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

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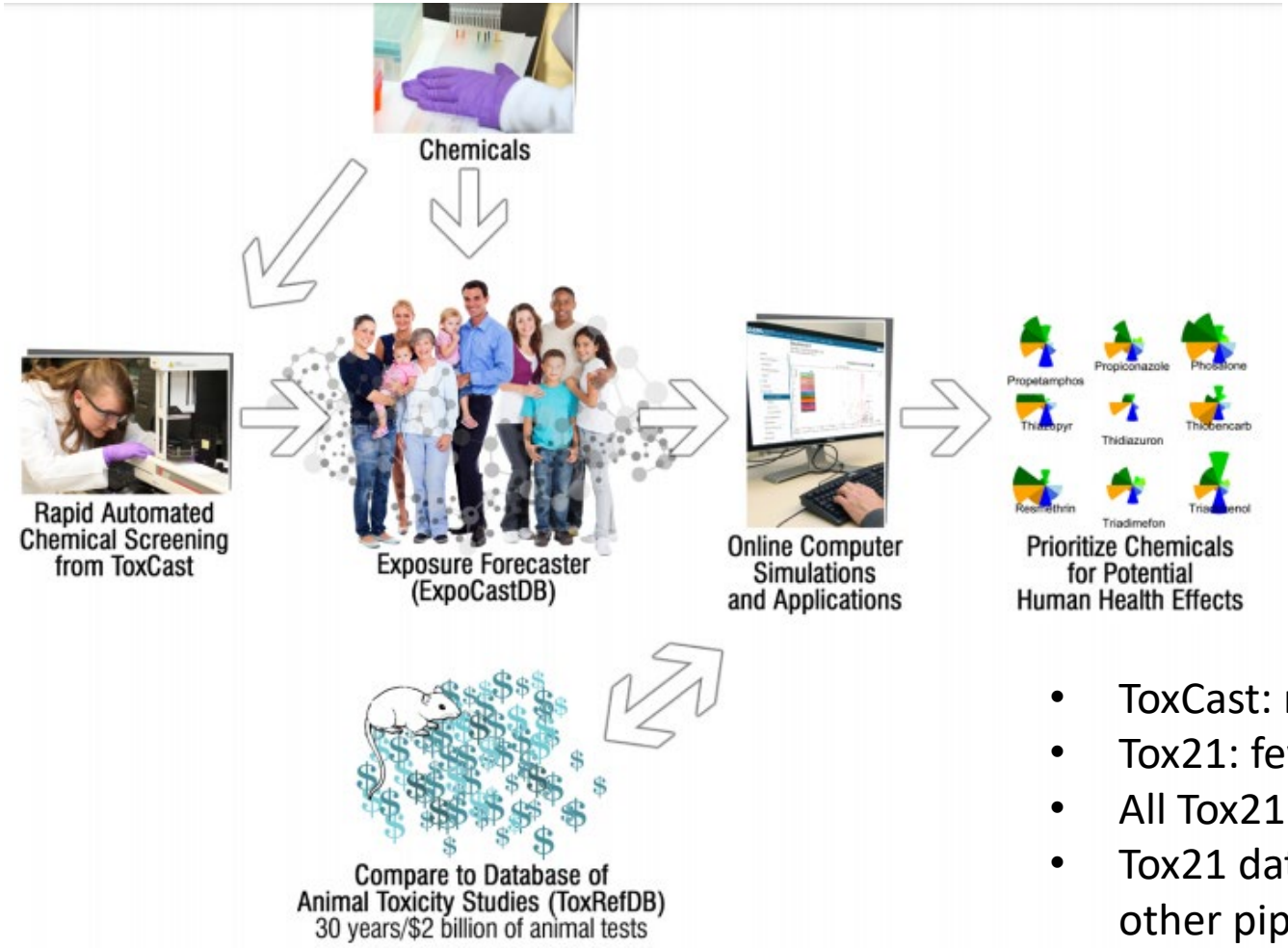
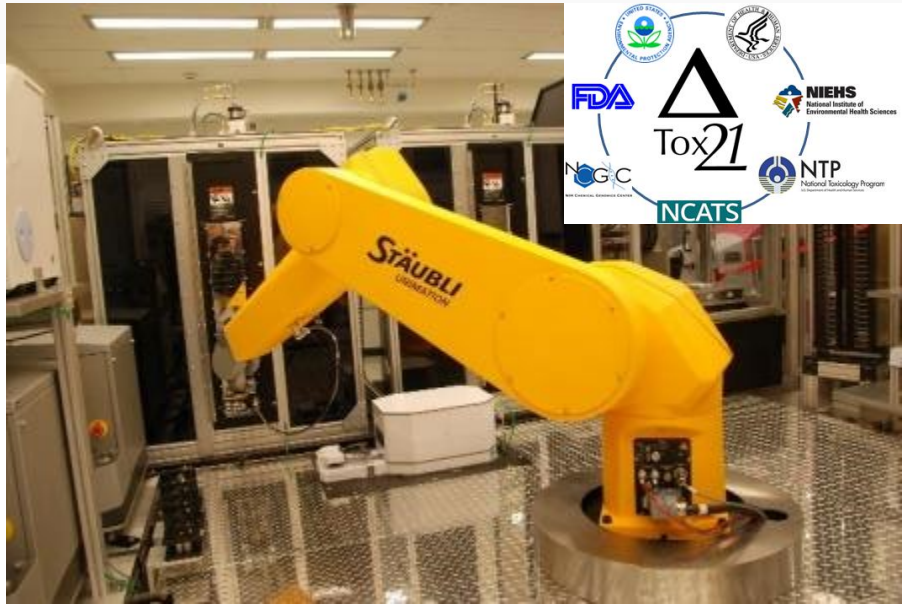
ToxCast and Tox21 have generated a lot of publicly available bioactivity data for hazard screening and prediction.

United States Environmental Protection Agency

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.

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

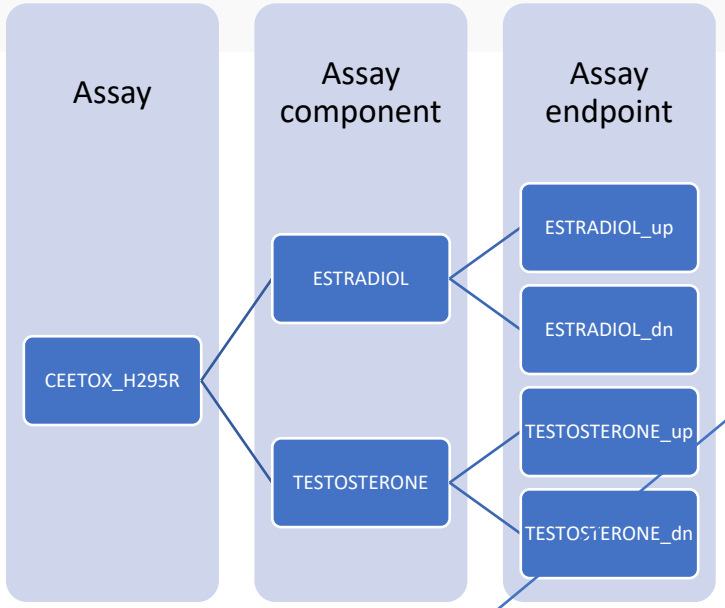
Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

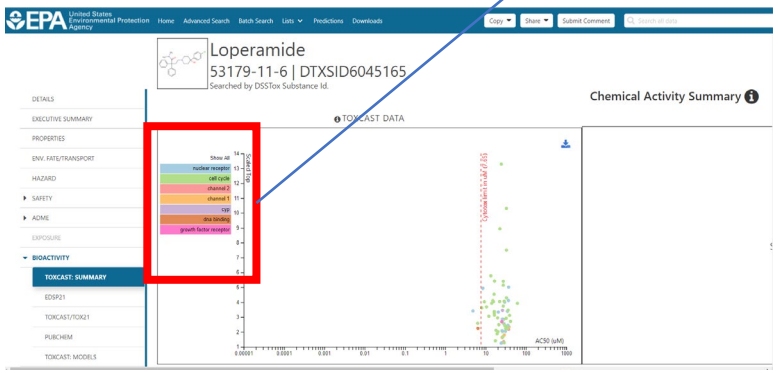
These assay endpoints were notable additions in invitrodb version 3.3.

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

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
  - 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

What can be done with ToxCast data?

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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.

