Biological Profiling of Endocrine Related Effects of Chemicals in ToxCast™

RJ Kavlock, D Dix, K Houck, R Judson, T Knudsen, D Reif and M Martin

National Center for Computational Toxicology, Office of Research and Development,
United States Environmental Protection Agency, Research Triangle Park, NC.

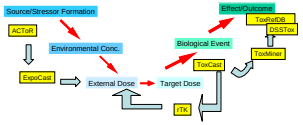
Abstract

The Food Quality Protection Act of 1996 mandates that EPA implement a validated screening program for detecting estrogenic chemicals, as well as other endocrine targets deemed appropriate by the Administrator. EPA's Endocrine Disruptor Screening Program (EDSP) has been developing and validating screening assays for disruption of estrogen (E), androgen (A) and thyroid (T) signaling pathways. The EDSP includes in vitro and in vivo assays for detecting E, A or T activity; and 73 chemicals have been proposed for initial screening. ToxCast is an EPA research program using a broad range of high-throughput screens to profile the bioactivity of chemicals and develop predictive signatures of toxicity, based on modeling in vitro assay data to in vivo toxicity phenotypes. ToxCast profiled 56 of the 73 EDSP chemicals using in vitro assays which characterized receptor binding, activation, inhibition and target gene regulation, providing biological fingerprints relevant to E, A, T and other endocrine related activities. Of the over 600 ToxCast assays, six assess E, and 5 each are related to A and T receptor signaling. In addition to E, A and T endpoints, ToxCast also measured interactions with progesterone, glucocorticoid and PPAR receptors, aromatase activity, and other nuclear receptors including AhR, CAR, FXR, LXR and PXR that may modulate endocrine metabolism. Many assay targets were human proteins, but in some cases rodent or other species were targeted, affording cross-species comparisons. Results for the prototypic xenoestrogen bisphenol A, and the anti-androgen vinclozolin support the ability of ToxCast to identify potential endocrine disruptors, while screening other endpoints beyond E, A and T offers broader insights into the bioactivity of the EDSP chemicals. *Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.*

Transforming Toxicology

Science, Feb 15, 2008

Applying Computational Toxicology Along the Source to Outcome Continuum



ToxCast Background

Prioritization Product Timeline

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
Ia	320	Data Rich (pesticides)	Signature Development	552	$25k	FY08
Ib	15	Nanomaterials	Pilot	366	$50K	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	~$80-150k	FY09
IIb	>300	Known Human Toxicants	Extrapolation	>400	~$80-150k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	~$80-150k	FY10
IId	>12	Nanomaterials	PMN	>300	~$15-20K	FY08-10
III	Thousands	Data poor	Prediction and Prioritization	>300	~$15-20k	FY11-12

FY07 FY08 FY09 FY10 FY11 FY12

Proof of Concept: ToxCast | Verification/Extension | Reduce to Practice

Tox21

ToxCast Phase I Datasets

- **ToxCast 1.0 (April, 2007)**
  - Enzyme inhibition/receptor binding HTS (Novascreen)
  - NR/transcription factors (Attagene, NCGC)
  - Cellular impedance (ACEA)
  - Complex cell interactions (BioSeek)
  - Hepatocellular HCS (Cellumen)
  - Hepatic, renal and airway cytotoxicity (IVAL)
  - In vitro hepatogenomics (IVAL, Expression Analysis)

19 assay sources, over 500 endpoints and continuing to expand

- **ToxCast 1.1 (January, 2008)**
  - Neurite outgrowth HCS (NHEERL)
  - Cell proliferation (NHEERL)
  - Zebrafish developmental toxicity (NHEERL)
- **ToxCast 1.2 (June, 2008)**
  - XME Gene Regulation (CellzDirect)
  - HTS Genotoxicity (Gentronix)
  - Organ toxicity: reverse dosimetry (Hamner Institutes)
  - Toxicity and signaling pathways (Invitrogen)
  - C. elegans WormTox (NIEHS)
  - Gene markers from microscale cultured hepatocytes (MIT)
  - 3D Cellular Zebrafish vascular/cardiotoxicity (Zygogen)
  - Microarrayed metabolic components (Solidus)
  - HTS stress response (NHEERL/NCGC)

