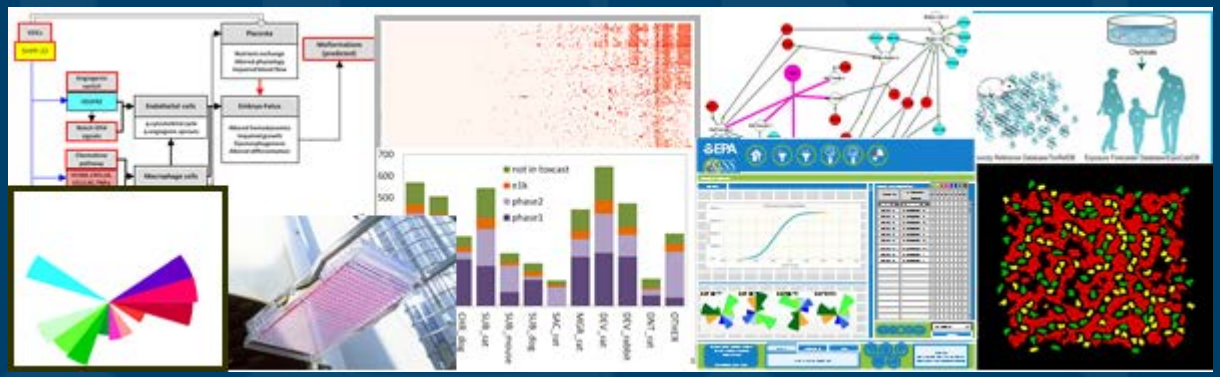
EPA United States Environmental Protection Agency

Developments in the EPA Computational Toxicology Program to Identify Environmental Endocrine Disruptors



Environmental Endocrine Disruptors Gordon Conference
June 22, 2016

Rusty Thomas
Director
National Center for Computational Toxicology

First, Let's Celebrate…

Today, President Obama signed a bill to reform the Toxic Substances Control Act (TSCA) that requires EPA to review existing chemicals on a specific, enforceable schedule, as well as to review new chemicals for health risks before they're allowed on the market.

Legislative Mandate

1996 Federal Food, Drug and Cosmetic Act, section 408(p)

Requires the U. S. EPA to develop a screening program using appropriate validated test systems and other scientifically relevant methods to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate.

1996 Safe Drinking Water Act Amendments, section 1457

Testing of chemical substances that may be found in sources of drinking water, if substantial human populations may be exposed.

Formation of the Initial Screening and Testing Strategy

- **Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Key Recommendations (1998):**
  - Expand protection to include human health and wildlife
  - Include estrogen, androgen and thyroid pathways
  - Develop a two-tiered screening and testing program
- **EDSTAC Conceptual Framework:**
  - Tier 1 screening for *potential* to interact with estrogen, androgen or thyroid hormone systems
  - Tier 2 testing to verify interaction with the endocrine system and quantify the dose-response relationship

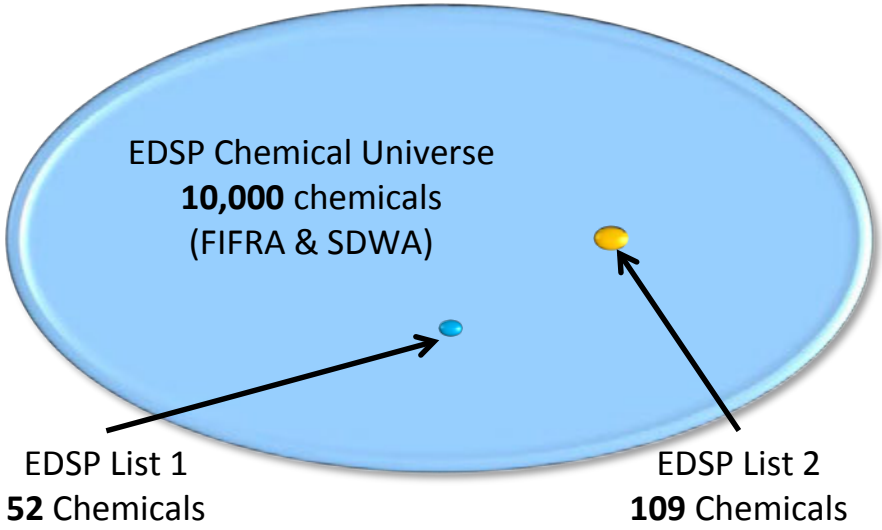
EDSP Screening Batteries

Endocrine Pathway	Tier 1 Screening Battery										Tier 2 Testing Assays			
	ER Binding	*ERα Transcriptional Activation**	*AR Binding*	*Aromatase Inhibition*	*Steroidogenesis**	Uterotrophic*	Hershberger*	Pubertal Male	Pubertal Female	Amphibian Metamorphosis*	Fish Short Term Reproduction*	Rat 2-gen/ Extended One-Gen*	Medaka Extended One-Gen Repro Test*	Amphibian Growth and Dev Assay*	Japanese Quail Two Gen Toxicity Test
E+	■	■			■	■			■		■	■	■	■	■
E-	■			■	■				■		■	■	■	■	■
A+			■		■		■	■			■	■	■	■	■
A-			■		■		■	■			■	■	■	■	■
HPT Axis								■	■	■		■		■	■

*OECD harmonized guidelines; *In vitro assays indicated by italic font*

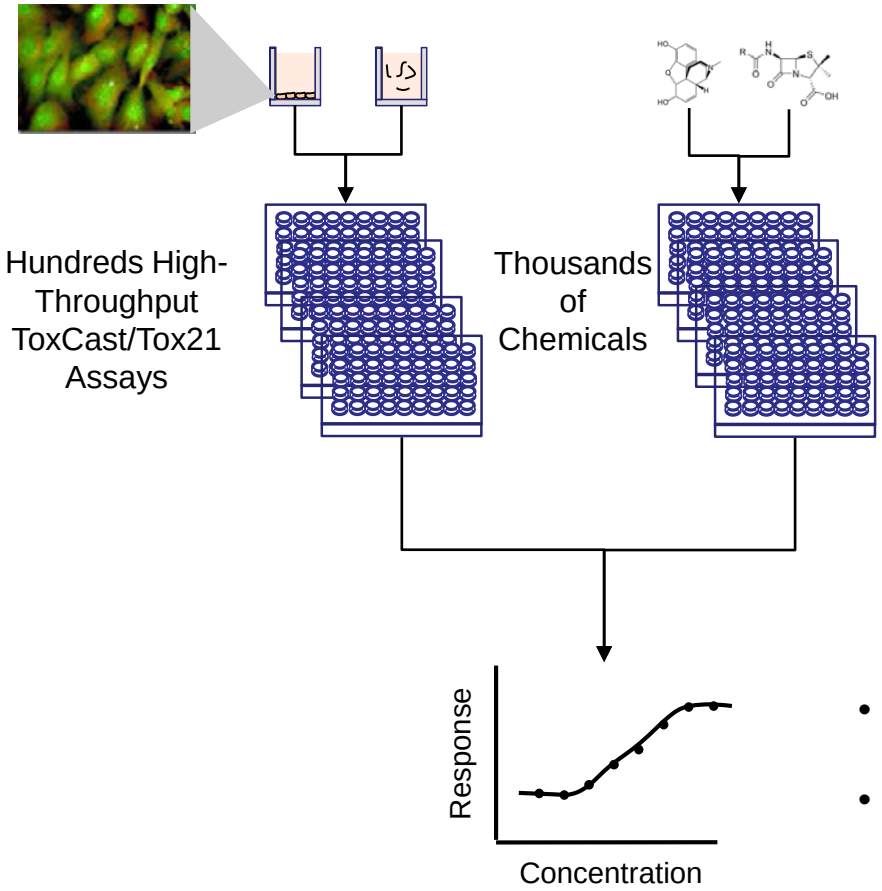
The Challenge

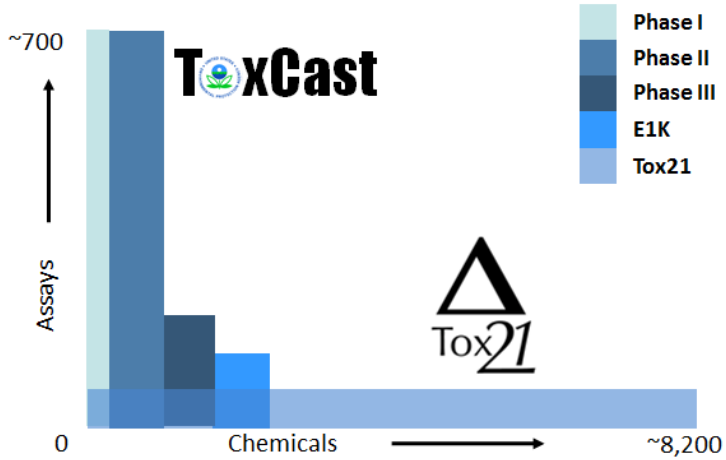
- In 2009, EPA published list of 67 pesticide chemicals (List 1) for Tier 1 screening (15 subsequently withdrawn)
- In 2013, EPA published a revised second list (List 2) of 109 chemicals for proposed Tier 1 screening
- In 2015, EPA issued EDSP ordered additional testing on positive List 1
- The cost of running the Tier 1 battery is ~$750,000 - $1 million
- At current rate, it would take decades and cost billions of dollars to screen all 10,000 chemicals of interest to EPA for potential endocrine activity



EDSP Chemical Universe List	Number
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616
TOTAL	**10,341**

