QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

Tutorial on how to predict Skin sensitization potential taking into account alert performance

Outlook

- **Background**
- Keywords
- Objectives
- Specific Aims
- Read across and analogue approach
- The exercise
- Workflow

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data filling exercise accounting fo alert performance.

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Keywords

TARGET CHEMICAL - chemical of interest

MODULE – a Toolbox module is a section dedicated to specific actions and options (e.g. Profiling)

WORKFLOW – the use, in combination, of the different modules (e.g. prediction workflow: from input to report)

PROFILER - algorithm (rule set) for the identification of specific features of the chemicals. Several types of profilers are available, such as structural (e.g. Organic functional groups), mechanistic (e.g. Protein binding by OECD) and endpoint-specific (e.g. in vitro in vitro mutagenicity (Ames test) alerts by ISS) profilers.

ALERT - the profilers consist of sets of rules or alerts. Each of the rules consists of a set of queries. The queries could be related to the chemical structure, physicochemical properties, experimental data, comparison with the target or list with substances and external queries from other predefined profilers (reference queries).

CATEGORY – "group" of substances sharing same characteristics (e.g. the same functional groups or mode of action). In a typical Toolbox workflow, it consists of the target chemical and its analogues gathered according to the selected profilers

ENDPOINT TREE – Endpoints are structured in a branched scheme, from a broader level (Phys-Chem properties, Environmental Fate and transport, Ecotoxicology, Human health hazard) to a more detailed one (e.g. EC3 in LLNA test under Human health hazard-Skin sensitization)

ALERT PERFORMANCE – is used to define how much relevant to a target endpoint an alert is. It reflect usability of category formation

DATA MATRIX – Table reporting the chemical(s) and data (experimental results, profilers outcomes, predictions). Each chemical is in a different column and each data in a different row

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Define target endpoint;
- Relevancy of profiles and data availability;
- Calculation of alert performance (AP).

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Specific Aims

- To introduce to the Toolbox user to the workflow for defining the target endpoint;
- To familiarize the user with the new interface of the Toolbox;
- To familiarize the user with the different highlighting of profiles and databases;
- To familiarize the user with the calculation of alert performance;
- To explain to the Toolbox user the rationale behind each step of the exercise.

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Alert performance
Overview

- Alert performance (AP) is used to define how relevant to a target endpoint an alert is;
- AP reflects the alerts usability for category formation;
- AP can be calculated for any endpoint and any profile; one should only have preliminary defined target endpoint;
- AP can be calculate for an alert with or without accounting for metabolism;

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The Exercise

- In this exercise we will illustrate how to calculate alert performance and to use this information for predicting skin sensitization potential (EC3 LLNA assay) for two untested compounds: *pyridaphenthion* [CAS# 119-12-0] and *2-methoxyphenol (guaiacol)* [CAS# 90-05-1] which will be the "target" chemicals.
- We will preliminary define the target endpoint.
- This prediction will be accomplished by calculation of alert performance.
- The category will be defined by the mechanism of protein binding common to all the chemicals in the category.
- The metabolic activation (skin sensitisation metabolism) will be taken into account in the prediction for the second target chemical [CAS# 90-05-1]
- The prediction itself will be made by "read-across".

The Exercise
Theoretical considerations on Skin Sensitization

- Allergic contact dermatitis that results from skin sensitization is a significant health concern.
- Skin sensitization is a toxicological endpoint that is complex and conceptually difficult.
- Many organic chemicals have been shown to induce skin sensitization after covalent binding to skin proteins[1].
- Therefore, mechanisms by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents.

[1] OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

Outlook

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Workflow

- **The Toolbox has six modules which are used in a sequential workflow:**
  - Input
  - Profiling
  - Data
  - Category Definition
  - Data Gap Filling
  - Report

Outlook

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- **Workflow**
  - **Input**

Input
Overview

- This module provides the user with several means of entering the chemical of interest or the target chemical.
- Since all subsequent functions are based on chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Input
Ways of Entering a Chemical

User Alternatives for Chemical ID:

A. Single target chemical

- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing chemical structure
- Select from User List/Inventory/Databases

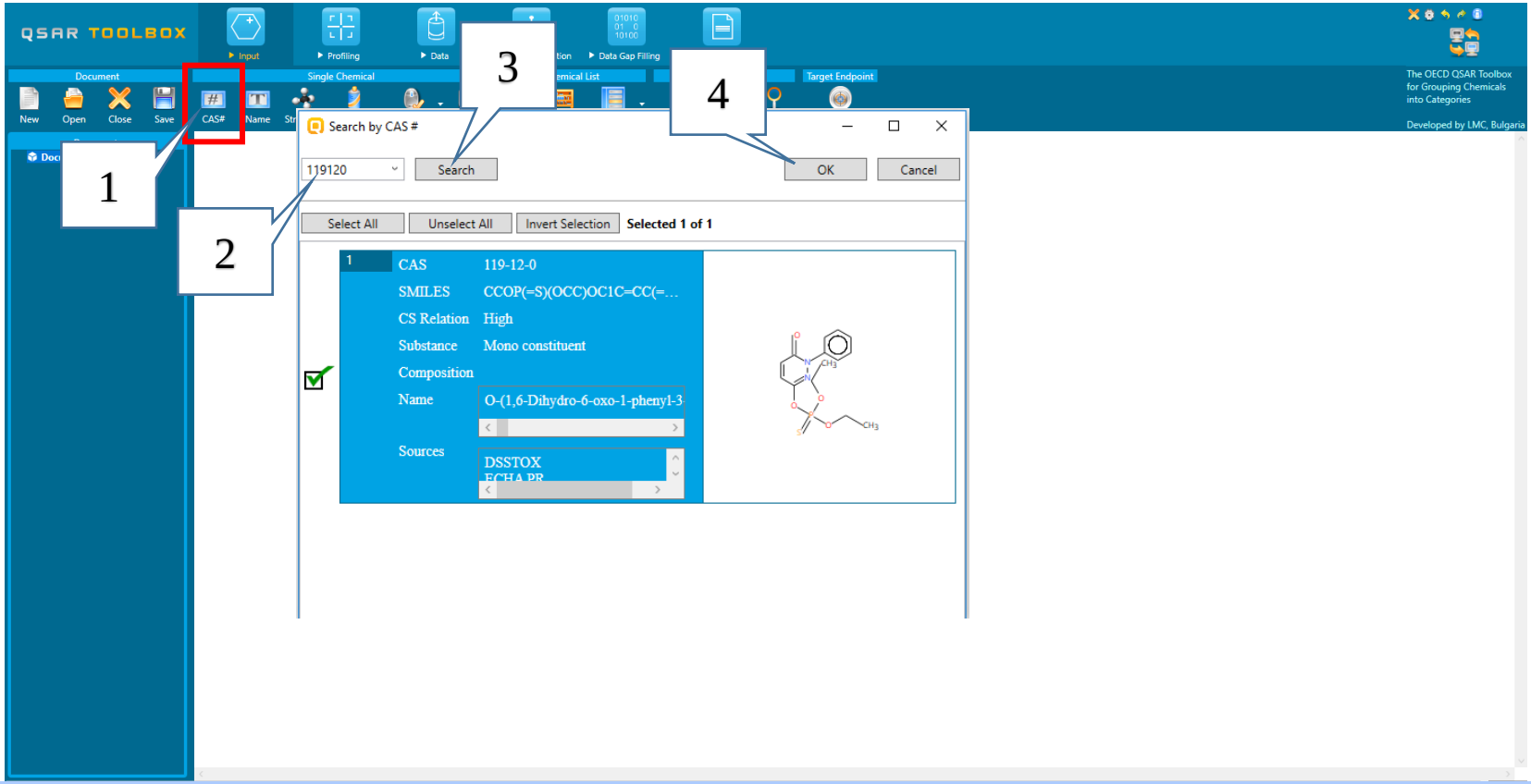
B. Group of chemicals

- User List/Inventory
- Specialized Databases

Input Screen

Input target chemical by CAS#

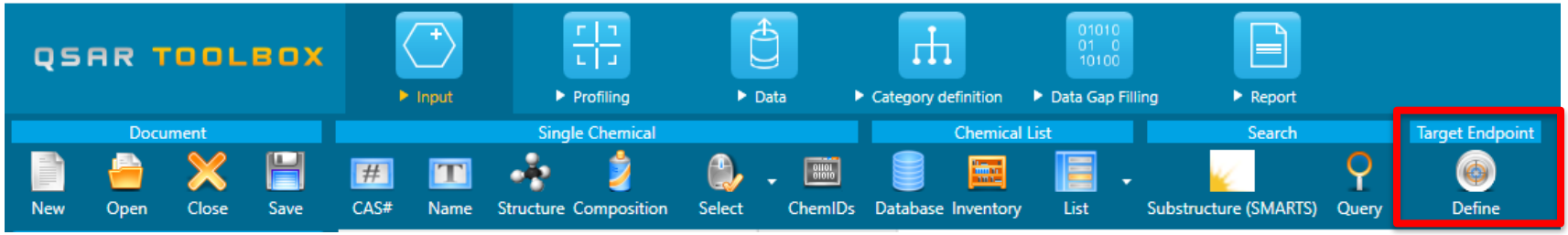
Exercise 1: ***CAS 119-12-0***



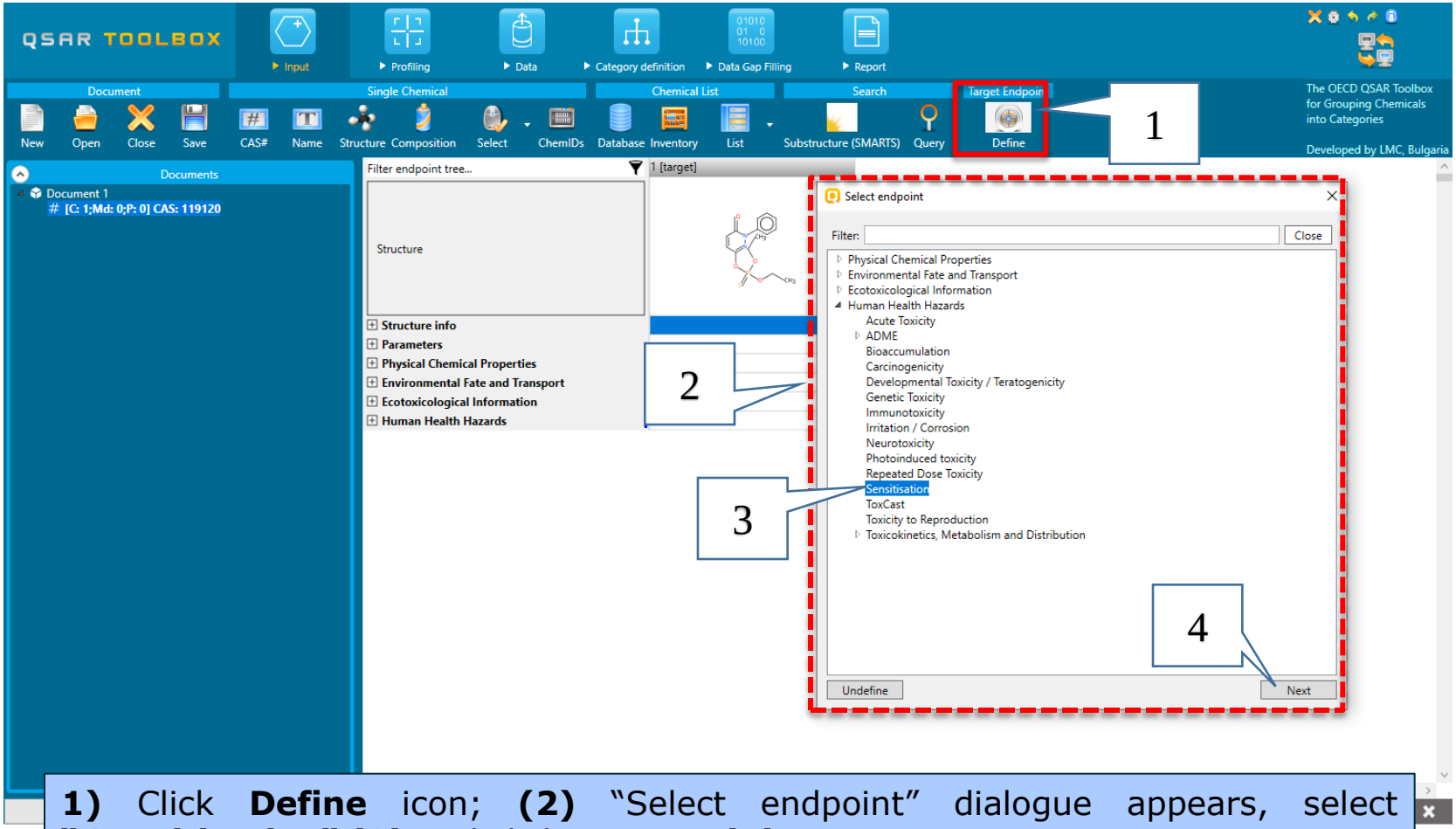
Click **CAS#** (1); Insert CAS **119-12-0** in the blank field (2) and click **Search** (3). When the structure appears, click **OK** (4).

Input
Define target endpoint

Calculation of alert performance (AP) is only possible if the target endpoint is preliminary defined.
Defining of the endpoint allows entering the endpoint of interest e.g. EC3, LC50, gene mutation etc., along with specific metadata information. Based on the metadata, different relevancy scores for profiles could be provided for the same endpoint.



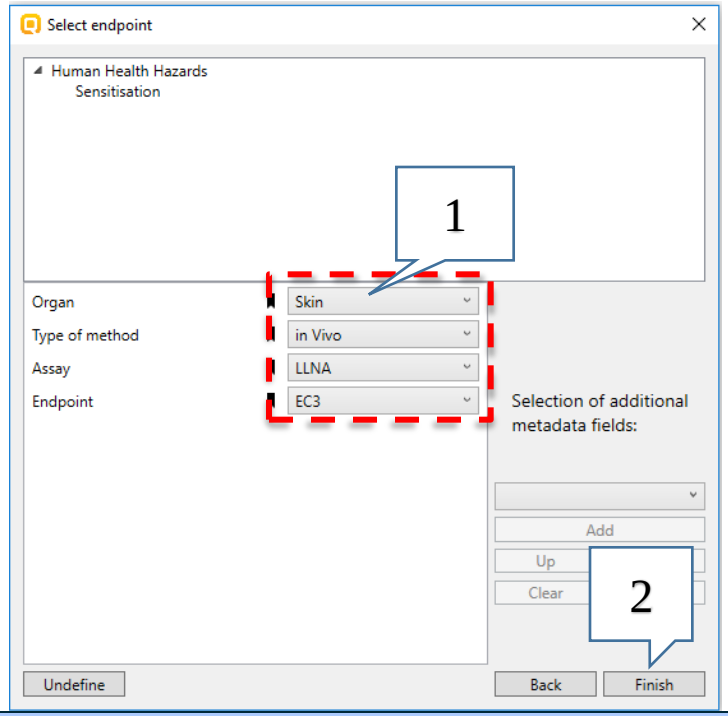
Input
Define target endpoint



1) Click **Define** icon; **(2)** "Select endpoint" dialogue appears, select "***Sensitization***" **(3)** and click on **Next (4)**

Input
Define target endpoint

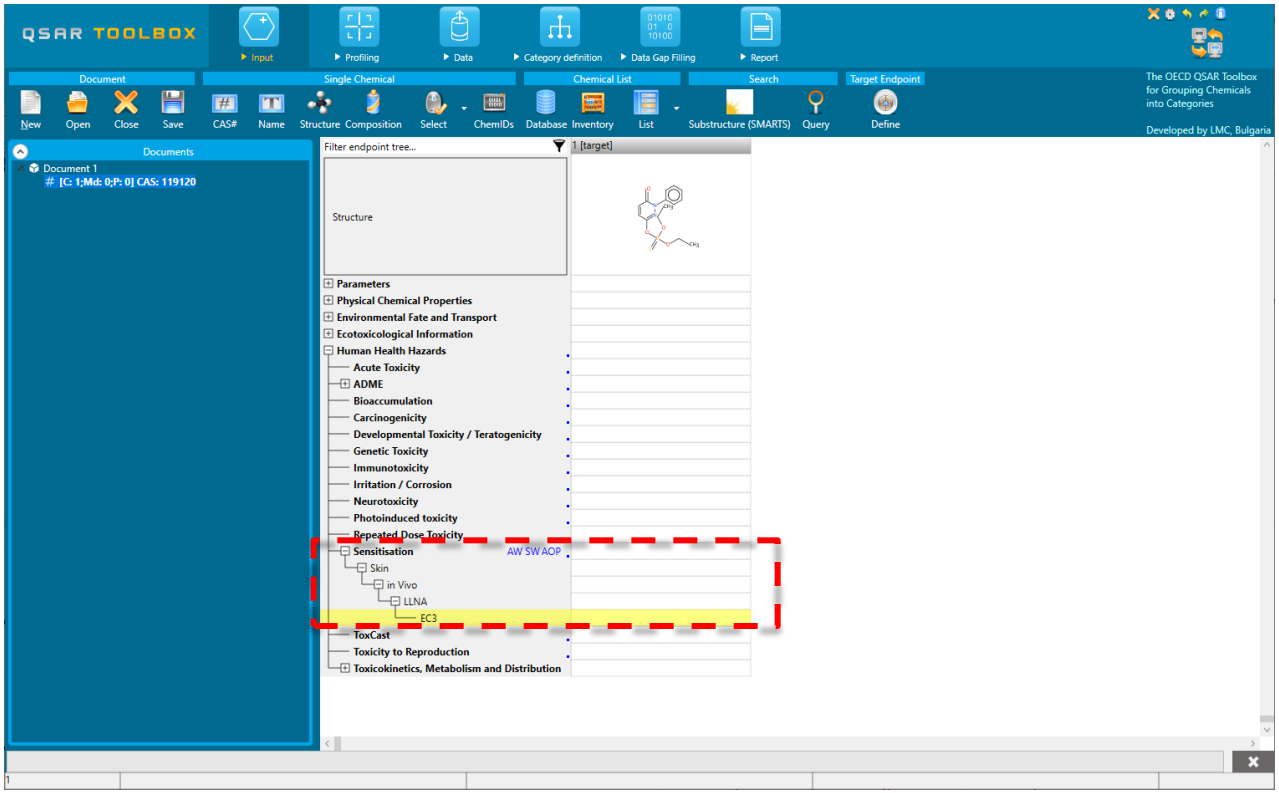
On the next step you have to select the endpoint of interest and additional metadata if needed.



A new dialogue for defining additional details to the selected target endpoint appears;
(1) From the drop-down menus select the specific information for the metadata fields as follows: Endpoint is **EC3**; Organ is **Skin**; Type of method is **in Vivo;** Assay is **LLNA** **(2)** Click **Finish**

Input
Define target endpoint

Once the endpoint is defined along with its metadata, they appear in the endpoint tree and the corresponding row of the data matrix is highlighted.



