Endocrine Disruptor Screening Program Update

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PPDC
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1996 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.

1998 Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC)

EDSTAC Key Recommendations:

- 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

Potential to interact with the estrogen, androgen or thyroid hormone systems

Tier 2 Testing to determine Interaction with the endocrine system

If endocrine-mediated adverse effects then quantify dose-response relationship

EDSP Chronology

1996 FFDCA and SDWA

1998 EDSTAC recommendations

1999 EPA established the EDSP

2008 Validated eleven Tier 1 assays

2009 Initial test orders for Tier 1 assays

2011 EDSP21 Work plan

2012 EDSP Comprehensive Mgmt Plan

2013 Scientific Advisory Panel Reviews

EDSP Universe of Chemicals

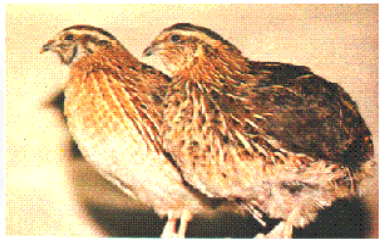
Chemical List	Number of Compounds
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616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

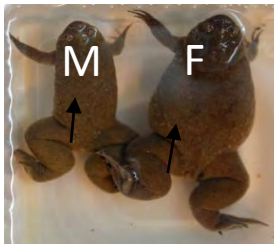
Tier 1 Screening Assays

					Steroid Synthesis			
	E	**E-**	**A**	**A-**	**T**	**E**	**HPG**	**HPT**
In vitro								
ER Binding	X	X						
ER Transcriptional Activation	X							
AR Binding			X	X				
Steroidogenesis (H295R)					X	X		
Aromatase (Recombinant)						X		
In vivo								
Uterotrophic	X							
Hershberger			X	X				
Pubertal male			X	X	X		X	X
Pubertal female	X	X				X	X	X
Fish Reproductive Screen	X	X	X	X	X	X	X	
Amphibian Metamorphosis								X

Tier 2 Test Methods

- Rat: Two-generation rat reproduction test (OECD TG 416)
  - Rat: Extended F1-Generation (OECD TG 443)
- Bird: determine long-term effects of maternal transfer and *in ovo* exposure – Japanese Quail
- Fish: Medaka Multi-generation Toxicity Test (MMT) and Medaka Reproduction Test (MRT) methods
- Frog: characterize perturbations of normal development and growth – Xenopus Laevis





EDSP Tier 1 Data Review: *Current* Pace

2009-2010

EDSP Issued Initial Tier 1 Test Orders on 67 chemicals

2012-2013

Tier 1 Data Being Submitted to the Agency on 52 chemicals

2013-2014

Agency Completes 52 Tier 1 Data Reviews

Evolution of the EDSP

- Based on current pace it could take decades to screen all 10,000 chemicals for potential to interact with the endocrine system.
- Recent advances in computational toxicology herald an important “evolutionary turning point” and an accelerated pace of screening and testing.
- To address thousands of chemicals for potential to interact with the endocrine system, we must implement a more strategic approach to prioritize chemicals for targeted screening.

EDSP21 Objectives

- Maximize use of extant data, current and emerging technologies.
- Strategic Testing: Targeted *in vivo* toxicity screening.
- Use a variety of tools in a multi-tiered testing and assessment framework.
- Systematically incorporate new tools and methods, measure performance and build confidence.
- Advance understanding of key events in toxicity pathways.

Chemical Prioritization: FIFRA SAP January 29-31, 2013

✓ **Focus and Objective**:

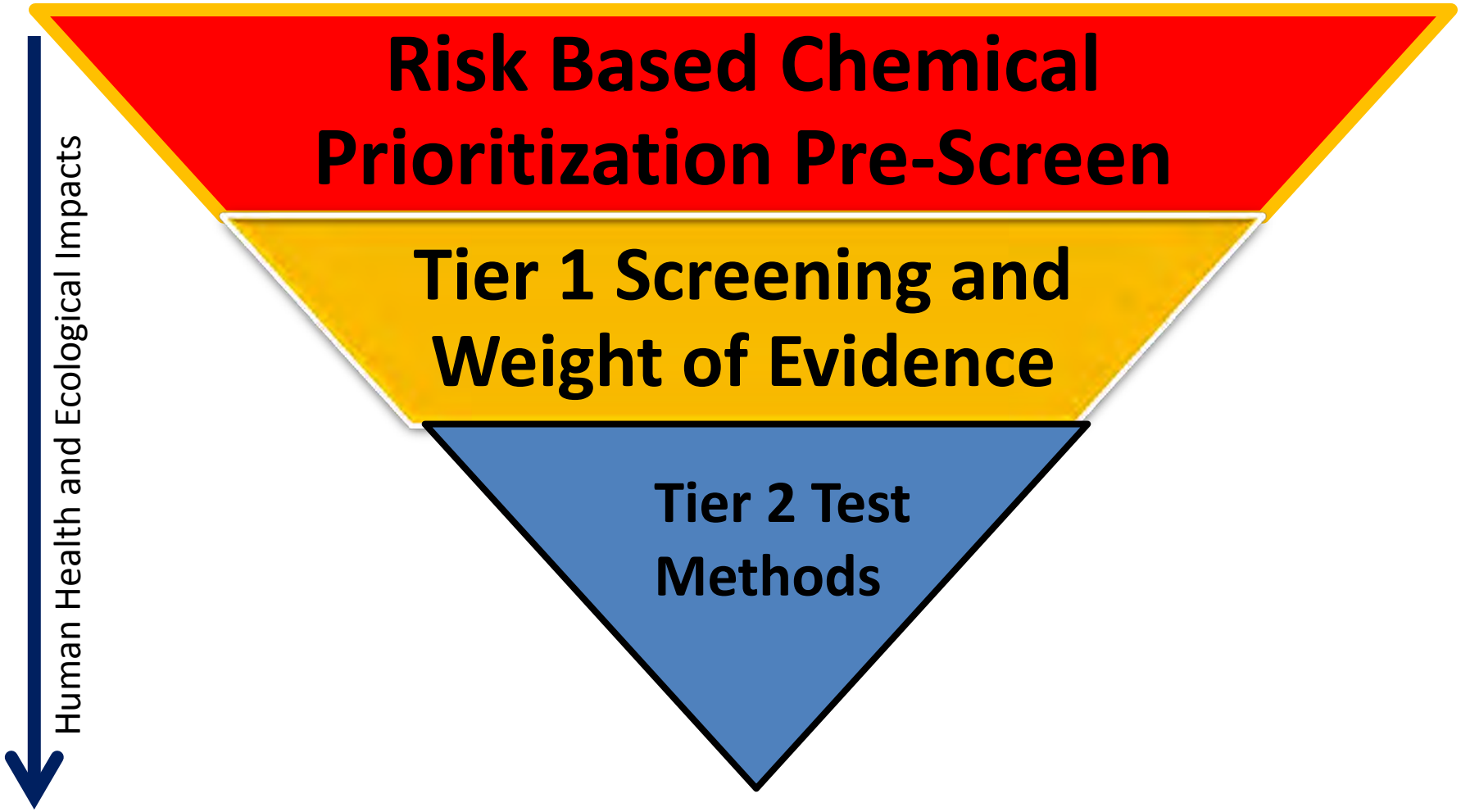
1. Prioritization of the universe of chemicals for estrogen receptor adverse outcome pathway using computational toxicology tools

2. To obtain input and recommendations on the scientific concepts, principles and processes used to prioritize chemicals for EDSP screening.

SAP 2013 Overall Recommendations

- Steps in the prioritization scheme were organized and clearly described, need to consider exposure earlier in the process
- Physico-chemical properties filters are founded on strong scientific principles and consistent with recommendations from 1998 EDSTAC.
- High throughput assays and QSARS are both useful in developing a risk-based “priority score” in combination with exposure determinations

Conceptual Framework: Strategic Testing Approach



Utility of Computational Toxicology

- Rapidly screen chemicals and use predictive models to evaluate thousands of chemicals for potential risk to human health and environment
- Increase capacity to prioritize, screen and predict chemical toxicity and exposure
- Overcome throughput limitations of traditional chemical toxicity testing, augmenting current data sources
- Eventual replacement of some existing tests with non-animal alternatives
- Partner across EPA, with other federal agencies, state agencies, industry and non-governmental organizations to validate and apply tools
- Provide open access to data and adverse outcome pathway (AOP) risk predictions

