United States Environmental Protection Agency

EPA's TOXCAST PROGRAM FOR PREDICTING TOXICITY AND PRIORITIZING CHEMICALS FOR FURTHER SCREENING AND TESTING

American College of Toxicology Annual Meeting
Tucson, AZ
9:40-10:05 am
November 19, 2008

Keith Houck, Ph.D.

National Center for Computational Toxicology, US EPA

Research Triangle Park, NC

Office of Research and Development
National Center for Computational Toxicology

ToxCast™ : a significant part of EPA's transformation

- 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 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/*

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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

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

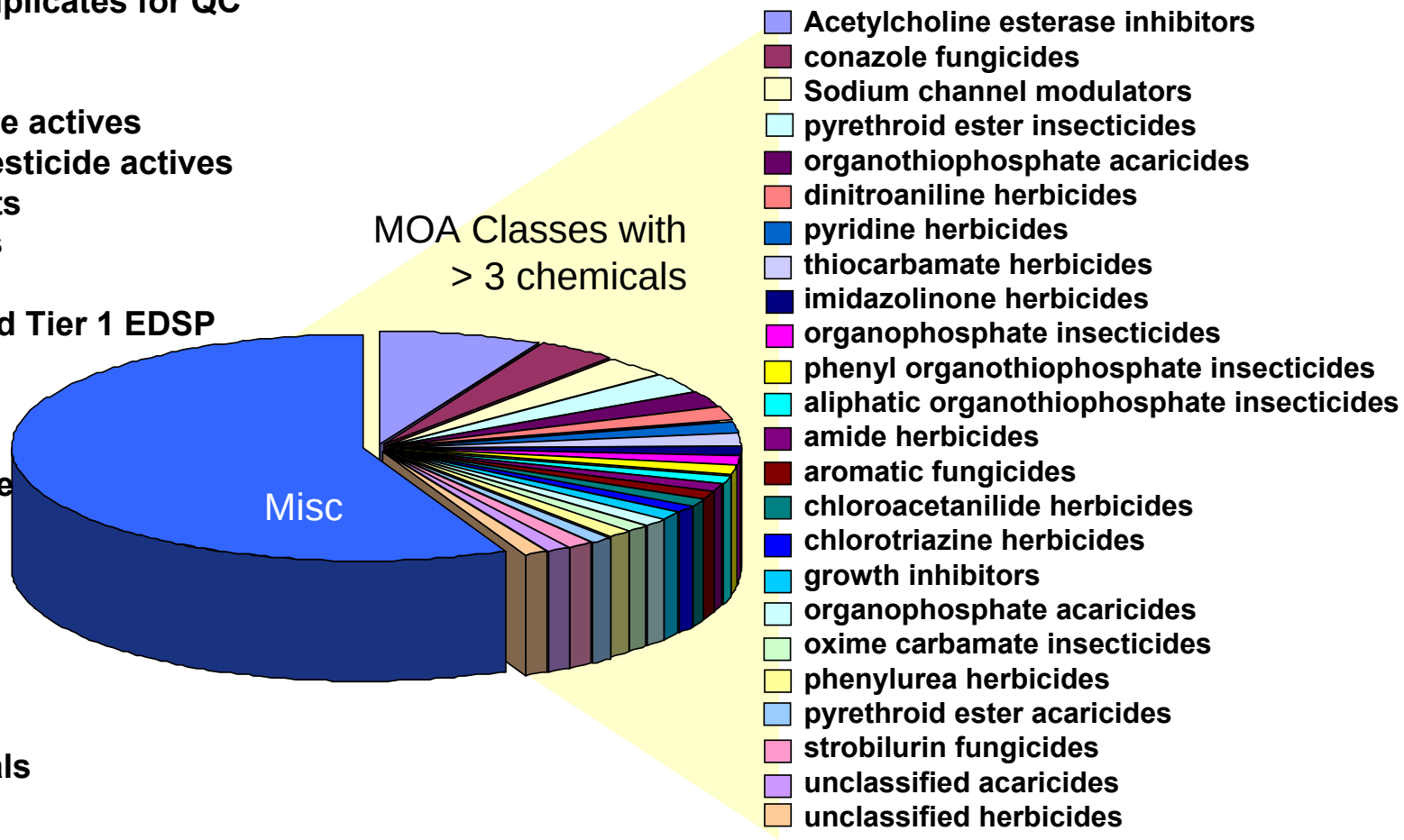
291 total pesticide actives
273 registered pesticide actives
22 pesticide inerts
33 antimicrobials

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

ToxCast Phase I Datasets

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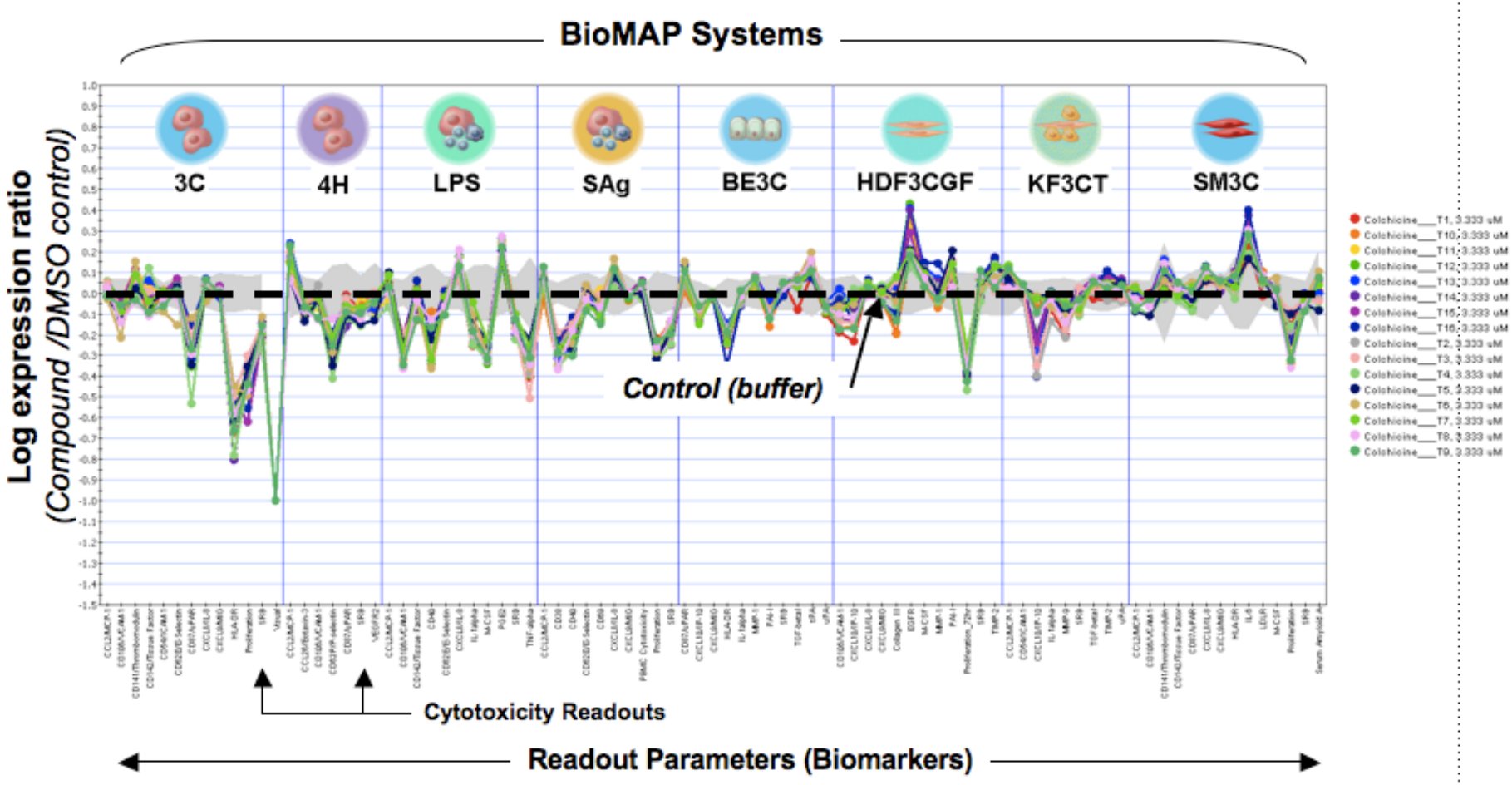
- **ToxCast 1.0 (April, 2007)**
  - *Enzyme inhibition/receptor binding HTS (Novascreen)*
  - *NR/transcription factors (Attagene, NCGC)*
  - *Cellular impedance (ACEA)*
  - *Complex cell interactions (BioSeek)*
  - *Hepatocelluar HCS (Cellumen)*
  - *Hepatic, renal and airway cytotoxicity (IVAL)*
 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

20 Assay sources
554 Endpoints

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)

