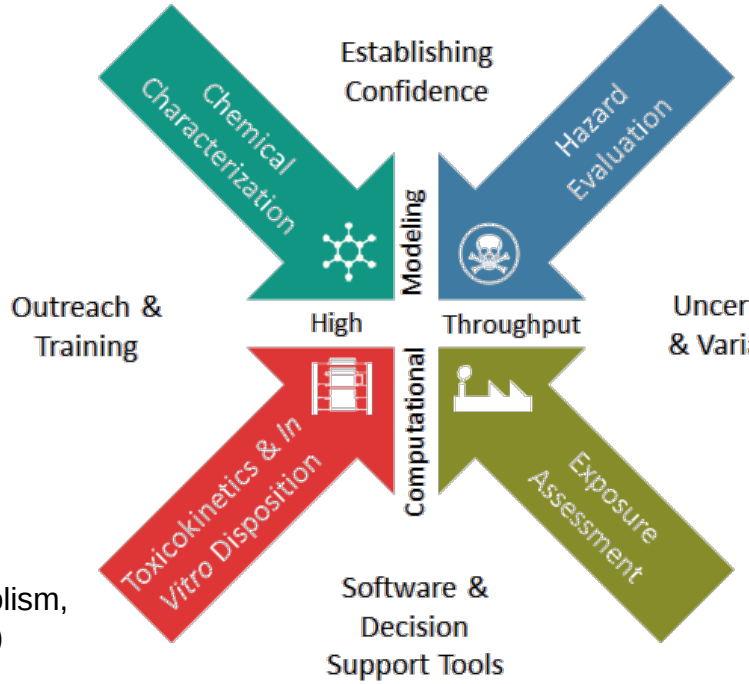
ABSTRACT

The U.S. Environmental Protection Agency (EPA) is faced with the challenge of efficiently and credibly evaluating chemical safety often with limited or no available toxicity data. The expanding number of chemicals found in commerce and the environment, coupled with time and resource requirements for traditional toxicity testing and exposure characterization,

- DSSTox
- Chemical library
- Read across
- SAR/QSAR modeling
- Chemotypes
- TTC

- Case Studies
- Reference Materials
- Reporting Templates

- In Vitro Assays (HTTr, HTPP, ToxCast)
- Tiered testing
- Organotypic models
- Addressing limitations (metabolism, chemical space)
- Statistical and Biologically-based Modeling
- AOPs



- Communities of Practice
- ToxCast Owners Manual
- Training courses/ videos

- SEEM
- ToxBoot
- HTTK
- ENTACT
- ToxRefDB

- HTTK assays (metabolism, bioavailability, binding)
- Partition coefficients
- HTTK R package
- Multi-route models
- Model verification (e.g., CvT)
- In vitro disposition

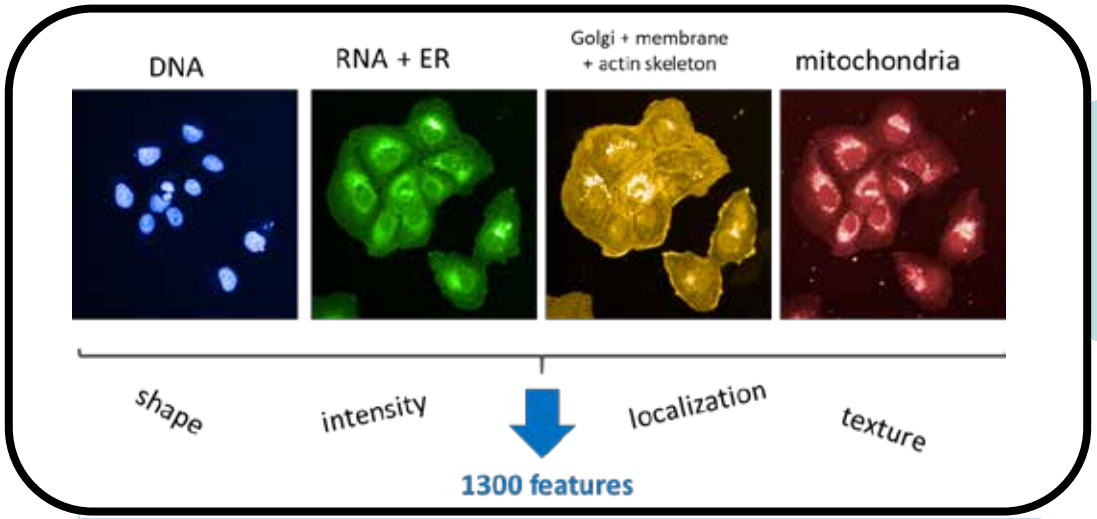
- CompTox Chemicals Dashboard
- RapidTox
- Factotum
- ECOTOX
- SeqAPASS

- ExpoCast
- NTA/SSA
- CPDat/CPCat
- Product emissivity

Application of Cellular Phenotypic Profiling for Mechanism of Action and POD/BER Estimation

EPA United States Environmental Protection Agency

Cell Painting assay (Bray *et al.* 2016)

