In Vitro Screening of 1877 Industrial and Consumer Chemicals, Pesticides, and Pharmaceuticals in up to 782 Assays: ToxCast Phase I and II

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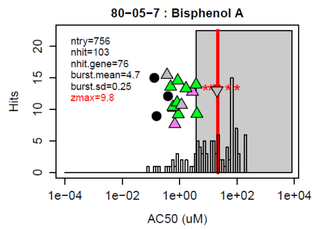
Abstract

In Phase II of the ToxCast program, the U.S. EPA and Tox21 partners screened 1,877 chemicals, including pesticides; food, cosmetics and personal care ingredients; pharmaceuticals; and industrial chemicals. Testing used 782 *in vitro* assays across 7 technologies and multiple biological formats (cell-free, cell lines and primary cells from multiple tissues). Assays were run in concentration-response format (from ~0.01 to ~100 µM) with replicates and controls. We report several key findings. First, in 95% of the genes assayed for which we have reference chemicals (defined as a chemical with a known molecular target), a reference chemical is in the top quartile of potency. Second, ~50% of chemicals show a "burst" of non-specific activity related to cell stress / cytotoxicity. On average, once ~5% of assays are activated by a chemical, there is a linear increase in the number of cytotoxicity/cell-stress-related hits relative to hits in other assays (R^2=0.84). This indicates a potential for assay activity which is not due to the chemical interacting with the intended assay target. Finally, chemicals were ranked by relative potency and target specificity to provide a quantitative metric to prioritize chemicals for further study. About ~1% of chemical-gene combinations show potent and specific activity, defined as AC50<10mM and below the burst, which is ~3 gene hits per chemical on average

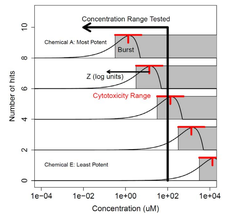
Separation of Selective from Non-Selective Activity



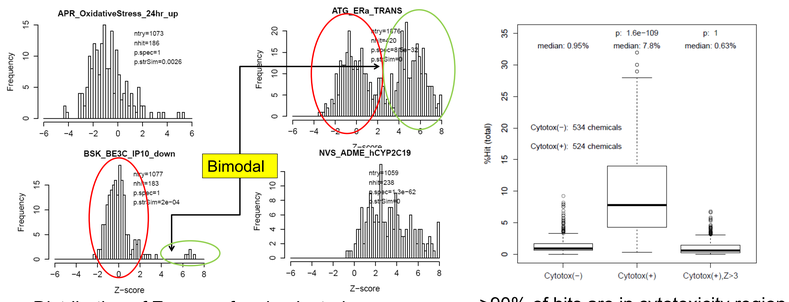
We observe a large number of assay hits in the region of cytotoxicity which we call the "burst".

Potency relative to cytotoxicity is quantified by the cytotoxicity Z-score

Z=N(MAD) below cytotoxicity median

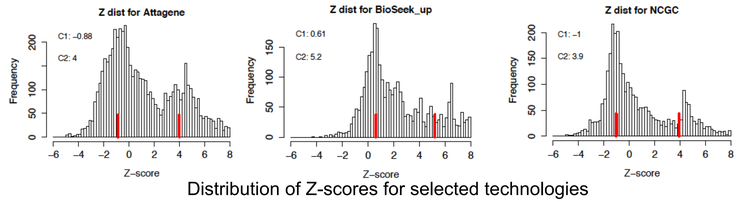


Z-score Examples



Distribution of Z-scores for 4 selected assays

>90% of hits are in cytotoxicity region



Distribution of Z-scores for selected technologies

Promiscuity Analysis by Chemical Class

After excluding low Z-score hits ("non-selective"), we analyzed frequency of hits by chemical class. There is a 10-fold difference in promiscuity between the most promiscuous and the least promiscuous chemical classes

Category	Nchem	Mean Hit Ratio	p-hot
conazole (triazoles)	13	0.034	3.5E-06
Pharma Class 4.86	10	0.031	1.1E-05
Pharma Class 4.58	11	0.029	4.1E-05
conazole (imidazoles)	6	0.031	0.003
Pharma Class 3.292	5	0.039	0.0049
steroid P	5	0.022	0.0052
Pharma Class 4.43	7	0.020	0.0067

Most Promiscuous Chemical Classes

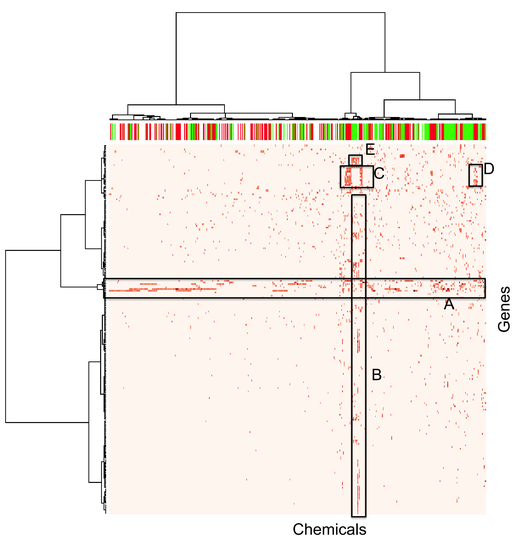
2-3% of assays are active

All designed to be bioactive

Category	Nchem	Mean Hit Ratio	p-cold
alcohol primary	10	0.0011	0.00021
phthalate	17	0.0032	0.00084
carboxylate di	15	0.0028	0.0029
carboxylate	7	0.0015	0.0042

