U.S. EPA Endocrine Disruptor Screening Program

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April 11th 2016
Expert Meeting to Reach Scientific Consensus on Endocrine Disruptors
Berlin, Germany
EDSP Prioritization, Screening & Testing

Pathways:
- Estrogen
- Androgen
- Thyroid

Prioritization
Bioactivity/Exposure

Relies on:
- QSARs
- ToxCast/ExpoCast
- Monitoring data
- OSRI

Screening
Bioactivity

Relies on:
- QSARs
- ToxCast
- EDSP Tier 1 data
- OSRI

Testing
Dose-Response/Adversity

Relies on:
- EDSP Tier 2 data
- OSRI

Prioritization and Screening for bioactivity
Testing for dose-response and adverse effects
EDSP Screening and Testing

Toxicant → Molecular Interaction → Cellular Response → Organ → Organ System → Organism → Population

MIE → Key Events → Adverse Outcome

Adverse Outcome Pathway

Toxicity Pathway (part of AOP)
## EDSP Screening and Testing by Pathway

### Endocrine Pathway

<table>
<thead>
<tr>
<th>Endocrine Pathway</th>
<th>Tier 1</th>
<th>Tier 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER Binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR Binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER Transcriptional Activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatase</td>
<td></td>
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<tr>
<td>Steroidogenesis</td>
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<tr>
<td>Uterotrophic</td>
<td></td>
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</tr>
<tr>
<td>Hershberger</td>
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<tr>
<td>Pubertal Male</td>
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<tr>
<td>Pubertal Female</td>
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</tr>
<tr>
<td>Amphibian Metamorphosis</td>
<td></td>
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</tr>
<tr>
<td>Fish Short Term Reproduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 2-gen/EOGRTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEOGRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAGDA</td>
<td></td>
<td></td>
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<tr>
<td>JQTT</td>
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</table>

<table>
<thead>
<tr>
<th>Tier 1</th>
<th>Tier 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E+</td>
<td></td>
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<tr>
<td>E-</td>
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<tr>
<td>A+</td>
<td></td>
</tr>
<tr>
<td>A-</td>
<td></td>
</tr>
<tr>
<td>HPT Axis</td>
<td></td>
</tr>
</tbody>
</table>

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Slide 4
EDSP Pivot Announcement

“Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment”

## Developing Alternative EDSP Assays

<table>
<thead>
<tr>
<th>EDSP Tier 1 Battery of Assays</th>
<th>Model Alternative Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen Receptor (ER) Binding</td>
<td>ER Model FY 2015</td>
</tr>
<tr>
<td>Estrogen Receptor Transactivation (ERTA)</td>
<td>ER Model FY 2015</td>
</tr>
<tr>
<td>Uterotrophic</td>
<td>ER Model FY 2015</td>
</tr>
<tr>
<td>Androgen Receptor (AR) Binding</td>
<td>AR Model FY 2016</td>
</tr>
<tr>
<td>Hershberger</td>
<td>AR Model FY 2016</td>
</tr>
<tr>
<td>Aromatase</td>
<td>STR Model FY 2016</td>
</tr>
<tr>
<td>Steroidogenesis (STR)</td>
<td>STR Model 2016</td>
</tr>
<tr>
<td>Female Rat Pubertal</td>
<td>ER, STR &amp; THY Models FY 2017</td>
</tr>
<tr>
<td>Male Rat Pubertal</td>
<td>AR, STR &amp; THY Models FY 2017</td>
</tr>
<tr>
<td>Fish Short Term Reproduction</td>
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<td>Amphibian Metamorphosis</td>
<td>THY Model FY 2017</td>
</tr>
</tbody>
</table>

ER = estrogen receptor; AR = androgen receptor; STR = steroidogenesis; THY = thyroid
The EDSP is Currently Working on the Individual Puzzle Pieces

- Estrogen
- Androgen
- Steroidogenesis
- Toxicokinetics
- Thyroid
- Exposure

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ER Model: Performance Based Approach to Establish Scientific Confidence

Judson et al. 2015, Tox Sci: “Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor"

