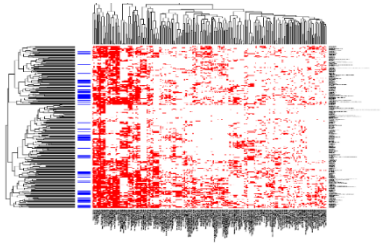
Tox21 Update: A Strategic Plan for Continued Leadership

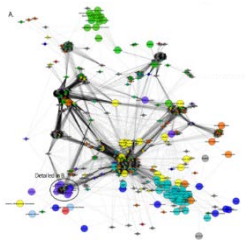
ICCVAM Public Forum
Thursday, May 24, 2018
NIH Natcher Center, Bethesda

U.S. Tox21 Federal Collaboration Goals

- Identify mechanisms of chemically-induced biological activity
- Prioritize chemicals for more extensive toxicological evaluation
- Develop more predictive models of human adverse responses

Central to this effort is the exploration of biological space through **screening chemicals** using **cell culture systems**, **non-mammalian species**, and **high-throughput methods**.



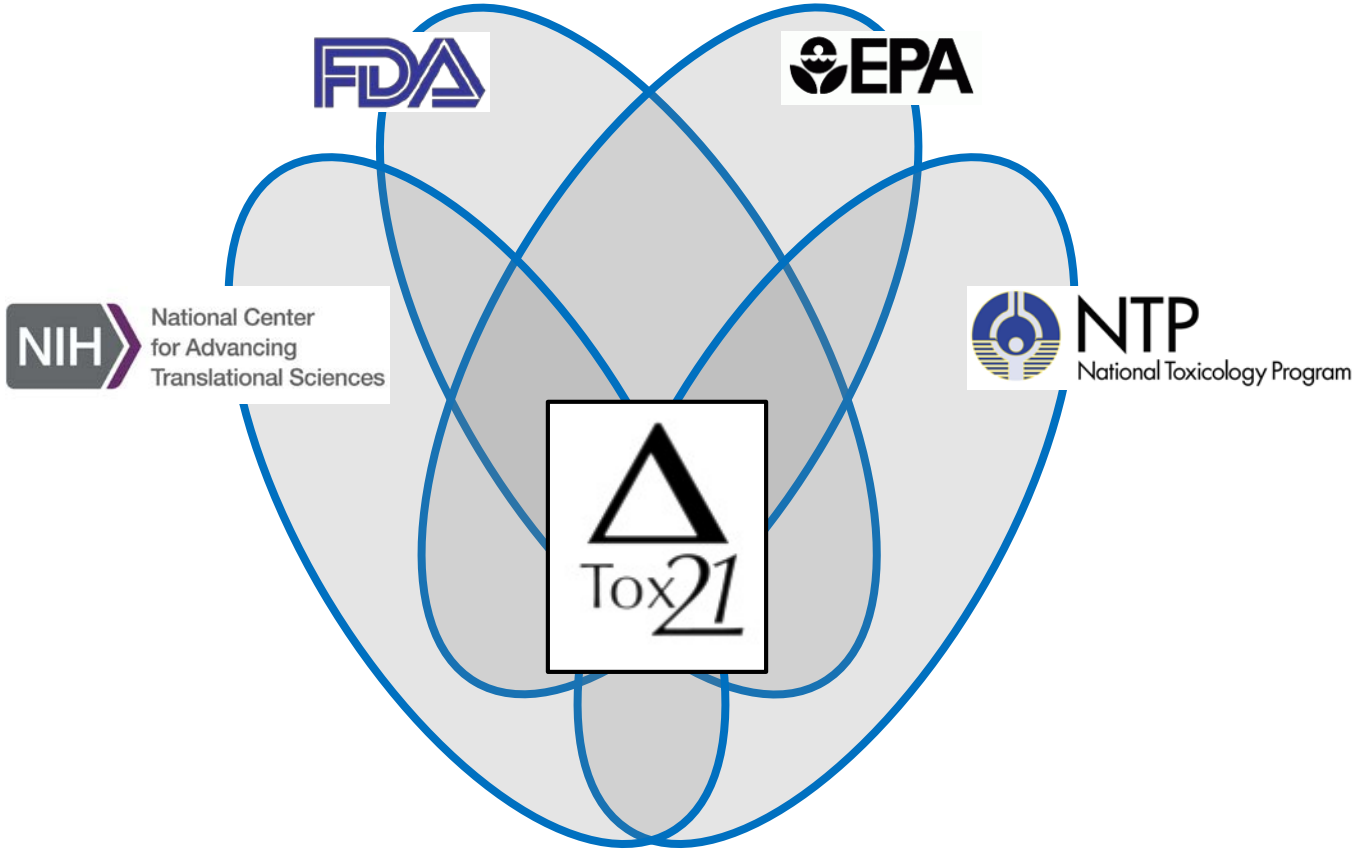


Reassessing Tox21

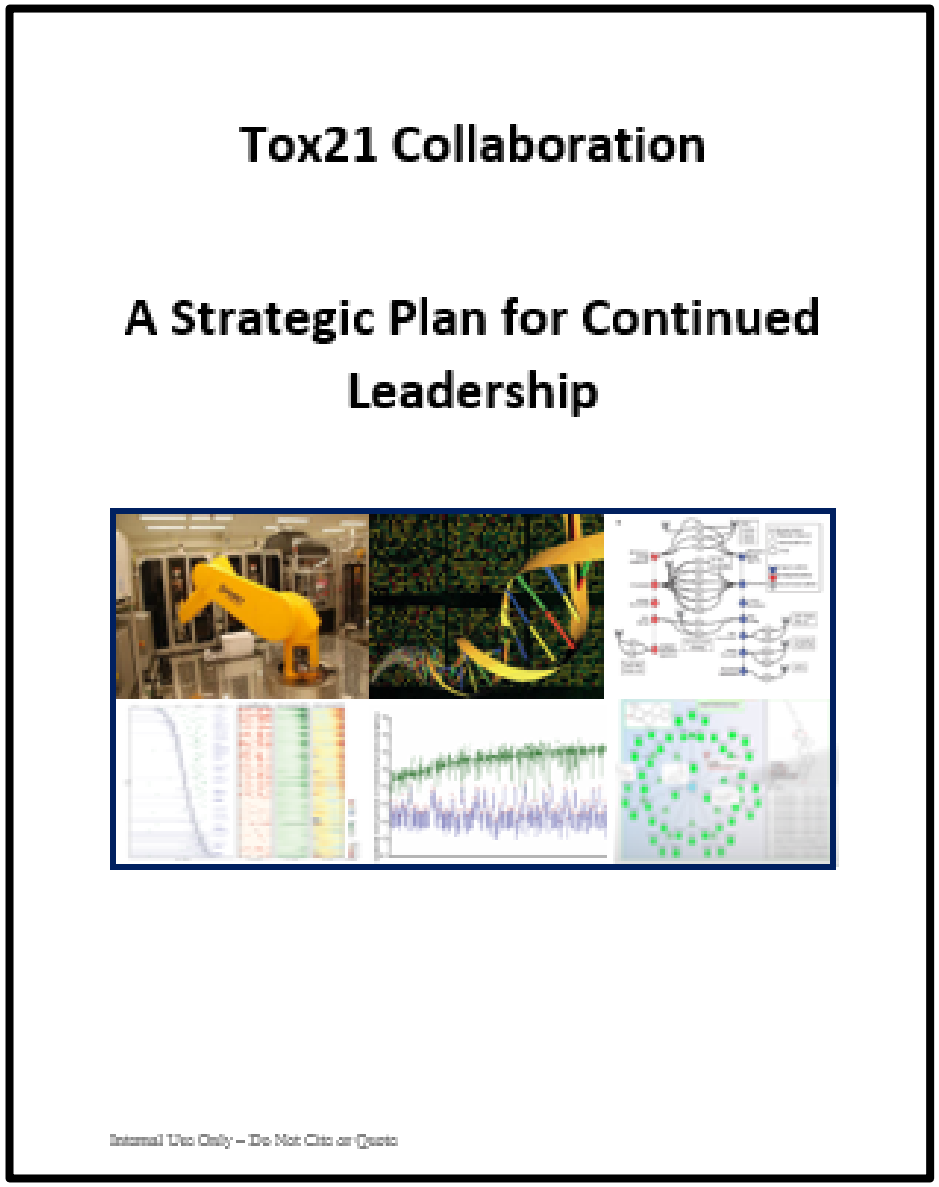
- Tox21 has been a very successful interagency collaboration
  - Thousands of chemicals tested in over 50 relevant pathways
  - Public release of millions of data points
  - Over 200 joint publications
  - Data now being used for regulatory decisions
- The decision was made to broaden the focus beyond developing and applying HTS to toxicology to developing toxicology approaches for the 21st century
- The reality is that the different partners have different missions
  - No single framework
  - No one-size-fits-all approach
- New strategic plan needs to focus on:
  - Key challenges in toxicology in the 21st century
  - Common goals that have substantial benefit in each organization regardless of mission.

