QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 4 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 assessing of the outcome whether read across is scientifically acceptable or not
- 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 a target endpoint;
- Relevancy of profiles and data availability;
- Searching of analogues accounting for metabolism;
- A category consistency check;
- Selection of a 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 specifically with Scenario 4;
- 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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- **Read Across Assessment Framework (RAAF)**
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Read Across Assessment Framework (RAAF)

Overview

- RAAF has been developed by ECHA as an internal tool providing 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 a particular aspects (assessment elements, AEs).
- Total six scenarios are available: two for an analogue approach and four for a 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

1. Distinguish whether an analogue or a category approach is decided based on the number (N) of analogues*:
   a) N of analogues ≤ 3 is an Analogue approach (scenario 1-2);
   b) N of analogues > 3 is a Category approach (scenario 3-6).
2. To identify the basis of the read across hypothesis
   a) (Bio)transformation to (a) common compound(s) – the read across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed to
   b) Different compounds have the same type of (an) 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**.
3. For a category approach (scenario 3-6) there is a need to take further into account whether or not quantitative variations in the properties are observed among the category members:
   a) There is a quantitative variation in the (eco)toxicity when it is more than 1 log units**(scenario 3 and 4);
   b) A quantitative variation is not expected in the (eco)toxicity when it is less or equal to 1 log unit (scenario 5-6).

*** The threshold for the number of analogues which distinguishes an analogue from a category approach is proposed by LMC**
****The quantitative variation in the (eco)toxicity of 1 log unit is proposed by LMC based on empirically observations.**

Read Across Assessment Framework (RAAF)
Selection of a RAAF scenario

- Each scenario consists of a pre-defined set of assessment elements (AEs) that, when taken together, covers 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 a 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 exercise**
- Workflow

The Exercise

- In this exercise we will predict *Repeated Dose Toxicity (RDT)* of 3,5-dimethyl-aniline [CAS# 108-69-0], which will be the "target" chemical.
- In this exercise the category will be defined based on the aniline functionality identified in the package: parent and metabolites based on Repeated dose toxicity profiler. This chemical class is related with *Hemolytic anemia with a methemoglobinemia* accounting. The effect is considered when *in vivo* Rat metabolism is taken into account;
- The read across approach will be used for the prediction. The read-across will be based on a category approach relying on a common metabolite generated for the 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.

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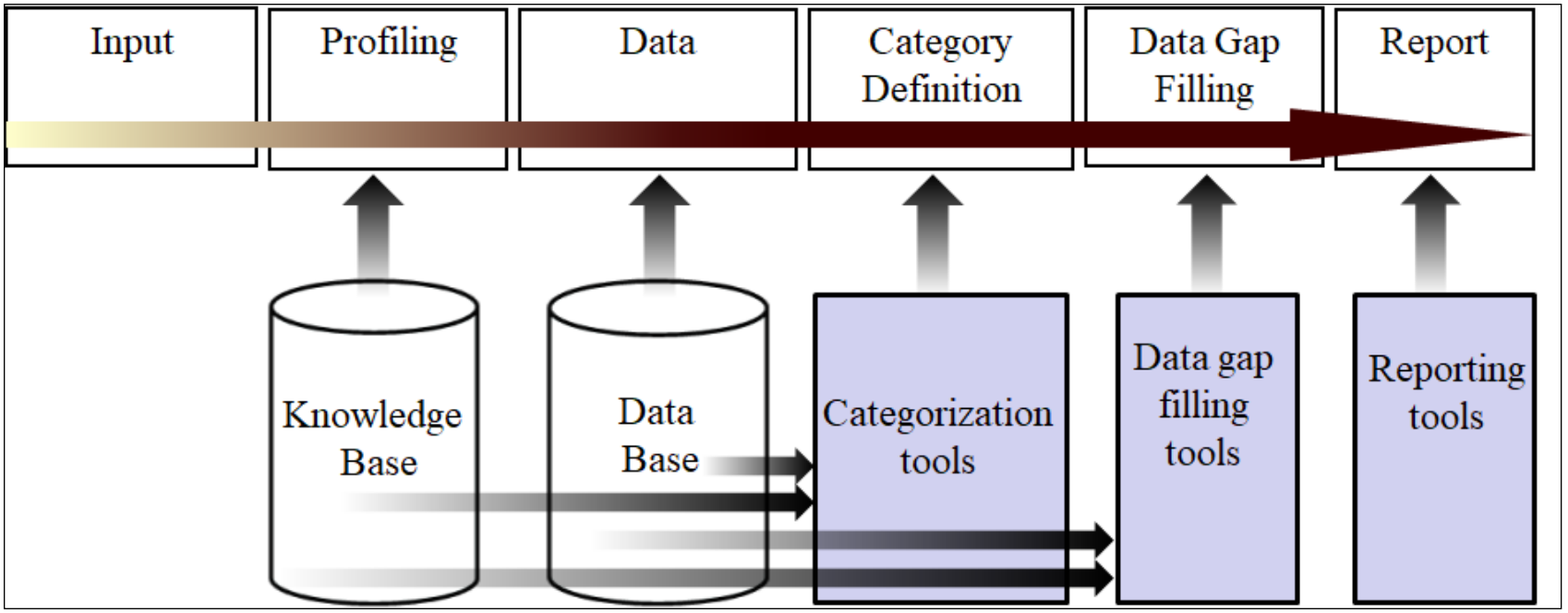
Workflow

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

Workflow

Scheme illustrating the Toolbox workflow

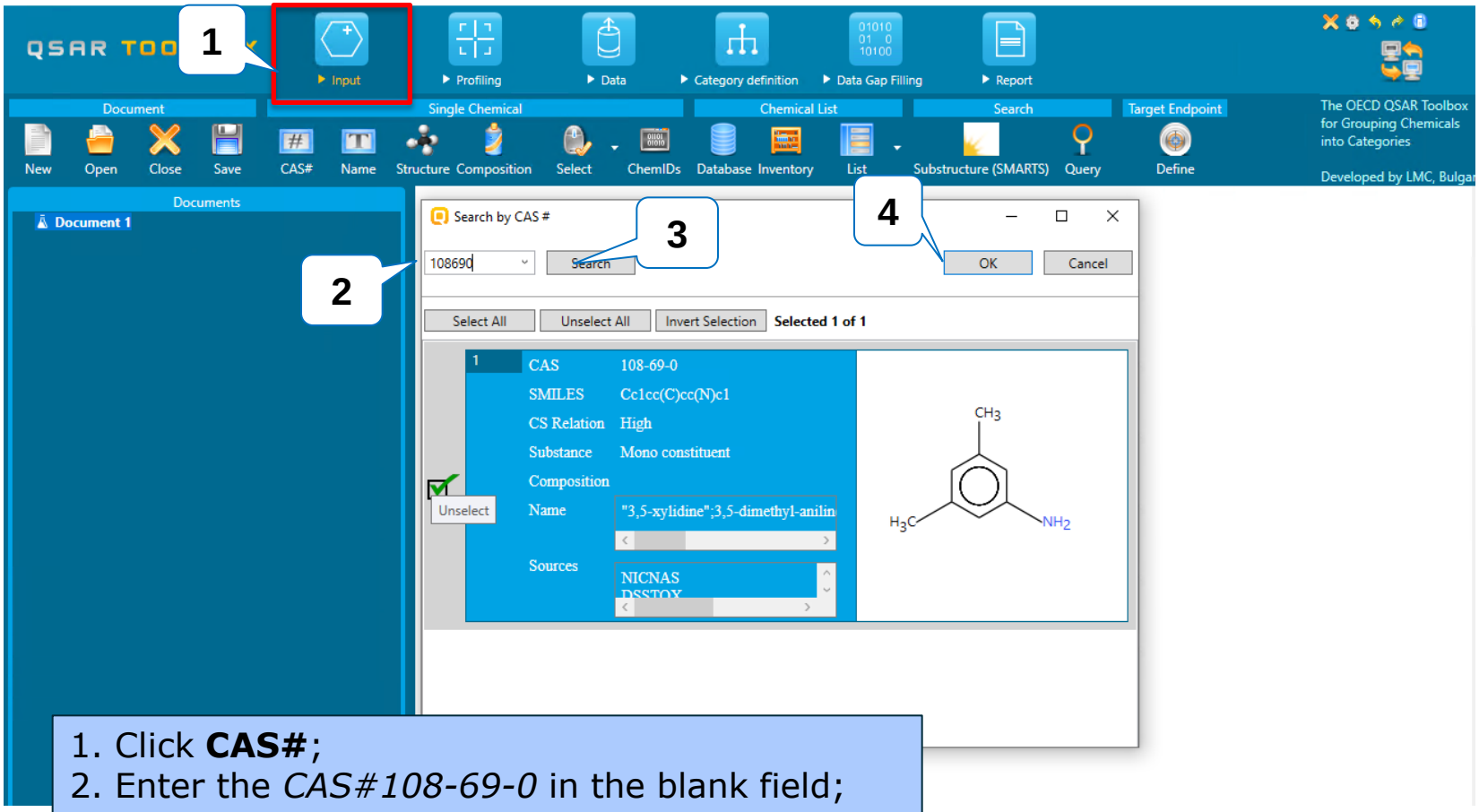


Input
Overview

- 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 a chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Input

Input the target chemical by CAS#

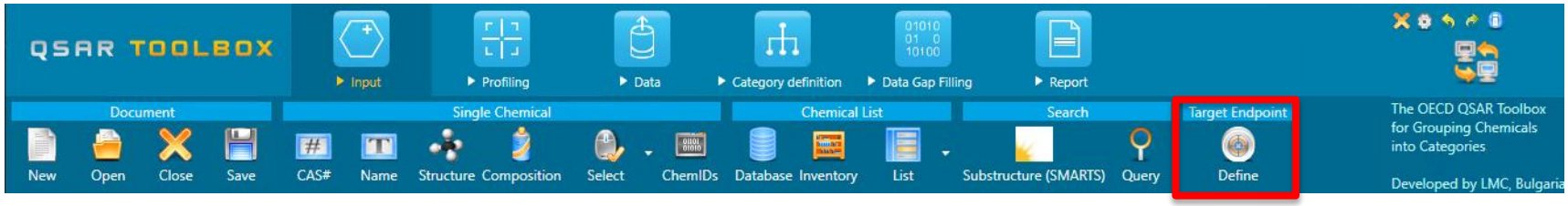


1. Click **CAS#**;
2. Enter the *CAS#108-69-0* in the blank field;
3. Click **Search**;
4. When the structure appears, click **OK.**

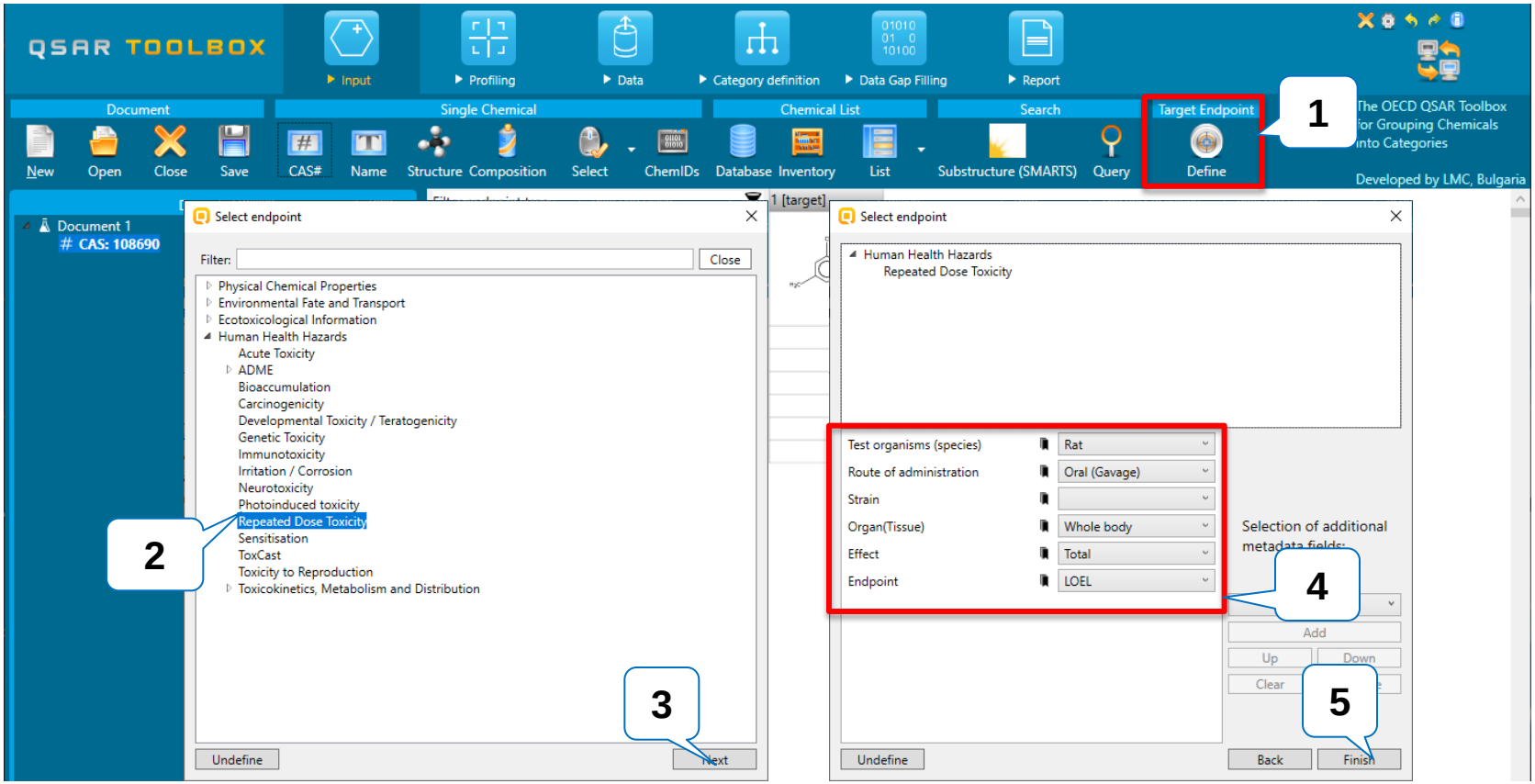
Input
Define the target endpoint

Defining of the endpoint allows entering the endpoint of interest e.g. EC3, LOEL, LC50 etc., along with specific metadata information. Based on the metadata, different relevancy scores for profiles could be provided for the same endpoint.

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



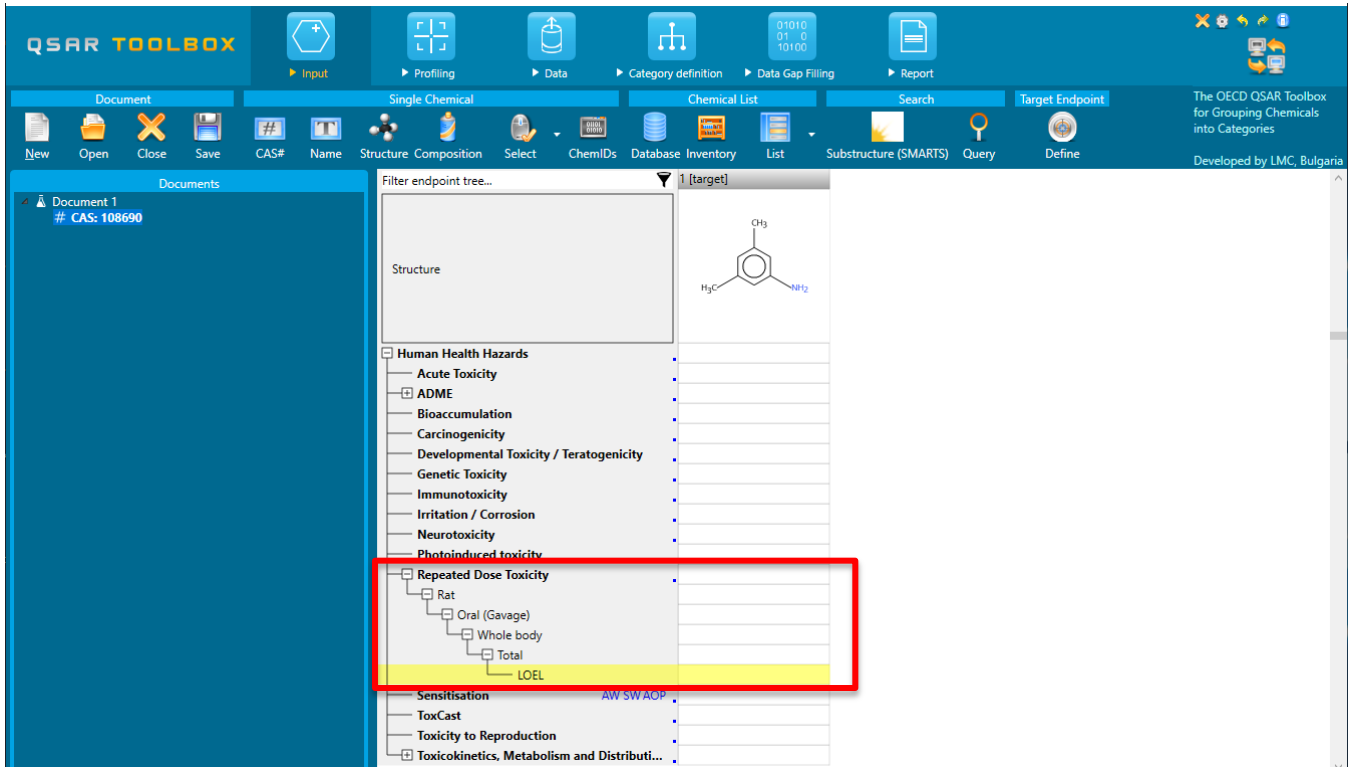
Input
Define the target endpoint



Click **Define** (1), select **Repeated Dose Toxicity** (2) and then click **Next** (3). Select **LOEL** as an endpoint from the drop-down menu and then consecutively the following metadata: *Effect*: **Total,** *Organism(tissue):* ***Whole body,*** *Test organism(species):* **Rat**, *Route of administration:* **Oral (gavage)** (4). Finally click **Finish** (5).

