

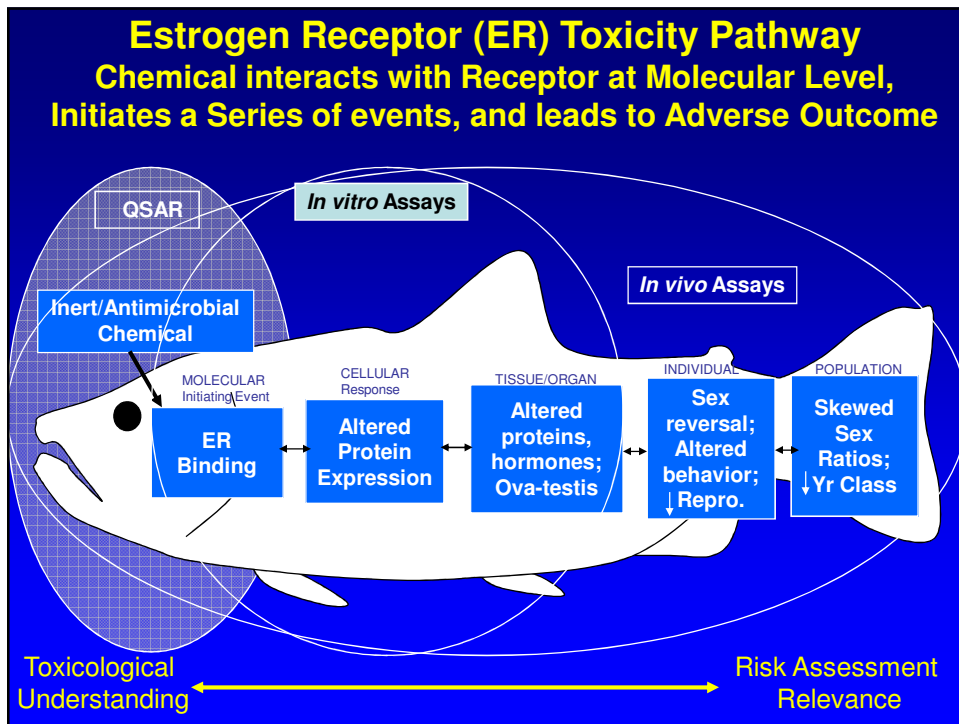
# Dose-Response for Reproductive Effects of ER Binders

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## Acknowledgements:

In Vitro- J. Denny; R. Kolanczyk; Barb Sheedy; Mark Tapper  
Students: R. Maciewski; W. Backe; M. Dybvig; M. Mereness  
Post-Doc: H. Aladjov

In Vivo- K. Flynn; D. Hammermeister; D. Lothenbach; F. Whiteman;  
Students: J. Nagel; W. Backe  
Post-Doc: M. Haasch

