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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.

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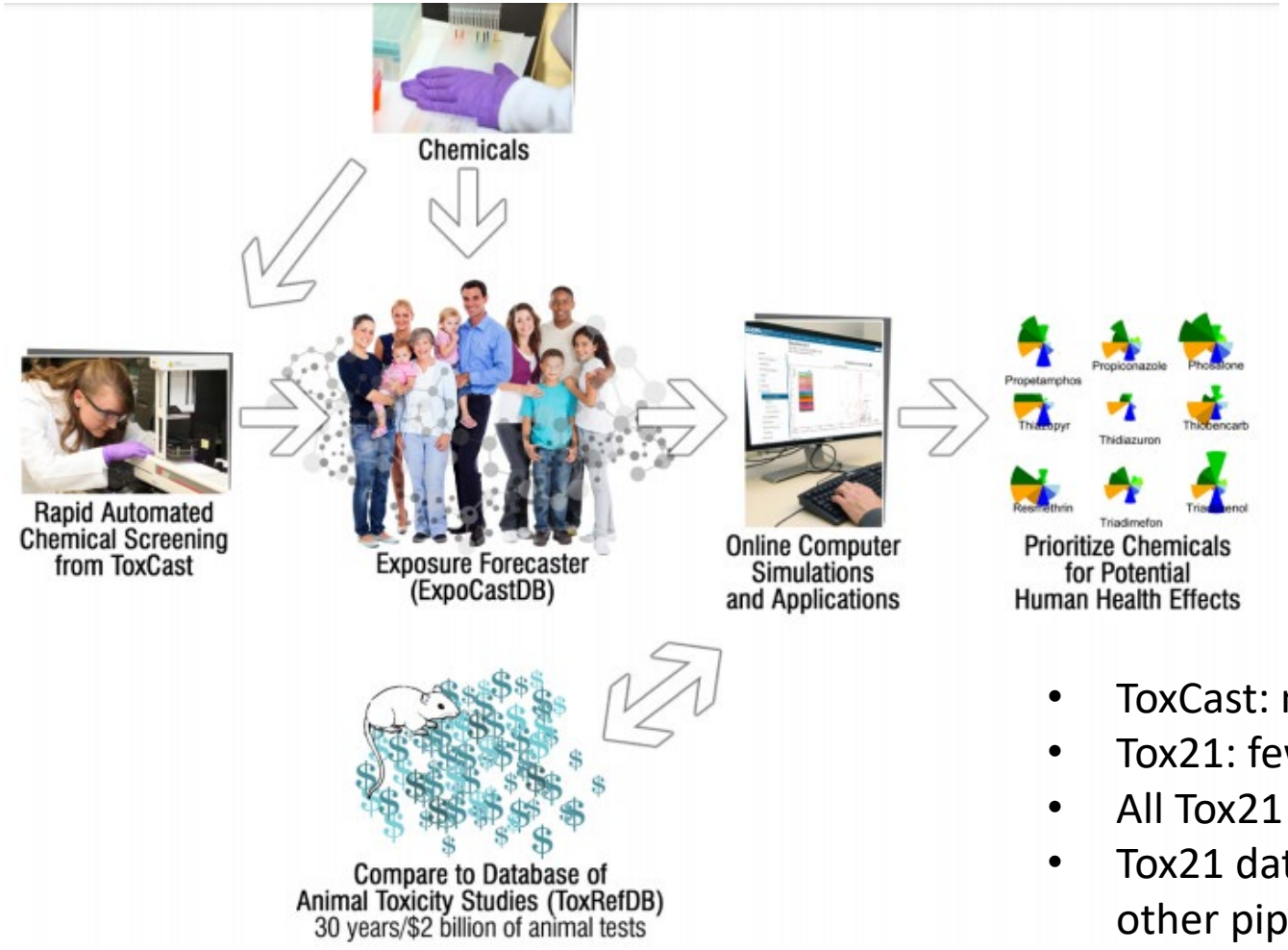
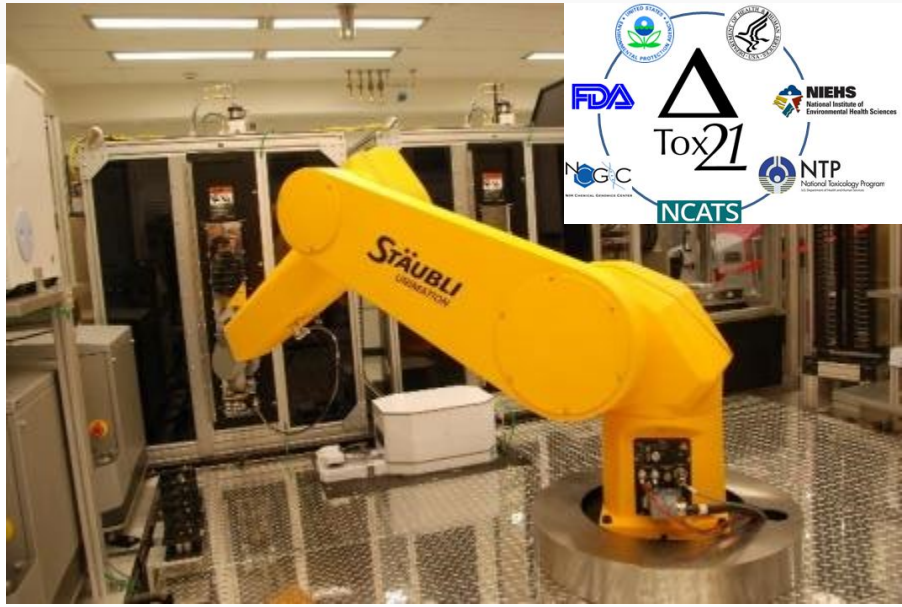
EPA's ToxCast program at a glance





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.

