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# Overview of the CompTox Chemicals Dashboard and ToxCast/Tox21 Screening Program: Tools for Users

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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*

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

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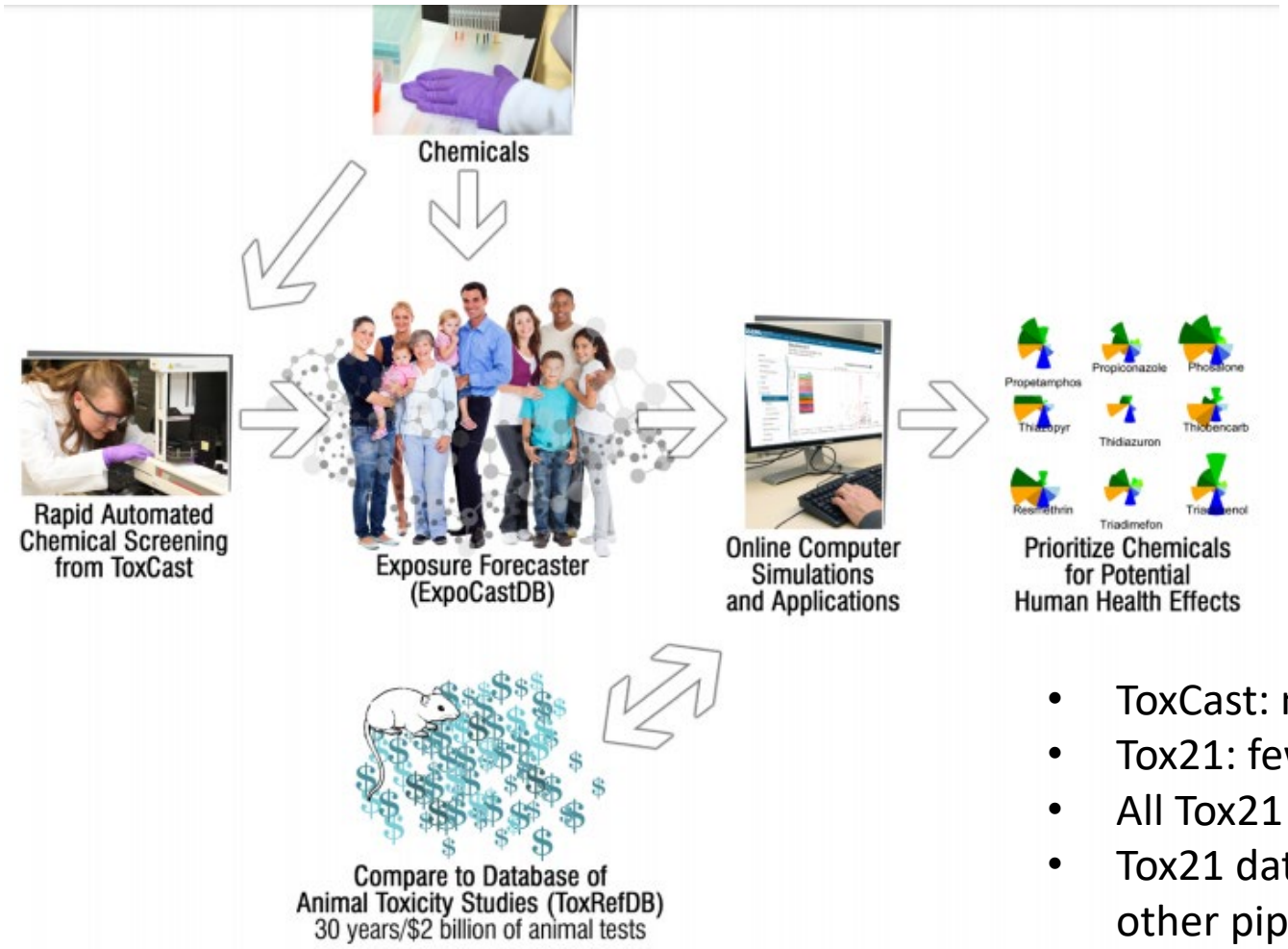
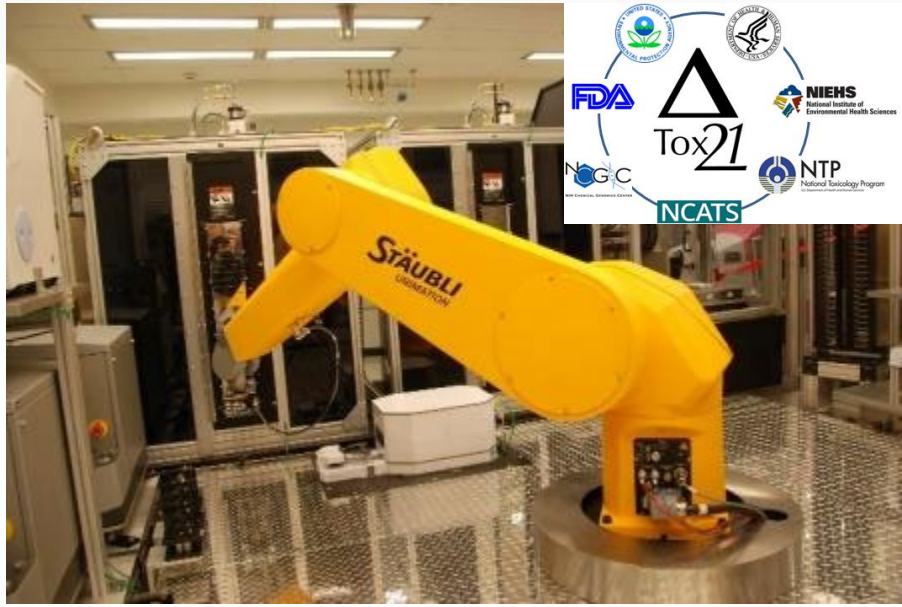
**EPA's ToxCast program at a glance**




**Tox21 robot**

- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, mostly 1536, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline and other pipelines as well

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

United States Environmental Protection Agency

*Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 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) |
| 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) |
| 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) |

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

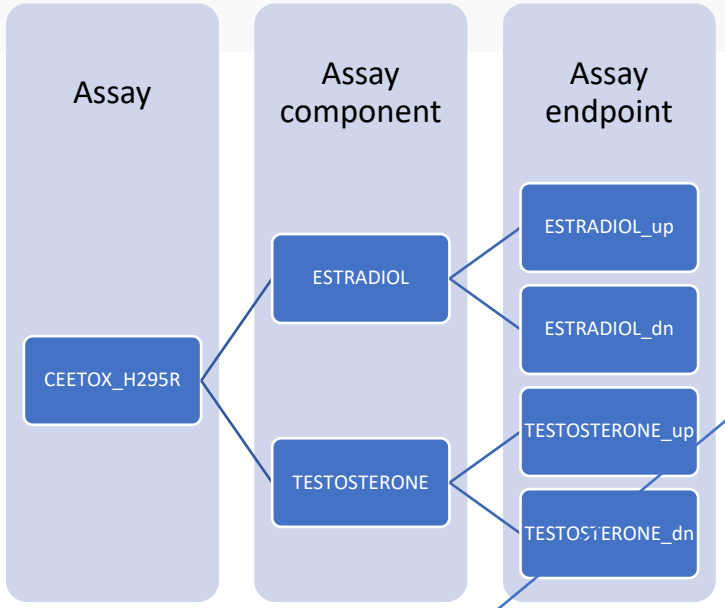
*Invitrodb version 3.3 (released August 2020) contained 17 different assay sources, covering (at least) 491 unique gene-related targets with 1600 unique assay endpoints. Varying amounts of data are available for 9949 unique substances.*

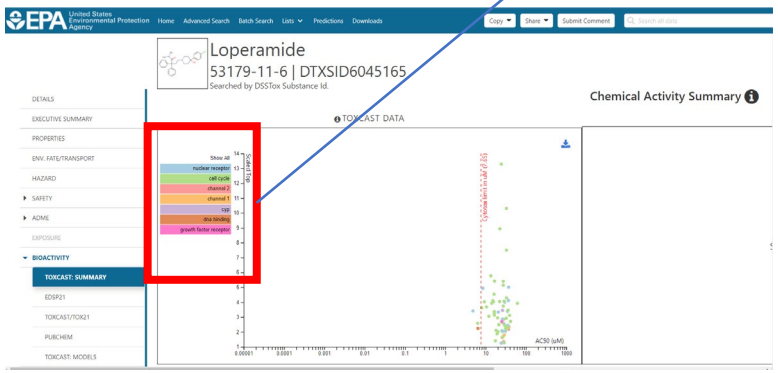
*These assay endpoints were notable additions in invitrodb version 3.3.*

| Assay source | Long name | Truncated assay source description | Some rough notes on the biology covered |
|---|---|---|---|
| NCCT_MITO | NCCT (now Center for Computational Toxicology and Exposure) Mitochondrial toxicity | Respirometric assay that measure mitochondrial function in HepG2 cells | Multiple assay endpoints to evaluate mitochondrial function https://doi.org/10.1093/toxsci/kfaa059. |
| 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 – hDIO1,2,3 inhibition) https://doi.org/10.1093/toxsci/kfy302 |
| STM | Stemina | Stem cell-based metabolomic indicator of developmental toxicity for screening. | Developmental toxicity screening – multiple assay endpoints https://doi.org/10.1093/toxsci/kfaa014 |
| 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 |

# Learning more about the assay endpoints and biology

Example assay annotation hierarchy




- Many assay endpoints are mapped to a gene, if applicable
- Assay endpoints now cover 1398 unique gene targets in invitrodb version 3.3, in addition to other processes
- 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
  - 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

# What can be done with ToxCast data?

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## Answering biological questions

- (*for example*) Does this substance have endocrine or liver-mediated bioactivity?
- Is there support for one or more adverse outcome pathways based on these data, or does the substance appear “non-selective?”

## Answering risk-related questions

- Can a protective bioactivity-based point-of-departure be calculated?
- What is the relative priority of this substance for additional evaluation?

# Using ToxCast Data in Weight of Evidence or Screening Level Assessment

- Vignette 1: Weight of evidence example
- Vignette 2: Risk-based approach that incorporates bioactivity and exposure, making the best use of new approach methodologies, for endocrine bioactivity.


*This presentation will demonstrate where to find these information and suggest an approach for utilizing them in screening level risk evaluation.*

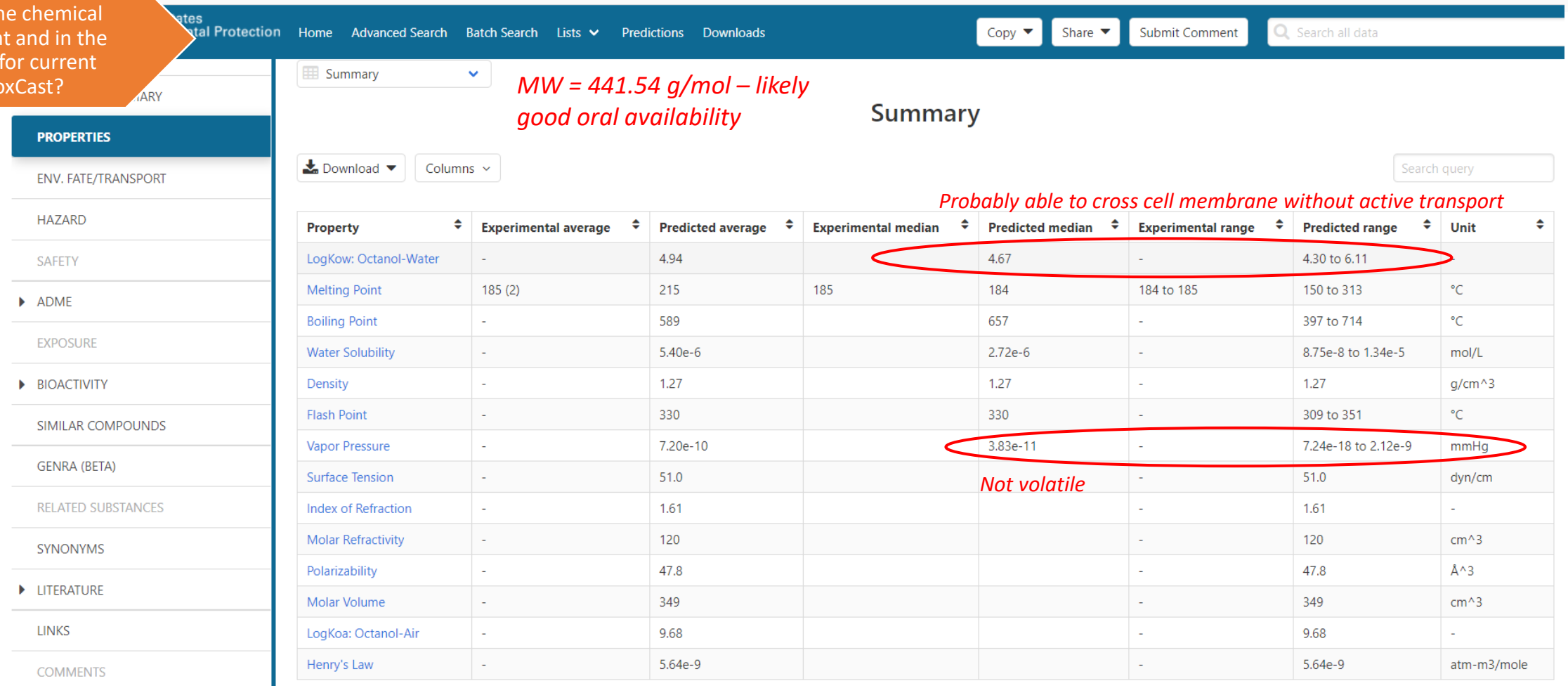
EPA United States Environmental Protection Agency

# Vignette one: bioactivity for weight-of-evidence/biological questions

Is mystery compound A toxic to liver and/or mitochondria?

