NTP
National Toxicology Program

NIEHS Update

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

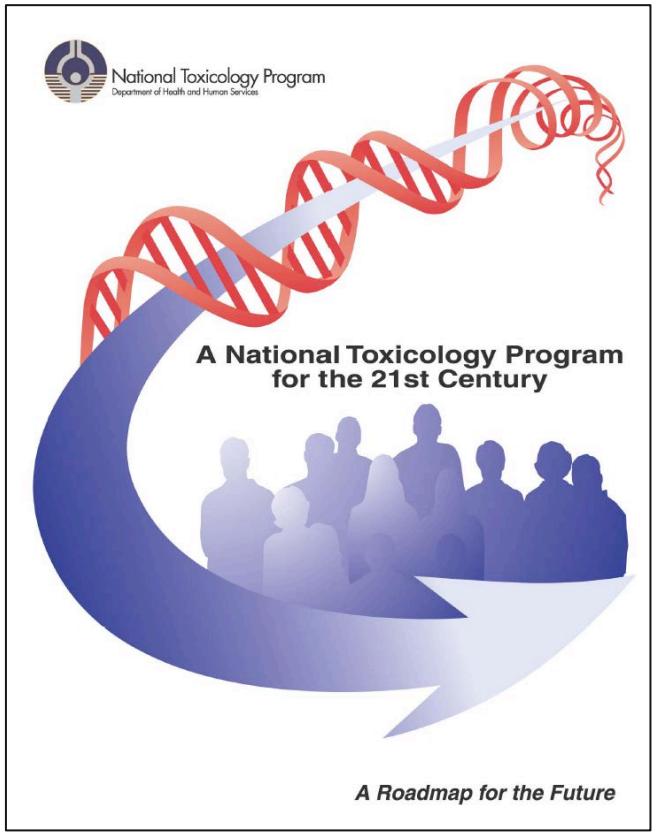
Scientific Advisory Committee on
Alternative Toxicological Methods (SACATM)
September 16, 2014

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

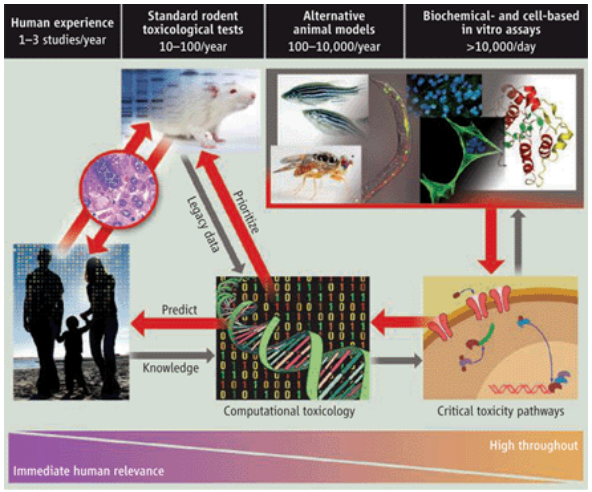
A National Toxicology Program for the 21st Century

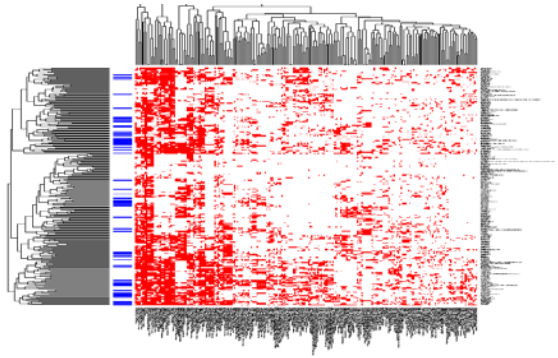
- Roadmap to Achieve the NTP Vision
  - Released November 2004
  - http://ntp.niehs.nih.gov/go/vision
- "*To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target specific, mechanism-based, biological observations.*"



Toxicology in the 21st Century (Tox21)

