NTP
National Toxicology Program

The NTP Biomolecular Screening Program

Raymond Tice, Ph.D.
National Institute of Environmental Health Sciences

Board of Scientific Counselors
November 21, 2008

Outline

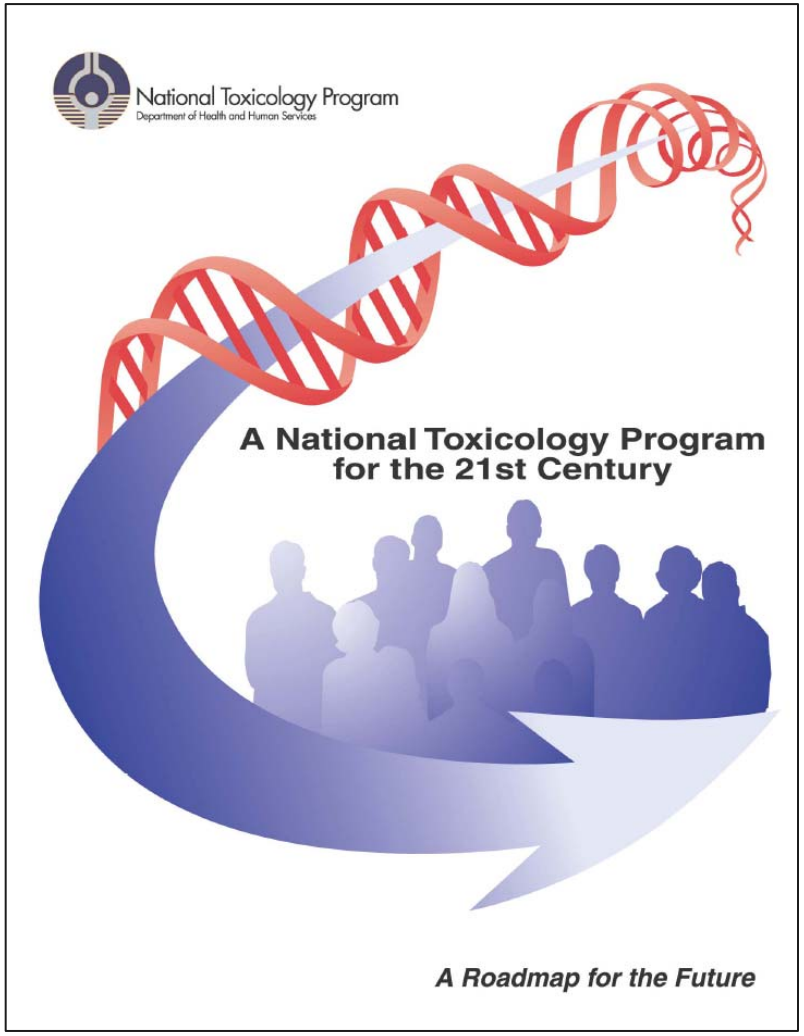
- The NTP and its High Throughput Screening (HTS) Initiative
- The National Academy of Sciences (NAS) Report “Toxicity Testing in the 21st Century: A Vision and Strategy”
- Interagency Coordination on Biomolecular Screening - the Tox21 Community
- Toxicity Pathways
- The Tox21 Compound Library
- HTS-Related Activities

NTP's Vision for the 21st Century

To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad array of target specific, mechanism-based, biological observations

NTP Roadmap - High Throughput Screening Goals

- Prioritize chemicals for further in-depth toxicological evaluation
- Identify mechanisms of toxicity
  - characterize toxicity pathways
  - facilitate cross-species extrapolation
  - provide input to models for low-dose extrapolation
- Develop predictive models for *in vivo* biological response in humans



Biomolecular Screening

Short-term Goals (2-3 years)

- Continue to develop tools and approaches
- Characterize and probe toxicity pathways
- Develop capabilities for including hepatic metabolism in *in vitro* cell-based assays
- Further develop and populate relational databases
- Prioritize chemicals for more in-depth and targeted testing

Long-term Goals (5+ years)

- Incorporate *in vitro* 3-D organ/tissue models into the biomolecular screening strategy
- Inform cross-species and low-dose extrapolation
- Continue to evaluate the genetic basis for variability in sensitivity to toxicants
- Develop predictive models for human diseases
- Establish its role in regulatory science via the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

This 2007 National Academy of Science report envisions a not-so-distant future in which virtually all routine toxicity testing would be conducted *in vitro* in human cells or cell lines by evaluating perturbations of cellular responses in a suite of toxicity pathway assays using high throughput robotic assisted methodologies.

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.

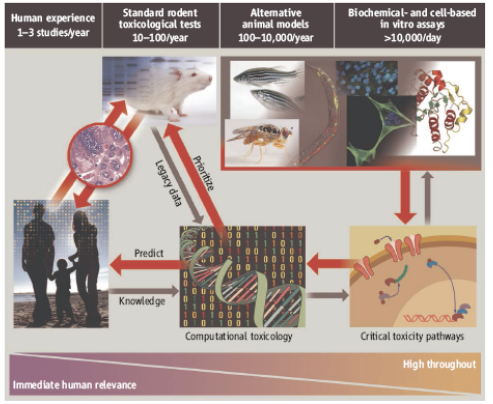
Francis S. Collins,*† George M. Gray,‡* John R. Bucher§*

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.

*Director, National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda, MD 20892; ‡Assistant Administrator for the Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460; §Associate Director, U.S. National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA.

