ToxCast Research Program Update

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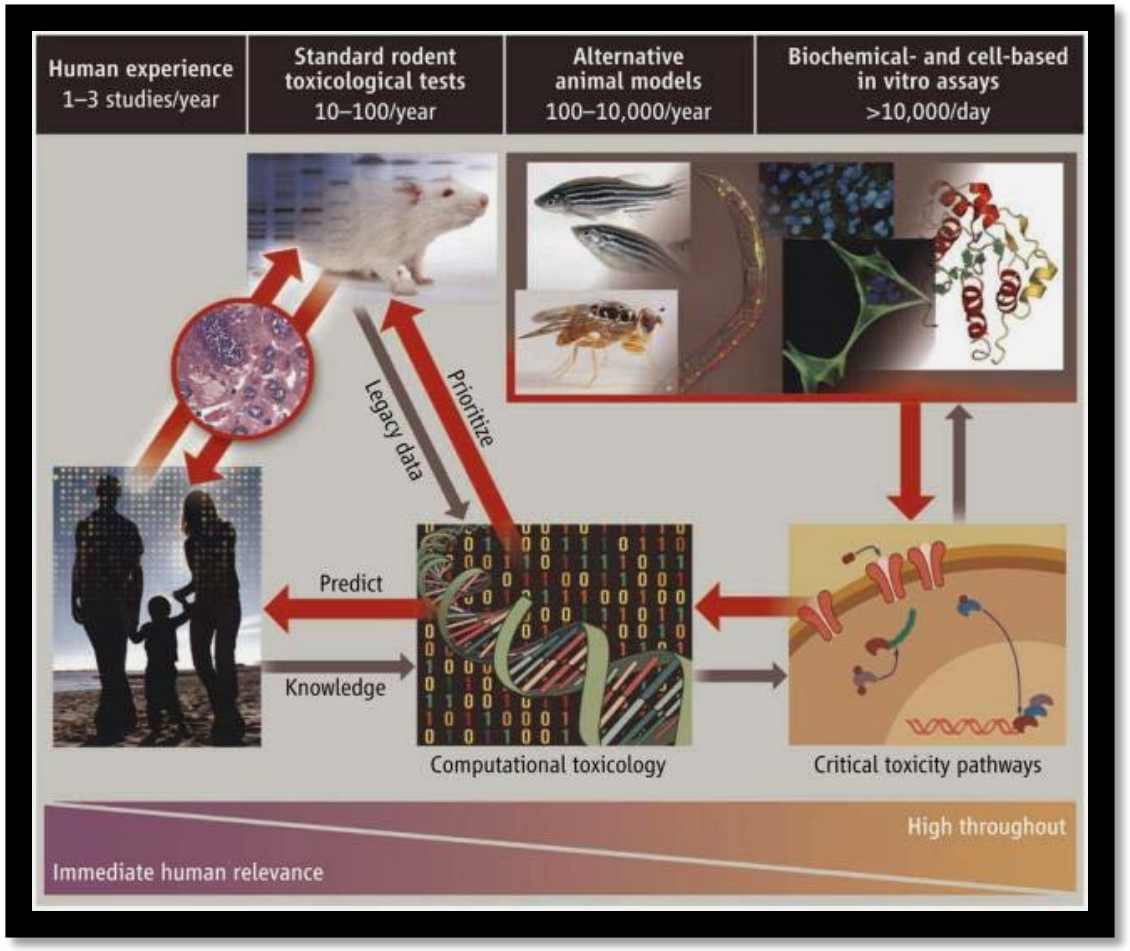
Tox21 Vision: Transforming Toxicity Testing



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

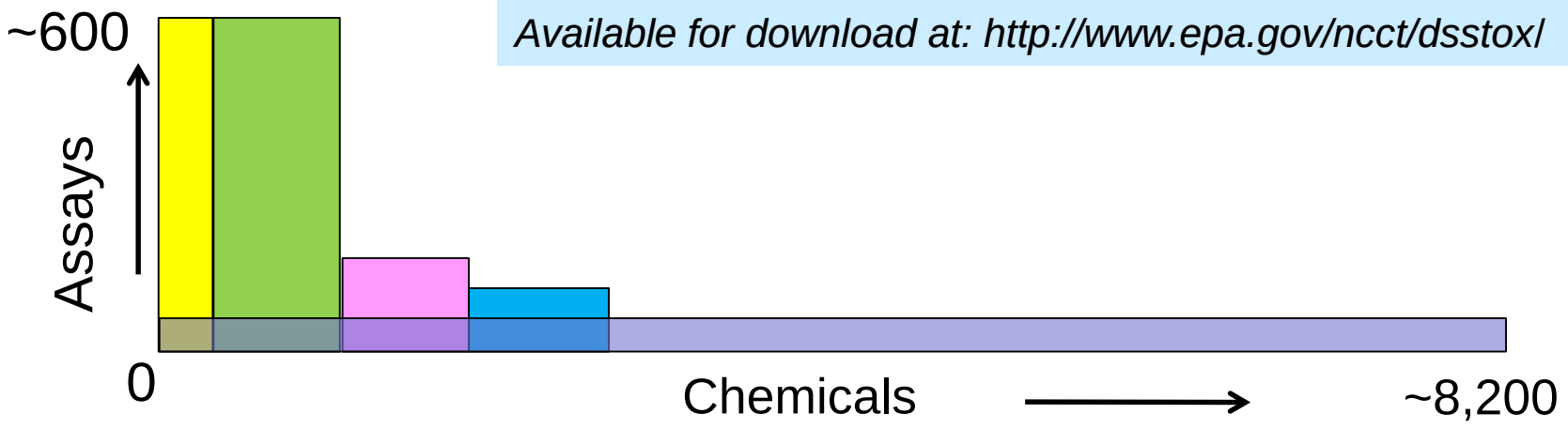
SOURCE: Collins, Gray and Bucher (2008) Toxicology. Transforming environmental health protection. Science 319: 906 [2]

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 (ExpoCast)*
- *High-throughput Risk Assessments (quantitative)*

Testing under ToxCast and Tox21 Chemicals, Data and Release Timelines

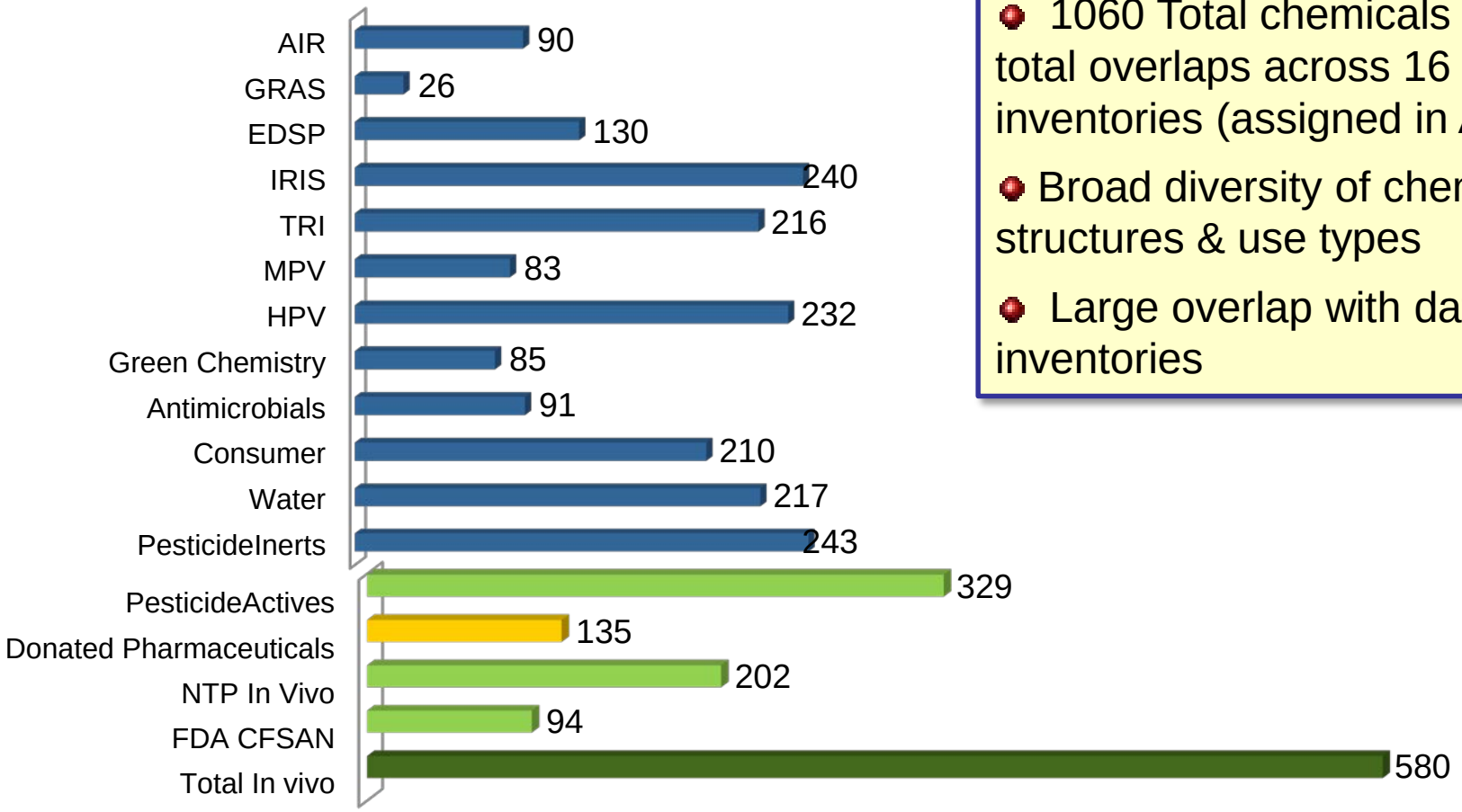
Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~700	2011	Now
ToxCast Phase II	767	~600	~700	03/2013	12/2013
ToxCast Phase IIIa	1001	~100	~100	Just starting	2014
E1K (endocrine)	880	~50	~120	03/2013	12/2013
Tox21	8,193	~25	~50	Ongoing	Ongoing



Available for download at: http://www.epa.gov/ncct/dsstox/

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

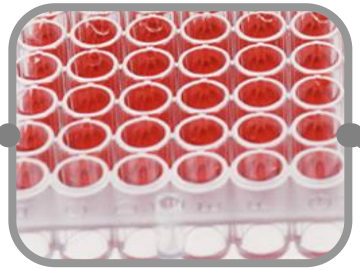


- 1060 Total chemicals → 2806 total overlaps across 16 diverse inventories (assigned in ACToR)
- Broad diversity of chemical structures & use types
- Large overlap with data-rich inventories

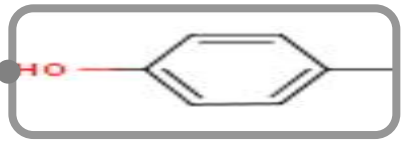
High-Throughput Screening 101 (HTS)

