CASE STUDY 1: Endocrine Disruptors: Estrogen Receptor Expert System (ERES)

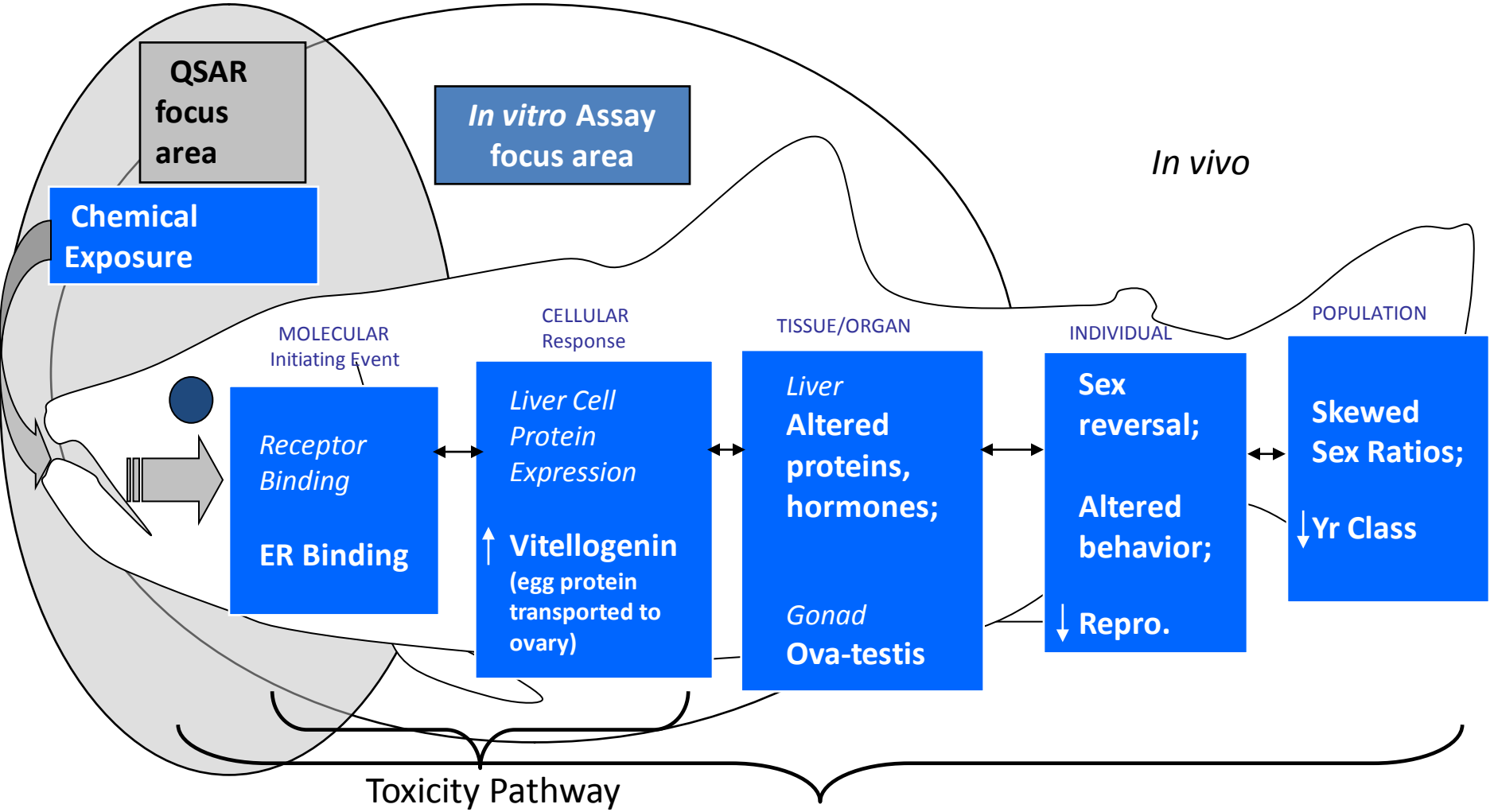
Mechanistic Basis of the Expert System

- Adverse Outcome Pathway (AOP) context
- OECD QSAR Validation Principles
- Expert System decision tree codes the measured data from *in vitro* assays designed for purpose
  - Assays optimized to detect any potential for ER interaction, including low affinity binding
  - Provides the data and expert interpretation in decision tree format; provides predictions based on extrapolation of measured data (displays training set used in prediction) so user can interpret and assess use for their purpose
    - Transparency
      - Can the QSAR estimate be explained mechanistically?
      - How reasonable is an estimate compared with data for similar chemicals?
    - Usefulness
      - Are the predictions applicable to all the chemicals of regulatory concern?
      - Does the model/expert system answer the regulatory question?

ER-mediated Reproductive Impairment Adverse Outcome Pathway

Chemical Effects Across Levels of Biological Organization

Mechanistic linkage between the risk assessment endpoint (ER-mediated reproductive impairment) and the hazard identification endpoint (MIE: ER binding)



ER Binding Affinity: An Indicator of Potential Reproductive Effects

- *In vitro* Assays used to build the Expert System are along an adverse outcome pathway (AOP) ending in reproductive impairment
  - The molecular initiating event (MIE) of the pathway is ER binding *(msrd)*
  - Tissue level response key event along the pathway confirms a higher level response *(msrd)*
  - The measured data identifies which chemical structures can initiate the pathway and subsequent key event
  - The Expert System extrapolates from the measured data to predict ER binding potential of unmeasured chemicals that are within the bounds of measurement
  - The Expert System also indicates when a chemical is outside the bounds of the measured data thus accurate predictions are not possible (Unknown Binding Potential)
  - AOP context provides conceptual model useful for generating testable hypotheses (e.g., prioritization for Tier 1 screening)
  - AOP context provides decision-making rationale for the regulatory community (e.g., knowns and unknowns along the AOP)