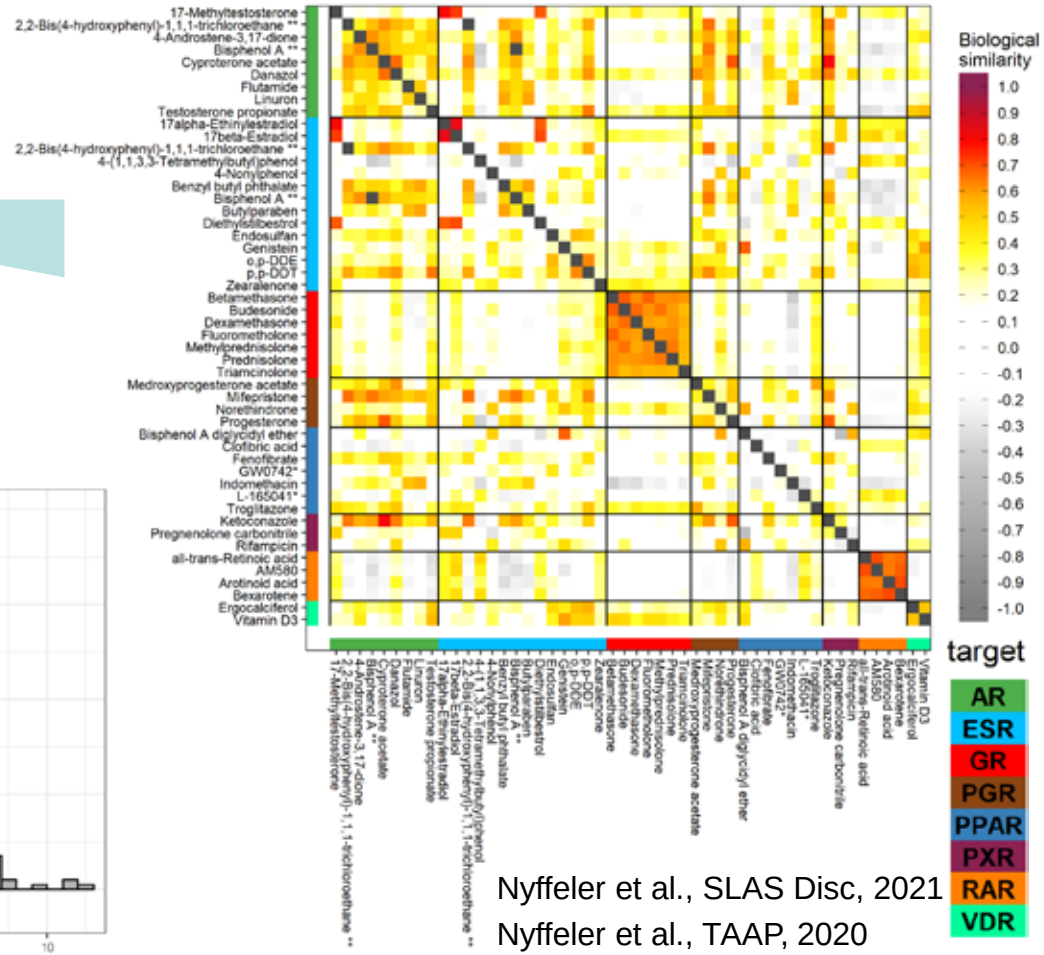


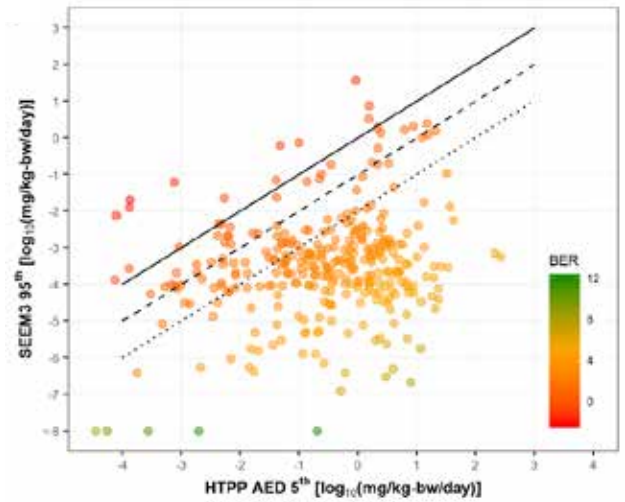
Application 1: POD and BER Estimation

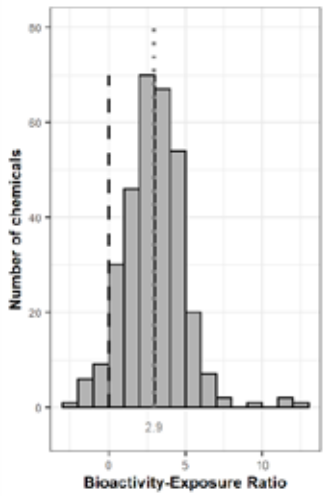
Application 2: Profile Comparison for Chemical Grouping & MIE Prediction