United States Environmental Protection Agency

Robots

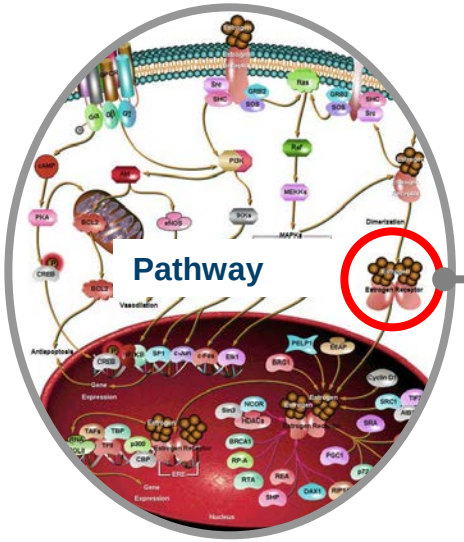


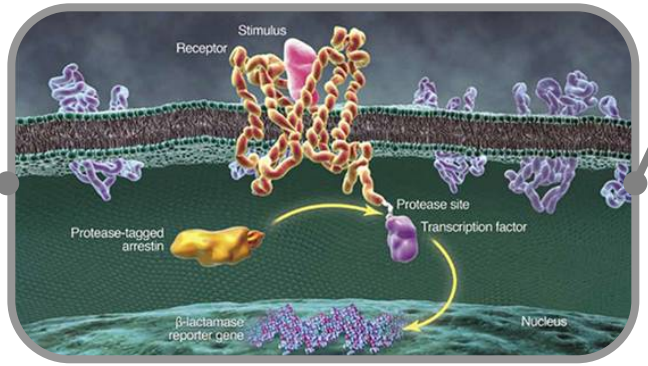
96-, 384-, 1536 Well Plates



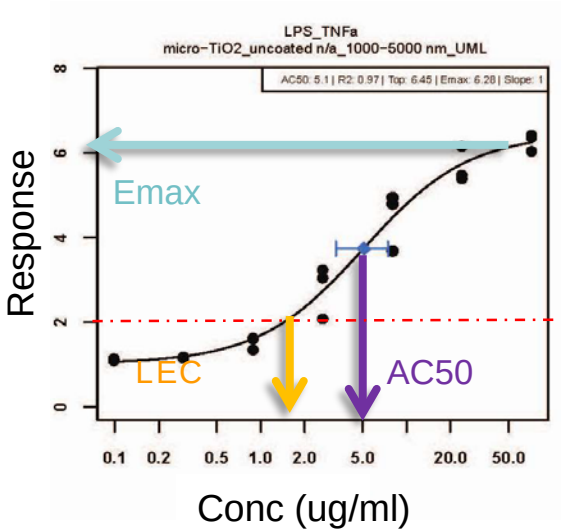
Chemical Exposure

Cell Population

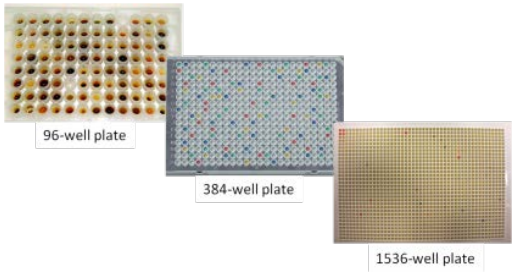




Target Biology (e.g., Estrogen Receptor)



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

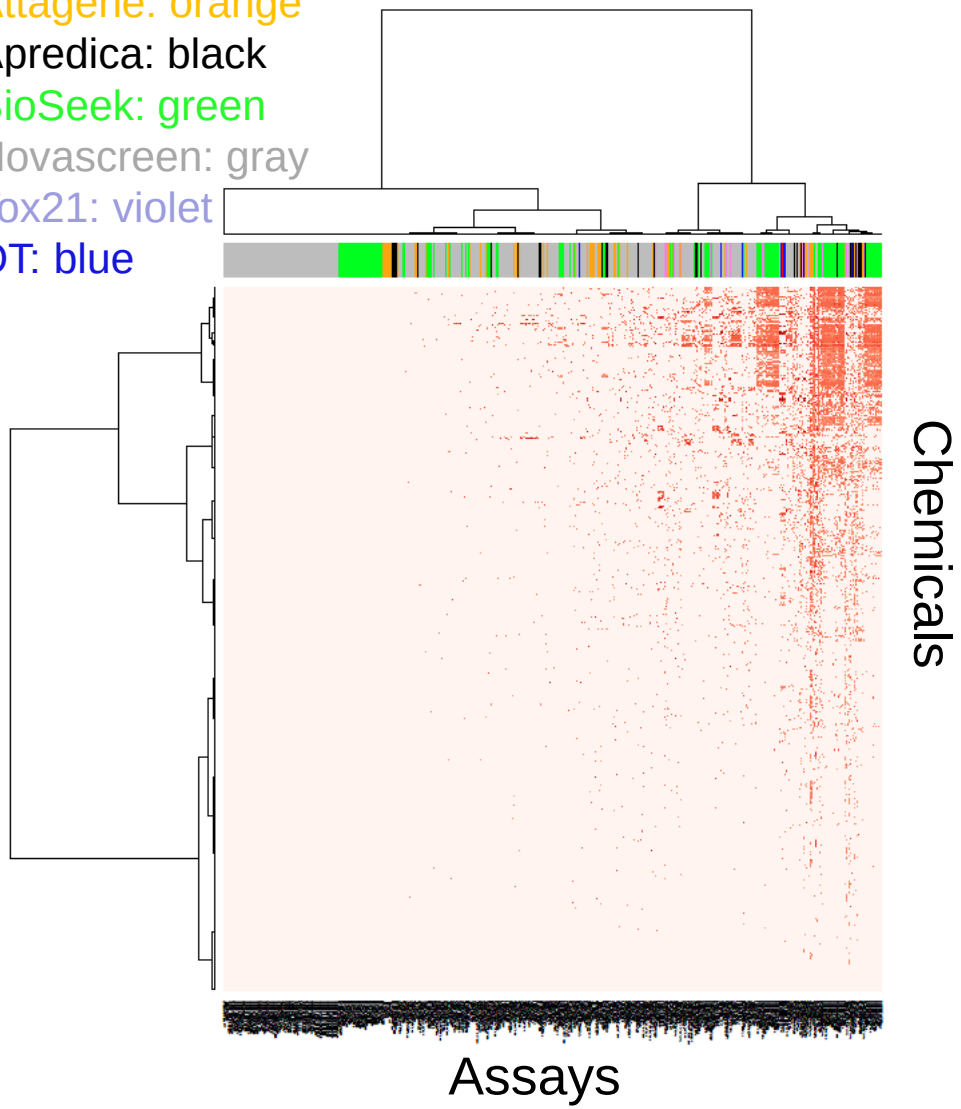
Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

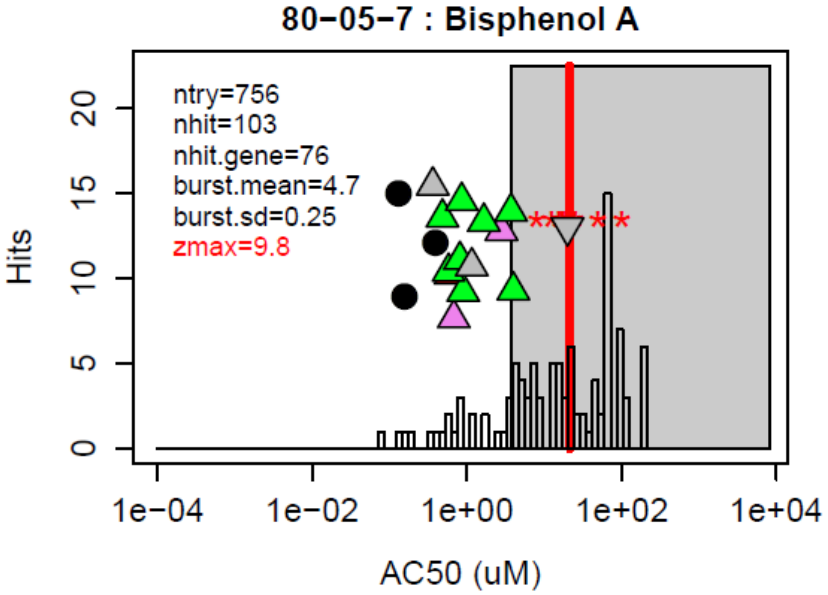
7

List of assays and related information at: **http://www.epa.gov/ncct/**

ToxCast Phase II: 1051 Chemicals x 791 Assay Readouts

ACEA: red
Attagene: orange
Apredica: black
BioSeek: green
Novascreen: gray
Tox21: violet
OT: blue





Most chemicals cause activity in many assays near the cytotoxicity threshold

Cell stress-related assay activity: “Burst”

“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
-Low Z: less likely to be specific

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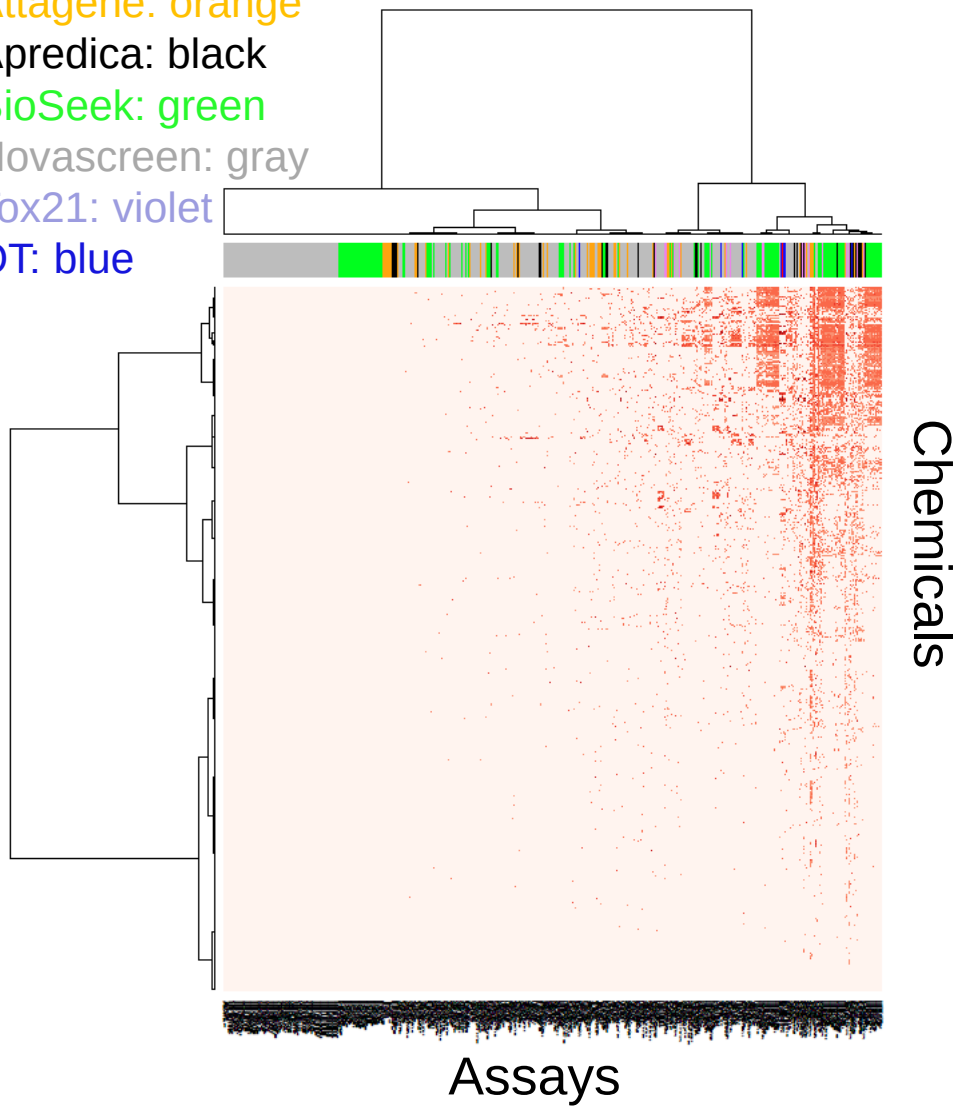

