An Update on Public Tools for Prediction of Endocrine Hazard and Risk

Katie Paul Friedman

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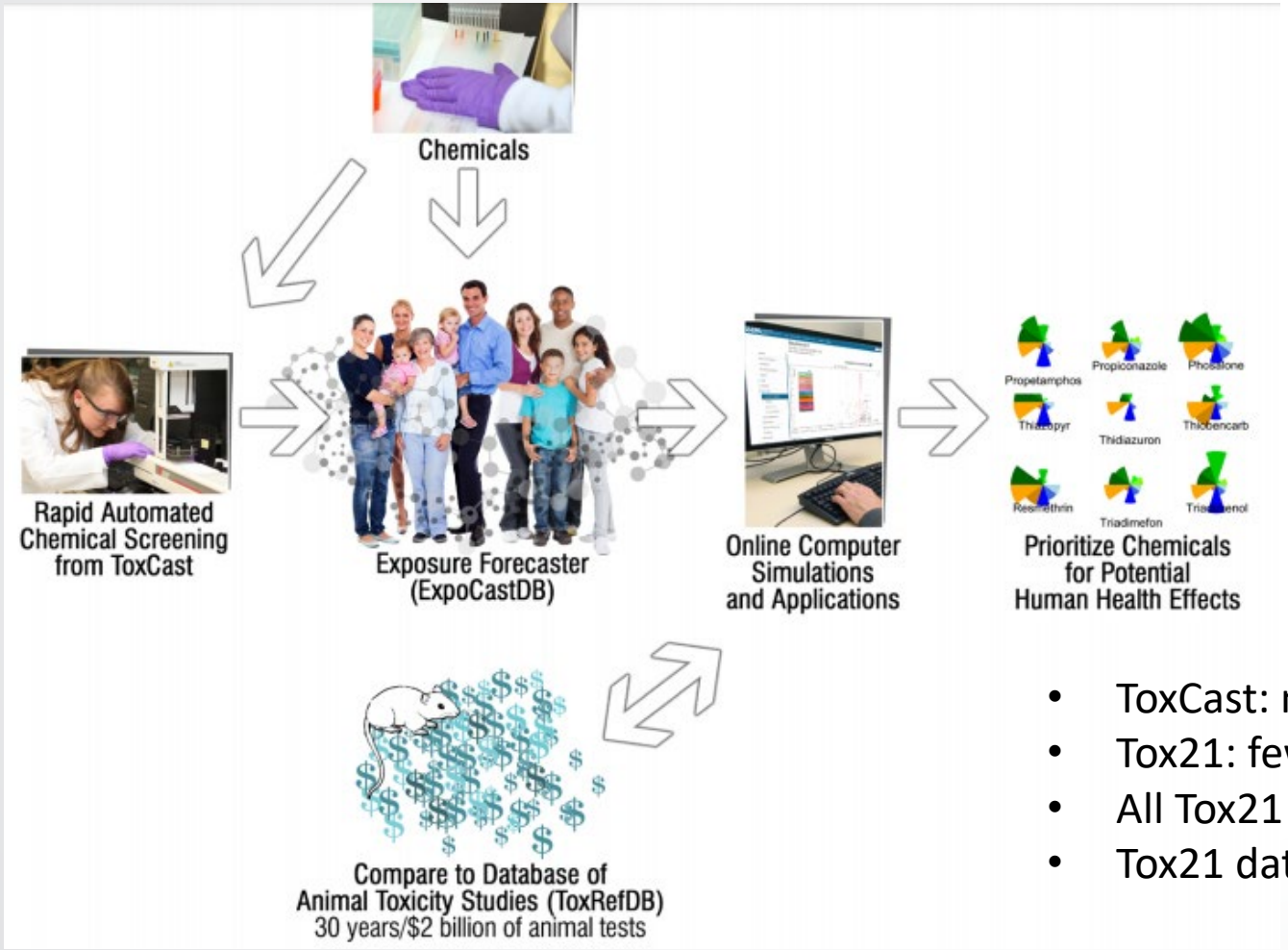
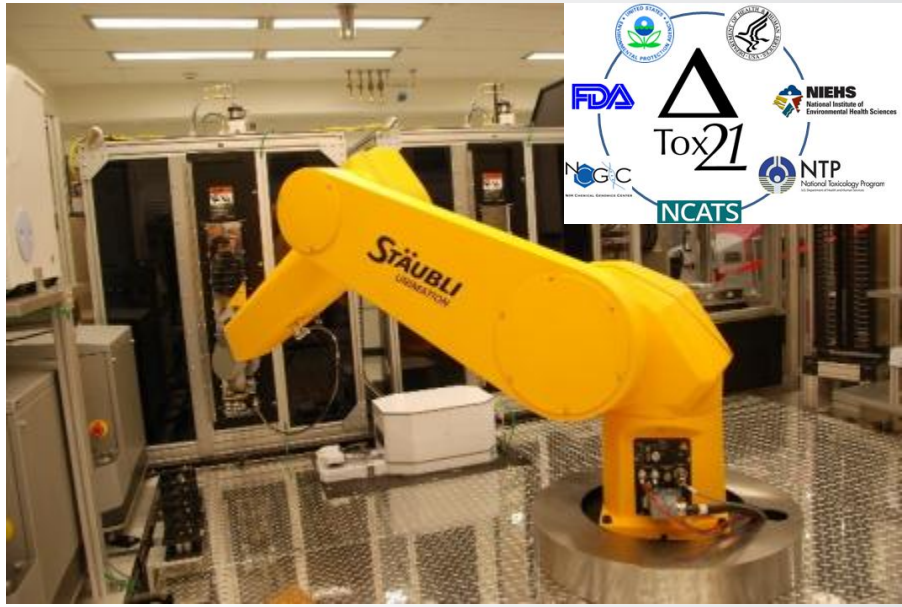
Paul-friedman.katie@epa.gov

