NIEHS Update

Nigel Walker, PhD, DABT
Deputy Director for Research
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
(walker3@niehs.nih.gov)

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Outline

• Alternative methods
  – Toxicology in the 21st Century (Tox21)

• Implementation of alternate approaches
  – Elk River chemical spill example
  – Flame retardants project

• Development of new alternative methods
  – NIEHS SBIR/STTR programs
  – Collaborative screening activities with new assays
A National Toxicology Program for the 21st Century

• Roadmap to Achieve the NTP Vision
  – Released November 2004

• “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 inclusion of target specific, mechanism-based, biological observations.”
Toxicology in the 21st Century (Tox21)

- NTP Roadmap 2004 goal
  - “Develop a HT capability for mechanistic targets”

- Interagency collaboration
  - NIEHS/DNTP, US EPA, NIH/NCATS, FDA

- Main goals
  - Identify mechanisms of action
  - Prioritize substances for further in-depth toxicological evaluation
  - Develop predictive models for in vivo biological response

- Revised 5-year MoU to add FDA signed on July 19, 2010
Tox21” - Phase II (2011-14)

- "EPA’s ToxCast™: ~700 compounds in ~700 assays, ~1000 compounds in endocrine activity assays
  - NCGC qHTS Phase II: 10K compound library: nuclear receptor activation or inhibition, induction of cellular stress response pathways, characterizing human variability in response

- Lessons learned paper
  - Tice RR, Austin CP et al. EHP 2013

- Systematic study of mitochondrial toxicity of environmental chemicals using quantitative high throughput screening.
  - Attene-Ramos MS, Huang R et al. 2013

- Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway.
  - Huang R, Sakamuru S et al. 2014
Tox21 Phase III

• Increased focus on tools for in vitro concentration to in vivo extrapolation

• Different cells systems
  – cells capable of xenobiotic metabolism (primary hepatocytes, HepaRG, HepG2 3D)
  – ES/iPSC derived differentiated cell populations

• Expanded utilization of lower organisms (zebrafish, C. elegans)
  – High content screening

• High-throughput transcriptomics project
  – Selection of 1500 “sentinel” genes
  – Genes are included to ensure maximal biological pathway coverage.
NIEHS Efforts to Incorporate Metabolism into Tox21

- Establish onsite in vitro lab focused on predictive toxicology screening
- Collaborate with NCATS on quantitative high throughput screening efforts incorporating xenobiotic metabolism
- Collaborate with EPA/NCCT on ToxCast chemical evaluations using metabolically-competent in vitro models for toxicity and pharmacokinetics
- Collaborate with FDA/NCTR on drug induced liver injury projects using metabolically-competent in vitro liver models
- Collaborate with new technology providers to evaluate the utility of developing in vitro model systems and assays
- Support laboratory efforts in Europe in this area to harmonize international research
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West Virginia Chemical Spill: NTP Research Response

• Jan 2014; Residents of Charleston, West Virginia began to notice a “sweet smell” (like licorice) in the air and reported it to the WV Department of Environmental Protection.

• 10,000 gallons of chemicals used to process coal spilled from a storage tank
  – Mixture of multiple chemicals including 4-methylcyclohexanemethanol (MCHM), propylene glycol phenyl ether (PPH)

• CDC issues a 1 ppm screening level based on limited information

• NTP asked to evaluate the point of departure used in the risk assessment, determine if there are life stage specific hazards and screen minor components of the mixture.
In silico SAR

In vitro HTS

Bacterial mutagenicinity

Nematode Toxicity

Zebrafish Embryotoxicity

Mouse Dermal Irritancy/Hypersensitivity

Rat repeat dose Toxicogenomics

Rat Prenatal Developmental Toxicity
## Proposed NTP Studies

<table>
<thead>
<tr>
<th>Test Article [Abbreviation, CAS Number]</th>
<th>Rat Prenatal Toxicity</th>
<th>Mouse Dermal Irritation and Hypersensitivity</th>
<th>5-Day Rat Toxicogenomic</th>
<th>Bacterial Mutagenicity</th>
<th>Zebrafish Developmental</th>
<th>Nematode Toxicity</th>
<th>High Throughput Screening</th>
<th>Structure Activity Relationship (SAR) Analysis</th>
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Aromatic Phosphate Flame retardants

• High production volume (HPV)
  – 10 -50 million pounds/year

• Nominated by Consumer Product Safety Commission
  – Neurotoxicity/reproductive/developmental toxicity
  – Have been identified by EPA as substitutes for some of the PBDEs

• Inadequacy and limitations in existing data sets from HPV program

• Associated with reproductive and neurologic and systemic effects

• Mixtures containing different compounds
Battery to Screen for Potential DNT/Neurotoxicity

**Toxicant**
- Chemical Property Profile
  - ? (many)

**Molecular Initiating Event**
- Key Events
  - proliferation
  - differentiation
  - migration
  - neurite growth
  - synaptogenesis

**Cellular Responses**
- Nervous System
  - Δ connectivity (morphology, neurochemistry, physiology)

**Organ Responses**
- Individual Responses
  - behavior cognition

**Population Responses**
- Structure Recruitment Extinction

**Biochemical/Molecular assays**
- Ion channels
- AChE
- Neurotransmitter receptors
- Thyroid hormone
- Metabolism
- Growth factor receptors
- Cell adhesion molecules
- Kinases

**Neurite outgrowth (High-Content Screening)**

**Neuronal Firing**
- Multi-Electrode Array (MEA)

**HTS/Tox 21**

**3-D aggregates**

**Zebrafish**
- C. Elegans
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NIEHS SBIR/STTR programs

• Emphasis is on the development of new and novel approaches using state-of-the-art technologies:
  – 6 active awards
  – 3D human tissue culture; Computational models; novel assays

• Novel Assays for Screening the Effects of Chemical Toxicants on Cell Differentiation (RFA-ES-13-003)
  – 7 awards
  – Reporter assays, metabolomics, microfluidics, epigenetics, stem cell differentiation

• Novel Methods for Obtaining Molecular Information from Archived Tissue Samples (RFA-ES-13-009)
  – 5 awards
Collaborative activities screening an 80 compound library

• Neurite outgrowth/mitochondrial membrane potential
  – Cellular Dynamics and Molecular Devices

• Human and rat neuronal cell culture systems
  – primary, embryonic stem cell–derived, induced pluripotent stem cell [iPSC]-
    derived, transformed neural cell lines
  – QPS, PhoenixSongs Biologicals, and the Hamner Institutes

• Migration of neural crest cells/neurite outgrowth in a human cell line.
  – Univ. Konstanz (Germany)

• iPSC-derived neural precursor cells (with a mitochondrial defect
  associated with Parkinson’s disease) vs isogenic wild-type.
  – Xcell

• hTERT astrocytic cell lines to identify senescence-inducing agents.
  – Buck Institution
More collaborative screening activities

• iPSC-derived human hematopoietic cell culture systems.
  – Primorigen

• iPSC-derived human cardiomyocyte cell culture systems
  – Vala Sciences, Primorigen

• Beating cardiomyocytes/mitochondrial membrane potential
  – Cellular Dynamics and Molecular Devices

• Drosophila intestinal stem cells to ascertain effects on the cell cycle, stem cell differentiation and the Notch, Jak-Stat, JNK signaling pathways.
  – U. Mass, Amherst