Predictive Signatures of Developmental Toxicity Modeled with HTS data from ToxCast™ Bioactivity Profiles


Disclaimer: views are those of the presenter and do not necessarily reflect Agency policy nor imply endorsement of software used here

SOT, abstract 1382, 03/09/2010
ToxCast™

http://www.epa.gov/ncct/toxcast/

- project to profile the bioactivity of hundreds to thousands of environmental chemicals using *in vitro* HTS assays,
- mine for *in vivo* correlations by training *in vitro* bioactivity profiles against compounds with evident toxicity,
- build and test computational (*in silico*) models for ‘toxicity signatures’ that predict subtending biological pathways,
- and prioritize chemicals that inform mechanistic models during chemical disruption (e.g., embryonic development)
ToxCast™ bioactivity profiling

### Biochemical HTS assays

- **Protein families**
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter

- **Assay formats**
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

### Cell-based assays

- **Cell lines**
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
  - J1 mouse ES cells (ACDC)

- **Primary cells**
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells

- **Biotransformation competent cells**
  - Primary rat hepatocytes
  - Primary human hepatocytes

- **Assay formats**
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

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**309 chemicals**

**471 endpoints**
**Profiling developmental toxicity**

ToxRefDB: >30 yrs of toxicity data worth >$2B

*in vivo* bioassays

(targe, description)

- **target:** kidney
  - description: absent renal papilla
  - code: UG_REN_3.1060.5013

- **target:** sternum
  - description: incomplete ossification
  - code: SK_AXL_2.1039.513D

- **target:** hindpaw
  - description: polydactyly (digit 1)
  - code: SK_APP_2.1051.5234

ToxRefDB 387 chemicals, 751 prenatal studies, 988 effects annotated

283 chemicals x 293 effects → 19 target systems from rat (■) and rabbit (□) studies

Developmental effects (ToxRefDB): cLEL based on mg/kg/day administered dose

<table>
<thead>
<tr>
<th>CRITICAL ENDPOINT</th>
<th>NUMBER of CHEMICALS</th>
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<tbody>
<tr>
<td></td>
<td>rabbit</td>
<td>rat</td>
<td>overlap</td>
</tr>
<tr>
<td>Developmental (global)</td>
<td>111</td>
<td>153</td>
<td>70</td>
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<tr>
<td>Skeletal_Axial</td>
<td>55</td>
<td>118</td>
<td>18</td>
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<tr>
<td>FetalWeightReduction</td>
<td>49</td>
<td>92</td>
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<tr>
<td>Skeletal_Appendicular</td>
<td>24</td>
<td>50</td>
<td>7</td>
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<tr>
<td>Skeletal_Cranial</td>
<td>21</td>
<td>41</td>
<td>1</td>
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<tr>
<td>Embryo-Fetal losses</td>
<td>33</td>
<td>35</td>
<td>5</td>
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<tr>
<td>Urogenital (renal, ureteric)</td>
<td>3</td>
<td>19</td>
<td>0</td>
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<tr>
<td>JawHyoid</td>
<td>8</td>
<td>14</td>
<td>0</td>
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<tr>
<td>CleftLipPalate</td>
<td>2</td>
<td>11</td>
<td>0</td>
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<tr>
<td>Neurosensory (brain and eye)</td>
<td>6</td>
<td>8</td>
<td>0</td>
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<tr>
<td>BodyWall (somatic)</td>
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<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Viscera (splanchnic)</td>
<td>9</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Cardiovascular (heart, major vessels)</td>
<td>6</td>
<td>3</td>
<td>0</td>
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</table>

SOURCE: http://www.epa.gov/NCCT/toxrefdb/
Assay-DevTox associations: distribution by HTS assay platform

894 total univariate DevTox associations from ToxMiner v16
DevTox targets in ToxCast™

nonredundant **assays** (154 annotated by target gene function tested) selected by significant AC50 - cLEL correlation and mapped across the prenatal ‘penetration spectrum’:

- **FWR** fetal weight reduction
- **MAL** abnormalities and variations
- **RES** resorptions-fetal death

**TARGETS**

<table>
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<tr>
<th>Assays</th>
<th>FWR</th>
<th>MAL</th>
<th>RES</th>
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<tr>
<td>21</td>
<td>98</td>
<td>104</td>
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</table>

**Pathways** inferred from ‘perturbation score’ (PS)

**Pathways**

<table>
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<tr>
<th>Pathways</th>
<th>FWR</th>
<th>MAL</th>
<th>RES</th>
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<td>75</td>
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*SOURCE: ToxMiner v16, NCCT*
Signature detection

SOURCE: NCCT – N Kleinstreuer, built with 'linmod from R Judson [presented 03/08/2010, abstract 96]
Multivariate - placeholder
Predictive signatures: *challenges*

- Predictive modeling of an effect is complicated by the inherent nonlinearity of biological systems.
- Even homogeneous cell populations *in vitro* can display complex responses to environmental chemicals.
- Toxicity in an intact organism results from numerous complex and inter-related events at a multi-cellular scale.
- Holy Grail: *in silico* reconstruction of tissues to evaluate biological plausibility of predictive signatures.
Cell networks - placeholder
Modeling Morphogenesis

-cell growth & death

induced by FGFs from the AER

Polarized limb outgrowth

Modeled in www.CompuCell3d.org environment
N Poplowski (chick limb) → M Rountree (mouse limb)
toxicity in the embryo is an expression of complex and interwoven events that follow from cellular perturbation

ToxCast™ is a resource to compile *in vitro* signatures into computational models that are diagnostic of *in vivo* toxicity

systems-level models that recapitulate *in vivo* biology can be used to assess the plausibility of diagnostic signatures

multicellular ‘virtual tissues’ can help bridge the gap between *in vitro* profiling and *in vivo* response
Research Network

Virtual Embryo (NCCT)
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Amar Singh (LHM)
Michael Rountree (SSC)
Richard Spencer (EMVL)
Rob DeWoskin (NCEA)
Nikal Keinstreuer
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Virtual Embryo (NHEERL)
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Crowley-Davis (Endogenics)
Richard Newman
Tim Otter
Jeff Habig

EU interactions
Virtual Physiome
ChemScreen (2010)

http://www.epa.gov/ncct/v-Embryo/