

Overview of the ToxCast Research Program: Applications to Predictive Toxicology and Chemical Prioritization

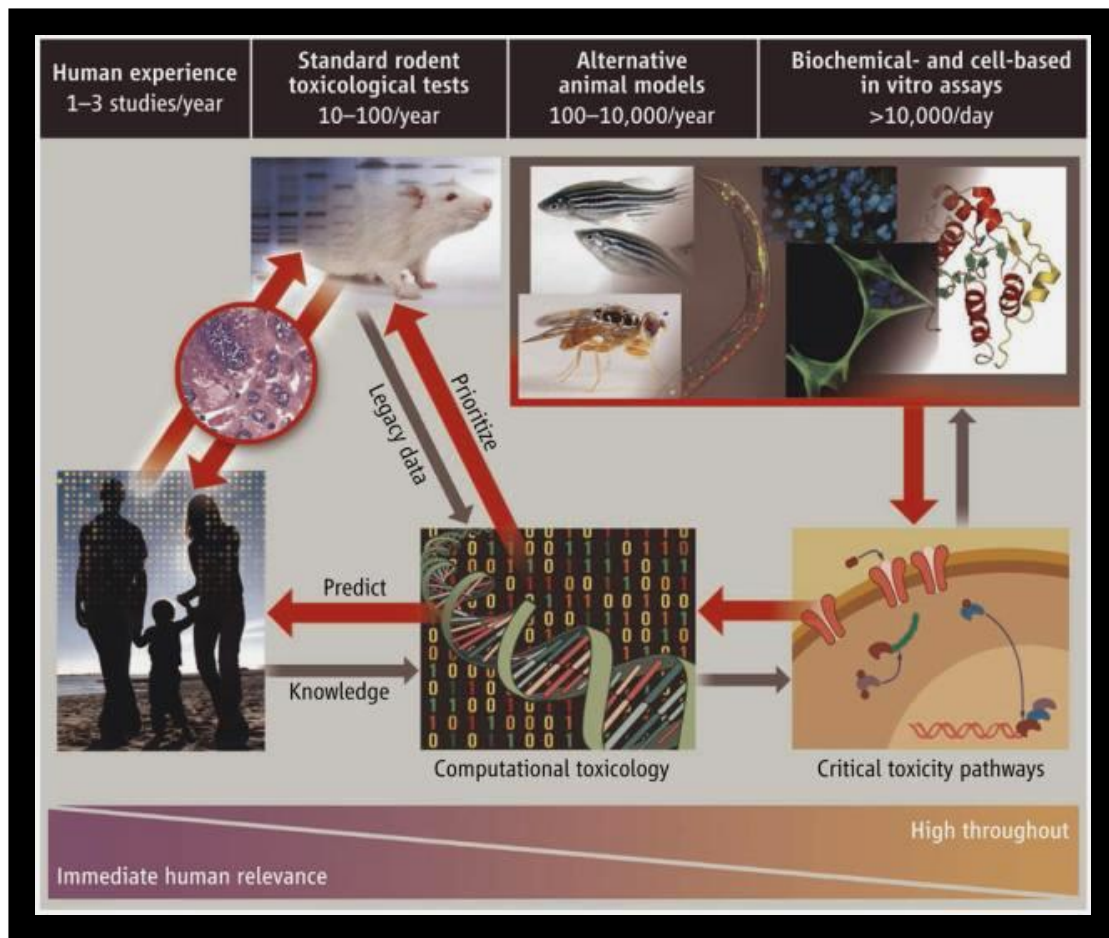
Keith Houck

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Office of Research and Development*



SETAC, Nashville, TN 2013

Tox21 Vision: Transforming Toxicity Testing



**National Center for Advancing
Translational Sciences (NCATS)**
<http://www.ncats.nih.gov/>

**SOURCE: Collins, Gray and Bucher (2008) Toxicology.
Transforming environmental health protection. ²
Science 319: 906**

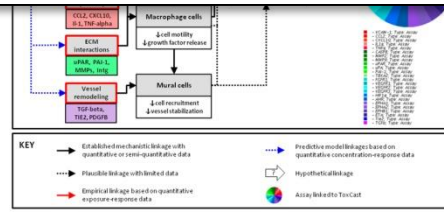
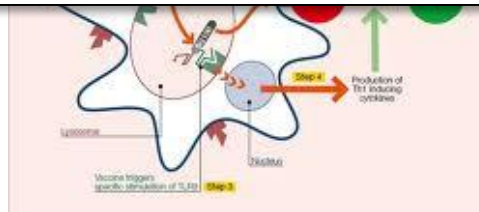
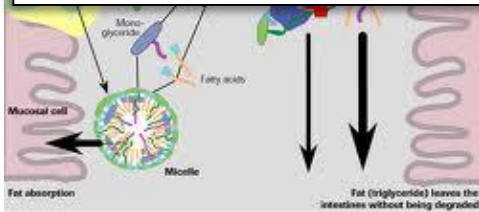
Problem Statement

Too many chemicals to test with standard animal-based methods

— Cost, time, animal welfare

Need for better mechanistic data

- Determine human relevance
- What is the Mode of Action (MOA) or Adverse Outcome Pathway (AOP)?








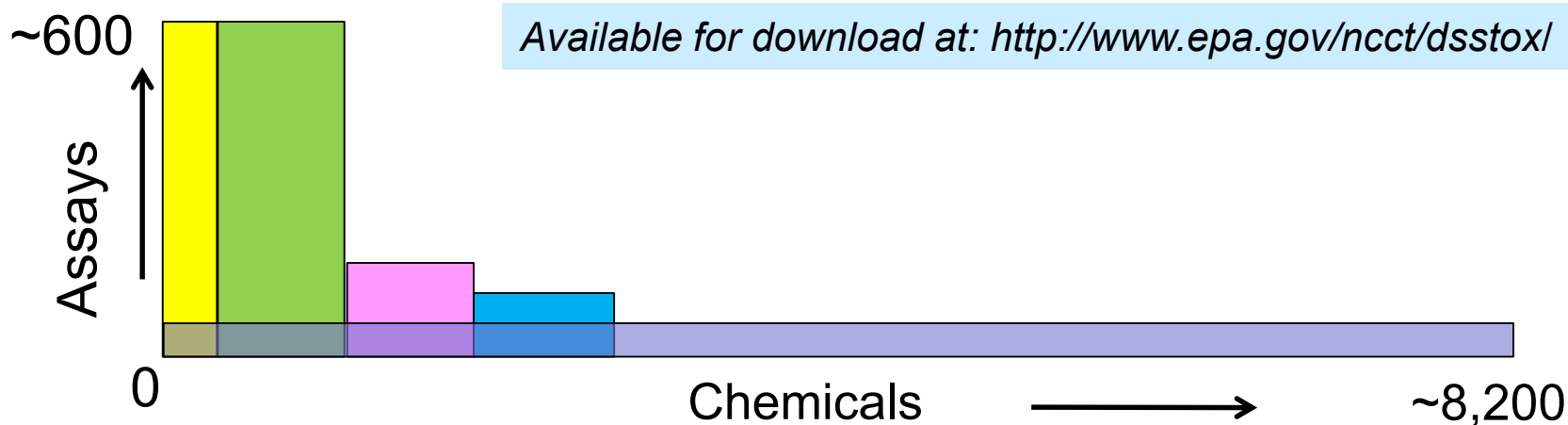
ToxCast /Tox21 Overall Strategy

- Identify targets or pathways linked to toxicity (AOP focus)
- Identify/develop high-throughput assays for these targets or pathways
- Develop predictive systems models
 - *in vitro* → *in vivo*
 - *in vitro* → *in silico*
- Use predictive models (qualitative):
 - Prioritize chemicals for targeted testing
 - Suggest / distinguish possible AOP / MOA for chemicals
- *High-throughput Exposure Predictions*
- *High-throughput Risk Assessments (quantitative)*

Testing under ToxCast and Tox21

Chemicals, Data and Release Timelines

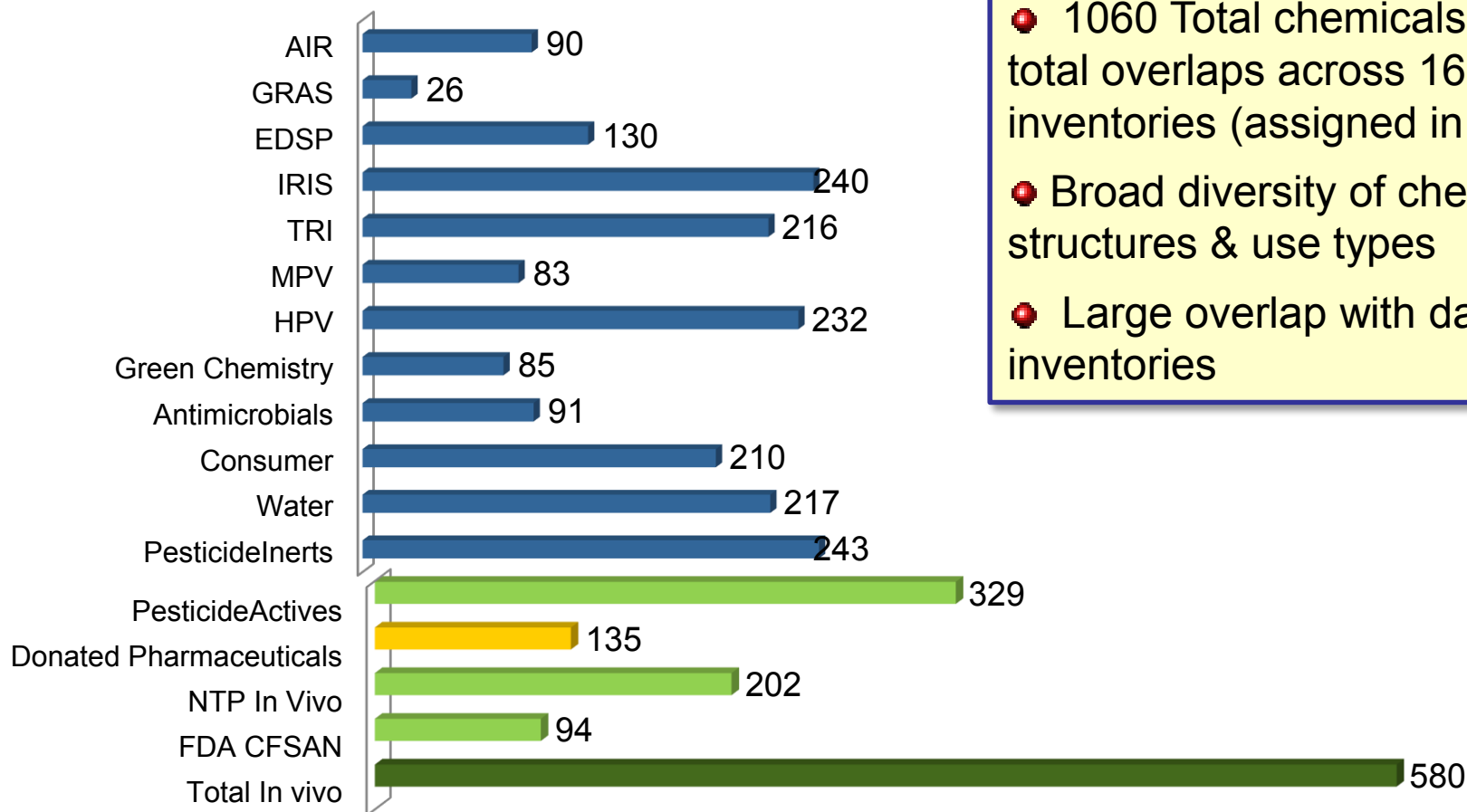
Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	 293	~600	~700	2011	Now
ToxCast Phase II	 767	~600	~700	03/2013	12/2013
ToxCast Phase IIIa	 1001	~100	~100	Just starting	2014
E1K (endocrine)	 880	~50	~120	03/2013	11/2013
Tox21	 8,193	~25	~50	Ongoing	Ongoing



Pesticides, antimicrobials, food additives, green alternatives, HPV, MPV, endocrine reference cmpds, other tox reference cmpds, failed drugs, NTP in vivo, EPA high interest compounds, industrial, marketed drugs, fragrances, ...

