United States Environmental Protection Agency

2008 HESI Emerging Issue: Identification of Pharmaceuticals for Validation of ToxCast

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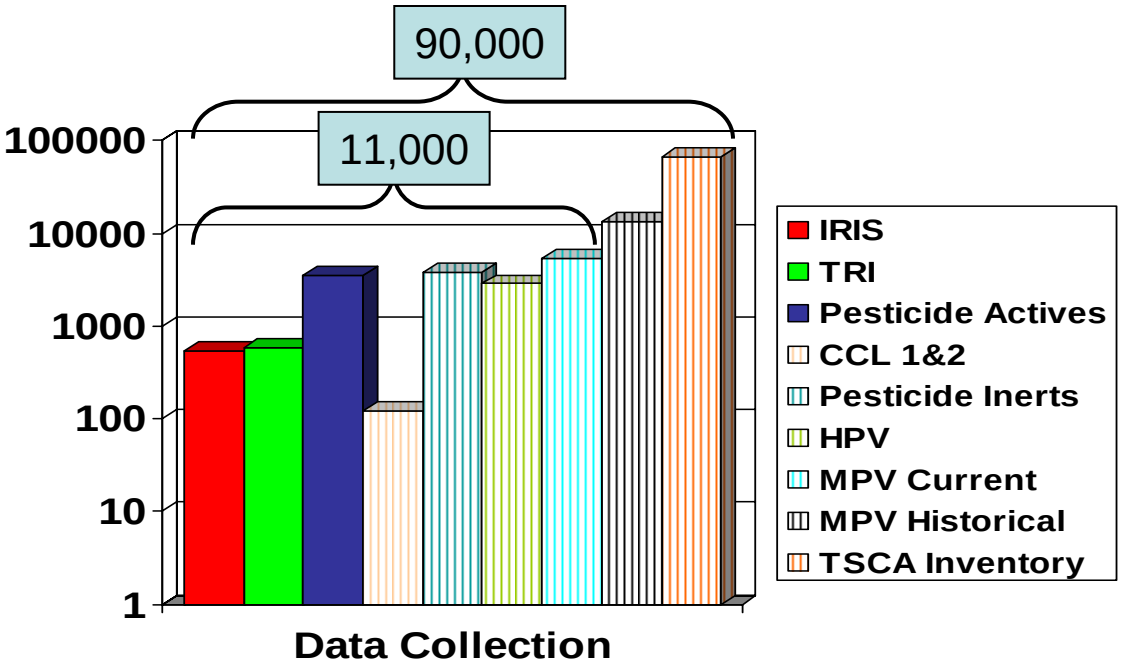
Office of Research and Development
National Center for Computational Toxicology

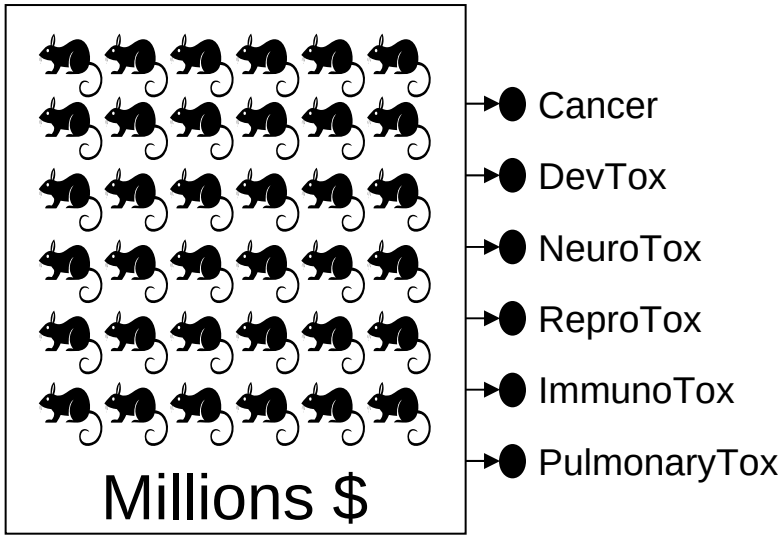
January 20, 2009

Change Needed Because …..