Outlook

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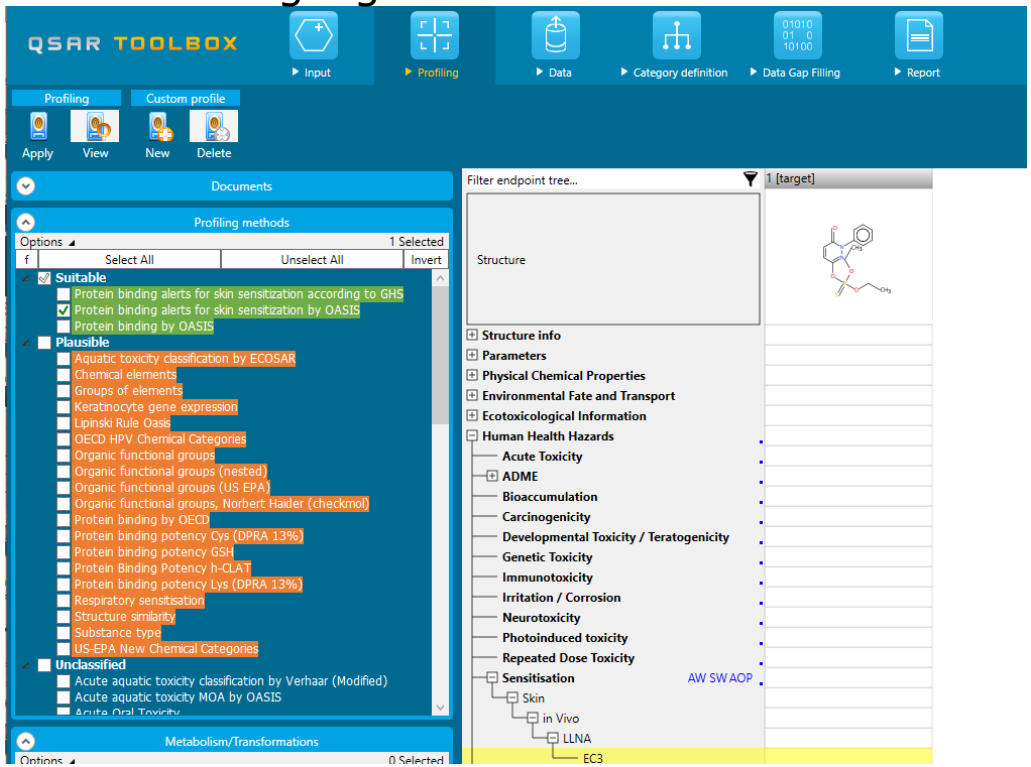
- **Workflow**
  - Input
  - **Profiling**

Profiling
Overview

- "Profiling" refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database.
- Available information includes likely mechanism(s) of action, as well as observed or simulated metabolites.

Profiling
Sidebar to profilers' relevancy

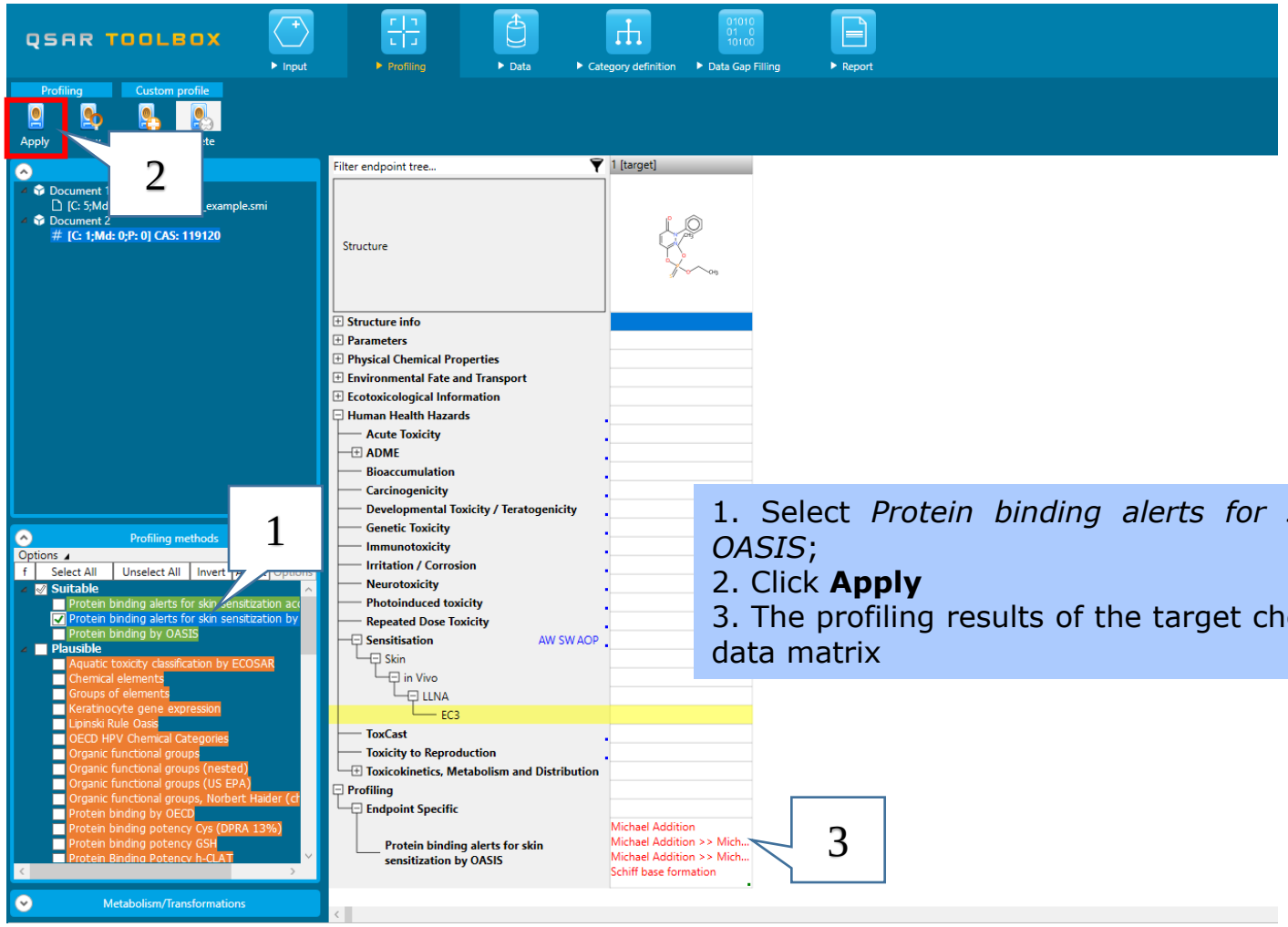
Once the endpoint is selected, the relevant profilers and metabolic transformations are highlighted.



- **Suitable** (in green) - developed using data/knowledge for the target endpoint;
- **Plausible** (in orange) - structure-based; form broader group of analogues;
- **Unclassified** (no color) – all profilers, which are not classified in any of the categories above.

Profiling

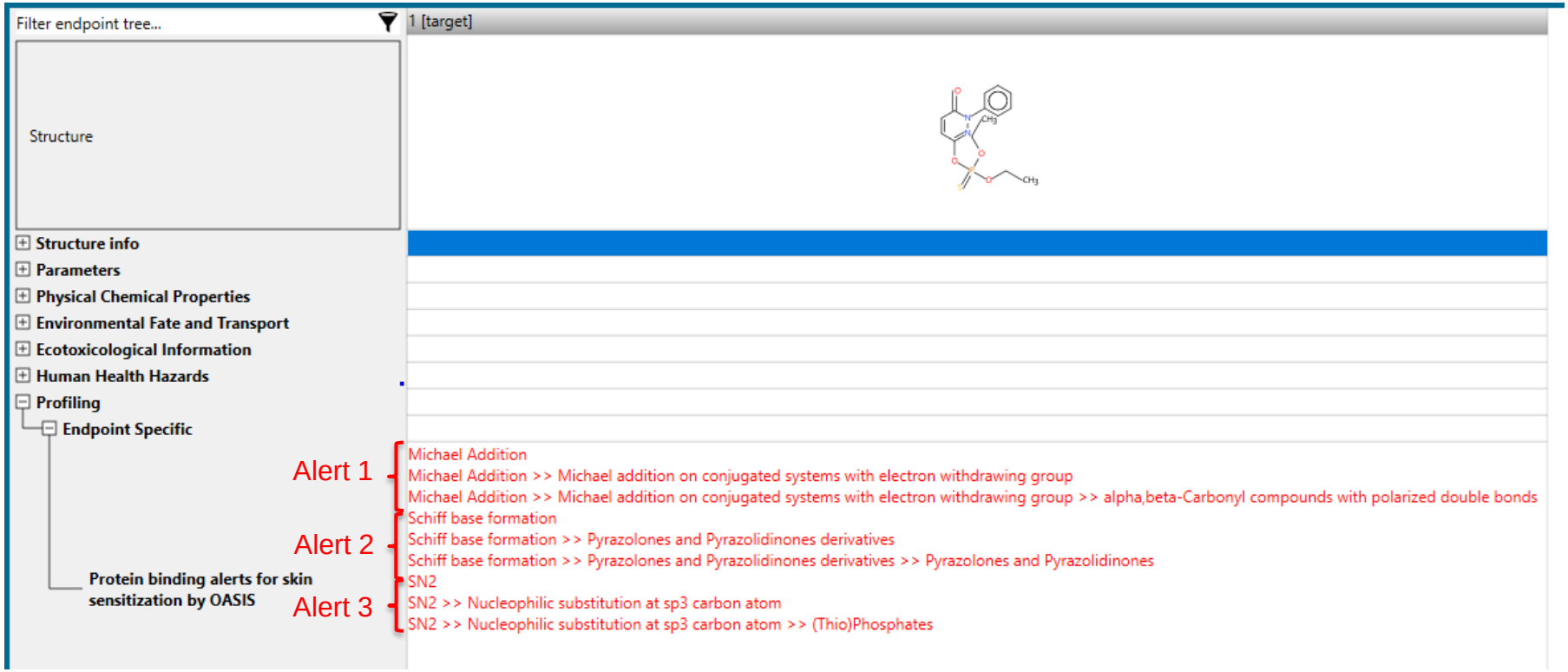
Profiling the target chemical



1. Select *Protein binding alerts for skin sensitization by OASIS*;
2. Click **Apply**
3. The profiling results of the target chemical appears in the data matrix

Profiling

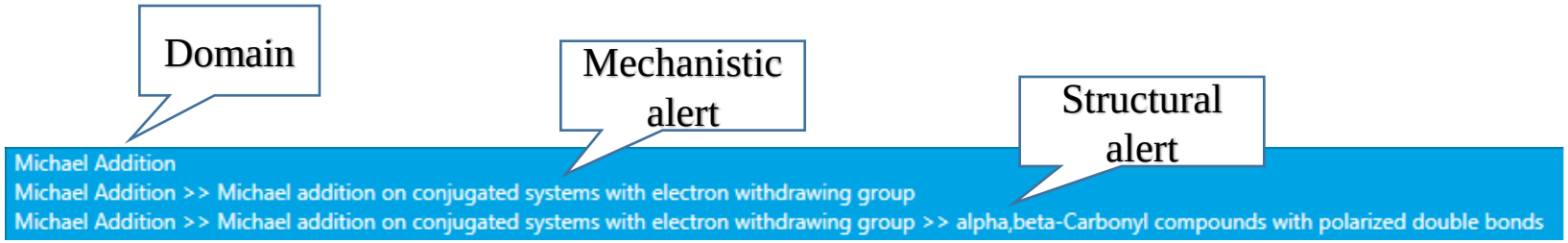
Profiling the target chemical



Three protein binding alerts for skin sensitization are found in the target chemical.

Profiling
Sidebar on the hierarchical type profiles

Protein binding alerts for skin sensitization by OASIS is a hierarchical profile. The organization of the hierarchical profiles includes three levels of information for each category – domain, mechanistic alert and structural alert.



Right-click over the structural alert and select *Explain* to open the profiling scheme, where the user can see more details about the current alert.

Outlook

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- **Workflow**
  - Input
  - Profiling
  - **Data**

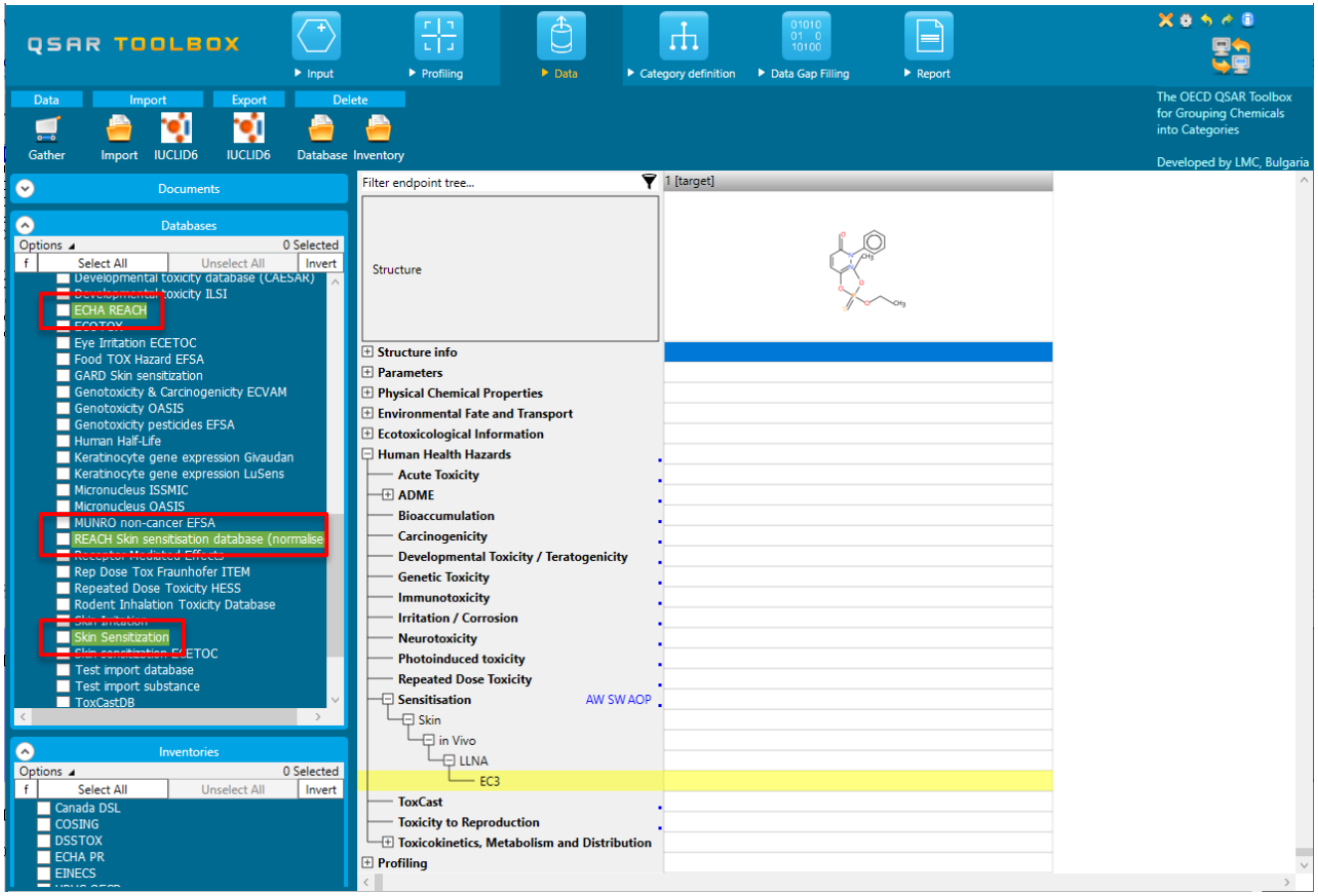
Data
Overview

- "Data" refers to the electronic process of retrieving the environmental fate, ecotoxicity and toxicity data that are stored in the Toolbox.
- Data gathering can be executed in a global fashion (i.e., collecting all data for all endpoints) or on a more narrowly defined basis (e.g., collecting data for a single or limited number of endpoints).

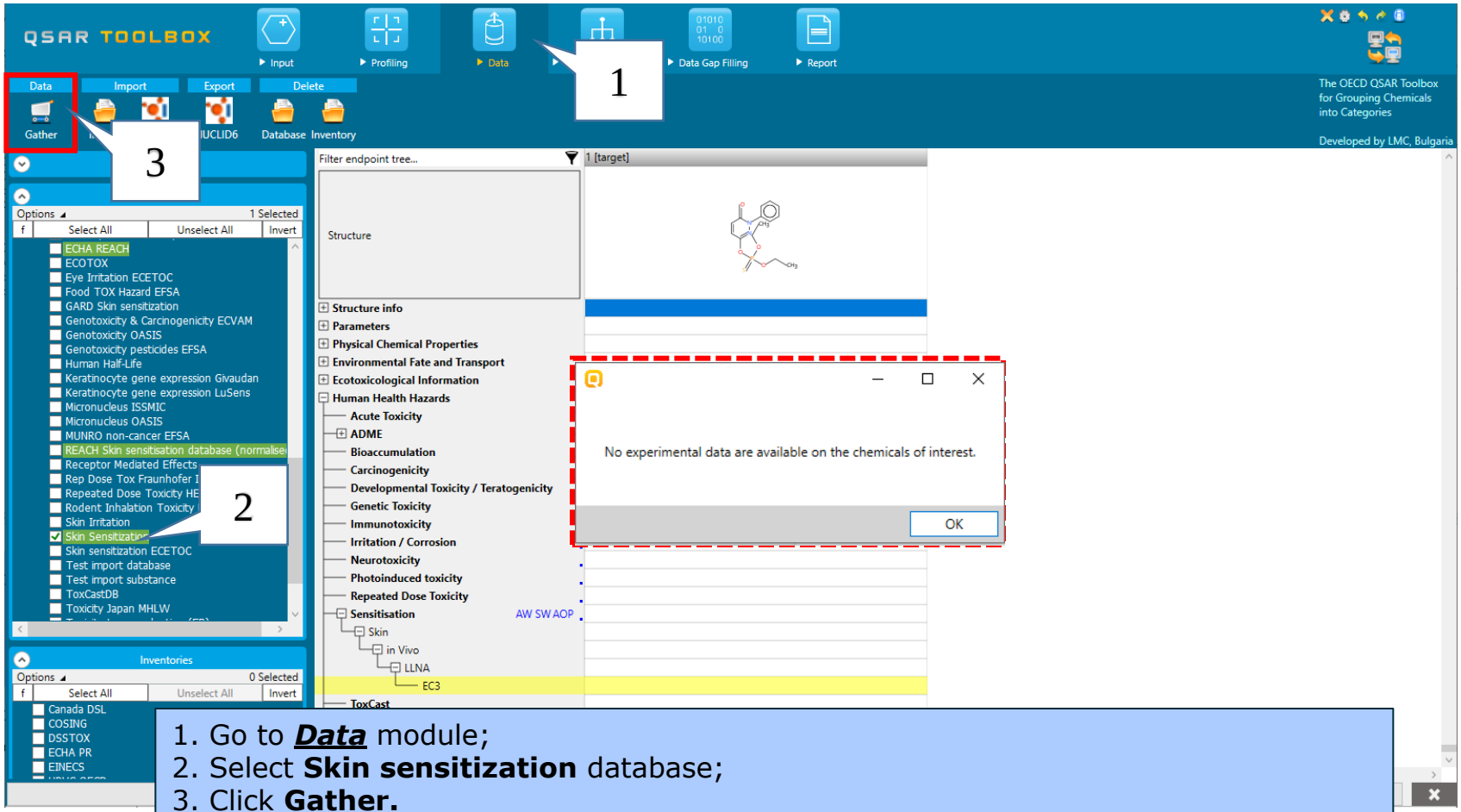
Data

Sidebar on Data availability

Once the endpoint is selected, the databases, which contain such type of data, are highlighted in green.