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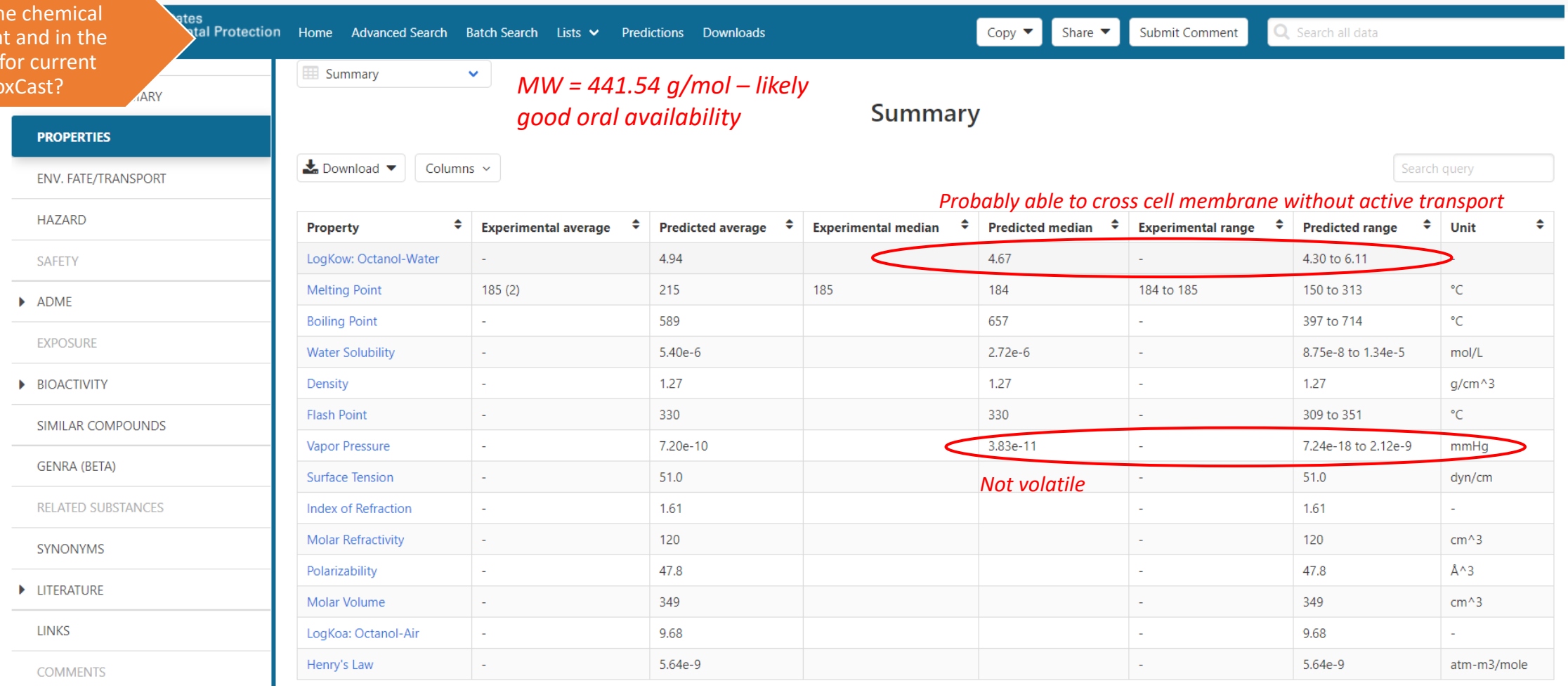
Vignette one: bioactivity for weight-of-evidence/biological questions

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

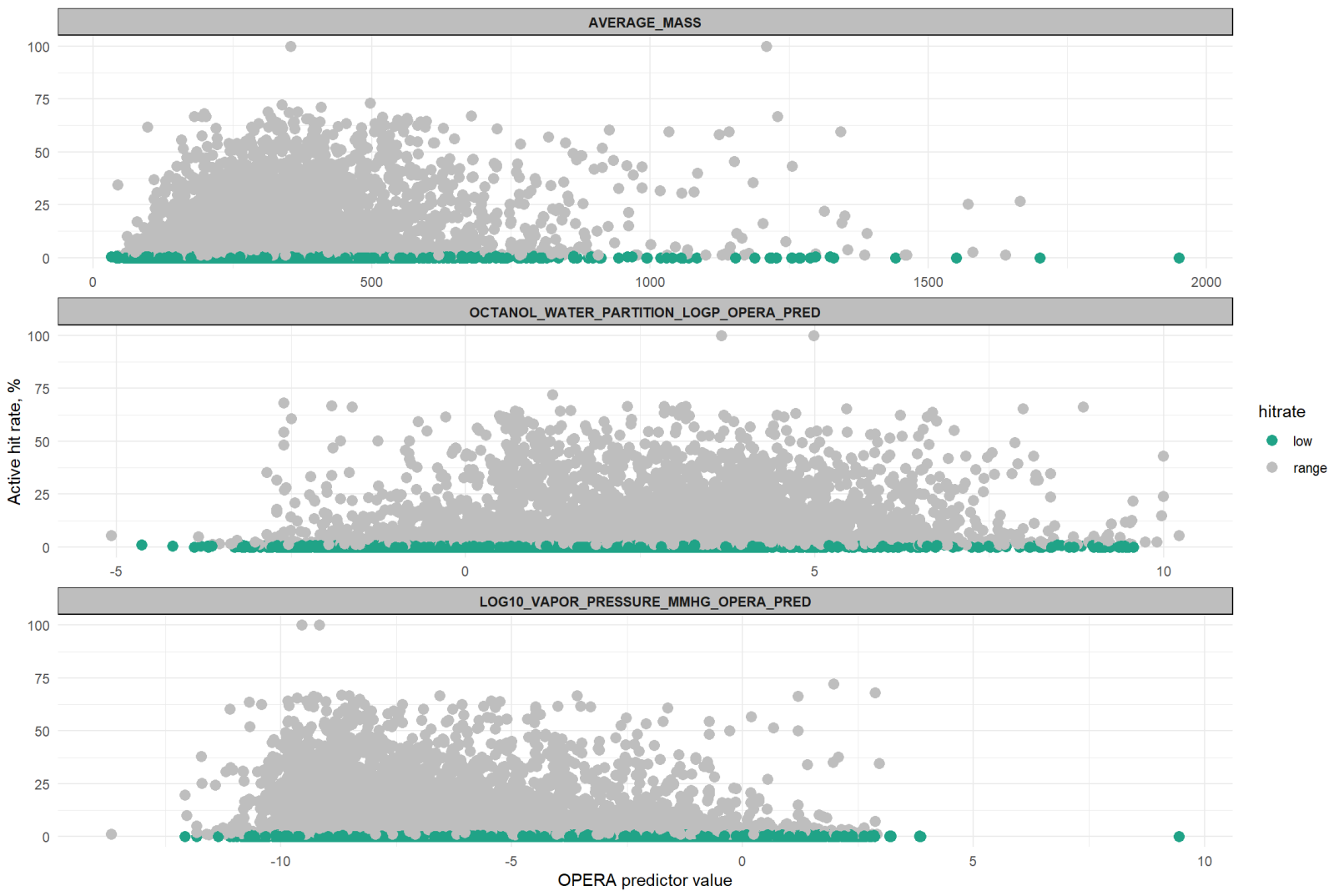
Mystery compound A: in domain of current screening?

