What are new approach methodologies and how can I use them?

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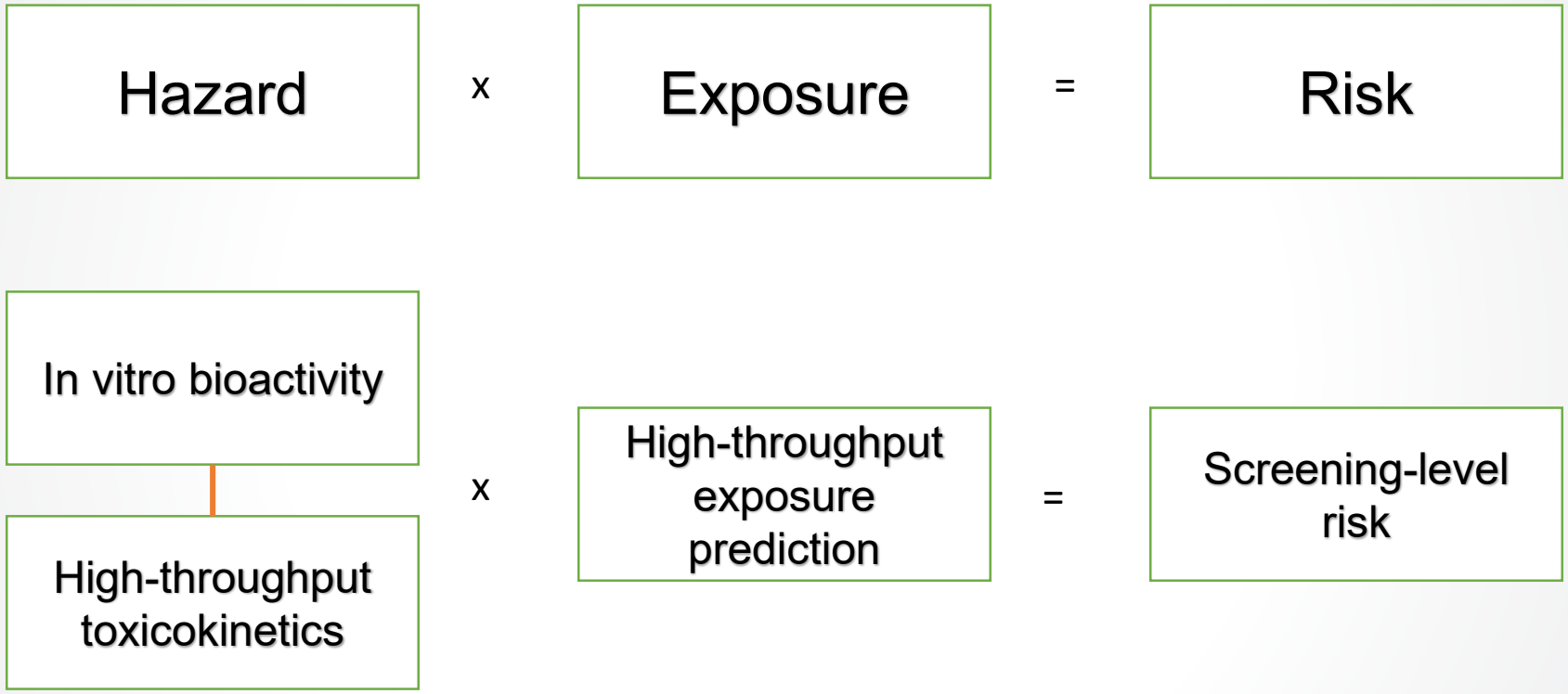
The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

Goals of computational toxicology

- Identify biological pathways of toxicity (AOPs)
- Develop high-throughput *in vitro* assays to test chemicals
- Identify “Human Exposure Chemical Universe” to test
- Develop models that link *in vitro* to *in vivo* hazard
- Use pharmacokinetic models to predict activating doses
- Develop exposure models for all chemicals
- Add uncertainty estimates
- Create high-throughput risk assessments

New approach methodologies (NAMs) are in silico or in vitro methods to predict components of risk.

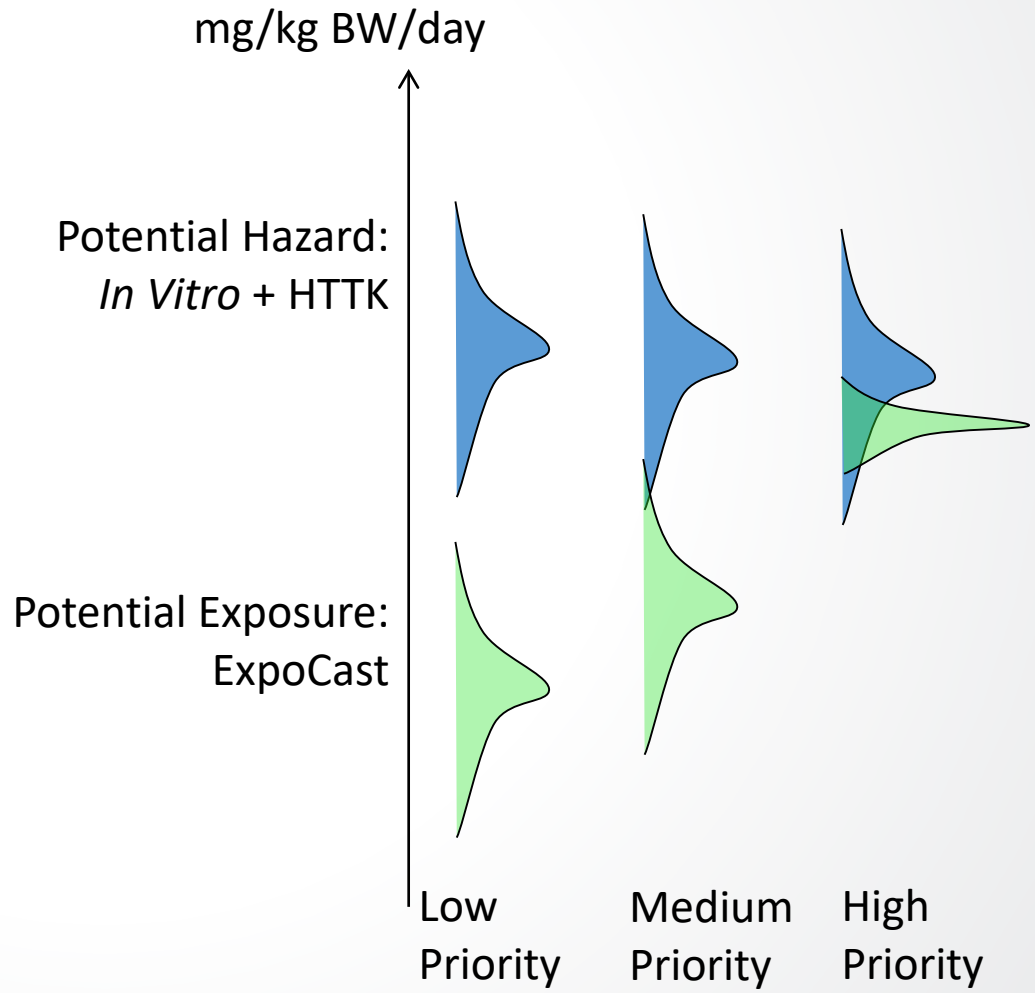


See EPA's strategic plan for using NAMs in chemical management under TSCA:

https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

As exposures approach doses with activity, priority for further review of the data.

Chemical concentrations active in ToxCast assays (in vitro) can be converted to mg/kg-bw/day doses using high-throughput toxicokinetic information (HTTK)



What are the regulatory drivers for using NAMs in risk assessment?

- In US, Section 4(h) in amended TSCA says –
  - "…Administrator shall reduce and replace, to the extent practicable and scientifically justified…the use of vertebrate animals in the testing of chemical substances or mixtures…"
  - New approach methods (NAMs) need to provide "information of equivalent or better scientific quality and relevance…" than the traditional animal models
- In Canada, Health Canada (HC) and Environment and Climate Change Canada (ECCC) are continuing work under the Chemicals Management Plan (CMP) to address human health and ecological concerns for approximately 4,300 prioritized substances on the Canadian Domestic Substances List (DSL) by the year 2020.
- In Europe, REACH says –
  - Article 13: "Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met (…) for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods…"
  - Annex XI: "Results obtained from suitable *in vitro* methods may indicate the presence of a certain dangerous property or may be important in relation to a mechanistic understanding, which may be important for the assessment…" BUT confirmation using standard *in vivo* tests are still required unless:
    - Results are derived from an in *vitro* method whose scientific validity has been established by a validation study, according to internationally agreed validation principles; AND
    - Results are adequate for the purpose of classification and labelling and/or risk assessment; AND
    - Adequate and reliable documentation of the applied method is provided.

What is needed to understand the acceptability of NAMs for risk assessment?

- In US, Section 4(h) in the Lautenberg amendment to TSCA:
  - "…Administrator shall reduce and replace, to the extent practicable and scientifically justified…the use of vertebrate animals in the testing of chemical substances or mixtures…"
  - New approach methods (NAMs) need to provide "information of equivalent or better scientific quality and relevance…" than the traditional animal models
- "Directive to Prioritize Efforts to Reduce Animal Testing" memorandum signed by Administrator Andrew Wheeler on September 10, 2019
  - "1. Validation to ensure that NAMs are equivalent to or better than the animal tests replaced."

How do we define expectations of *in silico, in chemico,* and *in vitro* models for predicting repeat-dose toxicity?

***In silico, in chemico,* and *in vitro* models cannot predict *in vivo* systemic effect values with greater accuracy than those animal models reproduce themselves.**

Some examples sources of NAM data include ToxCast and ExpoCast

CompTox Dashboard (many data streams, currently centered on chemistry; Williams et al. 2017 PMID 29185060): https://comptox.epa.gov/dashboard

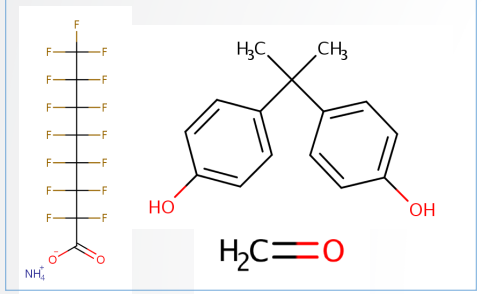
Data downloads (download databases and supporting data files):

https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

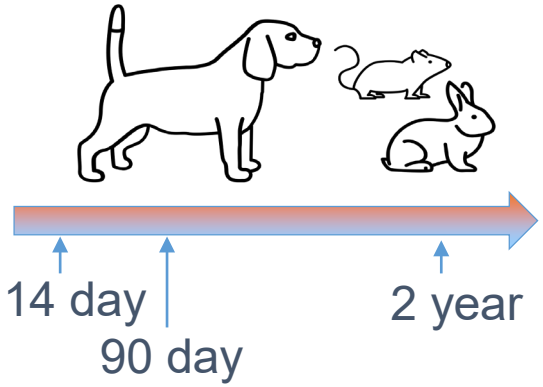
Why can't we just use traditional approaches?

