NTP
National Toxicology Program

HTS and Mixtures: Lessons Learned

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SRP Risk e-Learning Webinar
New Approaches and Alternatives for Toxicity Testing:
Session III - Modernizing Safety Testing
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Outline

- Introduction
  - Challenges Facing Toxicology and Hazard Assessment
  - Tox21 vs ToxCast vs Tox21 approaches
- Case studies
  - Evaluating dose addition in Tox21
  - Evaluating mixtures in Tox21

Toxicological Challenges in the 21st Century

- Too many chemicals.
  - Thousands of chemicals on the market with significant toxicological data gaps
- Too many commercial mixtures.
  - Botanicals
  - Pesticide formulations
  - PAHs
- Too many co-exposures.
  - We are exposed to mixtures of mixtures
- We cannot use traditional methods to test our way out of this!

Toxicity Testing in the 21st Century

- Early 2000's it became apparent to a number of organizations that our traditional testing approaches were unsustainable.
  - 2004
    - NTP Road Map
  - 2005
    - Tox21 initiated with NTP, NCGC, USEPA
    - USEPA implemented ToxCast
  - 2007
    - NAS Report: Toxicity Testing in the 21st Century: A Vision and a Strategy (2007)
  - 2010
    - US FDA Joins Tox21

Tox21 vs Tox21 Approaches

- Tox21
  - Focus on human biology/human cells/ tissues.
  - Initially focused on the 10K library and HTS methods using robotics.
    - Phase I and II
      - Screening one pathway at a time, but 75-100 different pathways.
    - Phase III
      - High Throughput Transcriptomics

- Tox21 Approaches
  - Focus on human biology/human cells/ tissues.
  - Smaller libraries – no robots but liquid handling stations using 384 well plates.
    - Hypothesis based screening; limited number of pathway-based assays but can do high throughput transcriptomics.

Mixtures Risk Assessment

How can we estimate human health risk from exposure to mixtures

Whole Mixtures

Requires toxicity data on whole mixtures

- Data on mixture of interest
- Data on "sufficiently similar" reference mixture

Component-based

Requires toxicity data for individual chemicals within the mixture

- Dose addition
  - Relative Potency Factor
- Response addition

Case Study 1: Evaluating Dose Addition in Tox21

- Focus on chemicals positive in Phase I of Tox21 in the Estrogen Receptor (10 chemicals) and Androgen Receptor (8 chemicals) assays.
- Made 67 mixtures of these 18 chemicals (used Ray Design).
  - ER agonists only
  - AR agonists only
  - Mixtures of ER/AR agonists
- All individual chemicals and mixtures were in phase II of Tox21 for all assays.
  - Initial analysis of two ER assays (BG1 whole receptor assay; B-Gal partial receptor assay.

Chemicals and mixtures

ER actives

- Zearalenone
- Bisphenol A
- Ethylenediamine
- Chlordecone
- Acetochlor
- Butylbenzylphtalate
- Dicumyl peroxide
- o,p-DDT
- P,n-nonylphenol
- alachlor

AR actives

- Oxymetholone
- Fluoxymestrone
- Progesterone
- Dexamethasone
- Medroxyprogesterone acetate
- O-methoxyphenol
- Hydroxyflutamide
- Androstenedione