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



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

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

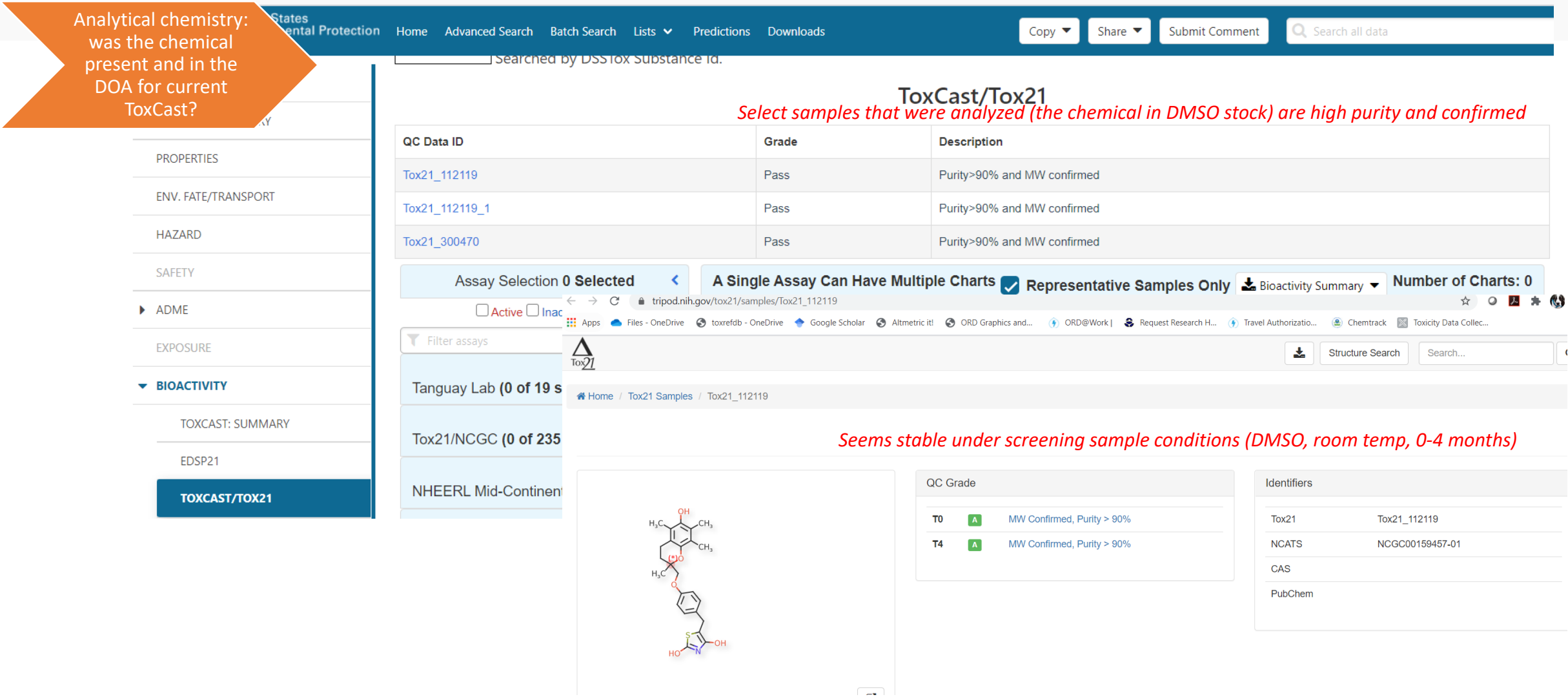


These physicochemical properties may be helpful in considering substances that look negative across ToxCast, but physicochemical properties don’t tell the entire story.

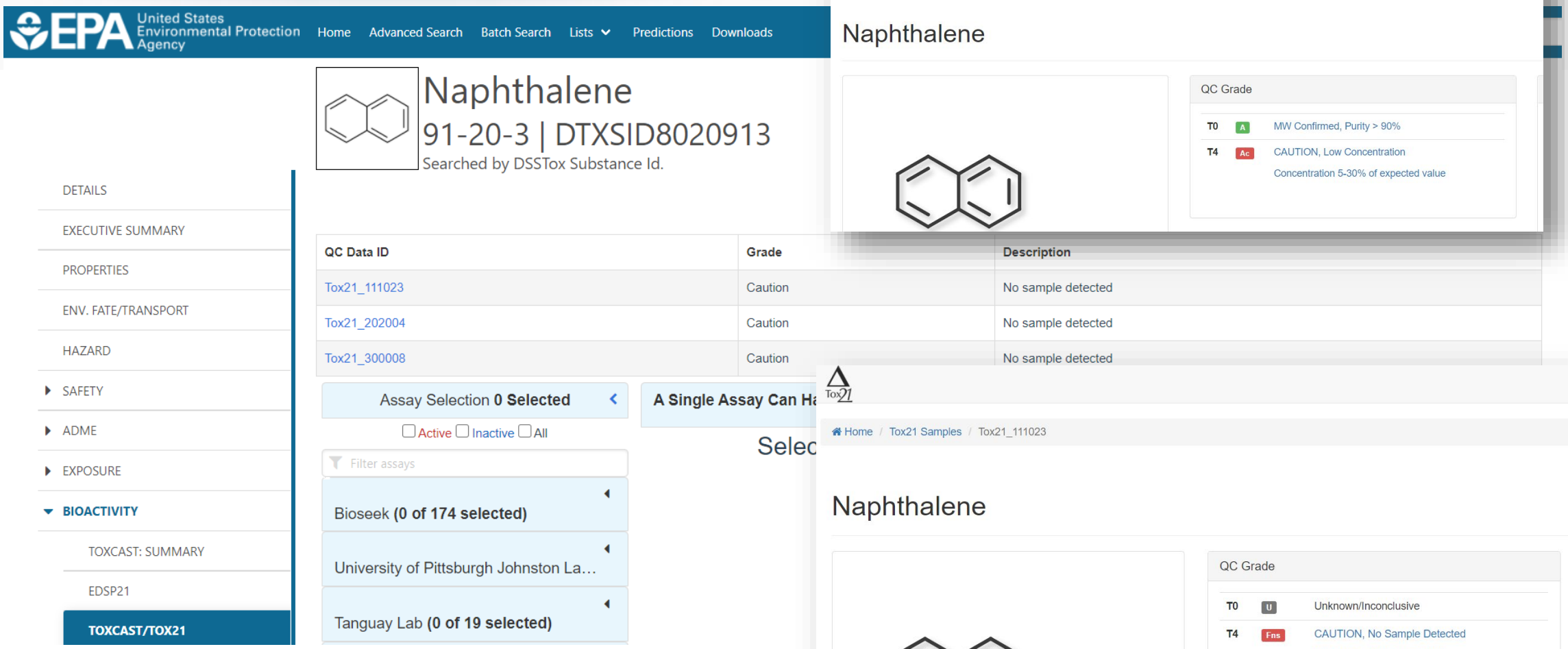
Substances with low hit-rate on the “fringe” of the distribution may need closer consideration to understand if they are within the domain of screening.

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

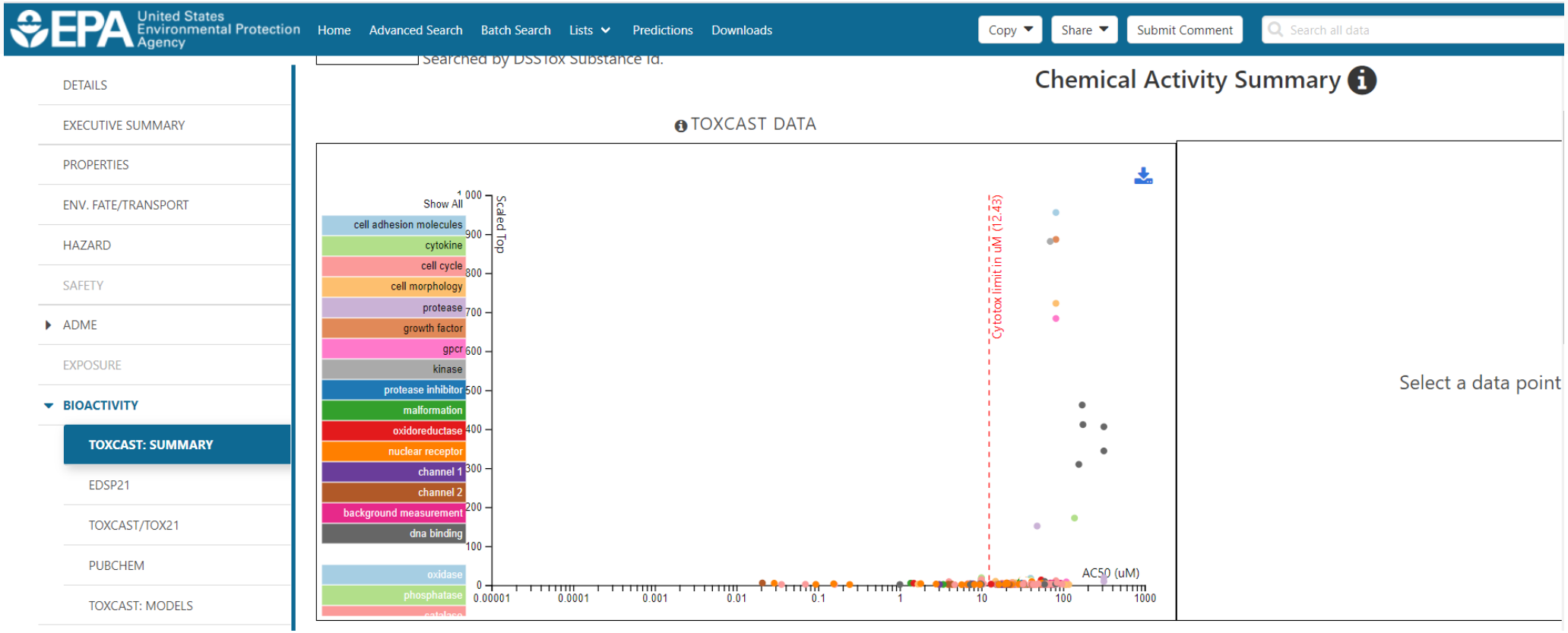
Analytical chemistry: was the chemical present and in the DOA for current ToxCast?



