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

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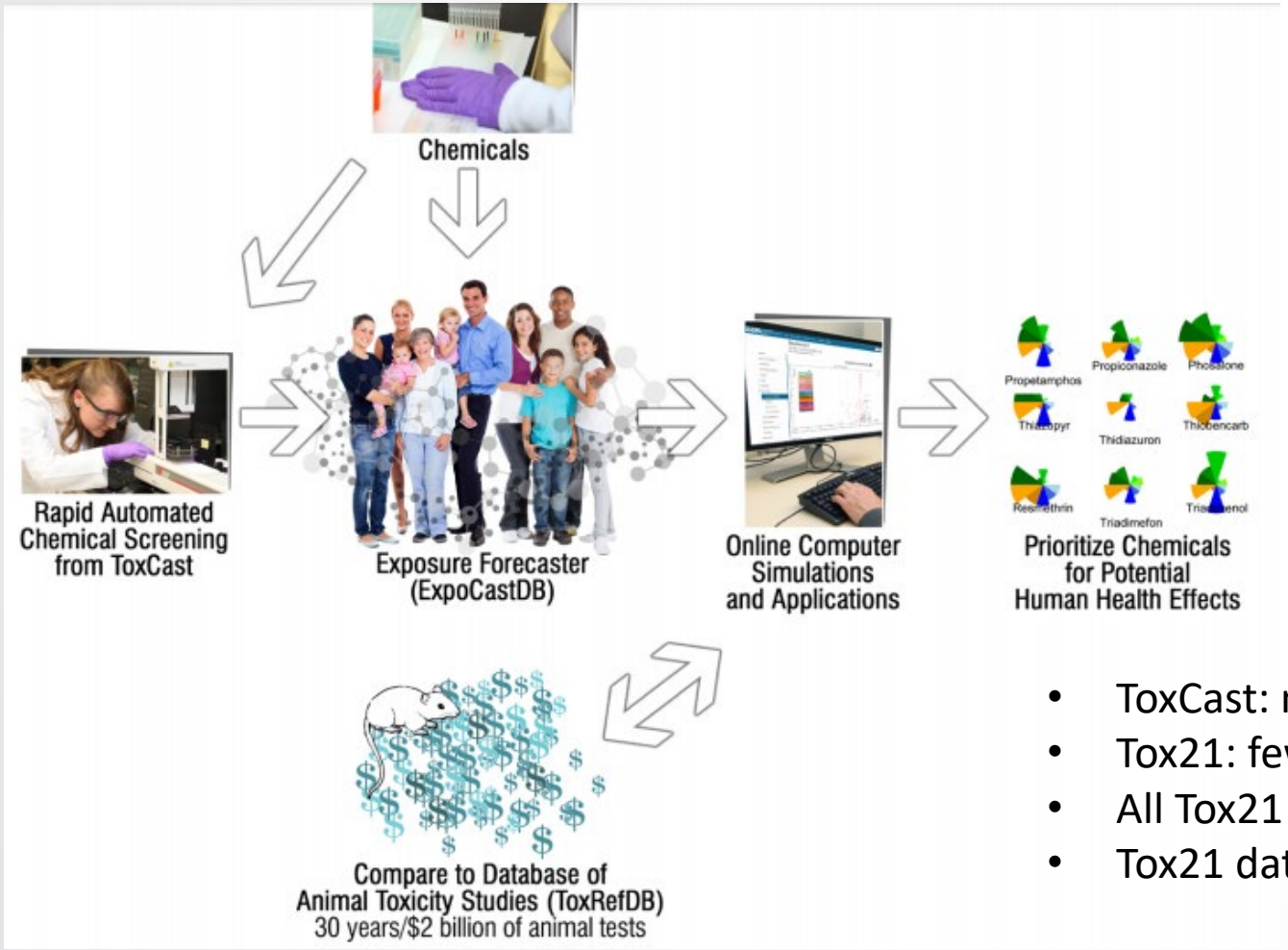
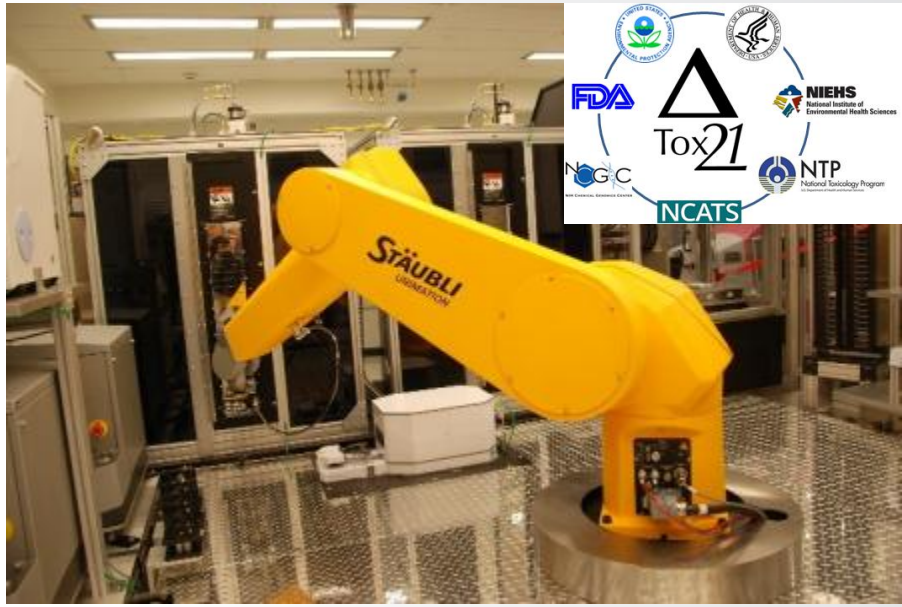
ToxCast and Tox21 have generated a lot of publicly available bioactivity data for hazard screening and prediction.

EPA's ToxCast program at a glance





Tox21 robot

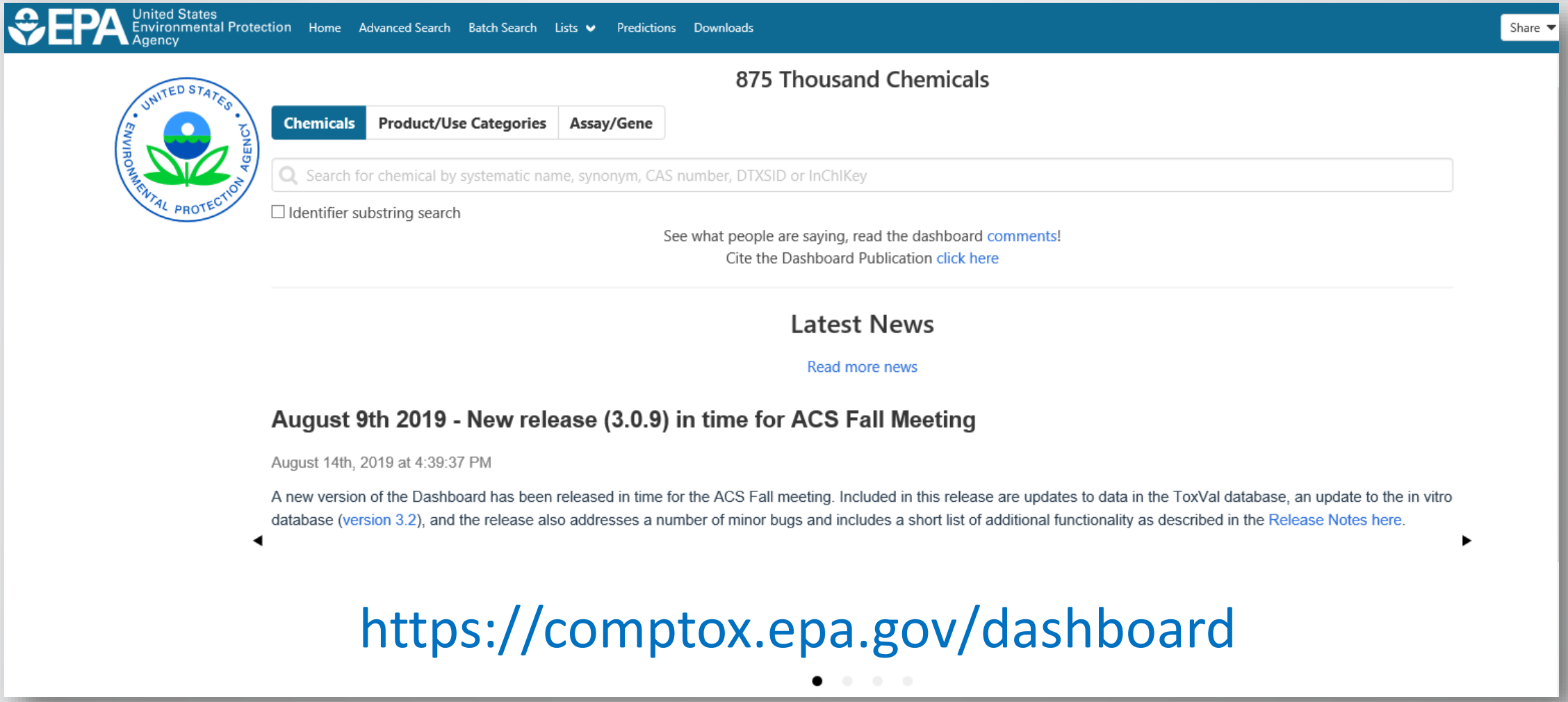
- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, all 1536, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline

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

EPA's CompTox Chemicals Dashboard

- **A publicly accessible website delivering:**
  - ~875,000 chemicals with related property data
  - Experimental and predicted physicochemical property data
  - Integration to "biological assay data" for 1000's of chemicals
  - Information regarding consumer products containing chemicals
  - Links to other agency websites and public data resources
  - "Literature" searches for chemicals using public resources
  - "Batch searching" for thousands of chemicals
  - Downloadable Open Data for reuse and repurposing
  - Many features (only highlighting a few)
  - Access to multiple tools (direct data interpolation and predictive) for multiple disciplines

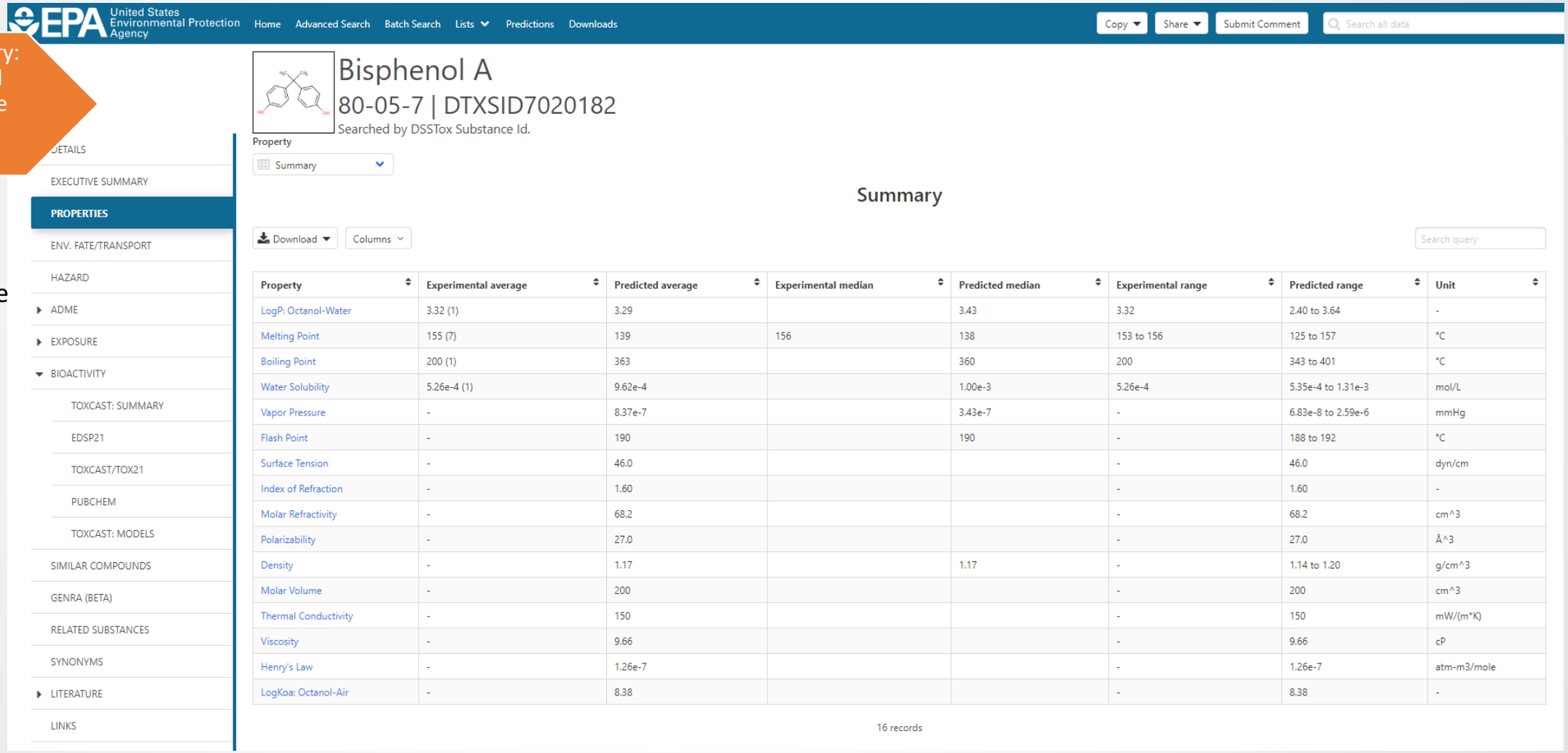
https://www.epa.gov/chemical-research/comptox-chemicals-dashboard

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?

Consider some aspects of the Lipinski's rules: logP -0.4 to 5.6 range; MW 180-480; Vapor Pressure < 1.