US Tox21 Federal Collaboration

Understanding the Intersection of Priorities for Toxicity Testing in the 21st Century for Federal Agencies in the Tox21 Consortium



Tox21 2017 Strategic Plan



Agency Representatives

Russell S. Thomas, Ph.D.
Director, National Center for Computational Toxicology
U.S. Environmental Protection Agency

Richard S. Paules, Ph.D.
Chief, Biomedical Screening Branch
National Toxicology Program
National Institutes of Environmental Health Sciences

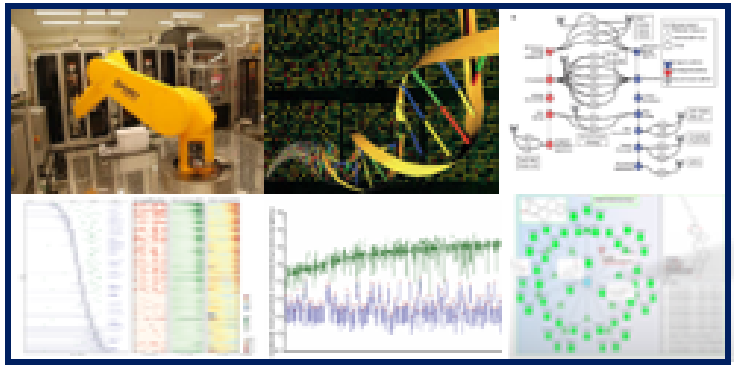
Anton Simeonov, Ph.D.
Scientific Director
National Center for Advancing Translational Sciences
National Institutes of Health

Suzanne C. Fitzpatrick, PhD, DABT, ERT
Senior Advisor for Toxicology
Senior Science Advisory Staff
Office of the Center Director
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration

Primary Goals for Tox21 in Next 5 Years

Tox21 Collaboration

A Strategic Plan for Continued Leadership



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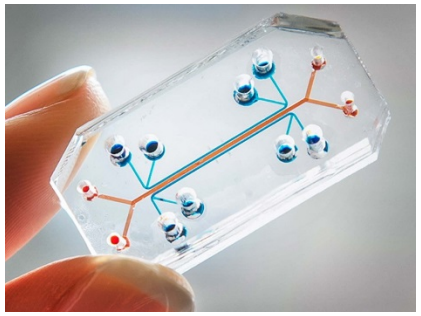
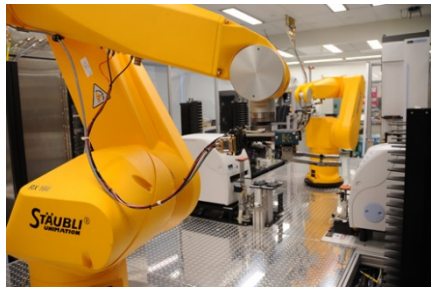
1. Develop and deploy alternative test systems that are predictive of human toxicity and dose response
2. Address key technical limitations of current *in vitro* test systems
3. Consolidate Chemical Compound Library Management
4. Curate and characterize legacy *in vivo* toxicity studies to serve as a resource for interpreting Tox21 data
5. Develop framework for efficient validation of Tox21 approaches
6. Refine and deploy *in vitro* methods for characterizing pharmacokinetics to increase predictivity and reduce uncertainty

