Incorporating New Approach Methodologies in Risk Assessments

Federal State Toxicology Risk Assessment Committee (FSTRAC)

April 21, 2021

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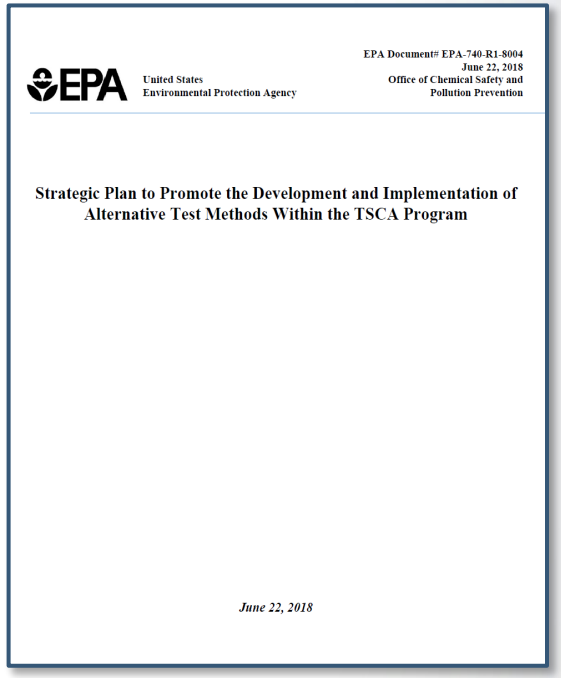
The views presented are those of the author and do not necessarily reflect the views of the US EPA.

Definition(s) of New Approach Methods (NAMs)

- Commonly defined to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment.

- Recently defined in the EPA's TSCA Alternative Toxicity Strategy as:
  - a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals.


EPA Document# EPA-740-R1-8004
June 22, 2018
Office of Chemical Safety and Pollution Prevention

EPA United States Environmental Protection Agency

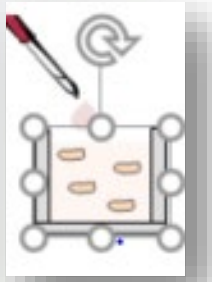
Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program

June 22, 2018

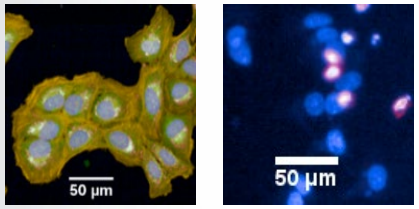
https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf

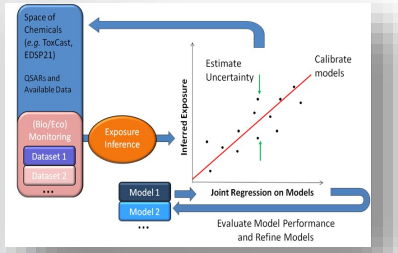
https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

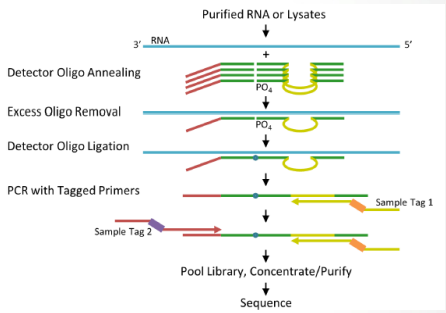
Examples of NAMs

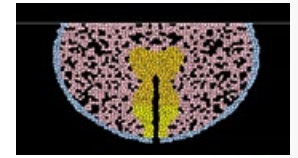


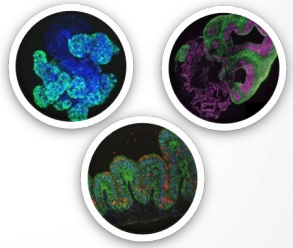
- In silico (e.g. QSAR and Read-across)
  - Estimate effects and doses
  - Consensus exposure modeling
- In vitro assays
  - Broad / screening (transcriptomics, cell painting)
  - Targeted (receptors, enzymes)
  - In vitro PODs, modes / mechanisms of action
- In vitro Toxicokinetics
  - Allow conversion of an in vitro POD to in vivo (IVIVE)
- High-throughput Exposure Measurements
  - To fill data gaps in monitoring data
- Computer models
  - Hazard models to integrate multiple in silico and in vitro data streams
  - Exposure models to increase information on different pathways of exposure







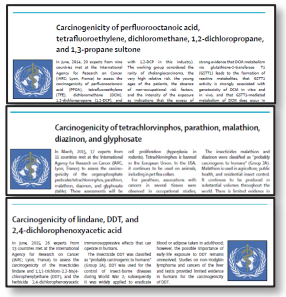




Where can NAMs "fit" in Risk Assessment?

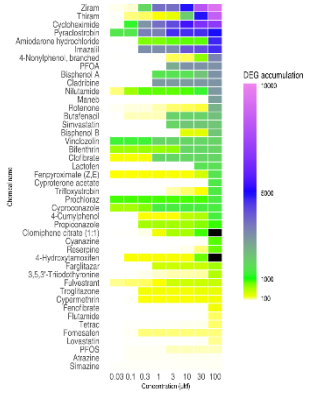
- Hazard characterization
- Dose-Response
- Exposure assessment

Provide Mechanistic Support for Hazard ID



IARC Monographs
110, 112, 113

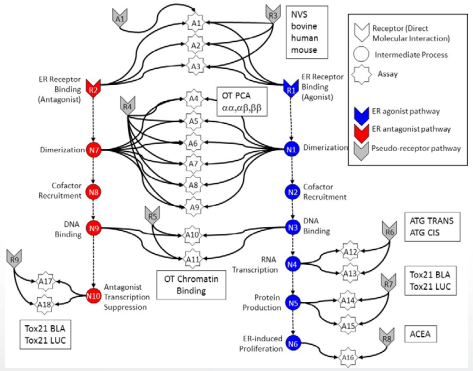
Tiered testing with High-throughput screening



Bundy et al unpublished

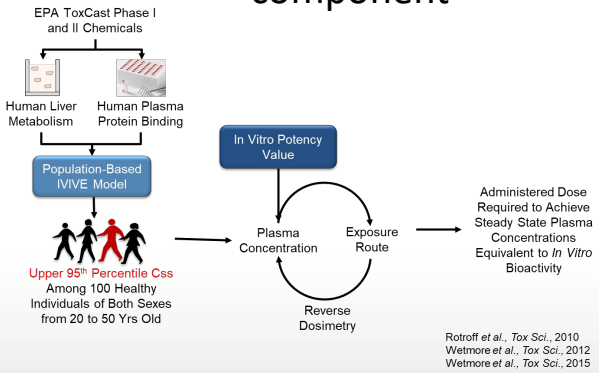
…and more!

