The Future of Endocrine Screening: An Animal Welfare Perspective

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Outline

- Animal use in the current EDSP design
- Need for a better approach
- Begin with modified tiered system + weight-of-evidence
- Move toward pathway-based approach
## Tier 1 Animal Use

<table>
<thead>
<tr>
<th>Assay</th>
<th># animals/test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
</tr>
<tr>
<td>Estrogen Receptor Binding – rat cytosol</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Androgen Receptor Binding - rat cytosol</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Estrogen Receptor Transcriptional Activation</td>
<td>—</td>
</tr>
<tr>
<td>Aromatase Recombinant</td>
<td>—</td>
</tr>
<tr>
<td>Steroidogenesis H295R</td>
<td>—</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
</tr>
<tr>
<td>Uterotrophic</td>
<td>18</td>
</tr>
<tr>
<td>Hershberger</td>
<td>48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male Pubertal</td>
<td>45</td>
</tr>
<tr>
<td>Female Pubertal</td>
<td>45</td>
</tr>
<tr>
<td>Fish Short-term Reproduction (&lt;♂ &amp; ♀)</td>
<td>72</td>
</tr>
<tr>
<td>Amphibian Metamorphosis</td>
<td>320</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>571</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> for receptor extract
<sup>b</sup> the TG assumes both agonist and antagonist methods are performed
## Putative Tier 2 tests

<table>
<thead>
<tr>
<th>Assay</th>
<th># animals/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian 2-Generation</td>
<td>1600 - 2400</td>
</tr>
<tr>
<td>Amphibian 2-Generation</td>
<td>400 P, plus progeny</td>
</tr>
<tr>
<td>Avian 2-Generation</td>
<td>1024</td>
</tr>
<tr>
<td>Fish Lifecycle</td>
<td>~1000*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>&gt; 4,000</td>
</tr>
</tbody>
</table>

*number varies by species
Data submitted and generated for 54 chemicals in Phase I: OSRI was submitted for all or some assays for 47 chemicals*

<table>
<thead>
<tr>
<th>Assay</th>
<th>Assays in Initial Test Plans</th>
<th>Assays in Final Test Plans</th>
<th># Animals Initial Test Plans</th>
<th># Animals Final Test Plans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER Binding</td>
<td>2</td>
<td>37</td>
<td>26</td>
<td>468</td>
</tr>
<tr>
<td>AR Binding</td>
<td>2</td>
<td>37</td>
<td>20</td>
<td>360</td>
</tr>
<tr>
<td>FRTA</td>
<td>3</td>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aromatase</td>
<td>10</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroidogenesis</td>
<td>17</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>34</td>
<td>187</td>
<td>46</td>
<td>828</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterotrophic</td>
<td>12</td>
<td>35</td>
<td>216</td>
<td>612</td>
</tr>
<tr>
<td>Hershberger</td>
<td>16</td>
<td>31</td>
<td>768</td>
<td>1,632</td>
</tr>
<tr>
<td>Male Pubertal</td>
<td>3</td>
<td>44</td>
<td>135</td>
<td>1,440</td>
</tr>
<tr>
<td>Female Pubertal</td>
<td>4</td>
<td>43</td>
<td>180</td>
<td>1,710</td>
</tr>
<tr>
<td>Fish Short-term Reproduction</td>
<td>21</td>
<td>26</td>
<td>1,512</td>
<td>3,168</td>
</tr>
<tr>
<td>Amphibian Metamorphosis</td>
<td>15</td>
<td>32</td>
<td>4,800</td>
<td>13,120</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>71</td>
<td>211</td>
<td>7,611</td>
<td>21,682</td>
</tr>
<tr>
<td>Other 7 chems</td>
<td>77</td>
<td>77</td>
<td>3,997</td>
<td>3,997</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182</td>
<td>501</td>
<td>11,508</td>
<td>26,507</td>
</tr>
<tr>
<td><strong>Total if all testing done for 54 chemicals</strong></td>
<td>30,834</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Bishop, Sullivan and Willett, submitted
Acceptance of existing information:

Generally in cases of identical or nearly identical information to that provided by a Tier 1 test

Or indicated positive result

• Same study showing negative result was rejected

Weight-of-evidence inconsistently applied

* Bishop, Sullivan and Willett, submitted.
Need for a better approach

Current program design is resource, labor and animal-intensive
Provides much data, only some of which may have regulatory use
Provides insufficient opportunity for chemically-relevant tailoring
Does not link mechanism to adverse outcome
Is not predictive
Does not fully cover “endocrine” effects
First step: a more refined tiered system

Initial tier takes into account all existing information, including physicochemical.

