U.S. EPA Endocrine Disruptor Screening Program

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April 11th 2016
Expert Meeting to Reach Scientific Consensus on
Endocrine Disruptors
Berlin, Germany

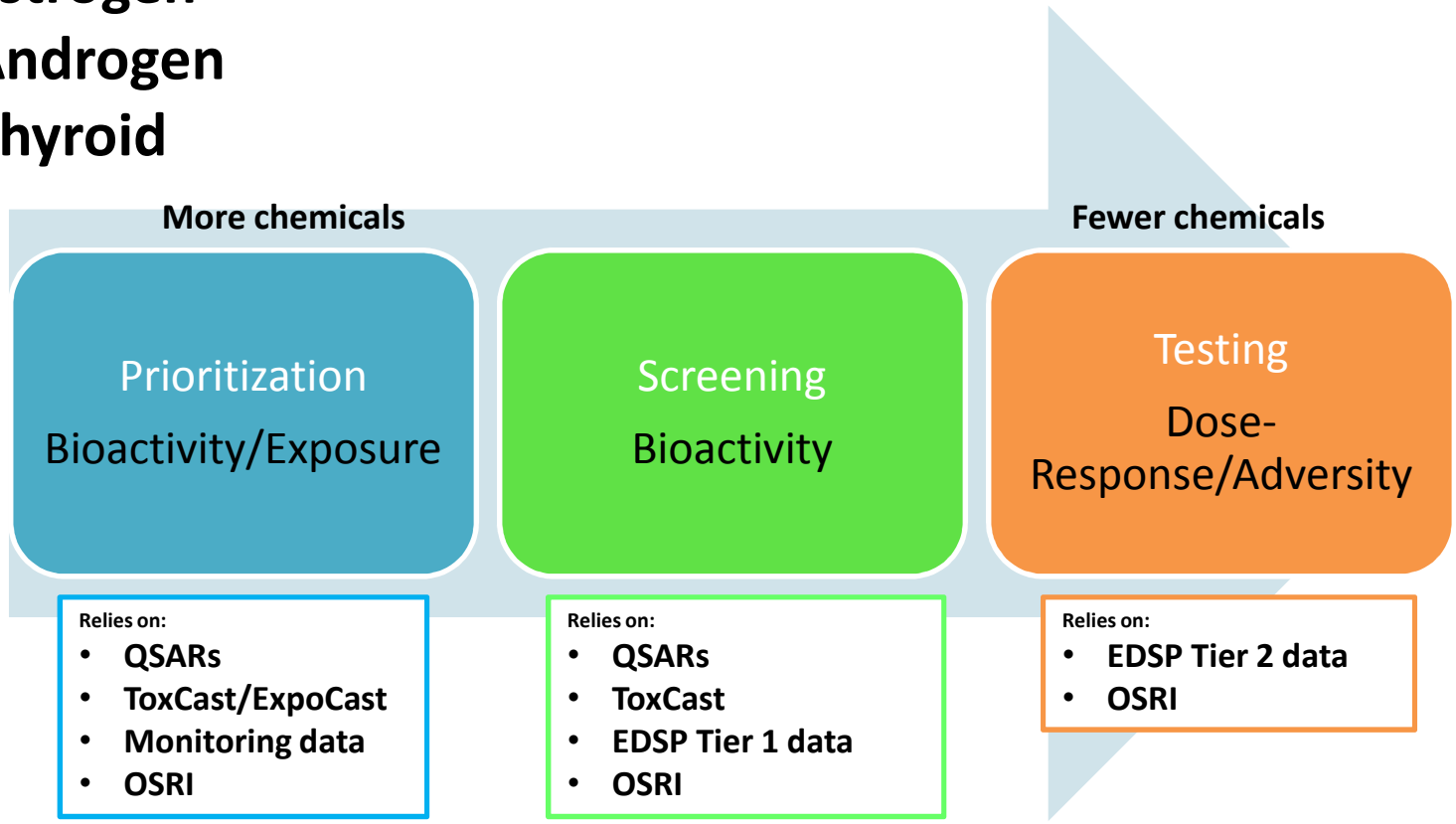
EDSP Prioritization, Screening & Testing

Pathways:

Estrogen

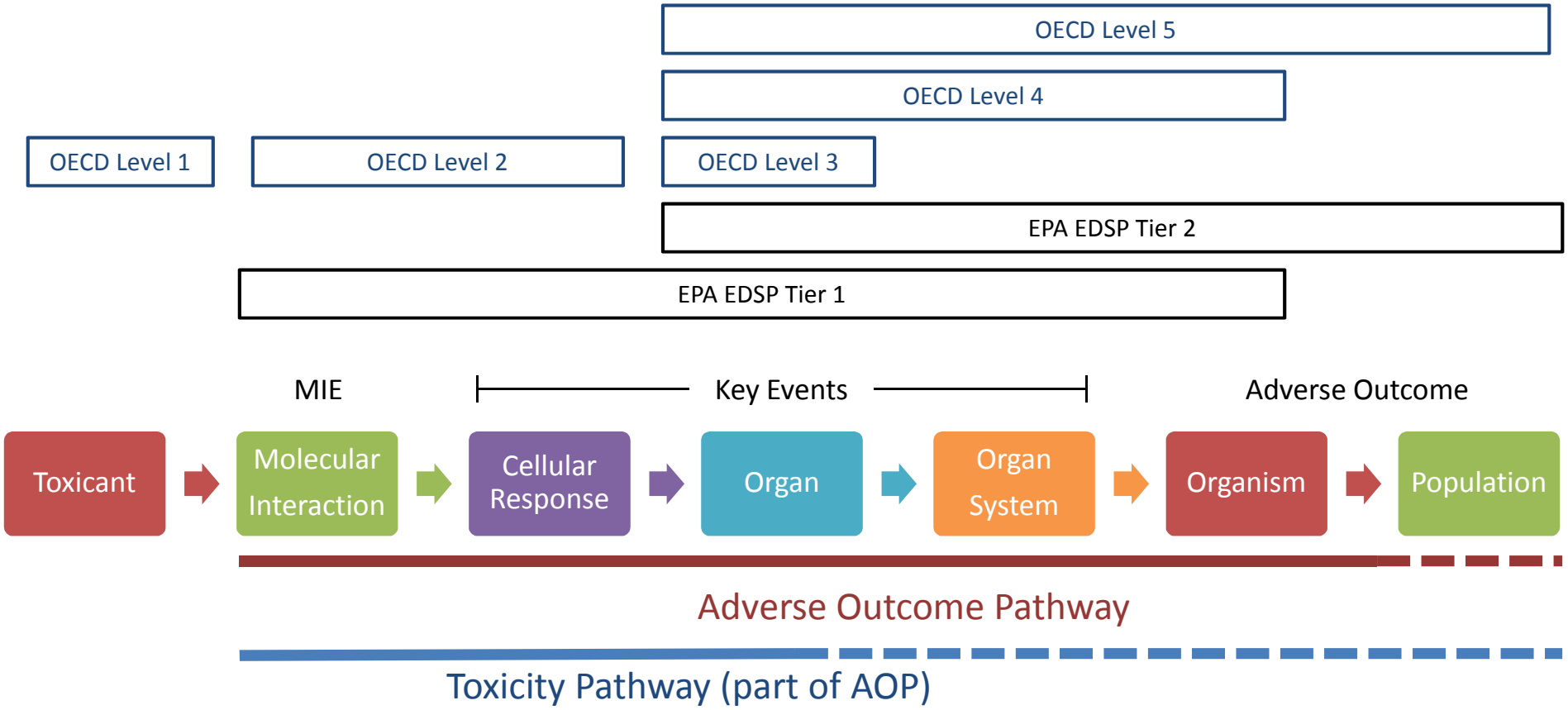
Androgen

Thyroid

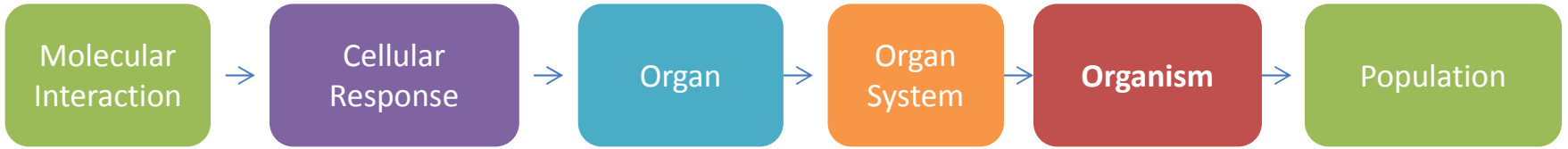


Prioritization and Screening for bioactivity
Testing for dose-response and adverse effects

EDSP Screening and Testing



EDSP Screening and Testing by Pathway



Endocrine Pathway	Tier 1											Tier 2			
	ER Binding	AR Binding	ER Transcriptional Activation	Aromatase	Steroidogenesis	Uterotrophic	Hershberger	Pubertal Male	Pubertal Female	Amphibian Metamorphosis	Fish Short Term Reproduction	Rat 2-gen/ EOGRTS	MEOGRT	LAGDA	JQTT
E+	■		■		■	■			■		■	■	■	■	■
E-	■			■	■				■		■	■	■	■	■
A+		■			■		■	■			■	■	■	■	■
A-		■			■		■	■			■	■	■	■	■
HPT Axis								■	■	■		■		■	■

EDSP Pivot Announcement

FEDERAL REGISTER
The Daily Journal of the United States Government

June 19, 2015
FRL-9928-69

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

https://www.federalregister.gov/articles/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice

35350 Federal Register / Vol. 80, No. 118 / Friday, June 19, 2015 / Notices

may claim all or part of a response confidential. EPA will disclose information that is covered by a claim of confidentiality only to the extent permitted by, and in accordance with, the procedures in TSCA section 14 and 40 CFR part 2.

Burden statement: The annual public reporting and recordkeeping burden for this collection of information is estimated to average 31.5 hours per response. Burden is defined in 5 CFR 1320.3(b).

The ICR, which is available in the docket along with other related materials, provides a detailed explanation of the collection activities and the burden estimate that is only briefly summarized here:

Respondents/Affected Entities: Entities potentially affected by this ICR are companies that manufacture, process or import chemical substances, mixtures or categories.