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



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?

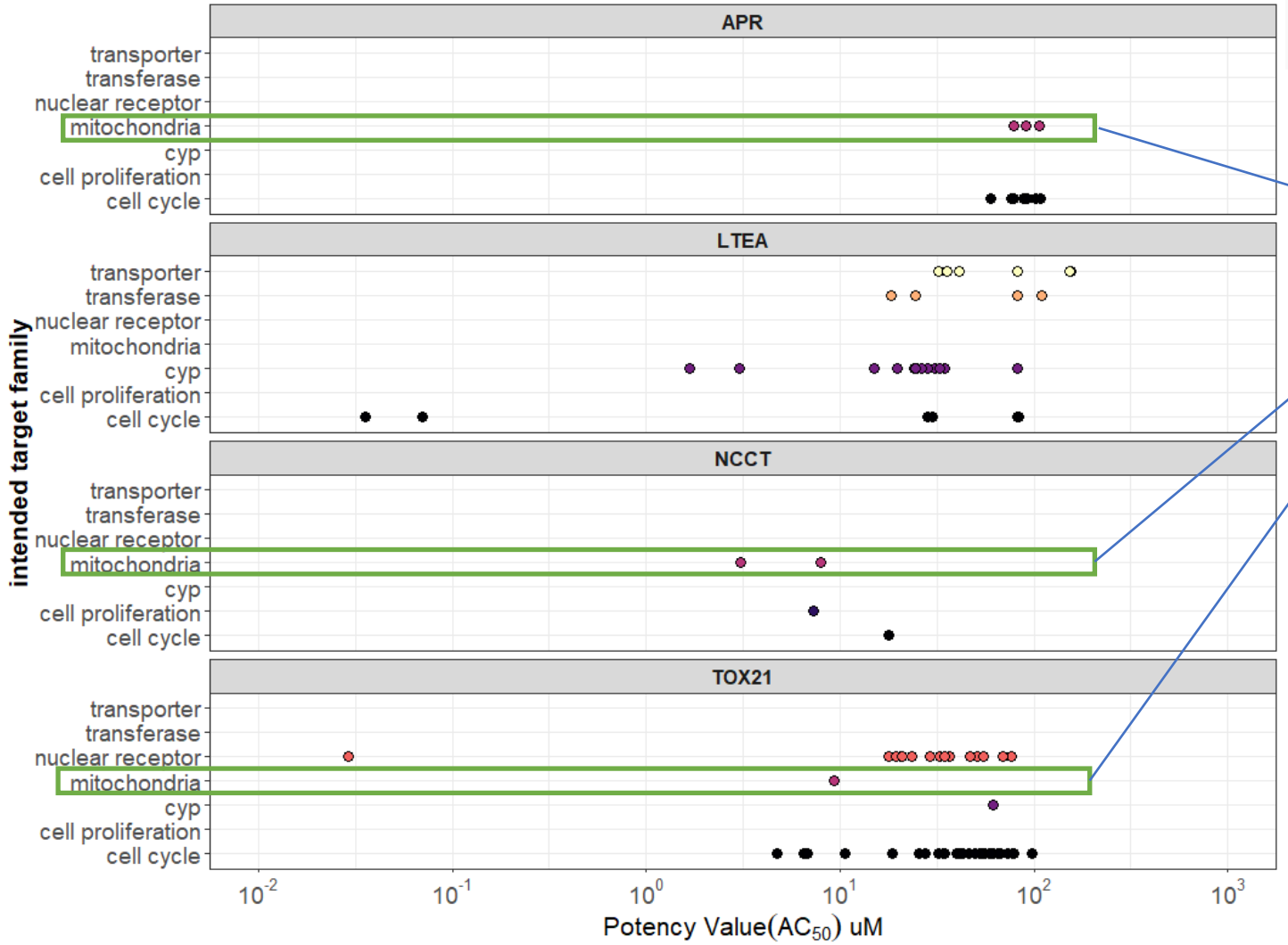
Selective or non-selective?

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 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

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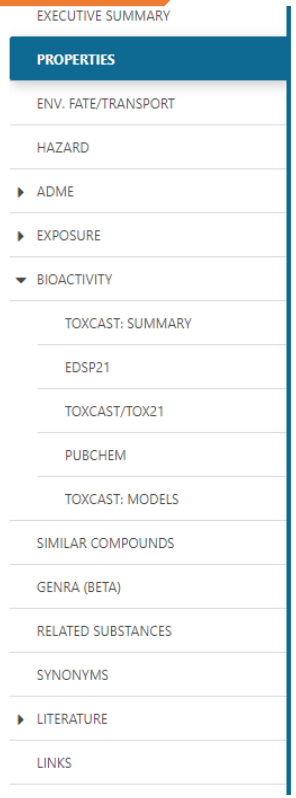
Vignette two: Screening-level endocrine bioactivity assessment

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)?



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	-

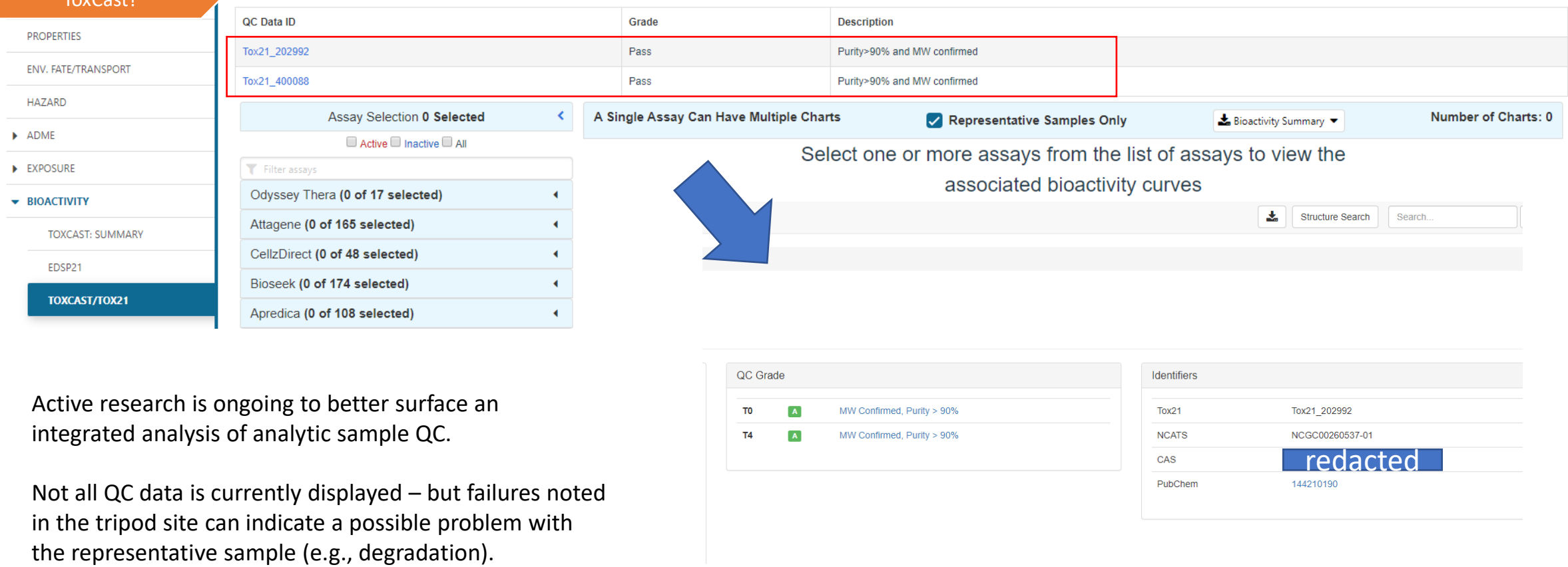
16 records

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

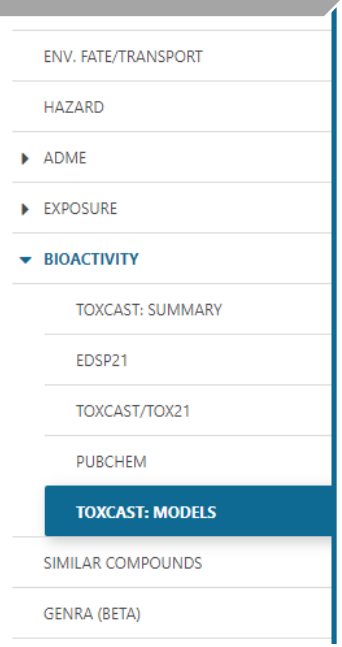


- Active research is ongoing to better surface an integrated analysis of analytic sample QC.
- Not all QC data is currently displayed – but failures noted in the tripod site can indicate a possible problem with the representative sample (e.g., degradation).