Challenge

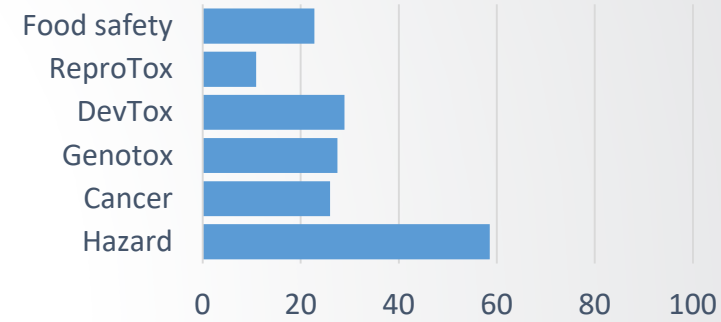
Too many chemicals



Traditional methods are slow, costly



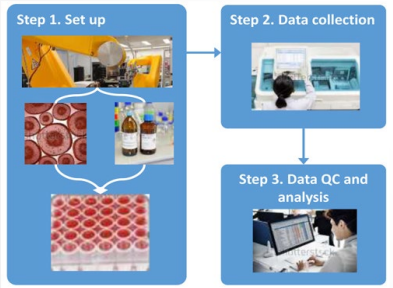
Not enough data

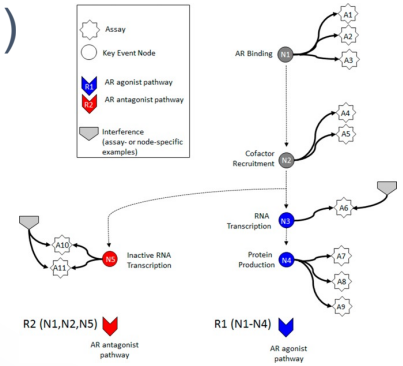


Judson *et al* 2009

Solution

New Approach Methods (NAMs)





Interagency Collaboration Toxicity Testing in the 21st century (Tox21)

- >10K chemicals
- >100 biological targets

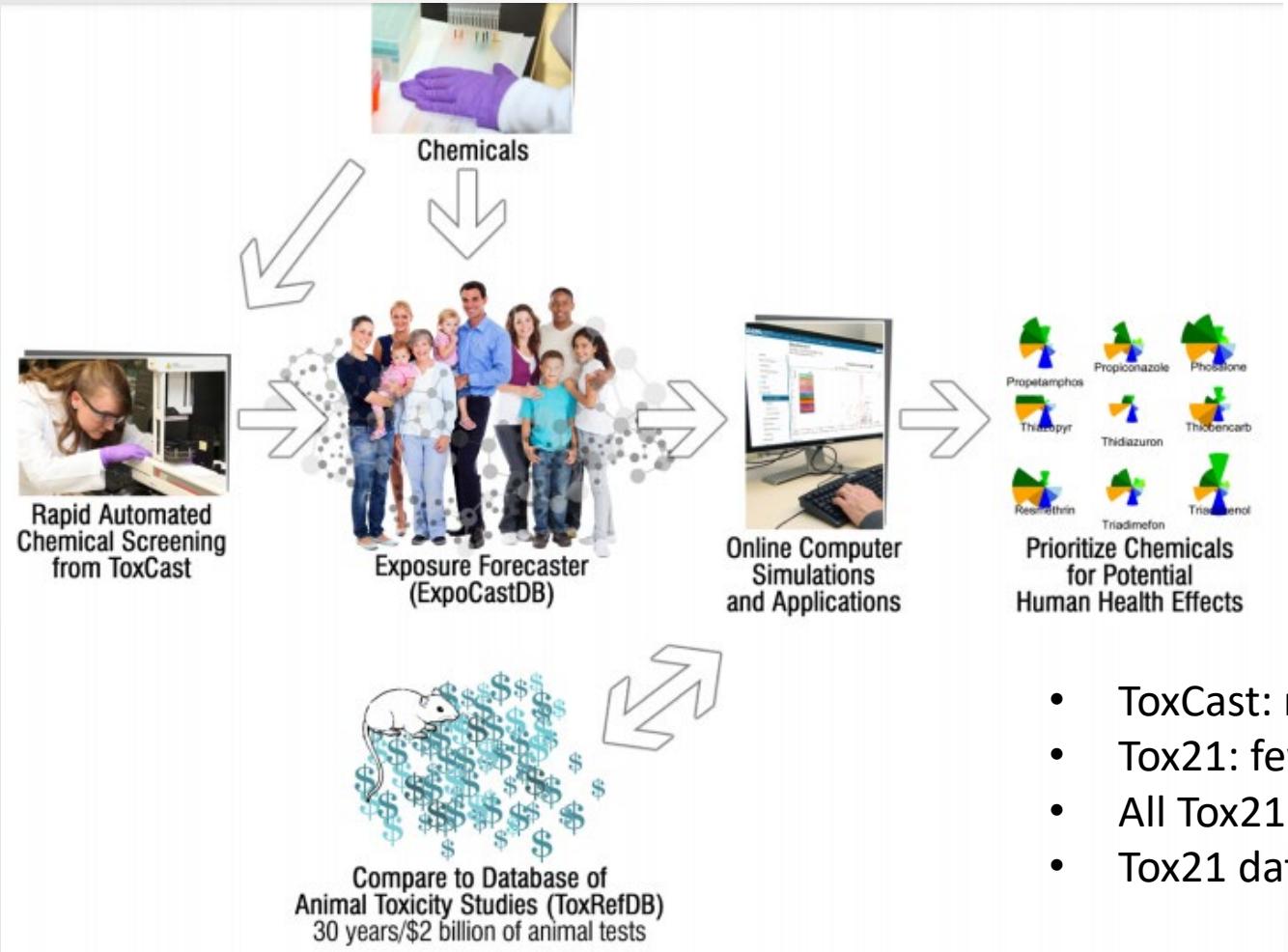
Cell painting, transcriptomics (HTTr, S1500+)

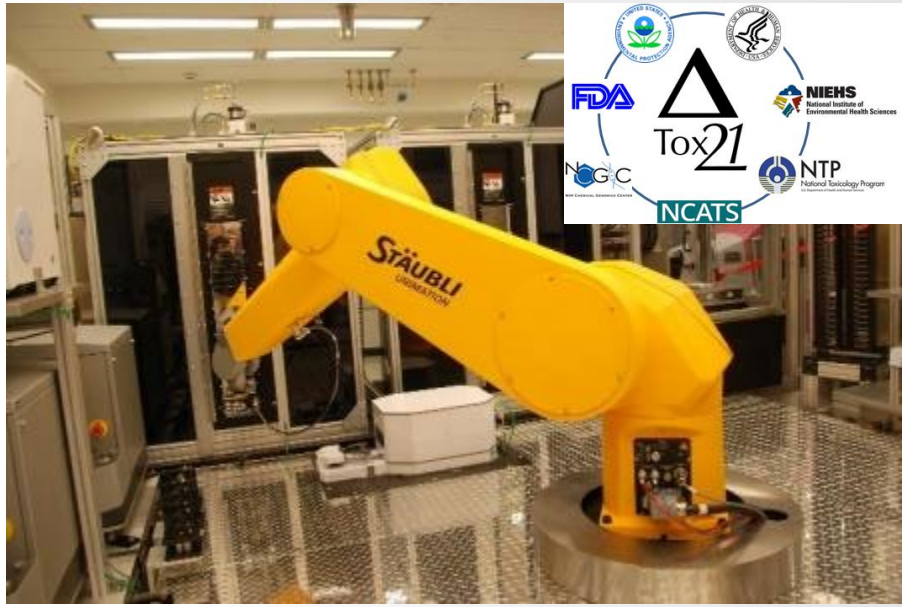
USEPA's Toxicity Forecaster (ToxCast) program

- > 3K chemicals screened in
- > 1K assays
  - ~400 biological targets

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

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

Invitrodb version 3.2 (released August 2019) contained 15 different assay sources, covering (at least) 443 unique gene-related targets with 1473 unique assay endpoints. Varying amounts of data are available for 9224 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, primary)
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)
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 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)

What can I do with ToxCast data?

Some of the tasks we can use ToxCast for:

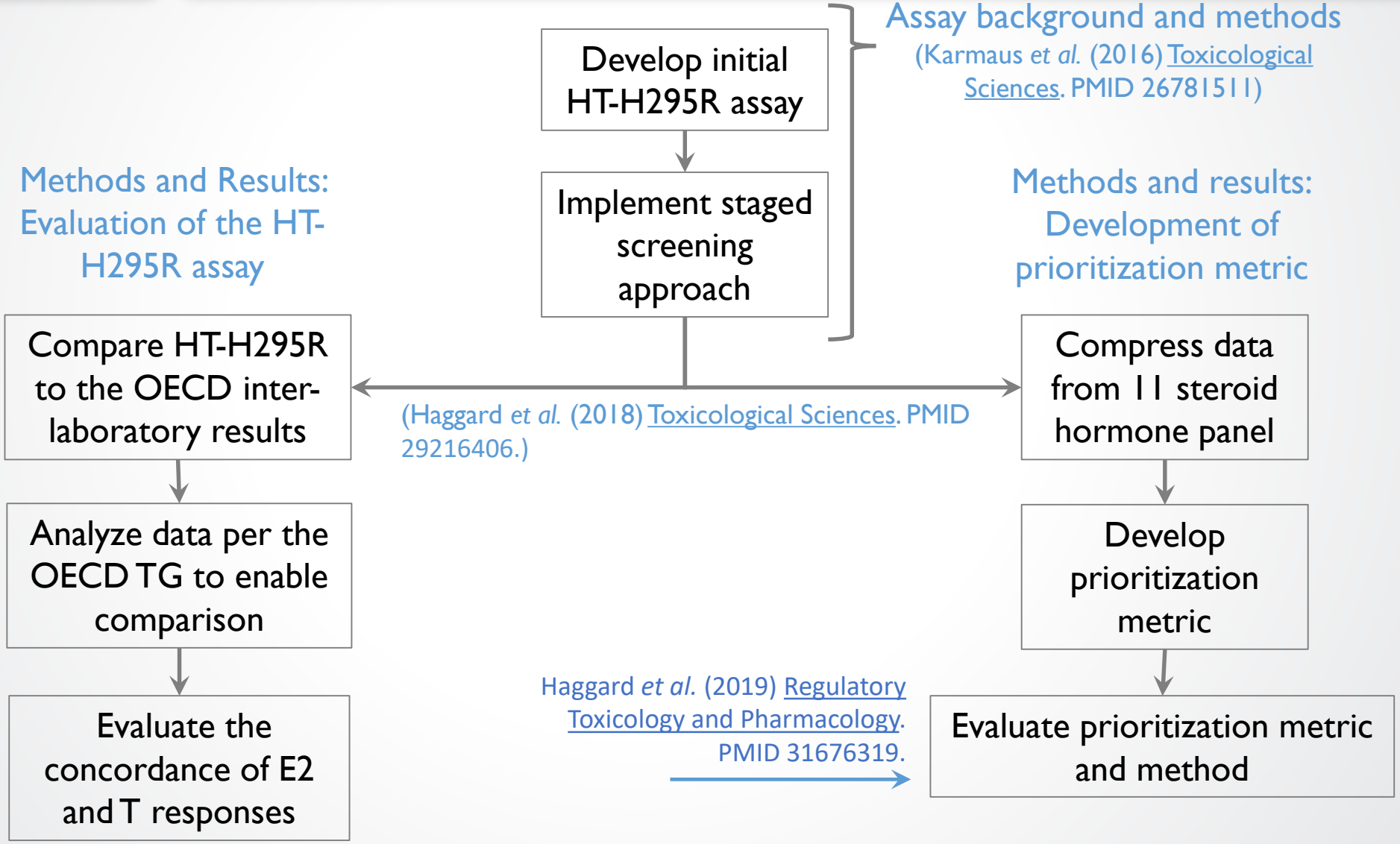
- *Qualitative (and perhaps quantitative) support for mode-of-action (MOA) or adverse outcome pathway (AOP);*
- Prediction of specific adverse outcomes, e.g. developmental toxicity or disruption of steroidogenesis;
- Estimation of a point-of-departure dose for use in screening level assessments or prioritization.

Task 2: Example of predicting a specific adverse outcome: perturbation of steroidogenesis

- Does a substance interfere with biosynthesis of steroid hormones *in vitro*?
- Step 1: collect *in vitro* data
  - In this case, it's multi-dimensional because multiple hormones are involved
- Step 2: build model to interpret those data
  - How to reduce an 11-dimensional problem to one dimension?
  - How to reduce noise in the system and find "true" signal?
- Step 3: develop context for understanding the model output
  - Layer in information about cytotoxicity, exposure, etc.