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

†Author for correspondence. E-mail: francisc@mail.nih.gov

Interagency Coordination on HTS and toxicity testing

- Memorandum of Understanding on "High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings"
  - Signed February 14, 2008 by:
    - NIH/NIEHS/NTP
    - NIH/NHGRI
    - EPA/ORD
- Leverage
  - Pools resources for common goal
  - Overcomes the resource limitations of a single agency
  - Builds on existing expertise
  - Avoids the need to create a new administrative and support structure

NIEHS
National Institute of Environmental Health Sciences

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

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Biomolecular Screening Branch

Raymond Tice*, acting Chief
Keith Shockley*
Cynthia Smith*
Tina Teng
Kristine Witt*

Jonathan Freedman*

C. elegans Screening Core
Windy Boyd
Paul Dunlap
Julie Rice
Daniel Snyder

*Secondary responsibility

Tox21 (MoU) Partners

NIH Chemical Genomics Center

Chris Austin, Ruili Huang, Jim Inglese, Noel Southall, Menghang Xia

United States Environmental Protection Agency

Bob Kavlock, David Dix, Keith Houck, Richard Judson, Ann Richard

Activities	NTP	NCGC	EPA
Historical Toxicology Data	✓		✓
Experimental Toxicology	✓		✓
Ultra High-Throughput Testing		✓	
Mid- to High Throughput Systems			✓
Lower Organism Model System	✓ *C. elegans*		✓ Zebrafish
In Vitro 3-D Model Systems	✓		✓
Effect of Human/Rodent Genetic Background on Toxic Effects	✓	✓	
Computational Toxicology	✓	✓	✓
Validation Experience	✓ (NICEATM-ICCVAM)	✓	✓

Tox21 Focus Groups

- **Pathways/Assays - K. Witt (NTP), K. Houck (EPA), M. Xia (NCGC)**
  - Identify key toxicity pathways/assays (with a focus on human cells) and prioritize assays for use at the NCGC
  - Identify assay gaps and consider methods for filling those gaps
  - Develop methods for incorporating hepatic metabolism into *in vitro* assays
  - Evaluate human and rodent genetic variability in response to toxicants
  - Consider compound, pathway, and cell-to-cell interactions

- **Compounds - C. Smith (NTP), D. Dix (EPA), N. Southall (NCGC)**
  - Establish a library ~10,000 compounds with known structures for testing at the NCGC
  - Establish procedures for determining the identity, purity, and stability of each compound
  - Establish a library of water soluble compounds for testing at the NCGC
  - Establish a library of mixtures for testing at the NCGC

Tox21 Focus Groups

- **Bioinformatics - K. Shockley (NTP), R. Judson (EPA), R. Huang (NCGC)**
  - Evaluate patterns of response and relationship to adverse health outcomes in experimental animals and humans
  - Evaluate consistency of response within assays and across related endpoints
  - *Make all data publicly accessible (PubChem, ACToR, CEBS)*

- **Targeted Testing - J. Bucher (NTP), S. Edwards (EPA), J. Inglese (NCGC)**
  - Prioritize substances for more complex testing, including the use of alternative assay platforms or species (e.g., *C. elegans*, zebrafish)

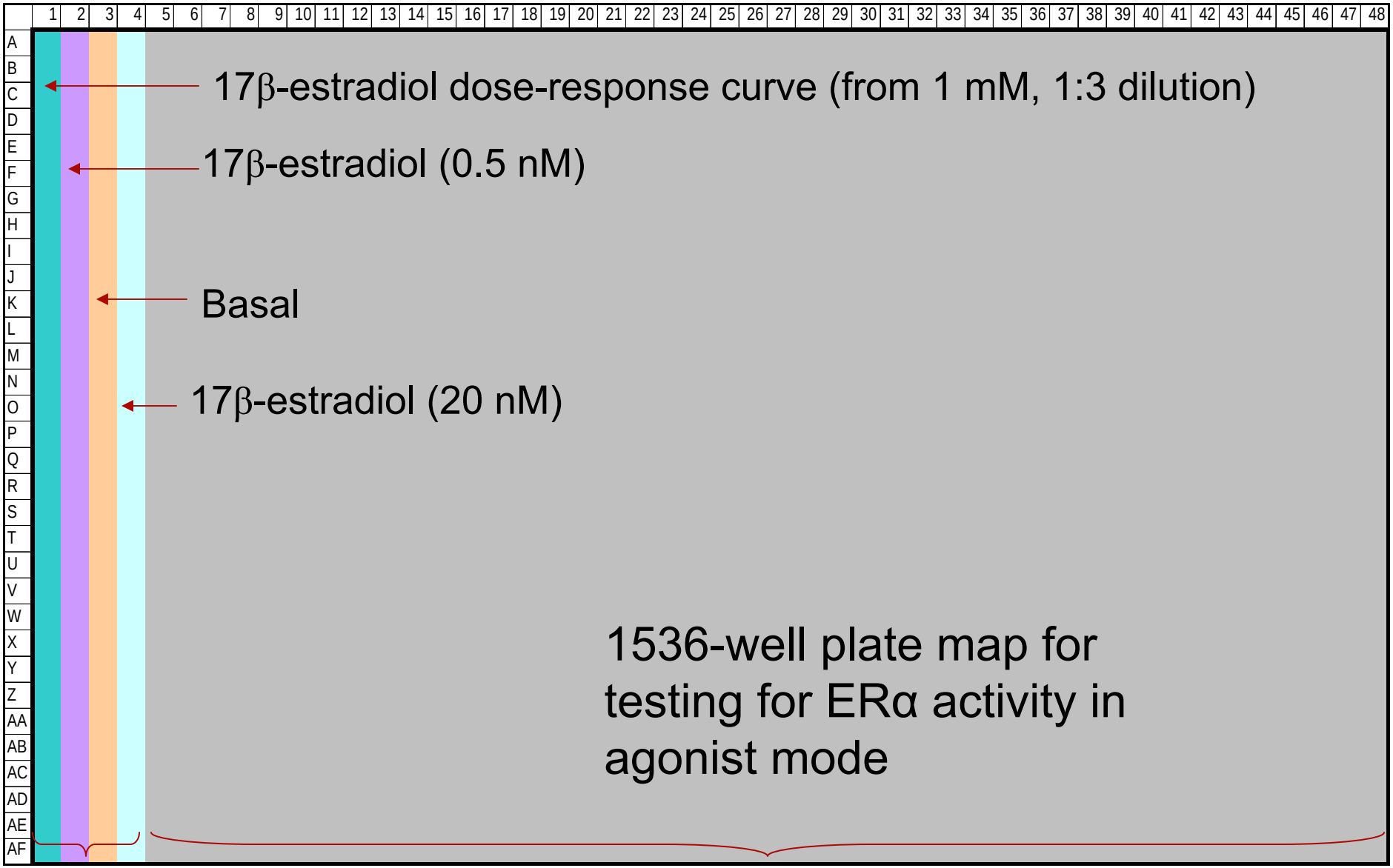
NIH Chemical Genomics Center

- Conducts quantitative high-throughput screening (qHTS)
  - >300,000 profiles/week
- qHTS profile
  - 1536-well plate format
  - 15-point concentration-response curve
  - 5 nM to 92 μM typical
  - ~5 μL assay volume
  - ~1000 cells/well
- Criteria for call based on response profile