Tox21 Methods

General Tox21 Methods

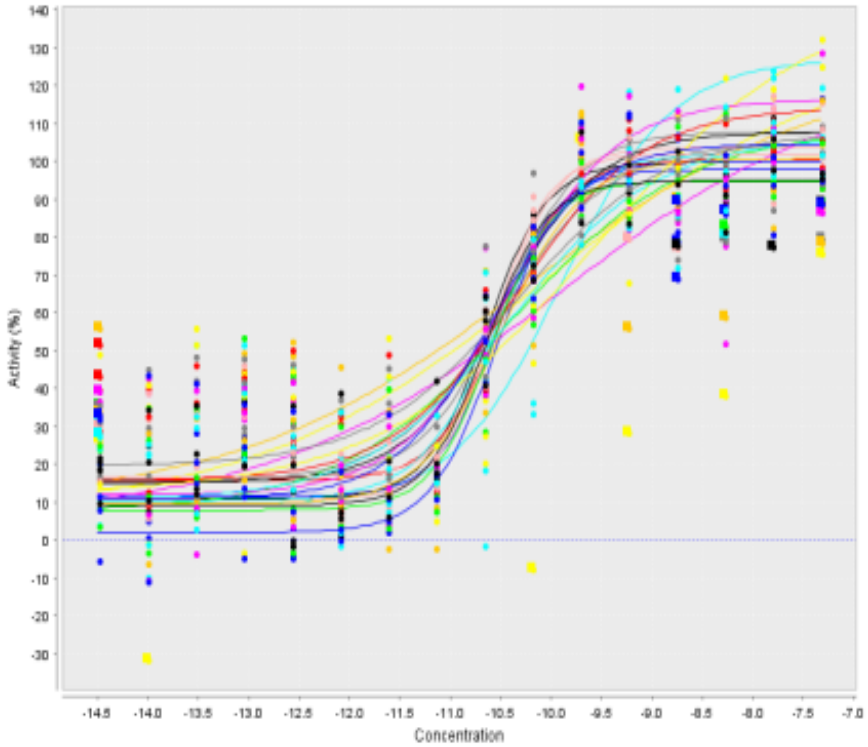
- 1536 well plates
- 15 point dose response curves for individual chemicals and mixtures
- All assays performed in triplicate on three consecutive days.
- Culture volume 5uL

ER-Luciferase Assay

- Assay provider: UC Davis
- Cell line name: BG1Luc4E2/(MCF-7)
- Compound treatment time: 22h
- Assay readout: Luc-reporter, luciferase readout
- Target: ER-alpha (full-length receptor, endogenous)
- Luminescence read out

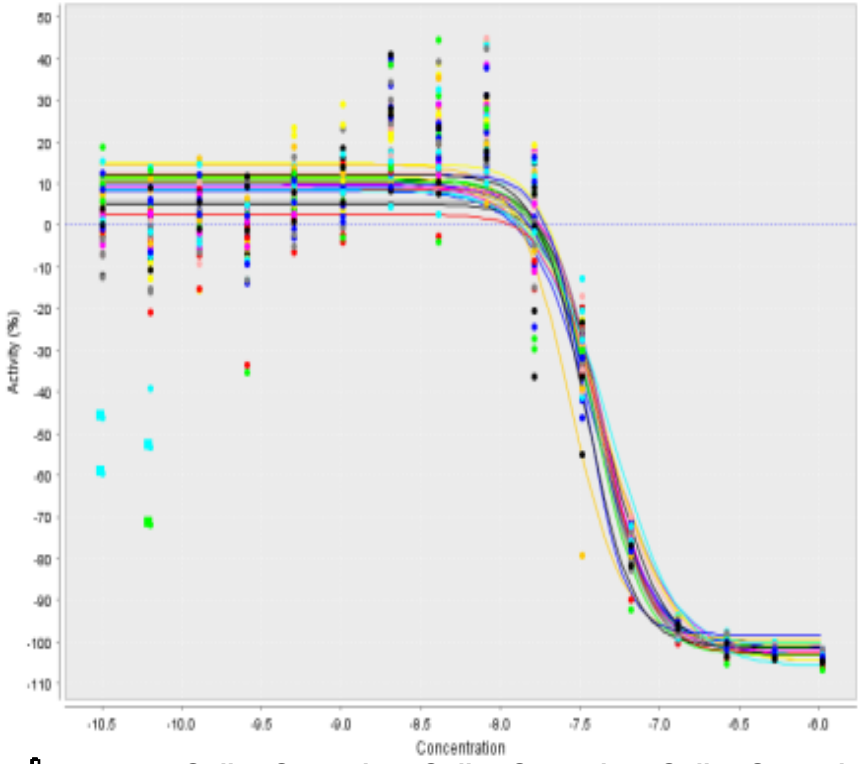
Estrogen Receptor alpha (ERα-BG1) (2)

