QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.2

An example illustrating RAAF Scenario 2 and related assessment elements

Outlook

- **Background**
- Objectives
- Specific Aims
- Read Across Assessment Framework (RAAF)
- The exercise
- Workflow

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and justification of the outcome.
- The read-across prediction will be justified by fulfilling all information requirements according to the Read Across Assessment Framework (RAAF).

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Define target endpoint;
- Calculation of alert performance (AP) accounting for metabolism;
- Searching of analogues accounting for metabolism;
- Category consistency check;
- Selection of RAAF scenario;
- Filling in the report sections related to each read-across assessment element.

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Specific Aims

- To familiarize the user with the Read-Across Assessment Framework (RAAF) and more specifically with Scenario 2;
- To introduce to the user the read across assessment elements;
- To introduce to the user the report basket;
- To provide sufficient information allowing a scientific assessment of the outcome;
- To explain to the Toolbox user the rationale behind each step of the exercise.

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- Specific Aims
- **Read-Across Assessment Framework (RAAF)**
- The exercise
- Workflow

Read-Across Assessment Framework (RAAF)

Overview

- RAAF was developed by ECHA as an internal tool which provides a framework for a consistent and structured assessment of grouping and read across approaches under REACH.
- The outcome of the assessment is a conclusion on whether the read across is scientifically acceptable or not.
- The RAAF defines different scenarios for different read-across approaches.
- Each scenario is associated with particular aspects (assessment elements, AEs).
- Total six scenarios are available: two for analogue approach and four for category approach.

Read-Across Assessment Framework (RAAF)

Selection of a RAAF scenario

SCENARIO	APPROACH	READ-ACROSS HYPOTHESIS BASED ON	QUANTITATIVE VARIATIONS
1	Analogue	(Bio)transformation to common compound(s)	Property of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach.
2	Analogue	Different compounds have qualitatively similar properties	Properties of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach.
3	Category	(Bio)transformation to common compound(s)	Variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach.
4	Category	Different compounds have qualitatively similar properties	Variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach.
5	Category	(Bio)transformation to common compound(s)	No relevant variations in properties observed among source substances and the same strength predicted for the target substance.
6	Category	Different compounds have qualitatively similar properties	No relevant variations in properties observed among source substances and the same strength predicted for the target substance

*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Read-Across Assessment Framework (RAAF)

Selection of a RAAF scenario

- Distinguish whether it is an analogue or a category approach*
- To identify the basis of the read across hypothesis
  - (Bio)transformation to common compound(s) – the read across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed
  - Different compounds have the same type of effect(s) – the read across hypothesis is that the organism is not exposed to common compounds but rather, as a result of similarity, that different compounds have similar (eco)toxicological and fate properties. These compounds may be the source and target substances themselves or one or more of their (bio)transformation products.
- For a category approach there is a need to take further account whether or not quantitative variations in the properties are observed among the category members

*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Read-Across Assessment Framework (RAAF)

Selection of RAAF scenario

- Each scenario consists of a pre-defined set of assessment elements (AEs) that, when taken together, cover all of the essential scientific aspects that need to be addressed in the read-across approach for a particular scenario.*
- Each AE reflects a critical scientific aspect of a read-across.
- The AEs could be:
  - **common** for all scenario within one approach - common AEs for Scenario 1 and 2 (analogue approach) and common AEs for Scenario 3, 4, 5 and 6 (category approach)
  - **specific** – addressing specific scenario.

*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

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- Read-Across Assessment Framework (RAAF)
- **The example**
- Workflow

The Example

- In this example we will predict the skin sensitization potential of *1,3-Propanediamine, N-(3-aminopropyl)-* [CAS# 56-18-8], which will be the "target" chemical;
- The category will be defined by the mechanism of protein binding accounting for metabolism common to all the chemicals in the category;
- A read-across approach will be used for the prediction. The read-across will be based on analogue approach expressed as common underlying mechanism for metabolites of source and target substances;
- Read-across assessment elements will be included to the report;
- Examples for the possible content of each of AEs will be provided.

The Example
Sidebar 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.
- However, there is growing agreement that most organic chemicals must react covalently with skin proteins in order to behave as skin sensitizers.
- Therefore, mechanisms by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents.

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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

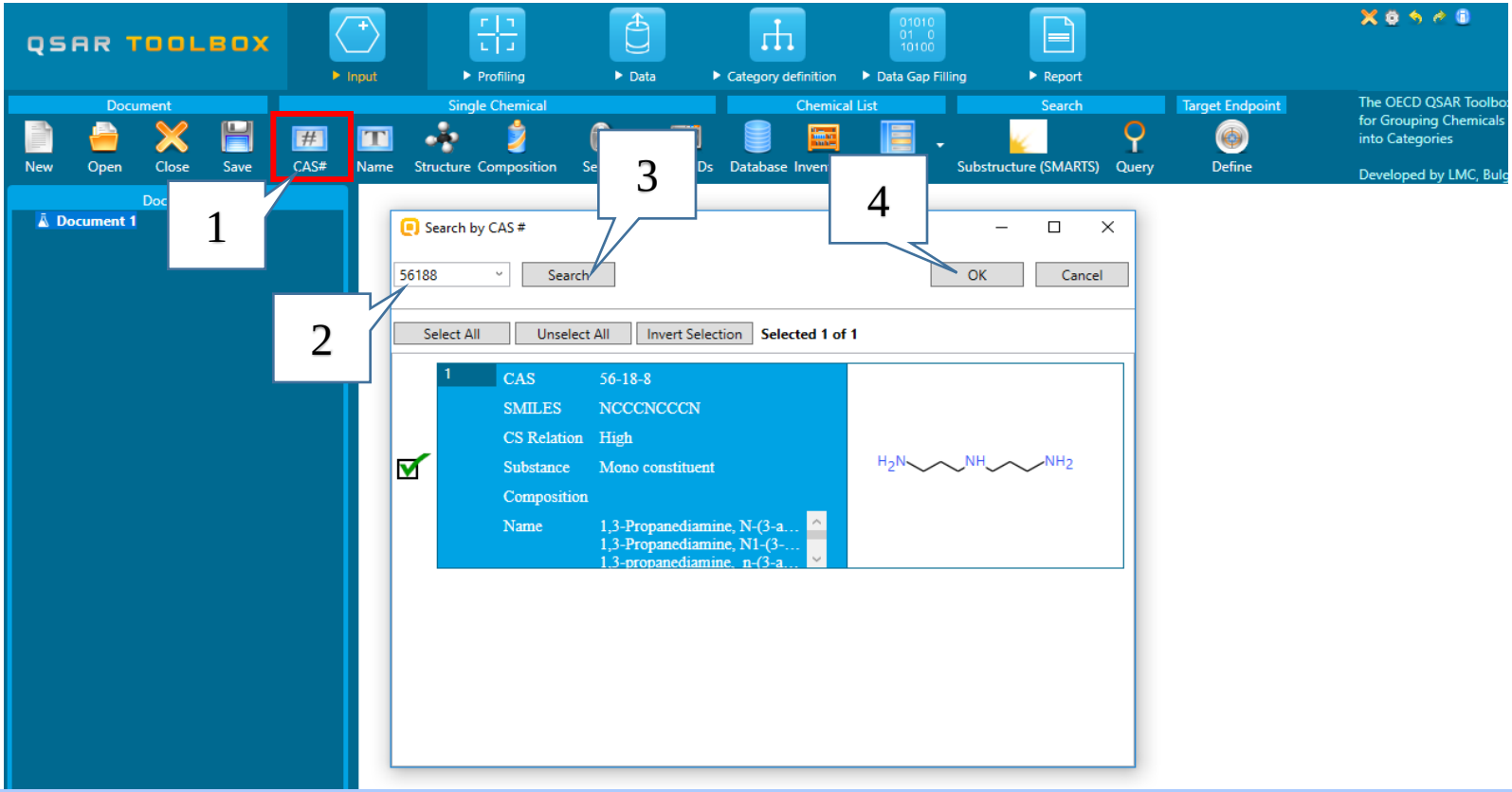
The modules will be presented in different sequence than the one showed above.

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 Screen

Input target chemical by CAS#

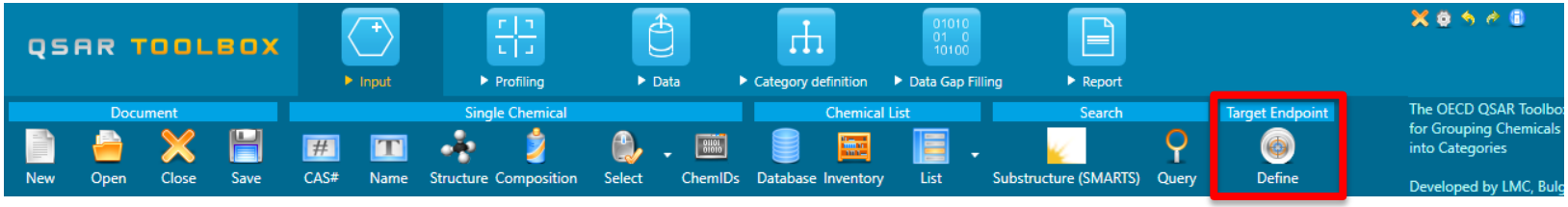


Click **CAS#** button (1); Type CAS **56-18-8** in the blank field (2) and click **Search** (3). When the structure appears, click **OK** (4).

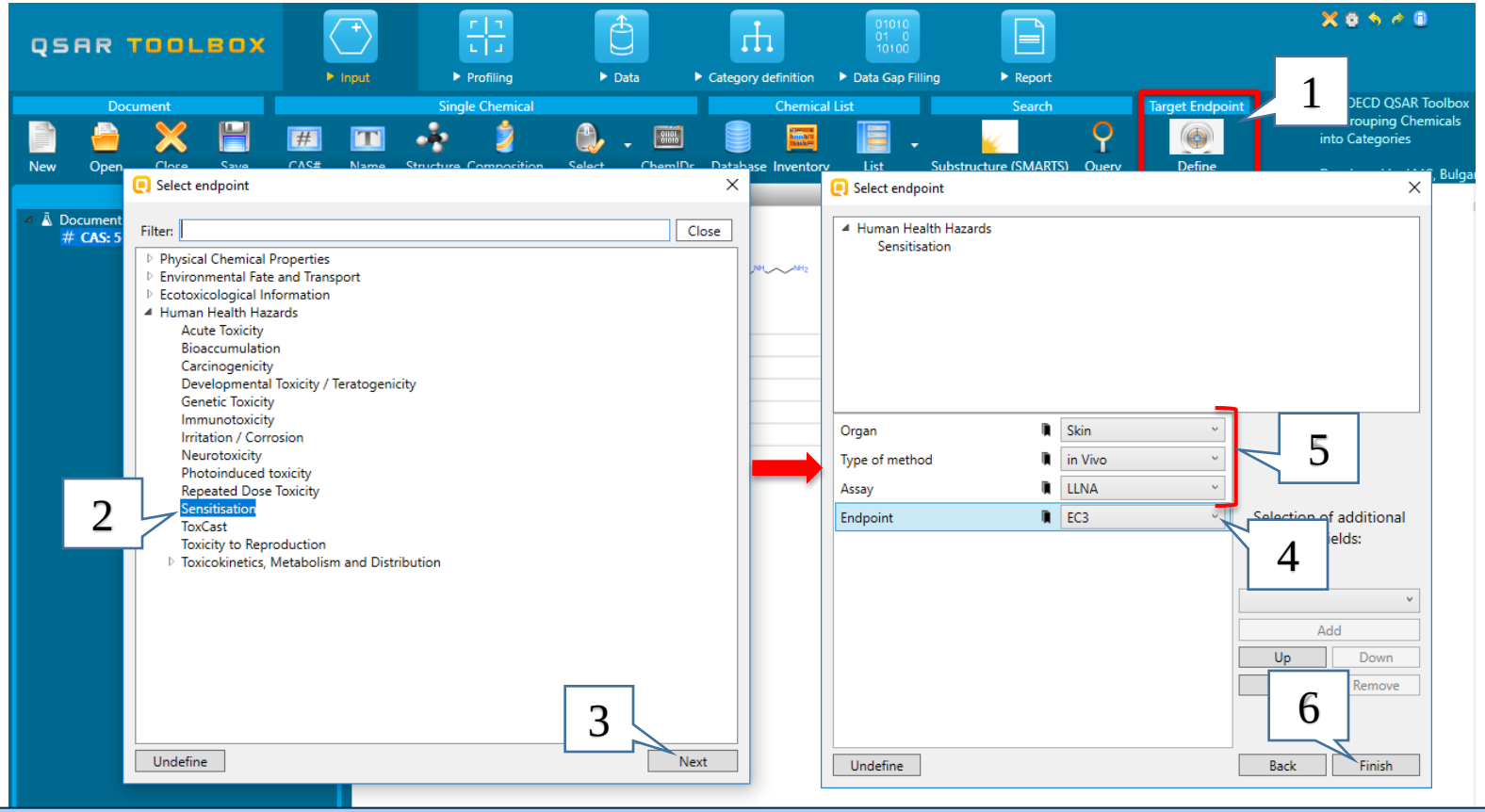
Input
Define target endpoint

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 same endpoint.

Calculation of alert performance (AP) is only possible if the target endpoint is preliminary selected.



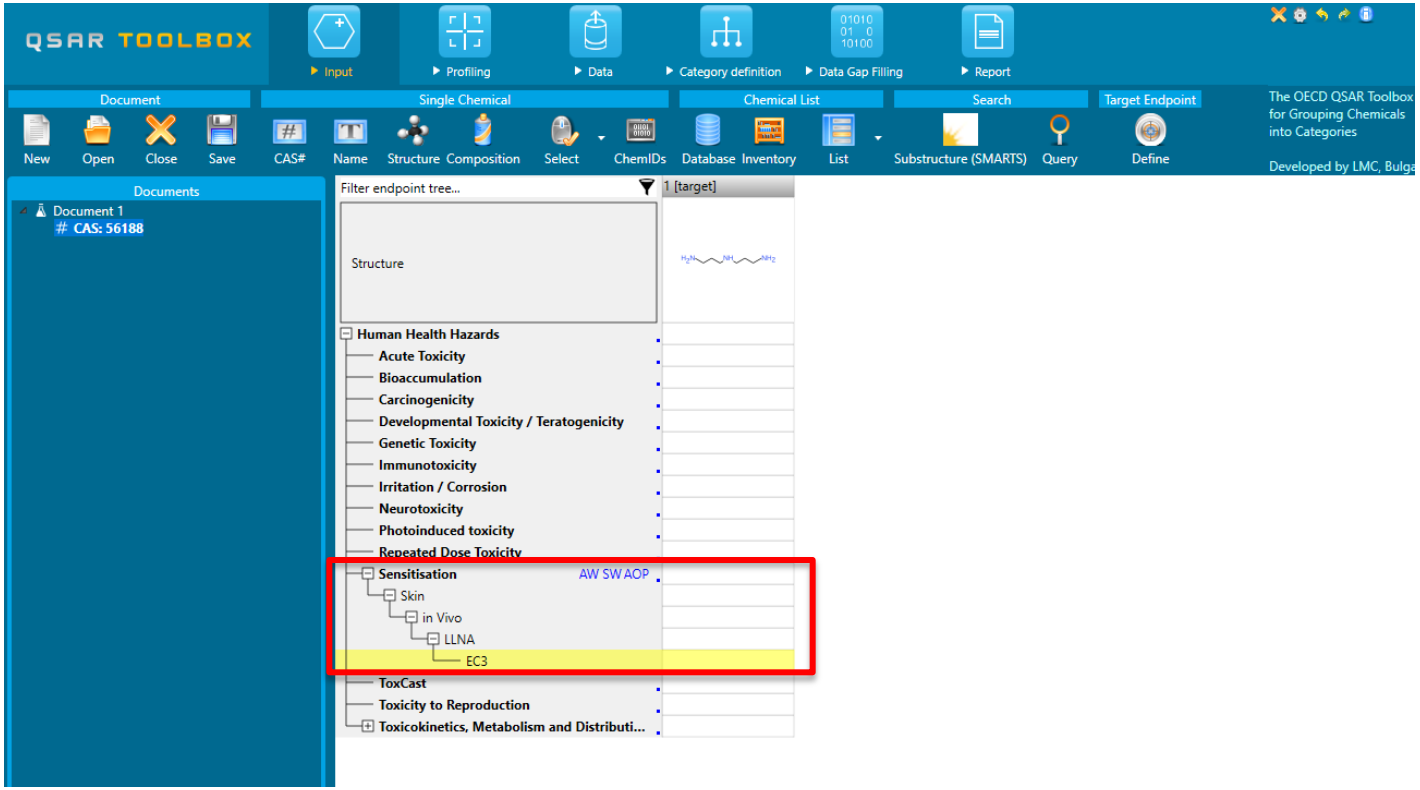
Input
Define target endpoint



By clicking **Define** (1) you could select the target endpoint. Select **Sensitisation** in the *Human health hazards* category (2) and click **Next** (3). Select **EC3** endpoint (4) from the drop-down menu and then consecutively the following metadata: *Assay*: **LLNA**, *Organ*: **Skin**, *Type of method*: **In Vivo** (5). Finally click on **Finish** (6).

