Quantitative Prediction of Phenotypic Change from High Throughput Assay Results

Ted W. Simon, Ph.D., DABT
Ted Simon LLC
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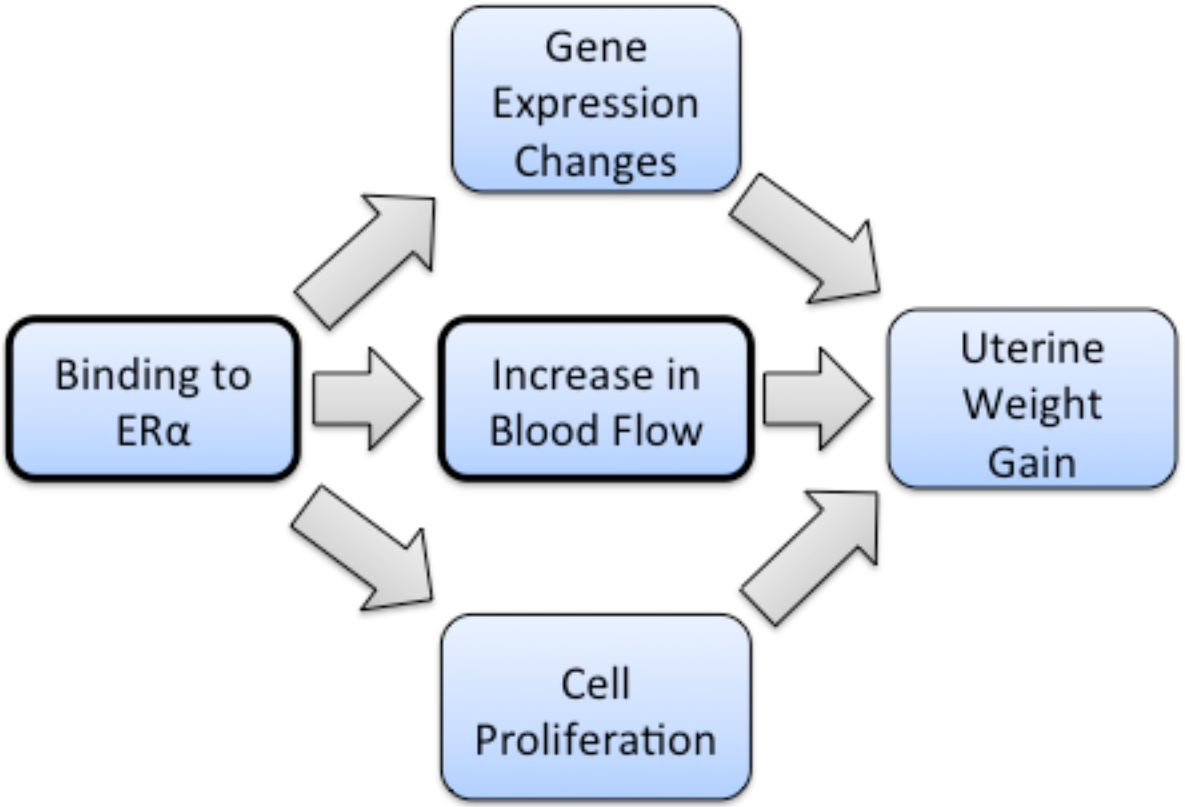
Road Map

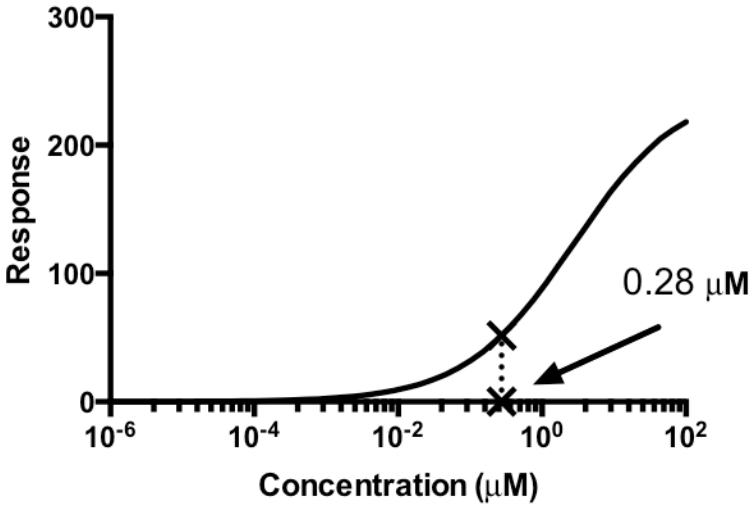
- For use in screening/prioritization schemes or even risk assessment, prediction models must be evaluated as fit for their intended purpose.
- Users need to have similar or greater confidence in the results as they do in current results
- Here we'll try to predict the occurrence of uterotrophy from 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



Transitional Dose Values occur for the Hill model at the 21% response level, the transition point to the rising phase of the curve.

