ToxCast and the Use of Human Relevant *In Vitro* Exposures: Incorporating high-throughput exposure and toxicity testing data for 21st century risk assessments

BTS
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National Center for Computational Toxicology

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA
Using the ‘Golden Circle’ for the Transition to 21st Century Risk Assessment

Why?

What?

How?
Understanding ‘Why’ We Need to Innovate In This Space...

Number of Chemicals /Combinations

Ethics Concerns

Lack of Data

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<th>Percent of Chemicals</th>
<th>Acute</th>
<th>Cancer</th>
<th>Gentox</th>
<th>Dev Tox</th>
<th>Repro Tox</th>
<th>EDSP Tier 1</th>
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Modified from Judson et al., EHP 2010

Why?

Economics

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<th>Reproductive Tox</th>
<th>Dev Neurotox</th>
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‘Golden Circle’ of 21st Century Risk Assessment

Why?

What?

How?
Risk Assessments Generally Contain a Standard Set of Components

New technologies and approaches will also have to cover these basic components:

- **Phys Chem**
- **Exposure**
- **Hazard**
- **Dose Response, PK, and PODs**
- **Risk Summary**
- **Uncertainty**

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**EXECUTIVE SUMMARY**

1. BACKGROUND AND SCOPE
   1.1 INTRODUCTION
   1.2 USES AND PRODUCTION VOLUMES
   1.3 USES AND PRODUCTION VOLUMES: History
   1.4 Scope of the Assessment

2. PROBLEM FORMULATION
   2.1 Physical and/or Chemical Properties
   2.2 Toxicological Properties
   2.3 Conceptual Models
   2.4 Analysis Plan

3. EXPOSURE ASSESSMENT
   3.1 OCCUPATIONAL EXPOSURES
   3.2 Approach and Methodology
   3.2.1 Approach for determining Occupational Exposure Data and Input Parameters for PBPK Modeling
   3.2.2 Use of Occupational Exposure Data and Input Parameters for PBPK Modeling
   3.2.3 Use of Consumer Exposure Data for PBPK Modeling
   3.2.4 Use of Consumer Exposure Data for PBPK Modeling

4. HAZARD IDENTIFICATION AND DOSE-RESPONSE
   4.1 APPROACH AND METHODOLOGY
   4.1.1 Selection of peer-reviewed assessments for hazard identification and dose-response analysis
   4.1.2 Hapten Summaries and Reactivity
   4.1.3 Selection of developmental data
   4.1.4 Conclusions and Selection of Key Exposures
   4.2 DOSE-RESPONSE ASSESSMENT AND STUDY SELECTION

5. CONCLUSIONS AND RECOMMENDATIONS
   5.1 Conclusions
   5.2 Recommendations

**REFERENCES**

APPENDIX A: ENVIRONMENTAL EFFECTS SUMMARY
- A-1 Acute Toxicity to Aquatic Organisms
- A-2 Chronic Toxicity to Aquatic Organisms
- A-3 Toxicity to Sepaete and Soil Organisms
- A-4 Toxicity to Plants
- A-5 Summary of Environmental Hazard Assessment

APPENDIX B: CHEMICAL REPORTING DATA
- B-1 Consumer Use
- B-2 Pesticide Use Applications

APPENDIX C: STATE NMP REGULATIONS

APPENDIX D: OCCUPATIONAL EXPOSURE ASSESSMENT SUPPORT INFORMATION
- D-1 Summary of Derived Exposure Parameters, Incidence and Exposure Concentrations
- D-2 Data Needs and Data Collection
- D-3 Data Needs and Data Collection
- D-4 Occupational Contact Studies: Industrial Workers
- D-5 Technical and Exposure Data and Derivation
- D-6 Technical and Exposure Data and Derivation

APPENDIX E: CONSUMER EXPOSURE ASSESSMENT
- E-1 Estimation of Exposure Doses and Routes
- E-2 Toxicity Abbott for Determination
- E-3 Toxicity Abbott for Determination
- E-4 Toxicity Abbott for Determination
- E-5 MCM Estimated No Observed Effect Concentrations

**3.2.3 CONCLUSIONS AND RECOMMENDATIONS**

- **Variability**
- **Risk Summary**
It All Starts With Chemistry…

- Chemical structure database of >700,000 unique substances with QC flags to link chemical structure with names and identifiers
- Consensus QSAR models for a range of physical chemical properties, environmental fate, and hazard characteristics
- Comprehensive physical-chemical property database (experimental and predicted)

https://comptox.epa.gov/dashboard
Adding the High-Throughput Hazard Screening Component

ToxCast

- ~600 Cell & biochemical assays
- ~1,000 Chemicals
- Concentration Response

Tox21

- ~30 Cell & biochemical assays
- ~8,000 Chemicals

<table>
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<tr>
<th>Set</th>
<th>Chemicals</th>
<th>Assays</th>
<th>Completion</th>
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<tr>
<td>ToxCast Phase I</td>
<td>293</td>
<td>~600</td>
<td>2011</td>
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<tr>
<td>ToxCast Phase II</td>
<td>767</td>
<td>~600</td>
<td>2013</td>
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<td>ToxCast Phase III</td>
<td>1001</td>
<td>~100</td>
<td>Ongoing</td>
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<td>E1K (endocrine)</td>
<td>880</td>
<td>~50</td>
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Broad Success Derived from High-Throughput Screening Approaches