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.



ToxCast: Models

ToxCast Model Predictions

Download ToxCast Model Predictions

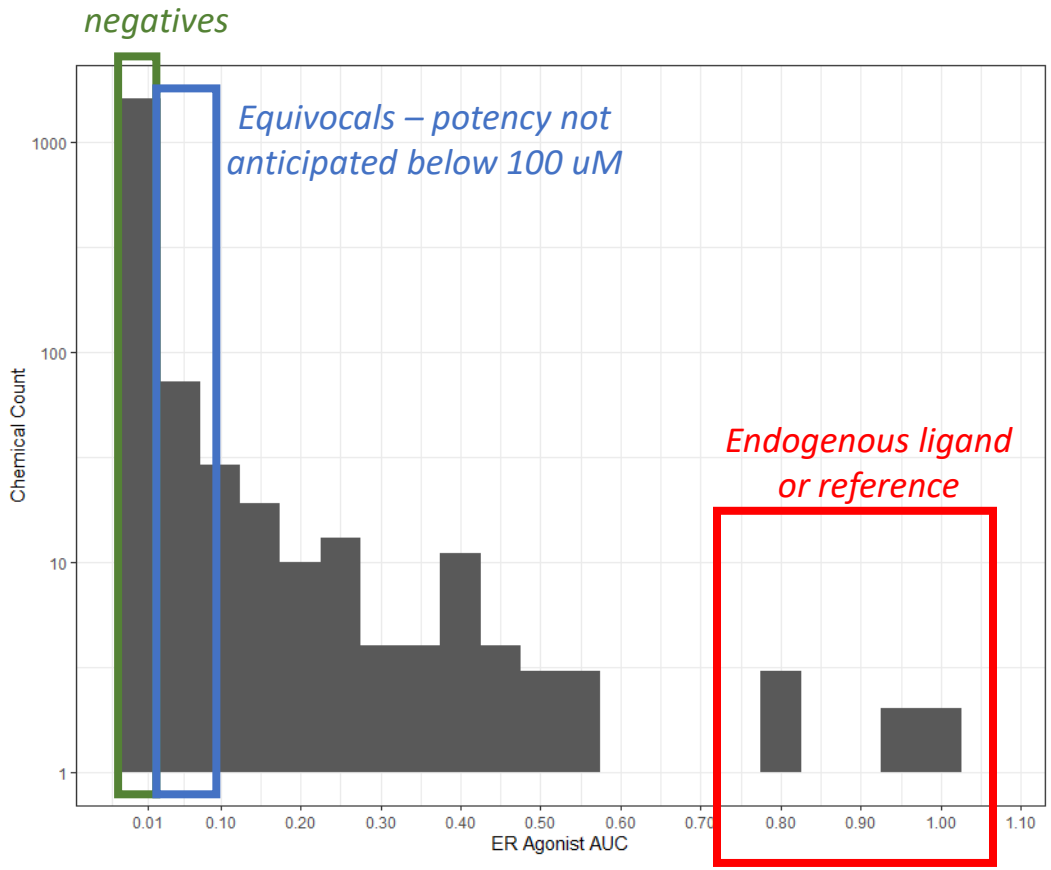
>0.1 = positive; 0.001-0.1 = equivocal

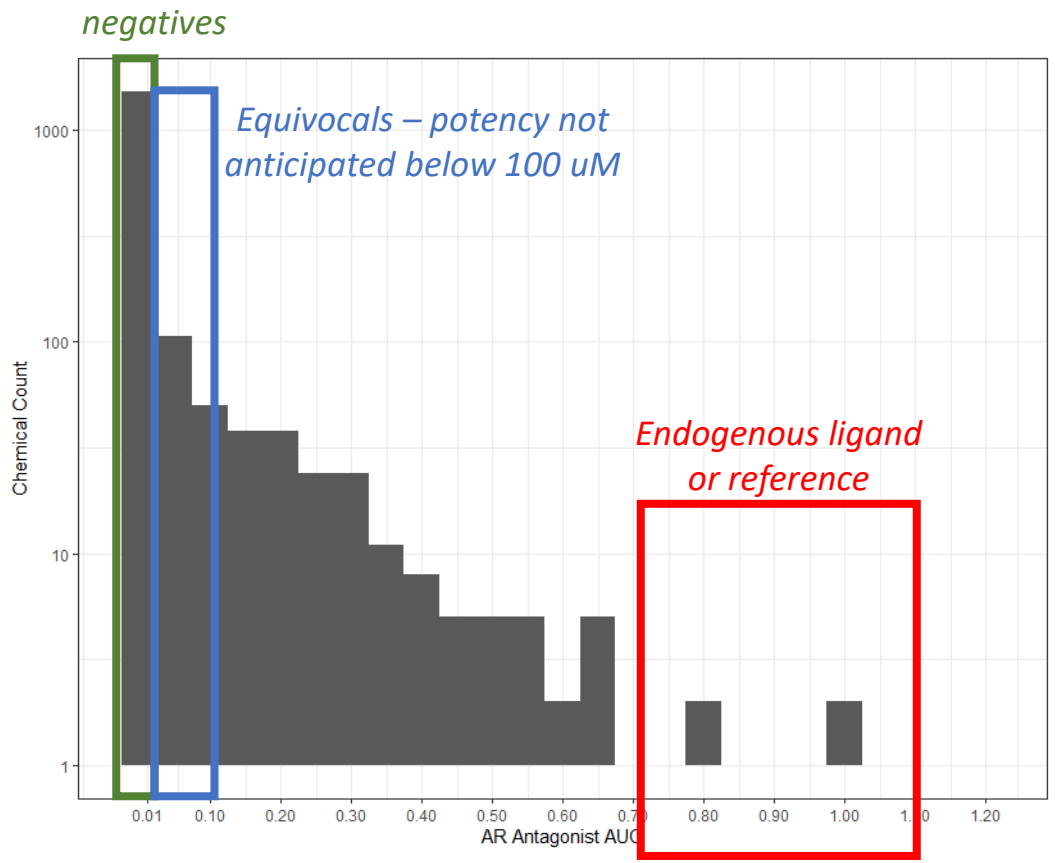
Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
COMPARA (Consensus)	Androgen	Inactive	Active	Active
CERAPP Potency Level (From Literature)	Estrogen	Active (Weak)	-	Active (Weak)
CERAPP Potency Level (Consensus)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

