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EPA United States Environmental Protection Agency

Applied Bioactivity Screening: What We Have Learned and Where We are Headed

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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

Overview of this presentation

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- Introduction to ToxCast/Tox21
- What biology is covered by ToxCast/Tox21?
- How are ToxCast/Tox21 data managed and what are the key data definitions for use?
- Key context: assay interference from cytotoxicity is related to selective and non-selective phenotypes in HTS
- Where to next?

Why can't we use traditional toxicology for all of our problems?

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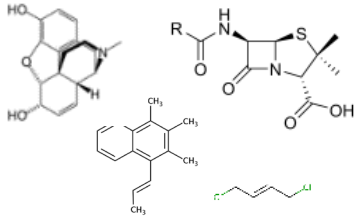
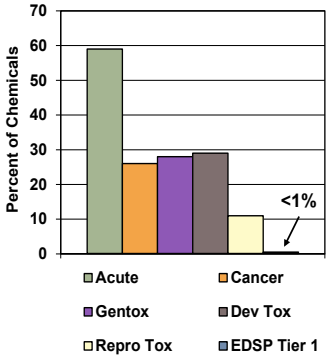
Number of Chemicals /Combinations



Lack of Data

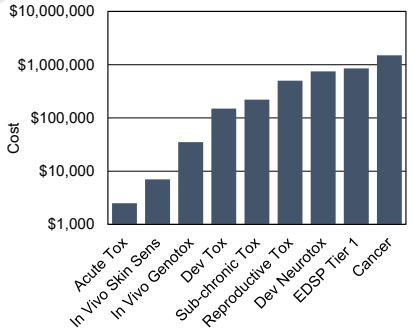


Modified from Judson *et al.*, EHP 2010

Why?

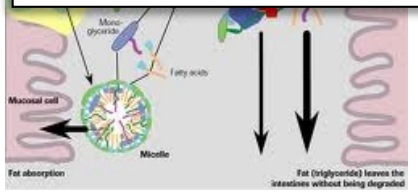
Ethics Concerns

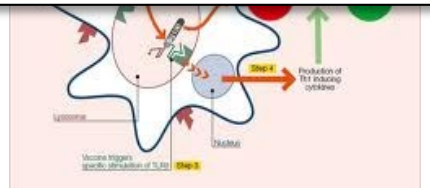
Economics

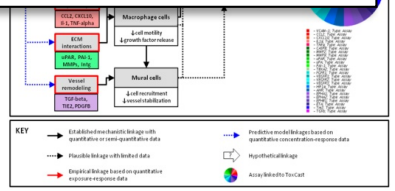


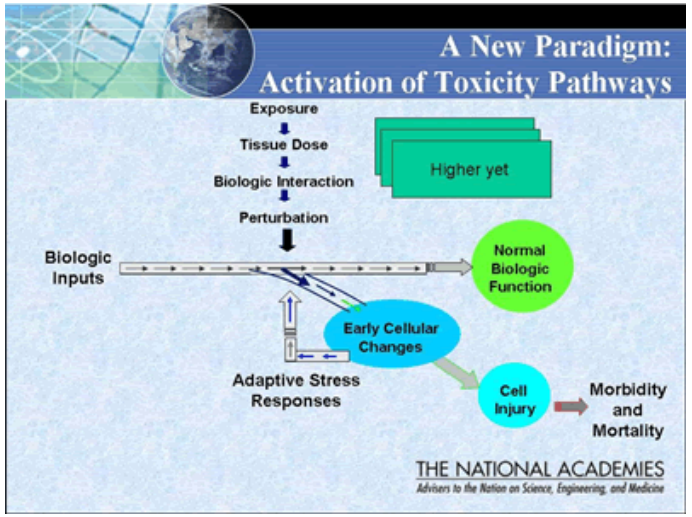
Need for better mechanistic data

- Determine human relevance
- What is the Mode of Action (MOA) or Adverse Outcome Pathway (AOP)?









TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY, NRC, 2007.

ToxCast / Tox21 Overall Strategy

- Identify targets or pathways linked to toxicity (AOP focus)
- Identify/develop high-throughput assays for these targets or pathways
- Develop predictive systems models
  - *in vitro/in silico→ in vivo*
  - human focus
- Use predictive models:
  - Prioritize chemicals for targeted testing
  - Suggest / distinguish possible AOP / MOA for chemicals
- *High-throughput Exposure Predictions*
- *High-throughput Risk Assessments*

ToxCast begins with chemistry

Chemical Research in Toxicology
Perspective
pubs.acs.org/cr

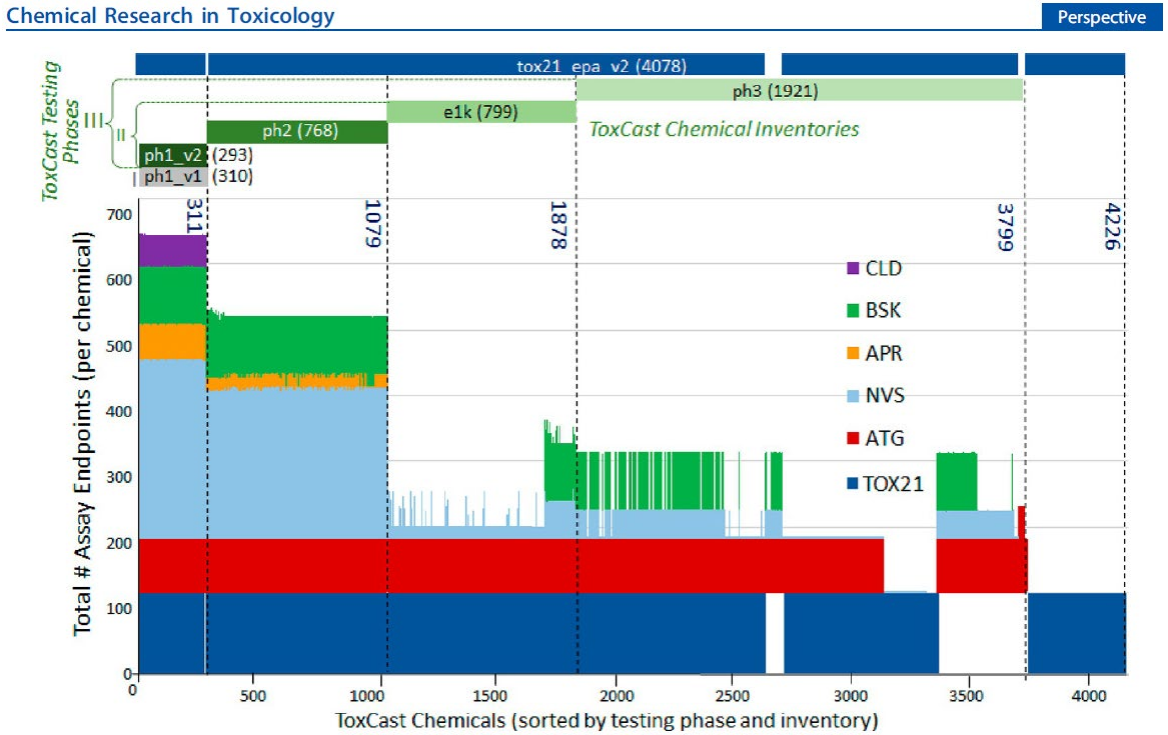
Richard *et al.*, 2016

https://comptox.epa.gov/dashboard/chemical_lists/toxcast

ToxCast Chemical Landscape: Paving the Road to 21st Century Toxicology

Ann M. Richard,[*,†] Richard S. Judson,[†] Keith A. Houck,[†] Christopher M. Grulke,[†] Patra Volarath,[‡] Inthirany Thillainadarajah,[§] Chihae Yang,[∥,⊥] James Rathman,[⊥,#] Matthew T. Martin,[†] John F. Wambaugh,[†] Thomas B. Knudsen,[†] Jayaram Kancherla,[▽] Kamel Mansouri,[▽] Grace Patlewicz,[†] Antony J. Williams,[†] Stephen B. Little,[†] Kevin M. Crofton,[†] and Russell S. Thomas[†]

- Include pesticides, antimicrobials, contaminants, industrial, high production volume, lists with regulatory interest, FDA *in vivo* data sets, FDA food additives, fragrances, plasticizers, drugs
- ToxCast total substances: approaches 4,000
- Tox21 total substances: approaches 10,000



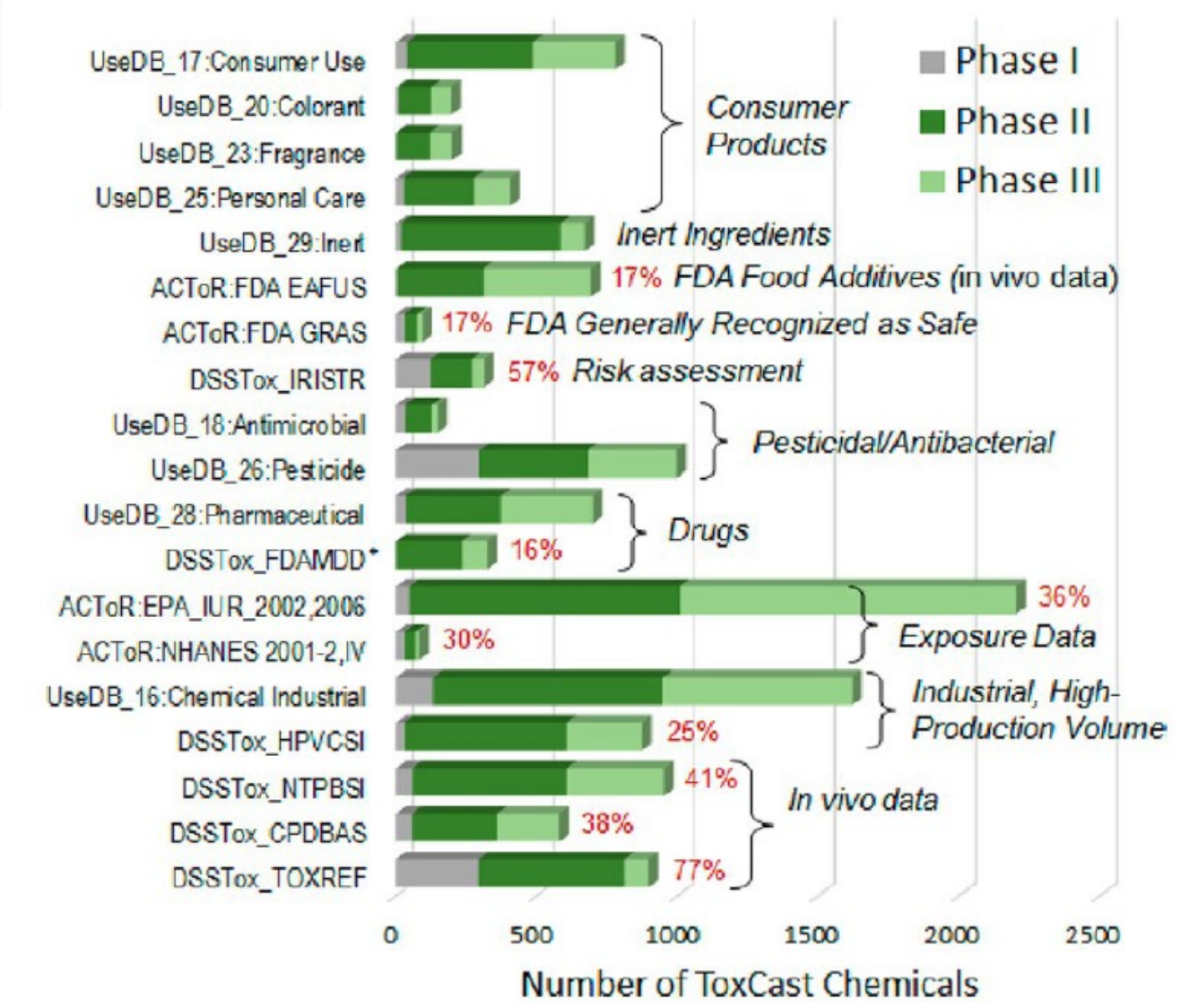
What did we learn about bioactivity from screening large numbers of substances (100s to 10,000)?

- *Assay performance could be defined*
- *New reference chemicals by target could be understood*
- *Integrated and predictive models could be built*
- *Prioritization based on bioactivity could be achieved*

Screening large numbers of substances for bioactivity can illustrate trends, define domain of applicability, and better highlight strengths and weaknesses of the assays.

Bottom-line: building confidence

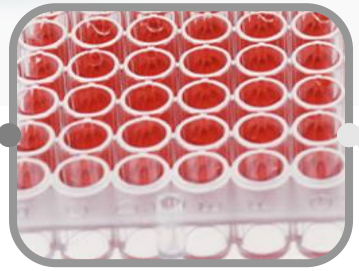
ToxCast PhI & PhII 1060: # Compounds per Inventory



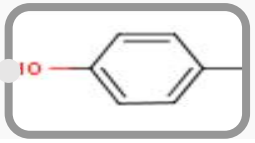
- Addressing chemicals of interest: Excellent coverage of multiple inventories; many chemicals appear on many lists
- Learnings for more than one class: broad diversity of chemical-use categories.
- Large overlap with data-rich *in vivo* inventories to build confidence/models.

Hazard Predictions: High-Throughput Screening (HTS)

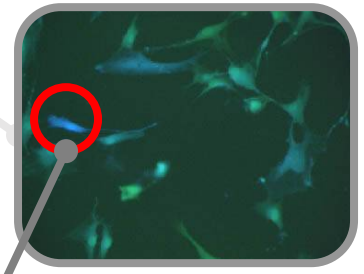
Robots



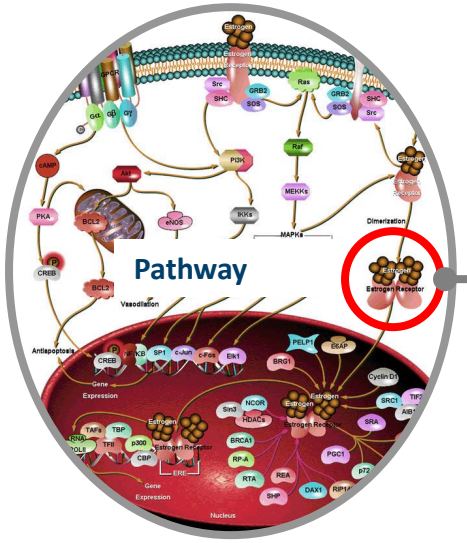
96-, 384-, 1536 Well Plates

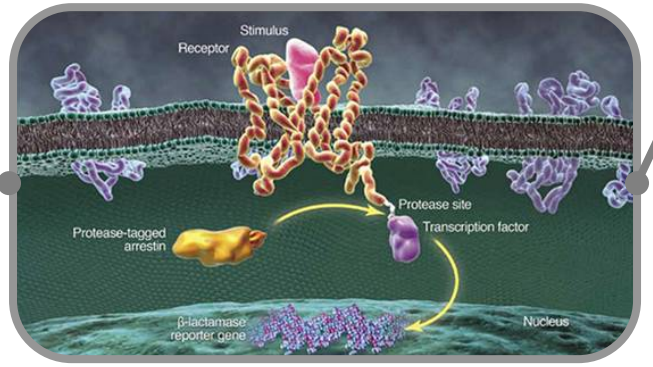


Chemical Exposure

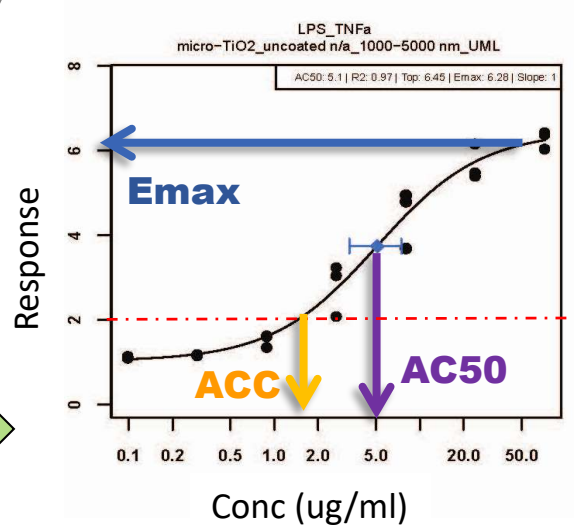


Cell Population

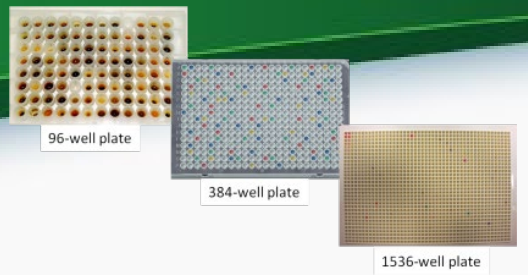




Target Biology (e.g., Estrogen Receptor)



ToxCast contains heterogeneous data



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Assay Sources
ACEA
Apredica
Attagene
BioSeek
CCTE/EPA ORD
CeeTox
CellzDirect
LifeTech Expression Analysis
NovaScreen (Perkin Elmer)
Odyssey Thera
Stemina
Tox21/NCATS
University Partners
Zebrafish: CCTE and Tanguay

Biological Response
cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA, RT-PCR)
receptor binding
receptor activity
Steroidogenesis
Metabolomic responses in stem cells

Target Family
response Element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases
XME metabolism
GPCRs
ion channels
ETC

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter
ETC

Readout Type
single
multiplexed
multiparametric

Cell Format
cell free
cell lines
primary cells
complex cultures
free embryos

Species
human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source
Lung
Liver
Skin
Cervix
Uterus
Intestinal
Bladder
Pancreas
Inflammatory
Breast
Vascular
Kidney
Testis
Brain
Spleen
Ovary
Prostate
Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
RT-PCR
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

List of assays and related information at: **https://www.epa.gov/chemical-research/toxcast-data-generation-toxcast-assays**

ToxCast and Tox21 have generated a lot of publicly available bioactivity data for hazard screening and prediction.

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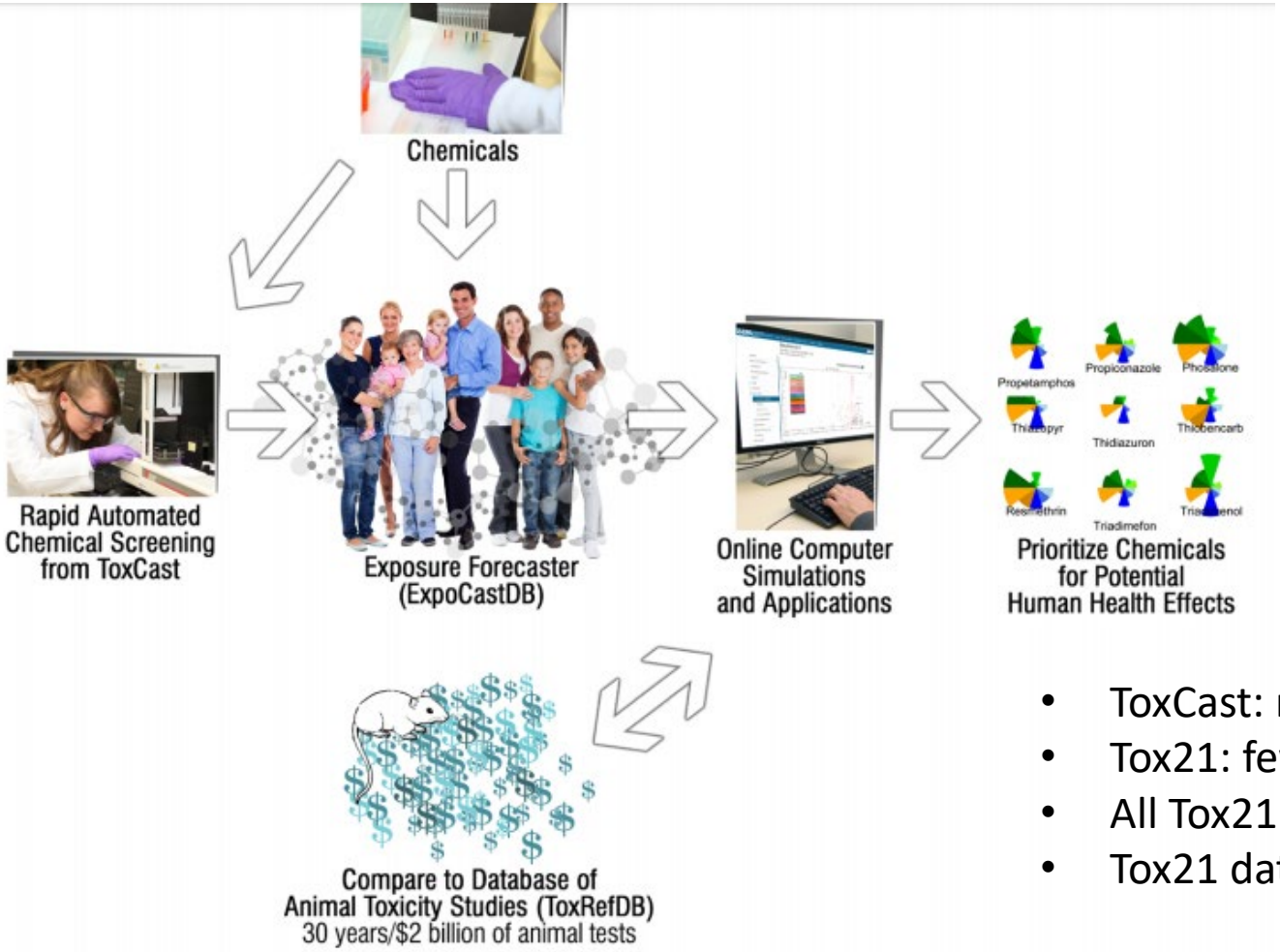
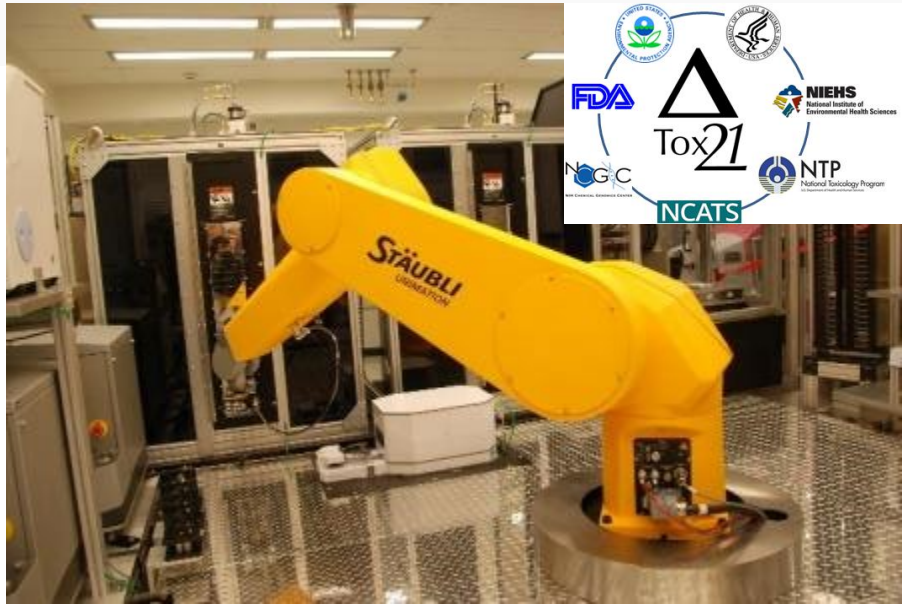
EPA's ToxCast program at a glance





Tox21 robot

- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, all 1536, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline

ToxCast covers a lot of biology but not all; and, ToxCast is growing over time.

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Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

Assay source	Long name	Truncated assay source description	Some rough notes on the biology covered
ACEA	ACEA Biosciences	real-time, label-free, cell growth assay system based on a microelectronic impedance readout	Endocrine (ER-induced proliferation)
APR	Apredica	CellCiphr High Content Imaging system	Hepatic cells (HepG2)
ATG	Attagene	multiplexed pathway profiling platform	Nuclear receptor and stress response profile
BSK	Bioseek	BioMAP system providing uniquely informative biological activity profiles in complex human primary co-culture systems	Immune/inflammation responses
NVS	Novascreen	large diverse suite of cell-free binding and biochemical assays.	Receptor binding; transporter protein binding; ion channels; enzyme inhibition; many targets
OT	Odyssey Thera	novel protein:protein interaction assays using protein-fragment complementation technology	Endocrine (ER and AR)
TOX21	Tox21/NCGC	Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high-throughput screening assays across a large interagency-developed chemical library	Many – with many nuclear receptors
CEETOX	Ceetox/OpAns	HT-H295R assay	Endocrine (steroidogenesis)
CLD	CellzDirect	Formerly CellzDirect, this Contract Research Organization (CRO) is now part of the Invitrogen brand of Thermo Fisher providing cell-based in vitro assay screening services using primary hepatocytes.	Liver (Phase I/Phase II/ Phase III expression)
NHEERL_PADILLA	NHEERL Padilla Lab	The Padilla laboratory at the EPA National Health and Environmental Effects Research Laboratory focuses on the development and screening of zebrafish assays.	Zebrafish terata
NCCT	NCCT Simmons Lab	The Simmons Lab at the EPA National Center for Computational Toxicology focuses on developing and implementing in vitro methods to identify potential environmental toxicants.	Endocrine (thyroid - thyroperoxidase inhibition)
TANGUAY	Tanguay Lab	The Tanguay Lab, based at the Oregon State University Sinnhuber Aquatic Research Laboratory, uses zebrafish as a systems toxicology model.	Zebrafish terata/phenotypes
NHEERL_NIS	NHEERL Stoker & Laws	The Stoker and Laws laboratories at the EPA National Health and Environmental Effects Research Laboratory work on the development and implementation of high-throughput assays, particularly related to the sodium-iodide cotransporter (NIS).	Endocrine (thyroid - NIS inhibition)
UPITT	University of Pittsburgh	The Johnston Lab at the University of Pittsburgh ran androgen receptor nuclear translocation assays under a Material Transfer Agreement (MTA) for the ToxCast Phase 1, Phase 2, and E1K chemicals.	Endocrine (AR related)

With each release, more assay endpoints and more chemical x endpoint data are released

Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

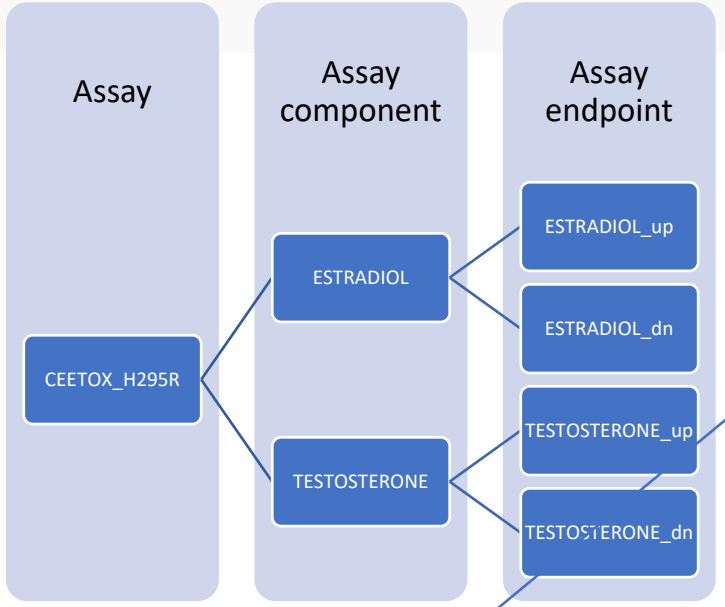
These assay endpoints were notable additions in invitrodb version 3.3.

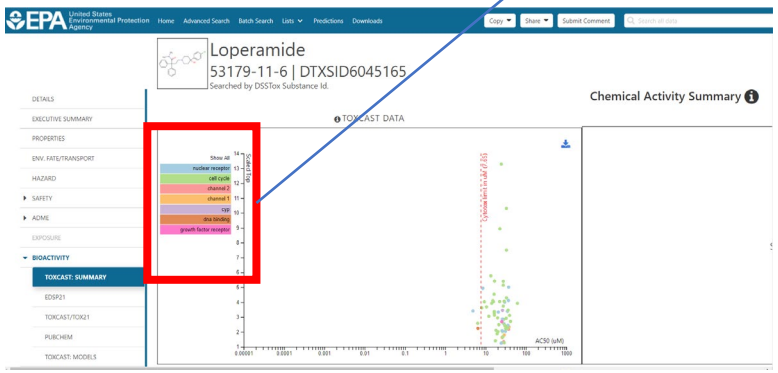
Assay source	Long name	Truncated assay source description	Some rough notes on the biology covered
NCCT_MITO	NCCT (now Center for Computational Toxicology and Exposure) Mitochondrial toxicity	Respirometric assay that measure mitochondrial function in HepG2 cells	Multiple assay endpoints to evaluate mitochondrial function https://doi.org/10.1093/toxsci/kfaa059.
NHEERL_MED	NHEERL Mid-Continent Ecology Division	The EPA Mid-Continent Ecology Division of the National Health and Environmental Effects Research Laboratory screened the ToxCast Phase 1 chemical library for hDIO1 (deiodinase 1) inhibition as part of an ecotoxicology effort.	Endocrine (thyroid – hDIO1,2,3 inhibition) https://doi.org/10.1093/toxsci/kfy302
STM	Stemina	Stem cell-based metabolomic indicator of developmental toxicity for screening.	Developmental toxicity screening – multiple assay endpoints https://doi.org/10.1093/toxsci/kfaa014
LTEA	Life Tech Expression Analysis	Gene expression measured in HepaRG cells following 48 hr exposure	Liver toxicity model via transcription factor regulated-metabolism and markers of oxidative/cell stress; multiple assay endpoints

What biology is covered currently (or in the near future) for ToxCast?

Learning more about the assay endpoints

Example assay annotation hierarchy





- Many assay endpoints are mapped to a gene, if applicable
- Assay endpoints now cover 1398 unique gene targets in invitrodb version 3.3, in addition to other processes
- Intended target family is one way to understand biological target (incomplete list here):
  - Apolipoprotein
  - Apoptosis
  - Background measurement
  - Catalase
  - Cell adhesion
  - Cell cycle
  - Cell morphology
  - CYP
  - Cytokine
  - Deiodinase
  - DNA binding
  - Esterase
  - Filaments
  - GPCR
  - Growth factor
  - Histones
  - Hydrolase
  - Ion channel
  - Kinase
  - Ligase
  - Lyase
  - Malformation (zebrafish)
  - Membrane protein
  - Metabolite (Stemina metabolomics)
  - Mitochondria
  - Methyltransferase
  - microRNA
  - Mutagenicity response
  - Nuclear receptor
  - Oxidoreductase
  - Phosphatase
  - Protease/inhibitor
  - Steroid hormone
  - Transferase
  - Transporter

https://comptox.epa.gov/dashboard/assay_endpoints/

Download summary information here: https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data

More information about assay endpoints

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EPA United States Environmental Protection Agency | Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Share | Search all data

Download | 10

Assay Component Endpoint Name	Details	Multi... Active
ACEA_ER_80hr		456 / 3024
APR_HepG2_CellCycleArrest_1h_dn		3 / 310

All Chemicals in Assay Endpoint: ACEA_ER_80hr

Excel

Annotations | Citations | tcpl Processing | Reagents | AOPs

Aeid	2
Assay Component Endpoint Name	ACEA_ER_80h
Assay Component Endpoint Desc	Data from the was analyzed growth repor they relate to produced mu targets, this a
Assay Function Type	signaling
Normalized Data Type	percent_activ
Analysis Direction	positive
Burst Assay	false
Key Positive Control	17b-estradiol
Signal Direction	gain
Intended Target Type	pathway
Intended Target Type Sub	pathway-specified
Intended Target Family	nuclear receptor
Intended Target Family Sub	steroidal
Assay Component Name	ACEA_ER_80hr
Assay Component Desc	ACEA_ER_80hr, is one of two assay component(s) measured or calculated from the ACEA_ER assay. It is designed to make measurements of real-time cell-growth kinetics, a form of growth reporter, as detected with electrical impedance signals by Real-

All Chemicals in Assay Endpoint: ACEA_ER_80hr

Excel

Annotations | Citations | tcpl Processing | Reagents | AOPs

	PMID	url	Title	Author	Citation	doi
1	16481145	PubMed URL	Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity	Xing JZ, Zhu L, Gabos S, Xie L	Xing JZ, Zhu L, Gabos S, Xie L. Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. Toxicol In Vitro. 2006 Sep;20(6):995-1004. Epub 2006 Feb 14. PubMed PMID: 16481145.	
2	23682706	PubMed URL	Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T-47D human ductal carcinoma cells	Rotroff DM, Dix DJ, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Reif DM, Richard AM, Sipes NS, Abassi YA, Jin C, Stampfl M, Judson RS	Rotroff DM, Dix DJ, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Reif DM, Richard AM, Sipes NS, Abassi YA, Jin C, Stampfl M, Judson RS. Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T-47D human ductal carcinoma cells. Chem Res Toxicol. 2013 Jul 15;26(7):1097-107. doi:10.1021/tx400117y. Epub 2013 Jun 10. PubMed PMID: 23682706.	doi: 10.1021/tx400117y

Biological coverage

- Not all assays can be mapped to a single gene as a surrogate for biology (e.g., cytotoxicity, mitochondrial toxicity)
- Large focus on nuclear receptors, cell cycle, cell stress, but many diverse assays
- *Revisit in next section: How can we better cover biological space in a Tier 1 screening, followed by targeted screening?*

In the following slide, some of the assays will be discussed briefly to help orient the user to the types of assay data in ToxCast.

ACEA: Real Time Cell Analysis Based on Electrical Impedance

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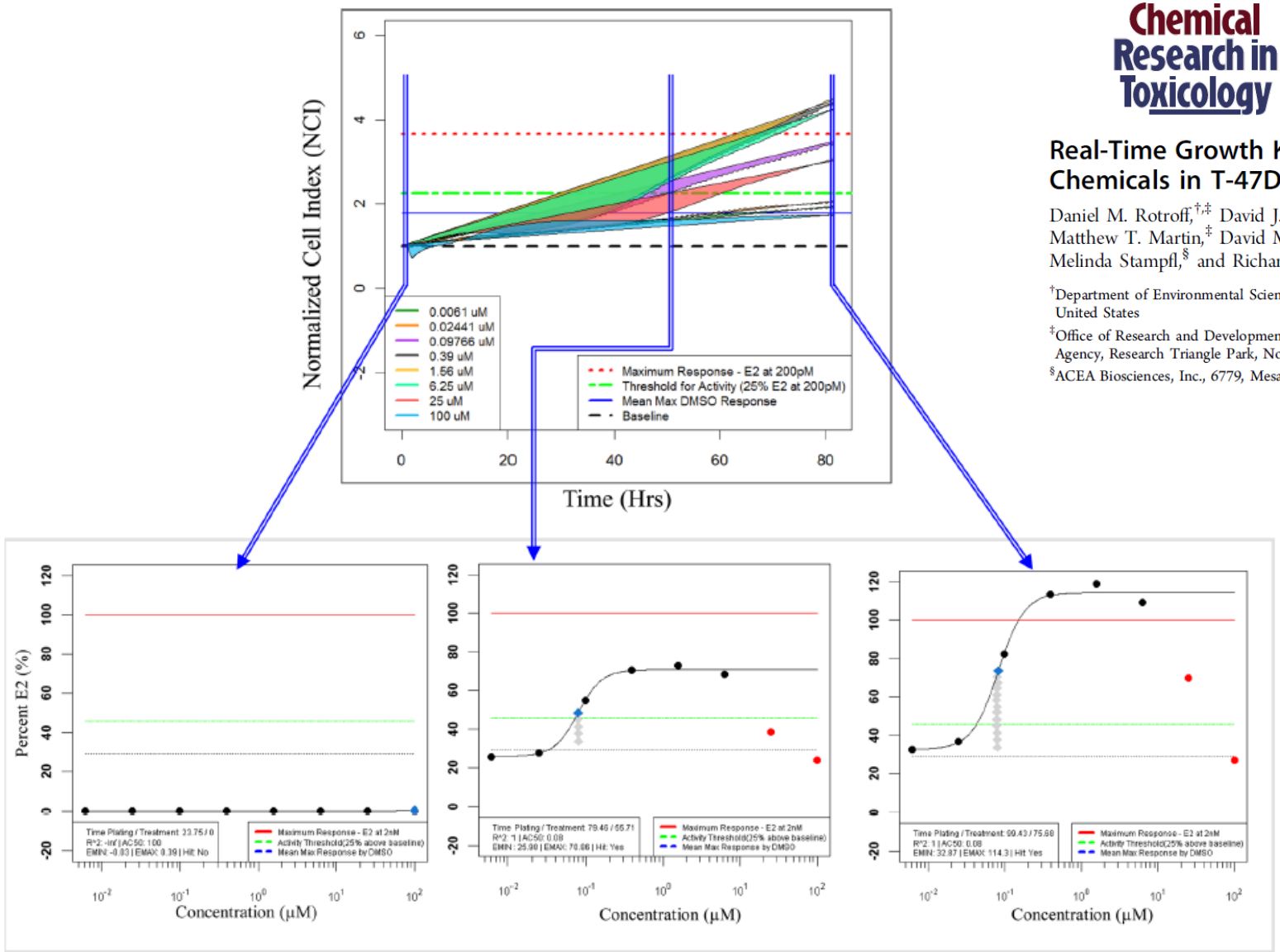


Chemical Research in Toxicology

Article

pubs.acs.org/crt

Real-Time Growth Kinetics Measuring Hormone Mimicry for ToxCast Chemicals in T-47D Human Ductal Carcinoma Cells

Daniel M. Rotroff,[†,‡] David J. Dix,[‡] Keith A. Houck,[‡] Robert J. Kavlock,[‡] Thomas B. Knudsen,[‡] Matthew T. Martin,[‡] David M. Reif,[‡] Ann M. Richard,[‡] Nisha S. Sipes,[‡] Yama A. Abassi,[§] Can Jin,[§] Melinda Stampfl,[§] and Richard S. Judson[*,‡]

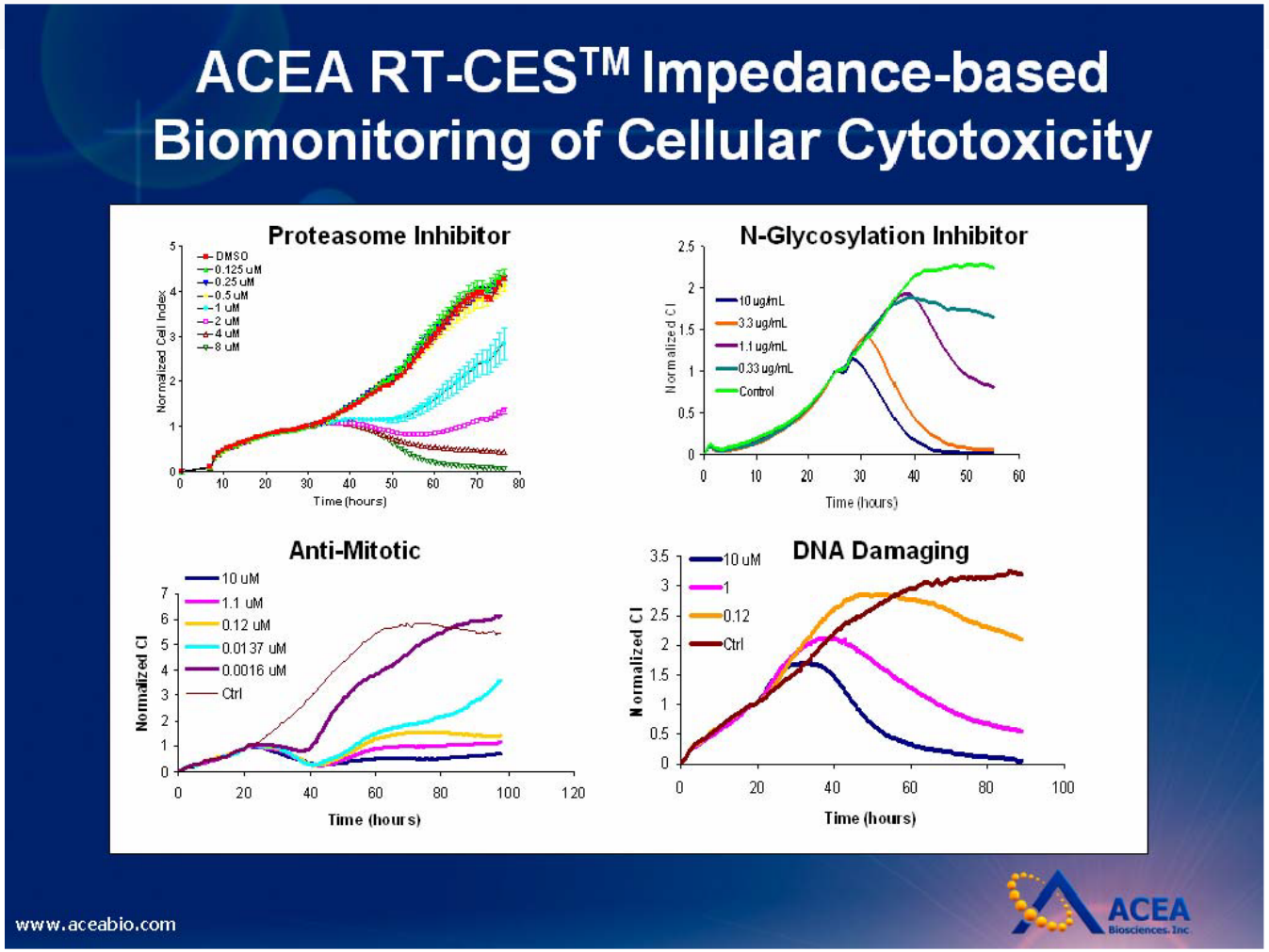
[†]Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina 27514, United States

[‡]Office of Research and Development, National Center for Computational Toxicology, United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

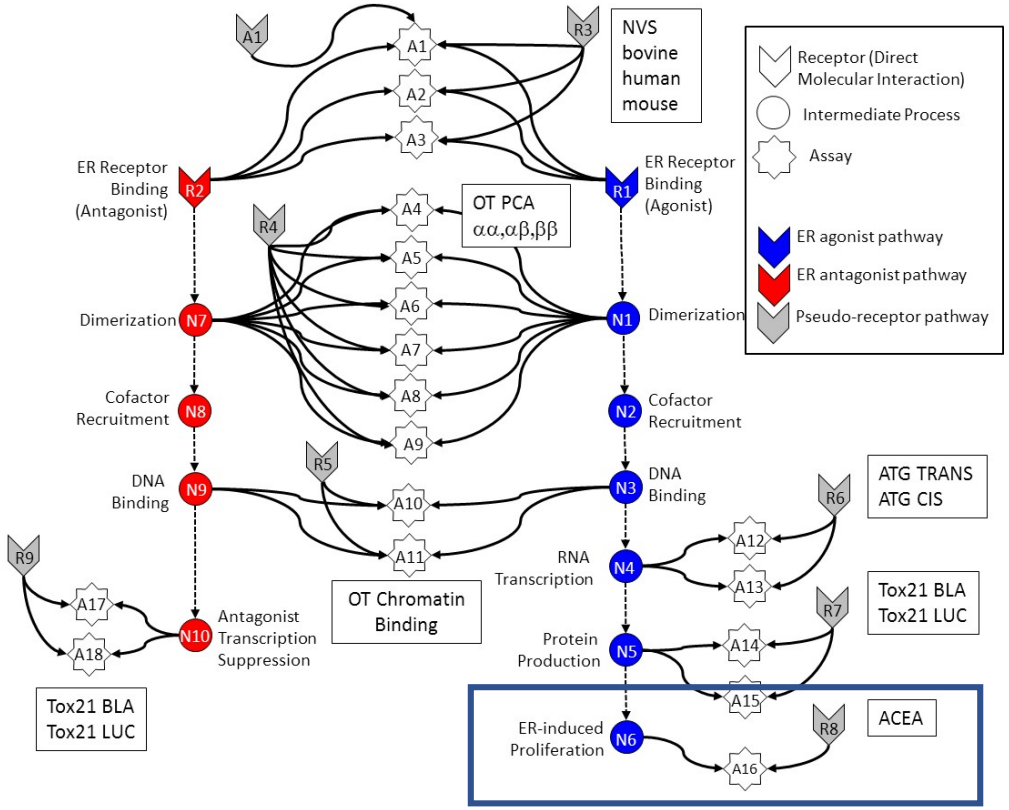
[§]ACEA Biosciences, Inc., 6779, Mesa Ridge Road, San Diego, California 92121, United States

- Can measure cell proliferation or cytotoxicity depending on the direction
- Electrical impedance measured over 80 hr
- ACEA ER assay uses T-47D breast cancer cells
- ACEA AR assay uses 22Rv1 human prostate cancer cell line

ACEA: ER and cytotoxicity examples



Judson et al. 2015



Apredica: High-content imaging of HepG2

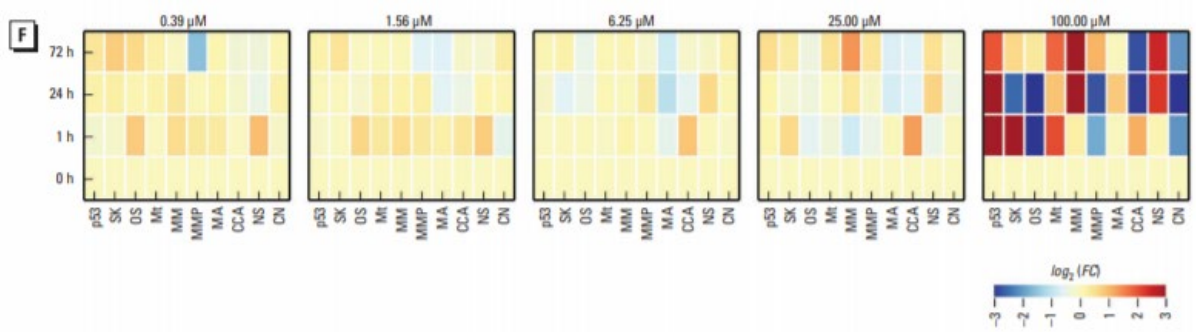
Research

A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1409029.