Group Chemicals by Similar Bioactivity and Predictive Modeling

Provide Mechanistic Support for Hazard ID

Prioritization of Chemicals for Further Testing

IARC Monographs 110, 112, 113

FIFRA SAP, Dec 2014
Selected Criticisms of ToxCast

- You don’t include metabolism in your *in vitro* assays
- You don’t measure my favorite endpoint
- You don’t cover all of biological space
- *In vitro* assays are not normal biology
- Assay (x) in your battery did not get the right answer for my chemical
- My assay disagrees with your assay (x), so your approach is flawed
- You can’t test my favorite chemicals because of limitations in your methods (e.g., solvents, high LogP)
- Your assay descriptions do not allow me to reproduce your results
- I get different answers when I analyze your data

Updated from Bob K’s original list
Beginning to Address Concerns for Increased Biological Coverage

Gene Coverage

Pathway Coverage*

*At least one gene from pathway represented

Gene Coverage

Requirements:
- Low cost
- Whole genome

Thousands of chemicals

Multiple Cell Types

ToxCast
Not in ToxCast

At least one gene from pathway represented
Comparing Sequencing Platforms and Developing Analysis Approach

- Large scale screen of 1,000 chemicals (ToxCast I/II) in single cell type this summer
- Additional screens across multiple cell types/lines
- Additional reference chemicals and genetic perturbations (RNAi/CRISPR/cDNA)

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

**TruSeq**
- \( r^2 \) 0.74

**Low Coverage**
- \( r^2 \) 0.83
Beginning to Address Metabolic Competence

“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

Collaboration with Unilever
Framework for Integrating Hazard Components...

Tier 1
- Tier 1: High-Throughput Transcriptomic Assay
  - No Defined Biological Target or Pathway
  - Defined Biological Target or Pathway

Tier 2
- Tier 2: Select In Vitro Assays
  - Orthogonal confirmation

Tier 3
- Tier 3: Estimate Point-of-Departure
  - Based on Pathway Transcriptional Perturbation
  - Based on AOP
  - Based on Likely Tissue- or Organ-level Effect without AOP

No Defined Biological Target or Pathway
- Tier 3: Organotypic Assays and Microphysiological Systems
- Estimated Point-of-Departure Based on Likely Tissue- or Organ-level Effect without AOP

Defined Biological Target or Pathway
- Tier 3: Organotypic Assays and Microphysiological Systems
- Estimated Point-of-Departure Based on Likely Tissue- or Organ-level Effect without AOP
Adding the High-Throughput Toxicokinetic Component

EPA ToxCast Phase I and II Chemicals

Human Liver Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

In Vitro Potency Value

Plasma Concentration

Oral Exposure

Reverse Dosimetry

Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to In Vitro Bioactivity

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through “httk” R package (https://cran.r-project.org/web/packages/httk/)

Rotroff et al., Tox Sci., 2010
Wetmore et al., Tox Sci., 2012
Comparing Dosimetry Adjusted Bioactivity with Exposure

Wetmore et al., Tox Sci., 2012
But, Exposure Information is Lacking on Most Chemicals

Bioactivity from Wetmore et al., Tox Sci., 2015
Adding the High-Throughput Exposure Component

Exposures estimated for >8,000 chemicals

4 product use categories plus production volume explain ~50% of the variance in the NHANES data

The same five variables work for all NHANES demographic groups analyzed – stratified by age, sex, and body-mass index

Uncertainty captured in exposure estimates

E.g., CDC NHANES study

Drug Monitoring

Dataset 1

Dataset 2

Inferred Exposure

Pharmacokinetic Models

Calibrate models

Predicted Exposures

Estimate Uncertainty

Predicted Exposure

Wambaugh et al., 2014
Comparing Bioactivity with Exposure Predictions for Risk Context

Wetmore et al., Tox Sci., 2015
Adding in Uncertainty and Variability for PD and PK

Propagation of Experimental Uncertainty in Models of ER Potency

- Chemical Rank vs. ER Model Score

Propagation of Experimental Uncertainty in High-Throughput Toxicokinetic Estimates

- Ratio of Css 95th Percentile to Median Estimate vs. Chemical Rank
  - Uncertainty Predominates
  - Variability Predominates
‘Golden Circle’ of 21st Century Risk Assessment

Why?

What?

How?
Regulatory Applications Require More Focus on Quality and Transparency

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Publicly available ToxCast data analysis pipeline
  - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
- Tox21 and ToxCast chemical libraries have undergone analytical QC and results publicly available
- Public posting of ToxCast procedures
  - Chemical Procurement and QC
  - Data Analysis
  - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline
- Migrating ToxCast assay annotations to OECD 211 compliant format
Application to Regulatory Decisions for Endocrine Screening
Beginning Application to Quantitative Risk Assessment Through New ‘RapidTox’ Dashboard

- Semi-automated decision support tool with dashboard interface for high-throughput risk assessments
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure
- Transparent and interactive enough to enable expert users to review the assumptions made and refine the predictions
- Deliver quantitative toxicity values with associated estimates of uncertainty
Thank You for Your Attention!

Tox21 Colleagues:
  NTP Crew
  FDA Collaborators
  NCATS Collaborators

EPA Colleagues:
  NERL
  NHEERL
  NCEA

Collaborators:
  Unilever