United States Environmental Protection Agency

Using high-throughput toxicology to develop tools for regulatory decision-making and screening-level human health risk assessment

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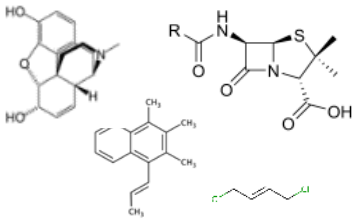
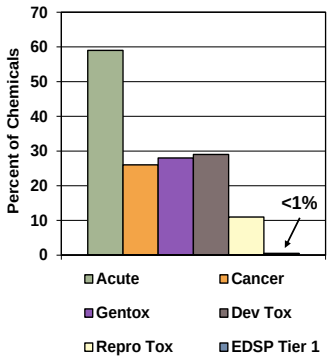
Why can't we use traditional toxicology for all of our problems?

Number of Chemicals /Combinations



Why?

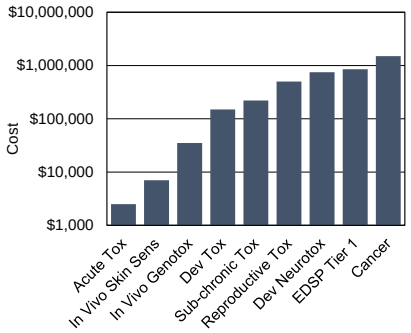
Lack of Data



Modified from Judson *et al.*, EHP 2010

Ethics Concerns

Economics



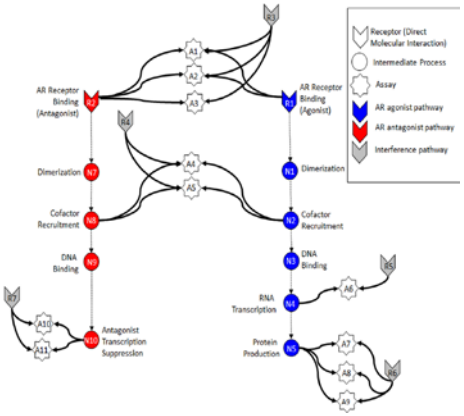
Goals of computational toxicology

- Identify biological pathways of toxicity (AOPs)
- Develop high-throughput *in vitro* assays to test chemicals
- Identify "Human Exposure Chemical Universe" to test
- Develop models that link *in vitro* to *in vivo* hazard
- Use pharmacokinetic models to predict activating doses
- Develop exposure models for all chemicals
- Add uncertainty estimates
- Create high-throughput risk assessments

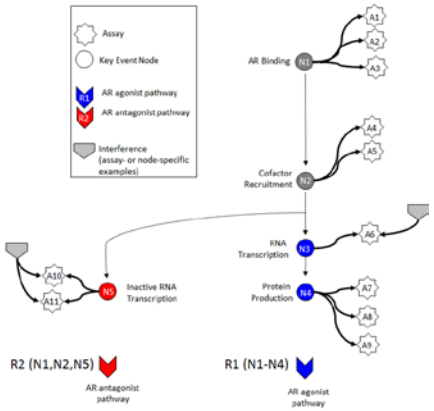
High-throughput toxicology answers scientific and regulatory needs

- We face many environmental challenges:
  - Chemicals, disease, crop-failure, climate change
- Data alone cannot answer all necessary questions:
  - Data can be expensive and noisy
  - Cause and effect relationships are multivariate and non-linear
- Needed: mathematical and statistical models, approximations, and other tools that increase safety and efficiency.

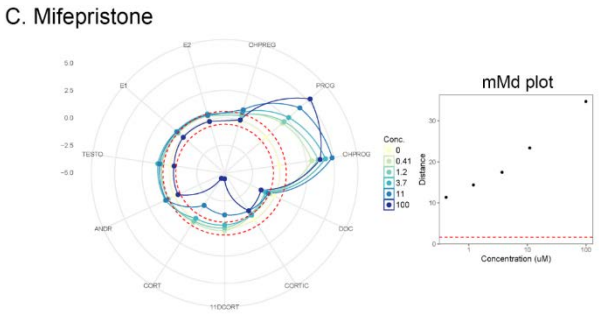
- *Example of a regulatory application: Endocrine Disruptor Screening Program (EDSP/EDSP21)*



Estrogen receptor pathway model

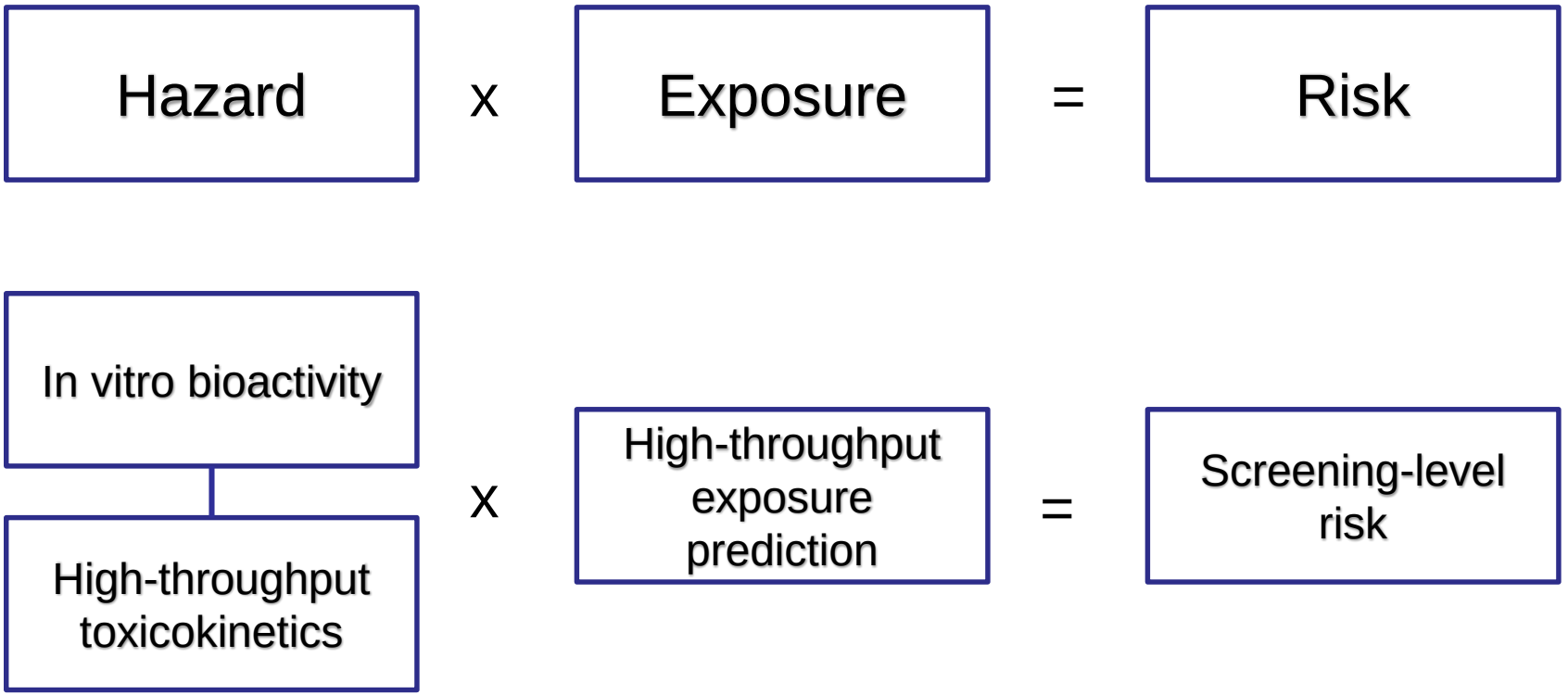


Androgen receptor pathway model

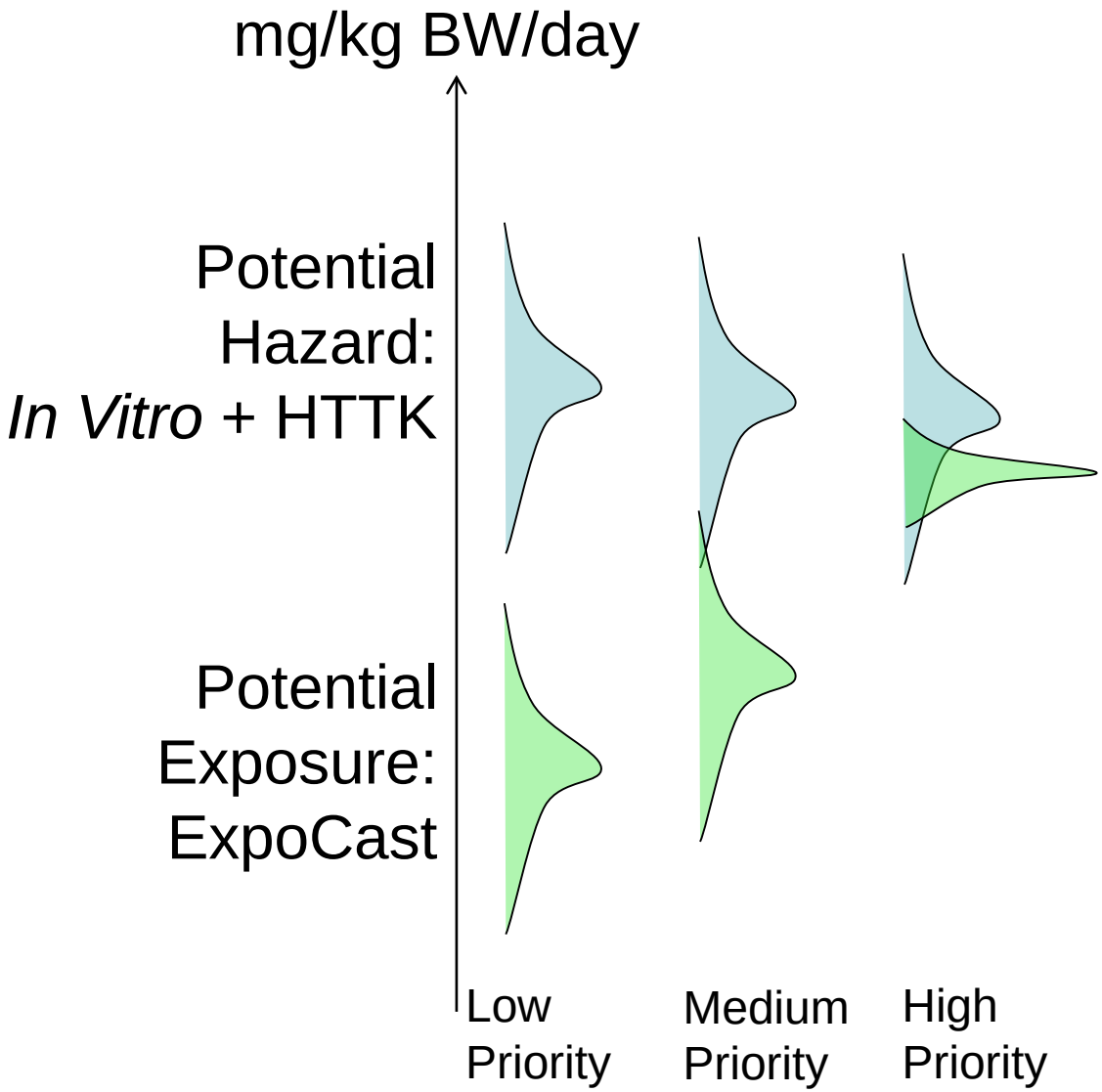


Steroidogenesis HT-H295R model

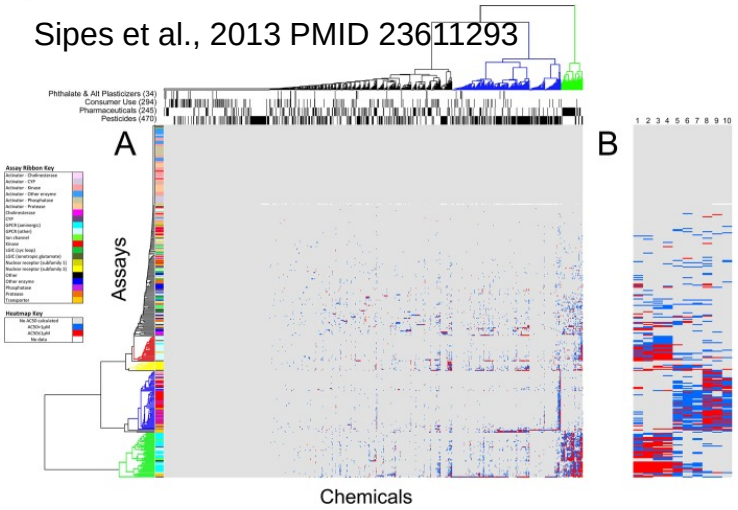
Chemical Risk = Hazard x Exposure



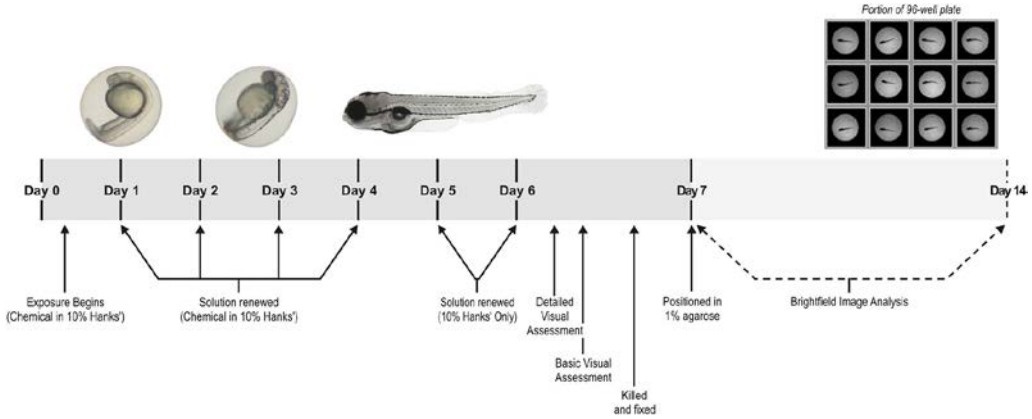
Screening level risk assessments depend on a bioactivity:exposure ratio