Data
Gather data



1. Go to ***Data*** module;
2. Select **Skin sensitization** database;
3. Click **Gather.**

A pop-up message informs that there is no experimental data for the target chemical.

Data
Gather data

- Toxicity information on the target chemical is electronically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues (and therefore calculation of AP) is performed only among the chemicals which are listed in the selected databases. In this example only the Skin sensitization database is selected.
- In this example, an insert window appears stating there was “no data found” for the target chemical.

Recap

- In module one, you have entered the target chemical and defined the target endpoint.
- In the second module, you have profiled the target chemical with a profiler, which is suitable for the selected target endpoint.
- In the third module, you have seen the database corresponding to the defined target endpoint. You have found that no experimental data is currently available in the database for the structure.
- In other words, you have identified a data gap which you would like to fill in.
- Click “Category Definition” to move to the next module.

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- **Workflow**
  - Input
  - Profiling
  - Data
  - **Category definition**

Category Definition
Overview

- This module provides the user with several means of grouping chemicals into a toxicologically meaningful category that includes the target molecule.
- This is the critical step in the workflow.
- Several options are available in the Toolbox to assist the user in refining the category definition.

Category Definition
Grouping methods

- The different grouping methods allow the user to group chemicals into chemical categories according to different measures of "similarity" so that within a category data gaps can be filled by read-across.
- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind by the same mechanism and for which experimental results are available.

Category Definition
Protein binding by OASIS grouping method

- This is one of the best grouping methods in the Toolbox. It is built on conventional organic chemical reactions and as such is qualitative in character.
- This method is particularly relevant for respiratory and skin sensitization and acute aquatic toxicity, but also for chromosomal aberration and acute inhalation toxicity.

Category Definition
Protein binding by OASIS grouping method

- This scheme includes 112 categories organized in three level of information:

  ✓ Level I: Mechanistic Domains (11 categories)
  ✓ Level II: Mechanistic alerts associated to each mechanistic domain are created on the basis of a common reactive centre being activated by a number of substituents (50 categories)
  ✓ Level III: A number of structural alerts specifying the substituents to a common reactive center are made up each mechanistic alert (112 categories)

Category Definition
Protein binding by OASIS grouping method

- Each category from level III is presented by defined 2-dimensional structural alerts that is responsible for eliciting the toxic effects, such as skin sensitization which are a result of protein binding.

- The associated chemical reactions are in accordance with existing knowledge on electrophilic interaction mechanisms of various structural functionalities.

Category Definition
Protein binding by OASIS grouping method

- There is an agreement that most organic chemicals must react covalently with skin proteins in order to behave as skin sensitizers.
- Therefore, chemical reactions by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents. So you have mechanistic plausibility for defining your category based on similar protein-binding mechanism.

Category Definition

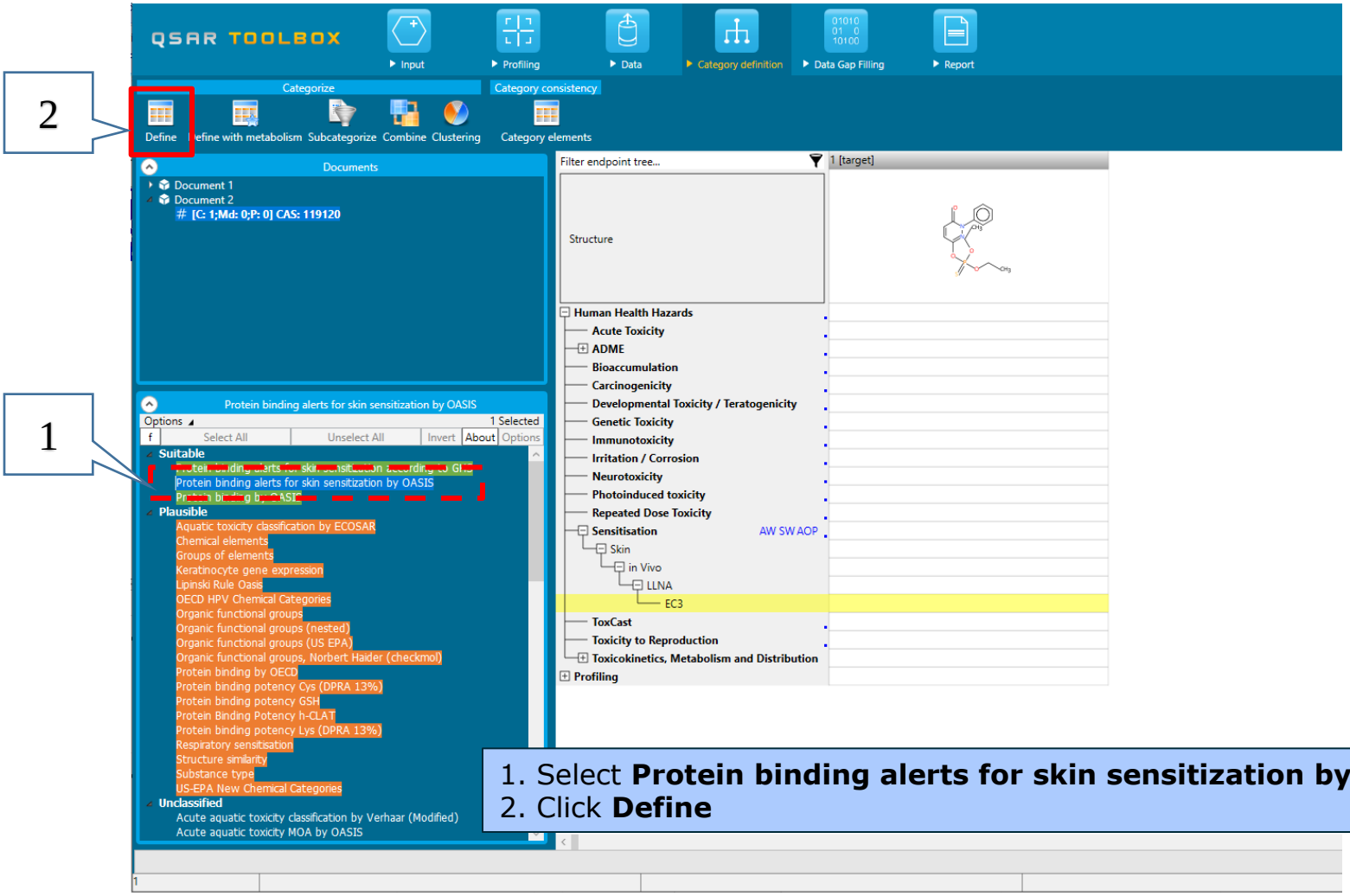
- When more than one alert is found in the target structure before or after metabolic activation, Alert performance could be used to define which of them is the most suitable for primary categorization.

Alert performance
Overview

The performance of an alert represents the number of chemicals with data related to the predefined scale across all chemicals from the selected databases, which have the same alert. It provides and distribution of data according to a given effect (e.g. positive, negative) in percentages.

Category Definition

Calculation of Alert performance



Category Definition
Calculation of Alert performance

After clicking ***Define***, the Categorization dialog appears. It consists of all protein binding alerts for SS found in the target structure.

The most suitable alert for category formation is determined by comparison of their alert performance.

Additional section for calculating "Alert performance" is designed in this dialogue, when the endpoint is preliminary defined (see on the next slide).

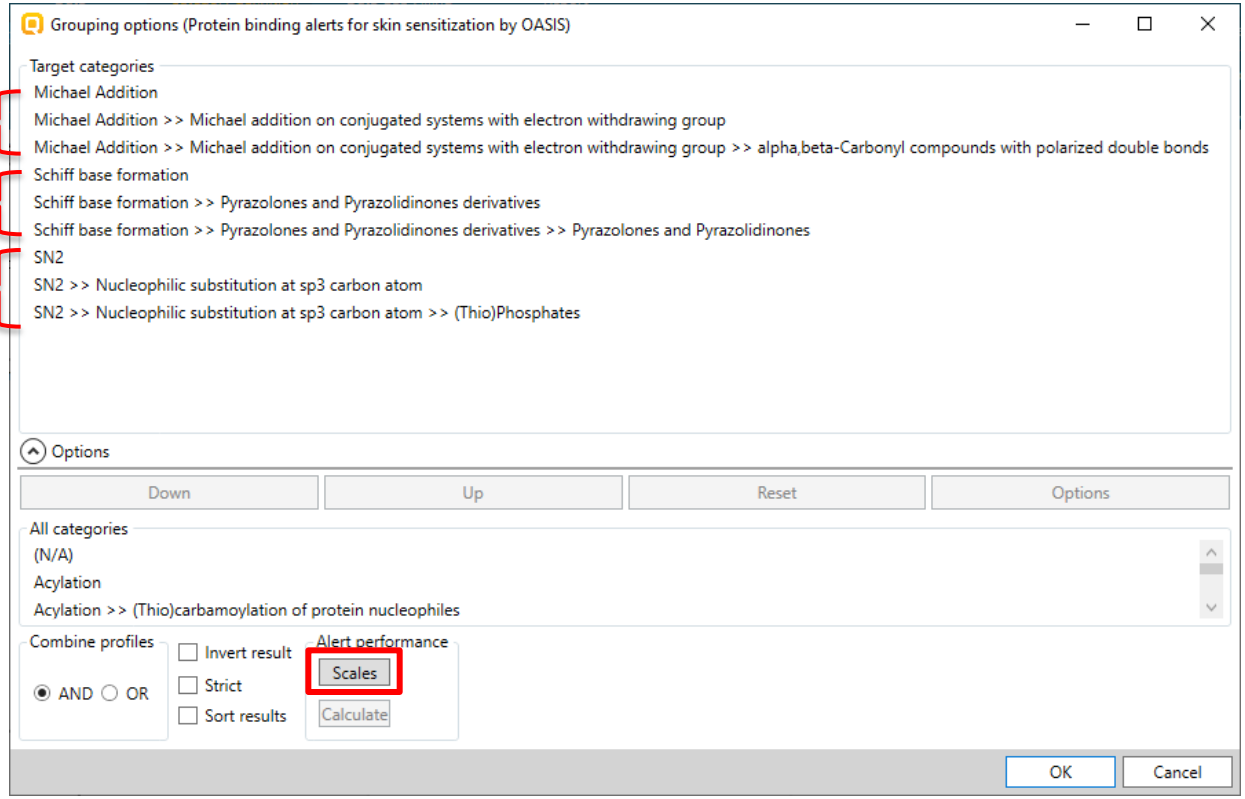
Alert performance can be calculated for only one alert, for combination of alerts or for all found alerts.

Category Definition
Calculation of Alert performance

alert 1

alert 2

alert 3



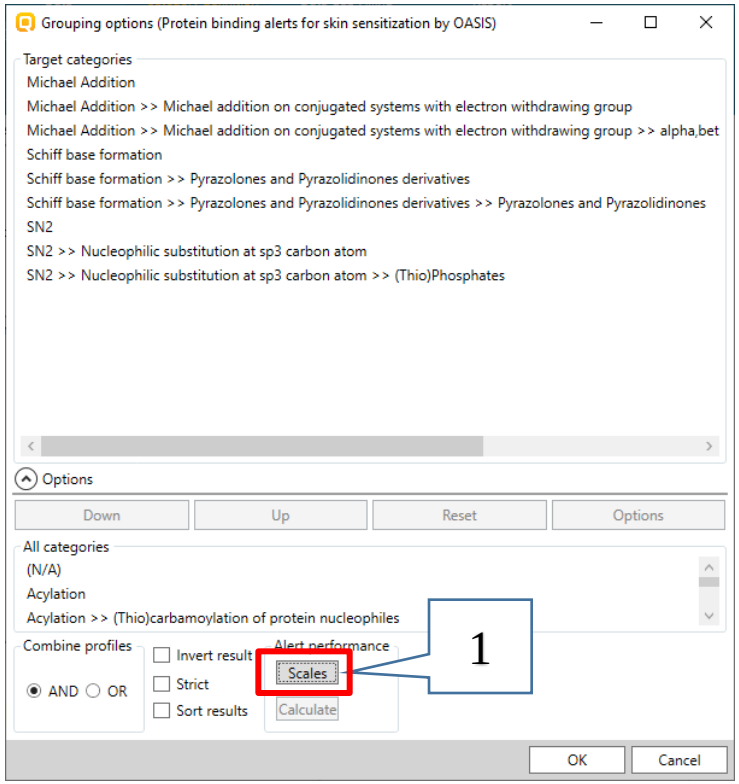
Category Definition
Calculation of Alert performance

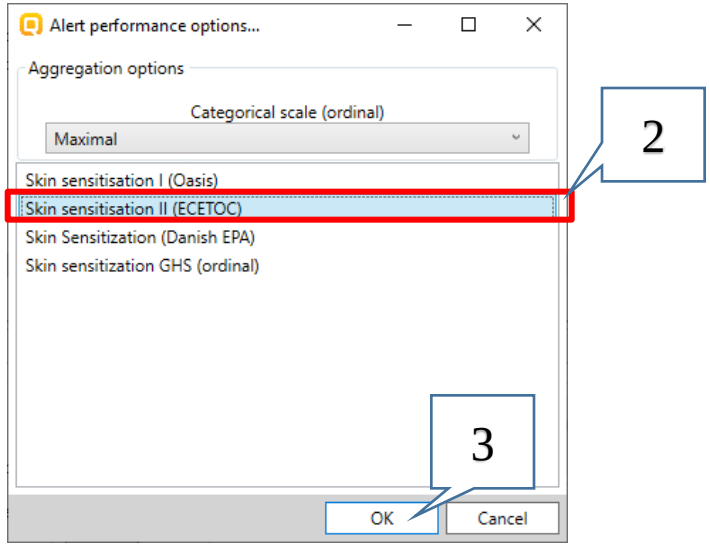
In order to calculate the performance of an alert, first of all you have to click on **Scales**.

The main purpose of the scales is to unify all data available in the Toolbox databases for a certain endpoint. Therefore, the most appropriate scale is "Skin Sensitisation II (ECETOC)". It is a dichotomous scale that converts all skin data into: Positive and Negative.

Additional option for applying different weight of the data that is available is also provided. Worst case scenario have been taken into account, i.e. "Maximal" data is set as default.

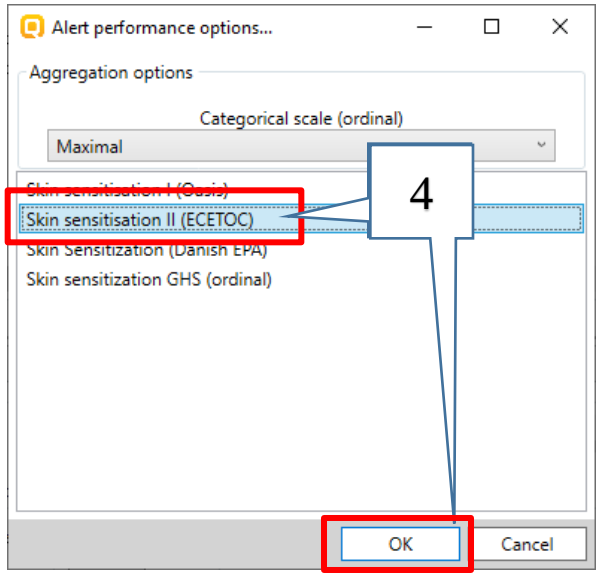
Category Definition
Calculation of Alert performance

