EPA
United States
Environmental Protection
Agency

ToxCast and Tox21:
Overview and Future Directions

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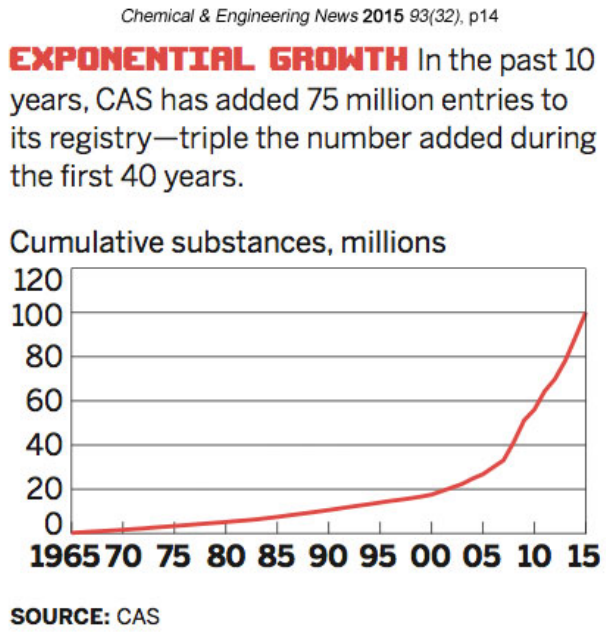
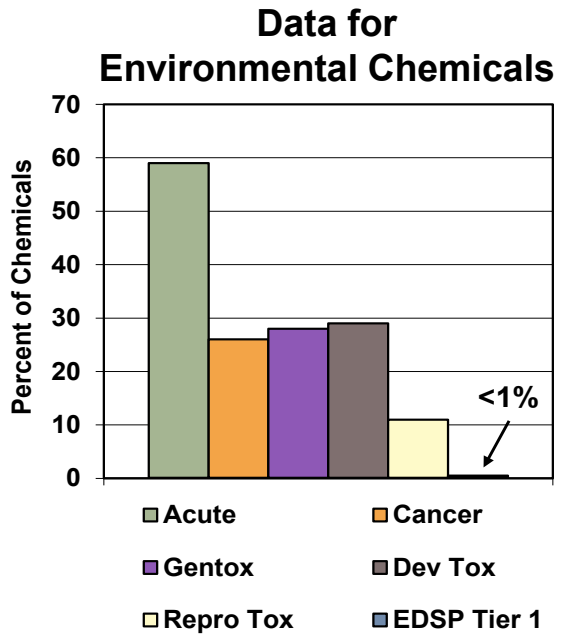
Challenge: Too Many Chemicals, too Few Resources

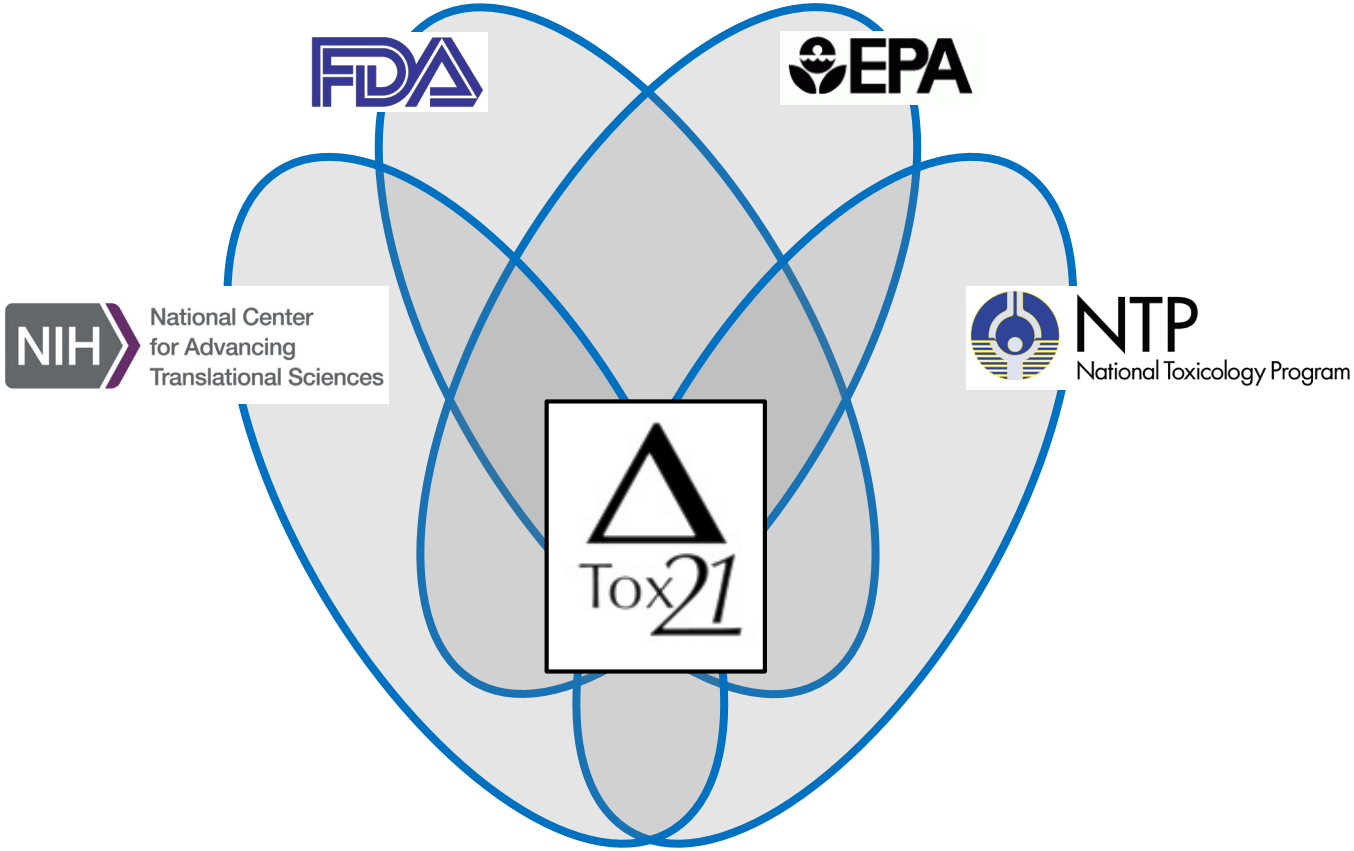
- **Timely characterization of human and ecological risk posed by thousands of existing and emerging chemicals is a critical challenge to protect public health and the environment**
  - Significant growth in the number of substances and chemicals / and the associated data
  - Nearly 60% of chemicals on various EPA lists have acute toxicity data, 30% have data on other types of toxicity