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

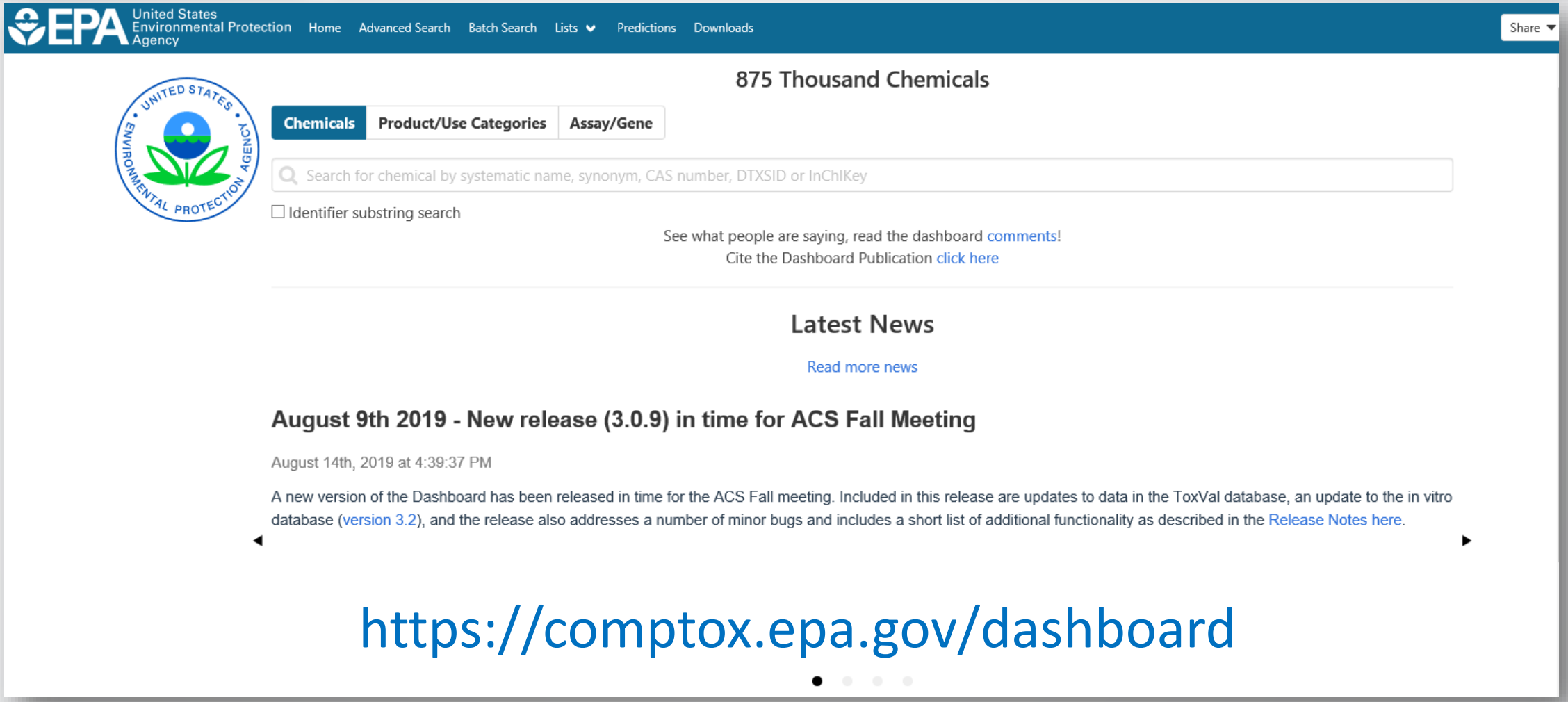
- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, all 1536, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline

Endocrine hazard and risk evaluation using public tools: approach outline

- Publicly available data from ToxCast is actively being applied to endocrine hazard labeling in the EU.
- Risk-based approaches that incorporate bioactivity and exposure make the best use of new approach methodologies.

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

CompTox Chemicals Dashboard



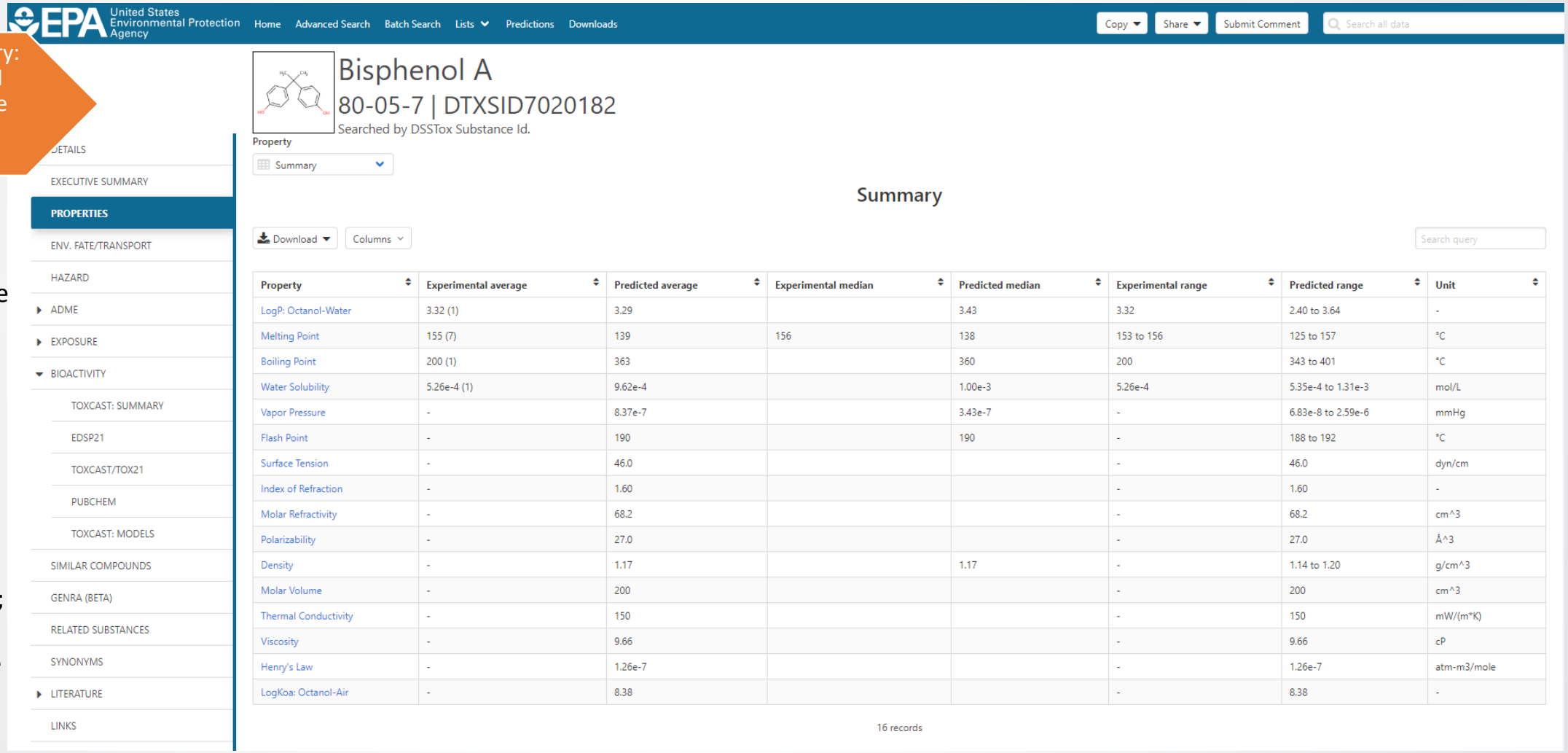
https://comptox.epa.gov/dashboard

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?