Input
Define target endpoint

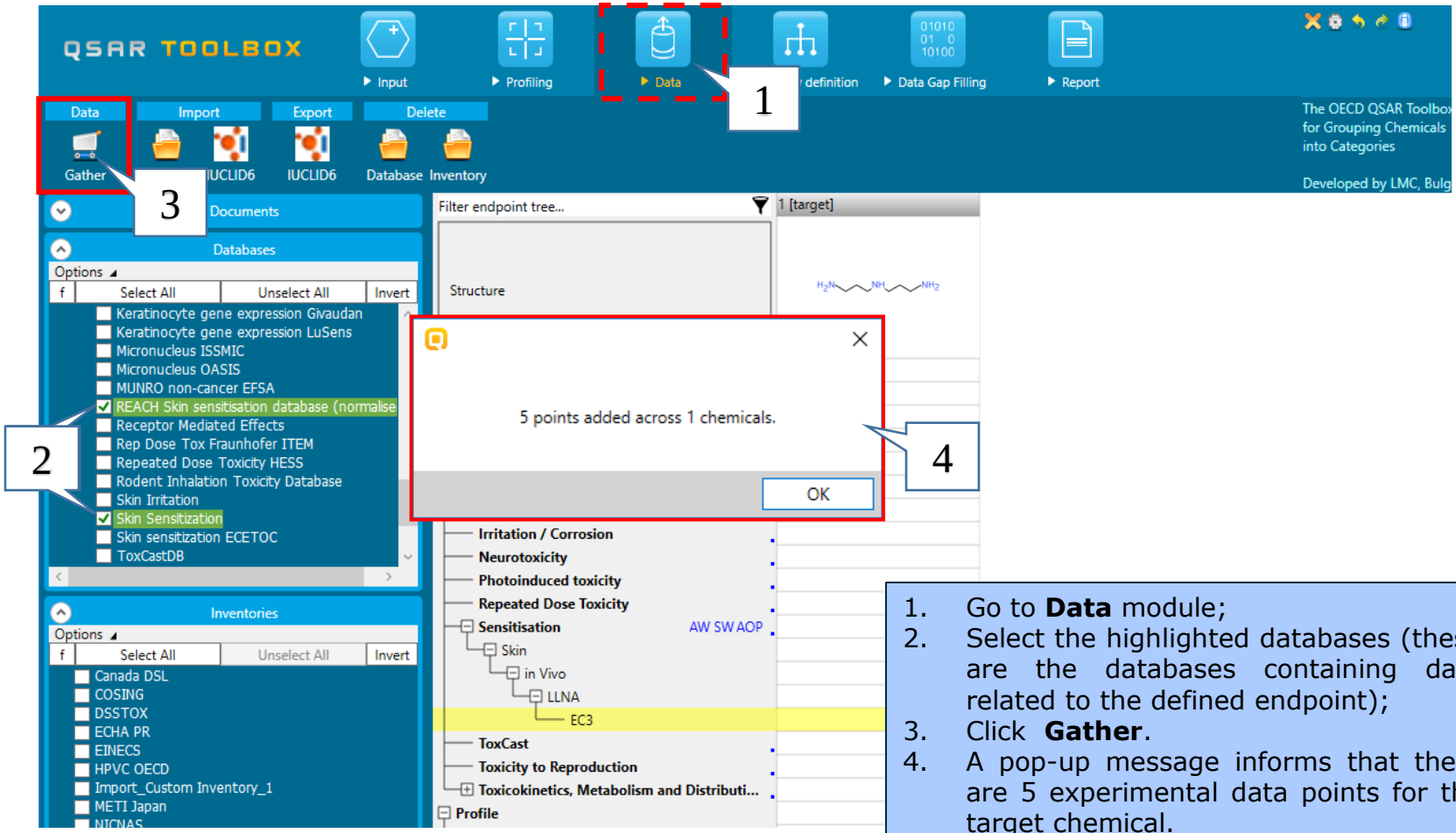
Once the endpoint is defined along with its metadata, they appear in the endpoint tree and the corresponding row of the data matrix is highlighted.



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
Gather data



1. Go to **Data** module;
2. Select the highlighted databases (these are the databases containing data related to the defined endpoint);
3. Click **Gather**.
4. A pop-up message informs that there are 5 experimental data points for the target chemical.

Data
Gather data

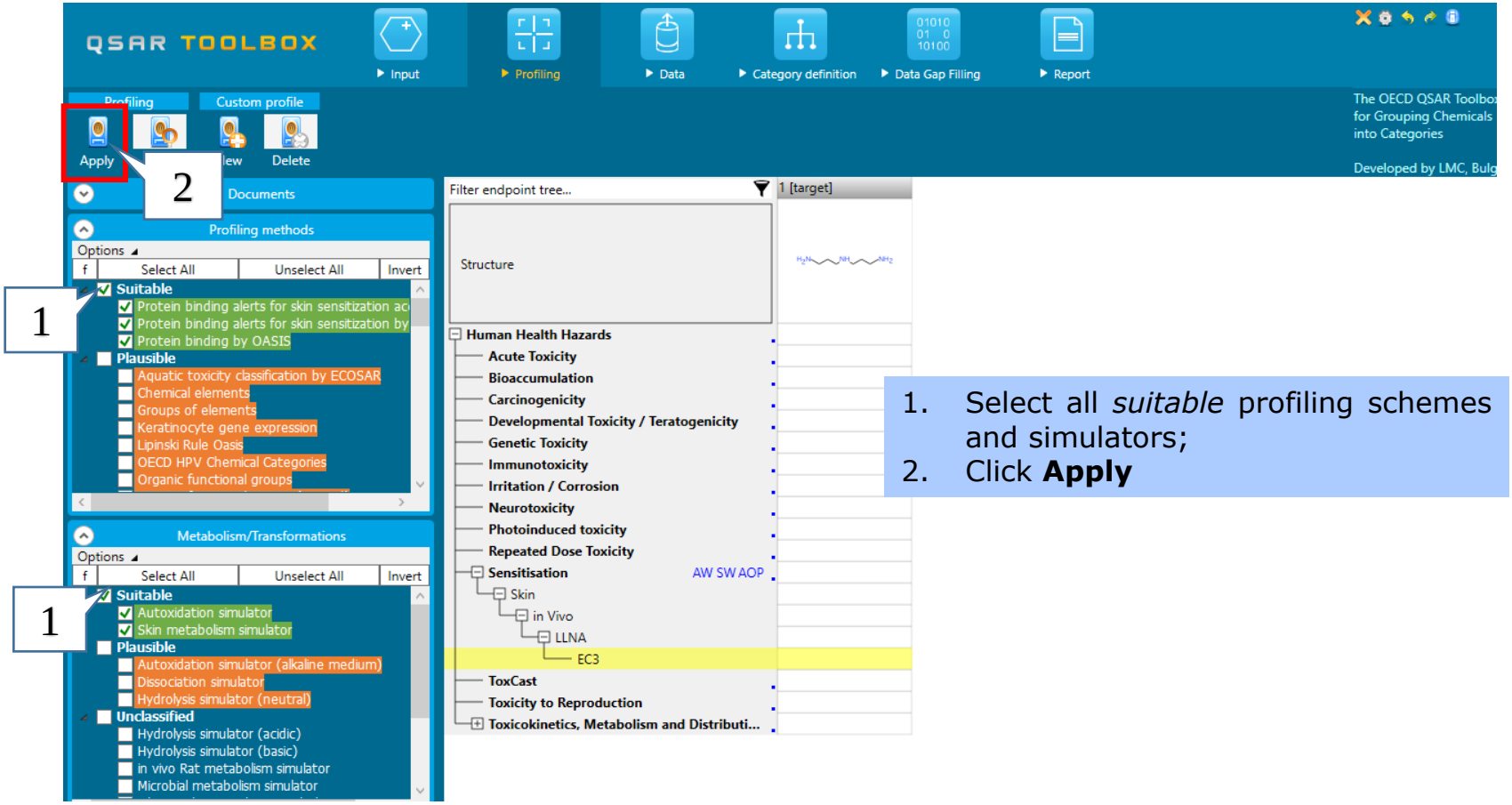
- Toxicity information on the target chemical is electronically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues (and further calculation of AP) is performed only among the chemicals which are listed in the selected databases. In this example only the *Skin sensitization database* and *REACH Skin sensitization database (normalized)* are selected.
- In this example, a pop-up window appears stating there are 5 experimental data points for the target chemical. Positive experimental data are available.
- Go to the *Profiling* module to check for the possible reasons of the positive effect (to check for an alert identified in the target chemical).

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

Profiling the target chemical



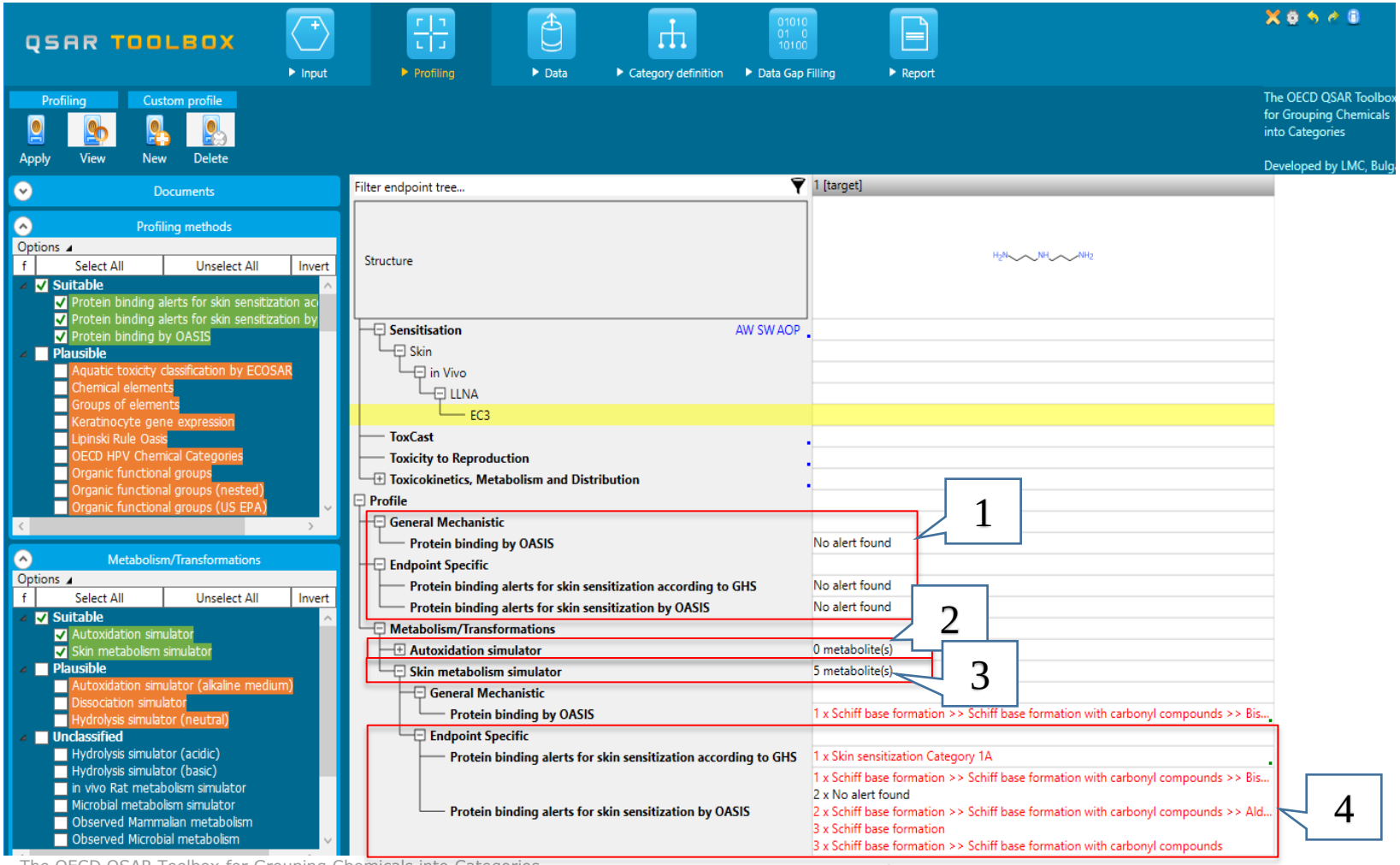
1. Select all *suitable* profiling schemes and simulators;
2. Click **Apply**

Profiling
Profiling results

1) No alerts are identified in the target structure as a parent;
2) No metabolites are produced as a result of abiotic activation (*Autoxidation simulator*);
3) 5 metabolites are produced as a result of biotic activation (*Skin metabolism simulator*);
4) Endpoint specific protein binding alerts are identified in the metabolites produced by the Skin metabolism simulator.

See on the next slide

Profiling
Profiling results



Recap

- In module one, you entered the target chemical and defined the target endpoint.
- In the *Data* module, you saw the database corresponding to the defined target endpoint. You also found some experimental data for the target available in the selected databases.
- In the *Profiling* module, you profiled the target chemical with profiling schemes and metabolic simulators, suitable for the selected target endpoint.
- Protein binding alerts for skin sensitization were identified for some of the metabolites produced by simulating of biotic activation.
- Click "Category Definition" to move to the next module.

Category Definition
Overview

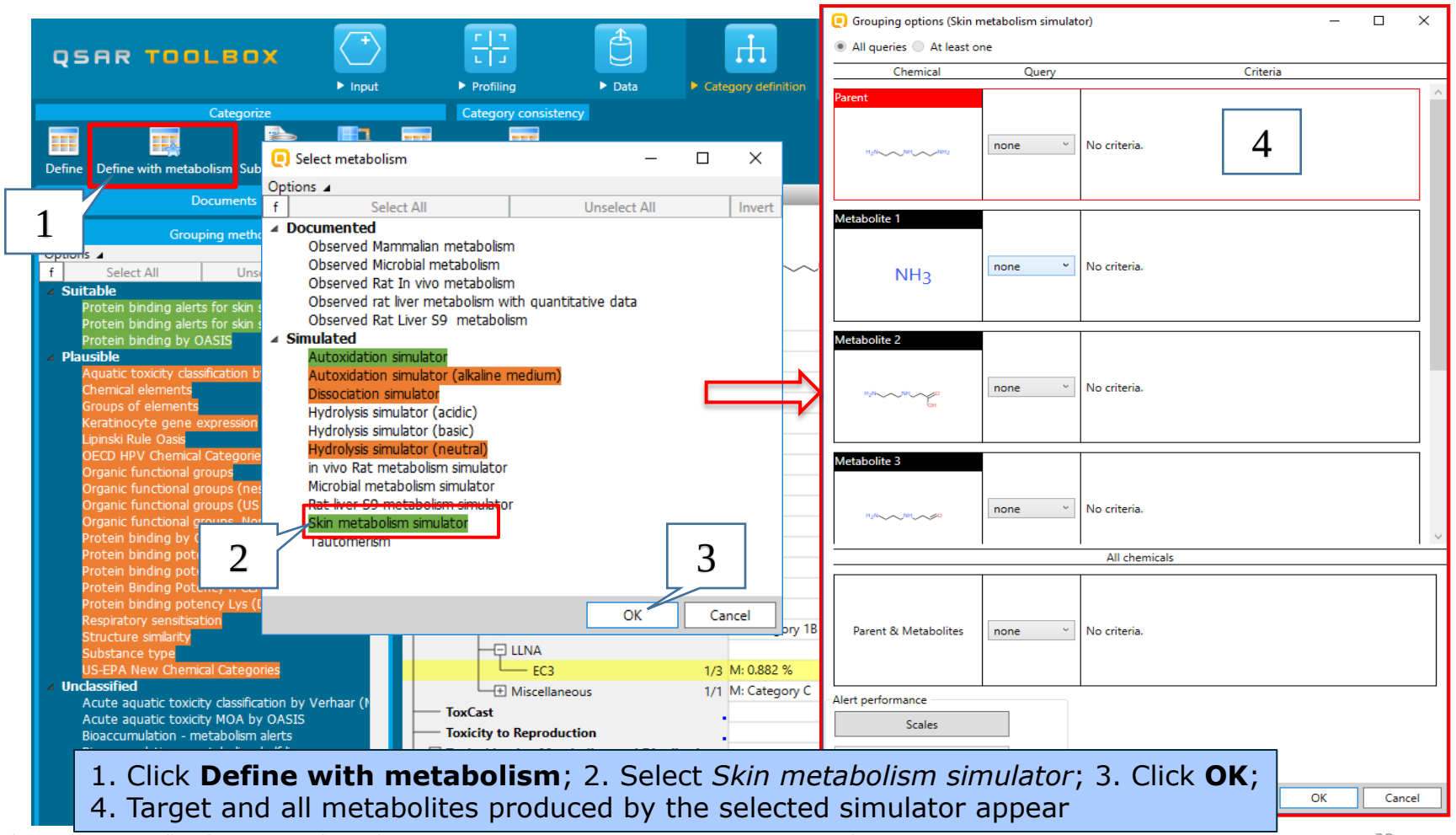
- This module provides the user with several means of grouping chemicals into a toxicologically meaningful category that includes the target molecule.
- This is the critical step in the workflow.
- Several options are available in the Toolbox to assist the user in refining the category definition.

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.
- If no alert is identified in the target structure, but is identified in its metabolites, analogues can be searched accounting for metabolism. In this way the target chemical and the identified analogues will have similar metabolic pattern.
- 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.

