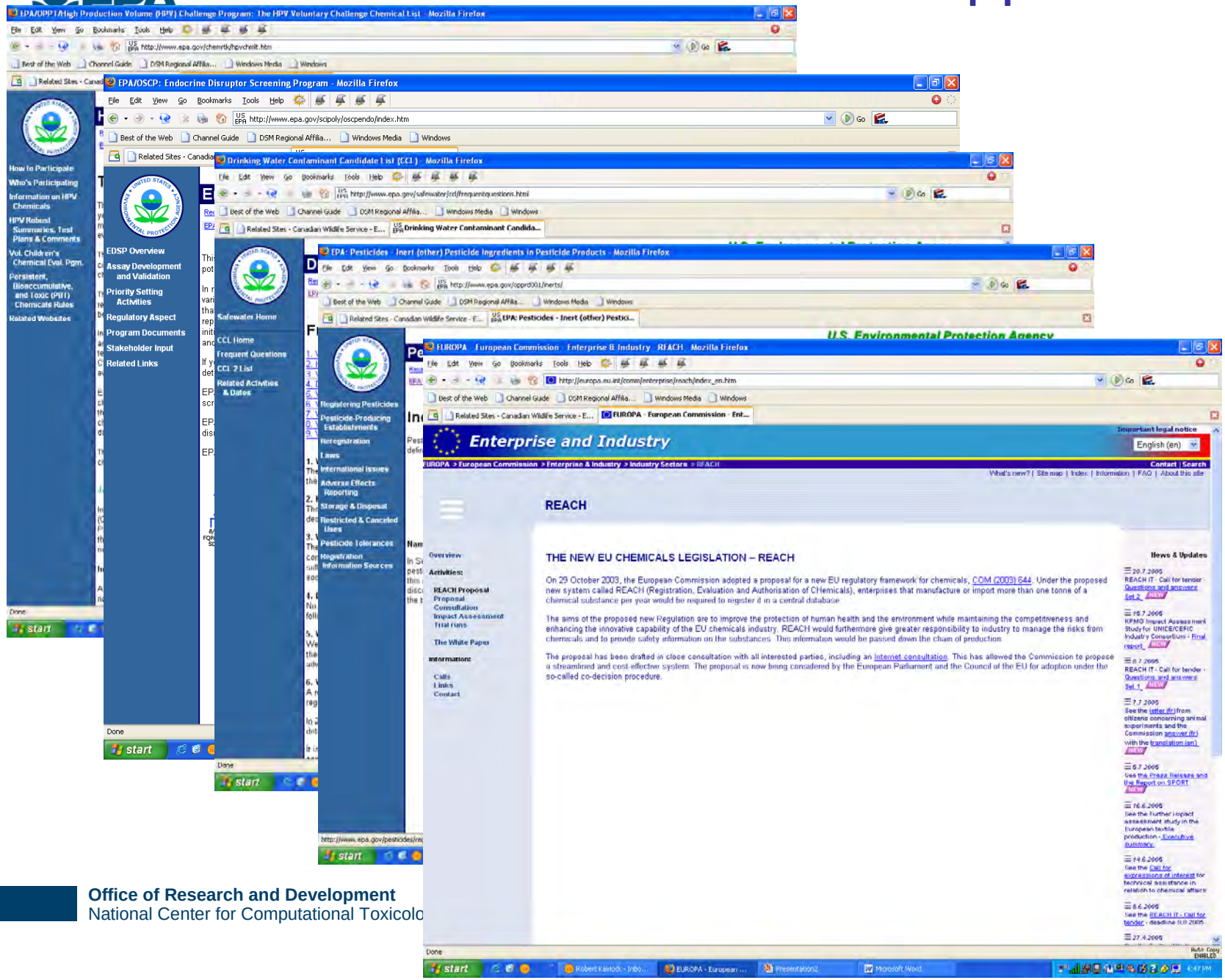
EPA
United States
Environmental Protection
Agency

The ToxCast™ Program – predicting hazard, characterizing toxicity pathways and prioritizing the toxicity testing of environmental chemicals

Office of Research and Development
National Center for Computational Toxicology

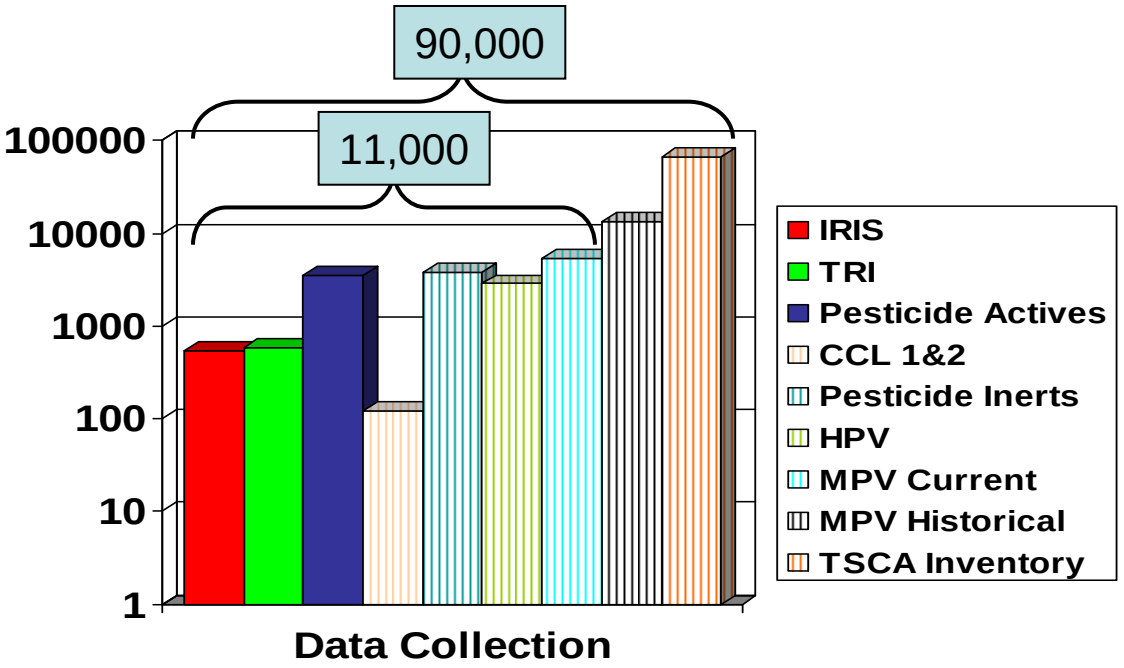
The Need For a New Approach



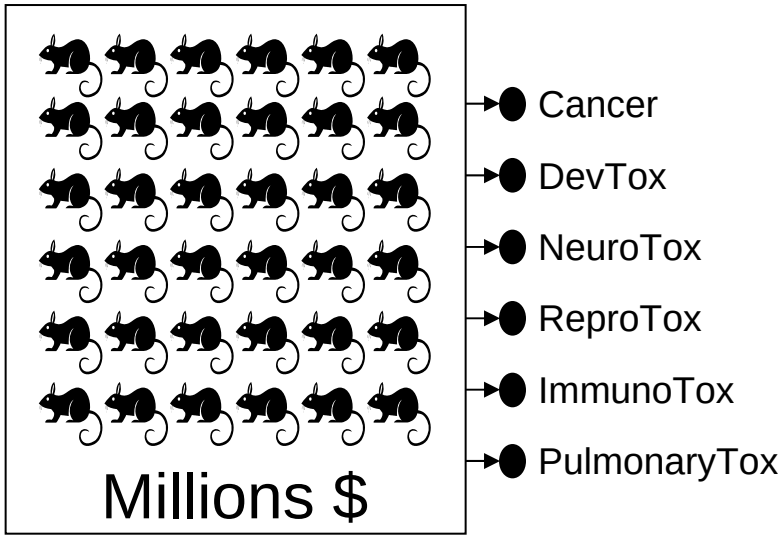
United States Environmental Protection Agency

EPA's Need for Chemical Prioritization

Too Many Chemicals



Too High a Cost



…and not enough data.

United States Environmental Protection Agency

Ways to Prioritize:

- Animal studies
  - cost, time, ethical considerations
- QSAR
  - domain of applicability, availability of models
- Bioactivity Profiling
  - biologically relevant chemical characterization
  - HTS methods
  - ToxCast

ToxCast™ : a computational toxicology approach based on high-throughput bioactivity profiling

- Research program of EPA's National Center for Computational Toxicology
- Addresses chemical screening and prioritization needs for pesticidal inerts, anti-microbials, CCLs, HPVs and MPVs
- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures
- Coordinated with NIH: NTP and NHGRI/NCGC via Tox21
- Committed to stakeholder involvement and public release of data
  - Communities of Practice- Chemical Prioritization; Exposure
  - NCCT website- http://www.epa.gov/ncct/toxcast
  - ACToR- Aggregated Computational Toxicology Resource *http://actor.epa.gov/actor/*

EPA United States Environmental Protection Agency

Phased Development of ToxCast

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
I	320	Data Rich (pesticides)	Signature Development	552	$20k	FY08
Ib	15	Nanomaterials	Pilot	166	$10K	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	~$20-25k	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	~$20-25k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	~$20-25k	FY10
IId	>12	Nanomaterials	PMN	>200	~$15-20K	FY09-10
III	Thousands	Data poor	Prediction and Prioritization	>300	~$15-20k	FY11-12

ToxCast_320 Phase I Chemicals

United States Environmental Protection Agency

309 unique structures
3 triplicates, 5 duplicates for QC
8 metabolites

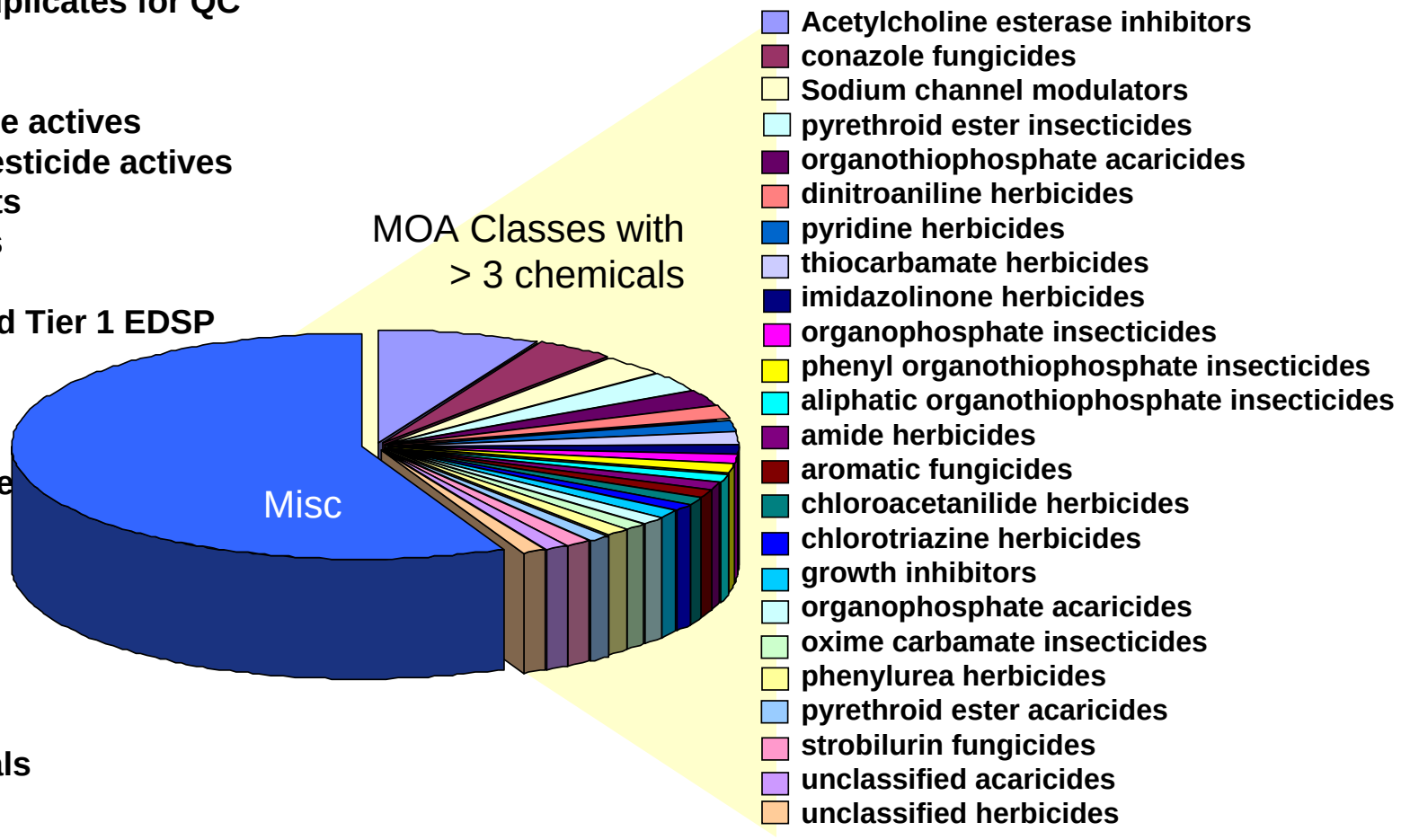
291 total pesticide actives
273 registered pesticide actives
22 pesticide inerts
33 antimicrobials

56 of 73 proposed Tier 1 EDSP

23 IUR
13 HPV
11 HPV Challenge

73 OW PCCL
11 CCL1
10 CCL2
25 CCL3

122 IRIS chemicals



Classification based on OPPIN

EPA United States Environmental Protection Agency

High-Throughput Screening Assays

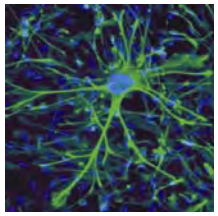
batch testing of chemicals for pharmacological/toxicological endpoints using automated liquid handling, detectors, and data acquisition