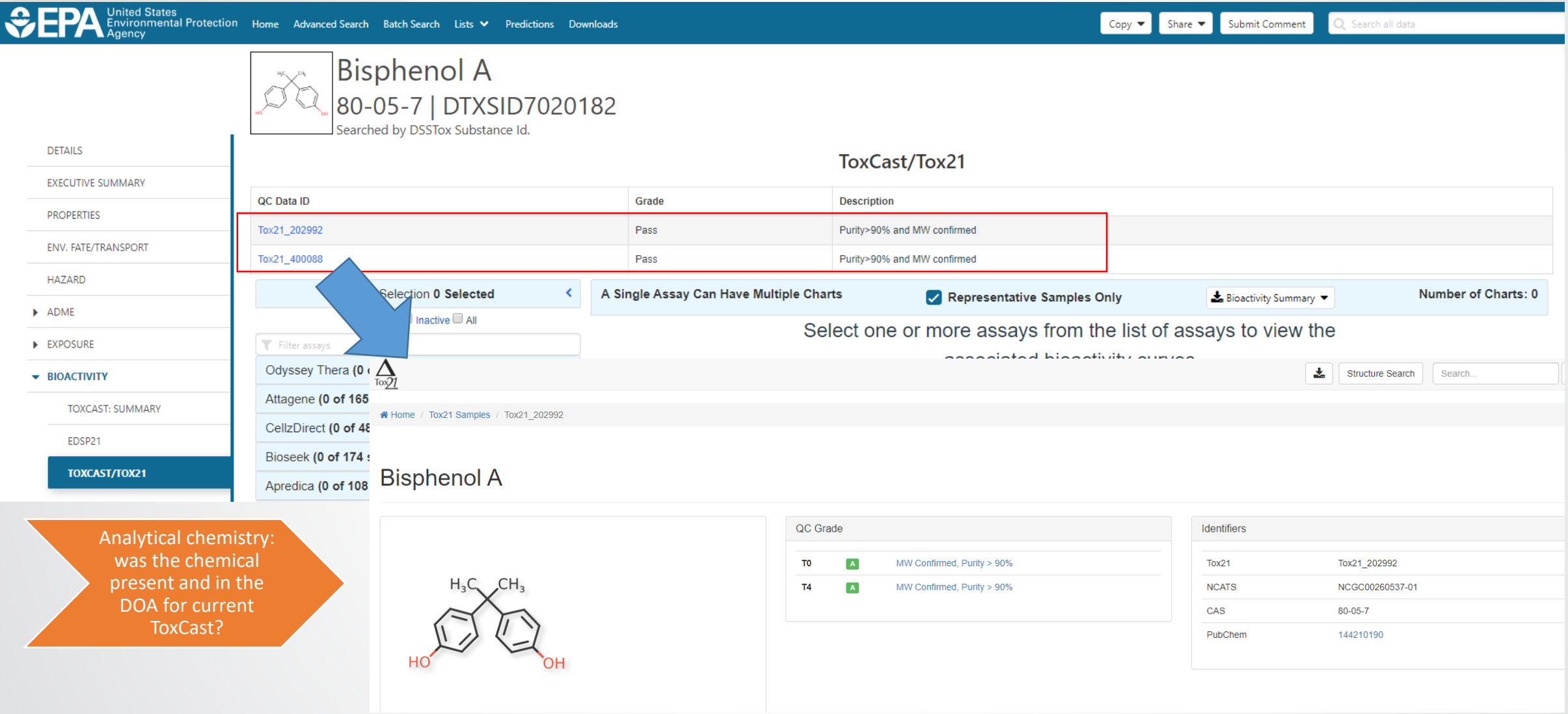
ToxCast negatives: what does a negative mean? Outside of domain of applicability (DOA)?

Many successfully screened chemicals have been:
logP -0.4 to 5.6 range;
MW 180-480;
log10 Vapor Pressure < 1.



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	-

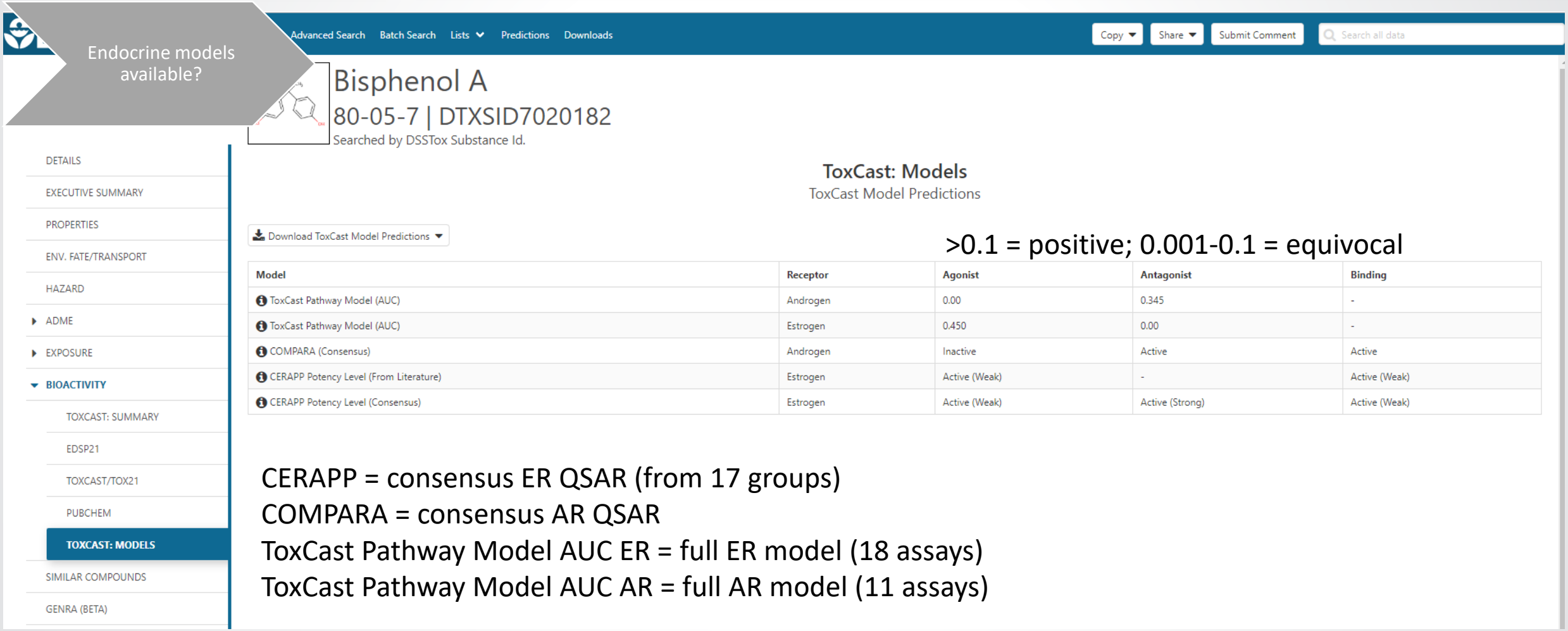
Examine QC data (if available) to see if we expect that the chemical was present for screening



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

Models >>> single assays. And equivocals happen.

Endocrine models available?



