Extending the Human-Relevant Potency-Threshold (HRPT) Concept to Ecological Species: Estrogen Receptor Alpha Agonists as Prototype for Hazard Identification of EDCs & Development of a Potency Threshold for Androgen-Receptor in Fish:

Christopher J. Borgert
Applied Pharmacology & Toxicology, Inc., Gainesville, FL
Department of Physiological Sciences, C.E.H.T.
University of Florida College of Veterinary Medicine, Gainesville, FL

John C. Matthews
University of Mississippi School of Pharmacy
University, MS, USA

Stephen P. Baker
Department of Pharmacology and Therapeutics
University of Florida College of Medicine, Gainesville, FL, USA

Steven L. Levine
Bayer U.S. - Crop Science
Chesterfield, MO

Audrey Bone
Bayer U.S. - Crop Science,
Research Triangle Park, N.C.
Key Points

Potency is a general expression of response per unit of chemical. BUT, it is not a single concept that can be applied uniformly to all aspects of chemical effects.

Potency for a MoA is conceptually different from potency for an adverse effect . . . these are measured and interpreted differently.

Potency for a MoA (mechanistic potency) is the fundamental consideration for biological plausibility of a link between the MoA and a physiological effect, including an adverse effect.

Potency for Adverse Effects pertains to Hazard Characterization, BUT
Potency for MoA pertains to Hazard Identification.

Potency ≠ Efficacy

- Potency: A measure of biological activity per unit of chemical
- Efficacy: A measure of the ability to produce a maximal response

![Diagram showing the difference between Potency and Efficacy]
The concepts and kinetics apply to any molecular interaction with conformational specificity, e.g., to receptors, enzymes, transporters, DNA response elements, etc.

**Crux of the Issue**

*Identifying Chemical Hazards according to their Mode of Action, rather than by the Adverse Effects they produce, requires an understanding of the potency by which a chemical can operate by any particular Mode of Action.*
Dose-Response, potency, and efficacy for a ANY response reveals little, if anything, about *HOW* that response is produced.

A flooded backyard is an adverse effect!

Potency of the adverse effect is measured in centimeters of water standing in the backyard.

Different mechanisms can flood the backyard.

Knowing the potency for a particular mechanism determines whether it can cause water to pool in the backyard.

Mechanistic potencies are measured differently from toxic potency.
Thesis

*There are levels of mechanistic potency too low to manifest biological effects.*

*We proposed a Human-Relevant Potency-Threshold (HPRT) for the Estrogen Receptor-alpha (ERα) mode of action.*

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**Human-Relevant Potency-Threshold (HRPT) for the ERα-Agonist MoA**


- Identify functional assays that provide robust potency data for the specific mechanism of interest (e.g., ERα- or ERβ-mediated)
- Identify chemicals that are:
  a) ineffective in producing mechanism-specific effects in intact animals – androgens; weakest botanical estrogens
  b) marginally effective – most potent botanical estrogens
  c) effective – endogenous hormones and human pharmaceuticals
The concept is being extended to other hormonal modes of action, e.g., androgen.

Conservatively identified an agonist potency via ER-α below which no estrogenic effects have been detected in any patient population = “HRPT.”

- The more potent members of the soy isoflavones define this mechanistic potency threshold.

- Applied to a negative case study chemical; result consistent with EDSP-21 Dashboard.

- The concept is being extended to other hormonal modes of action, e.g., androgen receptor agonists in fish.

- Session on potency approaches accepted for SETAC Helsinki.

Table 2: Uterotrophic Relative Potencies Calculated from Values Reported in the Literature

<table>
<thead>
<tr>
<th>Chemical</th>
<th>MOUSE</th>
<th>Ave. RP (^1) Mouse</th>
<th>RAT</th>
<th>Ave. RP (^1) Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylstilbestrol (DES)</td>
<td>4.0E-00 ± 1</td>
<td>2.3E-00 ± 1</td>
<td>3.3E-00</td>
<td>9.1E-04 ± 1</td>
</tr>
<tr>
<td>Estrone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>2.0E-03 ± 1</td>
<td>4.2E-03 ± 1</td>
<td>3.1E-03</td>
<td>7.6E-05 ± 1</td>
</tr>
<tr>
<td>Equol</td>
<td>5.3E-04 ± 1</td>
<td>1.3E-04 ± 1</td>
<td>3.0E-04</td>
<td>1.2E-04 ± 1</td>
</tr>
<tr>
<td>Genistein</td>
<td>1.0E-04 ± 1</td>
<td>4.0E-04 ± 1</td>
<td>5.0E-04</td>
<td>2.0E-04 ± 1</td>
</tr>
<tr>
<td>Daidzein</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochanin A</td>
<td>&lt; 1.0E-06 ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zearalenol</td>
<td>8.0E-05 ± 1</td>
<td>1.0E-05 ± 1</td>
<td>5.0E-05</td>
<td>1.0E-05 ± 1</td>
</tr>
<tr>
<td>Zearalnone</td>
<td>1.0E-06 ± 1</td>
<td>1.0E-06 ± 1</td>
<td>ND ± 1</td>
<td>6.7E-05 ± 1</td>
</tr>
<tr>
<td>Naringenin</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-DMA-Naringenin</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoxanthohumol</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hops Extract</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Clover Extract</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46% Soy Extract HIGH(^a)</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51% Soy Extract LOW(^b)</td>
<td>ND ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone Enanthate</td>
<td>1.2E-05 ± 1</td>
<td>1.2E-05 ± 1</td>
<td>5.0E-06</td>
<td>2.0E-04 ± 1</td>
</tr>
<tr>
<td>Dihydrotestosterone (DHT)</td>
<td>5.0E-06</td>
<td>5.0E-06</td>
<td>3.0E-04</td>
<td>7.9E-05</td>
</tr>
<tr>
<td>Methyltestosterone</td>
<td>1.2E-05 ± 1</td>
<td>1.2E-05 ± 1</td>
<td>1.7E-06 ± 1</td>
<td>2.5E-07 ± 1</td>
</tr>
<tr>
<td>ND</td>
<td>6.0E-06 ± 1</td>
<td>1.0E-06 ± 1</td>
<td>1.7E-06 ± 1</td>
<td>2.5E-07 ± 1</td>
</tr>
</tbody>
</table>