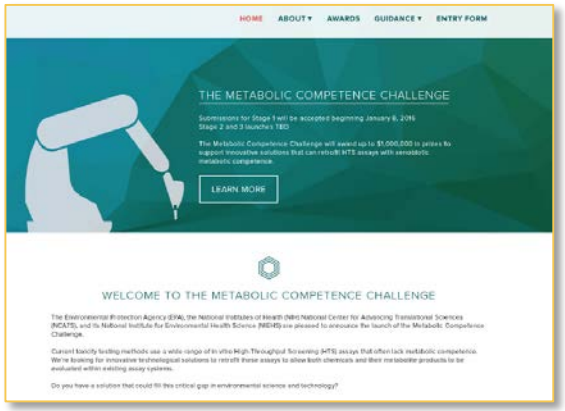
Goal 1: Predictive Alternative Test Systems



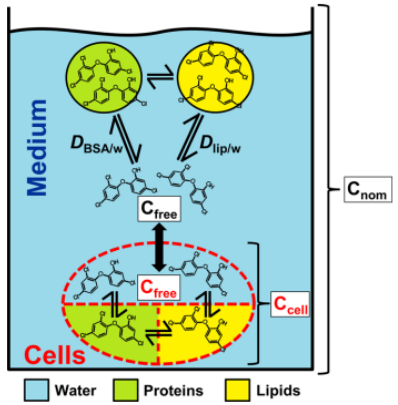


- Development and application of a screening platform that more comprehensively covers biological space
  - High-throughput transcriptomics
- Development and application of new screening platforms that capture biological complexities at tissue and organ level to bridge between the molecular perturbations identified in the high-throughput assays and phenotypic outcomes
  - Alternative species, organotypic culture models, microscale tissues, and microphysiological systems
- Incorporate variability in chemical responses from genetically diverse populations into high- and medium-throughput assays
  - iPSC cell systems
- Maintain capability for qHTS screening for evaluation of specific pathways or biological targets of relevance to partner organizations

Goal 2: Address Screening Limitations

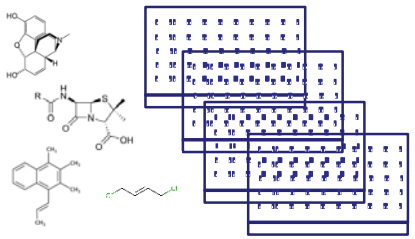


- Many technical limitations to *in vitro* chemical screening: lack of metabolism, inability to screen volatile or highly hydrophobic chemicals, and a lack of understanding of *in vitro* chemical biokinetics
- Address limitations by adapting existing methods or developing new methods and data that allow the consortium to address priority technical challenges.
- For example,
  - Retrofitting HTS assays for metabolic competence (Transform Tox Testing Challenge)
  - Develop organotypic *in vitro* models with physiologically relevant profiles of xenobiotic metabolism
  - Collate and screen water soluble library
  - Collect data and develop models for nominal vs. cellular chemical concentrations

