The U.S. EPA ToxCast Program: Moving from Data Generation to Application

Society of Toxicology Annual Meeting
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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
Current System for Chemical Testing is Antiquated and Inefficient

Current testing paradigm does not incorporate advances in technology and does not provide mechanistic data

Acute toxicity studies ($LD_{50}$) developed to standardized batches of pharmaceuticals

- Draize test introduced for eye irritants
- Thalidomide led to testing of pharmaceuticals for developmental toxicity
- Rodent cancer bioassay introduced

... and cannot efficiently assess safety of all the existing chemicals or keep pace with those being developed

EPA Formed the CompTox Center to Address this Challenge

Strategic Plan for CompTox Research Program
(November, 2003)

Celebratory Opening of NCCT
(March 1, 2005)
The Effort was Expanded with the Formation of Tox21

MEMORANDUM OF UNDERSTANDING

ON

High Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings

MOU Signed February, 2008; Revised July, 2010
Recent Highlights from a Decade of Progress

- High-throughput *in vitro* screening of ~2,000 chemicals across ~700 assay endpoints representing over 327 genes and 293 pathways
- High quality, curated chemical structure database of 22,000 molecules
- Legacy *in vivo* data from 5,891 animal toxicology studies on ~1,110 unique chemicals
- Exposure estimates for over 7,000 chemicals based on production volume and chemical use
- A database of chemical-product categories (CPCat) that maps over 45,000 chemicals to ~8,000 product uses or functions
- Steady-state IVIVE models for hundreds of chemicals based on high-throughput *in vitro* assays
- AOPs and computational models for embryonic vascular disruption, cleft palate, hypospadias, and limb (digit) defects
At the Beginning of Regulatory Application

Prioritization of the Endocrine Disruptor Screening Program Universe of Chemicals for an Estrogen Receptor Adverse Outcome Pathway Using Computational Toxicology Tools

U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program

Jointly developed by:
Office of Chemical Safety and Pollution Prevention (OCSPP)
Office of Science Coordination and Policy (OSCSP)
Office of Pollution Prevention and Toxics (OPPT)
Office of Water (OW)
Washington, DC 20460
Office of Research and Development (ORD)
National Environmental and Health Research Laboratory (NEERL) Mid-Continent Ecology Division (MED), Duluth, MN 55810
Toxicity Assessment Division (TAD), RTP, NC 27711
National Center for Computational Toxicology (NCCT)
Research Triangle Park, NC 27709

December 2012

Integrated Bioactivity and Exposure Ranking
A Computational Approach for the Prioritization and Screening of Chemicals in the Endocrine Disruptor Screening Program

U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program

Jointly developed by:
U.S. EPA Office of Chemical Safety and Pollution Prevention (OCSPP)
U.S. EPA Office of Research and Development (ORD)
U.S. EPA Office of Water (OW)
NIH National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Exposure SAP White Paper
New High-throughput Methods to Estimate Chemical Exposure

Scientific Advisory Panel Meeting, July 2014

FIFRA SAP December 2-5, 2014
Top 10 Lessons Learned

THE TOP 10 LESSONS FROM TOXCAST IN THE LAST DECADE...

10. Most chemicals are promiscuous
9. No assay is perfect
8. Quality and transparency matters
7. PK is cool again
6. Exposure is context
5. Embrace uncertainty
4. Look for local predictivity
3. Your CAS and structure may not be friends
2. Stay relevant
1. Old habits die hard
Lesson #10: Most Chemicals are Promiscuous

Thomas et al., Tox Sci., 2013
Non-Selectivity Frequently Occurs at Cytotoxic Concentrations

\[ Z \text{-score} = \#SD \text{ from Cytotox Region} \]
Lesson #9: No Assay is Perfect
(Search for Consensus)

18 In Vitro Assays Measure ER-Related Activity

80-05-7: Bisphenol A

10016-20-3: alpha-Cyclodextrin

ER Receptor Binding (Agonist)
ER Receptor Binding (Antagonist)
Dimerization
Cofactor Recruitment
DNA Binding
ER-induced Proliferation
Antagonist Transcription Suppression

Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
Noise Process
ER agonist pathway
ER antagonist pathway
Interference pathway

In Vitro Assays Measure ER-Related Activity
Lesson #9: No Assay is Perfect
(Search for Consensus)

18 *In Vitro* Assays Measure ER-Related Activity

Model scores show ~94% concordance with *in vitro* reference chemicals
Lesson #8: Quality and Transparency Matters

