Predicting Estrogen Receptor Binding within Categories

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Estrogen Receptor Toxicity Pathway

- Need is derived from Food Quality Protection Act – "estrogens"
- Chemical binding to ER is known to cause adverse effects
  - Toxicity Pathway
- Diverse chemicals bind to ER
- Drug bioassays for ER binding are not relevant
  - drug-design assays optimized for high affinity binders
  - environmental hazard may come from low affinity binding

Development and use of a QSAR 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)
- The chemicals of regulatory concern must be defined to develop a database of tested chemicals (training set) for QSAR development

EPA Office of Pesticide Programs:
Food Use Inerts & Antimicrobials

- Get 'lists'
- Characterize structures
- Assess coverage of existing data (training sets, TrSets)
- Select chemicals for testing to strategically expand structure space to maximize information gained from every structure tested

Defining the Problem:
EPA Office of Pesticide Programs
Food Use Inerts List - Structures

List from OPP/RD 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:
EPA Office of Pesticide Programs
Antimicrobials/Sanitizers - Structures

Lists from OPP/AD 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

In-lab Testing = Training Set

The Issue:

Assay protocols optimized to increase confidence in quantifying activity of low potency compounds

Compare EPA Office of Pesticide Programs chemical structures to existing ER data

The assays:

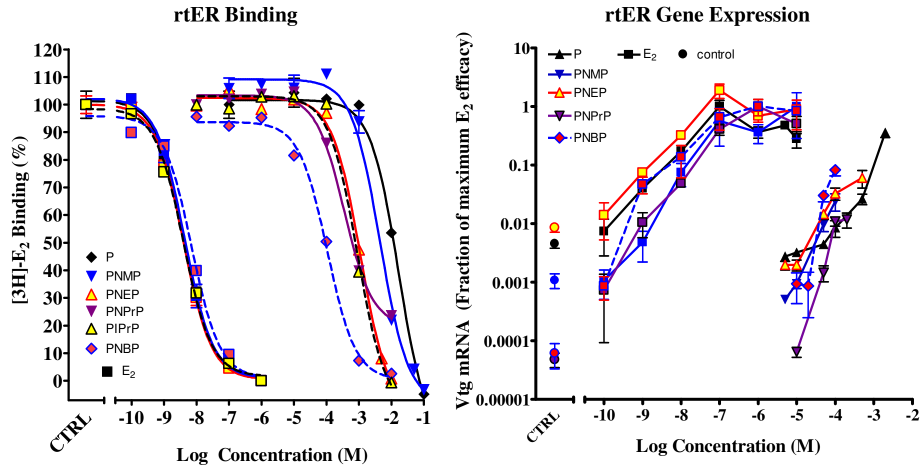
1) ER binding –

- standard competitive binding assay optimized for low affinity binders
- Rainbow trout liver cytosol (rtER)

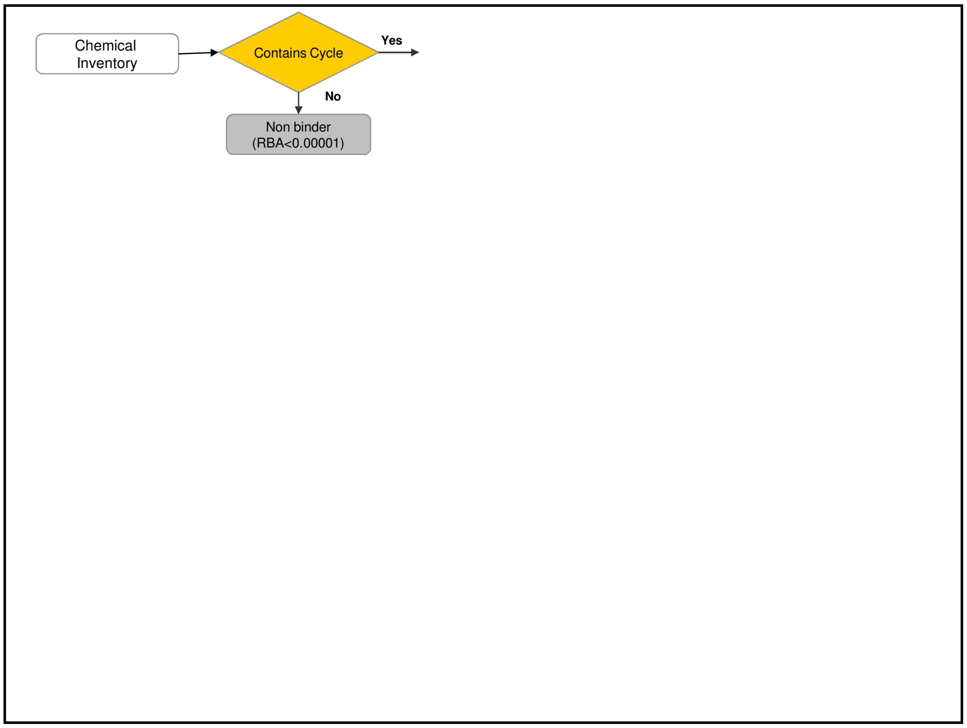
2) Gene Activation

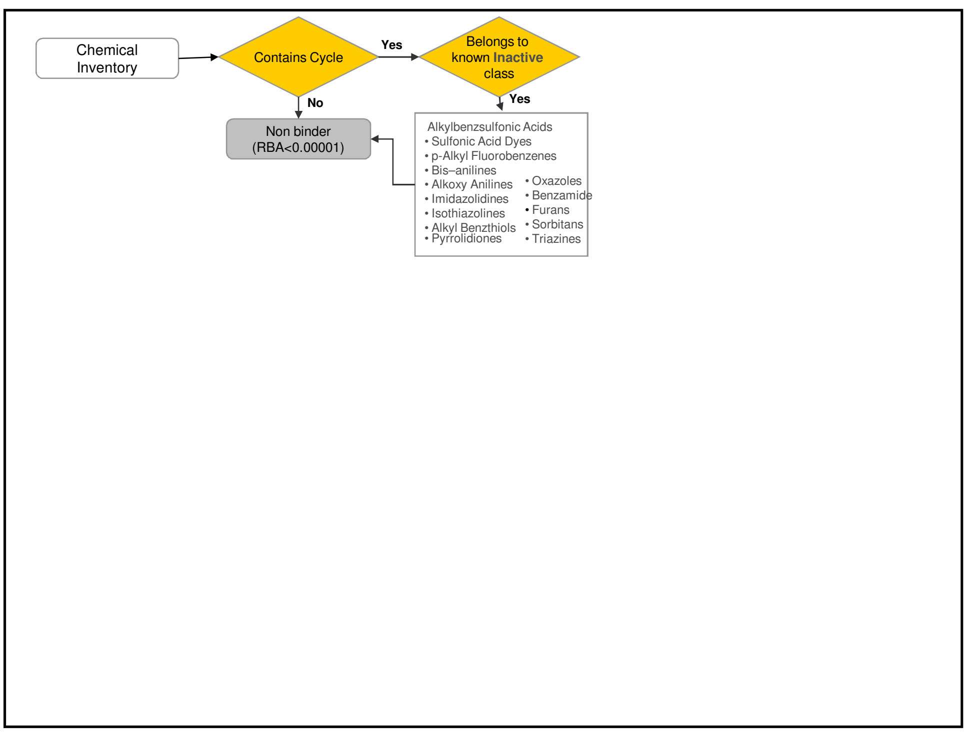
- trout liver slice - vitellogenin mRNA
- Endogenous metabolism

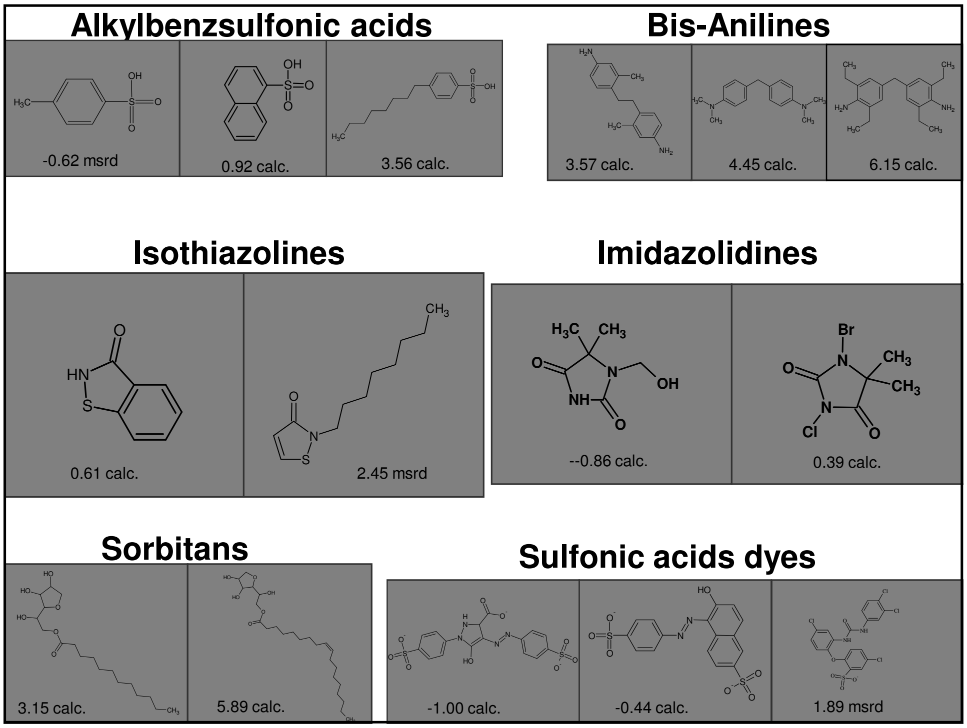
Data Example – Pos. Binding; Pos. Gene Activation

