ToxCast and Tox21: High Throughput Screening for Hazard & Risk of Environmental Chemicals

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This work was reviewed by EPA and approved for presentation but does not necessarily reflect official Agency policy.
Future of Toxicity Testing and Ultimately, Environmental Risk Assessments
Grand Challenge for Computational Toxicology: Predicting Human Toxicity

Office of Research and Development
Computational Toxicology Research Program
High Throughput Screening 101 (HTS)

Robotic Platform

96-, 384-, 1536 Well Plates

Chemical Exposure

Cell Population

Pathway

Target Biology (e.g., Estrogen Receptor)
HTS in Drug Development

Identify target, pathway, or cellular phenotype → Create testing system (aka, “assay”) → HTS tests >100,000 chemicals with no known activity for effect on target → Make modifications to most active chemicals to make suitable for in vivo testing → Test in animals for safety, effectiveness → Test in humans for safety, effectiveness
HTS in Toxicology

Test prioritized chemicals in animals

Categorize as inactive subject to further testing

Computational analysis & Synthesis of HTS results

HTS tests chemicals for effect on assays

Obtain or create testing systems (“assays”)

Identify toxicity pathways, cellular phenotypes

Chemicals with known or suspected toxicity/activity

Office of Research and Development
Computational Toxicology Research Program
**ToxCast and Tox21 High Throughput Screening of Chemical Bioactivity**

- Addresses chemical screening and prioritization needs for chemicals regulated by EPA

- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures

- Committed to stakeholder involvement and transparency
  - Communities of Practice- Chemical Prioritization; Exposure
  - Release of all data upon peer review publication
Goals of ToxCast and Tox21

- Identify toxicity pathways
- Obtain HTS assays for pathways
- Screen a large chemical library
- Initially link HTS results to adverse effects
  - Toxicity signatures
- Ultimately identify points of departure from HTS data
  - Toxicity pathways
Tox21 Screening Throughput

- 96-well plate
  - 8 rows x 12 columns
  - 88 test samples

- 384-well plate
  - 16 rows x 32 columns
  - 352 test samples
  - 4 x 96-well plates

- 1536-well plate
  - 32 rows x 48 columns
  - 1,408 test samples
  - 16 x 96-well plates

If @ 100 microtiter plates per day:

<table>
<thead>
<tr>
<th>Plate format</th>
<th>samples§/day (wells/day)</th>
<th>Time to screen 1 MM samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>8,800 (9,600)</td>
<td>4 months</td>
</tr>
<tr>
<td>384-well</td>
<td>35,200 (38,400)</td>
<td>4 weeks</td>
</tr>
<tr>
<td>1,536-well</td>
<td>140,800 (153,600)</td>
<td>7 days</td>
</tr>
</tbody>
</table>

§ wells remaining after subtraction of control wells; NCGC uses left 4 columns of a 1536-well plate for control wells
Comparison of Volumes for Screening Compounds in 7 Concentrations

Total Volume

96-well plate: 100 µl x 7 pts = 700 µl

384-well plate: 40 µl x 7 pts = 280 µl

1536-well plate: 5 µl x 7 pts = 35 µl
- 1536 well HTS
- 10,000 chemicals
- 25 assays per year
# ToxCast Phase I and II, Tox21

## Number of Chemicals

<table>
<thead>
<tr>
<th>Category</th>
<th>ToxCast Phase I</th>
<th>ToxCast Phase II</th>
<th>Tox21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actives</td>
<td>272</td>
<td>120</td>
<td>700</td>
</tr>
<tr>
<td>Inerts</td>
<td>24</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>Antimicrobials</td>
<td>33</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>HPV</td>
<td>35</td>
<td>170</td>
<td>1300</td>
</tr>
<tr>
<td>MPV</td>
<td>7</td>
<td>60</td>
<td>1500</td>
</tr>
<tr>
<td>Green</td>
<td>4</td>
<td>60</td>
<td>500</td>
</tr>
<tr>
<td>PCCL</td>
<td>73</td>
<td>150</td>
<td>500</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>0</td>
<td>100</td>
<td>2500</td>
</tr>
<tr>
<td>Consumer Products /Food</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>additives</td>
<td>0</td>
<td>0</td>
<td>1500</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>309</strong></td>
<td>~700</td>
<td>~10000</td>
</tr>
</tbody>
</table>

**Diagram:**
- **Phase I**
- **Phase II**
- **Tox21**

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ToxCast HTS Assays

Cellular Assays

- **Cell lines**
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney

- **Primary cells**
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
  - Rat hepatocytes
  - Mouse embryonic stem cells (Sid Hunter)

- **Biotransformation competent cells**
  - Primary rat hepatocytes
  - Primary human hepatocytes

- **Assay formats**
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

Biochemical Assays

- **Protein families**
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter

- **Assay formats**
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

**Primarily Human / Rat**

**Exception**: Zebrafish development (Stephanie Padilla)
The ToxCast In Vitro Data Set

Many hits – median value = 50

Fewer in cell-free HTS (Novascreen, red)