β-estradiol (agonist) Online Validation Positive Control Dose Response Curve



ERα-BG1	Online Validation Agonist (Mean ± SD)	Online Validation Antagonist (Mean ± SD)
EC50	0.17 ± 0.12 nM (n = 27)	0.04 ± 0.004 µM (n = 27)
S/B	2.58 ± 0.17	7.88 ± 0.39
CV (%)*	14.79 ± 4.65 (n = 18)	8.27 ± 5.78 (n = 18)
Z'	0.36 ± 0.16	0.73 ± 0.10

4-hydroxy tamoxifen (antagonist) Online Validation Positive Control Dose Response Curve



ERα-BG1	Online Screening Agonist (Mean ± SD)	Online Screening Antagonist (Mean ± SD)	Online Screening Viability (Mean ± SD)
IC50	0.082 ± 0.42 nM (n = 458)	73.6 ± 8.9 nM (N = 458)	NA
S/B	2.53 ± 0.29	8.02 ± 0.95	6.15 ± 0.85
CV (%)**	7.72 ± 1.60 (n = 54)	5.25 ± 0.97 (n = 54)	6.57 ± 0.93 (n = 54)
Z'	0.54 ± 0.14	0.77 ± 0.07	0.80 ± 0.06

*CV values shown represent average of DMSO plates and low concentration plates
**CV values shown represent average of DMSO plates only

Concentration Response Modeling and Mixture Modeling

- Individual chemical data fit to a Hill model.

$$f_i(x) = f_0 + v_i \frac{\frac{x}{k_i}}{1 + \frac{x}{k_i}}$$

- Mixtures we used two models
  - Independent Action or Response Addition

$$f(z_i) = dM\left[1 - \prod_j \left(1 - \frac{|f_j(x_{ij})|}{M}\right)\right]$$

  - Integrated concentration addition/independent action model (Howard and Webster, 2009).

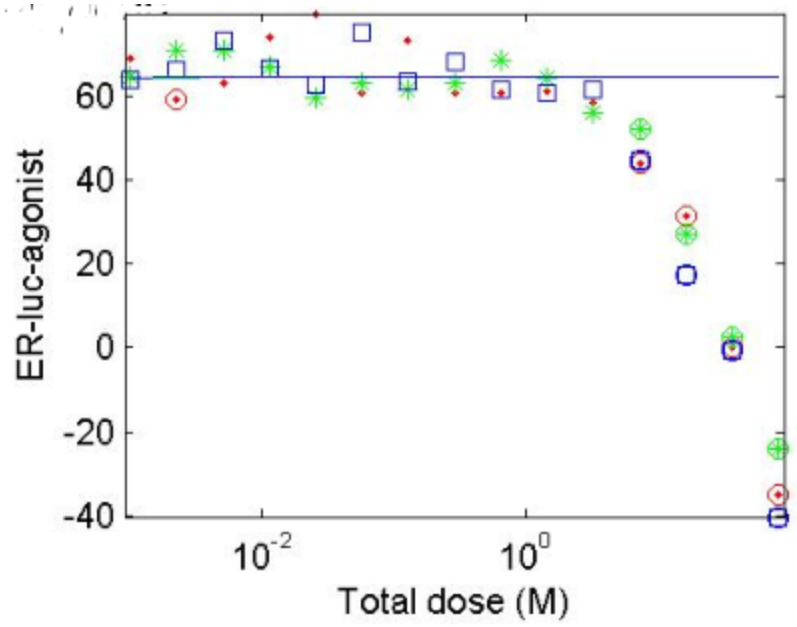
$$f(z_i) = \frac{\sum_j \frac{v_j x_{ij}}{k_j}}{1 + \sum_j \frac{x_{ij}}{k_j}}$$