Profile

Biological similarity





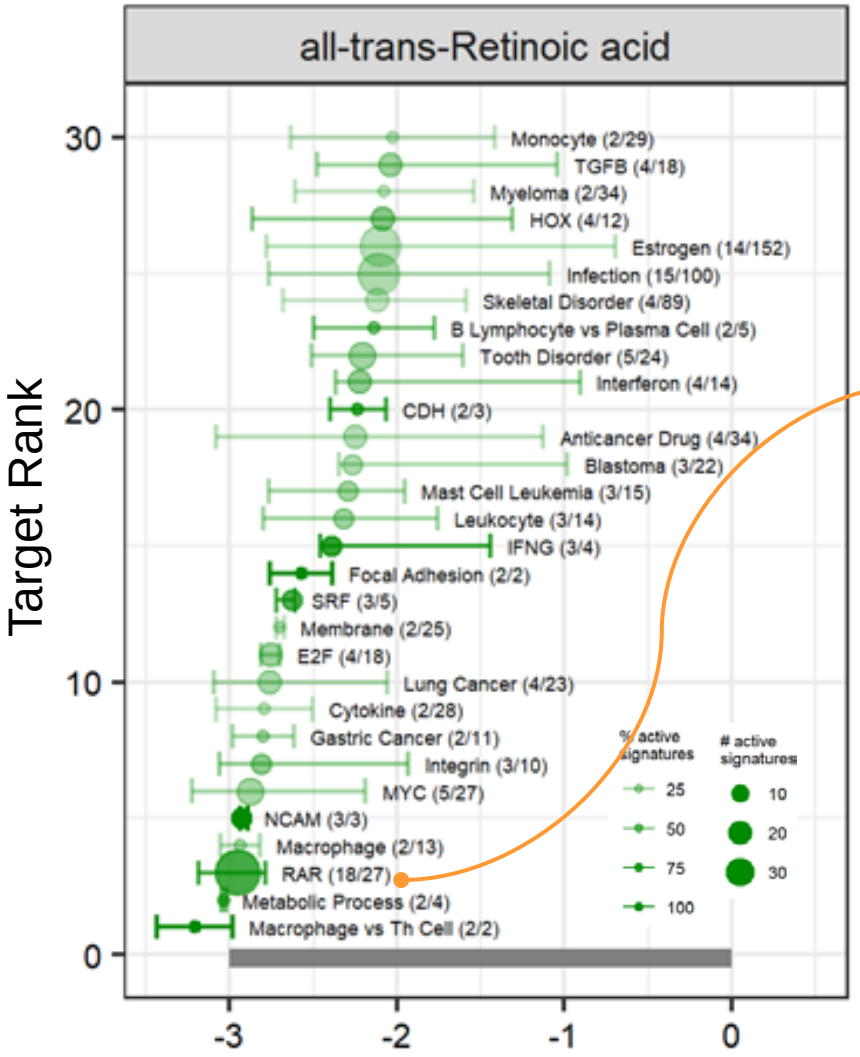


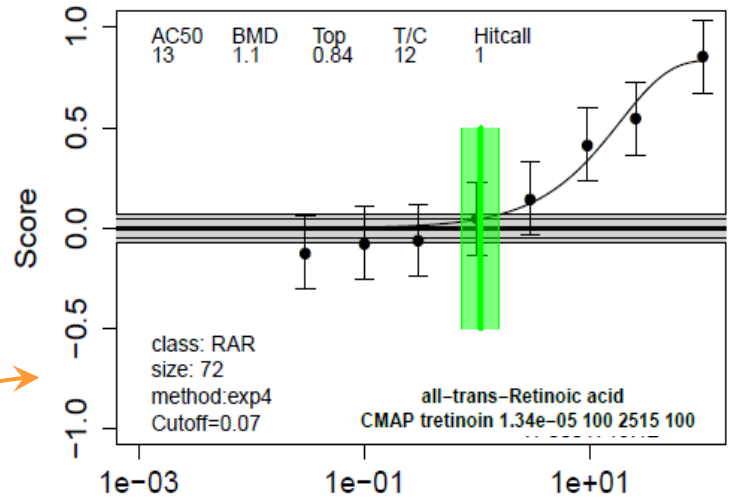
Nyffeler et al., SLAS Disc, 2021

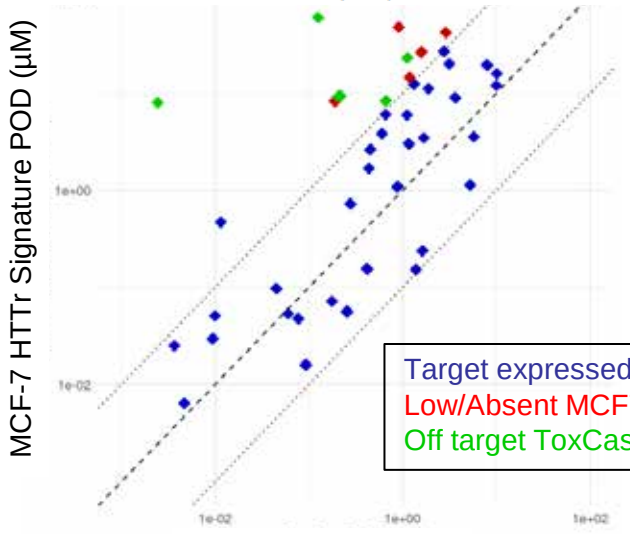
Nyffeler et al., TAAP, 2020

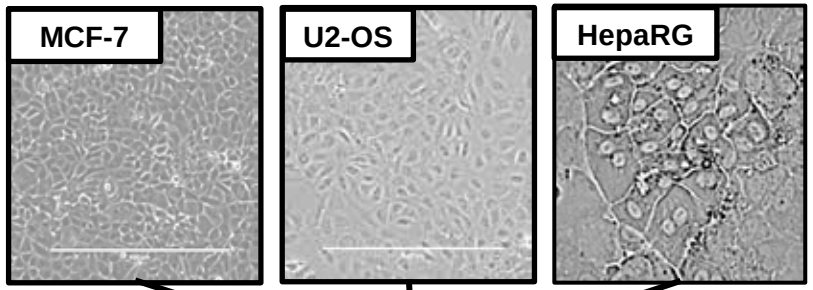
Nyffeler et al., Unpublished.

Application of High-Throughput Transcriptomics for Mechanism of Action and POD Estimation









> 1200 ToxCast Chemical
APCRA Case Study Chemicals
Pharmacological Reference Chemicals

Target expressed in MCF7
Low/Absent MCF7 target expression
Off target ToxCast hit most potent

Harrill *et al., Toxicol Sci.*, 2021
Harrill *et al., Unpublished*

Selecting Cell Types for Phenotypic Profiling and Transcriptomics to Maximize Biological Coverage

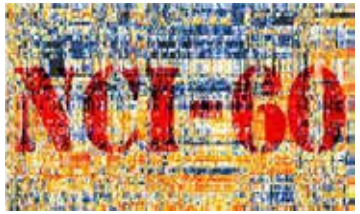
EPA United States Environmental Protection Agency

Public Gene Expression Databases

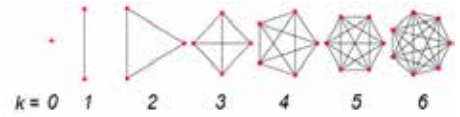
CCLE Cancer Cell Line Encyclopedia

RESEARCH ARTICLE Open Access

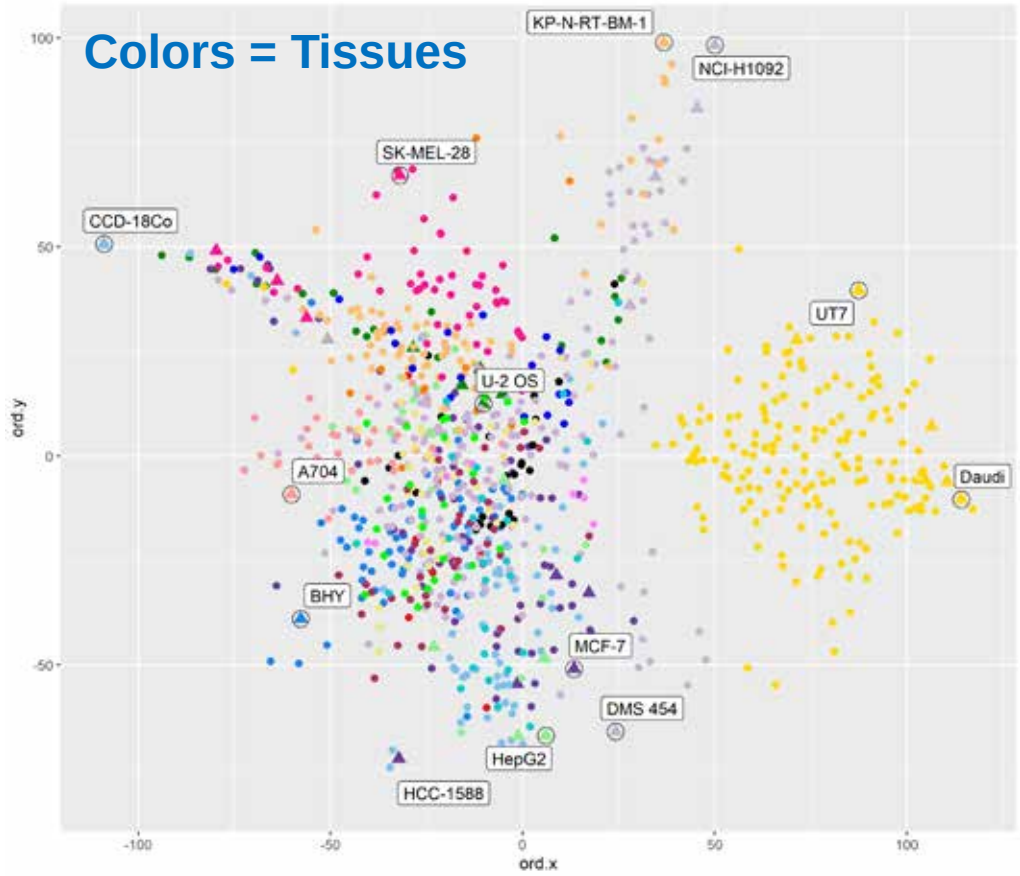
An expression atlas of human primary cells: inference of gene function from coexpression networks



Content Maximization



- Edge length = Euclidean distance
- Set seeds.
- Maximize volume of the hyperspace



