



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

Katie Paul Friedman

November 13, 2019

Presentation to 10th International Akademie Fresenius Conference

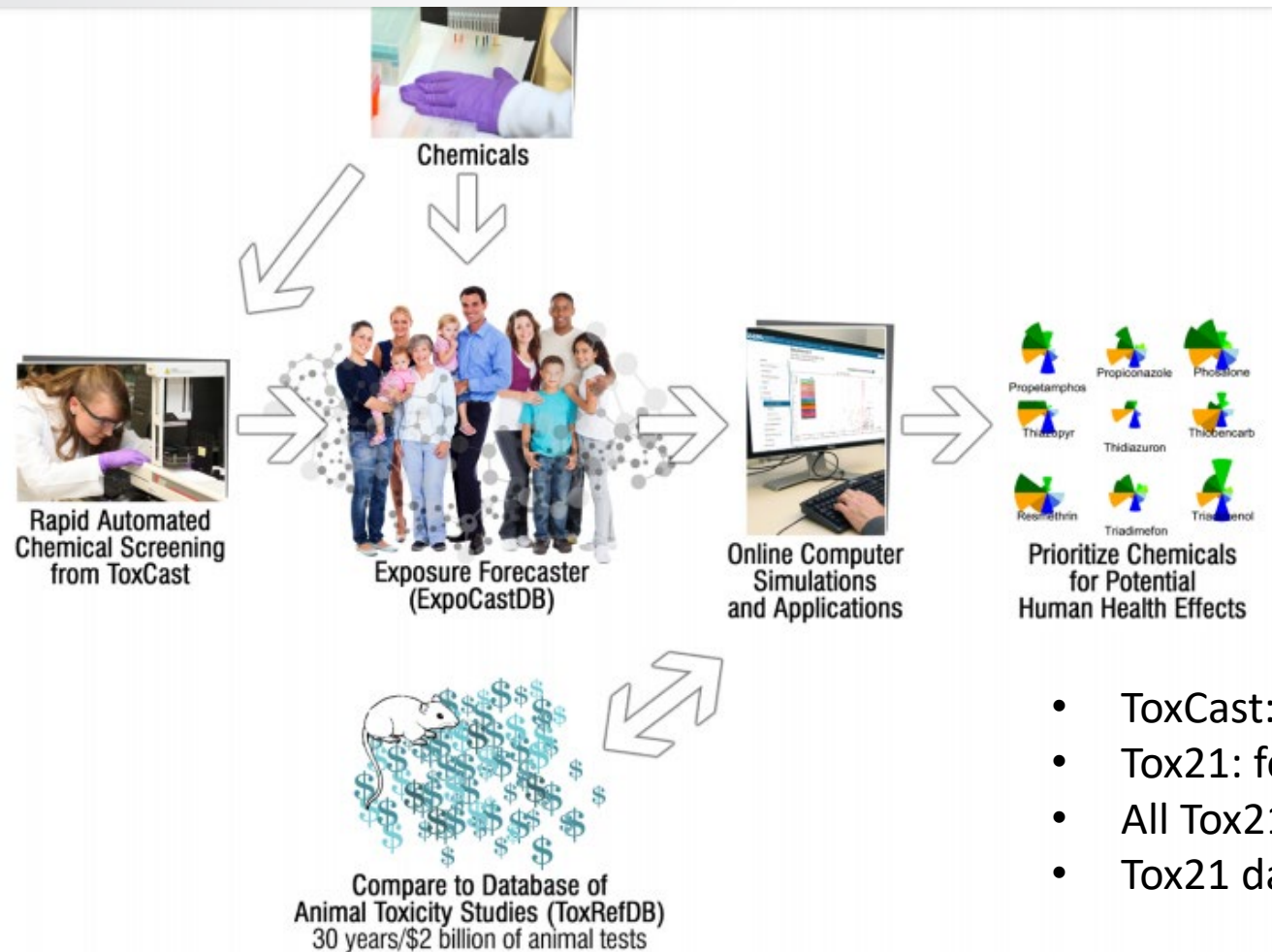
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ToxCast and Tox21 have generated a lot of publicly available bioactivity data for hazard screening and prediction.

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



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875 Thousand Chemicals

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August 9th 2019 - New release (3.0.9) in time for ACS Fall Meeting

August 14th, 2019 at 4:39:37 PM

A new version of the Dashboard has been released in time for the ACS Fall meeting. Included in this release are updates to data in the ToxVal database, an update to the in vitro database ([version 3.2](#)), and the release also addresses a number of minor bugs and includes a short list of additional functionality as described in the [Release Notes here](#).