Estimated total number of potential respondents: 1.

Frequency of response: On occasion.

Estimated total average number of responses for each respondent: 1.

Estimated total annual burden hours: 31.5 hours.

Estimated total annual costs: $2,388. This includes an estimated burden cost of $2,388 and an estimated cost of $0 for capital investment or maintenance and operational costs.

III. Are There Changes in the Estimates from the Last Approval?

There is a decrease of 916 hours in the total estimated respondent burden compared with that identified in the ICR currently approved by OMB. This decrease reflects additional both adjustment changes from a reduction in the assumed number of PAIR reports filed annually, and program changes resulting from mandatory electronic submissions of PAIR reports. In recent years (FY 2011–FY 2014), EPA has received no PAIR submissions and, for the purposes of this analysis, EPA assumes an annual rate of one submission per year. At the time OMB last renewed this ICR, EPA estimated an average of 33 reports from 14.8 submitters based on fiscal year 2006–2010 data. The ICR supporting statement provides a detailed analysis of the change in burden estimate. This change is both an adjustment and a program change.

IV. What is the Next Step in the Process for this ICR?

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

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

Dated: June 10, 2015.

James Jones,

Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

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

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[EPA–HQ–OPPT–2015–0305; FRL–9928–69]

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

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will improve the Agency's ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a computational model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and computational models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and efficient screening using alternative test methods to some assays in the Tier 1 battery to protect human health and the environment.

DATES: Comments must be received on or before August 18, 2015.

ADDRESSES: Submit your comments, identified by docket identification (ID) number EPA–HQ–OPPT–2015–0305, by one of the following methods:

- *Federal eRulemaking Portal: http://www.regulations.gov.* Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute.
- *Mail:* Document Control Office (7407M), Office of Pollution Prevention and Toxics (OPPT), Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001.
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For general information contact: The TSCA-Hotline, ABVI-Goodwill, 422 South Clinton Ave., Rochester, NY 14620; telephone number: (202) 554–1404; email address: *TSCA-Hotline@epa.gov.*

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this action apply to me?

This action is directed to the public in general, and may be of interest to a wide range of stakeholders including those interested in endocrine testing of chemicals (including pesticides), and the EDSP in general. Since others also may be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action.

B. What is the agency authority for taking this action?

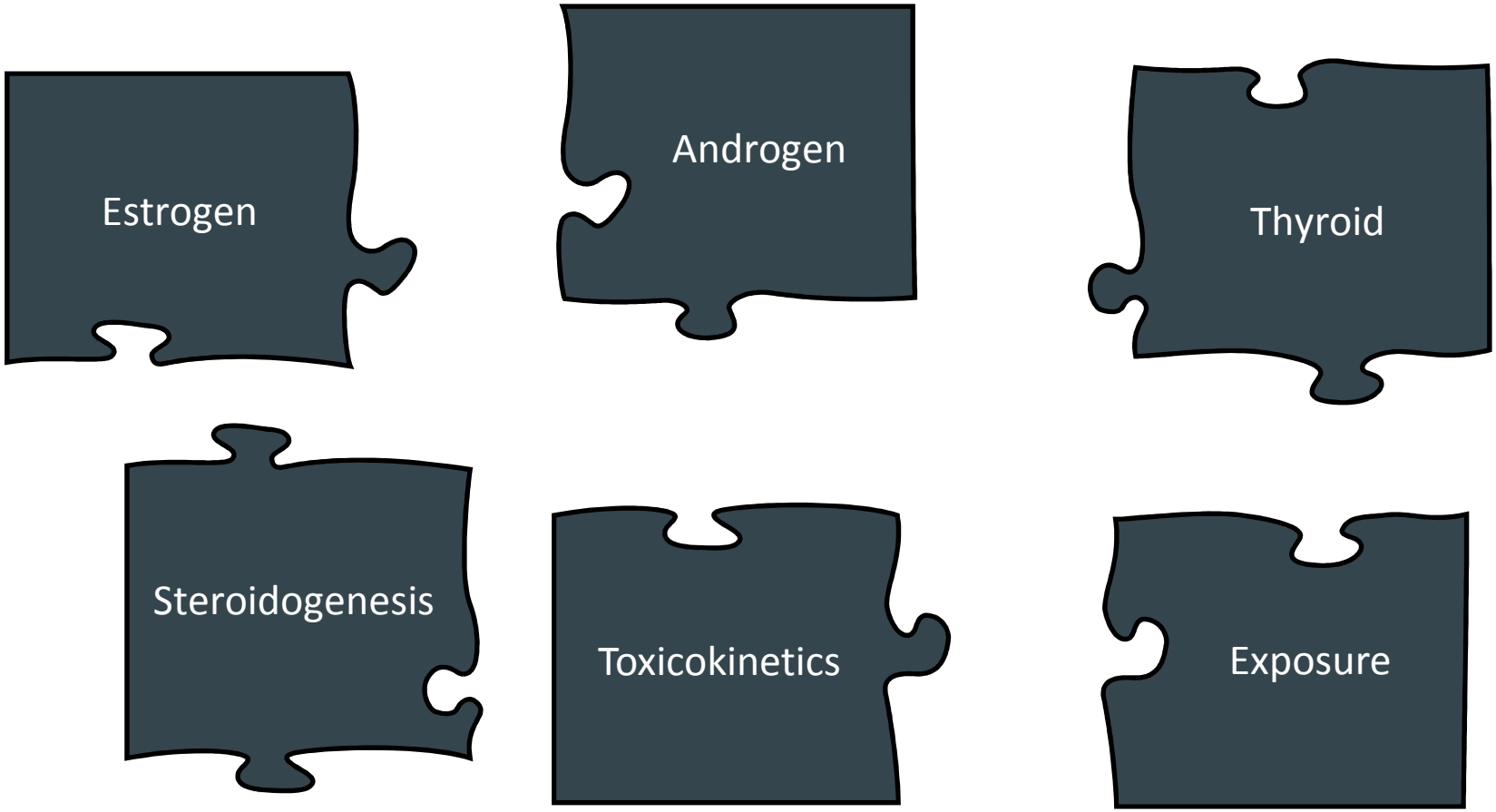
The EDSP is established under section 408(p) of the Federal Food, Drug and

Developing Alternative EDSP Assays

EDSP Tier 1 Battery of Assays	Model Alternative Development
Estrogen Receptor (ER) Binding	**ER Model FY 2015**
Estrogen Receptor Transactivation (ERTA)	**ER Model FY 2015**
Uterotrophic	**ER Model FY 2015**
Androgen Receptor (AR) Binding	AR Model FY 2016
Hershberger	AR Model FY 2016
Aromatase	STR Model FY 2016
Steroidogenesis (STR)	STR Model 2016
Female Rat Pubertal	**ER**, STR & THY Models FY 2017
Male Rat Pubertal	AR, STR & THY Models FY 2017
Fish Short Term Reproduction	**ER**, AR & STR Models FY 2017
Amphibian Metamorphosis	THY Model FY 2017

ER = estrogen receptor; AR = androgen receptor; STR = steroidogenesis; THY = thyroid

The EDSP is Currently Working on the Individual Puzzle Pieces



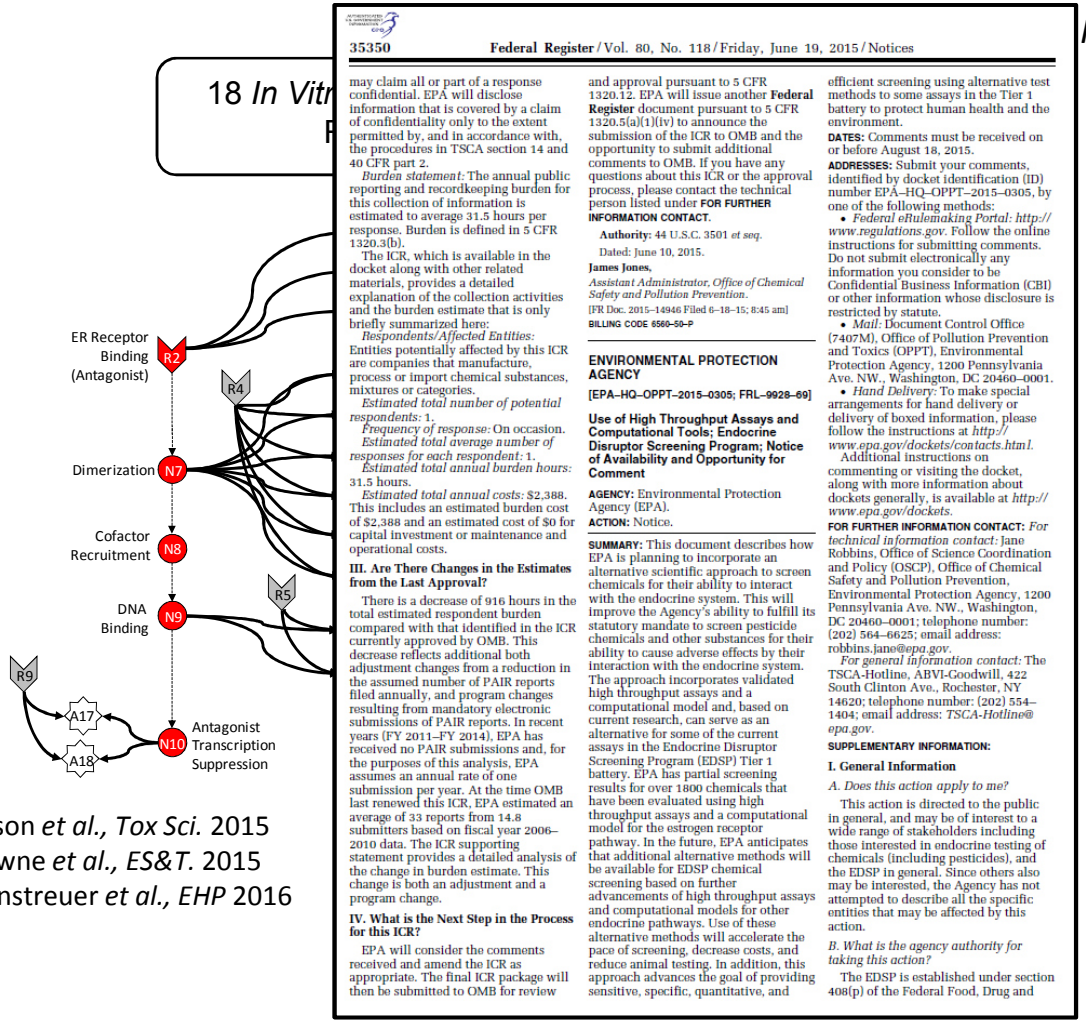
ER Model: Performance Based Approach to Establish Scientific Confidence