Cell Line Subset Selection

Cancer Cell Lines

Cell Line	Organ Lineage	Derivation
MCF-7 *	Breast	Cancer
U-2 OS *	Bone	Cancer
HepG2 *	Liver	Cancer
Daudi	Immune	Cancer
CCD-18Co	Fibroblast	Immortalized
NCI-H1092	Lung	Cancer
DV-90	Lung	Cancer
SET-2	Immune	Cancer
BHY	Skin	Cancer
SK-MEL-28	Skin	Cancer
KP-N-RT-BM-1	CNS	Cancer
DMS-454	Lung	Cancer
A-704	Kidney	Cancer

Immortalized Primary Cell Lines

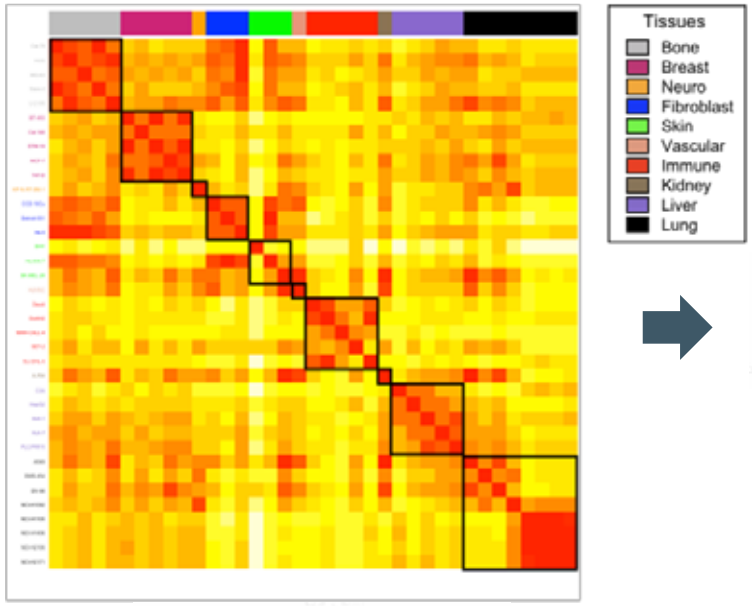
Cell Line	Organ Lineage	Derivation
HME-1	Breast	Immortalized
ASC52telo	Mesenchymal Stem Cell	Immortalized
CHON-001	Fibroblast	Immortalized
Ker-CT	Skin	Immortalized
HUVEC	Vascular	Immortalized
TeloHAEC	Vascular	Immortalized
TIME	Vascular	Immortalized
RPTEC	Kidney	Immortalized
HPNE	Pancreas	Immortalized
HBEC3-KT	Lung	Immortalized
HSAEC-1	Lung	Immortalized
RPE-1	Retina	Immortalized

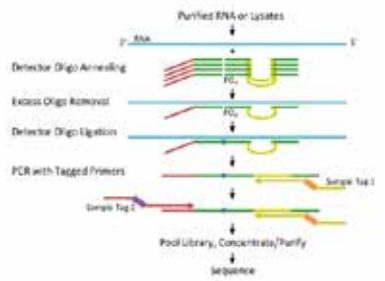
Harrill and Sipes, *Unpublished*

Selecting Cell Types for Phenotypic Profiling and Transcriptomics to Maximize Biological Coverage

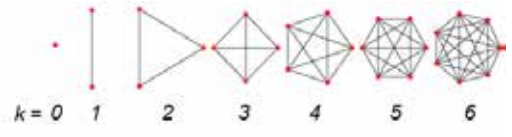
United States Environmental Protection Agency

Gene Expression Similarity Matrix with TempO-Seq





Content Maximization



- Euclidian distance used for edge length
- Maximize volume of the hyperspace

"Next-One-Up" Cell Line Selection

Immortalized Primary Cell Lines

Cell Type	Tissue Origin
MCF-7	Breast
HepaRG_2D	Liver
U-2 OS	Bone
HBEC3-KT	Lung
hNP1	CNS
CHON-001	Fibroblast
TeloHAEC	Vascular
RPTEC	Kidney
Ker-CT	Skin
ARPE-19	Retina
CCD-18Co	Fibroblast
ASC52telo	Mesenchymal Stem Cell
BJ-5ta	Fibroblast
HME-1	Breast
HPNE	Pancreas
TIME	Vascular
RPE-1	Retina
HUVEC	Vascular
HSAEC-1	Lung

Cancer Cell Lines

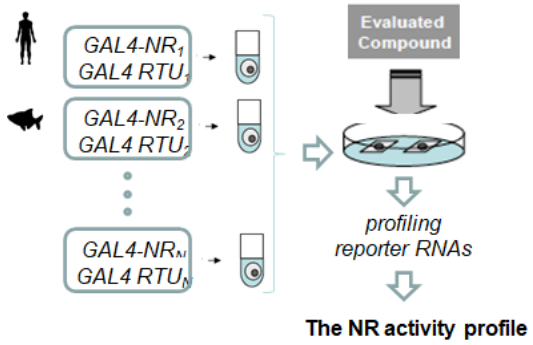
Cell Type	Tissue Origin
MCF-7	Breast
HepaRG_2D	Liver
U-2 OS	Bone
BHY	Skin
C3A	Liver
Detroit-551	Fibroblast
KP-N-RT-BM-1	CNS
DMS-454	Lung
DV-90	Lung
SK-MEL-28	Skin
BT-483	Breast
PLC/PRF/5	Liver
A-704	Kidney
Saos-2	Bone
MG-63	Bone
Huh-1	Liver
Huh-7	Liver
EFM-19	Breast
A549	Lung
Hs.839.T	Skin
HTB-9	Urinary Bladder
Cal-78	Bone
T47-D	Breast
HOS	Bone
HepG2	Liver
Hs-5	Fibroblast