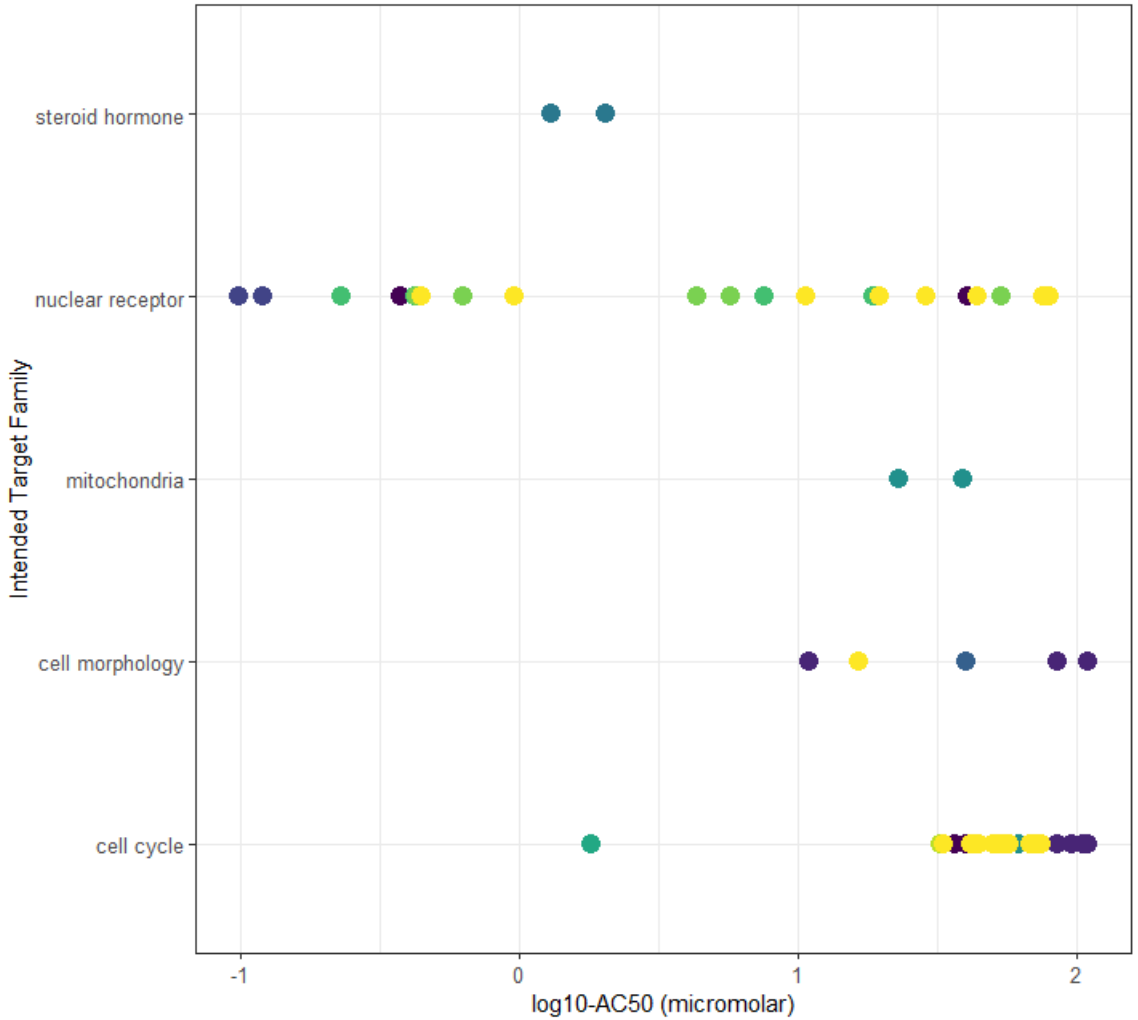
As of now, the models supported in the CompTox Chemicals Dashboard are endocrine-related but hope to expand to other published models in the future.
Consult the peer-reviewed literature for additional models and interpretations.

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

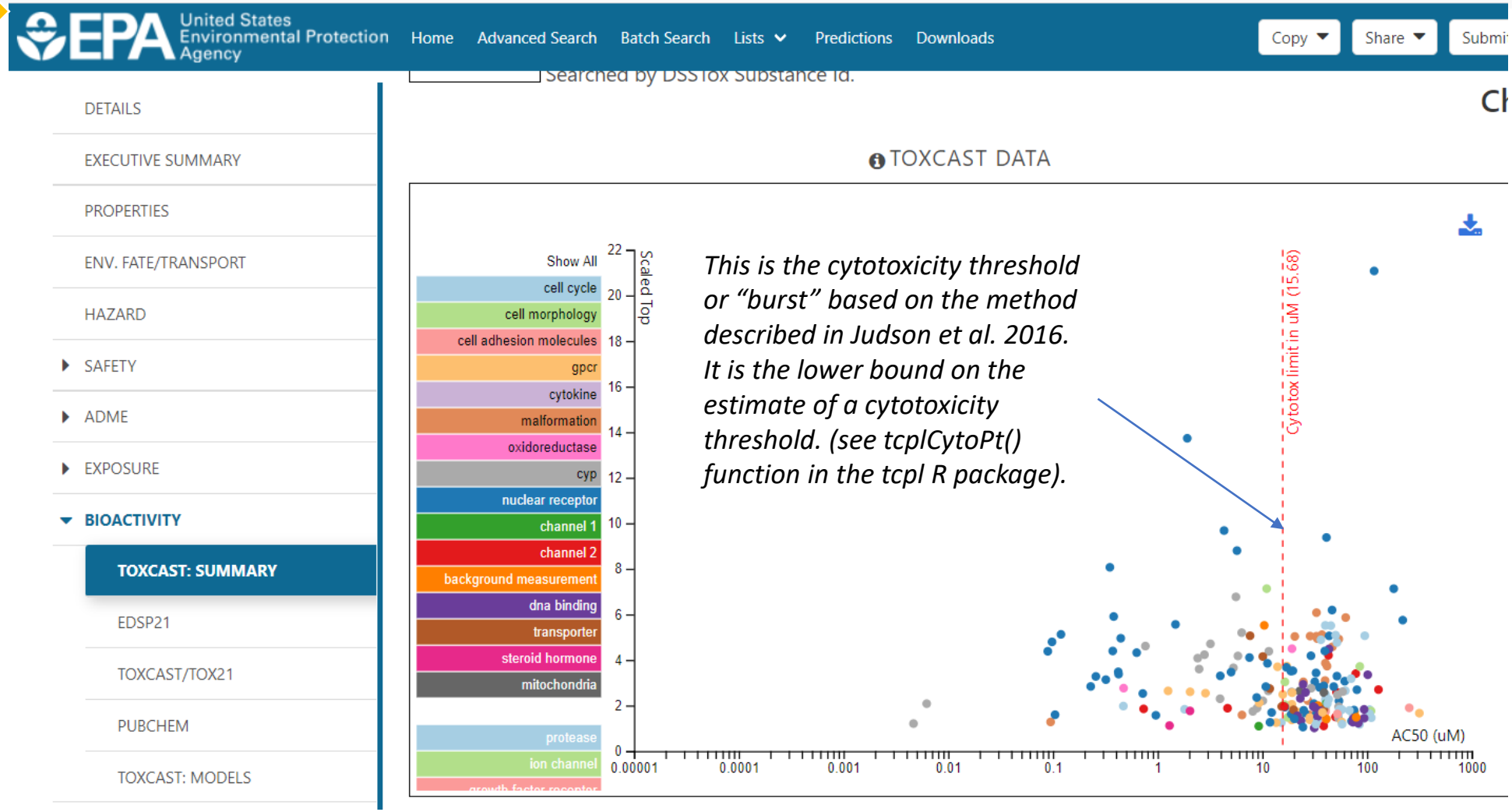


Downloaded ToxCast Summary from the CompTox Chemicals Dashboard, and filtered for one gene of interest

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_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

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?

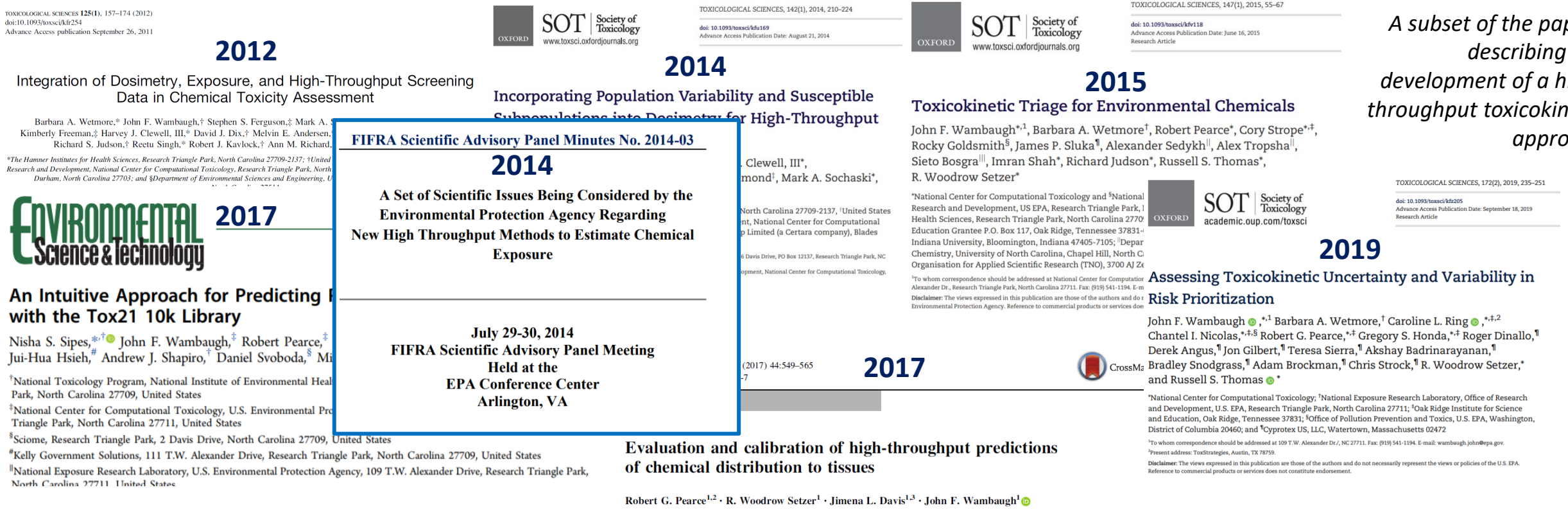
- AC50 < burst?
- AC50 0.5$\log_{10}$ distance from burst?
- AC50 < parallel viability assays? This makes sense if you have parallel viability assays.
- How else to filter ToxCast data: 3+ caution flags and curves with both low efficacy and potency values below the concentration range screened, certain 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?

