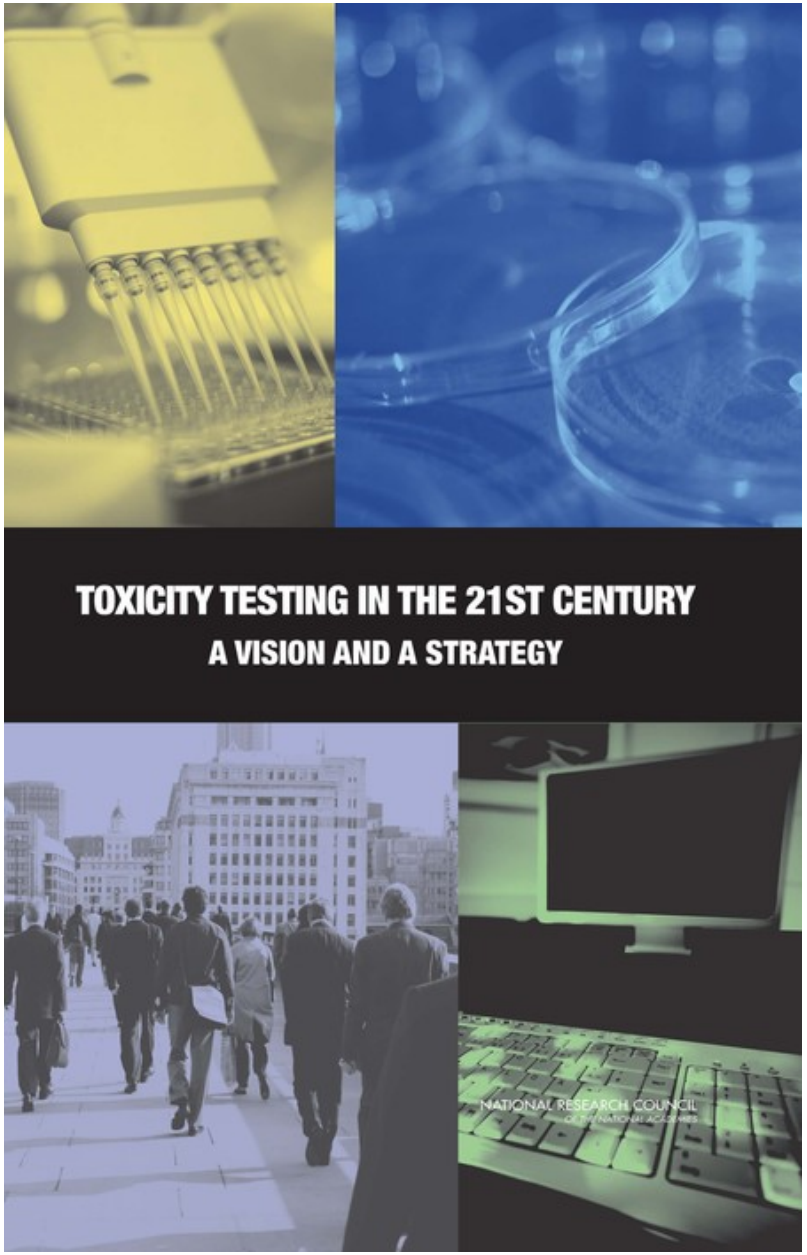
SETAC Europe Continuing Education Course: TC07-The Endocrine System: Global Perspectives on Testing Methods and Evaluation of Endocrine Activity (https://dublin.setac.org/programme/scientific-programme/training-courses/)

Slides given as part of this training course in a virtual training recorded on May 8, 2020

Katie Paul Friedman and Antony Williams

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

What does the future hold?



Goals

1. To provide broad coverage of chemicals, chemical mixtures, outcomes, and life stages

2. To reduce the cost and time of testing

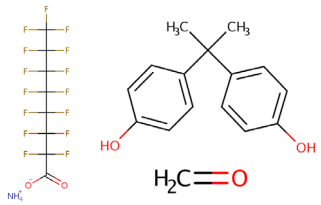
3. To use fewer animals and cause minimal suffering in the animals used

4. To develop a more robust scientific basis for assessing health effects of environmental agents

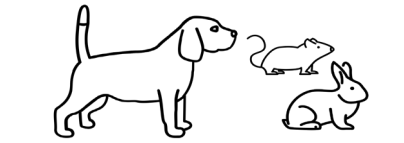
Why can't we just use traditional approaches?

Challenge

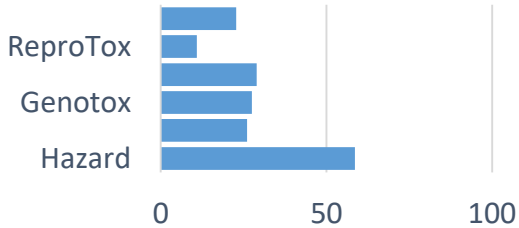
Too many chemicals



Traditional methods are slow, costly



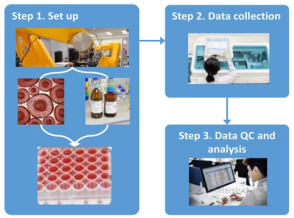
Not enough data

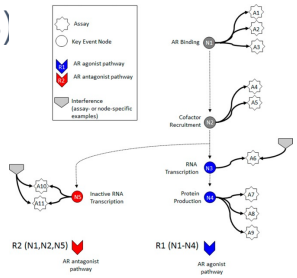


Judson *et al* 2009

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

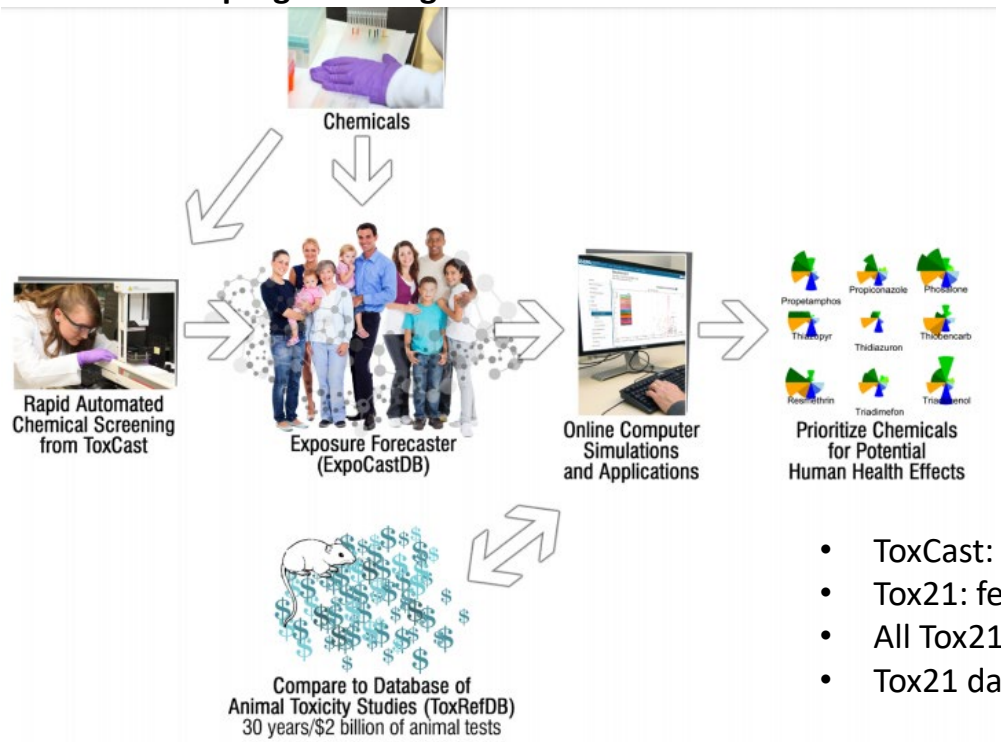
- > 3K chemicals screened in
- > 1K assays
  - ~400 biological targets

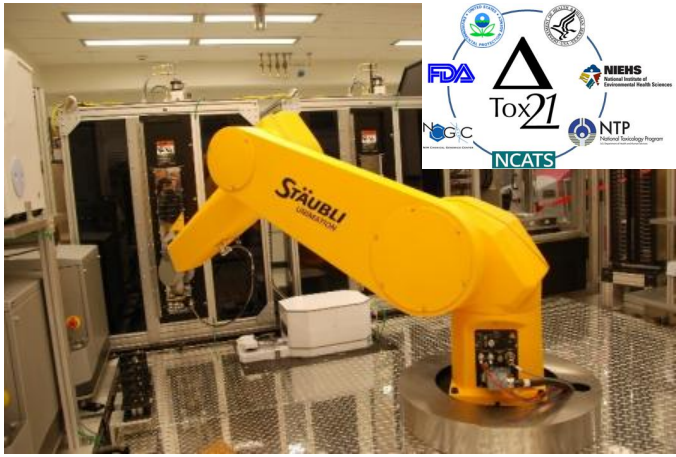
New Assessment Methodologies

- Animal testing isn't always the answer
  - Time consuming, expensive, ethically challenging
- New Assessment Methodologies (NAMs)
  - Categories, read across, (Q)SARs, and other model predictions
    - Need for regulatory acceptance (e.g., confidence in applying data to decisions)
    - Challenges in interpretation (e.g., linking molecular/cellular changes to adversity)
    - Need for flexibility (e.g., NAMs may be context/pathway specific)