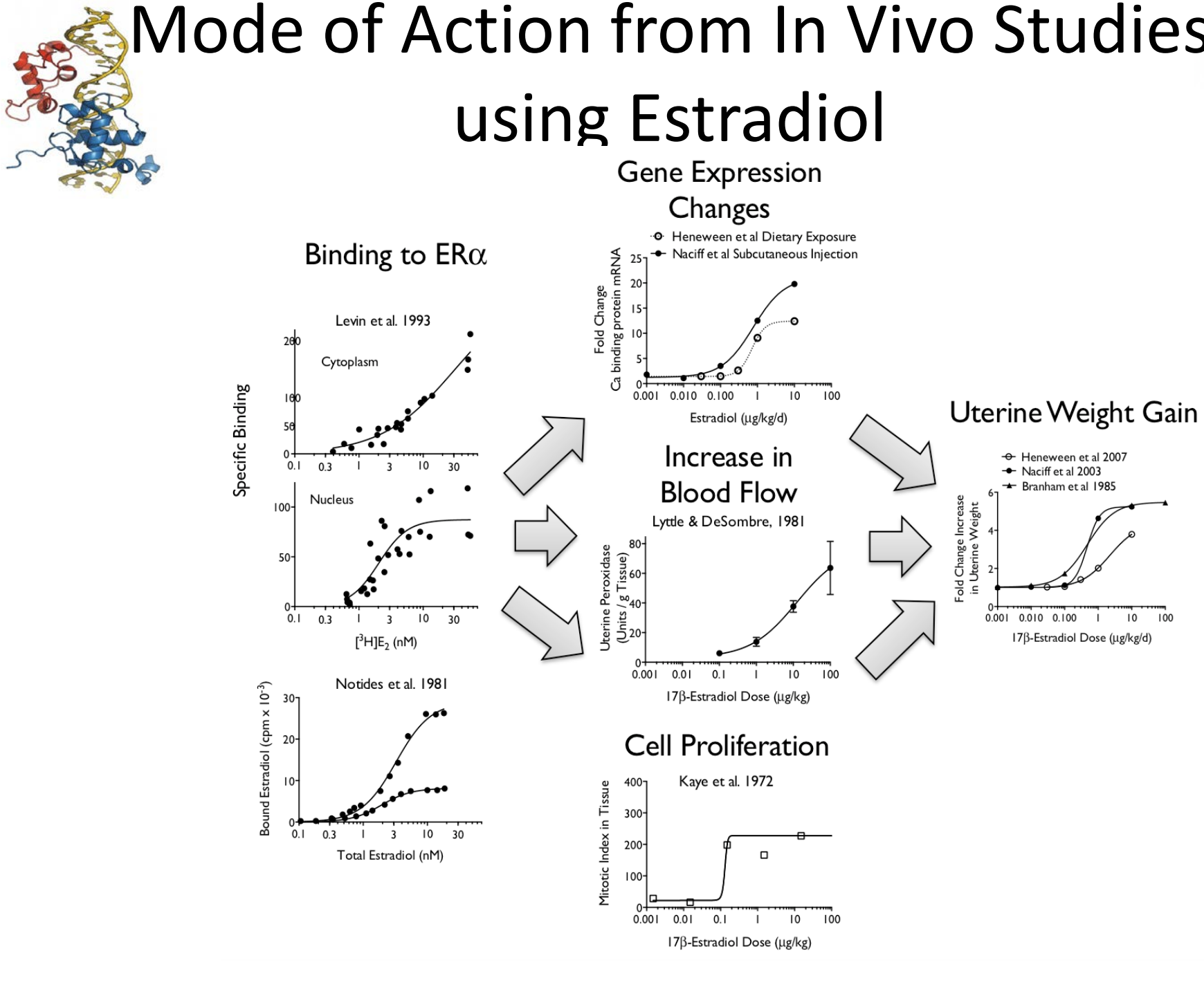
$$\text{Response} = \frac{1}{1+10^{gx(\log(ga)-\log(dose))}}$$