ER-mediated Reproductive Impairment Adverse Outcome Pathway

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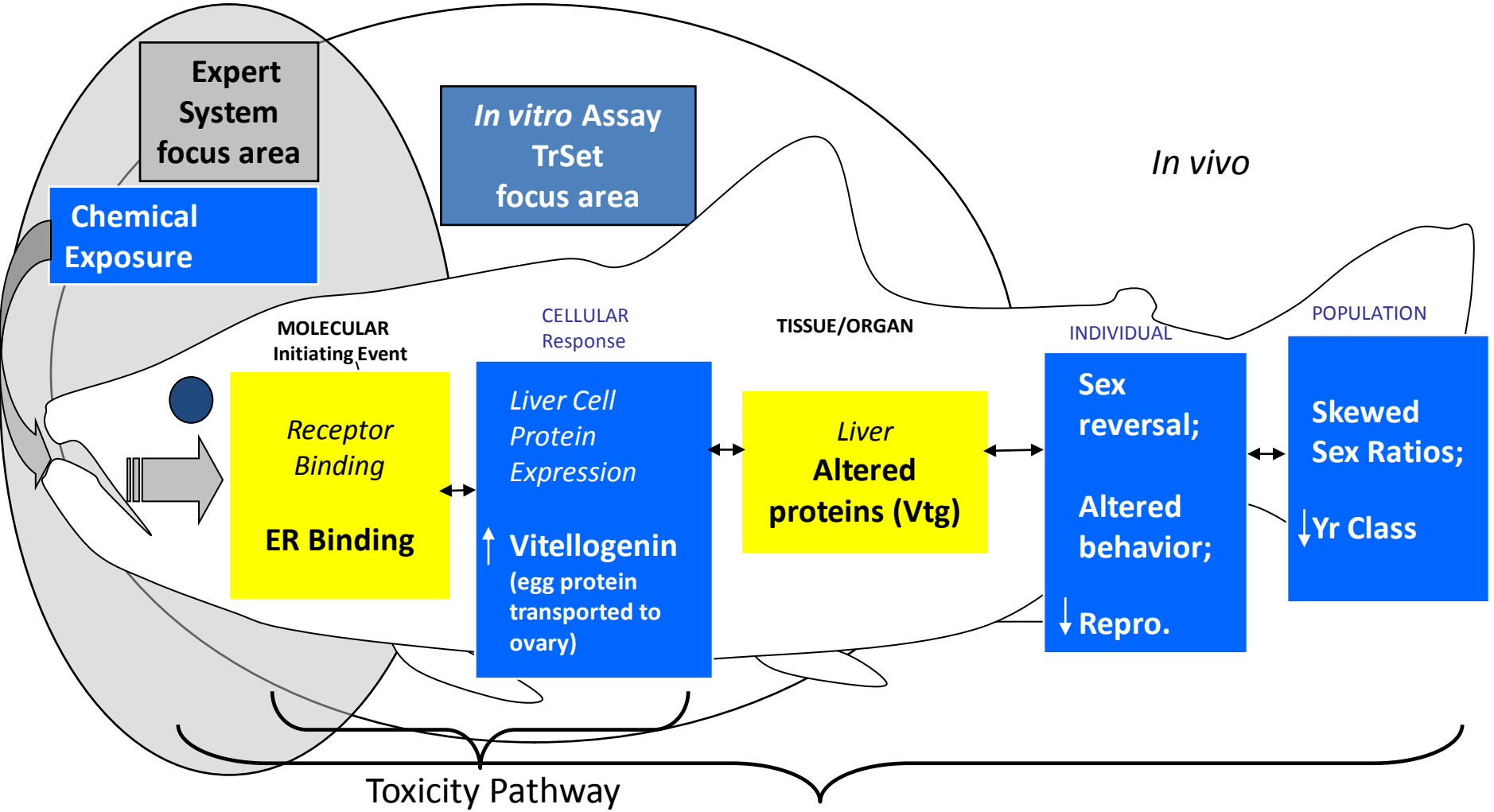


OECD Principles for QSAR Validation

- Well-Defined Endpoint – (*in vitro* assay domain)
  - Well-defined biological endpoint –
    - Informs important risk endpoint –
      - AOP ending in impaired reproduction provides plausible linkage of MIE to higher level adversity as basis for prioritizing chemicals for higher level assays
    - Interpreting the measurements
      - Measurement endpoint and confounding factors are discussed
  - Well-defined chemistry
    - Using *in vitro* assays that allow testing of the types of chemicals (range of properties) found on regulatory inventories
    - Understanding the chemical form and concentration in the assays

- Mechanistic interpretation
  - Being able to explain the predictions mechanistically
    - With respect to chemistry & biology in the assay system
  - Relationship of predicted parameter to regulatory question
    - Likelihood to initiate ER-mediated Reproductive Impairment AOP
  - Relationship of chemical parameters to biological activity

OECD Principles for QSAR Validation

- Defined Model Applicability Domain
  - Well-defined application
    - Regulatory question – priority setting not predicting adverse outcome
    - Expert System model domain coverage well-defined
      - Decision tree, logic rules, local *in vitro* TrSets upon which rules are based
    - Expert System model domain adequately covers the Regulatory Chemical domain
      - Fooduse pesticidal Inerts (FI) ; Antimicrobials (AM)
      - EDSP Universe

- Appropriate measures of goodness of fit, robustness, ability to predict
  - Measures appropriate for a regression model are not appropriate to evaluate an expert system logic rules
    - Series of local models, local *in vitro* TrSets, “unknown” structure compared to tested chemicals

- Unambiguous algorithm
  - Expert Systems – logic tree, rules/queries, supporting information

ER Expert System – Effects-Based Chemical Categories Approach

Chemical Similarity

<u>Building Effects-based Categories:</u>
Structural similarity defined by similar biological activity

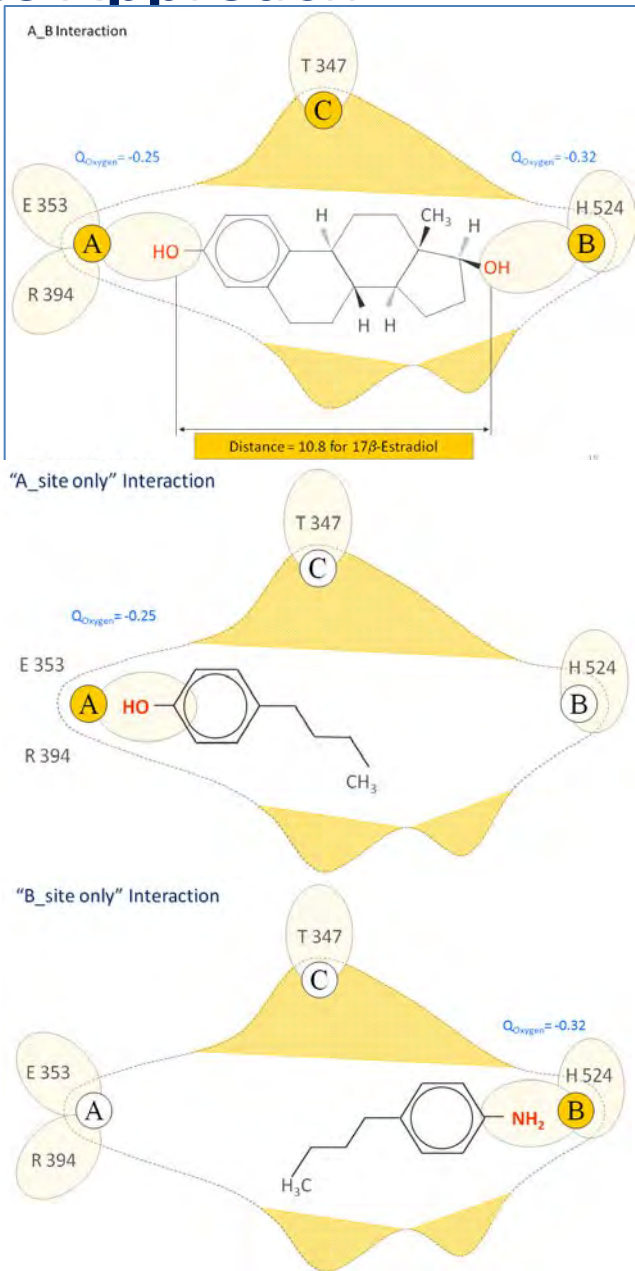
- MIE of ER-mediated AOP

Multiple ER-interaction types recognized

-chemicals can interact at different points ('A' or 'B'), depending on their properties

