IDENTIFICATION OF PHARMACEUTICALS FOR VALIDATION OF TOXCAST™

The HESI Emerging Issues Subcommittee on Identification of Pharmaceuticals for Validation of ToxCast™ was formed in March 2009. The Subcommittee held a webinar for interested parties on April 20, 2009. The purpose of the webinar was to provide background and present preliminary plans. The webinar presentation by Dr. Robert Kavlock (US EPA National Center for Computational Toxicology) is included below.

May 13, 2010: Press release from USEPA: HESI fosters partnership between EPA and Pharmaceutical Companies to provide data. Click here for more information.

2009 Participants
AstraZeneca
GlaxoSmithKline
Merck & Co. Inc.
Pfizer Inc.
sanofi-aventis
US Environmental Protection Agency
National Center for Computational Toxicology
2009 HESI Emerging Issue: Identification of Pharmaceuticals for Validation of ToxCast

Robert Kavlock
Director, National Center for Computational Toxicology, US EPA
EPA’s Need for Prioritization

Too Many Chemicals

Too Little Data (%)

Future of Toxicity Testing

Transforming Environmental Health Protection

By M. E. McFarland, C. K. Hecht, and S. M. Nemerow

In silico analysis

Cancer
ReproTox
DevTox
NeuroTox
PulmonaryTox
ImmuNoTox

$T

Tox21

www.epa.gov/ncct/toxcast

EPAs Contribution: The ToxCast Research Program
ToxCast™ Background

• Research program of EPA’s National Center for Computational Toxicology
• Addresses chemical screening and prioritization needs for pesticidal inerts, anti-microbials, CCLs, HPVs and MPVs
• Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures
• Coordinated with NTP and NHGRI/NCGC via Tox21
• Committed to stakeholder involvement and public release of data
  • Communities of Practice- Chemical Prioritization; Exposure
  • NCCT website- http://www.epa.gov/ncct/toxcast
  • ACToR- Aggregated Computational Toxicology Resource
    http://www.epa.gov/actor/
Key Challenges Of Pathway Profiling

- Find the Toxicity Pathways
  - Hepato vs developmental neurotoxicity

- Obtain HTS Assays for Them
  - Including metabolic capability

- Screen Chemical Libraries
  - Coverage of p-chem properties

- Link Results to in vivo Effects
  - Gold standard and dosimetry
Implications for Success

• Hazard Identification
  • Closing Data Gaps
  • Reductions in Cost
  • Hypothesis Generation
  • Reduced Animal Usage

• Ancillary Applications
  • Mixtures
  • Chirals
  • Nanomaterials
  • Green Chemistry
  • Lot variations

• Risk Assessment
  • Providing MOA(s)
  • Targeted Testing
  • Identifying Susceptible Populations
## Prioritization Product Timeline

<table>
<thead>
<tr>
<th>FY07</th>
<th>FY08</th>
<th>FY09</th>
<th>FY10</th>
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<td><strong>Proof of Concept: ToxCast</strong></td>
<td></td>
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<td><strong>Verification/Extension</strong></td>
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<td><strong>Reduce to Practice</strong></td>
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*Office of Research and Development*
National Center for Computational Toxicology
### Phased Development of ToxCast

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*January 2009*
ToxCast Phase I Datasets

- Released to Data Analysis Partners:
  - ACEA - Real-time Cell Electronic Sensing (7 assays)
  - Attagene - Transcription factor assays (81 assays)
  - BioSeek - Cell-based protein level assays (87 assays)
  - Cellumen - Cell imaging assays (11 assays)
  - CellzDirect – NR target-gene expression assays (16 assays)
  - Gentronix - GreenScreen GeneTox assay (1 assay)
  - NCGC - nuclear receptor assays (11 assays)
  - Novascreen / Caliper - receptor binding and enzyme inhibition assays (239 assays)
  - Solidus - P450 vs. cytotoxicity assays (4 assays)

- Upcoming Dataset Additions:
  - Neurite outgrowth HCS (NHEERL)
  - Cell proliferation (NHEERL)
  - Zebrafish developmental toxicity (NHEERL)
  - Organ toxicity; dosimetry (Hamner Institutes)
  - C. elegans WormTox (NIEHS)
  - Gene markers from microscale cultured hepatocytes (Hepregen)
  - 3D Cellular Zebrafish vascular/cardiototoxicity (Zygogen)
  - HTS stress response (NHEERL+NCGC)
  - Embryonic Stem Cells (NHEERL)
  - Metabolic Phenotyping (Biolog)
ToxCast Assays

Biochemical Assays

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

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

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

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

- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype
ToxCast Phase I Assays
500 endpoints