Second tier assesses potential mechanisms of action.

Following tiers address potential effects in more complex systems, on multiple modes of action.

Highest tiers address adverse outcome and dose-response.

WoE with clearly articulated criteria applied at each tier.

• Defines positive and negative cut-offs at the outset for each test and each Tier or Level.

• Results are used to design the strategy for further testing.

Strategy is designed to address information needs.
<table>
<thead>
<tr>
<th>Level</th>
<th>OECD Conceptual Framework</th>
</tr>
</thead>
</table>
| Level 1 | Sorting and prioritization based on existing information  
• physical and chemical properties  
• exposure  
• available toxicological data |
| Level 2 | *In vitro* assays providing mechanistic data  
• QSARs  
• binding and transcription assays  
• aromatase, steroidogenesis, thyroid function  
• high-throughput screens |
| Level 3 | *In vivo* assays addressing single mechanisms and effects  
• uterotrophic  
• Hershberger  
• amphibian metamorphosis  
• fish VTG (e.g. TG 230) |
| Level 4 | *In vivo* assays addressing multiple mechanisms and effects  
• enhanced 407  
• male and female pubertal assays  
• adult intact male  
• fish short-term reproductive assay (e.g. TG 299) |
| Level 5 | *In vivo* assays providing data on effects from endocrine + other mechanisms  
• 1 and 2 generation reproduction in rodents, birds, fish |
Lessons Learned, Challenges, and Opportunities: The US EDSP | April 23 - 24, 2013 | RTP, North Carolina

The future of EDSP: pathway-based approach

A conceptual construct that portrays existing knowledge of linkages between a direct molecular initiating event and an adverse outcome at a level of biological organization relevant to risk assessment.

ER-mediated reproductive impairment

Developed from:
- an understanding of the MIE plus
- a need for a strategy for measuring endocrine-mediated effects.
- Includes use of biomarker (vitellogenin) that is (in the case of females) or is not (in the case of males) directly linked to the adverse outcome
ER-mediated reproductive impairment

P. Schmieder, McKim conference 2008.
ER and AR pathway efforts

US EPA:
• AOP development
• HTS and other in vitro assays

The Hamner Institutes: “Tier 1 and Done”

CAAT: Pathways of Toxicity

OECD Validation Management Group – Non-animal
• Binding and TA assays

OECD Advisory Group on Molecular Screening and Toxicogenomics
• AOP development
Thyroid hormone pathway

Crofton, K. US EPA. Presented at DC area SOT, May 2012.

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Thyroid pathway efforts

Development of AOPs and relevant assays

- OECD Thyroid Scoping Effort Expert Group
- OECD Validation Management Group – Non-animal
- OECD Advisory Group on Molecular Screening and Toxicogenomics
- US EPA
Other necessary elements

- Build Biological and adverse-outcome “pathways”
  - OECD integration of AOPs into the Test Guidelines program
  - Guidance
- Improve predictive tools
  - NIH National Center for Advancing Translational Sciences
  - EPA’s Computational Toxicology Research
  - OECD QSAR tool box
  - Hamner Institute
- Develop assessment systems for complex endpoints
  - Reconstructed tissues and organ systems
    - Human skin, eye, lung
    - Liver-on-a-chip
    - Stem-cell derived
  - Integrate absorption, metabolism and distribution information
    - QSAR QIVIVE -
    - Liver cells, tissues, extracts, reconstructed tissues
- Integrated databases and “knowledge bases”
  - ACToR and MetaPath: EPA – all available chemical toxicity data on over 500,000 environmental chemicals searchable by chemical name and structure
  - Kegg pathway database: collection of manually drawn pathway maps representing current knowledge on the molecular interaction and reaction networks
  - AOP Wiki: EPA-European Commission joint project to house developing AOPs
The current EDSP design could be improved in terms of efficiency and utility of data generated. Could begin with:

- a more refined organization of tiered tests
- combined with an iterative weight-of-evidence analysis

As AOPs and tests are developed, move toward pathway-based approach.
Implementing the Science: Working together to accelerate technical and scientific advances in pathway-based approaches to chemical safety assessment.

Presentations · Workshops · Papers · Sponsorship

Articulating the Vision: Effective communication facilitates consensus building among stakeholders and is essential to building confidence in chemical and drug safety.

Website · Articles · Video

Lobbying/Funding: Advocate policy changes and cultivate funding opportunities that support pathway-based approaches in the U.S. and internationally.

Bill language · Appropriations
Thank You

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