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

EPA's ToxCast program at a glance

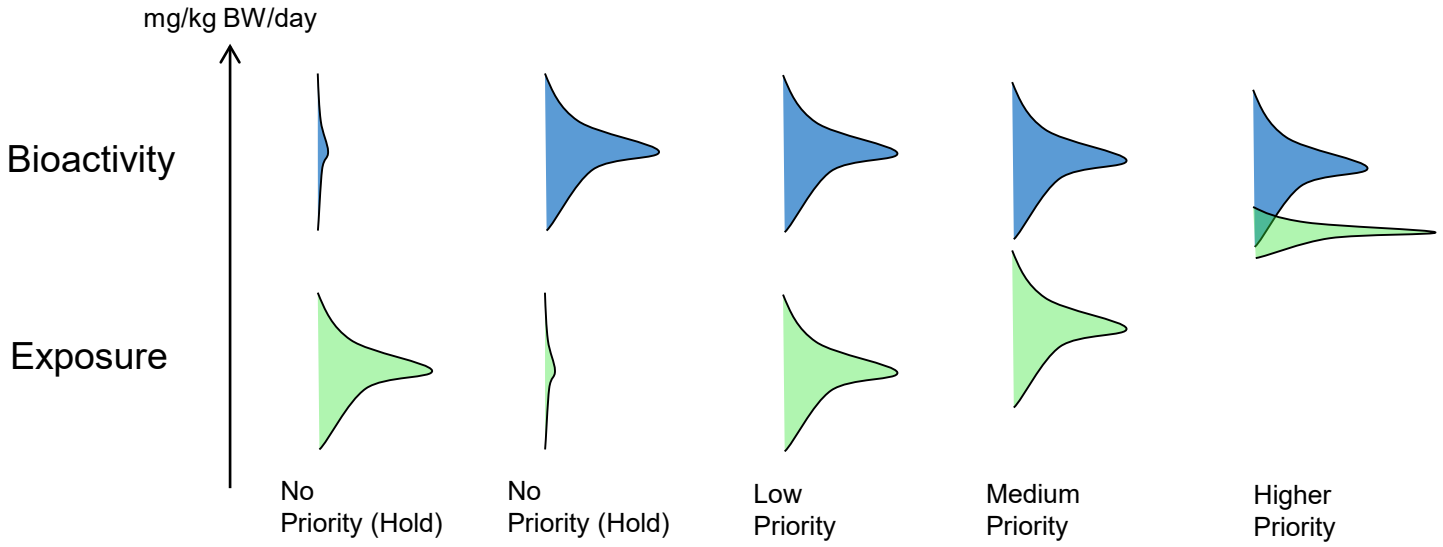




Tox21 robot

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

Exposure provides context for high-throughput science

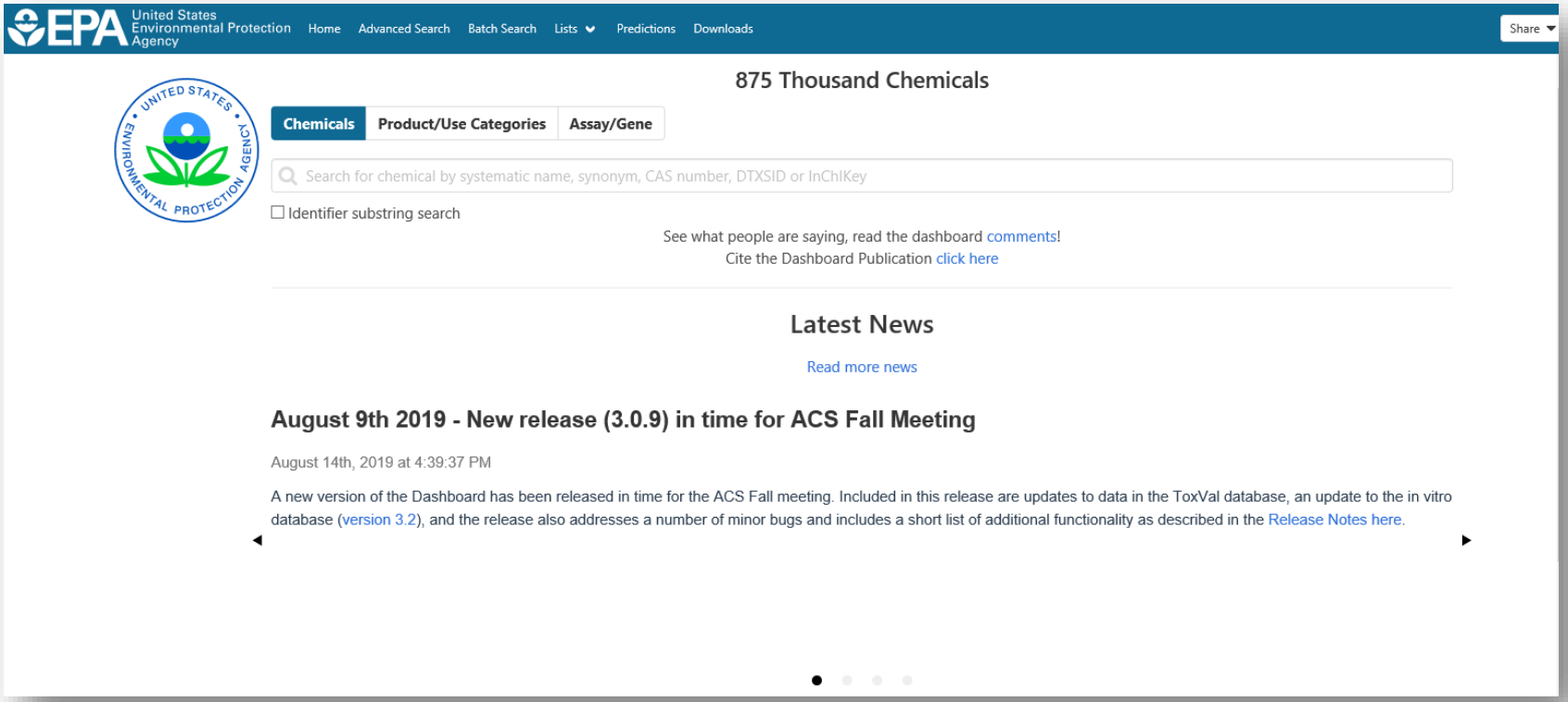


Endocrine hazard and risk evaluation using public tools: approach outline

- 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

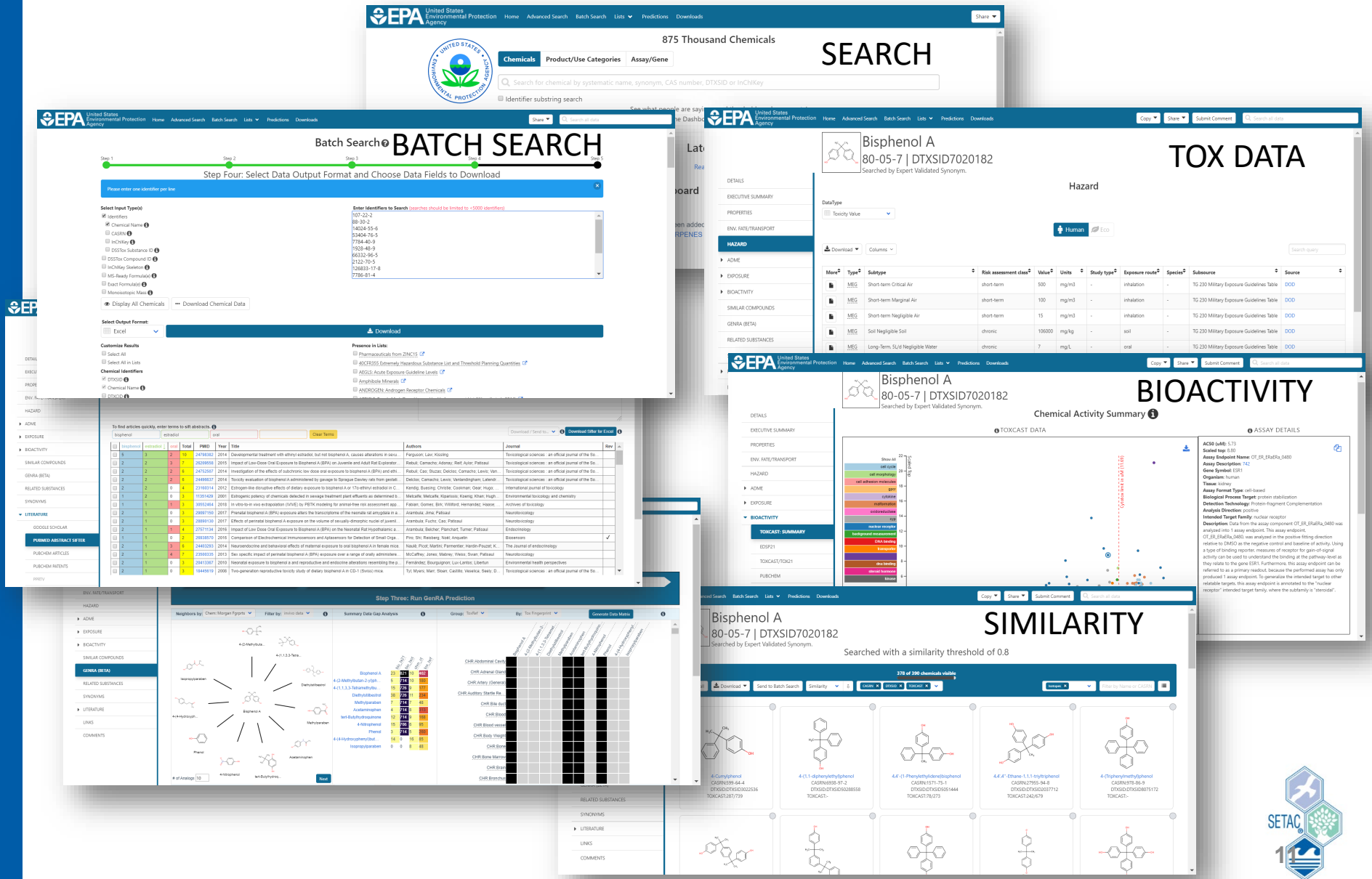


https://comptox.epa.gov/dashboard

The CompTox Chemical Dashboard

- **Freely accessible website and integration hub:**
  - Chemical substances – the majority with structures
  - Searchable by chemical, product use, and gene
  - Experimental and predicted physicochemical property data
  - Experimental and predicted fate and transport data
  - Information regarding consumer products containing chemicals
