

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

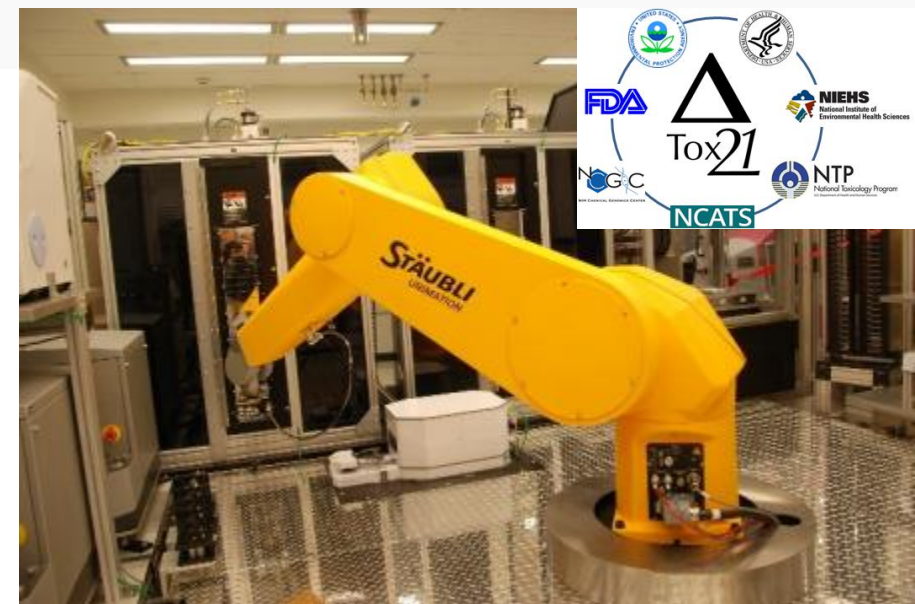
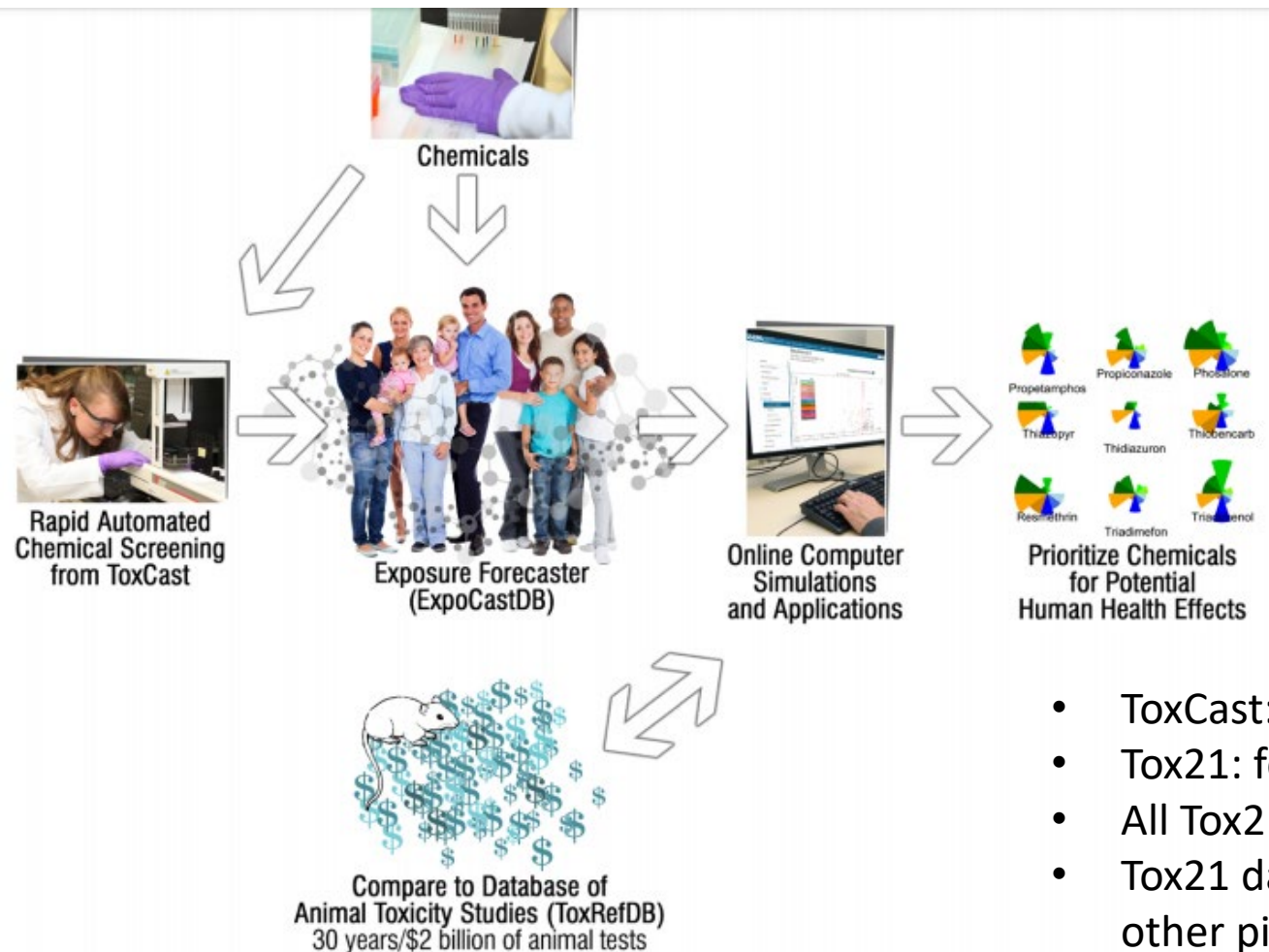
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*The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the
U.S. EPA*

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

EPA's ToxCast program at a glance



Tox21 robot

- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, mostly 1536, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline and other pipelines as well

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



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



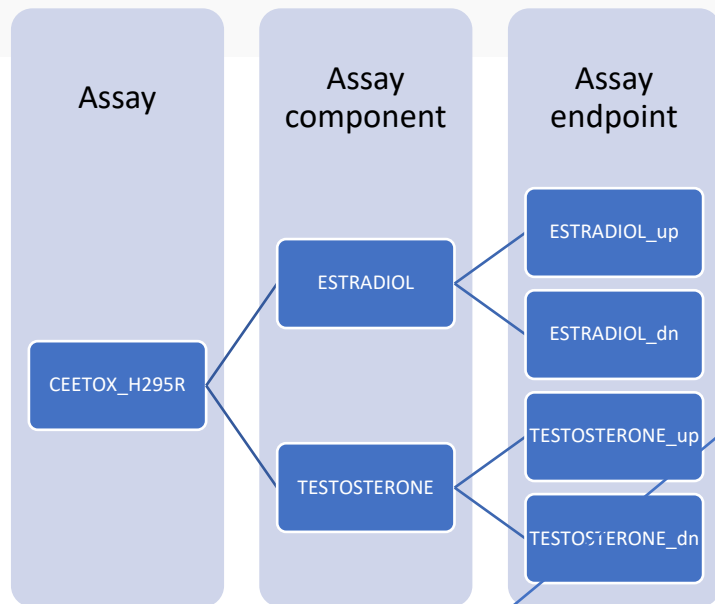
Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

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

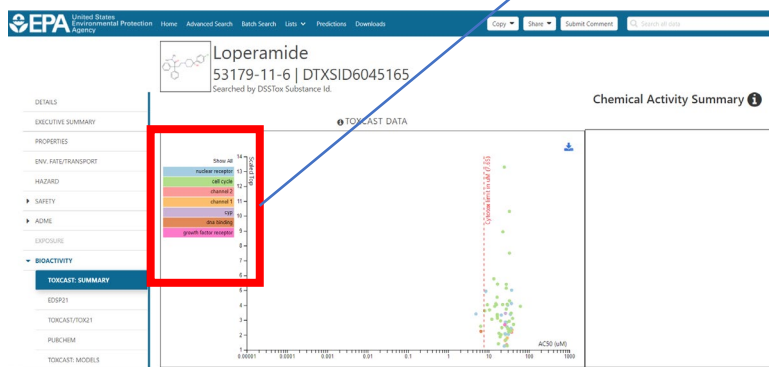
Learning more about the assay endpoints and biology