Too Many Chemicals



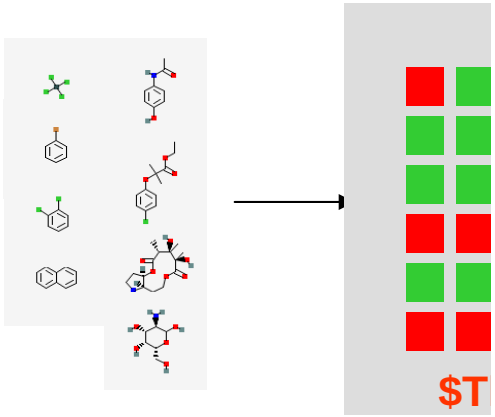
Too High a Cost



…and not enough data.

Judson, et al *EHP submitted*

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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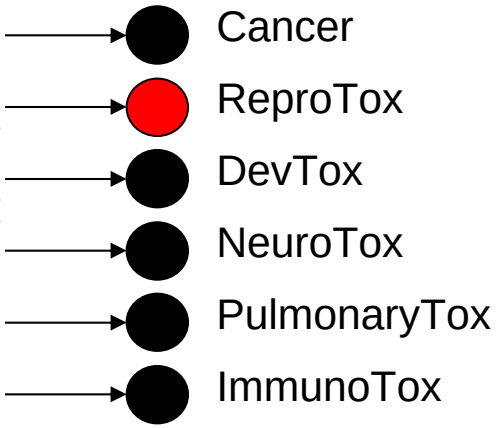
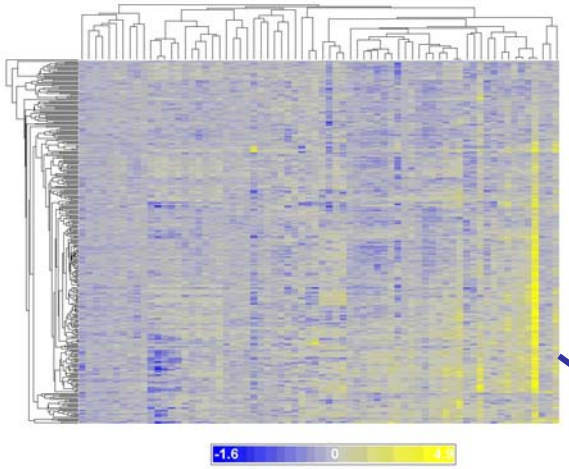


Tox21

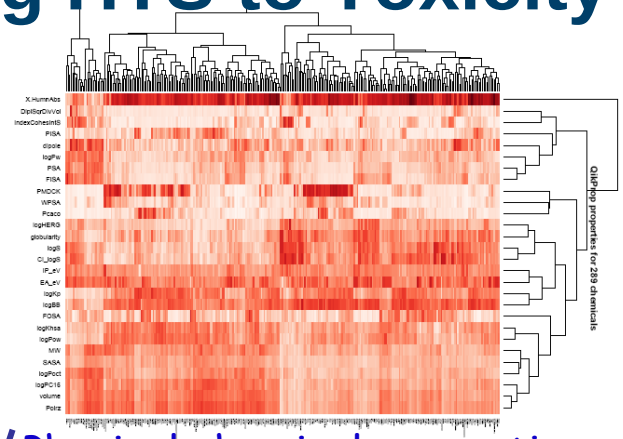
EPAs Contribution: The ToxCast Research Program

Correlating HTS to Toxicity

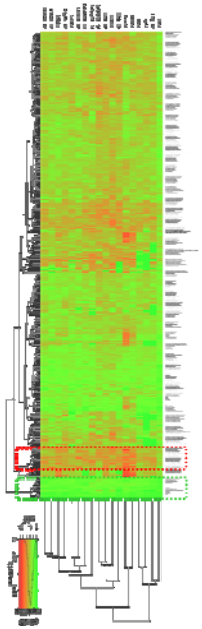
United States Environmental Protection Agency