Judson et al. 2015, Tox Sci: “Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor"

Kleinstreuer et al. 2015, EHP: “A Curated Database of Rodent Uterotrophic Bioactivity"

Browne et al. 2015, ES&T: “Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model"

Current Status on the Estrogen Piece of the Puzzle



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

In Vitro Reference Chemicals*

True Positive	26 (25)
True Negative	11 (11)
False Positive	1 (0)
False Negative	2 (2)
Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

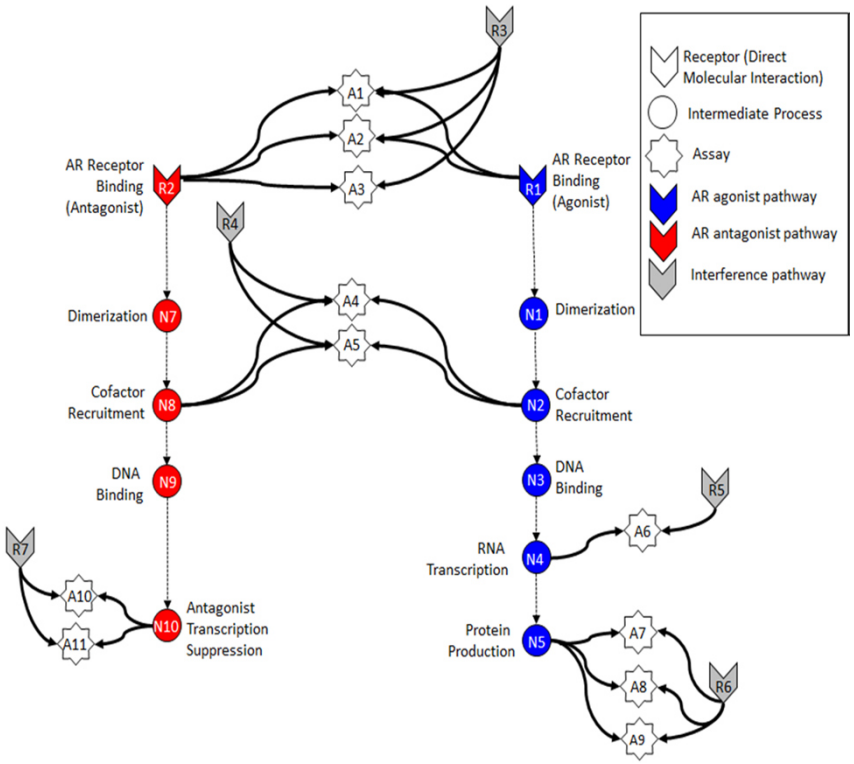
In Vivo Reference Chemicals*

True Positive	29 (29)
True Negative	8 (8)
False Positive	5 (1)
False Negative	1 (1)
Accuracy	**0.86 (0.95)**
Sensitivity	**0.97 (0.97)**
Specificity	**0.67 (0.89)**

*Values in parentheses exclude inconclusive chemicals

Current Status on the Androgen Piece of the Puzzle

11 *In Vitro* Assays Measure AR-Related Activity



Kleinstreuer et al. 2016 SOT poster #2651

Agonism

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

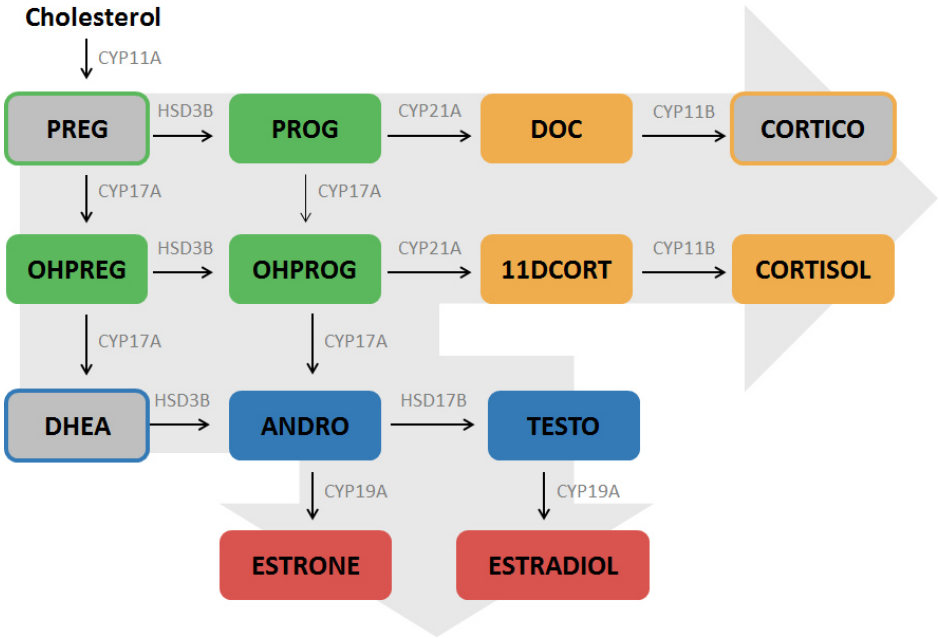
Antagonism

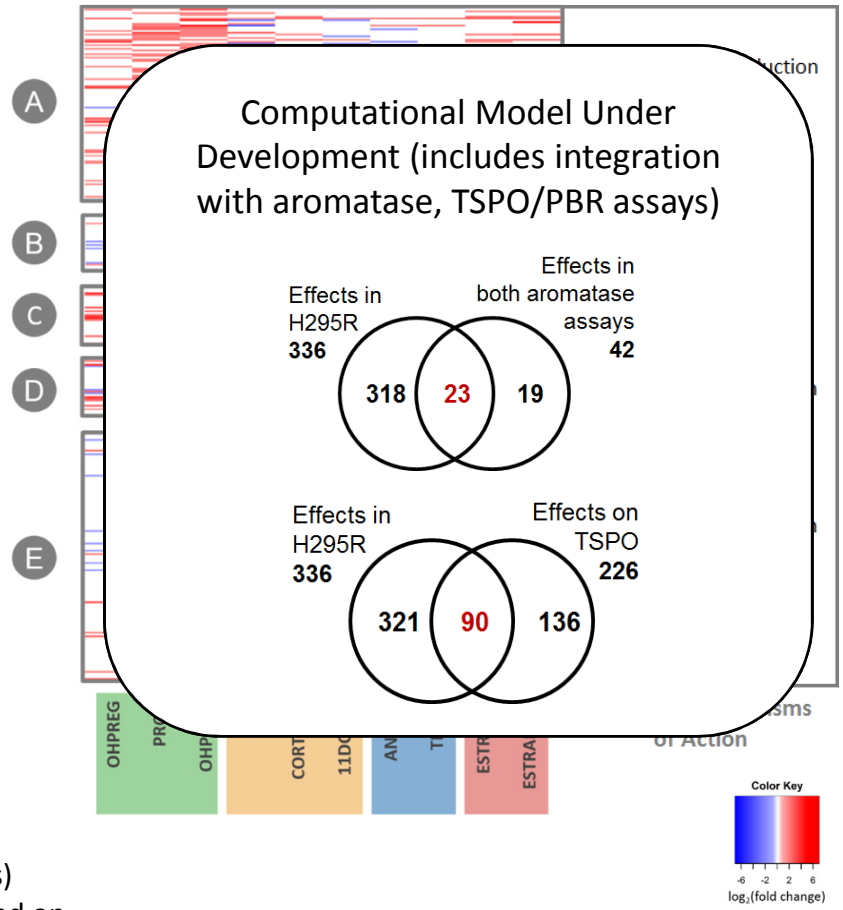
True Positives	18
True Negatives	8
False Positives	0
False Negatives	2*
Accuracy	0.93*
Sensitivity	0.90*
Specificity	1.00

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

Current Status on the Steroidogenesis Piece of the Puzzle

ToxCast H295R Steroidogenesis Assay

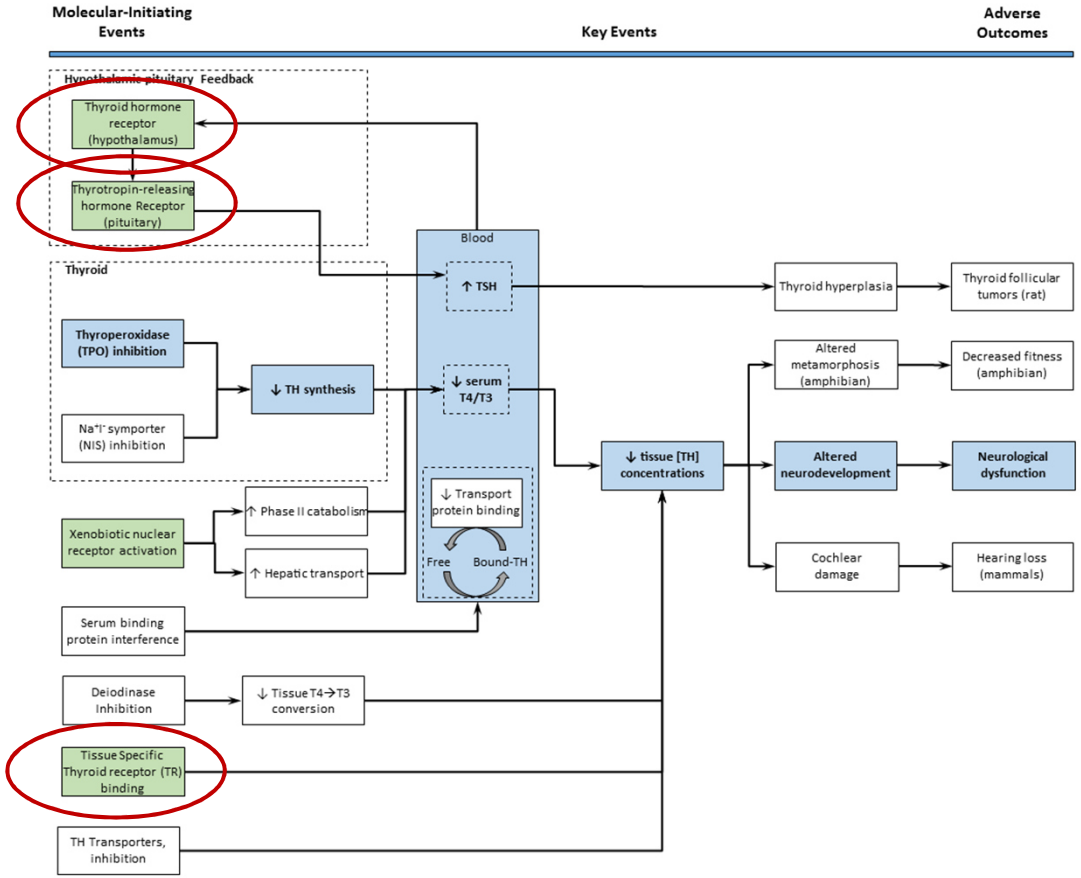




- 13 hormones were quantified using HPLC-MS/MS
- 2060 chemicals screened at single concentration
- 403 chemicals selected for concentration response (altered ≥ 4 hormones)
  - 120 additional chemicals selected for concentration response based on other needs