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 | • Filaments | • Methyltransferase |
| • Apoptosis | • GPCR | • microRNA |
| • Background measurement | • Growth factor | • Mutagenicity response |
| • Catalase | • Histones | • Nuclear receptor |
| • Cell adhesion | • Hydrolase | • Oxidoreductase |
| • Cell cycle | • Ion channel | • Phosphatase |
| • Cell morphology | • Kinase | • Protease/inhibitor |
| • CYP | • Ligase | • Steroid hormone |
| • Cytokine | • Lyase | • Transferase |
| • Deiodinase | • Malformation (zebrafish) | • Transporter |
| • DNA binding | • Metabolite (Stemina metabolomics) | |
| • Esterase | • Mitochondria | |



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

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

What can be done with ToxCast data?

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?

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?

Summary							
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

*MW = 441.54 g/mol – likely
good oral availability*

Summary

Probably able to cross cell membrane without active transport

Not volatile

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?

United States Environmental Protection Agency | Home | Advanced Search | Batch Search | Lists | Predictions | Downloads

Copy | Share | Submit Comment | Search all data

Searched by DSSTox Substance ID.

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 ☐ Inactive

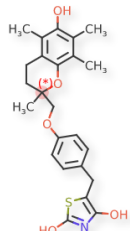
Filter assays

- Tanguay Lab (0 of 19 s
- Tox21/NCGC (0 of 235
- NHEERL Mid-Continen

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	

PROPERTIES

ENV. FATE/TRANSPORT

HAZARD

SAFETY

ADME

EXPOSURE

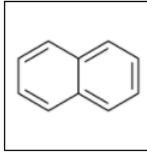
BIOACTIVITY

TOXCAS: SUMMARY

EDSP21

TOXCAS/TOX21

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



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

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 Ha

Select



Home / Tox21 Samples / Tox21_300008

Naphthalene



QC Grade

T0

A

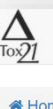
MW Confirmed, Purity > 90%

T4

Ac


CAUTION, Low Concentration

Concentration 5-30% of expected value



Home / Tox21 Samples / Tox21_111023

Naphthalene



QC Grade

T0

U

Unknown/Inconclusive

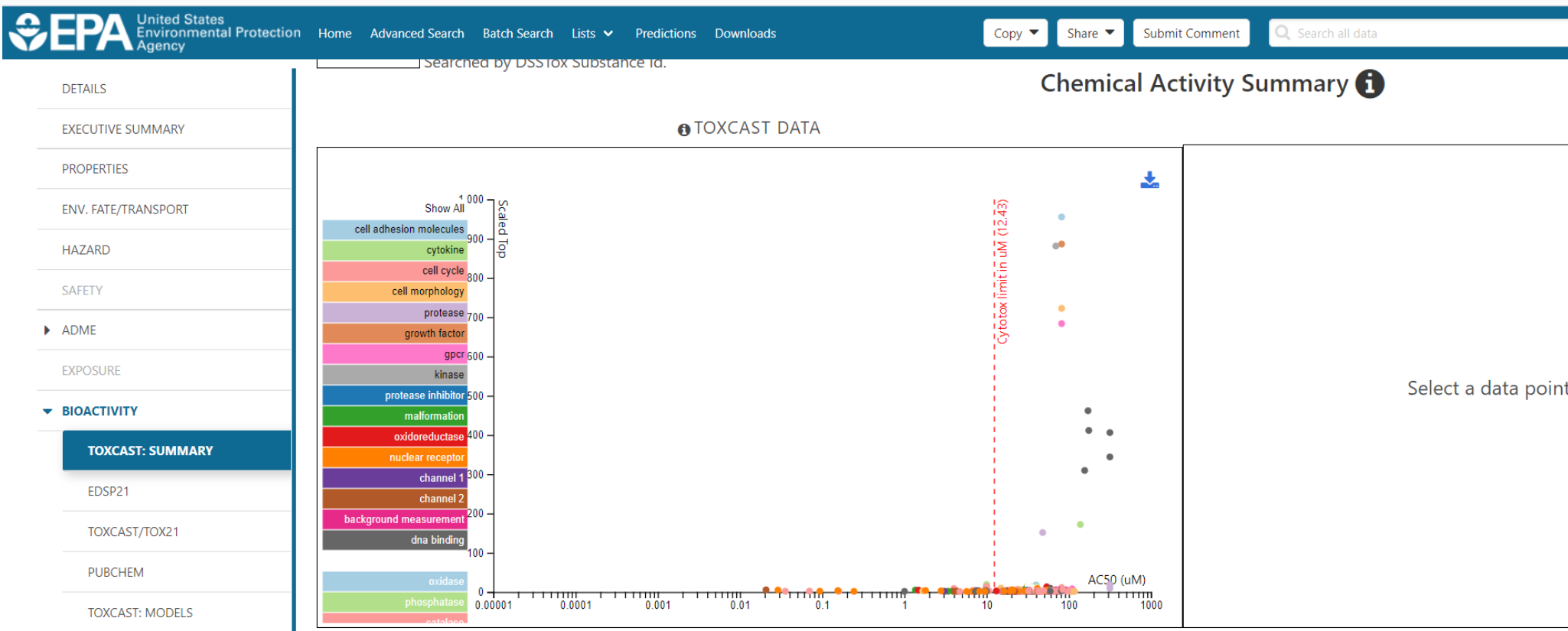
T4

Fns

CAUTION, No Sample Detected

Biological Activity Unreliable

But what bioactivity does Mystery Compound A have?



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

Models available?