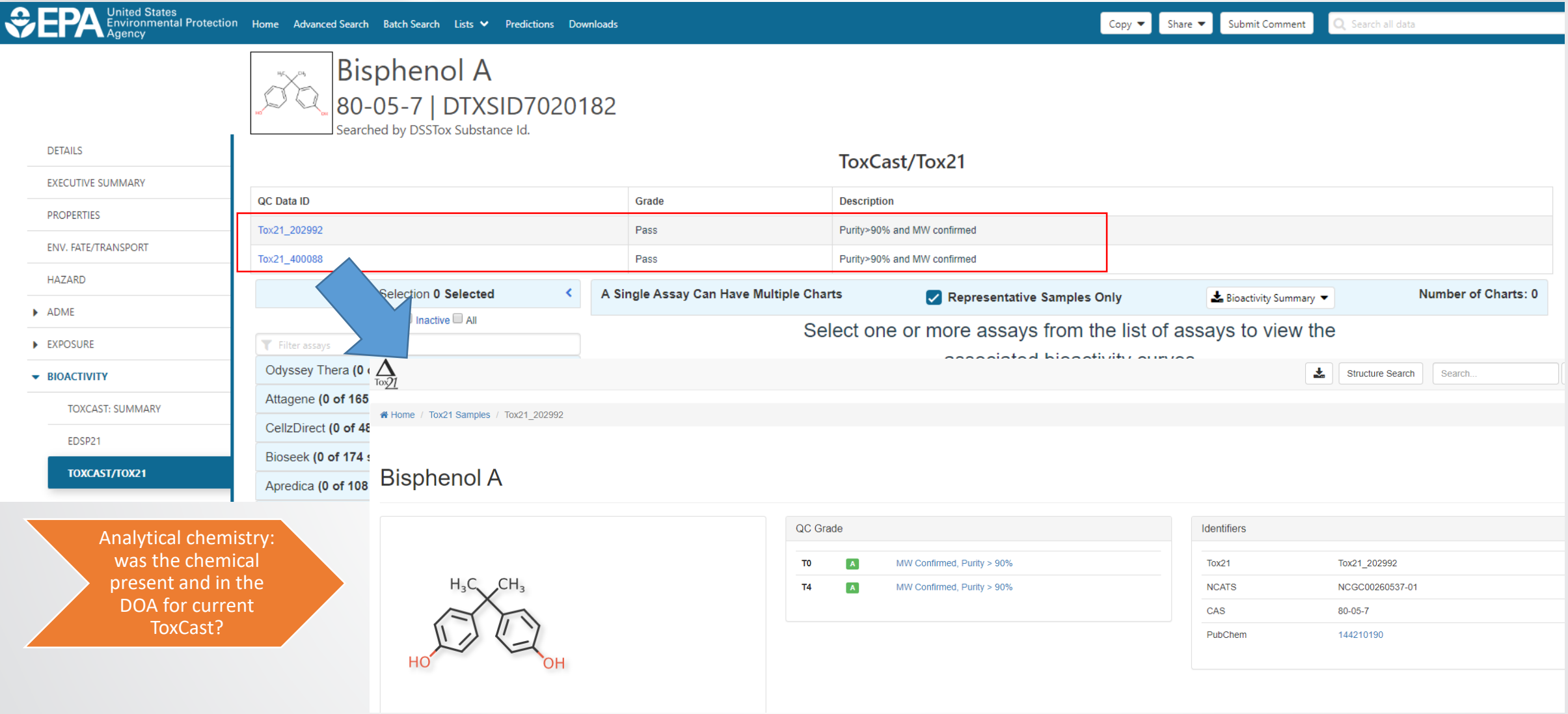


Property	Experimental average	Predicted average	Experimental median	Predicted median	Experimental range	Predicted range	Unit
LogP: Octanol-Water	3.32 (1)	3.29		3.43	3.32	2.40 to 3.64	-
Melting Point	155 (7)	139	156	138	153 to 156	125 to 157	°C
Boiling Point	200 (1)	363		360	200	343 to 401	°C
Water Solubility	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	-

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**

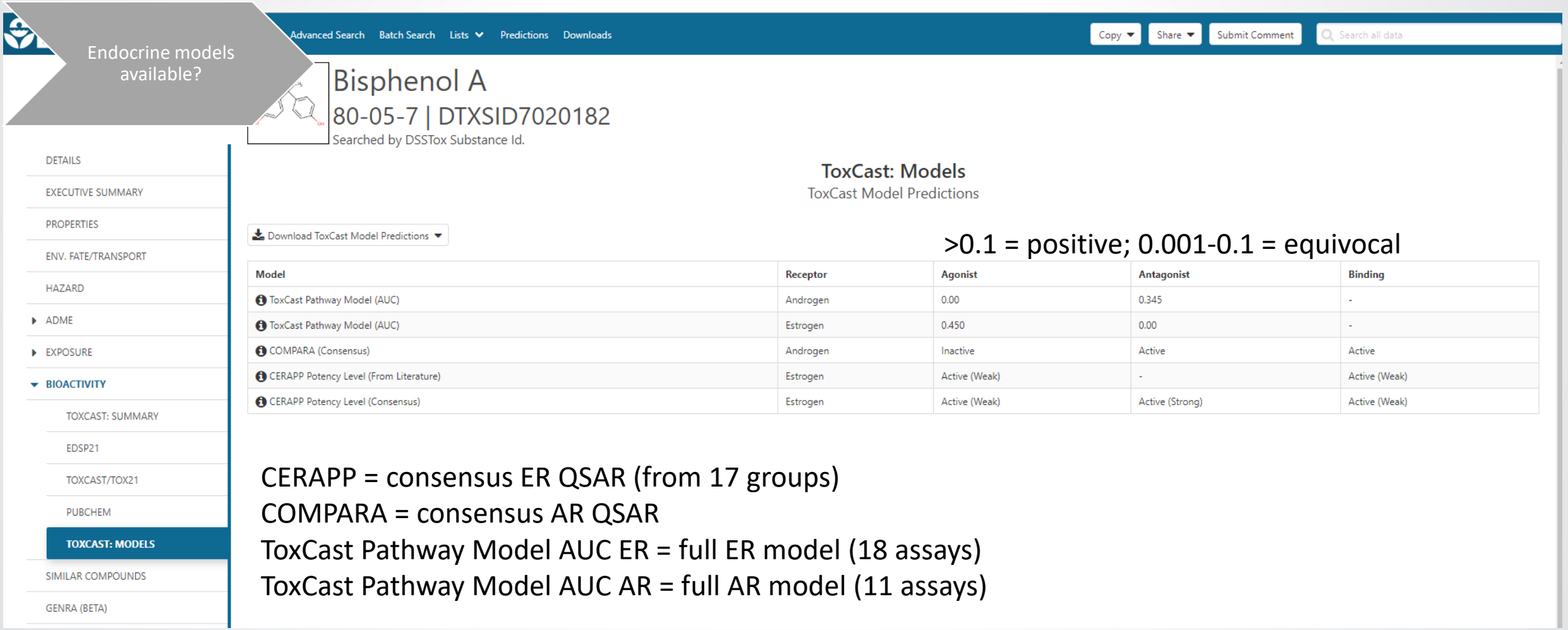
Examine QC data (if available) to see if we expect that the chemical was present for screening



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

Models >>> single assays. And equivocals happen.

Endocrine models available?



