Skin Sensitisation Examples

Introduction to OECD QSAR Toolbox

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Purpose of ECHA examples:

- To address specifically the REACH registrants
- To try to translate the science into regulatory language
- To increase the transparency of a complex tool
- To re-iterate the Toolbox philosophy for a prediction
- To illustrate the Toolbox flexibility
- To facilitate the development of adaptations
- To promote the use of alternatives for REACH

Outline of this presentation

On prediction of skin sensitisation

1) A straight-forward example

2) Example with activation:

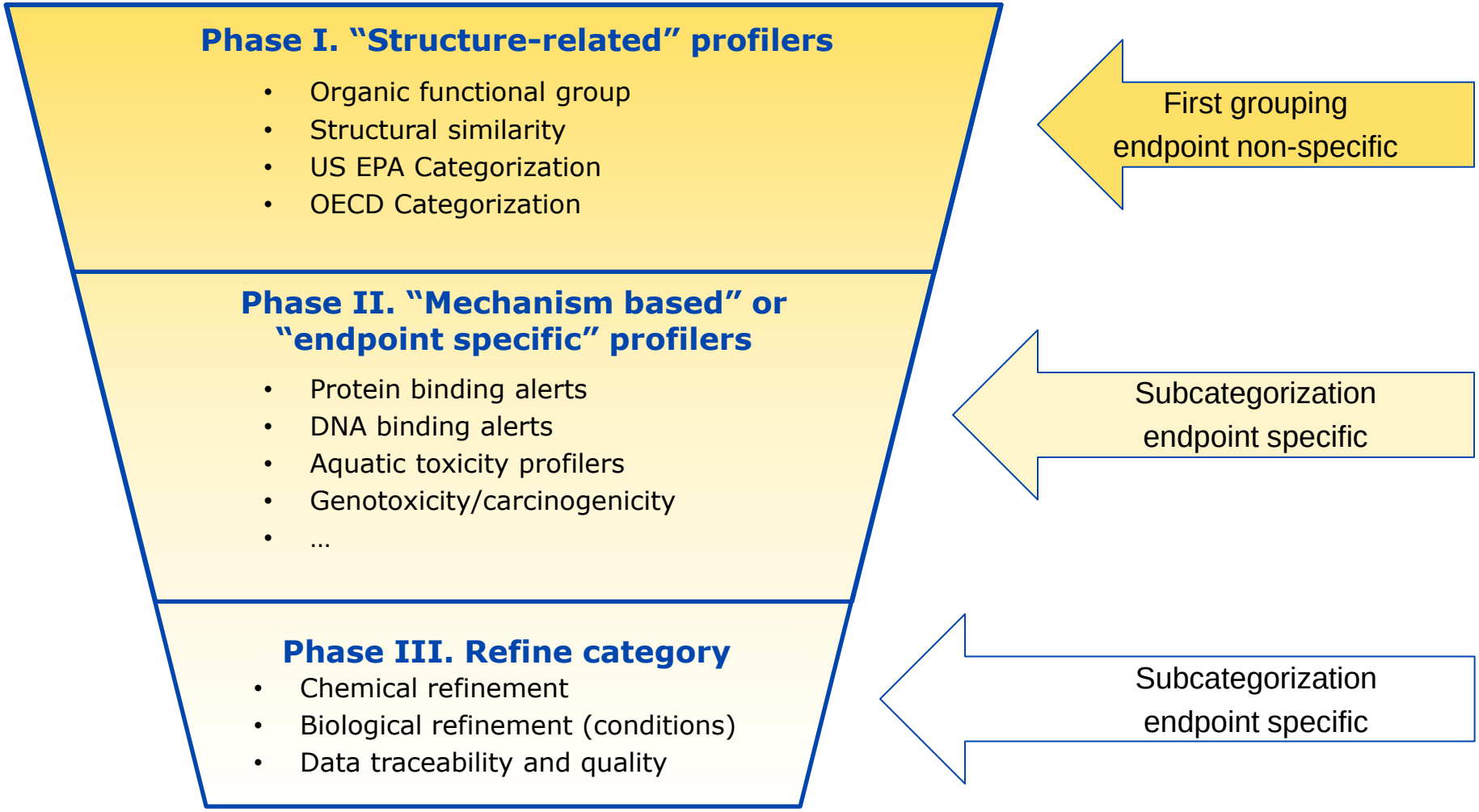
transformations including skin metabolism and auto-oxidation

Objectives of this presentation:

To demonstrate the following:

- Input and profiling the target chemical
- Identifying analogues of the target chemical
- Filling data gaps for target chemical by read-across
- Profiling target chemical taking into account its (a)biotic activation (by simulating skin metabolism and auto-oxidation products)
- Collect mechanistic analogues depending on the products
- Filling data gaps by read across when (a)biotic activation is taken into account (final structural refinement)

Recommended Category formation process



The skin sensitisation endpoint

- In Annex VII of REACH (for more than 1 tpa)
- The information requirement can be adapted:
  - According to column 2 of the Annex
  - According to Annex XI
- The Murine Local Lymph Node Assay (LLNA) is recommended
- Guinea Pig Maximisation Test (GPMT) is still sometimes used
- No requirement for testing proposal in Annex VII, BUT
- New animal studies to be conducted only as a last resort

Relevant databases and profilers

Relevant databases:

- "Skin sensitisation", which includes more than 1 035 chemicals (includes the OASIS skin sensitisation database)
- "Skin sensitisation ECETOC", with 39 chemicals
- ECHA Chem currently brings more than 1 000 studies to the Toolbox

For classification purposes, the thresholds in the **CLP** Regulation and the respective guidance should be checked.

Relevant profilers:

- Protein binding by OASIS (101 categories)
- Protein binding by OECD (102 categories)
- Protein binding potency (90 categories)
- Protein binding alerts for skin sensitisation by OASIS (100 categories)

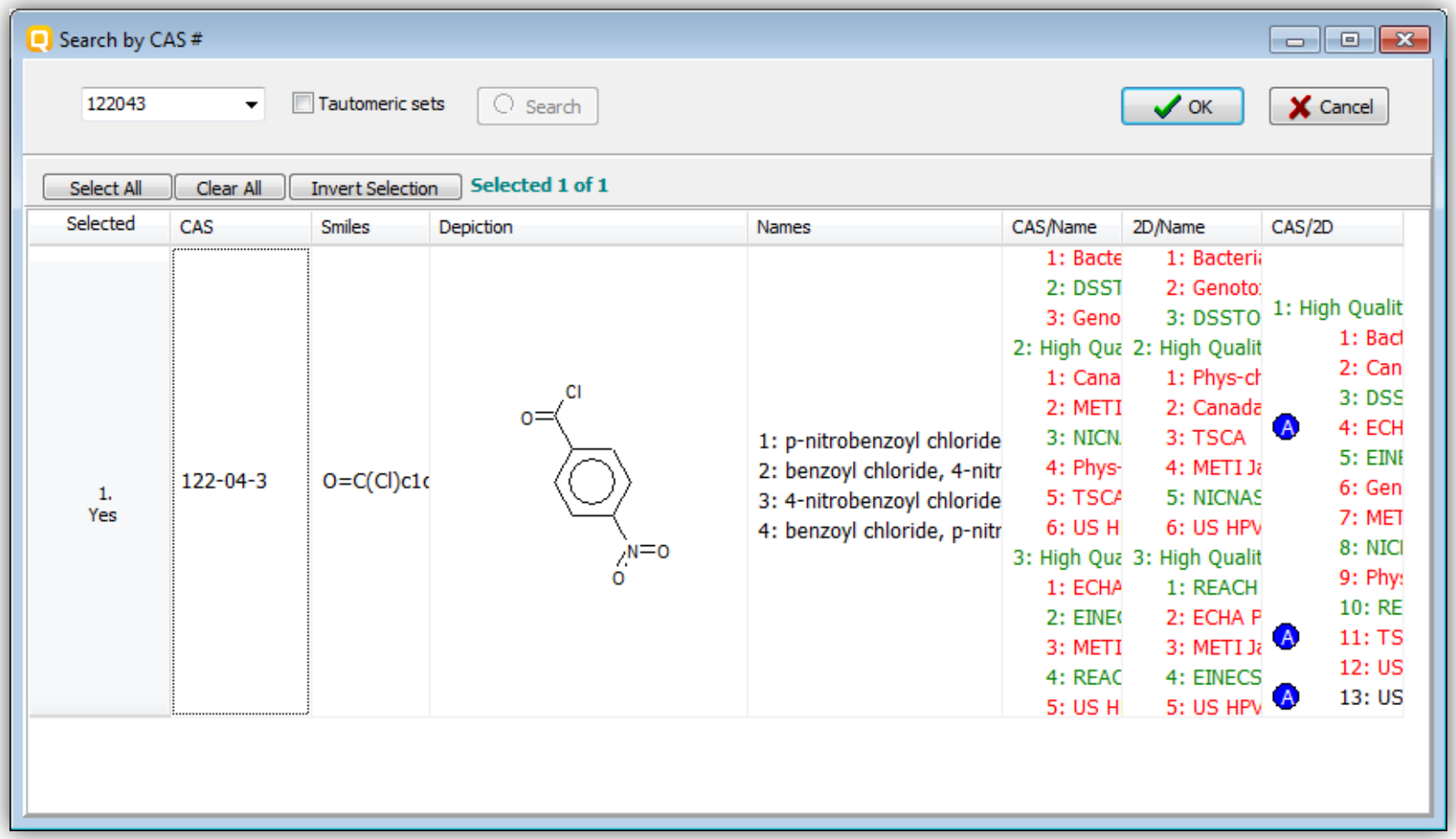
A straight-forward example

Step-by-step example on how to predict the skin sensitisation potential approach of a chemical by read-across based on an analogue approach (for beginners)