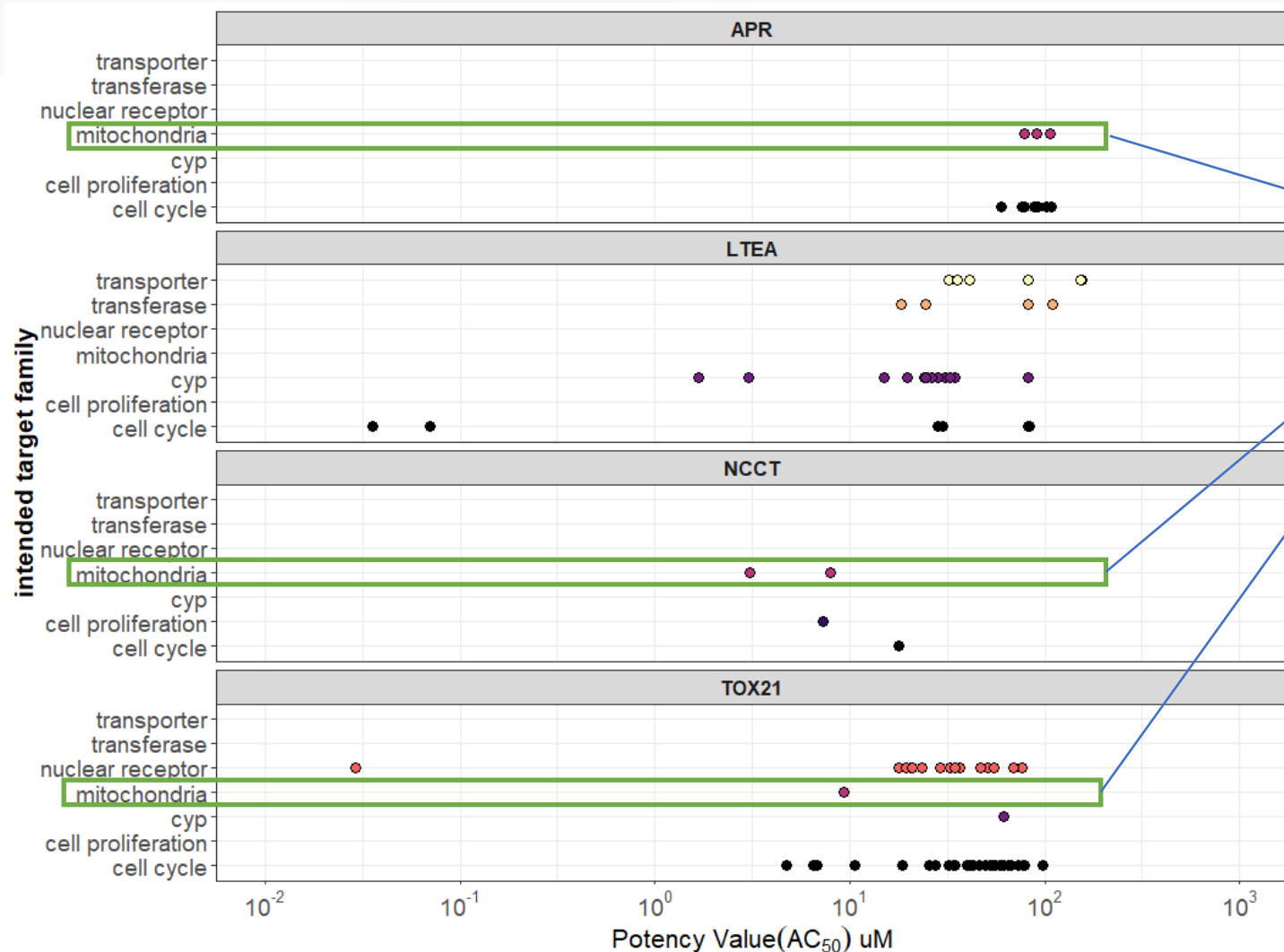
Selective or non-selective?

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 MitoMemPot	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
occurring*
May occur at concentrations
greater than mitochondria
or cell cycle bioactivity

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

- Troglitazone
- Treatment for Type II diabetes, works primarily by activating PPAR γ
 - 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							

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-m ³ /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...

- 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).

QC Grade			Identifiers	
T0	A	MW Confirmed, Purity > 90%	Tox21	Tox21_202992
T4	A	MW Confirmed, Purity > 90%	NCATS	NCGC00260537-01
			CAS	redacted
			PubChem	144210190

Mystery substance B: Models >>> single assays. And equivocal happens.

Models available?

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

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)

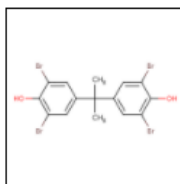
COMPARA = consensus AR QSAR

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.



3,3',5,5'-Tetrabromobisphenol A

79-94-7 | DTXSID1026081

Searched by DSSTox Substance Id.

DETAILS

EXECUTIVE SUMMARY

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BIOACTIVITY

TOXCAST: SUMMARY

EDSP21

TOXCAST/TOX21

PUBCHEM

TOXCAST: MODELS

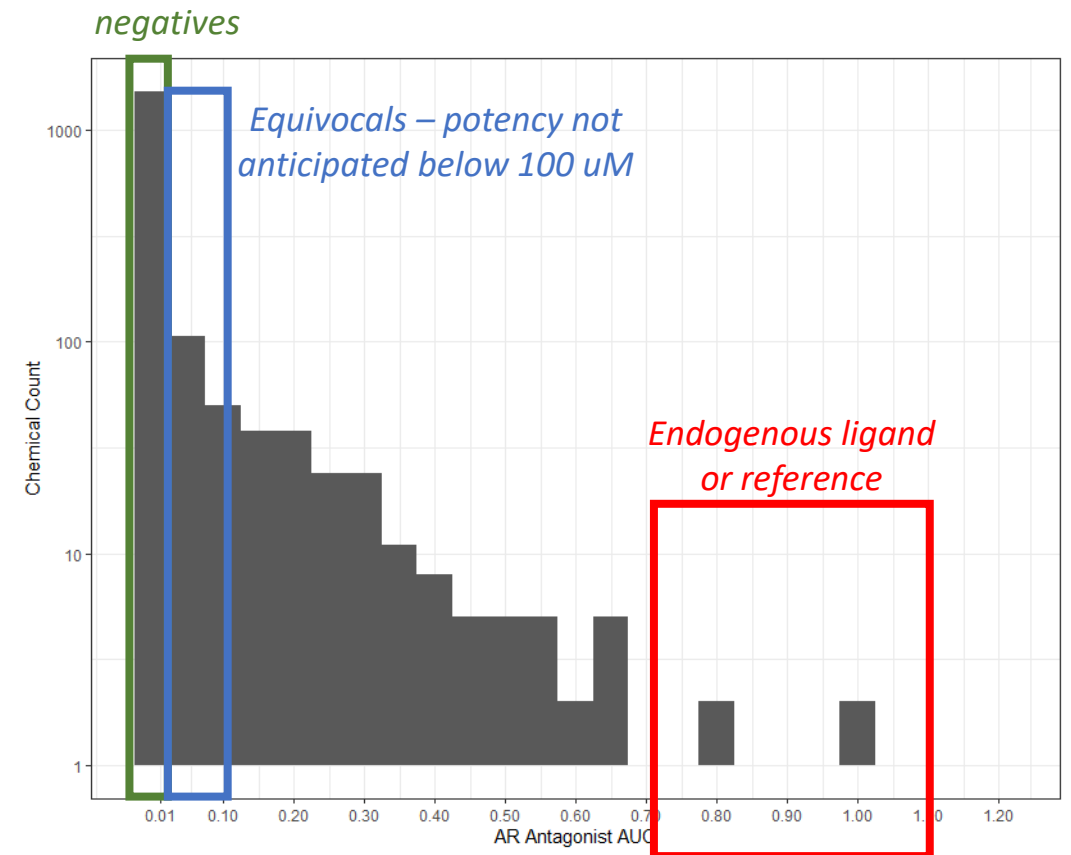
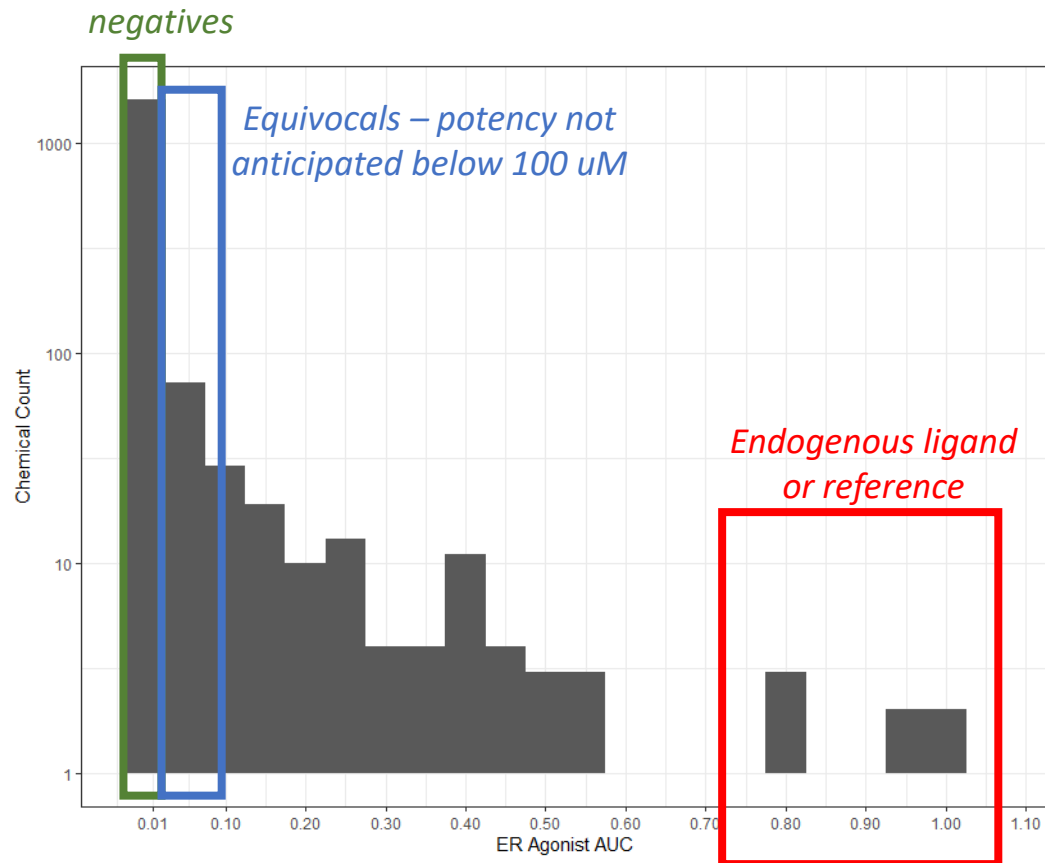
ToxCast: Models