Sipes et al., 2013 PMID 23611293



Padilla et al., 2015, 2016, in preparation



The ToxCast program and data pipeline

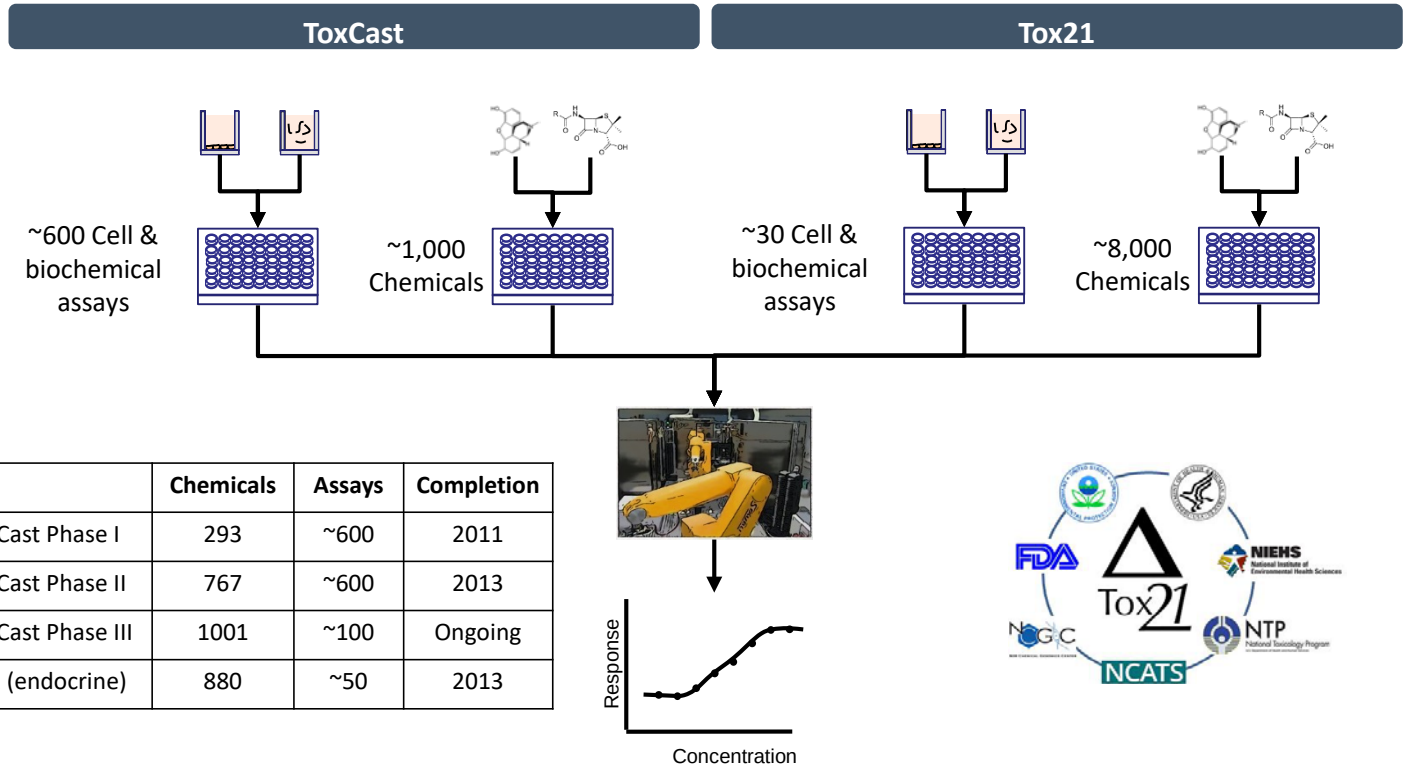
ToxCast Dashboard (current most-detailed assay information interface): https://actor.epa.gov/dashboard/

CompTox Dashboard (many data streams, currently centered on chemistry; Williams et al. 2017 PMID 29185060): https://comptox.epa.gov/dashboard

Data downloads (download databases and supporting data files):

https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

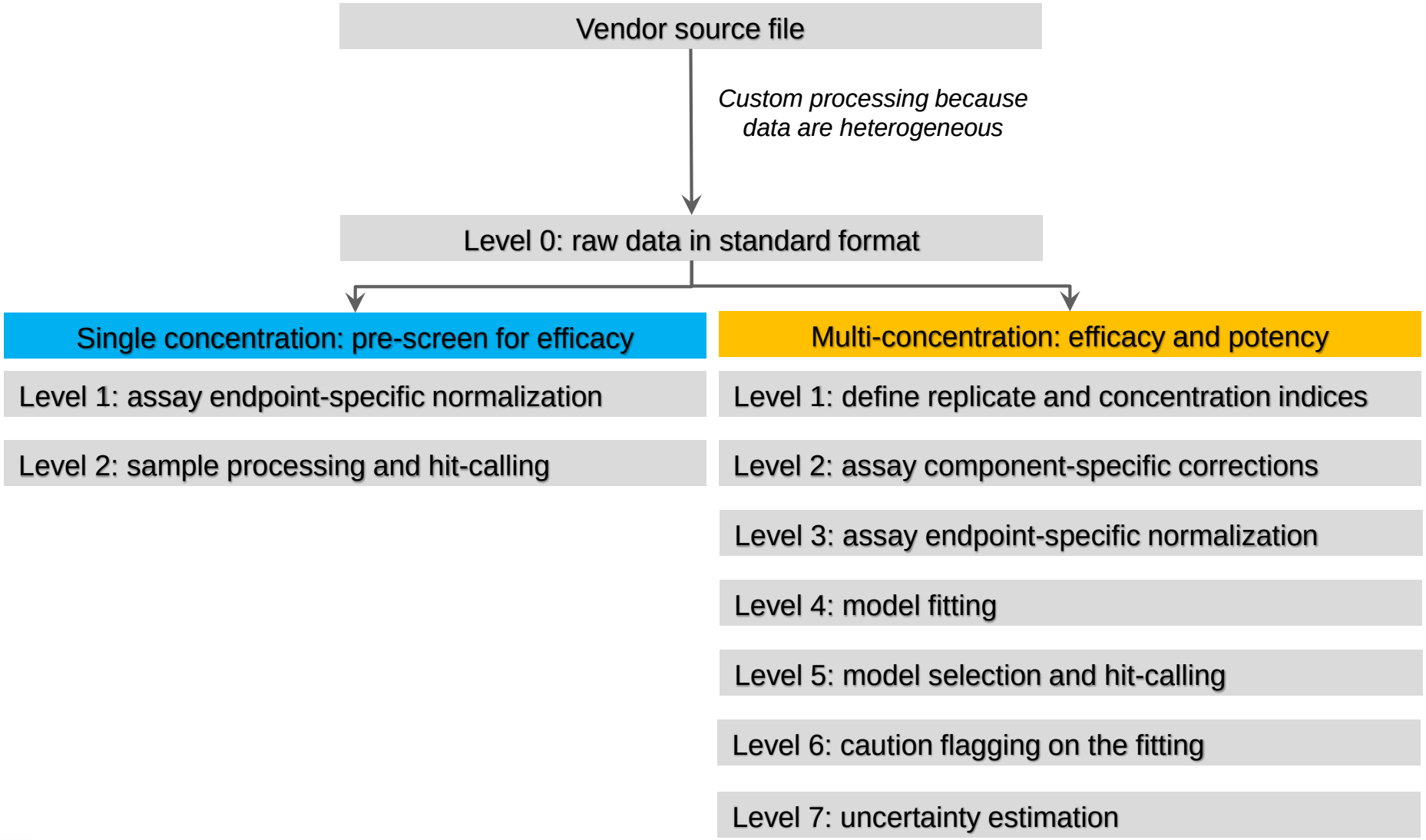
High-Throughput Hazard Screening Component: ToxCast and Tox21



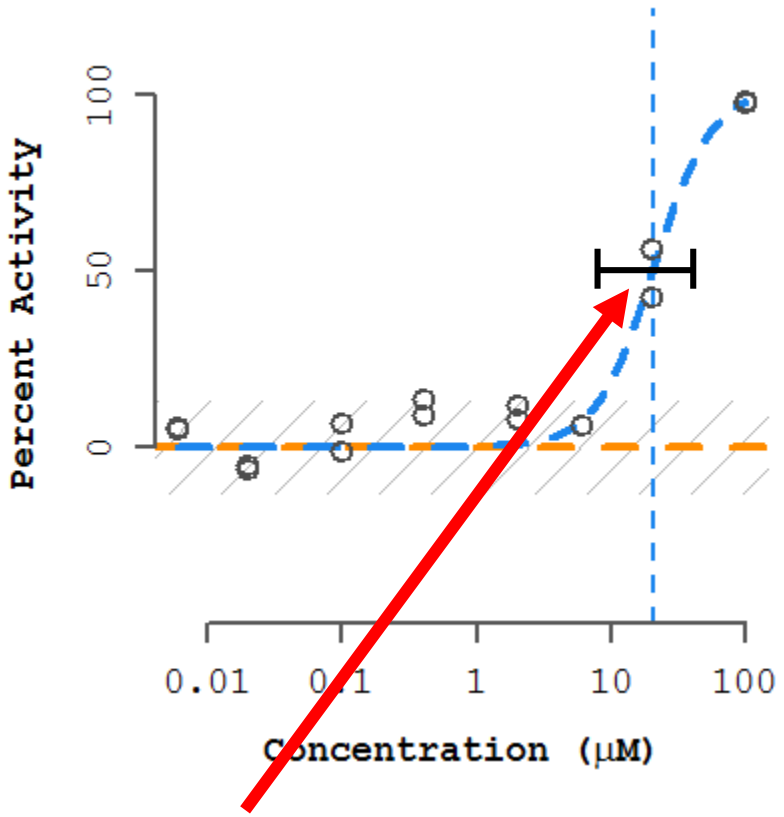
Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013

- All Tox21 data are analyzed by multiple partners
- Tox21 data is available analyzed in the ToxCast Data Pipeline

ToxCast: high-throughput bioactivity information

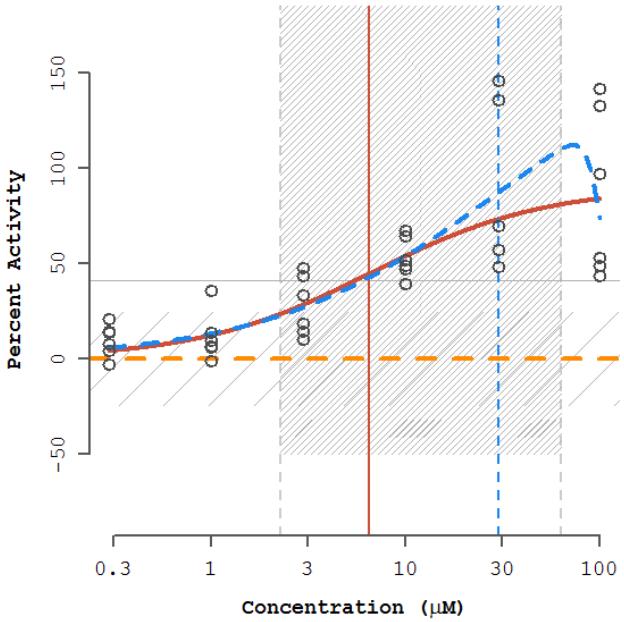


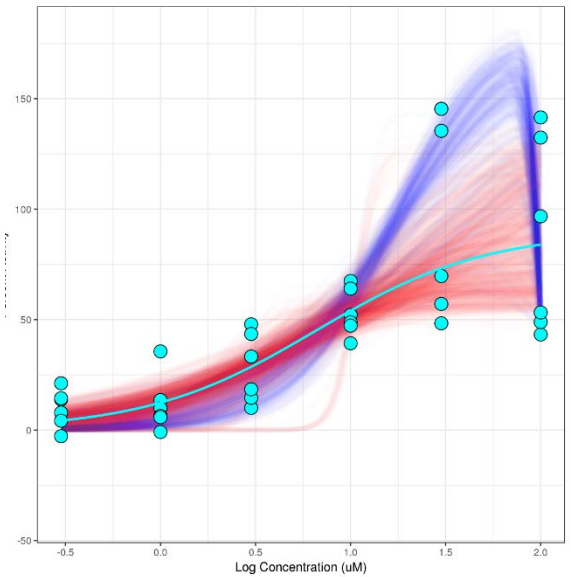
Uncertainty in bioactivity data



- Some sources of uncertainty in fitting high-throughput screening (HTS) data include:
  - Biological variance
  - Systematic error in measurement
  - Contribution of experimental design, e.g. dose-spacing and dose #
- Not quantified in tcpl currently.
- Uncertainty could be incorporated into predictive models, e.g. QSAR, hybrid descriptor sets, etc., and likely impacts predictivity of these models.
- Quantifying uncertainty may support more robust screening level risk assessment.

An example of uncertainty in ToxCast data

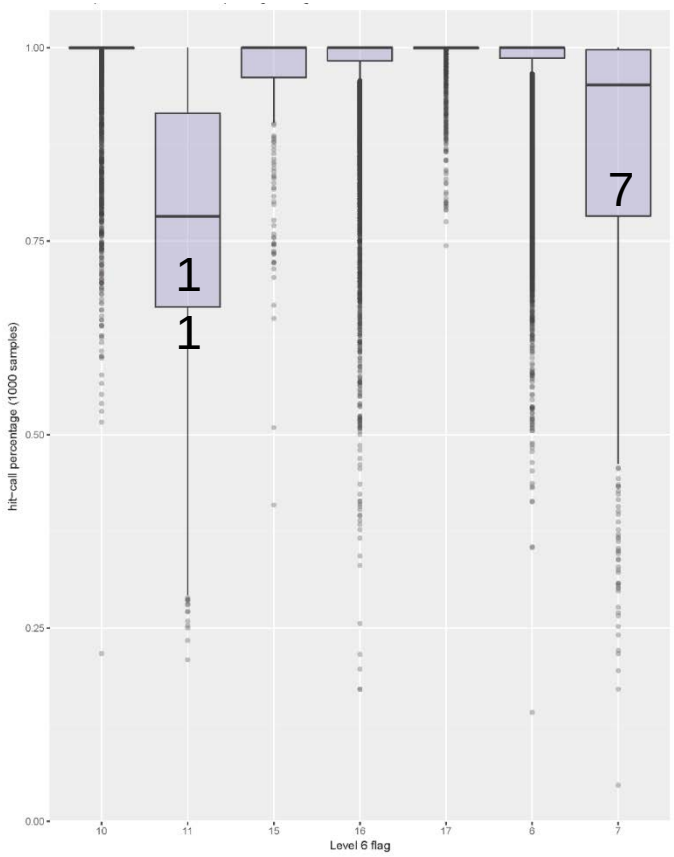


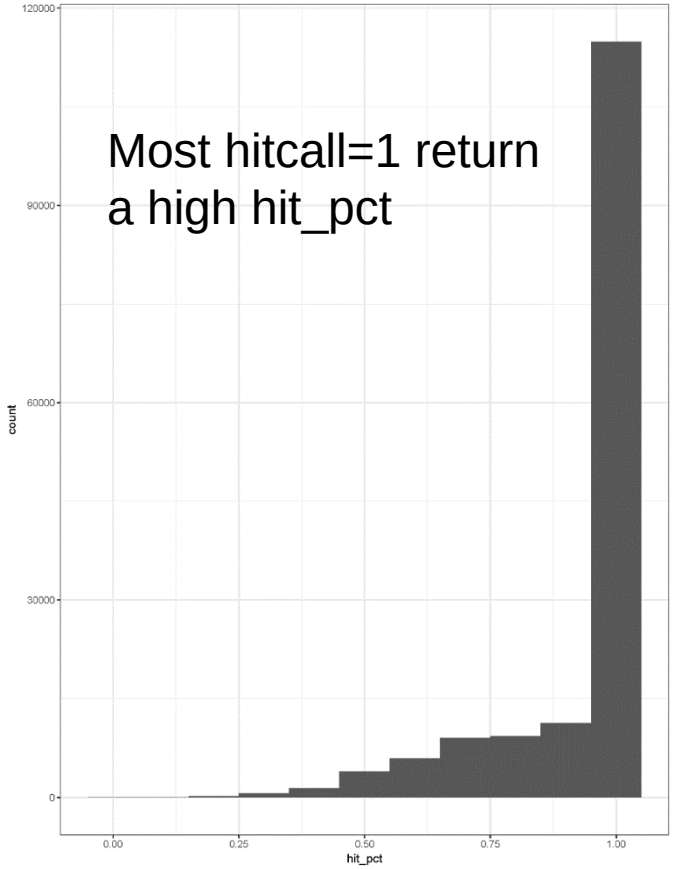



```
ASSAY:    AEID754 (OT_FXR_FXRSRC1_1440)

NAME:     Mifepristone
CHID:     23322    CASRN: 84371-65-3
SPID(S): TP0000759D12
M4ID:     8795482

HILL MODEL (in red):
       tp        ga        gw
val:   89.9      0.817     0.973
sd:    24.4      0.305     0.389

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   175       1.48      0.735     2.02      10.4
sd:    NaN       NaN       NaN       NaN       NaN

       CNST          HILL          GNLS
AIC:   403.91        345.89        348.64
PROB:  0             0.8           0.2
RMSE:  64.8          27.91         26.62

MAX_MEAN: 100        MAX_MED: 103        BMAD: 8.17

COFF: 40.9    HIT-CALL: 1    FITC: 42    ACTP: 1

FLAGS:

HIT-PCT: 1  MED-GA: 1.1354  GA-CI: 1.4462
```

- Toxboot: resamples datapoints from the curve for an m4id, with added noise (0 mean) (*Watt et al., submitted*).
- Tcpl level 4 (mc4) fitting of resampled data.
- Repeat x1000.
- Store the information from each resampled fit in ToxCast/invitrodb (*Brown et al., in prep*).