Reference Compound Variability



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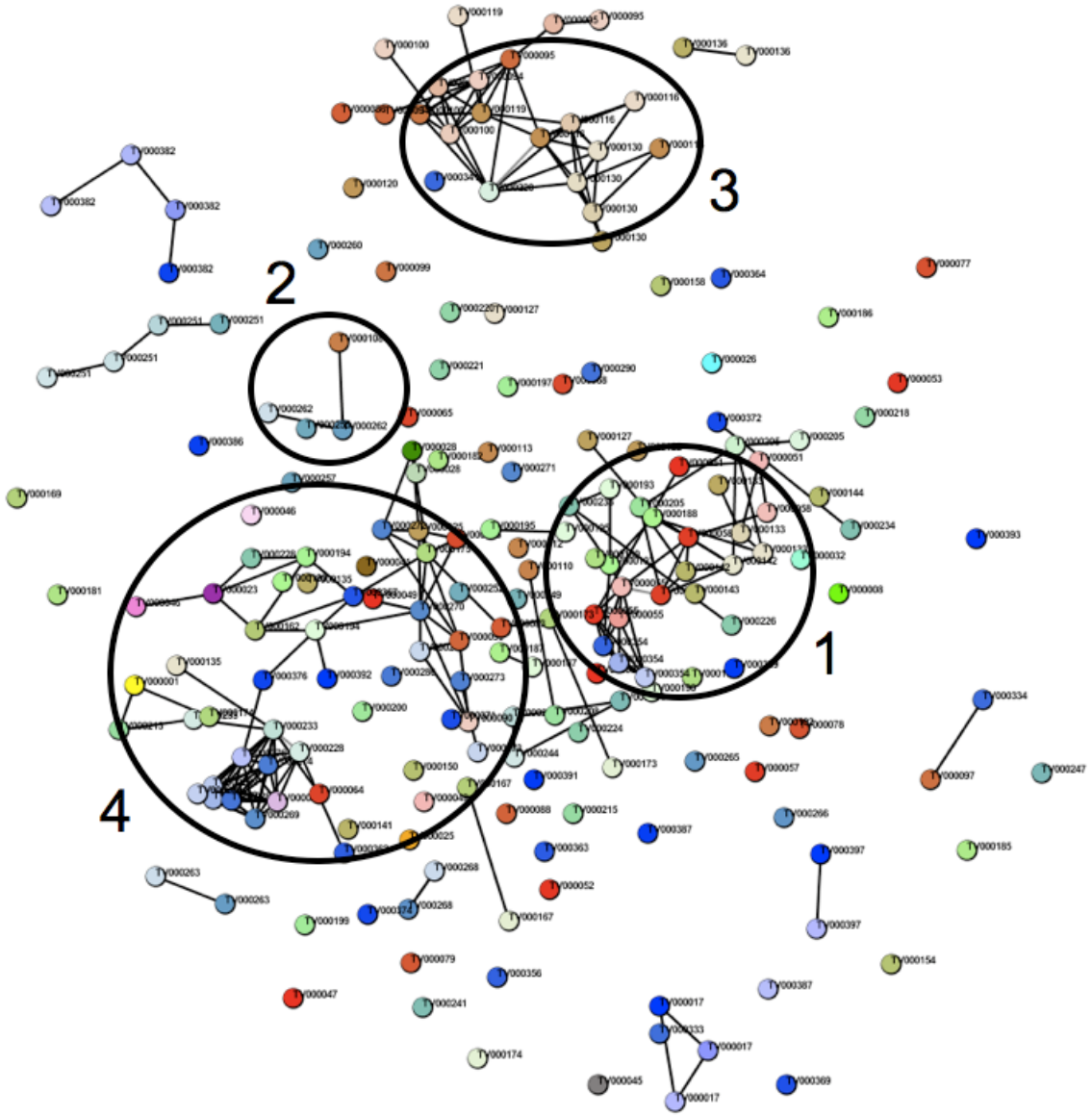
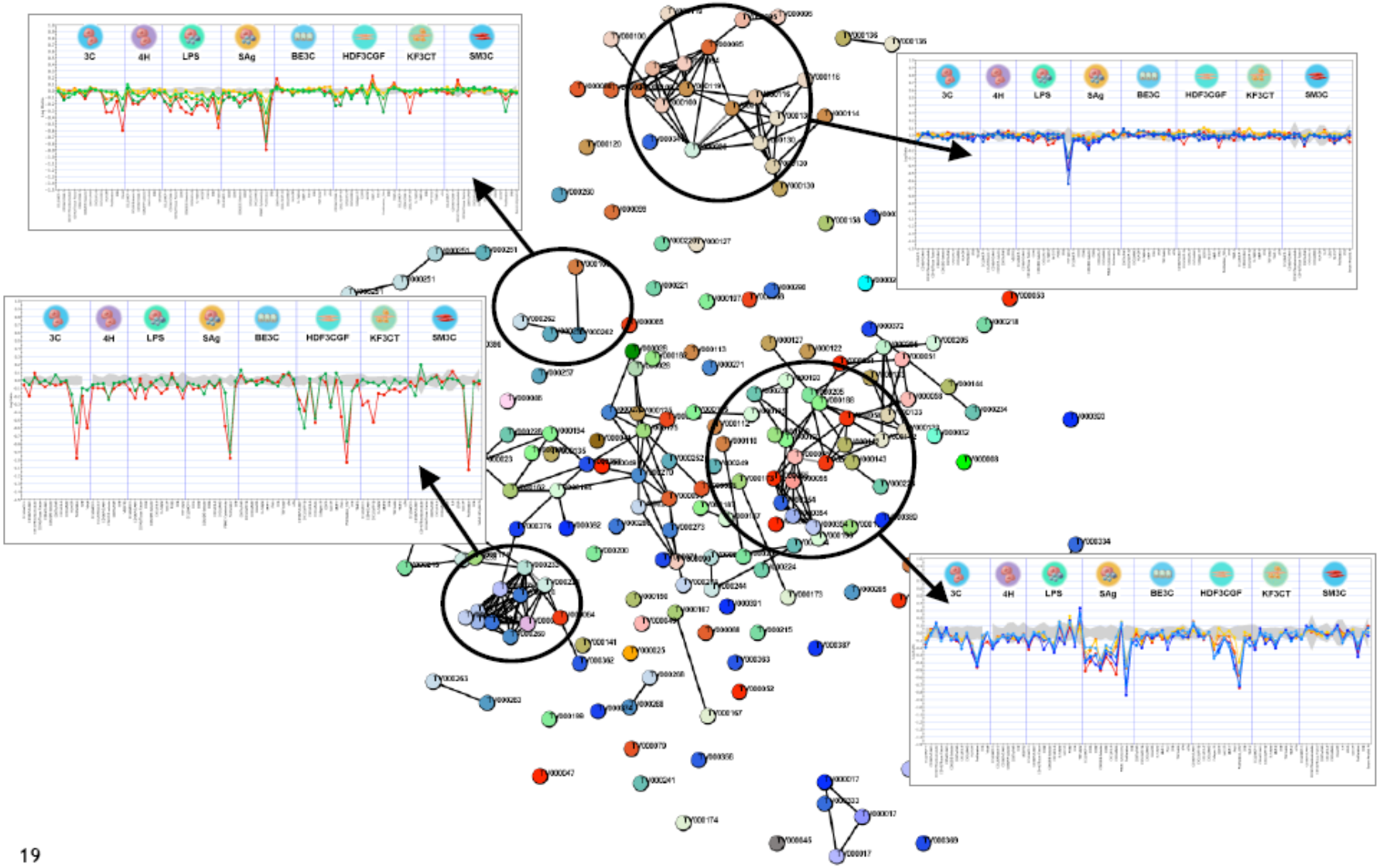
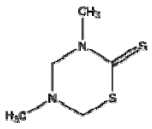
Functional Similarity Map of ToxCast Library



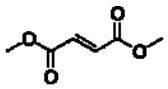
Functional Similarity Map of ToxCast Library



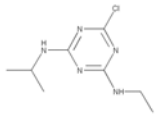
NFkB Inhibition and cAMP Stimulation Classes



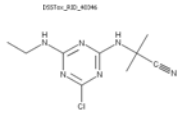
dazomet



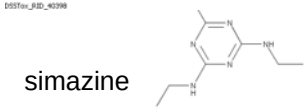
Dimethyl fumarate



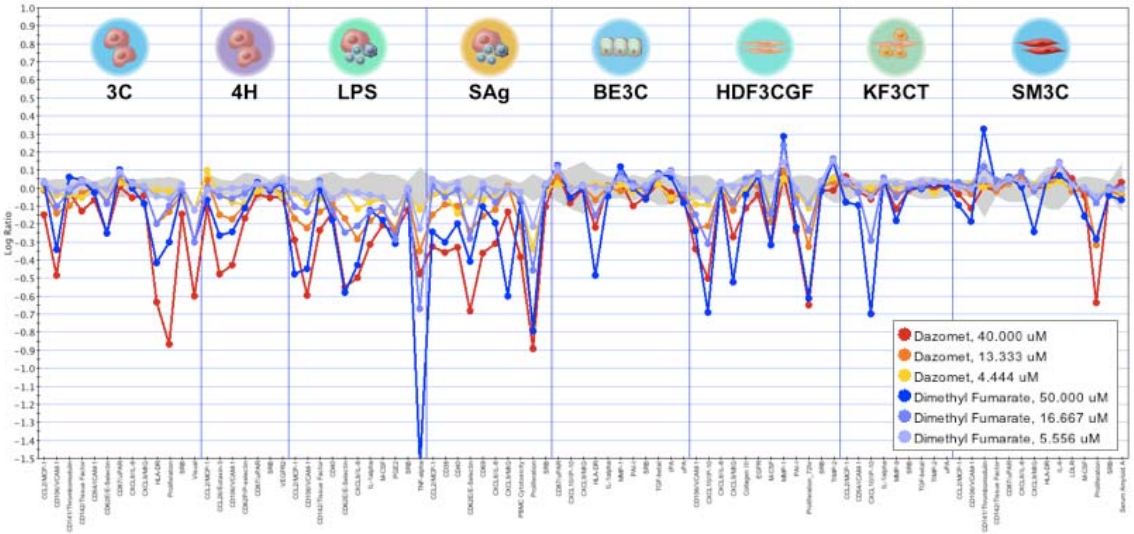
atrazine

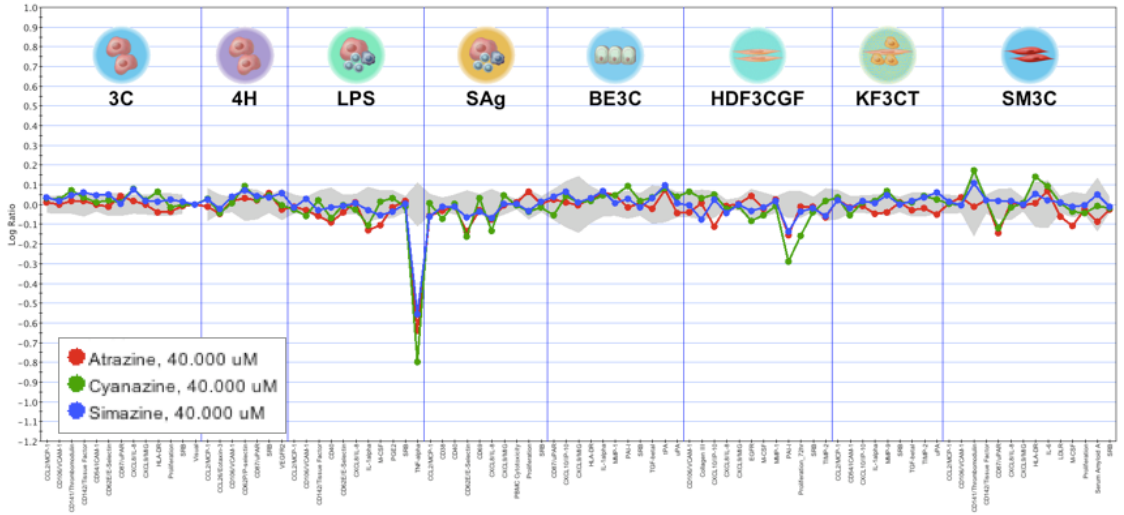


cyanazine



simazine





Mitochondrial Dysfunction and Endoplasmic Reticulum Stress Classes

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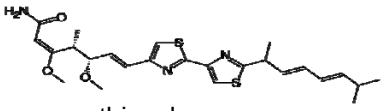
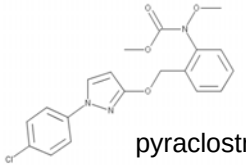
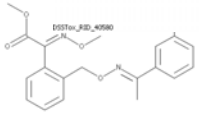