ToxCast Model Predictions

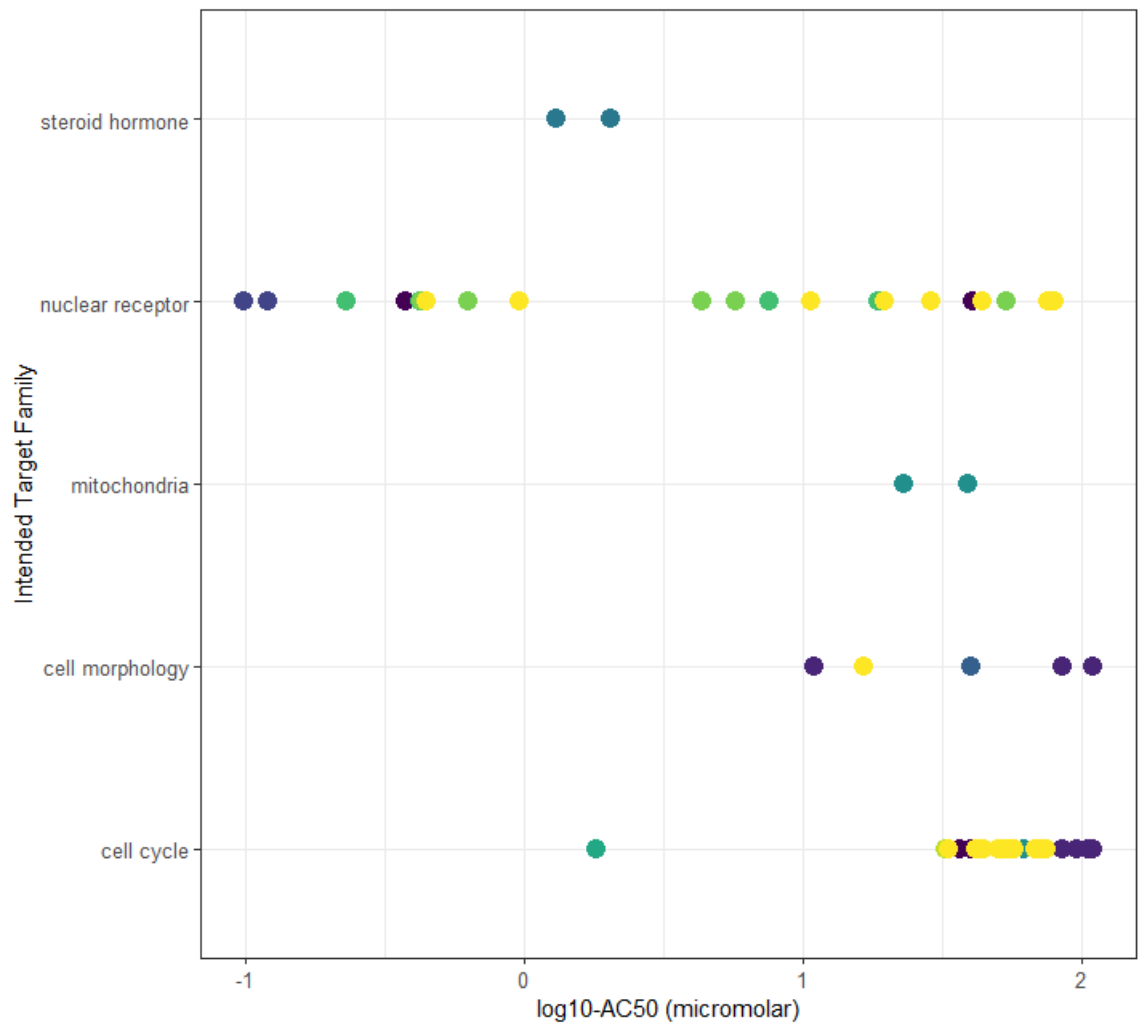
Download ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
i ToxCast Pathway Model (AUC)	Androgen	0.00	0.104	-
i ToxCast Pathway Model (AUC)	Estrogen	0.00	3.81e-7	-
i COMPARA (Consensus)	Androgen	Inactive	Active	Active
i CERAPP Potency Level (From Literature)	Estrogen	-	-	-
i CERAPP Potency Level (Consensus)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)

Interpreting and using ToxCast pathway model scores: relative activity



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_EREFGFP_0120	ESR1	ACTIVE	0.424
OT_ERa_EREFGFP_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

Bioactivity summary in the Dashboard

Selective or non-selective?

Searched by DSSTox Substance Id.