LTS | MTS | HTS | uHTS

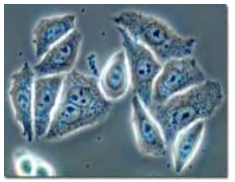
10s-100s/yr

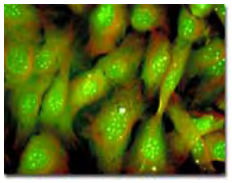
Gene-expression

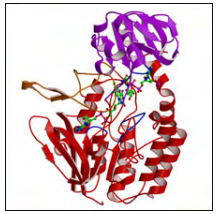
10s-100s/day



1000s/day







10,000s-
100,000s/day

Human Relevance/
Cost/Complexity

United States Environmental Protection Agency

ToxCast Phase I Datasets

- **ToxCast 1.0 (April, 2007)**
  - Enzyme inhibition/receptor binding HTS (Novascreen)
  - NR/transcription factors (Attagene, NCGC)
  - Cellular impedance (ACEA)
  - Complex cell interactions (BioSeek)
  - Hepatocelluar HCS (Cellumen)
  - Hepatic, renal and airway cytotoxicity (IVAL)
  - In vitro hepatogenomics (IVAL, Expression Analysis)
  - Zebrafish developmental toxicity (Phylonix)

20 Assay sources
554 Endpoints

- **ToxCast 1.1 (January, 2008)**
  - Neurite outgrowth HCS (NHEERL)
  - Cell proliferation (NHEERL)
  - Zebrafish developmental toxicity (NHEERL)

- **ToxCast 1.2 (June, 2008)**
  - XME Gene Regulation (CellzDirect)
  - HTS Genotoxicity (Gentronix)
  - Organ toxicity; dosimetry (Hamner Institutes)
  - Toxicity and signaling pathways (Invitrogen)
  - C. elegans WormTox (NIEHS)
  - Gene markers from microscale cultured hepatocytes (MIT)
  - 3D Cellular Zebrafish vascular/cardiotoxicity (Zygogen)
  - microarray with metabolism (Solidus)
  - HTS stress response (NHEERL+NCGC)

ToxCast Assays

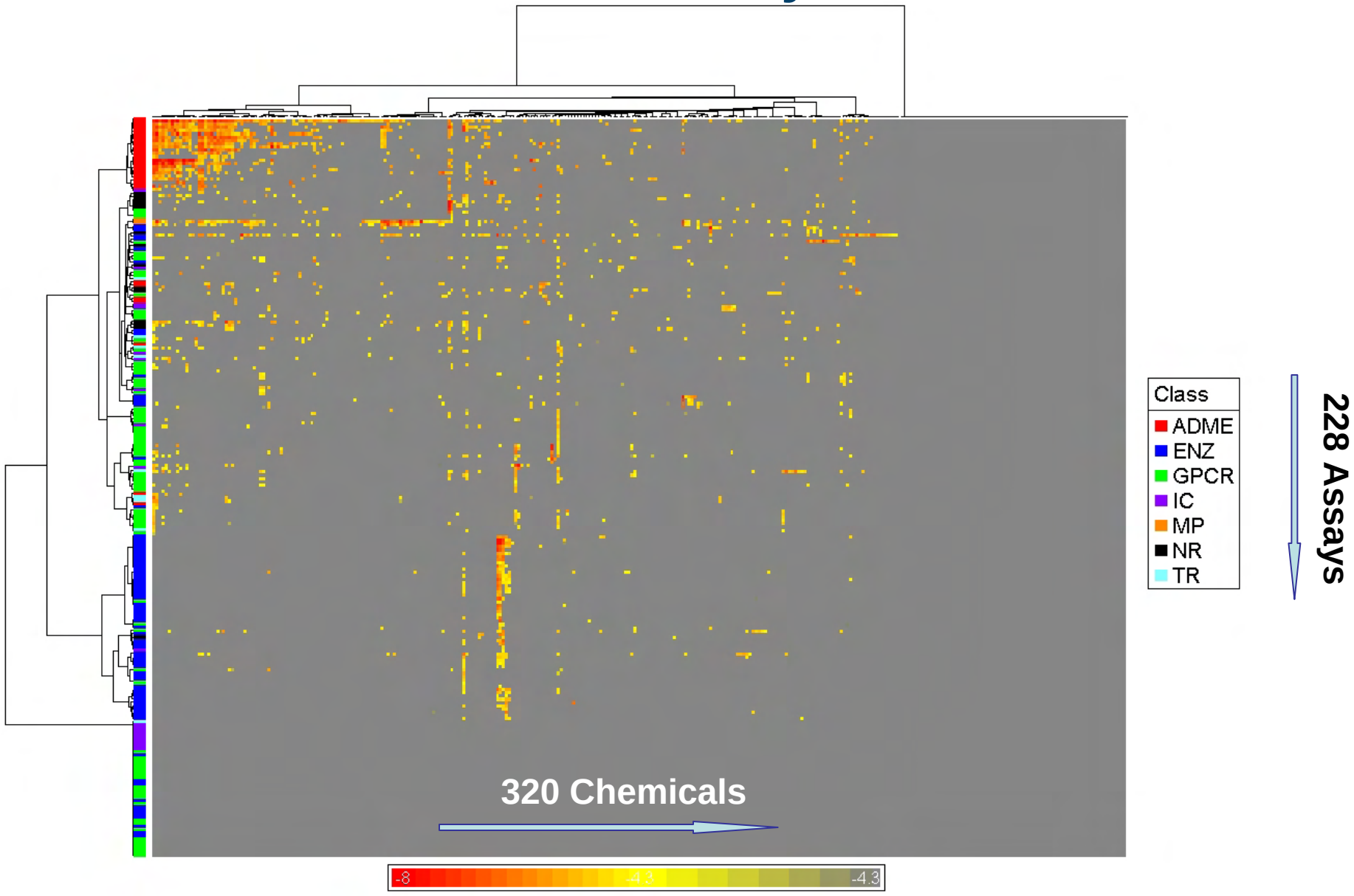
Biochemical Assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

Cellular Assays

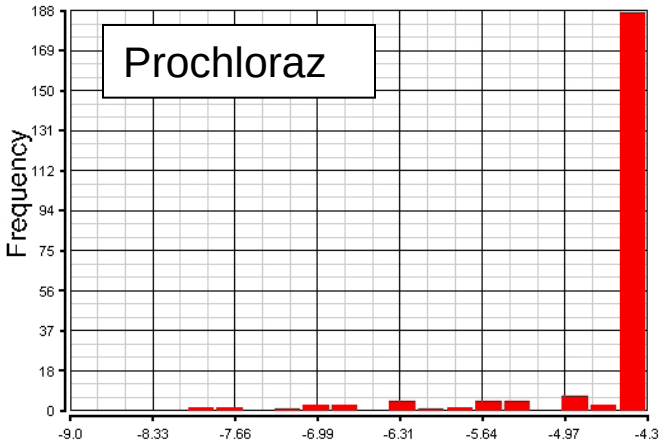
- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

Biochemical Assay Results

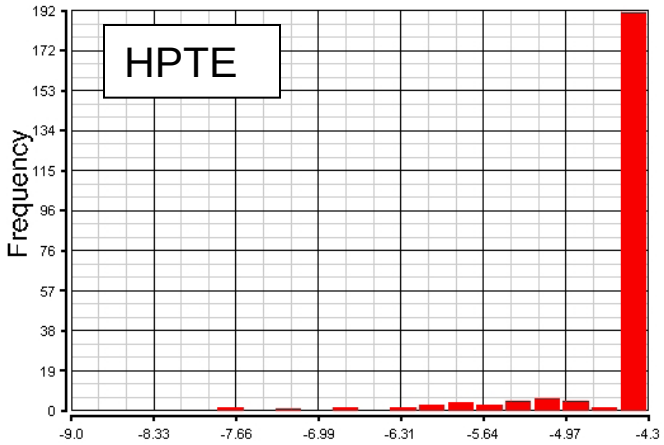


Log IC50 (M)

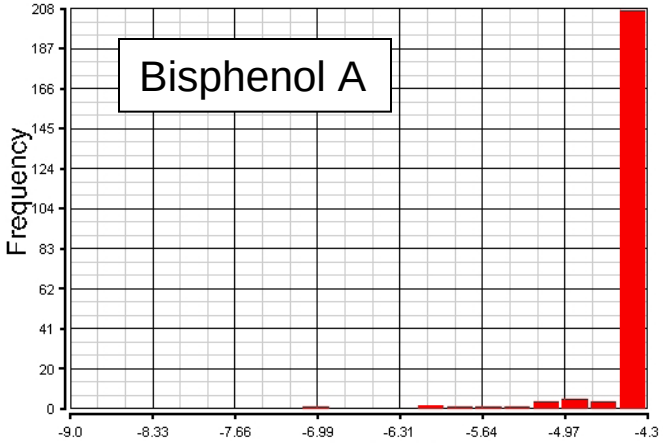
Examples of Chemical Activity in Biochemical Assays



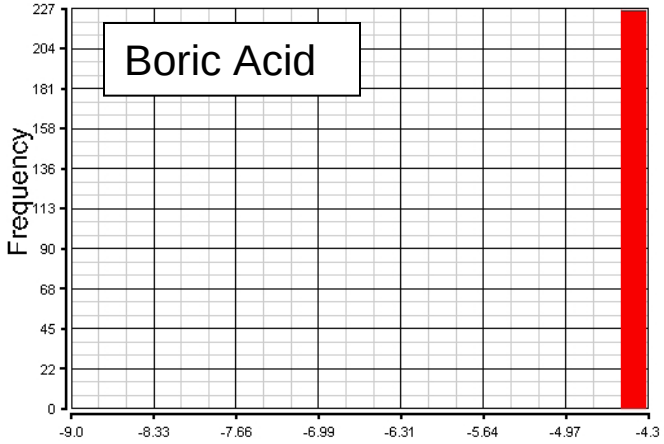
(IC50 log M)



(IC50 log M)



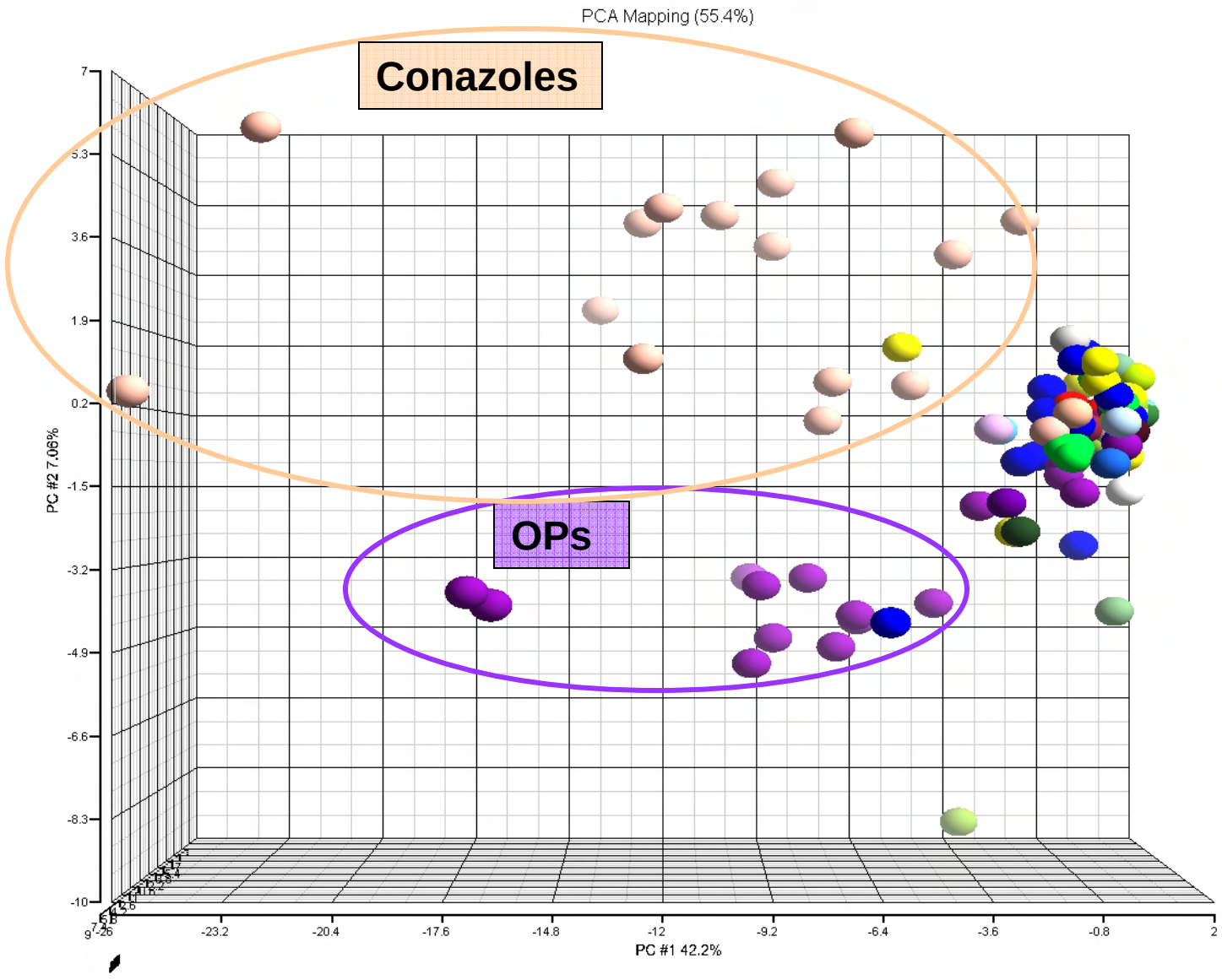
(IC50 log M)



