# Quantitative Prediction of Phenotypic Change from High Throughput Assay Results

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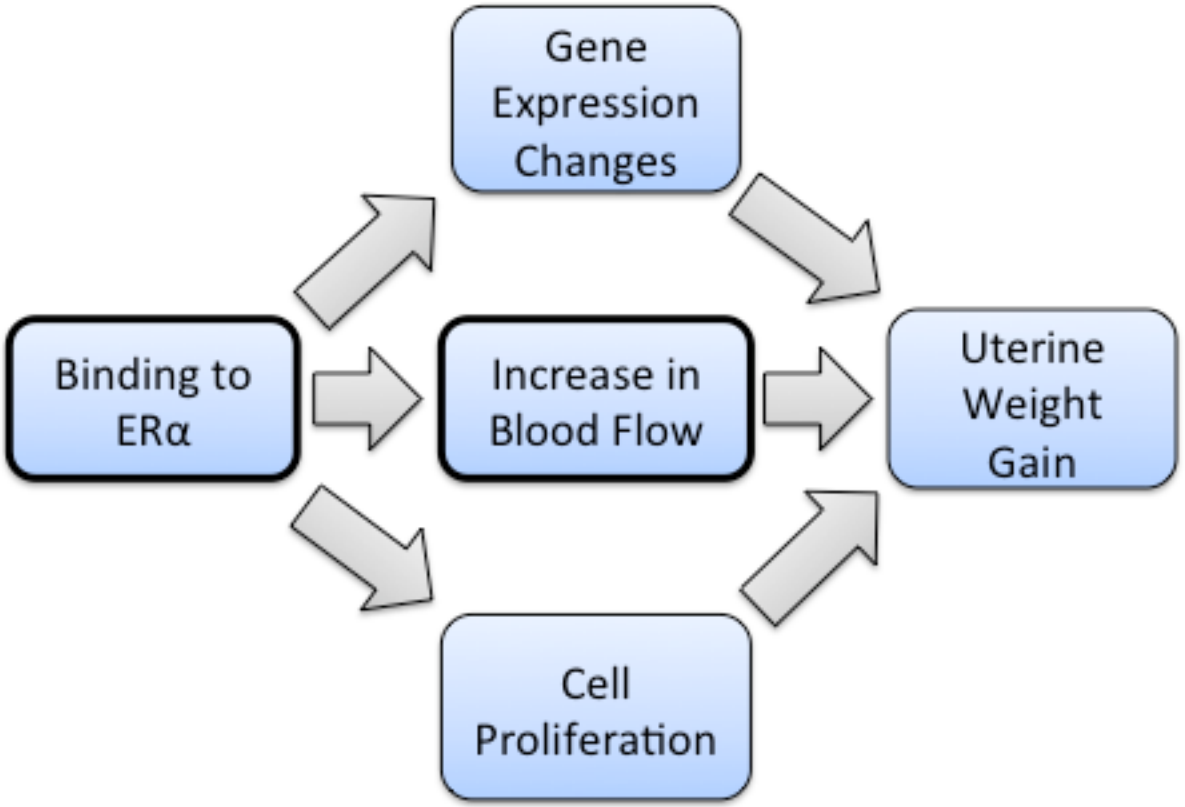
# Road Map

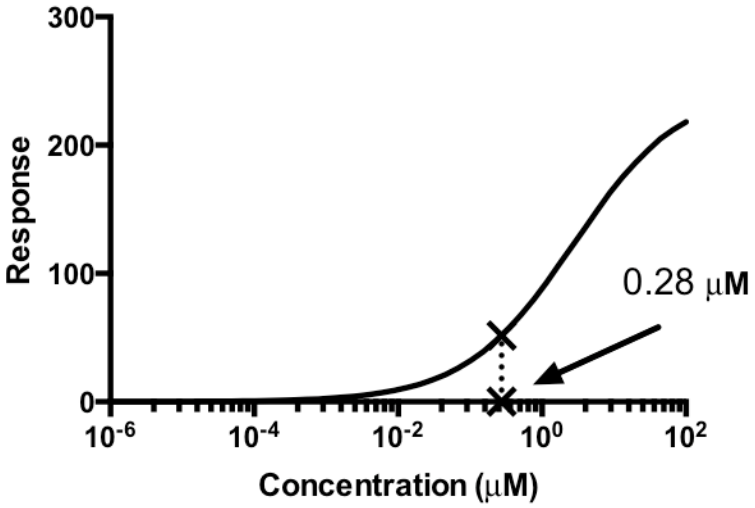
- For use in screening/prioritization schemes or even risk assessment, prediction models must be evaluated as fit for their intended purpose.
- Users need to have similar or greater confidence in the results as they do in current results
- Here we'll try to predict the occurrence of uterotrophy from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