cl

DETAILS

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EXPOSURE

BIOACTIVITY

TOXCAST: SUMMARY

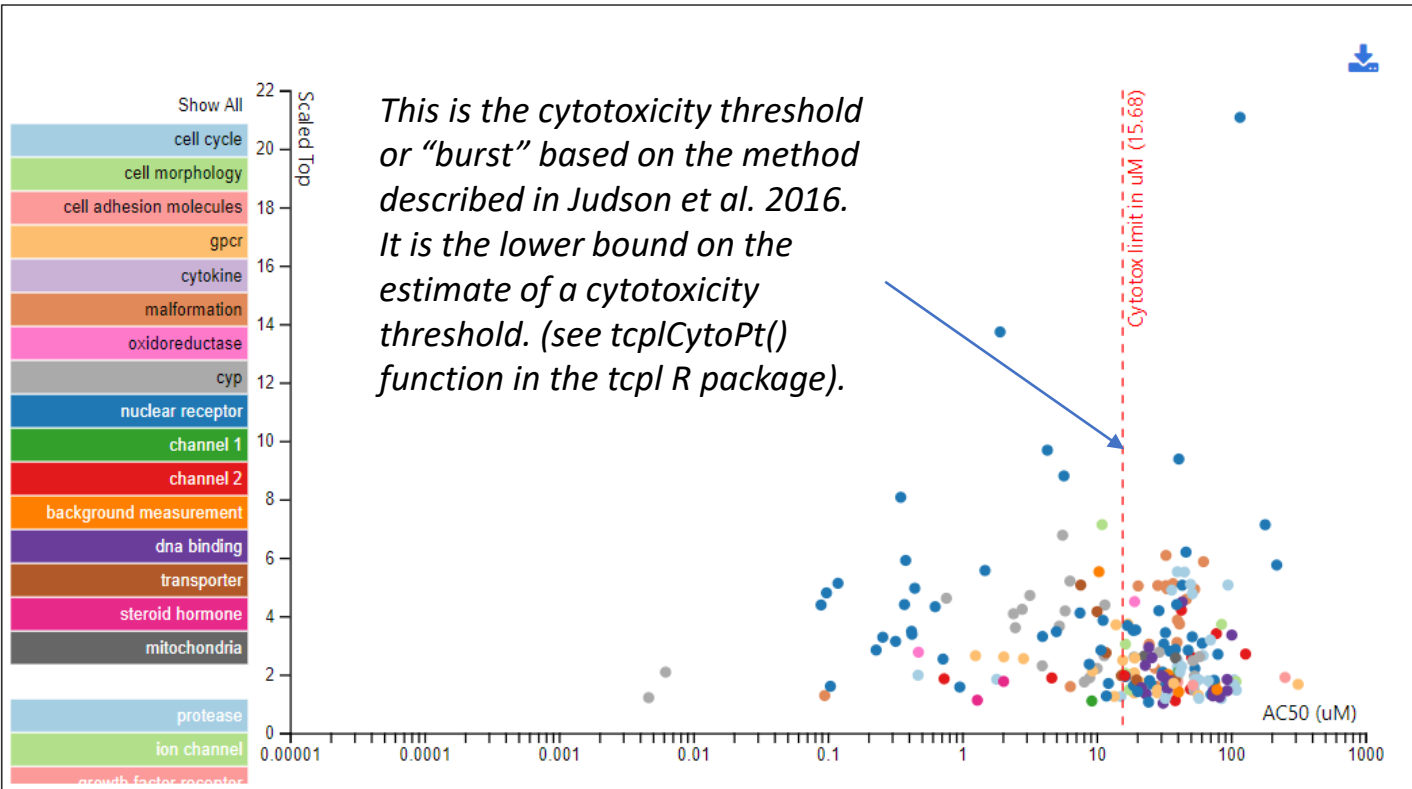
EDSP21

TOXCAST/TOX21

PUBCHEM

TOXCAST: MODELS

TOXCAST DATA



Selective or non-selective?

- $AC50 < \text{burst?}$
- $AC50 \ 0.5\log_{10}$ distance from burst?
- $AC50 < \text{parallel viability assays?}$ This makes sense if you have 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, certain curve properties (such as the maximum), etc.
- 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?

- 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.

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.

2012

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

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

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2017



An Intuitive Approach for Predicting Risk with the Tox21 10k Library

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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

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

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

Robert G. Pearce^{1,2} · R. Woodrow Setzer¹ · Jimena L. Davis^{1,3} · John F. Wambaugh¹



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^{*}

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2017

(2017) 44:549–565
doi:10.1093/toxsci/kfx077



Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization

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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.

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

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

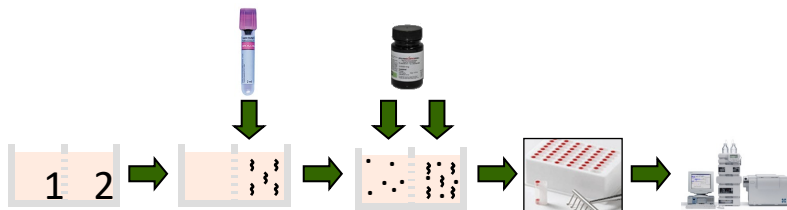
2019

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

High throughput toxicokinetics (HTTK)

in vitro data

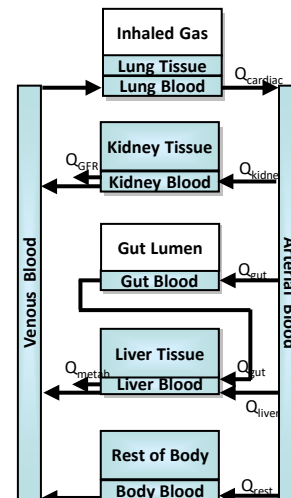
Hepatic clearance from suspended hepatocytes



Plasma protein binding



Generic toxicokinetic models



httk

Some high-level assumptions:


- (1) bioactive nominal *in vitro* assay concentration \sim *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.

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).
- AC_{50} or LEC (micromolar) * (1 mg/kg/day/ C_{ss} (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).

3.3 mg	g	mol	1e6 μmol	= 14.45523 μmol/L = μM	0.1 μM	1 mg/kg/day	= 0.007 mg/kg/day = AED95
L	1000 mg	228.291 g	mol			14.45523 μM	



United States
Environmental Protection
Agency

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Searched by DSS tox Substance Id.

IVIVE

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Search query

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


6 records

C_{ss} 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](#)".

 United States Environmental Protection Agency

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Searched by DSS Tox Substance Id.

Exposure Predictions (mg/kg-bw/day)

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Search query

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

10 records

0.007 mg/kg/day

0.0204 mg/kg/day

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

- 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.

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)*



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TOXICOLOGICAL SCIENCES, 2019, 1–24

doi: 10.1093/toxsci/kfz201

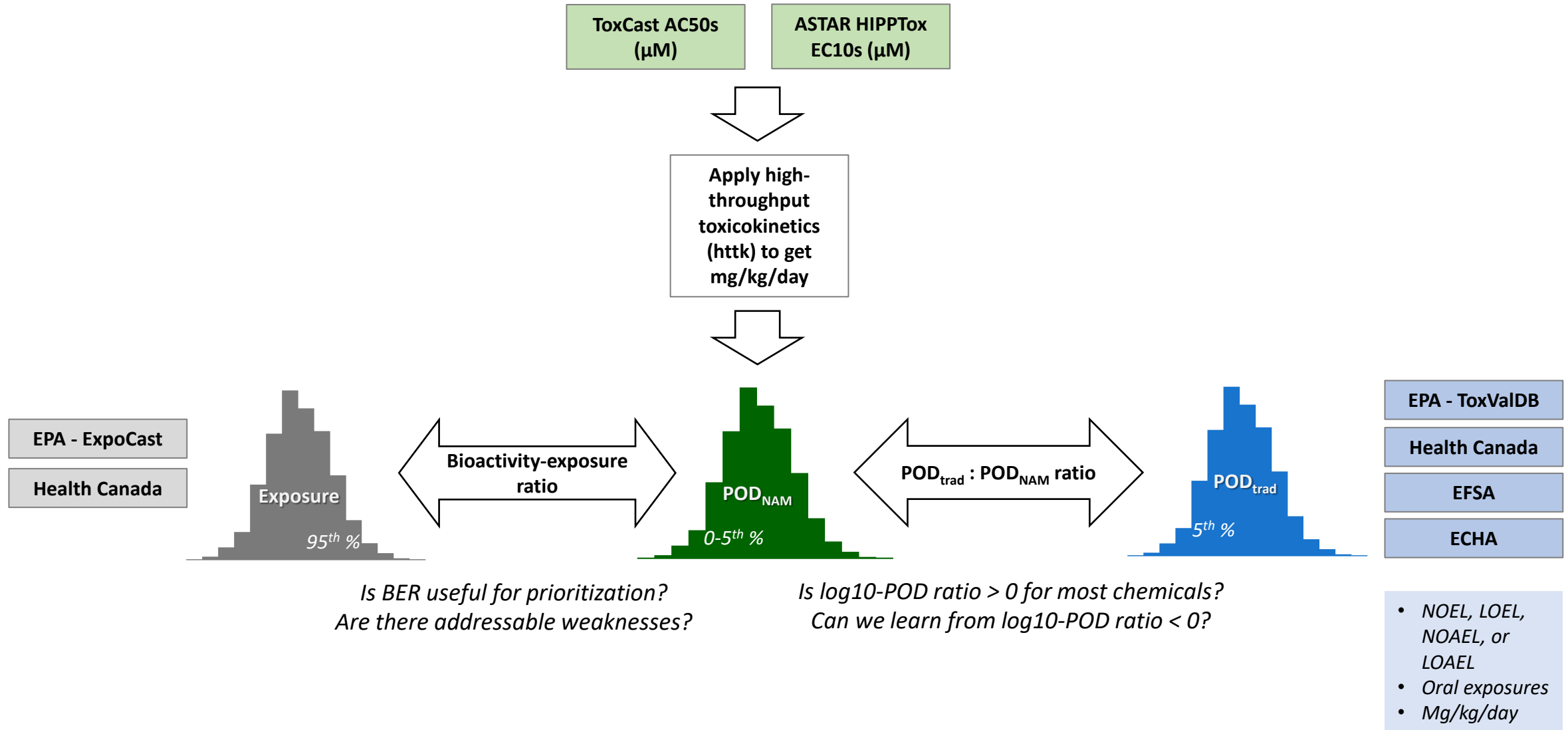
Advance Access Publication Date: September 18, 2019
Research Article