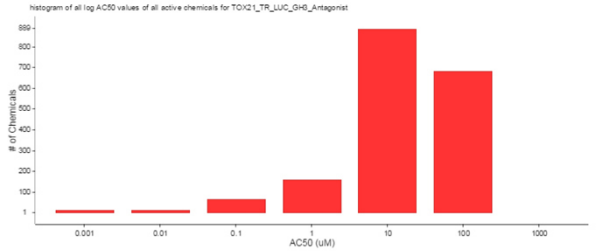
Karmaus et al. 2016 SOT poster #2637

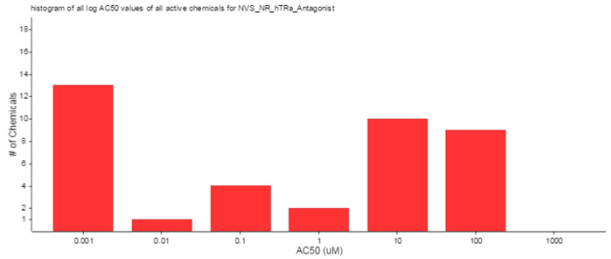
Current Status on the Thyroid Piece of the Puzzle



Paul *et al.,* In Review

THR Assays





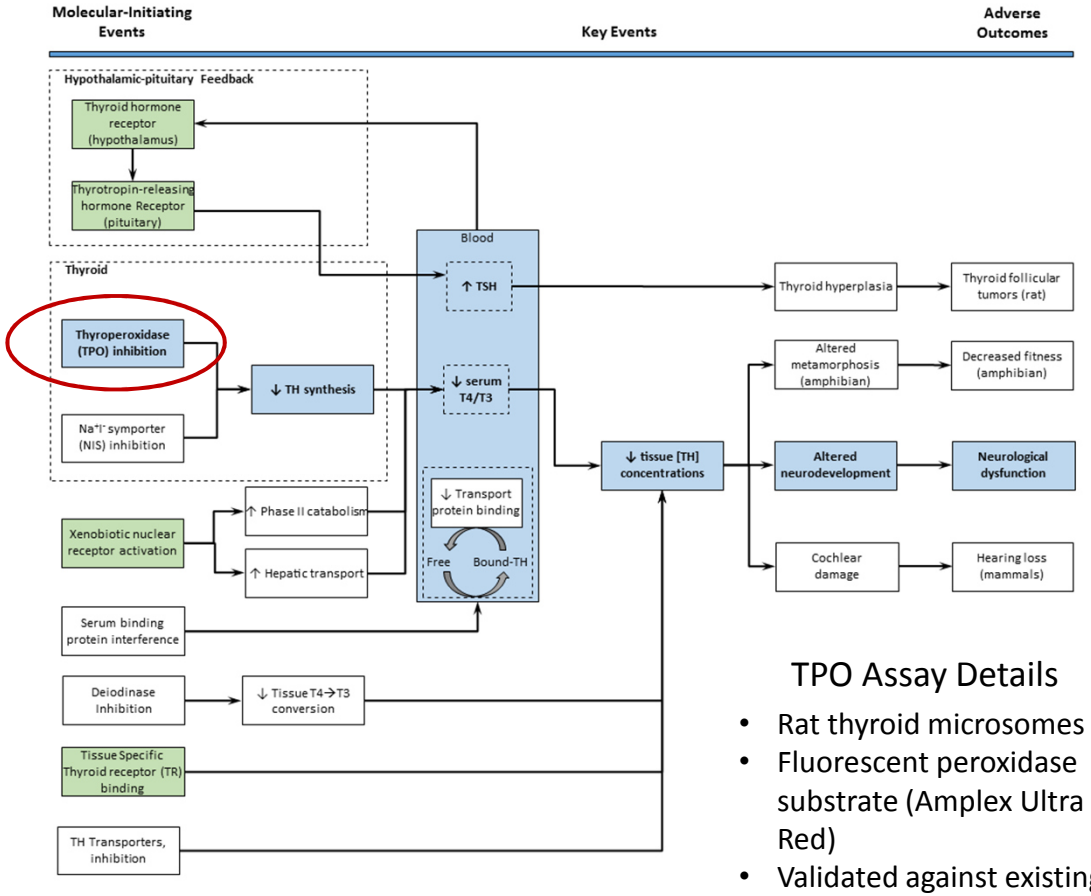
TRHR Assay

Undergoing Online Validation (Tox21)

TSHR Assay

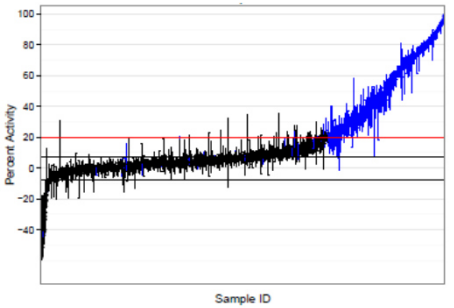
Undergoing Online Validation (Tox21)