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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

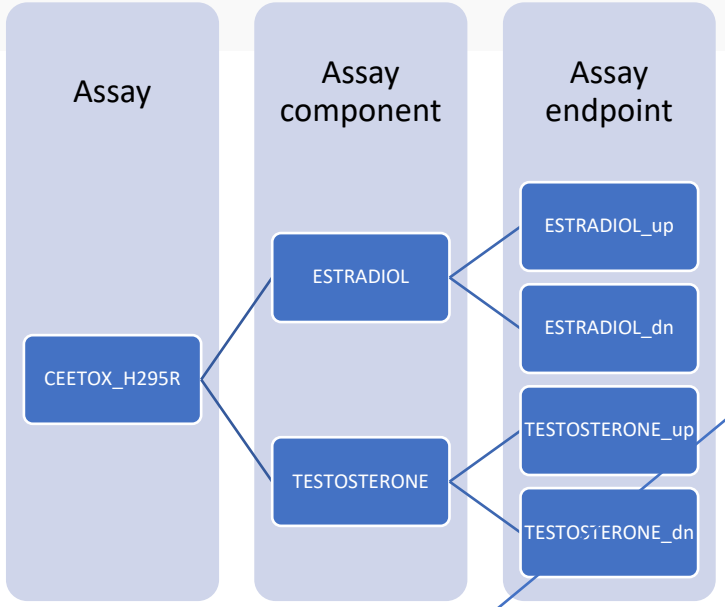
Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.

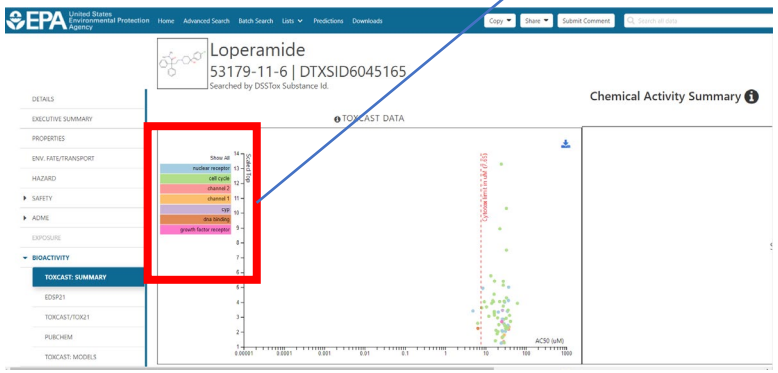
These assay endpoints were notable additions in invitrodb version 3.3.

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

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.

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

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

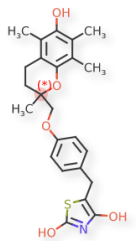
tripod.nih.gov/tox21/samples/Tox21_112119

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	

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

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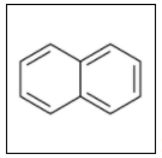
Home | Advanced Search | Batch Search | Lists | Predictions | Downloads



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)**

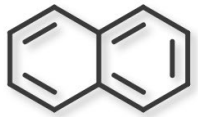
A Single Assay Can Ha

Selec

Tox21

Home / Tox21 Samples / Tox21_300008

Naphthalene



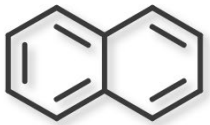
QC Grade

T0	A	MW Confirmed, Purity > 90%
T4	Ac	CAUTION, Low Concentration Concentration 5-30% of expected value

