Biochemical Activities of 320 ToxCast™ Chemicals Across 239 Functional Targets

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1. INTRODUCTION

ToxCast™: EPA's high-throughput chemical screening and prioritization research program, ToxCast™ was implemented to identify predictive 'toxicity signatures' for environmental chemicals. Phase-I explores 320 chemicals with rich *in vivo* toxicity data (www.epa.gov/ncct/toxcast/).

Study goal: To profile the 320 chemical library against a set of cell-free biochemical assays (NovaScreen) covering an array of molecular targets in metabolic and signaling pathways.

2. STUDY DESIGN

Chemical library: includes 309 unique structures, 5 duplicates that were differently sourced, and 3 triplicates as technical repeats. For a complete listing of the 320 chemicals (mostly pesticides, antimicrobials) visit the NCCT website at www.epa.gov/ncct/toxcast/. Chemical samples were QA'd by structure-annotation (www.epa.gov/ncct/dsstox/) and plated by BioFocus DPI (South San Francisco CA).

Assay portfolio: NovaScreen assays were run by Caliper Discovery Alliances and Services (Hanover MD). Details on individual assays, QA methods and literature references can be found at www.caliperls.com/products/contract-research/. The ToxCast™ assay portfolio included mostly human and rat orthologs distributed as follows:

- 79 G-protein coupled receptor binding assays (GPCR)
- 32 CYP450 related enzyme activities (ADME)
- 82 kinase-phosphatase-protease enzyme activities (ENZ)
- 15 nuclear receptor binding assays (NR)
- 18 ion channel activities (IC)
- 9 transporter proteins (TR)
- 2 mitochondrial protein transporters (MP)
- 2 other receptor types (OR)

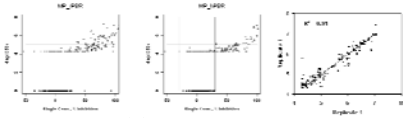
Assay series: preliminary screen for 320 chemical x 231 assay combinations (n=1) run at a single concentration (10 µM CYPs, 25 µM others); and definitive screen run on chemical-assay pairs selected from the range-finder, and eight new assays, run at 8-point concentration series (0.009 - 20 µM for CYP assays otherwise 0.023 - 50 µM).

3. NOVASCREEN

Preliminary screen: Based on analysis of the chemical replicates a 30% inhibition of activity was a conservative metric to define a chemical-assay 'hit'. The distribution of hits for 320 chemicals across 231 target assays is shown.

Assay Bins		*Chemical Bins*	
# target assays	*% active chemicals*	*# active chemicals*	*% assays hit*
40	0.0 %	72	0.0 %
160	0.1 - 2.5 %	153	0.1 – 2.5 %
14	2.6 – 5.0 %	39	2.6 – 5.0 %
9	5.1 – 10.0 %	33	5.1 – 10.0 %
6	10.1 – 20.0 %	15	10.1 – 15.0 %
7	20.1 – 30.0 %	4	15.1 – 20.0 %
3	30.1 – 60.0 %	4	20.1 – 30.0 %

Definitive screen: Selected chemical-assay pairs were re-tested in concentration range. IC50 (50% inhibition) was derived from a Hill function curve-fit with GeneData Screener software (GeneData, Inc., Basel). IC50 values were transformed to –log10(M).



Concordance: IC50 (definitive) versus %-inhibition (preliminary) screens shown for the mitochondrial peripheral-type benzodiazepine receptor (PBR) of rat (*left panel*) and human (*middle panel*) as an example. Chemicals not included in the definitive screen are assigned IC50 = 1 M. Global correlation (*right panel*) compares replicate IC50 across 132 assays (R^2 = 0.91).

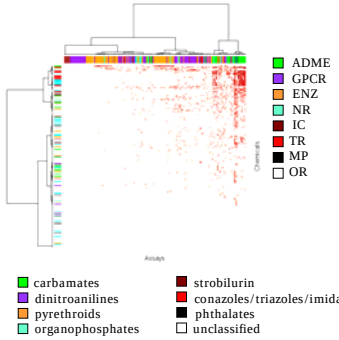
Chemicals with multi-site activity pesticidal mode of action

Chemical	Assays Hit [1]	Rank order in ToxCast_320
Mancozeb	60	1
Maneb	48	5
Metiram-zinc	38	8
Thiram	15	47
Captan	13	52
Chlorothalonil	10	68
Dichloran	9	76
Folpet	5	103
Chloroneb	0	249+

[1] rank in 320 is position in the ordered list by number of hits

4. CHEMICAL-ASSAY HIERARCHY

Data representation: hierarchical relationship for all 320 chemicals x 239 assay combinations based on –log10(IC50). Heatmap scales from 0 (pale) to 8 (dark). *Left color ribbon* indicates chemical rows by class, and *Upper color ribbon* indicates assay column by type.

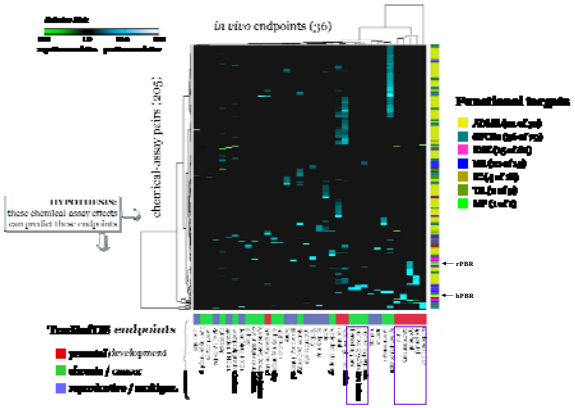


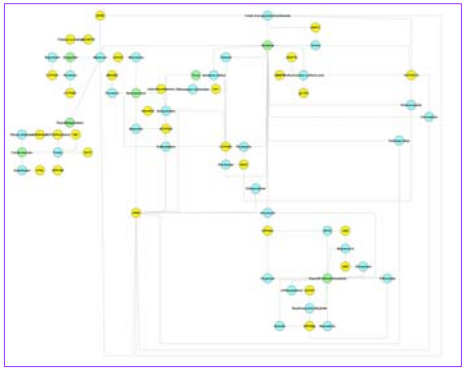
5. CORRELATION WITH *in vivo* TOXICITY

ToxRefDB: NovaScreen is one of several inputs being used to predict *in vivo* toxicity from LEL (lowest effect level) dose in ToxRefDB (www.epa.gov/ncct/toxrefdb/). This addresses two-year cancer / chronic studies, multigenerational reproductive studies, and prenatal developmental studies.

Relative Risk: computed by dichotomizing NovaScreen and ToxRefDB datasets in a 2x2 contingency table for each assay-endpoint pair, calculating a Fisher's exact test p-value to select all significant correlations ($p \leq 0.05$), and estimating *Relative Risk* for chemical-assay → chemical-endpoint correlation (see plots to the right).

SUMMARY: ADME and MP were *sensitive* targets (hit by many chemicals), and GPCR, NR, ENZ were *specific* targets (hit by few chemicals). Assays could be grouped by predictive capacity for ToxRefDB endpoints. The strongest predictors based on *Relative Risk*: chemicals → GPCRs → heart development; chemicals → NRs → thyroid hyperplasia / tumors.





This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.