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:

$$\log_{10}(dose) = \log_{10}(AC50) - \frac{0.575}{gx}$$

"tipping point" on the dose-response curve

Mode of Action from In Vivo Studies using Estradiol

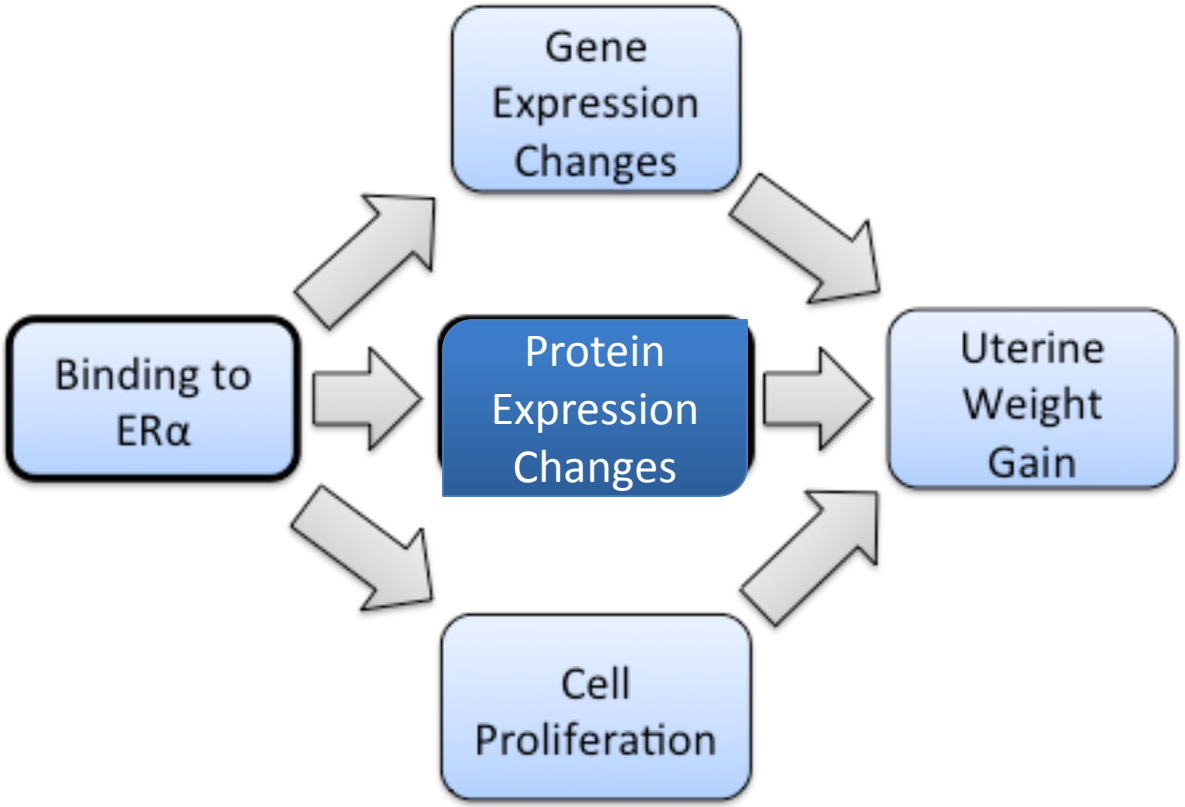


TDVs for Estradiol

- 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)

