The Tox21 Program

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Chief, Biomolecular Screening Branch

Accelerating Implementation of the NRC Vision for Toxicity Testing in the 21st Century

Gallaudet University,
Washington, DC
November 9 & 10, 2010
An Expanded Tox21 Community

• Revised MoU on “High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings” signed July 19, 2010 (http://ntp.niehs.nih.gov/go/28213) by:

  • **National Toxicology Program:** Linda S. Birnbaum, Ph.D., DABT, ATS Director National Institute of Environmental Health Sciences National Institutes of Health

  • **NIH Chemical Genomics Center:** Eric D. Green, M.D., Ph.D. Director National Human Genome Research Institute

  • **U.S. Environmental Protection Agency:** Paul T. Anastas, Ph.D. Assistant Administrator Office of Research and Development

  • **Food and Drug Administration:** Janet Woodcock, M.D., Director Center for Drug Evaluation and Research
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Partners

NTP: Raymond Tice, Alex Merrick, Keith Shockley, Cynthia Smith, Tina Teng, Kristine Witt
C. elegans Screening Core: Jon Freedman, Windy Boyd, Paul Dunlap, Julie Rice, Daniel Snyder
(http://ntp.niehs.nih.gov/go/28213)

Chris Austin, Ruili Huang, Jim Inglese, Noel Southall, Menghang Xia (http://www.ncgc.nih.gov/)

National Center for Computational Toxicology: Bob Kavlock, David Dix, Keith Houck, Richard Judson, Ann Richard (http://www.epa.gov/ncct/)

David Jacobson-Kram, Dan Benz, Kevin Gaido, Donna Mendrick, Weida Tong
Tox21 Goals

• Research, develop, validate, and translate innovative compound testing methods that characterize toxicity pathways

• Identify compounds, assays, informatic tools, and targeted testing needed for the innovative testing methods

• Prioritize compounds for more extensive toxicological evaluation

• Identify mechanisms of compound-induced biological activity in order to characterize toxicity pathways, facilitate cross-species extrapolation, and provide input to models for low-dose extrapolation

• Develop predictive models for biological response in humans
Agency Points of Contact

Christopher Austin, M.D. (NCGC)
David Jacobson-Kram, Ph.D., DABT (FDA)
Robert Kavlock, Ph.D. (EPA)
Raymond Tice, Ph.D. (NTP)

Informatics Working Group
Co-Chairs
Ruili Huang, Ph.D. (NCGC)
Richard Judson, Ph.D. (EPA)
Keith Shockley, Ph.D. (NIEHS)
Weida Tong, Ph.D. (FDA)

Compound Selection Working Group
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Donna Mendrick, Ph.D. (FDA)
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Cynthia Smith, Ph.D. (NTP)
Noel Southall, Ph.D. (NCGC)

Targeted Testing Working Group
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R. Daniel Benz, Ph.D. (FDA)
Kevin Crofton, Ph.D. (EPA)
James Inglese, Ph.D. (NCGC)
Scott Masten, Ph.D. (NTP)

Assays & Pathways Working Group
Co-Chairs
Kevin Gaido, Ph.D. (FDA)
Keith Houck, Ph.D. (EPA)
Menghang Xia, Ph.D. (NCGC)
Kristine Witt, M.S. (NTP)
The NCGC

• conducts quantitative high-throughput screening (qHTS)
  − >300,000 profiles/week

• qHTS profile
  − 1536-well plate format
  − 15-point concentration-response curve
  − DMSO soluble
  − 5 nM to 92 μM typical
  − ~5 μL assay volume
  − ~1000 cells/well
Tox21 Phase I Activities

- Screened ~2800 NTP/EPA compounds for activity in ~70 qHTS assays
- Compared the sensitivity of 76 HapMap cell lines to 240 toxic compounds
- EPA’s ToxCast™ Phase I screened 320 compounds (mostly pesticide actives) for activity in >500 assays (including zebrafish embryos and C. elegans)
- Developing statistical and informatic tools for analyzing the resulting data and for compound prioritization
- Developing databases for Tox21 and legacy data (EPA ACToR, NTP CEBS)
- Identifying “fingerprints” potentially indicative of in vivo toxic effects
- Supporting extramural interactions via material transfer agreements, NIEHS SBIR contracts, and EPA’s STAR program
Tox21 Phase II Activities

- Obtaining human toxicity data
- Establishing a library >10,000 DMSO-soluble compounds with known structures for screening at the NCGC (includes failed and approved drugs, EPA and NTP centric compounds, ICCVAM reference compounds, etc.)
- Establishing procedures for determining the identity, purity, and stability of each compound in the 10K library
- Developing a qHTS strategy for the 10K library
- Developing a strategy for mid-throughput assays
- Evaluating the relevance of prediction models developed from ToxCast™ data (e.g., rat liver nongenotoxic carcinogens)
Tox21 Phase II Activities (cont.)