High-Throughput Assays Used to Screen Chemicals for Bioactivity

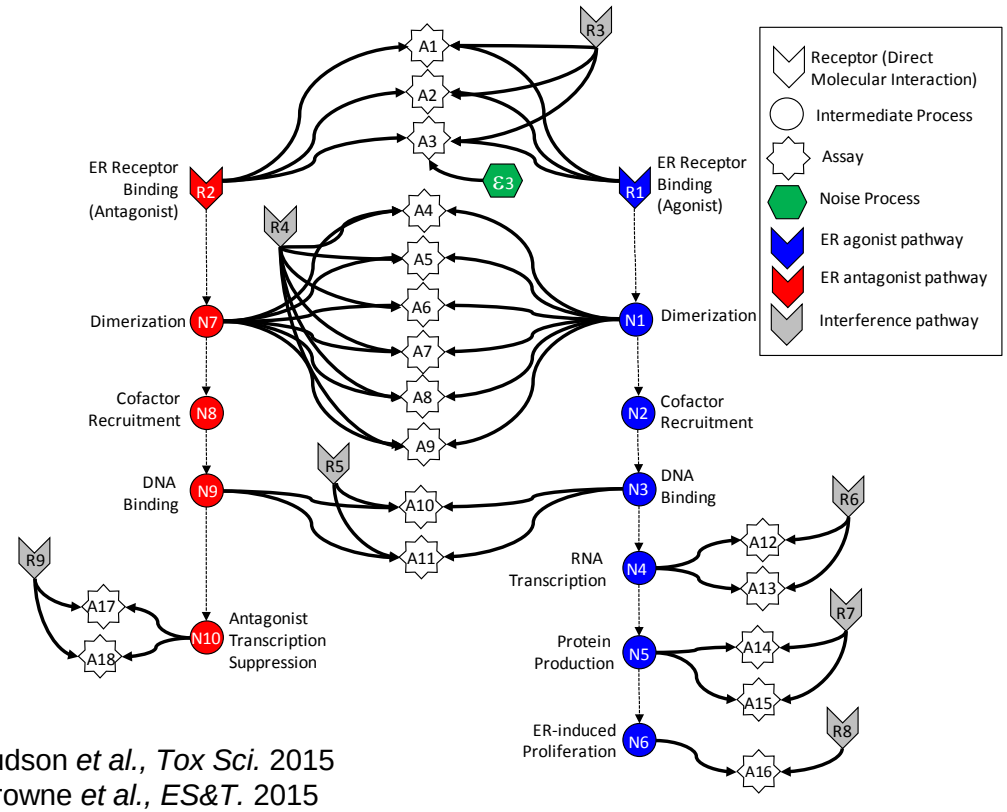


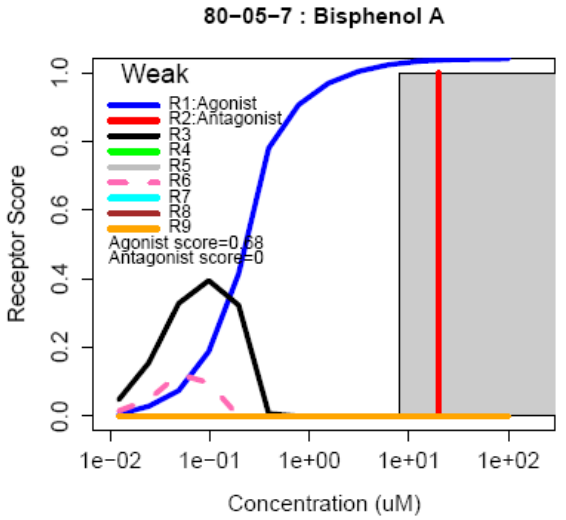


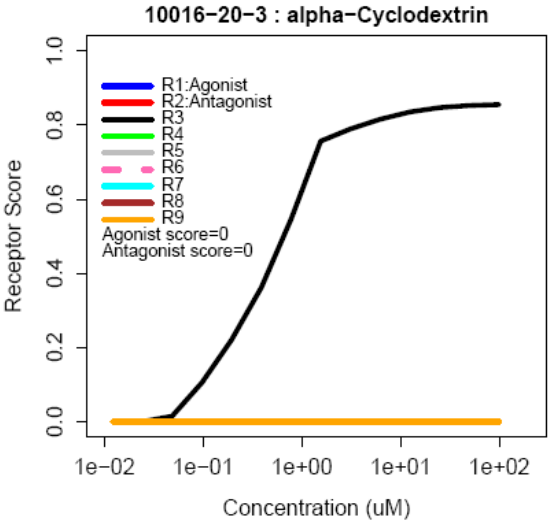
- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations

Current Application to Estrogen Signaling in EDSP

18 *In Vitro* Assays Measure ER-Related Activity

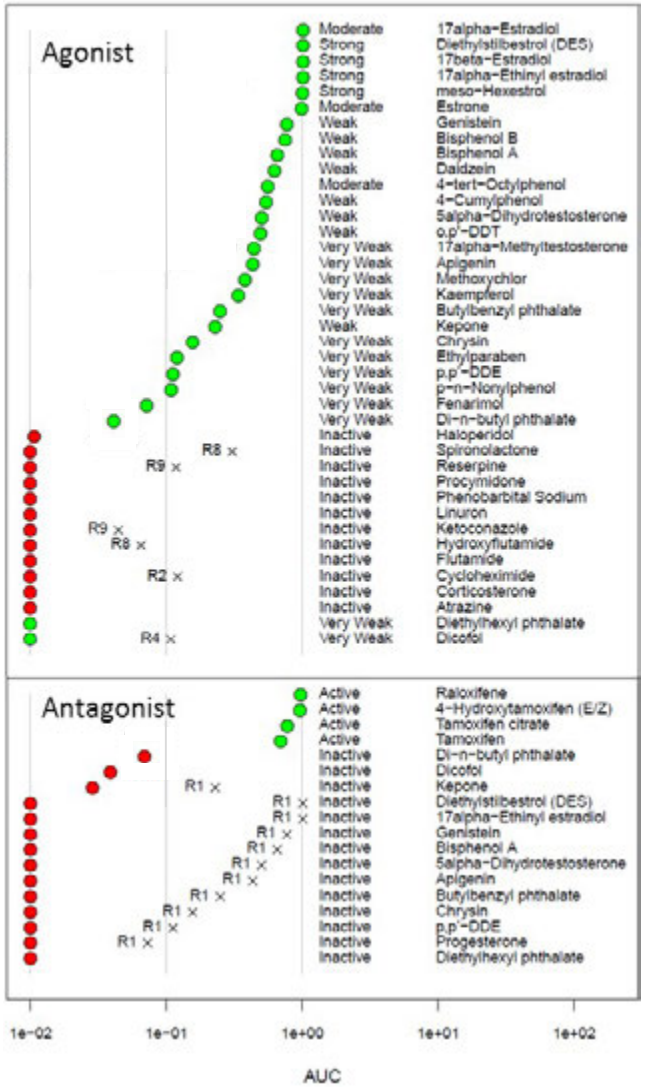


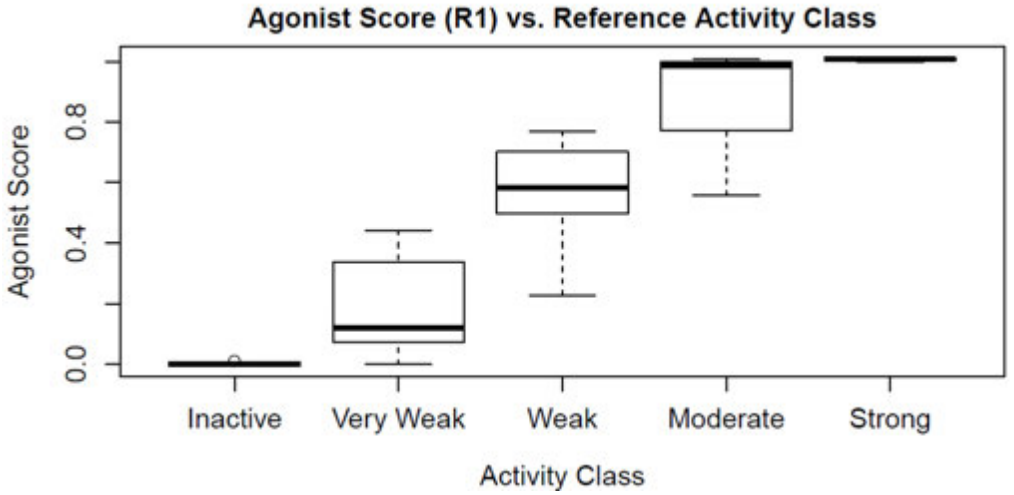




Judson *et al., Tox Sci.* 2015
Browne *et al., ES&T.* 2015
Kleinstreuer *et al., EHP* 2016

Validation With *In Vitro* Reference Chemicals



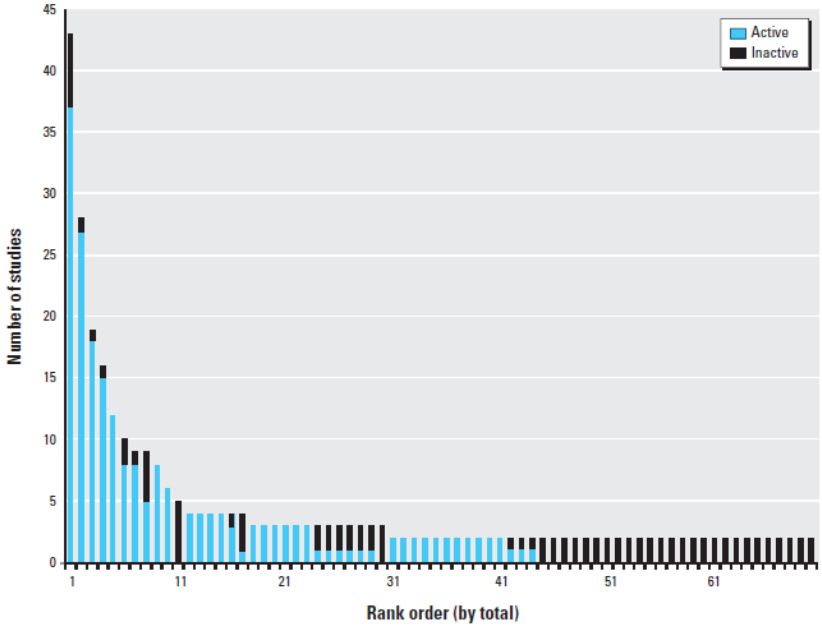


In Vitro Reference Chemicals*

Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

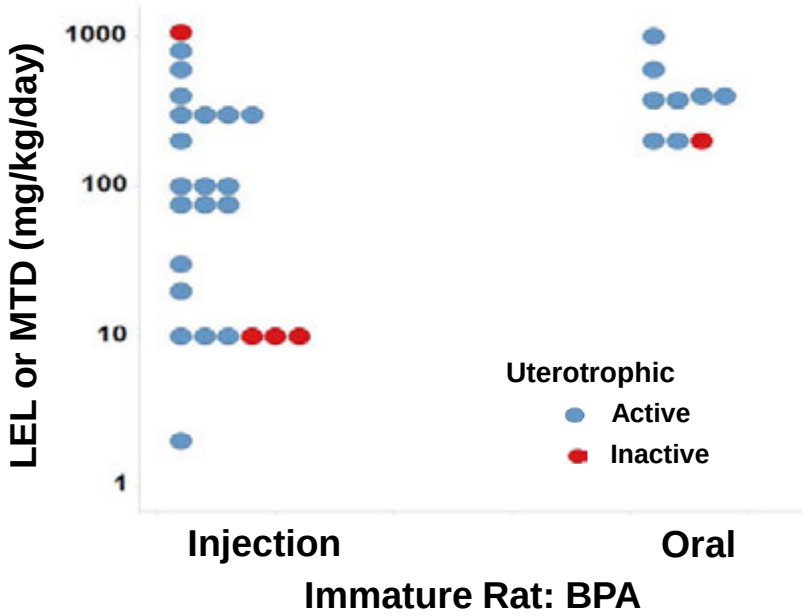
Validation With *In Vivo* Reference Chemicals