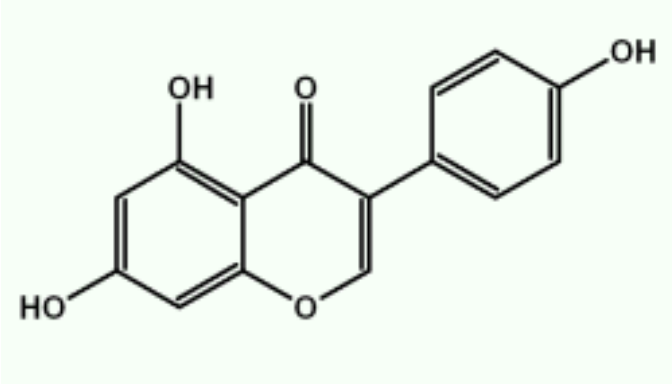
Far field
Near field
Use of “big” data

Assay comparison

Good for estimates of intake, not for steady state conc

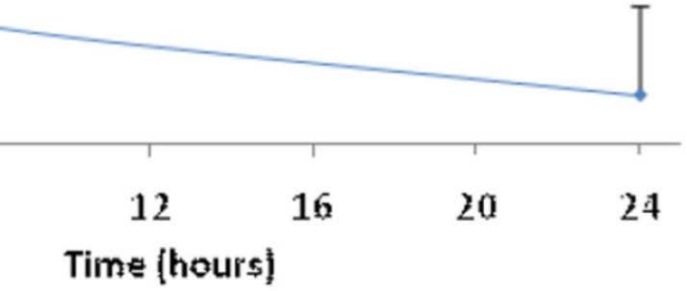
In Vitro In Vivo Extrapolation

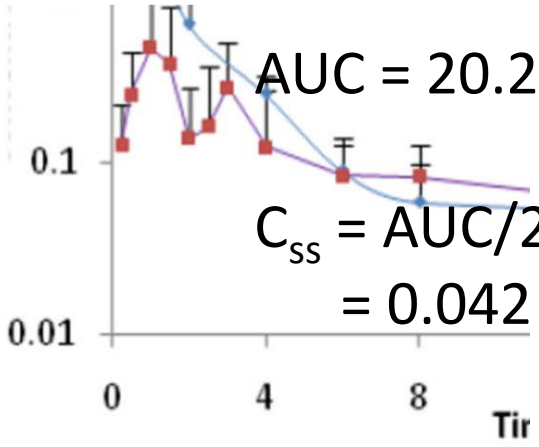
- IVIVE to obtain doses corresponding to effect concentrations
- Need IVIVE for both humans and mice
- Humans
  - Oral equiv. (mg/kg/d) = Conc (µM) / Css
  - Units of Css are (mg/kg/d) per µM
  - Wetmore et al. calculated Css values for many chemicals
  - Genistein
    - Css low = 1.49E-01
    - Css 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 µM-hr

