



NTP
National Toxicology Program

NIEHS Update

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Outline

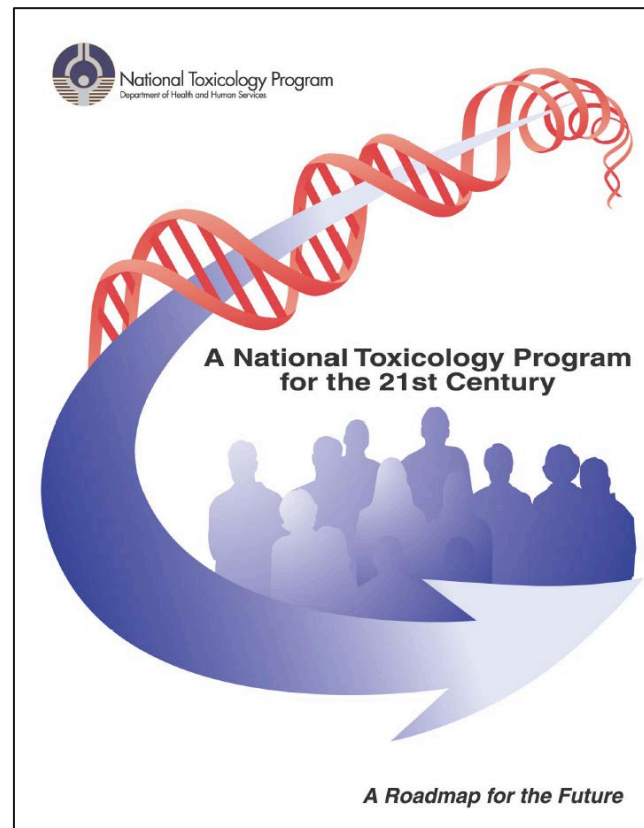
- Alternative methods
 - Toxicology in the 21st Century (Tox21)
- Implementation of alternate approaches
 - Elk River chemical spill example
 - Flame retardants project
- Development of new alternative methods
 - NIEHS SBIR/STTR programs
 - Collaborative screening activities with new assays





A National Toxicology Program for the 21st Century

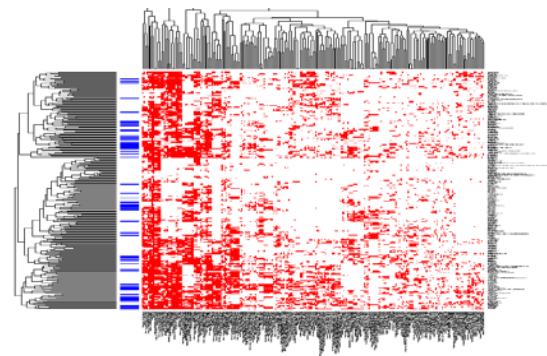
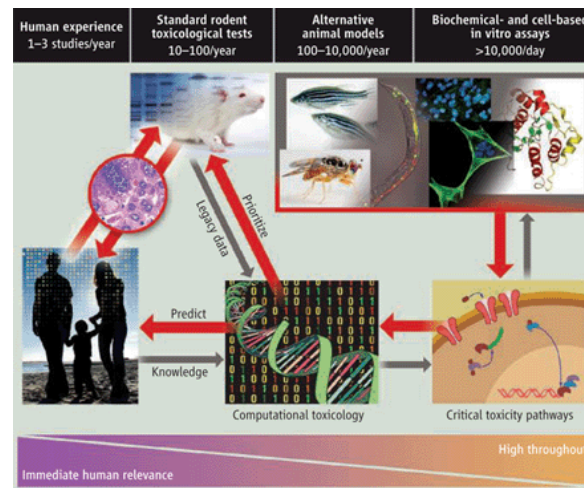
- Roadmap to Achieve the NTP Vision
 - Released November 2004
 - <http://ntp.niehs.nih.gov/go/vision>
- *“To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target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