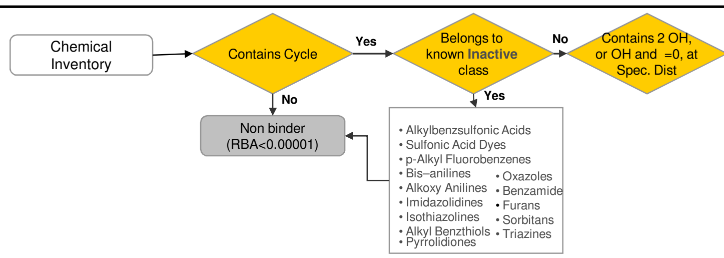


Building a Decision Tree with Categories

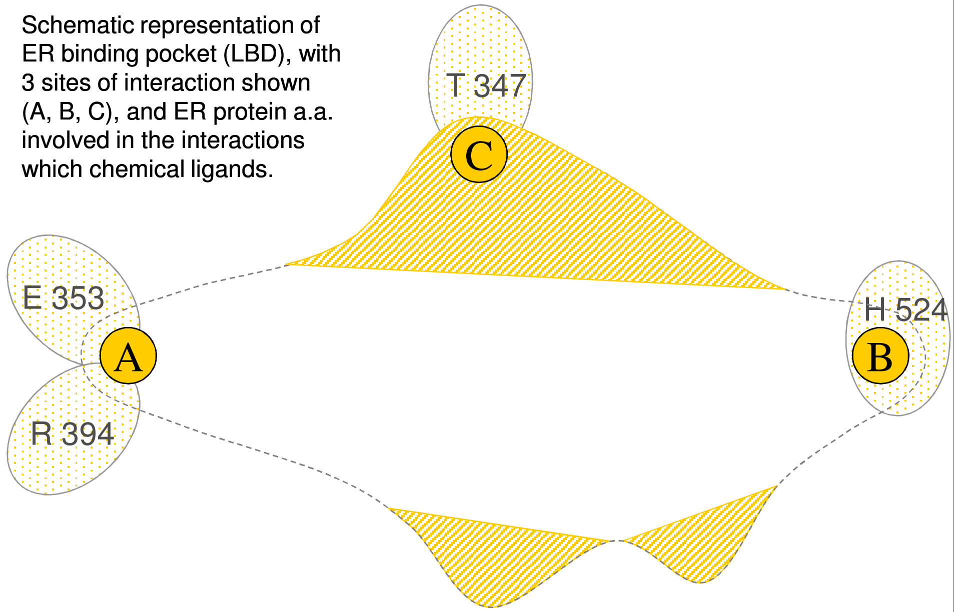

Chemical Inventory
Contains Cycle
Yes
No
Non binder
(RBA<0.00001)


Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
Yes
Non binder
(RBA<0.00001)
Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines


Alkylbenzsulfonic acids
-0.62 msrd
0.92 calc.
3.56 calc.
Bis-Anilines
3.57 calc.
4.45 calc.
6.15 calc.
Isothiazolines
0.61 calc.
2.45 msrd
Imidazolidines
--0.86 calc.
0.39 calc.
Sorbitans
3.15 calc.
5.89 calc.
Sulfonic acids dyes
-1.00 calc.
-0.44 calc.
1.89 msrd

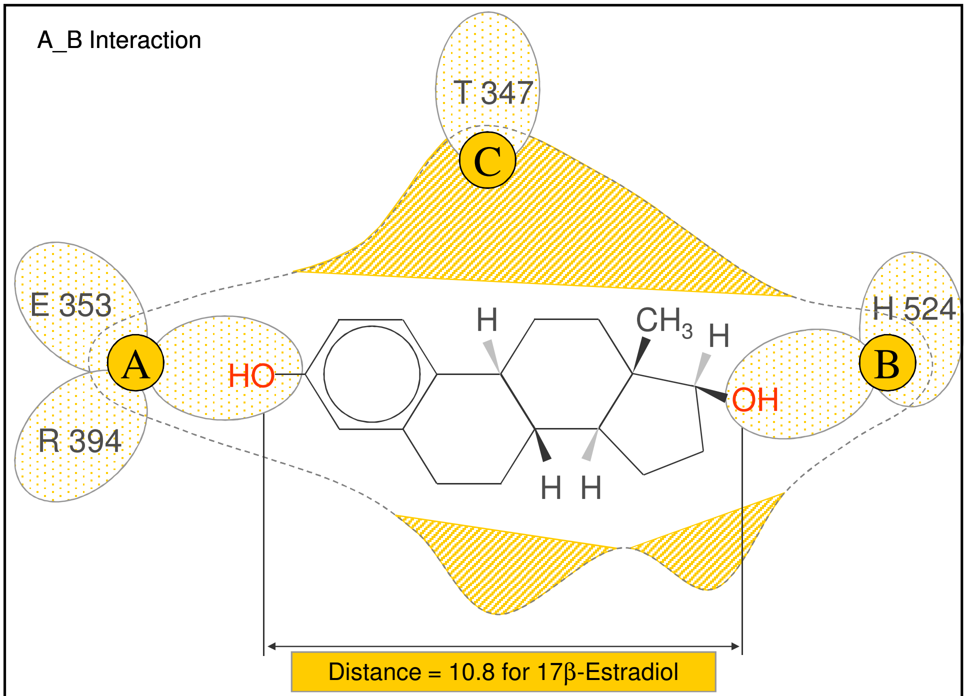

Chemical Inventory
Contains Cycle
Yes
No
Non binder (RBA<0.00001)
Belongs to known Inactive class
No
Yes
Contains 2 OH, or OH and =0, at Spec. Dist
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines

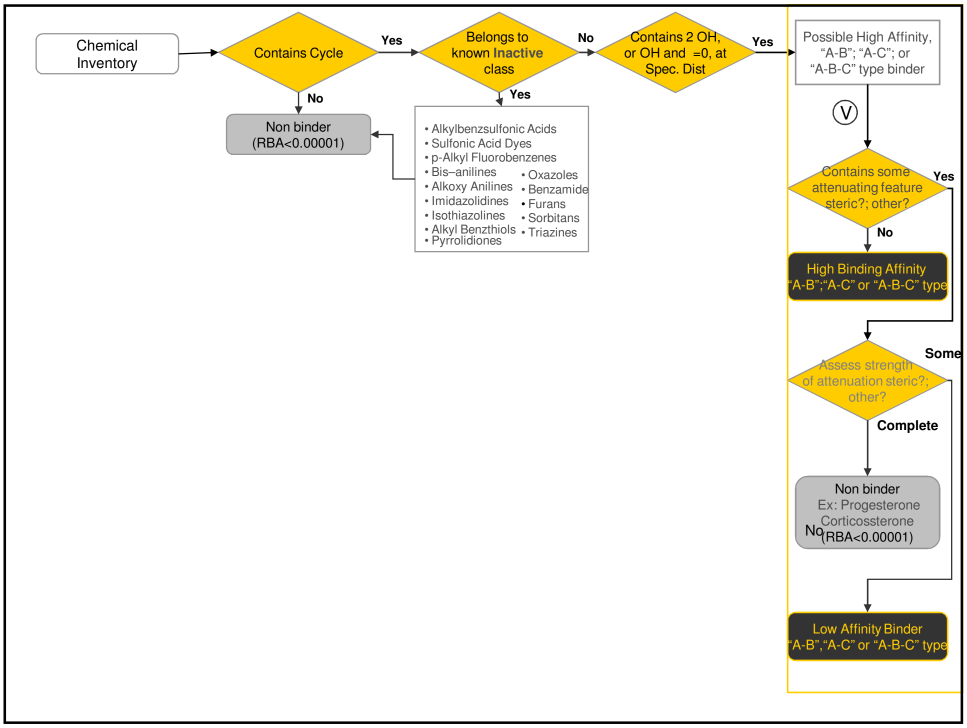
Schematic representation of ER binding pocket (LBD), with 3 sites of interaction shown (A, B, C), and ER protein a.a. involved in the interactions which chemical ligands.

