Tox 21
Developmental Toxicology

Susan Makris
US EPA, Office of Research and Development
National Center for Environmental Assessment
Washington, DC

The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the US Environmental Protection Agency.
Origins of Tox21

“Toxicity Testing in the 21st Century”

- Limitations in the current testing paradigm
  - Historical increase in:
    - Number of tests
    - Cost of testing
    - Use of laboratory animals
    - Time to develop and review data
  - Difficult to apply to risk assessment due to inability to fully address complex issues such as:
    - Life stage sensitivity
    - Mixtures and cumulative exposures
    - Varying exposure scenarios
    - Understanding of mechanism of toxicity and implications in assessing dose-response
    - Characterization of uncertainty

NRC recommended a transformation in toxicity testing and risk assessment that focuses on toxicity pathways

NRC, 2007
Formation of U.S. Tox21 Federal Partnership - 2008

• National Institute of Environmental Health Sciences (NIEHS)
• National Center for Advancing Translational Sciences (NCATS)
• U.S. Environmental Protection Agency (EPA)
• U.S. Food and Drug Administration (FDA)

A Successful Interagency Collaboration:
• Thousands of chemicals tested in 70 assays and over 50 relevant pathways (primarily HTP testing)
• Public release of millions of data points
• 200 peer reviewed articles in 56 journals
• Data now being used for regulatory decisions

https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21

MOU Signed February, 2008; Revised July, 2010
Tox21 Strategic and Operational Plan - 2018

Areas of Focus

- Developing and deploying alternative test systems that are predictive of human toxicity and dose response
- Addressing key technical limitations of current high throughput screening systems
- Consolidating chemical library management and developing more focused libraries
- Curating and characterizing legacy animal toxicity studies for continued comparison to high-throughput screening results
- Validating high-throughput assays, integrated assay batteries, computational models, 3-D organ-like model systems, and other emerging Tox21 approaches
- Refining and deploying high-throughput methods for characterizing pharmacokinetics to better predict the relationship between target tissue concentrations and external doses of chemicals.

Agency-Specific Roadmaps Published

FDA Predictive Toxicology Roadmap
(Dec 2017)

- Reliable interpretation and application for product development and/or regulatory decisions
- Clear context of use
- Importance of multi-sector partnerships and collaborations to identify, develop, validate, and integrate assays into risk assessment


ICCVAM Strategic Roadmap
(Jan 2018)

https://ntp.niehs.nih.gov/go/natl-strategy
Tox 21 Collaborative Projects

- Cell Line Selection for High-throughput Transcriptomics (Sipes, Harrill, Setzer)
- Acetylcholinesterase (AChE) Inhibitors Screening (Xia, Santillo)
- In Vitro Disposition of Tox21 Chemicals (DeVito, Friedman)
- High-Throughput (Ferguson, Harrill, Xia)
- Predictive Modeling of Developmental Toxicity with Human Pluripotent Stem Cells (Knudsen, Kleinstreuer, Lumen)
- Incorporating Genetic Susceptibility into Developmental Neurotoxicity Screening via Population Diversity (Harrill, Behl)
- Performance Based Validation of Tox21 Assays (Houck, Judson, Kleinstreuer)
- Retrofitting Existing Tox21 HTS Assays with Metabolic Capability (Xia, Witt, Simmons)

https://www.epa.gov/chemical-research/tox21-cross-program-projects
An EPA Statutory Mandate for Chemical Testing

The 1976 Toxic Substances Control Act (TSCA) was amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act in 2016:

- new requirements and deadlines for actions related to the regulation of new and existing chemical substances.
- new subsection under Section 4 (Testing of Chemical Substances and Mixtures); particularly, Section 4 (h) entitled Reduction of Testing on Vertebrates

4(h)(2) - **Implementation of Alternative Testing Methods**—To promote the development and timely incorporation of new scientifically valid test methods and strategies that are not based on vertebrate animals, the Administrator **shall**—

4(h)(2)(A) - “not later than 2 years after the date of enactment....develop a strategic plan to **promote the development and implementation of alternative test methods and strategies** to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment of chemical substances or mixtures through, …”

