EPA
United States
Environmental Protection
Agency

High-Throughput Screening in ToxCast/Tox21

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Office of Research and Development

FutureTox II
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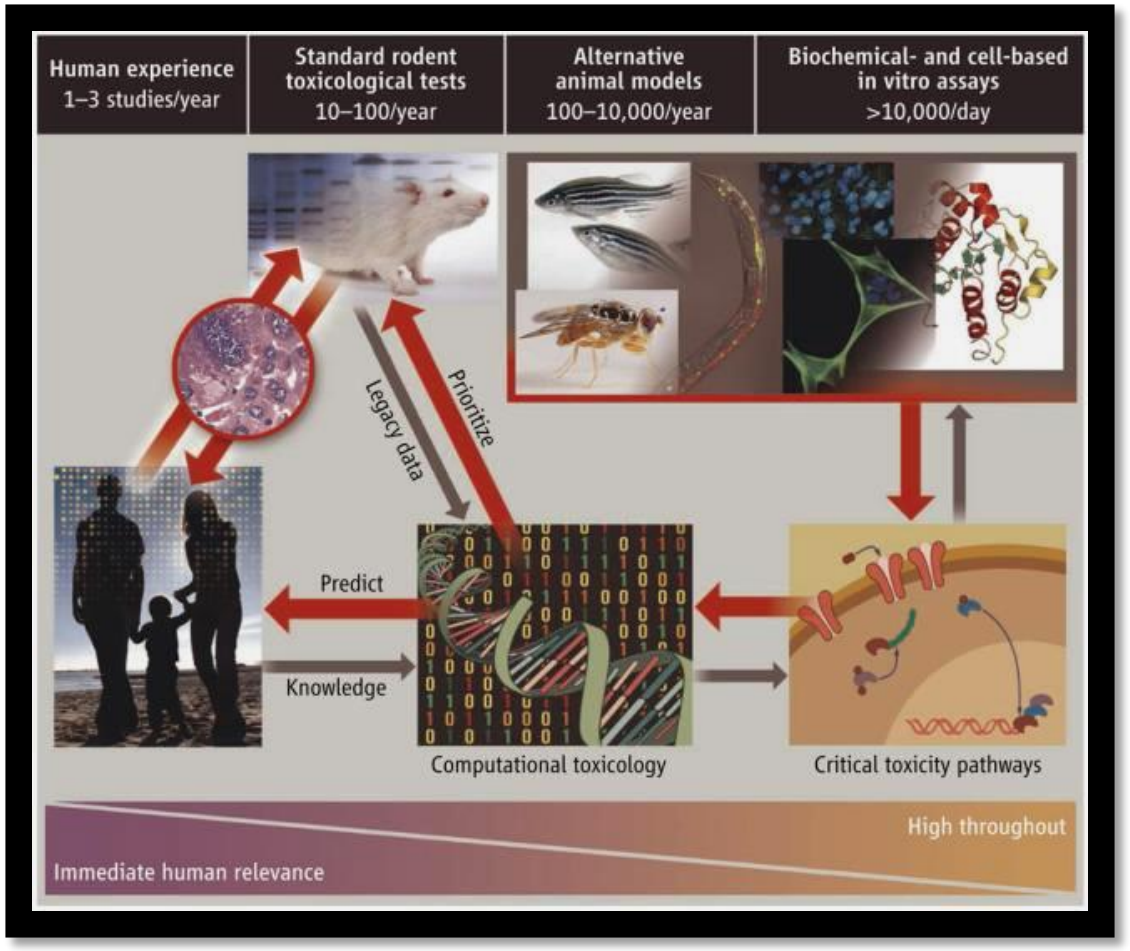
EPA United States Environmental Protection Agency

Outline

- Need for alternative toxicity testing
- Chemical libraries tested
- Biological assays for chemical profiling
- Tox21 Assay Example
- ToxCast Assay Platform Example
- Use of Data in Predictive Modeling
- Summary of Advantages/Challenges

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

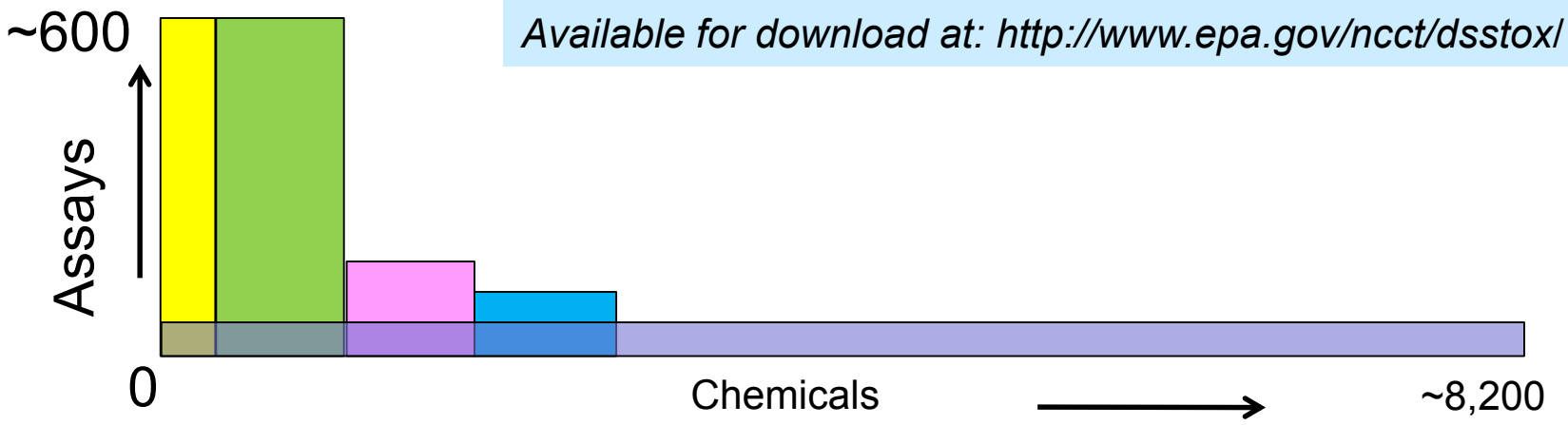
ToxCast /Tox21 Overall Strategy

- Identify targets or pathways linked to toxicity (AOP focus)
- Identify/develop high-throughput assays for these targets or pathways
- Develop predictive systems models: *in silico/in vitro → in vivo*
- 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 III	1001	~100	~100	Just starting	2014
E1K (endocrine)	880	~50	~120	03/2013	12/2013
Tox21	8,193	~25	~50	Ongoing	Ongoing

