United States Environmental Protection Agency

2009 HESI Emerging Issue: Identification of Pharmaceuticals for Validation of ToxCast

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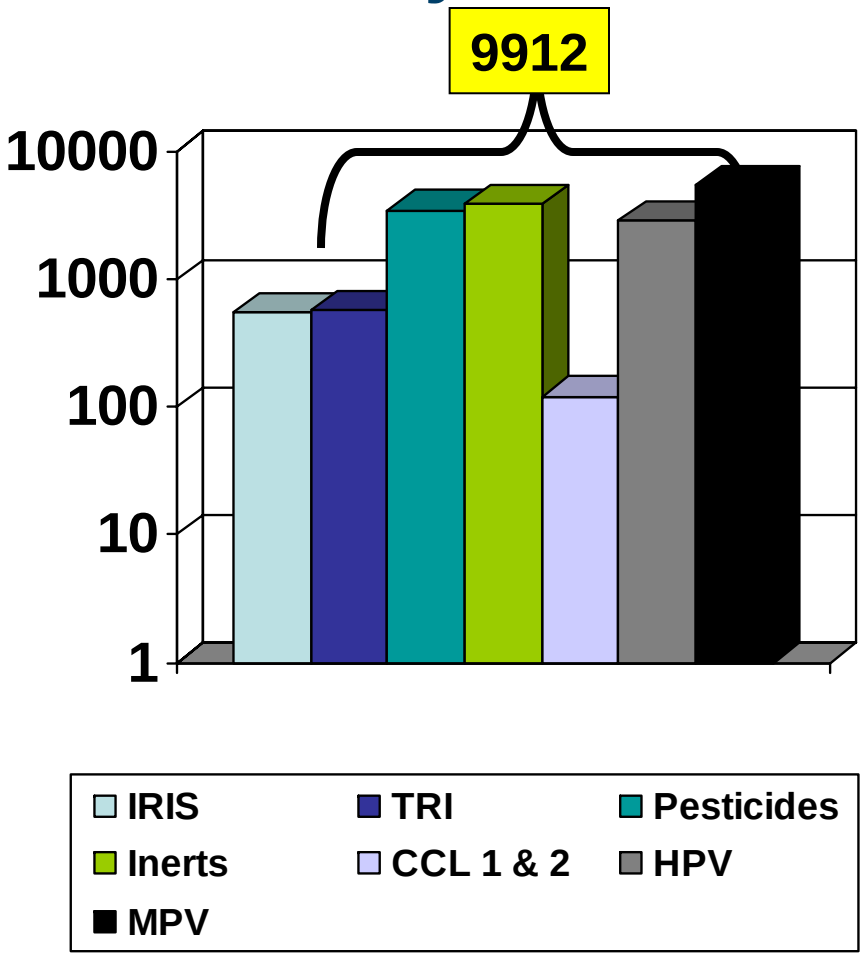
Office of Research and Development
National Center for Computational Toxicology

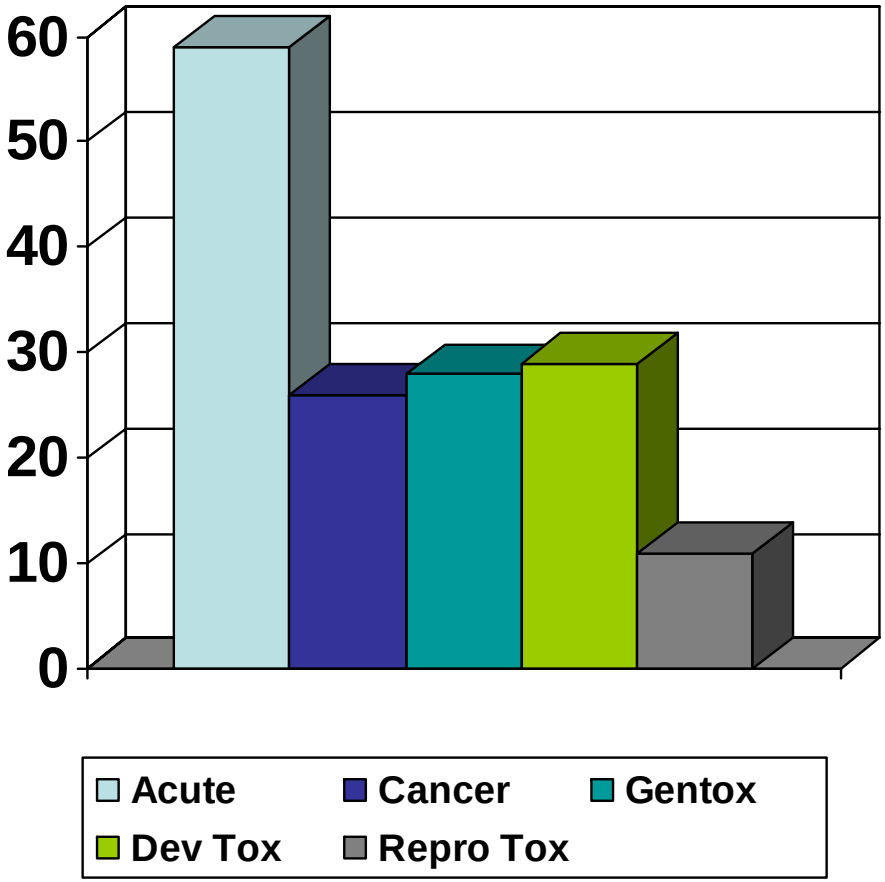
January 20, 2009

EPA's Need for Prioritization

Too Many Chemicals

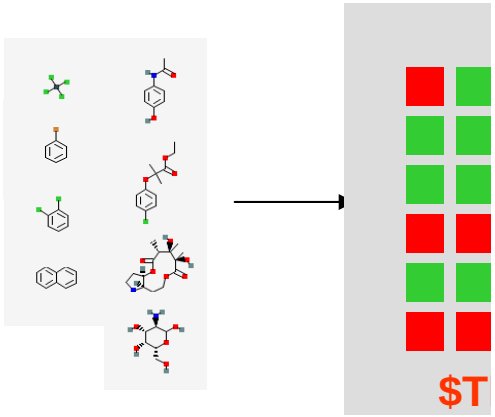


Too Little Data (%)