ToxCast and steroidogenesis

Derik Haggard, Katie Paul Friedman, and colleagues



Steroidogenesis is critical for several physiological processes and modeled in the H295R cell-based assay

Steroidogenesis pathway: relevant biology

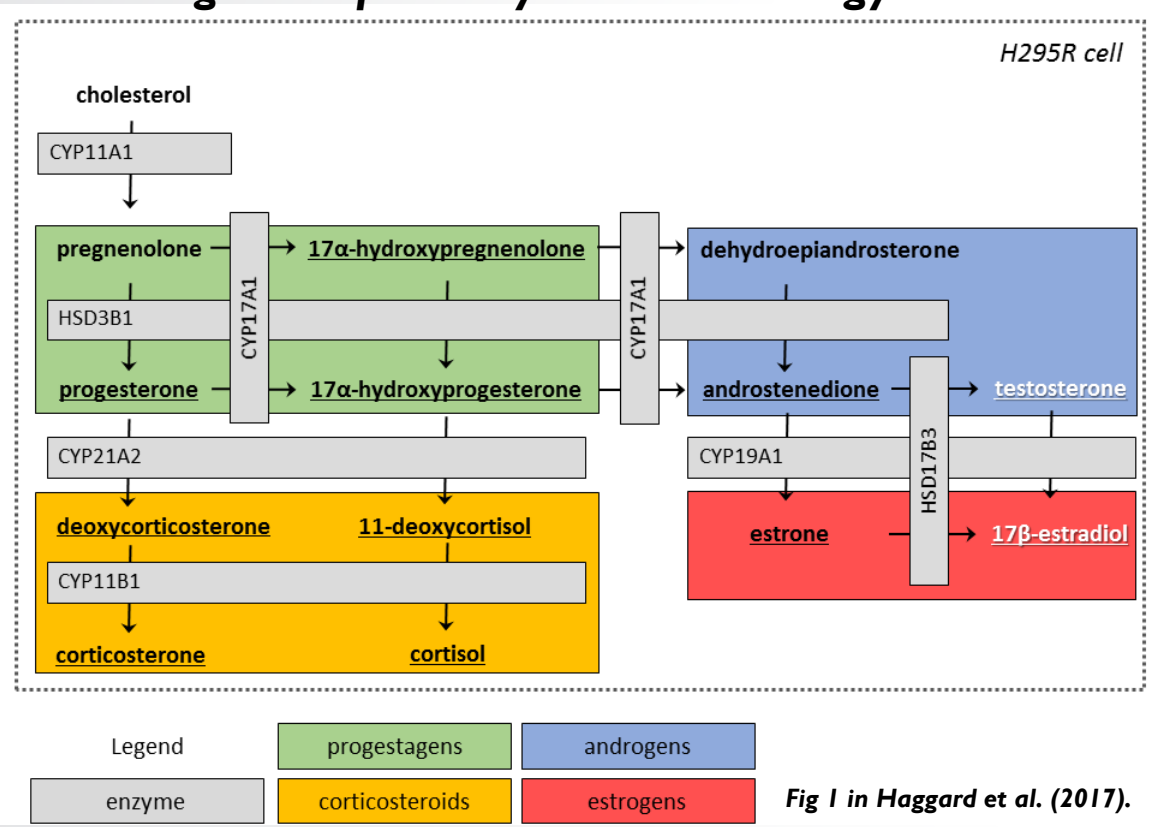
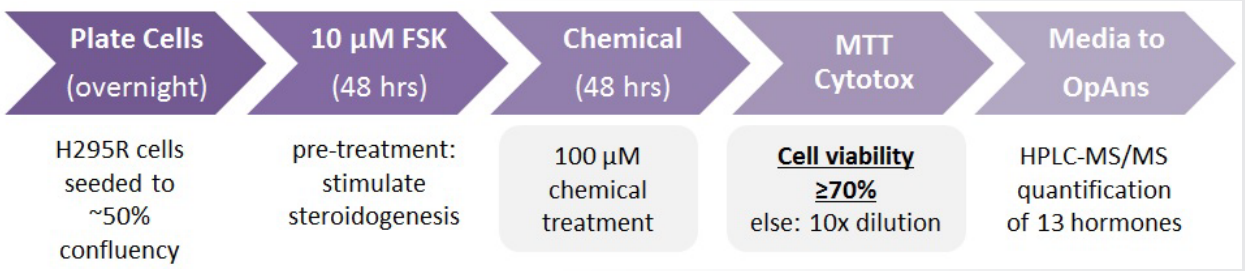


Fig 1 in Haggard et al. (2017).

High-throughput adaptation of H295R assay



- Maximized screening resource efficiency.
- 2012 unique test chemicals have been screened at a high concentration.
- # steroid hormones affected in single concentration (along with other considerations) were used to select 656 chemicals for multi-concentration screening.

Problem 1: Does HT-H295R perform like validated H295R for estradiol and testosterone synthesis?

- Comparison to the OECD-validated version of the H295R assay for a set of reference chemicals.
- This detailed, performance-based comparison highlights good concordance of results, with accuracies that range 0.80 – 0.95 for effects on E2 and T.

Effect	Revised Sensitivity	Revised Specificity	Revised Accuracy
Testosterone up	1.00	0.89	0.90
Testosterone dn	0.67	0.92	0.82
Estradiol up	0.75	0.83	0.80
Estradiol dn	0.80	1.00	0.95

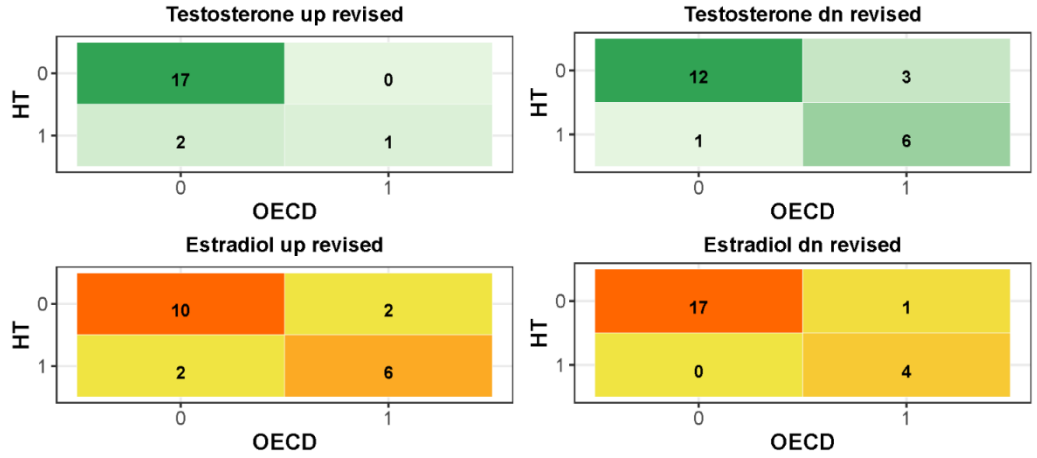


Figure 6 Haggard et al. (2017).

Problem 2: How to compress 11-dimensional data to a single prioritization metric for regulators?

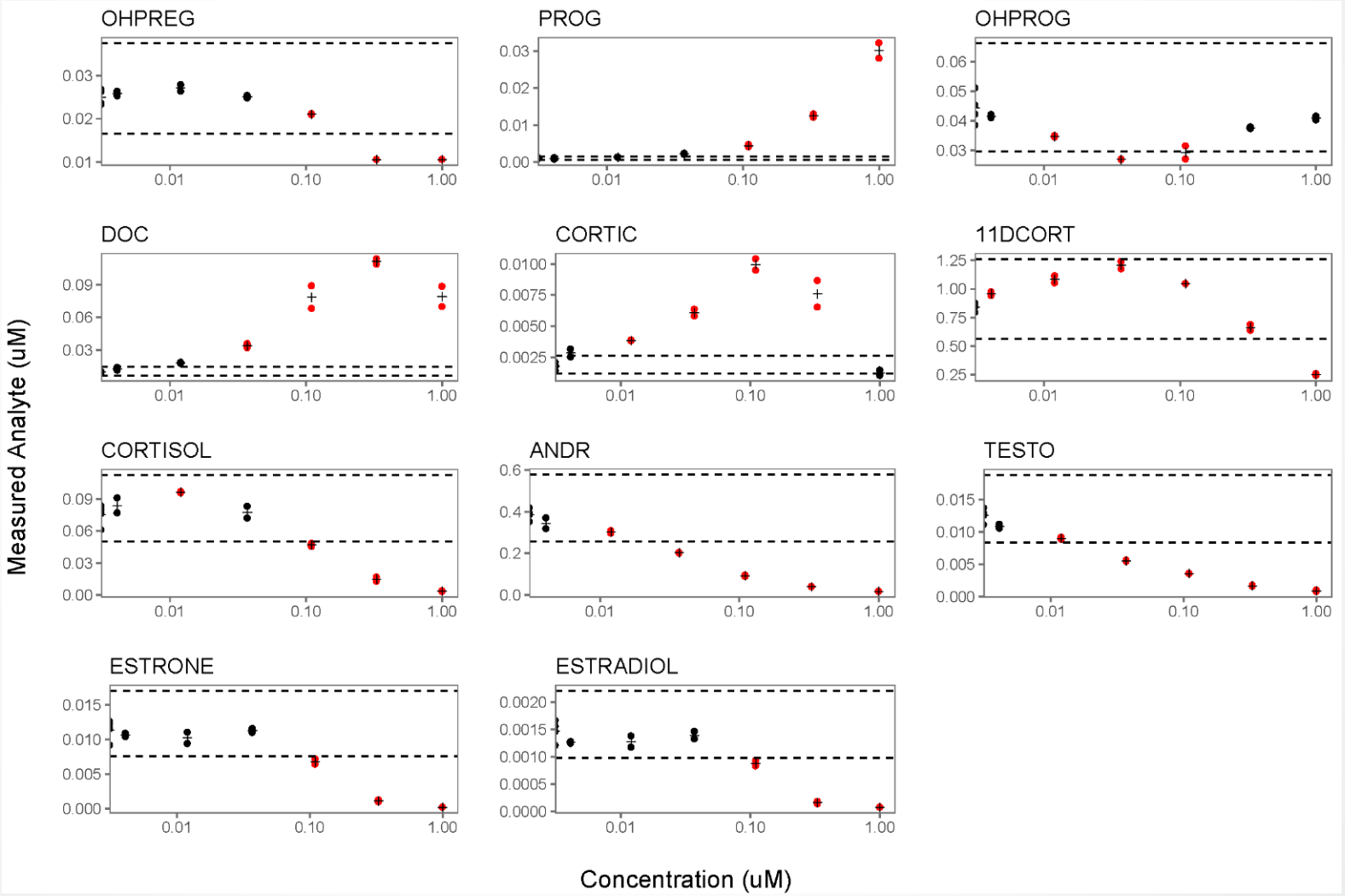
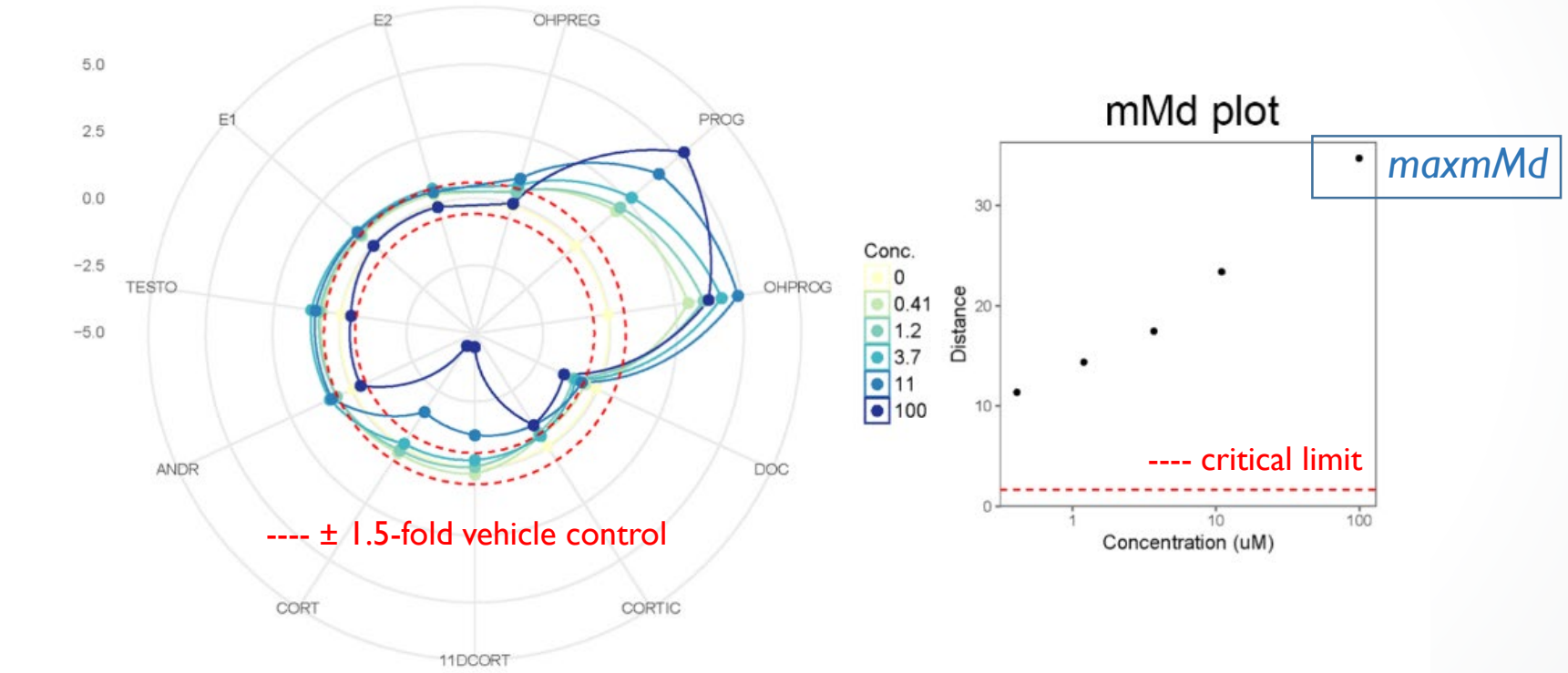


Figure 2 Haggard et al. (2017).