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

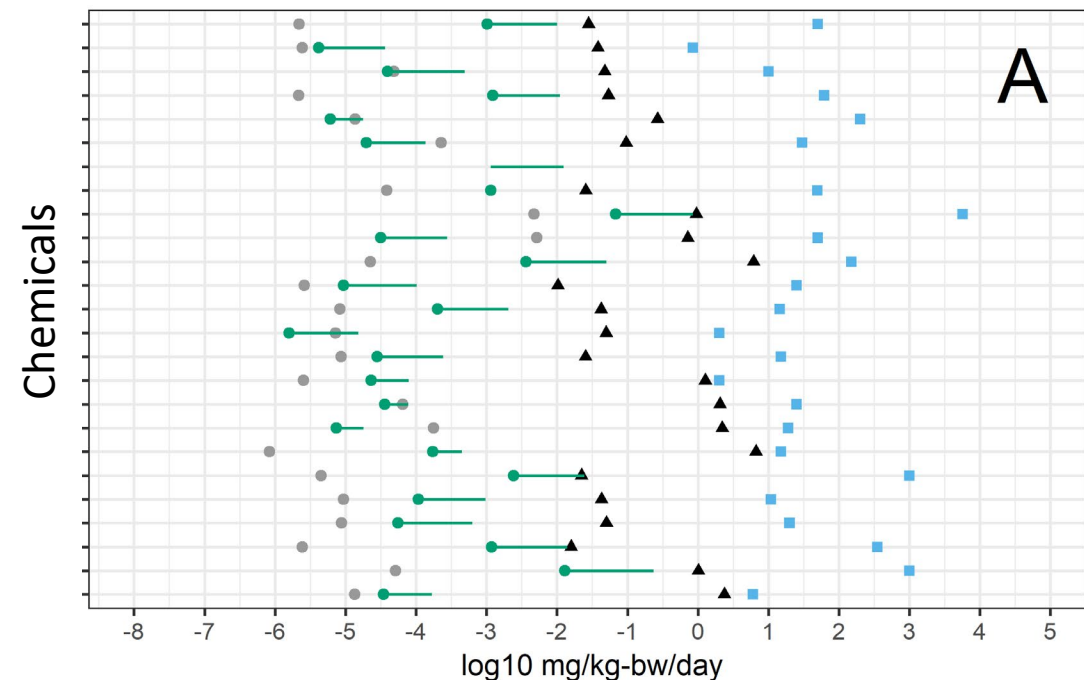
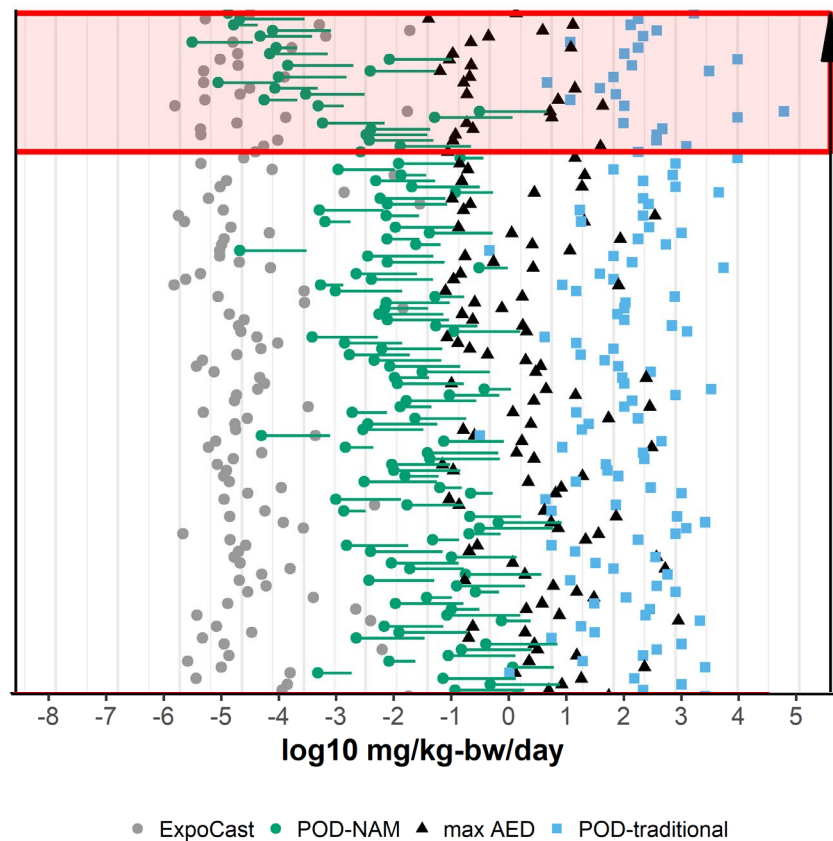


(APCRA partners for these two case studies)

Case study workflow



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.

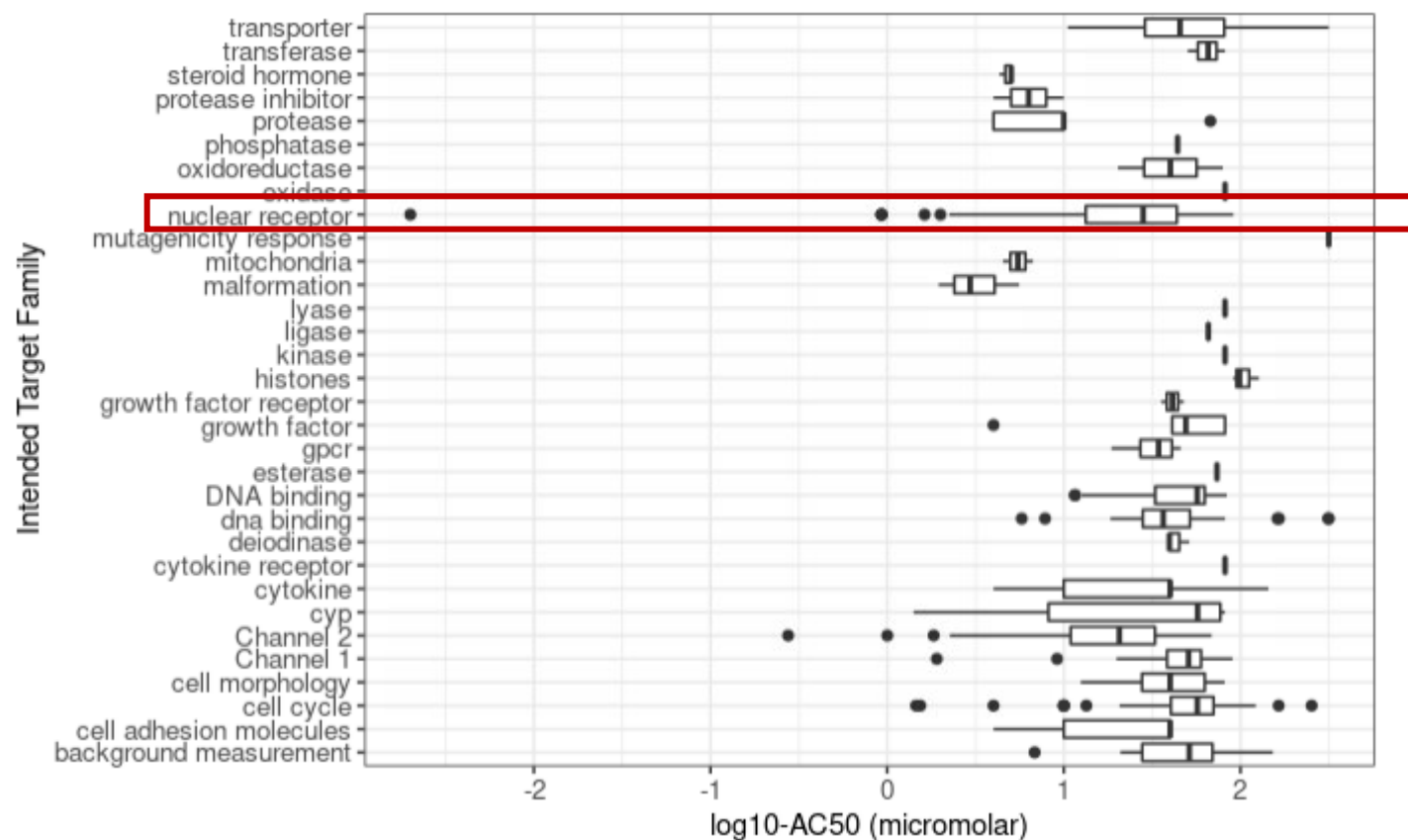
Figure 3 from Paul Friedman et al.