Input
Define the 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 yellow highlighted.



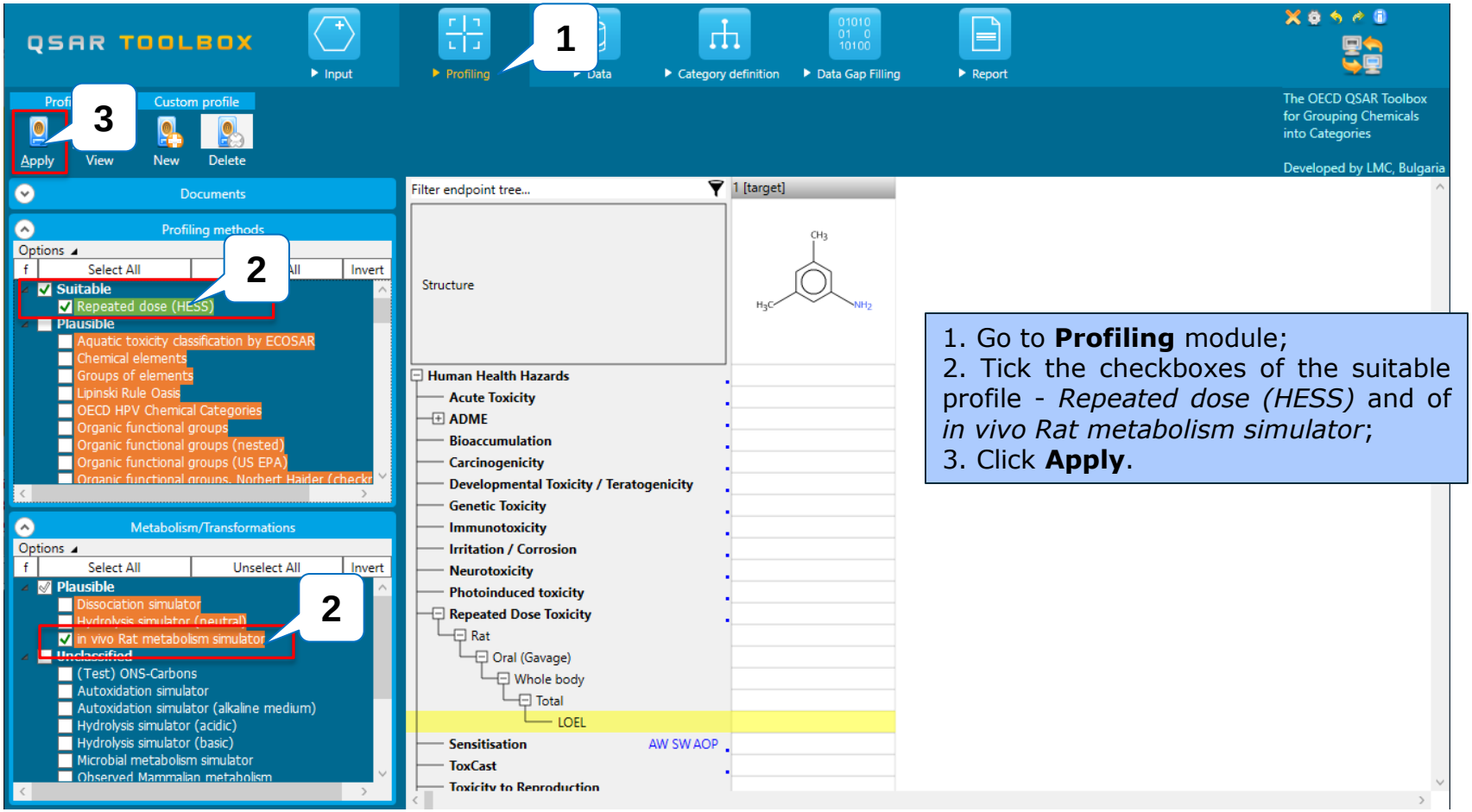
Profiling
Overview

- "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 (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 profiling schemes;
- 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

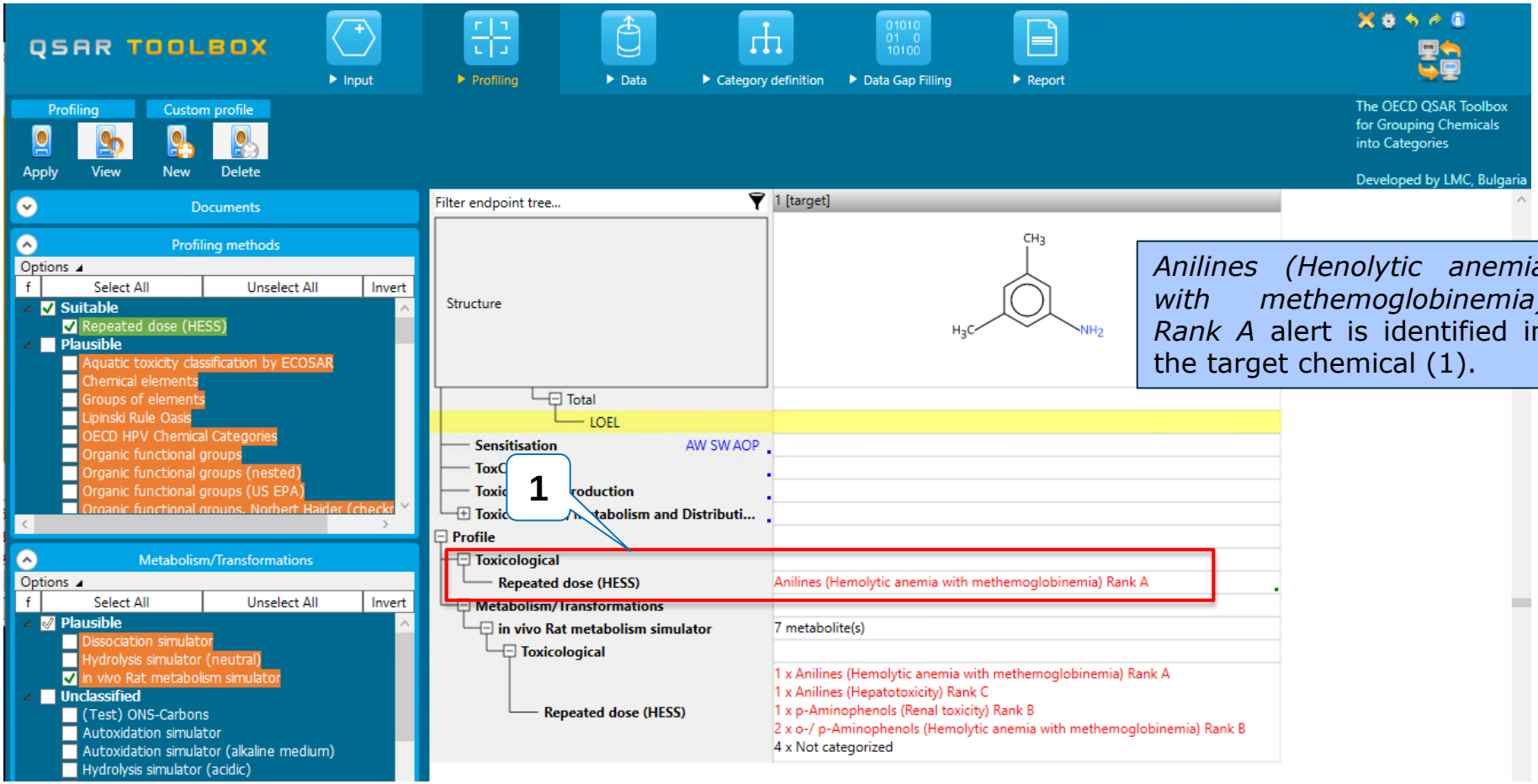
Profiling the target chemical



1. Go to **Profiling** module;
2. Tick the checkboxes of the suitable profile - *Repeated dose (HESS)* and of *in vivo Rat metabolism simulator*;
3. Click **Apply**.

Profiling

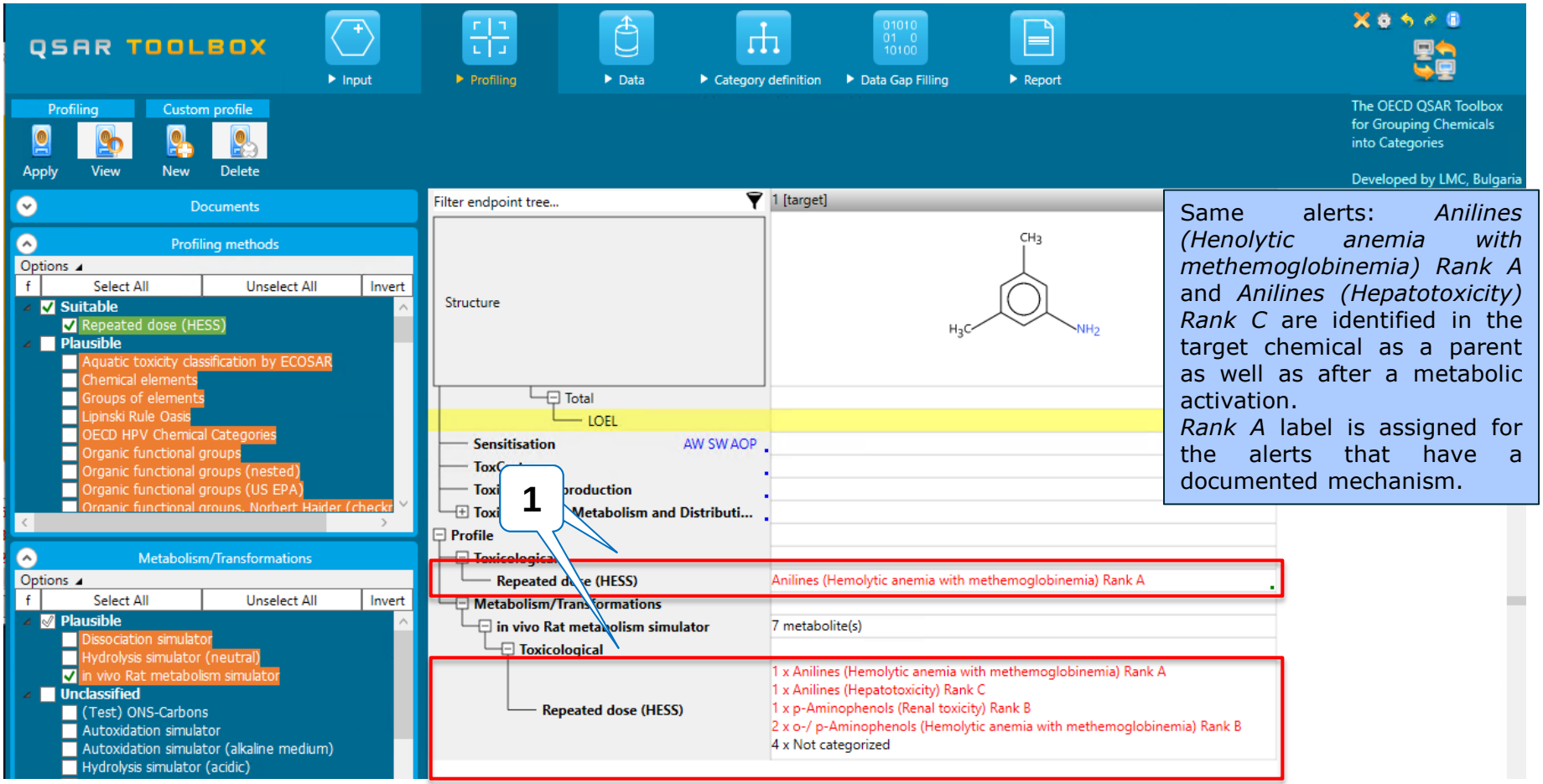
Profiling the target chemical



Anilines (Henolytic anemia with methemoglobinemia) Rank A alert is identified in the target chemical (1).

Profiling

Profiling the target chemical



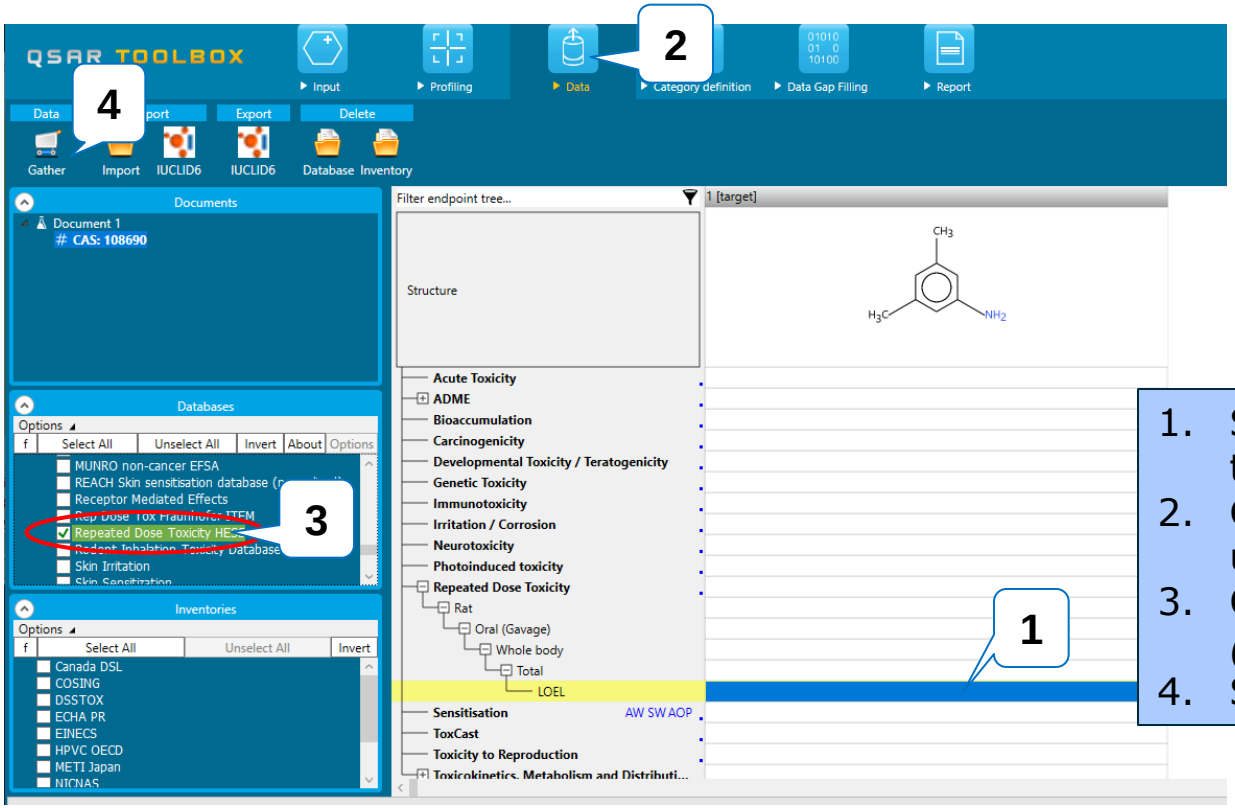
Same alerts: *Anilines (Henolytic anemia with methemoglobinemia) Rank A* and *Anilines (Hepatotoxicity) Rank C* are identified in the target chemical as a parent as well as after a metabolic activation.

Rank A label is assigned for the alerts that have a documented mechanism.