Cellular Assays

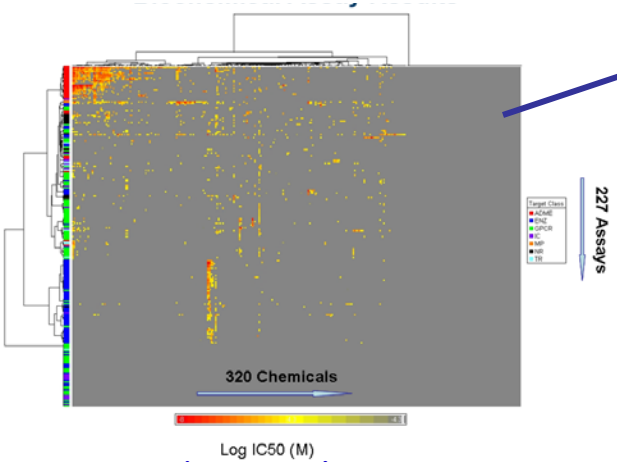


Physical chemical properties

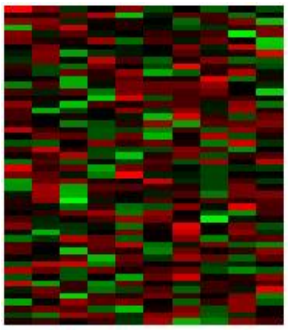


In silico Predictions

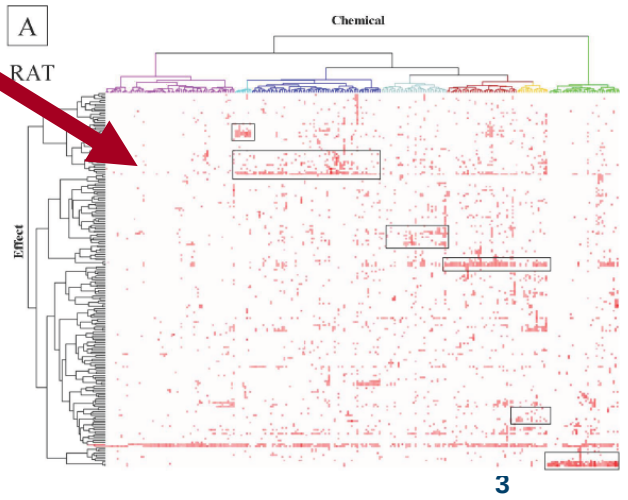
Profile Matching



Biochemical Assays



Genomic Signatures



Toxicology Endpoints

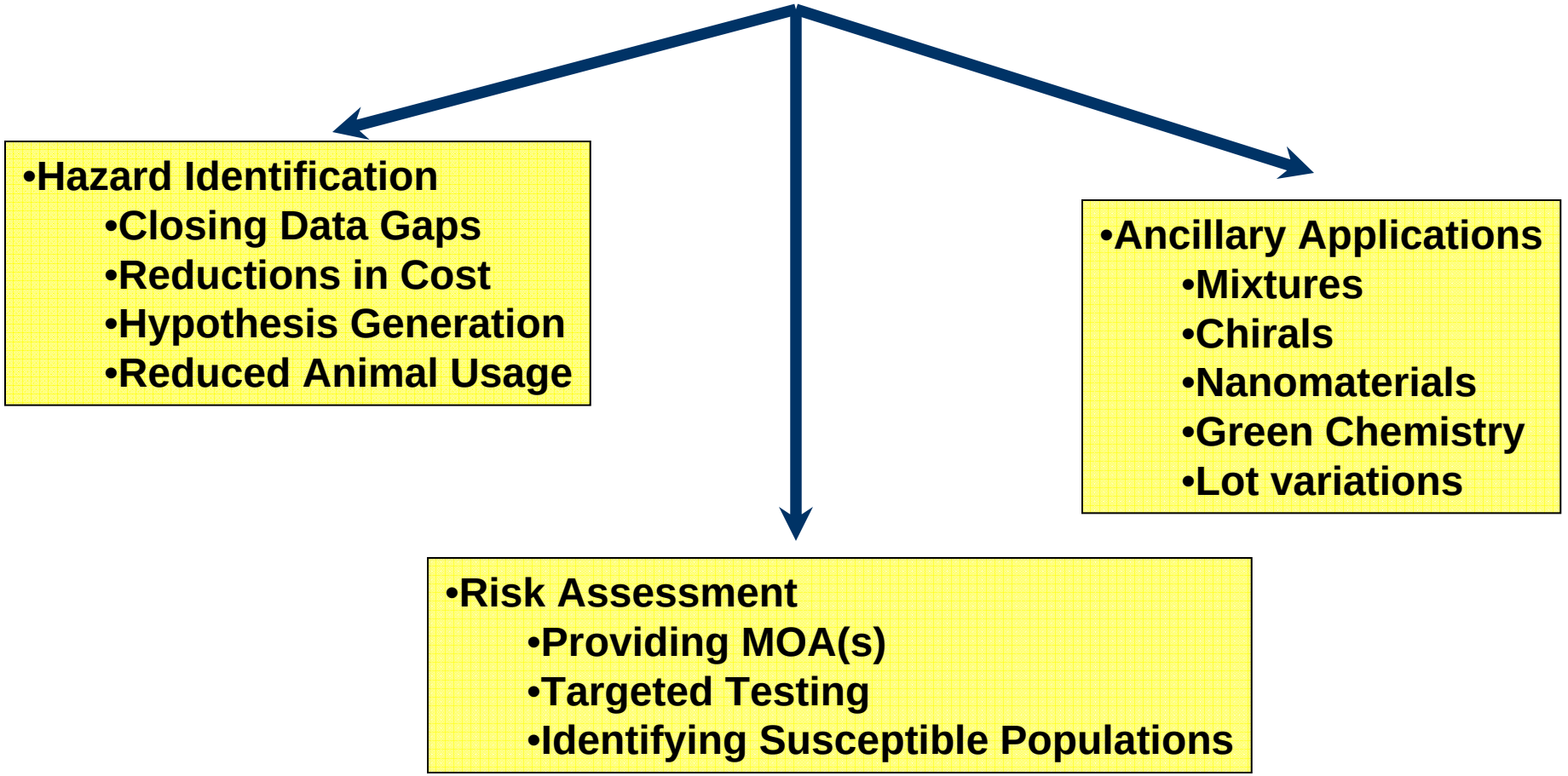
Office of Research and Development
National Center for Computational Toxicology