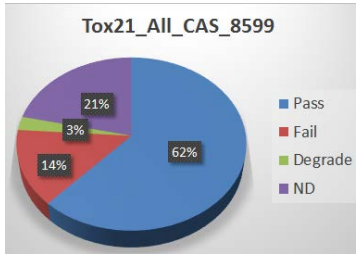


Fischer et al. 2017 PMID 25014875

Goal 3: Consolidate Chemical Compound Management



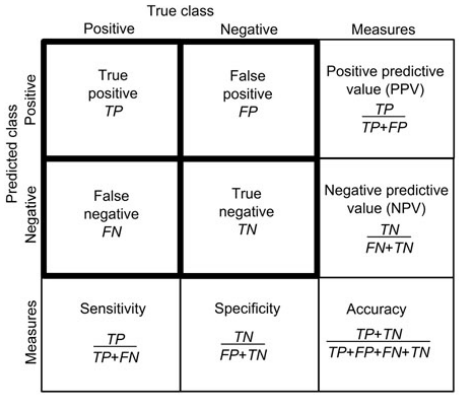
- Current Tox21 chemical library (Phase II) constructed as a one-time endeavor in 2011 and consists of 8,193 unique chemical substances
- Extend the lifespan of current library to 2019 by idling the screening for one four month period each year
- Consolidate library management under a single entity
- Any new Tox21 chemical libraries will need to support
  - Large-scale screens of the whole library by the consortium (e.g., high-throughput gene expression methods or qHTS screens)
  - Follow-up testing and evaluation of subsets of the library by the consortium or collaborators (e.g., testing in microphysiological systems)
  - Analytical validation activities using subsets of reference chemicals contained within the library



Goal 4: Curate Legacy *In Vivo* Studies

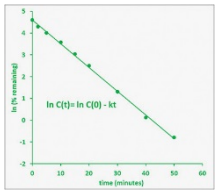
- Legacy *in vivo* toxicity studies form the basis for understanding the potential effects of chemicals and provide a resource to help interpret the *in vitro* screening efforts.
  - Link the effects observed at the molecular level to those observed at the tissue, organ, and organism level
- New efforts will need to
  - Identify and curate legacy *in vivo* toxicity studies
  - Enter data into a computable form
  - Harmonize ontologies used to characterize the toxicity studies
  - Quantify the inherent variability associated with traditional *in vivo* toxicity studies
  - Provide benchmarks for comparing with the *in vitro* testing approaches
  - Run additional *in vivo* studies to fill critical gaps

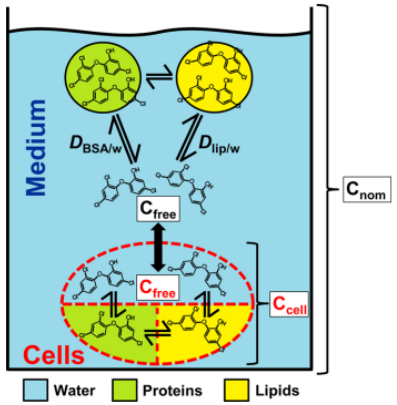
Goal 5: Validate Assays, Models, and Systems



- Traditional approach for validating *in vitro* assays takes many years to complete and has typically focused on a one-for-one replacement of a regulatory endpoint of interest
- Initial goal is to develop a framework for fit-for-purpose analytical validation of high-throughput screening data that is less time and resource intensive and is appropriate for a variety of regulatory decision contexts

Goal 6: Refine Methods for *In Vitro* PK





- High-throughput screening data and approaches typically summarized using potency and efficacy estimates based on nominal media concentrations
- Reliance on nominal concentrations does not take into account potentially important chemical and biological factors that can shift the effective concentration
- Improve methods and computational modeling approaches to better predict the relationship between target tissue concentrations and external doses of chemicals and incorporate *in vitro* biokinetics into estimates of effective potency and efficacy.

Fischer et al. 2017 PMID 25014875

Implementation – Progress to Date

- New Strategic Plan Published: *ALTEX*. 2018 Mar 8. doi: 10.14573/altex.1803011.
- Official roll-out with presentations at the SOT Annual Meeting March 2018
- Infrastructure Teams Formed and Active
  - Communications (https://tox21.gov)
  - Chemical Selection & Library
  - Assay Evaluation & Screening
- Cross Partner Projects – Initiated and Active
  - Cross-partner projects will be limited to 3 years terms
  - Driven by specific aims that match primary goals
  - Have proposed time-line with benchmarks for “Go – No Go” Decisions
  - Partner resource driven
  - Cross Partner Project members can recommend “No Go” based on science or resource priorities

Questions?

The Need for Change in Toxicology

The Challenge for Environmental Toxicology:

- There are tens of thousands of chemicals in use at significant levels for which there is little or no safety or toxicological information to evaluate the risk of adverse effects on human health from exposures.