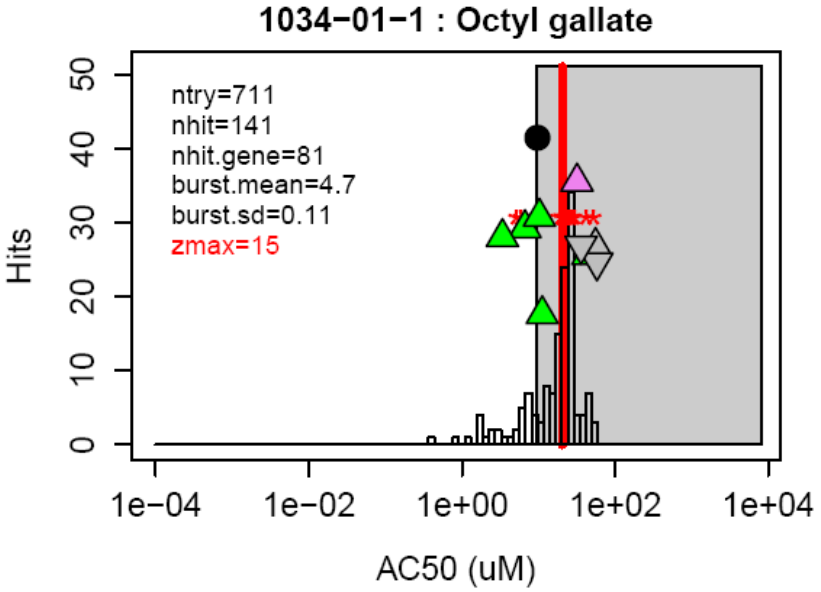

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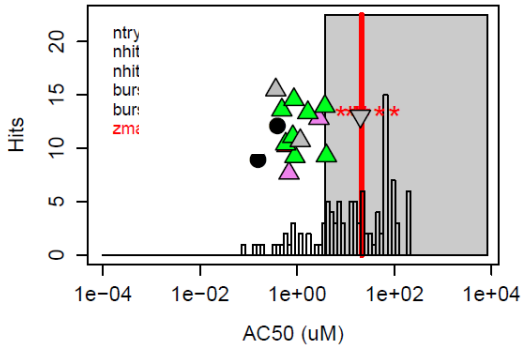
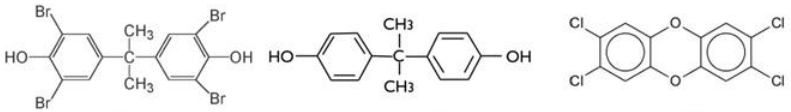
Significance of *In Vitro* Effects





Assay Target Class

Assessment

Molecular Target

EDC
Acetylcholinesterase Inhibition
Ion channel blocker
Genotoxicity

→ AOP Assessment
Targeted testing

Cell Stress Mediated

Oxidative stress
Membrane disruption
Nucleophiles
Electrophiles
Energy depletion

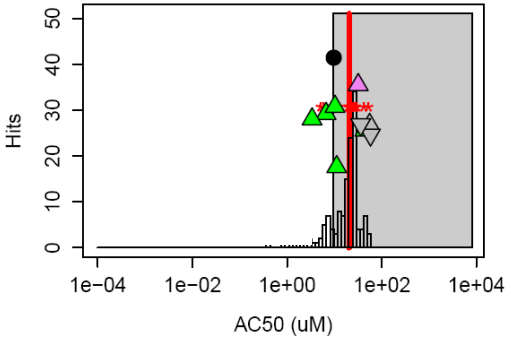
→ Estimate MTD
Estimate NOEL

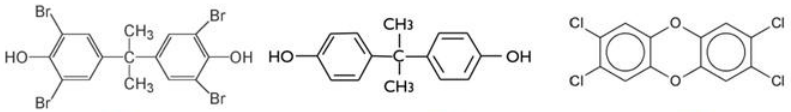
No Effect

Non-reactive chemical
Not bioactive
Effects would require high doses

→ Estimate NOEL

Significance of *In Vitro* Effects



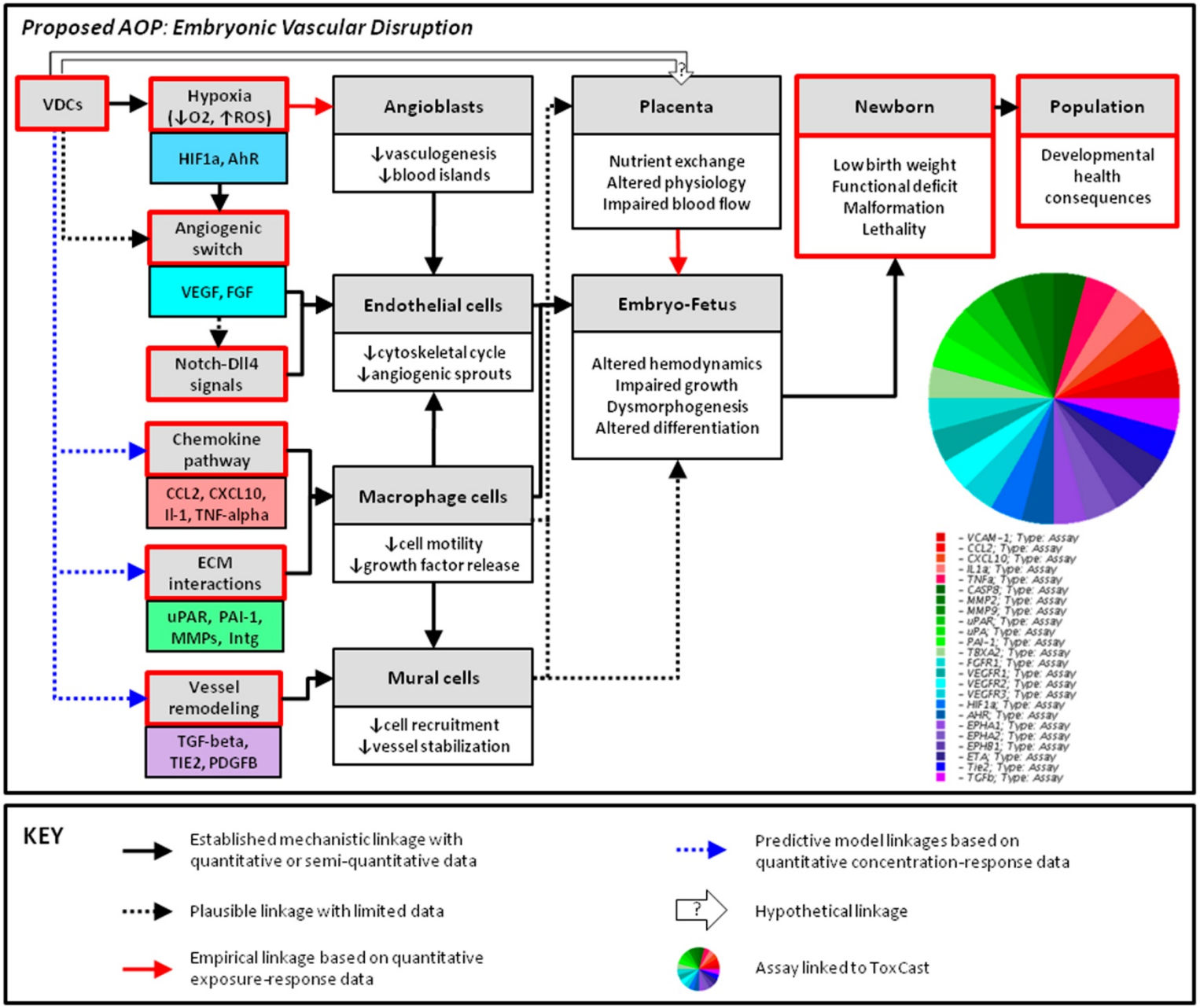


Assay Target Class		Assessment
Molecular Target	EDC Acetylcholinesterase Inhibition Ion channel blocker Genotoxicity	AOP Assessment Targeted testing
Cell Stress Mediated	Oxidative stress Membrane disruption Nucleophiles Electrophiles Energy depletion	Estimate MTD Estimate NOEL
No Effect	Non-reactive chemical Not bioactive Effects would require high doses	Estimate NOEL

How to summarize 1000s of chemicals x 100s of assays?

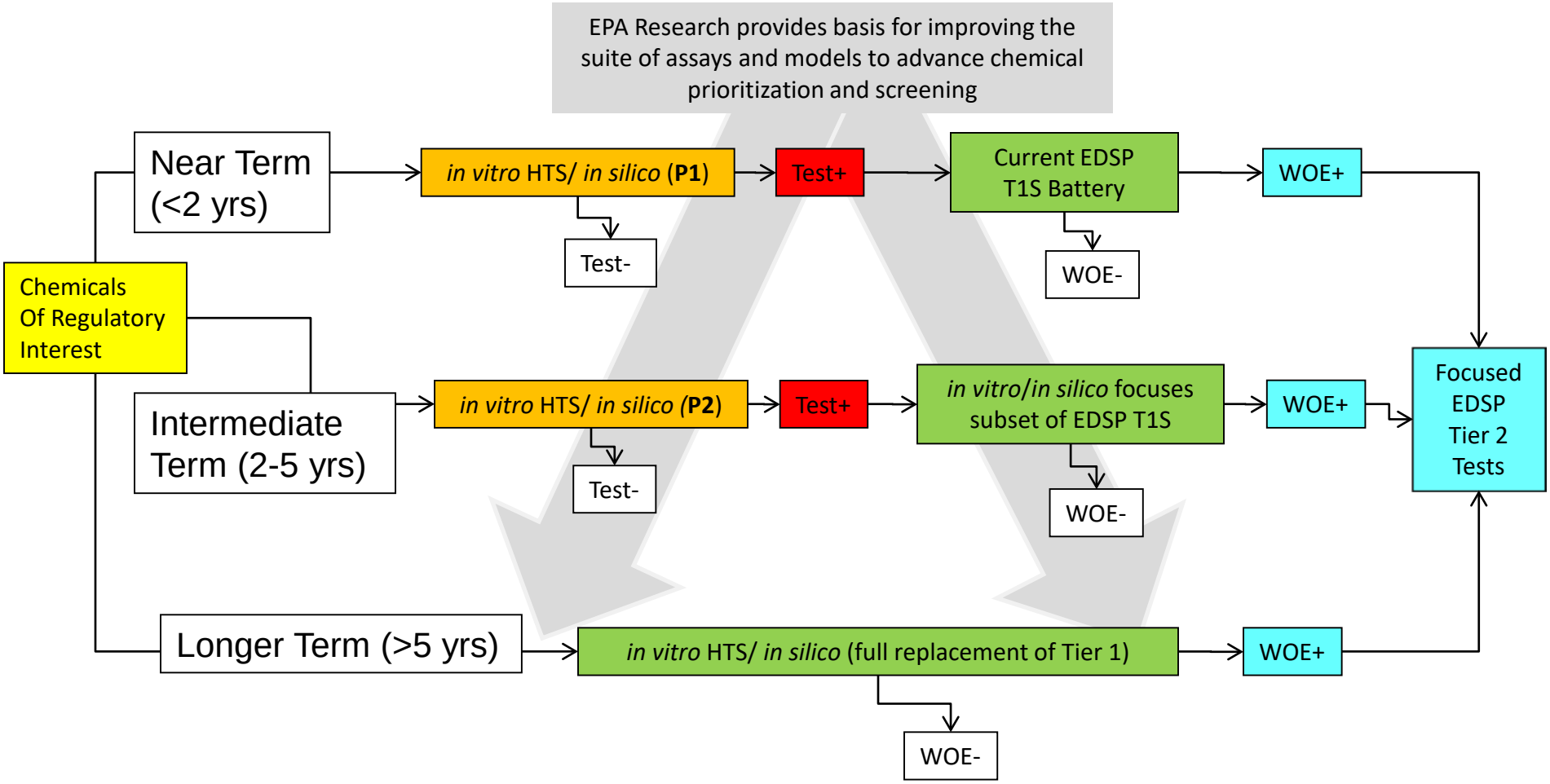
- Gene Score: Combine potency and specificity
  - Potency: -log(AC50)
  - Specificity: Z-score
  - Gene score = Potency + Specificity
    - average over assays for gene [-log(AC50) + Z-score]
  - Can be used to get quick ranking of chemicals
  - Gene Score > 7 are most interesting
    - Z-score=2 and AC50=10 μM
    - 5670 chemical-gene combinations >7 (~1%)
    - 281 Genes (out of 330)
    - 1231 Chemicals (out of 1877)