Inglese et al. (2006) PNAS 103:11473

NCGC HTS Assay Criteria

Criteria	Biochemical	Cell-based
Assay Steps	≤10	≤10
Minimum Time Increments/ Maximum Duration	5 min	5 min 48 hrs
Reagent Addition Steps	4 maximum	4 maximum
Reagent Removal Steps	None	None
Signal:Background Ratio	≥3-fold	≥3-fold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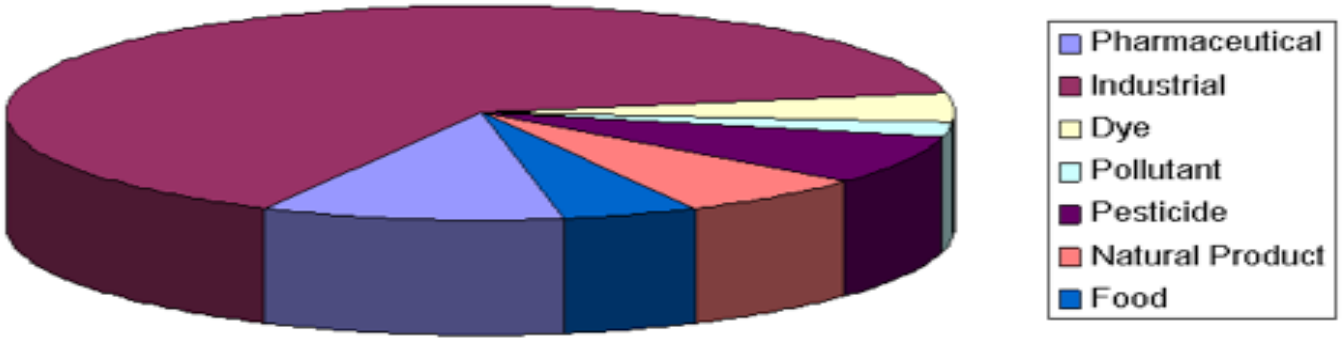
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z AA AB AC AD AE AF
17β-estradiol dose-response curve (from 1 mM, 1:3 dilution)
17β-estradiol (0.5 nM)
Basal
17β-estradiol (20 nM)
1536-well plate map for testing for ERα activity in agonist mode
Controls
Test compounds (1408 wells)

NTP and EPA “1408” compound libraries at the NCGC

- All have been evaluated in one or more toxicological tests
- NTP: 1353 unique compounds
  - 1206 with NTP test data, 147 ICCVAM reference substances
  - MW = 32-1168, calculated log p = -3 to 13.2
- EPA: 1330 unique compounds
  - MW = 58-516, calculated log p = -2.8 to 8.2
  - 400 compound overlap



NTP compounds

Current Testing Status of the NTP and EPA libraries at the NCGC

- CellTiter-Glo® Luminescent Cell Viability Assay (13 cell types - 9 human, 4 rodent; 2 with EPA; also in 40 lymphoblastoid cell lines from 20 sets of identical twins)
- Cytotox-ONE™Homogeneous Membrane Integrity Assay and a proteolytic release assay (both in 2 cell types)
- Caspase-Glo® Assays for caspases 3/7 (13 cell types), and 8 or 9 (6 cell types each)
- P53 signaling pathway in HCT-116 cells
- Nrf2/ARE signaling pathway in HepG2 cells
- Hsp70 stress protein pathway
- Differential cytotoxicity in chicken lymphoblastoid cell lines deficient in different DNA repair pathways (wild-type plus 7 repair deficient clones)
- 10 nuclear receptors (agonist & antagonist)- AR, ERα, FXR, GR, LXRβ, PPARδ, PPARγ, RXR, TRβ, VDR, RORα (35 screens: 96 protocols, 276 data sets, 4,119,808 data points)
- Human and Rat PXR reporter gene assays

EPA ToxCast™

- Research program of EPA's National Center for Computational Toxicology (NCCT- http://www.epa.gov/ncct/toxcast)
- Addresses chemical screening and prioritization needs for EPA
- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures
- Phase I started in 2007
- Committed to stakeholder involvement and public release of data
  - May 14-15 2009 - ToxCast Phase I Data Summit - EPA RTP campus

Phased Development of ToxCast™

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Target Date
I	320	Data Rich (pesticides)	Signature Development	552	FY08
Ib	15	Nanomaterials	Pilot	166	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	FY10
IId	>12	Nanomaterials	PMN	>200	FY09-10
III	Thousands	Data poor	Prediction and Prioritization	>300	FY11-12