  - Bioactivity data for the ToxCast/Tox21 project
  - "Literature" searches for chemicals using public resources
  - Links to other agency websites and public data resources
  - "Batch searching" for thousands of chemicals
  - Chemical lists of interest – pesticides, leachables, PFAS, (but not a list of endocrine disruptors)
  - Downloadable Open Data for reuse and repurposing

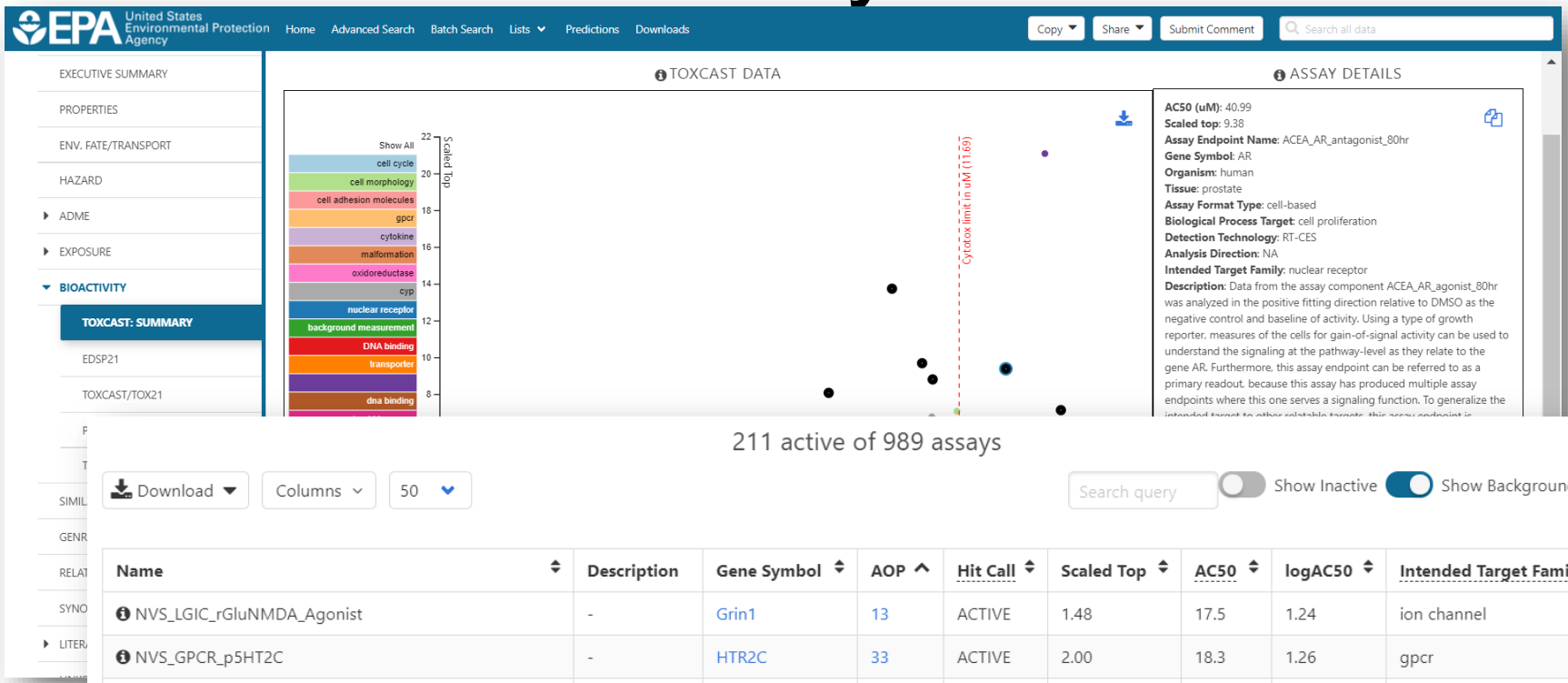
A single application integrating…



A data integration hub
LOTS of data!

- >875,000 chemicals curated over 20 years
- >700,000 toxicity data points from >30 sources
- Millions of synonyms and identifiers
- Tens of thousands of experimental data points
- Millions of QSAR prediction reports
- Millions of bioactivity data points for >4000 chemicals and hundreds of assay end points
- Searching of Pubmed's 30 million abstracts