Using ToxCast™ Data to Reconstruct Dynamic Cell State Trajectories and Estimate Toxicological Points of Departure

Imran Shah,[1] R. Woodrow Setzer,[1] John Jack,[2] Keith A. Houck,[1] Richard S. Judson,[1] Thomas B. Knudsen,[1] Jie Liu,[3] Matthew T. Martin,[1] David M. Reif,[4] Ann M. Richard,[1] Russell S. Thomas,[1] Kevin M. Crofton,[1] David J. Dix,[1] and Robert J. Kavlock[1]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [2]Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA; [3]Oak Ridge Institute for Science Education (ORISE), U.S. Department of Energy, Oak Ridge, Tennessee, USA; [4]Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina, USA



p53, stress kinase, oxidative stress, microtubules, mitochondrial mass, mitochondrial membrane potential, mitotic arrest, cell cycle arrest, nuclear size, cell number

- 1, 24, 72 hr of exposure in HepG2 cells x 384 wp
- Cell stress, mitochondrial toxicity, oxidative stress
- Applies automated image analysis techniques to capture multiple cytological features using fluorescent labels, to measure the concentration-dependent changes
- not fully metabolically capable, but HepG2 cells can undergo continuous proliferation in culture and have a demonstrated capacity to predict hepatotoxicity

Attagene: transcription factor activity profiling

EPA United States Environmental Protection Agency

SCIENCE ADVANCES | RESEARCH ARTICLE

SIGNAL TRANSDUCTION

Evaluating biological activity of compounds by transcription factor activity profiling

Alexander Medvedev[1], Matt Moeser[1]*, Liubov Medvedeva[1], Elena Martsen[1], Alexander Granick[1], Lydia Raines[1], Ming Zeng[1], Sergei Makarov Jr.[1], Keith A. Houck[2], Sergei S. Makarov[1†]

Assessing the biological activity of compounds is an essential objective of biomedical research. We show that one can infer the bioactivity of compounds by assessing the activity of transcription factors (TFs) that regulate gene expression. Using a multiplex reporter system, the FACTORIAL, we characterized cell response to a com-

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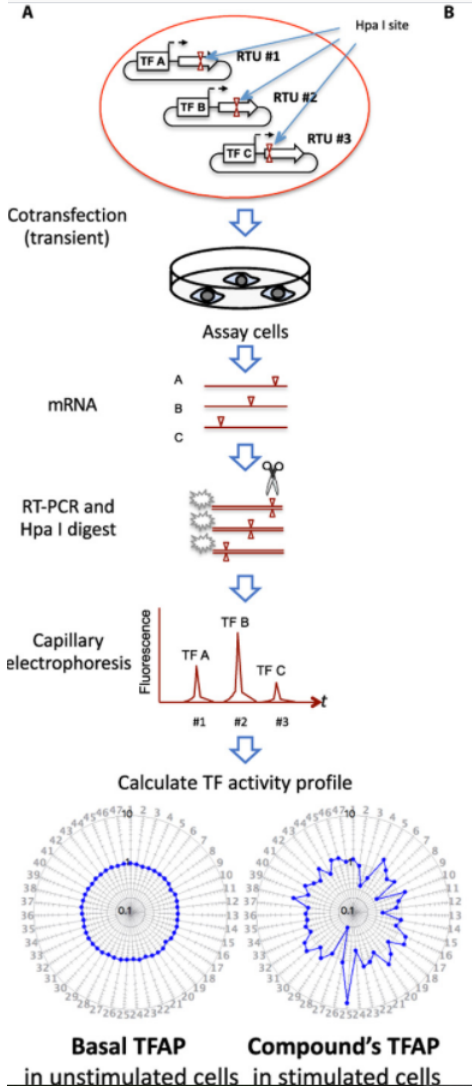
- HepG2 HG19 subclone for elevated xenobiotic metabolic capacity
- "CIS" assays: endogenous transcription factors that regulated transfected reporters (nuclear receptors, cell stress
- "TRANS" assays: exogenous receptor-reporter system is transfected in (xenobiotic nuclear receptors)
- Recently published (not yet in Dashboard): addition of TRANS-FACTORIAL nuclear receptor assays for multiple species (Houck et al. 2020)

Journal Pre-proof

Evaluation of a Multiplexed, Multispecies Nuclear Receptor Assay for Chemical Hazard Assessment

Keith A. Houck[1], Anita Simha[2], Audrey Bone[1], Jon A. Doering[3], Sara M.F. Vliet[4], Carlie LaLone[5], Alex Medvedev[6], Sergei Makarov[6]*

ATG "CIS" endpoints (endogenous signal)



RTU #	Name	Transcription factor
1	TGFRE	TGF beta response element (SMAD3/4)
2	HNF6	Hepatocyte nuclear factor 6
3	TCF	TCF/LEF
4	Ebox	Myc and upstream stimulatory factor 1 (USF-1)
5	PPAR	Peroxisome proliferator activating receptor
6	NF1	Nuclear factor 1
7	GR	Glucocorticoid receptor
8	AP-1	Activator protein 1
9	ISRE	Interferon regulatory factors IRF1, IFR3
10	MTF-1	The metal regulatory transcription factor 1 (MTF-1)
11	STAT3	Signal transducer and activator of transcription 3
12	TAL	A minimal promoter
13	NF-κB	Nuclear factor kappa B
14	FoxA2	Forkhead box protein A2
15	CMV	Cytomegalovirus promoter-enhancer
16	Xbp1	X-box protein 1
17	CREB	cAMP-response element binding protein
18	AhR	Aryl hydrocarbon receptor
19	EGR	Early growth response protein 1
20	NRF2	Nuclear factor (erythroid-derived 2)-like 2
21	TA	A minimal promoter
22	ER	Estrogen receptor
23	Oct	Octamer transcription factor
24	LXR	Liver X receptor
25	HSF-1	Heat shock factor-1 protein
26	SREBP	Sterol regulatory element-binding protein
27	p53	The p53 transcription factor
28	BMPRE	Bone morphogenetic protein response element (SMAD4/5)
29	Pax6	Transcription factor paired box 6
30	HIF-1α	Hypoxia-inducible factor-1 alpha
31	VDR	Vitamin D receptor
32	ROR	Retinoic acid receptor-related orphan receptor protein
33	Ets	E-twenty six transcription factor
34	GLI-1	Gli-1 transcription factor
35	NRF1	Nuclear respiratory factor 1
36	GATA	GATA transcription factor
37	E2F	E2F transcription factor
38	C/EBP	The CCAAT-enhancer-binding protein
39	Myb	Transcriptional activator Myb
40	PBREM	Phenobarbital responsive enhancer module /constitutive androstane receptor
41	FXR	Farnesoid X receptor
42	AP-2	Activating protein 2
43	RAR	Retinoic acid receptor
44	FoxO	Forkhead box proteins FOXO1 and FOXO3
45	SOX	SOX transcription factor
46	Sp1	Sp1 transcription factor
47	Myc	Myc transcription factor

BioSeek: co-culture models that provide phenotypic information

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Journal of Pharmacological and Toxicological Methods 53 (2006) 67 – 74

Journal of Pharmacological and Toxicological Methods

www.elsevier.com/locate/jpharmtox

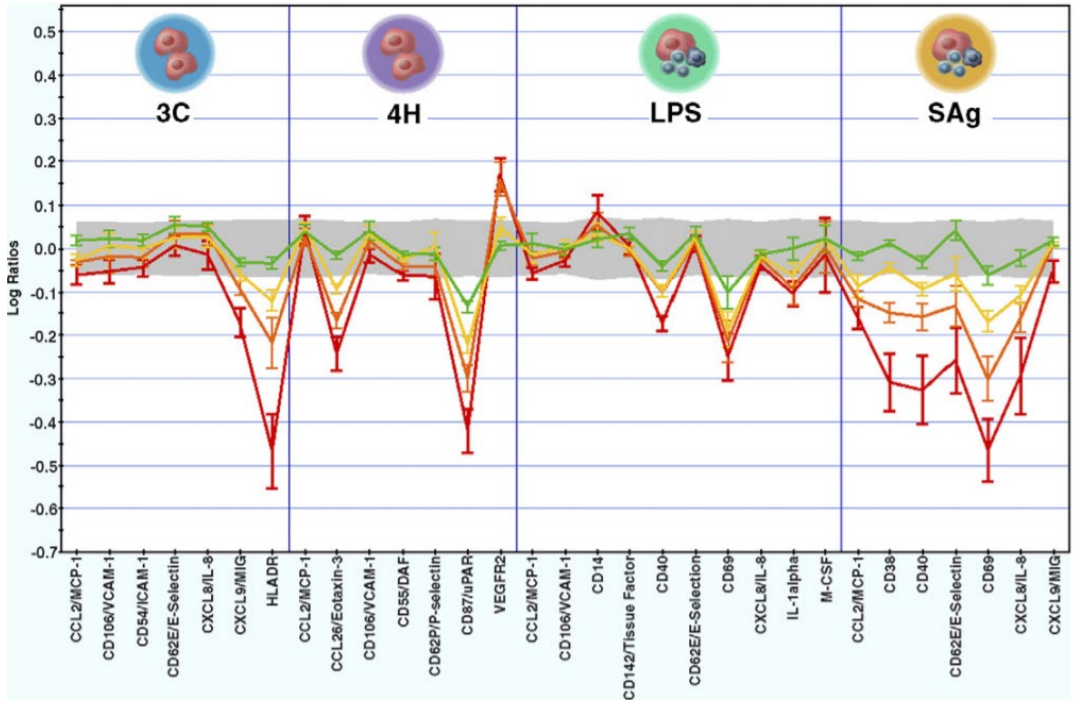
Original article

Characterization of compound mechanisms and secondary activities by BioMAP analysis

Ellen L. Berg *, Eric J. Kunkel, Evangelos Hytopoulos, Ivan Plavec

BioSeek, Inc., 863-C Mitten Rd., Burlingame, CA 94010, United States

Received 10 June 2005; accepted 14 June 2005



nature biotechnology

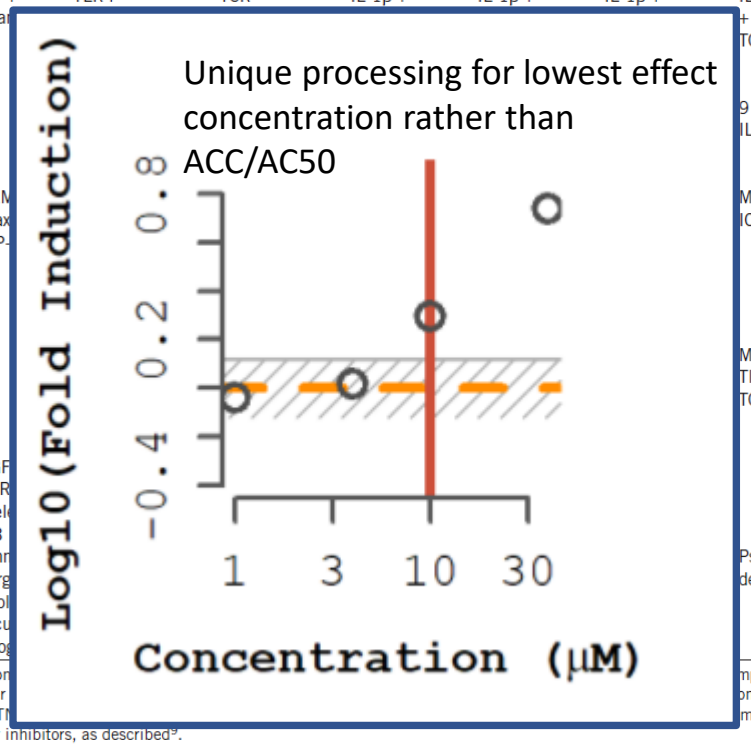
Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms

Nicole C Kleinstreuer[1], Jian Yang[2], Ellen L Berg[2], Thomas B Knudsen[1], Ann M Richard[1], Matthew T Martin[1], David M Reif[1], Richard S Judson[1], Mark Polokoff[2], David J Dix[1], Robert J Kavlock[1] & Keith A Houck[1]

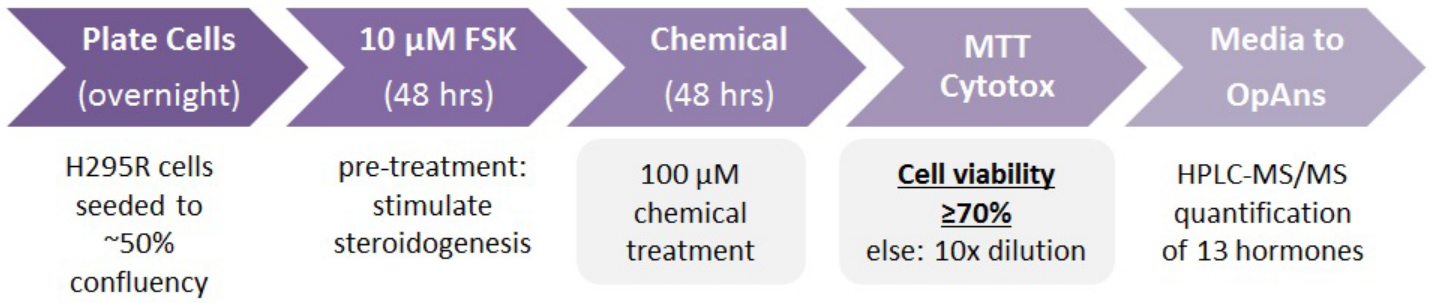
Table 1 Panel of 8 BioMAP systems used in this study

BioMAP system		3C	4H	LPS	SAg	BE3C	CASM3C	HDF3CGF	KF3CT
Primary human cell types		Venular endothelial cells	Venular endothelial cells	Peripheral blood mononuclear cells + endothelial cells	Peripheral blood mononuclear cells + endothelial cells	Bronchial epithelial cells	Coronary artery smooth muscle cells	Fibroblasts	Keratinocytes + fibroblasts
Stimuli		IL-1β + TNF-α + IFN-γ	IL-4 + hista	TLR4	TCR	IL-1β +	IL-1β +	IL-1β +	IL-1β + TNF-α + IFN-γ + TGF-β
Number of endpoints		13	7						9
Endpoint types	Acute inflammation	E-selectin, IL-8							IL-1α
	Chronic inflammation	VCAM-1, ICAM-1, MCP-1, MIG	VCAM Eotax MCP-						MCP-1, ICAM-1, IP-10
	Immune response	HLA-DR							
	Tissue remodeling								MMP-9, SRB, TIMP-2, uPA, TGF-β1
	Vascular biology	TM, TF, uPAR, EC proliferation, SRB, Vis	VEGF uPAR P-sel SRB						
Disease/tissue relevance		Cardiovascular disease, chronic inflammation	Asthr allerg oncol vascu biolog						Psoriasis, dermatitis, skin

BioMAP systems listed according to their short names con ... mpounds) for 24 h. For each system, the protein or mediator biomarker ... omarker endpoints measured were all cell-associated with the exception of T ... m were assessed by testing highly selective, pathway-specific activators or inhibitors, as described[9].



CeeTox/Cyprotex (HT-H295R assay)



Assay background and methods
(Karmaus *et al.* 2016)

Develop initial HT-H295R assay

Implement staged screening approach

Methods and Results: Evaluation of the HT-H295R assay

Compare HT-H295R to the OECD inter-laboratory results

Analyze data per the OECD TG to enable comparison

Evaluate the concordance of E2 and T responses

(Haggard *et al.* 2018 Toxicological Sciences.)

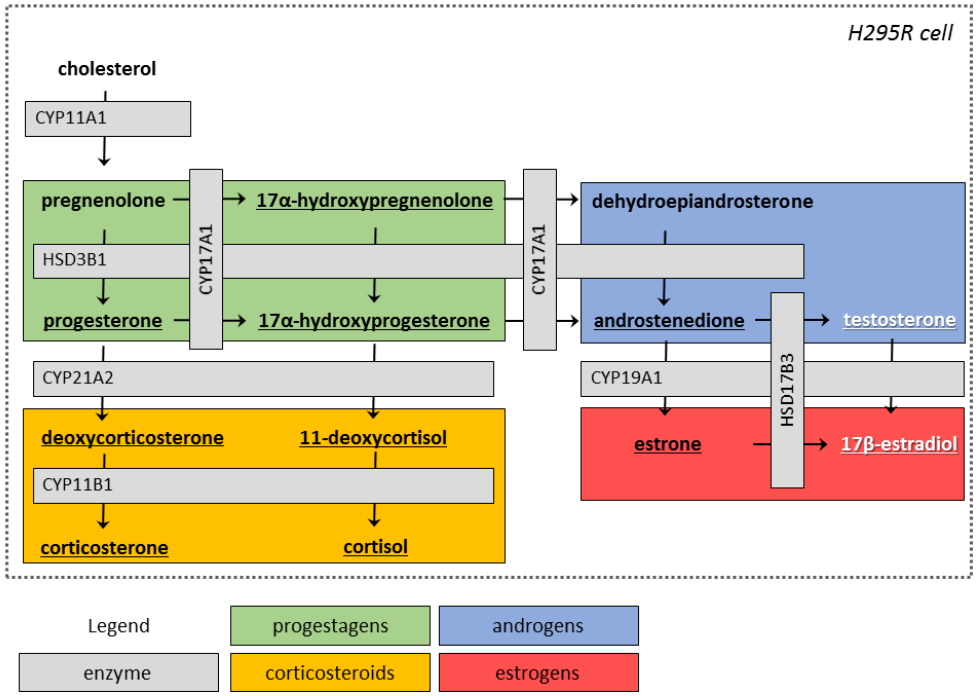
Methods and results: Development of prioritization metric

Compress data from 11 steroid hormone panel

Develop prioritization metric

Evaluate prioritization metric

(Haggard *et al.* 2019)



Published HT-H295R statistical model for prioritization

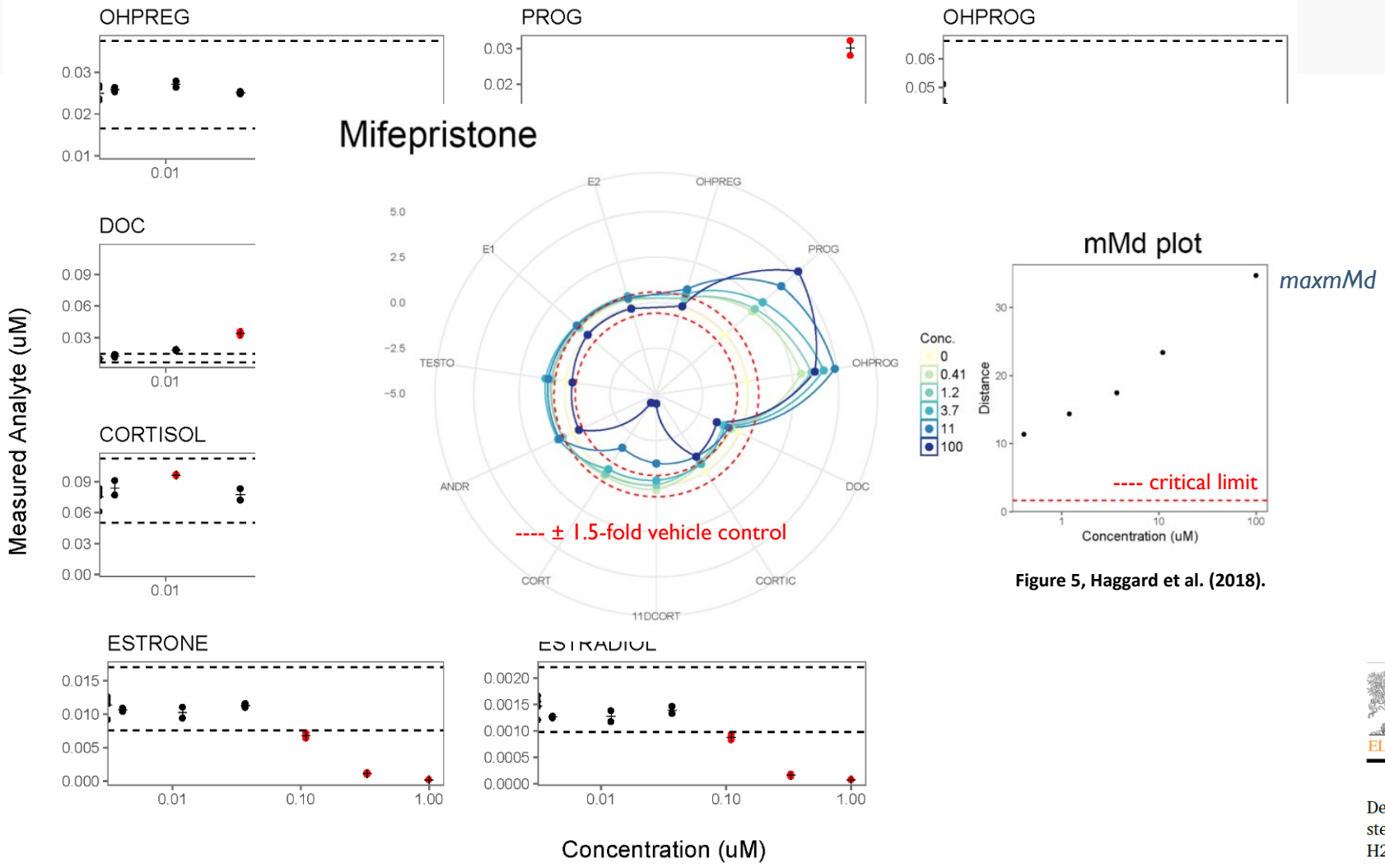


Figure 5, Haggard et al. (2018).

- Reduced an 11-dimensional question to a single dimension.
- Selection of the maxmMd appeared to provide a reproducible, quantitative approximation of the magnitude of effect on steroidogenesis.

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

TOXICOLOGICAL SCIENCES, 162(2), 2018, 509–534
doi: 10.1093/toxsci/kfx274
Advance Access Publication Date: December 1, 2017
Research Article

High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis

Derik E. Haggard,*,† Agnes L. Karmaus,*,†,1 Matthew T. Martin,†,2 Richard S. Judson,† R. Woodrow Setzer,† and Katie Paul Friedman†,3

*Oak Ridge Institute for Science and Education Postdoctoral Fellow, Oak Ridge, TN. 37831; and †National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection

Regulatory Toxicology and Pharmacology 109 (2019) 104510

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

Development of a prioritization method for chemical-mediated effects on steroidogenesis using an integrated statistical analysis of high-throughput H295R data

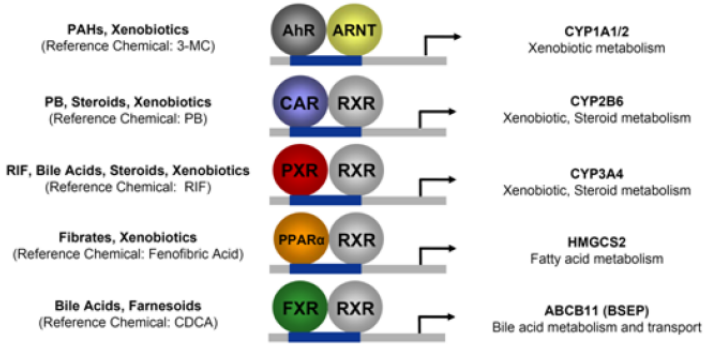
Derik E. Haggard[a,b], R. Woodrow Setzer[b], Richard S. Judson[b], Katie Paul Friedman[b,*]

[a] Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, TN, 37830, USA
[b] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA

Gene expression in models of the liver

CellzDirect (CLD): fewer genes, ToxCast Phase I only

- ToxCast 320 Chemical Library
- Fresh Primary Human Hepatocytes
- 2 human donors
- 6 Reference Chemicals (Rif, PB, 3-MC, Fenofibric Acid, CDCA, CITCO)
- 5 receptors targets (AhR, CAR, PXR, PPARα, FXR)
- 2 endogenous control gene targets (GAPDH, Actin)
- 14 relevant gene targets
- 3 Time Points (6,24,48 hours)
- 5 Concentrations (.004, .04,0.4, 4, 40 μM)



LifeTech Expression Analysis (LTEA): HepaRG cells, 1060 substances

- Newly released in invitrodb version 3.3
- ToxCast Phase I and Phase II Chemical library
- 189 assay endpoints, including ~93 genes: biotransformation, transporters, cell cycle, disease state markers (inc microRNA), etc.
- Paper forthcoming

NovaScreen (NVS)

- Cell-free assays
- Receptor binding, protein binding, transporter function, and enzyme activity for a substrate
- Typically performed in a tiered workflow

Chemical Research in Toxicology

ACS Author Choice

Article

pubs.acs.org/crt

Terms of Use

Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays

Nisha S. Sipes,* Matthew T. Martin, Parth Kothiya, David M. Reif, Richard S. Judson, Ann M. Richard, Keith A. Houck, David J. Dix, Robert J. Kavlock, and Thomas B. Knudsen*

EPA United States Environmental Protection Agency

Sipes et al. 2013 analysis

Table 1. Biochemical Activity Profiles by Assay Category[a]

assay category	assays[b]	actives[c]	actives %[c]	AC50s[d] ≤10 μM	AC50s[d] ≤1 μM
activator: cholinesterase	3	1	0.03	0	0
activator: CYP	10	10	0.10	10	7
activator: kinase	37	32	0.09	16	7
activator: other enzyme	16	2	0.01	1	0
activator: phosphatase	19	27	0.15	9	1
activator: protease	15	5	0.03	2	0
cholinesterase	3	151	5.16	50	15
CYP	10	843	8.64	450	129
GPCR (aminergic)	32	1579	5.06	540	148
GPCR (other)	45	1175	2.68	287	55
ion channel	7	226	3.31	83	17
kinase	37	277	0.77	49	10
LGIC (cys loop)	9	109	1.24	35	8
LGIC (ionotropic glutamate)	4	28	0.72	1	0
nuclear receptor (subfamily 1)	10	282	2.89	90	41
nuclear receptor (subfamily 3)	9	393	4.47	144	52
other	3	111	3.79	36	15
other enzyme	17	484	2.92	105	25
phosphatase	19	262	1.41	69	19
protease	15	351	2.40	81	14
transporter	11	787	7.33	271	61
total	**331**	**7135**	**53.19**	**2329**	**624**

Stemina (STM) devTOX quickPredict platform

- Human pluripotent stem cells
- Developmental toxicity predicted based on changes in cellular metabolism following chemical exposure.
- Multiple parameters measured; the ornithine/cystine ratio is the key assay endpoint, along with cytotoxicity for context.

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TOXICOLOGICAL SCIENCES, 174(2), 2020, 189–209

doi: 10.1093/toxsci/kfaa014
Advance Access Publication Date: February 19, 2020
Research Article

Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity

Todd J. Zurlinden ,* Katerine S. Saili,* Nathaniel Rush,* Parth Kothiya,* Richard S. Judson ,* Keith A. Houck,* E. Sidney Hunter,† Nancy C. Baker,‡ Jessica A. Palmer ,§ Russell S. Thomas ,* and Thomas B. Knudsen *,1

Thyroid-related molecular initiating events and key events as targets for HTS

EPA United States Environmental Protection Agency

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org
TOXICOLOGICAL SCIENCES, 151(1), 2016, 160–180
doi: 10.1093/toxsci/kfw034
Advance Access Publication Date: February 15, 2016
Research Article

Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries

Katie Paul Friedman,[*,†,2] Eric D. Watt,[*,‡,2] Michael W. Hornung,[§] Joan M. Hedge,[†] Richard S. Judson,[‡] Kevin M. Crofton,[‡] Keith A. Houck,[‡] and Steven O. Simmons[‡,1]

*Oak Ridge Institute
Toxicology Division
Development, U.S. I
Computational Tox
Research Triangle P
Effects Research Lal
Duluth, MN, 55804

he thyroid-related
s an outline for HTS

Toxicology in Vitro 40 (2017) 66–78
Contents lists available at ScienceDirect
Toxicology in Vitro
journal homepage: www.elsevier.com/locate/toxinvit
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Development of a screening approach to detect thyroid disrupting chemicals that inhibit the human sodium iodide symporter (NIS)

Daniel R. Hallinger [a], Ashley S. Murr [a], Angela R. Buckalew [a], Steven O. Simmons [b], Tammy E. Stoker [a,*], Susan C. Laws [a,*]

[a] Endocrine Toxicology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, Office of Research and Agency, Research Triangle Park, NC 27711, United States
[b] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park,

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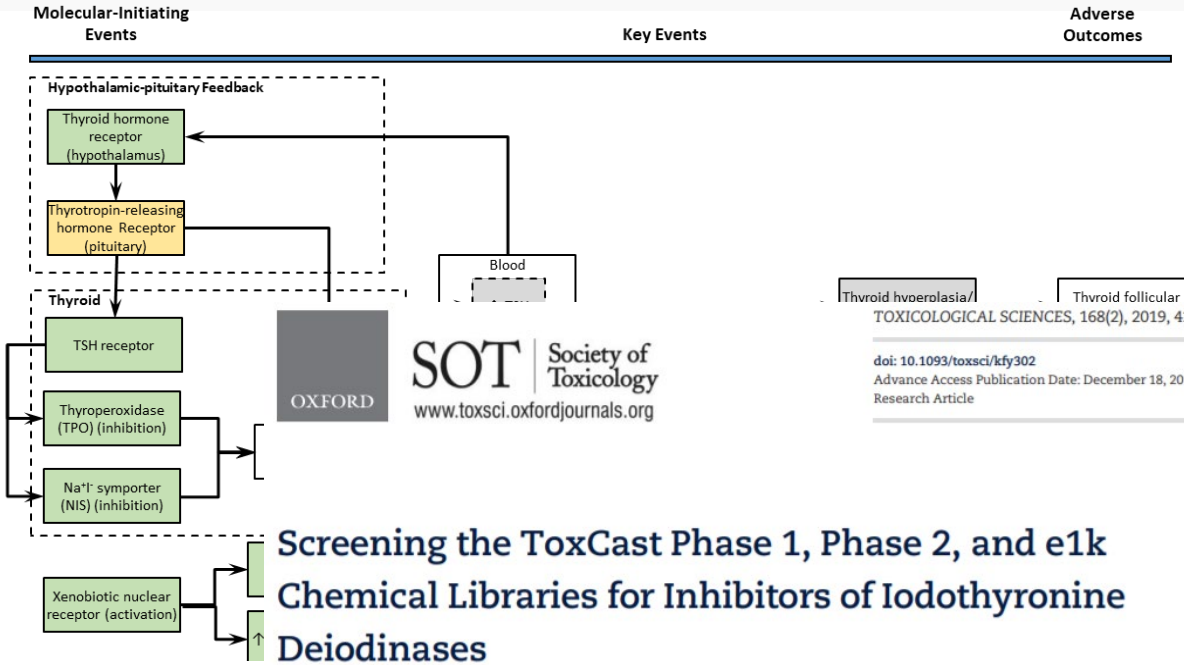

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TOXICOLOGICAL SCIENCES, 168(2), 2019, 430–442
doi: 10.1093/toxsci/kfy302
Advance Access Publication Date: December 18, 2018
Research Article
TOXICOLOGICAL SCIENCES, 2017, 1–12
doi: 10.1093/toxsci/kfx279
Advance Access Publication Date: December 7, 201
Research Article

Screening the ToxCast Phase 1, Phase 2, and e1k Chemical Libraries for Inhibitors of Iodothyronine Deiodinases

Jennifer H. Olker,[*,†,‡,§,1] Joseph J. Korte,[*,†,‡,§] Jeffrey S. Denny,[*,†,‡,§] Phillip C. Hartig,[*,†,‡,¶] Mary C. Cardon,[*,†,‡,¶] Carsten N. Knutsen,[ll] Paige M. Kent,[lll] Jessica P. Christensen,[lll] Sigmund J. Degitz,[*,†,‡,§] and Michael W. Hornung[*,†,‡,§]

HTS in dev.

EDSP Tier 1/2 measured endpoints

Screening the ToxCast Phase 1 Chemical Library for Inhibition of Deiodinase Type 1 Activity

Michael W. Hornung,[*,†,‡,§,1], Joseph J. Korte,[*,†,‡,§] Jennifer H. Olker,[*,†,‡,§] Jeffrey S. Denny,[*,†,‡,§] Carsten Knutsen,[*,†,‡,§] Phillip C. Hartig,[*,†,‡,¶] Mary C. Cardon,[*,†,‡,¶] and Sigmund J. Degitz[*,†,‡,§]

*US Environmental Protection Agency; †Office of Research and Development; ‡National Health and Environmental Effects Research Laboratory; §Mid-Continent Ecology Division, Duluth, Minnesota 55804; and ¶Toxicity Assessment Division, Research Triangle Park, North Carolina 27709

Recent publication of work to integrate 12 assay endpoints for the thyroid hormone receptor.

EPA United States Environmental Protection Agency

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP5314.

Limited Chemical Structural Diversity Found to Modulate Thyroid Hormone Receptor in the Tox21 Chemical Library

Katie Paul-Friedman,[1] Matt Martin,[1] Kevin M. Crofton,[1] Chia-Wen Hsu,[2] Srilatha Sakamuru,[3] Jinghua Zhao,[3] Menghang Xia,[3] Ruili Huang,[3] Diana A. Stavreva,[4] Vikas Soni,[4] Lyuba Varticovski,[4] Razi Raziuddin,[4] Gordon L. Hager,[4] and Keith A. Houck[1]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA
[2]Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Washington, DC, USA
[3]National Center for Advancing Translational Sciences, National Institutes of Health (NIH), Bethesda, Maryland, USA
[4]Center for Cancer Research, National Cancer Insti

Background: Thyroid hormone receptors (TRs
isms, and thyroid hormone disruption is of high
ber of chemical classes have been identified as
diversity. Thus, the question of whether TRs are
Objective: Our goal was to evaluate the hypo
modulating TR activity via the collaborative int
Methods: We screened the Tox21 chemical lib
agonist or antagonist activity. Active compoun
assays, coactivator recruitment assays, and a hig
Results: Known agonist reference chemicals w
the pharmaceutical betamipron. Indirect activatio
detected by confirmation in an RXR agonist ass
founding cytotoxicity and other, non-TR-specific
zuril, and risarestat—were confirmed as antagoni
Discussion: The results support limited structu
hormone axis should be a greater priority for bio

We tested the hypothesis that TR has a more restrictive ligand-binding pocket than estrogen and androgen receptors using Tox21 screening and follow-up assays.

Table 1. Assay names (aenm) and assay end point identification (aeid) values used in the text and invitrodb database together with mode and purpose of assay.

Assay short name	invitrodb: aenm	invitrodb: aeid	Cell line	Assay mode	Function
GH3-TRE-Ag	TOX21_TR_LUC_GH3_Agonist	803	GH3-TRE-Luc	Agonist	Primary qHTS
GH3-TRE-Antag	TOX21_TR_LUC_GH3_Antagonist	804	GH3-TRE-Luc	Antagonist	Primary qHTS
GH3-TRE-Via	TOX21_TR_LUC_GH3_Antagonist_viability	805	GH3-TRE-Luc	Viability	Cytotoxicity
GH3-TRE-Ag-Followup	TOX21_TR_LUC_GH3_Agonist_Followup	2226	GH3-TRE-Luc	Agonist	Confirmation
GH3-TRE-Antag-Followup	TOX21_TR_LUC_GH3_Antagonist_Followup	2227	GH3-TRE-Luc	Antagonist	Confirmation
TRb-bla	TOX21_TRB_BLA_Antagonist_Followup_ratio	2240	TRβ-UAS-bla HEK 293T	Antagonist	Specificity
RXRa-bla-Ag	TOX21_TR_RXR_BLA_Agonist_Followup_ratio	2253	RXRα-UAS-bla HEK 293T	Agonist	Specificity
RXRa-bla-Antag	TOX21_TR_RXR_BLA_Antagonist_Followup_ratio	2257	RXRα-UAS-bla HEK 293T	Antagonist	Specificity
RXRa-Via	TOX21_TR_RXR_BLA_Antagonist_Followup_viability	2258	RXRα-UAS-bla HEK 293T	Viability	Cytotoxicity
TRa-coa	TOX21_TRA_COA_Agonist_Followup_ratio	2230	NA	Agonist	Orthogonal
TRb-coa	TOX21_TRB_BLA_Agonist_Followup_ratio	2236	NA	Agonist	Orthogonal
GFP-GR-TRb	NA	NA	GFP-GR-TRβ MCF7	Agonist and antagonist	Orthogonal

Note: Ag, agonist; Antag, antagonist; bla, beta-lactamase; coa, coactivator; GFP, green fluorescent protein;GH3, rat pituitary cell line; GR, glucocorticoid receptor; HEK 293T, human embryonic kidney cell line; LUC, luciferase; MCF7, human breast cancer cell line; NA, not applicable; qHTS, quantitative high-throughput screen; RXRa, retinoid X receptor alpha; TRa, thyroid hormone receptor alpha; TRb, thyroid hormone receptor beta; TRE, thyroid hormone receptor response element; UAS, upstream activating sequence; Via, viability.

