United States Environmental Protection Agency

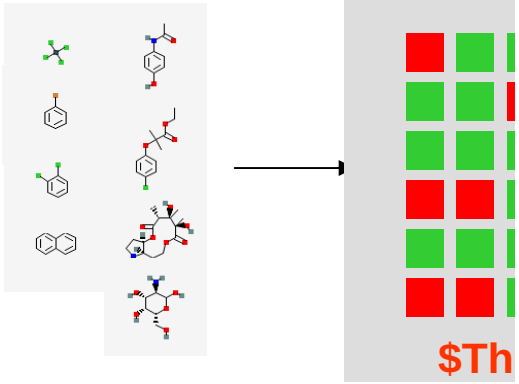
Overview of ToxCast™

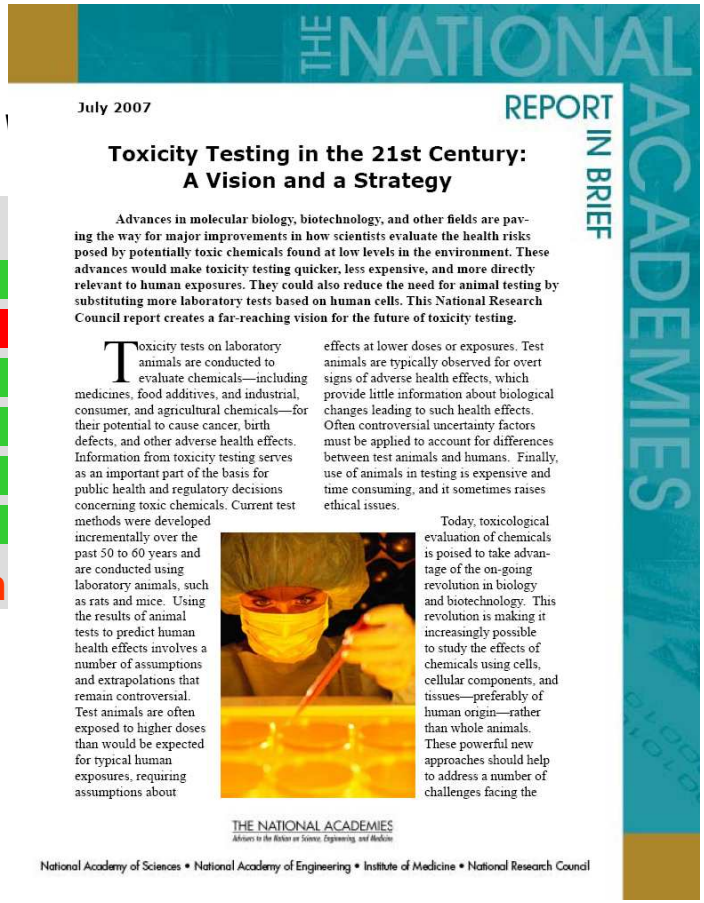
CarcinoGENOMICS Workshop
Brussels, Belgium

Office of Research and Development
National Center for Computational Toxicology

June 9, 2008

Future of Toxicity Testing





July 2007

REPORT IN BRIEF

THE NATIONAL ACADEMIES

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.

Toxicity tests on laboratory animals are conducted to evaluate chemicals—including medicines, food additives, and industrial, consumer, and agricultural chemicals—for their potential to cause cancer, birth defects, and other adverse health effects. Information from toxicity testing serves as an important part of the basis for public health and regulatory decisions concerning toxic chemicals. Current test methods were developed incrementally over the past 50 to 60 years and are conducted using laboratory animals, such as rats and mice. Using the results of animal tests to predict human health effects involves a number of assumptions and extrapolations that remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about effects at lower doses or exposures. Test animals are typically observed for overt signs of adverse health effects, which provide little information about biological changes leading to such health effects. Often controversial uncertainty factors must be applied to account for differences between test animals and humans. Finally, use of animals in testing is expensive and time consuming, and it sometimes raises ethical issues.