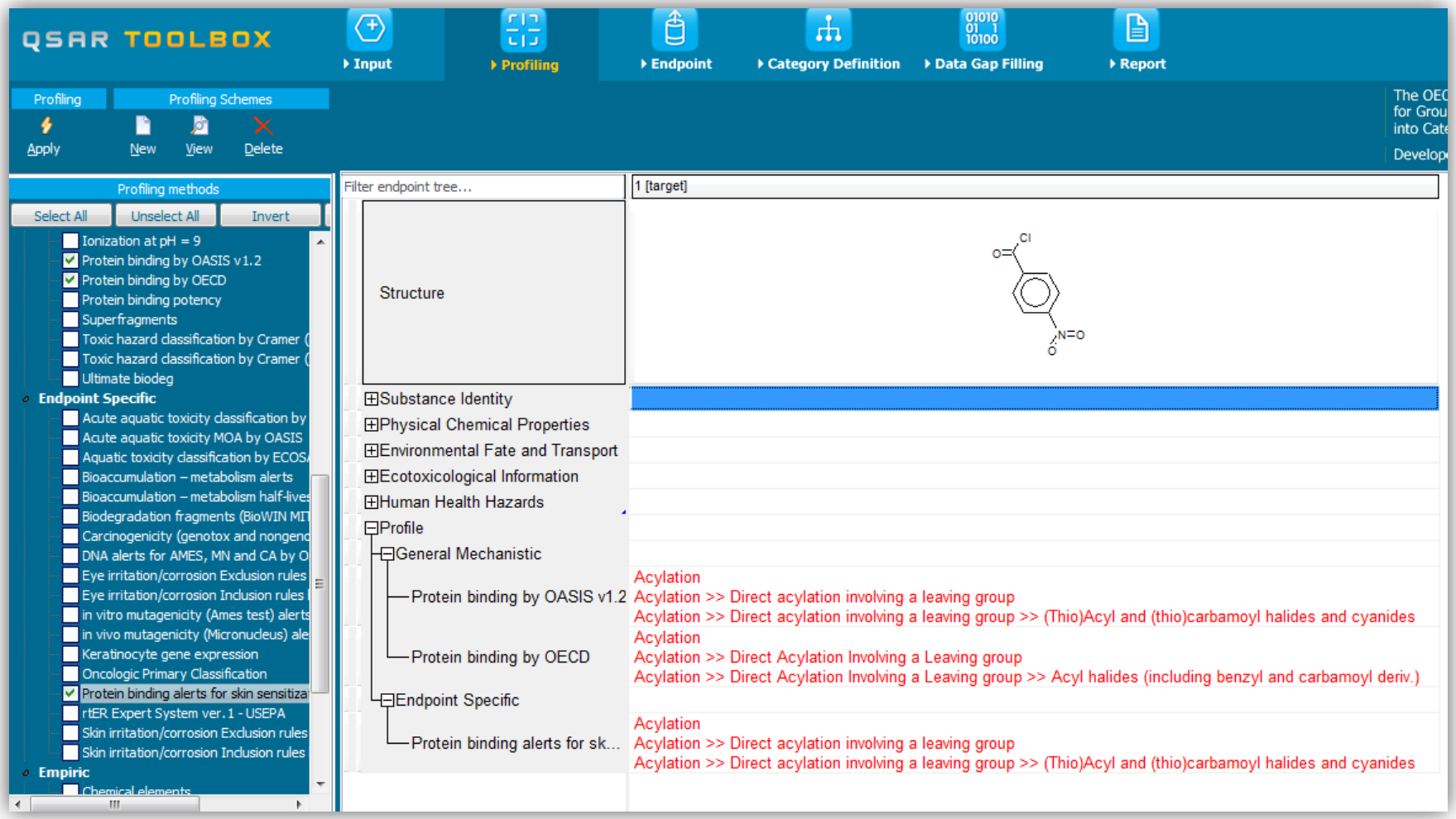
pdf

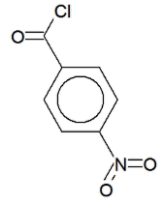
video

Input of chemical (CAS 122-04-3)



Profiling for protein binding





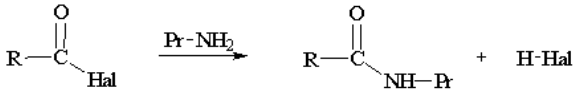
MoA Explanation: Acyl halides

Mechanistic Domain: Acylation
Mechanistic Alert: Direct acylation involving a leaving group

Structural Alert: Acyl halides

This category includes chemicals that potentially can cause skin sensitization effect as a result of protein conjugation via **Nucleophilic substitution on acyl halides.**

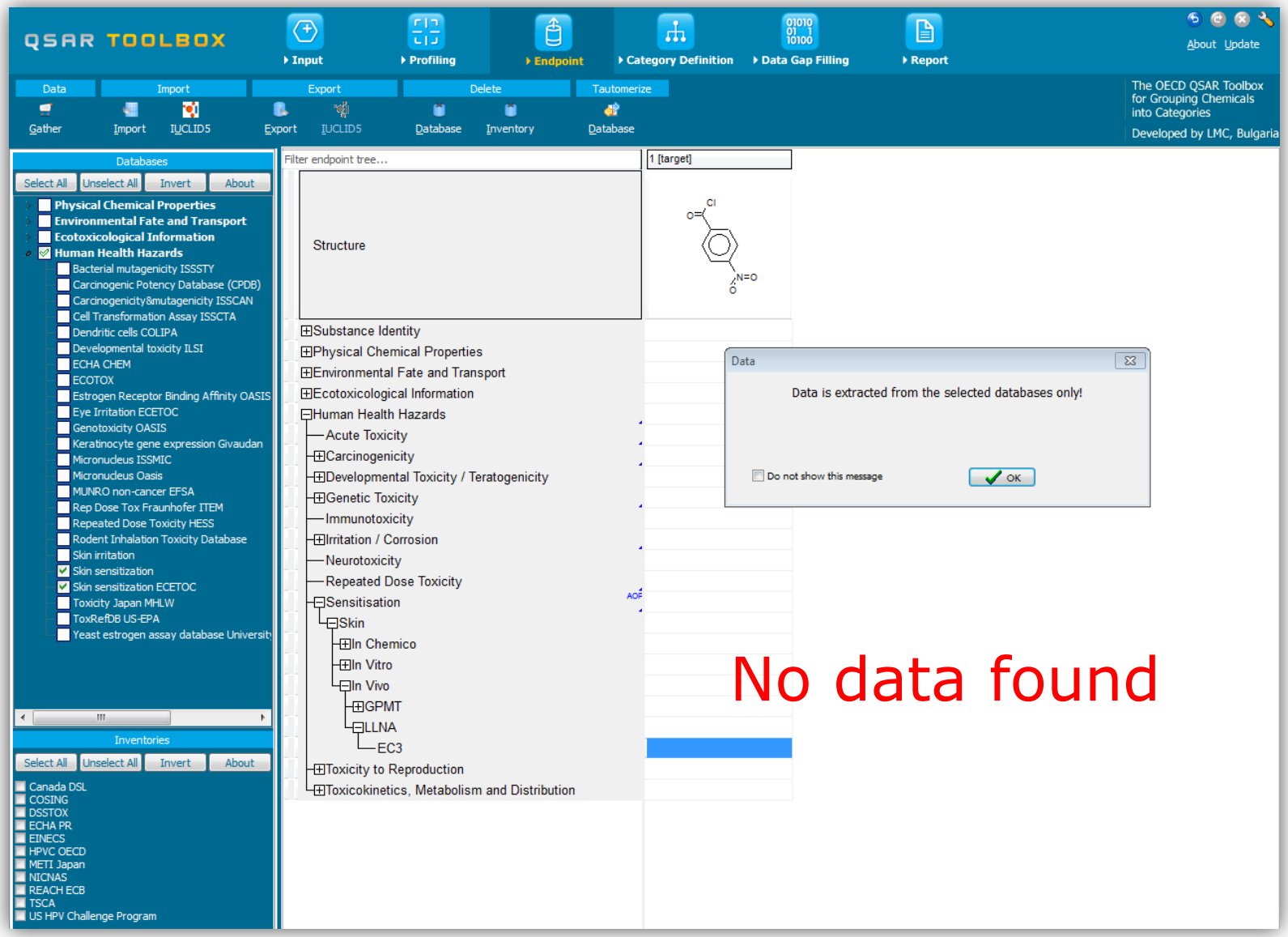
The possible structural alert acting by this mechanism is illustrated below:



Hal = F, Cl, Br, I
R = alkyl, aryl

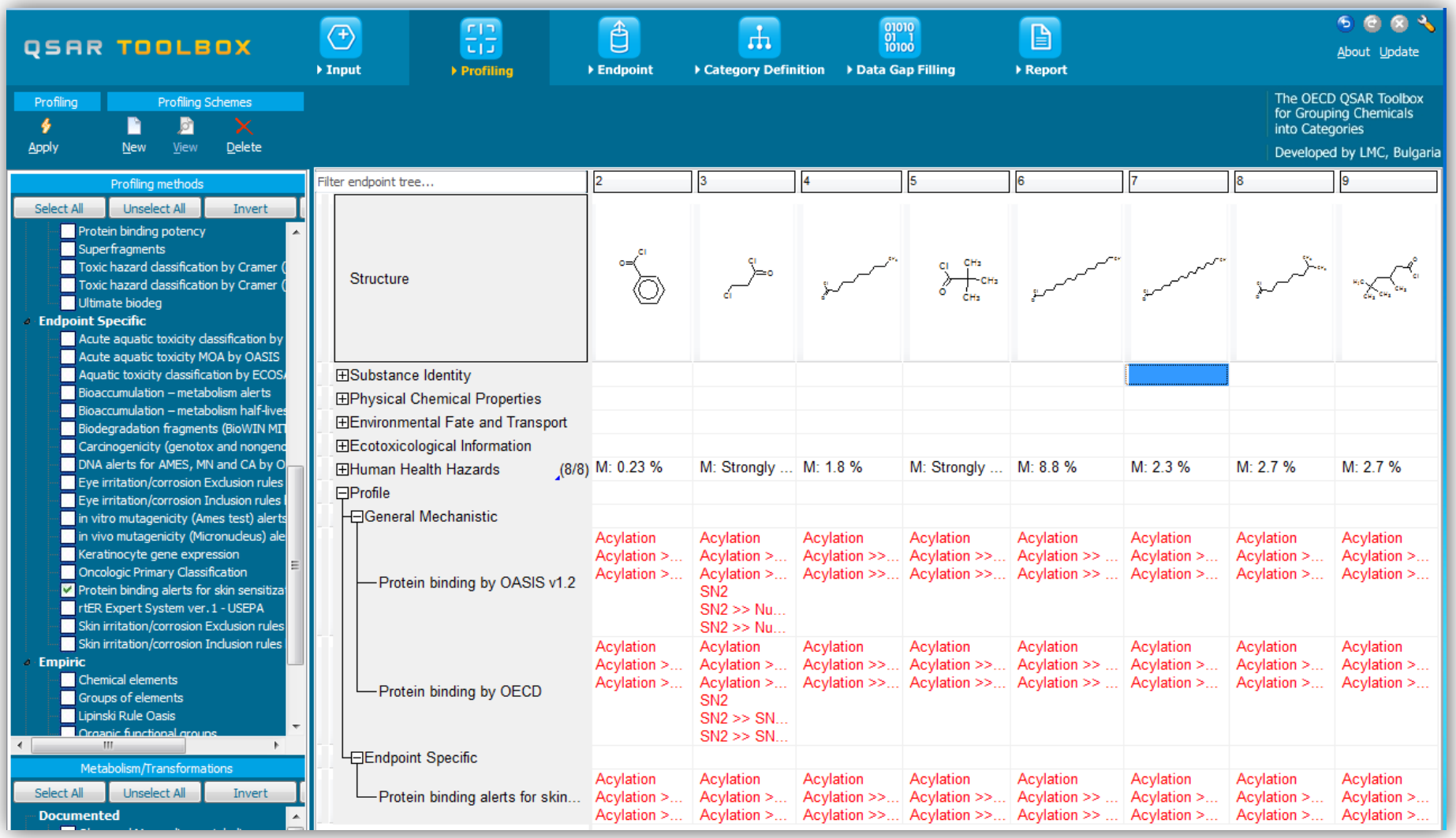
Acyl halides are compounds that have a halogen atom in place of OH group of acids. The nucleophile attacks the carbonyl carbon forming a tetrahedral intermediate. When the tetrahedral intermediate collapses, the weaker base is eliminated. If the nucleophile is neutral, the mechanism has an additional step. A proton is lost from the tetrahedral intermediate formed in the first step, resulting in a tetrahedral intermediate equivalent to the one formed by negatively charged nucleophiles. This tetrahedral intermediate expels the weaker of the two bases- the newly added group after it has lost a proton or the group that was attached to the acyl group in the reactant. Halogen ions are weaker bases than the amino groups in proteins.
These reactions are called nucleophilic acyl substitution reactions because a nucleophile (protein molecule) has replaced the substituent that was attached to the acyl group in the reactant. It is also called an acyl transfer reaction because an acyl group has been transferred from one group to another.