NCCT_MITO: mitochondrial function

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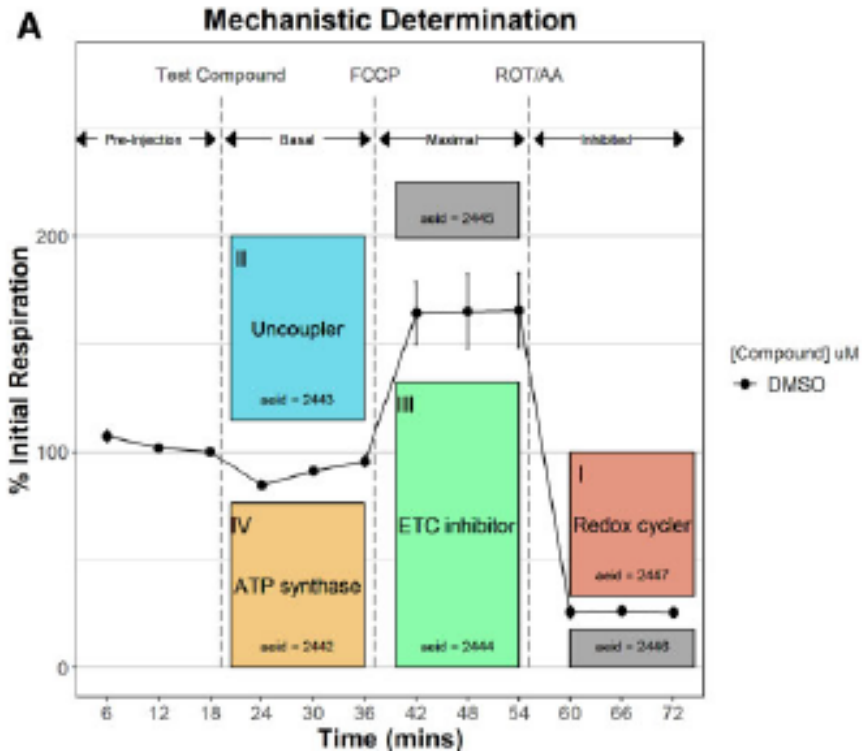
TOXICOLOGICAL SCIENCES, 176(1), 2020, 175–192

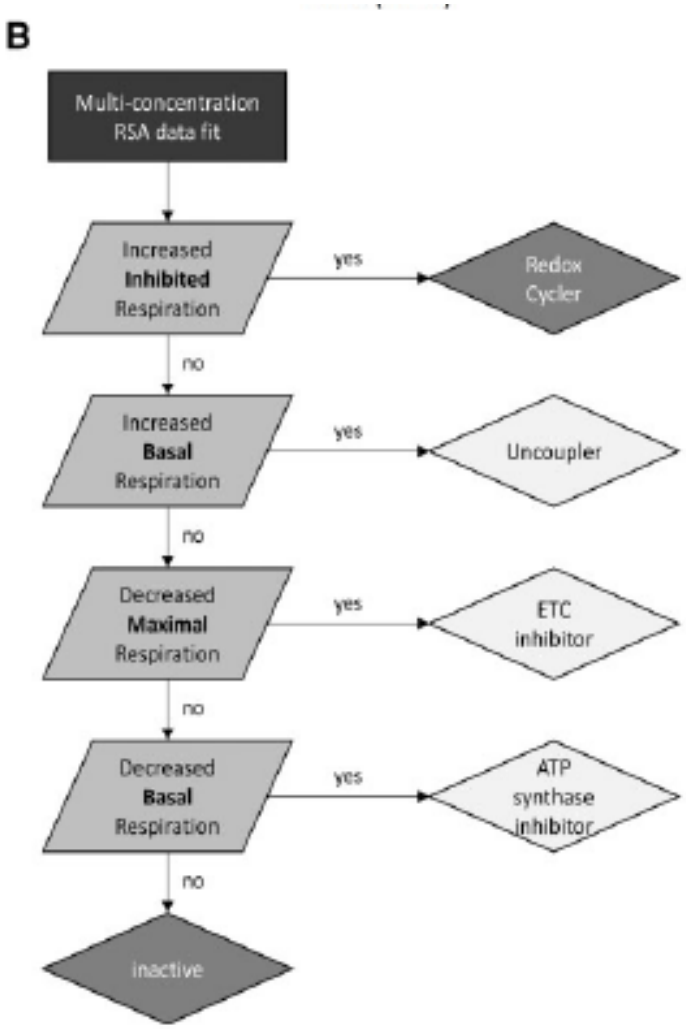
doi: 10.1093/toxsci/kfaa059
Dryad Digital Repository DOI: http://doi:10.5061/dryad.zkh189367
Advance Access Publication Date: May 6, 2020
Research article

EPA United States Environmental Protection Agency

Respirometric Screening and Characterization of Mitochondrial Toxicants Within the ToxCast Phase I and II Chemical Libraries

Daniel R. Hallinger,* Hayley B. Lindsay,† Katie Paul Friedman,* Danielle A. Suarez,‡ and Steven O. Simmons *,1





- Contrast to Tox21 and Apredica mitochondrial membrane permeability assay
- Apredica also has some additional mitochondrial morphology assays

Zebrafish developmental malformation screening: 2 labs, lots of peer-reviewed literature

Reproductive Toxicology 33 (2012) 174–187

Contents lists available at SciVerse ScienceDirect

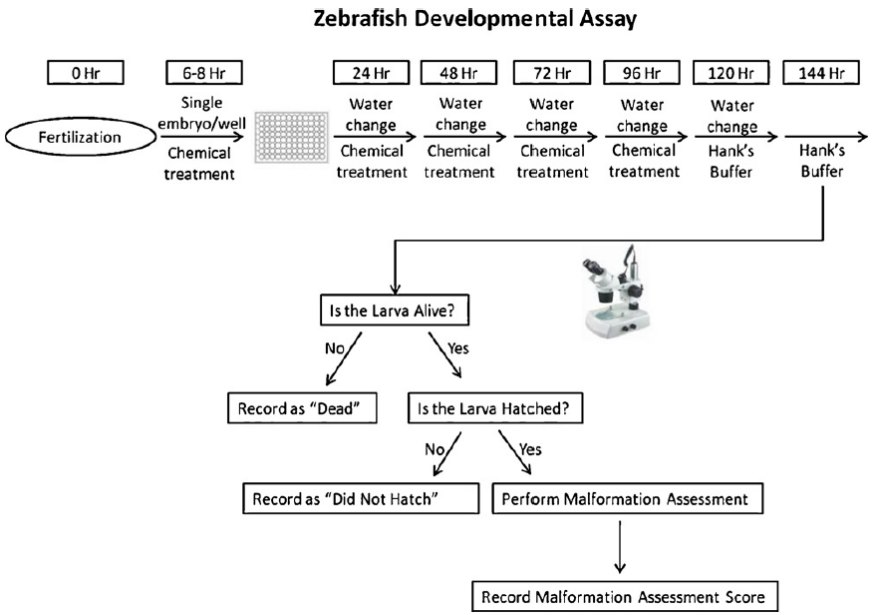
Reproductive Toxicology

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

Zebrafish developmental screening of the ToxCast™ Phase I chemical library

S. Padilla[a,*], D. Corum[b,1], B. Padnos[a], D.L. Hunter[a], A. Beam[b,2], K.A. Houck[b], N. Sipes[b], N. Kleinstreuer[b], T. Knudsen[b], D.J. Dix[b], D.M. Reif[b]

[a] National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA
[b] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA



- Integrated, highly conserved model of development
- Applicable to both human and eco toxicology
- Experimental design
  - Duration of experiment: 6 days with repeat dosing
  - Initial single dose testing (80 uM)
  - Dose-response for all actives plus a subset of inactives
  - 8 concentrations, 3 replicates
- Malformation visual assessment manually and by automated microscopy

Zebrafish developmental malformation screening: 2 labs, lots of peer-reviewed literature

EPA United States Environmental Protection Agency

Reproductive Toxicology 33 (2012) 174–187

Contents lists available at SciVerse ScienceDirect

Reproductive Toxicology

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

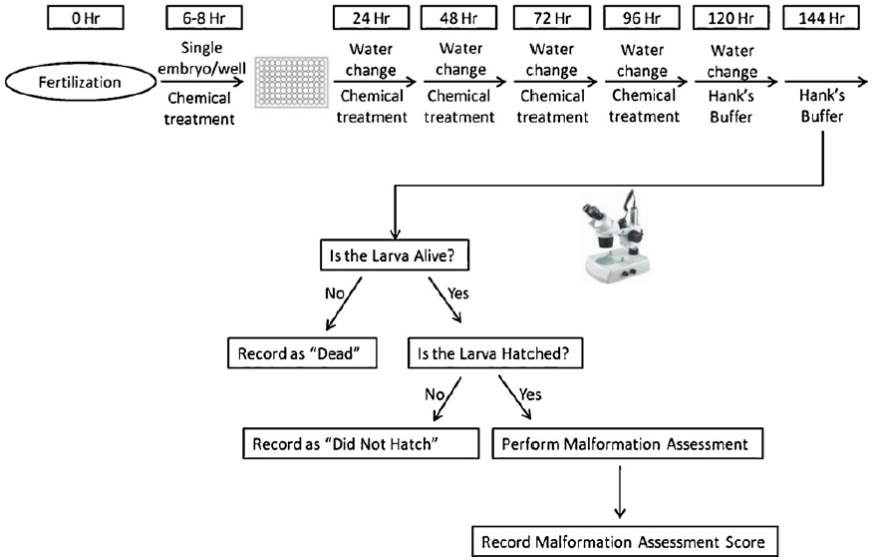
ELSEVIER

Zebrafish developmental screening of the ToxCast™ Phase I chemical library

S. Padilla[a,*], D. Corum[b,1], B. Padnos[a], D.L. Hunter[a], A. Beam[b,2], K.A. Houck[b], N. Sipes[b], N. Kleinstreuer[b], T. Knudsen[b], D.J. Dix[b], D.M. Reif[b]

[a] National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA
[b] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

Zebrafish Developmental Assay



TOXICOLOGICAL SCIENCES 137(1), 212–233 2014
doi:10.1093/toxsci/kft235
Advance Access publication October 17, 2013

Multidimensional *In Vivo* Hazard Assessment Using Zebrafish

Lisa Truong,* David M. Reif,† Lindsey St Mary,* Mitra C. Geier,* Hao D. Truong,* and Robert L. Tanguay*[,1]

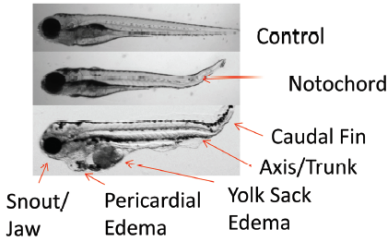
*Department of Environmental and Molecular Toxicology, the Sinnhuber Aquatic Research Laboratory and the Environmental Health Sciences Center at Oregon State University, Corvallis, Oregon 97333; and †Department of Biological Sciences, Bioinformatics Research Center, North Carolina State University, Raleigh, North Carolina 27695

At 5 Days (120 hpf)

Morphology Assessment

120 hpf End Points

End Point	Abbreviation
Mortality	MORT
Yolk Sac Edema	YSE_
Body Axis	AXIS
Eye Defect	Eye
Snout	SNOU_
Jaw	JAW_
Otic Vesicle	OTIC
Pericardial Edema	PE_
Brain	DRAI
Somite	SOMI
Pectoral Fin	PFIN
Caudal Fin	CFIN
Pigment	PIG_
Circulation	CIRC
Truncated Body	TRUN
Swim Bladder	SWIM
Notochord & Bent Tail	NC_
Touch Response	TR_



Larval Behavior

Using the Viewpoint Zebrabox, light-induced larval locomotor activity was measured. Locomotor activity is tracked for 25 minutes

NHEERL_PADILLA: not dechorinated; TANGUAY: dechorinated

Tox21 assays: a diverse suite.

- Most of these assays are in 1536 wp format, but not all.
- Typically 15 concentrations with n=3
- ~8500 unique chemical structures (~10,000 samples)
- Many are for nuclear receptors, stress pathways, assay interference.
  - E.g., Nuclear Receptors: AR, ERa, PPARg, GR, TR, AhR, PXR
  - GAL4 System (ligand detection assay) and full-length receptors
  - β-lactamase or luciferase reporter gene assays
  - **Agonist** and **antagonist** mode, sometimes with multiple concentrations of antagonist available
  - **Viability** assays measured in parallel
  - Other assays: mitochondrial toxicity, DNA damage, aromatase

This was an incomplete tour through much of what is in ToxCast, but not all.

- Biological gaps continue to be filled.
- E.g., developmental neurotoxicity new approach methodologies.

How are ToxCast/Tox21 data managed and what are the key data definitions for use?

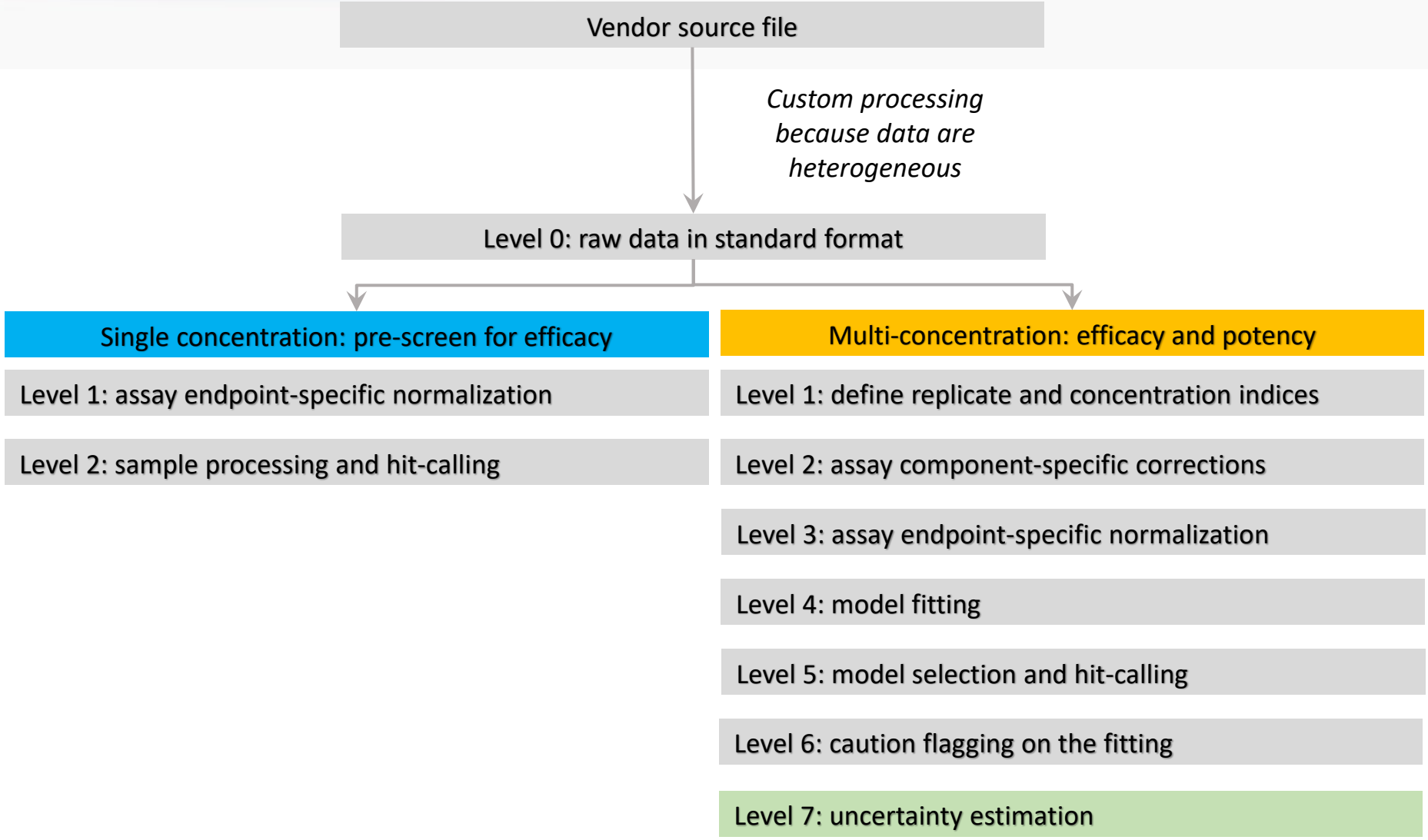
Summary information, datasets, and the full database (invitrodb version 3.3 August 2020 release) are available here:

https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data

Pipeline Overview

- Raw (source) data remains unaltered
- Storage of data at “levels” to standardize for any future analysis
- Use combination of statistics (x-MAD, AIC) and biology-based efficacy cutoffs
- Points of Departure (e.g. AC10, ACC) are included
- System of “caution flags” has been developed (continues to evolve)

ToxCast: high-throughput bioactivity information

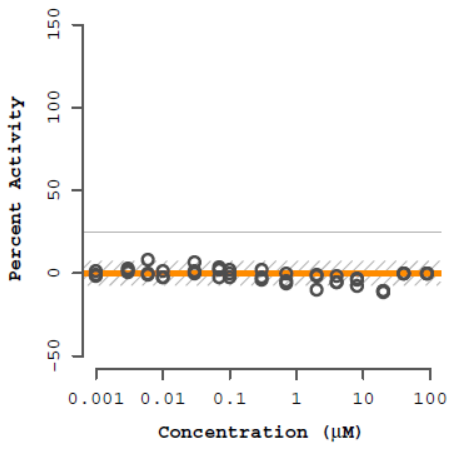


Pipeline Overview: Curve Fits

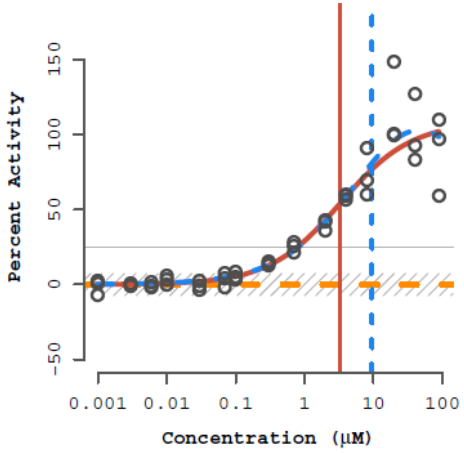
EPA United States Environmental Protection Agency

Winner determined by AIC

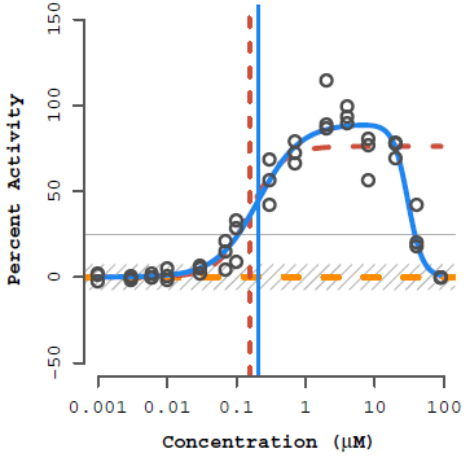
CONSTANT (cnst)



HILL(hill)



GAIN-LOSS (gnls)



Tcpl is on CRAN and GitHub with 1-2 updates a year

tcpl: ToxCast Data Analysis Pipeline

A set of tools for processing and modeling high-throughput and high-content chemical screening data. The package was developed for the the chemical screening data generated by the US EPA ToxCast program, but can be used for diverse chemical screening efforts.

Version:	2.0.2
Depends:	R (≥ 3.2.0)
Imports:	data.table (≥ 1.9.4), DBI, RMySQL, numDeriv, RColorBrewer, utils, stats, methods, graphics, grDevices, sqldf
Suggests:	roxygen2, knitr, prettydoc, rmarkdown, htmlTable
Published:	2019-07-26
Author:	Richard S Judson [cre, ths], Dayne L Filer [aut], Jason Brown [ctb], Todd Zurlinden [ctb], Parth Kothiya [ctb], Woodrow R Setzer [ctb], Matthew T Martin [ctb, ths], Katie Paul Friedman [ctb]
Maintainer:	Richard S Judson <Judson.Richard at epa.gov>
License:	GPL-2
URL:	https://github.com/USEPA/CompTox-ToxCast-tcpl
NeedsCompilation:	no
Materials:	NEWS
CRAN checks:	tcpl results

Vignettes on CRAN and peer-reviewed work

Bioinformatics, 33(4), 2017, 618–620
doi: 10.1093/bioinformatics/btw680
Advance Access Publication Date: 22 November 2016
Applications Note

OXFORD

Data and text mining

tcpl: the ToxCast pipeline for high-throughput screening data

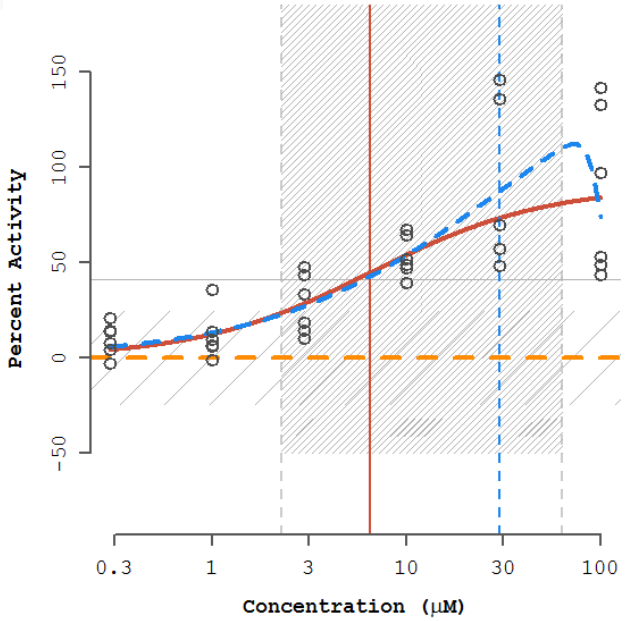
Dayne L. Filer[1], Parth Kothiya[1], R. Woodrow Setzer[2], Richard S. Judson[2] and Matthew T. Martin[2,*]

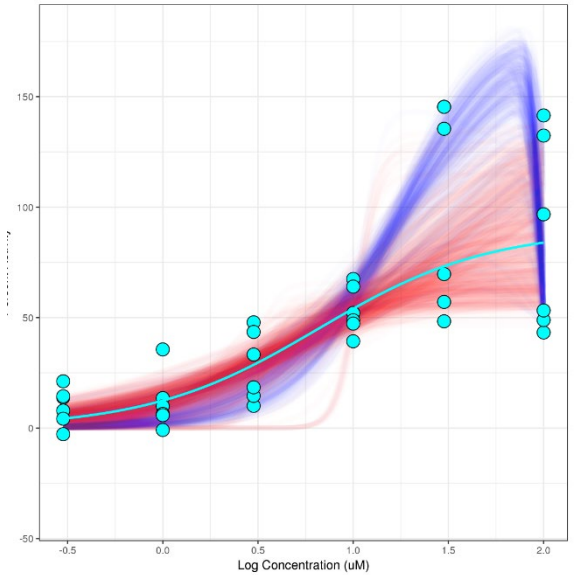
Key ToxCast vocabulary for using these data

Key vocabulary	Full description	Derivation	Use
AC50	50% activity concentration, often represented as log10-AC50 (micromolar units)	A stable point on the curve that is 50% of the maximal fitted response	Potency estimate
ACC	Activity concentration at the cutoff, often represented as log10-ACC (micromolar units)	Similar to a benchmark dose; variable efficacy across heterogeneous assays	Potency estimate
HITC	Hitcall: -1, 0, 1	Qualitative activity determination; hitc=-1 not enough data to fit; hitc=0 negative because model top does not exceed the coff and/or the winning model is constant; hitc=1 positive	Binary activity – pretty incomplete picture (think borderline efficacy)
COFF	Efficacy "cut-off"	Statistical or biology-based cut-off for a positive; assay endpoint-dependent	Determines positive/negative hitcall
BMAD	Baseline median absolute deviation	Median absolute deviation of data that approximate assay "baseline;" can be lowest two concentrations in the index (by plate), or can be DMSO or vehicle wells	3*BMAD is a common way to bound the "noise" in the assay baseline so that signal can be distinguished from noise
Flags	Caution flags on curve-fitting (from level 6)	Lots of different specific flags from "borderline activity" to "noisy fit"	Not all curve fits with flags are bad; some flags worse than others; >= 3 flags tend to indicate low quality curves
Model, winning model	Curve-fitting models (e.g., Hill, gain-loss, constant)	The winning model has the lowest AIC	the winning model determines the potency estimates reported

Improvements for 2021 and beyond

- New curve-fitting to incorporate BMDExpress curvefitting models (for tcpl version 3.0).
- Ongoing onsideration of uncertainty in potency values.





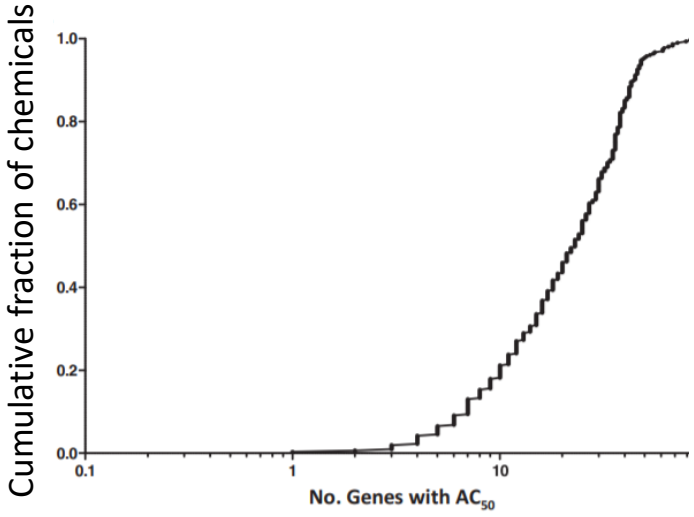
Key context: assay interference from cytotoxicity is related to selective and non-selective phenotypes in HTS

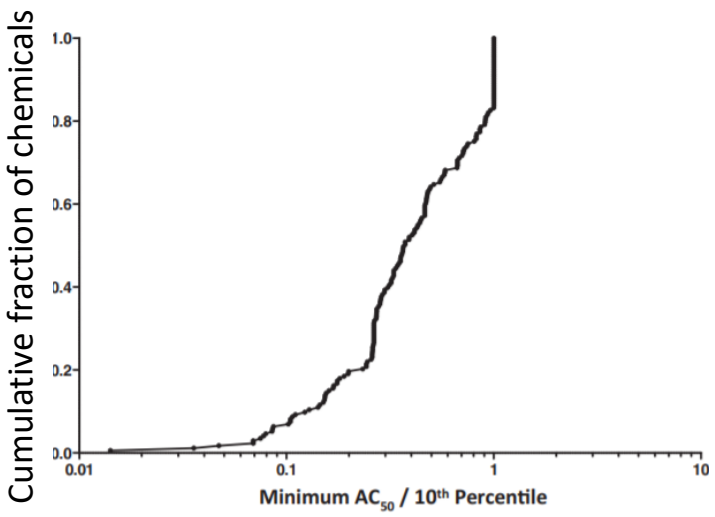
Many of the substances in ToxCast appear non-selective

TOXICOLOGICAL SCIENCES 136(1), 4–18 2013
doi:10.1093/toxsci/kft178
Advance Access publication August 19, 2013

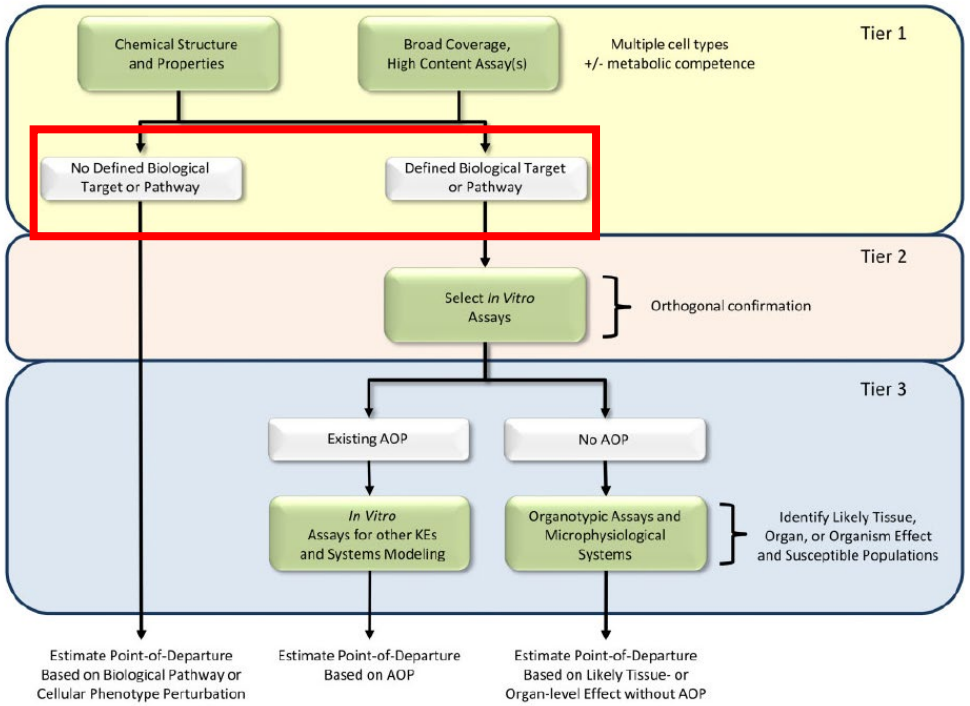
Incorporating New Technologies Into Toxicity Testing and Risk Assessment: Moving From 21st Century Vision to a Data-Driven Framework

Russell S. Thomas,*,1 Martin A. Philbert,† Scott S. Auerbach,‡ Barbara A. Wetmore,* Michael J. Devito,‡ Ila Cote,§ J. Craig Rowlands,¶ Maurice P. Whelan,‖ Sean M. Hays,‖‖ Melvin E. Andersen,* M. E. (Bette) Meek,‖‖ Lawrence W. Reiter,# Jason C. Lambert,** Harvey J. Clewell III,* Martin L. Stephens,†† Q. Jay Zhao,** Scott C. Wesselkamper,** Lynn Flowers,§ Edward W. Carney,¶ Timothy P. Pastoor,‡‡ Dan D. Petersen,** Carole L. Yauk,§§ and Andy Nong§§

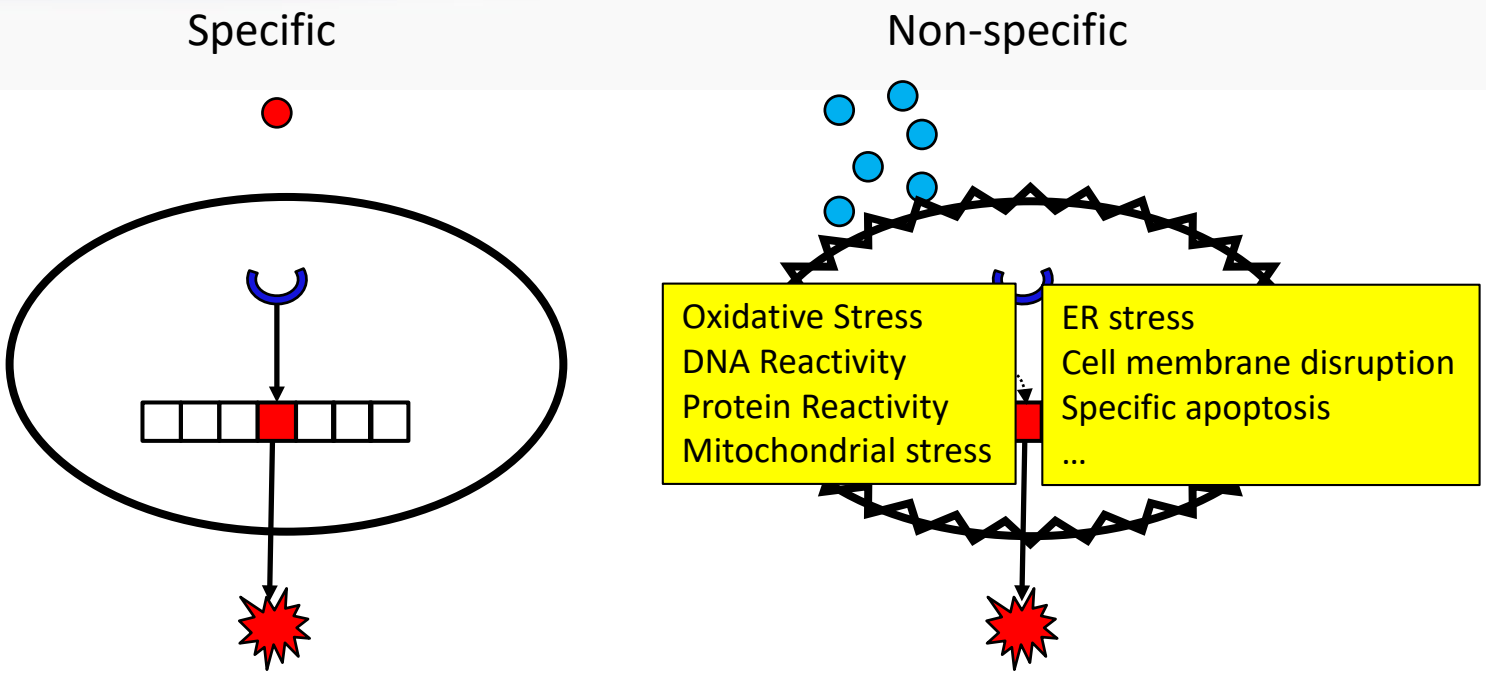




- Many chemicals appear to act at many targets, or be non-selective
- This could be used to subset chemicals into screening tracks

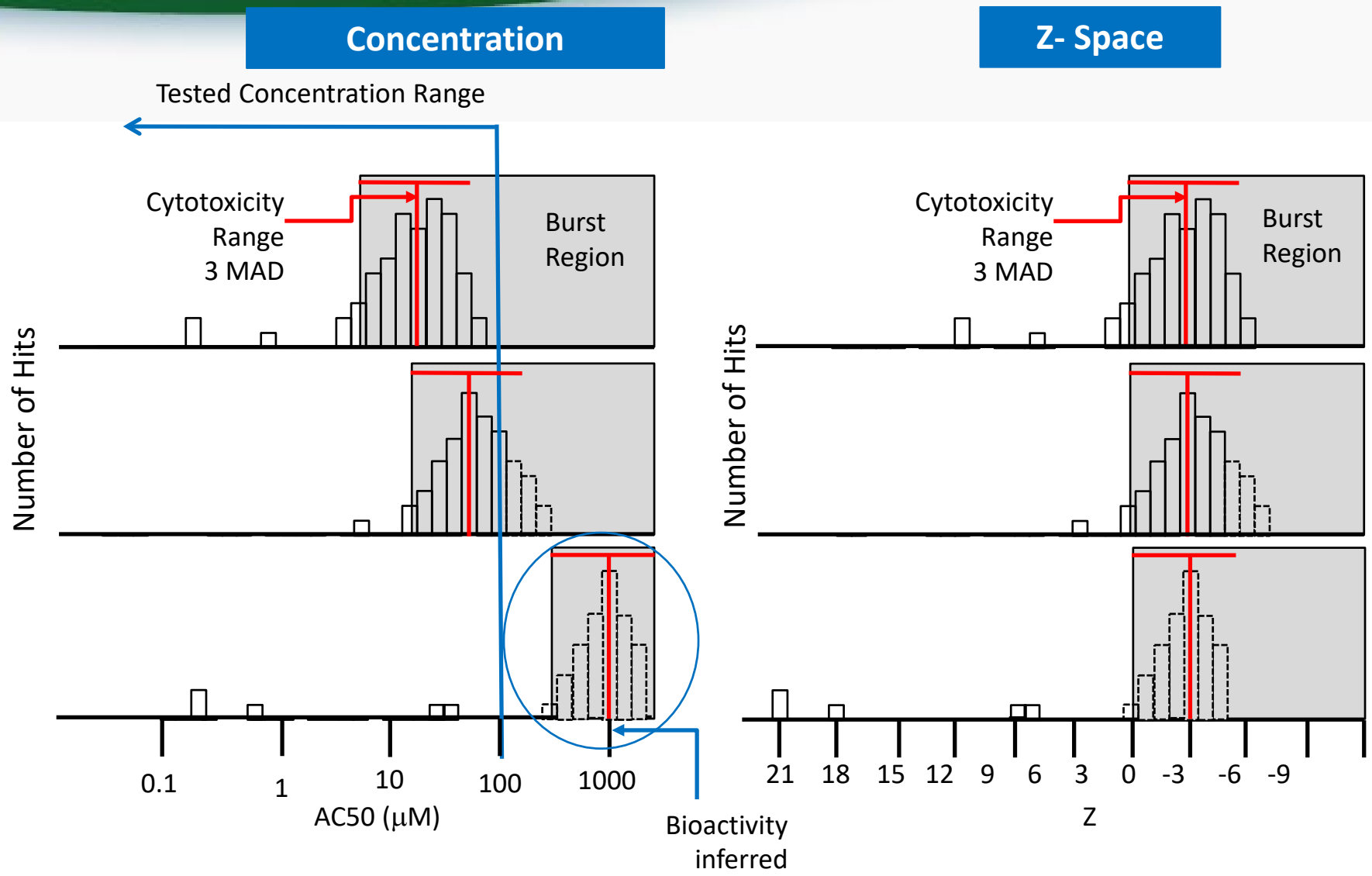


Schematic explanation of the burst



Judson et al. Tox.Sci. (2016); slide from Richard Judson

Most chemicals display a “burst” of potentially non-selective bioactivity near cytotoxity concentration



Judson *et al.* Tox.Sci. (2016)

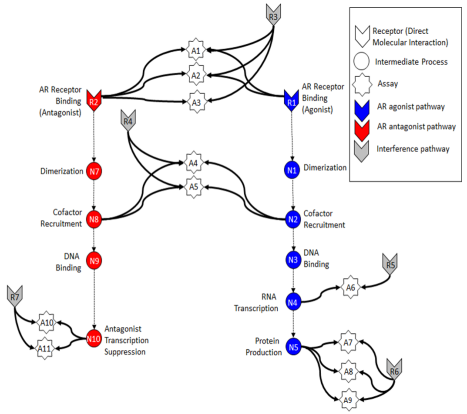
The cytotoxicity “burst” is useful for context

- The latest Comptox Chemicals Dashboard release (version 3.5, July 2020 release) demonstrates a cytotoxicity threshold based on the latest ToxCast database (invitrodb version 3.3, released Aug 2020). This value can change as more cytotoxicity data become available, curve-fitting approaches for existing data change, or the “burst” calculation approach is updated.
- In invitrodb version 3.3, 88 assays are considered for the cytotoxicity threshold. A positive hit must be observed in 5% of these assays (noting that not all chemicals are screened in all 88 assays) in order to assign a cytotoxicity threshold. The cytotoxicity threshold is a median of AC50 potency values from the N assays with a hit. The cytotoxicity threshold visualized in the Dashboard is a lower bound on this estimate, calculated as the median cytotoxicity potency minus 3 times the global median absolute deviation.
- This is discussed further in a publication (10.1093/toxsci/kfw148) and the ToxCast Pipeline R package (tcpl) function, tcplCytoPt() (available on CRAN: https://cran.r-project.org/web/packages/tcpl/index.html).
- If fewer than 5 cytotoxicity assays demonstrate a positive hit, a default of 1000 micromolar is assigned for the chemical.
- The lower bound estimate of the cytotoxicity threshold or “burst” is useful context for ToxCast results. Bioactivity observed below the cytotoxicity threshold may represent more specific activity that is less likely to be confounded by cytotoxicity.
- It is possible that AC50 values above the cytotoxicity threshold are informative. If an assay has a parallel cytotoxicity assay in the same cell type, that may be more informative for interpreting that assay. Or, if a result is consistent with an AOP relevant to the chemical with assay AC50 values above and below the cytotoxicity threshold, those data may be meaningful.

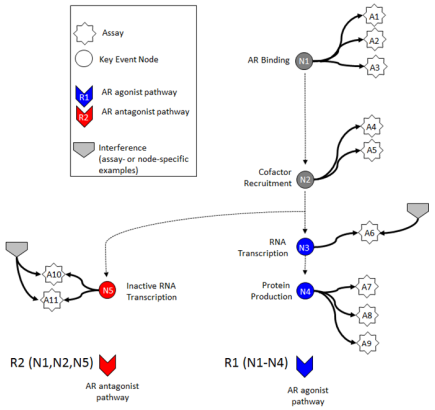
Where are we headed?

High-throughput toxicology answers scientific and regulatory needs

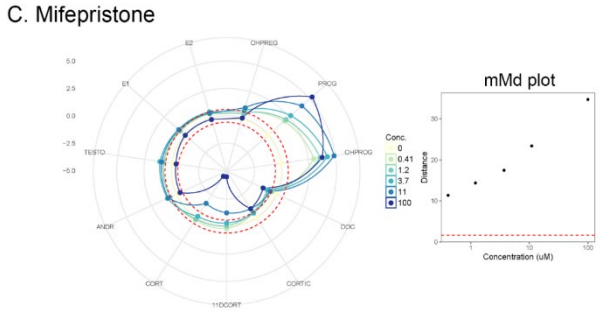
- We face many environmental challenges:
  - Chemicals, disease, crop-failure, climate change
- Data alone cannot answer all necessary questions:
  - Data can be expensive and noisy
  - Cause and effect relationships are multivariate and non-linear
- Needed: mathematical and statistical models, approximations, and other tools that increase safety and efficiency. Endocrine examples below, many more in the literature!
- Extension of HTS data to QSAR.



Estrogen receptor pathway model



Androgen receptor pathway model

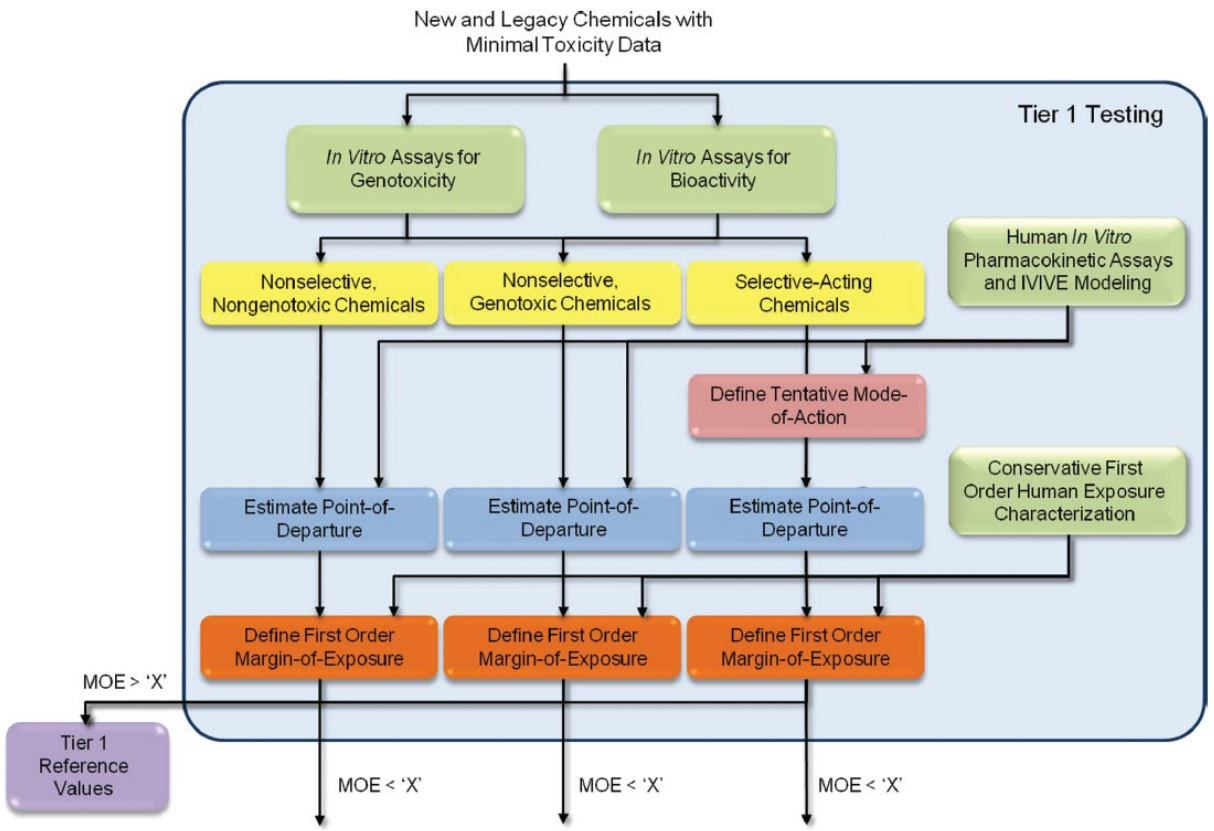


Steroidogenesis HT-H295R model

Use of predictive science in chemical safety should include risk-based approaches like BER

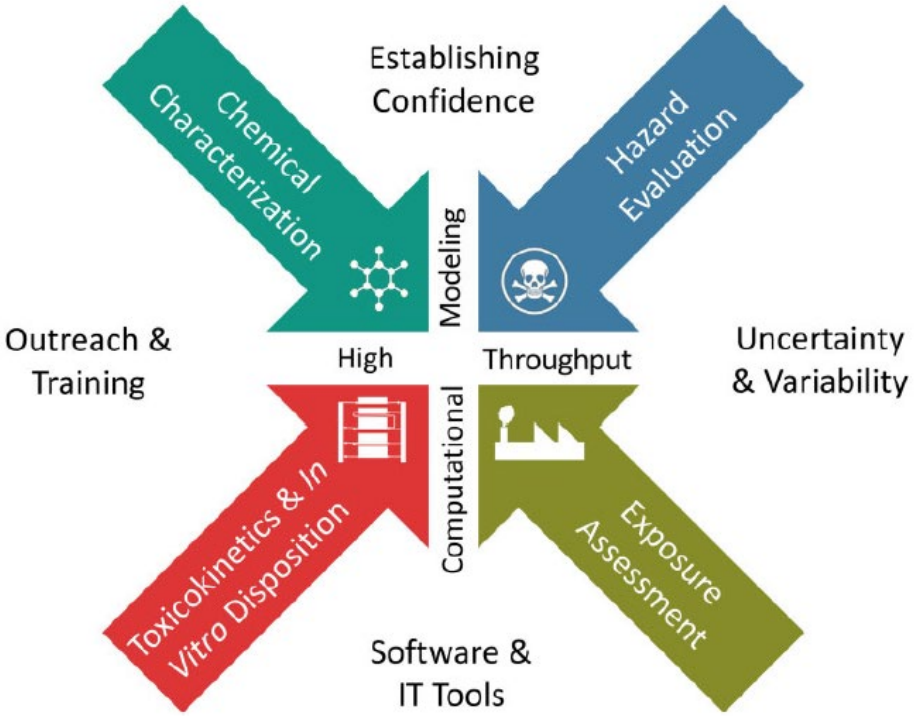
- Specific vs. nonspecific modes-of-action and the challenge of hazard labeling

Thomas et al. 2013 suggested a framework for hazard assessment that would be largely customized based on MOE (or now, BER).



