

# Biological Profiling of Endocrine Related Effects of Chemicals in ToxCast™



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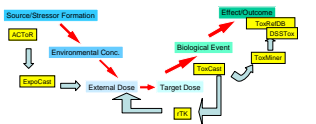
## Abstract

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



## 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	100	Data Rich (Screenable)	Signature Identification	500	100k	FY08
II	10	Non-screenable	Final	500	100k	FY08
III	1000	See-It-Now Chemicals	Validation	1000	100-200k	FY09
IV	1000	Human Protein Targets	Expansion	1000	100-200k	FY09
V	1000	Expanded Biotech and New Biotech	Expansion	1000	100-200k	FY10
VI	100	Non-screenable	Final	1000	100-200k	FY10-11
VII	10000	New year	Finalized Product Set	1000	100-200k	FY11-12



### ToxCast Phase I Datasets

- Released to Data Analysis Partners:
  - NCBA - Rodentia Cell Electronic Screening (7 assays)
  - NIH - Transcription factor assays (3 assays)
  - NIH - Cell based protein-protein assays (3 assays)
  - Colburn - Cell signaling assays (11 assays + 2 cell types)
  - CellDirect - Nucleosome gene expression assays (11 assays)
  - CellDirect - Downstream Gene Tox assays (1 assay)
  - NCSC - nuclear receptor assays (11 assays)
  - Novartis - Cdk2/cdk7 - receptor binding and enzyme inhibition assays (20 assays)
  - Novartis - P450 cytochrome assays (6 assays)
- Upcoming Dataset Additions:
  - Neuro - integrin - hCE (Novartis)
  - Cell proliferation (Novartis)
  - Daphnia developmental toxicity (Novartis)
  - Single receptor, signaling pathway (Novartis)
  - C. elegans WormTox (Novartis)
  - Gene regulation from transcriptional control (Novartis)
  - 21 Cellular pathways from transcriptome (Novartis)
  - HTS stress response (Novartis/NCSC)
  - Estrogen - Non-Cdk (Novartis)
  - Metabolic - Phenotyping (Novartis)

## Approach

- Extract *in vitro* endpoints from ToxCast
  - Restricted Assay Set - limited to estrogen, androgen and thyroid related assays, plus aromatase
    - Binding and reporter assays from NovaScreen, Attagene, CellDirect and the NCCG
    - 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 ToxReIDB

## Endocrine Screening

### Proposed EDSP Tier 1 Battery

In vitro
Estrogen receptor (ER) binding - rat uterus
Estrogen receptor alpha (hERα) transcriptional activation - Human cell line (HeLa-990)
Androgen receptor (AR) binding - rat prostate
17β-Steroidogenesis - Human cell line (H295R)
Aromatase - Human recombinant
In vivo
Ovariectomized (OVX)
Male/female (rat)
Pubertal male (rat)
Pubertal female (rat)
Ampibian metamorphosis (frog)
Fish short-term reproduction

### EDSP Priority Chemicals in ToxCast Phase 1 Library

Chemical	ER	AR	17β-Steroidogenesis	Aromatase	OVX	Pubertal male	Pubertal female	Amphibian	Fish
Bisphenol A	+	+	+	+	+	+	+	+	+
Vinclozolin	+	+	+	+	+	+	+	+	+
...	...	...	...	...	...	...	...	...	...

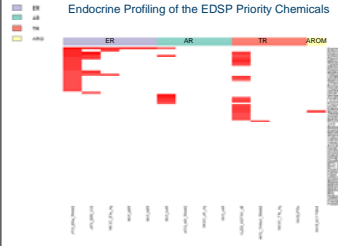
### Nuclear Receptor (NR) Assays in ToxCast



## ToxCast Profiling

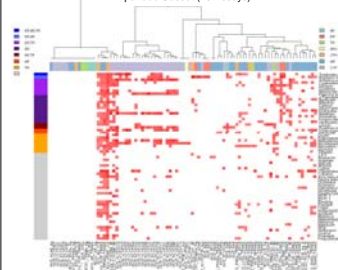
Restricted Set (14 Assays)

### Endocrine Profiling of the EDSP Priority Chemicals



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.

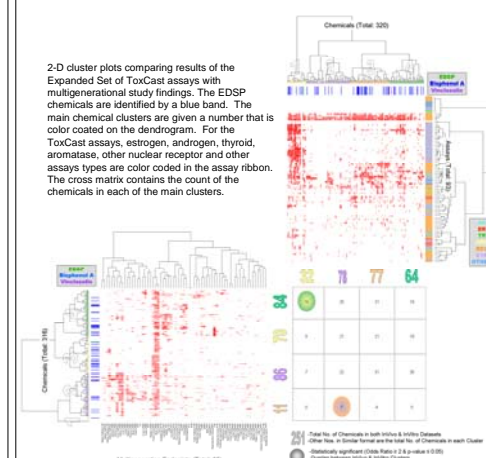
### Expanded Subset (78 Assays)



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 coded 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

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