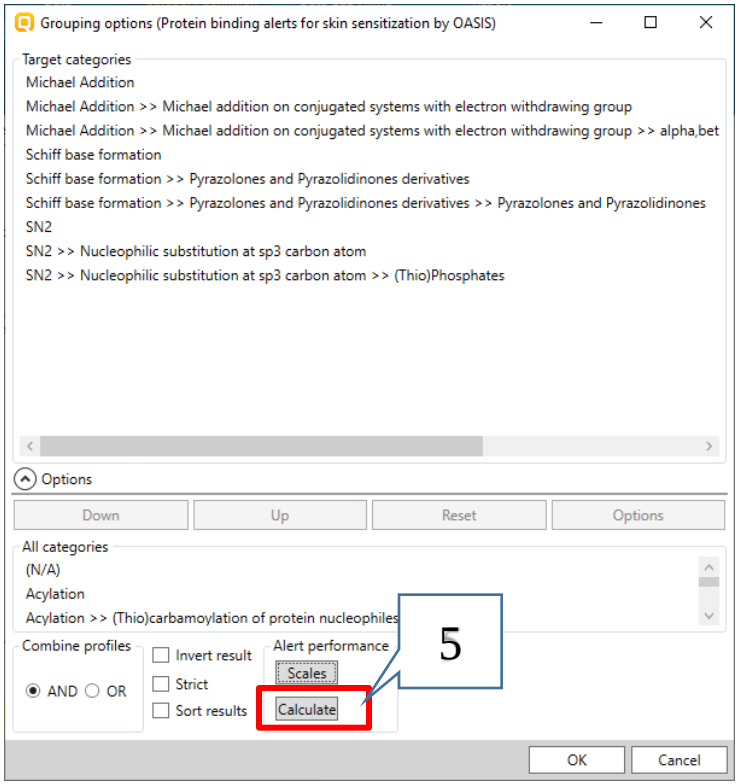




1. Click **Scales**;
2. Select **Skin sensitization II (ECETOC)** scale;
3. Confirm with "**OK**"

Category Definition
Calculation of Alert performance

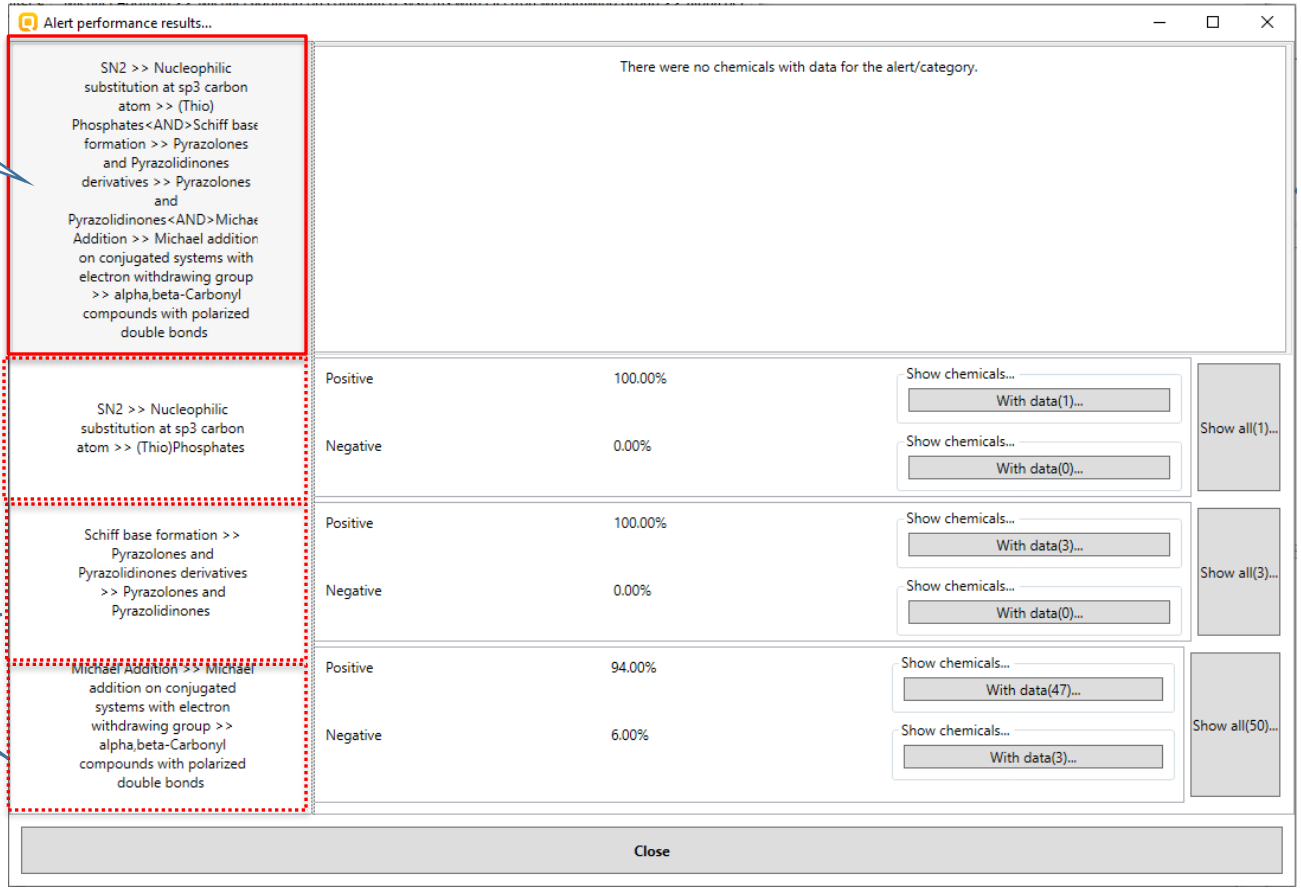




4. After selection and scale and confirming with **OK;**
5. Click **Calculate;**

Category Definition
Calculation of Alert performance

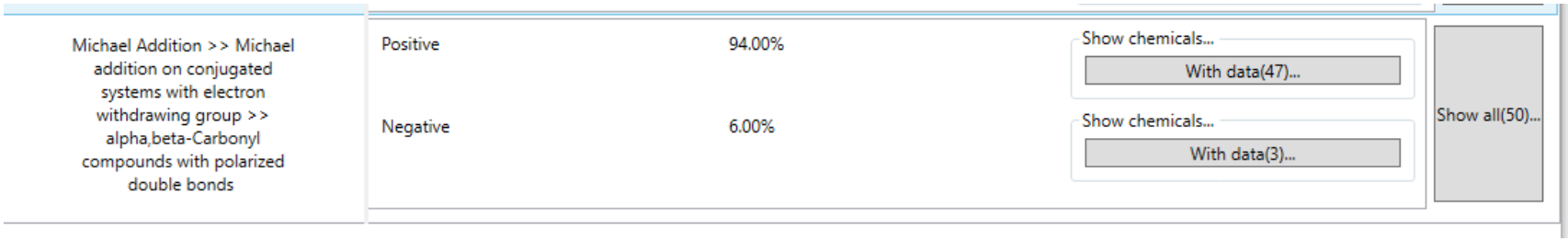
Information for the calculated AP for each of the alerts appears in the following window:



1. Alert performance is calculated for the combination of **all alerts** (alerts are AND-ed);
2. And for **each alert individually.**

Category Definition

Calculation of Alert performance for one alert



50 analogues having *alpha, beta-Carbonyl compounds with polarized double bonds* have been found in the database.

Of them:

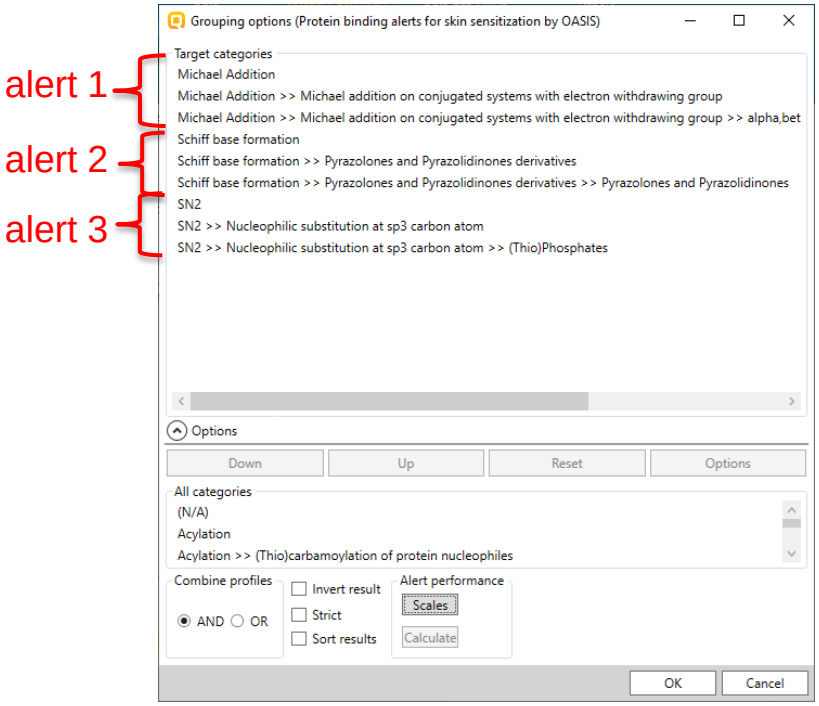
- 47 out of 50 chemicals have positive data (94%)
- 3 out of 50 chemicals have negative data (6%).

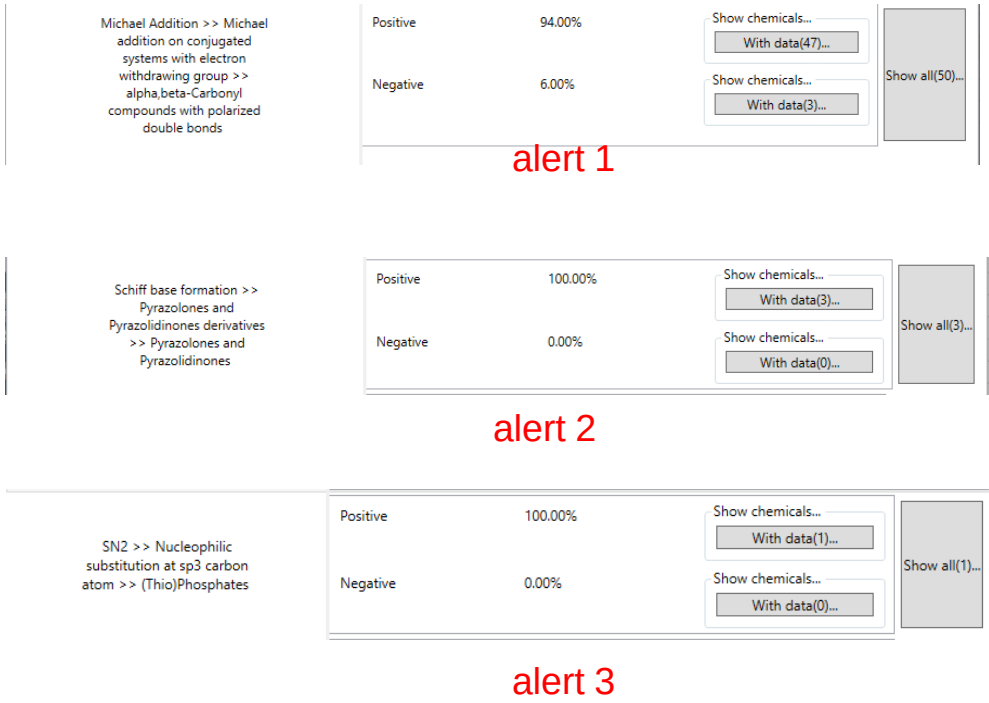
⚠ Keep in mind that the statistic is obtained from the chemicals and data, available in the selected databases

Category Definition

Calculation of Alert performance for one alert

In summary we see that the second alert (Schiff base formation >>….>> Pyrazolones and Pyrazolidinones) is the most suitable to define a category (100% positive alert). Read-across prediction is not illustrated in the forthcoming slides





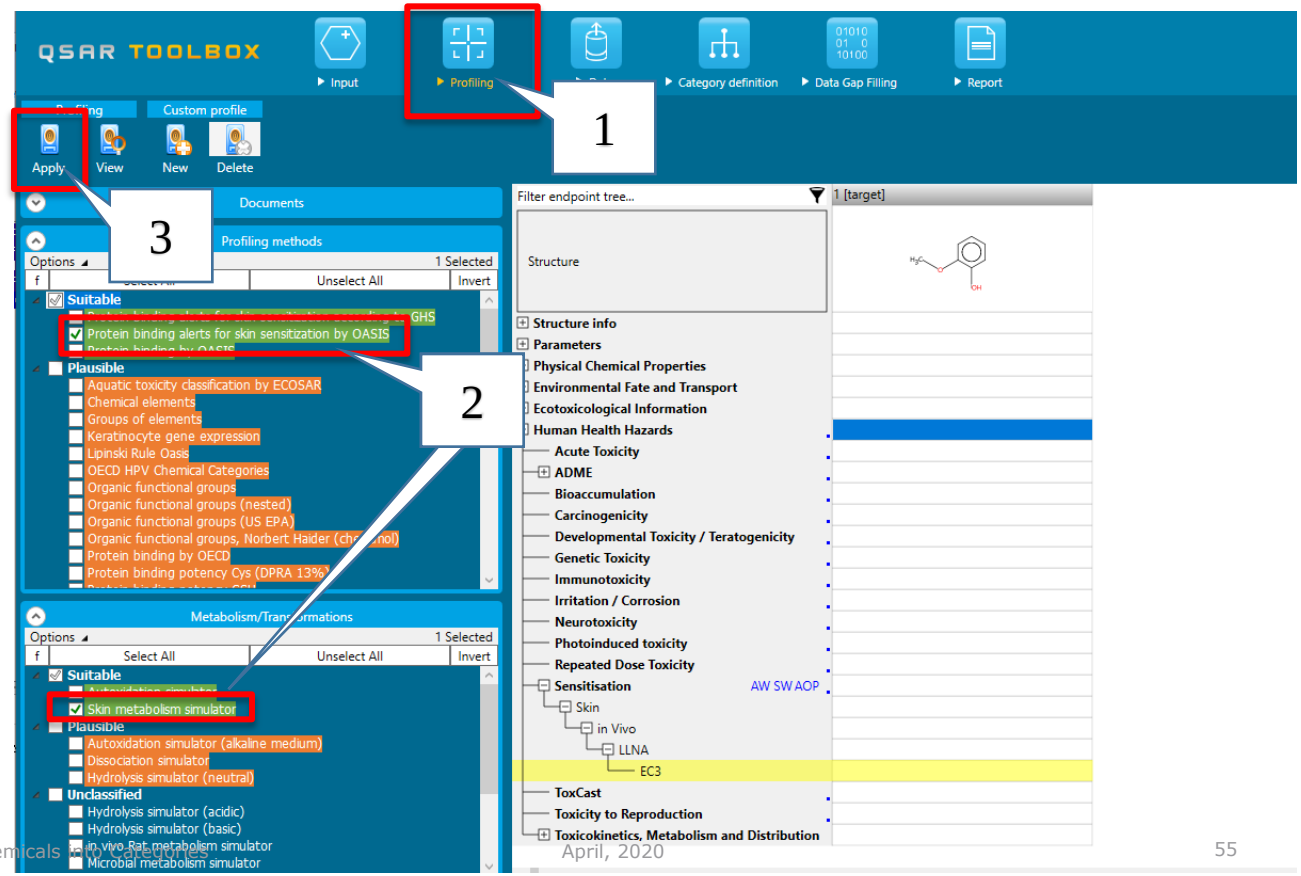
Category Definition

Alert performance accounting for metabolism

Exercise 2: CAS 90-05-1

AP can be also calculated for alert(s) identified in the simulated metabolites after autooxidation simulation or metabolic simulation.

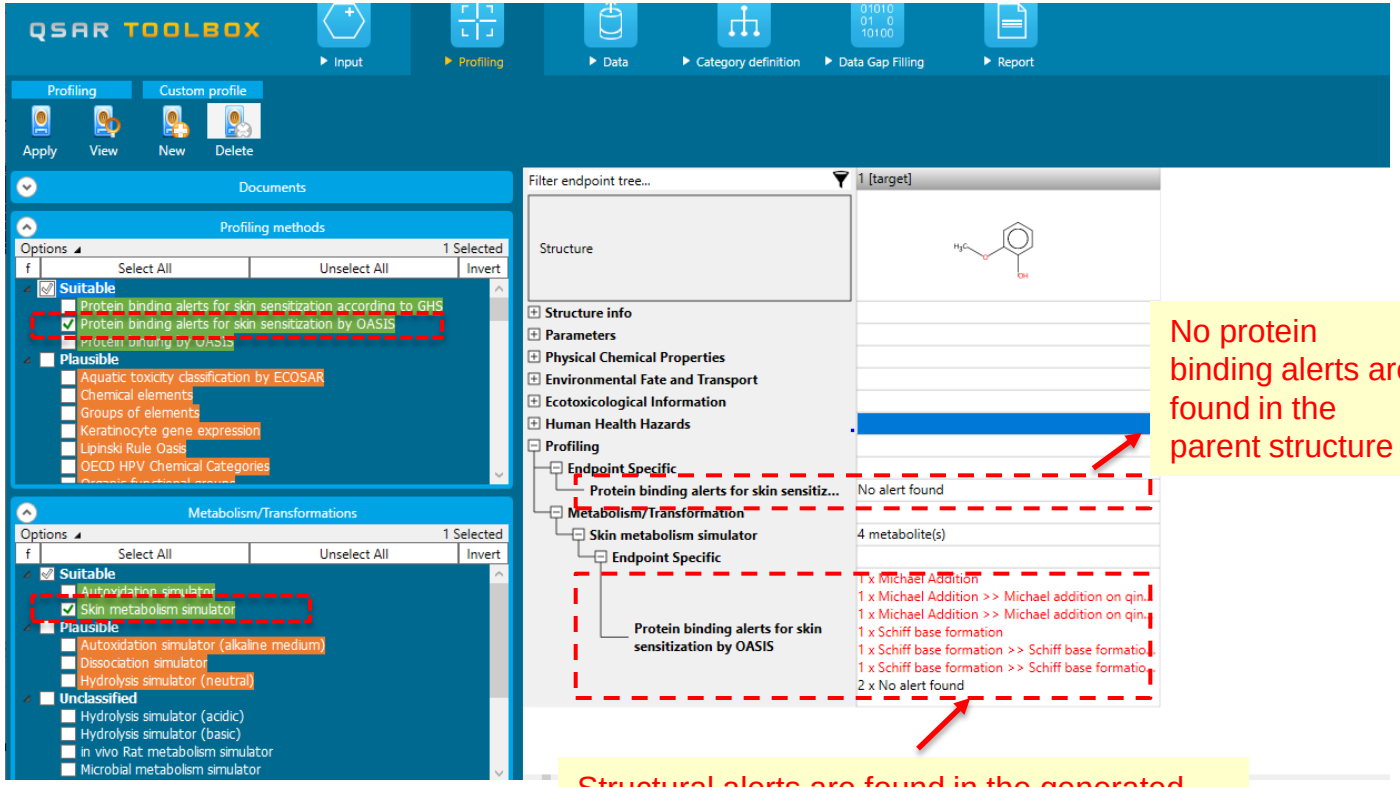
1. Click ***Profiling*** (1);
2. Check *Protein binding alerts for skin sensitization by OASIS and Skin metabolism simulator (2)*;
3. Click ***Apply*** (3).