Prioritization of Chemicals for Further Testing



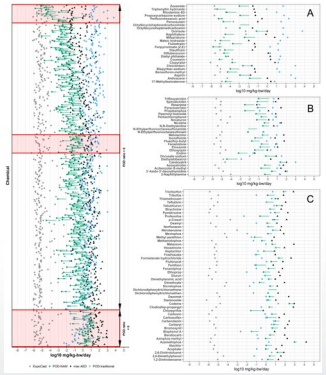
Judson et al., 2015

High-throughput toxicokinetic component



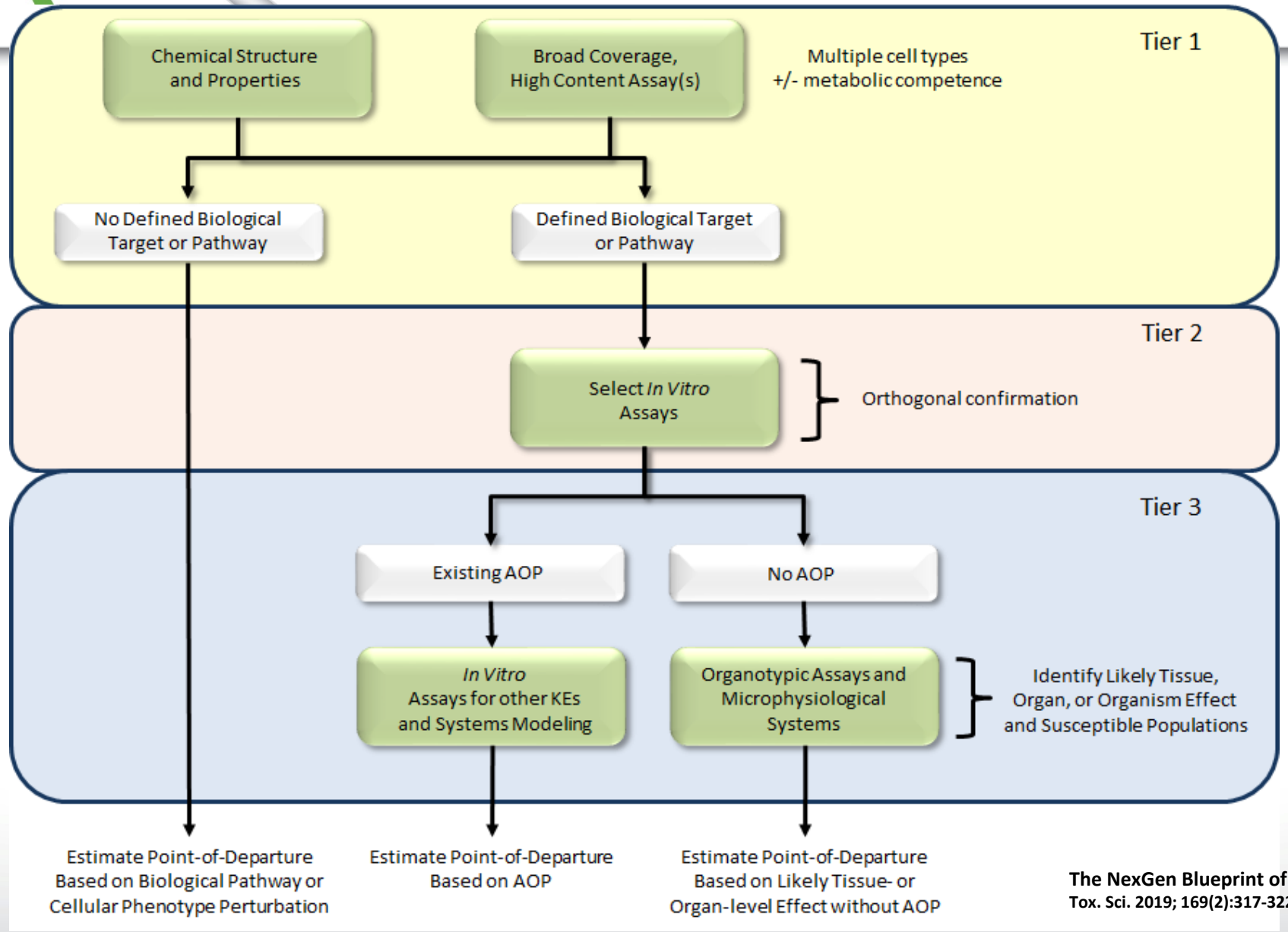
Rotroff *et al.*, *Tox Sci.*, 2010
Wetmore *et al.*, *Tox Sci.*, 2012
Wetmore *et al.*, *Tox Sci.*, 2015

In vitro point-of-departure development from NAMs



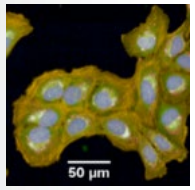
Paul-Friedman et al, 2020

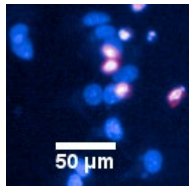
Tiered Hazard Evaluation Approach

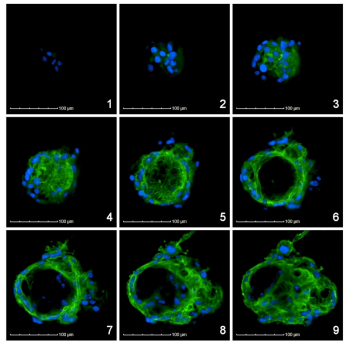


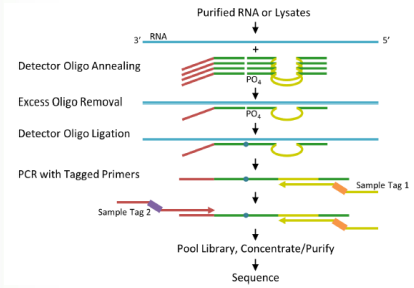
The NexGen Blueprint of CompTox at USEPA
Tox. Sci. 2019; 169(2):317-322