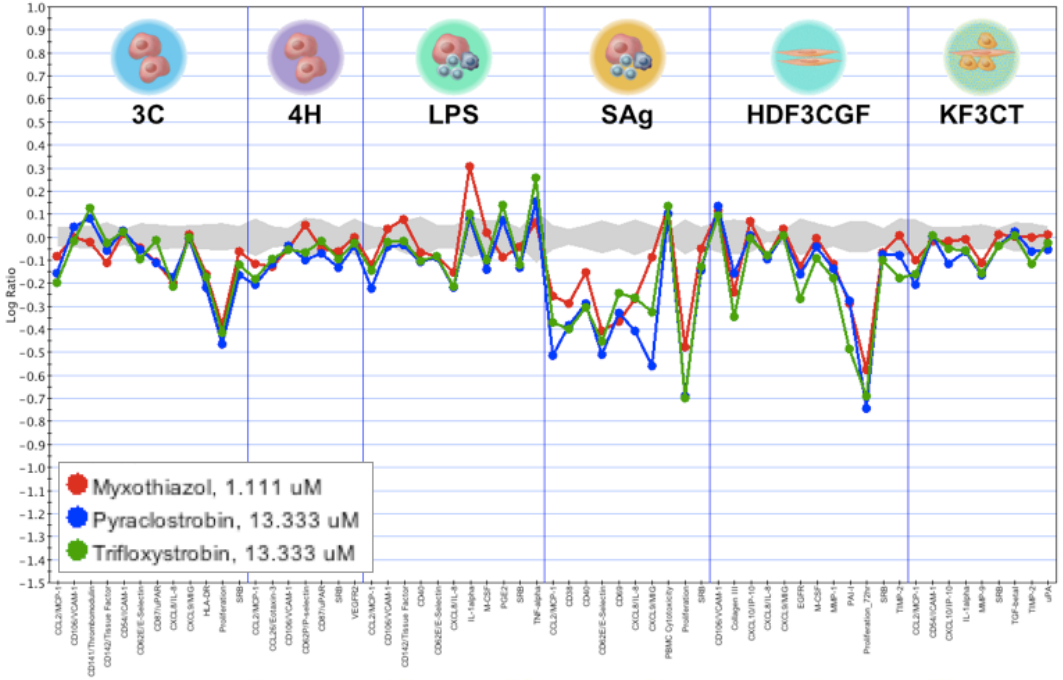
myxothiazol

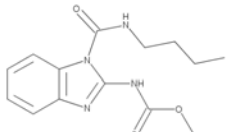


pyraclostrobin

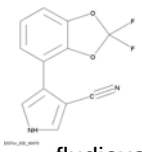


trifloxystrobin

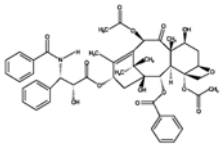




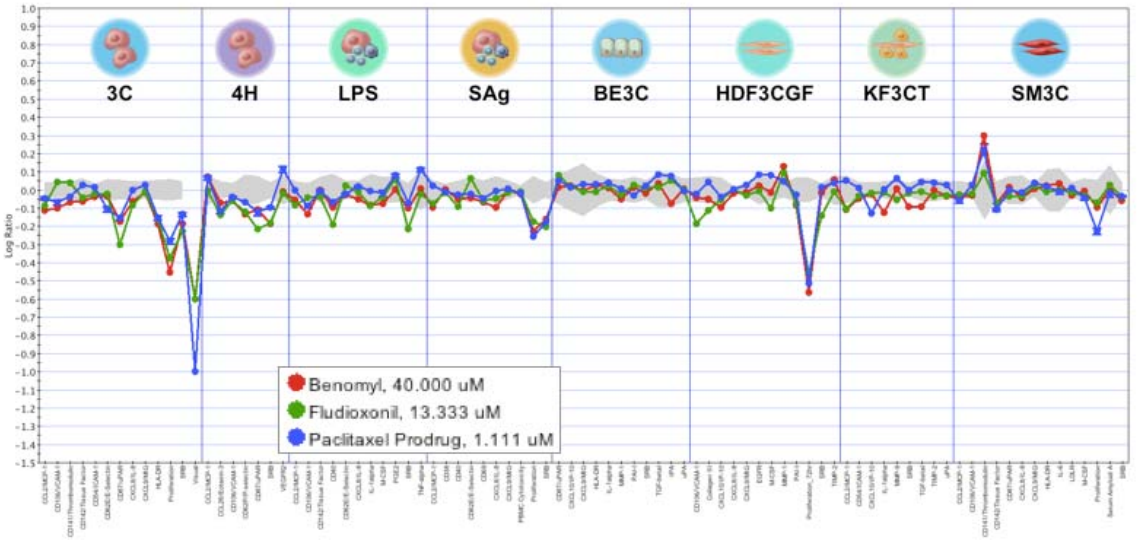
benomyl



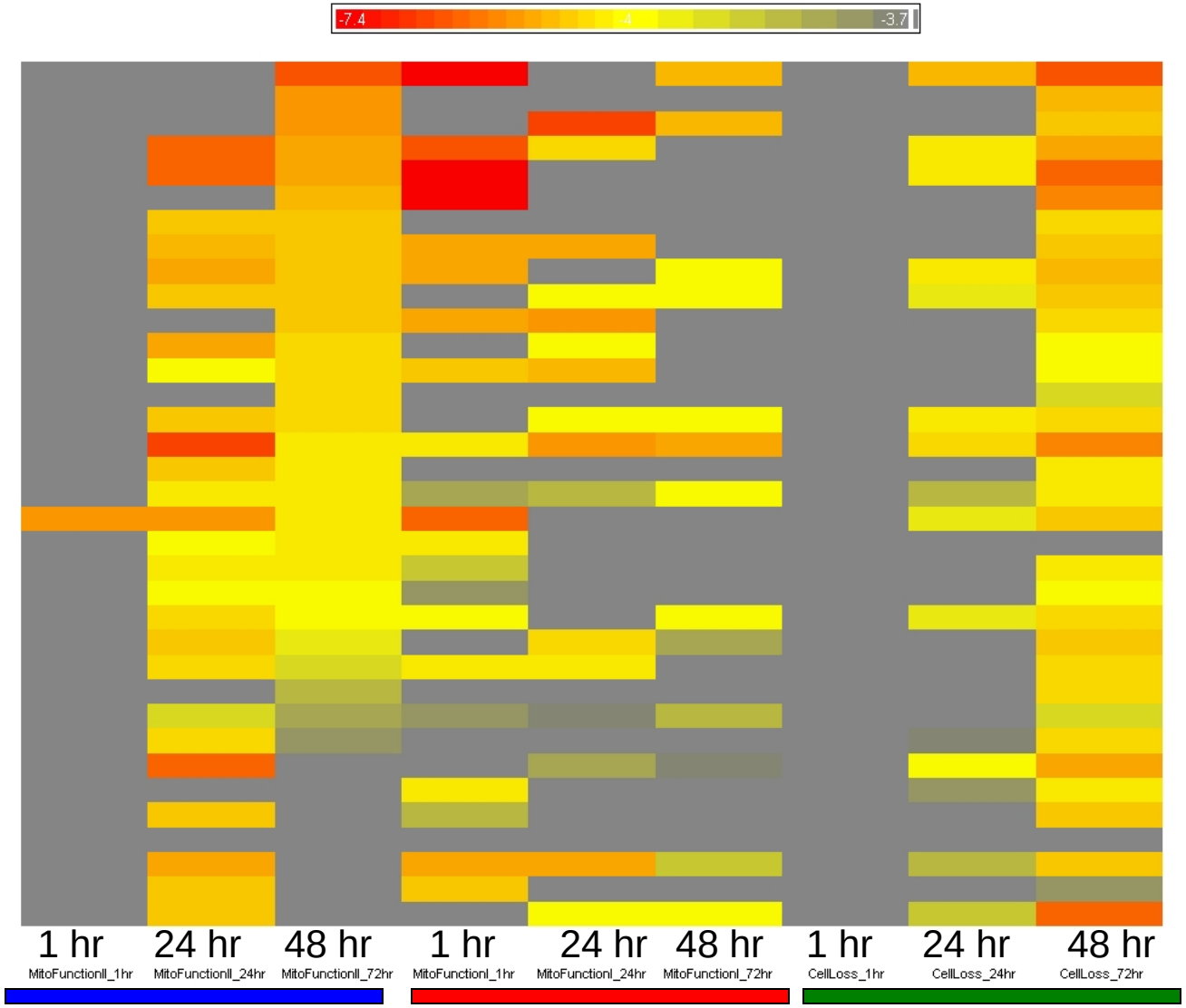
fludioxonil



paclitaxel



Correlation of BioSeek Mitochondrial Dysfunction Class with HCS Mitochondrial Function Endpoints



Multiplexed Reporter Gene Assay (Attagene , Inc.)

