



NTP

National Toxicology Program

The National Toxicology Program and Alternatives to Traditional Toxicity Testing

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for the Evaluation of
Alternative Toxicological Methods



Outline: NTP Alternatives to Traditional Toxicity Testing

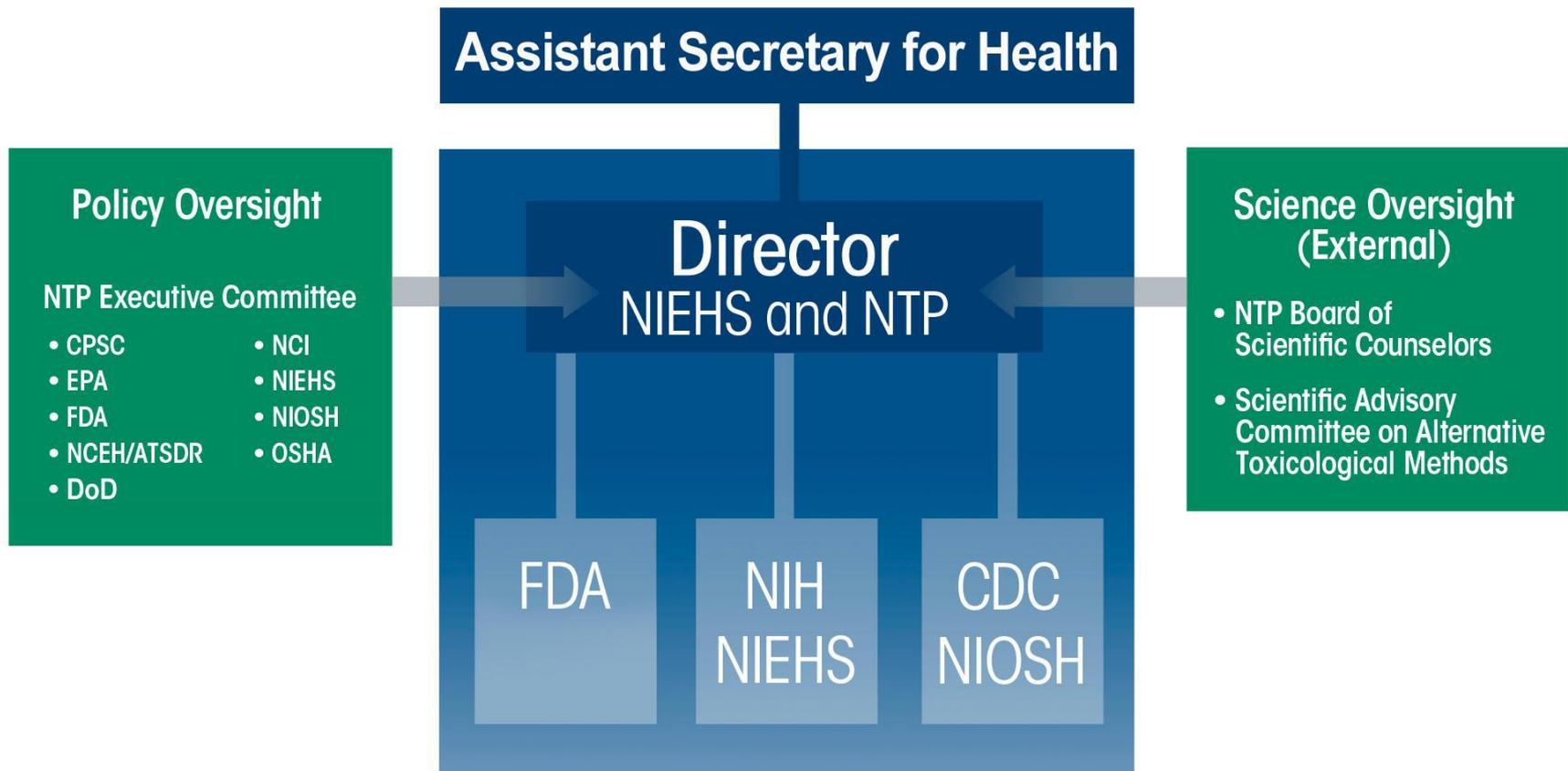
- National Toxicology Program – Big and Little
- Tox21 Program
- ICCVAM and NICEATM