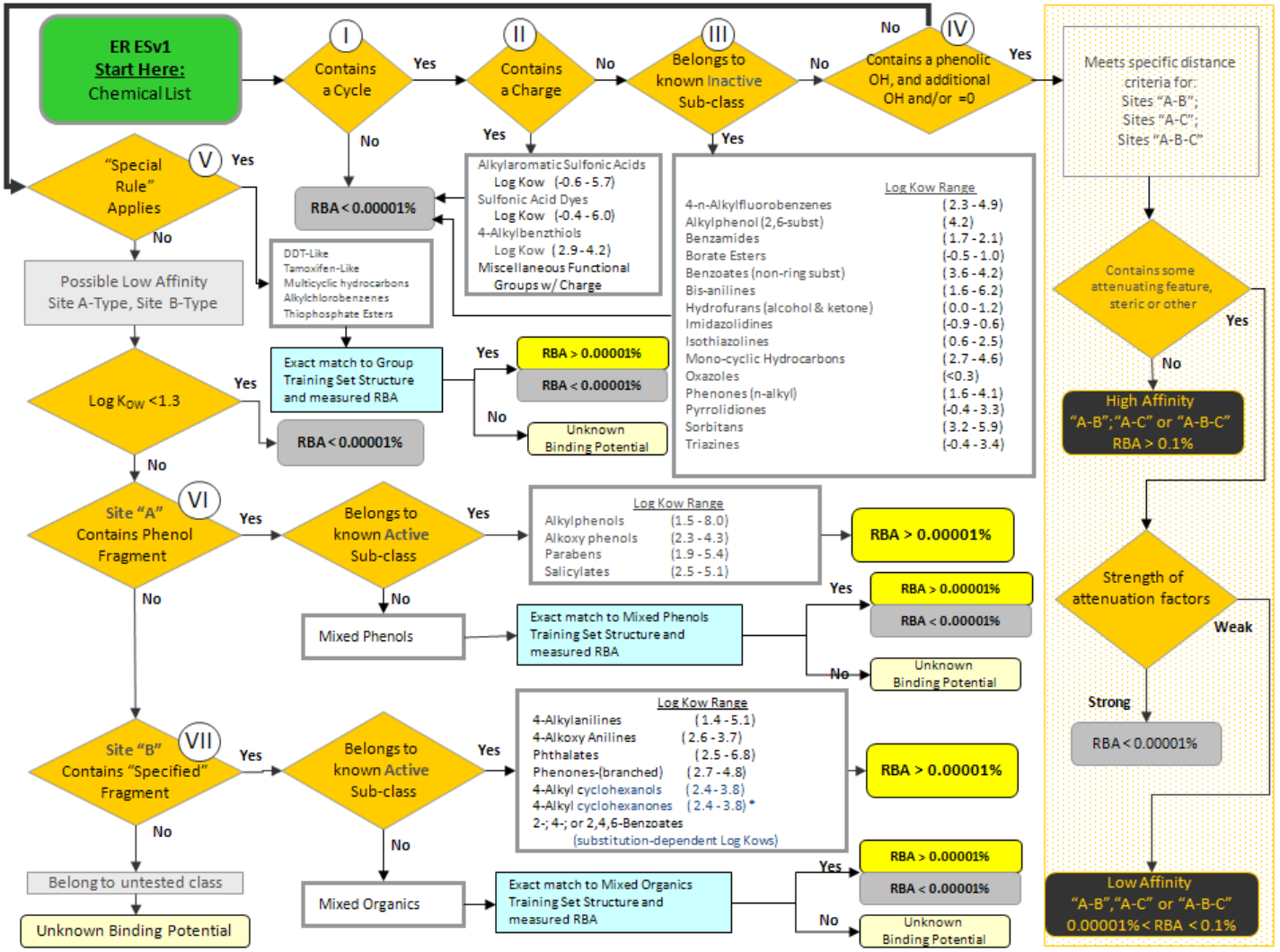
-chemicals initiating MIE at same point are 'similar'

Common biological activity within a chemical structural series is coded into ES logic rules in decision tree

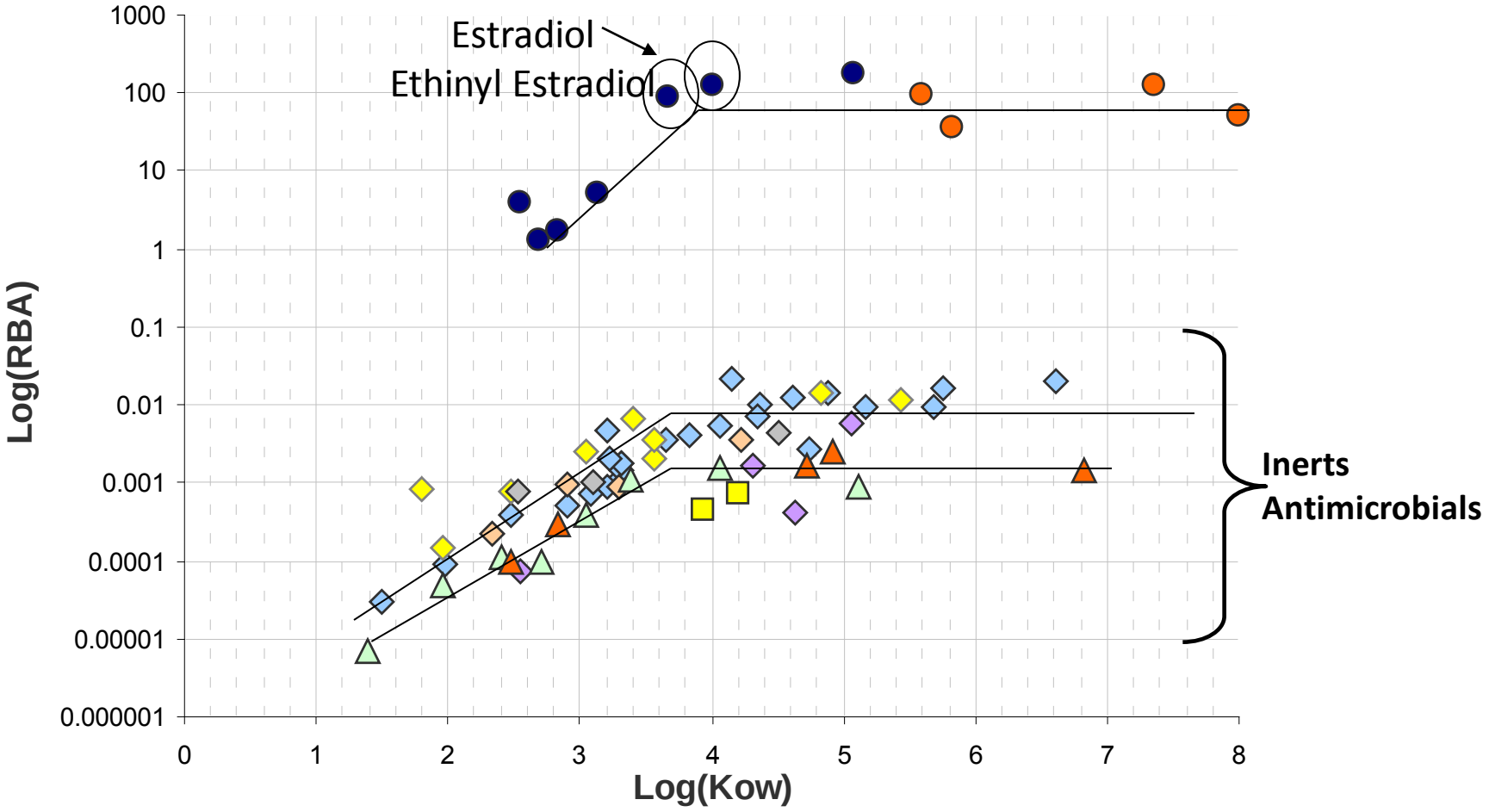


ER Expert System Decision Tree

- Expert System codes the measured data in a decision tree from the *in vitro* assays designed for purpose