Harrill and Sipes, *Unpublished*

Evaluating Cross-Species Sensitivity to Nuclear Receptor Activity

United States Environmental Protection Agency

Cross-Species Factorial Assay



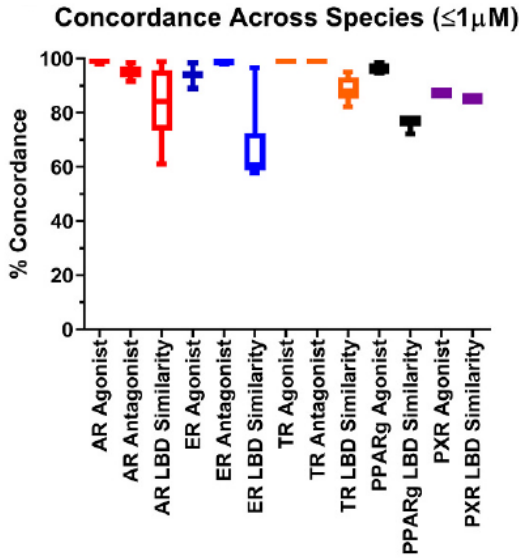
EcoTox Factorial Assay

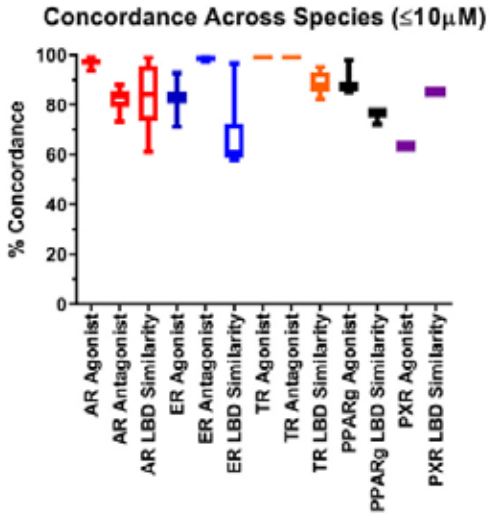
NR	Class	Species
ER1	Fish	Danio rerio
ER2α		Danio rerio
ER2β		Danio rerio
ER1	Amphibian	Xenopus laevis
ER2		Xenopus laevis
ER1	Reptilian	Chrysemys picta
ER1	Avian	Gallus gallus
ERα	Mammalian	Homo Sapiens
ERβ		Homo Sapiens
AR	Fish	Danio rerio
AR	Amphibian	Xenopus laevis
AR	Reptilian	Chrysemys picta
AR	Avian	Gallus gallus
AR	Mammalian	Homo Sapiens
TRα	Fish	Danio rerio
TRβ		Danio rerio
TRα	Amphibian	Xenopus laevis
TRα	Reptilian	Chrysemys picta
TRα	Mammalian	Homo Sapiens
TRβ		Homo Sapiens
PPARγ	Fish	Danio rerio
PPARγ	Mammalian	Mus musculus
PPARγ		Homo Sapiens
PXR	Mammalian	Mus musculus

XS-2 Factorial Assay

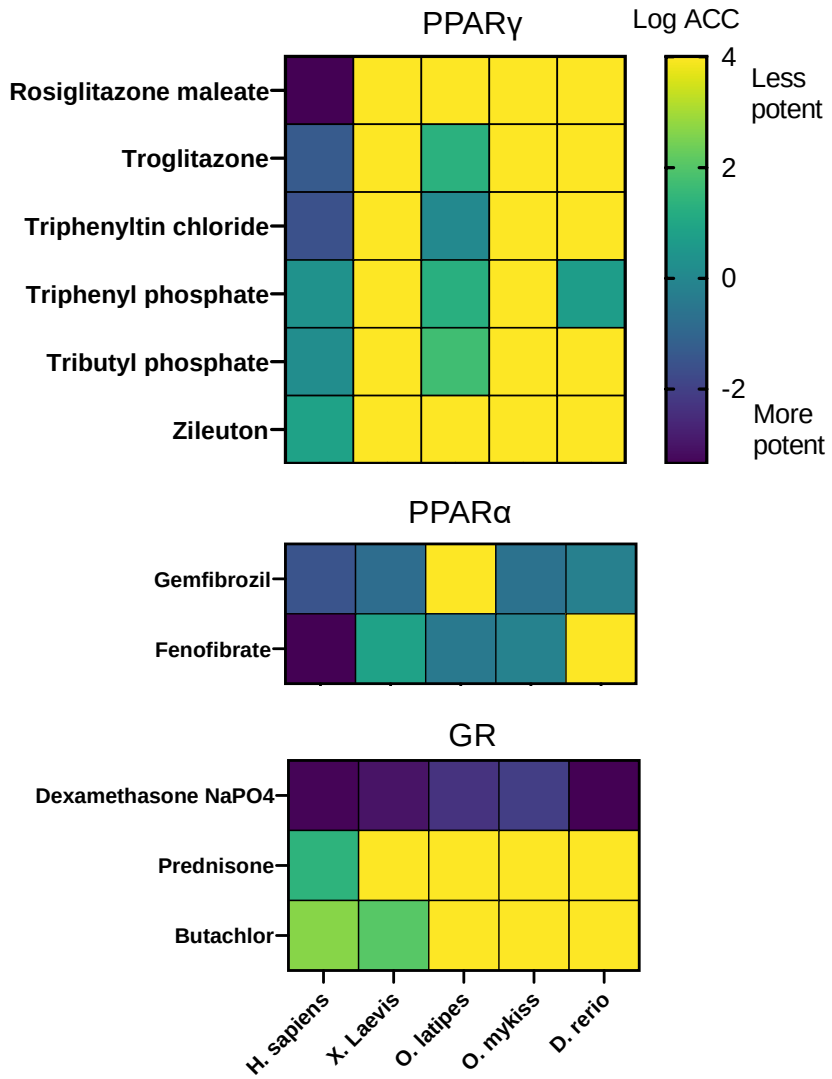
NR	Species	Latin names
GR	human	Homo Sapiens
GR	african clawed frog	Xenopus laevis
GR	rainbow trout	Oncorhynchus mykiss
GR	japanese medaka	Oryzias latipes
GR	Zebrafish	Danio rerio
PPARa	human	Homo Sapiens
PPARa	african clawed frog	Xenopus laevis
PPARa	rainbow trout	Oncorhynchus mykiss
PPARa	japanese medaka	Oryzias latipes
PPARa	Zebrafish	Danio rerio
PPARg	human	Homo Sapiens
PPARg	african clawed frog	Xenopus laevis
PPARg	rainbow trout	Oncorhynchus mykiss
PPARg	japanese medaka	Oryzias latipes
PPARg	Zebrafish	Danio rerio
RXRb	human	Homo Sapiens
RXRb	african clawed frog	Xenopus laevis
RXRb	rainbow trout	Oncorhynchus mykiss
RXRb	japanese medaka	Oryzias latipes
RXRb	Zebrafish	Danio rerio
ERa	human	Homo Sapiens
ER1	Zebrafish	Danio rerio
ER1	african clawed frog	Xenopus laevis
AR	human	Homo Sapiens
AR	Zebrafish	Danio rerio