Library of RTUs

Cell Transfection

Transcription

RNA Isolation

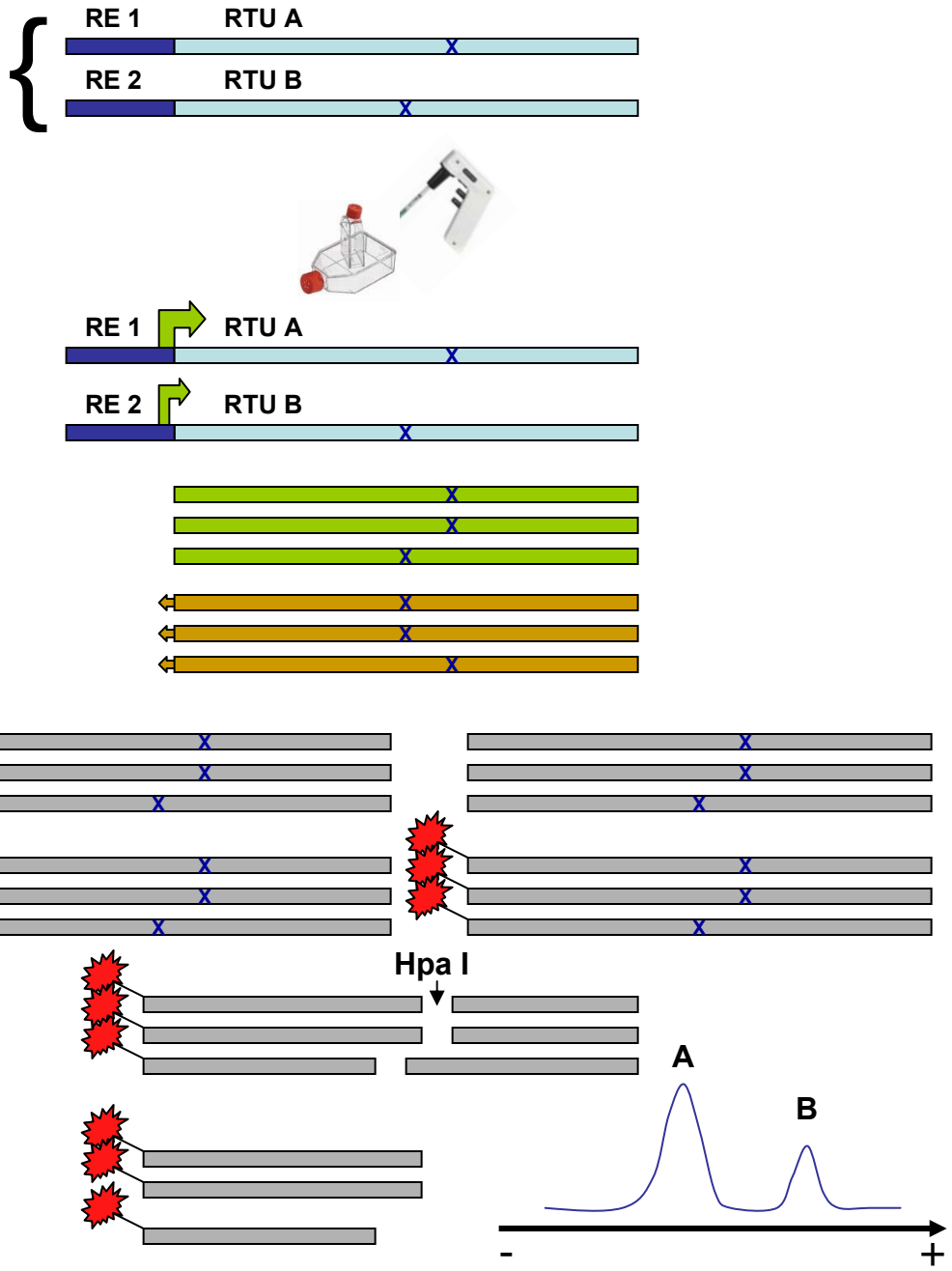
Reverse transcription

PCR amplification

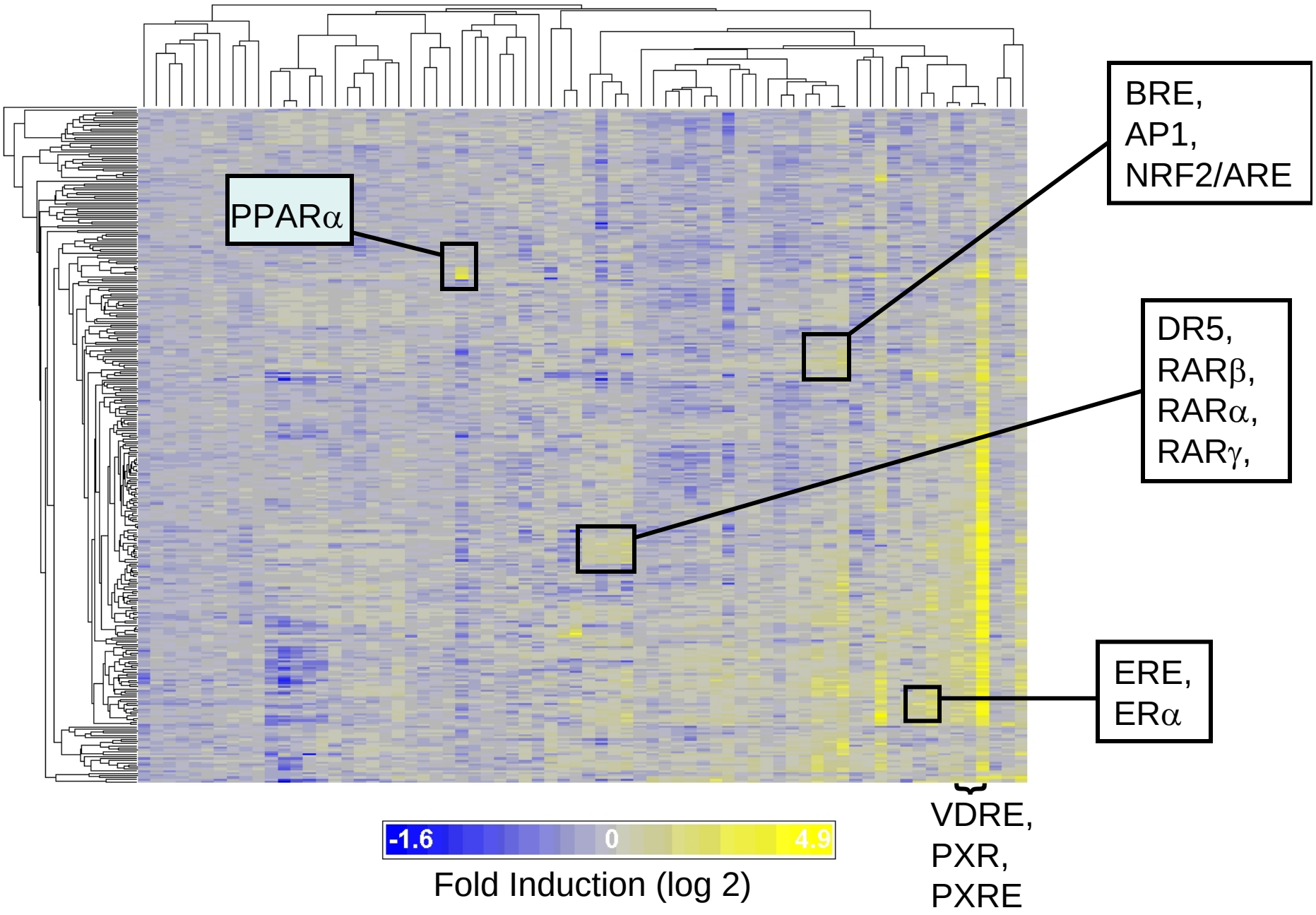
Labeling

Processing (Hpa I)

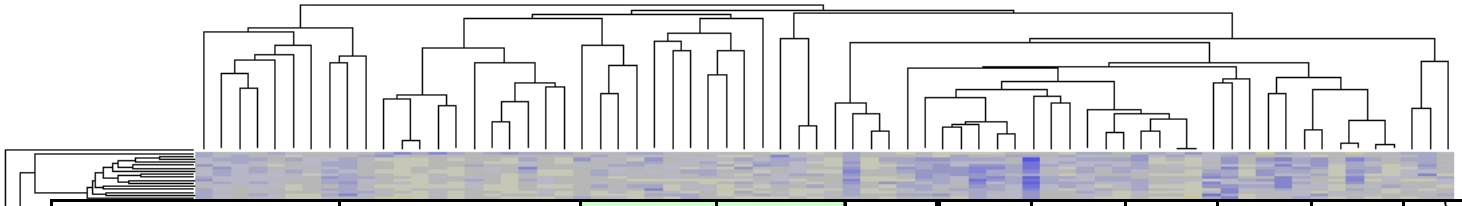
Separation and detection
(capillary electrophoresis)



Hierachical Cluster Attagene Results



Hierachical Cluster Attagene Results

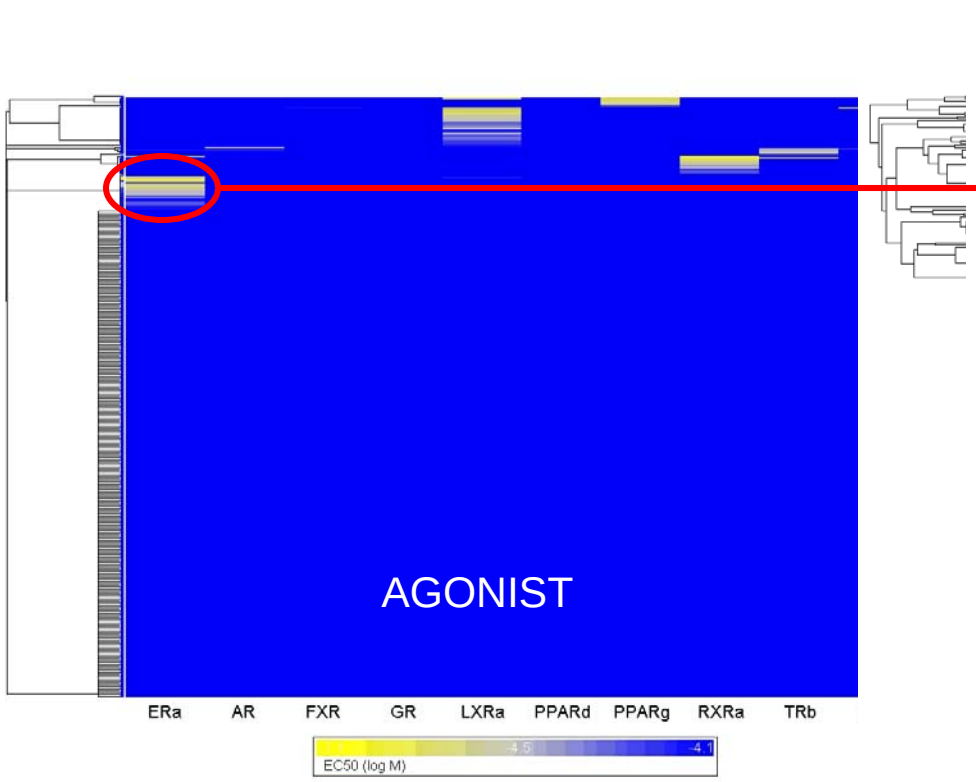


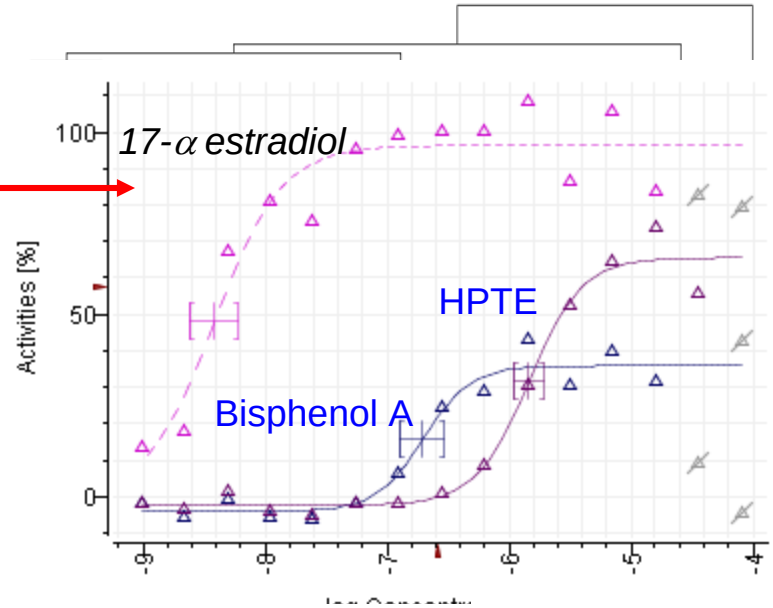
CAS No.	Chemical Name	PPARα	PPARγ	Rat Liver Tumors	Rat Tumorigen	Rat Multigender Tumorigen	Rat Multisite Tumorigen	Mouse Liver Tumors	Mouse Tumorigen	Mouse Multigender Tumorigen	Mouse Multisite Tumorigen	Multispecies Tumorigen
77501-63-4	Lactofen	X	X	76	19	76	76	7.1	7.1	7.1		19
51338-27-3	Diclofop-methyl	X	X	25	25	32	25	2.5	2.5			25
1689-84-5	Bromoxynil	X	X					3.1	3.1			
69377-81-7	Fluroxypyr	X			500							
10453-86-8	Resmethrin		X		400.9	450.3		169.3	169.3			400.9
82-68-8	Quintozene		X		150	300						
584-79-2	d-cis,trans-Allethrin		X									
66332-96-5	Flutolanil		X									
36734-19-7	Iprodione		X		69			604	604	793	793	604
19666-30-9	Oxadiazon		X	50.9	50.9			12	12	14		50.9
23031-36-9	Prallethrin		X									
62476-59-9	Acifluorfen, sodium		X					119	119	711		

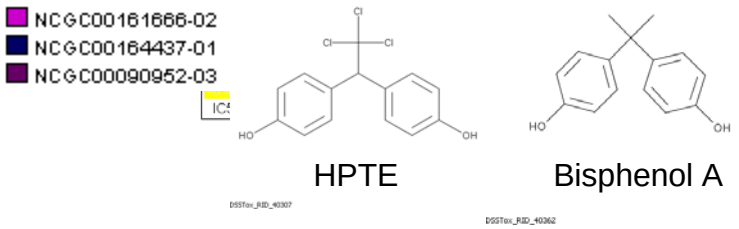
Fold Induction (log 2)

Nuclear Receptor Screening (NCGC)