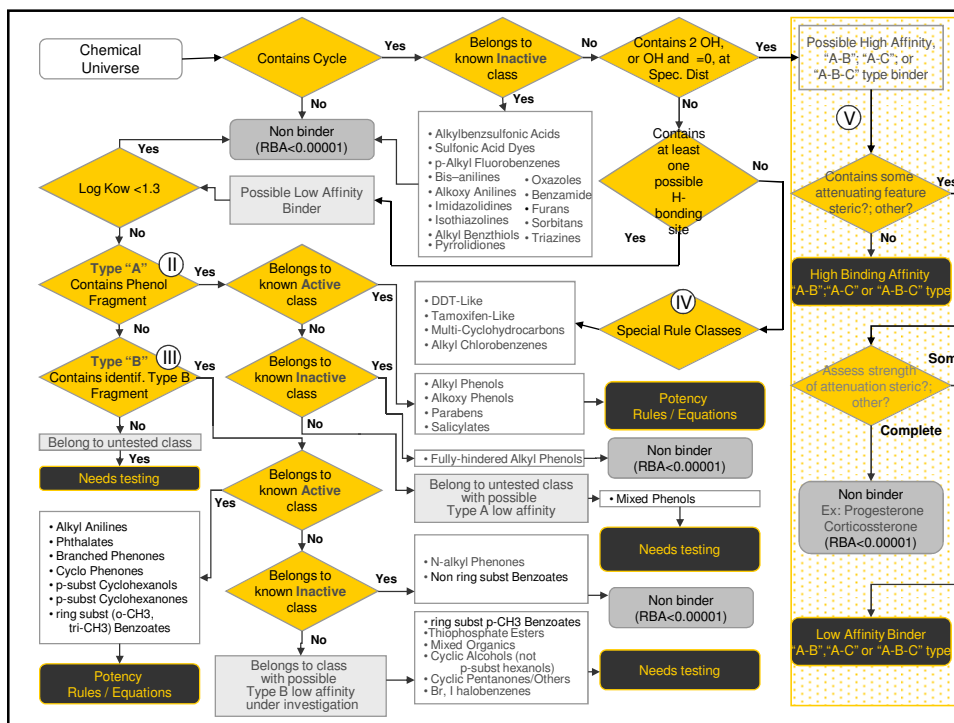
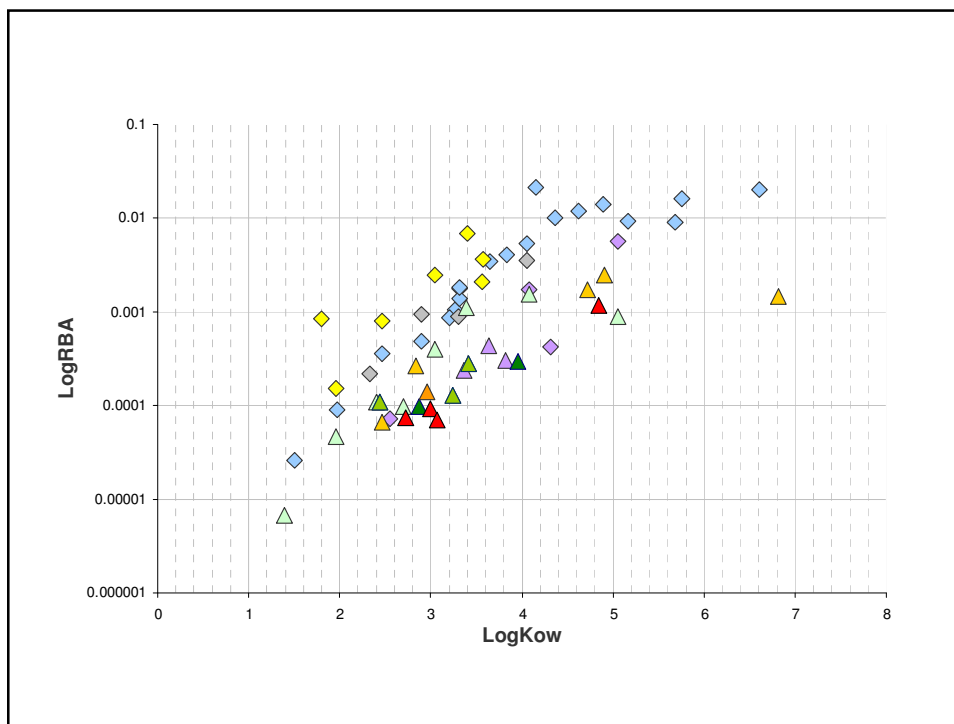