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



Knudsen and Kleinstreuer. Birth Def Res C. 2012

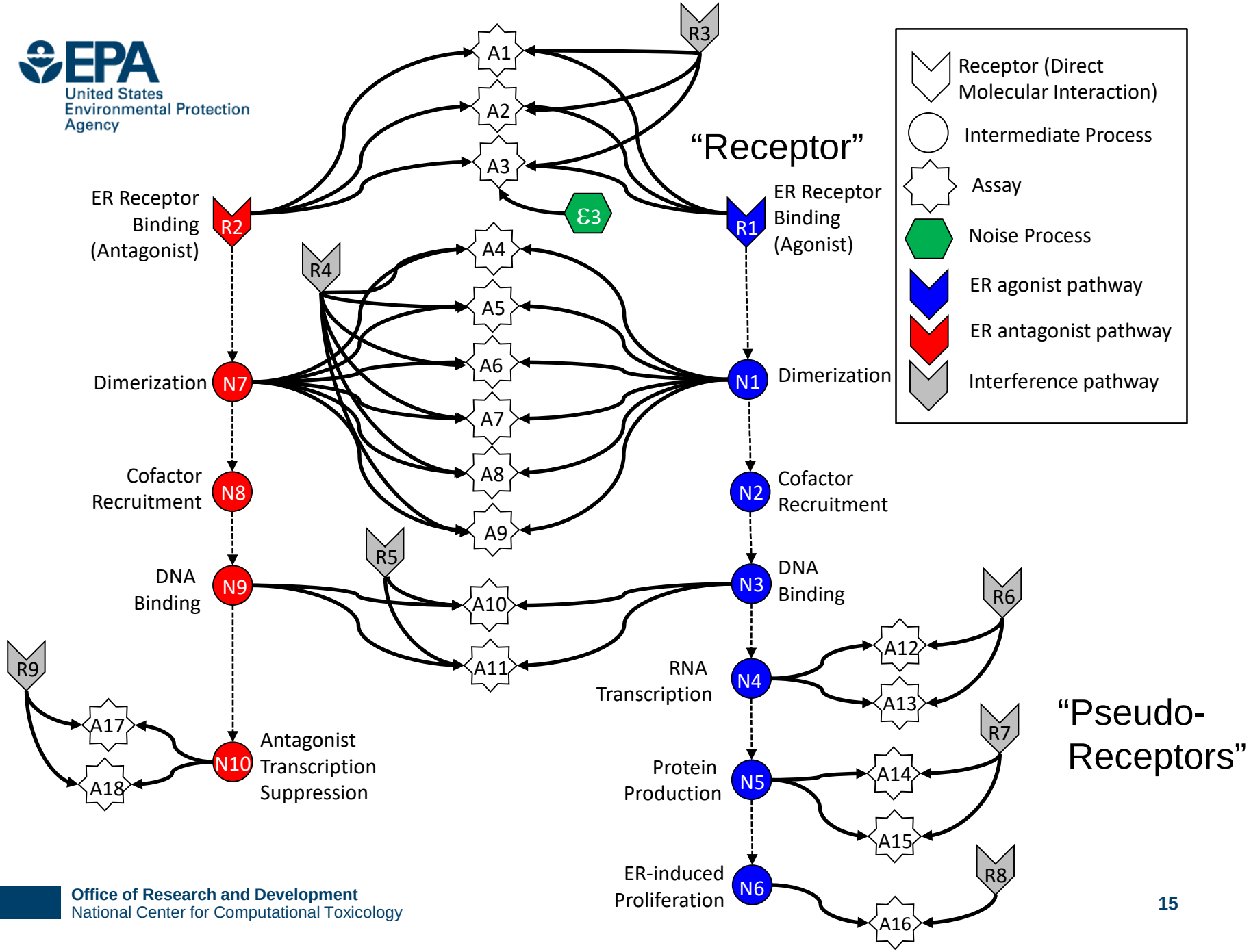
ToxCast and the Endocrine Disruptor Screening Program



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

http://www.epa.gov/endo/pubs/edsp21_work_plan_summary%20_overview_final.pdf


United States Environmental Protection Agency
"Receptor"
"Pseudo-Receptors"
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
Noise Process
ER agonist pathway
ER antagonist pathway
Interference pathway
ER Receptor Binding (Antagonist)
ER Receptor Binding (Agonist)
Dimerization
Cofactor Recruitment
DNA Binding
RNA Transcription
Protein Production
ER-induced Proliferation
Antagonist Transcription Suppression
R1
R2
R3
R4
R5
R6
R7
R8
R9
N1
N2
N3
N4
N5
N6
N7
N8
N9
N10
A1
A2
A3
ε3
A4
A5
A6
A7
A8
A9
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A11
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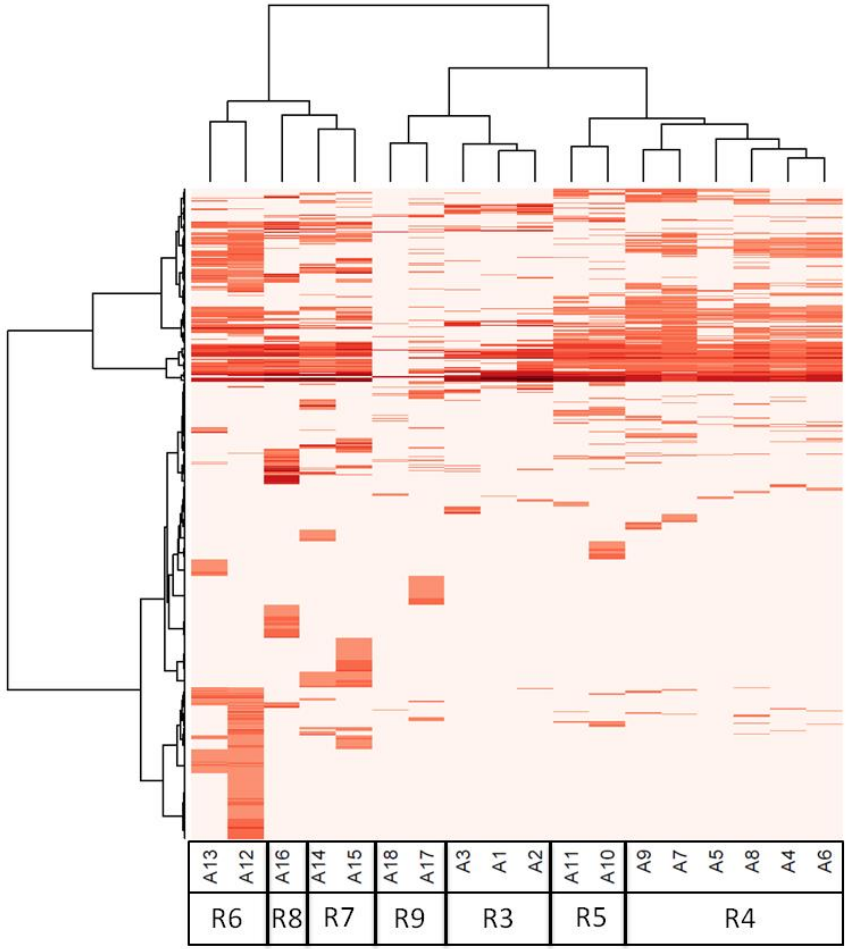
A14
A15
A16
A17
A18

Major theme – all assays have false positives and negative

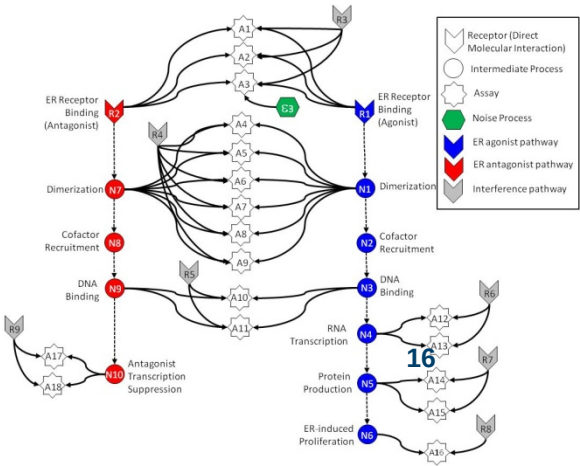
Assays cluster by technology, suggesting technology-specific non-ER activity



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

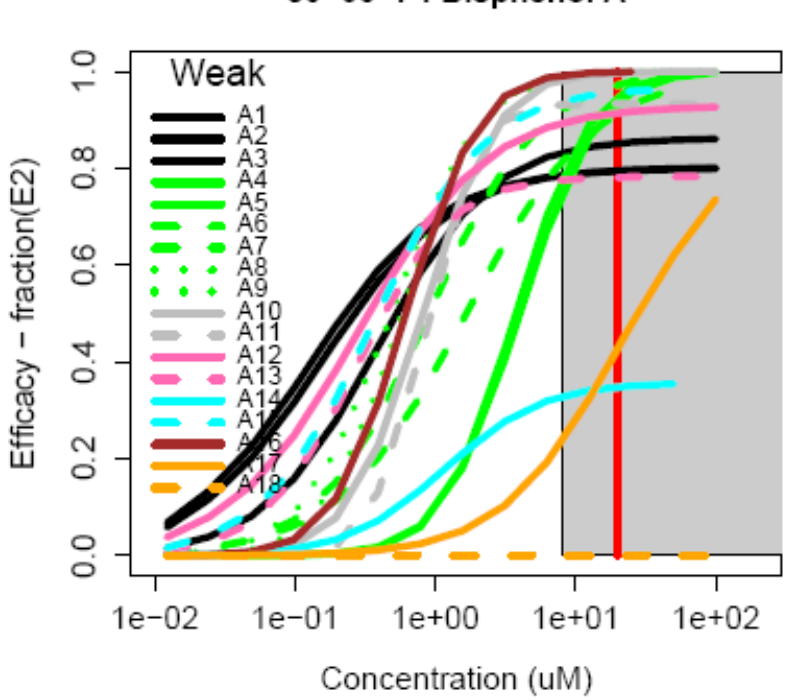
Result of interaction of chemical with complex biology in the assay