Qualitative Concordance of Uterotrophic Studies



Kleinstreuer *et al., EHP* 2016

Quantitative Concordance of Uterotrophic Studies



Kleinstreuer *et al., EHP* 2016

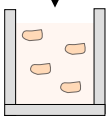
Predictive Performance for *In Vivo* Uterotrophic Studies

Accuracy	**0.86 (0.95)**
Sensitivity	**0.97 (0.97)**
Specificity	**0.67 (0.89)**

*Values in parentheses exclude inconclusive chemicals

Incorporating a High-Throughput Toxicokinetic Approach

EPA ToxCast Phase I and II Chemicals



Human Liver Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

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

ToxCast AC_{50} Value

Plasma Concentration

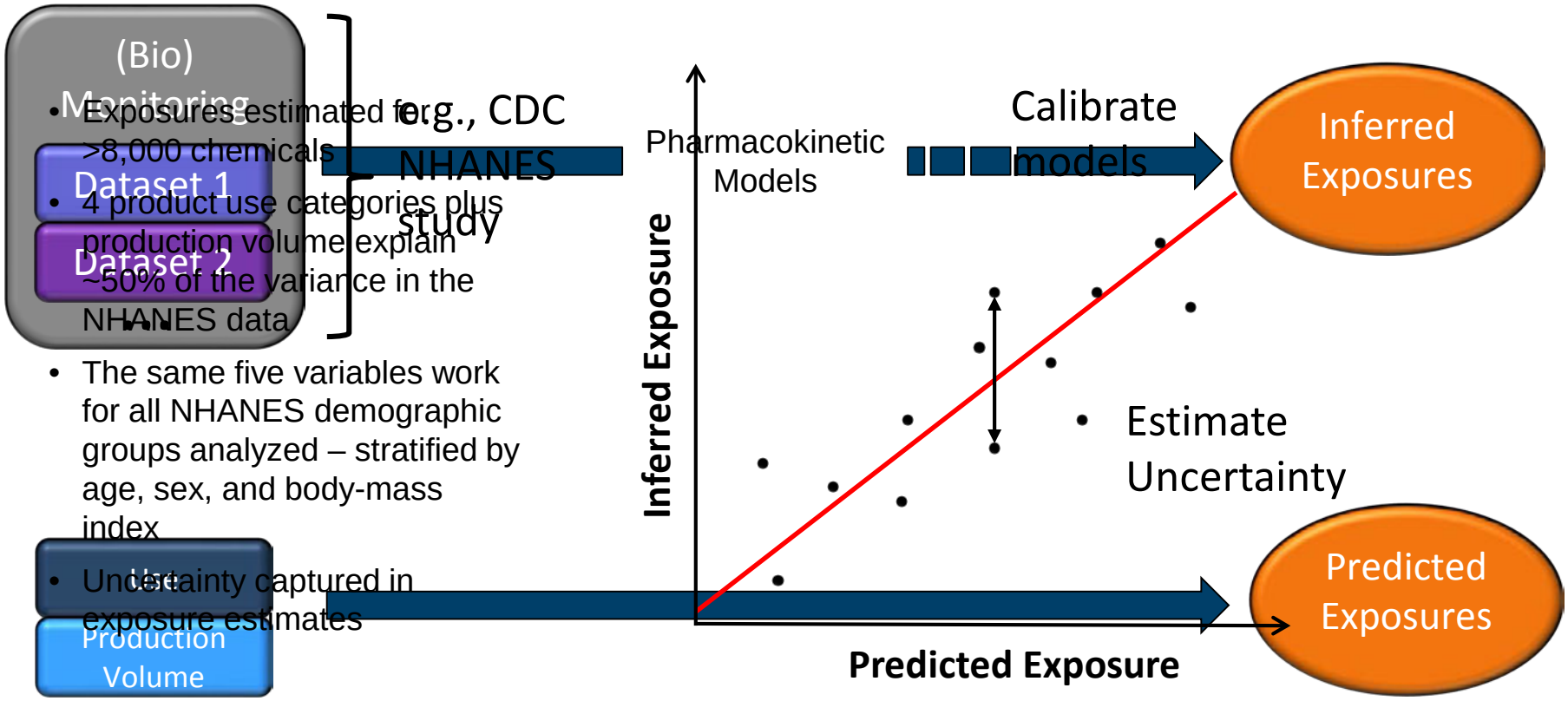
Oral Exposure

Reverse Dosimetry

Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

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

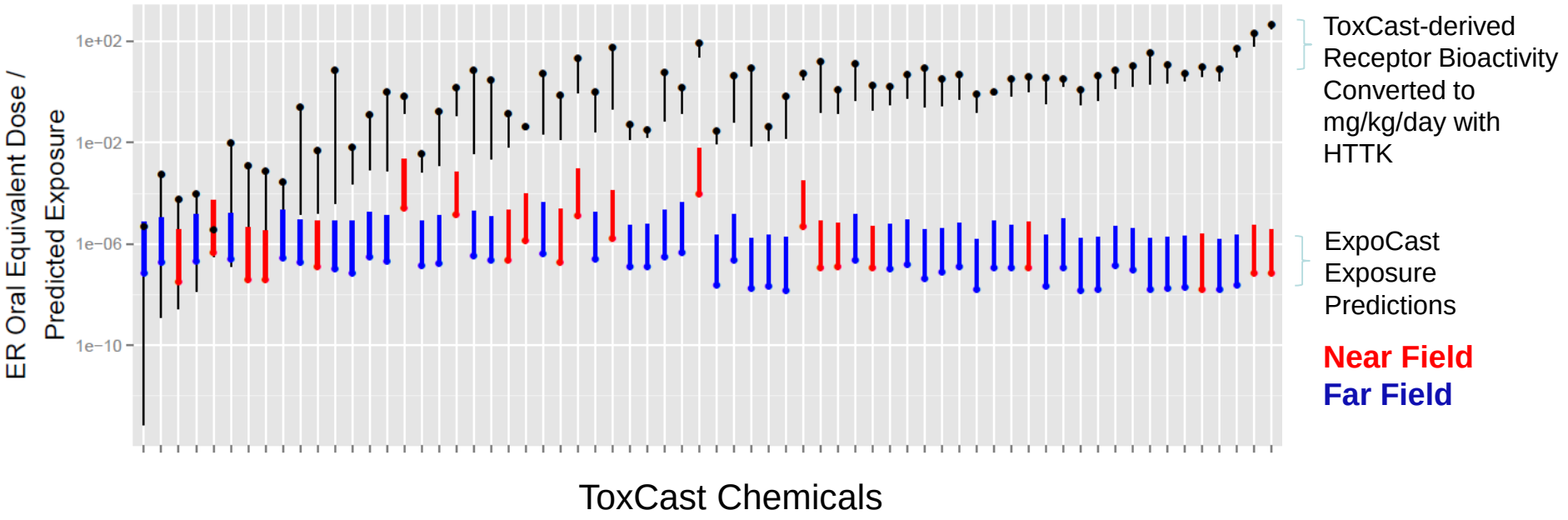
Adding on High-Throughput Exposure



- Exposures estimated for >8,000 chemicals
- 4 product use categories plus production volume explain ~50% of the variance in the NHANES data
- The same five variables work for all NHANES demographic groups analyzed – stratified by age, sex, and body-mass index
- Uncertainty captured in exposure estimates