Circa 2009
ToxCast_320 Phase I Chemicals

ACEA
Attagene
Bioseek
Cellumen
CellzDirect
Gentronix
NovaScreen
Solidus

CYP
ToxRefDB

- Relational phenotypic/toxicity database
- Provides in vivo anchor for ToxCast predictions

Three study types
- Chronic/Cancer rat and mouse (Martin, et al, EHP 2008)
- Rat & Rabbit developmental (Knudsen, et al, Repro Tox, in press)

Two types of synthesis
- Supervised (common individual phenotypes)
- Unsupervised (machine based clustering of phenotype patterns)
> $1B in Toxicology Now Stored in ToxRefDB

Martin, et al *EHP*, 2009
ToxCast In Vivo Data from ToxRefDB

Red: Chronic/Cancer
White: Multigen
Blue: Developmental
ToxCast In vitro data

- Novascreen
- Attagene
- BioSeek
- Cellumen
- CellzDirect
- NCGC
- Gene assays
- Pathway assays

Promiscuous Chemicals

Chemicals
2-D cluster plots comparing results of the Expanded Set of ToxCast assays with multigenerational study findings. The EDSP chemicals are identified by a blue band. The main chemical clusters are given a number that is color coated on the dendrogram. For the ToxCast assays, estrogen, androgen, thyroid, aromatase, other nuclear receptor and other assays types are color coded in the assay ribbon. The cross matrix contains the count of the chemicals in each of the main clusters.
XME / Nuclear Receptor Pathway
Covered by ToxCast
ToxCast™ Program
Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

ToxCast™ Data Analysis Summit

Click here to go to registration and travel information page for the TDAS Meeting

Meeting Notice and Call for Abstracts

Transforming Toxicity Testing From In Vivo to In Vitro:
A Computational Toxicology Challenge

The First ToxCast™ Data Analysis Summit
Hosted by U.S. EPA’s National Center for Computational Toxicology
EPA Campus, Research Triangle Park NC
May 14-15, 2009

Overview: The U.S. EPA ToxCast™ Program is developing approaches to predict chemical toxicity using data from high-throughput and high content in vitro assays. Phase I of ToxCast has produced data from 320 chemicals, ~500 in vitro assays and ~100 in vivo endpoints, providing a powerful dataset for evaluating the applicability of various analytic approaches for predicting the potential for an adverse response.

The goal of ToxCast is to develop and verify “toxicity signatures,” which are algorithms using in vitro and in silico data to predict in vivo toxicities. These signatures will be used to screen and prioritize chemicals for targeted toxicity testing, and over the next several years EPA would like to screen thousands of compounds. However, successful predictive models will depend on robust and reliable methods that EPA can rely on for making decisions about further testing of environmental chemicals.

This first ToxCast Data Analysis Summit is designed to bring together experts in machine learning, computational chemistry, statistics, high-throughput screening and computational toxicology, with toxicologists and regulatory staff. Plenary talks will describe the ToxCast Program and a series of issues related to toxicity prediction, both from a scientific and regulatory standpoint. Speakers will be selected from abstract submitters to describe algorithmic, computational or systems biology approaches to solving these issues.

To further this aim, we invite interested researchers to submit abstracts and present their analyses, using the ToxCast Phase I dataset, at the First ToxCast Data Analysis Summit

Topics of Interest Include:

- Machine learning or statistical approaches for signature generation
- Systems biology/pathway modeling approaches
- Issues of statistical power for prediction and verification.
- Models to address prediction of metabolism and biotransformation

The analyses described in the abstracts must make use of the ToxCast Phase I dataset, although additional data may also be included.

This is not a “Critical Assessment” workshop with an outcome of “best” prediction methods. That will be the goal of a follow-up meeting in 2010, for which a significant body of blinded validation data will be available from later phases of ToxCast.

Data Overview: The collection of ToxCast Phase I chemicals were chosen because high quality, guideline-based animal toxicity data were available. These chemicals are mostly pesticide active compounds for which we have rat and mouse 2-year chronic/cancer, 2-generation reproductive, and developmental toxicity data.

For analysis, we will provide ~100 toxicity endpoints from these study types whose value is a “LEL” or lowest effective level at which the endpoint was observed—these are the values to predict. In addition, we will provide other aggregated endpoints derived from clustering analyses. Analysis groups (or analysis partners) are also free to develop other endpoints to predict from the data that we will provide.

