QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 2 and related assessment elements

Outlook

- **Background**
- Keywords
- 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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Keywords

TARGET CHEMICAL - chemical of interest

MODULE – a Toolbox module is a section dedicated to specific actions and options

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)

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;
- 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 to the user allowing for scientific assessment of the outcome;
- To explain to the Toolbox user the rationale behind each step of the exercise.

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

- In this example we will predict the skin sensitization potential of chemical: *1,3-Propanediamine, N-(3-aminopropyl)* - [CAS# 56-18-8], which will be the "target" chemical;
- The category will be defined based on protein binding mechanism identified in the target chemical after skin metabolism is taken into account. The identified protein binding mechanism is 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**

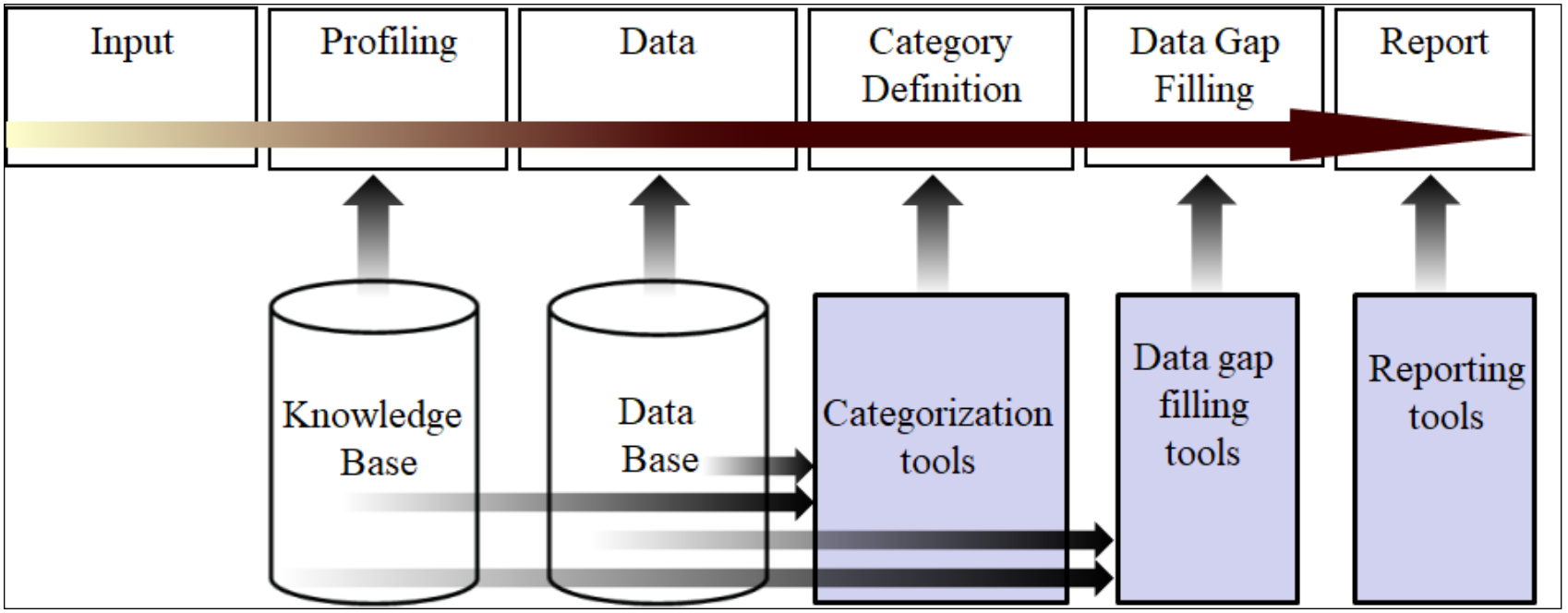
Workflow

- **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.

Workflow

Scheme illustrating the Toolbox workflow

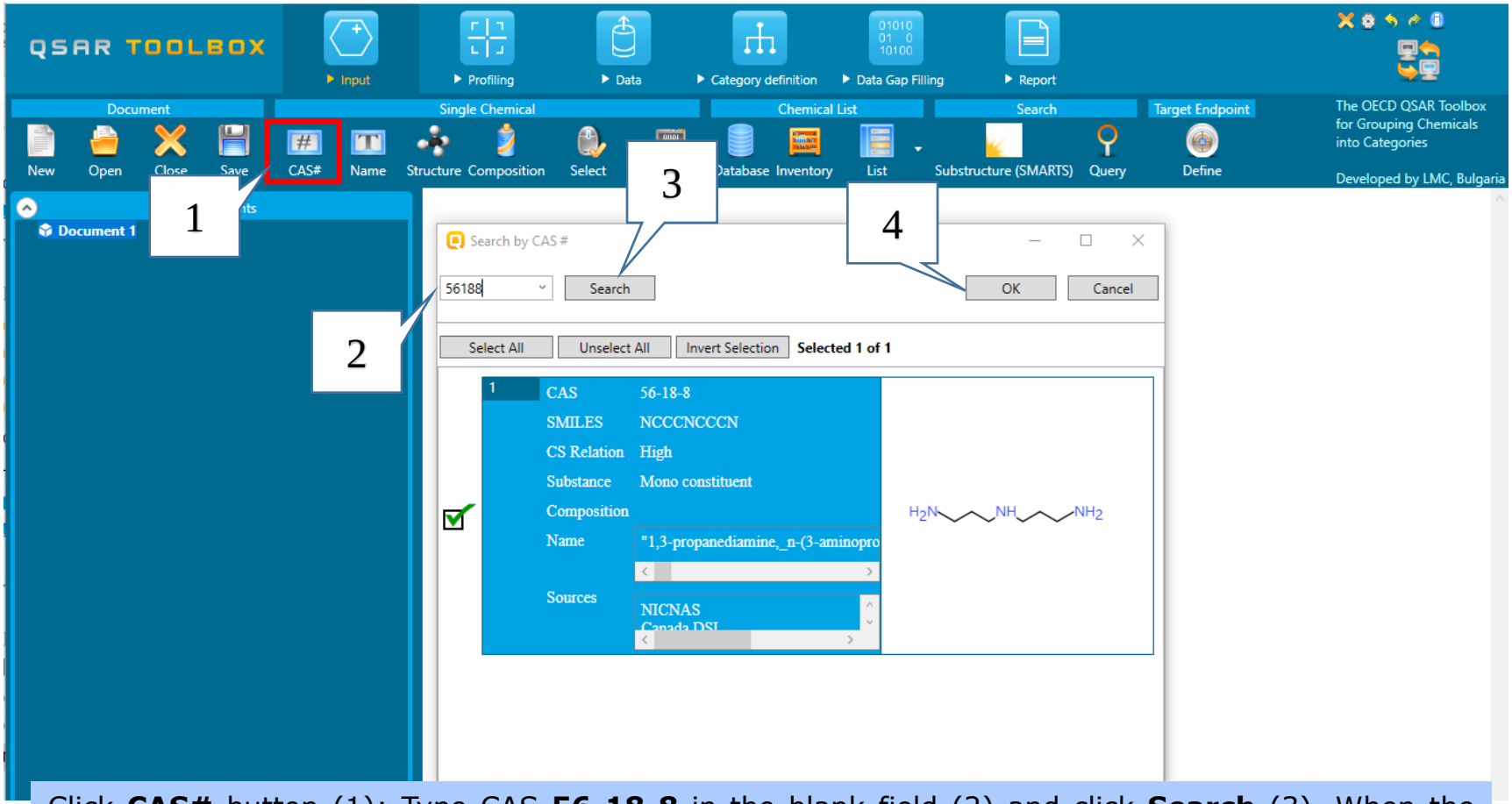


Input
Overview

- 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#

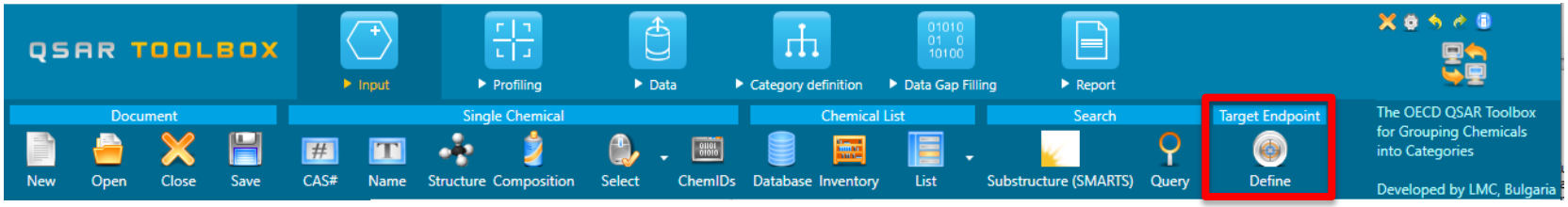


Click **CAS#** button (1); Type CAS **56-18-8** in the blank field (2) and click **Search** (3). When the structure appears, click **OK** (4).

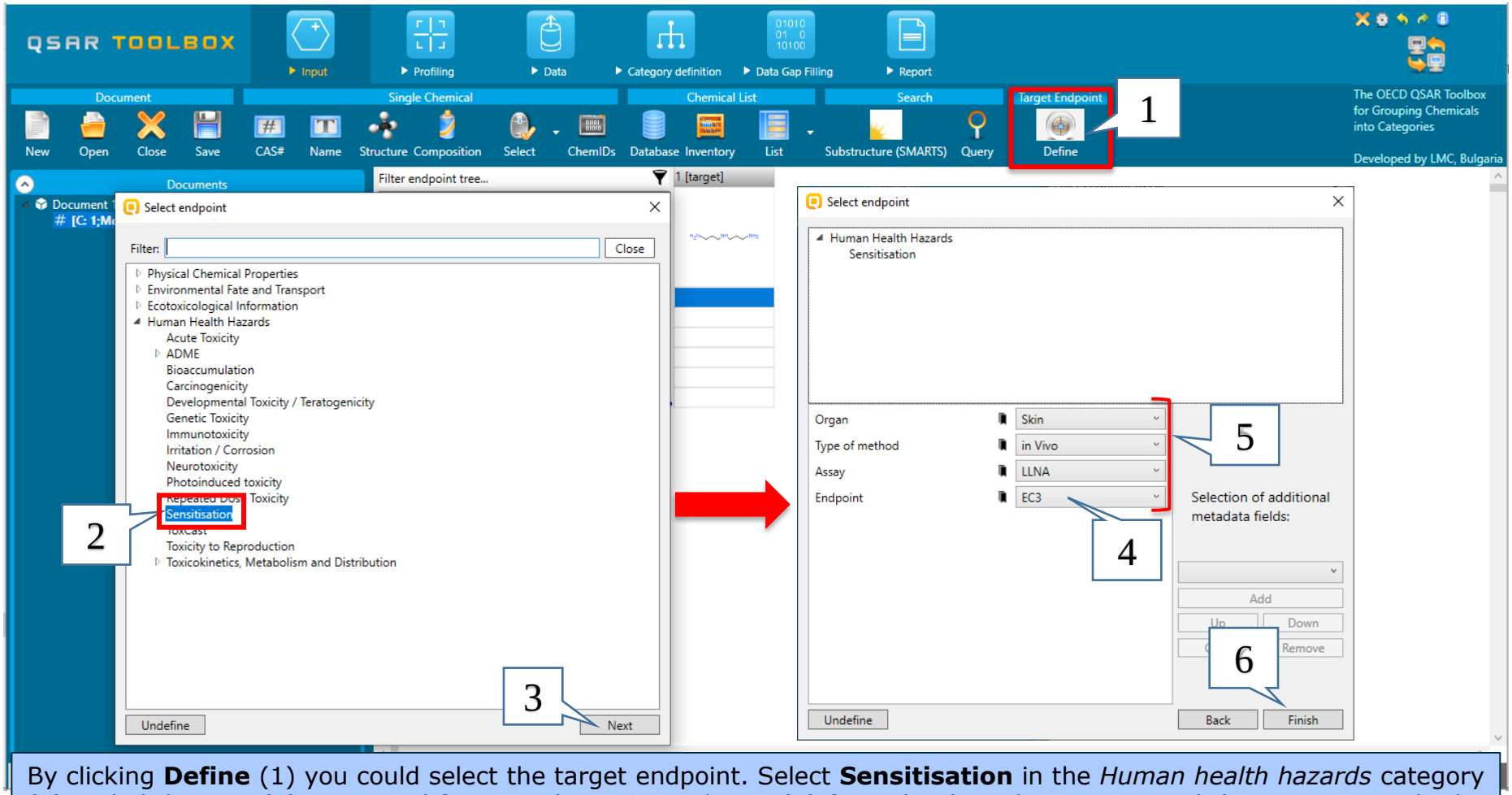
Input
Define target endpoint

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, relevancy of the profiles is provided expressed in different highlighting.

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



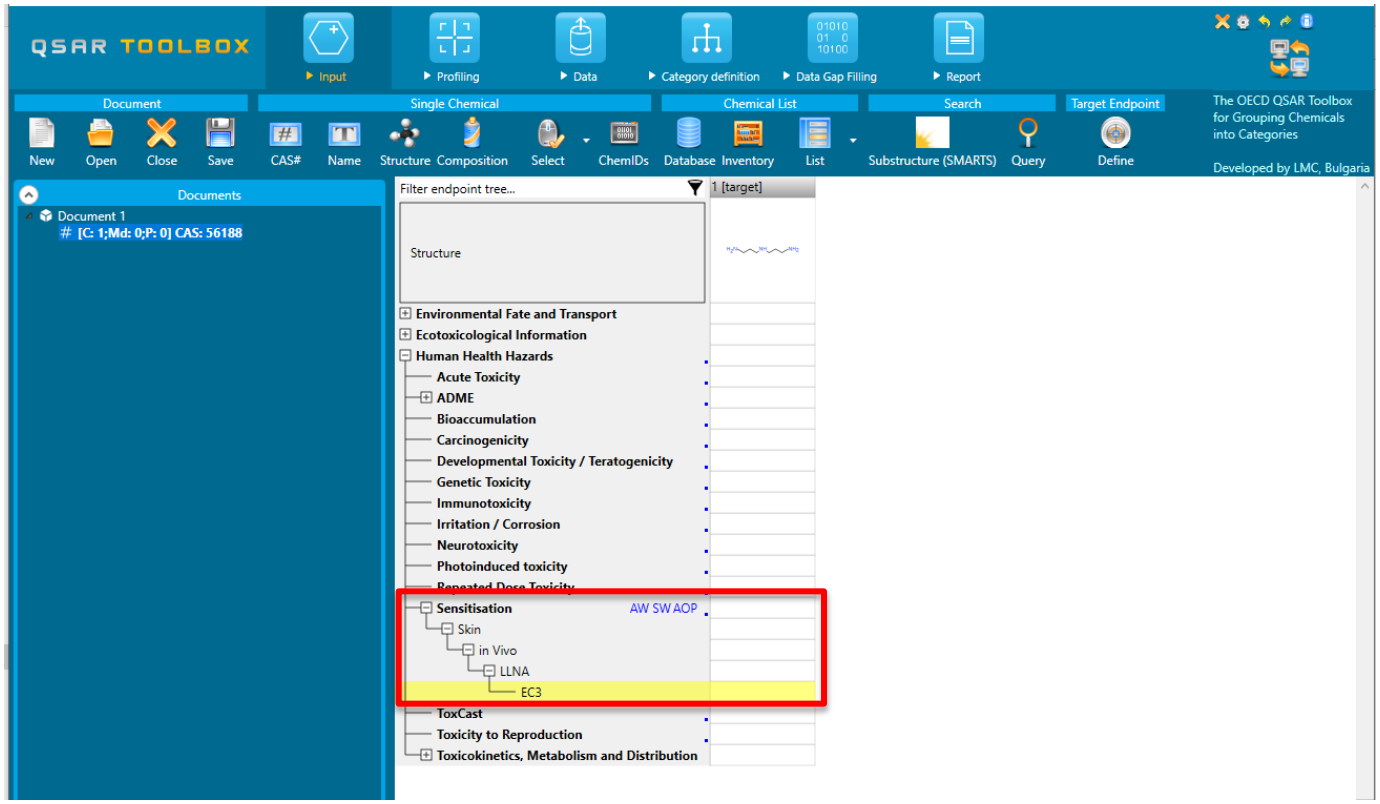
Input
Define target endpoint



By clicking **Define** (1) you could select the target endpoint. Select **Sensitisation** in the *Human health hazards* category (2) and click **Next** (3). You need first to 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

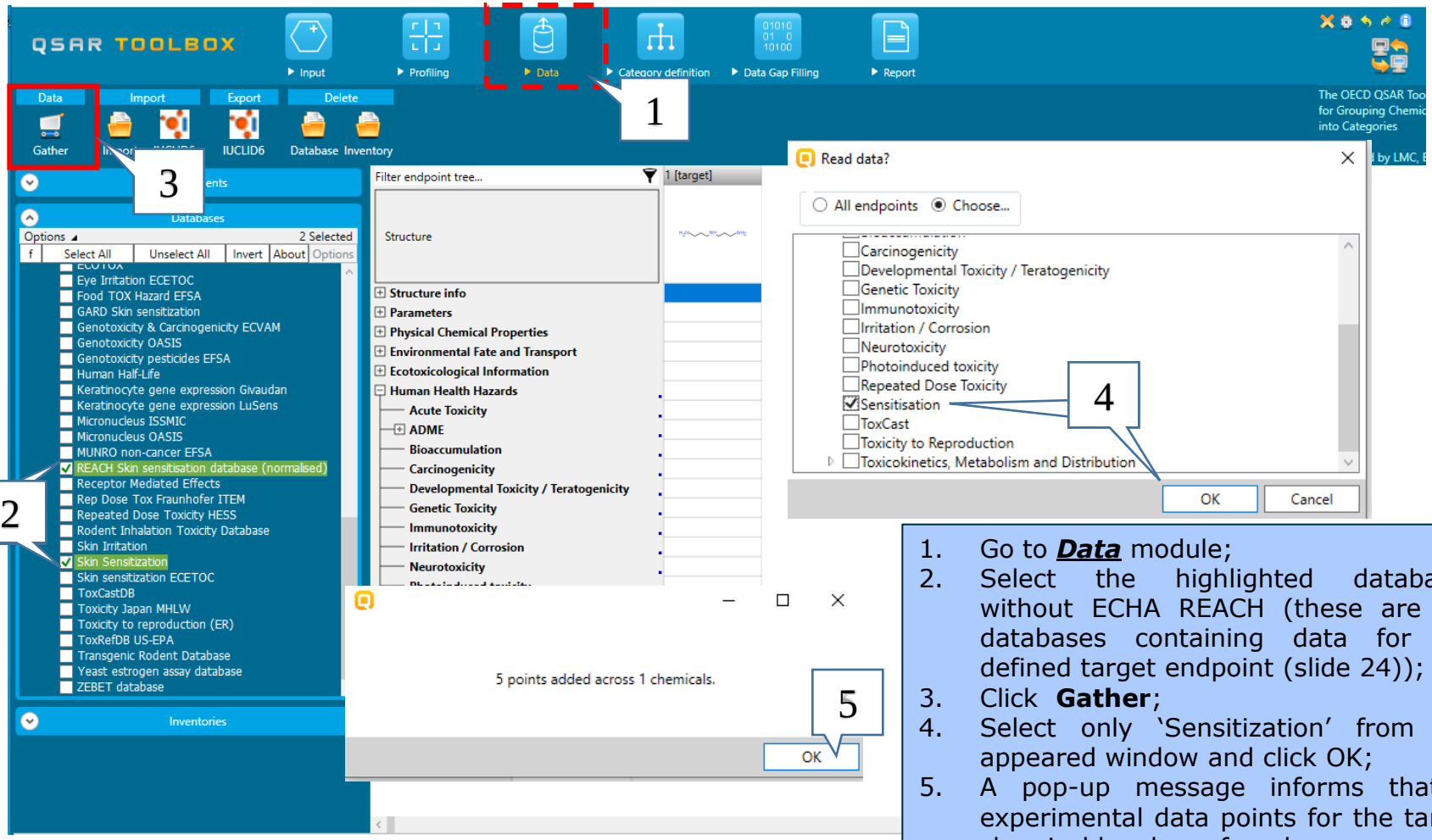
Once the endpoint is defined along with its metadata, they appear in the endpoint tree and the corresponding row of the data matrix is highlighted.