ToxCast 320 Phase I Chemicals

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

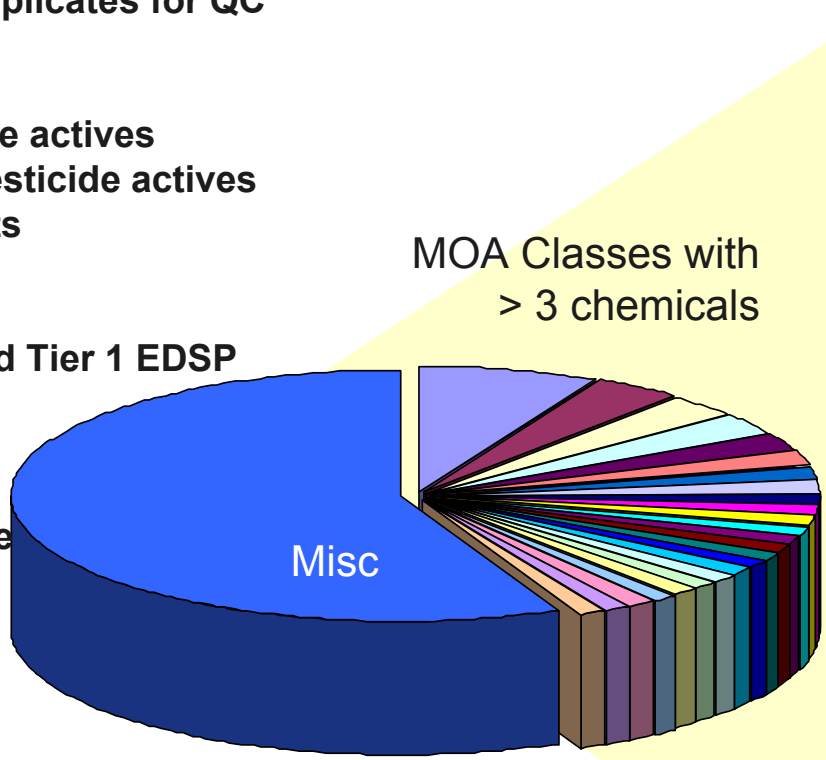
291 total pesticide actives
273 registered pesticide actives
22 pesticide inerts
33 antimicrobials

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



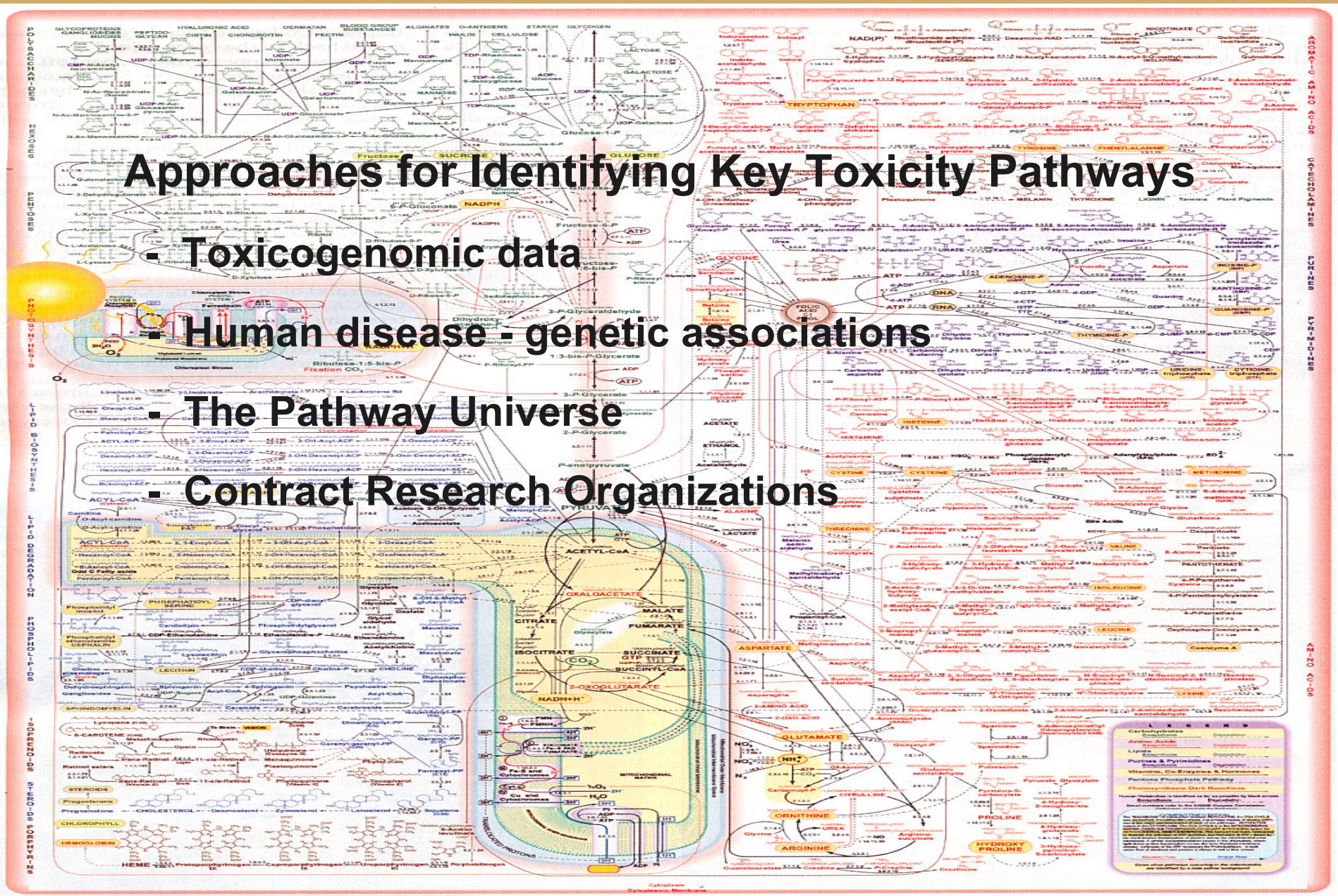
- Acetylcholine esterase inhibitors
- conazole fungicides
- Sodium channel modulators
- pyrethroid ester insecticides
- organothiophosphate acaricides
- dinitroaniline herbicides
- pyridine herbicides
- thiocarbamate herbicides
- imidazolinone herbicides
- organophosphate insecticides
- phenyl organothiophosphate 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 Office of Pesticide Programs Information Network

- **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)

20 Assay sources
554 Endpoints



Approaches for Identifying Key Toxicity Pathways

- Toxicogenomic data
- Human disease - genetic associations
- The Pathway Universe
- Contract Research Organizations