Data
Overview

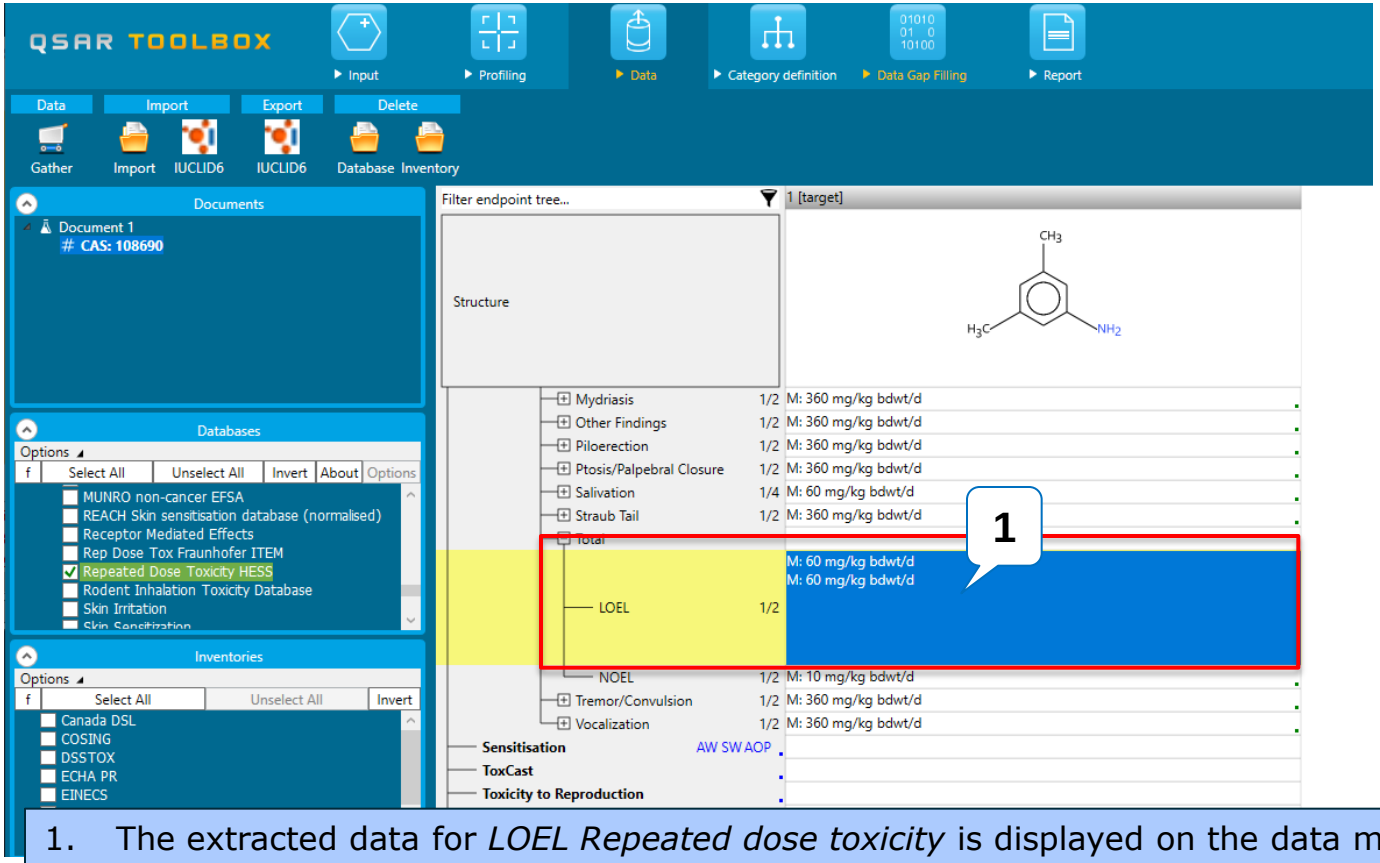
- "Data" refers to the electronic process of retrieving the environmental fate, eco-toxicity and toxicity data that are stored in the Toolbox.
- Data gathering can be executed in a global fashion (i.e., collecting all data for all endpoints) or on a more narrowly defined basis (e.g., collecting data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

Data
Collecting experimental data



1. Select the cell corresponding to the target endpoint;
2. Go to module ***Data*** and unselect all databases;
3. Check **Repeated *dose (HESS)* database**;
4. Select ***Gather***.

Data
Collecting experimental data



1. The extracted data for *LOEL Repeated dose toxicity* is displayed on the data matrix; Both experimental data for target chemical are equal (*60 mg/kg bdw/d*).

Data
Collecting experimental data

- Toxicity information on the target chemical is automatically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues is performed only among the chemicals which are listed in the selected database(s), which in this example is *Repeated dose (HESS).*
- Two experimental data related to the defined target endpoint are found.

Based on the observed data (60 mg/kg bw/d) the target chemical is classified as *Category 2* regarding GHS classification [1]

Table 3.9.2: Guidance values to assist in Category 2 classification

Route of exposure	Units	Guidance value range (dose/concentration)
Oral (rat)	mg/kg bw/d	10 - 100
Dermal (rat or rabbit)	mg/kg bw/d	20 - 200
Inhalation (rat) gas	ppm/6h/d	50 - 250
Inhalation (rat) vapour	mg/litre/6h/d	0.2 - 1.0
Inhalation (rat) dust/mist/fume	mg/litre/6h/d	0.02 - 0.2

See on the next slide

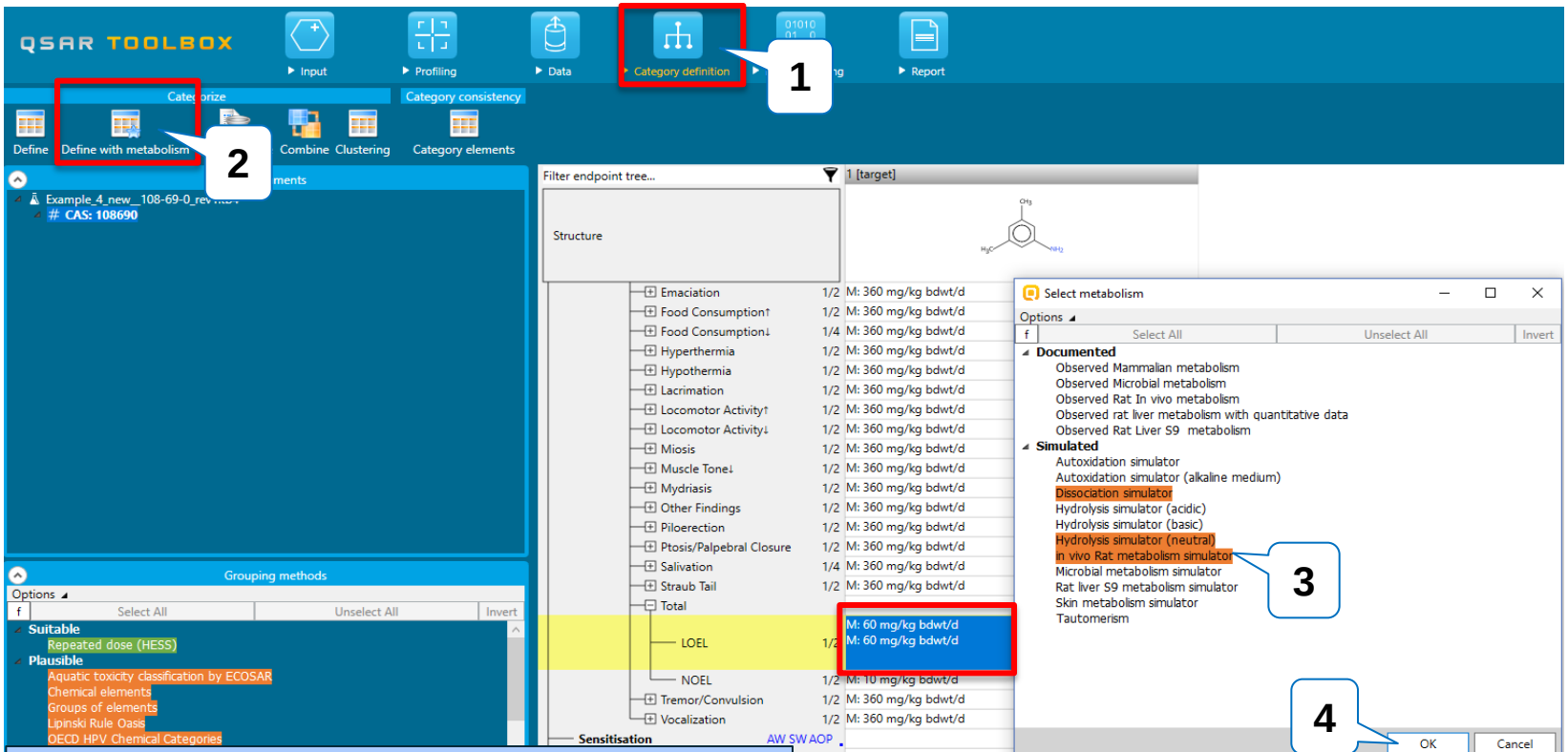
[1] Globally Harmonized System of Classification and Labeling of Chemicals (GHS):
http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf

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.
- As the RDT is a systemic endpoint the metabolism could take place. The primary category in the current example will be defined accounting for an *in vivo rat* metabolism.

Category Definition

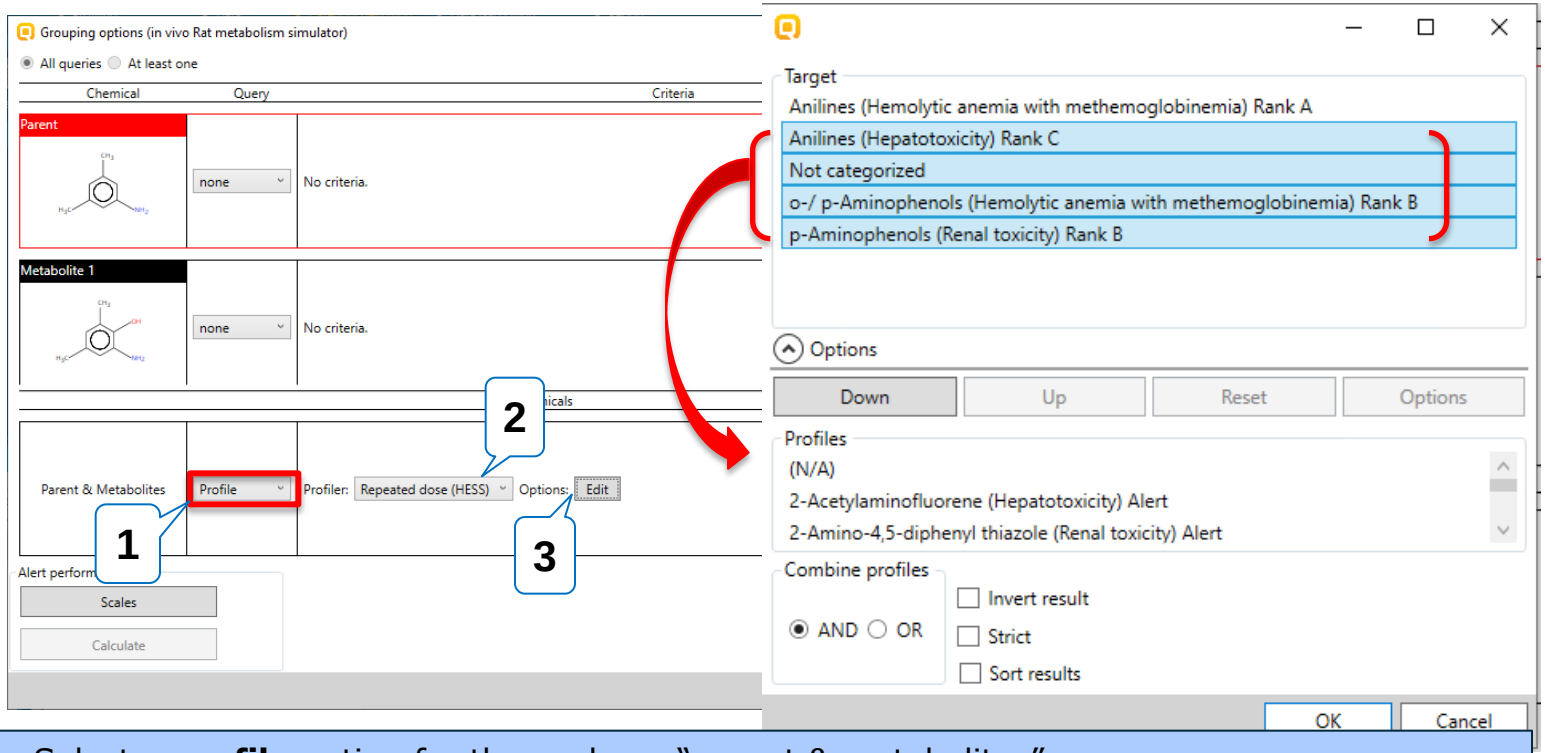
Searching for analogues accounting for an *in vivo* Rat metabolism



1. Go to the **Category definition** module;
2. Click **Define with metabolism**;
3. Select **in vivo Rat metabolism simulator**;
4. Click **OK**.

Category Definition

Searching for analogues accounting for an *in vivo* Rat metabolism

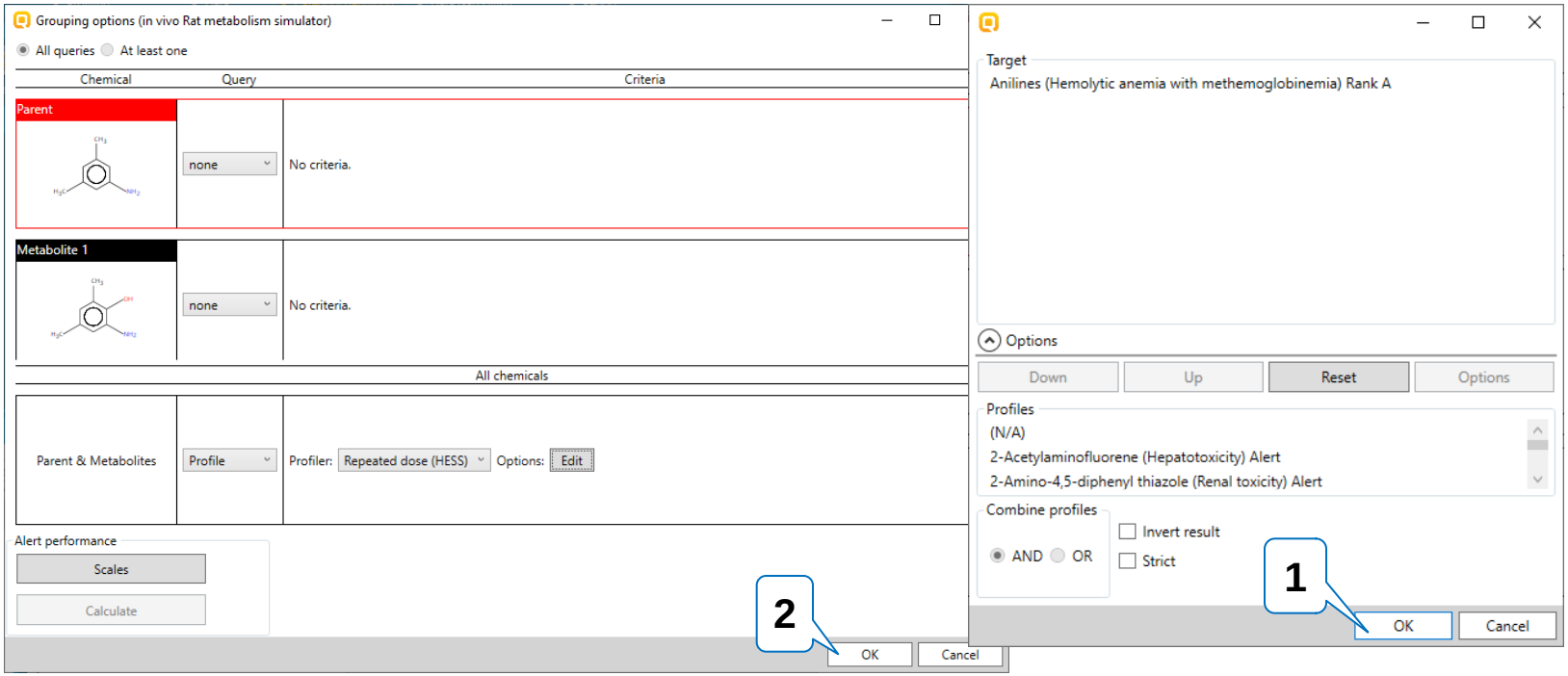


1. Select a **profile** option for the package "parent & metabolites";
2. Select "*Repeated dose (HESS)*" profile;
3. Click the **Edit** button. Remove all categories except *Anilines (Hemolytic anemia with a methemoglobinemia) Rank A** category by double click or using "Down" button;

*The categories with *Rank A* are supported with training sets chemicals having reliable experimental data.

