Quantitative Prediction of Phenotypic Change from High Throughput Assay Results

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Road Map

• For use in screening/prioritization schemes or even risk assessment, prediction models must be evaluated as fit for their intended purpose.
• Users need to have similar or greater confidence in the results as they do in current results
• Here we’ll try to predict the occurrence of uterotrophy from a common phytoestrogen using ToxCast assay results
• This is the essence of KERs as part of AOPs
• Using the Transitional Dose Value or BMD21 for MOA evaluation
• The need for IVIVE
Mode of Action for Uterotrophy

Actual Sequence of Events in the MOA not entirely clear
Transitional Dose Values occur for the Hill model at the 21% response level, the transition point to the rising phase of the curve.

\[
\text{Response} = \frac{1}{1 + 10^{g \chi (\log(ga) - \log(dose))}}
\]

Murell et al. (1998) Risk Anal 18:13-26 suggested estimating the slope at the EC50 and projecting down to the baseline to identify the TDV. Sand et al. (2006) Toxicol Sci 90:241-51 identified the BMR21 at the point at which the third derivative is equal to zero. Use inverse with Response = 0.21 as follows:

\[
\log_{10}(dose) = \log_{10}(AC50) - \frac{0.575}{g \chi}
\]

“tipping point” on the dose-response curve
Mode of Action from In Vivo Studies using Estradiol

Gene Expression Changes
- Levin et al. 1993
- Notides et al. 1981

Fold Change in Gene Expression (protein mRNA)
- Henewen et al. Dietary Exposure
- Naciff et al. Subcutaneous Injection

Uterine Weight Gain
- Henewen et al. 2007
- Naciff et al. 2003
- Brazham et al. 1985

Increase in Blood Flow
- Lytle & DeSombre, 1981

Cell Proliferation
- Kaye et al. 1972

Binding to ERα
- Levin et al. 1993
- Notides et al. 1981
TDVs for Estradiol

• ER binding: TDV = 0.001 – 0.005 μM
• Gene Expression: 0.14 – 5.15 μg/kg/d
• Cell Proliferation: 0.44 μg/animal/d
• Blood Flow Increase: 1.64 μg/animal/d
• Uterine wt. gain: 0.014 μg/animal/d

With IVIVE, all these doses could be compared!
Mode of Action for Uterotrophy using ToxCast™ Results

Actual Sequence of Events in the MOA not entirely clear
The example chemical will be Genistein, a soy isoflavone.
Basic IVIVE Model

External Dose (mg/kg/d) → Steady State Plasma Conc. (μM) → Urinary Conc. (mg/L)

Far field
Near field
Use of “big” data

Assay comparison

Good for estimates of intake, not for steady state conc
In Vitro In Vivo Extrapolation

- IVIVE to obtain doses corresponding to effect concentrations
- Need IVIVE for both humans and mice
- Humans
  - Oral equiv. (mg/kg/d) = Conc (μM) / Css
  - Units of Css are (mg/kg/d) per μM
  - Wetmore et al. calculated Css values for many chemicals
    - Genistein
      - Css low = 1.49E-01
      - Css med = 3.10E-01
      - Css hi = 6.01E-02
In Vivo In Vitro Extrapolation in Mice for Uterotrophic Response

• What is the equivalent steady state concentration ($C_{ss}$) to an IV or SC dose of GEN?
  – From Yang et al. 2010 20 mg/kg IV in mice at steady state for aglycone

\[
AUC = 20.25 \, \mu M\cdot hr
\]
\[
C_{ss} = \frac{AUC}{24/20} = 0.042 \, \mu M/mg/kg/d
\]
ToxCast Assay Data

NVS_NR_hER

ACEA_T47D_80hr_Positive

Data pipeline or flat files;
How else to get the raw data?
Download these images and use GraphClick, now free from Arizona software
Genistein – Dietary Phytoestrogen

From OECD Studies on mice

Uncertain fits for gene expression and proliferation
Assay Responses would be needed to trigger a Uterotrophic Response?

<table>
<thead>
<tr>
<th>Dose at</th>
<th>1) ER binding</th>
<th>2) Gene Expression</th>
<th>3) Protein Expression</th>
<th>4) Cell Proliferation</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. at Tipping Point for Uterotrophy</td>
<td>0.003</td>
<td>0.025</td>
<td>0.28</td>
<td>0.04</td>
<td>0.89</td>
</tr>
<tr>
<td>In Vivo % Response</td>
<td>0.03%</td>
<td>0.4%</td>
<td>6.5%</td>
<td>0.7%</td>
<td>NC</td>
</tr>
</tbody>
</table>

ACEA T-47D cell proliferation assay doesn’t fit. Due to measuring impedance as representative of proliferation?

Using $KE_{upstream}$ to predict $KE_{downstream}$ is the goal of KERs as the central point of AOPs.
**Comparison with Steady State**

**Genistein Aglycone Blood Levels**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.005 μM</td>
</tr>
<tr>
<td>P01</td>
<td>1.5E-05 μM</td>
</tr>
<tr>
<td>P05</td>
<td>4.8E-04 μM</td>
</tr>
<tr>
<td>P95</td>
<td>0.66 μM</td>
</tr>
<tr>
<td>P99</td>
<td>1.76 μM</td>
</tr>
</tbody>
</table>

From 17 human studies

**Assay Tipping Points**

- Binding = 0.003μM
- Gene Exp. = 0.025μM
- Protein. Exp. = 0.28μM
- Prolif. = 0.04μM
- Uterotrophy = 0.9μM
Comparing with the TTC

- Median Css from Wetmore et al. = 0.309 μM per mg/kg/d
- Cramer Class I: TTC = 1800 μg/person/day or 1.8 mg/d ÷ 60 kg = 0.03 mg/kg/d
- Cramer Class III: TTC = 90 μg/person/day or 0.09 mg/d ÷ 60 kg = 0.0015 mg/kg/d
- Hence, the internal bioequivalent dose representing the external TTC for GEN would be
  - Class I: 0.309 x 0.03 = 0.0093 μM
  - Class III: x 0.0015 = 4.6E-04 μM
Thank you for your attention! Questions?