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.

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.



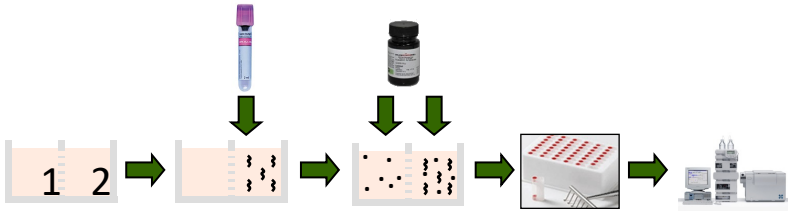
A subset of the papers describing the development of a high-throughput toxicokinetic approach

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

High throughput toxicokinetics (HTTK)

in vitro data

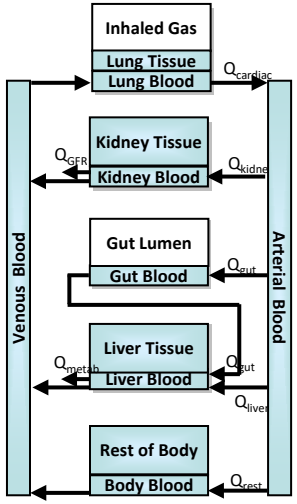
Hepatic clearance from suspended hepatocytes



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

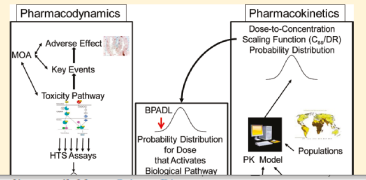
Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

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*

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Toxicology in Vitro 47 (2018) 213–227

Contents lists available at ScienceDirect

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

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 C
[d] L'Oréal Safety
[e] L'Oréal Safety
[f] Currently at G

Environment International 137 (2020) 105470

Contents lists available at ScienceDirect

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 Sobanski,§ Jill A. Francone,¶ Ann M. Richa
Angrish,
Bahadori
Rasenbe

PLOS ONE

2020

Toxicology and Applied Pharmacology 387 (2020) 114774

Contents lists available at ScienceDirect

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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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

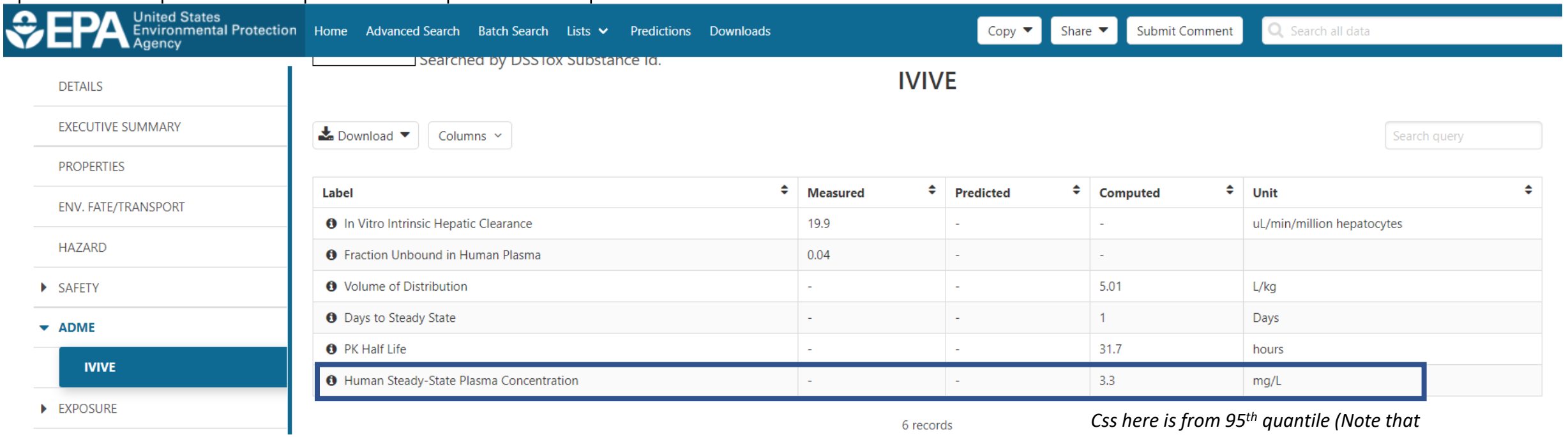
IVIVE via high-throughput toxicokinetic data and models

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).

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

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

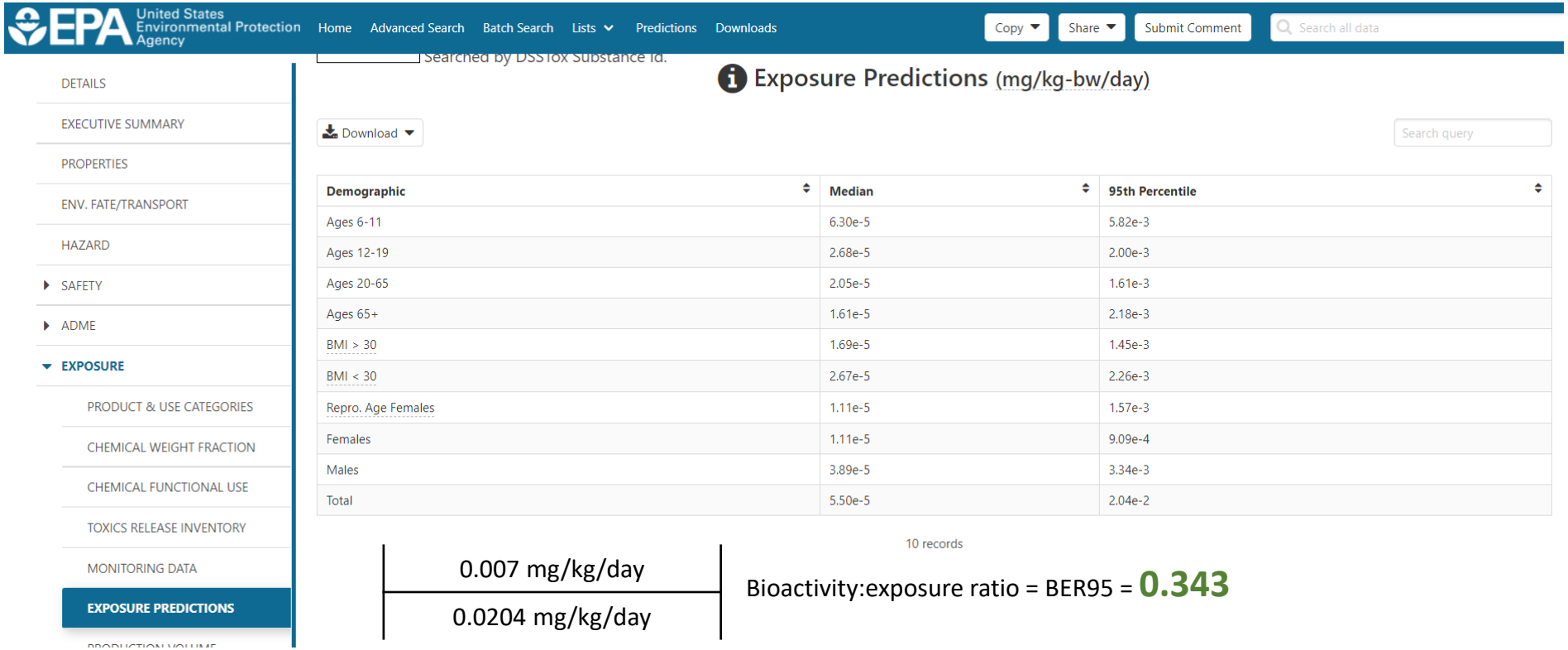


Css here is from 95th quantile (Note that 95th concentration quantile is the same population as the 5th dose quantile).

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".



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.