Category Definition

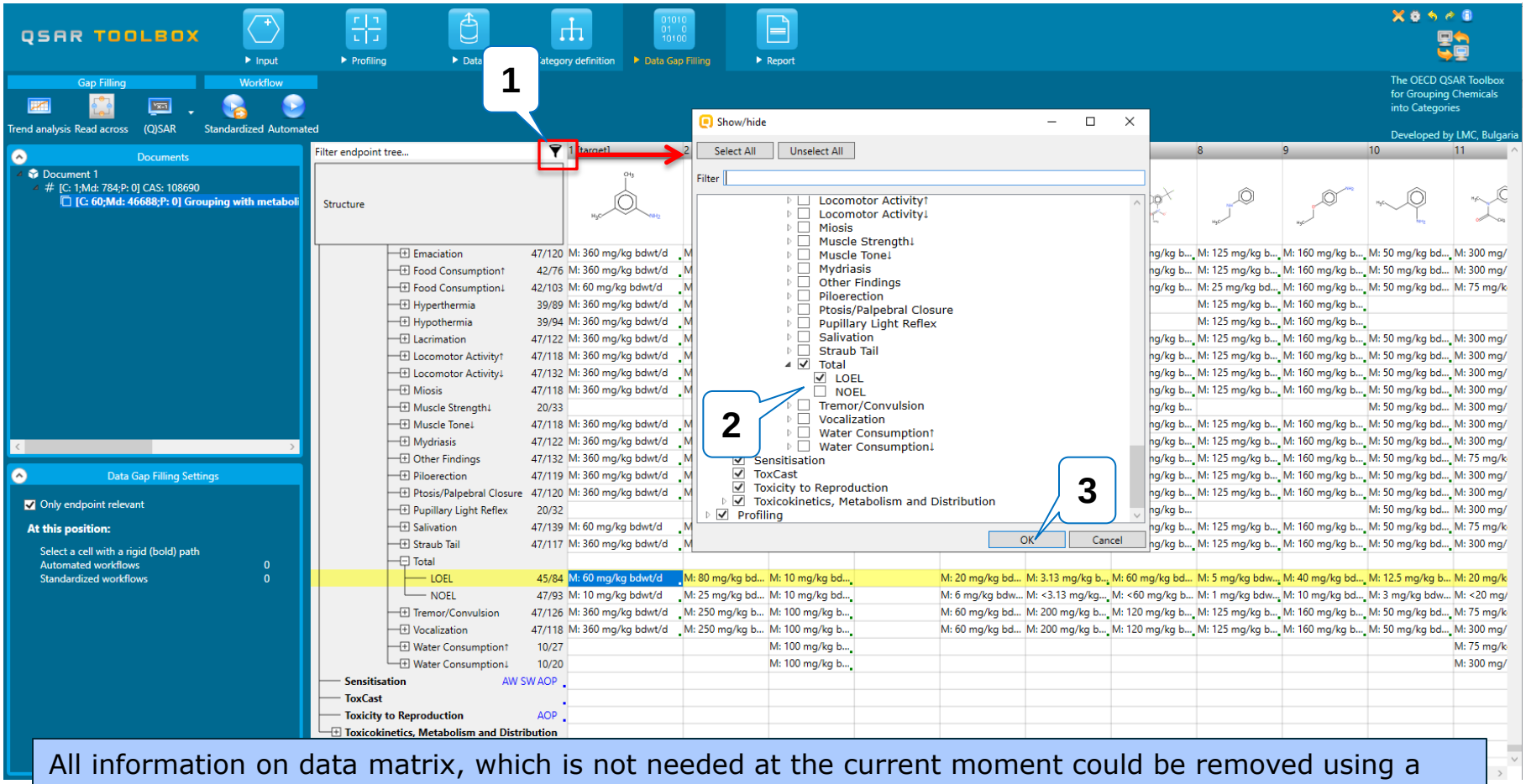
Searching for analogues accounting for an *in vivo* Rat metabolism



1. Click **OK** to confirm the defined search criteria.
2. Click **OK** in Map similarity options window to execute the search.

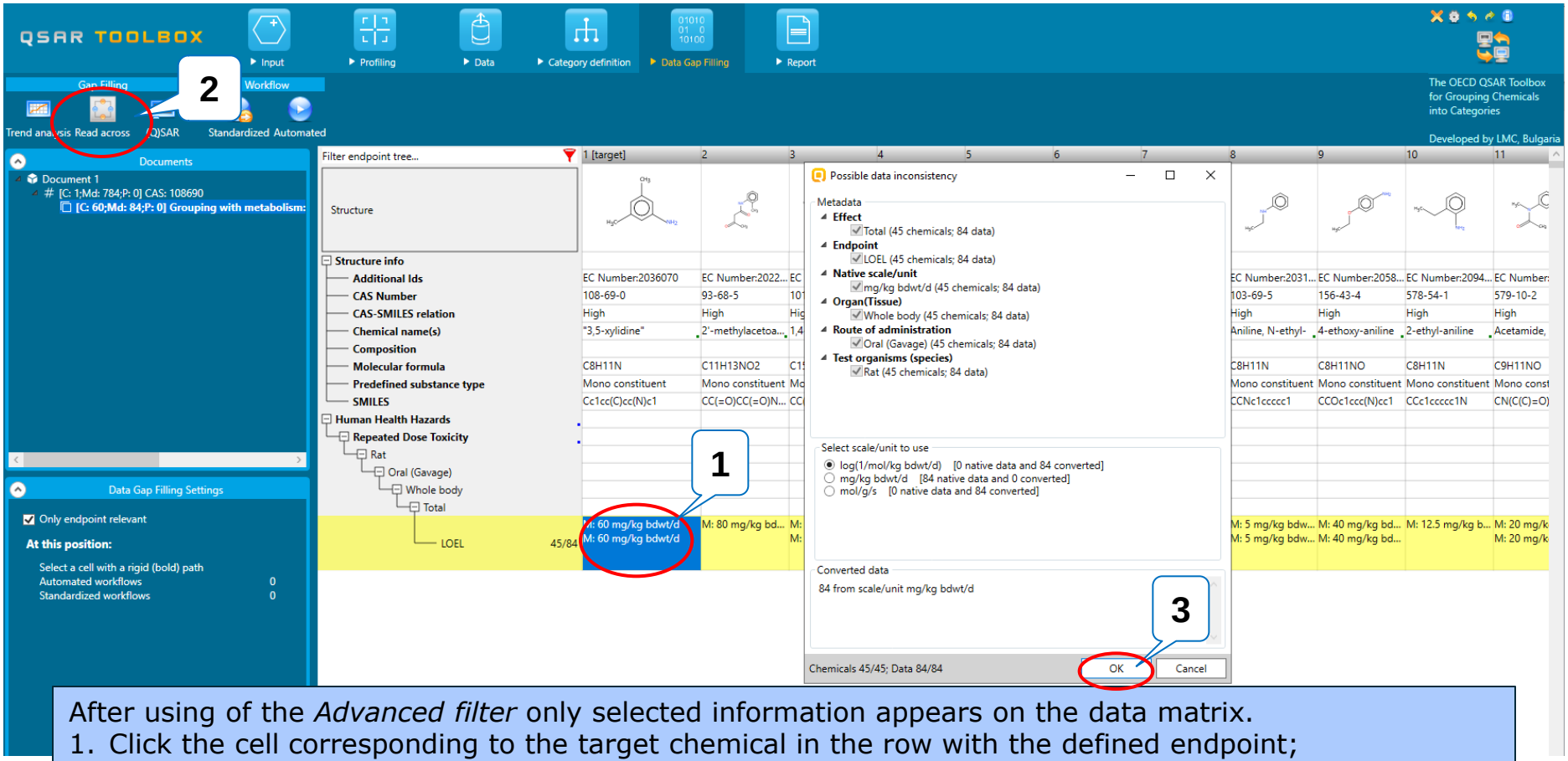
In this way we will search for analogues that have this alert as a parent or as a metabolite.

Filter data matrix



All information on data matrix, which is not needed at the current moment could be removed using a filter.
Click the ***Advanced filter*** icon (1). A window with the endpoint tree organization appears. Select only the nodes which you want to see in the data matrix and confirm by clicking OK (3).

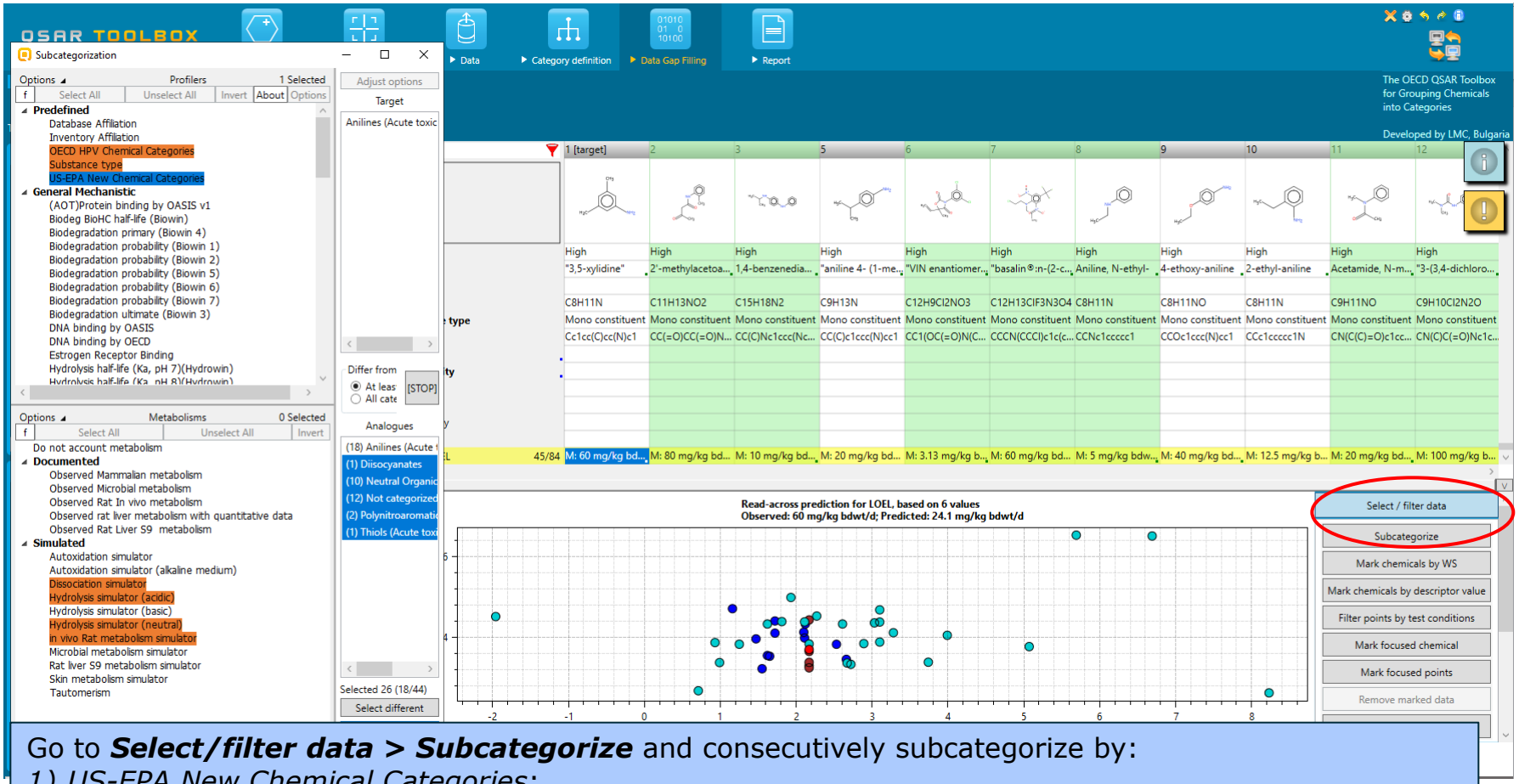
Data Gap Filling
Apply Read across



After using of the *Advanced filter* only selected information appears on the data matrix.

1. Click the cell corresponding to the target chemical in the row with the defined endpoint;
2. Click **Read-across**;
3. **Possible data inconsistency** window appears, keep the default selection. Click **OK**.

Data Gap Filling
Subcategorizations



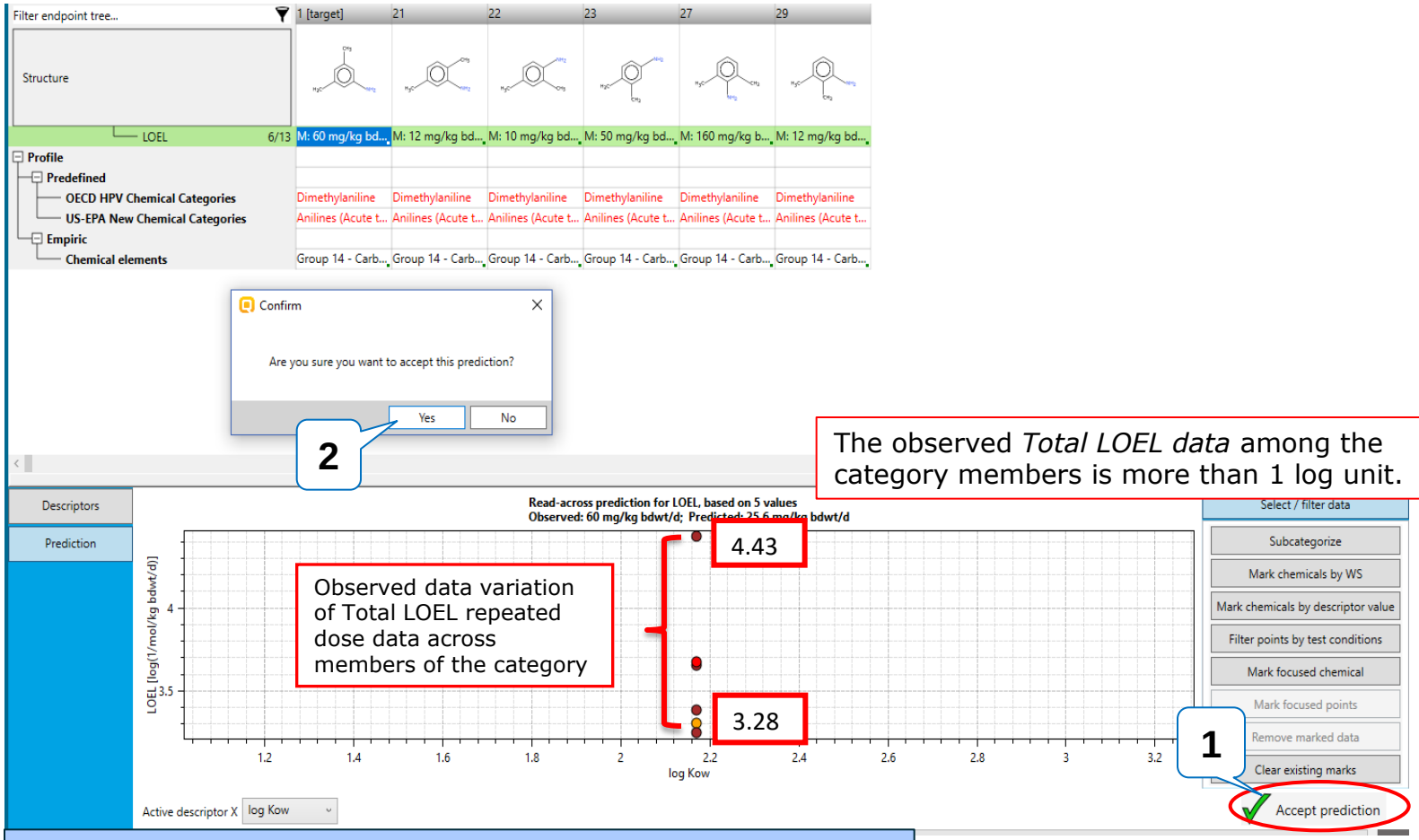
Go to ***Select/filter data > Subcategorize*** and consecutively subcategorize by:

1) US-EPA New Chemical Categories;

2) Chemical elements;

3) OECD HPV Chemical Categories.

Data Gap Filling
Subcategorizations



1. Click ***Accept prediction*** *(25.6 mg/kg bdw/d)*;
2. Click ***Yes*** to confirm the prediction.

Data Gap Filling
RA prediction for CAS 108-69-0
Results

Observed data:
Total LOEL - 60 mg/kg bdw/day

Prediction:
Total LOEL – 25.6 mg/kg bdw/day

Based on the predicted data (for Total LOEL) the target chemical is classified as *Category 2* regarding GHS classification [1]. The RA results is in accordance with the observed data.

Table 3.9.2: Guidance values to assist in Category 2 classification

Route of exposure	Units	Guidance value range (dose/concentration)
Oral (rat)	mg/kg bw/d	10 - 100
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Inhalation (rat) dust/mist/fume	mg/litre/6h/d	0.02 - 0.2

[1] **Globally Harmonized System of Classification and Labeling of Chemicals (GHS):**
http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf

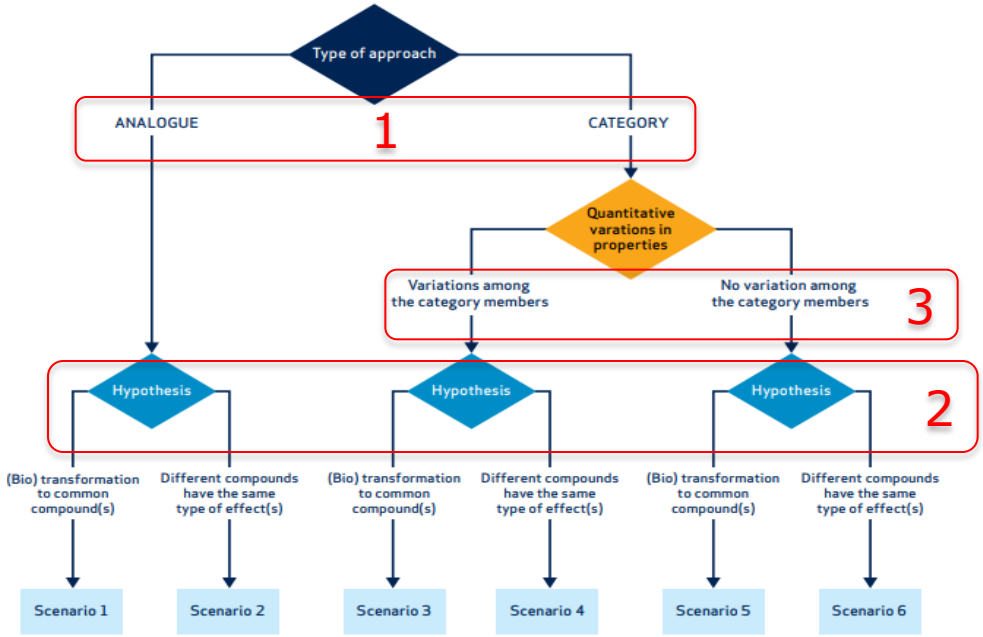
Report
Overview

- The report module allows generating a report for predictions performed within the Toolbox.
- The report module contains a predefined report template which the users can customize.
- Additionally a 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 a RAAF scenario

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

1) the type of approach applied - an analogue approach or a 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.



