Endocrine Assessment and Prioritization using Bioactivity: Exposure Ratio

NAMs Training Workshop

RTP, NC

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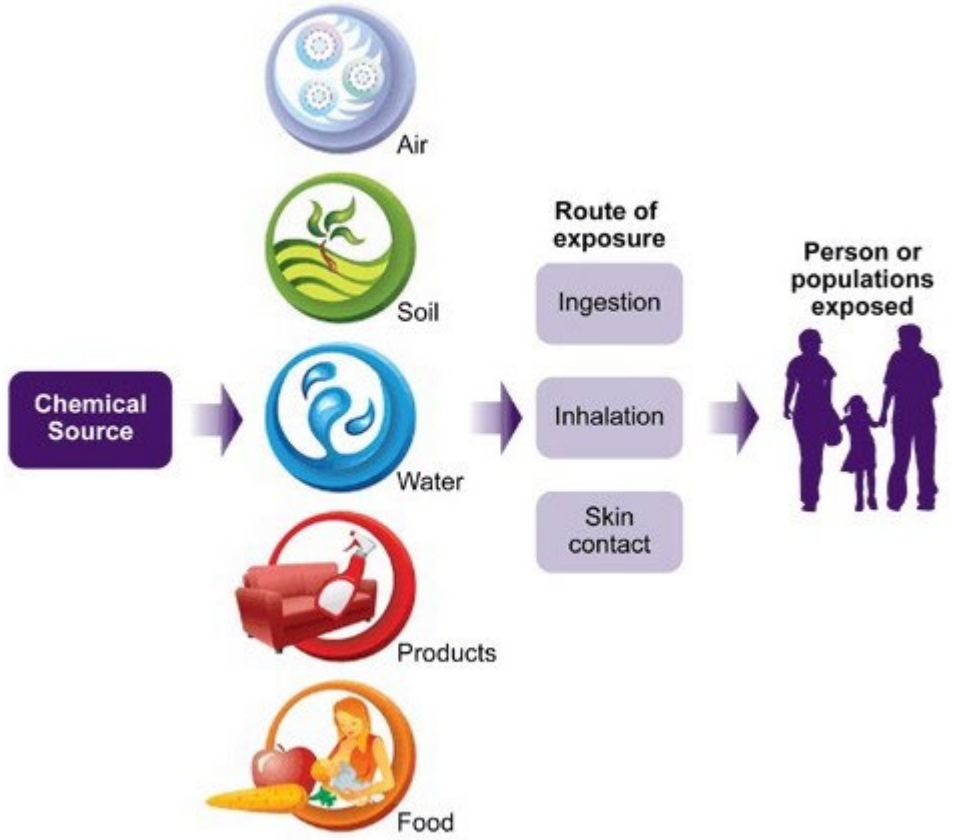
Disclaimer

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

What is hazard in toxicology?

Hazard x **Exposure** = **Risk**

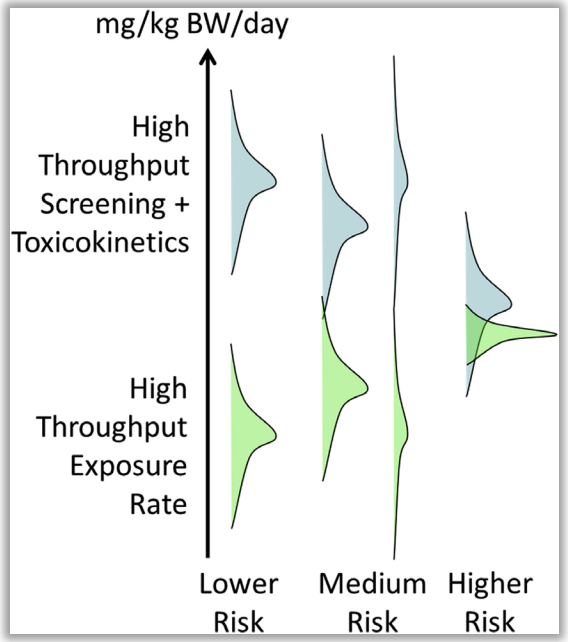
Something with the potential to cause biological harm



https://www.canada.ca/en/health-canada/services/home-garden-safety/measuring-your-exposure-chemicals.html

Hazard can be quantitative and qualitative

Risk-based regulatory paradigms tend to emphasize quantitative estimates of point-of-departure (POD) to enable derivation of a reference dose.



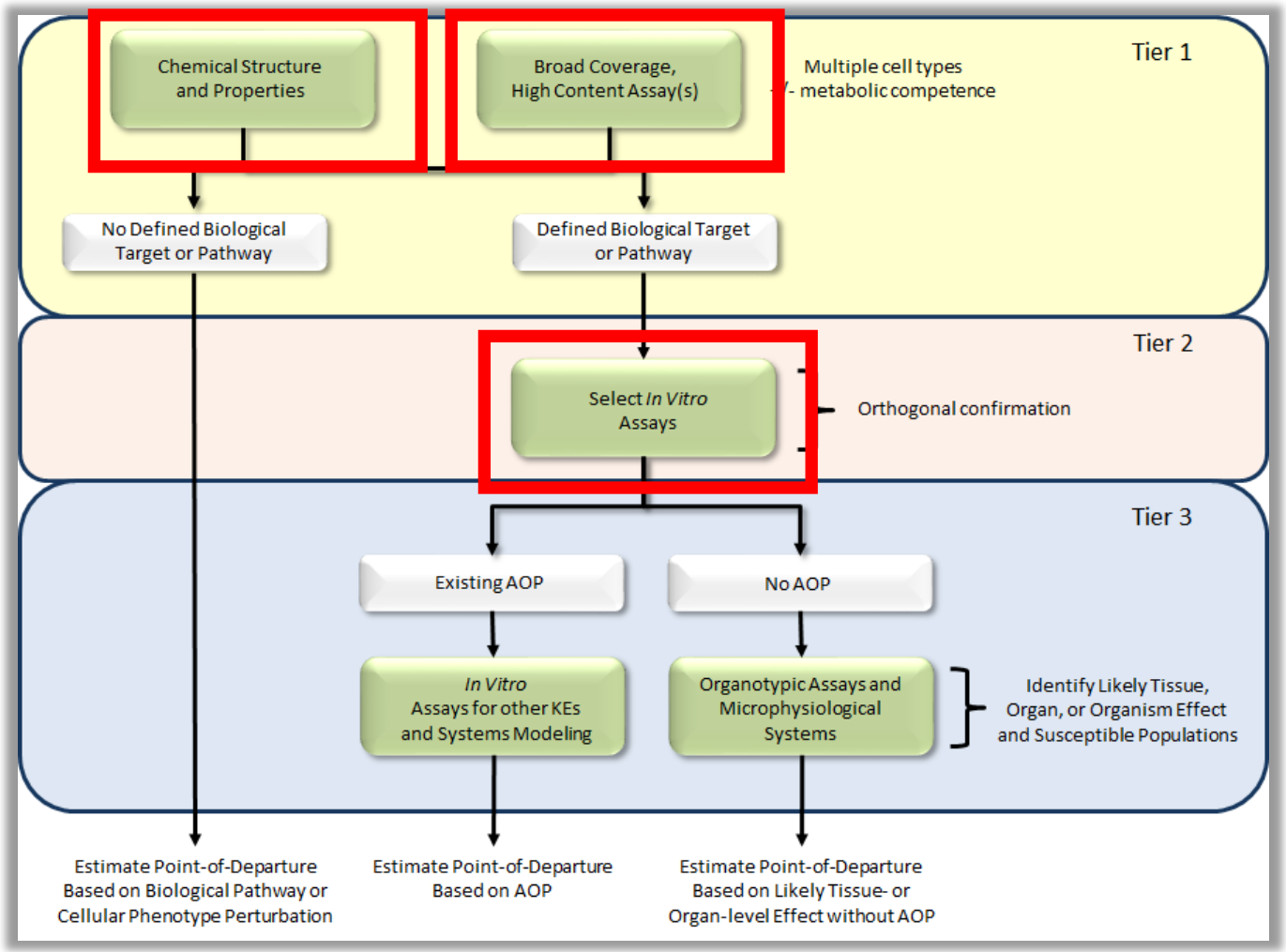
Wambaugh et al. (2019)
https://doi.org/10.1016/j.cotox.2019.07.001

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

Hazards of interest for regulatory toxicology commonly include, depending on statute :

- Acute
- Repeated dose/ systemic
- Genotoxicity
- Carcinogenicity
- Developmental and reproductive toxicity
- Neurotoxicity
- Immunotoxicity

NAMs for hazard include broad profiling and targeted NAMs



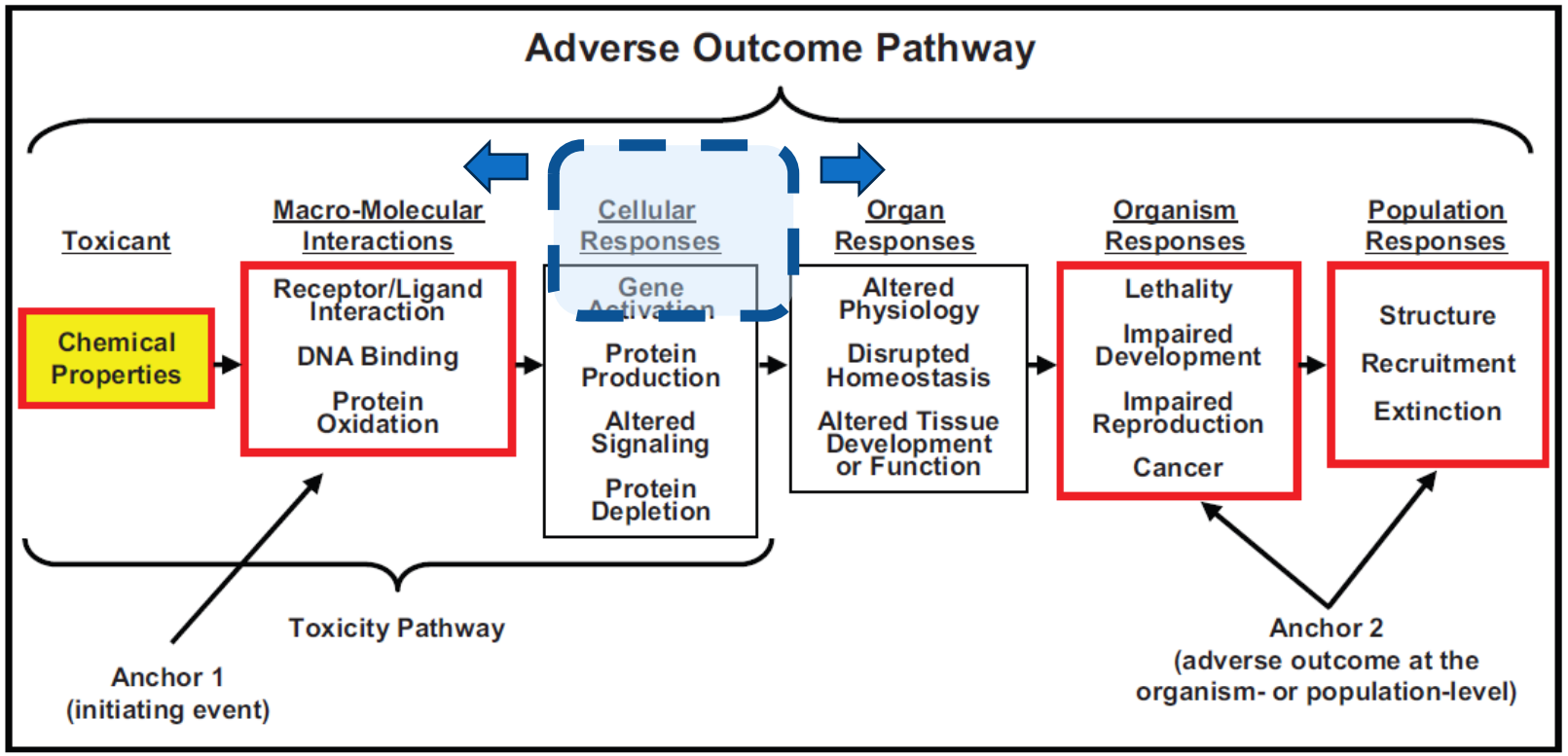
The NexGen Blueprint of CompTox at US EPA

Thomas et al. (2019) 10.1093/toxsci/kfz058

Brief introduction to 3 types of NAMs for hazard currently available to users in multiple formats, including the CompTox Chemicals Dashboard (CCD):

(1) In silico NAMs (cheminformatics)
(2) Broad profiling NAMs
(3) Targeted NAMs (ToxCast)

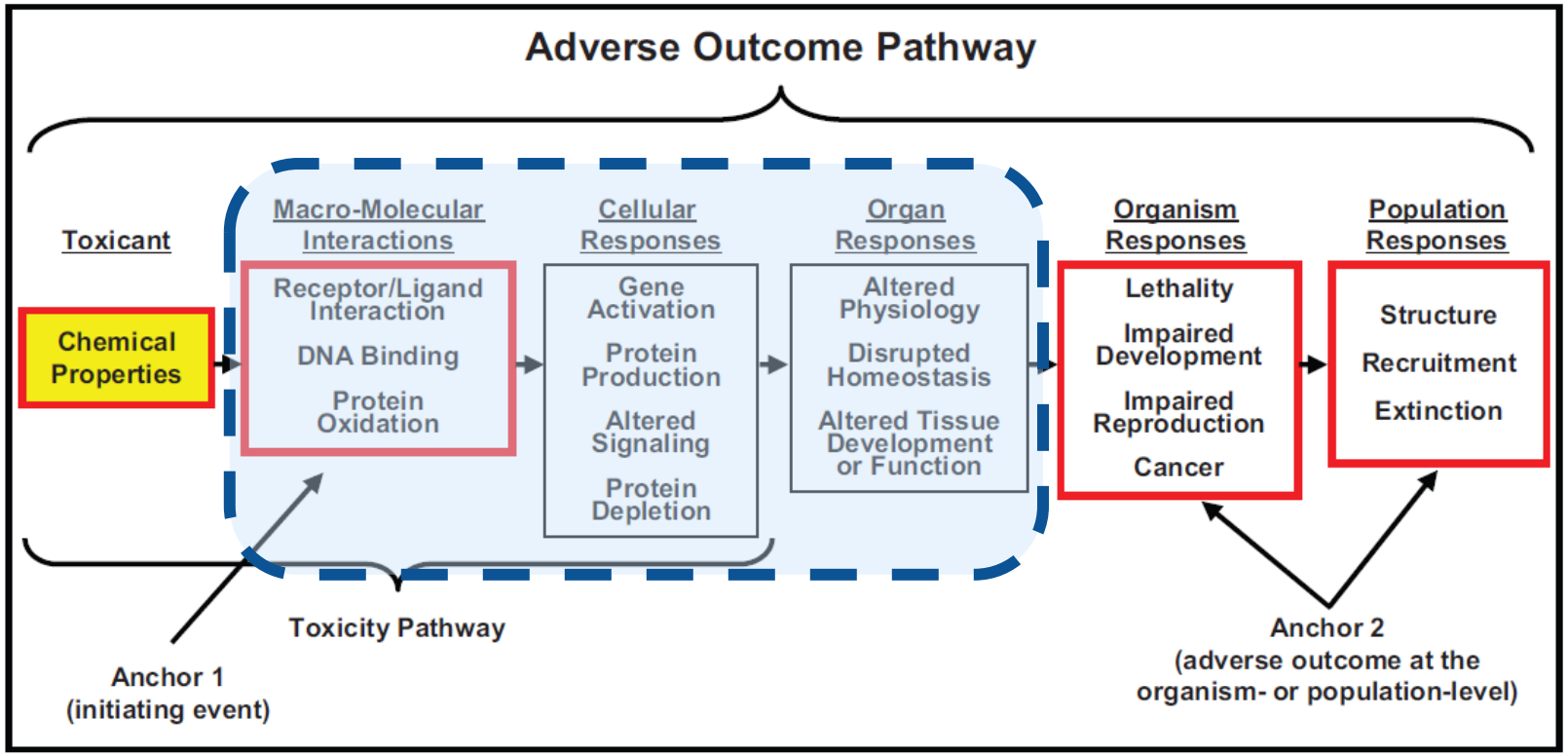
Broad profiling NAMs tend to probe cellular responses



Ankley et al. (2010)
10.1002/etc.34

- Broad profiling NAMs in use interrogate gene expression and cell morphological responses
- These data may be used to infer upstream interactions or downstream organ responses

Heterogeneous targeted NAMs in ToxCast address a range of event types in the AOP framework



Ankley et al. (2010)
10.1002/etc.34

Target NAMs: ToxCast Database

The **Toxicity Forecaster (ToxCast)** program curates and makes publicly available targeted bioactivity screening data. Latest database release (v4.1) includes:

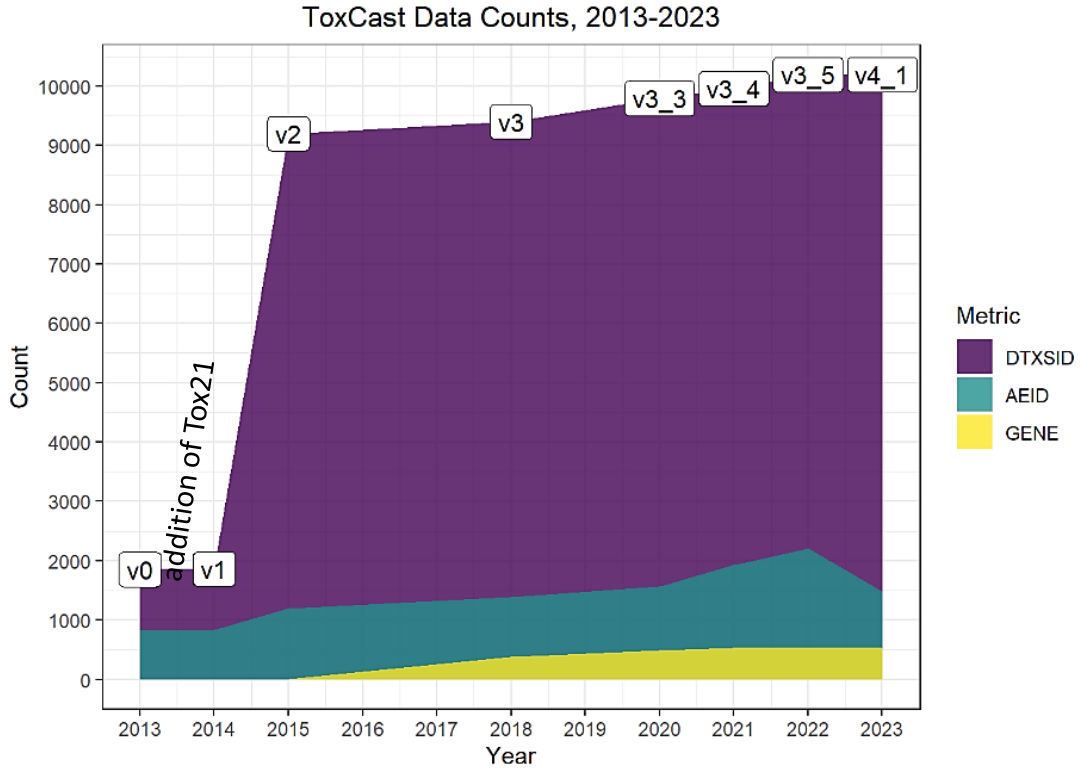
26 Assay Sources

Including a TOX21 assay source for data generated by the TOX21 program

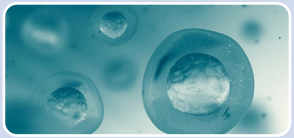
623 Unique Assays

1496 Unique Endpoints

9559 Chemicals



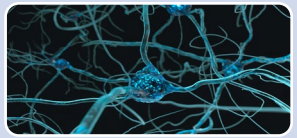
Diverse biology with ***over 500 mapped gene targets***, including:



Endocrine-Related: Estrogen Receptor, Androgen Receptor, Thyroid, Steroidogenesis

Cellular Signaling Pathways: Cytotoxicity, Proliferation, Stress, Mitochondrial Toxicity

Protein Interactions: Receptors, Transporters, Ion Channels, Enzymes



Complex Responses, e.g. Immune Response, Development, Neurotoxicity, etc.