**Insufficient Potency:**

**Flaxseed / Linoleic Acid: In Vitro vs. In Vivo [5 Published Studies]**

<table>
<thead>
<tr>
<th>In Vitro Response</th>
<th>In Vivo Response Rodents</th>
<th>In Vivo Response Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 of 6 Endpoints</td>
<td>1 of 5 Endpoints</td>
<td>2* of 10 Endpoints</td>
</tr>
</tbody>
</table>

*subjective observation or self-reported impression

**Marginal Efficacy: Soy foods & Soy Isoflavones**


~ 25 mg soy Isoflavones per serving Intake Ranges from ~ 10 – 40 mg / day in Asian Countries
Electronic Supplementary material contains the complete Relative Potency Calculations for 144 Data Points.

Relative Potency
Range

- 3.5E-02 – 1.0E+00 Hormones (11)
- 2.6E-02 – 2.0E+00 Drugs (19)
- 3.3E-05 to 7.0E-02 Botanicals (109)
- 2.5E-06 to 7.1E-06 Androgens (5)

Figure 1: Human-Relevant Potency-Threshold (HRPT) for the Erα-Agonist MoA

EDSP-21
D4 Inactive Via Estrogen Pathway Based on Potency

https://actor.epa.gov/edsp21/
Results for 556-67-2
(Octamethylcyclotetrasiloxane)

Conclusions Regarding the HRPT

1. The HRPT is the first step in Hazard Identification for endocrine MoAs.
2. The HRPT approach is consistent with the US EPA’s potency-based AUC value derived using ToxCast assays for specific endocrine MoAs.
3. An Endocrine Hazard is biologically plausible for chemicals that significantly exceed the HPRT (green).
4. For chemicals marginally exceeding the HRPT (yellow), additional WoE considerations related to biological plausibility would be necessary to conclude Endocrine Hazard.
5. Chemicals below the HRPT (pink) lack biological plausibility to pose an Endocrine Hazard.
6. HRPTs can be extended to or derived for ecological receptors.
7. Failure to evaluate mechanistic potency for endocrine MoAs leads to illogical and scientifically untenable conclusions regarding Endocrine Hazards.
Additional Uses Of Potency-Based Thresholds

A. HRPT concept applies far beyond hormonal MoAs; it applies to any MoA that involves a stereo-selective interaction of a chemical with a biologically active macromolecule: receptors, enzymes, transporters, transcriptional activation sites, etc.

• Needs to be extended to ecological receptors (Fish would be a logical extension for ERα agonist pathway, as extensive comparative data are available).

B. Should be a requirement for developing an AOP or other predictive model that involves a stereo-selective molecular interaction.

C. Should be a required for inclusion criterion for chemicals assessed in common assessment groups for mixtures risk assessment.

D. Can be used to interpret in-house screening data.

• In combination with QSAR models, to evaluate untested chemicals.

Sequence Similarity can be the Basis for Extrapolation

• Many structural and functional aspects of the vertebrate HPG axis are highly conserved but the significance from a toxicological perspective has received little attention.

• LaLone et al. 2013 suggested that sequence similarity can be the basis for extrapolation across species to assess the risks with known modes of action.

• The Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) application was developed to assess protein sequence/structural similarity across taxa to predict intrinsic susceptibility.
The AR sequence is Highly Homologous

- There is a relatively high degree of AR sequence homology across vertebrates.
  - Fathead minnow and humans have 64% homology in the AR binding domain
  - Zebrafish and humans have 68% homology in the AR binding domain
  - Medaka and humans have 70% and 64% homology in the α and β domains

- AR binding affinity and spectrum for AR agonists are highly comparable between fish and mammalian ARs.

A Cross Taxa Comparison: 17β-Trenbolone

- There was a high concordance between the fish and rat assays with respect to identifying chemicals AR agonists.

- How about relative sensitivity in vivo?

- FSTRA for 17β-trenbolone:
  - LOEC = 0.050 µg/L

- AG distance in rats:
  - LOEC = 0.5 mg/kg/day repeat dose

- Attempting to compare daily doses between rats and fish to inform an in vivo comparison of relative potencies.