Kleinstreuer et al. 2015, EHP: “A Curated Database of Rodent Uterotrophic Bioactivity"

Browne et al. 2015, ES&T: “Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model"
Current Status on the Estrogen
Piece of the Puzzle

**In Vitro Reference Chemicals**

<table>
<thead>
<tr>
<th></th>
<th>True Positive</th>
<th>True Negative</th>
<th>False Positive</th>
<th>False Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 (25)</td>
<td>11 (11)</td>
<td>1 (0)</td>
<td>2 (2)</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>0.93 (0.95)</td>
<td>0.93 (0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>0.93 (0.93)</td>
<td>0.93 (0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>0.92 (1.0)</td>
<td>0.92 (1.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**In Vivo Reference Chemicals**

<table>
<thead>
<tr>
<th></th>
<th>True Positive</th>
<th>True Negative</th>
<th>False Positive</th>
<th>False Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29 (29)</td>
<td>8 (8)</td>
<td>5 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>0.86 (0.95)</td>
<td>0.97 (0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>0.97 (0.97)</td>
<td>0.97 (0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>0.67 (0.89)</td>
<td>0.67 (0.89)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values in parentheses exclude inconclusive chemicals
Current Status on the Androgen Piece of the Puzzle

11 *In Vitro* Assays Measure AR-Related Activity

**Agonism**
- True Positives: 8
- True Negatives: 20
- False Positives: 1
- False Negatives: 0
- Accuracy: 0.97
- Sensitivity: 1.00
- Specificity: 0.95

**Antagonism**
- True Positives: 18
- True Negatives: 8
- False Positives: 0
- False Negatives: 2*
- Accuracy: 0.93*
- Sensitivity: 0.90*
- Specificity: 1.00

*Two false negatives were correctly identified when Tox21 AR antagonist screen was evaluated at both agonist concentrations

Kleinstreuer et al. 2016 SOT poster #2651
Current Status on the Steroidogenesis Piece of the Puzzle

ToxCast H295R Steroidogenesis Assay

- 13 hormones were quantified using HPLC-MS/MS
- 2060 chemicals screened at single concentration
- 403 chemicals selected for concentration response (altered ≥ 4 hormones)
  - 120 additional chemicals selected for concentration response based on other needs

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Current Status on the Thyroid Piece of the Puzzle

THR Assays

Undergoing Online Validation (Tox21)

TRHR Assay

Undergoing Online Validation (Tox21)

TSHR Assay

Undergoing Online Validation (Tox21)

Molecular-Initiating Events & Key Events

TRHR Assay

Undergoing Online Validation (Tox21)

Paul et al., In Review

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Current Status on the Thyroid: Piece of the Puzzle

TPO Assay Details
- Rat thyroid microsomes
- Fluorescent peroxidase substrate (Amplex Ultra Red)
- Validated against existing kinetic guaiacol assay
- Luciferase, cytotoxicity counterscreens

Simmons et al. 2016 SOT poster #1886

Paul et al., In Review

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Current Status on the Thyroid

Piece of the Puzzle

High-Throughput Human NIS Screen
(with Secondary Screen in FRTL-5 rat follicular cells)

Buckalew et al. 2016 SOT poster #1887
Murr et al. 2016 SOT poster #1888
Hallinger et al. 2016 SOT poster #1889

High-Throughput Deiodinase Screen
(In Development)

M. Hornung
Current Status on the Thyroid Piece of the Puzzle

**SLCO1B1:** thyroid hormone transporter in the liver
**SULT2A1:** not main SULT that metabolizes TH, but demonstrated to have some activity
**UGT1A1/1A6:** mediate T(4) glucuronidation
**THRSP:** thyroid hormone-inducible hepatic protein
**HIF1a:** downstream to TRB1 activation via T3 or T4 signaling

**HepaRG Gene Expression Assay**

- HepaRG cells
- Treated for 48 h
- Gene expression measured using Fluidigm 96.96
- Cytotoxicity measured in parallel

**LTEA Assay Details**

Paul et al., In Review

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Slide 15
Addressing the Metabolic Competence Challenge

Alginate Immobilization of Metabolic Enzymes (AIME)