United States Environmental Protection Agency

Example of an ER Pathway Model Based on ToxCast Data

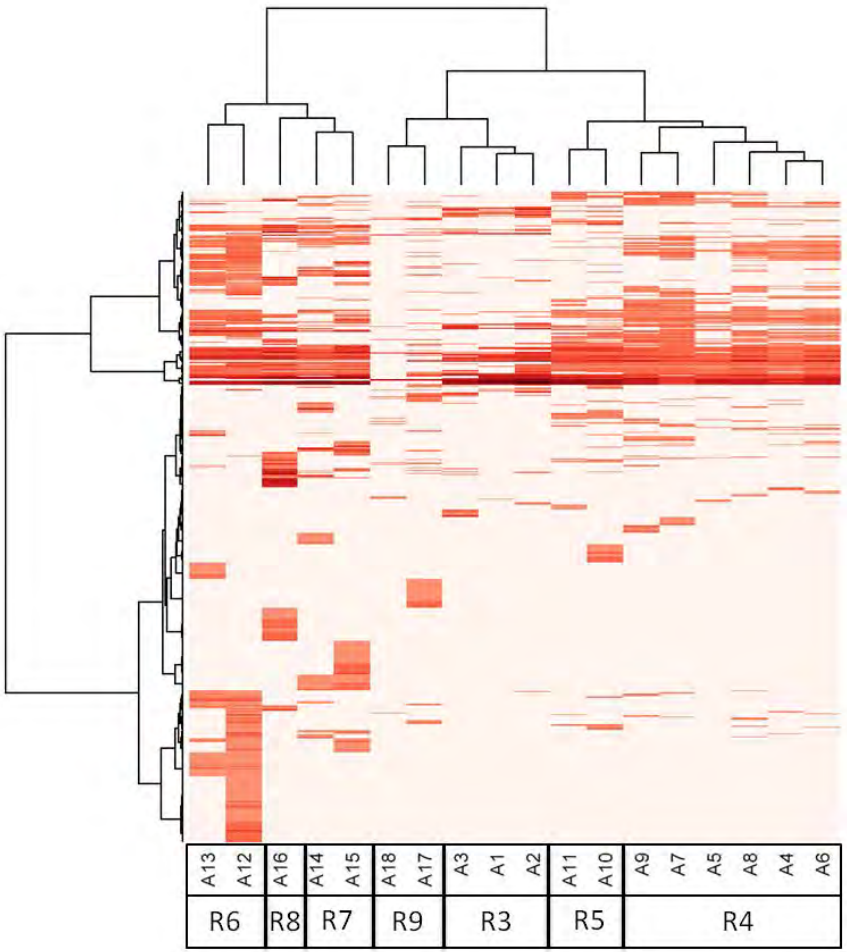
- For 1800 chemicals with ER data
- Having over 700 overall assays allows us to look for assay interference issues
  - Assays turn on at ~same concentration as cytotoxicity
  - The “burst”, aka cytotoxicity, aka general toxicity
- Also allows us to see what other pathways are active at ER-activity concentrations

ToxCast Assays for the ER Pathway

ID	Assay Name	Source	Gene	Species	Type
1	NVS bovine ER	Novascreen	ESR1	Bos taurus	Receptor Binding
2	NVS human ER	Novascreen	ESR1	Homo sapiens	Receptor Binding
3	NVS mouse ERa	Novascreen	Esr1	Mus musculus	Receptor Binding
4	OT ERa-ERa (8 h)	Odyssey Thera	ESR1	Homo sapiens	Dimerization
5	OT ERa-ERa (24 h)	Odyssey Thera	ESR1	Homo sapiens	Dimerization
6	OT ERa-ERb (8 h)	Odyssey Thera	ESR1, ESR2	Homo sapiens	Dimerization
7	OT ERa-ERb (24 h)	Odyssey Thera	ESR1, ESR2	Homo sapiens	Dimerization
8	OT ERb-ERb (8 h)	Odyssey Thera	ESR2	Homo sapiens	Dimerization
9	OT ERb-ERb (24 h)	Odyssey Thera	ESR2	Homo sapiens	Dimerization
10	OT GFP ERa-ERE (2 h)	Odyssey Thera	ESR1, ERE	Homo sapiens	DNA Binding
11	OT GFP ERa-ERE (8 h)	Odyssey Thera	ESR1, ERE	Homo sapiens	DNA Binding
12	ATG ERa (TRANS)	Attagene	ESR1	Homo sapiens	RNA Reporter Gene
13	ATG ERE (CIS)	Attagene	ESR1	Homo sapiens	RNA Reporter Gene
14	Tox21 ERa BLA Agonist ratio	NCGC	ESR1	Homo sapiens	Reporter Gene
15	Tox21 ERa LUC BG1 Agonist	NCGC	ESR1	Homo sapiens	Reporter Gene
16	ACEA T47D (80 h)	ACEA	ESR1	Homo sapiens	Proliferation
17	Tox21 ERa BLA Antagonist ratio	NCGC	ESR1	Homo sapiens	Reporter Gene
18	Tox21 ERa LUC BG1 Antagonist	NCGC	ESR1	Homo sapiens	Reporter Gene