Category Definition

Alert performance accounting for metabolism

Exercise 2:
CAS 90-05-1

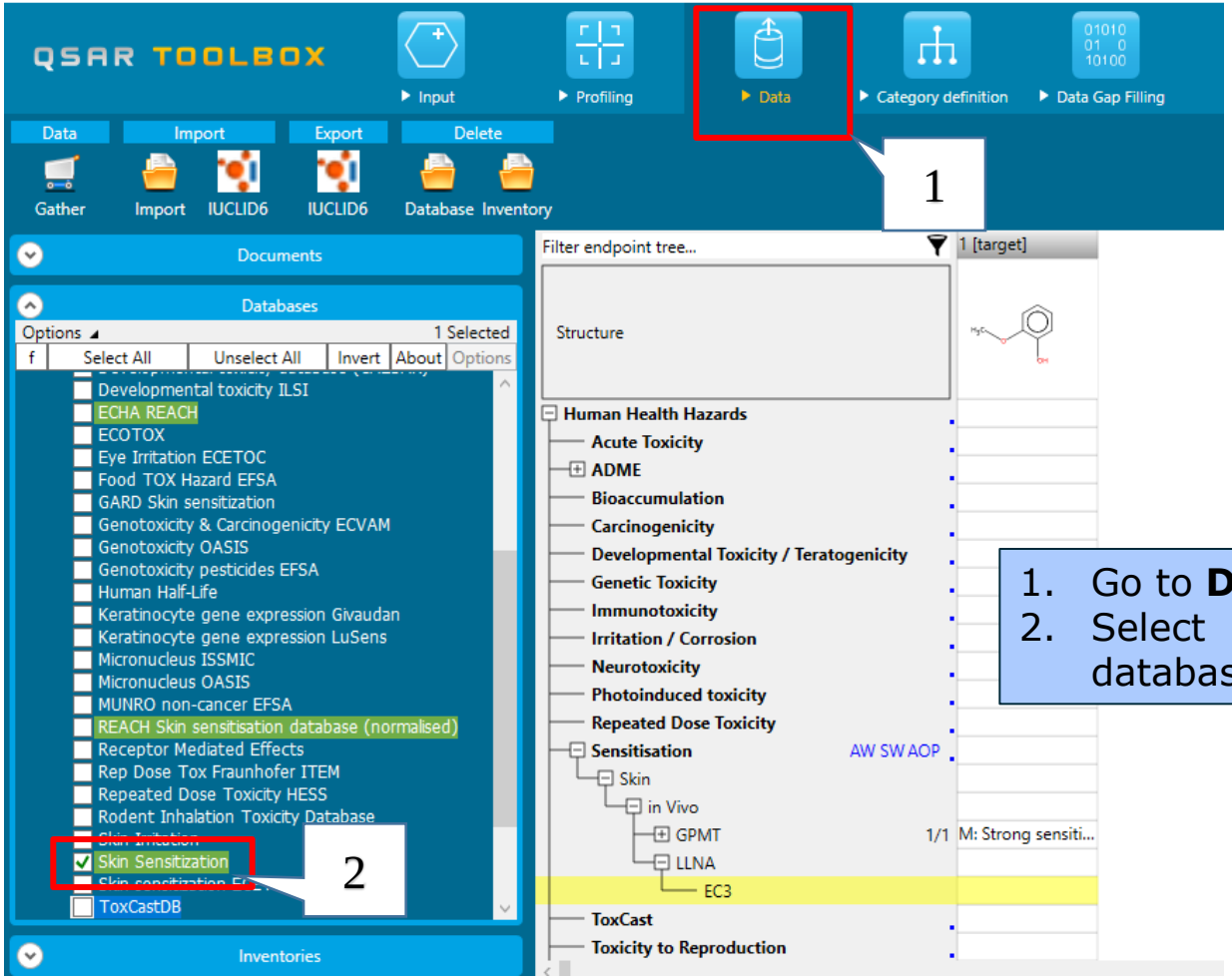


No protein binding alerts are found in the parent structure

Structural alerts are found in the generated metabolites after applying Skin metabolism simulator

Category Definition

Alert performance accounting for metabolism



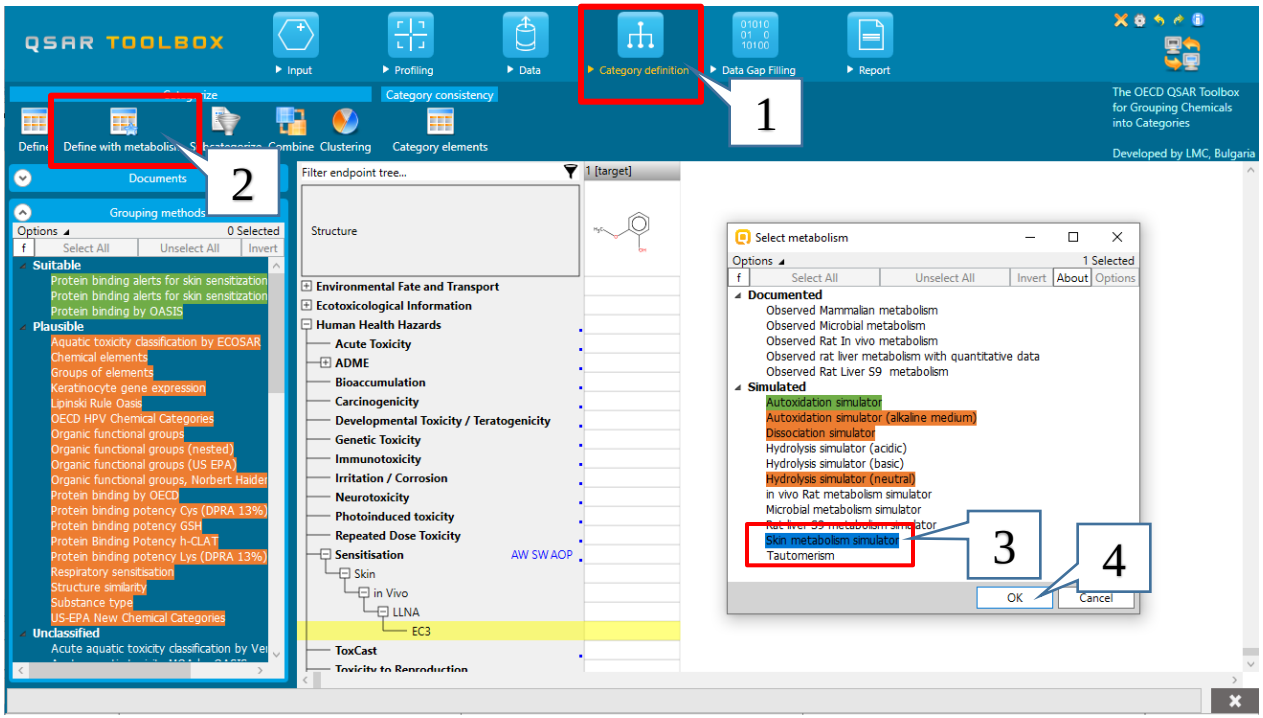
1. Go to **Data**;
2. Select **Skin sensitization** database;

Category Definition

Alert performance accounting for metabolism

The target chemical has no alert for protein binding as parent but it is activated as a result of skin metabolism. In this respect, the primary category will be defined with accounting for the metabolic activation.

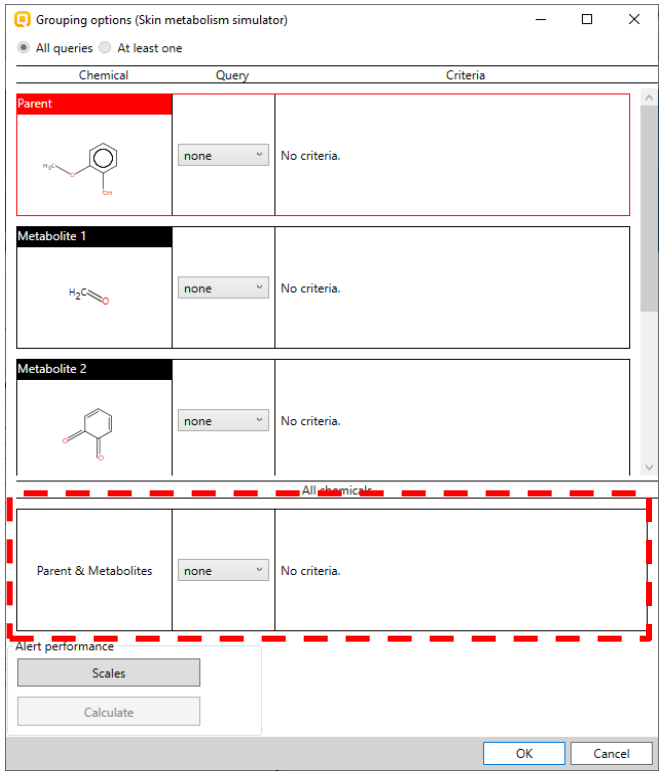
1. Go to ***Category definition*** module;
2. Click **Define with metabolism**;
3. Select *Skin metabolism simulator*.
4. Click **OK**.



Category Definition

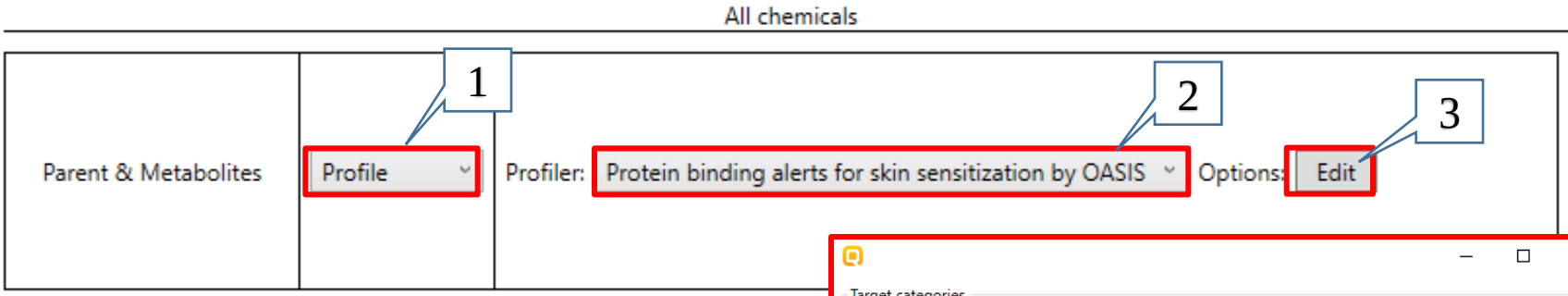
Alert performance accounting for metabolism

The system will search for chemicals which have similar distribution of the protein binding alerts as identified in the generated package parent and metabolites, accounting for the skin metabolism activation for the analogues.

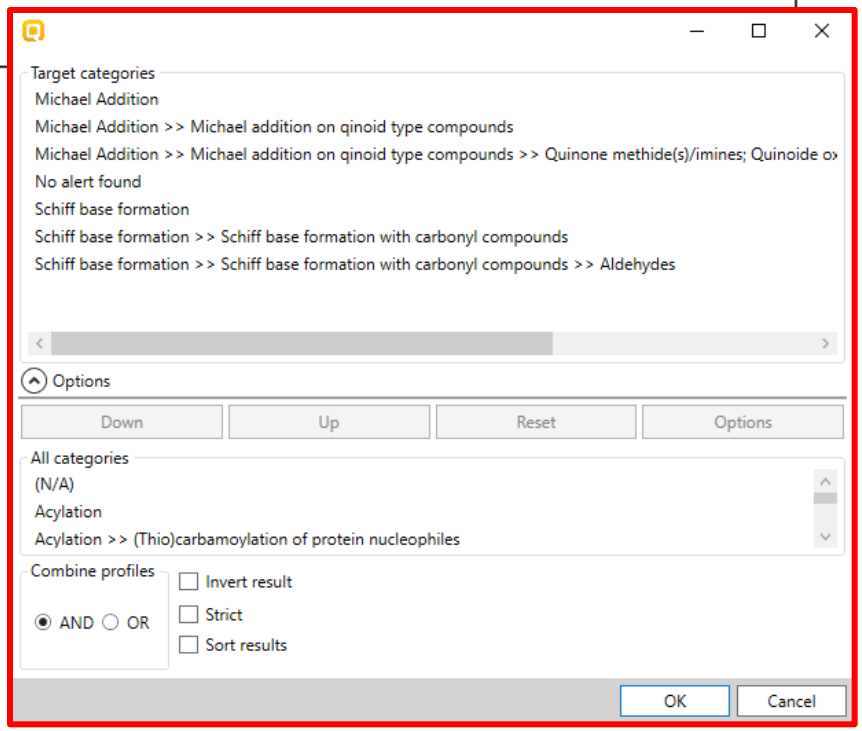


Category Definition

Alert performance accounting for metabolism

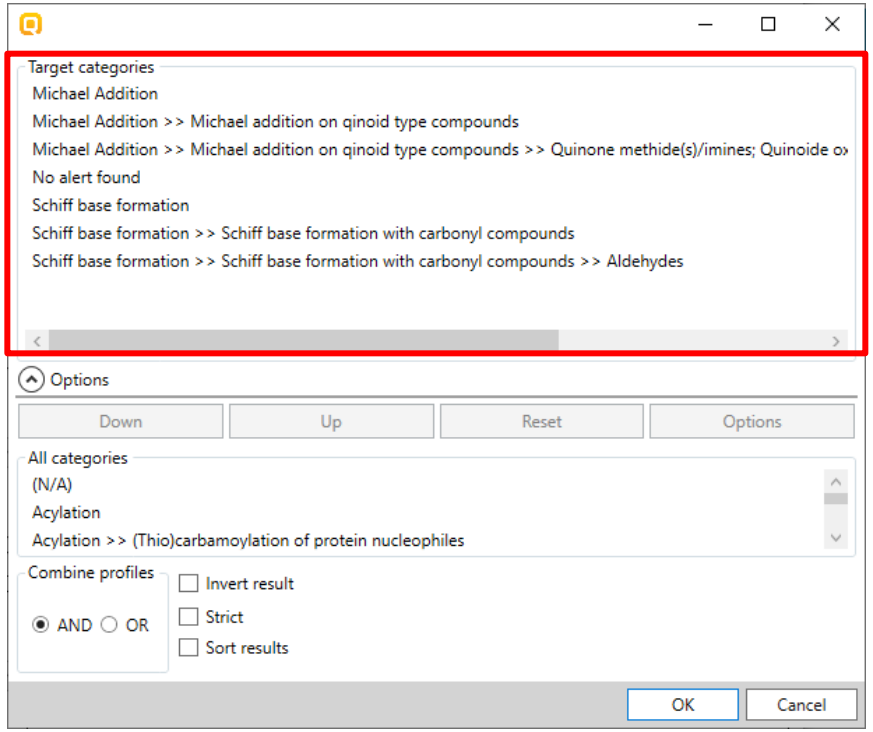


1. Select **Profile** from the drop-down
2. Select **Protein binding alerts for skin sensitization by OASIS** from the drop-down menu ;.
3. Click **Edit.**



Category Definition
Alert performance accounting for metabolism

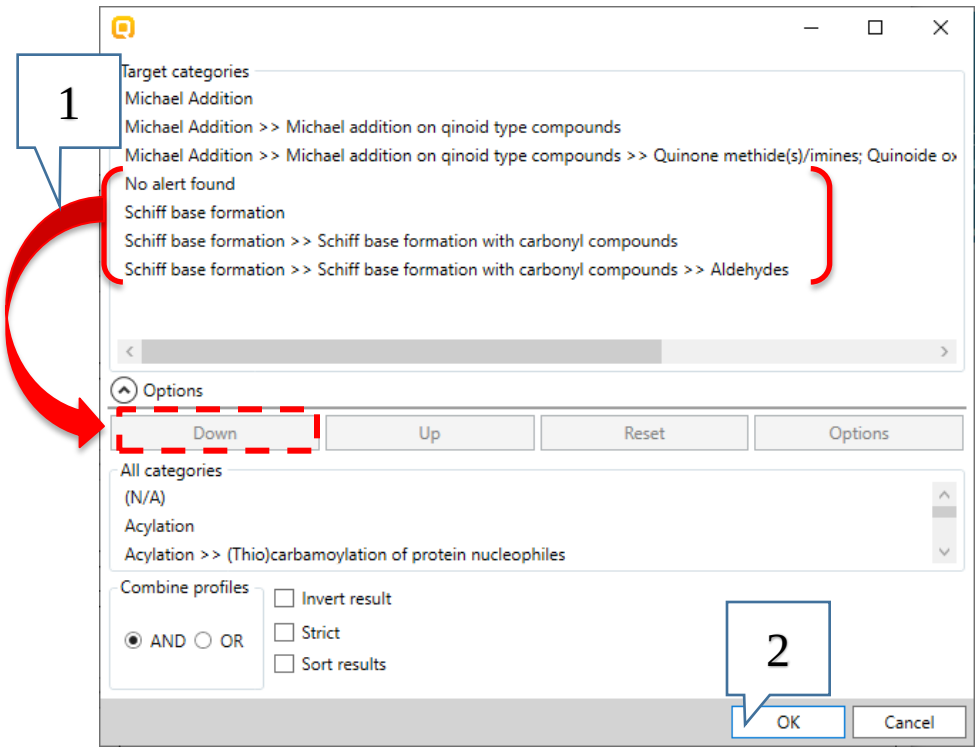
The found alerts could be seen by click on the **Edit** button. To calculate AP for one alert – remove all alerts except the alert for which AP will be calculated (three levels of mechanistic information are required – domain, mechanistic and structural alert), select a scale in the **Options** and click on **Calculate** (see next slide).

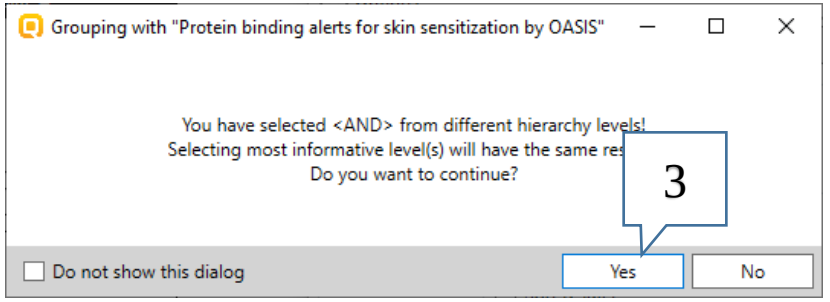


Category Definition

Calculation of Alert performance accounting metabolism for one alert

Now we will calculate AP for each of the alerts in order to see which of them is the most suitable for category formation.



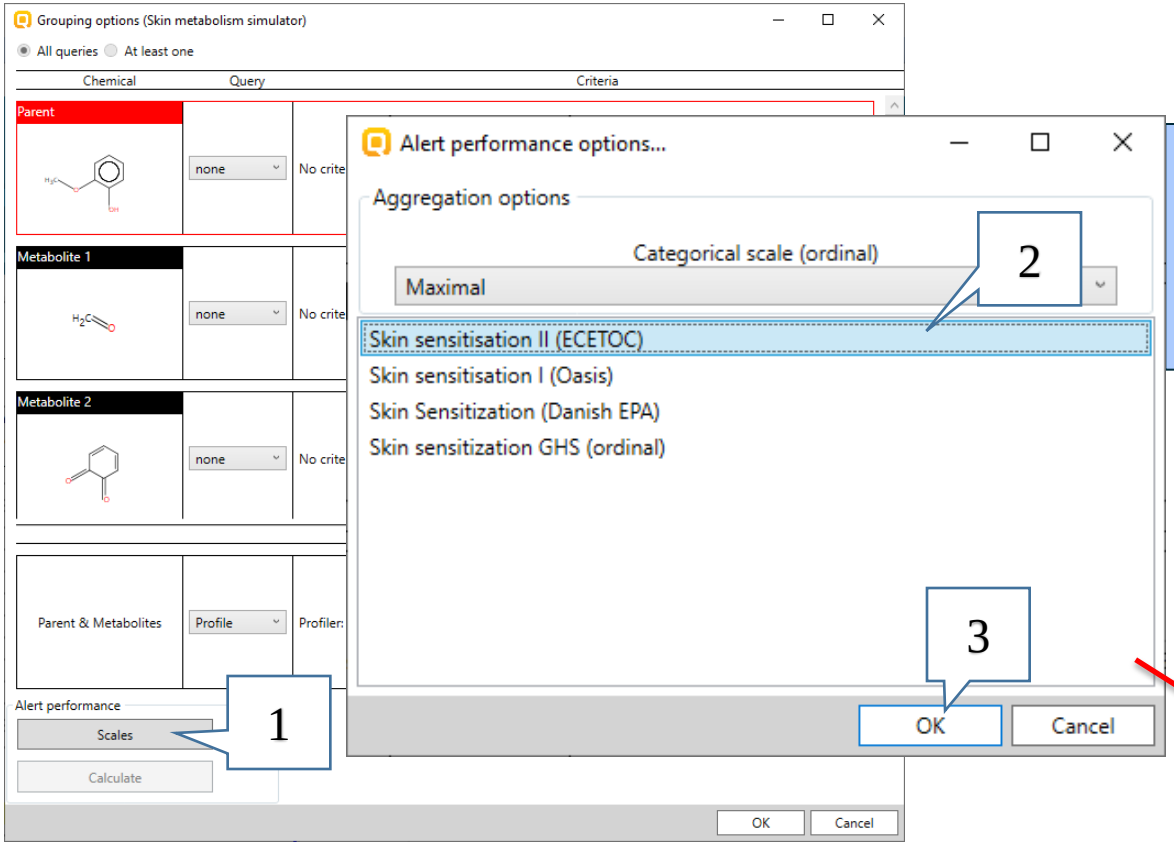


1. Remove ***No alert found*** and the second alert by double click or using **"Down"** button;
2. Click ***OK***.
3. Click ***OK*** in the Warning message.