Category Definition

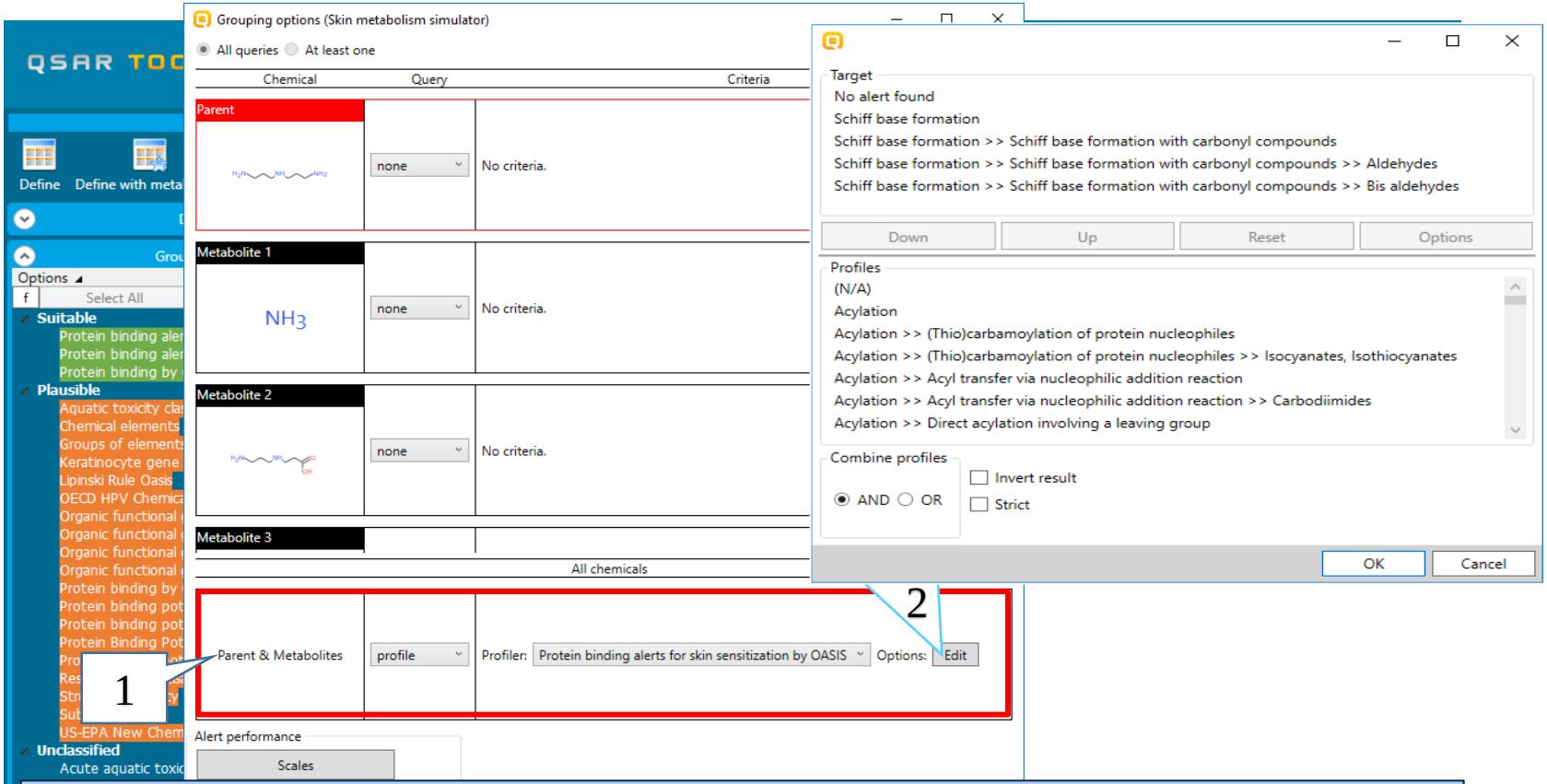
Searching for analogues accounting for skin metabolism



1. Click **Define with metabolism**; 2. Select *Skin metabolism simulator*; 3. Click **OK**;
4. Target and all metabolites produced by the selected simulator appear

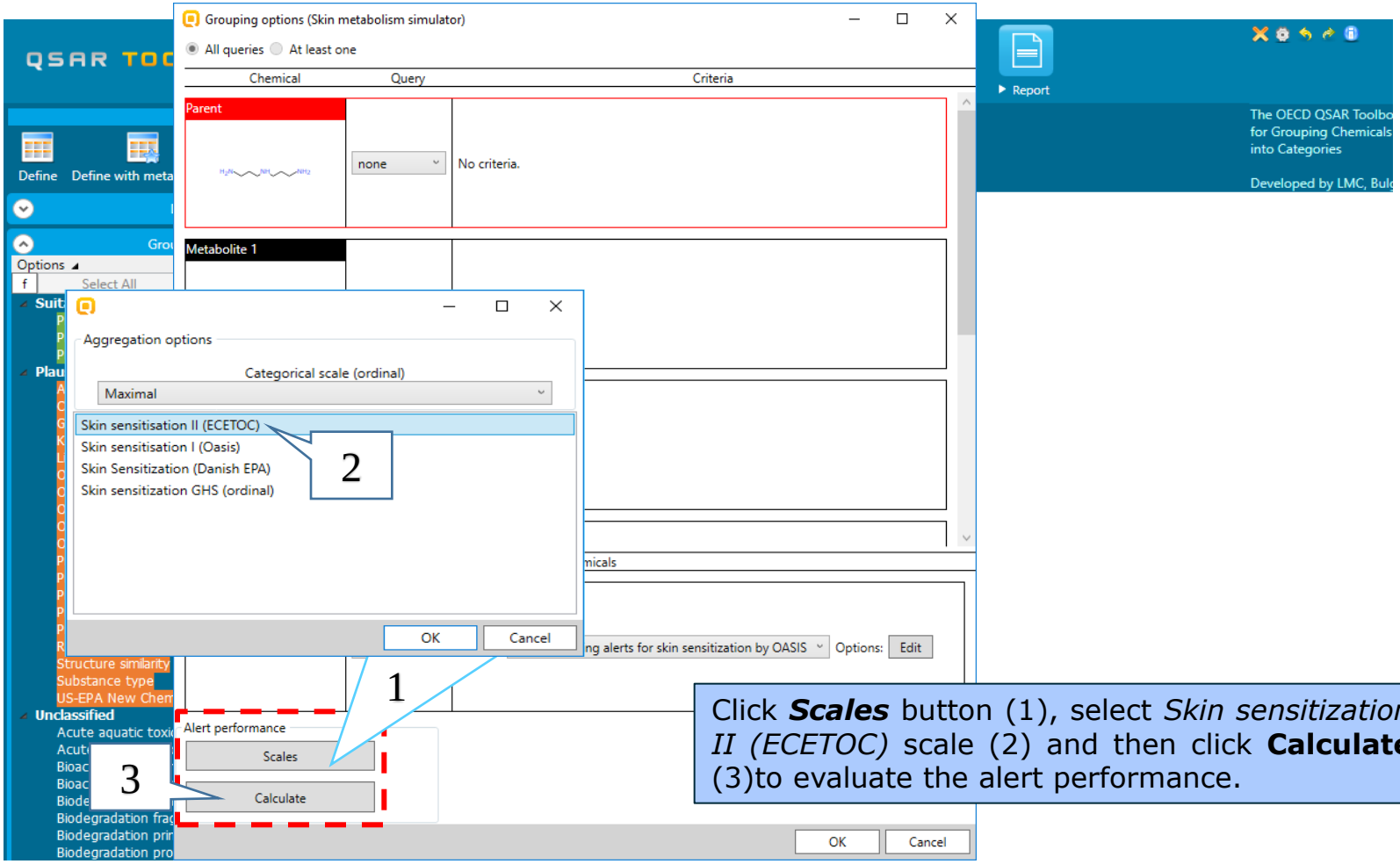
Category Definition

Searching for analogues accounting for skin metabolism



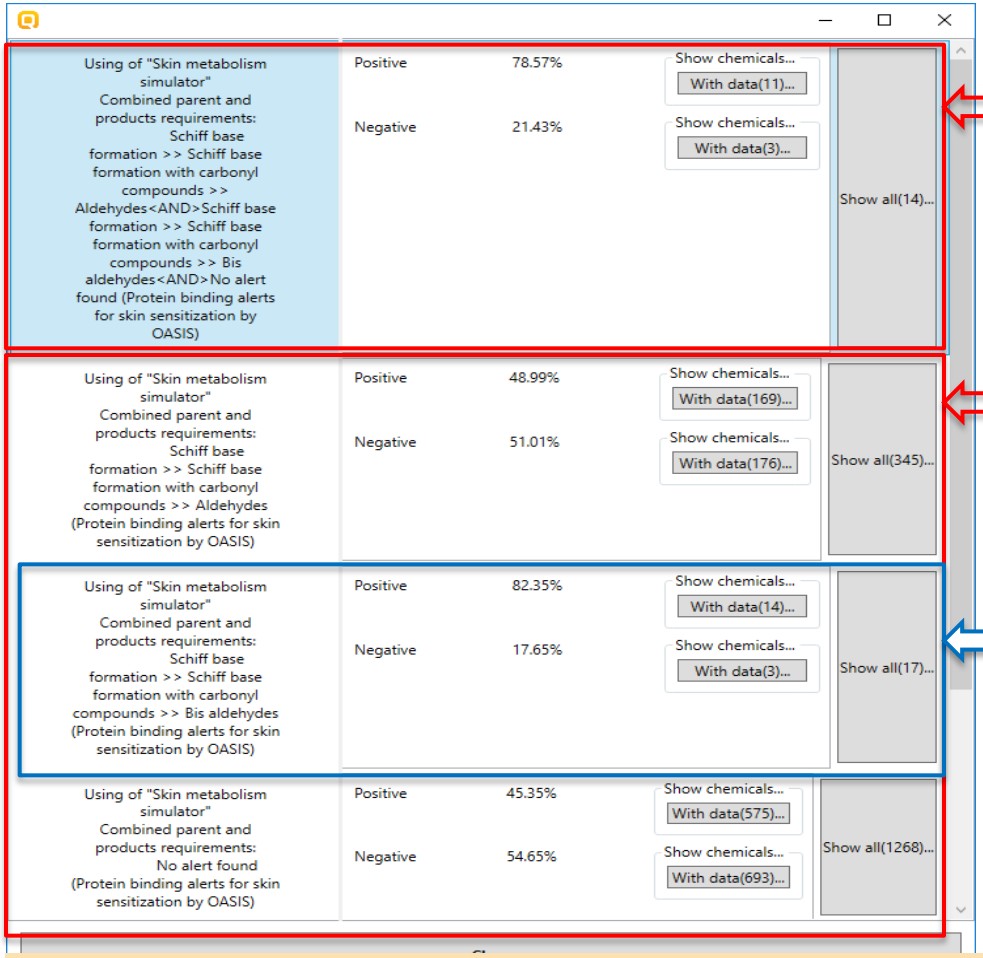
1. Define *profile* query for the package – "Parent & Metabolites" and then select *Protein binding alerts for skin sensitization by OASIS*.
2. Click **Edit** to see all identified alerts in the parent and metabolites.

Category Definition
Alert performance calculation



Click ***Scales*** button (1), select *Skin sensitization II (ECETOC)* scale (2) and then click **Calculate** (3)to evaluate the alert performance.

Category Definition
Alert performance calculation



Statistic for **all** alerts identified in the package "Parent & Metabolites"

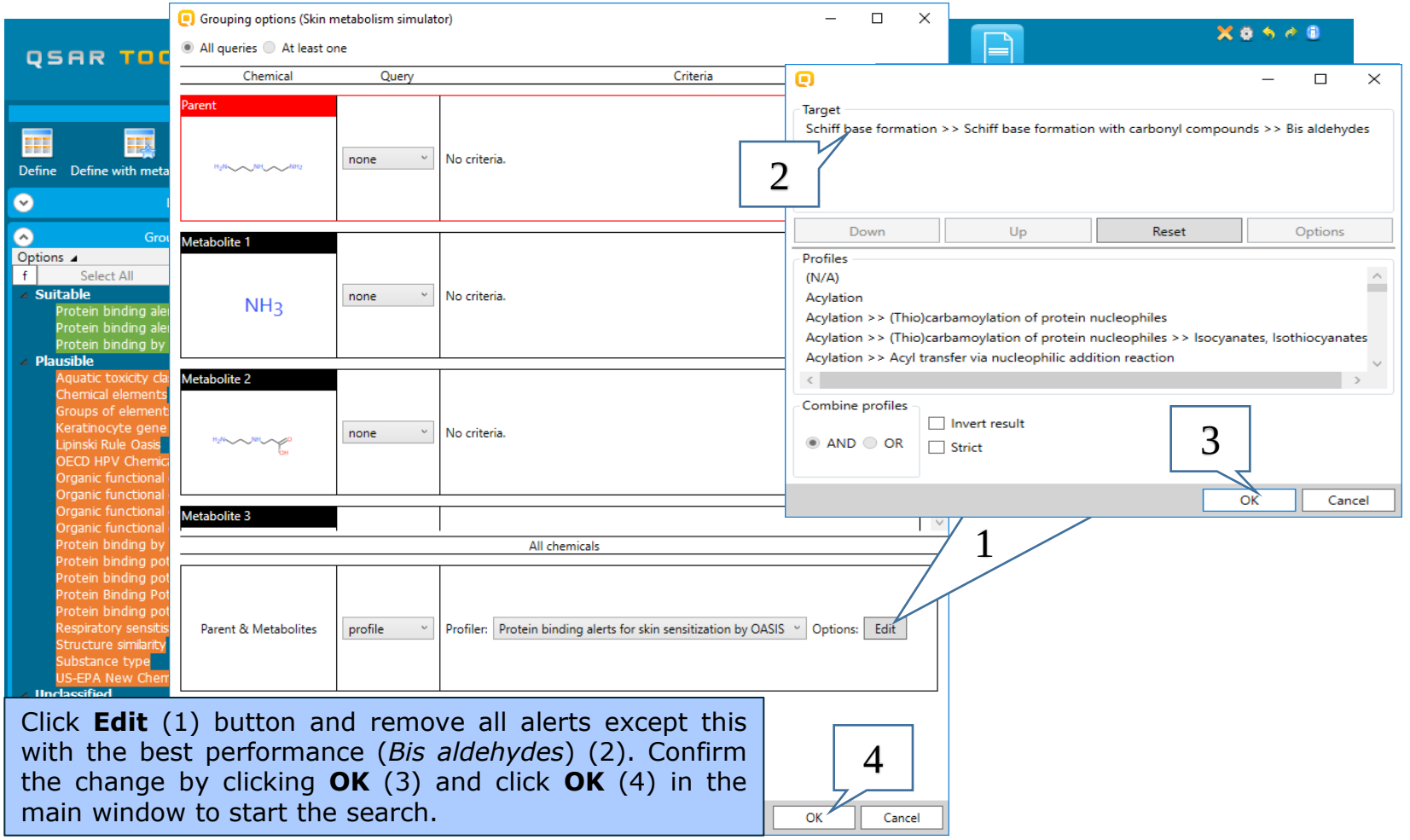
Statistic for **each** of the alerts identified in the package "Parent & Metabolites"

The alert with the best performance in this case. "*Bis aldehydes*" alert will be used for searching for analogues.

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

Category Definition

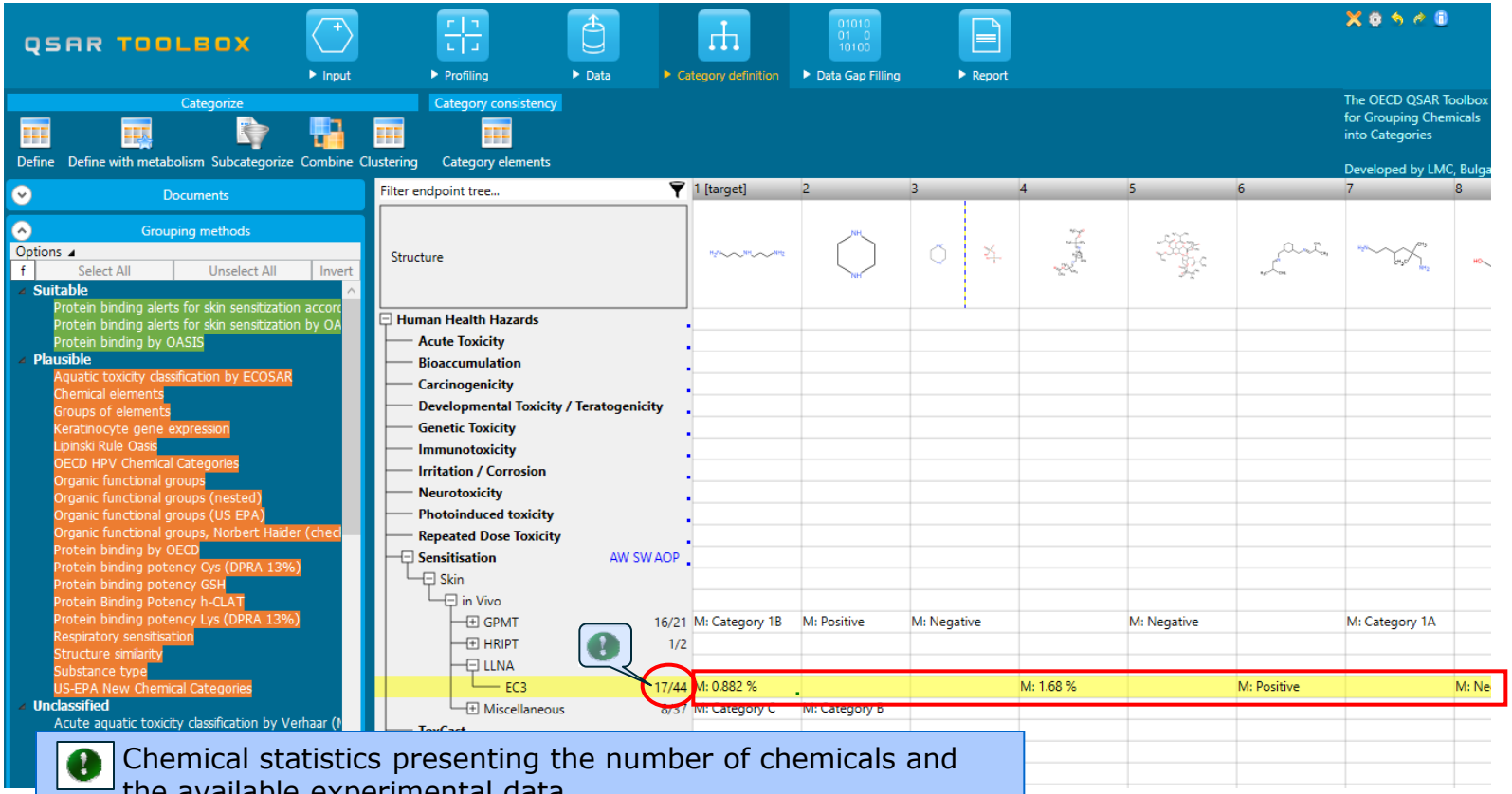
Searching for analogues accounting for skin metabolism



Click **Edit** (1) button and remove all alerts except this with the best performance (*Bis aldehydes*) (2). Confirm the change by clicking **OK** (3) and click **OK** (4) in the main window to start the search.

Category Definition
Summary information for Analogues

17 chemicals with 44 experimental results related to the defined target endpoint are found across all 29 analogues.