Modified from Judson *et al.*, EHP 2010

Tox21 Partnership



Tox21 Strategic and Operational Plan

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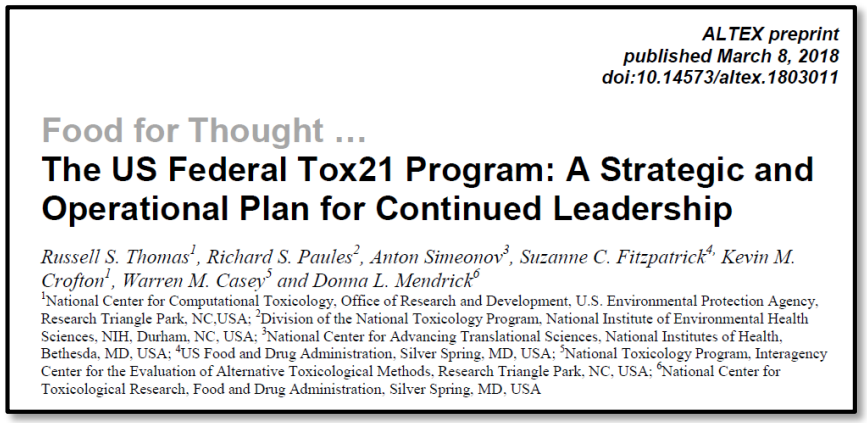

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Food for Thought …

The US Federal Tox21 Program: A Strategic and Operational Plan for Continued Leadership

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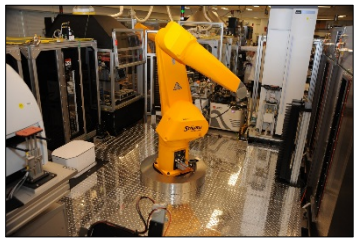
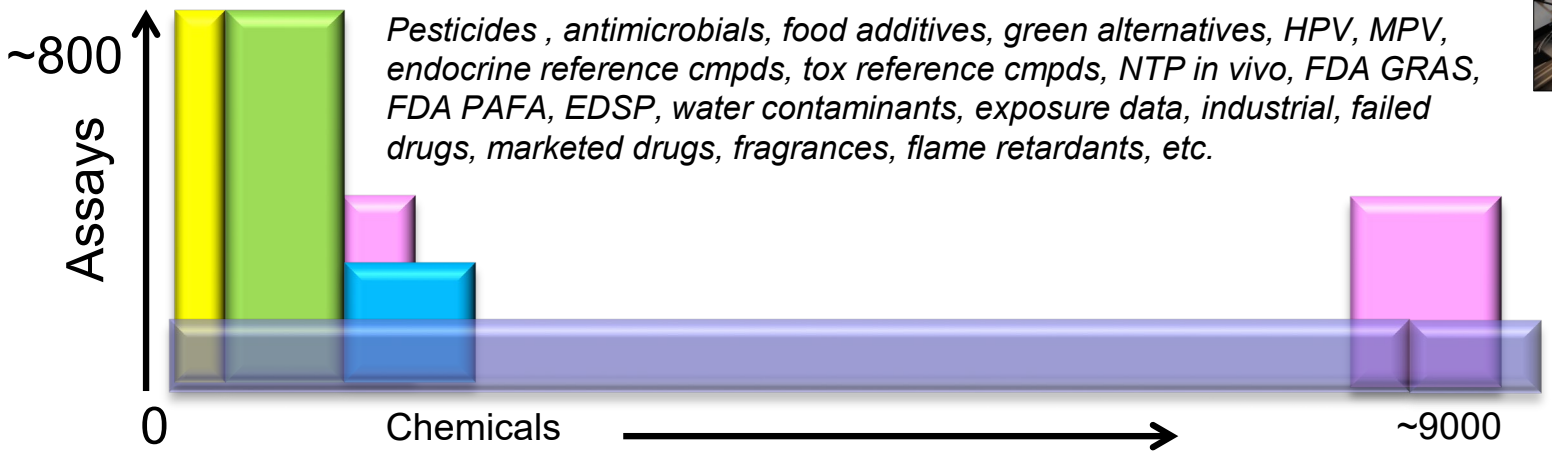
Areas of Focus

