United States Environmental Protection Agency

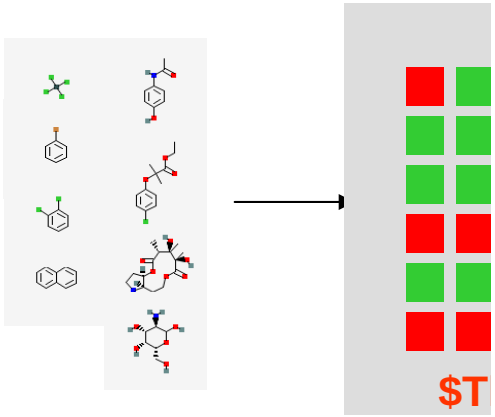
ToxCast: Developing Predictive Signatures of Chemically Induced Toxicity

11th SAC Seminar: New Trends in Chemical Toxicology
Moscow, Russian Federation

Office of Research and Development
National Center for Computational Toxicology

September 22, 2008

Future of Toxicity Testing



POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

Francis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,

Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

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*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

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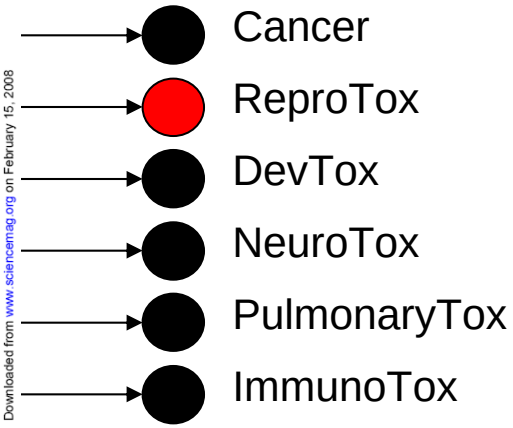
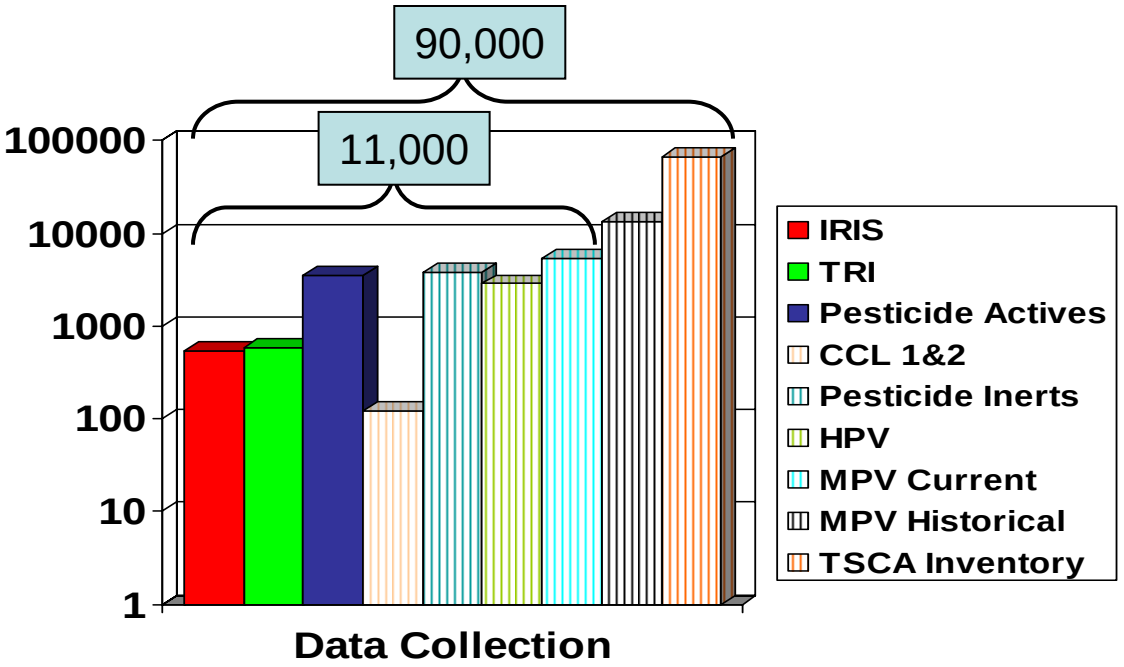


NCGC Tox21 EPA

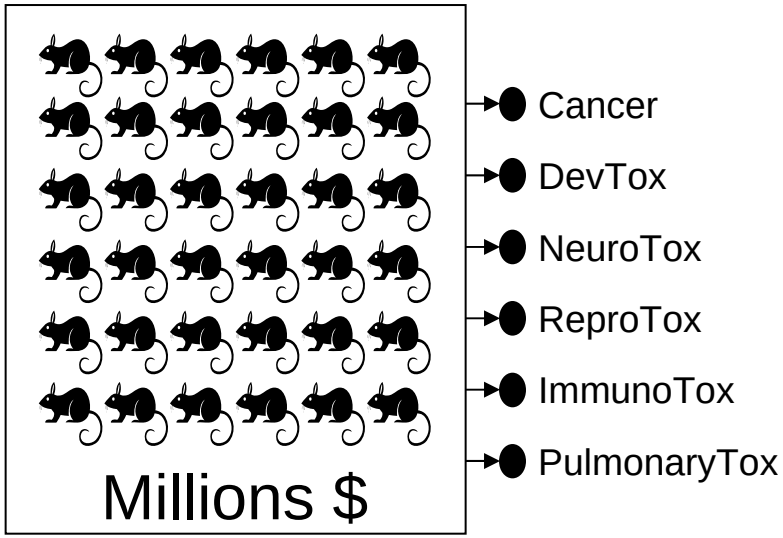
EPAs Contribution: The ToxCast Research Program

Change Needed Because …..

Too Many Chemicals



Too High a Cost

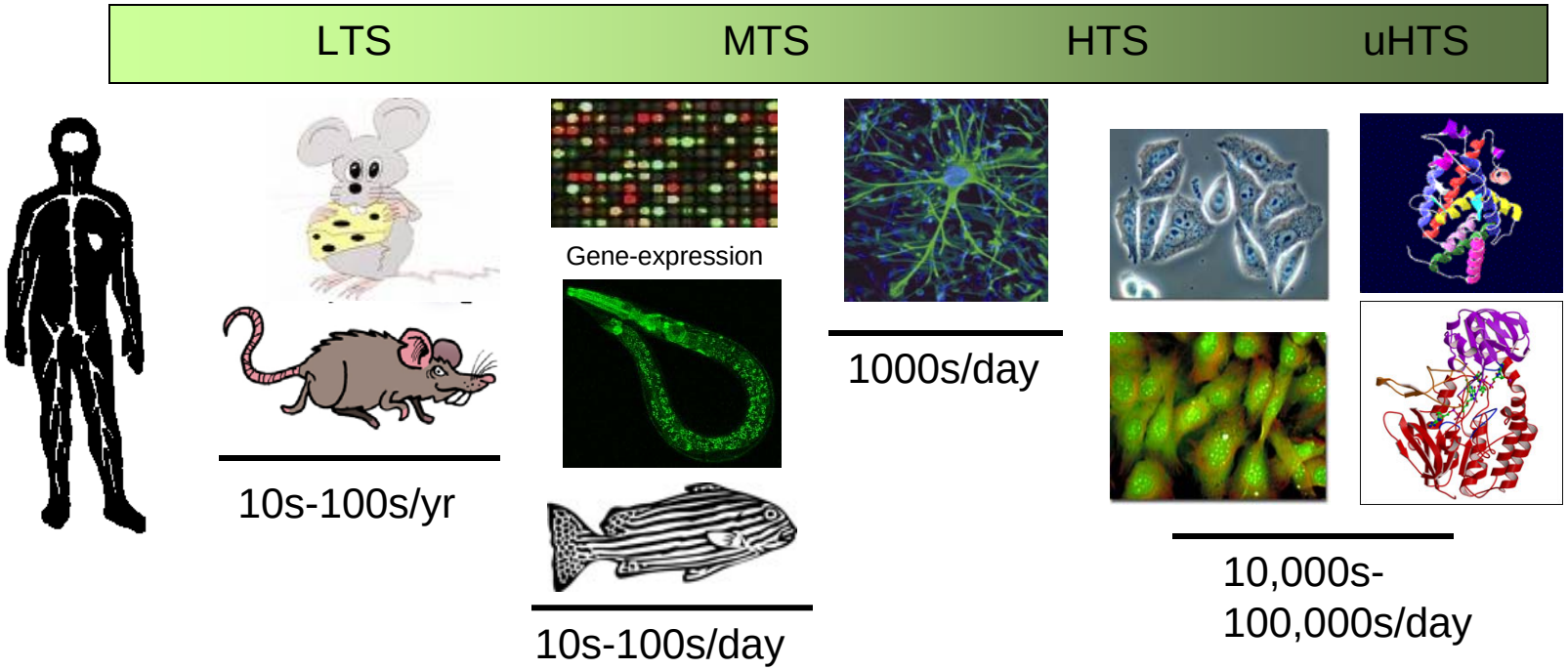


…and not enough data.