Judson, et al *EHP* (2009)

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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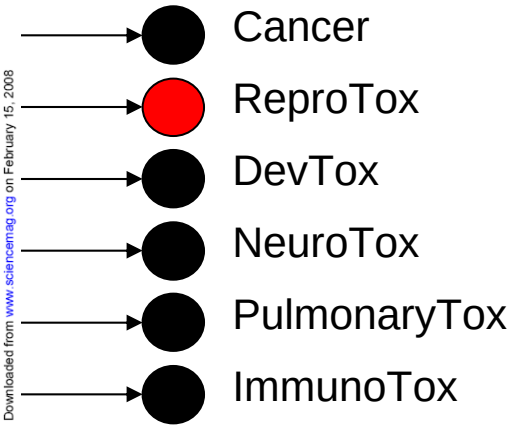

Tox21

EPAs Contribution: The ToxCast Research Program

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://www.epa.gov/actor/

Key Challenges Of Pathway Profiling

- Find the Toxicity Pathways
  - Hepato vs developmental neurotoxicity
- 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

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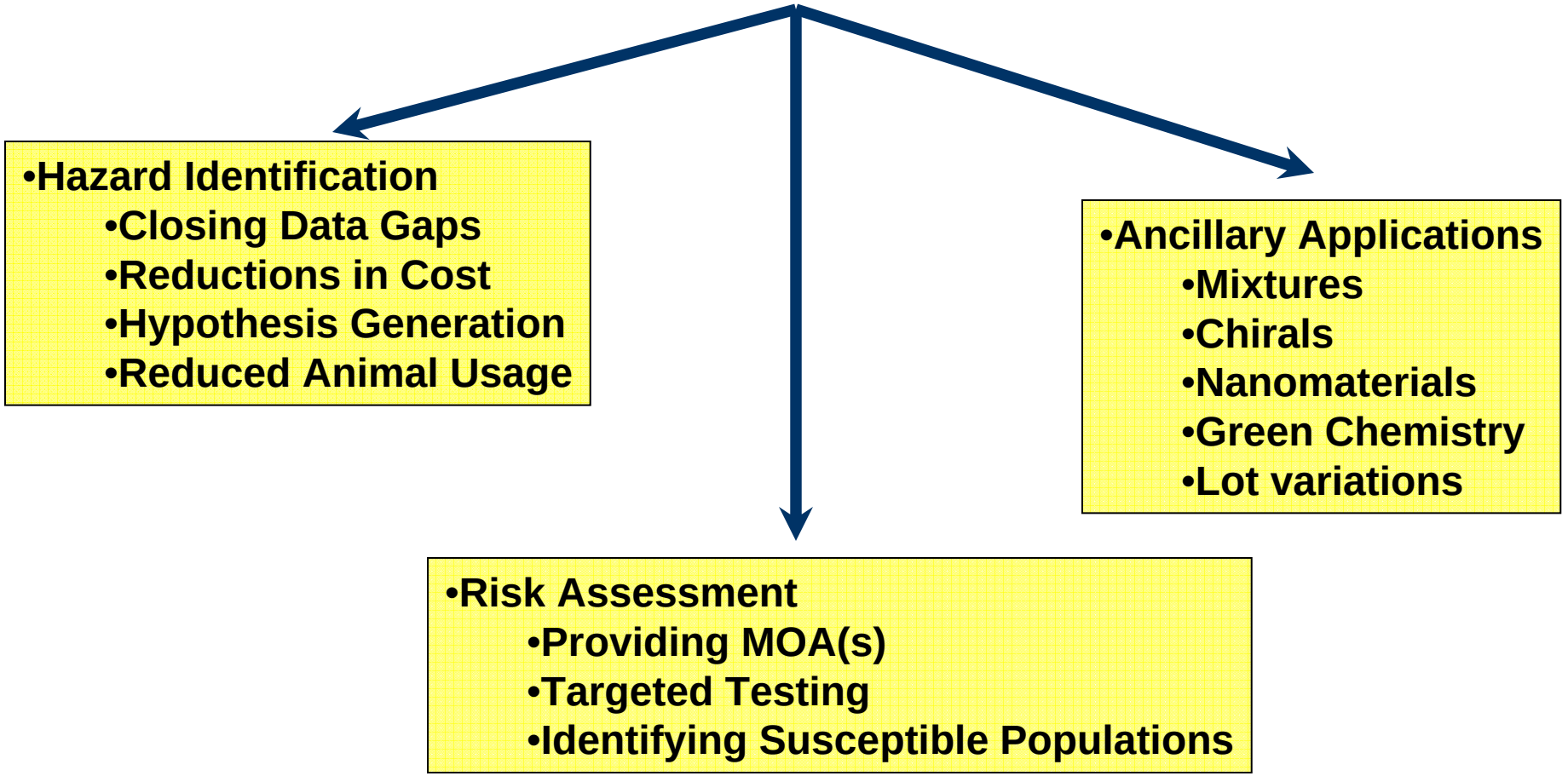
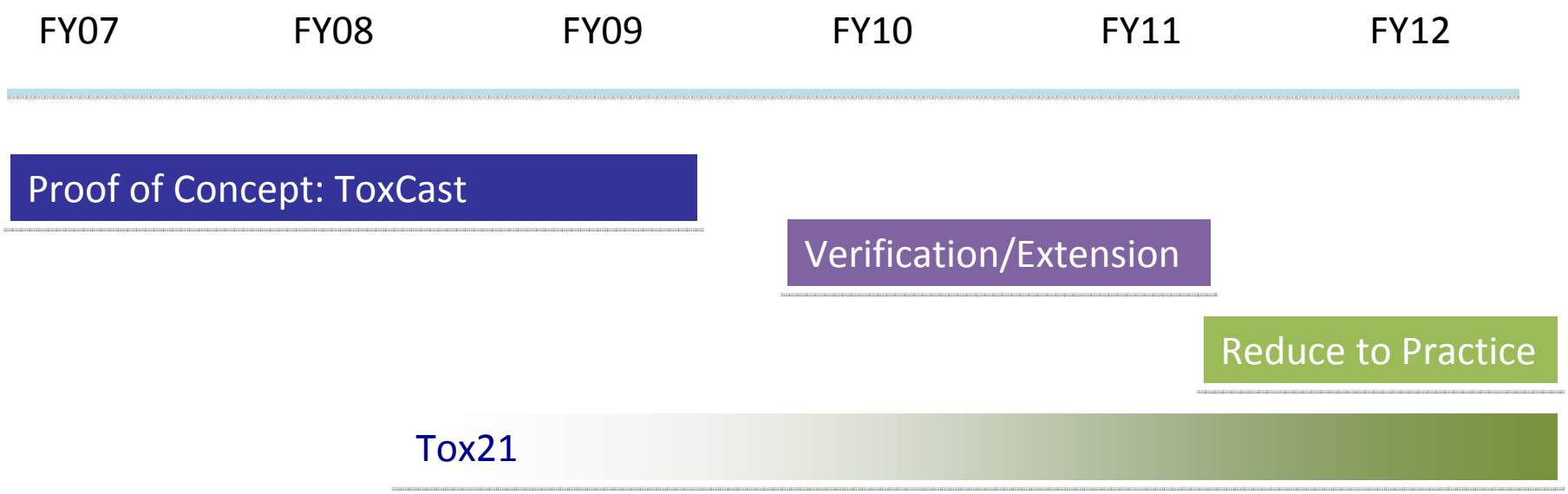
Implications for Success



