NIEHS Update

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Scientific Advisory Committee on
Alternative Toxicological Methods (SACATM)
September 16, 2014
Outline

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

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

• Refined toxicology testing methods
  – NTP Modified one-generation study

• 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.
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West Virginia Chemical Spill: NTP Research Response

• 10,000 gallons of chemicals used to process coal spilled from a storage tank into the Elk River in West Virginia (January 2014)
  – 4-methylcyclohexanemethanol (MCHM)
  – Others including dipropylene glycol phenyl ether, and propylene glycol phenyl ether

• NTP approach
  – SAR models to predict potential adverse effects from the chemicals
  – Alternative models, including zebrafish to assess developmental effects
  – Short term toxicogenomic studies to identify biological systems affected and at which dose levels no effects are seen.
  – MCMH teratology study in rodents to assess developmental effects
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 → Molecular Initiating Event

Key Events
proliferation
differentiation
migration
neurite growth
synaptogenesis

Nervous System
Δ connectivity
(morphology, neurochemistry, physiology)

behavior
cognition

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

Zebrafish
C. Elegans

3-D aggregates
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NTP Modified One–generation study (MOG)

• Outcomes from NTP workshops and NIEHS Strategic plan identified the need for better evaluation of impact of early-life exposures
  – Inclusion of perinatal exposure in chronic studies
  – Assessment of endocrine-related responses in subchronic studies

• NTP developing and evaluating a new design—the MOG
  – Continuous exposure from GD6 (implantation) through to sexual maturity
  – Uses various toxicity “cassettes” incorporated from other standard regulatory studies to make better use of animals already incorporated into the study.
  – Positive advantages over conducting individual DART and range finding studies or multiple traditional “segmented” study designs
  – Foster PM 2014 Toxicol Pathol DOI: 10.1177/0192623314534920
Timed – Pregnant Female Rats - 3 dose groups + control
Continuous dosing

Seg III

G L

Select 4♂ and 4♀ / litter

Necropsy ~PND 110

Select 2♂ and 2♀
[sub-chronic & Special tox cohort]

Select 1♂ and 1♀
[Teratology Cohort]

Select 1♂ and 1♀
[Breeding &/or Littering Cohort]

Necropsy GD 20
Fetal Pathology

F₂ pups
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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
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