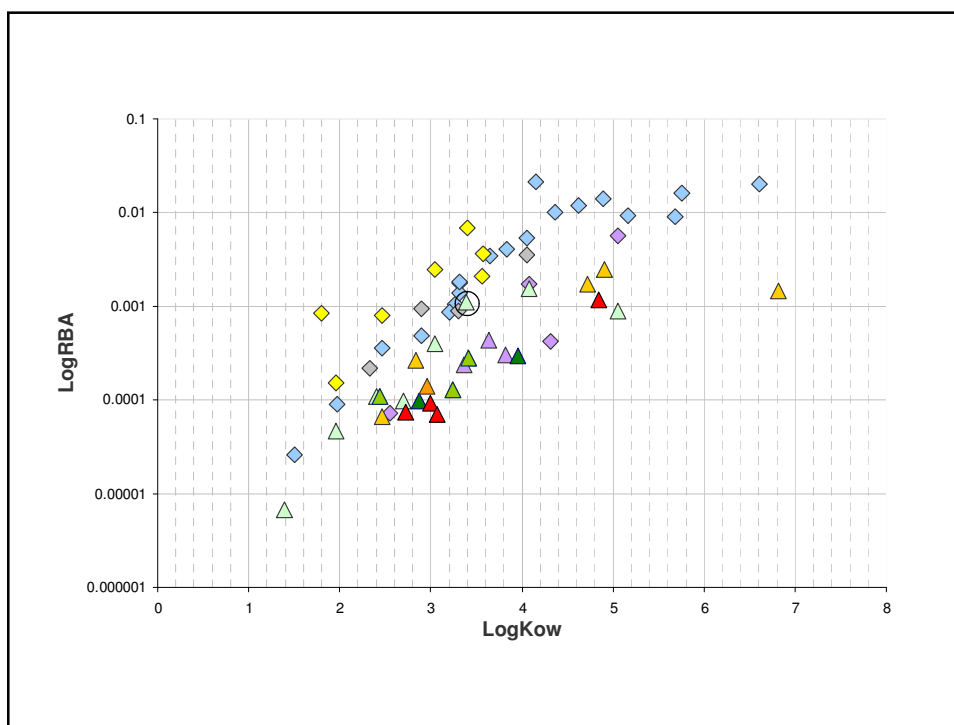
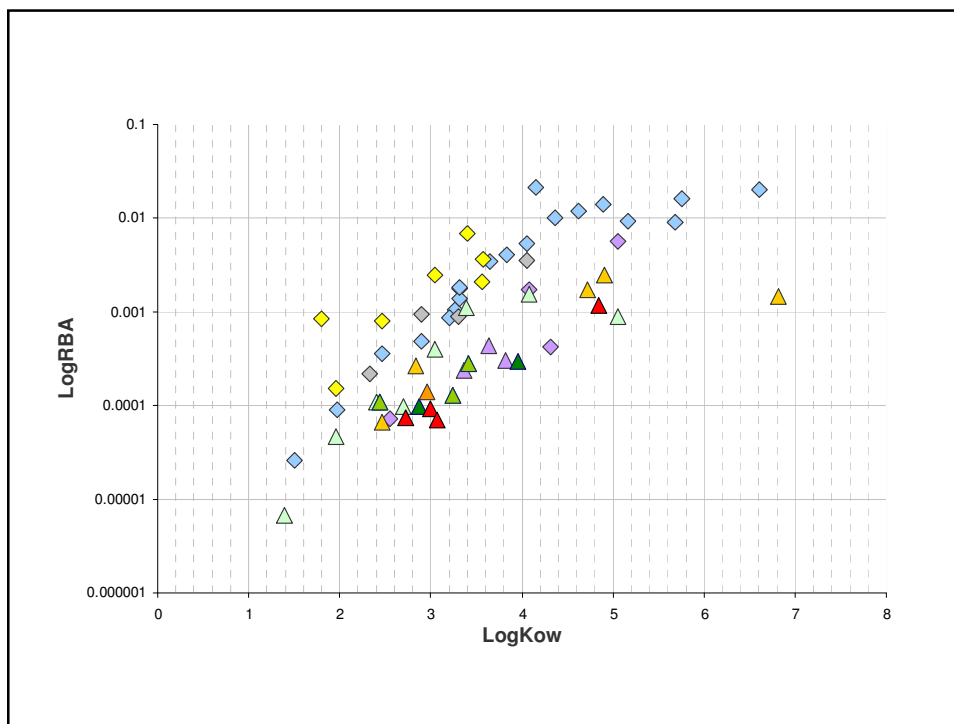
### ER Prioritization System – Establishing Chemical Categories

Test chemicals within FI and AM inventories for potential to bind ER to prioritize which chemicals, of hundreds EPA is required to evaluate, should go on for further testing. Focus in on the chemical likely to have the activity.