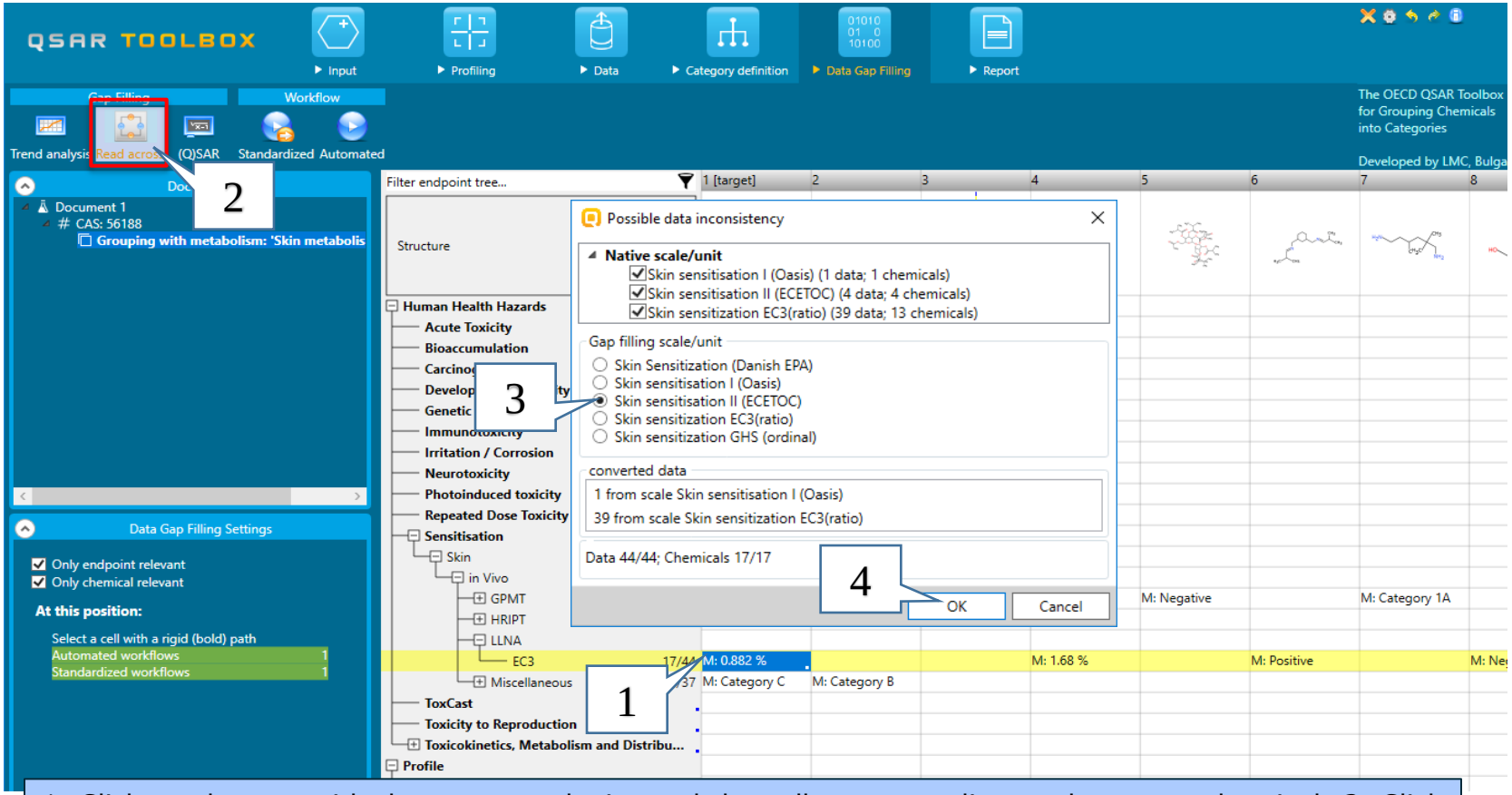
Chemical statistics presenting the number of chemicals and the available experimental data.

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.

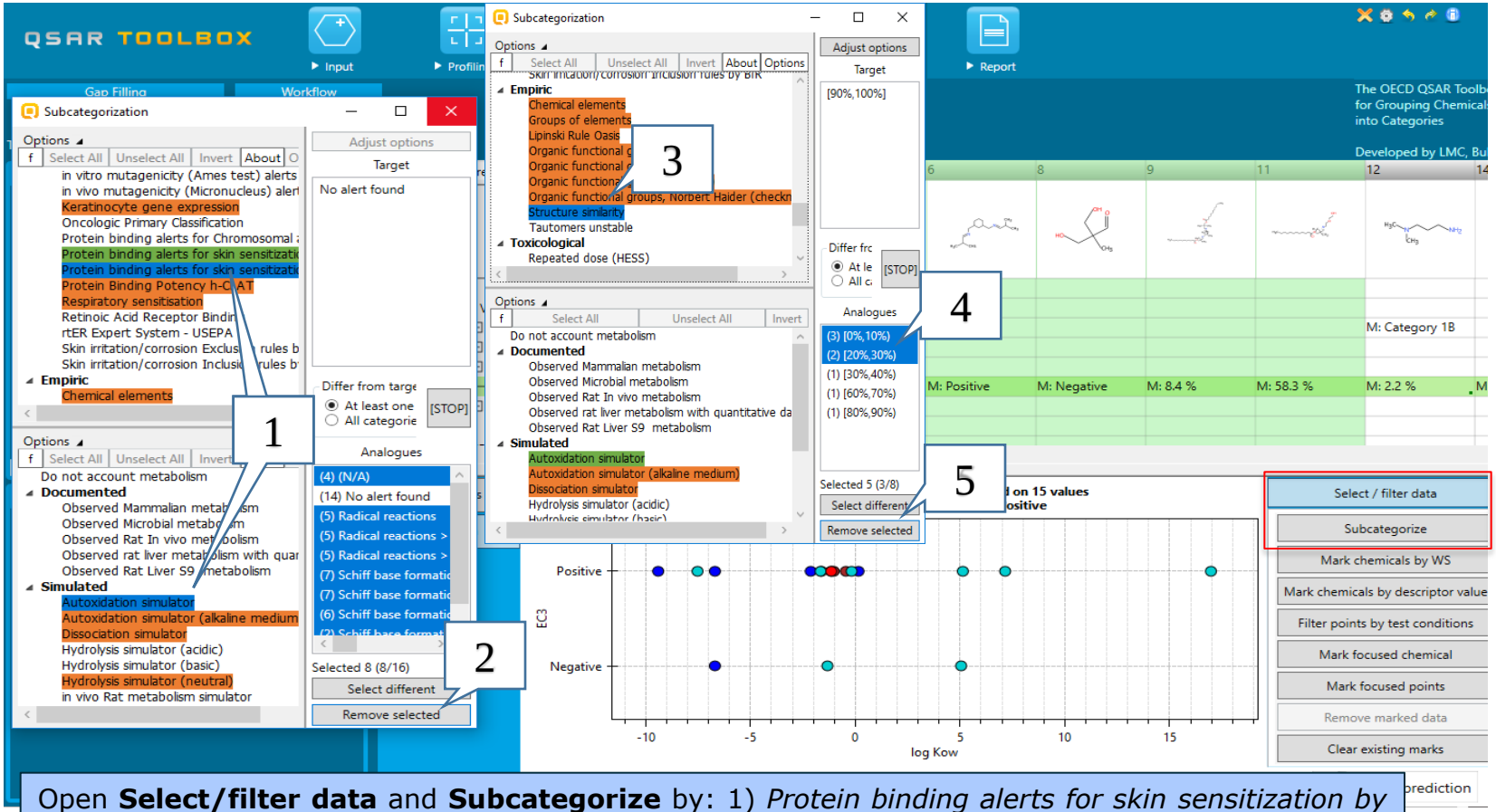
In this example we will use the read-across approach.

Data Gap Filling
Apply Read-across



1. Click on the row with the target endpoint and the cell corresponding to the target chemical; 2. Click **Read-across** button; 3. Select *Skin sensitisation II (ECETOC);* 4. Click **OK**

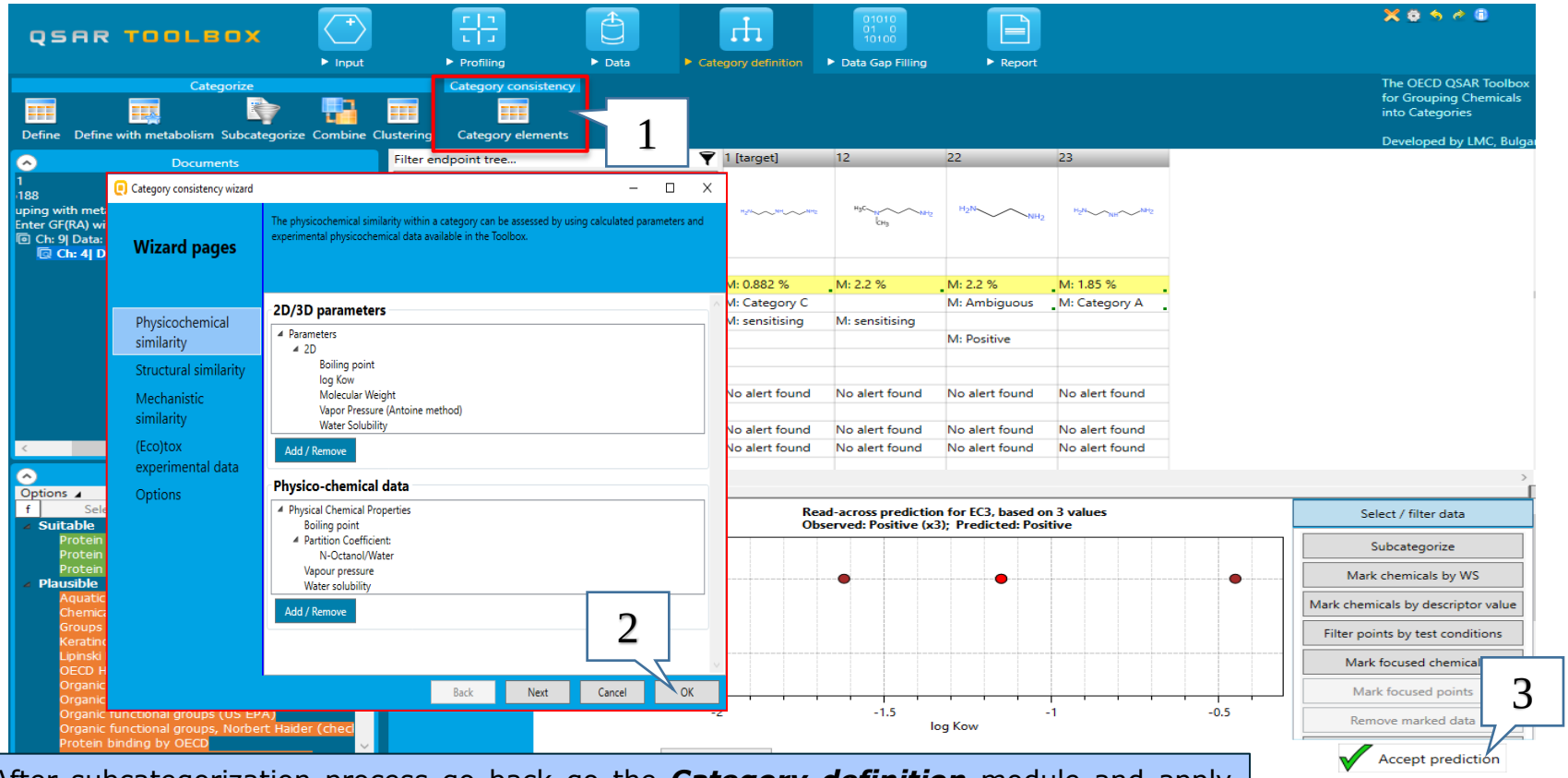
Data Gap Filling
Apply Read-across



Open **Select/filter data** and **Subcategorize** by: 1) *Protein binding alerts for skin sensitization by OASIS* profiler in combination with *Autoxidation simulator, 2)* Remove the different analogues; 3) *Structural similarity 4) Select* all analogues (3) similar less than 30% to the target chemical, by hold Ctrl button; 5) Click *Remove*

Data Gap Filling

Apply Category consistency elements



After subcategorization process go back go the ***Category definition*** module and apply category elements* (1). No different selection than the default is needed – click **OK** (2). Once the category elements are applied **accept the prediction** (3).

*For more information on category elements see *Tutorial_1_TB 4.2. Category consistency*

Recap

- In the *Category definition* module you found analogues based on the alert with the best performance accounting for skin metabolism.
- In *Data gap filling* module you applied a read-across approach. 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.
- Category consistency was checked by applying the category elements.
- You are now ready to complete the final module and to create the report.
- Click "Report" to proceed to the last module.

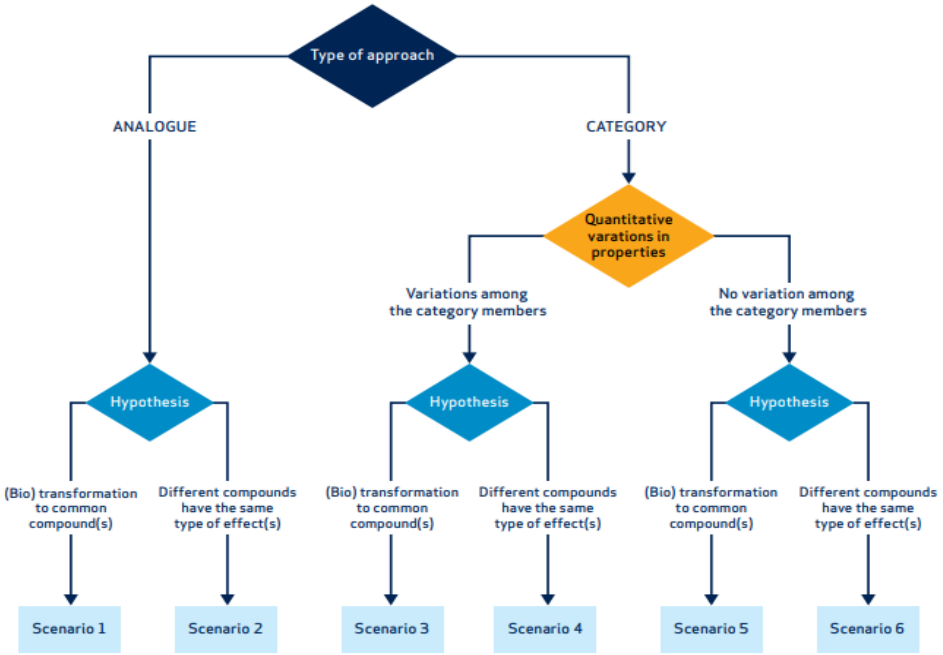
Report
Overview

- Report module allows generating a report for any of the predictions performed within the Toolbox.
- The report module contains predefined report template which users can customize.
- Additionally specific RAAF scenario could be chosen. Selection of one of the scenarios will append automatically the related assessment elements related to the corresponding report sections.

Report
Selection of RAAF scenario

To select the applicable RAAF scenario for assessment, the following aspect should be identified*:

- the type of approach applied - analogue approach or category approach;
- the read-across hypothesis;
- For category approach - whether quantitative variations in the properties are observed among the category members must be considered.



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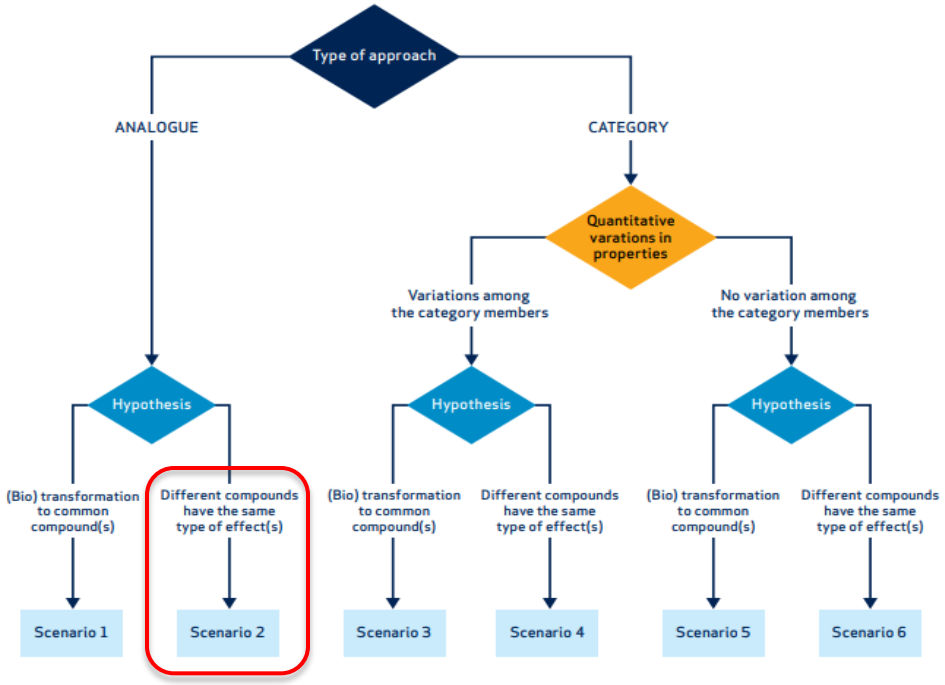
Report
Selection of RAAF scenario

For the current example:

- the type of approach applied - analogue approach is used (threshold of ≤3 analogues is proposed by LMC for the analogue approach) ;
- the read-across hypothesis – different compounds with common underlying mechanism for metabolites of source and target substances ;

Based on that Scenario II was identified as appropriated for the current example.