Major theme – all assays have false positives and negative

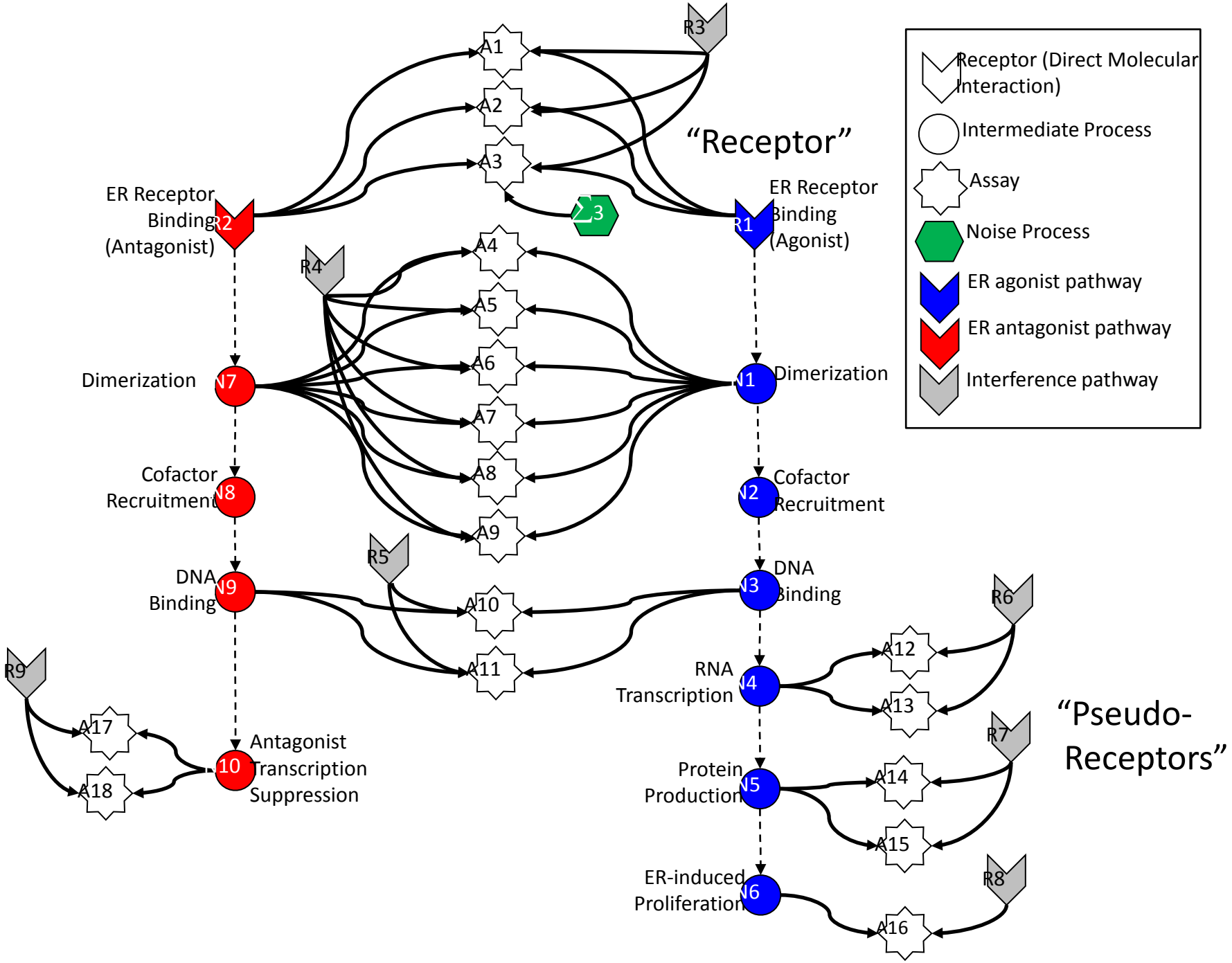
Assays cluster by technology, suggesting technology-specific non-ER activity



Much of this “noise” is reproducible, i.e. it is “assay interference”

Result of interaction of chemical with complex biology in the assay

Our chemical library is only partially “drug-like”
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics


Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
Noise Process
ER agonist pathway
ER antagonist pathway
Interference pathway
"Receptor"
"Pseudo-Receptors"
ER Receptor Binding (Antagonist)
ER Receptor Binding (Agonist)
Dimerization
Cofactor Recruitment
DNA Binding
Antagonist Transcription Suppression
RNA Transcription
Protein Production
ER-induced Proliferation
R1
R2
R3
R4
R5
R6
R7
R8
R9
A1
A2
A3
A4
A5
A6
A7
A8
A9
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A11
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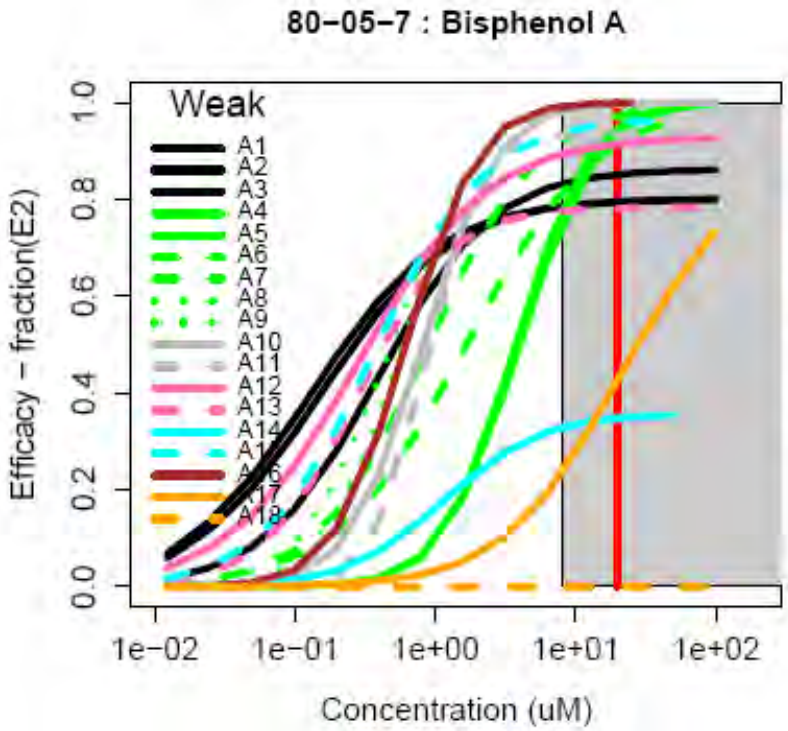
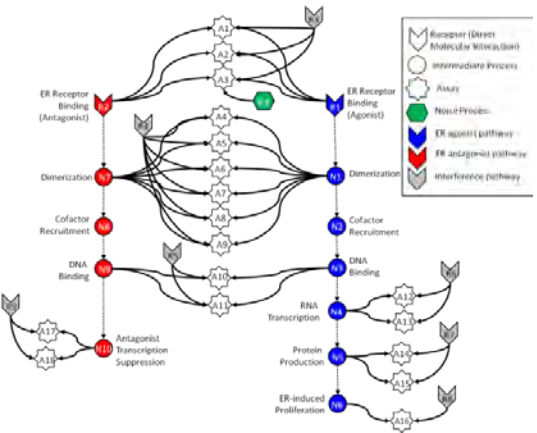
A15
A16
A17
A18

Example 1 – BPA – true agonist (AUC=0.66)

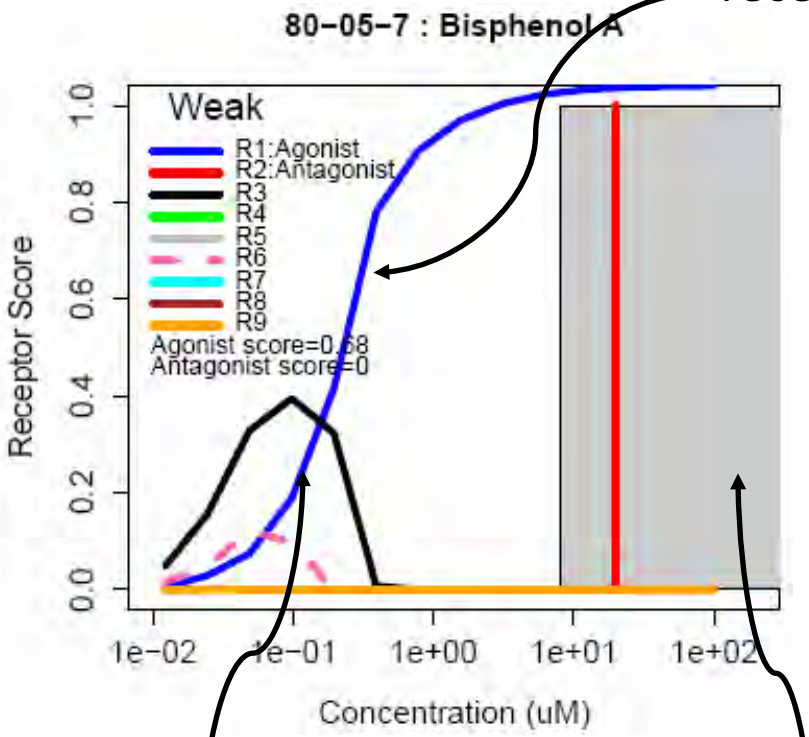
Assays





“Receptors”

Blue: agonist “receptor”