Key Challenges

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

United States Environmental Protection Agency

Implications for Success



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/

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
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

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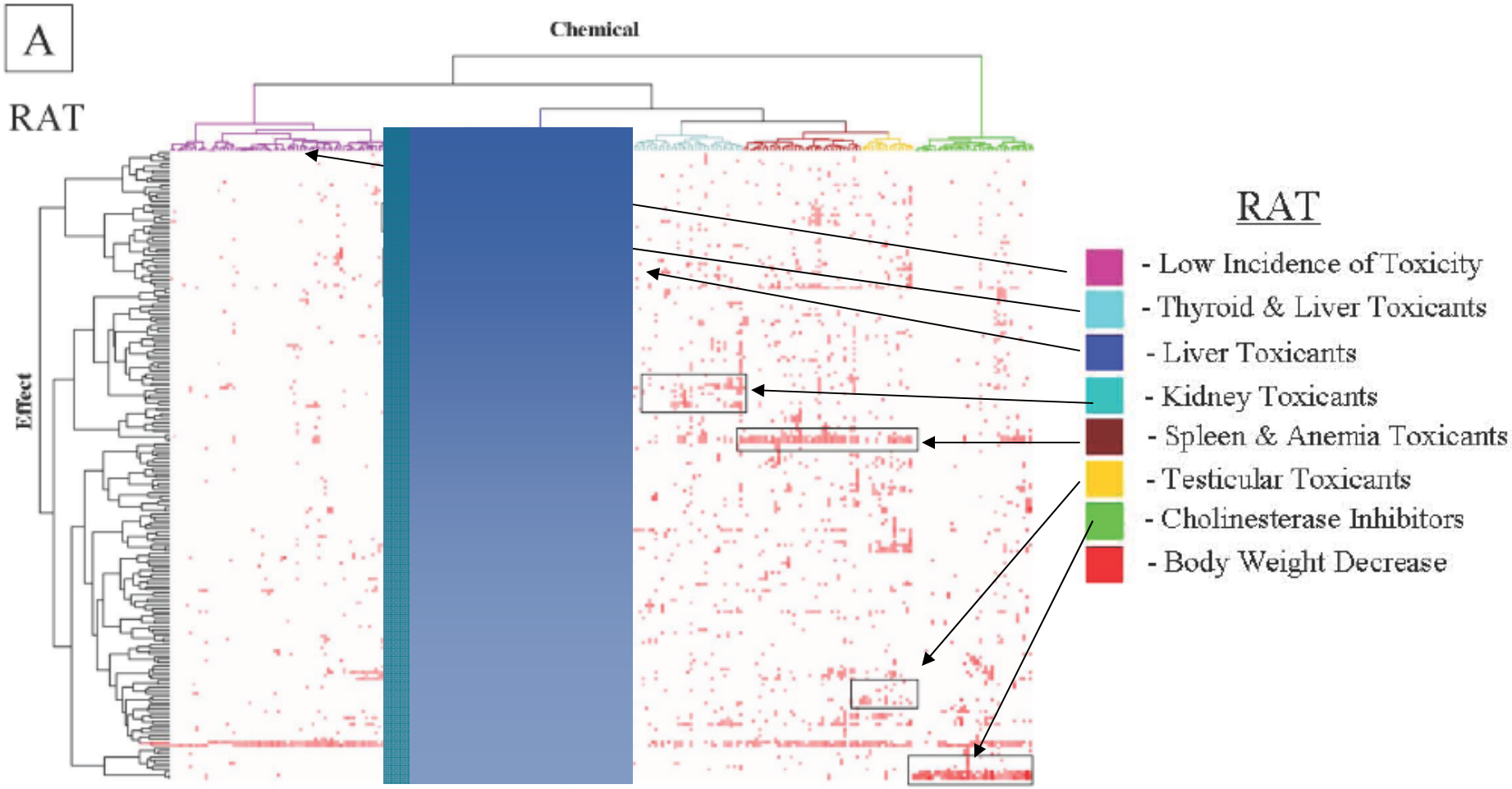
$1B in Toxicology Now Stored in ToxRefDB