Can level 5 fit information, level 6 caution flags, level 7 uncertainty information, and human curation help to build a model to predict data that is fit “well?”





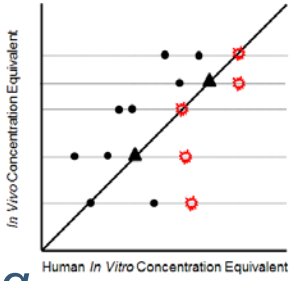
- Several patterns of caution flags evident, but hard to use flag patterns alone to remove fits based on noise or overfitting.
- Most hitcall=1 return a high hit_pct, but some borderline candidates could easily be removed.

Brown et al., in prep

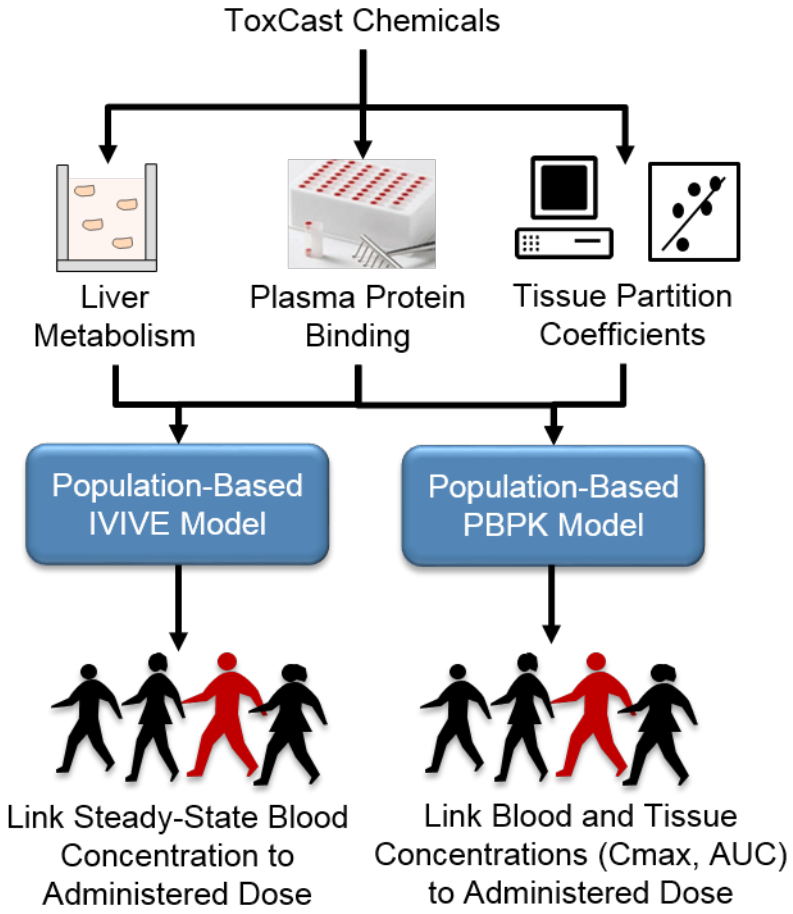
EPA United States Environmental Protection Agency

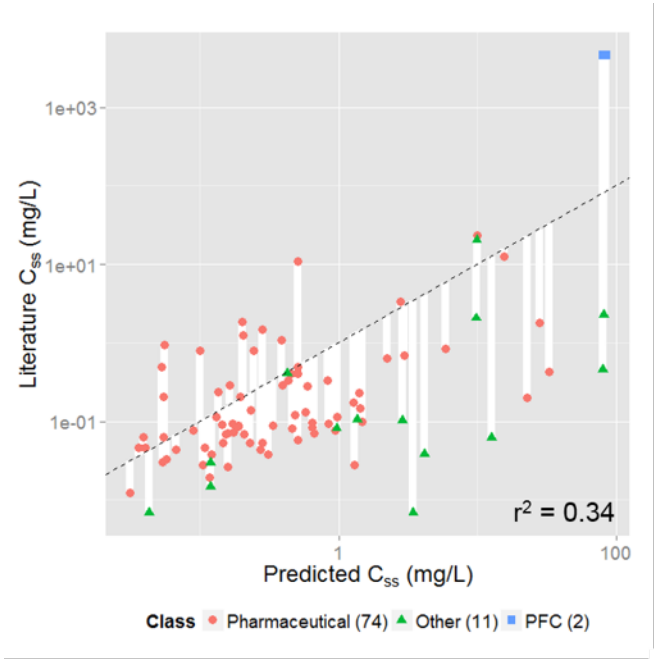
Connecting *in vitro* bioactivity to an administered dose equivalent and to exposure

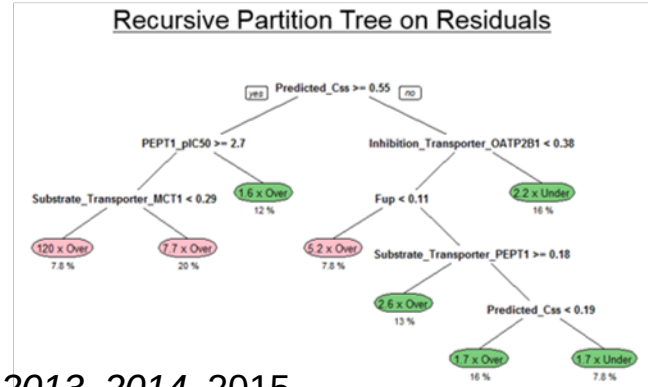
Toxicokinetics Modeling



Incorporating Dosimetry and Uncertainty into In Vitro Screening

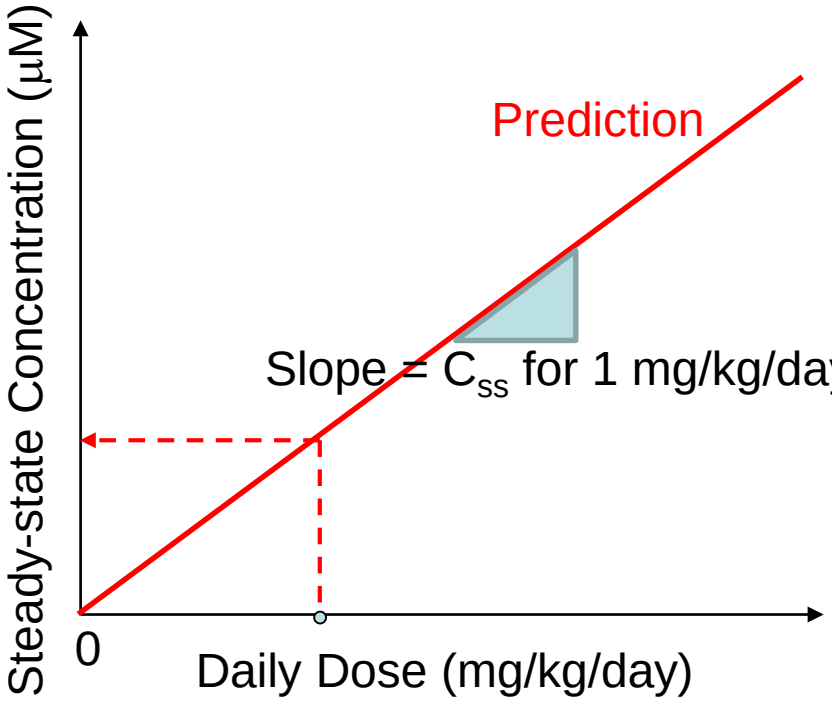


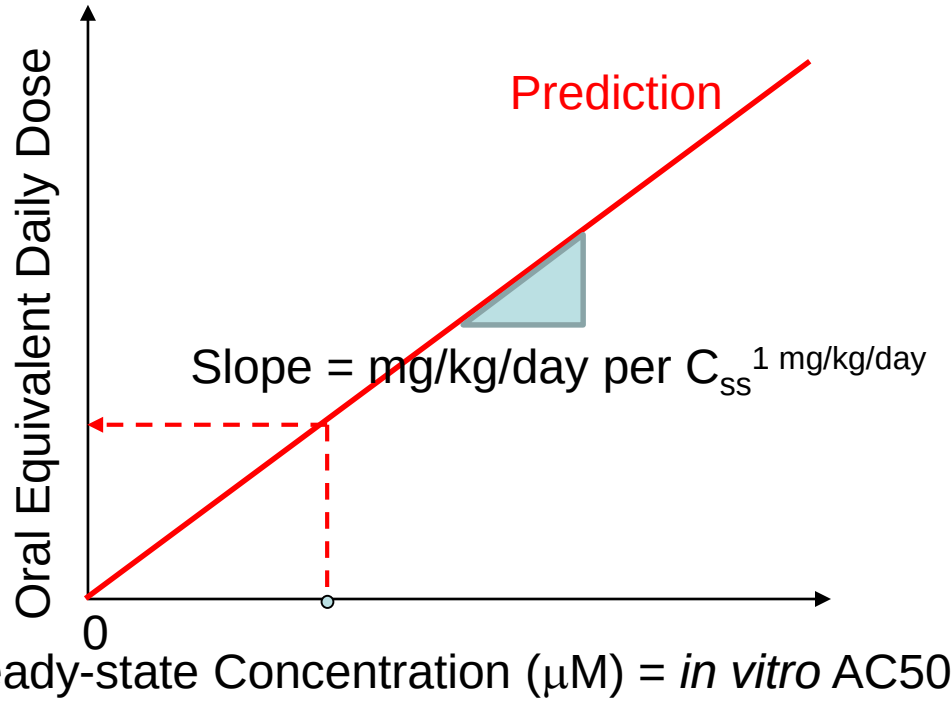




Wetmore, Rotroff, Wambaugh *et al., 2013, 2014,* 2015

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





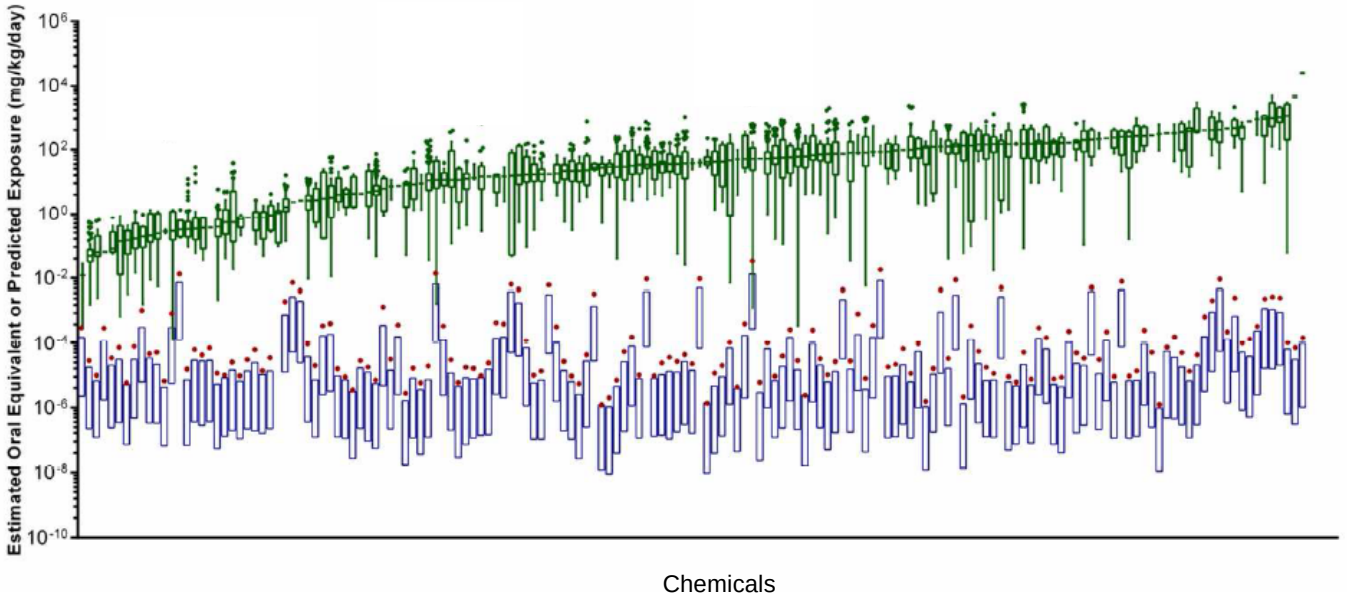
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

Wetmore *et al*. (2012)

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

Slide from John Wambaugh

Comparing Bioactivity with Exposure Predictions for Risk Context



Wetmore *et al., Tox Sci.,* 2015

United States Environmental Protection Agency

EDSP21: example of fit-for-purpose tools

ER Pathway Model (Judson et al., 2015; Browne *et al.* 2015)

AR Pathway Model (Kleinstreuer et al., 2017)

Steroidogenesis Model (Karmaus et al., 2016; Haggard et al., 2017)

EDSP21 Project: Major Points

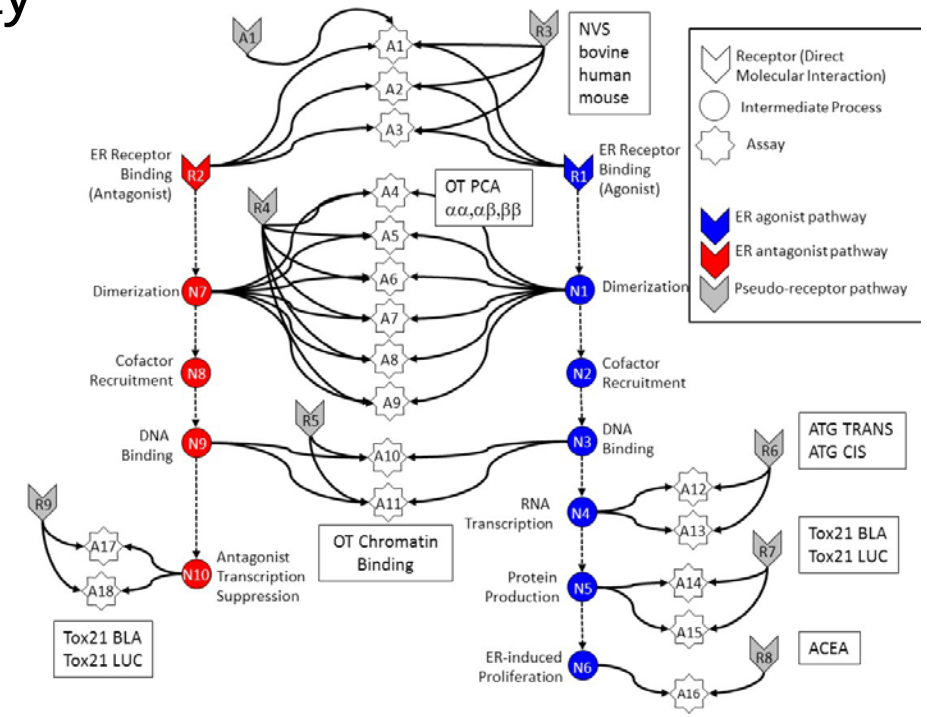
- EDSP: Endocrine Disruptor Screening Program
  - Mandated by U.S. Congress
  - "Tier 1 battery" – 11 in vitro and in vivo assays (estrogen, androgen, thyroid)