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 without ECHA REACH (these are the databases containing data for the defined target endpoint (slide 24));
3. Click **Gather**;
4. Select only 'Sensitization' from the appeared window and click OK;
5. A pop-up message informs that 5 experimental data points for the target chemical has been found.

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 found for the target chemical. There are three out of five ppositive experimental data for the target chemical.
- 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).

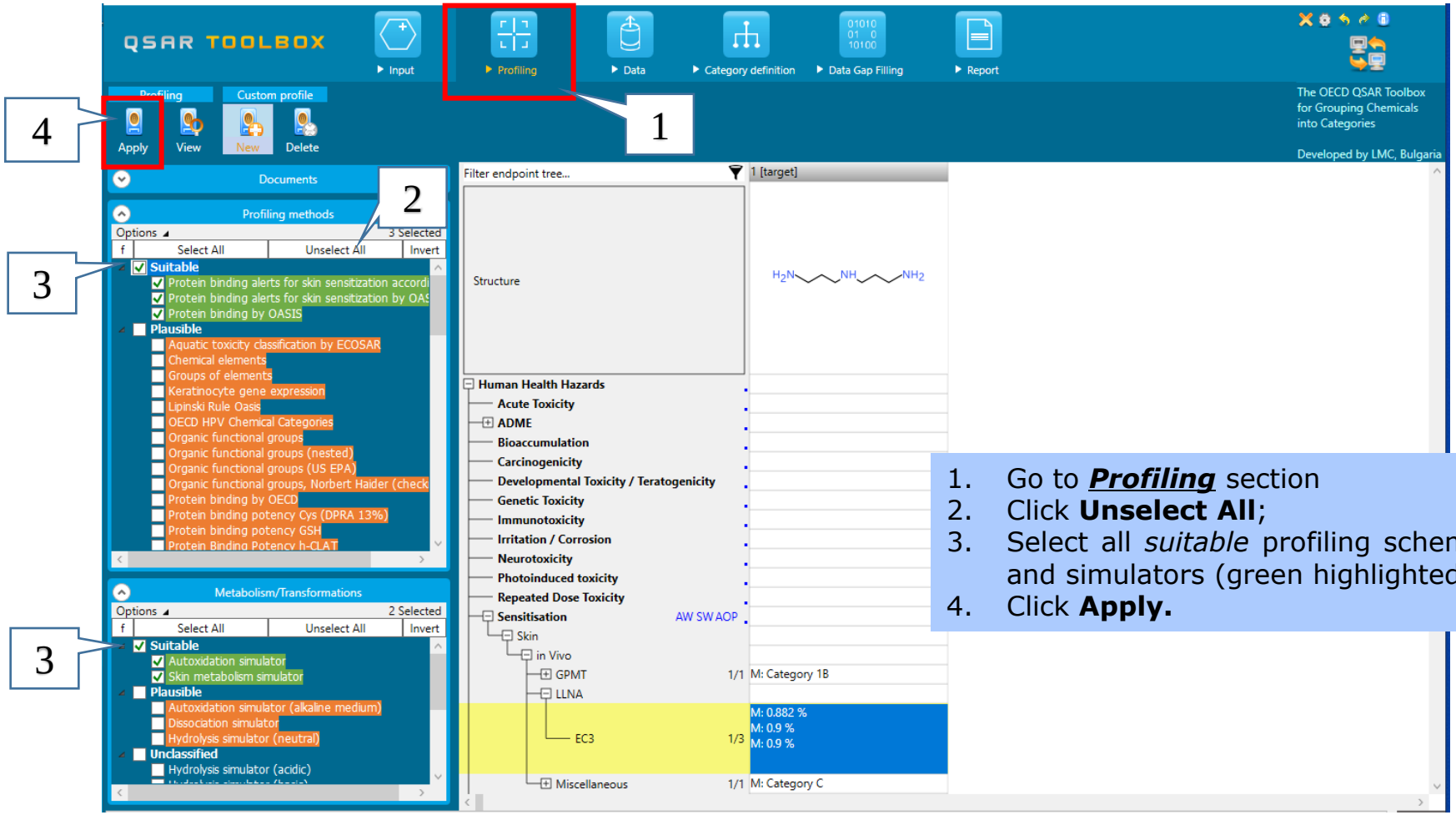
Profiling
Overview

- "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;
- "Profiling" module contains all the knowledge in the system coded in profiling schemes (i.e. profilers);
- "Profilers" are a collection of empirical and mechanism knowledge (expertly derived) which could be used to analyse the structural properties of chemicals;
- The "profilers" identify the affiliation of the target chemical(s) to preliminary defined categories (functional groups/alerts);
- The "Profiling" module contains also observed and simulated metabolisms/transformations, which could be used in combination with the profilers;
- The outcome of the profiling determines the most appropriate way to search for analogues, but they are also useful for preliminary screening or prioritization of substances;
- The "profilers" are not (Q)SARs, i.e. they are not prediction models themselves;
- Based on the "profilers' relevancy" (determined by the defined target endpoint), the most suitable once are getting colour highlighted*.

*For more details regarding relevancy of the profilers see ppt: Example for predicting skin sensitization taking into account alert performance

Profiling

Profiling the target chemical



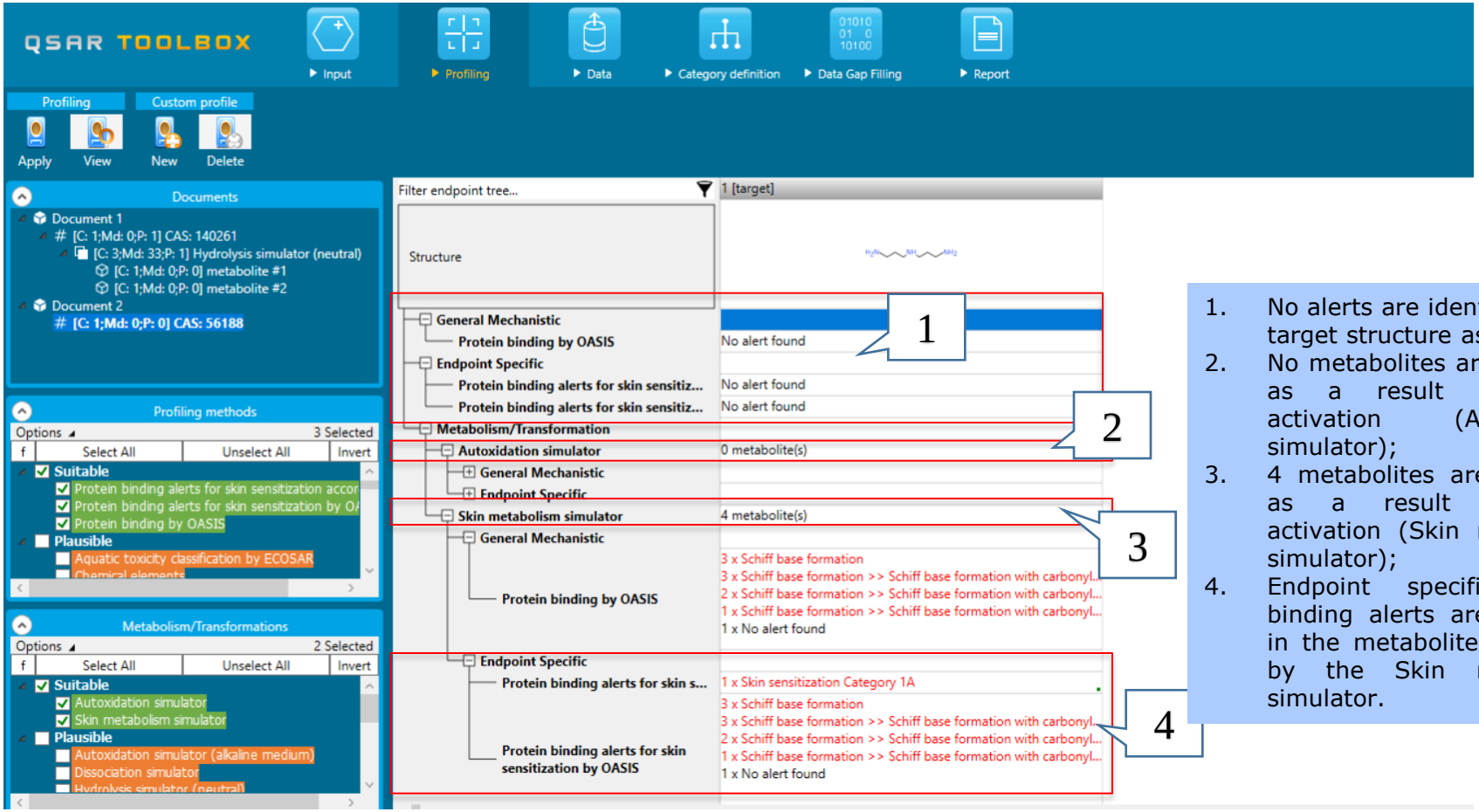
1. Go to ***Profiling*** section
2. Click **Unselect All**;
3. Select all *suitable* profiling schemes and simulators (green highlighted);
4. Click **Apply.**

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



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. 4 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.

Recap

- In module *Input*, you have 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 chemical 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 (skin metabolism).
- Click "Category Definition" to move to the next module.

Category Definition
Overview

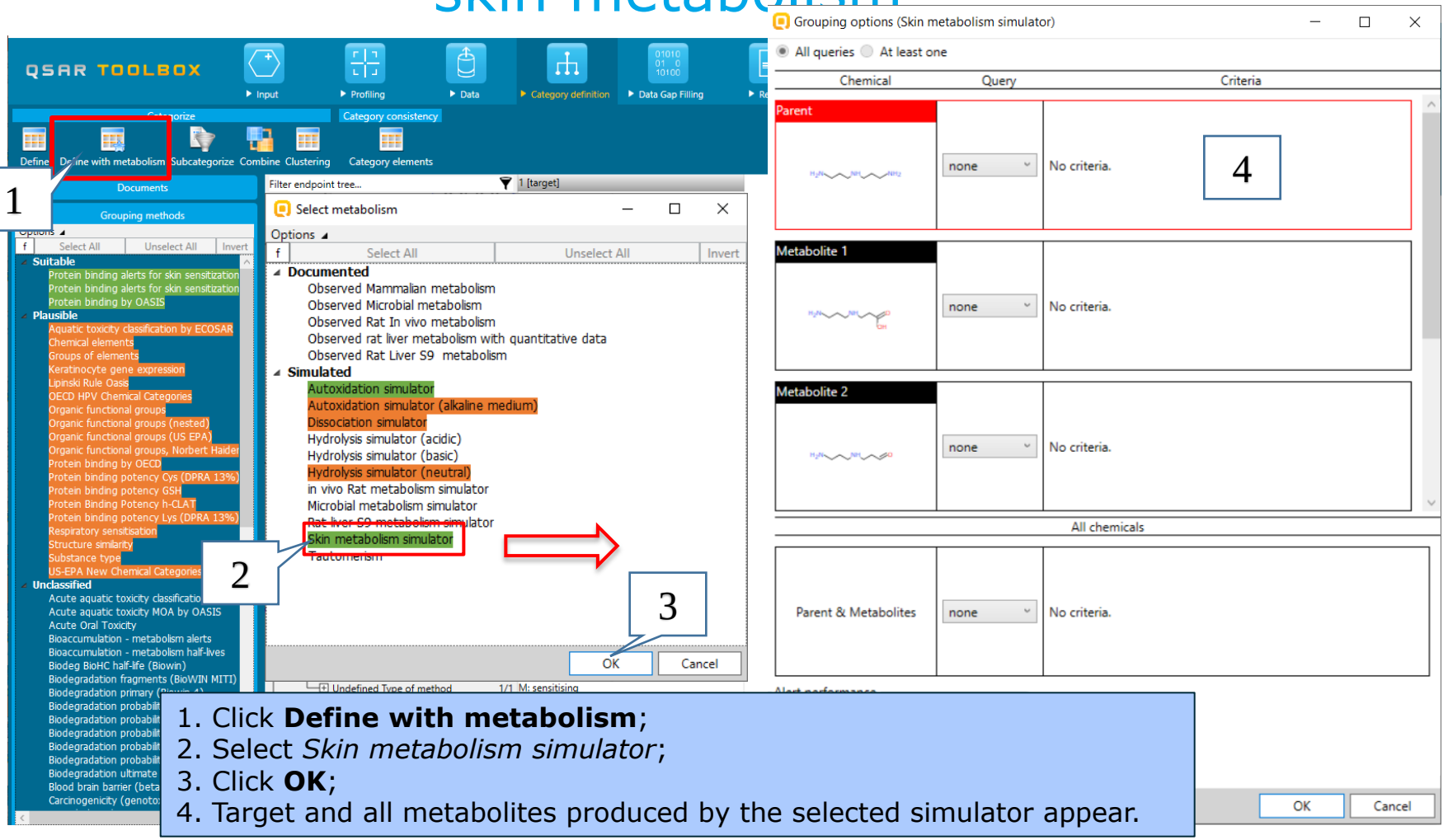
- This module provides the user with several means of grouping chemicals into a toxicologically meaningful category that includes the target molecule.
- This is the critical step in the workflow.
- Several options are available in the Toolbox to assist the user in refining the category definition.

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 a metabolism (e.g. metabolites of the analogues to have same metabolic pattern as metabolites of the parent chemical). 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' functionality could help for defining which of them is the most suitable for primary categorization. The latter is shown on the next few slides.

Category Definition

Searching for analogues accounting for skin metabolism

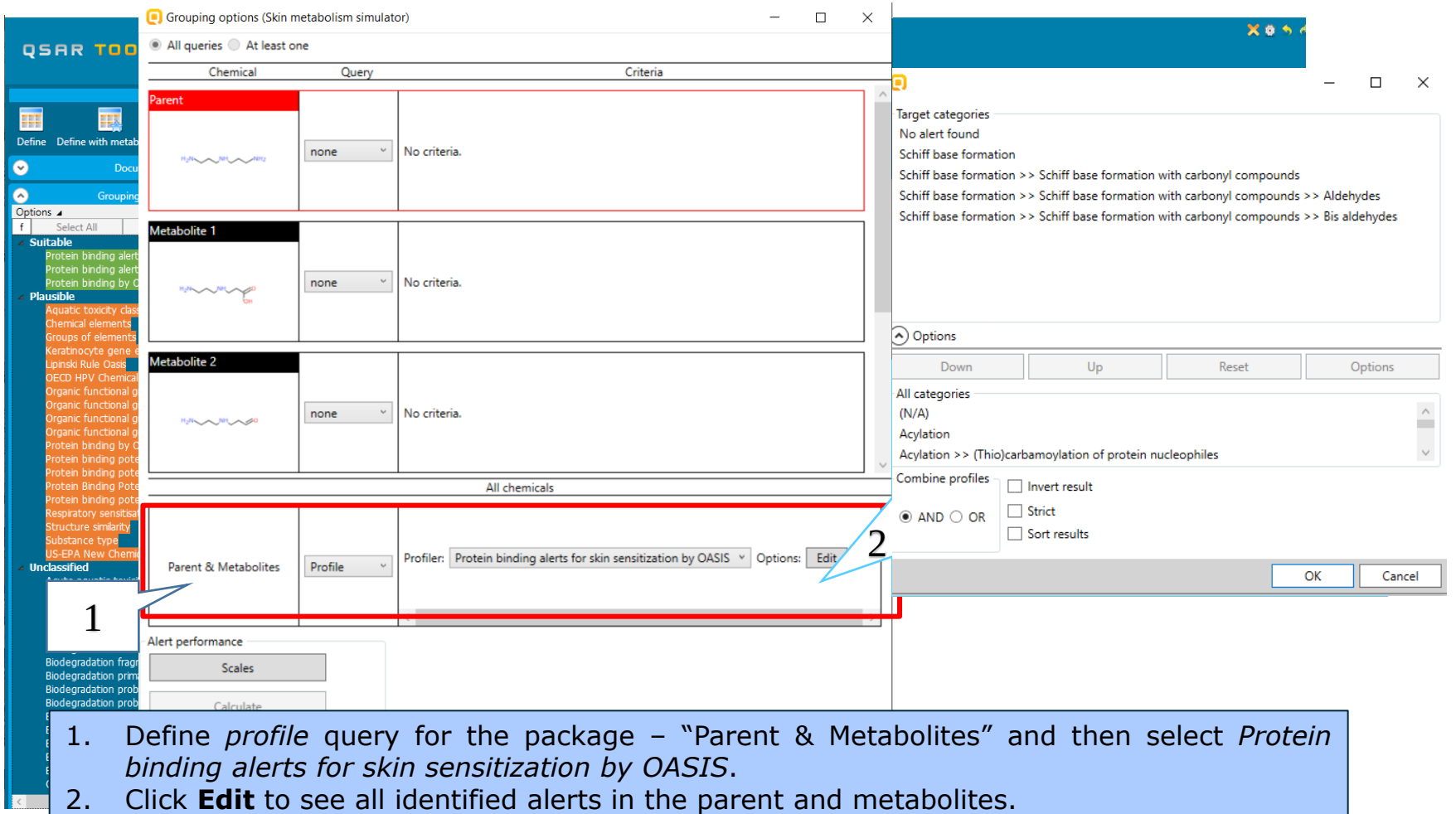


1. Click **Define with metabolism**;
2. Select *Skin metabolism simulator*;
3. Click **OK**;
4. Target and all metabolites produced by the selected simulator appear.