Using ToxCast: from hazard to risk

- **Hypotheses about mode-of-action or adverse outcome pathways**
- **Contributions to “weight of evidence” for mechanistic inference**
- **Endocrine assessments using models and assay data**
- **Calculation of a bioactivity-based point of departure**
- **Quantitative estimation of a risk metric: bioactivity:exposure ratio (BER)**

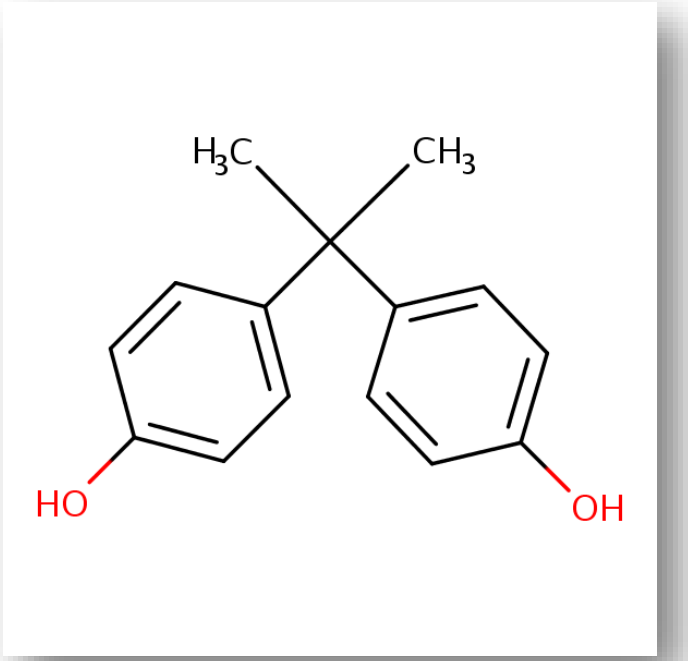
Examining a single substance for endocrine bioactivity

A generic workflow is illustrated which suggests examining:

- the amenability of the substance for HTS screening and sample quality;
- Models or single assays available; and,
- Whether the activity is likely to be selective or not.

- Quantitative structure-activity relationship (QSAR) (CERAPP and COMPARA)
- Computational model of bioactivity data (ToxCast ER and AR pathway models)
- Statistical model of steroidogenesis activity (ToxCast HT-H295R model)

Bisphenol A (BPA)

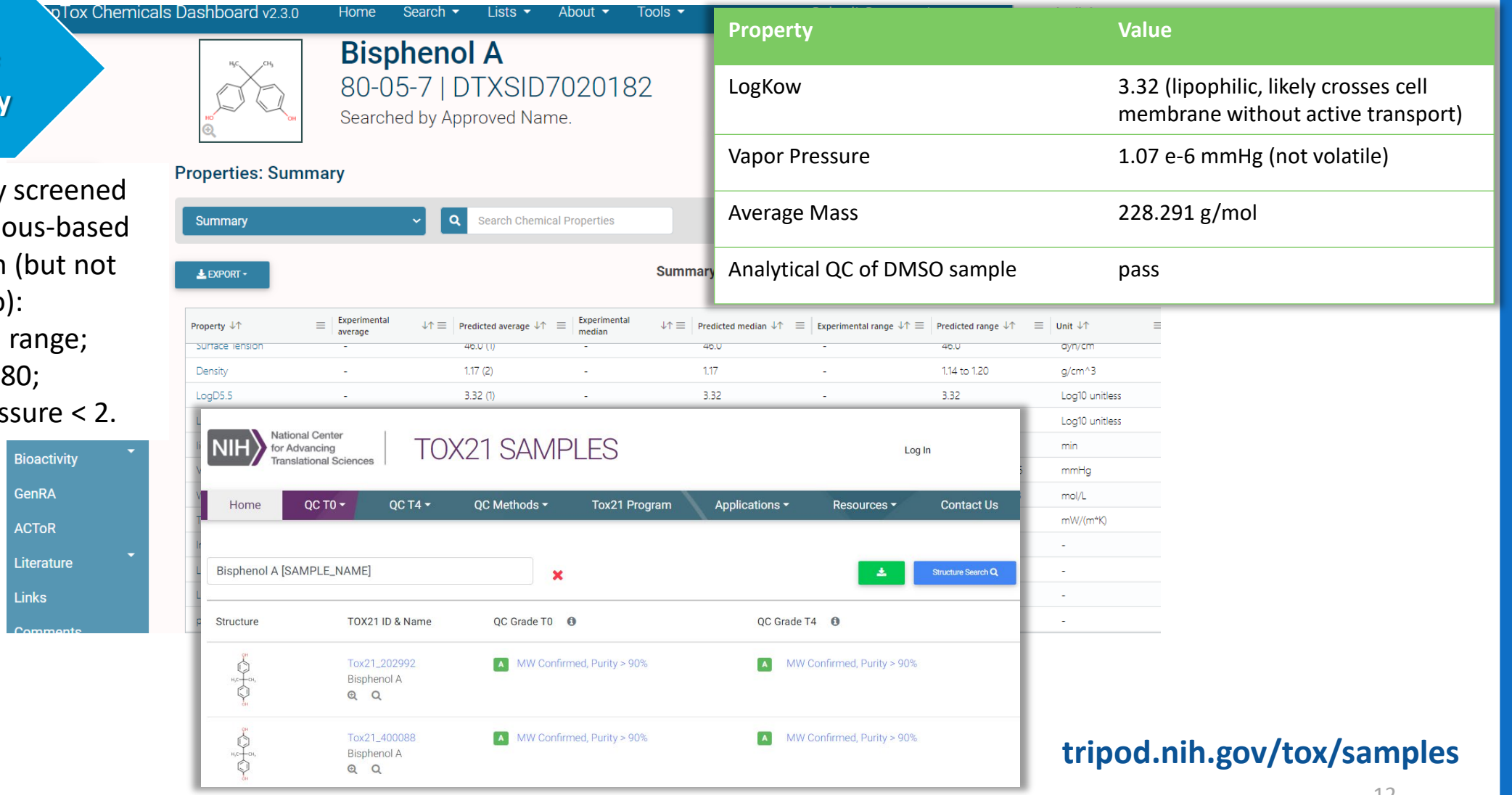


- Primarily industrial chemical
- Used in production of polycarbonate plastics, epoxy resins, and vinyl ester resins (used as food container liners, eyewear, pipes, sealants, dental composite, printed circuit boards)
  - Minor uses in producing flame retardants used in plastic, antioxidant in brake fluid, thermal paper
  - Through recycling, BPA can reach other products
- In the early 1990s, scientists discovered this chemical was leaching from plastic (in a lab)
- No longer permitted in baby bottles, sippy cups, or infant formula packaging (FDA 2012)
- Note that rapid metabolism in humans to an inactive form greatly lowers internal exposure of BPA
- BPA remains prevalent in biomonitoring data (from sources such as NHANES) suggesting continuous exposure (largely food and water) due to lack of bioaccumulation

Bisphenol A: in domain of aqueous cell-based screening?

Chemical domain of applicability

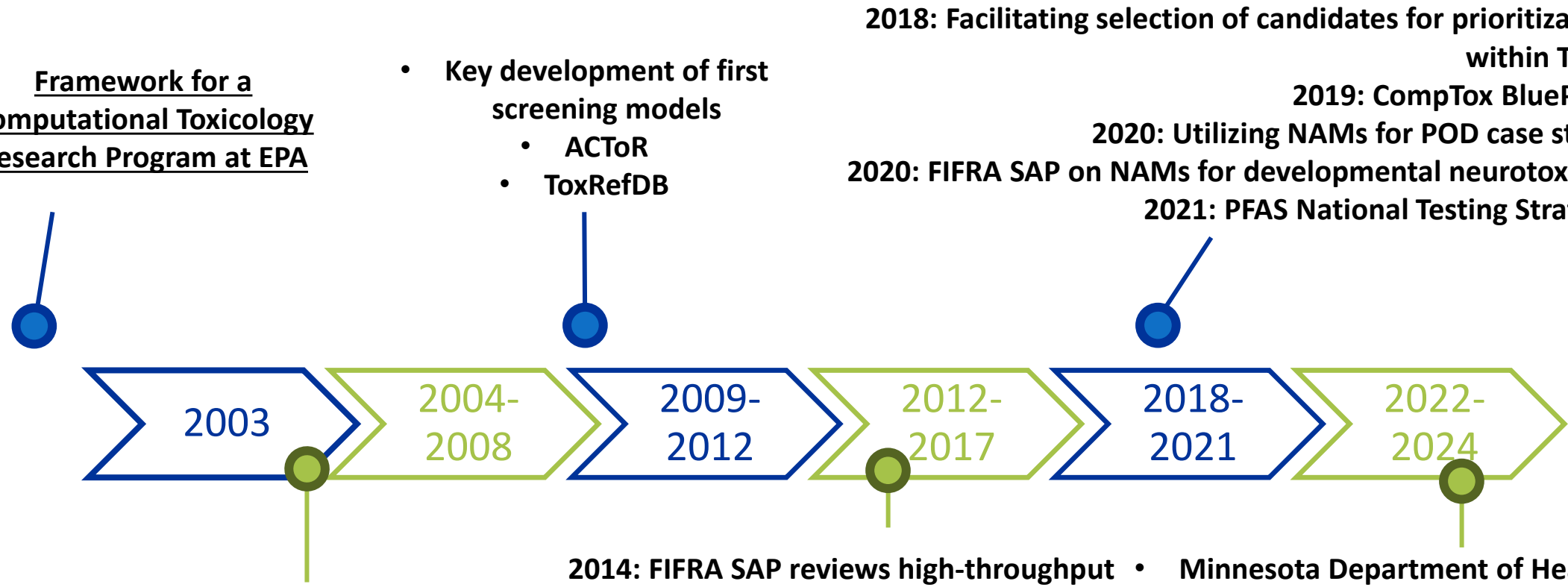
Many successfully screened chemicals in aqueous-based assays have been (but not limited to):
logP -1 to ~6.5 range;
MW 140-480;
log10 Vapor Pressure < 2.



Property	Value
LogKow	3.32 (lipophilic, likely crosses cell membrane without active transport)
Vapor Pressure	1.07 e-6 mmHg (not volatile)
Average Mass	228.291 g/mol
Analytical QC of DMSO sample	pass

tripod.nih.gov/tox/samples

Key milestones in NAM development and application to regulatory challenges



Overview of endocrine models available

Consensus QSARs (*in silico*)

CERAPP: Collaborative Estrogen Receptor Activity Prediction Project for agonist, antagonist, and binding prediction [Mansouri *et al.*, 2016, http://dx.doi.org/10.1289/ehp.1510267]

COMPARA: Collaborative Modeling Project for Androgen Receptor Activity for agonist, antagonist, and binding prediction [Mansouri *et al.*, 2020, https://doi.org/10.1289/EHP5580]

ToxCast ER and AR pathway models (based on *in vitro* data for multiple assays)

Original models using 18 and 12 assays, respectively, have results on the CompTox Chemicals Dashboard

Confidence score and examination of selectivity can be important for interpreting the overall results

Work is currently in progress to create a set of assays to inform a prospective model with fewer assays

ToxCast HT-H295R statistical model (based on *in vitro* data for multiple hormones in H295R cells)

Similar to OECD Test Guideline 456

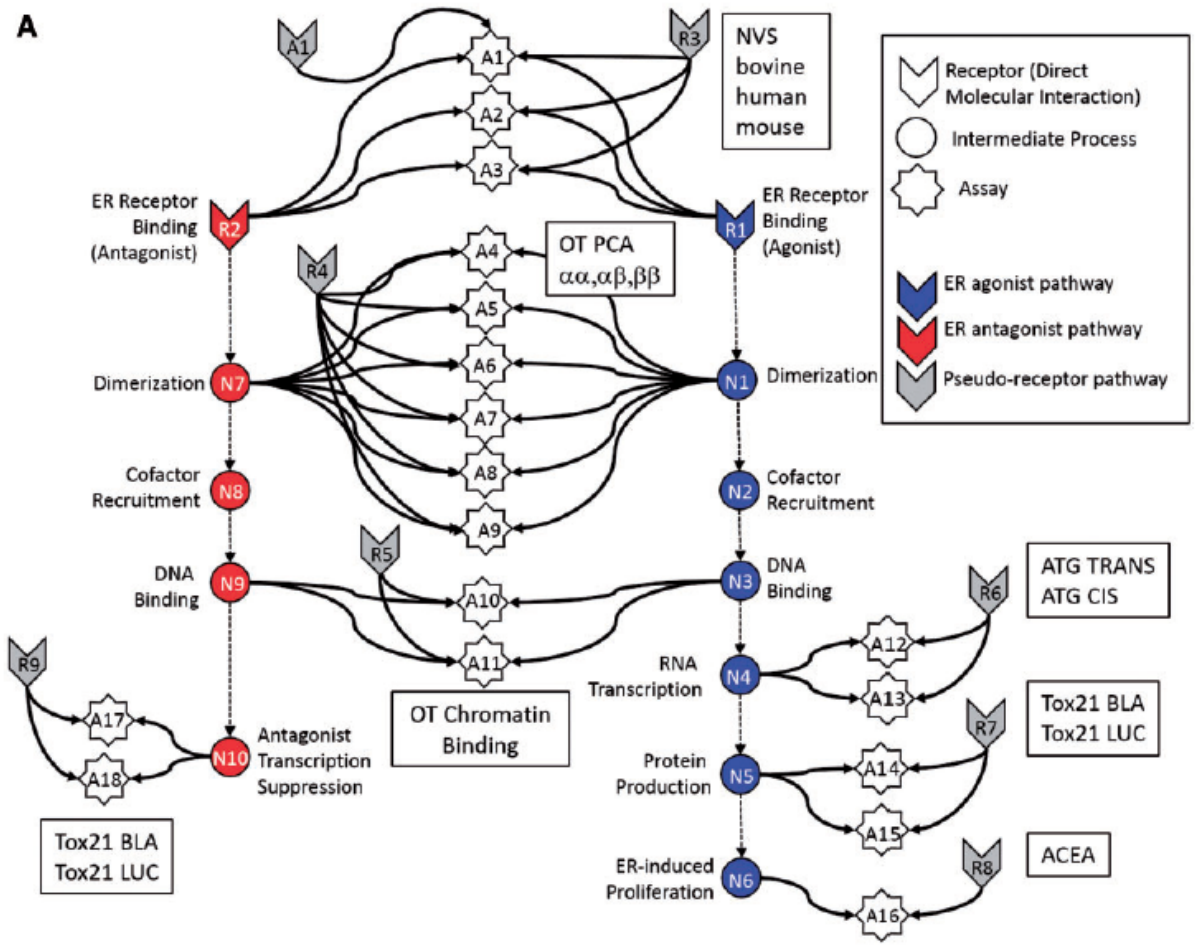
Maximum mean Mahalanobis distance, which compresses the 11-hormone responses into a single value to determine if steroidogenesis has been perturbed in the H295R system

Log2-fold changes by hormone are available in the publication(s)

ER and AR are unique targets in their assay redundancy, facilitating this approach

- Developed multiple high-throughput screening assays
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use a systems biology model to integrate assays
  - Model creates a composite dose-response curve for each chemical to summarize results from all assays
  - Includes penalization for cytotoxicity

ToxCast ER pathway model

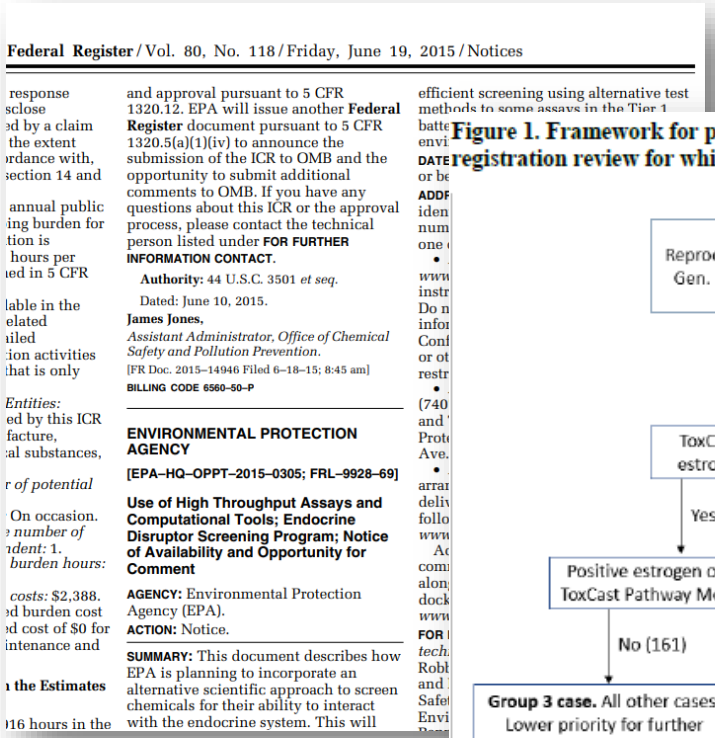


- The current model in the CompTox Chemicals Dashboard (v2.3.0) is an update of the 2015 published model but still includes all 18 assays for agonist mode.
- This model has been accepted as an alternative for the ER binding, ER-TA, and uterotrophic assays in the EDSP Tier 1 (https://www.federalregister.gov/documents/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice).
- Only 4 assays that cover key “receptors” or events in the activation of ER can achieve similar performance as the full model (10.1016/j.yrtph.2017.09.022).

https://doi.org/10.1093/toxsci/kfv168

Regulatory use of endocrine bioactivity models

2015 US Federal Register notice: **ToxCast ER pathway model** is a suitable alternative to 3 ER assays in EDSP Tier 1



Federal Register / Vol. 80, No. 118 / Friday, June 19, 2015 / Notices

ENVIRONMENTAL PROTECTION AGENCY

[EPA–HQ–OPPT–2015–0305; FRL–9928–69]

Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will

and approval pursuant to 5 CFR 1320.12. EPA will issue another **Federal Register** document pursuant to 5 CFR 1320.5(a)(1)(iv) to announce the submission of the ICR to OMB and the opportunity to submit additional comments to OMB. If you have any questions about this ICR or the approval process, please contact the technical person listed under **FOR FURTHER INFORMATION CONTACT**.