# Mystery compound A: in domain of current screening?

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




| Property | Experimental average | Predicted average | Experimental median | Predicted median | Experimental range | Predicted range | Unit |
|---|---|---|---|---|---|---|---|
| LogKow: Octanol-Water | - | 4.94 | | 4.67 | - | 4.30 to 6.11 | |
| Melting Point | 185 (2) | 215 | 185 | 184 | 184 to 185 | 150 to 313 | °C |
| Boiling Point | - | 589 | | 657 | - | 397 to 714 | °C |
| Water Solubility | - | 5.40e-6 | | 2.72e-6 | - | 8.75e-8 to 1.34e-5 | mol/L |
| Density | - | 1.27 | | 1.27 | - | 1.27 | g/cm^3 |
| Flash Point | - | 330 | | 330 | - | 309 to 351 | °C |
| Vapor Pressure | - | 7.20e-10 | | 3.83e-11 | - | 7.24e-18 to 2.12e-9 | mmHg |
| Surface Tension | - | 51.0 | | | - | 51.0 | dyn/cm |
| Index of Refraction | - | 1.61 | | | - | 1.61 | - |
| Molar Refractivity | - | 120 | | | - | 120 | cm^3 |
| Polarizability | - | 47.8 | | | - | 47.8 | Å^3 |
| Molar Volume | - | 349 | | | - | 349 | cm^3 |
| LogKoa: Octanol-Air | - | 9.68 | | | - | 9.68 | - |
| Henry's Law | - | 5.64e-9 | | | - | 5.64e-9 | atm-m3/mole |

# Mystery compound A seems to fit into the domain of screening based on chemistry

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

States
ental Protection | Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Copy | Share | Submit Comment | Search all data

Searched by DSSTox Substance Id.

- PROPERTIES
- ENV. FATE/TRANSPORT
- HAZARD
- SAFETY
- ADME
- EXPOSURE
- **BIOACTIVITY**
  - TOXCAST: SUMMARY
  - EDSP21
  - **TOXCAST/TOX21**

## ToxCast/Tox21

*Select samples that were analyzed (the chemical in DMSO stock) are high purity and confirmed*

| QC Data ID | Grade | Description |
|---|---|---|
| Tox21_112119 | Pass | Purity>90% and MW confirmed |
| Tox21_112119_1 | Pass | Purity>90% and MW confirmed |
| Tox21_300470 | Pass | Purity>90% and MW confirmed |

Assay Selection **0 Selected** | **A Single Assay Can Have Multiple Charts** | **Representative Samples Only** | Bioactivity Summary | **Number of Charts: 0**

Active | Inac

Filter assays

Tanguay Lab **(0 of 19 s**

Tox21/NCGC **(0 of 235**

NHEERL Mid-Continen

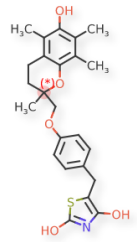
tripod.nih.gov/tox21/samples/Tox21_112119

Apps | Files - OneDrive | toxrefdb - OneDrive | Google Scholar | Altmetric it! | ORD Graphics and... | ORD@Work | Request Research H... | Travel Authorizatio... | Chemtrack | Toxicity Data Collec...

Tox21 | Structure Search | Search...

Home / Tox21 Samples / Tox21_112119

*Seems stable under screening sample conditions (DMSO, room temp, 0-4 months)*



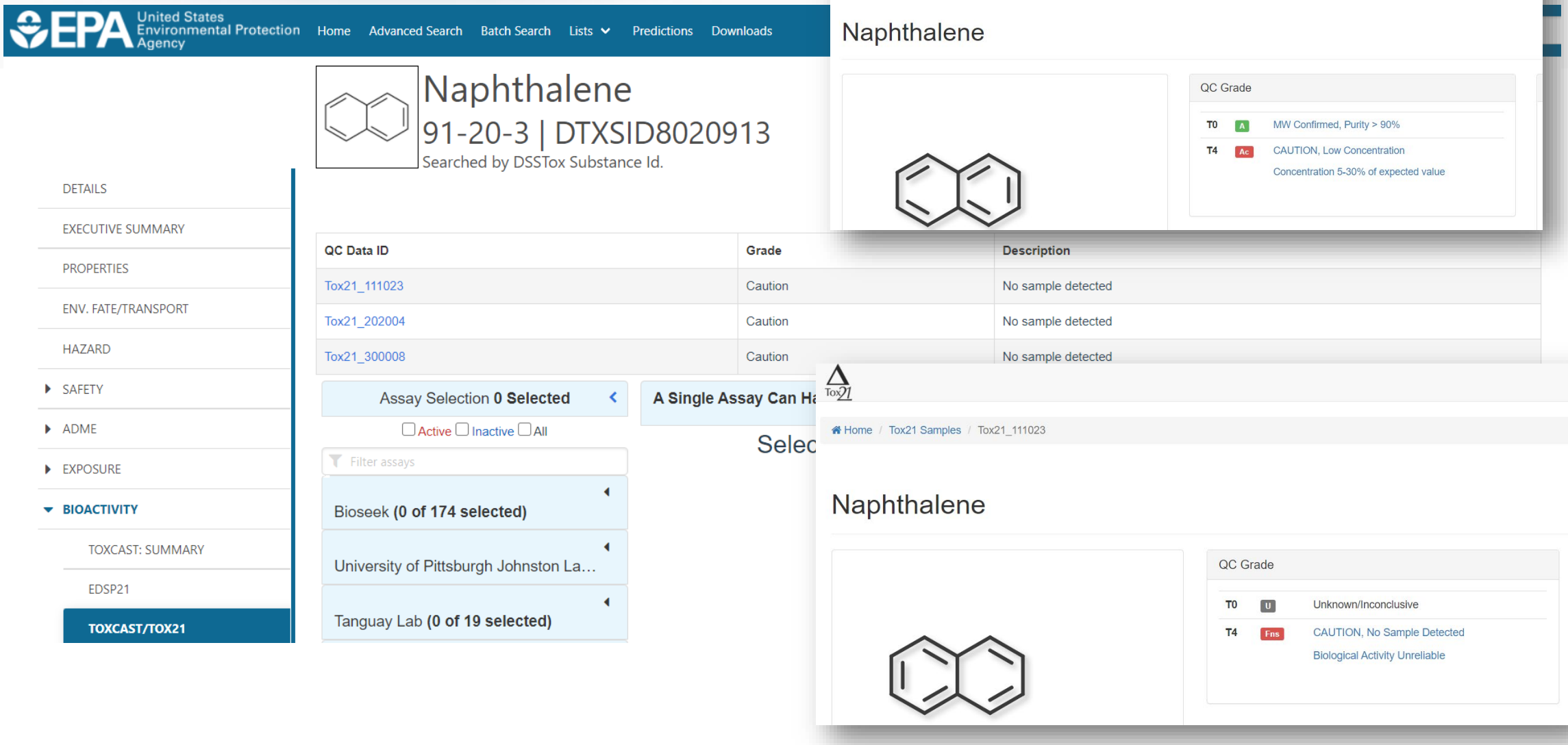
**QC Grade**

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

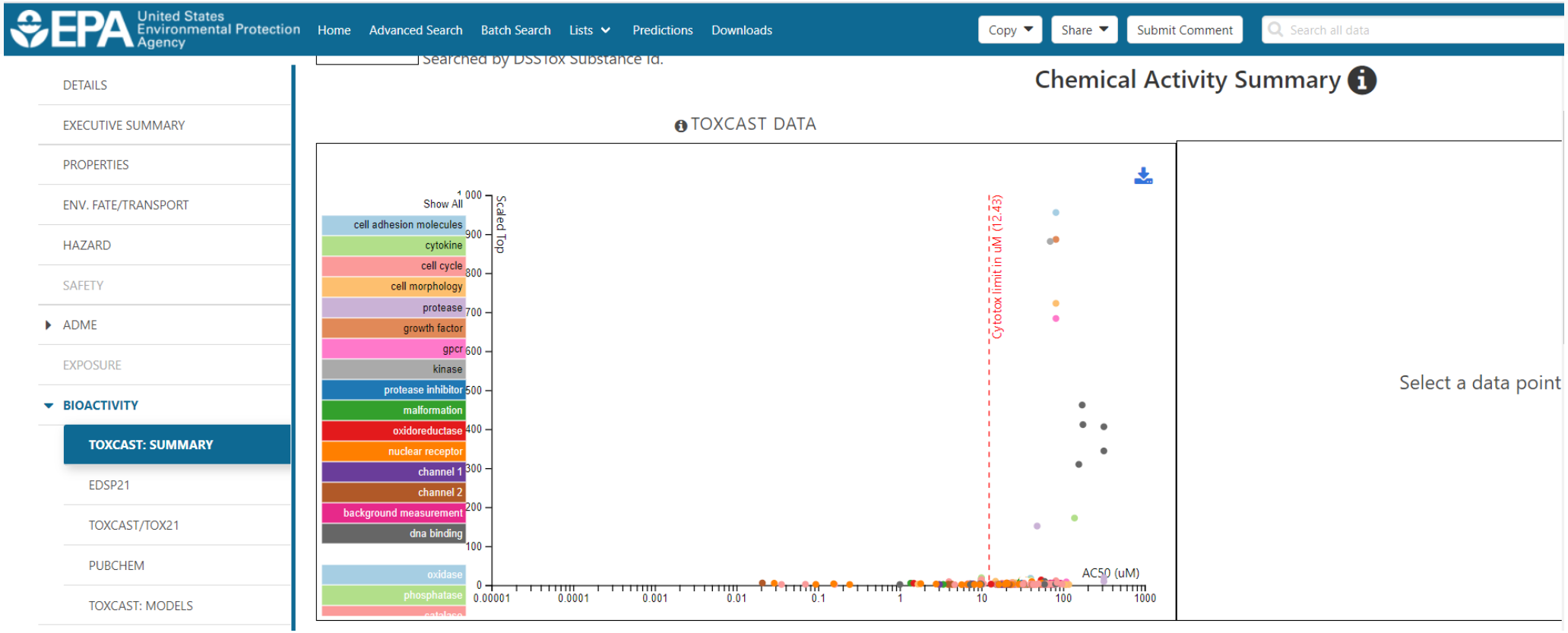
**Identifiers**

| | |
|---|---|
| Tox21 | Tox21_112119 |
| NCATS | NCGC00159457-01 |
| CAS | |
| PubChem | |

# *What is an example of a substance that QC might tip us off we need different NAMs from what is currently in ToxCast?*



# But what bioactivity does Mystery Compound A have?



# Each assay platform or source can be a surrogate for one or more collections of AOPs

Models available?