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. Curate and characterize legacy *in vivo* toxicity studies to serve as a resource for interpreting Tox21 data
4. Develop framework for efficient validation of Tox21 approaches
5. Refine and deploy *in vitro* methods for characterizing pharmacokinetics to increase predictivity and reduce uncertainty

TEN YEARS of Assay Measurements: ToxCast & Tox21

Set	Chemicals	Assays	Endpoints	Completion
ToxCast Phase I	293	~600	~700	2011
ToxCast Phase II	767	~600	~700	03/2013
ToxCast E1K	800	~50	~120	03/2013
ToxCast Phase III	~900	~300	~300	In progress
Tox21	~9000	~80	~150	In progress





Pesticides , antimicrobials, food additives, green alternatives, HPV, MPV, endocrine reference cmpds, tox reference cmpds, NTP in vivo, FDA GRAS, FDA PAFA, EDSP, water contaminants, exposure data, industrial, failed drugs, marketed drugs, fragrances, flame retardants, etc.

Infrastructure Teams and Example Cross Partner Projects

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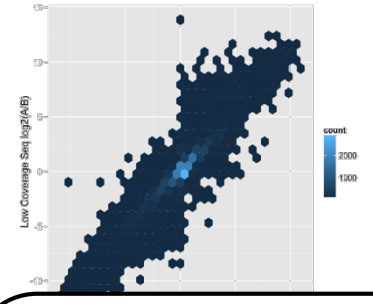
Infrastructure Teams

- **Chemical Library Management**
- **Communications**
- **Assay Evaluation and Screening**

Cross-Partner Projects

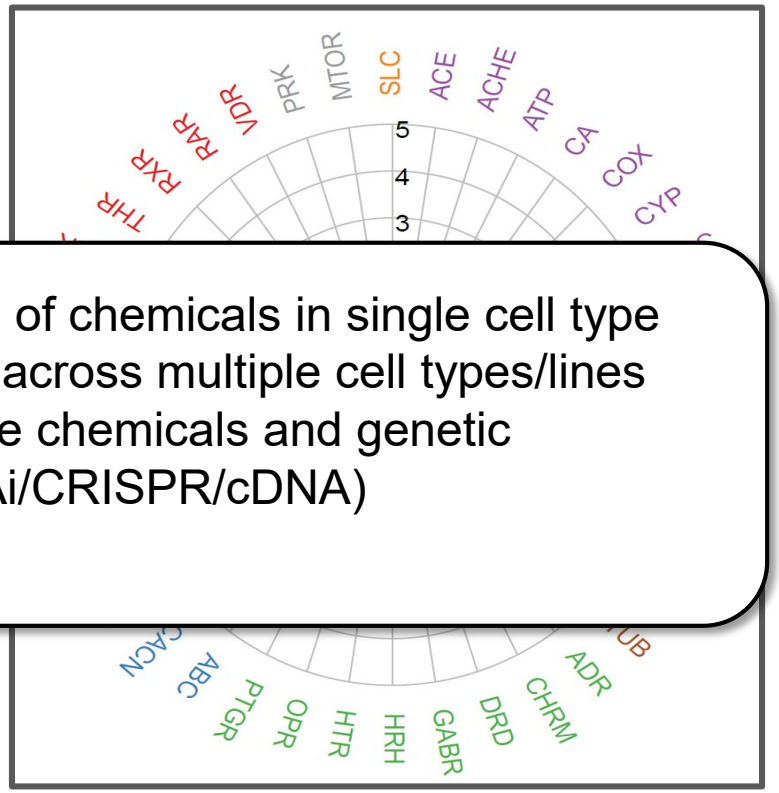
- ***In Vitro* Disposition of Tox21 Chemicals**
- Performance Based Validation of Tox21 Assays
- **Development of a Reference Chemical Dataset for Interpretation of High-Throughput Transcriptomic Screening Data**
- Incorporating Genetic Susceptibility into Developmental Neurotoxicity Screening
- **Cell Line Selection for High-Throughput Transcriptomic Screening**
- Predictive Modeling of Developmental Toxicity with Human Pluripotent Stem Cells
- Development of a High-Throughput Assay to Identify Acetylcholinesterase Inhibitors

Transcriptomics Data will Deliver Terabytes of Data for Analysis



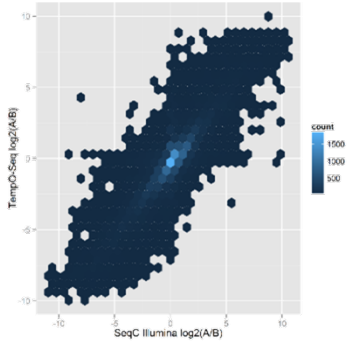
TruSeq
r^2 0.74

MOA Analysis Pipeline



Currently capable of assigning to >40 MOAs based on transcriptional responses

- Large scale screen of chemicals in single cell type
- Additional screens across multiple cell types/lines
- Additional reference chemicals and genetic perturbations (RNAi/CRISPR/cDNA)



Low Coverage
r^2 0.83

Addressing Metabolic Competence in HTS

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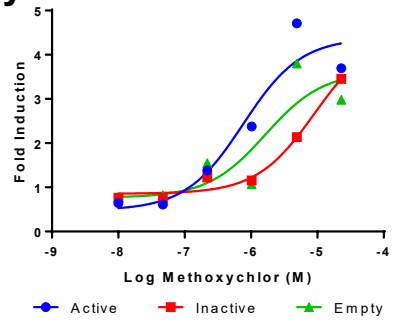
"Extracellular" Approach