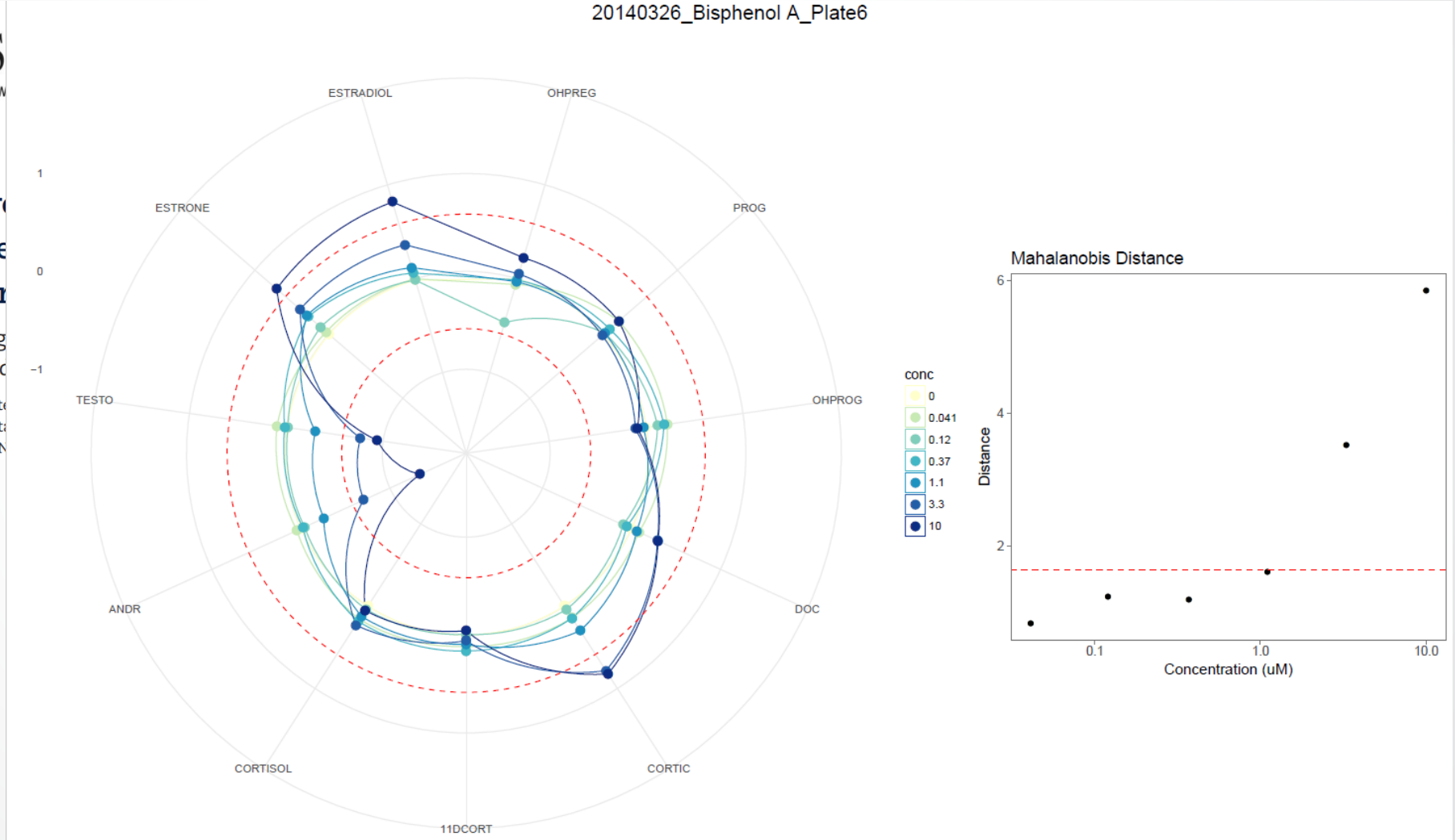
>0.1 = positive; 0.001-0.1 = equivocal

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)

HT-H295R model for steroidogenesis

Endocrine models available?

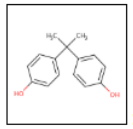


- Supplemental File 4 has fold-change by hormone
- Supplemental File 9 has mMd (model values)
- Invitrodb v3.2 has a hth295r model table with both of these included in it.
- Hope to include this in future release of the Dashboard.

Bioactivity summary in the Dashboard

Selective or non-selective?

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



Bisphenol A

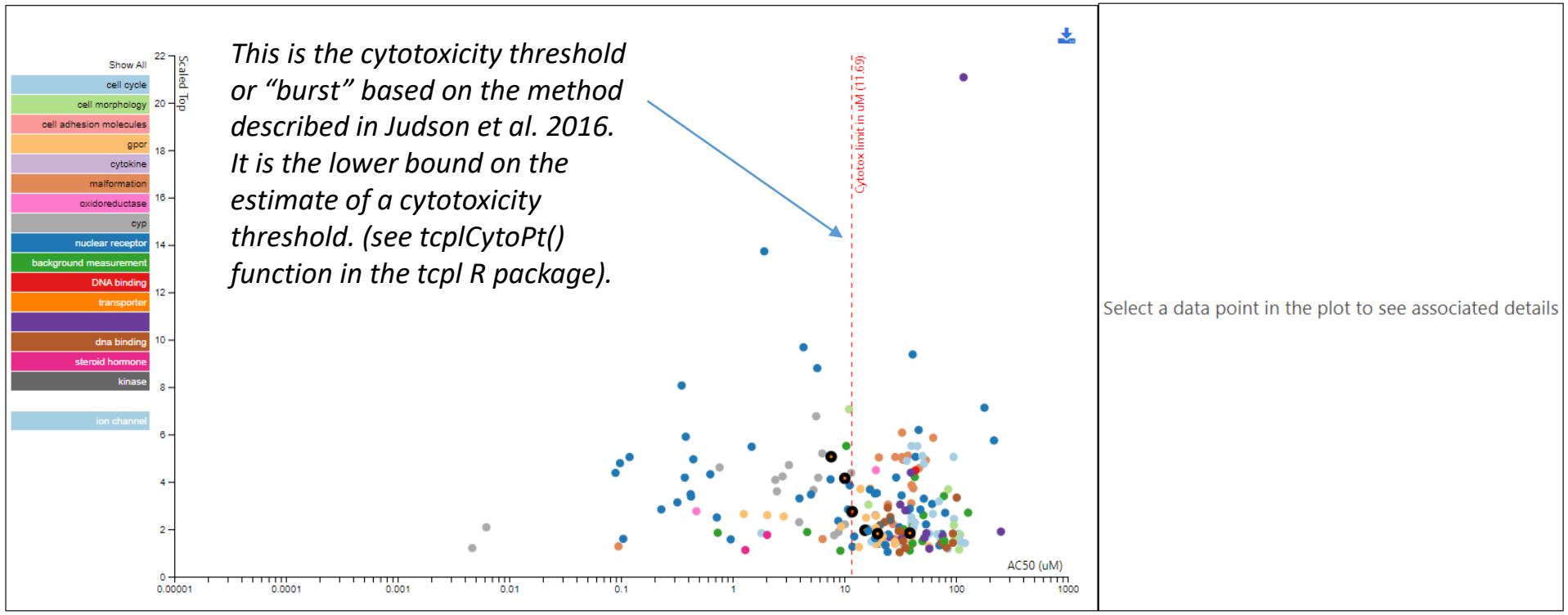
80-05-7 | DTXSID7020182

Searched by DSSTox Substance Id.

- DETAILS
- 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

Chemical Activity Summary

TOXCAST DATA | ASSAY DETAILS



This is the cytotoxicity threshold or "burst" based on the method described in Judson et al. 2016. It is the lower bound on the estimate of a cytotoxicity threshold. (see tcplCytoPt() function in the tcpl R package).