Data gathering

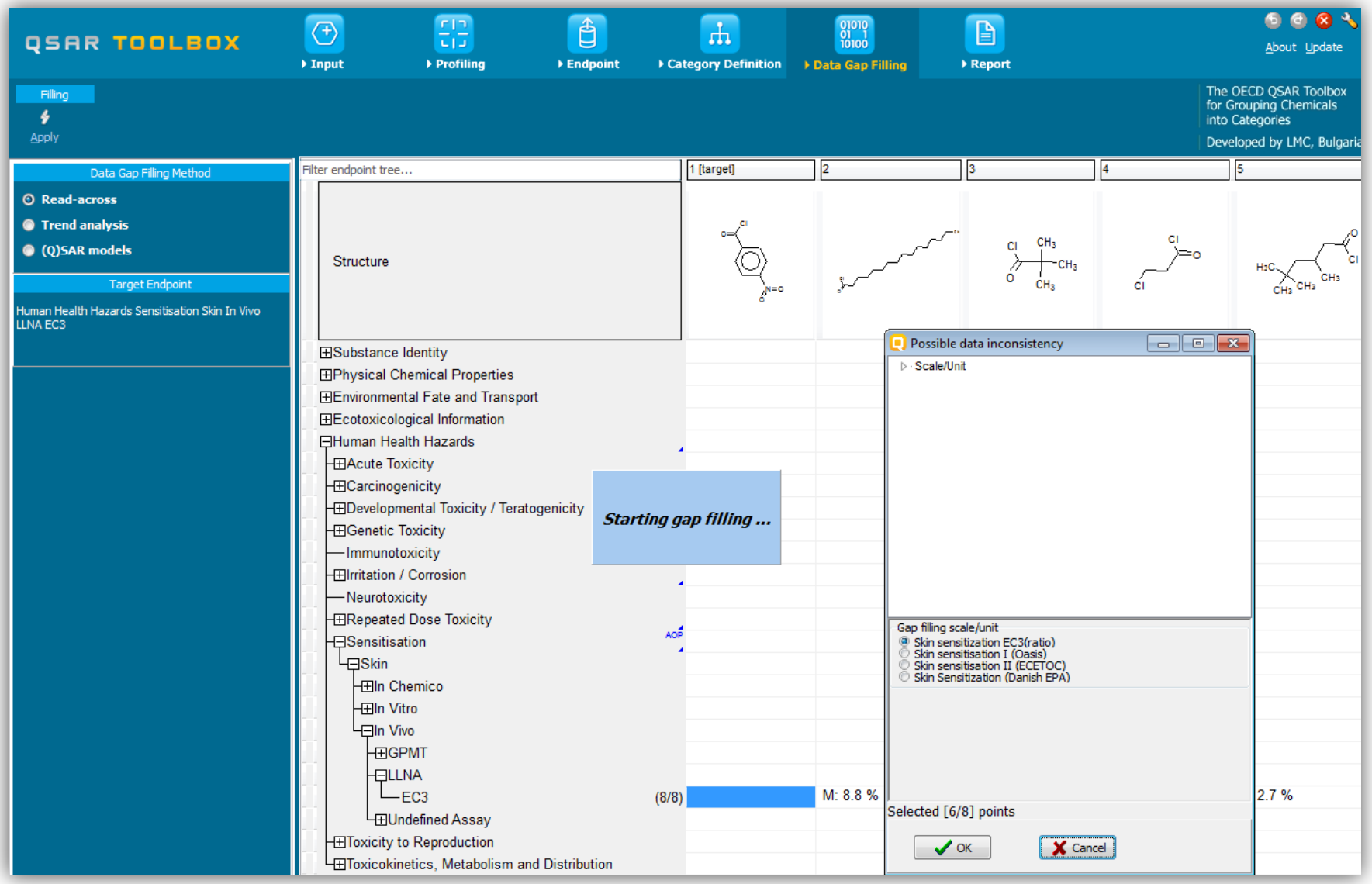


No data found

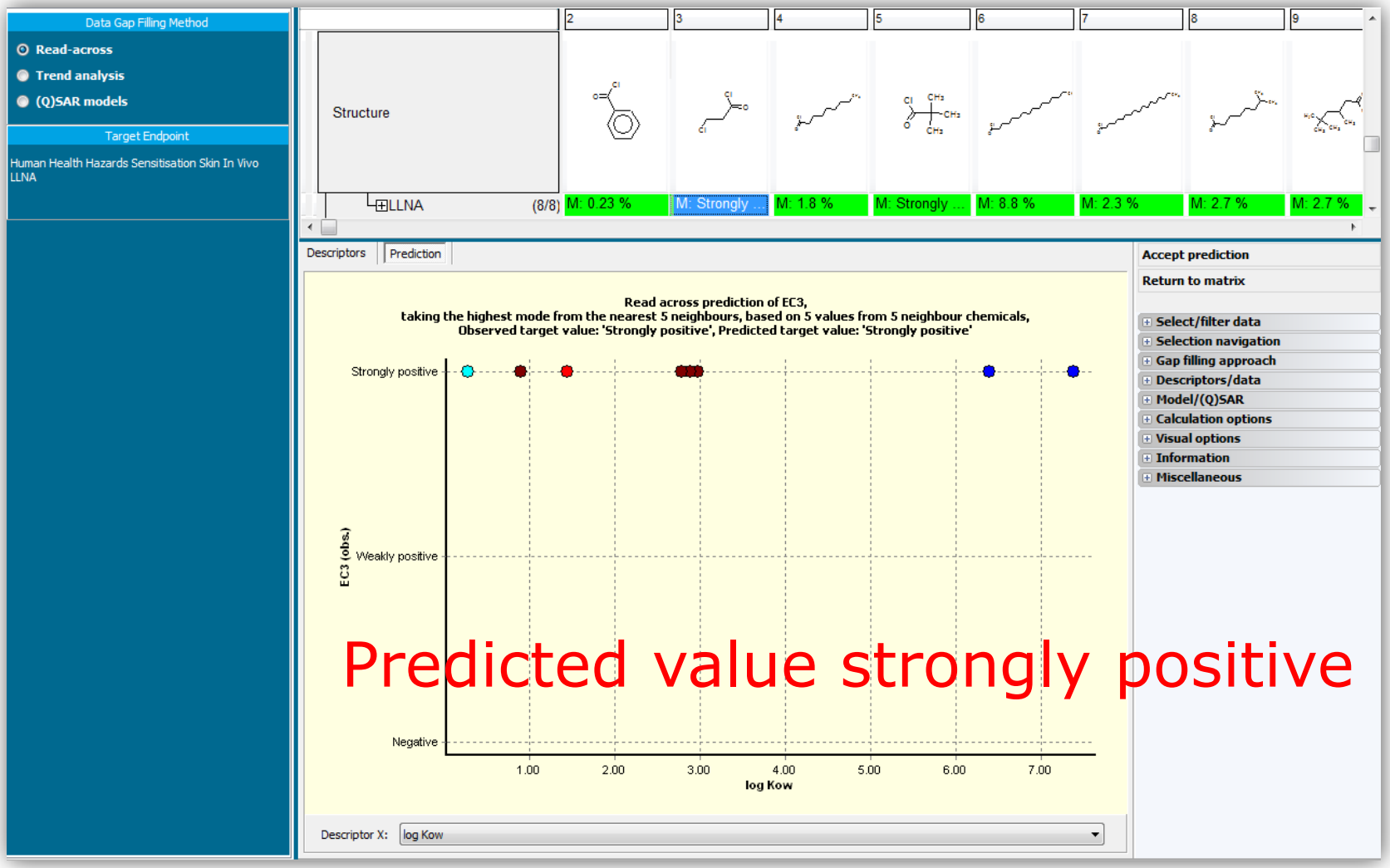
Structure based grouping and profiling of the analogues