Review of Bioassay Data

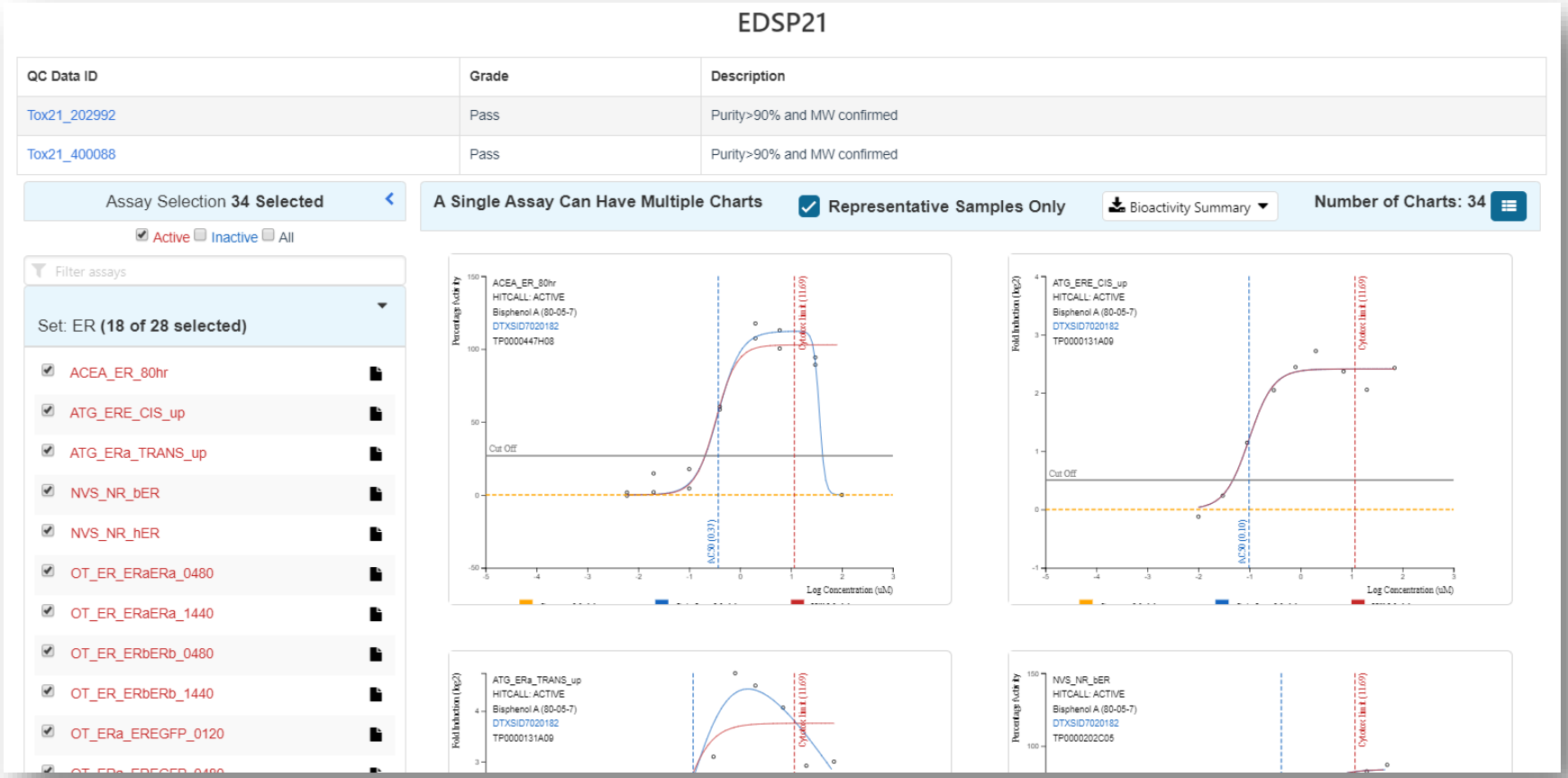


211 active of 989 assays

Download | Columns | 50 | Search query | Show Inactive | Show Background

Name	Description	Gene Symbol	AOP	Hit Call	Scaled Top	AC50	logAC50	Intended Target Family
NVS_LGIC_rGluNMDA_Agonist	-	Grin1	13	ACTIVE	1.48	17.5	1.24	ion channel
NVS_GPCR_p5HT2C	-	HTR2C	33	ACTIVE	2.00	18.3	1.26	gpcr
TOX21_PPARd_BLA_antagonist_ratio	1125	PPARD	36	ACTIVE	2.80	37.2	1.57	nuclear receptor
ATG_PPRE_CIS_up	102	PPARA	58	ACTIVE	1.04	24.4	1.39	nuclear receptor
NVS_NR_hPPARa	718	PPARA	58	ACTIVE	1.59	0.105	-0.979	nuclear receptor
ATG_DR4_LXR_CIS_dn	-	NR1H3	58	ACTIVE	1.78	24.8	1.40	nuclear receptor
ATG_PXRE_CIS_up	-	NR1I2	60	ACTIVE	5.48	1.48	0.171	nuclear receptor
ATG_PXR_TRANS_up	-	NR1I2	60	ACTIVE	2.49	0.722	-0.141	nuclear receptor
NVS_NR_hPXR	-	NR1I2	60	ACTIVE	1.69	12.3	1.09	nuclear receptor
NVS_NR_hFXR_Antagonist	716	NR1H4	61	ACTIVE	1.93	16.4	1.22	nuclear receptor
OT_FXR_FXRSRC1_0480	753	NR1H4	61	ACTIVE	2.08	31.1	1.49	nuclear receptor
OT_FXR_FXRSRC1_1440	754	NR1H4	61	ACTIVE	3.43	32.6	1.51	nuclear receptor

Endocrine-related subset of assays

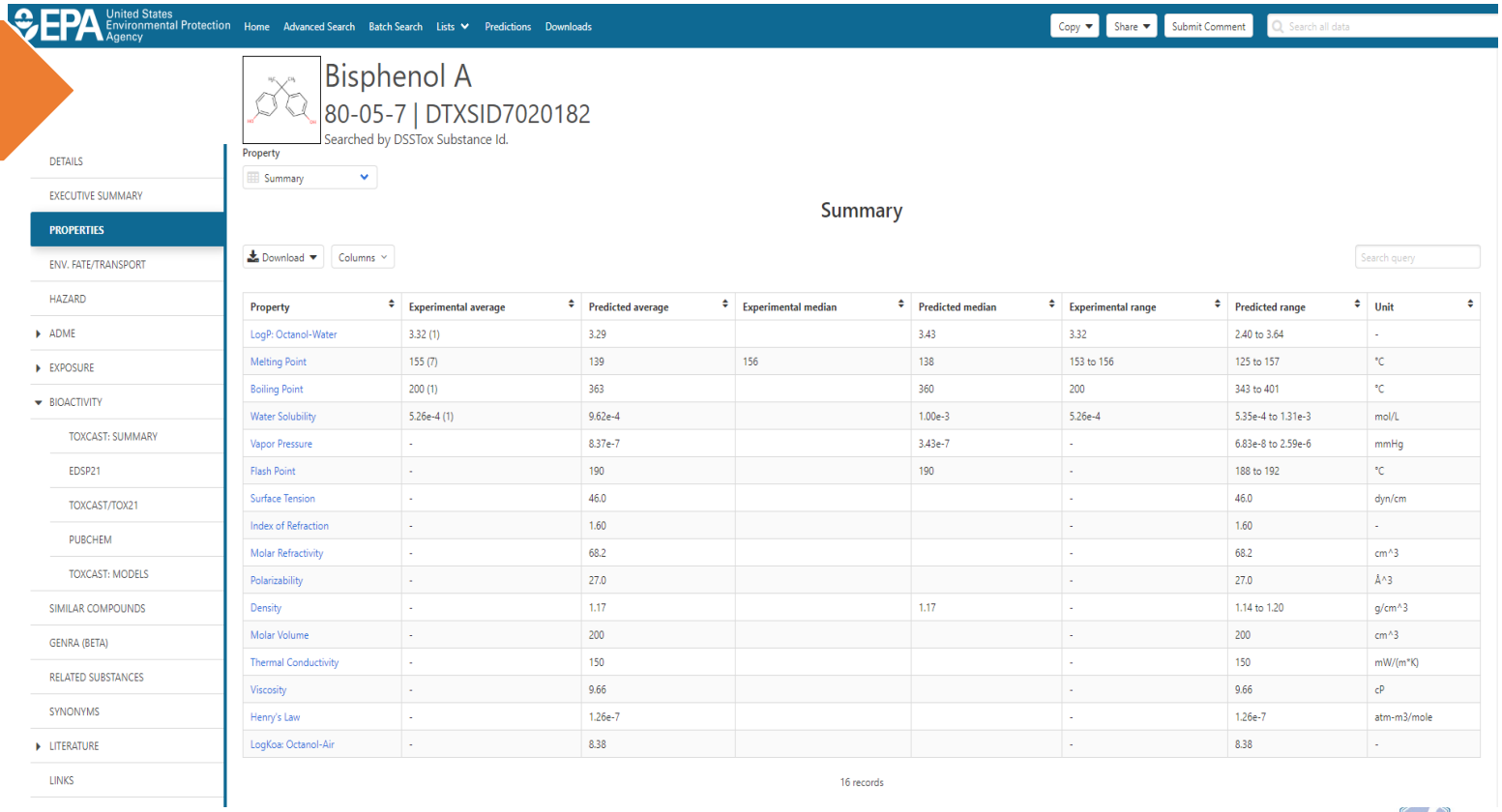


Examine physicochemical properties such as logP, vapor pressure, and MW to get a better sense of whether the chemical was suitable for the current *in vitro* assay suite