Challenges in Hypothesis Testing in Tox21

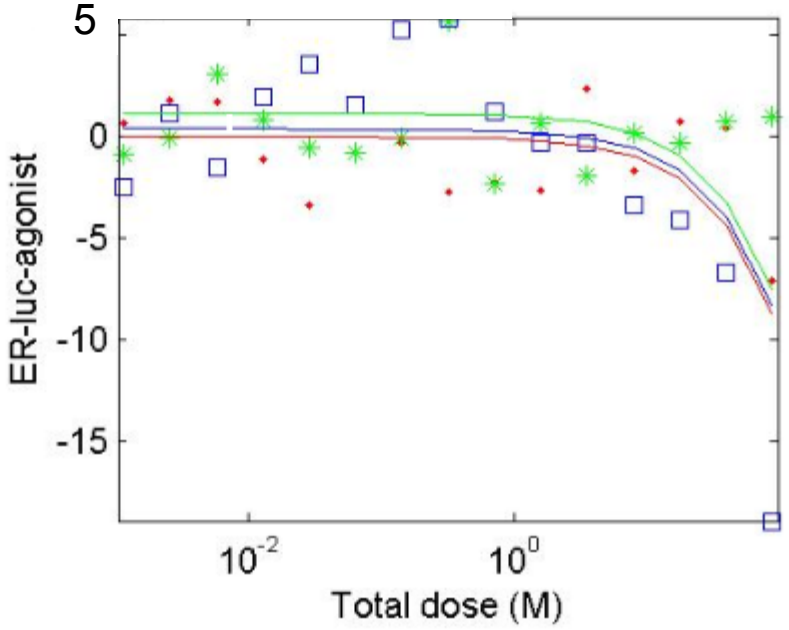
- In for a penny, in for a pound
  - Once the chemicals are on the plate, they are going to be run on every assay (>75 assays)
- No going back!
  - Think about the 10K library and HTS as a ship leaving port. You are either on it or you are at the dock. Once you leave port you do not get off the ship until the trip is finished.
- Data inconsistencies between phase I and II data.
  - All chemicals tested were positive in phase I and about half were positive in phase II.
  - All concentrations of zearalenone tested were at maximal responses