- EDSP has a mismatch between resources needed for Tier 1 and number of chemicals to be tested
  - ~10,000 chemicals in EDSP Universe
  - ~$1M per chemical for Tier 1, 50-100 year backlog

- Demonstrate new approach: Estrogen Receptor (ER)
  - Multiple high-throughput *in vitro* assays
  - Prioritize chemicals and replace selected Tier 1 assays

In Vitro Estrogen Receptor Model

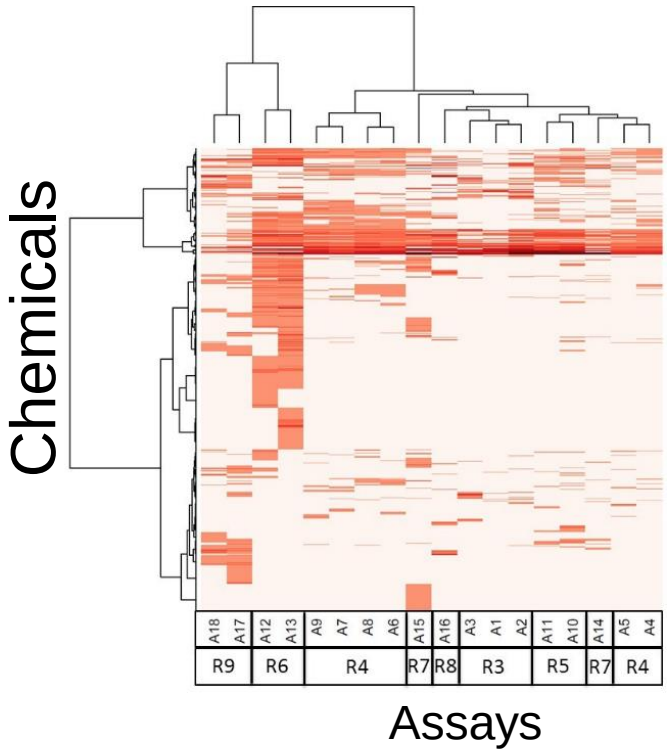
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (EHP 2015)

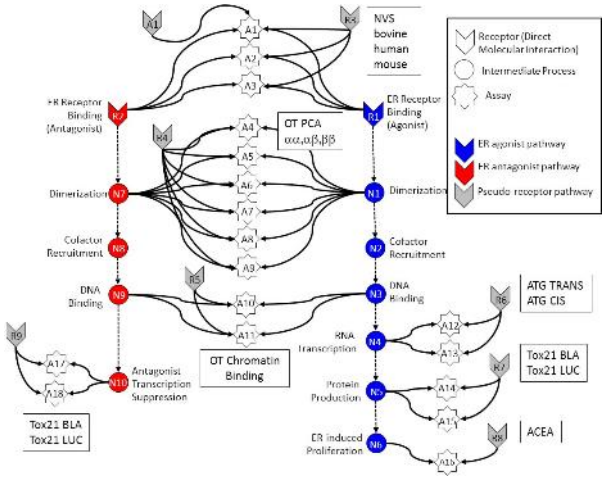
All *in vitro* assays have false positives and negatives

Assays cluster by technology, suggesting technology-specific non-ER bioactivity



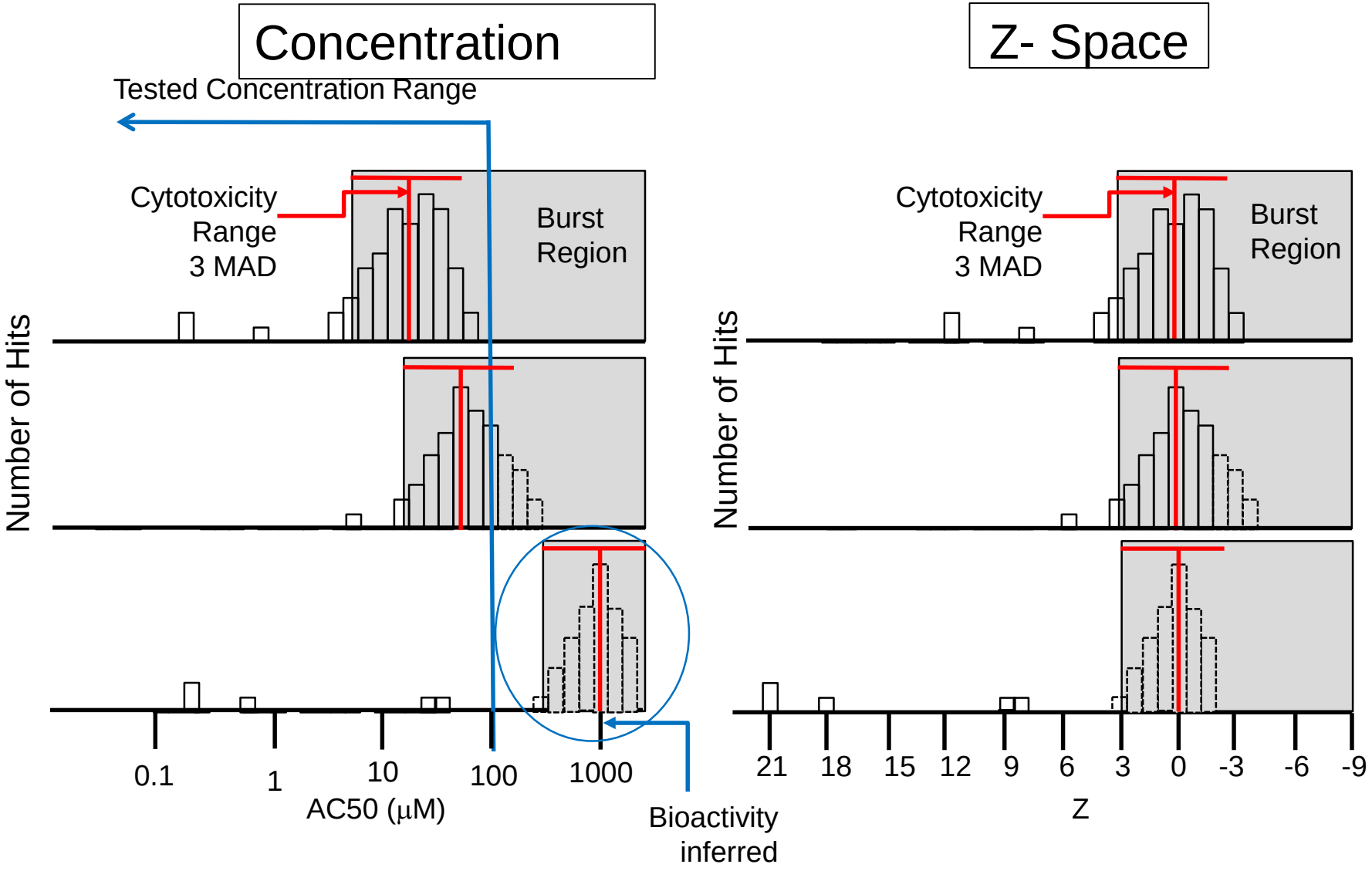
Much of this “noise” is reproducible
- “assay interference”
- Result of interaction of chemical with complex biology in the assay

EDSP chemical universe is structurally diverse
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



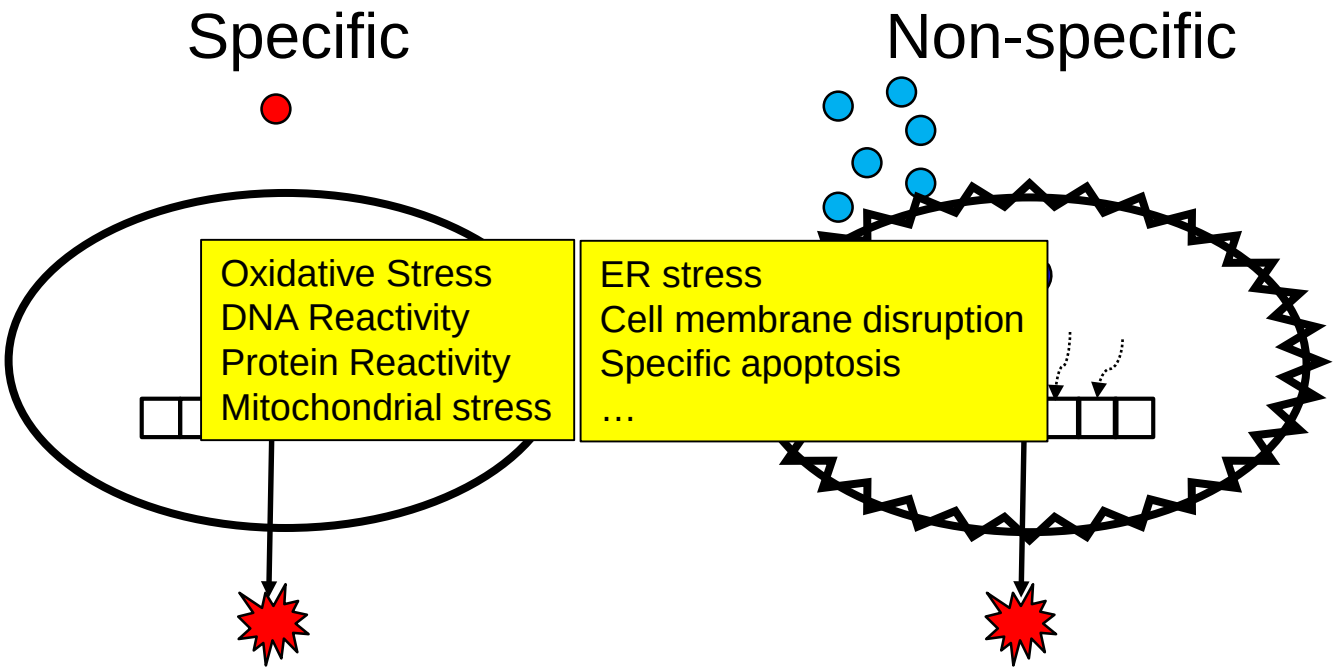
Judson et al: ToxSci (2015); slide from Richard Judson

Most chemicals display a “burst” of potentially non-selective bioactivity near cytotoxity concentration