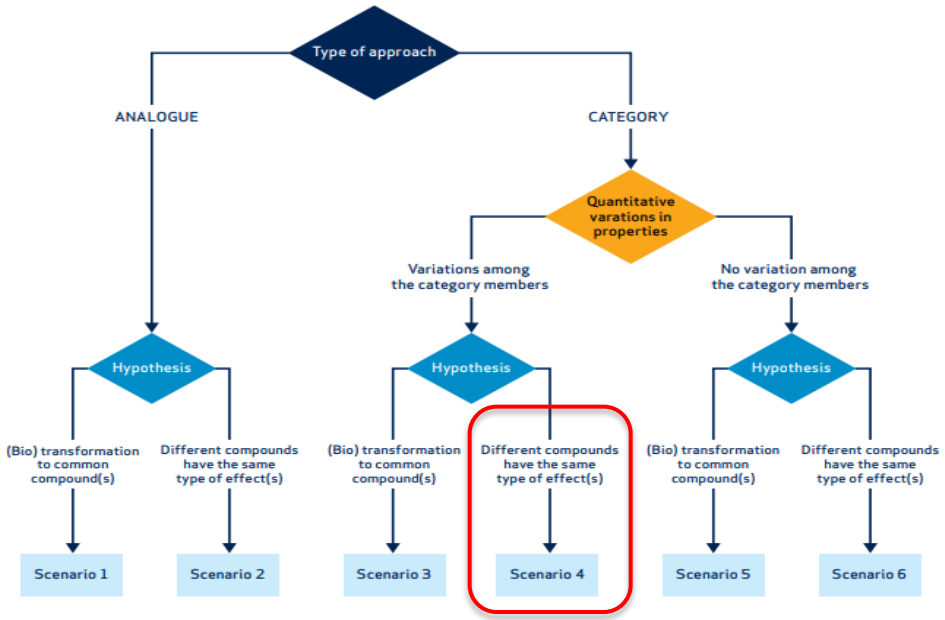
*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Report
Selection of a RAAF scenario

For the current example:

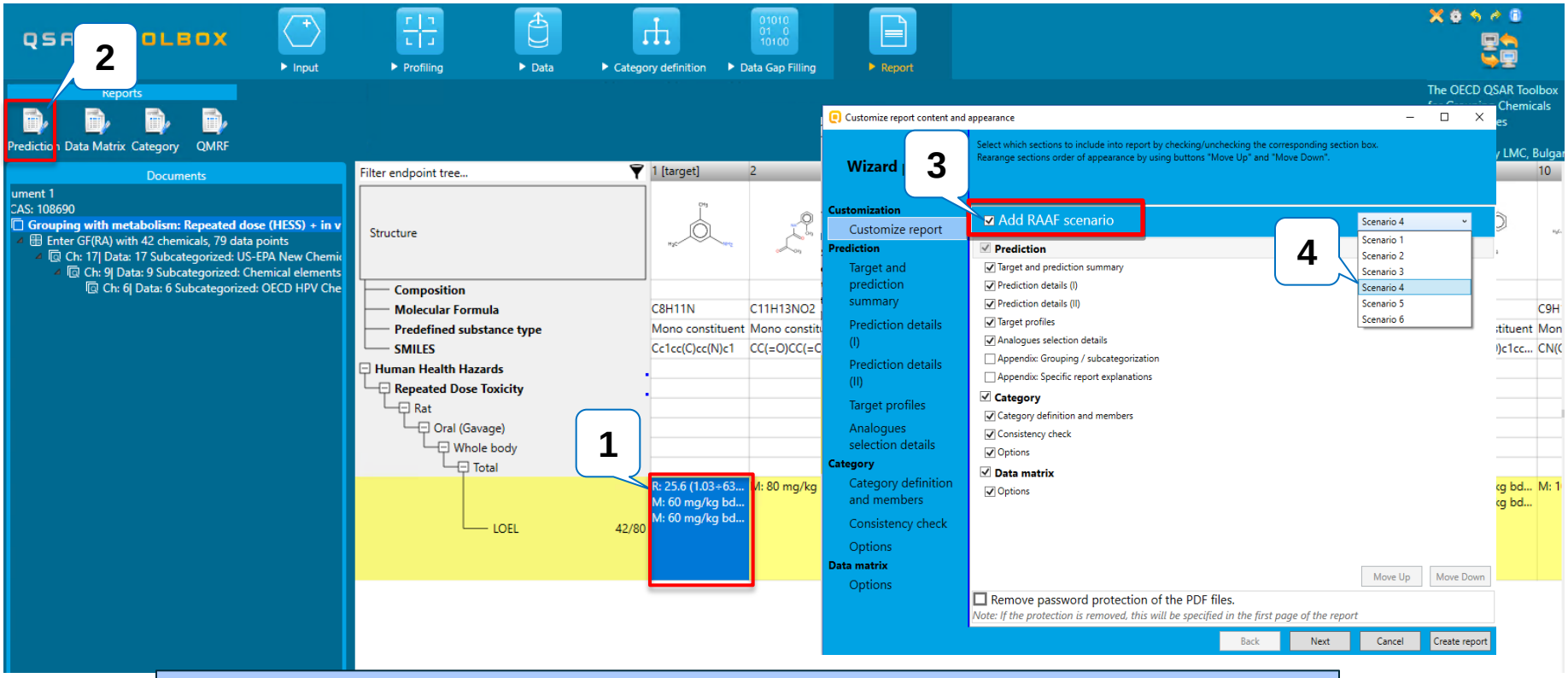
- the type of approach applied - a category approach is used (a threshold of >3 analogues is proposed by LMC for the category approach);
- the read-across hypothesis – different compounds with a common underlying mechanism for metabolites of source and target substances;
- For a category approach - The observed quantitative variation of *Total LOEL* among the category members is more than 1 log unit*.

Scenario 4 was selected for the current example based on the RAAF selection criteria.



***The range of quantitative variation in the (eco)toxicity among the category members of 1 log unit is proposed by LMC based on empirically observations.**

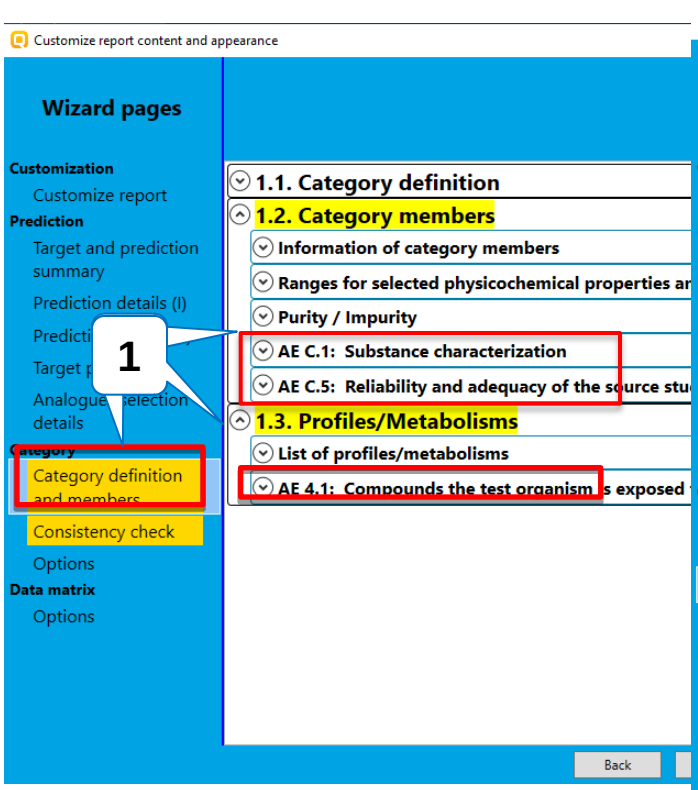
Reporting
Report Generation according to RAAF-Scenario 4

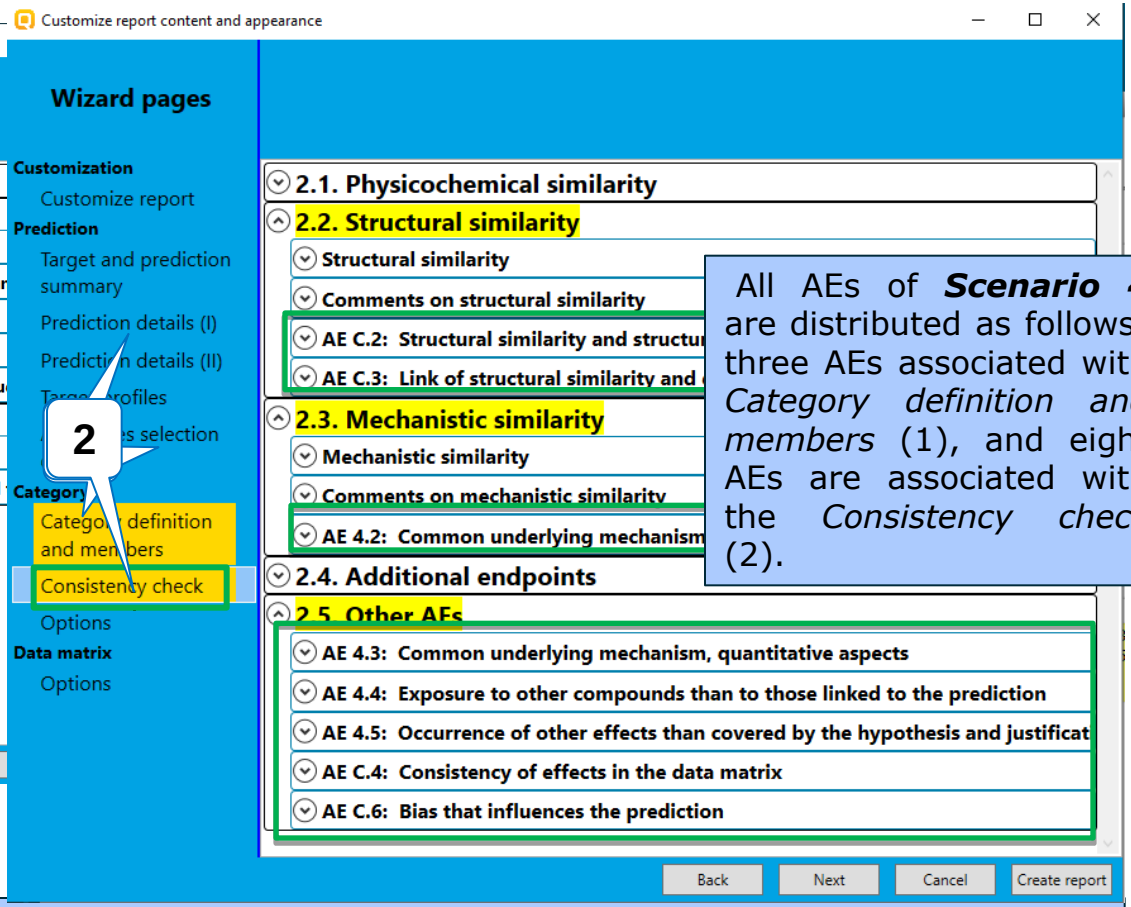


1. Go to the ***Report module*** and click on the cell with the prediction (***R:25.6 mg/kg bdwt/d)***;
2. Click the **Prediction** button;
3. Check the box at the top to add a **RAAF scenario**;
4. Select **Scenario 4** from the drop-down menu.

Reporting

Report Generation according to RAAF-Scenario 4





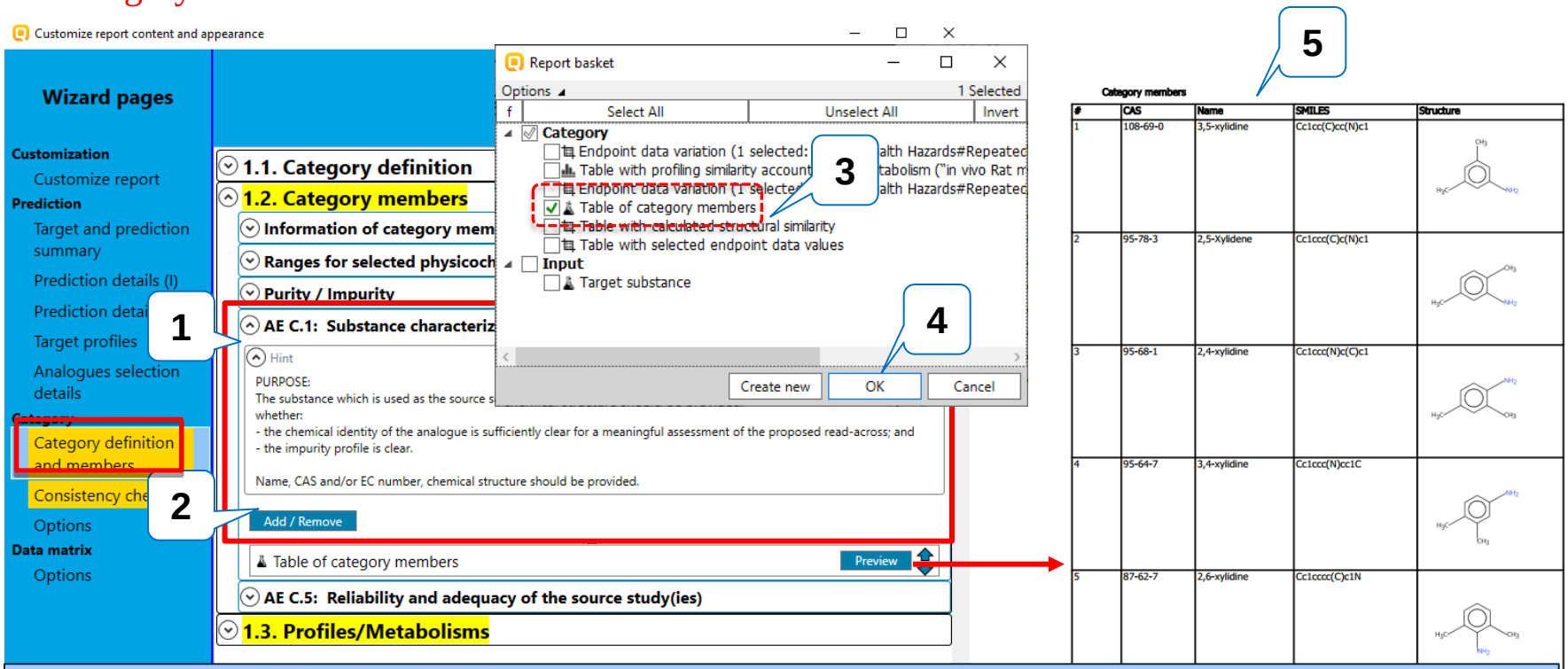
All AEs of ***Scenario 4*** are distributed as follows: three AEs associated with *Category definition and members* (1), and eight AEs are associated with the *Consistency check* (2).

Once the RAAF scenario is selected 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***. ***Category definition and members*** and ***Consistency check***.