For more details regarding grouping with metabolism see tutorial Tutorial_20_TB_4.4_New options for grouping with metabolism.pdf

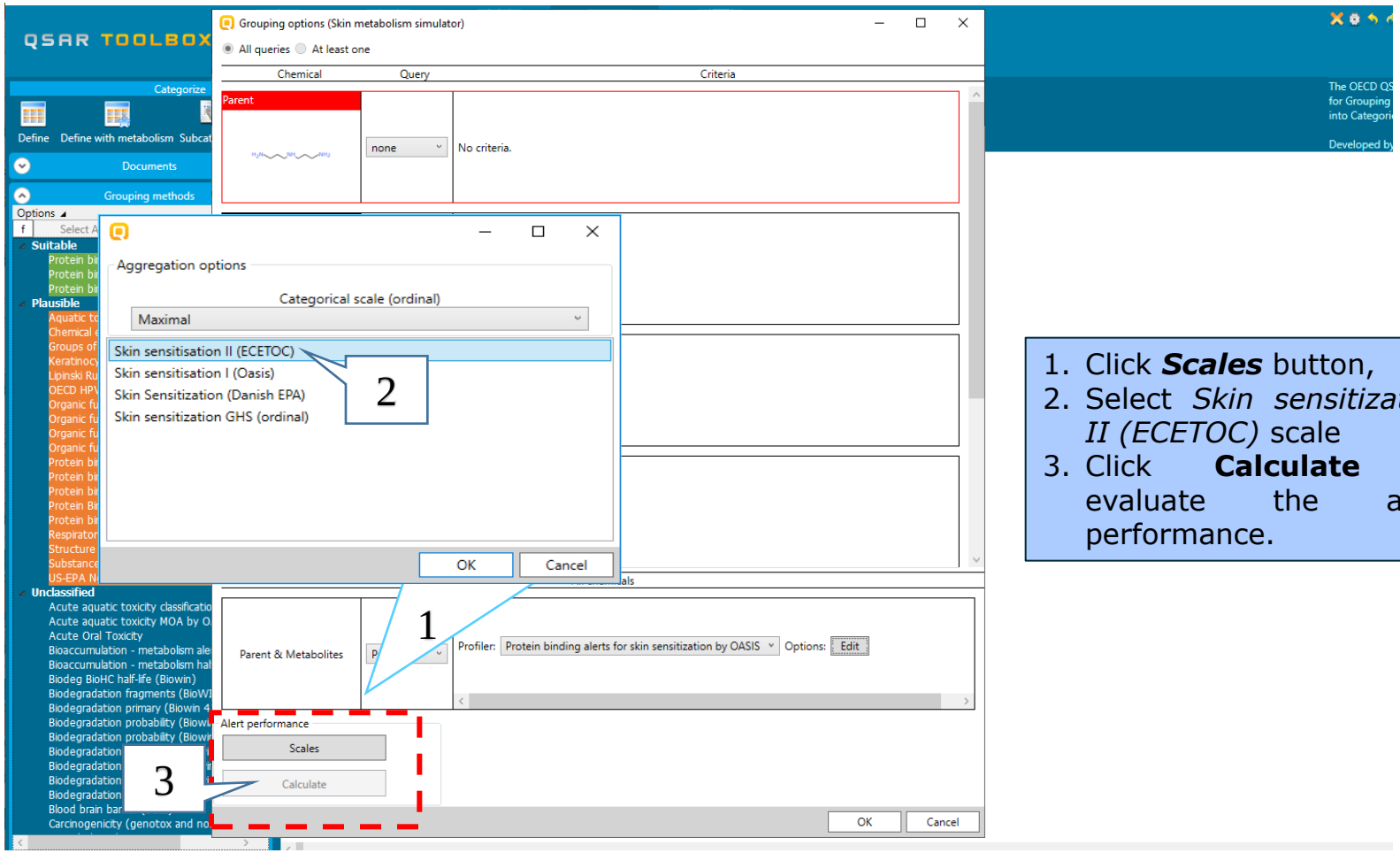
Category Definition

Searching for analogues accounting for skin metabolism



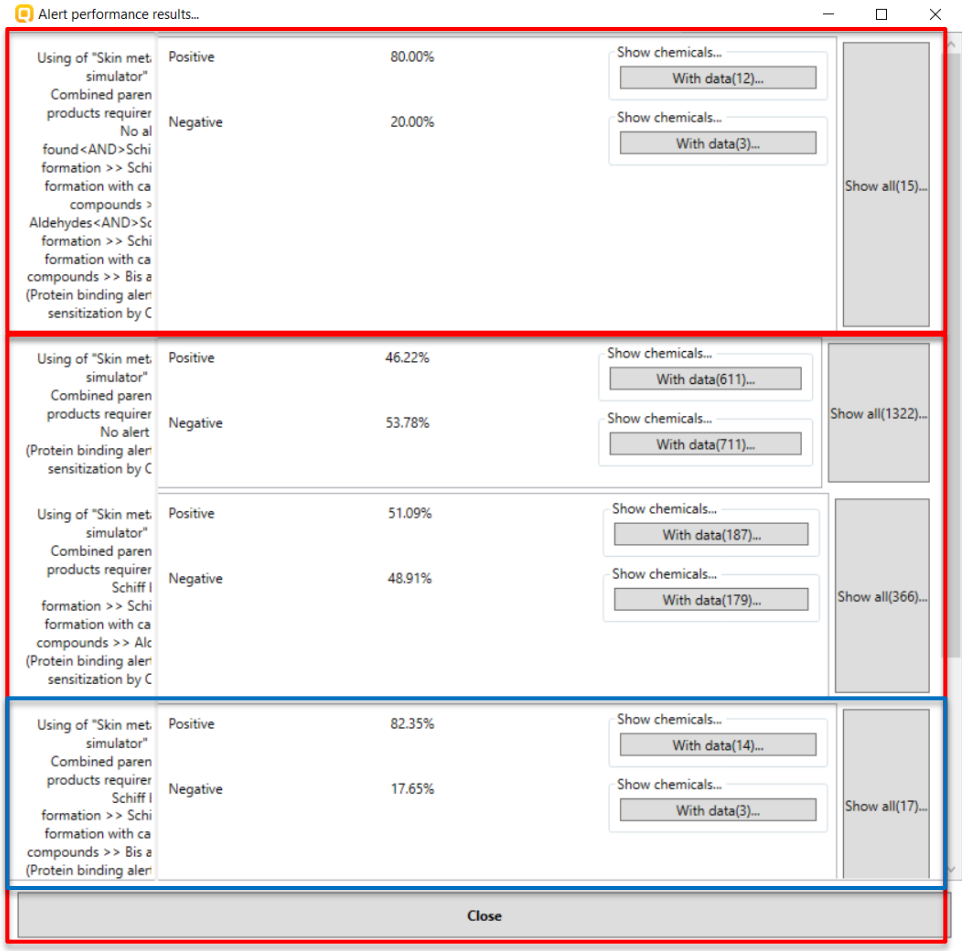
1. Define *profile* query for the package – "Parent & Metabolites" and then select *Protein binding alerts for skin sensitization by OASIS*.
2. Click **Edit** to see all identified alerts in the parent and metabolites.

Category Definition
Alert performance calculation



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

Category Definition
Alert performance calculation



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

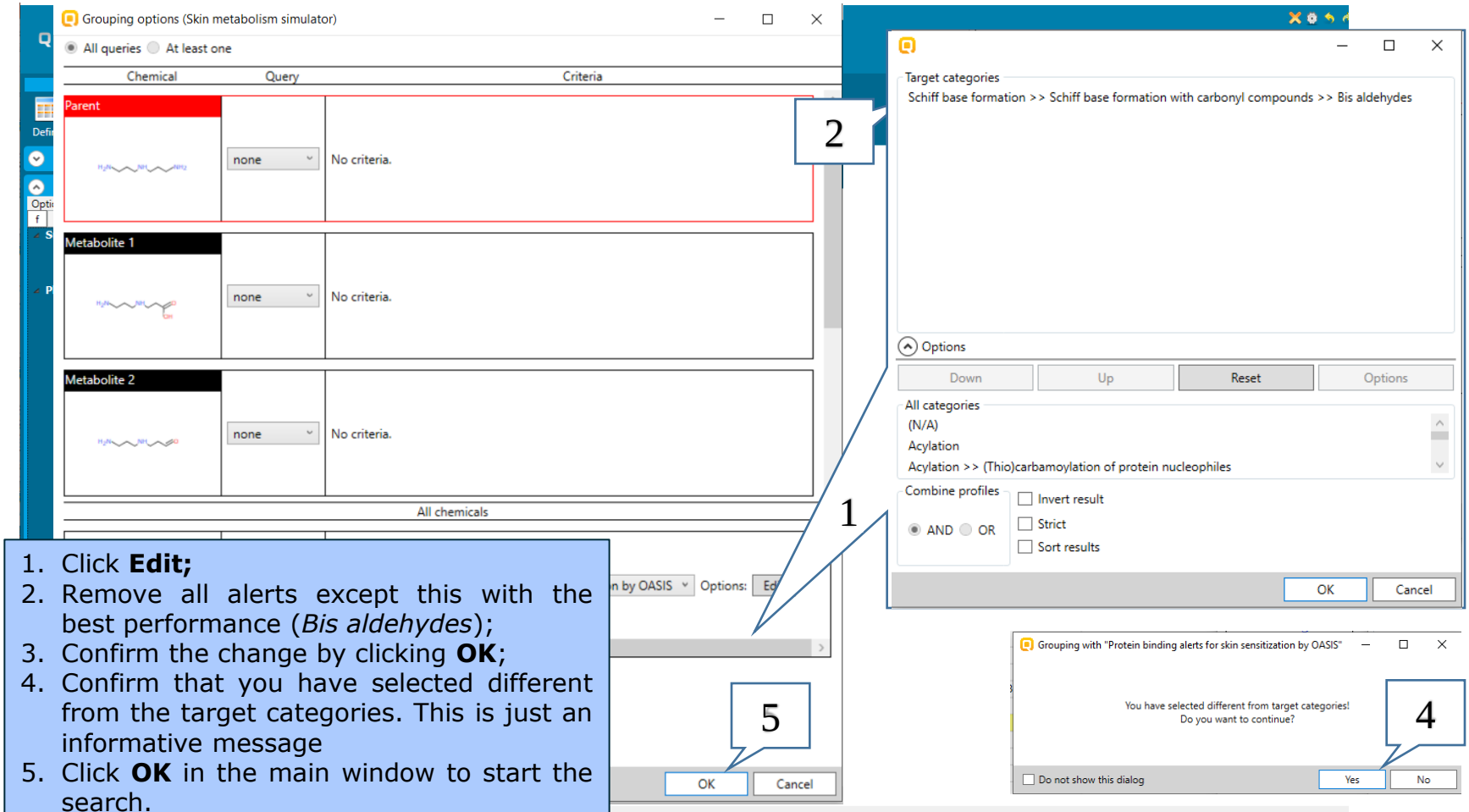
⇦ Statistic for **each** of the alerts identified in the package "Parent & Metabolites"

⇦ The alert with the best performance in this case.
"*Bis aldehydes*" alert will be used for searching for analogues (see next slides).

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

Category Definition

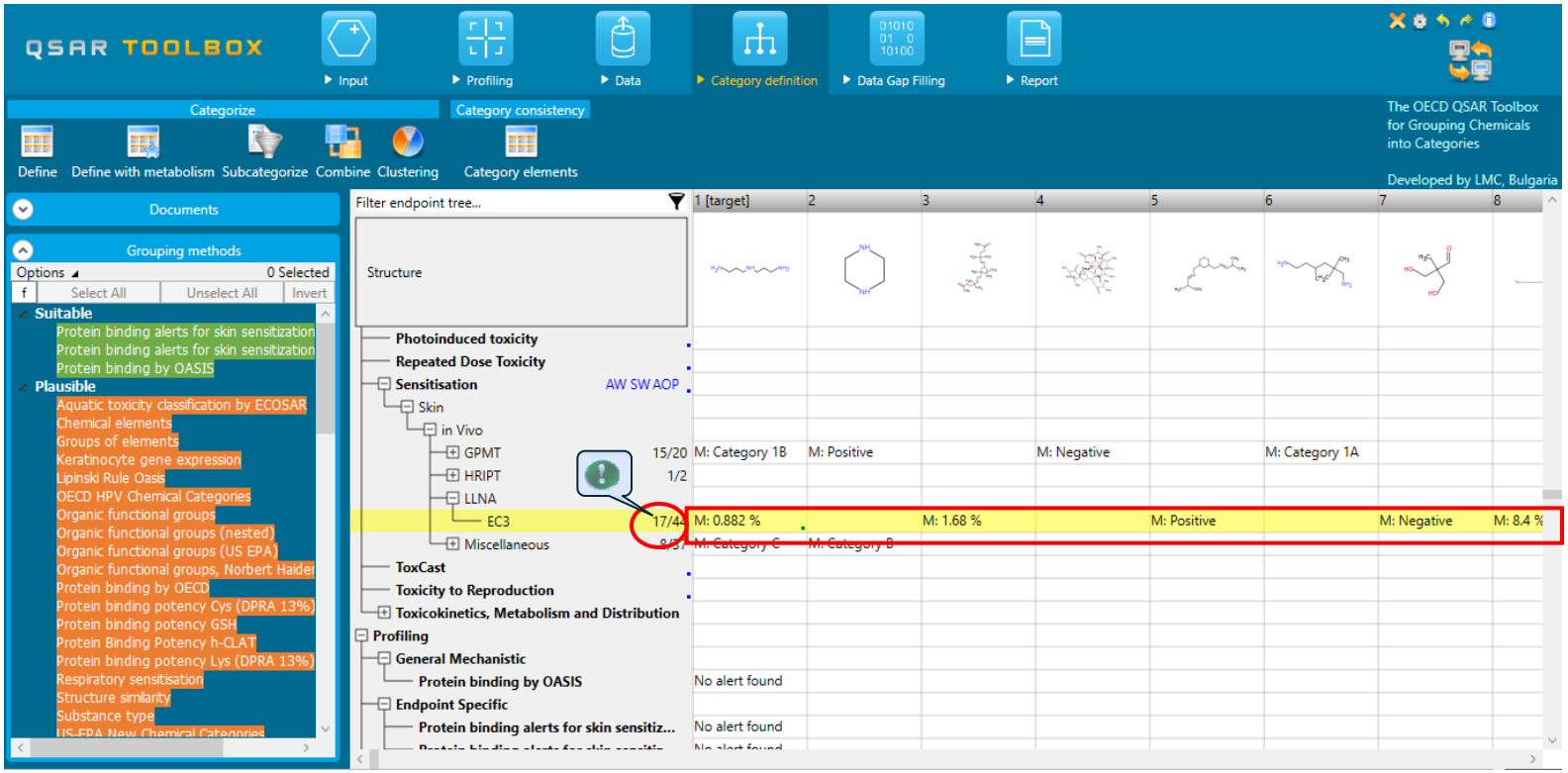
Searching for analogues accounting for skin metabolism



1. Click **Edit;**
2. Remove all alerts except this with the best performance (*Bis aldehydes*);
3. Confirm the change by clicking **OK**;
4. Confirm that you have selected different from the target categories. This is just an informative message
5. Click **OK** in the main window to start the search.

Category Definition
Summary information for Analogues

17 chemicals with 44 experimental results are found across all 28 analogues related to the defined target endpoint (data for skin sensitization is selected only).



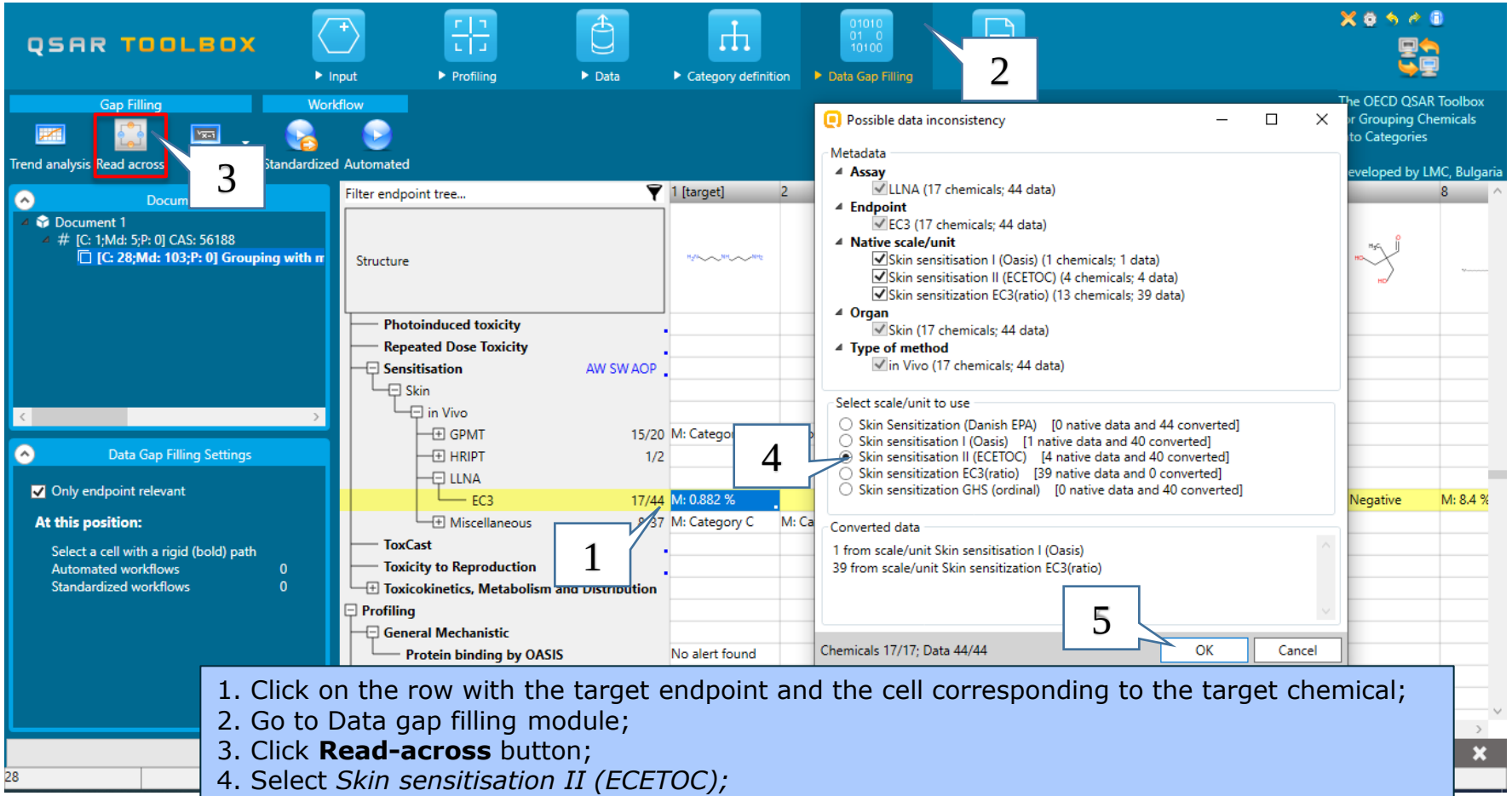
Chemical statistics representing the number of chemicals and the available experimental data for them.

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.