Judson, et al *EHP submitted*

High-Throughput Screening Assays

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



Human Relevance/
Cost/Complexity

Throughput/
Simplicity

ToxCast™ Background

- 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://134.67.216.45:22722/servlet/ActorPrototype2008Q1?page=0

    http://www.epa.gov/actor/

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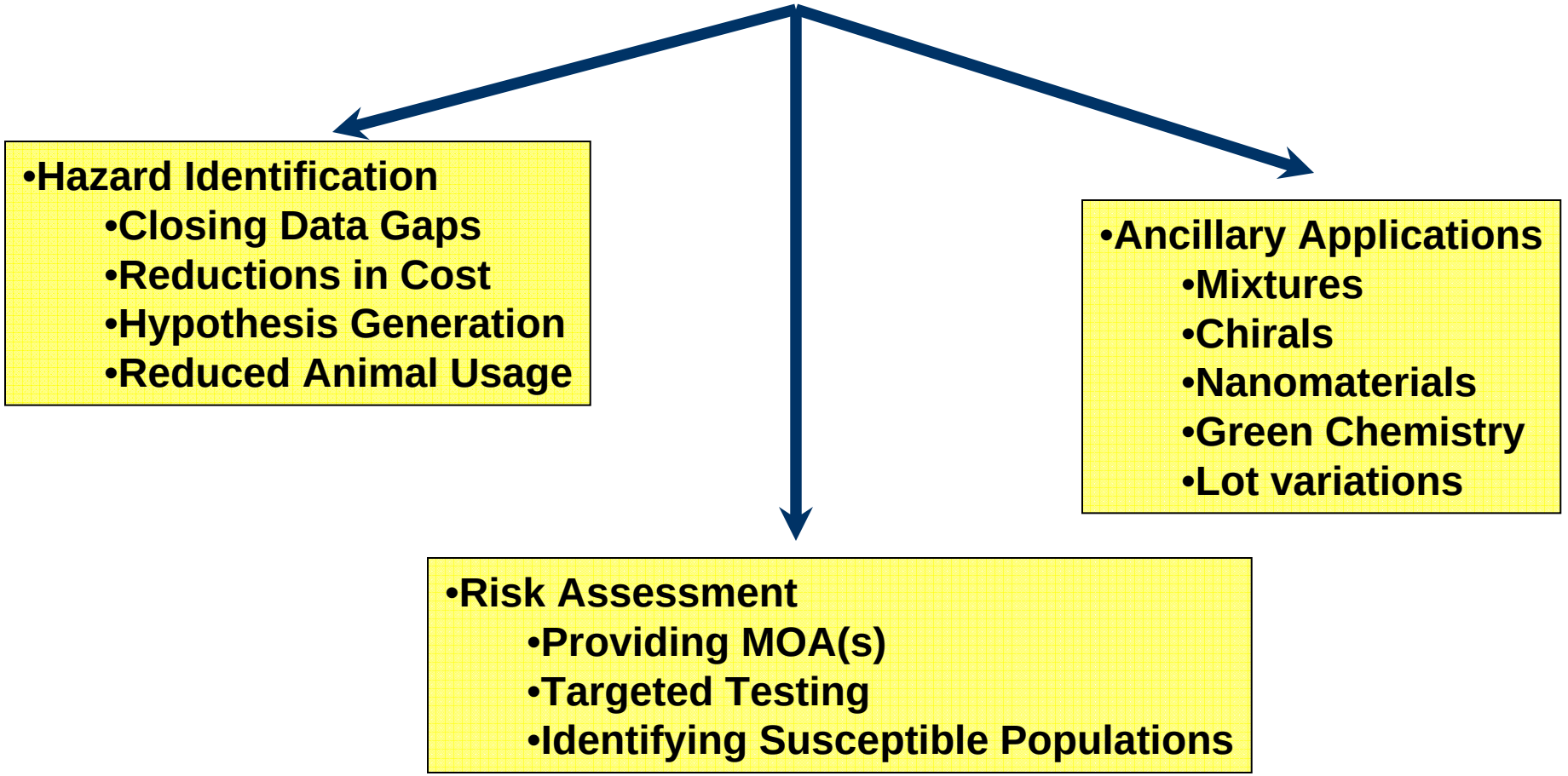
Implications for Success



Key Challenges

- Find the Toxicity Pathways
  - Hepato vs developmental
- Obtain HTS Assays for Them
  - Including metabolic capability
- Screen Chemical Libraries
  - Coverage of p-chem properties
- Link Results to in vivo Effects
  - Gold standard and dosimetry

Phased Development of ToxCast

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
Ia	320	Data Rich (pesticides)	Signature Development	552	$20k	FY08[1]
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	Reducing to Practice	>300	~$15-20k	FY11-12

[1]Initiated April 2007

The ToxCast_320

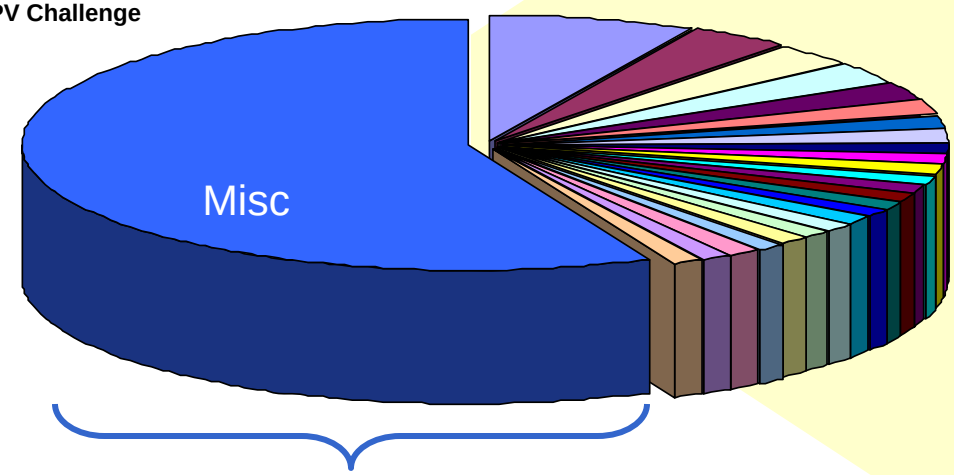
309 Unique Structures

Replicates for QC

291 Pesticide Actives
9 Industrial Chemicals
8 Metabolites

56/73 Proposed Tier 1 EDSP

14 HPV
11 HPV Challenge



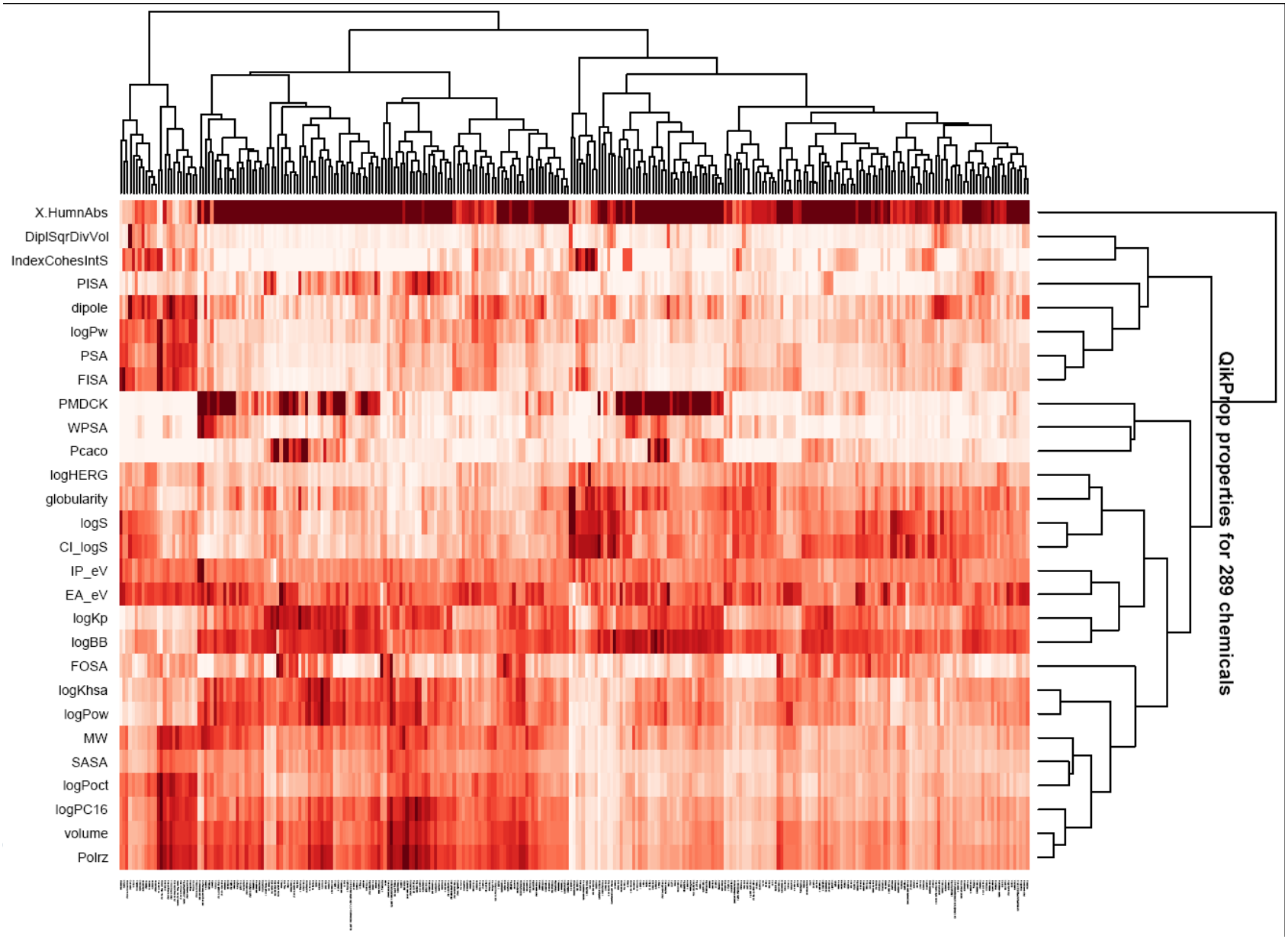
Misc MOA classes with
3 or fewer representatives