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



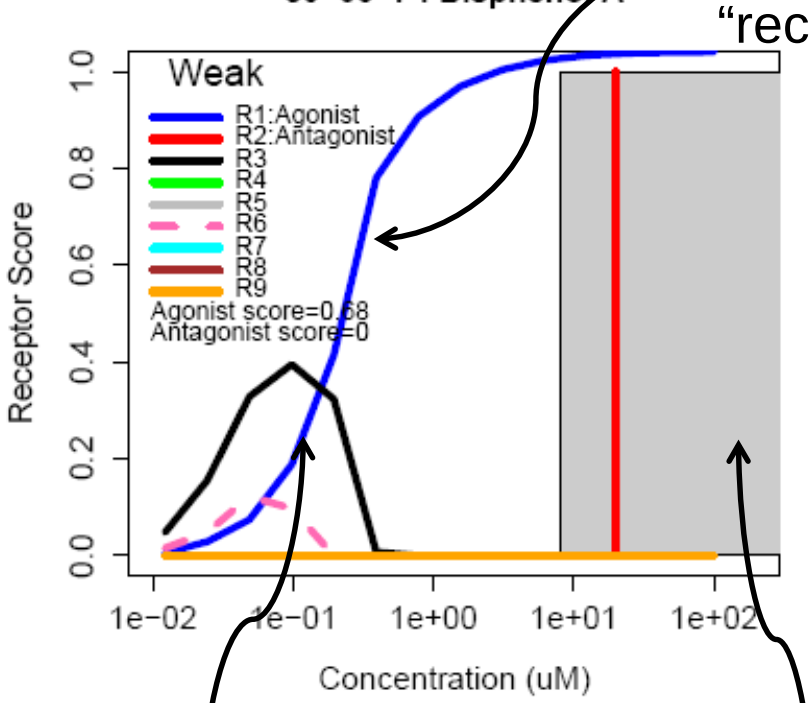
Example 1 – BPA – true agonist (AUC=0.66)

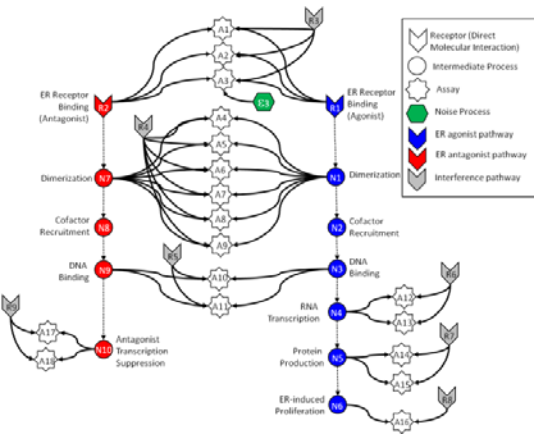
Assays



"Receptors"

Blue: agonist "receptor"





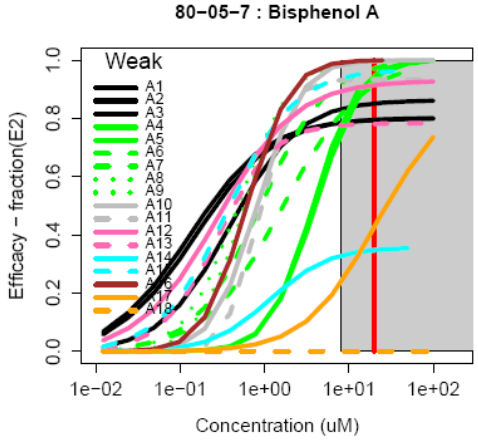
Binding assays active at lowest concentration