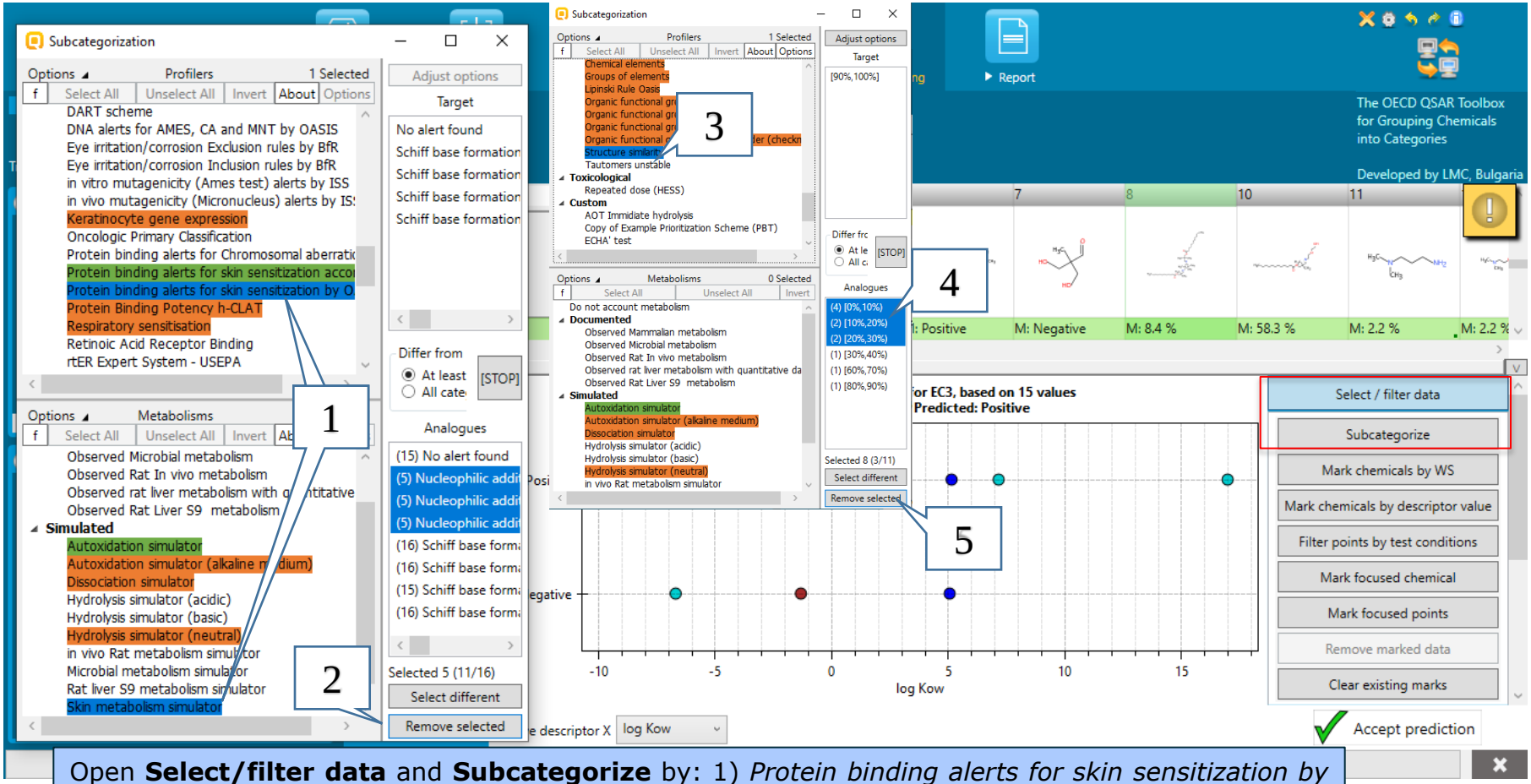
In this example we will use the read-across approach.

Data Gap Filling
Apply Read-across



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

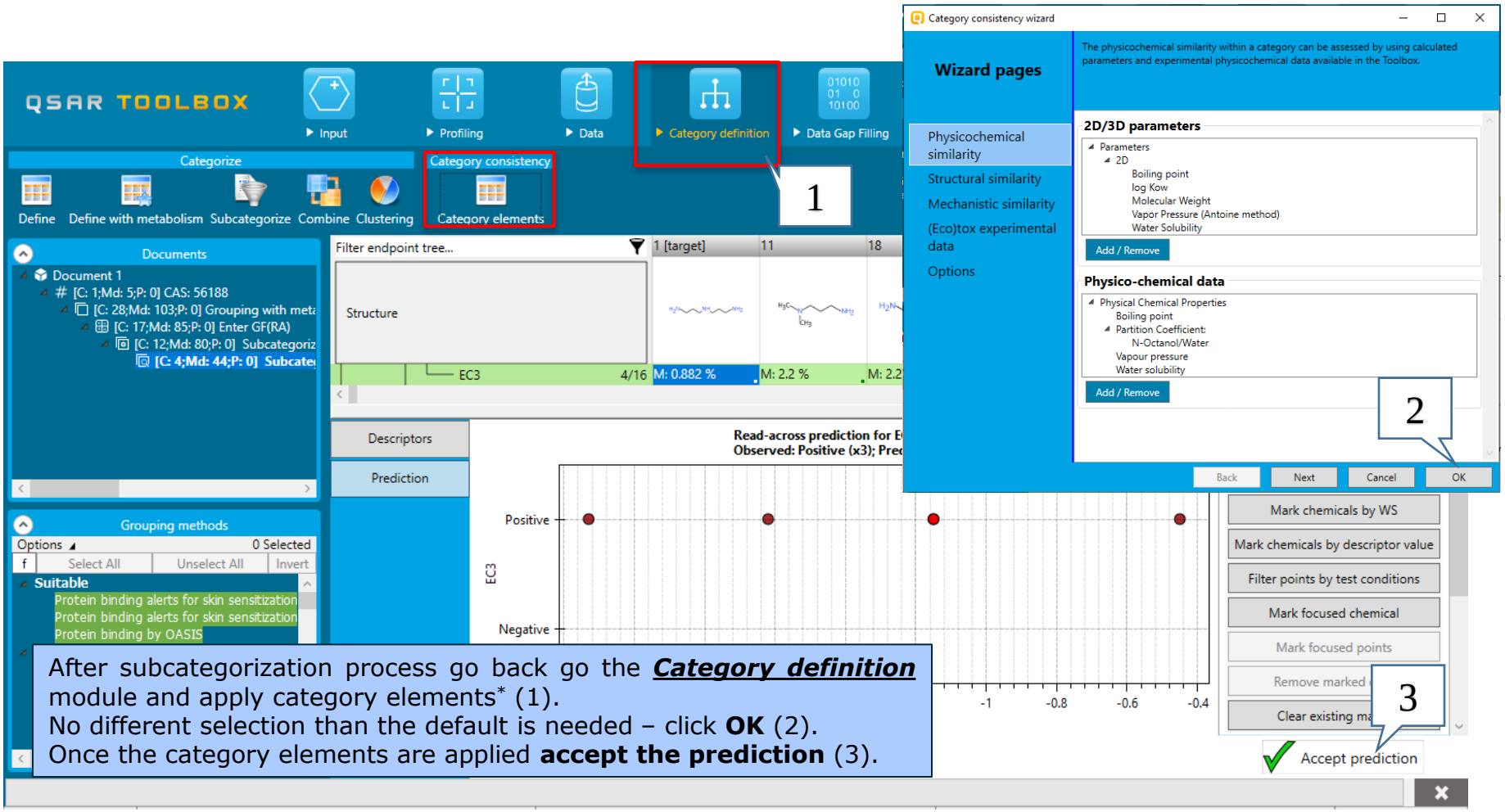
Data Gap Filling
Apply Read-across



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) Select *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_27_TB 4.4. Category elements for assessing Category consistency.pdf

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 accept 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.

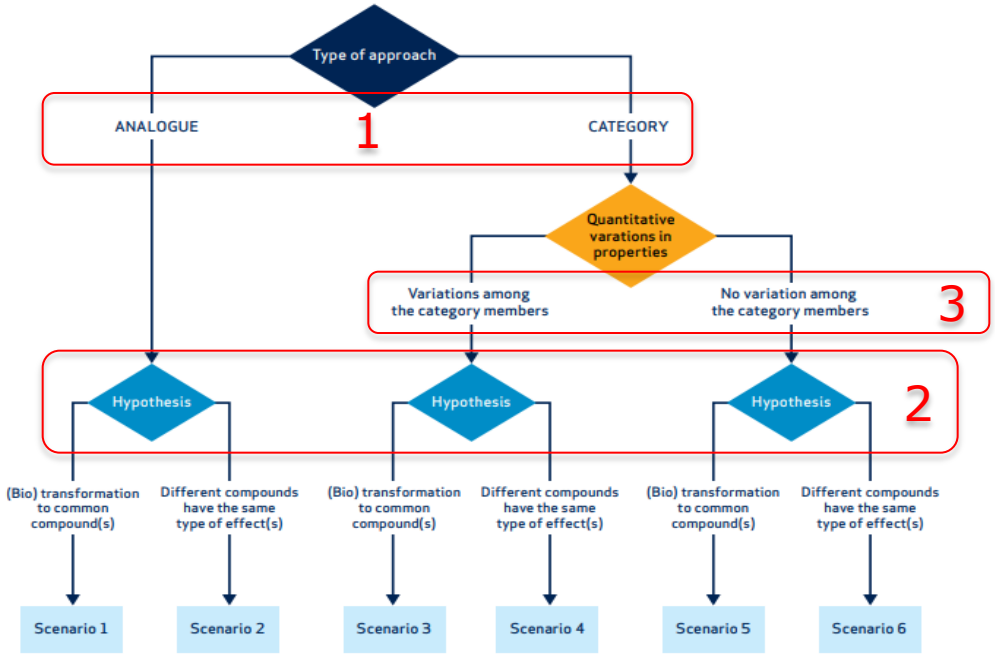
Report
Overview

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

Report
Selection of RAAF scenario

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

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



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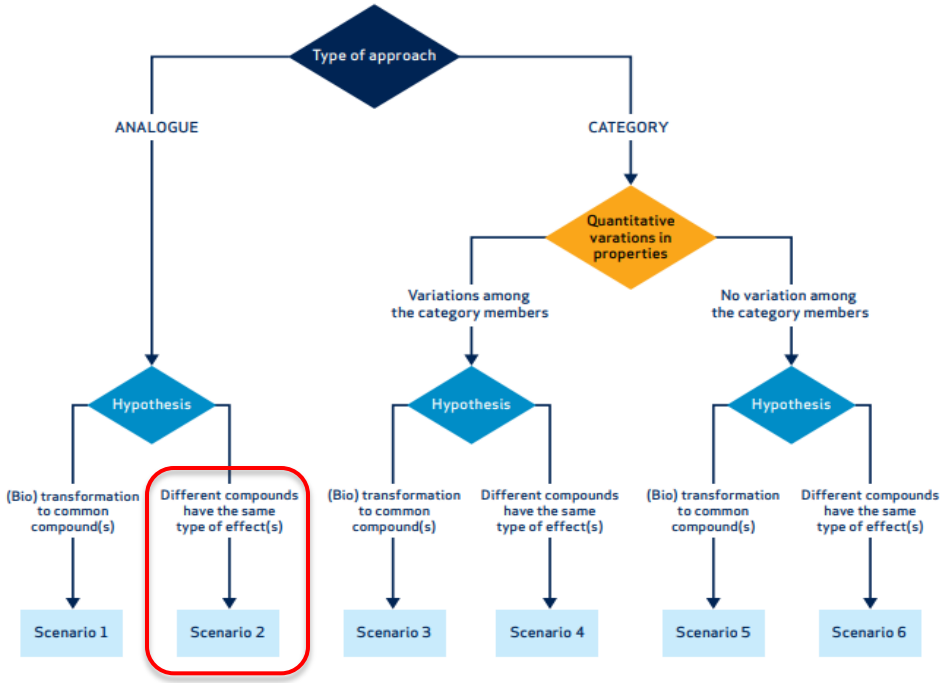
Report
Selection of RAAF scenario

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

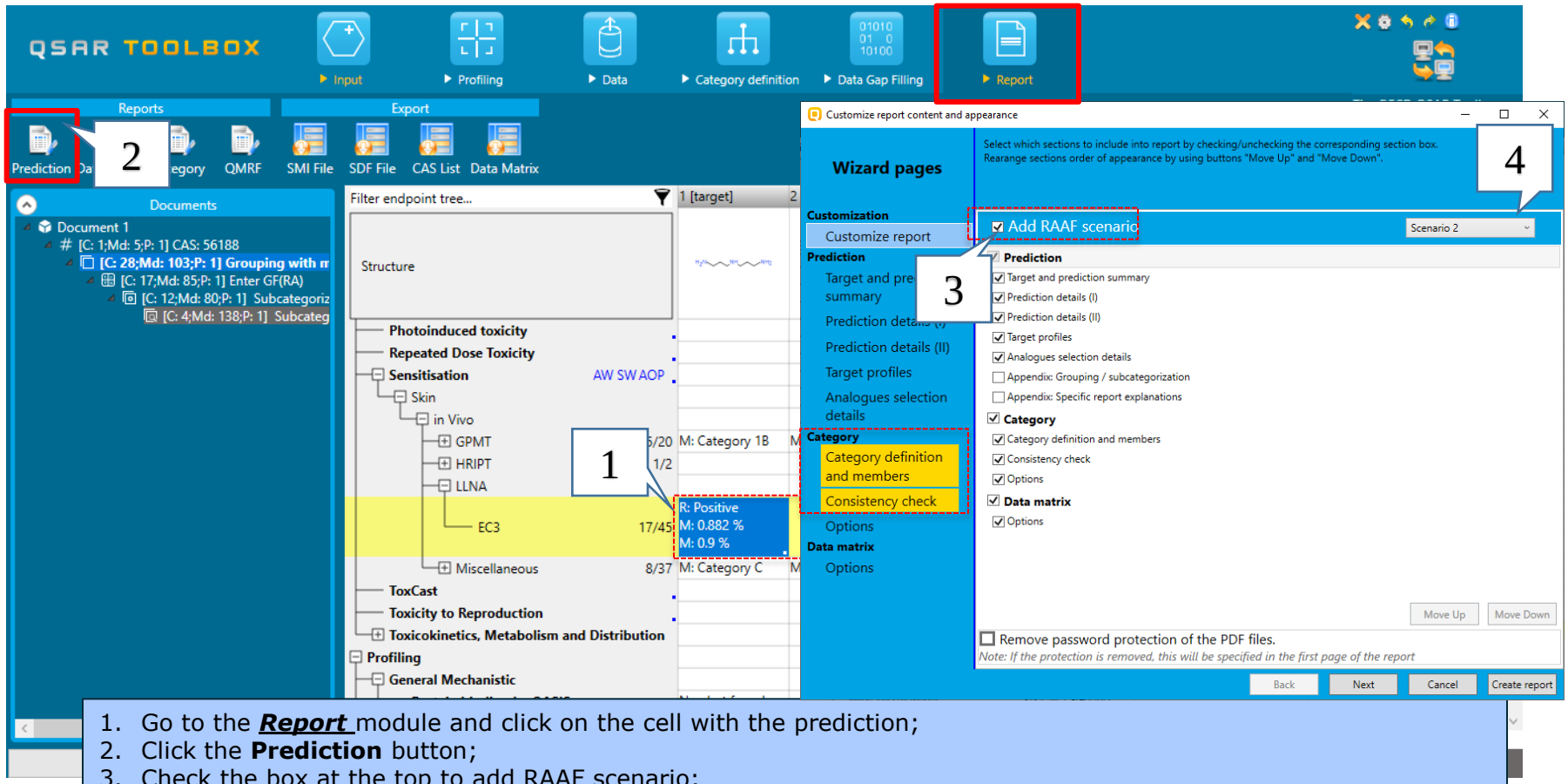
- Scenario 2 covers the analogue approach for which the read-across hypothesis is based on different compounds with qualitatively similar properties.
- For the REACH information requirement under consideration, the property investigated in a study conducted with one source substance is used to predict 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

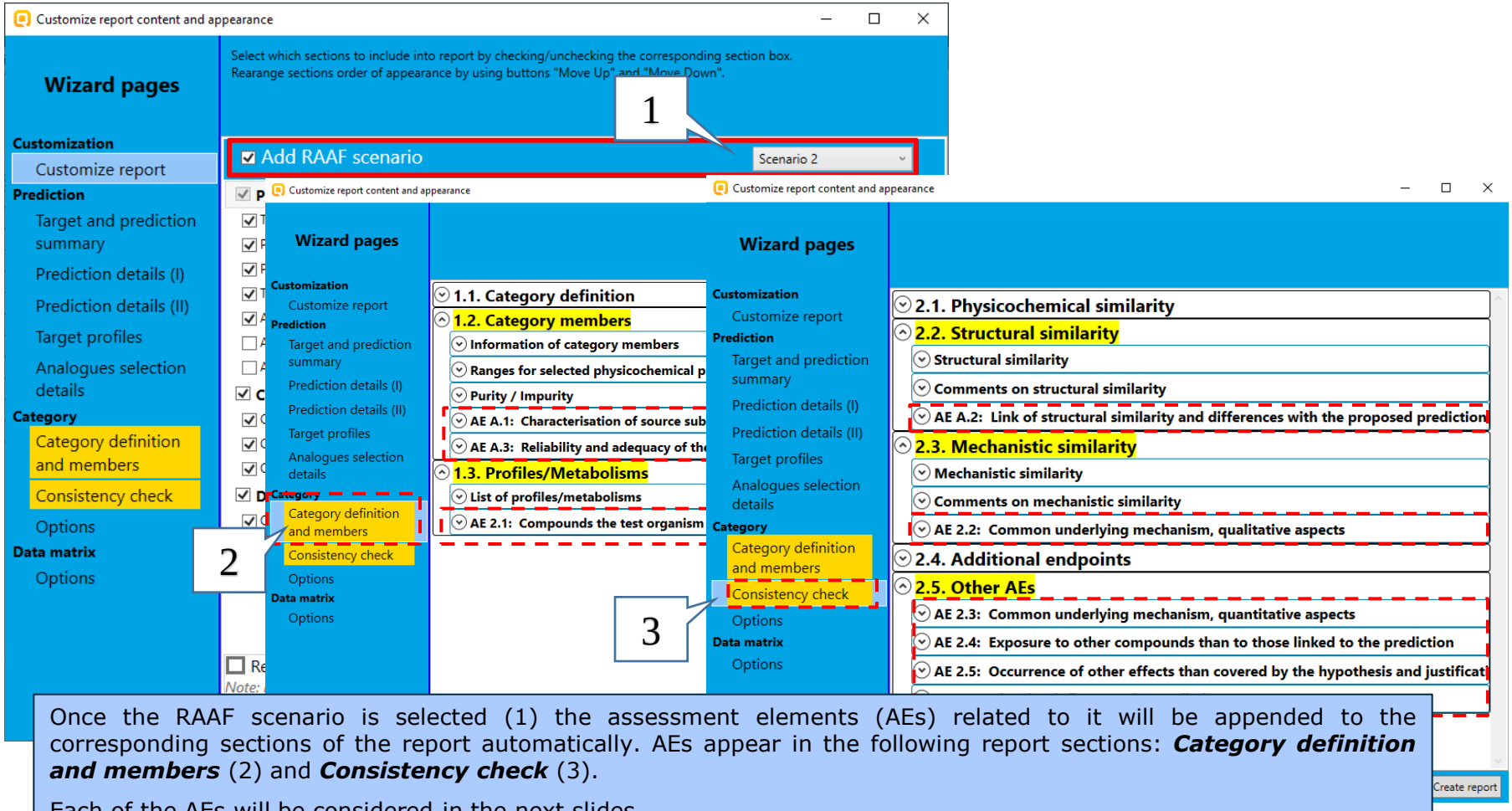
Report

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. Section of the report to which the related AE automatically appeared are getting yellow highlighted.