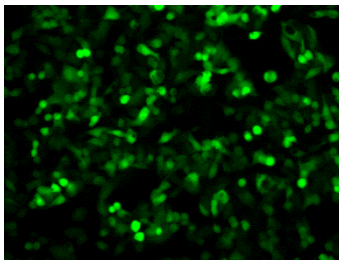
Chemicals metabolism in the media or buffer of cell-based and cell-free assays

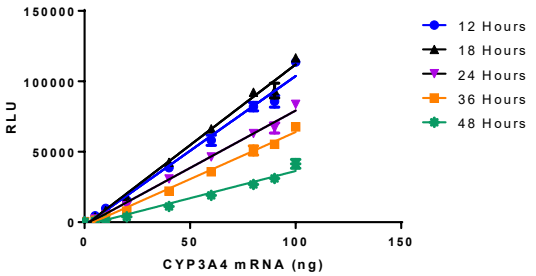


More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Approach

Capable of metabolizing chemicals inside the cell in cell-based assays





More closely models effects of target tissue metabolism

Integrated approach to model *in vivo* metabolic bioactivation and detoxification

Retrofitting Metabolism to an Estrogen Receptor Transactivation Assay

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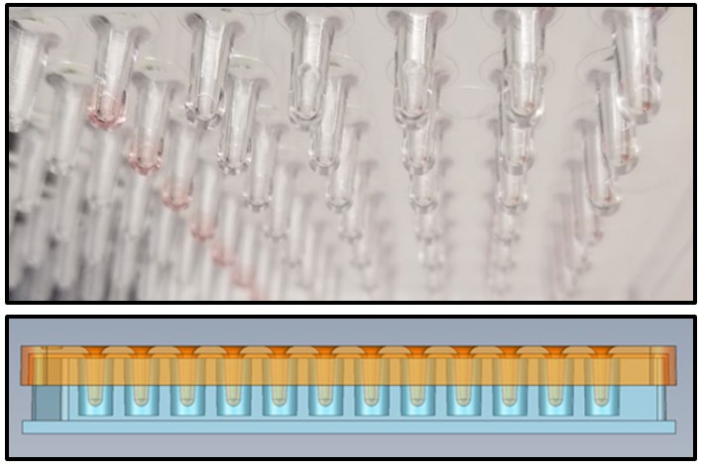
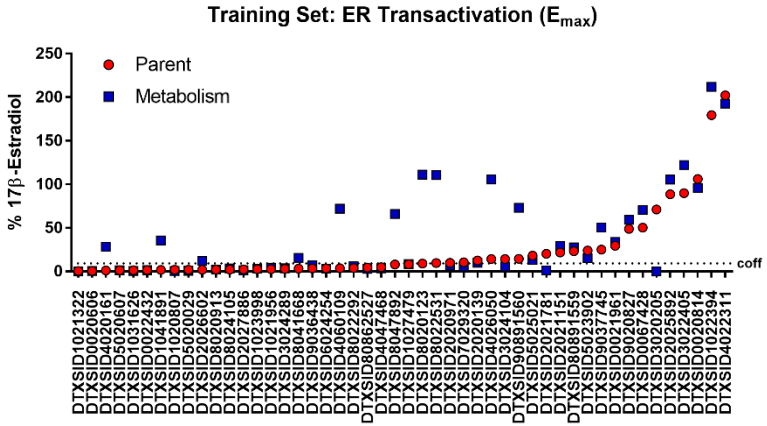


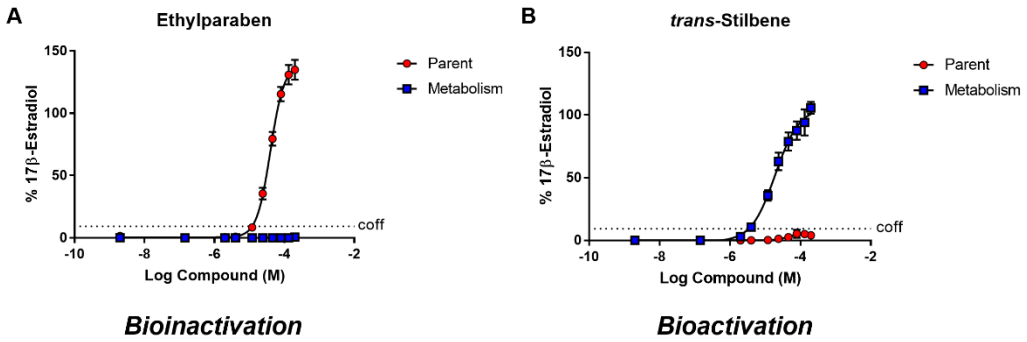
AIME Method: S9 fraction immobilization in alginate microspheres on 96- or 384-well peg lids

		Metabolism	
		Neg	Pos
NRS	Neg	0.91	0.89
	Pos	0.91	0.71
		Z'	

The AIME method applied to the ERTA assay provides a robust screening window



Metabolism impacts estrogen receptor bioactivity in a subset of compounds from Pinto et al., 2016



Bioinactivation *Bioactivation*

Parallel evaluation of parent compound and biotransformed metabolites

- **Retrofitting Metabolism: AIME method suitable for biochemical- and cell-based HTS assays**
- **Screening Throughput: Adaptable to 96- and 384-well screening platforms**
- **Regulatory Relevance: Integration of phase I liver metabolism for hazard identification of parent and metabolite endocrine activity**