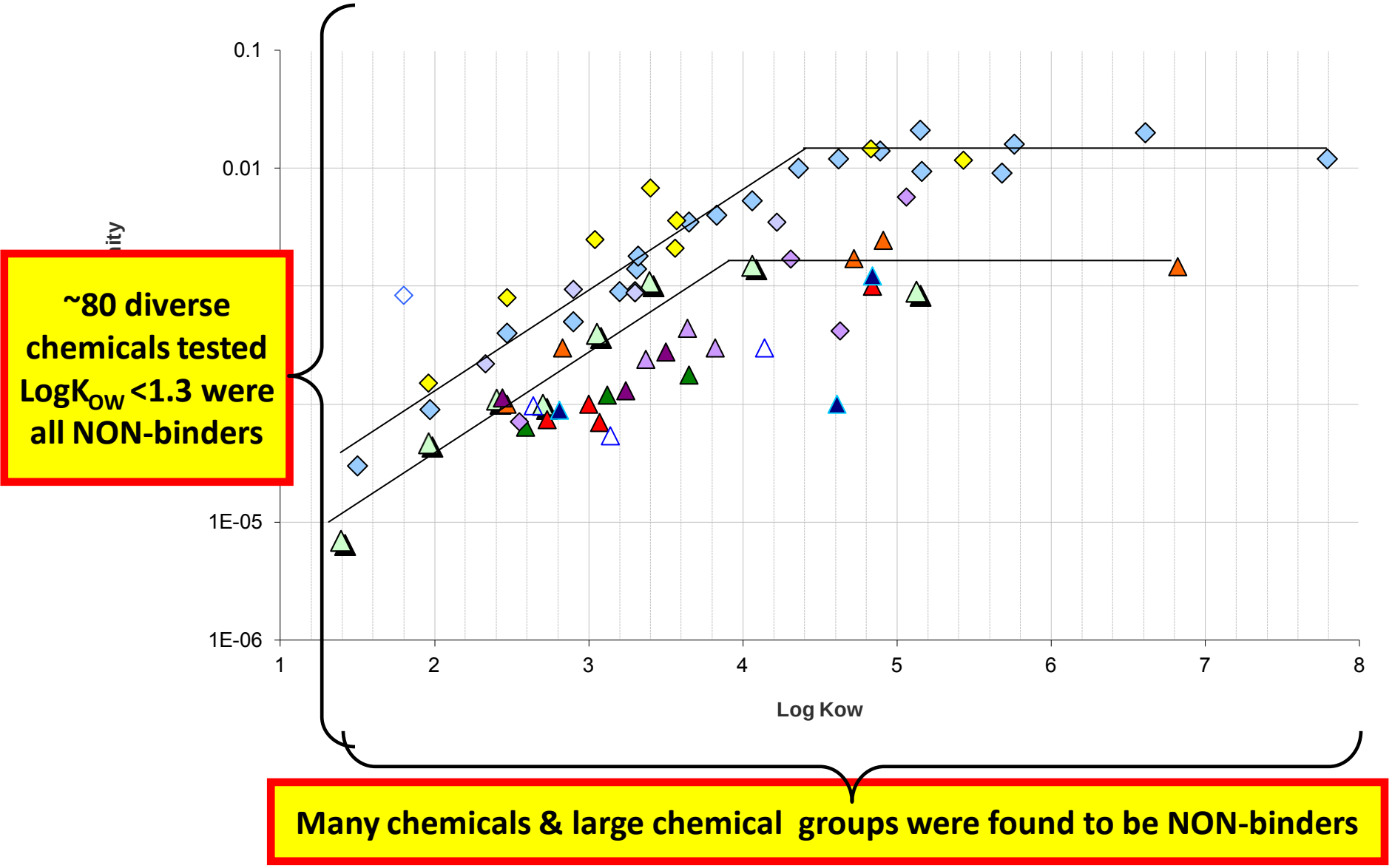


ER Binders


Estradiol
Ethinyl Estradiol
Inerts
Antimicrobials
Log(RBA)
Log(Kow)
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0
1
2
3
4
5
6
7
8

Trout ER Relative Binding Affinity vs. Log Kow