Read-Across Assessment Framework (RAAF)

Scenario 2

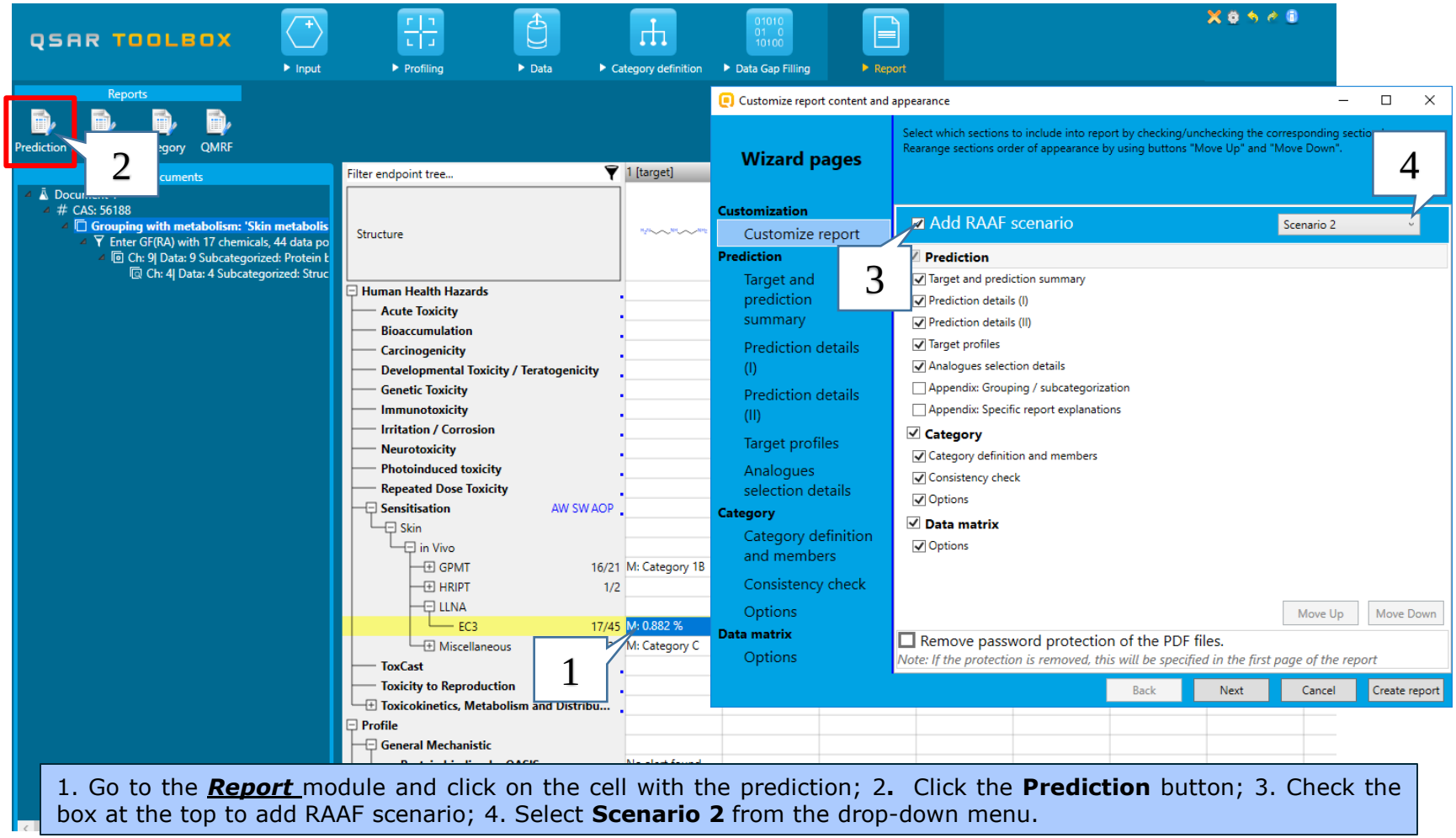
- Scenario 2 covers the analogue approach for which the read-across hypothesis is based on different compounds with qualitatively similar properties.
- For the REACH information requirement under consideration, the property investigated in a study conducted with one source substance is used to predict properties that would be observed in a study with the target substance if it were to be conducted.
- The current case corresponds to Example 2 for Scenario 2 of the RAAF*. The target (B) and the source chemicals (A) are biotransformed to substances causing the same type of effects through a common mechanism (A1 and B1). The rest of the obtained compounds, non-common for the target and the source substance does not influence the prediction of the property under the consideration.

	PARENT SUBSTANCES	(BIO)TRANSFORMATION	COMMON COMPOUND	NON-COMMON COMPOUNDS
SOURCE	A	A → A1+ A2	A1	A2
TARGET	B	B → B1	B1	-

*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

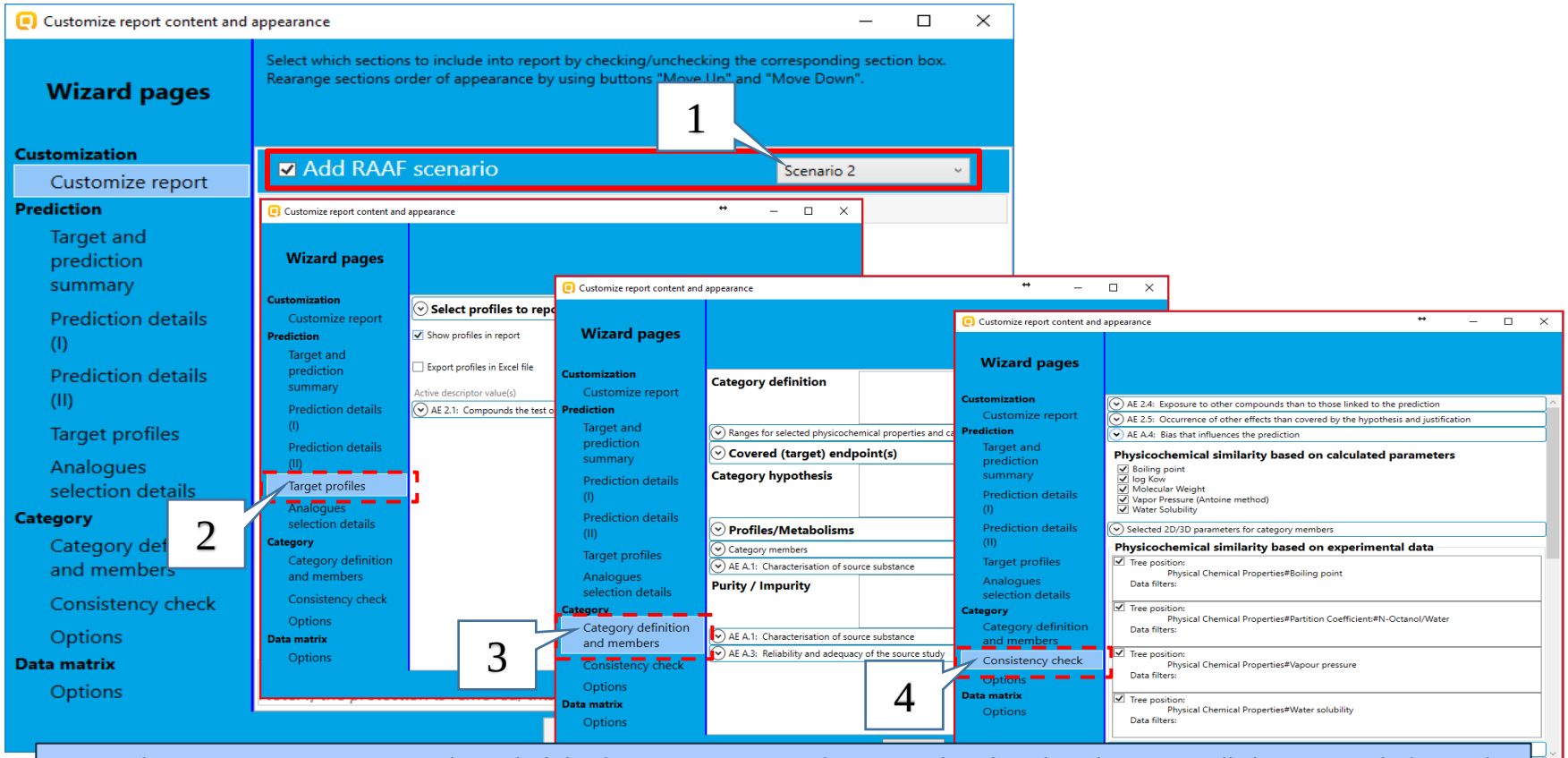
Report

Generation report according to RAAF-Scenario 2



1. Go to the ***Report*** module and click on the cell with the prediction; 2. Click the **Prediction** button; 3. Check the box at the top to add RAAF scenario; 4. Select **Scenario 2** from the drop-down menu.

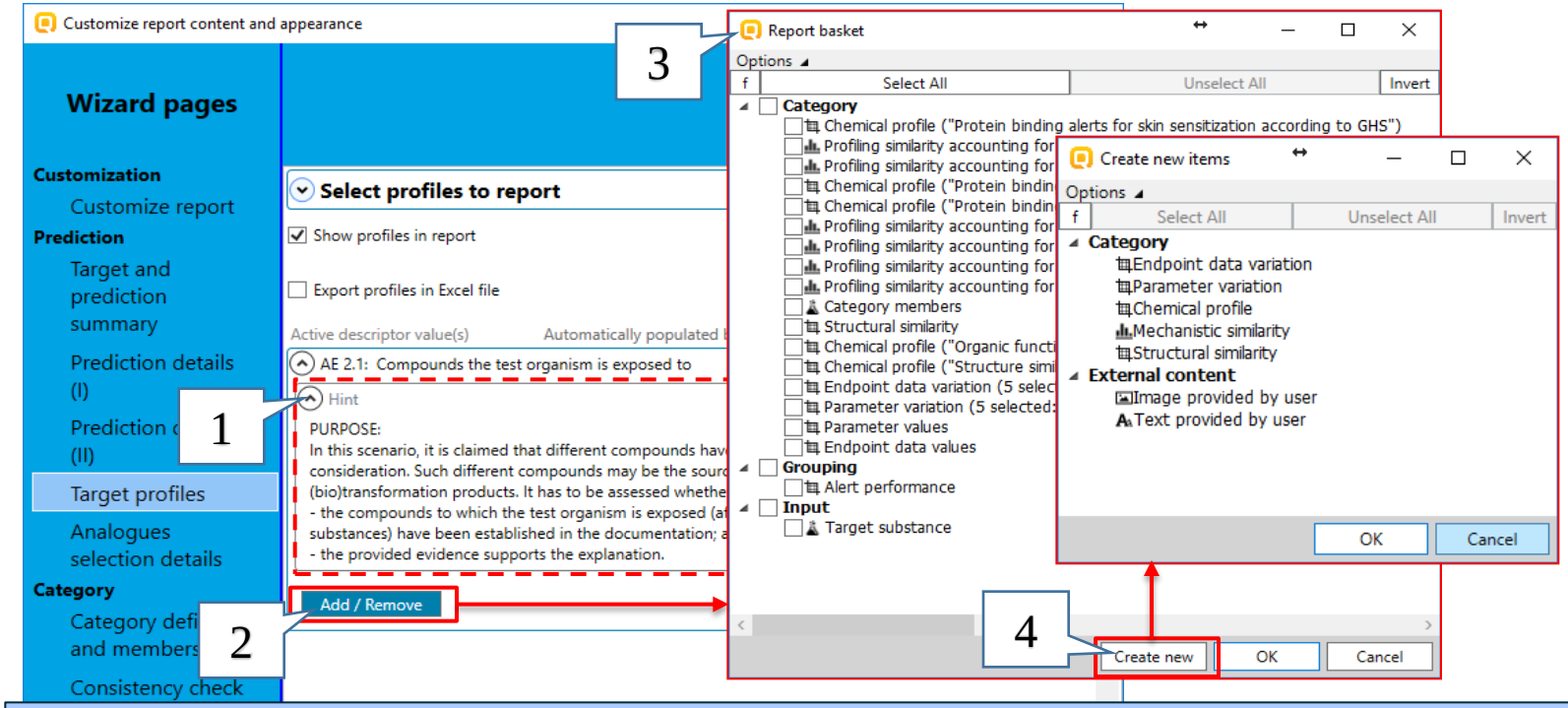
Report
Generation report according to RAAF-Scenario 2



Once the RAAF scenario is selected (1) the assessment elements (AEs) related to it will be appended to the corresponding sections of the report automatically. AEs appear in the following report sections: ***Target profiles*** (2). ***Category definition and members*** (3) and ***Consistency check*** (4).

Each of the AEs will be considered in the next slides.

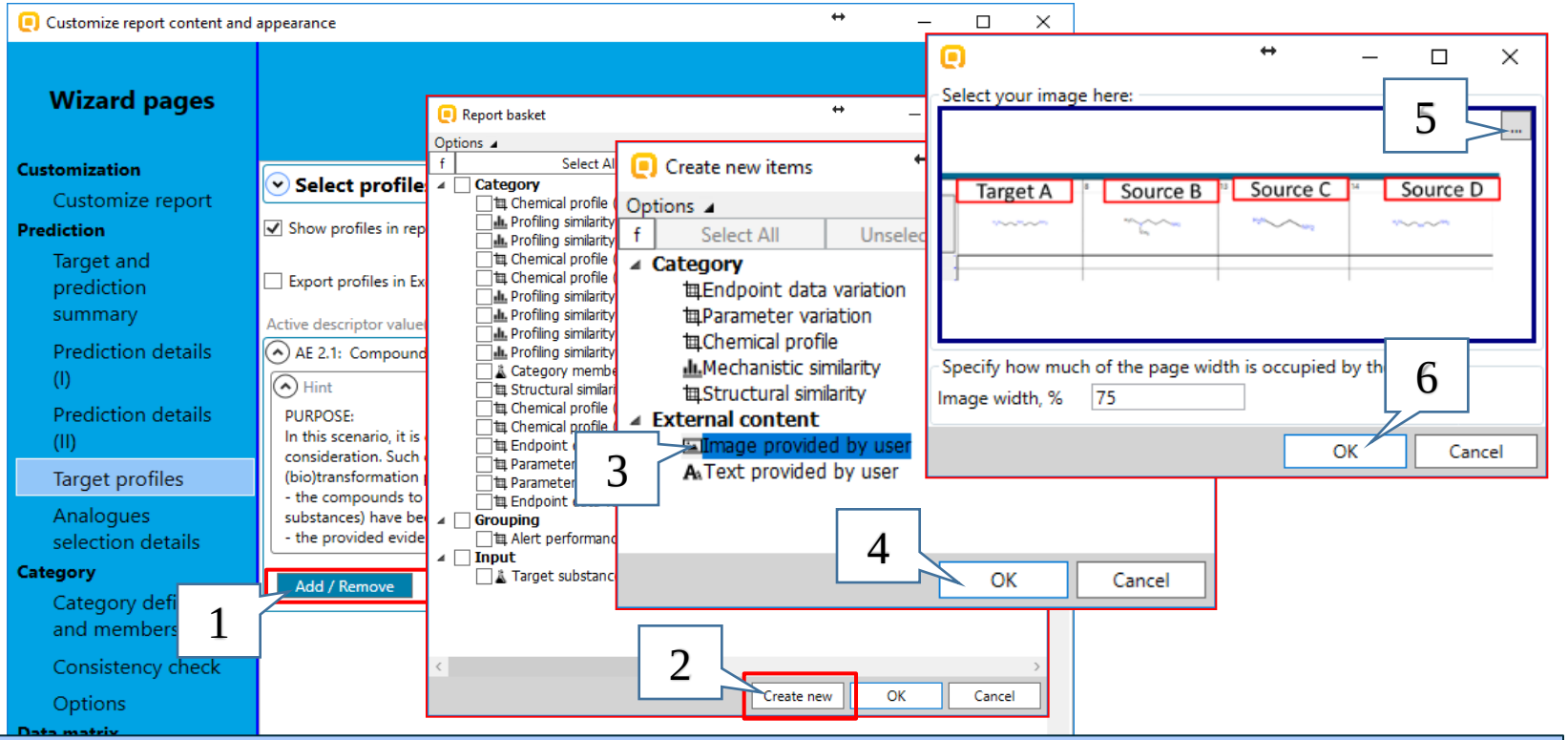
Report
Assessment elements of Scenario 2



Hint for each of the assessment elements is available (1). Information can be included by clicking the **Add/Remove** button (2) located below the corresponding AE. The *Add/Remove* button invokes the so-called "***Report basket***" (3). The latter contain different items triggered by the actions of the user during the workflow (e.g. Alert performance calculation, applying of category elements, etc.).
Additionally, new items (including items with external content) can be created (4).

Items with external content (picture and text) will be added for **AE 2.1**. **Compounds the test organism is exposed to**

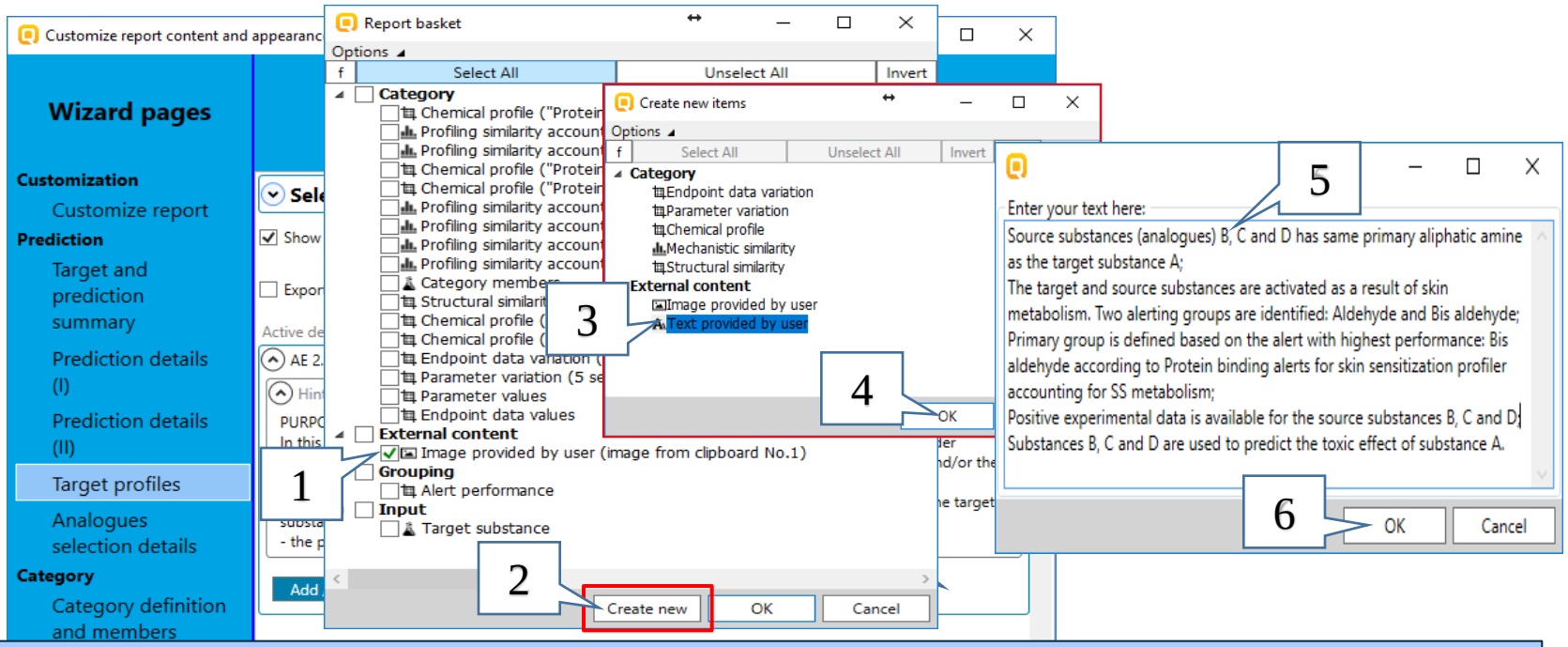
Report
Assessment elements of Scenario 2



Click the **Add/Remove** button (1) and then **Create new** (2). Select to create an item with external content – ***Image provided by user*** (3) and click **OK** (4). New window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture is saved*. Finally confirm by **OK** (6).

