Application of computational and high-throughput *in vitro* screening for prioritization

Richard Judson  
*U.S. EPA, National Center for Computational Toxicology  
Office of Research and Development*

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The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA
Major Points

• EDSP has a mismatch between resources needed for Tier 1 and number of chemicals to be tested
  – ~10,000 chemicals in EDSP Universe
  – ~$1M per chemical for Tier 1, 50-100 year backlog

• Need new approach
  – Prioritize chemicals
  – Replace low-throughput assays with high-throughput variants

• Demonstrate new approach: Estrogen receptor
  – Multiple high-throughput in vitro assays
  – Demonstrate use to prioritize chemicals and replace selected Tier 1 assays
In Vitro Estrogen Receptor Model
Combines results from multiple in vitro assays

- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway

- No assay is perfect
  - Assay Interference
  - Noise

- Use model to integrate assays

- Evaluate model against reference chemicals

- Methodology being applied to other pathways

**In vivo guideline study uncertainty**

26% of chemicals tested multiple times in the uterotrophic assay gave discrepant results.

**Immature Rat: BPA**

- **Phenotype X**

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<th>species / study 2</th>
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<th>Does Not Reproduce</th>
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Kleinstreuer et al. EHP 2015
In vitro assays also have false positives and negatives

Assays cluster by technology, suggesting technology-specific non-ER bioactivity

Much of this “noise” is reproducible
- “assay interference”
- Result of interaction of chemical with complex biology in the assay

EDSP chemical universe is structurally diverse
- Solvents
- Surfactants
- Intentionally cytotoxic compounds
- Metals
- Inorganics
- Pesticides
- Drugs

Assay-to-assay variation

All appropriate assays are active but efficacy and potency vary

“Noise” or real variation in biology between cell types?

**In Vitro Reference Chemical Performance**

**Agonist**

- Moderate: 17α-Estradiol
- Strong: Diethylstilbestrol (DES)
- Strong: 17β-Estradiol
- Strong: 17α-Ethynyl estradiol
- Strong: mestranol
- Moderate: Estrone
- Weak: Genistein
- Weak: Bisphenol B
- Weak: Bisphenol A
- Weak: Daidzein
- Moderate: 4-tert-Octylphenol
- Weak: 4-Cumylphenol
- Weak: 5α-Dihydrotestosterone
- Weak: p,p’-DDT
- Very Weak: 17α-Methyltestosterone
- Very Weak: Apigenin
- Very Weak: Methoxychlor
- Very Weak: Kaempferol
- Very Weak: Butylbenzyl phthalate
- Weak: Kepone
- Very Weak: Chrysin
- Very Weak: Ethylparaben
- Very Weak: p,p’-DDE
- Very Weak: p-n-Nonylphenol
- Very Weak: Fenarimol
- Very Weak: Di-n-butyl phthalate
- Inactive: Haloperidol
- Inactive: Spirolactone
- Inactive: Reserpine
- Inactive: Procymidine
- Inactive: Phenobarbital Sodium
- Inactive: Linuron
- Inactive: Ketoconazole
- Inactive: Hydroxyflumethamide
- Inactive: Flutamide
- Inactive: Cycloheximide
- Inactive: Corticosterone
- Inactive: Atazanavir
- Very Weak: Diethylhexyl phthalate
- Very Weak: Diclof

**Antagonist**

- Active: Raloxifene
- Active: 4-Hydroxytamoxifen (E/Z)
- Active: Tamoxifen citrate
- Active: Tamoxifen
- Inactive: Di-n-butyl phthalate
- Inactive: Diclofenac
- Inactive: Kepone
- Inactive: Diethylstilbestrol (DES)
- Inactive: 17α-Ethynyl estradiol
- Inactive: Genistein
- Inactive: Bisphenol A
- Inactive: Safflower-Clinium
- Active: Apigenin
- Inactive: Butylbenzyl phthalate
- Inactive: Chrysin
- Inactive: p,p’-DDE
- Inactive: Progesterone
- Inactive: Diethylhexyl phthalate
Identifying Uterotrophic Reference Chemicals from the Literature

Literature Searches: 1800 Chemicals

Data Review: 700 Papers, 42 Descriptors, x2

Uterotrophic Database
98 Chemicals
442 GL uterotrophic bioassays

High-Level Filter

6 Minimum Criteria

"Guideline-Like" (GL)

Selection Criteria

In Vivo ER Reference Chemicals
30 Active, 13 Inactive

Kleinstreuer et al: “A Curated Database of Rodent Uterotrophic Bioactivity” (submitted)
Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic.