Prioritization Product Timeline



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	>500	$20k	FY07-08
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 Phase I Datasets

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- Released to Data Analysis Partners:
  - ACEA - Real-time Cell Electronic Sensing (7 assays)
  - Attagene - Transcription factor assays (81 assays)
  - BioSeek - Cell-based protein level assays (87 assays)
  - Cellumen - Cell imaging assays (11 assays)
  - CellzDirect – NR target-gene expression assays (16 assays)
  - Gentronix - GreenScreen GeneTox assay (1 assay)
  - NCGC - nuclear receptor assays (11 assays)
  - Novascreen / Caliper - receptor binding and enzyme inhibition assays (239 assays)
  - Solidus - P450 vs. cytotoxicity assays (4 assays)

467 Endpoints

- Upcoming Dataset Additions:
  - Neurite outgrowth HCS (NHEERL)
  - Cell proliferation (NHEERL)
  - Zebrafish developmental toxicity (NHEERL)
  - Organ toxicity; dosimetry (Hamner Institutes)
  - C. elegans WormTox (NIEHS)
  - Gene markers from microscale cultured hepatocytes (Hepregen
  - 3D Cellular Zebrafish vascular/cardiotoxicity (Zygogen)
  - HTS stress response (NHEERL+NCGC)
  - Embryonic Stem Cells (NHEERL)
  - Metabolic Phenotyping (Biolog)

New contract proposals under review

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

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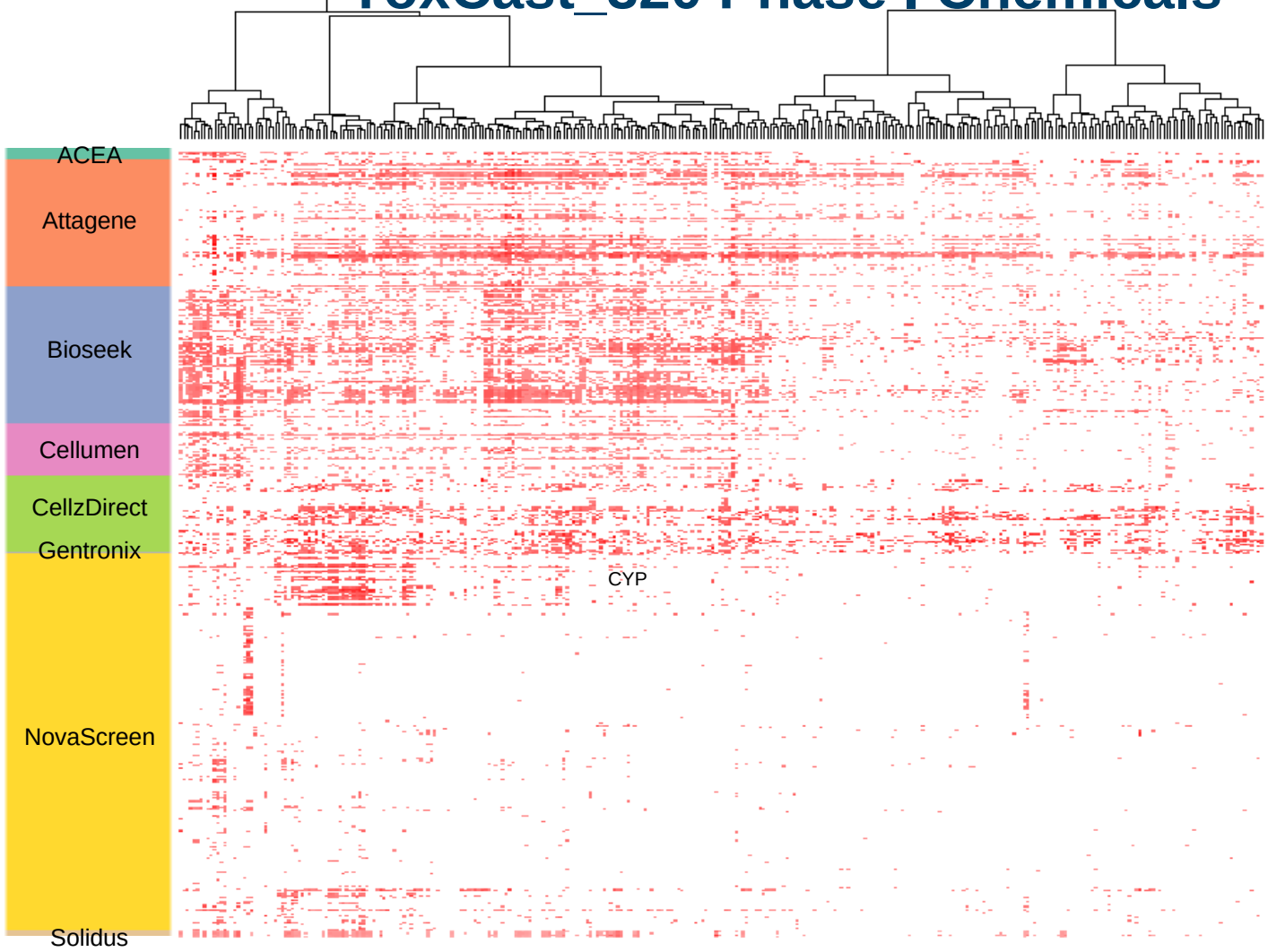
Circa 2009