Data gap filling



Structure based grouping (acyl halide) and prediction

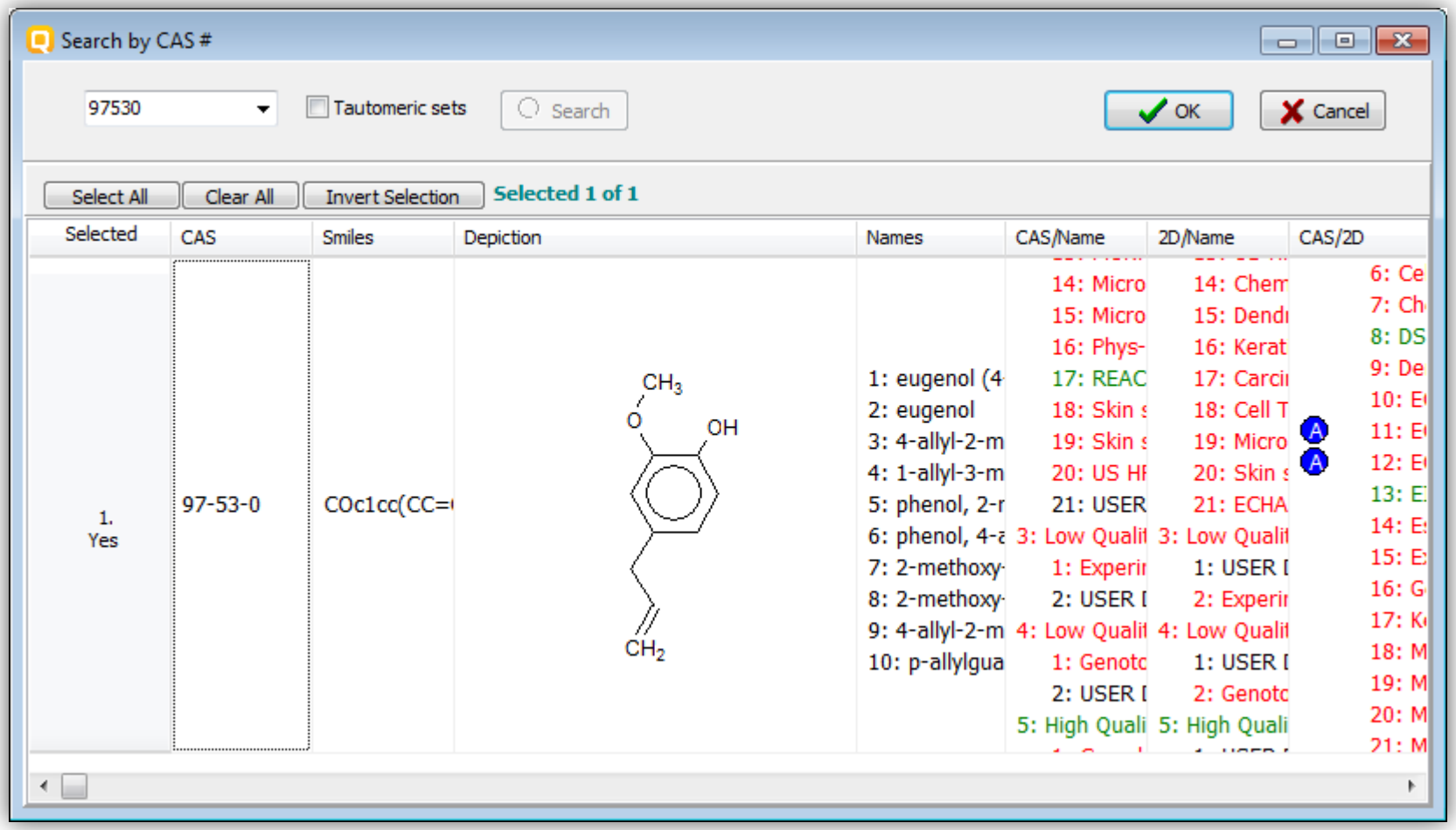


An example with (bio)activation

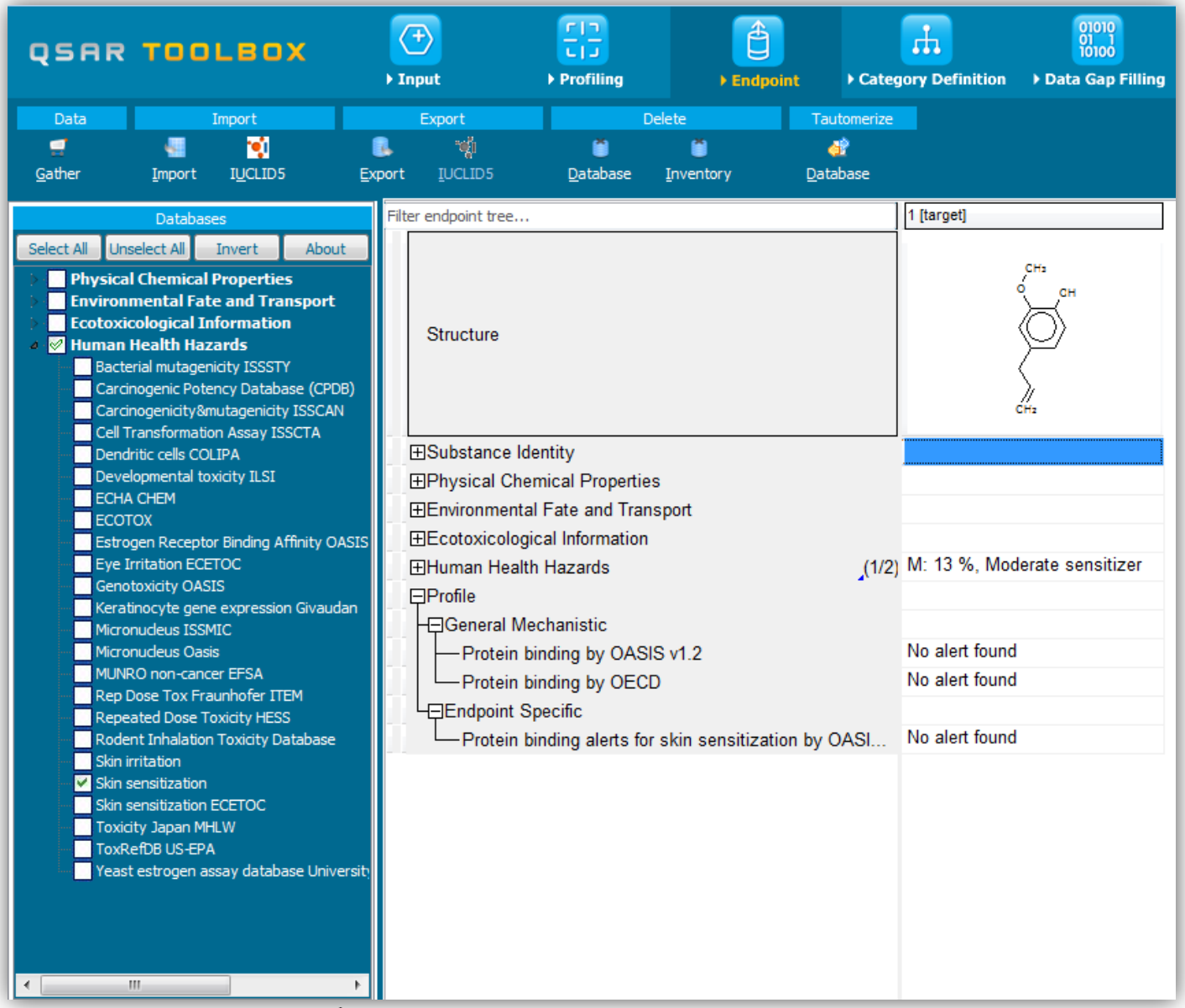
Step-by-step example for predicting skin sensitization accounting for skin metabolism

pdf

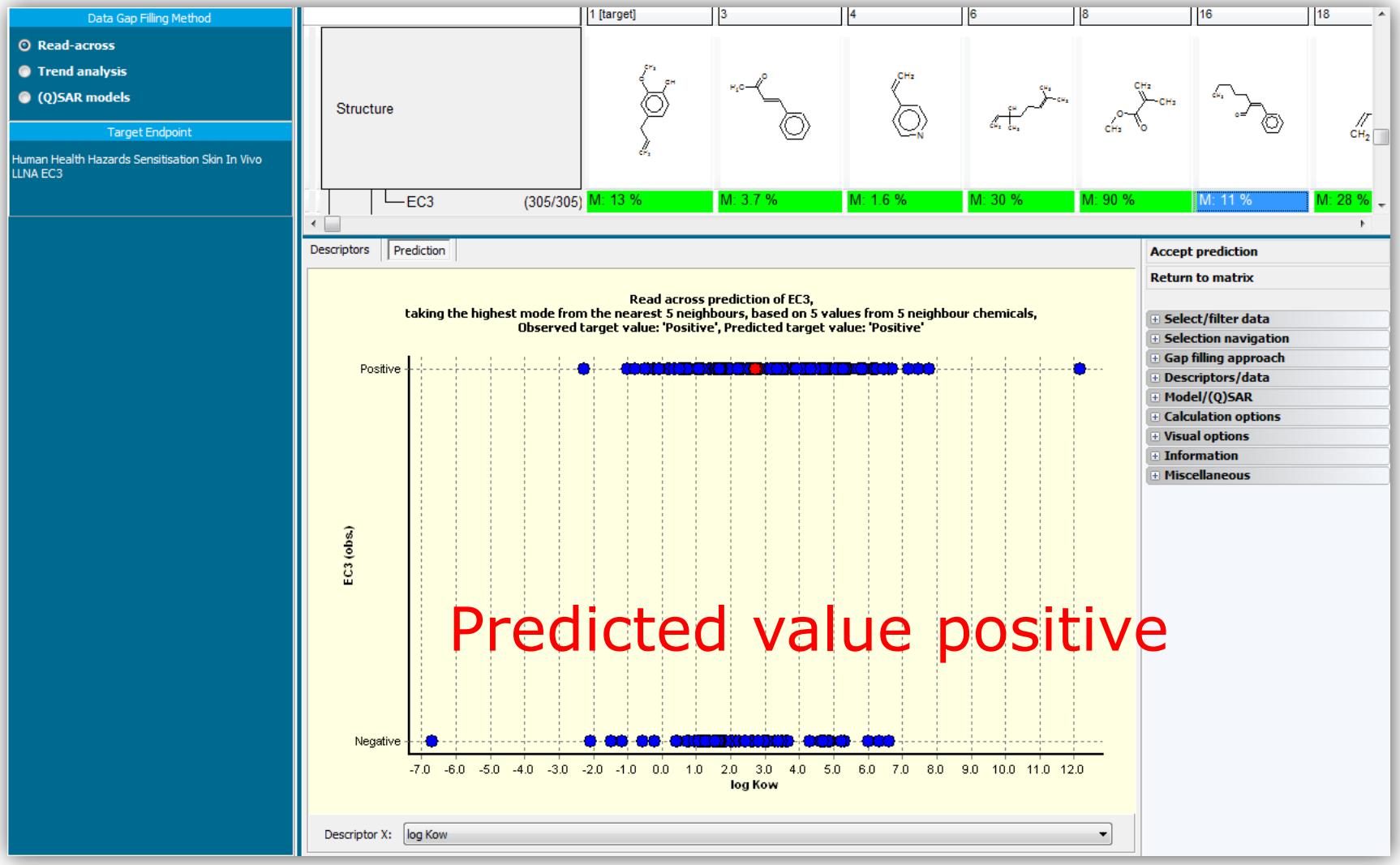
Input of chemical (CAS 97-53-0)