Using our maximum Mahalanobis distance approach to get a single prioritization metric



Mifepristone strongly modulated progestagens with significant effects on progesterone and OH-progesterone and moderate but non-significant trends on corticosteroids and androgens, resulting in a relatively high adjusted maxmMd of 33.

Figure 5, Haggard et al. (2017).

- Reduced an 11-dimensional question to a single dimension.
- Selection of the maxmMd appeared to provide a reproducible, quantitative approximation of the magnitude of effect on steroidogenesis.

For hazard characterization, many want to know: does the bioactivity occur at concentrations lower than nonspecific activity (cytotoxicity)?

Trying to distinguish highly “selective” steroidogenesis perturbation, i.e. activity on steroidogenesis happens at concentrations lower than cytotoxicity

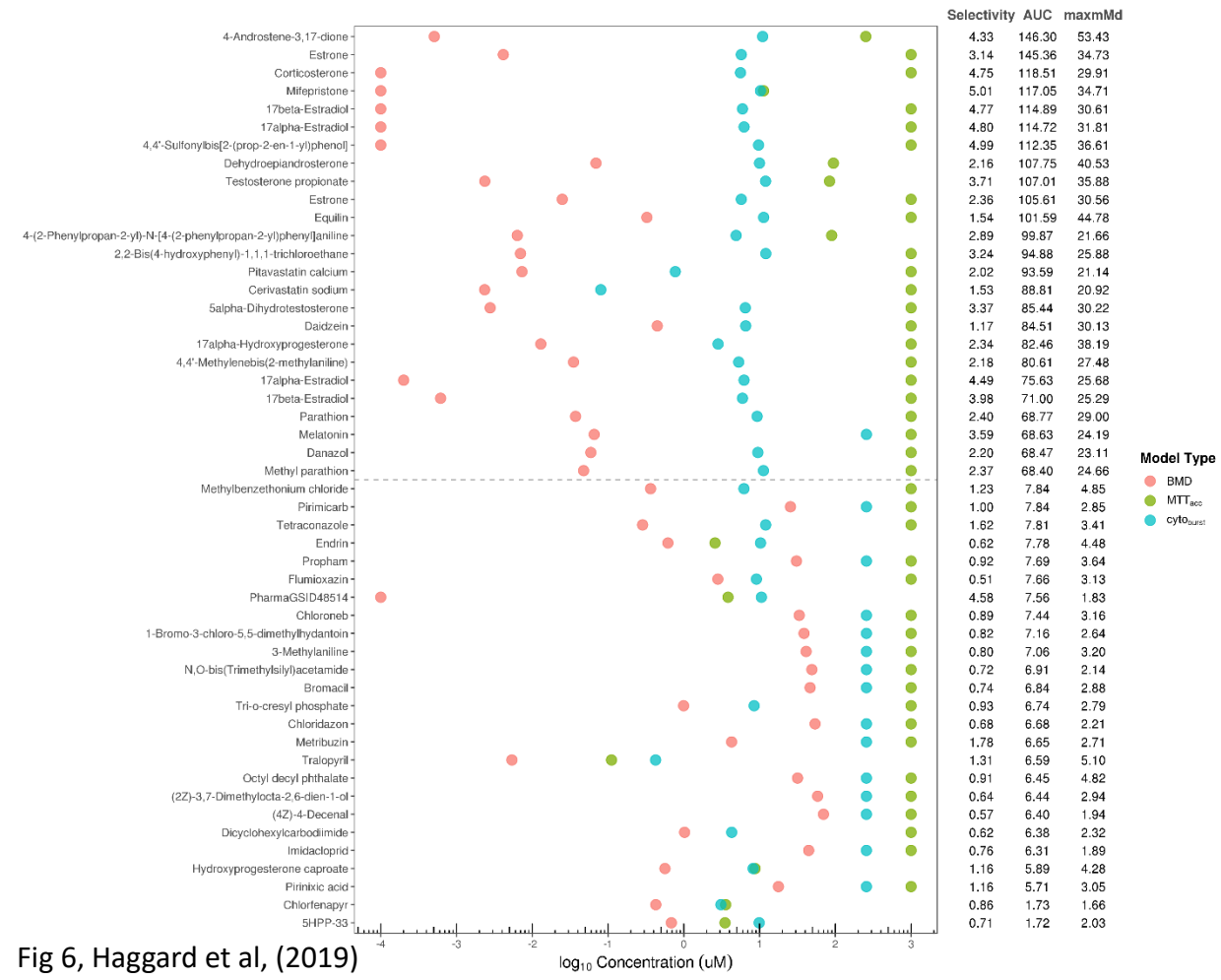


Fig 6, Haggard et al, (2019)

Task 3: Getting a point-of-departure for screening level assessments from bioactivity

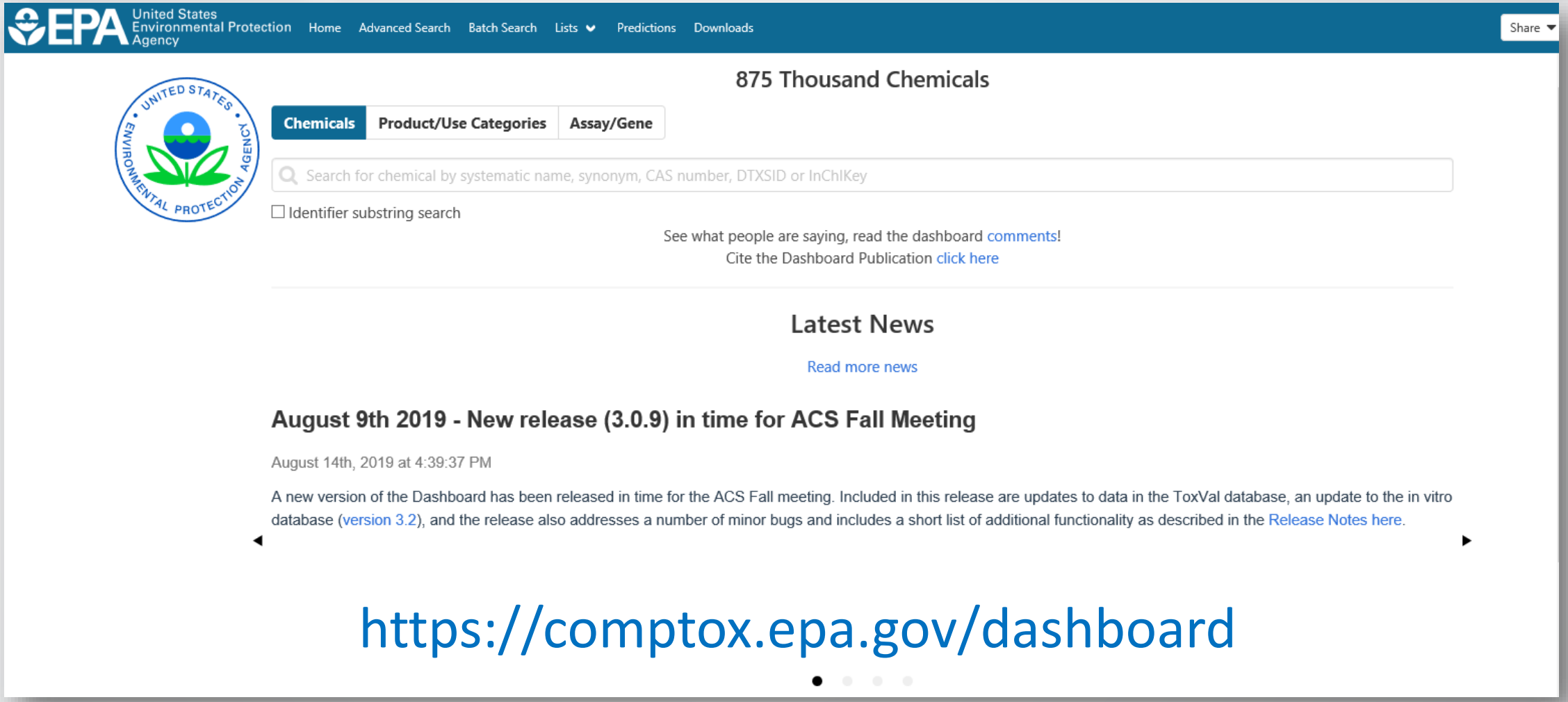
- This is important especially for TSCA
- 1000's of chemicals that lack a full hazard assessment
- We can use bioactivity as one source of information to derive a POD (other sources could be things like the threshold of toxicological concern, for example)

Using ToxCast Data in Screening Level Assessment

- A common question is how to approach the use of ToxCast information in a screening level assessment.
- 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 screening level risk evaluation.

CompTox Chemicals Dashboard



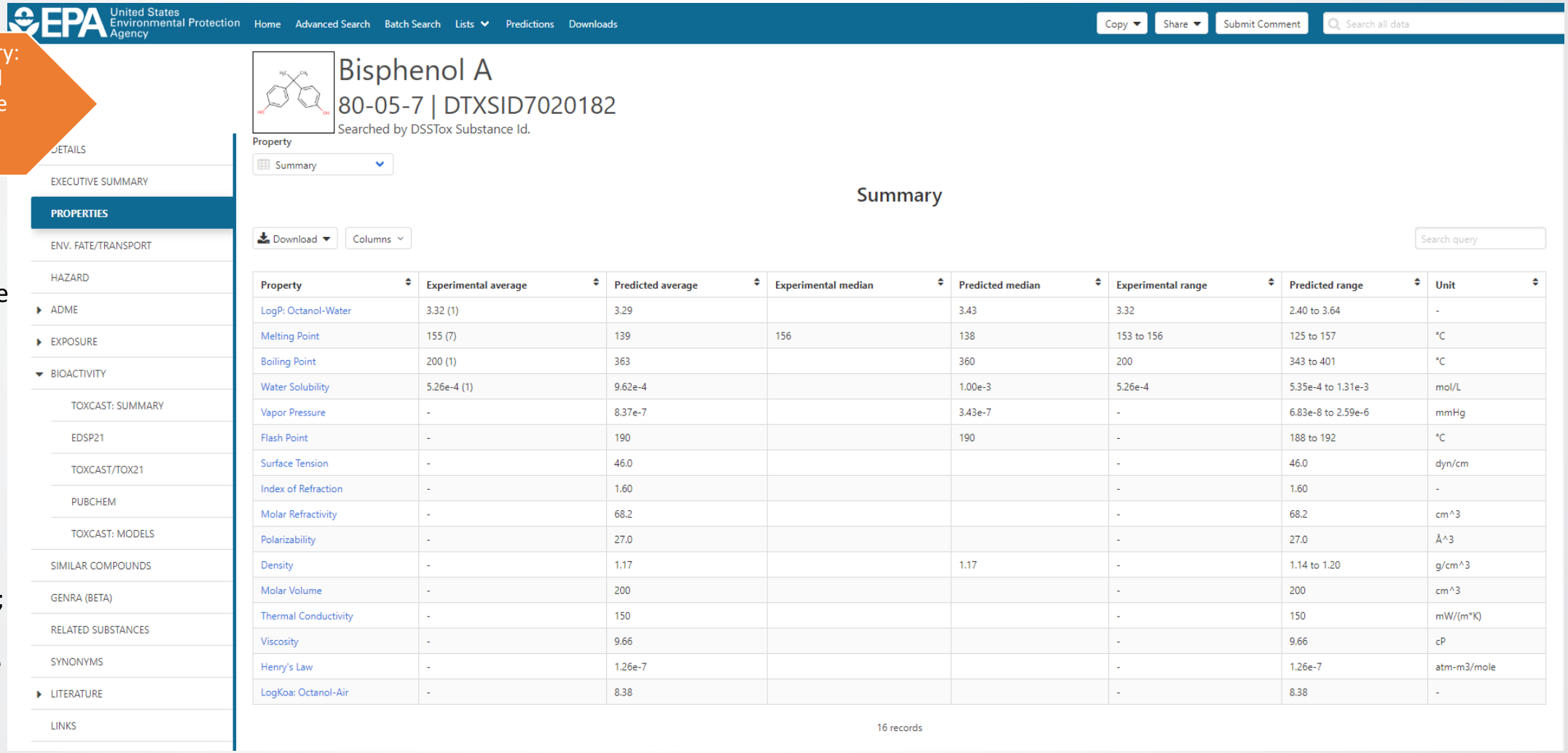
https://comptox.epa.gov/dashboard

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?