Authority: 44 U.S.C. 3501 *et seq.*

Dated: June 10, 2015.

James Jones,

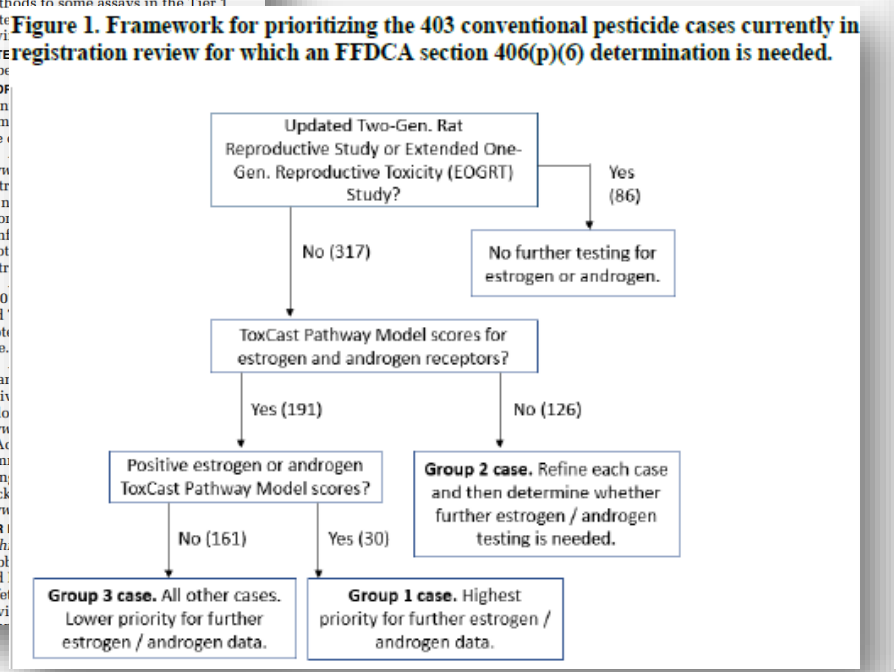
Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

[FR Doc. 2015–14946 Filed 6–18–15; 8:45 am]

BILLING CODE 6560–50–P

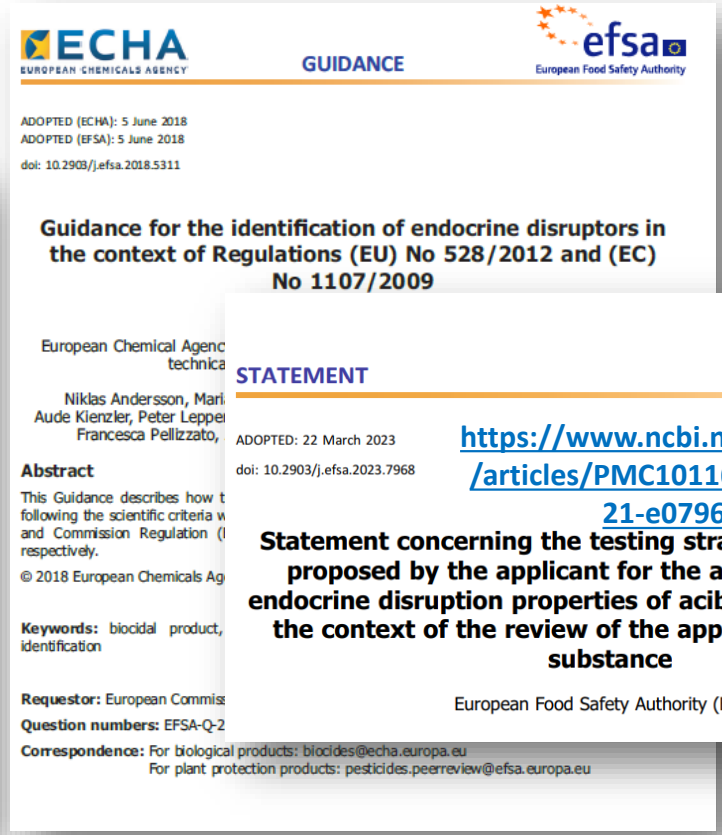
https://www.govinfo.gov/content/pkg/FR-2015-06-19/pdf/2015-15182.pdf

2023 EPA-OCSPP applied strategy for using **ToxCast ER and AR pathway models** to fill data gaps



https://www.regulations.gov/document/EPA-HQ-OPP-2023-0474-0002

2018 ECHA/EFSA guidance on using ToxCast ER pathway model and other bioactivity data



ECHA EUROPEAN CHEMICALS AGENCY

GUIDANCE

efsa European Food Safety Authority

ADOPTED (ECHA): 5 June 2018

ADOPTED (EFSA): 5 June 2018

doi: 10.2903/j.efsa.2018.5311

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

European Chemical Agenc technica

Niklas Andersson, Mari Aude Kienzler, Peter Leppe Francesca Pellizzato,

Abstract

This Guidance describes how t following the scientific criteria w and Commission Regulation (respectively.

© 2018 European Chemicals Ag

Keywords: biocidal product, identification

Requestor: European Commis

Question numbers: EFSA-Q-2

Correspondence: For biological products: biocides@echa.europa.eu
For plant protection products: pesticides.peerreview@efsa.europa.eu

https://www.efsa.europa.eu/en/efsajournal/pub/5311

2023 Examples of use by EFSA

efsa JOURNAL

STATEMENT

ADOPTED: 22 March 2023

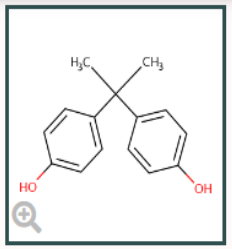
doi: 10.2903/j.efsa.2023.7968

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10116401/pdf/EFS2-21-e07968.pdf

Statement concerning the testing strategy and timelines proposed by the applicant for the assessment of the endocrine disruption properties of acibenzolar-S-methyl in the context of the review of the approval of the active substance

European Food Safety Authority (EFSA)

Where available, the Bioactivity > ToxCast Models provide the most reliable ER and AR predictions



Bisphenol A

80-05-7 | DTXSID7020182

Searched by Approved Name.

- Chemical Details
- Executive Summary
- Physchem Prop.
- Env. Fate/Transport
- Hazard Data
- Safety > GHS Data
- ADME > IVIVE
- Exposure
- Bioactivity

Bioactivity - ToxCast: Models

EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	1.00	1
ToxCast Pathway Model (AUC)	Estrogen	0.450	0.00	-
CERAPP Potency Level (From Literature)	Estrogen	Weak	Strong	Weak
CERAPP Potency Level (Consensus)	Estrogen	1.00	1.00	1
ToxCast Pathway Model (AUC)	Androgen	0.00	0.345	-

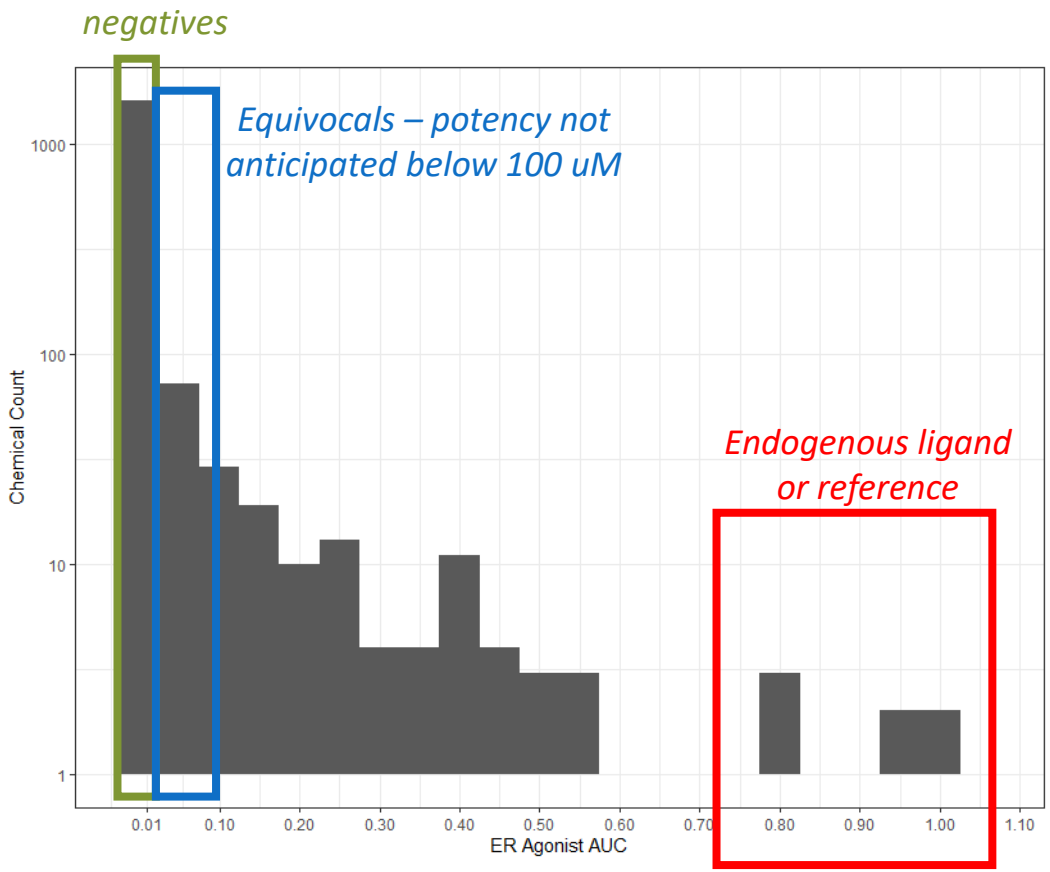
- 2 kinds of models are represented here: *in silico* consensus (Q)SARs and bioactivity-based ToxCast models
- For ToxCast models, >0.1 is positive; 0.001-0.1 is equivocal

CERAPP (literature and model), and ToxCast ER pathway model, suggest estrogen receptor agonism

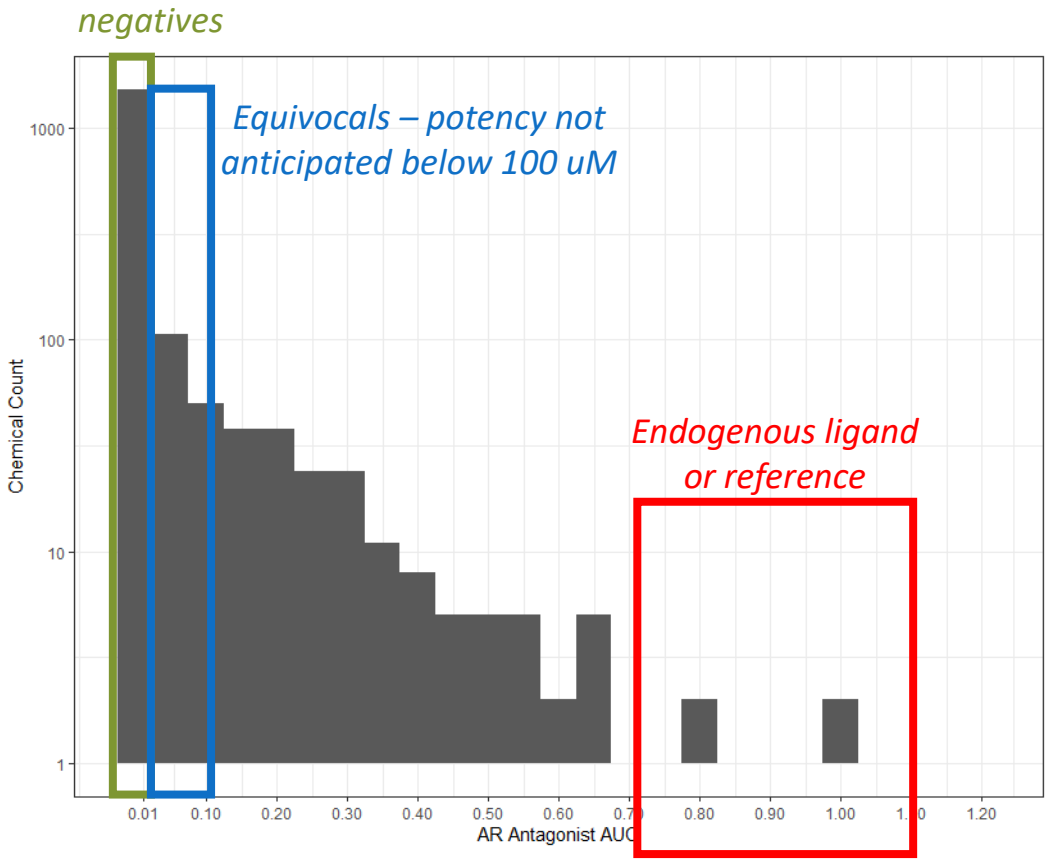
COMPARA and ToxCast AR pathway model suggest androgen receptor antagonism

Interpreting and using ToxCast pathway model scores: relative activity

Distribution of ToxCast ER Pathway Agonist Scores



Distribution of ToxCast AR Pathway Antagonist Scores



Bisphenol A is active in the HT-H295R model for steroidogenesis

Endocrine models available?

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

TOXICOLOGICAL SCIENCES, 162(2), 2018, 509–534

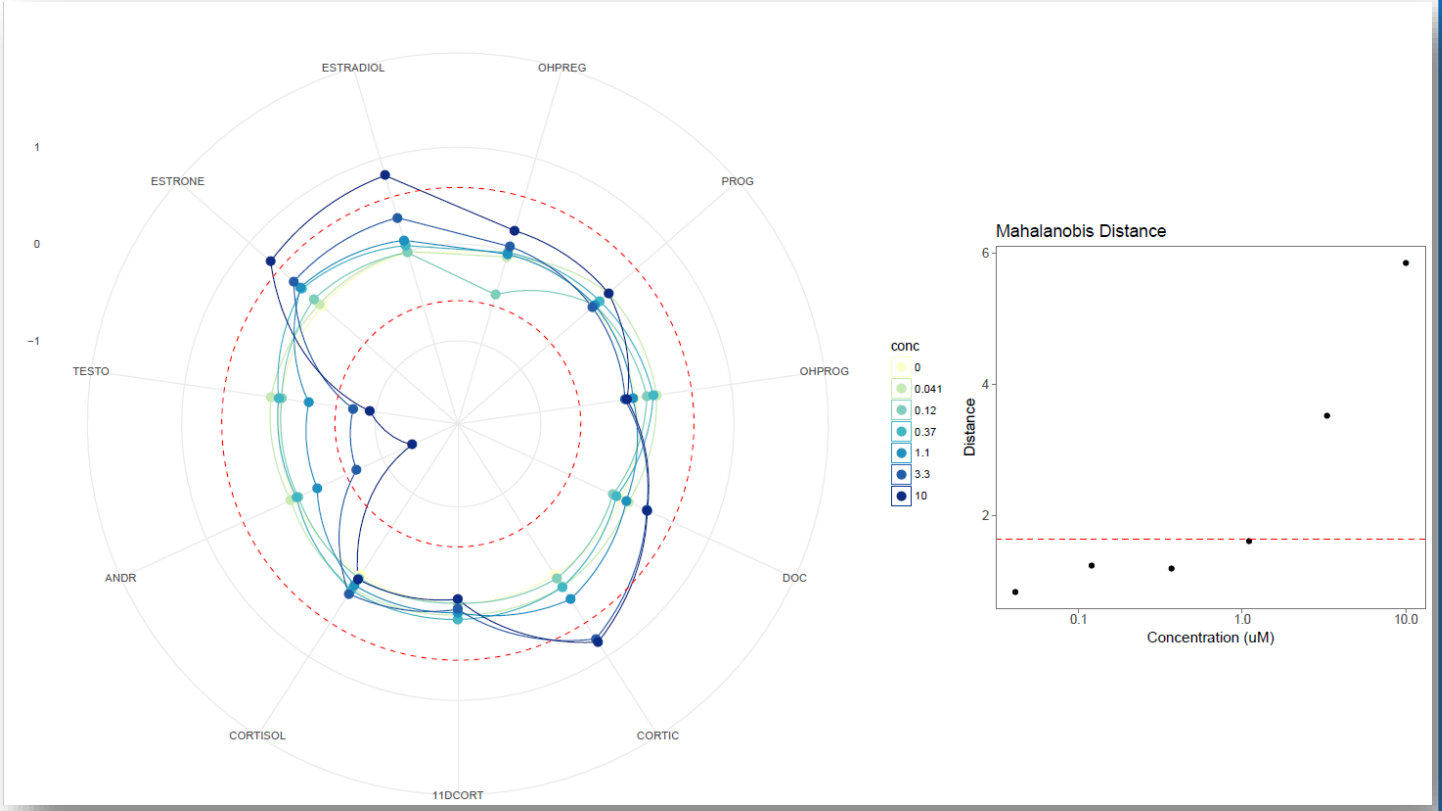
doi: 10.1093/toxsci/kfx274
Advance Access Publication Date: December 1, 2017
Research Article