The Problem:

- Current approaches are:
  - **Lengthy** (taking anywhere from 5 – 10 years to report the final findings)
  - **Costly** – both in terms of resources and numbers of animals used
  - **Limited** in their ability to be extrapolated to human health or to demonstrate clear human relevance

The Formation of Tox21

The Response:

- **2004 NTP** *Vision and Roadmap for the 21st Century*
- **2003-2004 EPA** *Computational Toxicology Vision*
- **2007 NRC** Report on *Toxicity Testing in the 21st Century*
- **Establishment** of the **"Tox21" Federal Collaboration** through a **Memorandum of Understanding (MOU)** with **NTP, EPA, NHGRI NIH Chemical Genomics Center (now NCATS)**
- **Signed in 2008, revised with FDA 2010, renewed 2015**

Tox21 Phase I – Proof of Principle Pilot

(2005 – 2010)

Tox21 Phase I:

- **Developed protocols** for environmental chemical screening
  - Compiled **~ 3000** compound library from **NTP** and **EPA**
  - Screened at **NCATS** using ultra high speed robot (1536 well plates)
  - 140 predominantly cell-based reporter gene assays
- Successfully **established best practices** for Tox21 **qHTS**
  - **Chemical library** acquisition and handling approaches developed
  - **Assay optimization** and performance standards developed
  - **Data analysis** approaches developed

Tox21 Phase II – Expanded Screening

(2011 – Present)

Tox21 Phase II:

- Screened using high-throughput robotics **10K compound library**
  (8,948 unique; 13,129 unique solution IDs)
  ~ 3,000 each from **NTP, EPA, NCATS (drugs)**
  - Screened 3 independent runs at 15 concentrations for each "quantitative High-Throughput Screen" (**qHTS**) assay
  - qHTS cell-based assays (> 80 assays) focused on:
    - **Nuclear receptor** activation or inhibition and **cytotoxicity**
    - **Cellular stress response** pathways and **cytotoxicity**
  - More than **70 million data points** generated in qHTS

Tox21 Cross Partner Projects

CPP 1 ***Project Title*****: Development of a High-throughput Assay to Identify 5-α Reductase Inhibitors for Orthogonal Evaluation in an Androgen-dependent Human 3D Prostate Microtissue**

- *Project Partner Leads*: **Chad Deisenroth (EPA), Joshua Harrill (EPA), Menghang Xia (NCATS)**

CPP 2 ***Project Title*****: Cell Line Selection for HT Transcriptomics**

- *Project Partner Leads*: **Nisha Sipes (NTP), Joshua Harrill (EPA), Woody Setzer (EPA)**

CPP 3 ***Project Title:*** **Profiling environmental and drug/food-related chemicals that inhibit acetylcholinesterase activity**

- *Project Partner Leads*: **Menghang Xia (NCATS), Michael Santillo (FDA)**

CPP 4 ***Project Title*****: *In vitro* disposition of Tox21 chemicals**

- *Project Partner Leads*: **Mike DeVito (NTP)**, **Katie Paul-Friedman (EPA)**

CPP 5 ***Project Title*****: Development of a Common Reference Chemical Dataset for Interpretation of High-Throughput Transcriptomic Screening Data**

- *Project Partner Leads*: **Stephen Ferguson (NTP)**, **Josh Harrill (EPA), Menghang Xia (NCATS)**

CPP 6 ***Project Title*****: Predictive Modeling of Developmental Toxicity with Human Pluripotent Stem Cells**

- *Project Partner Leads*: **Thomas Knudsen (EPA), Nicole Kleinstreuer (NTP), Annie Lumen (FDA)**

CPP 7 ***Project Title*****: Incorporating genetic susceptibility into developmental neurotoxicity (DNT) screening via population diversity**

- *Project Partner Leads*: **Alison Harrill (NTP), Mamta Behl (NTP), Syed Imam (FDA)**

CPP 8 ***Project Title*****: Performance Based Validation of Tox21 Assays**

- *Project Partner Leads*: **Keith Houck (EPA), Richard Judson (EPA), Nicole Kleinstreuer (NTP)**