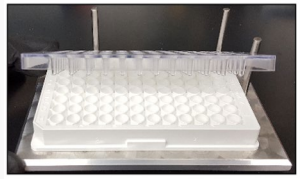
Potential Challenges with New Approach Methods

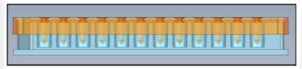


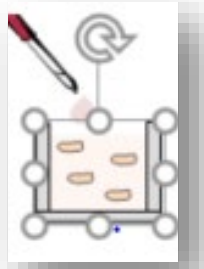


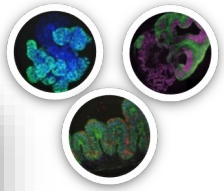






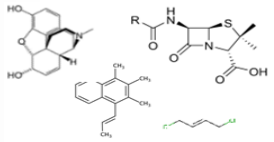




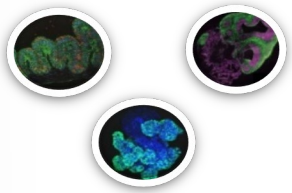


- Incomplete coverage of important pathways (i.e., biological space)
- Limited higher order biological interactions (i.e., cell-cell, tissue, and organ-level)
- Limited or lack of relevant metabolism
- Addressing uncertainties

Incorporating High-Content Technologies to Increase Biological Coverage

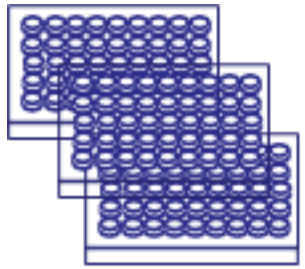


Thousands of Chemicals

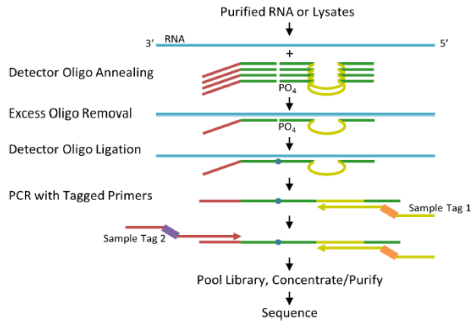


Multiple Cell Types

Concentration Response Screening

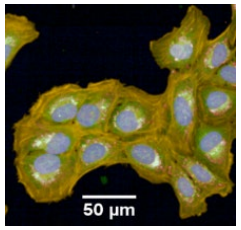


Whole Genome Transcriptomics

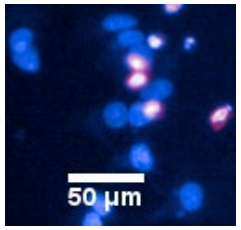


Multi-Parameter Cellular Phenotypic Profiling

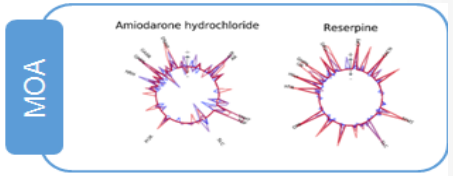
DNA RNA/ER AGP Mito



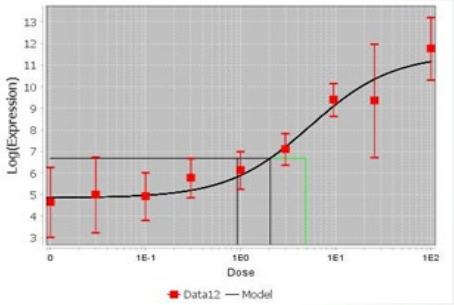
H-33342 Casp3/7 PI



Mode-of-Action Identification



Concentration Response Modeling

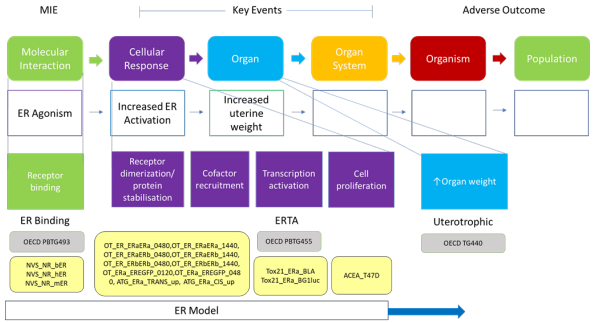


- 384-well, laboratory automation compatible
- Relatively inexpensive ($2.50 - $1,500 per chemical)
- Broad complementary coverage of molecular and phenotypic responses
- Integration of reference materials and controls for performance standards

Nyffeler et al. SLAS Discov. 2021 Feb;26(2):292-308. doi: 10.1177/2472555220950245
Harrill et al. Toxicol Sci. 2021 Feb 4;kfab009. doi: 10.1093/toxsci/kfab009. Online ahead of print

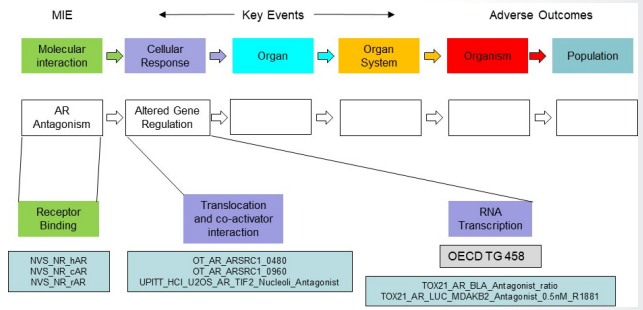
Orthogonal In Vitro Assays and Computational Modeling

- 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 computational model to integrate assays
  - Model creates a composite dose-response curve for each chemical to summarize results from all assays