<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.

EPA United States Environmental Protection Agency

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Bisphenol A
80-05-7 | DTXSID7020182
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Property
Summary

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Summary

Search query


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-m ³ /mole
LogKoa: Octanol-Air	-	8.38			-	8.38	-

16 records

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



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TOXCAST: SUMMARY

EDSP21

TOXCAST/TOX21



Bisphenol A

80-05-7 | DTXSID7020182

Searched by DSSTox Substance Id.

ToxCast/Tox21

QC Data ID	Grade	Description
Tox21_202992	Pass	Purity>90% and MW confirmed
Tox21_400088	Pass	Purity>90% and MW confirmed

Selection 0 Selected

A Single Assay Can Have Multiple Charts

☒ Representative Samples Only

Bioactivity Summary

Number of Charts: 0

Select one or more assays from the list of assays to view the associated bioactivity curves

Filter assays

Odyssey Thera (0 of 165)

Attagene (0 of 165)

CellzDirect (0 of 48)

Bioseek (0 of 174)

Apredica (0 of 108)

Home / Tox21 Samples / Tox21_202992

Bisphenol A



QC Grade

T0	A	MW Confirmed, Purity > 90%
T4	A	MW Confirmed, Purity > 90%

Identifiers

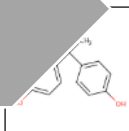
Tox21	Tox21_202992
NCATS	NCGC00260537-01
CAS	80-05-7
PubChem	144210190

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



Models >>> single assays. And equivocals happen.

Endocrine models available?



Bisphenol A
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 - 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)

Endocrine models
available?