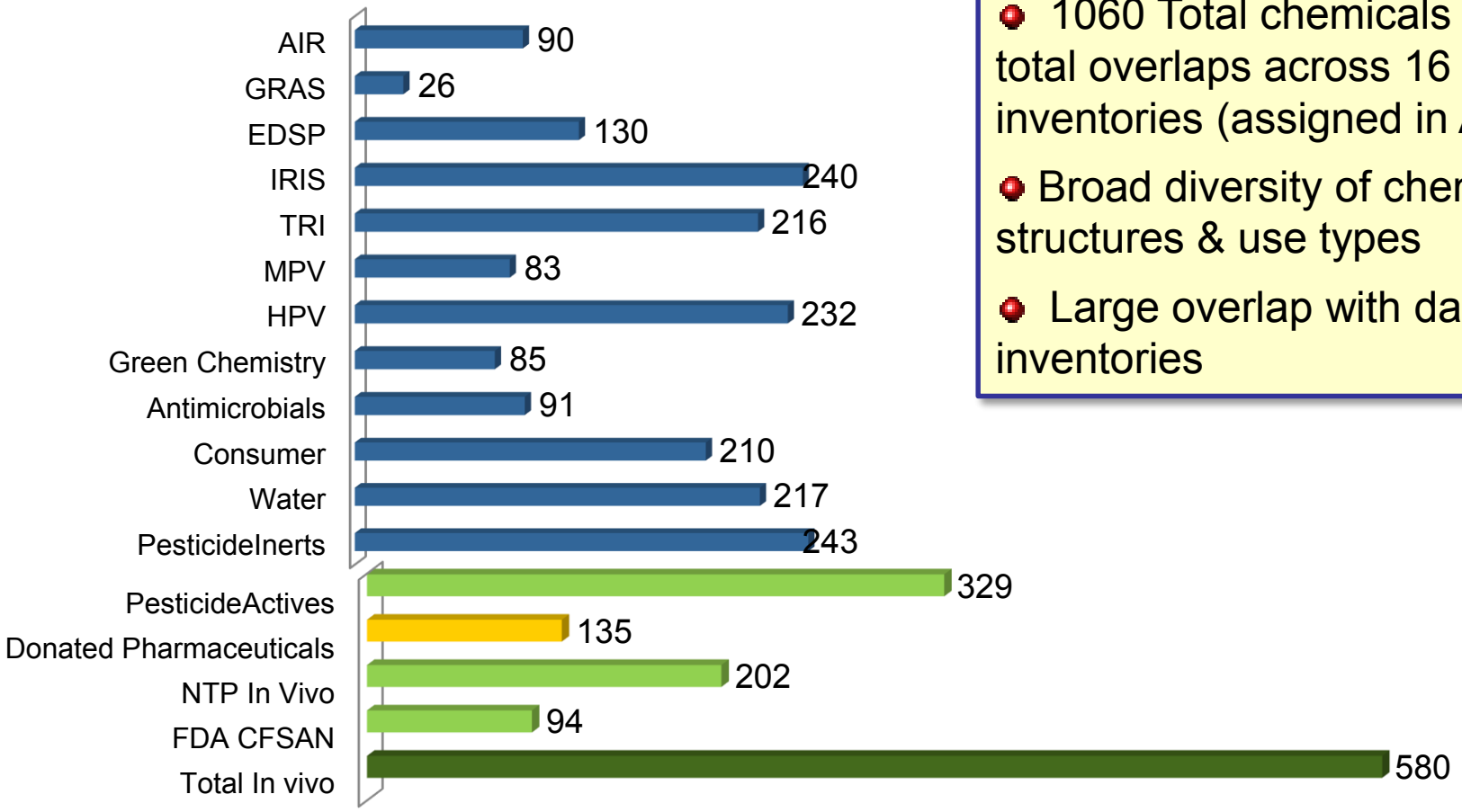


Available for download at: http://www.epa.gov/ncct/dsstox/

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)

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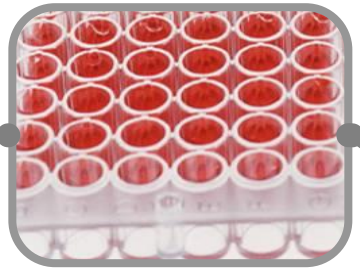
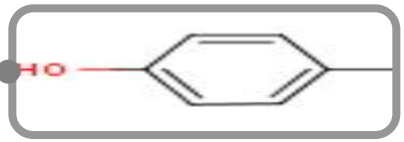
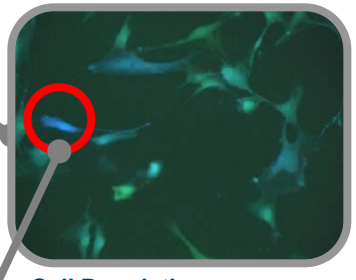
Robots



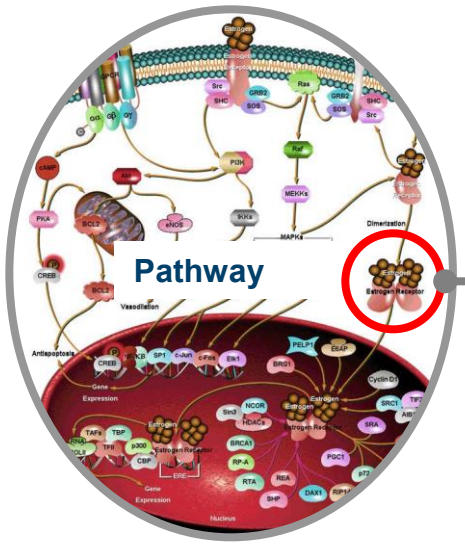
96-, 384-, 1536 Well Plates

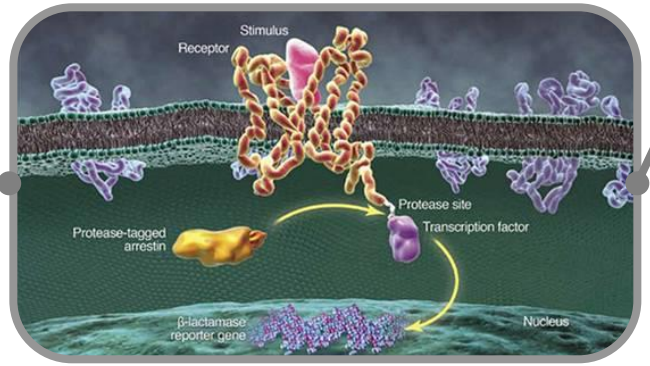


Chemical Exposure

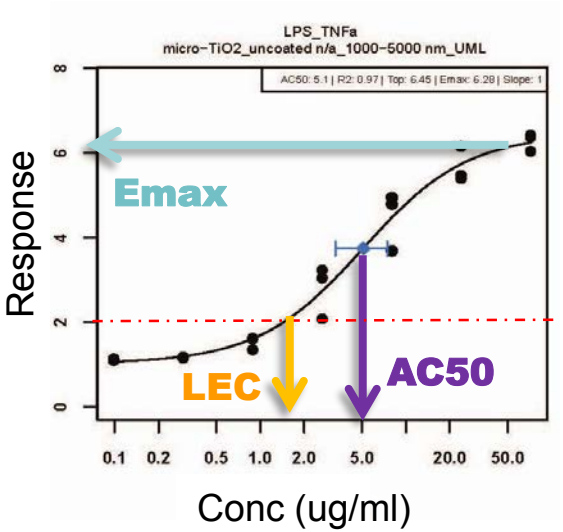


Cell Population

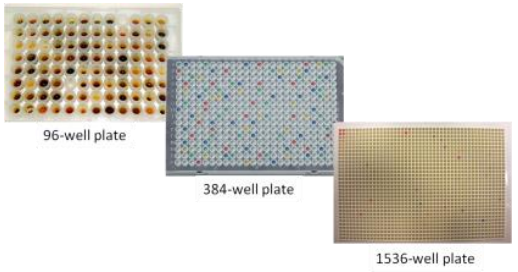




Target Biology (e.g., Estrogen Receptor)



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

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

ToxCast Phase II: 1051 Chemicals x 791 Assay Readouts

United States Environmental Protection Agency

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

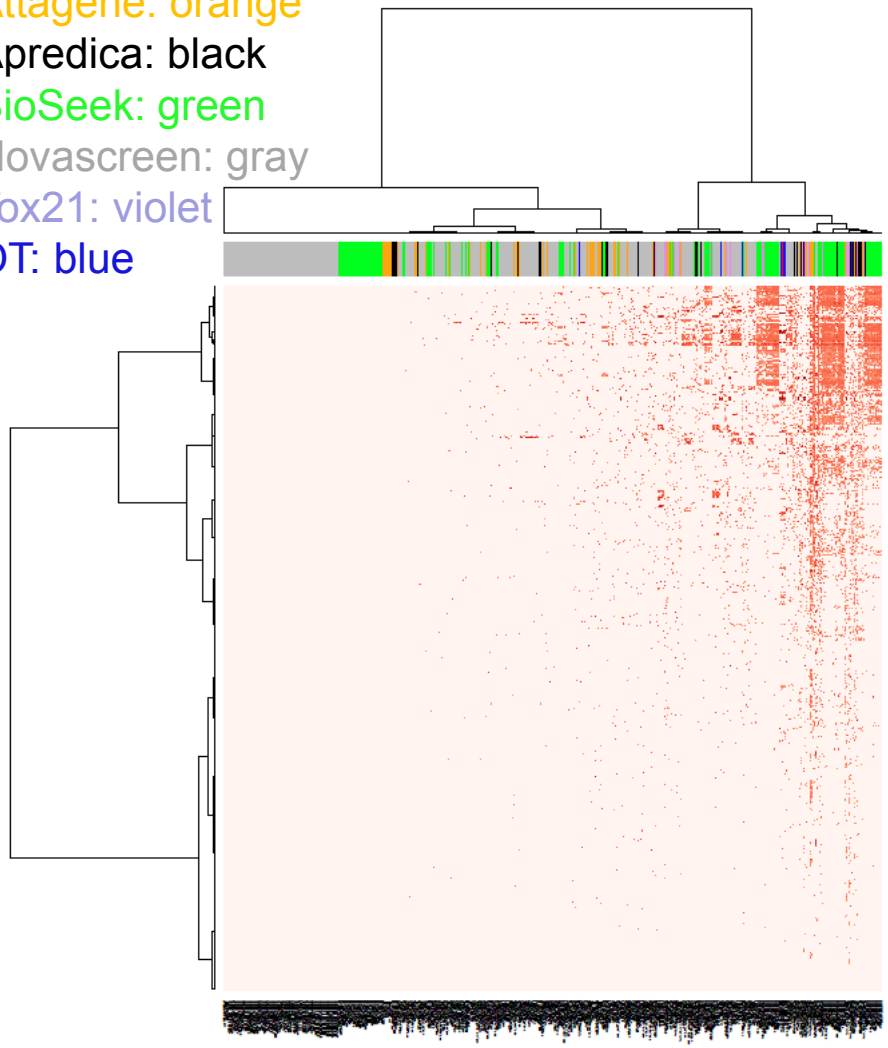


Table 2 Top 20 most promiscuous chemicals[a]