Tox21

Home / Tox21 Samples / Tox21_111023

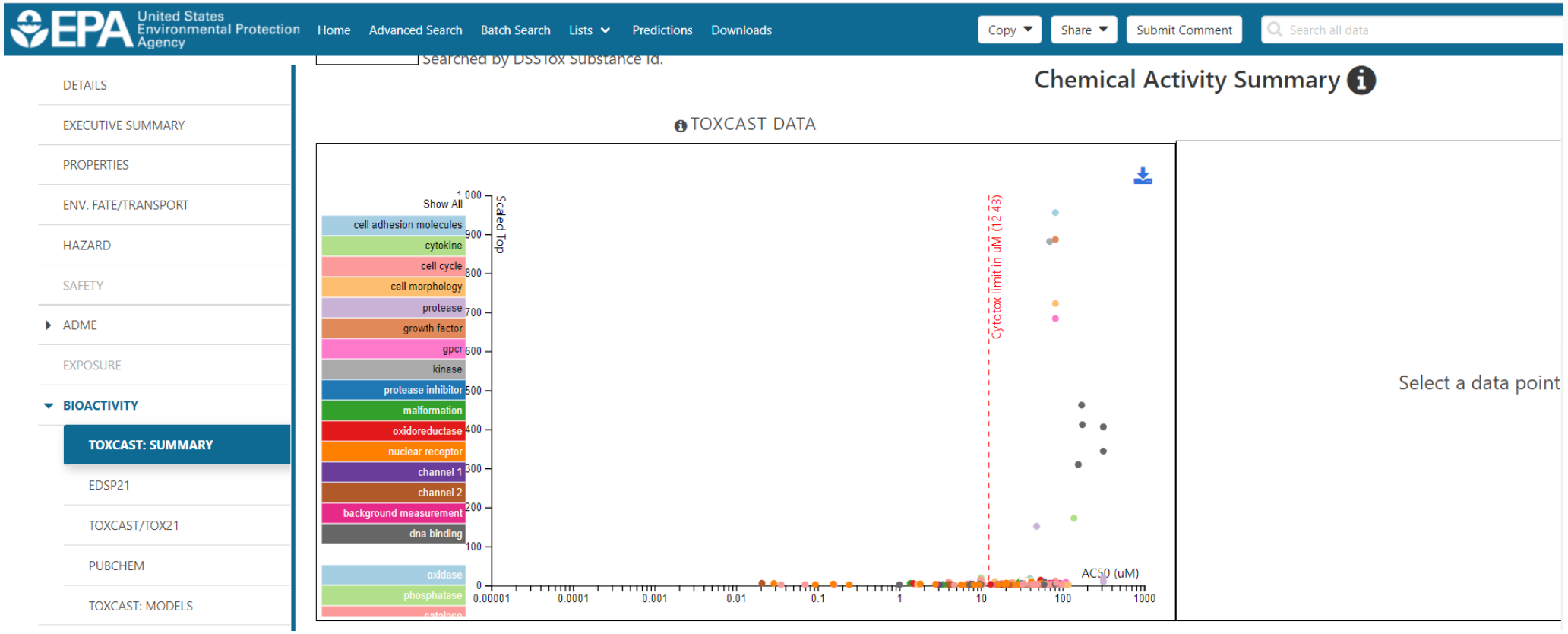
Naphthalene



QC Grade

T0	U	Unknown/Inconclusive
T4	Fns	CAUTION, No Sample Detected Biological Activity Unreliable

But what bioactivity does Mystery Compound A have?



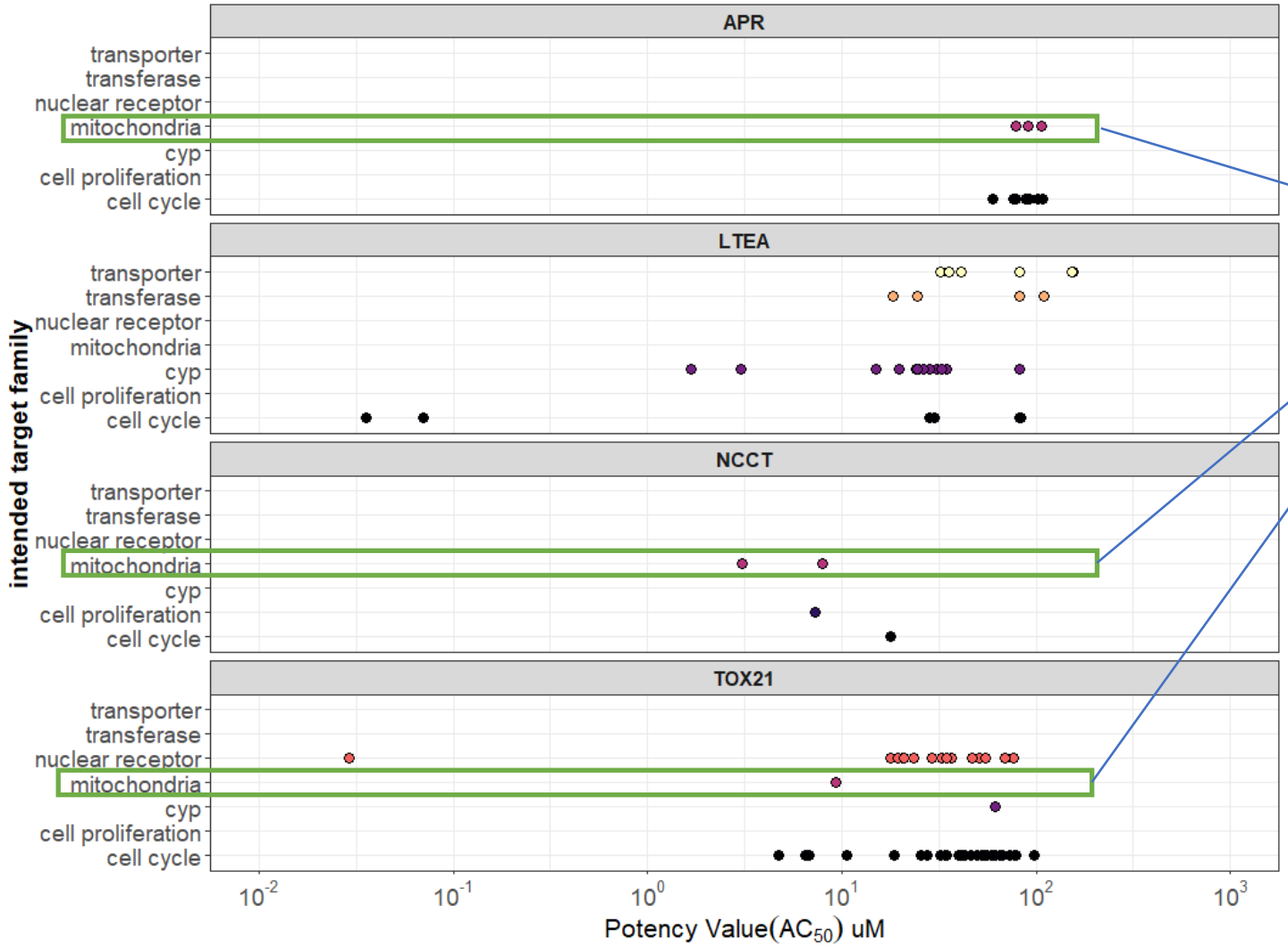
Each assay platform or source can be a surrogate for one or more collections of AOPs