High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis

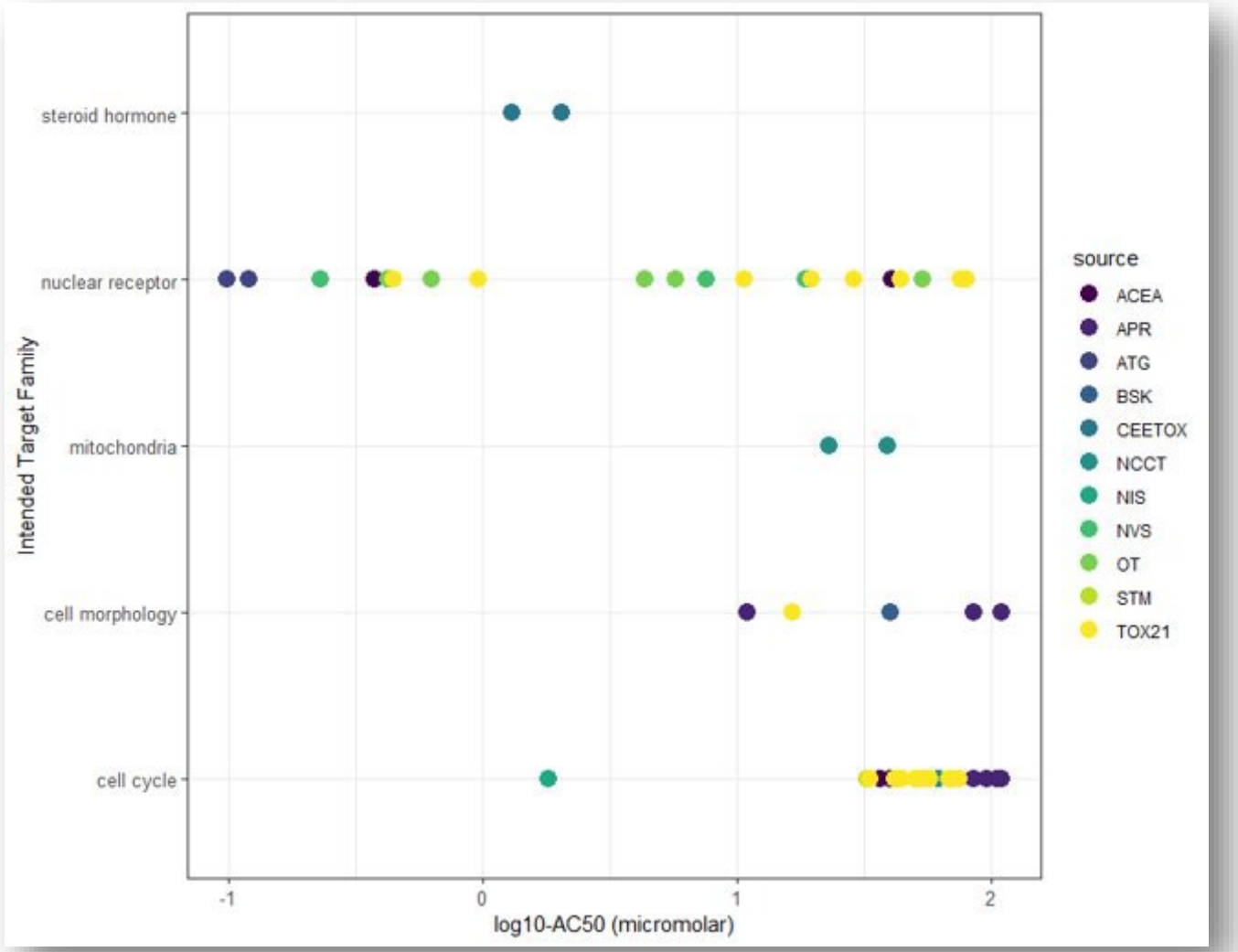
Derik E. Haggard,*,† Agnes L. Karmaus,*,†,1 Matthew T. Martin,†,2 Richard S. Judson,† R. Woodrow Setzer,† and Katie Paul Friedman†,3

*Oak Ridge Institute for Science and Education Postdoctoral Fellow, Oak Ridge, TN. 37831; and †National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, Durham, NC 27711

- Supplemental File 4 has fold-change by hormone
- Supplemental File 9 has mMd (model values)
- Invitrodb v4.1 has a hth295r model table with both of these included in it.
- Hope to include this in future release of the Dashboard.



Some of Bisphenol A's most potent *in vitro* activity is for the estrogen receptor



Exporting data from CCD, and then plotting by intended target family can be a helpful way of understanding the overall activity of the chemical

Examining ToxCast data for ESR1 using an export from CCD

NAME	ASSAY_COMPONENT_NAME	ASSAY_LISTS	GENE_SYMBOL	HIT_CALL	AC50	ACC
ATG_ERa_TRANS	ATG_ERa_TRANS	EDSP ER	ESR1	Active	0.09	0.03
ATG_ERE_CIS	ATG_ERE_CIS	EDSP ER	ESR1	Active	0.1	0.05
NVS_NR_mERa	NVS_NR_mERa	EDSP ER	Esr1	Active	0.14	0.02
NVS_NR_hER	NVS_NR_hER	EDSP ER	ESR1	Active	0.23	0.16
OT_ER_ERbERb_1440	OT_ER_ERbERb_1440	EDSP ER	ESR2	Active	0.35	0.12
OT_ERa_GFPERaERE_0120	OT_ERa_GFPERaERE_0120	EDSP ER	ESR1	Active	0.37	0.3
OT_ER_ERbERb_0480	OT_ER_ERbERb_0480	EDSP ER	ESR2	Active	0.37	0.1
ACEA_ER_80hr	ACEA_ER_80hr	EDSP ER	ESR1	Active	0.37	0.2
NVS_NR_bER	NVS_NR_bER	EDSP ER	ESR1	Active	0.42	0.19
TOX21_ERa_LUC_VM7_Agonist	TOX21_ERa_LUC_VM7_Agonist	EDSP ER	ESR1	Active	0.43	0.12
OT_ER_ERaERb_0480	OT_ER_ERaERb_0480	EDSP ER	ESR1 | ESR2	Active	0.5	0.28
OT_ERa_GFPERaERE_0480	OT_ERa_GFPERaERE_0480	EDSP ER	ESR1	Active	0.65	0.38
TOX21_ERa_BLA_Agonist_ratio	TOX21_ERa_BLA_Agonist_ratio	EDSP ER	ESR1	Active	0.96	1.37
OT_ER_ERaERb_1440	OT_ER_ERaERb_1440	EDSP ER	ESR1 | ESR2	Active	1.92	0.09
OT_ER_ERaERa_0480	OT_ER_ERaERa_0480	EDSP ER	ESR1	Active	4.03	0.68
OT_ER_ERaERa_1440	OT_ER_ERaERa_1440	EDSP ER	ESR1	Active	4.31	1.05
TOX21_ERa_BLA_Antagonist_ratio	TOX21_ERa_BLA_Antagonist_ratio	EDSP ER	ESR1	Active	31.34	9.16

Depending on your use case, 0.1 uM appears to be a threshold AC50 value for BPA in estrogen receptor (ESR1) related assays

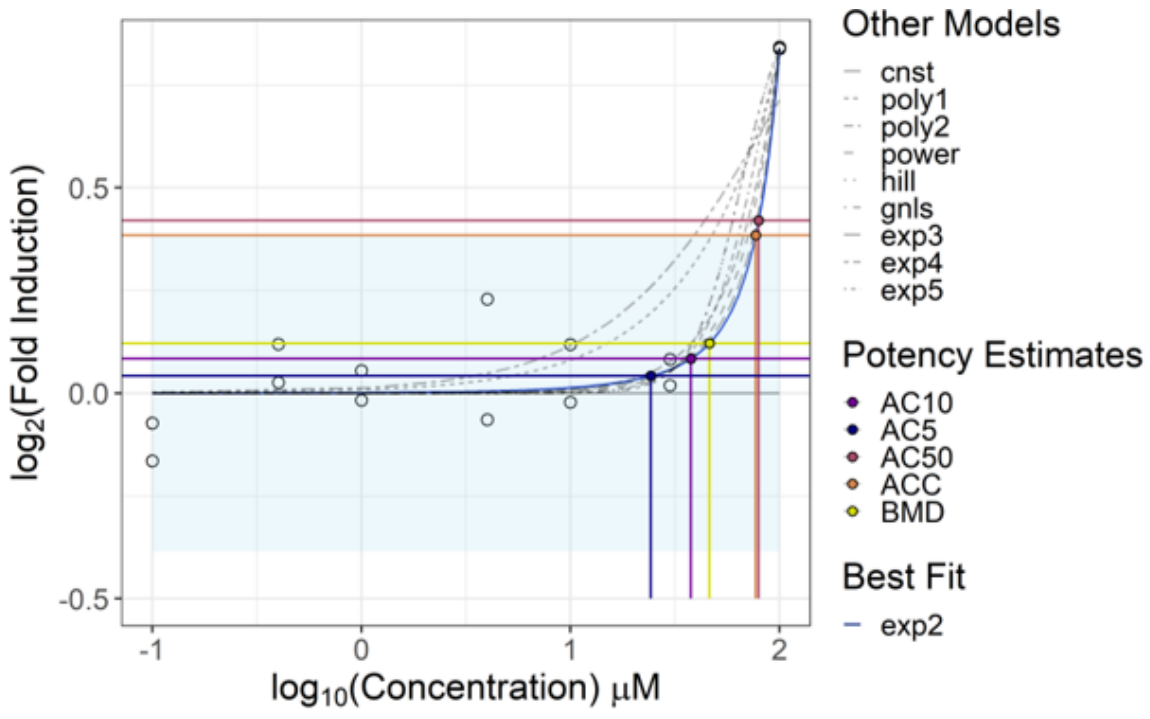
It is important to cite the data and explain the derivation of your point-of-departure

Calculating a bioactivity-based point-of-departure (POD) and bioactivity:exposure ratio (BER)

First, we need to convert the bioactive concentrations from micromolar to external dose estimates, termed “administered equivalent doses” in mg/kg/day (at least, for oral exposure)

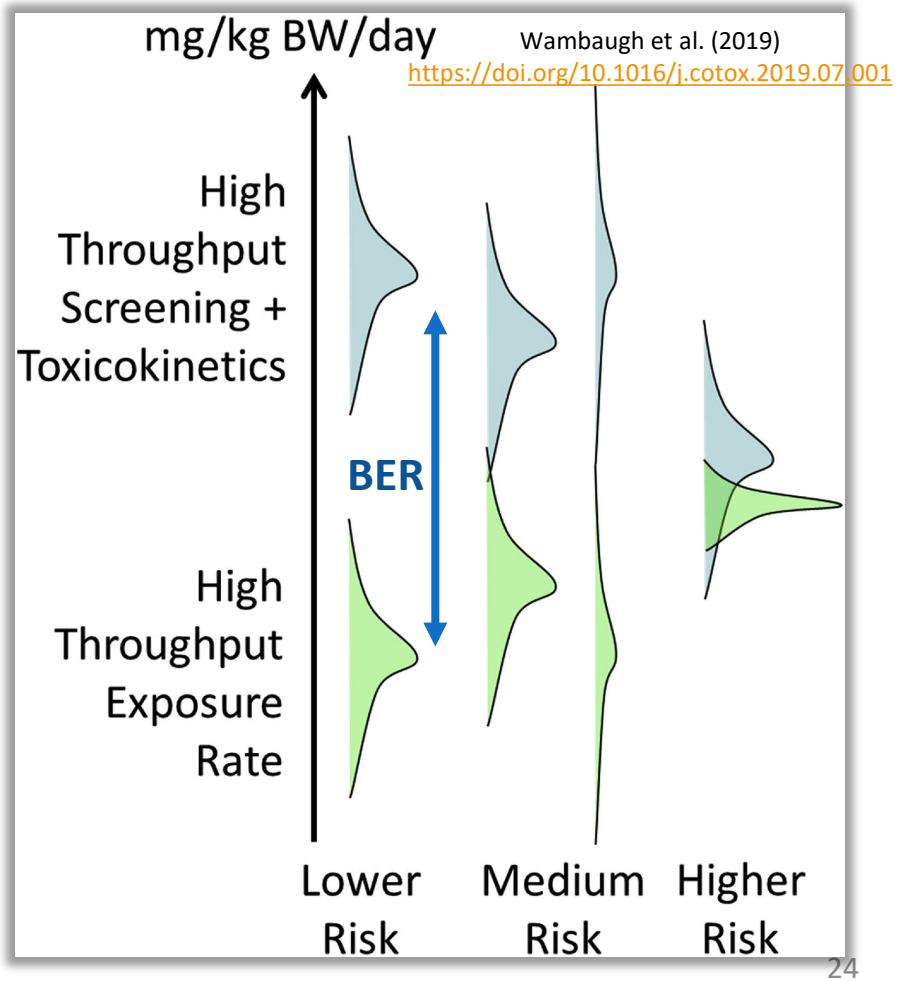
Defining POD and BER

A point-of-departure describes a point on a concentration (or dose) response curve where the activity moves away from the background and can be a first basis for setting health-protective limits



Feshuk et al (2023)
https://doi.org/10.3389/ftox.2023.1275980

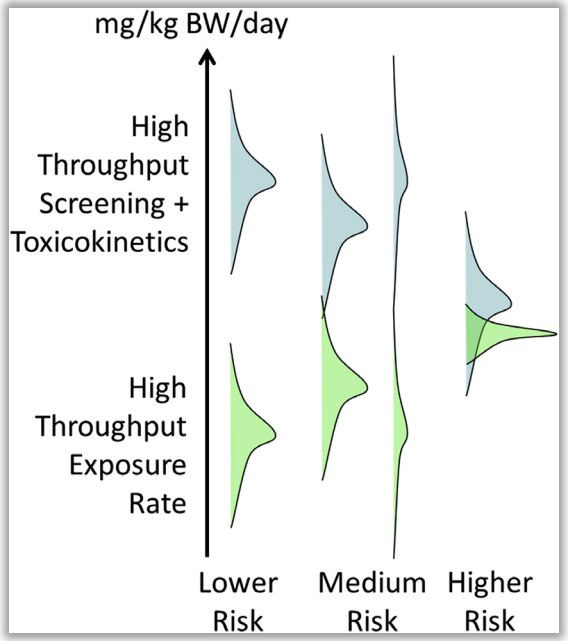
Bioactivity:exposure ratio: quantitative difference between bioactive dose and possible exposure dose

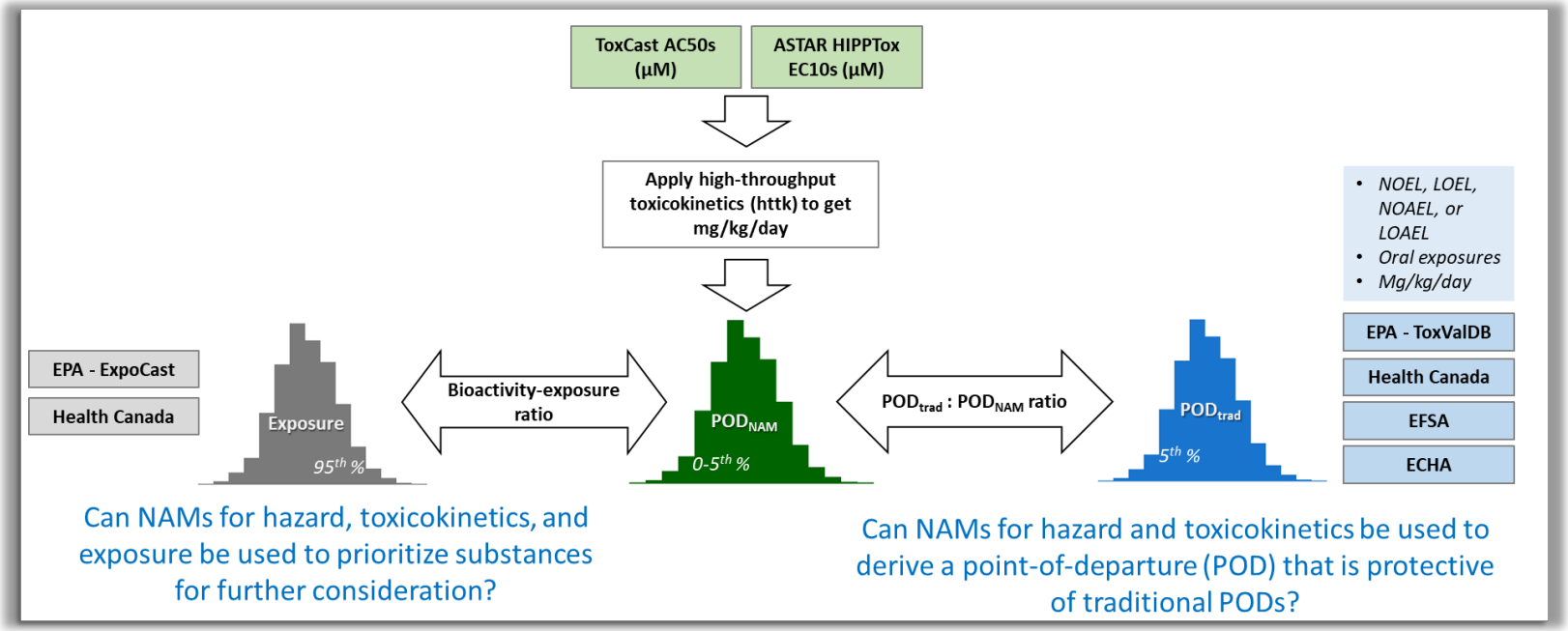


Wambaugh et al. (2019)
https://doi.org/10.1016/j.cotox.2019.07.001

Bioactivity:exposure ratio is similar to a margin of exposure

In vitro bioactive concentrations would be useful to compare to predicted exposures in humans, i.e. for derivation of a bioactivity:exposure ratio (BER).