Jones et al. (2008) **Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses** (www.sciencexpress.org / 4 September 2008 / Page 1 / 10.1126/science.1164368)

- Performed a genetic analysis of **24 pancreatic cancers** **by first determining** the sequences of **23,219 transcripts, representing 20,661 protein-coding genes**
- Searched for homozygous deletions and amplifications in the tumor DNA by using microarrays containing probes for ~106 single nucleotide polymorphisms (SNPs)
- Ppancreatic cancers contain an average of **63 genetic alterations, the majority of which are point mutations**
- These alterations defined a **core set of 12 cellular signaling pathways and processes that were each genetically altered in 67% to 100% of the tumors**
- **Dysregulation of these core pathways and processes through mutation can explain the major features of pancreatic tumorigenesis**

C. Portier and Colleagues (NIEHS)

Identifying Critical Pathways for Human Diseases

Genetic Association database (geneticassociationdb.nih.gov)

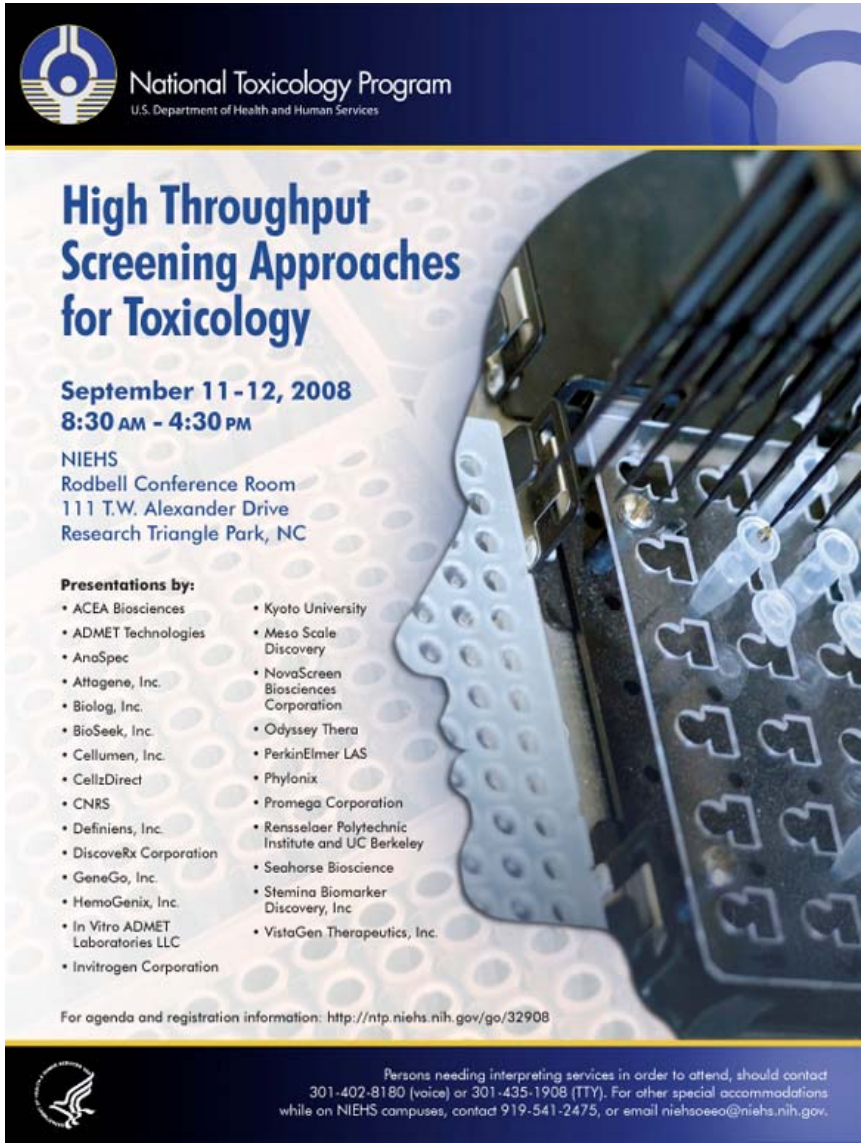
- an archive of genetic association studies of complex diseases/disorders
- the database contains **28,347 records on human polymorphisms** analyzed in the context of a particular disease or disorder
- **6,134** were considered statistically significant **gene-disease associations**

Individual diseases/disorders/traits are grouped into 15 categories:

Cancer
Cardiovascular
Chemical dependency
Developmental
Immune
Infection
Hematological
Longevity
Metabolic
Neurodegenerative
Normal Variation (e.g. elite athletes)
Pharmaceutical sensitivity/metabolism
Psychological
Renal
Vision

The Pathway Universe

- BioCarta : 314 pathways
  (http://cgap.nci.nih.gov/Pathways/BioCarta_Pathway)
- GeneGo: >500 pathways
  (http://www.genego.com/)
- Invitrogen: 194 pathways
  (http://escience.invitrogen.com/ipath/browse.do)
- KEGG: 134 pathways
  (http://cgap.nci.nih.gov/Pathways/Kegg_Standard_Pathways)



Purpose of the meeting was to obtain information on:

- critical toxicity pathways and useful molecular targets
- technologies and assay systems that might be used in the development of a comprehensive approach to biomolecular screening

Attending BSC member - W. Janzen

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

NTP Expectations for the 21st Century

- Continue to refine traditional methods and develop new methods to provide basic toxicology information for public health protection
  - Mechanistic information
  - Exposure-response information
  - Predictive of toxicity to humans, animals. and the environment
  - Life stage susceptibility
  - Genetic susceptibility
- Reconcile results from new “data rich” techniques (e.g., genomics, proteomics, high throughput screens) with existing testing information for **conceptual validation**
- Develop approaches to accomplish **formal validation** of new methods for human hazard and risk estimations