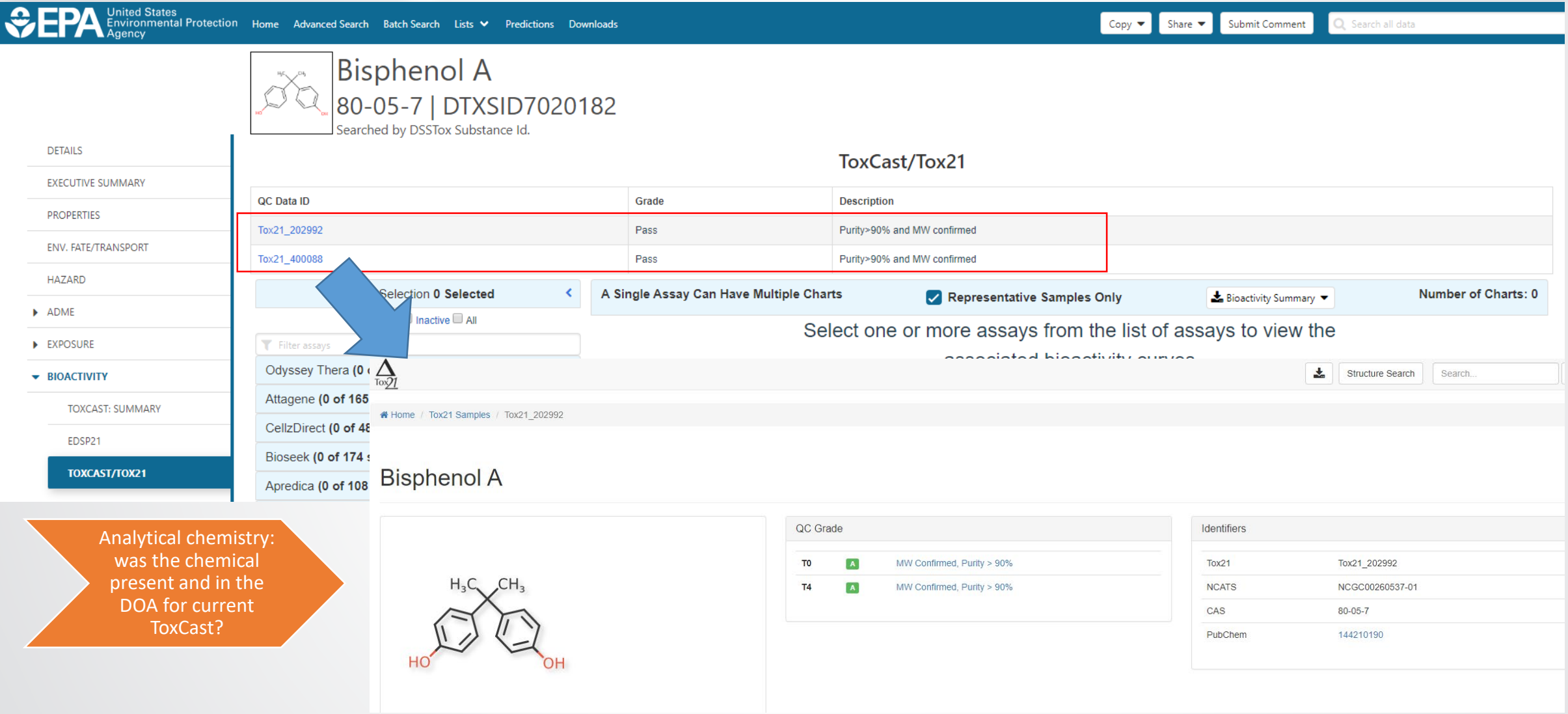
ToxCast negatives: what does a negative mean? Outside of domain of applicability (DOA)?

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	-

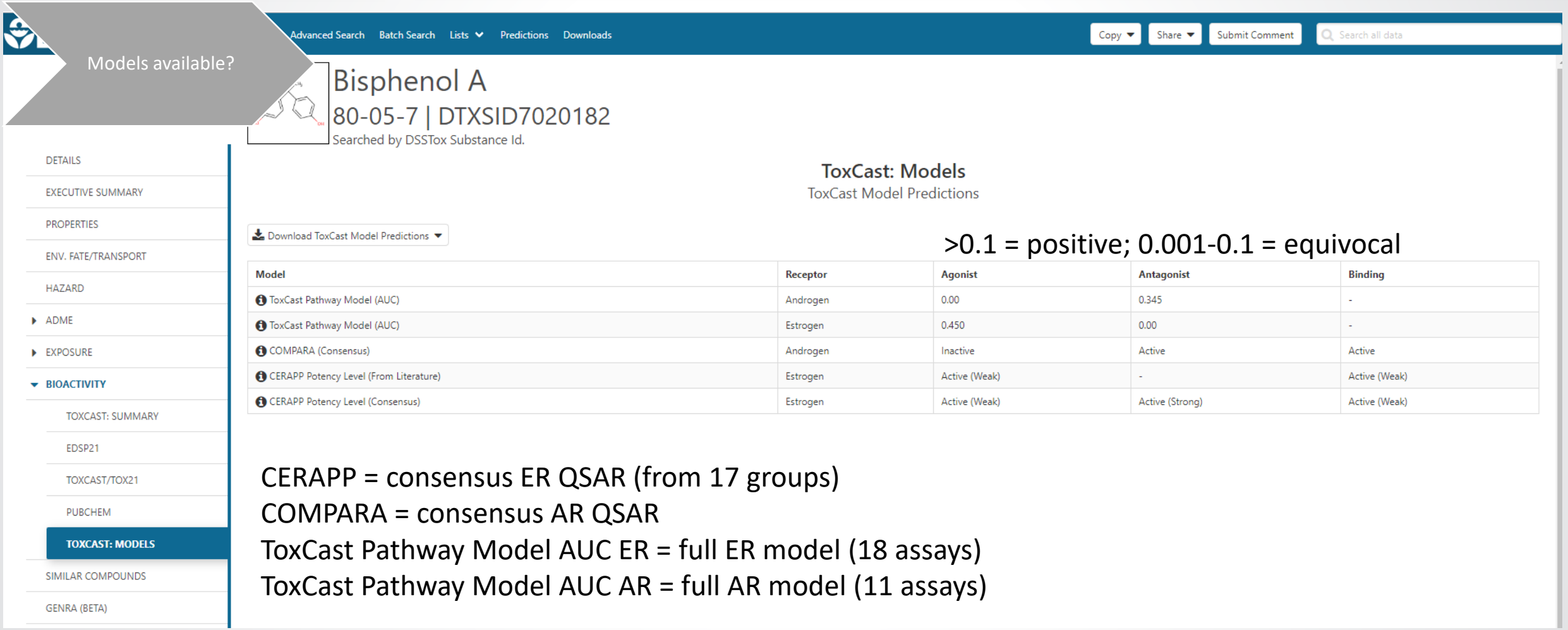
Examine QC data (if available) to see if we expect that the chemical was present for screening



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

Models >>> single assays. And equivocals happen.

Models available?



>0.1 = positive; 0.001-0.1 = equivocal

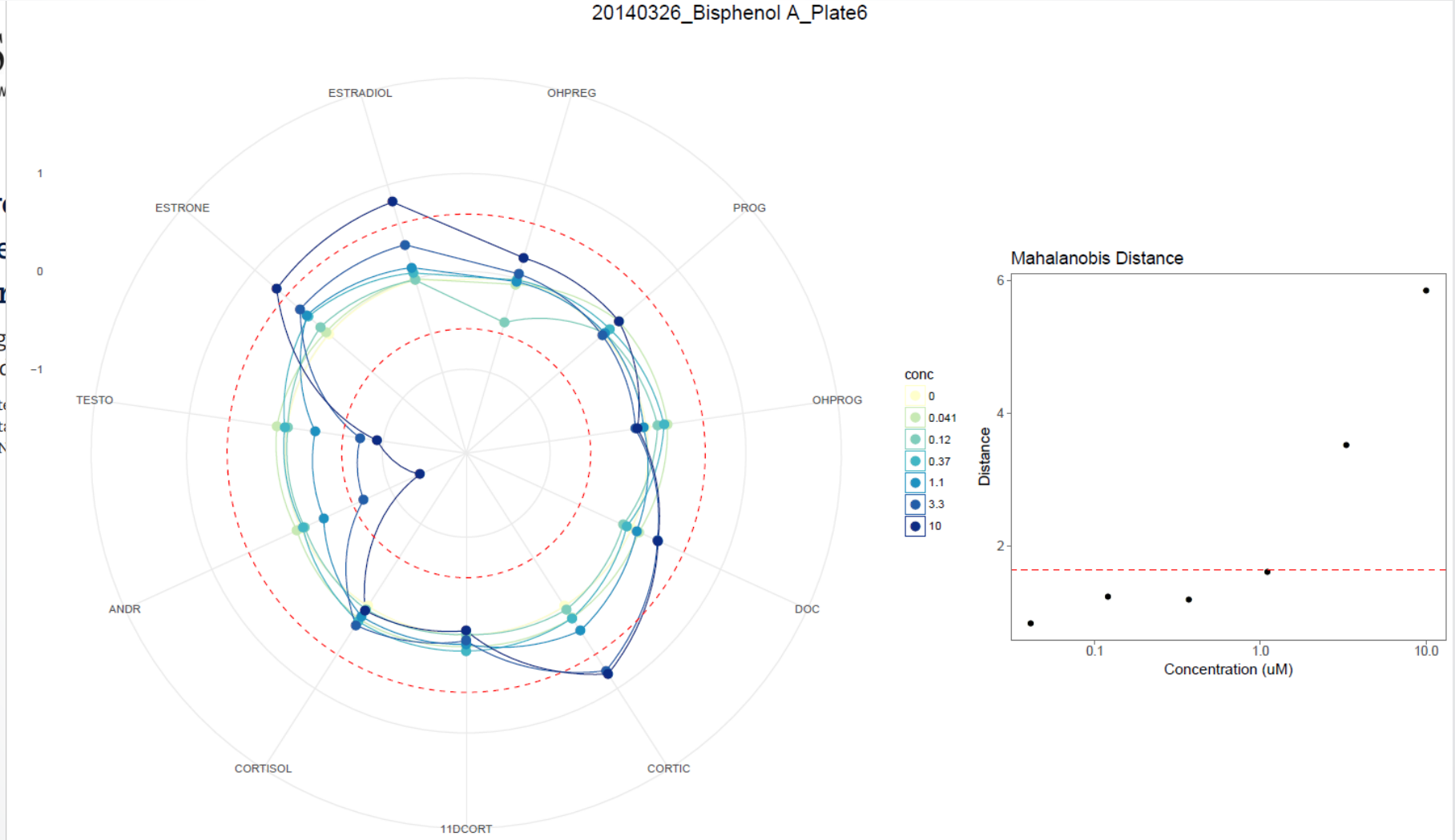
Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
COMPARA (Consensus)	Androgen	Inactive	Active	Active
CERAPP Potency Level (From Literature)	Estrogen	Active (Weak)	-	Active (Weak)
CERAPP Potency Level (Consensus)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

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.