National Toxicology Program

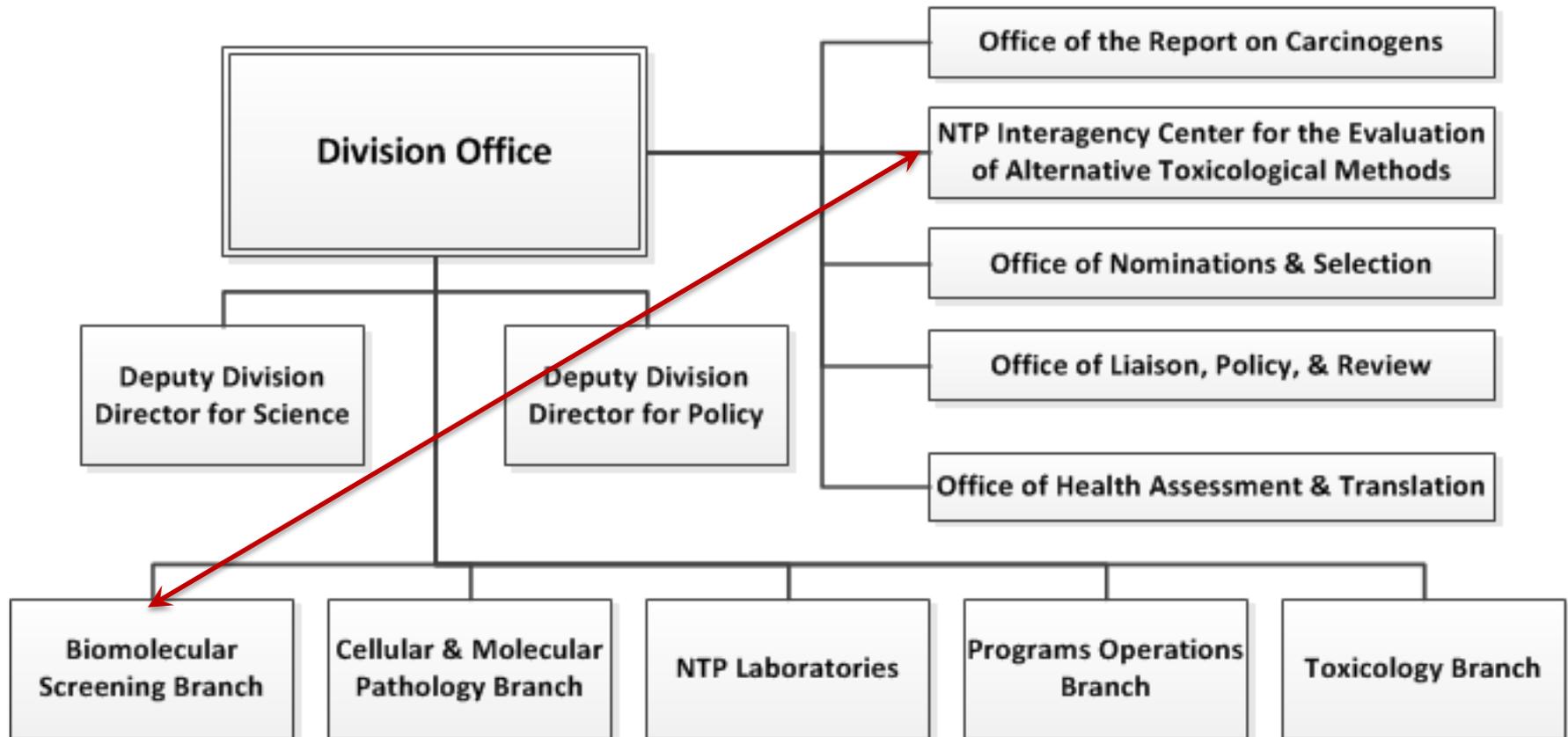
- Established in 1978
- Mission
 - To evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology
- Goals
 - Coordinate toxicology testing programs within the federal government.
 - Strengthen the science base in toxicology.
 - Develop and validate improved testing methods.
 - Provide information about potentially toxic chemicals to health, regulatory, and research agencies, scientific and medical communities, and the public.

BIG NTP

National Toxicology Program, DHHS (Headquartered at the NIEHS)

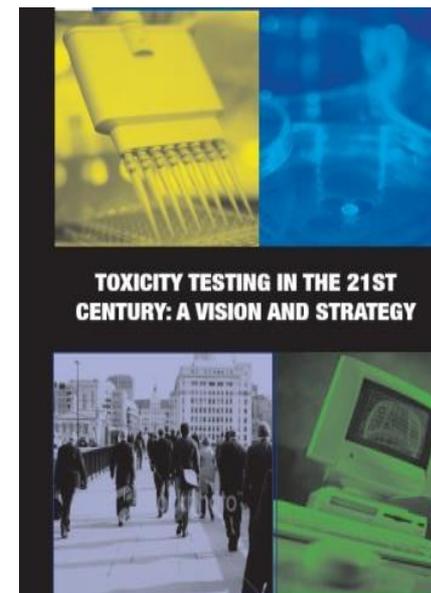


NIEHS Division of the National Toxicology Program



Tox21

- An interagency collaboration between:
 - NIEHS (NTP), EPA (NCCT), NCATS (NCGC), FDA
- Goal is to shift the assessment of chemical hazards away from traditional experimental animal toxicology studies to one based on target-specific, mechanism-based, biological observations largely obtained using *in vitro* assays.
- Achieved through high throughput screening, toxicity pathway profiling, computational toxicology, and biological interpretation of findings.