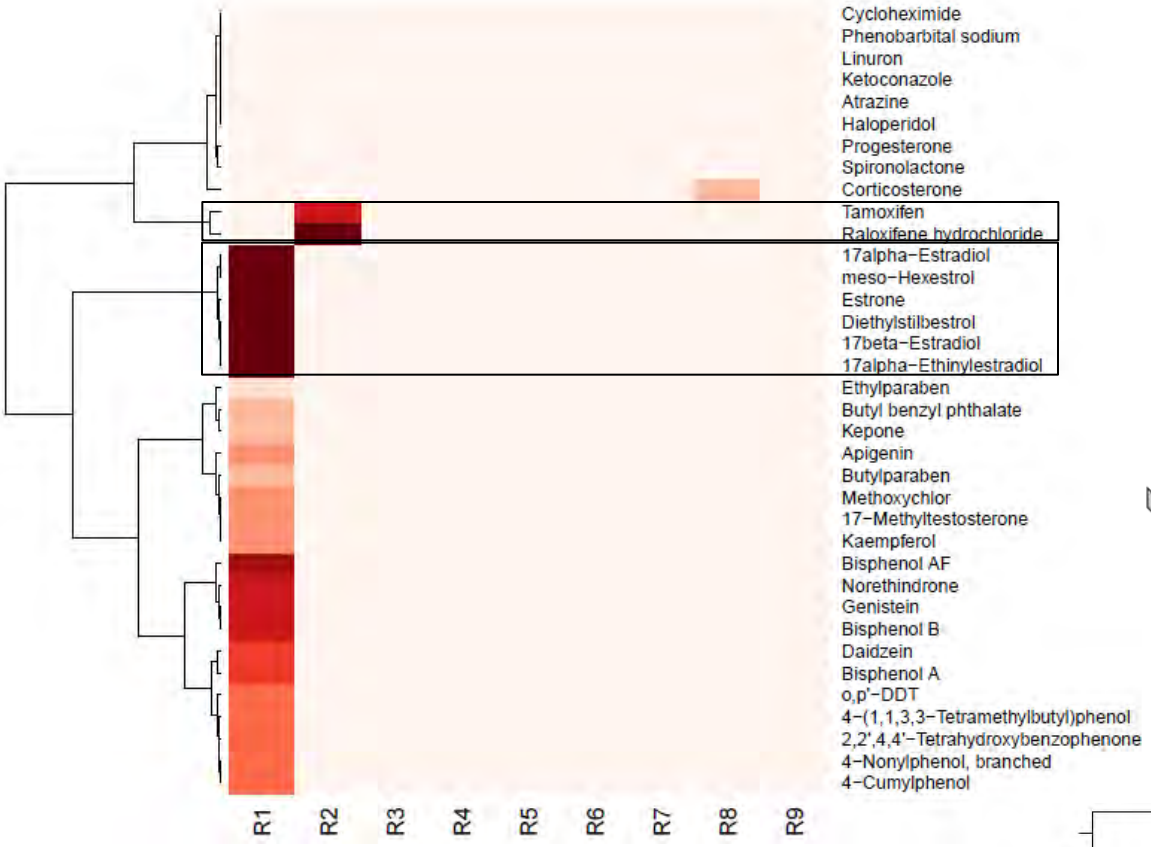


Binding assays active at lowest concentration

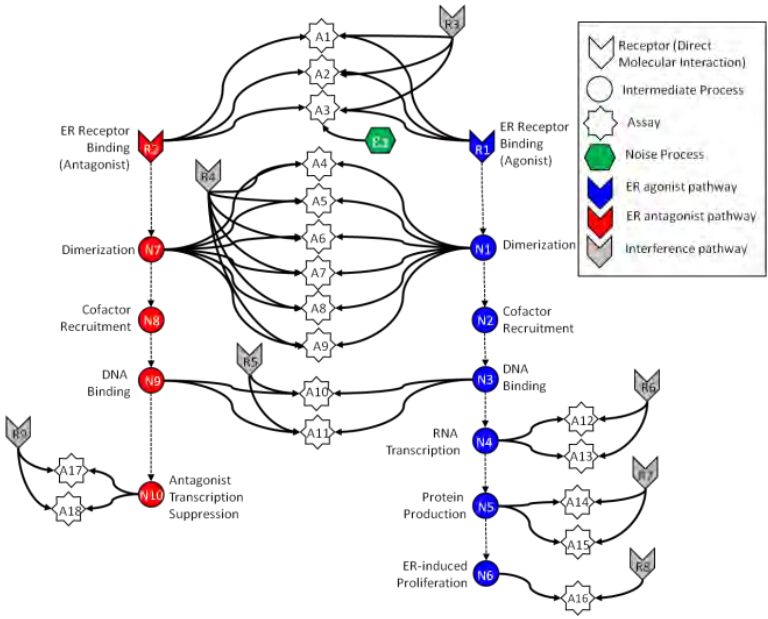
AUC “sign” feature will discount this

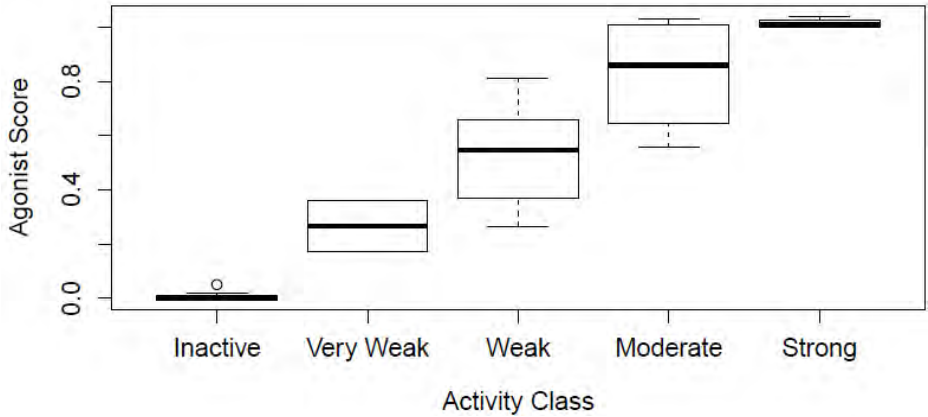
Cytotoxicity Region: red line is median cytotox AC50