RBA = relative binding affinity compared to Estradiol at 100%



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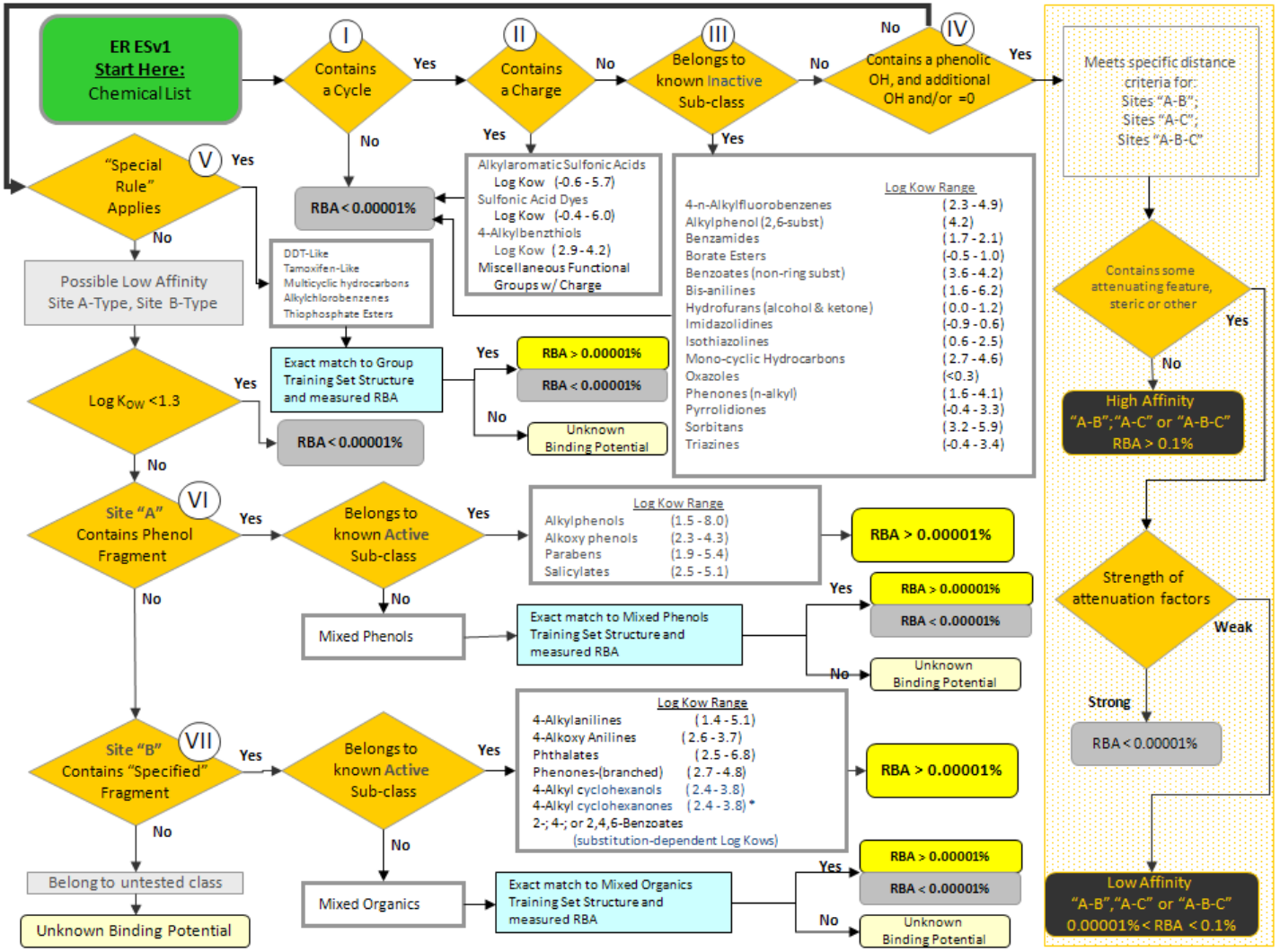
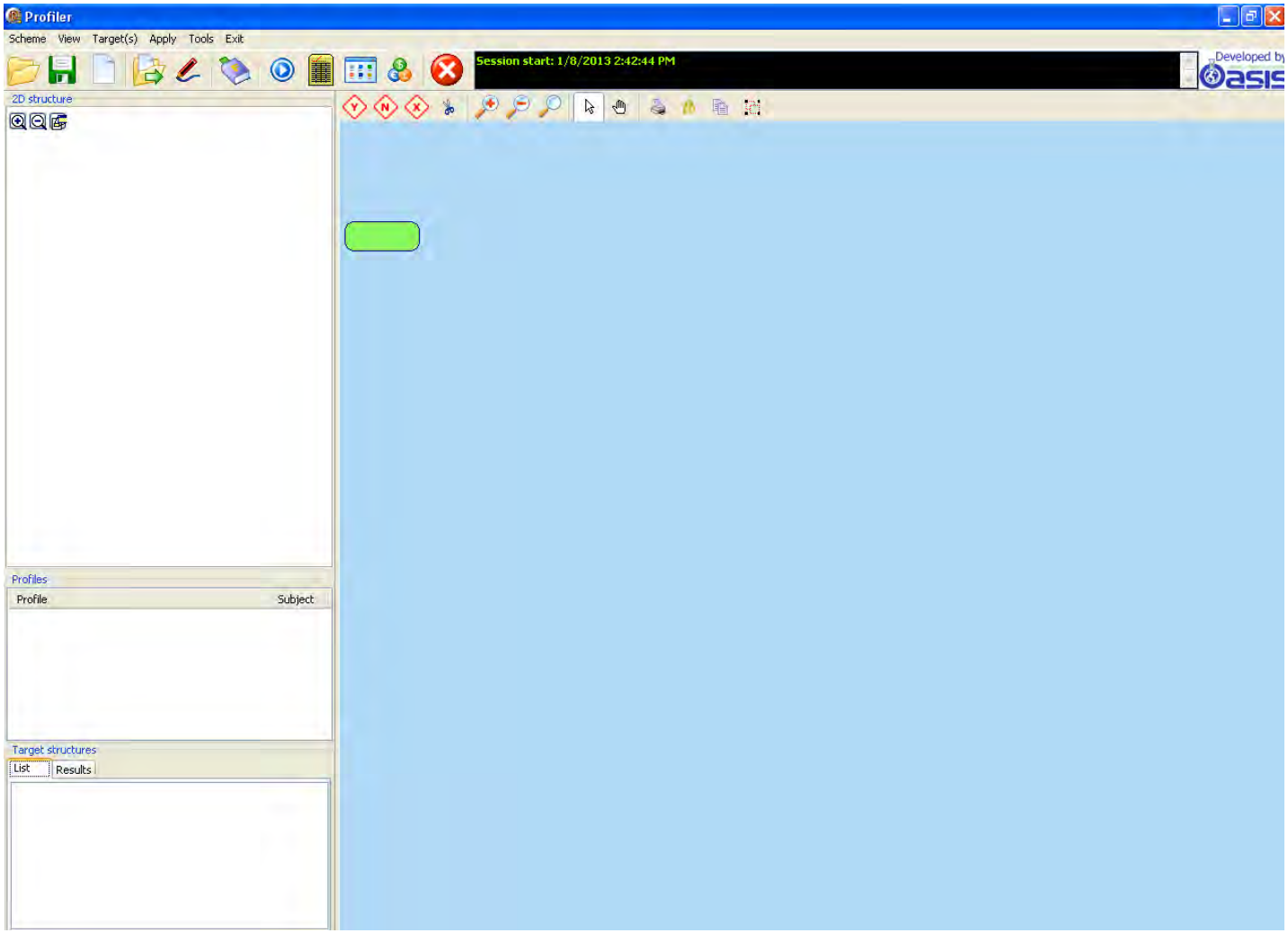