D. DeGroot, C. Deisenroth

Engineering an Organotypic 3D Model of the Human Thyroid

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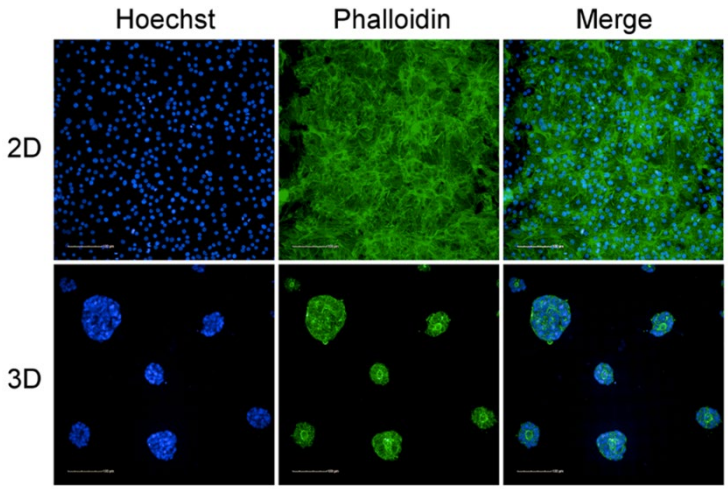
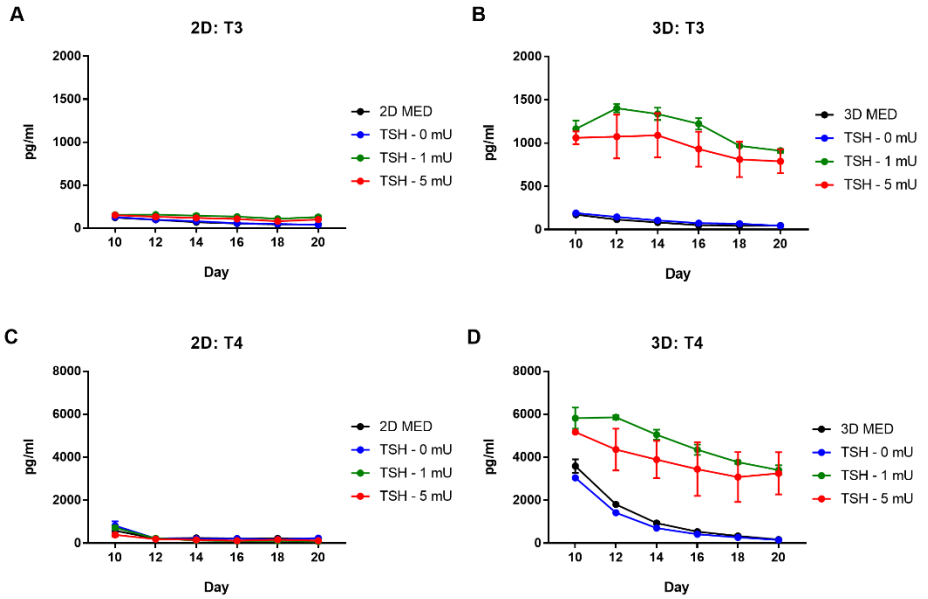
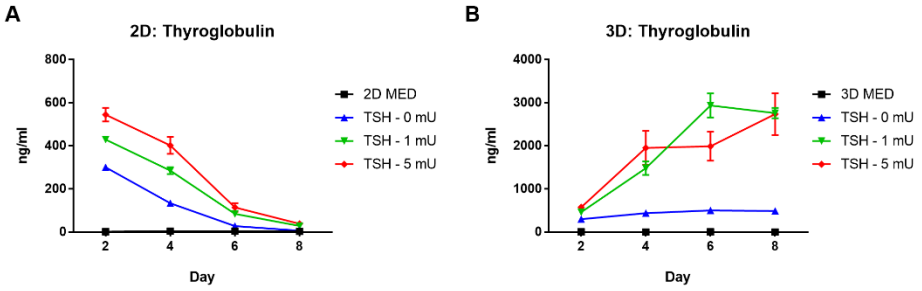