Reference Chemical Classification

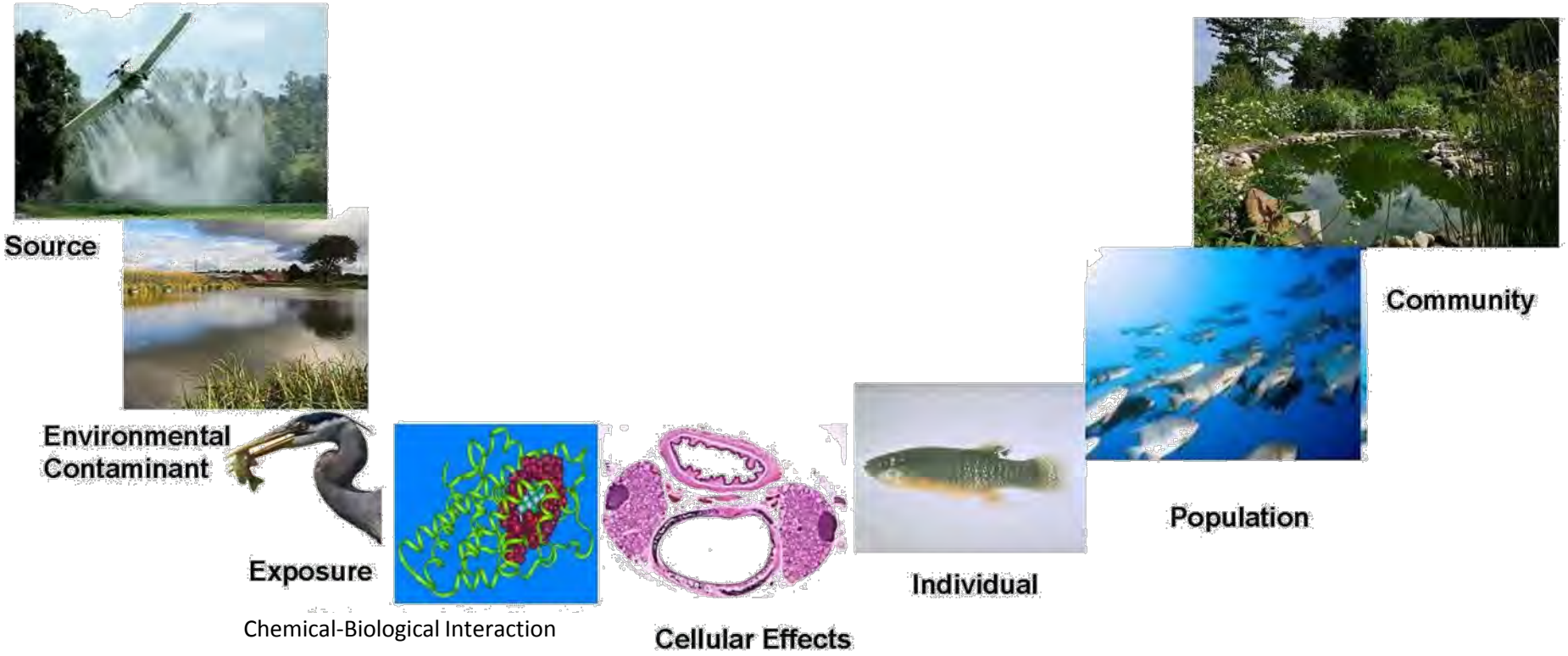


AUC heat map for
Reference chemicals



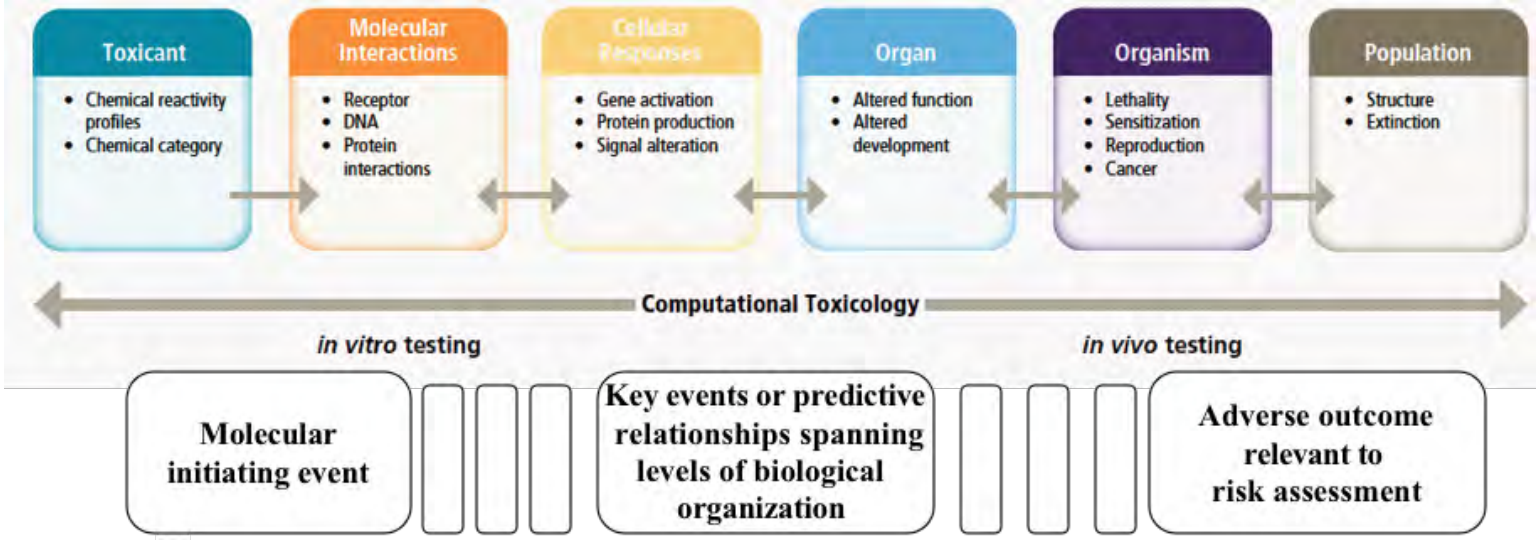


Adverse Outcome Pathways (AOP)



- Peer reviewed method integrating chemical, biological and toxicological data relevant to exposure and effects
- Captures information across source-to-outcome continuum and efficiently informs various steps of risk assessment process
- AOP development supports broad stakeholder input and transparency
- Part of EPA's strategy for Integrated Approaches to Testing and Assessment (IATA)

Adverse Outcome Pathways (AOP)



AOP Support:

- Developing and applying lower tiered tests & non-animal models (e.g., QSAR, *in vitro*, HTS)
- Forming Chemical Categories & Read Across methods
- Better dosimetry and biomarkers in experimental studies, epidemiology, population monitoring
- Species extrapolation

EDSP Relevant AOP

- Estrogen, Androgen and Thyroid (EAT)

- Risk-based AOP prioritization and assessment requires
  - measurement or prediction of in life dose-response
  - monitoring or prediction of real-world exposures

- Consist with 2013 SAP recommendations on use of Physical Chemical Properties, QSAR/HTS, and Exposure Predictions

- EDSP21 is focused on developing high throughput, risk-based AOP methods to prioritize targeted testing

Current Status of EDSP Prioritization and Screening

- 52 List 1 chemicals with complete Tier 1 datasets undergoing weight-of-evidence determination of EAT endocrine activity and possible Tier 2 testing
- 109 List 2 chemicals going through OMB review for Tier 1 screening
- EDSP Universe of chemicals being prioritized for EDSP screening using computational toxicology and other tools
- Science Advisory Panel peer reviews being planned 1) Exposure Prediction Models, and 2) Risk-Based Prioritization

Key Points

- Significant progress has been made in Computational Toxicology, particularly in high throughput screening of thousands of chemicals for bioactivity
- Pathway models based on biology, chemistry, toxicology and statistics are being developed to predict and quantify hazard, exposure and risk
- Initial EPA OCSPP application of predictive models is underway for EDSP chemical prioritization
- Positive outcomes:
  - Increase certainty, predictive ability and timeliness
  - Better utilize testing resources and reduce reliance on animals
  - Harmonize requirements across chemical programs
  - Improve risk management decisions