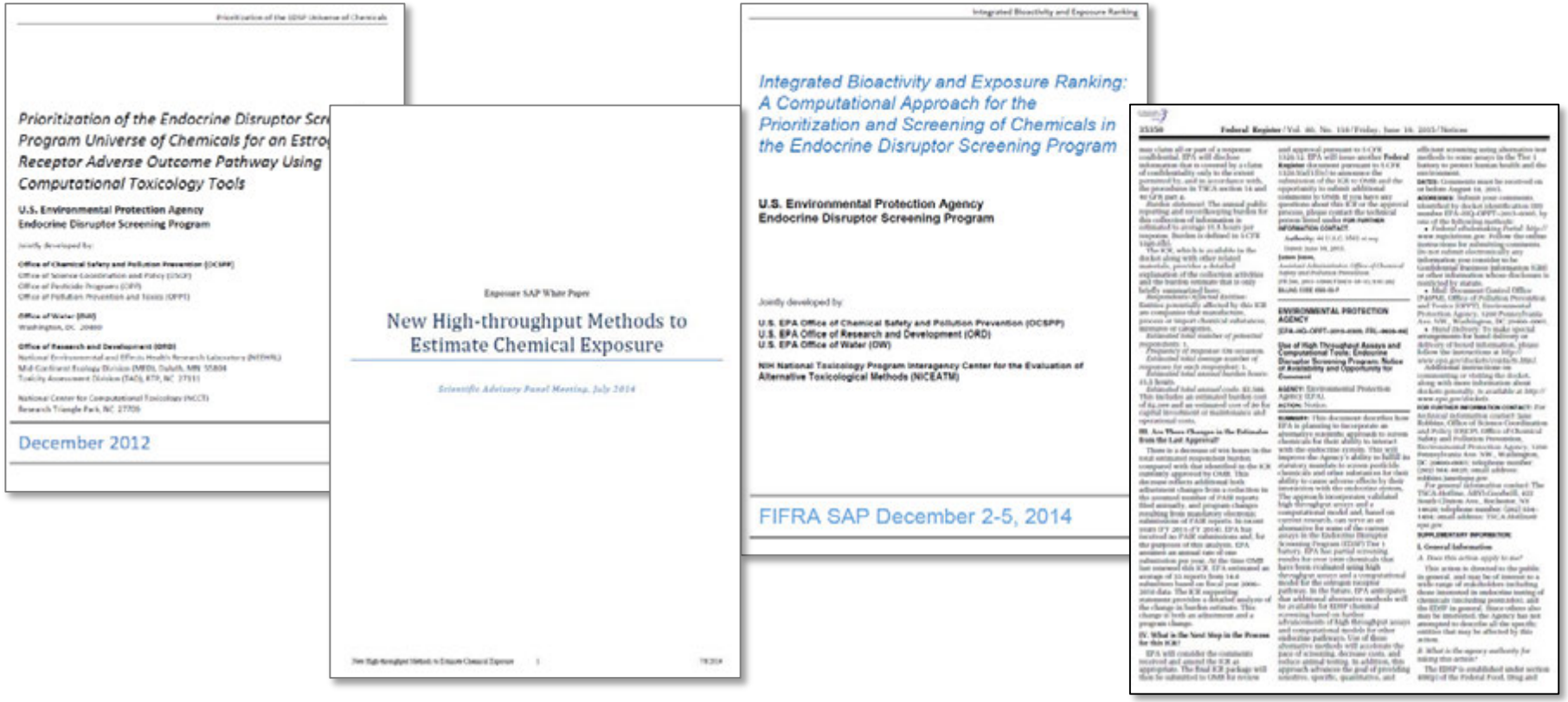
Wambaugh *et al.*, 2014

Integrating All Three for Risk-Based Prioritization and Decision Making

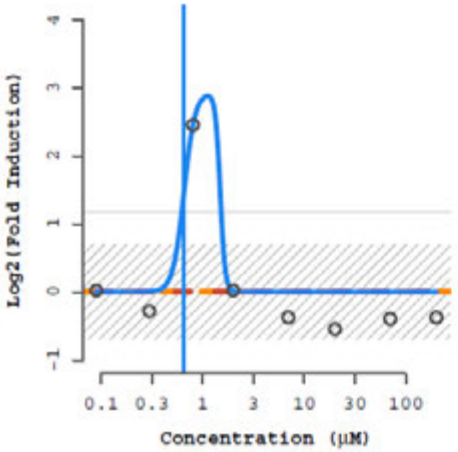


United States Environmental Protection Agency

Peer Review of New Approaches and Adoption by Regulatory Partners



Efforts to Ensure Data Quality and Increase Transparency



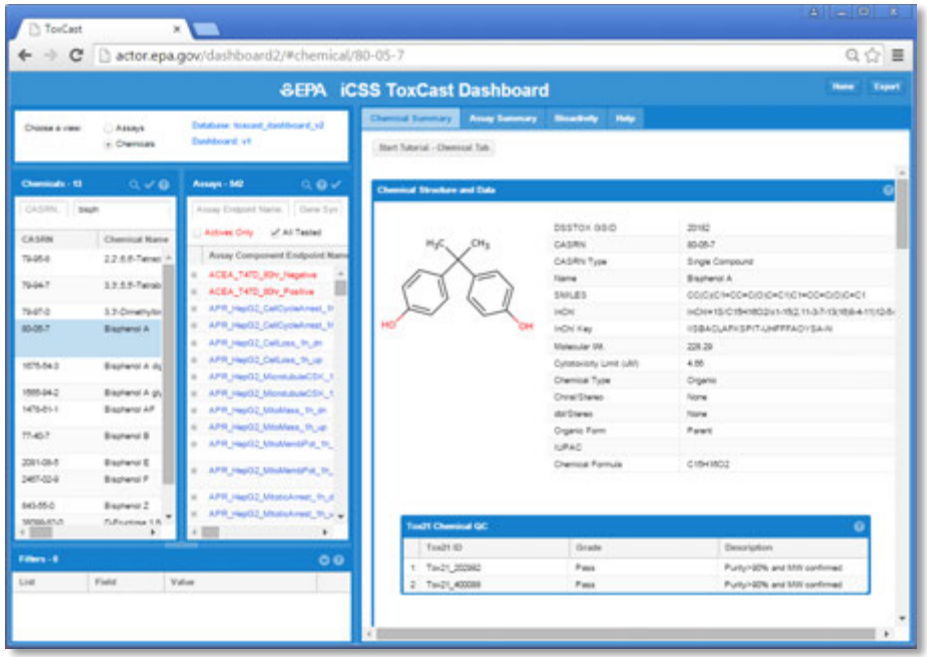

```
ASSAY:    ARID117 (ATG_ERa_TRANS)

NAME:     Thioglycolic acid
CHID:     26141    CASRN: 68-11-1
SPID(S): TX007664
L4ID:     420385

HILL MODEL (in red):
          tp          ga          gw
val:   3.1e-11     -2.15       0.416
sd:    NaN         NaN         NaN

GAIN-LOSS MODEL (in blue):
          tp          ga          gw          la          lw
val:   2.93        -0.184      8           0.173       18
sd:    3.56        0.334       9.48        5.82        814

          CNST        HILL        GNLS
AIC:   20.14       26.14       17.79
PROB:  0.23        0.01        0.76
RMSE:  0.92        0.92        0.32

MAX_MEAN: 2.45      MAX_MED: 2.45      BMAD: 0.233

COFF: 1.17    HIT-CALL: 1    FITC: 50    ACTP: 0.77
```

FLAGS:
Only one conc above baseline, active
Borderline active

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Transparent ToxCast data analysis pipeline
  - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
  - Publicly available as an R package
- Tox21 and ToxCast chemical libraries have undergone analytical QC and results publicly available
- Public posting of ToxCast procedures
  - Chemical Procurement and QC
  - Data Analysis
  - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline
- Multiple webinars and workshops to educate stakeholders on high-throughput screening data analysis and interpretation

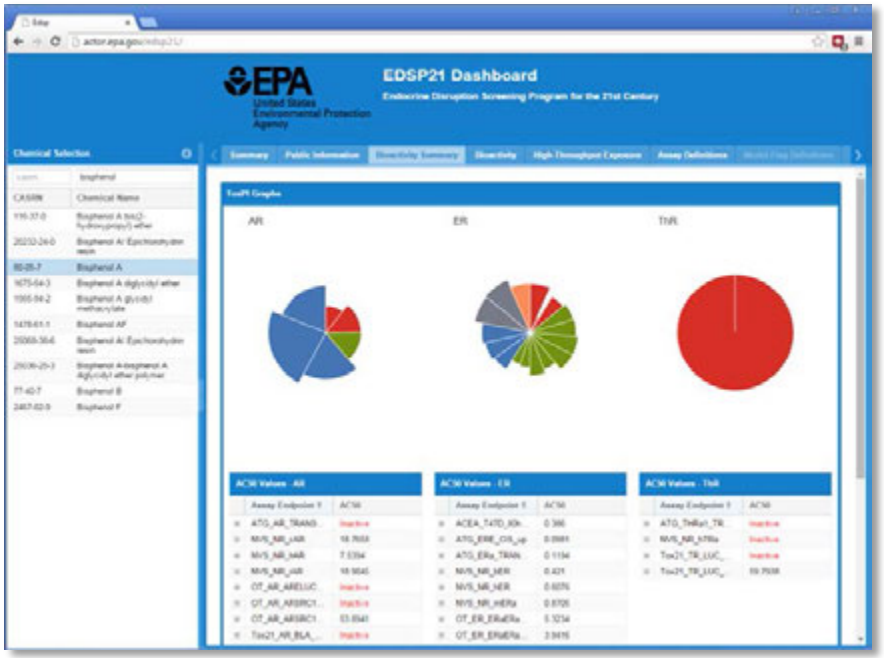
Enabling Stakeholders to Use ToxCast/Tox21 Data

iCSS Dashboard



http://actor.epa.gov/dashboard2/

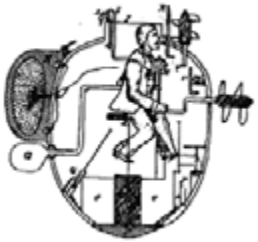
EDSP21 Dashboard

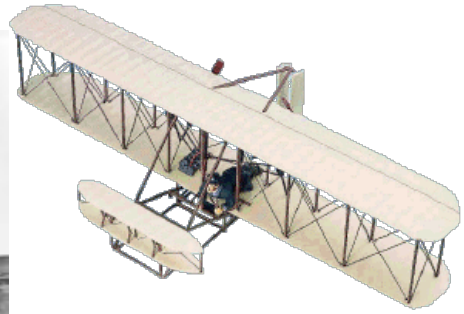


http://actor.epa.gov/edsp21/

EPA United States Environmental Protection Agency

But, Version 1.0 is Never Perfect…





What is Version 2.0 Looking Like?

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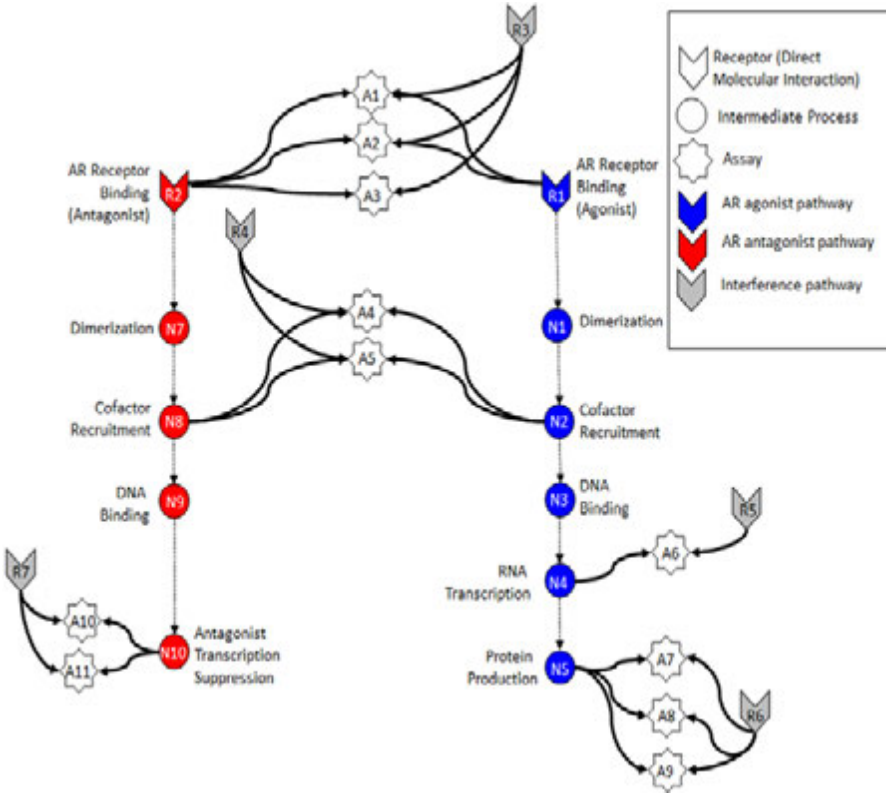
Expanding Screening and Modeling to the Androgen Pathway