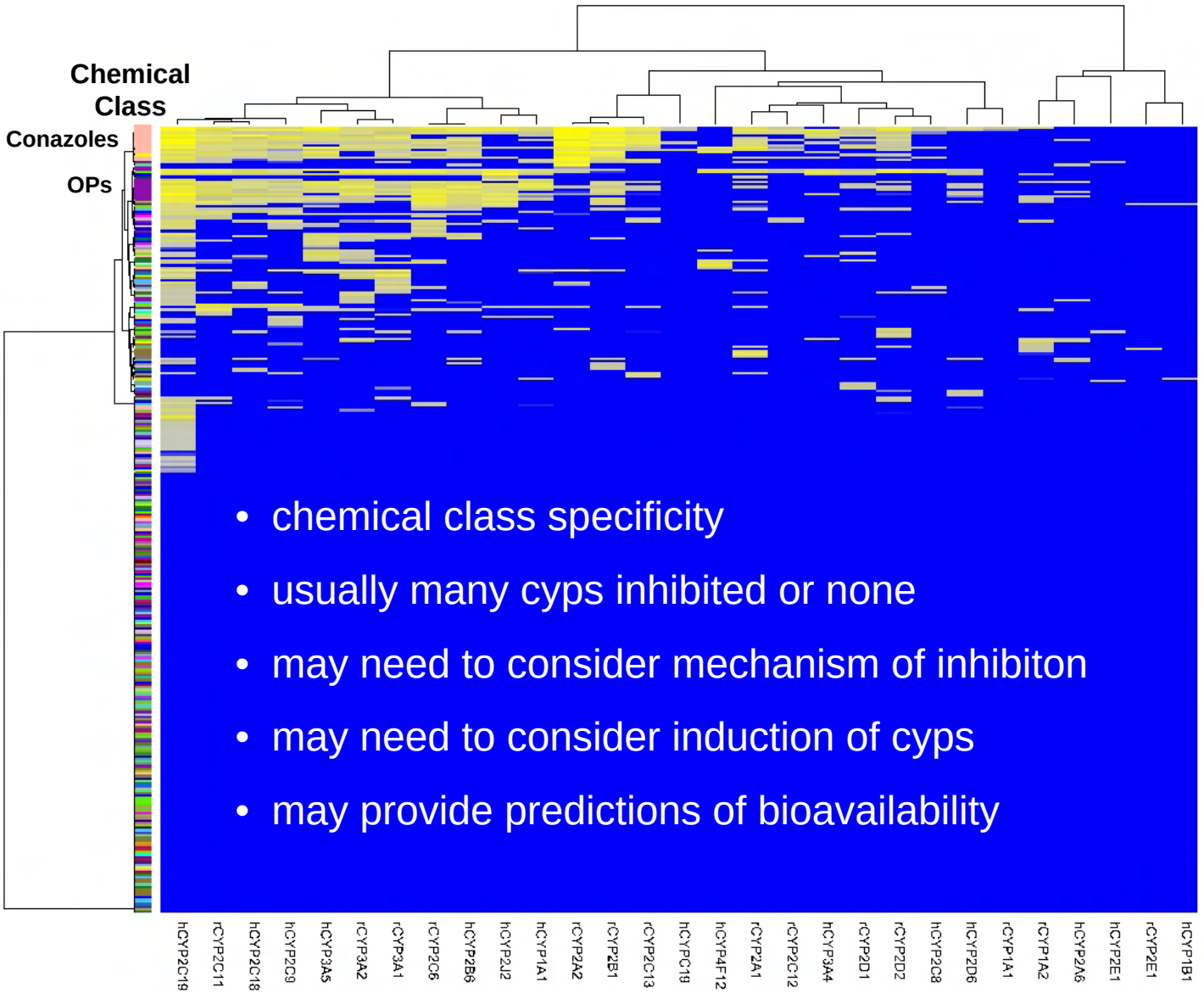
(IC50 log M)

United States Environmental Protection Agency

PCA Mapping of CYP Inhibition

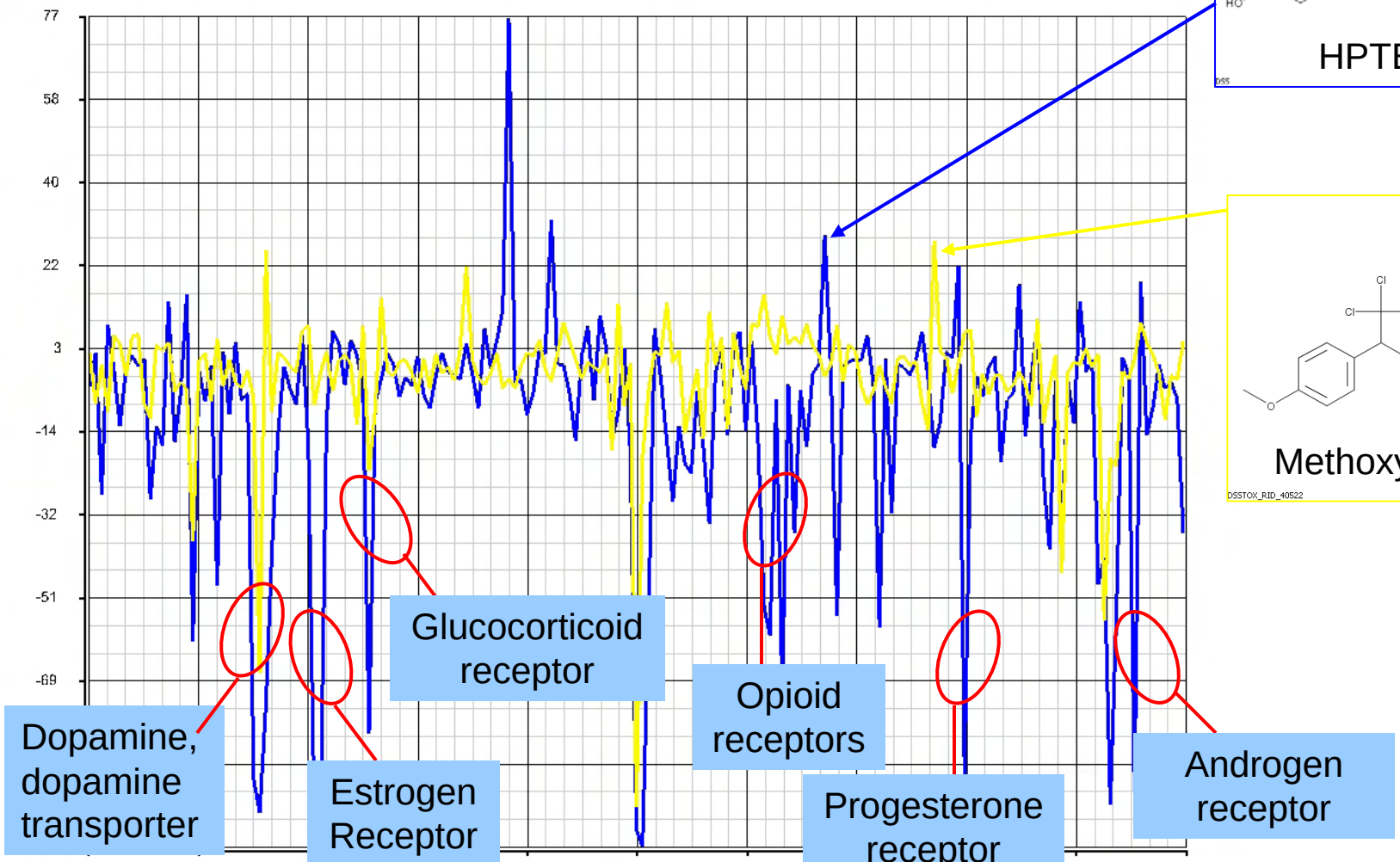


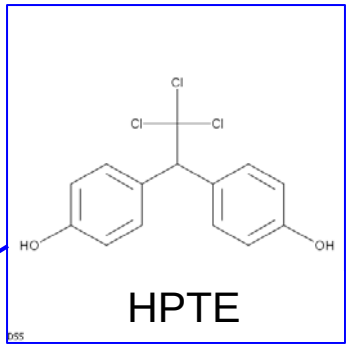
CYP Inhibition



- chemical class specificity
- usually many cyps inhibited or none
- may need to consider mechanism of inhibiton
- may need to consider induction of cyps
- may provide predictions of bioavailability

ToxCast and Biotransformation





United States Environmental Protection Agency

Cellular Assays

- Types of Assays
  - Known toxicity pathways and targets
    - biomarker measurements
    - reporter gene assays
  - General cytotoxicity
  - Toxicity cellular phenotypes
- Cell lines and primary cells
- Generally screened at up to 100 μM or used maximally tolerated concentration defined by general cytotoxicity determination
- Concentration-response format used and EC_{50} generated

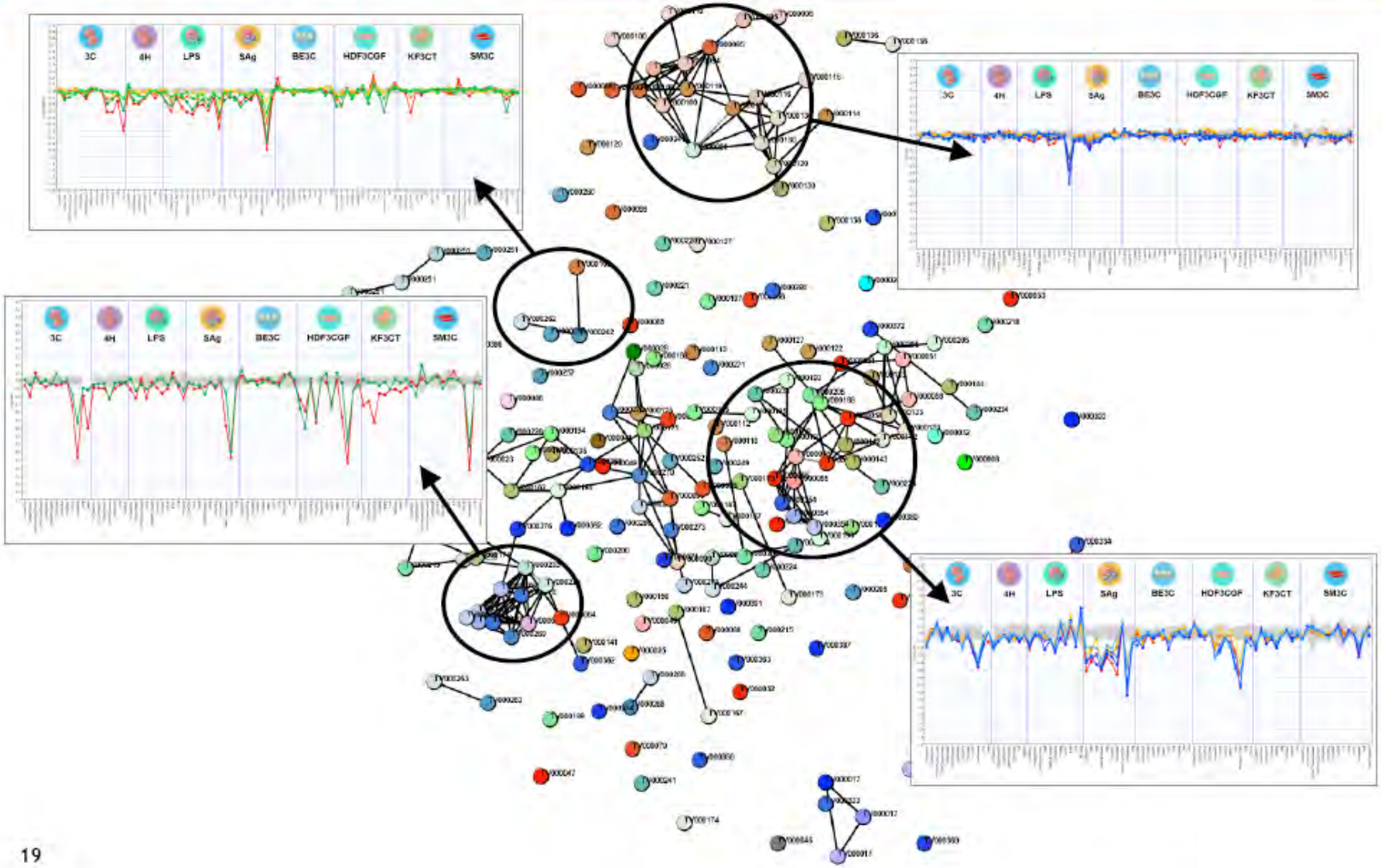
EPA United States Environmental Protection Agency

Primary Human Cell Systems (BioSeek, Inc.)

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)
SM3C 	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)

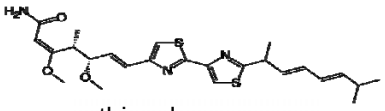
- 8 Assay systems
- 87 endpoints
- 4 concentrations

Functional Similarity Map of ToxCast Library

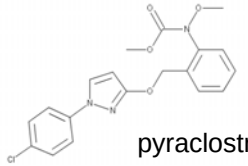


Mitochondrial Dysfunction and Endoplasmic Reticulum Stress Classes

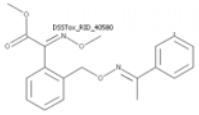
EPA United States Environmental Protection Agency