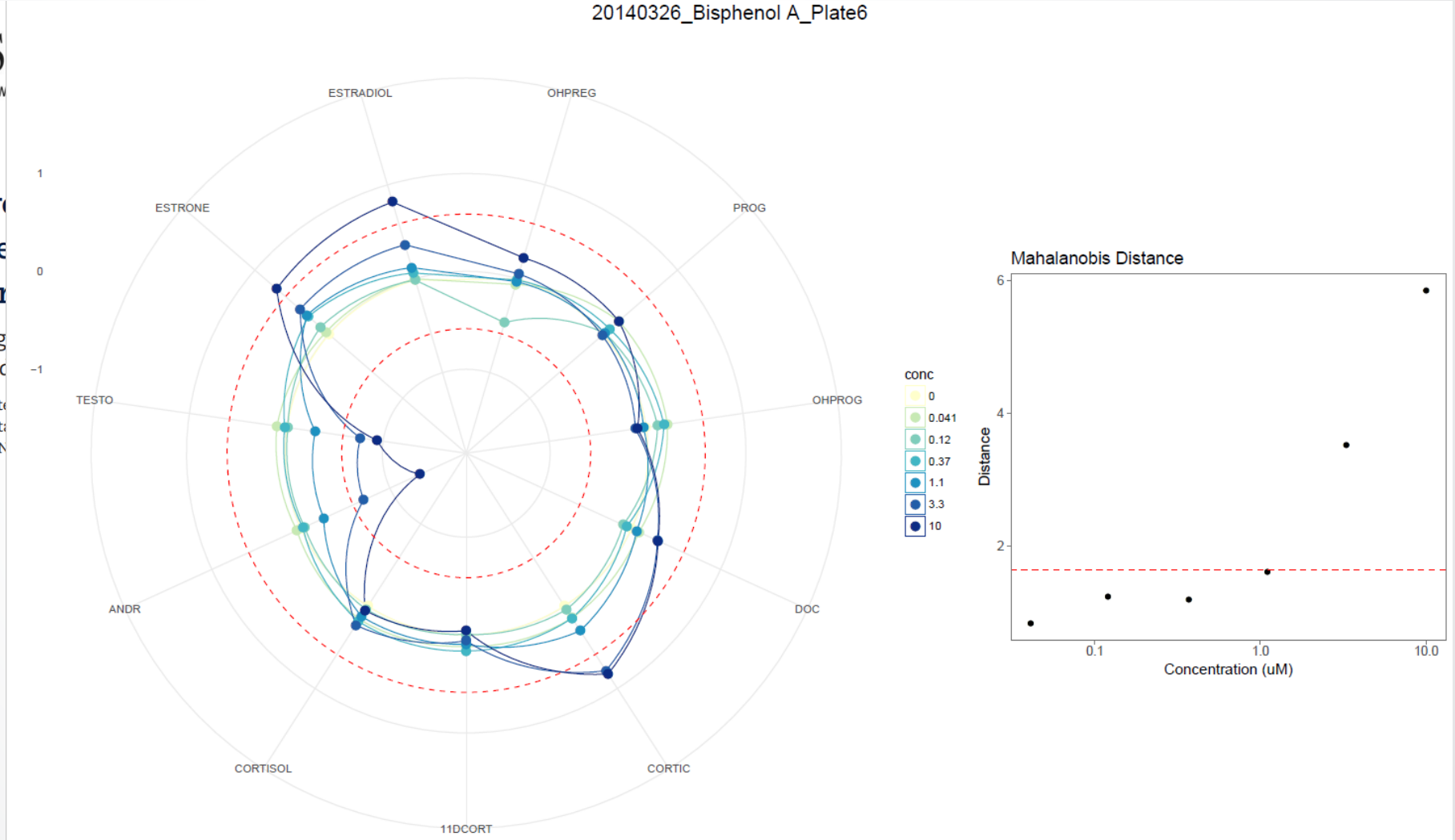
>0.1 = positive; 0.001-0.1 = equivocal

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

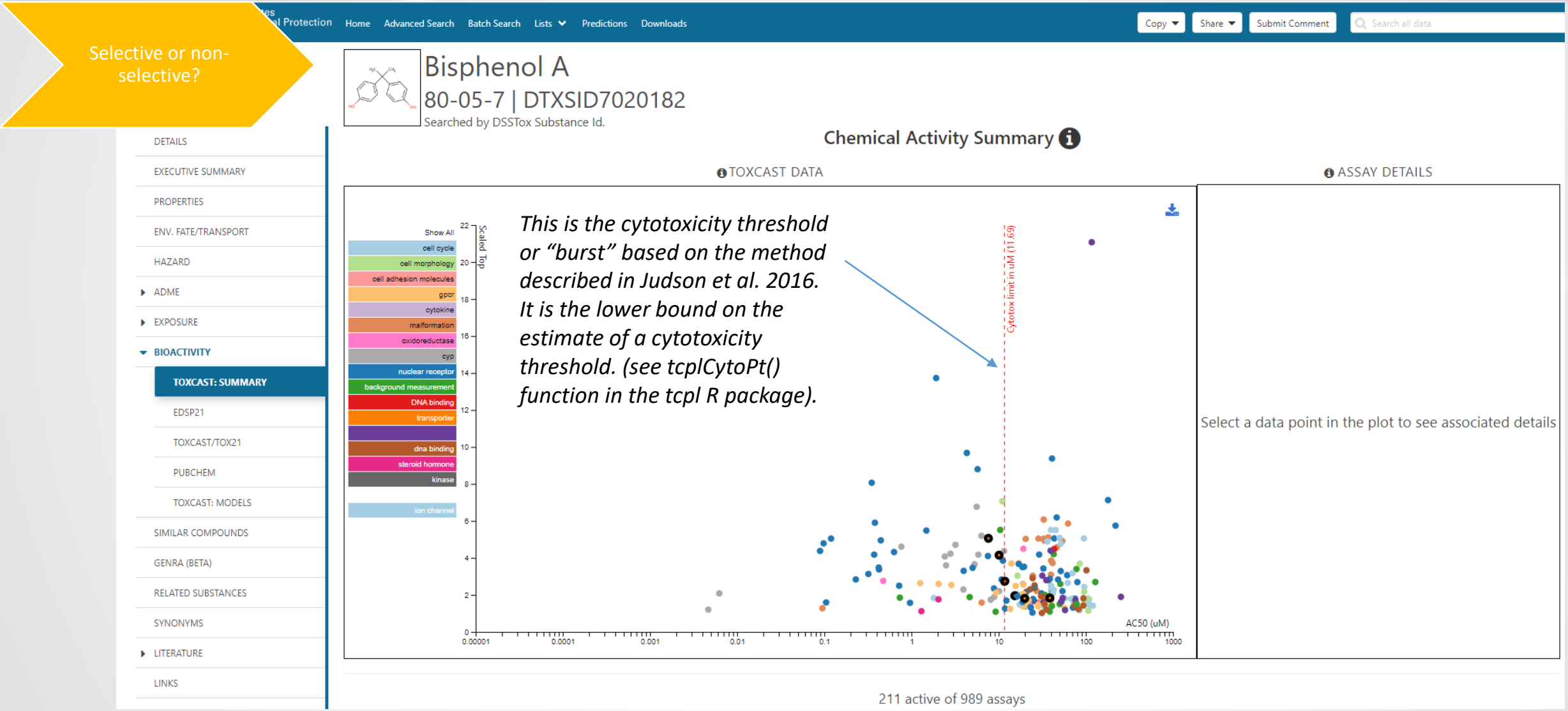
Endocrine models available?



- 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?



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).

Summary of the assay data is in a table

Selective or non-selective?

211 active of 989 assays

Search query | Show Inactive | Show Background

	Modal	Description	SeqAPASS	AOP	Event	Hit Call	Top	Scaled Top	AC50	logAC50	Bmad	MaxMed	MaxMedConc	Cutoff	Flags	ModlAcc	ModlAc10	ModlAcb	Intended Target Family
ACEA_ER_80hr		2	NP_000116.2	200	1181	ACTIVE	112	4.18	0.373	-0.428	8.96	113 - percent_activity	0.301	26.9		-0.686	113	-0.686	nuclear receptor
APR_HepG2_CellLoss_24h_dn		-	-	-	-	ACTIVE	1.20	1.81	106	2.02	6.63e-2	1.20 - log2_fold_induction	2.30	0.663		2.04	1.20	1.94	cell cycle
APR_HepG2_MitoMass_24h_dn		-	-	-	-	ACTIVE	0.874	1.76	109	2.04	4.96e-2	0.867 - log2_fold_induction	2.30	0.496		2.05	0.867	1.95	cell morphology
APR_HepG2_MitoMembPot_24h_dn		-	-	-	-	ACTIVE	5.92	7.07	11.0	1.04	8.38e-2	6.45 - log2_fold_induction	1.70	0.838		0.813	6.45	0.646	cell morphology
APR_HepG2_OxidativeStress_24h_up		-	-	-	-	ACTIVE	1.20	1.47	110	2.04	8.19e-2	1.19 - log2_fold_induction	2.30	0.819		2.08	1.19	1.97	cell cycle
APR_HepG2_CellLoss_72h_dn		-	-	-	-	ACTIVE	4.49	5.05	95.2	1.98	8.89e-2	4.43 - log2_fold_induction	2.30	0.889		1.75	4.43	1.52	cell cycle
APR_HepG2_MitoMembPot_72h_dn		-	-	-	-	ACTIVE	2.71	3.69	85.3	1.93	7.33e-2	2.26 - log2_fold_induction	2.30	0.733		1.70	2.26	1.36	cell morphology
APR_HepG2_MitoticArrest_72h_up		-	-	-	-	ACTIVE	1.66	1.17	84.7	1.93	0.142	1.44 - log2_fold_induction	2.30	1.42	Borderline active	2.29	1.44	1.71	cell cycle
APR_HepG2_OxidativeStress_72h_up		-	-	-	-	ACTIVE	1.80	1.65	106	2.02	0.110	1.60 - log2_fold_induction	2.30	1.10		2.08	1.60	1.82	cell cycle
ATG_Ahr_CIS_up		-	NP_001612.1	150	18	ACTIVE	1.31	1.32	23.4	1.37	0.199	1.28 - log2_fold_induction	2.00	0.994		1.56	1.28	1.34	dna binding

First << < 1 2 3 4 5 6 7 8 9 10 > >> Last

Showing 1 to 10 of 211 records

“Burst:” thinking and updates

Selective or non-selective?