Paul Friedman et al. (2020)
https://doi.org/10.1093/toxsci/kfz201

In vitro to in vivo extrapolation (IVIVE) of dose can be a simple approximation

$$[Conc]_{SS} = \frac{\text{Dose Rate} * \text{Body Weight}}{CL_{WholeBody}}$$

Steady State Blood Concentration

CL: clearance

Rowland *et al.,* 1973
Wilkinson and Shand, 1975
Gillette, 1980
Wilkinson, 1987

Slide adapted from Barbara Wetmore

In vitro to in vivo extrapolation (IVIVE) of dose can be a simple approximation

Assumptions:
100% absorption
Linear kinetics
No extrahepatic metabolism

Whole Body Clearance (CL) = Considering renal and hepatic clearance (CL_R, CL_H) – adjusted for blood binding (F_{ub})

$$[Conc]_{SS} = \frac{\text{Dose Rate} * \text{Body Weight}}{CL_{WholeBody}}$$

Steady State Blood Concentration

$$CL_{WholeBody} = CL_R + CL_H$$

$$CL_R = F_{ub} * GFR$$

$$CL_H = \frac{F_{ub} * Q_L * CL_{Int}}{Q_L + F_{ub} * CL_{Int}}$$

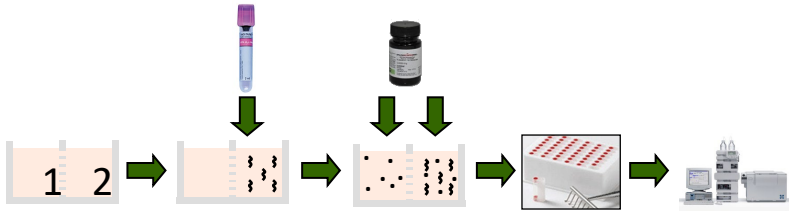
GFR = glomerular filtration rate
F_{ub} = fraction unbound in blood
Q_L = hepatic blood flow
Cl_{int} = intrinsic clearance

Slide adapted from Barbara Wetmore

High throughput toxicokinetics (HTTK)

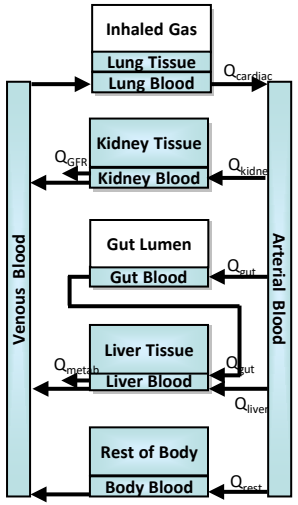
in vitro toxicokinetic data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



= *httk*

Some high-level assumptions commonly employed:

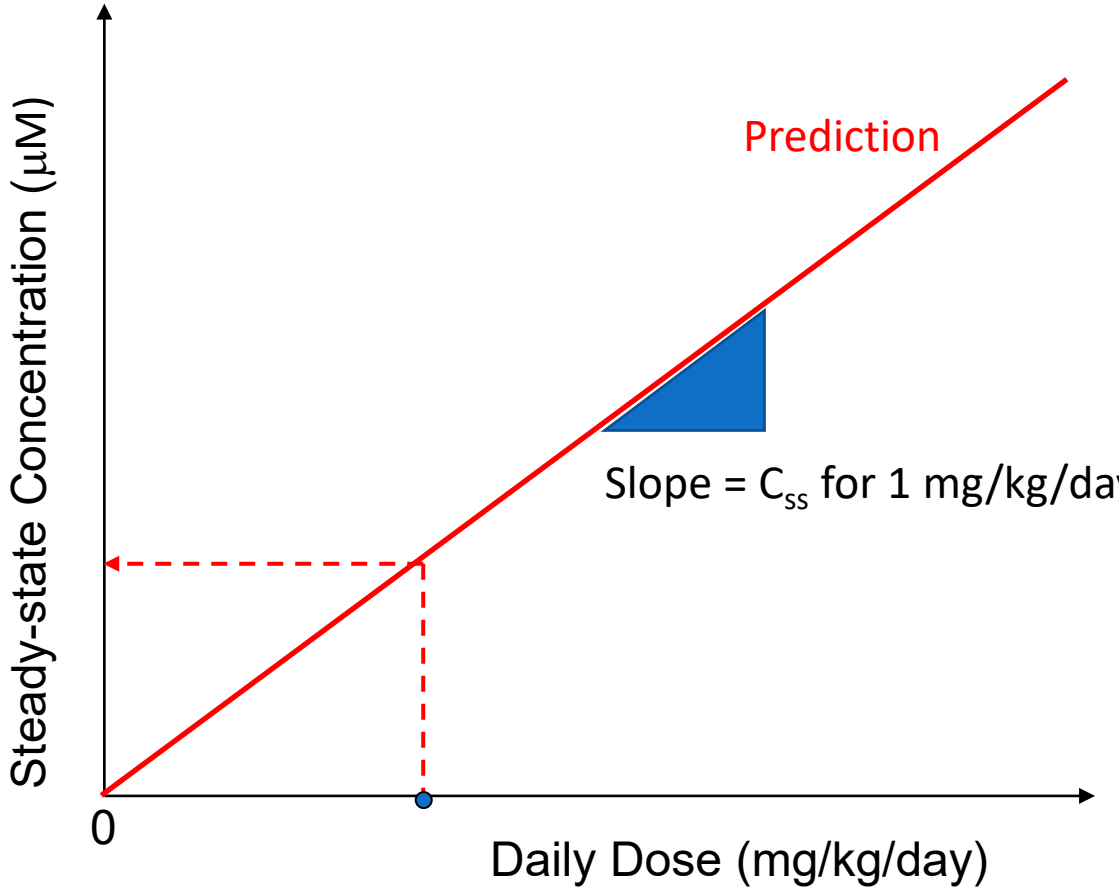
(1) bioactive nominal *in vitro* assay concentration ~ *in vivo* plasma concentration that would correspond to a similar effect;

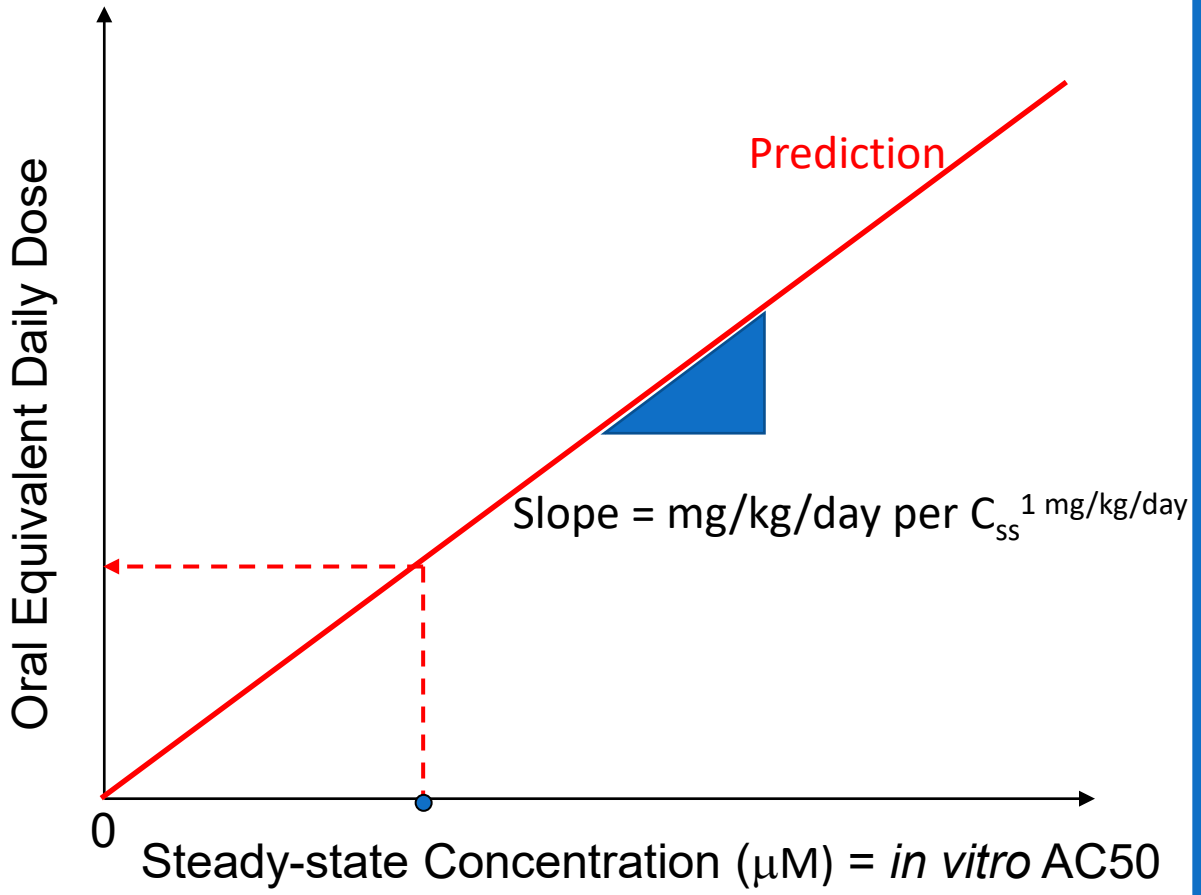
(2) external exposures (in mg/kg/day units) that may have resulted in that plasma concentration can be constructed using estimates of species-specific physiology and Phase I and Phase II enzyme-driven hepatic clearance; and,

(3) Often, we expect that plasma concentration can be approximated by steady-state kinetics (unless we have enough information to use other dose metrics).

Led by John Wambaugh, Barbara Wetmore, Caroline Ring, and colleagues

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

Slide from John Wambaugh

Simple calculation of AED based on 3 compartment steady state model

AED values in mg/kg/day units can be calculated from bioactivity using the following equation:

$$Eq: \quad AED_{50}\left(\frac{\frac{mg}{kg}}{day}\right) = AC_{50}(\mu M) * \frac{1\frac{\frac{mg}{kg}}{day}}{Css_{50}}$$

Where the Css (steady-state concentration) values for the median individual based on Monte Carlo simulation of species-specific physiological parameters (Css_{50}) (Pearce et al. 2017) were generated using the 3-compartment steady state model

HTTK performance is good despite many generalized assumptions

- HTTK models have demonstrated reasonable accuracy in predicting relevant TK endpoints, for example plasma concentrations over time (AUC) (R^2 = 0.62) and maximum plasma concentrations (Cmax) (R^2 = 0.48) (Wambaugh et al., 2018, 10.1093/toxsci/kfy020).
  - For these 45 pharmaceutical and non-pharmaceutical chemicals, this equates to an RMSE of 2.2-fold for Cmax and 1.64-fold for AUC.
  - The same work also found that httk predicted human volume of distribution with an RMSE of 0.48 (3-fold).
- Linakis, Sayre et al. (2020, 10.1038/s41370-020-0238-y) demonstrated that a generic PBTK model for 40 volatile, non-pharmaceutical chemicals found Cmax was predicted within 2.9-fold (RMSE = 0.46) ad 3.2-fold for AUC RMSE=0.5)
- HTTK predictions of Css are within a factor of 3 for many chemicals (many references, as reviewed in Breen et al. 2021, 10.1080/17425255.2021.1935867)

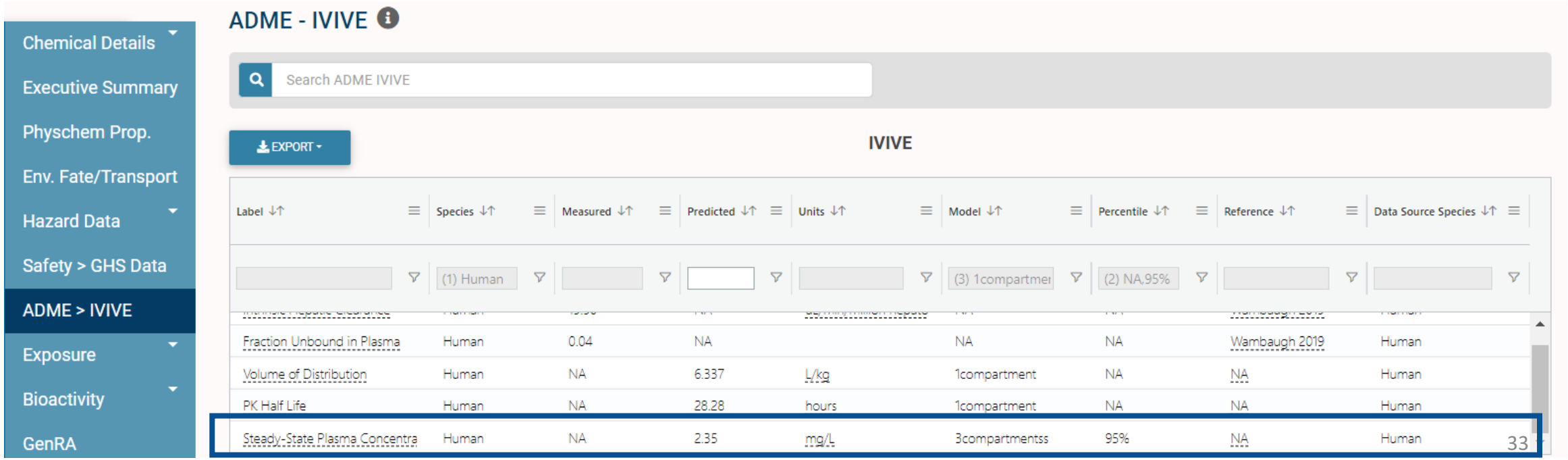
A simple approach for using the CompTox Chemicals Dashboard to estimate a POD

- 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 (e.g., using pbtk model or including interindividual toxicokinetic variability).

$$\frac{2.35\ \cancel{mg}}{L} \times \frac{\cancel{g}}{1000\ \cancel{mg}} \times \frac{\cancel{mol}}{228.291\ \cancel{g}} \times \frac{1e6\ \mu mol}{\cancel{mol}} = 10.2938793\ \mu mol/L = \mu M$$

Bioactive concentration

$$\frac{0.1\ \cancel{\mu M}}{} \times \frac{1\ mg/kg/day}{10.2938793\ \cancel{\mu M}} = \mathbf{0.0097\ mg/kg/day = AED95}$$

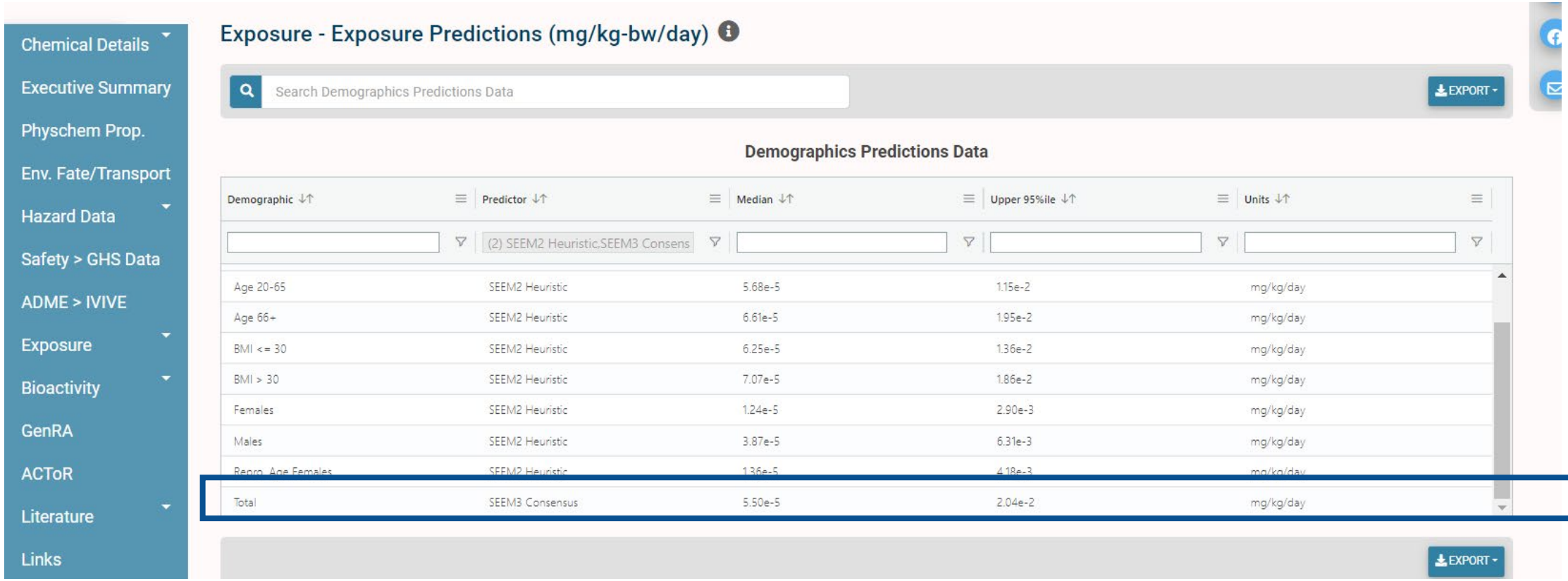


Calculate the bioactivity:exposure ratio (BER)

BER = bioactive dose/exposure = 0.0097/0.0204 = 0.476

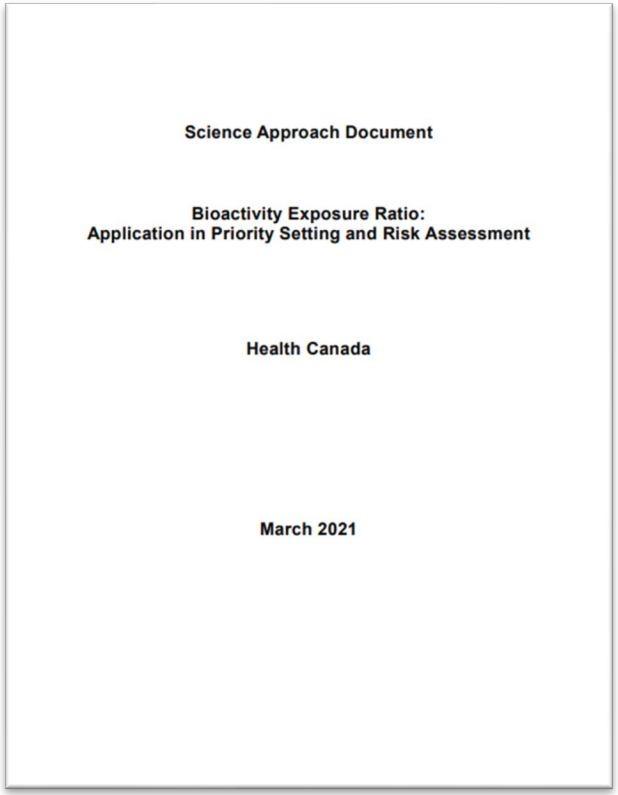
or log10(AED) – log10(exposure) = -2.01 - -1.69 = -0.322

where both bioactive dose and exposure are in the same units



Examples of BER in the regulatory toxicology

In managing risk evaluations of large inventories of chemicals, such as Canada's Domestic Substances List