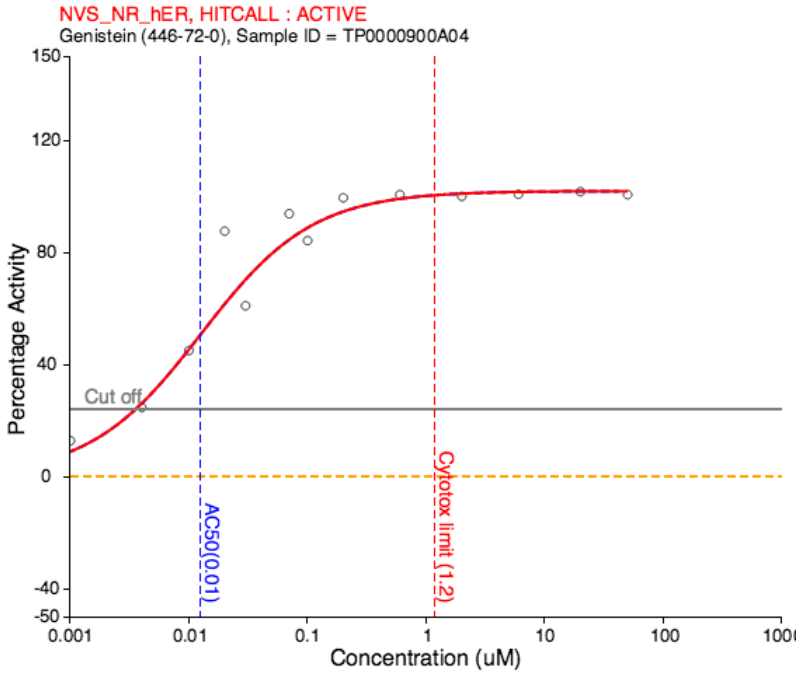
C_{ss} = AUC/24/20
= 0.042 µM/mg/kg/d

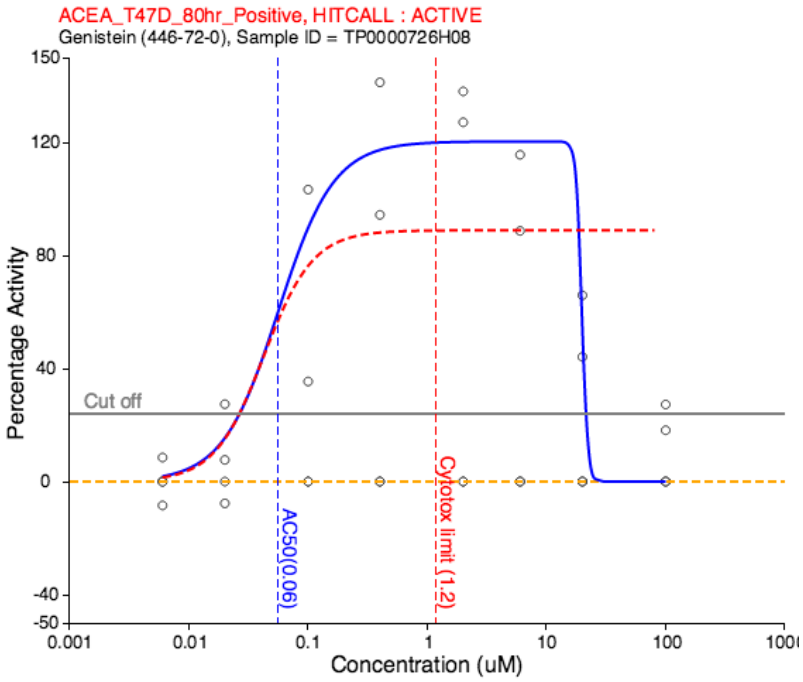
ein 4'-O-sulfate

100

Genistein

ToxCast Assay Data



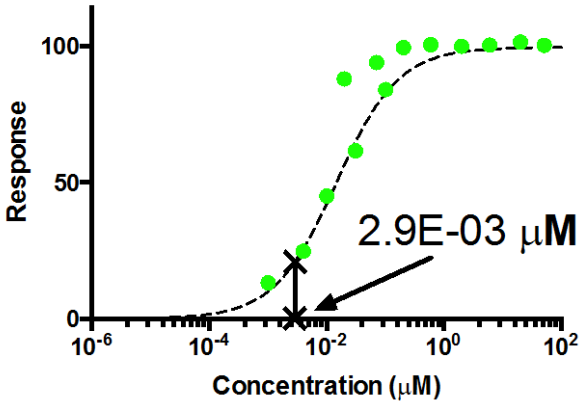


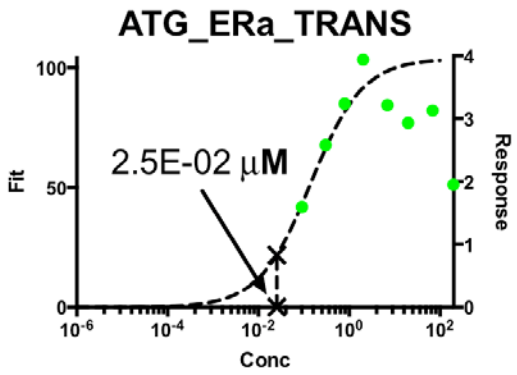
NVS_NR_hER

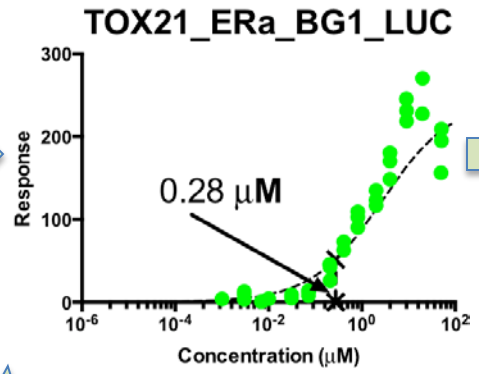
ACEA_T47D_80hr_Positive

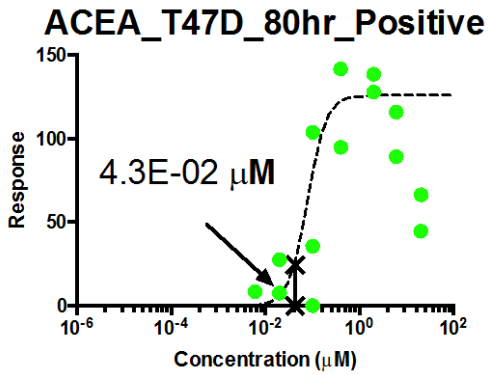
Data pipeline or flat files;
How else to get the raw data?
Download these images and use GraphClick, now free from Arizona software