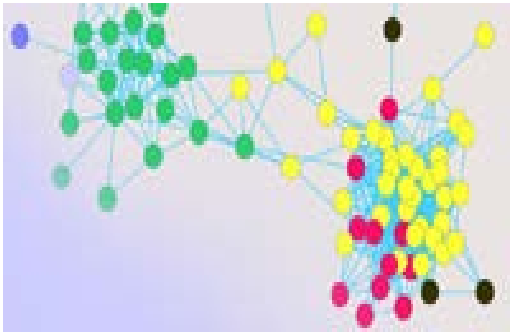
- NTP Roadmap 2004 goal
  - "Develop a HT capability for mechanistic targets"
- Interagency collaboration
  - NIEHS/DNTP, US EPA, NIH/NCATS, FDA
- Main goals
  - Identify mechanisms of action
  - Prioritize substances for further in-depth toxicological evaluation
  - Develop predictive models for in vivo biological response
- Revised 5-year MoU to add FDA signed on July 19, 2010





Tox21” - Phase II (2011-14)

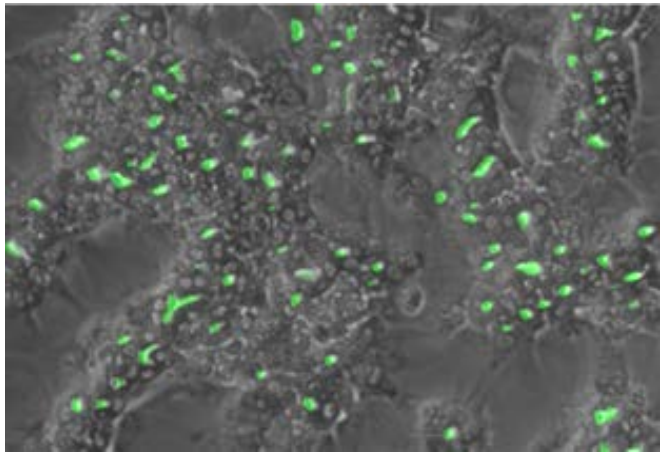
- "EPA’s ToxCast™: ~700 compounds in ~700 assays, ~1000 compounds in endocrine activity assays
  - NCGC qHTS Phase II: 10K compound library: nuclear receptor activation or inhibition, induction of cellular stress response pathways, characterizing human variability in response
- Lessons learned paper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

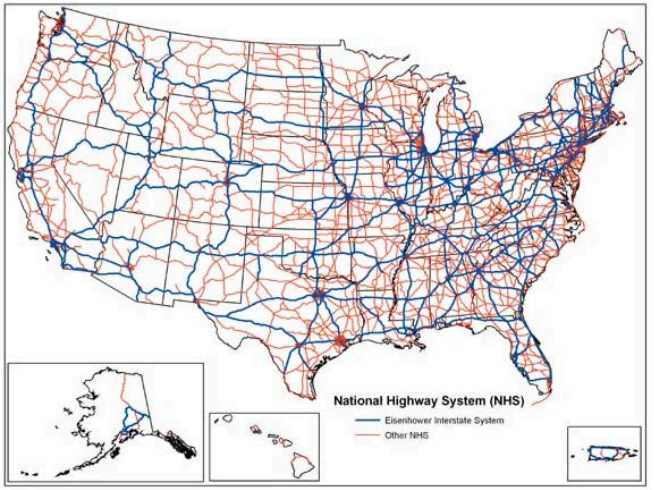


Tox21 Phase III

- 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.

HepaRG Cells





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West Virginia Chemical Spill: NTP Research Response

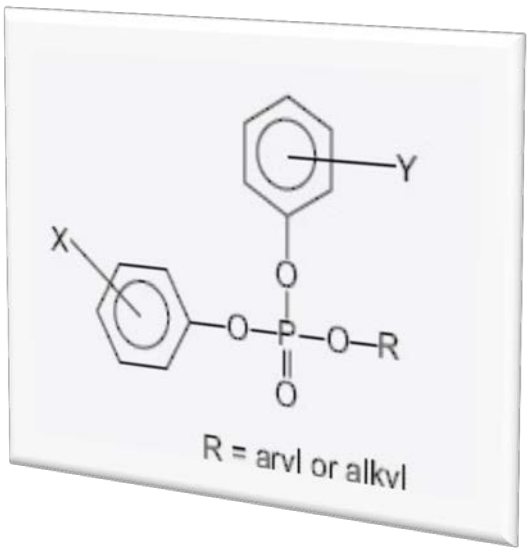
- 10,000 gallons of chemicals used to process coal spilled from a storage tank into the Elk River in West Virginia (January 2014)
  - 4-methylcyclohexanemethanol (MCHM)
  - Others including dipropylene glycol phenyl ether, and propylene glycol phenyl ether