Chemical Name	Total	AC50s <=10µM	AC50s <=1µM
Phenylmercuric acetate	90	47	20
Mancozeb	88	41	13
Gentian violet	86	51	5
Sodium dodecylbenzenesulfonate	82	19	0
Tributyltin methacrylate	79	48	12
Tributyltin chloride	77	45	9
Mercuric chloride	73	45	14
Perfluorooctane sulfonic acid	72	13	2
{4-[3-(aminomethyl)phenyl]piperidin-1-yl}{5-[(2-fluorophenyl)ethynyl]furan-2-yl}methanone (pharma)	71	25	4
Dodecylbenzene sulfonate triethanolamine (1:1)	66	7	1
SSR241586 (pharma)	66	30	8
Emamectin benzoate	65	14	2
{4-[5-(aminomethyl)-2-fluorophenyl]piperidin-1-yl}(4-bromo-3-methyl-5-propoxythiophen-2-yl)methanone hydrochloride (pharma)	64	19	2
(1R)-1-[(ethoxycarbonyl)oxy]ethyl 1-{[5-(5-chlorothiophen-2-yl)-1,2-oxazol-3-yl]methyl}-2-{[1-(propan-2-yl)piperidin-4-yl]carbamoyl}-1H-indole-5-carboxylate hydrochloride(pharma)	63	29	2
Maneb	62	31	16
SSR150106 (pharma)	62	41	13
Didecyl dimethyl ammonium chloride	62	30	2
Zamifenacin (pharma)	60	27	11
SSR125047 (pharma)	59	16	3
Metiram	56	16	4

Sipes et al., Chem Res Toxicol. 26:878-95, 2013

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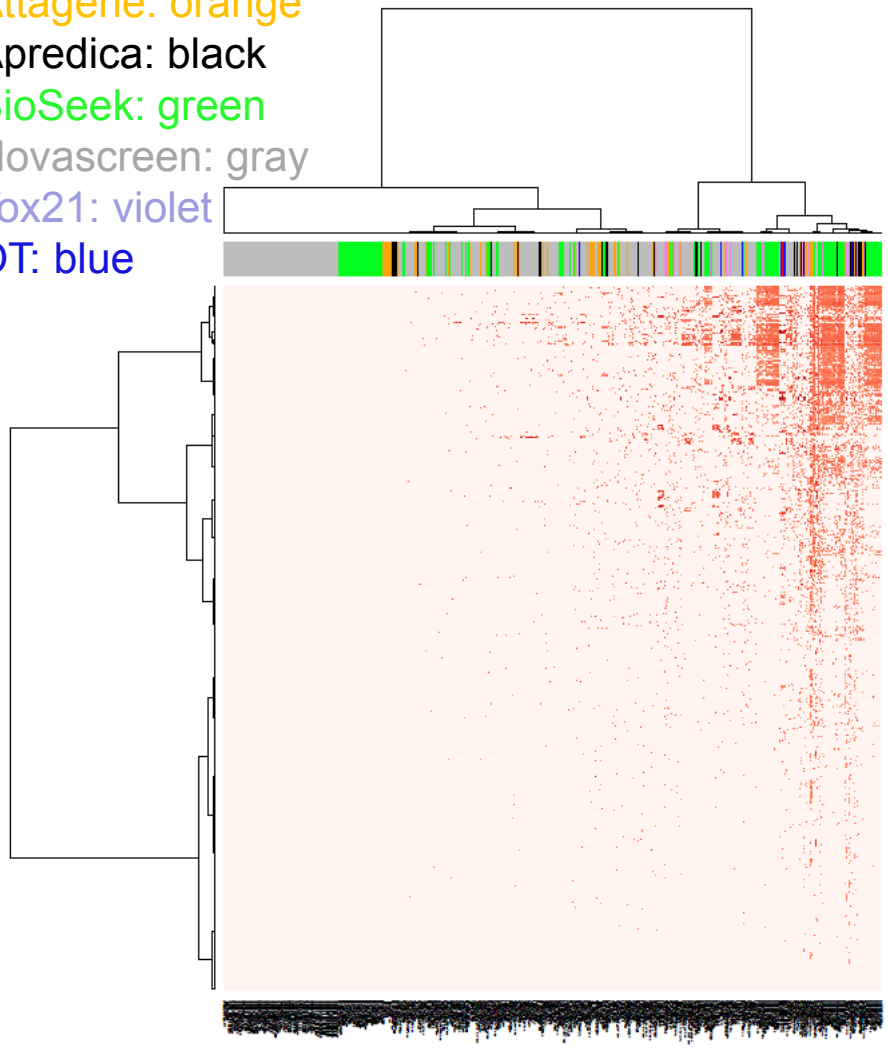

Table 3 Top 20 most promiscuous assays[a]

Assay target	Assay category	AC50s Total	AC50s <=10μM	AC50s <=1μM
hCYP2C19	CYP	264	144	53
hCYP2C9	CYP	152	81	19
rPBR	Transporter	147	62	18
hPXR	Nuclear receptor (subfamily 1)	140	73	35
hNET	Transporter	136	48	13
hPBR	Transporter	117	36	5
hDAT	Transporter	117	45	7
hCYP1A2	CYP	108	60	16
gDAT	Transporter	98	26	4
h5HT7	GPCR (aminergic)	96	35	13
hGR	Nuclear receptor (subfamily 3)	96	35	6
hOpiate_mu	GPCR (other)	92	27	5
hDRD1	GPCR (aminergic)	89	36	9
rNaCh_site2	Ion channel	87	37	13
hCYP2B6	CYP	81	43	16
gSIGMA_NonSelective	Other	80	31	13
gOpiateK	GPCR (other)	75	18	4
rMAOAC	Other enzyme	73	15	6
hAR	Nuclear receptor (subfamily 3)	73	33	8
hBACE	Protease	73	28	3

Sipes et al., Chem Res Toxicol. 26:878-95, 2013

Tox21 qHTS Screen
Aryl hydrocarbon Receptor (AhR) Signaling

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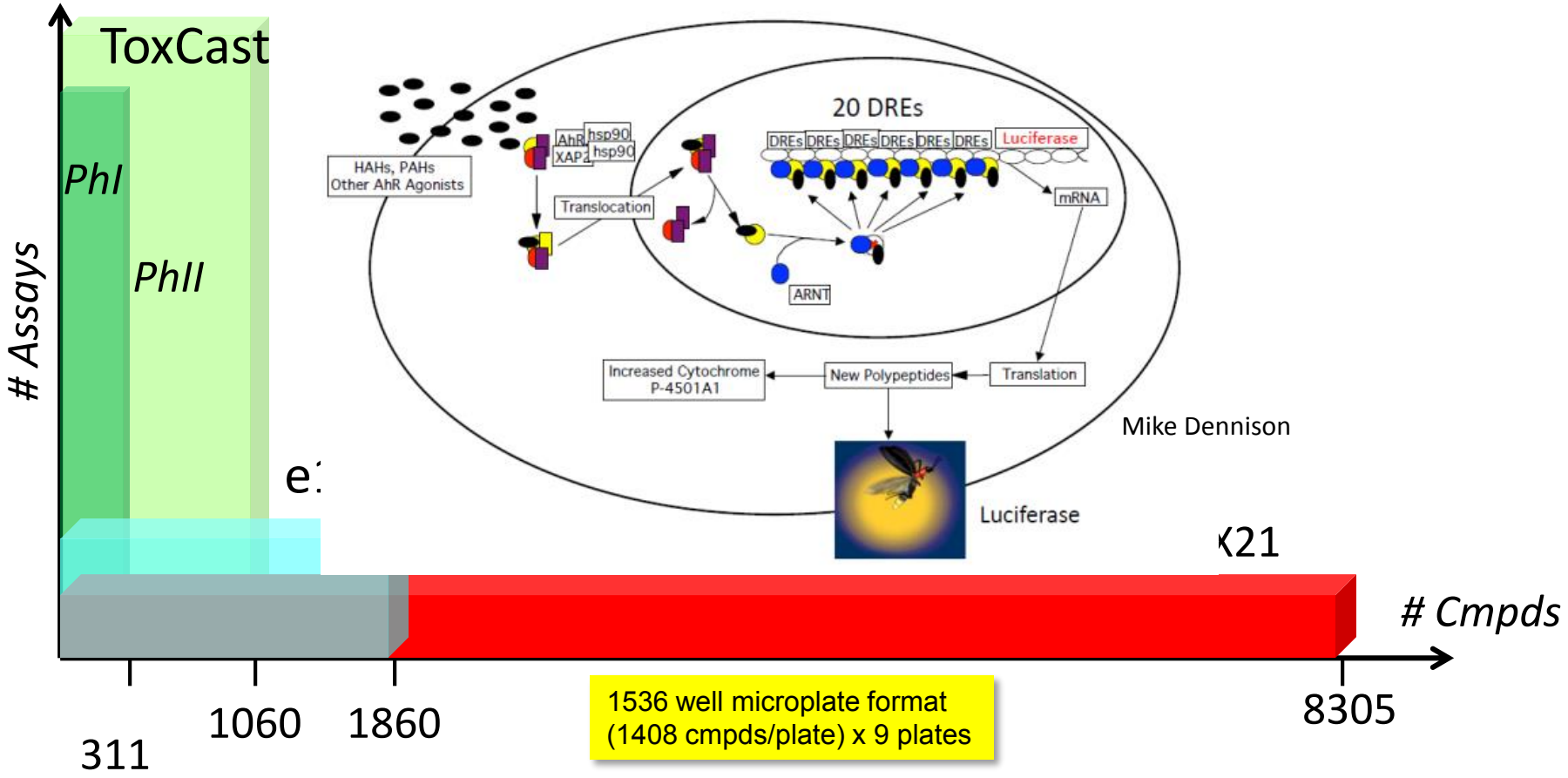
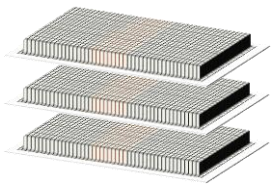



