

Update on Research Using *in vitro* and Computer-based Tools for Screening Potential Estrogenic Activity

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Quantitative Structure-Activity Relationships

Assumptions

- A chemical's structure imparts properties
- A group of chemicals that produce the same biological activity (toxicity; adverse effect) have something similar about their chemistry (structure)
- Goal is to quantify 'structural similarity' imparting biological activity; identify which other chemicals may be 'similar' with the assumption that an untested chemical may produce the same activity

Chemical similarity is defined in the context of biological similarity

- Robustness Depends on:
 - Well-defined biological system; Well-characterized chemistry
 - Well-defined application –
 - Risk context - What's the question being asked - problem definition

QSAR Assumption

Chemical
Structure/
Property



Toxicological
Potential

Δ Chemical
Structure/
Property



Δ Dose
Metric
(kinetics/
metabolism)



Δ Endpoint
Potency

Toxic potency is correlated to chemical concentration at the site of action

-C. Hansch

Well-defined system (chemistry and biology)

Well-Defined Biological System

(What do you know and what are you assuming)

- Is the chemical administered what you thought it was
 - Impurities
- Metabolism
 - Is the system used for collection of empirical data capable of xenobiotic metabolism?
 - Is what you're measuring due to parent chemical or to a metabolite?
- Kinetics
 - What do you understand about the chemical kinetics within the system?
 - Is the chemical in solution
 - Bound and unavailable
 - Loss to hydrolysis

Has chemical form and/or concentration been measured in the biological system upon which the QSAR is based

QSAR Approach

- QSAR is approach to help think about, hypothesize, and investigate, in a systematic manner how a chemical is most likely to interact with a biological system and what adverse effect might be the consequence of that interaction
- QSAR depends upon a well-defined biological system
- QSAR for large diverse chemical inventories is an iterative process
- How QSAR used depends upon the regulatory context
 - Defining the regulatory domain is non-trivial; identify the exact chemicals and verify structures
 - Defining the regulatory question is essential; regulatory acceptance criteria are dependent upon the use

Risk Context

Development and use of a QSAR in regulatory risk assessment requires clear problem definition

- The purpose of the QSAR application must be well-defined (e.g., priority setting for testing, and chemical-specific risk assessment are two very different purposes – different acceptance criteria)
- The chemicals of regulatory concern must be defined to establish an appropriate training set for QSAR development and/or to assess appropriateness of QSAR application
 - Regulatory Domain
 - Applicability Domain of QSAR (dependent on Training Set)

A QSAR can only be as good as the underlying toxicological understanding and data it is based upon

- Toxicological activity is assessed based on a well-defined endpoint in a well-defined assay
 - e.g., chemical dosimetry –
 - if you assume parent chemical is responsible for biological activity but in fact a metabolite produced toxicity, then you're working from wrong structure
 - If you assume chemical was 100% available in your system but in fact 80% was loss due to volatility, or binding to glassware, unavailable in vehicle administered, etc then your concentration may have to be corrected

Today's Research Update: Developing the Tools to move EPA toward the New Paradigm

- Use screening and priority setting to focus on the most plausible toxicological potential for chemical or group of chemicals, not all possible adverse outcomes.
- Challenge of implementing FQPA
 - Endocrine Disruptors - How to prioritize and efficiently test a large number of chemicals while still carrying out existing chemical (new and old) evaluation programs
- Hypothesis-driven approach

QSARs for Prioritization

Food Quality Protection Act –

Need to prioritize *in vivo* testing options for classes of compounds where ‘endocrine data’ is lacking:

- Inert ingredients used in formulations of pesticides used on crops
- Antimicrobial active ingredient pesticides

Prioritize -

- Based on effect endpoint(s) in combination with existing exposure estimates
- Use QSARs to estimate potential for ‘estrogenic activity’ for untested inerts and antimicrobial pesticides

Research Focus:

- **Adverse outcome pathway:**
 - Reproductive impairment through the ER-mediated pathway
- **Chemicals:**
 - Inert ingredients
 - Antimicrobials
- **Hypothesis-driven approach:**
 - Chemicals that have similar activity will have similar structure; quantifying the structural similarity will allow extrapolation across chemicals

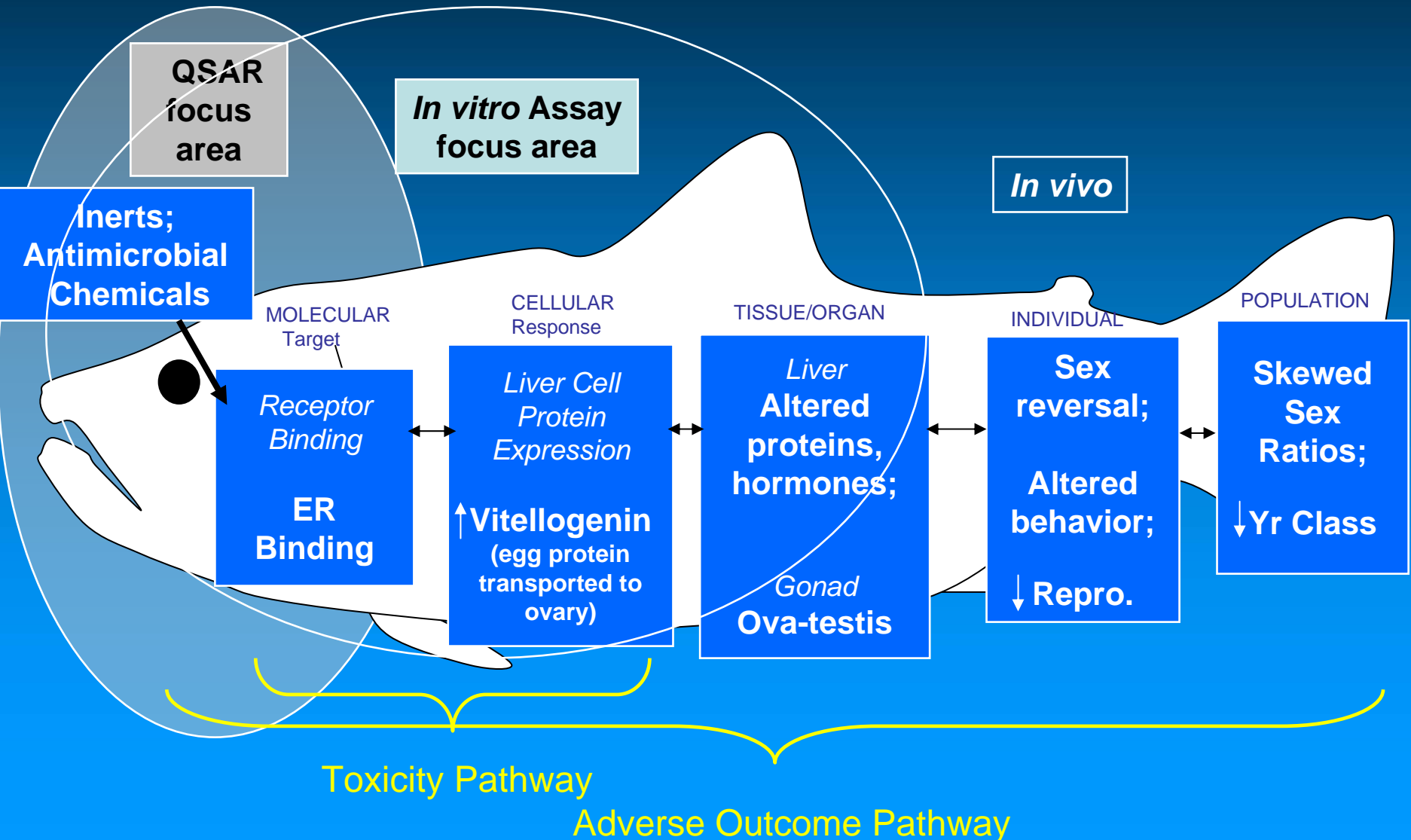
Research Approach:

- Test a 'representative' chemicals *in vitro* to extrapolated potential for activity to untested
- Chemical Class Approach based on mechanism:
 - What types of chemicals can interact with the ER and which ones can't
- *in vitro* assays:
 - ER binding displacement
 - ER-mediated gene activation

Adverse Outcome Pathway

ER-mediated Reproductive Impairment

Measurements made across levels of biological organization



Defining the Problem:

Prioritizing estrogenic potential of Food Use Inert Ingredients

Inert chemicals in Pesticides used on Food Crops
The 2004 List included:

893 entries = **393** discrete chemicals + **500** non-discrete substances
(44% discrete : 56% non-discrete)

393 discrete chemicals include:
366 organics (93%)
24 inorganics (6%)
3 organometallics (1%)

500 non-discrete substances include:
147 polymers of mixed chain length
170 mixtures
183 undefined substances

Defining the Problem: *Prioritizing Estrogenic Potential of Antimicrobial Pesticides*

Antimicrobials and Sanitizers

List included:

299 = **211** discrete chemicals + **88** non-discrete substances
(71% discrete : 29% non-discrete)

211 discrete chemicals include:

153 organics (72%)

52 inorganics (25%)

6 organometallics-acyclic (3%)