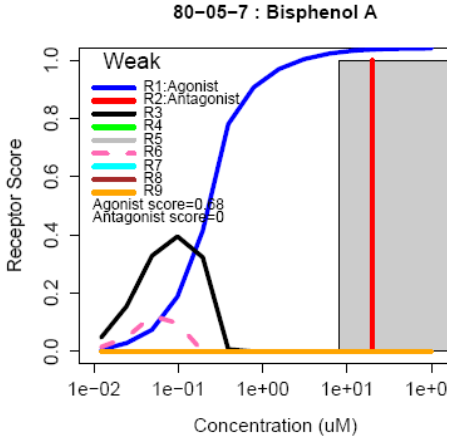
AUC "sign" feature will discount this

Cytotoxicity Region: red line is median cytotox AC50

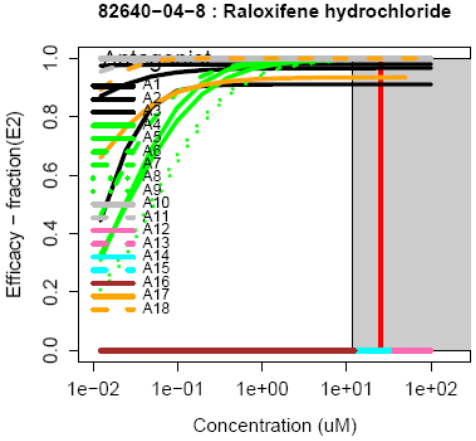
Example curves

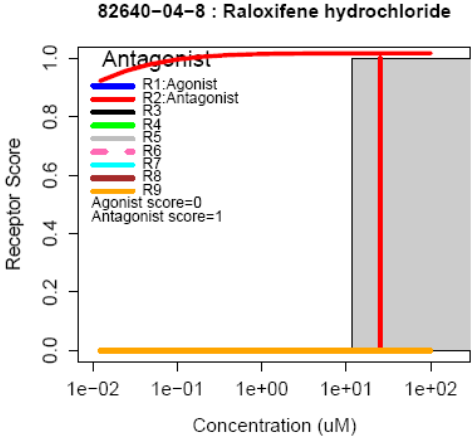
True Agonist



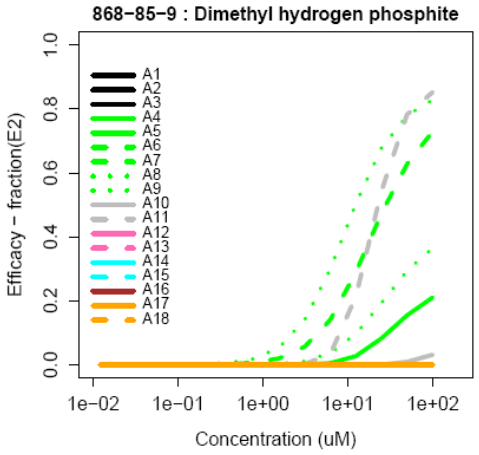


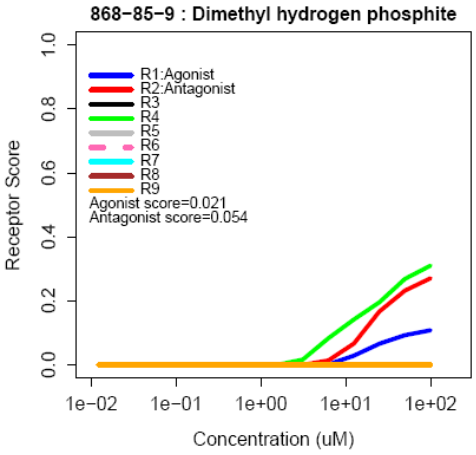
True Antagonist



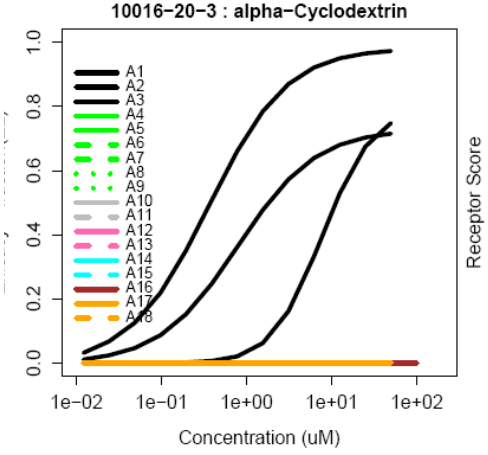


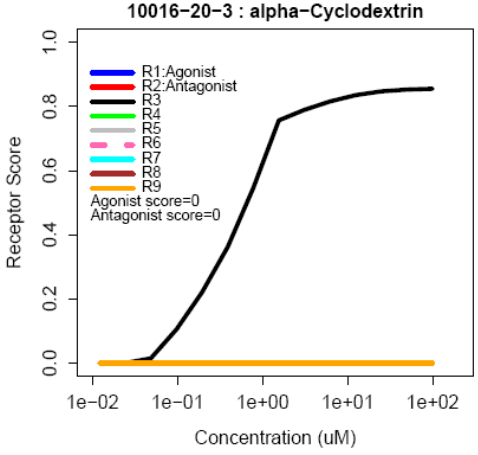
Negative-Broad Assay Interference



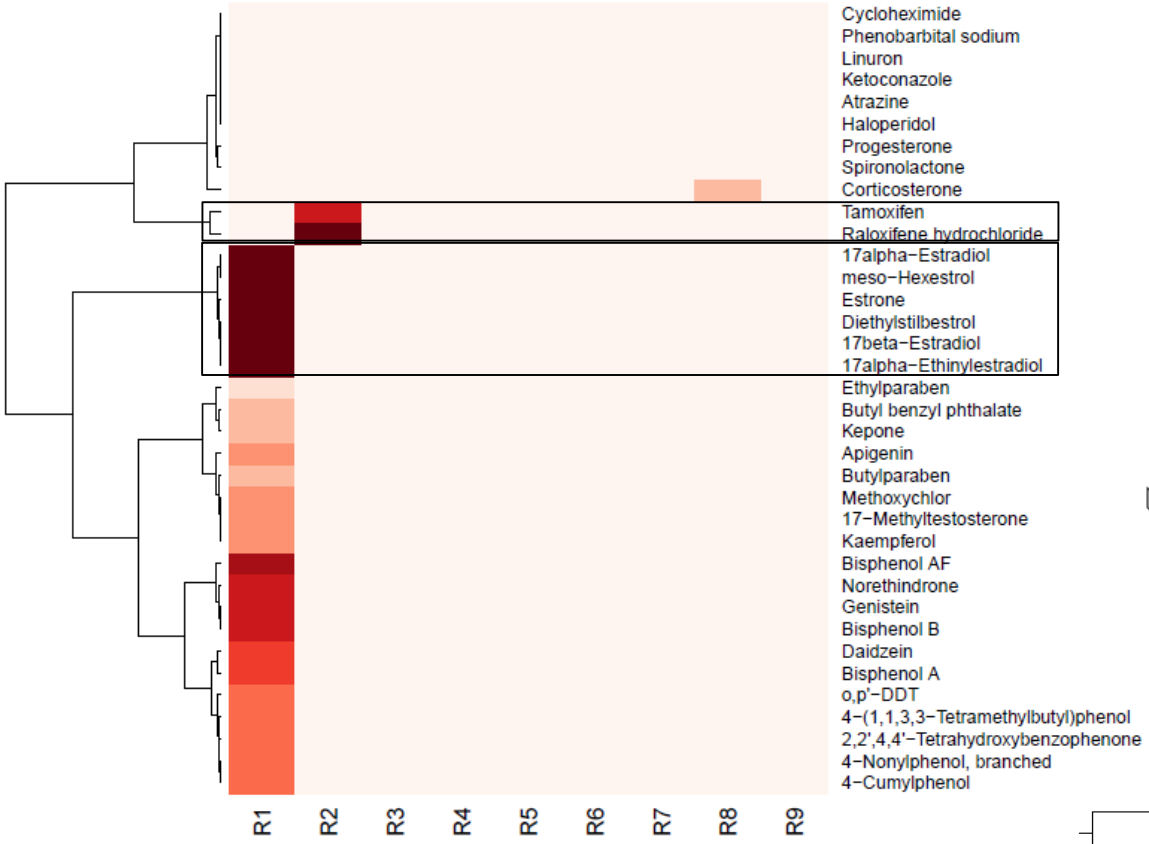


Negative-Narrow Assay Interference

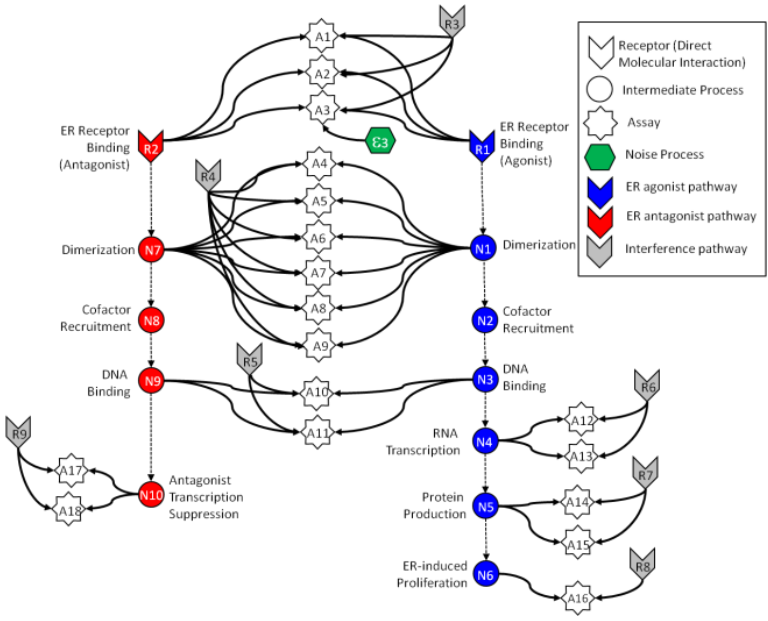


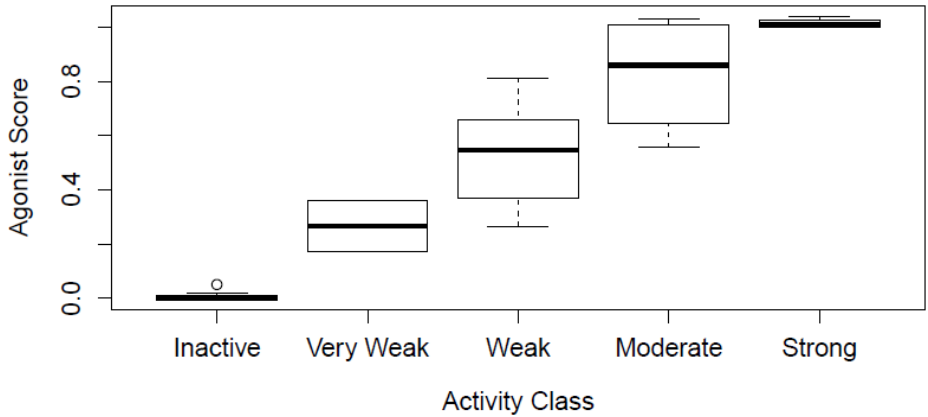


Reference Chemical Classification



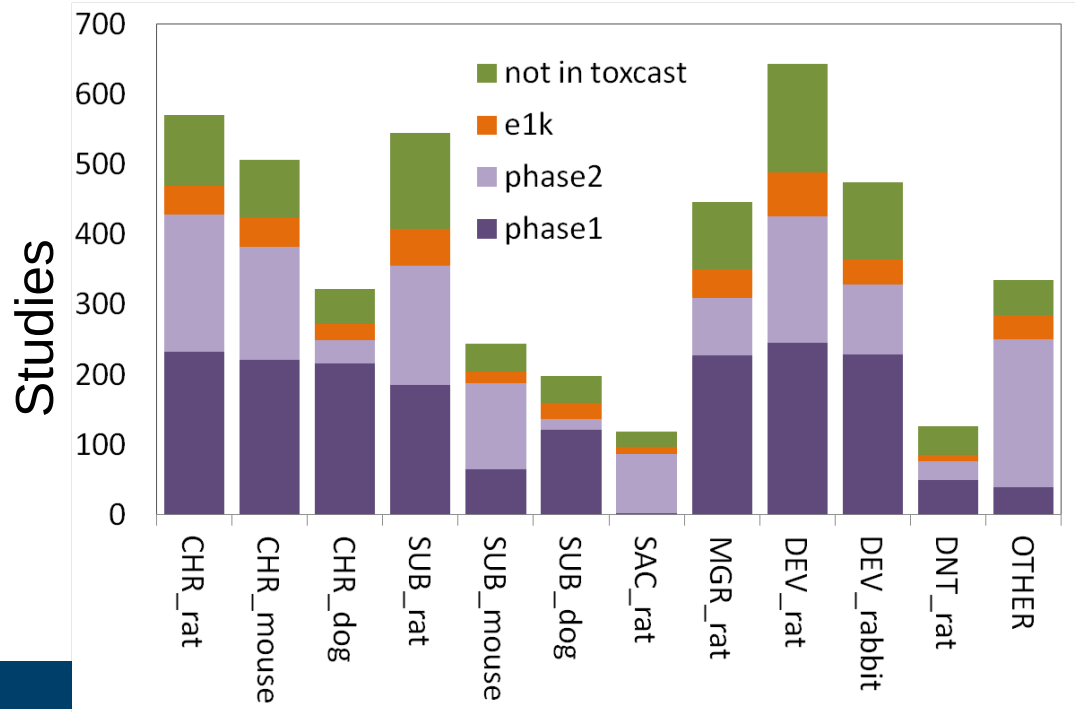
AUC heat map for Reference chemicals





Toxicity Reference Database (ToxRefDB)

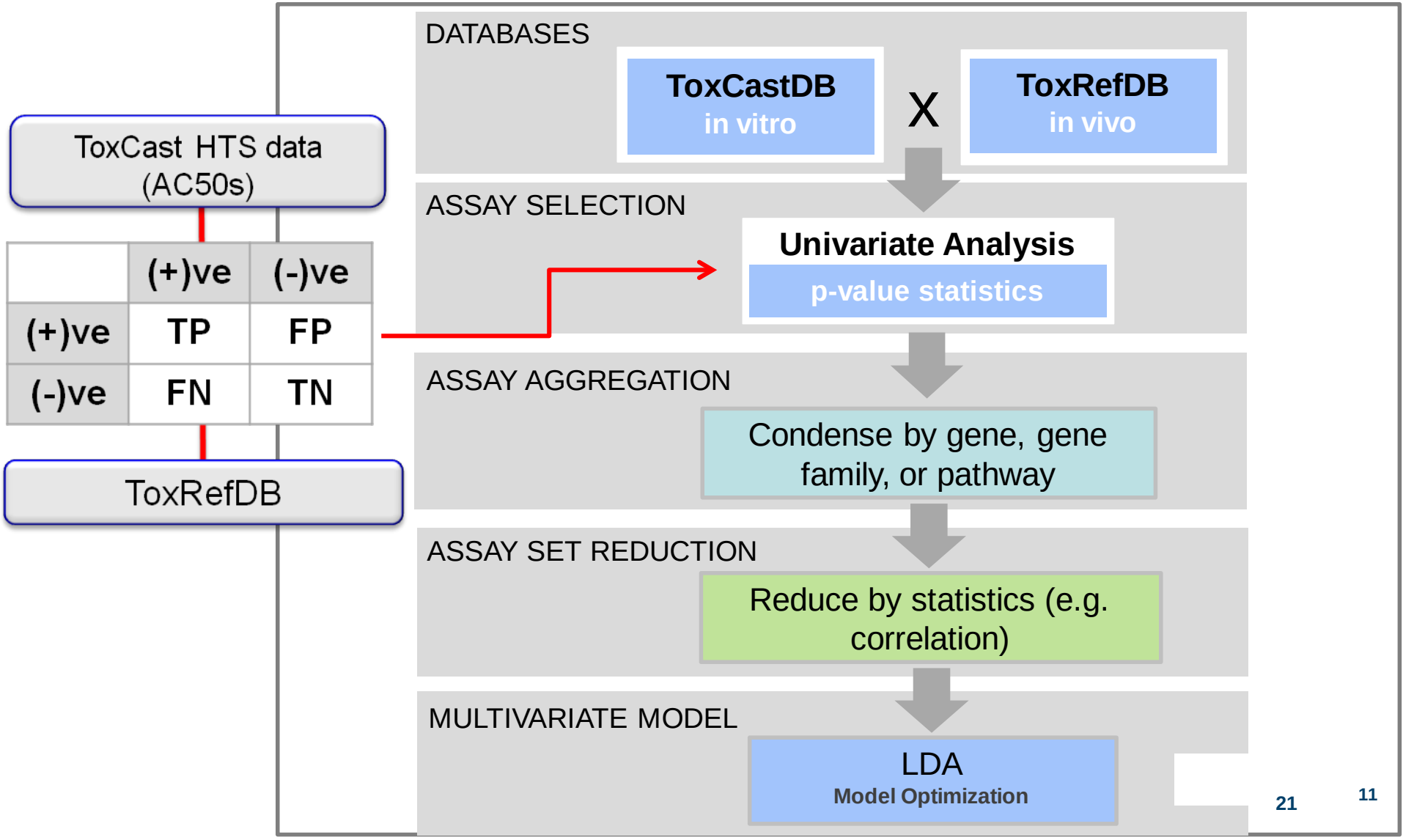
- Holds *in vivo* endpoint data from animal toxicology studies
- Currently at 5567 studies on 1049 unique chemicals