HT-H295R model for steroidogenesis

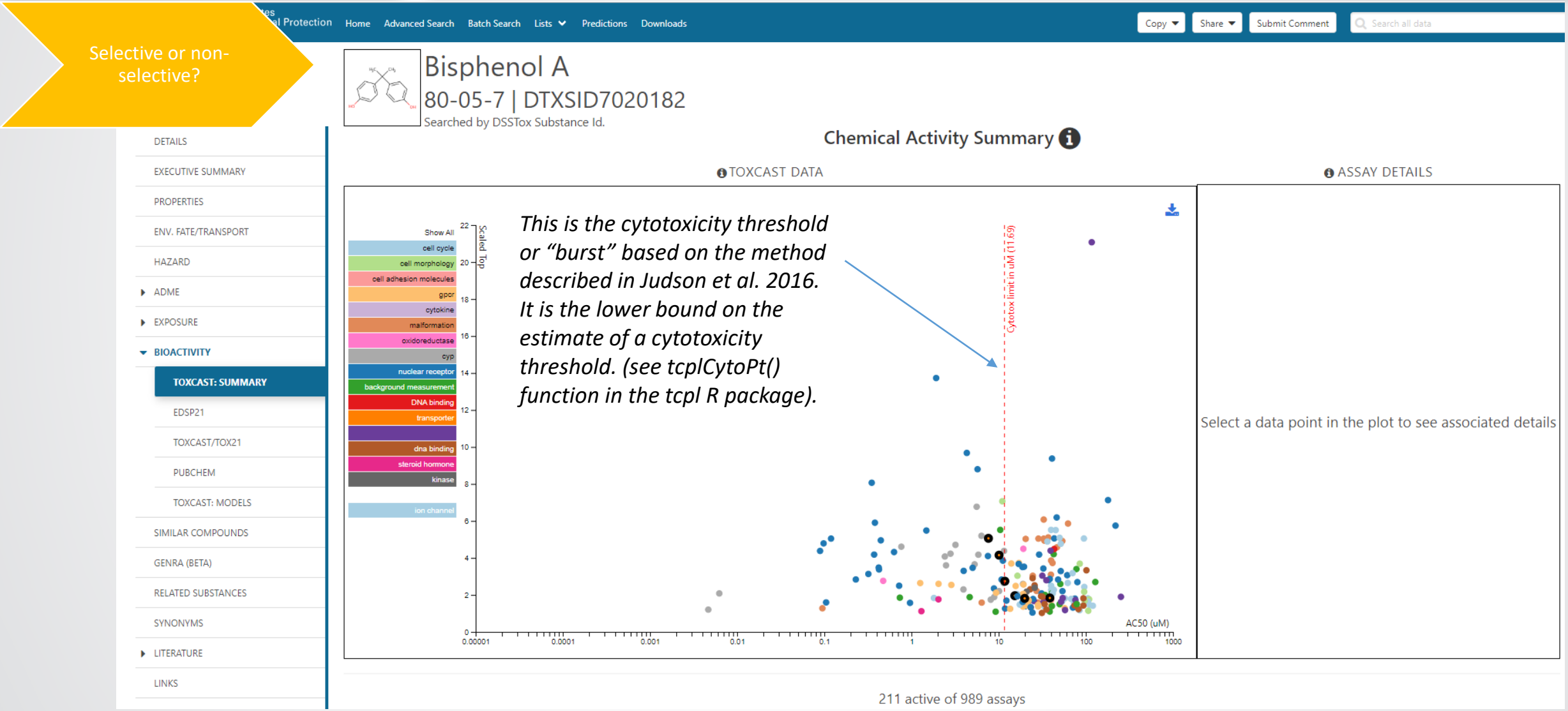
Endocrine models available?



- 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).

User application dictates “selectivity”

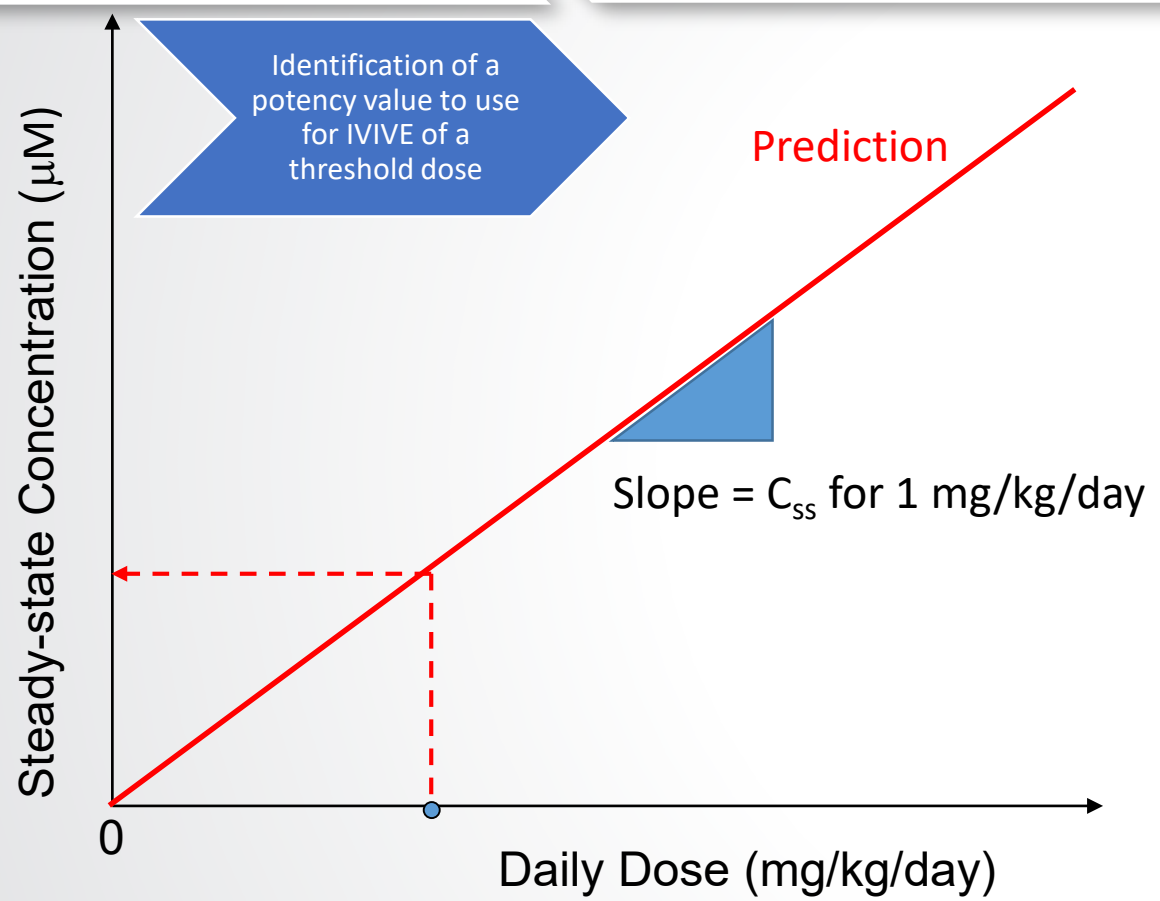
Selective or non-selective?

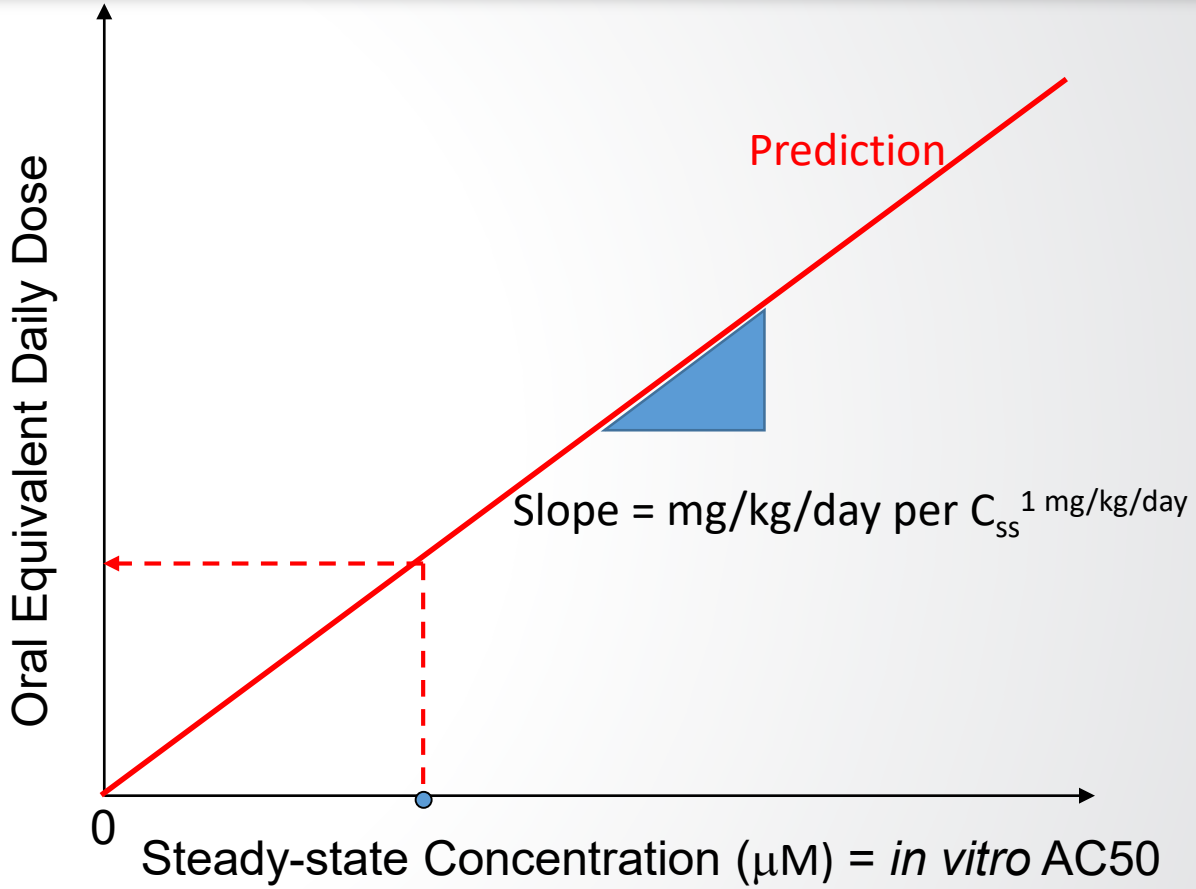
- AC50 < burst?
- AC50 0.5$\log_{10}$ distance from burst?
- AC50 < parallel viability assays?
- How else to filter ToxCast data: 3+ caution flags & hit-percent
- Other related ideas:
  - What other assays appear active in a similar concentration range?
  - Is there consistent support for MOA(s), or 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 CCTE: **https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data**
- We anticipate a new ToxCast release around March 2020.