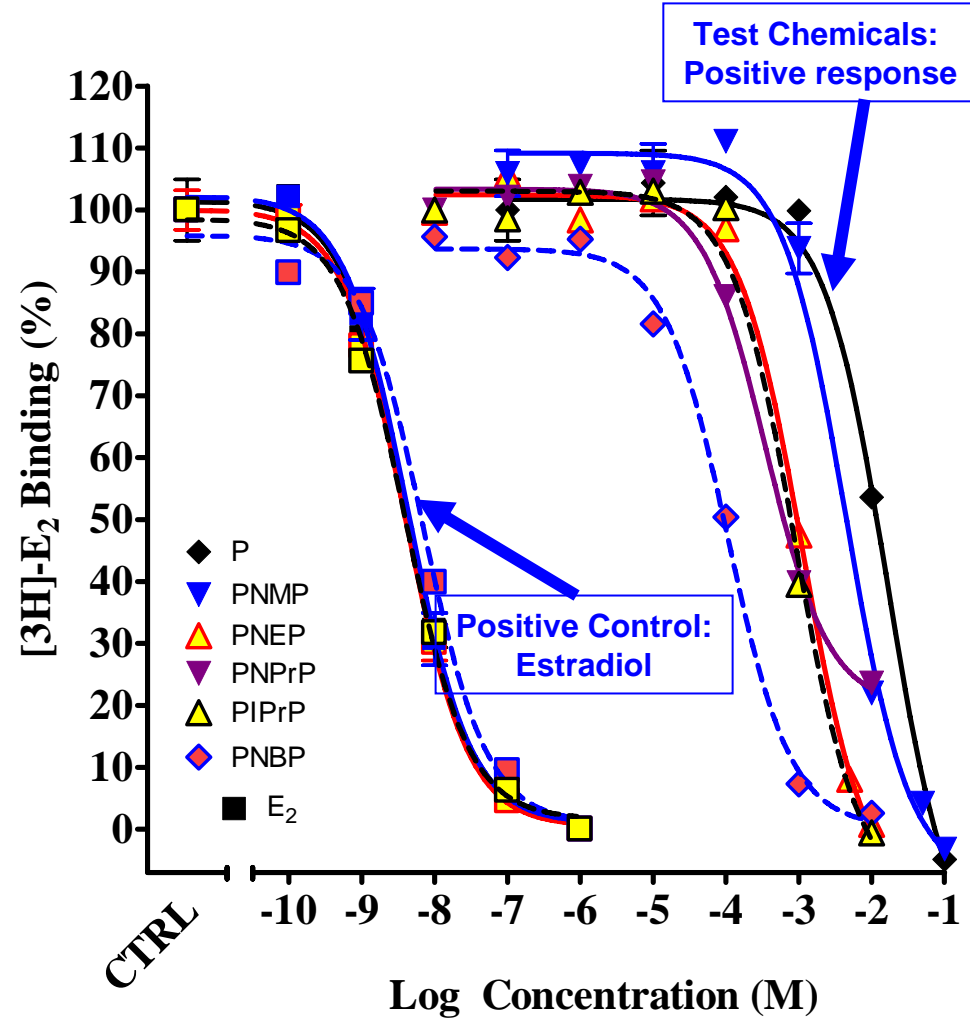
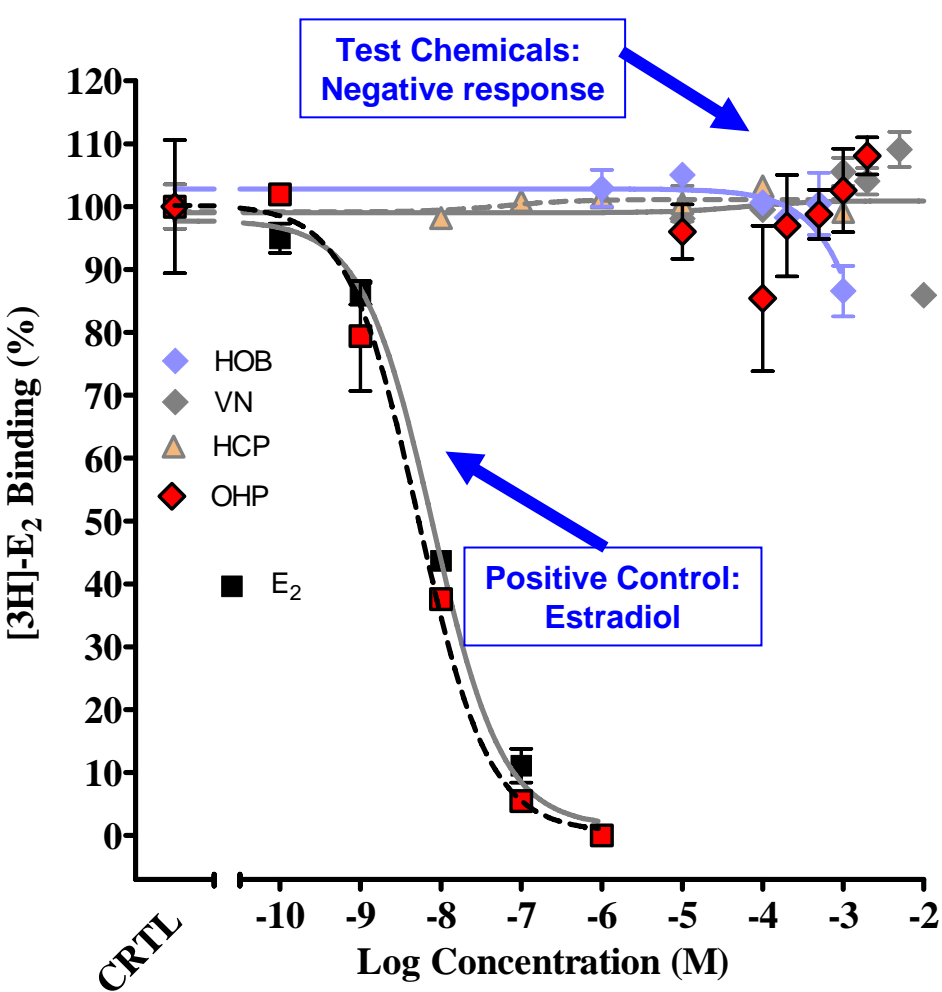
88 non-discrete substances include:

25 polymers of mixed chain length

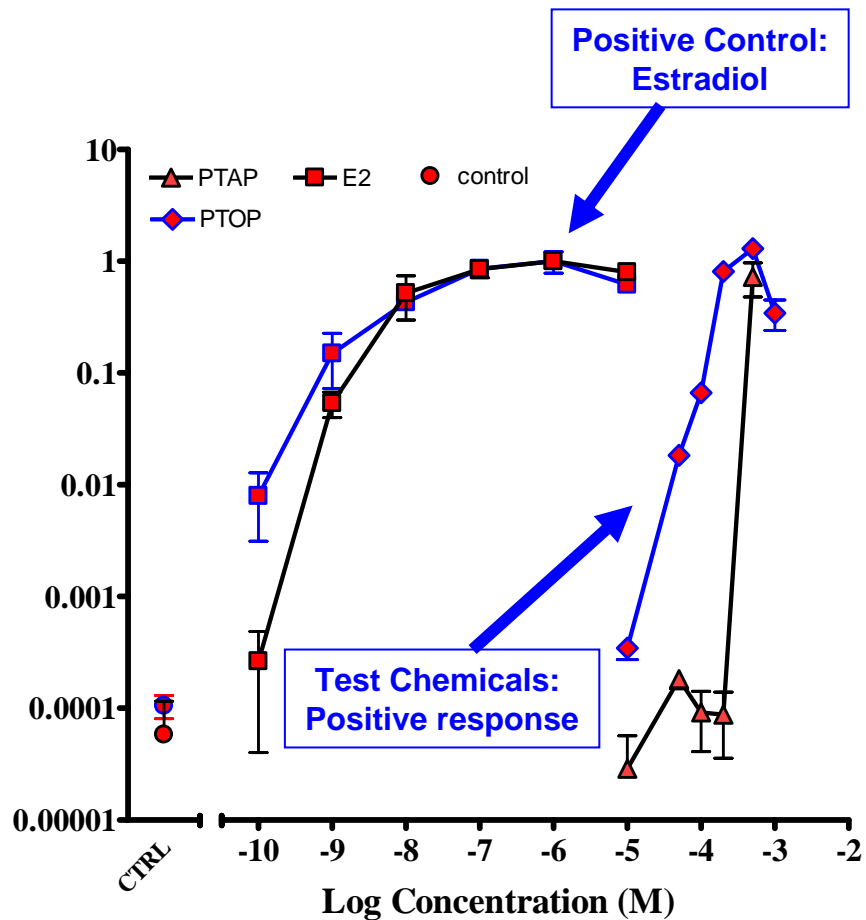
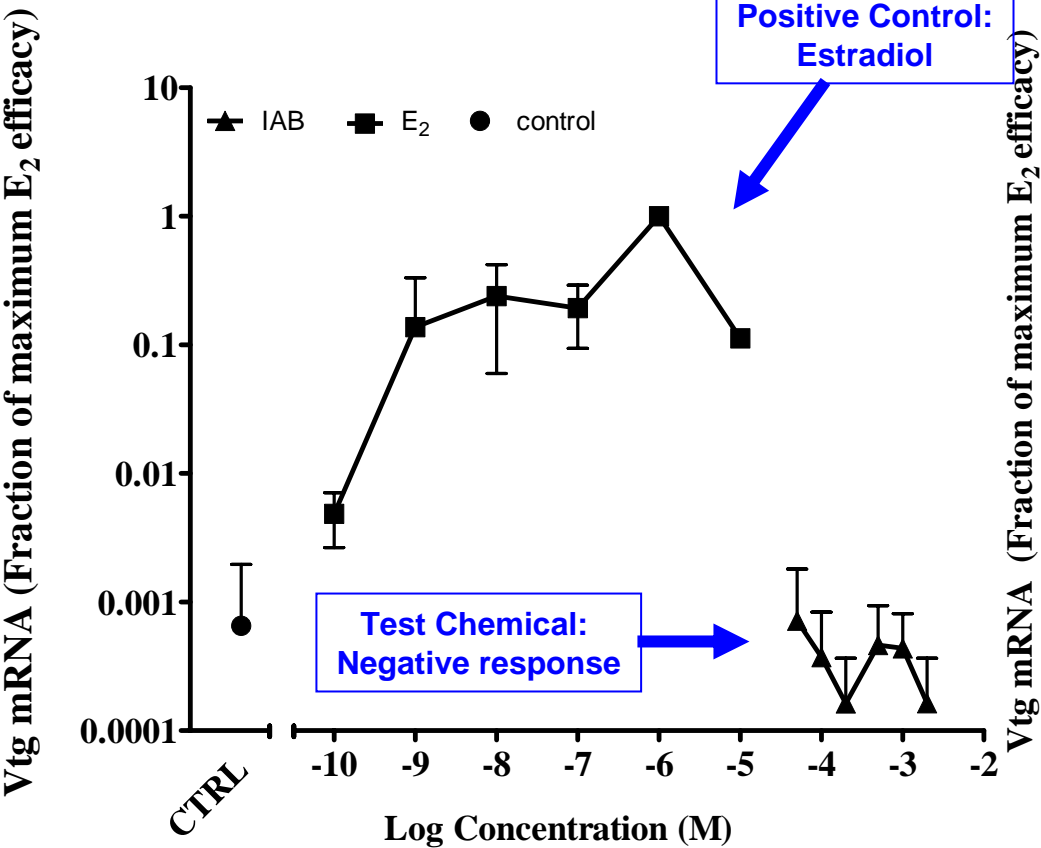
35 mixtures