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

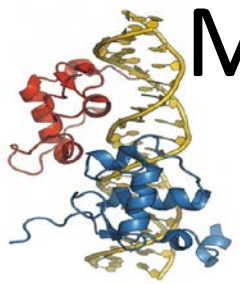
$$\text{Response} = \frac{1}{1+10^{gx(\log(ga)-\log(dose)}}$$

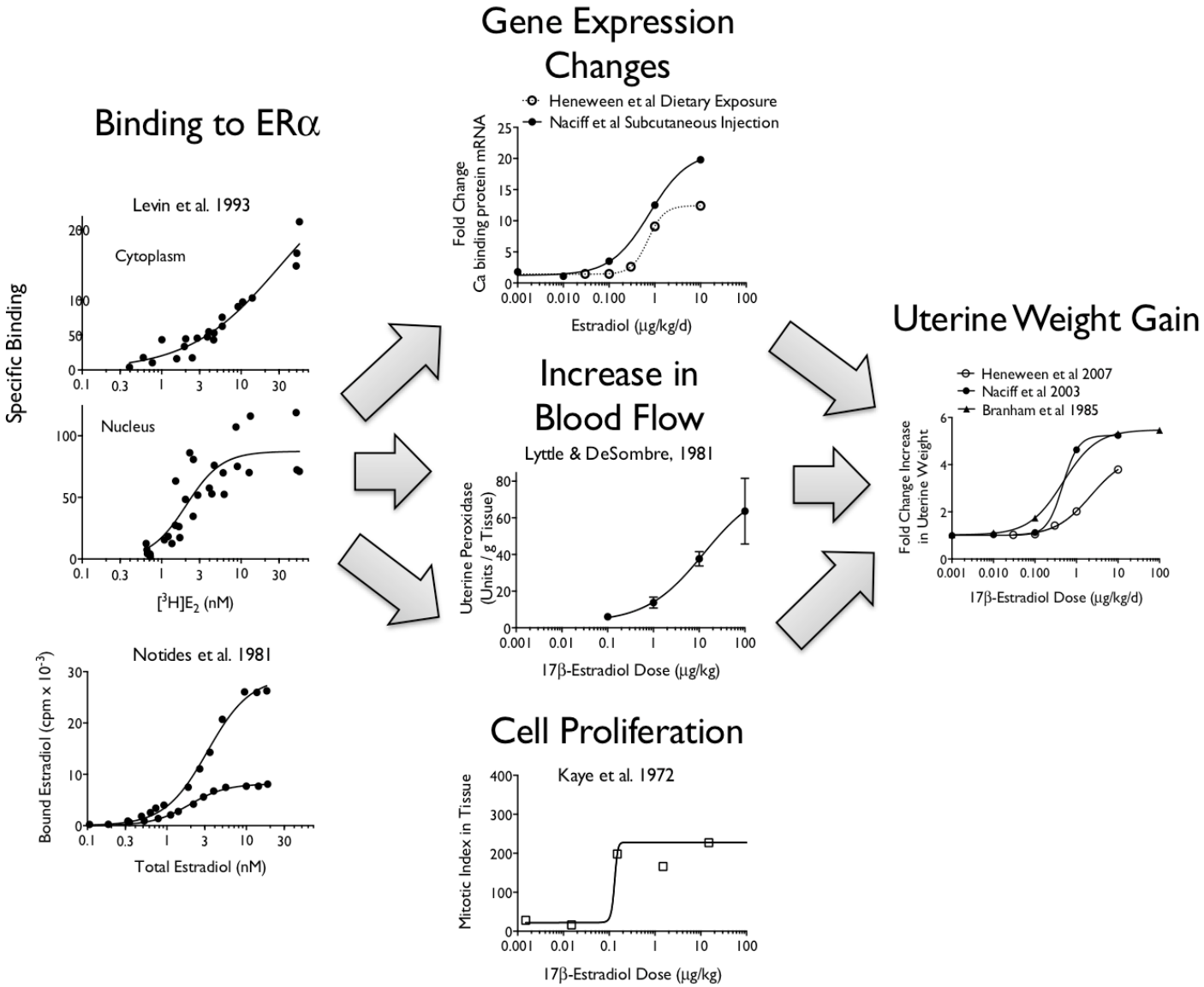
Murell et al. (1998) Risk Anal 18:13-26 suggested estimating the slope at the EC50 and projecting down to the baseline to identify the TDV. Sand et al. (2006) Toxicol Sci 90:241-51 identified the BMR21 at the point at which the third derivative is equal to zero. Use inverse with Response = 0.21 as follows:

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

"tipping point" on the dose-response curve

# Mode of Action from In Vivo Studies using Estradiol



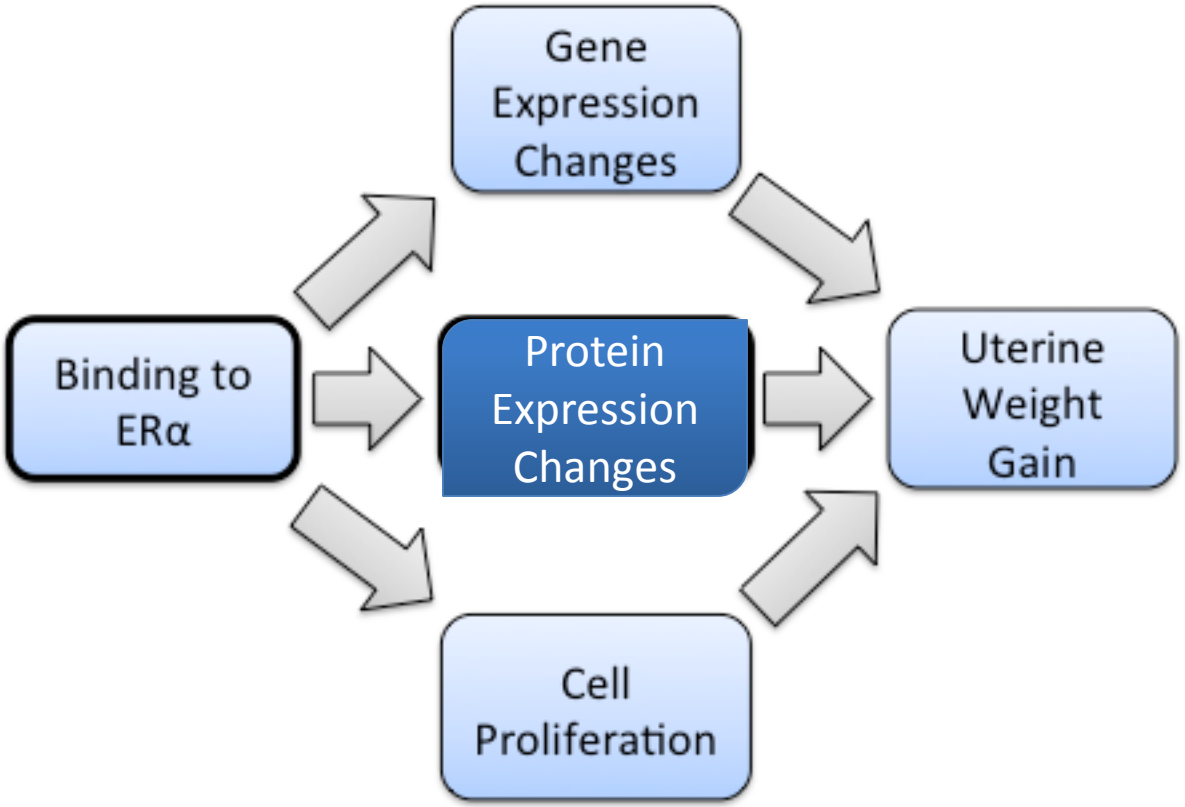


# TDVs for Estradiol

- 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)