Today, toxicological evaluation of chemicals is poised to take advantage of the on-going revolution in biology and biotechnology. This revolution is making it increasingly possible to study the effects of chemicals using cells, cellular components, and tissues—preferably of human origin—rather than whole animals. These powerful new approaches should help to address a number of challenges facing the

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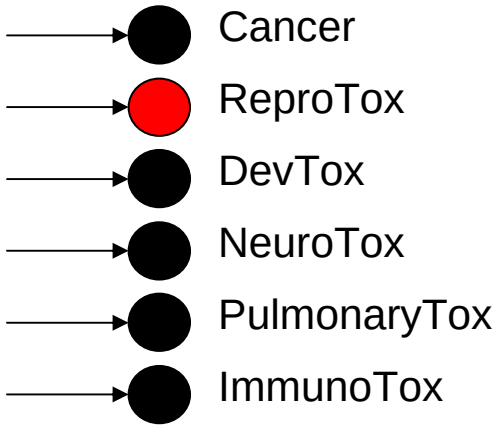


EPAs Approach: The ToxCast Research Program

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
I	320	Data Rich (pesticides)	Signature Development	>400	$20k	FY07-08
IIa	>300	Data Rich Chemicals	Validation	>400	$15-20k	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	$15-20k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	$15-20k	FY10
III	Thousands	Data poor	Prediction and Prioritization	???	$10-15k	FY11-12

- Affordable science-based system for categorizing chemicals
- Increasing confidence as database grows
- Identifies potential mechanisms of action
- Refines and reduces animal use for hazard ID and risk assessment