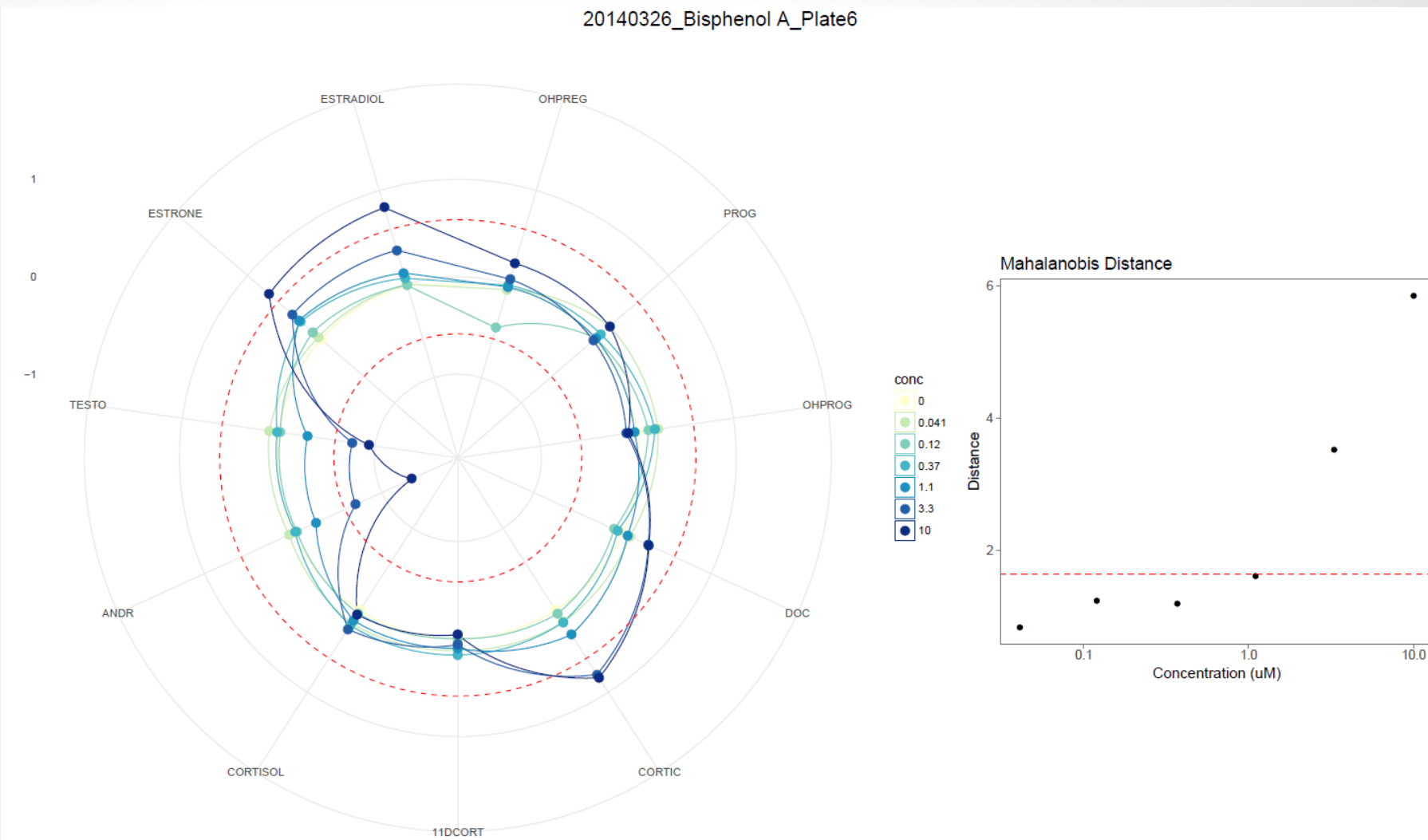


High-Throughput as an Alternative Characterization

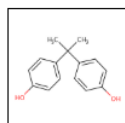
Derik E. Hagg
Richard S. Juc

*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.



Selective or non-selective?



Bisphenol A

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TOXCAST: MODELS

SIMILAR COMPOUNDS

GENRA (BETA)

RELATED SUBSTANCES

SYNONYMS

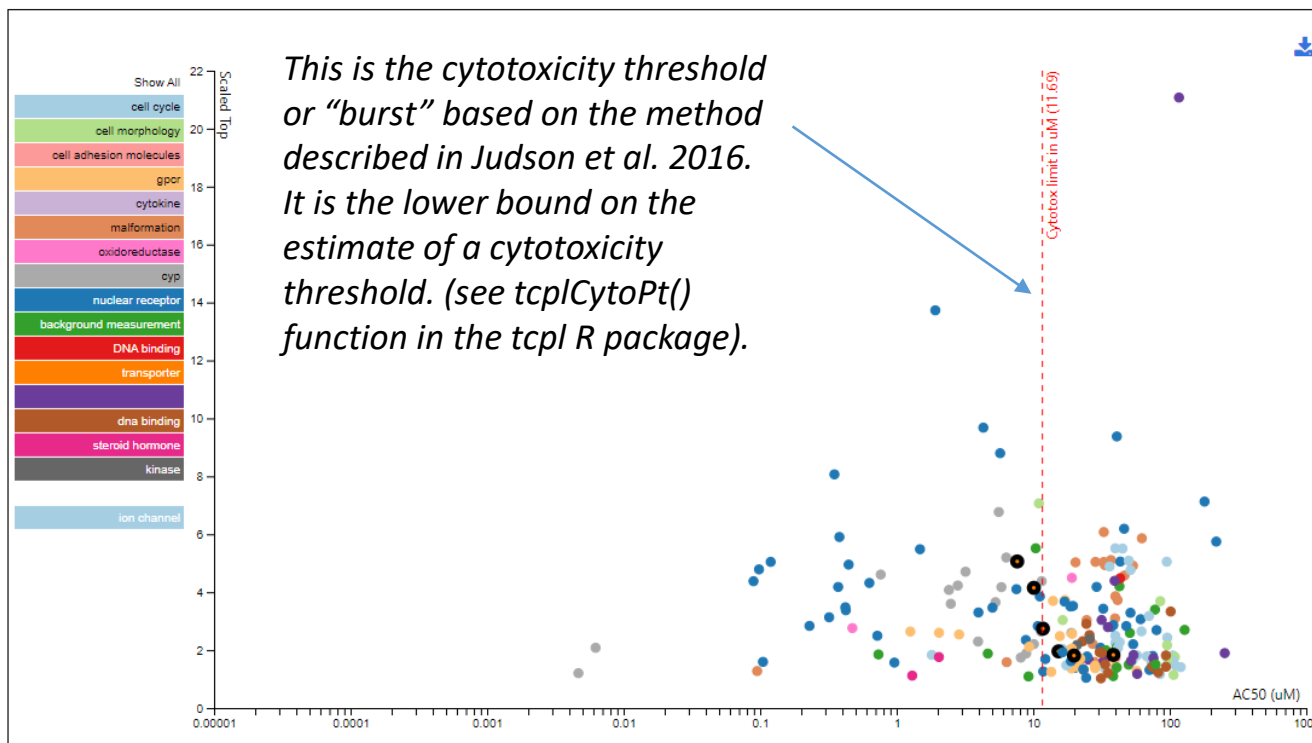
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LINKS

Chemical Activity Summary

TOXCAST DATA

ASSAY DETAILS



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?

