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
  – http://ntp.niehs.nih.gov/go/vision

• “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 mutagenicity

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

**Molecular Initiating Event**
? (many)

**Cellular Responses**
- Key Events
  - proliferation
  - differentiation
  - migration
  - neurite growth
  - synaptogenesis

**Organ Responses**
- Nervous System
  - Δ connectivity
  - morphology
  - neurochemistry
  - physiology

**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

**HTS/ Tox 21**

**Zebrafish C. Elegans**

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

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

**3-D aggregates**
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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