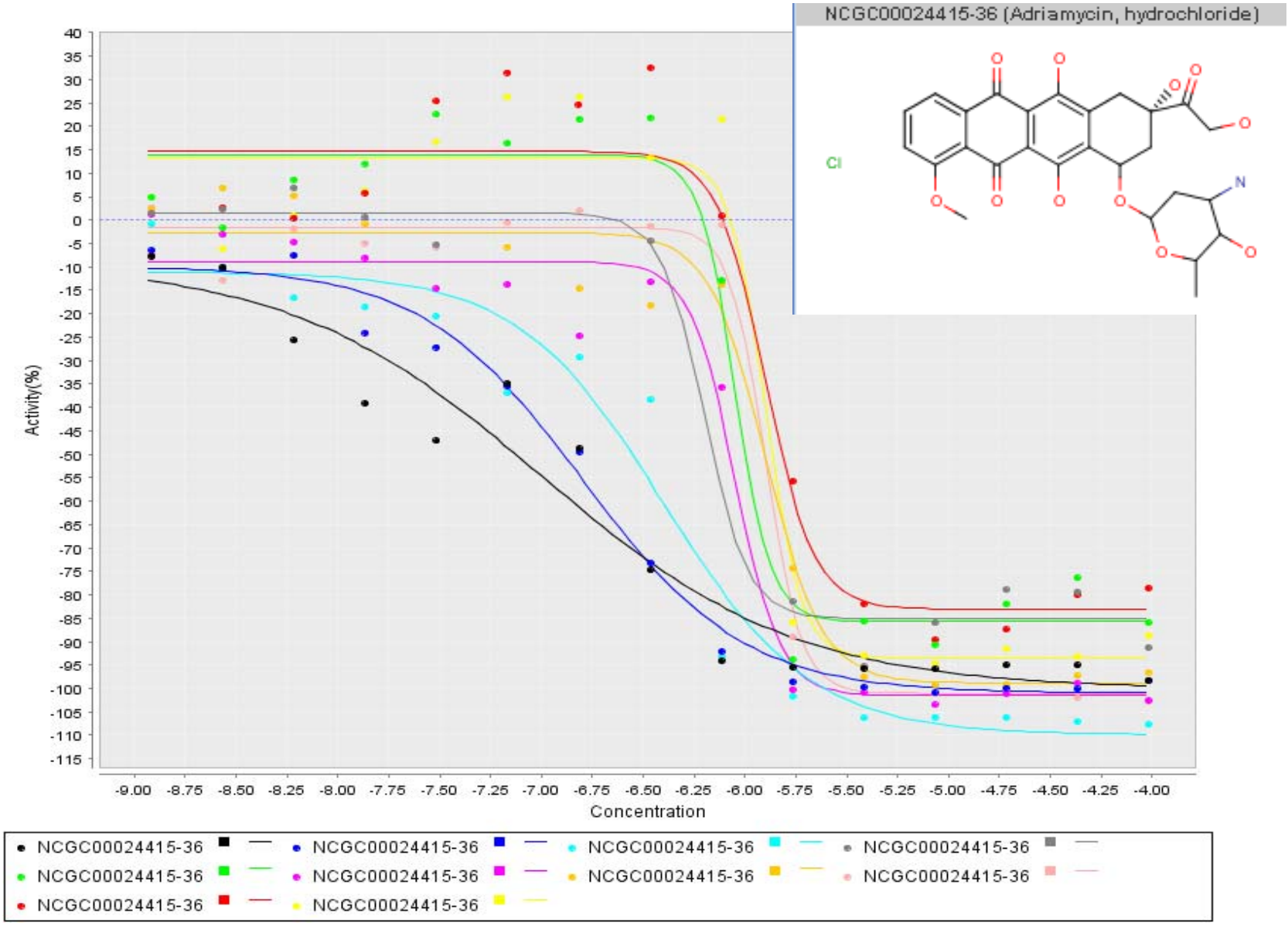
HTS-Related Activities

- Extended OECD/IPCS Advisory Group on Toxicogenomics
- NIEHS SBIR/STTR Program Topic "Predictive Test Systems for Safety Evaluation Program" (http://www.niehs.nih.gov/research/supported/programs/sbir/pts.cfm)
- EU 7th Framework Programme Calls for Proposals (115 M euros) - High Throughput Research and Systems Biology
- EU WP ENV.2009.3.3.1.1 (193.5 M euros) - Screening Methods for Assessing the Toxicological and Eco-toxicological Properties of Chemicals
- EPA Proposal to ILSI-HESI for "Identification of Pharmaceuticals for Validation of ToxCast" - emerging issue the request to pharmaceutical companies to provide failed drugs for screening

DNA Damage Assessment Using Chicken DT40 (B-lymphocyte) Cells

Dr. S. Takeda, Kyoto University

Clone #	Name	Function
653	Wild type	
915	pol β (-/-) clone#1	DNA polymerase β repairs base damage and single-strand break
916	pol β (-/-) clone#2	
1384	FANCc (-/-)	Required for eliminating inter-strand crosslinks
100	ku70/rad54 (-/-)	Ku70 is required for repairing chromosomal breaks, Rad54 and XRCC3 are involved in repairing chromosomal breaks that occur during DNA replication
657	rev3 (-/-)	Rev3 have multiple functions in cellular tolerance to a variety of DNA damage
1782	ubc13 (-/-)	UBC13 have multiple functions in cellular tolerance to a variety of DNA damage
245	ATM(-/-)	ATM arrests cell cycle when chromosomal breaks are present, makes cells tolerant to reactive O2 species


