Application of ToxCast High Throughput Screening to Green Chemical Design

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This work was reviewed by EPA and approved for presentation but does not necessarily reflect official Agency policy.
Why ToxCast?

Too Many Chemicals

- IRIS
- TRI
- Pesticides
- Inerts
- CCL 1 & 2
- HPV
- MPV

Too Little Data (%)

- Acute
- Cancer
- Gentox
- Dev Tox
- Repro Tox

Office of Research and Development
National Center for Computational Toxicology
High Throughput Screening 101 (HTS)

- 96-, 384-, 1536 Well Plates
- Target Biology (e.g., Estrogen Receptor)
- Robots
- Pathway
- Chemical Exposure
- Cell Population
- Target Biology (e.g., Estrogen Receptor)
Pesticides (active and inert), industrial chemicals, consumer products, marketed and failed pharmaceuticals, food additives, water contaminants, natural human metabolites.
Over 500 ToxCast HTS Assays from Contractors and Collaborators

Compound Focus, Inc.
a subsidiary of BioFocus DPI

EPA/ORD/NHEERL
Deepwater Horizon

Oil Exploration Platform Explodes April 20, 2010
• Estimated 4.9 million barrels of South Louisiana Crude released

1.8 million gallons of dispersant used
• 1072K surface; 771K subsea
• Corexit 9500A (9527 early in spill)

EPA Administrator call for less toxic alternative
• Verification of toxicity information on NCP Product Schedule
• ORD involvement in assessments of dispersant toxicity
Goals of the NCCT Oil Dispersants Project

• Test 8 candidate dispersants for endocrine (ER, AR, TR) activity
  – Driven by fact that some dispersants contain nonylphenol ethoxylates, known ER agonists

• Evaluate relative cytotoxicity

• Look for other types of bioactivity using broad in vitro screen

• Return analysis in ~6 weeks
## The Dispersants

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Volume Received</th>
<th>Comments</th>
<th>Date Received</th>
<th>Manufacturer/ Source</th>
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<tbody>
<tr>
<td>Corexit 9500</td>
<td>1 L</td>
<td>hazy yellow</td>
<td>11-May-10</td>
<td>Nalco</td>
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<tr>
<td>JD 2000</td>
<td>10 ml</td>
<td>clear yellow</td>
<td>27-May-10</td>
<td>Ethox Chemicals, LLC</td>
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<td>DISPERSIT SPC 1000</td>
<td>10 ml</td>
<td>clear amber</td>
<td>27-May-10</td>
<td>Polychem</td>
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<td>Sea Brat #4</td>
<td>10 ml</td>
<td>hazy yellow</td>
<td>27-May-10</td>
<td>Alabaster Corp</td>
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<tr>
<td>Nokomis 3-AA</td>
<td>10 ml</td>
<td>clear light color</td>
<td>27-May-10</td>
<td>MAR-LEN Supply inc.</td>
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<td>MAR-LEN Supply inc.</td>
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<td>ZI-400</td>
<td>25 ml</td>
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<td>ZI Chemical</td>
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<tr>
<td>SAF-RON GOLD</td>
<td>500 ml</td>
<td>silver iridescent</td>
<td>4-June-10</td>
<td>Sustainable Environmental Technologies, Inc.</td>
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</table>
Dispersant Cytotoxicity Results

Significantly more cytotoxic (statistically but not biologically)

- More potent
- Less potent

Bottom line: no significant difference across products

- Attagene: HepG2
- NCGC: Bla ER/AR
- NHEERL: T47D, MDA, CV1
Concentration-Response Profiles for ER

**Estradiol and Nonylphenol compounds**

ERa.T = Attagene ERa TRANS
ERE.C = Attagene ERa CIS

CIS Efficacy less than half TRANS efficacy for reference compounds

**Dispersant Positives**

TRANS assay efficacy near detection threshold for these dispersants
Bottom line: 2 products show weak estrogen activity
“Not Significant Biologically”
Dispersant Conclusions

• Weak evidence of ER activity in 2 dispersants
  – Seen in single, perhaps over-sensitive assay (1 of 6)
  – Not of biological significance
  – Consistent with presence of NPE
  – Activity only at concentrations >> seen in Gulf after dilution

• No AR activity

• No ER activity seen in Corexit 9500

• Corexit is in the middle of the pack for cytotoxicity

• No worrisome activity seen in other NR assays

Analysis of Eight Oil Spill Dispersants Using In Vitro Tests for Endocrine and Other Biological Activity, June 30, 2010
(PDF 47pp)
Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity.
ToxPi: Prioritizing Chemicals for EDSP21 and Potential Endocrine Activity