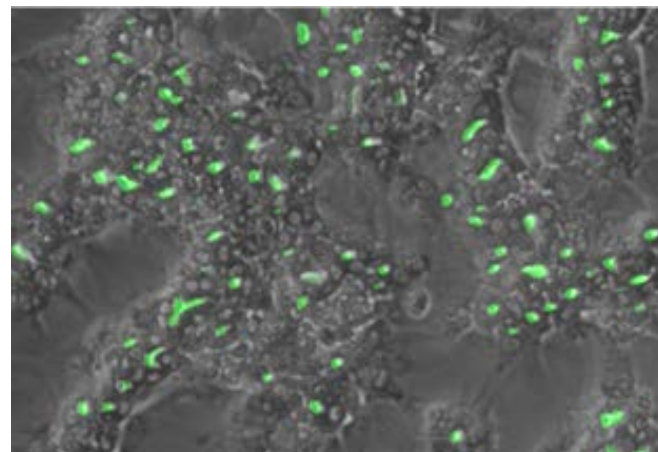




Tox21 Phase III

- 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.

HepaRG Cells





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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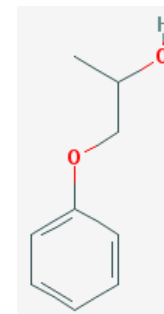
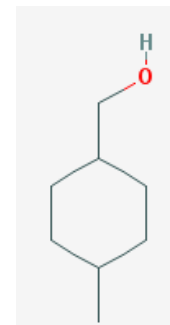
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West Virginia Chemical Spill: NTP Research Response

- 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.





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In silico SAR

In vitro HTS

Bacterial mutagenicity

Nematode Toxicity

Zebrafish Embryotoxicity

Mouse Dermal Irritancy/Hypersensitivity

Rat repeat dose Toxicogenomics

Rat Prenatal Developmental Toxicity

Compounds evaluated

Biological Complexity

Proposed NTP Studies

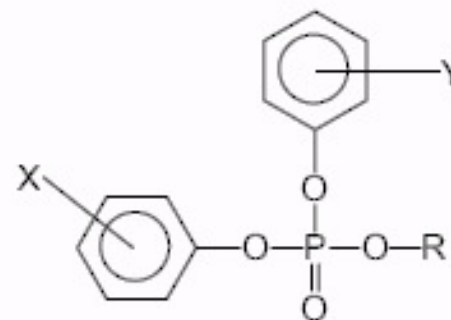
Test Article [Abbreviation, CAS Number]	Rat Prenatal Toxicity	Mouse Dermal Irritation and Hypersensitivity	5-Day Rat Toxicogenomic	Bacterial Mutagenicity	Zebrafish Developmental	Nematode Toxicity	High Throughput Screening	Structure Activity Relationship (SAR) Analysis
4-Methylcyclohexanemethanol [MCHM, 34885-03-5]	X	X	X	X	X	X	X	X
Dipropylene glycol phenyl ether [DiPPH, 51730-94-0]			X	X	X	X		X
Propylene glycol phenyl ether [PPH, 770-35-4]			X	X	X	X	X	X
1,4-Cyclohexanedimethanol (CHDM; 105-08-8)				X	X	X	X	X
2-Methylcyclohexanemethanol [2MCHM, 2105-40-0]				X	X	X		X
4-(Methoxymethyl)cyclohexanemethanol [MMCHM, 98955-27-2]				X	X	X		X
4-Methylcyclohexanecarboxylic acid [4331-54-8]					X	X		X
Cyclohexanemethanol, 4-[(ethenyloxy)methyl]- [114651-37-5]					X	X	X	X
Cyclohexanemethanol, alpha,alpha,4-trimethyl- [498-81-7]					X	X		X
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				X	X	X	X	X
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	X	X		X
Phenoxyisopropanol [4169-04-4]					X	X	X	X
Technical product ["crude MCHM"]		X	X	X	X	X		