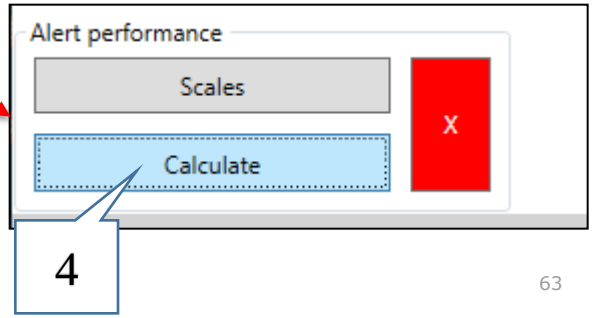
Category Definition

Calculation of Alert performance accounting metabolism for one alert

Now we will calculate AP for each of the alerts in order to see which of them is the most suitable for category formation.



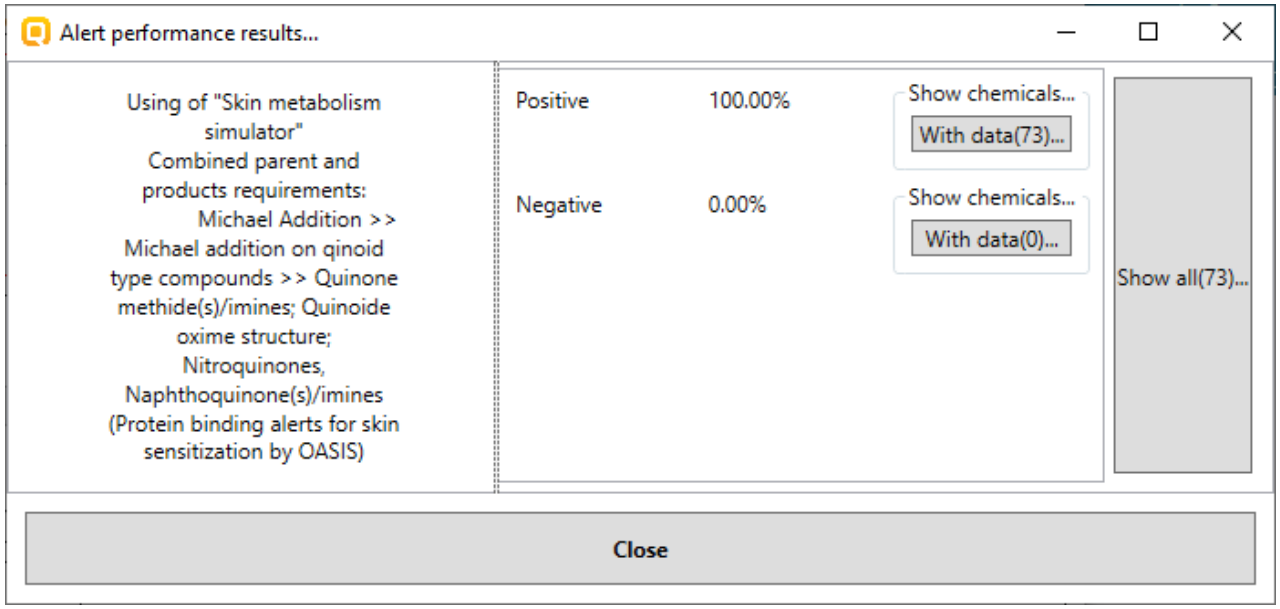
1. Click ***Scales;***
2. Select Skin sensitization II (ECETOC)
3. Click ***OK.***
4. Then click ***Calculate***



Category Definition

Calculation of Alert performance accounting metabolism for one alert

Performance of the first alert appears in the following window:



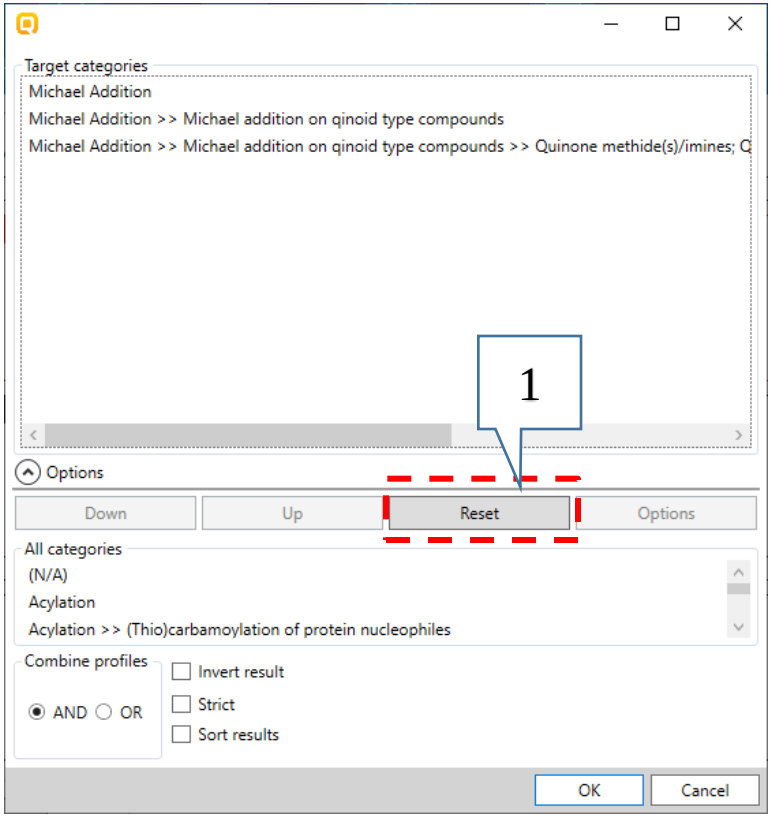
The system informs that 73 analogues with the searched alert accounting for skin metabolism have been found. All the chemicals have positive data (100%).

⚠ Keep in mind that the statistic is obtained from the chemicals and data, available in the selected databases

Category Definition

Calculation of Alert performance accounting metabolism for one alert

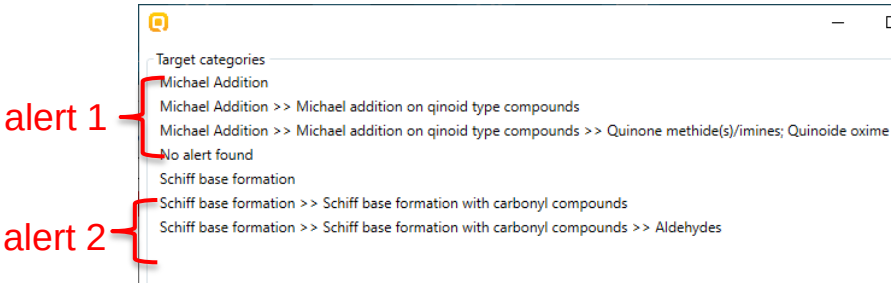
Click on the **Reset (1)** button and repeat the alert performance calculation steps for the second alert.

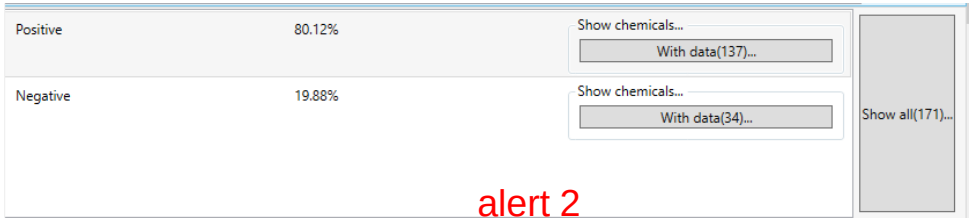


Category Definition

Calculation of Alert performance accounting metabolism for one alert

In summary we see that the first alert (alert 1) is the most suitable to define a category (because of its higher performance).

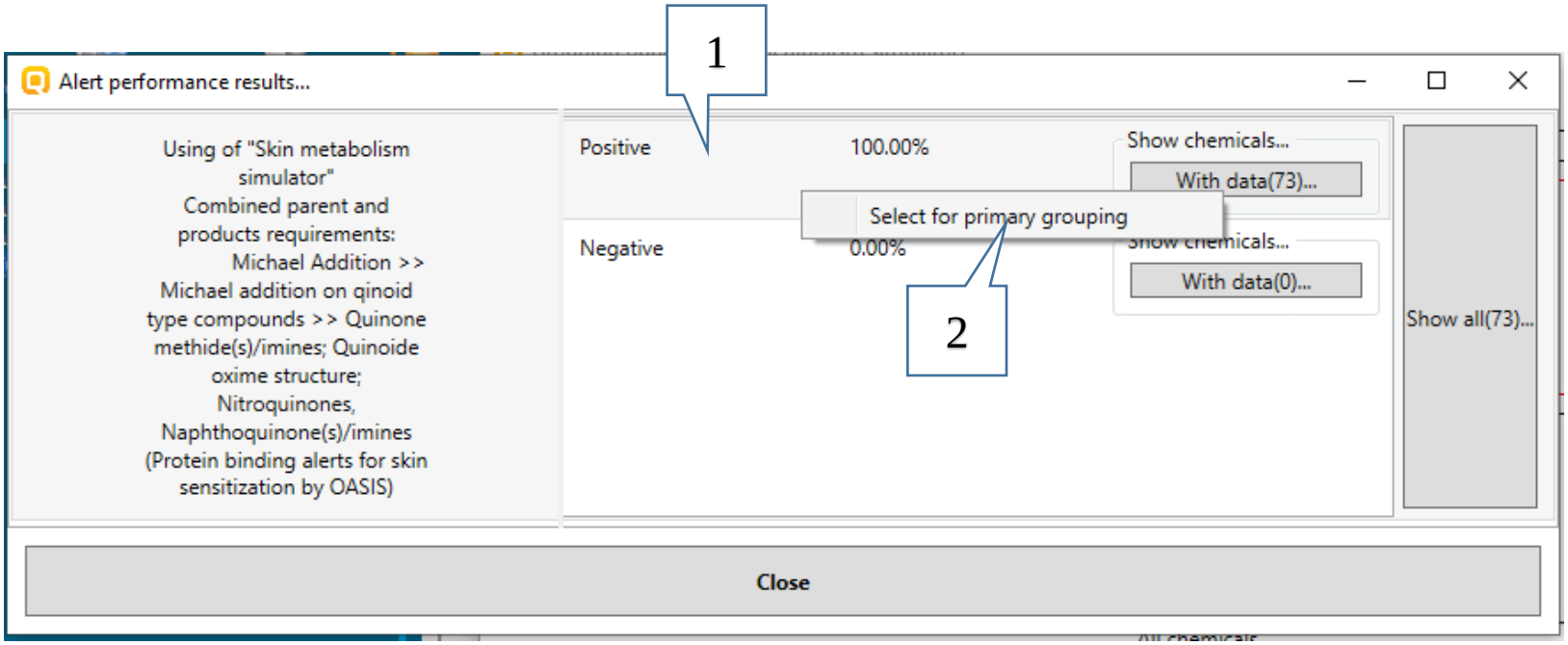




Category Definition

Calculation of Alert performance accounting metabolism for one alert

Right-click on the results (Positive/Negative) (1) to display ***Select for primary grouping***, select ***Select for primary grouping*** (2)



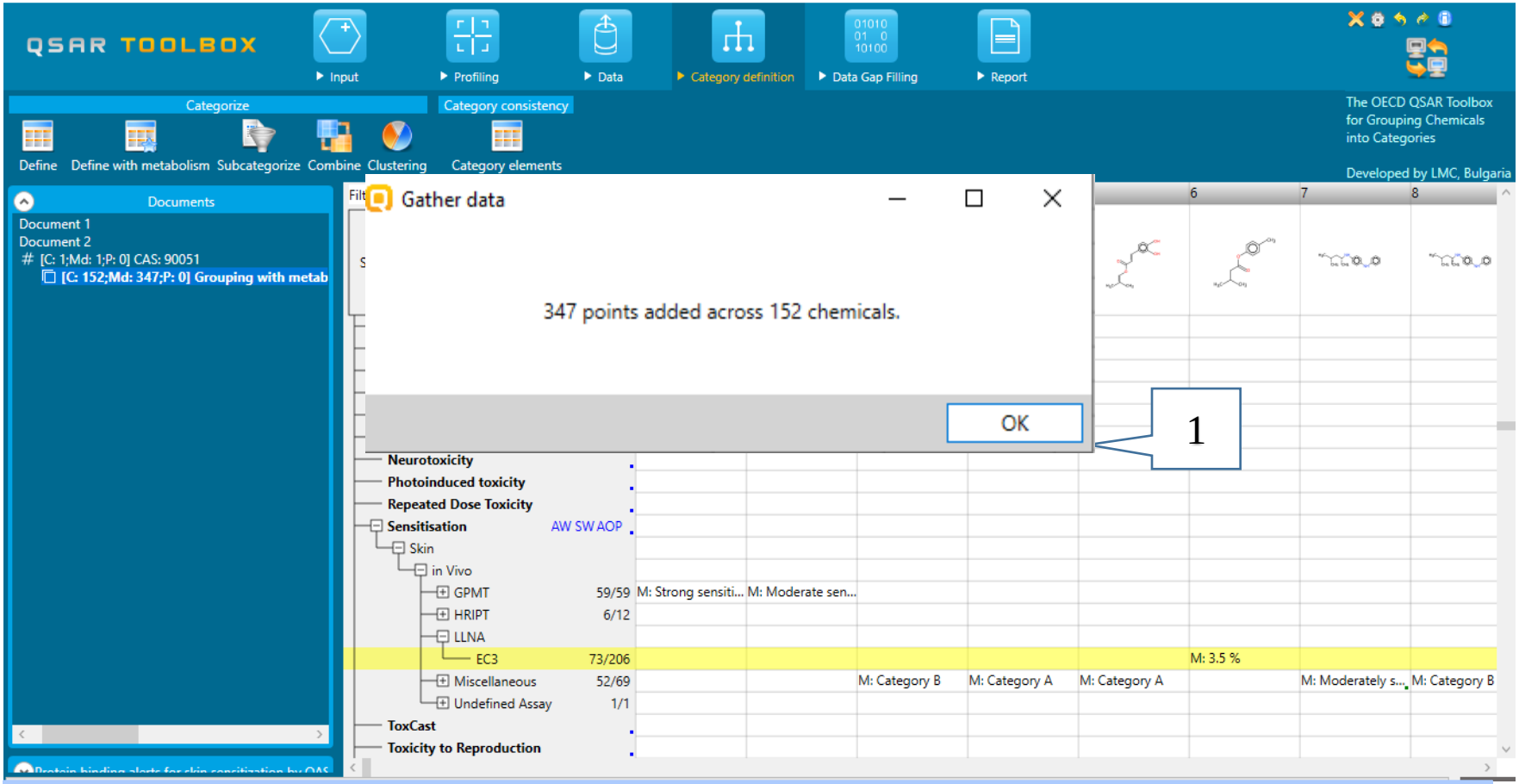
Category Definition
Analogues

- Based on the defined category (Michael Addition > Michael addition on quinoid type compounds > Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphtoquinone(s)/imines) 152 analogues have been identified (including the target chemical CAS: 90-05-1).

- The Toolbox automatically requests the user to select the endpoint data that should be retrieved.
- The user can either select the specific endpoint or by default choose to retrieve data on all endpoints (see below).

Only Skin sensitization database is selected in this example and we click **OK**.

Category Definition
Analogues

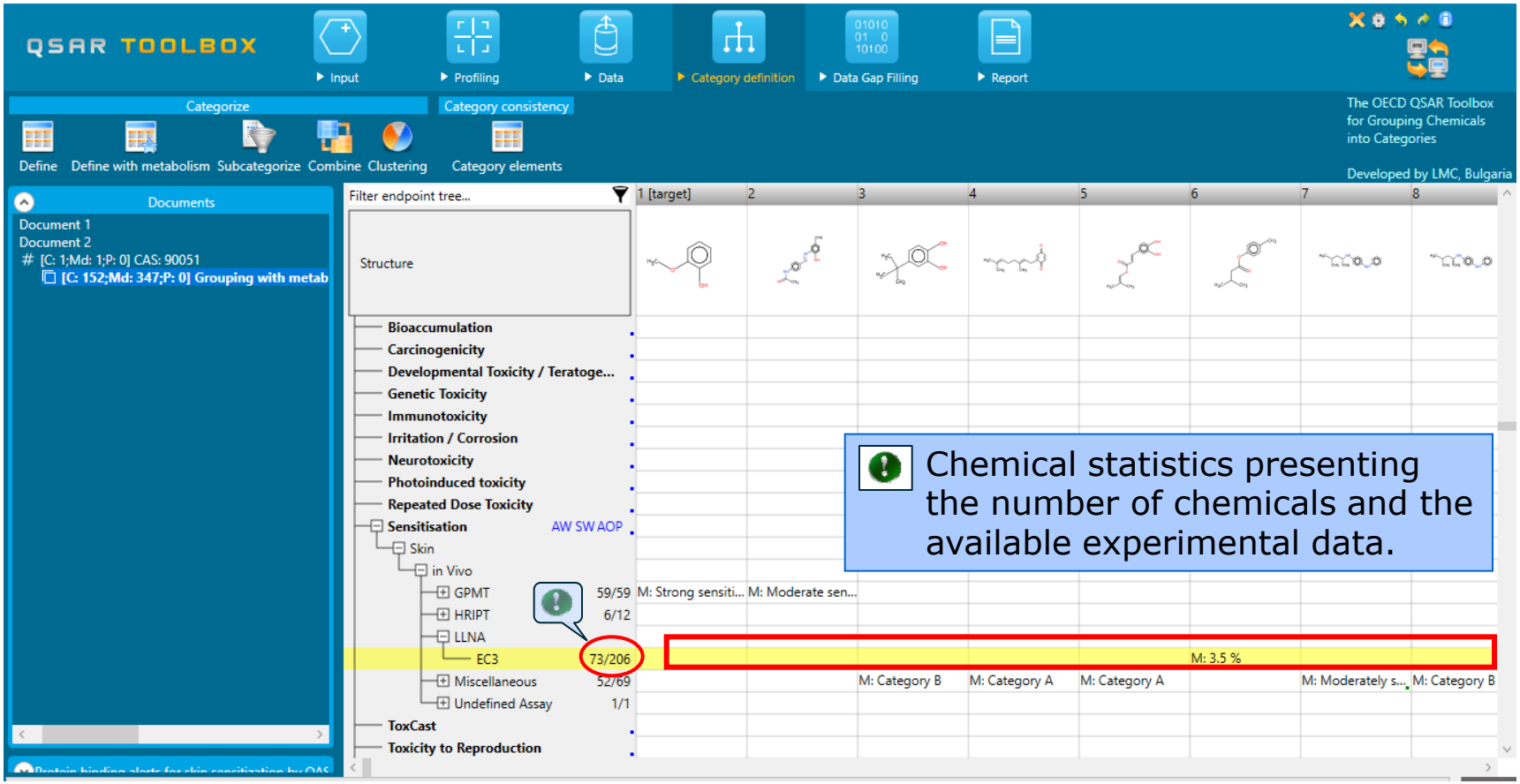


1. The Toolbox automatically informs the user for the number of collected data points across the chemicals in the category. Click **OK** to confirm.