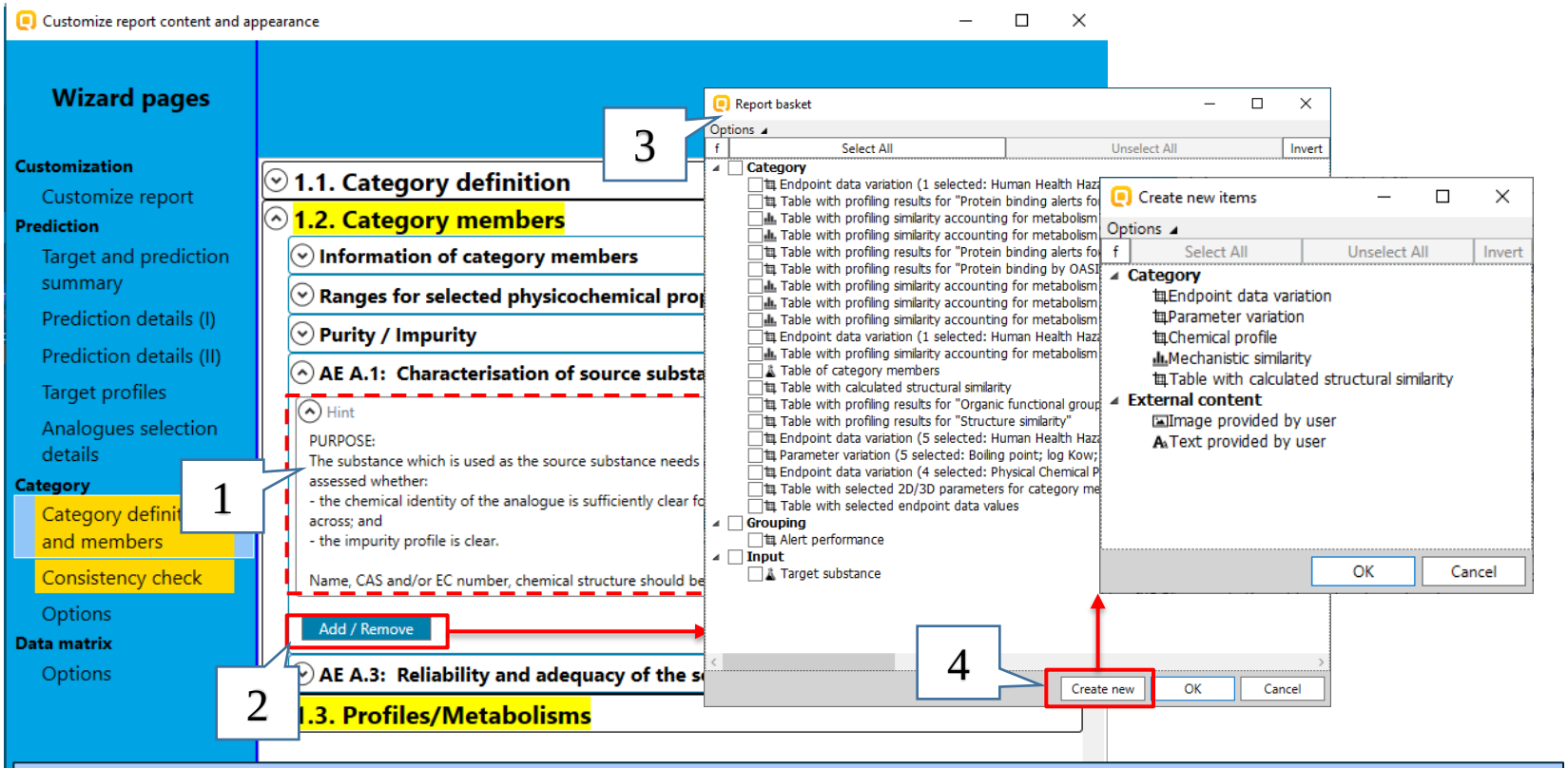
Report
Generation report according to RAAF-Scenario 2



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: ***Category definition and members*** (2) and ***Consistency check*** (3).

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

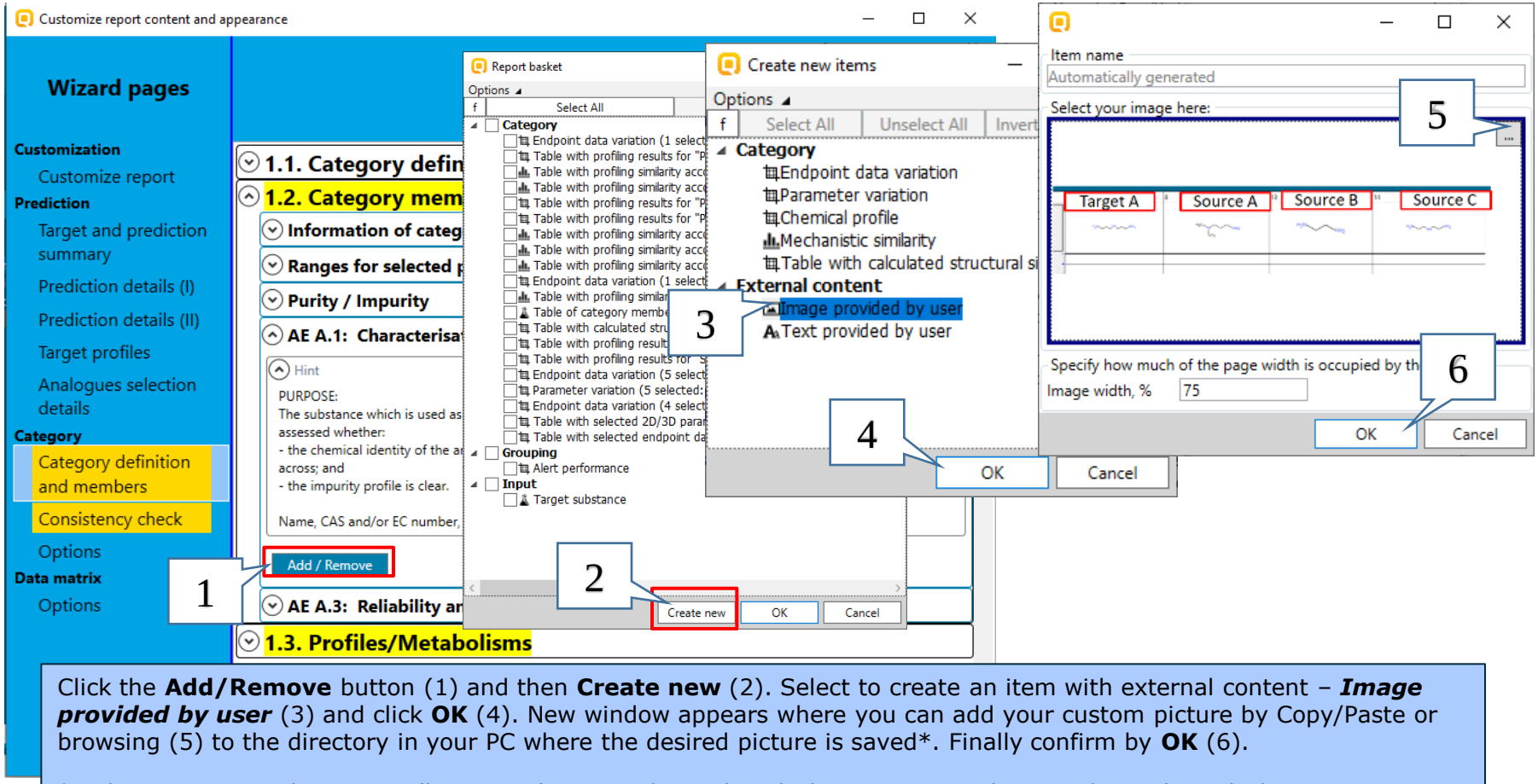
Report
Assessment elements of Scenario 2



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**

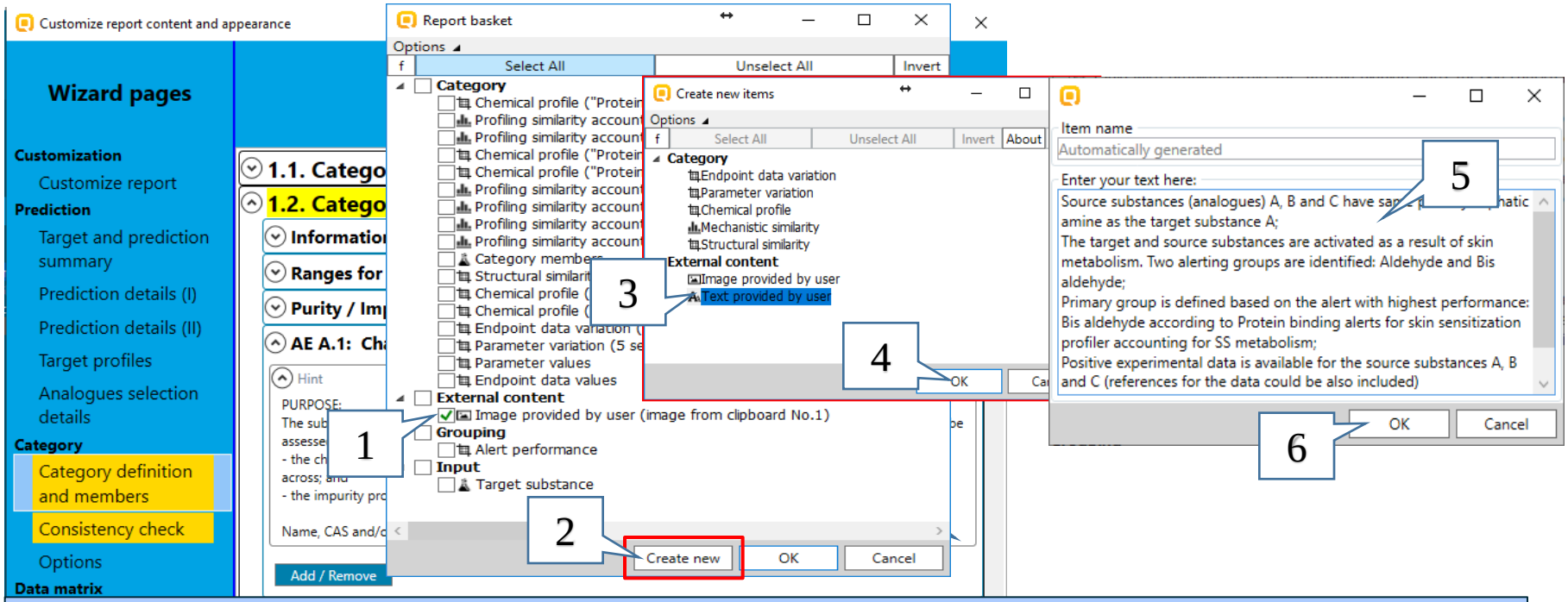
Report
Assessment elements of Scenario 2



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

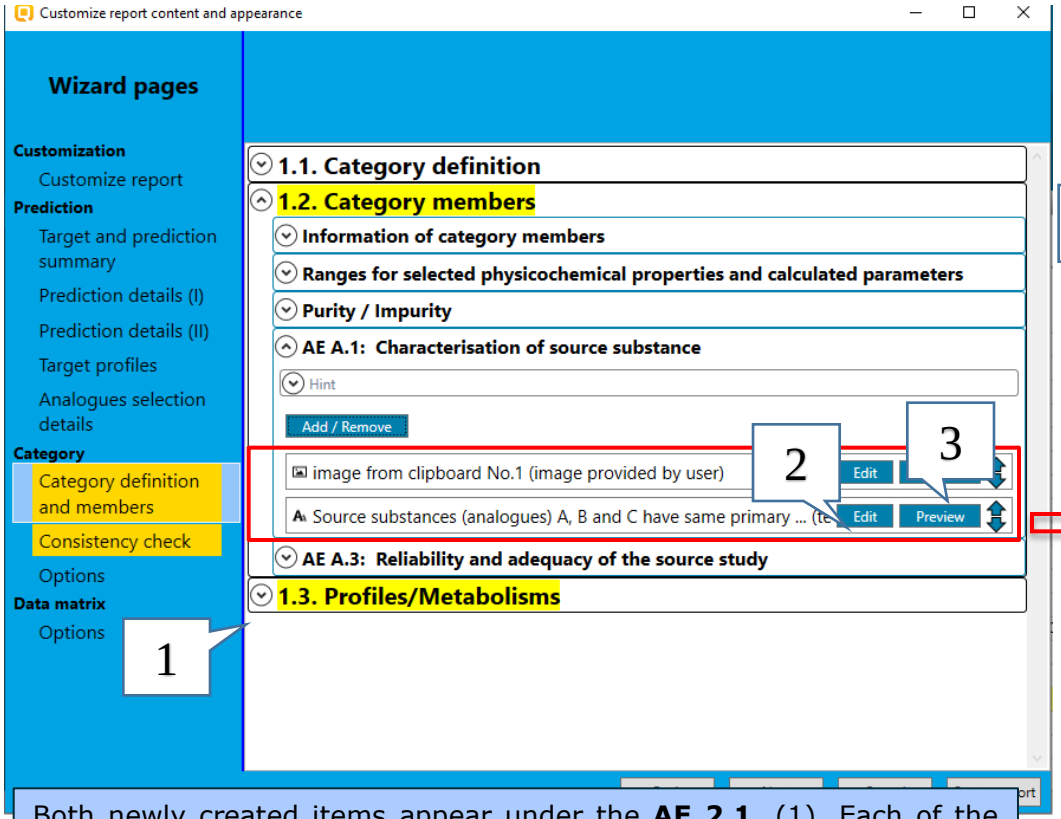


The newly created item appears in the *Report basket* (1). Now text will be also included. Click **Create new** (2), select ***Text provided by user*** (3) and click **OK** (4). Copy the following example text:

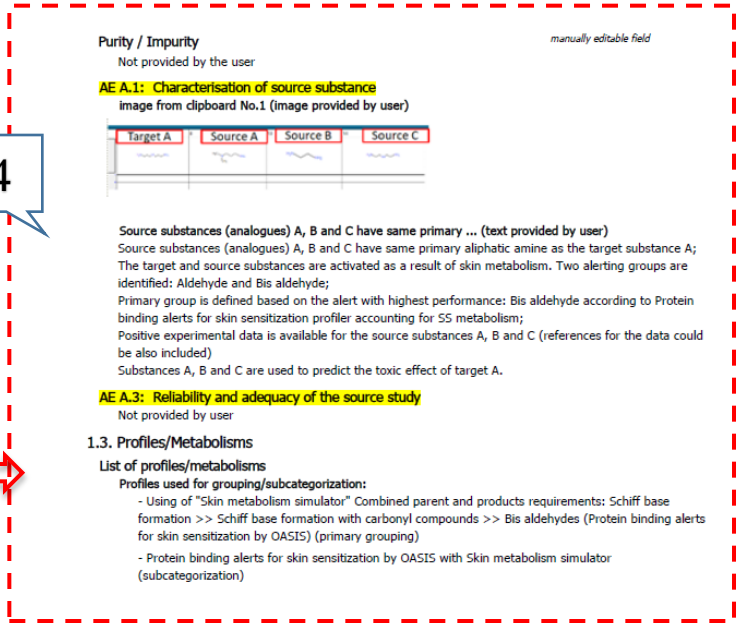
- *Source substances (analogues) A, B and C have same primary aliphatic amine as the target substance A;*
- *The target and source substances are activated as a result of skin metabolism. Two alerting groups are identified: Aldehyde and Bis aldehyde;*
- *Primary group is defined based on the alert with 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 A, B and C* (references for the data could be also included)
- *Substances A, B and C are used to predict the toxic effect of target A.*

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

Report
Assessment elements of Scenario 2



4



Purity / Impurity *manually editable field*

Not provided by the user

AE A.1: Characterisation of source substance

image from clipboard No.1 (image provided by user)

Source substances (analogues) A, B and C have same primary ... (text provided by user)

Source substances (analogues) A, B and C have 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 A, B and C (references for the data could be also included)
Substances A, B and C are used to predict the toxic effect of target A.

AE A.3: Reliability and adequacy of the source study

Not provided by user

1.3. Profiles/Metabolisms

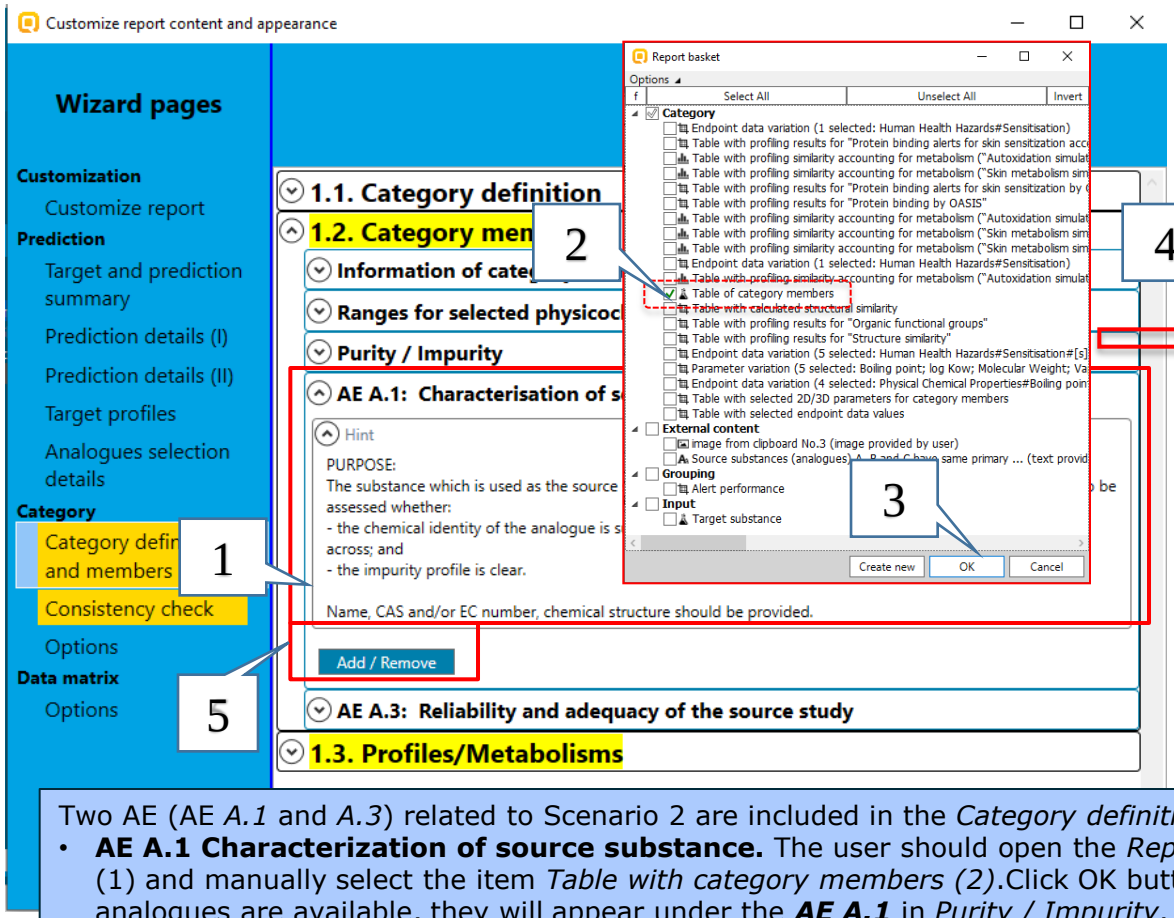
List of profiles/metabolisms

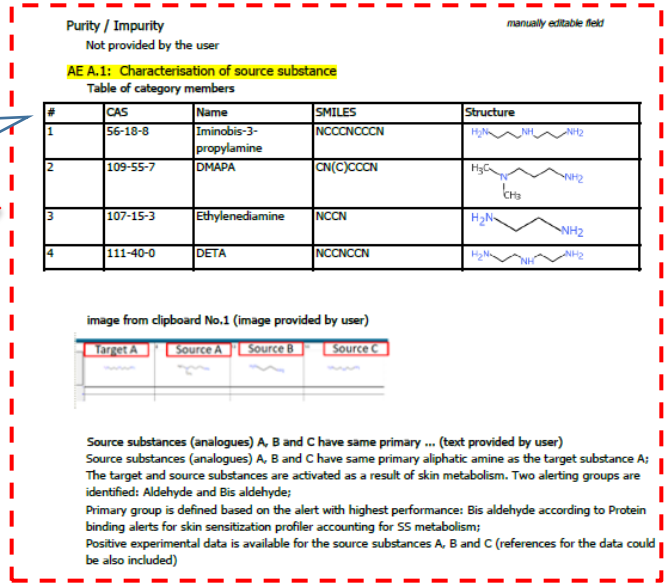
Profiles used for grouping/subcategorization:

- Using of "Skin metabolism simulator" Combined parent and products requirements: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes (Protein binding alerts for skin sensitization by OASIS) (primary grouping)
- Protein binding alerts for skin sensitization by OASIS with Skin metabolism simulator (subcategorization)

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. The order of the appearance in the report could also be changed
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





Purity / Impurity *manually editable field*

Not provided by the user

AE A.1: Characterisation of source substance

Table of category members

#	CAS	Name	SMILES	Structure
1	56-18-8	Iminobis-3-propylamine	NCCCNCCCN	
2	109-55-7	DMAPA	CN(C)CCCN	
3	107-15-3	Ethylenediamine	NCCN	
4	111-40-0	DETA	NCCNCCN	

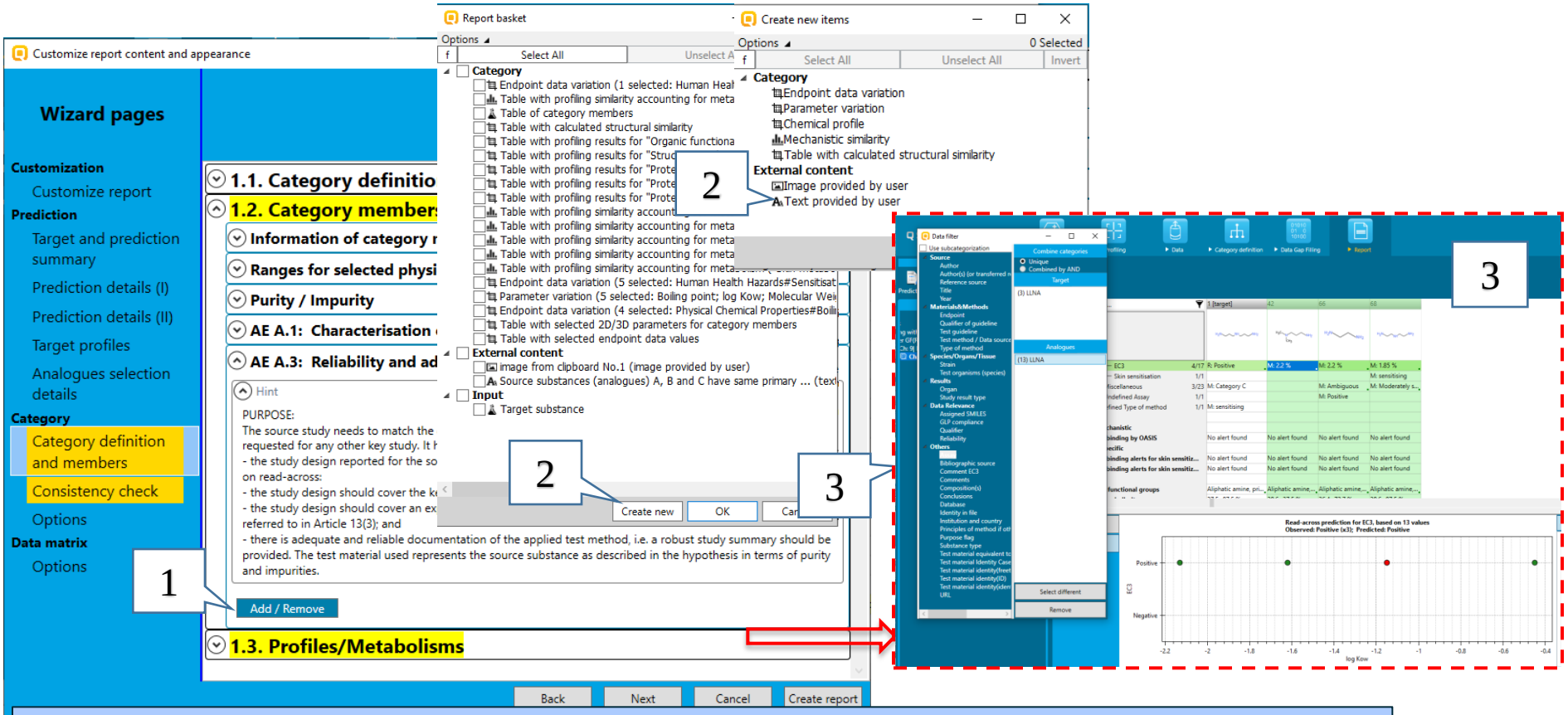
image from clipboard No.1 (image provided by user)

Source substances (analogues) A, B and C have same primary ... (text provided by user)
Source substances (analogues) A, B and C have 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 A, B and C (references for the data could be also included)

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.** The user should open the *Report basket* by clicking the *Add/Remove* button (1) and manually select the item *Table with category members (2)*.Click OK button (3). If impurities/additives of the used analogues are available, they will appear under the ***AE A.1*** in *Purity / Impurity*. 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(4).
- **AE A.3 Reliability and adequacy of the source study** should be filled in manually (5) *(see on the next slide)*

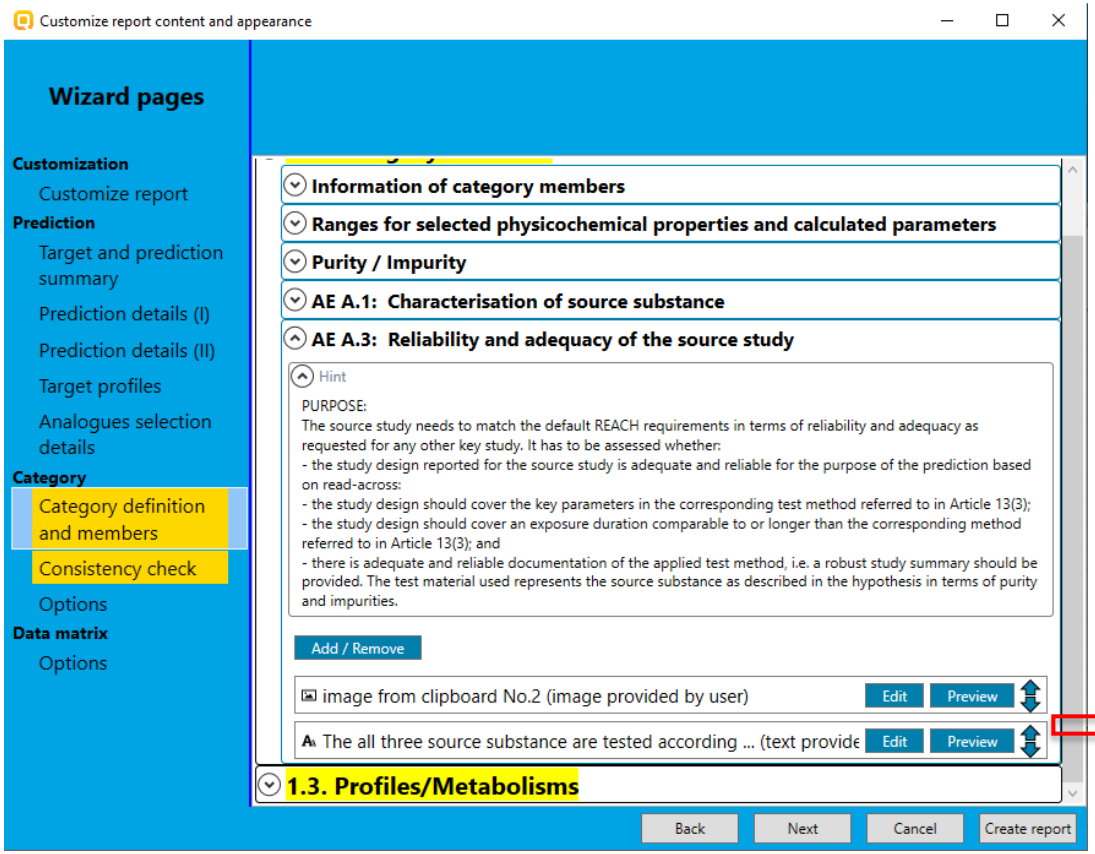
Report
Assessment elements of Scenario 2

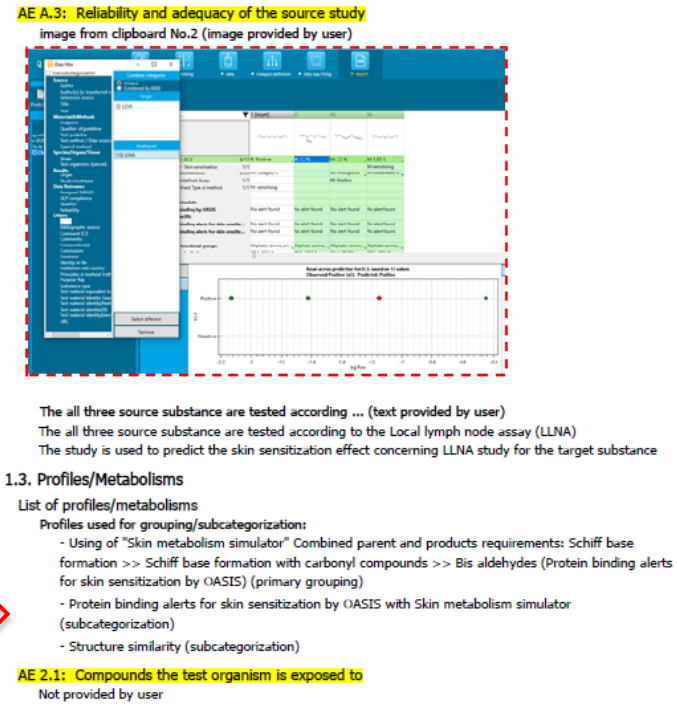


AE A.3: Click the **Add/Remove** button (1) and create new item with textual content (2) (see slide 55).
In the text field paste the following example text:
"*The all three source substance are tested according to the Local lymph node assay (LLNA)*
The study is used to predict the skin sensitization effect concerning LLNA study for the target substance"

Additionally a snapshot of the filter by test conditions window (3) could be added to confirm the consistency regarding the assay (create new image item).

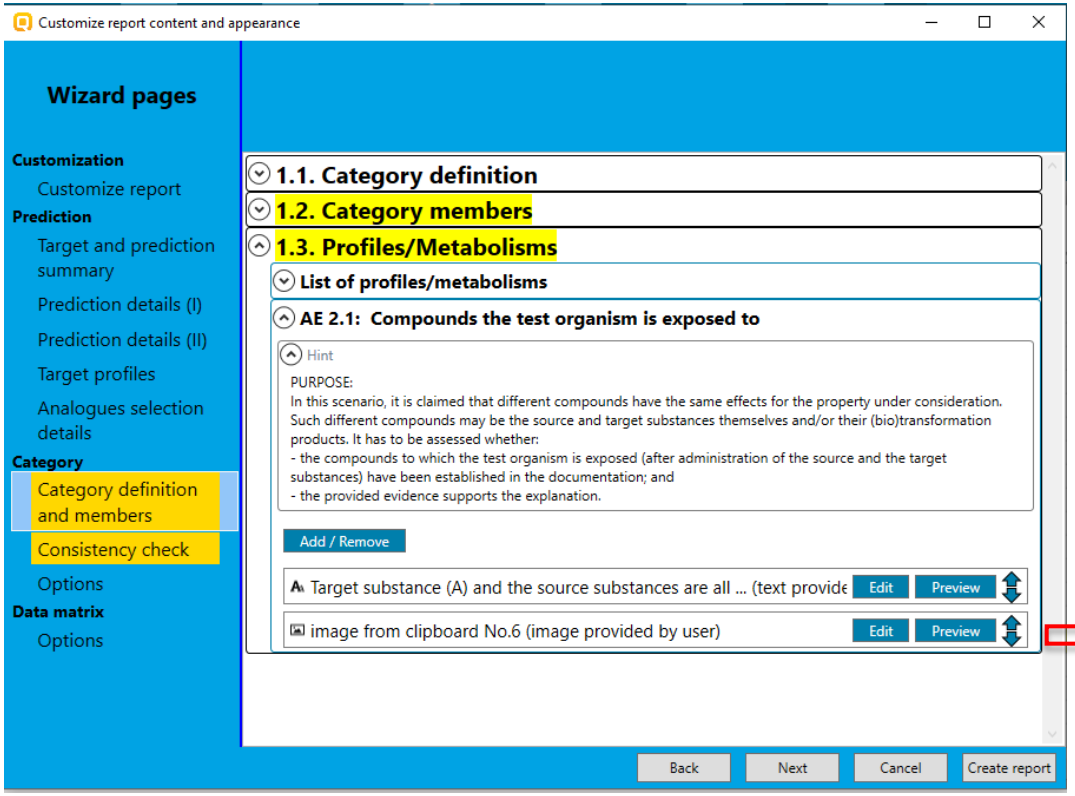
Report
Assessment elements of Scenario 2

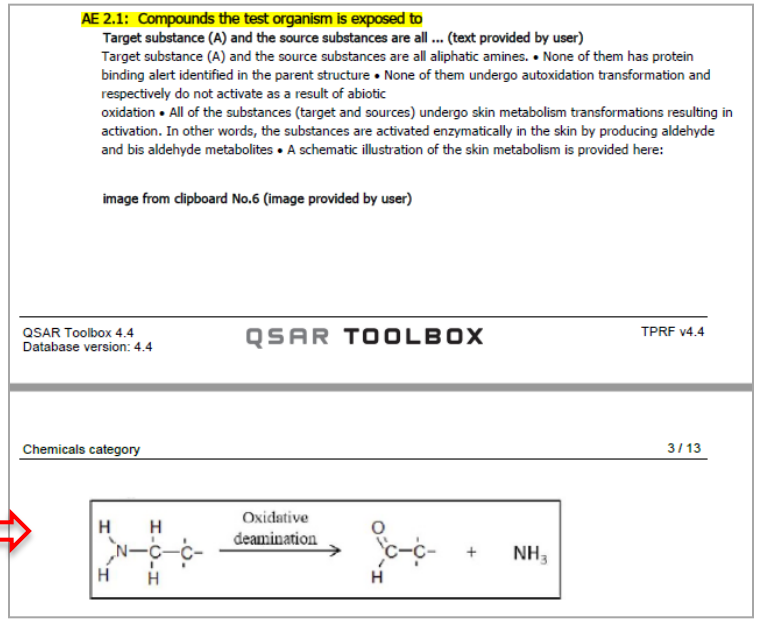




Example of how the **AE A.3**. will look in the generated report is shown on the right.

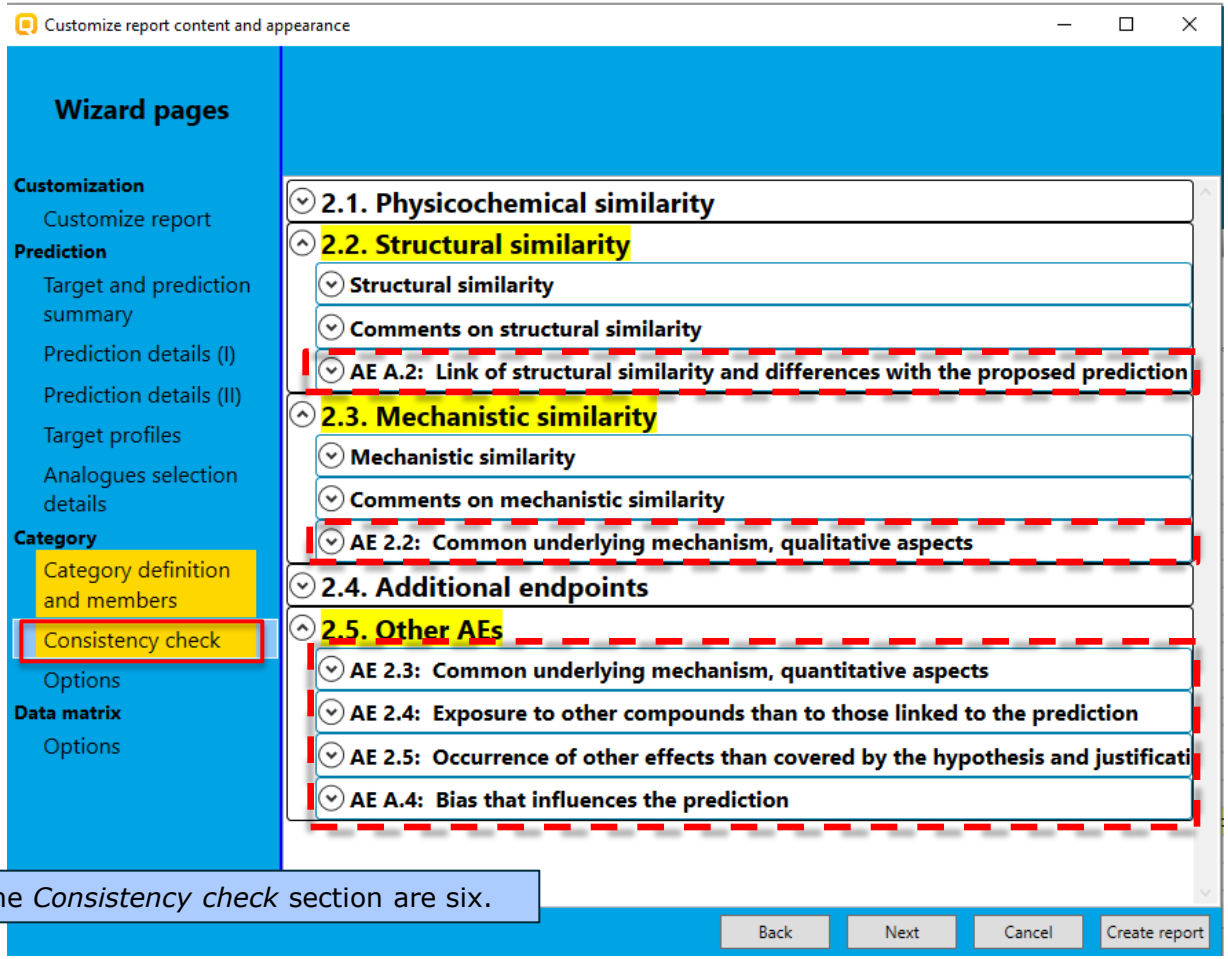
Report
Assessment elements of Scenario 2





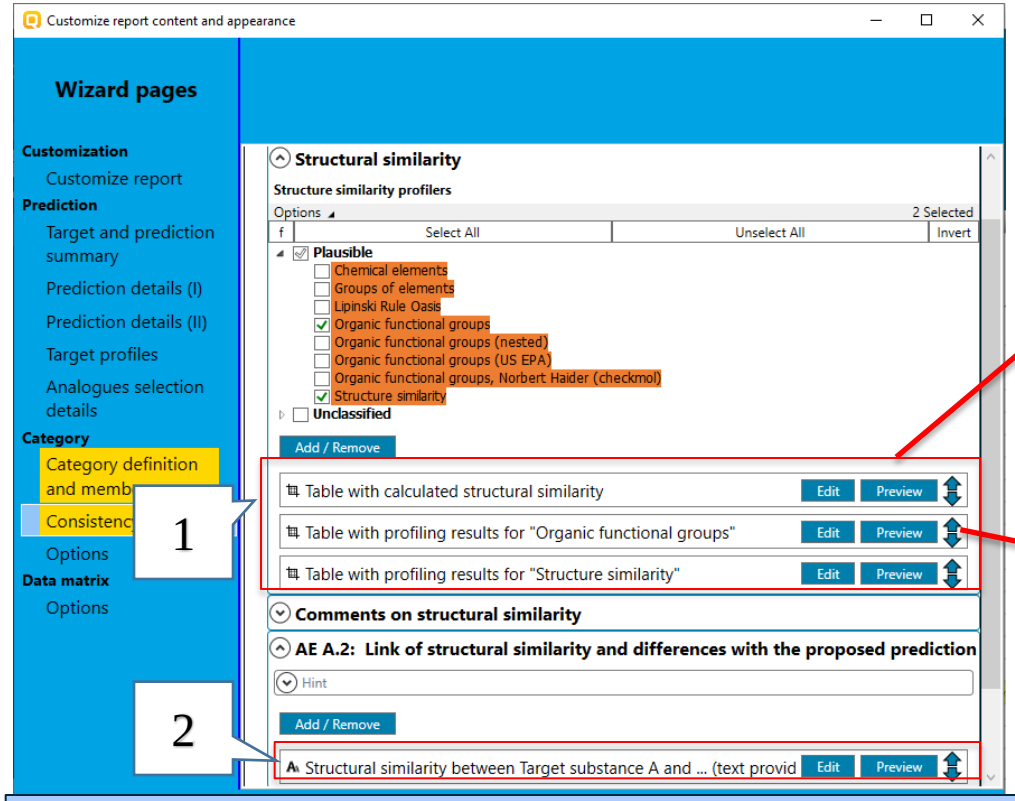
An example text and illustration related to **AE 2.1: Compounds the test organism is exposed to** is shown above.

