The U.S. "Tox21 Community" and the Future of Toxicology

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History

2004 NTP publishes a Vision and Roadmap for Toxicology in the 21st Century
the NCGC founded

2005 NTP and NCGC began their HTS collaboration
EPA established the NCCT

2006 the NCGC begins testing NTP 1408 compounds
EPA joins collaboration with assays and 1462 compounds

2007 NCCT starts the ToxCast™ Program
NAS publishes *Toxicology in the 21st Century*

2008 NTP, NCGC, EPA "Tox21" collaboration established via a Memorandum of Understanding (MoU)

2009 ToxCast™ Phase I completed
NCGC completes first phase of testing with 2870 compounds

The U.S. Tox21 Community

An MoU on "High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings" signed February 14, 2008 (http://ntp.niehs.nih.gov/go/282130) by:

- the U.S. Environmental Protection Agency (Dr. G. Gray, Assistant Administrator for the Office of Research & Development)
- the NIH National Institute of Environmental Health Sciences/National Toxicology Program (Dr. S. Wilson, Acting Director)
- the NIH Human Genome Research Institute (Dr. F. Collins, Director)

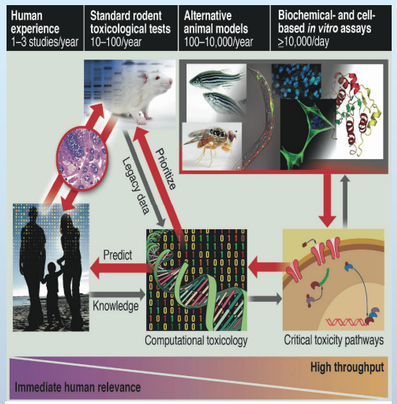
The MoU builds on existing expertise and overcomes the resource limitations of a single agency.

The three organizations agreed to collaborate on the research, development, validation, and translation of new and innovative test methods that characterize key steps in toxicity pathways.

Ultimately, the data generated by these new tools is to be provided to risk assessors to use in the protection of human health and the environment.

Goals are to investigate the use of these new tools to:

- identify mechanisms of chemically induced biological activity,
- prioritize chemicals for more extensive toxicological evaluation, and
- develop more predictive models of *in vivo* (human) biological response.



The Tox21 Paradigm

Collins, FS, Gray, GM, Bucher, JR (2008) Transforming Environmental Health Protection. *Science* 319:906.

Tox21 Working Groups

Pathways/Assays - K. Houck (EPA), K. Witt (NTP), M. Xia (NCGC)

- Identify key toxicity pathways/assays (with a focus on human cells) and prioritize assays for use at the NCGC
- Identify methods for incorporating hepatic metabolism into *in vitro* assays
- Evaluate human and rodent genetic variability in response to toxicants

Compounds - A. Richard (EPA), N. Southall (NCGC), C. Smith (NTP)

- Establish a library ~10,000 compounds(~1/3 approved drugs) 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 and one for mixtures for testing at the NCGC

Bioinformatics - R. Huang (NCGC), R. Judson (EPA), K. Shockley (NTP)

- Evaluate patterns of response and relationship to adverse health outcomes in experimental animals and humans
- Evaluate consistency of response within and across assays/endpoints
- Make all data publicly accessible (ACToR, CEBS, PubChem)

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)
- Consider approaches for evaluating compound, pathway, and cell-to-cell interactions

The NIH Chemical Genomics Center

The NCGC conducts quantitative high throughput screens (qHTS) (Inglese et al. 2006 PNAS 103:11473)

- >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
- 1408 wells used for test compounds, 128 for negative and positive control wells