ToxCast_320 Phase I Chemicals

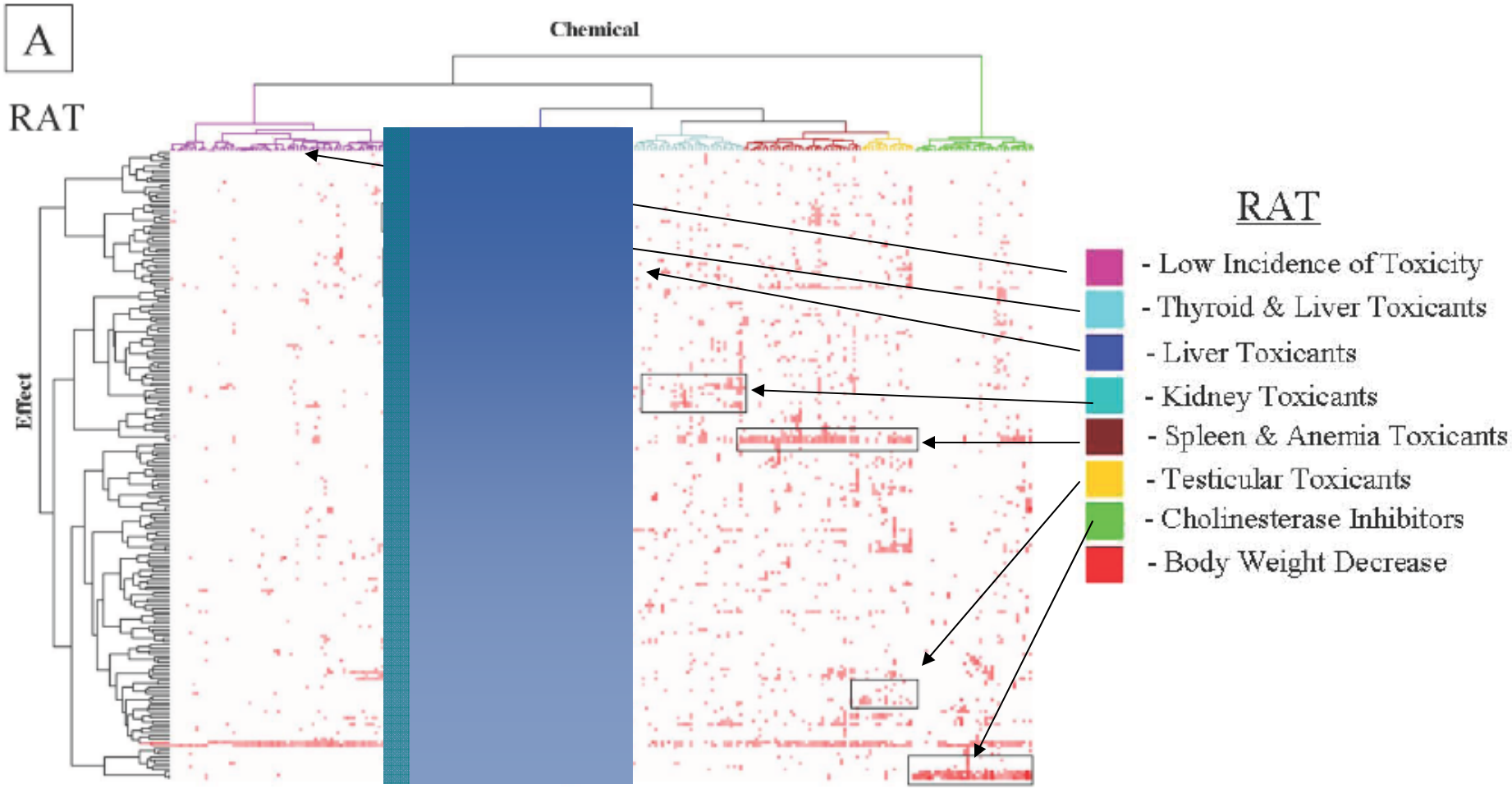
ToxCast Phase I Assays
500 endpoints



ToxRefDB

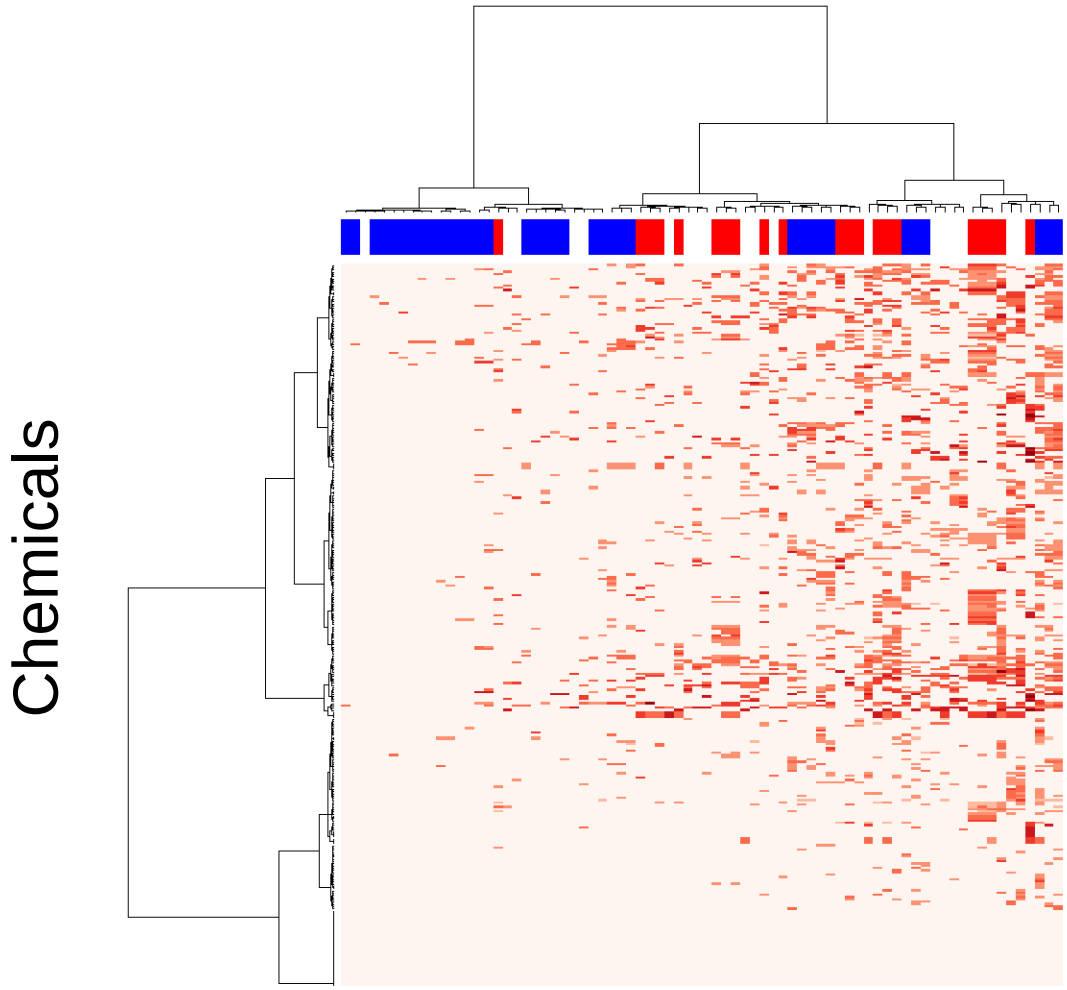
- Relational phenotypic/toxicity database
- Provides in vivo anchor for ToxCast predictions
- Three study types
  - Chronic/Cancer rat and mouse (Martin, et al, EHP 2008)
  - Rat multigenerational Reproduction (Martin, et al, Tox Sci, in press)
  - Rat & Rabbit developmental (Knudsen, et al, Repro Tox, in press)
- Two types of synthesis
  - Supervised (common individual phenotypes)
  - Unsupervised (machine based clustering of phenotype patterns)