Evolution of Phase I

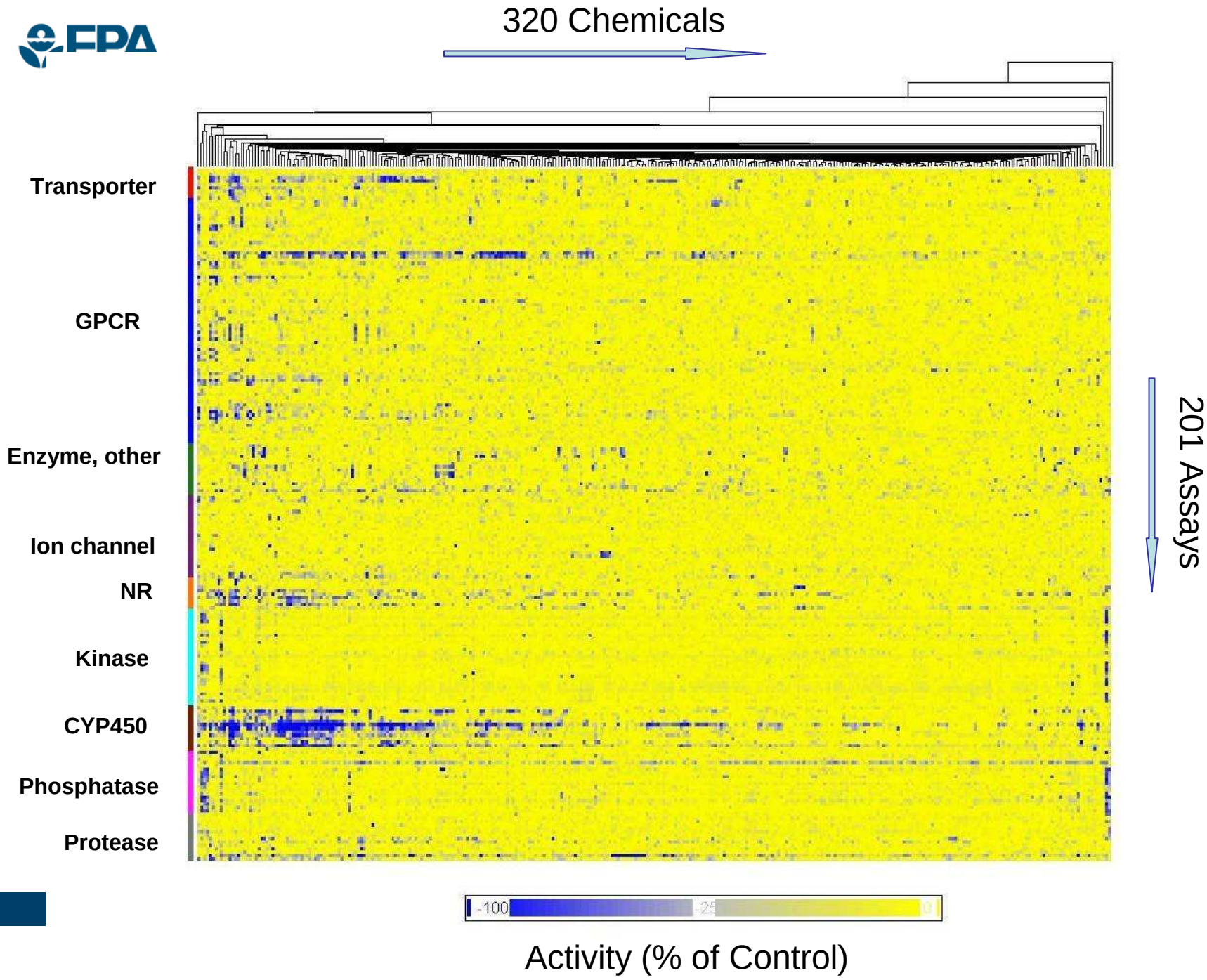
- **ToxCast 1.0 (April, 2007)**
  - *Enzyme inhibition/receptor binding HTS (Novascreen)*
  - *NR/transcription factors (Attagene, NCGC)*
  - *Cellular impedance (ACEA)*
  - *Complex cell interactions (BioSeek)*
  - *Hepatocelluar HCS (Cellumen)*
  - *Hepatic, renal and airway cytotoxicity (IVAL)*
  - *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 (March, 2008)**
  - *Organ culture: liver, kidney, lung (Hamner Institutes)*
  - *HTS Genotoxicity (Gentronix)*
  - *Toxicity and signaling pathways (Invitrogen)*
  - *NR Activation and translocation (CellzDirect)*
  - *3D Cellular microarray with metabolism (Solidus)*
  - *C. elegans (NIEHS)*
  - *Functional markers from microscale cultured hepatocytes (MIT)*