28 undefined substances

Data Example - primary *In vitro* assay used : Estrogen Receptor Binding Displacement Assay



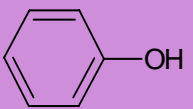
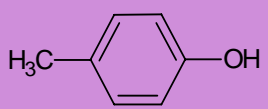
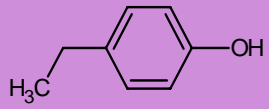
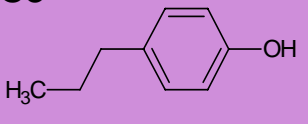
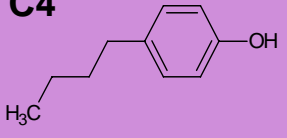
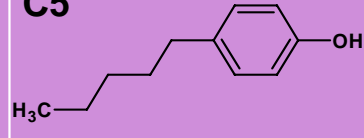
Data example – Confirmatory *in vitro* Assay: Gene Activation

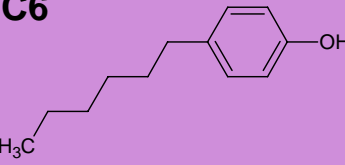
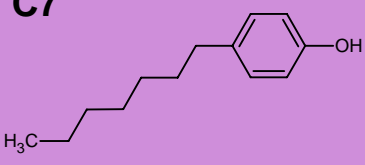
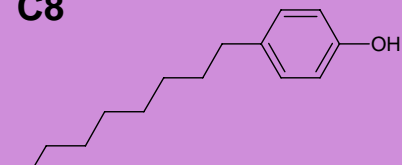
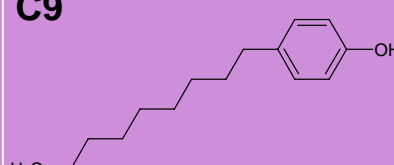


Research Approach:

- Test a few 'representative' chemicals *in vitro* to extrapolate to others
- Chemical Class Approach based on mechanism:
 - What types of chemicals can interact with the ER and which ones can't
 - chemicals selected to investigate mechanisms of binding the ER
 - chemicals selected to cover classes found on list

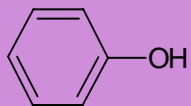
Homologous Series Alkylphenols

| | | | | | |
|--|---|---|--|---|---|
| C0  Log Kow = 1.50 msrd | C1  1.97 msrd | C2  2.47 msrd | C3  3.20 msrd | C4  3.65 msrd | C5  4.06 msrd |
|--|---|---|--|---|---|

| | | | |
|---|---|--|---|
| C6  4.62 calc | C7  4.15 msrd | C8  5.68 calc | C9  5.76 msrd |
|---|---|--|---|