Automation of the ER Expert System

Built through collaborative effort between EPA, OECD and LMC

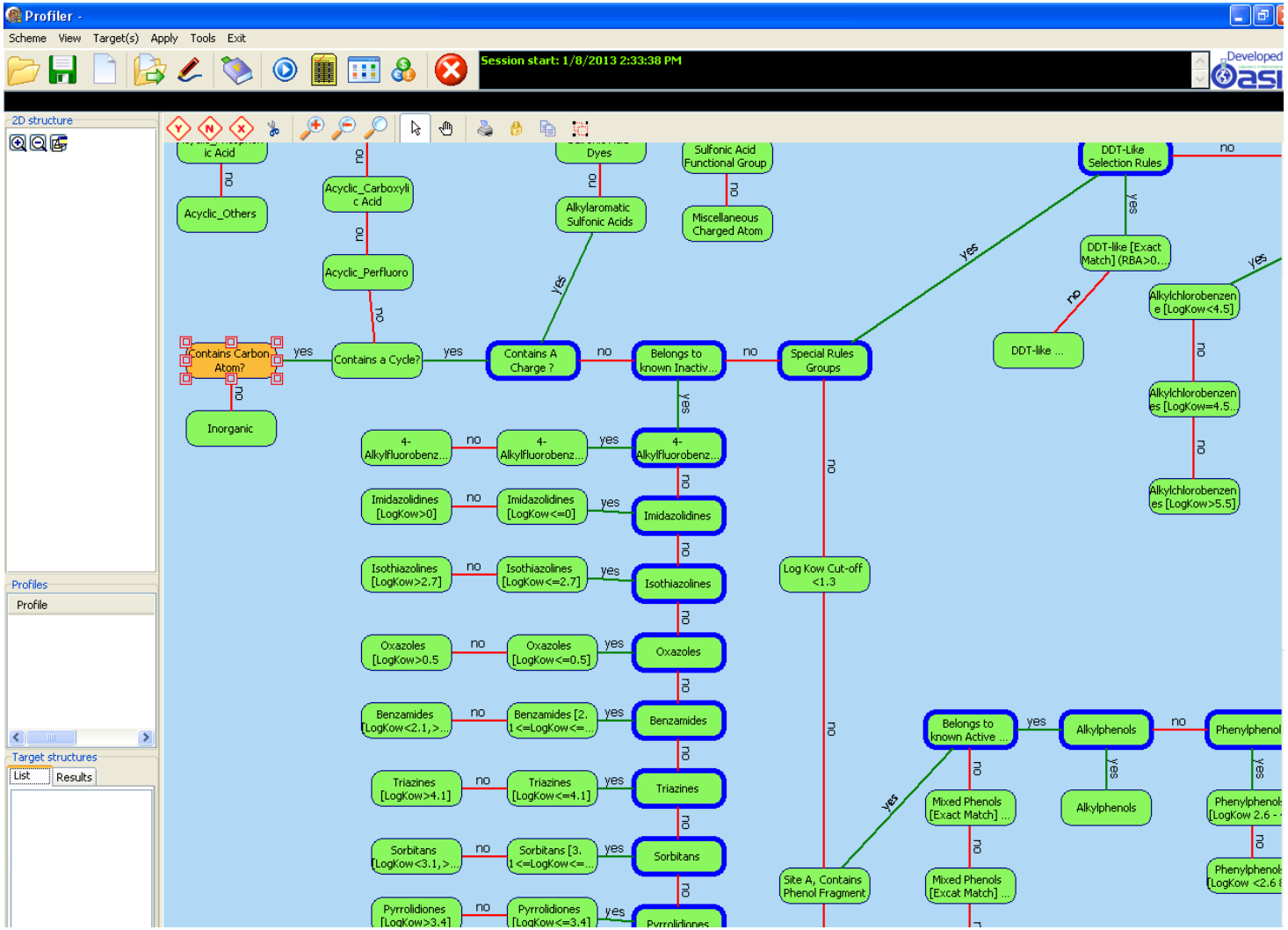
http://www.qsartoolbox.org/



Automation of the ER Expert System

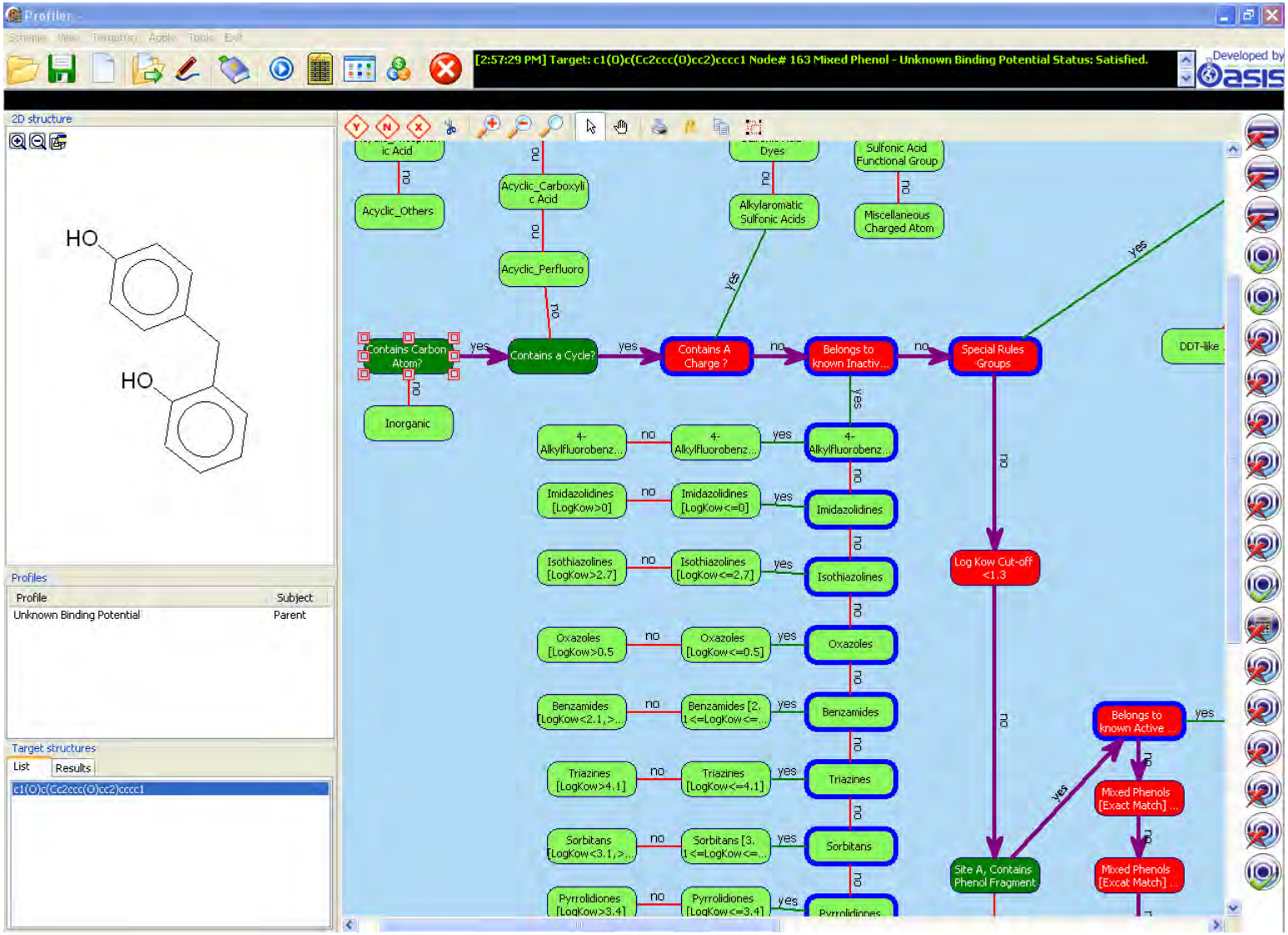
- Decision Tree

Yes/No decision-based dendroid logic scheme



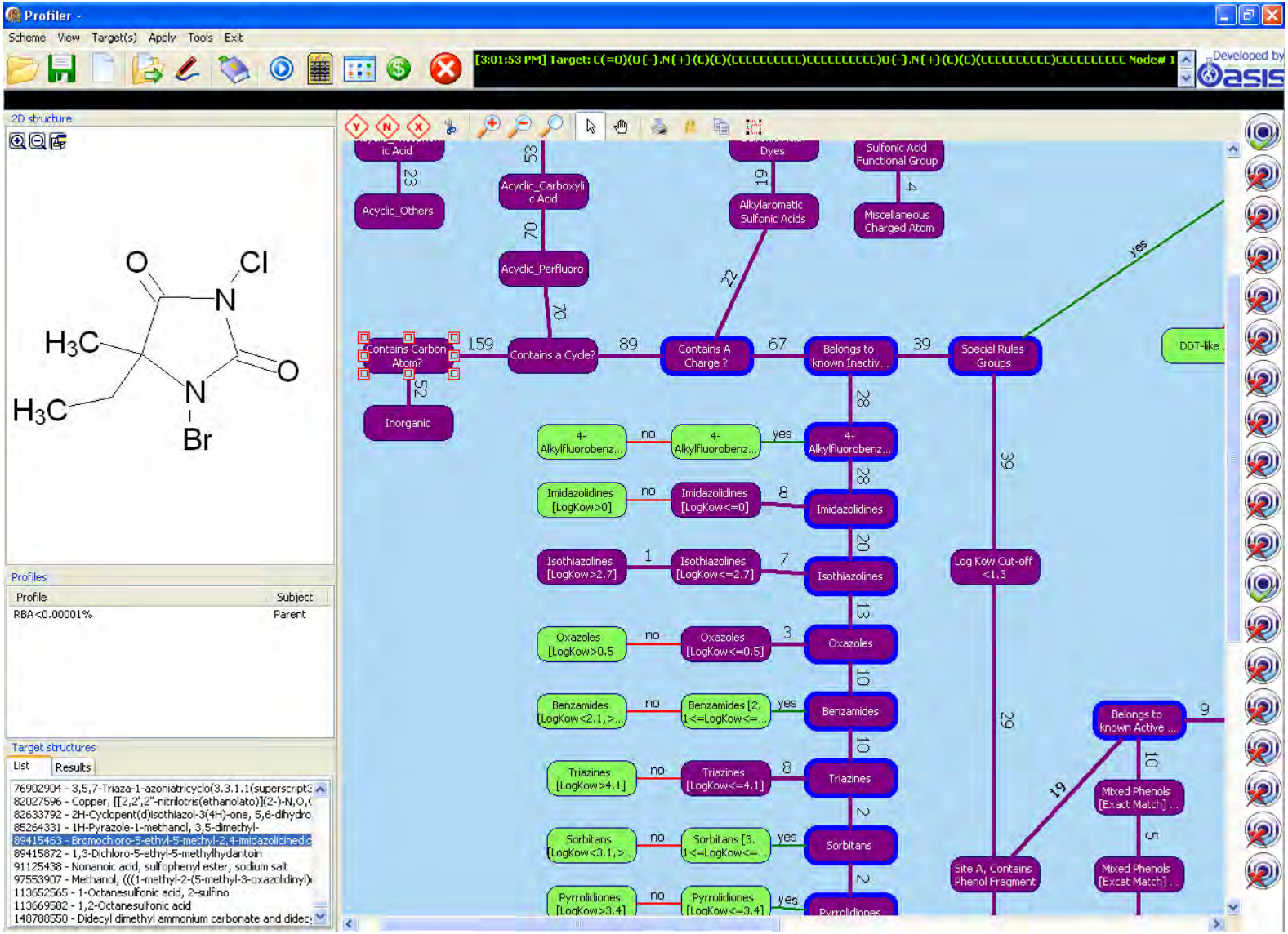
Automation of the ER Expert System

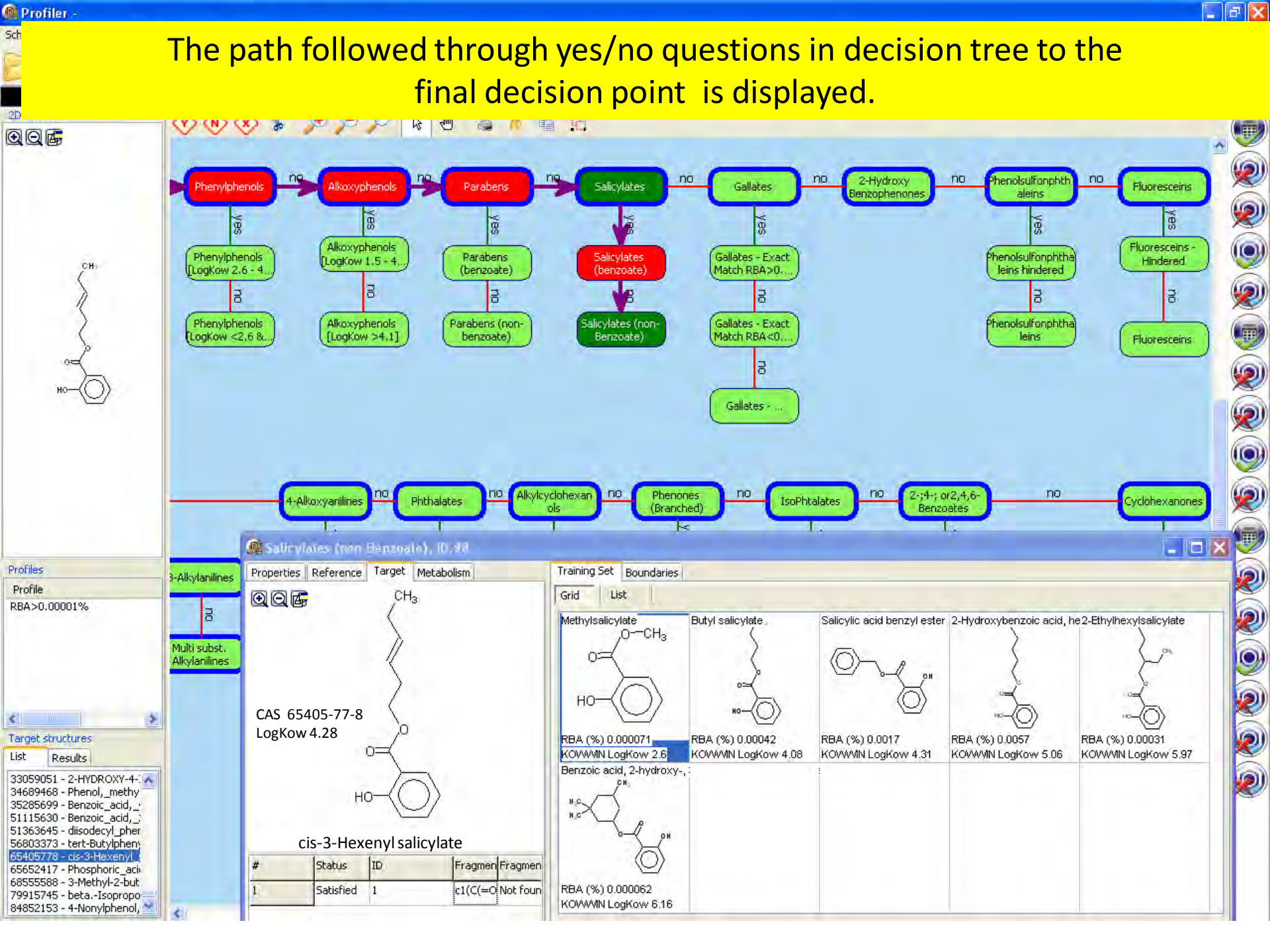
- Single chemical profiling….



Automation of the ER Expert System

- Or batch profiling, e.g., inventory list




Profiler -
The path followed through yes/no questions in decision tree to the final decision point is displayed.
Phenylphenols
Alkoxyphenols
Parabens
Salicylates
Gallates
2-Hydroxy Benzophenones
Phenolsulfonphthaleins
Fluoresceins
4-Alkoxyanilines
Phthalates
Alkylcyclohexanols
Phenones (Branched)
IsoPhtalates
2-;4-; or2,4,6-Benzoates
Cyclohexanones
Salicylates (non Benzoate), ID 98
Properties
Reference
Target
Metabolism
Training Set
Boundaries
Grid
List
CAS 65405-77-8
LogKow 4.28
cis-3-Hexenyl salicylate
Methylsalicylate
Butyl salicylate
Salicylic acid benzyl ester
2-Hydroxybenzoic acid, he
2-Ethylhexylsalicylate