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: 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

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

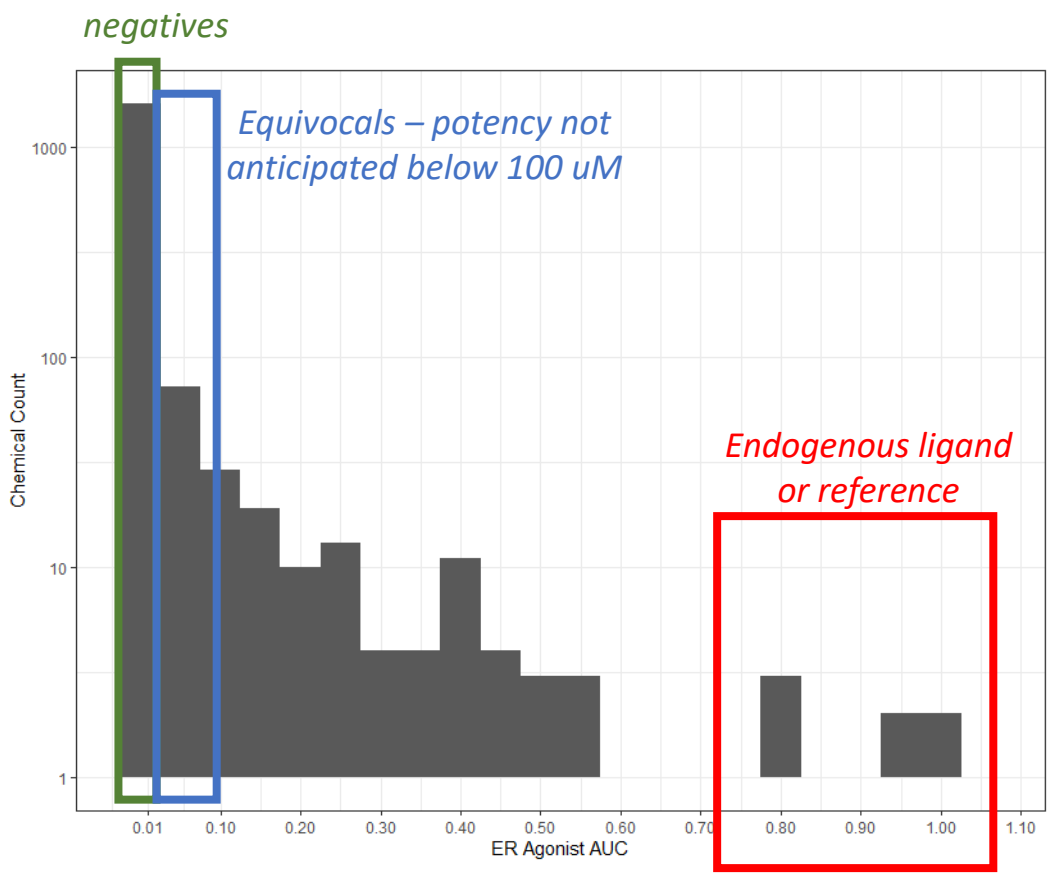
>0.1 = positive; 0.001-0.1 = equivocal

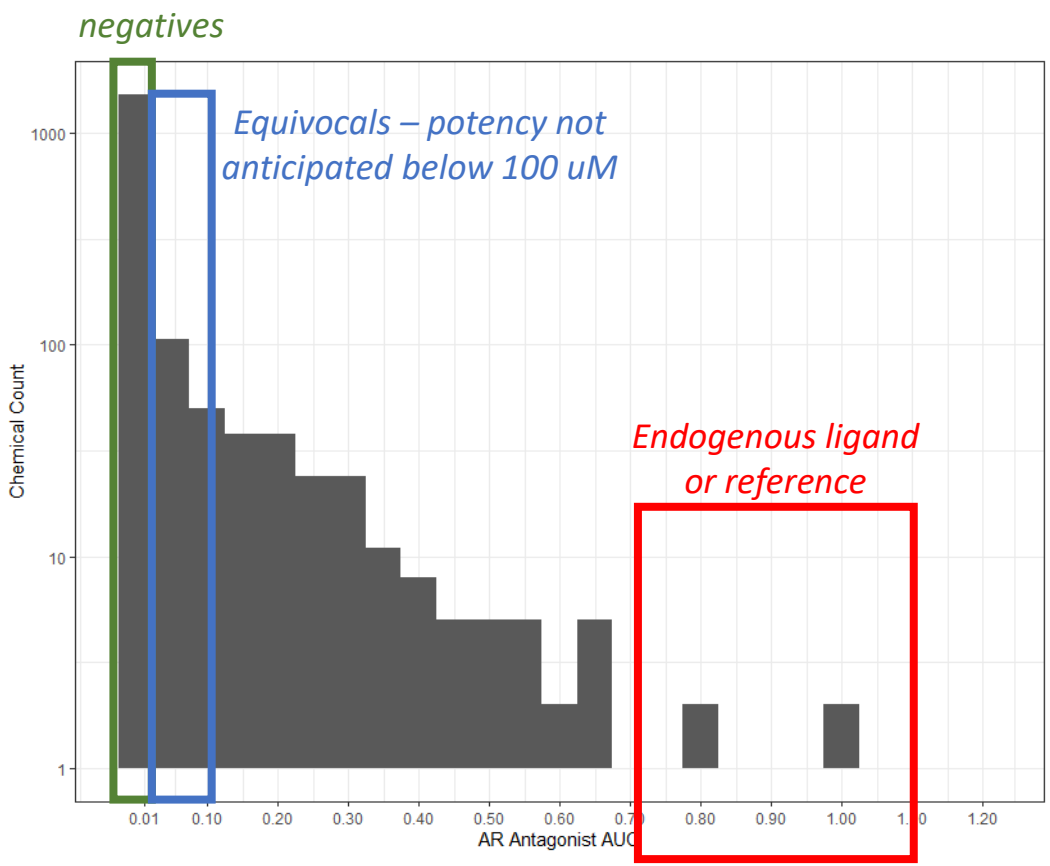
Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
COMPARA (Consensus)	Androgen	Inactive	Active	Active
CERAPP Potency Level (From Literature)	Estrogen	Active (Weak)	-	Active (Weak)
CERAPP Potency Level (Consensus)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