Judson et al. Tox.Sci. (2016); slide from Richard Judson

Schematic explanation of the burst



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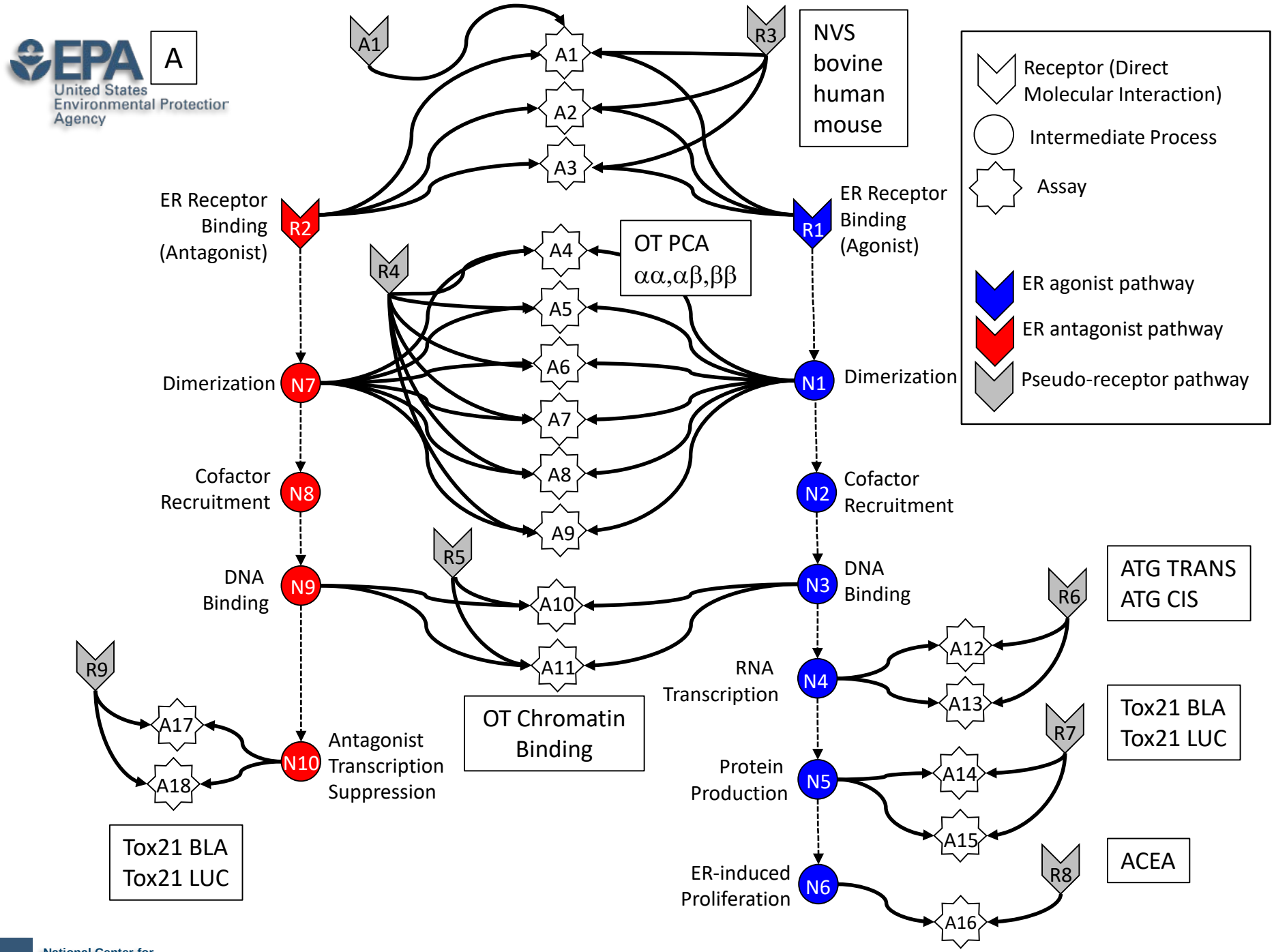
A
United States Environmental Protection Agency
NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
R1
R2
R3
R4
R5
R6
R7
R8
R9
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A4
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A14
A15
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A18
N1
N2
N3
N4
N5
N6
N7
N8
N9
N10
ER Receptor Binding (Agonist)
ER Receptor Binding (Antagonist)
OT PCA
αα,αβ,ββ
Dimerization
Dimerization
Cofactor Recruitment
Cofactor Recruitment
DNA Binding
DNA Binding
RNA Transcription
Protein Production
ER-induced Proliferation
Antagonist Transcription Suppression
OT Chromatin Binding
ATG TRANS
ATG CIS
Tox21 BLA
Tox21 LUC
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ACEA
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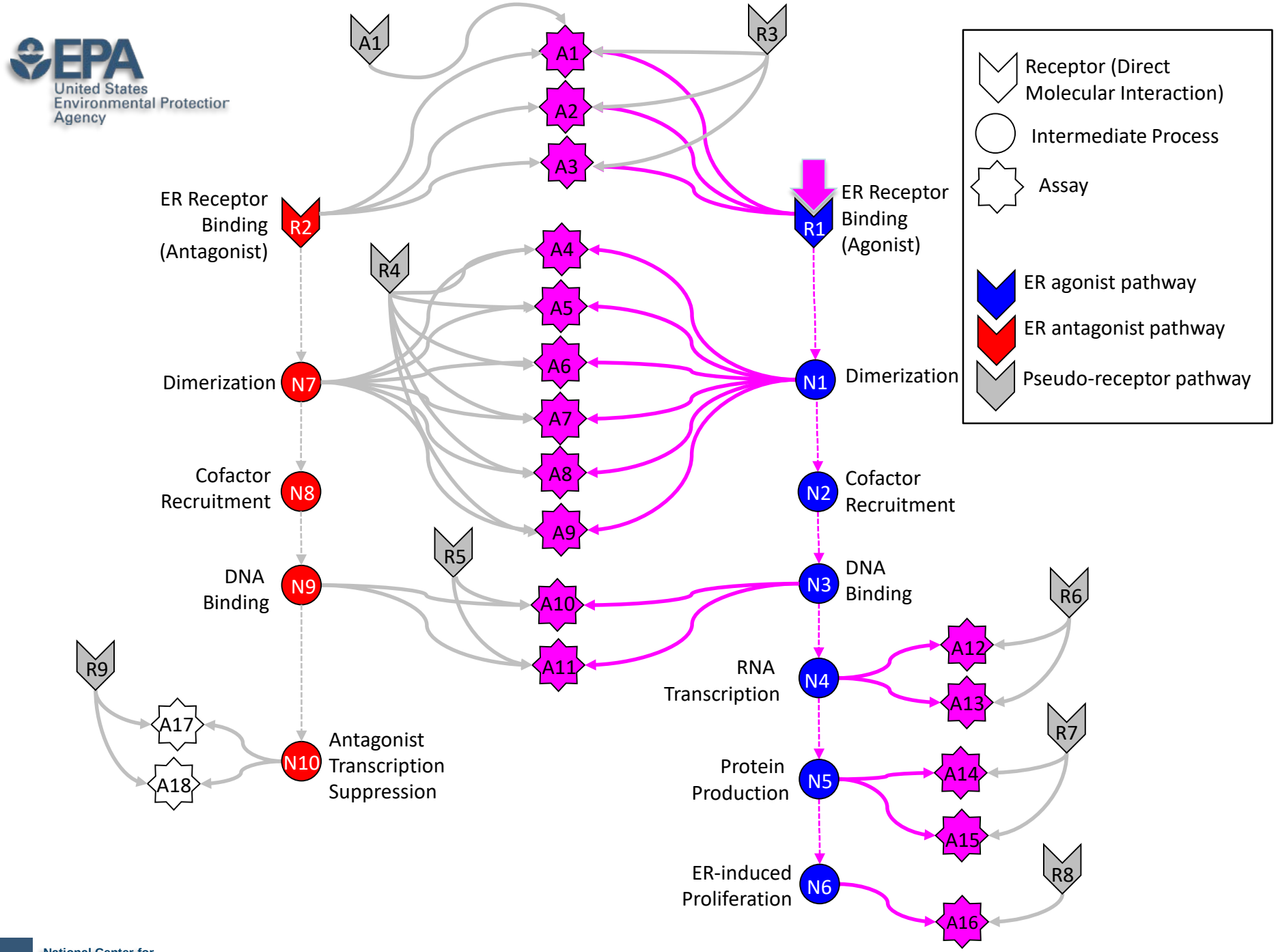
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(Agonist)
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Dimerization
Cofactor
Recruitment
Cofactor
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Binding
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Transcription
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Production
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Proliferation
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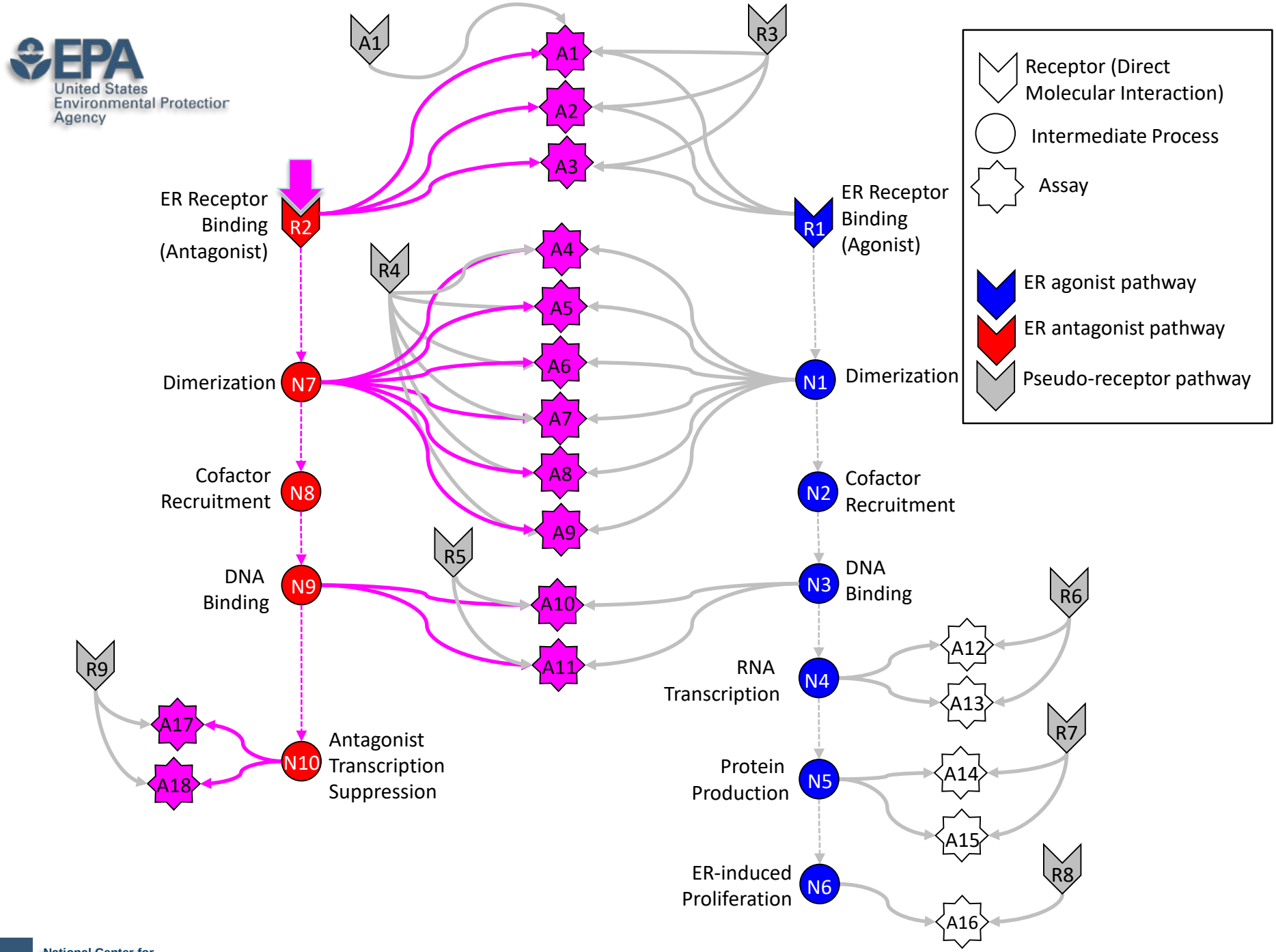
National Center for
Computational Toxicology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Binding
N3
Antagonist
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Suppression
N10
RNA
Transcription
N4
Protein
Production
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ER-induced
Proliferation
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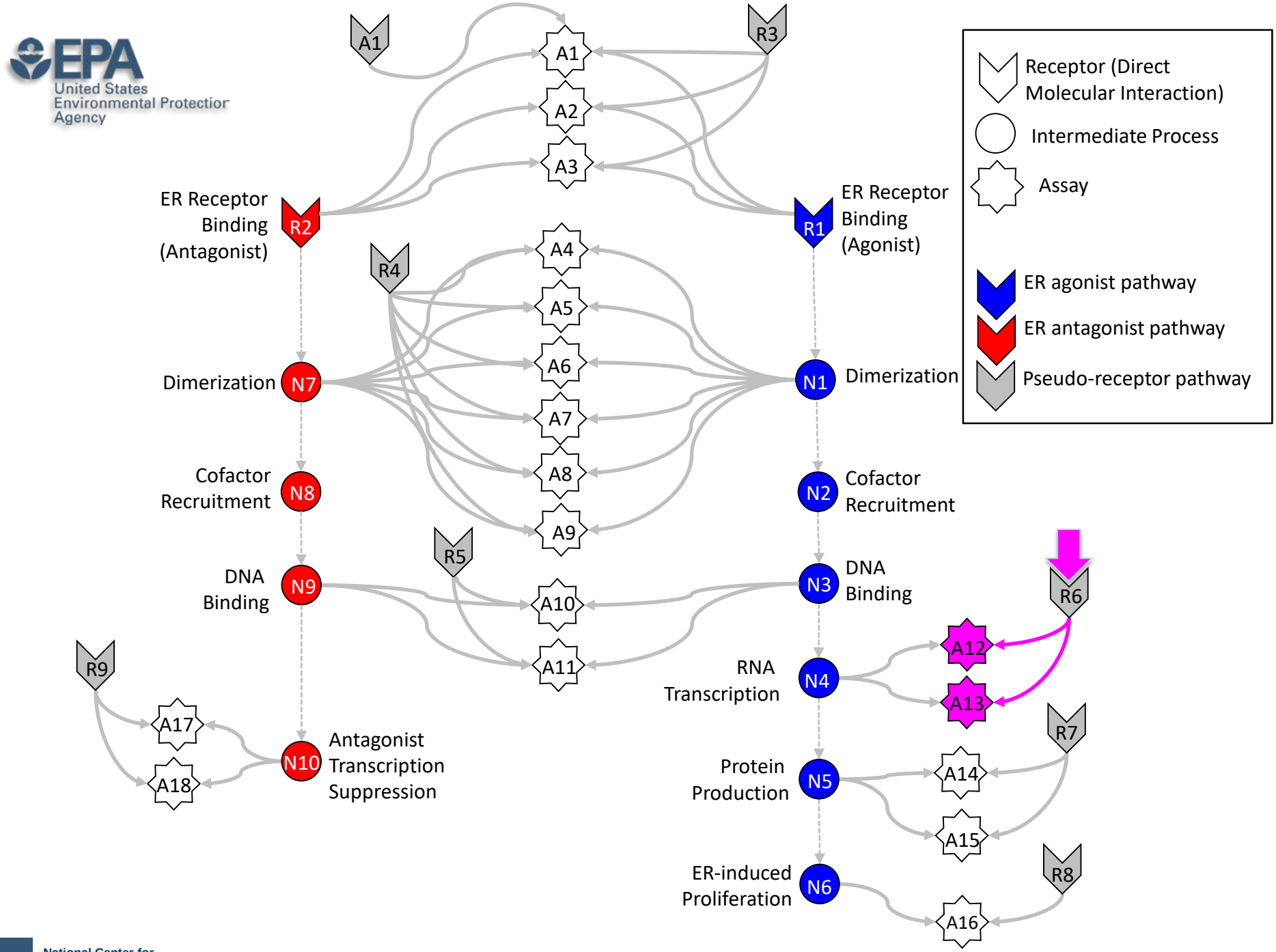
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Antagonist
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R1
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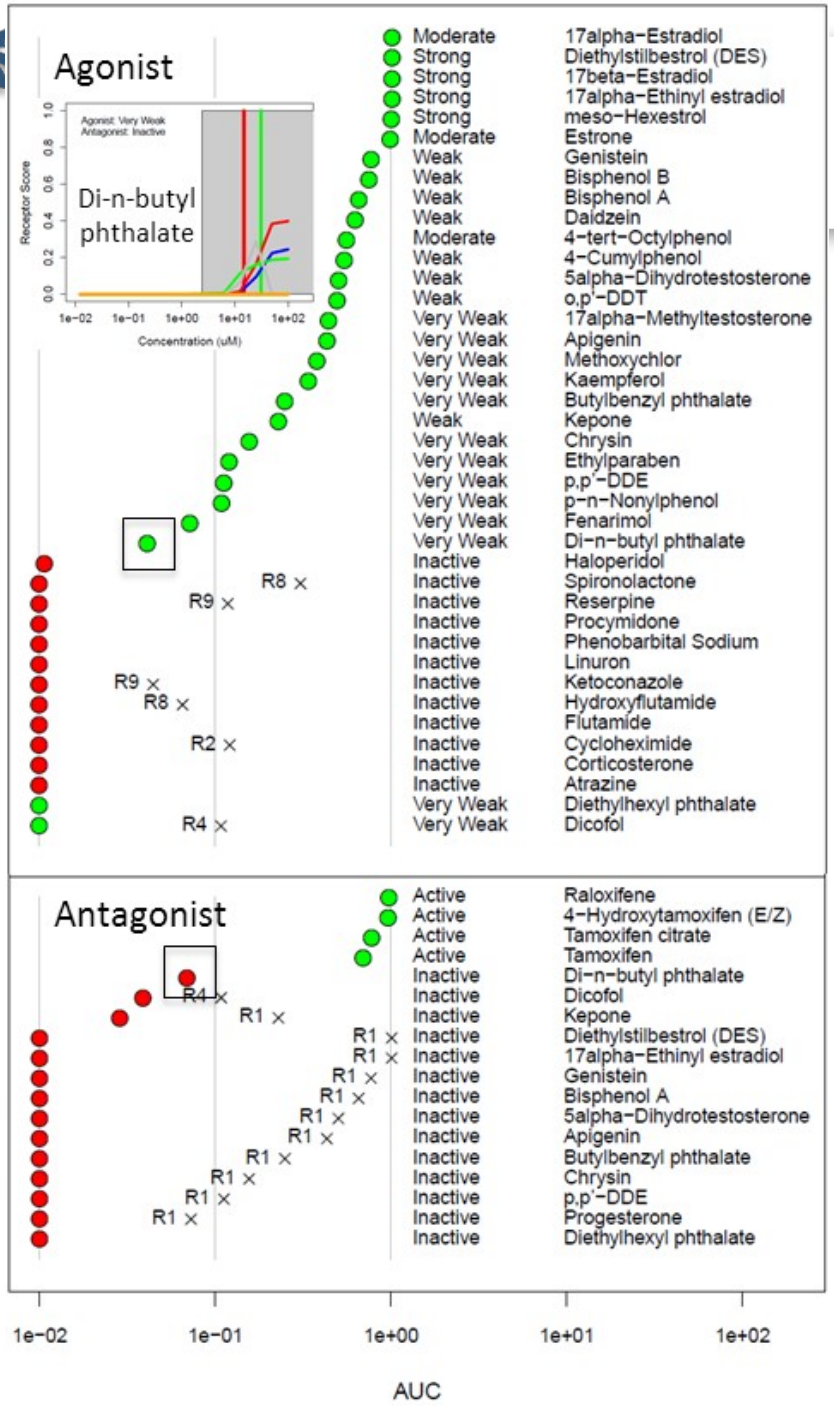
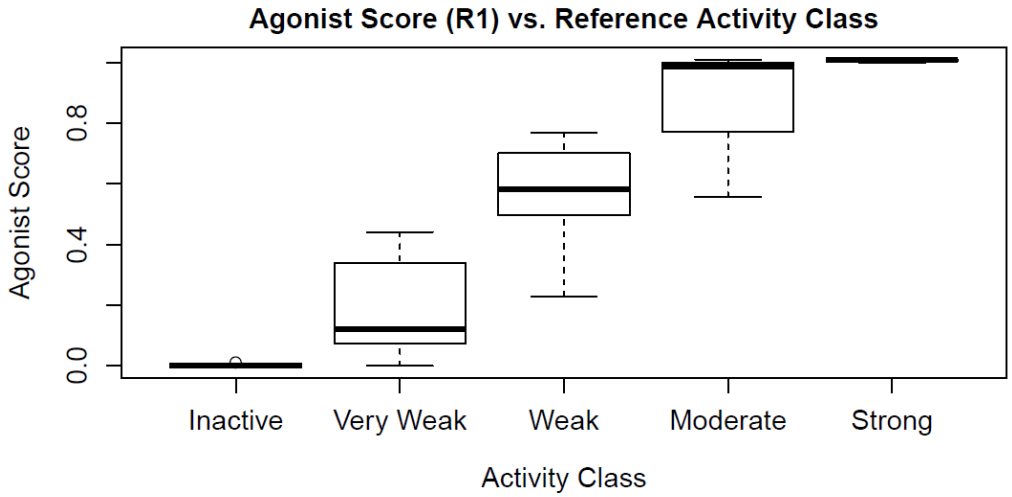
N10
National Center for
Computational Toxicology