Category Definition

Summary information for Analogues

73 chemicals with 206 experimental results related to the defined target endpoint are found.



Recap

- You have identified two protein binding alerts for the target chemical (2-methoxyphenol (guaiacol), CAS 90-05-1).
- You have calculated and compared the alert performance for each of the alerts.
- You have now retrieved in the available experimental results on skin sensitisation (EC3) values for 73 chemicals with the same mechanism of protein binding as the target compound, which were found in the "Skin Sensitisation" database.
- The user can now proceed to the next module; click on "*Data Gap Filling*".

Outlook

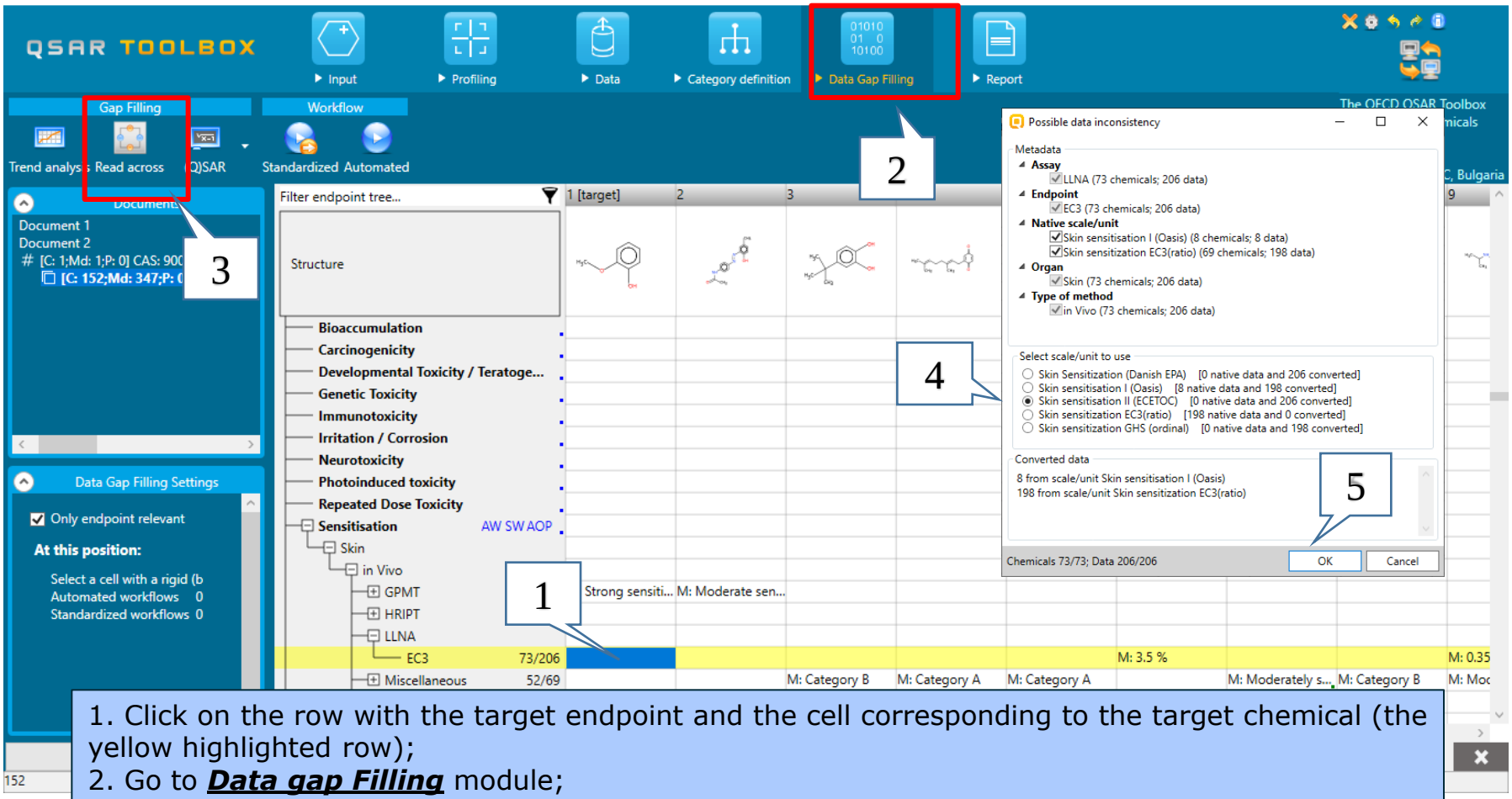
- Background
- Keywords
- Objectives
- Specific Aims
- Read across and analogue approach
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Data
  - Category definition
  - **Data Gap Filling**

Data Gap Filling
Overview

- "Data Gap Filling" module give access to five different data gap filling tools:
  - Read-across
  - Trend analysis
  - (Q)SAR models
  - Standardized workflow
  - Automated workflow
- Depending on the situation, the most relevant data gap mechanism should be chosen, taking into account the following considerations:
  - Read-across is the appropriate data-gap filling method for "qualitative" endpoints like skin sensitisation or mutagenicity for which a limited number of results are possible (e.g. positive, negative, equivocal). Furthermore read-across is recommended for "quantitative endpoints" (e.g., 96h-LC50 for fish) if only a low number of analogues with experimental results are identified.
  - Trend analysis is the appropriate data-gap filling method for "quantitative endpoints" (e.g., 96h-LC50 for fish) if a high number of analogues with experimental results are identified.
  - "(Q)SAR models" can be used to fill a data gap if no adequate analogues are found for a target chemical.
  - Standardized and Automated workflows are developed to facilitate the users work. Once started, they follow the implemented logic and finish with prediction. The general differences between the two type of workflows are represented on the next slide.

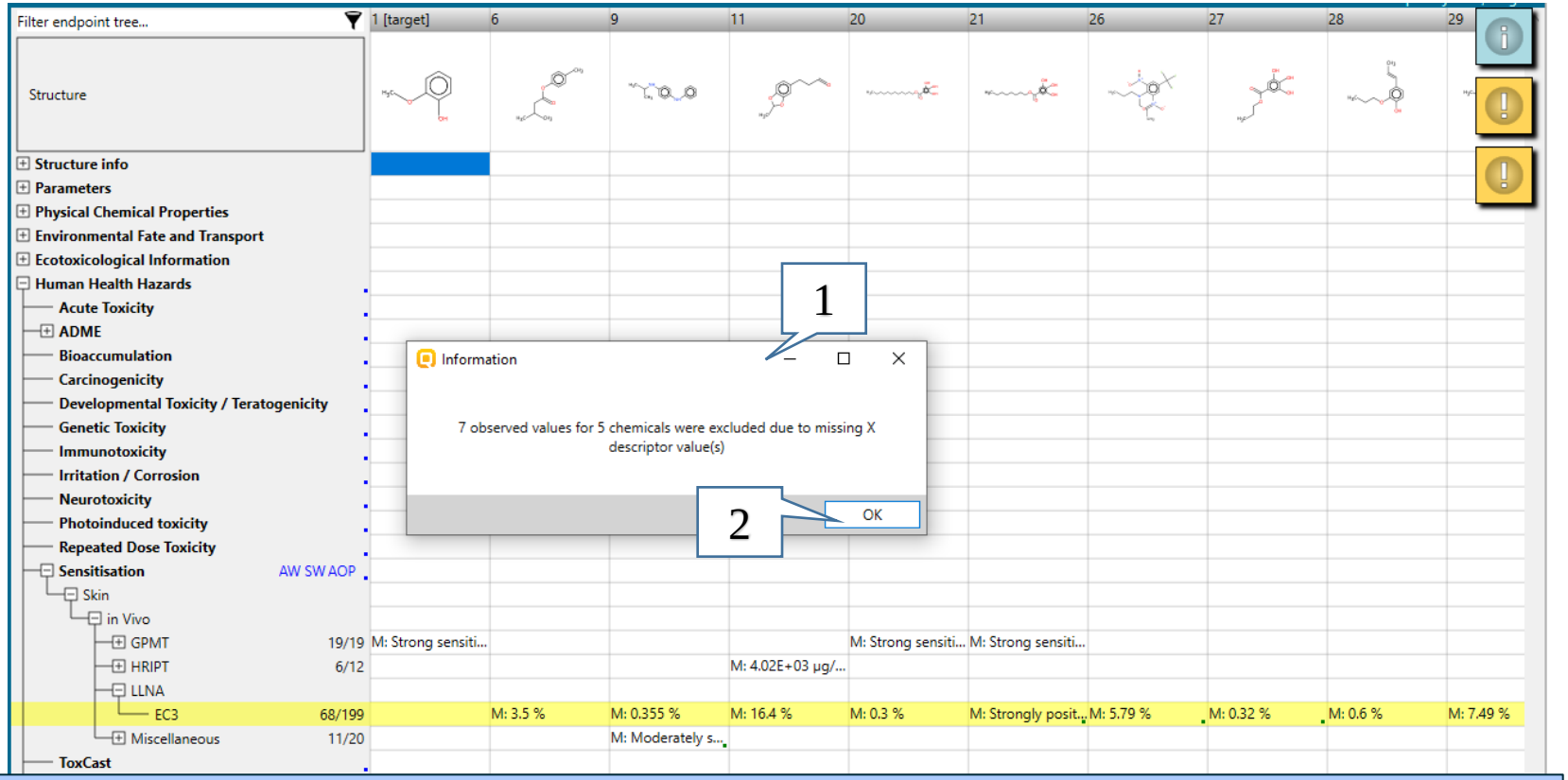
In this example we will use the manual read-across approach.

Data Gap Filling
Apply Read across



1. Click on the row with the target endpoint and the cell corresponding to the target chemical (the yellow highlighted row);
2. Go to ***Data gap Filling*** module;
3. Click on **Read-across** button;
4. Select ***Skin sensitisation II (ECETOC);***
5. Click **OK.**

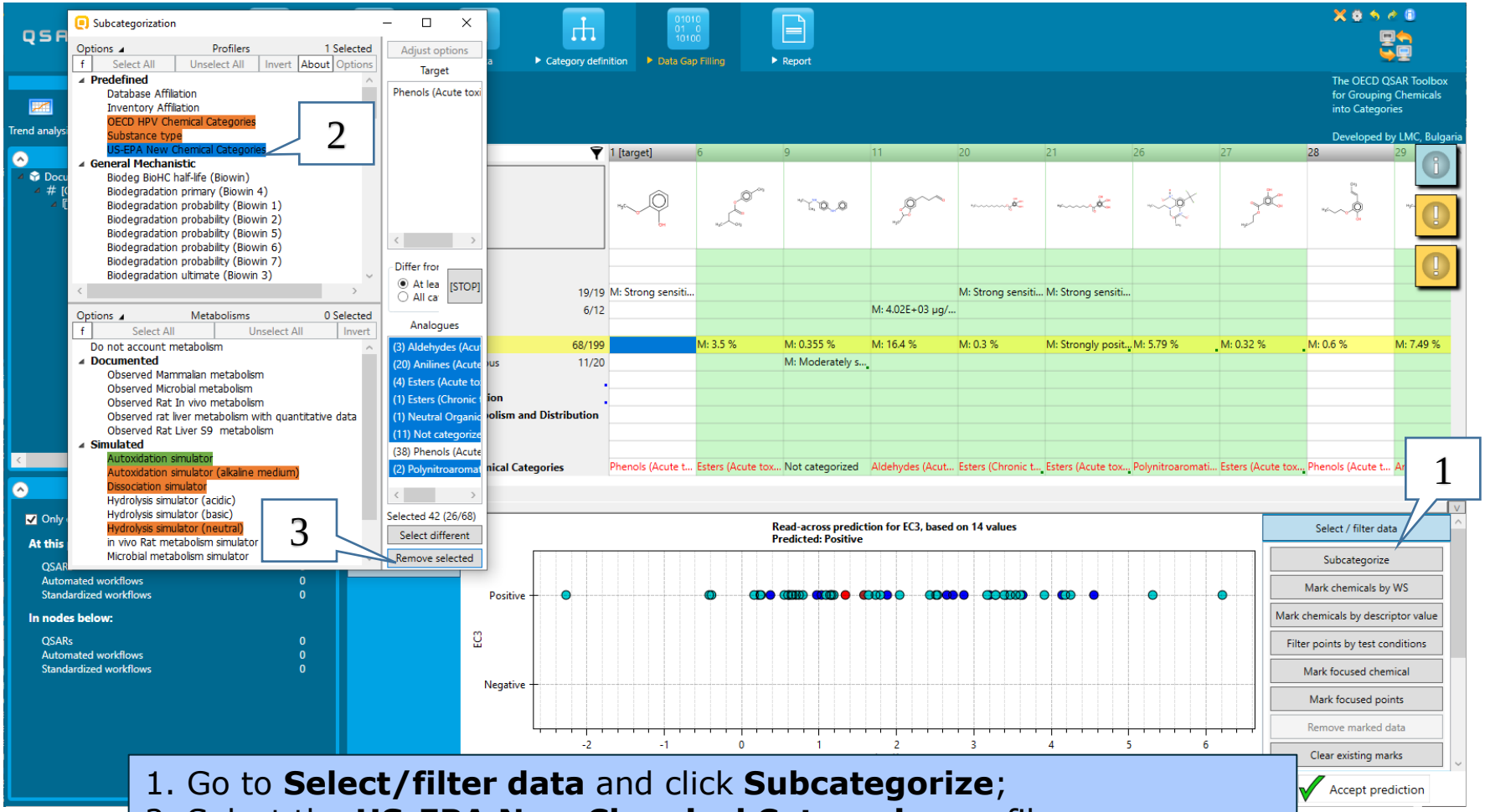
Data Gap Filling
Apply Read across



1. The Toolbox informs the user that 7 observed values for 5 chemicals were excluded due to missing X descriptor values. The reason for exclusion is that log Kow for these chemicals cannot be calculated (most probably these are mixtures, UVCB substances or some metal containing chemicals for which EPISUITE program cannot return result). The log Kow is the default X-descriptor for the read-across approach.
2. Click **OK.**

Data Gap Filling

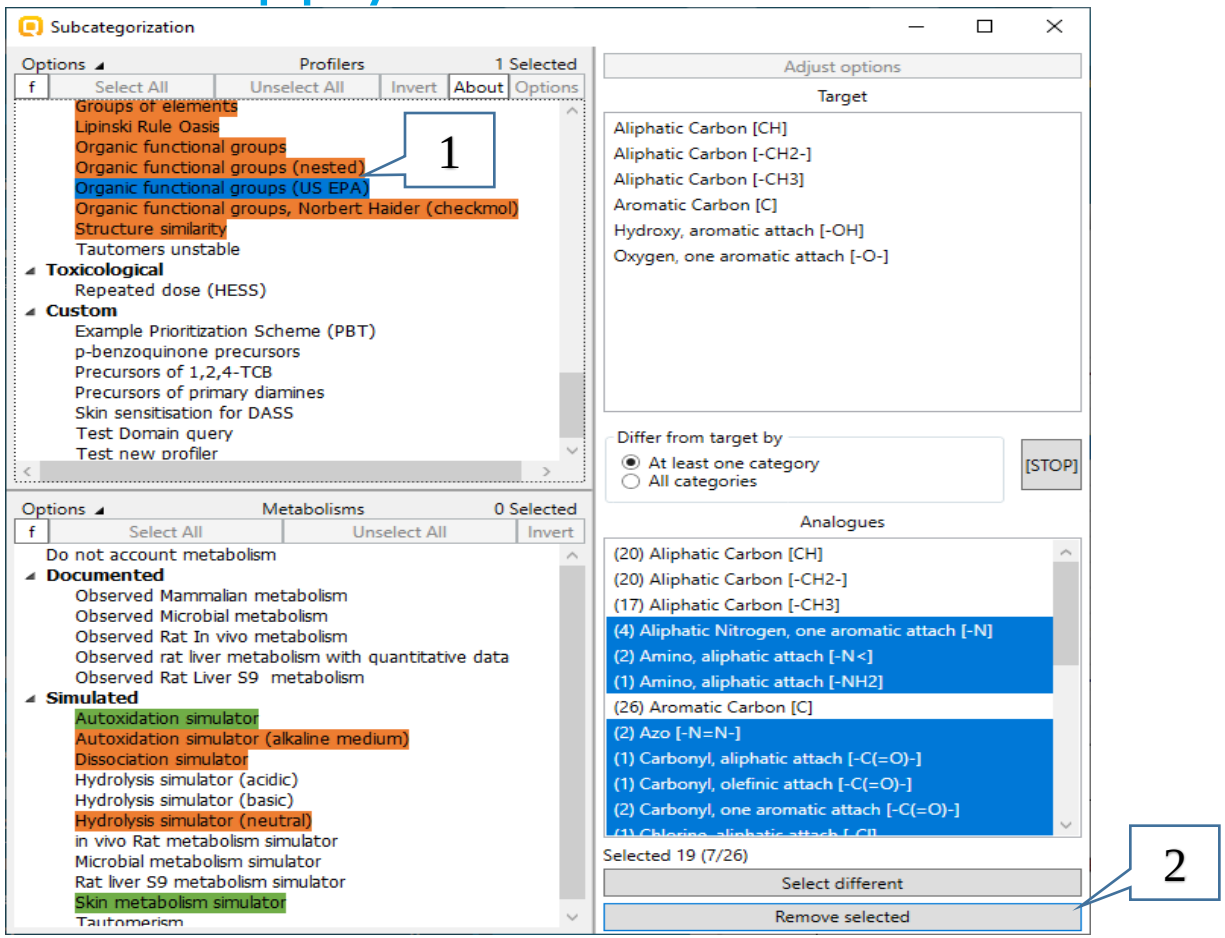
Apply Read across



1. Go to **Select/filter data** and click **Subcategorize**;
2. Select the **US-EPA New Chemical Categories** profiler;
3. Click **Remove selected**