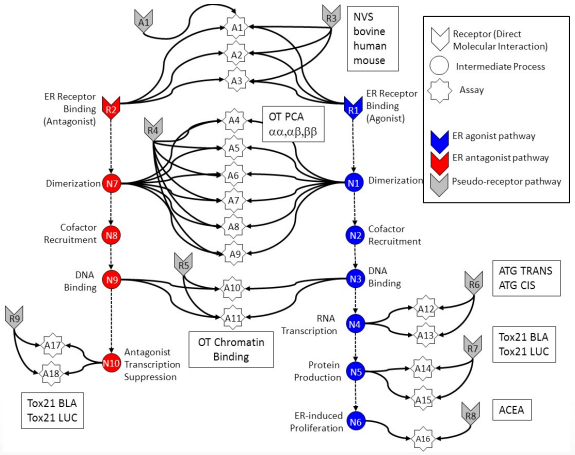
Estrogen Receptor Computational Model

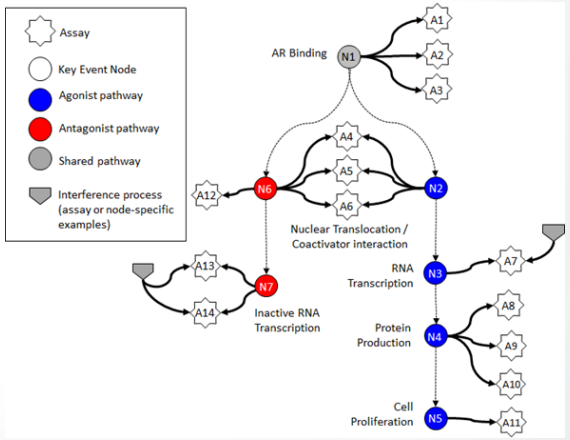
Judson et al., Envi Health Pers (2015)



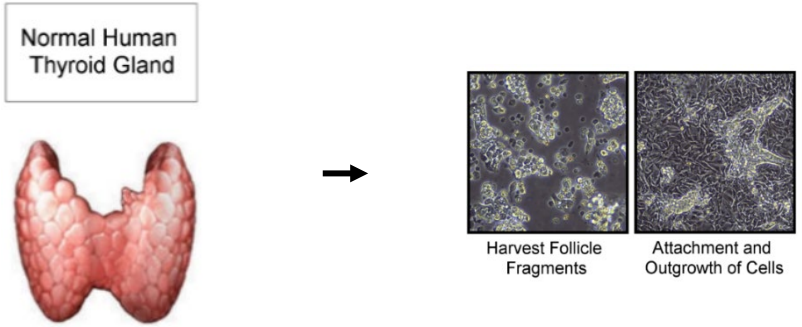
Androgen Receptor Computational Model

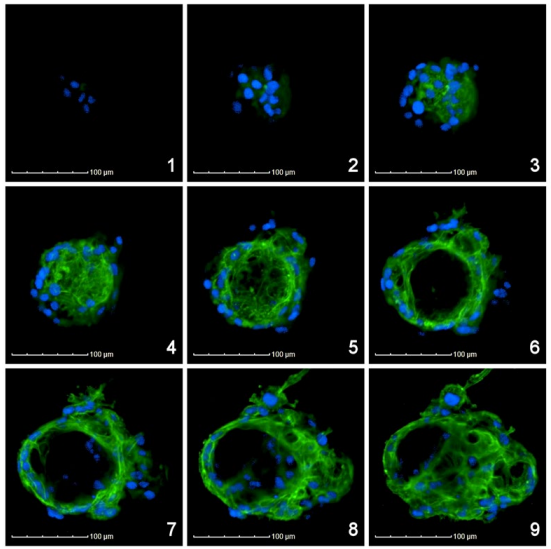
Kleinstreuer et al., Chem Res Toxicol (2017)



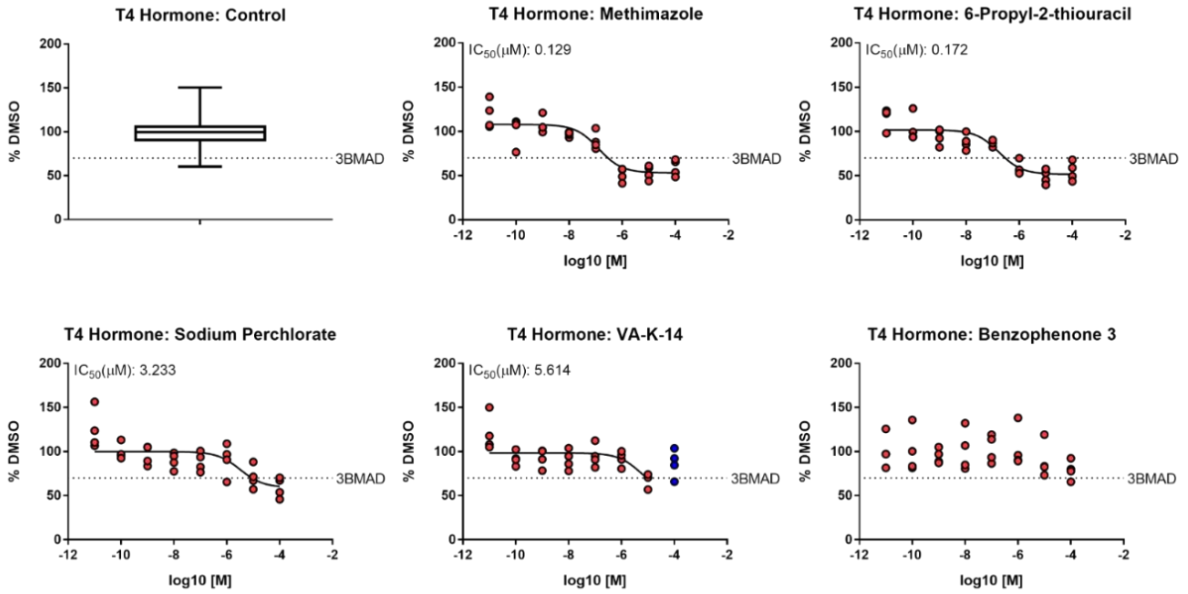


Developing Organotypic Culture Models to Identify Tissue/Organ Effects



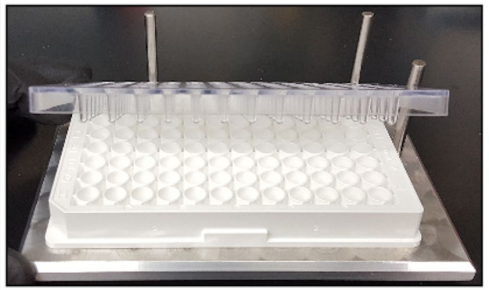


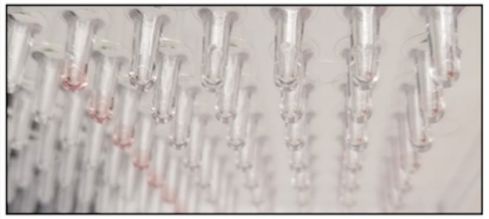
Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin

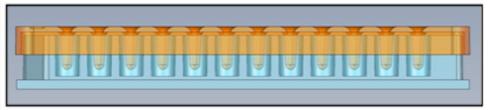


Deisenroth et al. Toxicological Sciences, Volume 174, Issue 1, March 2020, Pages 63-78, https://doi.org/10.1093/toxsci/kfz238

Metabolic Competence

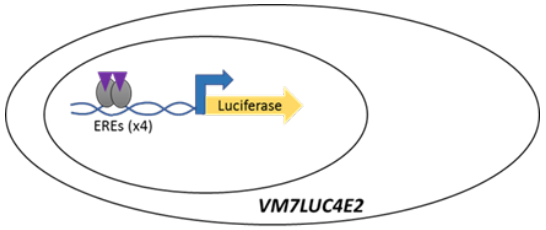


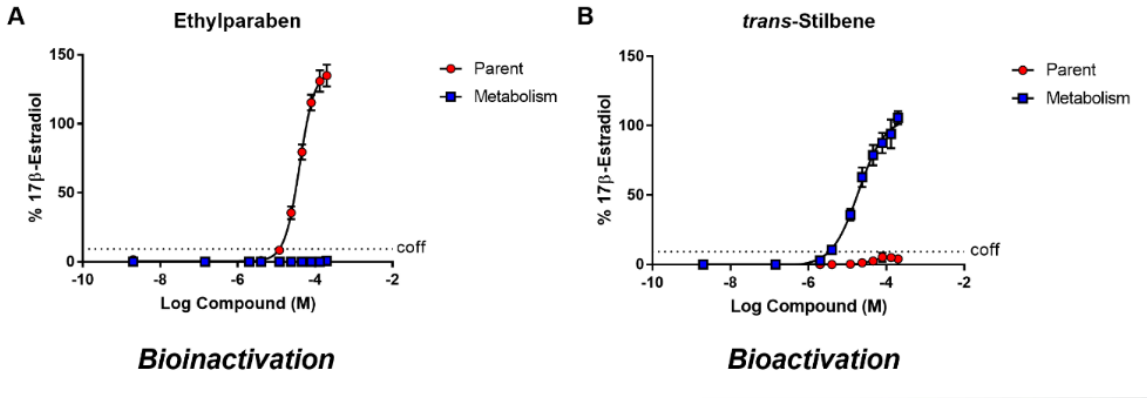




Alginate Immobilization of Metabolic Enzymes (AIME) Method: S9 fraction immobilization in alginate microspheres on 96- or 384-well peg lids

- **Retrofitting Metabolism**: AIME method suitable for biochemical- and cell-based HTS assays
- **Screening Throughput**: Adaptable to 96- and 384-well screening platforms
- **Regulatory Relevance**: Integration of phase I liver metabolism for hazard identification of parent and metabolite endocrine activity
- **Results:** Evaluation of a 63 chemical test set supports metabolic screening for -
  - Refinement of prioritization for ER-active substances based on metabolite effects
  - In some cases, supports more accurate prediction of *in vivo* effects for biotransformed substances





Parallel evaluation of parent compound and metabolites identifies false positive and false negative effects

Collaboration with Unilever

Deisenroth et al. Toxicological Sciences, Volume 178, Issue 2, December 2020, Pages 281–301, https://doi.org/10.1093/toxsci/kfaa147

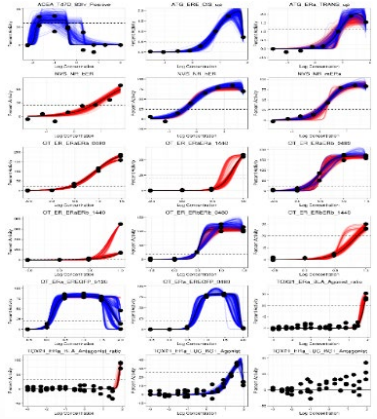
Uncertainty Analysis

Major sources of uncertainty:

1. Qualitative: is an assay "hit" really due to ER/AR activity, or assay interference?
2. Quantitative: uncertainty around the true potency value (AC50)

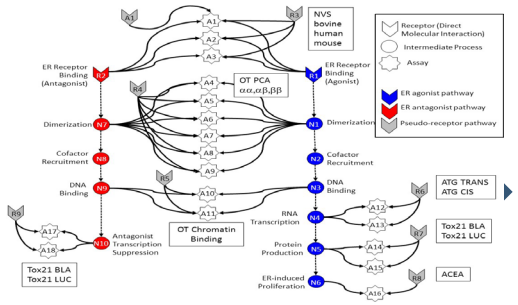
Both are now incorporated into the ER and AR model results through the development of statistical methods have been developed to establish uncertainty bounds around potency and efficacy values. These statistical methods involve resampling the data and refitting the concentration response curves thousands of times to quantitatively estimate the uncertainty.