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

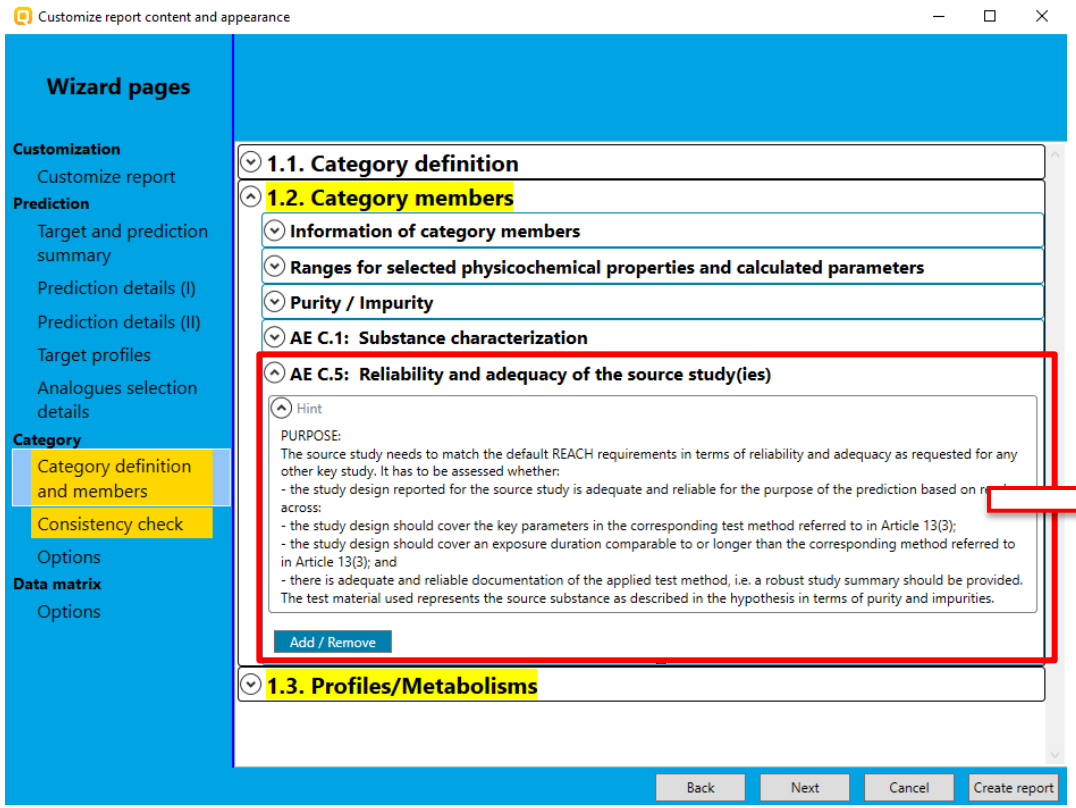
Reporting
Report Generation according to RAAF-Scenario 4

- Category definition and members section



One AE (1) related to the characterization of the category members (target and source substances) is included in the *Category definition and members* section. The **AE C.1** should be manually populated with items available in the *Report basket*. Click Add/Remove (2) button and select item Table of category members (3). Click **OK (4)**; If impurities/additives of the used analogues are available, they will be also included. The current analogues have no additives/impurities. The selected report item appears under the Add/remove button. Click **Preview** button in order to see how the AE C.1. will look in the generated report (5).

Reporting
Report Generation according to RAAF-Scenario 4



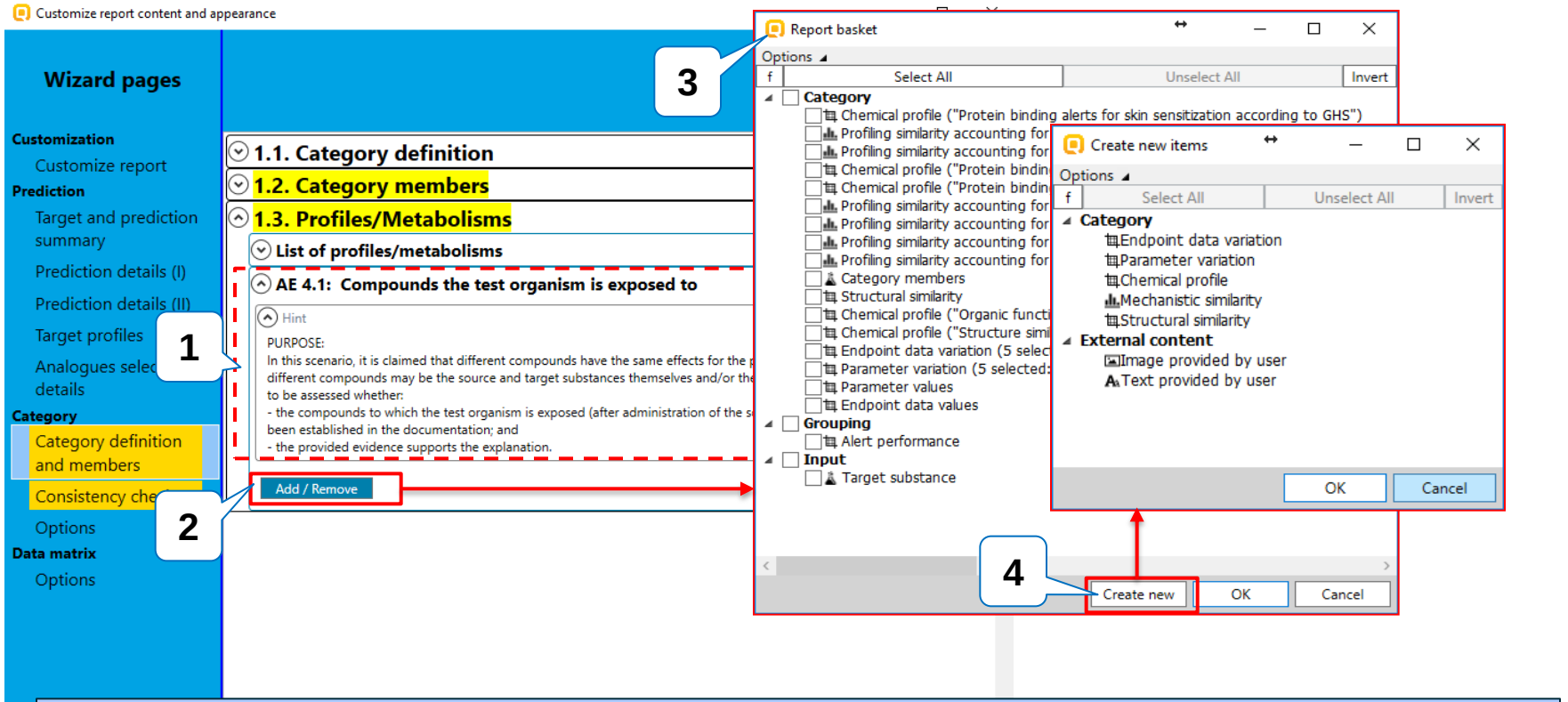
Possible content of AE C.5: Reliability and adequacy of the source study(ies)

- The target substance has been tested according to test guideline 407
- All of the five source substances with one exception (substance E) has been tested based on test guideline 407: Repeated does 28-day oral toxicity study in Rodents
- For the source substances E and B the study was based on report NTP Long term and OECD: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

A snapshot from *Filter points by test conditions* could be provided.

Reporting

Report Generation according to RAAF-Scenario 4

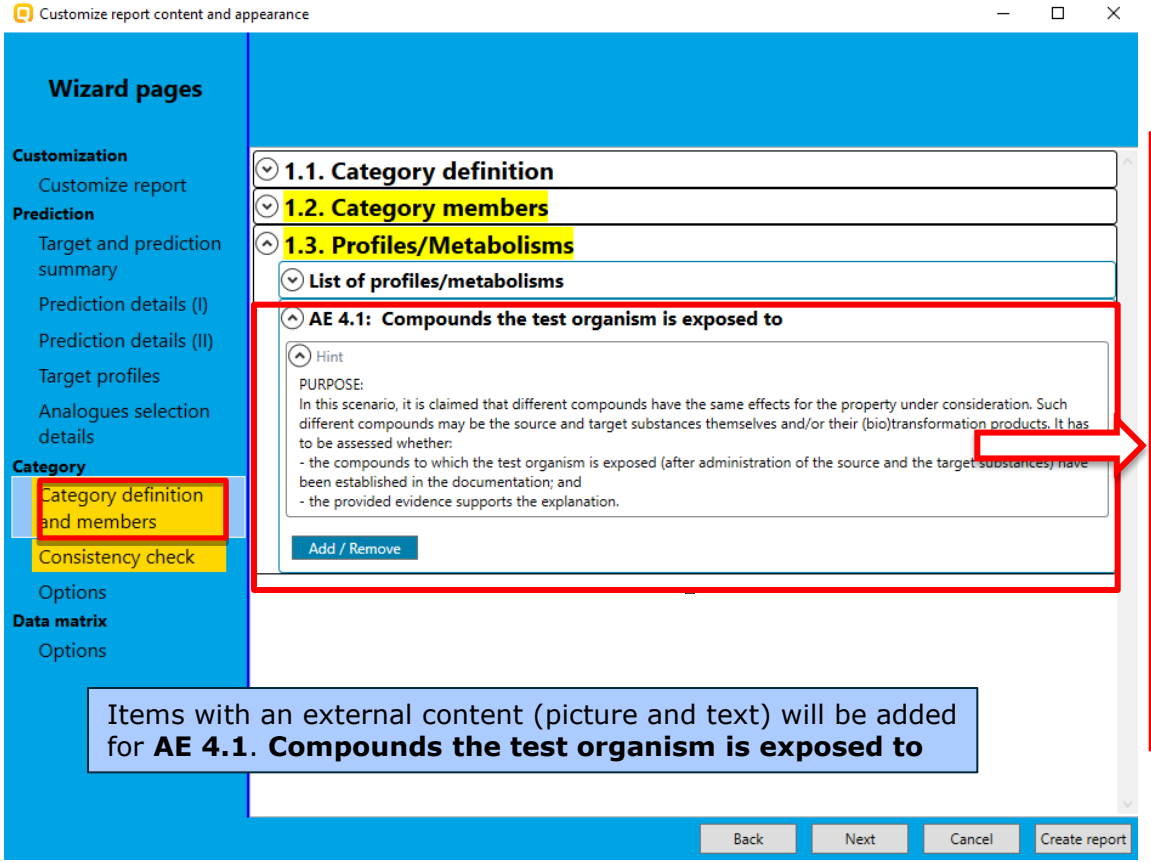


A hint for each of the assessment elements is available (1). Information can be included by the **Add/Remove** button (2) located below the corresponding AE. The *Add/Remove* button invokes so called "***Report basket***" (3). The latter contains different items triggered by the actions of the user during 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 an external content (picture and text) will be added for **AE 4.1**. **Compounds the test organism is exposed to**

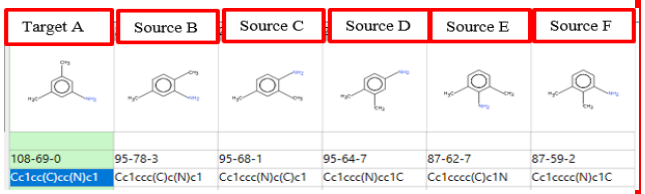
Reporting

Report Generation according to RAAF-Scenario 4



Items with an external content (picture and text) will be added for **AE 4.1. Compounds the test organism is exposed to**

Possible content of AE 4.1: Compounds the test organism is exposed to

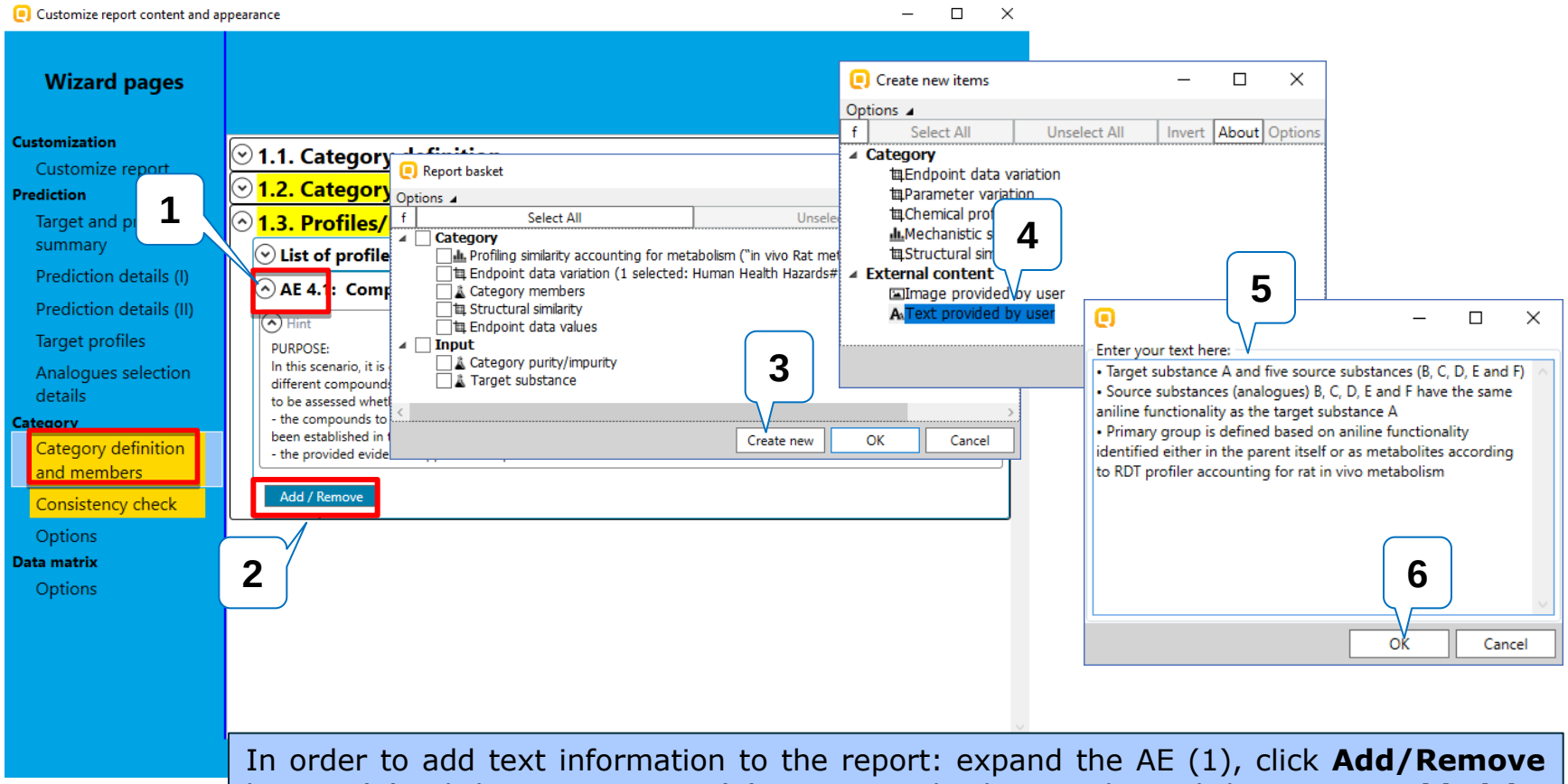


- There are target substance A and five source substances (B, C, D, E and F)
- The source substances (analogues) B, C, D, E and F have the same aniline functionality as the target substance
- A primary group is defined based on aniline functionality identified either in the parent itself or as metabolites according to RDT profiler accounting for the rat in vivo metabolism.

See how to add the textual content of AE 4.1 along with an illustrative picture of the target and source substances in the next slides.

Reporting

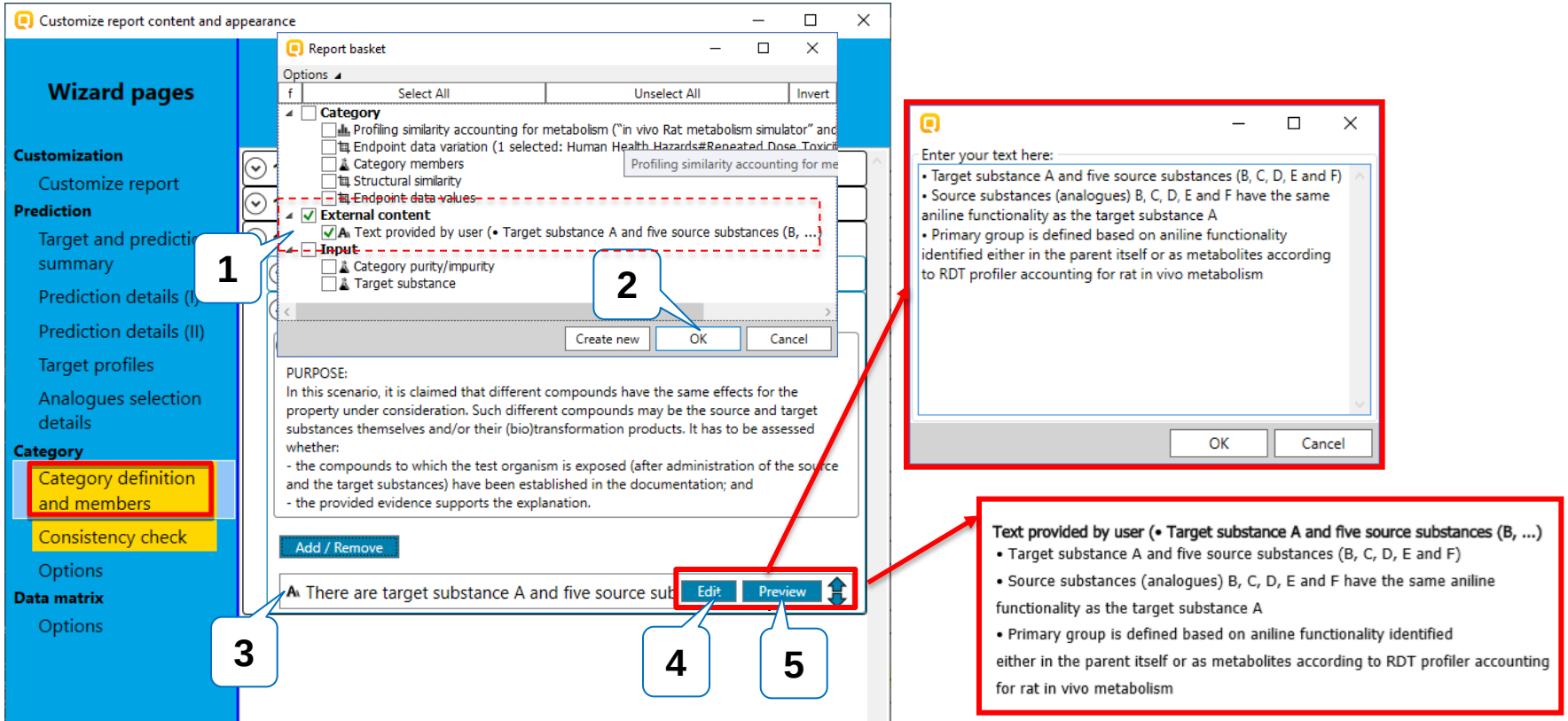
Report Generation according to RAAF-Scenario 4



In order to add text information to the report: expand the AE (1), click **Add/Remove** button (2), click **Create new** (3) in Report basket window, click **Text provided by user** (4), write in or paste the text in the empty field (5), click **OK** (6).