8 Assay Sources & 412 Endpoints

+3 Assay Sources & 16 Endpoints

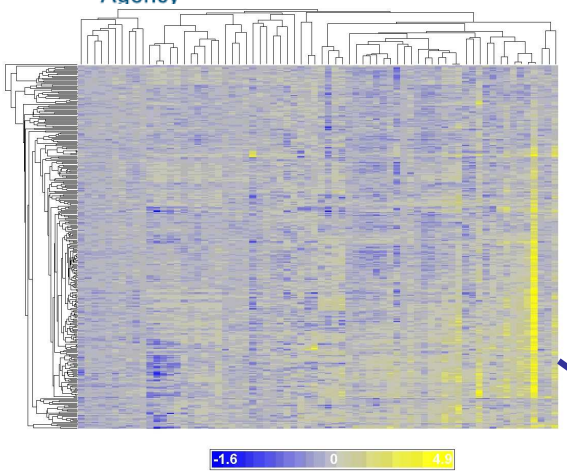
+7 Assay Sources & 123 Endpoints

18 Assay Sources, 551 Endpoints

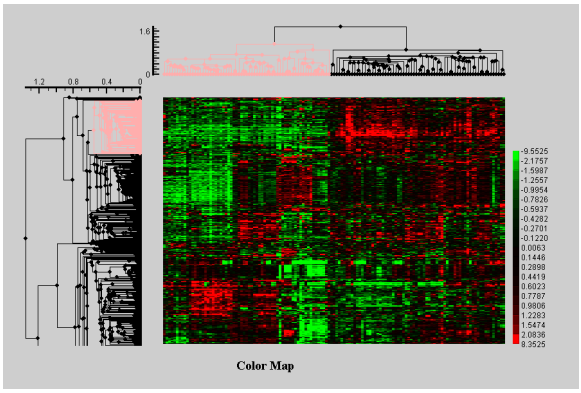

320 Chemicals
Transporter
GPCR
Enzyme, other
Ion channel
NR
Kinase
CYP450
Phosphatase
Protease
201 Assays
-100
Activity (% of Control)

Correlating HTS to Toxicity

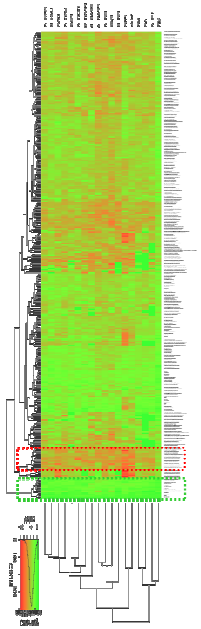
United States Environmental Protection Agency



Cellular Assays

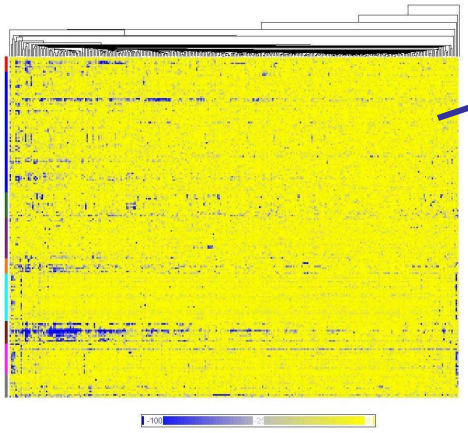


Physical chemical properties

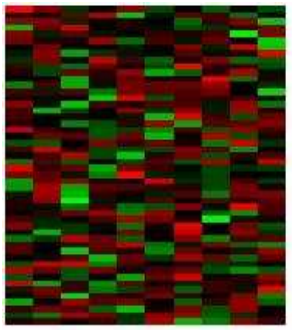


In silico Predictions

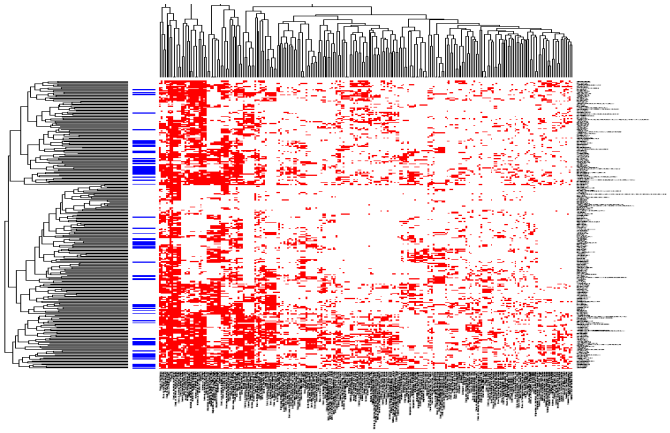
Profile Matching



Biochemical Assays



Genomic Signatures



Toxicology Endpoints

Moving Forward

- Completion of Data Acquisition and Data Mining for Phase I
- Publication and Public Release of all Data
- OECD Molecular Screening Initiative (June, Bilthoven)
- Data Summit, Fall/Winter 2008
- Tox21 MOU partnership with NTP/NIEHS and NCGC/NHGRI
  - Total of ~7000 chemicals for screening
  - Subset to feed Phase II of Toxcast
- EPA Research Strategy and FY10 Research Initiative

Toxicity Pathways in Prioritization ⟷ Toxicity Pathways in Risk Assessment ⟷ Institutional Transition