Data Gap Filling

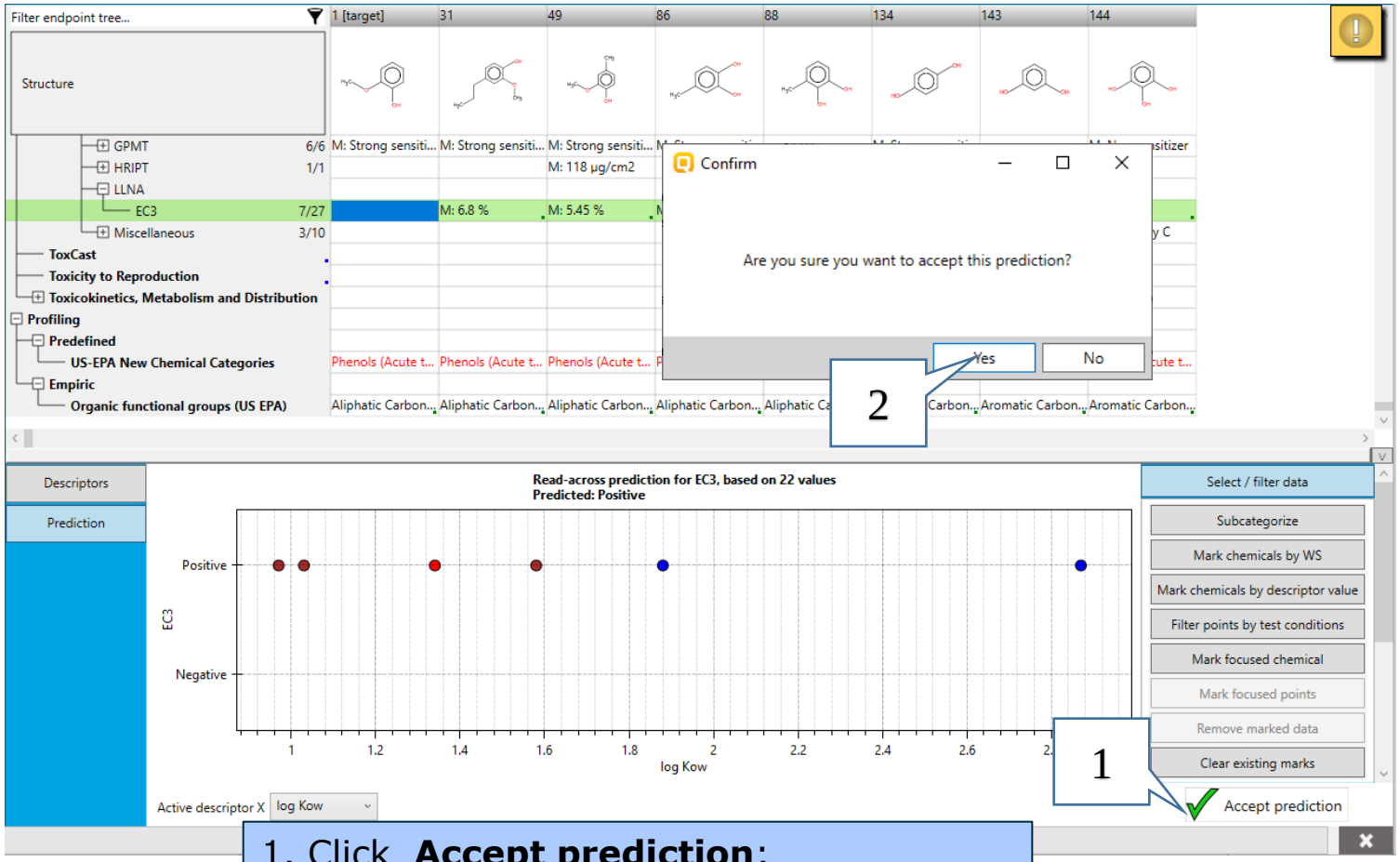
Apply Read across



1. Select the **Organic functional groups (US EPA)** profiler;
2. Click **Remove selected**

Data Gap Filling

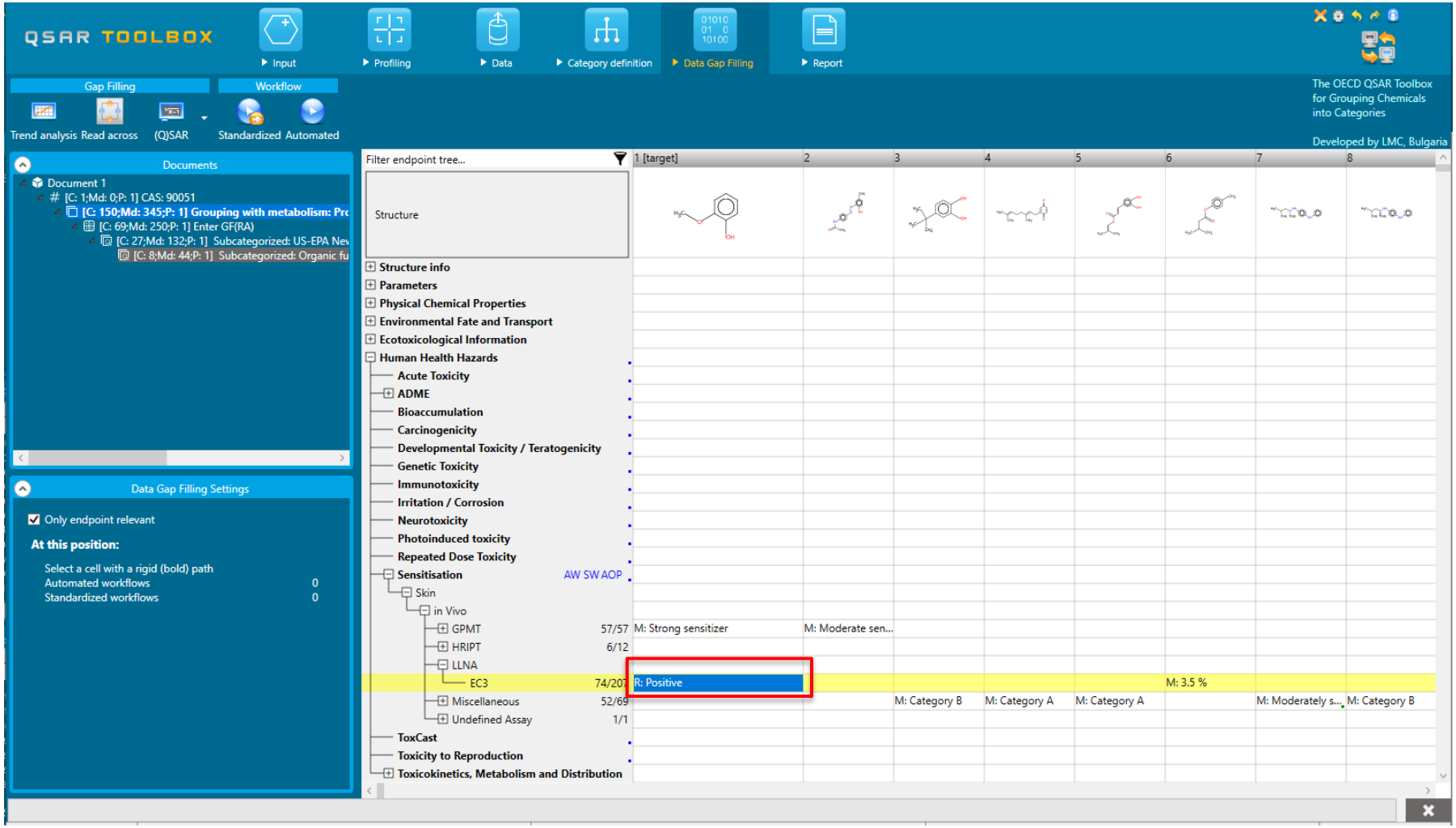
Accepting the predicted result



1. Click **Accept prediction**;
2. Confirm with "**Yes**"

Data Gap Filling

Accepting the predicted result



Recap

- Read-across is the appropriate data-gap filling method for "qualitative" endpoints like skin sensitisation. Since the most of the analogues and all five neighbouring tested chemicals in the category were positive, it was easy to accepting the prediction of positive for the target chemical.
- You are now ready to complete the final module and to create the report.
- Click on "Report" to proceed to the last module.

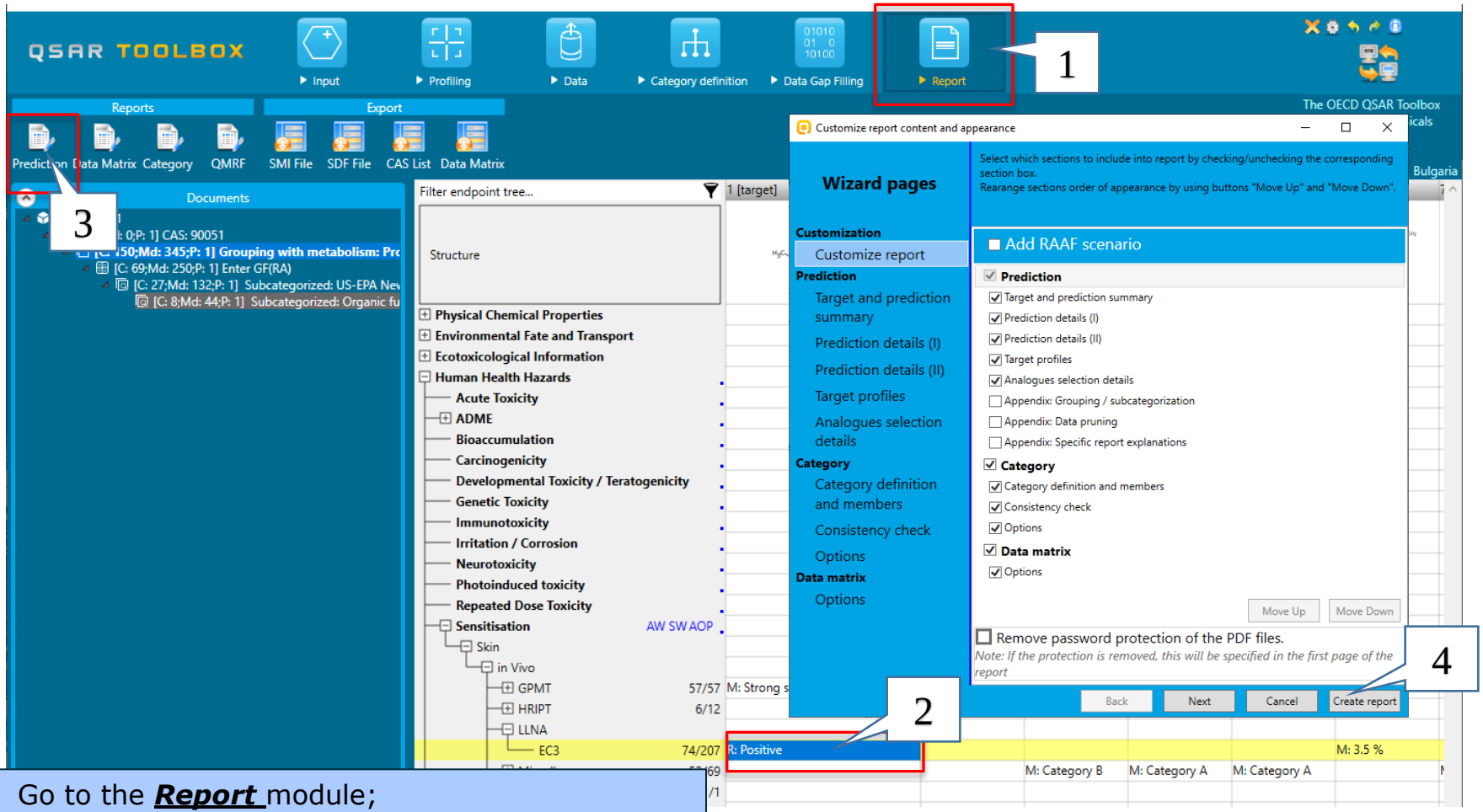
Outlook

- Background
- Keywords
- Objectives
- Specific Aims
- Read across and analogue approach
- The exercise
- **Workflow**
  - Chemical Input
  - Profiling
  - Endpoint
  - Category definition
  - Data Gap Filling
  - **Report**

Report
Overview

- The report module can generate reports of predictions performed with the Toolbox.
- The report module contains a predefined report template which users can customize.

Report
Generation report

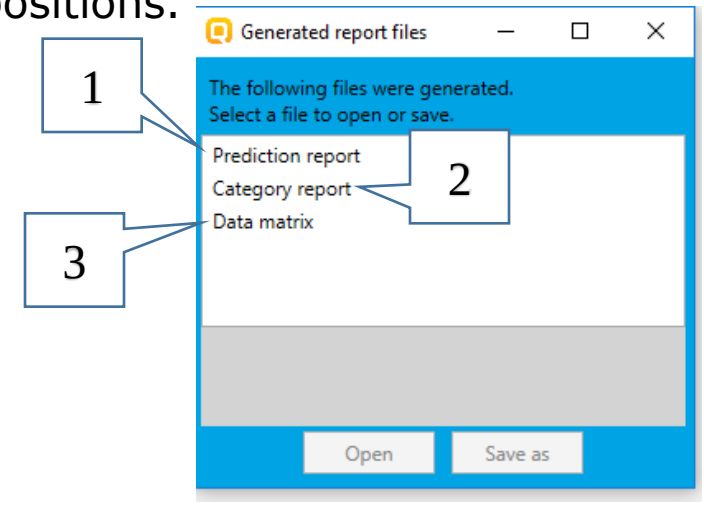


1. Go to the ***Report*** module;
2. Click on the cell with the read-across prediction;
3. Click **Prediction**;
4. Click **Create report.**

Report
Generation report

After clicking **Create report** button, *Generated report files* window appears. It contains three type of files:

1) **Prediction report** - a PDF file containing the prediction information related to the target.
2) **Category report** - a PDF file containing the justification for the consistency of the category with respect to the defined endpoint.
3) **Data matrix** - a MS Excel file containing chemicals used for prediction along with their data for selected parameters, profiles and endpoint tree positions.



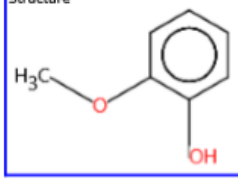
Report
Generated report files

Prediction report

QSAR Toolbox prediction for single chemical

Date: 15 Apr 2020
Author(s):
Contact details:

Target information

Structural information	Numerical identifiers	Chemical names
SMILES: COc1ccccc1O Structure 	CAS#: 90-05-1 Other: EC Number:2019647	"2-methoxyphenol (guaiacol);guaiacol;phenol, 2-methoxy-;2-methoxy-phenol;2-methoxyphenol;o-methoxyphenol;o-methoxy phenol;phenol, o-methoxy-;guaiacol [jan]" 2-methoxy-phenol 2-Methoxyphenol

Prediction summary

Predicted endpoint: EC3; No effect specified; No species specified; No duration specified; No guideline specified
Predicted value: Positive
Unit/scale: Skin sensitisation II (ECETOC)
Data gap filling method: Read-across analysis
Summary: *manually editable field*
Not provided by the user

Category report

QSAR Toolbox report for category

1. Category definition

1.1. Category definition

Category name *manually editable field*
Not provided by the user

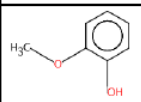
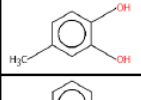
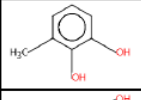
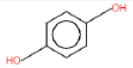
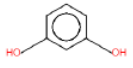
Covered (target) endpoint(s)
- Human Health Hazards/Sensitisation: EC3, LLNA, in Vivo, Skin

Category hypothesis *manually editable field*
Not provided by the user

1.2. Category members

Information of category members

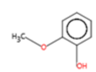
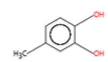
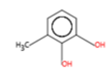
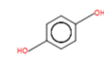
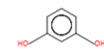
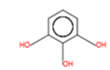
Table of category members

#	CAS	Name	SMILES	Structure
1	90-05-1	guaiacol	COc1ccccc1O	
2	452-86-8	4-Methylcatechol	Cc1ccc(O)c(O)c1	
3	488-17-5	3-methylcatechol	Cc1cccc(O)c1O	
4	123-31-9	p-Quinol	Oc1ccc(O)cc1	
5	108-46-3	resorcin	Oc1cccc(O)c1	

Report

Generated report files

Data matrix report

	Target chemical	Neighbour #1	Neighbour #2	Neighbour #3	Neighbour #4	Neighbour #5
Substance identity						
Structure						
CAS number	90-05-1	452-86-8	488-17-5	123-31-9	108-46-3	87-66-1
Chemical name	guaiacol	4-Methylcatechol	3-methylcatechol	p-Quinol	resorcin	Pyrogallol
Other identifier						
SMILES	COc1ccccc1O	Cc1ccc(O)c(O)c1	Cc1cccc(O)c1O	Oc1ccc(O)cc1	Oc1cccc(O)c1	Oc1cccc(O)c1O
Profilers						
Profiles used for grouping/subcategorization						
Using of "Skin metabolism simulator" Combined parent and products requirements: Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines (Protein binding alerts for skin sensitization by OASIS) (primary grouping)	Parent and 4 metabolite(s):; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on qinoid type compounds, Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes	Parent and 2 metabolite(s):; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on qinoid type compounds, Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found	Parent and 2 metabolite(s):; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on qinoid type compounds, Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found	Parent and 1 metabolite(s):; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on qinoid type compounds, Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found	Parent and 9 metabolite(s):; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on qinoid type compounds, Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found	Parent and 5 metabolite(s):; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on qinoid type compounds, Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found
US-EPA New Chemical Categories	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)
Organic functional groups (US EPA) (subcategorization)	Aliphatic Carbon [CH]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [-CH3]; Aromatic Carbon [C]; Hydroxy, aromatic attach [-OH]; Oxygen, one aromatic attach [-O-]	Aliphatic Carbon [CH]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [-CH3]; Aromatic Carbon [C]; Hydroxy, aromatic attach [-OH]; Oxygen, one aromatic attach [-O-]	Aliphatic Carbon [CH]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [-CH3]; Aromatic Carbon [C]; Hydroxy, aromatic attach [-OH]; Oxygen, one aromatic attach [-O-]	Aromatic Carbon [C]; Hydroxy, aromatic attach [-OH]; Oxygen, one aromatic attach [-O-]	Aromatic Carbon [C]; Hydroxy, aromatic attach [-OH]; Oxygen, one aromatic attach [-O-]	Aromatic Carbon [C]; Hydroxy, aromatic attach [-OH]; Oxygen, one aromatic attach [-O-]
Endpoint Specific						
Protein binding alerts for skin sensitization by						

		Target chemical			Neighbour #1			Neighbour #2			Neighbour #3			Neighbour #4			Neighbour #5		
Measured and predicted data																			
Data used for prediction																			
sublevel	*endpoint*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*	*value*	*unit*	*species, duration, test type, type of method, assay, strain, test guideline, year, reference, database*
Sensitisation	EC3				Strongly positive	Skin sensitisation l (Oasis)	Test organisms (species): mouse Endpoint: EC3 Type of method: in Vivo Assay: LLNA Year: 2002 Reference source: Test organisms	1	%	Test organisms (species): mouse Endpoint: EC3 Type of method: in Vivo Assay: LLNA Year: 2005 Reference source:	1.67	%	Test organisms (species): mouse Endpoint: EC3 Type of method: in Vivo Assay: LLNA Year: 1999 Author: Lea	5.92	%	Test organisms (species): mouse Endpoint: EC3 Type of method: in Vivo Assay: LLNA Year: 2008 Author: ICCVAM, 2008. Test organisms	1.4	%	Test organisms (species): mouse Endpoint: EC3 Type of method: in Vivo Assay: LLNA Author: Submitted by National Toxicology

Congratulations!

- You have now been introduced to the definition of target endpoint;
- You have now been familiarized with the meaning of the different colouring of the profilers and databases.
- You have now been introduced to the consecutive steps of the calculation of alert performance.
- Note, proficiency comes with practice!