Benzoic acid, 2-hydroxy-,
Profiles
Profile
RBA>0.00001%
Target structures
List
Results

4-Alkylanilines 1.3<LogKow<5.1, ID:86

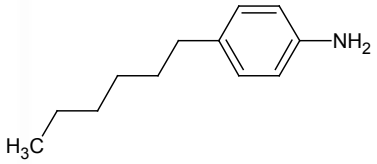
Properties | Reference | Target

Caption: 4-Alkylanilines 1.3<LogKow<5.1

YES Category Name: RBA>0.00001%

NO Category Name: Label to assign to the target if it doesn't meet the criteria

Description

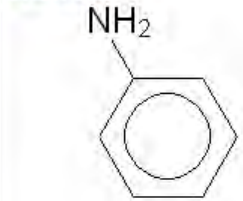


LogKow = 4.06 est
CAS#

YES Example | NO Example

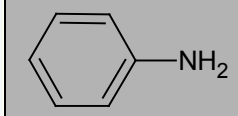
Boundaries | Training Set

Chemical name	SMILES	RBA (%)	LogKow	LogKow ...	Acronym	CAS No.
Aniline	c1(N)ccccc1	<0.000001	0.90	msrd	ANL	62533
4-Methylaniline, p-Toluidine	c1(N)ccc(C)cc1	0.000007	1.39	msrd	PT	106490
4-Ethylaniline	c1(N)ccc(CC)cc1	0.000047	1.96	msrd	EA	589162
4-Propylaniline	c1(N)ccc(CCC)cc1	0.00011	2.40	msrd	PA	2696846
4-tert-butylaniline	C(C)(C)(C)c1ccc(N)cc1	0.000098	2.70	msrd	TBA	769926
4-n-butylaniline	c1(N)ccc(CCCC)cc1	0.00040	3.05	msrd	BA	104132
4-n-amylaniline	c1(N)ccc(CCCCC)cc1	0.0011	3.39	msrd	AAN	33228443
4-hexylaniline	c1(N)ccc(CCCCCC)cc1	0.0015	4.06	ClogP	HAL	33228454
4-n-octylaniline	c1(N)ccc(CCCCCCCC)cc1	0.00089	5.12	ClogP	OA	16245797