Primary human thyrocytes self-assemble into microtissues in a 3D culture model



Thyroid hormone is synthesized and secreted over time in a 3D culture model

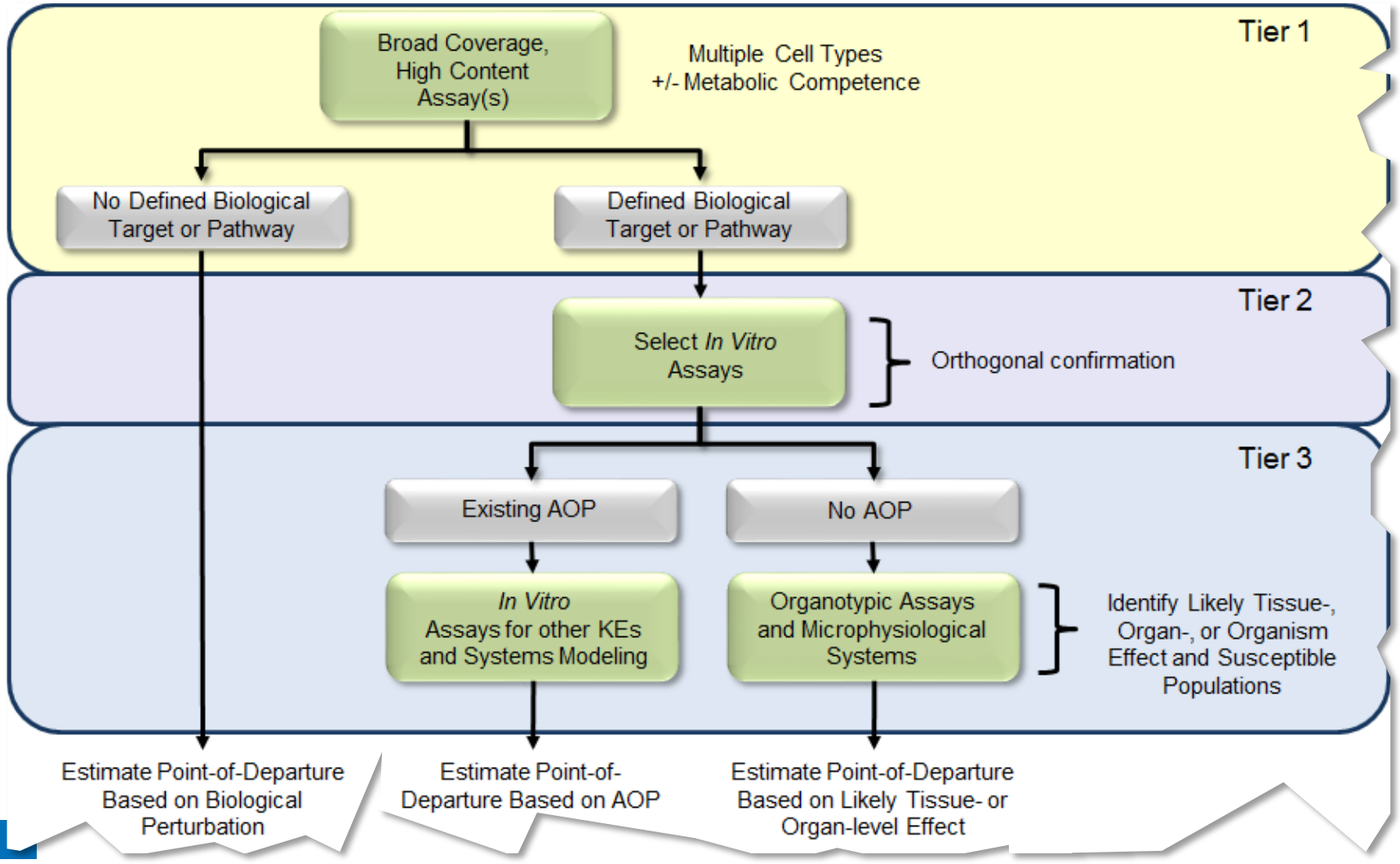


Thyroglobulin secretion is enhanced over time in a 3D culture model

- **MIEs: All molecular components of the system are present in a physiological balance**
- **Key Event: Experimental evaluation of thyroid hormone synthesis perturbation**
- **Screening Throughput: Amenable to medium-throughput screening for dozens of HTS prioritized hits**
- **Regulatory Relevance: Interpretation of chemical mediated effects on hormone output in the human thyroid gland**

C. Deisenroth

Framework for Integrating NAMs Based on Chemical Characteristics



HTS Data in Context

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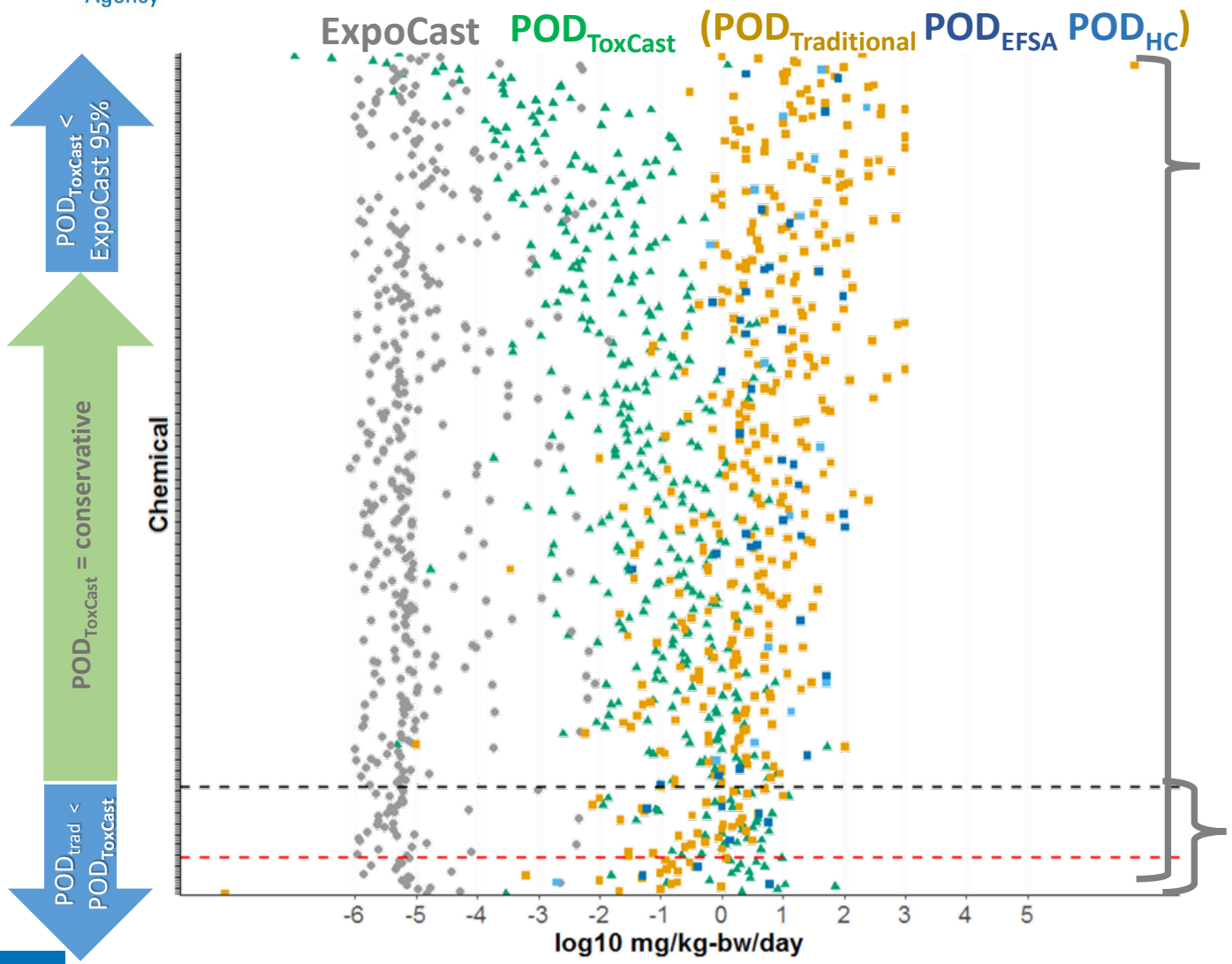