Tox21 Overview

- Phase I Proof of Concept / Optimize qHTS platform
- Phase II Screening of 10K library
- Phase III Follow up screening in low/med throughput assays

Tox21 Phase I

- ~2800 Chemicals from NTP and EPA
- 1536-well plates, qHTS
- 13 Cell types (9 Human, 2 Rat, 2 Mouse)
- >100 Assays
- EPA via ToxCast™ Phase I screened 320 compounds (309 unique, primarily pesticide actives and some endocrine active compounds) in ~550 assays
- Data released to the scientific community via:
 - EPA ACToR (Aggregated Computational Toxicology Resource; <http://epa.gov/actor>)
 - NLM PubChem (<http://pubchem.ncbi.nlm.nih.gov/>)
 - NTP CEBS (Chemical Effects in Biological Systems; <http://www.niehs.nih.gov/research/resources/databases/cebs/>)

Tox21 Phase I



Improving the Human Hazard Characterization of Chemicals: A Tox21 Update

**Raymond R. Tice, Christopher P. Austin,
Robert J. Kavlock, and John R. Bucher**

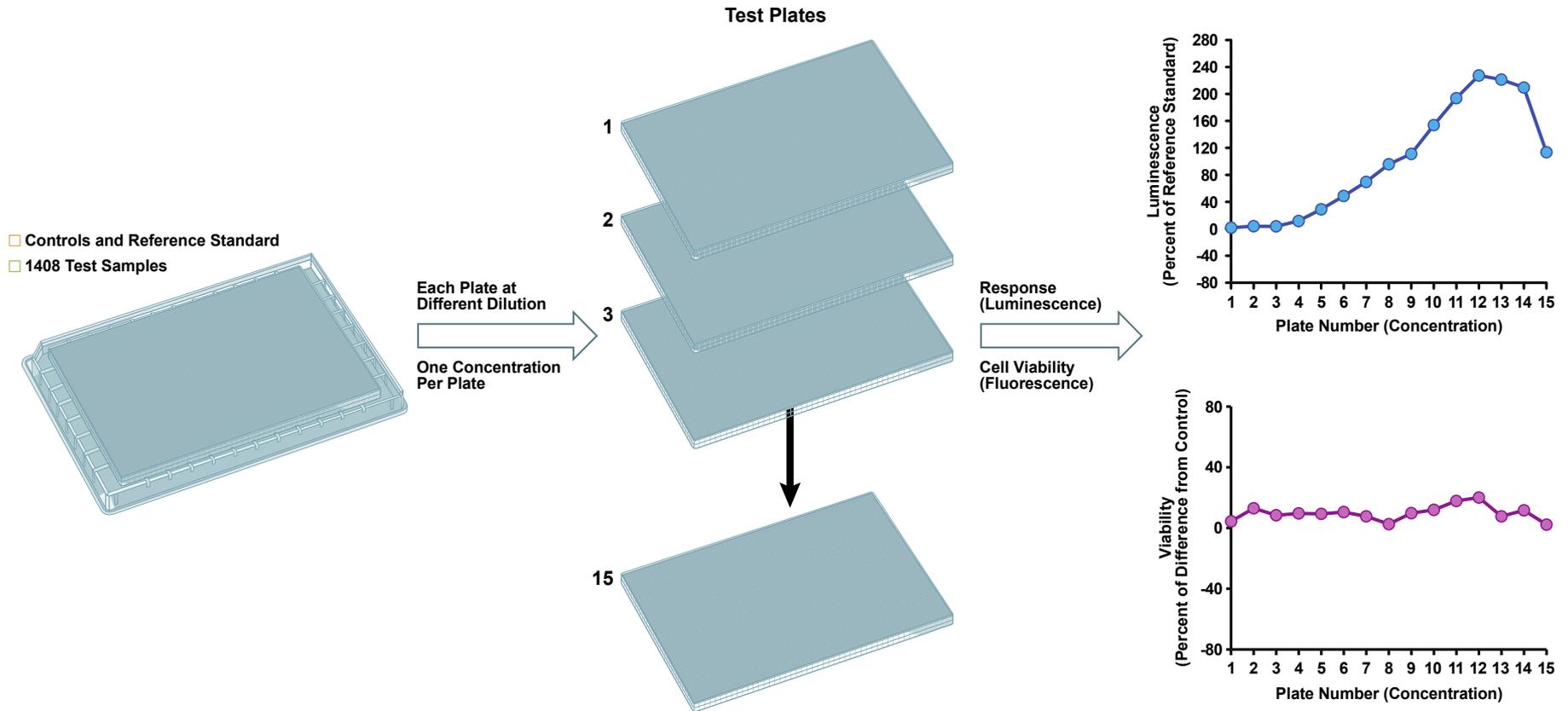
Citation: Tice RR, Austin CP, Kavlock RJ, Bucher JR. Environ Health Perspect ():
.doi:10.1289/ehp.1205784

Received: July 18, 2012; **Accepted:** April 18, 2013; **Published:** April 19, 2013

Tox21 Phase II

- ~10,000 (8,000 unique) chemicals from NTP, EPA, and NCGC
- Library Completed in Dec 2011
- NCGC goal is ~30 Assays per year
- Initial focus on Stress Response and NR Signaling pathways.
- EPA's ToxCast™ Phase II: ~700 compounds in >700 assays, ~1000 compounds in endocrine activity assays (e1K)