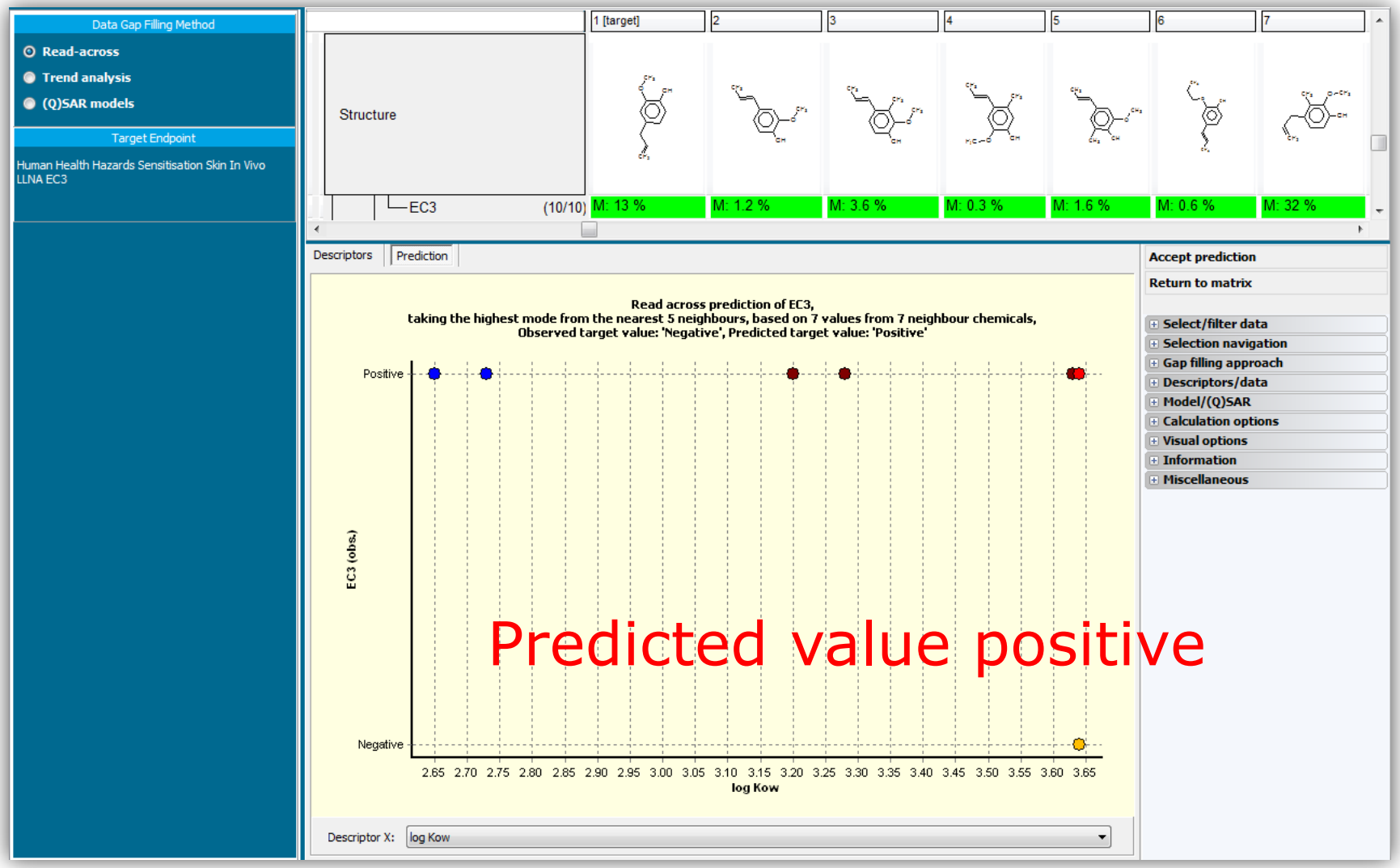
Profiling and data gathering



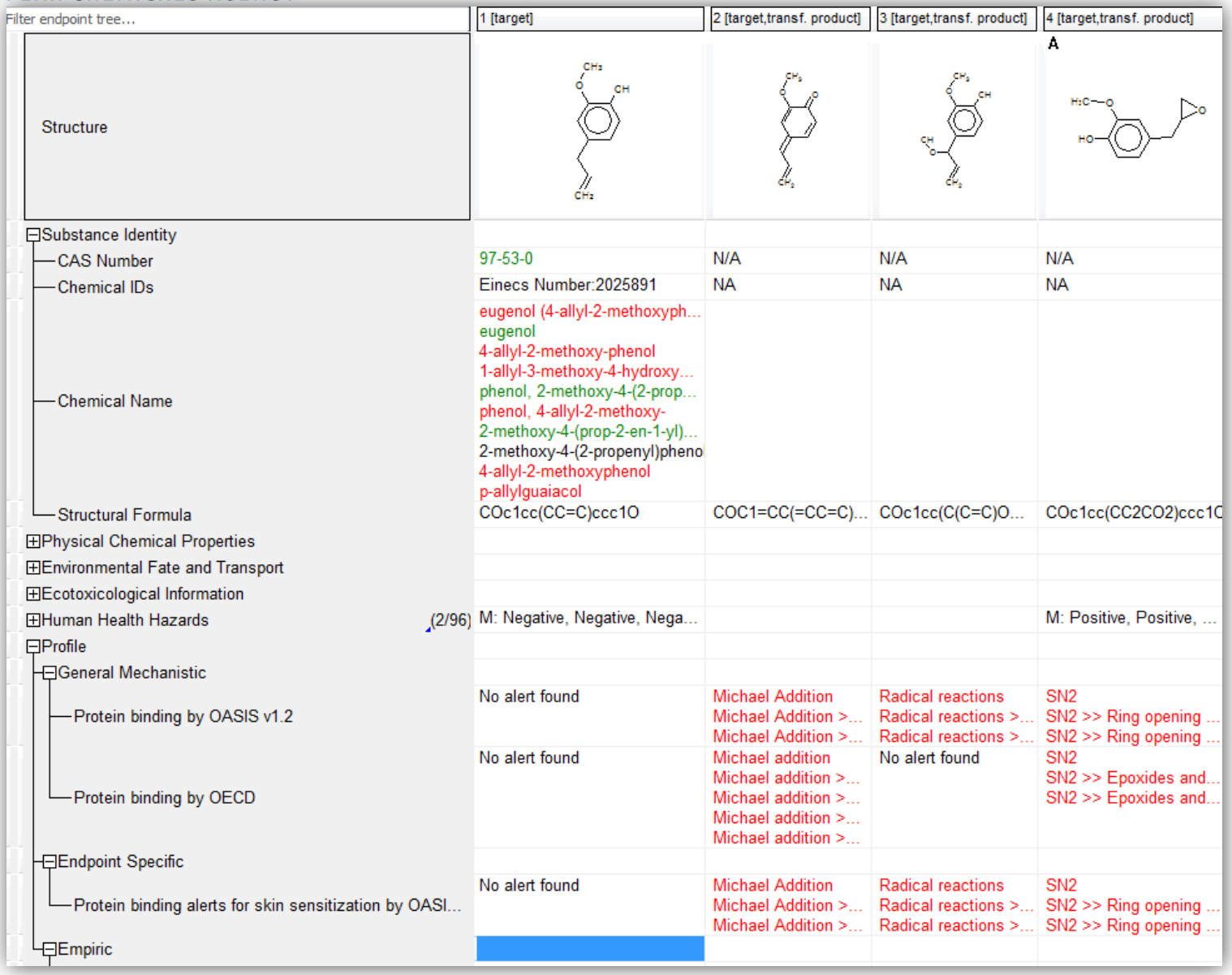
Grouping by organic functional groups (OFG)



Grouping by OFG (nested)