Classes with
> 3 chemicals

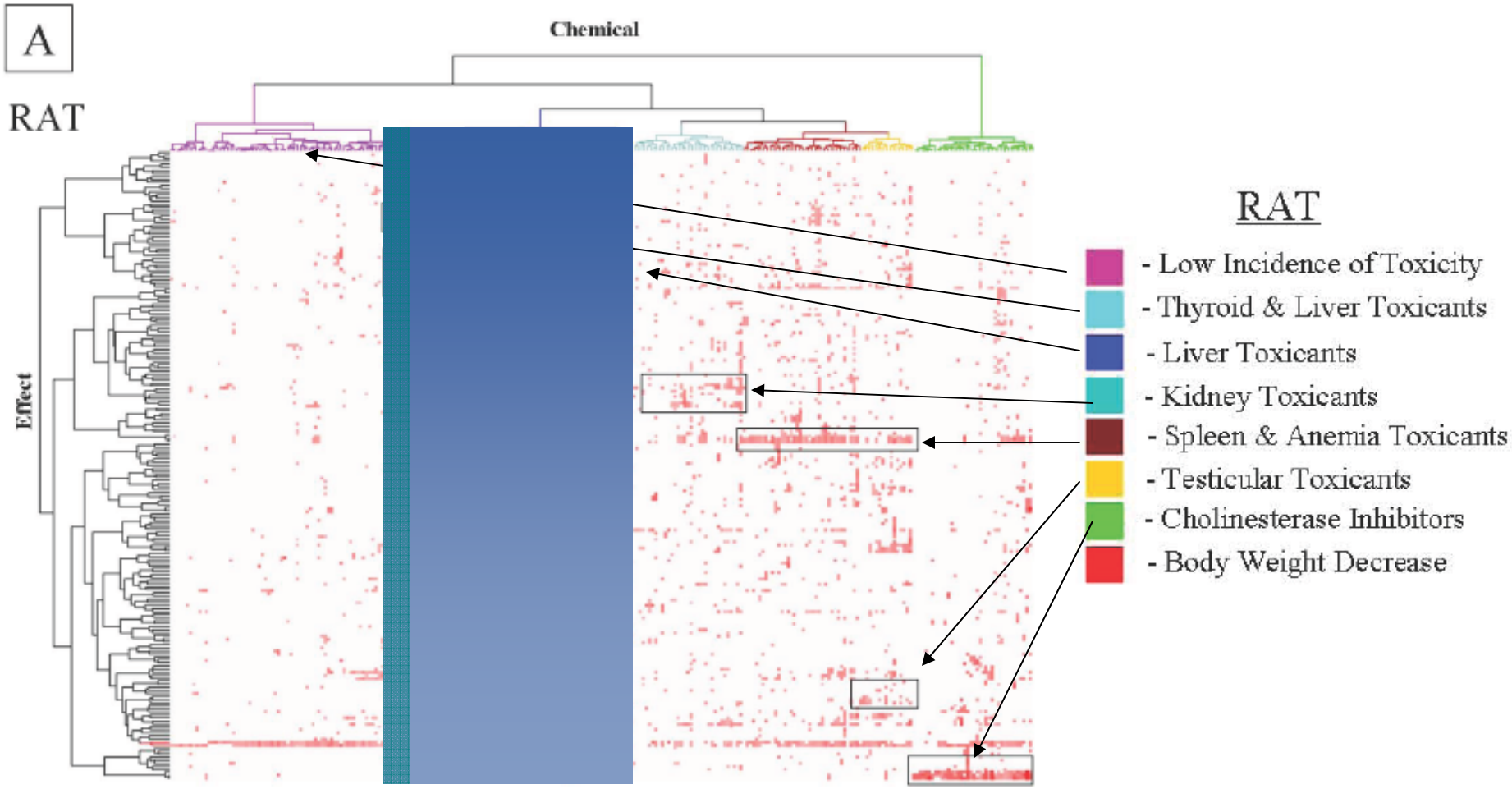
- **Acetylcholine esterase inhibitors**
- **conazole fungicides**
- **Sodium channel modulators**
- **pyrethroid ester insecticides**
- **organothiophosphate acaricides**
- **dinitroaniline herbicides**
- **pyridine herbicides**
- **thiocarbamate herbicides**
- **imidazolinone herbicides**
- **organophosphate insecticides**
- **phenyl organothiophosphate insecticides**
- **aliphatic organothiophosphate insecticides**
- **amide herbicides**
- **aromatic fungicides**
- **chloroacetanilide herbicides**
- **chlorotriazine herbicides**
- **growth inhibitors**
- **organophosphate acaricides**
- **oxime carbamate insecticides**
- **phenylurea herbicides**
- **pyrethroid ester acaricides**
- **strobilurin fungicides**
- **unclassified acaricides**
- **unclassified herbicides**