Quantitative High Throughput Screening (qHTS)



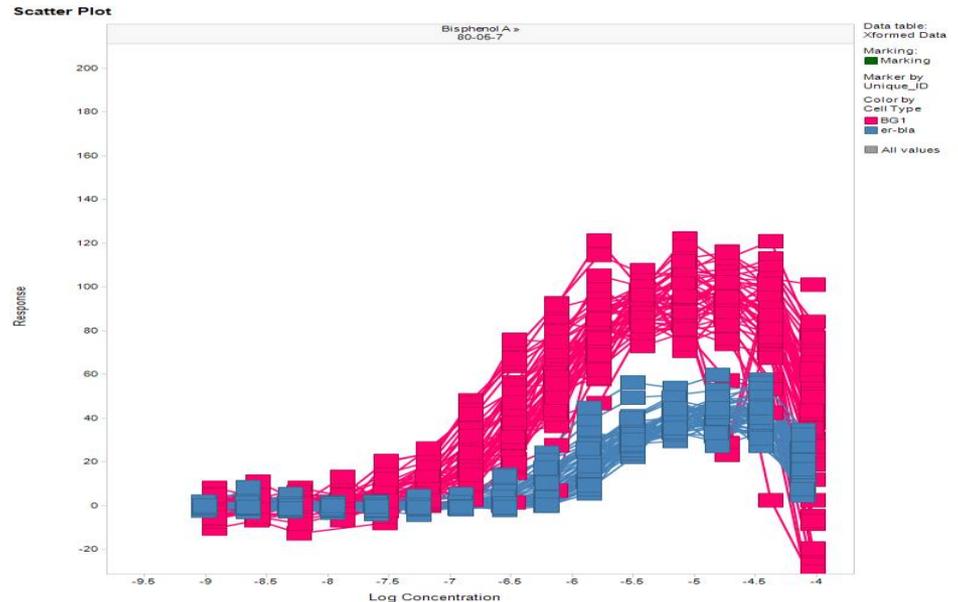
Tox21 Assays Completed*

ATAD5/ELG1	PPARg
Mitochondrial membrane potential	Aromatase
P53	Hsp70
Estrogen receptor alpha, LBD	AhR
Estrogen receptor alpha, full	PPARg
Androgen receptor, LBD	PPARd
Glucocorticoid receptor, full	Nrf2/ARE
Thyroid receptor, full	HSE
Estrogen receptor alpha, full	FXR
Glucocorticoid receptor, full	RXR
Thyroid receptor, full	DNA repair, Rev3 (-/-)
Androgen receptor, full	DNA repair, wild type
NFkB	

***Data will be released to Public ~1 Year after assay completion**

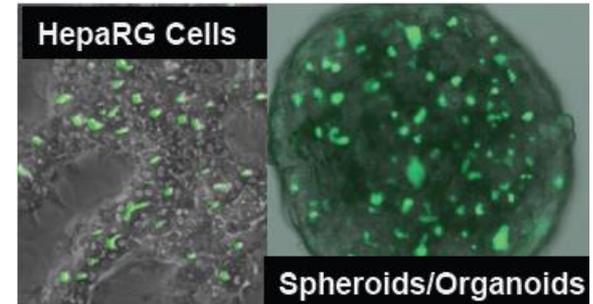
qHTS Assay Limitations

- DMSO soluble (20 mM stock)
- Fixed concentration range, typically 5 nM to 92 μ M
- ~5 μ L assay volume
- ~3000 cells/well
- Addition only – no wash steps



Tox21 Phase III – Improving on Biological Coverage and Relevance (2014 - ?)

- Focus on high content assays and high throughput transcriptomics platforms using:
 - cells capable of hepatic metabolism
 - ES/iPSC derived differentiated cell populations (e.g., cardiomyocytes, neurocytes, hepatocytes) from human and mouse representing healthy and disease models.
- Increased use of *in silico* models (e.g., metabolite prediction) and extrapolation models (e.g., reverse toxicokinetics)
- Expanded utilization of lower organism model systems (zebrafish, *C. elegans*)
- Use of 3D tissue models
- Integrate AOP concept into Tox21
- Expand collaborations and interactions



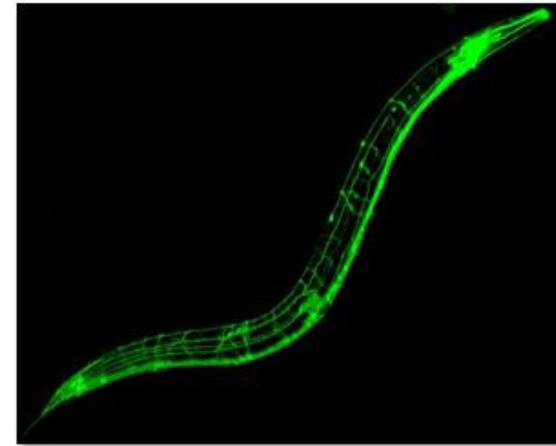
Targeted Assays

- High Content screening
 - Hoechst: Cell loss & nuclear size
 - DHE: Oxidative stress/ROS
 - p53: DNA damage
 - pH2A.X: Genotoxicity
 - JC-10: Mitochondrial damage (MMP)
 - Caspase 3: Apoptosis
 - Lipitox: Steatosis & Phospholipidosis
 - Reactive metabolites/ROS: GSH depletion
- Receptor Activation via Induction of gene expression
 - AhR, CAR, PXR, PPAR α , FXR
- Necrosis
 - miR-122 leakage or LDH leakage