Select a data point in the plot to see associated details

211 active of 989 assays

User application dictates “selectivity”

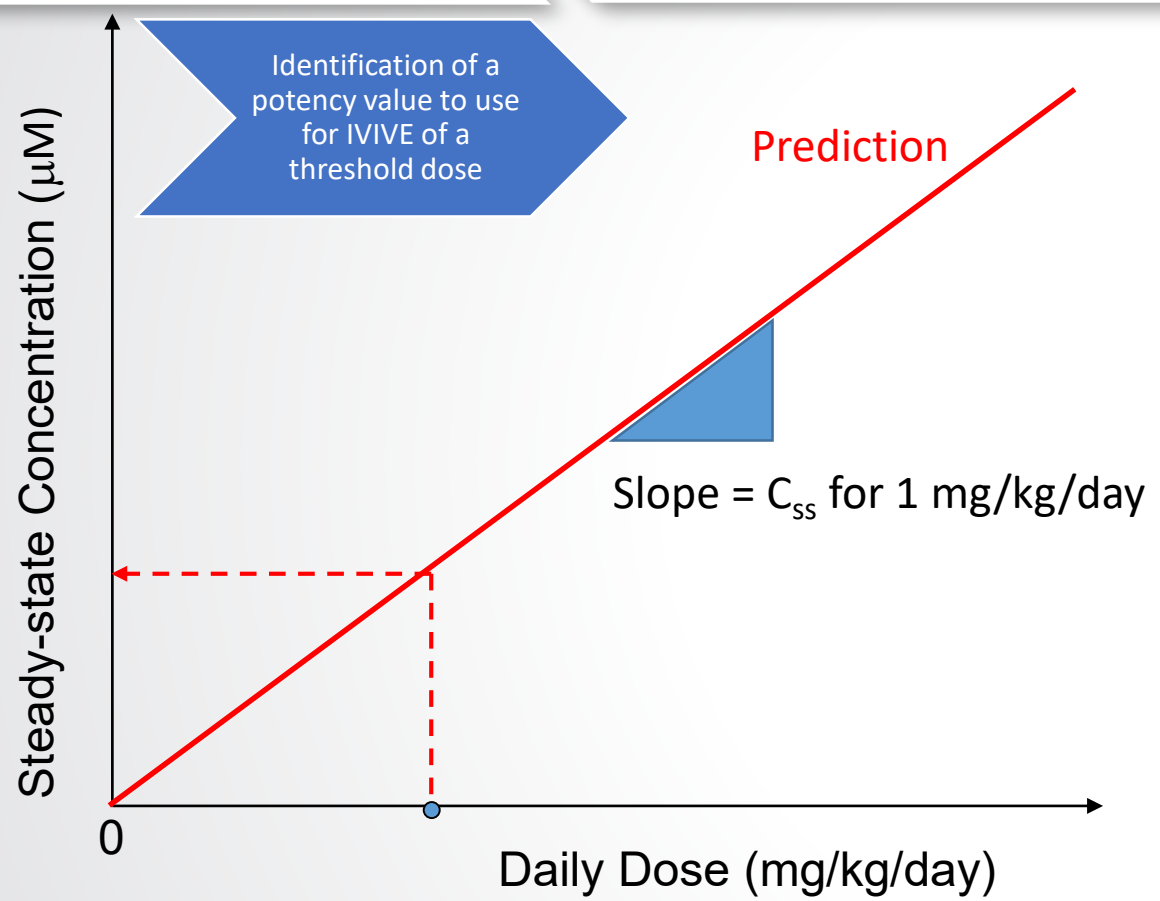
Selective or non-selective?

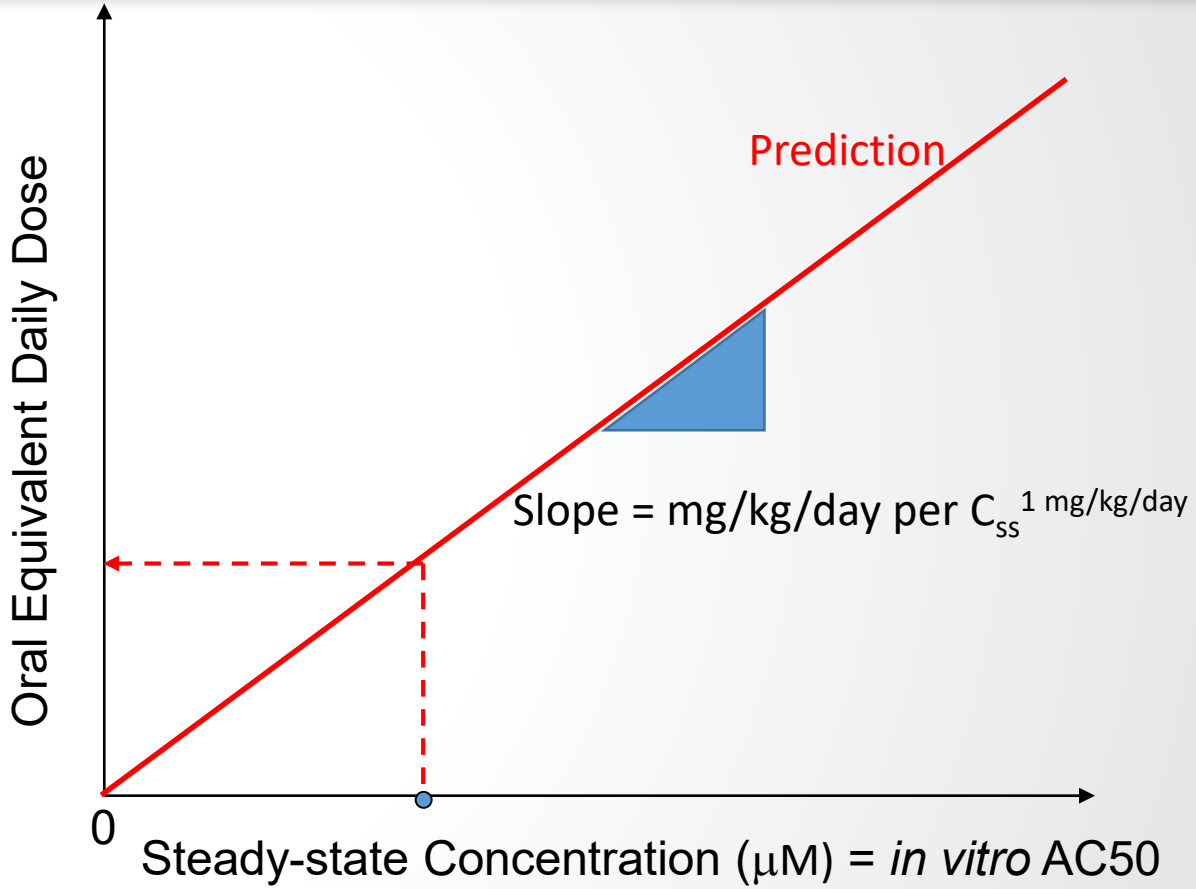
- AC50 < burst?
- AC50 0.5$\log_{10}$ distance from burst?
- AC50 < parallel viability assays?
- How else to filter ToxCast data: 3+ caution flags & hit-percent
- Other related ideas:
  - What other assays appear active in a similar concentration range?
  - Is there consistent support for MOA(s), or 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 August 9, 2019 is now using ToxCast invitrodb version 3.2: **https://doi.org/10.23645/epacomptox.6062623.v4**
- 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**