Bootstrap Uncertainty in *In Vitro* Potency Values



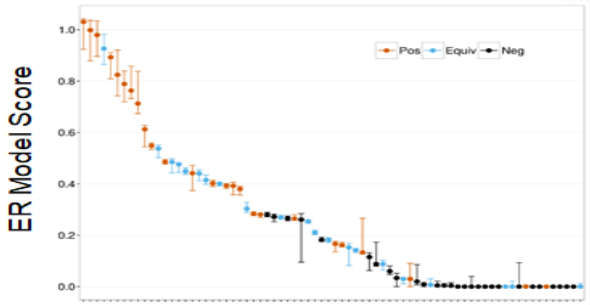
18 ER *In Vitro* Assays

Computational Modeling



ER Pathway Model

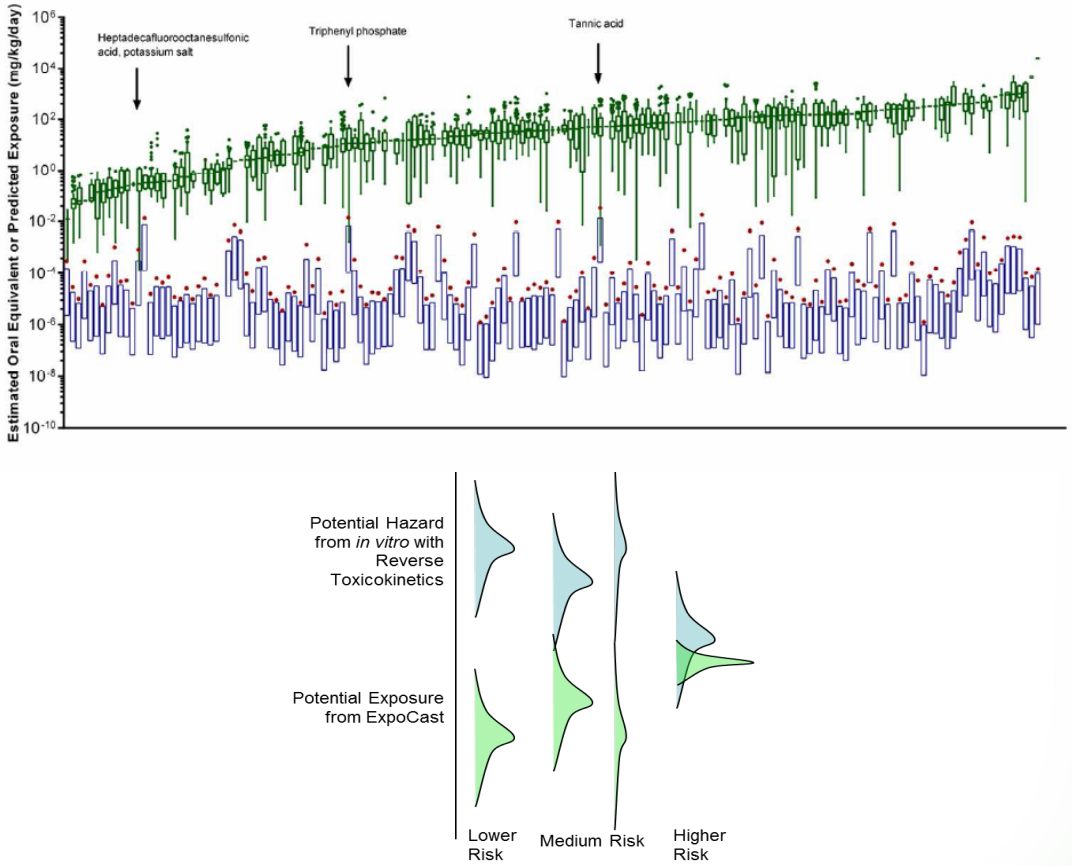
Propagation of Uncertainty in Modeling Output



Watt and Judson, PLOS One 2018 doi.org/10.1371/journal.pone.0196963

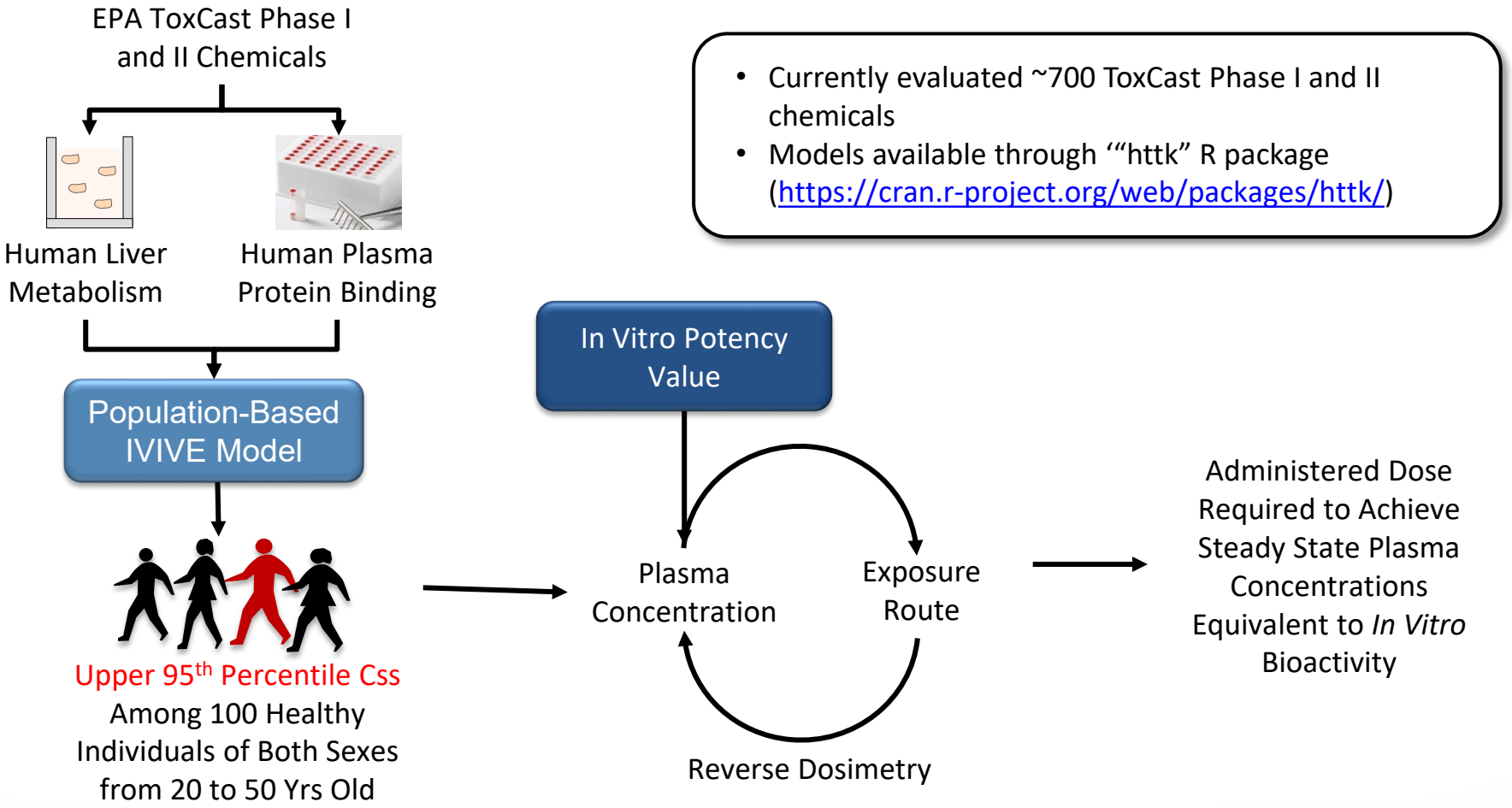
Linking Bioactivity and Exposure (*i.e.* Risk)