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

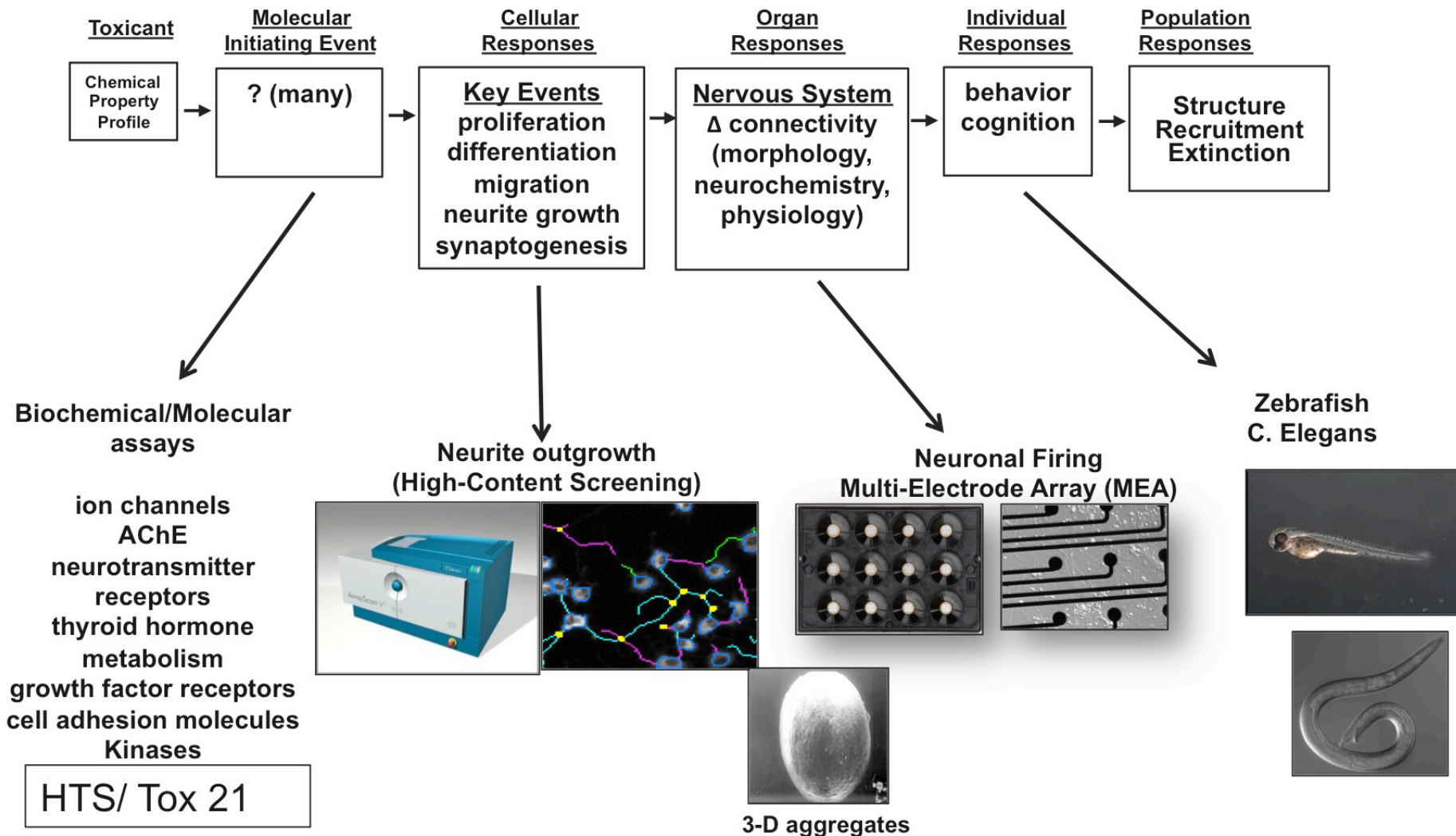
	X	Y	R
Triphenyl phosphate (TPP)	-H	-H	-Ph
<i>tert</i> -Butylphenyl diphenyl phosphate (BPDP)	-H	-H	- <i>t</i> -BuPh
Tricresyl phosphate (TCP)	-Me	-Me	-MePh
2-Ethylhexyl diphenyl phosphate (EHDP)	-H	-H	-2-Ethx
Isodecyl diphenyl phosphate (IDDP)	-H	-H	-IDecyl
Isopropylated triphenyl phosphate (IPP)	-H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



R = aryl or alkyl



Battery to Screen for Potential DNT/Neurotoxicity





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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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U.S. Department of Health and Human Services

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