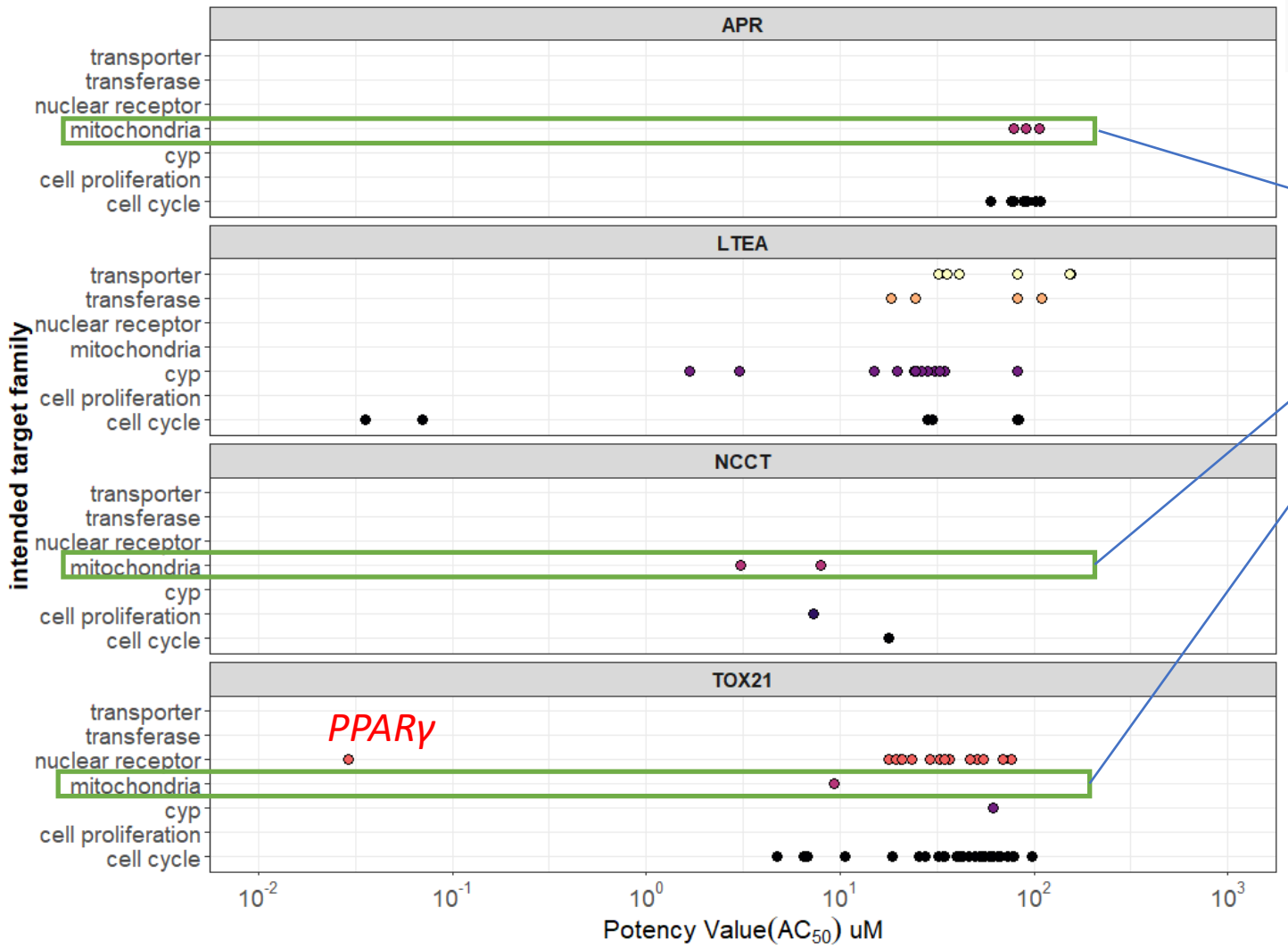
Selective or non-selective?

Consider some of the information that might inform about liver toxicity:

- Mechanistic information on mitochondrial toxicity, oxidative stress, nuclear receptor transcription factor activity, markers of injury in liver-specific models, cell stress and cytotoxicity (inexhaustive listing here):

| Biological process | Assay technologies | Details |
|---|---|---|
| Mitochondrial toxicity | TOX21_MMP | Mitochondrial membrane permeability (HepG2) |
| | NCCT_MITO | Multiple assay endpoints that measure oxygen consumption and respiration via Seahorse; can distinguish mechanism (HepG2) |
| | Apredica MitoMembPot | High content imaging, mitochondrial membrane permeability (HepG2) |
| | Apredica MitoMass | High content imaging, mitochondrial mass (HepG2) |
| Nuclear receptors and oxidative stress | ATG | Transcription factor activity, including nuclear receptor and cell stress panel (CIS by endogenous expression and TRANS by GAL4-NR receptor modules); HG19 subclone of HepG2 cells (for elevated metabolism) |
| | LTEA | mRNA expression in HepaRG for nuclear-receptor regulated metabolism/oxidative stress |
| | CLD | mRNA expression in sandwich-cultured primary human hepatocytes for Phase I-II metabolism and transport |
| | Tox21 NR assays | LUC and BLA nuclear receptor reporter assays |
| | NVS NR and transporter assays | Cell-free binding |
| | Odyssey Thera | Receptor complexes and stabilization of coactivator interaction |
| Cell stress and cytotoxicity | Viability and cell stress assays across platforms | 88+ assays |

# Looking for consistency in MOA and concentration ranges (this is just a subset of assay technologies for demonstration)




Mitochondria:
*Consistency in MOA*
Concentration ranges by technology; the NCCT Seahorse technology suggests 1-10 uM, similar to Tox21 MMP assay

Liver:
*Clearly CYPs, Phase II transferases, and nuclear receptor interactions occuring*
May occur at concentrations greater than mitochondria or cell cycle bioactivity

*Consider reviewing the curves more specifically for a single chemical weight-of-evidence.*

# Mystery substance A: revealed

- Troglitazone
- Treatment for Type II diabetes, works primarily by activating PPARγ
  - Also involved in immune response via decrease in NF-KB
- Drug removed from market due to DILI, with several proposed mechanisms, including:
  - Mitochondrial toxicity [Electron transport chain inhibitor (Complex I) at low micromolar concentrations]
  - Inhibits of bile acid transport/cholestatic effects (e.g., BSEP)
  - Apoptosis
  - Formation of reactive metabolites/oxidative stress

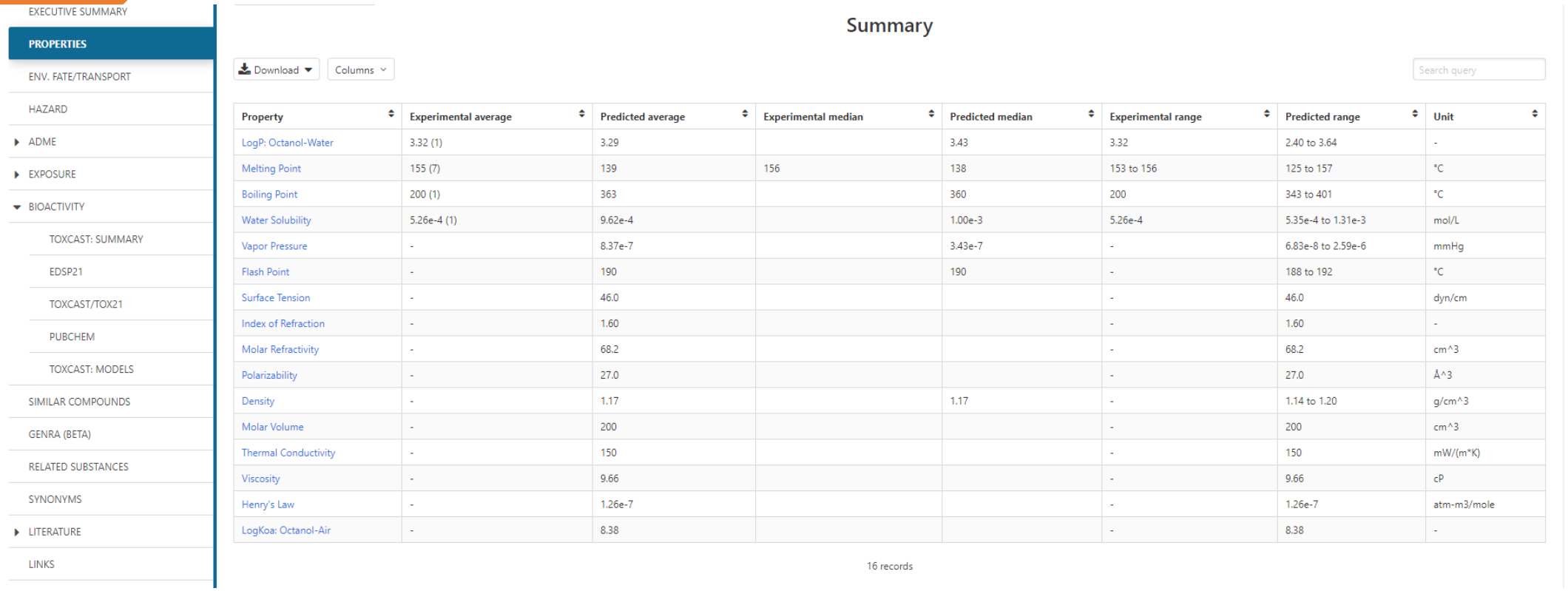
# Vignette two: Screening-level endocrine bioactivity assessment

Evaluate mystery compound B for endocrine bioactivity risk

# 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)?




## Summary

Download | Columns | 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-m3/mole |
| LogKoa: Octanol-Air | - | 8.38 | | | - | 8.38 | - |

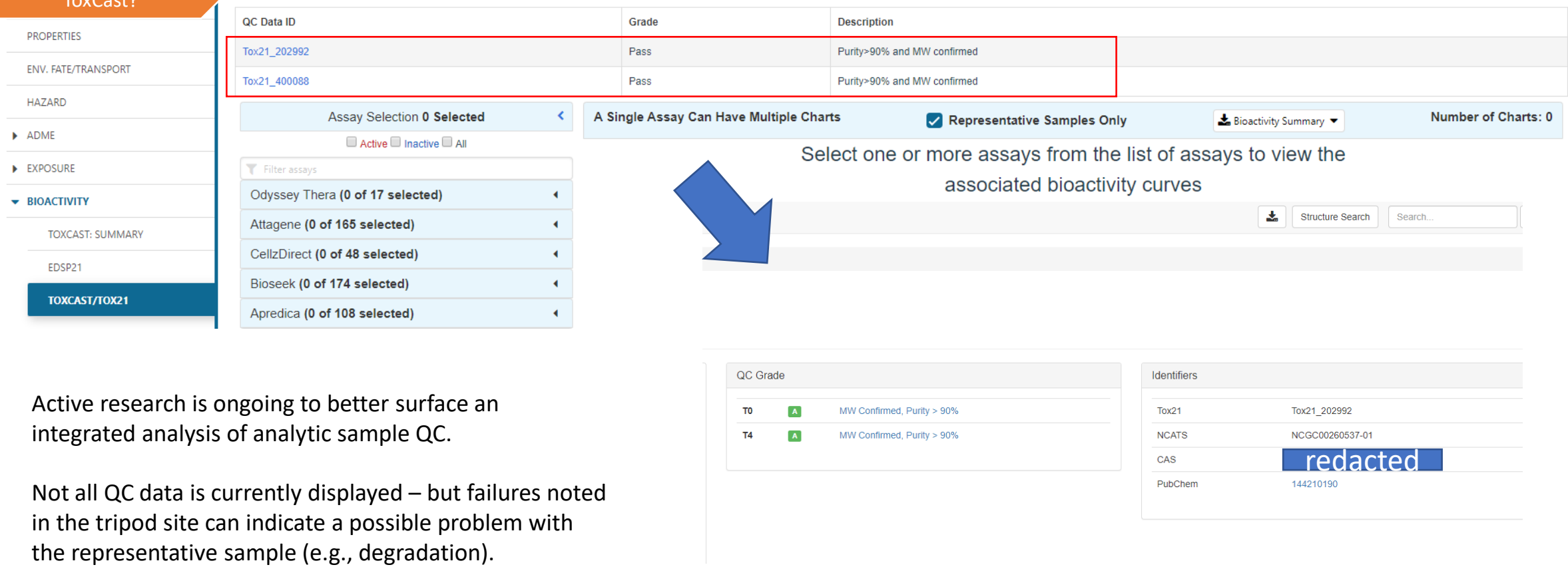
16 records

Many successfully screened chemicals have been (but not limited to):
logP -0.4 to 5.6 range; MW 180-480;
log10 Vapor Pressure < 1.