Classification based on OPPIN

Physical-Chemical Properties

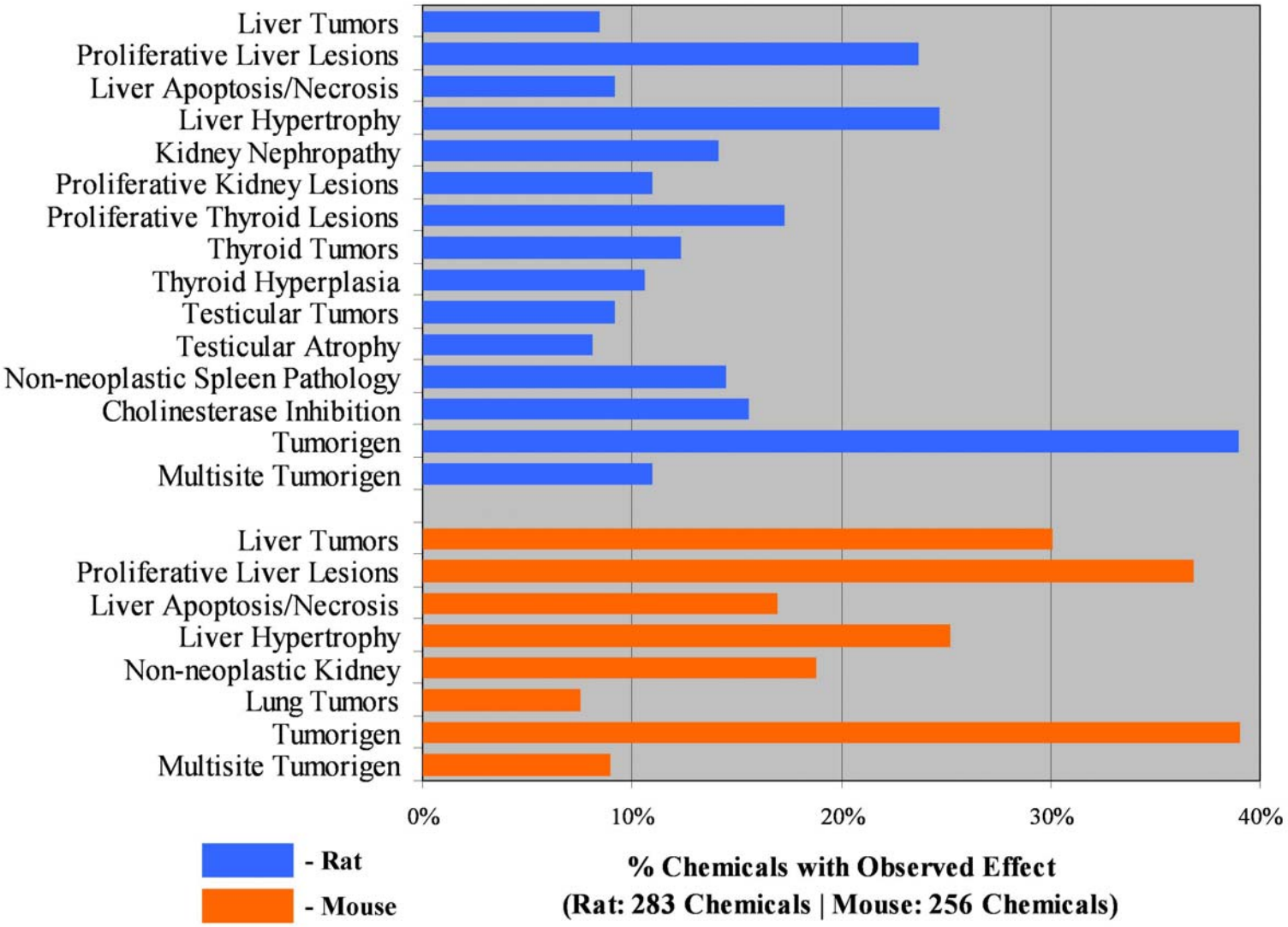


$1B in Toxicology Now Stored in ToxRefDB

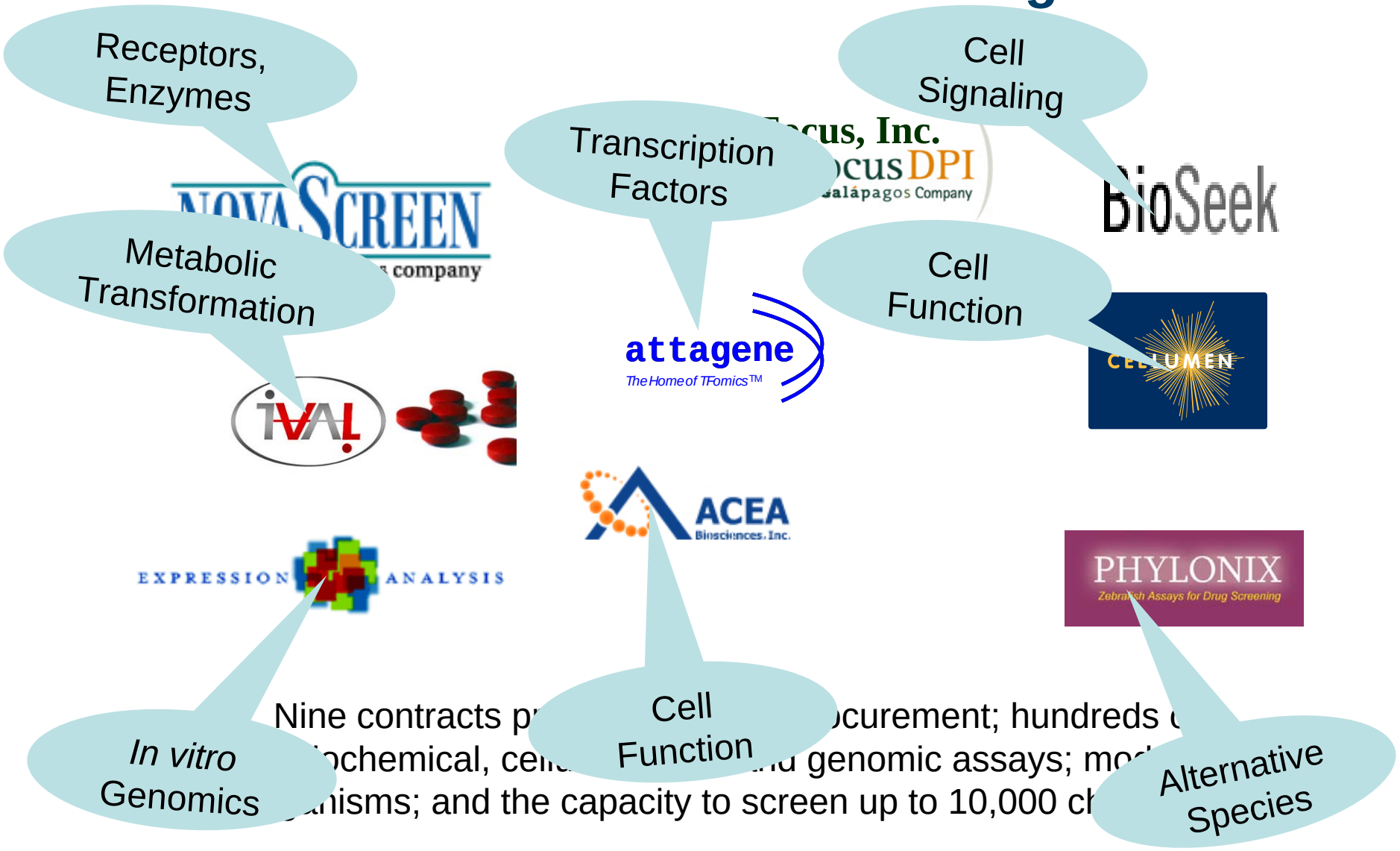


Martin, et al *EHP*,*submitted*

ToxRefDB Endpoints from Chronic Rodent Studies for Training ToxCast Predictions



ToxCast Contracts for Generating HTS Data



Nine contracts p… …ocurement; hundreds … …ochemical, cell… …nd genomic assays; mo… …anisms; and the capacity to screen up to 10,000 ch…

Transcription Factor Activity Profiling

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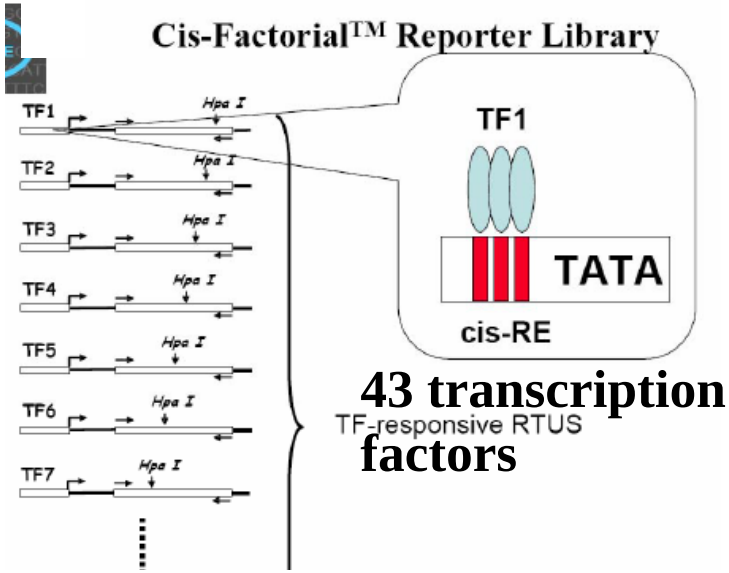
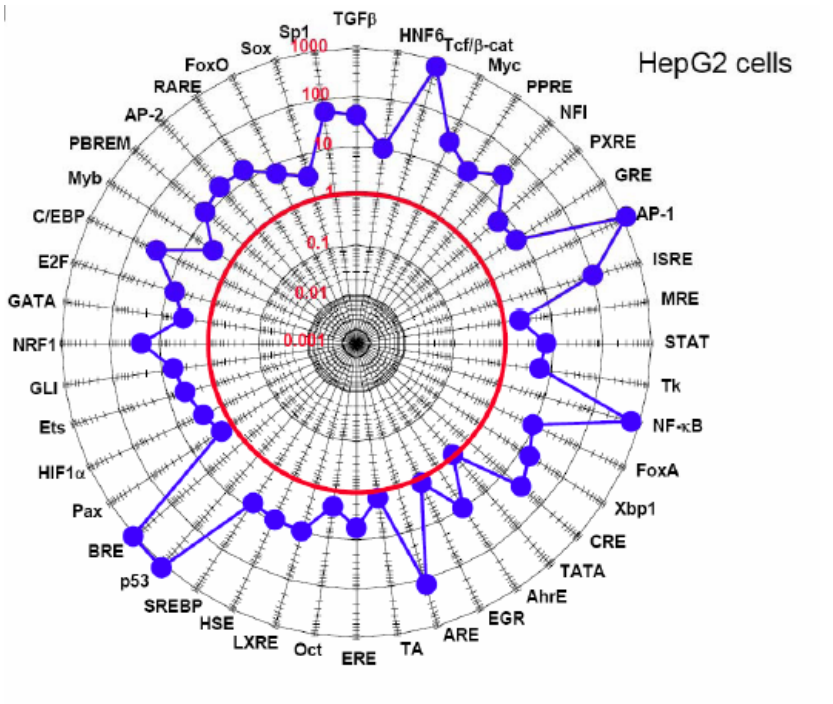
attagene
The Home of TFomics™