EcoTox Factorial Assay



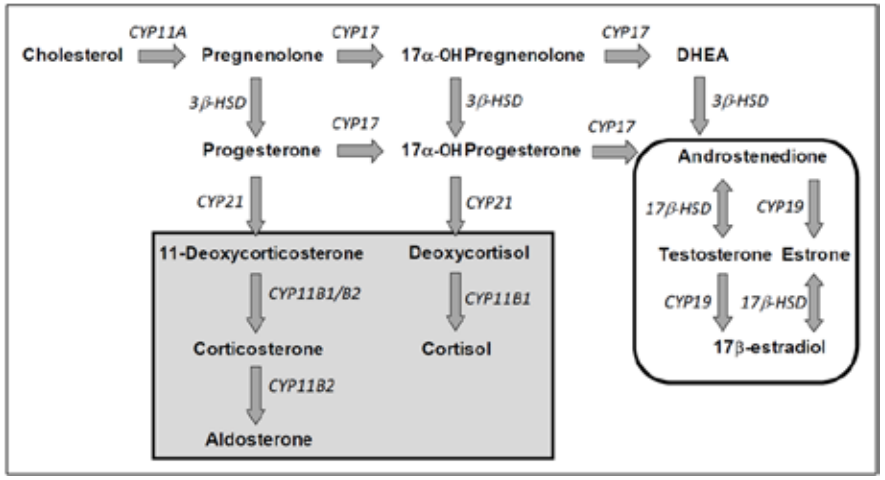


XS-2 Factorial Assay



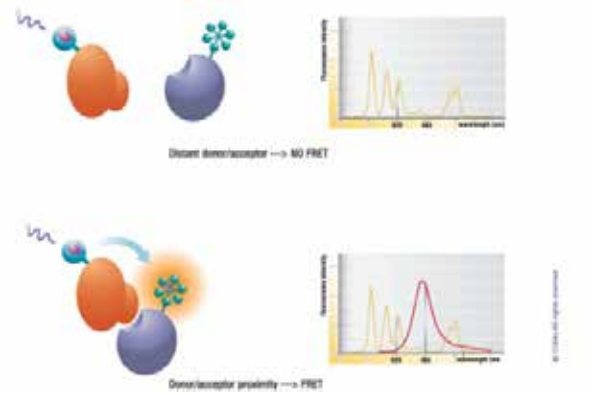
Modifying H295R Steroidogenesis Assay for Increased Scalability and Reduced Cost

Steroidogenic Pathway in H295R Cells

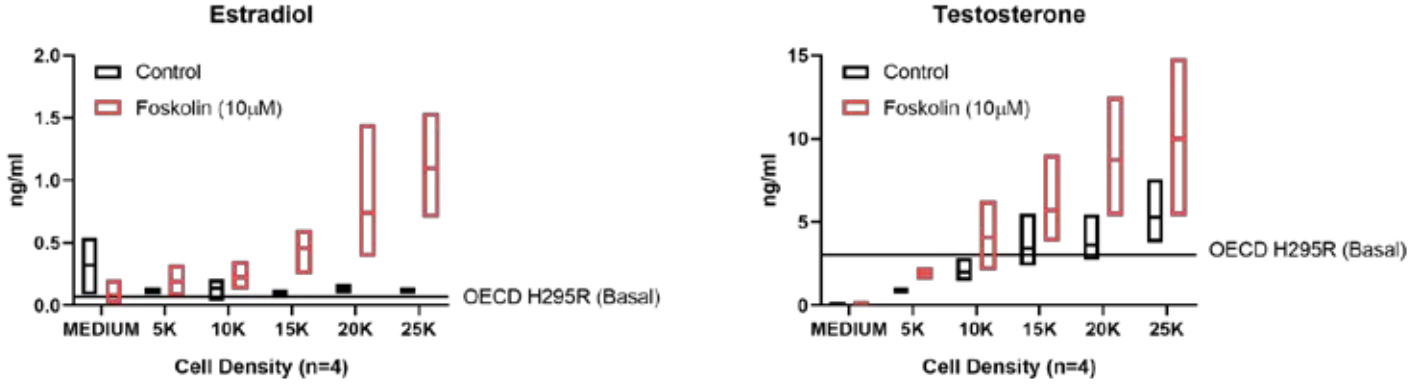


- Guideline H295R steroidogenesis assay (OECD 456) evaluates 17β-estradiol (E2) and testosterone (T) synthesis.
- ToxCast HT-295R assay evaluated 11 hormones including androgens, estrogens, progestagens, and corticosteroids using analytical chemistry methods and was time and resource intensive.
- ToxCast HT-295R assay performed initial testing at single concentration with limited replicates followed by concentration response on positive hits.
- Adapting H295R assay to 384-well format using Homogenous Time Resolved Fluorescence (HTRF) to inexpensively evaluate E2 and T endpoints enabling full concentration response testing with replicates.

Homogenous Time-resolved Fluorescence (HTRF) can Rapidly Measure E2 and T concentrations



Preliminary Basal and Induced Analyte Levels in 384-well H295R assay



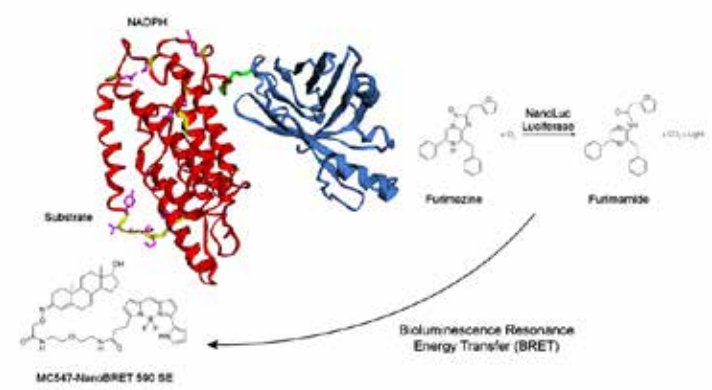
Deisenroth *et al.*, Unpublished

Development and Application of a 5α-Reductase Assay for Androgen Steroidogenesis

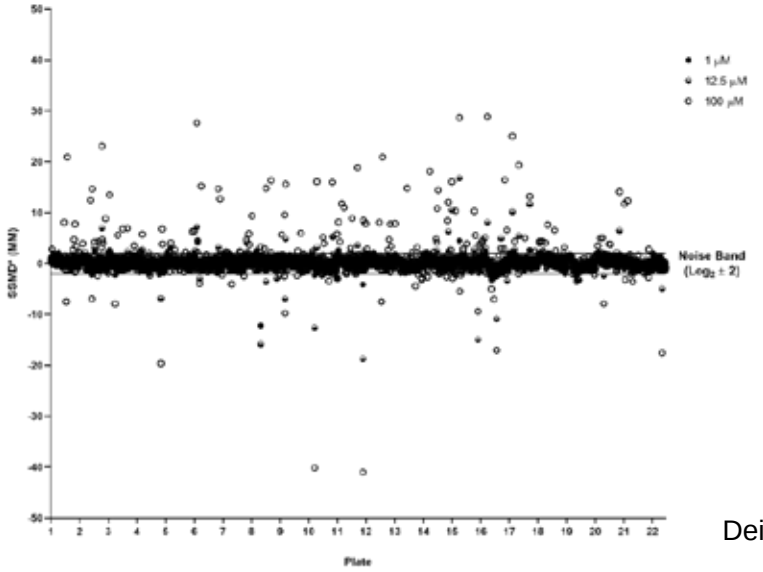
Data gaps for a Comprehensive *in vitro* AR Assay Battery

- Target tissue androgen steroidogenesis (conversion of testosterone to dihydrotestosterone) represents an *in vitro* testing gap relative to *in vivo* testing.
- A custom cell-based NanoBRET assay was developed that demonstrates selectivity for 5$^{\alpha}$-reductase substrates and inhibitors.
- Evaluation of ToxCast chemical library identifies putative inhibitors of enzyme activity.