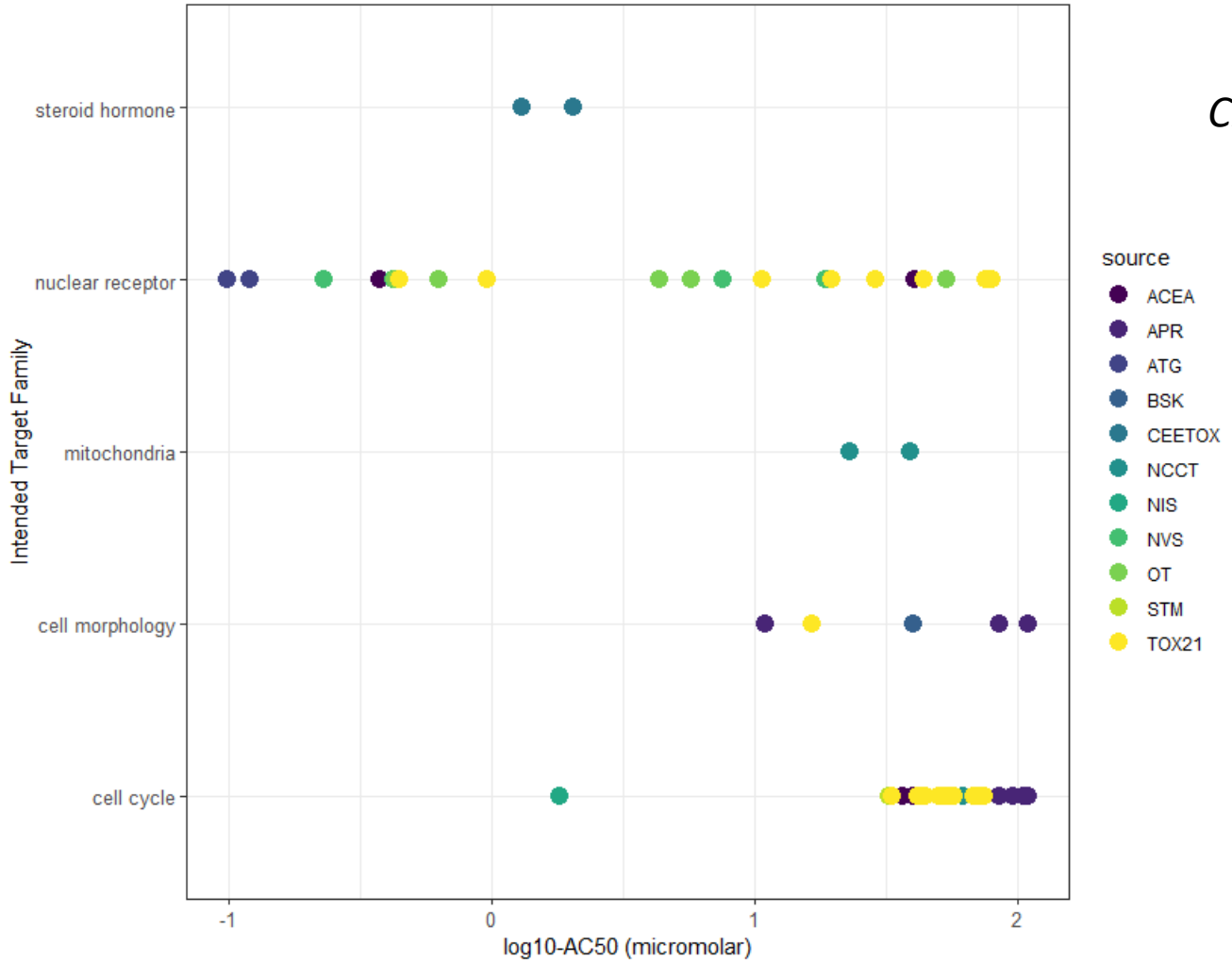
As of now, the models supported in the CompTox Chemicals Dashboard are endocrine-related but hope to expand to other published models in the future.
Consult the peer-reviewed literature for additional models and interpretations.