Cis-Factorial™ Biosensors

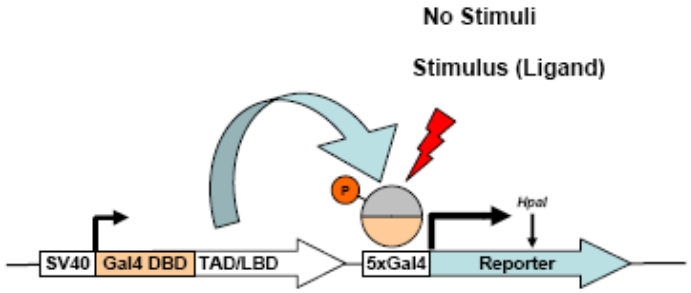


43 transcription factors

TF-responsive RTUS

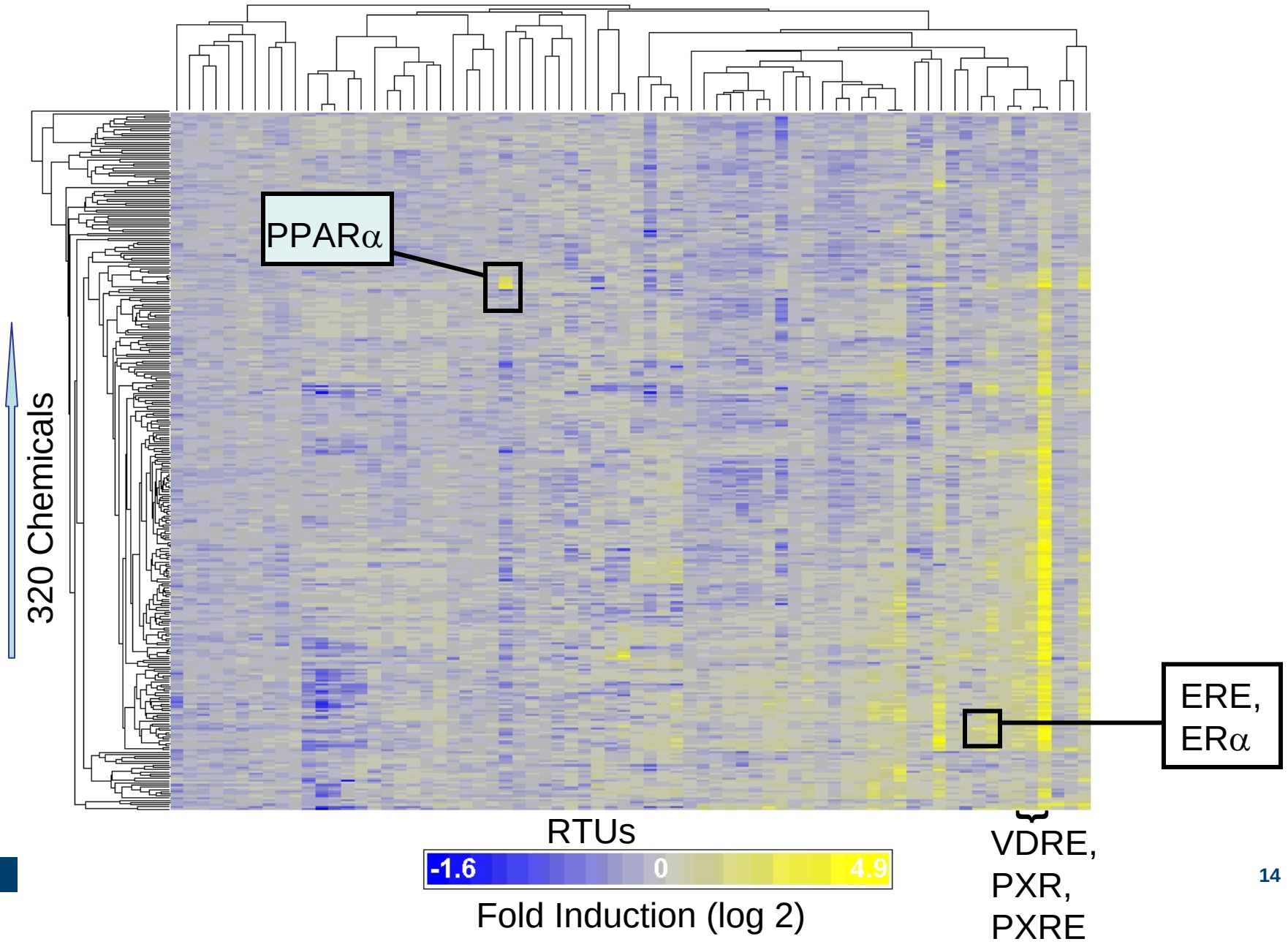


Trans-Factorial™ Biosensors



24 nuclear receptors

Hierachical Cluster Attagene Results



ToxCast Phase I Assays/Datasets/Publications

- **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)**
  - ★ *NR Activation and translocation (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 microarray with metabolism (Solidus)*
  - *Zebrafish vascular/cardiotoxicity (Zygogen)*
  - *HTS stress response (NHEERL+NCGC)*

21 Assay Sources
>550 Endpoints

★ Nearing publication

Correlating HTS to Toxicity

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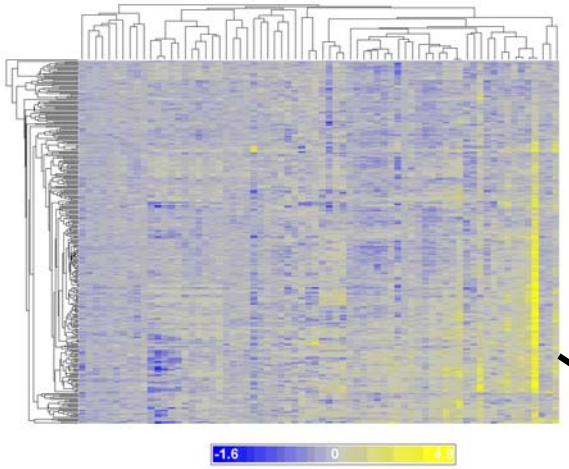
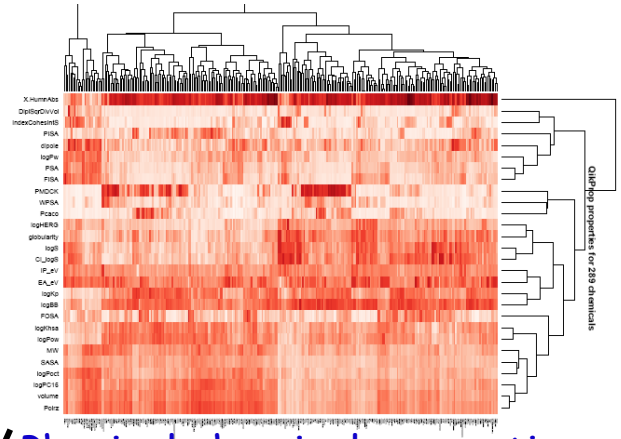
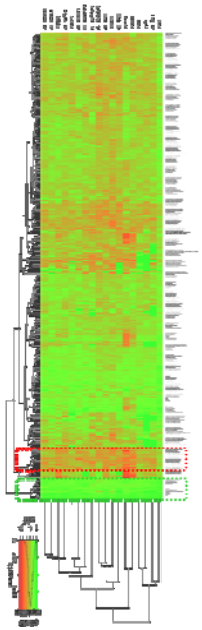