NCCT/EPA


NIEHS/NTP


NIH/NCATS


FDA

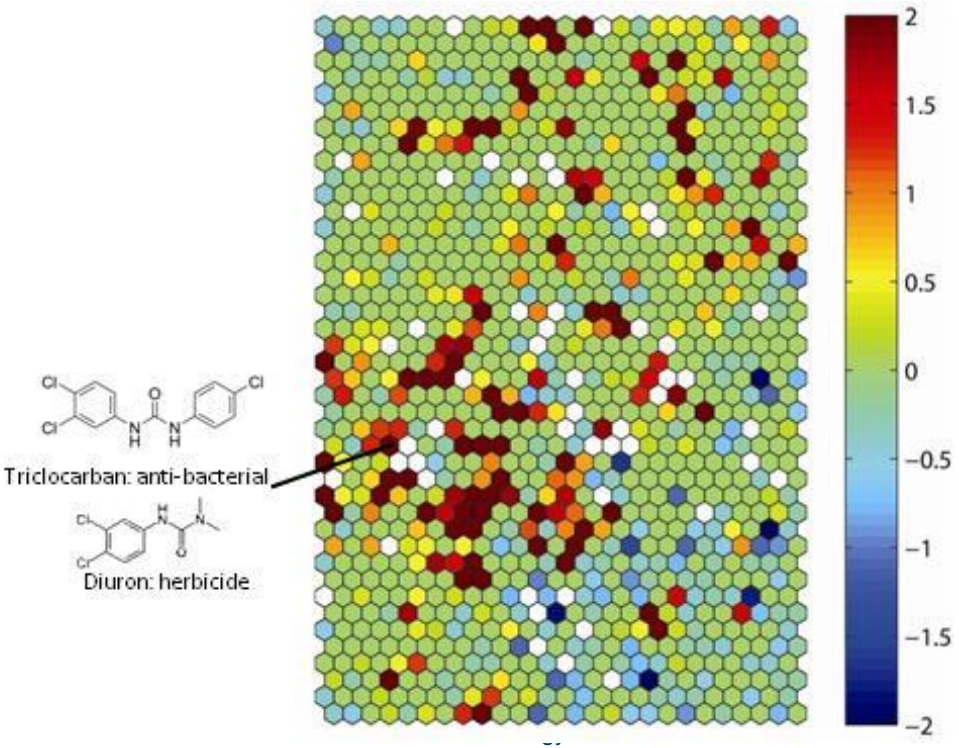
Environmental
Industrial
Pesticides
Food Use
Drugs
Toxicology

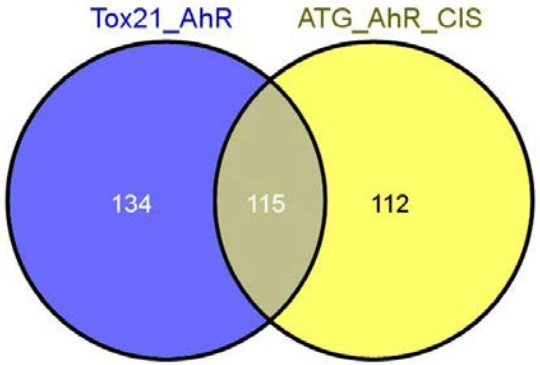
Chemical Space of Actives

United States Environmental Protection Agency

Level 8 Results Summary	
No. of HITS	768
% HITS	9.2
% Concordance	94.3

Compound Features	Frequency	Frequency	Z-Score	-LOG(AC50)
All Compounds		764		
Amino acids		26	-0.465	
Bases, nucleosides		3	-0.1735	
Benzenes		676	3.307	
Carbocycles		24	0.8749	
Carbohydrates		5	-0.6666	
Elements		31	1.598	
Functional groups		726	-1.759	
Heterocycles		378	4.896	
Naphthalenes		55	0.3566	
Natural products		8	0.7849	
Peptidomimetics		0		
Pharmacophores		747	-2.234	
Protective groups		41	-1.701	
Spacer groups		177	-1.309	
Compounds Without Features		6	-1.802	

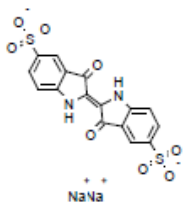
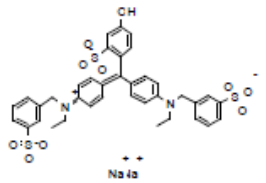
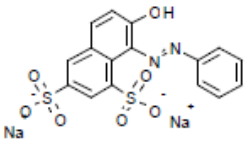
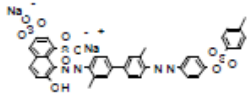
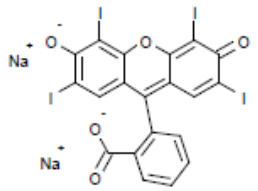
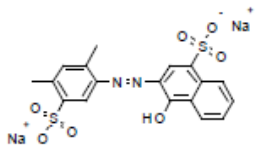
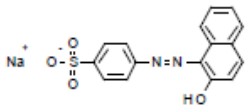
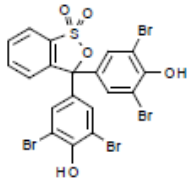
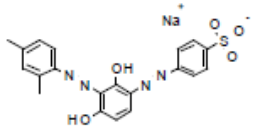
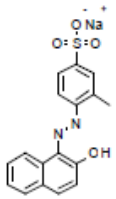
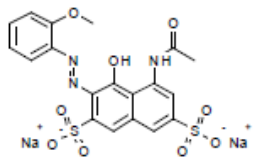
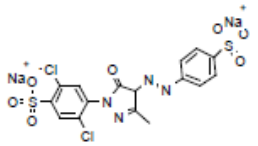
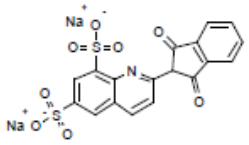
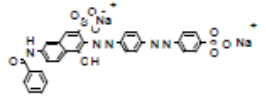
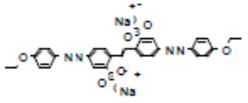
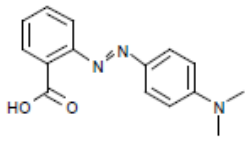
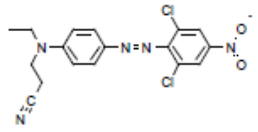
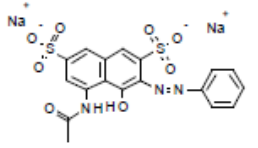
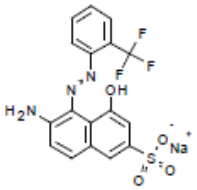
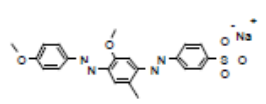
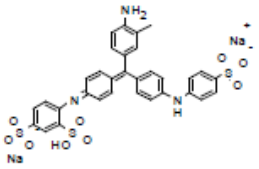
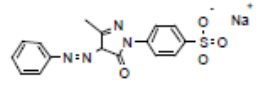
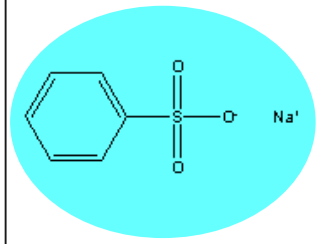




Comparison with actives from AhR reporter in Attagene assays

Source: Ruili Huang/NCATS

Active Structures: Dyes

 DSSTox_GSID_20190	 DSSTox_GSID_20673	 DSSTox_GSID_21082	 DSSTox_GSID_21224	 DSSTox_GSID_21233	 DSSTox_GSID_21234
 DSSTox_GSID_38881	 DSSTox_GSID_41682	 DSSTox_GSID_41708	 DSSTox_GSID_41709	 DSSTox_GSID_41715	 DSSTox_GSID_41719
 DSSTox_GSID_41721	 DSSTox_GSID_41726	 DSSTox_GSID_41728	 DSSTox_GSID_42154	 DSSTox_GSID_44538	 DSSTox_GSID_44663
 DSSTox_GSID_44713	 DSSTox_GSID_44718	 DSSTox_GSID_44904	 DSSTox_GSID_47451		Tox21 ATG Tox21 & ATG