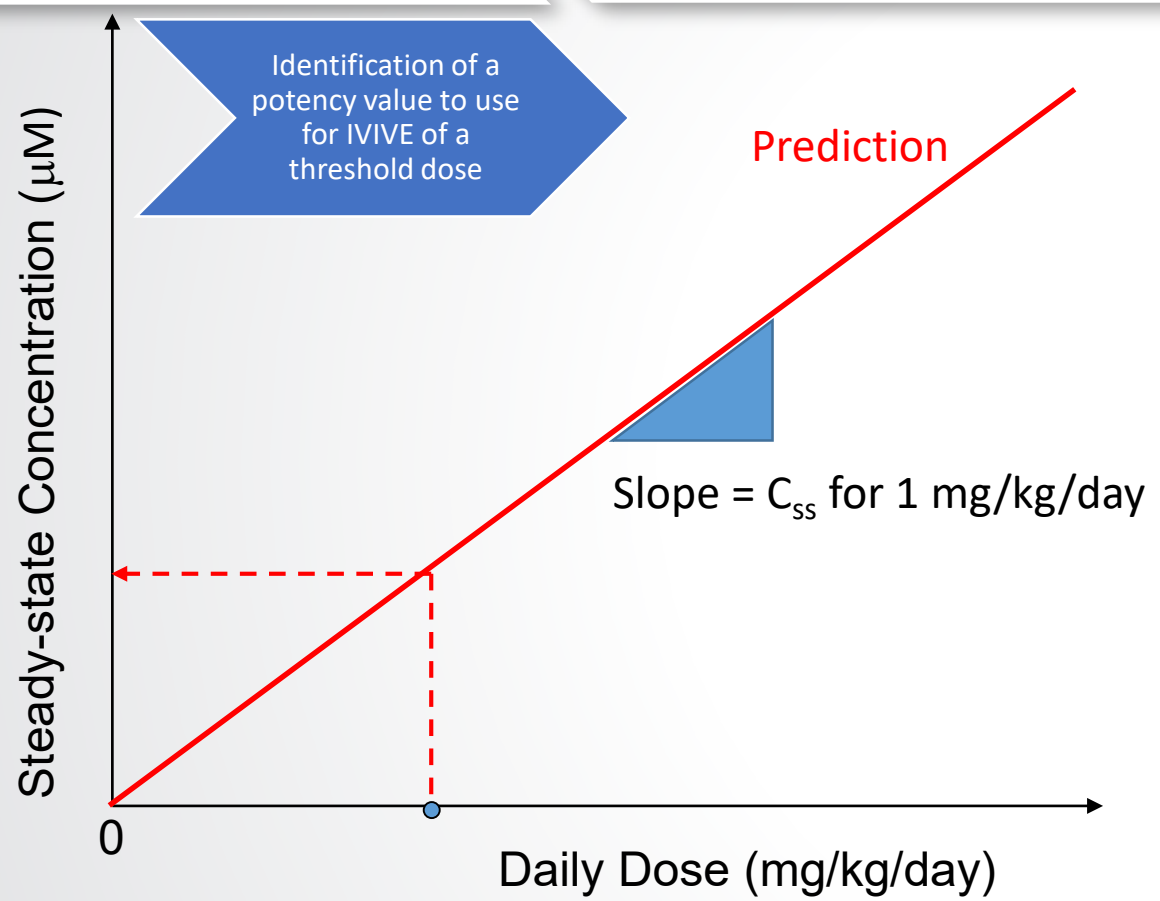
- In the Comptox Chemicals Dashboard released March 7, 2019 (version 3.0.5), the cytotoxicity threshold was erroneously displayed as the median. The value that should have been displayed was the lower bound on the estimate of cytotoxicity. The median would appear much higher than the anticipated lower bound (note that both the median and lower bound values were in the ToxCast database, invitrodb). The Dashboard was subsequently corrected in a bug fix release (version 3.0.8, May 10, 2019) to again show the lower bound estimate for the cytotoxicity threshold.
- 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 or curve-fitting approaches for existing data change.
- 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.

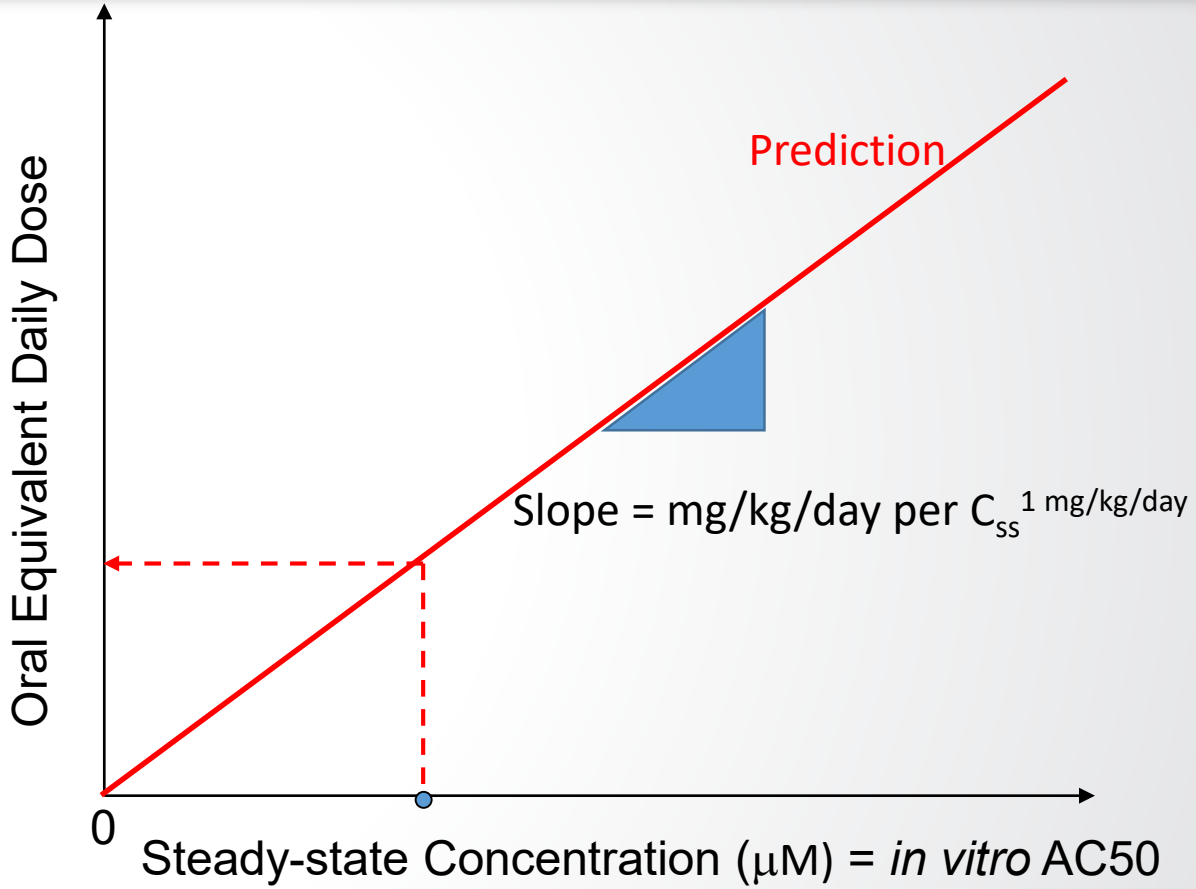
User application dictates "selectivity"

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

- 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?