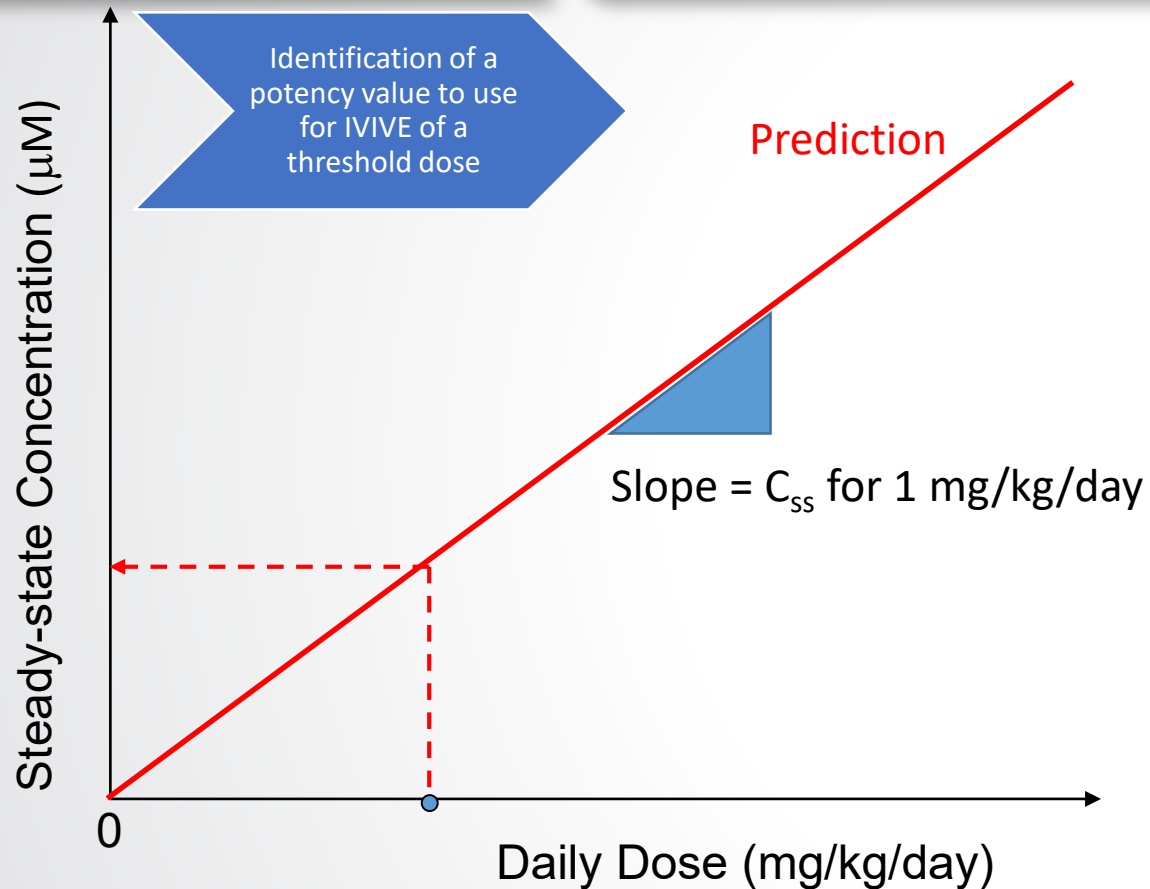


A note on ToxCast versioning

- 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>

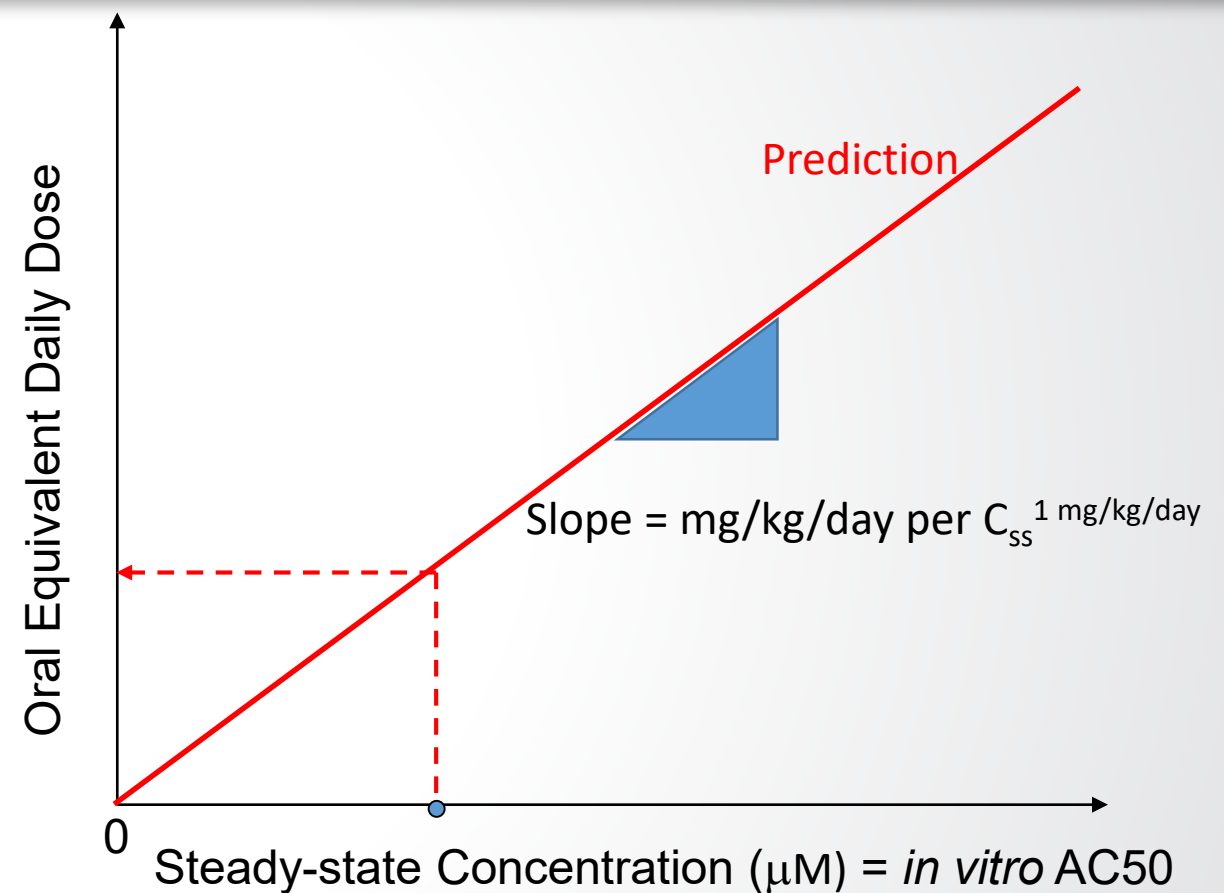


Steady state in vitro-in vivo extrapolation assumption: blood::tissue partitioning \approx cells::medium partitioning



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

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 C_{ss} and other values needed (no models). More chemicals have information in the httk package.
- AC₅₀ or LEC (micromolar) * (1 mg/kg/day/C_{ss} (micromolar)) = AED prediction
- Httk package optionally implements multiple models that can have increasing complexity based on data available

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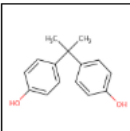
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SIMILAR COMPOUNDS



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Label	Measured	Predicted	Computed	Unit
In Vitro Intrinsic Hepatic Clearance	19.29	-	-	uL/min/million hepatocytes
Fraction Unbound in Human Plasma	0.07	-	-	
Volume of Distribution	-	-	6.69	L/kg
Days to Steady State	-	-	8	Days
PK Half Life	-	-	29.83	hours
Human Steady-State Plasma Concentration	-	-	1.98	mg/L

6 records



Comparison to
exposure predictions
for a
bioactivity:exposure
ratio

Bioactivity:exposure ratio requires exposure

- Currently the Dashboard shows SEEM2 (2014) values

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PRODUCT & USE CATEGORIES

CHEMICAL WEIGHT FRACTION

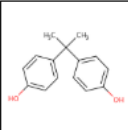
CHEMICAL FUNCTIONAL USE

TOXICS RELEASE INVENTORY

MONITORING DATA

EXPOSURE PREDICTIONS

PRODUCTION VOLUME



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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	2.11e-5	2.00e-3

10 records



Comparison to
exposure predictions
for a
bioactivity:exposure
ratio

Consensus modeling of chemical exposure based on pathways: ExpoCast SEEM3

- “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.