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





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

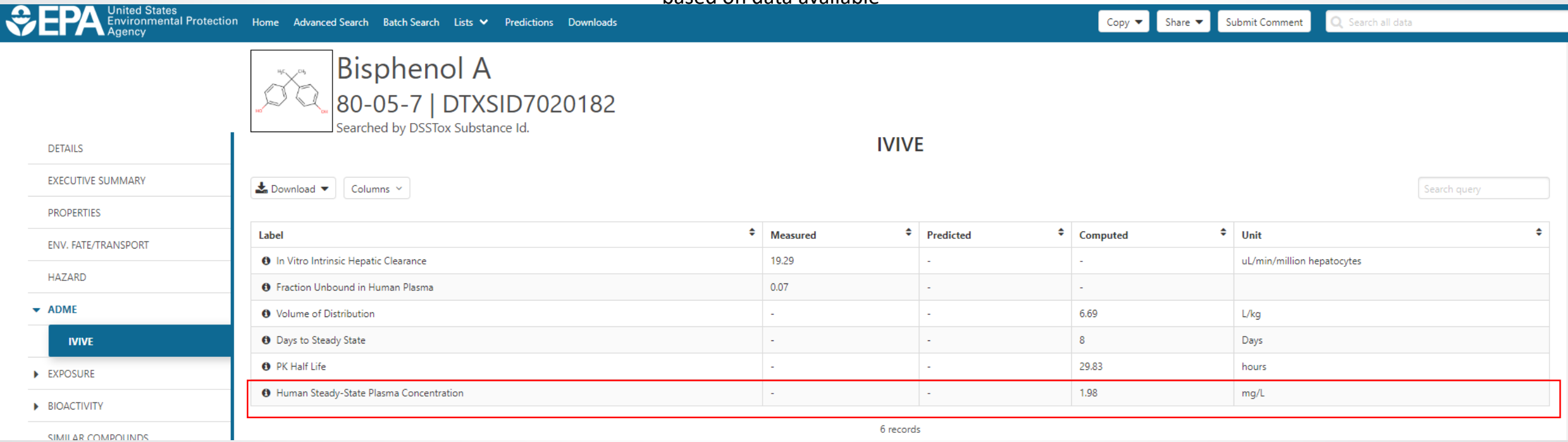
Wetmore *et al*. (2012)

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

IVIVE via high-throughput toxicokinetic data and models

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

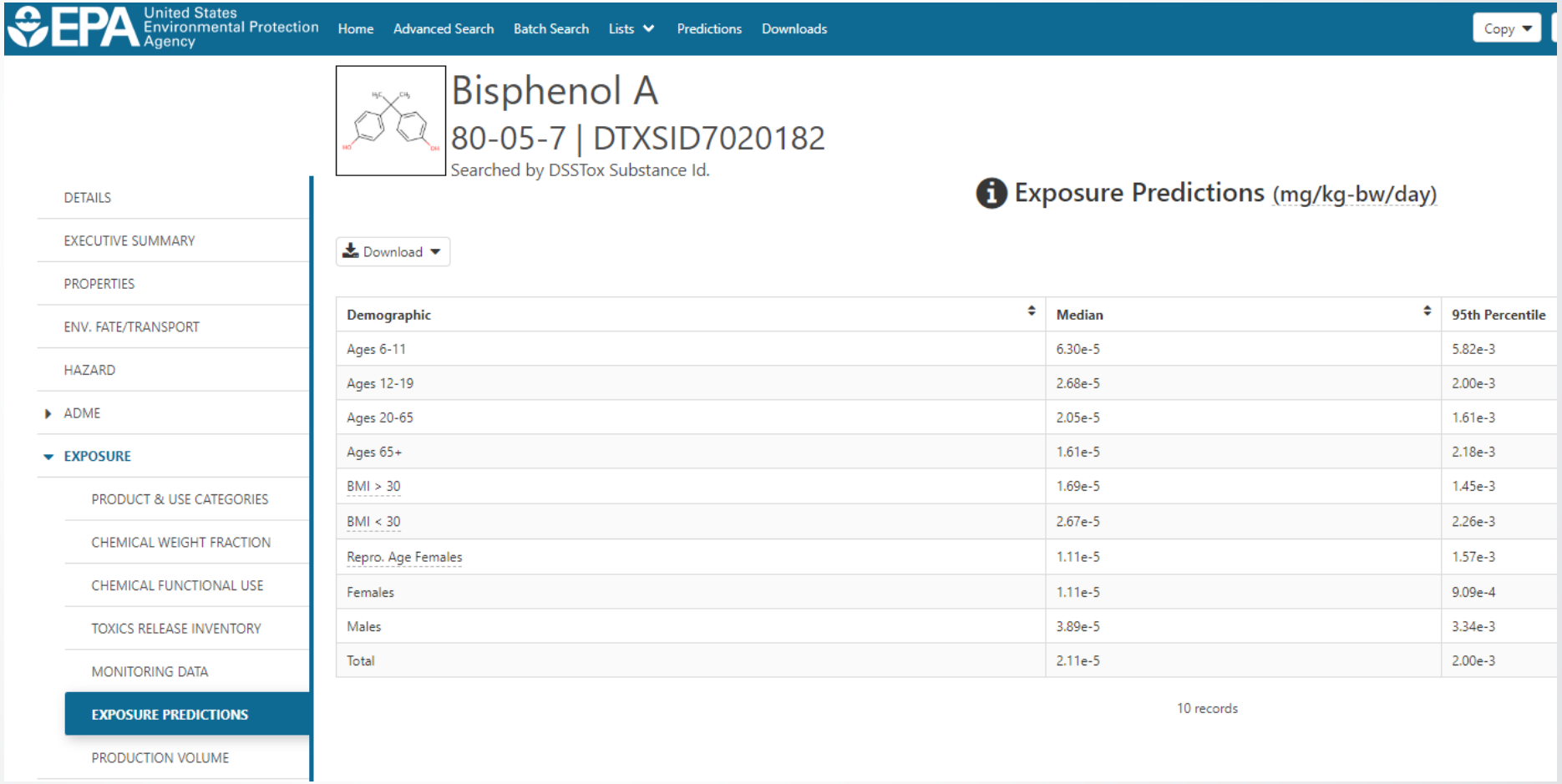
- Operationally, the httk R package (v 1.10.0) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs)
- For some substances, there is a beta tab in the Dashboard with Css and other values needed (no models). More chemicals have information in the httk package.
- 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