ToxCast PhI&PhII chemicals:

Spanning diverse inventories of EPA interest

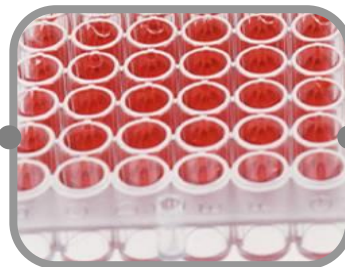


- 1060 Total chemicals → 2806 total overlaps across 16 diverse inventories (assigned in ACToR)
- Broad diversity of chemical structures & use types
- Large overlap with data-rich inventories

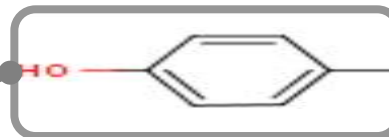
High Throughput Screening 101 (HTS)



Robots



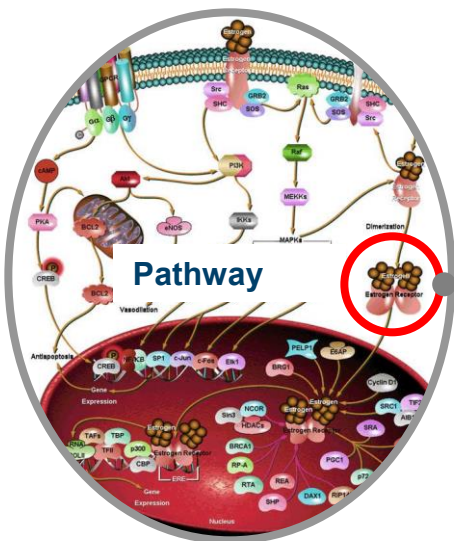
96-, 384-, 1536 Well Plates



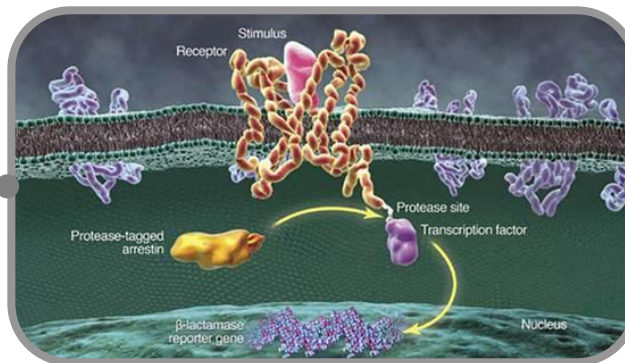
Chemical Exposure



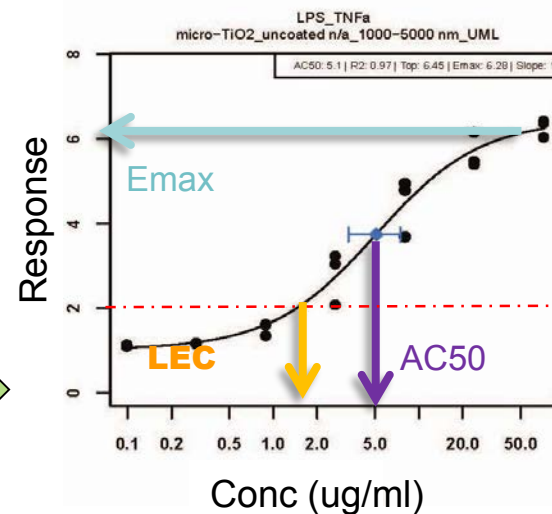
Cell Population



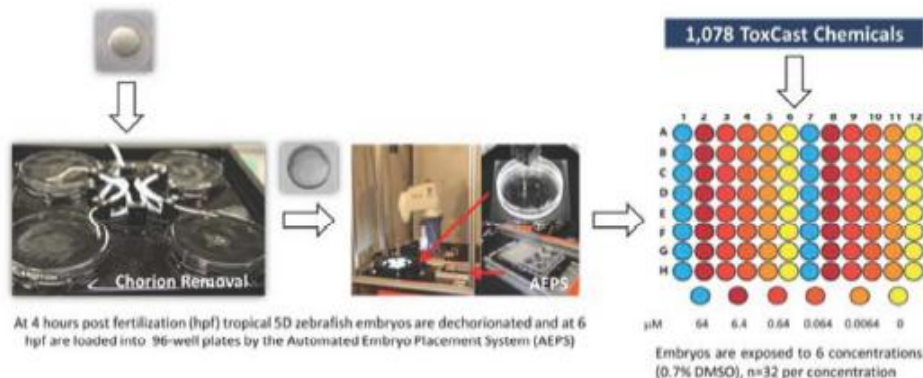
Pathway



Target Biology (e.g.,
Estrogen Receptor)



Zebrafish Development Screen



24 hours later...

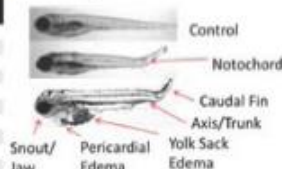
Morphology Assessment	
24 hpf End Points	
End Point	Abbreviation
Mortality	MO24
Developmental Delay	DP24
Spontaneous Movement	SM24
Notochord	NC24



24hpf embryonic behavior is assessed by the Photo-motor Response Assessment Tool (PRAT). PRAT quantifies an individual embryo's photo-motor response to two distinct pulses of light

At 5 Days (120 hpf)

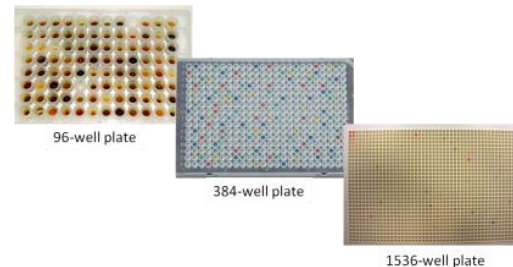
Morphology Assessment	
120 hpf End Points	
End Point	Abbreviation
Mortality	MORT
Yolk Sac Edema	YSE
Body Axis	AXIS
Eye Defect	EYE
Snout	SNOUT
Jaw	JAW
Otic Vesicle	OTIC
Pericardial Edema	PE
Brain	BRAI
Somite	SOMI
Pectoral Fin	PFIN
Caudal Fin	CFIN
Pigment	PIG
Circulation	CIRC
Truncated Body	TRUN
Swim Bladder	SWIM
Notochord & Bent Tail	NC
Touch Response	TR



Using the Viewpoint Zebrabox, light-induced larval locomotor activity was measured. Locomotor activity is tracked for 25 minutes

Truong et al., Tox.
Sci, *in press*

ToxCast Assays (>700 endpoints)



Assay Provider

ACEA
Apredica
Attagene
BioReliance
BioSeek
CeeTox
CellzDirect
Tox21/NCATS
NHEERL MESC
NHEERL Zebrafish
NovaScreen (Perkin Elmer)
Odyssey Thera
Vala Sciences

Biological Response

cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor binding
receptor activity
steroidogenesis

Target Family

response Element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases
XME metabolism
GPCRs
ion channels

Assay Design

viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type

single
multiplexed
multiparametric

Cell Format

cell free
cell lines
primary cells
complex cultures
free embryos

Species

human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

Detection Technology

qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arraysan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

ToxCast Phase II: 1051 Chemicals x 791 Assay Readouts

ACEA: red

Attagene: orange

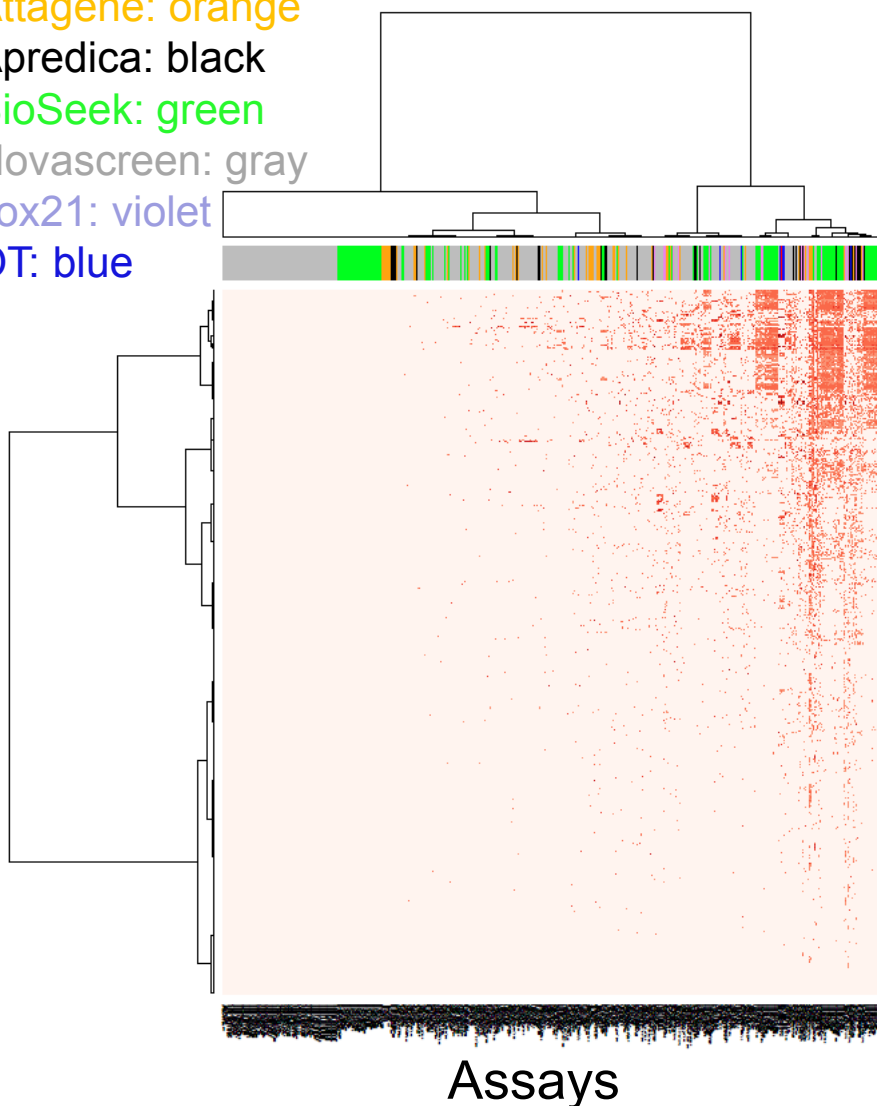
Apredica: black

BioSeek: green

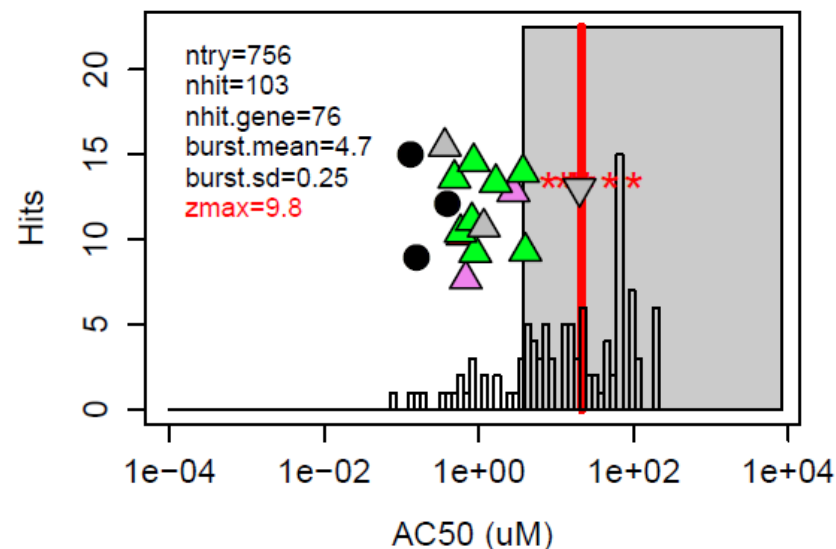
Novascreen: gray

Tox21: violet

OT: blue



80-05-7 : Bisphenol A



Most chemicals cause activity in many assays near the cytotoxicity threshold

Cell stress-related assay activity

“Hit” (AC50) in burst region is less likely to result from specific activity
(e.g. binding to receptor or enzyme)