Customized 5α-Reductase NanoBRET Assay



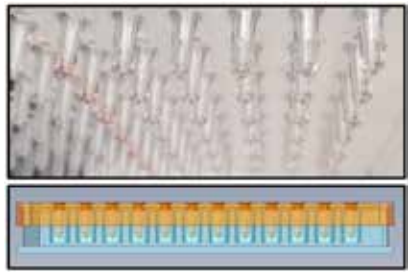
Preliminary Screening Results for 1803 ToxCast and E1K Chemicals



Deisenroth *et al.*, Unpublished

Retrofitting *In Vitro* Assays with Metabolic Competence

"Extracellular" Approach



Chemical metabolism in the media or buffer of cell-based and cell-free assays

More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Approach

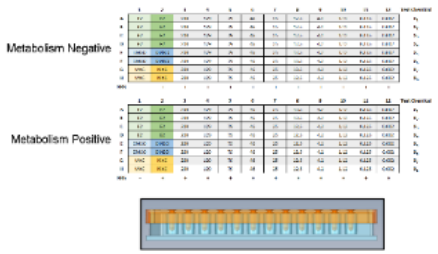
Metabolizing chemicals inside the cell in cell-based assays

More closely models effects of target tissue metabolism

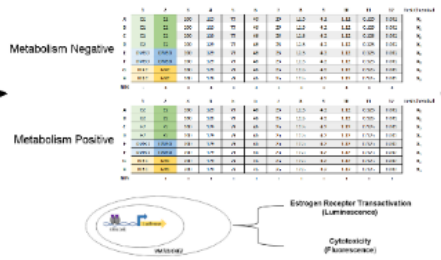
Integrated approach to model *in vivo* metabolic bioactivation and detoxification

Application of the Extracellular Approach to Estrogen Receptor Testing

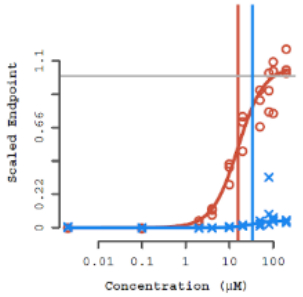
AIME Metabolism Assay



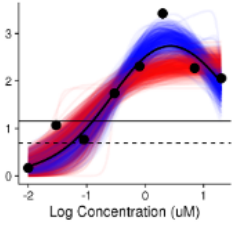
Estrogen Receptor Transactivation Assay



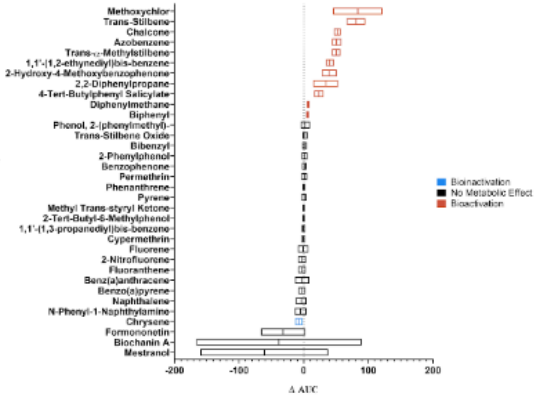
ToxCast Pipeline

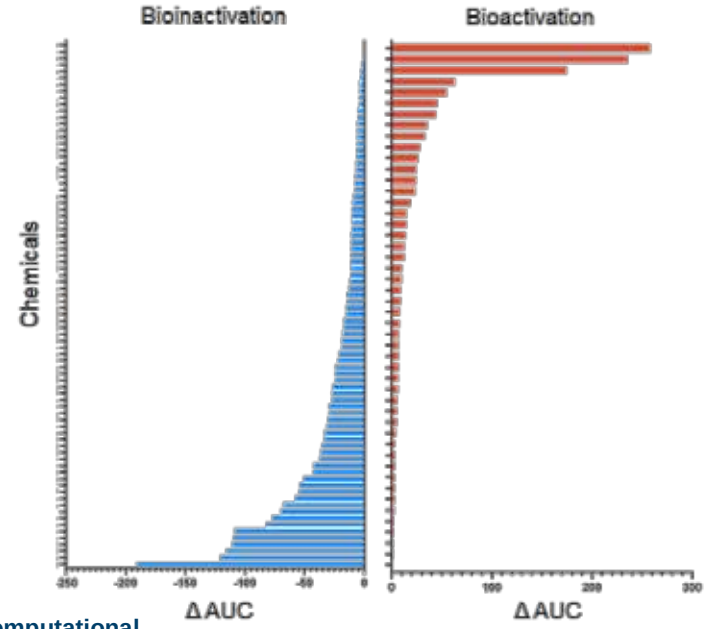


Toxboot Uncertainty Quantification



Rank Metabolism-dependent Bioactivity





Preliminary Analysis of 768 ToxCast Chemical Screen

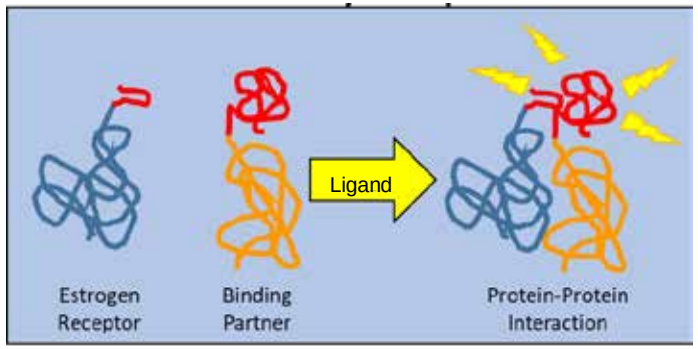
- Application of Deisenroth et al., *Toxicol Sci.,* 2020
- 11% of chemicals exhibit metabolism-dependent changes in ER bioactivity. Most are estrogenic ± metabolism.
- False positive and false negative chemicals represent 3.6% of total chemicals screened.
- Profiles of predicted routes of biotransformation and potential metabolites.

Deisenroth *et al.,* Unpublished

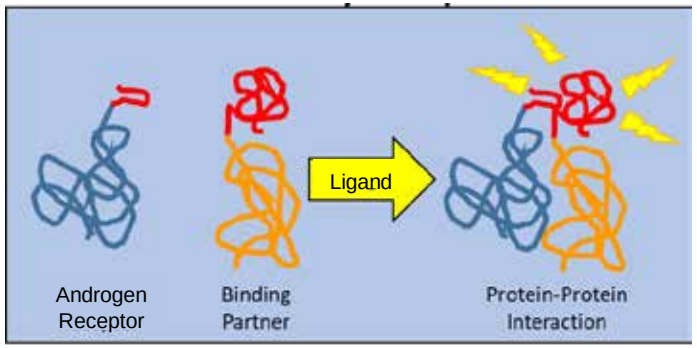
Application of the Intracellular Approach to Estrogen and Androgen Receptor Testing