11 *In Vitro* Assays Measure AR-Related Activity



Kleinstreuer et al. Unpublished

Agonism

True Positives	8
True Negatives	20
False Positives	1
False Negatives	0
Accuracy	0.97
Sensitivity	1.00
Specificity	0.95

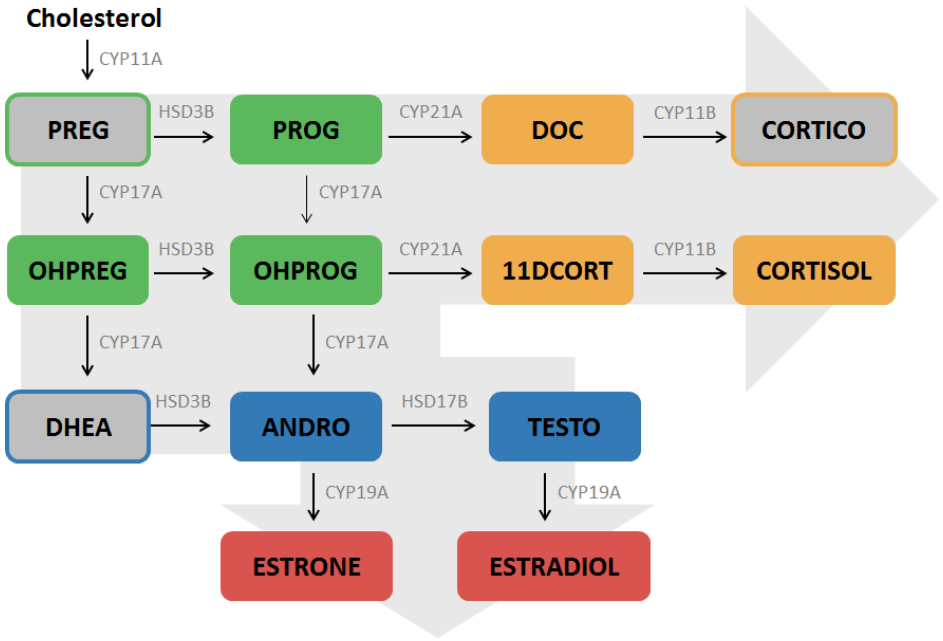
Antagonism

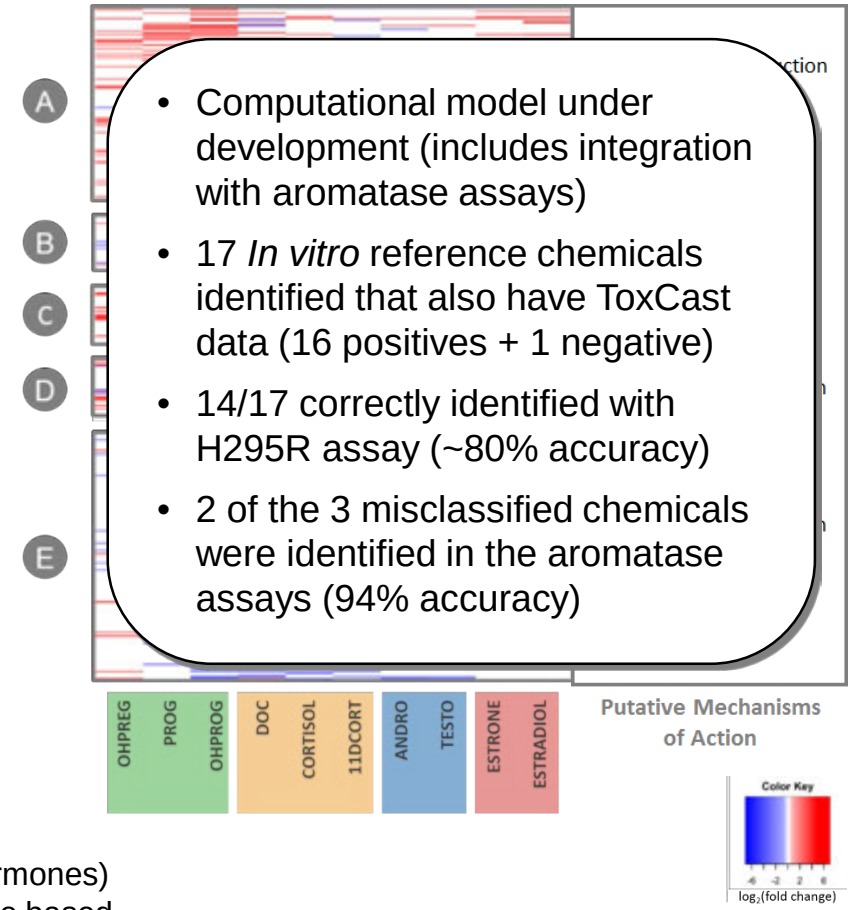
True Positives	18
True Negatives	8
False Positives	0
False Negatives	2*
Accuracy	0.93*
Sensitivity	0.90*
Specificity	1.00

*Two false negatives were correctly identified when Tox21 AR antagonist screen was evaluated at both agonist concentrations

Adding Screening for Disruption of Steroidogenesis

ToxCast H295R Steroidogenesis Assay





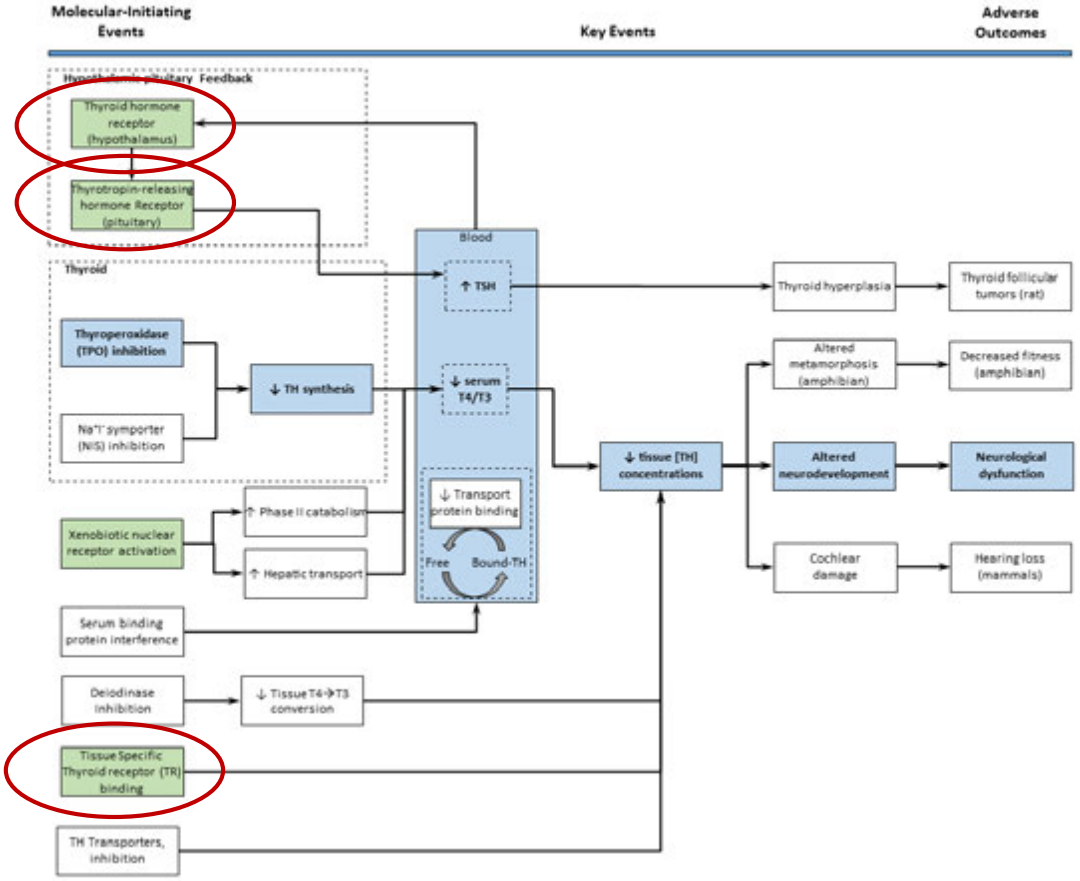
- Computational model under development (includes integration with aromatase assays)
- 17 *In vitro* reference chemicals identified that also have ToxCast data (16 positives + 1 negative)
- 14/17 correctly identified with H295R assay (~80% accuracy)
- 2 of the 3 misclassified chemicals were identified in the aromatase assays (94% accuracy)

- 13 hormones were quantified using HPLC-MS/MS
- 2060 chemicals screened at single concentration
- 403 chemicals selected for concentration response (altered ≥ 4 hormones)
  - 120 additional chemicals selected for concentration response based on other needs