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

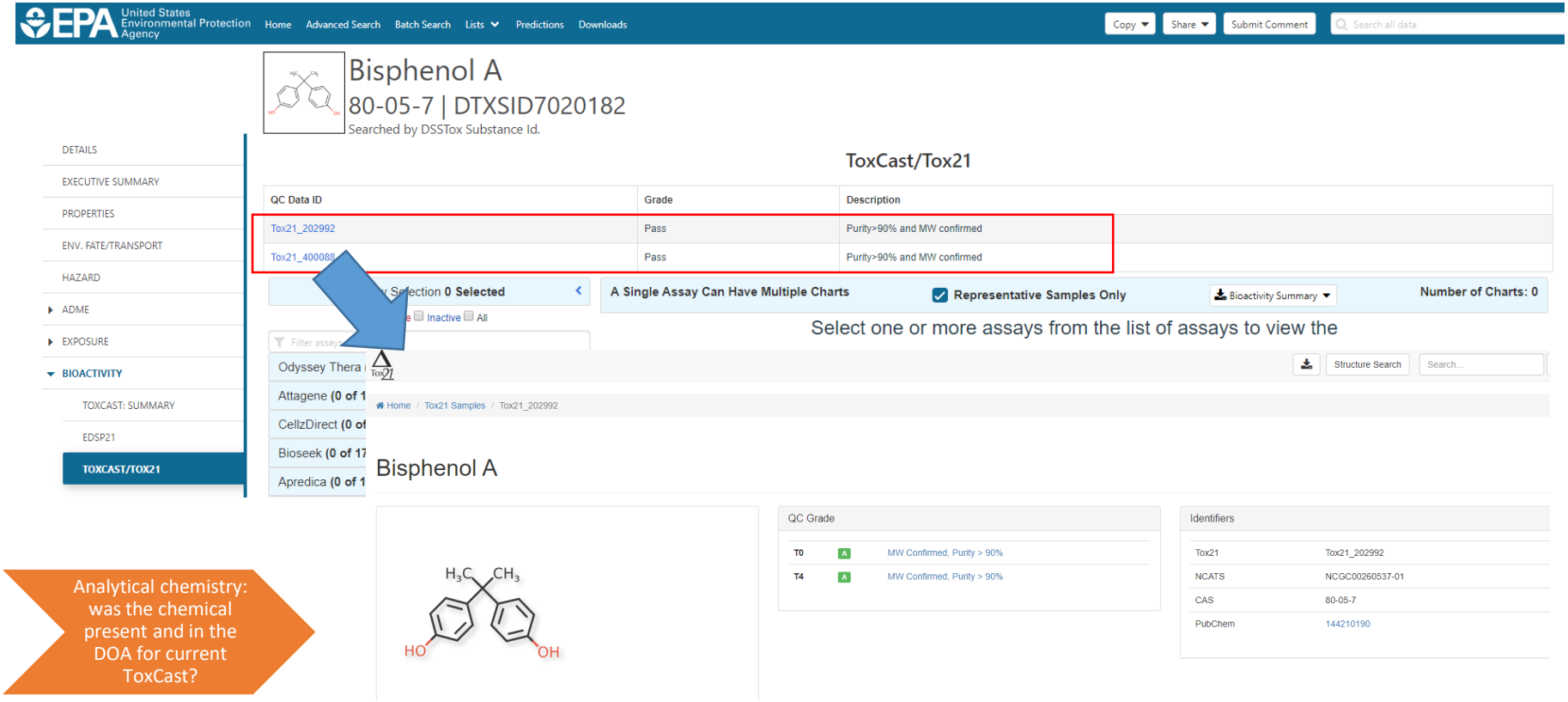
ToxCast negatives: what does a negative mean? Outside of domain of applicability?

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	-

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



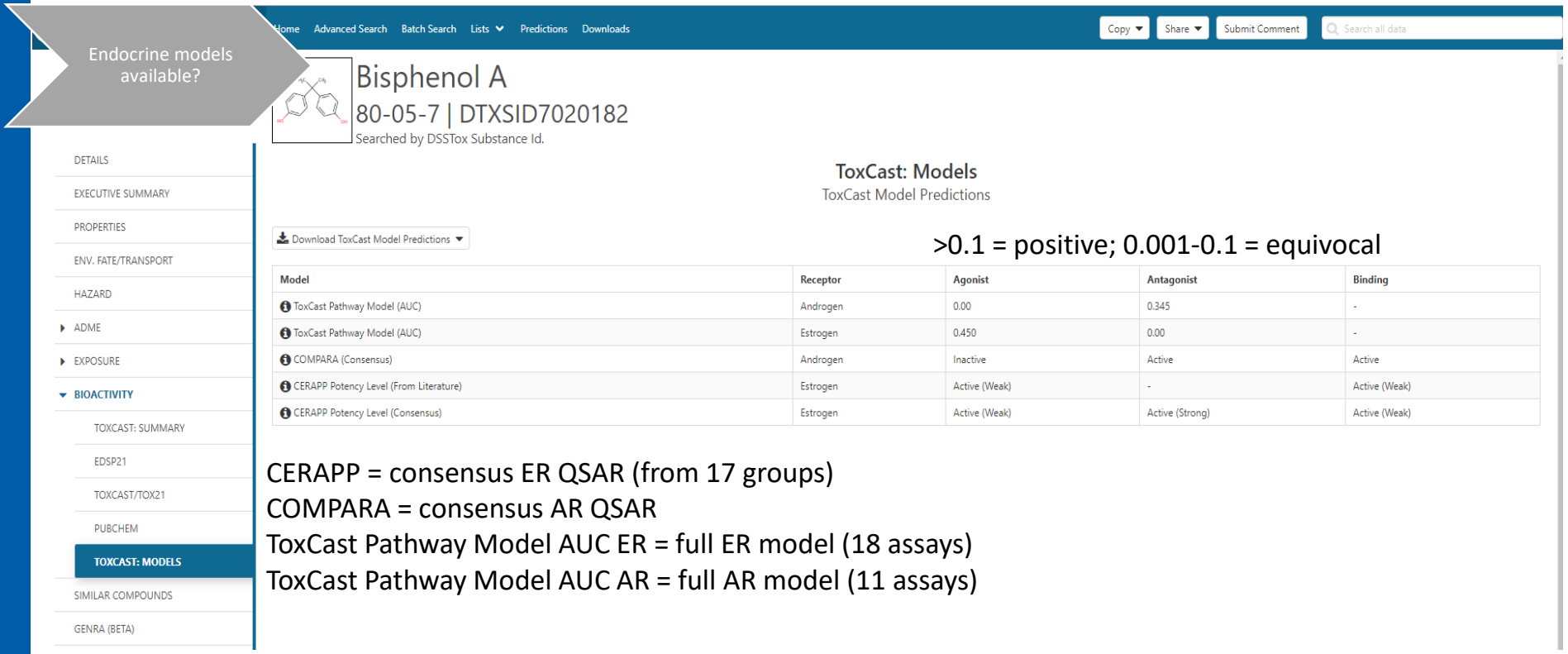
Analytical chemistry: was the chemical present and in the DOA for current ToxCast?

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**

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)

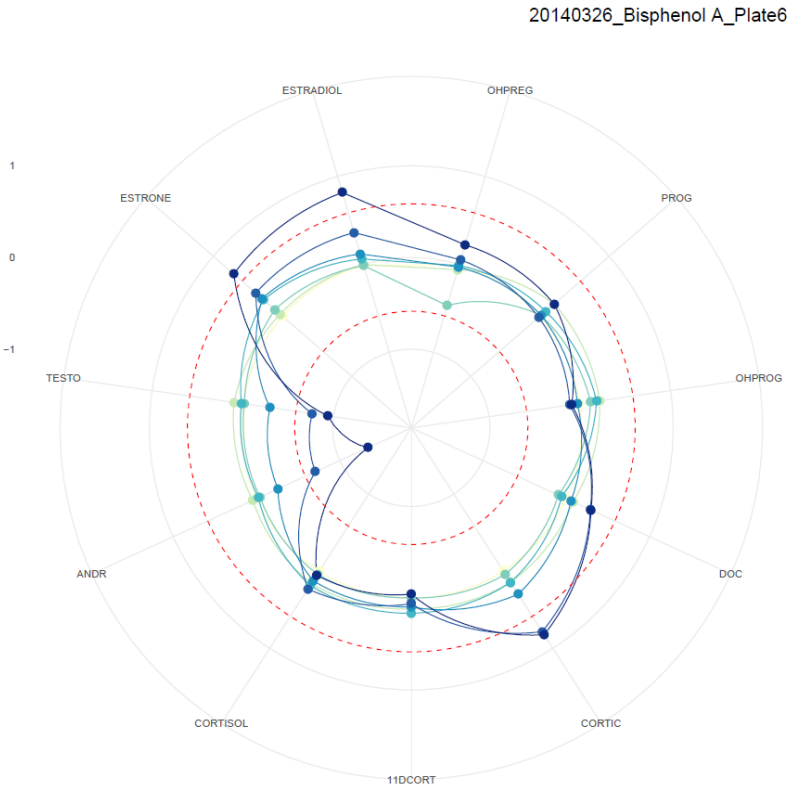
HT-H295R model for steroidogenesis

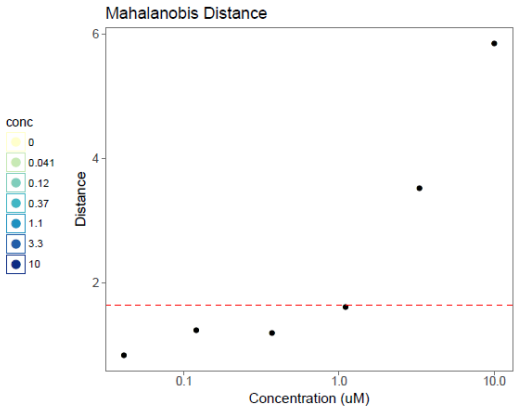
Endocrine models available?