Total =

380 chemicals

httk, ToxCast data, and POD value(s) currently available

For ~91.3% of the chemicals, $POD_{ToxCast}$ was conservative.

(~100-fold with human HTTK

~50-fold with rat HTTK)

Missing an important component of biology?

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA

Integration of Data for Environmental Decision Making

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ADME
- In vitro dosimetry data
- In vitro High Throughput Toxicokinetics (HTTK) data
- Physiologically Based Pharmacokinetic (PBPK) models

Exposure
- National Health and Nutrition Examination Survey (NHANES) biomonitoring
- Functional Use Categories
- Non-targeted Screening
- Models

Human Hazard
- Toxicity Reference (ToxRef) Database (legacy in vivo data)
- Toxicity Value (ToxVal) Database
- Toxicity Forecasting (ToxCast) data
- Many other models

Chemistry Data
- Laboratory Information Management System (LIMS) chemical library
- Distributed Structure Searchable (DSSTox) Library
- Physchem prop modeling

Other Data
- European Chemicals Agency (ECHA)
- Office of Pollution Prevention and Toxics (OPPT)
- Health Canada (HC)

Data for Chemical Safety Decision

Data Delivery

- Dashboards

- Chemical Prioritization
- Screening level Risk
- User-specific workflows

Working With Regulatory Partners to Incorporate Data into Decision Making

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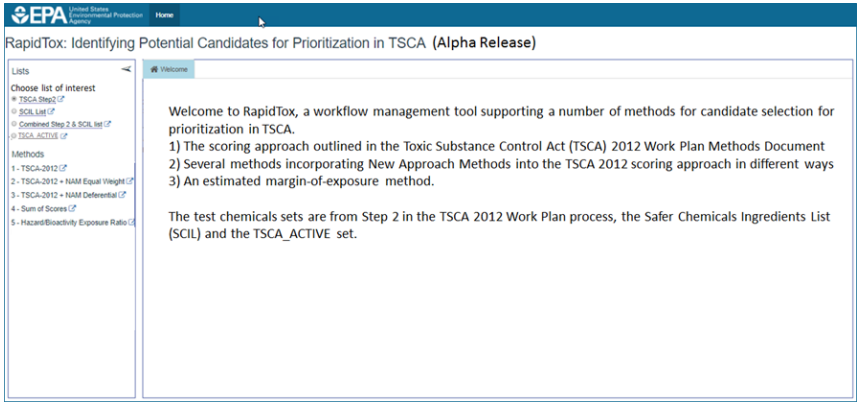
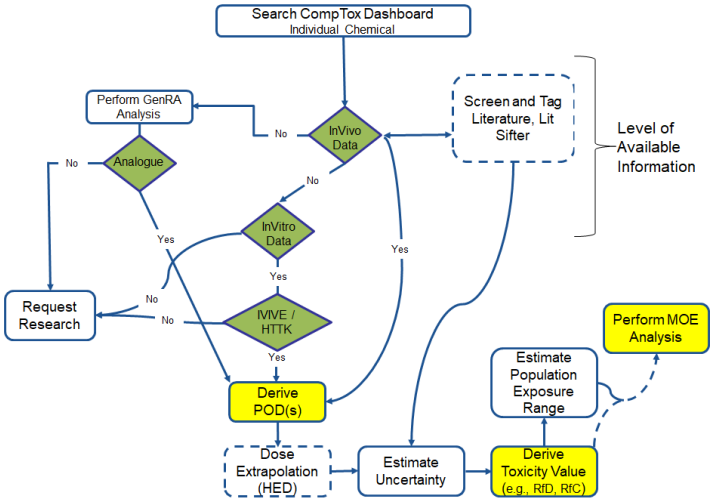
RapidTox Prioritization Workflow



- Decision support tool for prioritization with dashboard interface
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure
- Transparent and interactive
- Working with EPA Office of Chemical Safety and Pollution Prevention