Reporting

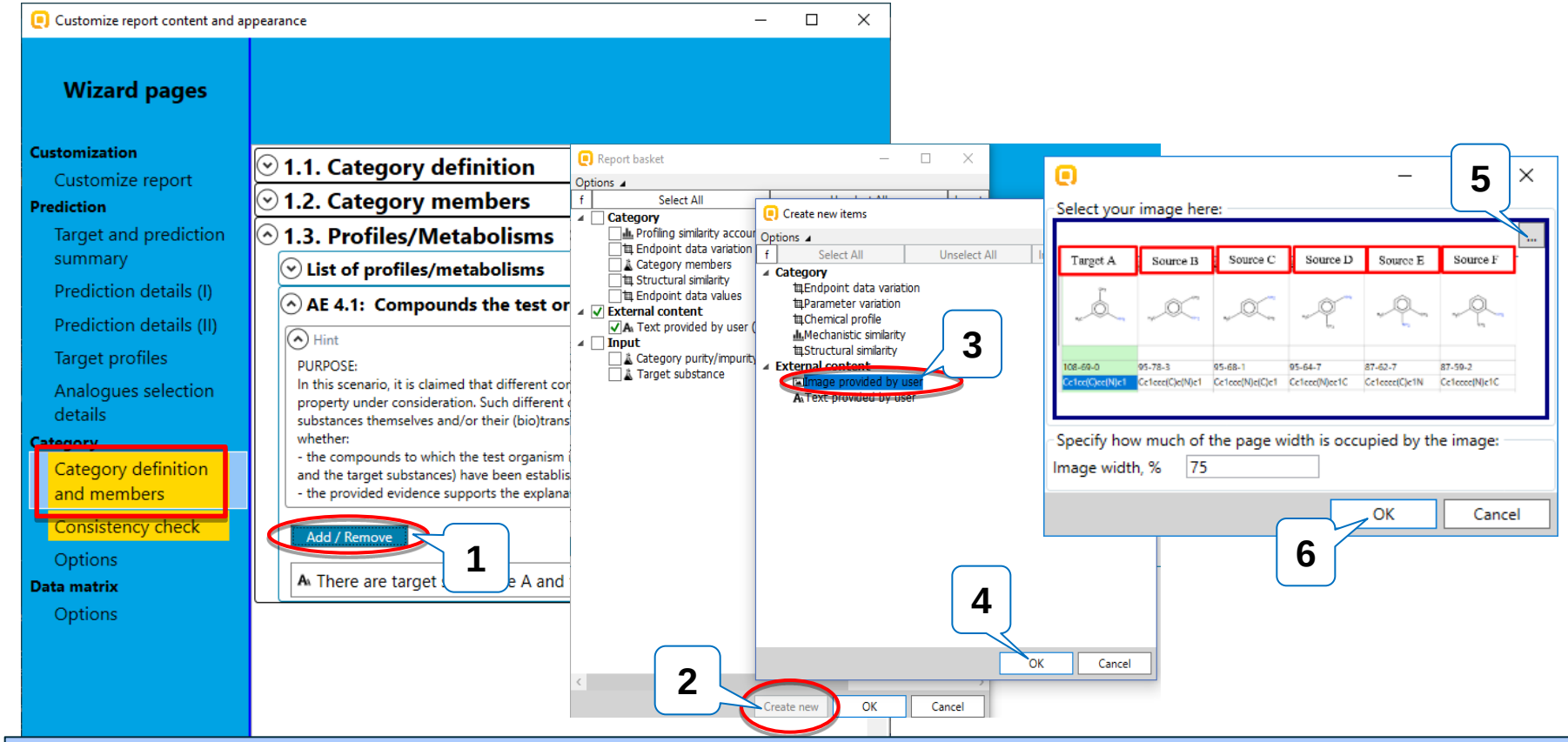
Report Generation according to RAAF-Scenario 4



The entered text is listed in the **Report basket** under *External content* section and the check box is ticked (1). Click **OK** (2). The new item is added under the corresponding AE (3). There are two options for each of the report items - **edit** (4) (if you want to change the content) and **preview** (5) (if you want to see the information provided by this item).

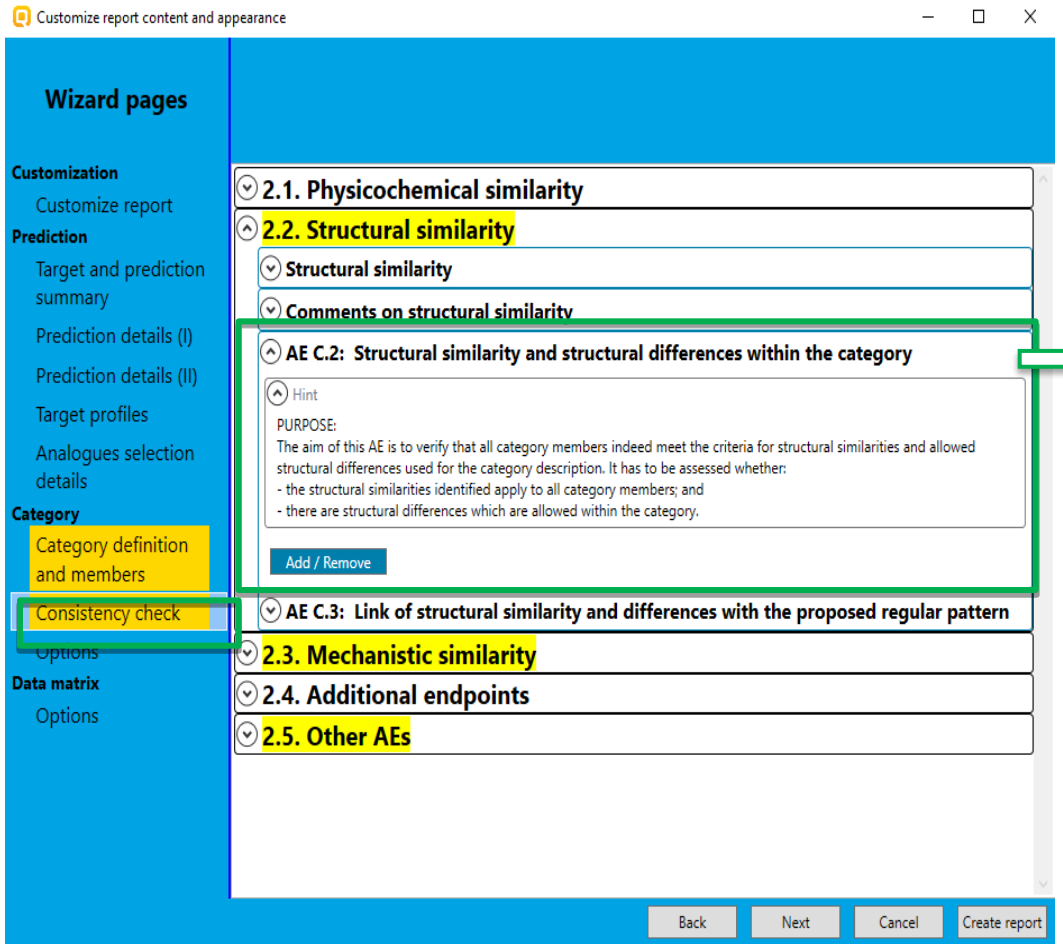
Reporting

Report Generation according to RAAF-Scenario 4



In order to also add an image: click **Add/Remove** button again (1), *create a new* item (2) and select **Image provided by user** (3) and click **OK** (4). A 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 clicking **OK** (6).

Reporting
Report Generation according to RAAF-Scenario 4

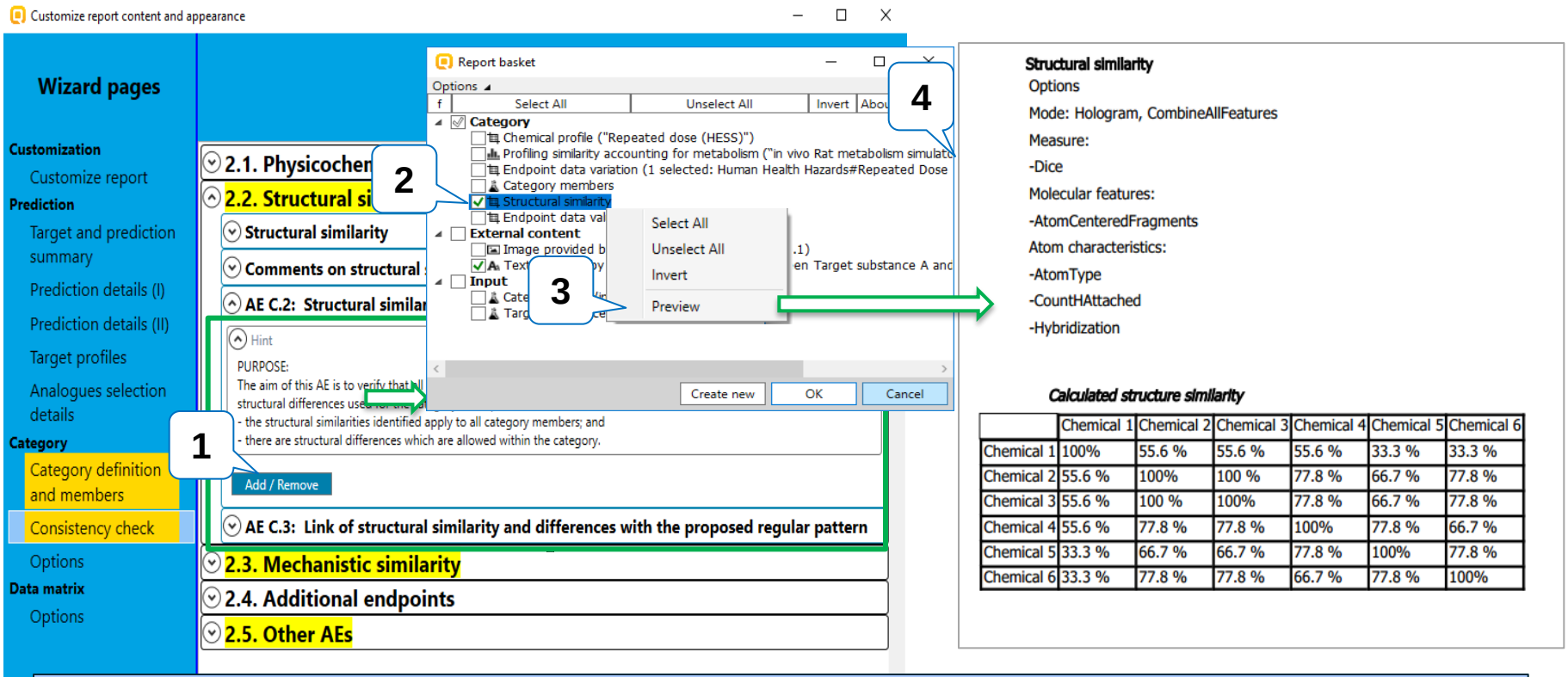


Possible content of AE C.2: Structural similarity and structural differences within the category

- The structural similarity between the Target substance A and the five source substances (B, C, D, E and F) according to Str. similarity profiler is in the range of [33-78%]
- Target A and substances B, F have the same reactivity pattern with respect to the OFG profiler
- The source substances C and D have the same reactivity pattern as source E, with one additional group: precursor quinoid compound

Reporting

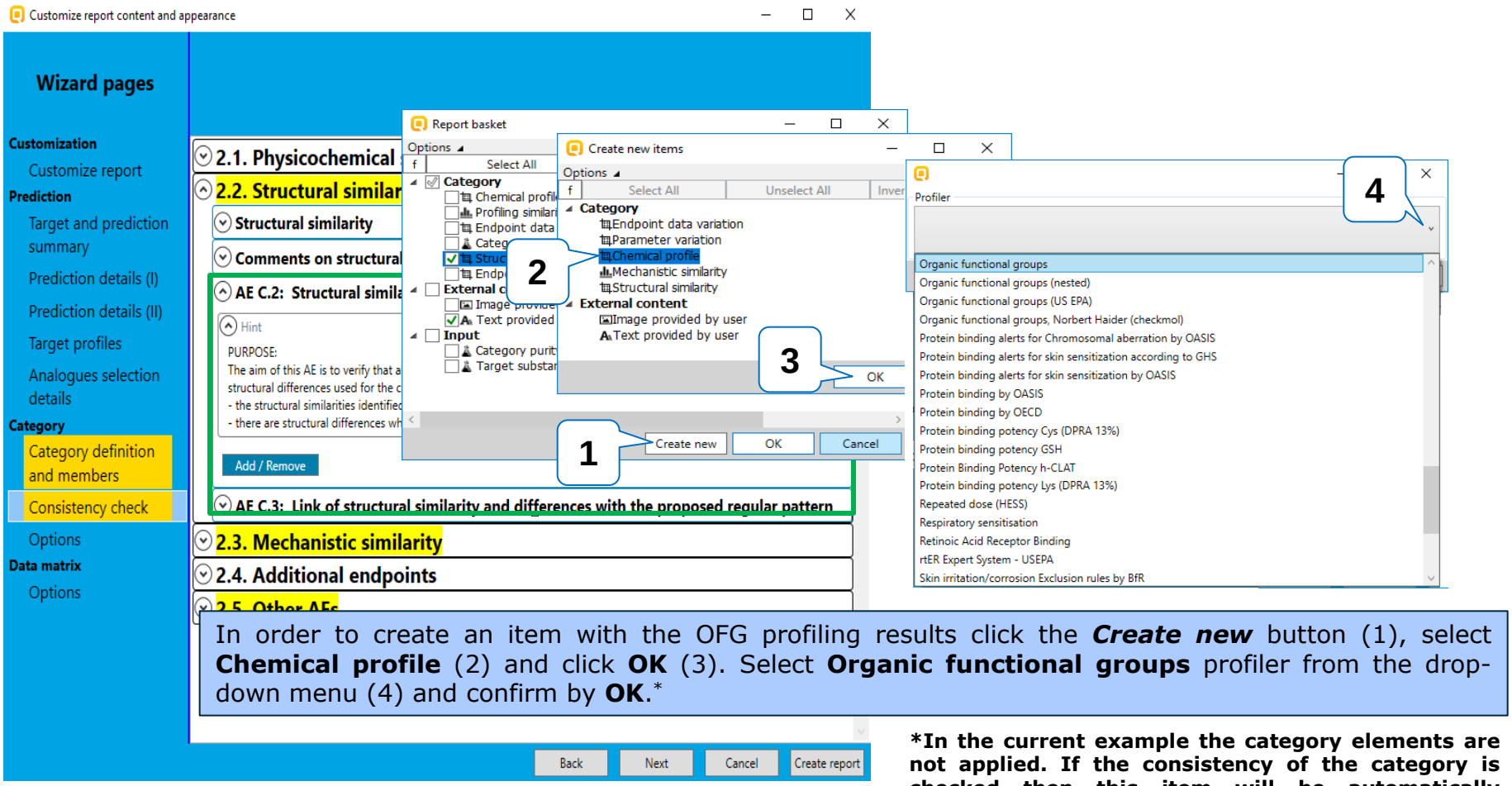
Report Generation according to RAAF-Scenario 4



Two additional items have to be added in order to support the textual information: *A structural similarity* item and an item with the results of the OFG profiler.
Click ***Add/Remove*** button (1) and check the *Structural similarity* item (2) which is stored in the *Report basket*. Right click and preview the item (3). A table providing structural similarity between each of the category members is shown (4).