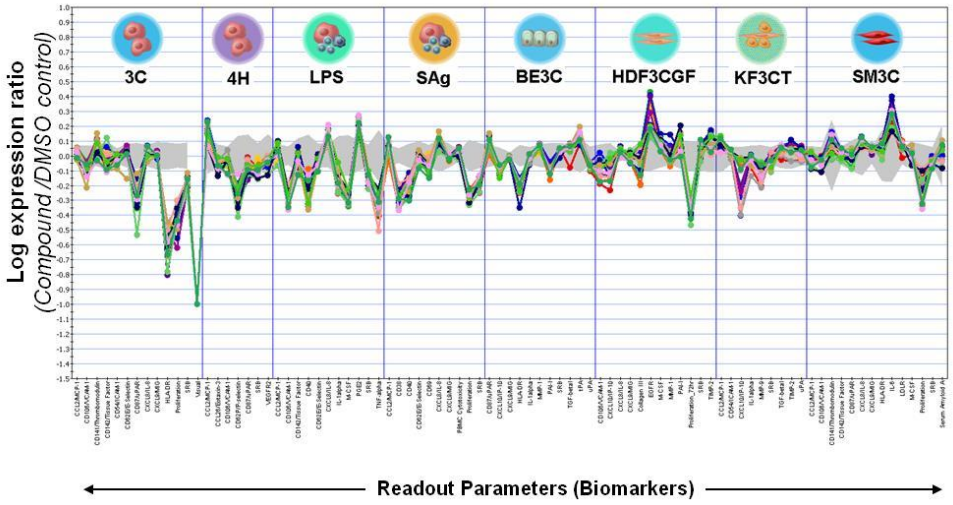
Extensively used as colorants in food, cosmetics, waxes, solvents, textiles and so on

Franzosa et al., in prep

Insights in to Mechanisms: BioMap Profiling Assays

Table 1. Diversity 8 panel of BioMAP Systems utilized in this study.

System	Cell Types	Environment	Readouts
3C	Endothelial cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, ICAM-1, Thrombomodulin, Tissue Factor, E-selectin, uPAR, IL-8, MIG, HLA-DR, Prolif., Vis., SRB (13)
4H	Endothelial cells	IL-4+histamine	VEGFRII, P-selectin, VCAM-1, uPAR, Eotaxin-3, MCP-1, SRB (7)
LPS	Peripheral Blood Mononuclear Cells + Endothelial cells	TLR4	CD40, VCAM-1,Tissue Factor, MCP-1, E-selectin, IL-1a, IL-8, M-CSF, TNF-a, PGE2, SRB (11)
SAg	Peripheral Blood Mononuclear Cells + Endothelial cells	TCR	MCP-1, CD38, CD40, CD69, E-selectin, IL-8, MIG, PBMC Cytotox., SRB, Proliferation (10)
BE3C	Bronchial epithelial cells	IL-1β+TNF-α+IFN-γ	uPAR, IP-10, MIG, HLA-DR, IL-1a, MMP-1, PAI-1, SRB, TGF-b1, tPA, uPA (11)
HDF3CGF	Fibroblasts	IL-1β+TNF-α+IFN-γ +bFGF+EGF+PDGF-BB	VCAM-1, IP-10, IL-8, MIG, Collagen III, M-CSF, MMP-1, PAI-1, Proliferation, TIMP-1, EGFR, SRB (12)
KF3CT	Keratinocytes + Fibroblasts	IL-1β+TNF-α+IFN-γ +TGF-β	MCP-1, ICAM-1, IP-10, IL-1a, MMP-9, TGF-b1, TIMP-2, uPA, SRB (9)
CASM3C	Coronary artery vascular smooth muscle cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, Thrombomodulin, Tissue Factor, IL-6, LDLR, SAA, uPAR, IL-8, MIG, HLA-DR, M-CSF, Prolif., SRB (14)



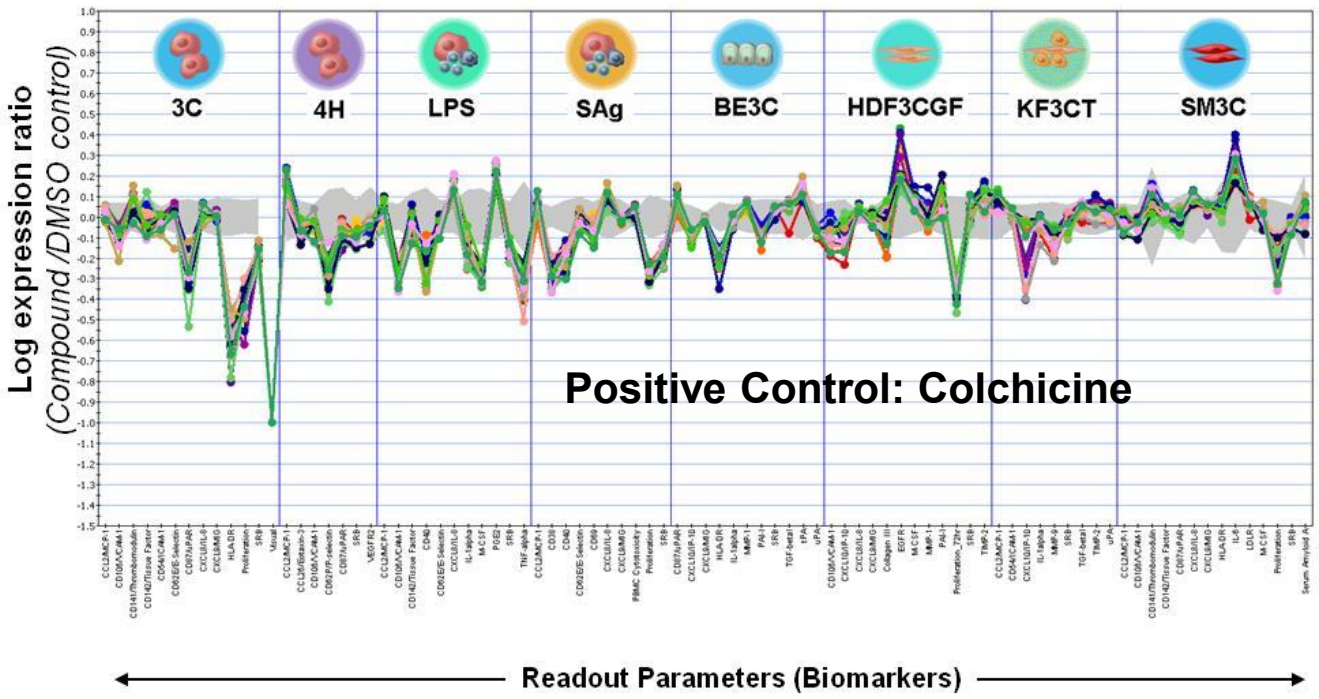
DiscoveRx BioMAP®
High Throughput Human Biology

Insights in to Mechanisms: BioMap Profiling Assays

EPA United States Environmental Protection Agency

Table 1. Diversity 8 panel of BioMAP Systems utilized in this study.