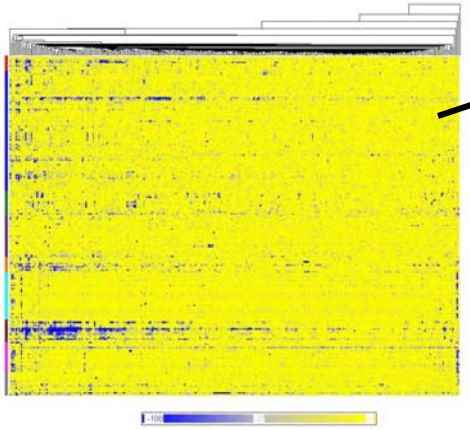
Cellular Assays



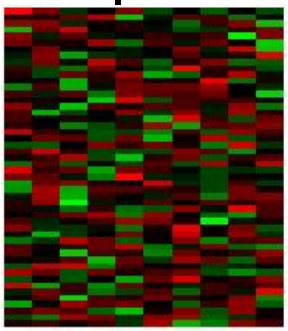
Physical chemical properties



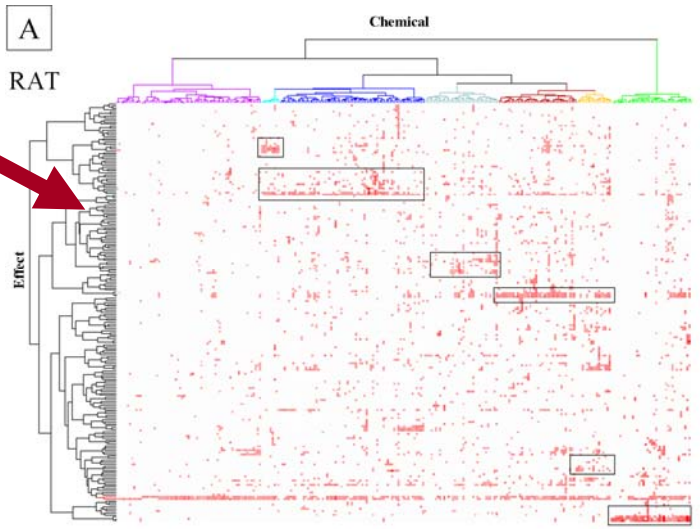
In silico Predictions



Biochemical Assays

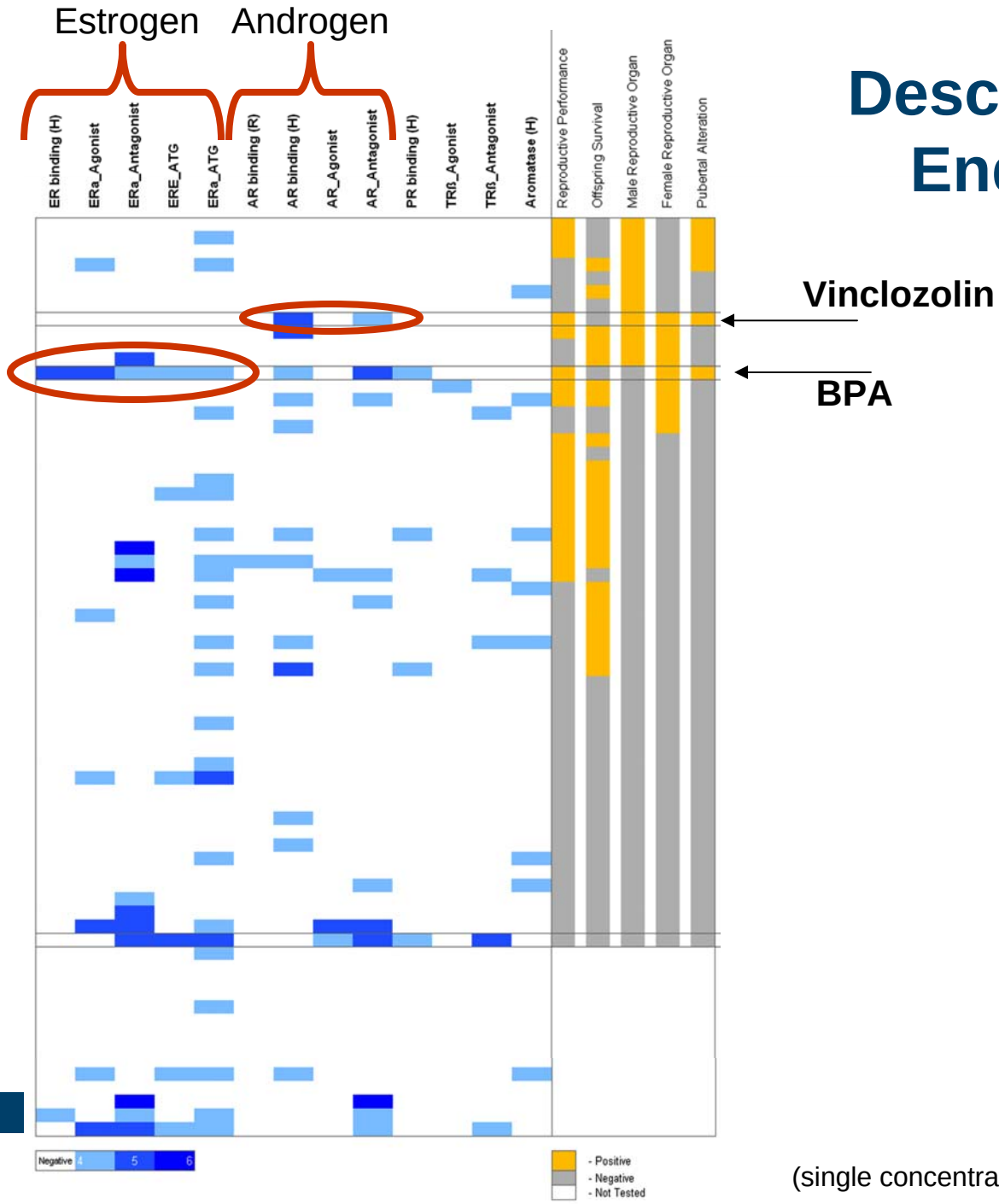


Genomic Signatures



Toxicology Endpoints

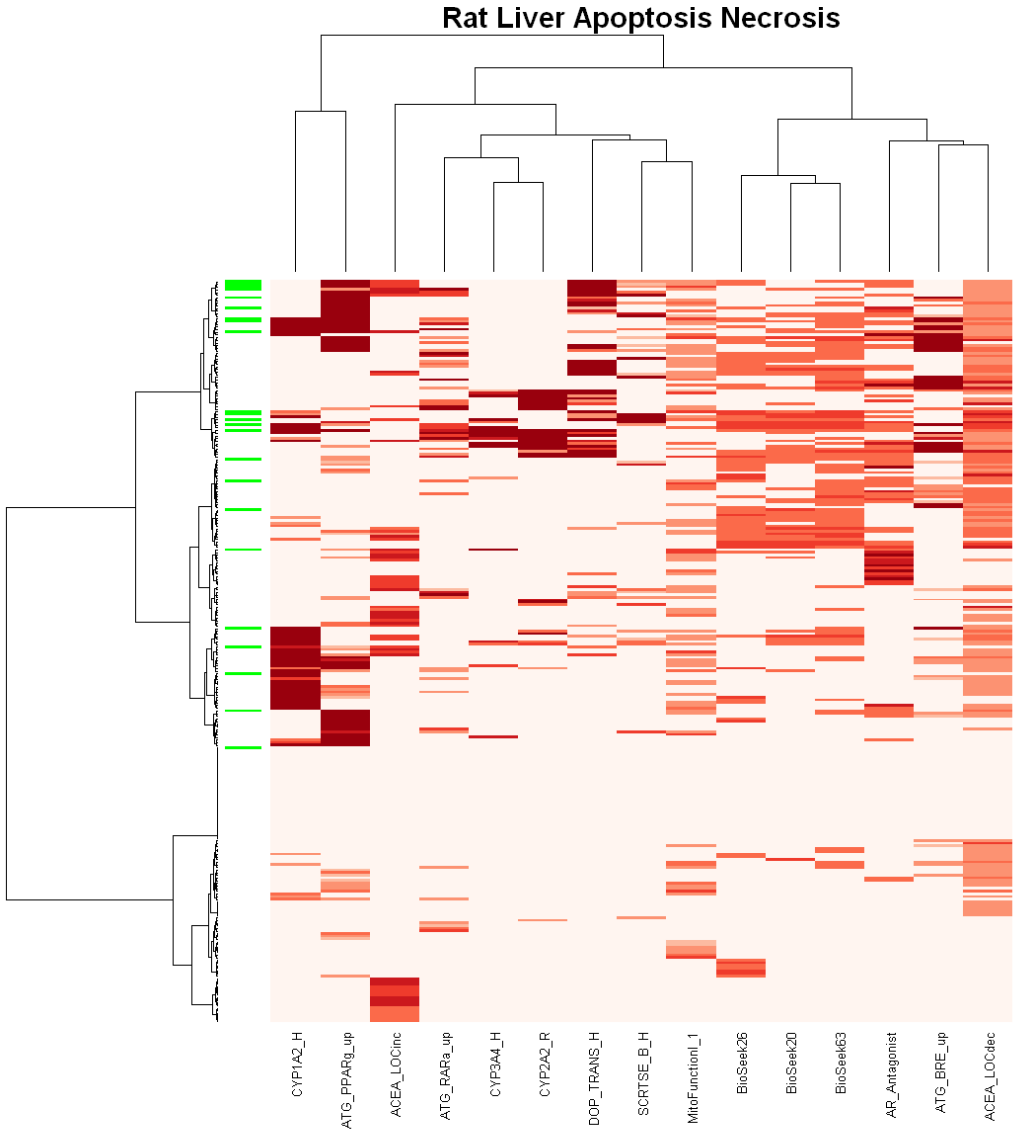
Descriptive Profiling of Endocrine Activity



A number of ToxCast assays examine the ability of a chemical to interact with hormone systems, including binding to hormone receptors and activating, or inhibiting, hormonally sensitive genes. Here is the profile for 56 of the 73 proposed EDSP priority chemicals in a number of relevant assays.