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



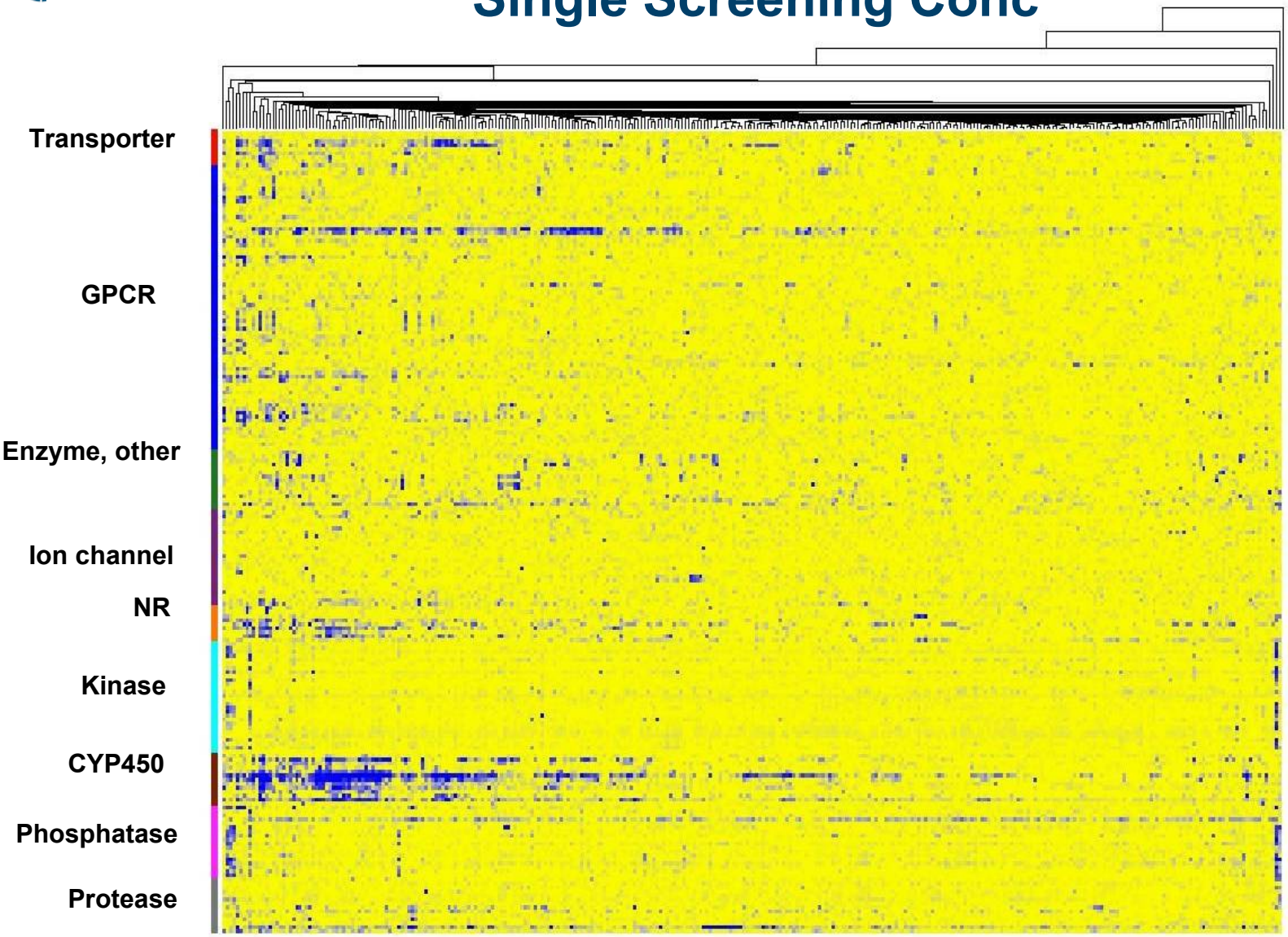




HPTE

Bisphenol A

Biochemical Assay Results
Single Screening Conc



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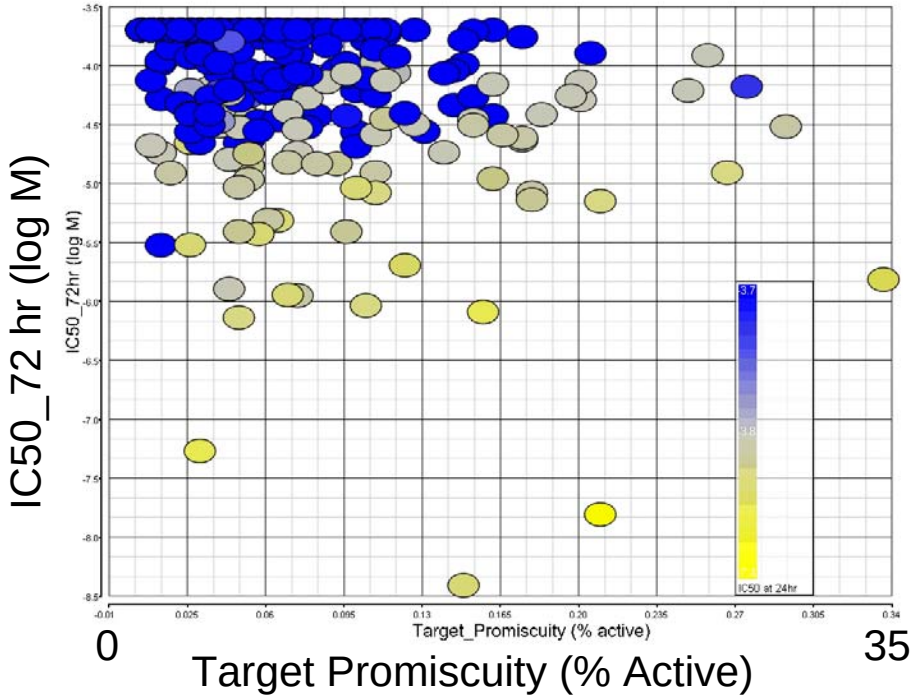
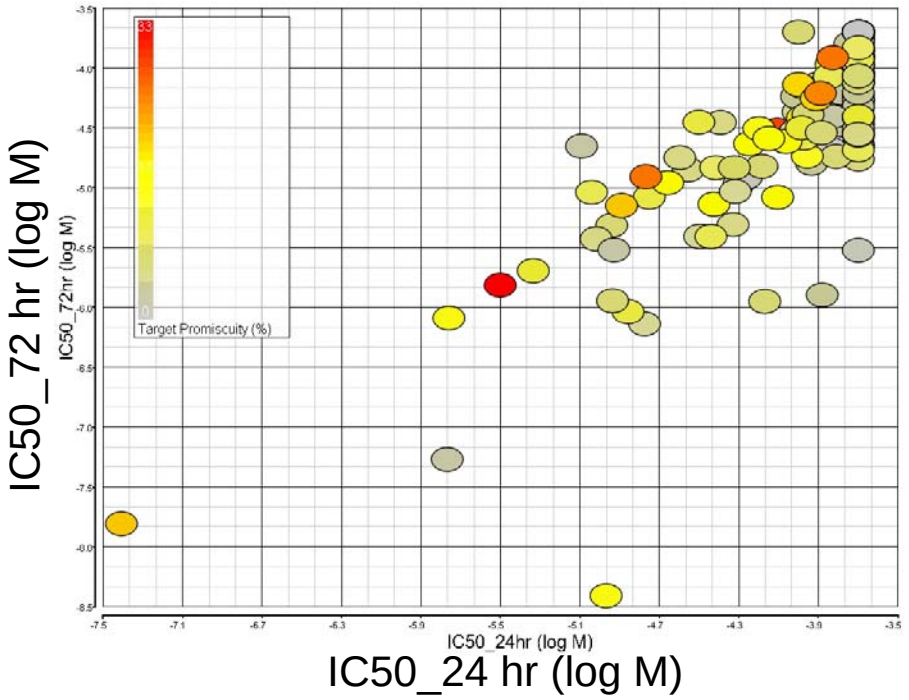
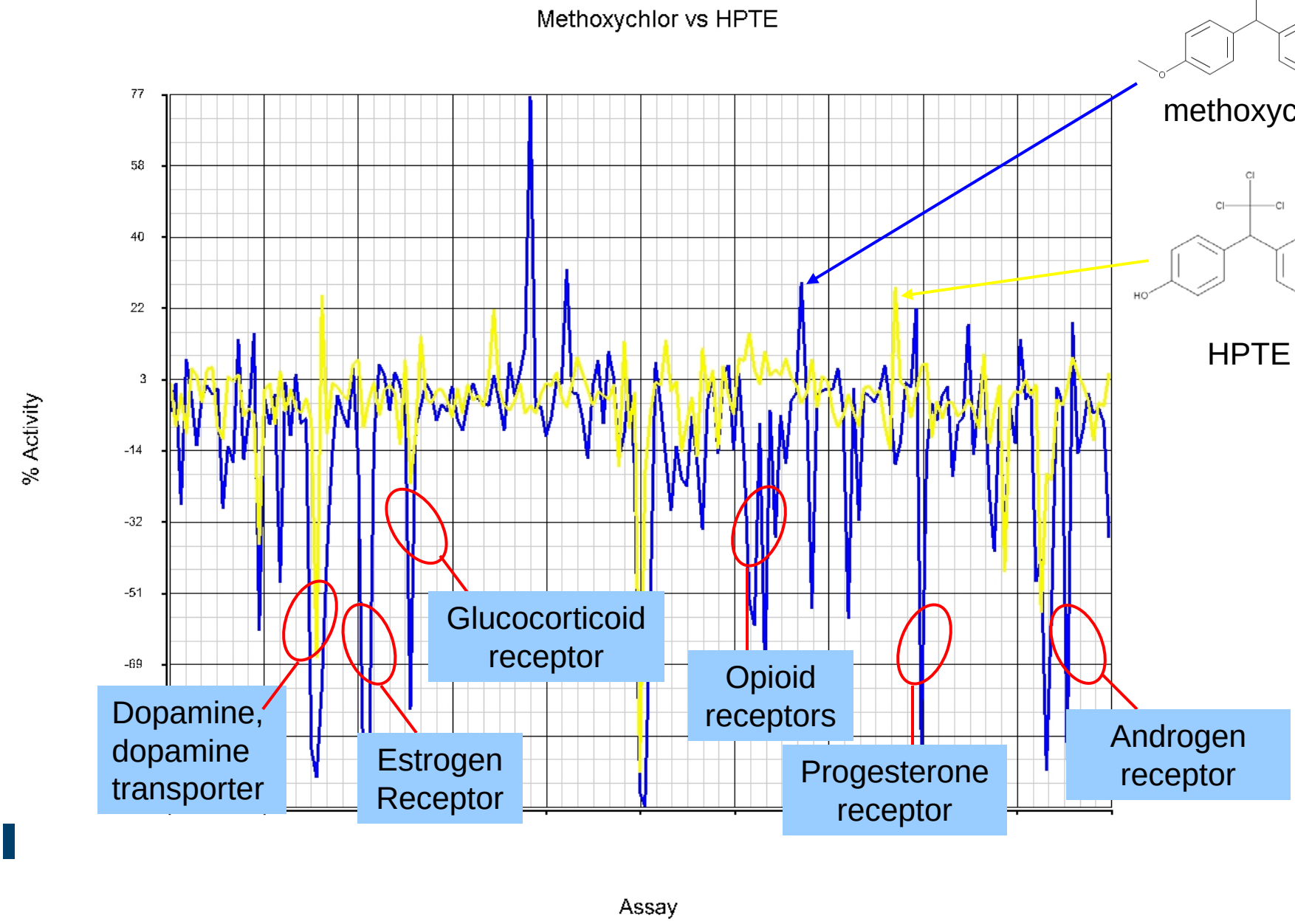
Relationship Between Biochemical Target Promiscuity and Cytotoxicity



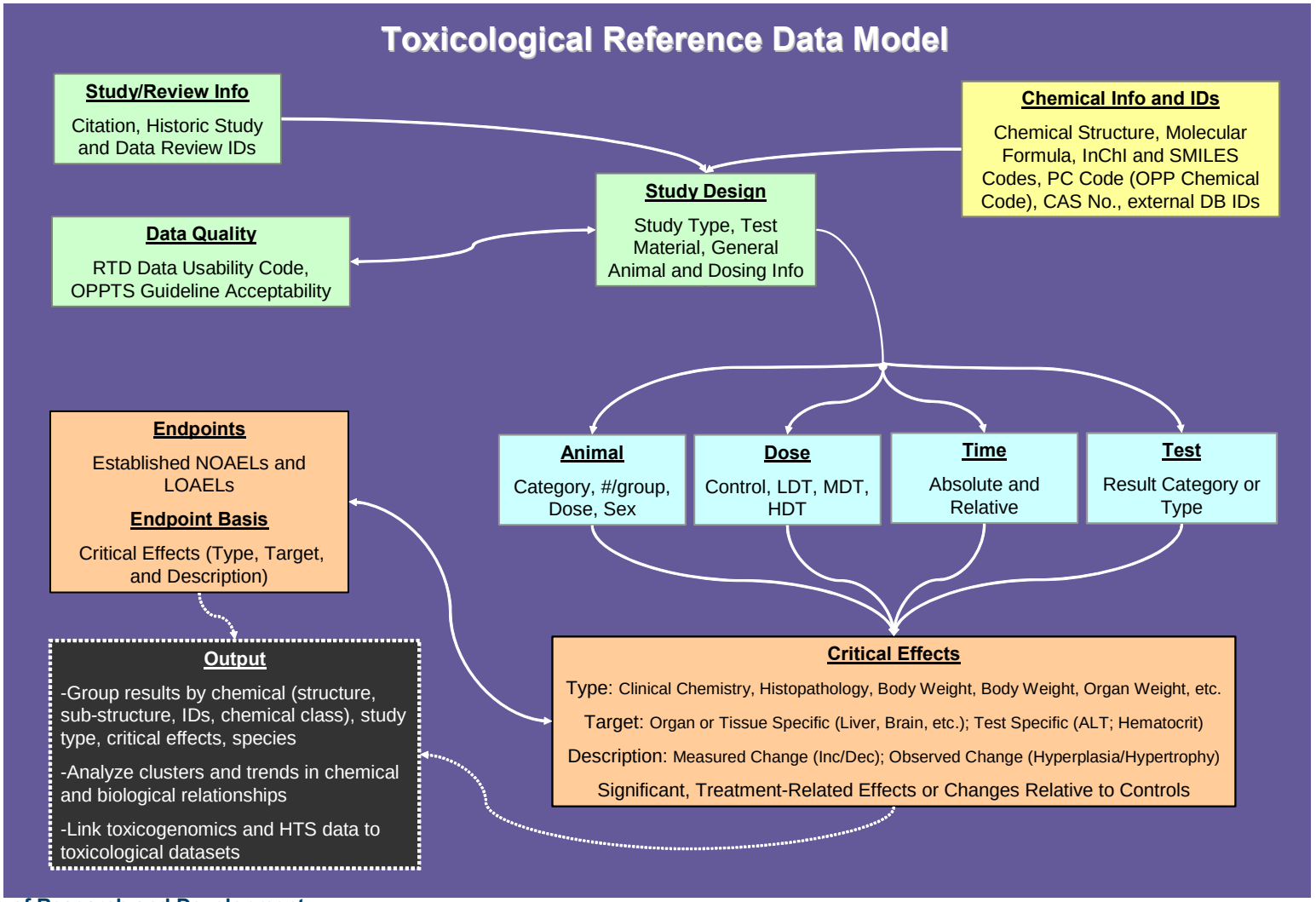


No good correlation between target promiscuity and cytotoxicity; however most chemicals hitting >10% targets are cytotoxic