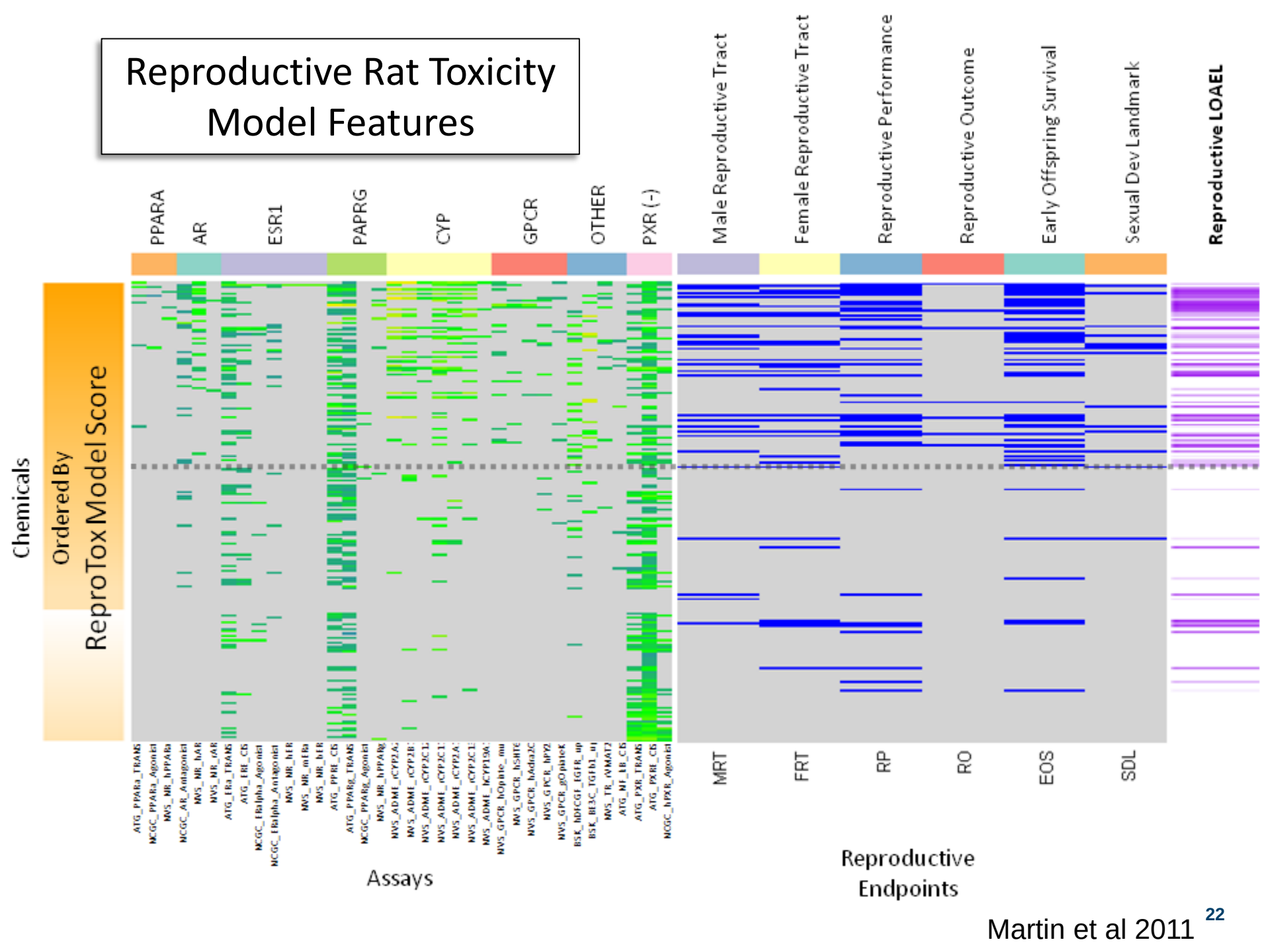
Data Source	Study Count
EPA OPP_der	3279
Open Literature	731
National Toxicol Program	666
Sanofi_pharma	222
Unpublished_submissions	50
GSK_pharma	38
Health Canada PMRA_der	23

http://actor.epa.gov/toxrefdb/

Predictive Model Development from ToxCast and Other Data

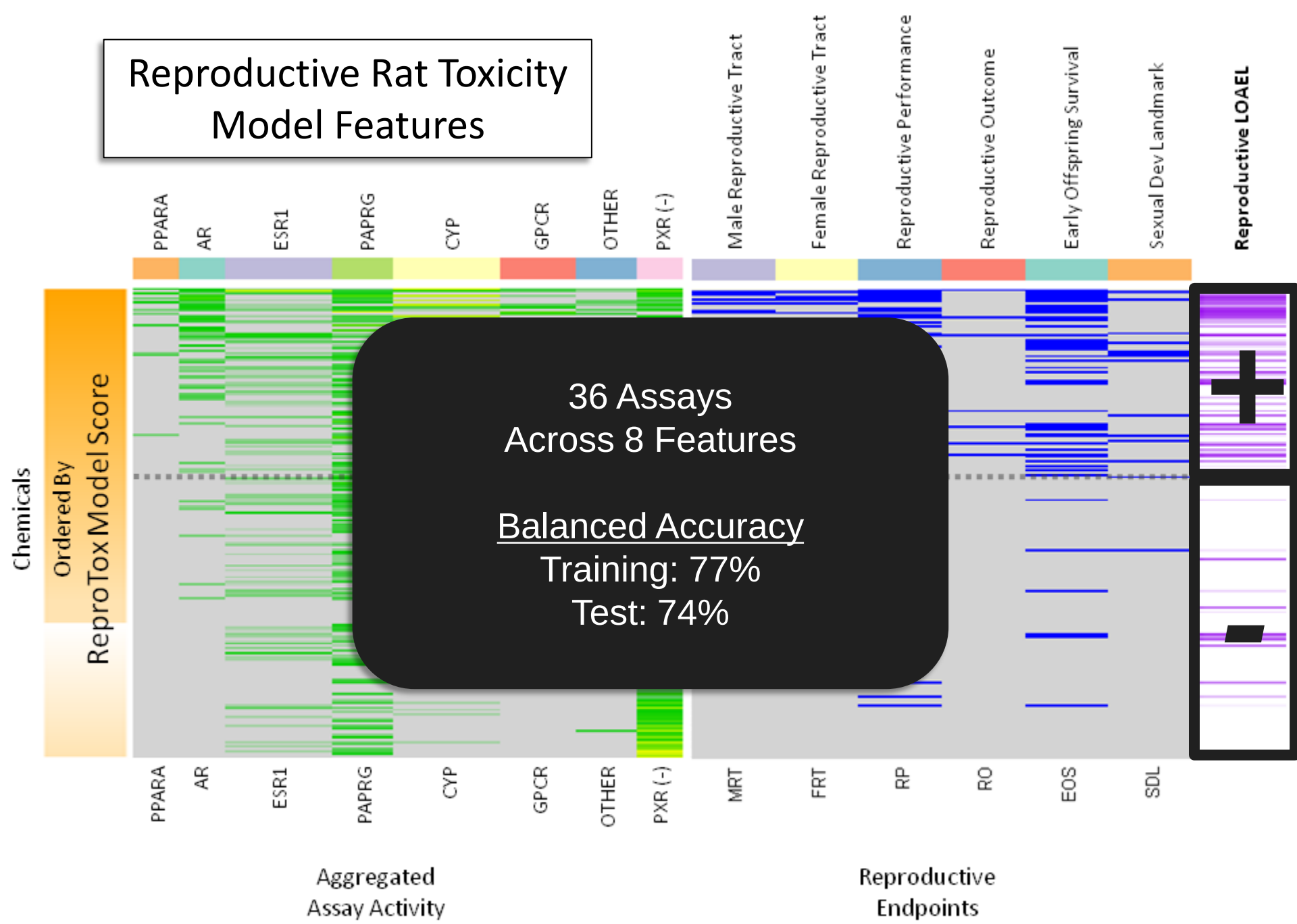


Reproductive Rat Toxicity Model Features



Martin et al 2011

Reproductive Rat Toxicity Model Features



36 Assays
Across 8 Features

Balanced Accuracy
Training: 77%
Test: 74%

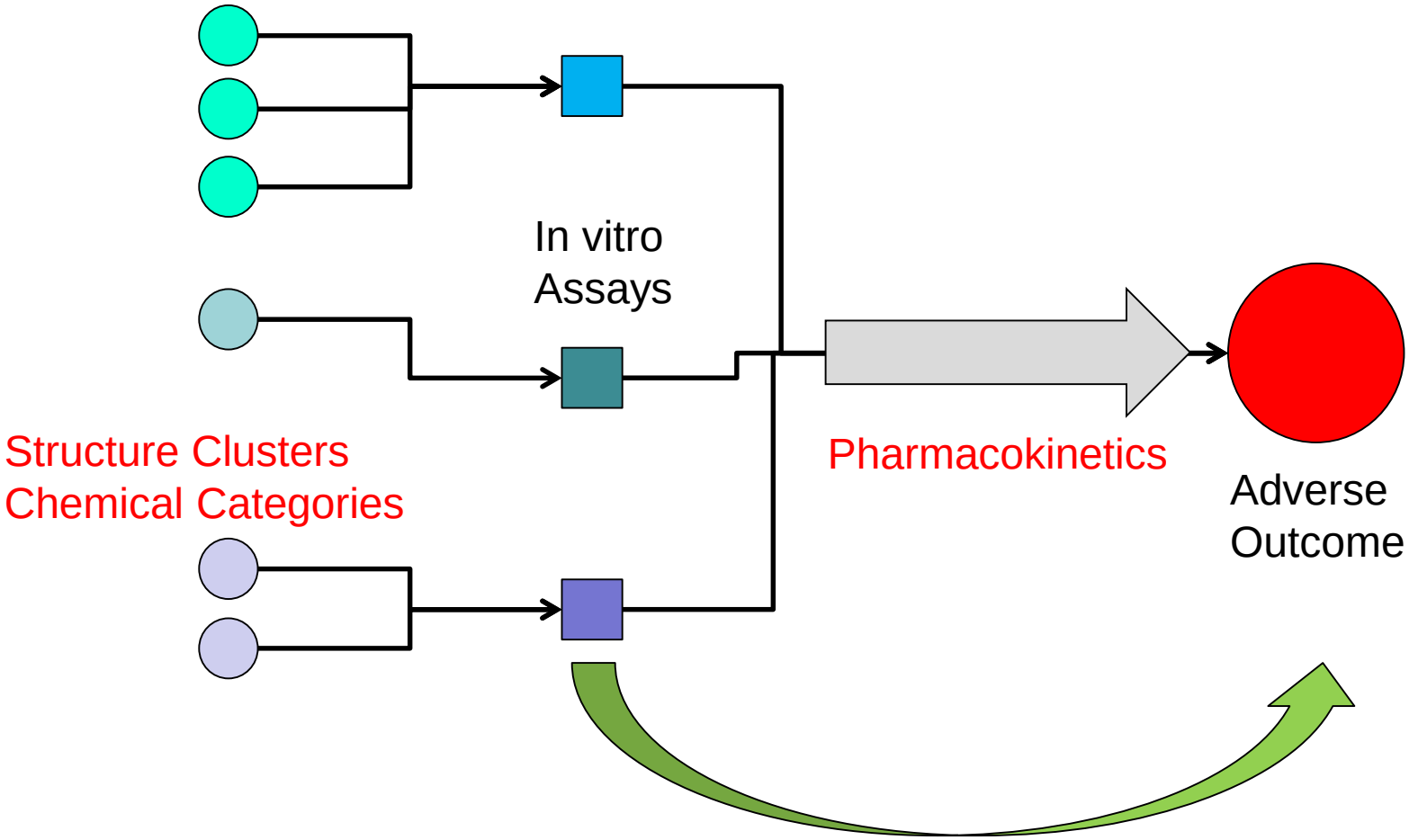
Martin et al 2011

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**

Beyond *in vitro* to *in vivo* signatures



Why is Chemistry needed?