# Available QC data suggests that the substance is present in DMSO sample and stable over 4 months

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

*Representative samples that were analyzed (the chemical in DMSO stock) are high purity and confirmed*

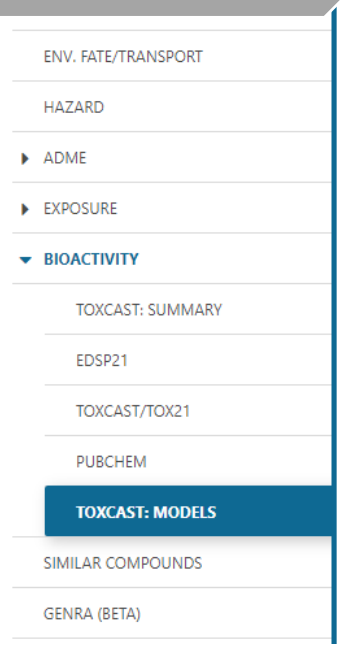



- Active research is ongoing to better surface an integrated analysis of analytic sample QC.
- Not all QC data is currently displayed – but failures noted in the tripod site can indicate a possible problem with the representative sample (e.g., degradation).

# Mystery substance B: Models >>> single assays. And equivocals happen.

Models available?

*Mystery substance B has positive ToxCast ER pathway agonist and ToxCast AR antagonist scores.*




**ToxCast: Models**

ToxCast Model Predictions

Download ToxCast Model Predictions

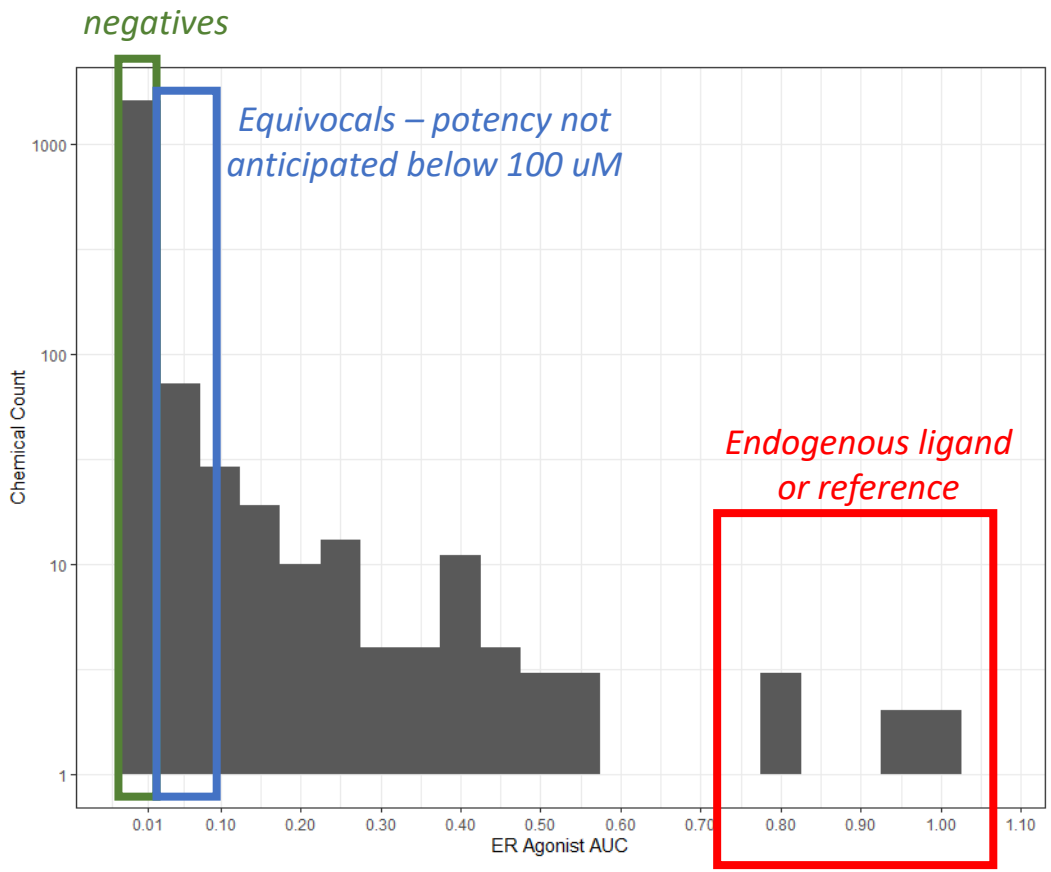
>0.1 = positive; 0.001-0.1 = equivocal

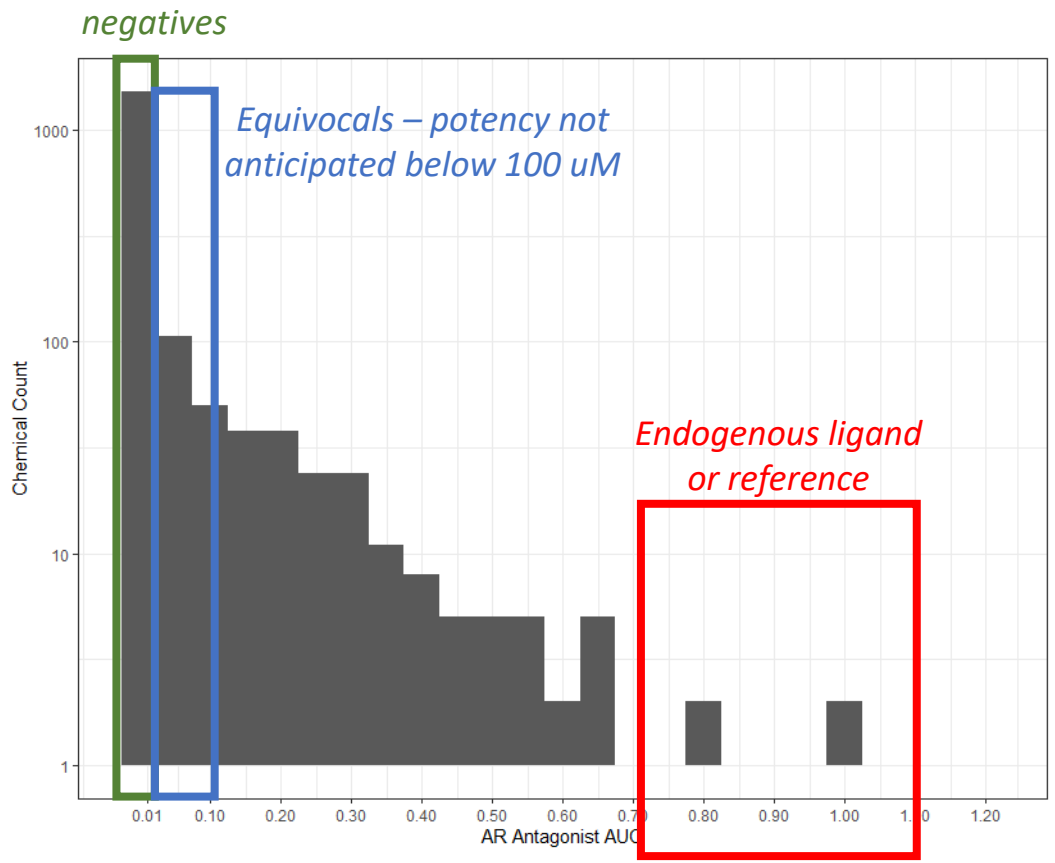
| Model | Receptor | Agonist | Antagonist | Binding |
|---|---|---|---|---|
| ToxCast Pathway Model (AUC) | Androgen | 0.00 | 0.345 | - |
| ToxCast Pathway Model (AUC) | Estrogen | 0.450 | 0.00 | - |
| COMPARA (Consensus) | Androgen | Inactive | Active | Active |
| CERAPP Potency Level (From Literature)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

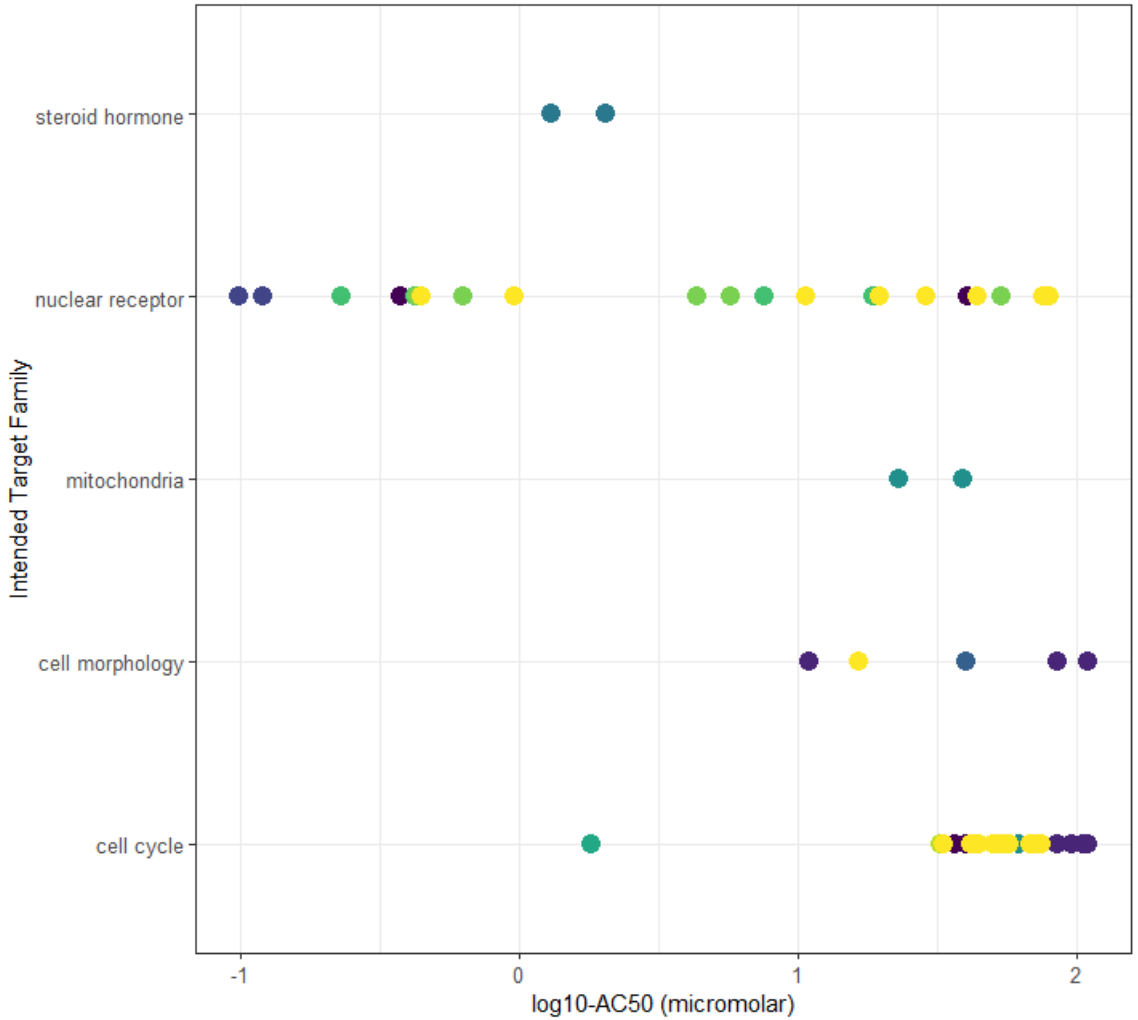
*As of now, the models supported in the CompTox Chemicals Dashboard are endocrine-related but hope to expand to other published models in the future.*
*Consult the peer-reviewed literature for additional models and interpretations.*