HTS assays used at the NCGC

Cell Viability
- ATP
- LDH
- Protease release

Caspases
- 3/7, 8, & 9

Pathways
- AP1
- ARE
- CRE
- HRE
- NFκB

DNA damage
- p53
- Multiple repair gene-deficient cell lines

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

Inter-individual variation in response using 20 sets of identical twins

NCGC Robotics Facility

EPA's ToxCast™ Program

- Research program of EPA's National Center for Computational Toxicology (NCCT) (see 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 ToxCast™ Data Summit - May 14-15 2009 - EPA RTP campus
- Data released via ACToR (Aggregated Computational Toxicology Resource) (http://epa.gov/actor)
  - Contains data on ~500,000 environmental chemicals
  - Multiple Domains - Physchem, biological, use levels, regulations
  - Brings together data from >200 sources

Phased Development of ToxCast™

Phase	# of Cmpds	Cmpd Criteria	Purpose	# of Assays	Target Date
I	*320*	*Data Rich (pesticides)*	*Signature Development*	*554*	*FY08*
Ib	15	Nano-materials	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	Nano-materials	PMN	>200	FY09-10
III	1000s	Data poor	Prediction and Prioritization	>300	FY11-12

ToxCast™ 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)

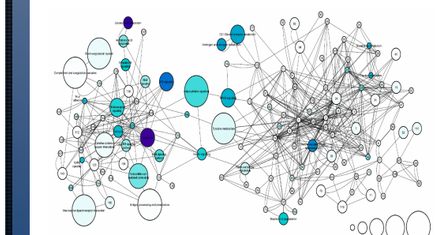
ToxCast™ Phase I (cont'd)

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)

Tox21 (Near-Term) Goals

- Complete the ~10000 compound library and determine the identity, purity, and stability of each compound
- Establish water-based and mixture compound libraries
- Evaluate performance of assays conducted to date
- Expand and prioritize assays that assess key ("toxicity") pathways involved in human disease (e.g., Gohlke et al., 2009) and test main library
- Explore new platforms for HTS and high content screening that use primary human cells and that incorporate organ-specific metabolism
- Conduct ToxCast™ Phase II with >750 compounds (50 from NTP) and >400 assays
- Make all data publicly accessible and mineable (EPA ACToR, NTP CEBS, NLM PubChem)
- Start to evaluate human and rodent genetic variability in response to toxicants
- Continue to refine traditional methods and develop new methods to provide basic toxicology information for public health protection
  - Mechanistic information
  - Life stage susceptibility
  - Genetic susceptibility
- Expand the Tox21 community nationally and internationally (FDA, EU Joint Research Centre)



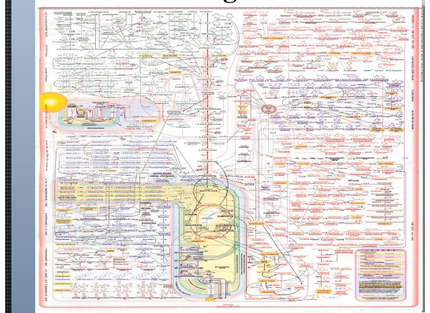
Gohlke et al. (2009) Genetic and environmental pathways to complex diseases. BMC Systems Biology, Vol. 3, article 46.

Issues

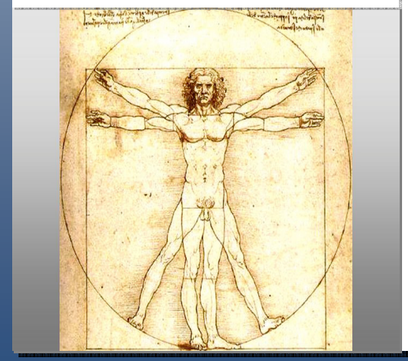
- How do we deal with genetic differences in sensitivity and environmental modulation of response?
- What is the optimal testing prioritization scheme?
- How do we achieve a fully integrated testing strategy that takes into account ADMR as well as exposure data?
- How can biomolecular screening data be used for making regulatory decisions?
- Two cautionary notes:
  - Not all compounds can be tested *in vitro*.
  - A gene is not a pathway, a pathway is not a cell, a cell is not a tissue or organ, an organ is not an organism, an organism is not a species,…

or

how do we go from this



to us?



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