- **High throughput risk characterization** relies on three components:
  1. High throughput **hazard** (*i.e.* bioactivity) characterization
  2. High throughput **exposure** forecasts
  3. High throughput **toxicocokinetics** (*i.e.* dosimetry)



SAP Dec 2014: http://www2.epa.gov/sap/meeting-materials-december-2-4-2014-scientific-advisory-panel
ExpoCast: http://www2.epa.gov/chemical-research/rapid-chemical-exposure-and-dose-research
Wambaugh 2015. "A Systems Approach to Exposure Modeling (ExpoCast)"

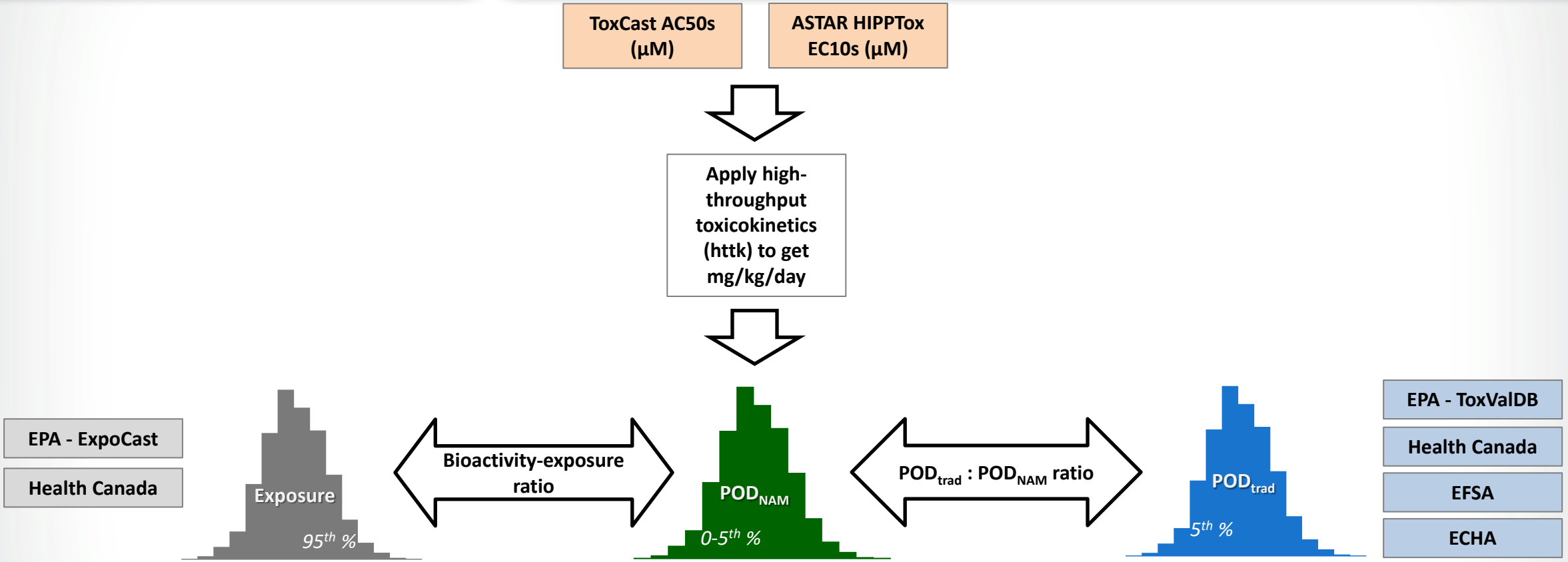
Adding the High-Throughput Toxicokinetic Component



- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through '"httk" R package (https://cran.r-project.org/web/packages/httk/)

Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore *et al., Tox Sci.*, 2015