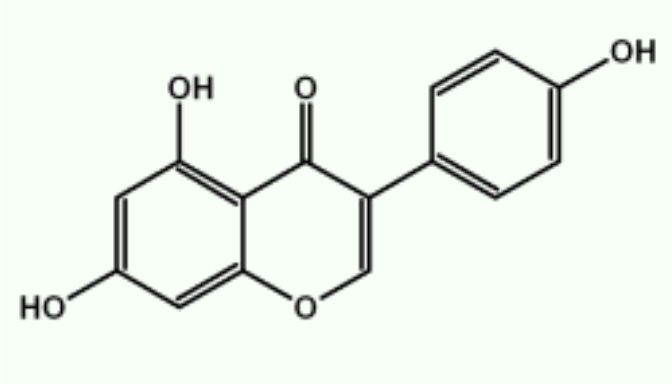
Far field
Near field
Use of “big” data

Assay comparison

Good for estimates of intake, not for steady state conc

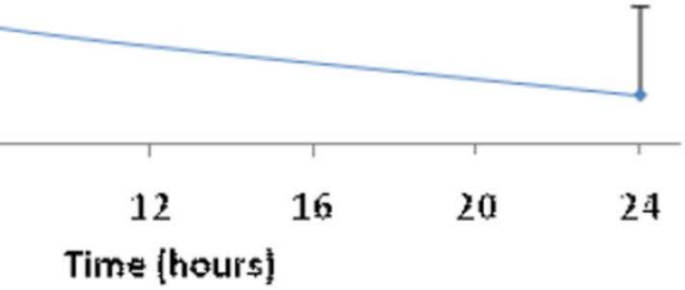
# In Vitro In Vivo Extrapolation

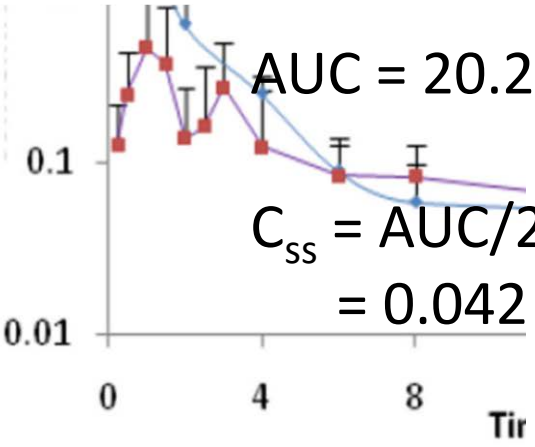
- IVIVE to obtain doses corresponding to effect concentrations
- Need IVIVE for both humans and mice
- Humans
  - Oral equiv. (mg/kg/d) = Conc (µM) / Css
  - Units of Css are (mg/kg/d) per µM
  - Wetmore et al. calculated Css values for many chemicals
  - Genistein
    - Css low = 1.49E-01
    - Css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