*In the current example a picture illustrating the target chemical marked as **Target A** and source chemicals marked as **Source A**, **B** and **C** was prepared in advance.

Report
Assessment elements of Scenario 2

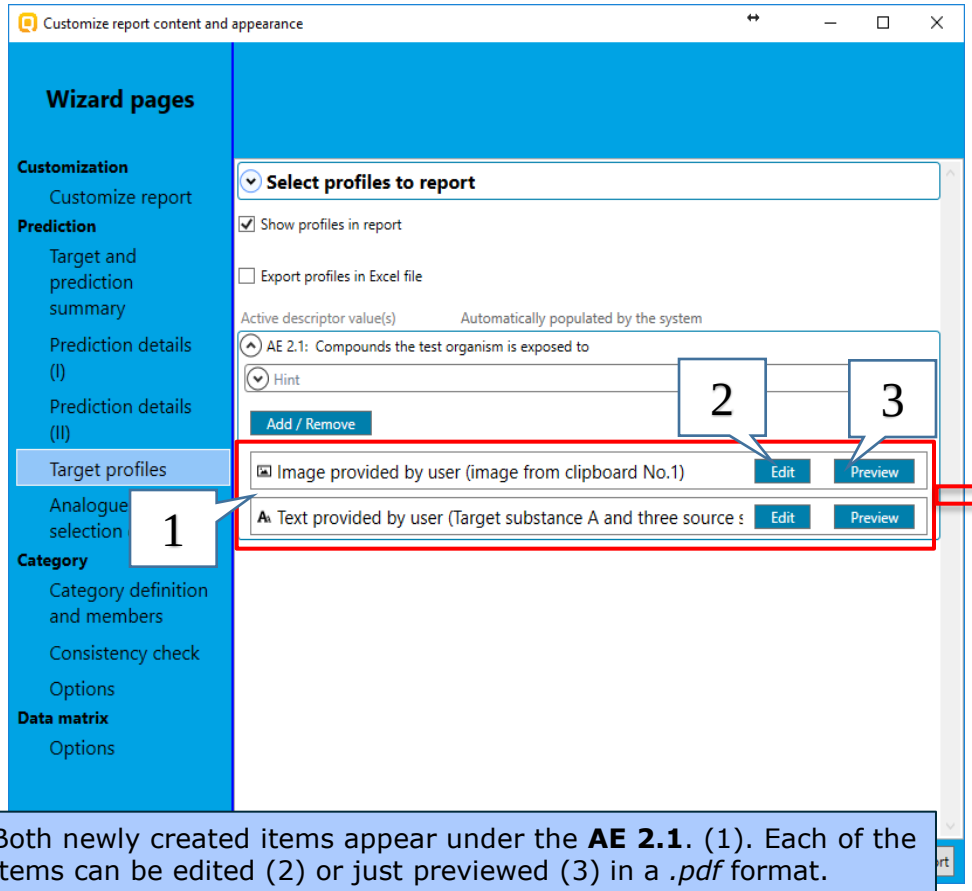


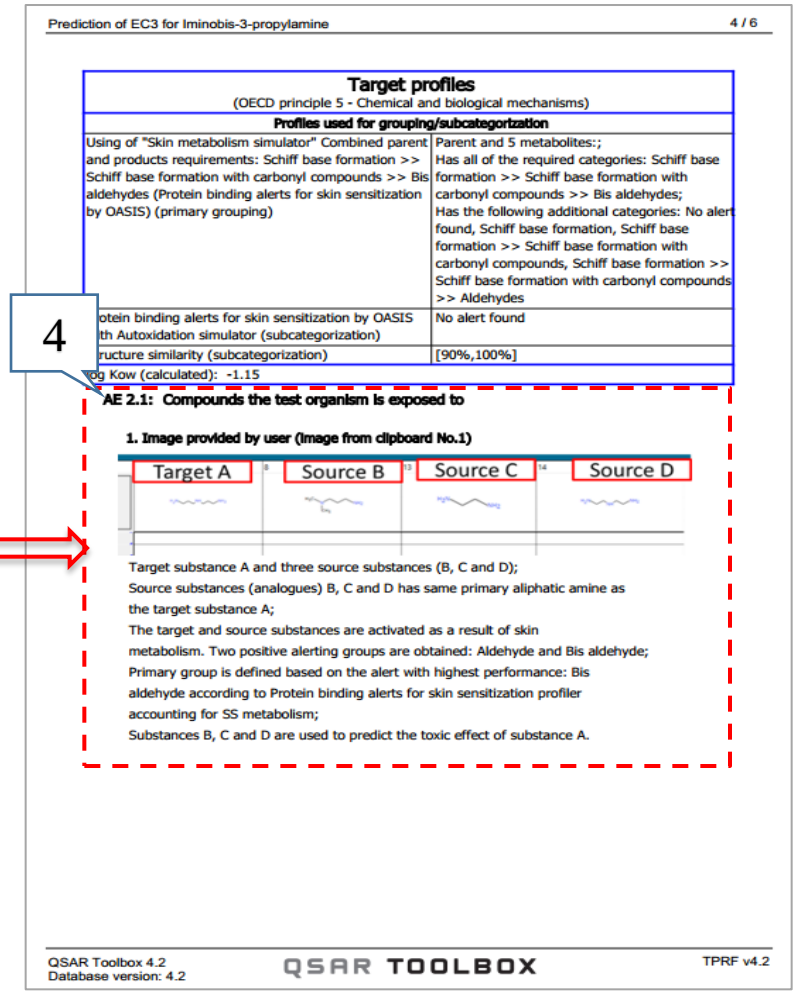
The newly created item appears in the *Report basket* (1). Now text will be also included. Click **Create new** (2), select ***Text provided by user*** (3) and click **OK** (4). Copy the following example text:

- *Source substances (analogues) B, C and D has same primary aliphatic amine as the target substance A;*
- *The target and source substances are activated as a result of skin metabolism. Two alerting groups are identified: Aldehyde and Bis aldehyde;*
- *Primary group is defined based on the alert with highest performance: Bis aldehyde according to Protein binding alerts for skin sensitization profiler accounting for SS metabolism;*
- *Positive experimental data is available for the source substances B, C and D* (references for the data could be also included)
- *Substances B, C and D are used to predict the toxic effect of substance A.*

and paste it in the new window (5). Finally confirm by **OK** (6).

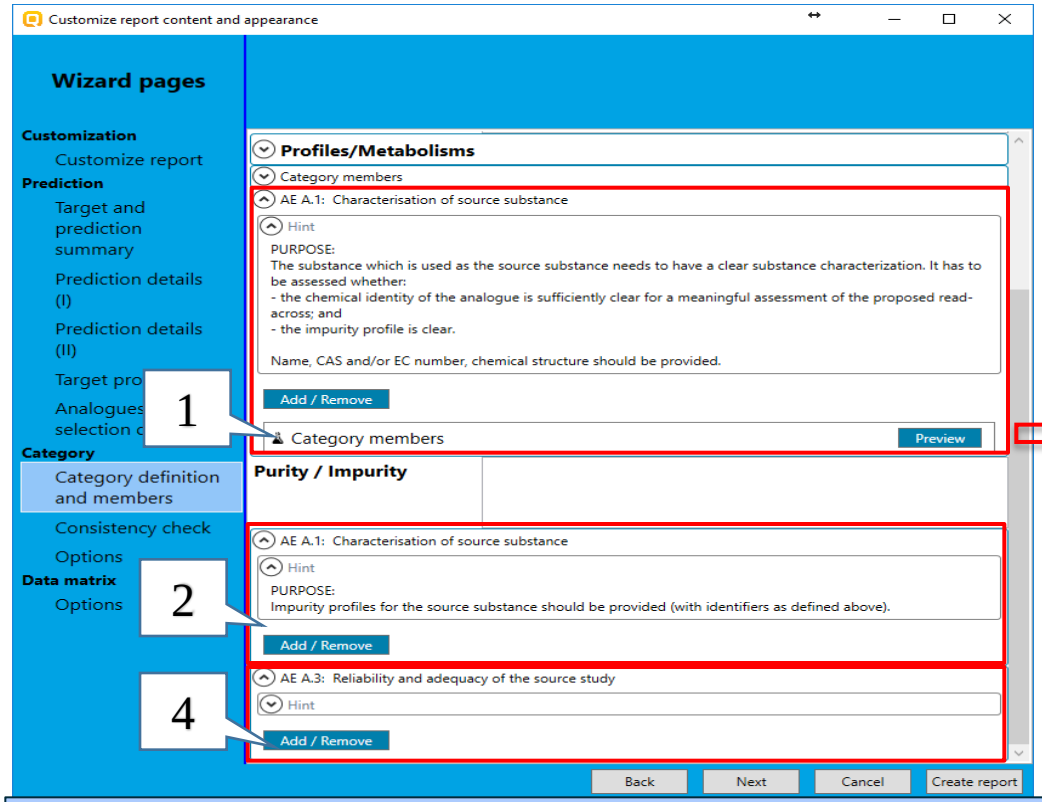
Report
Assessment elements of Scenario 2

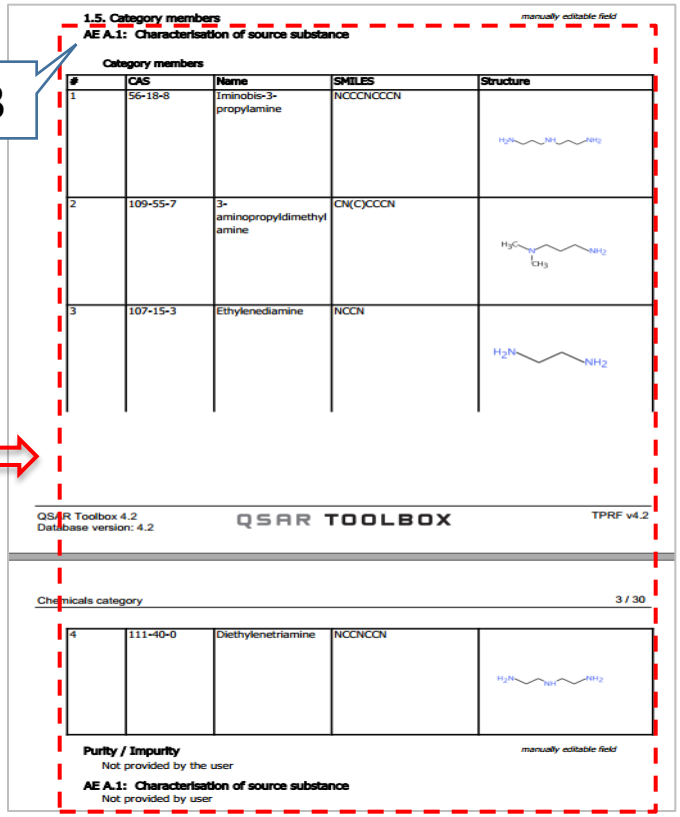




Both newly created items appear under the **AE 2.1**. (1). Each of the items can be edited (2) or just previewed (3) in a *.pdf* format. Example of how the AE 2.1. and related description will look in the generated report is shown on the right (4).

Report
Assessment elements of Scenario 2

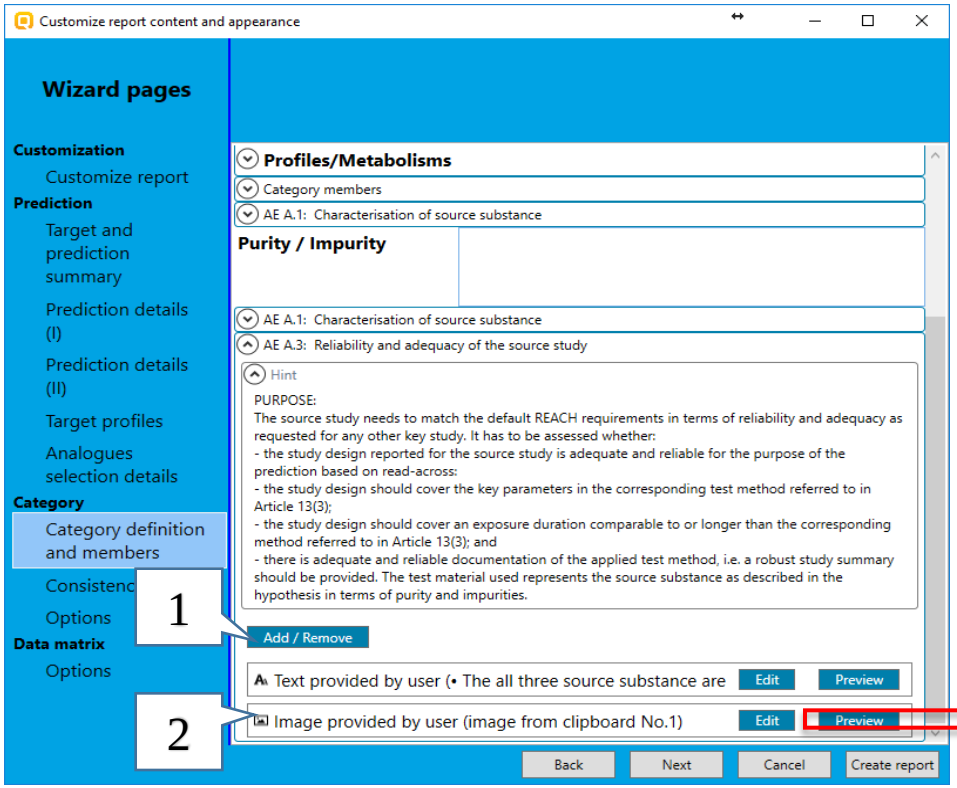


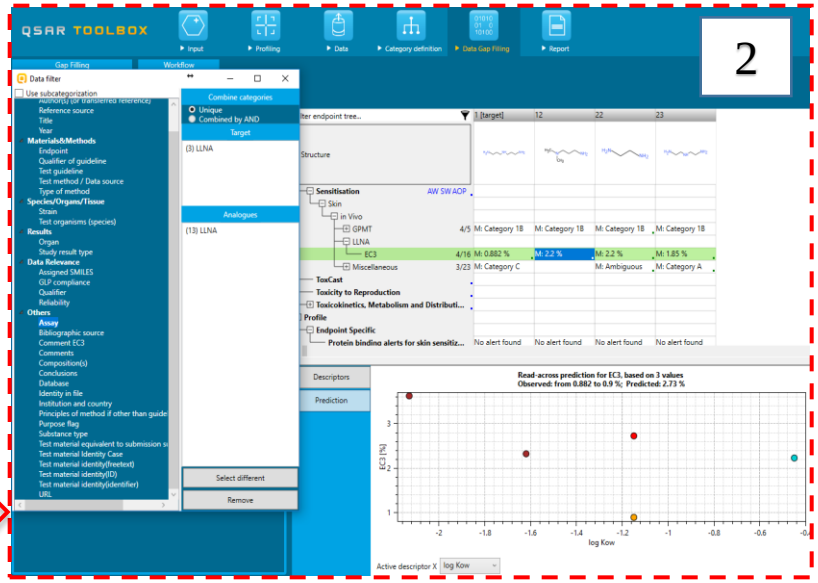


Two AE (AE *A.1* and *A.3*) related to Scenario 2 are included in the *Category definition and members* section.