Current Status on the Thyroid Piece of the Puzzle

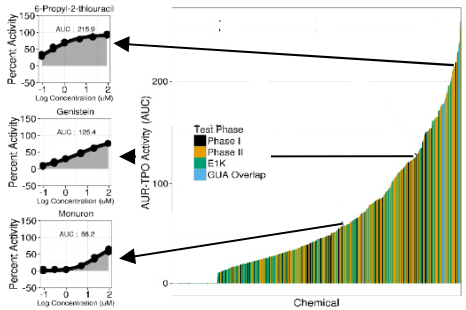


Paul *et al.*, In Review

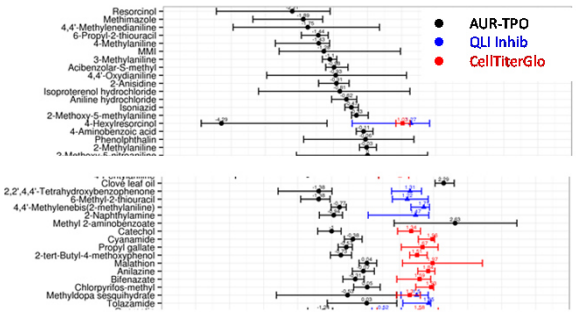
Single-concentration Screen



Concentration Response Screen



Stratify by Selectivity

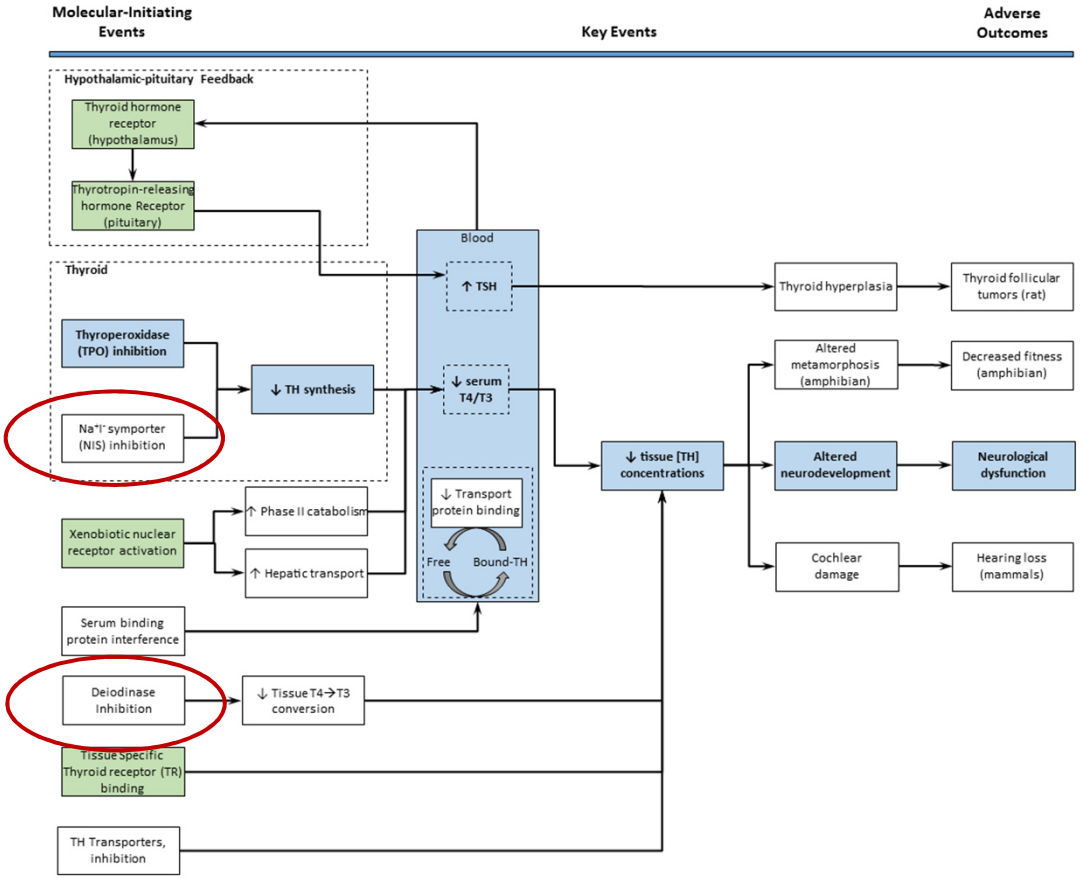


TPO Assay Details

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

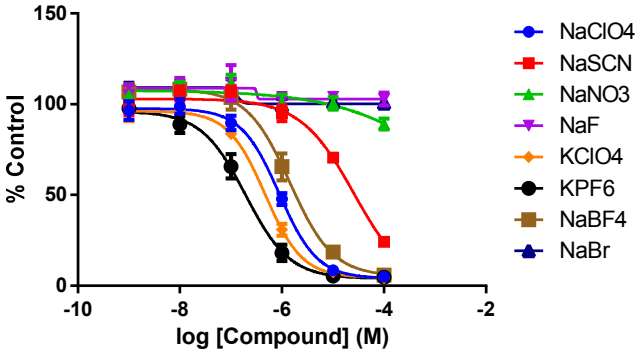
Simmons et al. 2016 SOT poster #1886

Current Status on the Thyroid Piece of the Puzzle



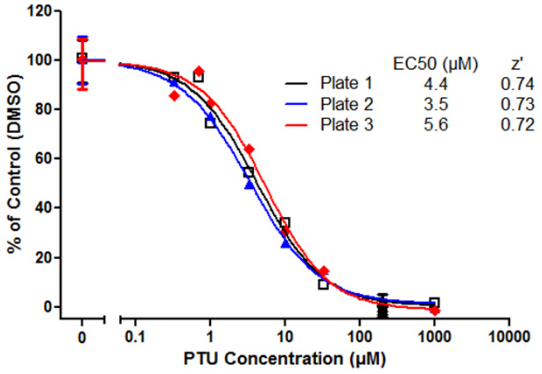
Paul *et al.,* In Review

High-Throughput Human NIS Screen
(with Secondary Screen in FRTL-5 rat follicular cells)



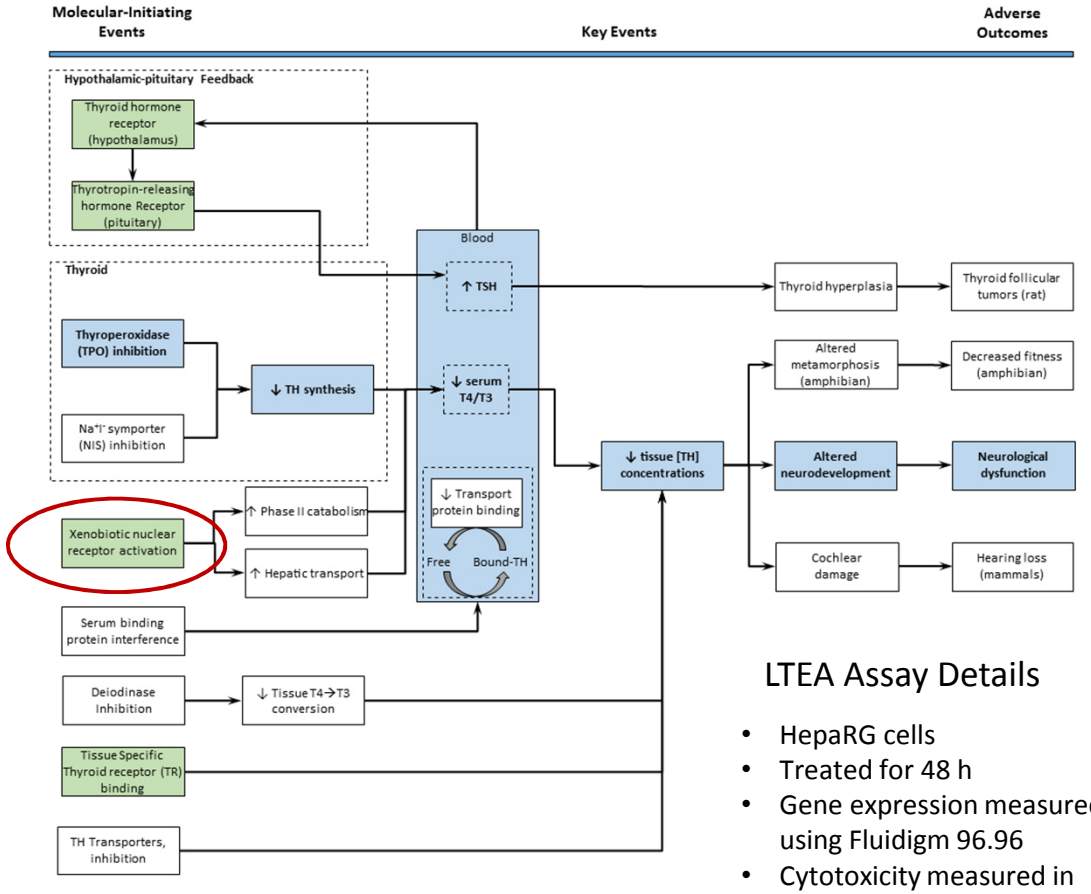
Buckalew et al. 2016 SOT poster #1887
Murr et al. 2016 SOT poster #1888
Hallinger et al. 2016 SOT poster #1889

High-Throughput Deiodinase Screen
(In Development)



M. Hornung

Current Status on the Thyroid Piece of the Puzzle

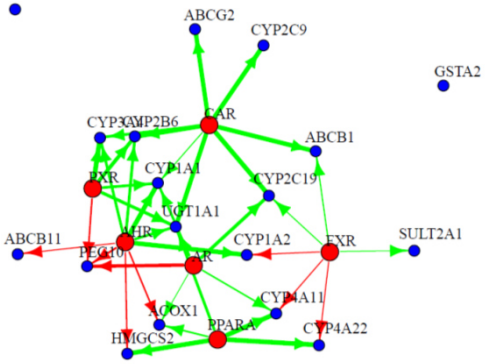


Paul *et al.,* In Review

LTEA Assay Details

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

HepaRG Gene Expression Assay



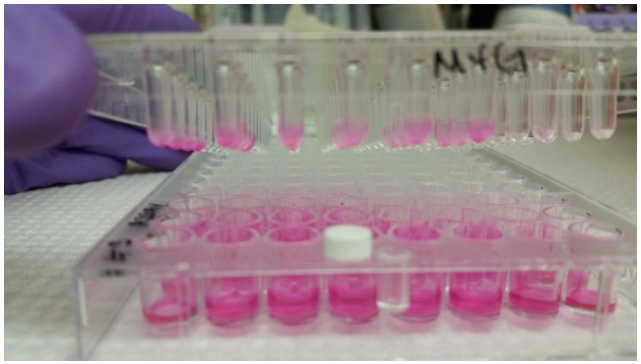
Bayesian model for NR regulation

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

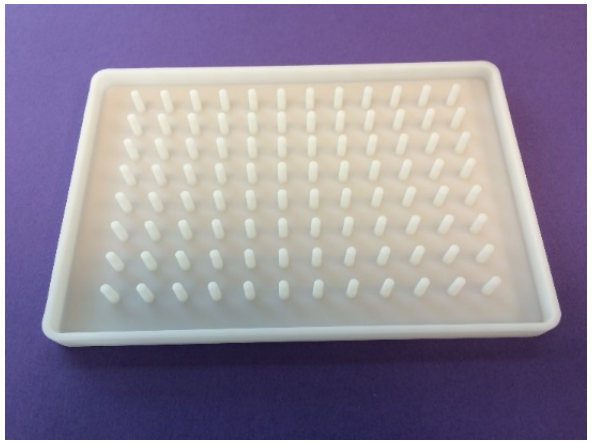
J. Franzosa
J. Wambaugh

Addressing the Metabolic Competence Challenge

Alginate Immobilization of Metabolic Enzymes (AIME)