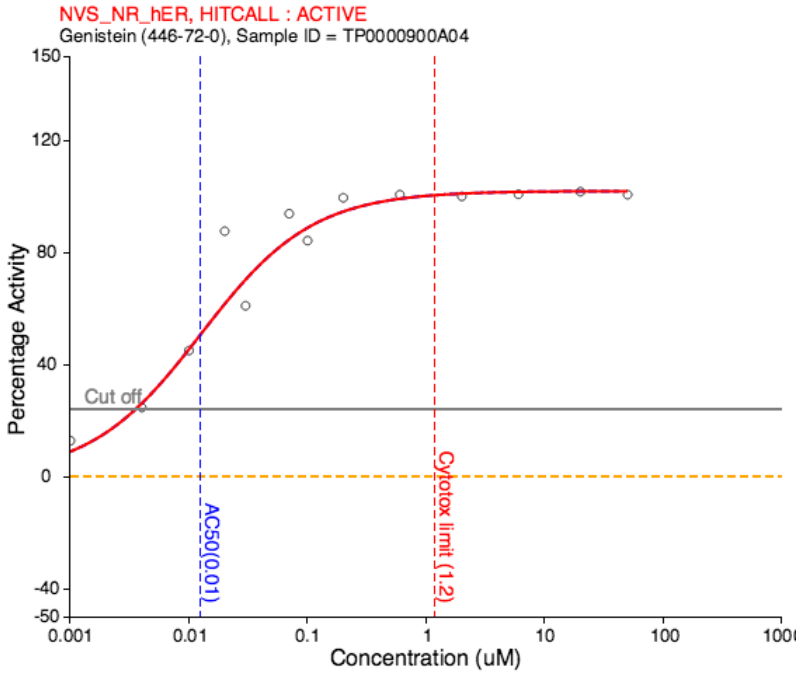
$C_{ss}$ = AUC/24/20
= 0.042 µM/mg/kg/d

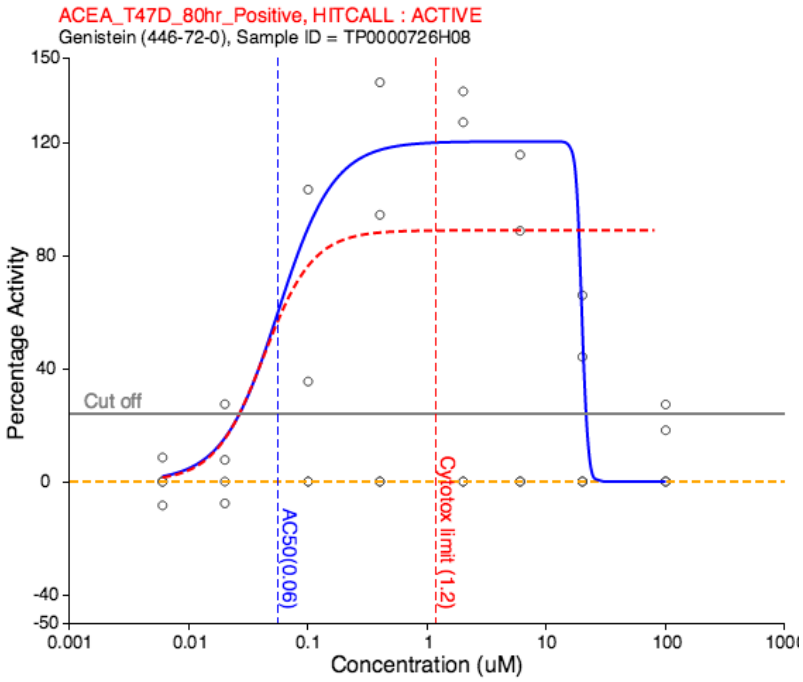
ein 4'-O-sulfate

100

Genistein

# ToxCast Assay Data



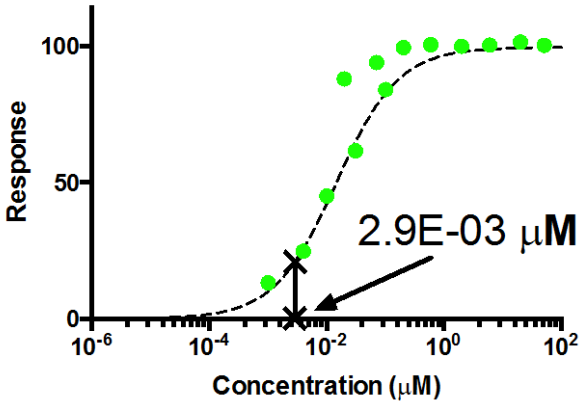



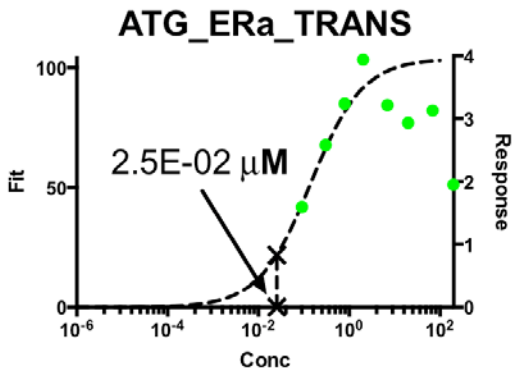

NVS_NR_hER

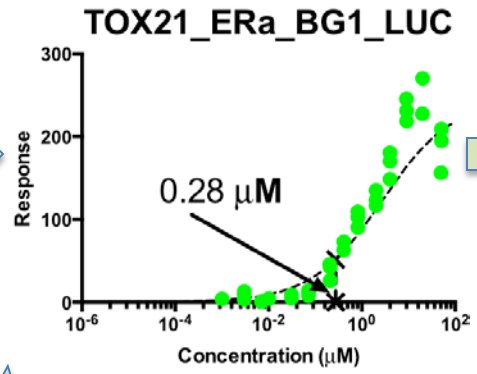
ACEA_T47D_80hr_Positive

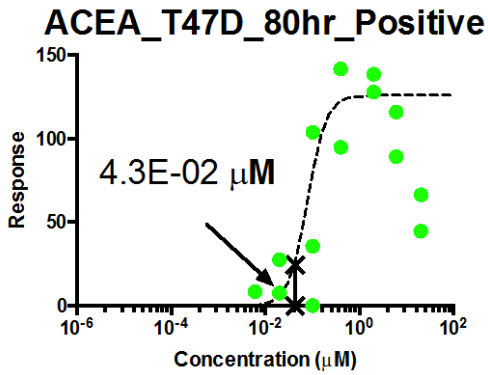
Data pipeline or flat files;
How else to get the raw data?
Download these images and use GraphClick, now free from Arizona software

