Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays

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EPA's Computational Toxicology Communities of Practice
US EPA, May 23, 2012
ToxCast™ Program

Chemical prioritization using in vitro to in vivo correlations

Chemical library

1000s chemicals
• food use pesticides, failed pharmaceuticals, plasticizers, and food additives

in vivo data

in vitro testing

NovaScreen Biochemical Platform

More chemicals screened
Less time and cost
Fewer animals
Based on in vitro targets, pathways and processes

Bioinformatics

www.epa.gov/ncct/toxrefdb/

www.epa.gov/ncct/toxcast/
Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays


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Supporting Information

ABSTRACT: Understanding potential health risks is a significant challenge due to the large numbers of diverse chemicals with poorly characterized exposures and mechanisms of toxicities. The present study analyzes 976 chemicals (including failed pharmaceuticals, alternative plasticizers, food additives, and pesticides) in Phases I and II of the U.S. EPA’s ToxCast project across 331 cell-free enzymatic and ligand-binding high-throughput screening (HTS) assays. Half-maximal activity concentrations (AC50) were identified for 729 chemicals in 256 assays (7,135 chemical–assay pairs). Some of the most commonly affected assays were CYPs (CYP2C9 and CYP2C19), transporters (mitochondrial TSPO, norepinephrine, and dopaminergic), and GPCRs (aminergic). Heavy metals, surfactants, and diisocyanate fungicides showed promiscuous but distinctly different patterns of activity, whereas many of the pharmaceutical compounds showed promiscuous activity across GPCRs. Literature analysis confirmed >50% of the activities for the most potent chemical–assay pairs (54) but also revealed 10 missed interactions. Twenty-two chemicals with known estrogenic activity were correctly identified for the majority (77%), missing only the weaker interactions. In many cases, novel findings for previously unreported chemical–target combinations clustered with known chemical–target interactions. Results from this large inventory of chemical–biological interactions can inform read-across methods as well as link potential targets to molecular initiating events in adverse outcome pathways for diverse toxicities.

Phase I & II, NS Sipes et al 2013, Chemical Research in Toxicology Article ASAP, DOI: 10.1021/tx400021f
NovaScreen Biochemical Assays
-Caliper Life Sciences (a PerkinElmer company)

Purified or recombinant protein

Species
• Human (73%)
• Rat (18%)
• Other (9%)

Binding and enzymatic activity
• Radioligand receptor binding
• Fluorescent receptor binding
• Fluorescent enzyme substrate - intensity quench
• Fluorescent enzyme substrate - mobility shift

http://www.perkinelmer.com/services/contractresearch/default.xhtml
976 chemicals in 331 biochemical assays

<table>
<thead>
<tr>
<th>Protein families</th>
<th># assays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Binding</strong></td>
<td></td>
</tr>
<tr>
<td>G-protein-coupled-receptors</td>
<td>77</td>
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<tr>
<td>Ion channels</td>
<td>20</td>
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<tr>
<td>Nuclear receptors</td>
<td>19</td>
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<tr>
<td>Transporters</td>
<td>11</td>
</tr>
<tr>
<td>Other receptors</td>
<td>3</td>
</tr>
<tr>
<td><strong>Enzymatic activity</strong></td>
<td></td>
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<tr>
<td>Kinases</td>
<td>74</td>
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<tr>
<td>Phosphatases</td>
<td>38</td>
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<tr>
<td>Proteases</td>
<td>30</td>
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<tr>
<td>Cytochrome P450 enzymes</td>
<td>20</td>
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<tr>
<td>Cholinesterase</td>
<td>6</td>
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<tr>
<td><strong>Inhibition &amp; Activation</strong></td>
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<tr>
<td>Other enzyme (COX, HDAC, MAO, PDE, SIRT)</td>
<td>33</td>
</tr>
</tbody>
</table>

Sipes et al 2013, CRT
NovaScreen Workflow
-Caliper Life Sciences (a PerkinElmer company)

Chemical selection
Experimental design
Ordering
Assay annotation

Step 1

Chemical
Assay
single concentration

323,056 chemical-assay pairs
NovaScreen Workflow
-Caliper Life Sciences (a PerkinElmer company)

Chemical selection
Experimental design
Ordering
Assay annotation

Step 1
Raw data → QC → Processing to a usable matrix → Curve-fitting

Did we get what we ordered?

Custom R scripts

24,118 chemical-assay pairs

Chemical
Assay
multi concentration

x 8
Example Chemical-Response Plot

7,135 chemical-assay pairs
- 75% chemicals (729/976)
- 77% assays (256/331)

Assay
Chemical name
CASRN
AC50
Min conc tested
Max conc tested
Emax
Slope
R-squared
Fit p-value

Sipes et al 2013, CRT
NovaScreen Workflow
-Caliper Life Sciences (a PerkinElmer company)

Chemical selection
Experimental design
Ordering
Assay annotation

Did we get what we ordered?