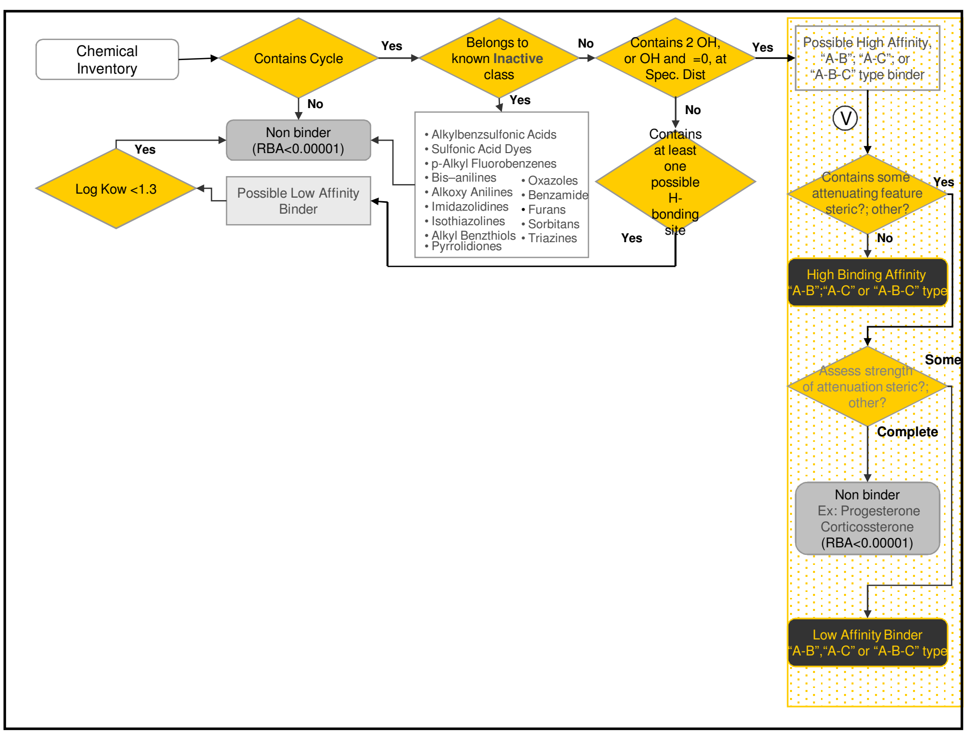


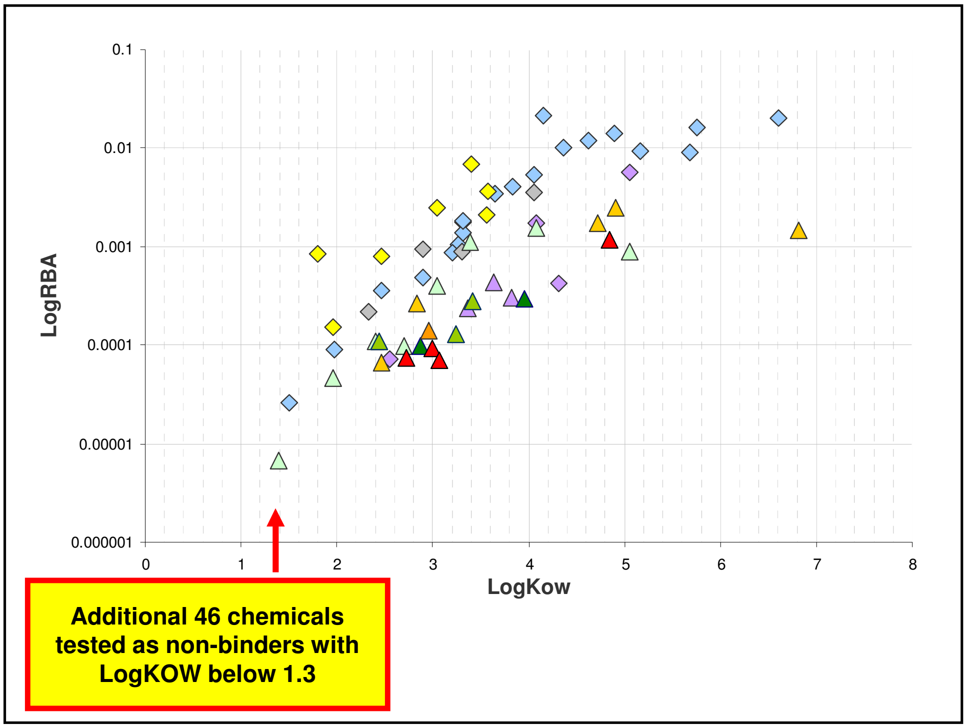
J. Katzenellenbogen

A_B Interaction

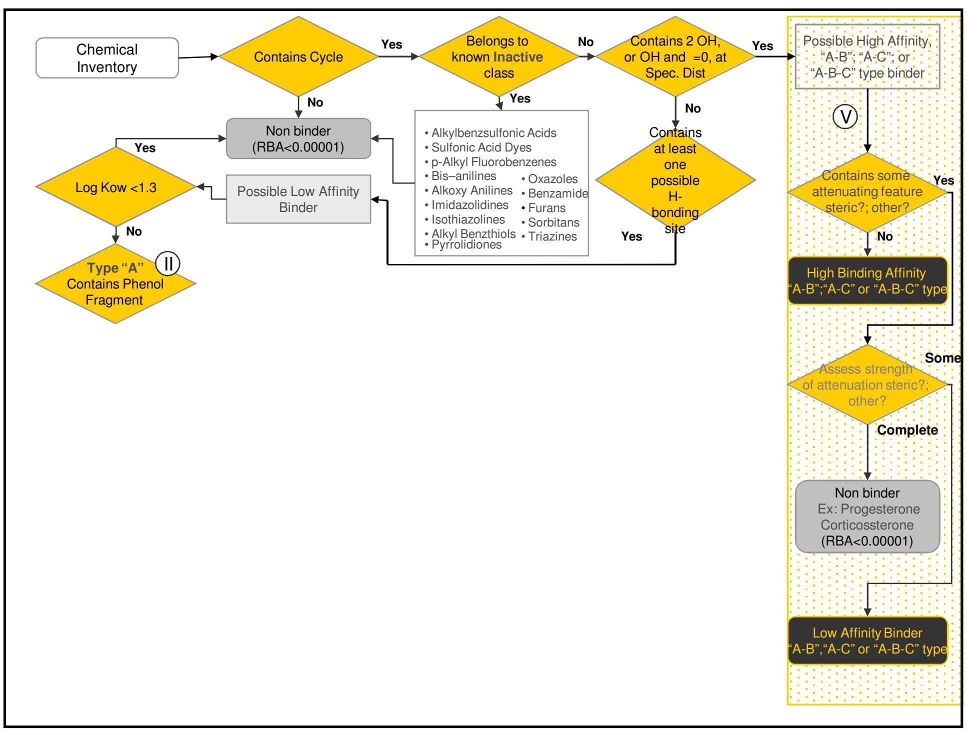



Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
Possible High Affinity, "A-B"; "A-C"; or "A-B-C" type binder
Non binder (RBA<0.00001)
Yes
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
V
Contains some attenuating feature steric?; other?
Yes
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
Assess strength of attenuation steric?; other?
Some
Complete
Non binder Ex: Progesterone Corticossterone (RBA<0.00001)
No
Low Affinity Binder "A-B","A-C" or "A-B-C" type

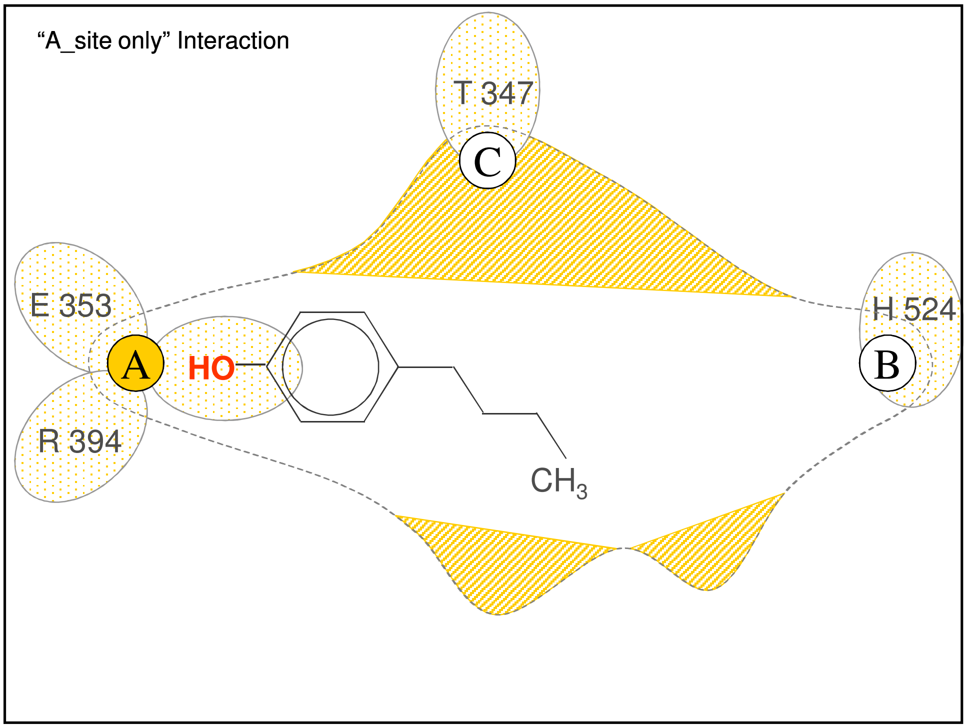

Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
Possible High Affinity, "A-B"; "A-C"; or "A-B-C" type binder
Non binder (RBA<0.00001)
Yes
Log Kow <1.3
Possible Low Affinity Binder
Yes
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
No
Contains at least one possible H-bonding site
Yes
V
Contains some attenuating feature steric?; other?
Yes
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
Assess strength of attenuation steric?; other?
Some
Complete
Non binder Ex: Progesterone Corticossterone (RBA<0.00001)
Low Affinity Binder "A-B","A-C" or "A-B-C" type