A total of 9 in vitro datasets have been produced, reviewed and cleaned for research use. These include biochemical receptor and enzyme assays; and cell-based
Beyond the Proof of Concept

Chemicals

ToxRef in vivo bioassay data

ToxCast_320

HighThroughput Assay Data
# Tox21 Existing and Candidate Chemicals*

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<td>EPA</td>
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<tr>
<td>NCGC</td>
<td>~3000 drugs</td>
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<tr>
<td>Target library, Summer 2009</td>
<td>~10,000</td>
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* Sources include NTP, EPA HPV, CCL, OPPIN, OW, Inerts, ToxCast, DSSTox, EU Carcinogenomics, Pharmaceuticals, others
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January 2009
Emerging Issues Proposal

• SPECIFIC ACTIONS

  – Coordinate Public-Private sector involvement in ToxCast predictions
  – Scoping meeting to articulate needs, timelines and boundaries of involvement by participants
  – Organization commitment to effort by early June 2009
  – Delivery of chemicals as soon as possible
  – Coordinate data extraction template

• DESIRED OUTCOME

  – Successful deliberations and negotiations would result in:
    • Identification and provisioning of chemicals (~100mg) for screening
    • Sharing of relevant pre-clincal and clinical data
    • Co-publications on predictive models
MATERIALS TRANSFER AGREEMENT

EPA:

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Pfizer:
Pfizer Inc, having a principal place of business at 235 East 42nd Street, New York,
(“Pfizer”) New York, 10017 and its Affiliates

WHEREAS the EPA wishes to obtain Pfizer Compounds to use in certain test assay
panels, and whereas Pfizer wishes to have Pfizer Compounds evaluated on such test
panels, the parties agree as follows:

“Affiliate” means any corporation, firm partnership or other entity which directly or
indirectly controls, is controlled by, or is under common control with either of the parties.

1. EPA agrees to receive Pfizer’s compounds, listed in Exhibit B, in any form or any of
its intermediates and derivatives (“Pfizer Compound”), in order to perform the research
activities, further described in Exhibit A, and known as the “ToxCast™ Program.”

2. The Pfizer Compounds:
   a. are the property of Pfizer and all existing rights including, without limitation,
      patent rights in or to the Pfizer Compounds will remain the property of the Pfizer.
   b. will be used with caution and for research purposes only, and shall not be used
      for research involving human subjects.
   c. will be used only by the EPA in the ToxCast™ Program described below, under
      suitable containment conditions.
   d. will not be used for screening, production or sale, for which a commercialization
      license may be required.

Both Pfizer and EPA agree to comply with all applicable laws, rules, guidelines and
regulations applicable to the use, storage, shipping and the handling of the Pfizer
Compounds and ToxCast™ Program.
Benefits of Proposal

• Draws on unique position of HESI in bringing the public and private sectors together for progress in science

• Enables utilization of a unique private sector knowledge

• Builds on the experience of EPA in computational toxicology

• Brings direct human relevance to HTS screening on environmental chemicals, which already involves the use of many human protein targets and cell types

• HESI would be intimately associated and linked with progress at reaching the vision of toxicity testing in the 21st envisioned by the National Research Council
2009 ACTIVITIES AND ACCOMPLISHMENTS
In 2007, the EPA launched ToxCast to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time. Using data from state-of-the-art high-throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast is building computational models to forecast the potential human toxicity of chemicals. These hazard predictions will provide EPA regulatory programs with science-based information that will be helpful in prioritizing chemicals for more detailed toxicological evaluations, and will lead to more efficient use of animal testing.

In the proof-of-concept phase of ToxCast, the EPA completed work on 320 well-characterized chemicals, primarily pesticides. Phase II of the project, currently underway, will include screening of additional compounds representing broader chemical structures and classes to evaluate the predictive bioactivity signatures that were developed in Phase I.

HESI engaged in Phase II of the ToxCast program by contributing data on chemicals that have failed in drug development because of manifestations of toxicity in humans. The data generated from this effort will become part of the Tox21 consortium and screened in the ultra-HTS assays at the National Chemical Genomics Center (NCGC) and in the ToxCast program. The consortium will be composed of the EPA, National Toxicology Program (NTP)/NIEHS, and the NCGC/National Human Genome Research Institute (NHGRI). The results will be made publicly available.

The HESI project was announced in March 2009 at the Society of Toxicology Annual Meeting in Baltimore, Maryland. A webinar was held in April 2009 to outline the purpose of the project and to solicit company participation. By the fall of 2009, five HESI member companies from the pharmaceutical sector had completed agreements or were actively engaged in negotiations with the EPA to contribute data sets.

FUTURE ACTIVITIES
The HESI ToxCast Subcommittee has officially sunset at the close of 2009. The EPA will acknowledge the subcommittee’s contributions in its future scientific publications on the ToxCast program.

2009 PARTICIPANTS
GOVERNMENT
US Environmental Protection Agency
National Center for Computational Toxicology

INDUSTRY
AstraZeneca
GlaxoSmithKline
Merck & Co. Inc.
Pfizer Inc.
sanofi-aventis

MISSION
The mission of the Subcommittee on Identification of Pharmaceuticals for Validation of ToxCast was to contribute data on failed drugs with known human toxicity to the US EPA as a mechanism to extend and further validate the EPA’s ToxCast program.