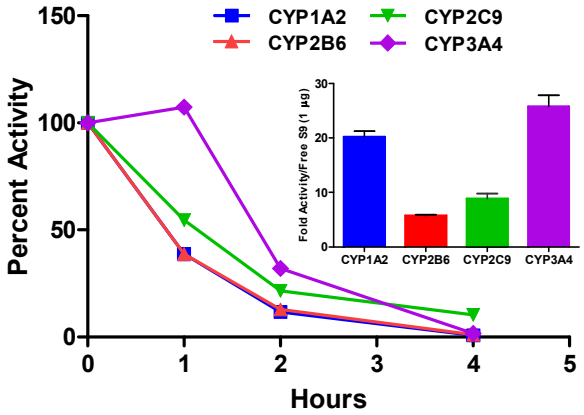


Prototype Lids

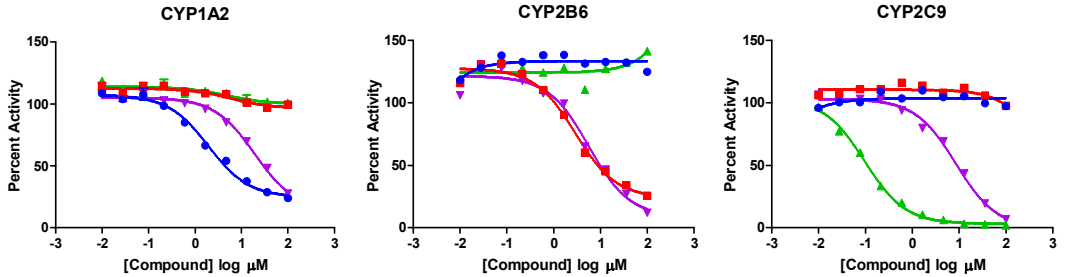


DeGroot et al. 2016 SOT poster #3757

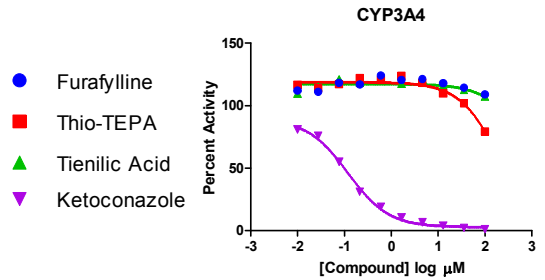
Amount of XME Activity in Microspheres



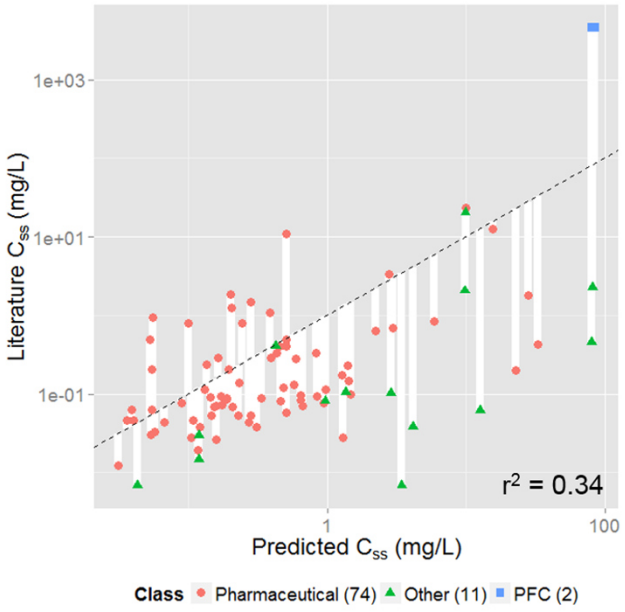
Small Molecule Inhibition of XME Activity

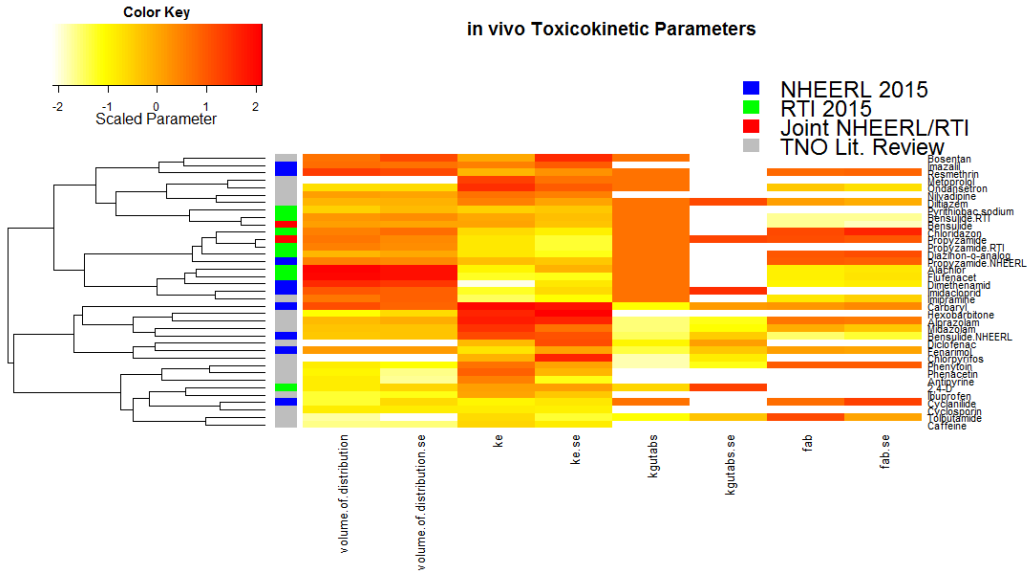


Compound	Mol. Wt. (g/mol)	Targeted P450	IC50 Free S9 (µM)	IC50 AIME (µM)
Furafylline	260.25	1A2	2.39	1.92
Thio-TEPA	189.22	2B6	7.46	2.86
Tienilic Acid	331.17	2C9	.053	.096
Ketoconazole	531.43	3A4	.086	0.12



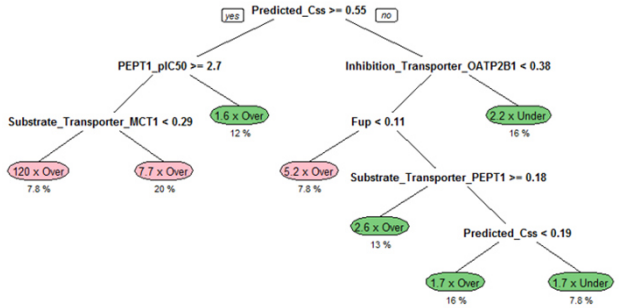
The Toxicokinetics Piece of the Puzzle



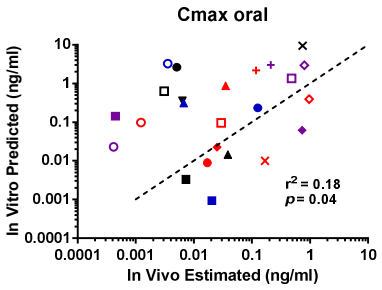


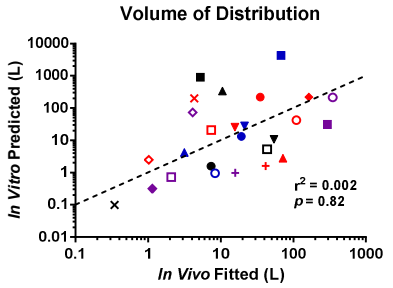
- Additional *in vivo* rat TK data collected for 26 chemicals by NHEERL collaborators and RTI contractors
- Literature *in vivo* rat TK data curated by TNO collaborators

Recursive Partition Tree on Residuals



Wambaugh *et al.*, *Toxicol Sci*, 2015





Hughes et al. 2016 SOT poster #3436

Current Status on the Exposure Piece of the Puzzle

3rd Gen

SHEDS-HT

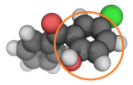
Literature Models

CPcat Database

NHANES Urine, Blood and Serum Data

- Averaging multiple exposure models
- Challenge is to know which exposure model to use for which chemical
- Chemical use identifies relevant pathways

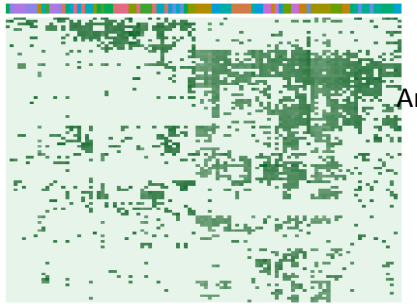
Use Database (FUSE)



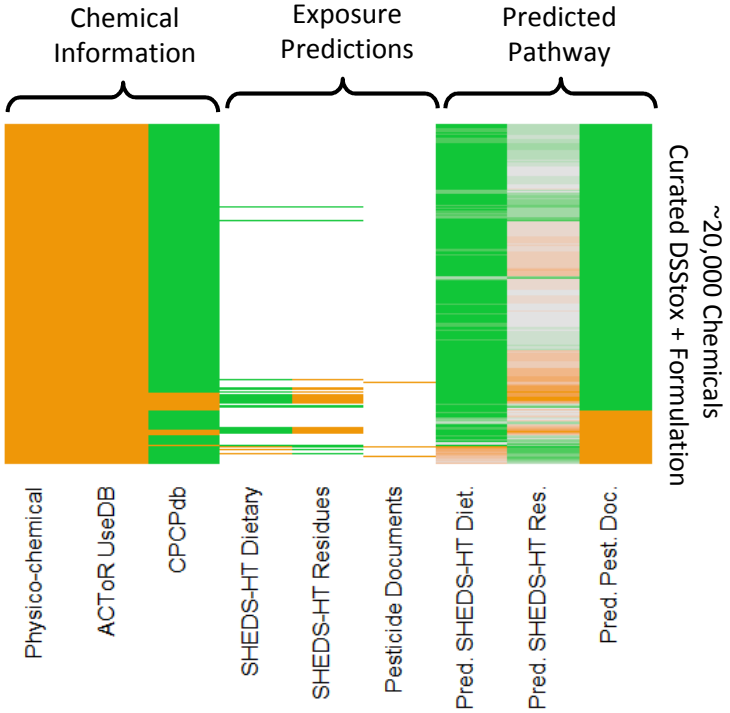
Machine Learning Approaches to Predict Chemical Use

Pilot Study

- 20 Product types x 5 products/type
- 2D GCxTOF/MS



Non-Targeted Analytical Screening of Consumer Product Composition

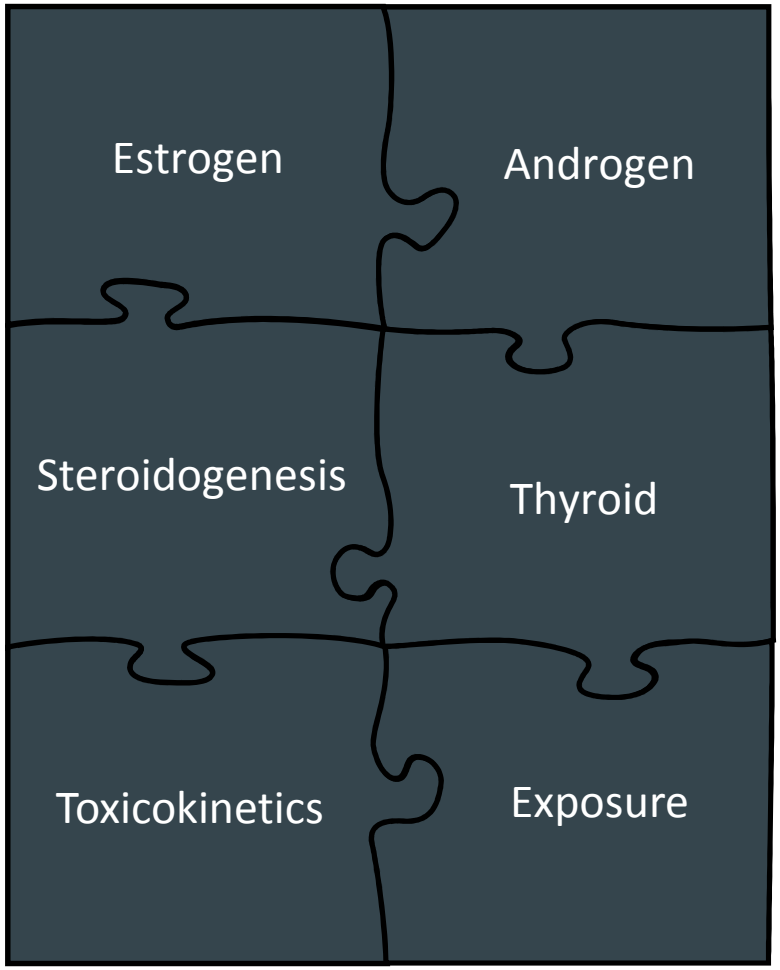


Isaacs et al. 2016 SOT poster #1679

Phillips et al. 2016 SOT poster #1680

Wambaugh et al. 2016 SOT poster #1682

Working to Assemble the EDSP Screening Puzzle



Validation

OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Validation is a process by which the reliability and relevance of a test method are established for a specific purpose.