(single concentration only for binding and transcription factors)

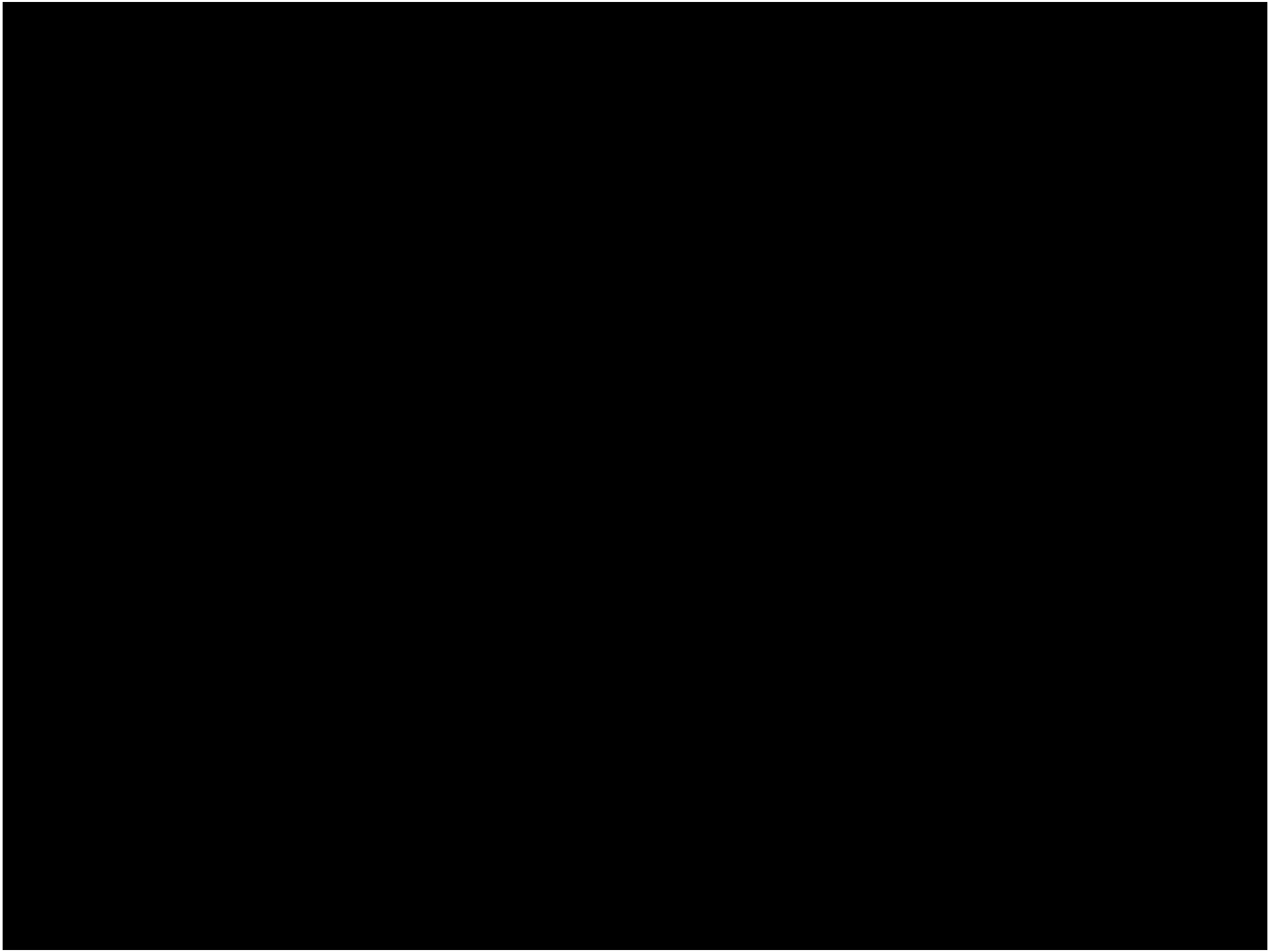
Predictive (Meta) Analysis of HTS Results Against a Liver Phenotype