Martin, et al *EHP*, 2008

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

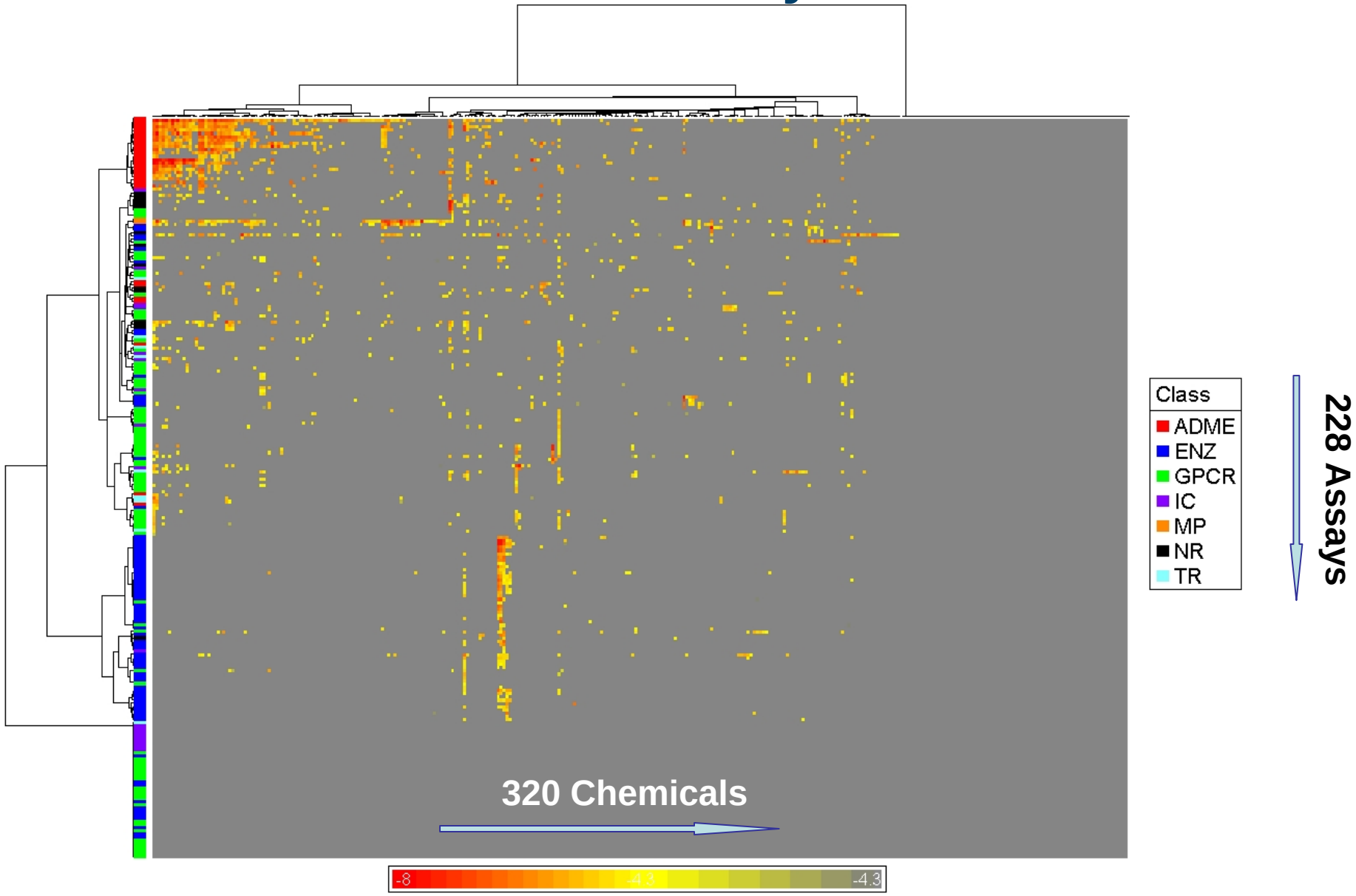
Cellular Assays

- 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

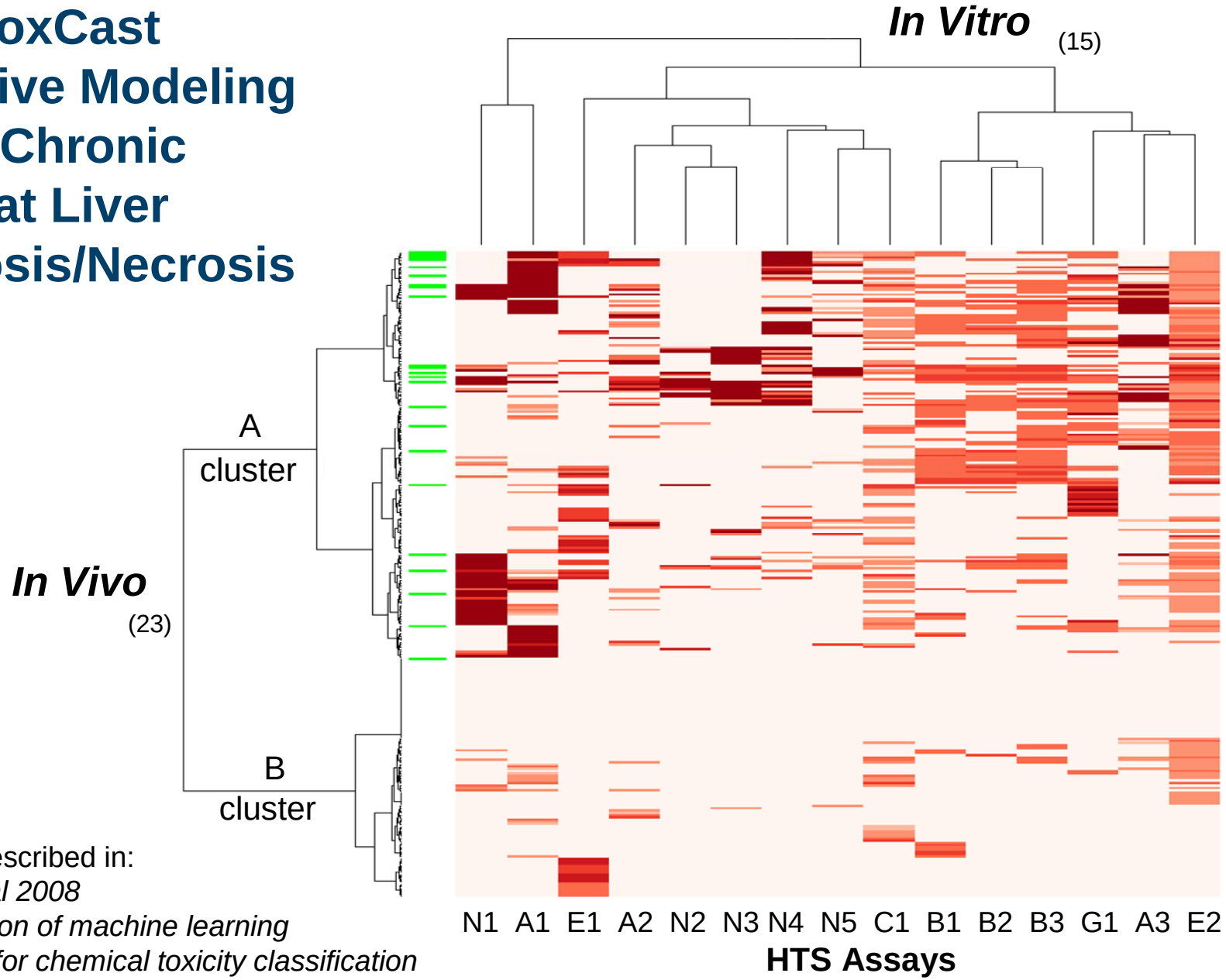
Cellular Assays

- 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

Biochemical Assay Results

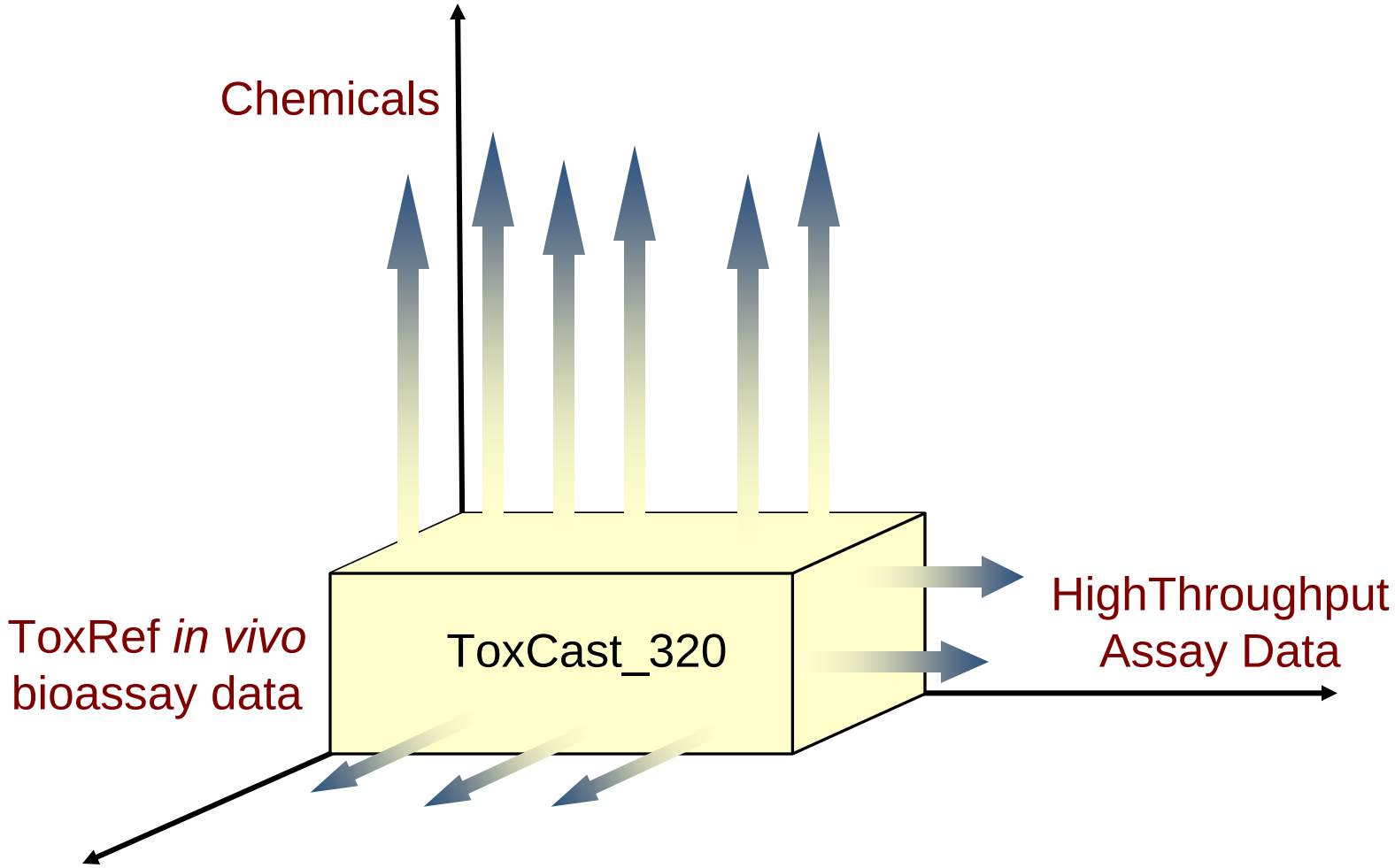


ToxCast Predictive Modeling of Chronic Rat Liver Apoptosis/Necrosis



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

Beyond the Proof of Concept




NIEHS
National Institute of
Environmental Health Sciences

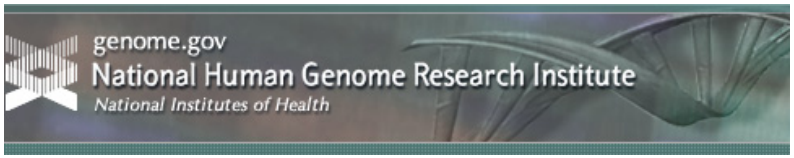

UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY


National Toxicology Program
U.S. Department of Health and Human Services


UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
COMPUTATIONAL
TOXICOLOGY


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

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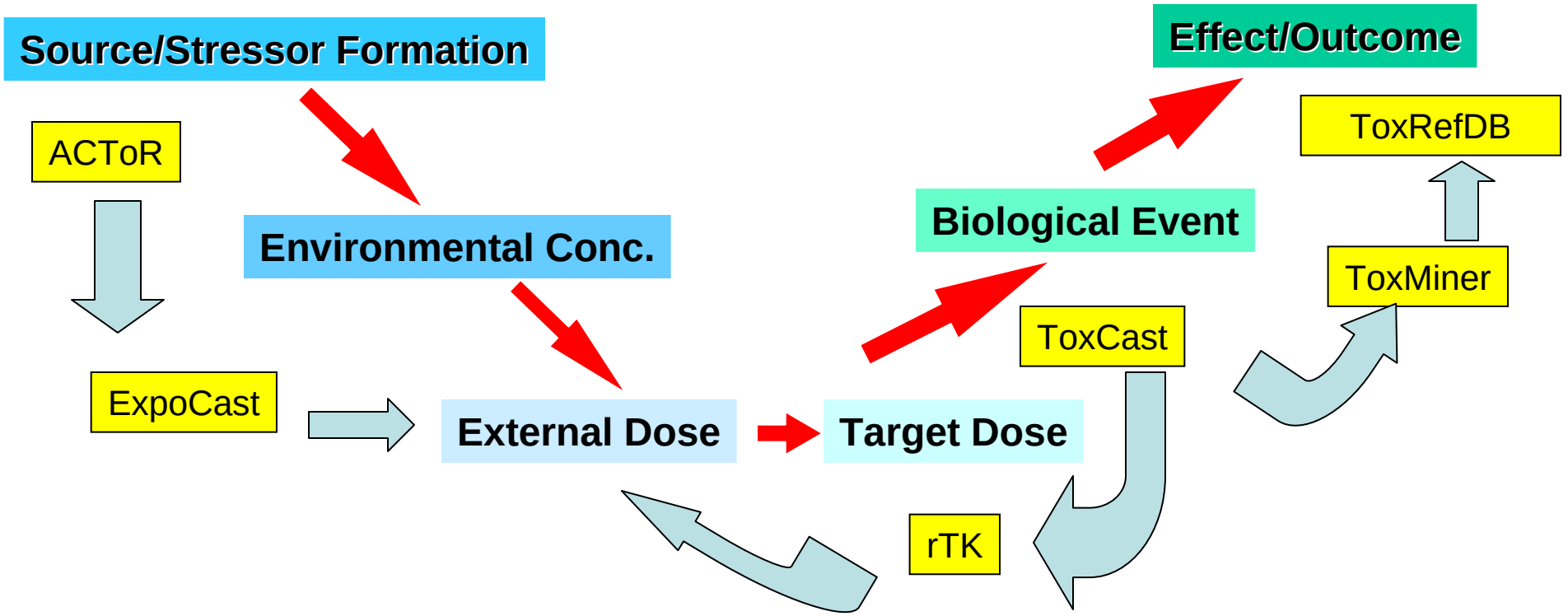
Source to Outcome Continuum



Current Status of ToxCast and Tox21

- ToxRefDB - Relational phenotypic databases
  - Chronic rat and mouse studies (Martin, et al, EHP 2008)
  - Rat multigenerational studies (Martin, et al, submitted)
  - Rat and Rabbit developmental studies (Knudsen, et al, internal review)
- ToxCast
  - Submit manuscripts on v1.0 by Feb 1 2009
  - Data Summit
    - RTP, May 14-15
  - Phase II launch
    - Mid summer 2009
    - Major Pharma is considering supply +100 candidate drugs
- ACToR (Aggregated Computational Toxicology Resource) - www.epa.gov/actor
  - Released Jan 2009
  - Portal for public toxicity information, ToxRef and ToxCast data
- Tox21
  - qHTS on +6000 chemicals starting in mid 2009
    - Includes large collection of pharmaceuticals
    - One to two assays per week

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
- DESIRED OUTCOME
  - Successful deliberations and negotiations would result in:
    - Identify and provide chemicals (~100mg) for screening
    - Sharing of relevant pre-clincal and clinical data
    - [Cost sharing of screening costs]
    - Co-publications on predictive models

Benefits of Proposal

- Creates a government – private sector effort with potential to significantly improve predictive basis of toxicity evaluations
- 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
- Significantly enables progress at reaching the vision of toxicity testing in the 21st envisioned by the National Research Council