Z-score: # of SD from burst center

-High Z: more likely to be specific ¹⁰

-Low Z: less likely to be specific

ToxCast Phase II: 1051 Chemicals x 791 Assay Readouts

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Attagene: orange

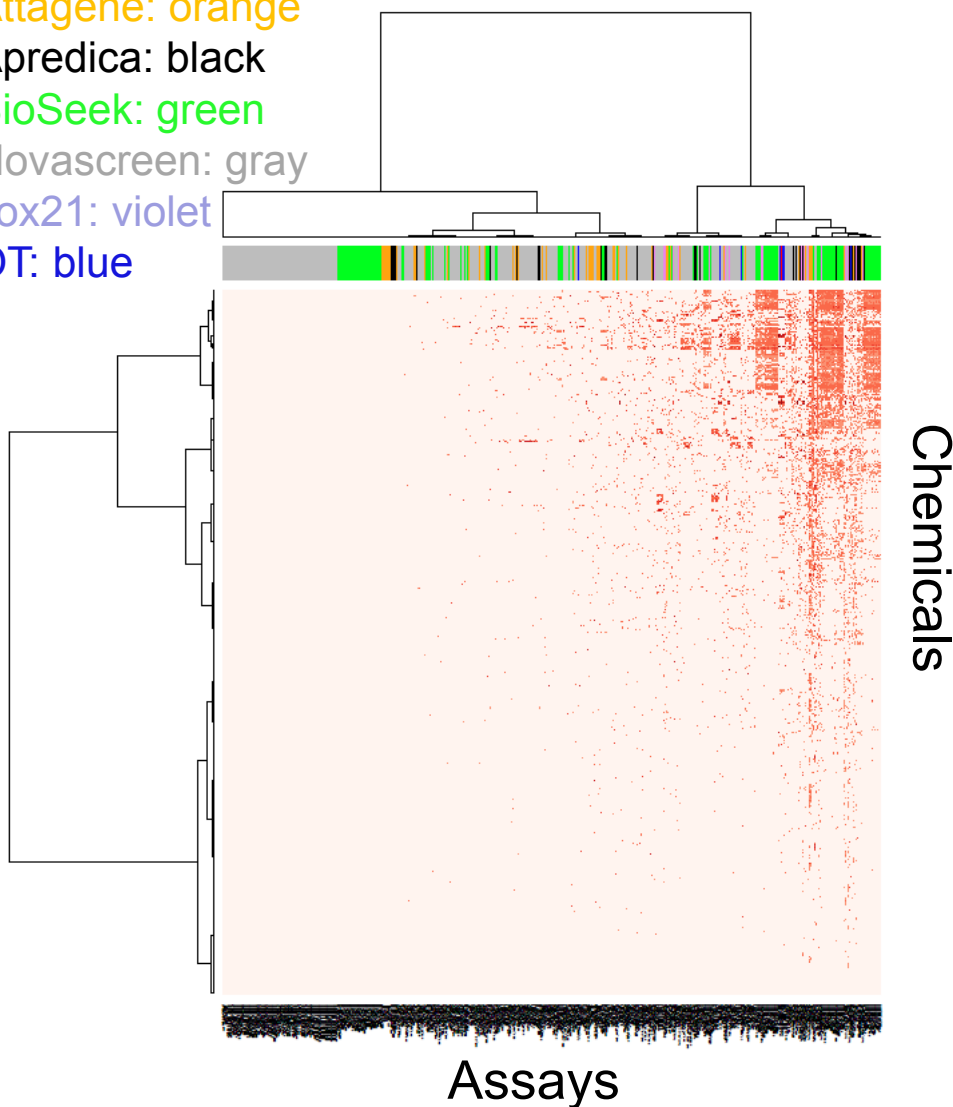
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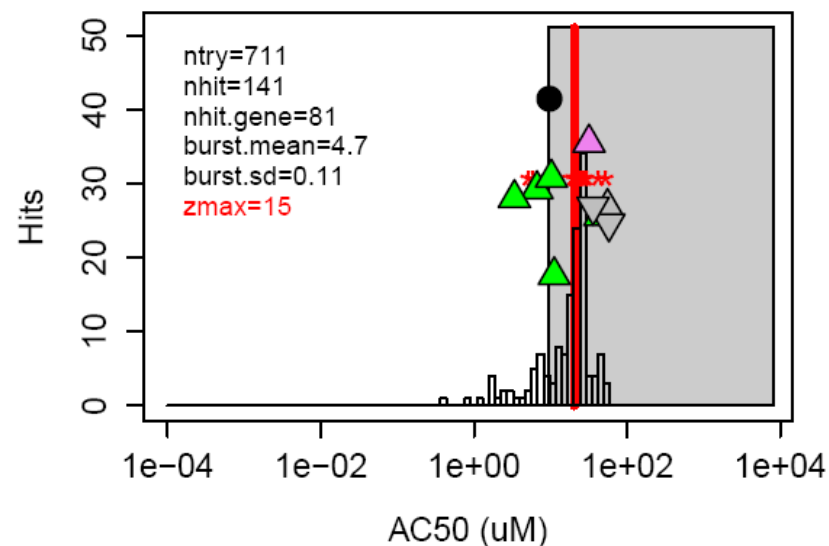
Novascreen: gray

Tox21: violet

OT: blue



1034-01-1 : Octyl gallate



Most chemicals cause activity in many assays near the cytotoxicity threshold

Cell stress-related assay activity

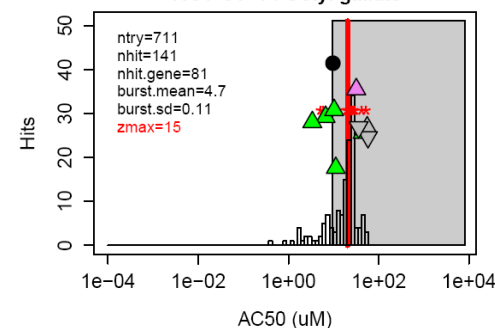
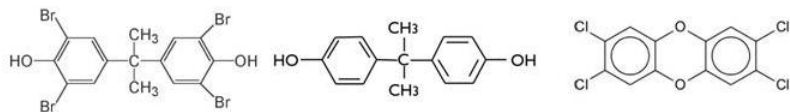
“Hit” (AC50) in burst region is less likely to result from specific activity
(e.g. binding to receptor or enzyme)

Z-score: # of SD from burst center

-High Z: more likely to be specific ¹¹

-Low Z: less likely to be specific

Significance of In Vitro Effects



Assay Target Class

Molecular Target

EDC
Acetylcholinesterase Inhibition
Ion channel blocker
Genotoxicity

Assessment

AOP Assessment
Targeted testing

Cell Stress Mediated

Oxidative stress
Membrane disruption
Nucleophiles
Electrophiles
Energy depletion

Estimate MTD
Estimate NOEL

No Effect

Non-reactive chemical
Not bioactive
Effects would require high doses

Estimate NOEL



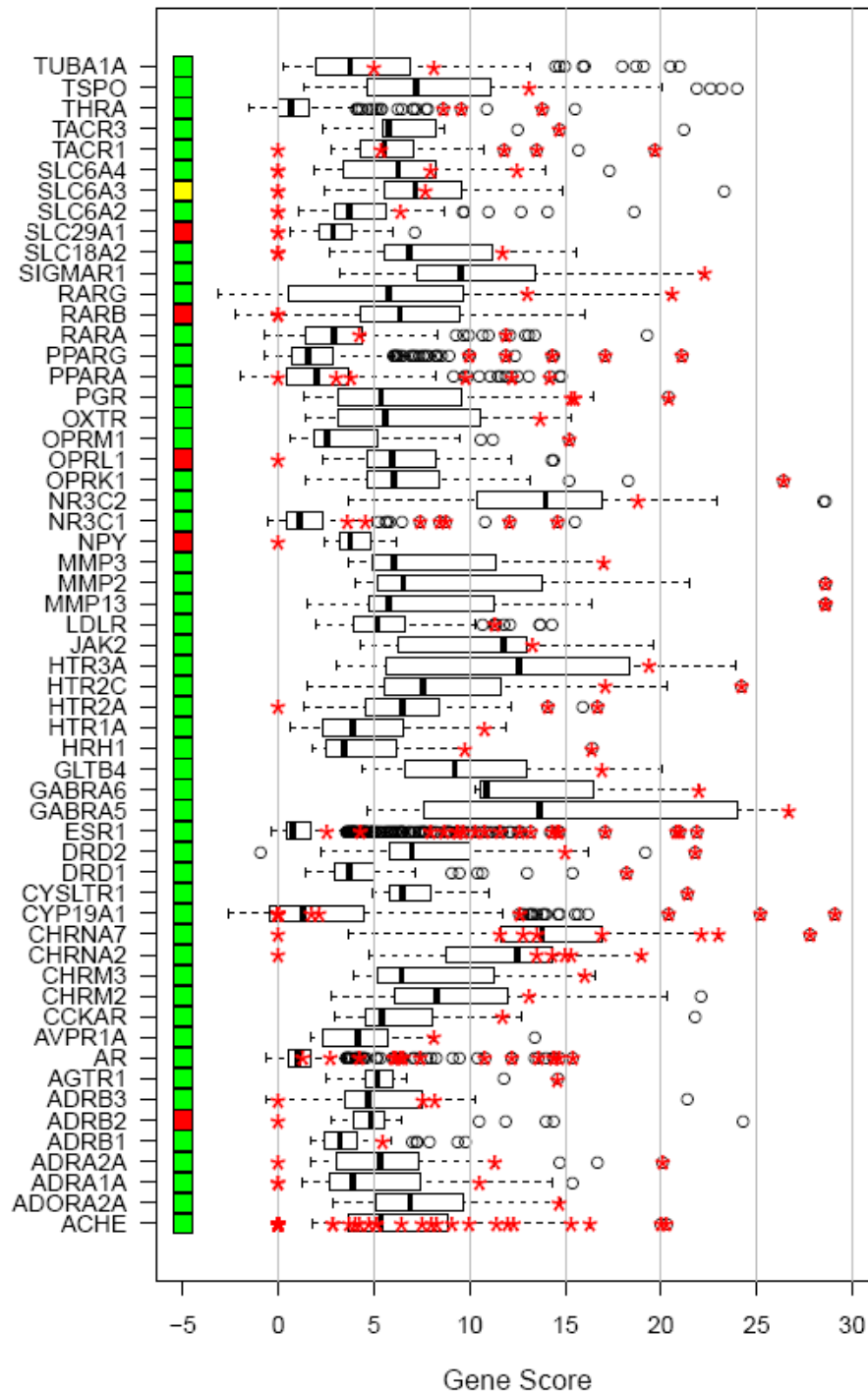
Gene Score: Combine potency and specificity

- How to summarize 1000s of chemicals x 100s of assays?
 - Potency: $-\log(\text{AC}_{50})$
 - Specificity: Z-score
 - Gene score = Potency + Specificity
 - average over assays for gene $[-\log(\text{AC}_{50}) + \text{Z-score}]$
 - Can be used to get quick ranking of chemicals
 - Gene Score > 7 are most interesting
 - Z-score=2 and $\text{AC}_{50}=10 \mu\text{M}$
 - 5670 chemical-gene combinations >7 (~1%)
 - 281 Genes (out of 330)
 - 1231 Chemicals (out of 1877)

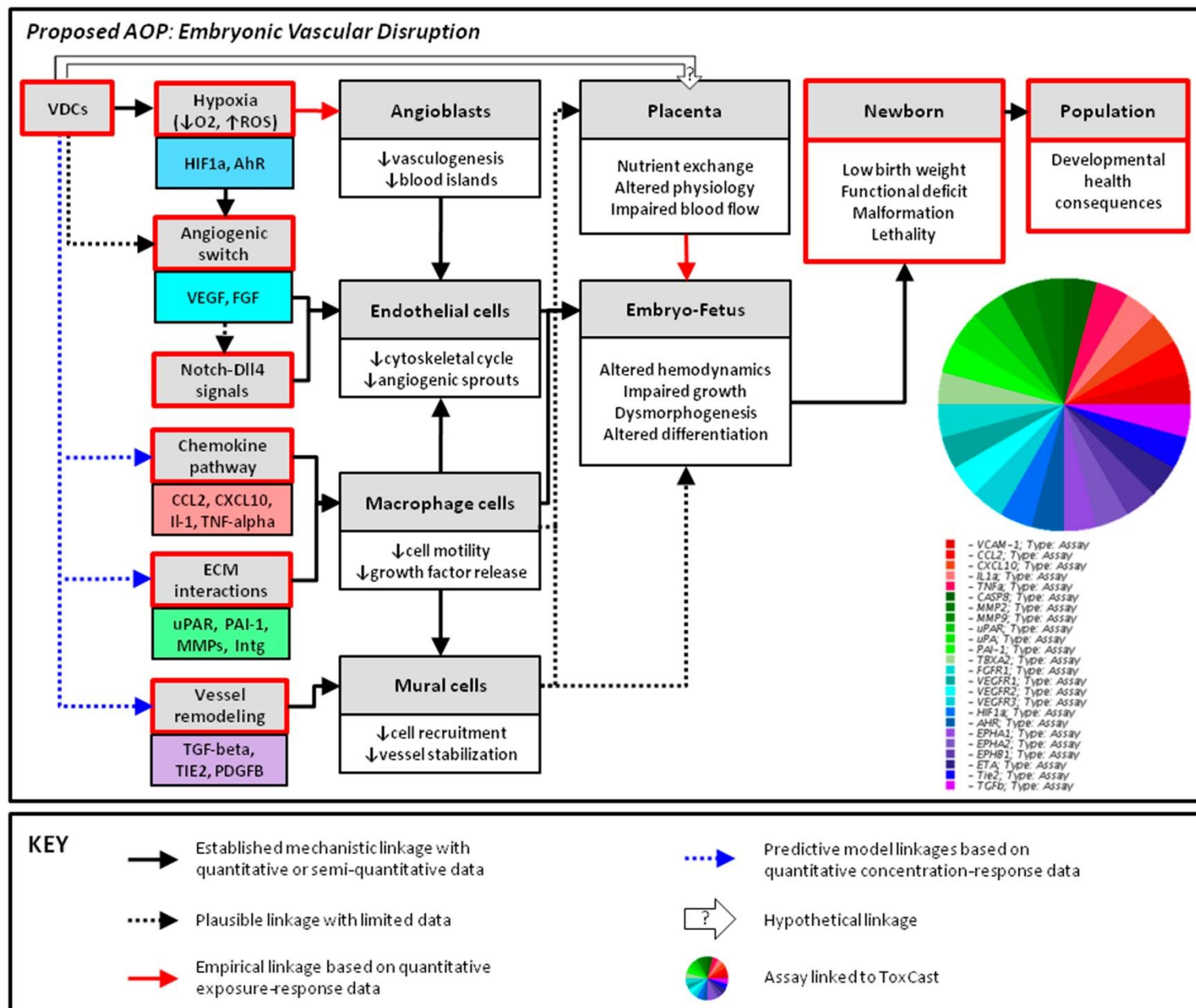
Do Assays Detect Potent Reference Chemicals?