High-Thr
as an Alte
Character

Derik E. Hagg
Richard S. Juc

*Oak Ridge Institute
Center for Computa
Agency, Durham, N

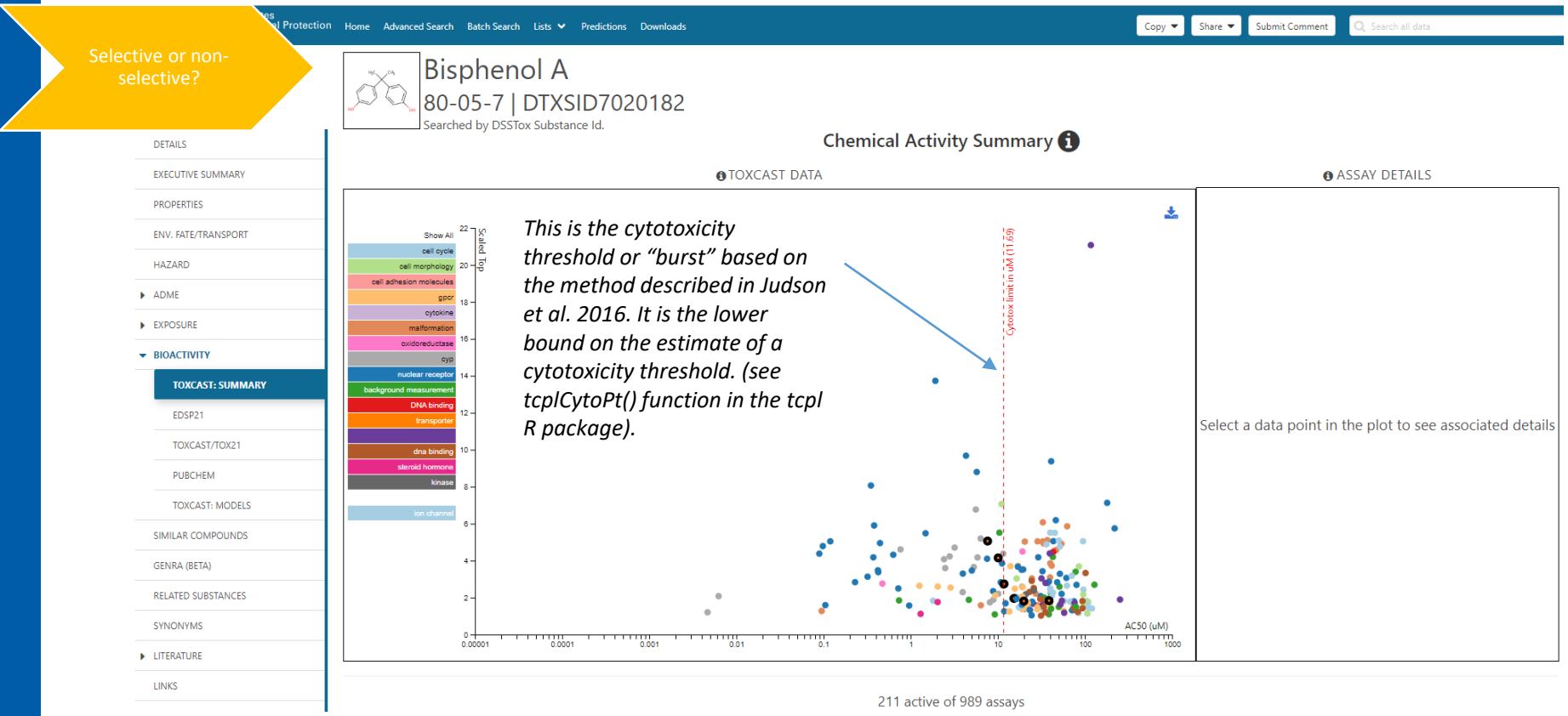




- Supplemental File 4 has fold-change by hormone
- Supplemental File 9 has mMd (model values)
- Invitrodb v3.2 has a hth295r model table with both of these included in it.
- Hope to include this in future release of the Dashboard.

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?

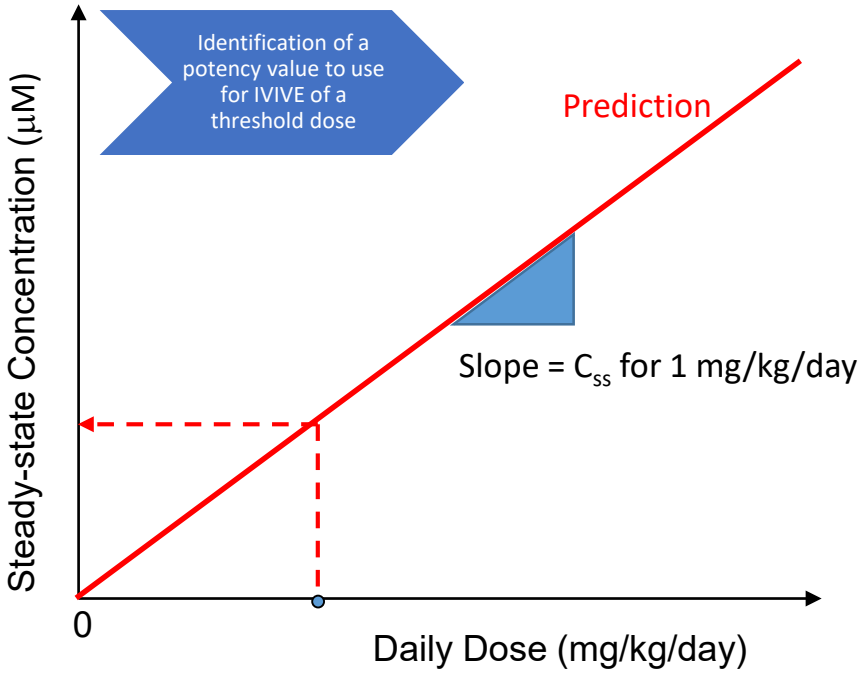
- The latest Comptox Chemicals Dashboard release (version 3.0.9, August 9, 2019) demonstrates a cytotoxicity threshold based on the latest ToxCast database (invitrodb version 3.2, released August 2019). This value can change as more cytotoxicity data become available, curve-fitting approaches for existing 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.

User application dictates “selectivity”

Selective or non-selective?

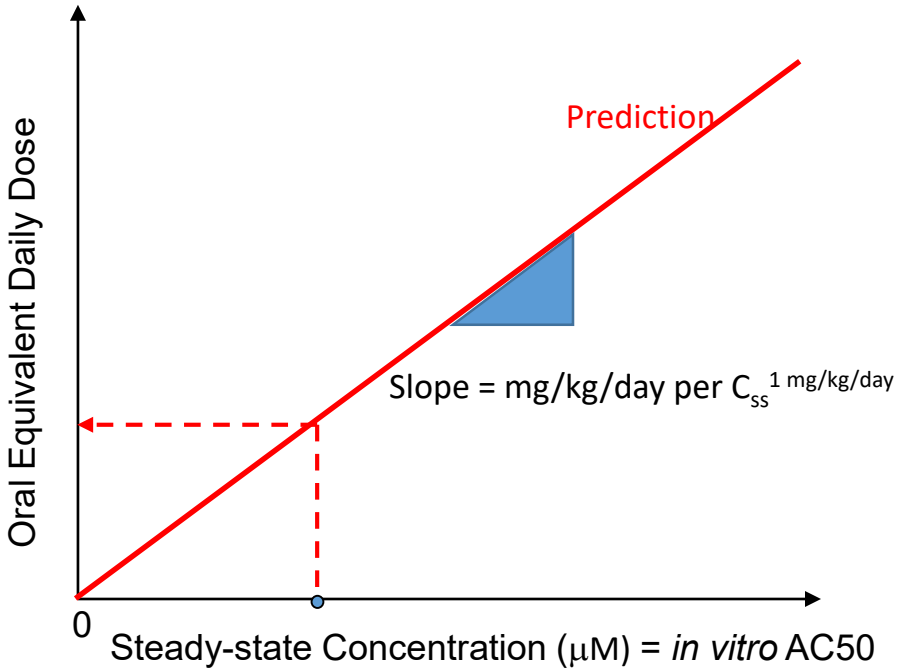
- AC50 < burst?
- AC50 0.5$\log_{10}$ distance from burst?
- AC50 < parallel viability assays?
- How else to filter ToxCast data: 3+ caution flags & hit-percent
- Other related ideas:
  - What other assays appear active in a similar concentration range?
  - Is there consistent support for MOA(s), or is it nonspecific activity?