Predictions are needed for chemicals with little of no data

EPA problem is low affinity chemicals





Will a chemical with rtER binding affinity 0.001 in our assay have ER-mediated effects in vivo?

How do you compare dosimetry and effects across in vitro and in vivo assay systems?

Dosimetry:

Chemical concentration – How much? Where?  
Total Chemical Conc; Bound vs Free – internal bioavailability  
Chemical Solubility

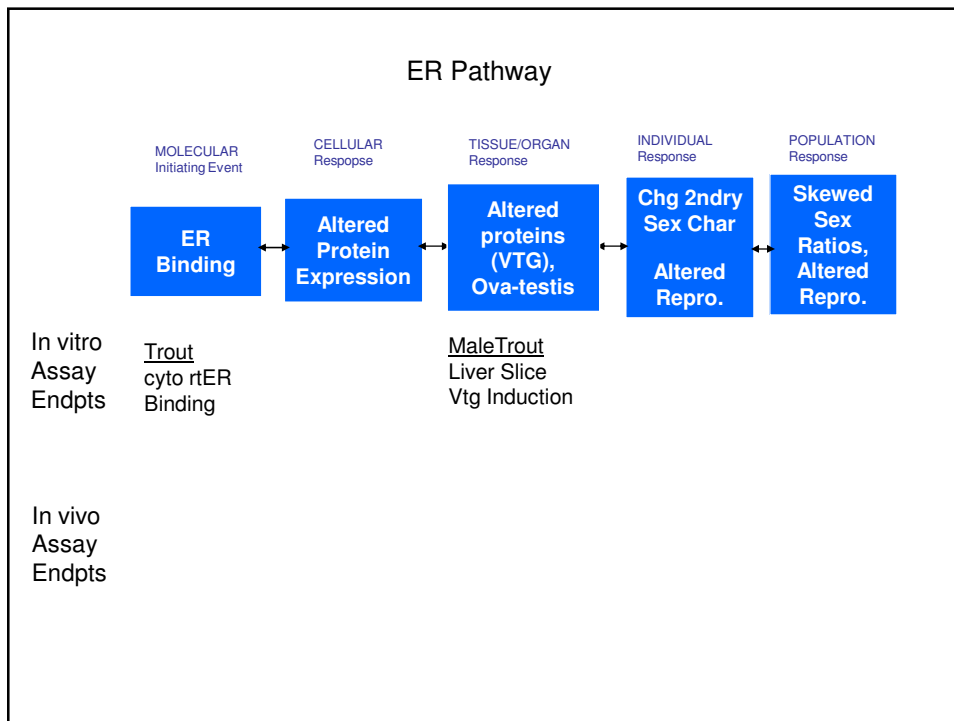
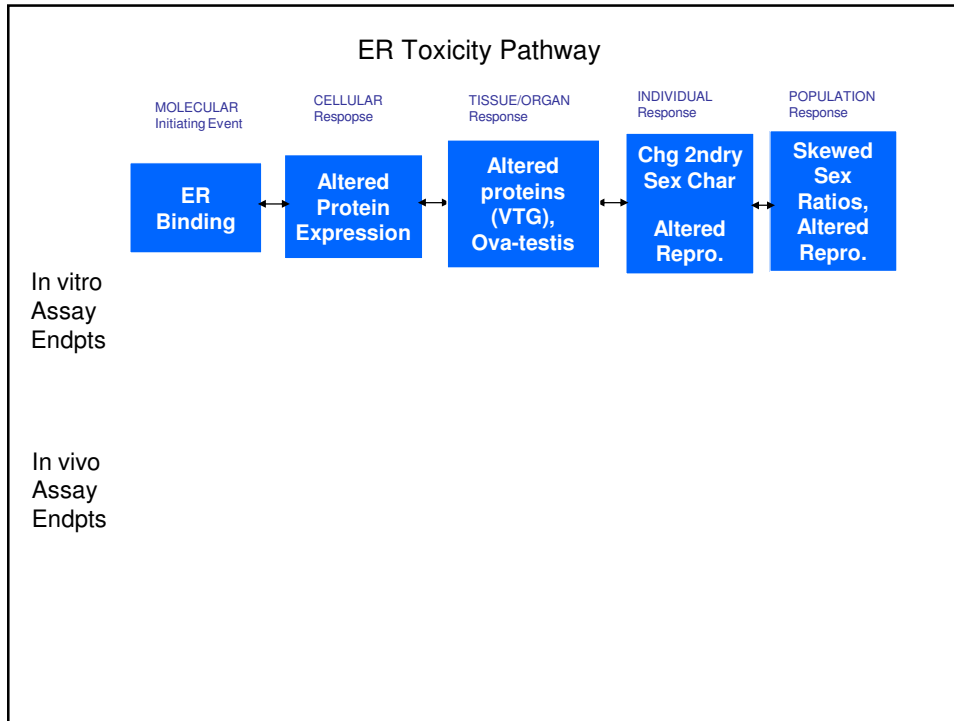
Effects:

Biological Response; Toxicity

How do you compare data across levels of biological organization?

Comparing Biological Response

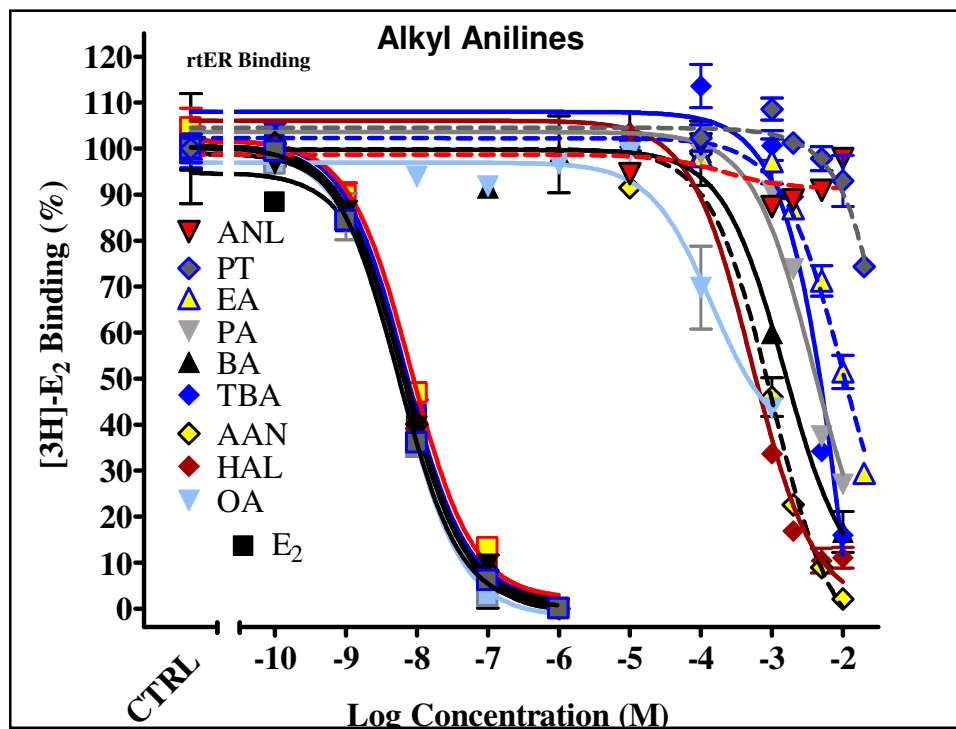
Comparing Dosimetry

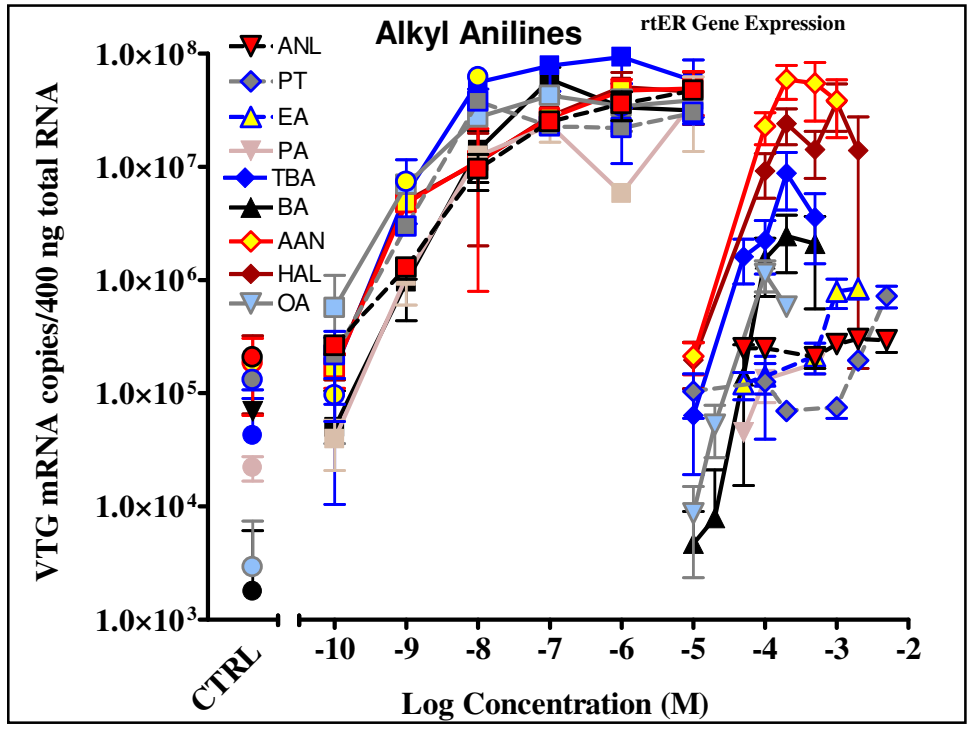


### The in vitro Assays

rtER Binding –  $^3\text{H}$ -E<sub>2</sub> displacement  
cytosolic fraction from M, F trout livers  
cytosol diluted in TEDG buffer (Tris, EDTA, dithiothreitol, glycerol);  
avg protein = 4.6 mg/ml,  $\pm$  1.2, (range 3.4 - 8.8) N=97

rtER Liver Slice Vtg Induction  
precision cut trout liver slices  
incubated in 1.4ml of L-15 buffer (10% FBS);  
 $\sim$  3 mg/ml protein