* =Reference chemicals

- These chemicals should be near the right of the gene score distribution
- Most assays show reference chemicals to be potent and specific
- Gives confidence that novel chemicals active in the assay are perturbing that pathway



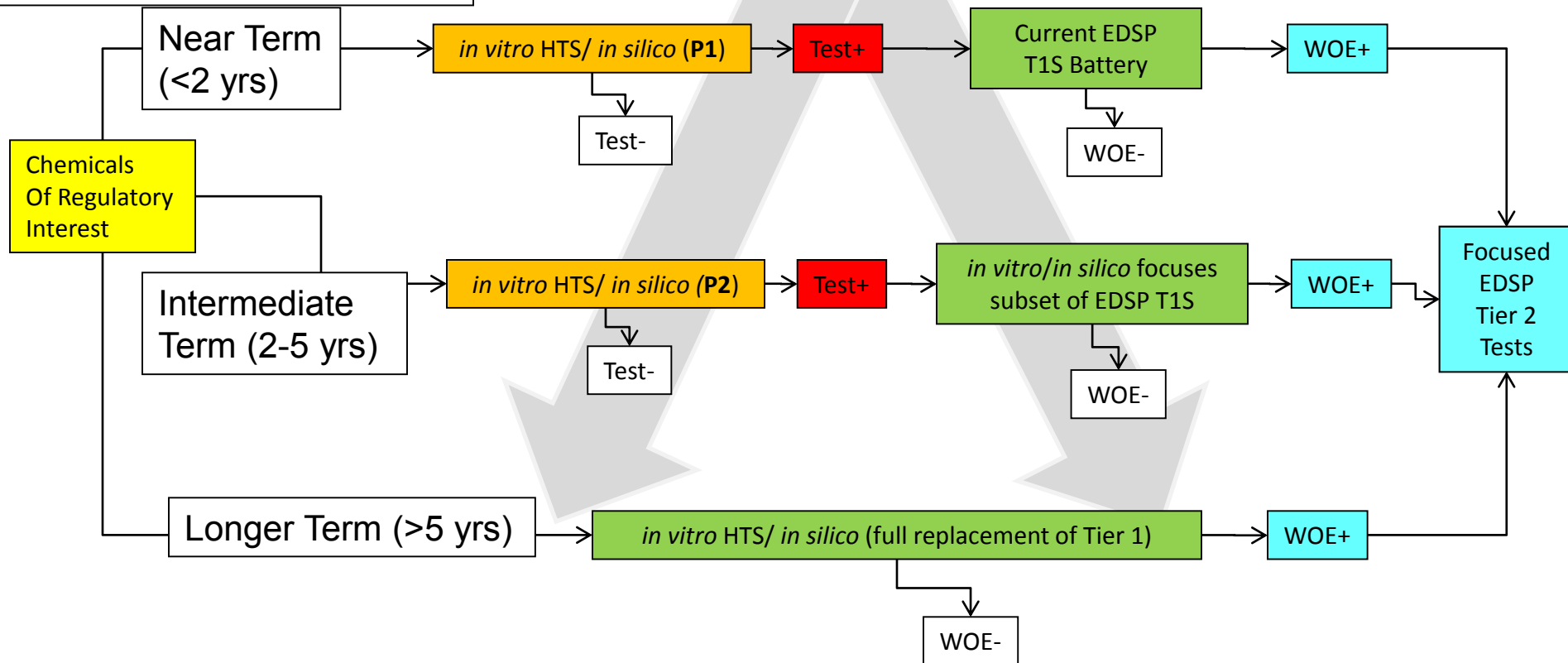
Use of HTS Results in an Adverse Outcome Pathway (AOP)



ToxCast and the Endocrine Disruptor Screening Program

The universe of chemicals passes through each version of the HTS/*in silico* pipeline to evaluate chemicals in refined tests, or for new pathways, to evaluate improve and validate methods.

EPA Research provides basis for improving the suite of assays and models to advance chemical prioritization and screening

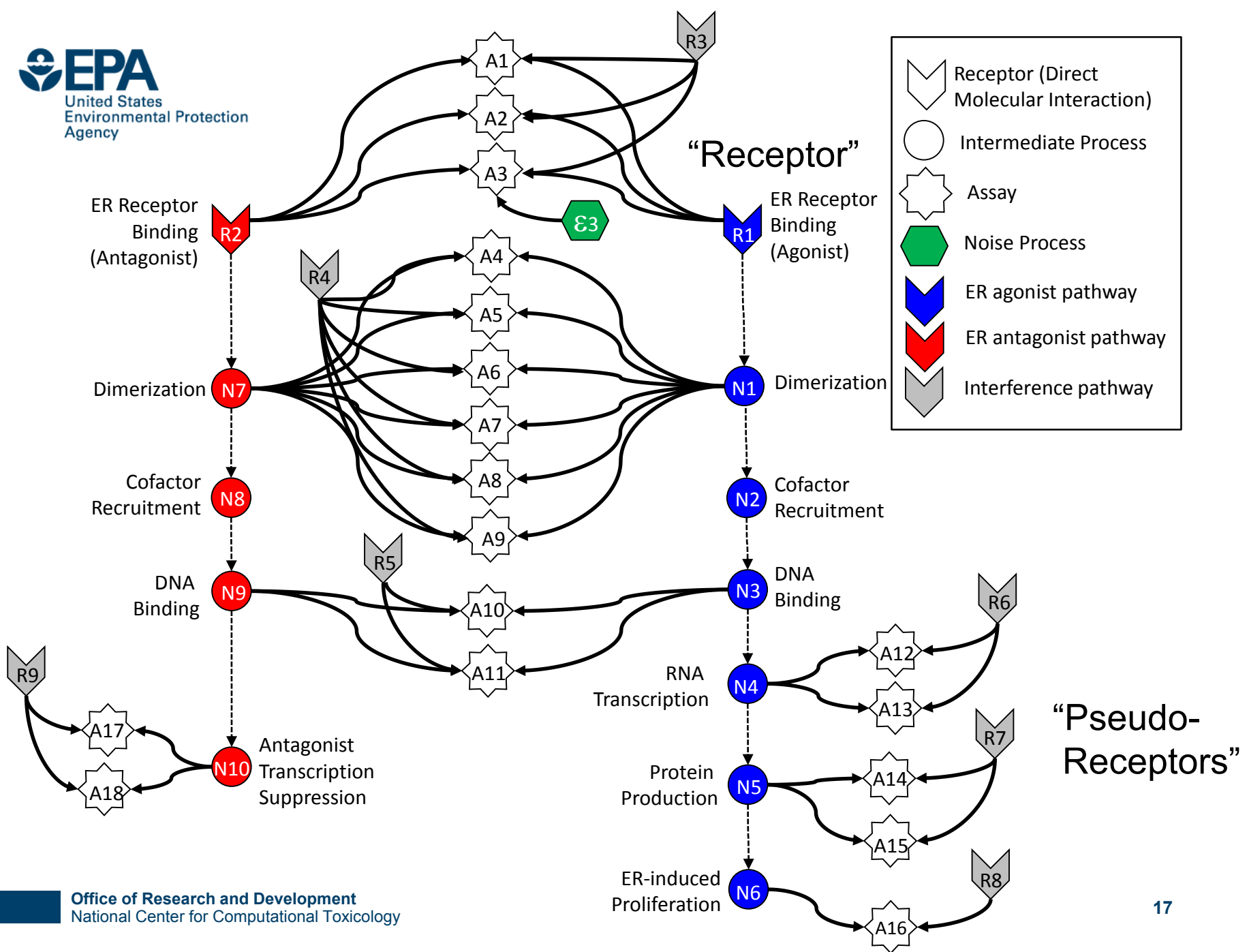


Chemical Prioritization

Includes registration review timeline, physico-chemical properties, exposure estimates, *in vitro* assays and computer models (QSAR, expert systems, systems biology models).

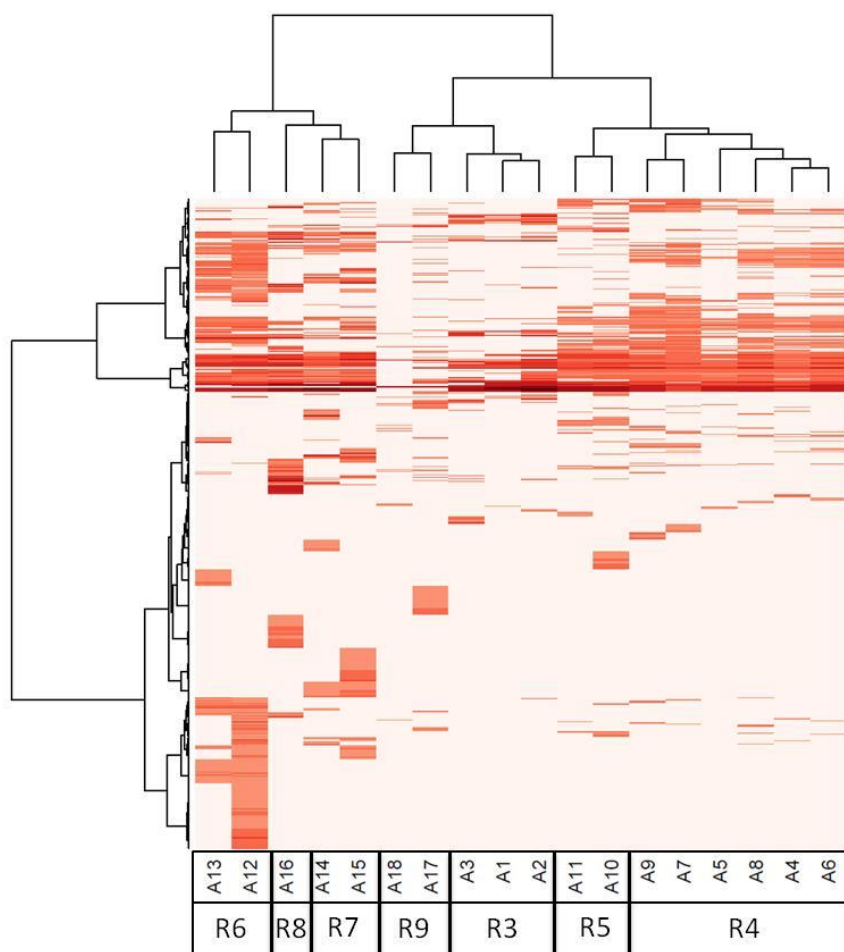
Screening Decisions

Near Term = Incorporates HTS/*in silico* prioritization methods for post EDSP List 2
Intermediate = Run subset of T1S assays indicated by HTS and *in silico* predictions
Long Term = Full replacement of EDSP T1S Battery



Major theme – all assays have false positives and negative

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

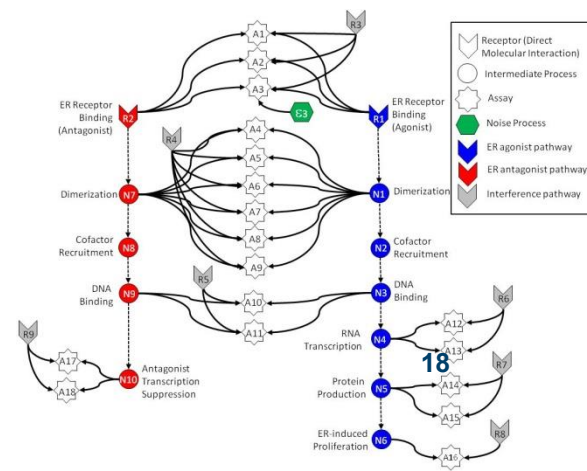


Much of this “noise” is reproducible, i.e. it is “assay interference”

Result of interaction of chemical with complex biology in the assay

Our chemical library is only partially “drug-like”