Step 1
Raw data
QC
Processing to a usable matrix
Curve-fitting

Custom R scripts

Data Transformation

- AC50s

Data Analysis

- Statistical software
- Hierarchical clustering
- Correlation matrices
Data Analysis Workflow

Are phylogenetically similar proteins being affected by similar chemicals?

Chemical fragment similarity

Sipes et al 2013, CRT
Hierarchical Clustering

No hits

75% chems (729)
77% assays (256)

Most promiscuous

Sipes et al 2013, CRT
Hierarchical Clustering

High Potency

Assays

Heatmap Key
- No IC50 calculated
- ACS20-pH2
- ACS20-pH4
- No data

Chemicals

Sipes et al 2013, CRT
Mostly pharma

Mostly pesticides

Hierarchical Clustering

Top Promiscuous Chemicals
• Pharma
• Phenylmercuric acetate (fungicide)
• Mancozeb, Maneb, Metiram (fungicide)
• Crystal violet (fungicide/bactericide)
• Sodium dodecylbenzenesulfonate (surfactant)
• Tributyltin methacrylate
• Tributyltin chloride
• Mercuric chloride

Top 20 Promiscuous Assays
• CYPs - 2C19, 2C9, 2B6
• Transporters - TSPO/PBR, NET, DAT
• Receptors – PXR, GR, AR
• GPCR – 5HT7, Opiate µ/K, DRD1
• Other – NaCh_site2, SIGMA, MAOAC, BACE

Sipes et al 2013, CRT
Hierarchical Clustering

**Top Promiscuous Chemicals**

**Heavy metals**
- 1-Phenylmercuric acetate
- 2-Mercuric chloride
- 3-Tributyltin methacrylate
- 4-Tributyltin chloride

**Surfactants**
- 5-Sodium dodecylbenzenesulfonate
- 6-Perfluorooctane sulfonic acid
- 7-Dodecylbenzene sulfonate triethanolamine (1:1))

**Dithiocarbamate fungicides**
- 8-Mancozeb
- 9-Maneb
- 10-Metiram

Sipes et al 2013, CRT
DEHP 5 assays

DEGDB 5 assays

TOTM DOTP DEHA 1/2 assays

DINCH DIDP 0 assays

Phthalate & Alt Plasticizers (34)

Assay Ribbon Key

Activator - Cholinesterase
Activator - CYP
Activator - Kinase
Activator - Other enzyme
Activator - Phosphatase
Activator - Prelease
Cholinesterase
CYP
GPCR (aminergic)
GPCR (other)
Ion channel
Kinase
LGIC (cyc-loop)
LGIC (onconorphic glutamate)
Nuclear receptor (subfamily 1)
Nuclear receptor (subfamily 3)
Other
Other enzyme
Phosphatase
Prelease
Transporter

Heatmap Key

No AUC calculated
AUC0-3h/ugM
AUC0-24h/ugM
No data

Chemicals

Sipes et al 2013, CRT
Anthralin

- Anti-proliferative, anti-inflammatory
- Treat psoriasis
- Unknown mechanism

- 22 assays
- Inflammatory caspases
- MMPs
- Cox1 & 2

Perfluorinated compounds

(13 compounds)

- Lipophilic surfactants

- Leukotriene B4 GPCR (target)
  - 80% of PFCs
  - Fatty acid signaling molecules

Chemicals

Sipes et al 2013, CRT
Most Potent Chemical-Assay Pairs

AC50s @ 23nM (9nM for CYPs)