CHEMICAL OVERLAP WITH CARCINOGENOMICS

CASRN	Name	EU CARCINOGENOMICS	ToxCast_320	EPA-A 1408 @NCGC	NTP-A 1408 @ NCGC	EPA-A or NTP-A
51-03-6	Piperonyl butoxide	1	1	1	1	1
1897-45-6	Chlorothalonil	1	1	1	0	1
150-68-5	Monuron	1	0	1	1	1
64-77-7	Tolbutamide	1	0	1	1	1
50892-23-4	[4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid	1	0	1	0	1
607-57-8	2-Nitrofluorene	1	0	1	0	1
69-65-8	D-Mannitol	1	0	1	0	1
303-47-9	Ochratoxin A	1	0	1	0	1
10108-64-2	Cadmium dichloride	1	0	0	1	1
75-27-4	Dichlorobromomethane	1	0	0	1	1
21829-25-4	Dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxy	1	0	0	1	1
542-56-3	Isobutyl nitrite	1	0	0	1	1
62-75-9	N-Nitrosodimethylamine	1	0	0	1	1
		34	307	1327	1351	2325

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Transforming Toxicology

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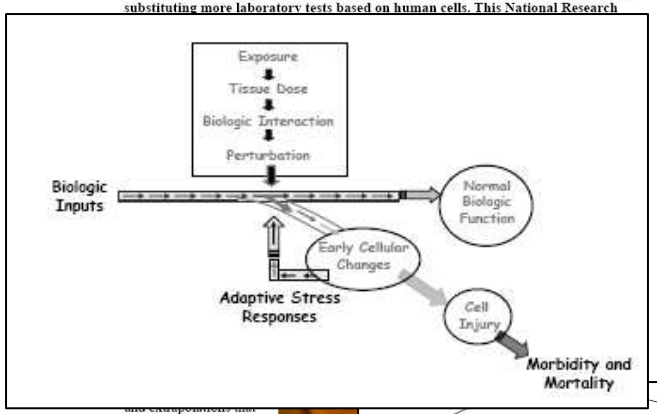

remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about

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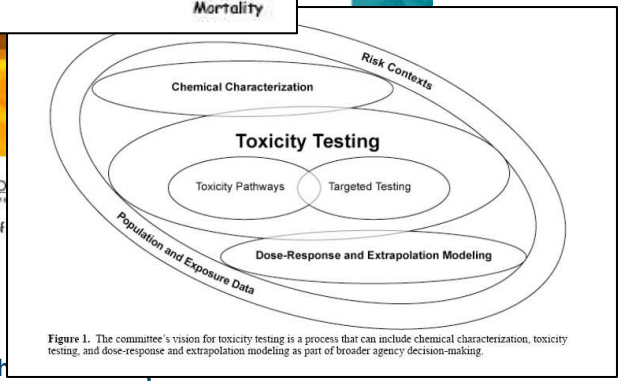


Figure 1. The committee's vision for toxicity testing is a process that can include chemical characterization, toxicity testing, and dose-response and extrapolation modeling as part of broader agency decision-making.

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.

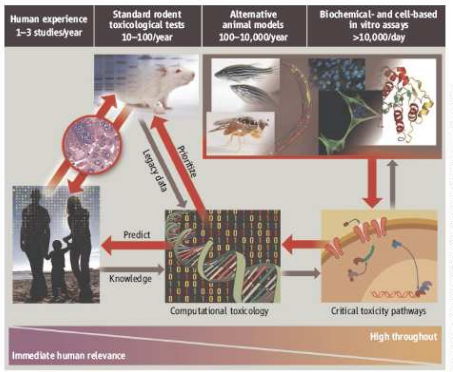
Francis S. Collins,*† George M. Gray,‡* John R. Bucher§*