- 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

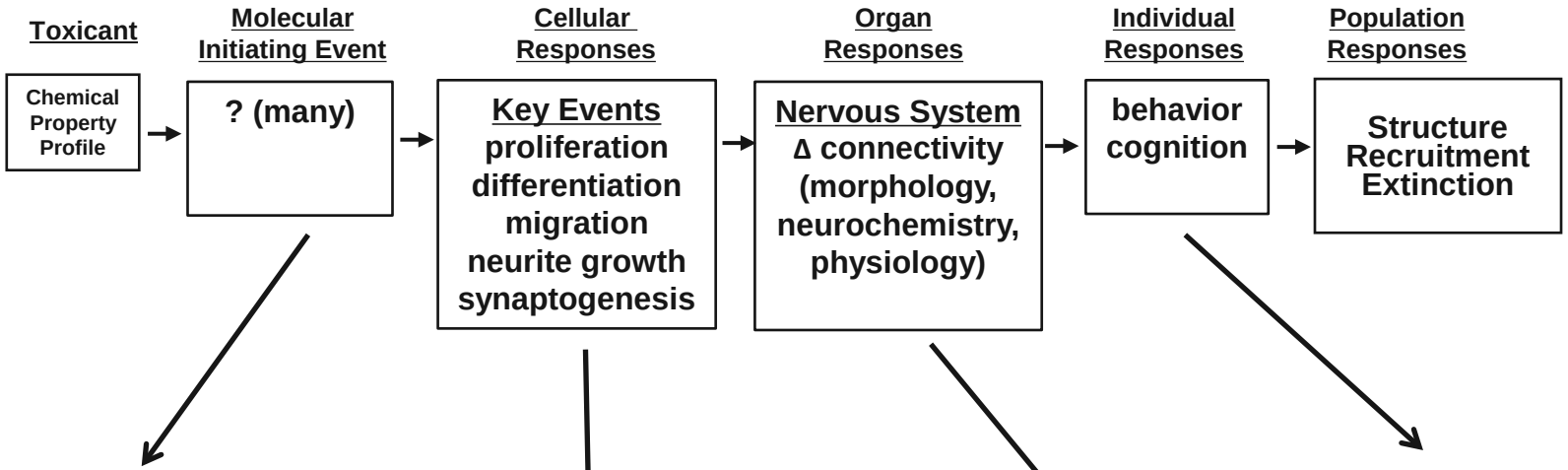
Aromatic Phosphate Flame retardants

- 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

	X	Y	R
Triphenyl phosphate (TPP)	-H	-H	-Ph
tert-Butylphenyl diphenyl phosphate (BPDP)	-H	-H	-t-BuPh
Tricresyl phosphate (TCP)	-Me	-Me	-MePh
2-Ethylhexyl diphenyl phosphate (EHDP)	-H	-H	-2-EtHx
Isodecyl diphenyl phosphate (IDDP)	-H	-H	-IDecyl
Isopropylated triphenyl phosphate (IPP)	-H or -iPr	-H or -iPr	-iPrPh



Battery to Screen for Potential DNT/Neurotoxicity

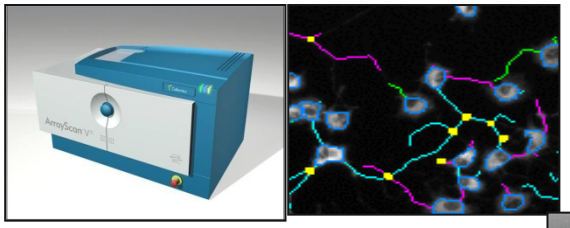


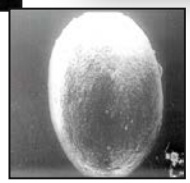
Biochemical/Molecular assays

ion channels
AChE
neurotransmitter receptors
thyroid hormone metabolism
growth factor receptors
cell adhesion molecules
Kinases

HTS/ Tox 21

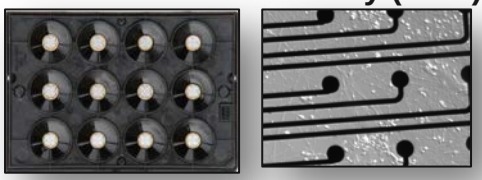
Neurite outgrowth (High-Content Screening)