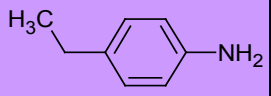
Details

Training Set for: Aniline (RBA<0.00001%), and 4,n-alkylanilines 1.3<LogKow<5.1

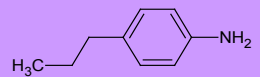


LogKow = 0.90 m
CAS# 62533
RBA<0.000001%

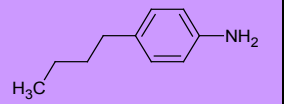
LogKow = 1.39 m
CAS# 106-49-0
RBA = 0.000007



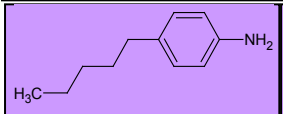
LogKow = 1.96 m
CAS# 589-16-2
RBA = 0.000047



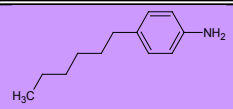
LogKow = 2.40 m
CAS# 2696-84-6
RBA = 0.00011



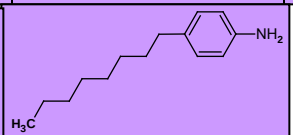
LogKow = 3.05 m
CAS# 104-13-2
RBA = 0.00040



LogKow=3.39 m
CAS# 33228443
RBA = 0.0011

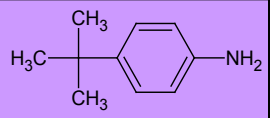


Log Kow = 4.06 est
CAS# 33228454
RBA=0.0015

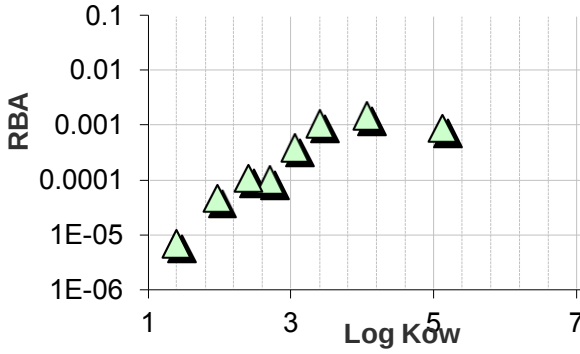


LogKow = 5.12 est
CAS# 16245-79-7
RBA = 0.00089

Training Set for related group: 4,t-alkylanilines:



LogKow = 2.70 m
CAS# 769926
RBA=0.000098

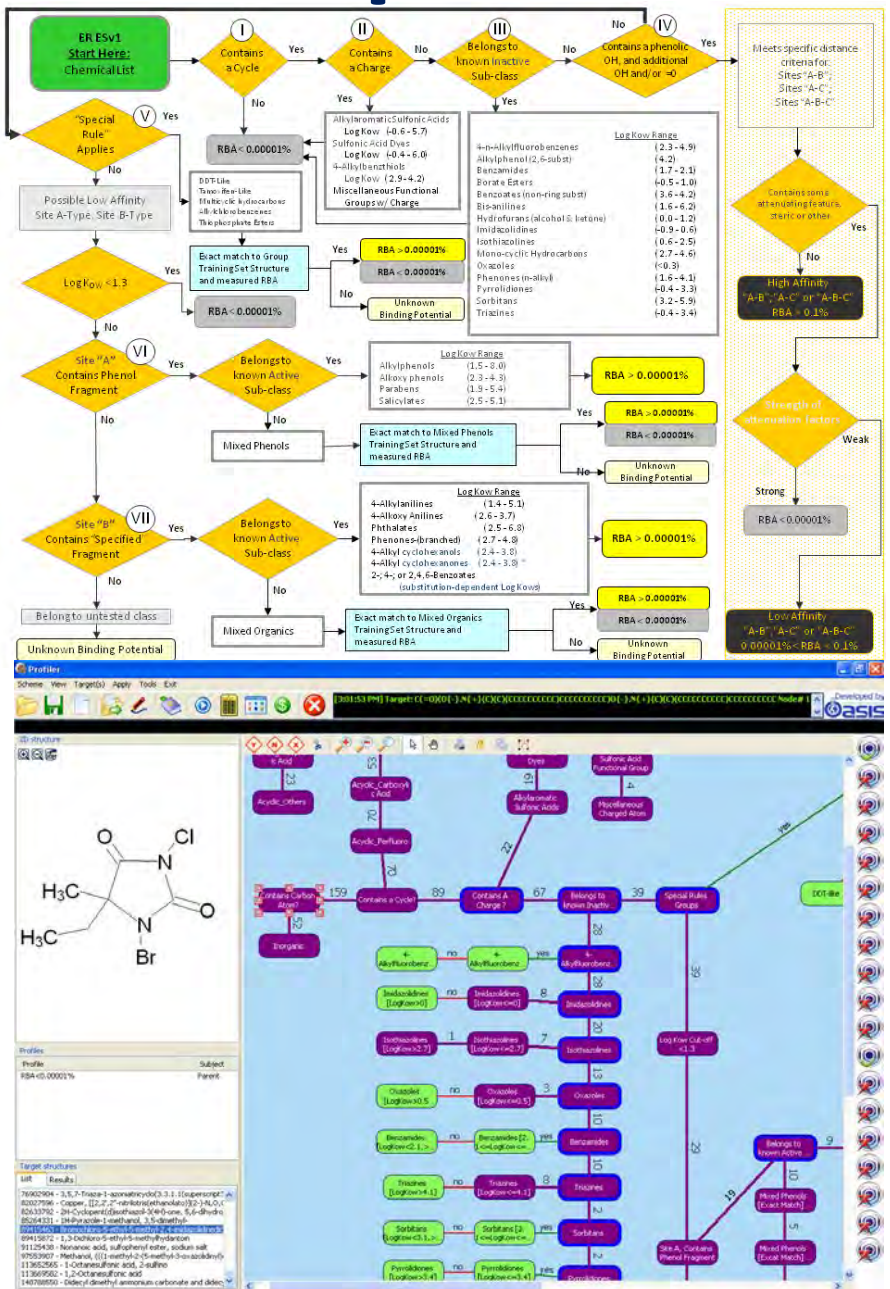


Notes on Interpretation: Aniline and a series of 4,n-alkylanilines were tested for rainbow trout ER affinity and gene activation. Aniline (LogKow=0.90) did not bind while 4,n-methyl-, ethyl-, propyl-, butyl-, pentyl-, and octylaniline all bound rtER. Additionally, 4,t-butylaniline also bound rtER. All 8 alkylanilines also induced VTG mRNA in trout liver slices to varying extents, with at least one concentration significantly different from controls. Alkylanilines of LogKow 3 to 4 yielded the most potent VTG induction. Therefore, 4-alkylanilines of LogKow between 1.3 and 5.2 are predicted to bind ER, with a good degree of confidence; 4,t-substituted alkylanilines in this LogKow range are also likely to bind.

Alkylanilines of Log Kow greater than that of p,n-octylaniline (5.12), or with ortho- or meta-alkylsubstituents are outside of the domain of the training set.

Expert System Development

- 2009 SAP review
  - Model Domain - **ER ESv1**
  - Regulatory Domains
    - Coverage: Food-use Inerts (FI) and Antimicrobials (~95% NON-Binders; ~5% prioritized Binders)
- 2013 SAP review:
  - Automated **ER ESv1**
  - Expanding Domain Coverage with *in vitro* testing; build effects-based chemical categories:
    - Non Food-use inerts (NFI)
  - Evaluate ES Coverage of EDSP Universe = 71%; (~5% prioritized Binders)
- Additional Work:
  - Complete **ER ESv2**
  - Expand Domain (*in vitro* testing) to cover remaining EDSP Universe; build **ER ESv3**



Thank you!

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U. S. Environmental Protection Agency