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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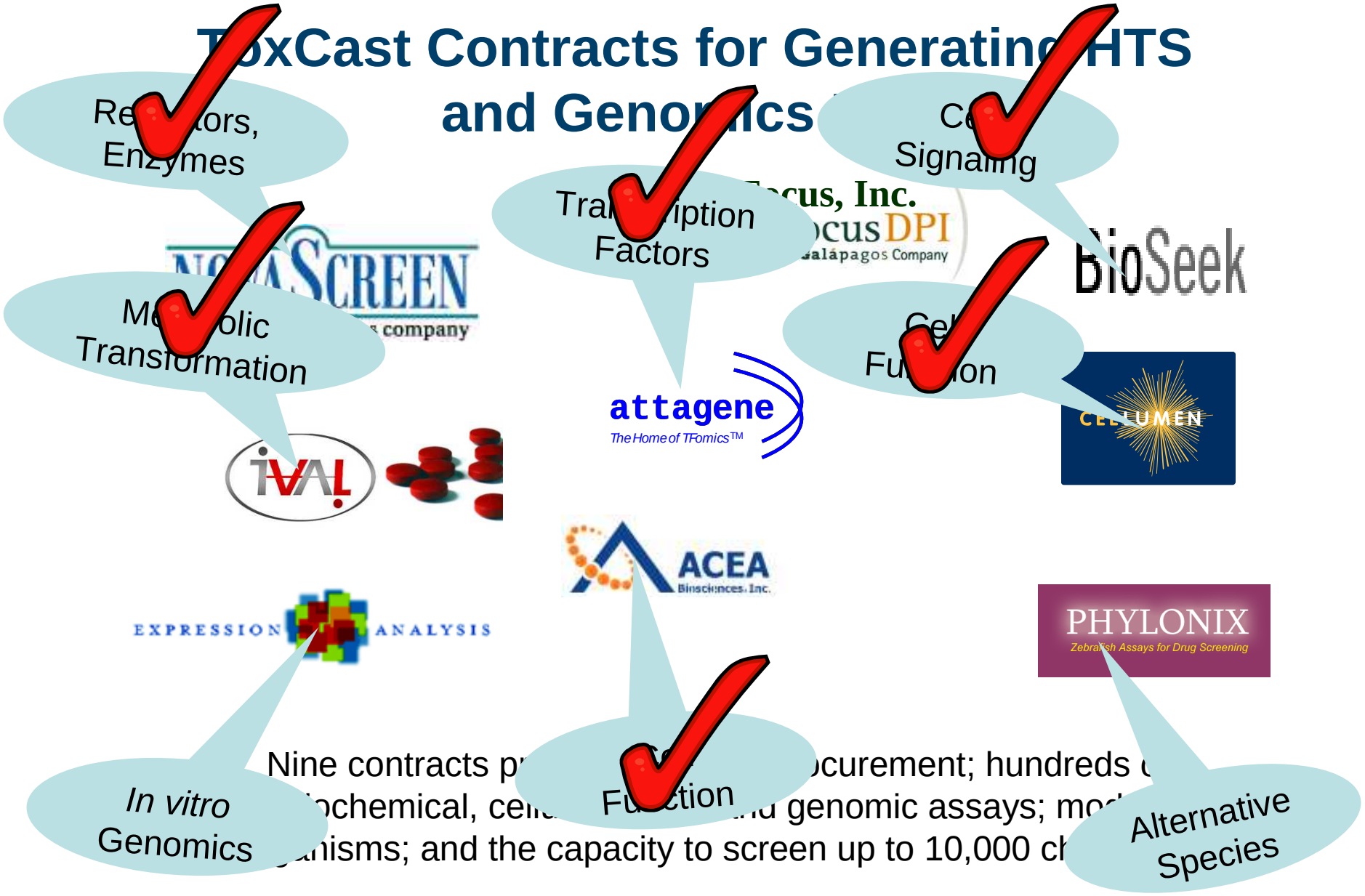
Science, Feb 15, 2008