Examples of Dose Response of individual chemicals

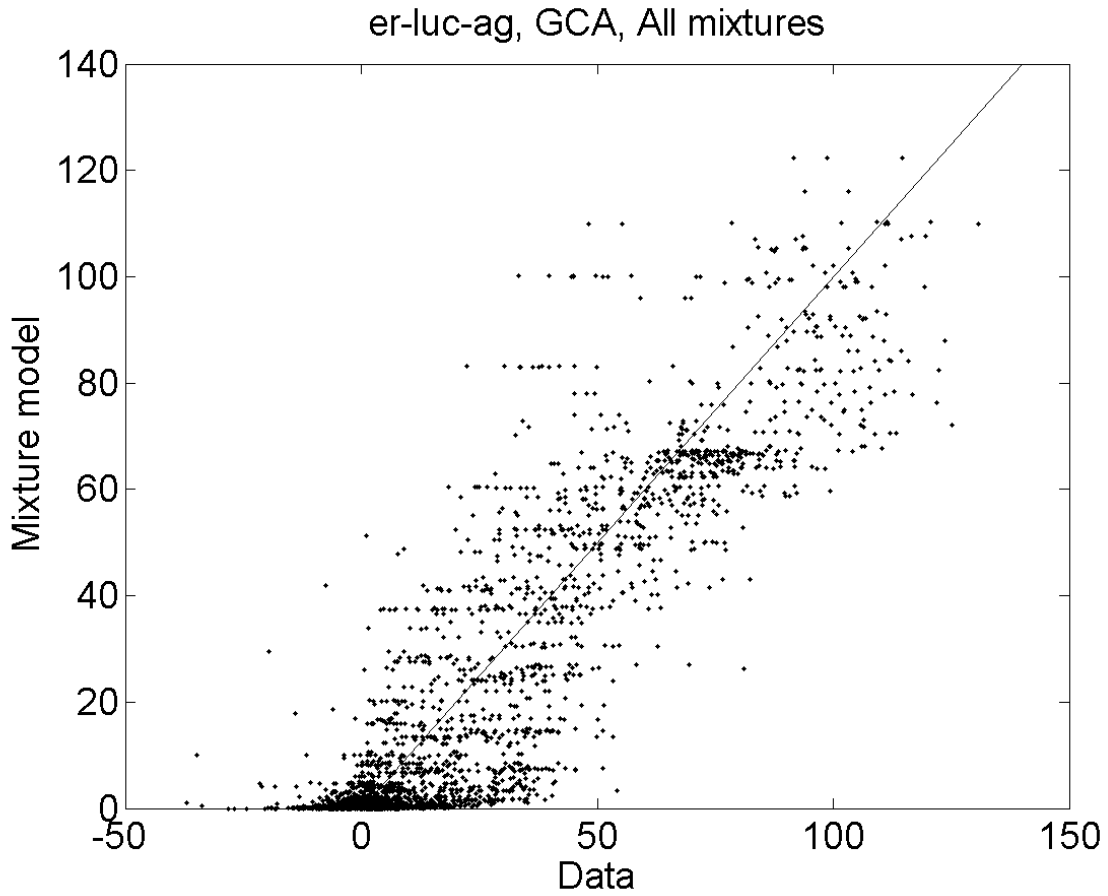
Zearalenone



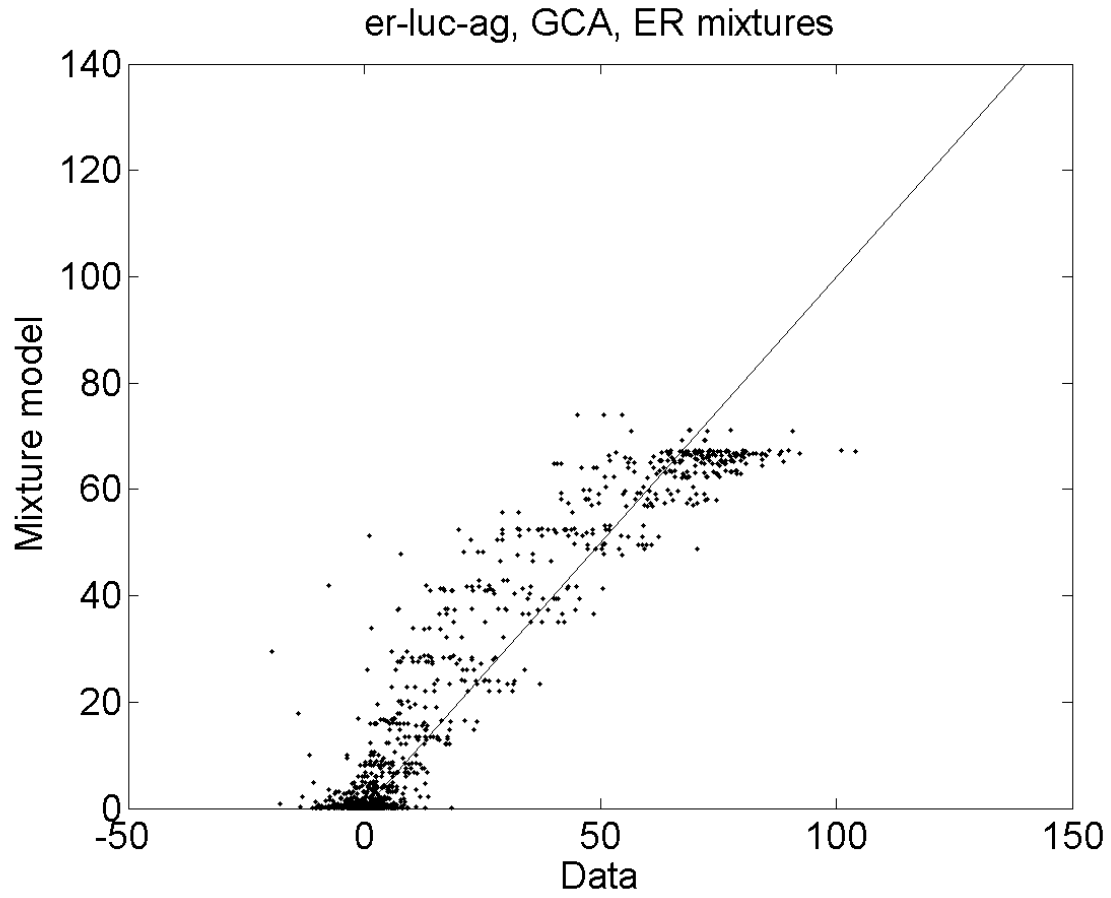