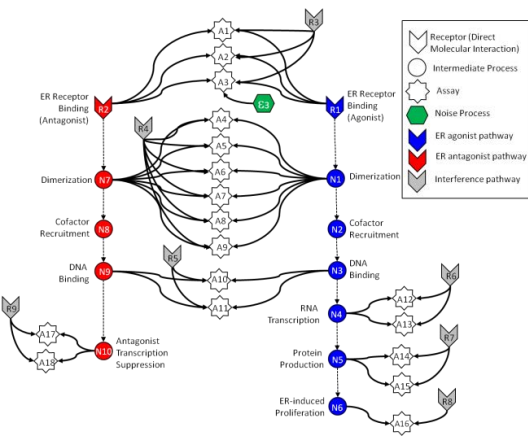
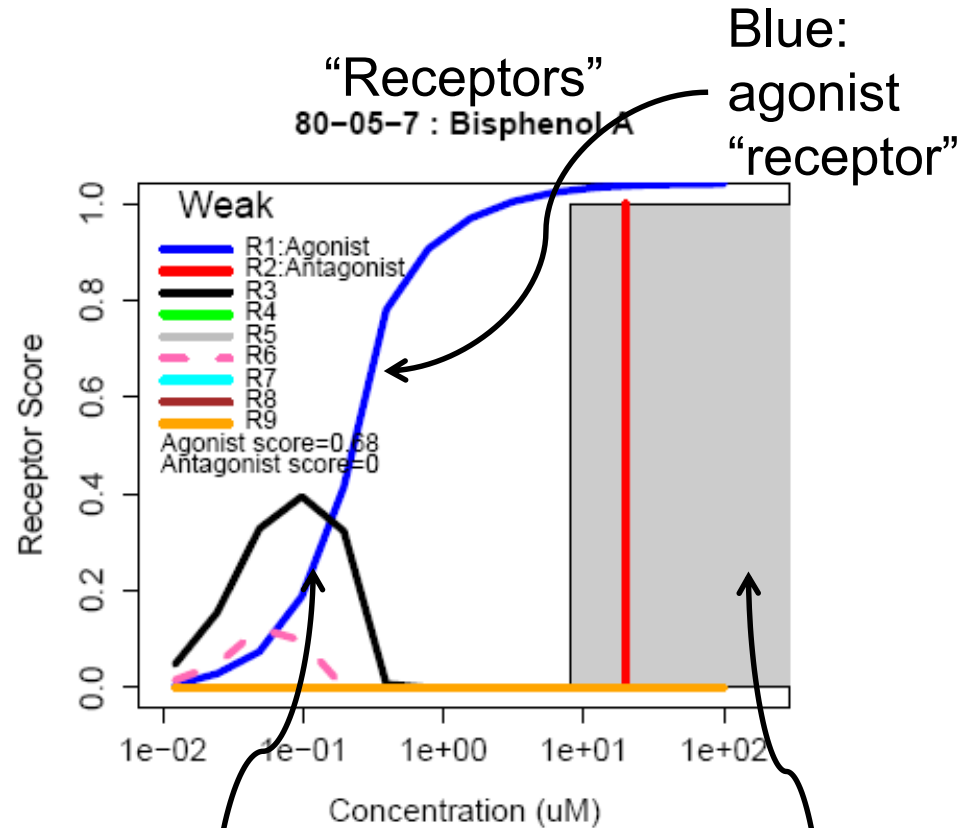
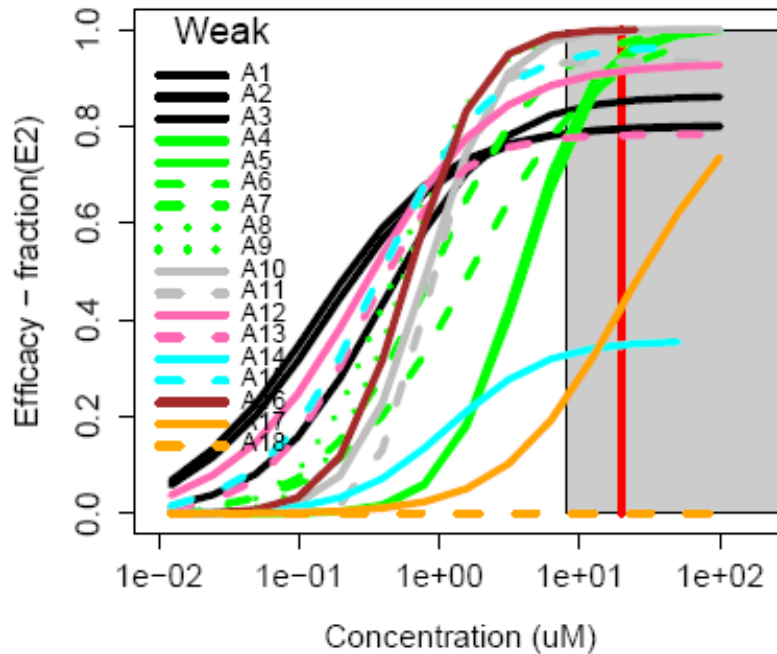
- Solvents
- Surfactants
- Intentionally cytotoxic compounds
- Metals
- Inorganics



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

Assays

80-05-7 : Bisphenol A



Binding assays active at lowest concentration

AUC “sign” feature will discount this

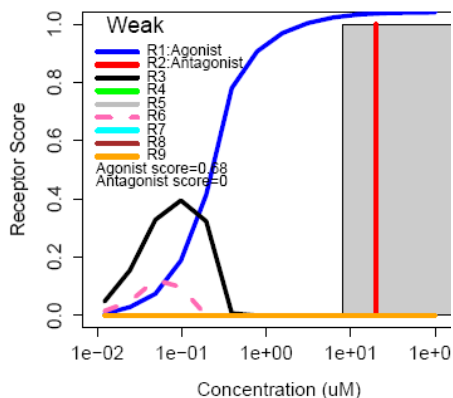
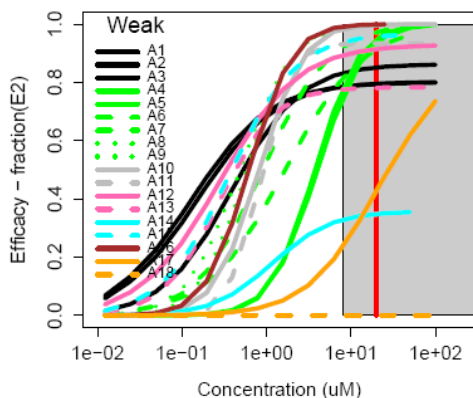
Cytotoxicity Region: red line is median cytotox AC50

Example curves

True Agonist

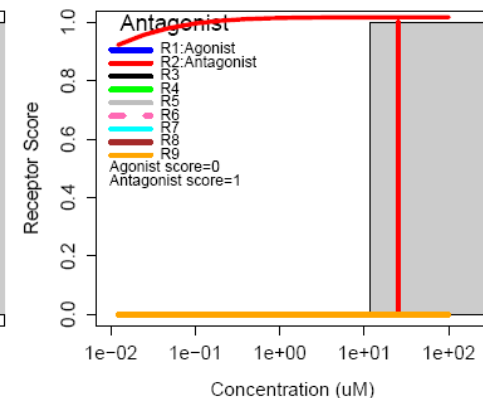
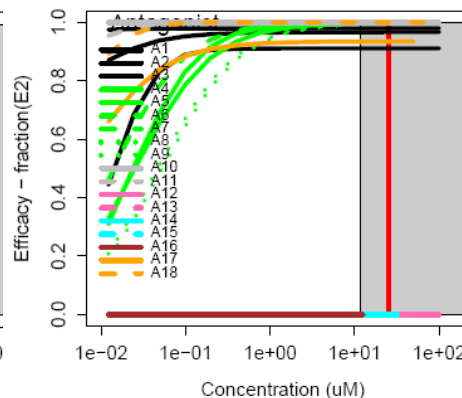
80-05-7 : Bisphenol A

80-05-7 : Bisphenol A



82640-04-8 : Raloxifene hydrochloride

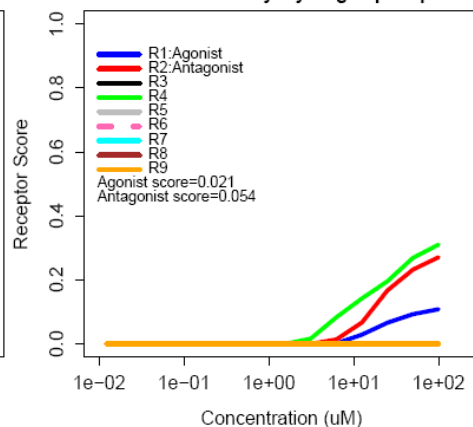
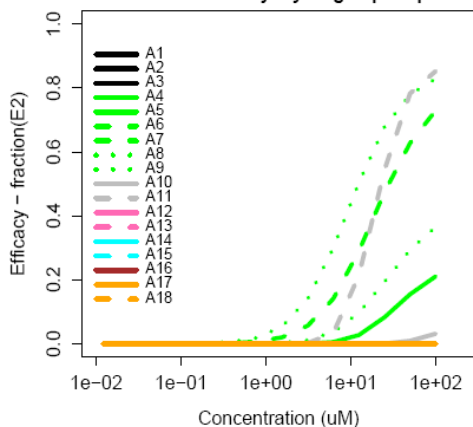
82640-04-8 : Raloxifene hydrochloride



Negative-Broad Assay Interference

868-85-9 : Dimethyl hydrogen phosphite

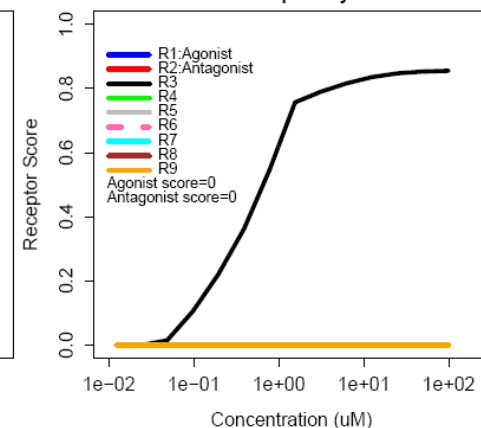
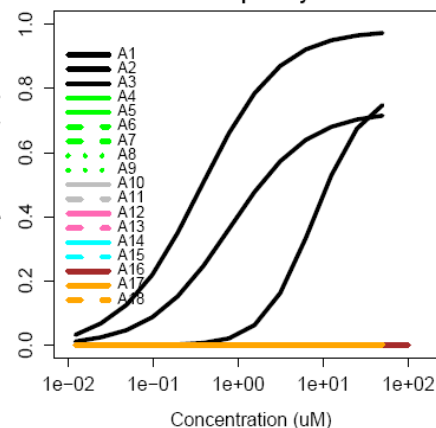
868-85-9 : Dimethyl hydrogen phosphite



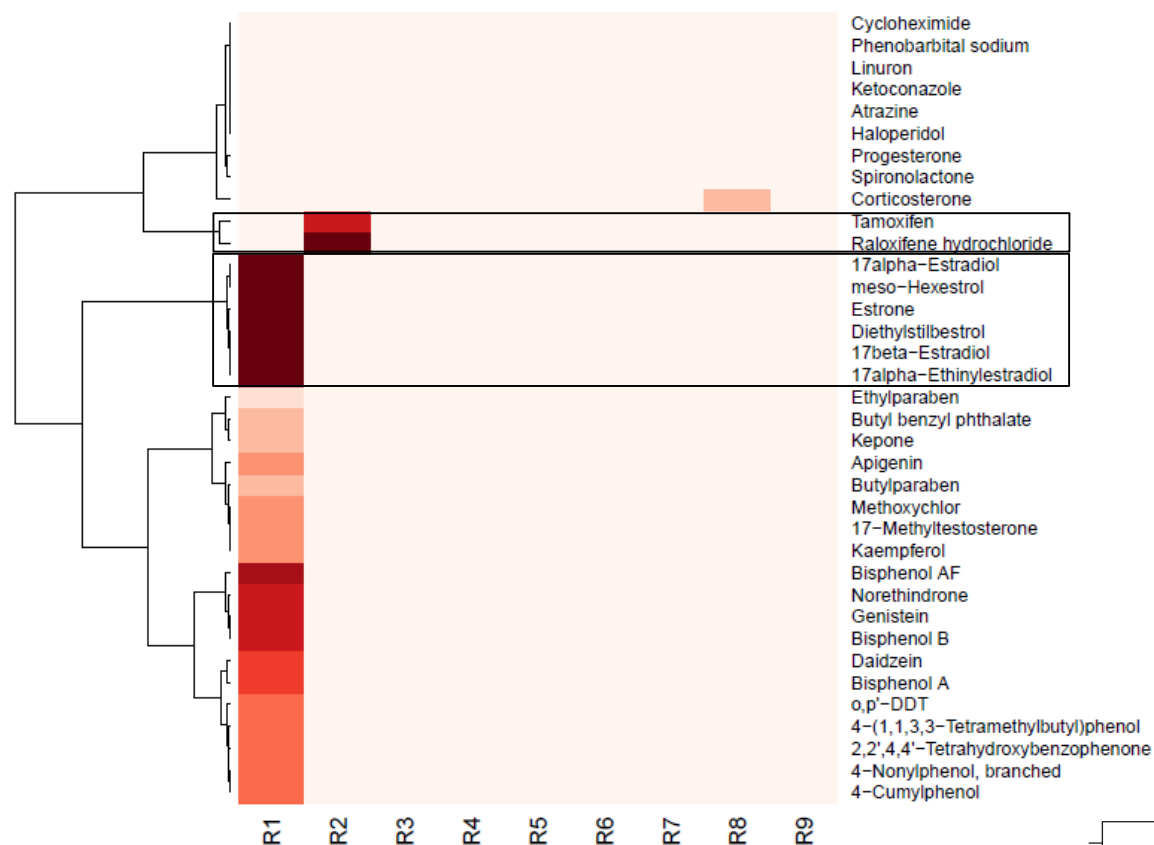
Negative-Narrow Assay Interference

10016-20-3 : alpha-Cyclodextrin

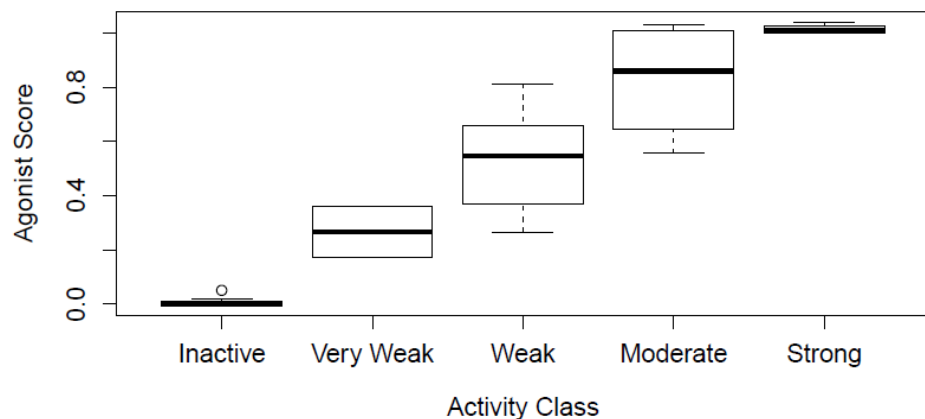
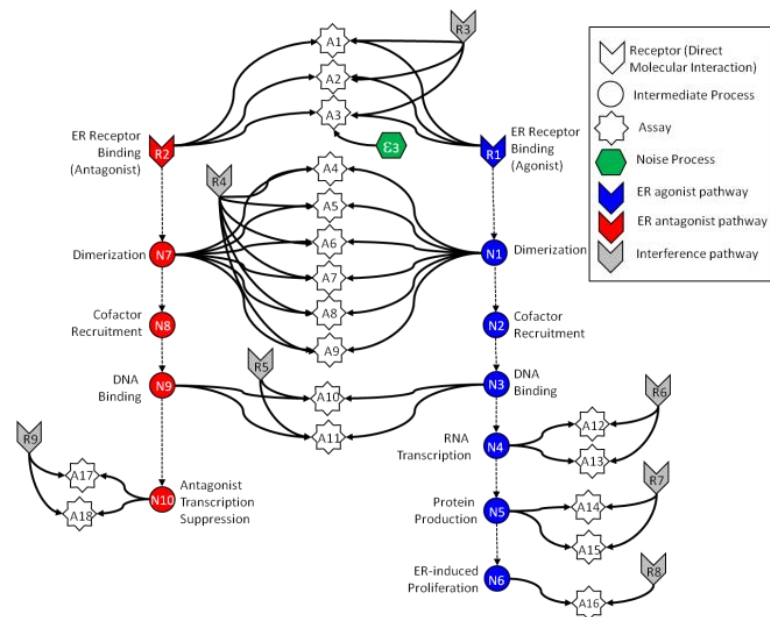
10016-20-3 : alpha-Cyclodextrin