- **AE A.1 Characterization of source substance** is automatically filled by the system using the available items in the *Report basket*. In this example *Category members* item (1) is appended, only. If impurities/additives of the used analogues are available, they will appear under the ***AE A.1*** in *Purity / Impurity* (2). The current analogues have no additives/impurities. Example of how the AE A.1. will look in the generated report is shown on the right(3).
- **AE A.3 Reliability and adequacy of the source study** should be filled in manually (4) *(see on the next slide)*

Report
Assessment elements of Scenario 2

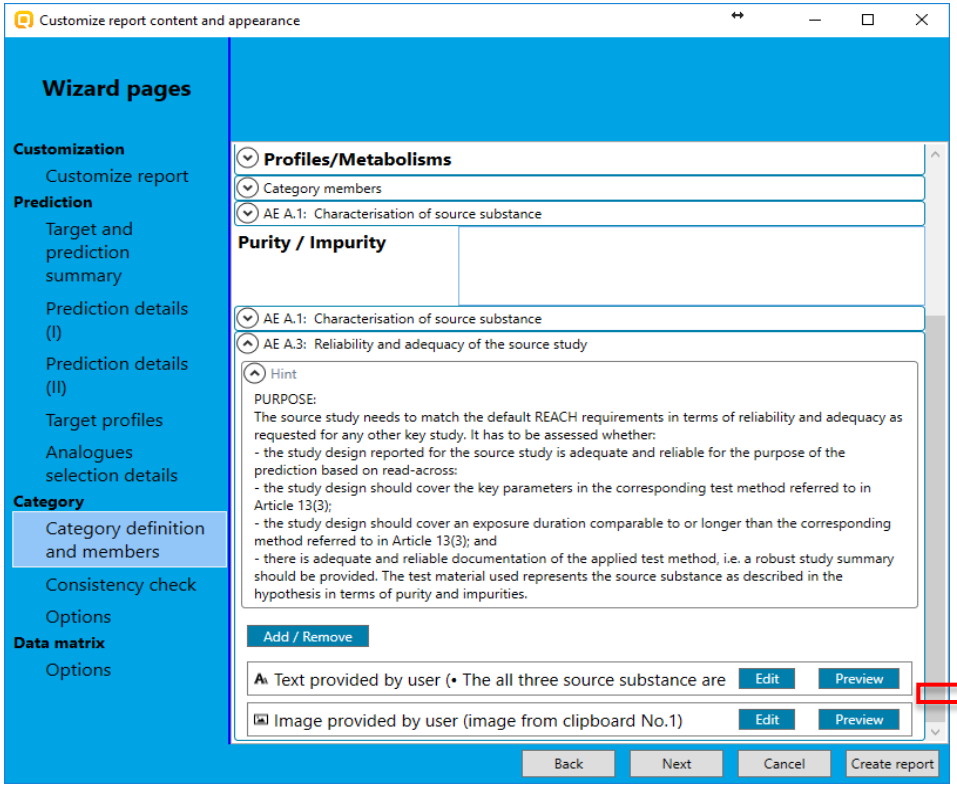


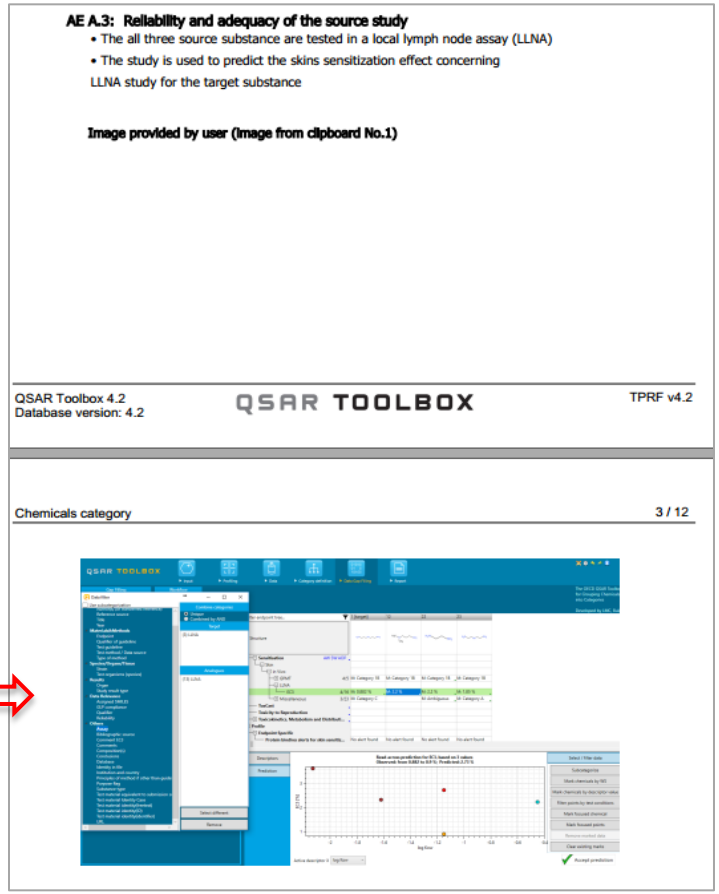


AE A.3: Click the **Add/Remove** button (1) and create new item with textual content (2) (see slide 52).
In the text field paste the following example text:
"*The all three source substance are tested according to the Local lymph node assay (LLNA)*
The study is used to predict the skin sensitization effect concerning LLNA study for the target substance"

Additionally a snapshot of the filter by test conditions window (2) could be added to confirm the consistency regarding the assay.

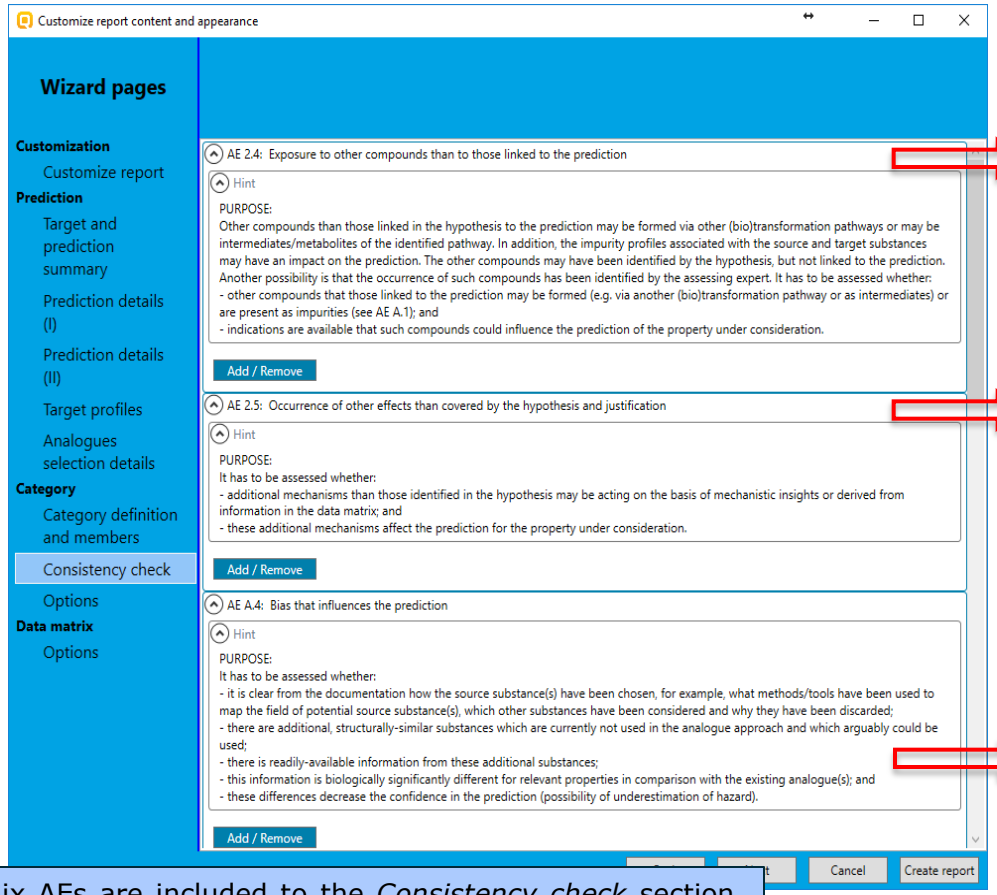
Report
Assessment elements of Scenario 2





Example of how the **AE A.3.** will look in the generated report is shown on the right.

Report
Assessment elements of Scenario 2



Six AEs are included to the *Consistency check* section. Example content for the first three AEs is given.

Example text for **AE 2.4. Exposure to other compounds than to those linked to the prediction:**

•Target substance A and source substances B,C and D are all metabolized to aldehydes and bis aldehydes;
•Bis aldehydes functionality is responsible for the toxic effect;
•Aldehydes are not expected to cause skin sensitization effect by the expert knowledge;
•The latter is confirmed with the smaller value of alert performance of the group

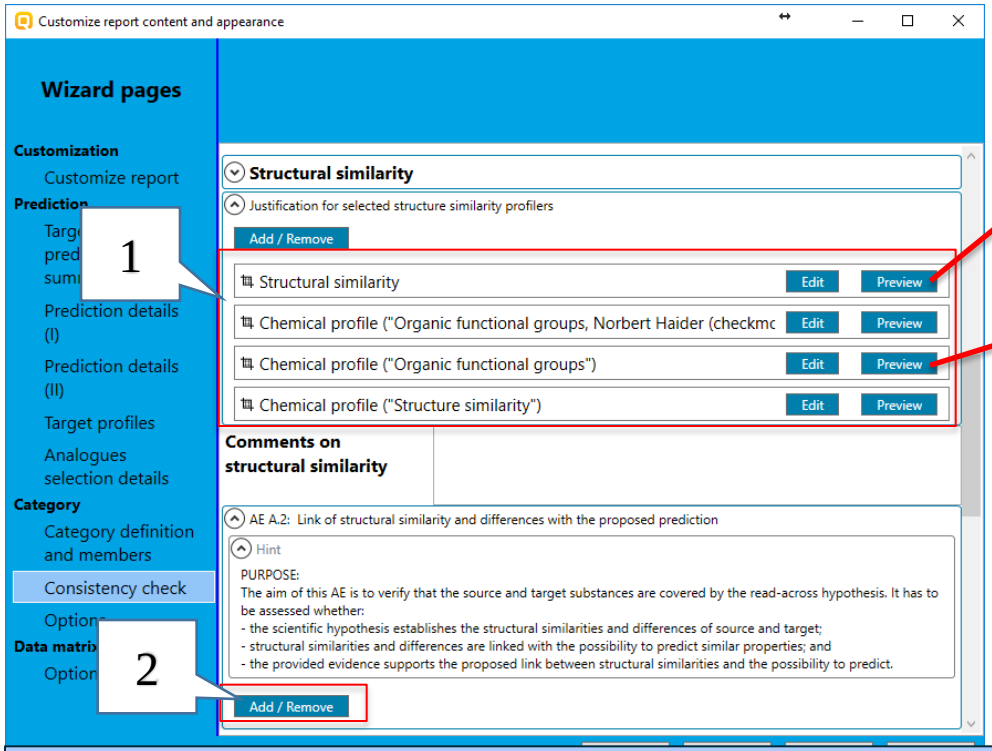
Example text for **AE 2.5. Occurrence of other effects than covered by the hypothesis and justification:**

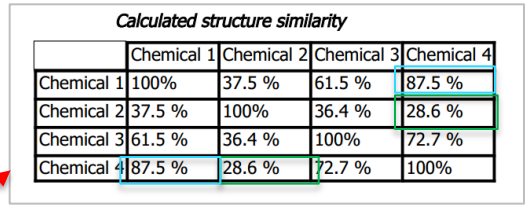
•Target substance A and source substances B,C and D are metabolized by skin metabolism simulator;
• Protein binding alerts (PBA) for skin sensitization are identified in the metabolites of the target and source substances;
• No PBA for chromosomal aberration are identified in the target and source substances, nor in the structures of their metabolites.

Example text for **AE A.4. Bias that influences the prediction:**

•Alert performance is used to define the alert with the best prediction purposes with respect to the target endpoint. Bis aldehyde functionality is selected for searching for analogues.
•All identified analogues that have PBA as a result of Autoxidation simulator are removed. The most dissimilar chemicals (with similarity below 30%) are also removed.
Expert can provide additional literature search of similar analogues with similar effects.

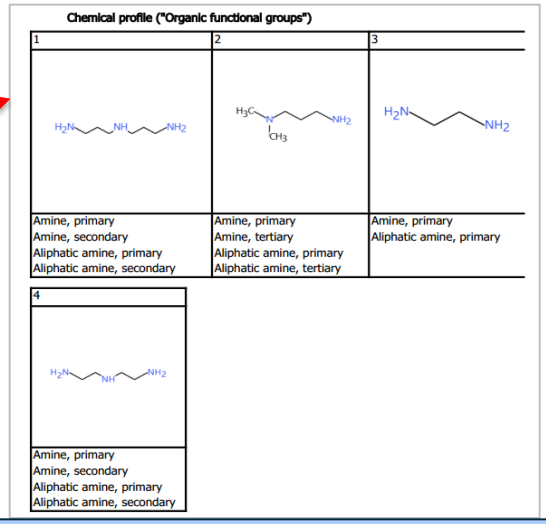
Report
Assessment elements of Scenario 2





Calculated structure similarity

	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Chemical 1	100%	37.5 %	61.5 %	87.5 %
Chemical 2	37.5 %	100%	36.4 %	28.6 %
Chemical 3	61.5 %	36.4 %	100%	72.7 %
Chemical 4	87.5 %	28.6 %	72.7 %	100%



Chemical profile ("Organic functional groups")

1	2	3
Amine, primary Amine, secondary Aliphatic amine, primary Aliphatic amine, secondary	Amine, primary Amine, tertiary Aliphatic amine, primary Aliphatic amine, tertiary	Amine, primary Aliphatic amine, primary

4
Amine, primary Amine, secondary Aliphatic amine, primary Aliphatic amine, secondary

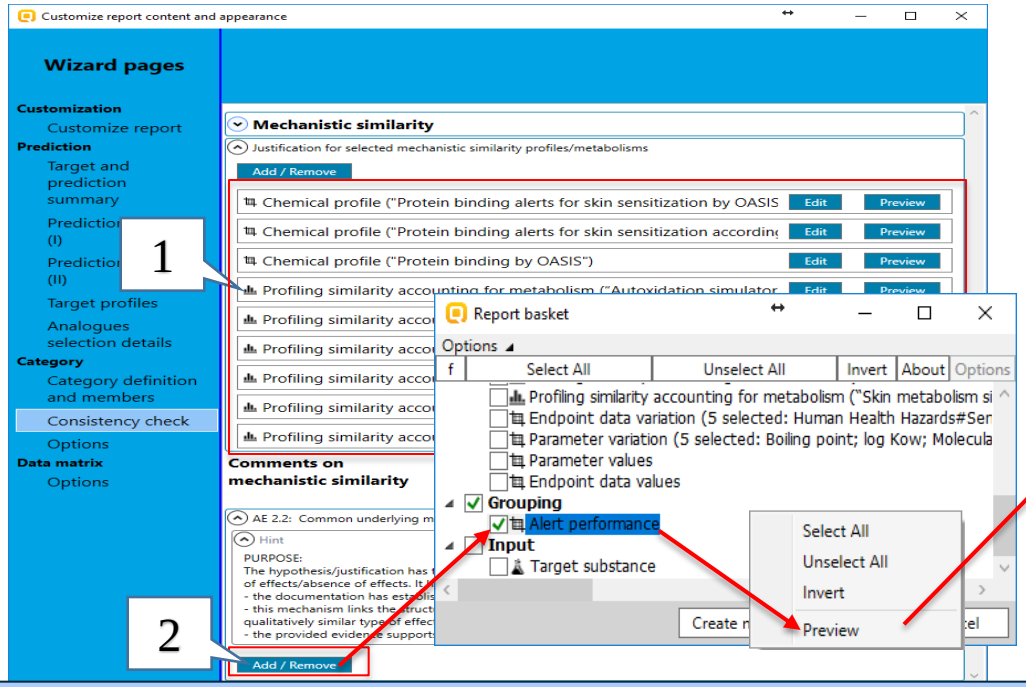
AE A.2. Link of structural similarity and differences with the proposed prediction is related to the structural similarity of the final category.

All items in the report basket related to the structural consistency of the category (1) are added automatically.

The following example text can be added for AE A.2. (2) by analyzing the structural similarity items:

- *Structural similarity between Target substance A and 3 source substances B, C and D according to Str.similarity profiler is in the range of [29-88%]*
- *They all have primary aliphatic amine based on the OFG profiler, while the target substance A and source substance D have additional secondary aliphatic amine and the source substance B has additional tertiary amine functional group.*

Report
Assessment elements of Scenario 2



Alert performance

Scale=Skin sensitisation II (ECETOC); Endpoint=EC3; Metabolism=Skin metabolism simulator

#	Alert name	Alert performance, %		Number of chemicals	
		Positive	Negative	Positive	Negative
1	Using of "Skin metabolism simulator" Combined parent and products requirements: No alert found<AND>Aldehydes<AND>Bis aldehydes (Protein binding alerts for skin sensitization by OASIS)	78.57	21.43	11	3
2	Using of "Skin metabolism simulator" Combined parent and products requirements: No alert found (Protein binding alerts for skin sensitization by OASIS)	45.35	54.65	575	693
3	Using of "Skin metabolism simulator" Combined parent and products requirements: Aldehydes (Protein binding alerts for skin sensitization by OASIS)	48.99	51.01	169	176
4	Using of "Skin metabolism simulator" Combined parent and products requirements: Bis aldehydes (Protein binding alerts for skin sensitization by OASIS)	82.35	17.65	14	3

AE A.2.2. Common underlying mechanism, qualitative aspects is related to the mechanistic similarity of the final category.

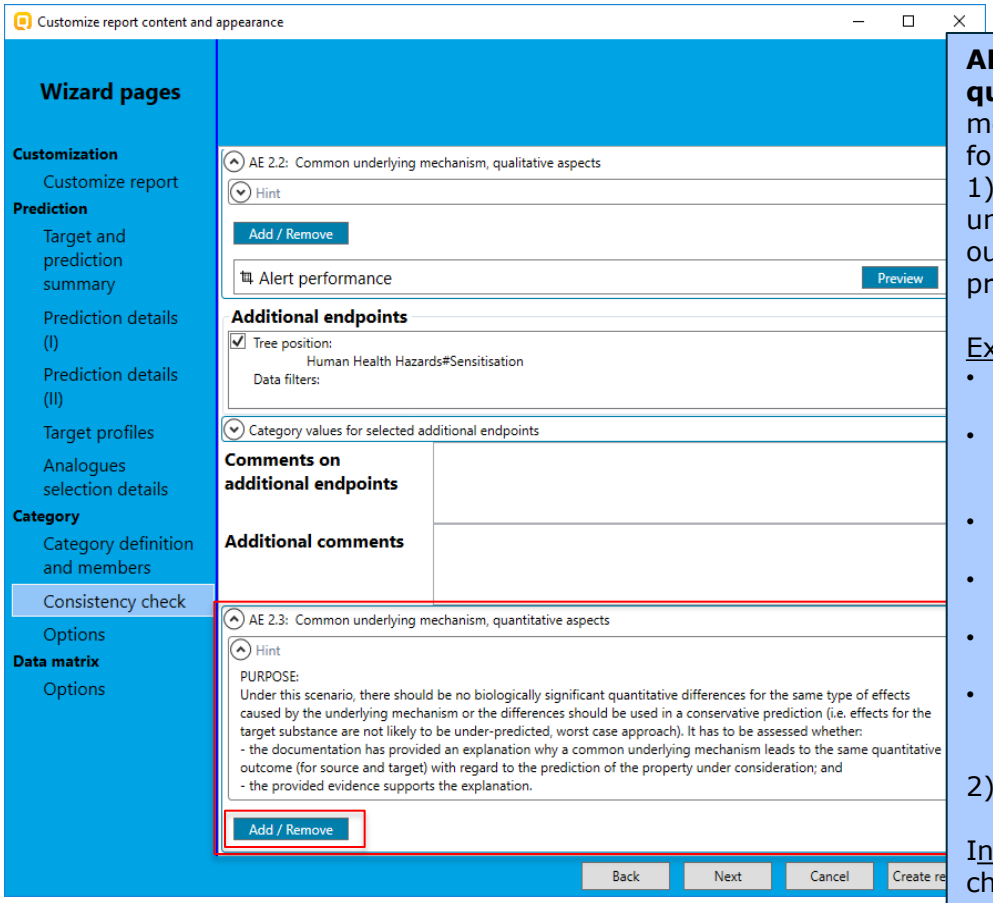
All items in the report basket related to the mechanistic consistency of the category (1) are added automatically. Only the Alert performance item have to be included here manually (2). Right-click on Alert performance in order to preview it.