Development of a POD_{NAM}

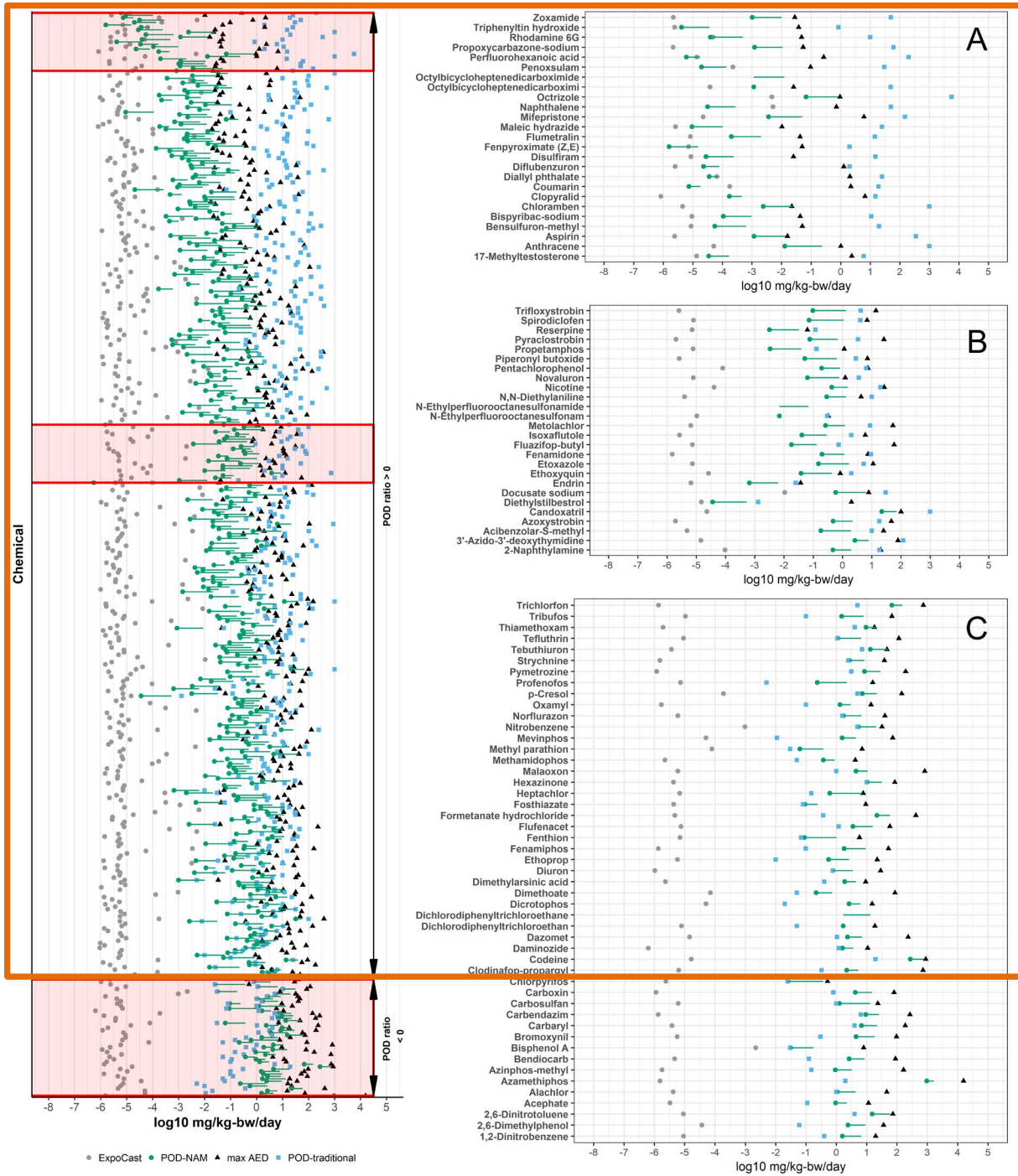


Is BER useful for prioritization?
Are there addressable weaknesses?

Is log10-POD ratio > 0 for most chemicals?
Can we learn from log10-POD ratio < 0?

- *NOEL, LOEL, NOAEL, or LOAEL*
- *Oral exposures*
- *Mg/kg/day*

Paul Friedman et al. *2020 Toxicol Sci. 2020 Jan 1;173(1):202-225. doi: 10.1093/toxsci/kfz201.*



400/448 chemicals =
89% of the time this naïve approach appears conservative

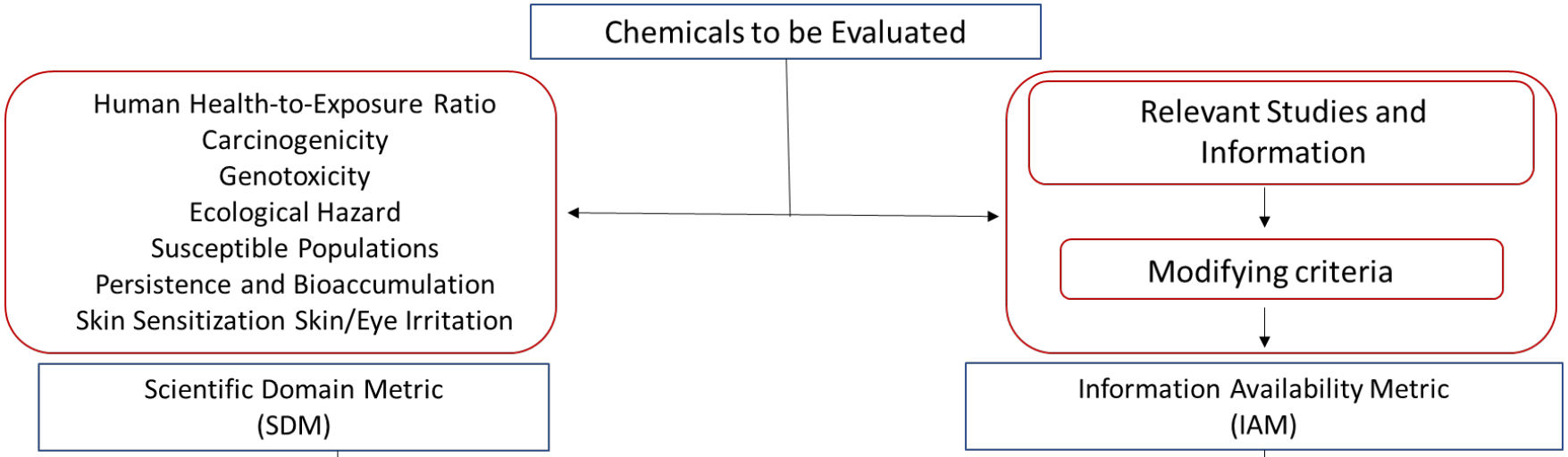
$POD_{NAM} < POD_{traditional}$

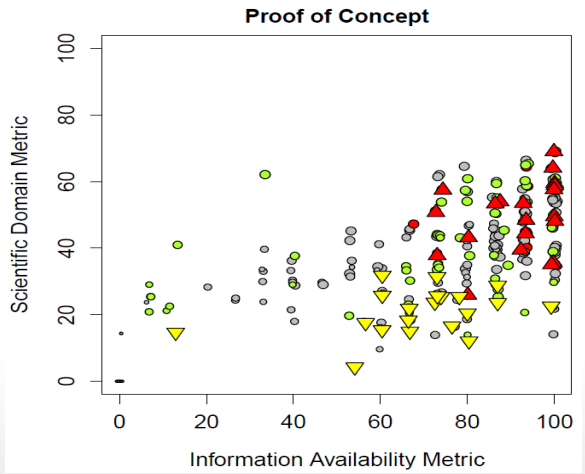
(most of the time)

48/448 chemicals =
11% where $POD_{NAM} > POD_{traditional}$

Paul Friedman et al. *2020 Toxicol Sci. 2020 Jan 1;173(1):202-225. doi: 10.1093/toxsci/kfz201.*

Public Information Curation and Synthesis (PICS) Approach





Summary

- Incorporating new technologies and innovations in toxicology can more rapidly and inexpensively screen chemicals for potential adverse biological effects.
- EPA has made great advances in the development of NAMs for filling information gaps for decision-making and integrating those tools and data streams into chemical risk assessment.
- EPA has worked with other stakeholders to leverage resources and develop NAMs that can support different regulatory contexts.
- Building confidence in the use of NAMs for regulatory decision-making is key to the increased implementation of these methods.

Questions?