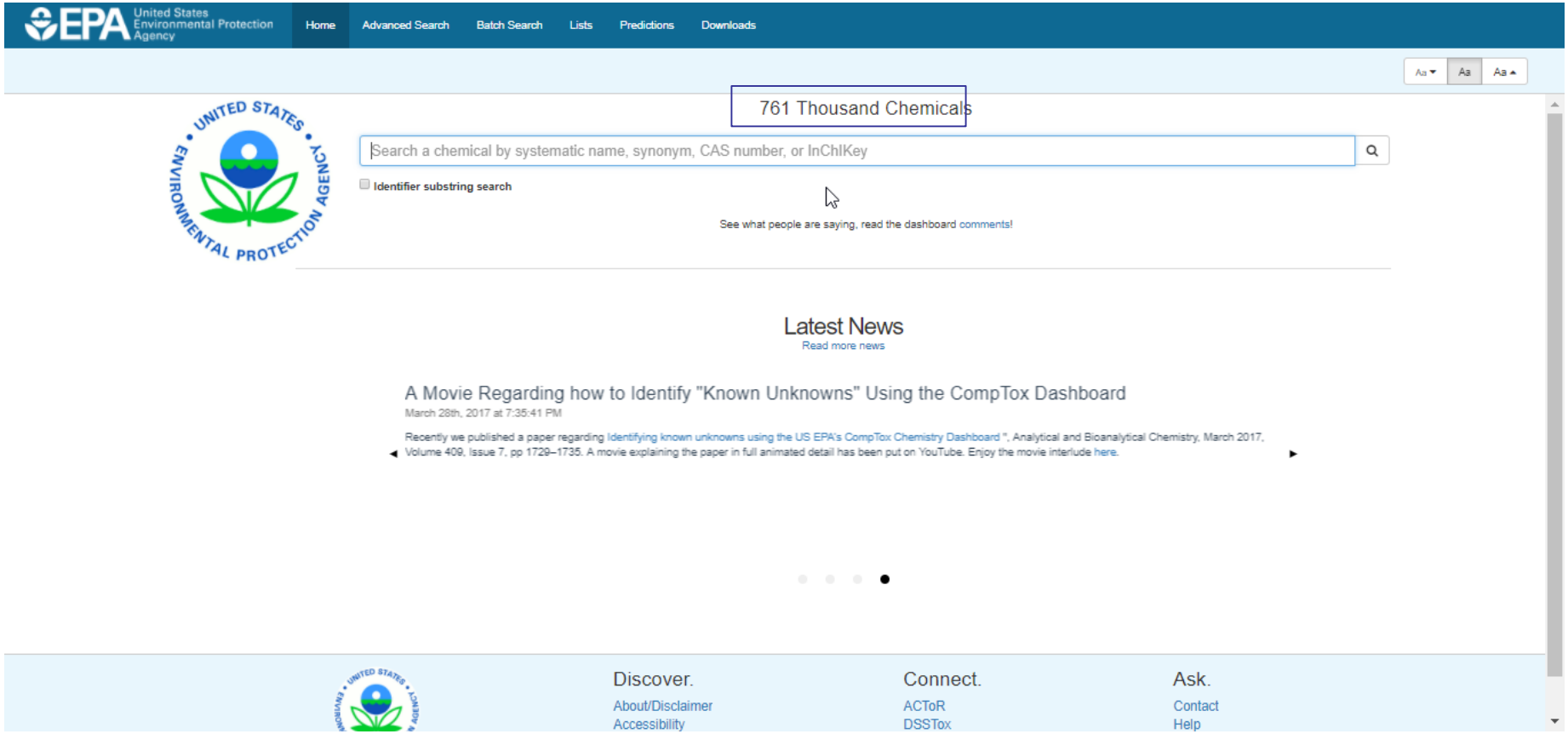
RapidTox Screening Level Assessment Workflow



- Decision support tool for screening level chemical assessments
- Integrate a range of traditional and NAM data related to hazard, and exposure
- Transparent and interactive
- Derive screening level points of departure and margins-of-exposure
- Working with EPA Office of Land and Emergency Management

CompTox Chemicals Dashboard

https://comptox.epa.gov/dashboard



EPA's CompTox Chemicals Dashboard

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A publicly accessible website delivering:

- ~760,000 chemicals with related property data
- Experimental and predicted physicochemical property data
- Integration to "biological assay data" for 1000's of chemicals (includes ToxCast and Tox21)
- Information regarding consumer products containing chemicals
- Generalized Read-Across (GenRA) module
- Links to other agency websites and public data resources
- "Literature" searches for chemicals using public resources
- "Batch searching" for thousands of chemicals
- Downloadable Open Data for reuse and repurposing
- Many features (only highlighting a few)
- Access to multiple tools (direct data interpolation and predictive) for multiple disciplines

Summary

- **Continue data generation through Tox21 and ToxCast**
  - Address assay limitations and uncertainties
  - Refine approaches to estimate human and ecological effects
- **Develop a "one-stop-shop" for data as an integration node for environmental chemical data to support EPA and partner decision making:**
  - Centralized location for relevant chemical safety data:
    - Chemistry, exposure, hazard, dosimetry
  - Combination of existing data and predictive models
  - Publicly accessible, periodically updated, curated
  - Develop user-specific workflows
- **Ease of access to data results in efficiency and accelerates chemical risk assessment**

Acknowledgements and Questions

Tox21 Colleagues:

- FDA
- NCATS
- NTP

EPA Colleagues:

- NERL
- NHEERL
- NCEA

Collaborative Partners:

- Unilever
- A*STAR
- ECHA
- EFSA
- Health Canada

EPA's National Center for Computational Toxicology