Validation

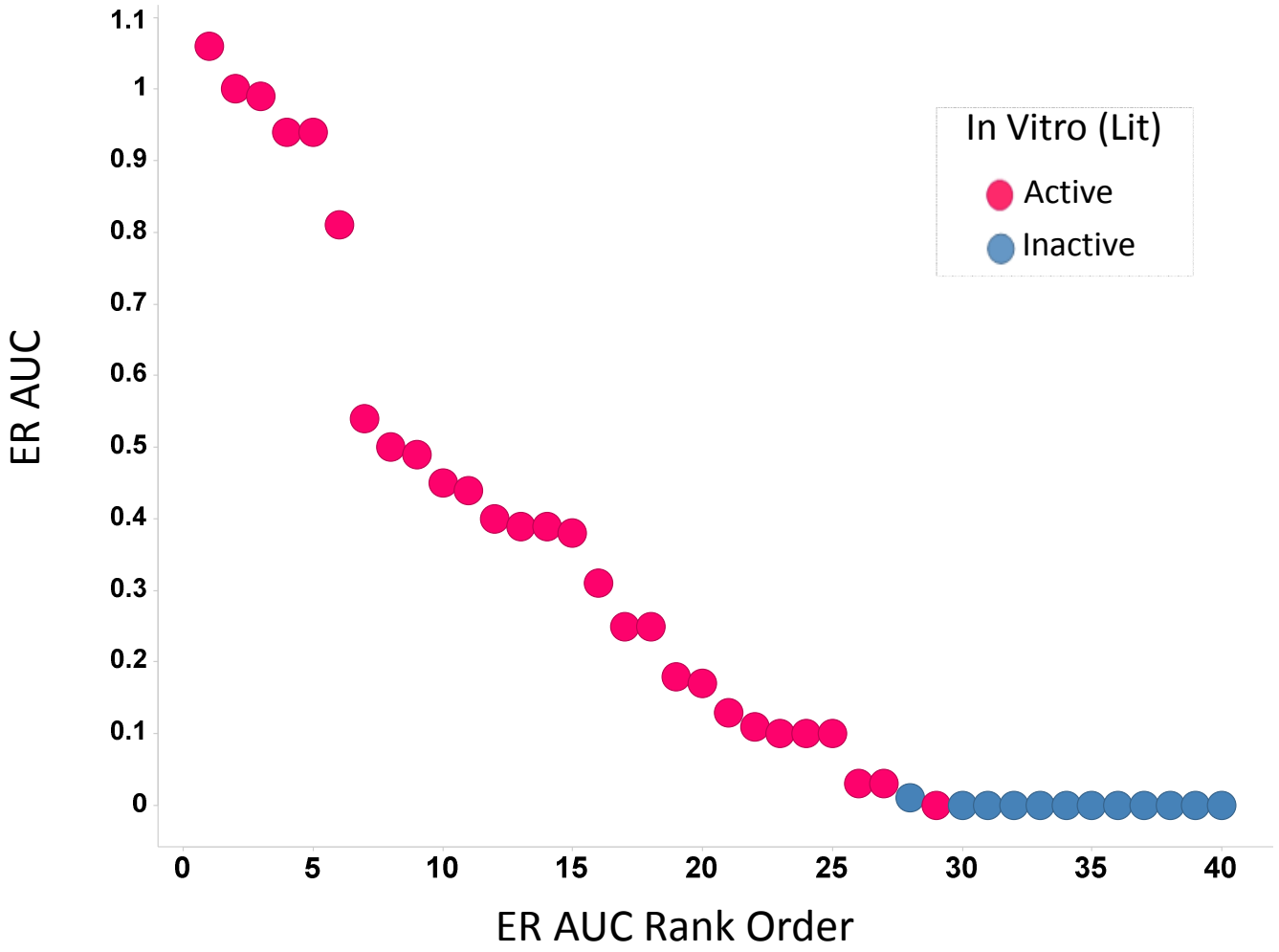
OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Relevance and reliability should be characterized against data generated with a list of reference chemicals (tested in the original methods) accepted by regulatory agencies.

Reference chemicals: Chemicals selected for use in the validation process, for which responses in the in vitro or in vivo reference test system or the species of interest are already known.

ER Model Validation- In Vitro

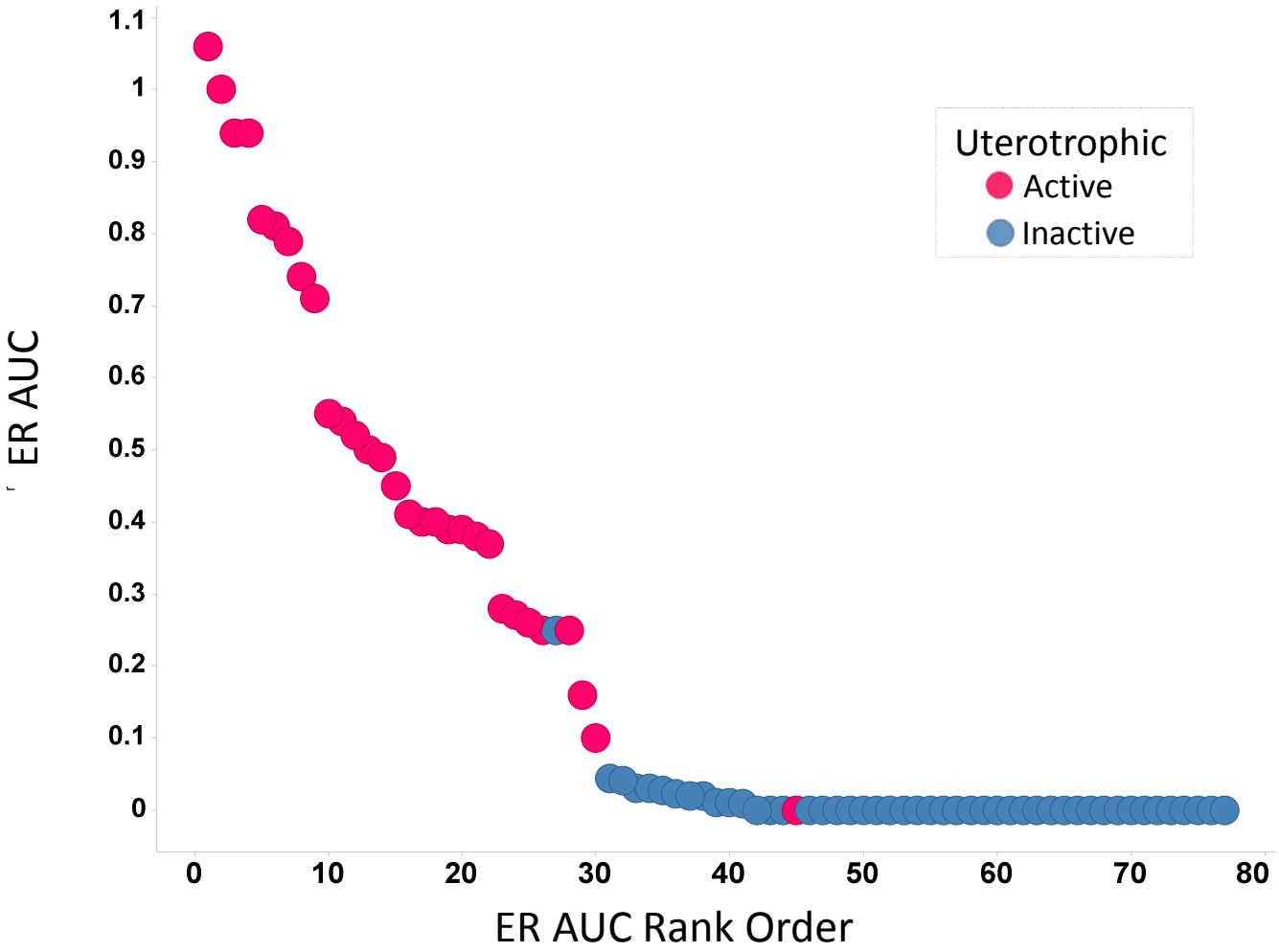
40 In Vitro Reference Chemicals



True Positive	25
True Negative	12
False Positive	0
False Negative	3
Accuracy	**0.95**
Sensitivity	**0.89**
Specificity	**1.00**