# Interpreting and using ToxCast pathway model scores: relative activity



# A deeper dive into the intended target family categories relevant for ER/AR activity and selectivity

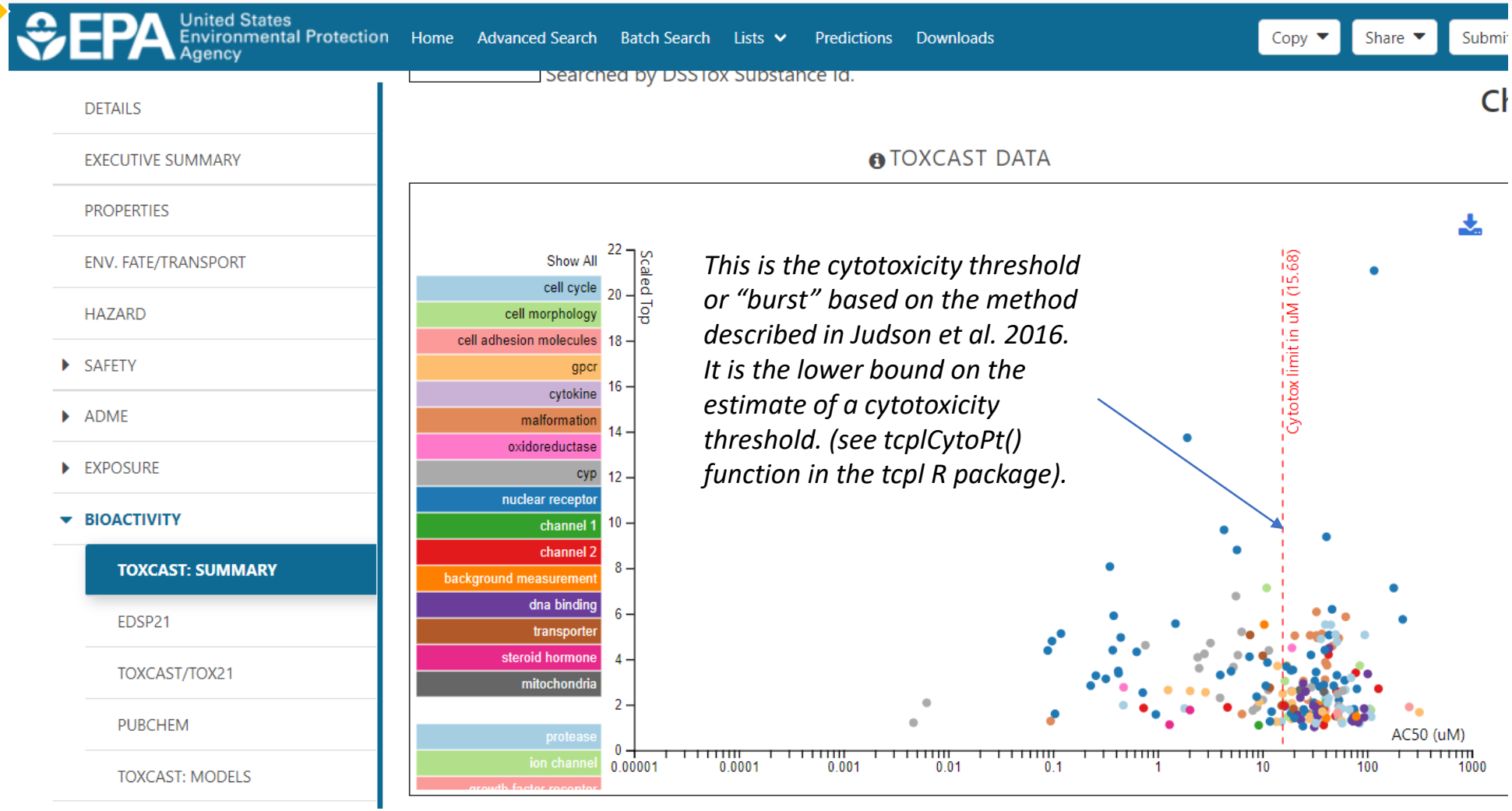



*Downloaded ToxCast Summary from the CompTox Chemicals Dashboard, and filtered for one gene of interest*

| NAME | GENE_SYMBOL | HIT_CALL | AC50 |
|---|---|---|---|
| ACEA_ER_80hr | ESR1 | ACTIVE | 0.373 |
| ATG_ERE_CIS_up | ESR1 | ACTIVE | 9.81E-02 |
| ATG_ERa_TRANS_up | ESR1 | ACTIVE | 0.119 |
| NVS_NR_bER | ESR1 | ACTIVE | 0.421 |
| NVS_NR_hER | ESR1 | ACTIVE | 0.23 |
| NVS_NR_mERa | Esr1 | ACTIVE | 0.257 |
| OT_ER_ERaERa_0480 | ESR1 | ACTIVE | 5.73 |
| OT_ER_ERaERa_1440 | ESR1 | ACTIVE | 4.31 |
| OT_ERa_EREGFP_0120 | ESR1 | ACTIVE | 0.424 |
| OT_ERa_EREGFP_0480 | ESR1 | ACTIVE | 0.631 |
| TOX21_ERa_BLA_Agonist_ratio | ESR1 | ACTIVE | 0.962 |
| TOX21_ERa_BLA_Antagonist_ratio | ESR1 | ACTIVE | 43.5 |
| TOX21_ERa_LUC_VM7_Agonist | ESR1 | ACTIVE | 0.445 |
| TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2 | ESR1 | ACTIVE | 75.1 |
| TOX21_ERa_LUC_VM7_Agonist_10nM_ICI182780 | ESR1 | ACTIVE | 19.6 |

# 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?

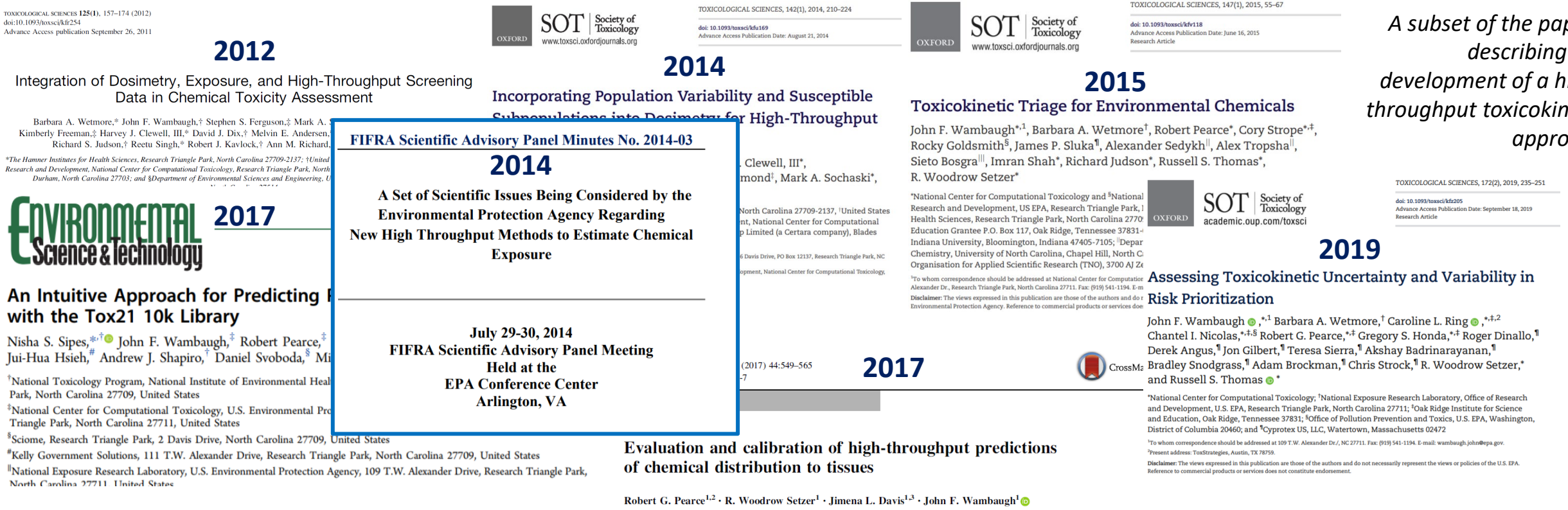
- AC50 < burst?
- AC50 0.5$\log_{10}$ distance from burst?
- AC50 < parallel viability assays? This makes sense if you have parallel viability assays.
- How else to filter ToxCast data: 3+ caution flags and curves with both low efficacy and potency values below the concentration range screened, certain curve properties (such as the maximum), etc.
- 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 July 2020 is now using ToxCast invitrodb version 3.3: **https://doi.org/10.23645/epacomptox.6062479.v5**
- 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**
- We anticipate a new ToxCast release in 2021.

# An IVIVE approach based reverse toxicokinetics has been developed

High-throughput toxicokinetic (HTTK) approaches make it possible to predict doses corresponding to in vitro bioactivity for thousands of chemicals.



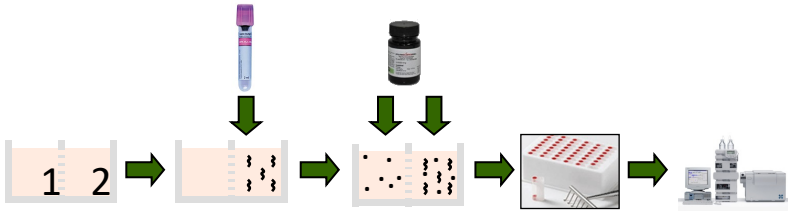

*A subset of the papers describing the development of a high-throughput toxicokinetic approach*

***Reverse dosimetry can be leveraged in IVIVE to estimate the exposure that would produce the plasma concentration corresponding to bioactivity***

# High throughput toxicokinetics (HTTK)

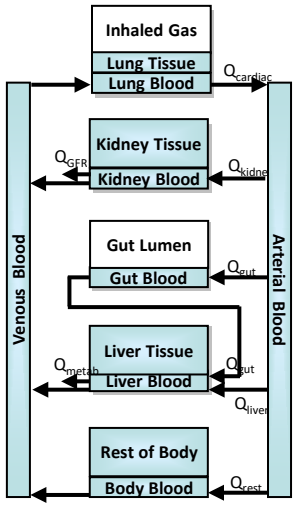
## *in vitro* data

*Hepatic clearance from suspended hepatocytes*




*Plasma protein binding*

## Generic toxicokinetic models




= ***httk***

## Some high-level assumptions:

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

(2) plasma concentration can be approximated by steady-state kinetics; and,

(3) 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.

*Slide modified from John Wambaugh*

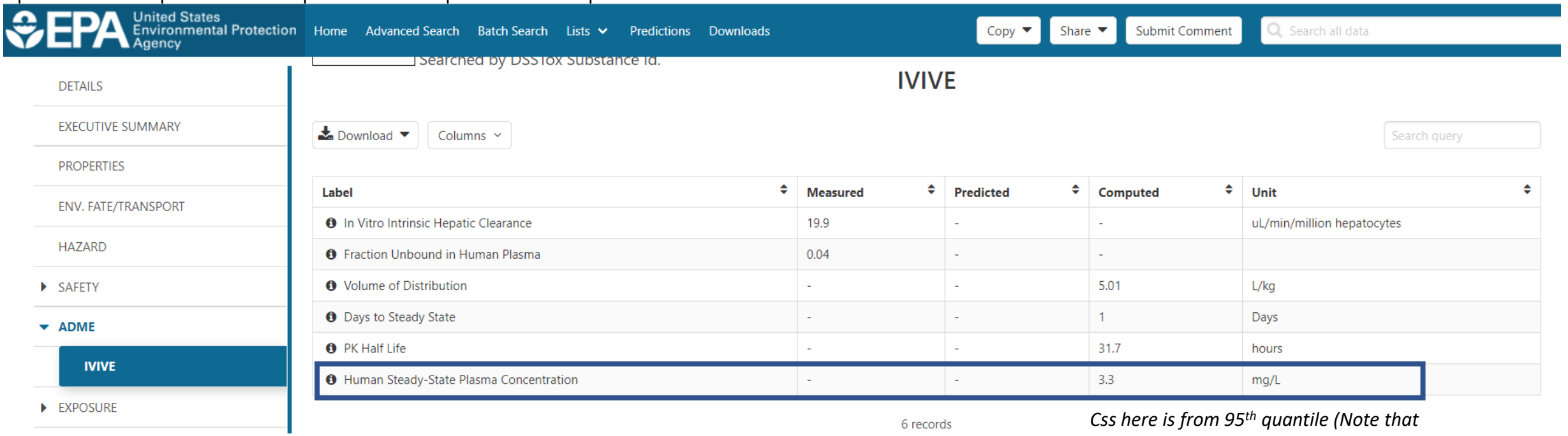
# IVIVE via high-throughput toxicokinetic data and models