> $1B in Toxicology Now Stored in ToxRefDB



Martin, et al *EHP*, 2009

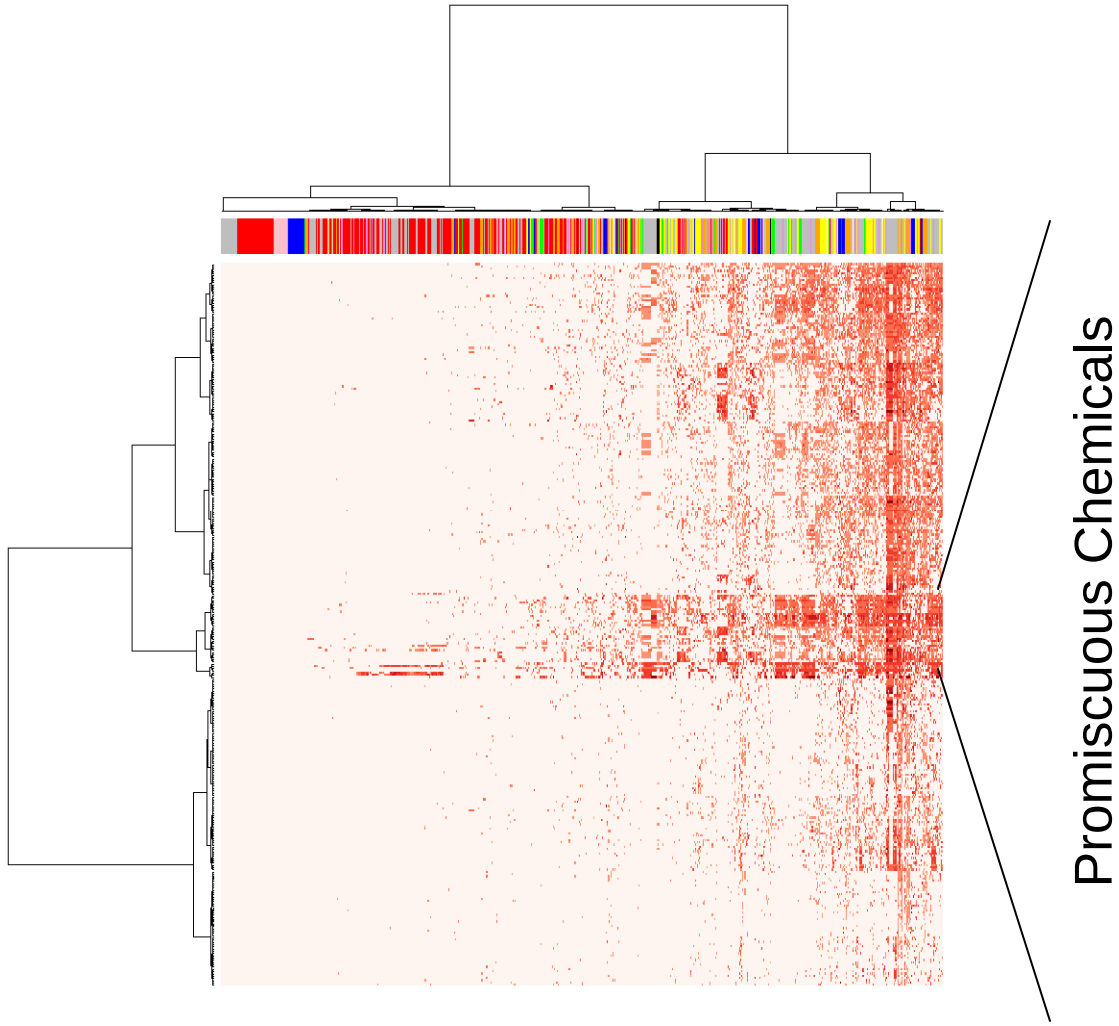
ToxCast In Vivo Data from ToxRefDB



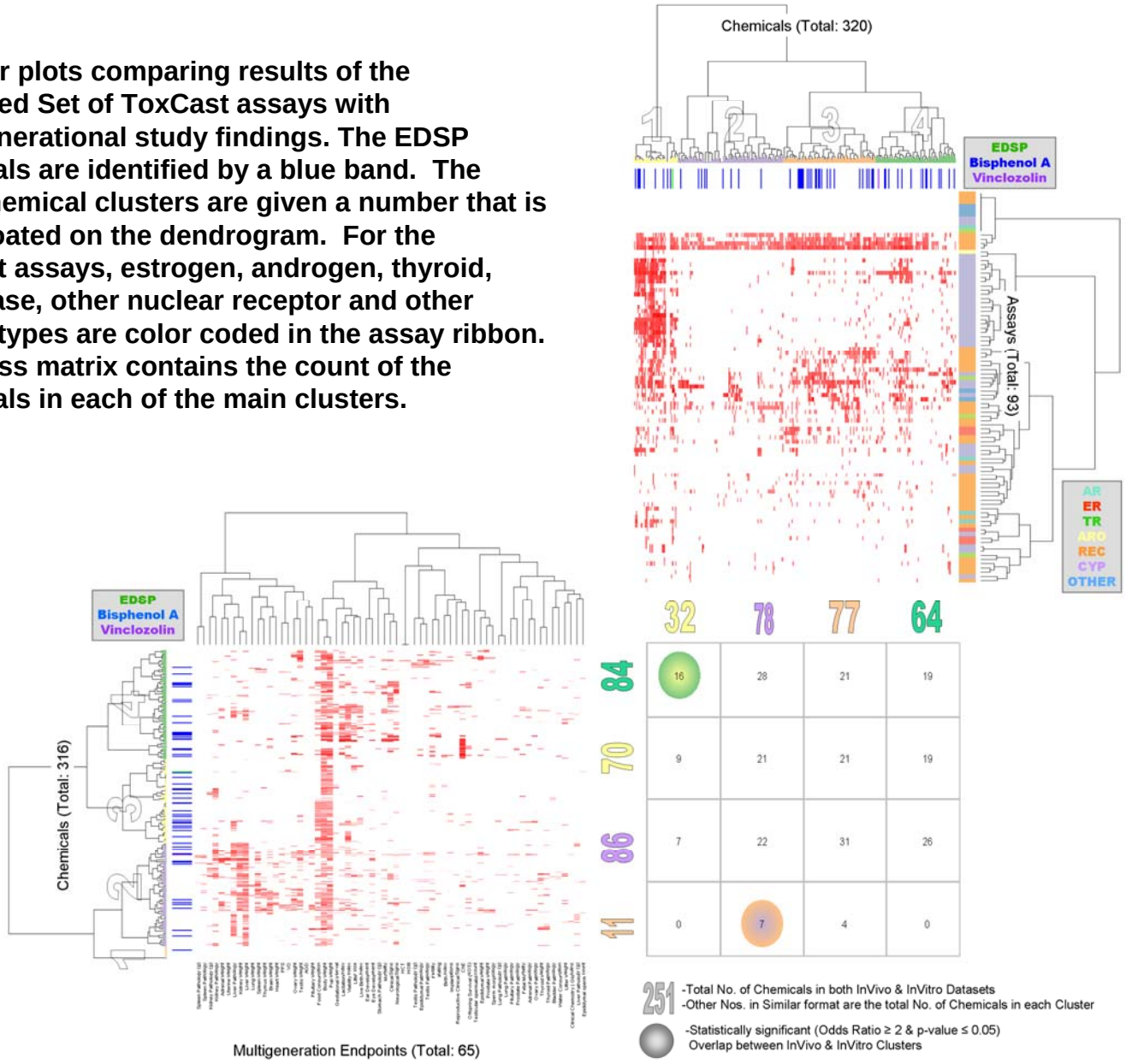
Red: Chronic/Cancer