NCGC00024415-36 (Adriamycin, hydrochloride)
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O
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Activity(%)
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30
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-65
-70
-75
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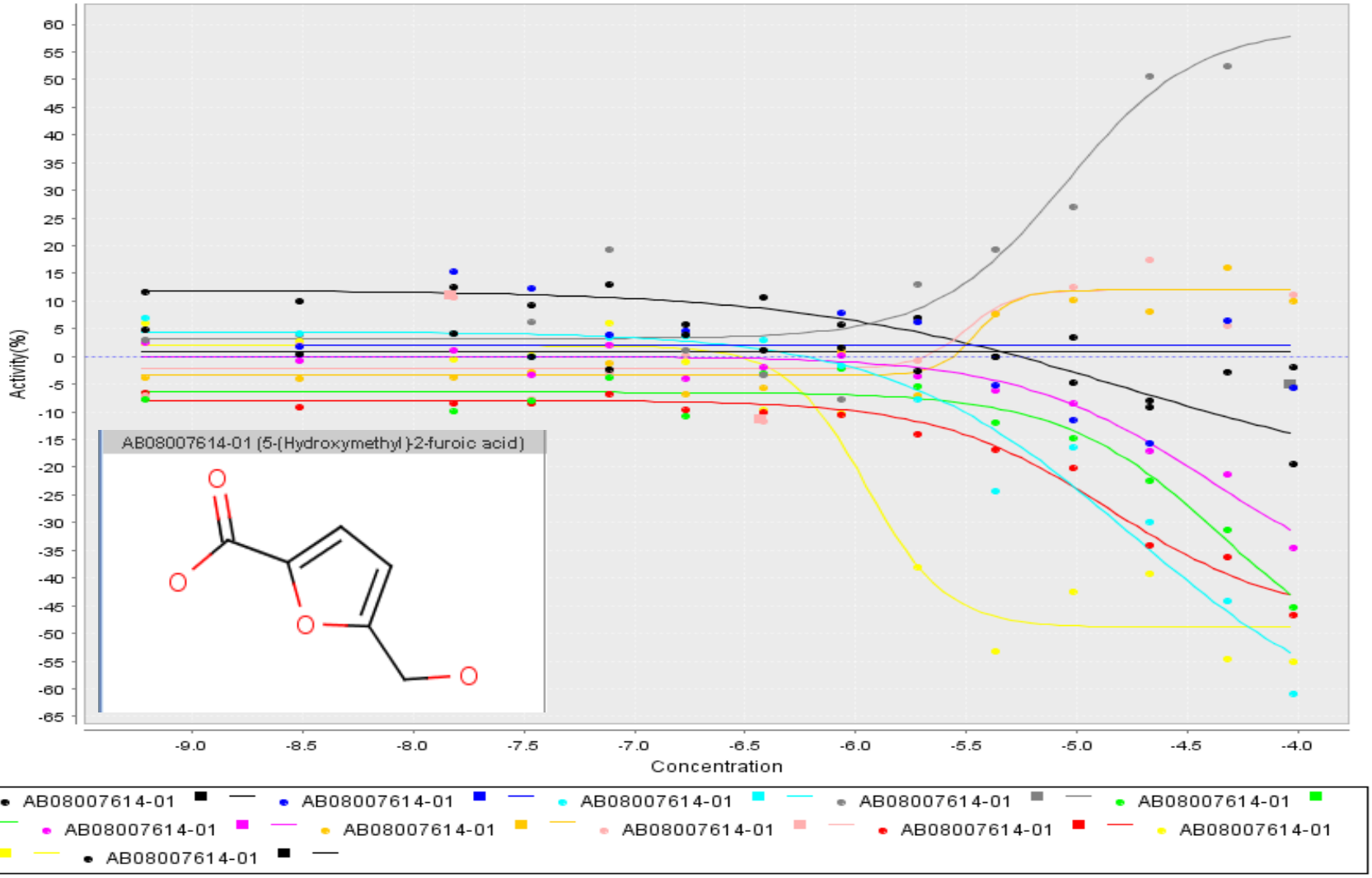
Activity(%)
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-30
-35
-40
-45
-50
-55
-60
-65
AB08007614-01 (5-(Hydroxymethyl)-2-furoic acid)
-9.0
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
-5.5
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Concentration
AB08007614-01