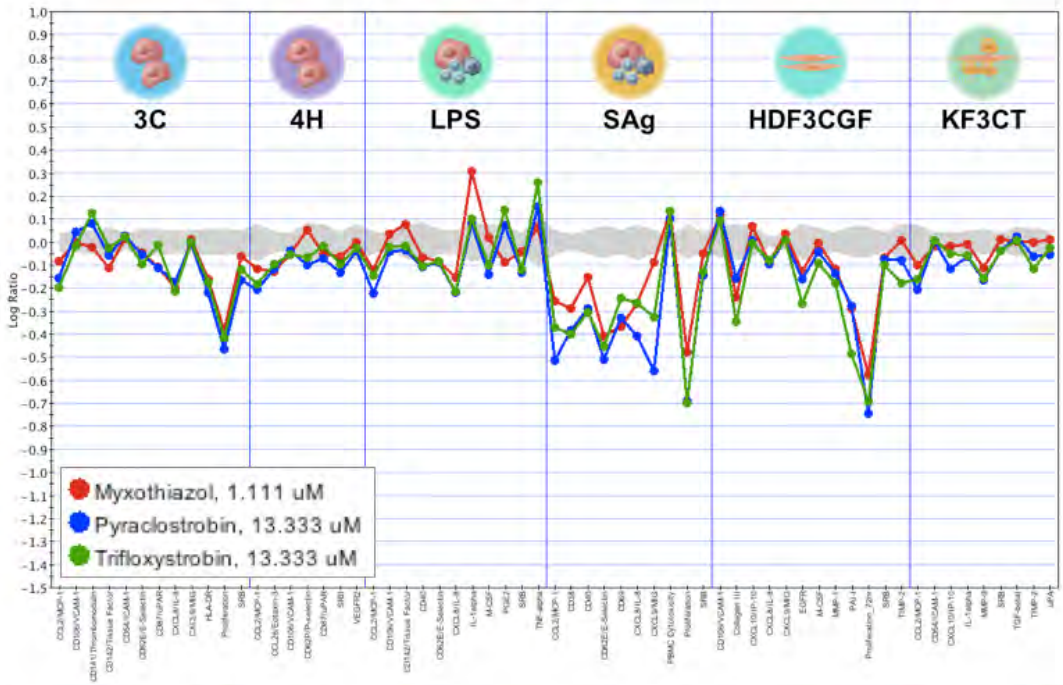
myxothiazol

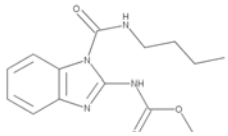


pyraclostrobin

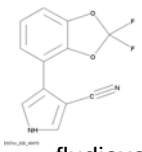


trifloxystrobin

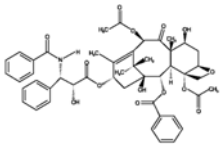




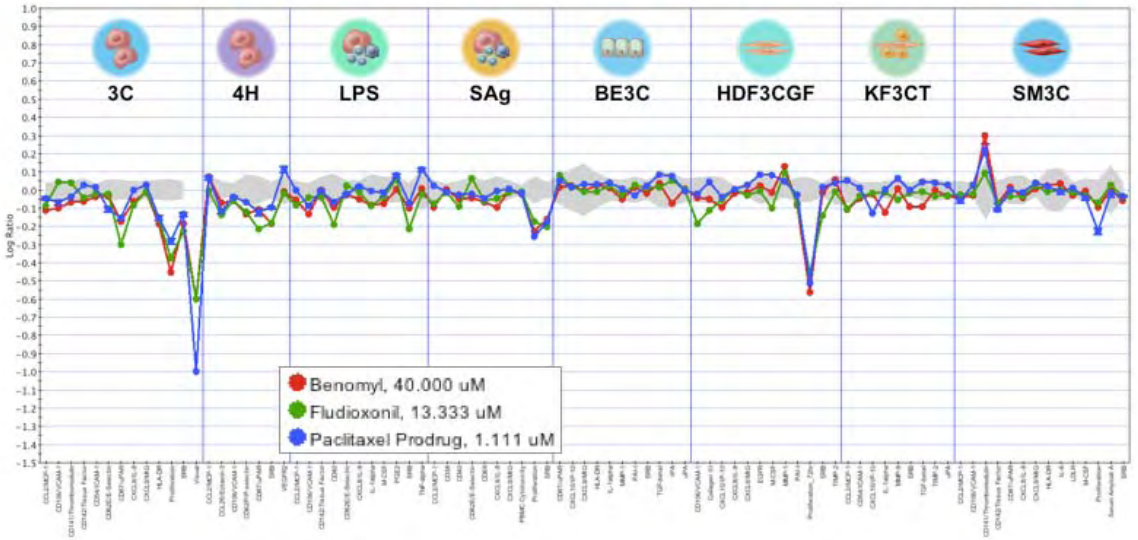
benomyl



fludioxonil



paclitaxel



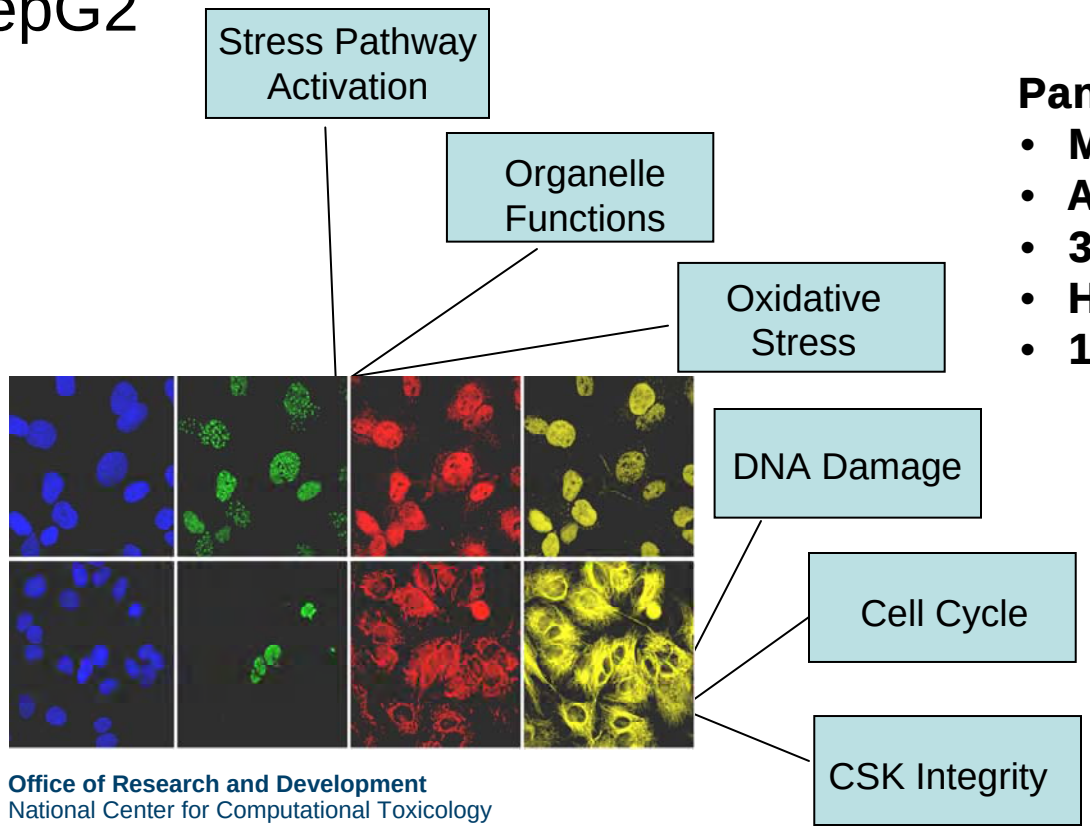
United States Environmental Protection Agency

Use of BioSeek Data in ToxCast

- Individual assay endpoints become part of larger ToxCast data set for developing predictive models
- BioMAP signatures used to provide mechanistic understanding of potential mechanism/mode of action
- May be able to validate signatures with other phenotypic assays

High-Content Screening of Cellular Phenotypic Toxicity Parameters (Cellumen, Inc.)

- Technology: automated fluorescent microscopy
- Objective: Determine effects of chemicals on toxicity biomarkers in a cell culture of human liver hepatoma HepG2



Panel 1 design*:

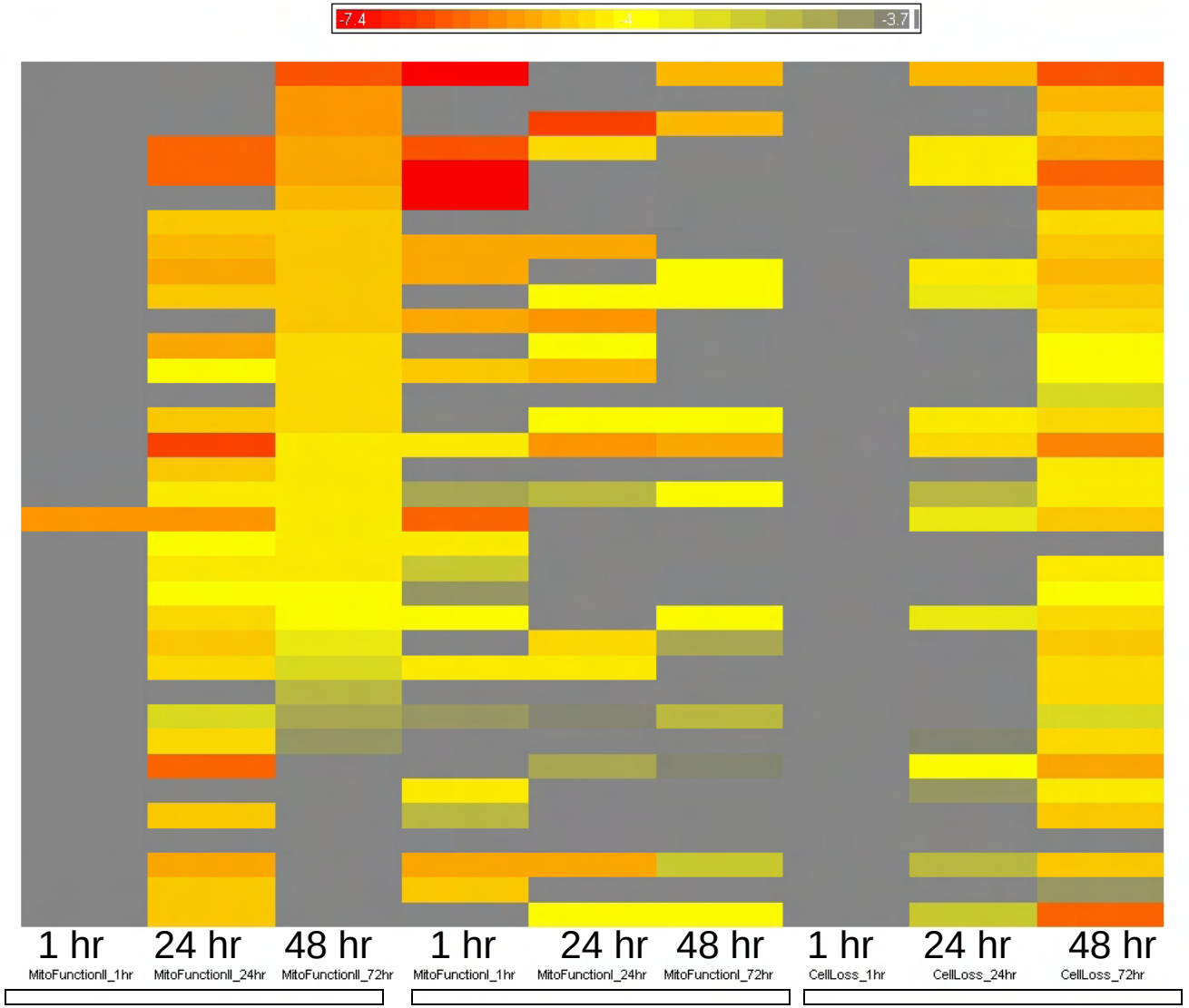
- **Multiple mechanisms of toxicity**
- **Acute, early & chronic exposure**
- **384-well capacity**
- **HepG2**
- **1º rat hepatocytes**

CellCiphr™ Cytotoxicity Panel

- 10-point conc-response (200 μM-39 nM)
- Three time points (1 hr, 24 hr, 72 hr)
- 11 endpoints per assay

Biomarker	Positive Control	Z'
Stress Pathway	Anisomycin	.63
Oxidative Stress	Camptothecin	.7
Mitochondrial Function	CCCP	.55
Mitochondrial Mass	CCCP	.35
Cell Loss	Camptothecin	.56
Cell Cycle	Paclitaxel	.54
DNA Degradation	Paclitaxel	.6
Nuclear Size	Paclitaxel	.63
DNA Damage	Camptothecin	.43
Mitotic Arrest	Paclitaxel	.63
Cytoskeletal Integrity	Paclitaxel	.3

Correlation of BioSeek Mitochondrial Dysfunction Class with HCS Mitochondrial Function Endpoints

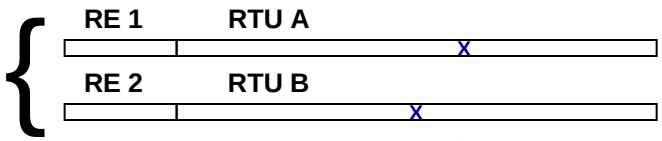


Multiplexed Reporter Gene Assay (Attagene, Inc.)

- Measures activation/inhibition of transcription factors (TF)
- TF integrate signals arising from changing cellular environments and coordinate cellular response to such change
- Similar to genomics but many fewer TF than genes
- Compounds with similar mechanism of toxicity should bear similar patterns
- Patterns should reflect the changes that precede or accompany the compounds' toxicity
- Use signatures for prediction of toxicological outcomes of compounds