White: Multigen
Blue: Developmental

ToxCast In vitro data

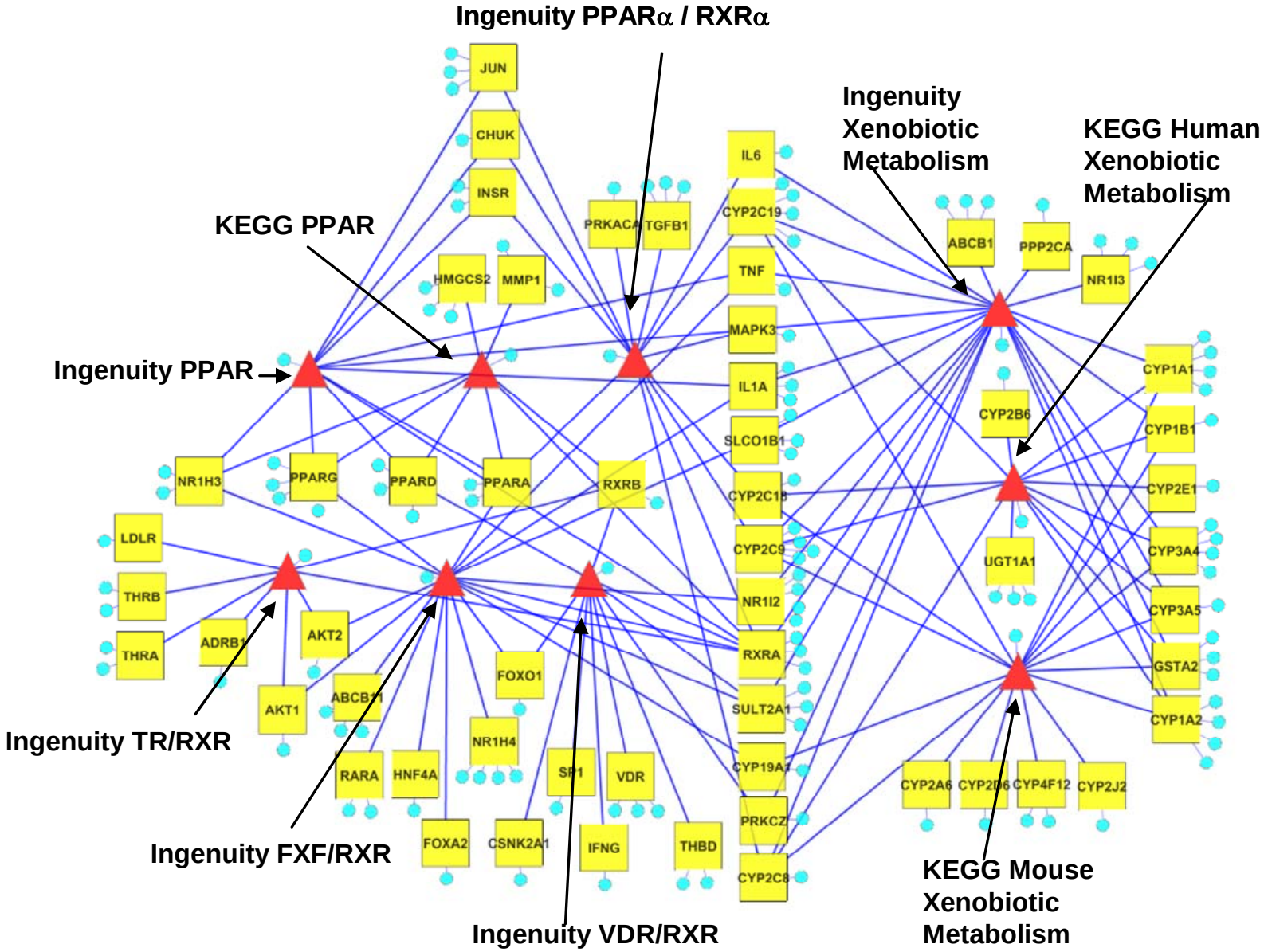
- Novascreen
- Attagene
- BioSeek
- Cellumen
- CellzDirect
- NCGC
- Gene assays
- Pathway assays