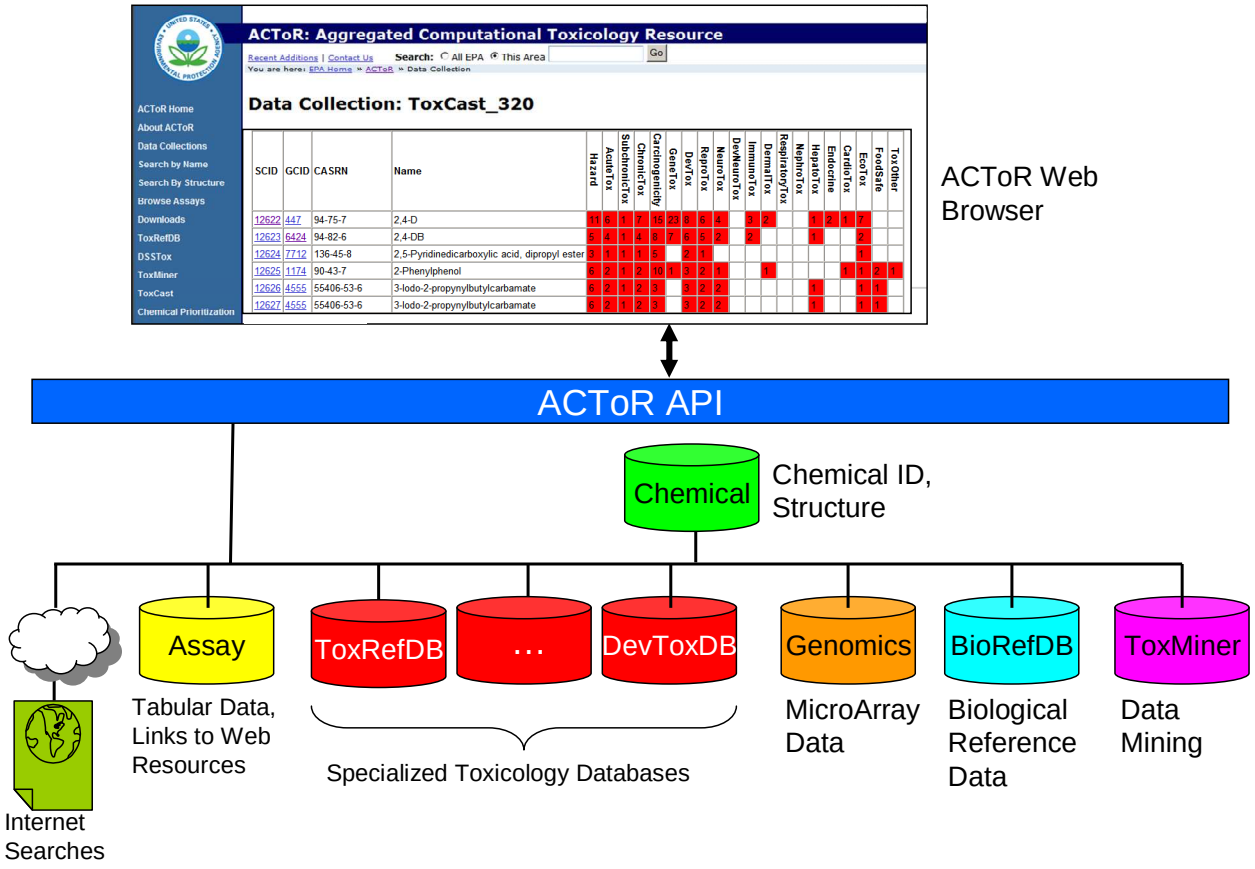
ToxCast Contracts for Generating HTS and Genomics