Approach

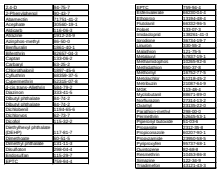
- Extract in vitro endpoints from ToxCast
  - Retricted Assay Set – limited to estrogen, androgen and thyroid related assays, plus aromatase
    - Binding and reporter assays from NovaScreen, Attagene, CellzDirect and the NCGC
    - Flag a pathway if any component assay is considered to have a 'hit'
  - Expanded Assay Set – Restricted Set plus assays covering other nuclear receptors (e.g., CAR, PXR, PPAR) and cytochromes
- Filter chemical library for Tier 1 priority chemicals (n=55 of 73 covered), plus Bisphenol A and vinclozolin as positive controls
- Compare Restricted and Expanded assay clusters with multigeneration clusters derived from ToxRefDB

Endocrine Screening

Proposed EDSP Tier 1 Battery

In vitro
[1]Estrogen receptor (ER) binding – rat uterus
Estrogen receptor a (hERa) transcriptional activation - Human cell line (HeLa-9903)
Androgen receptor (AR) binding – rat prostate
[1,2]Steroidogenesis – Human cell line (H295R)
[2]Aromatase – Human recombinant
In vivo
Uterotrophic (rat)
Hershberger (rat)
Pubertal female (rat)
Pubertal male (rat)
[3]Amphibian metamorphosis (frog)
[3]Fish short-term reproduction

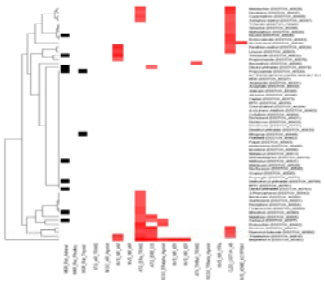
EDSP Priority Chemicals in ToxCast Phase 1 Library



Nuclear Receptor (NR) Assays in ToxCast

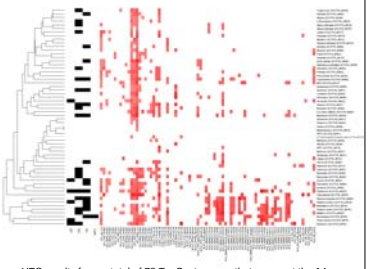
ToxCast Profiling

Restricted Set (14 Assays)



HTS results from 14 ToxCast assays directly related to E/A/T activity. Assay are grouped left to right as androgen (4 assays), estrogen (5 assays), thyroid (4 assays) and aromatase (1 assay) related. The black bars on the left side designate occurrence of a few selected endocrinopathies seen in multi-generation studies.

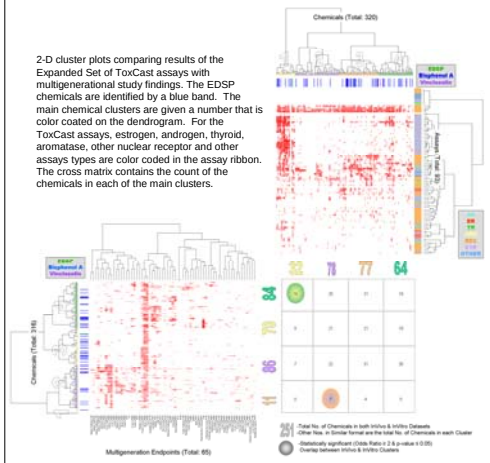
Expanded Subset (78 Assays)



HTS results from a total of 78 ToxCast assays that augment the 14 directly related to androgen, estrogen, thyroid and aromatase activities (results from those are displayed as composite pathway calls in the black bars on the left side). These new assays include a number of other nuclear receptors and cytochrome P450s. The assays are grouped by vendor source and listed alphabetically on the x-axis.

In vivo-in vitro Linkages

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.



Strengths and Limitations of ToxCast Endocrine Profiling

Strengths
- Inexpensive coverage of multiple endocrine pathways
- Many assays involve human derived endocrine targets
- Utilization of different technology platforms for redundancy
- Built in redundancy supports weight of evidence interpretations
- Readily scalable to large numbers of chemicals
- Facilitates prioritization based on biological measures
- Adaptable to changes in technology
- Current effort provides *a priori* predictions of Tier 1 results

Limitations
- Lack of robust intrinsic metabolic capability in most assays
- Determination of a 'hit' in any assay dependent on a number of aspects such as potency, efficacy and curve fitting techniques
- Exclusion of endocrine feedback loops present *in vivo*
- Limited coverage of thyroid toxicity pathways
- *In vivo* relevance of effective *in vitro* concentrations unknown
- Current predictive power for Tier 1 results is unknown