2-D cluster plots comparing results of the Expanded Set of ToxCast assays with multigenerational study findings. The EDSP chemicals are identified by a blue band. The main chemical clusters are given a number that is color coated on the dendrogram. For the ToxCast assays, estrogen, androgen, thyroid, aromatase, other nuclear receptor and other assays types are color coded in the assay ribbon. The cross matrix contains the count of the chemicals in each of the main clusters.



XME / Nuclear Receptor Pathway Covered by ToxCast



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ToxCast™ Program

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

ToxCast™ Data Analysis Summit

ToxCast™ Navigation

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Click here to go to registration and travel information page for the TDAS Meeting

Meeting Notice and Call for Abstracts

Transforming Toxicity Testing From In Vivo to In Vitro:
A Computational Toxicology Challenge

The First ToxCast™ Data Analysis Summit
Hosted by U.S. EPA"s National Center for Computational Toxicology
EPA Campus, Research Triangle Park NC
May 14-15, 2009

Overview: The U.S. EPA ToxCastTM Program is developing approaches to predict chemical toxicity using data from high-throughput and high content in vitro assays. Phase I of ToxCast has produced data from 320 chemicals, ~500 in vitro assays and ~100 in vivo endpoints, providing a powerful dataset for evaluating the applicability of various analytic approaches for predicting the potential for an adverse response.

The goal of ToxCast is to develop and verify "toxicity signatures," which are algorithms using in vitro and in silico data to predict in vivo toxicities. These signatures will be used to screen and prioritize chemicals for targeted toxicity testing, and over the next several years EPA would like to screen thousands of compounds. However, successful predictive models will depend on robust and reliable methods that EPA can rely on for making decisions about further testing of environmental chemicals.

This first ToxCast Data Analysis Summit is designed to bring together experts in machine learning, computational chemistry, statistics, high-throughput screening and computational toxicology, with toxicologists and regulatory staff. Plenary talks will describe the ToxCast Program and a series of issues related to toxicity prediction, both from a scientific and regulatory standpoint. Speakers will be selected from abstract submitters to describe algorithmic, computational or systems biology approaches to solving these issues.

To further this aim, we invite interested researchers to submit abstracts and present their analyses, using the ToxCast Phase I dataset, at the First ToxCast Data Analysis Summit

Topics of interest include:

- Machine learning or statistical approaches for signature generation
- Systems biology / pathway modeling approaches
- Issues of statistical power for prediction and verification.
- Models to address prediction of metabolism and biotransformation

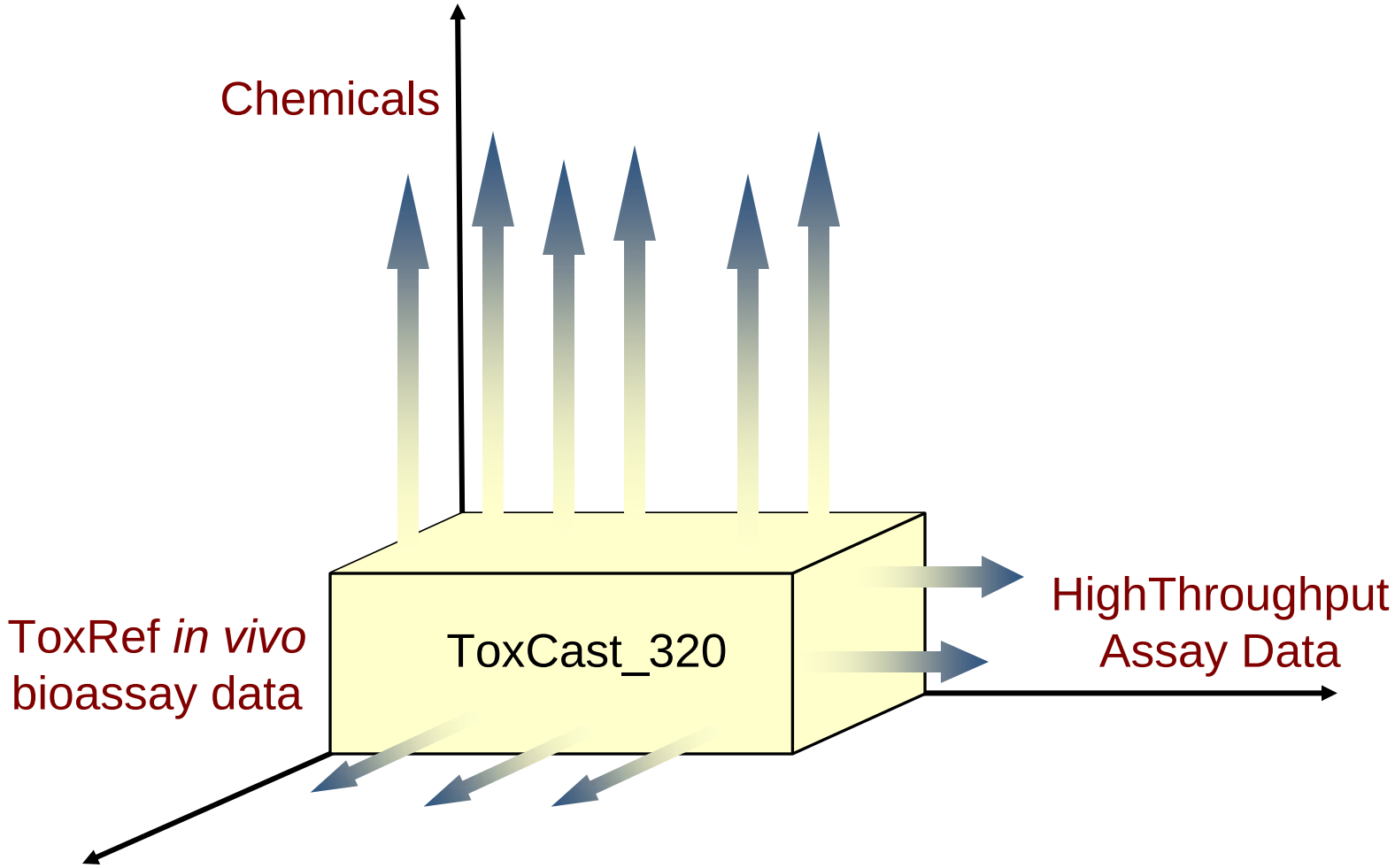
The analyses described in the abstracts must make use of the ToxCast Phase I dataset, although additional data may also be included.