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

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

United States Environmental Protection Agency

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. …
Kimberly Freeman,‡ Harvey J. Clewell, III,* David J. Dix,† Melvin E. Andersen,
Richard S. Judson,† Reetu Singh,* Robert J. Kavlock,† Ann M. Richard,

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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 …

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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 … Research and Development, US EPA, Research Triangle Park, … Health Sciences, Research Triangle Park, North Carolina 2770… Education Grantee P.O. Box 117, Oak Ridge, Tennessee 37831-… Indiana University, Bloomington, Indiana 47405-7105; ‖Depar… Chemistry, University of North Carolina, Chapel Hill, North C… Organisation for Applied Scientific Research (TNO), 3700 AJ Ze…

†To whom correspondence should be addressed at National Center for Computatio… Alexander Dr., Research Triangle Park, North Carolina 27711. Fax: (919) 541-1194. E-m…

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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

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

Environmental Science & Technology

2017

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

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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]

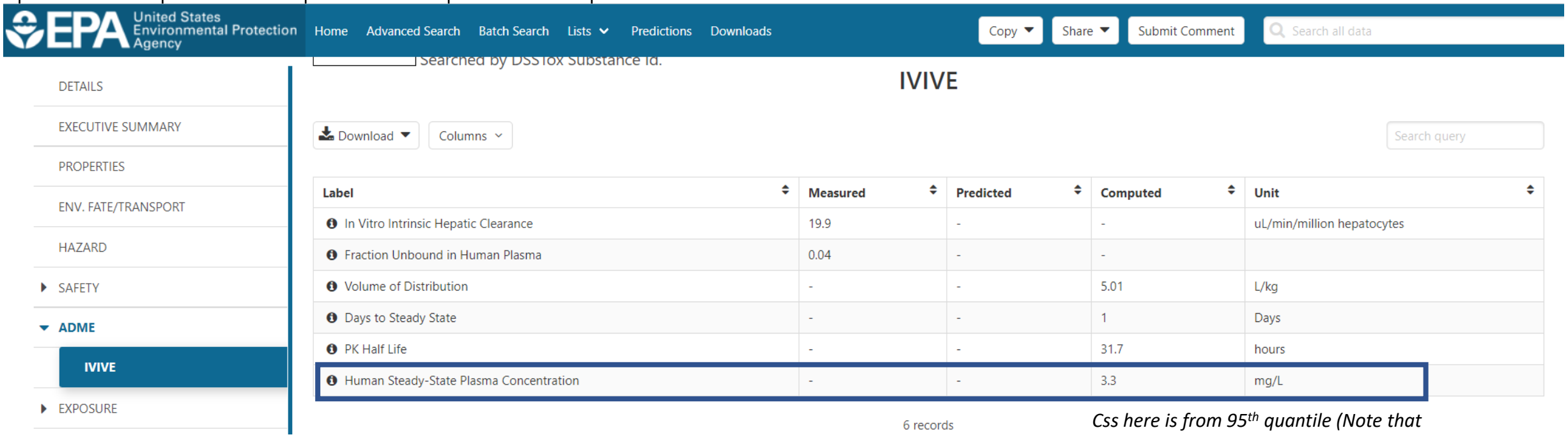
Reverse dosimetry can be leveraged in IVIVE to estimate the exposure that would produce the plasma concentration corresponding to bioactivity

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).

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~~	



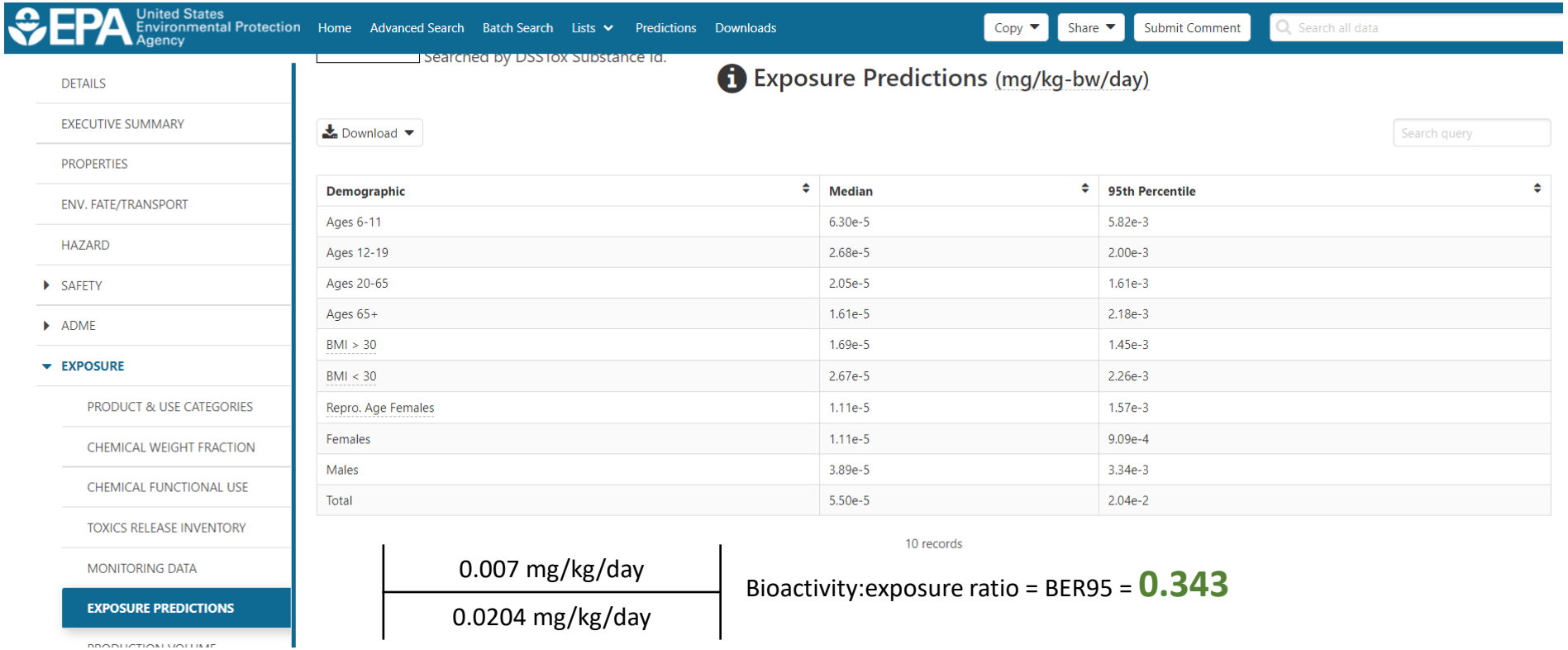
Label	Measured	Predicted	Computed	Unit
In Vitro Intrinsic Hepatic Clearance	19.9	-	-	uL/min/million hepatocytes
Fraction Unbound in Human Plasma	0.04	-	-	
Volume of Distribution	-	-	5.01	L/kg
Days to Steady State	-	-	1	Days
PK Half Life	-	-	31.7	hours
Human Steady-State Plasma Concentration	-	-	3.3	mg/L