Alachlor



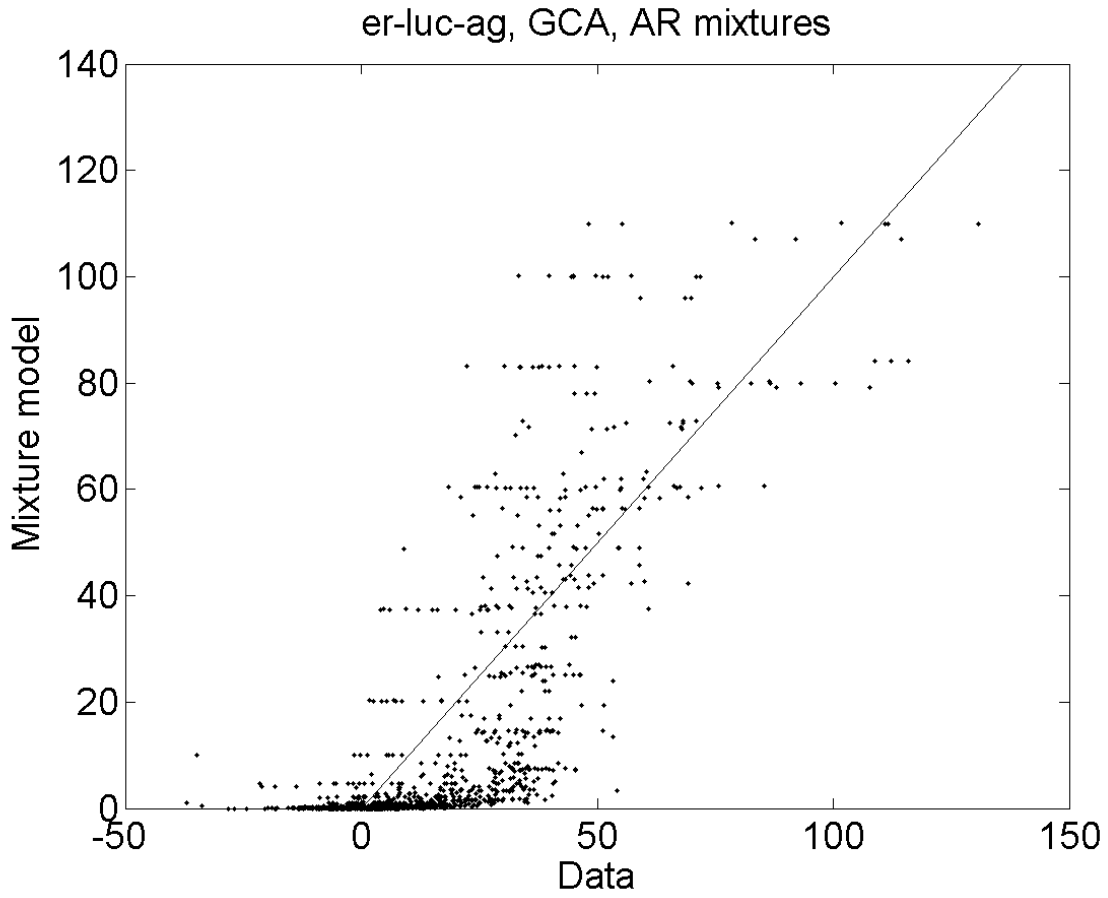
Evaluation of Concentration Addition Models with Mixtures in a high throughput ER Luciferase Assay