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

- 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.

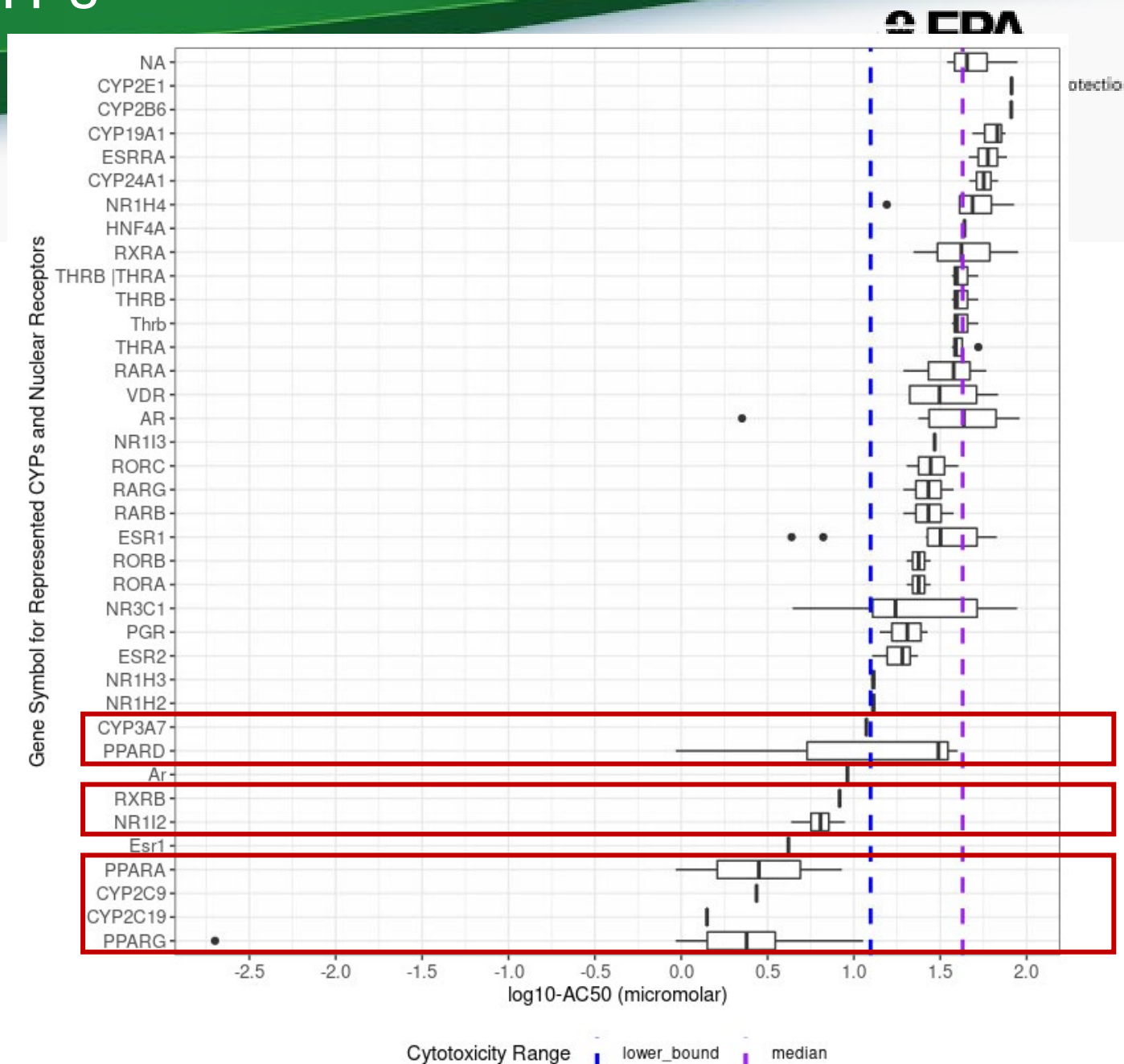
What about TBBPA?

- TBBPA is a fairly promiscuous substance (hit rate > 30%), with clear cytotoxicity across platforms at ~40 μM (lower bound estimate of ~12 μM).
- It's possible that some nuclear receptor activity precedes effects on other targets.