Report
Assessment elements of Scenario 2



AEs included to the *Consistency check* section are six.

Report
Assessment elements of Scenario 2



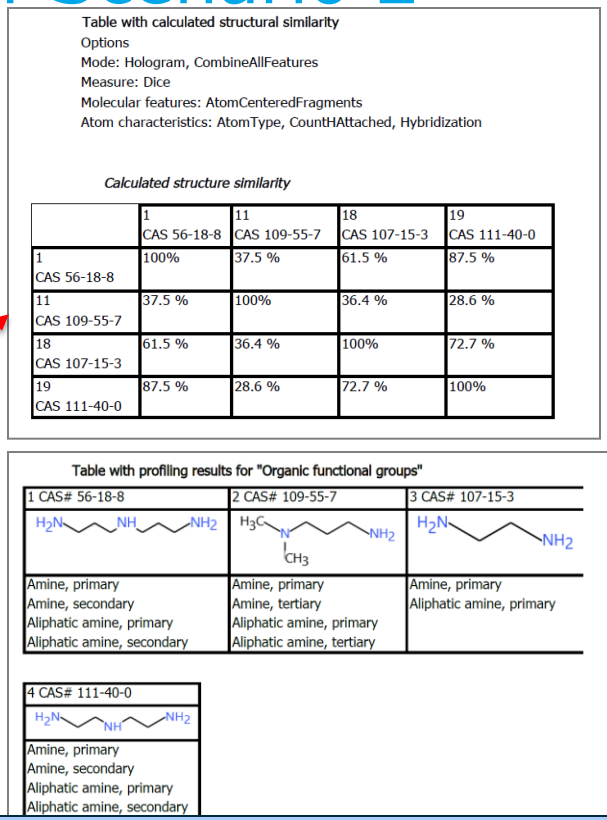


Table with calculated structural similarity
Options
Mode: Hologram, CombineAllFeatures
Measure: Dice
Molecular features: AtomCenteredFragments
Atom characteristics: AtomType, CountHAttached, Hybridization

Calculated structure similarity

	1 CAS 56-18-8	11 CAS 109-55-7	18 CAS 107-15-3	19 CAS 111-40-0
1 CAS 56-18-8	100%	37.5 %	61.5 %	87.5 %
11 CAS 109-55-7	37.5 %	100%	36.4 %	28.6 %
18 CAS 107-15-3	61.5 %	36.4 %	100%	72.7 %
19 CAS 111-40-0	87.5 %	28.6 %	72.7 %	100%

Table with profiling results for "Organic functional groups"

1 CAS# 56-18-8	2 CAS# 109-55-7	3 CAS# 107-15-3
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 CAS# 111-40-0
Amine, primary Amine, secondary Aliphatic amine, primary Aliphatic amine, secondary

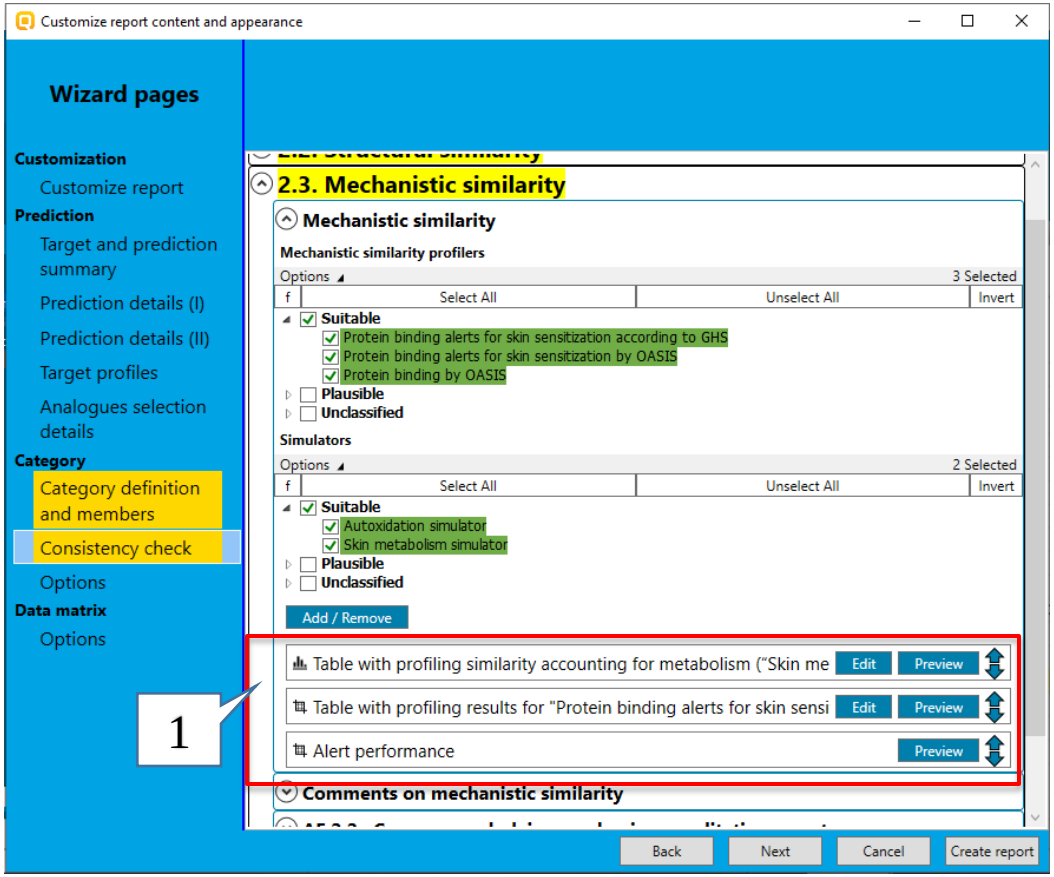
AE A.2. **Link of structural similarity and differences with the proposed prediction** is related to the structural similarity of the final category.

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:

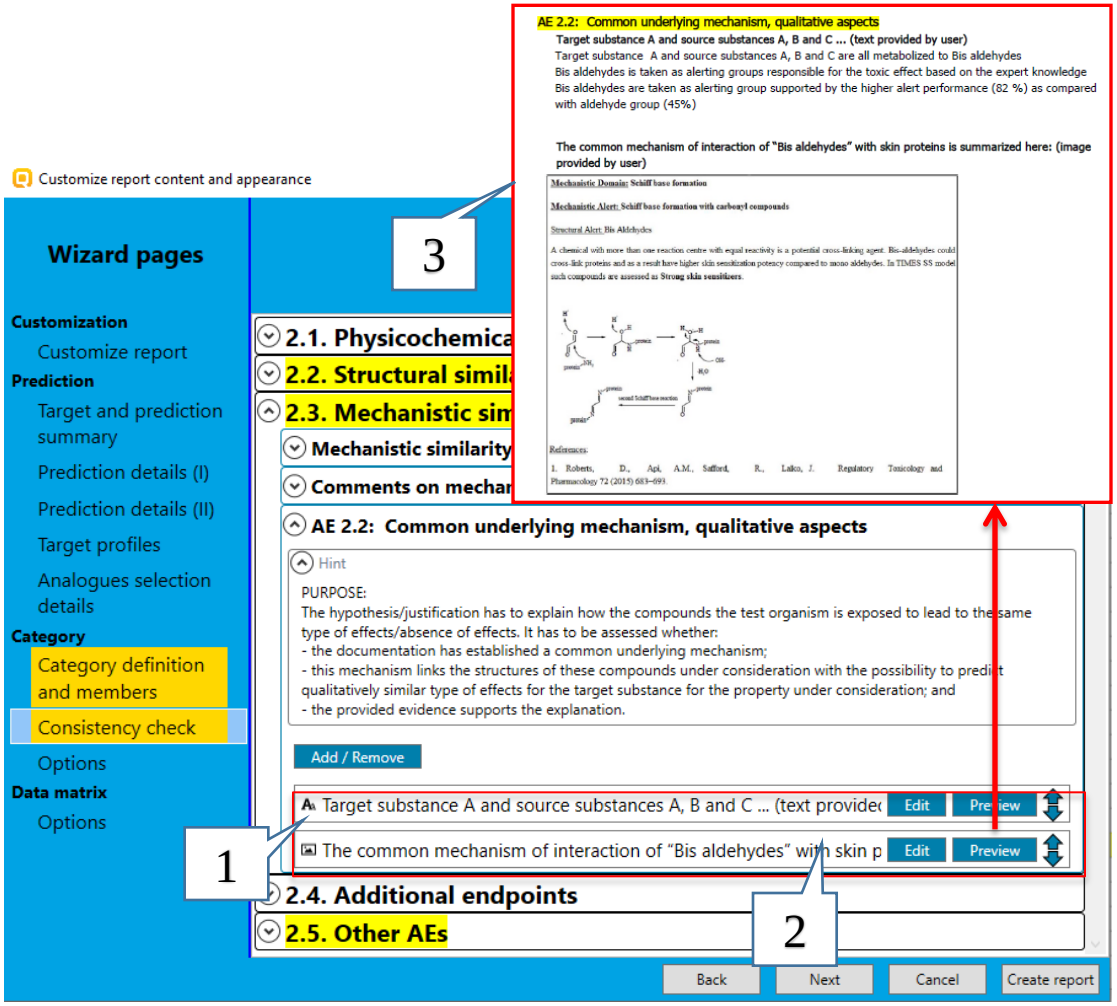
- *Structural similarity between Target substance A and 3 source substances A, B and C according to Str.similarity profiler is in the range of [29-88%]*
- *They all have primary aliphatic amine based on the OFG profiler, while the target substance A and source substance B have additional secondary aliphatic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

Report
Assessment elements of Scenario 2



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

Report
Assessment elements of Scenario 2

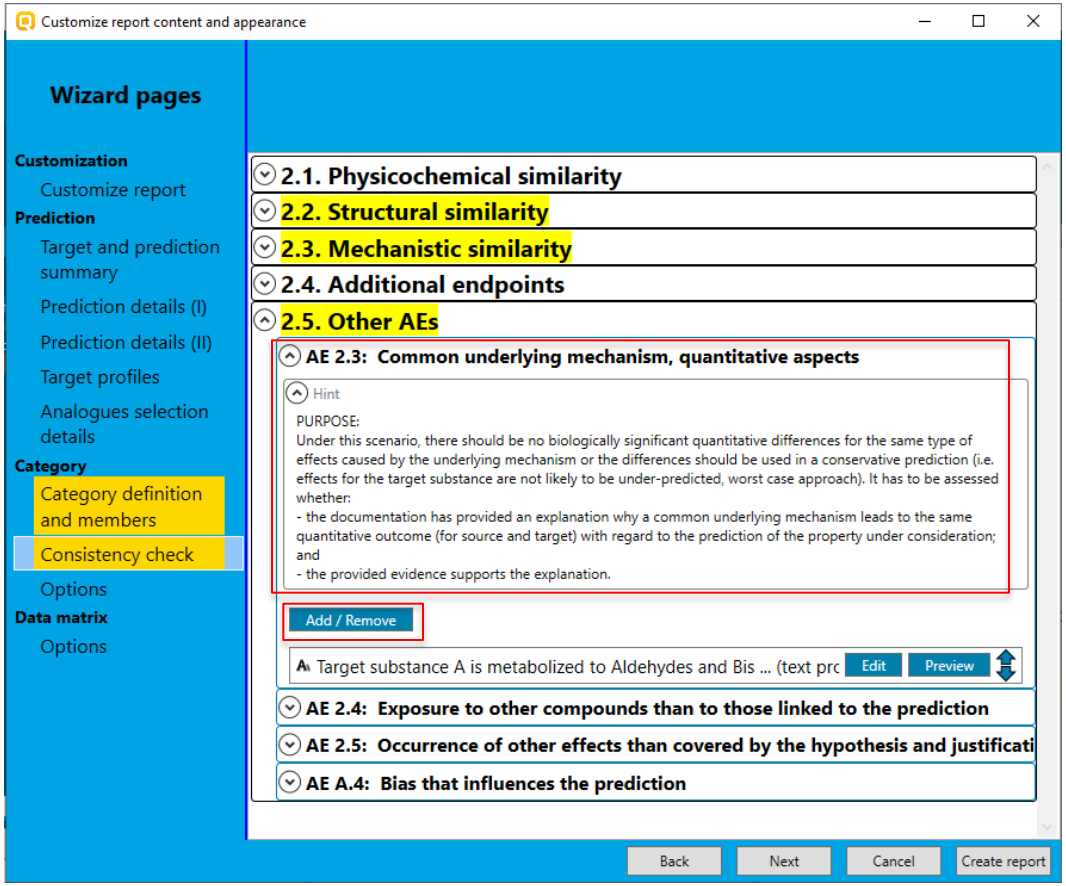


AE 2.2. Common underlying mechanism, qualitative aspects is related to the mechanistic similarity of the final category.
The following example text summarizing the results of the provided mechanistic similarity items can be added (1):

- *Target substance A and source substances A, B and C all produce metabolite that is recognized as "Bisaldehyde" according to the "Protein binding alerts for skin sensitization by OASIS" profiler*
- *As a result of skin metabolism, along with the Bisaldehyde metabolite there are also generated simple aldehydes.*
- *By applying evaluation of alert performance the results showed that the "Bis aldehyde" has higher positive performance (82%) as compared with the simple aldehyde group (45%).*

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. In the current case mechanism of interaction of "Bis aldehyde" with skin proteins is added as external report item (2). How the AE looks like in the report is given too (3)

Report
Assessment elements of Scenario 2



AE 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

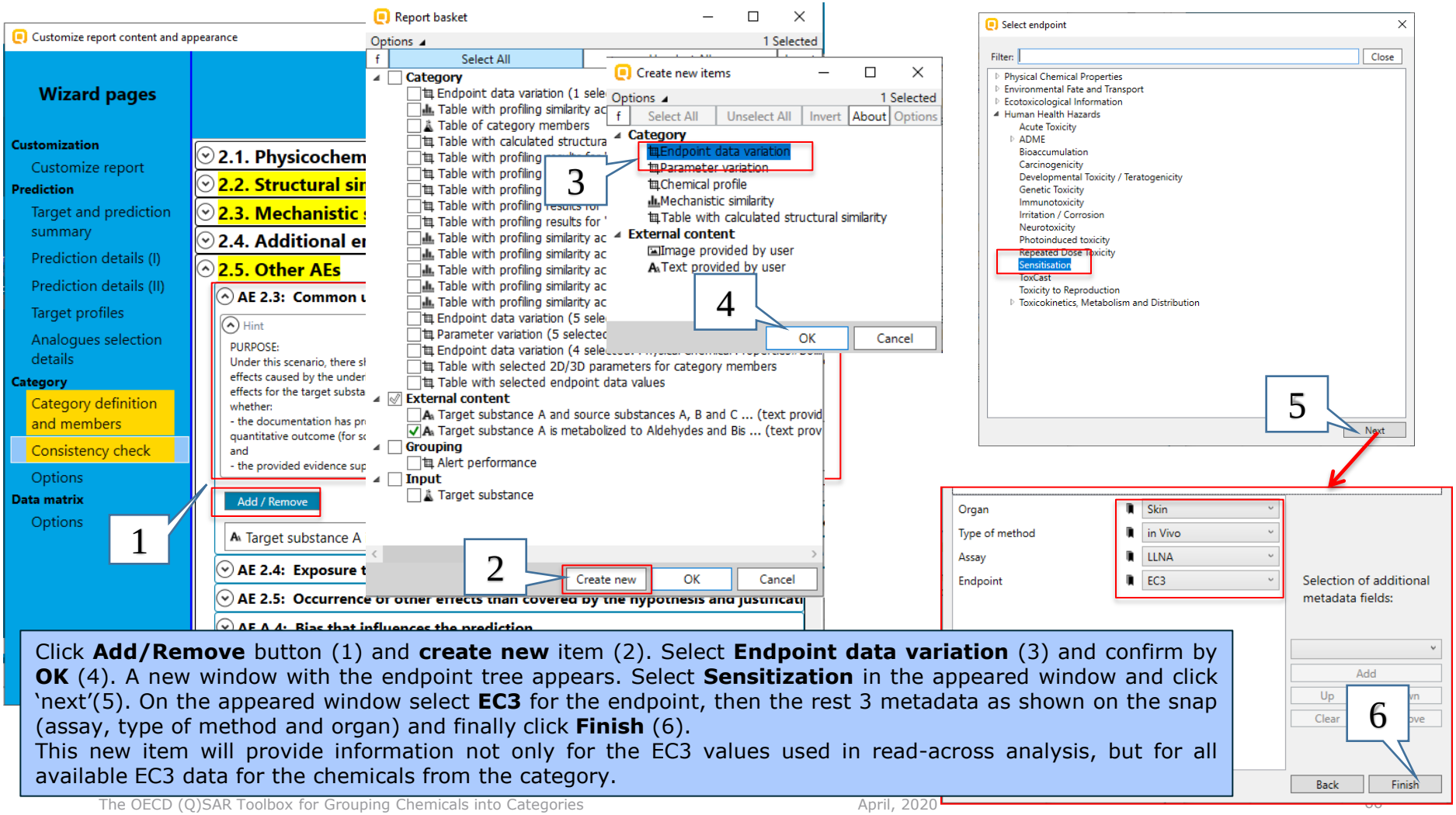
Example text:

- *Target substance A is metabolized to Aldehydes and Bis aldehydes*
- *It is expected that Bis-aldehydes as the alert with higher alert performance is responsible for the toxic effect*
- *Source substances A, B and C are metabolized to aldehydes and bis-aldehydes, too*
- *The available experimental EC3 values for the source substances corresponds to the 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.