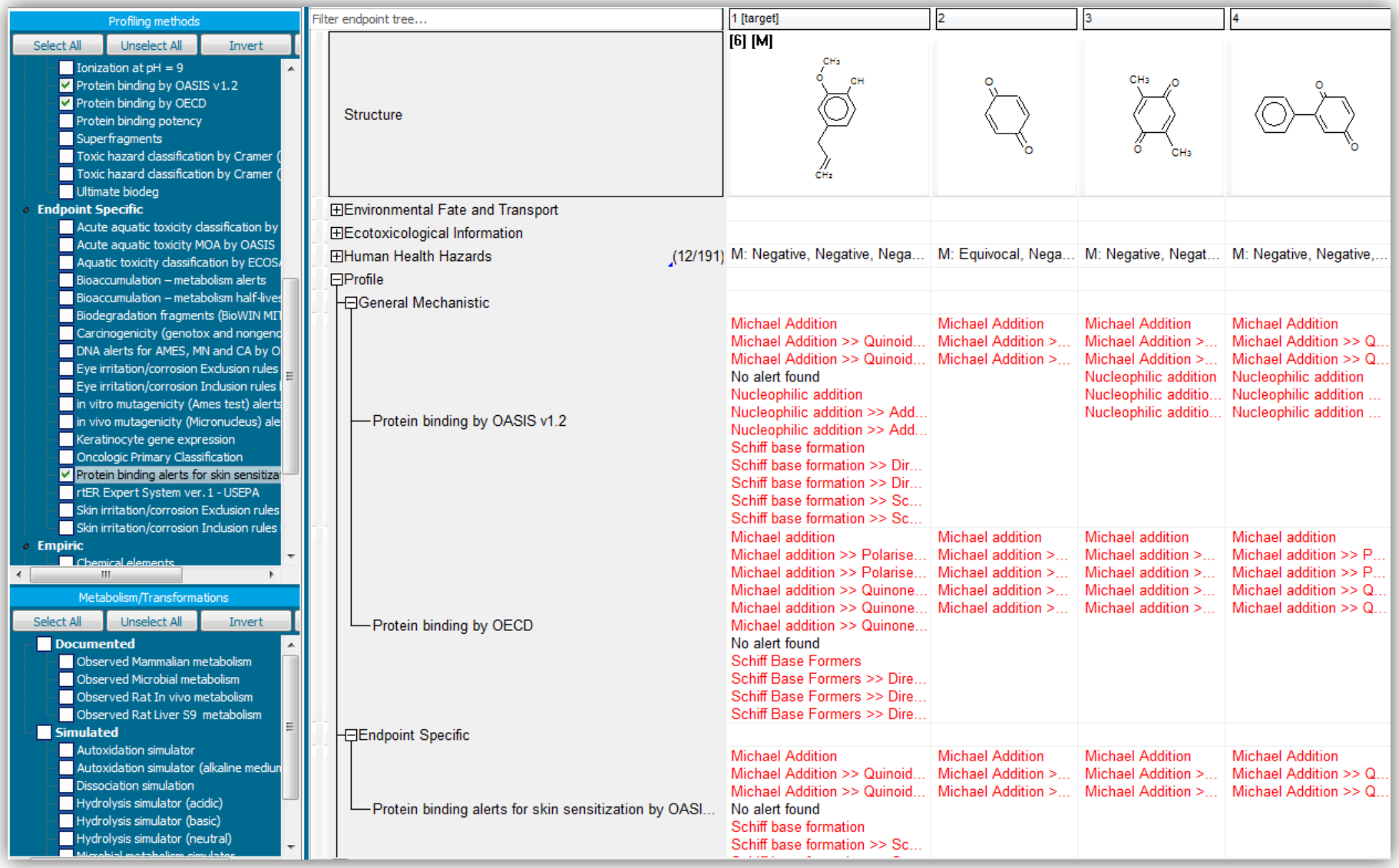


Auto-oxidation products

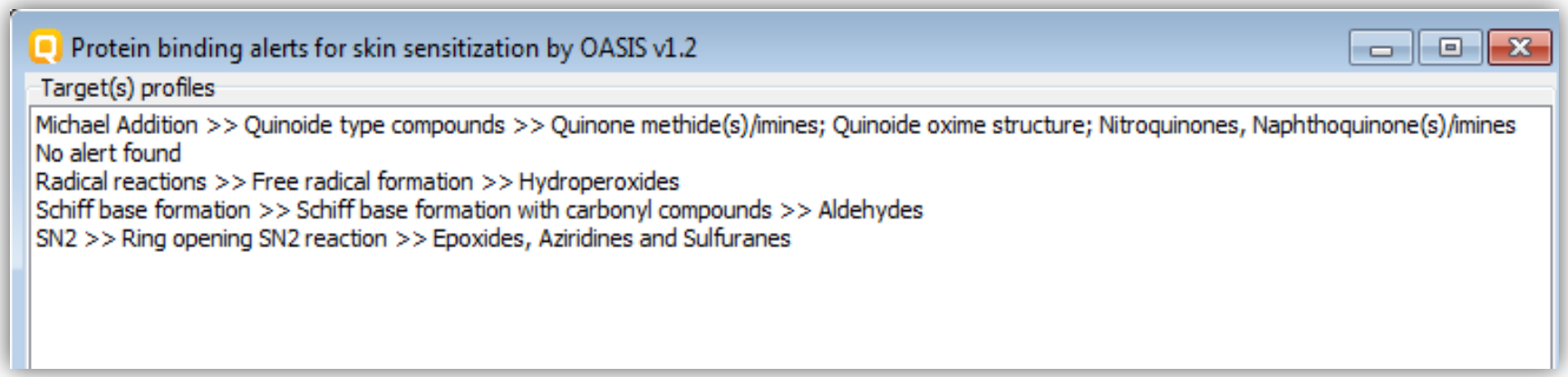


Filter endpoint tree...	1 [target]	2 [target,transf. product]	3 [target,transf. product]	4 [target,transf. product]
Structure				A
Substance Identity				
CAS Number	97-53-0	N/A	N/A	N/A
Chemical IDs	Einecs Number:2025891	NA	NA	NA
Chemical Name	eugenol (4-allyl-2-methoxyph... eugenol 4-allyl-2-methoxy-phenol 1-allyl-3-methoxy-4-hydroxy... phenol, 2-methoxy-4-(2-prop... phenol, 4-allyl-2-methoxy- 2-methoxy-4-(prop-2-en-1-yl)... 2-methoxy-4-(2-propenyl)pheno 4-allyl-2-methoxyphenol p-allylguaiacol			
Structural Formula	COc1cc(CC=C)ccc1O	COC1=CC(=CC=C)...	COc1cc(C(C=C)O...	COc1cc(CC2CO2)ccc1O
Physical Chemical Properties				
Environmental Fate and Transport				
Ecotoxicological Information				
Human Health Hazards (2/96)	M: Negative, Negative, Nega...			M: Positive, Positive, ...
Profile				
General Mechanistic				
Protein binding by OASIS v1.2	No alert found	Michael Addition Michael Addition >... Michael Addition >...	Radical reactions Radical reactions >... Radical reactions >...	SN2 SN2 >> Ring opening ... SN2 >> Ring opening ...
Protein binding by OECD	No alert found	Michael addition Michael addition >... Michael addition >... Michael addition >... Michael addition >...	No alert found	SN2 SN2 >> Epoxides and... SN2 >> Epoxides and...
Endpoint Specific				
Protein binding alerts for skin sensitization by OASI...	No alert found	Michael Addition Michael Addition >... Michael Addition >...	Radical reactions Radical reactions >... Radical reactions >...	SN2 SN2 >> Ring opening ... SN2 >> Ring opening ...
Empiric				