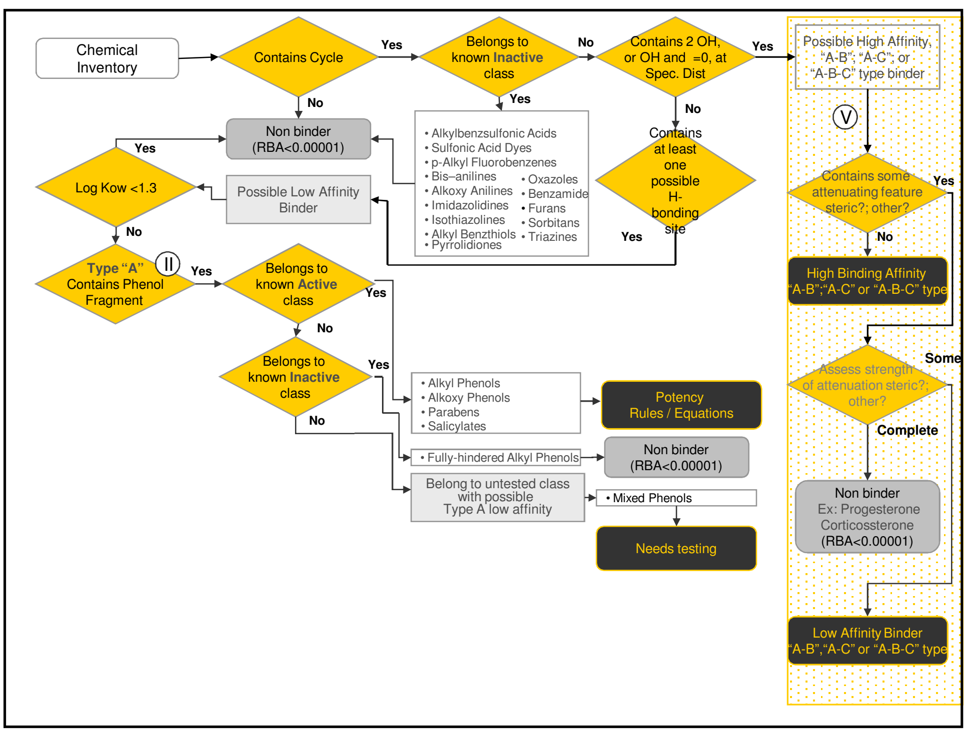
0.1
0.01
0.001
0.0001
0.00001
0.000001
LogRBA
0
1
2
3
4
5
6
7
8
LogKow
Additional 46 chemicals tested as non-binders with LogKOW below 1.3



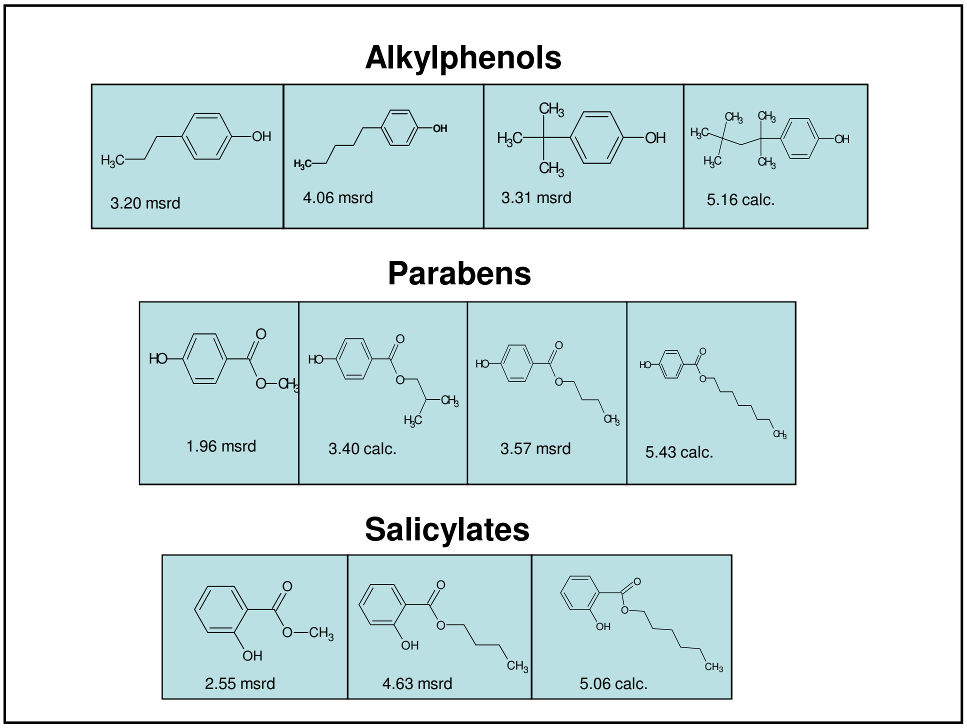
Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Yes
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
No
Possible High Affinity, "A-B"; "A-C"; or "A-B-C" type binder
V
Non binder (RBA<0.00001)
Yes
Log Kow <1.3
No
Possible Low Affinity Binder
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
Contains at least one possible H-bonding site
Yes
Type "A" Contains Phenol Fragment
II
Contains some attenuating feature steric?; other?
Yes
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
Assess strength of attenuation steric?; other?
Some
Complete
Non binder Ex: Progesterone Corticossterone (RBA<0.00001)
Low Affinity Binder "A-B","A-C" or "A-B-C" type



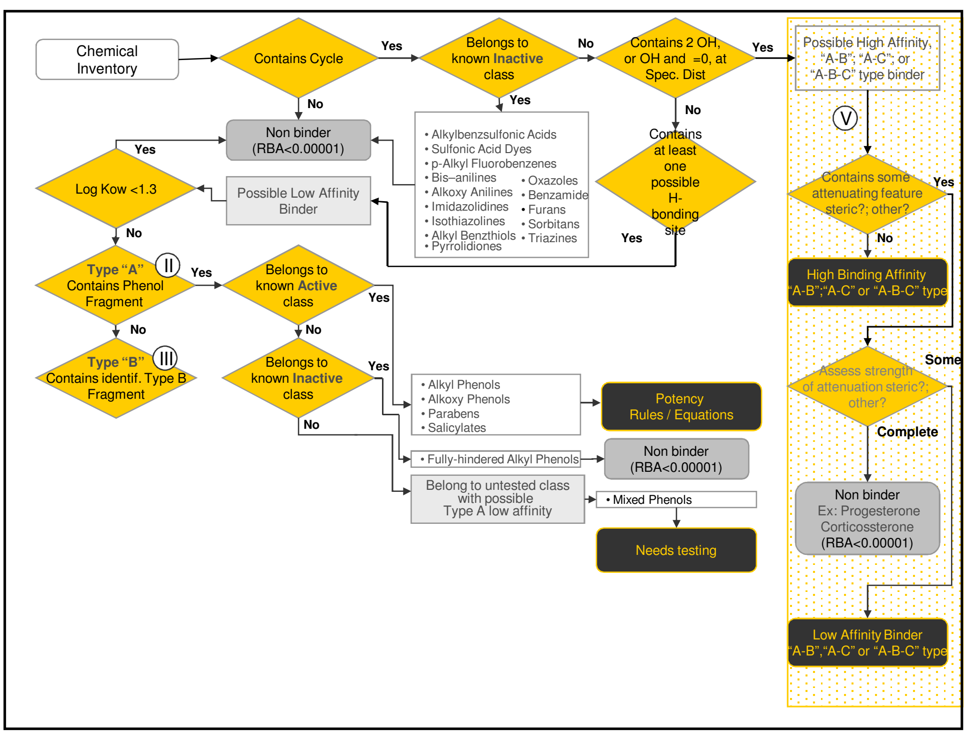
"A_site only" Interaction
T 347
C
E 353
A
HO
R 394
H 524
B
CH_3