ER Model Validation- In Vivo

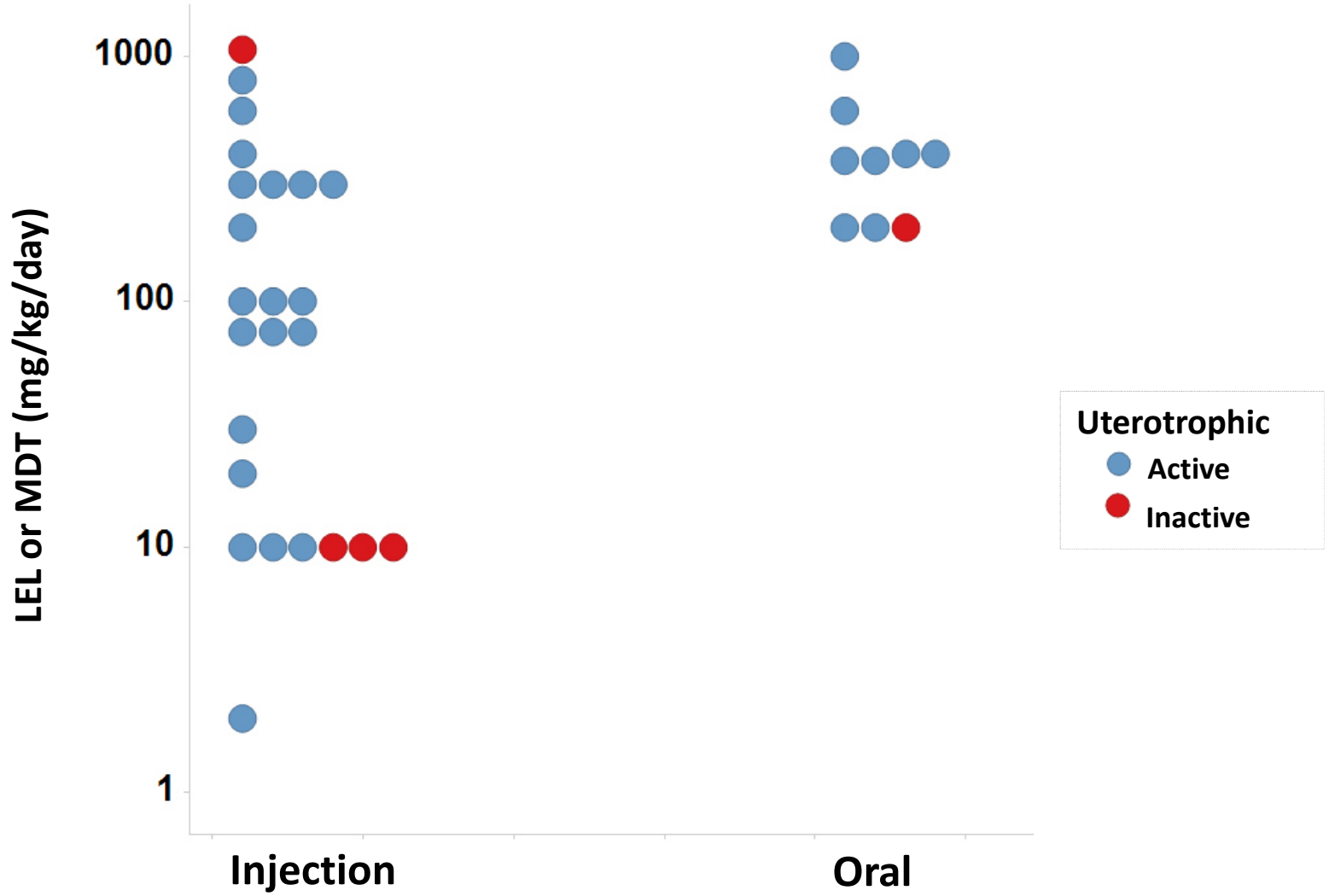
77 In Vivo Reference Chemicals



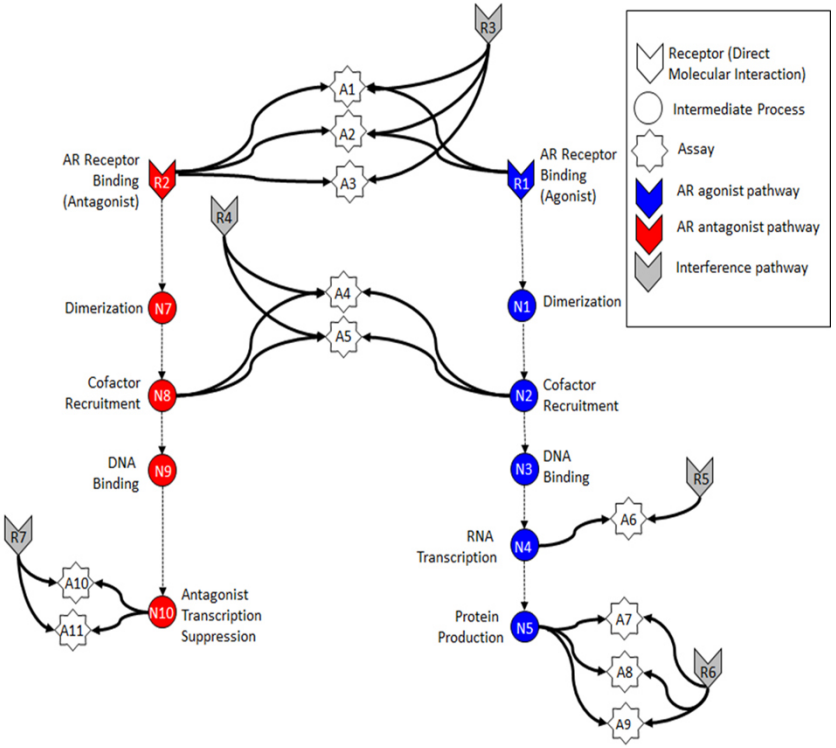
True Positive	29
True Negative	46
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.97**

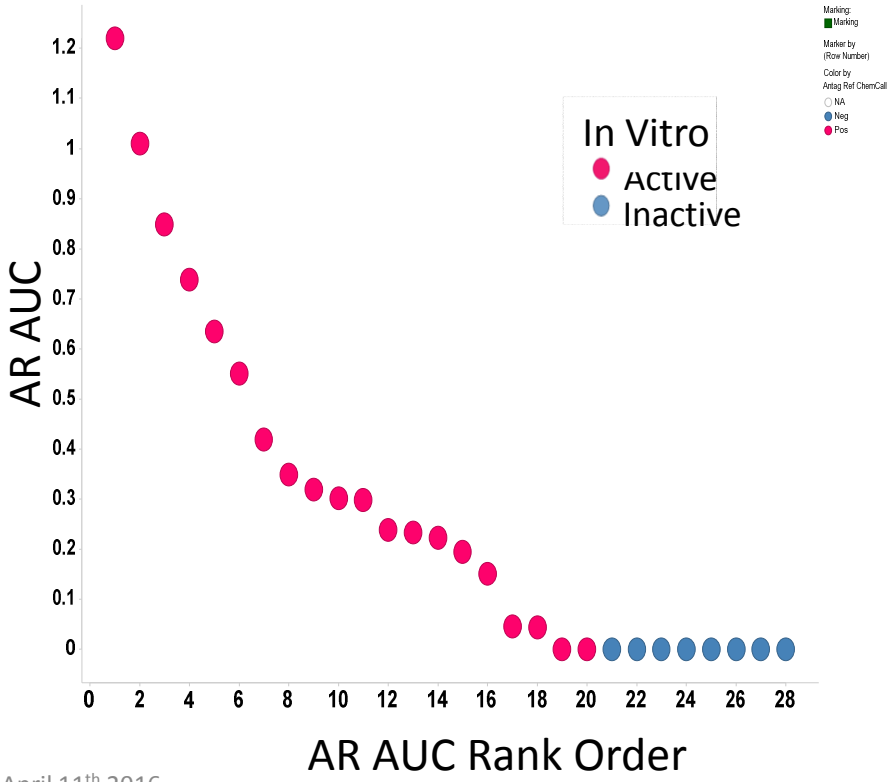
Uterotrophic Reproducibility

Same Study Design (Immature Rat): BPA

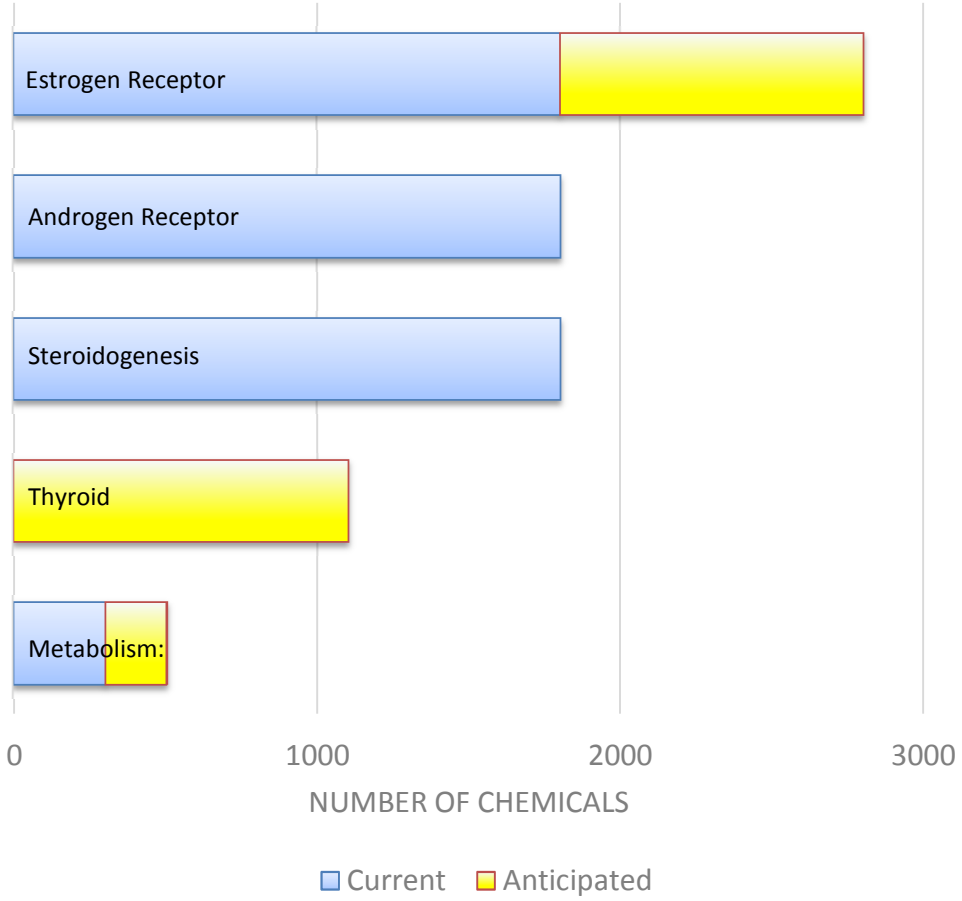


Androgen Receptor





Chemicals with High Throughput Data for Endocrine Screening



- Pathway based
- Ongoing data generation, analysis and validation
- Performance-Based Test Guidelines being developed