Restrict to chemicals with consistent results from the literature.

<table>
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<tr>
<th></th>
<th>Value</th>
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<tbody>
<tr>
<td>True Positive</td>
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</tr>
<tr>
<td>True Negative</td>
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</tr>
<tr>
<td>False Positive</td>
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</tr>
<tr>
<td>False Negative</td>
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</tr>
<tr>
<td>Accuracy</td>
<td>0.97</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.97</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.98</td>
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</table>

Explicitly Add Uncertainty to In Vitro Assay Data

Watt et al. (in prep)
CERAPP: using QSAR for further prioritization

• Collaborative Estrogen Receptor Activity Prediction Project

• Goals:
  – Use ToxCast ER score (or other data) to build many QSAR models
  – Use consensus of models to prioritize chemicals for further testing

• Assumptions
  – ToxCast chemicals cover enough of chemical space to be a good “global” training set
  – Consensus of many models will be better than any one individually

• Process
  – Curate chemical structures
  – Curate literature data set
  – Build many models
  – Build consensus model
  – Evaluate models and consensus
CERAPP Consensus evaluation

**Key point:** As greater consistency is required from literature sources, QSAR consensus model performance improves.

Total Database
Binders: 3961
Agonists: 2494
Antagonists: 2793
CERAPP Summary

- EDSP Universe (10K)
- Chemicals with known use (40K) (CPCat & ACToR)
- Canadian Domestic Substances List (DSL) (23K)
- EPA DSSTox – structures of EPA/FDA interest (15K)
- ToxCast and Tox21 (In vitro ER data) (8K)

~32K unique structures
5-10% predicted to be ER-active
Prioritize for further testing
ER Phenol Read-Across Model

Accuracy increases as
1. Better data is used in the evaluation
2. Neighbors are closer (structure and physchem)

Filtering 1 (Log $P_{kow}$ & MV)

Filtering 2 (No. of Literature Sources $\geq$ 3)
Can the ER Model be used for prioritization?

- “… the ER AUC appears to be an appropriate tool for chemical prioritization for … the EDSP universe compounds.”

Can the ER model substitute for the Tier 1 ER in vitro and uterotrophic assays?

- “… replacement of the Tier 1 in vitro ER endpoints …with the ER AUC model will likely be a more effective and sensitive measure for the occurrence of estrogenic activity…”
- “… the Panel did not recommend that the uterotrophic assay be substituted by the AUC model at this time. The Panel suggested that the EPA considers: 1) conducting limited uterotrophic and other Tier 1 in vivo assay testing, using the original Tier 1 Guidelines (and/or through literature curation)”

Based on follow-up presented here (FR notice, June 18 2015) …

- “EPA concludes that ER Model data are sufficient to satisfy the Tier 1 ER binding, ERTA and uterotrophic assay requirements.”
Data Transparency: EDSP21 Dashboard

• Goal: To make EDSP21 data easily available to all stakeholders
  – Assay-by-assays concentration-response plots
  – Model scores – AUC agonist and antagonist
  – ER QSAR calls
  – Other relevant data

• [https://actor.epa.gov/edsp21](https://actor.epa.gov/edsp21)
Summary

• EDSP is in need of new approach to handle large testing universe
  – Reduce cost, speed throughput

• Estrogen Receptor Model is first example of this
  – 54 chemicals in low-throughput Tier 1 assays
  – 1800 chemicals tested and published in high-throughput
  – 1000 more in queue – 2016 planned release

• Next steps
  – Androgen receptor (1800 chemicals tested, modeling and validation in progress)
  – Steroidogenesis (1000 chemicals with preliminary data)
  – Thyroid – assay development and testing underway for several targets (THR, TPO, deiodinases, ...)

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