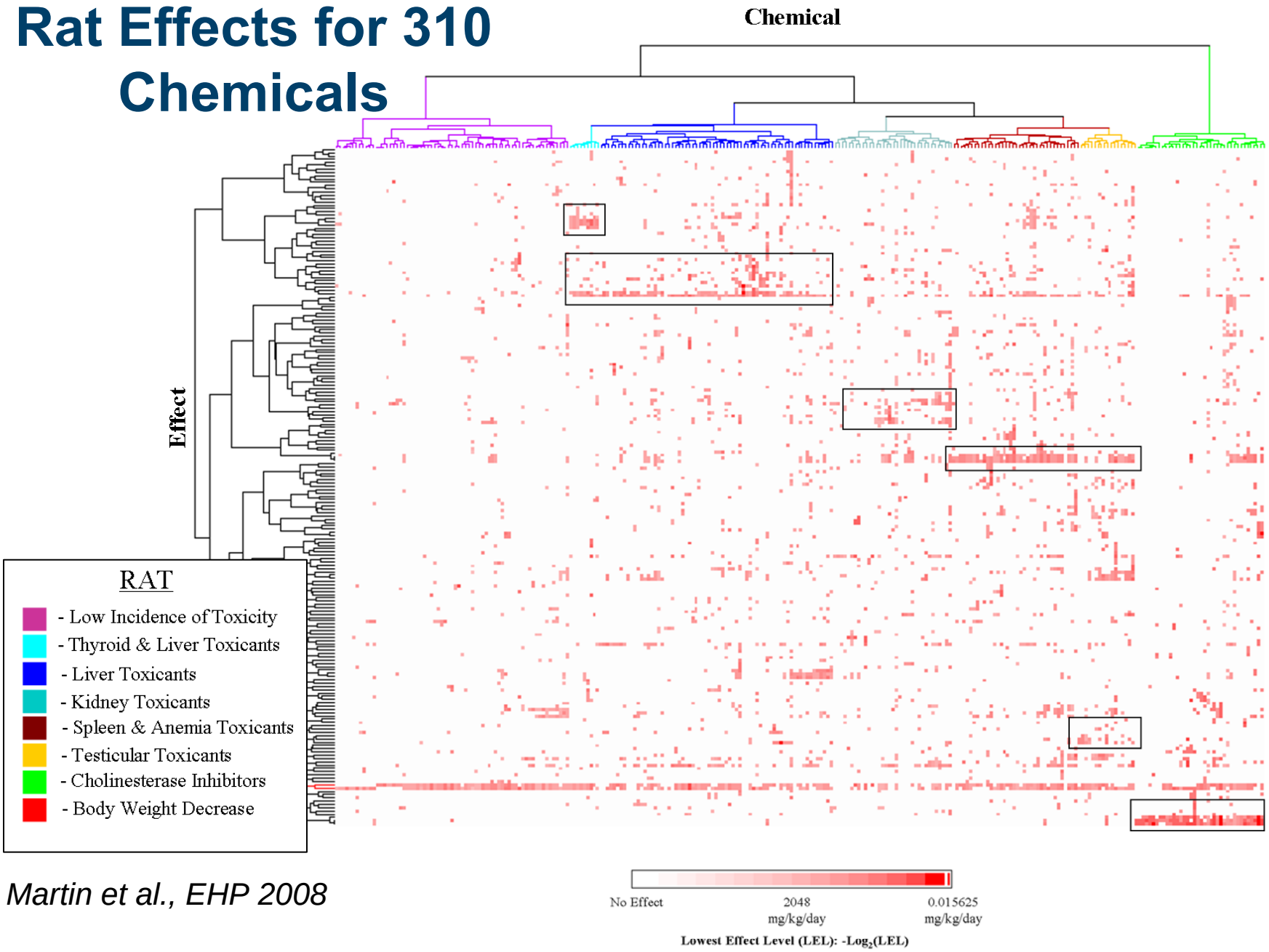
Parent vs Metabolite Activity in Biochemical Assays



Capturing Legacy Toxicity Data: ToxRefDB



ToxRefDB Chronic Rat Effects for 310 Chemicals



Martin et al., EHP 2008

ToxCast Data Analysis

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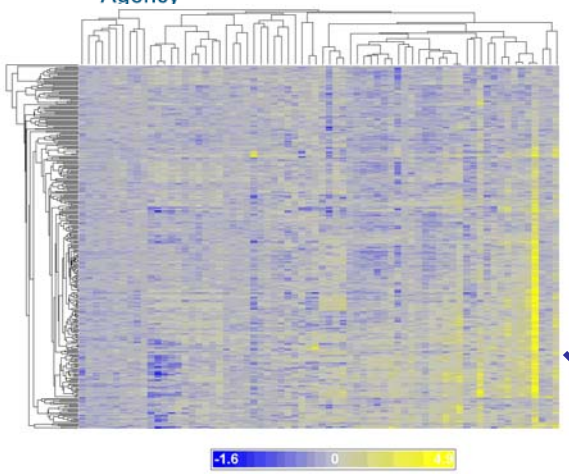
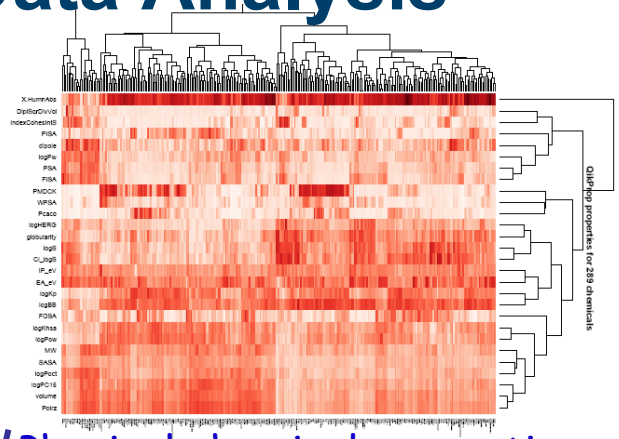
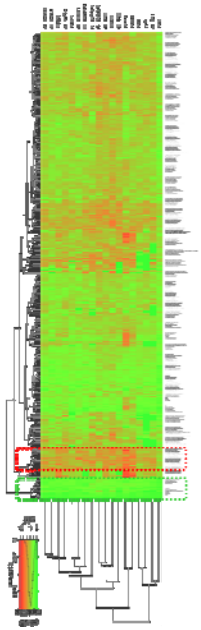
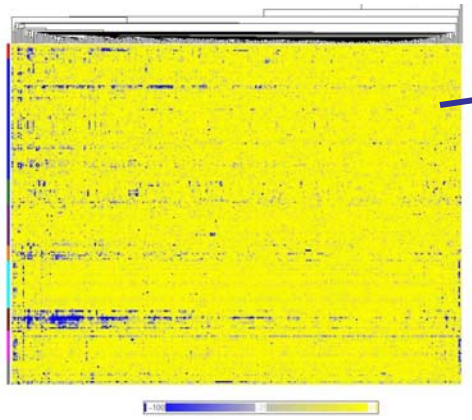