United States Environmental Protection Agency

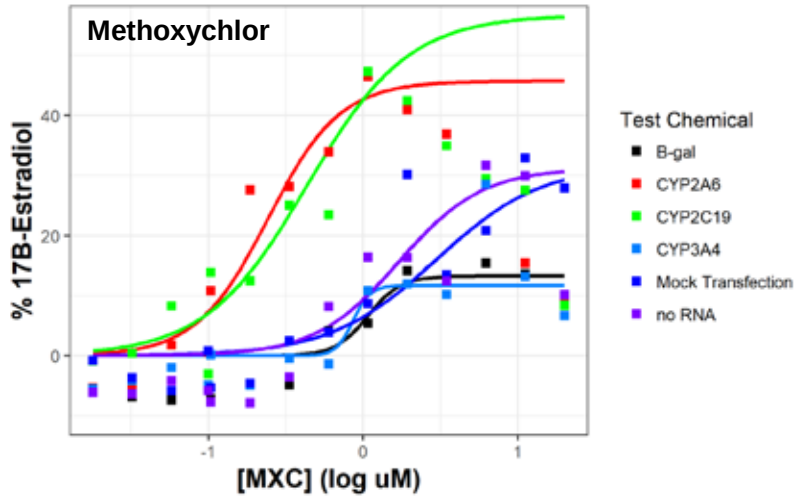
ER Protein-Protein-Interaction Assay (ERPPI)

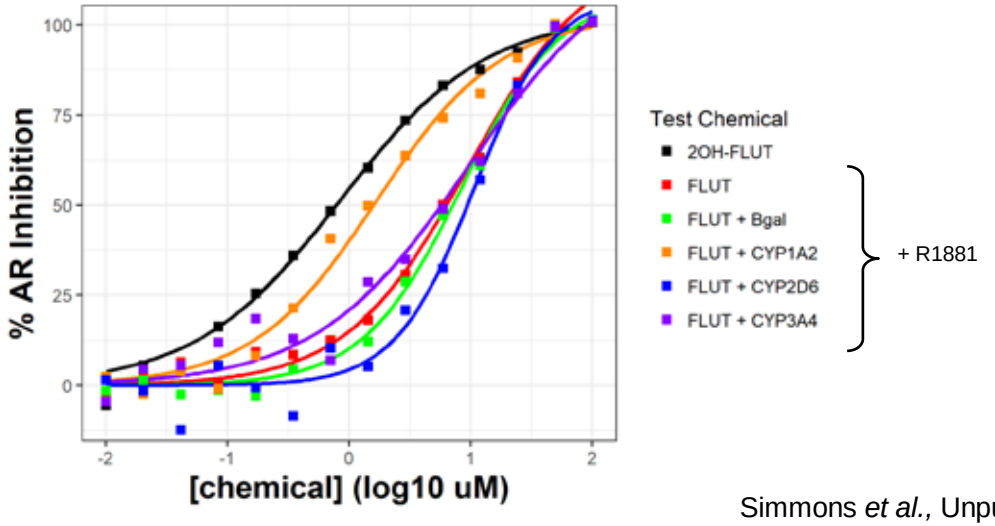


AR Protein-Protein-Interaction Assay (ARPPI)



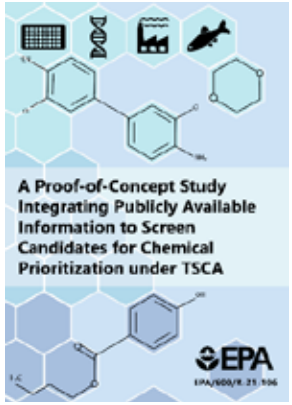
Preliminary Testing Results

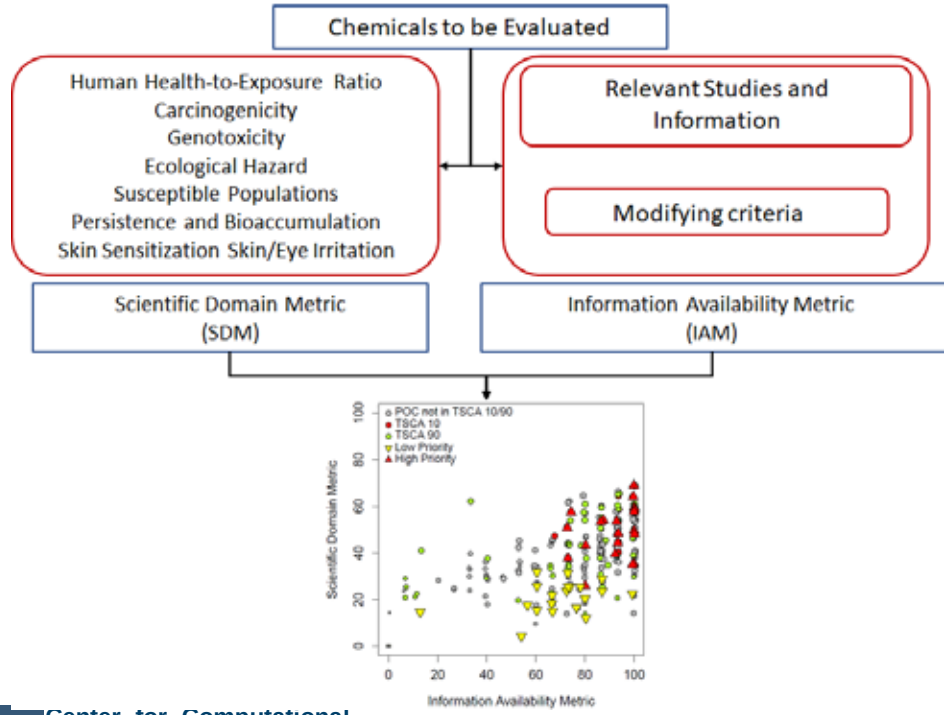




Simmons *et al.*, Unpublished

Integration of NAMs and Traditional Data for Prioritizing Large Chemical Inventories





- Proof-of-concept study demonstrating integration of publicly available information hazard, exposure, persistence, and bioaccumulation information for a large number of chemicals.
- Evaluates chemicals based on both potential concern related to human health and the environment and information availability.
- The study was intended to:
  - Be generally consistent with previous TSCA workplan process.
  - Provide a transparent and reproducible process for integrating available information and identifying potential information gaps.
  - Increase efficiency and manage workload by focusing expert review on substances that may have a greater potential for selection as high- or low-priority candidates.
  - Create a flexible and sustainable process that can adapt to scientific advances and continual generation of new safety-related information.
  - Organize the process into modular workflows that can be readily updated or adapted to address scientific advances and prioritization needs under other mandates.

Take Home Messages…

- The EPA NAM Work Plan and CompTox Blueprint provide strategic and operational direction for research and translation of NAMs
- ORD is working on a diverse portfolio of research activities to meet the address information gaps and build scientific confidence in NAMs
- Continued development and refinement of new technologies and analysis approaches will help comprehensively evaluate potential toxicological effects for both humans and ecological species
- Systematically addressing technical limitations such as a lack of metabolism, testing challenging chemicals, and filling important information gaps will enable important information gaps to be filled
- Partnering with regulators and national and international partners on proof-of-concepts and case studies will increase confidence in alternatives and accelerate application for a range of decision contexts

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