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





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

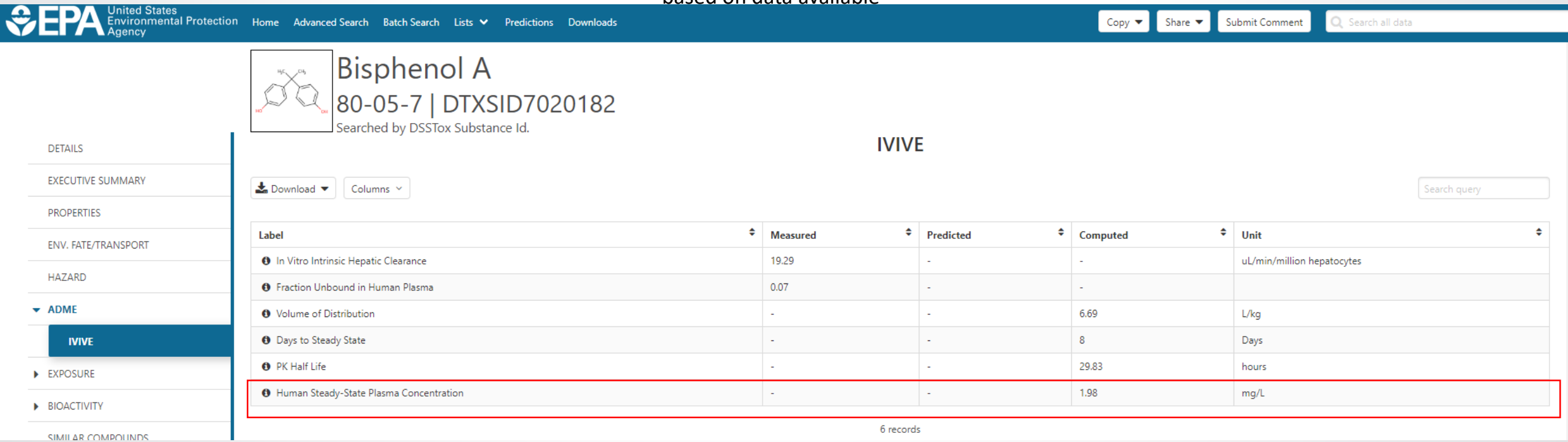
Wetmore *et al*. (2012)

- 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

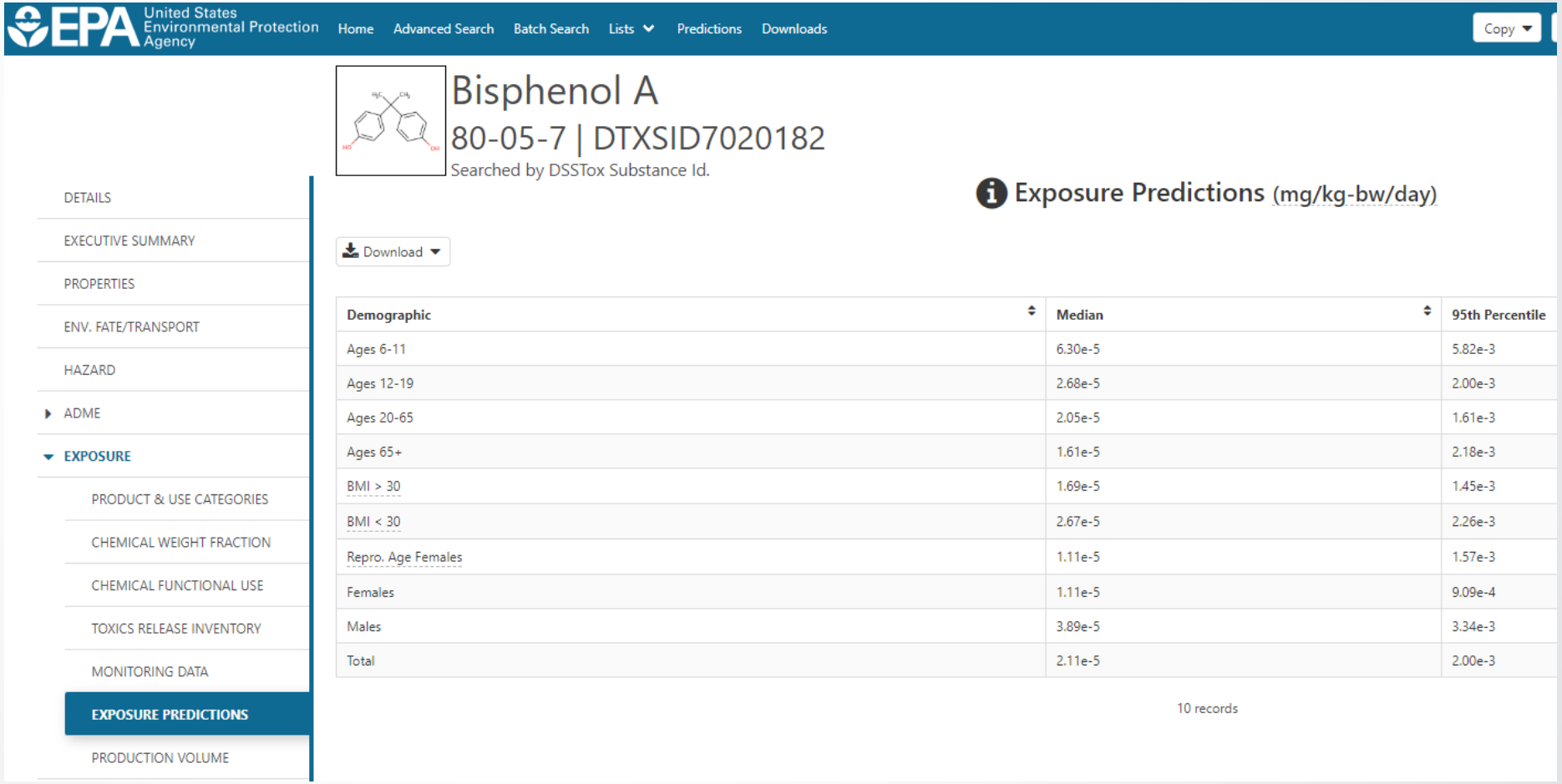
- Operationally, the httk R package (v 1.10.0) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs)
- For some substances, there is a beta tab in the Dashboard with Css and other values needed (no models). More chemicals have information in the httk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

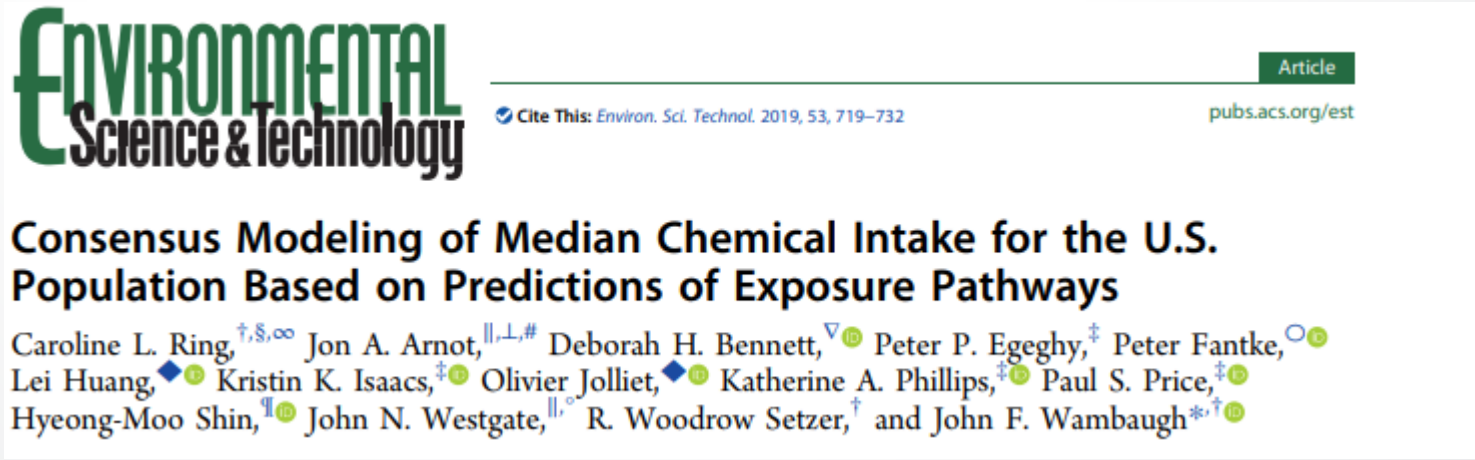
- Currently the Dashboard shows SEEM2 (2014) values



Consensus modeling of chemical exposure based on pathways: ExpoCast SEEM3

Comparison to exposure predictions for a bioactivity:exposure ratio

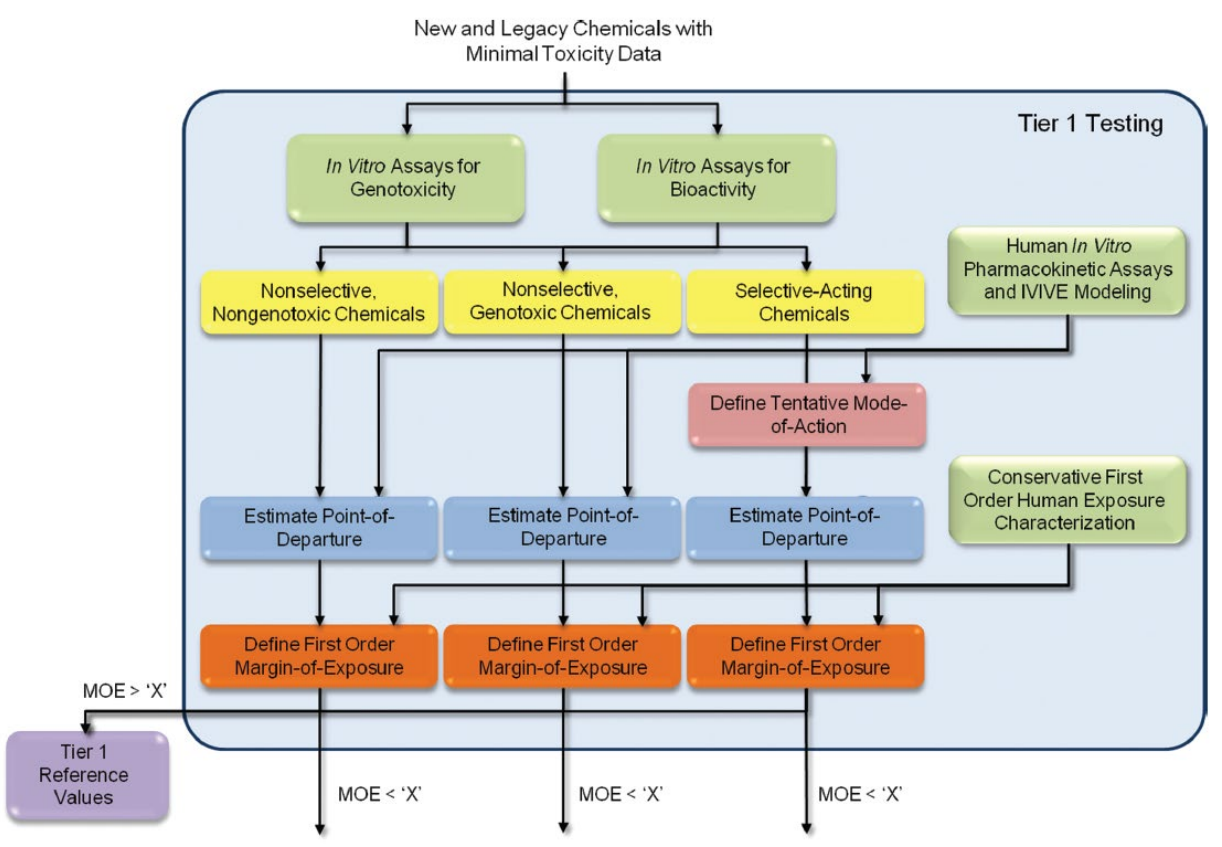
- "ExpoCast SEEM3" model:
  - uses twelve different exposure predictors including both near- and far-field models;
  - covers four distinct exposure pathways: non-pesticidal dietary, consumer products, far-field pesticide, and far-field industrial.
  - In SEEM3 each exposure predictor is scaled and centered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



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

- Now, ~6 years later, Thomas et al. (2019) suggest a computational toxicology blueprint that represents evolution of the same concept