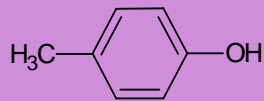
Alkylphenols

C0



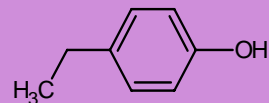
Log Kow = 1.50 msrd

C1



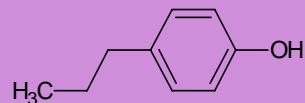
1.97 msrd

C2



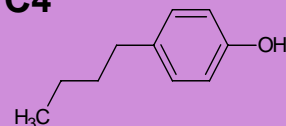
2.47 msrd

C3



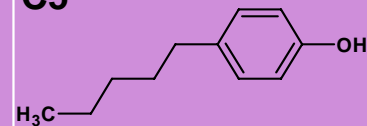
3.20 msrd

C4



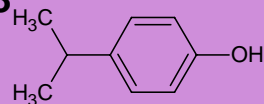
3.65 msrd

C5



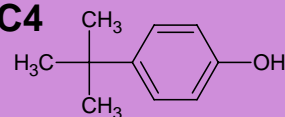
4.06 msrd

C3



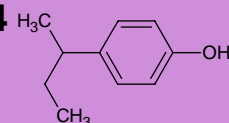
2.90 msrd

C4



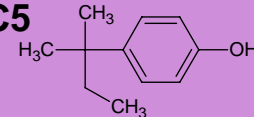
3.31 msrd

C4



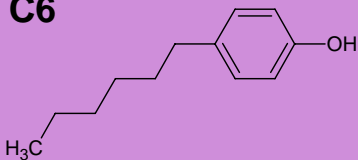
3.32 msrd

C5



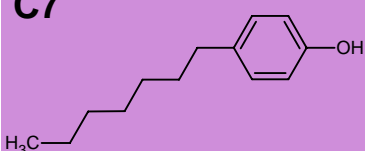
3.83 msrd

C6



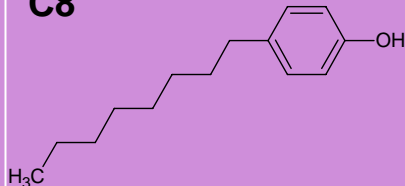
4.62 calc

C7



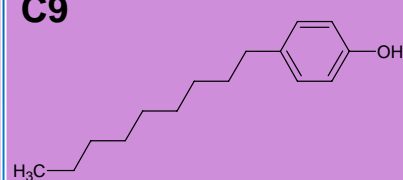
4.15 msrd

C8



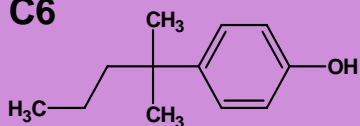
5.68 calc

C9



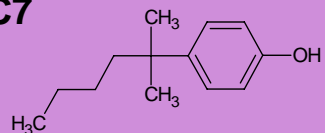
5.76 msrd

C6



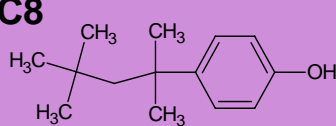
4.36 clog

C7



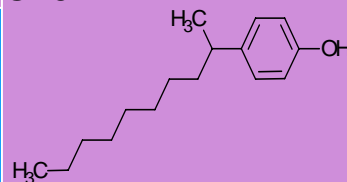
4.89 clog

C8



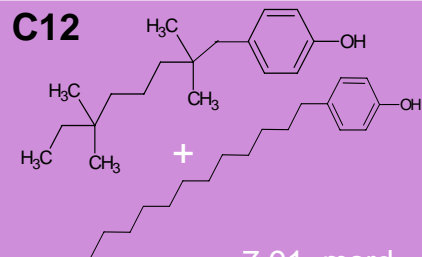
5.16 clog

C10

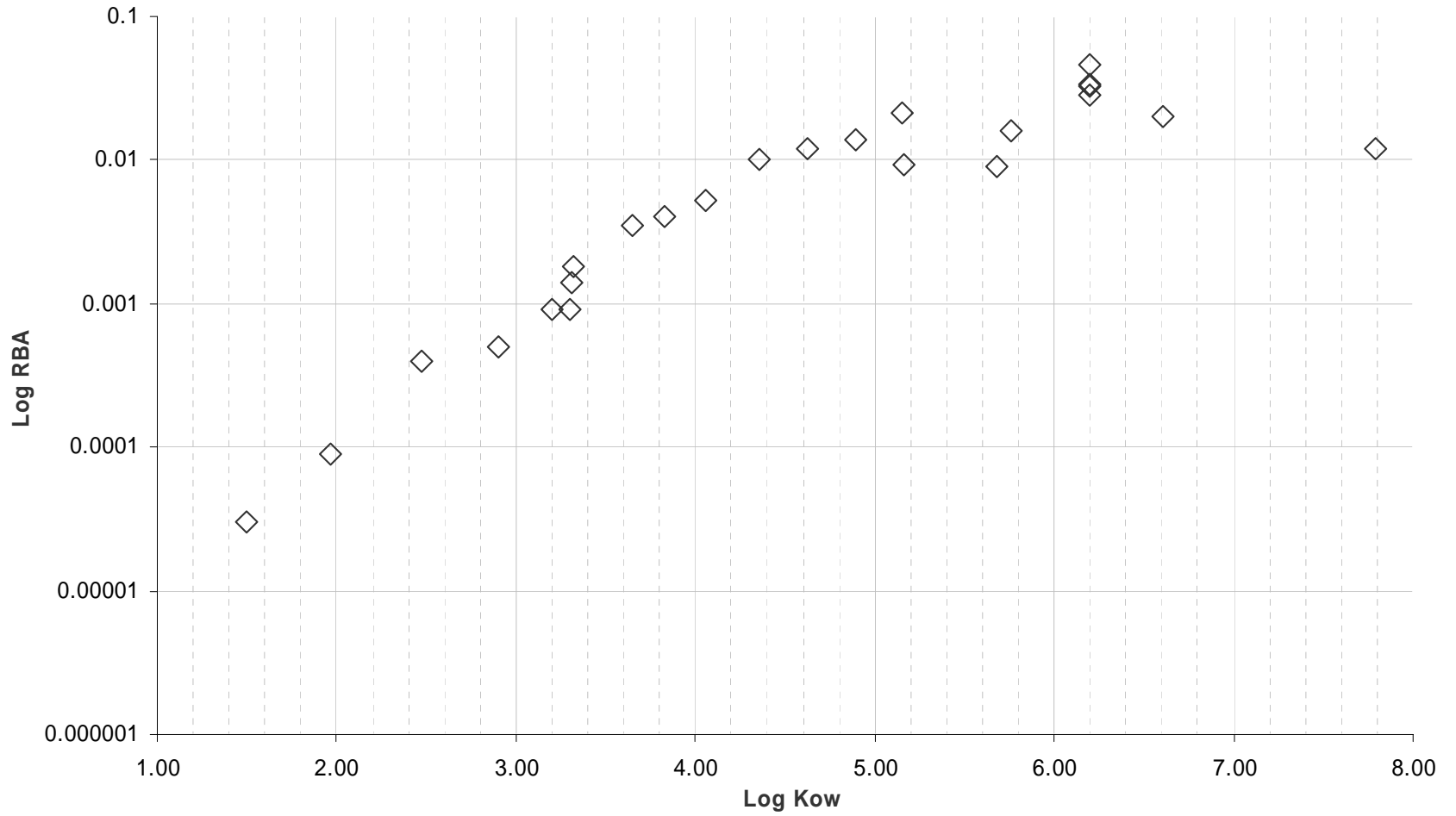


6.61 clog

C12

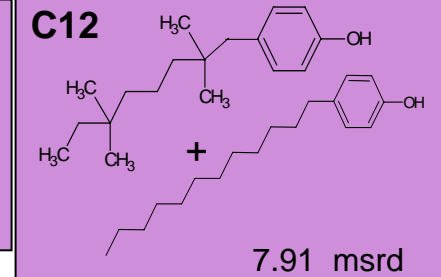
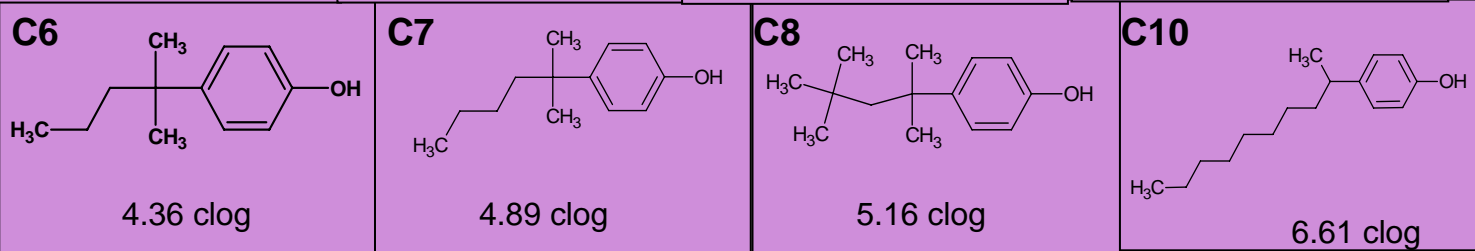
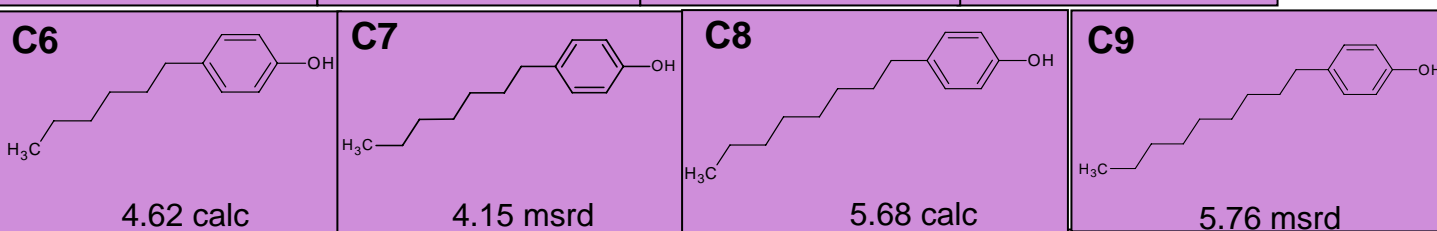
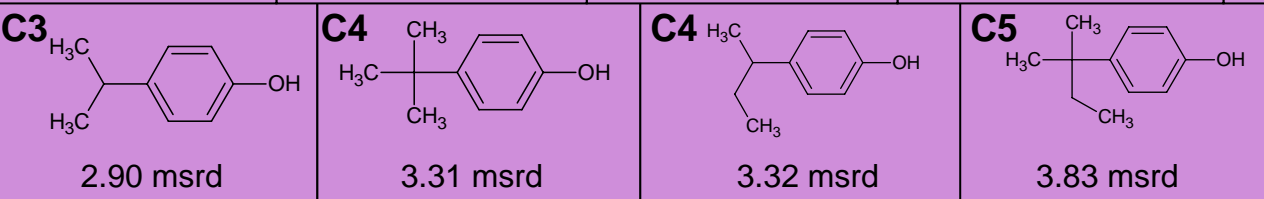
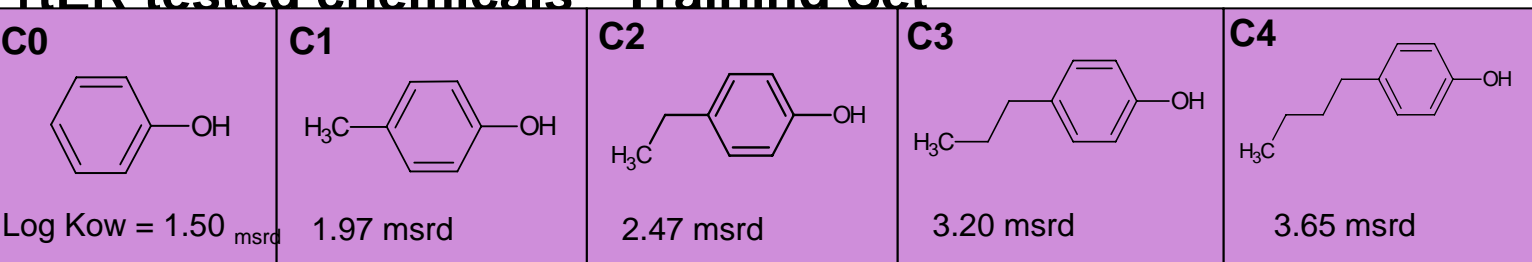


7.91 msrd

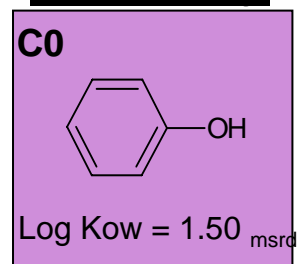


Alkylphenols – (p-branched chain)