The following example text summarizing the results of the provided mechanistic similarity items can be added:

- *Target substance A and source substances B, C and D are all metabolized to Bis aldehydes*
- *Bis aldehydes is taken as alerting groups responsible for the toxic effect based on the expert knowledge*
- *Bis aldehydes are taken as alerting group supported by the higher alert performance (82 %) as compared with aldehyde group (47%)*

Additionally, metabolic maps (for each of the analogues), produced by external software or found in the literature, could be included to AE in order to support the mechanistic similarity of the category.

Report
Assessment elements of Scenario 2



AE A.2.3. Common underlying mechanism, quantitative aspects is also related to the mechanistic similarity of the final category. The following information could be included here:

1) textual or illustrated explanation why the common underlying mechanism leads to the same quantitative outcome (for source and target) with regard to the prediction of the property under consideration; and

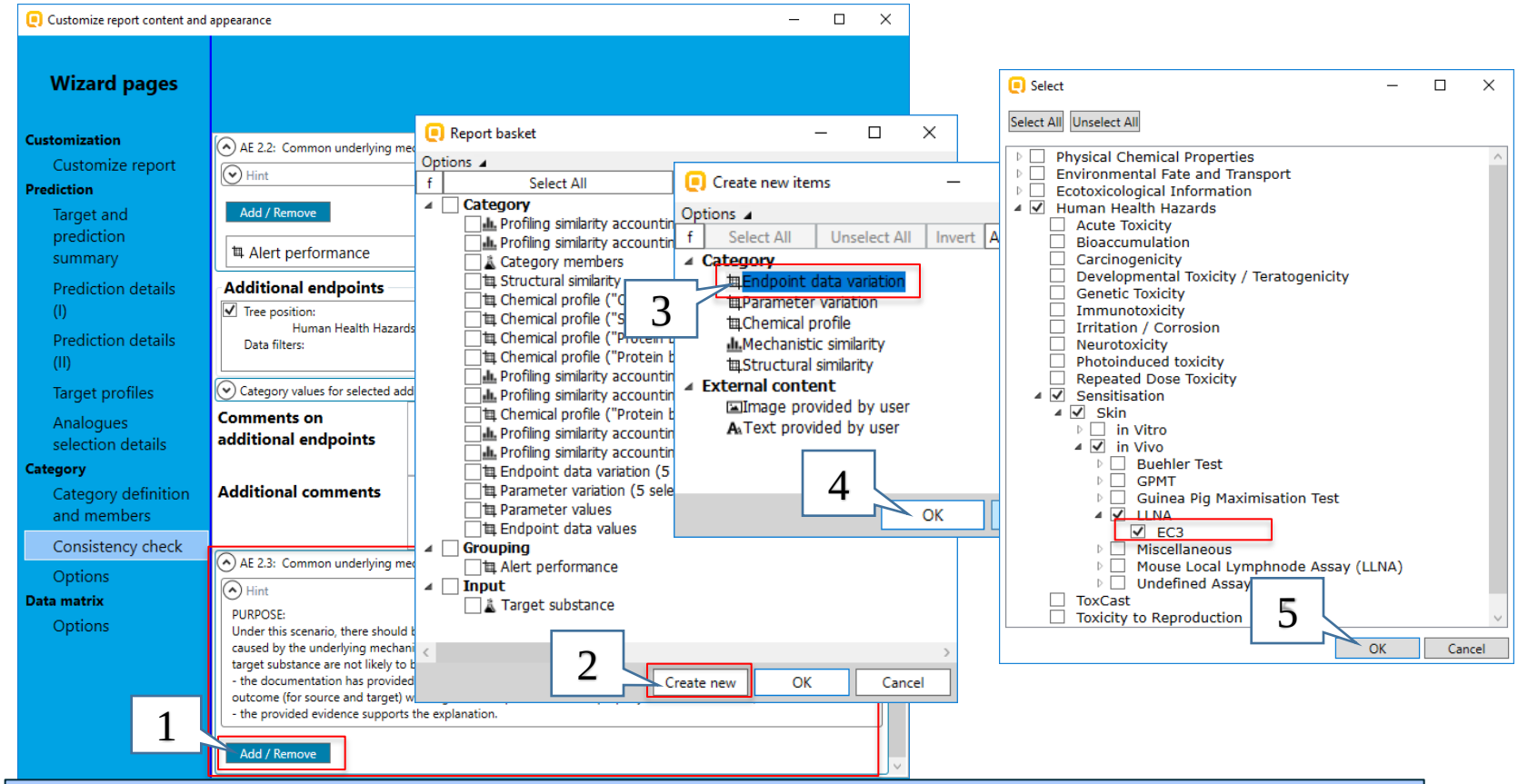
Example text:

- *Target substance A is metabolized to Aldehydes and Bis aldehydes*
- *It is expected that Bis-aldehydes as the alert with higher alert performance is could be responsible for the toxic effect*
- *Source substances B, C and D are metabolized to aldehydes and bis-aldehydes, too*
- *The available experimental EC3 values for the source substances corresponds to a positive effect.*
- *Similar toxic effects observed in sources substances supports the prediction for the target*
- *Toxic effects of all source substances and target are supported by the identified additional SS data*

2) evidences supporting the explanation.

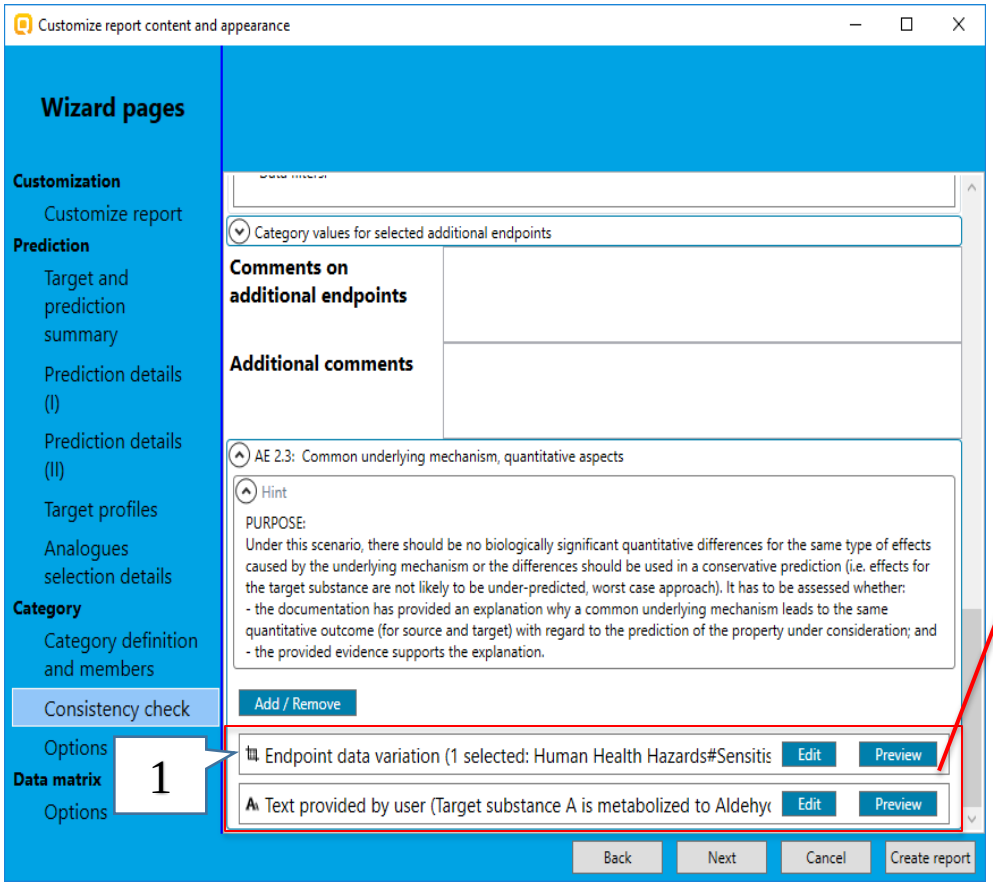
Include all available SS EC3 data for the target chemical and the source substances in all Toolbox database. See how to do this on the next two slides.

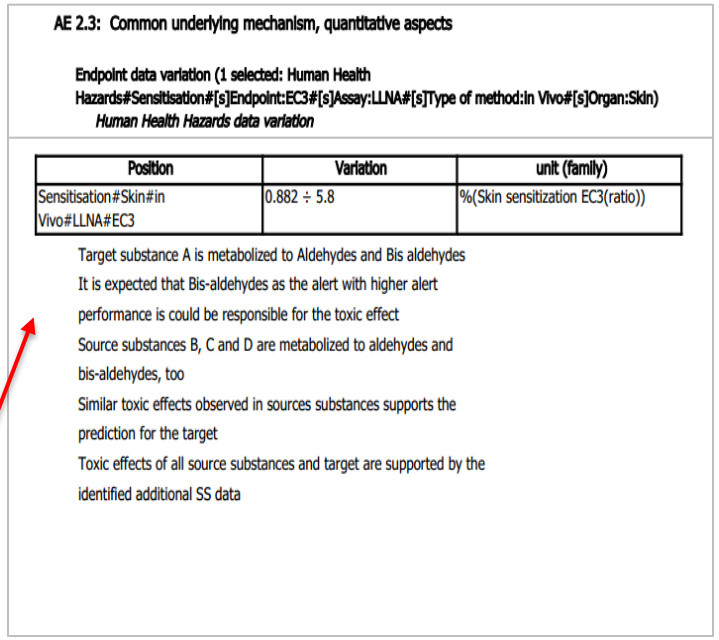
Report
Assessment elements of Scenario 2



Click **Add/Remove** button (1) and **create new** item (2). Select **Endpoint data variation** (3) and confirm by **OK** (4). A new window with the endpoint tree organization appears. Select **EC3** and click **OK** (5).
This new item will provide information not only for the used, but for all available EC3 data for the chemicals of the category.

Report
Assessment elements of Scenario 2





AE 2.3: Common underlying mechanism, quantitative aspects

Endpoint data variation (1 selected: Human Health Hazards#Sensitisation#[s]Endpoint:EC3#[s]Assay:LLNA#[s]Type of method:In Vivo#[s]Organ:Skin)

Human Health Hazards data variation

Position	Variation	unit (family)
Sensitisation#Skin#in Vivo#LLNA#EC3	0.882 ÷ 5.8	%(Skin sensitization EC3(ratio))

Target substance A is metabolized to Aldehydes and Bis aldehydes

It is expected that Bis-aldehydes as the alert with higher alert performance is could be responsible for the toxic effect

Source substances B, C and D are metabolized to aldehydes and bis-aldehydes, too

Similar toxic effects observed in sources substances supports the prediction for the target

Toxic effects of all source substances and target are supported by the identified additional SS data

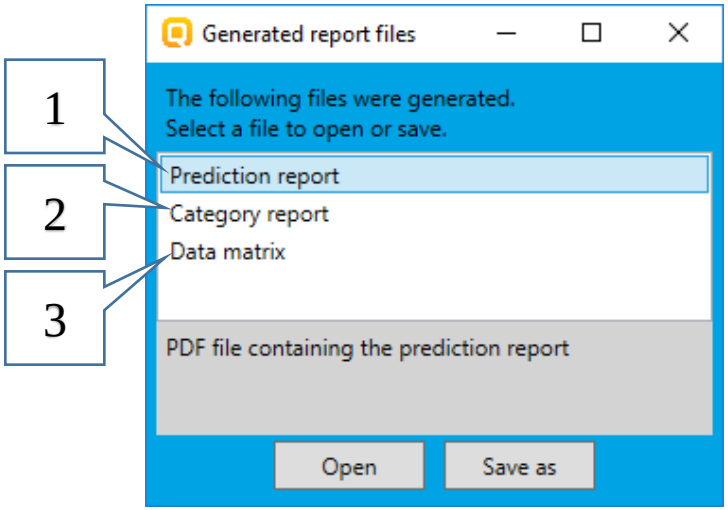
After creating of the new item, it appears below the AE 2.3. along with the created text item (1). Example on how the **AE 2.3**. will look in the generated report is shown in right (2).

Report Generation

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 information for the consistency of the final category (target plus used analogues)
3) **Data matrix** - a MS Excel file containing chemicals used for prediction along with their data for selected parameters, profiles and endpoint tree positions.

RAAF AEs are included in the first two files.
All generated files should be provided when submit a prediction.



Report
Generated report files

Prediction report

Prediction of EC3 for Iminobis-3-propylamine 1 / 6

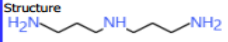
QSAR Toolbox prediction for single chemical

(In accordance with RAAF scenario 2)

The selected RAAF scenario is specified in the first page

Date: 20 Mar 2018
Author(s):
Contact details:

Target information

Structural information	Numerical identifiers	Chemical names
SMILES: NCCCNCCCN Structure 	CAS#: 56-18-8 Other: EC Number:3774080	1,3-Propanediamine, N-(3-aminopropyl)- 1,3-Propanediamine, N1-(3-aminopropyl)- 1,3-propanediamine,_ n-(3-aminopropyl)-

Prediction summary

Predicted endpoint: EC3; No effect specified; No species specified; No duration specified; No guideline specified

Category report

Chemicals category 1 / 31

QSAR Toolbox report for category

(In accordance with RAAF scenario 2)

1. Category definition

1.1. Category definition *manually editable field*

Not provided by the user

Ranges for selected physicochemical properties and calculated parameters

Parameter variation (5 selected: Boiling point; log Kow; Molecular Weight; Vapor Pressure (Antoine method); Water Solubility)

2D parameters data variation

Parameter name	Variation	unit (family)
Boiling point	103 ÷ 228	°C
log Kow	-2.13 ÷ -0.45	<no units>
Molecular Weight	60.1 ÷ 131	Da
Vapor Pressure (Antoine method)	0.0913 ÷ 19	mm Hg
	1E+06	mg/L

ariation (4 selected: Physical Chemical Properties#Boiling point; Physical Chemical our pressure; Physical Chemical Properties#Water solubility; Physical Chemical tition Coefficient:#N-Octanol/Water)

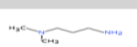
emical Properties data variation

n	Variation	unit (family)
	117 ÷ 207	°C(Temperature)
	NOT_SPECIFIED	HT Version 20120101 phrasegroup_C47
	0.0126 ÷ 99.8	bar(Pressure)
	5.9 ÷ 1.25E+03	Pa(Pressure)
	0.232 ÷ 12	mm Hg(Pressure)
	1E+06	mg/L(Mass concentration)
	1E+06	mg/L(Mass concentration)
N-	-4.96 ÷ -0.352	

get) endpoint(s) *manually editable field*

QSAR TOOLBOX TPRF v4.2

Data matrix report

	Target chemical	Neighbour #1	Neighbour #2	Neighbour #3
Substance identity				
Structure				
CAS number	56-18-8	109-55-7	107-15-3	111-40-0
Chemical name	Iminobis-3-propylamine	3-aminopropyldimethylamine	Ethylenediamine	Diethylenetriamine
Other identifier				
SMILES	NCCCNCCCN	CN(C)CCCN	NCCN	NCCNCCN
Parameters	**unit**			
Vapor Pressure (Antoine method)	mm Hg	0.0913	9.41	19	0.274
log Kow		-1.15	-0.45	-1.62	-2.13
Molecular Weight	Da	131	102	60.1	103
Boiling point	°C	228	134	103	189
Water Solubility	mg/L	1E+06	1E+06	1E+06	1E+06
Profilers					
Profiles used for grouping/subcategorization					
Using of "Skin metabolism simulator" Combined		Parent and 5 metabolites:;	Parent and 12 metabolites:;	Parent and 5 metabolites:;	Parent and 5 metabolites:;
Protein binding alerts for skin sensitization by		No alert found	No alert found	No alert found	No alert found
Structure similarity (subcategorization)		[90%,100%]	[30%,40%]	[60%,70%]	[80%,90%]
Endpoint Specific					
Protein binding alerts for skin sensitization by		No alert found	No alert found	No alert found	No alert found
Protein binding alerts for skin sensitization		No alert found	No alert found	No alert found	No alert found
Protein binding alerts for skin sensitization by		No alert found	No alert found	No alert found	No alert found
Empiric					
Organic functional groups		Amine, primary; Amine, secondary;	Amine, primary; Amine, tertiary;	Amine, primary; Aliphatic amine, primary	Amine, primary; Amine, secondary;
Measured and predicted data					
Data used for prediction					

environment	endpoint	value	unit	species, duration, test type, type of method, assay, strain, test	value	unit	species, duration, test type, type of method, assay, strain, test	value	unit	species, duration, test type, type of method, assay, strain, test	value	unit	species, duration, test type, type of method, assay, strain, test
Sensitisation	EC3				2.32	%	mouse in Vivo	2.7	%	mouse in Vivo	5.8	%	mouse in Vivo
Sensitisation	EC3				2.2	%	mouse in Vivo	2.2	%	mouse in Vivo	3.31	%	mouse in Vivo

Sheet1

READY

Congratulation

- You have now been introduced to the RAAF scenario;
- You have now been introduced to the *Report basket*.
- You have now been introduced to the AEs related to Scenario 2.
- Note proficiency comes with practice.