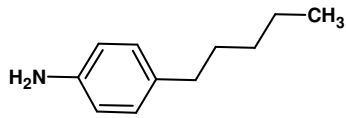




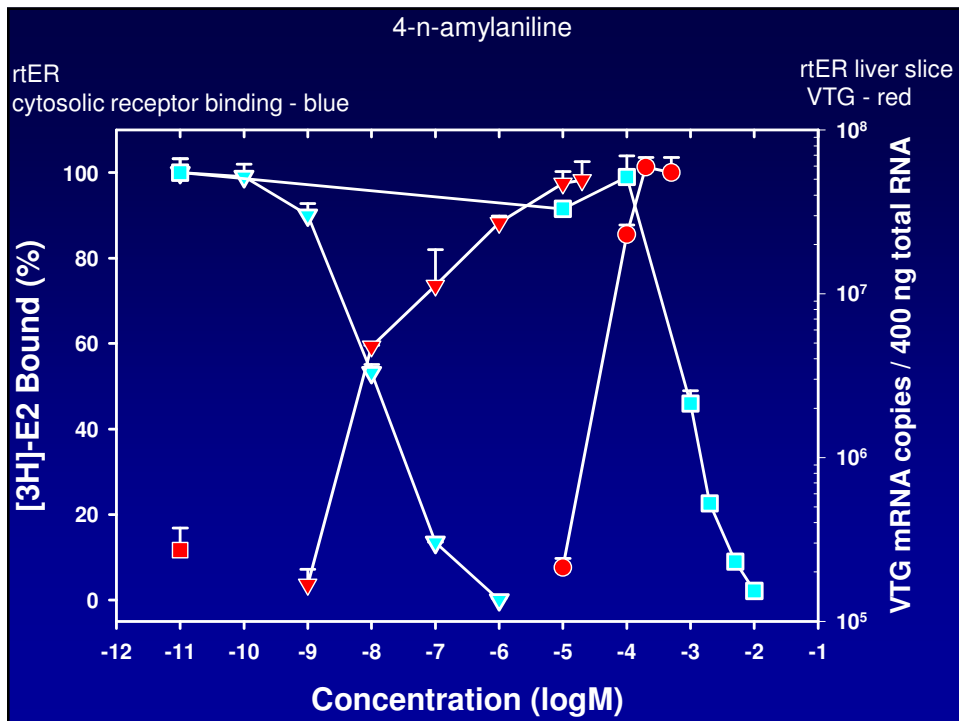
Comparing Biological Response



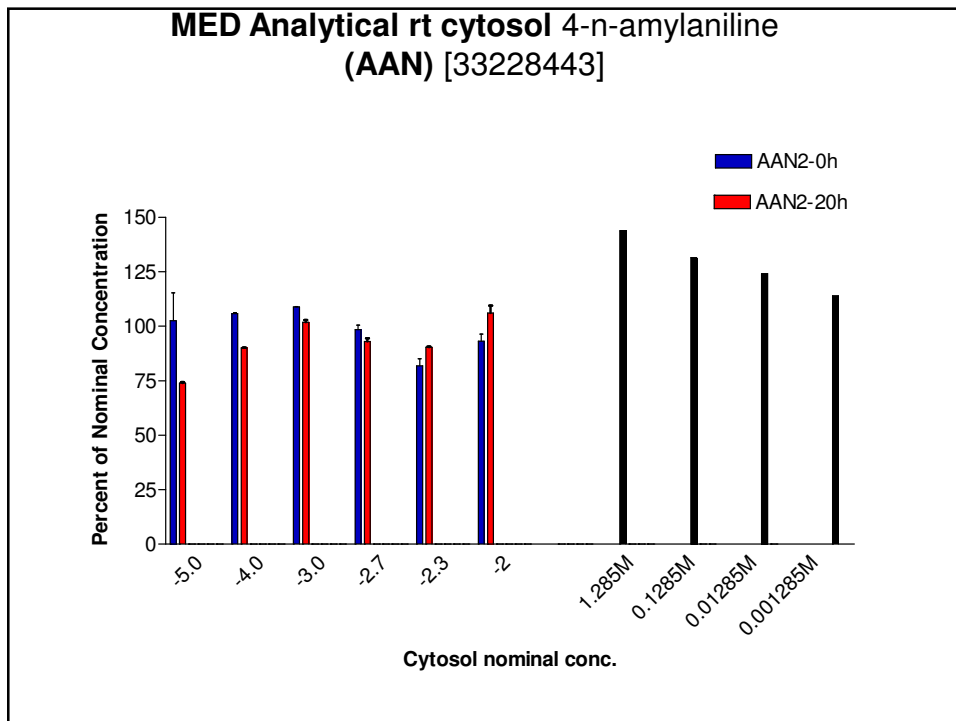
Comparison are made across the assay systems using one chemical  
RBA = 0.001