### Federal Register Notice, June 2015

“Endocrine Disruptor Screening Program: Use of High Throughput Assays and Computational Tools”


<table>
<thead>
<tr>
<th>EDSP Tier 1 Battery of Assays</th>
<th>Model Alternative Development</th>
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<tbody>
<tr>
<td>Estrogen Receptor (ER) Binding</td>
<td>ER Model</td>
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<td>Fish Short Term Reproduction</td>
<td>ER, AR &amp; STR Models</td>
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<td>Amphibian Metamorphosis</td>
<td>THY Model</td>
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Issues Typically Considered in the Evaluation and Interpretation of Regulatory Developmental Toxicity Data

- Bioavailability in the maternal animal (systemic exposure)
- Route(s) of exposure of the agent in vivo and any route-related differences in metabolism
- Ability of the agent or active metabolite to cross the placenta: fetal exposure
- Potential for the agent to cause death, altered growth structural abnormalities or functional effects in the offspring; effects of maternal toxicity or stress as a mitigating factor
- Life stage sensitivity: quantitative or qualitative effects
- Differences in toxicokinetic parameters (absorption, distribution, metabolism, storage, or excretion) in the fetus compared to adults
- Differences in toxicodynamic parameters in the fetus compared to adults: different targets or level of response
- Windows of susceptibility in the developing organism
- Dose-response (NOAELs/LOAELs/BMDs)
EPA IRIS Systematic Review Process

IRIS = Integrated Risk Information System

Examples of chemicals with mechanistic information integrated into deprotox characterization:

- TCE: putative AOP for disruption of valvulo-septal morphogenesis was integrated into the WOE evaluation supporting TCE-related cardiac malformations (Makris et al., 2016)
- Phthalates: testosterone reduction during critical period in late gestation resulted in malformations of the male reproductive system
Developmental Toxicity Ontology

- Ontology = a way to classify terms, how they relate to broader concepts and their interrelationships

- A developmental toxicity ontology can span multiple levels of organization and is based on:
  - Knowledge of developmental biology
  - Mode of action/ adverse outcome pathways

- Challenges
  - Role of potency (separating adaptive vs. adverse responses)
  - Maternal toxicity as a driver or confounder of in vivo responses
  - Importance of developmental stage susceptibility

Building a developmental toxicity ontology

Nancy Baker1 | Alan Boobis2 | Lyle Burgoon3 | Edward Carney4a | Richard Currie5 | Ellen Fritsche6 | Thomas Knudsen7 | Madeleine Laffont8 | Aldert H. Piersma9 | Alan Poole9 | Steffen Schneider10 | George Daston11

Baker et al. 2018
Moving Toward a ‘Virtual Embryo’

- Ahir et al. (MS in preparation)

- Zurlinden/Saili et al. (on-going)
- Hunter et al. (on-going)
- (Future research)
IATA: Computational Synthesis and Integration

HTS – high throughput screen
HTK – high throughput kinetics
SAR – structure activity relationship
MPS – microphysiological systems
AOP – adverse outcome pathway
ABM – agent based model

bioactivity profiles
kinetics & dosimetry
computational chemistry
microphysiological systems
computational dynamics
pathways & networks
Performance Check: In Vitro to In Vivo

- ToxCastSTM anchored to 42 DevTox benchmark compounds aimed at assessing alternative models\(^1\) and having information on pregnancy risk.

- Overall accuracy of 78.6% (0.65 sensitivity, 1.00 specificity, MCC = 0.647).

- Consistent with Palmer et al. (2013) pharma-trained model 77% accuracy (0.57 sensitivity, 1.00 specificity).