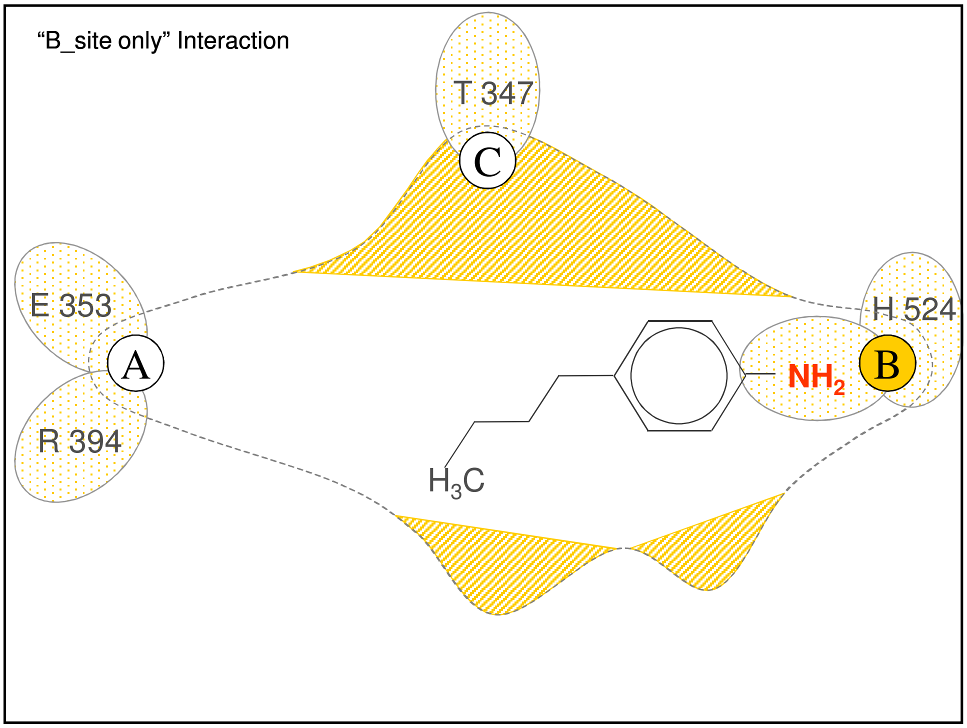
Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Yes
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
No
Possible High Affinity, "A-B"; "A-C"; or "A-B-C" type binder
V
Non binder (RBA<0.00001)
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolidines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
Contains at least one possible H-bonding site
Yes
Log Kow <1.3
Yes
No
Possible Low Affinity Binder
Contains some attenuating feature steric?; other?
Yes
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
Type "A" Contains Phenol Fragment
II
Yes
Belongs to known Active class
Yes
No
Assess strength of attenuation steric?; other?
Some
Complete
Belongs to known Inactive class
Yes
No
• Alkyl Phenols
• Alkoxy Phenols
• Parabens
• Salicylates
Potency Rules / Equations
• Fully-hindered Alkyl Phenols
Non binder (RBA<0.00001)
Belong to untested class with possible Type A low affinity
• Mixed Phenols
Needs testing
Non binder Ex: Progesterone Corticosterone (RBA<0.00001)
Low Affinity Binder "A-B","A-C" or "A-B-C" type



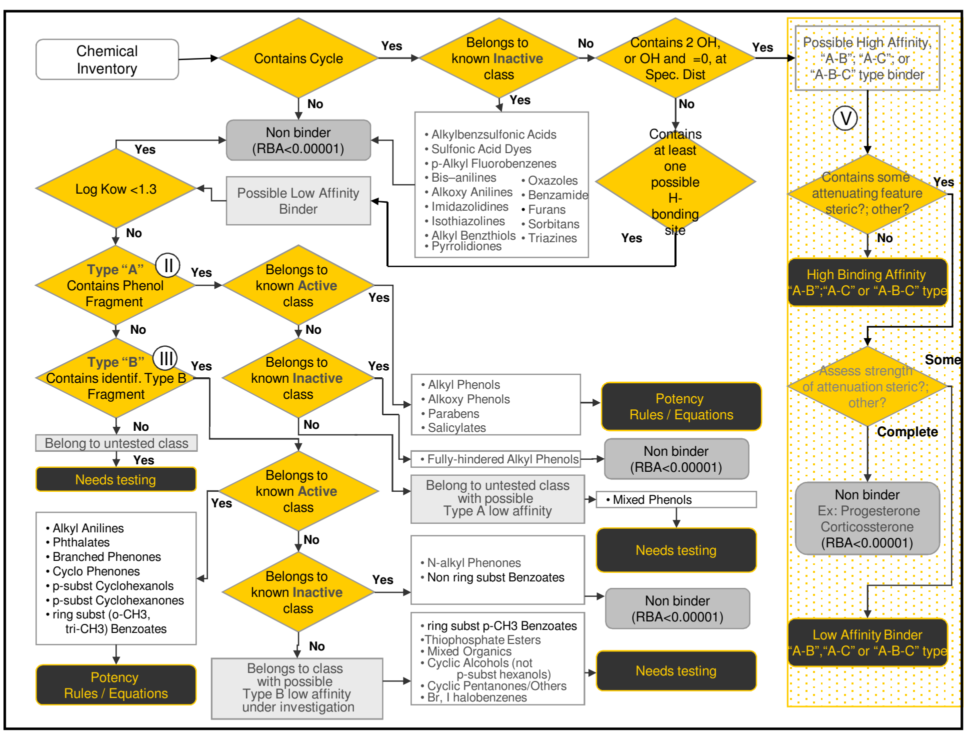
Alkylphenols
3.20 msrd
4.06 msrd
3.31 msrd
5.16 calc.
Parabens
1.96 msrd
3.40 calc.
3.57 msrd
5.43 calc.
Salicylates
2.55 msrd
4.63 msrd
5.06 calc.



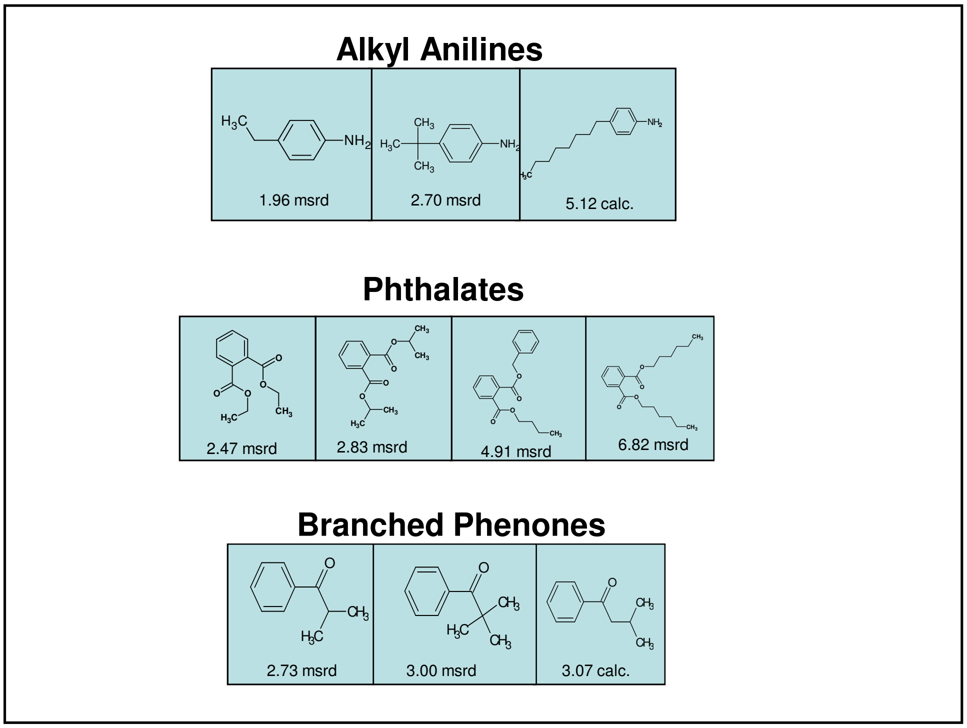
Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Yes
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
No
Possible High Affinity, "A-B", "A-C", or "A-B-C" type binder
V
Non binder (RBA<0.00001)
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
Contains at least one possible H-bonding site
Yes
Contains some attenuating feature steric?; other?
Yes
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
Yes
Log Kow <1.3
No
Possible Low Affinity Binder
Type "A" Contains Phenol Fragment
II
Yes
No
Belongs to known Active class
Yes
No
Type "B" Contains identif. Type B Fragment
III
Belongs to known Inactive class
Yes
No
• Alkyl Phenols
• Alkoxy Phenols
• Parabens
• Salicylates
Potency Rules / Equations
• Fully-hindered Alkyl Phenols
Non binder (RBA<0.00001)
Belong to untested class with possible Type A low affinity
• Mixed Phenols
Needs testing
Some
Assess strength of attenuation steric?; other?
Complete
Non binder Ex: Progesterone Corticosterone (RBA<0.00001)
Low Affinity Binder "A-B","A-C" or "A-B-C" type