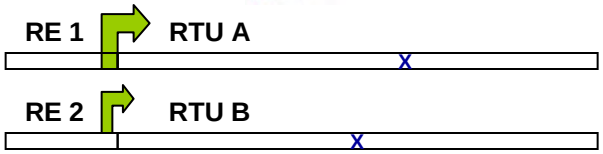
Multiplexed Reporter Gene Assay

Library of RTUs



Cell Transfection

Transcription

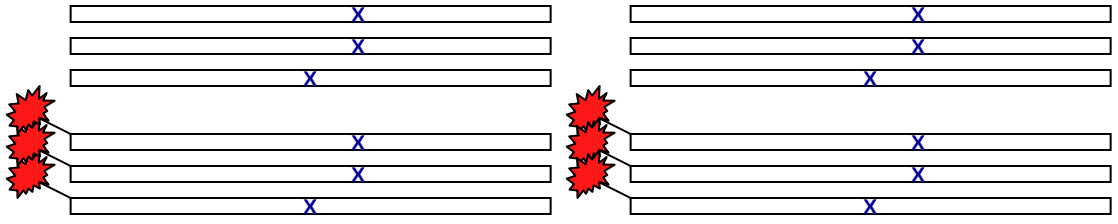


RNA Isolation

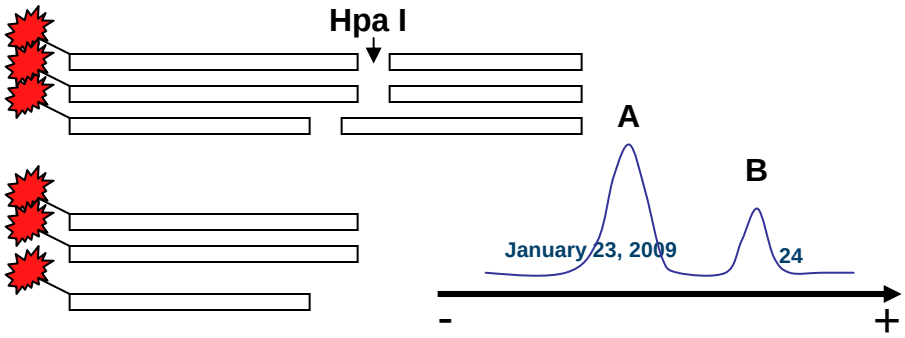
Reverse transcription

PCR amplification

Labeling

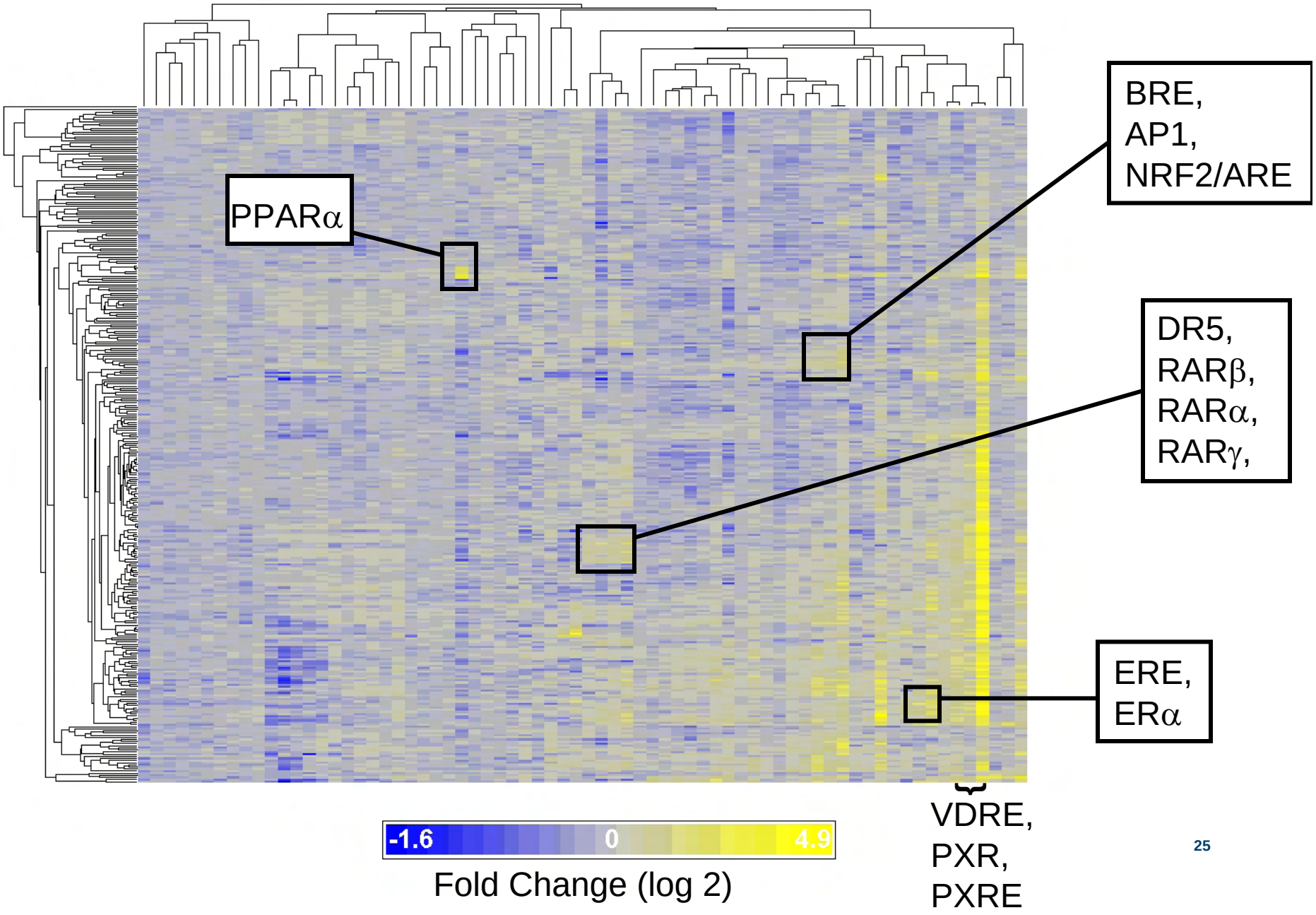


Processing (Hpa I)



Separation and detection
(capillary electrophoresis)

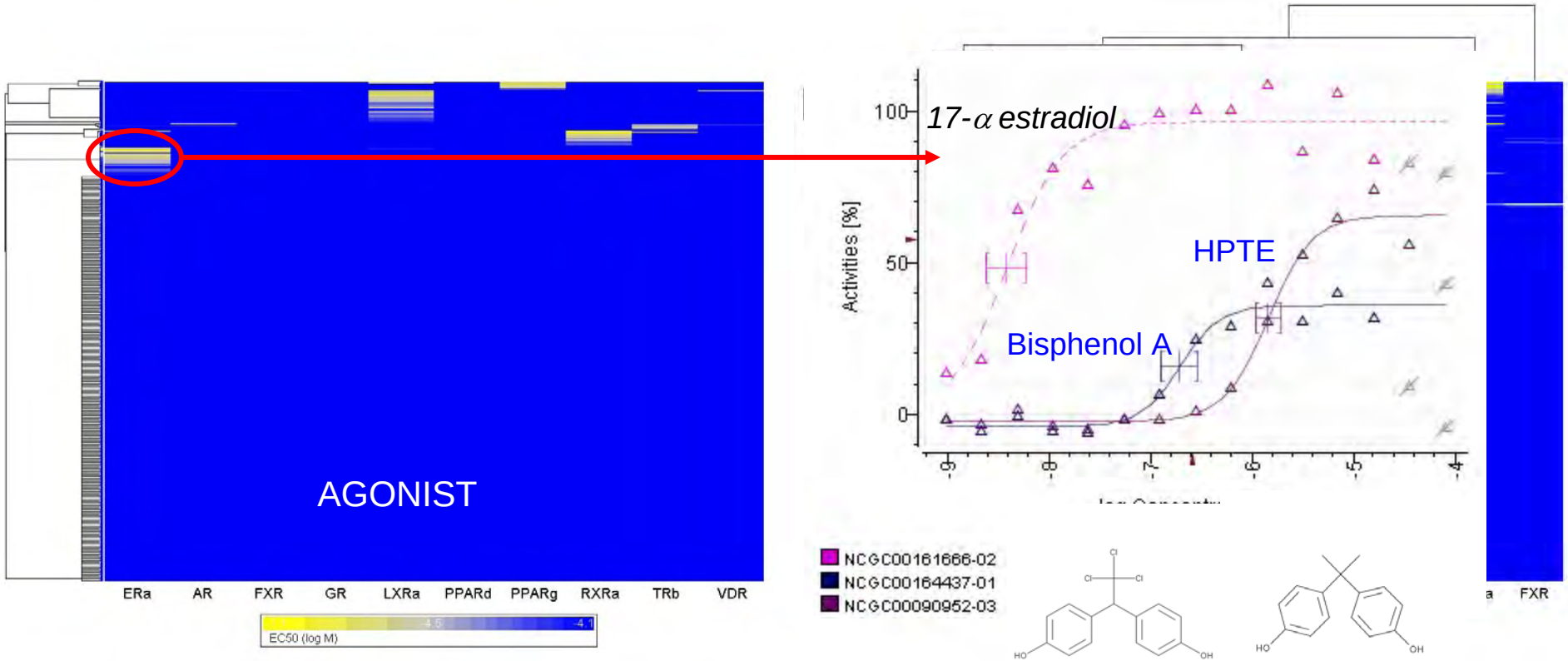
Hierachical Cluster Attagene Results



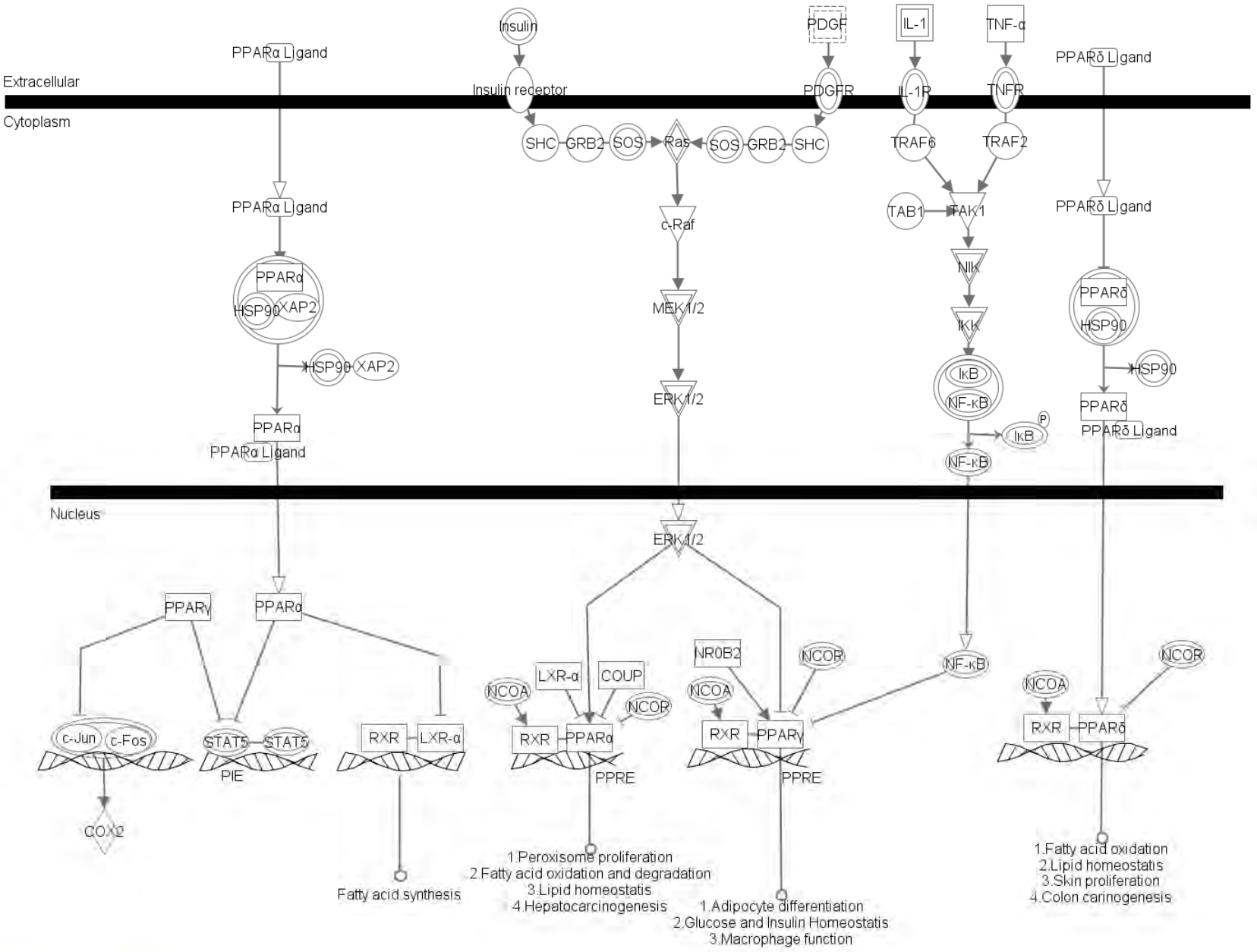
EPA United States Environmental Protection Agency

Nuclear Receptor Screening (NCGC)

- 10 Nuclear Receptors (more in queue)
- Cellular Reporter Assays
- Agonist and Antagonist modes
- Concentration-Response Format (15 conc)



PPAR Signaling



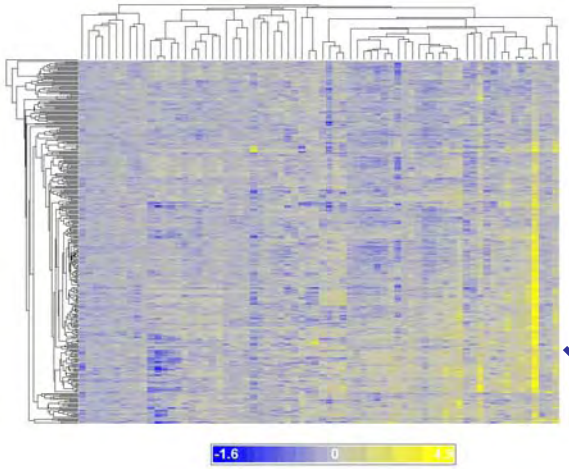
ToxCast Covers a Wide Swath of Biological Space

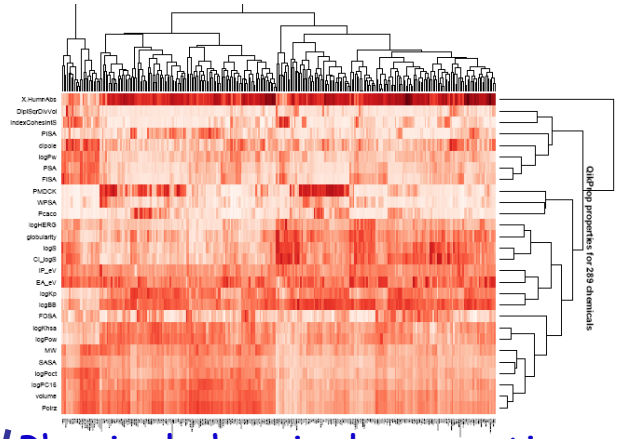
Molecular Pathways Identified by Analyses of ToxCast Assays