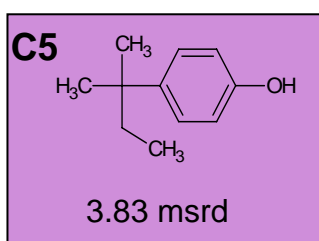
rtER tested chemicals - Training Set



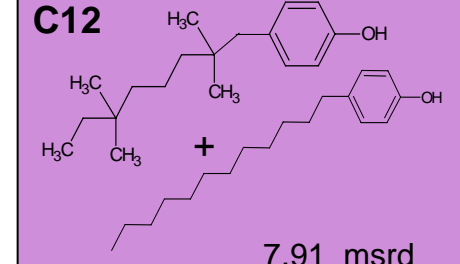
Inventory



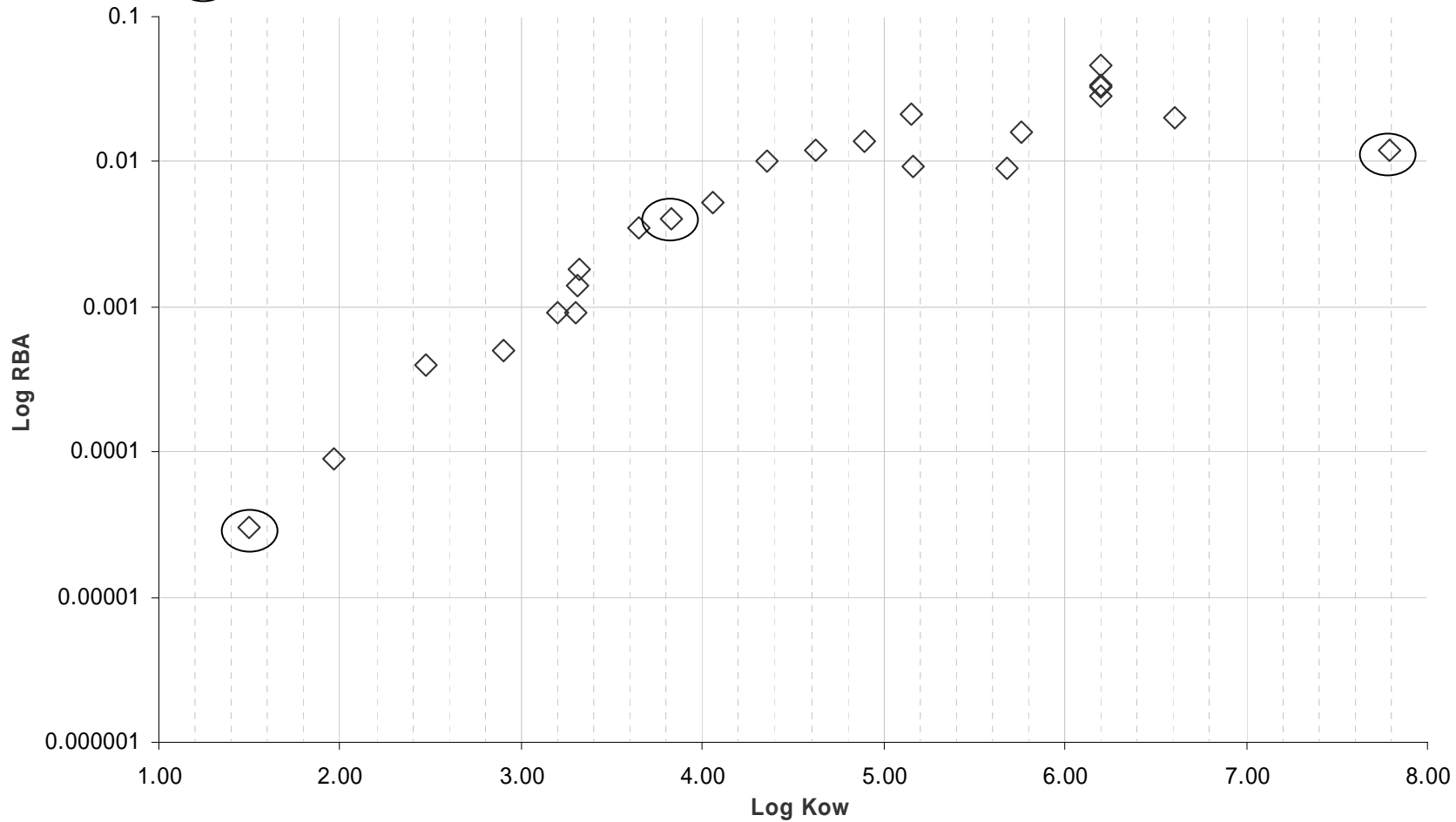
Inventory



Inventory



○ = Inventory chemical in the Alkylphenols group



Alkylanilines – (p-n chain)

rtER tested chemicals - Training Set

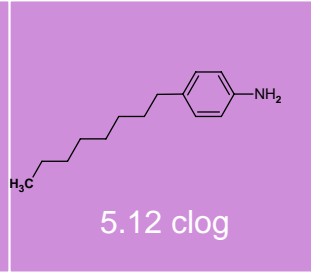
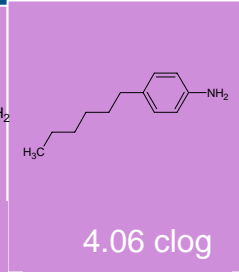
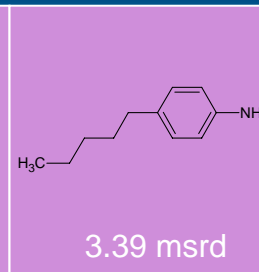
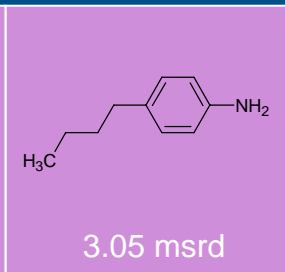
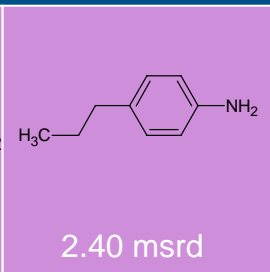
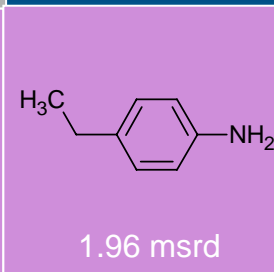
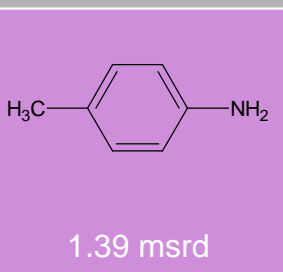
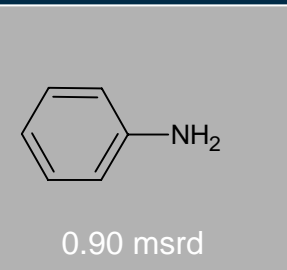
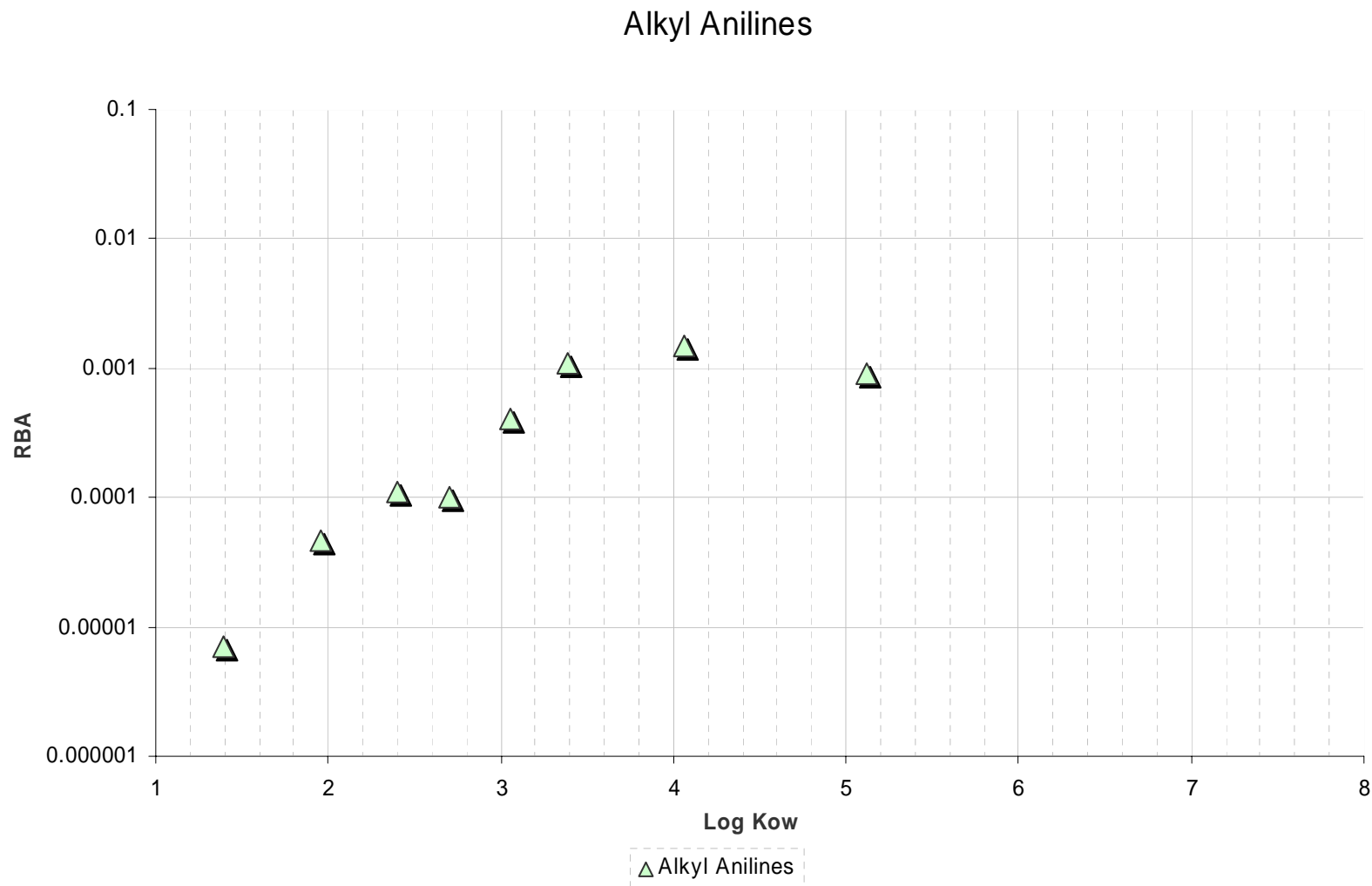
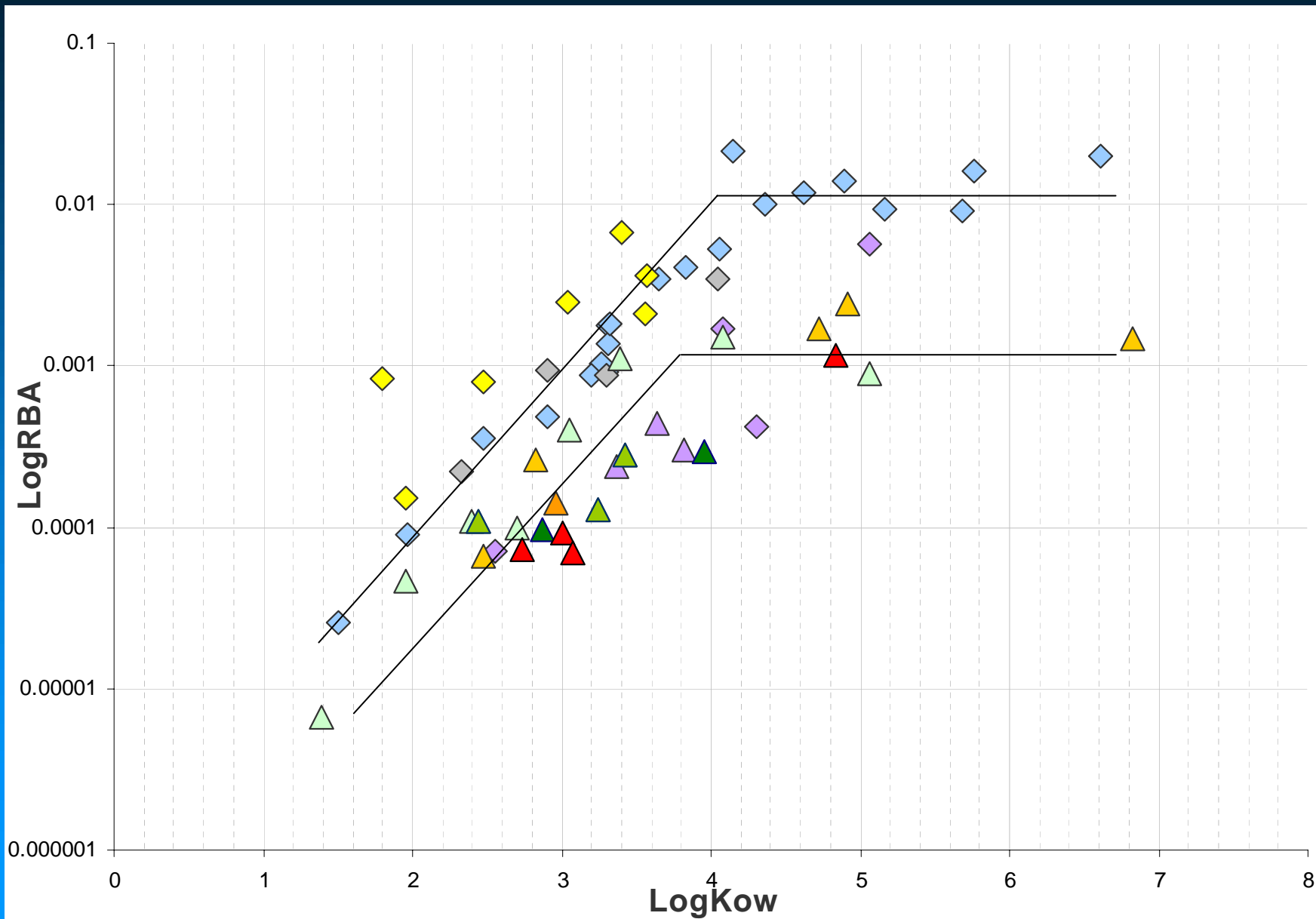