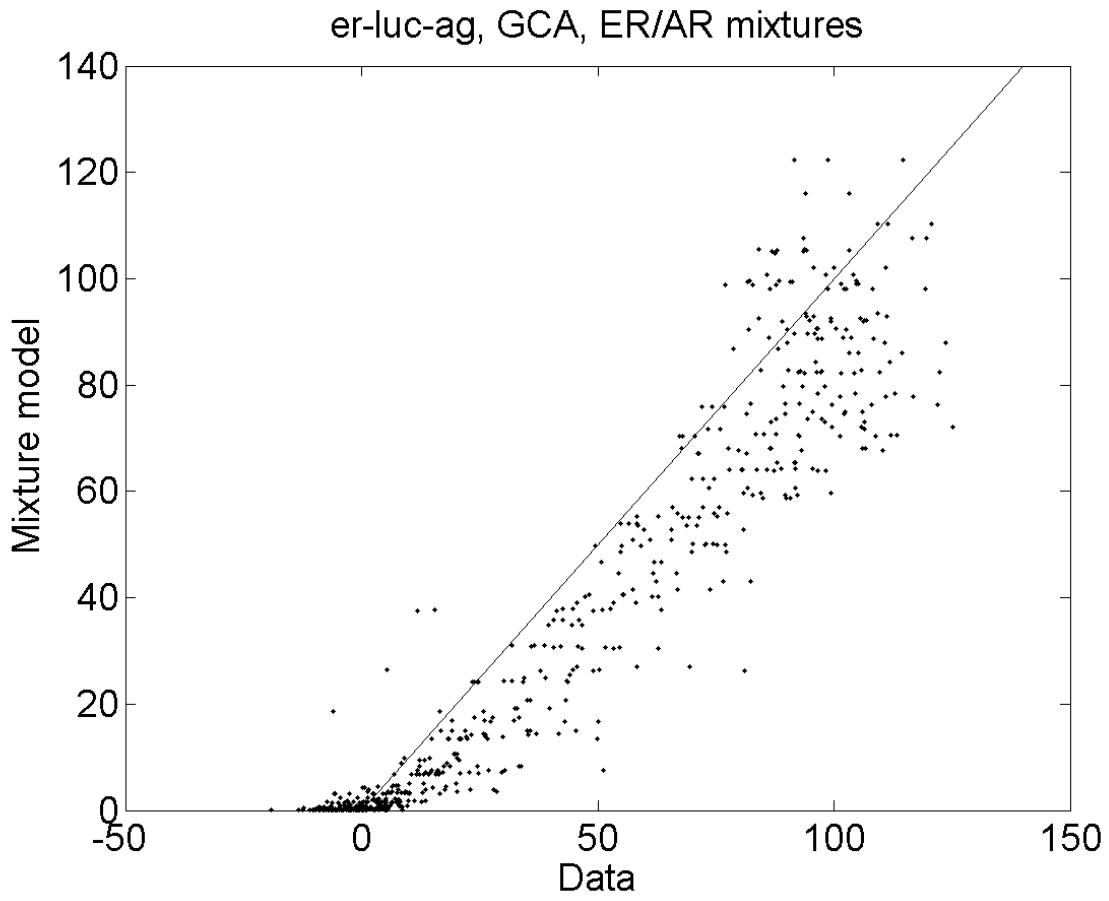
Evaluation of Concentration Addition Models with ER Agonist Mixtures in a High Throughput ER Luciferase Assay