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





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

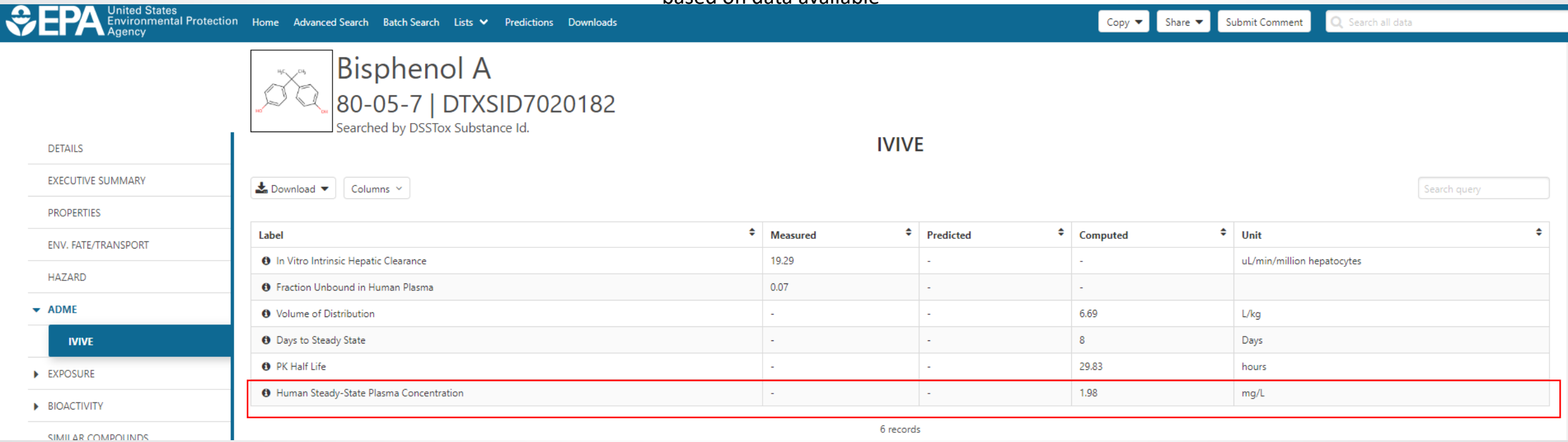
Wetmore *et al*. (2012)

- 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

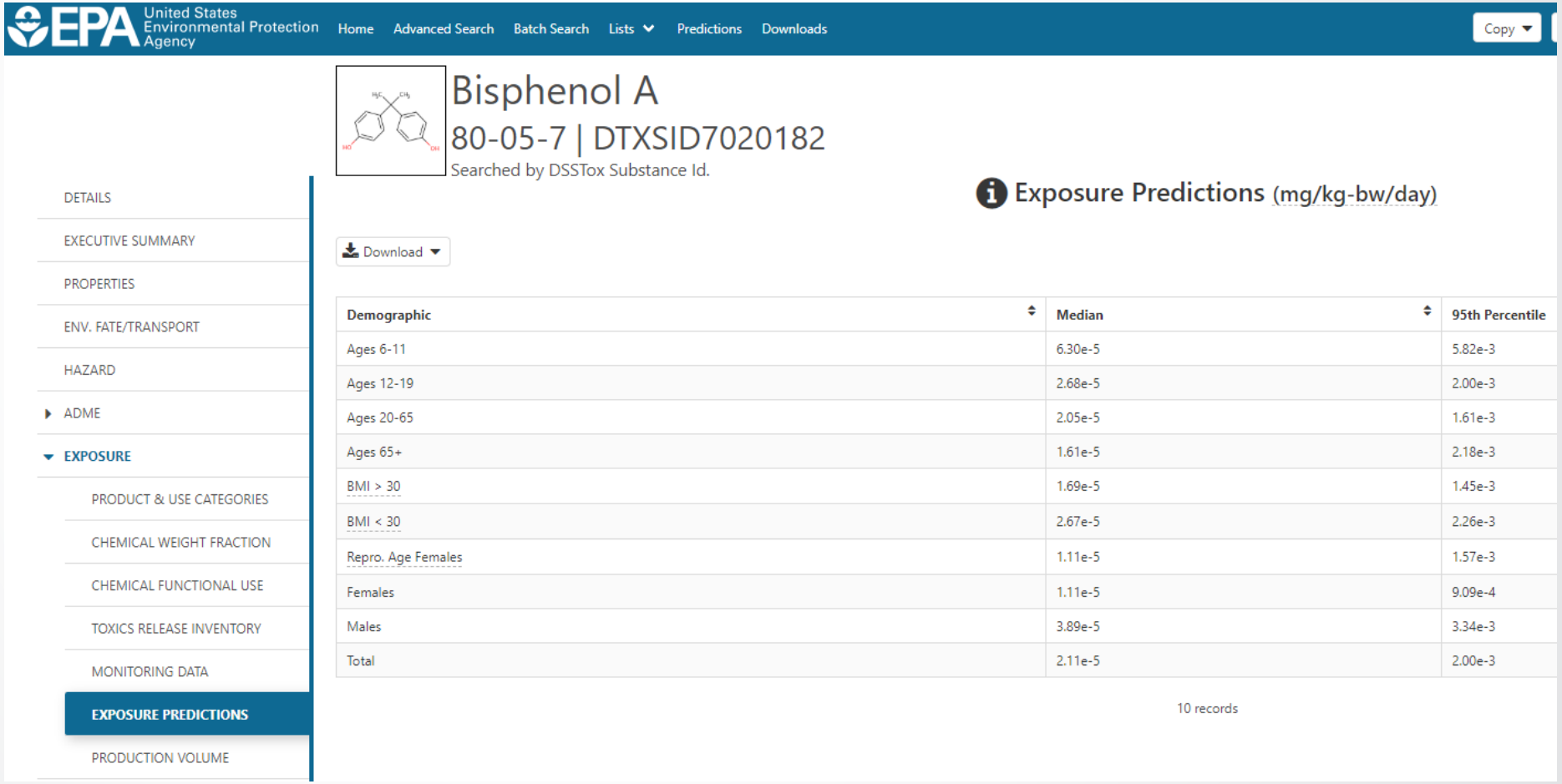
- Operationally, the httk R package (v 1.10.0) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs)
- For some substances, there is a beta tab in the Dashboard with Css and other values needed (no models). More chemicals have information in the httk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