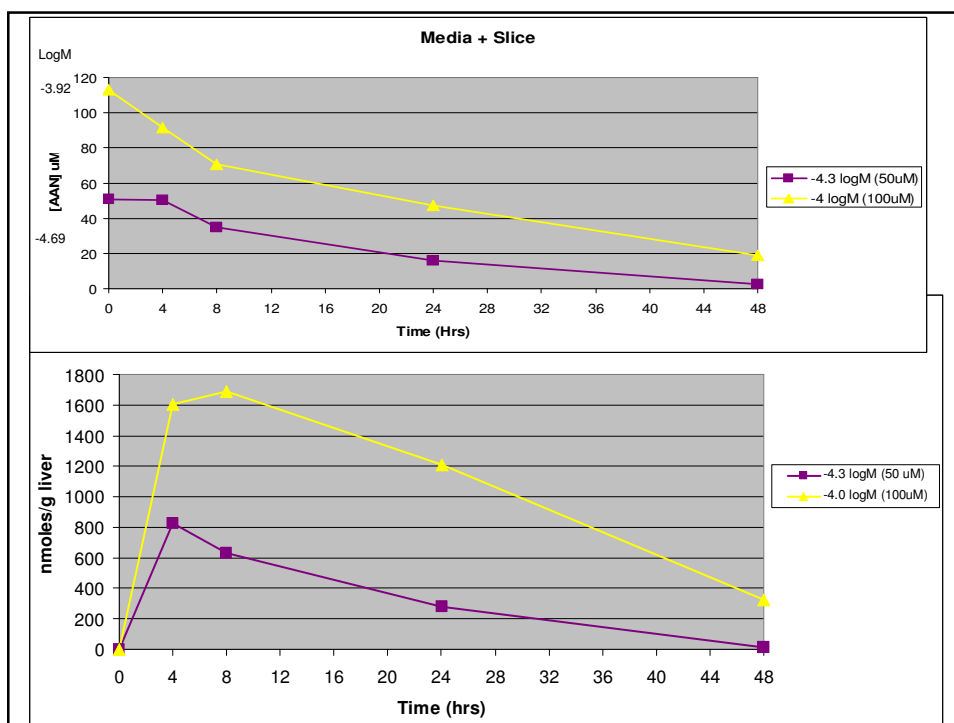


4-n-amylniline

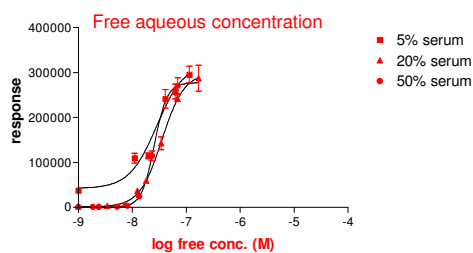
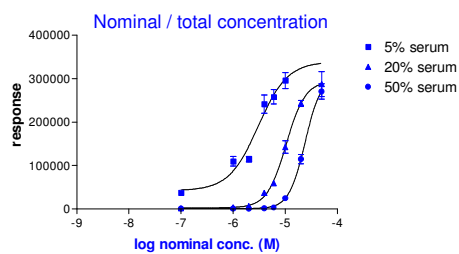
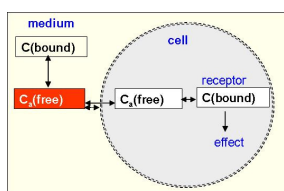


## Comparing Dosimetry





### Total vs. free concentration of octylphenol in an estrogenicity reporter gene assay using SPME to measure "free" chemical



Heringa M.B. et al. Environ. Sci. Technol. 2004, 38, 6263-6270  
 Picture from J. Hermens, Utrecht University

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