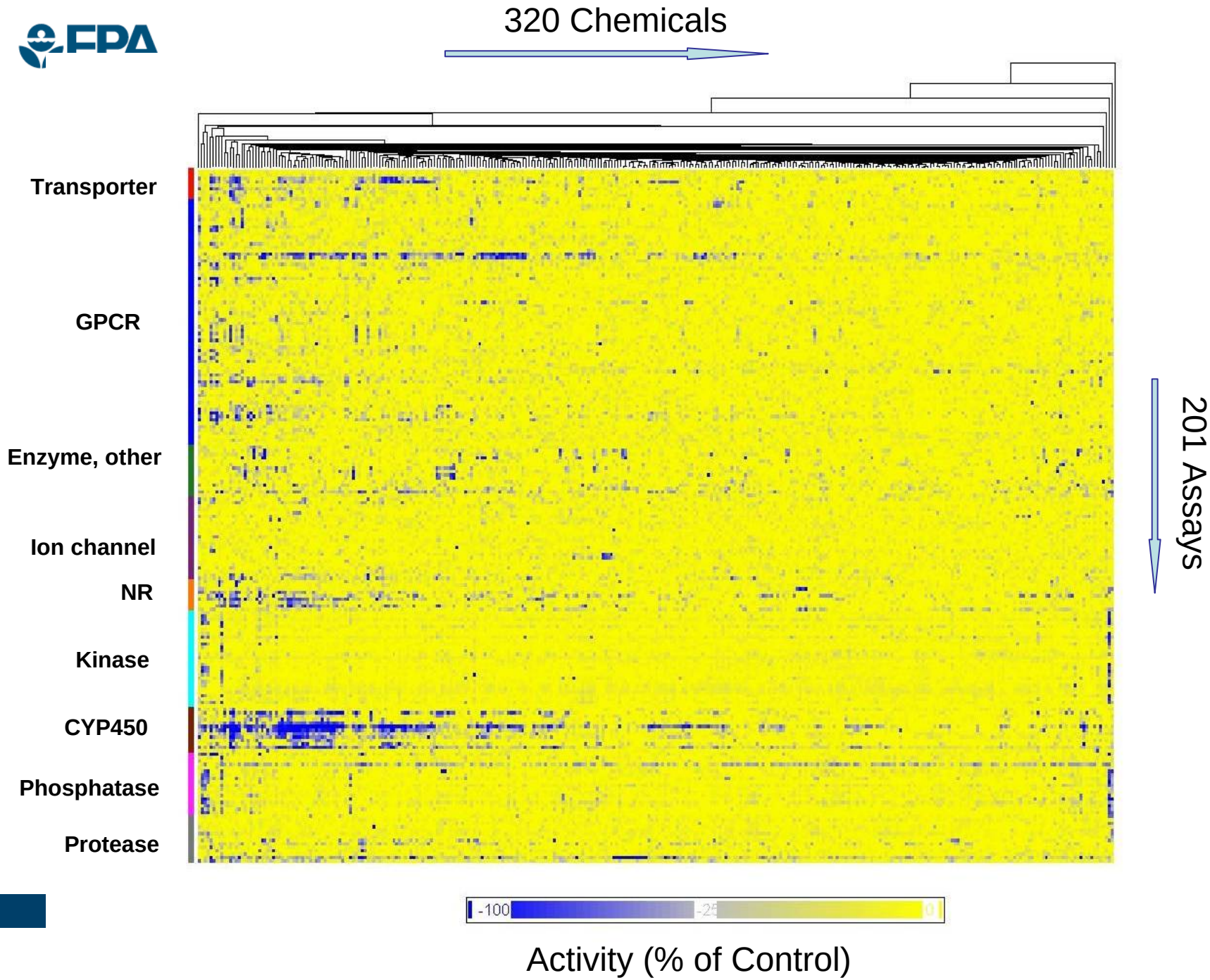


Summary

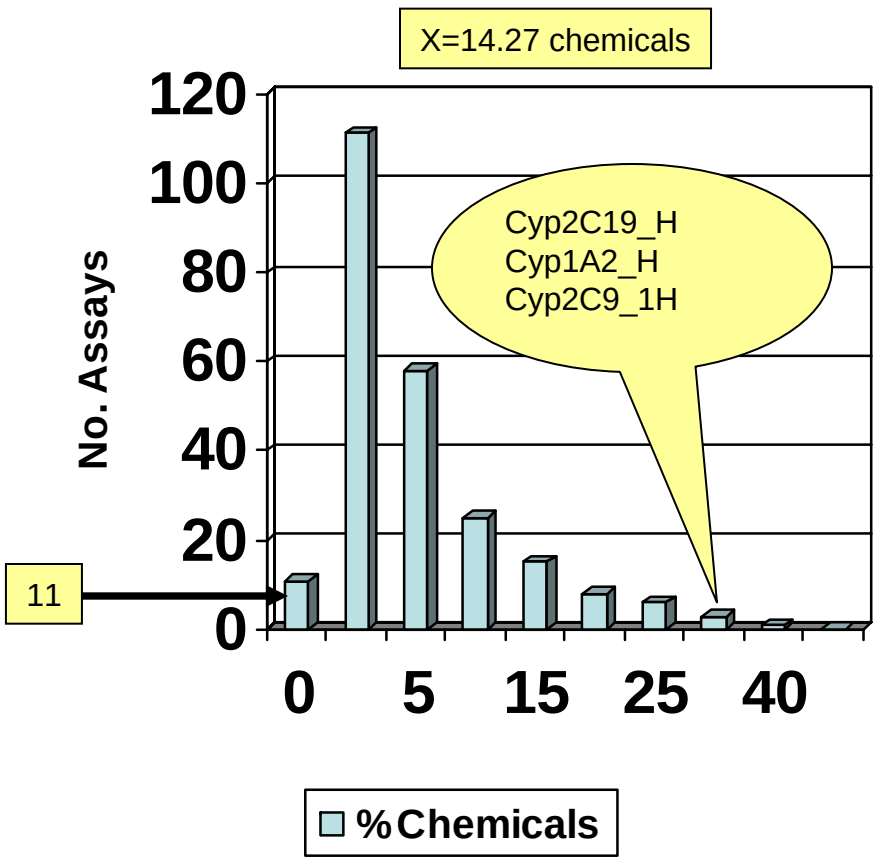
- The international community needs better predictive tools for assessing the hazards and risks of chemicals
- It is technically feasible to collect bioactivity data on virtually all chemicals of potential concern
- ToxCast is providing a proof of concept for obtaining predictive, broad-based spectra of bioactivity
- A critical need remains the elucidation of the majority of key biological processes involved in toxic responses
- The time is right to rapidly move this field along

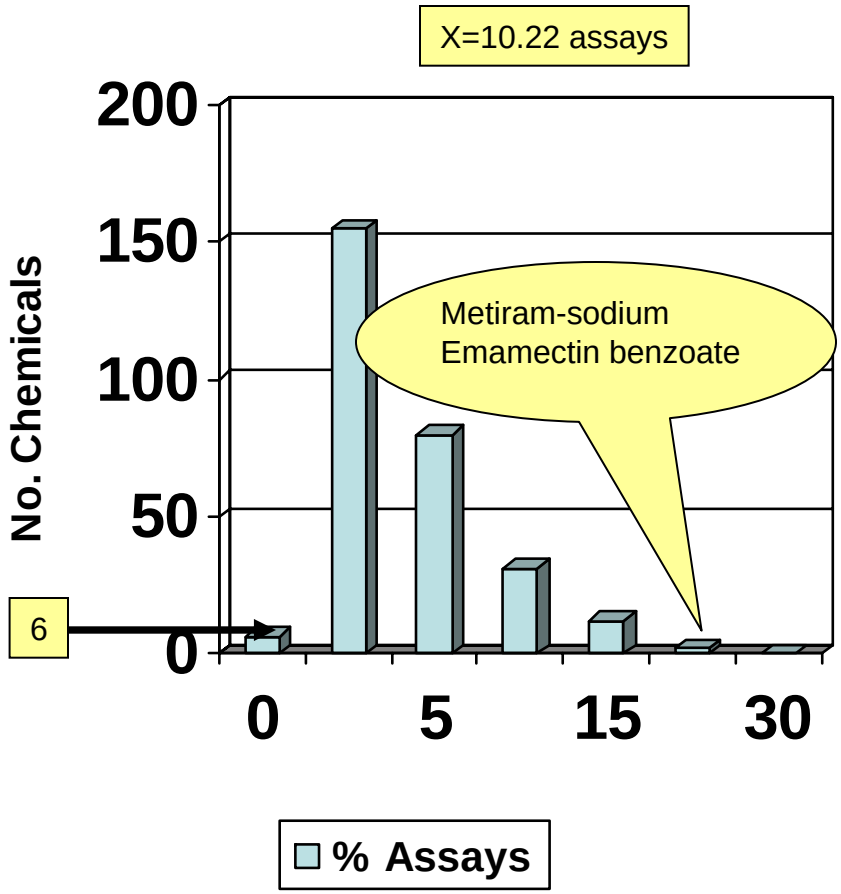
- **ToxCast Website: www.epa.gov/ncct/toxcast**




320 Chemicals
Transporter
GPCR
Enzyme, other
Ion channel
NR
Kinase
CYP450
Phosphatase
Protease
201 Assays
-100
-25
0
Activity (% of Control)

NovaScreen Descriptive Statistics (30% Cutoff)





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Real-time Cell Electronic Sensing (RT-CES) Assay

Judson et al., in preparation

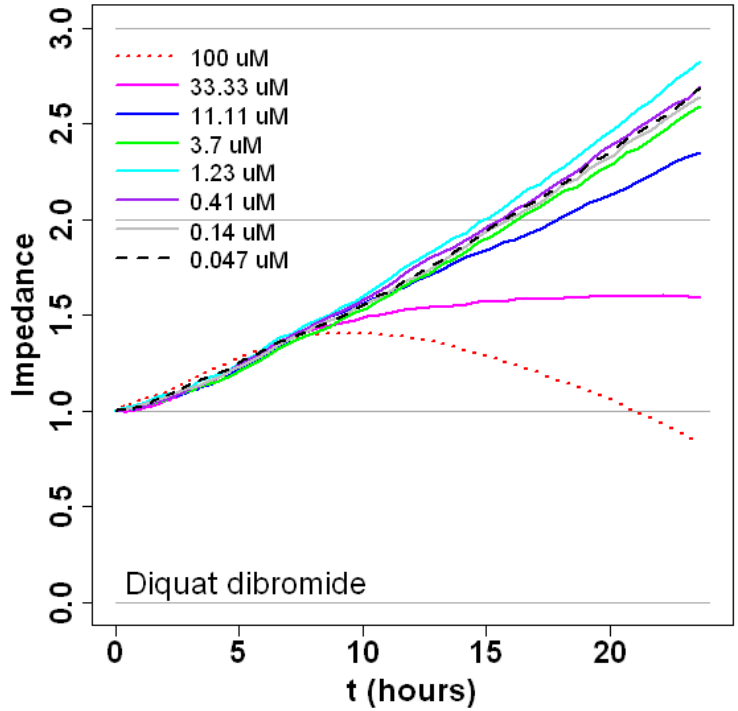

Figure 2 – Cell growth curve for Diquat Dibromide.

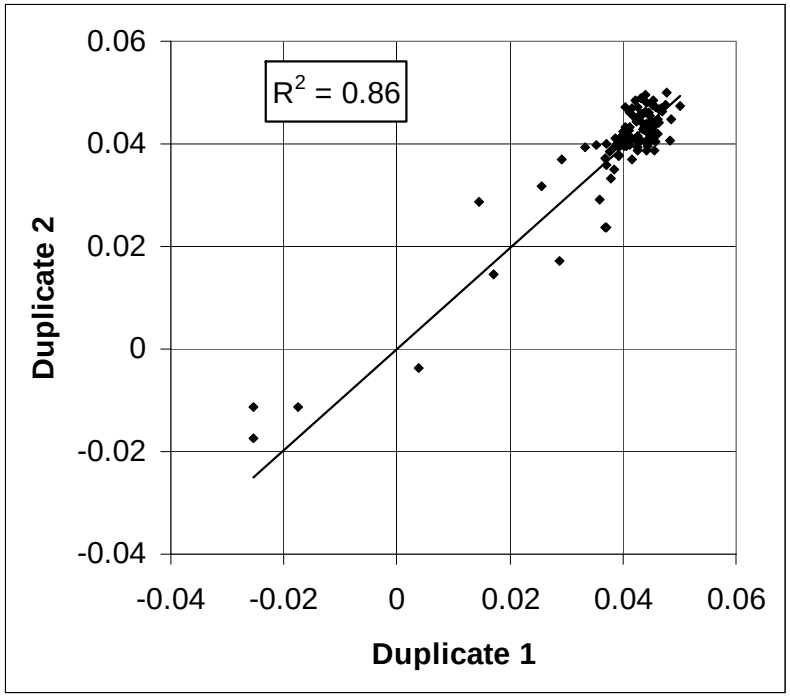

Figure 7 – Comparison of average growth rate values for replicates chemicals across all concentrations.

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

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National Center for Computational Toxicology

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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

ToxCast™ Navigation

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

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.