Physical chemical properties

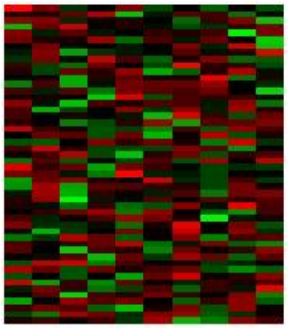


In silico Predictions

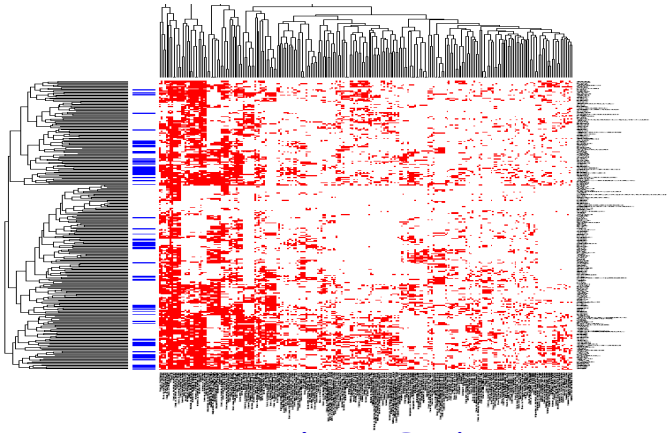


Biochemical Assays

Profile Matching



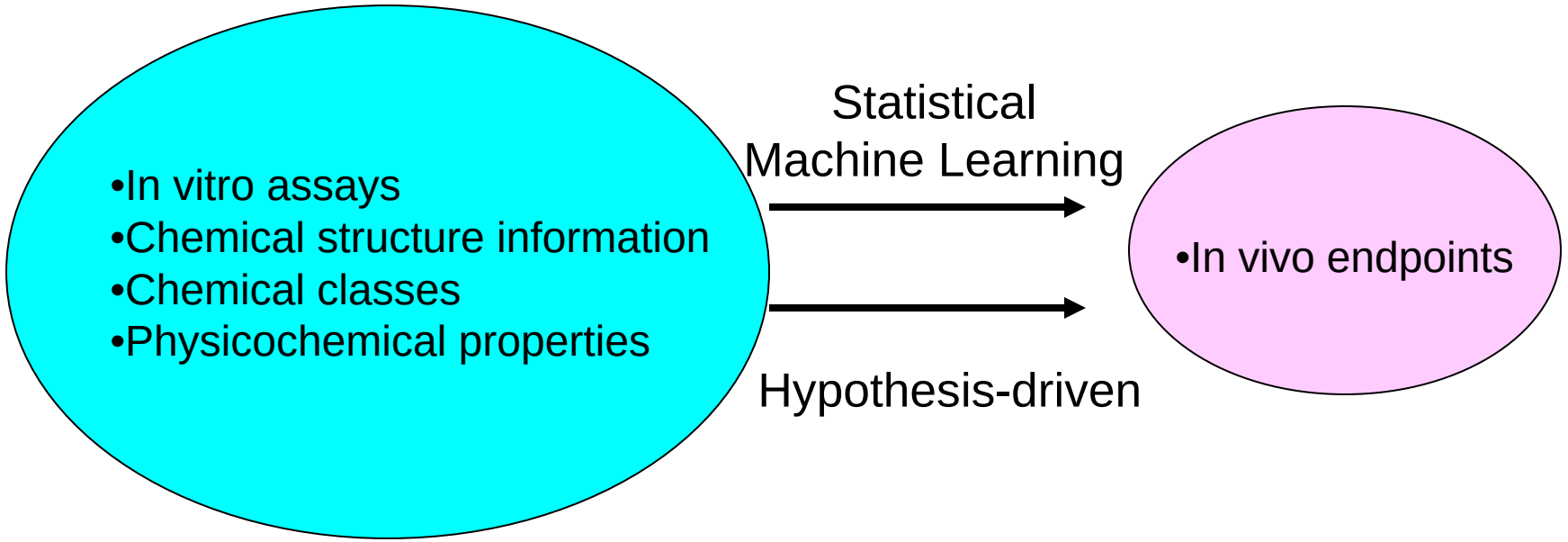
Genomic Signatures



Toxicology Endpoints

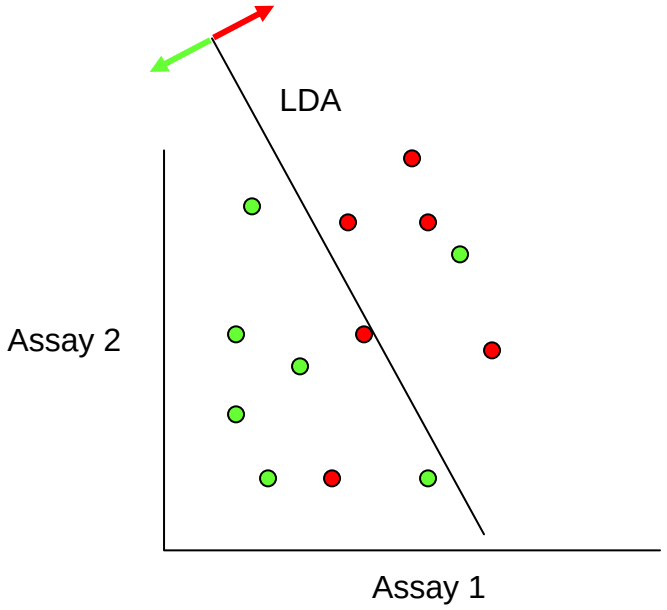
Find “Signatures” from *in vitro* & *in silico* assays that predict *in vivo* endpoints.

Analysis Approaches



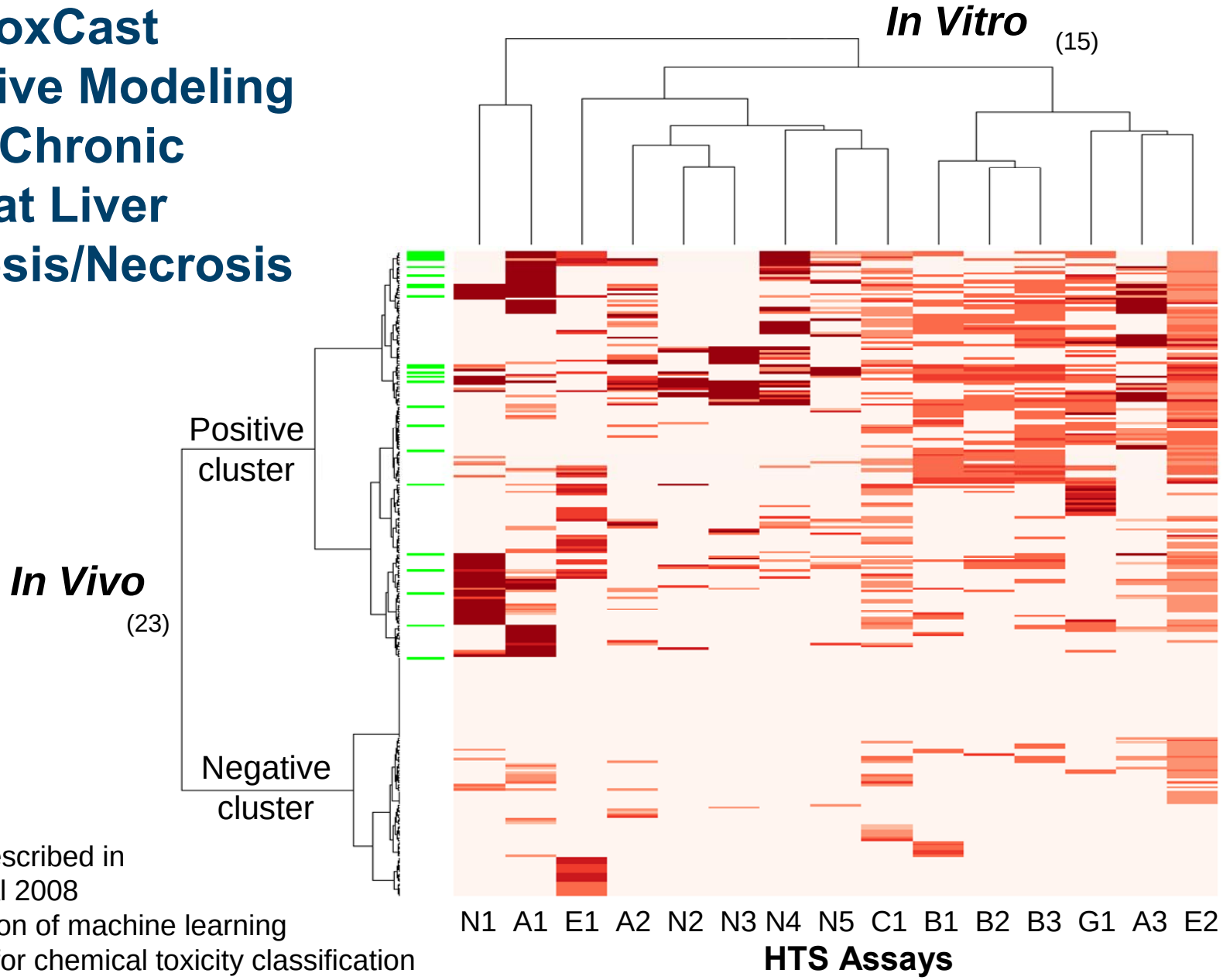
Association Analysis / Signatures

- 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



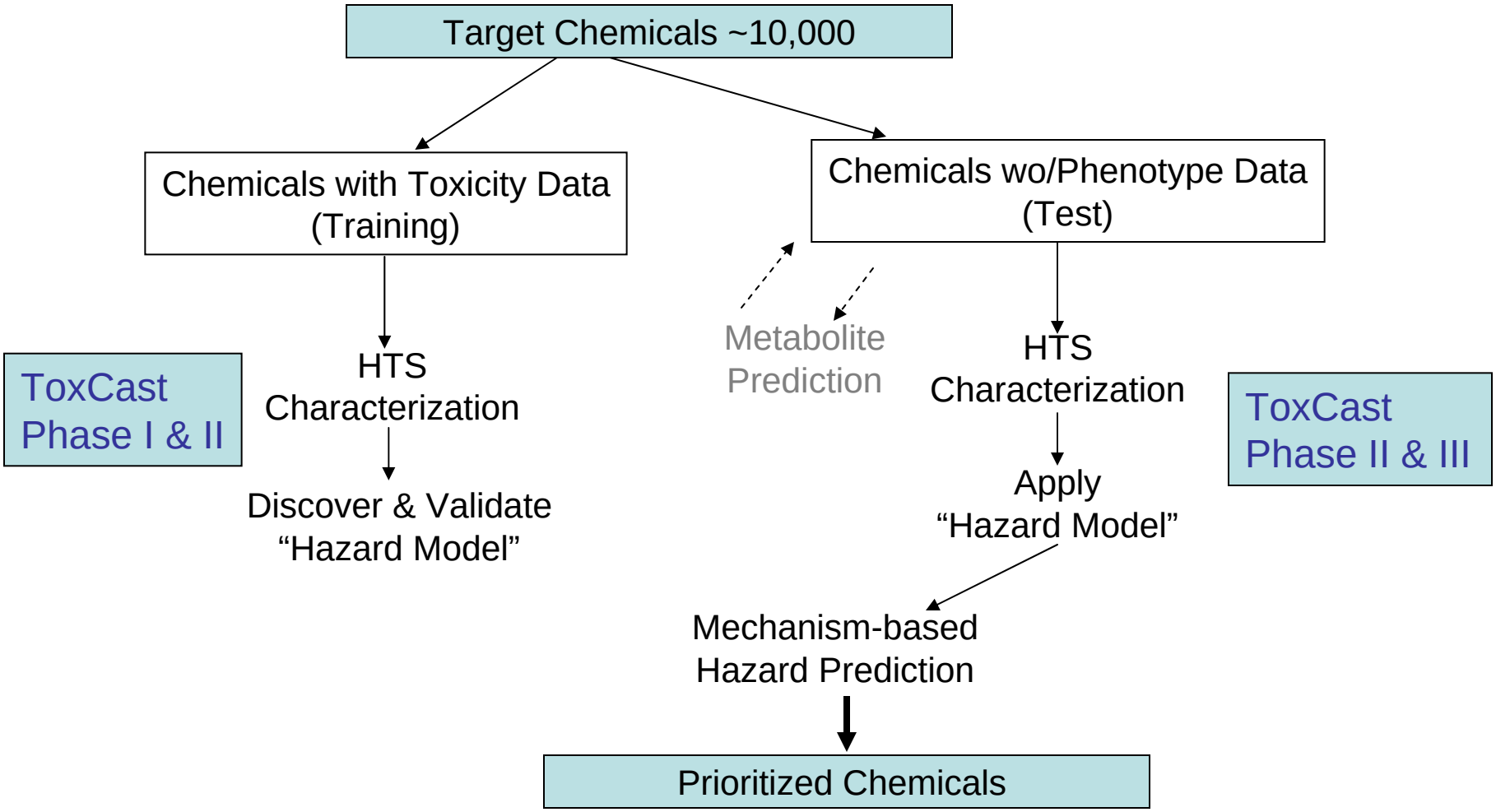
Test \ Truth	+	-
+	TP	FP
-	FN	TN

ToxCast Predictive Modeling of Chronic Rat Liver Apoptosis/Necrosis



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

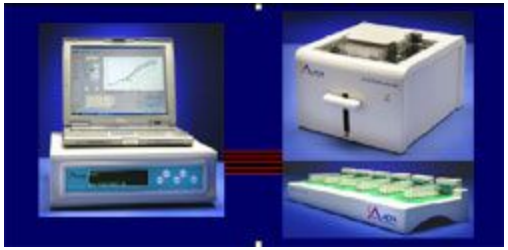
ToxCast Screening and Prioritization

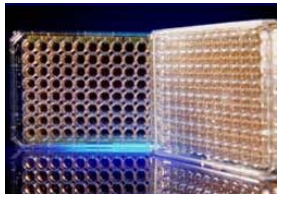


The ToxCast Team

Microelectronic Kinetic Cell Growth Analysis ACEA Biosciences

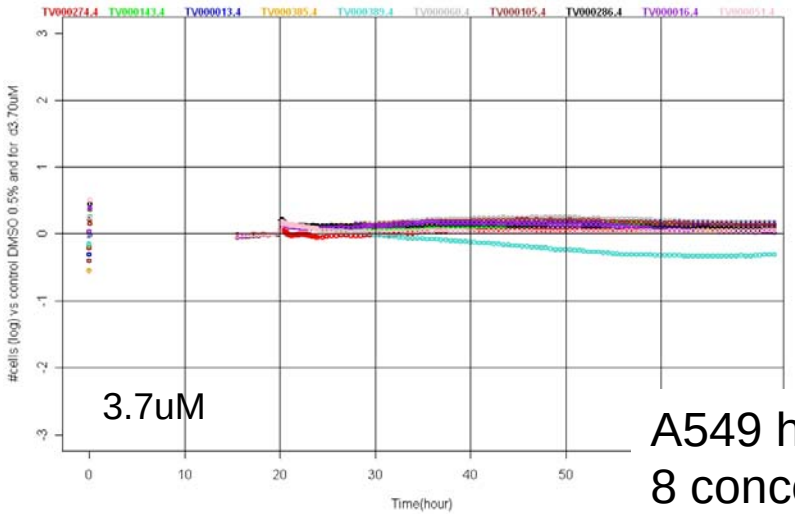
- Objective: Determine kinetic effects of chemicals on cell growth/phenotype
- Technology: Real-Time Cell Electronic Sensing (RT-CES) microelectrodes in microtiter plate monitoring cell effects on electrical impedance
- Methods:
  - Monitor growth of A549 human lung carcinoma cell line
  - 8-point, half-log dilution series starting at 100 μM
  - Cells grown 24 hr in plate, treated with chemical and impedance monitored for 72 hr
  - Analyze growth pattern and cluster with toxicants having known mechanism of cytotoxicity