	GeneGO	Ingenuity	David-KEGG	Total GeneID
Human	81	60	42	317
Rat	34	39	9	51

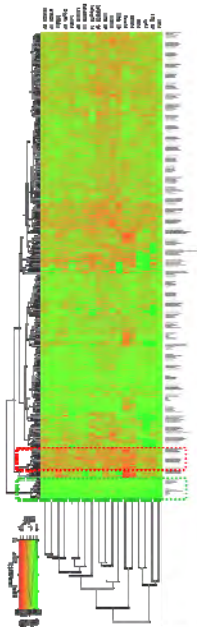
ToxCast Data Analysis

EPA United States Environmental Protection Agency



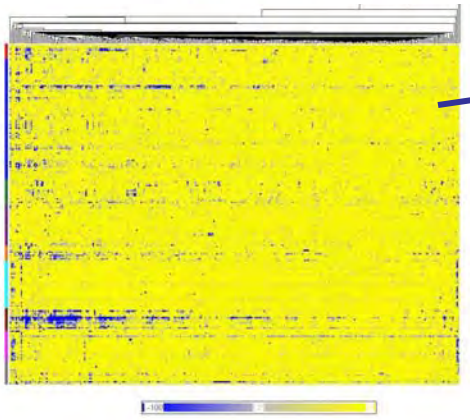


Physical chemical properties

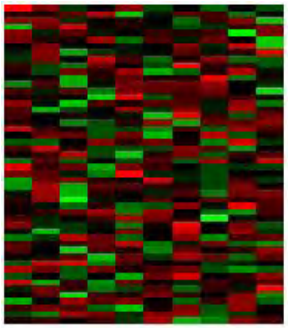


In silico Predictions

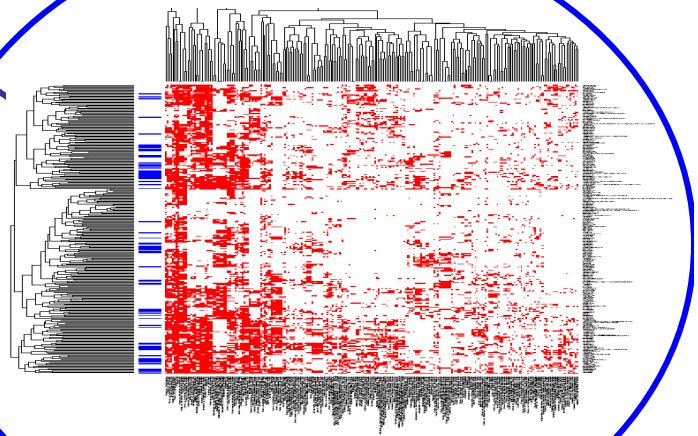
Profile Matching



Biochemical Assays

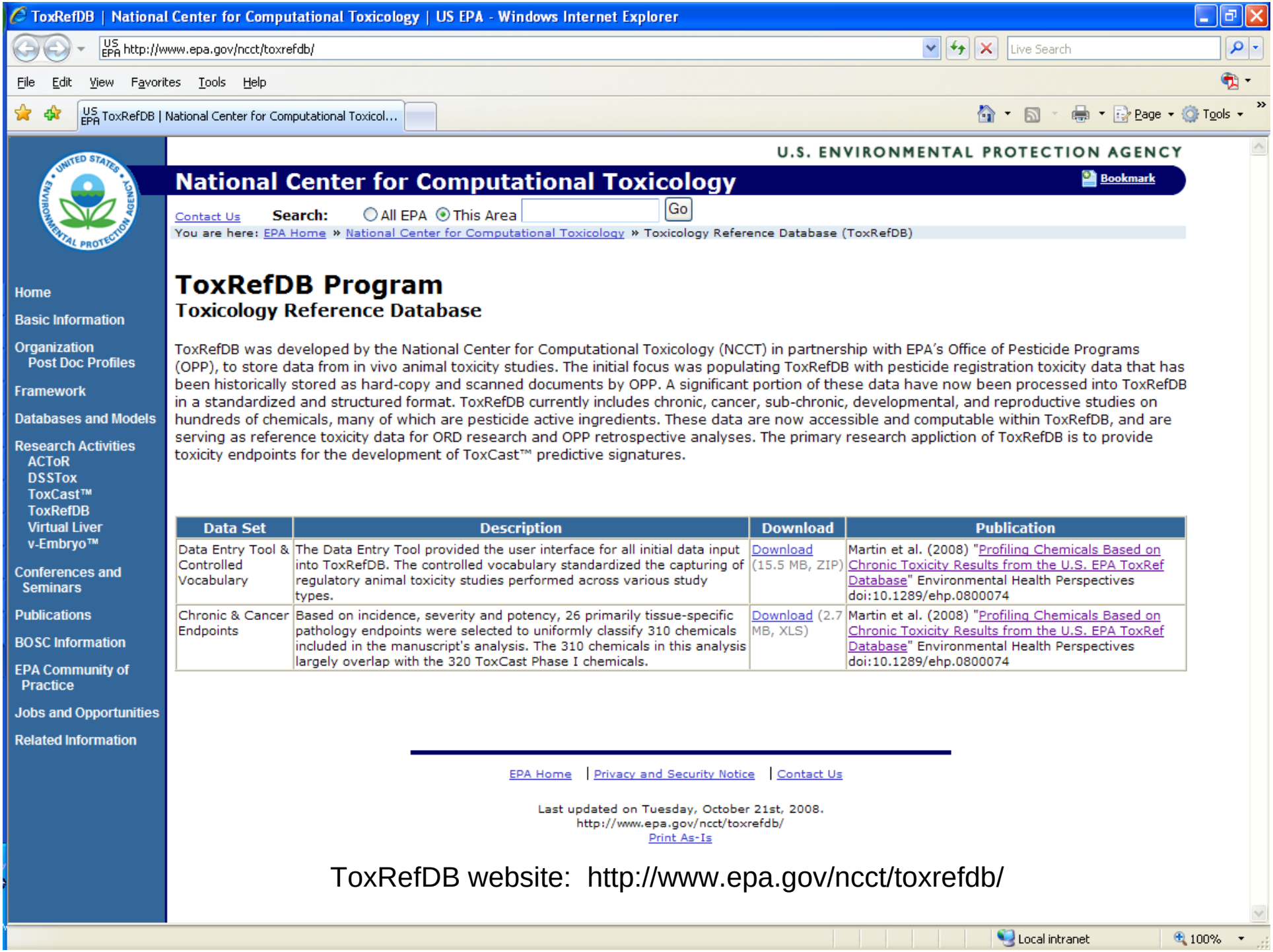


Genomic Signatures



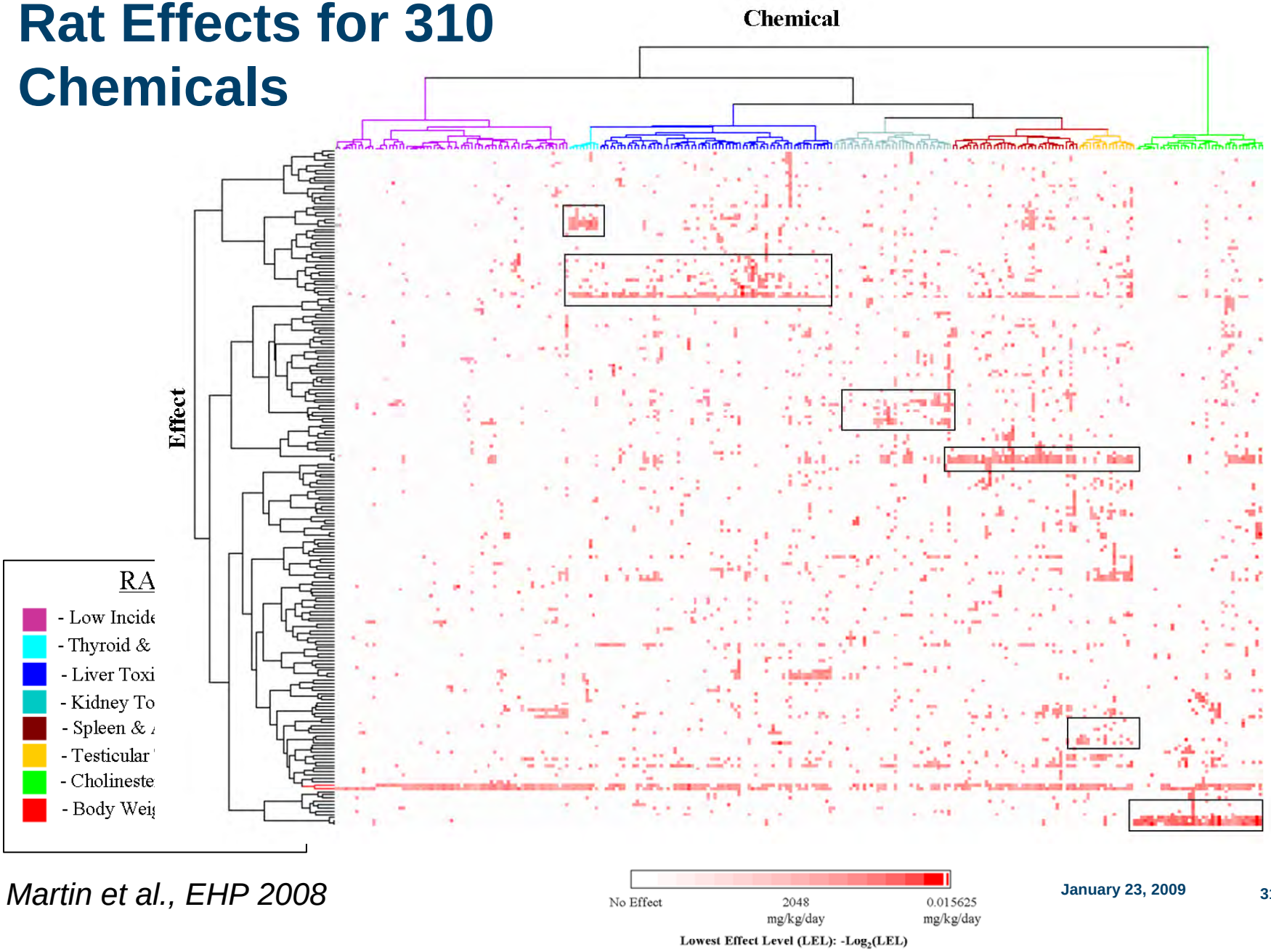
Toxicology Endpoints

Find “Signatures” from *in vitro* & *in silico* assays that predict *in vivo* endpoints.



ToxRefDB website: http://www.epa.gov/ncct/toxrefdb/

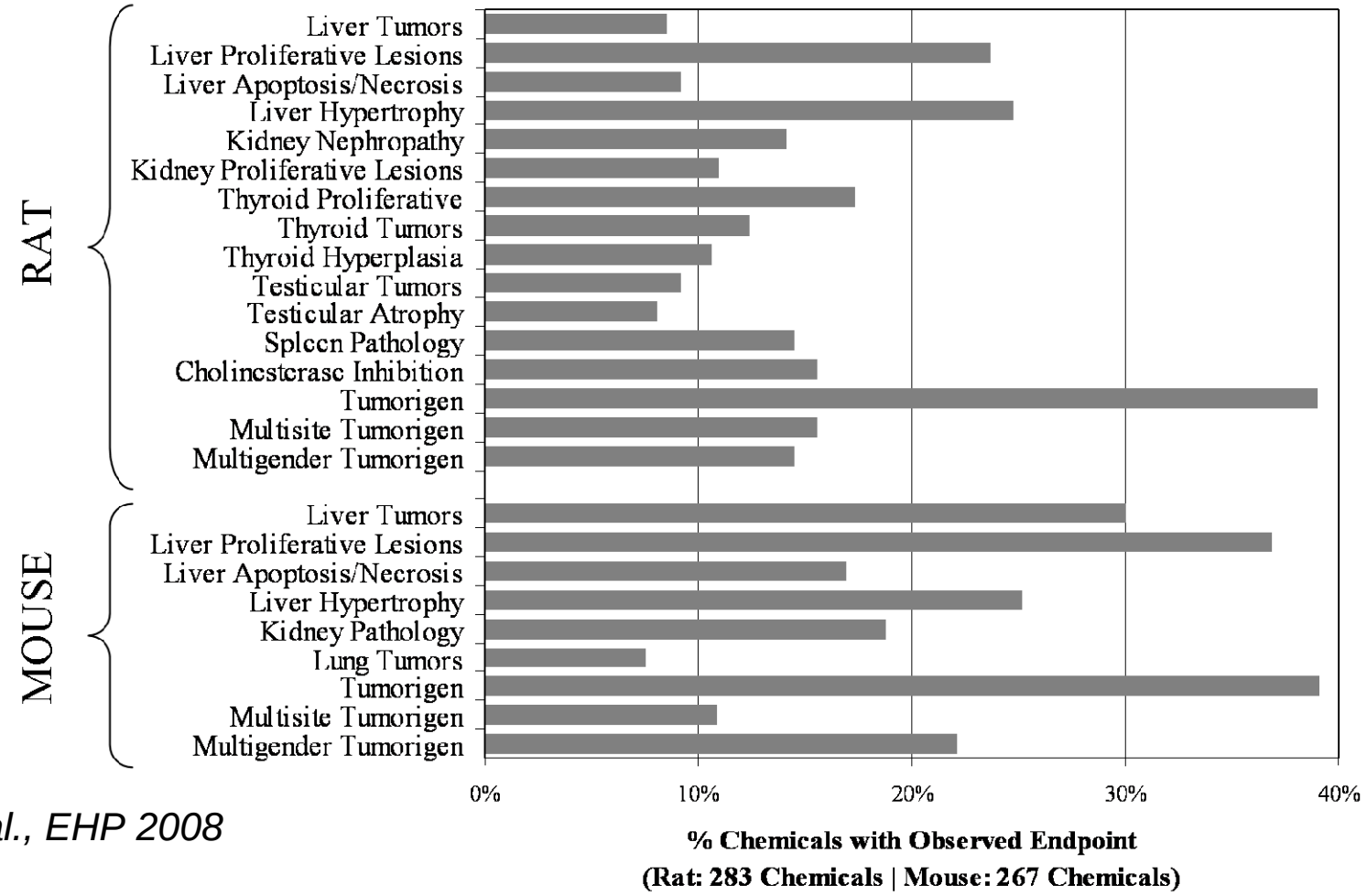
ToxRefDB Chronic Rat Effects for 310 Chemicals