Evaluation of Concentration Addition Models with AR Agonist Mixtures in a High Throughput ER Luciferase Assay

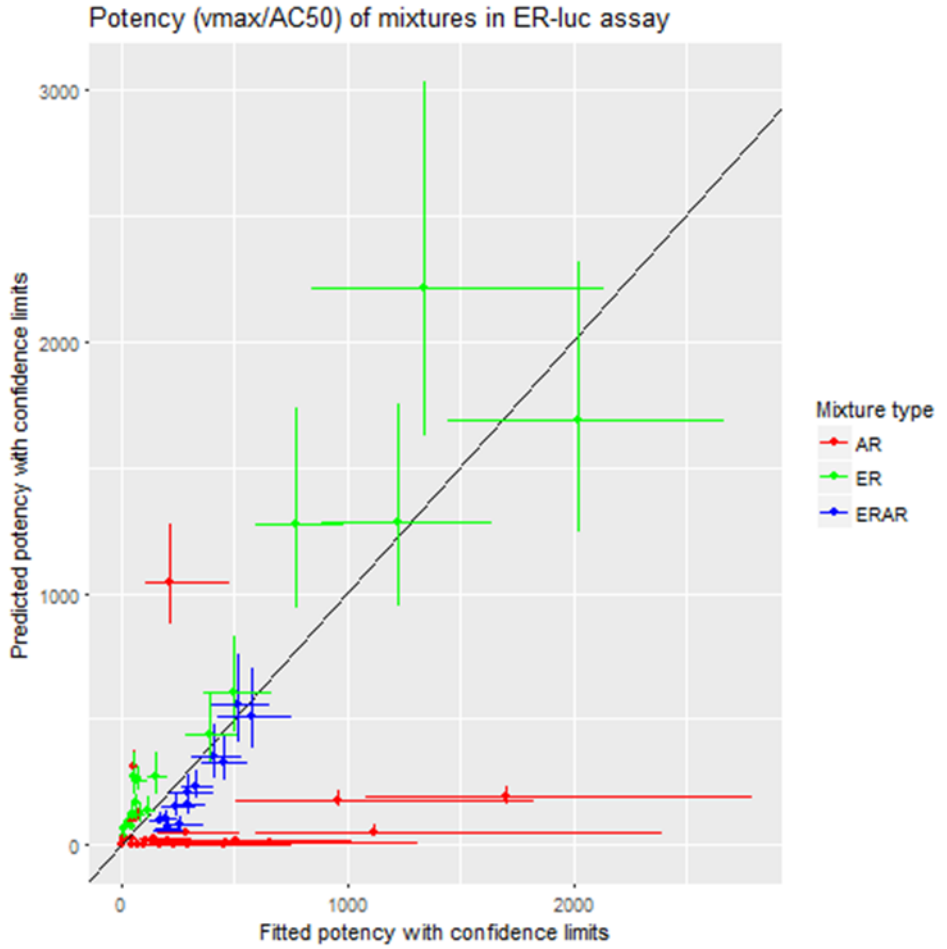


Evaluation of Concentration Addition Models with ER/AR Agonist Mixtures in a High Throughput ER Luciferase Assay



Results of Dose Addition Predictions

- Mixtures of ER agonists alone or ER/AR agonists with predicted low responses were well predicted.
- Mixtures of ER agonists with predicted high response were less well predicted due to uncertainty of zearalenone dose response relationship.
- Mixtures of AR agonists were poorly predicted, but predictions were highly uncertain.



Summary and Conclusions

- HTS can provide screening level information on biological activity.
- Mixtures containing either ER agonists or ER/AR agonists were well predicted in the low dose region.
- Concentration response addition models underestimated the mixtures containing AR agonists for their ER agonist effects.
- We are still analyzing the antagonist mode and the ER-BLA assay.

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