Science Approach Document

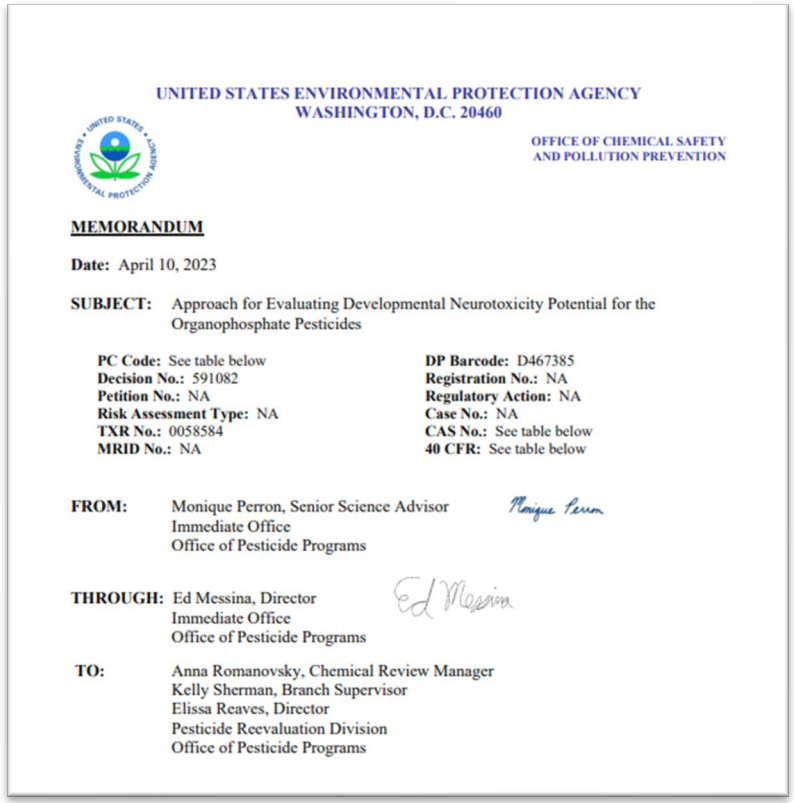
Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021

https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/science-approach-document-bioactivity-exposure-ratio-application-priority-setting-risk-assessment.html

In addressing data gaps within a weight-of-evidence for risk of developmental neurotoxicity of organophosphate insecticides



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

Date: April 10, 2023

SUBJECT: Approach for Evaluating Developmental Neurotoxicity Potential for the Organophosphate Pesticides

PC Code: See table below
Decision No.: 591082
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0058584
MRID No.: NA

DP Barcode: D467385
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: See table below
40 CFR: See table below

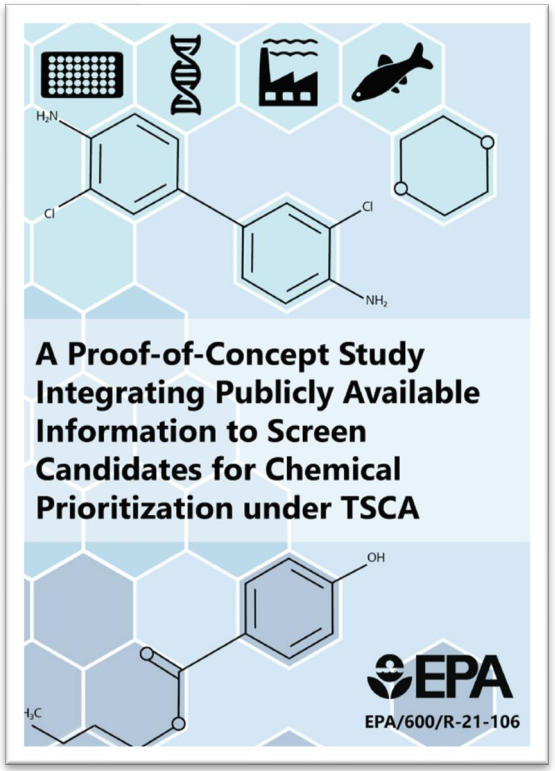
FROM: Monique Perron, Senior Science Advisor
Immediate Office
Office of Pesticide Programs

THROUGH: Ed Messina, Director
Immediate Office
Office of Pesticide Programs

TO: Anna Romanovsky, Chemical Review Manager
Kelly Sherman, Branch Supervisor
Elissa Reaves, Director
Pesticide Reevaluation Division
Office of Pesticide Programs

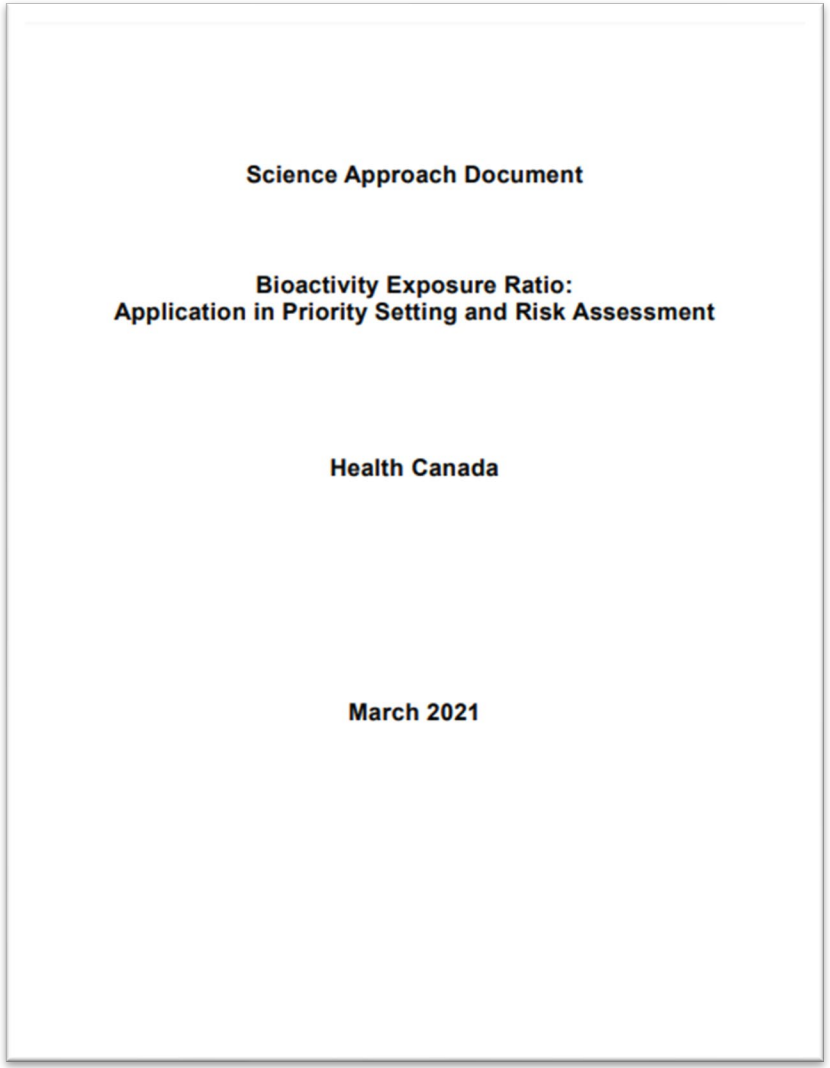
https://www.regulations.gov/document/EPA-HQ-OPP-2008-0915-0056

In proof-of-concept work to identify existing chemicals for further evaluation under the Toxic Substances Control Act



https://www.epa.gov/sciencematters/proof-concept-case-study-integrating-publicly-available-information-screen

Health Canada Scientific Approach Document used BER for prioritization with familiar methods

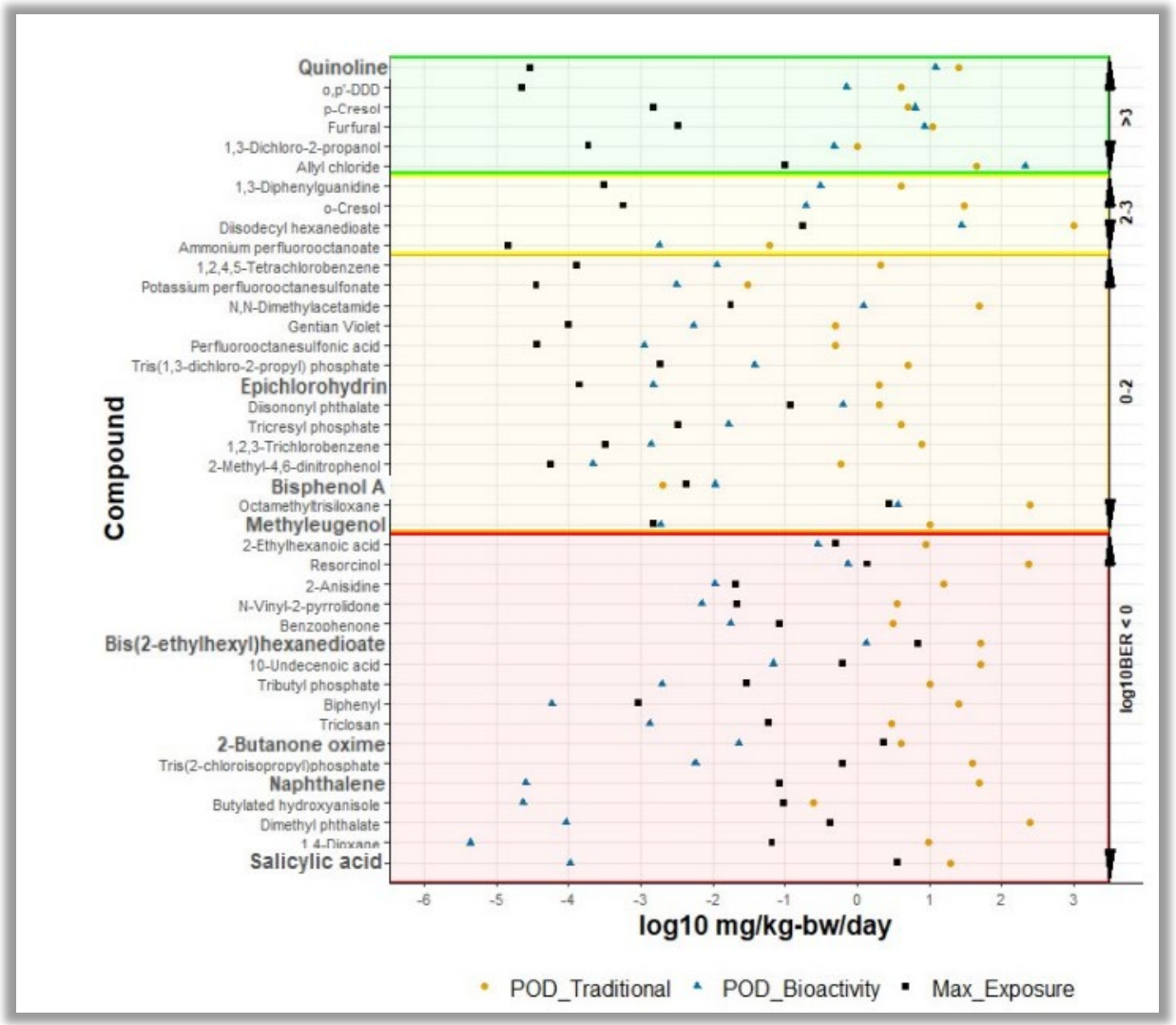


Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021



Much of the Health Canada SciAD for BER in priority setting paralleled APCRA paper

Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

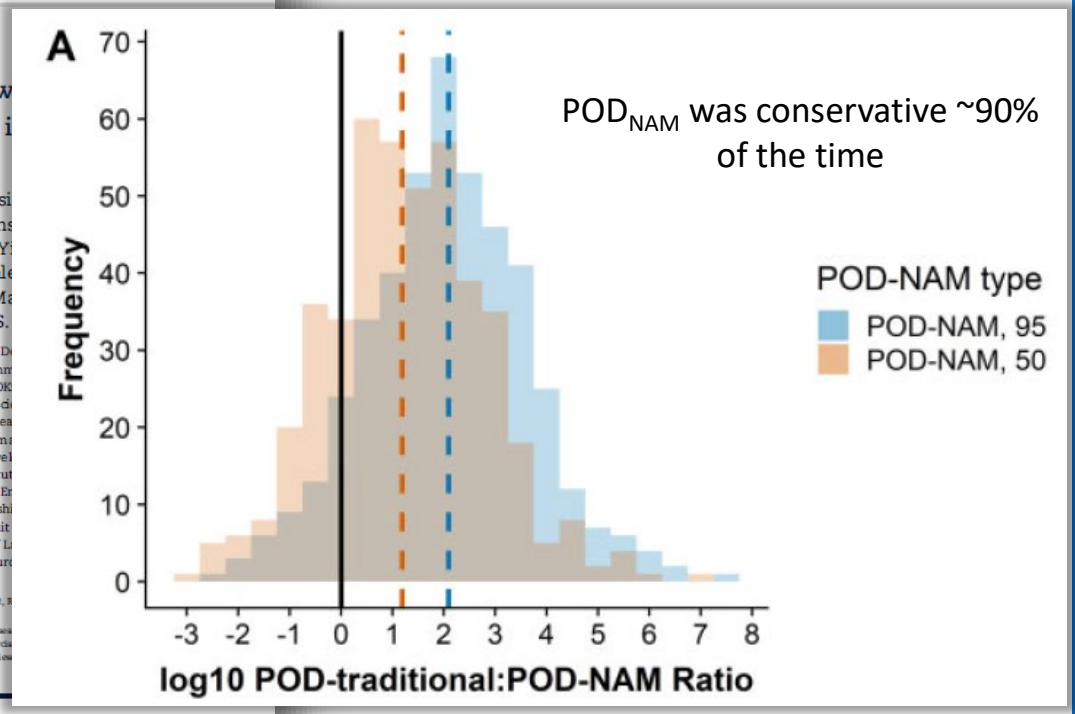
March 2021

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2019, 1–24

Utility of *In Vitro* Bioactivity as a Low
of *In Vivo* Adverse Effect Levels and i
Prioritization

Katie Paul Friedman ,*,1 Matthew Gagne,† Lit-Hsi
Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobans
M. Richard,* Ryan R. Lougee,*,|| Andrea Gissi,§ Jia-Y
Angrish,¶ Jean Lou Dorne,|| Stiven Foster,# Kathl
Bahadori,|| Maureen R. Gwinn,* Jason Lambert,* Ma
Rasenberg,§ Tara Barton-Maclaren,† and Russell S.



POD_{NAM} was conservative ~90% of the time

ABSTRACT

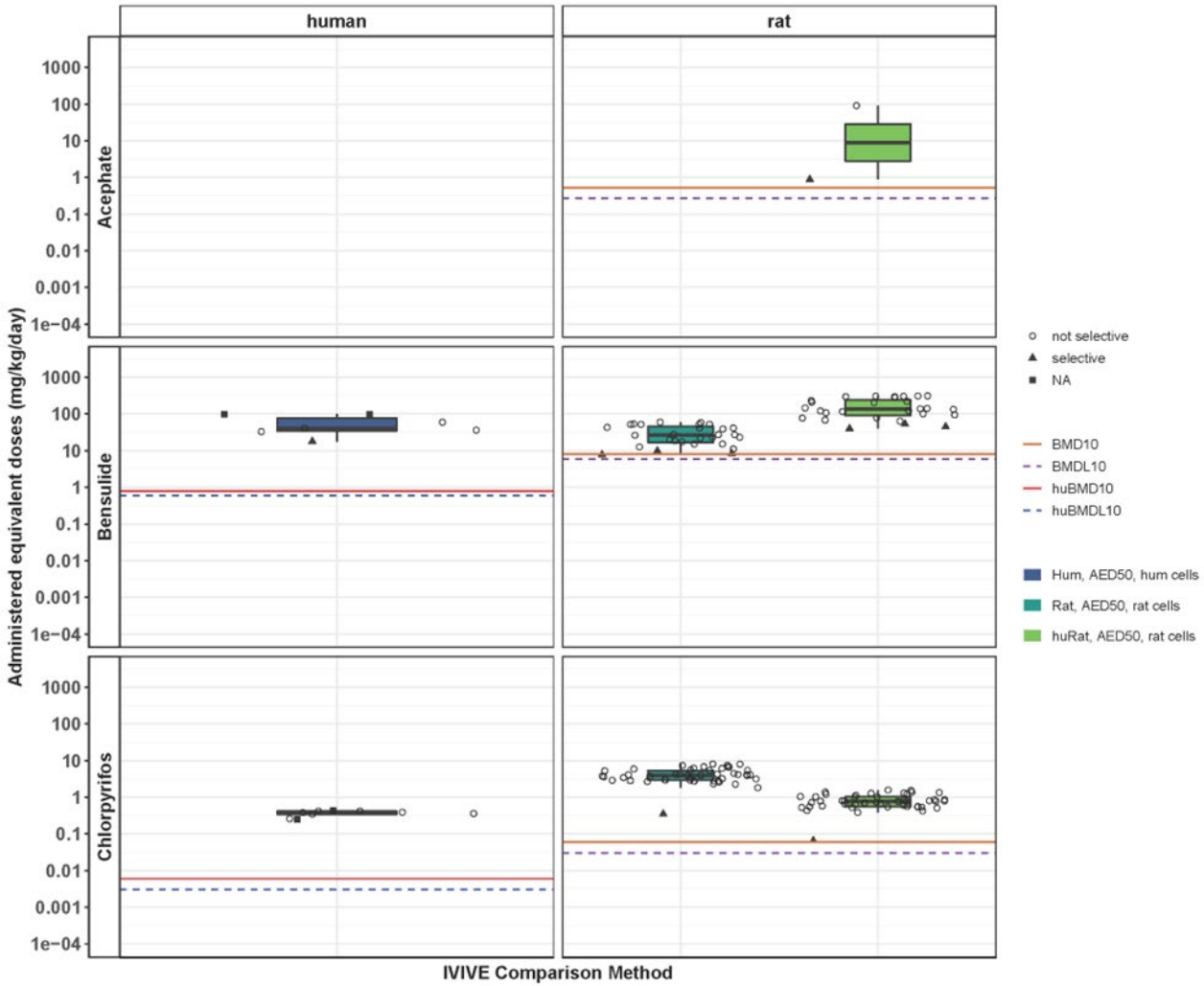
Use of high-throughput, in vitro bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health safety evaluation by informing screening-level assessments. The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals. PODs derived from new approach methodologies (NAMs) were obtained for this comparison using the 50th ($POD_{NAM,50}$) and the 95th ($POD_{NAM,95}$) percentile credible interval estimates for the steady-state plasma

Published by Oxford University Press on behalf of the Society of Toxicology 2019.