Martin et al., EHP 2008

United States Environmental Protection Agency

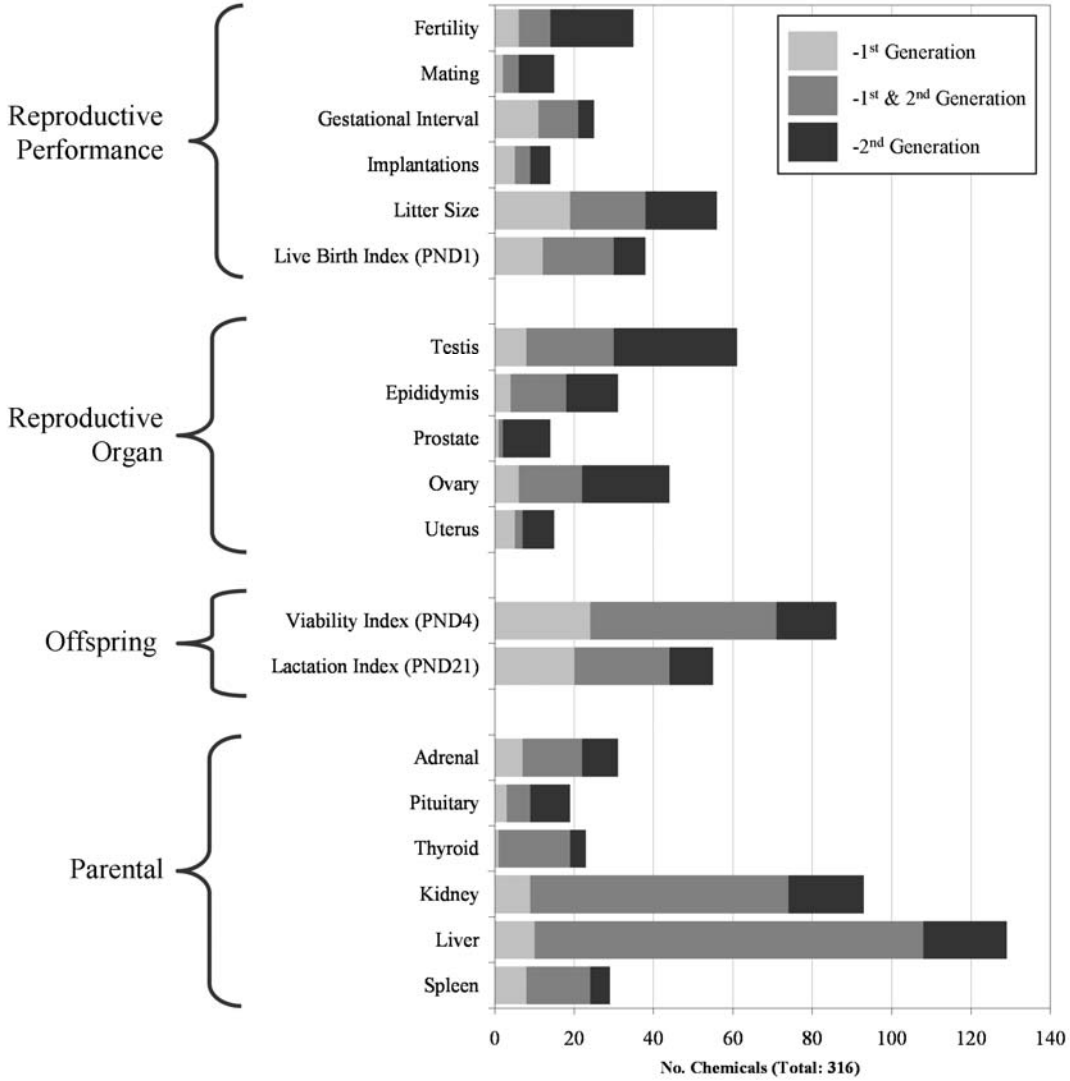
Selected Chronic Rat & Mouse Endpoints for ToxCast Predictive Modeling



Martin et al., EHP 2008

EPA United States Environmental Protection Agency

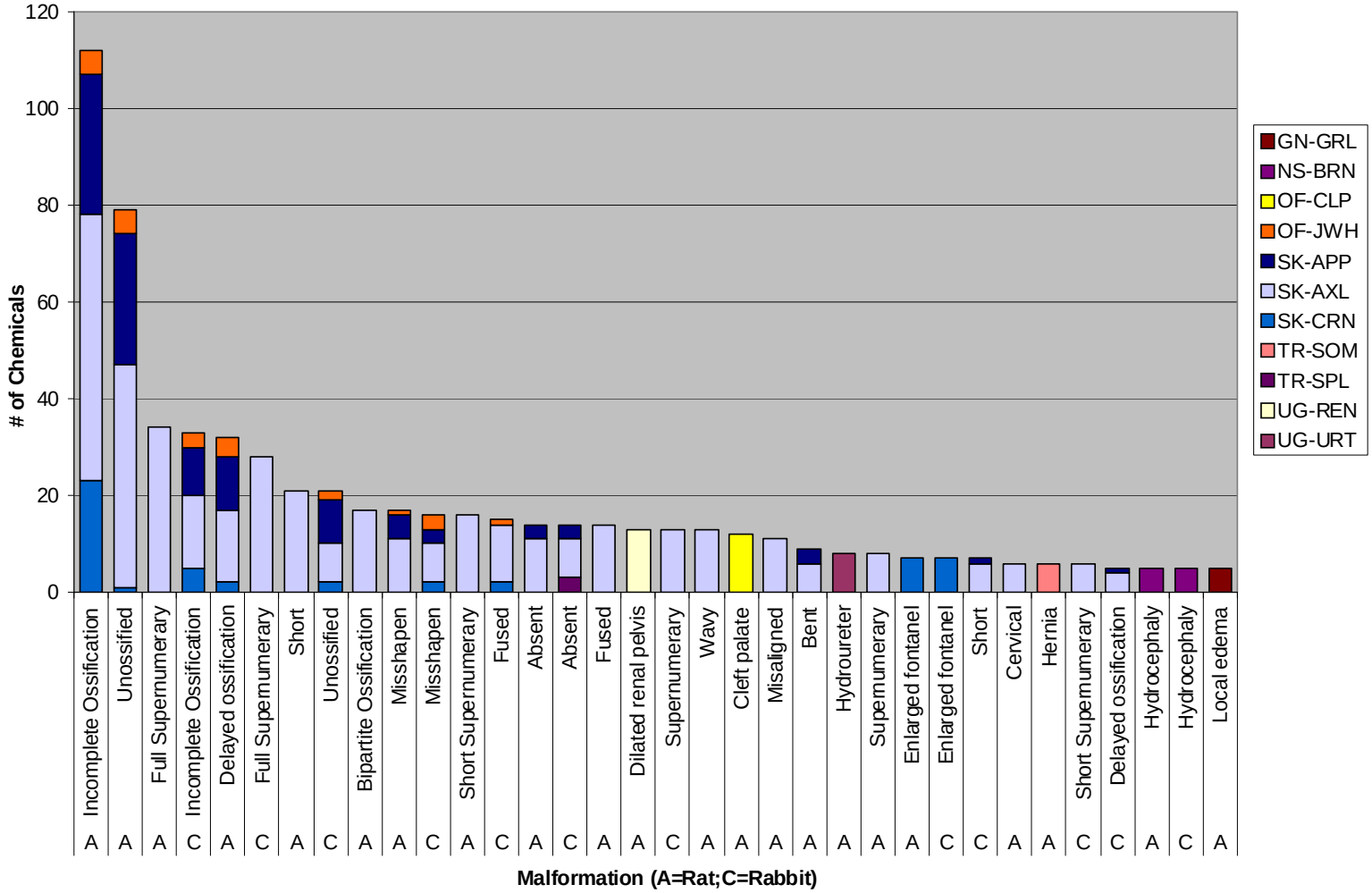
Selected Rat Reproductive Endpoints for ToxCast Predictive Modeling



Martin et al., subl

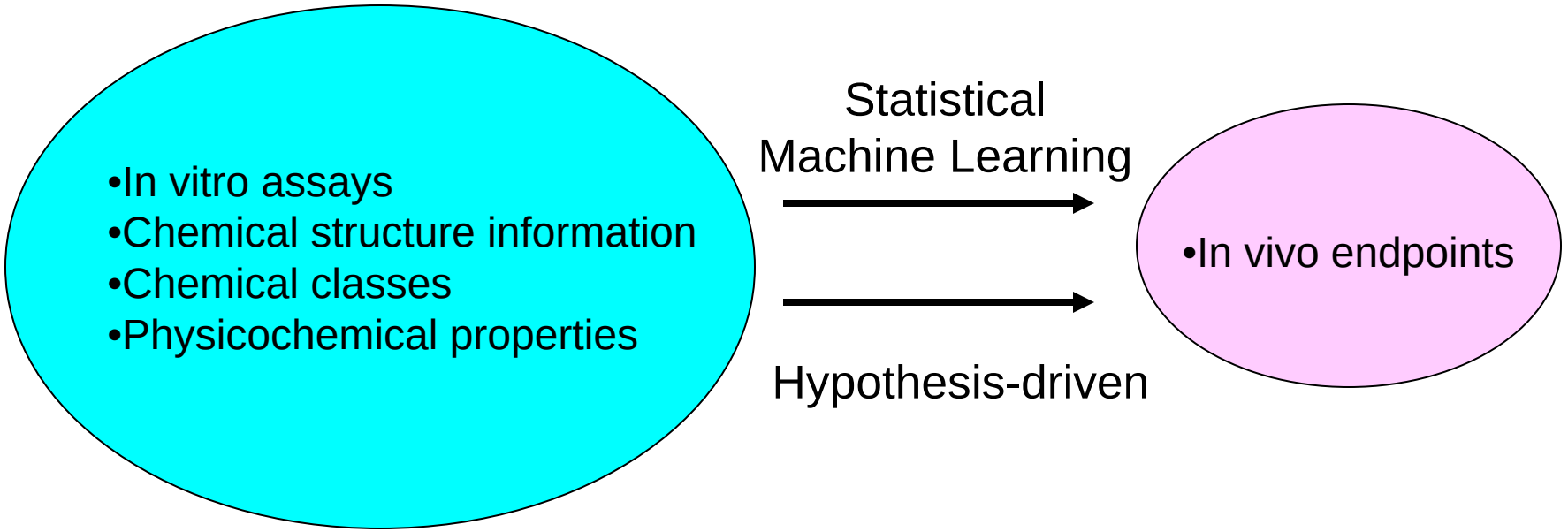
United States Environmental Protection Agency

Selected Developmental Rat & Rabbit Endpoints for ToxCast Predictive Modeling



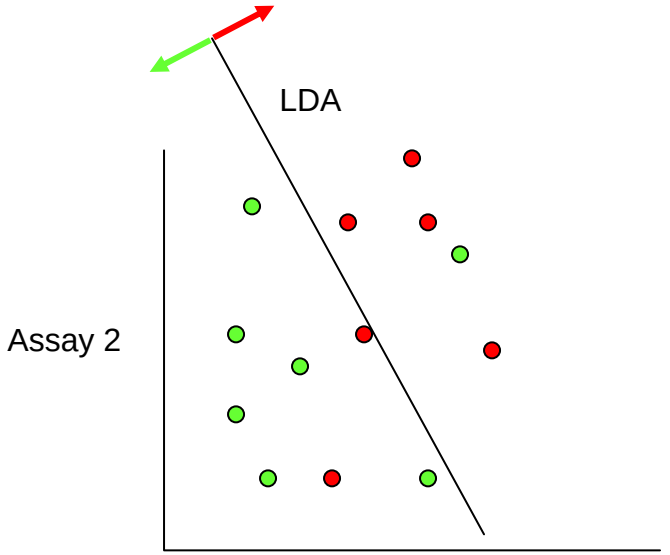
United States Environmental Protection Agency

ToxCast Analysis Approaches



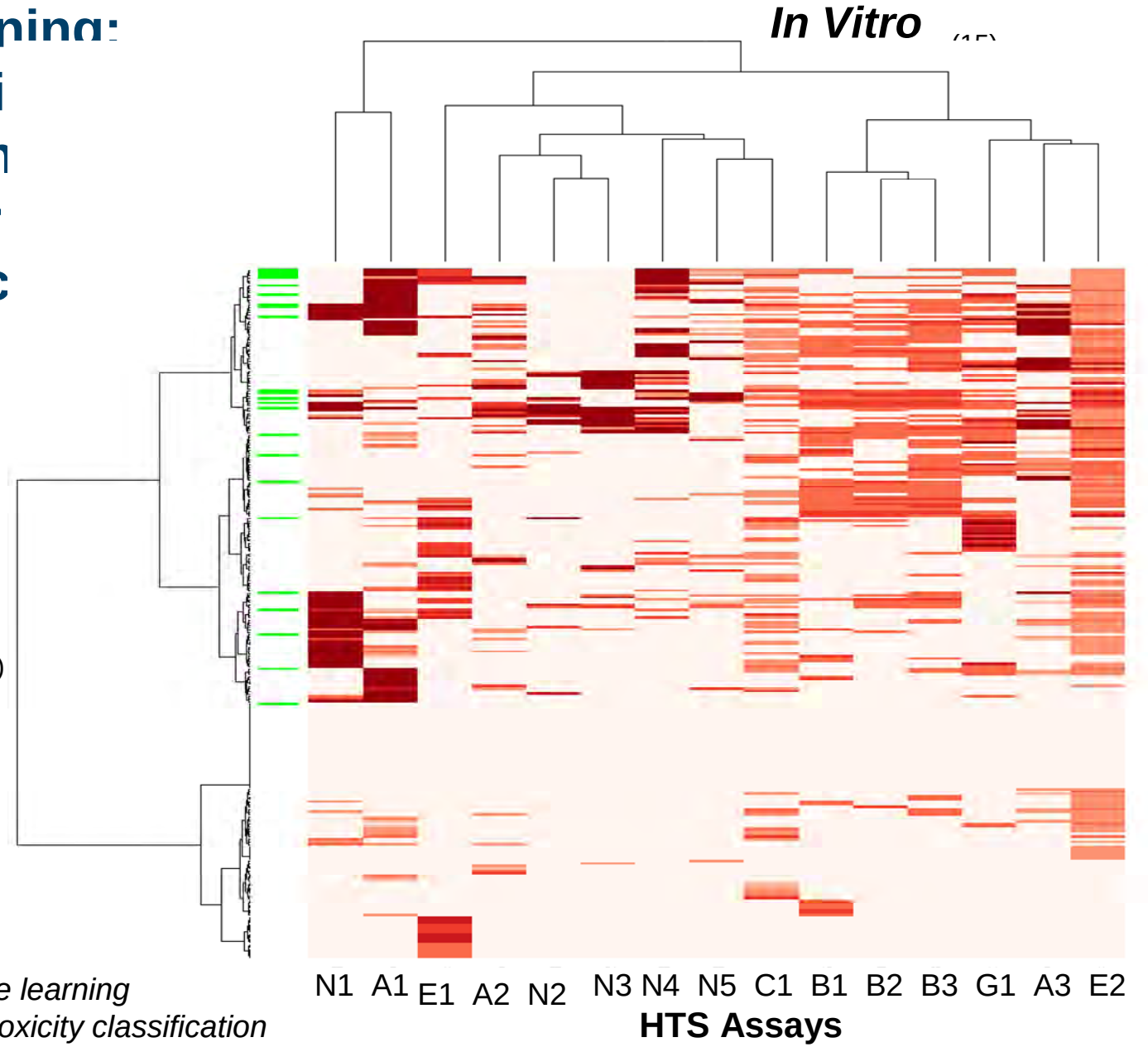
Association Analysis / Signatures

- Use Machine Learning methods
  - SLR: Stepwise Logistic Regression
  - LDA: Linear Discriminant Analysis
  - SVM: Support Vector Machines
  - Many others
- For each binary endpoint, build models of form
  - *Predictor* = *F*(assay values)
  - If
    - *Predictor* for a chemical meets criteria
  - Then
    - Predict endpoint to be positive for the chemical