# Genistein – Dietary Phytoestrogen



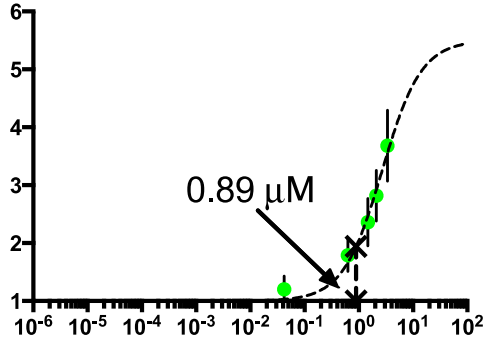




From OECD Studies on mice

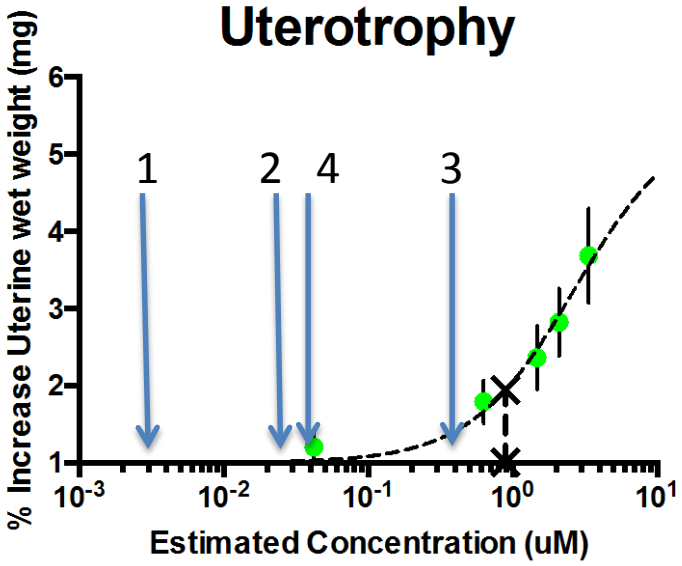
**ophy**




Uncertain fits for gene expression and proliferation

# Assay Responses would be needed to trigger a Uterotrophic Response?

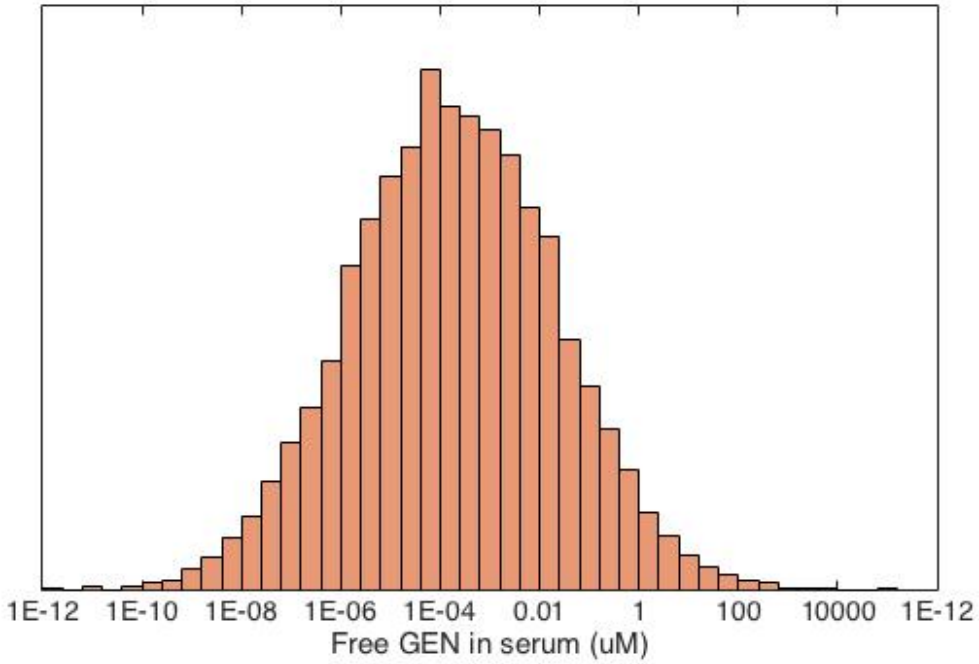
| Dose at | 1) ER binding | 2) Gene Expression | 3) Protein Expression | 4 ) Cell Proliferation | In vivo |
|---|---|---|---|---|---|
| Conc. at Tipping Point for Uterotrophy | 0.003 | 0.025 | 0.28 | 0.04 | 0.89 |
| In Vivo % Response | 0.03% | 0.4% | 6.5% | 0.7% | NC |




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?