Least Promiscuous Chemical Classes
0.1-0.3% of assays are active

None designed to be bioactive



Heat map of activity in 1000 chemicals x 782 assays, showing promiscuous chemicals and assays, with assays mapped to genes

(A) Most promiscuous genes (COL3A1, SAA1, PTGER2, CYP2C19, NFE2L2, CYP27B1, H2AFX, NR1I2/PXR)

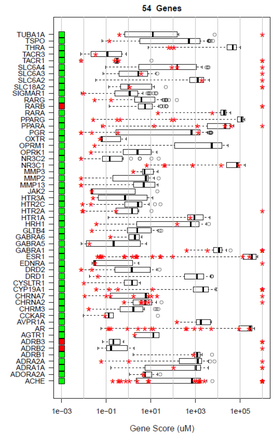
(B) Most promiscuous chemicals (Mancozeb, Titanium chelator, Maneb, Raloxifene, Imazalil, Chlorpromazine, Prochloraz, SSR150106, SSR146977)

(C, D) CYP-450 genes and conazoles

(E) pharmaceuticals and drug targets: CHRM2, SLC6A3, HRH2, ADRA2C, HTR2C, HTR7

Mapping Assays to Genes: Gene Score

Potency: -log(AC50)
log(Gene Score) = mean potency across all assays for a gene



Gene Score Distribution for genes with reference chemicals

Box and whiskers show distribution of Gene Scores (boxes are two inner quartiles, whiskers indicate 95% range)

Red stars show Gene Scores for reference chemicals

95% of genes with good reference chemicals can have those chemicals detected by the relevant assays. This is an indication of the overall reliability of the assay set.

Highest gene scores are mostly pharmaceuticals and pesticidal active ingredients

Chemical Name	Use Category	IntendedTarget	Gene	Gene Score
PharmaGSID_48521	Pharmaceutical		SAA1	12.9
Tributyltin methacrylate	Microbicide		H2AFX	12.8
Cladribine	Pharmaceutical	DNA	H2AFX	12.8
Cytarabine hydrochloride	Pharmaceutical	DNA	H2AFX	12.8
Tributyltin chloride	Microbicide		H2AFX	12.6
Mancozeb	Microbicide		SRC	12.6
Ziram	Fungicide		H2AFX	12.3
Sodium dimethyldithiocarbamate	Chelator		H2AFX	12.1
Simvastatin	Pharmaceutical	HMGCR	THBD	12
FR150011	Pharmaceutical	CYSLTR1	COL3A1	11.9
Pentachlorophenol	Wood preservative		COL3A1	11.7
tert-Butylhydroquinone	Antioxidant		SAA1	11.6
3,3,5,5-Tetraiodothyroacetic acid	Pharmaceutical	THRA	COL3A1	11.5
4-Chloro-1,2-diaminobenzene	Chemical intermediate/dye additive		H2AFX	11.5
2-Aminoanthraquinone	chemical intermediate (dyes and pharmaceuticals)		SAA1	11.5
Dichlorvos	Insecticide	ACHE	SAA1	11.5
Corticosterone	Pharmaceutical	NR3C1	COL3A1	11.4
Tebufenozide	Insecticide	Ecdysone receptor	COL3A1	11.2
Clotrimazole	Fungicide	Yeast 14 demethylase	PTGER2	11.2
Cariporide mesylate	Pharmaceutical	Ion channel Na	COL3A1	11
Triglycidyl isocyanurate	Epoxy hardener		H2AFX	11
Diethyl phthalate	Plastics		COL3A1	10.7
YM218	Pharmaceutical	AVPR1A	SAA1	10.7
Tebuthiuron	Herbicide		COL3A1	10.6
Octhilinone	Fungicide		H2AFX	10.6
PharmaGSID_47261	Pharmaceutical	HIV nucleocapsid protein	PTGER2	10.5
Imazethapyr	Herbicide	ALS	SAA1	10.4
4-Cyclohexylcyclohexanol	Chemical reactant		SAA1	10.3
Cycloate	Herbicide	cyp19a1 (?)	COL3A1	10.2
UK-373911	Pharmaceutical	SLCxAy	H2AFX	10.2
3,5,3'-Triiodothyronine	Pharmaceutical	THRB	SAA1	10.2
Cloprop	Herbicide		COL3A1	10.1
FR900409	Pharmaceutical		COL3A1	10.1
Norflurazon	Herbicide		COL3A1	10.1

Conclusions

The ToxCast program is generating a large set of high-throughput screening data on thousands of chemicals of environmental. This current analysis has shown that active assay information can be roughly divided into two types: selective, meaning occurring at concentrations well below cytotoxicity, and non-selective, which may be a reflection of cell stress or an overwhelming number of chemical-protein interactions within a narrow concentration range. After excluding the non-selective activity, we show that activity for reference compounds is almost always as expected, lending confidence in the data set. These analyses pave the way for the use of the Phase II ToxCast data in more detailed toxicology modeling efforts and evaluating chemical mode-of-action.

U.S. Environmental Protection Agency
Office of Research and Development