System	
3C	
4H	
LPS	
SAg	
BE3C	
HDF3CGF	
KF3CT	
CASM3C	



DiscoveRx BioMAP®
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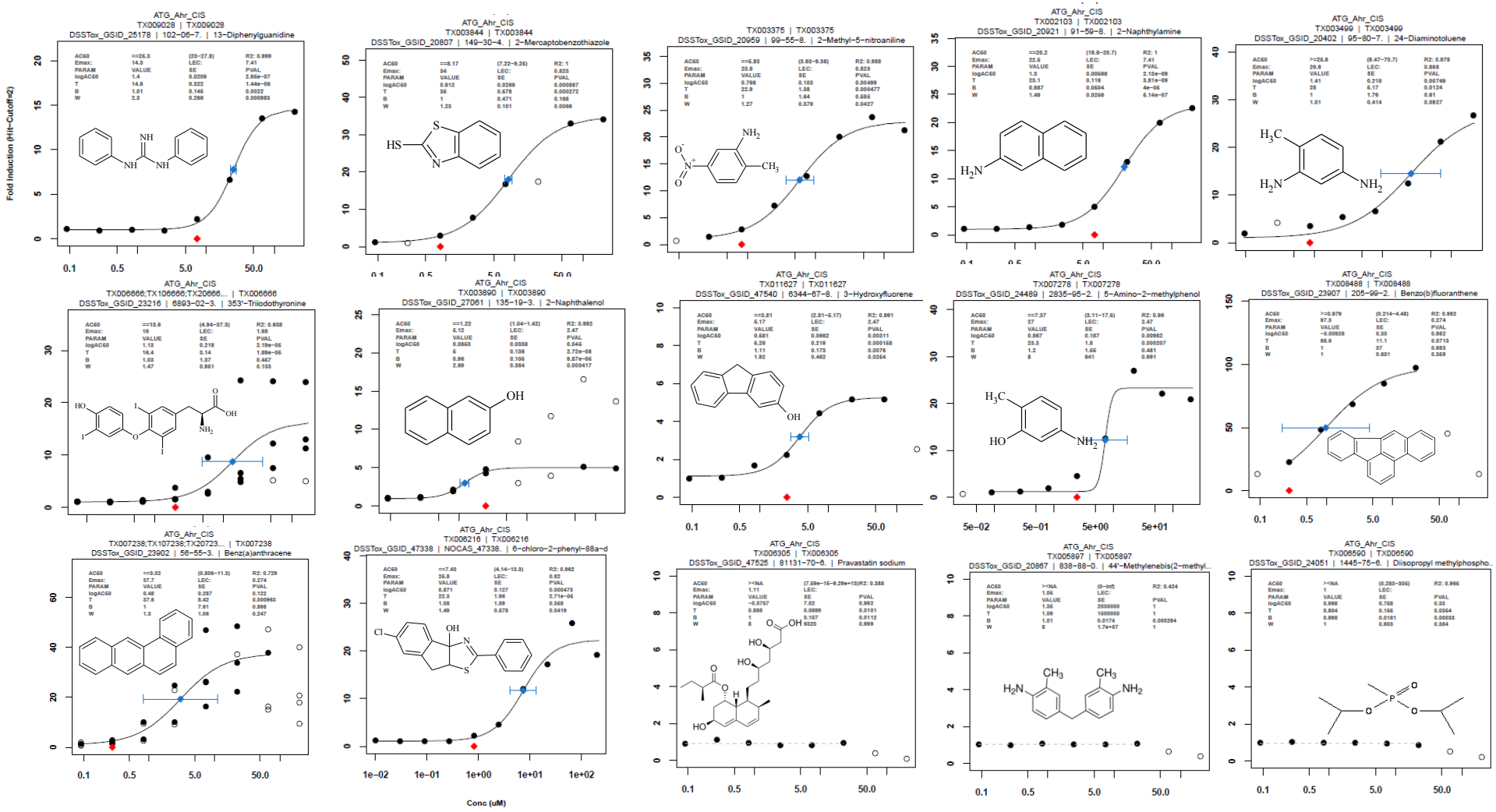
Unsupervised Clustering Analysis

- What can we learn about mechanisms of activity through similarity of activity?
- Chemicals analyzed at single conc level to minimize polypharmacology effect
- Self Organizing Maps (SOM): 10X10 Array/100 Clusters
- Identify clusters enriched with chemicals with known activity
- Two examples:

Cluster	Cluster Count	Common Activity	Example Compounds known associations	Example Compounds novel associations
57,67	52	AhR ligands	Hydroquinone 4-Chloro-1,2-diaminobenzene 1,2-Phenylenediamine Fenaminosulf	C.I. Solvent yellow 14
48	27	ER antagonists	Clomiphene citrate Tamoxifen citrate Fulvestrant Raloxifene Tamoxifen 4-Hydroxytamoxifen	Cyclopamine Amiodarone hydrochloride Haloperidol Reserpine Donated pharma: NK1 receptor antagonist Bradykinin B1 receptor antagonist Lipid-lowering agent

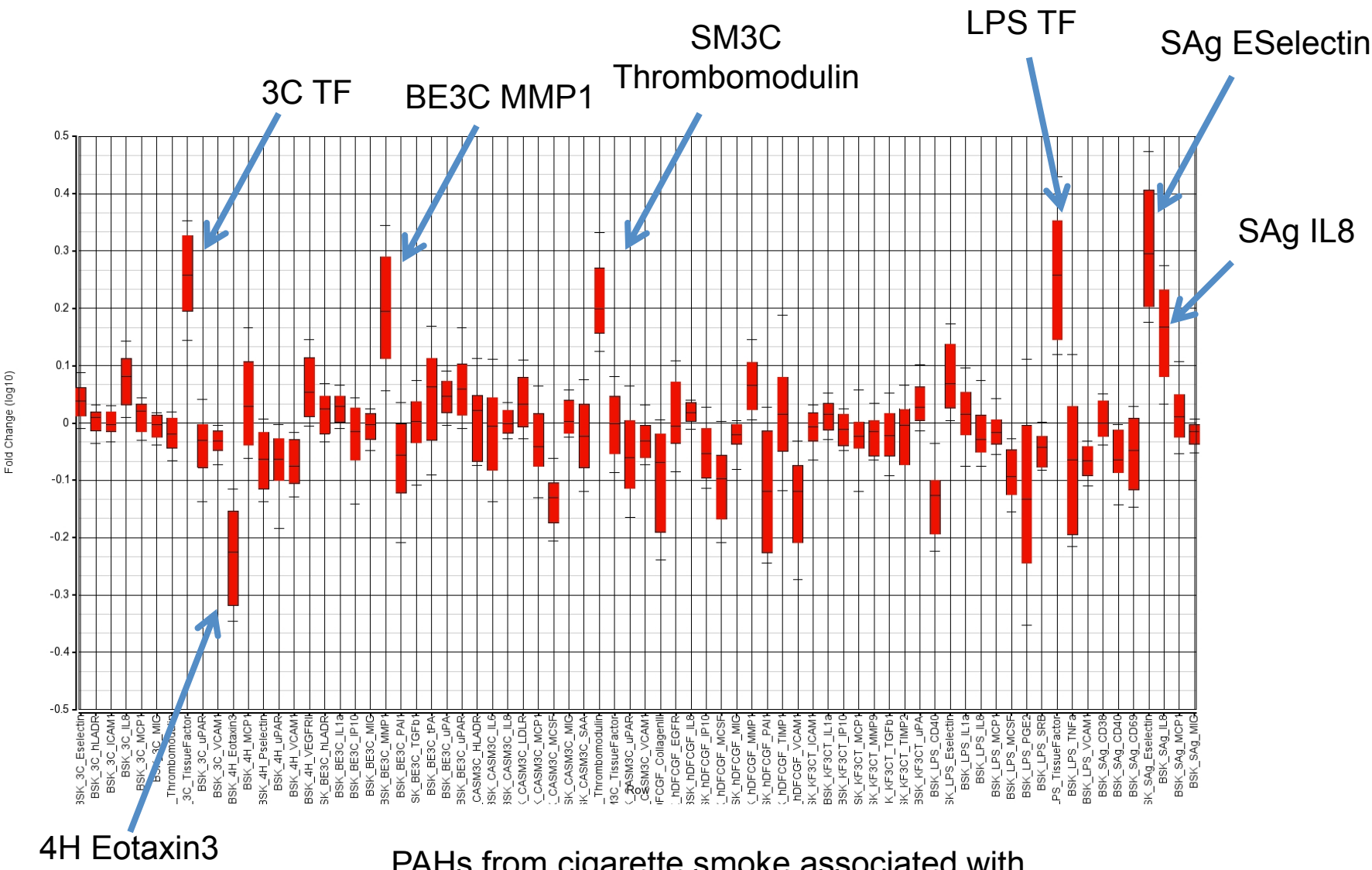
Clusters 57/67 and Relationship to ATG AhR Activity (85% positive)