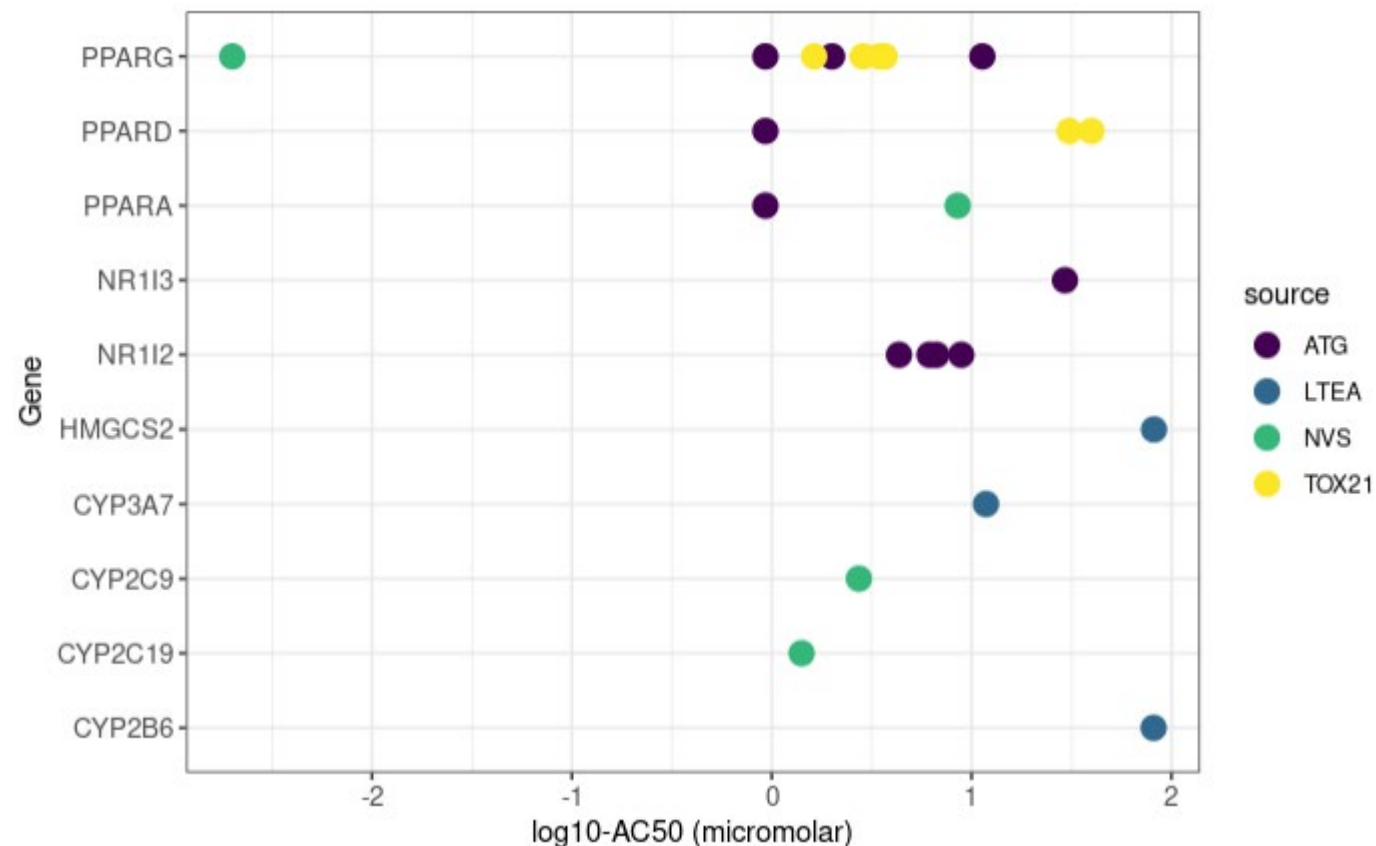


TBBPA: nuclear receptors and CYPs

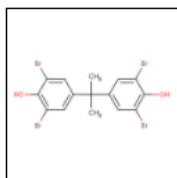
- Looking more specifically at nuclear receptors and related CYPs, there is good support for activity at multiple PPAR, CAR/PXR, at concentrations lower than other receptors and/or cytotoxicity.



- Looking at just PPAR, PXR, and CAR related endpoints, there is redundant support across assay technologies.



- TBBPA is not very active in the ToxCast ER and AR pathway models; a borderline hit for the AR antagonist model.



3,3',5,5'-Tetrabromobisphenol A






79-94-7 | DTXSID1026081

Searched by DSSTox Substance Id.

ToxCast: Models

ToxCast Model Predictions

 Download ToxCast Model Predictions ▼

Model	Receptor	Agonist	Antagonist	Binding
 ToxCast Pathway Model (AUC)	Androgen	0.00	0.104	-
 ToxCast Pathway Model (AUC)	Estrogen	0.00	3.81e-7	-
 COMPARA (Consensus)	Androgen	Inactive	Active	Active
 CERAPP Potency Level (From Literature)	Estrogen	-	-	-
 CERAPP Potency Level (Consensus)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)

TBBPA: HT-H295R model



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20 Years

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Advance Access Publication Date: December 1, 2017

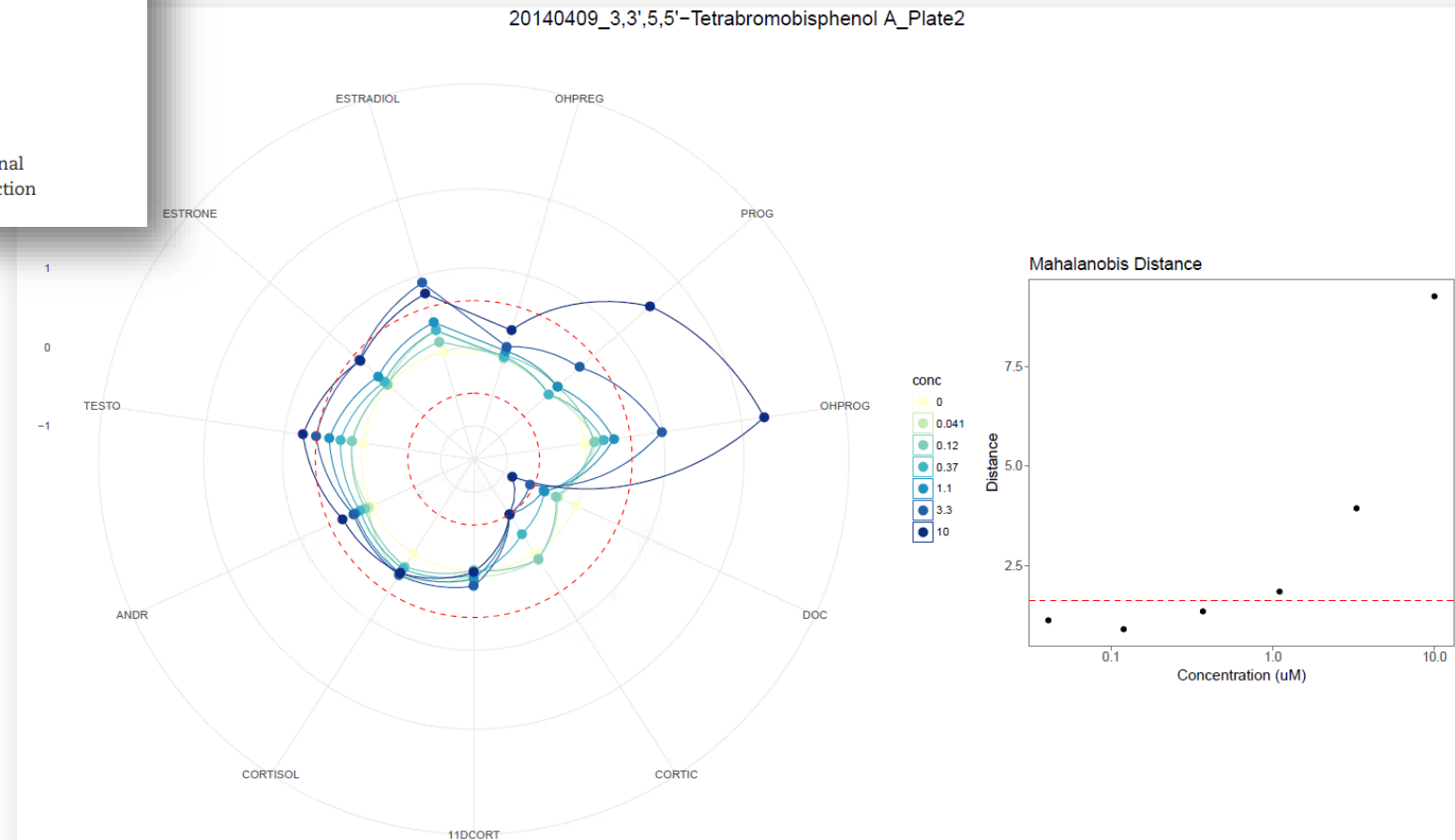
Research Article

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}

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- However, TBBPA has a moderate effect on the HT-H295R Mahalanobis distance model due to moderate effect sizes on progesterone synthesis.



TBBPA: with respect to thyroid-related targets

- It seems that TBBPA may participate in a “permissive heterodimer” effect for TR and possibly other RXR heterodimer partners.
- Several cell-free, non-receptor thyroid hormone synthesis-related targets (TPO, NIS, and DIO) are inhibited by TBBPA at concentrations (20-50 μ M) that approach cytotoxicity and/or exceed PPAR-related effects (1-10 μ M). The parallel cytotoxicity/non-specific protein inhibition assays were negative.
- Could be interesting to see TBBPA in a thyroid microtissue model of thyroid hormone synthesis to see if effects were observed.

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2	2309	NHEERL_MED_hDIO1_dn	39.5589136388614	deiodinase
3	2533	NHEERL_MED_hDIO3_dn	39.5937278083094	deiodinase
4	2037	NIS_RAIU_inhibition	40.2393801696582	transporter
5	2532	NHEERL_MED_hDIO2_dn	51.2827337018911	deiodinase

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- 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



EPA's Center for Computational Toxicology and Exposure