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



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Wetmore *et al*. (2012)

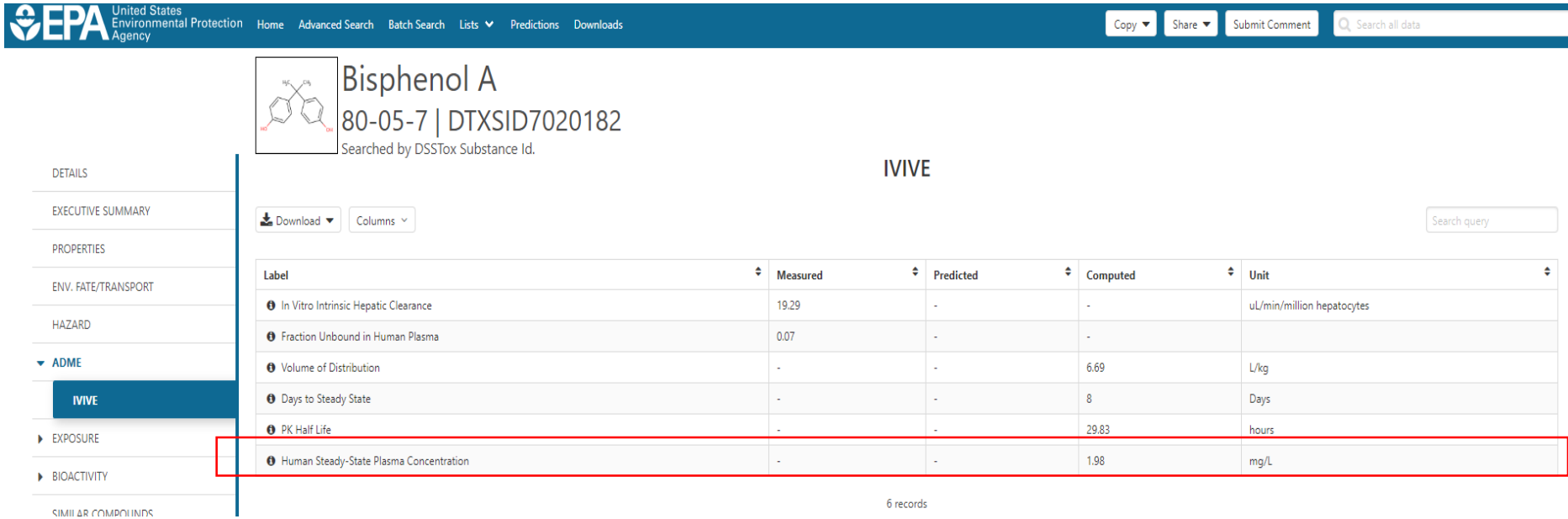


- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

IVIVE via high-throughput toxicokinetic data and models

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

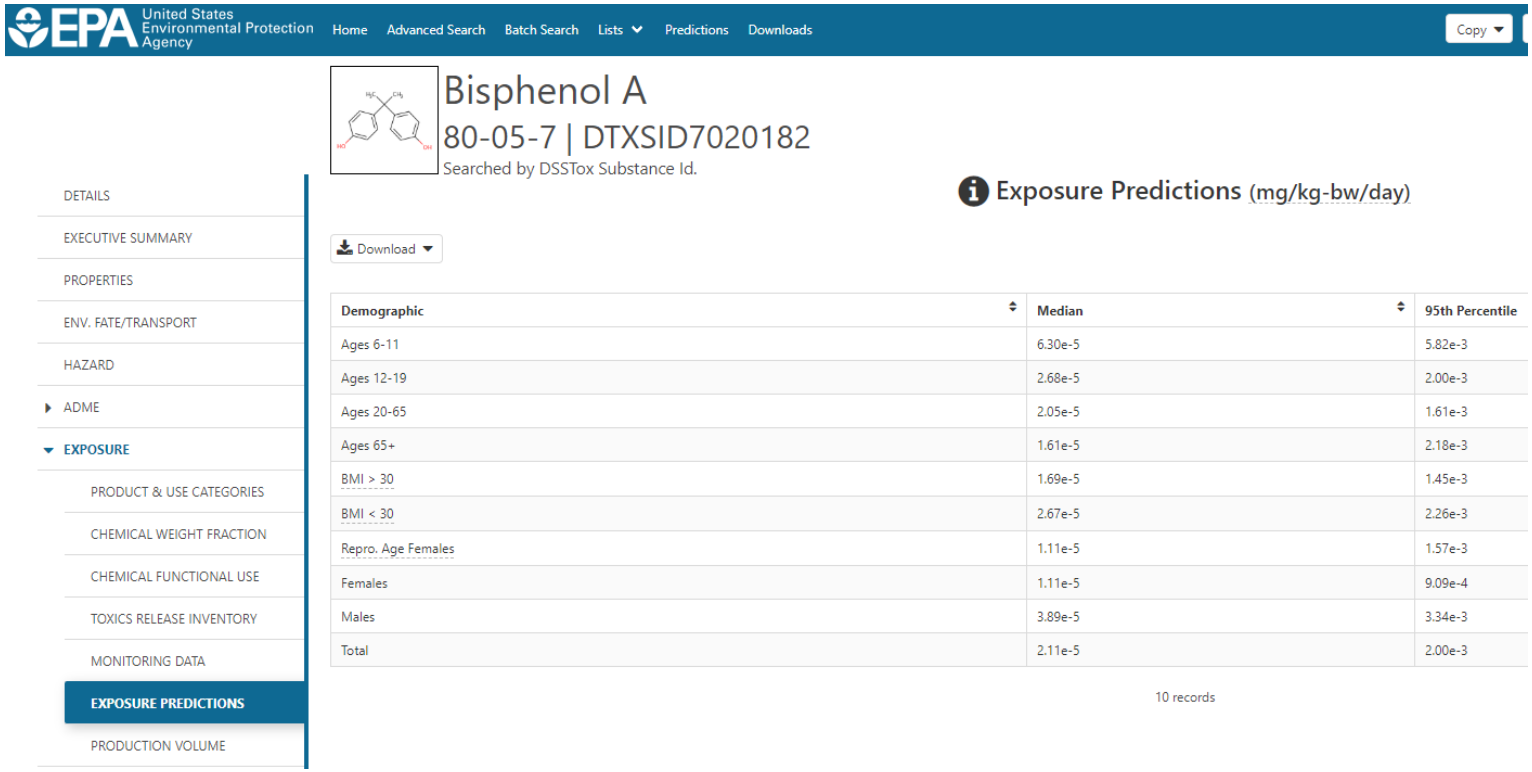
- Operationally, the httk R package (v 1.10.0) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs)
- For some substances, there is a beta tab in the Dashboard with Css and other values needed (no models). More chemicals have information in the httk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

Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2019, 53, 719−732

pubs.acs.org/est

Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways

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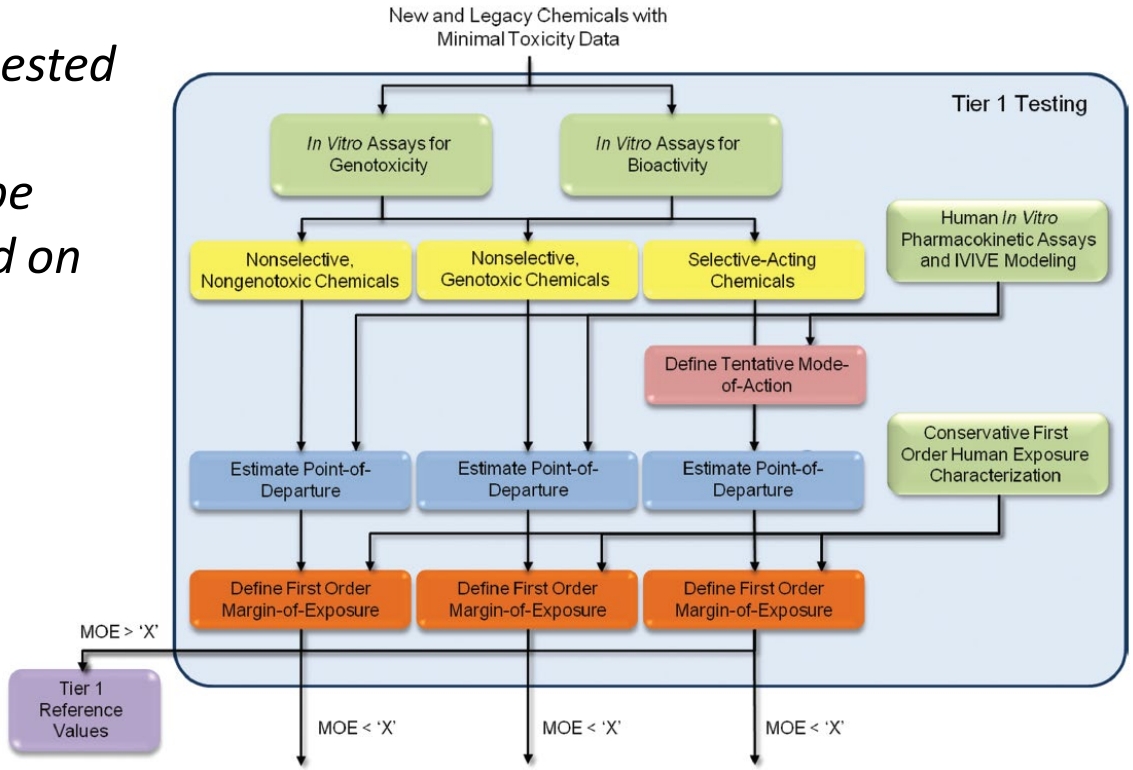
Including risk-based approaches like BER in chemical safety decisions