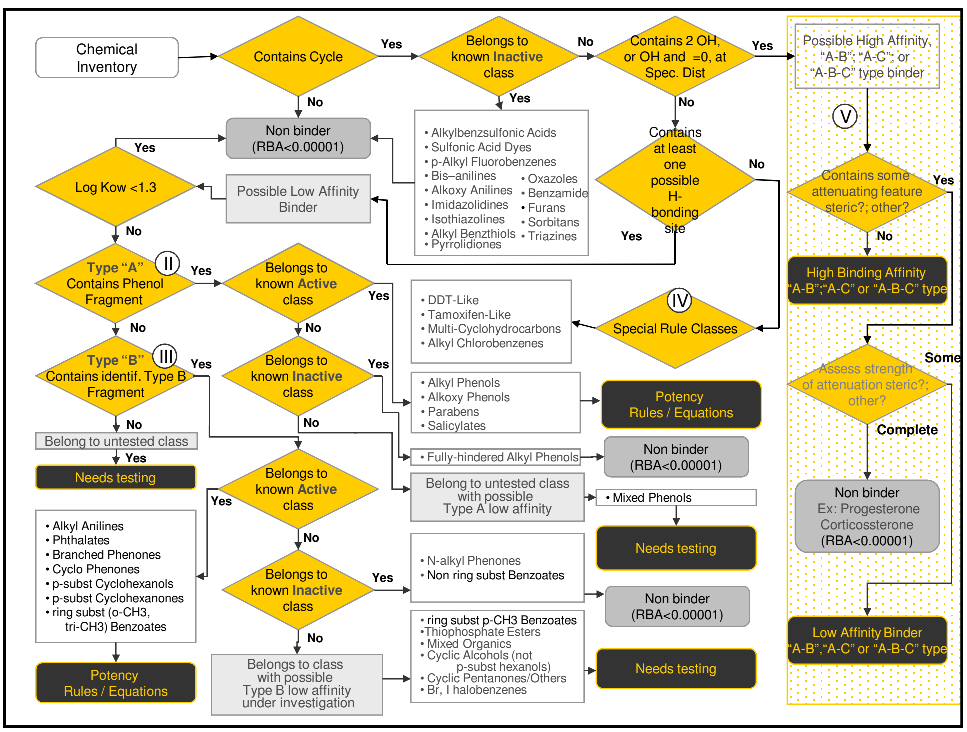


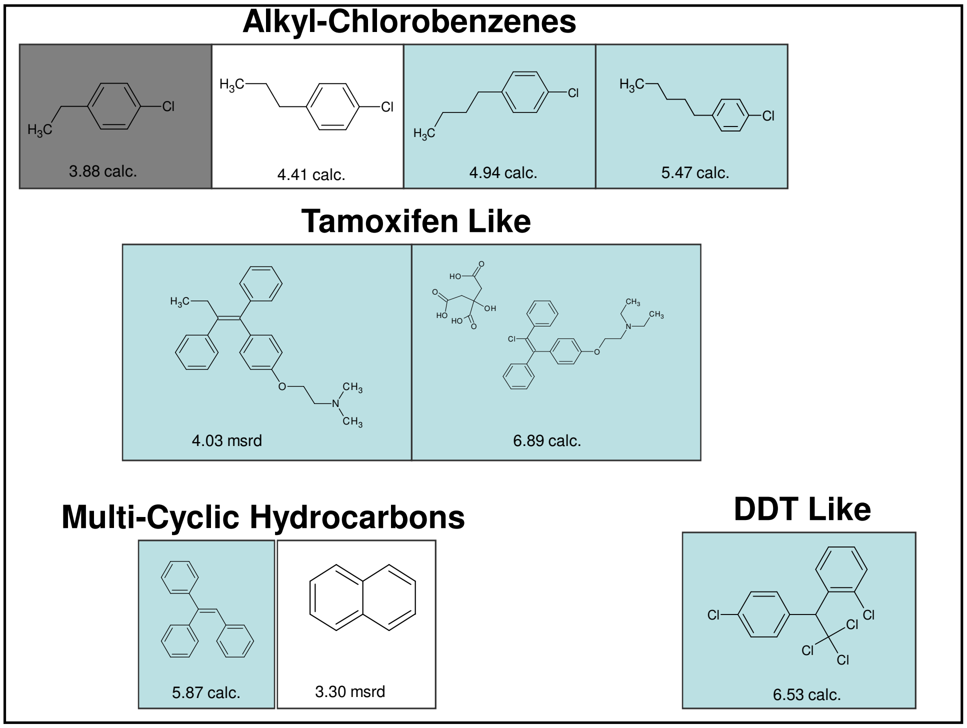
“B_site only” Interaction
T 347
C
E 353
A
R 394
H 524
B
NH_2
H_3C



Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Yes
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
No
Possible High Affinity, “A-B”, “A-C”, or “A-B-C” type binder
Non binder (RBA<0.00001)
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolidines
• Alkyl Benzthiols
• Pyrrolidines
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
Contains at least one possible H-bonding site
Yes
V
Contains some attenuating feature steric?; other?
Yes
No
Log Kow <1.3
Yes
No
Possible Low Affinity Binder
High Binding Affinity “A-B”;“A-C” or “A-B-C” type
Type “A” Contains Phenol Fragment
II
Yes
No
Belongs to known Active class
Yes
No
Assess strength of attenuation steric?; other?
Some
Complete
Type “B” Contains identif. Type B Fragment
III
Yes
No
Belongs to known Active class
Yes
No
• Alkyl Phenols
• Alkoxy Phenols
• Parabens
• Salicylates
Potency Rules / Equations
Belong to untested class
Yes
Needs testing
• Fully-hindered Alkyl Phenols
Non binder (RBA<0.00001)
Non binder Ex: Progesterone Corticosterone (RBA<0.00001)
Belongs to known Active class
Yes
No
Belong to untested class with possible Type A low affinity
• Mixed Phenols
Needs testing
• Alkyl Anilines
• Phthalates
• Branched Phenones
• Cyclo Phenones
• p-subst Cyclohexanols
• p-subst Cyclohexanones
• ring subst (o-CH3, tri-CH3) Benzoates
Belongs to known Inactive class
Yes
No
• N-alkyl Phenones
• Non ring subst Benzoates
Non binder (RBA<0.00001)
Low Affinity Binder “A-B”,“A-C” or “A-B-C” type
• ring subst p-CH3 Benzoates
•Thiophosphate Esters
• Mixed Organics
• Cyclic Alcohols (not p-subst hexanols)
• Cyclic Pentanones/Others
• Br, I halobenzenes
Needs testing
Potency Rules / Equations
Belongs to class with possible Type B low affinity under investigation


Alkyl Anilines
1.96 msrd
2.70 msrd
5.12 calc.
Phthalates
2.47 msrd
2.83 msrd
4.91 msrd
6.82 msrd
Branched Phenones
2.73 msrd
3.00 msrd
3.07 calc.


Chemical Inventory
Contains Cycle
Yes
No
Belongs to known Inactive class
No
Yes
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
No
Possible High Affinity, "A-B", "A-C", or "A-B-C" type binder
V
Non binder (RBA<0.00001)
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolidines
• Alkyl Benzthiols
• Pyrrolidines
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
Contains at least one possible H-bonding site
No
Yes
Contains some attenuating feature steric?; other?
Yes
No
Log Kow <1.3
Yes
No
Possible Low Affinity Binder
Type "A" Contains Phenol Fragment
II
Yes
No
Belongs to known Active class
Yes
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
• DDT-Like
• Tamoxifen-Like
• Multi-Cyclohydrocarbons
• Alkyl Chlorobenzenes
IV
Special Rule Classes
Type "B" Contains identif. Type B Fragment
III
Yes
No
Belongs to known Active class
Yes
No
• Alkyl Phenols
• Alkoxy Phenols
• Parabens
• Salicylates
Potency Rules / Equations
Assess strength of attenuation steric?; other?
Some
Complete
Belong to untested class
Yes
Needs testing
• Fully-hindered Alkyl Phenols
Non binder (RBA<0.00001)
Belongs to known Active class
Yes
No
Belong to untested class with possible Type A low affinity
• Mixed Phenols
Non binder Ex: Progesterone Corticosterone (RBA<0.00001)
• Alkyl Anilines
• Phthalates
• Branched Phenones
• Cyclo Phenones
• p-subst Cyclohexanols
• p-subst Cyclohexanones
• ring subst (o-CH3, tri-CH3) Benzoates
Needs testing
Belongs to known Inactive class
Yes
No
• N-alkyl Phenones
• Non ring subst Benzoates
Non binder (RBA<0.00001)
Low Affinity Binder "A-B","A-C" or "A-B-C" type
Potency Rules / Equations
Belongs to class with possible Type B low affinity under investigation
• ring subst p-CH3 Benzoates
• Thiophosphate Esters
• Mixed Organics
• Cyclic Alcohols (not p-subst hexanols)
• Cyclic Pentanones/Others
• Br, I halobenzenes
Needs testing