All 3 negatives were present at only one conc in the SOM cluster

AhR Signature

SOM Cluster 57



PAHs from cigarette smoke associated with atherogenesis/thrombosis

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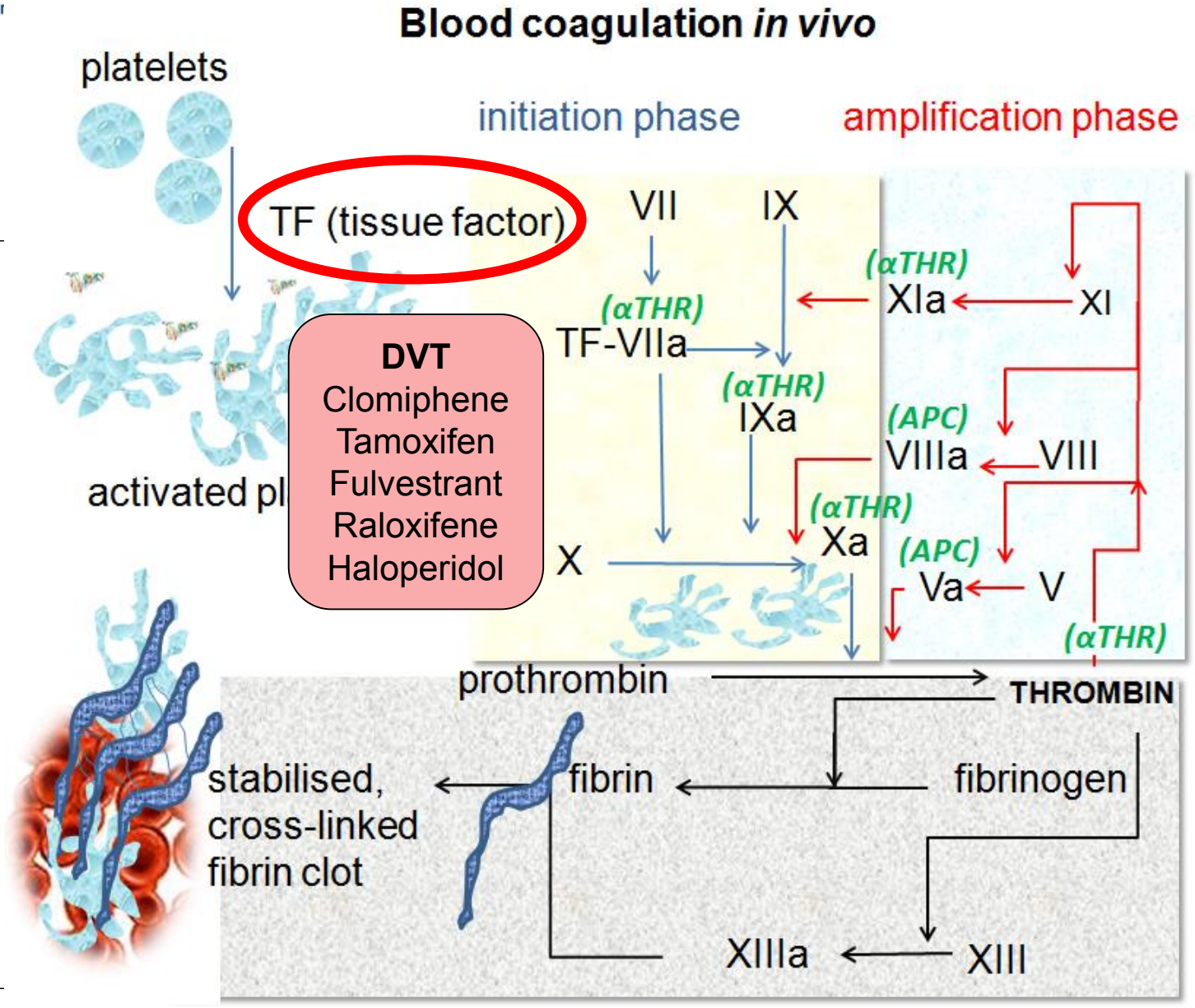
Hypothesis Generation Example

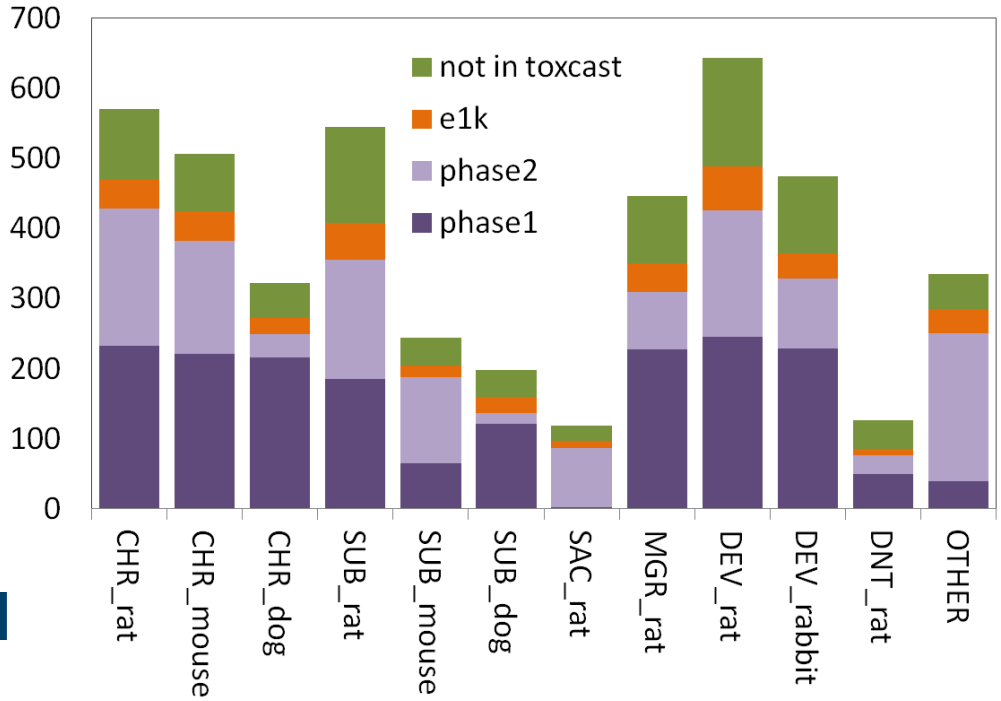


Dr Graham Beards, Wikipedia Commons

Use of Data in Predictive Modeling

United States Environmental Protection Agency

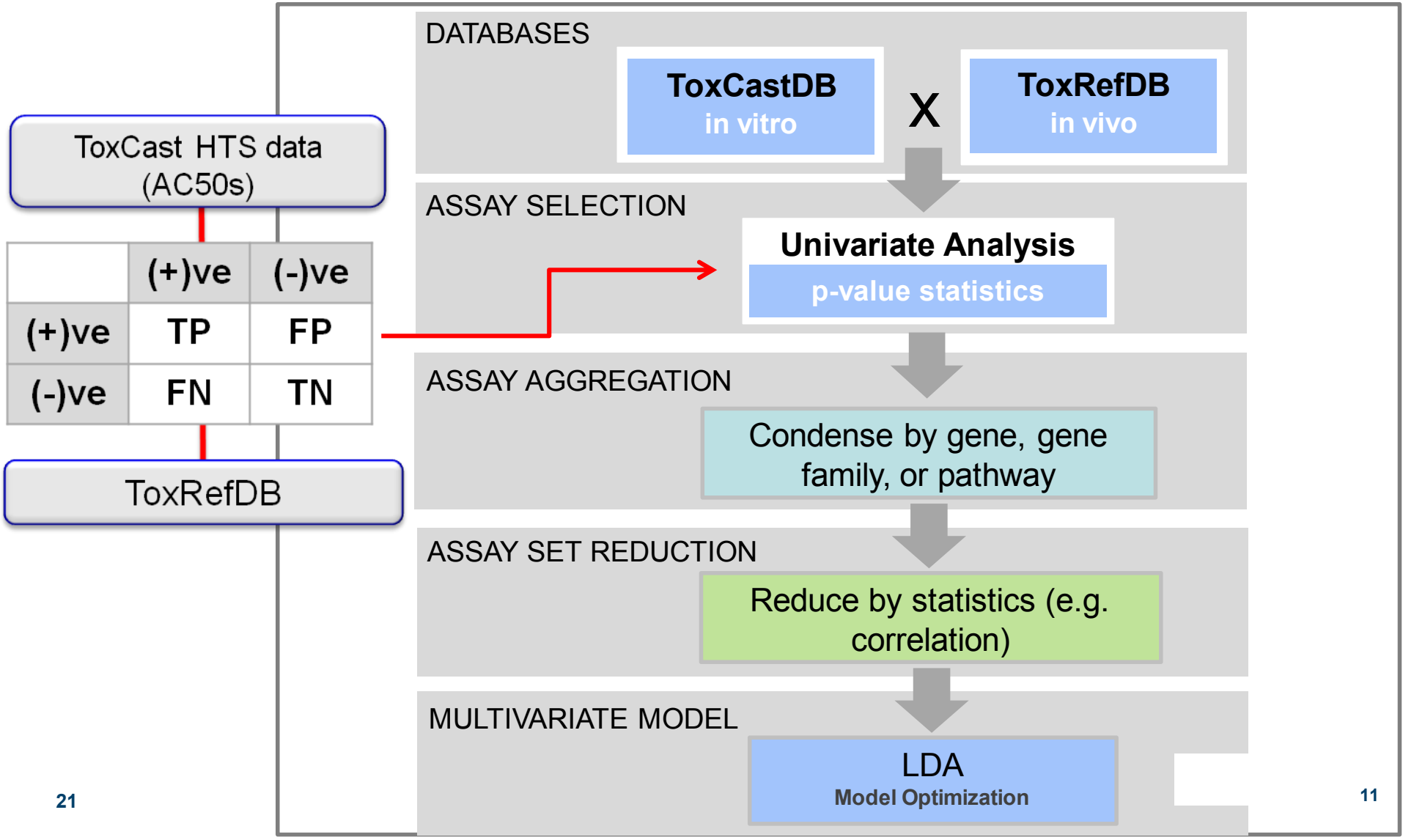
- **Combine all in vitro assay data following standardization/normalization—ToxCastDB**
- **Use *in vivo* data to anchor models—ToxRefDB**
  - **Holds in vivo endpoint data from animal toxicology studies**
  - **Currently at 5567 studies on 1049 unique chemicals**