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Article

pubs.acs.org/est

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

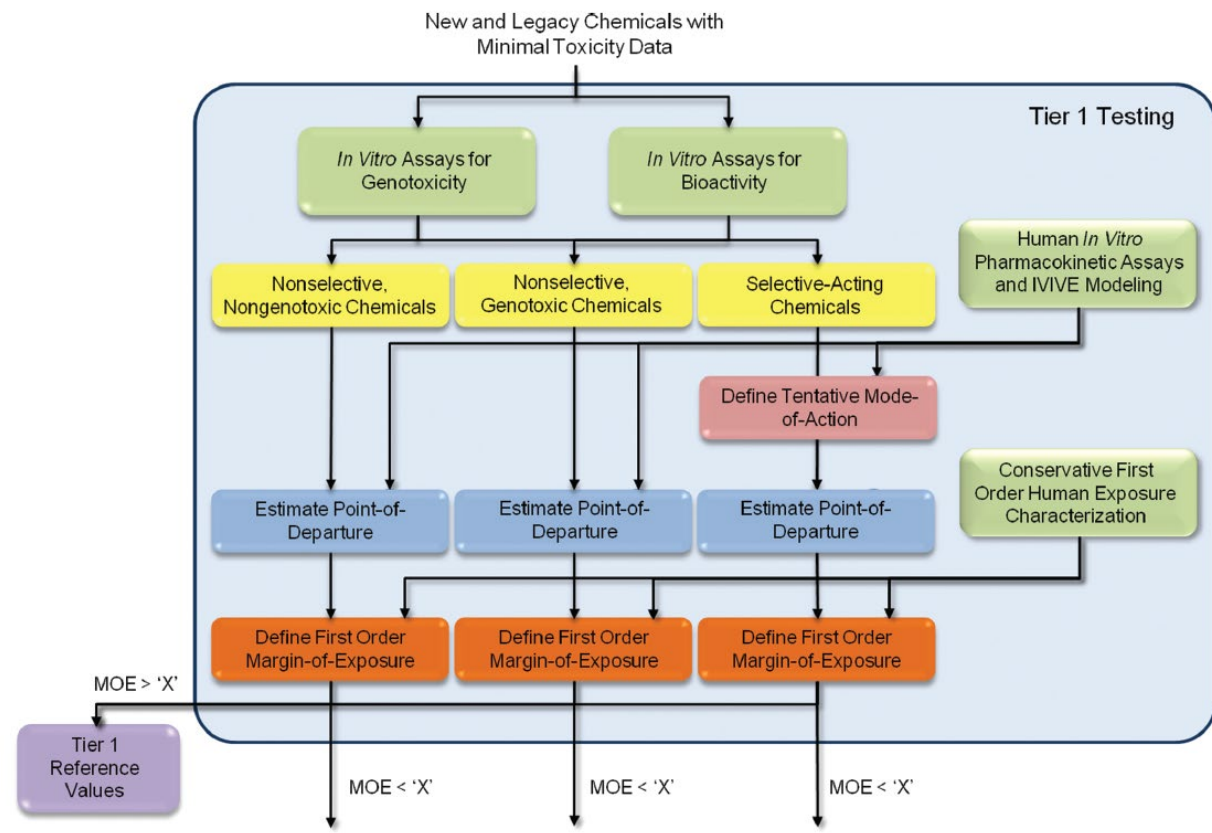
Caroline L. Ring,^{†,§,∞} Jon A. Arnot,^{||,⊥,#} Deborah H. Bennett,^{▽,Ⓜ} Peter P. Egeghy,[‡] Peter Fantke,^{○,Ⓜ} Lei Huang,^{◆,Ⓜ} Kristin K. Isaacs,^{‡,Ⓜ} Olivier Jolliet,^{◆,Ⓜ} Katherine A. Phillips,^{‡,Ⓜ} Paul S. Price,^{‡,Ⓜ} Hyeon-Moo Shin,^{¶,Ⓜ} John N. Westgate,^{||,Ⓜ} R. Woodrow Setzer,[†] and John F. Wambaugh^{*,†,Ⓜ}



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).