Alternative Organisms – *C. elegans* and Zebrafish

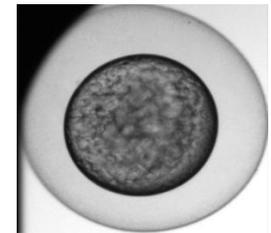
C. elegans (NIEHS/NTP) - J. Freedman/W. Boyd

- Screened ToxCast Phase II compounds in growth assay
- Screening subsets of compounds in assays that measure
 - feeding
 - larval lethality
 - reproduction



Zebrafish – R. Tanguay (Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR)

- Screened ToxCast Phase II compounds
- Screening 3455 NTP compounds at ~ 64 μ M
- Assays include
 - 1 day photo induced behavior
 - 1 day assessment of mortality/developmental progression
 - 5 day photo motor response
 - 5 day assessment of 20 morphological endpoints



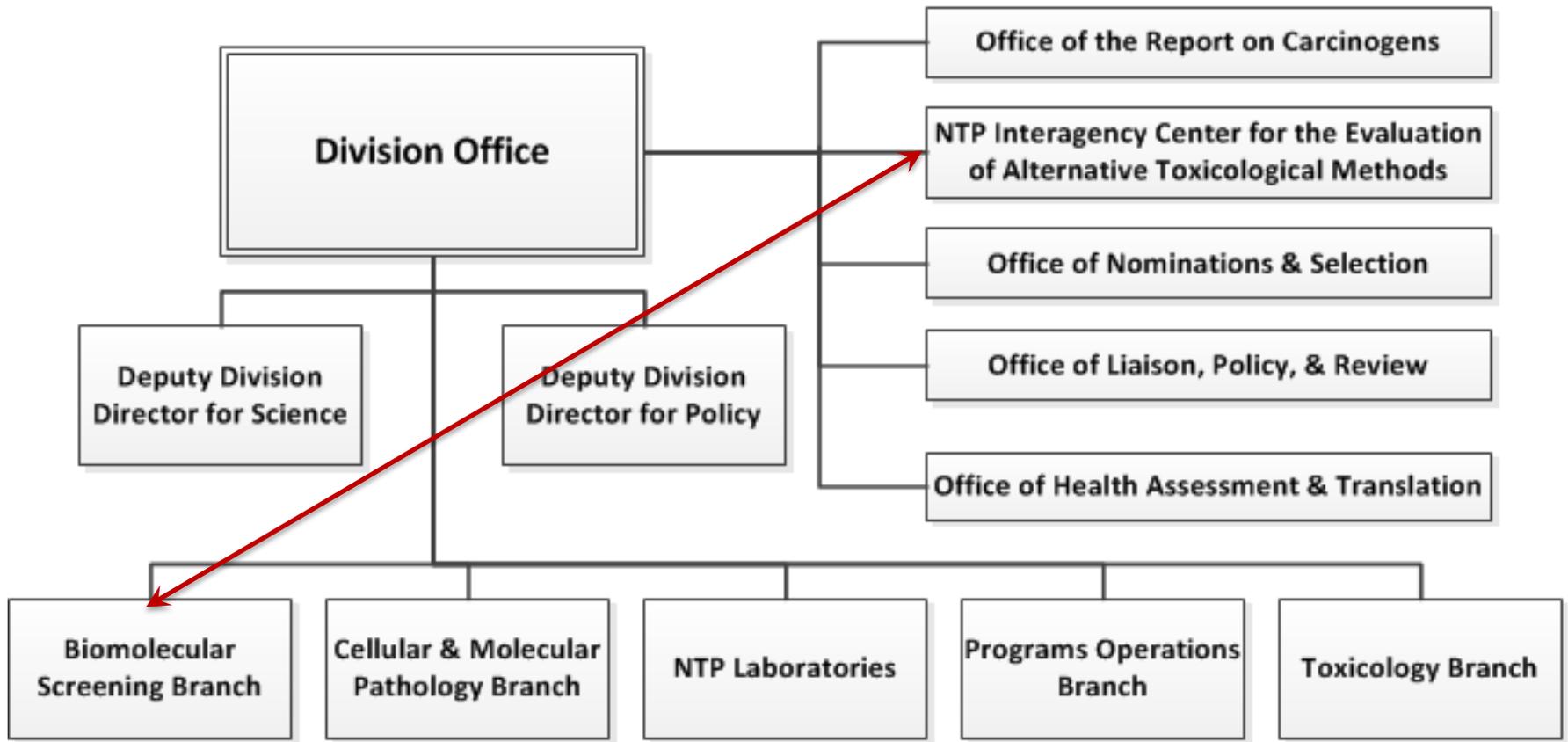
5 days

Tox21 Summary

- Phase I (2800 Chemicals x 100 Assays) is completed and data are publically available.
- Phase II (10,000 Chemicals x 30 assays/year) is ongoing, data available 1 year after assay completion.
- Phase III (follow up in Low/Med throughput systems) is ongoing, data available as projects are completed and published.
- Many challenges remain (validation, metabolism, systemic exposure, environmental exposure, complexity of mammalian physiology....)