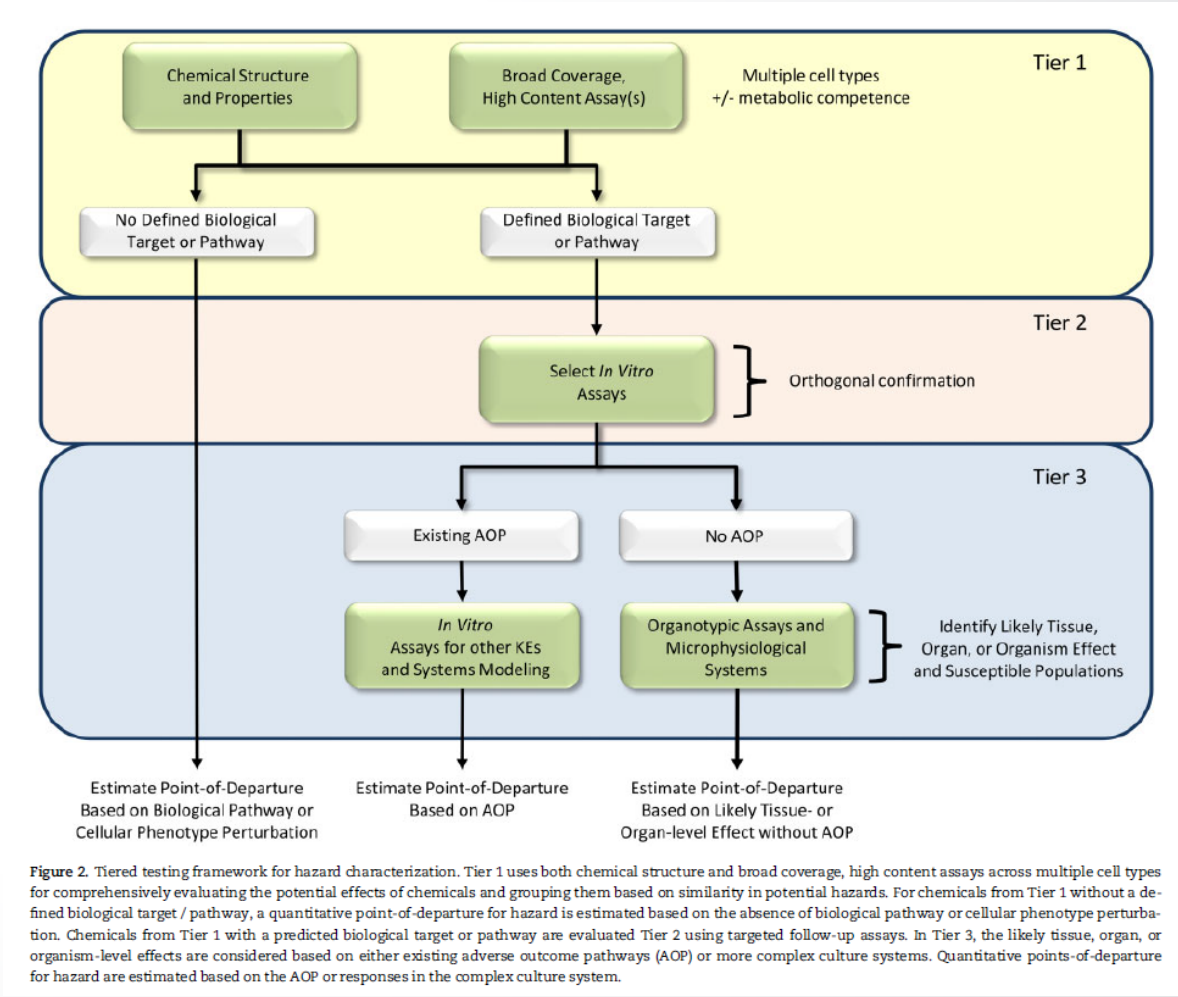


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

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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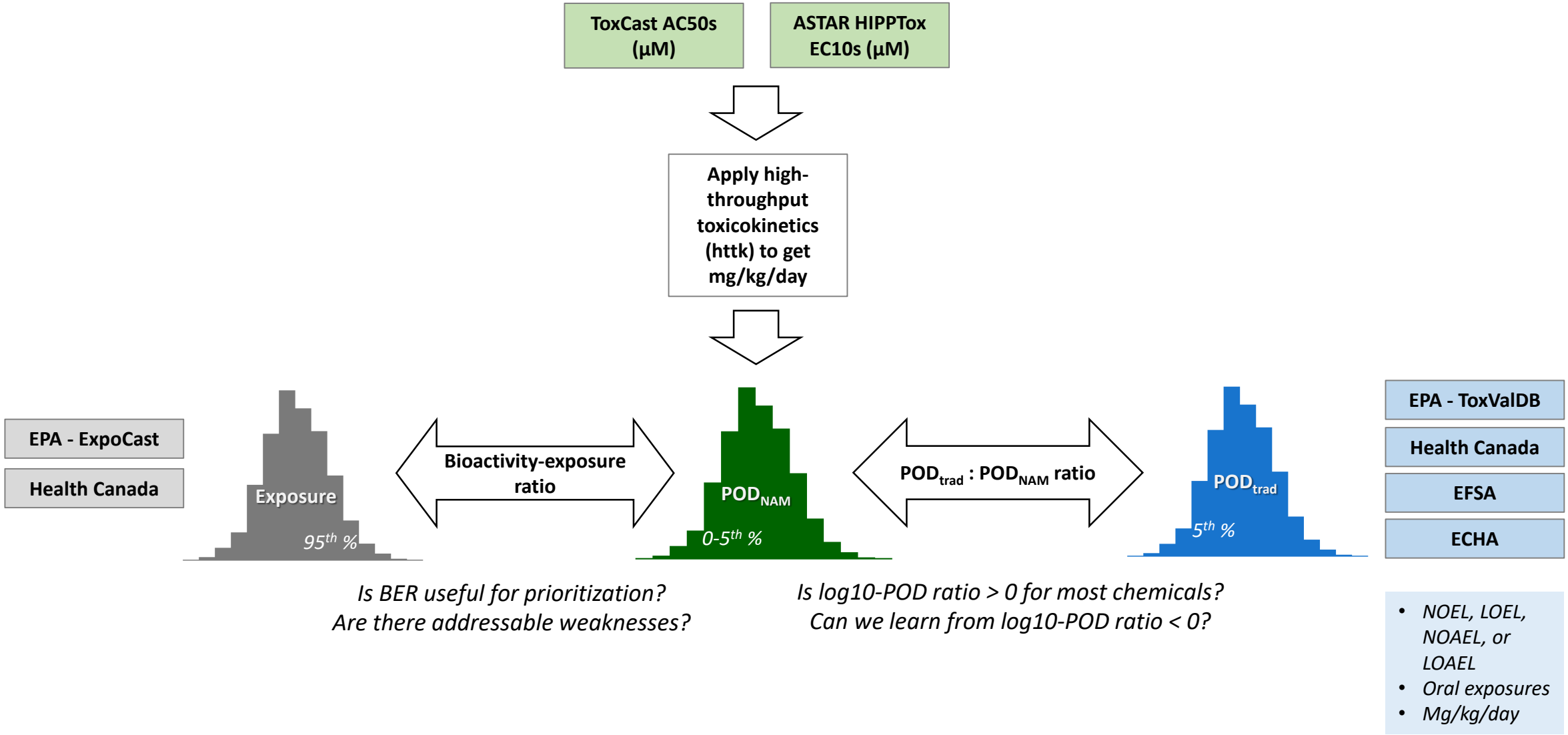
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

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

(APCRA partners for these two case studies)

Case study workflow

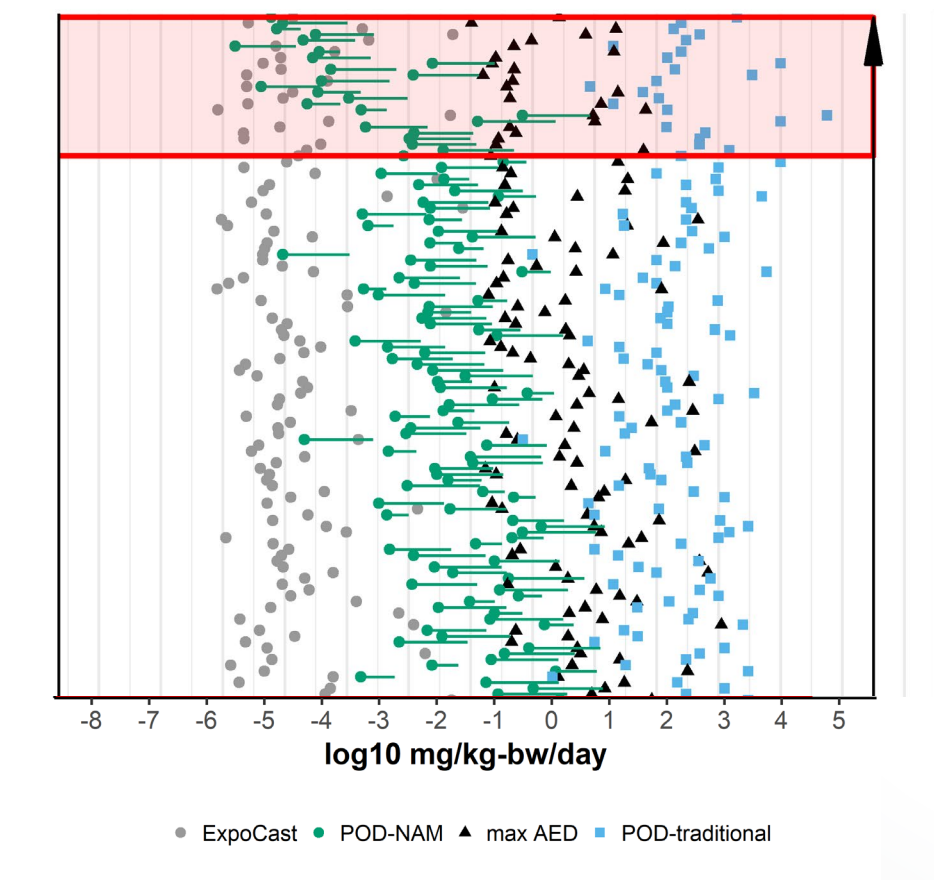


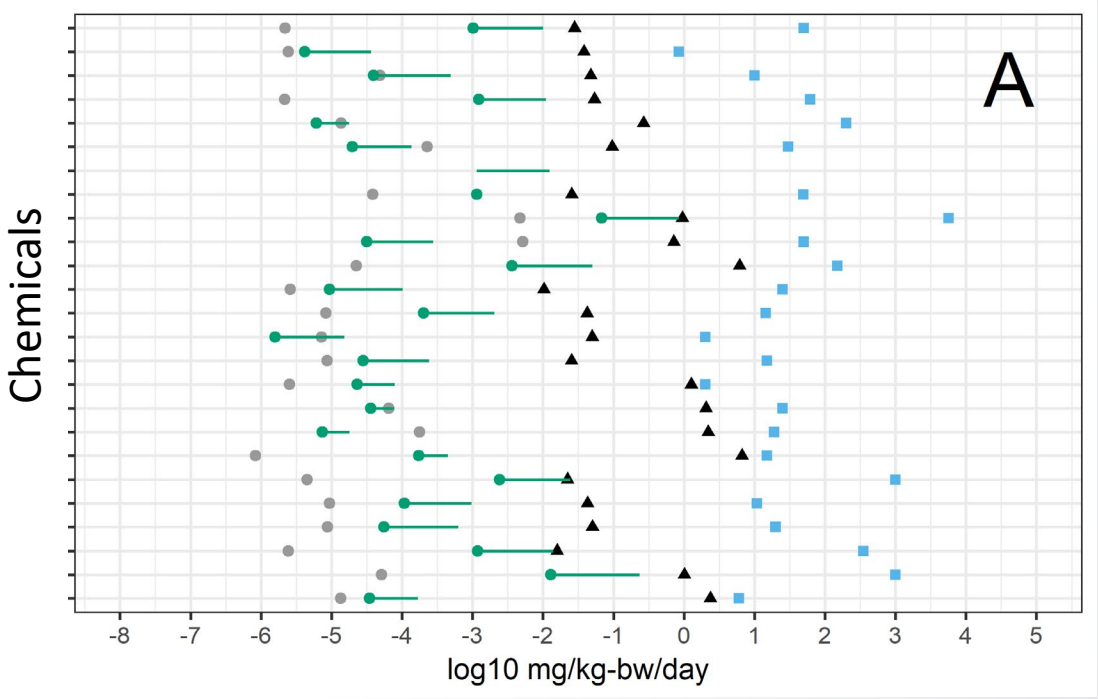
Is BER useful for prioritization?
Are there addressable weaknesses?

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

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- 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