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.

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

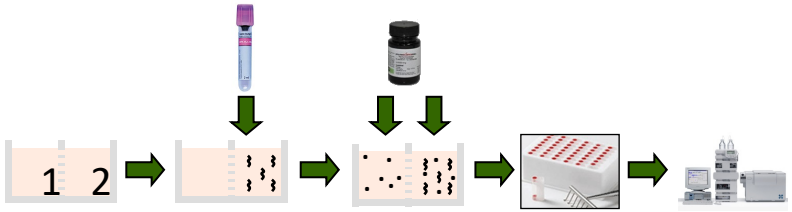
EPA's Center for Computational Toxicology and Exposure

Appendix slides for additional reference on bioactivity:exposure ratio

High throughput toxicokinetics (HTTK)

in vitro data

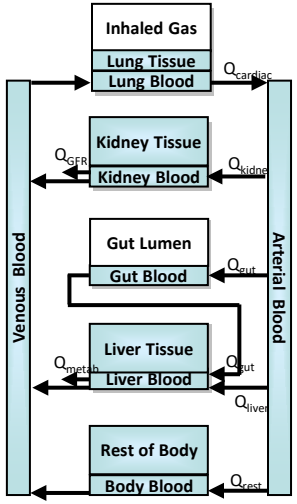
Hepatic clearance from suspended hepatocytes



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

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

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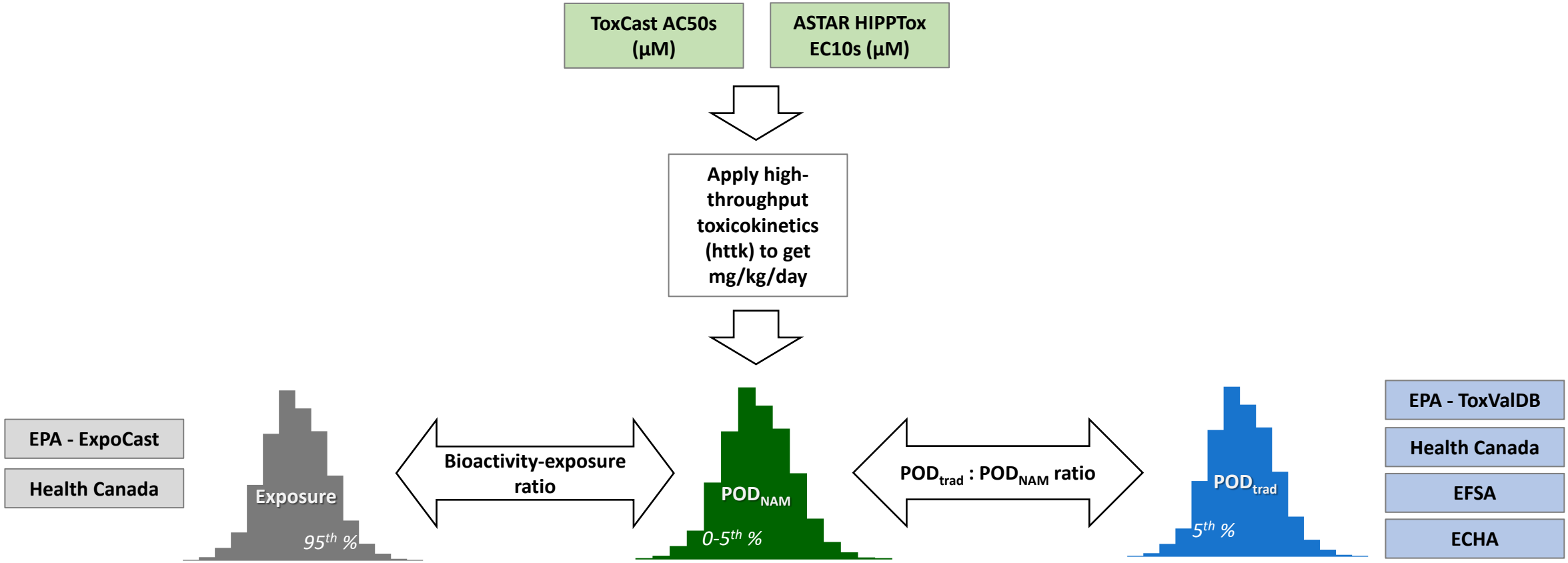
Research Article