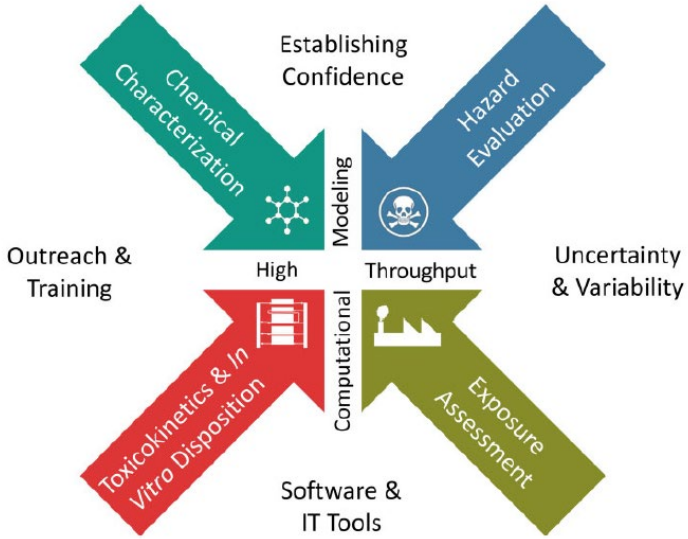
- Specific vs. nonspecific modes-of-action and the challenge of hazard labeling

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



Including risk-based approaches like BER in chemical safety decisions

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



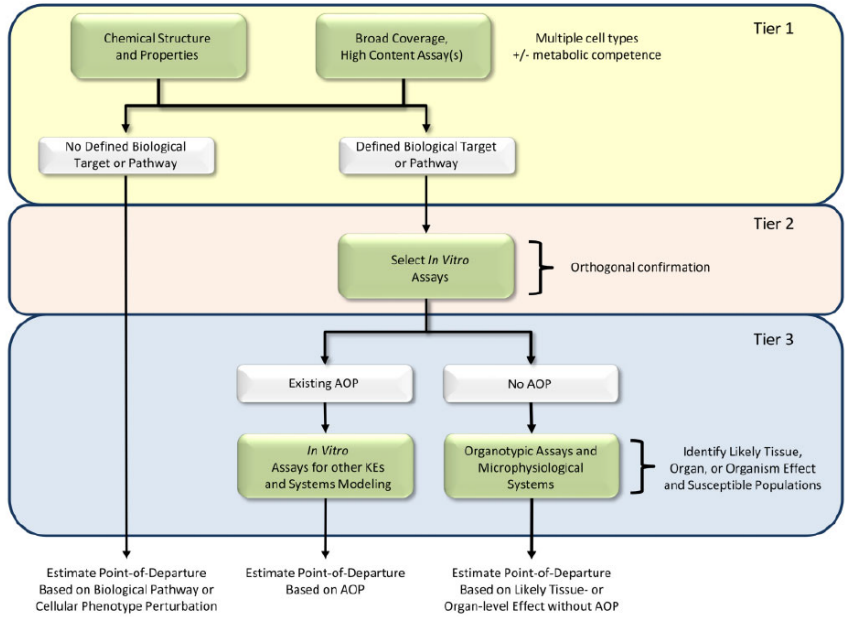


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals 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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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

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*

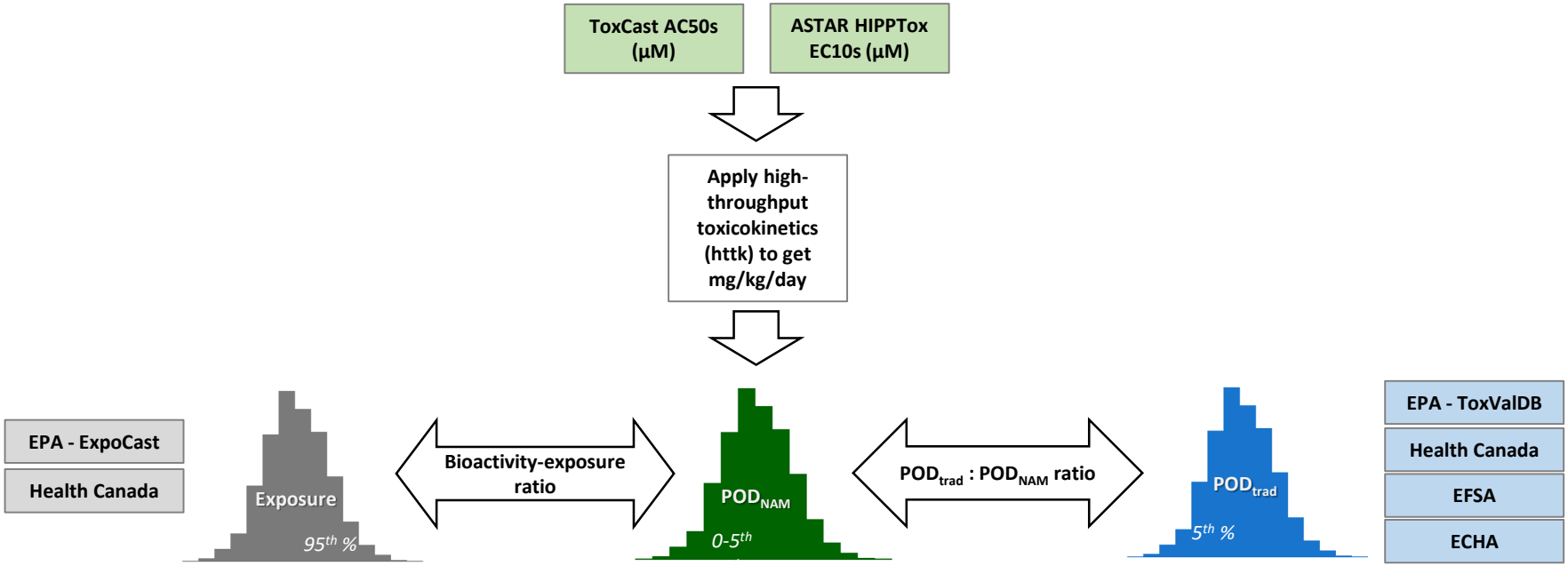
NTP National Toxicology Program U.S. Department of Health and Human Services

UNIVERSITY OF BIRMINGHAM

(APCRA partners for these two case studies)

SETAC

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*

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

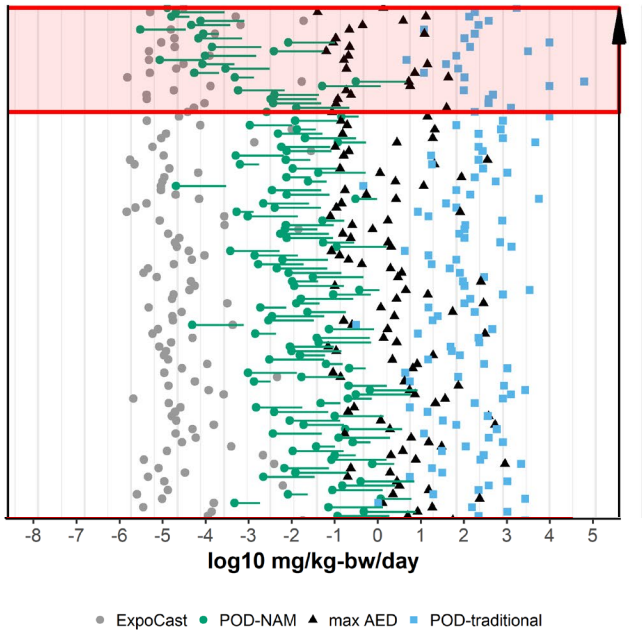
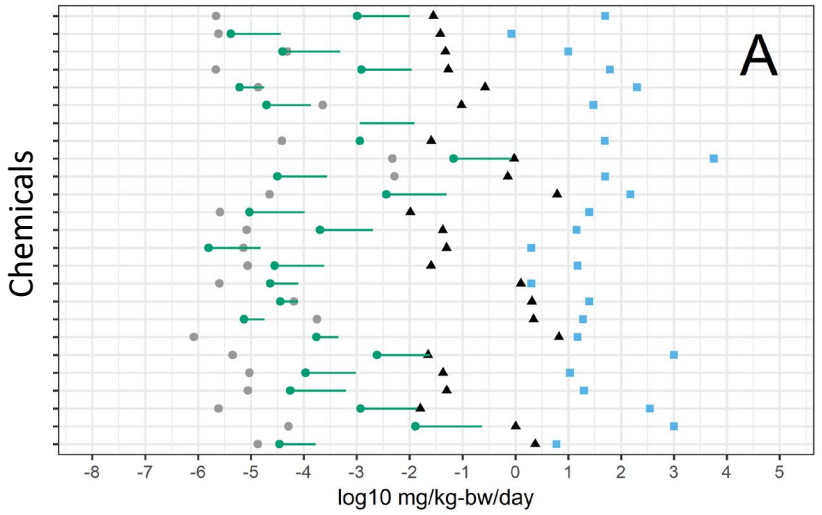


Figure 3 from Paul Friedman et al.
https://doi.org/10.1093/toxsci/kfz201



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.