1

10.1093/toxsci/kfz201

For developmental neurotoxicity, we examined AED50 values vs. the POD for acute neurotoxicity



- Similar to a BER, we looked for the DNT:acute ratio
- For many organophosphates, DNT NAM bioactivity occurred at AEDs that were greater than the POD associated with neurotoxicity

https://www.regulations.gov/docket/EPA-HQ-OPP-2020-0263

Conclusions for endocrine assessment and BER

- Always understand the chemical and its properties first
- Look to use models that integrate multiple descriptors over single assay endpoint data because no assay is perfect
- Single assay data can be examined – look at the curves
- Endeavor to make hypotheses or statements about what appears plausible, with greater certainty of possible activity when multiple assays confirm
- Can also look to include *in vivo* hazard data when available
- Calculation of a BER may help with understanding the priority for further examination of endocrine activity
- BER can help with many different types of regulatory toxicology questions

The ToxCast Team

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- *Lead, tcplfit2 Development and Statistics*

Carter Thunes
- *tcpl Development and Pipelining*
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- *SME, ToxCast Project Lead*

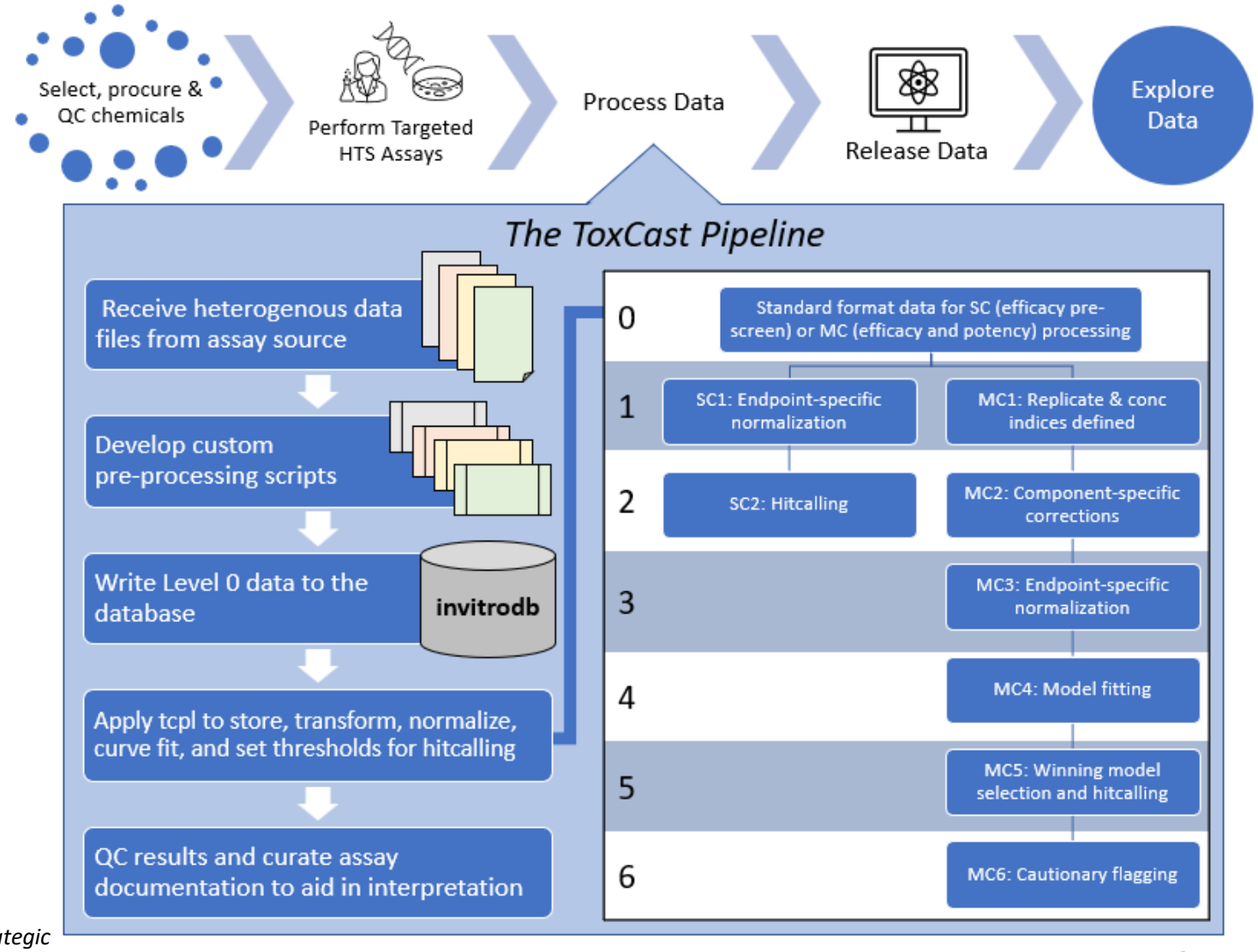
Richard Judson
- *SME*

Colleen Elonen
- *Project Liaison*

Additional Appendices for ToxCast Data and Retrieval

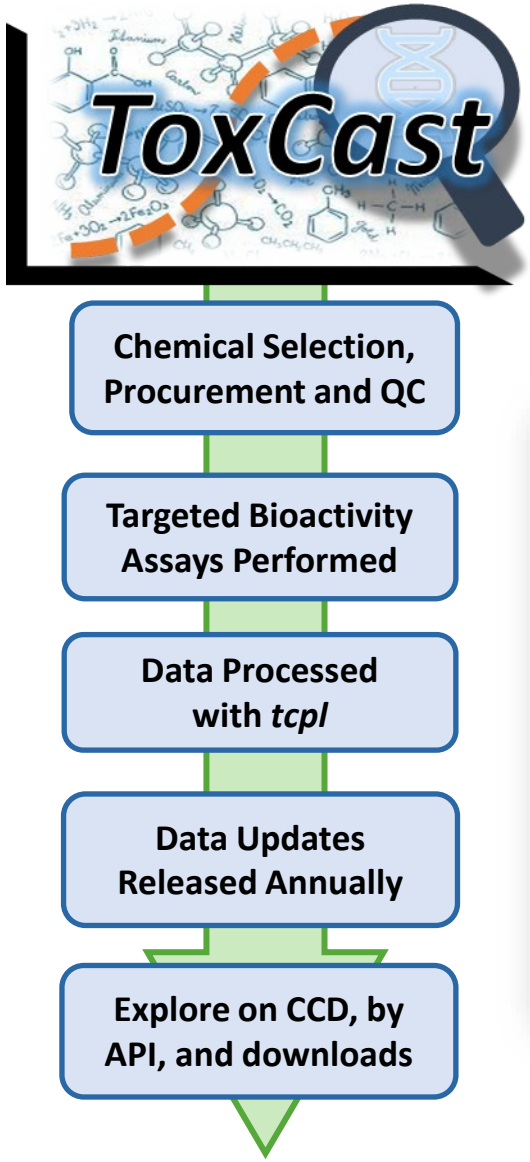
Process Overview

- EPA will **Select, Procure, and QC chemicals,** then ship the chemicals to different *assay sources*, such as EPA labs, contract vendors, or other partners*, who **Perform Targeted HTS Assays**
- Heterogeneous assays = heterogenous assay readouts
- Once the output files are received from the assay source, ToxCast team can **Process Data** with tcpl
  - The ToxCast Pipeline (tcpl) R software package to populate its linked MySQL database, invitrodb
  - Tcpl is a flexible analysis pipeline capable of processing and storing large volumes of data in addition to all processing decisions and metadata
- After additional QC and curation, the ToxCast team **Release data** annually via the ToxCast Downloadable Data page
- EPA & the public can **Explore data** through data downloads or via the CompTox Chemicals Dashboard

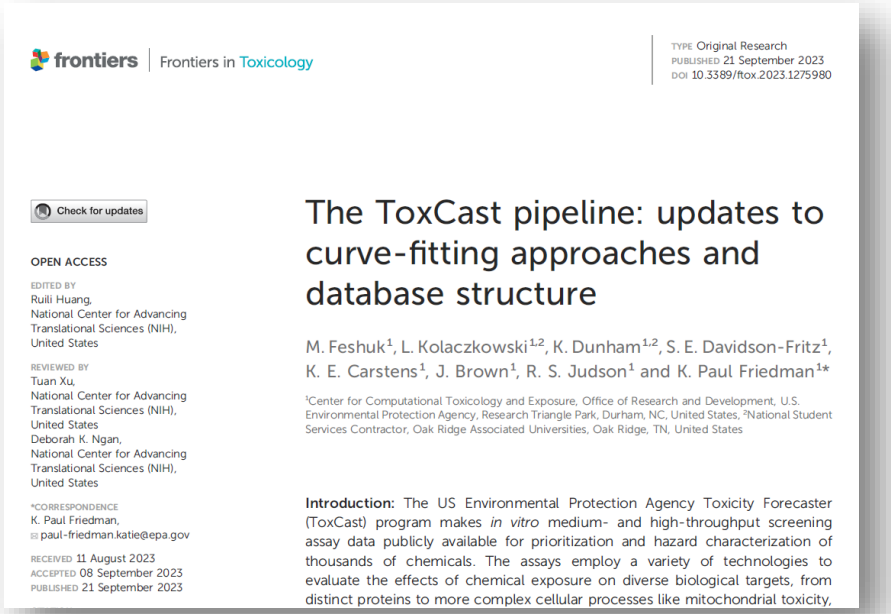


**initiated via Material Transfer Agreements (MTAs) according to the Agency's strategic research needs under Chemical Safety for Sustainability (CSS) Research Program*

ToxCast: Accessible bioactivity data for toxicology with big updates for invitrodb v4.1



The ToxCast program makes targeted in vitro screening assay data publicly available for prioritization and hazard characterization.



frontiers | Frontiers in Toxicology

TYPE Original Research
PUBLISHED 21 September 2023
DOI 10.3389/ftox.2023.1275980

Check for updates

OPEN ACCESS

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PUBLISHED 21 September 2023

The ToxCast pipeline: updates to curve-fitting approaches and database structure

M. Feshuk[1], L. Kolaczkowski[1,2], K. Dunham[1,2], S. E. Davidson-Fritz[1], K. E. Carstens[1], J. Brown[1], R. S. Judson[1] and K. Paul Friedman[1]*

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Introduction: The US Environmental Protection Agency Toxicity Forecaster (ToxCast) program makes *in vitro* medium- and high-throughput screening assay data publicly available for prioritization and hazard characterization of thousands of chemicals. The assays employ a variety of technologies to evaluate the effects of chemical exposure on diverse biological targets, from distinct proteins to more complex cellular processes like mitochondrial toxicity,

10.3389/ftox.2023.1275980

- Data needs in next generation risk assessment necessitated software and database updates for consistent and reproducible curve-fitting and data management across screening efforts.
- Updates include additional models, bidirectional curve-fitting, and continuous hit calling.
- Annotation structure, fit categories, and cautionary flags on curve-fitting behavior were modified for future invitrodb v4.1 release.
- Curve-fitting updates resulted in small changes in activity hit calls and potency estimates but without a uniform trend.

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

Invitrodb version 4.1 (released August 2022) contains 26 different assay sources that have been pipelined into nearly 1500 assay endpoints. > 500 target gene annotations have been applied, though not all endpoints can map to a single target.

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)
ARUNA	ArunA Biomedical	Human H9- derived embryonic stem cell models of neurodevelopment processes including neuronal migration	Cell cycle, neurodevelopment, neurovascular unit
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/cardiovascular responses
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)
LTEA	Life Tech Expression Analysis	Gene expression measured in HepaRG cells following 48 hr exposure	Liver toxicity model via transcription factor regulated-metabolism and markers of oxidative/cell stress; multiple assay endpoints

Assay sources (continued)

Assay source	Long name	Truncated assay source description	Some rough notes on the biology covered
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)
STM	Stemina	Stem cell-based metabolomic indicator of developmental toxicity for screening.	Developmental toxicity screening – multiple assay endpoints
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
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
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)
VALA	VALA Sciences	High content screening of cell-based models of development, neurodevelopment, and viability	Development, neurodevelopment, wound recovery

With each release, more assay endpoints and more chemical x endpoint data are released

Some assay sources are "in-house" in the EPA Office of Research and Development (subset shown here). Many of these targeted assays were developed to fill specific data gaps of interest.

Assay source	Truncated assay source description	Some rough notes on the biology covered
CCTE_DEISENROTH_5AR, AIME, DEVTOX	Targeted assays for androgen, estrogen + metabolism (AIME), and developmental toxicity	Endocrine/development https://doi.org/10.3390/toxics10070392 https://doi.org/10.1093/toxsci/kfac019
CCTE_GLTED	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 (many human and xenopus thyroid-related molecular targets) https://doi.org/10.1093/toxsci/kfy302
CCTE_PADILLA_ZF_TERATOSCORE	The Padilla laboratory at the EPA National Health and Environmental Effects Research Laboratory focuses on the development and screening of zebrafish assays.	Zebrafish terata https://doi.org/10.1016/j.reprotox.2011.10.018
CCTE_MUNDY_HCI	High content imaging of neurodevelopmental processes in cell-based models of neurodevelopment	Neurodevelopment https://doi.org/10.1016/j.taap.2018.04.001
CCTE_Shafer_MEA_acute, MEA_dev	Neuronal network function and/or development in neuronal cell models on microelectrode arrays (i.e., electrical function)	Neuroactivity, neurodevelopment (function) https://doi.org/10.1007/s00204-019-02636-x https://doi.org/10.1093/toxsci/kfac018
CCTE_SIMMONS_MITO	Respirometric assay that measure mitochondrial function in HepG2 cells	Multiple assay endpoints to evaluate mitochondrial function https://doi.org/10.1093/toxsci/kfaa059.
CPHEA_STOKER_NIS	High-throughput assay system for the the sodium-iodide cotransporter (NIS).	Endocrine (thyroid - NIS inhibition) https://doi.org/10.1007/s00204-021-03006-2

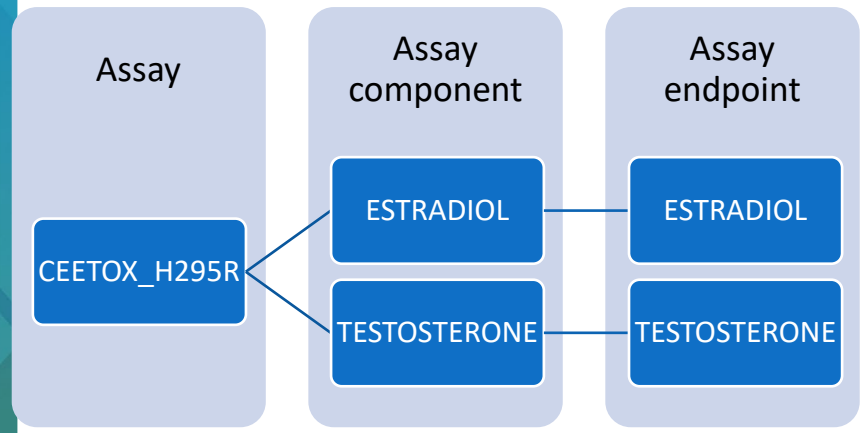
CompTox Chemicals Dashboard (CCD)

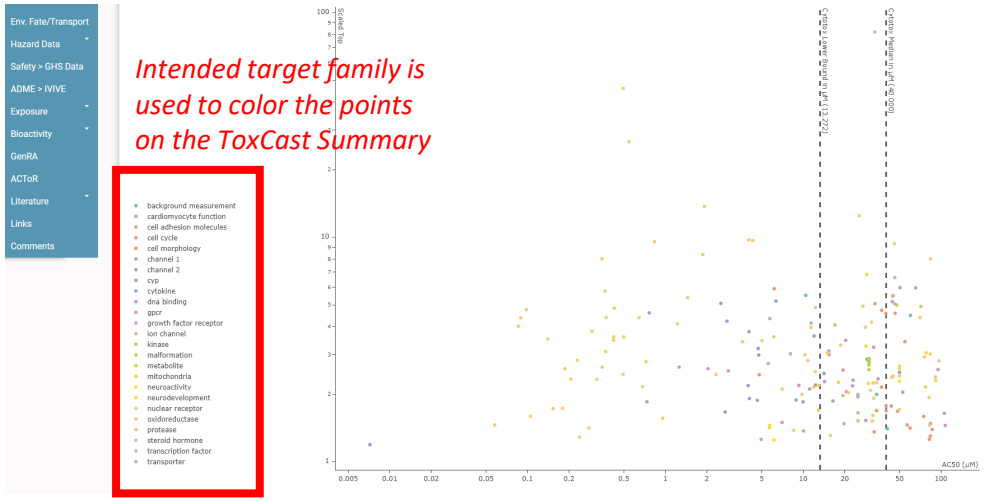
https://comptox.epa.gov/dashboard

- CCD's ToxCast bioactivity module presents a view of potency and relative efficacy metrics across ToxCast endpoints for chemicals of interest
- Users can easily sort, filter, and export ToxCast results and assay descriptions
- Notable updates in the CCD v2.3 release (December 2023) include:
  - Data was refreshed to invitrodb v4.1
  - ToxCast Summary tab is now a single tab that combines the previous ToxCast Summary and ToxCast Conc. Response tabs
  - Bioactivity Summary Grid includes v4.1 information in new columns, including benchmark dose (BMD), benchmark response (BMR), and Continuous Hitcall
- *Example on right: Bisphenol A*
  https://comptox.epa.gov/dashboard/chemical/invitrodb/DTXSID7020182