- Evaluating the relationships between compounds, genes, pathways, and disease (e.g., NTP Workshop on “State-of-the Science Evaluation of Environmental Exposures and Diabetes/Obesity”)
- Identifying methods for incorporating hepatic metabolism into qHTS assays
- Supporting the development of virtual organ and \textit{in vitro} 3D organ models
- Initiate EPA’s ToxCast Phase II
- Making all data publicly accessible (ACToR, CEBS, PubChem)
- Extending collaborations and interactions nationally and internationally
- Outreach (SOT, workshops, interactions with regulators)
qHTS assays used at the NCGC: Phase I

**Apoptosis**
- 3/7, 8, & 9

**Cell Viability**
- ATP
- LDH
- Protease release

**DNA damage**
- ELG1
- p53
- Multiple repair-deficient DT40 cell lines

**Epigenetics**
- LDR

**Mitochondrial toxicity**

**Nuclear Receptors**
- hAR
- hERα
- hFXR
- hGR
- hLXRβ
- hPPARα
- hPPARγ
- hPPARδ
- hPXR
- rPXR
- hROR
- hRXR
- hTRβ
- hVDR

**Pathways**
- AP1
- ARE/Nrf2
- CRE
- hERG
- HRE
- HSP
- JNK3
- NFkB

![Pie chart showing the distribution of qHTS assays by category.](chart.png)
qHTS assay strategy proposed for Tox21 Phase II

Stress response pathways
- Oxidative stress (ARE, Nrf2)
- Mitochondrial damage
- AP-1 (JNK, ERK, p38 signaling)
- NrfB (inflammatory response)
- Heat shock protein (Hsp70)
- DNA damage
  - p53
  - ELG1 DNA damage protein
  - DT40 chicken isogenic cell clones
- Cytotoxicity (decreased ATP)

Nuclear receptor activity
- ERα (agonist, antagonist)
- AR (agonist, antagonist)
- PPARλ
- FXR
- VDR
- TRβ
- GR
- PXR (rat and human)
- AhR
Gohlke et al. (BMC Systems Biology 2009, 3:46)

- MAP Kinase pathway
- p53 signaling pathway
- ErbB signaling pathway
- Starch and Sucrose Metabolism
- Signal transduction
- Cell cycle

Avg. of 16 diseases associated with each signaling pathway
Avg. of 4 diseases associated with each metabolic pathway

Gohlke et al. (BMC Systems Biology 2009, 3:46)
The NCGC BioPlanet

• Hosts the universe of pathways

• All pathway annotations from manually curated, public sources

• Integrates pathways from different data sources

• Annotates pathways by source, species, biological function/process, disease/toxicity relevance, assay availability

• Easy visualization, browsing, analysis of pathways

• Facilitates pathway assay selection/prioritization for Tox21

• Will be made publicly available
~1100 human pathways mapped to the pathway globe
CEPH cell lines
(collaboration with Ivan Rusyn, UNC-Chapel Hill)

- 87 cell lines (29 families, Parent-child trios)
- Large, renewable resource
- Publicly available information
- Easy to manipulate
- Ability to control environment
- Evidence of potential for use in heritability studies
Inter-individual Variability in Response To Toxic Compounds

O’Shea et al. (2010) In vitro screening for population variability in chemical toxicity.
TOXSCI-10-0537.R1
Mining the NTP Tissue Archives for Gene Signatures Associated with Disease

- >2,000 NTP studies
- >7.5 million histological glass slides
- >4.6 million paraffin-embedded tissue blocks
- >230 thousand sealed bags of formalin-preserved tissue
- >54 thousand frozen specimens
- Histopathology images that include >50 thousand 2x2 kodachrome slides and >20,000 digital images
- Study data that include >3.5 million pages, >10 million pages of microfiche data, >1.5 million pages of digital or electronic records
DrugMatrix

Gene Expression
- CodeLink RU1 10K rat array
- Affymetrix Whole Genome Arrays

Pathology Profiles

Pharmacology Profiles

Benchmark Drugs and Compounds

Literature Profiles

Pathology Assays
- Histopathology
- Clinical chemistry
- Hematology
- Body and organ weights

Pharmacology Assays
- Binding, Enzyme, ADME

Drug Literature
- Pharmacology
- Human Toxicology
- Structure
- Pathways

A rat toxicogenomic database
DrugMatrix Content

- ~ 640 compounds
- ~ 4,000 dose-time-tissue combinations (biological triplicates)
- ~ 2,000,000 dosed tissue samples
- ~ 15,000 Codelink RU1 Microarrays
- ~ 5,000 Affymetrix RG230-2 Arrays

345 compounds in LIVER
Gene expression
Blood Chemistry
Histopathology
Pharmacology
Literature

LIVER
KIDNEY
HEART
RAT PRIMARY HEPATOCYTES
Marrow
Spleen
Brain, Intestine
Muscle
The NTP Center for the Evaluation of Risks to Human Reproduction

Workshop on
“State-of-the Science Evaluation of Environmental Exposures and Diabetes/Obesity”

Jan 11-13, 2011
RTP
Critical Issues

• Human toxicity data
• Xenobiotic metabolism
• Interactions between chemicals, cells, tissues
• Statistical versus biological significance
• Extrapolation from *in vitro* to *in vivo*
• Acute vs chronic effects
• Non-adverse perturbations versus adverse perturbations
• Making sense
Tox21: Expectations for 21st Century Toxicology

- 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

- Results from new “data rich” techniques: genomics, proteomics, HTS, must be reconciled with existing testing information for conceptual validation

- Approaches to accomplish formal validation of new methods for human hazard and risk estimations must be concurrently developed

- Better coordination across the Federal government

- Increasing collaborations with major stakeholders

- ALL data will be made publicly accessible
NTP Board of Scientific Counselors Meeting

Rodbell Auditorium, Rall Building
National Institute of Environmental Health Sciences (NIEHS)
Research Triangle Park, NC
November 30 – December 1, 2010

Review of the Biomolecular Screening Branch and Tox21-Related Activities

http://ntp.niehs.nih.gov/go/165

To be webcast