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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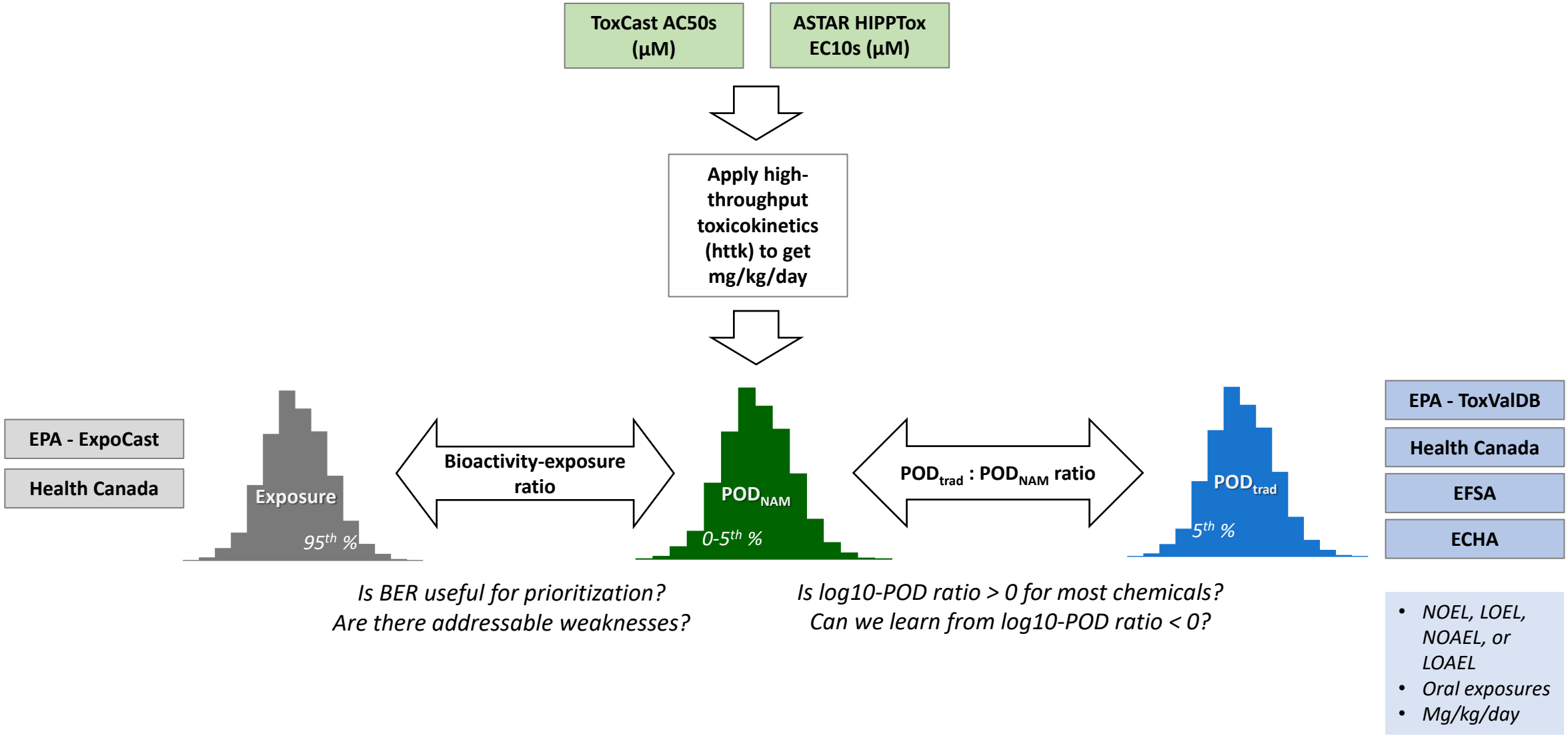
Research Article

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

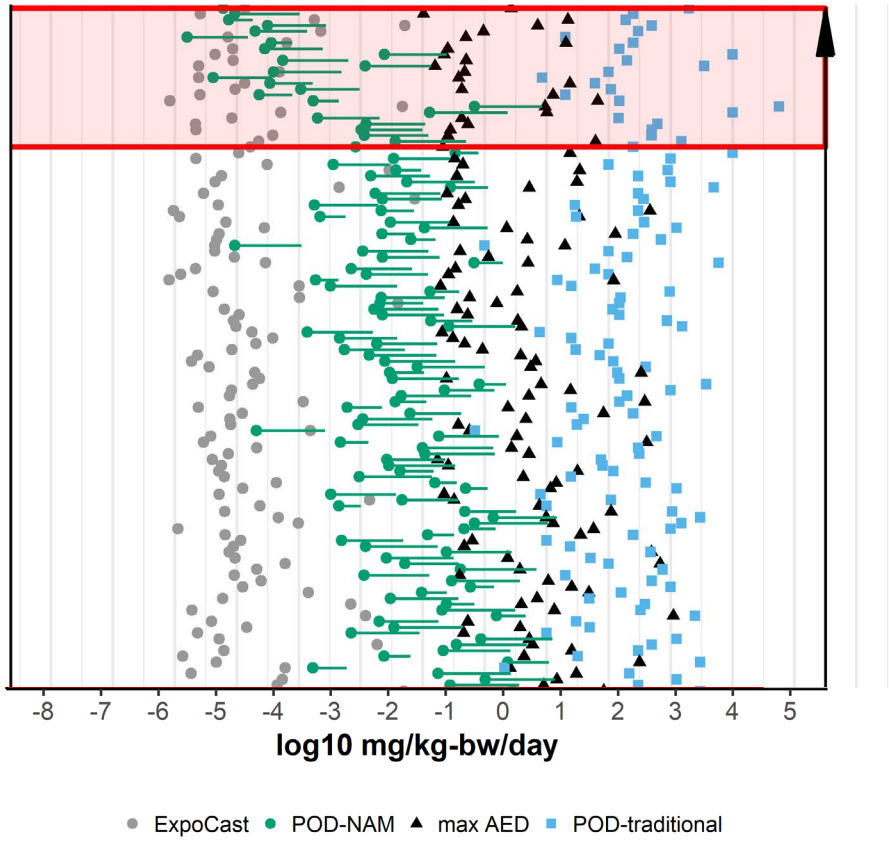


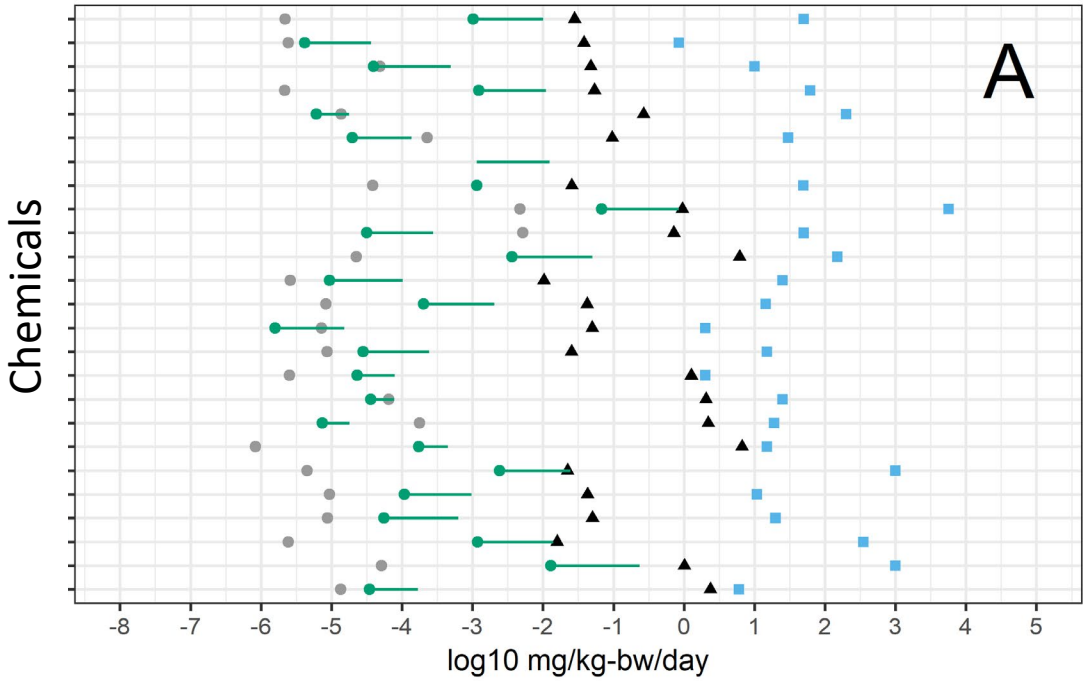
Is BER useful for prioritization?
Are there addressable weaknesses?

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

EPA's Center for Computational Toxicology and Exposure