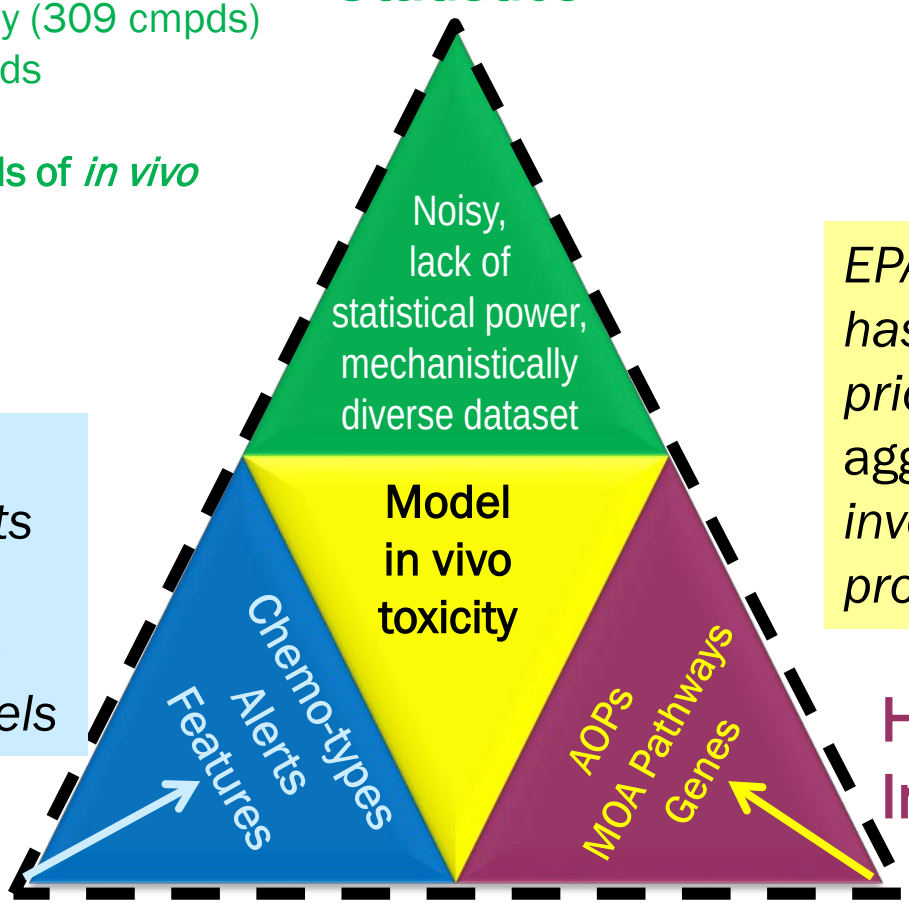
Thomas et al, 2012, Tox Sci

- ToxCast Phase I library (309 cmpds)
- >80 statistical methods
- ToxRef DB endpoints

→ No successful models of *in vivo* endpoints

Prior knowledge of chemical determinants of reactivity & toxicity are needed to build & refine predictive models

Chemistry



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

HTS (>500 assays)
In vitro Biology

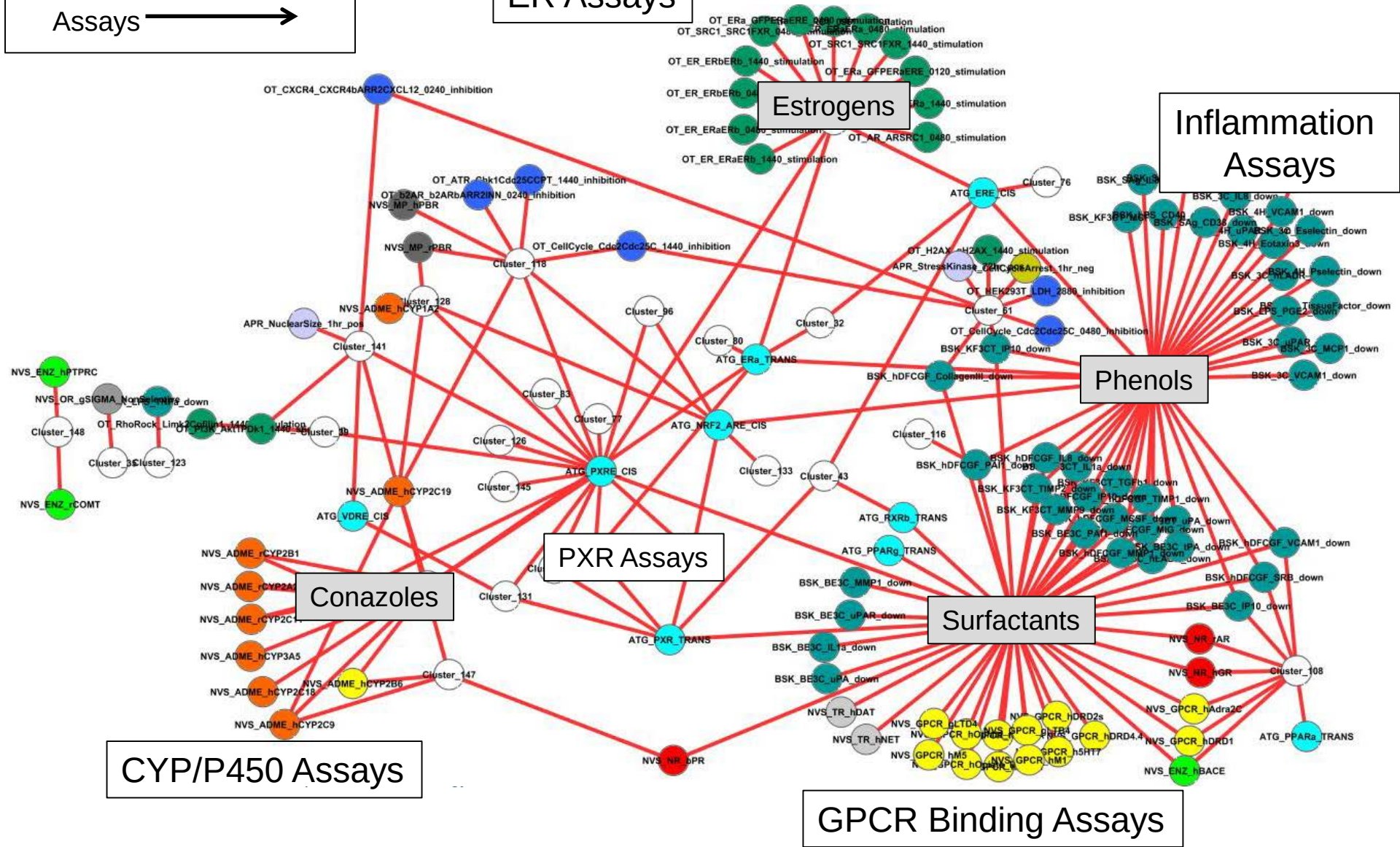
Data Set Incomplete

Chemical Set 1

Chemical Set 2

Assays →

Clusters 80% predictive of assay hit



Toxicity Prediction Challenge:

Bringing all knowledge & data to bear on problem

Data-mining

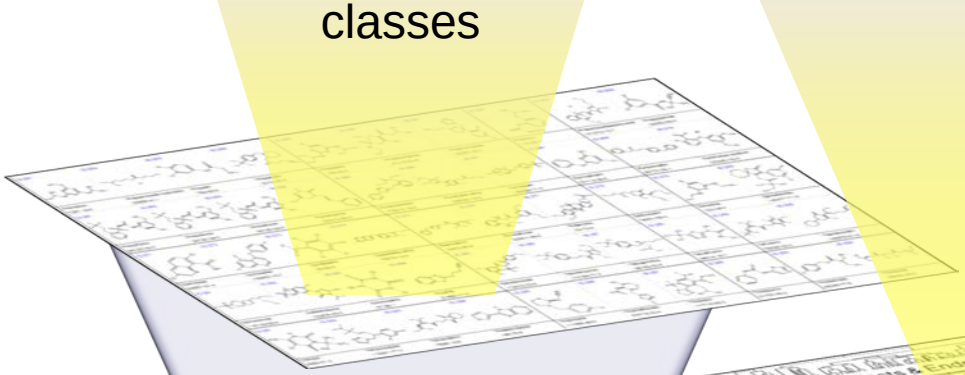
Biologically-based QSAR & Cheminformatics

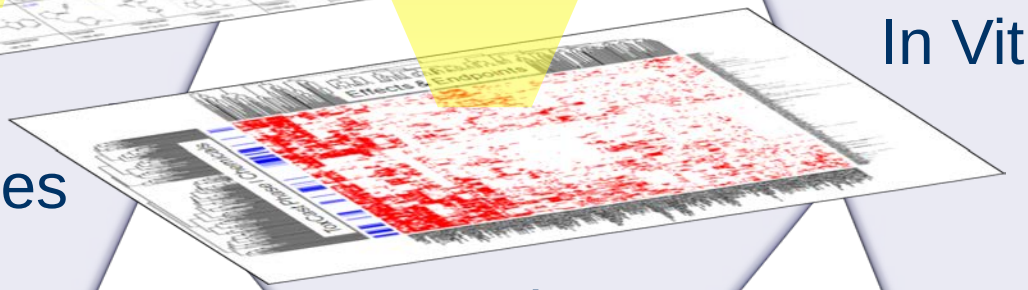
Aggregation

Adverse Outcomes:
> Pathways
> Genes
> Assays
+ Statistical associations

Reactivity & toxicity-informed features & classes

Mechanistically well-defined toxicity endpoint





Structures

In Vitro/HTS

In Vivo

Existing knowledge

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: December 2013

- 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

EPA United States Environmental Agency

EPA iCSS

TOXCAST HOME | DATA SELECTION | CHEMICAL SELECTION | DATA EXPLORER | CHEMICAL EXPLORER | PRIORITIZATION COMING SOON!

DATA EXPLORER

Assay List

Data Class	Assay Endpoint
ATG	ATG_ERa_TRANS
ATG	ATG_ERE_CIS
ATG	ATG_PPARg_TRANS
ATG	ATG_PPRE_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
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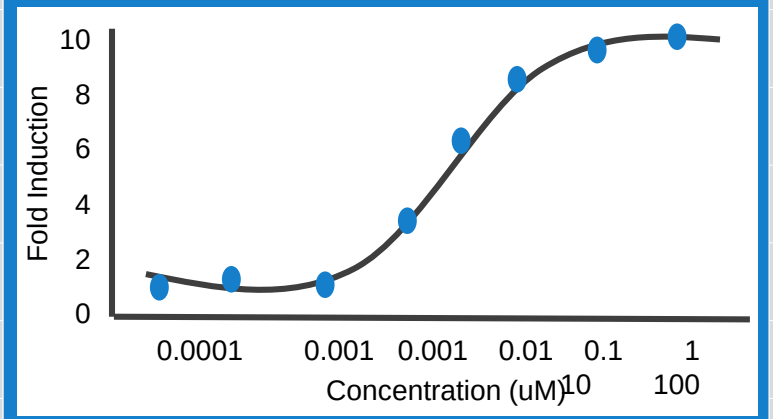
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS
ATG	ATG_ERa_TRANS

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						
80-05-3	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
February 5, 2013