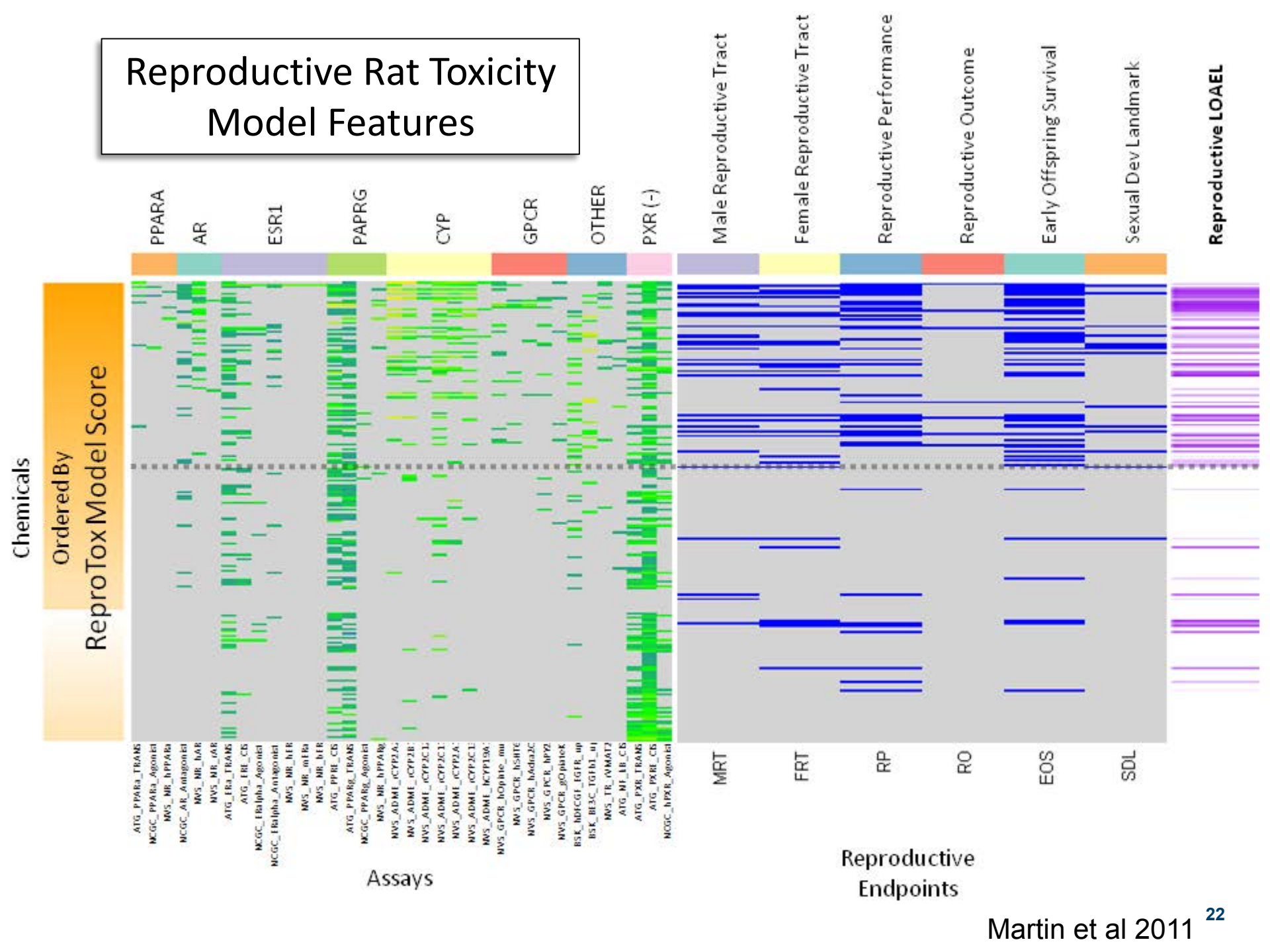
Data Source	Study Count
EPA OPP_der	3279
Open Literature	731
National Toxicol Program	666
Sanofi_pharma	222
Unpublished_submissions	50
GSK_pharma	38
Health Canada PMRA_der	23

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

Predictive Model Development from ToxCast and Other Data

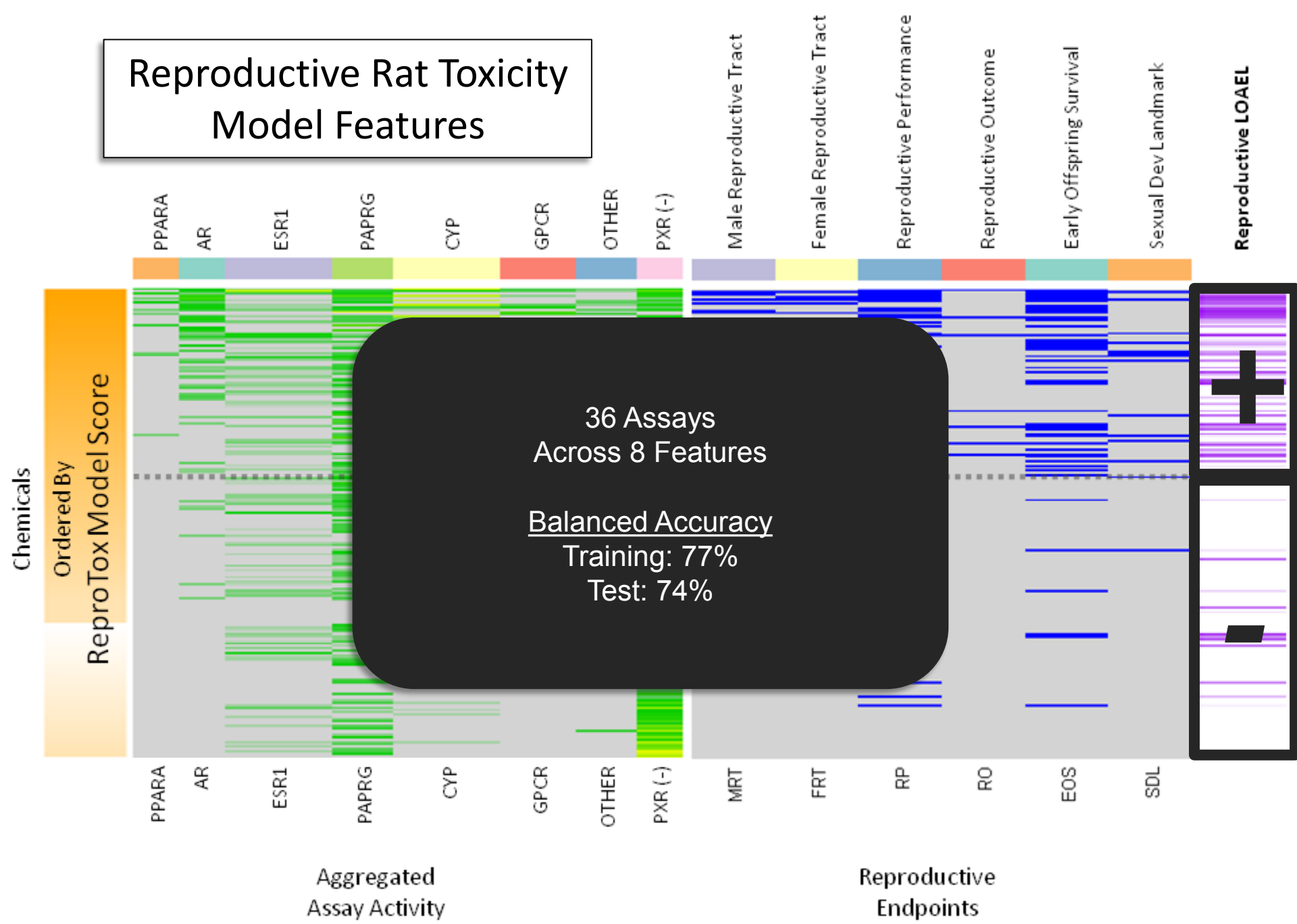


Reproductive Rat Toxicity Model Features



Martin et al 2011

Reproductive Rat Toxicity Model Features



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. 2013, Toxicol Sci 131:40-55.
  - **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 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)**

Summary

- Methods developed to use *in vitro* assays to screen and prioritize many data-poor chemicals
- Signature generation uses combination of biological insight and statistics
- Public release of Phase II data will provide opportunity for others to analyze
  - Innocentive & TopCoder Challenges
  - data summit in Spring ‘14
- Further refinements are in the works
  - More chemicals and assays
  - Use of chemoinformatics
  - Systems-level models
  - Targeted testing approaches

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1. ORISE
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