Reference Chemical Classification



AUC heat map for
Reference chemicals

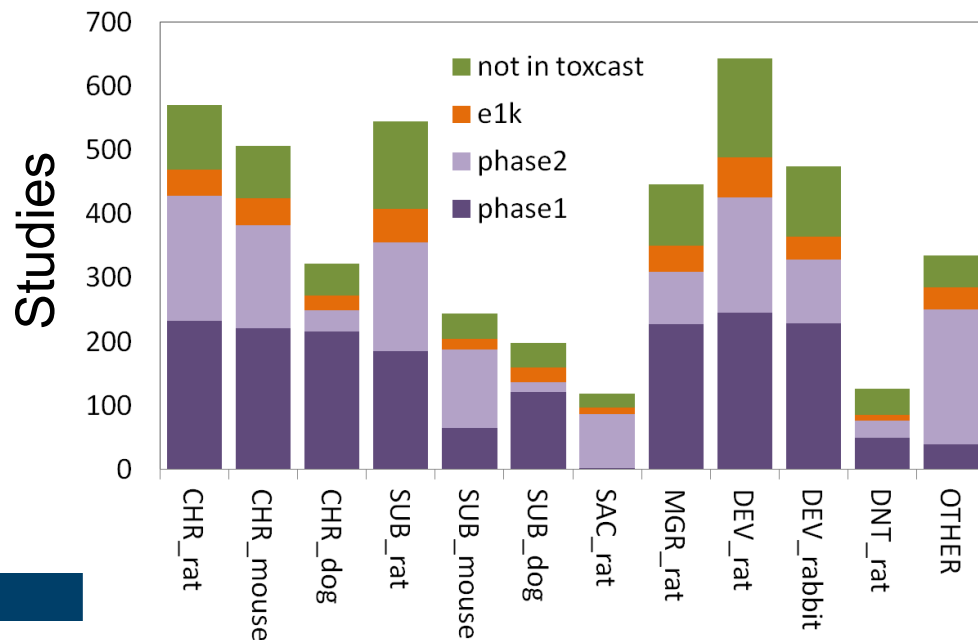


Predictive Models/Signatures

- Need to anchor to in vivo
- Guideline toxicity studies useful
 - EPA has extensive reports in support of registrations (pesticides)
 - Standardized
 - EPA regulates using these
- Recent incorporation of failed human drugs will provide more human-relevant in vivo

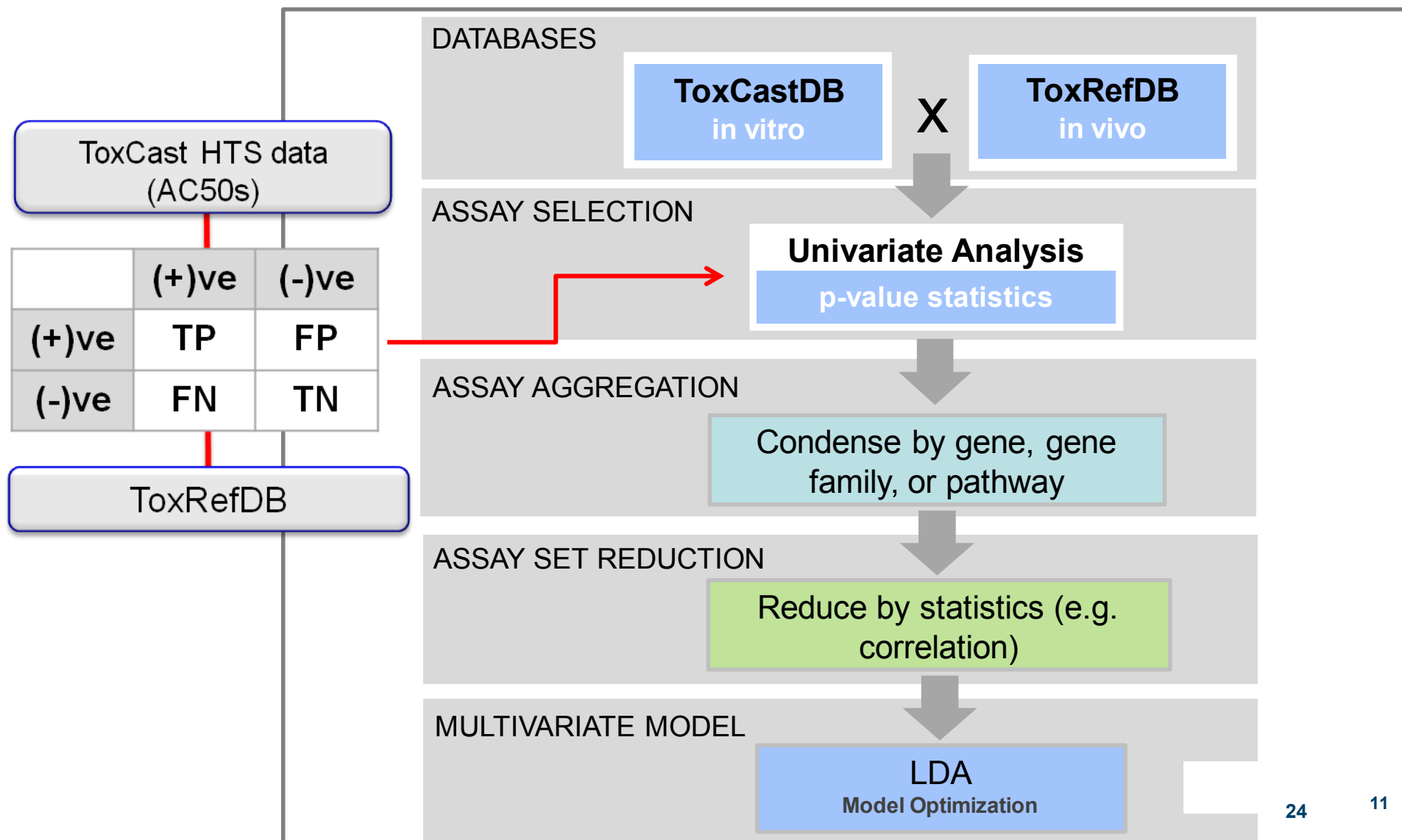
Toxicity Reference Database (ToxRefDB)

- ToxRefDB holds in vivo endpoint data from animal toxicology studies (DERs, NTP, open literature, pharma)
- Currently at 5567 studies on 1049 unique chemicals
- Used by:
 - ORD in predictive modeling (prospective)
 - e.g., multigen reproductive effects Martin et al., 2009)
 - OPP & OECD for assessing the impact of guideline studies on risk assessments (retrospective)
 - Public as a general chemical toxicity data resource



Data Source	Study Count
EPA OPP_der	3279
Open Literature	731
National Toxicol Program	666
Sanofi_pharma	222
Unpublished_submissions	50
GSK_pharma	38
Health Canada PMRA_der	23

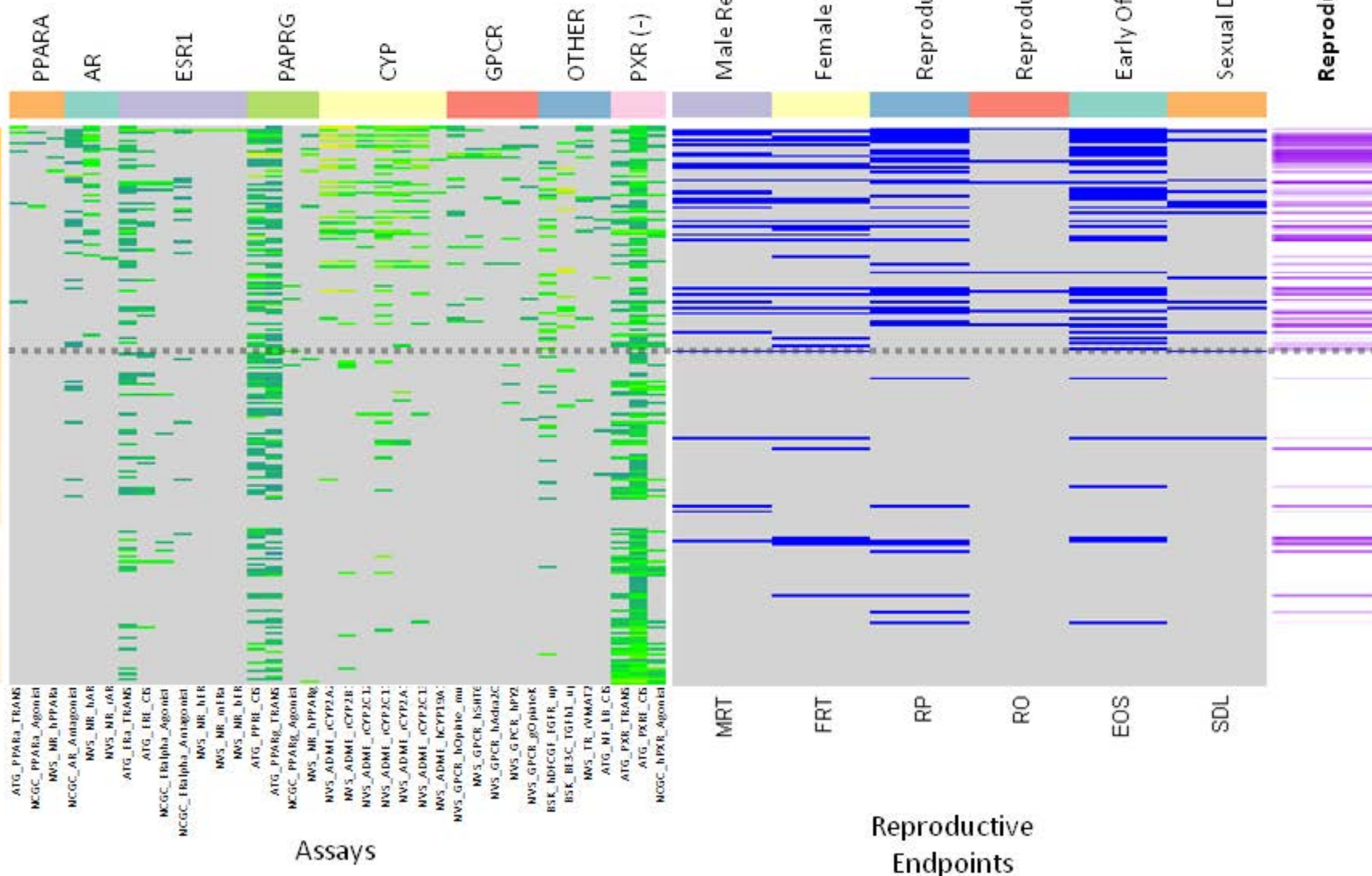
Predictive Model Development from ToxCast and Other Data