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- ToxCast data analysis pipeline has been completely revamped
  - More rigorous statistically and robust to outliers
  - Data quality flags to indicate concerns with chemical purity and identity, noisy data, systematic assay errors, and activity in range of cytotoxicity
- Tox21 and ToxCast chemical libraries undergoing analytical QC and results will be publicly released
- Release of ToxCast “Owner’s Manual”
  - Chemical Procurement and QC
  - Data Analysis
  - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline
- Multiple webinars and workshops to educate stakeholders on high-throughput screening data analysis and interpretation
Lesson #8: Quality and Transparency Matters

iCSS Dashboard

EDSP21 Dashboard

http://actor.epa.gov/dashboard/

http://actor.epa.gov/edsp21/

*Get dashboard demos at the EPA booth #2133
Lesson #7: PK is Cool Again

- 309 EPA ToxCast Phase I 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
- ToxCast AC₅₀ Value
  - Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to In Vitro Bioactivity
  - Plasma Concentration
  - Oral Exposure
  - Reverse Dosimetry

Wetmore et al., Tox Sci., 2012
Rotroff et al., Tox Sci., 2010
Lesson #6: Exposure is Context

What are humans exposed to?  

309 EPA ToxCast Phase I Chemicals

- Human Liver Metabolism
- Human Plasma Protein Binding
- Population-Based IVIVE Model

Oral Equivalent Dose (mg/kg/day)

~700 In Vitro ToxCast Assays

~In Vitro ToxCast AC50 Value

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

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

Reverse Dosimetry

Chemical

Rotroff et al., Tox Sci., 2010
Wetmore et al., Tox Sci., 2012
Lesson #6: Exposure is Context

Oral Equivalent Dose or Estimated Exposure (mg/kg/day)

Assay Bioactivity

Exposure Range

National Center for Computational Toxicology

Wetmore et al., Tox Sci., 2012
Lesson 5: Embrace Uncertainty

(Bio) Monitoring
Dataset 1
Dataset 2
...

Inferred Exposures

Use
Production Volume
...

e.g., CDC NHANES study

Pharmacokinetic Models

Calibrate models

Inferred Exposures

Predicted Exposures

*See talk by J. Wambaugh Thursday morning, Abstract #2445

Wambaugh et al., Environ Sci Technol., 2014
Lesson 5: Embrace Uncertainty

Oral Equivalent Dose or Exposure

Bioactivity data courtesy of Barbara Wetmore
Lesson 4: Look for Local Predictivity

Recursive Partition Tree on Residuals

*Transporter substrate predicted with QSAR (Sedykh et al, 2013)

*See talk by J. Wambaugh Tuesday afternoon, Abstract #1607
Lesson #3: Your CAS and Structure May Not Be Friends

DSSTox Database

(≈22,000 curated chemical structures)

Quality of CAS-Structure Mappings

- DSSTox_High: 40%
- DSSTox_Low: 40%
- Public_High: 13%
- Public_Med: 1%
- Public_Low: 2%
- Public_Conflicts: 4%
Lesson #2: Stay Relevant

- Undifferentiated human embryonic stem cells (H9 cells) treated for 72 hours
- Conditioned media from the final 24h treatment period was analyzed by LC-MS-based metabolomics to determine ornithine/cystine (o/c) ratio
- Teratogenicity defined by the o/c ratio ≤ 0.88
- Concurrent cell viability measured
- Rapid screen of 1066 ToxCast chemicals produced 15-16% actives


Cytotoxicity by itself only had an estimated predictive accuracy of 30-40% using a subset of ToxCast chemicals

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*See poster (late breaking) by T. Knudsen Thursday morning, Board #414
Lesson #1: Old Habits Die Hard

**Original OECD TG 440 Validation**


**Concordance of *In Vivo* Uterotrophic Studies**

Graph courtesy of Warren Casey

**Predictive Performance of *In Vitro* Assays for *In Vivo* Uterotrophic Studies**

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Acknowledgements

Tox21 Colleagues:
  NTP Crew
  FDA Collaborators
  NCATS Collaborators
Visit EPA’s Exhibit Booth #2133

EPA’s Demo Sessions
- CPCat- Chemical and Product Categories Database
- iCSS Dashboard
- ToxCast Data Download Page
- AOP Wiki
- EDSP21 Dashboard
- More!

Booth Materials
- Project and Program Factsheets
- Information on Funding, Grants, and Employment Opportunities

Meet the Directors Sessions
- EPA Lab, Center and Office Directors
- Informal- 1 Hour Sessions

[epa.gov/research/sot](http://epa.gov/research/sot) For full calendar of events and materials