Karmaus et al., 2016

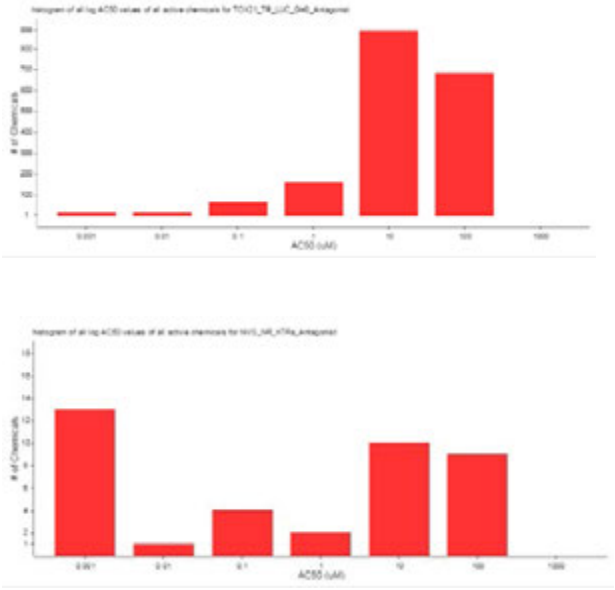
Making Progress on Thyroid

Thyroid Toxicity AOP Network



Paul-Friedman *et al.*,2016

THR Assays



TSHR Assay

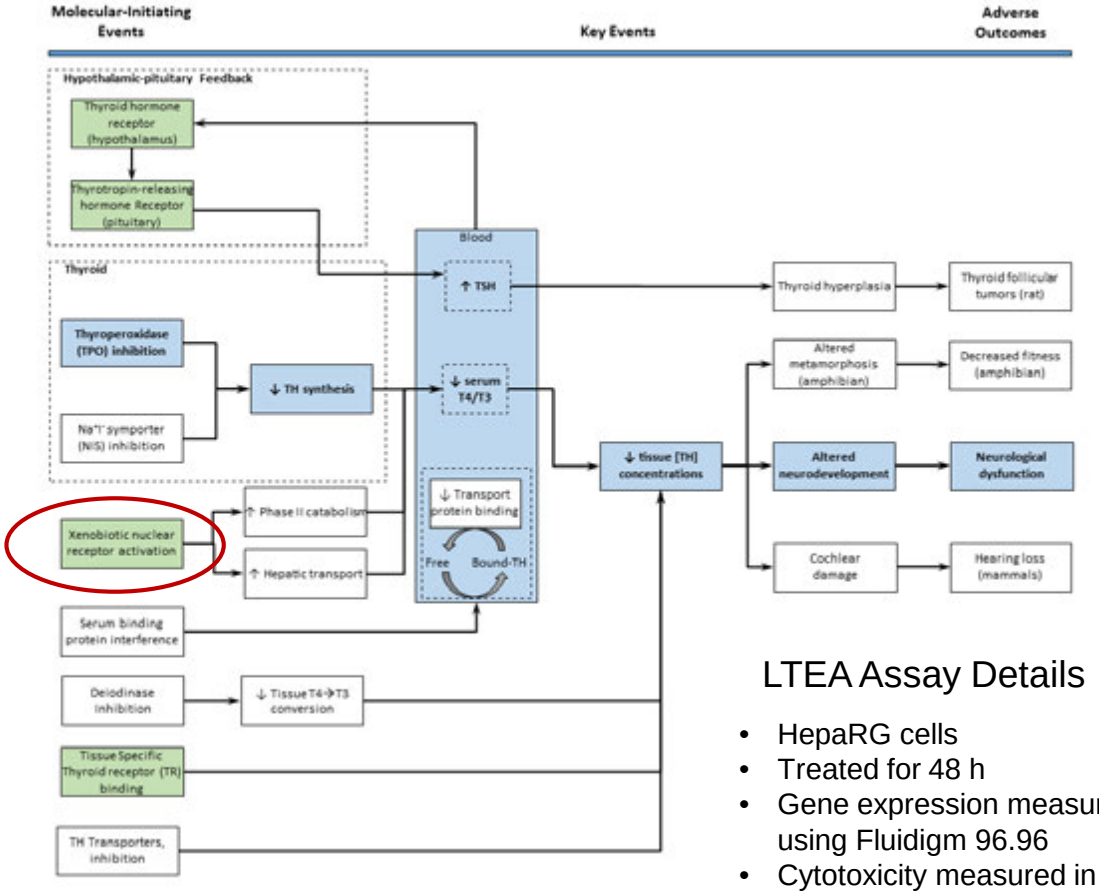
Completed Agonist (Nov ‘15)
Completed Antagonist (April 16)

TRHR Assay

Undergoing Online Validation (Tox21)

Making Progress on Thyroid

Thyroid Toxicity AOP Network

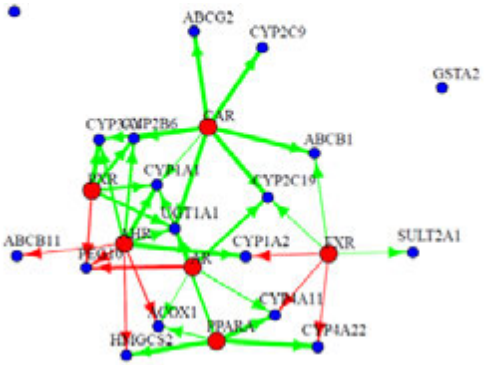


Paul-Friedman *et al.*,2016

LTEA Assay Details

- HepaRG cells
- Treated for 48 h
- Gene expression measured using Fluidigm 96.96
- Cytotoxicity measured in parallel

HepaRG Gene Expression Assay



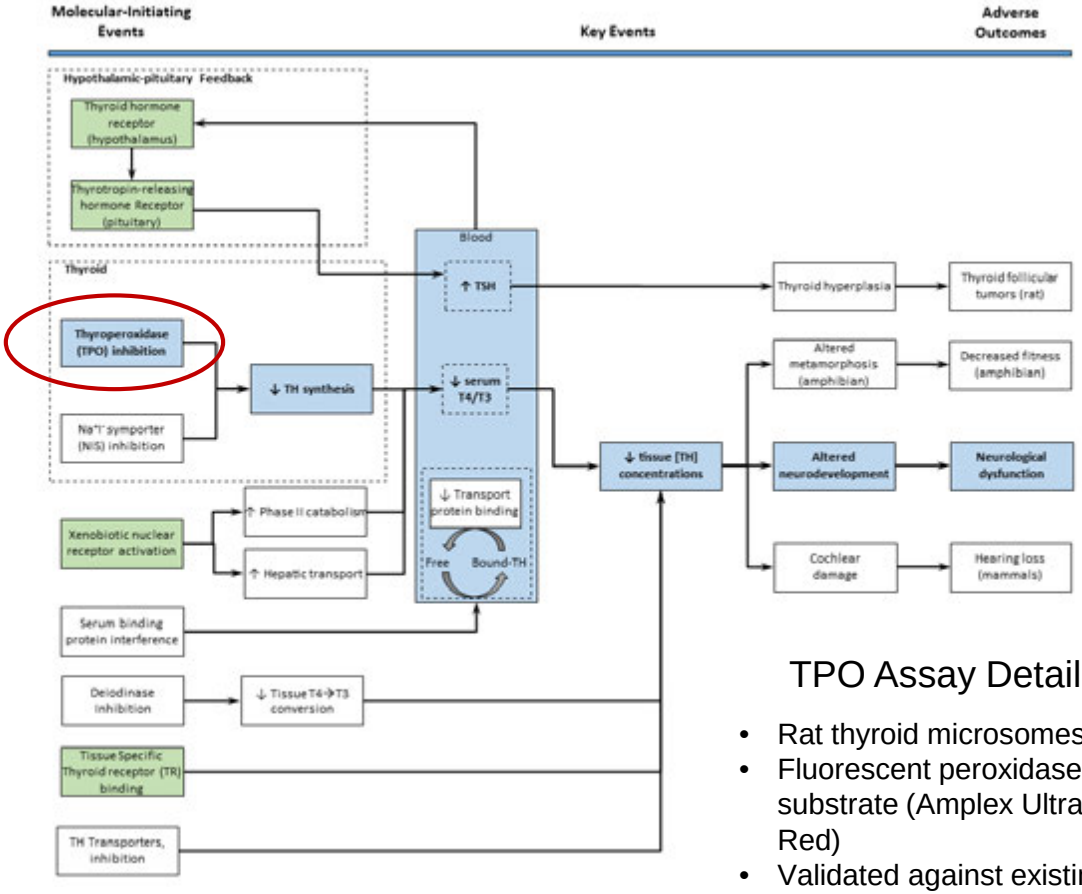
Bayesian model for NR regulation

SLCO1B1: thyroid hormone transporter in the liver
SULT2A1: not main SULT that metabolizes TH, but demonstrated to have some activity
UGT1A1/1A6: mediate T(4) glucuronidation
THRSP: thyroid hormone-inducible hepatic protein
HIF1a: downstream to TRB1 activation via T3 or T4 signaling

Franzosa and Wambaugh, Unpublished

Making Progress on Thyroid

Thyroid Toxicity AOP Network

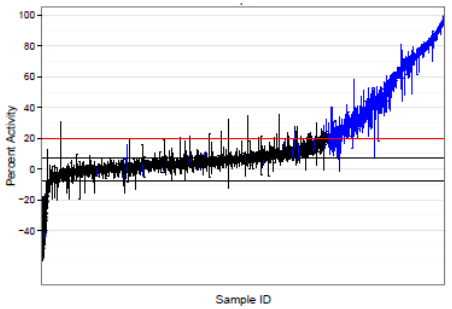


Paul-Friedman *et al.*,2016

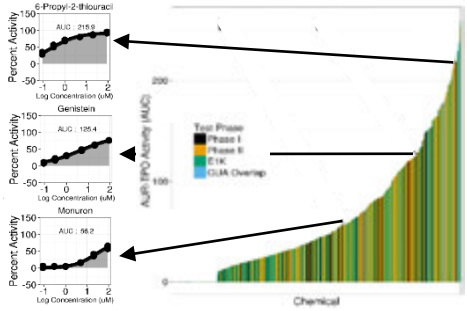
TPO Assay Details

- Rat thyroid microsomes
- Fluorescent peroxidase substrate (Amplex Ultra Red)
- Validated against existing kinetic guaiacol assay
- Luciferase, cytotoxicity counterscreens