<table>
<thead>
<tr>
<th>chemical name</th>
<th>AC50s</th>
<th>assay target(s)</th>
<th>refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorpromazine hydrochloride</td>
<td>55</td>
<td>TR_hNET, GPCR_hDRD1/2s, p5HT2C, hH1, rAdr1A/1B</td>
<td>55-58</td>
</tr>
<tr>
<td>haloperidol</td>
<td>42</td>
<td>OR_gSIGMA_NonSelective, GPCR_hDRD1/2s/4.4, GPCR_rAdr1_NonSelective, GPCR_bDR_NonSelective</td>
<td>59-61</td>
</tr>
<tr>
<td>trelanserin (SL650472 pharma)</td>
<td>24</td>
<td>GPCR_h5HT2A/7, p5HT2C, r5HTNonSelective, GPCR_hDRD1/4</td>
<td>62</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>9</td>
<td>NR_hAR, bPR, mERa, hER, bER</td>
<td>63,64</td>
</tr>
<tr>
<td>17α-ethinylestradiol</td>
<td>24</td>
<td>NR_hAR, mERa, hER, bER</td>
<td>65</td>
</tr>
<tr>
<td>CP-471358 pharma</td>
<td>6</td>
<td>ENZ_hMMP13/2/9</td>
<td>66</td>
</tr>
<tr>
<td>CP-544439 pharma</td>
<td>10</td>
<td>ENZ_hMMP13/2/9</td>
<td>67</td>
</tr>
<tr>
<td>diethylstilbestrol</td>
<td>31</td>
<td>NR_mERa, hER, bER</td>
<td>68</td>
</tr>
<tr>
<td>2,2-bis(4-hydroxyphenyl)-1,1,1-trichloroethane</td>
<td>36</td>
<td>NR_mERa, bER, hCAR_Antagonist</td>
<td>69</td>
</tr>
<tr>
<td>zamifenacin (pharma)</td>
<td>60</td>
<td>GPCR_gmPeripheral_NonSelective, hM3/5</td>
<td>70,71</td>
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<tr>
<td>flufenacet</td>
<td>10</td>
<td>MP_rFBR, NR_hPXR</td>
<td></td>
</tr>
<tr>
<td>maneb</td>
<td>62</td>
<td>ENZ_hPTPN9/4</td>
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<tr>
<td>methadone hydrochloride</td>
<td>37</td>
<td>GPCR_rOpiate_NonSelective/Na</td>
<td>72</td>
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<tr>
<td>progesterone</td>
<td>11</td>
<td>NR_hAR, NR_bPR</td>
<td>73</td>
</tr>
<tr>
<td>SR144190 pharma</td>
<td>24</td>
<td>GPCR_hNK2, NR_hPXR</td>
<td>74</td>
</tr>
</tbody>
</table>

• >50% had literature evidence for these associations
  • limited by publically available information

• 20% had literature evidence for additional chemical-target associations missed
  • bioavailability, conc not high enough, species differences, single screen miss

Sipes et al 2013, CRT
Assessing Reliability  EDSP Chemicals

EDSP reference chemicals
22 chemicals
bovine, human, mouse ER

- inactives (6) 100%
- very weak (3) 0%
- weak (9) 78%
- strong actives (3) 100%
- antagonists (1) 100%

Sipes et al 2013, CRT
Assay Similarity Analysis

Number of Chemicals (n)
- $x \leq 10$
- $10 < x \leq 20$
- $20 < x \leq 40$
- $40 < x \leq 100$
- $x < 100$

High Similarity

Low Similarity

GPCR (other)

GPCR (aminergic)

Kinase Phosphatase

Assays

Sipes et al 2013, CRT
GPCR Family Tree

- Not in cluster
- Group 1
- Group 2

**Peptide GPCRs**
- Vasoactive intestinal peptide
- Neuropeptide Y
- Endothelin B
- Serotonin

**Opiate**
- Neuropeptide Y

**Histamine**
- Endothelin B

**Biogenic Amine Receptors**
- Glutamate
- Dopamine
- Adrenergic

http://dx.doi.org/10.1016/j.str.2011.05.012
Chemical Similarity Analysis

Example cluster
- cyanazine
- prometryn
- caffeine
- theophylline
- cladribine

Triazine herbicides
Adenosine signaling or metabolism

Activator - Cholinesterase
Activator – CYP
Activator – Kinase
Activator - Other enzyme
Activator – Phosphatase
Activator – Protease
Cholinesterase
CYP
GPCR (aminergic)
GPCR (Other)
Ion channel
Kinase
LGIC (cys loop)
LGIC (ionotropic glutamate)
Nuclear receptor (subfamily 1)
Nuclear receptor (subfamily 3)
Other
Other enzyme
Phosphatase
Protease
Transporter

What about chemical fragment similarity?

Chemicals

Assay Enrichment

Sipes et al 2013, CRT
Chemical Similarity Analysis

Activator - Cholinesterase
Activator – CYP
Activator – Kinase
Activator - Other enzyme
Activator – Phosphatase
Activator – Protease
Cholinesterase
CYP
GPCR (aminergic)
GPCR (Other)
Ion channel
Kinase
LGIC (cys loop)
LGIC (ionotropic glutamate)
Nuclear receptor (subfamily 1)
Nuclear receptor (subfamily 3)
Other
Other enzyme
Phosphatase
Protease
Transporter

Assay Enrichment

Sipes et al 2013, CRT
Chemical Fragment-Assay Category Associations

- Simply a description of the features within chemicals preferentially affecting these assay groups
- Fragments are not indicating causal association
- Better inform chemical structure models

Sipes et al 2013, CRT
Summary

- ToxCast Phase I & II includes biochemical assay data for 1000 chemicals in >300 assays
- Unique dataset can be used to evaluate additivity of effects across concentration range in combination with cell-based data
- Associations may help inform chemical structure models for predicting chemical-target interactions.
- A combination of these *in vitro* results along with *in vivo* toxicity data are being used in building predictive models for chemical prioritization
Thank you!

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