Use of predictive science in chemical safety should include risk-based approaches like BER

- Now, ~6 years later, Thomas et al. (2019) suggest a computational toxicology blueprint that represents evolution of the same concept



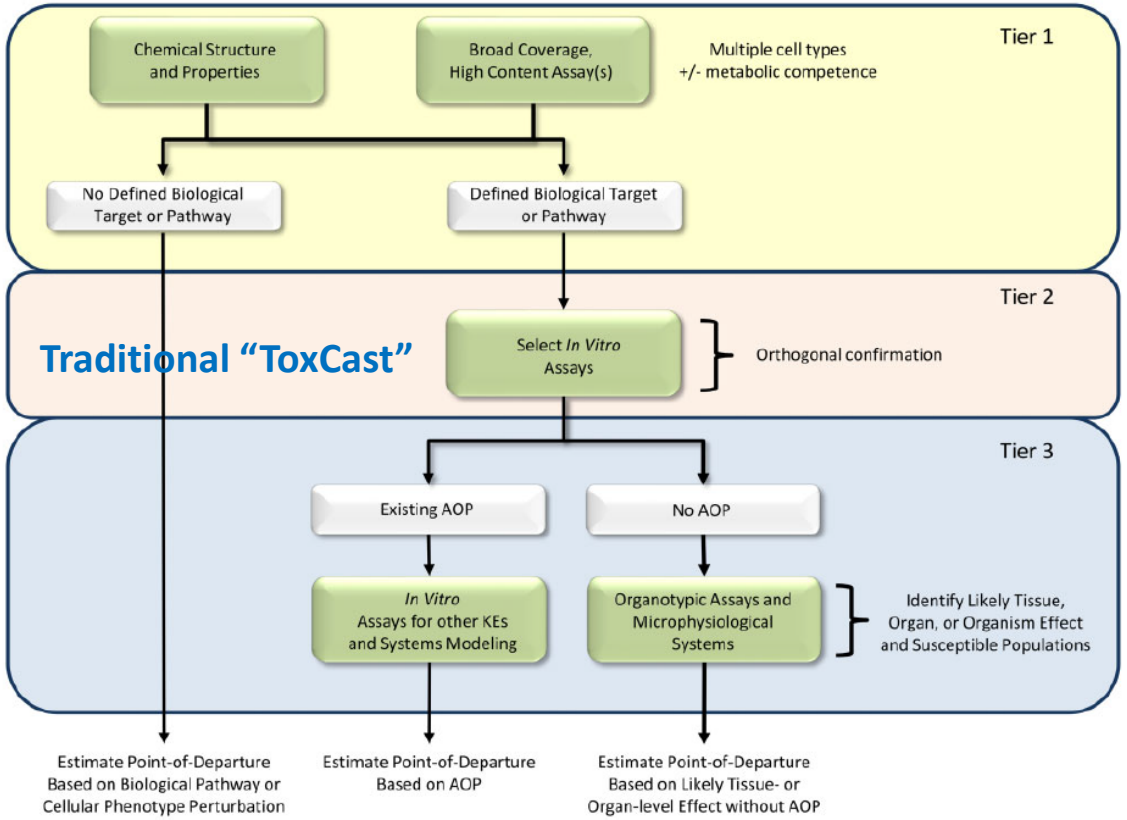


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

Tier 1 becomes a broad-based screening that segues to Tier 2 (targeted screening).

High-throughput phenotypic-profiling

Toxicology and Applied Pharmacology 389 (2020) 114876

Contents lists available at ScienceDirect

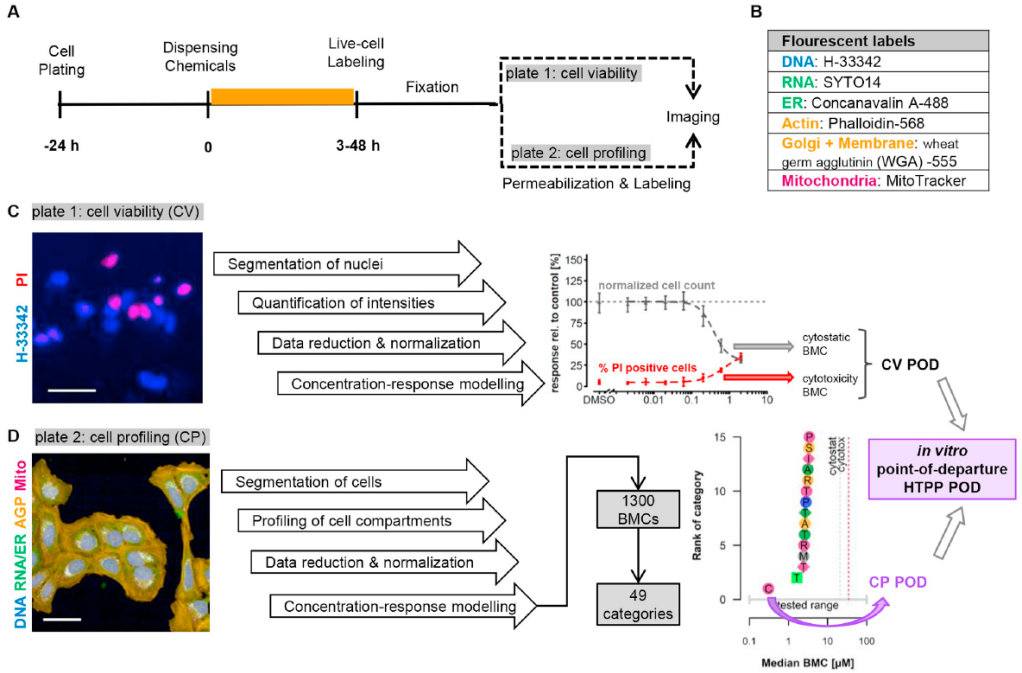
Toxicology and Applied Pharmacology

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

Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling

Johanna Nyffeler[a,b], Clinton Willis[a,c], Ryan Lougee[a,b], Ann Richard[a], Katie Paul-Friedman[a], Joshua A. Harrill[a,*]

[a] Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Durham, NC 27711, United States of America
[b] Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831, United States of America
[c] Oak Ridge Associated Universities (ORAU) National Student Services Contractor, Oak Ridge, TN 37831, United States of America



High-throughput transcriptomics

EPA Public Access
Author manuscript
Curr Opin Toxicol. Author manuscript; available in PMC 2020 January 01.

About author manuscripts | Submit a manuscript

Published in final edited form as:
Curr Opin Toxicol. 2019 ; 15: 64–75. doi:10.1016/j.cotox.2019.05.004.

Considerations for Strategic Use of High-Throughput Transcriptomics Chemical Screening Data in Regulatory Decisions

Joshua Harrill[1], Imran Shah[1], R. Woodrow Setzer[1], Derik Haggard[2], Scott Auerbach[3], Richard Judson[1], Russell S. Thomas[1]

- High-throughput phenotypic profiling and high-throughput transcriptomics will provide broad screening coverage
- Points-of-departure based on these techniques could then be augmented/refined using targeted screens (e.g., subsets of existing ToxCast assays and new assays to fill gaps)

Acknowledgments

- Thank you for listening.
- Thank you: Keith Houck and Richard Judson along with many others in CCTE who contribute to ToxCast.
- Please reach out to us if you need support or explanations for a specific case, or if you find issues.
- Paul-friedman.katie@epa.gov

EPA's Center for Computational Toxicology and Exposure

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EPA United States Environmental Protection Agency

Overview of the CompTox Chemicals Dashboard and ToxCast/Tox21 Screening Program: Tools for Users

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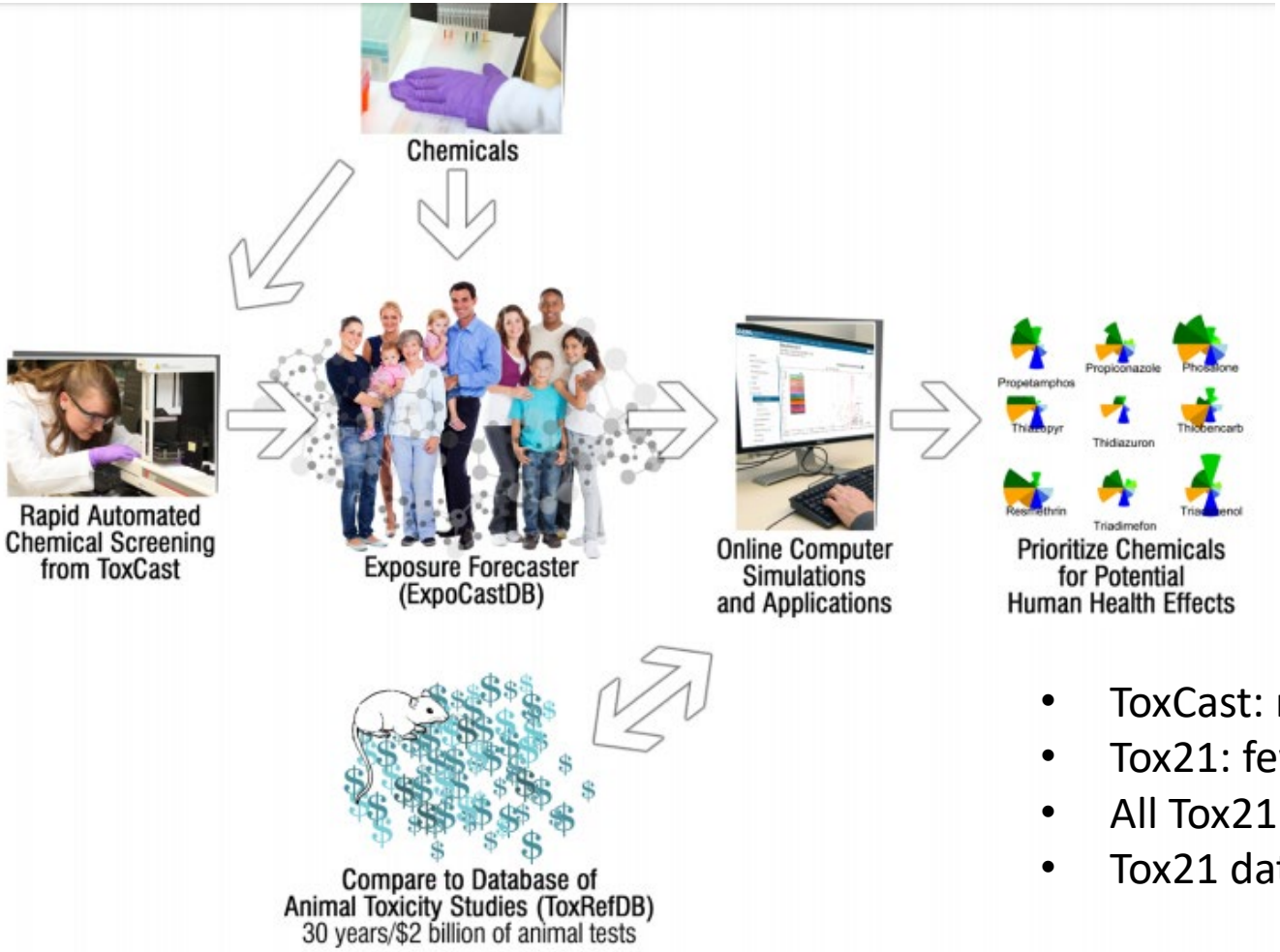
Center for Computational Toxicology and Exposure, US-EPA, RTP, NC

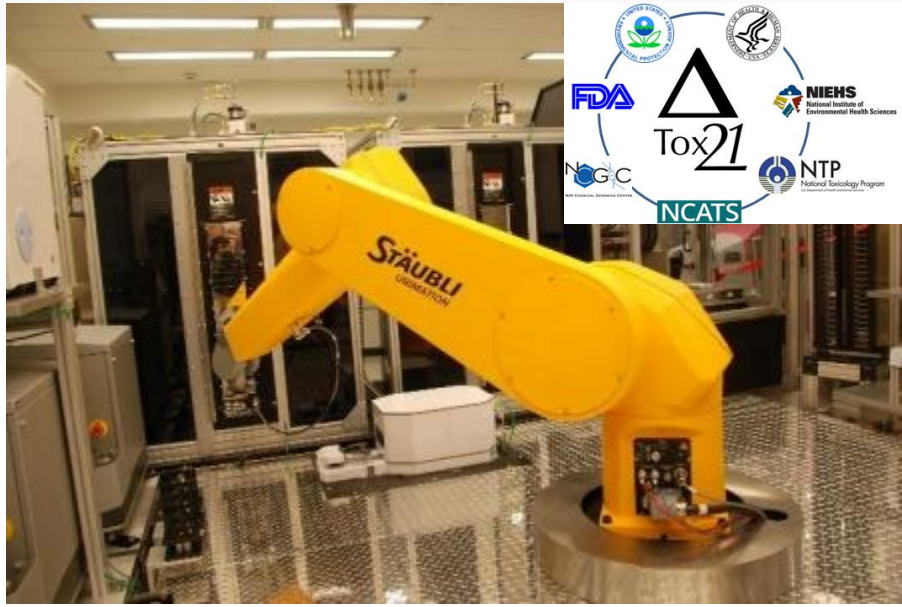
The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

ToxCast and Tox21 have generated a lot of publicly available bioactivity data for hazard screening and prediction.

EPA United States Environmental Protection Agency

EPA's ToxCast program at a glance





Tox21 robot

- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, all 1536, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline

ToxCast covers a lot of biology but not all; and, ToxCast is growing over time.

EPA United States Environmental Protection Agency

Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

Assay source	Long name	Truncated assay source description	Some rough notes on the biology covered
ACEA	ACEA Biosciences	real-time, label-free, cell growth assay system based on a microelectronic impedance readout	Endocrine (ER-induced proliferation)
APR	Apredica	CellCiphr High Content Imaging system	Hepatic cells (HepG2)
ATG	Attagene	multiplexed pathway profiling platform	Nuclear receptor and stress response profile
BSK	Bioseek	BioMAP system providing uniquely informative biological activity profiles in complex human primary co-culture systems	Immune/inflammation responses
NVS	Novascreen	large diverse suite of cell-free binding and biochemical assays.	Receptor binding; transporter protein binding; ion channels; enzyme inhibition; many targets
OT	Odyssey Thera	novel protein:protein interaction assays using protein-fragment complementation technology	Endocrine (ER and AR)
TOX21	Tox21/NCGC	Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high-throughput screening assays across a large interagency-developed chemical library	Many – with many nuclear receptors
CEETOX	Ceetox/OpAns	HT-H295R assay	Endocrine (steroidogenesis)
CLD	CellzDirect	Formerly CellzDirect, this Contract Research Organization (CRO) is now part of the Invitrogen brand of Thermo Fisher providing cell-based in vitro assay screening services using primary hepatocytes.	Liver (Phase I/Phase II/ Phase III expression)
NHEERL_PADILLA	NHEERL Padilla Lab	The Padilla laboratory at the EPA National Health and Environmental Effects Research Laboratory focuses on the development and screening of zebrafish assays.	Zebrafish terata
NCCT	NCCT Simmons Lab	The Simmons Lab at the EPA National Center for Computational Toxicology focuses on developing and implementing in vitro methods to identify potential environmental toxicants.	Endocrine (thyroid - thyroperoxidase inhibition)
TANGUAY	Tanguay Lab	The Tanguay Lab, based at the Oregon State University Sinnhuber Aquatic Research Laboratory, uses zebrafish as a systems toxicology model.	Zebrafish terata/phenotypes
NHEERL_NIS	NHEERL Stoker & Laws	The Stoker and Laws laboratories at the EPA National Health and Environmental Effects Research Laboratory work on the development and implementation of high-throughput assays, particularly related to the sodium-iodide cotransporter (NIS).	Endocrine (thyroid - NIS inhibition)
UPITT	University of Pittsburgh	The Johnston Lab at the University of Pittsburgh ran androgen receptor nuclear translocation assays under a Material Transfer Agreement (MTA) for the ToxCast Phase 1, Phase 2, and E1K chemicals.	Endocrine (AR related)

With each release, more assay endpoints and more chemical x endpoint data are released

Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

These assay endpoints were notable additions in invitrodb version 3.3.

Assay source	Long name	Truncated assay source description	Some rough notes on the biology covered
NCCT_MITO	NCCT (now Center for Computational Toxicology and Exposure) Mitochondrial toxicity	Respirometric assay that measure mitochondrial function in HepG2 cells	Multiple assay endpoints to evaluate mitochondrial function https://doi.org/10.1093/toxsci/kfaa059.
NHEERL_MED	NHEERL Mid-Continent Ecology Division	The EPA Mid-Continent Ecology Division of the National Health and Environmental Effects Research Laboratory screened the ToxCast Phase 1 chemical library for hDIO1 (deiodinase 1) inhibition as part of an ecotoxicology effort.	Endocrine (thyroid – hDIO1,2,3 inhibition) https://doi.org/10.1093/toxsci/kfy302
STM	Stemina	Stem cell-based metabolomic indicator of developmental toxicity for screening.	Developmental toxicity screening – multiple assay endpoints https://doi.org/10.1093/toxsci/kfaa014
LTEA	Life Tech Expression Analysis	Gene expression measured in HepaRG cells following 48 hr exposure	Liver toxicity model via transcription factor regulated-metabolism and markers of oxidative/cell stress; multiple assay endpoints

What can be done with ToxCast data?

EPA United States Environmental Protection Agency

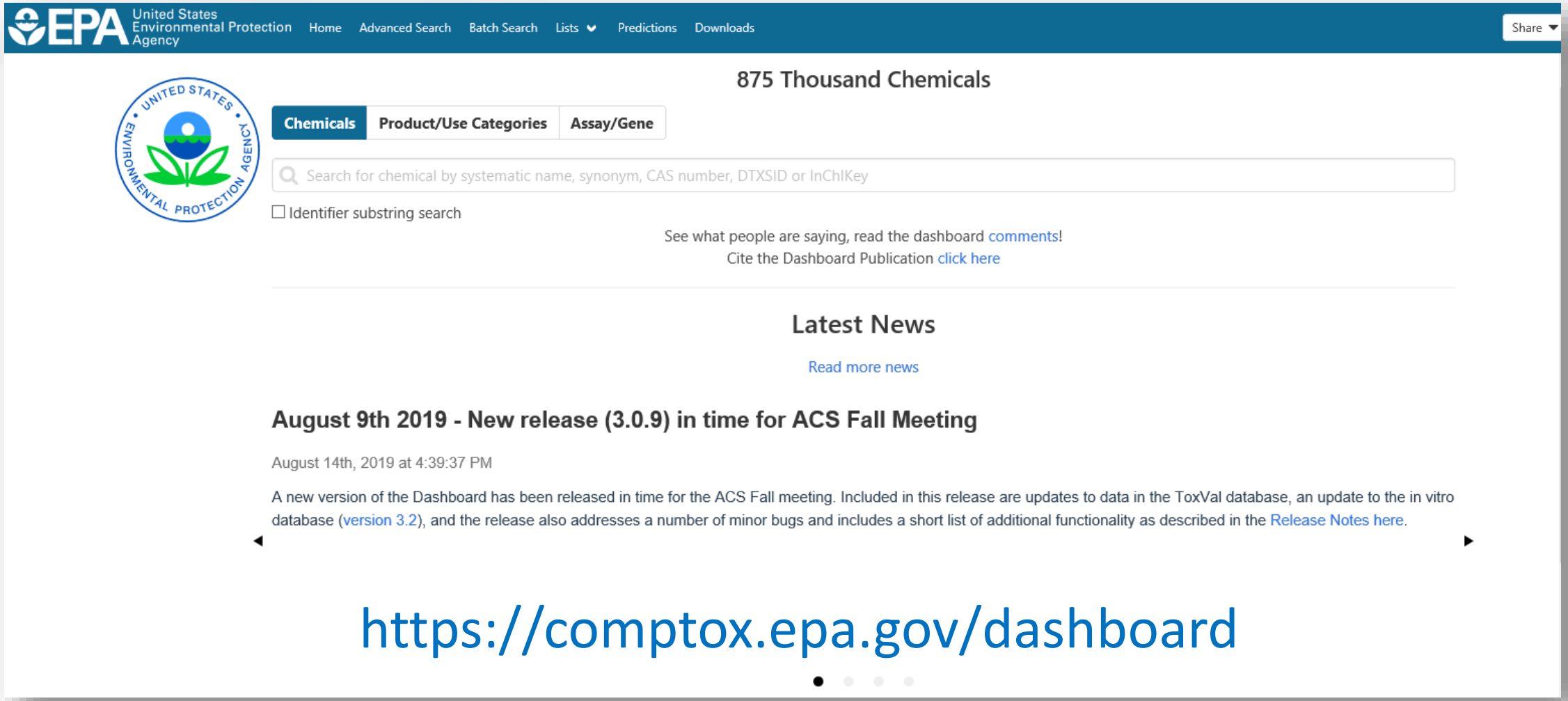
Answering biological questions

- (*for example*) Does this substance have endocrine or liver-mediated bioactivity?
- Is there support for one or more adverse outcome pathways based on these data, or does the substance appear “non-selective?”

Answering risk-related questions

- Can a protective bioactivity-based point-of-departure be calculated?
- What is the relative priority of this substance for additional evaluation?

A user interface to browse and download data: CompTox Chemicals Dashboard



https://comptox.epa.gov/dashboard

Using ToxCast Data in Weight of Evidence or Screening Level Assessment

- Vignette 1: Weight of evidence example
- Vignette 2: Risk-based approach that incorporates bioactivity and exposure, making the best use of new approach methodologies, for endocrine bioactivity.

This presentation will demonstrate where to find these information and suggest an approach for utilizing them in screening level risk evaluation.

Vignette one: bioactivity for weight-of-evidence/biological questions

Is mystery compound A toxic to liver and/or mitochondria?

Mystery compound A: in domain of current screening?

Analytical chemistry: was the chemical present and in the DOA for current ToxCast?

Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Copy | Share | Submit Comment | Search all data

- PROPERTIES
- ENV. FATE/TRANSPORT
- HAZARD
- SAFETY
- ADME
- EXPOSURE
- BIOACTIVITY
- SIMILAR COMPOUNDS
- GENRA (BETA)
- RELATED SUBSTANCES
- SYNONYMS
- LITERATURE
- LINKS
- COMMENTS

Summary

MW = 441.54 g/mol – likely good oral availability

Summary

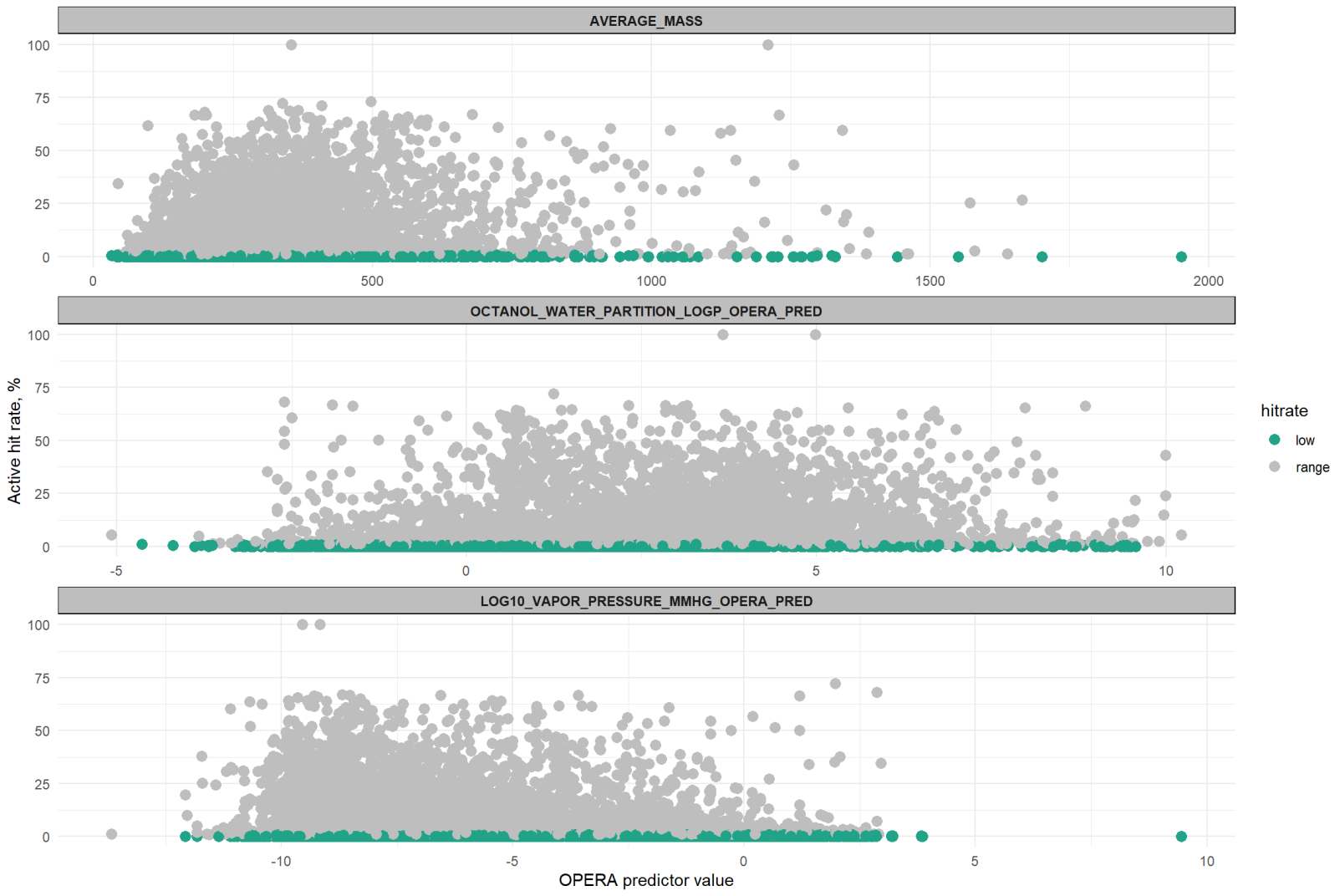
Download | Columns | Search query

Probably able to cross cell membrane without active transport

Property	Experimental average	Predicted average	Experimental median	Predicted median	Experimental range	Predicted range	Unit
LogKow: Octanol-Water	-	4.94		4.67	-	4.30 to 6.11	
Melting Point	185 (2)	215	185	184	184 to 185	150 to 313	°C
Boiling Point	-	589		657	-	397 to 714	°C
Water Solubility	-	5.40e-6		2.72e-6	-	8.75e-8 to 1.34e-5	mol/L
Density	-	1.27		1.27	-	1.27	g/cm^3
Flash Point	-	330		330	-	309 to 351	°C
Vapor Pressure	-	7.20e-10		3.83e-11	-	7.24e-18 to 2.12e-9	mmHg
Surface Tension	-	51.0			-	51.0	dyn/cm
Index of Refraction	-	1.61			-	1.61	-
Molar Refractivity	-	120			-	120	cm^3
Polarizability	-	47.8			-	47.8	Å^3
Molar Volume	-	349			-	349	cm^3
LogKoa: Octanol-Air	-	9.68			-	9.68	-
Henry's Law	-	5.64e-9			-	5.64e-9	atm-m3/mole

Not volatile

“Low” hit-rate substances in ToxCast are distributed across physicochemical properties



These physicochemical properties may be helpful in considering substances that look negative across ToxCast, but physicochemical properties don’t tell the entire story.

Substances with low hit-rate on the “fringe” of the distribution may need closer consideration to understand if they are within the domain of screening.

Mystery compound A seems to fit into the domain of screening based on chemistry

Analytical chemistry: was the chemical present and in the DOA for current ToxCast?

States ental Protection | Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Copy | Share | Submit Comment | Search all data

Searched by DSSTox Substance Id.

PROPERTIES
ENV. FATE/TRANSPORT
HAZARD
SAFETY
ADME
EXPOSURE
BIOACTIVITY
TOXCAST: SUMMARY
EDSP21
TOXCAST/TOX21

ToxCast/Tox21

Select samples that were analyzed (the chemical in DMSO stock) are high purity and confirmed

QC Data ID	Grade	Description
Tox21_112119	Pass	Purity>90% and MW confirmed
Tox21_112119_1	Pass	Purity>90% and MW confirmed
Tox21_300470	Pass	Purity>90% and MW confirmed

Assay Selection 0 Selected | A Single Assay Can Have Multiple Charts | Representative Samples Only | Bioactivity Summary | Number of Charts: 0

Active Inac

Filter assays

Tanguay Lab (0 of 19 s

Tox21/NCGC (0 of 235

NHEERL Mid-Continent

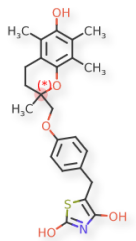
tripod.nih.gov/tox21/samples/Tox21_112119

Apps | Files - OneDrive | toxrefdb - OneDrive | Google Scholar | Altmetric it! | ORD Graphics and... | ORD@Work | Request Research H... | Travel Authorizatio... | Chemtrack | Toxicity Data Collec...

Tox21 | Structure Search | Search...

Home / Tox21 Samples / Tox21_112119

Seems stable under screening sample conditions (DMSO, room temp, 0-4 months)



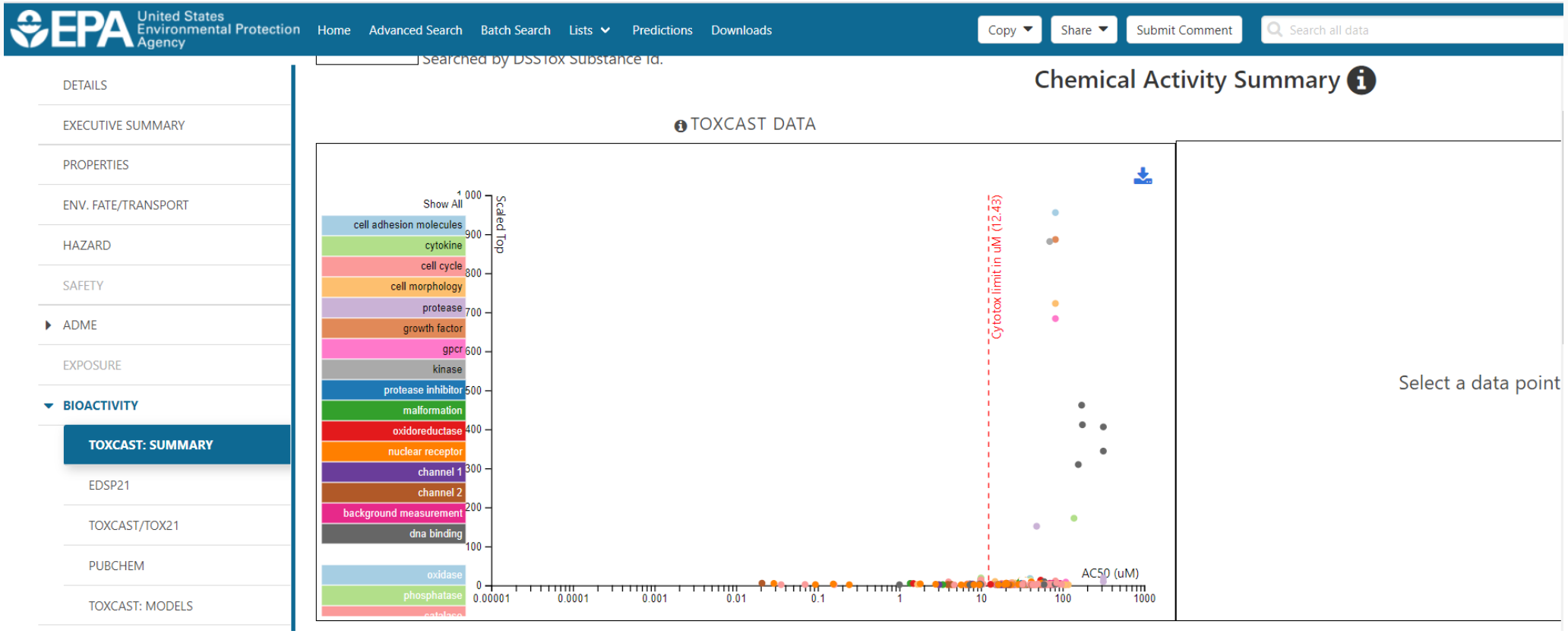
QC Grade

T0	A	MW Confirmed, Purity > 90%
T4	A	MW Confirmed, Purity > 90%

Identifiers

Tox21	Tox21_112119
NCATS	NCGC00159457-01
CAS	
PubChem	

But what bioactivity does Mystery Compound A have?



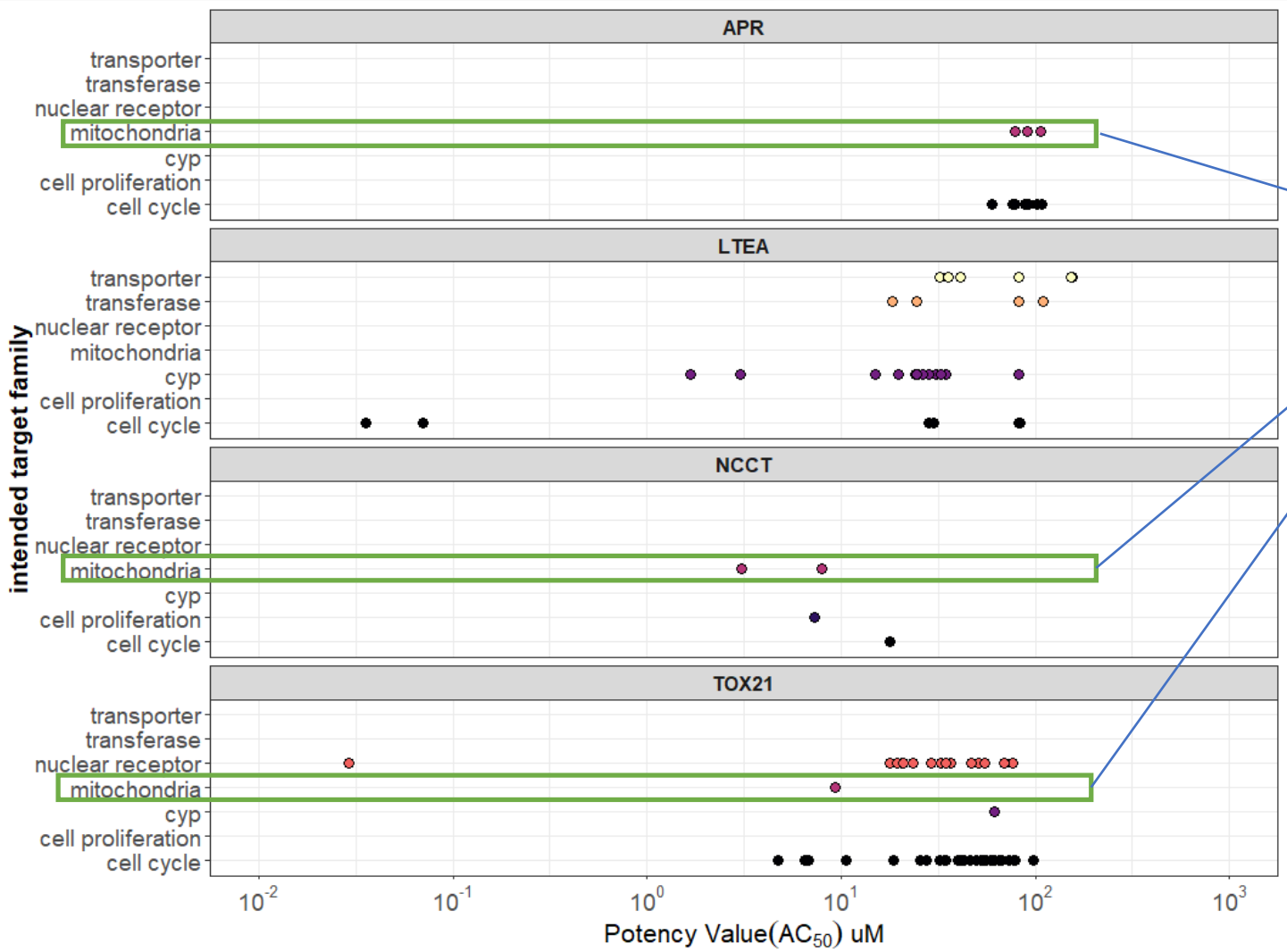
Each assay platform or source can be a surrogate for one or more collections of AOPs

Consider some of the information that might inform about liver toxicity:

- Mechanistic information on mitochondrial toxicity, oxidative stress, nuclear receptor transcription factor activity, markers of injury in liver-specific models, cell stress and cytotoxicity (inexhaustive listing here):

Biological process	Assay technologies	Details
Mitochondrial toxicity	TOX21_MMP	Mitochondrial membrane permeability (HepG2)
	NCCT_MITO	Multiple assay endpoints that measure oxygen consumption and respiration via Seahorse; can distinguish mechanism (HepG2)
	Apredica MitoMembPot	High content imaging, mitochondrial membrane permeability (HepG2)
	Apredica MitoMass	High content imaging, mitochondrial mass (HepG2)
Nuclear receptors and oxidative stress	ATG	Transcription factor activity, including nuclear receptor and cell stress panel (CIS by endogenous expression and TRANS by GAL4-NR receptor modules); HG19 subclone of HepG2 cells (for elevated metabolism)
	LTEA	mRNA expression in HepaRG for nuclear-receptor regulated metabolism/oxidative stress
	CLD	mRNA expression in sandwich-cultured primary human hepatocytes for Phase I-II metabolism and transport
	Tox21 NR assays	LUC and BLA nuclear receptor reporter assays
	NVS NR and transporter assays	Cell-free binding
	Odyssey Thera	Receptor complexes and stabilization of coactivator interaction
Cell stress and cytotoxicity	Viability and cell stress assays across platforms	88+ assays

Looking for consistency in MOA and concentration ranges (this is just a subset of assay technologies for demonstration)



Mitochondria:
Consistency in MOA
Concentration ranges by technology; the NCCT Seahorse technology suggests 1-10 uM, similar to Tox21 MMP assay

Liver:
Clearly CYPs, Phase II transferases, and nuclear receptor interactions occuring
May occur at concentrations greater than mitochondria or cell cycle bioactivity

Consider reviewing the curves more specifically for a single chemical weight-of-evidence.

Mystery substance A: brief consideration of weight of evidence

- 282/919 assays active: high hit-rate; consider that ToxCast contains a focus on NR-related processes, cell stress, and liver.
- Mitochondrial endpoint notes:
  - NCCT_MITO positive, suggests decrease in basal oxygen consumption and max respiration – indicative of Complex I inhibition (~3-7 uM)
  - TOX21 MMP assay positive (~9 uM)
  - APR_HepG2 mito assays – several positive – much higher concentrations (50 uM+).
  - Cytotoxicity limit is estimated at ~12 uM.
- Liver/cell stress endpoints:
  - LTEA
    - LDH assay in LTEA system suggests AC50 ~83 uM.
    - Effects on multiple transporters in LTEA (BSEP, MRP3, MRP2, OCT1, OATP1B1,etc.) (20-40 uM)
    - Effects on multiple Phase I enzyme expression inc CYP3A, CYP4A in LTEA (20-40 uM)
    - Acox1 expression altered in LTEA (suggests hepatic mitochondrial activity altered), along with other indicators of stress/apoptosis (BAX/BCL2-like 11) (~60+ uM)
    - Multiple inflammatory markers upregulated in LTEA and BSK
    - It is difficult to discern if effects on mitochondria and cell cycle precede or coincide with effects on Phase I-II metabolism and transport.
  - TOX21 and ATG suggest consistent PPAR activity (gamma), possibly PXR, GR, and other nuclear receptors (ToxCast AR model is equivocal).

Mystery substance A: revealed

- Troglitazone
- Treatment for Type II diabetes, works primarily by activating PPARγ
  - Also involved in immune response via decrease in NF-KB
- Drug removed from market due to DILI, with several proposed mechanisms, including:
  - Mitochondrial toxicity [Electron transport chain inhibitor (Complex I) at low micromolar concentrations]
  - Inhibits of bile acid transport/cholestatic effects (e.g., BSEP)
  - Apoptosis
  - Formation of reactive metabolites/oxidative stress

Vignette two: Screening-level endocrine bioactivity assessment

Evaluate mystery compound B for endocrine bioactivity risk

Examine physicochemical properties such as logP, vapor pressure, and MW to get a better sense of whether the chemical was suitable for the current *in vitro* assay suite

Analytical chemistry: was the chemical present and in the DOA for current ToxCast?

ToxCast negatives: what does a negative mean? Outside of domain of applicability (DOA)?

EXECUTIVE SUMMARY
PROPERTIES
ENV. FATE/TRANSPORT
HAZARD
ADME
EXPOSURE
BIOACTIVITY
TOXCAST: SUMMARY
EDSP21
TOXCAST/TOX21
PUBCHEM
TOXCAST: MODELS
SIMILAR COMPOUNDS
GENRA (BETA)
RELATED SUBSTANCES
SYNONYMS
LITERATURE
LINKS

Summary

Download | Columns | Search query

Property	Experimental average	Predicted average	Experimental median	Predicted median	Experimental range	Predicted range	Unit
LogP: Octanol-Water	3.32 (1)	3.29		3.43	3.32	2.40 to 3.64	-
Melting Point	155 (7)	139	156	138	153 to 156	125 to 157	°C
Boiling Point	200 (1)	363		360	200	343 to 401	°C
Water Solubility	5.26e-4 (1)	9.62e-4		1.00e-3	5.26e-4	5.35e-4 to 1.31e-3	mol/L
Vapor Pressure	-	8.37e-7		3.43e-7	-	6.83e-8 to 2.59e-6	mmHg
Flash Point	-	190		190	-	188 to 192	°C
Surface Tension	-	46.0			-	46.0	dyn/cm
Index of Refraction	-	1.60			-	1.60	-
Molar Refractivity	-	68.2			-	68.2	cm^3
Polarizability	-	27.0			-	27.0	Å^3
Density	-	1.17		1.17	-	1.14 to 1.20	g/cm^3
Molar Volume	-	200			-	200	cm^3
Thermal Conductivity	-	150			-	150	mW/(m*K)
Viscosity	-	9.66			-	9.66	cP
Henry's Law	-	1.26e-7			-	1.26e-7	atm-m3/mole
LogKoa: Octanol-Air	-	8.38			-	8.38	-

16 records

Many successfully screened chemicals have been (but not limited to):
logP -0.4 to 5.6 range; MW 180-480;
log10 Vapor Pressure < 1.

Available QC data suggests that the substance is present in DMSO sample and stable over 4 months

Analytical chemistry: was the chemical present and in the DOA for current ToxCast?

Representative samples that were analyzed (the chemical in DMSO stock) are high purity and confirmed

PROPERTIES
ENV. FATE/TRANSPORT
HAZARD
ADME
EXPOSURE
BIOACTIVITY
TOXCAST: SUMMARY
EDSP21
TOXCAST/TOX21

QC Data ID	Grade	Description
Tox21_202992	Pass	Purity>90% and MW confirmed
Tox21_400088	Pass	Purity>90% and MW confirmed

Assay Selection **0 Selected**

Active Inactive All

Filter assays

Odyssey Thera **(0 of 17 selected)**
Attagene **(0 of 165 selected)**
CellzDirect **(0 of 48 selected)**
Bioseek **(0 of 174 selected)**
Apredica **(0 of 108 selected)**

A Single Assay Can Have Multiple Charts **Representative Samples Only** Bioactivity Summary **Number of Charts: 0**

Select one or more assays from the list of assays to view the associated bioactivity curves

Structure Search Search...