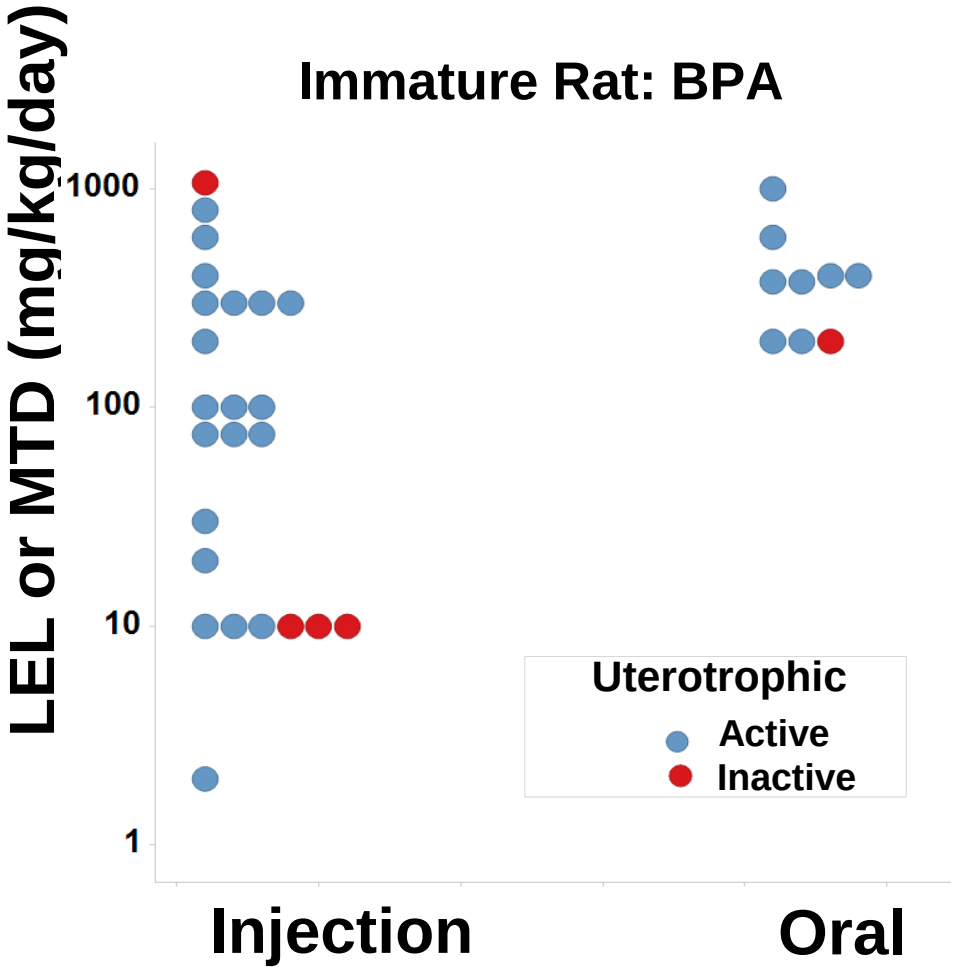
In Vitro Reference Chemical Performance





In vivo guideline study uncertainty

26% of chemicals tested multiple times in the uterotrophic assay gave discrepant results

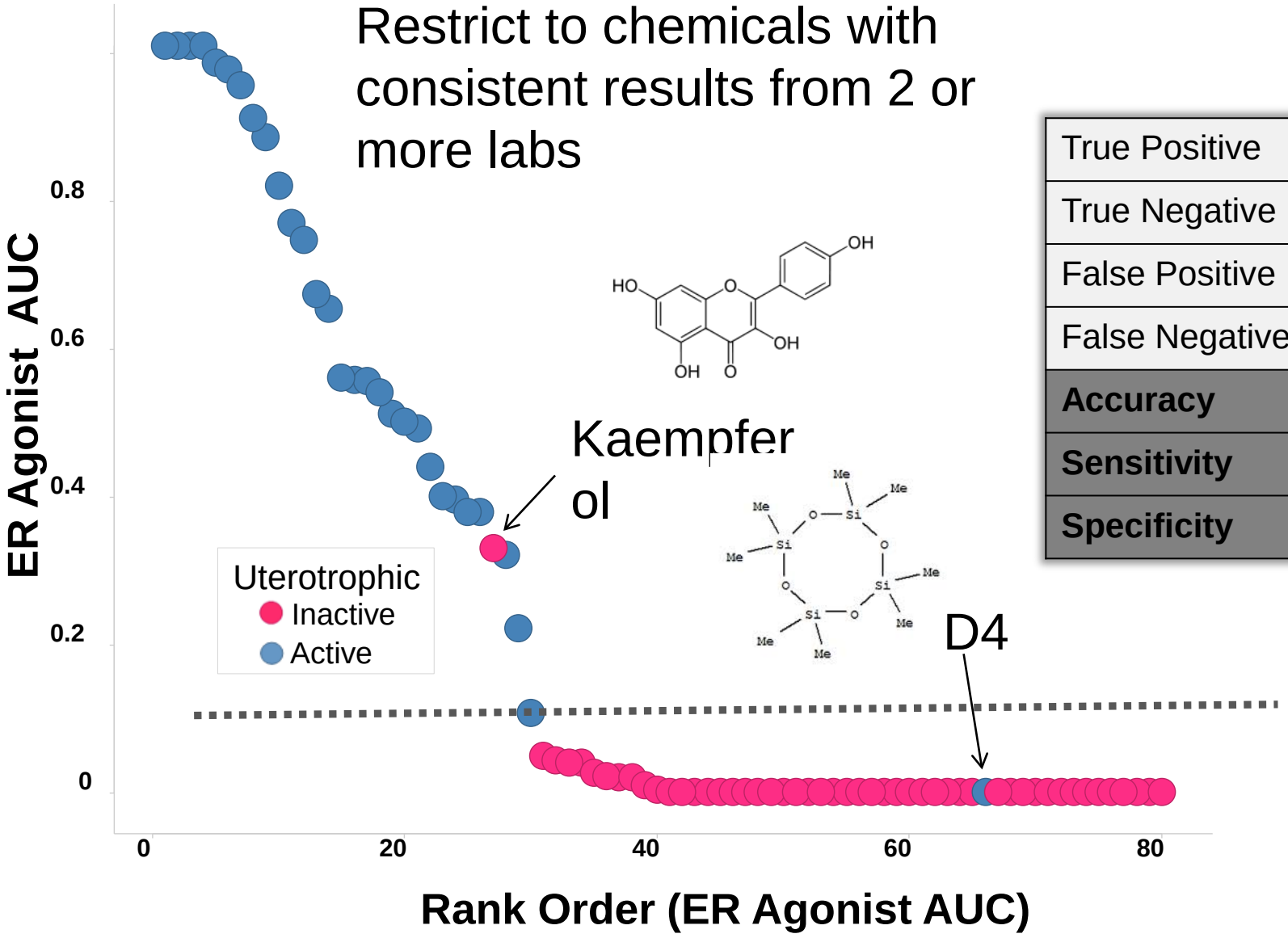


Phenotype X

species / study 1	species / study 2	Reproduce	Does Not Reproduce	Fraction Reproduce
rat SUB	rat CHR	18	2	0.90
rat CHR	dog CHR	13	2	0.87
rat CHR	rat SUB	18	4	0.82
rat SUB	rat SUB	16	4	0.80
rat SUB	dog CHR	11	4	0.73
mouse CHR	rat CHR	11	4	0.73
mouse CHR	rat SUB	13	7	0.65
dog CHR	rat SUB	11	6	0.65
dog CHR	rat CHR	13	8	0.62
rat CHR	mouse CHR	11	11	0.50
mouse CHR	dog CHR	6	6	0.50
rat SUB	mouse CHR	13	14	0.48
dog CHR	mouse CHR	6	8	0.43
mouse CHR	mouse CHR	2	3	0.40

Kleinstreuer et al. EHP 2016

Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic

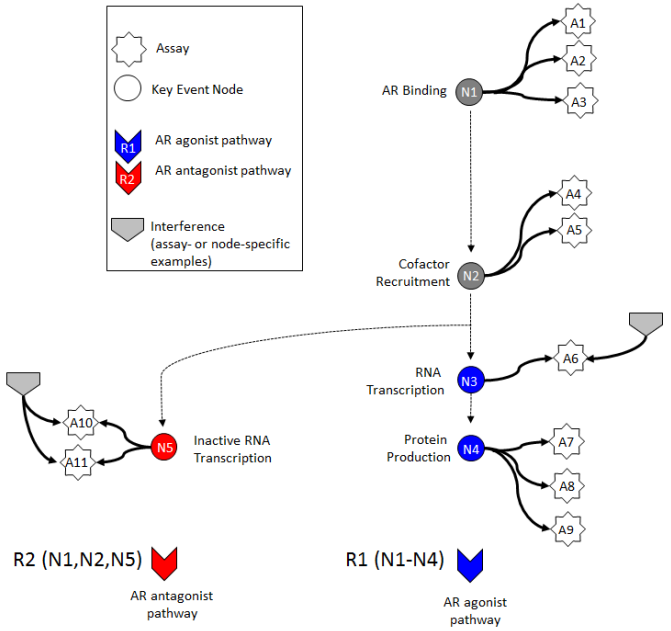


True Positive	29
True Negative	50
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.98**

Browne et al. ES&T (2015)

AR Pathway Model (Kleinstreuer et al., 2017); very similar to ER Pathway Model

- No assay is perfect
  - Test different biology
    - Cell system
    - Signaling mechanism
    - Differential sensitivity
  - Assay Interference
  - Noise
- Here, different technologies cover different points on AR pathway
- Use a mathematical model to integrate data from assays
- Model creates a composite dose-response curve for each chemical to summarize results from all assays



Key Points of the AR (and ER) Model

- Beginning Question: If any one AR assay is active, is the chemical an AR agonist/antagonist?
  - No: there can be false positive (and negative) activity
- Goal of the model is to distinguish true AR activity from false activity
- Mathematically / statistically test multiple sources of activity:
  - True agonist, true antagonist, several interference modes
  - Quantify each mode by AUC value (area under the dose-response curve)
  - Mode with the highest AUC is selected
  - AUC is not potency, but potency values are provided

United States Environmental Protection Agency

Steroidogenesis: progress of current tool development

See also: Haggard et al., 2017; Karmaus et al., 2016; and EDSP SAP documents from November 2017.

Steroidogenesis is critical for several physiological processes.

- Steroidogenesis: cholesterol → steroid hormones.
- Important physiology: sexual differentiation and development, reproduction, metabolism, etc.
- 4 major classes of steroid hormones synthesized largely in separate tissues *in vivo*: progestagens, corticosteroids, androgens, and estrogens.

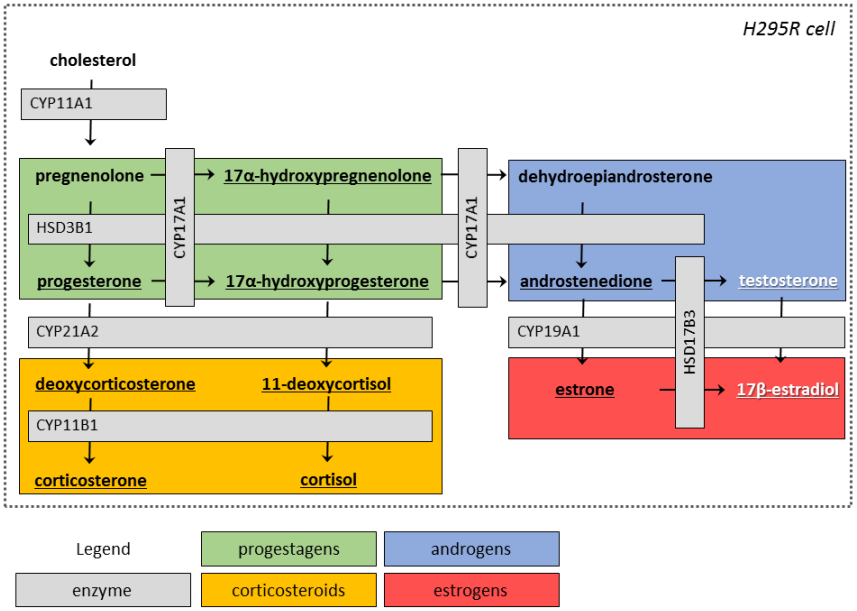
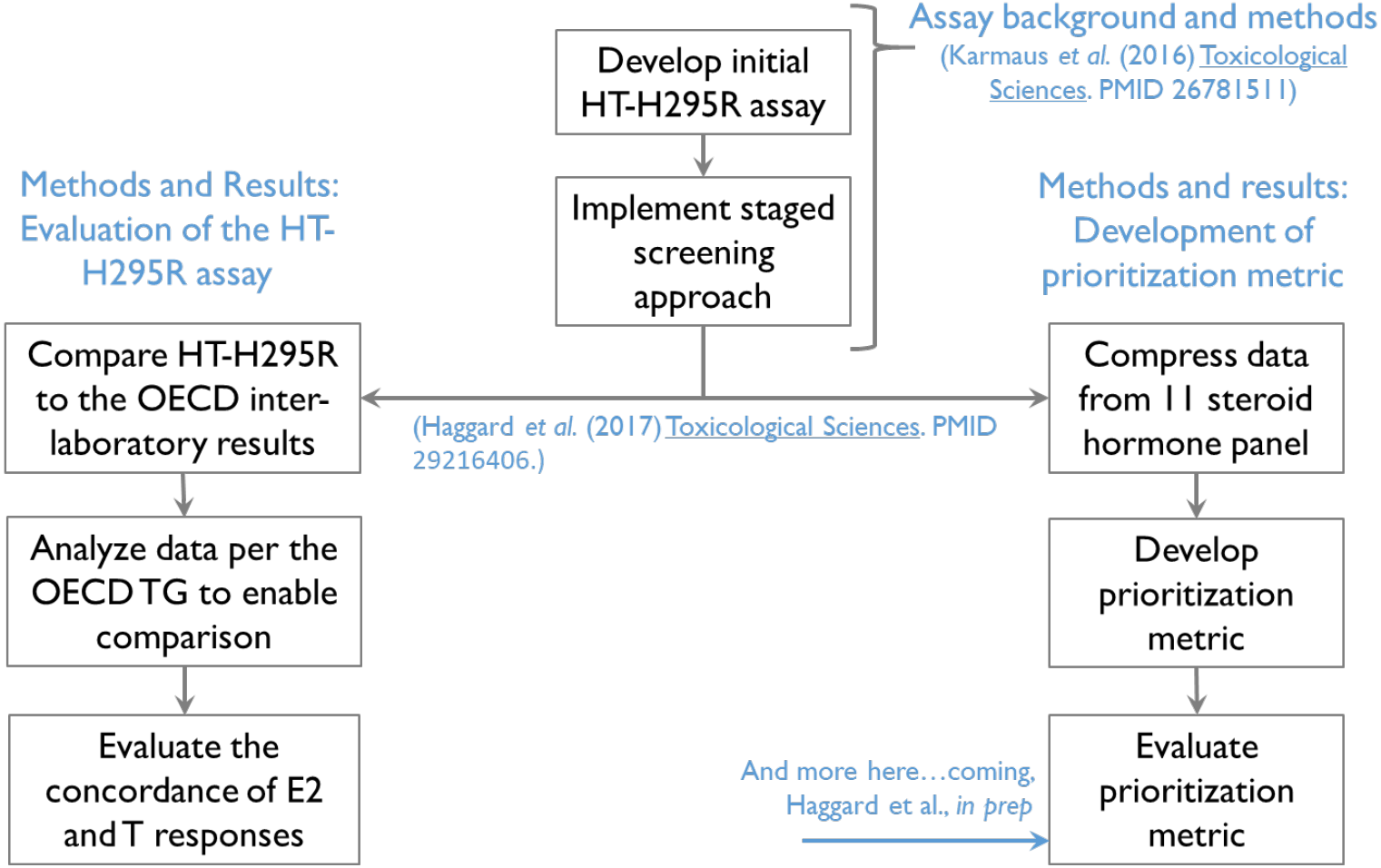
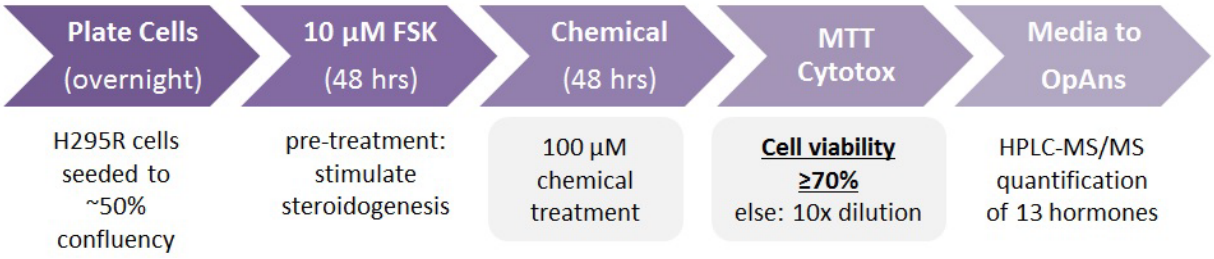


Fig 1 in Haggard et al. (2017).