This is not a "Critical Assessment" workshop with an outcome of "best" prediction methods. That will be the goal of a follow-up meeting in 2010, for which a significant body of blinded validation data will be available from later phases of ToxCast.

Data Overview: : The collection of ToxCast Phase I chemicals were chosen because high quality, guideline-based animal toxicity data were available. These chemicals are mostly pesticide active compounds for which we have rat and mouse 2-year chronic/cancer, 2-generation reproductive, and developmental toxicity data. For analysis, we will provide ~100 toxicity endpoints from these study types whose value is a "LEL" or lowest effective level at which the endpoint was observed- these are the values to predict. In addition, we will provide other aggregated endpoints derived from clustering analyses. Analysis groups (or analysis partners) are also free to develop other endpoints to predict from the data that we will provide.

A total of 9 in vitro datasets have been produced, reviewed and cleared for research use. These include biochemical receptor and enzyme assays; and cell-based

Beyond the Proof of Concept

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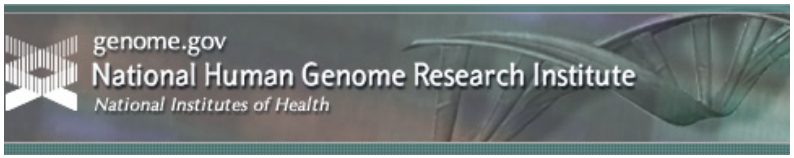
Tox21


NCGC
NIH Chemical Genomics Center


genome.gov
National Human Genome Research Institute
National Institutes of Health

Tox21 Existing and Candidate Chemicals*

Universe	**13,247**
With structures	**8,277**
Plausible P-chem (logP)	**7,116**

	Current	**Additional**
NTP	**1353**	**~1400**
EPA	**1330**	**~2800**
NCGC	**~3000 drugs**	**-**
Target library, Summer 2009		**~10,000**

* Sources include NTP, EPA HPV, CCL, OPPIN, OW, Inerts, ToxCast, DSSTox, EU Carcinogenomics, Pharmaceuticals, others

Tox21

Phased Development of ToxCast

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III	Thousands	Data poor	Prediction and Prioritization	>300	~$15-20k	FY11-12

January 2009

Emerging Issues Proposal

- SPECIFIC ACTIONS
  - Coordinate Public-Private sector involvement in ToxCast predictions
  - Scoping meeting to articulate needs, timelines and boundaries of involvement by participants
  - Organization commitment to effort by early June 2009
  - Delivery of chemicals as soon as possible
  - Coordinate data extraction template
- DESIRED OUTCOME
  - Successful deliberations and negotiations would result in:
    - Identification and provisioning of chemicals (~100mg) for screening
    - Sharing of relevant pre-clincal and clinical data
    - Co-publications on predictive models

MATERIALS TRANSFER AGREEMENT

EPA:

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Pfizer:
Pfizer Inc, having a principal place of business at 235 East 42nd Street, New York, ("Pfizer") New York, 10017 and its Affiliates

WHEREAS the EPA wishes to obtain Pfizer Compounds to use in certain test assay panels, and whereas Pfizer wishes to have Pfizer Compounds evaluated on such test panels, the parties agree as follows:

"Affiliate" means any corporation, firm partnership or other entity which directly or indirectly controls, is controlled by, or is under common control with either of the parties.

1. EPA agrees to receive Pfizer's compounds, listed in Exhibit B, in any form or any of its intermediates and derivatives ("Pfizer Compound"), in order to perform the research activities, further described in Exhibit A, and known as the "ToxCast™ Program."

2. The Pfizer Compounds:
   a. are the property of Pfizer and all existing rights including, without limitation, patent rights in or to the Pfizer Compounds will remain the property of the Pfizer.
   b. will be used with caution and for research purposes only, and shall not be used for research involving human subjects.
   c. will be used only by the EPA in the ToxCast™ Program described below, under suitable containment conditions.
   d. will not be used for screening, production or sale, for which a commercialization license may be required.

Both Pfizer and EPA agree to comply with all applicable laws, rules, guidelines and regulations applicable to the use, storage, shipping and the handling of the Pfizer Compounds and ToxCast™ Program.

Benefits of Proposal

- Draws on unique position of HESI in bringing the public and private sectors together for progress in science
- Enables utilization of a unique private sector knowledge
- Builds on the experience of EPA in computational toxicology
- Brings direct human relevance to HTS screening on environmental chemicals, which already involves the use of many human protein targets and cell types
- HESI would be intimately associated and linked with progress at reaching the vision of toxicity testing in the 21st envisioned by the National Research Council