QC Grade

T0	A	MW Confirmed, Purity > 90%
T4	A	MW Confirmed, Purity > 90%

Identifiers

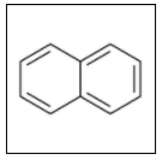
Tox21	Tox21_202992
NCATS	NCGC00260537-01
CAS	redacted
PubChem	144210190

- Active research is ongoing to better surface an integrated analysis of analytic sample QC.
- Not all QC data is currently displayed – but failures noted in the tripod site can indicate a possible problem with the representative sample (e.g., degradation).

What is an example of a substance that QC might tip us off we need different NAMs from what is currently in ToxCast?

EPA United States Environmental Protection Agency

EPA United States Environmental Protection Agency | Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Copy | Share | Submit Comment | Search all data



Naphthalene

91-20-3 | DTXSID8020913

Searched by DSSTox Substance Id.

- DETAILS
- EXECUTIVE SUMMARY
- PROPERTIES
- ENV. FATE/TRANSPORT
- HAZARD
- SAFETY
- ADME
- EXPOSURE
- **BIOACTIVITY**
  - TOXCAST: SUMMARY
  - EDSP21
  - **TOXCAST/TOX21**

ToxCast/Tox21

QC Data ID	Grade	Description
Tox21_111023	Caution	No sample detected
Tox21_202004	Caution	No sample detected
Tox21_300008	Caution	No sample detected

Assay Selection **0 Selected**

☐ Active ☐ Inactive ☐ All

Filter assays

Bioseek **(0 of 174 selected)**

University of Pittsburgh Johnston La…

Tanguay Lab **(0 of 19 selected)**

A Single Assay Can Have Multiple Charts ☑ **Representative Samples Only** Bioactivity Summary **Number of Charts: 0**

Select one or more assays from the list of assays to view the associated bioactivity curves

Mystery substance B: Models >>> single assays. And equivocals happen.

Models available?

Mystery substance B has positive ToxCast ER pathway agonist and ToxCast AR antagonist scores.

- ENV. FATE/TRANSPORT
- HAZARD
- ADME
- EXPOSURE
- BIOACTIVITY
  - TOXCAST: SUMMARY
  - EDSP21
  - TOXCAST/TOX21
  - PUBCHEM
  - TOXCAST: MODELS
- SIMILAR COMPOUNDS
- GENRA (BETA)

ToxCast: Models

ToxCast Model Predictions

Download ToxCast Model Predictions

>0.1 = positive; 0.001-0.1 = equivocal

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
COMPARA (Consensus)	Androgen	Inactive	Active	Active
CERAPP Potency Level (From Literature)	Estrogen	Active (Weak)	-	Active (Weak)
CERAPP Potency Level (Consensus)	Estrogen	Active (Weak)	Active (Strong)	Active (Weak)

CERAPP = consensus ER QSAR (from 17 groups)
COMPARA = consensus AR QSAR
ToxCast Pathway Model AUC ER = full ER model (18 assays)
ToxCast Pathway Model AUC AR = full AR model (11 assays)

As of now, the models supported in the CompTox Chemicals Dashboard are endocrine-related but hope to expand to other published models in the future.
Consult the peer-reviewed literature for additional models and interpretations.

HT-H295R model for steroidogenesis

Endocrine models available?

OXFORD

S

ww

High-Thr

as an Alte

Character

Derik E. Hagg

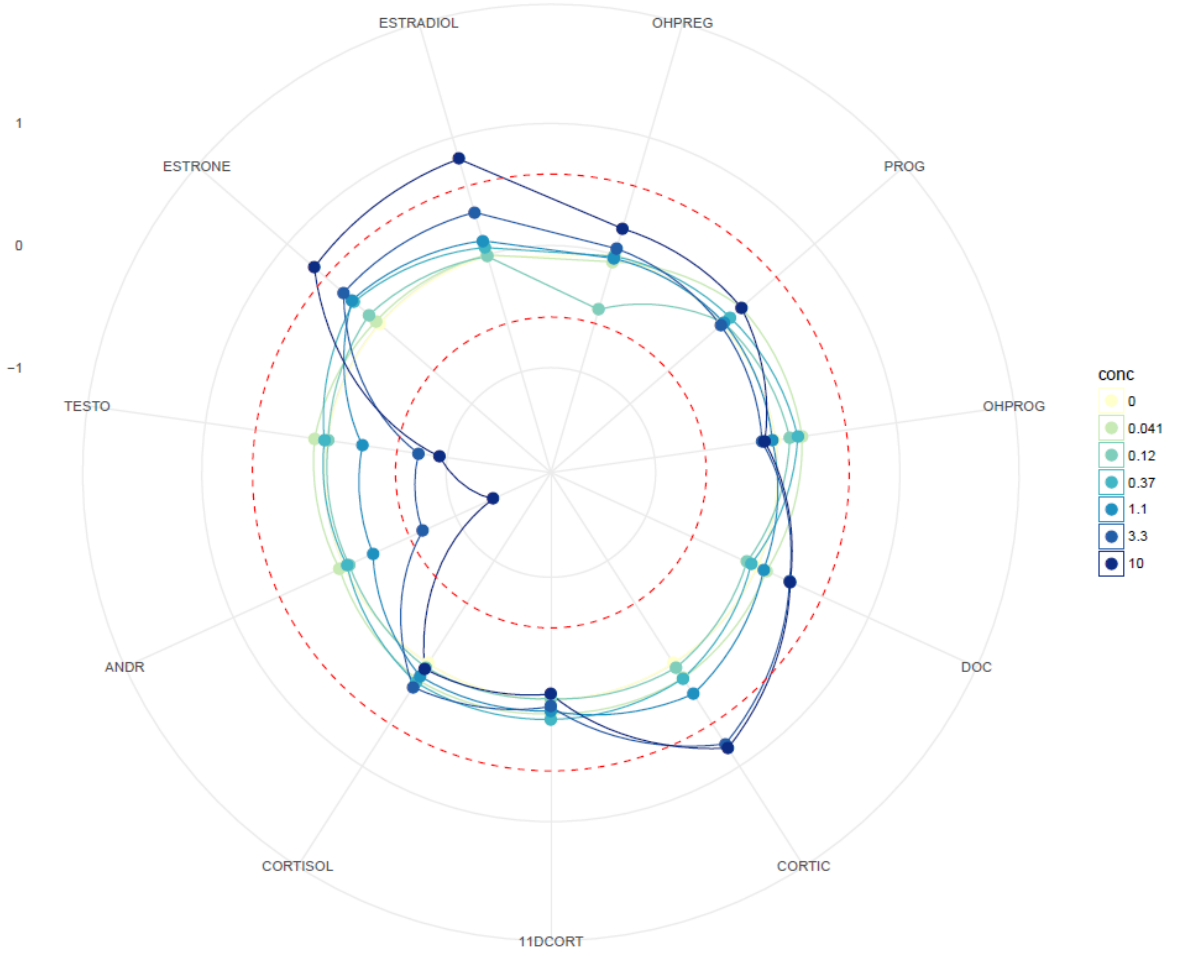
Richard S. Juc

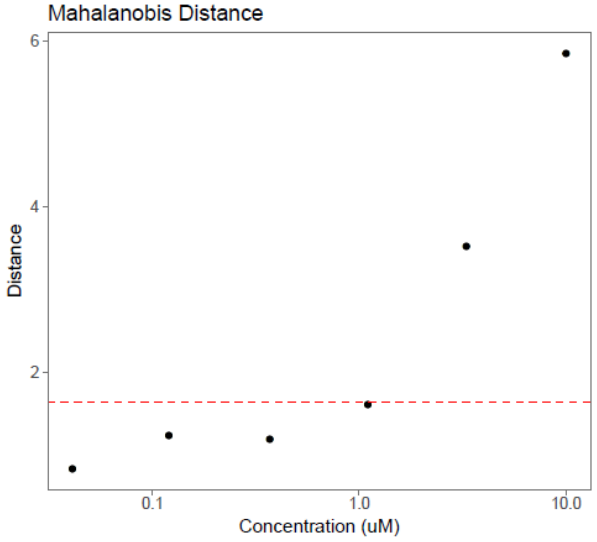
*Oak Ridge Institute

Center for Computa

Agency, Durham, N

TOXICOLOGICAL SCIENCES, 162(2), 2018, 509–534

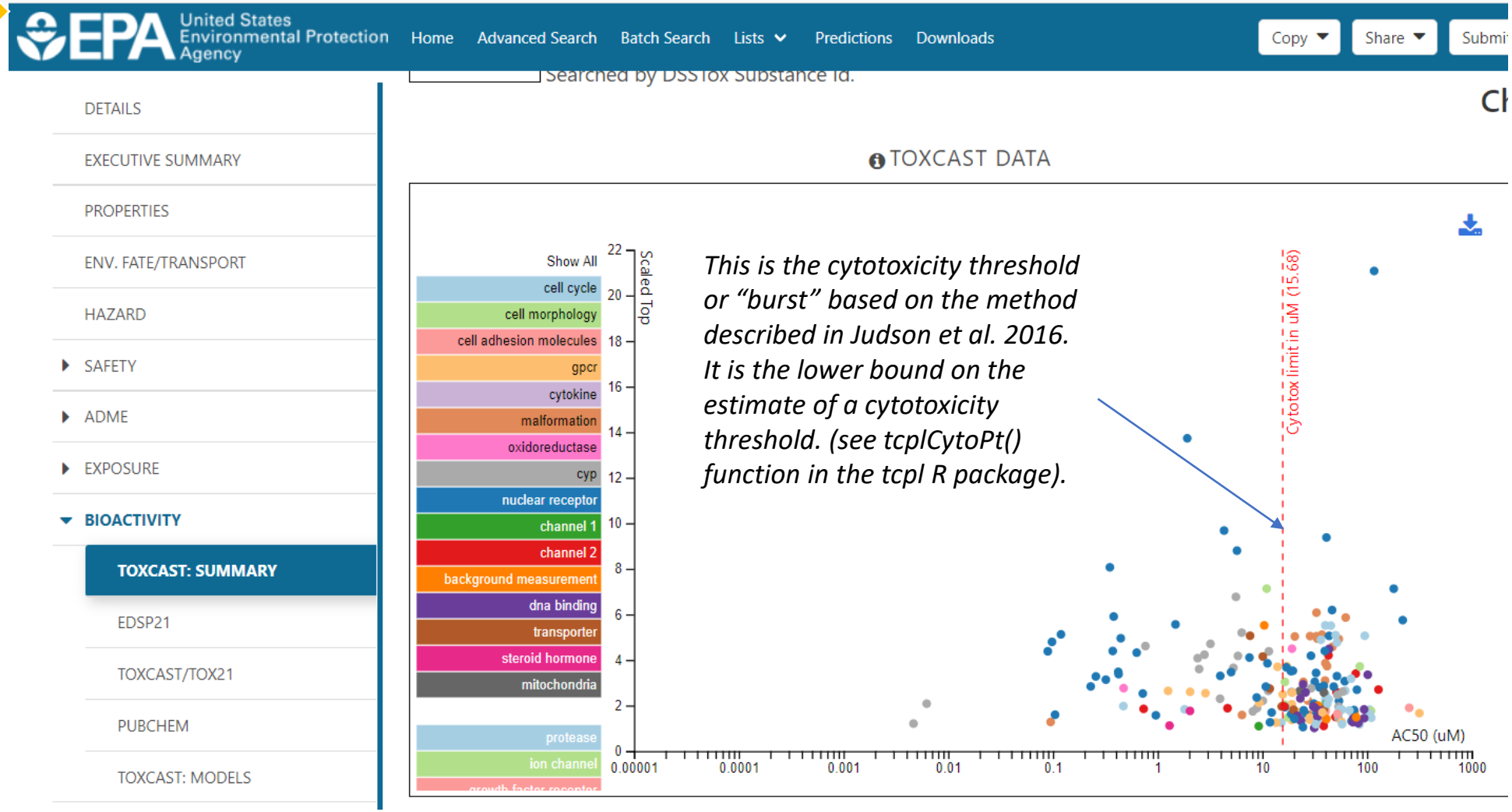




- Supplemental File 4 has fold-change by hormone
- Supplemental File 9 has mMd (model values)
- Invitrodb v3.2 has a hth295r model table with both of these included in it.
- Hope to include this in future release of the Dashboard.

Bioactivity summary in the Dashboard

Selective or non-selective?



The cytotoxicity “burst” is useful for context.

Selective or non-selective?

- The latest Comptox Chemicals Dashboard release (version 3.5, July 2020 release) demonstrates a cytotoxicity threshold based on the latest ToxCast database (invitrodb version 3.3, released Aug 2020). This value can change as more cytotoxicity data become available, curve-fitting approaches for existing data change, or the “burst” calculation approach is updated.
- In invitrodb version 3.3, 88 assays are considered for the cytotoxicity threshold. A positive hit must be observed in 5% of these assays (noting that not all chemicals are screened in all 88 assays) in order to assign a cytotoxicity threshold. The cytotoxicity threshold is a median of AC50 potency values from the N assays with a hit. The cytotoxicity threshold visualized in the Dashboard is a lower bound on this estimate, calculated as the median cytotoxicity potency minus 3 times the global median absolute deviation.
- This is discussed further in a publication (10.1093/toxsci/kfw148) and the ToxCast Pipeline R package (tcpl) function, tcplCytoPt() (available on CRAN: https://cran.r-project.org/web/packages/tcpl/index.html).
- If fewer than 5 cytotoxicity assays demonstrate a positive hit, a default of 1000 micromolar is assigned for the chemical.
- The lower bound estimate of the cytotoxicity threshold or “burst” is useful context for ToxCast results. Bioactivity observed below the cytotoxicity threshold may represent more specific activity that is less likely to be confounded by cytotoxicity.
- It is possible that AC50 values above the cytotoxicity threshold are informative. If an assay has a parallel cytotoxicity assay in the same cell type, that may be more informative for interpreting that assay. Or, if a result is consistent with an AOP relevant to the chemical with assay AC50 values above and below the cytotoxicity threshold, those data may be meaningful.

User application dictates “selectivity”

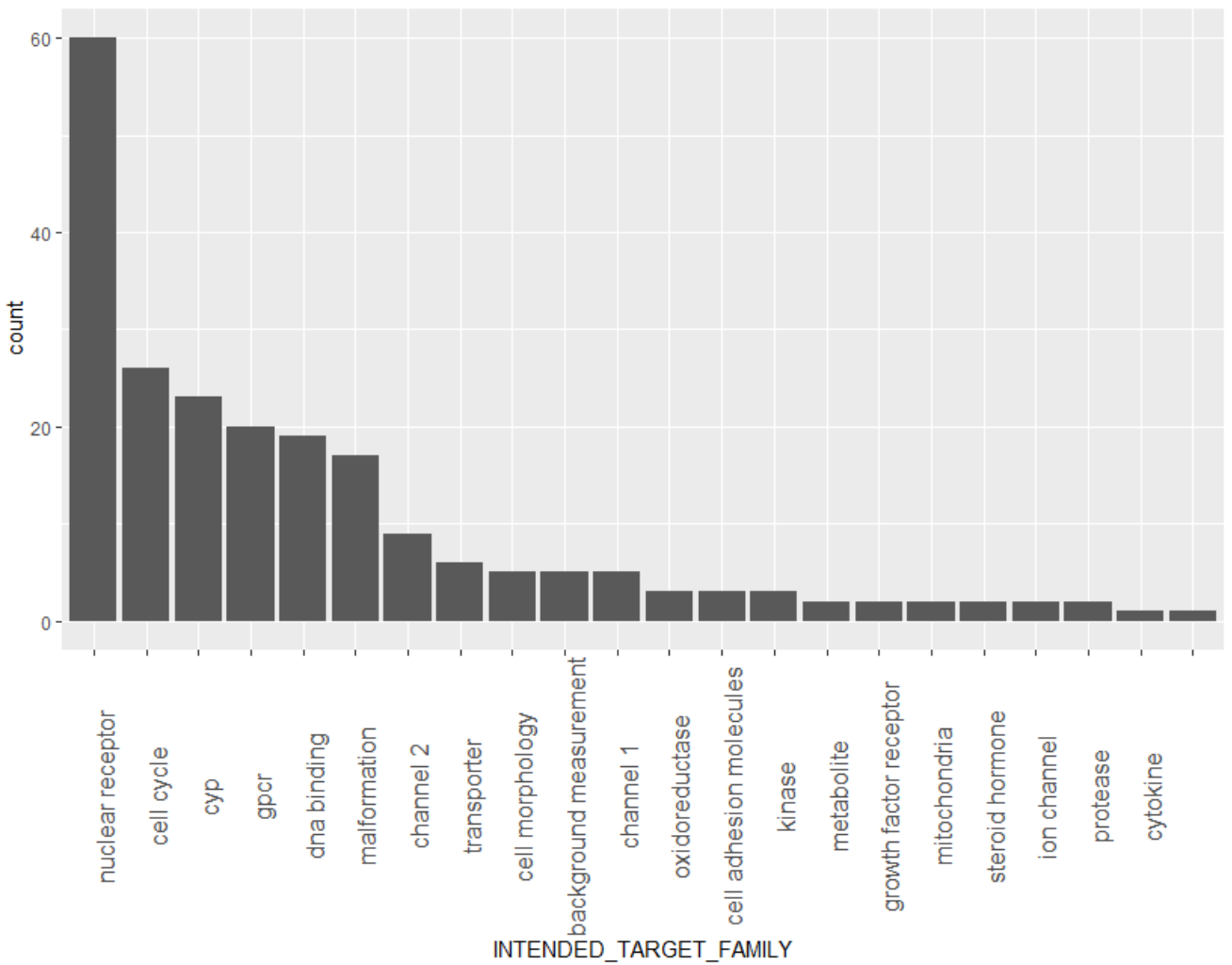
Selective or non-selective?

- AC50 < burst?
- AC50 0.5$\log_{10}$ distance from burst?
- AC50 < parallel viability assays?
- How else to filter ToxCast data: 3+ caution flags and curves with both low efficacy and potency values below the concentration range screened
- Other related ideas:
  - What other assays appear active in a similar concentration range?
  - Is there consistent support for MOA(s), or is it nonspecific activity?

A note on ToxCast versioning

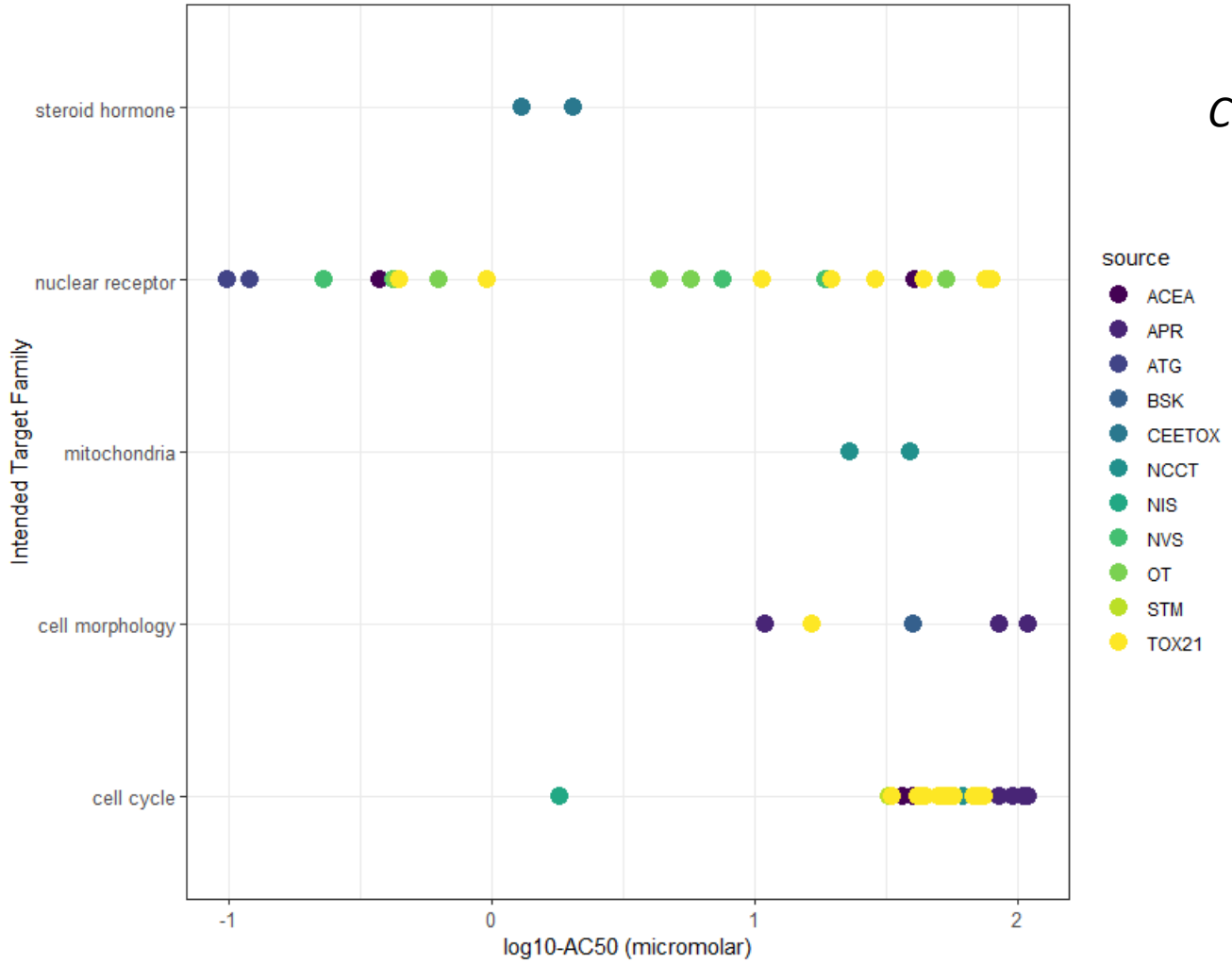
- Data change: curve-fitting, addition of new data
- Models change: improvements, more data, etc.
- The CompTox Chemicals Dashboard release from July 2020 is now using ToxCast invitrodb version 3.3: **https://doi.org/10.23645/epacomptox.6062479.v5**
- All ToxCast data and endocrine models (CERAPP, COMPARA, ER, AR, steroidogenesis) can currently be accessed from within invitrodb.
- Data downloads for NCCT: **https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data**
- We anticipate a new ToxCast release in 2021.

Mystery compound B has a lot of activity.



If endocrine bioactivity is of interest, examining some of these intended target families more closely would be helpful for understanding possible “selective” endocrine bioactivity.

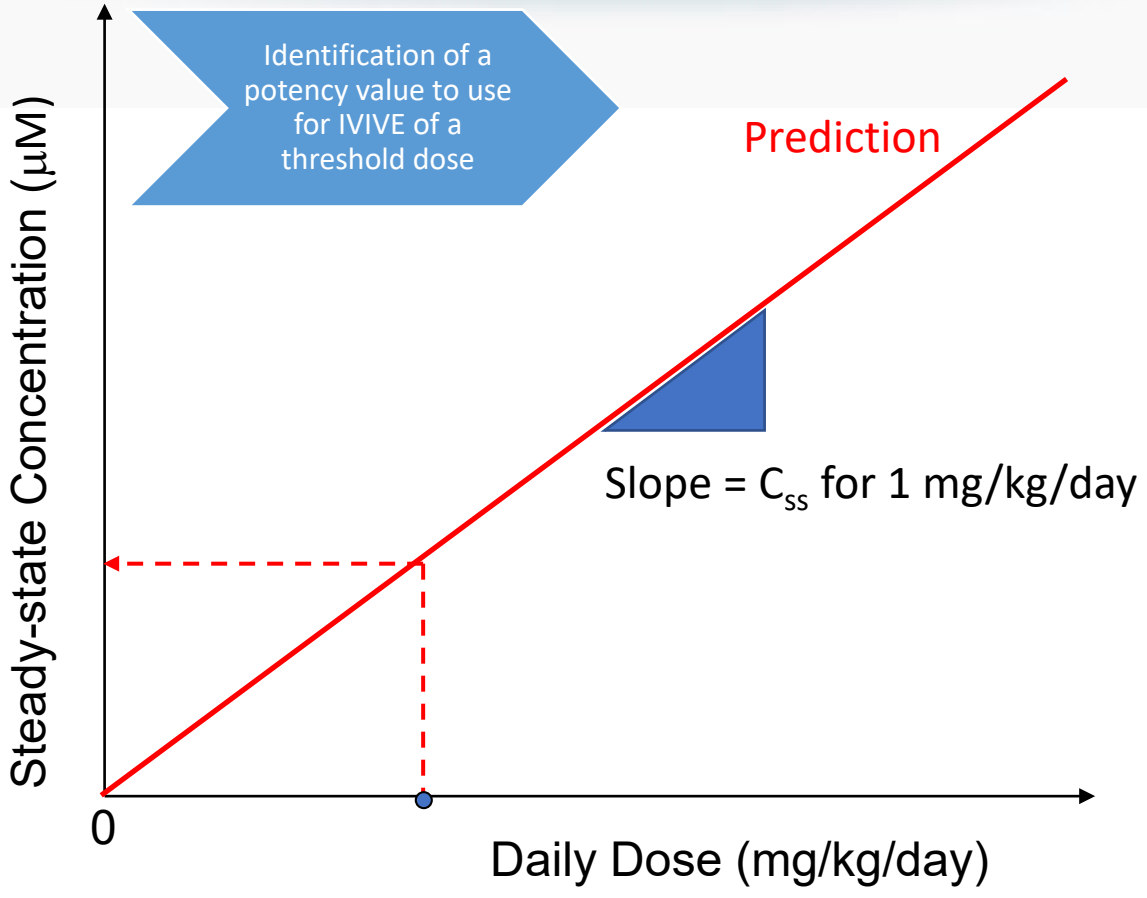
A deeper dive into the intended target family categories relevant for ER/AR activity and selectivity

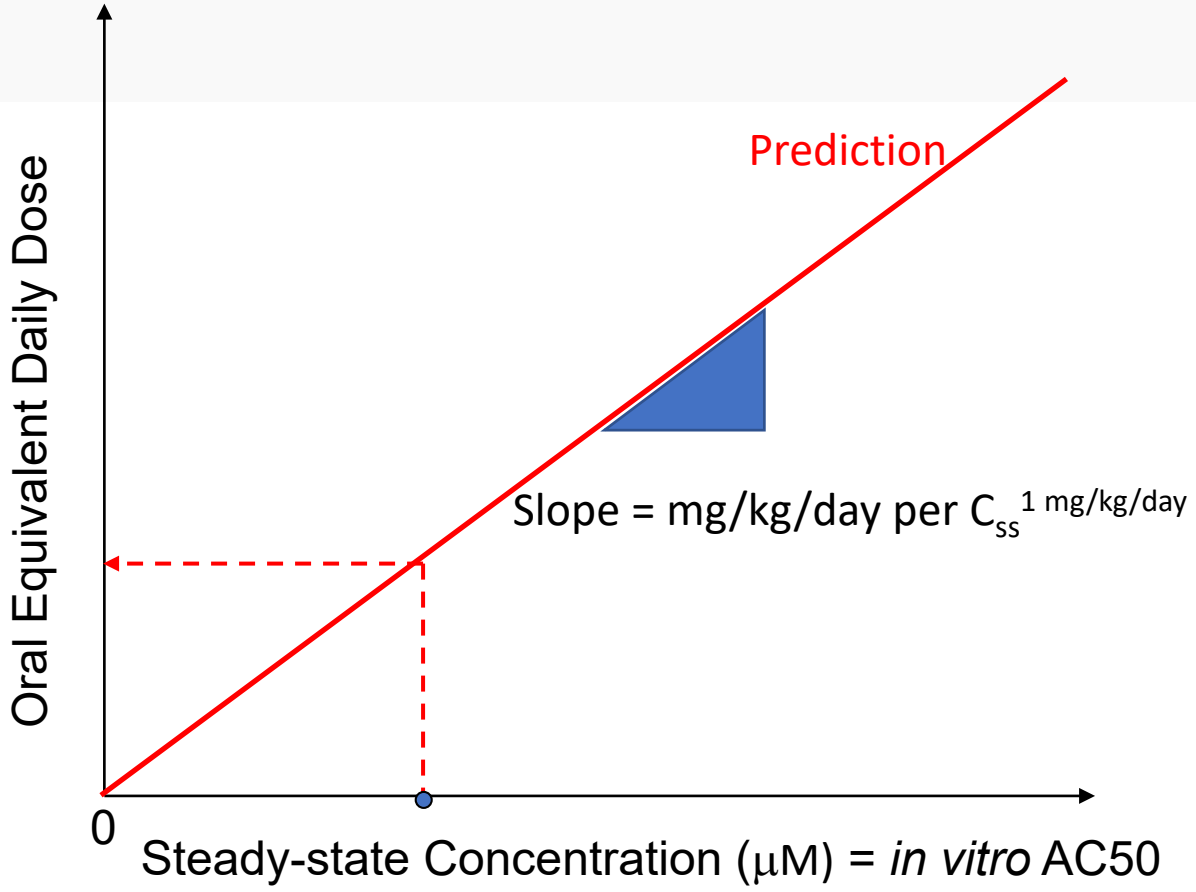


Downloaded ToxCast Summary from the CompTox Chemicals Dashboard, and filtered for one gene of interest

NAME	GENE_SYMBOL	HIT_CALL	AC50
ACEA_ER_80hr	ESR1	ACTIVE	0.373
ATG_ERE_CIS_up	ESR1	ACTIVE	9.81E-02
ATG_ERa_TRANS_up	ESR1	ACTIVE	0.119
NVS_NR_bER	ESR1	ACTIVE	0.421
NVS_NR_hER	ESR1	ACTIVE	0.23
NVS_NR_mERa	Esr1	ACTIVE	0.257
OT_ER_ERaERa_0480	ESR1	ACTIVE	5.73
OT_ER_ERaERa_1440	ESR1	ACTIVE	4.31
OT_ERa_EREGFP_0120	ESR1	ACTIVE	0.424
OT_ERa_EREGFP_0480	ESR1	ACTIVE	0.631
TOX21_ERa_BLA_Agonist_ratio	ESR1	ACTIVE	0.962
TOX21_ERa_BLA_Antagonist_ratio	ESR1	ACTIVE	43.5
TOX21_ERa_LUC_VM7_Agonist	ESR1	ACTIVE	0.445
TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2	ESR1	ACTIVE	75.1
TOX21_ERa_LUC_VM7_Agonist_10nM_ICI182780	ESR1	ACTIVE	19.6

Steady state in vitro-in vivo extrapolation assumption: blood::tissue partitioning ≈ cells::medium partitioning





$$C_{ss} = \frac{\text{oral dose rate}}{(\mathrm{GFR} * \mathrm{F_{ub}}) + \left(\mathrm{Q_l} * \mathrm{F_{ub}} * \frac{Cl_{\mathrm{int}}}{\mathrm{Q_l} + \mathrm{F_{ub}} * Cl_{\mathrm{int}}}\right)}$$

Wetmore *et al*. (2012)

- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

An IVIVE approach based reverse toxicokinetics has been developed

High-throughput toxicokinetic (HTTK) approaches make it possible to predict doses corresponding to in vitro bioactivity for thousands of chemicals.

TOXICOLOGICAL SCIENCES 125(1), 157–174 (2012)
doi:10.1093/toxsci/kfr254
Advance Access publication September 26, 2011

2012

Integration of Dosimetry, Exposure, and High-Throughput Screening Data in Chemical Toxicity Assessment

Barbara A. Wetmore,* John F. Wambaugh,† Stephen S. Ferguson,‡ Mark A. S
Kimberly Freeman,‡ Harvey J. Clewell, III,* David J. Dix,† Melvin E. Andersen,
Richard S. Judson,† Reetu Singh,* Robert J. Kavlock,† Ann M. Richard,

*The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709-2137; †United
Research and Development, National Center for Computational Toxicology, Research Triangle Park, North
Durham, North Carolina 27703; and §Department of Environmental Sciences and Engineering, U

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 142(1), 2014, 210–224
doi: 10.1093/toxsci/kfu169
Advance Access Publication Date: August 21, 2014

2014

Incorporating Population Variability and Susceptible Subpopulations into Dosimetry for High-Throughput

. Clewell, III*,
mond†, Mark A. Sochaski*,

North Carolina 27709-2137, †United States
nt, National Center for Computational
p Limited (a Certara company), Blades

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opment, National Center for Computational Toxicology,

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 147(1), 2015, 55–67
doi: 10.1093/toxsci/kfv118
Advance Access Publication Date: June 16, 2015
Research Article

2015

Toxicokinetic Triage for Environmental Chemicals

John F. Wambaugh*,1, Barbara A. Wetmore†, Robert Pearce*, Cory Strope*,‡, Rocky Goldsmith§, James P. Sluka¶, Alexander Sedykh||, Alex Tropsha||, Sieto Bosgra||, Imran Shah*, Richard Judson*, Russell S. Thomas*, R. Woodrow Setzer*

*National Center for Computational Toxicology and §National
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Education Grantee P.O. Box 117, Oak Ridge, Tennessee 37831-
Indiana University, Bloomington, Indiana 47405-7105; ||Depar
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Organisation for Applied Scientific Research (TNO), 3700 AJ Ze

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Disclaimer: The views expressed in this publication are those of the authors and do
Environmental Protection Agency. Reference to commercial products or services doe

OXFORD SOT Society of Toxicology academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 172(2), 2019, 235–251
doi: 10.1093/toxsci/kfz205
Advance Access Publication Date: September 18, 2019
Research Article

2019

Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization

John F. Wambaugh,*,1 Barbara A. Wetmore,† Caroline L. Ring,*,‡,2 Chantel I. Nicolas,*,‡,§ Robert G. Pearce,*,‡ Gregory S. Honda,*,‡ Roger Dinallo,¶ Derek Angus,¶ Jon Gilbert,¶ Teresa Sierra,¶ Akshay Badrinarayanan,¶ Bradley Snodgrass,¶ Adam Brockman,¶ Chris Strock,¶ R. Woodrow Setzer,* and Russell S. Thomas *

*National Center for Computational Toxicology; †National Exposure Research Laboratory, Office of Research and Development, U.S. EPA, Research Triangle Park, North Carolina 27711; ‡Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee 37831; §Office of Pollution Prevention and Toxics, U.S. EPA, Washington, District of Columbia 20460; and ¶Cyprotex US, LLC, Watertown, Massachusetts 02472

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2Present address: ToxStrategies, Austin, TX 78759.

Disclaimer: The views expressed in this publication are those of the authors and do not necessarily represent the views or policies of the U.S. EPA. Reference to commercial products or services does not constitute endorsement.

2017

Environmental Science & Technology

An Intuitive Approach for Predicting
with the Tox21 10k Library

Nisha S. Sipes,*,† John F. Wambaugh,‡ Robert Pearce,‡
Jui-Hua Hsieh,# Andrew J. Shapiro,† Daniel Svoboda,§ Mi

†National Toxicology Program, National Institute of Environmental Heal
Park, North Carolina 27709, United States
‡National Center for Computational Toxicology, U.S. Environmental Pro
Triangle Park, North Carolina 27711, United States
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#Kelly Government Solutions, 111 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709, United States
||National Exposure Research Laboratory, U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, Research Triangle Park,
North Carolina 27711, United States

FIFRA Scientific Advisory Panel Minutes No. 2014-03

2014

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding New High Throughput Methods to Estimate Chemical Exposure

July 29-30, 2014
FIFRA Scientific Advisory Panel Meeting
Held at the
EPA Conference Center
Arlington, VA

(2017) 44:549–565
-7

2017

CrossMa

Evaluation and calibration of high-throughput predictions of chemical distribution to tissues

Robert G. Pearce[1,2] · R. Woodrow Setzer[1] · Jimena L. Davis[1,3] · John F. Wambaugh[1]

A subset of the papers describing the development of a high-throughput toxicokinetic approach

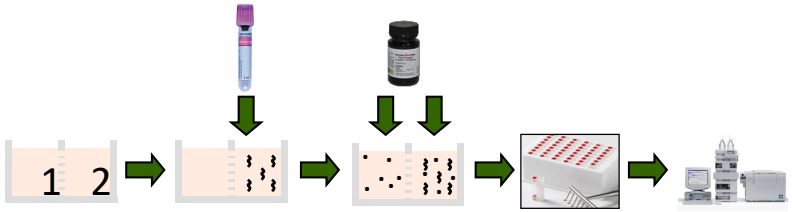
Reverse dosimetry can be leveraged in IVIVE to estimate the exposure that would produce the plasma concentration corresponding to bioactivity

High throughput toxicokinetics (HTTK)

EPA United States Environmental Protection Agency

in vitro data

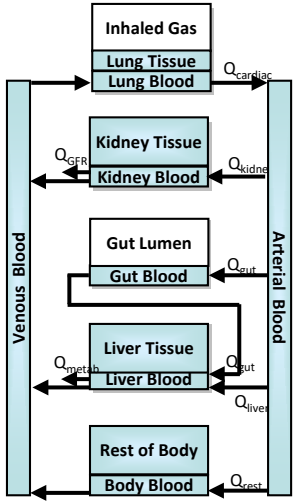
Hepatic clearance from suspended hepatocytes



Plasma protein binding

+

Generic toxicokinetic models



= ***httk***

Some high-level assumptions:

(1) bioactive nominal *in vitro* assay concentration ~ *in vivo* plasma concentration that would correspond to a similar effect;

(2) plasma concentration can be approximated by steady-state kinetics; and,

(3) external exposures (in mg/kg/day units) that may have resulted in that plasma concentration can be constructed using estimates of species-specific physiology and Phase I and Phase II enzyme-driven hepatic clearance.

Slide modified from John Wambaugh

Many works apply HTTK to prioritization and assessment case studies

EPA United States Environmental Protection Agency

Chemical Research in Toxicology — PERSPECTIVE — pubs.acs.org/crt — 2011

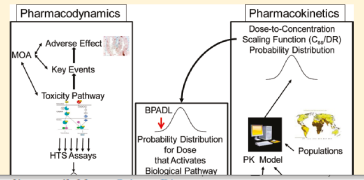
Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

Richard S. Judson,[*,†] Robert J. Kavlock,[†] R. Woodrow Setzer,[†] Elaine A. Cohen Hubal,[†] Matthew T. Martin,[†] Thomas B. Knudsen,[†] Keith A. Houck,[†] Russell S. Thomas,[‡] Barbara A. Wetmore,[‡] and David J. Dix[†]

[†]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

[‡]The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709, United States

ABSTRACT: We describe a framework for estimating the human dose at which a chemical significantly alters a biological pathway *in vivo*, making use of *in vitro* assay data and an *in vitro*-derived pharmacokinetic model, coupled with estimates of population variability and uncertainty. The quantity we calculate, the biological pathway altering dose (BPAD), is analogous to current risk assessment metrics in that it combines dose–response data with analysis of uncertainty and population variability to arrive at conservative exposure limits. The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome.