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

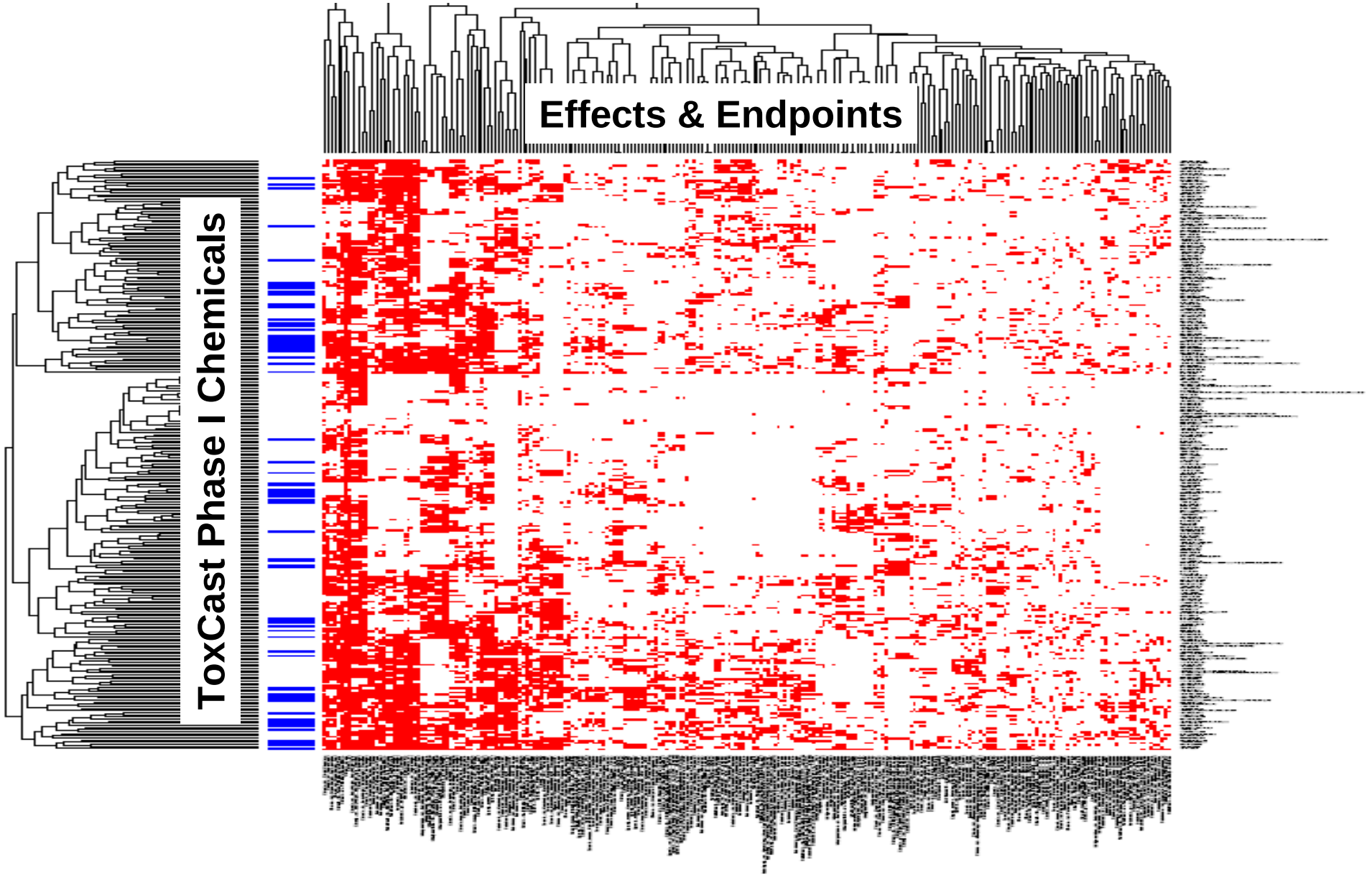
United States Environmental Protection Agency

ACToR: Aggregated Computational Toxicology Resource



$400 Million Dollars Worth of *In Vivo* Chronic/Cancer Bioassay Effects and Endpoints

EPA United States Environmental Protection Agency



The ToxCast Team