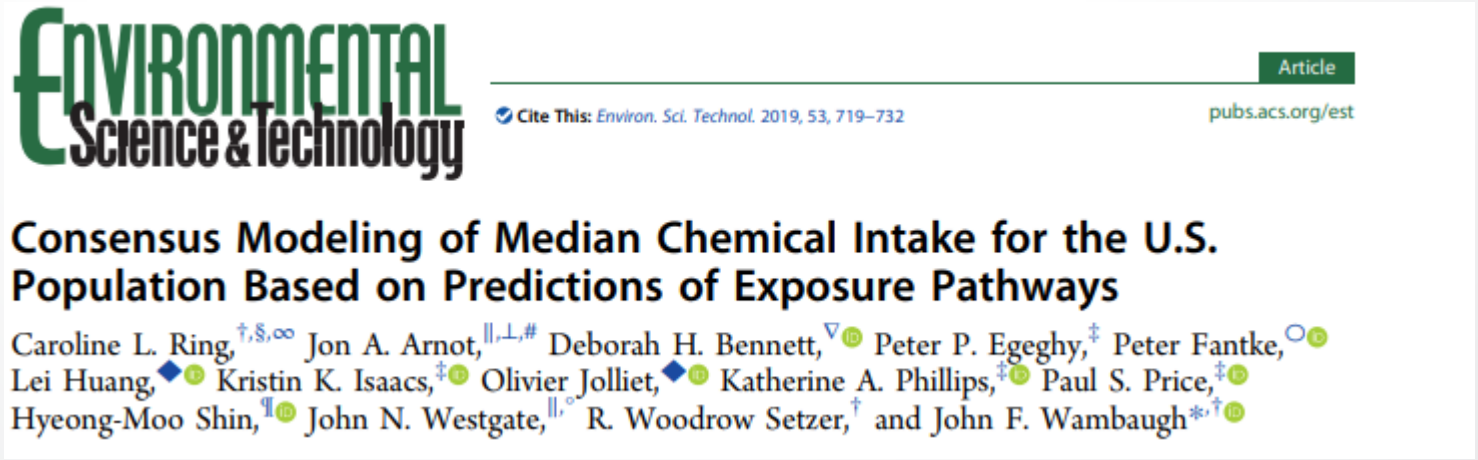
- Currently the Dashboard shows SEEM2 (2014) values



Consensus modeling of chemical exposure based on pathways: ExpoCast SEEM3

Comparison to exposure predictions for a bioactivity:exposure ratio

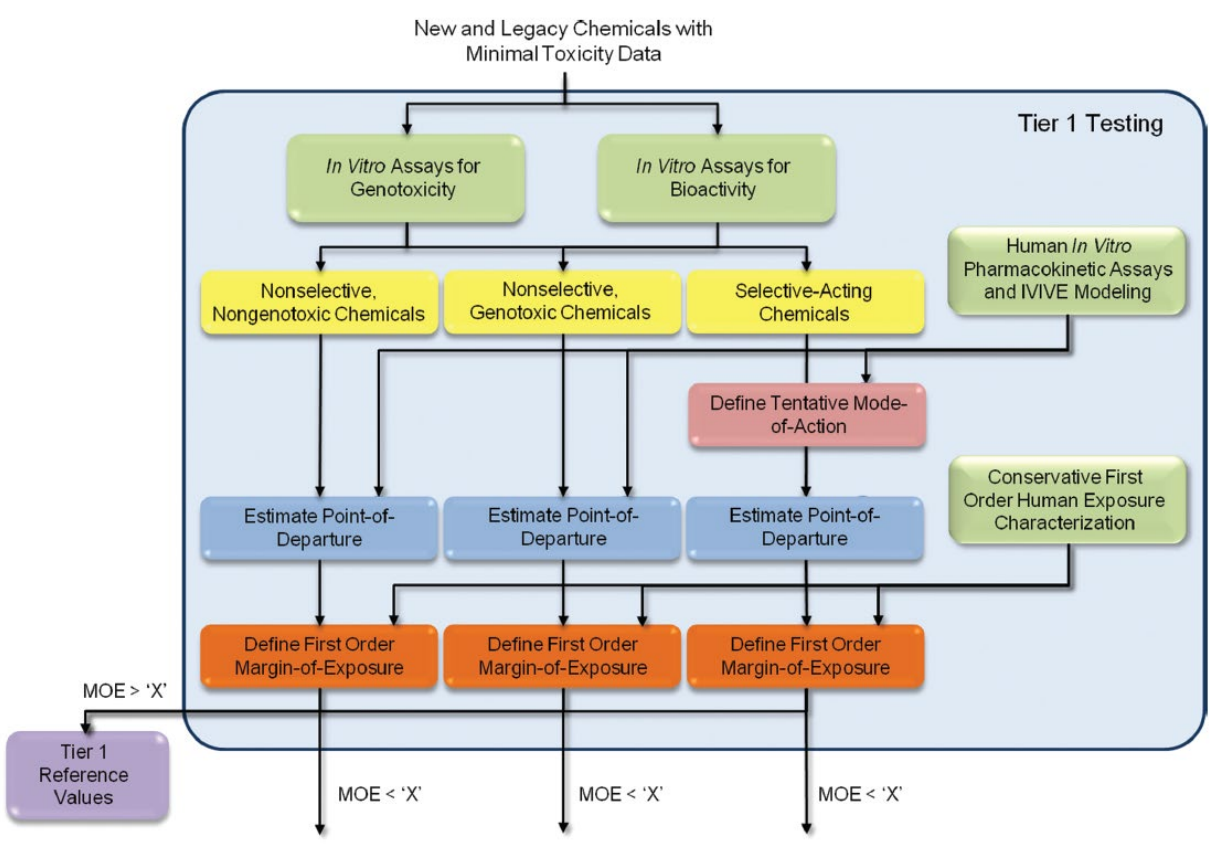
- "ExpoCast SEEM3" model:
  - uses twelve different exposure predictors including both near- and far-field models;
  - covers four distinct exposure pathways: non-pesticidal dietary, consumer products, far-field pesticide, and far-field industrial.
  - In SEEM3 each exposure predictor is scaled and centered 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).



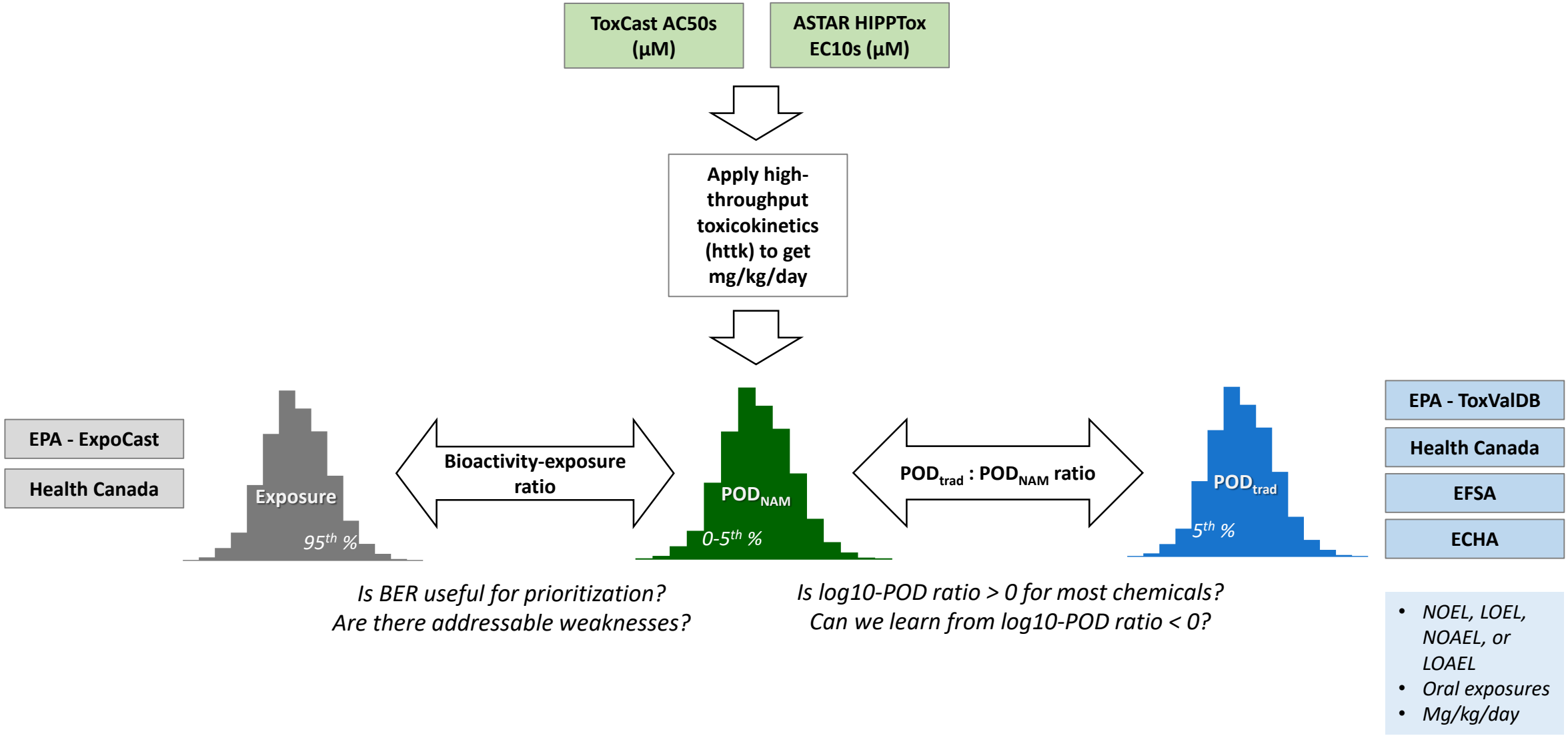
Screening level assessment: combine NAMs for exposure, *in vitro* bioactivity, and toxicokinetics