Bioactivity:exposure ratio requires exposure

Comparison to exposure predictions for a bioactivity:exposure ratio

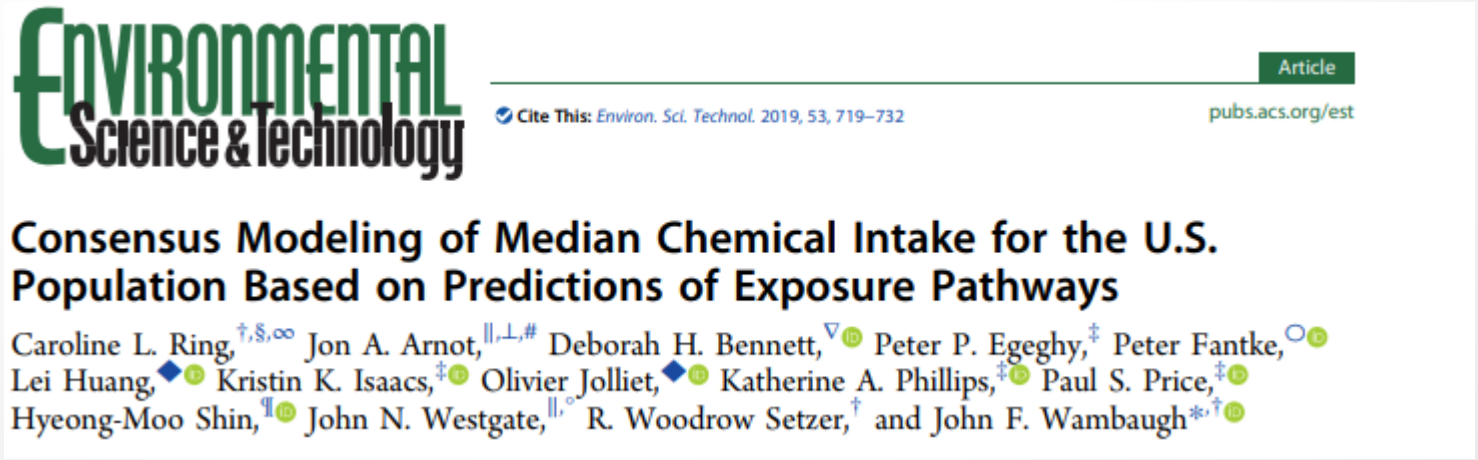
- Currently the Dashboard shows SEEM2 (2014) values



Consensus modeling of chemical exposure based on pathways: ExpoCast SEEM3

Comparison to exposure predictions for a bioactivity:exposure ratio

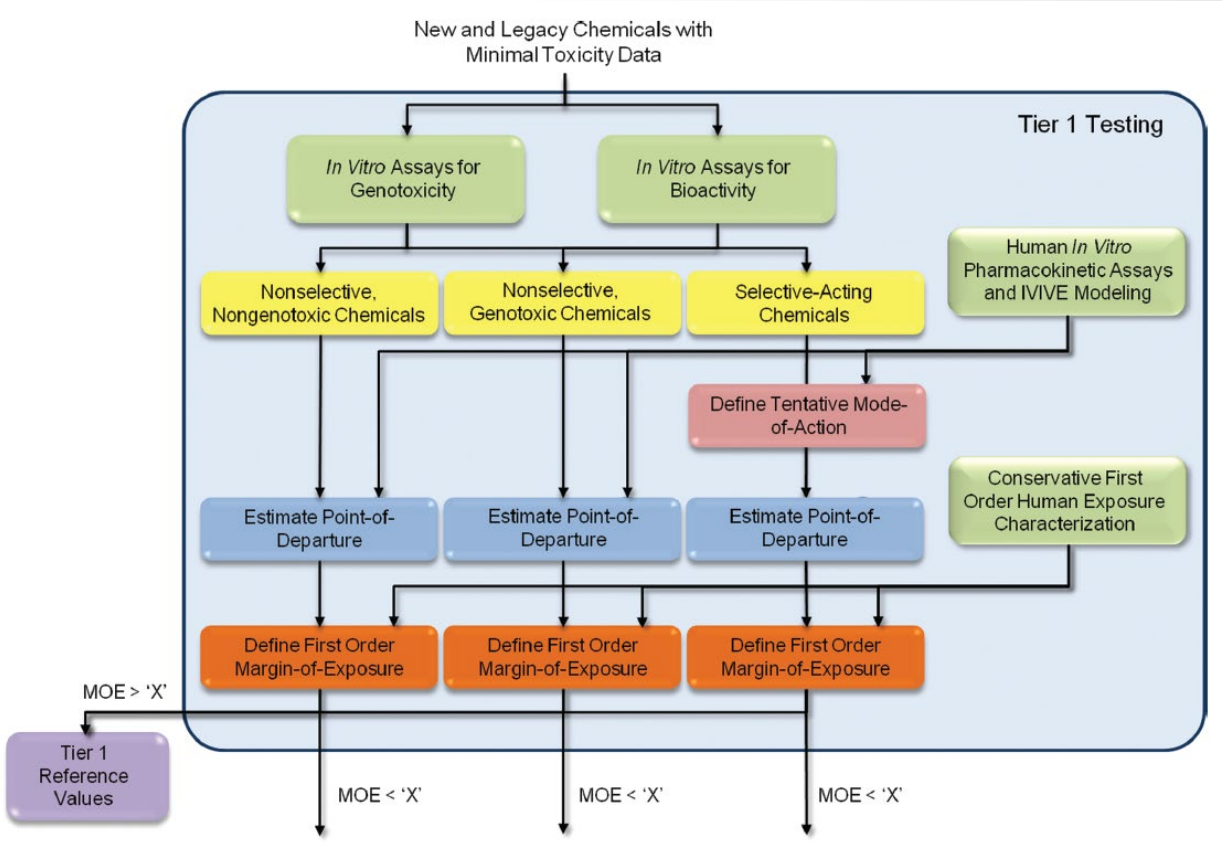
- "ExpoCast SEEM3" model:
  - uses twelve different exposure predictors including both near- and far-field models;
  - covers four distinct exposure pathways: non-pesticidal dietary, consumer products, far-field pesticide, and far-field industrial.
  - In SEEM3 each exposure predictor is scaled and centered such that chemicals without a value for a predictor relevant to its exposure pathways are assigned the average value.