Tox21 data belongs to the public!

NIEHS Division of the National Toxicology Program



Brief History of ICCVAM & NICEATM

- 1993 **NIH Revitalization Act**
- To establish criteria for the validation and regulatory acceptance of alternative toxicological test methods
 - To recommend a process through which scientifically valid alternative methods can be accepted for regulatory use
- 1994 Director, NIEHS, established ad hoc ICCVAM with 14 other agencies to address the mandates of the NIH Revitalization Act
- 1997 Standing ICCVAM established to achieve regulatory acceptance of valid alternative methods
- 1998 **NICEATM** created to administer and support ICCVAM
- 2000 **ICCVAM Authorization Act (PL 106-545)** established ICCVAM as a permanent interagency committee under NICEATM
- 2013 15 Years Out: Reinventing ICCVAM – EHP Editorial

What is ICCVAM?

Interagency Coordinating Committee on the Validation of Alternative Methods

- **Mission** – to facilitate development, validation, and regulatory acceptance of new and revised test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health and the environment
- **Abridged Duties:**
 - Facilitate interagency and international* collaboration to promote the development, regulatory acceptance, and use of alternative tests that encourage the reduction, refinement, or replacement of animal test methods
 - Provide guidance to test method developers
 - Consider results from expert peer reviews of alternative toxicological test methods that may be acceptable for regulatory use and make recommendations on their use to appropriate Federal Agencies

* International Cooperation on Alternative Toxicological Methods (ICATM)

* Organization for Economic Co-operation and Development (OECD)

ICCVAM:

- Is an Interagency Committee

7 Regulatory Agencies

Consumer Product Safety
Commission
Department of Agriculture
Department of the Interior
Department of Transportation
Environmental Protection Agency
Food and Drug Administration
Occupational Safety and Health
Administration

8 Research Agencies

Center for Disease Control and
Prevention
Agency for Toxic Substances and
Disease Registry
National Institute for Occupational
Safety and Health-CDC
National Institutes of Health
National Institutes of Health, Office of
the Director
National Cancer Institute
National Institute of Environmental
Health Sciences
National Library of Medicine
Department of Defense
Department of Energy



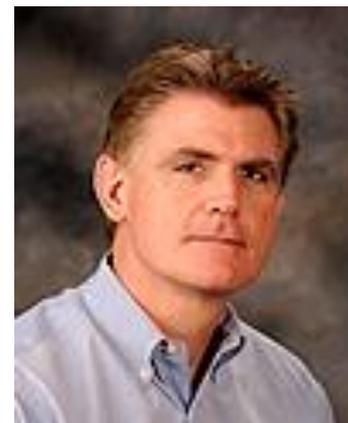
ICCVAM:

- Is an Interagency Committee
- ICCVAM does not have laboratories, dedicated FTEs, or budget
- ICCVAM does not conduct alternative test method R & D or validation studies
 - Federal agencies and other stakeholders conduct alternative test method R&D and validation studies and are encouraged to submit results to ICCVAM for evaluation

What is NICEATM?

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

- Organized as an Office under the Division of the National Toxicology Program
- Provides scientific and operational support to ICCVAM, NTP, and Tox21