Figure 5. Relationship between Log Kow and RBA for alkylanilines.

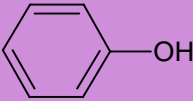

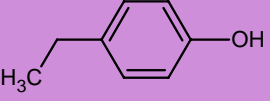
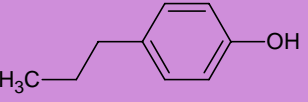
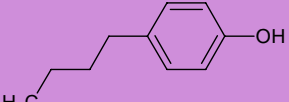
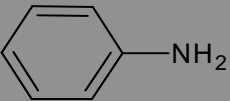

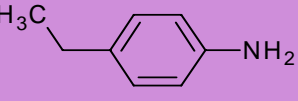
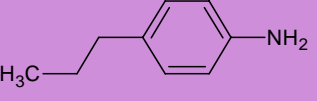
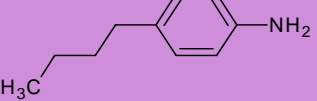
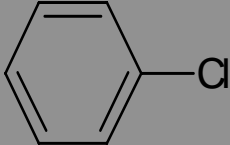
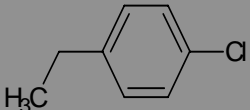
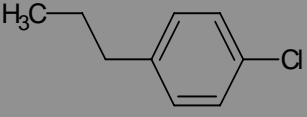
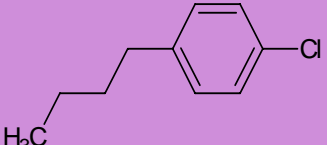
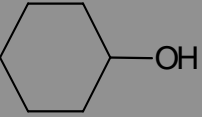
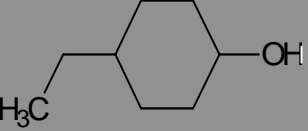
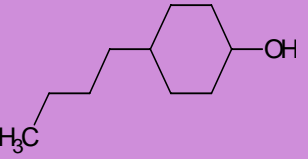