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

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

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

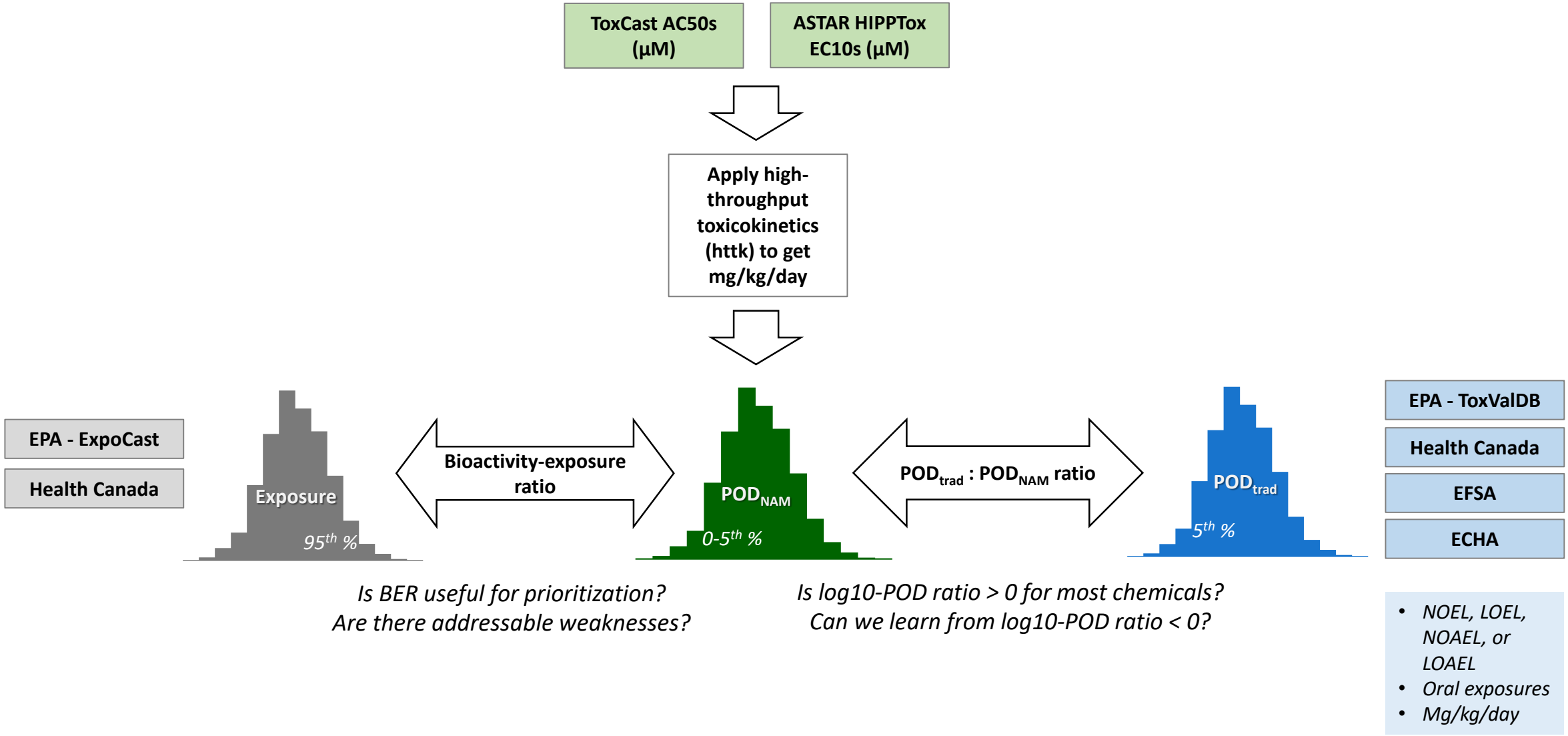


Screening level assessment example: combine NAMs for exposure, *in vitro* bioactivity, and toxicokinetics

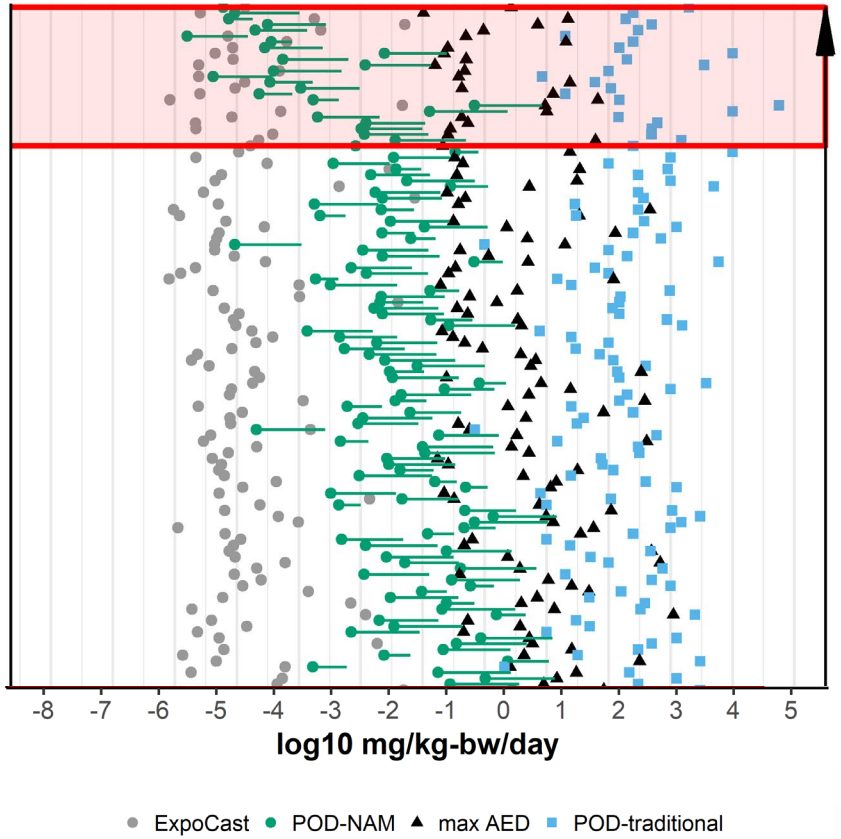
- Conducted by Accelerating the Pace of Chemical Risk Assessment (APCRA)
  - *"international cooperative collaboration of government agencies convened to address barriers and opportunities for the use of new approach methodologies (NAMs) in chemical risk assessment" (Paul Friedman et al., accepted)*

(APCRA partners for these two case studies)

Case study workflow



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



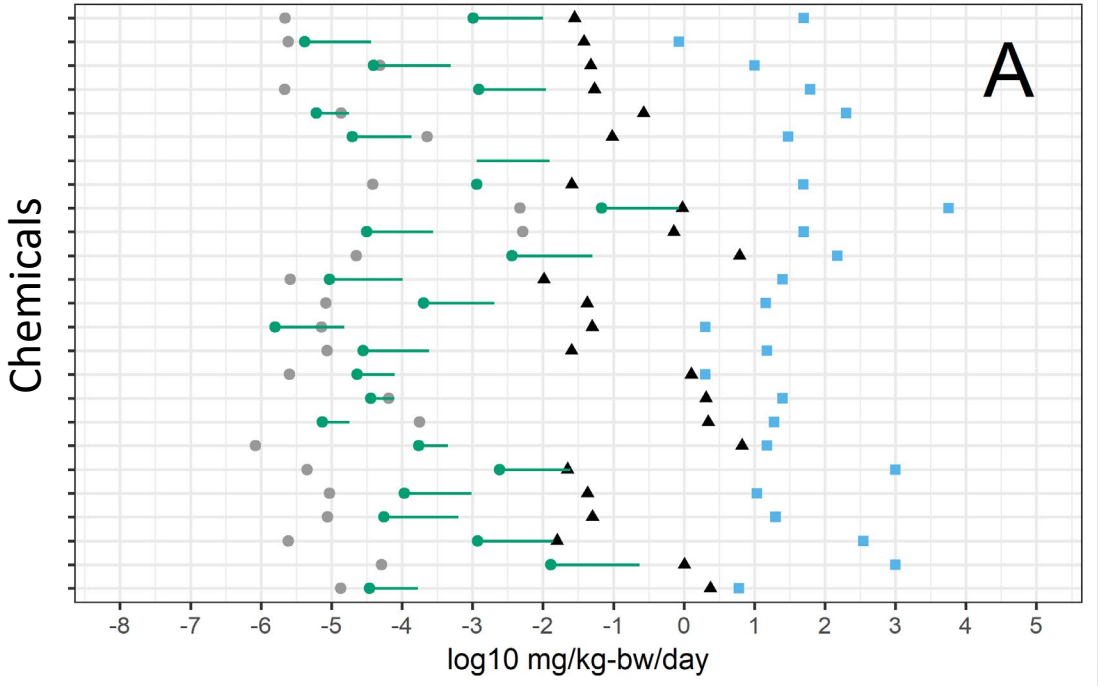


Figure 3 from Paul Friedman et al.

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

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

The cytotoxicity “burst” is useful for context.

Selective or non-selective?

- The latest Comptox Chemicals Dashboard release (version 3.0.9, August 9, 2019) demonstrates a cytotoxicity threshold based on the latest ToxCast database (invitrodb version 3.2, released August 2019). 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 3.2, 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.