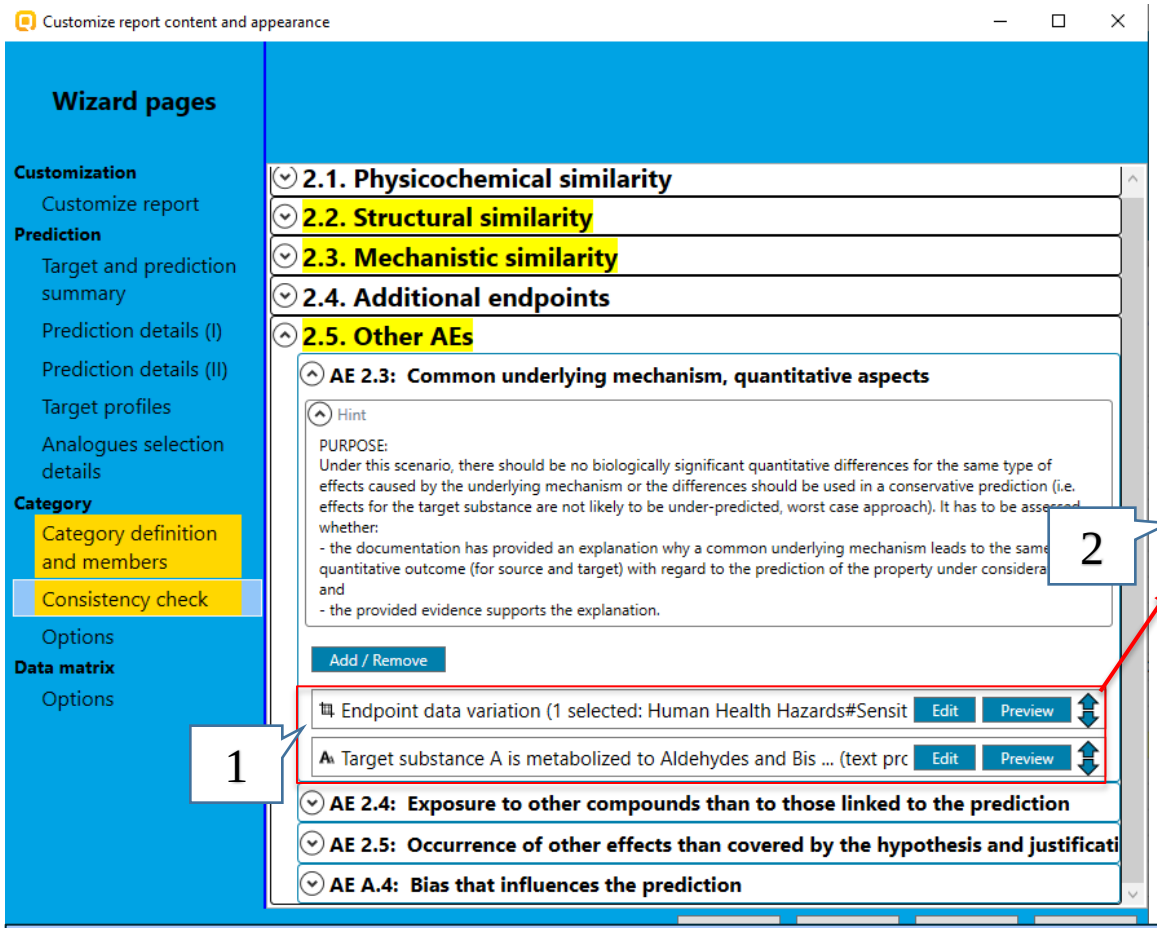
Include all available SS EC3 data for the target chemical and the source substances in all Toolbox database. See how to do this on the next two slides.

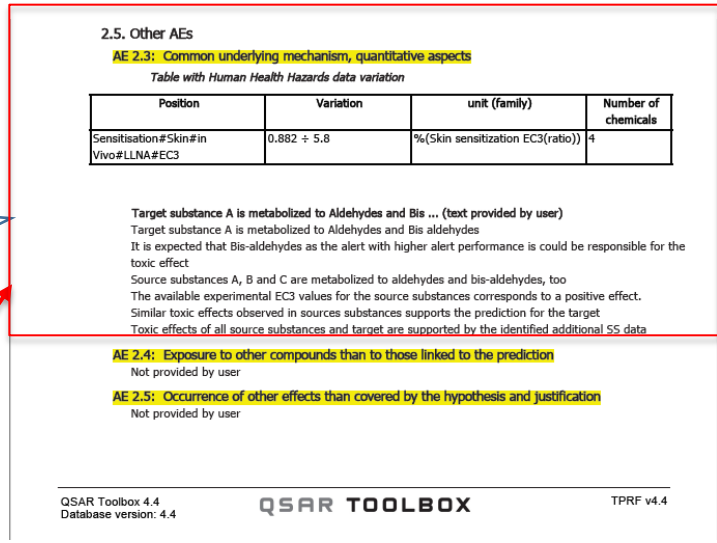
Report
Assessment elements of Scenario 2



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 appears. Select **Sensitization** in the appeared window and click 'next'(5). On the appeared window select **EC3** for the endpoint, then the rest 3 metadata as shown on the snap (assay, type of method and organ) and finally click **Finish** (6).
This new item will provide information not only for the EC3 values used in read-across analysis, but for all available EC3 data for the chemicals from the category.

Report
Assessment elements of Scenario 2

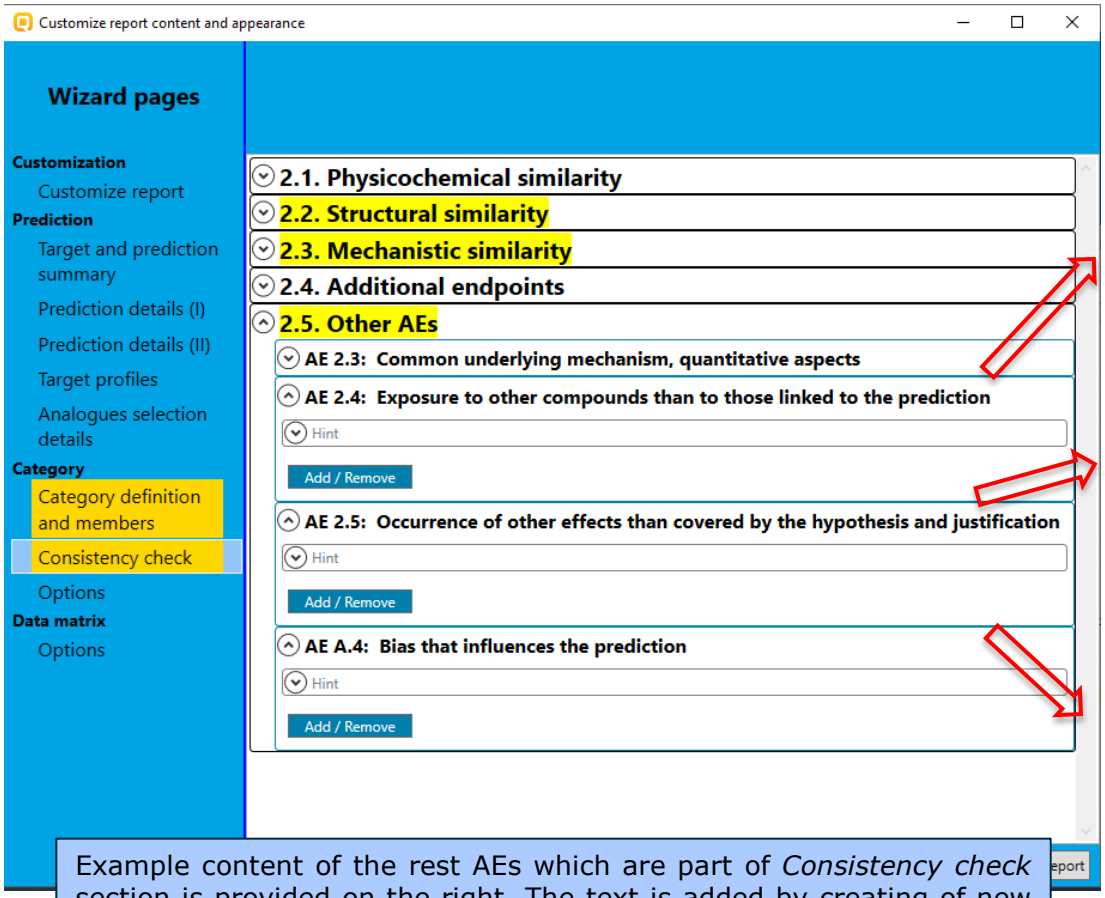




2

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
Assessment elements of Scenario 2



Example content of the rest AEs which are part of *Consistency check* section is provided on the right. The text is added by creating of new textual report item already explained in the previous slides.

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

- *No impurities are available for the Source substances.*
- *The target substance A and the source substances A,B and C are enzymatically transformed to the reactive species "bisaldehyde" and simple "aldehyde".*
- *Aldehydes are not expected to cause skin sensitization effect by the expert knowledge;*
- *No other reactive metabolites are produced based on the enzymatic transformations.*

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 metabolize to "Bis aldehyde".*
- *"Bis aldehyde" is responsible for the skin sensitization effect of the source substances.*
- *No PBA for chromosomal aberration are identified in the target and source substances, nor in the structures of their metabolites.*
- *In general, the "aldehyde" moiety is well known alerting group for protein binding. In this respect, all toxicity effects which are based on covalent interaction with protein molecules could be considered as relevant.*

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

•Target chemical is activated as result of skin metabolism by producing reactive metabolites – Bisaldehyde and simple aldehyde.
• The source chemicals have been selected based on the same reactivity pattern discovered for the target chemical, i.e. all source chemicals are activated as a result of skin metabolism producing same reactive metabolites – Bis aldehyde and simple aldehyde.
• The mechanism of interaction with skin proteins is Schiff base formation.
• Illustration of the enzymatic transformation is provided to AE 2.1.
• Illustration of the mechanism of interaction of Bis aldehyde to skin proteins is available in AE 2.2.

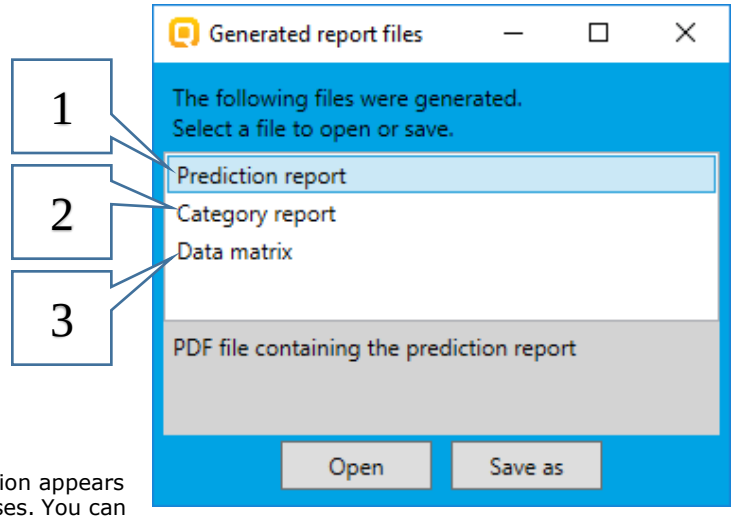
Report
Generation

After clicking the *Create report* button, the *Generated report files* window appears*. It contains three types 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 second file.

All generated files should be provided when submitting a prediction.

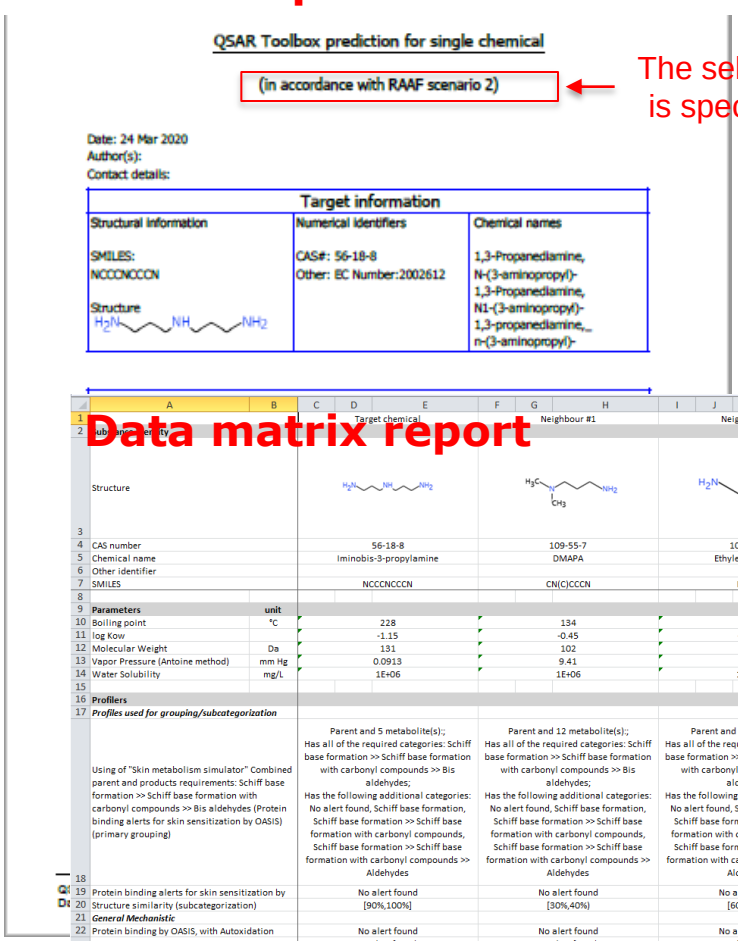


*Before appearing of the window with the report files additional window with information appears including how many chemicals used in the prediction belongs to the restricted databases. You can close it.

Report
Generated report files

Prediction report

Category report



QSAR Toolbox prediction for single chemical

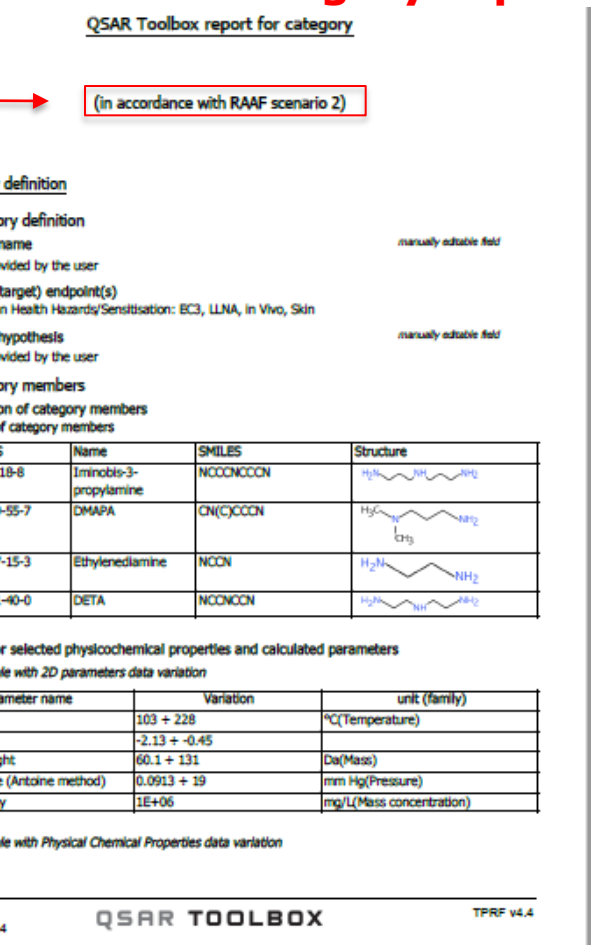
(in accordance with RAAF scenario 2)

Date: 24 Mar 2020
Author(s):
Contact details:

Target information

Structural Information	Numerical Identifiers	Chemical names
SMILES: NCCCNCCCN Structure	CAS#: 56-18-8 Other: EC Number:2002612	1,3-Propanediamine, N-(3-aminopropyl)- 1,3-Propanediamine, N1-(3-aminopropyl)- 1,3-propanediamine,_ n-(3-aminopropyl)-

The selected RAAF scenario is specified in the first page



QSAR Toolbox report for category

(in accordance with RAAF scenario 2)

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

iation of category members
le of category members

CAS	Name	SMILES	Structure
56-18-8	Iminobis-3-propylamine	NCCCNCCCN	
109-55-7	DMAPA	CN(C)CCCN	
107-15-3	Ethylenediamine	NCCN	
111-40-0	DETA	NCCNCCN	

s for selected physicochemical properties and calculated parameters

Table with 2D parameters data variation

Parameter name	Variation	unit (family)
it	103 + 228	°C(Temperature)
	-2.13 + -0.45	
Weight	60.1 + 131	Da(Mass)
sure (Antoine method)	0.0913 + 19	mm Hg(Pressure)
bility	1E+06	mg/L(Mass concentration)

Table with Physical Chemical Properties data variation

QSAR TOOLBOX TPRF v4.4

Data matrix report

	Target chemical	Neighbour #1	Neighbour #2	Neighbour #3
Structure				
CAS number	56-18-8	109-55-7	107-15-3	111-40-0
Chemical name	Iminobis-3-propylamine	DMAPA	Ethylenediamine	NCCNCCN
Other identifier				
SMILES	NCCCNCCCN	CN(C)CCCN	NCCN	NCCNCCN
Parameters	**unit**			
Boiling point	°C	228	134	103	189
log Kow		-1.15	-0.45	-1.62	-2.13
Molecular Weight	Da	131	102	60.1	103
Vapor Pressure (Antoine method)	mm Hg	0.0913	9.41	19	0.274
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 products requirements: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes (Protein binding alerts for skin sensitization by OASIS) (primary grouping)		Parent and 5 metabolite(s):; Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; 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 12 metabolite(s):; Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; 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 5 metabolite(s):; Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; 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 5 metabolite(s):; Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; 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
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%)
General Mechanistic					
Protein binding by OASIS, with Autoxidation		No alert found No alert found; Schiff base formation >> Schiff base	No alert found No alert found; Schiff base formation >> Schiff base	No alert found No alert found; Schiff base formation >> Schiff base	No alert found No alert found; Schiff base formation >> Schiff base

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!