Genistein – Dietary Phytoestrogen









From OECD Studies on mice

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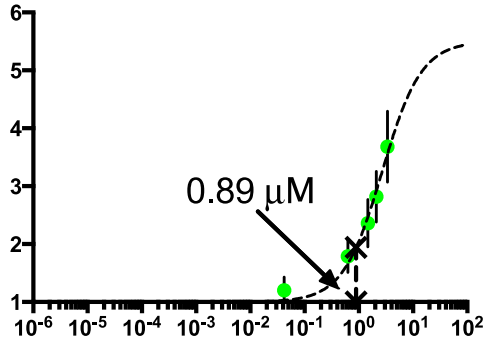
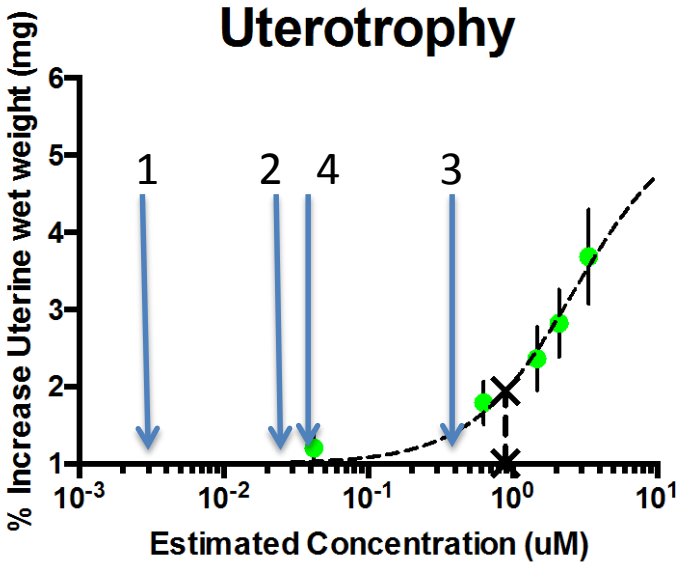


Uncertain fits for gene expression and proliferation

Assay Responses would be needed to trigger a Uterotrophic Response?

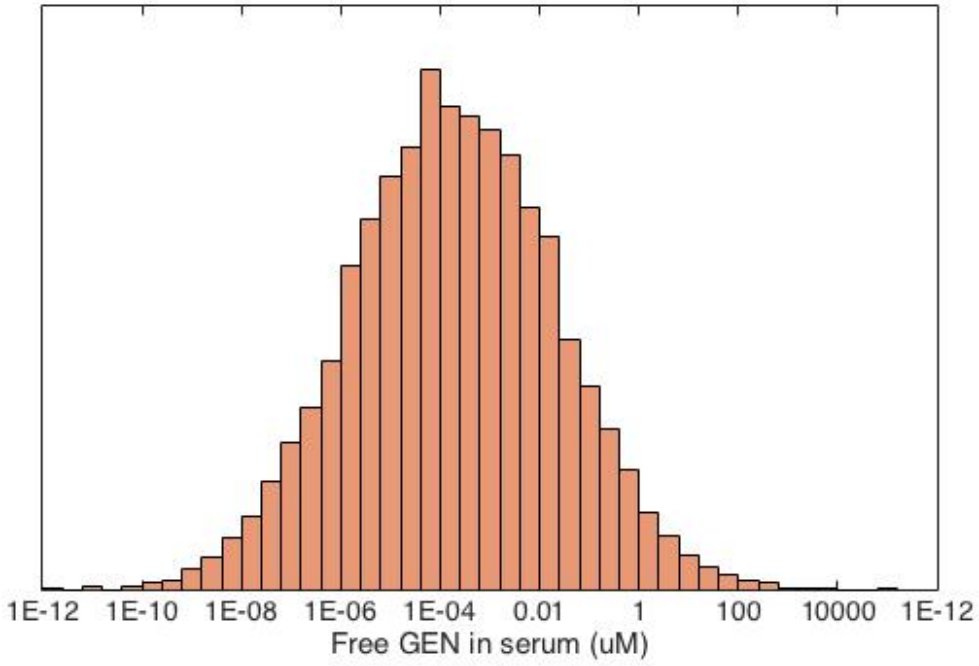
Dose at	1) ER binding	2) Gene Expression	3) Protein Expression	4) Cell Proliferation	In vivo
Conc. at Tipping Point for Uterotrophy	0.003	0.025	0.28	0.04	0.89
In Vivo % Response	0.03%	0.4%	6.5%	0.7%	NC



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



Blood Concentration

Median = 0.005 µM

P01 = 1.5E-05 µM

P05 = 4.8E-04 µM

P95 = 0.66 µM

P99 = 1.76 µM

From 17 human studies

Assay Tipping Points

Binding = 0.003µM

Protein. Exp. = 0.28µM

Gene Exp. = 0.025µM

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 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?