Rainbow Trout ER binding Affinity vs. Log Kow

RBA = relative binding affinity compared to Estradiol at 100%

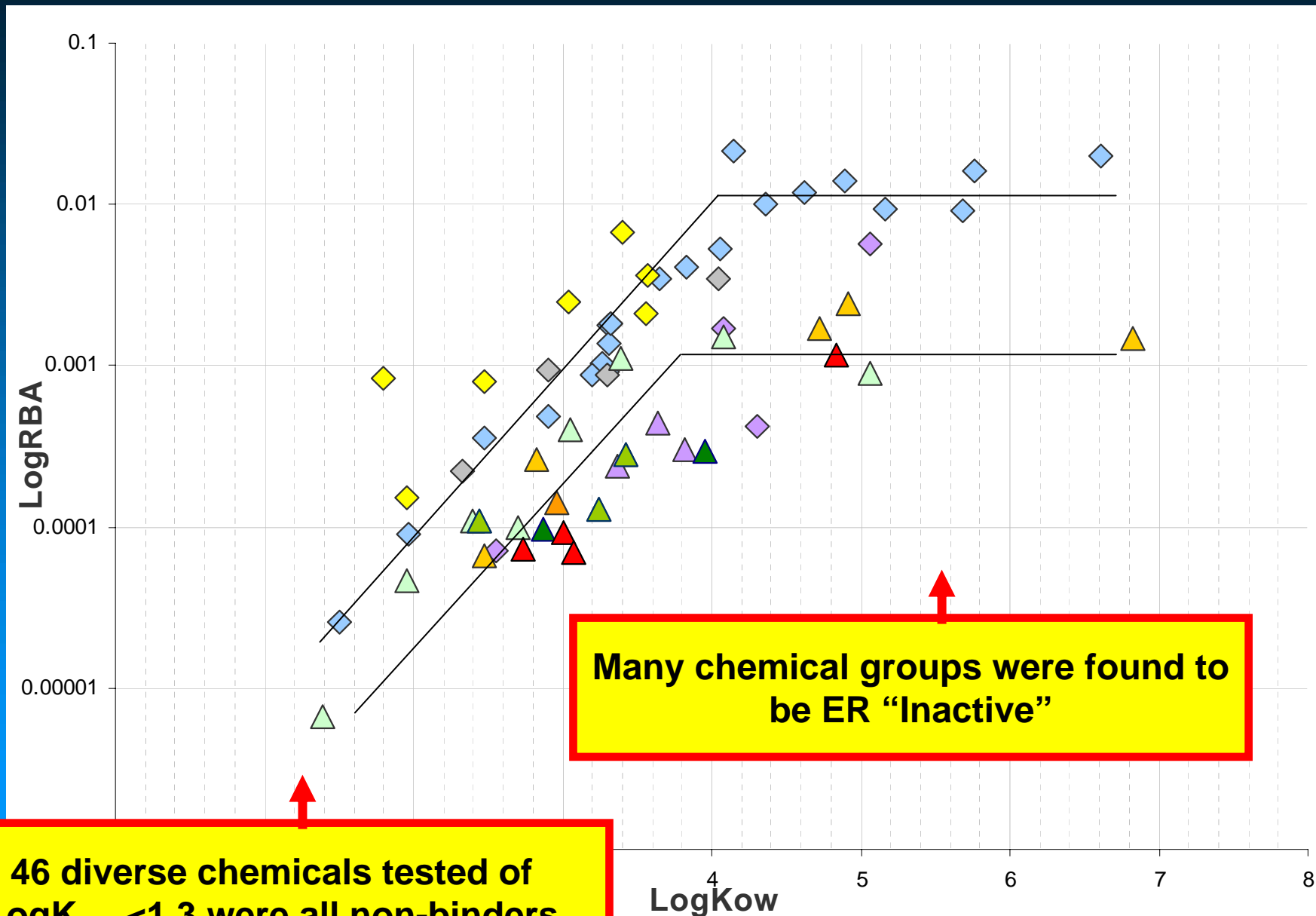


LogK_{ow} Cutoffs vary with Chemical Subgroups

| | C0 | C1 | C2 | C3 | C4 |
|-----------------------------------|--|---|--|---|---|
| <i>p,n</i> -Alkyl Phenols |  LogKow=1.50 m |  1.97 m |  2.47 m |  3.20 m |  3.65 m |
| <i>p,n</i> -Alkyl Anilines |  0.90 m |  1.39 m |  1.96 m |  2.40 m |  3.05 m |
| <i>p,n</i> -Alkyl Chloro benzenes |  2.84 m | |  3.88 c |  4.41 c |  4.94 c |
| <i>p,n</i> -Alkyl Cyclo hexanols |  1.23 m | |  2.32 c | |  3.37 c |

Rainbow Trout ER binding Affinity vs. Log Kow

RBA = relative binding affinity compared to Estradiol at 100%

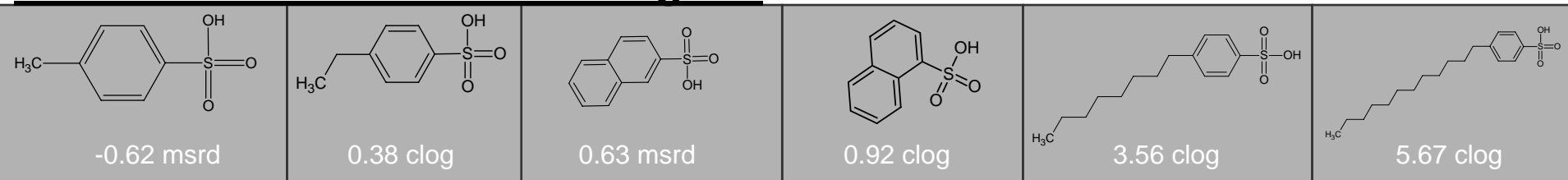


Many chemical groups were found to be ER "Inactive"

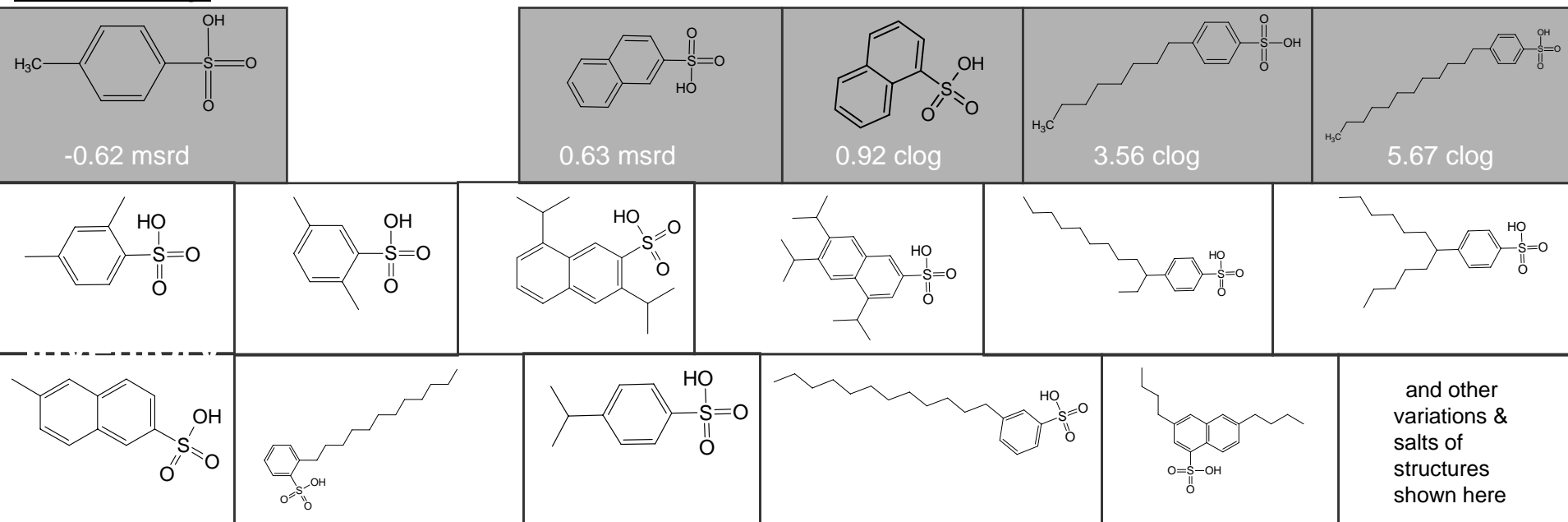
46 diverse chemicals tested of LogK_{ow} < 1.3 were all non-binders

Alkylaromatic sulfonic acids

rtER tested chemicals - Training Set



Inventory



Results:

Chemical has Low Potential for Activity if:

- Belongs to a group where testing showed no evidence of ER interaction ($RBA < 0.00001$);
- LogKow < 1.3 , or meets other group-specific LogKow cutoffs

General characteristics of these chemicals:

- Acyclic (e.g., no benzene rings)
- Cyclic, but does not contain a likely H-bonding group;

Results:

Chemical has Higher Potential for Activity if:

- Belongs to chemical group with evidence of ER interaction, ($RBA > 0.00001$), and:
- $\text{LogKow} > 1.3$, and $<$ any chemical group-specific high LogKow cutoff

General characteristics of these chemicals:

- Contains at least one cycle (e.g., benzene ring);
- Contains a possible H-bonding group;

Food Use Inerts, and Antimicrobials

Food Use Inerts

393

378 (96%)

15 (4%)

Total Chemicals

Lower Probability

Higher Probability

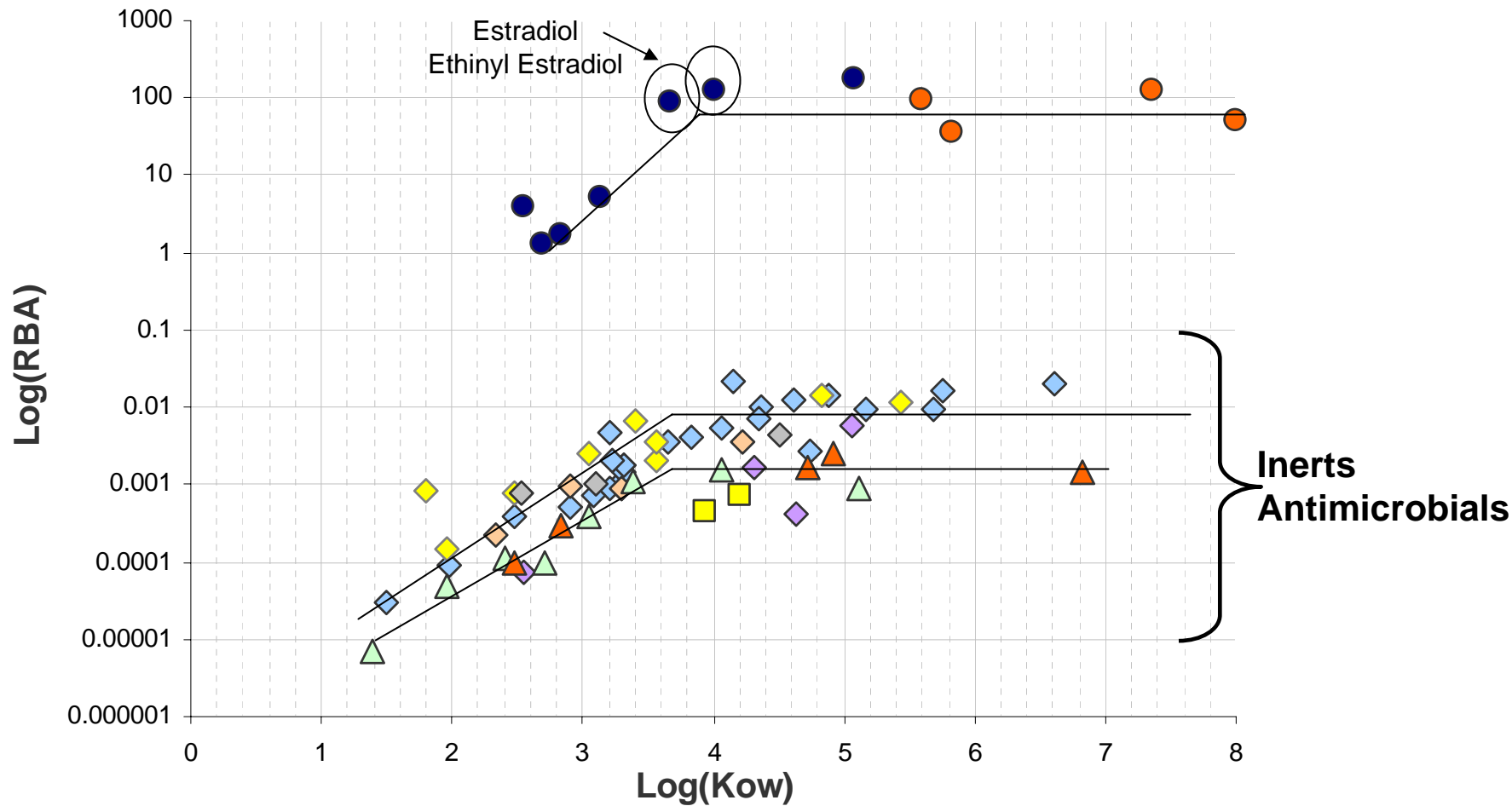
Antimicrobials

211

196 (93%)