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

Agency for Science, Technology and Research (SINGAPORE)
ECHA EUROPEAN CHEMICALS AGENCY
European Commission ENVIRONMENT
efsa European Food Safety Authority
EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing
EPA
Health Canada / Santé Canada
National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport
NTP National Toxicology Program, U.S. Department of Health and Human Services
UNIVERSITY OF BIRMINGHAM

(APCRA partners for these two case studies)

Case study workflow

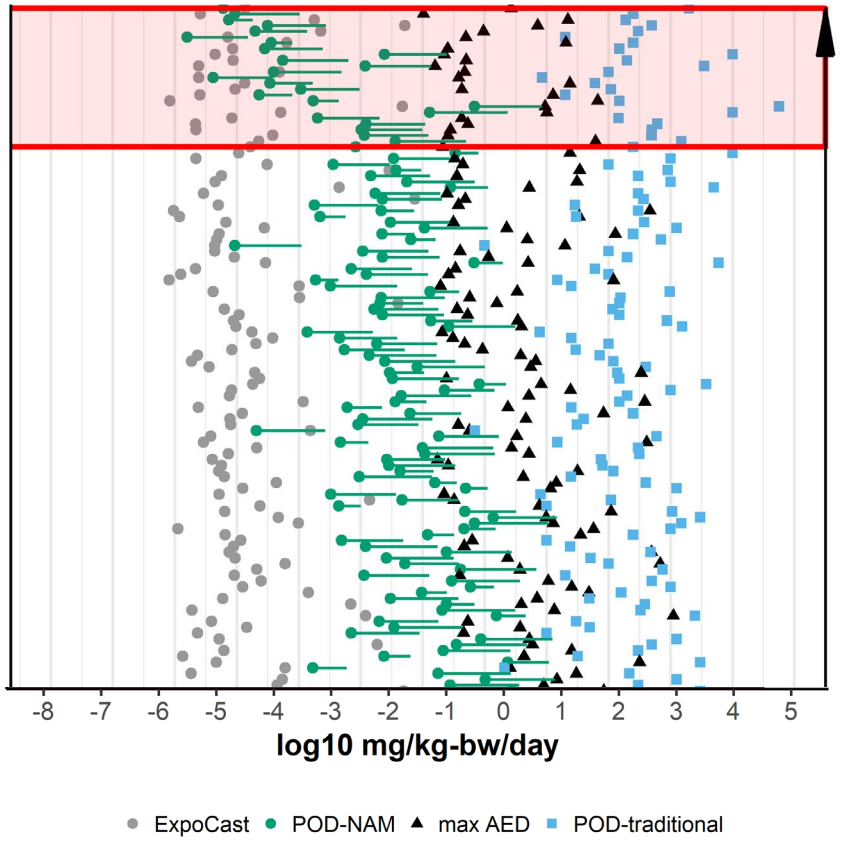


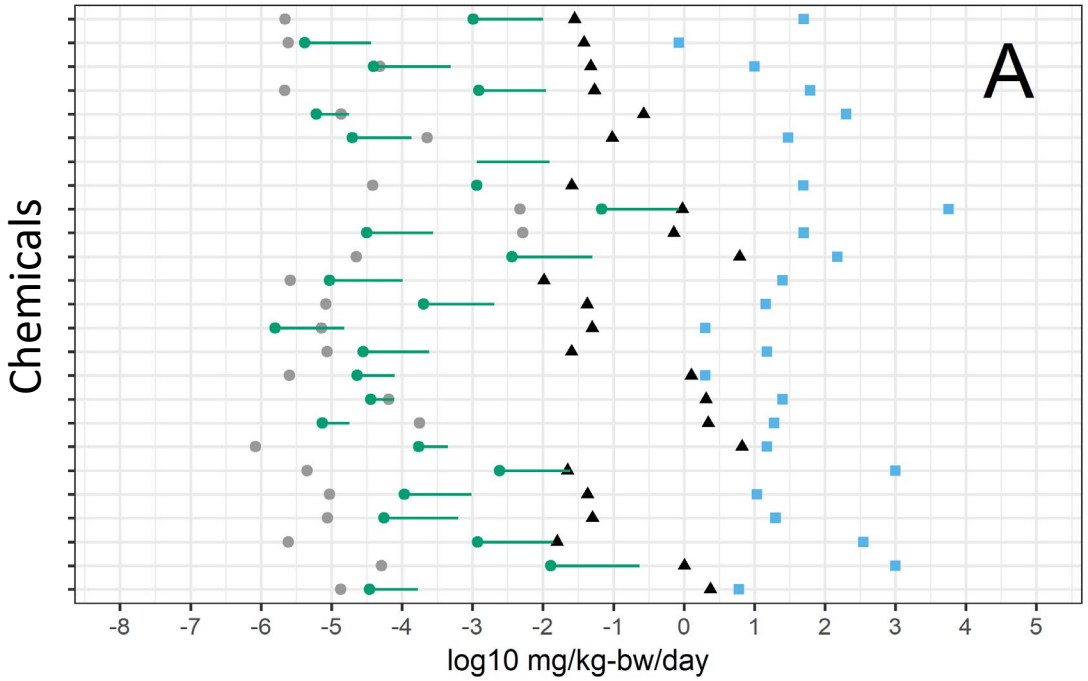
Is BER useful for prioritization?
Are there addressable weaknesses?

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