Steroidogenesis approach – a little different from ER and AR pathway models



HT-H295R assay method



- Maximized screening resource efficiency.
- 2012 unique test chemicals have been screened at a high concentration.
- # steroid hormones affected in single concentration (along with other considerations) were used to select 656 chemicals for multi-concentration screening.

Confusion matrices demonstrate good sensitivity, specificity, and accuracy for reference chemicals.

Effect	Revised Sensitivity	Revised Specificity	Revised Accuracy
Testosterone up	1.00	0.89	0.90
Testosterone dn	0.67	0.92	0.82
Estradiol up	0.75	0.83	0.80
Estradiol dn	0.80	1.00	0.95

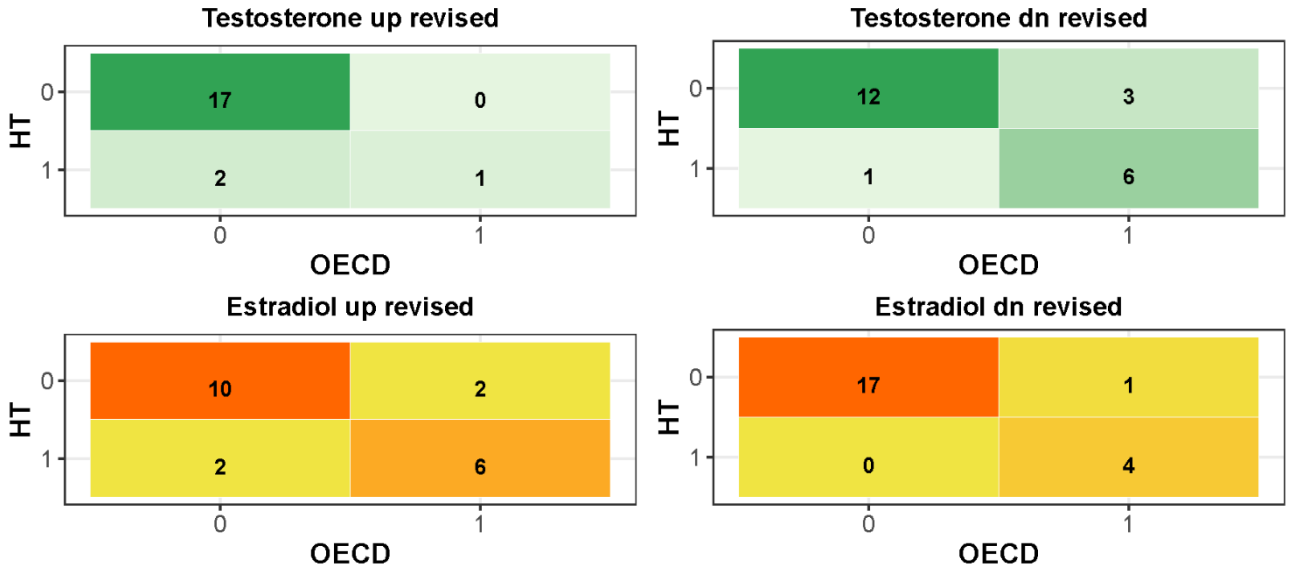


Figure 6 Haggard et al. (2017).

Agreement among labs in the inter-laboratory validation: compounding the lesson that one must consider variance in the reference data

- For any effect on testosterone:
  - Average concordance among labs was 0.88, 0.91, and 0.90 for the 12 core reference chemicals only, the 16 supplemental reference chemicals only, and the entire set.

- For any effect on estrogen:
  - Average concordance among labs was 0.95, 0.84, and 0.89 for the 12 core reference chemicals only, the 16 supplemental reference chemicals only, and the entire set.

Example of the 11-dimensional results for prochloraz

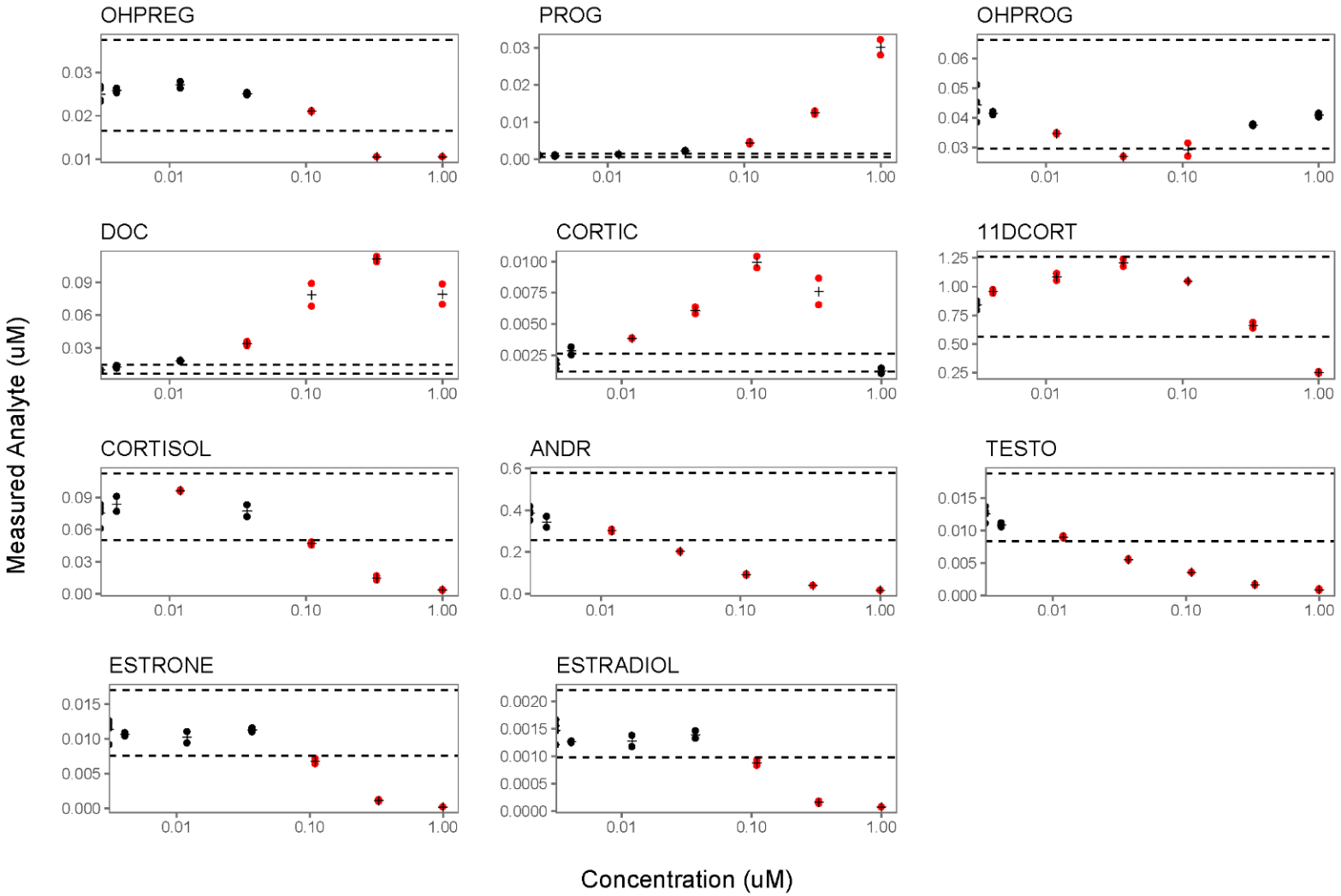


Figure 2 Haggard et al. (2017).

Using our maximum mean Mahalanobis distance approach to get a single prioritization metric

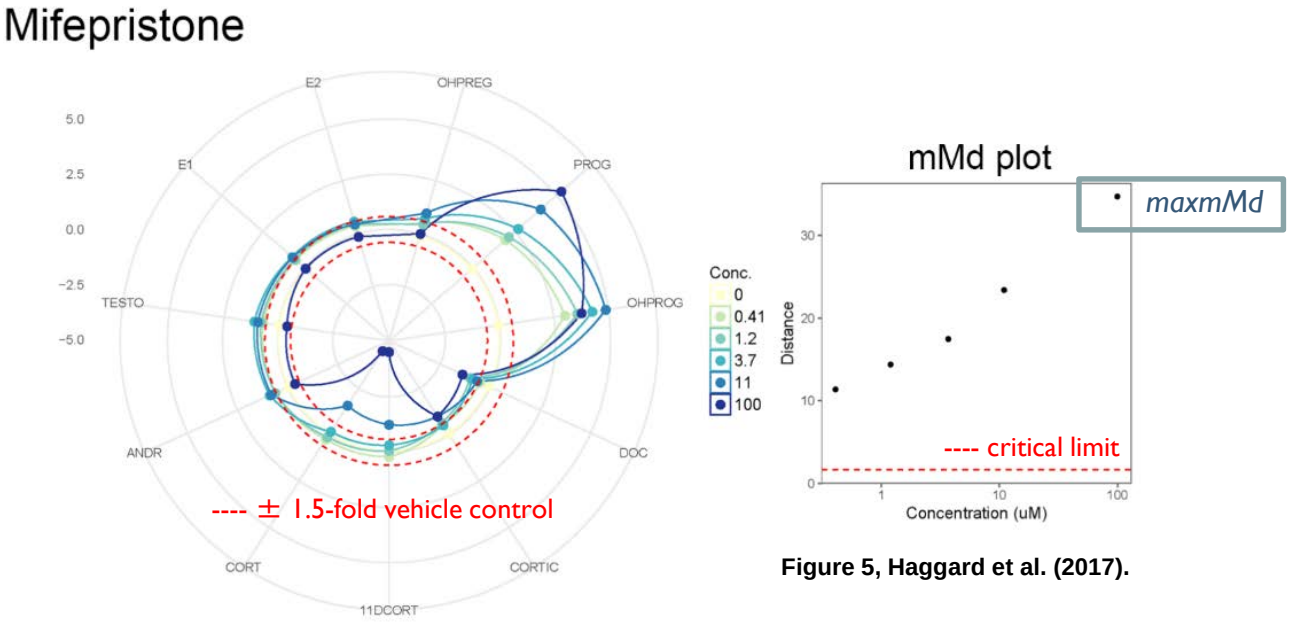


Figure 5, Haggard et al. (2017).

Mifepristone strongly modulated progestagens with significant effects on progesterone and OH-progesterone and moderate but non-significant trends on corticosteroids and androgens, resulting in a relatively high adjusted maxmMd of 33.

- Reduced an 11-dimensional question to a single dimension.
- Selection of the maxmMd appeared to provide a reproducible, quantitative approximation of the magnitude of effect on steroidogenesis.

MaxmMd was reproducible and quantitatively distinguished chemicals with larger effects.

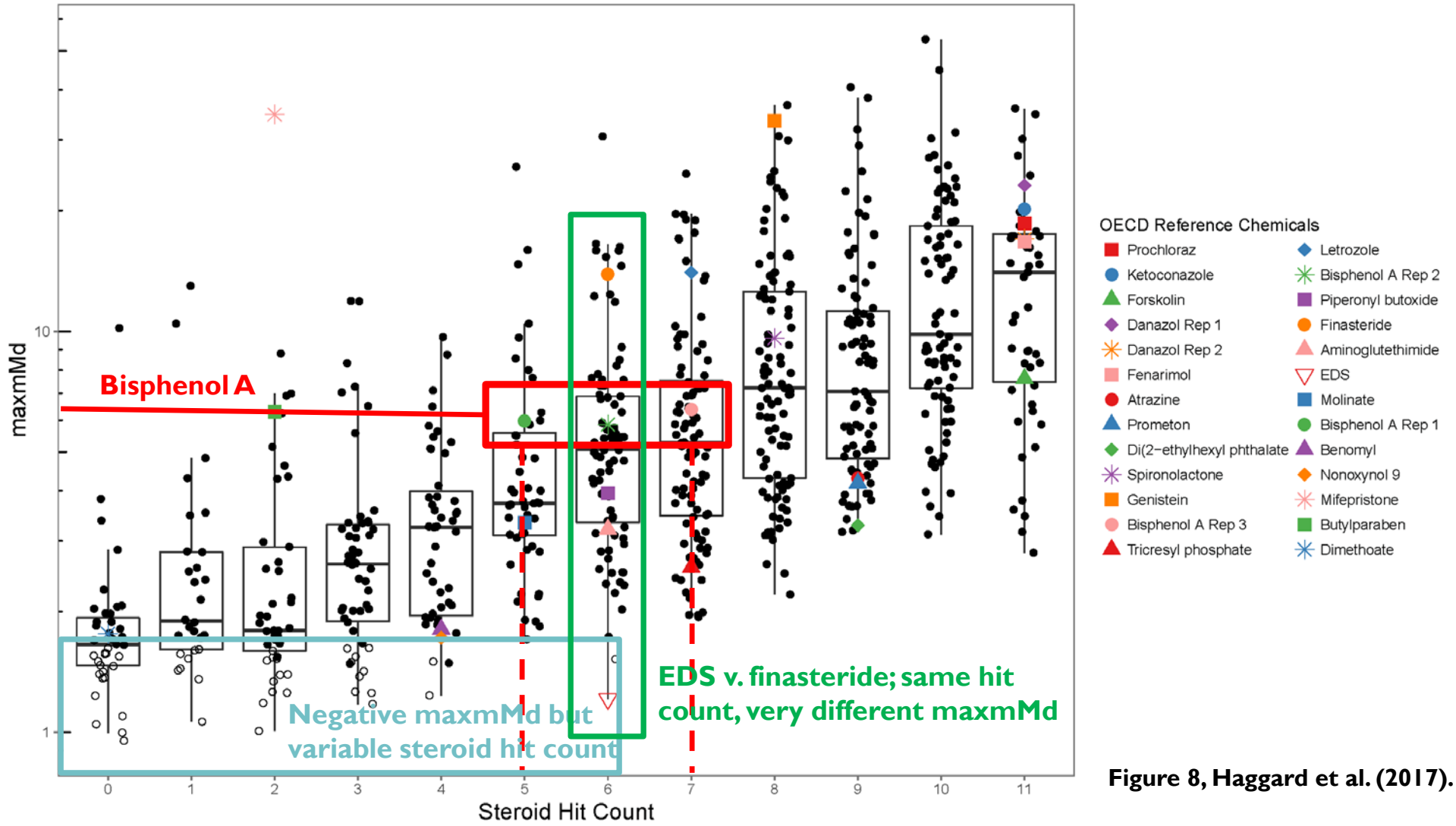


Figure 8, Haggard et al. (2017).