Prioritization Index = ToxPi = f(HTS assays + Chemical properties + Pathways)

ToxPi Ranking of Chemicals

309 Chemicals sorted by ToxPi

Linuron

Methoxychlor

Bisphenol A

Pyrimethanil

Tebuthiuron

EDSP chemical
ToxCast chemical (not on EDSP list)

Reif et al, 2010
Bioactivity Signature Workflow

ToxCast HTS data (AC50s)

<table>
<thead>
<tr>
<th>(+)ve</th>
<th>(-)ve</th>
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</thead>
<tbody>
<tr>
<td>TP</td>
<td>FP</td>
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<tr>
<td>FN</td>
<td>TN</td>
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</table>

ToxRefDB

UNIVARIATE ASSOCIATIONS
- t-test (continuous assay data)
- classifier (2x2 contingency table)
- Fisher’s exact test (dichotomous data)

MULTIVARIATE MODELS
- machine learning
- 5-fold cross-validation (80/20 split)
- sensitivity analysis (ROC curves)

MULTICELLULAR MODELS
- knowledge-base (VT-KB)
- computer simulations (VT-SE)
- biological inferences / mechanisms
Predictive model of rat reproductive toxicity from ToxCast high throughput screening.
Martin MT, Knudsen TB, Reif DM, Houck KA, Judson RS, Kavlock RJ, Dix DJ.
Predictive models of prenatal developmental toxicity from ToxCast high-throughput screening data.
Sipes NS, Martin MT, Reif DM, Kleinstreuer NC, Judson RS, Singh AV, Chandler KJ, Dix DJ, Kavlock RJ, Knudsen TB.
Endothelial proliferation, migration & sprouting (VEGF, Chemokines)

- Extracellular matrix degradation (uPAR, PAI-1, MMPs)

- Neovascular stabilization (Ang/Tie2)

Cell-agent-based models

- stochastic cellular behaviors
- specified cellular activities
- PDE solvers for biochemical gradients
- toolbox of morphogenetic processes
- executes collective cell behavior
- enables emergent properties

CompuCell3D.org
# Molecular Signals and Cellular Behaviors of Angiogenesis

<table>
<thead>
<tr>
<th>Molecular Signal</th>
<th>Expression</th>
<th>Chemotaxis</th>
<th>Proliferation</th>
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<tbody>
<tr>
<td>VEGF165</td>
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<td></td>
<td></td>
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<td>Proteases</td>
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<td></td>
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<td>Ang1</td>
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<tr>
<td>Tie2</td>
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</tr>
<tr>
<td>uPAR</td>
<td></td>
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<td></td>
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<tr>
<td>KDR (VEGFR2)</td>
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</tbody>
</table>

- **Endothelial Stalk (ECs)**
- **Mural Cell (MC)**
- **Endothelial Tip (ECT)**
- **Inflammatory Cell (IC)**
Angiogenetic field at time=0

SOURCE: Kleinstreuer et al. (in preparation)
Virtual angiogenesis

VEGF 165

Ang1/Tie2

MMPs

CXCL10

VEGF 121

CCL2

sFlt1
**In vitro**


(experimental, 100 uM)

**In silico**

*SOURCE: Kleinstreuer et al. 2011, in preparation* (ToxCastDB, 40 uM)

TEST CASE: Thalidomide and 5HPP-33

Quantitative prediction emergent from ToxCast HTS data input (AC50s)
Inhibition of Endothelial Cell Proliferation

HUVEC Proliferation: PFAA_Alt1

Inhibition of Endothelial Cell Proliferation

HUVEC Proliferation: PFAA_Alt2

HUVEC Proliferation: PFOS

HUVEC Proliferation: PFOA
PFOA, PFOS, and PFAA Alternatives: *in silico* vasculogenesis assay

- PFOA
- PFOS
- PFAA_Alt1
- PFAA_Alt2
Quantitative Effects: in silico chemical exposure

Total Cell Number

Total VEGF Concentration
Green Chemistry and CompTox

- Less expensive, higher throughput approach to hazard prediction
- Prioritization of green alternative chemicals for further development and testing
- High throughput exposure predictions also needed

Prioritization, Predictive and Systems Models

In vitro testing

Toxicity Pathways

BPA

TGSA