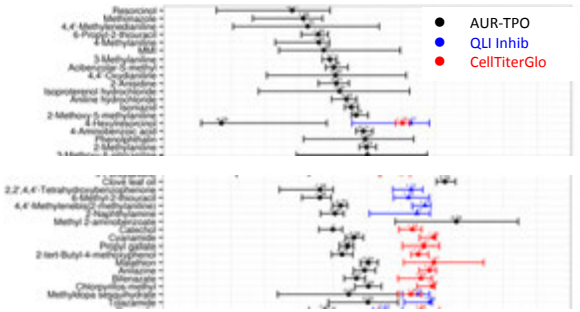
Single-concentration Screen



Concentration Response Screen



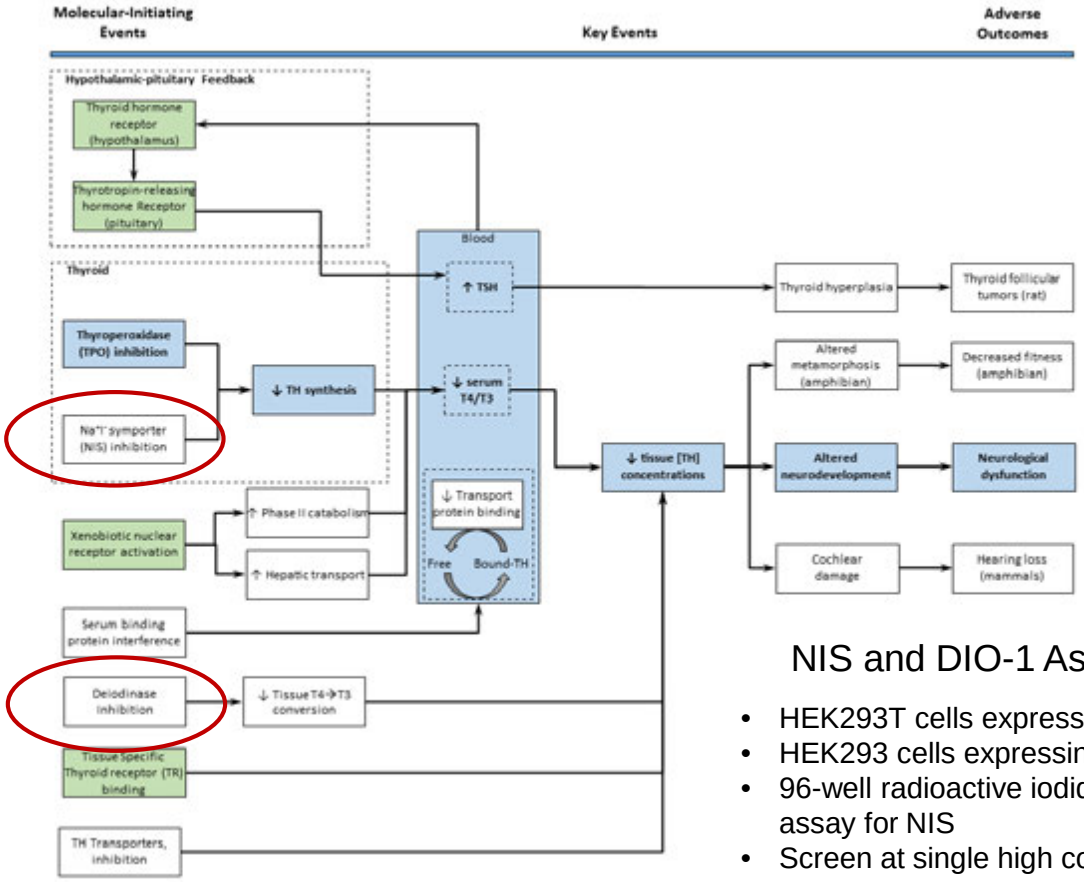
Stratify by Selectivity



Paul-Friedman et al., 2016

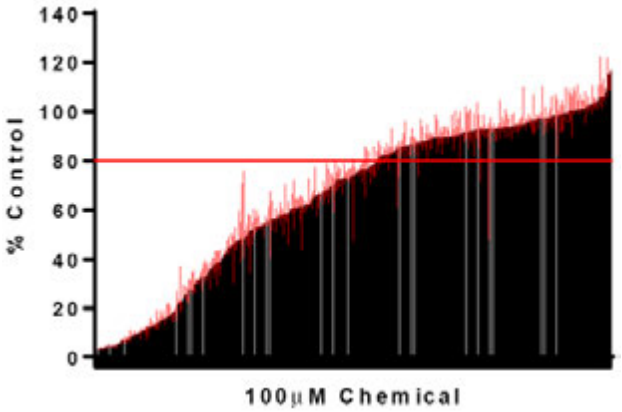
Making Progress on Thyroid

Thyroid Toxicity AOP Network



Paul-Friedman *et al.*,2016

NIS Assay (300 ToxCast cmpds)

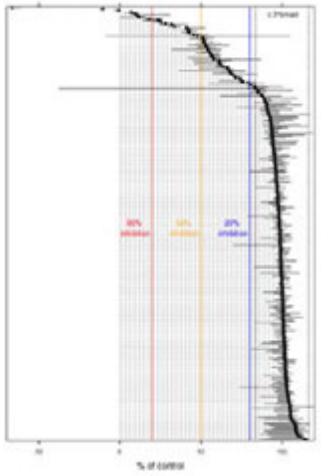


T. Stoker (NHEERL)

NIS and DIO-1 Assay Details

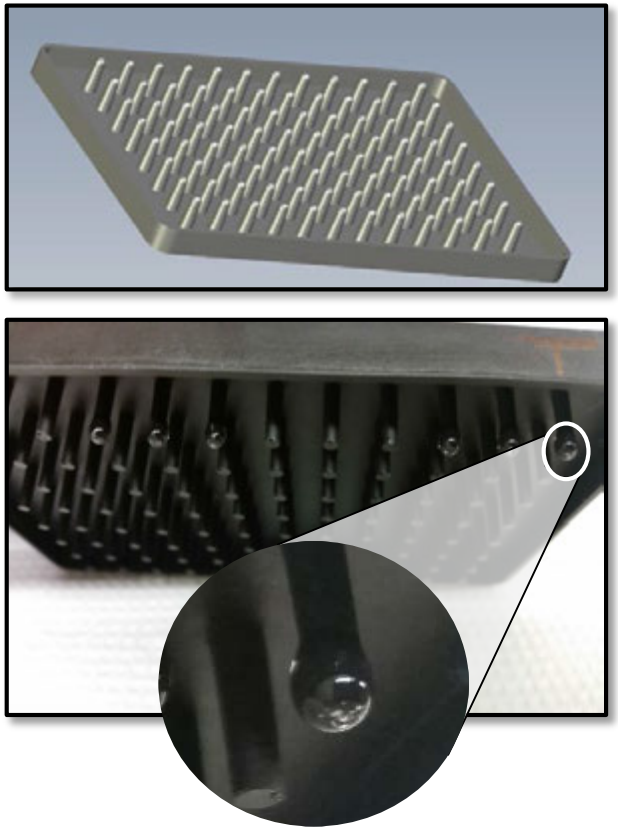
- HEK293T cells expressing NIS
- HEK293 cells expressing DIO-1
- 96-well radioactive iodide uptake assay for NIS
- Screen at single high concentration screen
- Hit follow-up at >20% inhibition for NIS and >50% inhibition for DIO-1

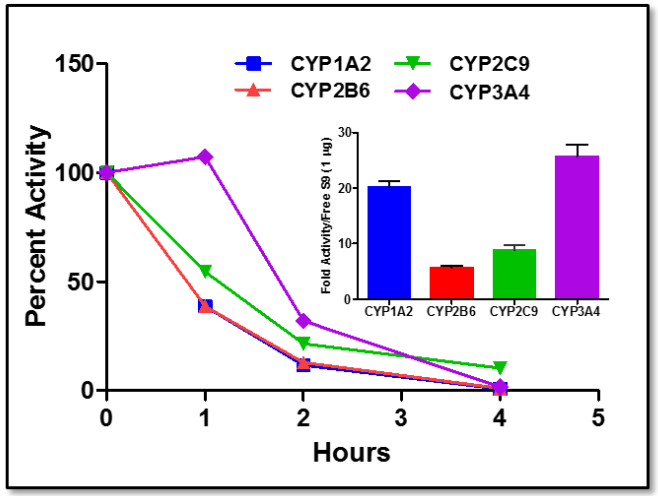
Deiodinase (DIO-1) Assay (300 ToxCast cmpds)