OXFORD — SOT Society of Toxicology — www.toxsci.oxfordjournals.org — 2015

TOXICOLOGICAL SCIENCES, 148(1), 2015, 121–136
doi: 10.1093/toxsci/kfv171
Advance Access Publication Date: August 6, 2015
Research Article

Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing

Barbara A. Wetmore,[*,1] John F. Wambaugh,[†] Brittany Allen,[*] Stephen S. Ferguson,[†,2] Mark A. Sochaski,[*] R. Woodrow Setzer,[†] Keith A. Houck,[†] Cory L. Strope,[*] Katherine Cantwell,[*] Richard S. Judson,[†] Edward LeCluyse,[*] Harvey J. Clewell,[*] Russell S. Thomas,[*,†,3] and Melvin E. Andersen[*]

[*]The Hamner Institutes for Health Sciences, Institute for Chemical Safety Sciences, Research Triangle Park, North Carolina 27709-2137; [†]United States Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, North Carolina 27711; and [‡]Life Technologies, ADME/Tox Division of the Primary and Stem Cell Systems Business Unit, Durham, North Carolina 27703

Toxicology in Vitro 47 (2018) 213–227
Contents lists available at ScienceDirect
ELSEVIER — 2018 — Toxicology in Vitro
journal homepage: www.elsevier.com/locate/toxinvit

Review

In vitro to in vivo extrapolation for high throughput prioritization and decision making

Shannon M. Bell[a], Xiaoqing Chang[a], John F. Wambaugh[b], David G. Allen[a], Mike Bartels[c,1], Kim L.R. Brouwer[d], Warren M. Casey[e], Neepa Choksi[a], Stephen S. Ferguson[f], Grazyna Fraczkiewicz[g], Annie M. Jarabek[b], Alice Ke[h], Annie Lumen[i], Scott G. Lynn[j], Alicia Paini[k], Paul S. Price[b], Caroline Ring[l,2], Ted W. Simon[m], Nisha S. Sipes[f], Catherine S. Sprankle[a], Judy Strickland[a], John Troutman[n], Barbara A. Wetmore[o,3], Nicole C. Kleinstreuer[e,*]

Contents lists available at ScienceDirect
ELSEVIER — 2019 — Food and Chemical Toxicology
journal homepage: www.elsevier.com/locate/foodchemtox

Profiling 58 compounds including cosmetic-relevant chemicals using ToxRefDB and ToxCast

Ly L. Pham[a,b], Lisa Truong[a,b,c], Gladys Ouedraogo[d], Sophie Loisel-Joubert[e], Matthew T. Martin[a,f], Katie Paul Friedman[a,*]

[a] National Cent
[b] ORISE Postdo
[c] Currently at G
[d] L'Oréal Safety
[e] L'Oréal Safety
[f] Currently at G

Environment International 137 (2020) 105470
Contents lists available at ScienceDirect
ELSEVIER — 2020 — Environment International
journal homepage: www.elsevier.com/locate/envint

High-throughput screening tools facilitate calculation of a combined exposure-bioactivity index for chemicals with endocrine activity

Susanna H. Wegner[a,b,*], Caroline L. Pinto[a,b], Caroline L. Ring[a,c], John F. Wambaugh[c]

[a] Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN, United States
[b] Office of Science Coordination and Policy, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC, United States
[c] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States

OXFORD — SOT Society of Toxicology — academic.oup.com/toxsci — 2019

TOXICOLOGICAL SCIENCES, 2019, 1–24
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman,[*,1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Schenki,[§] Jill A. Franzosa,[¶] Ann M. Richa
Angrish,
Bahadori
Rasenbe

Toxicology and Applied Pharmacology 387 (2020) 114774
Contents lists available at ScienceDirect
ELSEVIER — 2020 — Toxicology and Applied Pharmacology
journal homepage: www.elsevier.com/locate/taap

The role of fit-for-purpose assays within tiered testing approaches: A case study evaluating prioritized estrogen-active compounds in an in vitro human uterotrophic assay

Tyler Beames[a,*,1], Marjory Moreau[a,1], L. Avery Roberts[b], Kamel Mansouri[b], Saad Haider[a], Marci Smeltz[a], Chantel I. Nicolas[b], Daniel Doheny[b], Martin B. Phillips[a], Miyoung Yoon[b,2], Richard A. Becker[c], Patrick D. McMullen[a], Melvin E. Andersen[a], Rebecca A. Clewell[b,3], Jessica K. Hartman[a,*]

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[c] American Chemistry Council (ACC), Washington, DC 20002, USA

PLOS ONE — 2020

RESEARCH ARTICLE

Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions

Gregory S. Honda[1,2], Robert G. Pearce[1,2], Ly L. Pham[1,2], R. W. Setzer[1], Barbara A. Wetmore[3], Nisha S. Sipes[4], Jon Gilbert[5], Briana Franz[5], Russell S. Thomas[1], John F. Wambaugh[1]*

1 National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, North Carolina, United States of America, 2 Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee, United States of America, 3 National Exposure Research Laboratory, U.S. EPA, Research Triangle Park, North Carolina, United States of America, 4 Division of the National Toxicology Program, NIEHS, Research Triangle Park, North Carolina, United States of America, 5 Cyprotex, Watertown, MA, United States of America

A subset of the papers describing the application of a high-throughput toxicokinetic approach – too many to fit

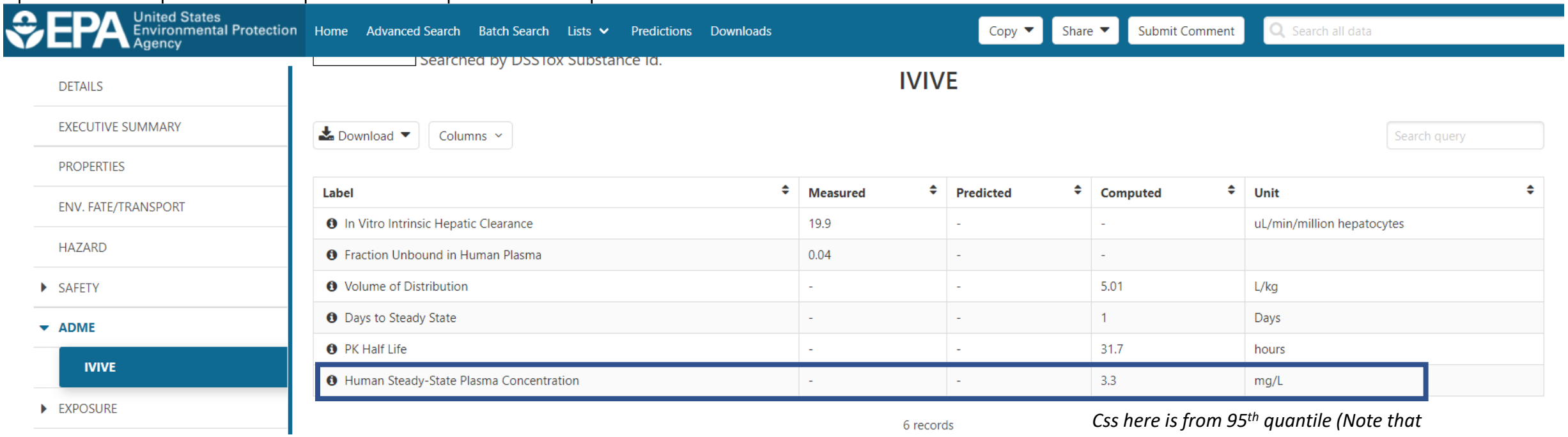
IVIVE via high-throughput toxicokinetic data and models

Identification of a potency value to use for IVIVE of a threshold dose

- Operationally, the httk R package (v 2.0.2) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs).
- AC50 or LEC (micromolar) * (1 mg/kg/day/Css (micromolar)) = AED prediction
- Httk package optionally implements multiple models that can have increasing complexity based on data available (e.g., using pbtk model or including interindividual toxicokinetic variability).

$$\frac{3.3\ \cancel{mg}}{L} \cdot \frac{\cancel{g}}{1000\ \cancel{mg}} \cdot \frac{\cancel{mol}}{228.291\ \cancel{g}} \cdot \frac{1e6\ \mu mol}{\cancel{mol}} = 14.45523\ \mu mol/L = \mu M$$

$$\frac{0.1\ \cancel{\mu M}}{} \cdot \frac{1\ mg/kg/day}{14.45523\ \cancel{\mu M}} = \mathbf{0.007\ mg/kg/day = AED95}$$



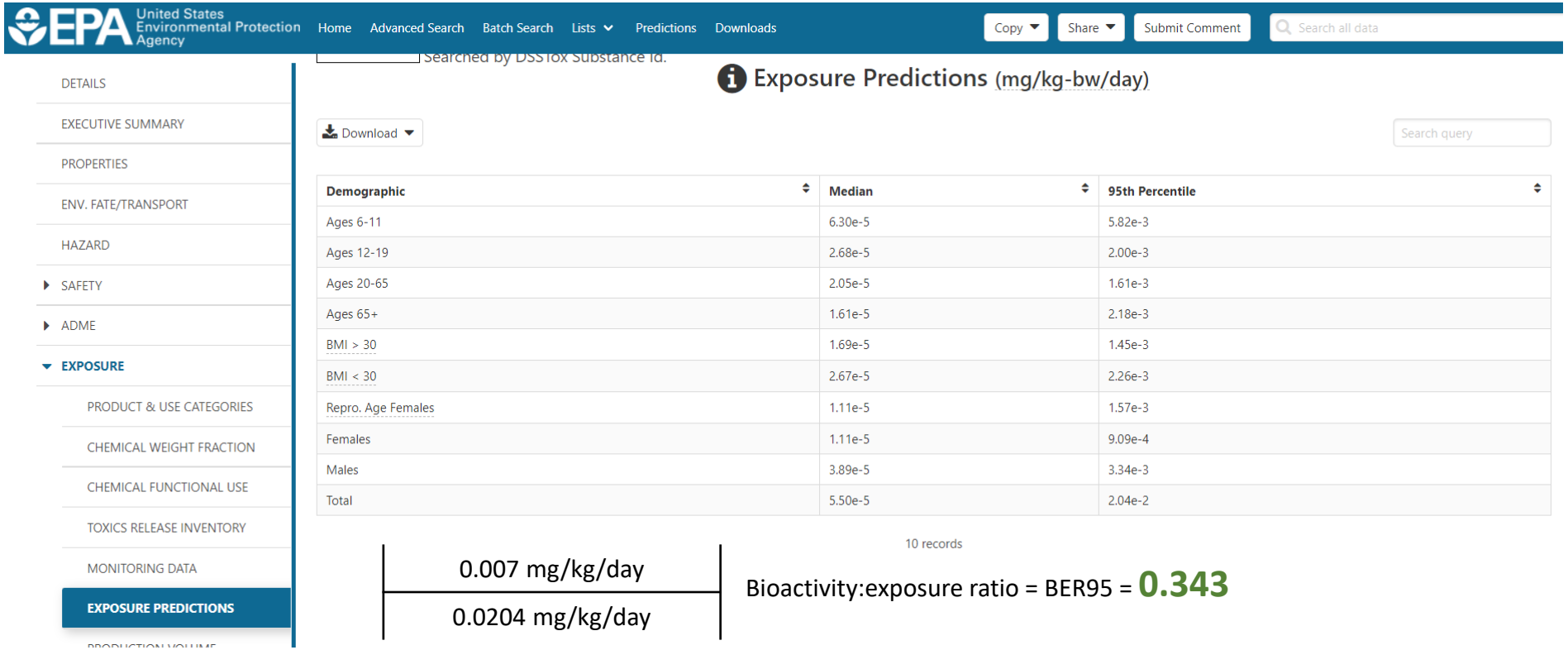
Label	Measured	Predicted	Computed	Unit
In Vitro Intrinsic Hepatic Clearance	19.9	-	-	uL/min/million hepatocytes
Fraction Unbound in Human Plasma	0.04	-	-	
Volume of Distribution	-	-	5.01	L/kg
Days to Steady State	-	-	1	Days
PK Half Life	-	-	31.7	hours
Human Steady-State Plasma Concentration	-	-	3.3	mg/L

Css here is from 95th quantile (Note that 95th concentration quantile is the same population as the 5th dose quantile).

Bioactivity:exposure ratio requires exposure

Comparison to exposure predictions for a bioactivity:exposure ratio

- Total population predictions are based upon consensus exposure model predictions and the similarity of the compound to those chemicals monitored by NHANES. The method for the total U.S. population was described in a 2018 publication, "Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways".
- When available, demographic-specific predictions are based upon a simpler, heuristic model described in the 2014 publication "High Throughput Heuristics for Prioritizing Human Exposure to Environmental Chemicals".



Demographic	Median	95th Percentile
Ages 6-11	6.30e-5	5.82e-3
Ages 12-19	2.68e-5	2.00e-3
Ages 20-65	2.05e-5	1.61e-3
Ages 65+	1.61e-5	2.18e-3
BMI > 30	1.69e-5	1.45e-3
BMI < 30	2.67e-5	2.26e-3
Repro. Age Females	1.11e-5	1.57e-3
Females	1.11e-5	9.09e-4
Males	3.89e-5	3.34e-3
Total	5.50e-5	2.04e-2

$$\frac{0.007 \text{ mg/kg/day}}{0.0204 \text{ mg/kg/day}}$$

Bioactivity:exposure ratio = BER95 = **0.343**

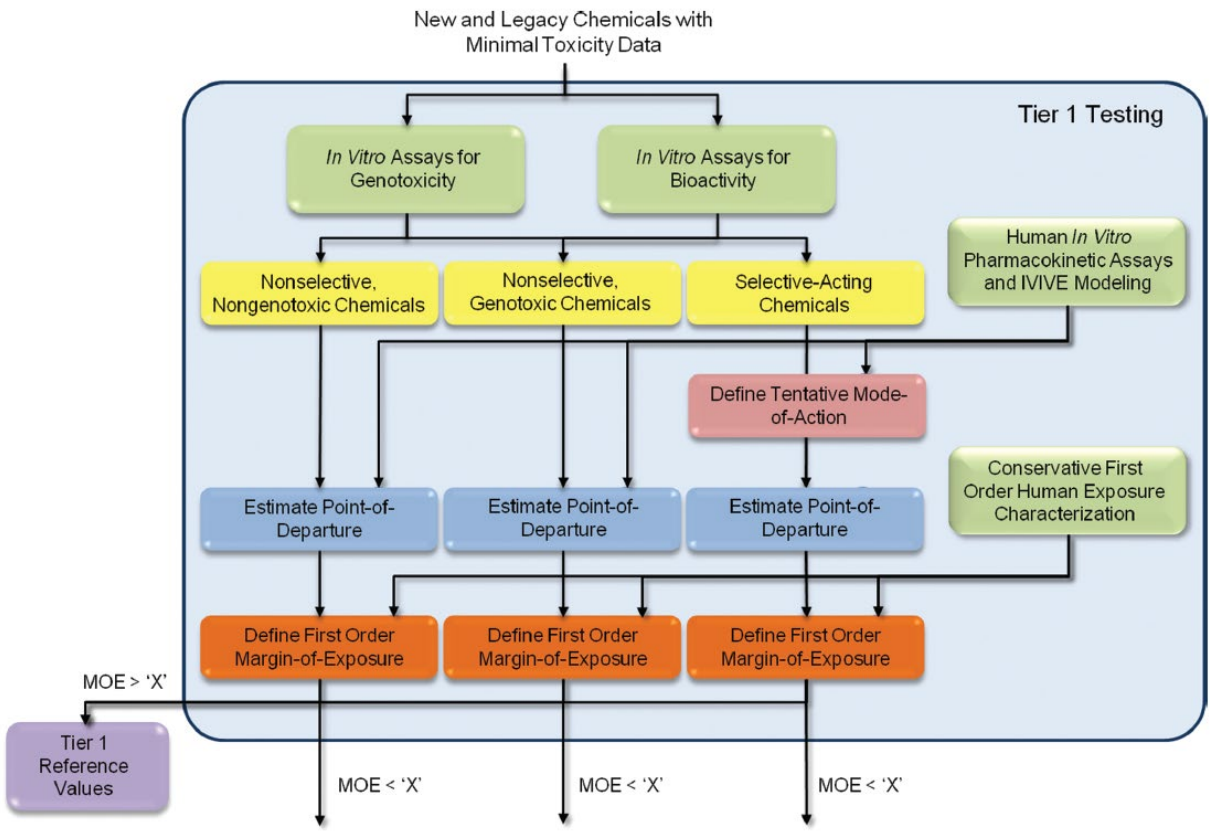
What to make of Mystery Substance B

- Mystery substance B is Bisphenol A, which clearly has some *in vitro* nuclear receptor activity at concentrations that may be below or near cytotoxicity.
  - It has moderate ToxCast ER agonist and AR antagonist scores.
  - The cytotoxicity threshold or “burst” seems to support selectivity of some nuclear receptor responses.
  - Diving a little deeper into the intended target family supports this analysis.

Use of predictive science in chemical safety should include risk-based approaches like BER

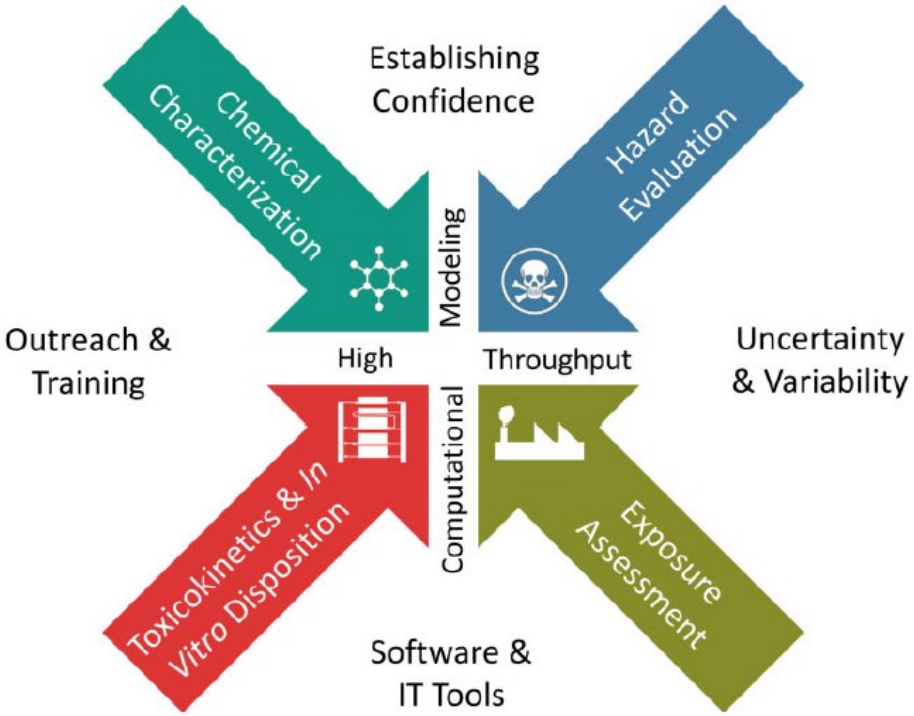
- Specific vs. nonspecific modes-of-action and the challenge of hazard labeling

Thomas et al. 2013 suggested a framework for hazard assessment that would be largely customized based on MOE (or now, BER).



Use of predictive science in chemical safety should include risk-based approaches like BER

- Now, ~6 years later, Thomas et al. (2019) suggest a computational toxicology blueprint that represents evolution of the same concept



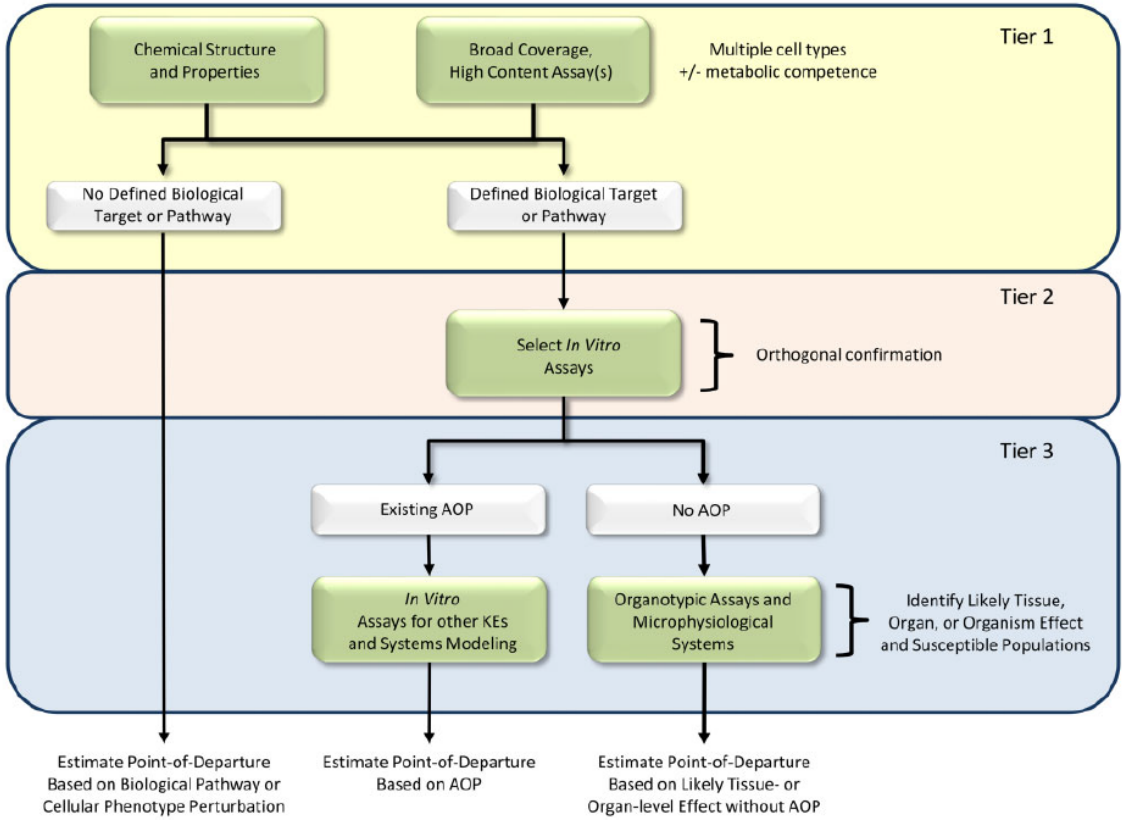


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

Screening level assessment example: combine NAMs for exposure, *in vitro* bioactivity, and toxicokinetics

- Conducted by Accelerating the Pace of Chemical Risk Assessment (APCRA)
  - *"international cooperative collaboration of government agencies convened to address barriers and opportunities for the use of new approach methodologies (NAMs) in chemical risk assessment" (Paul Friedman et al., accepted)*

OXFORD SOT Society of Toxicology academic.oup.com/toxsci

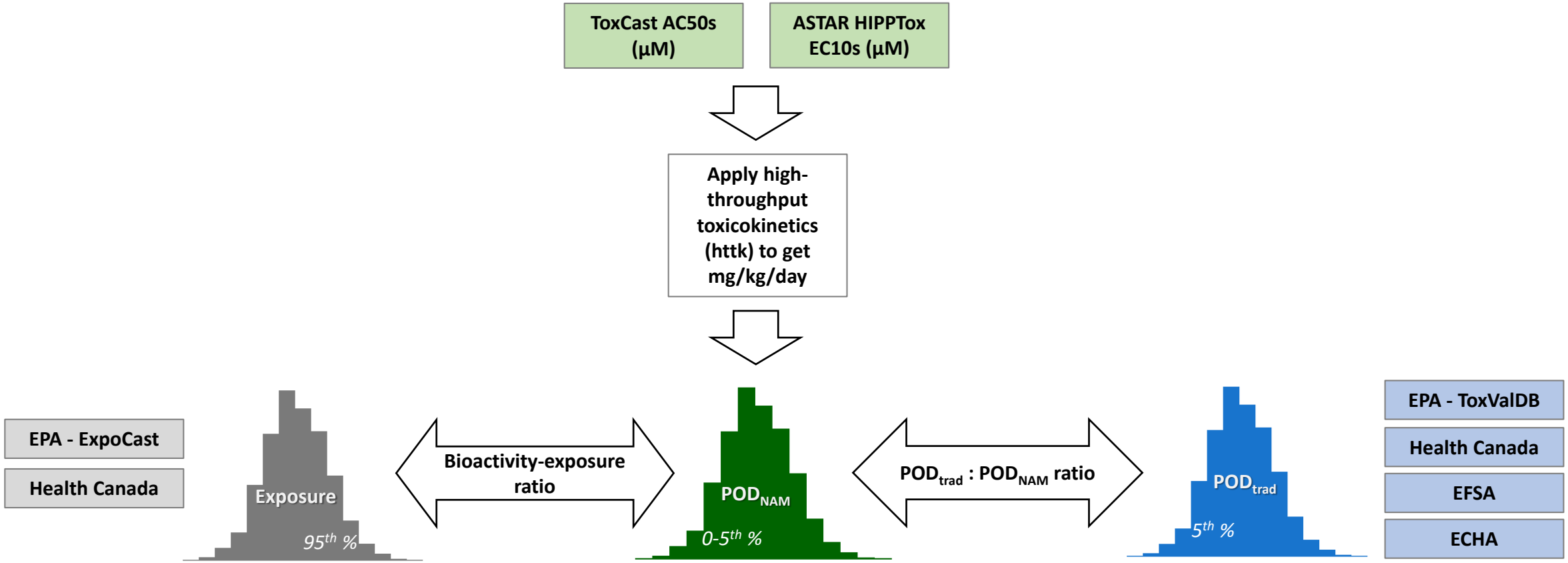
TOXICOLOGICAL SCIENCES, 2019, 1–24

doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

(APCRA partners for these two case studies)

Case study workflow

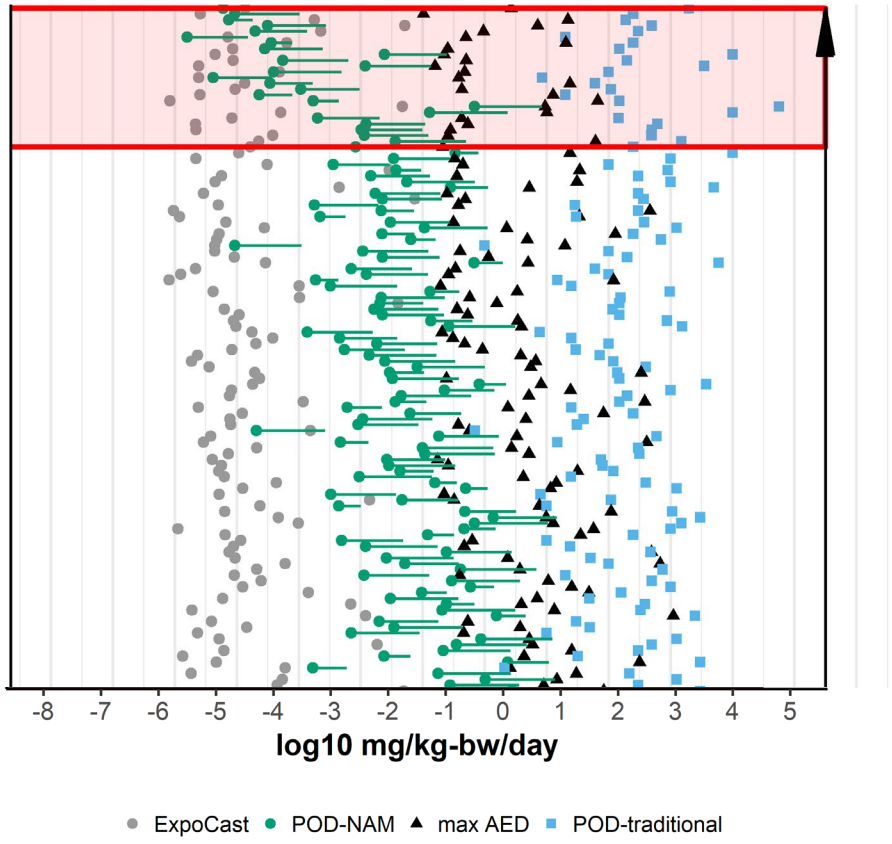


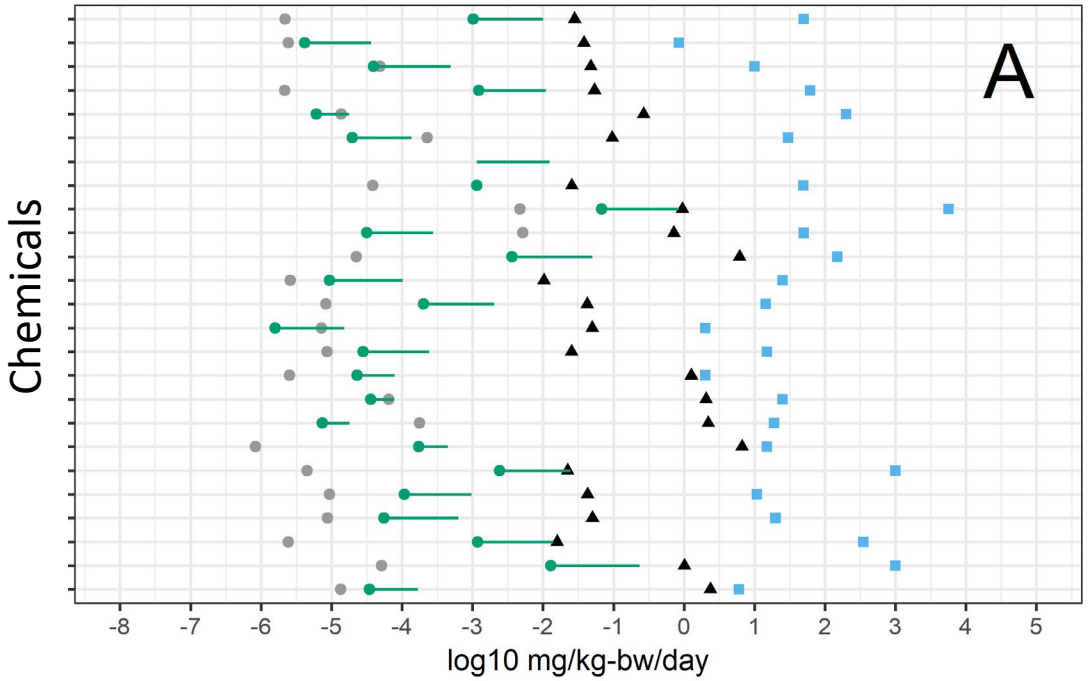
Is BER useful for prioritization?
Are there addressable weaknesses?

Is log10-POD ratio > 0 for most chemicals?
Can we learn from log10-POD ratio < 0?

- *NOEL, LOEL, NOAEL, or LOAEL*
- *Oral exposures*
- *Mg/kg/day*

Prioritize chemicals based on BER for all bioactivity or for some target bioactivity





For 448 substances, ~89% of the time, the point-of-departure based on ToxCast (POD-NAM) was less than the NOAEL/LOAEL values available from animals.

Figure 3 from Paul Friedman et al.

https://doi.org/10.1093/toxsci/kfz201

Conclusions

- Bioactivity data, including ToxCast, may help inform hazard prediction for weight-of-evidence, screening, and new approach methodologies-based points-of-departure for risk assessment.
- A high-throughput toxicokinetic approach to in vitro to in vivo extrapolation can translate bioactivity data in micromolar concentrations to administered equivalent doses for comparison to exposure or other *in vivo* data.
- The Comptox Chemicals Dashboard provides a data browsing and downloading capability to support weight-of-evidence evaluations and screening.
  - Consider that operationally, the steps taken to prepare a dataset for a single chemical weight-of-evidence evaluation may be different from preparation of a dataset for many chemicals.

Acknowledgments

- Thank you for listening.
- Thank you: Tony Williams, John Wambaugh, and Richard Judson.
- Please reach out to us if you need support or explanations for a specific case, or if you find issues.
- Paul-friedman.katie@epa.gov

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EPA United States Environmental Protection Agency

Identifying endocrine disrupting chemicals using *in vitro* and computational approaches

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Center for Computational Toxicology and Exposure, US-EPA, RTP, NC

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

Overview of this presentation

- EPA-specific catalysts for endocrine-related new approach methodologies
- Estrogen receptor and androgen receptor models
- Steroidogenesis
- Thyroid
- Ongoing research

EPA specific catalyst for endocrine new approach methodologies

- The US Environmental Protection Agency's (EPA) Endocrine Disrupting Screening Program (EDSP)
  - established in response to Congressional mandates in the Federal Food Quality Protection and Safe Water Drinking Acts
  - evaluating potential risk of endocrine disruption in humans and wildlife from exposure to pesticide chemicals and drinking water contaminants
  - recommendations from an expert advisory committee established a two tiered system
    - Tier 1 screening for *potential* to interact with the estrogen, androgen or thyroid hormone systems
    - Tier 2 testing to verify interaction and quantify dose-response relationship
  - In 2011, EPA began a multiyear transition to prioritize and screen thousands of EDSP chemicals using high-throughput *in vitro* assays and computational modeling approaches

https://www.federalregister.gov/articles/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice

FEDERAL REGISTER

The Daily Journal of the United States Government

permitted by, and in accordance with, the procedures in TSCA section 14 and 40 CFR part 2.

Burden statement: The annual public reporting and recordkeeping burden for this collection of information is estimated to average 31.5 hours per response. Burden is defined in 5 CFR 1320.3(b).

The ICR, which is available in the docket along with other related materials, provides a detailed explanation of the collection activities and the burden estimate that is only briefly summarized here:

Respondents/Affected Entities: Entities potentially affected by this ICR are companies that manufacture, process or import chemical substances, mixtures or categories.

Estimated total number of potential respondents: 1.

Frequency of response: On occasion.

Estimated total average number of responses for each respondent: 1.

Estimated total annual burden hours: 31.5 hours.

Estimated total annual costs: $2,388. This includes an estimated burden cost of $2,388 and an estimated cost of $0 for capital investment or maintenance and operational costs.

III. Are There Changes in the Estimates from the Last Approval?

There is a decrease of 916 hours in the total estimated respondent burden compared with that identified in the ICR currently approved by OMB. This decrease reflects additional both adjustment changes from a reduction in the assumed number of PAIR reports filed annually, and program changes resulting from mandatory electronic submissions of PAIR reports. In recent years (FY 2011–FY 2014), EPA has received no PAIR submissions and, for the purposes of this analysis, EPA assumes an annual rate of one submission per year. At the time OMB last renewed this ICR, EPA estimated an average of 33 reports from 14.8 submitters based on fiscal year 2006–2010 data. The ICR supporting statement provides a detailed analysis of the change in burden estimate. This change is both an adjustment and a program change.

IV. What is the Next Step in the Process for this ICR?

EPA will consider the comments received and amend the ICR as appropriate. The final ICR package will then be submitted to OMB for review

submission of the ICR to OMB and the opportunity to submit additional comments to OMB. If you have any questions about this ICR or the approval process, please contact the technical person listed under **FOR FURTHER INFORMATION CONTACT.**

Authority: 44 U.S.C. 3501 *et seq.*

Dated: June 10, 2015.

Jones,

Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

[FR Doc. 2015–14946 Filed 6–18–15; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[EPA–HQ–OPPT–2015–0305; FRL–9928–69]

Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will improve the Agency's ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a computational model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and computational models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and

DATES: Comments must be received on or before August 18, 2015.

ADDRESSES: Submit your comments, identified by docket identification (ID) number EPA–HQ–OPPT–2015–0305, by one of the following methods:

- *Federal eRulemaking Portal: http://www.regulations.gov.* Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute.
- *Mail:* Document Control Office (7407M), Office of Pollution Prevention and Toxics (OPPT), Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001.
- *Hand Delivery:* To make special arrangements for hand delivery or delivery of boxed information, please follow the instructions at *http://www.epa.gov/dockets/contacts.html.*

Additional instructions on commenting or visiting the docket, along with more information about dockets generally, is available at *http://www.epa.gov/dockets.*

FOR FURTHER INFORMATION CONTACT: *For technical information contact:* Jane Robbins, Office of Science Coordination and Policy (OSCP), Office of Chemical Safety and Pollution Prevention, Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001; telephone number: (202) 564–6625; email address: robbins.jane@*epa.gov.*

For general information contact: The TSCA-Hotline, ABVI-Goodwill, 422 South Clinton Ave., Rochester, NY 14620; telephone number: (202) 554–1404; email address: *TSCA-Hotline@epa.gov.*

SUPPLEMENTARY INFORMATION:

I. General Information

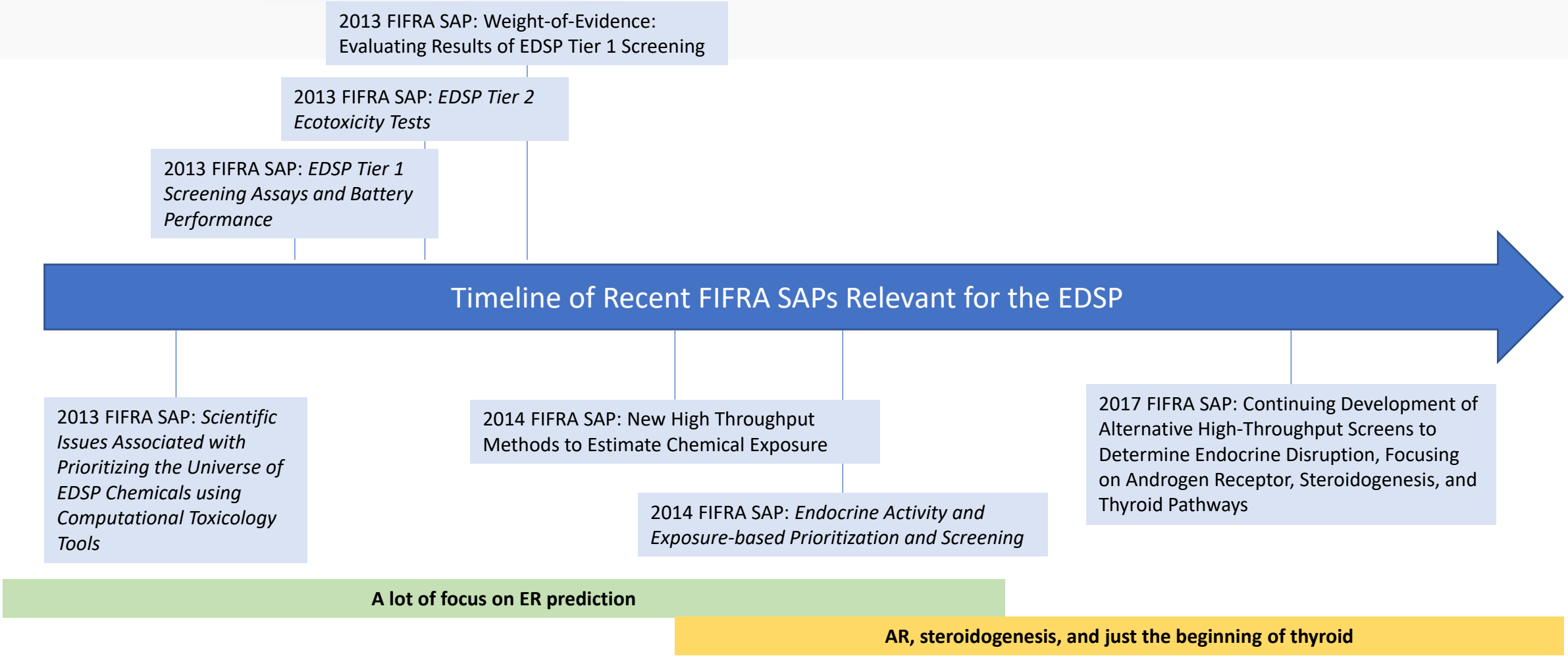
A. Does this action apply to me?

This action is directed to the public in general, and may be of interest to a wide range of stakeholders including those interested in endocrine testing of chemicals (including pesticides), and the EDSP in general. Since others also may be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action.

B. What is the agency authority for taking this action?

The EDSP is established under section 408(p) of the Federal Food, Drug and

Regulatory needs have driven a large research investment in endocrine-related bioactivity prediction

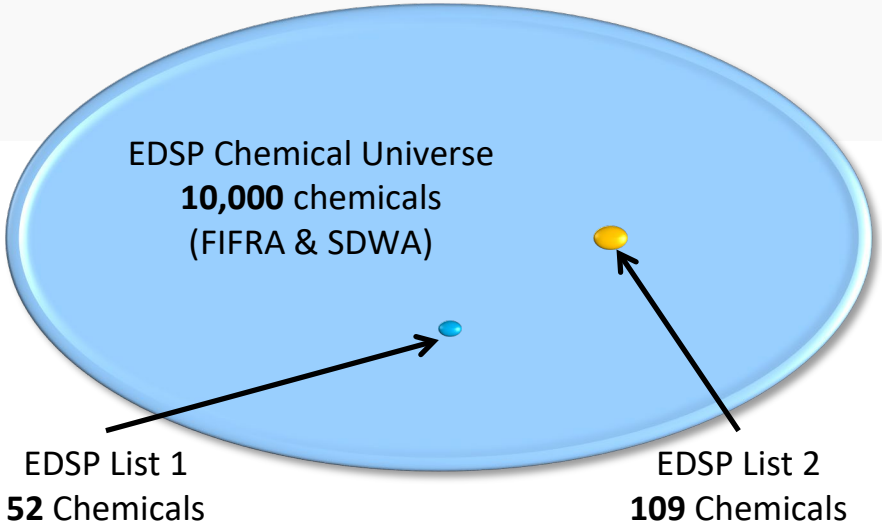


Note: *Prioritize* and *screen* have separate and distinct meanings in this context. Prioritization is a first step (think QSARs and bioactivity models with higher uncertainty). Screening is Tier 1 or Tier 1 equivalents (think the ER model as a substitute for the estrogen screens in Tier 1).

FIFRA SAP = Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel

EDSP to EDSP21

- In 2009, EPA published list of 67 pesticide chemicals (List 1) for Tier 1 screening (15 subsequently withdrawn).
- In 2013, EPA published a revised second list (List 2) of 109 chemicals for proposed Tier 1 screening.
- In 2015, EPA issued EDSP ordered additional testing on positive List 1 chemicals.
- The cost of running the Tier 1 battery is ~$1 million per chemical.
- The number of animals saved using alternative high throughput testing approach for EDSP tier 1 battery is approximately 600 animals for one chemical (~200 Rats, 80 fish and 320 frogs).
- At current rate, it would take decades and cost billions of dollars to screen all 10,000 chemicals of interest to EPA for potential endocrine activity.