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

- Operationally, the httk R package (v 2.0.4) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs).
- 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).

$$\frac{3.3\ \text{mg}}{\text{L}} \times \frac{\text{g}}{1000\ \text{mg}} \times \frac{\text{mol}}{228.291\ \text{g}} \times \frac{1e6\ \mu\text{mol}}{\text{mol}} = 14.45523\ \mu\text{mol/L} = \mu\text{M}$$

$$\frac{0.1\ \mu\text{M}}{} \times \frac{1\ \text{mg/kg/day}}{14.45523\ \mu\text{M}} = \textbf{0.007 mg/kg/day = AED95}$$

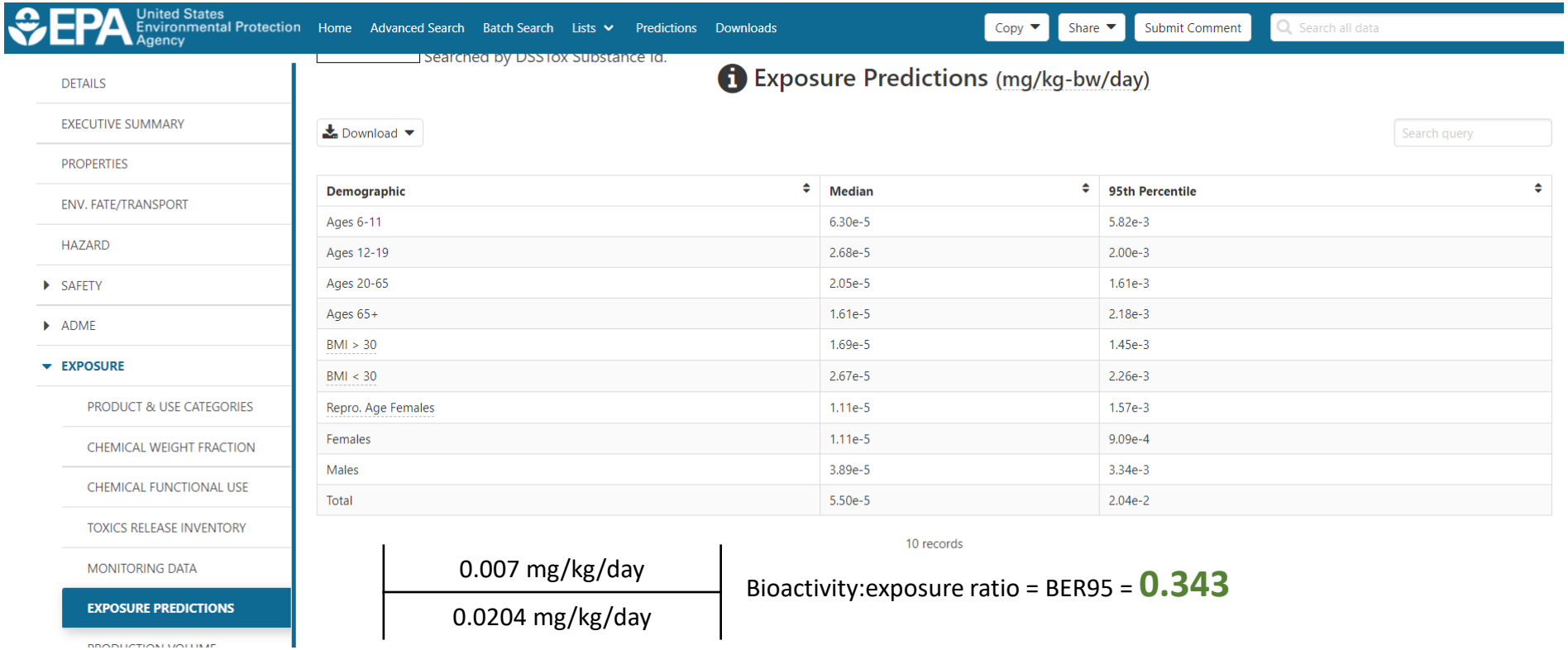



*Css here is from 95th quantile (Note that 95th concentration quantile is the same as the 5th dose quantile).*

# Bioactivity:exposure ratio requires exposure

Comparison to exposure predictions for a bioactivity:exposure ratio

- Total population predictions are based upon consensus exposure model predictions and the similarity of the compound to those chemicals monitored by NHANES. The method for the total U.S. population was described in a 2018 publication, "Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways".
- When available, demographic-specific predictions are based upon a simpler, heuristic model described in the 2014 publication "High Throughput Heuristics for Prioritizing Human Exposure to Environmental Chemicals".



# What to make of Mystery Substance B

- Mystery substance B is Bisphenol A, which clearly has some *in vitro* nuclear receptor activity at concentrations that may be below or near cytotoxicity.
  - It has moderate ToxCast ER agonist and AR antagonist scores.
  - The cytotoxicity threshold or “burst” seems to support selectivity of some nuclear receptor responses.
  - Diving a little deeper into the intended target family supports this analysis.

# 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)*

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

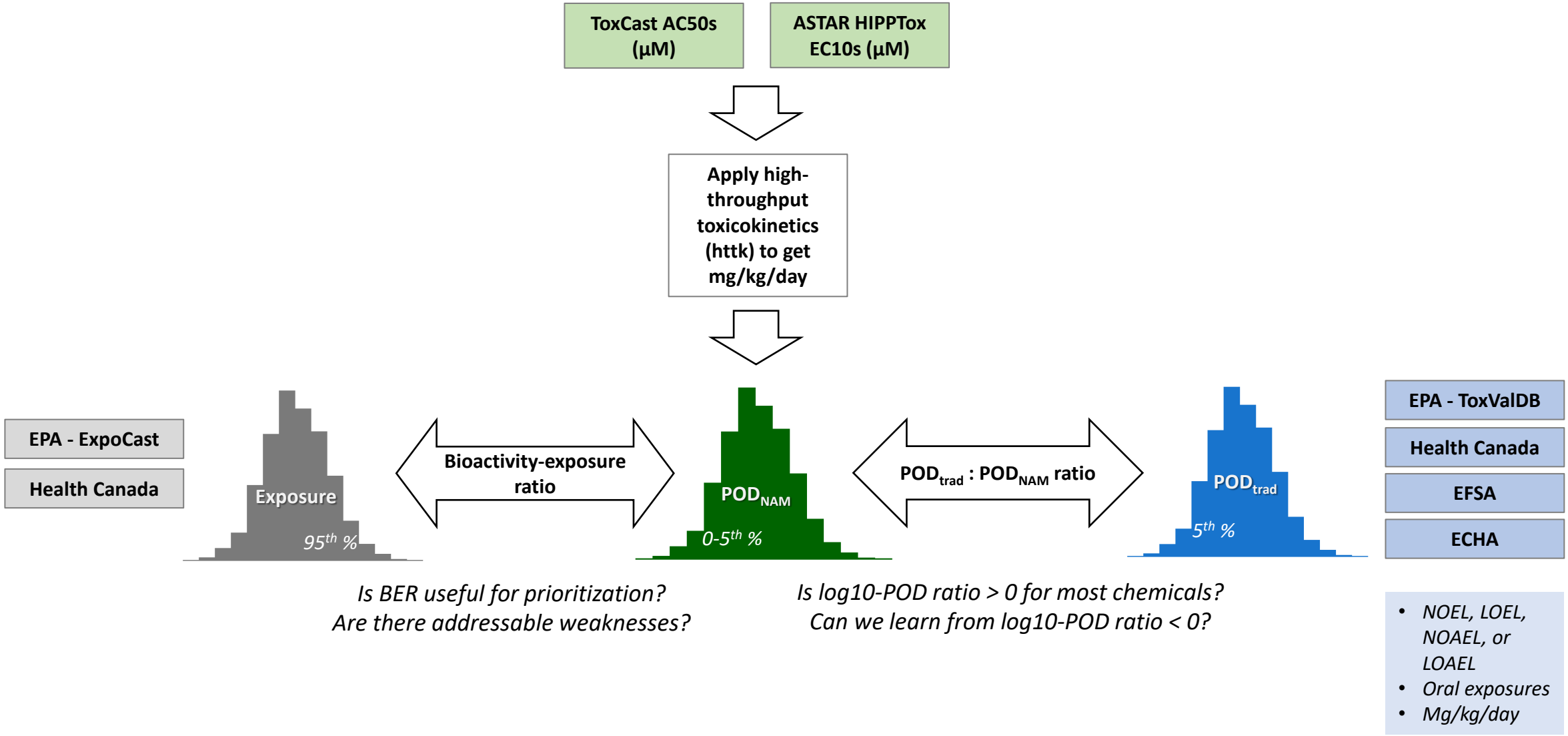
TOXICOLOGICAL SCIENCES, 2019, 1–24

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

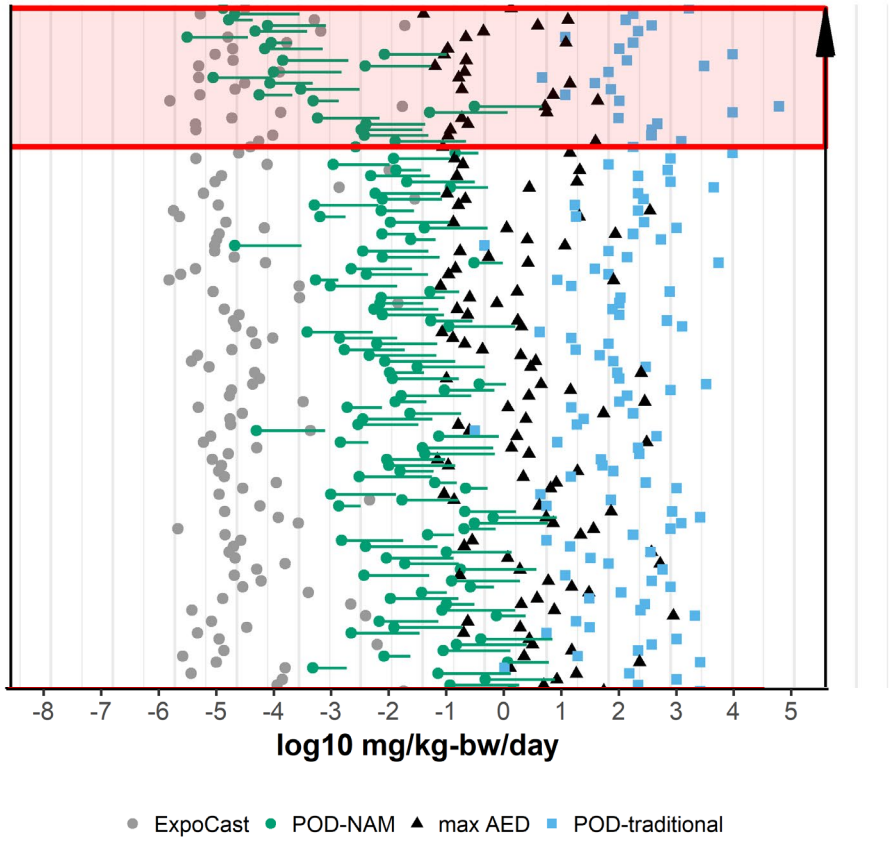
**Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization**