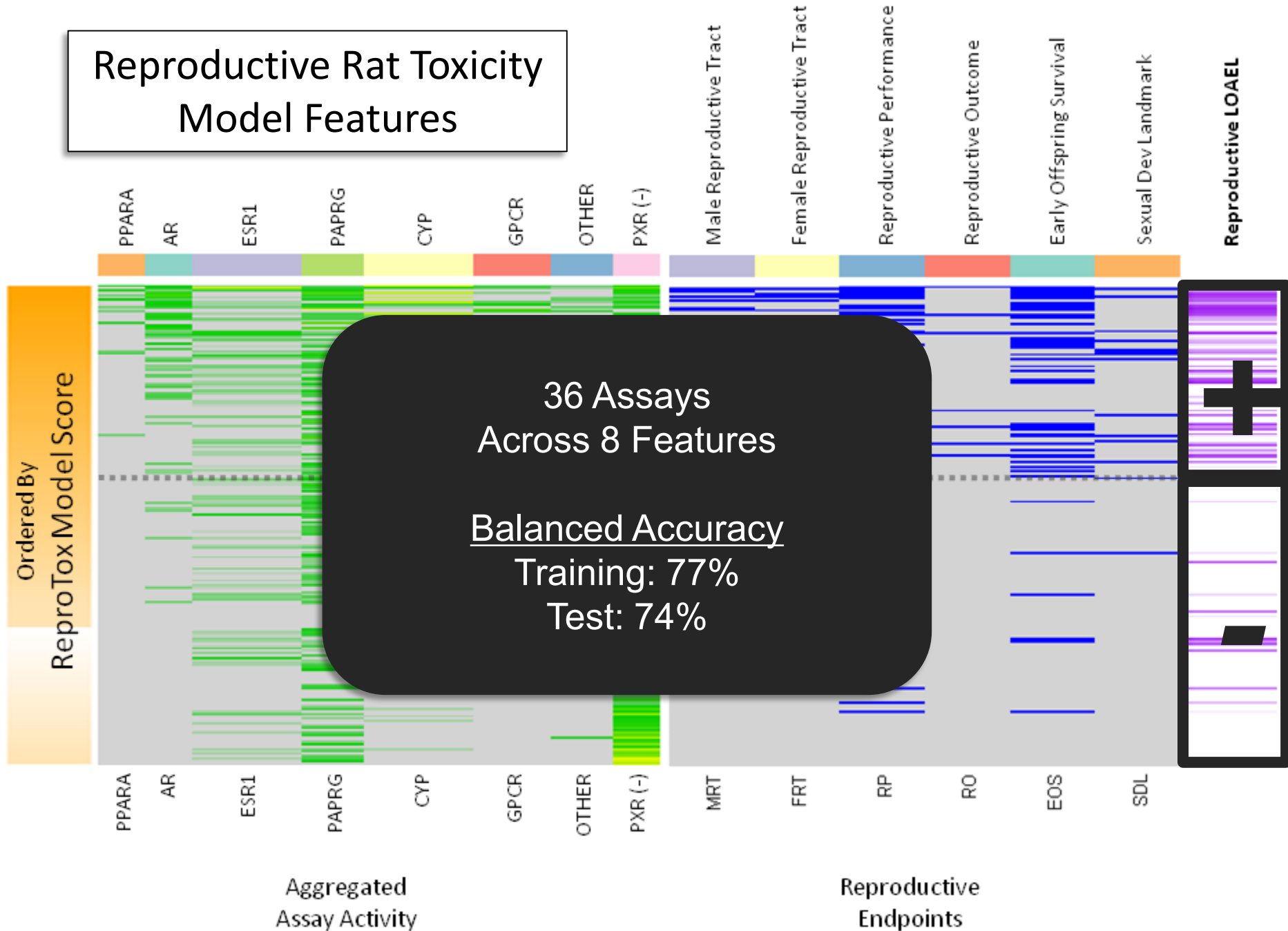
Reproductive Rat Toxicity Model Features

Chemicals

Ordered By
ReproToxModelScore



Reproductive Rat Toxicity Model Features



Predictive Toxicity Modeling Based on ToxCast Data

❖ Predictive models: **endpoints**

liver tumors: Judson et al. 2010, Env Hlth Persp 118: 485-492

hepatocarcinogenesis: Shah et al. 2011, PLoS One 6(2): e14584

cancer: Kleinstreuer et al. 2012, submitted

rat fertility: Martin et al. 2011, Biol Reprod 85: 327-339

rat-rabbit prenatal devtox: Sipes et al. 2011, Toxicol Sci 124: 109-127

zebrafish vs ToxRefDB: Sipes et al. 2011, Birth Defects Res C 93: 256-267

❖ Predictive models: **pathways**

endocrine disruption: Reif et al. 2010, Env Hlth Persp 118: 1714-1720

microdosimetry: Wambaugh and Shah 2010, PLoS Comp Biol 6: e1000756

mESC differentiation: Chandler et al. 2011, PLoS One 6(6): e18540

HTP risk assessment: Judson et al. 2011, Chem Res Toxicol 24: 451-462

angiogenesis: Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603

❖ Continuing To Expand & Validate Prediction Models

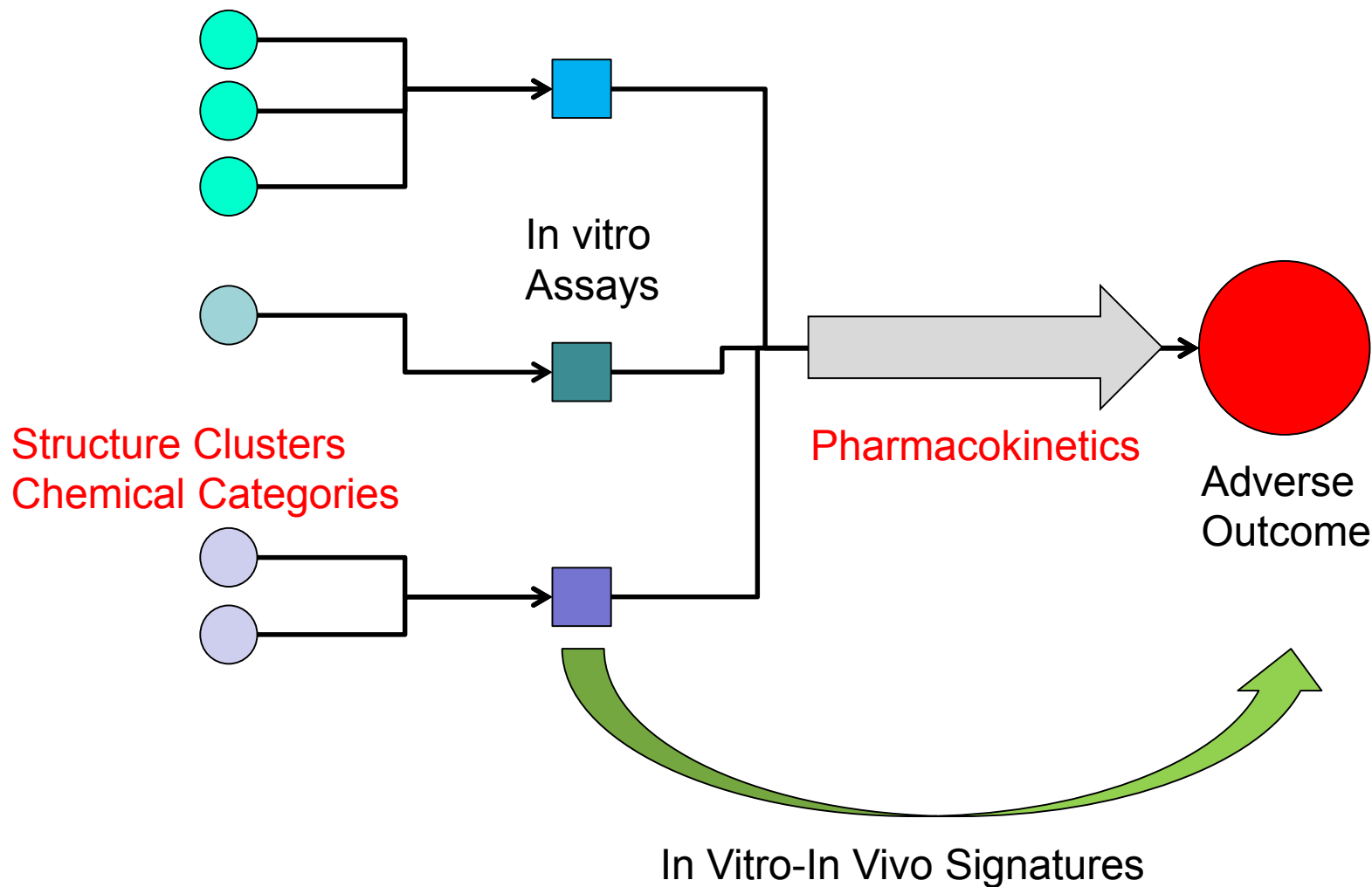
❖ Generally moving towards more mechanistic/AOP-based models

Understanding Success and Failure

- Why *In vitro* to *in vivo* can work:
 - Chemicals cause effects through direct molecular interactions that we can measure with *in vitro* assays
- Why *in vitro* to *in vivo* does not always work:
 - ★ – Pharmacokinetics issues: biotransformation, clearance (FP, FN)
 - ★ – Assay issues: don't have all the right assays (FN)
 - ★ – Tissue issues: may need multi-tissue signaling networks (FN)
 - ★ – Statistical power issues: need enough chemicals acting through a given MOA to be able to build and test model (FN)
 - ★ – Compensation: system may adapt to initial insult (FP)
 - *In vitro* assays are not perfect! (FP, FN)
 - *In vivo* rodent data is not perfect! (FP, FN)

Systems
Models

Beyond *in vitro* to *in vivo* signatures



Why is Chemistry needed?

Thomas et al, 2012, Tox Sci

- ToxCast Phase I library (309 cmpds)
- >80 statistical methods
- ToxRef DB endpoints
- No successful models of *in vivo* endpoints

Statistics

Noisy,
lack of
statistical power,
mechanistically
diverse dataset

Model
in vivo
toxicity

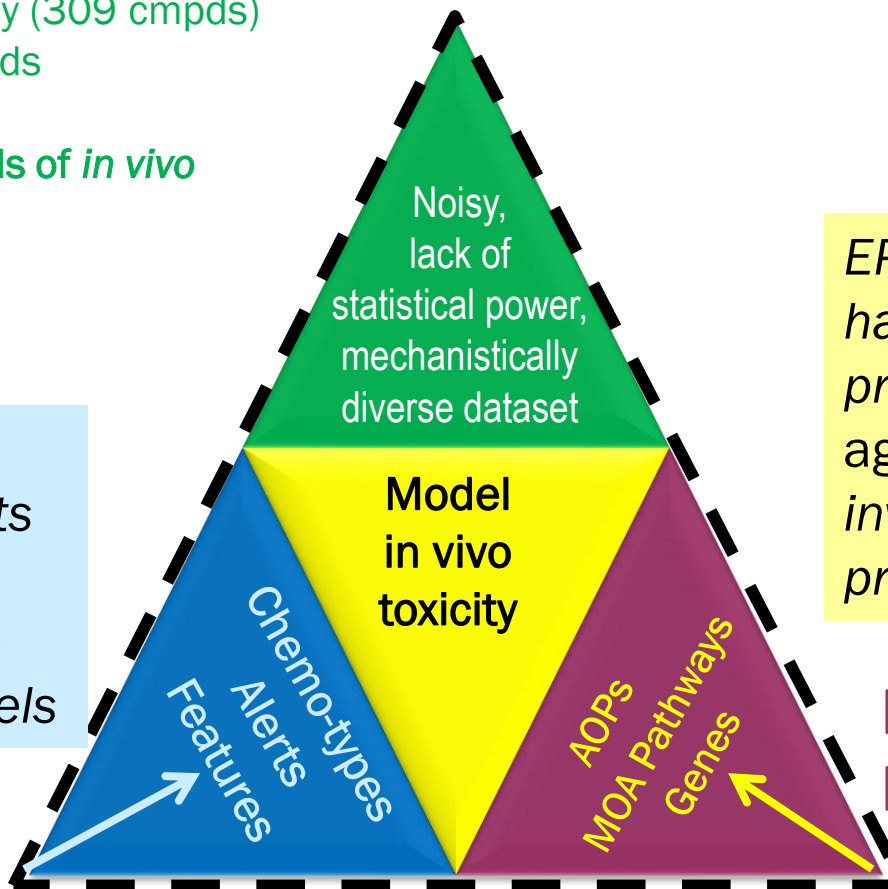
EPA's modeling success
has relied upon use of
prior knowledge &
aggregation to focus
investigations into
productive areas

HTS (>500 assays)
In vitro Biology

Chemistry

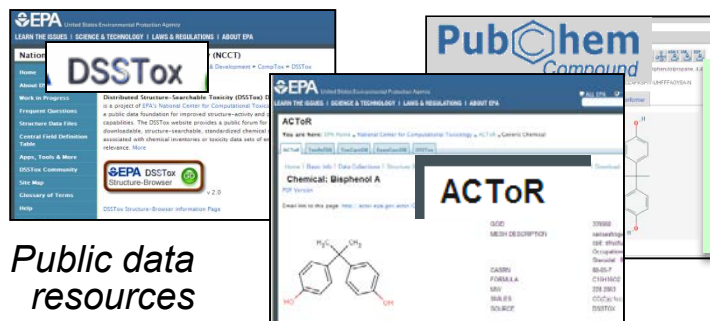
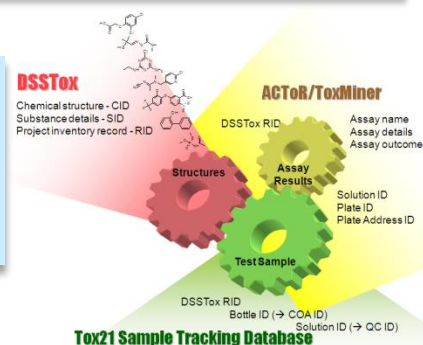
Chemo-types
Alerts
Features

AOPs
MOA Pathways
Genes



Chemistry: What's needed?