- 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)*
- Two case studies including a large retrospective analysis and a prospective analysis
- A poster on these two case studies won the Top Abstract Award from the Risk Assessment Specialty Section at SOT 2019
- First case study paper just accepted at Toxicological Sciences

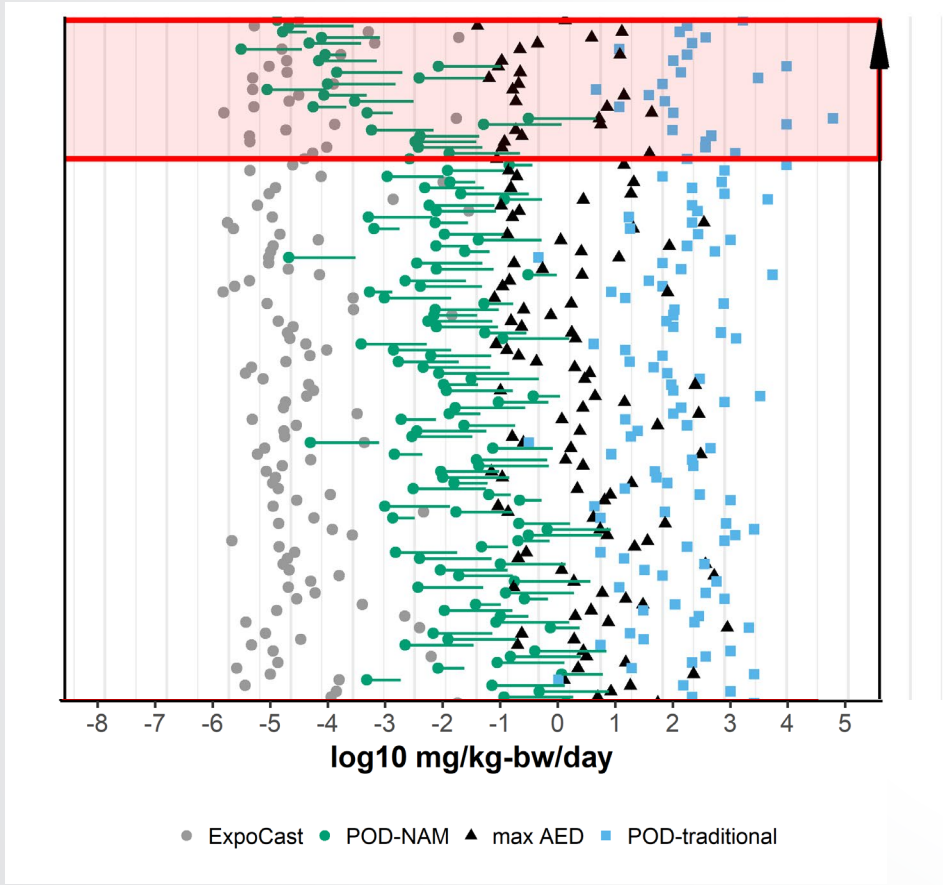
(APCRA partners for these two case studies)

Tune in for our Communities of Practice Webinar on 9/26/19, 11:00 AM- 12:00 PM EST

Case study workflow



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



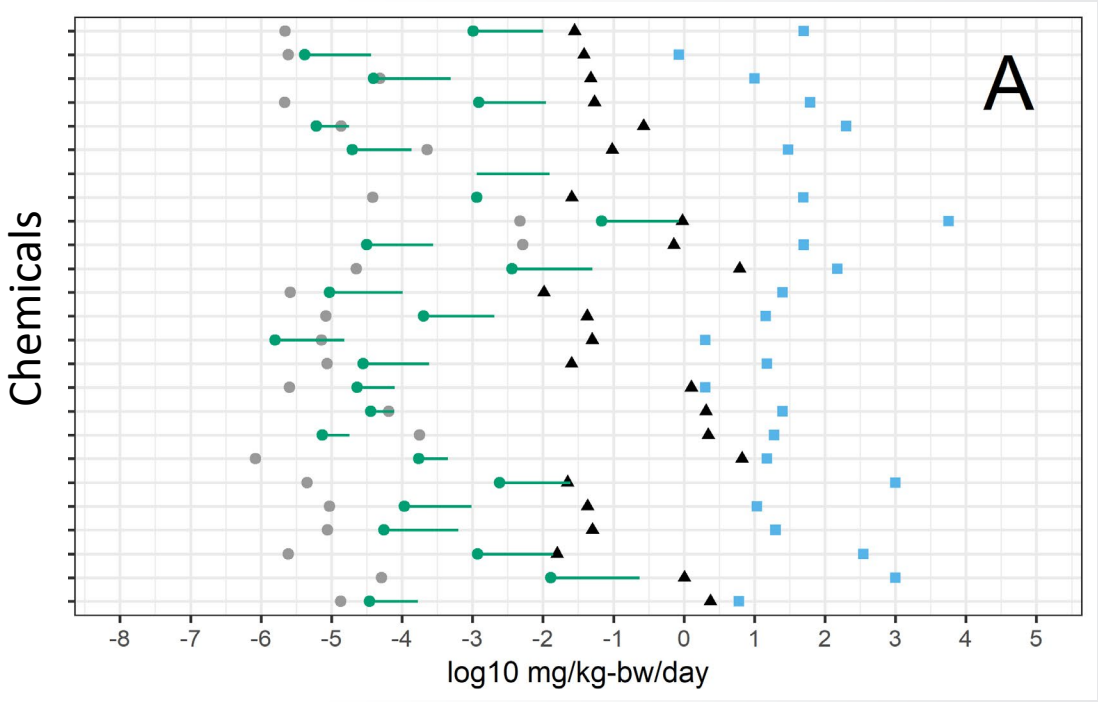


Figure 3 from Paul Friedman et al. accepted.

Acknowledgments

- Thank you for listening.
- Please reach out to us if you need support or explanations for a specific case, or if you find issues.

EPA's National Center for Computational Toxicology