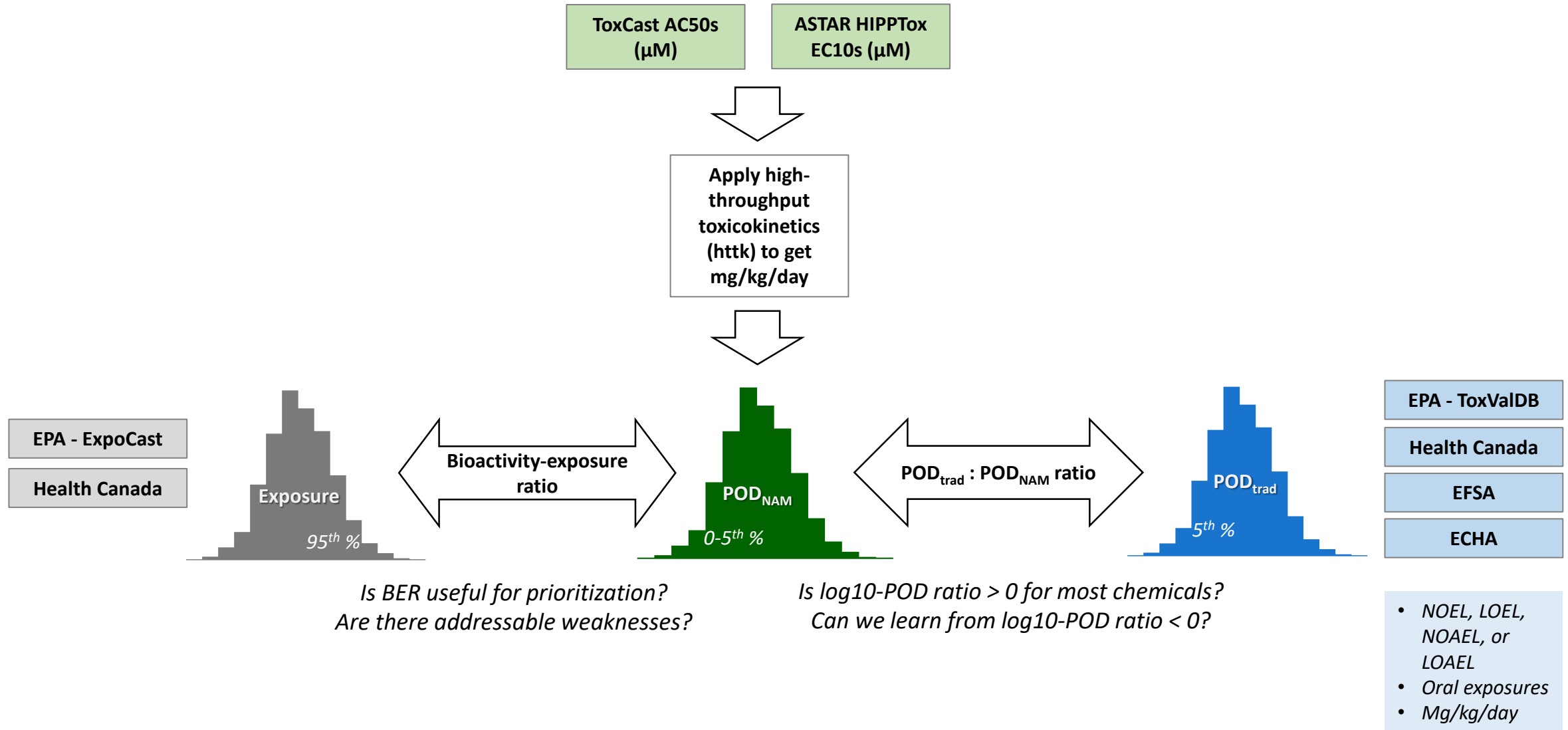
Screening level assessment example: combine NAMs for exposure, *in vitro* bioactivity, and toxicokinetics