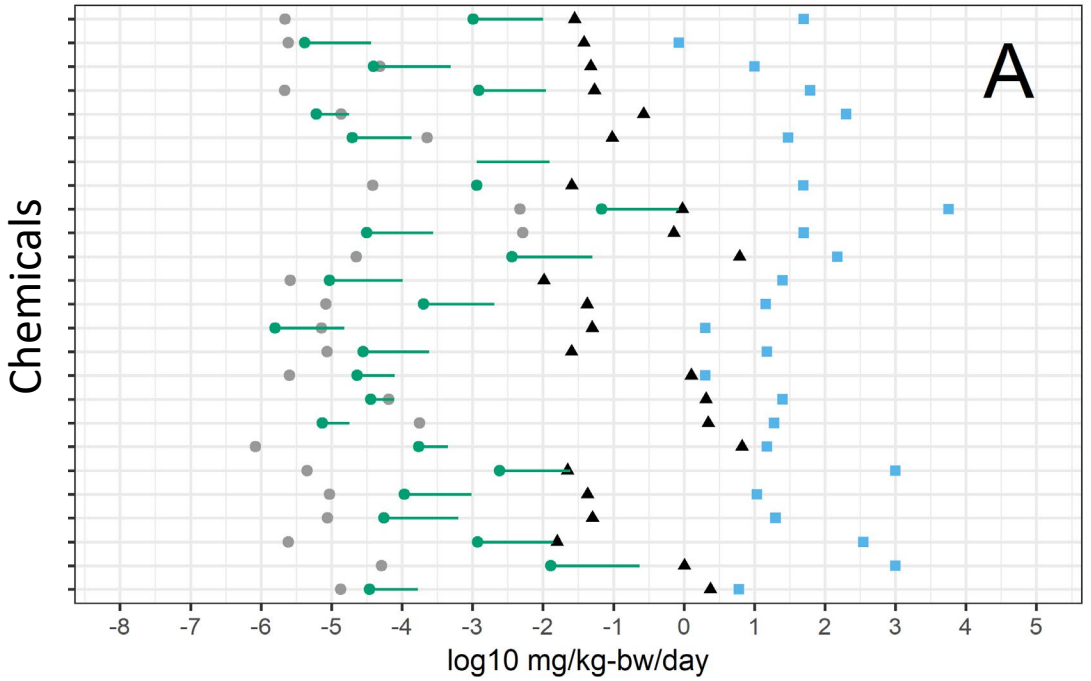
(APCRA partners for these two case studies)

# Case study workflow



# 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

# How to blend bioactivity-exposure ratios (BERs) and specific NAM-informed hazard indications?

| Challenges | Opportunities |
|---|---|
| NAM-based hazard indications may be “semi-quantitative” or relative to the screened set | Use comparisons to known reference chemicals to inform uncertainties on the relative potency, efficacy, or phenotype for a given new chemical |
| NAM-based hazard indications may not cover all of biology | Use broader screening platforms to try to cover more breadth and use “Tier 2” and “Tier 3” platforms to further target specific hazards of interest |
| Many NAM-based hazards may appear to occur at similar concentration ranges | Qualitatively flagging possible hazards and also quantitatively identifying a “lead” hazard may be informative. Seeking “selectivity” may be useful. |

**Thomas et al. 2019 further evolves a tiered screening strategy that adds in broader biological coverage but also suggests that we can strive to do more than BER in some cases.**

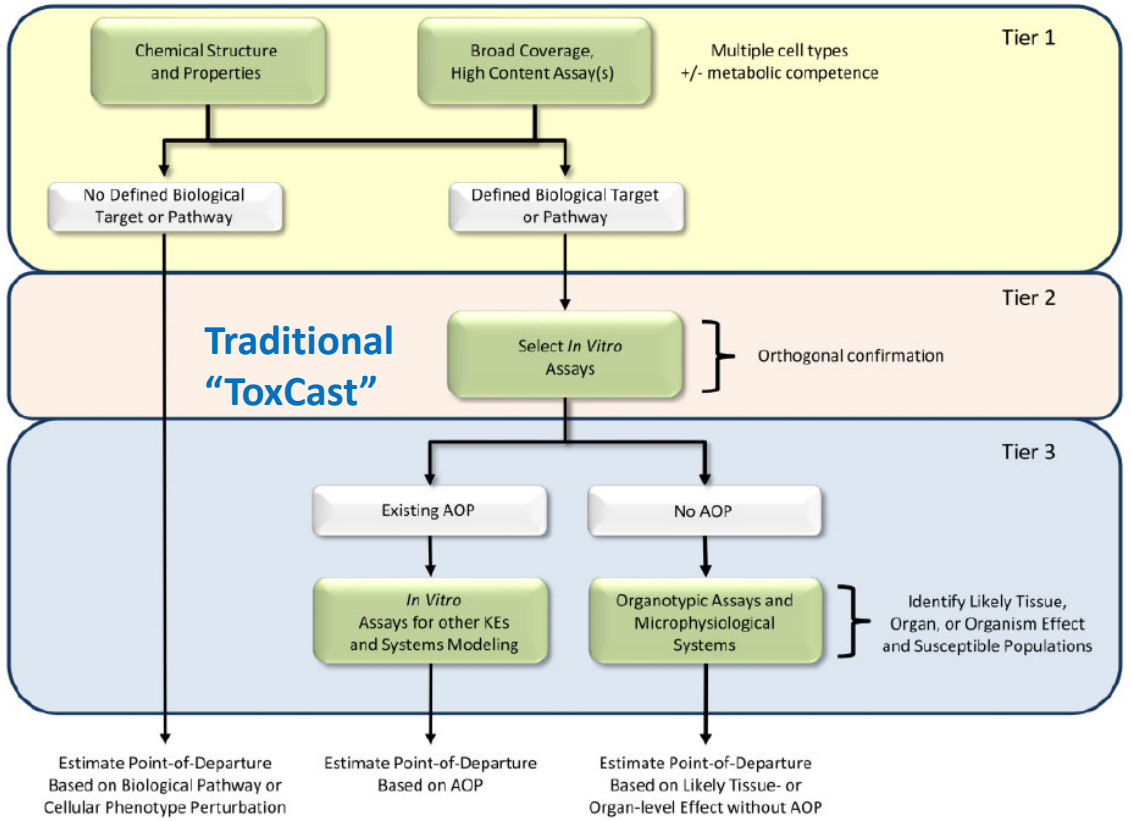



**Figure 2.** Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For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

# Conclusions

- Bioactivity data, including ToxCast, may help inform hazard prediction for weight-of-evidence, screening, and new approach methodologies-based points-of-departure for risk assessment.
- A high-throughput toxicokinetic approach to in vitro to in vivo extrapolation can translate bioactivity data in micromolar concentrations to administered equivalent doses for comparison to exposure or other *in vivo* data.
- The Comptox Chemicals Dashboard provides a data browsing and downloading capability to support weight-of-evidence evaluations and screening.
  - Consider that operationally, the steps taken to prepare a dataset for a single chemical weight-of-evidence evaluation may be different from preparation of a dataset for many chemicals.

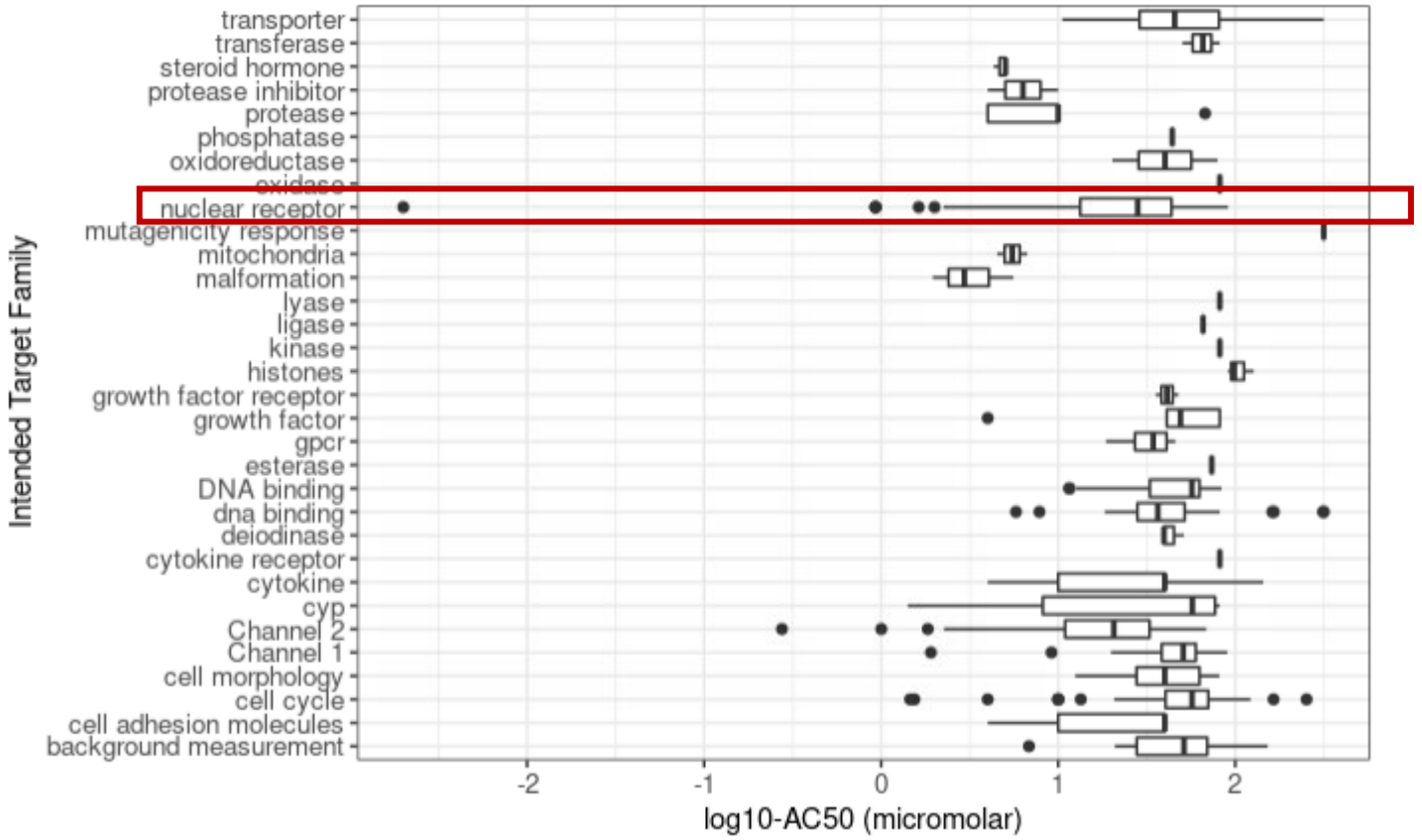
# Acknowledgments

- 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

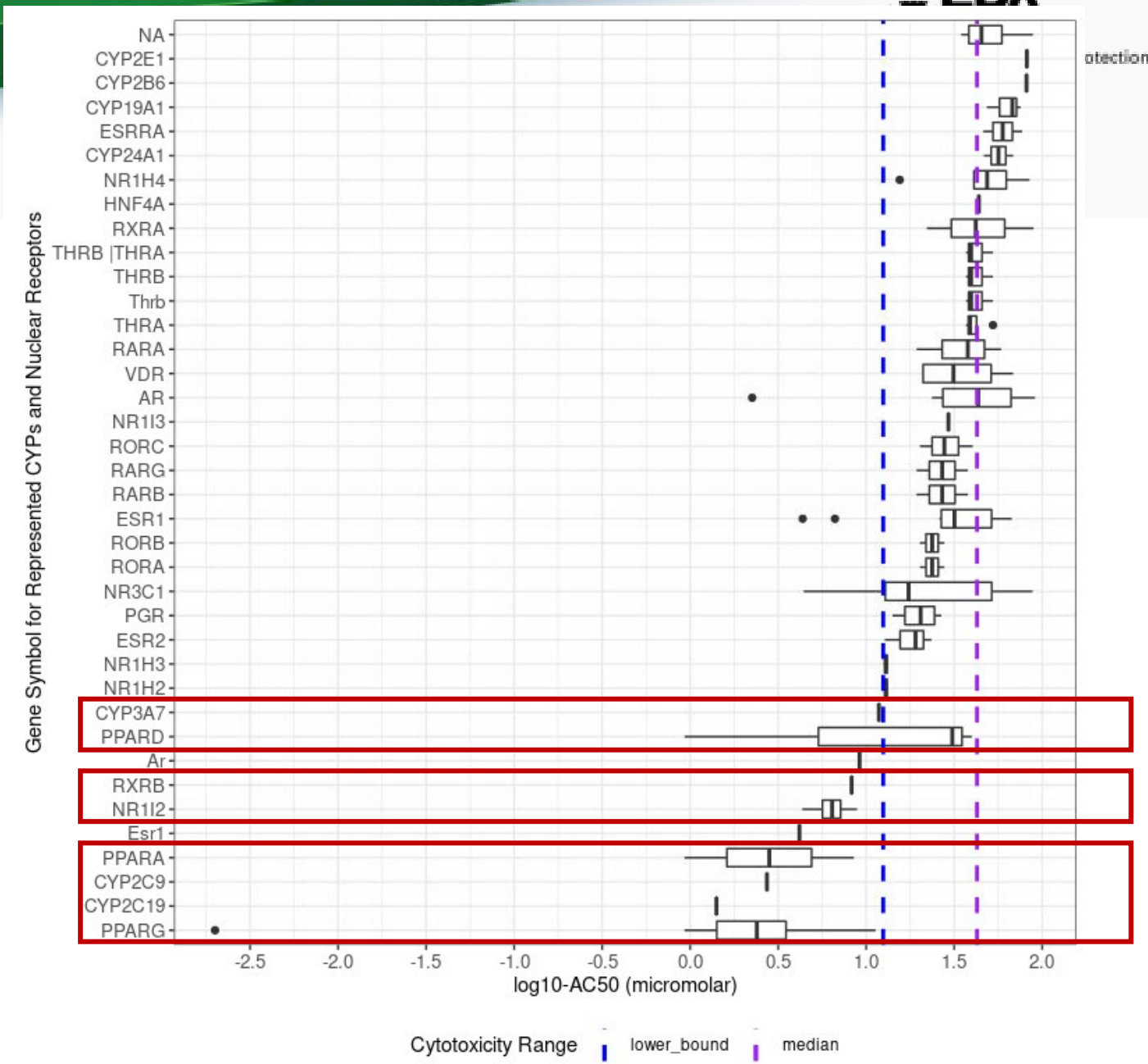
# What about one more example: TBBPA?