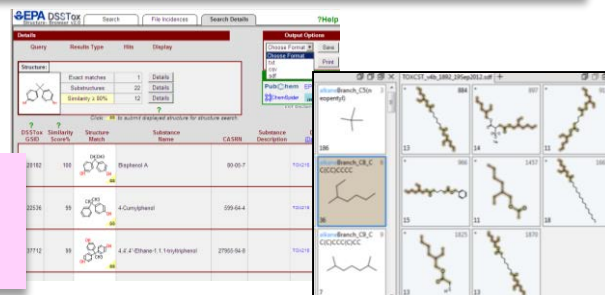
- Accurate chemical annotations of testing libraries (e.g., ToxCast & Tox21), transparency, & reporting of error sources
- Cheminformatics foundation to enable structure modeling



Public data
resources

Public data release: ability of non-chemists (biologists, statisticians) to access & utilize chemical information

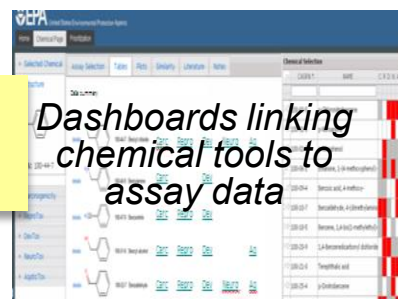
Structure-
similarity
searching



Use all available data (HTS+chemistry) to guide & inform analog selection & model predictions

Incorporate chemical information into usable tools for chemical prioritization & safety assessments

Dashboards linking
chemical tools to
assay data



Informed
feature-
grouping &
highlighting

DSSTox <http://www.epa.gov/ncct/dsstox/>

U.S. Environmental Protection Agency
Distributed Structure-Searchable Toxicity (DSSTox) Public Database Network

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DSSTox

Distributed Structure-Searchable Toxicity (DSSTox) Database Network is a project of EPA's [Computational Toxicology Program](#), helping to build a public data foundation for improved structure-activity and predictive toxicology capabilities. The DSSTox website provides a public forum for publishing downloadable, structure-searchable, standardized chemical structure files associated with toxicity data. [More](#)

Chemical Structures + **Toxicity Data**

EPA DSSTox Structure-Browser v1.0

[DSSTox Structure-Browser information](#)

Recent Additions: 27 Septemb

DSSTox Chemical Text Search

Choose search: Enter search text:

Data Files to Search

☒ All DSSTox Files
☒ Selected DSSTox Files

☒ ARYEXP_v1a
☒ ARYEXP_v1b
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Tox Chemical Structure Search

SMILES string:

Draw a molecule or substructure using the JME editor:

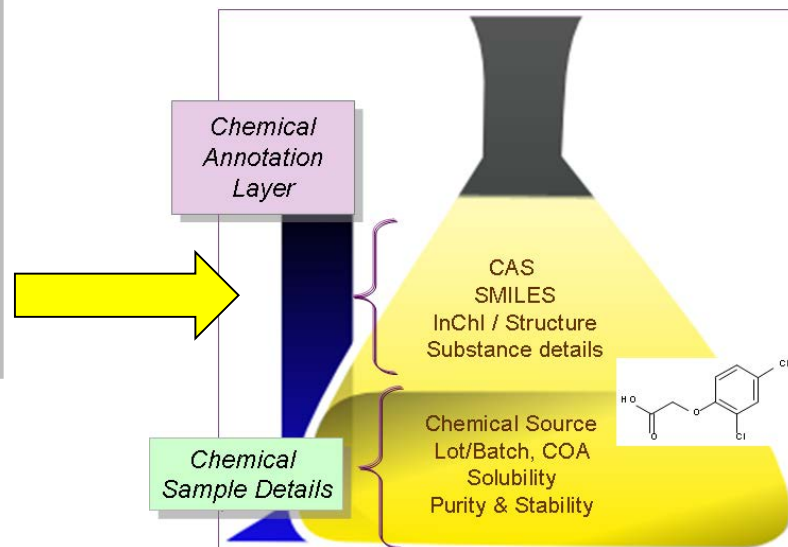
Search Options

☒ Exact match
☒ Substructure
☒ Similarity
Threshold: 80 %

DSSTox Master DB (Access → MySQL)

- ✓ 16K structures (mol, SMILES, InChI)
- ✓ 18K registered substances
- ✓ 22K CAS (6K STN-checked, 3K deleted)
- ✓ 36 inventories (45K record IDs)

ToxCast & Tox21 chemical QC & data management



- Public chemical-toxicity (SAR) data resource
- Source of high quality chemical structures across high-interest EPA inventories

- Public (open source) structure-browser provides inventory-specific structure-similarity searches & external linkages

Toxicity Prediction Challenge:

Bringing all knowledge & data to bear on problem

Biologically-based QSAR & Cheminformatics

Reactivity & toxicity-
informed
features &
classes

Aggregation

Mechanistically
well-defined
toxicity endpoint

Data-mining

Adverse Outcomes:

- > Pathways
- > Genes
- > Assays
- + Statistical
associations

Structures

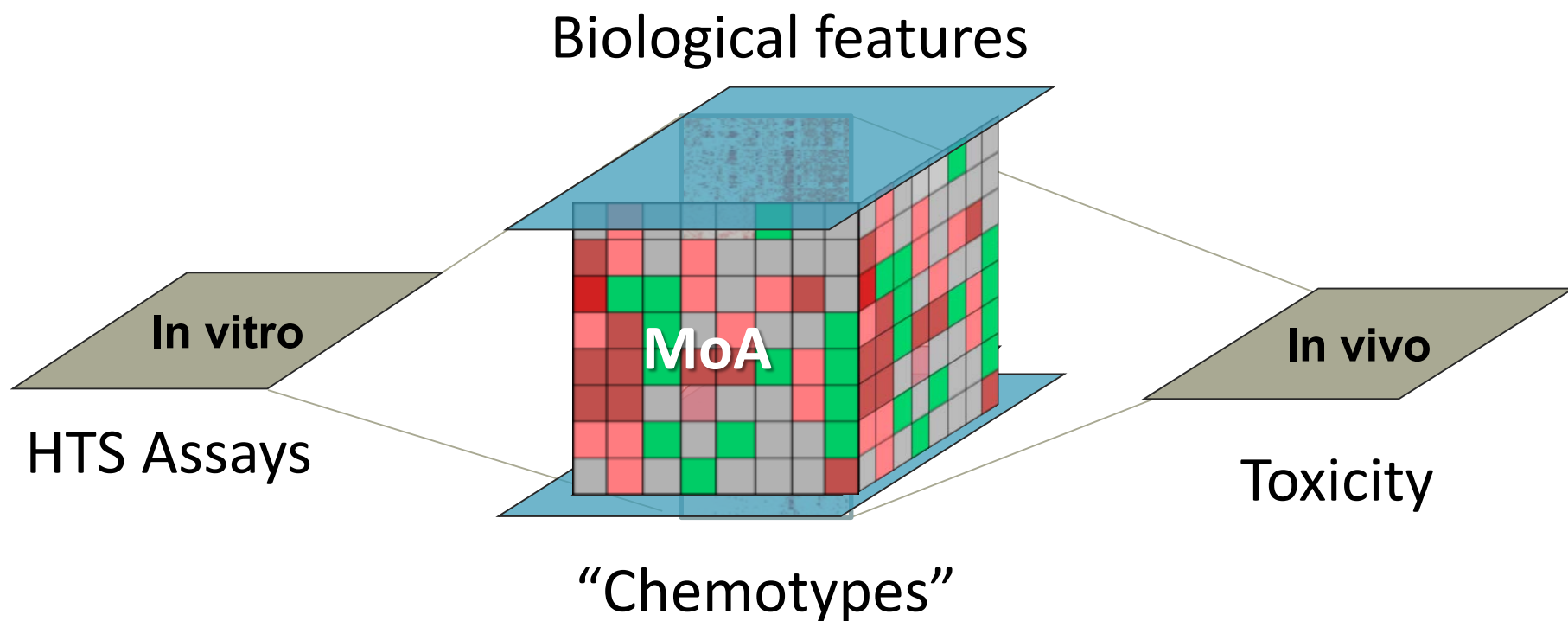
In Vitro/HTS

In Vivo

Existing knowledge

QSAR using biologically informed chemical features

“MoA QSAR”



*HTS results are used to inform feature selection, linking chemical features to putative toxicity Mode of Action (MoA) of toxicity*³⁴

Understanding Success and Failure

- Why *In vitro* to *in vivo* can work:
 - Chemicals cause effects through direct molecular interactions that we can measure with *in vitro* assays
- Why *in vitro* to *in vivo* does not always work:
 - ★– Pharmacokinetics issues: biotransformation, clearance (FP, FN)
 - ★– Assay coverage: don't have all the right assays (FN)
 - ★– Tissue issues: may need multi-cellular networks and physiological signaling (FN)
 - ★– Statistical power issues: need enough chemicals acting through a given MOA to be able to build and test model (FN)
 - ★– Homeostasis: A multi-cellular system may adapt to initial insult (FP)
 - *In vitro* assays are not perfect! (FP, FN)
 - *In vivo* rodent data is not perfect! (FP, FN)

Systems
Models

ToxCast Phase II Data Release

- ToxCast Assay Summary Activity Files (toxminer_v19b)
 - Rows of Chemicals, Columns of Assays, Intersection of AC50, EMAX
- ToxCast Assay Annotation Files (toxcast_assay_annotation_v1)
 - Assignment of assay design information
 - Assignment of target information (gene target)
- ToxCast Chemical Library & Structure Files (dsstox)
- ToxCast Concentration Response Data Files (toxminer_v19b)
 - Detailed files of normalized data (>50M Rows)
- ToxRefDB Effect & Endpoint Data Files (toxrefdb)
 - Flattened version of ToxRefDB with all effect information (>6000 studies & 1000 chemicals)
 - Endpoint summary file that has NEL/LEL and NOAEL/LOAEL across all studies

HOME

ToxCast Data Overview Video



ToxCast Dashboard v0.5 provides users with the ability to perform basic data and chemical selection as well as simple data exploration in a seamless environment. We will be striving to continuously add functionality and improve overall utility and performance. The initial release also intends to convey the conceptual framework and design of the iCSS web application with the intention of producing updated versions of the ToxCast Dashboard as well as additional Dashboards.

The ToxCast Dashboard contains the results of over 800 Assay Endpoints (High Throughput Screening (HTS) Data) across over 1800 chemicals from 7 primary HTS assay sources. The release of the ToxCast Dashboard coincides with the release of the ToxCast Phase II data, which is available below.

The Interactive Chemical Safety for Sustainability (iCSS) web application is releasing its first dashboard, called the ToxCast Dashboard. The ToxCast Dashboard is intended to provide an interactive data exploration tool. We are currently releasing ToxCast Dashboard version 0.5, a beta version of the application.

If you would like to provide feedback or be on a mailing list that provides updates on new releases of the ToxCast Dashboard as well as ToxCast data

ToxCast Phase II Data Release:

[ToxCast Assay Summary Activity Files \(toxminer v19b\)](#)

[ToxCast Assay Annotation Files \(toxcast assay annotation v1\)](#)

[ToxCast Chemical Library & Structure Files \(dsstox\)](#)

[ToxCast Concentration Response Data Files \(toxminer v19b\)](#)

[ToxRefDB Effect & Endpoint Data Files \(toxrefdb\)](#)

Disclaimer:

ToxCast Data will change over time as our understanding of

DATA EXPLORER

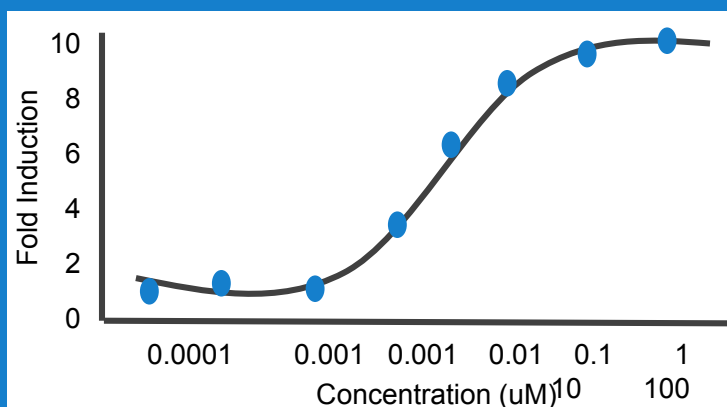
Assay List

Data Class	Assay Endpoint
ATG	ATG_ERa_TRANS
ATG	ATG_ERE_CIS
ATG	ATG_PPARG_TRANS
ATG	ATG_PPRES_CIS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS

Summary Activity Table

CASRN	Chemical Name	Q	AC50	T	B	W	Emax
80-05-7	Bisphenol A						
80-05-1	Bisphenol B						
80-05-2	Bisphenol C						
80-05-2	Bisphenol D						
80-05-4	Bisphenol E						
80-05-5	Bisphenol F						
80-05-6	Bisphenol G						
80-05-8	Bisphenol H						
80-05-9	Bisphenol I						

CONC RESPONSE PLOT



Summary

- Goal: use *in vitro* assays to screen and prioritize many data-poor chemicals
- Signature generation uses combination of biological insight and statistics
- Initial models point the way to real-world applications
- Further refinements are in the works
 - More chemicals and assays
 - Use of chemoinformatics
 - Systems-level models
 - Targeted testing approaches



EPA NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY STAFF
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