Steroidogenesis summary

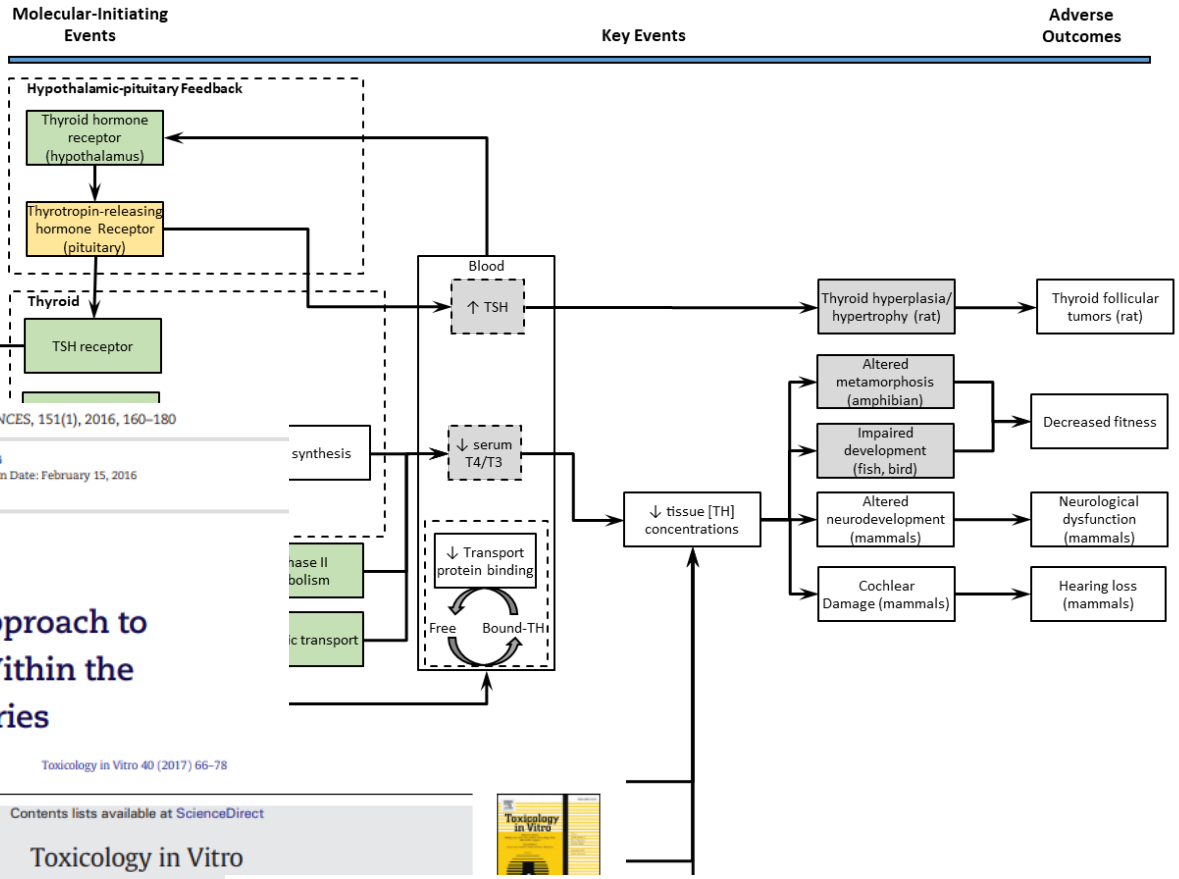
- HT-H295R screening assay as an alternative for the OECD-validated, low throughput H295R assay.
  - The ANOVA analysis and logic used herein for the HT-H295R dataset to determine effects on the steroid biosynthesis pathway enabled a direct comparison of the OECD inter-laboratory validation data and the HT-H295R data.
- Novel integration of 11 steroid hormone analytes for pathway-level analysis using the HT-H295R assay data.
  - A mean Mahalanobis distance (mMd) was computed for each chemical concentration screened.
  - The mMd provided a set of unitless values from which the maximum mean Mahalanobis distance (maxmMd) could be calculated across the concentration range screened. This maxmMd may be a useful prioritization metric.

Status of acceptance of these models

- EDSP FIFRA SAP Meeting in December 2014 (ER and AR pathway models)
- 2015 FR Notice: **“EPA concludes that ER Model data are sufficient to satisfy the Tier 1 ER binding, ERTA and uterotrophic assay requirements.”**
- AR Pathway model and HT-H295R model were reviewed at a recent SAP (November 2017), awaiting report.

United States Environmental Protection Agency

Considering the thyroid-related AOP network as an outline for HTS screening



OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 151(1), 2016, 160–180

doi: 10.1093/toxsci/kfw034
Advance Access Publication Date: February 15, 2016
Research Article

Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries

Katie Paul Friedman,*,† Joan M. Hedge,† Richar Steven O. Simmons†,1

*Oak Ridge Institute for Science E Toxicology Division, National He Development, U.S. Environmenta Computational Toxicology, Offic Research Triangle Park, NC, 2771 Effects Research Laboratory, Offic Duluth, MN, 55804

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journal homepage: www.elsevier.com

Development of a screening approach to detect thy chemicals that inhibit the human sodium iodide sy

Daniel R. Hallinger [a], Ashley S. Murr [a], Angela R. Buckalew [a], Steve Tammy E. Stoker [a,*], Susan C. Laws [a,*]

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[b] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protectio

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TOXICOLOGICAL SCIENCES, 2017, 1–12

doi: 10.1093/toxsci/kfx279
Advance Access Publication Date: December 7, 2017
Research Article

Screening the ToxCast Phase 1 Chemical Library for Inhibition of Deiodinase Type 1 Activity

Michael W. Hornung,*,†,‡,§,1, Joseph J. Korte,*,†,‡,§ Jennifer H. Olker,*,†,‡,§ Jeffrey S. Denny,*,†,‡,§ Carsten Knutsen,*,†,‡,§ Phillip C. Hartig,*,†,‡,¶ Mary C. Cardon,*,†,‡,¶ and Sigmund J. Degitz*,†,‡,§

*US Environmental Protection Agency; †Office of Research and Development; ‡National Health and

Continuing challenges for all high-throughput toxicology

- Technical limitations/obstacles associated with each technology (e.g., metabolism, volatiles, etc.)
- Moving from an apical to a molecular paradigm and defining adversity
- Predicting human safety vs. toxicity
- Combining new approaches to have adequate throughput and sufficiently capture higher levels of biological organization
- Systematically integrating multiple data streams from the new approaches in a risk-based, weight of evidence assessment
- Quantifying and incorporating uncertainty and variability
- Dealing with the validation
  - Defining a fit-for-purpose framework(s) that is time and resource efficient
  - Performance-based technology standards vs. traditional validation
  - Role of *in vivo* rodent studies and understanding their inherent uncertainty
- Legal defensibility of new methods and assessment products

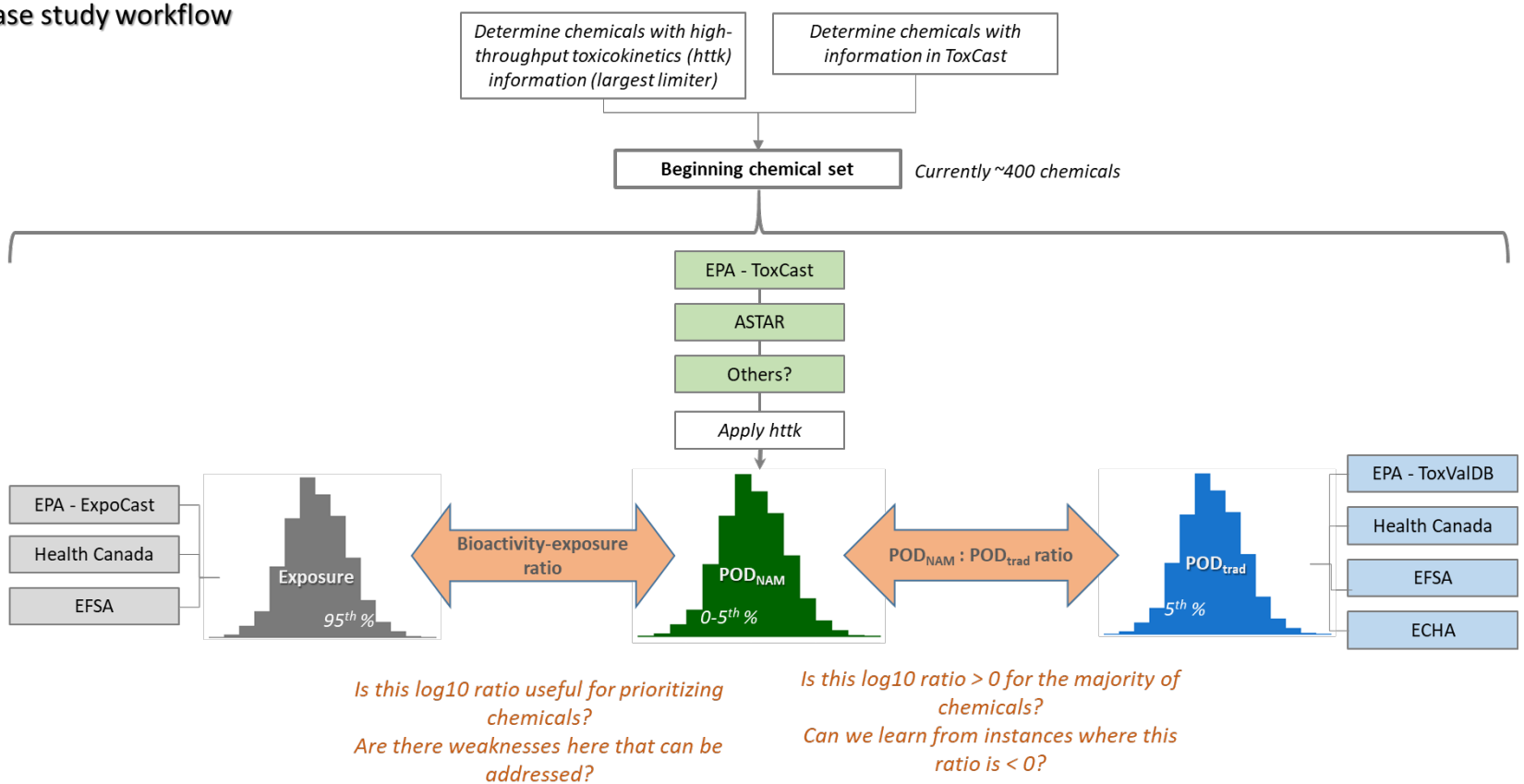
EPA United States Environmental Protection Agency

The big question:

Can in vitro bioactivity be used to derive a conservative point-of-departure (POD) for prioritization and risk assessment?

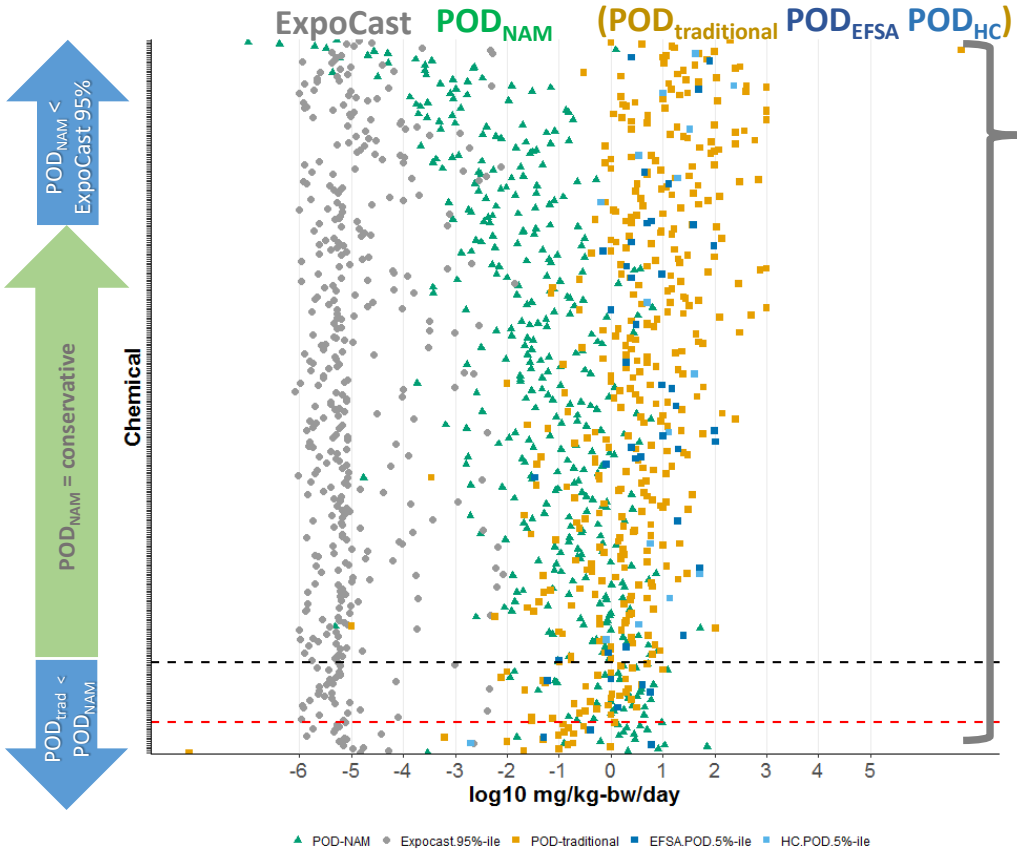
A retrospective case study in screening level risk assessment

Case study workflow



Paul Friedman et al., 2018; in prep

Preliminary work to compare traditional PODs and new approach method PODs demonstrates the possibility and challenges



Total =

380 chemicals

httk, ToxCast data, and POD value(s) currently available

So for ~92% of the chemicals, without modifying simplistic assumptions in the workflow, POD$_{NAM}$ was conservative.

POD ratio ≤ 0
31/380 = 8%

POD ratio < -1
8/380 = 2%

All but one chemical had a POD ratio > -2, which might suggest a UF of 100 (?) might be conservative.

Paul Friedman et al., 2018; in prep

United States Environmental Protection Agency

Thank You for Your Attention!

Tox21 Colleagues:
- NTP Crew
- FDA Collaborators
- NCATS Collaborators

EPA Colleagues:
- NERL
- NHEERL
- NCEA

Advancing the Pace of Chemical Risk Assessment Collaborators from EPA, Health Canada, ECHA, EFSA, and A*STAR

EPA's National Center for Computational Toxicology