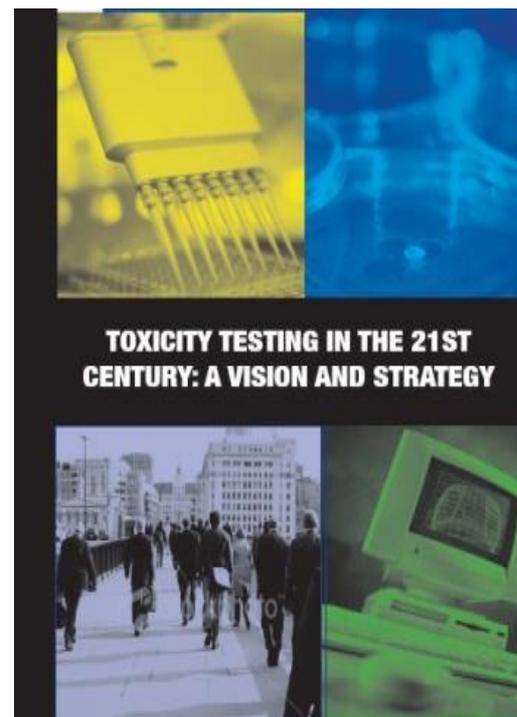


Reinventing ICCVAM: A New Vision and Direction

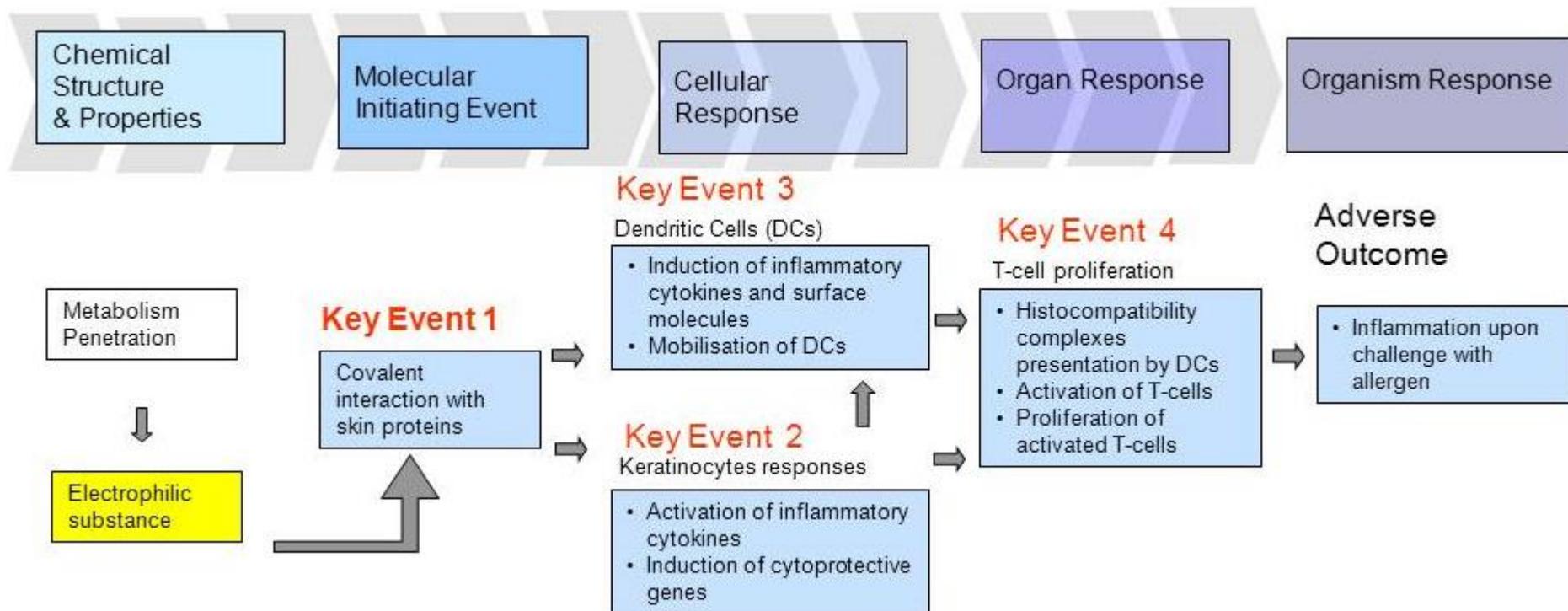
- ICCVAM priority setting and current science focus areas:
 - Member agencies are taking a more active role in priority setting and operations of the Committee
 - Streamline the number of active projects where the science has advanced
 - Maintain flexibility to reorient efforts to maximize potential progress towards use of alternative approaches
- Short-term priorities focus on identified Biologics:
 - Leptospira vaccine potency
 - Skin sensitization
 - Acute oral and dermal toxicity testing

Exploring New Paradigms

- ICCVAM has recognized the need for an evolving definition / concept of “validation” that is responsive to new technologies and on-going paradigm shifts in toxicity testing
- Better alignment with the vision laid out by the National Academy of Sciences in the 2007 NRC Report on Toxicity Testing in the 21st Century (NRC, 2007) while simultaneously fulfilling the mission of ICCVAM to implement the 3Rs of toxicity