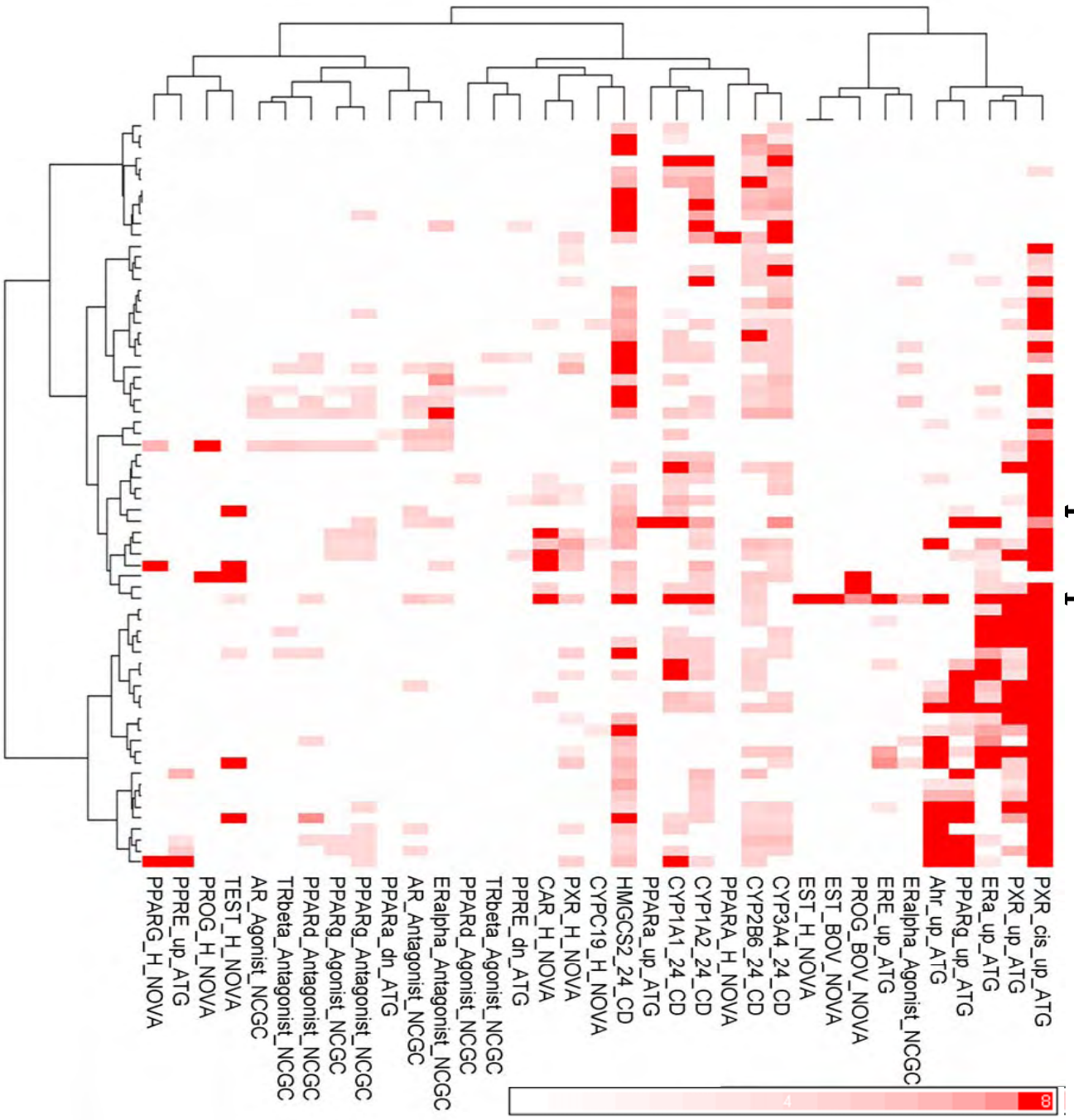
Test \ Truth	+	-
+	TP	FP
-	FN	TN

Machine Learning: ToxCast Predi Modeling of Ch Rat Liver Apoptosis/Nec



Methods described in:
Judson et al 2008
A comparison of machine learning algorithms for chemical toxicity classification using a simulated multi-scale data model.
BMC Bioinformatics 9:241

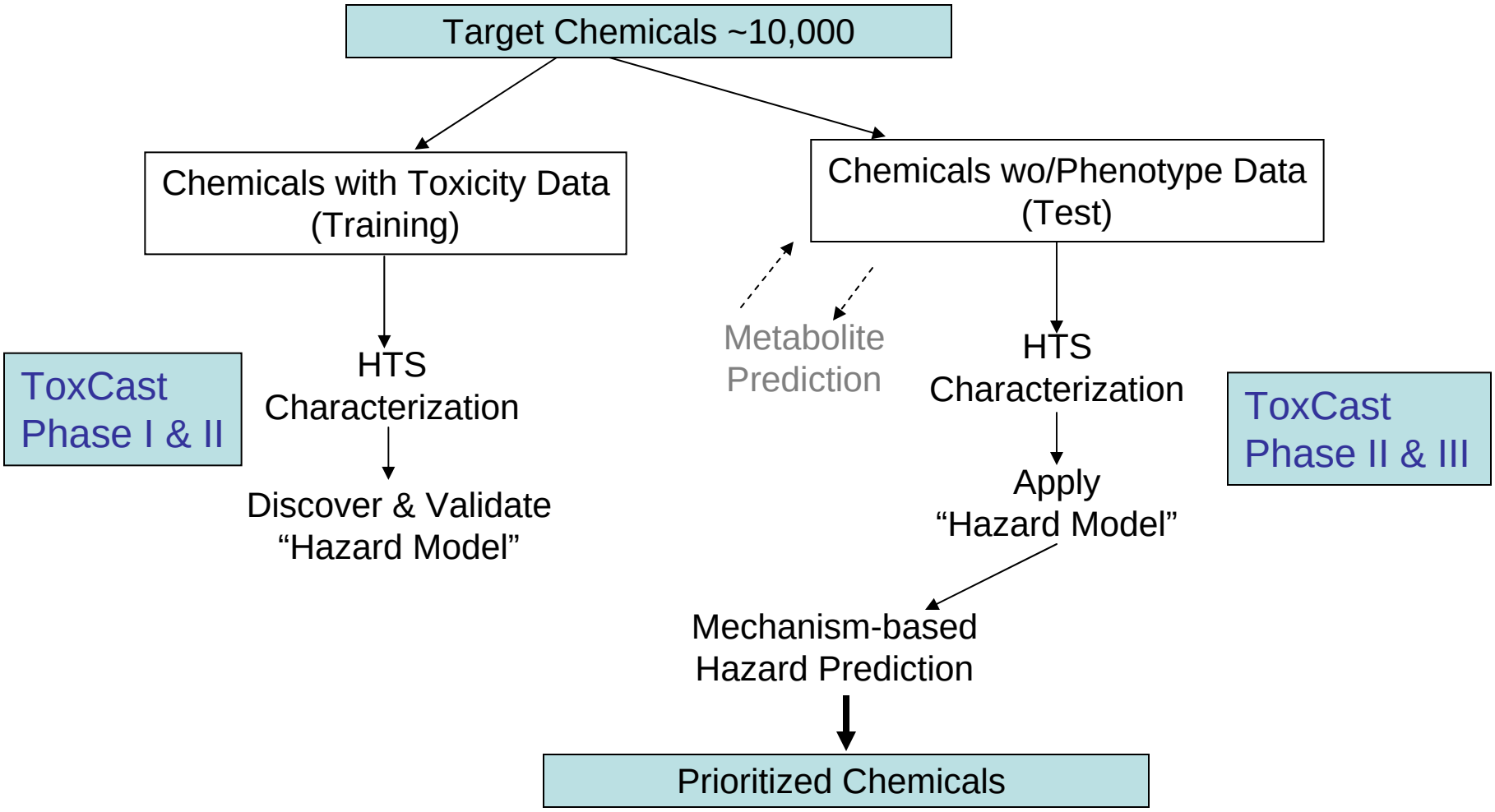
Hypothesis Driven: ToxCast Endocrine Profiling



Vinclozolin

Bisphenol A

ToxCast Screening and Prioritization



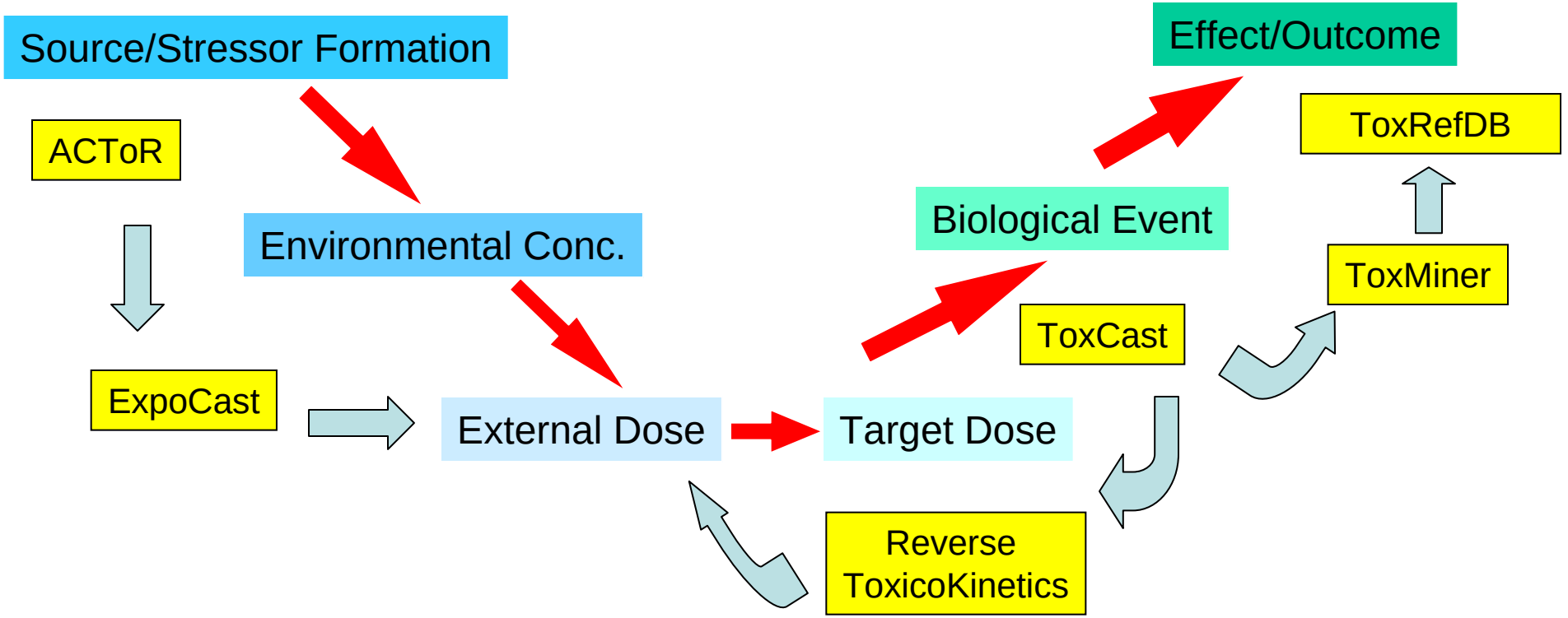
EPA United States Environmental Protection Agency

Moving Forward with ToxCast

- First predictive toxicity signatures based on ToxCast data submitted for publication April 2009
- ToxCast data available to collaborators now, publicly available May 2009 at 1st ToxCast Data Analysis Summit
- EPA & partners examining methods for analyzing ToxCast data, identifying predictive signatures from Phase I for validation in Phase II
- Phase II testing will commence June 2009 on upwards of 700 additional chemicals.

United States Environmental Protection Agency

Applying Computational Toxicology Along the Source to Outcome Continuum



ToxCast Website: www.epa.gov/ncct/toxcast

United States Environmental Protection Agency

National Center for Computational Toxicology

Contact Us Search: All EPA This Area Go

You are here: EPA Home » National Center for Computational Toxicology » ToxCast™ Program

ToxCast™ Program

Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

Introduction

In 2007, EPA launched ToxCast™ in order to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time. Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast™ is building computational models to forecast the potential human toxicity of chemicals. These hazard predictions will provide EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, and lead to more efficient use of animal testing.

In its first phase, ToxCast™ is profiling over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints. These endpoints include biochemical assays of protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebrafish embryos. Almost all of the compounds being examined in Phase 1 of ToxCast™ have been tested in traditional toxicology tests, including developmental toxicity, multi-generation studies, and sub-chronic and chronic rodent bioassays. ToxRefDB, a relational database being created to house this information, will contain nearly $1B worth of toxicity studies in animals when completed. ToxRefDB is integrated into a more comprehensive data management system developed by NCCT called ACToR (Aggregated Computational Toxicology Resource), that manages the large-scale datasets of ToxCast™. ACToR is comprised of several independent data repositories linked to a common database of chemical structures and properties, and to tools for development of predictive HTS and genomic bioactivity signatures that strongly correlate with specific toxicity endpoints from ToxRefDB. These ToxCast™ signatures will be defined and evaluated by their ability to predict outcomes from existing mammalian toxicity testing, and identify toxicity pathways that are relevant to human health effects.

The second phase of ToxCast™ will screen additional compounds representing broader chemical structure and use classes, in order to evaluate the predictive bioactivity signatures developed in Phase I. Following successful conclusion of Phases I and II, ToxCast™ will provide EPA regulatory programs an efficient tool for rapidly and efficiently screening compounds and prioritizing further toxicity testing.

ToxCast™ Navigation

- Introduction
- ToxCast™ Chemicals
- ToxCast™ Assays
- ToxCast™ Information Management
- ToxCast™ Partnerships
- ToxCast™ Contractors
- ToxCast™ Presentations
- ToxCast™ Publications
- ToxCast™ News