Prototype Lids

DeGroot et al. 2016 SOT poster #3757

Amount of XME Activity in Microspheres

Small Molecule Inhibition of XME Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. Wt. (g/mol)</th>
<th>Targeted P450</th>
<th>IC50 Free S9 (µM)</th>
<th>IC50 AIME (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furaflline</td>
<td>260.25</td>
<td>1A2</td>
<td>2.39</td>
<td>1.92</td>
</tr>
<tr>
<td>Thio-TEPA</td>
<td>189.22</td>
<td>2B6</td>
<td>7.46</td>
<td>2.86</td>
</tr>
<tr>
<td>Tienilic Acid</td>
<td>331.17</td>
<td>2C9</td>
<td>0.053</td>
<td>0.96</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>531.43</td>
<td>3A4</td>
<td>0.086</td>
<td>0.12</td>
</tr>
</tbody>
</table>
The Toxicokinetics Piece of the Puzzle

- Additional in vivo rat TK data collected for 26 chemicals by NHEERL collaborators and RTI contractors
- Literature in vivo rat TK data curated by TNO collaborators

Wambaugh et al., Toxicol Sci, 2015

Hughes et al. 2016 SOT poster #3436

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Current Status on the Exposure Piece of the Puzzle

- Averaging multiple exposure models
- Challenge is to know which exposure model to use for which chemical
- Chemical use identifies relevant pathways

3rd Gen

- SHEDS-HT
- Literature Models
- CPcat Database

NHANES
Urine, Blood and Serum Data

Use Database (FUSE)

Pilot Study
- 20 Product types x 5 products/type
- 2D GCxTOF/MS

Chemical Information
Exposure Predictions
Predicted Pathway

- Physico-chemical
- ACToR UseDB
- CPCat
- SHEDS-HT Dietary
- SHEDS-HT Residues
- Pesticide Documents
- Pred SHEDS-HT Diet.
- Pred SHEDS-HT Res.
- Pred Pest Doc.

~20,000 Chemicals

Curated DSStox + Formulation

Machine Learning Approaches to Predict Chemical Use

Non-Targeted Analytical Screening of Consumer Product Composition

Isaacs et al. 2016 SOT poster #1679
Phillips et al. 2016 SOT poster #1680
Wambaugh et al. 2016 SOT poster #1682

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Working to Assemble the EDSP Screening Puzzle

Estrogen
Androgen
Steroidogenesis
Thyroid
Toxicokinetics
Exposure
Validation

OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Validation is a process by which the reliability and relevance of a test method are established for a specific purpose.
OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Relevance and reliability should be characterized against data generated with a list of reference chemicals (tested in the original methods) accepted by regulatory agencies.

Reference chemicals: Chemicals selected for use in the validation process, for which responses in the in vitro or in vivo reference test system or the species of interest are already known.
ER Model Validation - In Vitro

40 In Vitro Reference Chemicals

In Vitro (Lit)
- Active
- Inactive

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>True Positive</td>
<td>25</td>
</tr>
<tr>
<td>True Negative</td>
<td>12</td>
</tr>
<tr>
<td>False Positive</td>
<td>0</td>
</tr>
<tr>
<td>False Negative</td>
<td>3</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.95</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.89</td>
</tr>
<tr>
<td>Specificity</td>
<td>1.00</td>
</tr>
</tbody>
</table>

ER AUC Rank Order

0 5 10 15 20 25 30 35 40

ER AUC

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1

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ER Model Validation - In Vivo

77 In Vivo Reference Chemicals

- True Positive: 29
- True Negative: 46
- False Positive: 1
- False Negative: 1
- Accuracy: 0.97
- Sensitivity: 0.97
- Specificity: 0.97
Uterotrophic Reproducibility

Same Study Design (Immature Rat): BPA

Graph showing the LEL or MDT (mg/kg/day) for injection and oral routes. The graph indicates that active and inactive responses are observed across different doses for both injection and oral routes.

Legend:
- Active
- Inactive
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Chemicals with High Throughput Data for Endocrine Screening

- Pathway based
- Ongoing data generation, analysis and validation
- Performance-Based Test Guidelines being developed