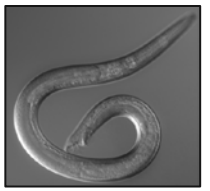


3-D aggregates

Neuronal Firing Multi-Electrode Array (MEA)



Zebrafish
C. Elegans



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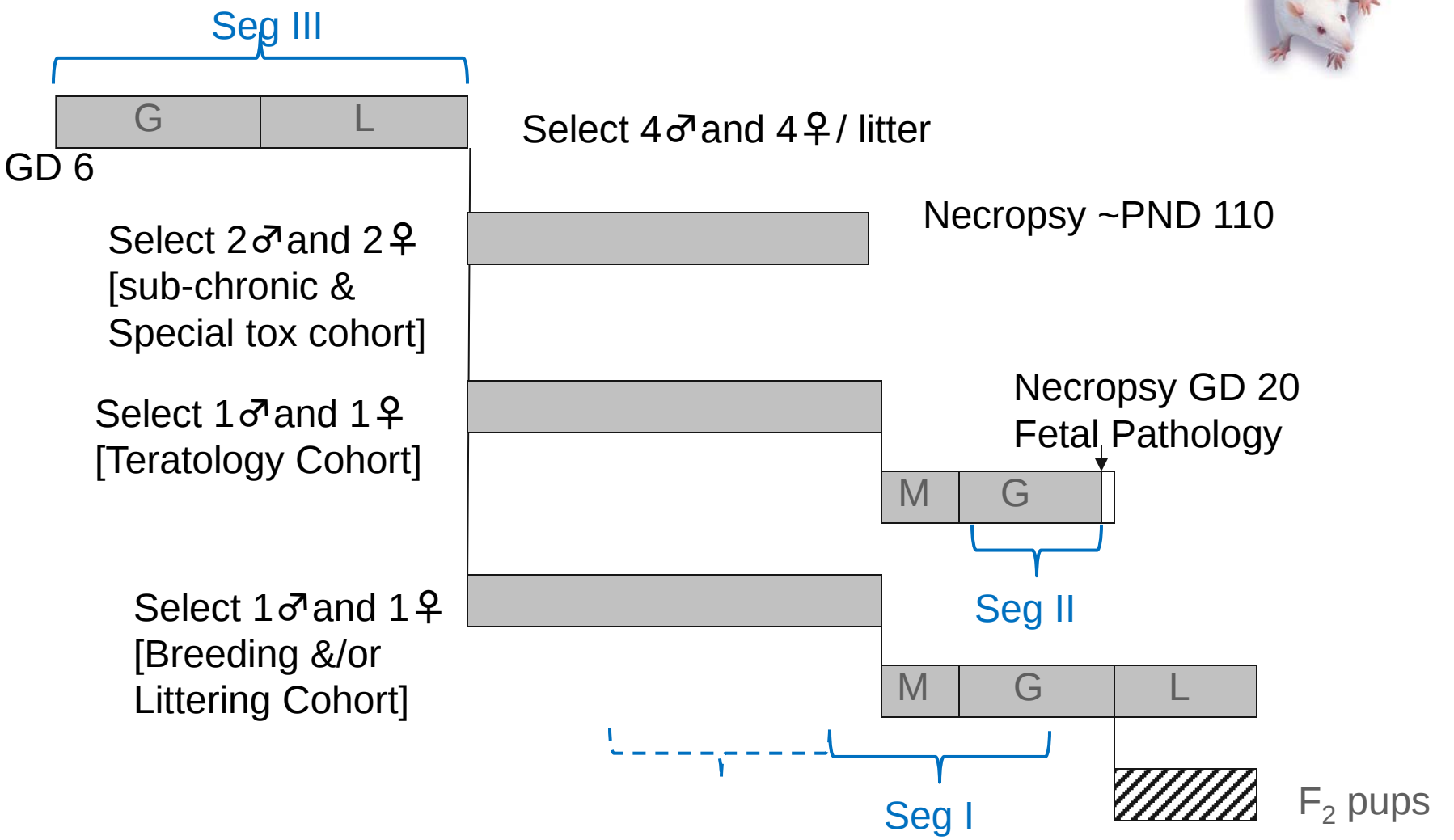
NTP Modified One–generation study (MOG)

- 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



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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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walker3@niehs.nih.gov

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U.S. Department of Health and Human Services