Setting Priority for Further Testing: Food Use Inert and Antimicrobial Lists

Lower Priority if:

-Chemical belongs to a class where testing showed no evidence of ER interaction (RBA < 0.00001);
-LogP <1.3, or meets other class specific LogP cutoffs

General characteristics of these chemicals:
-Acyclic (e.g., no benzene rings)
-Cyclic but does not contain a likely H-bonding group;

RBA = relative binding affinity; (a ratio of measured chemical affinity for the ER relative to 17-beta-Estradiol = 100%)
Log P = log of octanol/water partition coefficient (also known as Log Kow); is an indicator of lipophilicity

Setting Priority for Further Testing: Food Use Inert and Antimicrobial Lists

Higher Priority if:

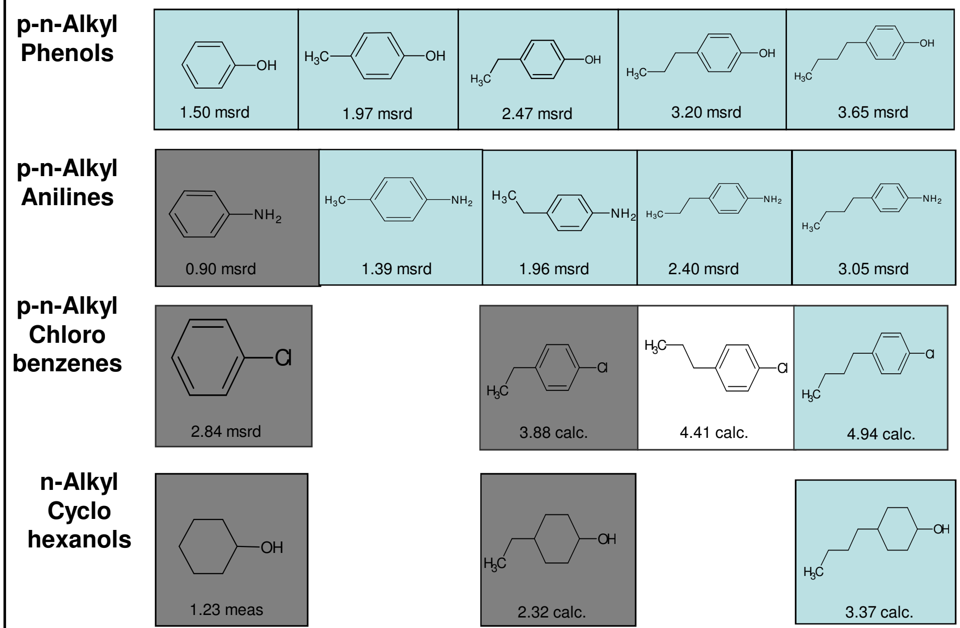
-Chemical belongs to class with evidence of ER interaction, and:
-LogP > 1.3 (or other class-specific cutoffs)

General characteristics of these chemicals:
-Contains at least one cycle (e.g., benzene ring);
-Contains a possible H-bonding group;

Inactive Classes – Lower Priority

Inactive Classes	FI	AM
Acyclics	230	122
Alkylbenzenesulfonic Acids	78	3
Sulfonic Acid Dyes	9	1
Sorbitans	7	0
Monocyclic Hydrocarbons	7	0
Cyclic caged Hydrocarbon	1	0
Pyrrolidines	3	0
Furans	3	0
N-chain Phenones	1	0
Oxazoles	0	3
Triazines	1	13
Isothiazolines	3	7
Imidazolidines	0	8
Cyclic Inorganics	0	3
Fully-Hindered Alkylphenols	1	0
Cyclic Pentanones/Others	2	0
Inactive ranges in Otherwise Positive Classes		
Cyclic Hexanones of LogP<1.5	1	0
Cyclic Hexanols of LogP<1.5	1	0
Chlorobenzenes of LogP<4	1	0
Inactives in Mixed Functional Grps/Heteratom Classes		
Mixed Phenols	4	1
Mixed Organics	14	13
Organometallics	0	1
Total Inactives	367	175

Classwise Differences in Lower LogKow Cutoff



Active Classes – High Priority

Active Classes	FI	AM
Alkyl Phenols	**4**	**9**
Parabens	**3**	**0**
Salicylates	**1**	**0**
Phthalates	**3**	**0**
Actives in Mixed Functional /Heteratom Classes		
Mixed Phenols	**1**	**6**
Mixed Organics	**1**	**0**
Total Positives	13	15

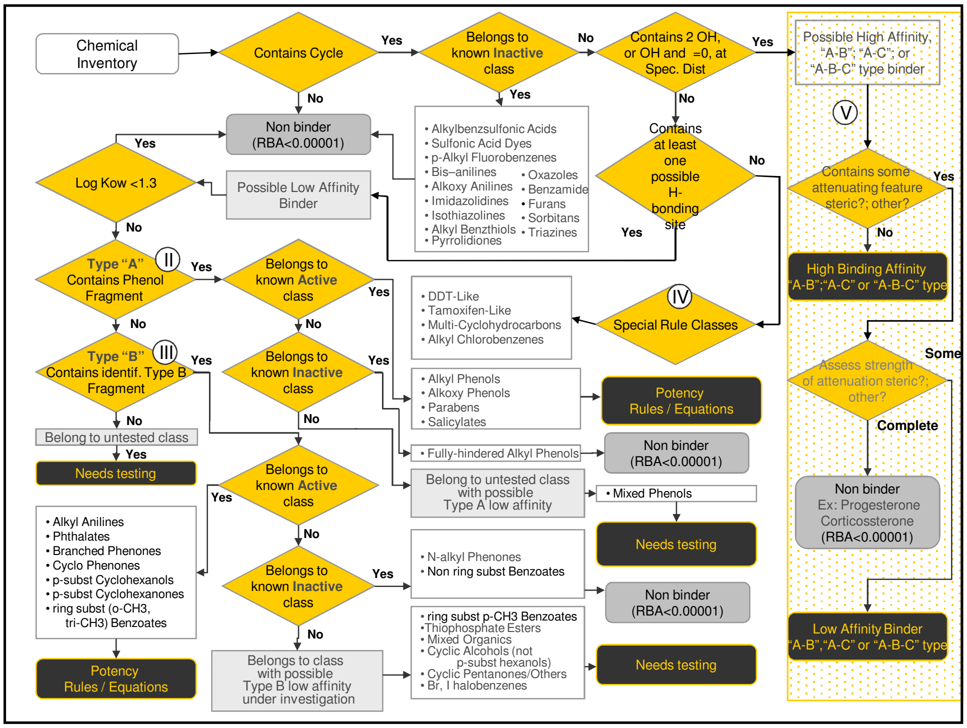
Classes – Under Investigation

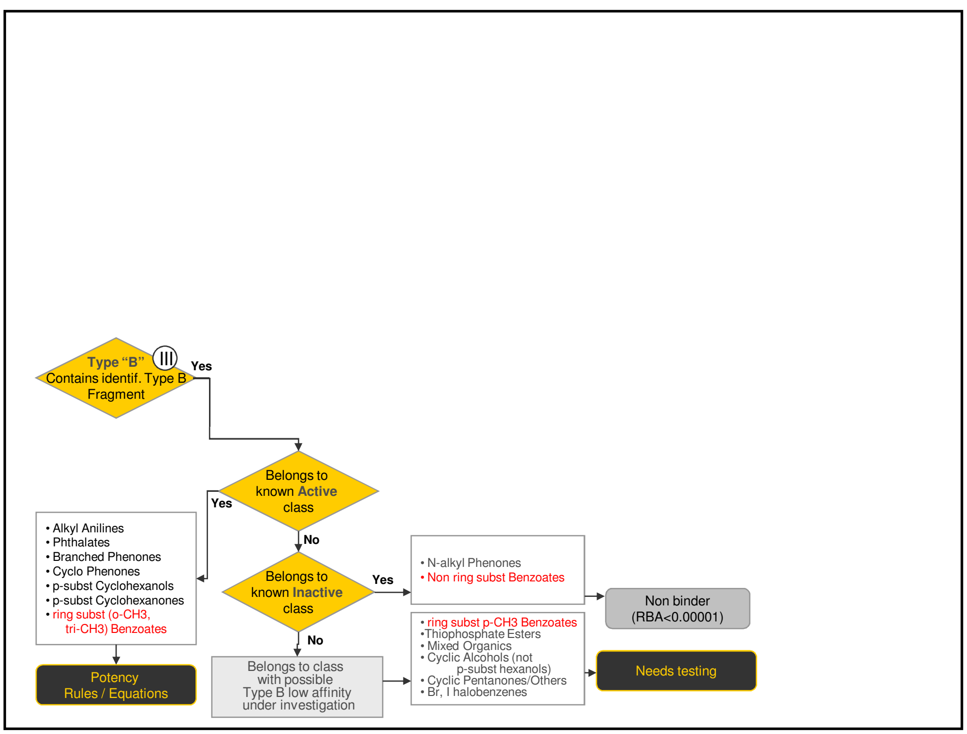
Classes		FI	AM
Mixed Phenols	(?)	3	2
Mixed Organics	(?)	7	16
Organometallics	(?)	2	3
Thiophosphate Esters	(?)	1	0
Total Under Investigation		13	21