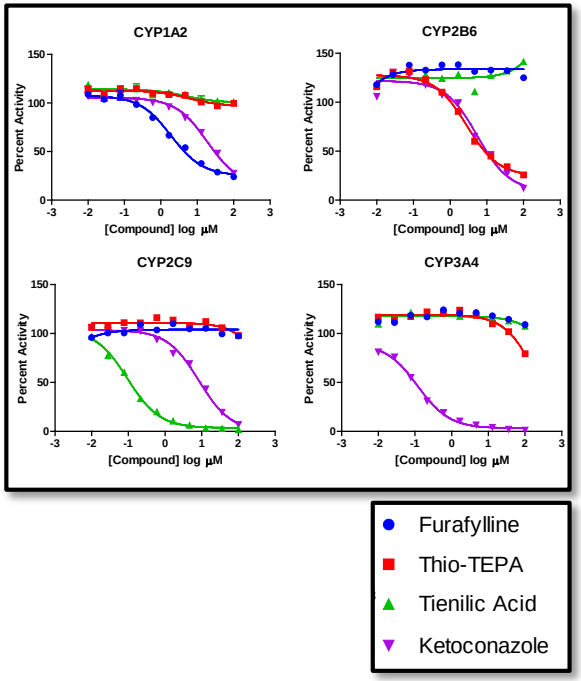


M. Hornung (NHEERL)

Adding Metabolic Competence



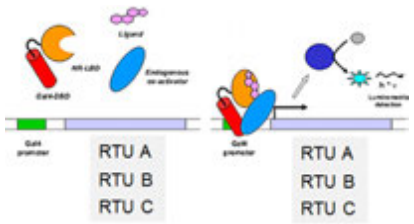


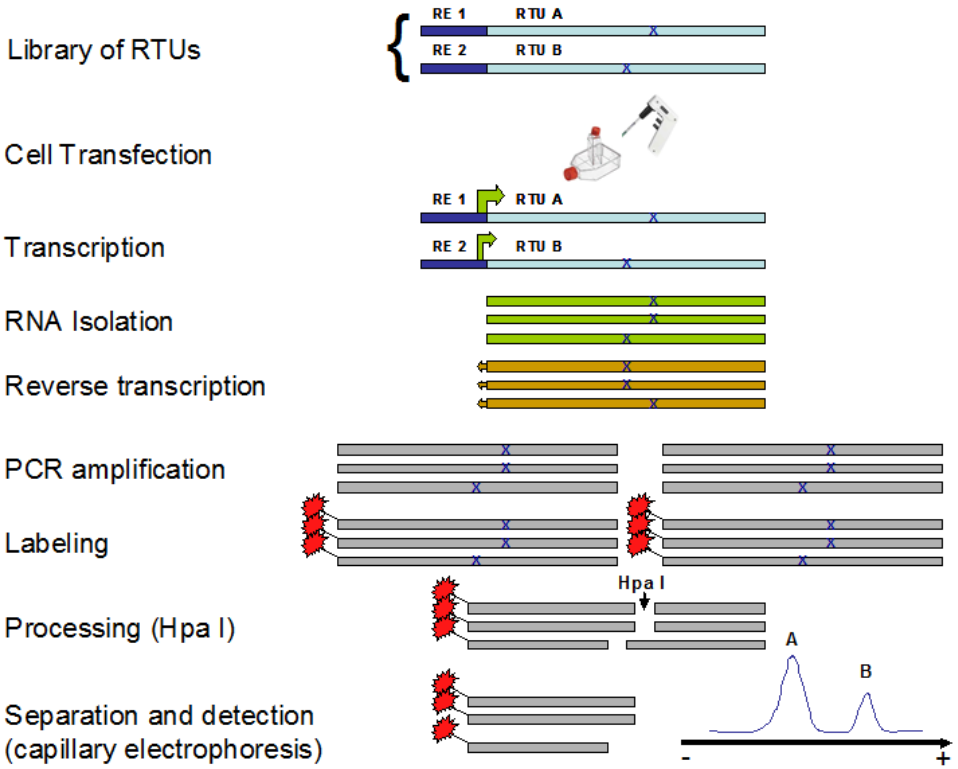


DeGroot and Simmons, Unpublished

Assessing Cross-Species Conservation of Response

Multispecies Attagene *Trans* Reporter Assay



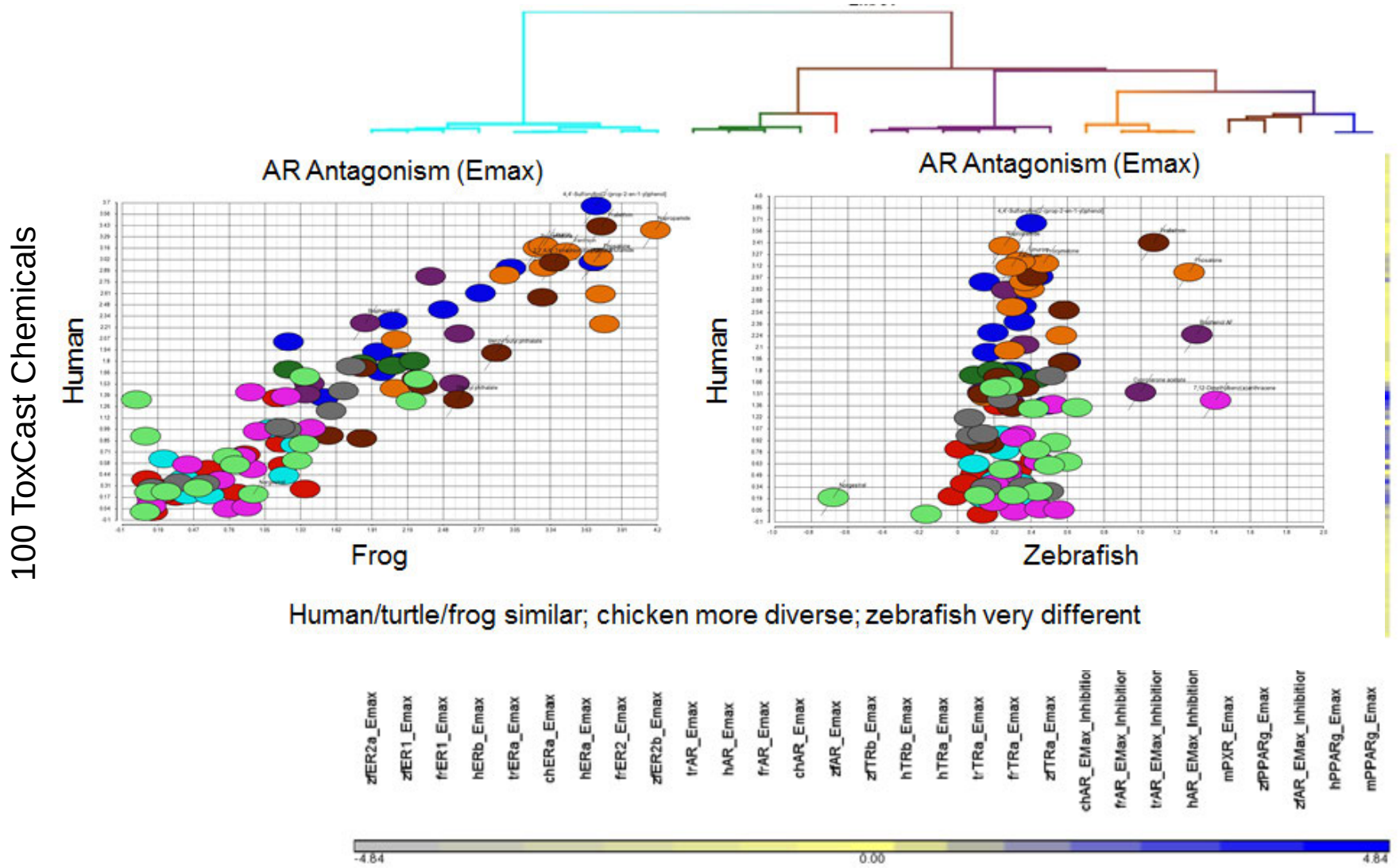


Receptor Family	Receptor Name	Species
Estrogen Receptor	ERa	Human
Estrogen Receptor	ERb	Human
Estrogen Receptor	ER1	Zebrafish
Estrogen Receptor	ER2a	Zebrafish
Estrogen Receptor	ER2b	Zebrafish
Estrogen Receptor	ERa	Chicken
Estrogen Receptor	ER1	Frog
Estrogen Receptor	ER2	Frog
Estrogen Receptor	ERa	Turtle
Estrogen Receptor	AR	Human
Estrogen Receptor	AR	Chicken
Estrogen Receptor	AR	Turtle
Estrogen Receptor	AR	Frog
Estrogen Receptor	AR	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPARg	Mouse
Peroxisome Proliferator Activated Receptor γ	PPARg	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPARg	Human
Pregnane X Receptor	PXR	Mouse
Thyroid Receptor	TRa	Turtle
Thyroid Receptor	TRb	Zebrafish
Thyroid Receptor	TRb	Zebrafish
Thyroid Receptor	TRa	Frog
Thyroid Receptor	TRa	Human
Thyroid Receptor	TRb	Human
Controls	M-06	NA
Controls	GAL4	NA
Controls	M-19	NA
Controls	m-32	NA
Controls	m-61	NA

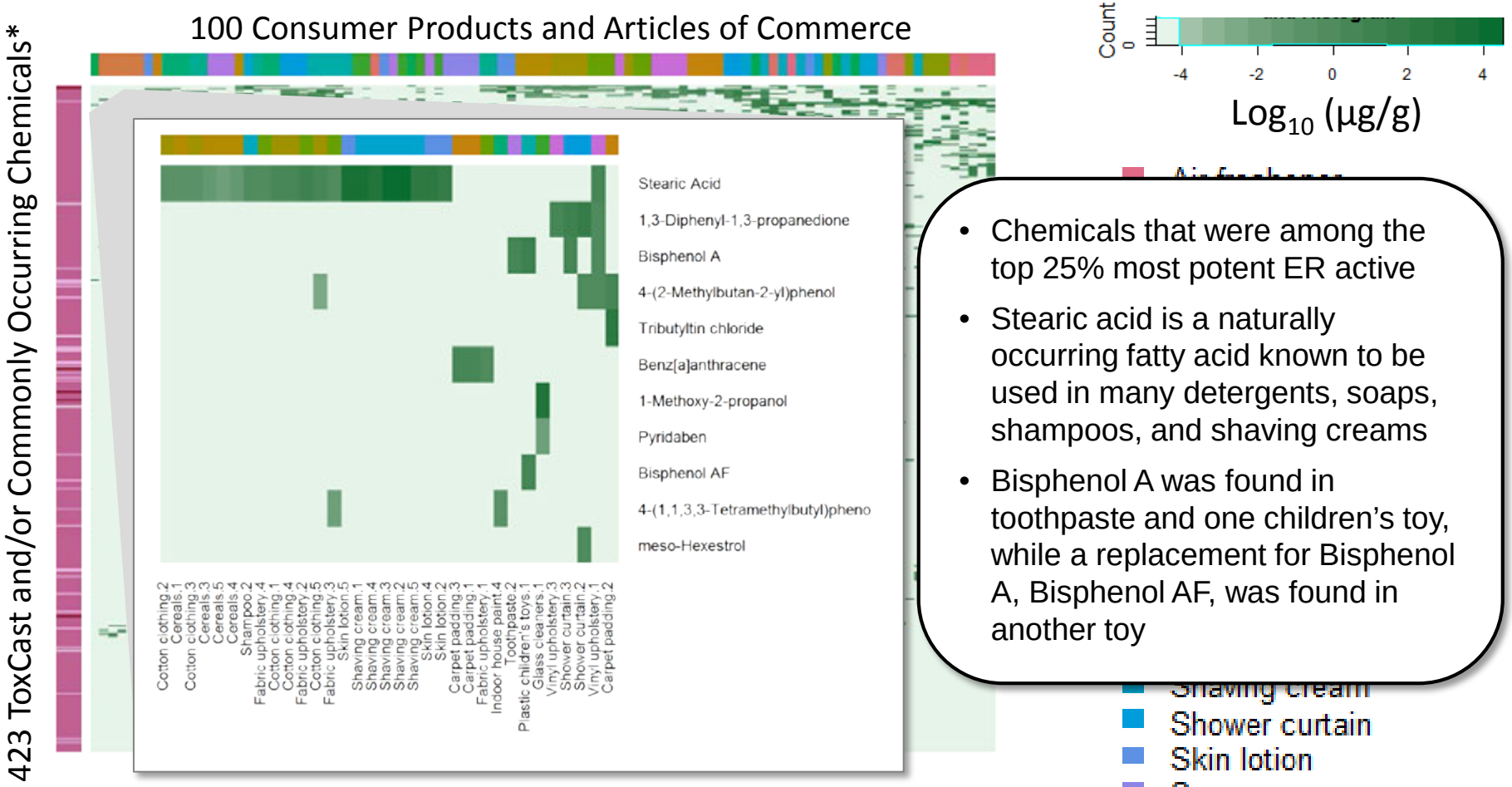
- Host cell: human HepG2
- Stimulation with EC20 of 6a-fluorotestosterone for detection of androgen receptor antagonists
- 100 chemicals with ER, AR, PPAR activity tested in concentration-response
- Data calculated as fold-change over control (6a-fluorotestosterone/DMSO)

Houck et al., Unpublished

Cross-Species Conservation of Efficacy



Improving Exposure Estimates and Characterization



- Chemicals that were among the top 25% most potent ER active
- Stearic acid is a naturally occurring fatty acid known to be used in many detergents, soaps, shampoos, and shaving creams
- Bisphenol A was found in toothpaste and one children's toy, while a replacement for Bisphenol A, Bisphenol AF, was found in another toy

- GCXGC-MS with DCM Extraction
- 1606 tentative and confirmed chemical identifications

Wambaugh et al. Unpublished

What Does Version 2.0 Look Like?

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Acknowledgements and Questions

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

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- NERL
- NHEERL
- NCEA

NICEATM Colleagues

OSCP Colleagues

EPA's National Center for Computational Toxicology