Skin metabolism products



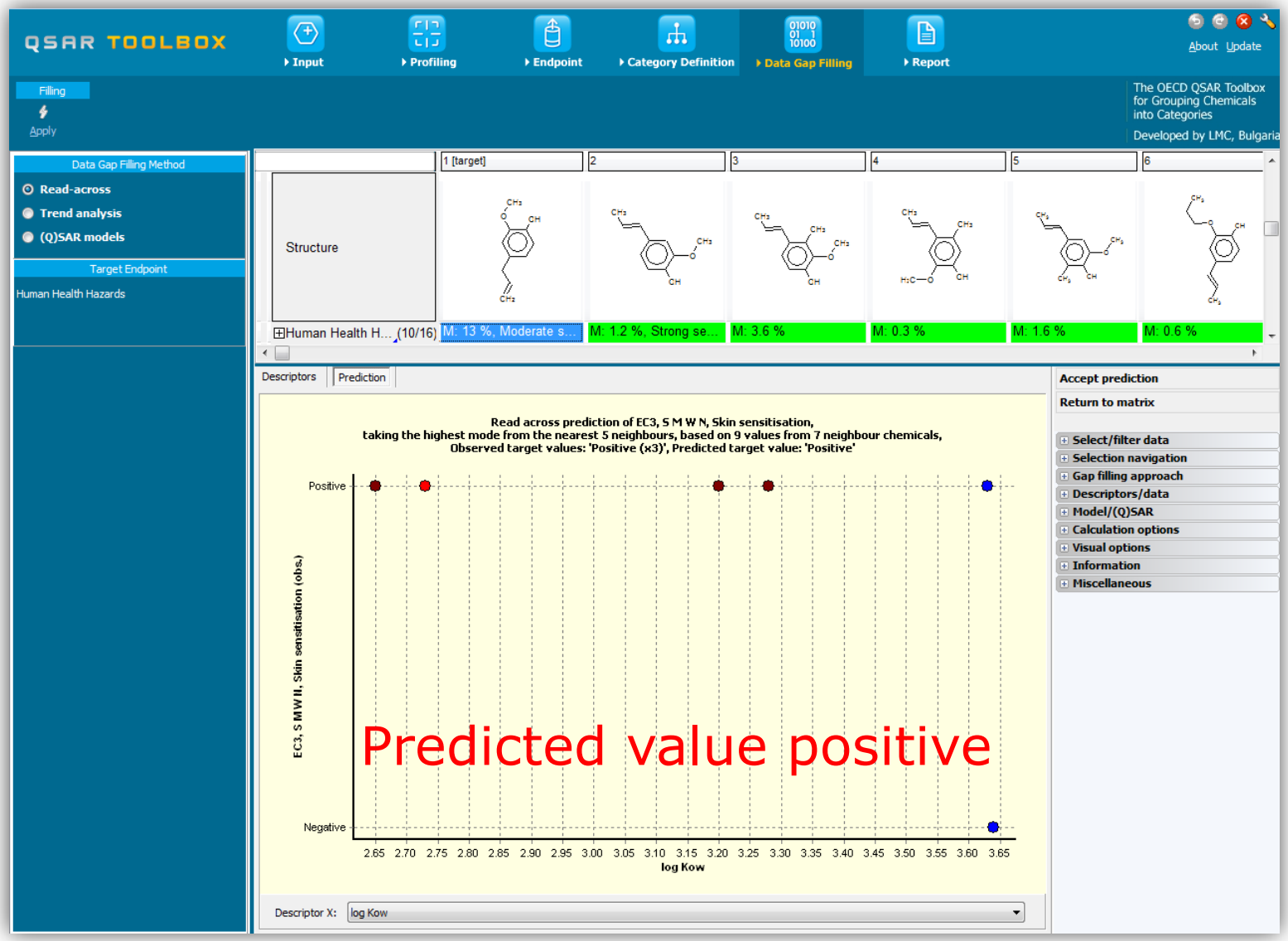
Combining transformation products



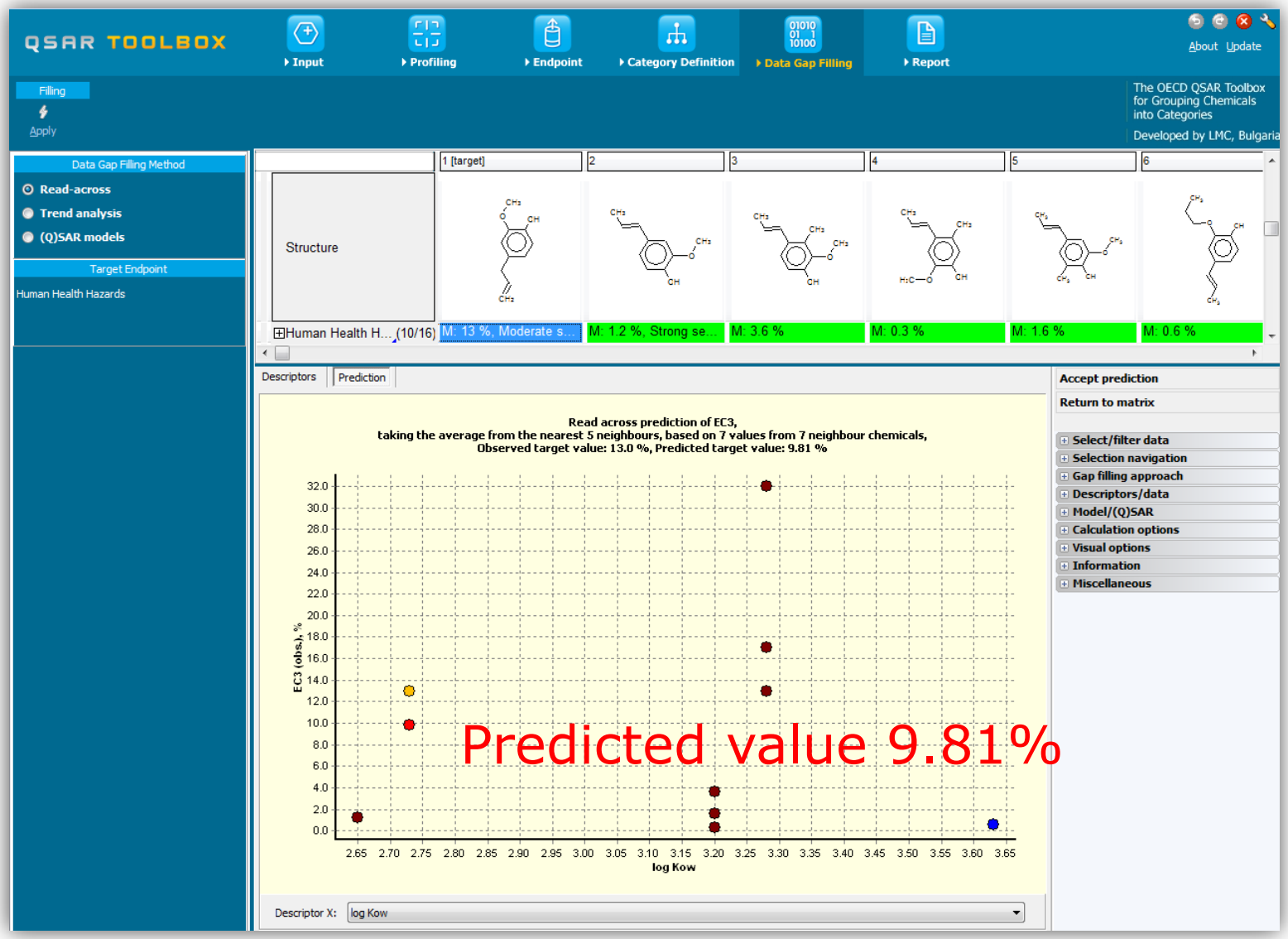
Combining compounds with the same mechanisms as the target products from skin metabolism and auto-oxidation resulted in:

- More than 900 structures, and
- More than 1 000 data points

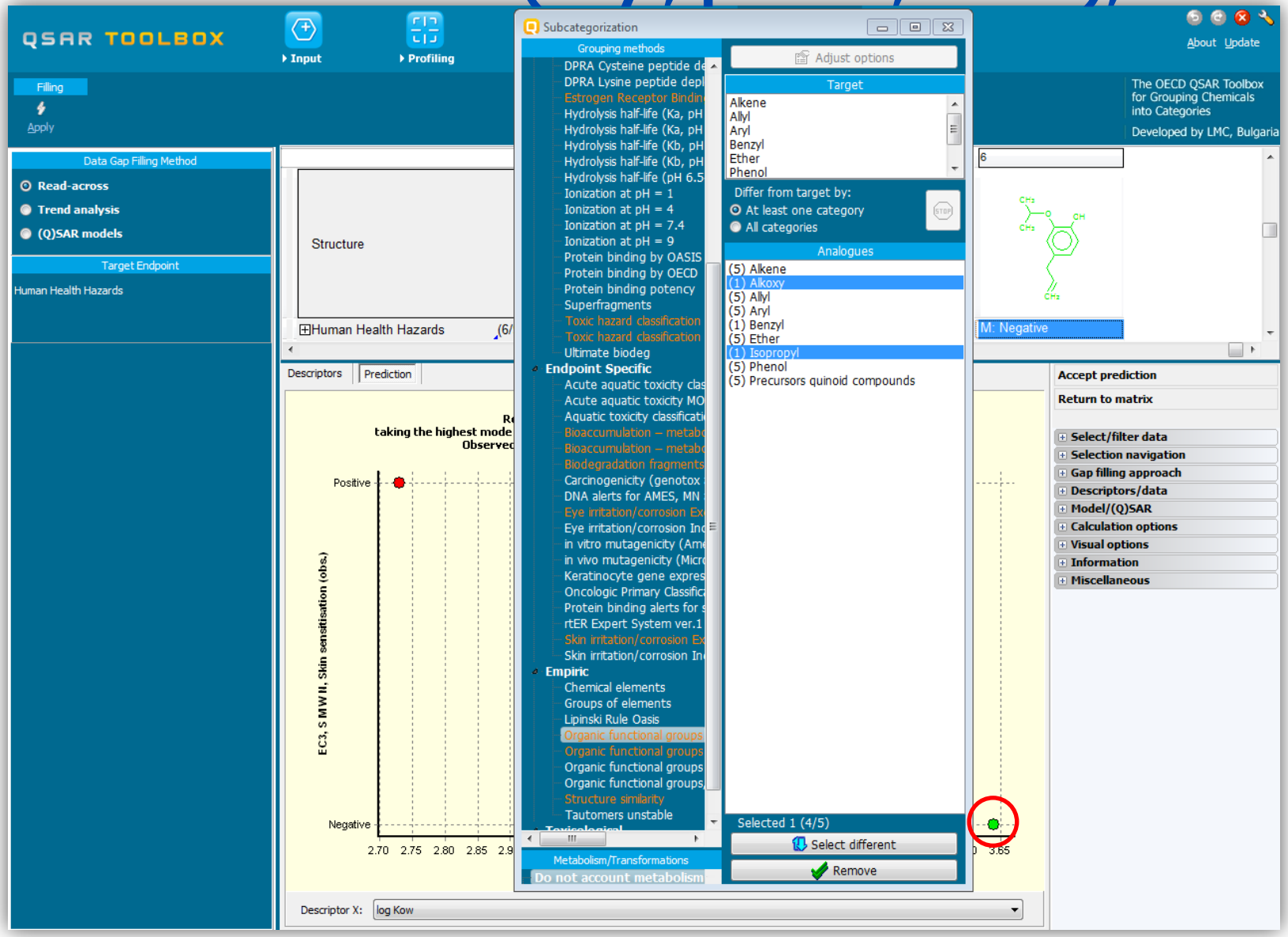
After chemical refinement OFG (allyl, phenol, ether), ECETOC



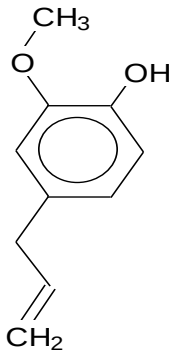
After chemical refinement OFG (allyl, phenol, ether), EC3 (%)



After chemical refinement OFG (allyl, phenol, ether), OASIS



Summary



Structural analogues → Too many or too little analogues, no mechanistic explanation → Positive read-across prediction

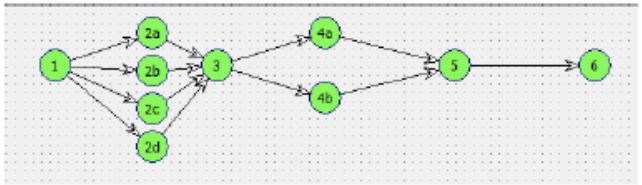
Protein binding alerts: No alert found → How to select mechanistic analogues? → Is prediction possible as stand-alone result?

Autoxidation Simulator/Skin metabolism → Protein binding alert found for many products (mixed pool) → Grouping with (bio)transformation: Identifying protein binding analogues of the target taking into account its (a)biotic activation → Chemical refinement → Positive read-across prediction with mechanistic explanation

Step-by-step example for how to use the Toolbox AOP workflow for Skin Sensitization(pdf)

QSAR TOOLBOX

Overview of implemented AOP scheme



Key node		Key event
1	Protein binding alerts	Protein binding – in silico/theoretical
2a	*in chemico* Peptide depletion assay DPRA (Cys)	Protein binding potency in chemico
2b	*in chemico* Peptide depletion assay DPRA (Lys)	
2c	*in chemico* Glutathione depletion assay GSH (RC50)	
2d	*in chemico* Adduct formation assay LC-MS	
3	*in vitro* Keratinocyte ARE (EC1.5, EC2, EC3)	Cellular response
4a	*in vitro* Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)	
4b	*in vitro* Dendritic cell activity assay MUSST (expression of CD86)	
5	*in vivo* Organ response (LLNA)	Organ response
6	*in vivo* Organism response (GPMT)	Organism response

The OECD QSAR Toolbox for Grouping Chemicals into Categories 28.01.2014 8

Some learnings:

- The prediction could be relatively simple, sometimes is more difficult, and sometimes looks impossible.
- Check for experimental data (all data principle) first, EC3?
- Source of data should be traceable, data of good quality
- Select analogues by broad structural similarity first
- Consider further (sub)categorisation for consistent mechanism
- If the prediction seems negative, try transformation to check
- Check for data for the predicted transformation products
- Make a conservative estimation – **toxicological hazard should not be underestimated** and the prediction should be useful for C&L and/or risk assessment (consider cut-offs!)

Thank you!

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