- TBBPA is a fairly promiscuous substance (hit rate > 30%), with clear cytotoxicity across platforms at ~40 µM (lower bound estimate of ~12 µM).
- It's possible that some nuclear receptor activity precedes effects on other targets.



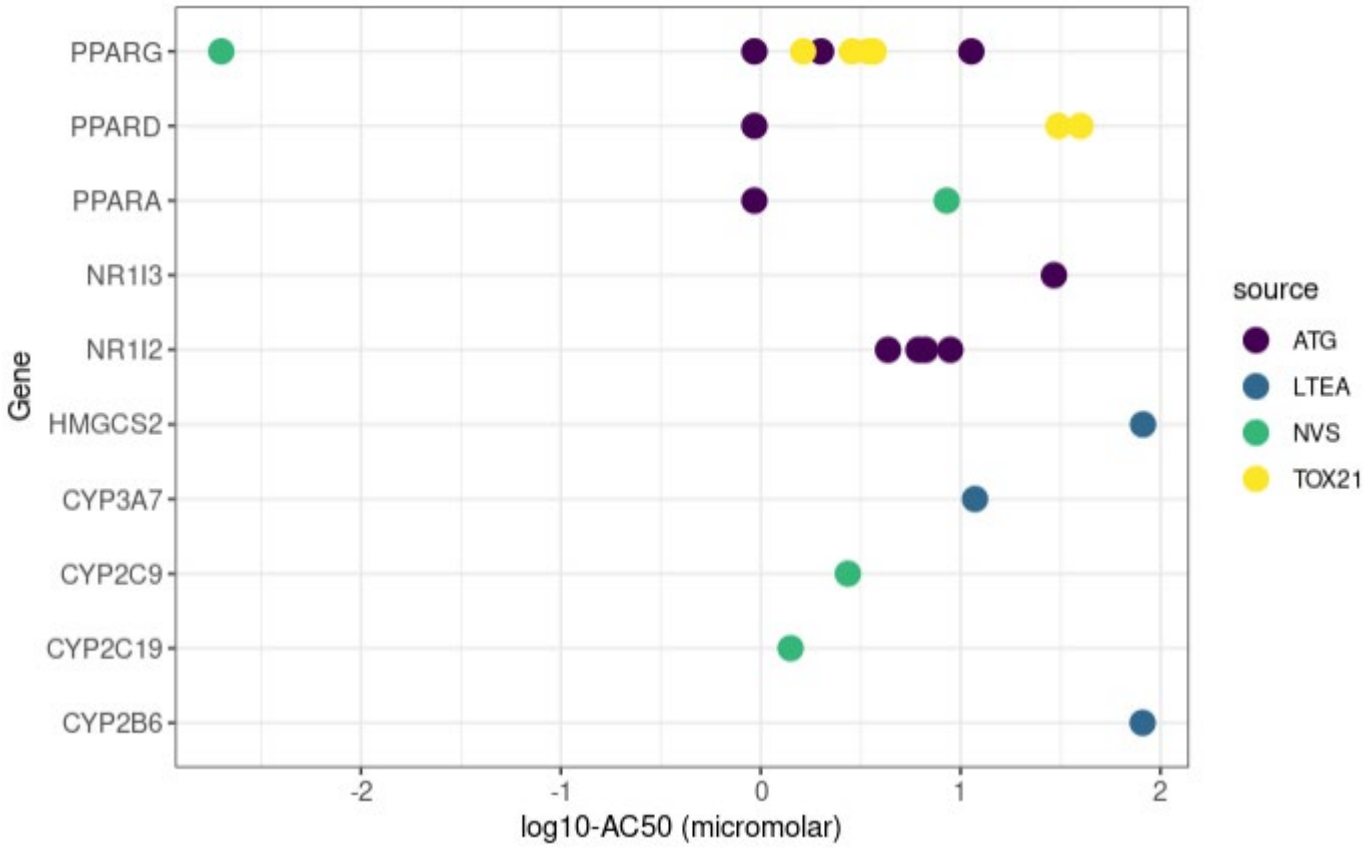
# TBBPA: nuclear receptors and CYPs

- Looking more specifically at nuclear receptors and related CYPs, there is good support for activity at multiple PPAR, CAR/PXR, at concentrations lower than other receptors and/or cytotoxicity.



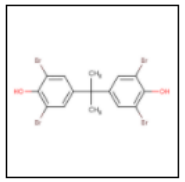
# TBBPA: PPAR, PXR, CAR endpoints

- Looking at just PPAR, PXR, and CAR related endpoints, there is redundant support across assay technologies.



# TBBPA: endocrine effects

- TBBPA is not very active in the ToxCast ER and AR pathway models; a borderline hit for the AR antagonist model.



3,3',5,5'-Tetrabromobisphenol A

79-94-7 | DTXSID1026081

Searched by DSSTox Substance Id.

**ToxCast: Models**

ToxCast Model Predictions

Download ToxCast Model Predictions

| Model | Receptor | Agonist | Antagonist | Binding |
|---|---|---|---|---|
| ToxCast Pathway Model (AUC) | Androgen | 0.00 | 0.104 | - |
| ToxCast Pathway Model (AUC) | Estrogen | 0.00 | 3.81e-7 | - |
| COMPARA (Consensus) | Androgen | Inactive | Active | Active |
| CERAPP Potency Level (From Literature) | Estrogen | - | - | - |
| CERAPP Potency Level (Consensus) | Estrogen | Inactive (Inactive) | Inactive (Inactive) | Inactive (Inactive) |

# TBBPA: HT-H295R model

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

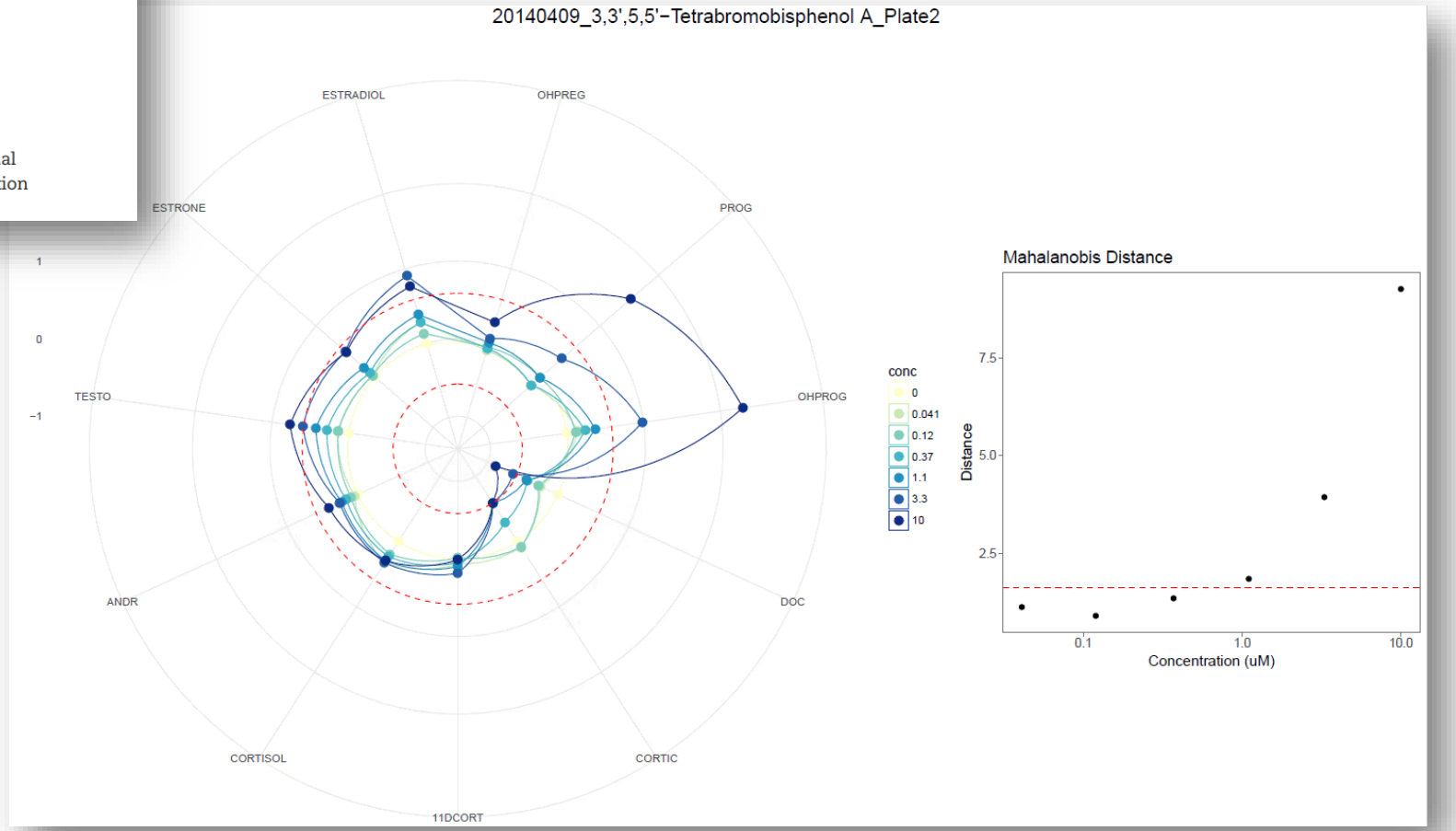
TOXICOLOGICAL SCIENCES, 162(2), 2018, 509–534
doi: 10.1093/toxsci/kfx274
Advance Access Publication Date: December 1, 2017
Research Article

## 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

- However, TBBPA has a moderate effect on the HT-H295R Mahalanobis distance model due to moderate effect sizes on progestogen synthesis.



# TBBPA: with respect to thyroid-related targets

- It seems that TBBPA may participate in a “permissive heterodimer” effect for TR and possibly other RXR heterodimer partners.
- Several cell-free, non-receptor thyroid hormone synthesis-related targets (TPO, NIS, and DIO) are inhibited by TBBPA at concentrations (20-50 µM) that approach cytotoxicity and/or exceed PPAR-related effects (1-10 µM). The parallel cytotoxicity/non-specific protein inhibition assays were negative.
- Could be interesting to see TBBPA in a thyroid microtissue model of thyroid hormone synthesis to see if effects were observed.

| | aeid | assay_component_endpoint_name | ac50_uM | intended_target_family |
|---|---|---|---|---|
| | | All | Al | All |
| 1 | 1508 | NCCT_TPO_AUR_dn | 20.2516904360491 | oxidoreductase |
| 2 | 2309 | NHEERL_MED_hDIO1_dn | 39.5589136388614 | deiodinase |
| 3 | 2533 | NHEERL_MED_hDIO3_dn | 39.5937278083094 | deiodinase |
| 4 | 2037 | NIS_RAIU_inhibition | 40.2393801696582 | transporter |
| 5 | 2532 | NHEERL_MED_hDIO2_dn | 51.2827337018911 | deiodinase |