Summary of Current Findings
Food Use Inert and Antimicrobial Lists

Inventory Status – September 20, 2007

Food Use Inventory		Antimicrobials
393	*Total Chemicals*	211
367 (94%)	*Lower Priority*	175 (82%)
13 (3%)	*Higher Priority*	15 (7%)
13 (3%)	*Under Investigation*	21 (10%)


Chemical Inventory
Contains Cycle
Yes
Belongs to known Inactive class
No
Contains 2 OH, or OH and =0, at Spec. Dist
Yes
Possible High Affinity, "A-B", "A-C", or "A-B-C" type binder
V
No
Non binder (RBA<0.00001)
Yes
• Alkylbenzsulfonic Acids
• Sulfonic Acid Dyes
• p-Alkyl Fluorobenzenes
• Bis–anilines
• Alkoxy Anilines
• Imidazolidines
• Isothiazolidines
• Alkyl Benzthiols
• Pyrrolidiones
• Oxazoles
• Benzamide
• Furans
• Sorbitans
• Triazines
No
Contains at least one possible H-bonding site
No
Yes
Contains some attenuating feature steric?; other?
Yes
No
Yes
Log Kow <1.3
Possible Low Affinity Binder
No
High Binding Affinity "A-B";"A-C" or "A-B-C" type
Type "A" II Contains Phenol Fragment
Yes
Belongs to known Active class
Yes
• DDT-Like
• Tamoxifen-Like
• Multi-Cyclohydrocarbons
• Alkyl Chlorobenzenes
IV
Special Rule Classes
No
No
Some
Assess strength of attenuation steric?; other?
Type "B" III Contains identif. Type B Fragment
Yes
Belongs to known Active class
Yes
• Alkyl Phenols
• Alkoxy Phenols
• Parabens
• Salicylates
Potency Rules / Equations
Complete
No
No
Belong to untested class
Yes
Needs testing
• Fully-hindered Alkyl Phenols
Non binder (RBA<0.00001)
Belongs to known Active class
Yes
Belong to untested class with possible Type A low affinity
• Mixed Phenols
Non binder Ex: Progesterone Corticosterone (RBA<0.00001)
Needs testing
• Alkyl Anilines
• Phthalates
• Branched Phenones
• Cyclo Phenones
• p-subst Cyclohexanols
• p-subst Cyclohexanones
• ring subst (o-CH3, tri-CH3) Benzoates
No
Belongs to known Inactive class
Yes
• N-alkyl Phenones
• Non ring subst Benzoates
Non binder (RBA<0.00001)
Low Affinity Binder "A-B","A-C" or "A-B-C" type
No
• ring subst p-CH3 Benzoates
•Thiophosphate Esters
• Mixed Organics
• Cyclic Alcohols (not p-subst hexanols)
• Cyclic Pentanones/Others
• Br, I halobenzenes
Potency Rules / Equations
Belongs to class with possible Type B low affinity under investigation
Needs testing


Type "B" III Contains identif. Type B Fragment
Yes
Belongs to known Active class
Yes
• Alkyl Anilines
• Phthalates
• Branched Phenones
• Cyclo Phenones
• p-subst Cyclohexanols
• p-subst Cyclohexanones
• ring subst (o-CH3, tri-CH3) Benzoates
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Belongs to known Inactive class
Yes
• N-alkyl Phenones
• Non ring subst Benzoates
Non binder (RBA<0.00001)
No
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Potency Rules / Equations
Belongs to class with possible Type B low affinity under investigation
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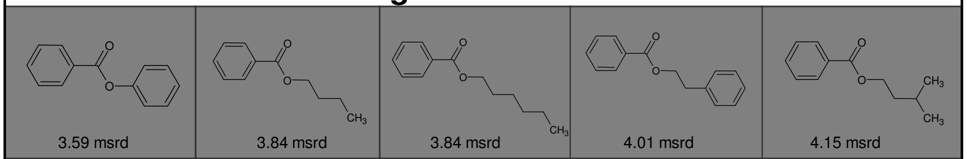
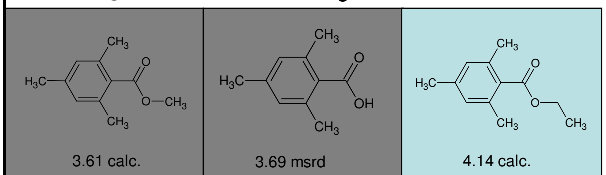
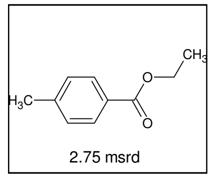
Non Ring-subst. Benzoates



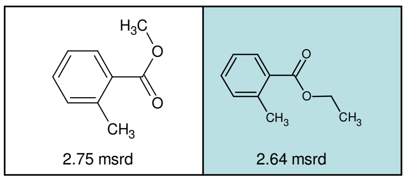
Ring-subst. (tri-CH_3) Benzoates



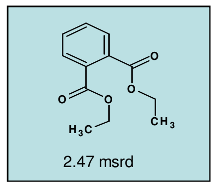
Ring-subst. (p-CH_3) Benzoates



Ring-subst. (o-CH_3) Benzoates



Phthalate



Summary

- The decision support system has been coded to perform an automatic classification of discrete chemical inventories.
- Decision support system was designed to categorize chemicals with similar activity (ER binding potential) to determine common aspects of structure.
- Modifications to the decision tree are made as needed as new inventories are examined which contain new structure/activity classes

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Assessment	GROUP	Total	Binders	Non-binders	Testing
(+)	A_B or A_C Type	31	26	5	0
(+)	Alkyl Anilines	9	8	1	0
(+)	Alkyl Phenols - Alkoxy	5	5	0	0
(+)	Alkyl Phenols - m-Branched	1	1	0	0
(+)	Alkyl Phenols - o-Branched	2	2	0	0
(+)	Alkyl Phenols - p-Branched	8	8	0	0
(+)	Alkyl Phenols - Phenyl	4	4	0	0
(+)	Alkyl Phenols - p-nChain	10	10	0	0
(+)	Benzoates - ortho-CH3	2	1	0	1
(+)	DDT Like	9	9	0	0
(+)	Parabens - monohydroxy	8	8	0	0
(+)	Parabens - trihydroxy	2	1	0	1
(+)	Phenones - Branched	7	5	2	0
(+)	Phthalates	8	5	3	0
(+)	Salicylates	5	4	1	0
(+)	Tamoxifen Like	3	3	0	0
?	Alkyl Phenols - Hindered	2	1	1	0
?	Benzoates - para-CH3	1	0	0	1
?	Benzoates - tri-CH3	3	1	2	0
?	Cyclic Alcohols - hexyl	9	3	2	4
?	Cyclic Hydrocarbons - Multi	10	3	5	2
?	Cyclic Ketone - hexyl	6	3	2	1
?	Halobenzenes	8	2	5	1
?	Mixed Organics	58	3	33	22
?	Mixed Phenols	16	4	8	4
?	Organometallic	6	0	1	5
?	Steroidal Backbone	9	3	6	0
?	Thiophosphate Esters	4	1	2	1
negative	Acetanilides	4	0	2	2
negative	Acyclic	26	0	23	3
negative	Alkyl Anilines - Alkoxy	4	0	2	2
negative	Alkyl Benzthiols	2	0	2	0
negative	Alkylbenzenesulfonic acids	6	0	6	0
negative	Benzamide	2	0	2	0
negative	Benzoates - not subst	5	0	5	0
negative	Bis-Anilines	8	0	8	0
negative	Cyclic Alcohols - other	2	0	1	1
negative	Cyclic Hydrocarbons - Mono	4	0	4	0
negative	Cyclic Ketones - other	4	0	4	0
negative	Furans	3	0	3	0
negative	Imidazolidines	4	0	4	0
negative	Isothiazolines	2	0	2	0
negative	Oxazoles	2	0	2	0
negative	Phenones - n-chain	3	0	3	0
negative	Pyrrolidiones	2	0	2	0
negative	Sorbitans	2	0	2	0
negative	Sulfonic Acid Dyes	9	0	9	0
negative	Triazines	4	0	4	0
	TOTALS	344	124	169	51