15 (7%)

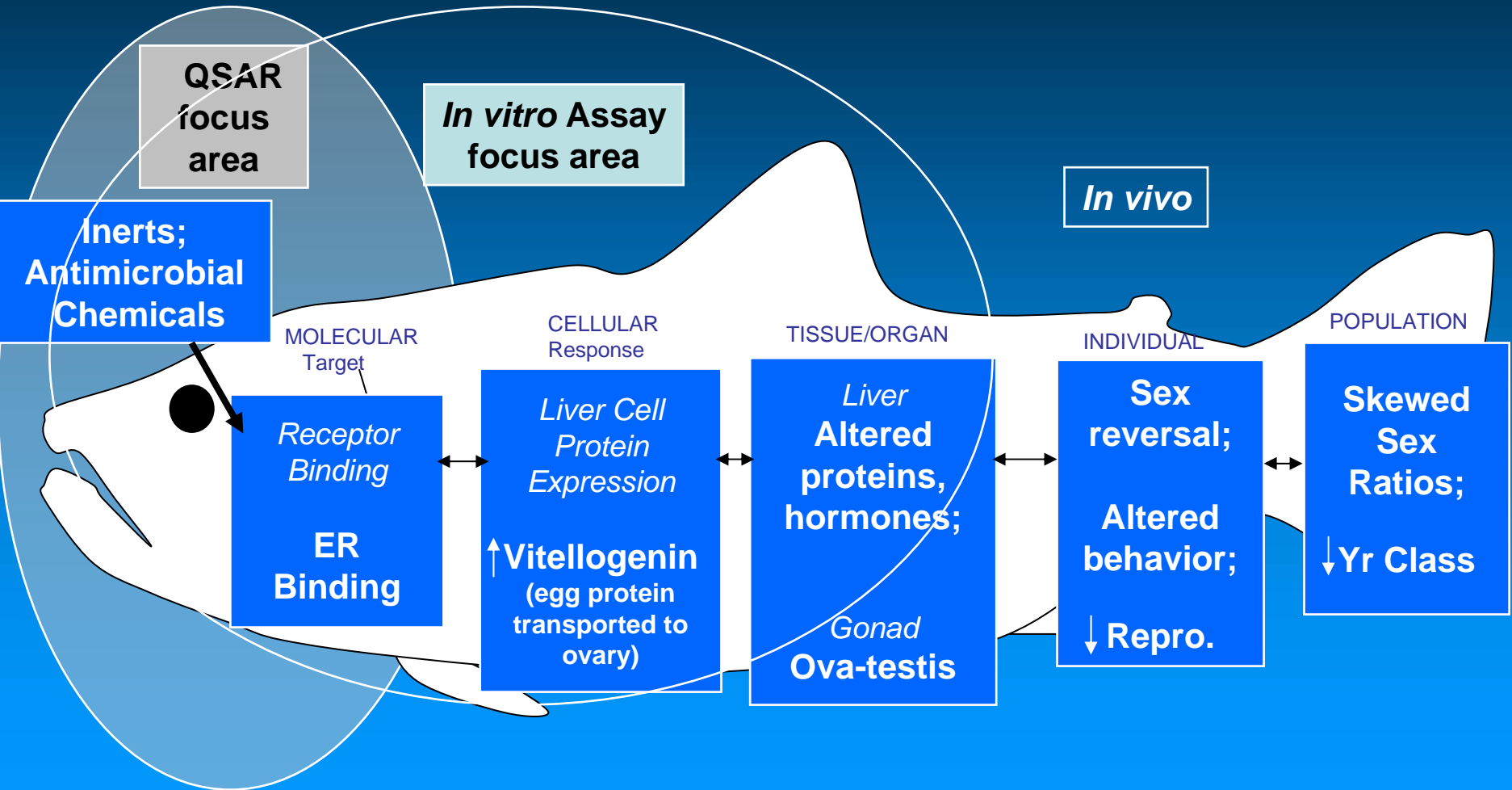
ER Binders

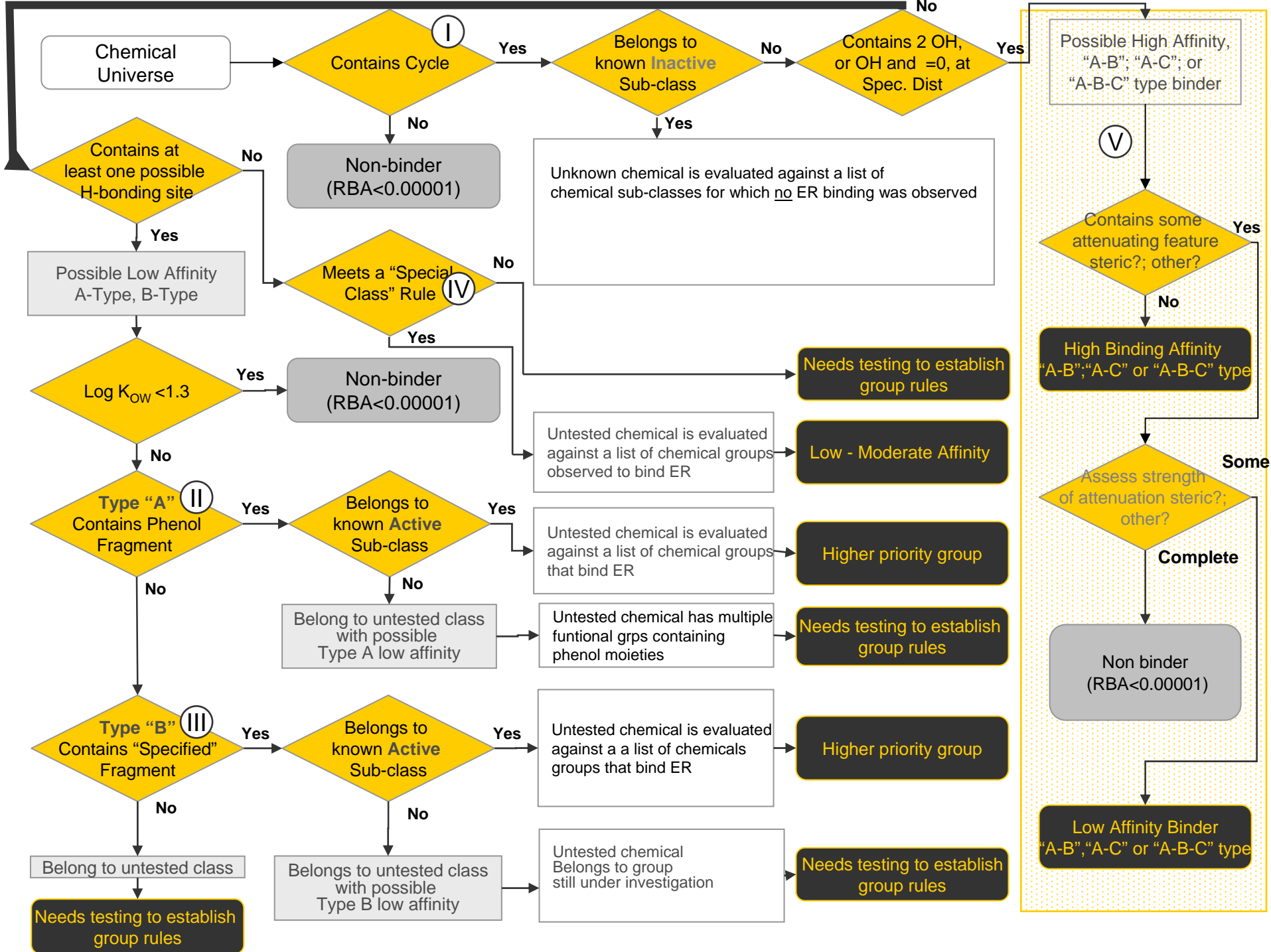


Adverse Outcome Pathway

ER-mediated Reproductive Impairment

Measurements made across levels of biological organization





Summary

- Hypothesis-driven approach
 - Adverse Outcome pathway (*in vitro* linked to *in vivo*)
 - Strategic chemical selection and testing to cover types of chemicals found on the list that needed prioritizing
 - Mechanistic hypothesis (LogKow; low affinity binding types)
- Derived a QSAR-based Decision Support System that can be applied to next chemical list, and expanded where needed (chemical classes not yet tested)
- Developed priority setting tool to focus on the 4 to 7% of chemicals with plausible toxicological potential for an important adverse outcome.

**Developing an Approach and Tools
to move EPA toward the new paradigm**