- 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

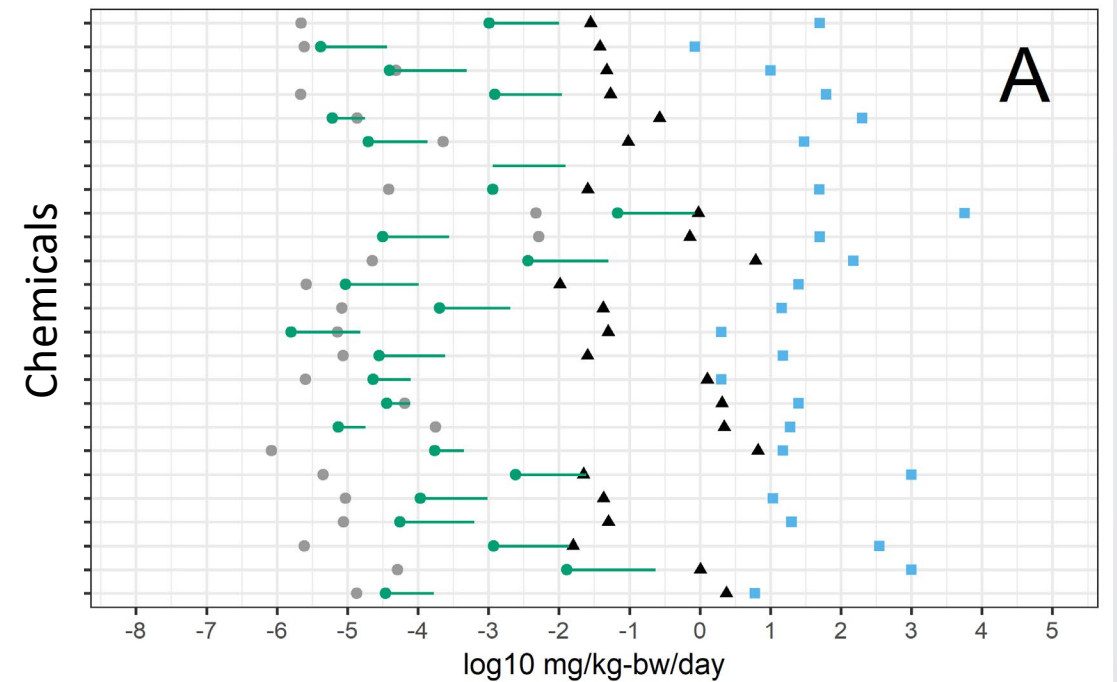
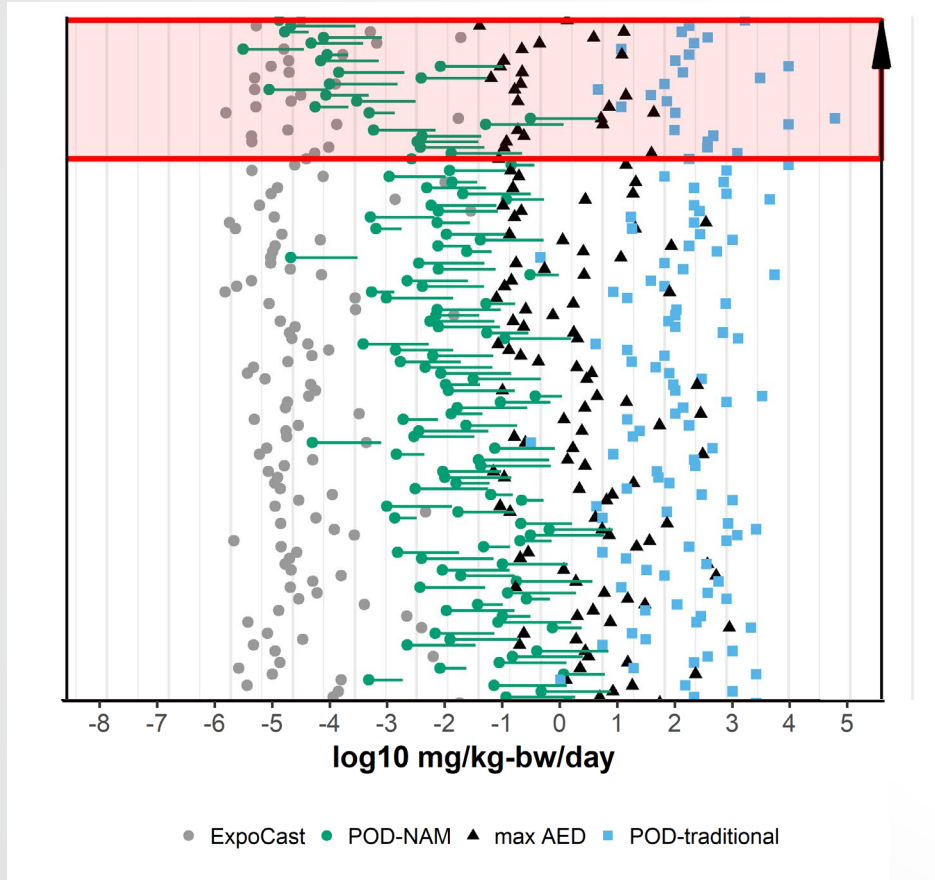


Figure 3 from Paul Friedman et al.

<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](https://doi.org/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.