\(^1\) Genschow et al. 2002; West et al. 2010; Daston et al. 2014; Augustine-Rauch et al. 2016; Wise et al. 2016

\(\text{NCCT, manuscript in preparation}\)
Performance Check: In Silico to In Vitro

Computational prediction (cNVU)

Biomimetic reconstruction (hNVU)

Critical concentration:
- predicted in silico \(~0.5\ \mu M\)
- observed in vitro \(~0.3\ \mu M\)

Todd Zurlinden, Kate Salli - NCCT

Murphy, W Daly, G Kaushick – U Wisconsin (HMAPS)
Performance Check: Cross Assays

Introduction

ToxCast chemicals were profiled for developmental toxicity potential in two embryonic stem cell assays and processed in the ToxCast data analysis pipeline (tcp)

1] human pluripotent h9 stem cell-based [hESC] assay
2] mouse differentiating embryonic stem cell (mESC) adherent assay [Harrair et al. 2011. Brocod Tox]

hESC (pluripotent) assay

mESC (differentiation) assay

Effects of ToxCast chemicals on mESC endpoints

MCC is a measure of model performance: MCC = (TP x TN) / sqrt((TP+FN) x (TP+FP) x (TN+FP) x (TN+FN))

To gain insight into the biological pathways and targets associated with the stem cell responses, machine-learning was used to mine correlations to 337 enzymatic and receptor signaling assays in the ToxCast NovaScreen database (NV5). Each NV5 assay was enriched for an AC50 correlation against a hESC-positive or hESC-negative outcome, weighted by an assay-specific logistic regression model, processed through the Reactome HSA Pathway Browser (v3.5, database release 63), and independently enriched for significant pathway associations with the ClueGO plug-in to Cytoscape v3.4 (Bonferroni-corrected p < 0.05, minimum 3 genes for a pathway identifier).

ToxCast modeling framework

mESC modeling framework

Example: Methotrexate (TI = 0.059 μM)

• ↓ ornithine/cysteine in the day 3 secretome predicts μM threshold for teratogenicity [TI] [12];
• point of departure for cell viability equates to 11% reduction in cell number.

• It was recovered for 181 chemicals (17% of 1065 tested); model performance used 42 benchmark compounds and ToxRefDB prenatal studies in rats and/or rabbits [DEE < 200 μg/kg/day] [manuscript in preparation]

NESC model performance... sensitivity filter applied to the in vivo anchor

MCC results:

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MCC modifiability for hESC

MCC modifiability for mESC

Profiling the ToxCast library with pluripotent embryonic stem cell assays

Thomas B. Knudsen1, Todd J. Zurlinden1, and E. Sidney Hunter2

U.S. EPA, Office of Research and Development 1NCCT and 2NHEERL

MCC:

• enriched pathway interactions mapped with the Cluepedia plug-in to Cytoscape.
• positive-response examples: inhibition of BHF signaling, adrenocorticoids (GR, MR).
• negative-response examples: female hormone receptors (ESR1, ER), melanocyte receptors (M43, M5N).

Summary

• mESC examples: p53
• ToxRefDB chemicals were classified for potential developmental toxicity using the NESC platform from Stemina Biomarker Discovery [1] or an adherent mESC assay [2].
• Performance against prenatal animal studies (ToxRefDB) improved from 62% to >84% accuracy as the level of confidence in the in vivo anchoring result (DEE) increased.
• Characterizing the applicability domain at a pathway level sets the stage for new approach methodologies predicting developmental toxicity without vertebrate animal testing.

Figure 6

MCC results:

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**EPA Developmental Neurotoxicity Research Plan**

**Goal I:** Compile existing data and identify gaps (focus on chemicals with in vivo data: Mundy et al. 2015)

**Goal II:** Expand the universe of compounds tested

**Goal III:** Data translation and accessibility

**Goal IV:** Provide a biological context for enhanced interpretation of DNT

Source: Tim Shafer, EPA NHEERL
Special Thanks

- Tom Knudsen (EPA/NCCT)
- Todd Zurlinden (EPA/NCCT)
- Katerine Saili (EPA/NCCT)
- Tim Shafer (EPA/NHEERL)
- Louis (Gino) Scarano (EPA/OPPT)
- Suzanne Fitzpatrick (FDA)

ToxCast/Tox21 data are located in the **CompTox Chemicals Dashboard**: https://actor.epa.gov/dashboard/