Single assay endpoint data are findable on the CCD

Example assay annotation hierarchy





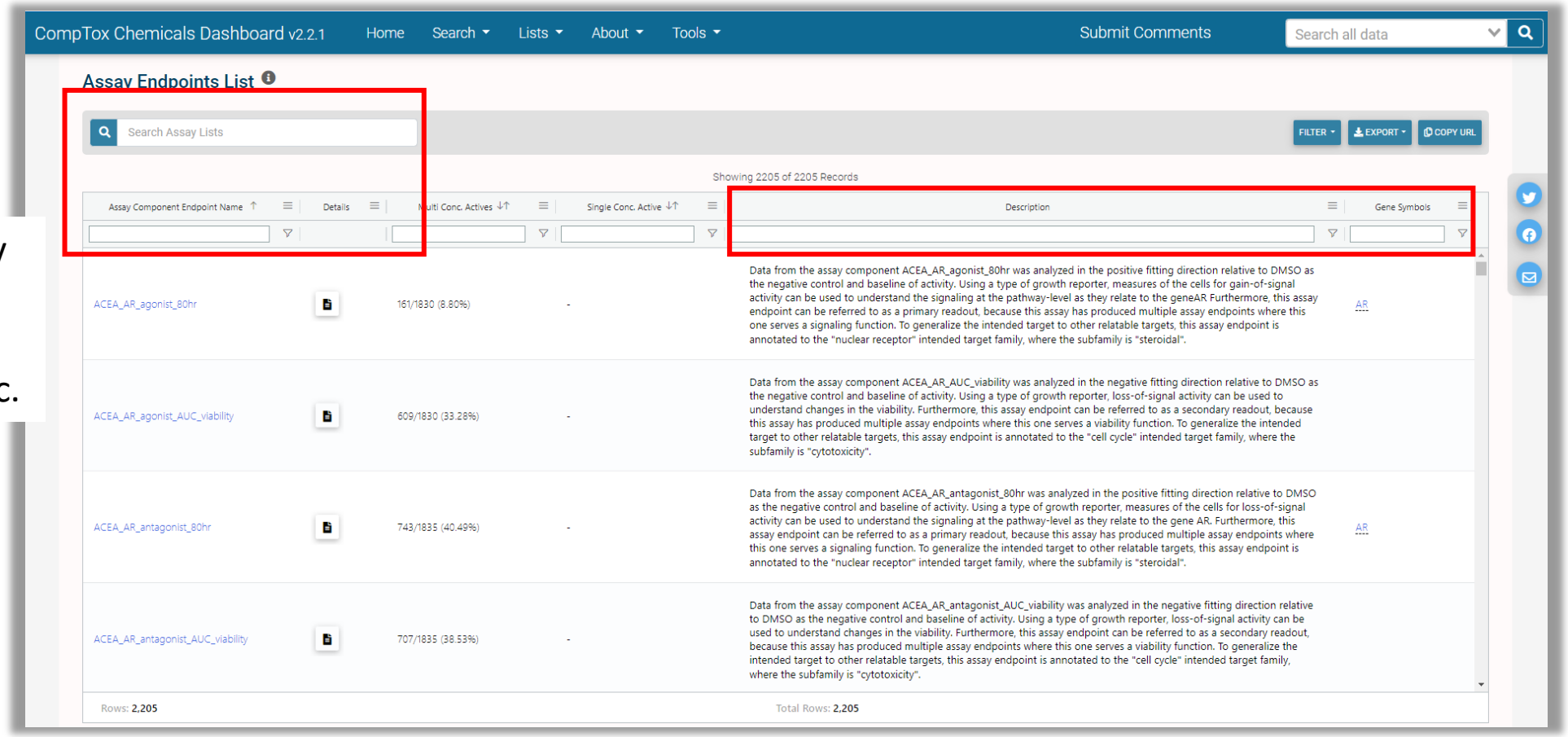
- Invitrodb v4.1 has 1485 assay endpoints
- At least one data point for 10,196 chemicals
- Many assay endpoints are mapped to a gene, if applicable
- Assay endpoints now cover 539 unique annotated genes
- Intended target family is one way to understand biological target (incomplete list here):
  - Apolipoprotein
  - Apoptosis
  - Background measurement
  - Catalase
  - Cell adhesion
  - Cell cycle
  - Cell morphology
  - CYP
  - Cytokine
  - Deiodinase
  - DNA binding
  - Esterase
  - Filaments
  - GPCR
  - Growth factor
  - Histones
  - Hydrolase
  - Ion channel
  - Kinase
  - Ligase
  - Lyase
  - Malformation (zebrafish)
  - Membrane protein
  - Metabolite (Stemina metabolomics)
  - Mitochondria
  - Methyltransferase
  - microRNA
  - Mutagenicity response
  - Neuroactivity/DNT
  - Nuclear receptor
  - Oxidoreductase
  - Phosphatase
  - Protease/inhibitor
  - Steroid hormone
  - Transferase
  - Transporter

https://comptox.epa.gov/dashboard/assay_endpoints/

Download summary information here: https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data

ToxCast data are publicly accessible from the CompTox Chemicals Dashboard

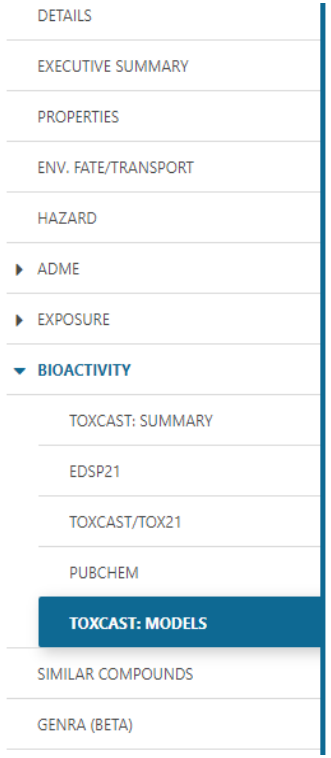
Search by gene, vendor name, etc.



Many users are accustomed to viewing data per substance (as identified by a DSSTox identifier, or DTXSID), but you can also identify assay endpoint data by entering from Lists > Lists of Assays. These data can be exported after loading the data for the assay.

https://comptox.epa.gov/dashboard/assay-endpoints

Model scores as available in the CompTox Chemicals Dashboard in addition to the publications



ToxCast: Models

ToxCast Model Predictions

Download ToxCast Model Predictions

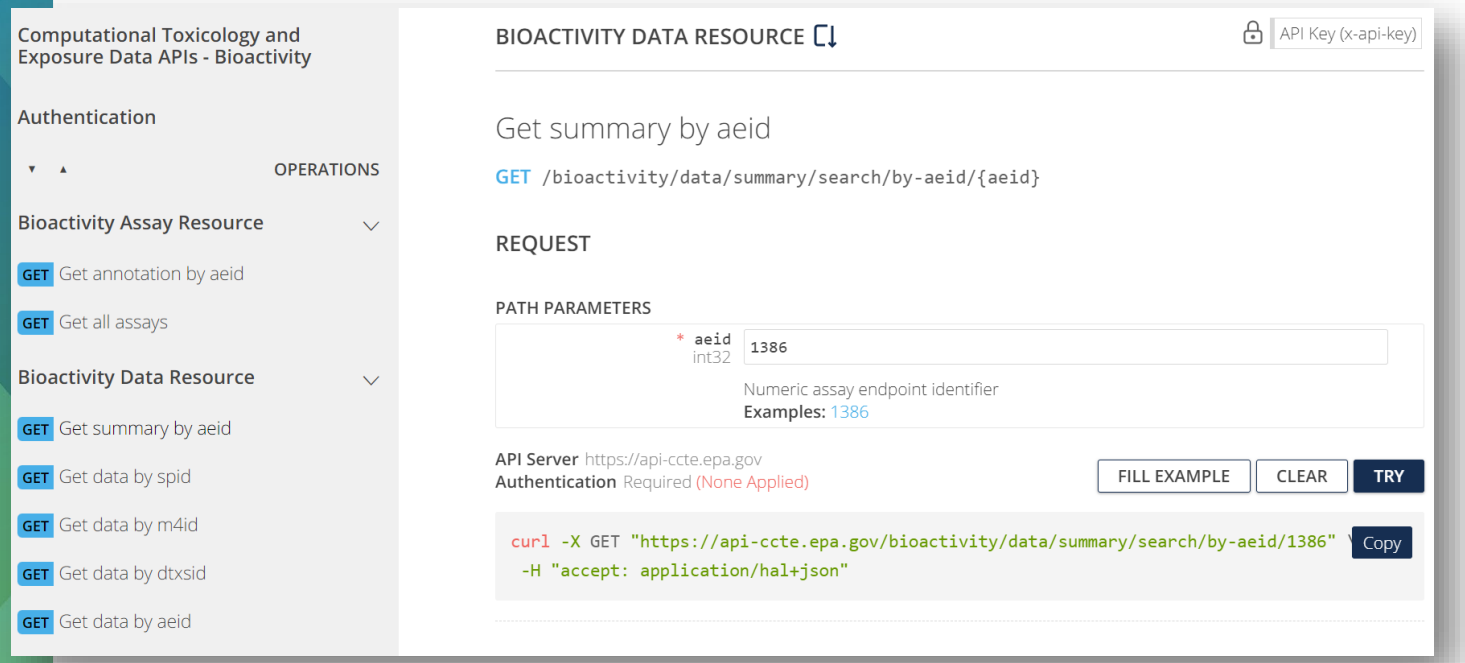
>0.1 = positive; 0.001-0.1 = equivocal

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)

ToxCast Pathway Model AUC ER = full ER model (18 assays)
ToxCast Pathway Model AUC AR = full AR model (11 assays)
CERAPP = consensus ER QSAR (from 17 groups)
COMPARA = consensus AR QSAR

Application Programming Interfaces (APIs)

https://api-ccte.epa.gov/docs/bioactivity.html

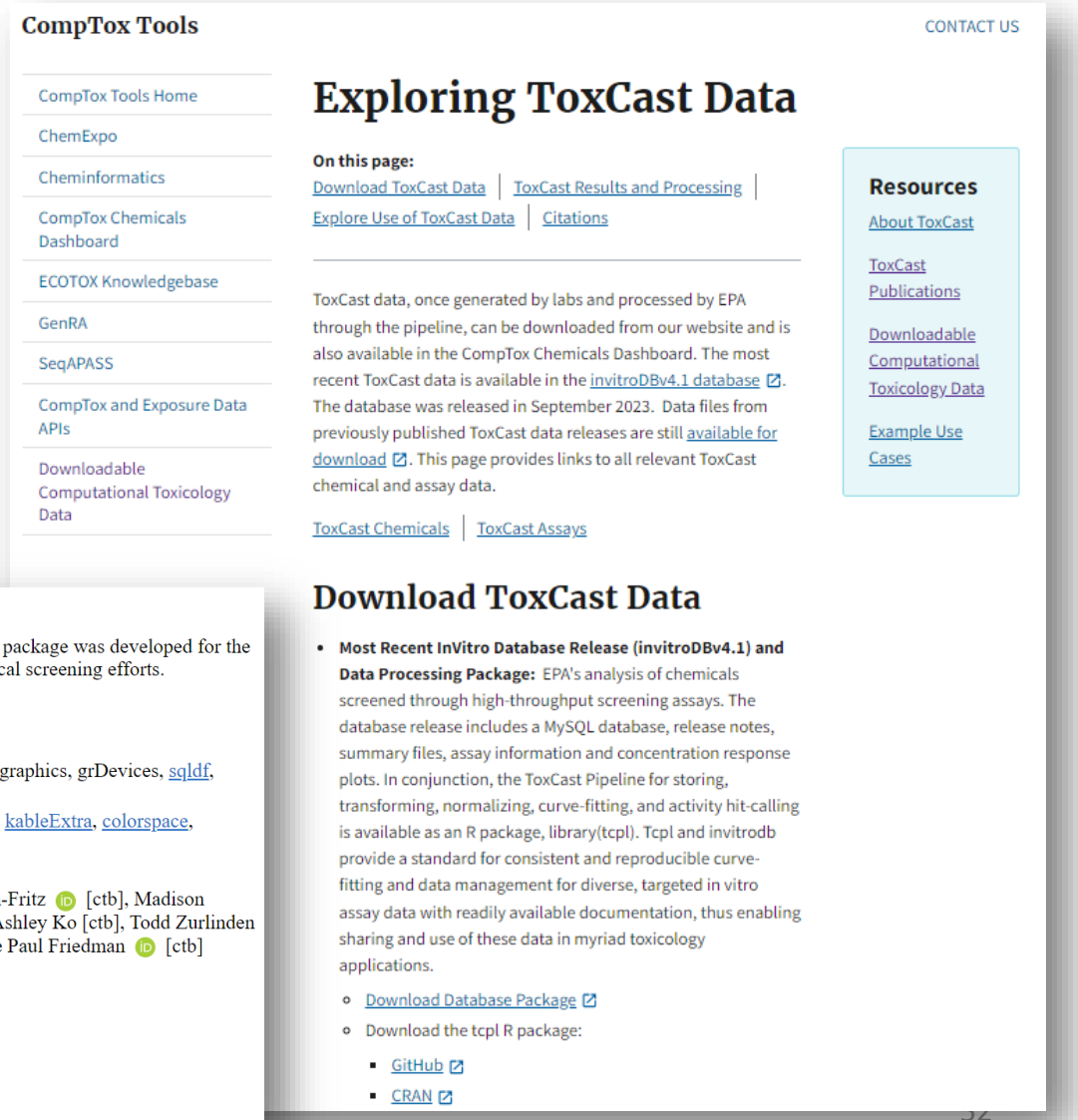


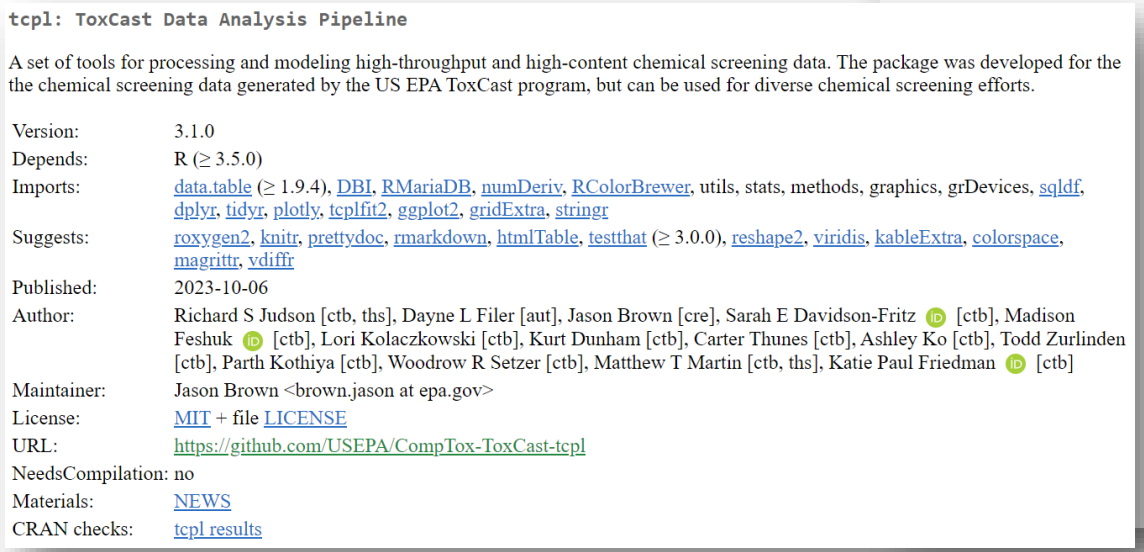
- APIs provide data for various use cases, including research and applications with user interfaces
- Users can avoid large data downloads by accessing invitrodb programmatically via an API
- This is a great read-only solution for users who require more flexibility than the CCD can provide
- More integration with tcpl is coming soon and for additional documentation, check out the CCTE API Home Page: https://api-ccte.epa.gov/docs/index.html

ToxCast Data Downloads

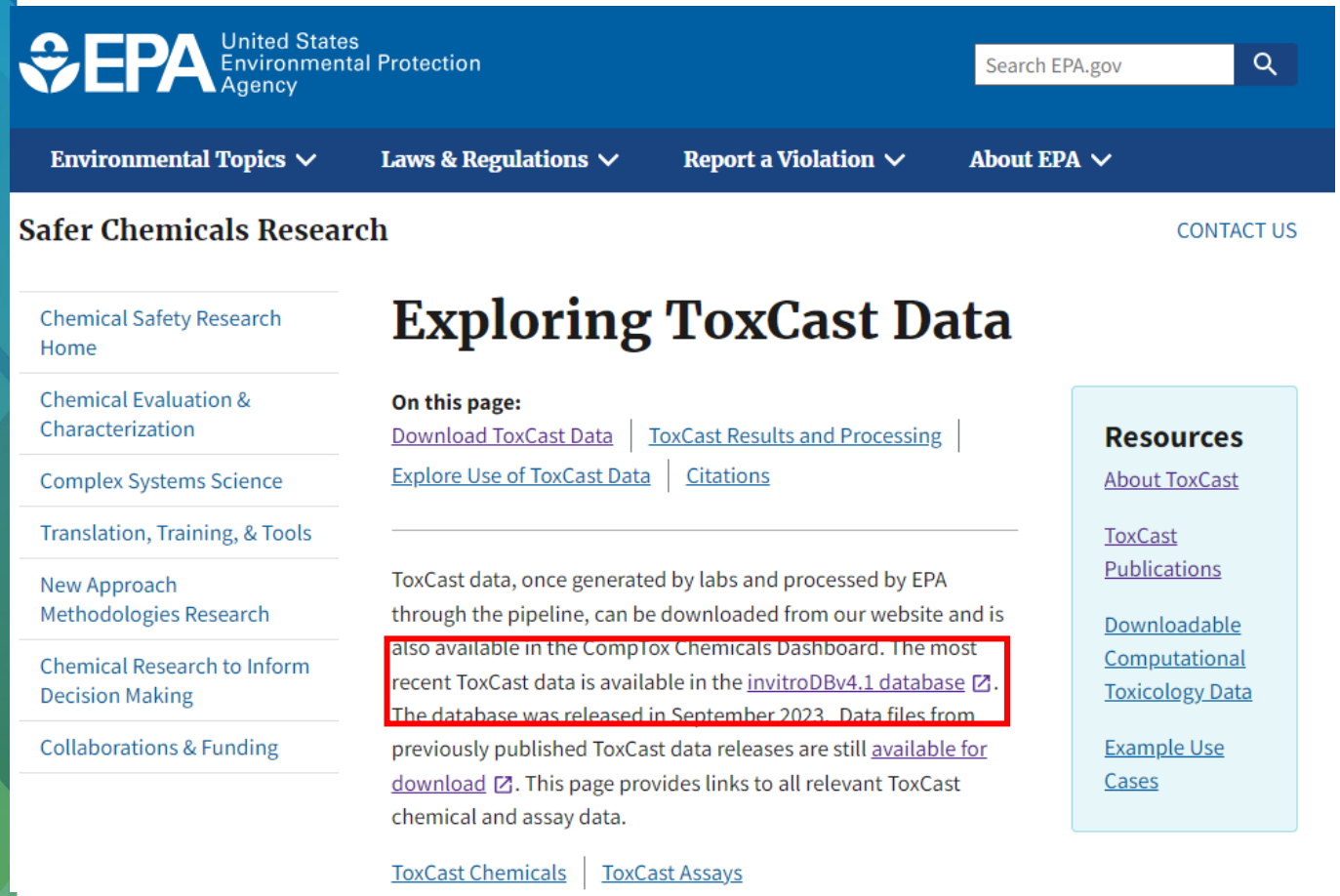
https://www.epa.gov/comptox-tools/exploring-toxcast-data

- Data downloads allow users to set up their own personal instance of the invitrodb MySQL database and interact with the data directly via the tcpl R package.
- This is a preferred option for more customized or programmatic ToxCast data needs, or if users want to do their own data processing.





More specifics on downloading



https://www.epa.gov/chemical-research/exploring-toxcast-data#Download

- Download “Summary_Files” .zip with README

Locate by experimental design features

- See “assay_annotation_information_invitrodbV4_1_SEPT2023” in the file format of your preference
- Locate assays by *organism*, *tissue*, *cell format*, *cell line*, *cell growth*, *detection technology*, *intended target family*, etc.

Locate by gene mappings

- See “assay_gene_mappings_invitrodb_v4_1_SEPT2023” in the file format of your preference
- Locate assays by nearest relevant *gene*(s) annotated for the target of the assay (if available)