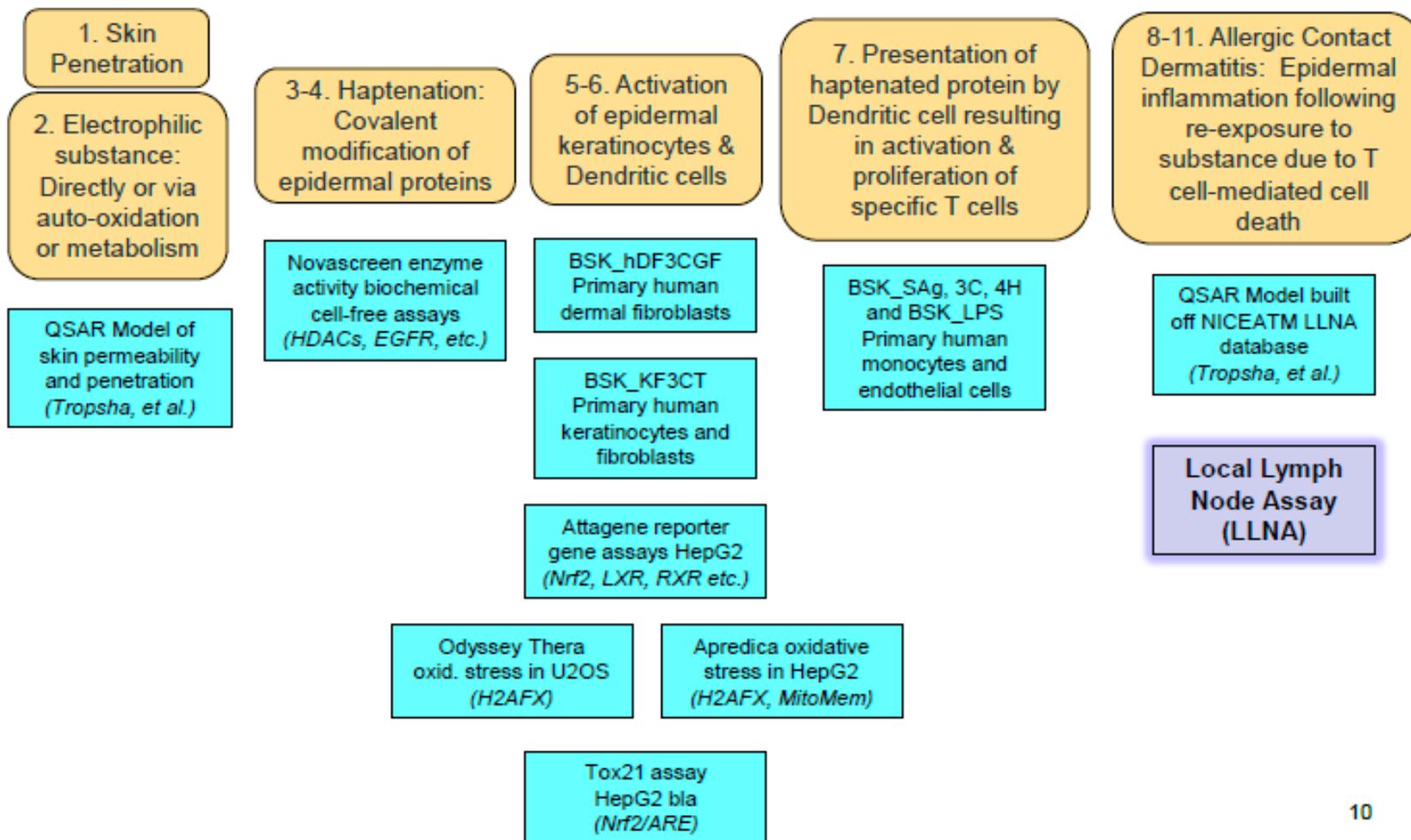


OECD Adverse Outcome Pathway (AOP) for Skin Sensitization¹



¹ For sensitization that is initiated by covalent binding to proteins.

Tox21 Assays Aligned to Key AOP Events



NICEATM Summary

- ICCVAM changing the way it does business but consistent with its mandate
- Incorporating new and emerging concepts
 - Characterizing Adverse Outcome Pathways
 - Aligning assays with key events within the AOPs
 - Mapping *in vitro* assays to AOP based on biological knowledge
- There remain many challenges in the validation of these AOP

Links

- National Toxicology Program:
 - <http://ntp.niehs.nih.gov/>
- NICEATM:
 - <http://ntp.niehs.nih.gov/?objectid=720160EB-BDB7-CEBA-F4A14A3D4AFF4B28>
- ICCVAM:
 - <http://ntp.niehs.nih.gov/?objectid=61BA4EF0-C880-CB0E-6C33B90B6612ABCF>
- Tox21:
 - <http://ntp.niehs.nih.gov/?objectid=06002ADB-F1F6-975E-73B25B4E3F2A41CB>

All work done by a Cast of Characters!

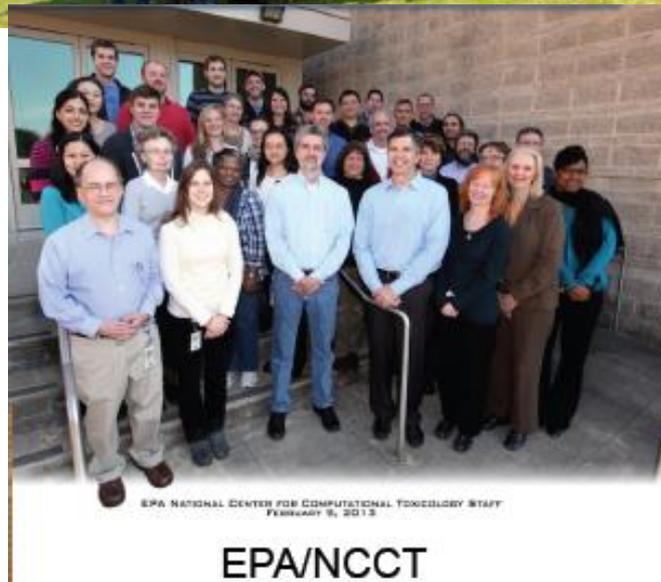


BSB, NICEATM & Contract Staff

NCATS/NCGC



DNTP



EPA NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY STAFF
FEBRUARY 9, 2013

EPA/NCCT