Many hits are at or near top of tested concentration range

A few are in nanomolar range
ToxCast Data Analysis

828 Assay-Chemical Pairs had AC50s of less than 1µM

500 Assays X 320 Chemicals X \{5-18\} Concentrations X \{1-3\} Replicates X \{1-3\} Time Points ≈ 3.2 Million Data Points
Digitizing Legacy *in Vivo* Data in ToxRefDB

30 years and more than $2B worth of data

Martin et al 2009a,b
Knudsen et al 2009

Chronic/Cancer
Multigenation
Developmental
Predicting Toxicity and Disease from HTS Data

ToxRefDB ↔ ToxCast → Human Disease
ToxCast: Multiple Assays and Technologies per Target
ToxCast: Multiple Targets per Pathway

- Biologically Multiplexed Activity Profiling (BioMAP)
- Multiplex Transcription Reporter Assay
- Cell-based HTS Assays
- Cell-free HTS Assays
- High Content Cell Imaging Assays

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Building Toxicity Signatures for Rat Liver Histopathology from Chronic Bioassays

- No Pathology: 68
- Proliferative Lesions: 37
- Pre-neoplastic Lesions: 21
- Neoplastic Lesions: 21

N = 248 Chemicals in ToxRefDB
Judson et al, EHP (2010)
Toxicity Pathways

Receptors / Enzymes / etc.
Direct Molecular Interaction

Pathway Regulation / Genomics

Cellular Processes

Tissue / Organ / Organism Tox Endpoint
Predictive Signatures from ToxCast for Chronic, Developmental and Reproductive Toxicity

- Chronic/Cancer endpoints from rat, mouse and dog
- Developmental endpoints from rat and rabbit
- Reproductive endpoints from rat
ToxPi: Prioritizing Chemicals for Potential Endocrine Activity

Prioritization Index = ToxPi = f(HTS assays + Chemical properties + Pathways)

Reif et al, *EHP*, 2010
Expanding the ToxPi Approach for Prioritization of Toxicity Testing Based on ToxCast Chemical Profiling

Identify *in vitro* assays, targets, genes and pathways associated with multiple sectors of *in vivo* toxicity.
ToxPi Scores for Antimicrobial Pesticide Actives

One of the methods be developed to use ToxCast data for classifying and prioritizing antimicrobials and inerts.

Scores for Antimicrobials in the ToxCast Phase-I Chemical Set

- Chlorothalonil
- Propiconazole
- 3-iodo-2-propynylbutylcarbamate
- Thiam
- TCMTB
- Cyproconazole
- Chlorophene
- Triclosan
- Dazomet
- Methylene bis(thiocyanate)
- Diuron
- Folpet
- Methyl isothiocyanate
- Thiabendazole
- 2-Phenylphenol
- Sancobene
- Boric acid
- Thiamethoxam
Future ToxPi (Toxicological Prioritization Index)

\[ \text{ToxPi} = f(\text{Exposure} + \text{Chemical properties} + \text{In vitro assays} + \text{Pathways}) \]

Incorporate additional components (slices) from other domains:
- Exposure
- Chemical properties
- QSAR
Distributions of Oral Equivalent Values and Predicted Chronic Exposures

Range of *in vitro* AC50 values converted to human *in vivo* daily dose

Estimated Exposure

Margin

Estimated Exposure

Systems Approaches to Modeling Toxicity: From Pathways to Virtual Tissues

Moving beyond empirical models, to multi-scale models of complex biological systems: vLiver, vEmbryo…

Identify Key Targets and Pathways For Prioritization

Quantitative Dose-Response Models

Next Generation Risk assessments
HIGH THROUGHPUT SCREENING and CHARACTERIZATION OF HAZARD & RISK

Exposure → Tissue Dose → Molecular Targets

IN VITRO: concentration response

Human IN SILICO: exposure dose response

Animal IN VIVO: exposure dose response

Tissues

Cellular Systems

Cell Changes

Cellular Networks

Toxicity
ToxCast™ Program
Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

In 2007, EPA launched ToxCast™ to develop a cost-effective approach for efficiently prioritizing the toxicity testing of thousands of chemicals.

- Uses data from state-of-the-art high throughput screening (HTS) bioassays.
- Builds computational models to forecast potential chemical toxicity in humans.
- Provides EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations and more efficient use of animal testing.
- Phase I profiled over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints. Endpoints include biochemical assays of protein function, cell-based transcriptional reporter and gene expression, cell line and primary cell functional, and developmental endpoints in zebrafish embryos and embryonic stem cells.
- Phase 1 chemicals have already been tested using traditional toxicity methods including developmental toxicity, multigeneration reproductive studies, and subchronic and chronic rodent bioassays. ToxRefDB is the relational database storing this information—nearly 32 billion worth of animal toxicity studies.
- Phase II of ToxCast will screen additional chemical compounds representing broader chemical structure and use classes to evaluate the predictive signatures developed in Phase I.
- Toxicity signatures from ToxCast will be defined and evaluated by how well they predict outcomes from mammalian toxicity tests and identify toxicity pathways.

http://epa.gov/ncct/toxcast/
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