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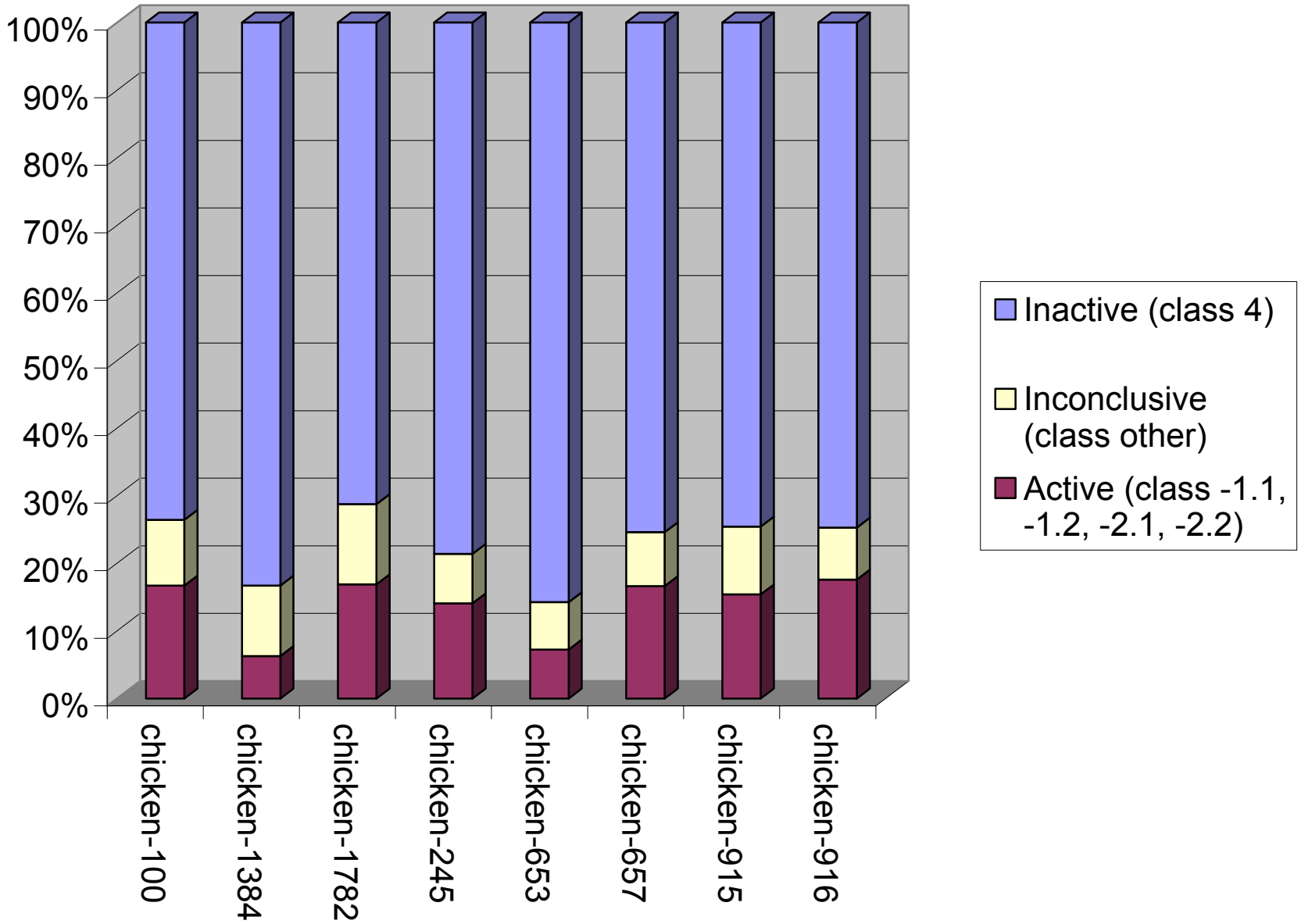
100%
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80%
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50%
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30%
20%
10%
0%
chicken-100
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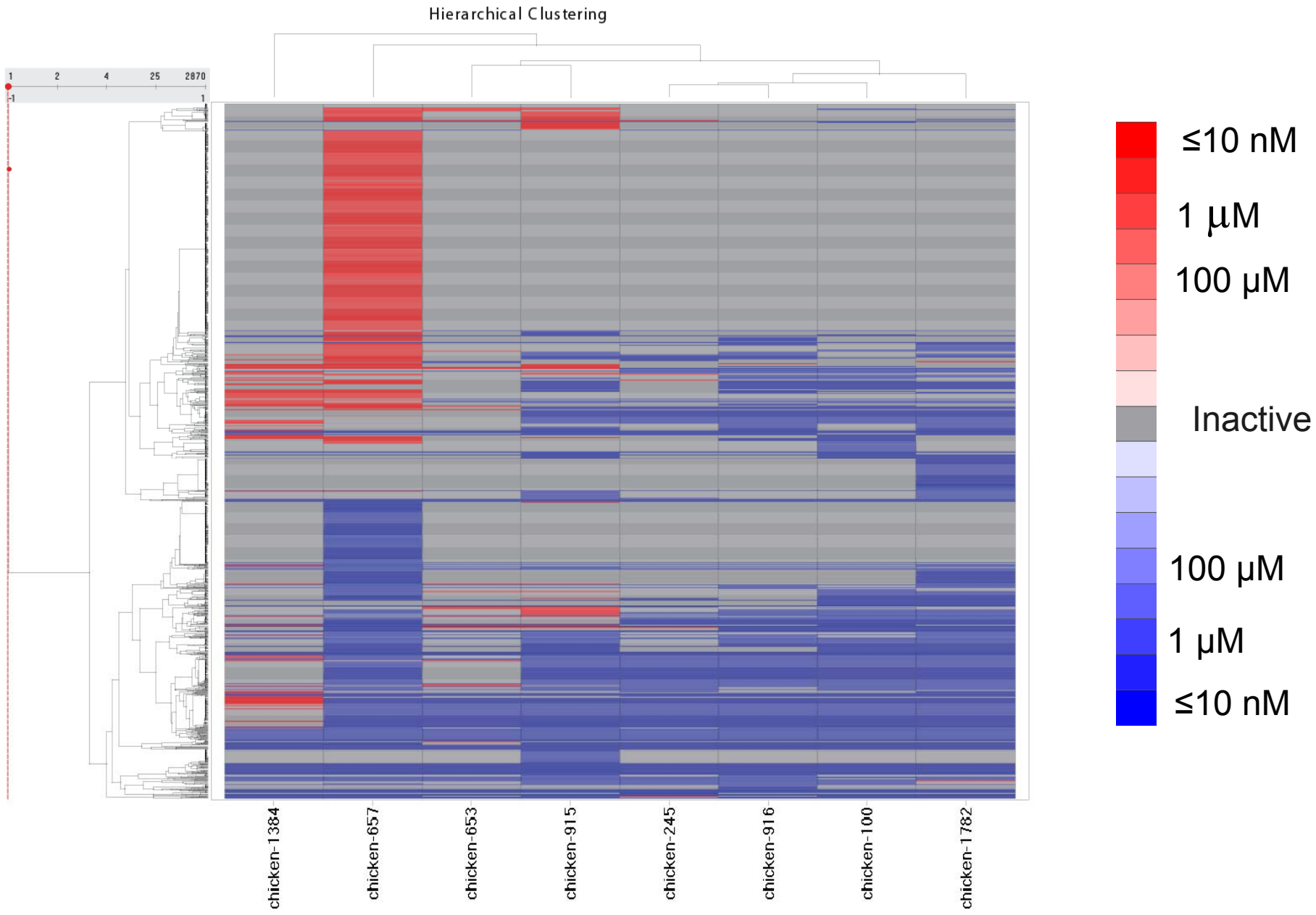
chicken-1782
chicken-245
chicken-653
chicken-657
chicken-915
chicken-916
Inactive (class 4)
Inconclusive (class other)
Active (class -1.1, -1.2, -2.1, -2.2)


Hierarchical Clustering
1
2
4
25
2870
-1
1
chicken-1384
chicken-657
chicken-653
chicken-915
chicken-245
chicken-916
chicken-100
chicken-1782
≤10 nM
1 µM
100 µM
Inactive
100 µM
1 µM
≤10 nM