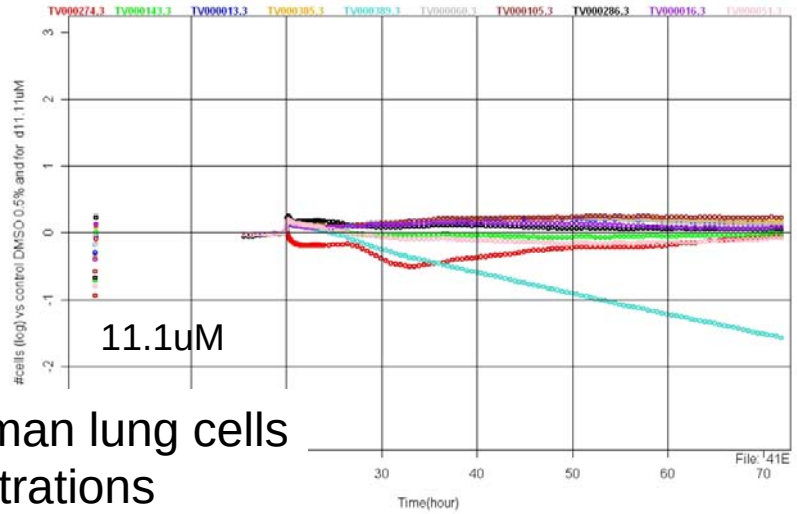




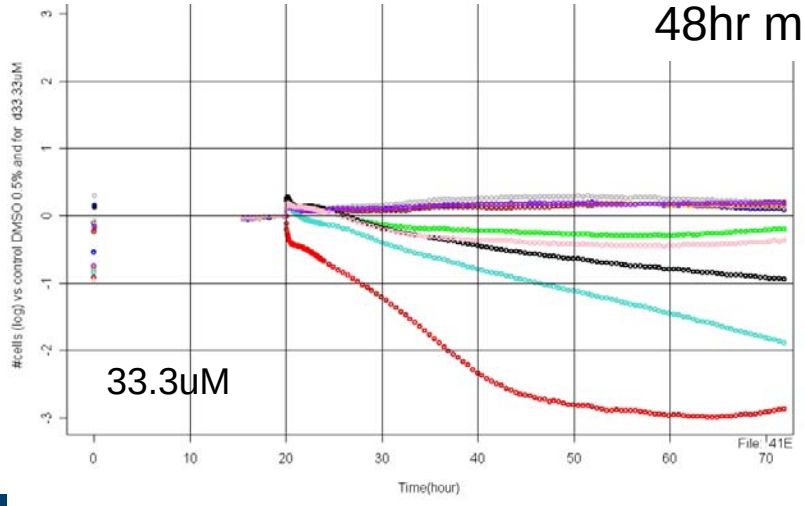
Examples of Kinetic Growth Response

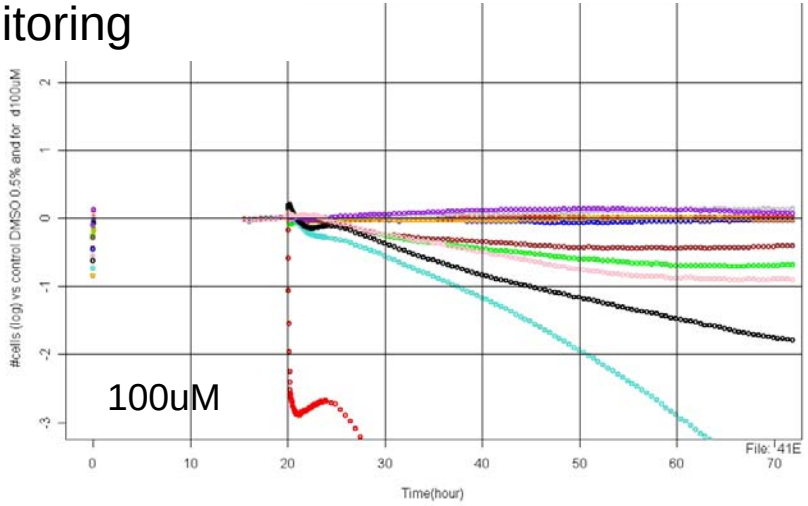
United States Environmental Protection Agency





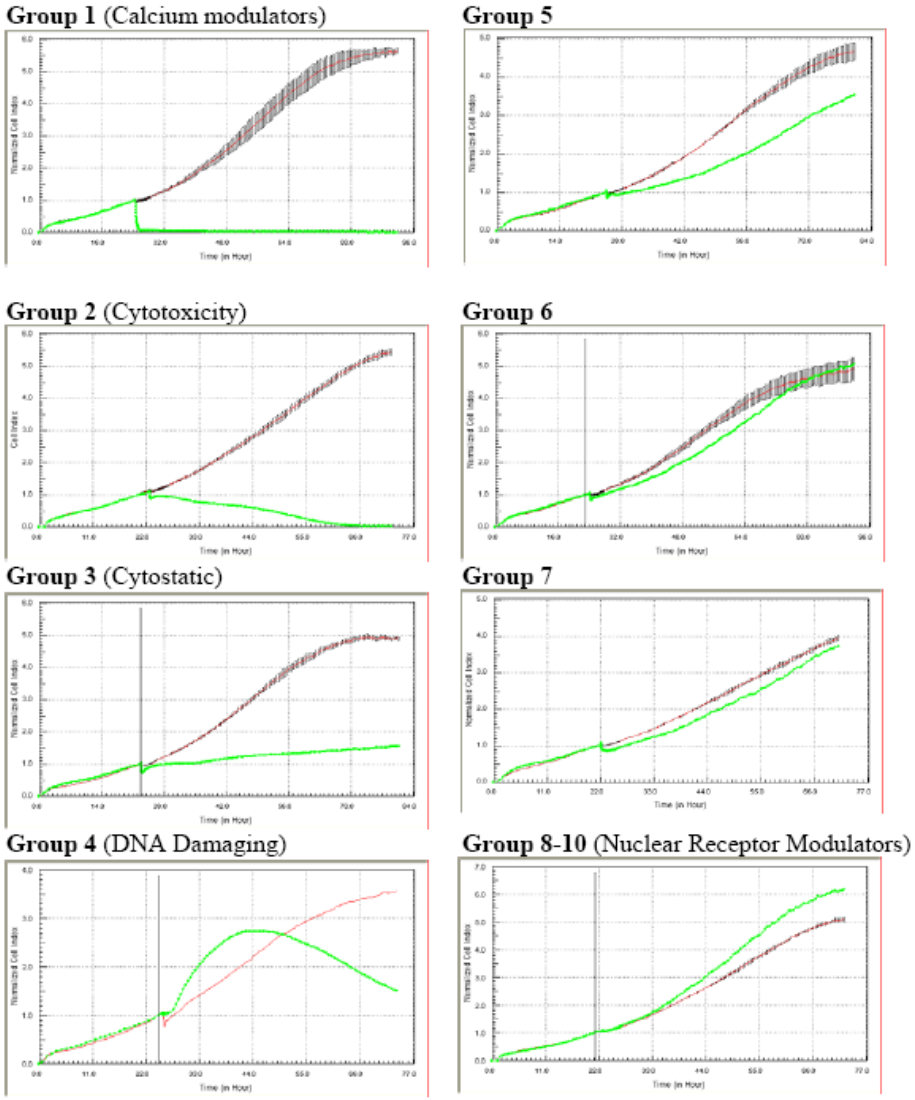
A549 human lung cells
8 concentrations
48hr monitoring





Categories of ACEA Kinetic Response Profiles

United States Environmental Protection Agency

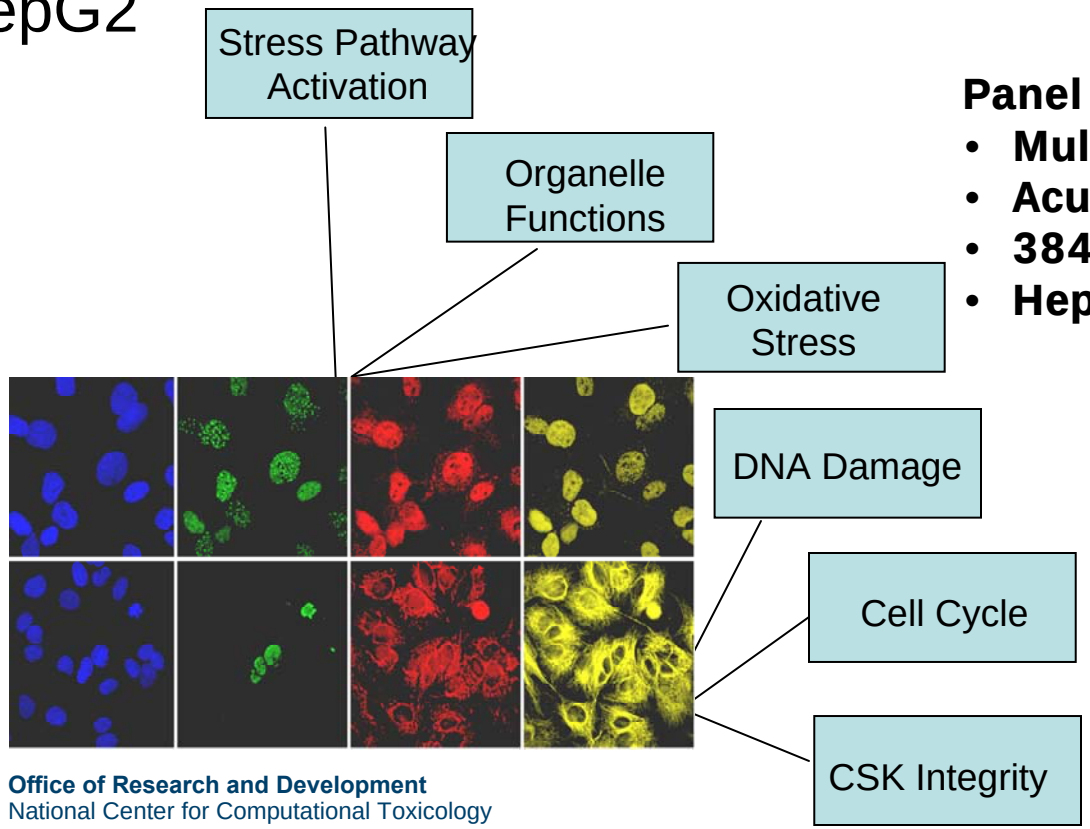


Designation	Compound	Mechanism
A	Emetine	Protein Synthesis Inhibitor
B	Cycloheximide	Protein Synthesis Inhibitor
G	Hydrocortisone	Nuclear Receptor Modulator
H	Fludrocortisone	Nuclear Receptor Modulator
C	Fluphenazine	Calcium modulator
D	Tamoxifen	Calcium modulator
E	Carmofur	DNA Damaging
F	5-Fluorouracil	DNA Damaging
I	Picropodophyllotoxin	Anti-mitotic
J	Nacadazole	Anti-mitotic
K	Oloumoucine	Cytotstatic

Figure 13. Representative cell response curves from the different groups

High-Content Screening of Cell Health 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 (1˚ rat hepatocytes in dev.)**

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