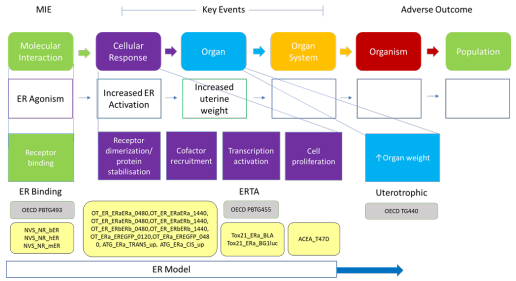


EDSP Chemical Universe List	Number
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616
TOTAL	**10,341**

Approaches for predicting estrogen and androgen receptor (ER, AR) activity

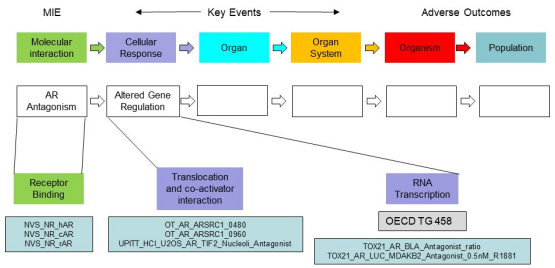
Approach using *in vitro* ToxCast data

- Developed multiple high-throughput screening assays
  - Use multiple assays per pathway
    - Different technologies
    - Different points in pathway
  - No assay is perfect
    - Assay Interference
    - Noise
- Use a systems biology model to integrate assays
  - Model creates a composite dose-response curve for each chemical to summarize results from all assays



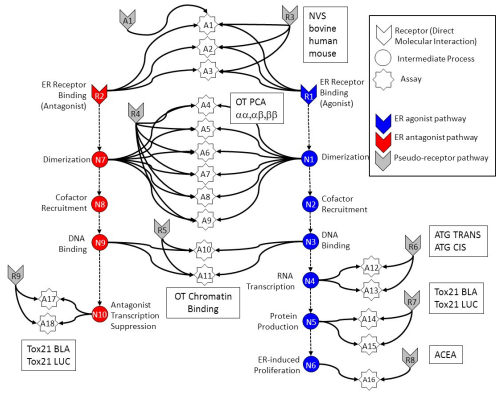
Estrogen Receptor Computational Model

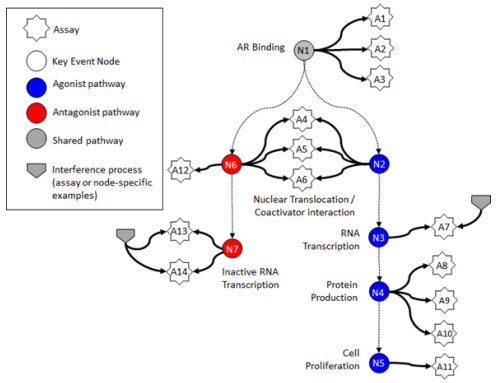
Judson et al., Envi Health Pers (2015)



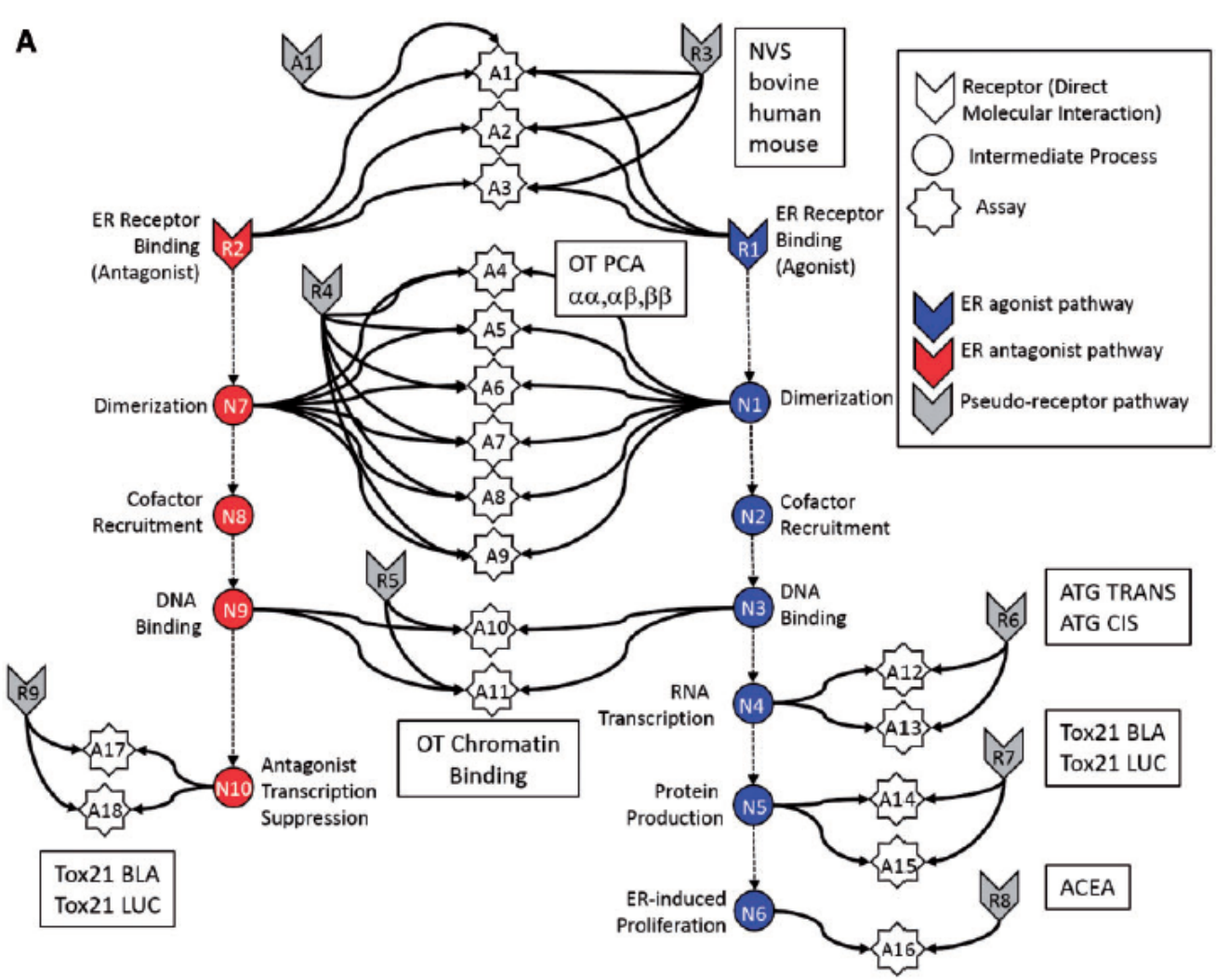
Androgen Receptor Computational Model

Kleinstreuer et al., Chem Res Toxicol (2017)





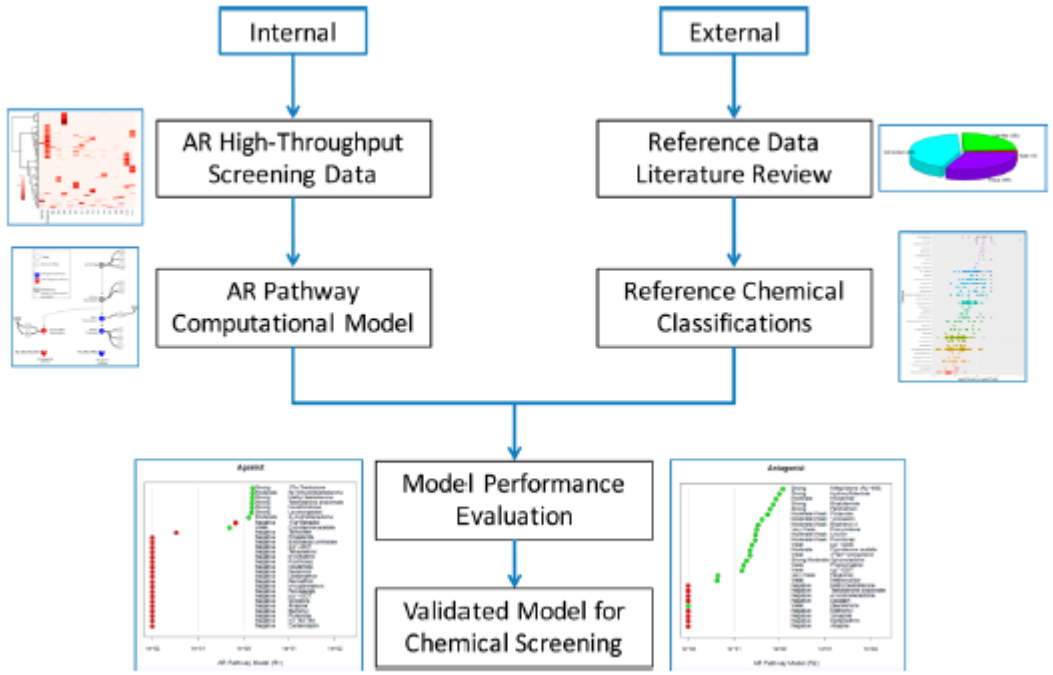
ToxCast ER model

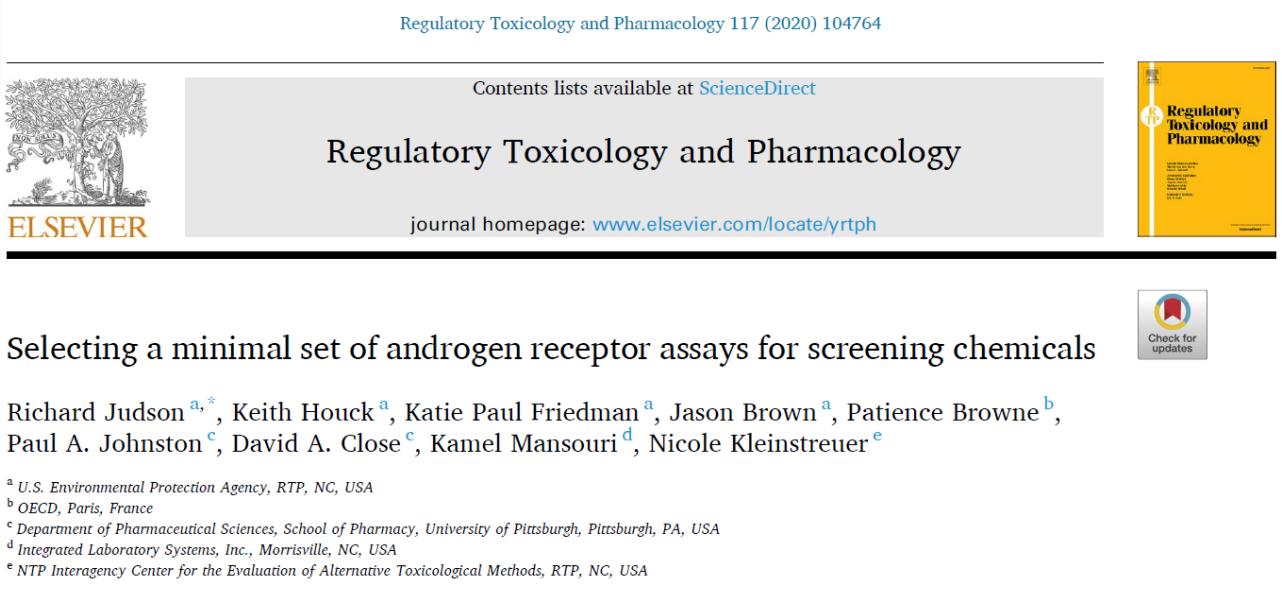


- The current model in the CompTox Chemicals Dashboard is an update of the 2015 published model but still includes all 18 assays for agonist mode.
- This model has been accepted as an alternative for the ER binding, ER-TA, and Uterotrophic assays in the EDSP Tier 1 (https://www.federalregister.gov/documents/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice).
- A newer publication describes how only 4 assays that cover key "receptors" or events in the activation of ER can achieve similar performance as the full model (10.1016/j.yrtph.2017.09.022).

https://doi.org/10.1093/toxsci/kfv168

ToxCast AR model




Regulatory Toxicology and Pharmacology 117 (2020) 104764

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Selecting a minimal set of androgen receptor assays for screening chemicals

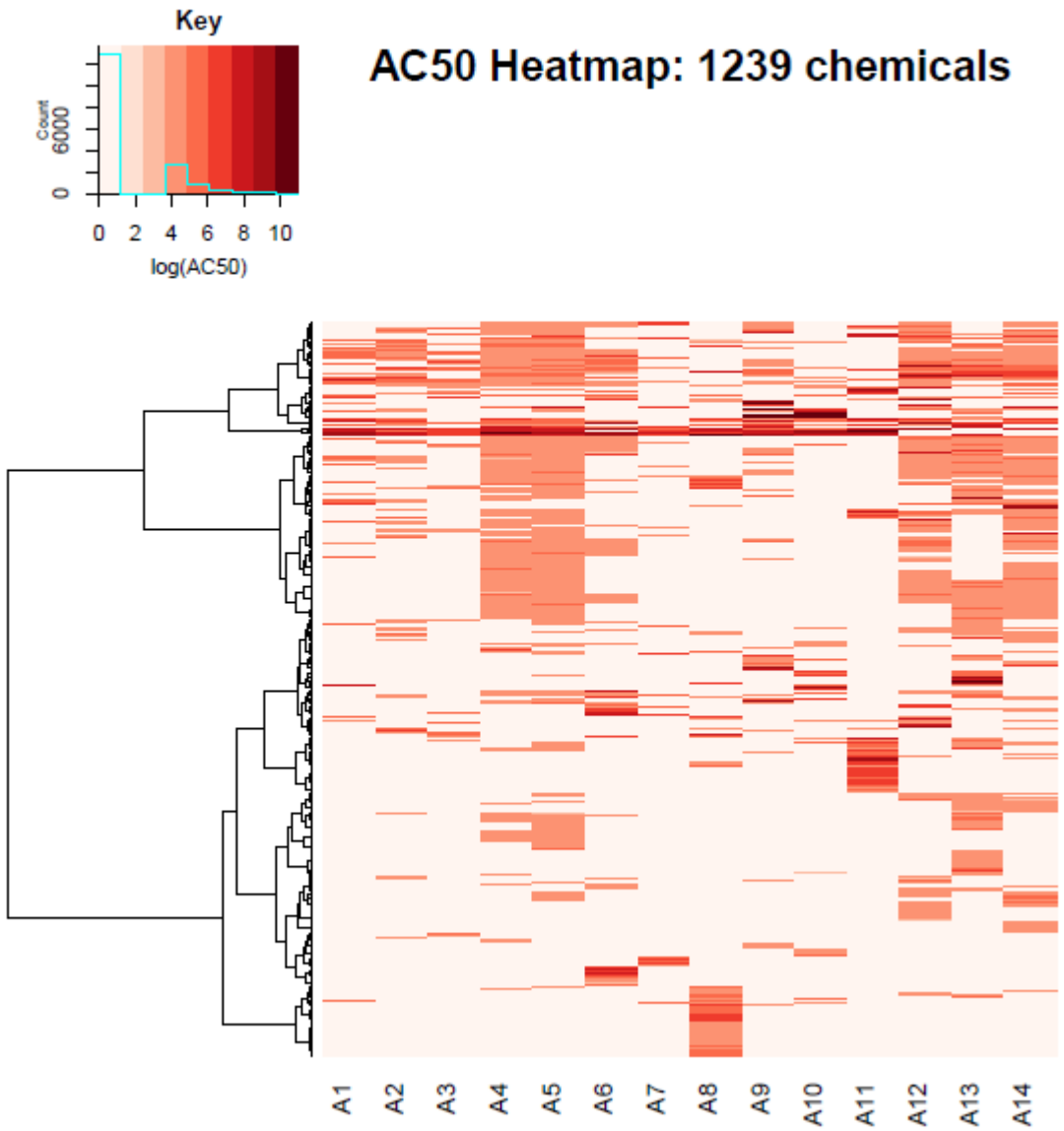
Richard Judson [a,*], Keith Houck [a], Katie Paul Friedman [a], Jason Brown [a], Patience Browne [b], Paul A. Johnston [c], David A. Close [c], Kamel Mansouri [d], Nicole Kleinstreuer [e]

[a] U.S. Environmental Protection Agency, RTP, NC, USA
[b] OECD, Paris, France
[c] Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA
[d] Integrated Laboratory Systems, Inc., Morrisville, NC, USA
[e] NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, RTP, NC, USA

10.1021/acs.chemrestox.6b00347

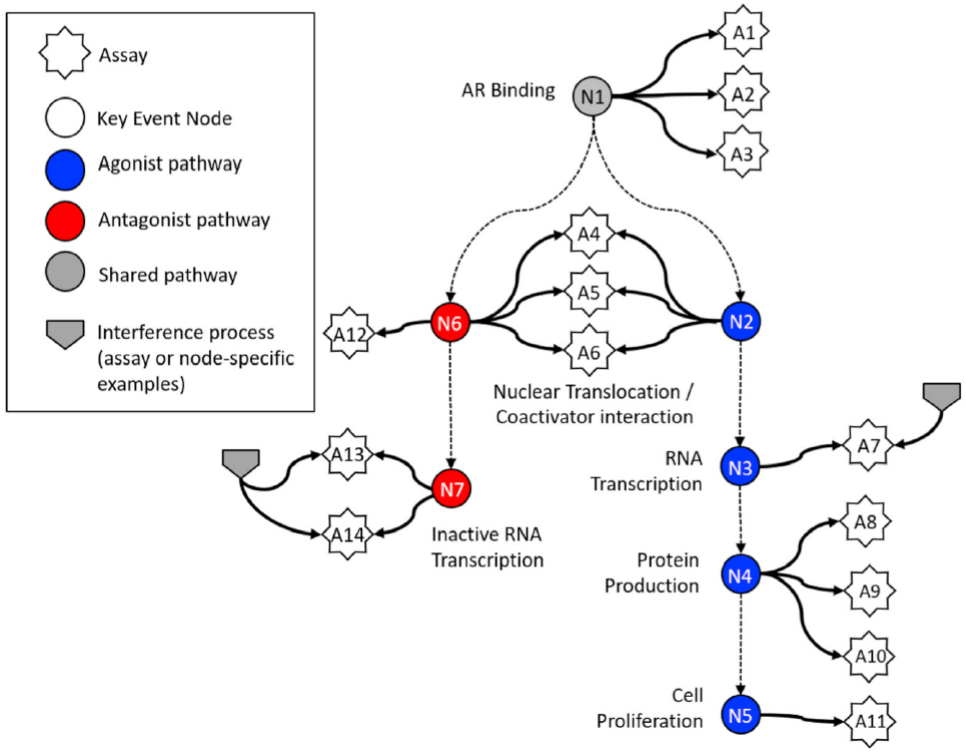
- Reviewed by Scientific Advisory Panels in 2014 and 2017.
- The Dashboard provides values from the original model published in 2017; new full AR model presented in 2020 publication on minimal assay set (with more assays – now 14).
- The use of the uncertainty bounds around both the ER and AR model scores can be helpful in understanding weak or borderline scores.
- Both the ER and AR models are most helpful in understanding relative bioactivity.

No assay is perfect (ToxCast AR model, 2020)

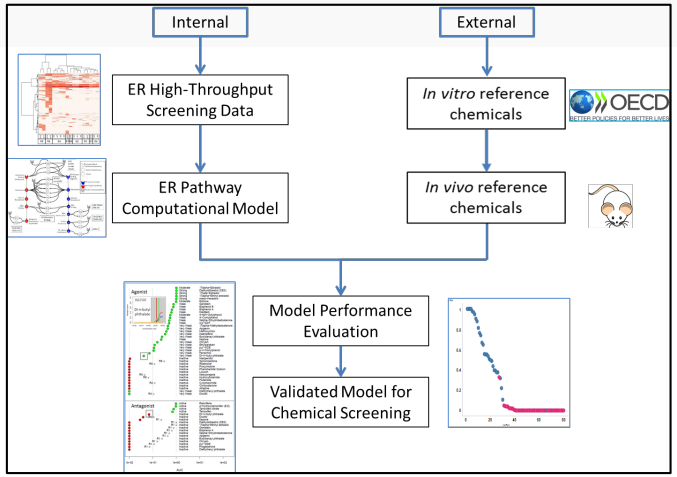


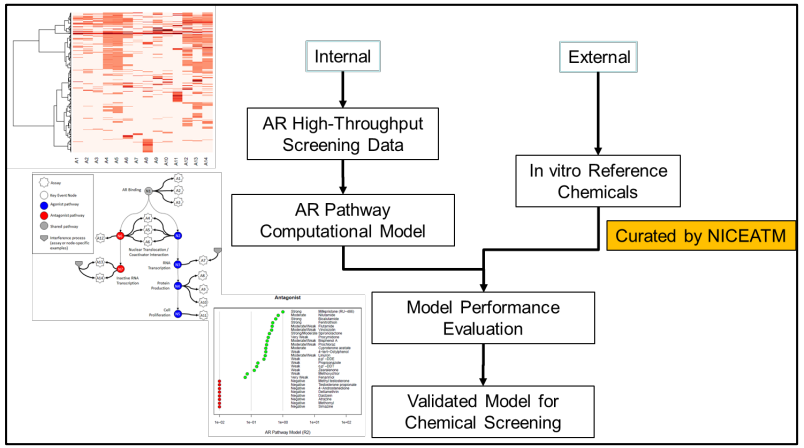
Consider the subset of 1239 substances for which at least on AR assay endpoint in the set of 14 is positive.

Not all assay endpoint positives are specific to the pathway (interference processes), and selectivity (distance from cytotoxicity) can be helpful in distinguishing AR antagonism from cytotoxicity.



Ongoing evaluation of these approaches

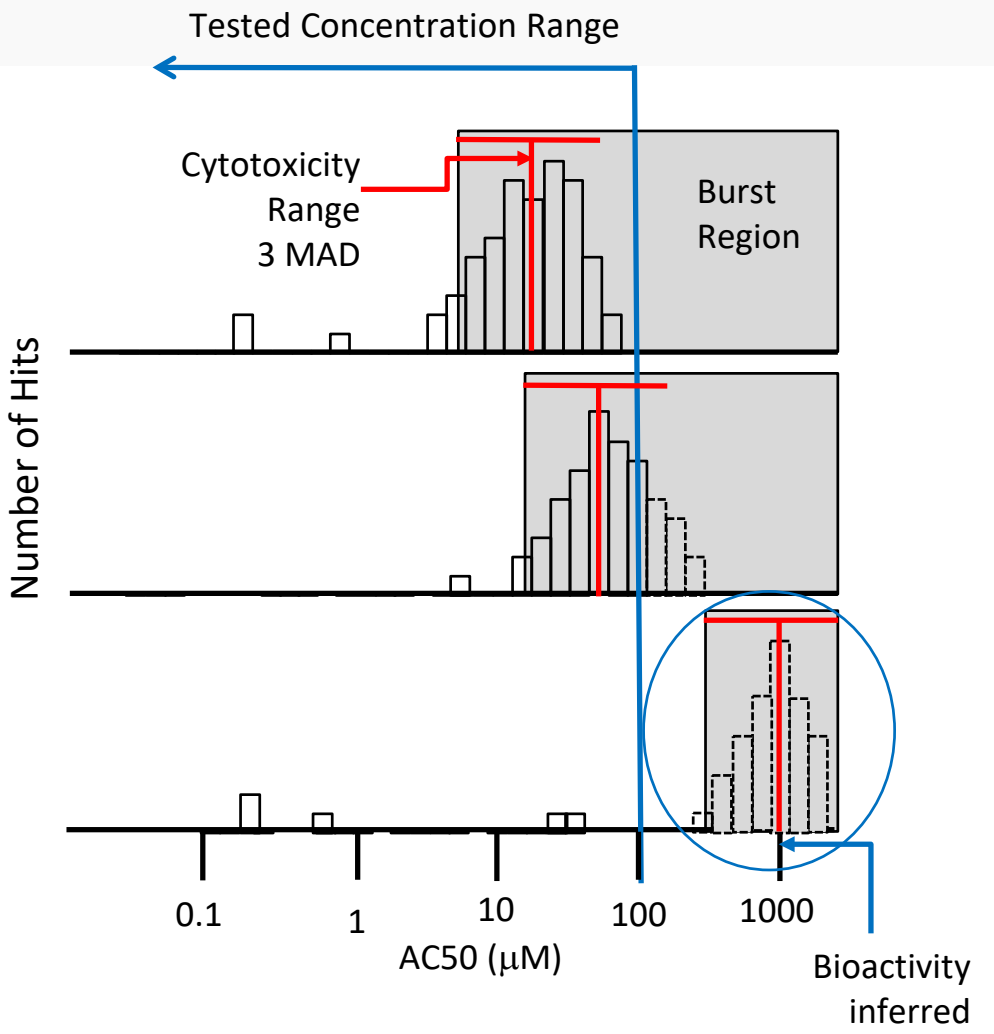




- Comparison to existing literature studies
- Comparison to curated reference chemicals
- Peer-reviewed publications
- FIFRA Scientific Advisory Panel (SAP)
- Organization of Economic Cooperation and Development (OECD) review

Cytotoxicity threshold or "burst" is incorporated into the ToxCast ER/AR models

- Most chemicals display a "burst" of potentially non-selective bioactivity near the cytotoxicity concentration.
- This is often "false positive" activity
  - E.g. Activity in an ER assay in the "burst" region is likely due to cell stress and not true ER binding activity
- "Z-score" method can be used to filter out this false positive activity before drawing conclusions about ER, AR (or other specific target) activity



Judson et al. Tox.Sci. (2016)

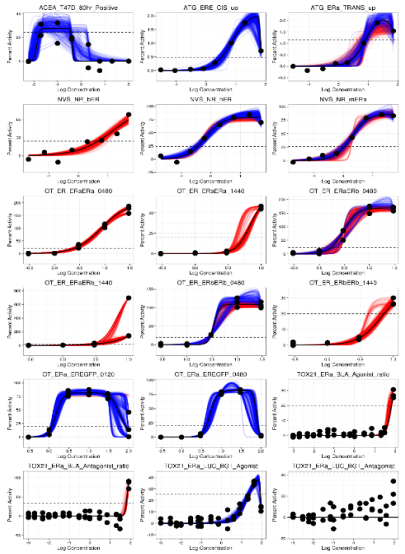
Uncertainty analysis for the ER and AR models

Major sources of uncertainty:

1. Qualitative: is an assay "hit" really due to ER/AR activity, or assay interference?
2. Quantitative: uncertainty around the true potency value (AC50)

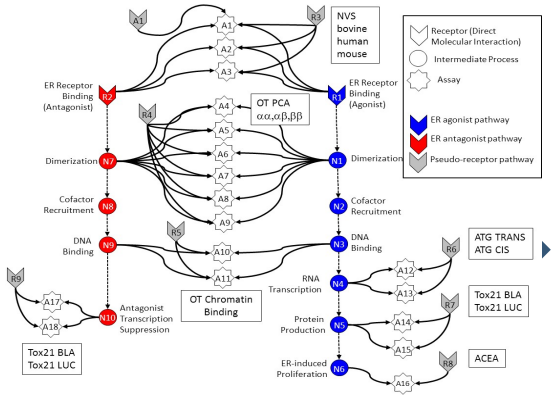
Both are now incorporated into the ER and AR model results

Bootstrap Uncertainty in *In Vitro* Potency Values



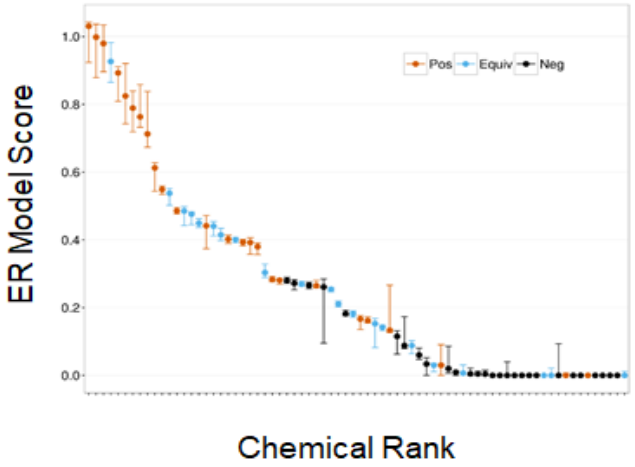
18 ER *In Vitro* Assays

Computational Modeling



ER Pathway Model

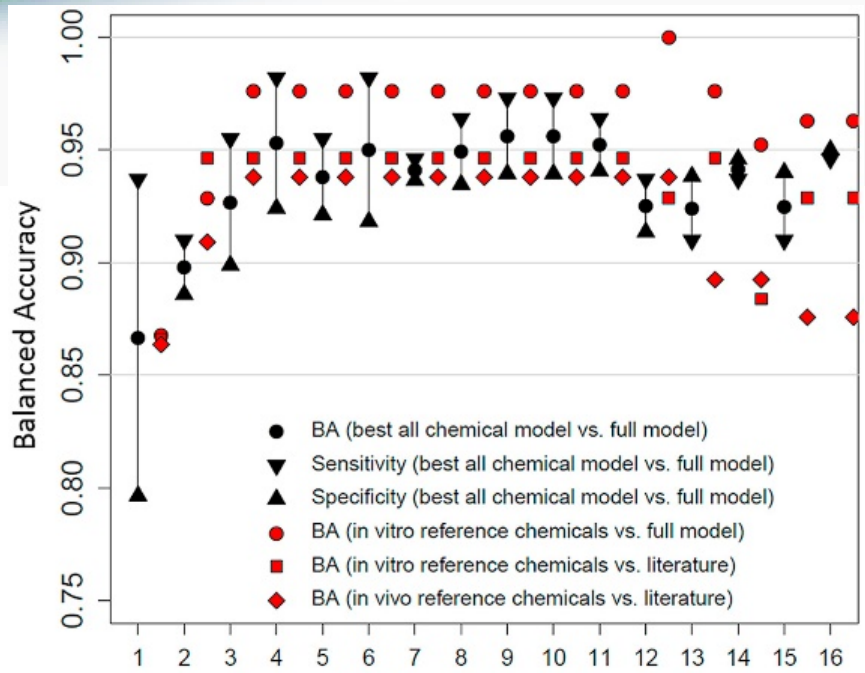
Propagation of Uncertainty in Modeling Output

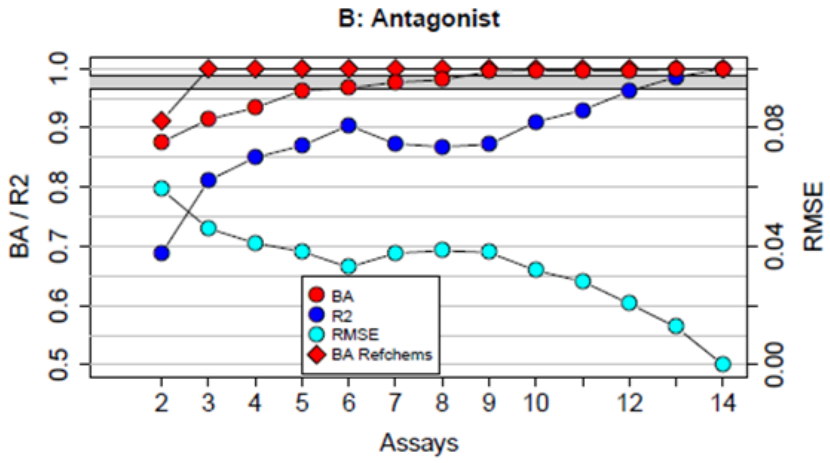


Watt and Judson, PLOS One 2018

Practically, how many assay endpoints are needed to maintain model performance?

- Original ER and AR models used many redundant assays to help understand the types of noise and assay interference occurring in *in vitro* assays
- "Subset models" were developed: Rebuild the original models using all subsets of assays (2, 3, 4, … n assays)
- Results show that subsets with fewer assays have acceptable performance against the full model, and the *in vitro* and *in vivo* reference chemicals.
- The acceptable subsets all have assays that:
  - probe diverse points in the pathway
  - use diverse assay reporting technologies
  - use diverse cell types
- ER Agonist: 4 or more assays
- AR Antagonist: 5 or more assays





Judson et al., Reg. Tox. Pharm. (2017) (ER)
Judson, et al. Reg. Tox. Pharm. (2020) AR)

Lessons learned

- Impact of cytotoxicity: Analysis and incorporation of cytotoxic 'burst'

- Flexibility in assay selection: Developed smaller subset pathway models and criteria for assay selection in the subset to allow use of existing/preferred assays

- Metabolic Competence: Lack metabolic competence in in vitro HTS Assays may lead to over- or underestimation of chemical hazard.

- In Vitro HTS Assays and the Pathway Model Analysis: In the analysis of the HTS assays, there is a need to establish uncertainty bounds around potency and efficacy values.

Approach using *in silico* methods: CERAPP and COMPARA

- Large scale QSAR modeling projects to predict ER and AR activity
- CERAPP - Collaborative Estrogen Receptor Activity Prediction Project
- CoMPARA : Collaborative Modeling Project for Androgen Receptor Activity
- Use ER and AR Pathway model results to train QSAR models
- Use data from the open literature to evaluate
- Many expert groups from US, Europe, Japan and China submitted models, from which consensus models were derived
- Modes: Binding, Agonist, Antagonist
- Model types:
  - Qualitative (active, inactive),
  - Semi-quantitative (inactive, very weak, weak, moderate, strong)
- Results available through the CompTox Chemicals Dashboard

CERAPP consensus validation

	Binding		Agonist		Antagonist	
	Training	Validation	Training	Validation	Training	Validation
Sn	0.93	0.58	0.85	0.94	0.67	0.18
Sp	0.97	0.92	0.98	0.94	0.94	0.90
BA	0.95	0.75	0.92	0.94	0.80	0.54

CoMPARA consensus validation

	Binding		Agonist		Antagonist	
	Training	Validation	Training	Validation	Training	Validation
Sn	0.99	0.69	0.95	0.74	1.00	0.61
Sp	0.91	0.87	0.98	0.97	0.95	0.87
BA	0.95	0.78	0.97	0.86	0.97	0.74

Forward Prediction Results

	CERAPP		CoMPARA	
	Active	Inactive	Active	Inactive
Binding	4001	28463	8202	40656
Agonist	2475	29989	1764	47094
Antagonist	2793	29671	9899	38959
Total	4001	28463	10623	47613

Mansouri et al., Environmental Health Perspectives (2016)
Mansouri et al., Environmental Health Perspectives (in press 2019).

Model scores as available in the CompTox Chemicals Dashboard

EPA United States Environmental Protection Agency

- DETAILS
- EXECUTIVE SUMMARY
- PROPERTIES
- ENV. FATE/TRANSPORT
- HAZARD
- ADME
- EXPOSURE
- BIOACTIVITY
  - TOXCAST: SUMMARY
  - EDSP21
  - TOXCAST/TOX21
  - PUBCHEM
  - TOXCAST: MODELS
- SIMILAR COMPOUNDS
- GENRA (BETA)

ToxCast: Models

ToxCast Model Predictions

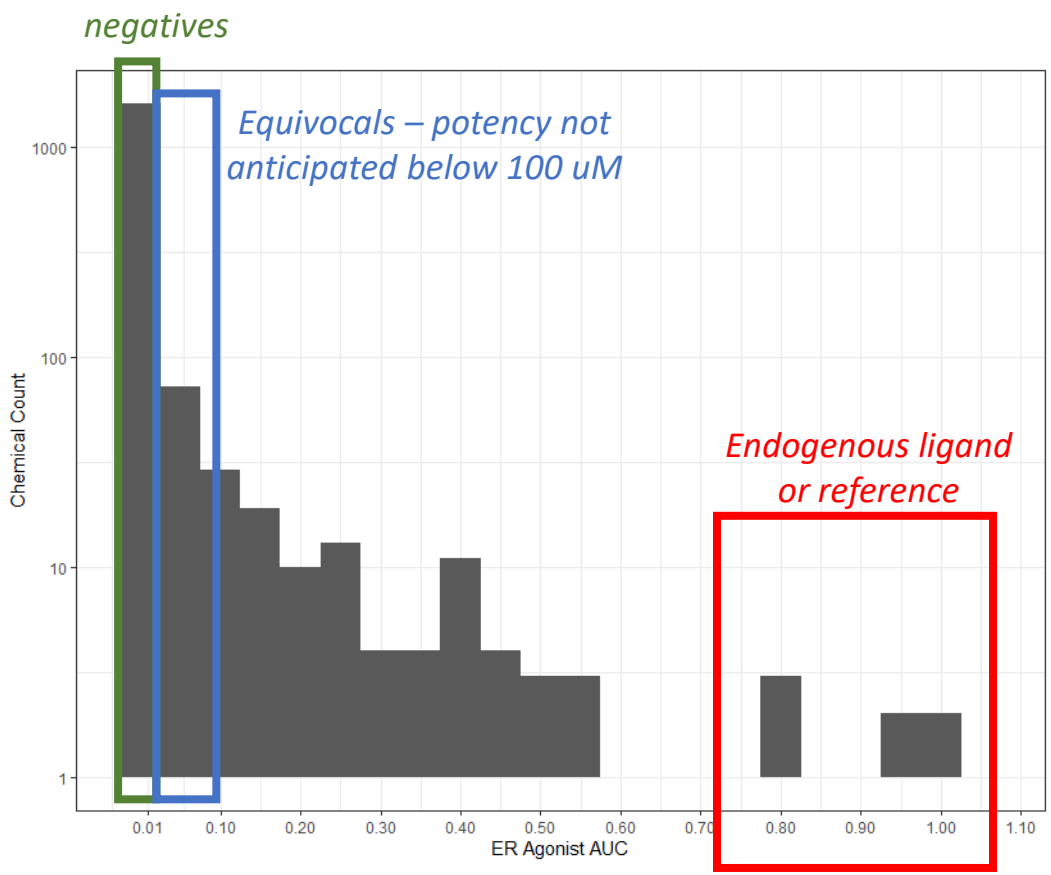
Download ToxCast Model Predictions

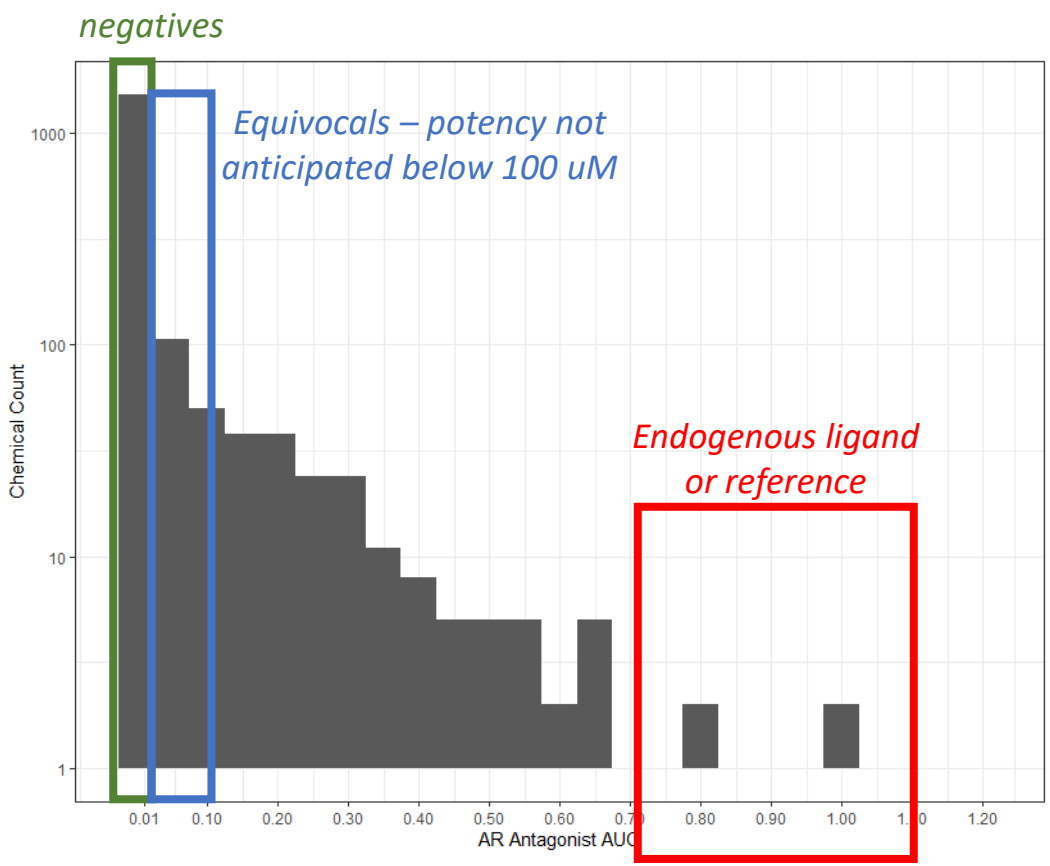
>0.1 = positive; 0.001-0.1 = equivocal

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
COMPARA (Consensus)	Androgen	Inactive	Active	Active
CERAPP Potency Level (From Literature)	Estrogen	Active (Weak)	-	Active (Weak)
CERAPP Potency Level (Consensus)	Estrogen	Active (Weak)	Active (Strong)	Active (Weak)

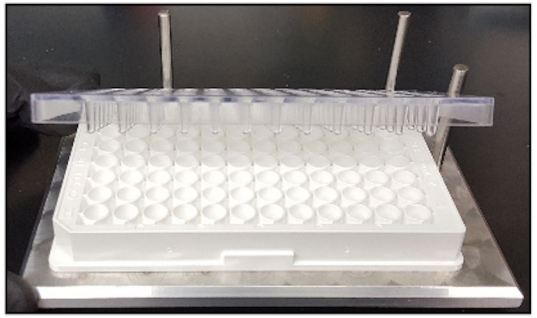
ToxCast Pathway Model AUC ER = full ER model (18 assays)
ToxCast Pathway Model AUC AR = full AR model (11 assays)
CERAPP = consensus ER QSAR (from 17 groups)
COMPARA = consensus AR QSAR

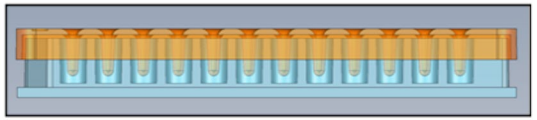
Interpreting and using ToxCast pathway model scores: relative activity





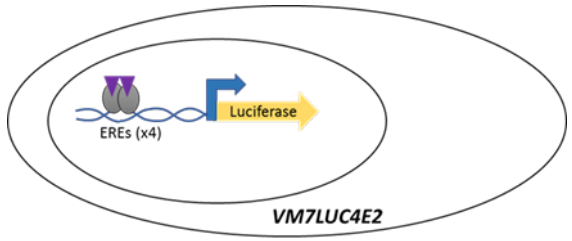
Future: Retrofitting Metabolism to an Estrogen Receptor Transactivation Assay

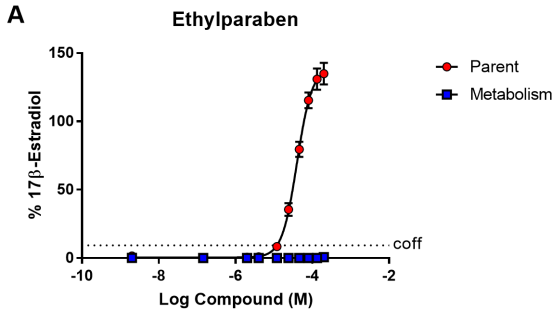




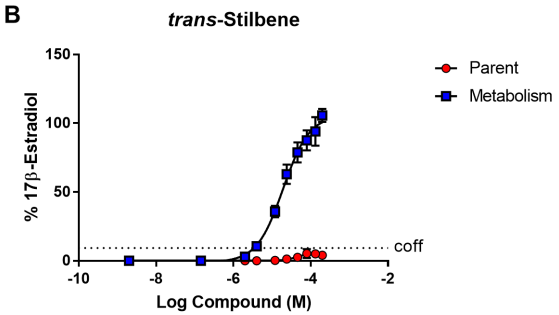
AIME Method: S9 fraction immobilization in alginate microspheres on 96- or 384-well peg lids

- **Retrofitting Metabolism**: AIME method suitable for biochemical- and cell-based HTS assays
- **Screening Throughput**: Adaptable to 96- and 384-well screening platforms
- **Regulatory Relevance**: Integration of phase I liver metabolism for hazard identification of parent and metabolite endocrine activity
- **Results:** Evaluation of a 63 chemical test set supports metabolic screening for -
  - Refinement of prioritization for ER-active substances based on metabolite effects
  - In some cases, supports more accurate prediction of *in vivo* effects for biotransformed substances





Bioinactivation



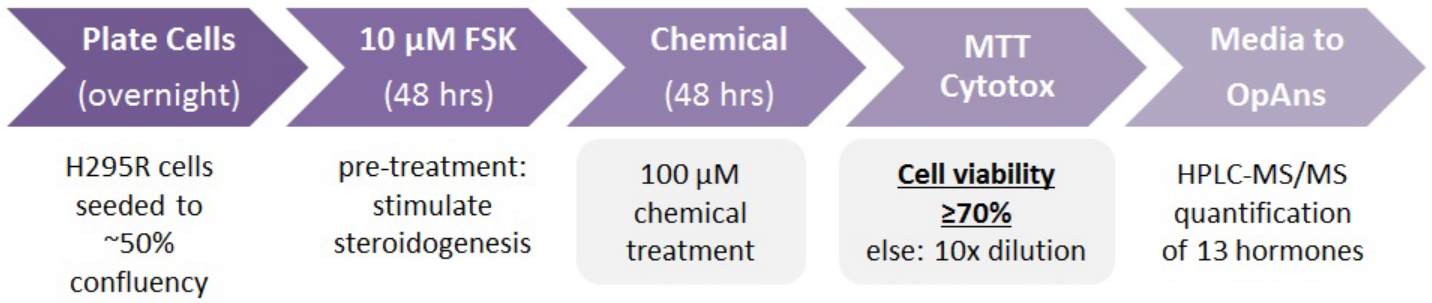
Bioactivation

Parallel evaluation of parent compound and metabolites identifies false positive and false negative effects

Deisenroth and colleagues, unpublished (forthcoming).

Predicting disruption of steroidogenesis: investigating NAMs for the H295R assay

CeeTox/Cyprotex (HT-H295R assay)



Assay background and methods
(Karmaus *et al.* 2016)

Develop initial HT-H295R assay

Implement staged screening approach

Methods and Results: Evaluation of the HT-H295R assay

Compare HT-H295R to the OECD inter-laboratory results

Analyze data per the OECD TG to enable comparison

Evaluate the concordance of E2 and T responses

(Haggard *et al.* 2018 Toxicological Sciences.)

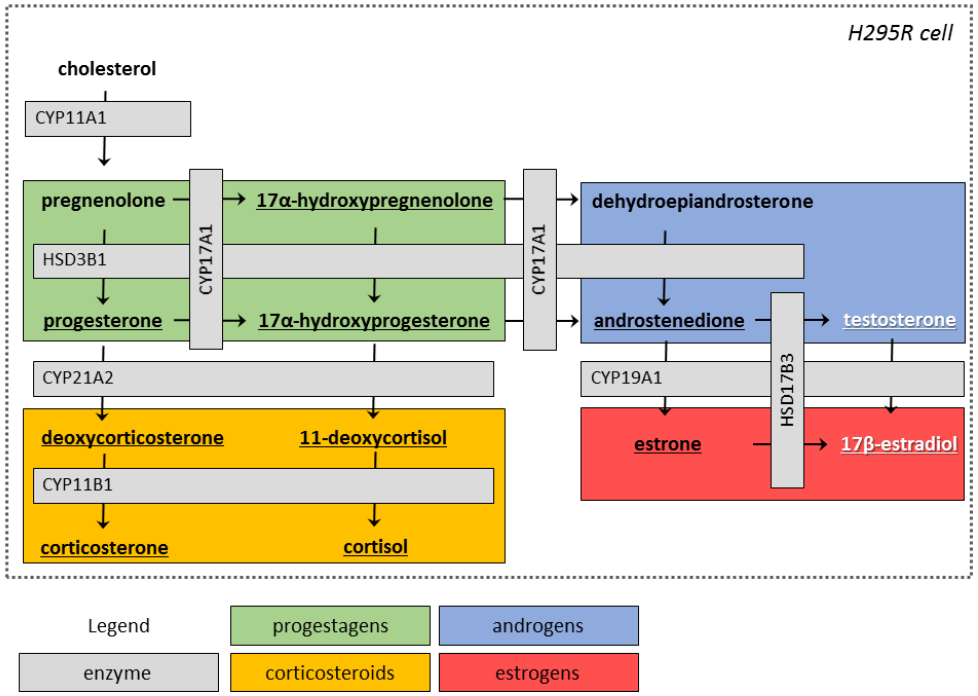
Methods and results: Development of prioritization metric

Compress data from 11 steroid hormone panel

Develop prioritization metric

Evaluate prioritization metric

(Haggard *et al.* 2019)



Confusion matrices demonstrate good sensitivity, specificity, and accuracy for reference chemicals.

Effect	Revised Sensitivity	Revised Specificity	Revised Accuracy
Testosterone up	1.00	0.89	0.90
Testosterone dn	0.67	0.92	0.82
Estradiol up	0.75	0.83	0.80
Estradiol dn	0.80	1.00	0.95

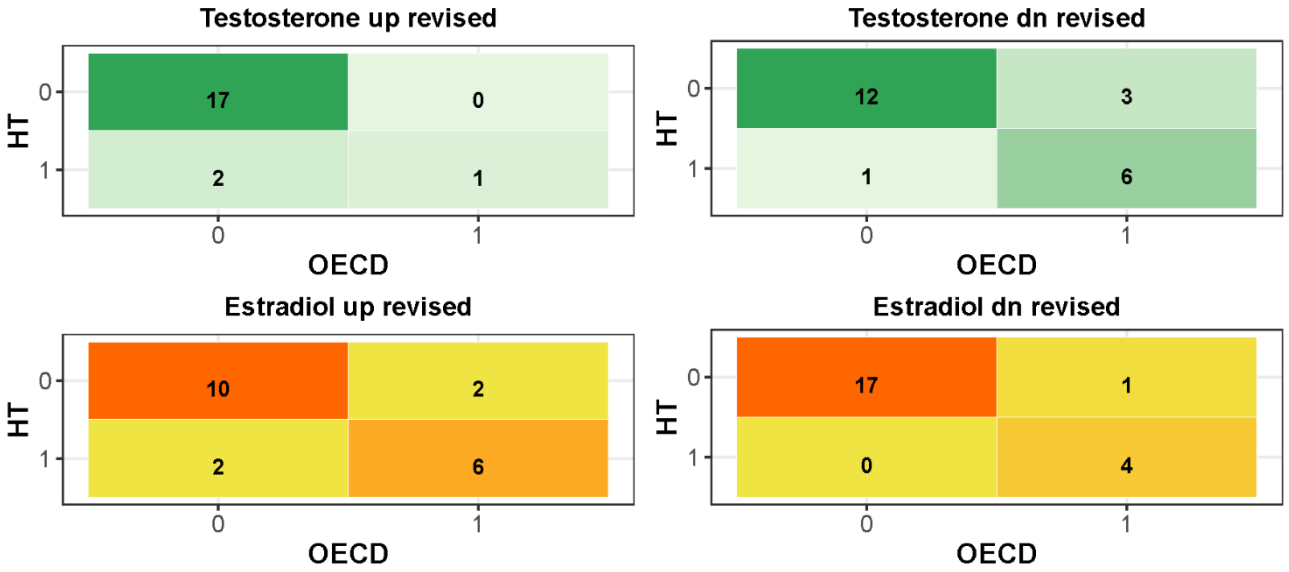


Figure 6 Haggard et al. (2017).

Agreement among labs in the OECD inter-laboratory validation

- For any effect on testosterone:
  - Average concordance among labs was 0.88, 0.91, and 0.90 for the 12 core reference chemicals only, the 16 supplemental reference chemicals only, and the entire set.

- For any effect on estrogen:
  - Average concordance among labs was 0.95, 0.84, and 0.89 for the 12 core reference chemicals only, the 16 supplemental reference chemicals only, and the entire set.

Example of the 11-dimensional results for prochloraz

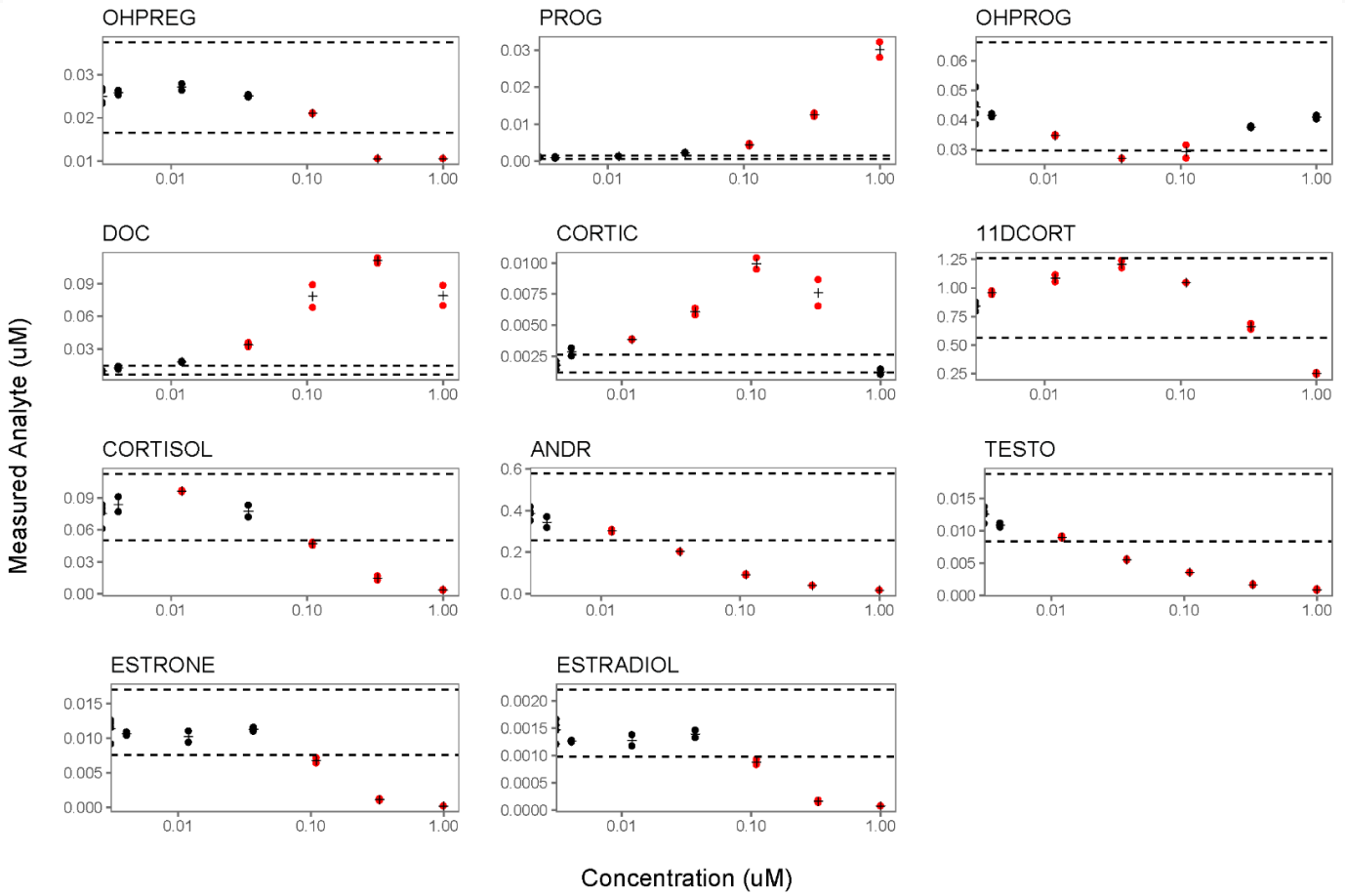


Figure 2 Haggard et al. (2017).

Mahalanobis distance compressed 11-dimensional data to 1.

- Hormones were measured from the same experimental well, and the synthesis of these steroid hormones is interdependent.
- The Mahalanobis distance adjusts the distances, or effect sizes, for the variance and covariance among the hormone measures at each concentration, thereby accounting for knowledge of the interrelatedness of the steroid hormone measurements.
- To calculate the Mahalanobis distance, the response at each concentration of a test chemical was considered as a point in an 11-dimensional space.
  - Each axis corresponds to the natural logarithm of the measured concentration of one of the hormones included in this analysis.
- Method in brief:
  - (1) the degree to which variation among replicates is correlated across hormones was estimated
  - (2) Covariance matrix that characterizes both the noise variance and correlation among hormone levels across replicates, after taking chemical and concentration into account, was constructed
  - (3) Computation of the mean Mahalanobis distance at each concentration of chemical screened

Using our maximum mean Mahalanobis distance approach to get a single prioritization metric

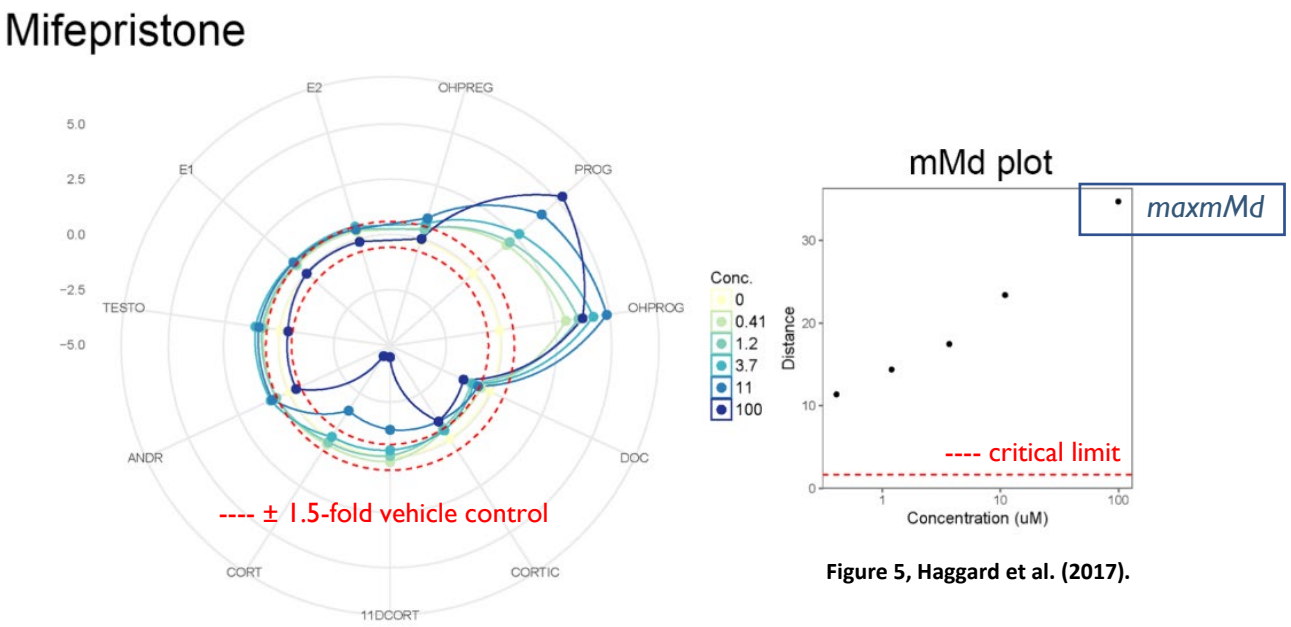


Figure 5, Haggard et al. (2017).

Mifepristone strongly modulated progestagens with significant effects on progesterone and OH-progesterone and moderate but non-significant trends on corticosteroids and androgens, resulting in a relatively high adjusted maxmMd of 33.

- Reduced an 11-dimensional question to a single dimension.
- Selection of the maxmMd appeared to provide a reproducible, quantitative approximation of the magnitude of effect on steroidogenesis.

MaxmMd was reproducible and quantitatively distinguished chemicals with larger effects.

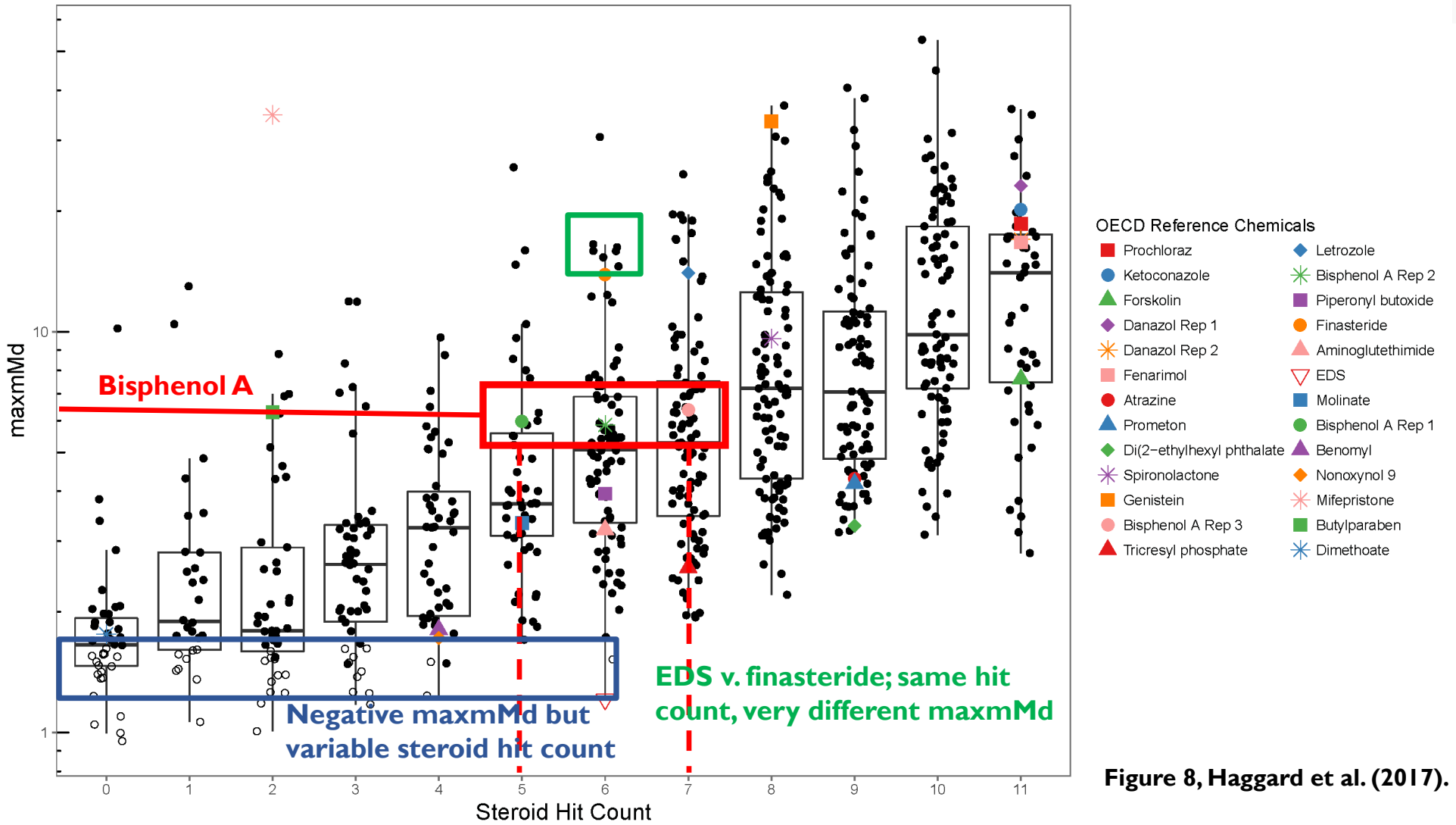


Figure 8, Haggard et al. (2017).

HT-H295R model for steroidogenesis: follow-up analysis

Regulatory Toxicology and Pharmacology 109 (2019) 104510

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Development of a prioritization method for chemical-mediated effects on steroidogenesis using an integrated statistical analysis of high-throughput H295R data

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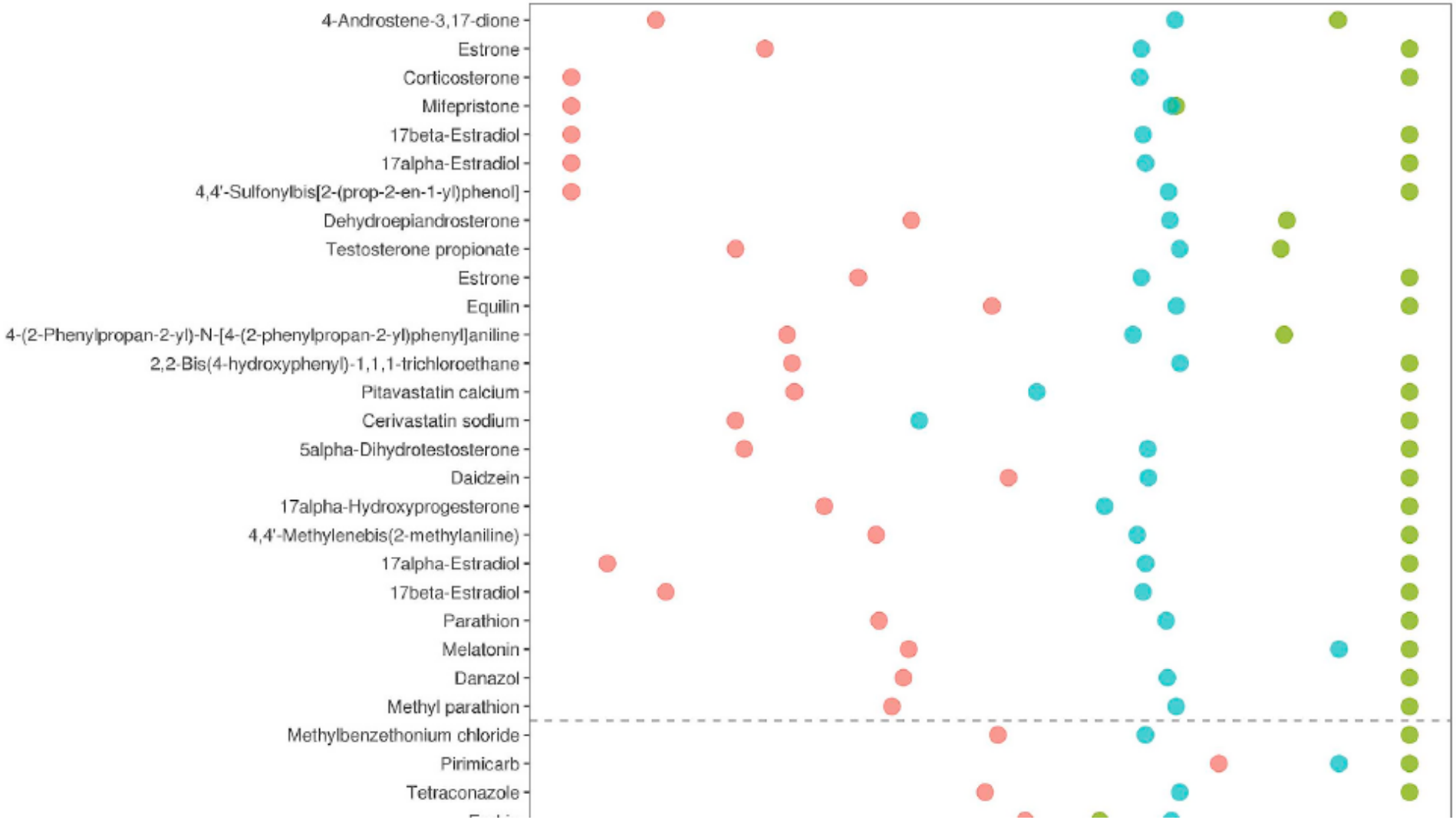
- Evaluated the robustness, reproducibility, and power of the HT-H295R statistical model per feedback received at Scientific Advisory Panel review.
- Considered a case study: does the HT-H295R assay and model detect aromatase inhibitors?
- Demonstrated the use of the HT-H295R statistical model in a selectivity-based prioritization exercise.

Parallel cytotoxicity (MTT assay) and cytotoxicity threshold estimates may help rank positives by selectivity



	Selectivity	AUC	maxmMd
4-Androstene-3,17-dione	4.33	146.30	53.43
Estrone	3.14	145.36	34.73
Corticosterone	4.75	118.51	29.91
Mifepristone	5.01	117.05	34.71
17beta-Estradiol	4.77	114.89	30.61
17alpha-Estradiol	4.80	114.72	31.81
4,4'-Sulfonylbis[2-(prop-2-en-1-yl)phenol]	4.99	112.35	36.61
Dehydroepiandrosterone	2.16	107.75	40.53
Testosterone propionate	3.71	107.01	35.88
Estrone	2.36	105.61	30.56
Equilin	1.54	101.59	44.78
4-(2-Phenylpropan-2-yl)-N-[4-(2-phenylpropan-2-yl)phenyl]aniline	2.89	99.87	21.66
2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane	3.24	94.88	25.88
Pitavastatin calcium	2.02	93.59	21.14
Cerivastatin sodium	1.53	88.81	20.92
5alpha-Dihydrotestosterone	3.37	85.44	30.22
Daidzein	1.17	84.51	30.13
17alpha-Hydroxyprogesterone	2.34	82.46	38.19
4,4'-Methylenebis(2-methylaniline)	2.18	80.61	27.48
17alpha-Estradiol	4.49	75.63	25.68
17beta-Estradiol	3.98	71.00	25.29
Parathion	2.40	68.77	29.00
Melatonin	3.59	68.63	24.19
Danazol	2.20	68.47	23.11
Methyl parathion	2.37	68.40	24.66
Methylbenzethonium chloride	1.23	7.84	4.85
Pirimicarb	1.00	7.84	2.85
Tetraconazole	1.62	7.81	3.41

Model Type

- BMD
- MTT_{acc}
- $cyto_{burst}$

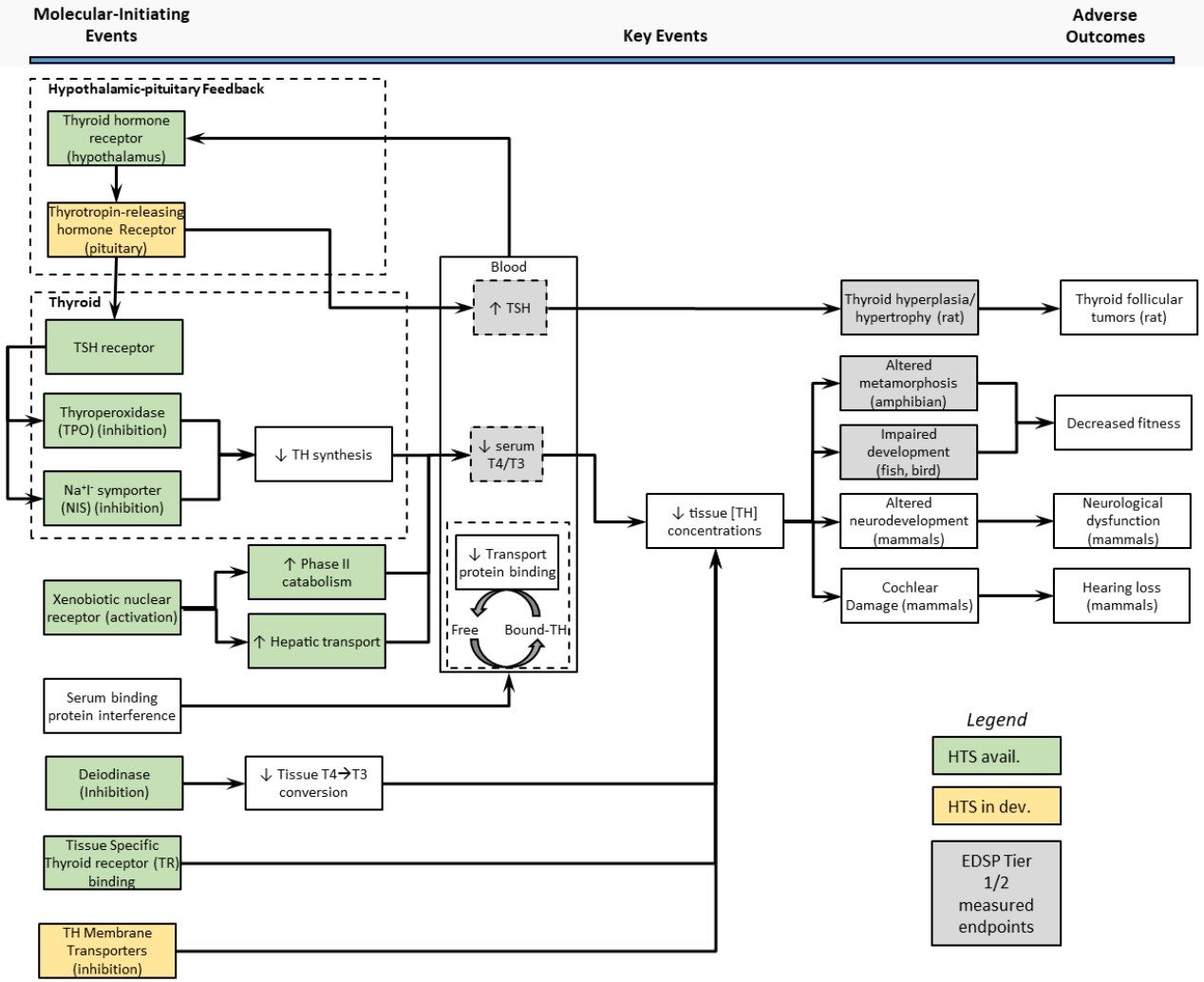
Steroidogenesis summary

- HT-H295R screening assay as an alternative for the OECD-validated, low throughput H295R assay.
  - The ANOVA analysis and logic used herein for the HT-H295R dataset to determine effects on the steroid biosynthesis pathway enabled a direct comparison of the OECD inter-laboratory validation data and the HT-H295R data.
- Novel integration of 11 steroid hormone analytes for pathway-level analysis using the HT-H295R assay data.
  - A mean Mahalanobis distance (mMd) was computed for each chemical concentration screened.
  - The mMd provided a set of unitless values from which the maximum mean Mahalanobis distance (maxmMd) could be calculated across the concentration range screened. This maxmMd may be a useful prioritization metric.
- How can we extend information about ~2000 substances in the HT-H295R assay to larger chemical inventories of interest? Ongoing development of structure-based activity prediction.

Progress on thyroid-relevant bioactivity screening in ToxCast

Progress in HTS assay development for targets in the AOP network

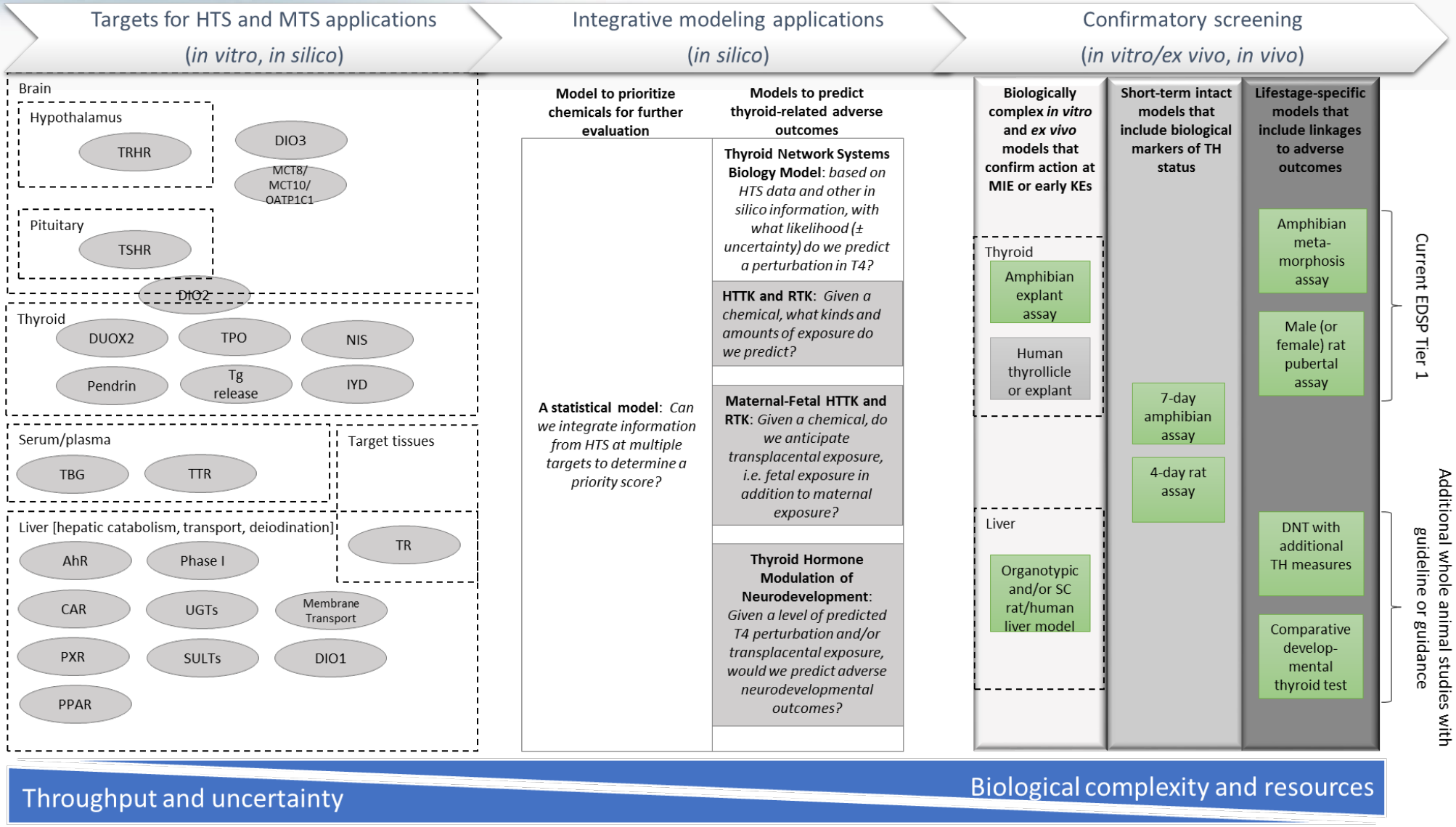
- Considering the thyroid-related AOP network as an outline for HTS screening
  - Ongoing research on the development of screening assays for molecular initiating events and key events
  - Includes development of confirmatory approaches that could be used in a future model



Paul-Friedman et al., 2016; Noyes *et al.*,2019 doi: 10.1289/EHP5297

A possible outline of thyroid screening, model development, and confirmatory screening

Many of the MIE targets have MTS and HTS assays, but efforts to evaluate the screening sensitivity and specificity of those screens are still in progress (e.g., TR, TRHR, TSHR).



Approach to thyroid will be different

- Many molecular-initiating event and key event targets for assay development.
- Uncertainties regarding species sensitivity.
- Less redundancy at each screening target.
- Uncertainties regarding the importance of all possible screening targets for modulation by xenobiotics.
- Understanding target tissue dose and critical windows of susceptibility will be key to any modeling approach.

Integrating multiple assay endpoints: agonism and antagonism of thyroid hormone receptor (TR) occurs with a limited number of substances

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP5314.

Limited Chemical Structural Diversity Found to Modulate Thyroid Hormone Receptor in the Tox21 Chemical Library

Katie Paul-Friedman,[1] Matt Martin,[1] Kevin M. Crofton,[1] Chia-Wen Hsu,[2] Srilatha Sakamuru,[3] Jinghua Zhao,[3] Menghang Xia,[3] Ruili Huang,[3] Diana A. Stavreva,[4] Vikas Soni,[4] Lyuba Varticovski,[4] Razi Raziuddin,[4] Gordon L. Hager,[4] and Keith A. Houck[1]

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Background: Thyroid hormone receptors (TRs
isms, and thyroid hormone disruption is of high
ber of chemical classes have been identified as
diversity. Thus, the question of whether TRs are
Objective: Our goal was to evaluate the hypo
modulating TR activity via the collaborative int
Methods: We screened the Tox21 chemical lib
agonist or antagonist activity. Active compoun
assays, coactivator recruitment assays, and a hig
Results: Known agonist reference chemicals w
the pharmaceutical betamipron. Indirect activatio
detected by confirmation in an RXR agonist ass
founding cytotoxicity and other, non-TR-specific
zuril, and risarestat—were confirmed as antagoni
Discussion: The results support limited structu
hormone axis should be a greater priority for bio

We tested the hypothesis that TR has a more restrictive ligand-binding pocket than estrogen and androgen receptors using Tox21 screening and follow-up assays.

Table 1. Assay names (aenm) and assay end point identification (aeid) values used in the text and invitrodb database together with mode and purpose of assay.

Assay short name	invitrodb: aenm	invitrodb: aeid	Cell line	Assay mode	Function
GH3-TRE-Ag	TOX21_TR_LUC_GH3_Agonist	803	GH3-TRE-Luc	Agonist	Primary qHTS
GH3-TRE-Antag	TOX21_TR_LUC_GH3_Antagonist	804	GH3-TRE-Luc	Antagonist	Primary qHTS
GH3-TRE-Via	TOX21_TR_LUC_GH3_Antagonist_viability	805	GH3-TRE-Luc	Viability	Cytotoxicity
GH3-TRE-Ag-Followup	TOX21_TR_LUC_GH3_Agonist_Followup	2226	GH3-TRE-Luc	Agonist	Confirmation
GH3-TRE-Antag-Followup	TOX21_TR_LUC_GH3_Antagonist_Followup	2227	GH3-TRE-Luc	Antagonist	Confirmation
TRb-bla	TOX21_TRB_BLA_Antagonist_Followup_ratio	2240	TRβ-UAS-bla HEK 293T	Antagonist	Specificity
RXRa-bla-Ag	TOX21_TR_RXR_BLA_Agonist_Followup_ratio	2253	RXRα-UAS-bla HEK 293T	Agonist	Specificity
RXRa-bla-Antag	TOX21_TR_RXR_BLA_Antagonist_Followup_ratio	2257	RXRα-UAS-bla HEK 293T	Antagonist	Specificity
RXRa-Via	TOX21_TR_RXR_BLA_Antagonist_Followup_viability	2258	RXRα-UAS-bla HEK 293T	Viability	Cytotoxicity
TRa-coa	TOX21_TRA_COA_Agonist_Followup_ratio	2230	NA	Agonist	Orthogonal
TRb-coa	TOX21_TRB_BLA_Agonist_Followup_ratio	2236	NA	Agonist	Orthogonal
GFP-GR-TRb	NA	NA	GFP-GR-TRβ MCF7	Agonist and antagonist	Orthogonal

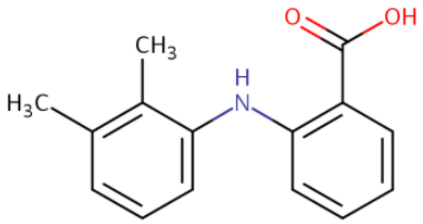
Note: Ag, agonist; Antag, antagonist; bla, beta-lactamase; coa, coactivator; GFP, green fluorescent protein;GH3, rat pituitary cell line; GR, glucocorticoid receptor; HEK 293T, human embryonic kidney cell line; LUC, luciferase; MCF7, human breast cancer cell line; NA, not applicable; qHTS, quantitative high-throughput screen; RXRa, retinoid X receptor alpha; TRa, thyroid hormone receptor alpha; TRb, thyroid hormone receptor beta; TRE, thyroid hormone receptor response element; UAS, upstream activating sequence; Via, viability.

TR agonism and antagonism

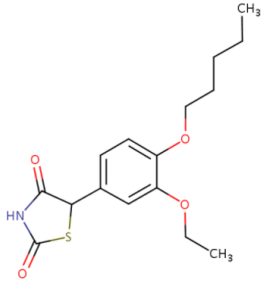
- 11 chemicals identified of 8,305 unique substances as putative direct TR ligands
  - 8 agonists
    - T3 analogs (see table to right)
  - Additional 9 chemicals, largely pharmaceuticals, that agonize RXR through TR:RXR heterodimer resulting in partial agonism in the transactivation assays (permissive heterodimer effect); no activity when RXR not present
  - 3 antagonists of higher confidence: pharmaceuticals, at concentrations exceeding therapeutic concentrations

Table 2 from Paul Friedman et al. 2019 EHP

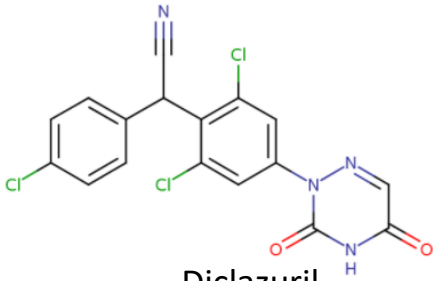
Chemical name
CP-634384
3,5,3′-Triiodothyronine
Levothyroxine
Tetrac
3,3′,5′-Triiodo-L-thyronine
Tiratricol
3,3′,5-Triiodo-L-thyronine sodium salt
Betamipron



Mefenamic acid
(NSAID, some evidence of plasma TH effects in rats)



Risarestat
(aldose reductase inhibitor for hypoglycemia assoc. with diabetes)



Diclazuril
(anticoccidal used in poultry)

Overall conclusion: work supports the hypothesis that TR is a very selective nuclear receptor.

Thyroid hormone synthesis targets: new assays in ToxCast

EPA United States Environmental Protection Agency

TPO inhibition

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 151(1), 2016, 160–180

doi: 10.1093/toxsci/kfw034
Advance Access Publication Date: February 15, 2016
Research Article

Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries

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NIS inhibition

Toxicology in Vitro 40 (2017) 66–78

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Toxicology in Vitro

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Development of a screening approach to detect thy chemicals that inhibit the human sodium iodide sy

Daniel R. Hallinger [a], Ashley S. Murr [a], Angela R. Buckalew [a], Steve
Tammy E. Stoker [a,*], Susan C. Laws [a,*]

[a] Endocrine Toxicology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research L
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OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 20

doi: 10.1093/toxsci/kfx279
Advance Access Publication Date: D
Research Article

DIO1 inhibition

Screening the ToxCast Phase 1 Chemical Library for Inhibition of Deiodinase Type 1 Activity

Michael W. Hornung,[*,†,‡,§,1], Joseph J. Korte,[*,†,‡,§] Jennifer H. Olker,[*,†,‡,§]
Jeffrey S. Denny,[*,†,‡,§] Carsten Knutsen,[*,†,‡,§] Phillip C. Hartig,[*,†,‡,¶]
Mary C. Cardon,[*,†,‡,¶] and Sigmund J. Degitz[*,†,‡,§]

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TOXICOLOGICAL SCIENCES, 168(2), 2019, 430–442

doi: 10.1093/toxsci/kfy302
Advance Access Publication Date: December 18, 2018
Research Article

DIO1, DIO2, DIO3 inhibition

Screening the ToxCast Phase 1, Phase 2, and e1k Chemical Libraries for Inhibitors of Iodothyronine Deiodinases

Jennifer H. Olker,[*,†,‡,§,1] Joseph J. Korte,[*,†,‡,§] Jeffrey S. Denny,[*,†,‡,§]
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Michael W. Hornung[*,†,‡,§]

- These assays have relatively high hit-rates.
- Determining selectivity of the response will be critical.

Ongoing work on the hypothalamic-pituitary regulatory targets

- Confirmation and followup on Tox21 TSHR and TRHR assay endpoints: the hits currently reported are not filtered for selectivity and assay interference.
- These assays use indirect readouts of TSHR and TRHR agonist and antagonist activity (cAMP and Ca^{2+}).

Putting the HPT all together

A critical component of integrating these assay information into models for prioritization or hazard prediction will be internal dosimetry during the critical neurodevelopmental window.

PLOS ONE

RESEARCH ARTICLE

Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation

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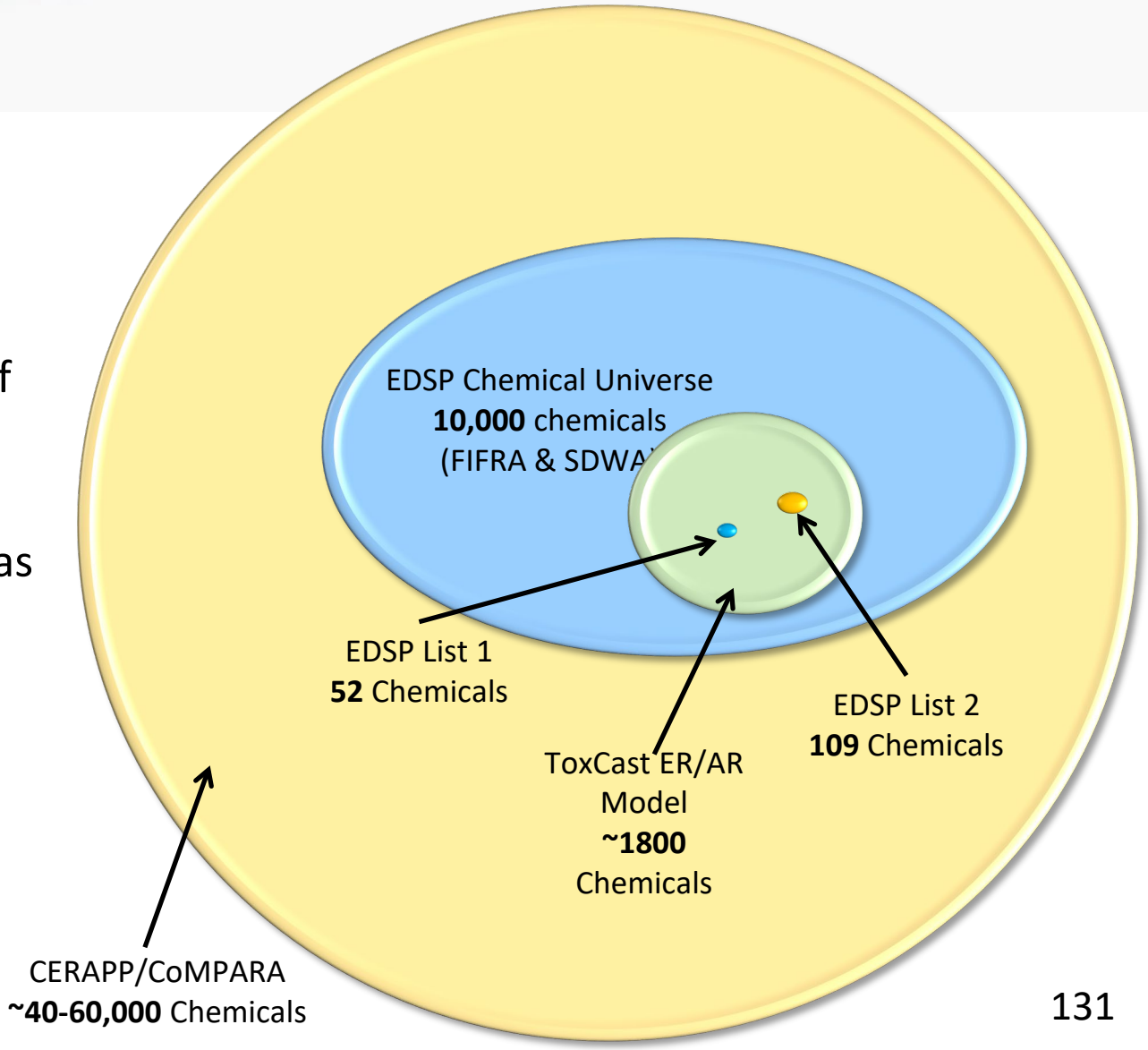
Take Home Messages

- EPA has addressed the need to screen and prioritize thousands of chemicals quickly and without the use of animals through:
  - Development of high-throughput screening assays
  - Integrated computational models
  - Development of in silico consensus models
- EPA has made great advances on including uncertainty and metabolic competence in analysis of high-throughput assays and computational approaches.
- An important component of scientific confidence in these approaches is performance-based evaluation as compared to curated reference chemicals.
- Current approaches can be applied more broadly beyond what is described here and can be used across testing laboratories and decision contexts.



Questions?

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Unilever

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US Environmental Protection Agency