Reporting

Report Generation according to RAAF-Scenario 4

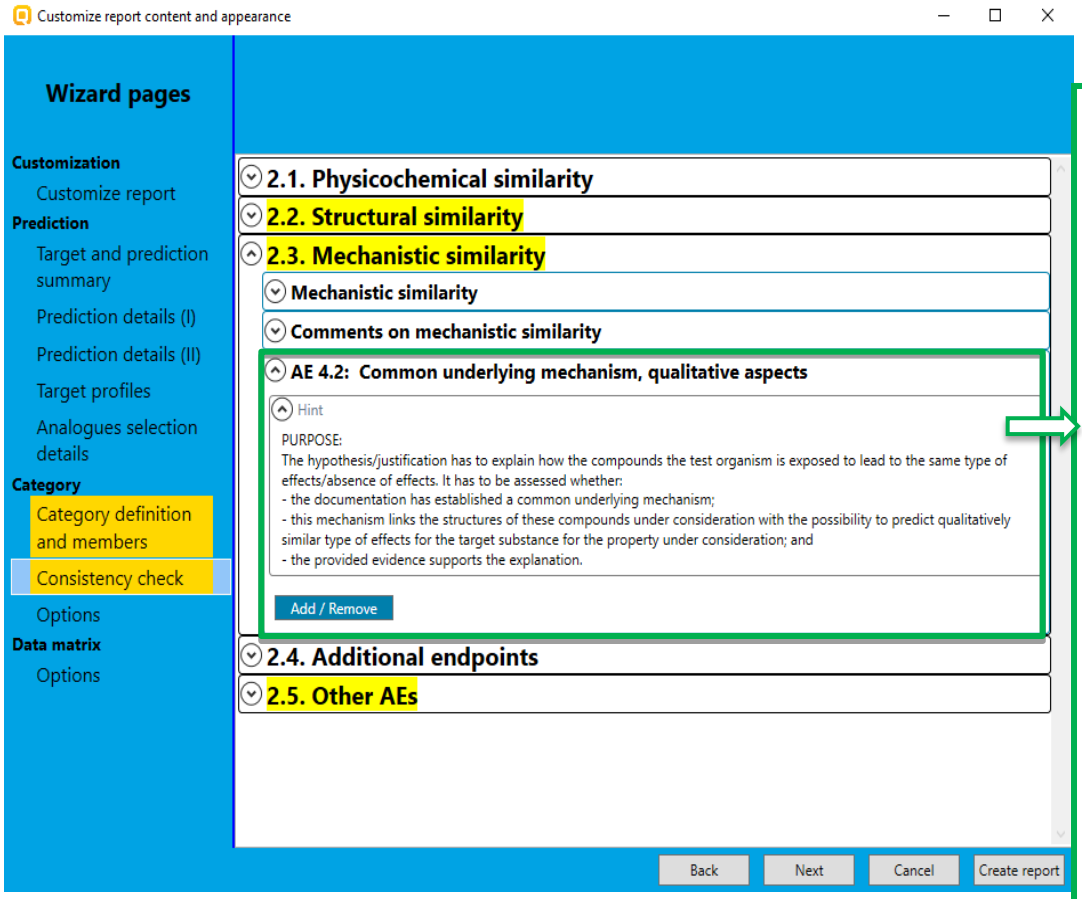


In order to create an item with the OFG profiling results click the ***Create new*** button (1), select **Chemical profile** (2) and click **OK** (3). Select **Organic functional groups** profiler from the drop-down menu (4) and confirm by **OK**.*

***In the current example the category elements are not applied. If the consistency of the category is checked then this item will be automatically generated by the system.**

Reporting

Report Generation according to RAAF-Scenario 4



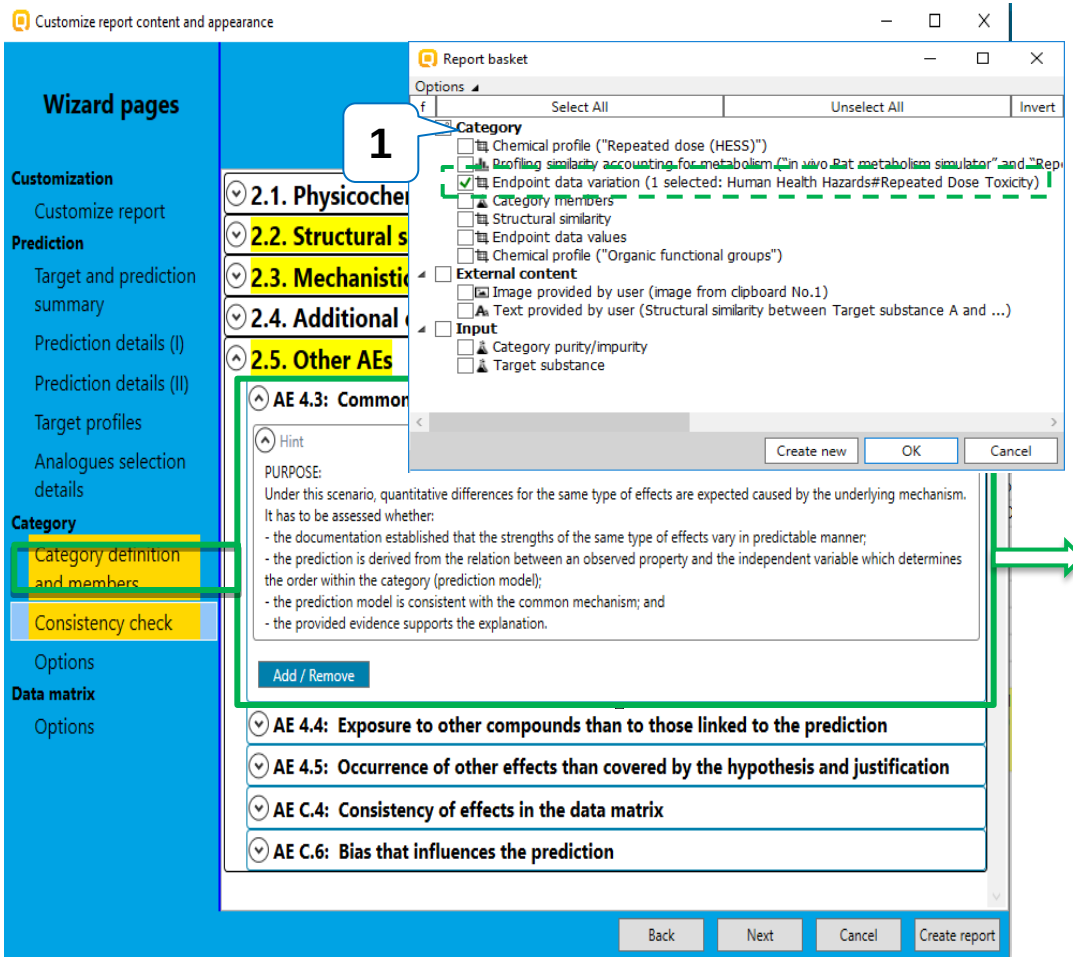
Possible content of AE 4.2. A common underlying mechanism, qualitative aspects

- The Target substance A and the source substances B, C, D, E and F all react via a common underling mechanism according to the RDT profiler
- They all have anilines functionality either as a parent or after a metabolic activation
- The similarity with respect to the metabolic pattern could be seen in **AE 4.5**. above.

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

Reporting

Report Generation according to RAAF-Scenario 4



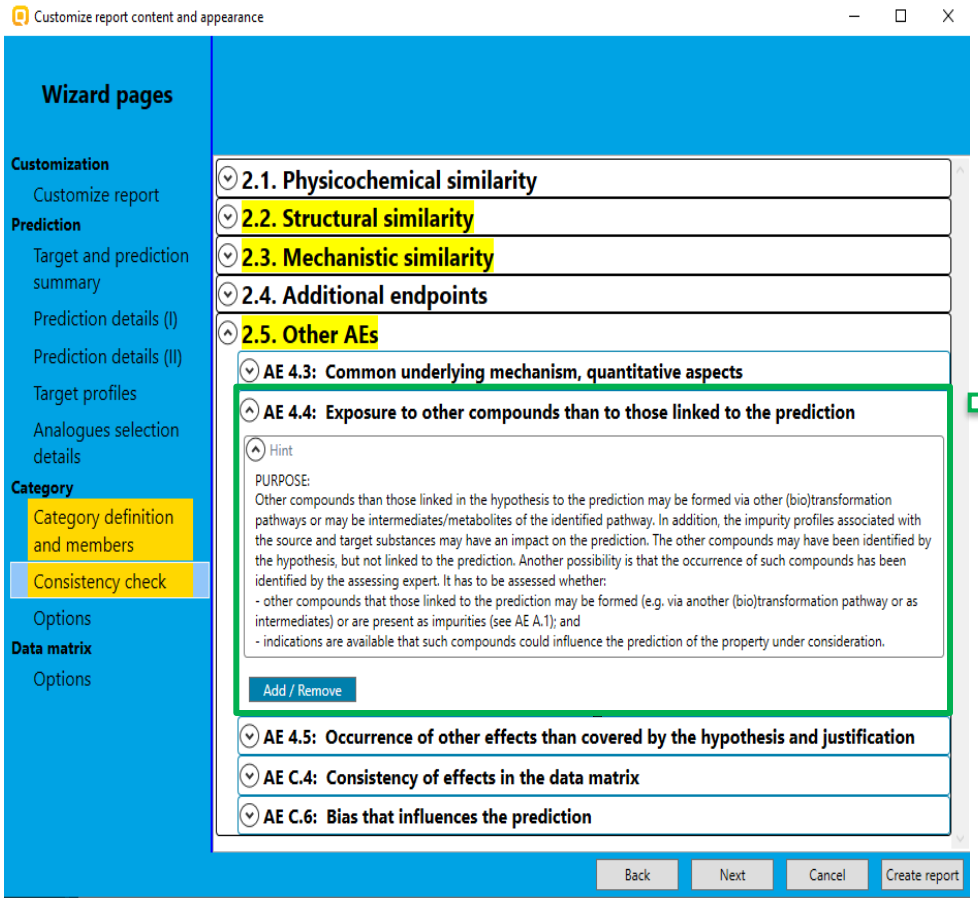
Possible content of AE 4.3. A common underlying mechanism, quantitative aspects

- The target substance A and the five source substances have a common reactivity pattern.
- They all formed aniline functionality either as parents or as metabolites responsible for the toxicity effects
- Similar toxic effects observed in sources substances support the prediction for the target
- Toxic effects of all source substances and target are supported by the identified additional RDT data (references could be included).
- The range of variation of the LOEL experimental data for all category members is shown below:

After the last bullet include the Endpoint data variation item stored in the report basket (1).

Reporting

Report Generation according to RAAF-Scenario 4

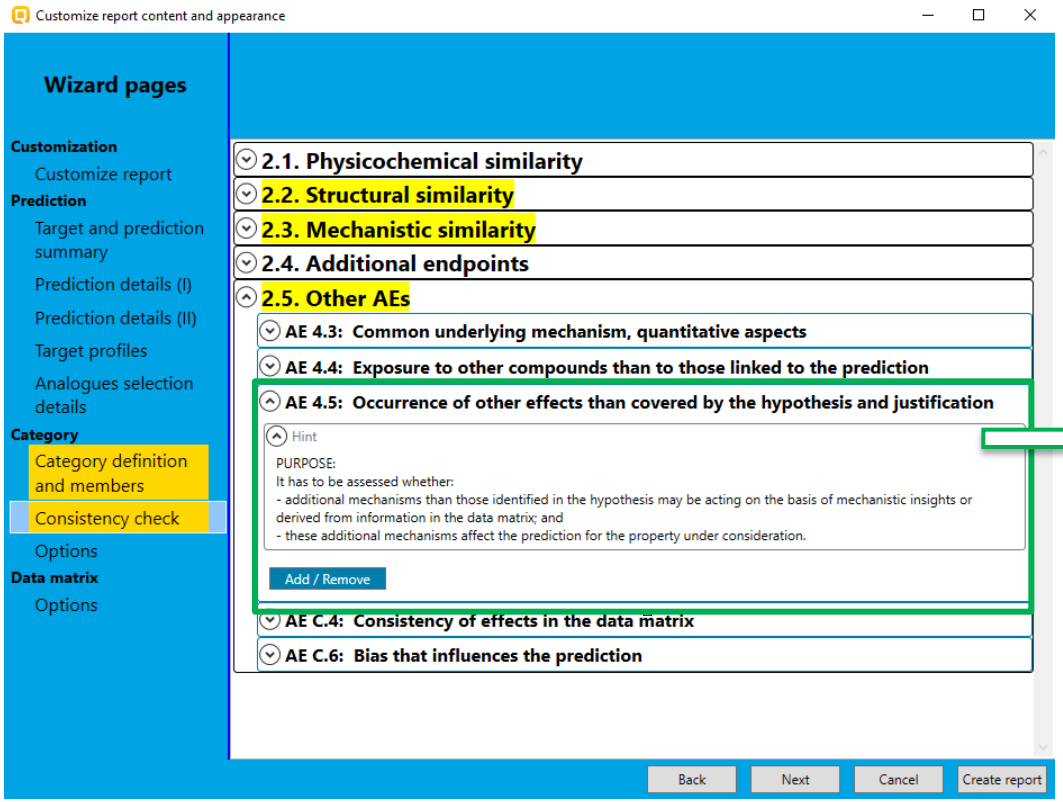


Possible content of AE 4.4: Exposure to other compounds than to those linked to the prediction

- Target substance A and source substances B, C, D, E and F all have common reactivity pattern. They all formed anilines responsible for the toxicity effects (refer to Appendix Metabolites/Profiling):

- The metabolism in vivo shows formation of other reactive groups (reference to Appendix Metabolites/Profiling) such as:
  - ✓ p-Aminophenols (Rank B)
  - ✓ o/p-Aminophenols (Rank B)
  - ✓ Acetaminophen (Alert)
- Being less reliable (Rank B, C or Alert only) these categories gave enough prove that they do not have significant impact over the influence of the toxic effect
- Our assumption is that Anilines is the functionality responsible for the toxic effect.

Reporting
Report Generation according to RAAF-Scenario 4



Possible content of AE 4.5: Occurrence of other effects than covered by the hypothesis and justification

- The target substance A and the source substances B,C, D, E and F have a common reactivity pattern based on Anilines functionality
- Additional alerts for repeated dose toxicity have been identified in the parents and their metabolites. The additional mechanisms are with Rank B and Rank C.
- Rank A is assigned only to the used *Anilines (Hemolytic anemia with methemoglobinemia)* category. The categories with *Rank A* are supported with training sets chemicals having reliable experimental data.
- It is assumed that the additional mechanism will not affect the prediction for the property under consideration.

Reporting
Report Generation according to RAAF-Scenario 4

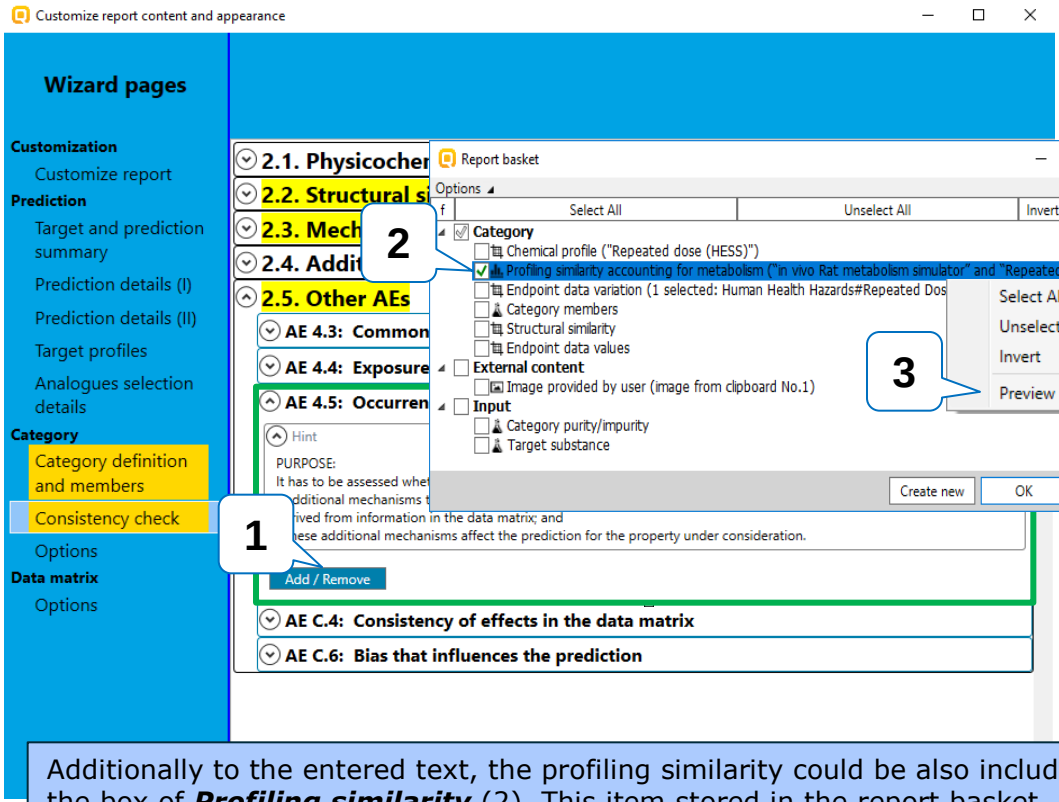
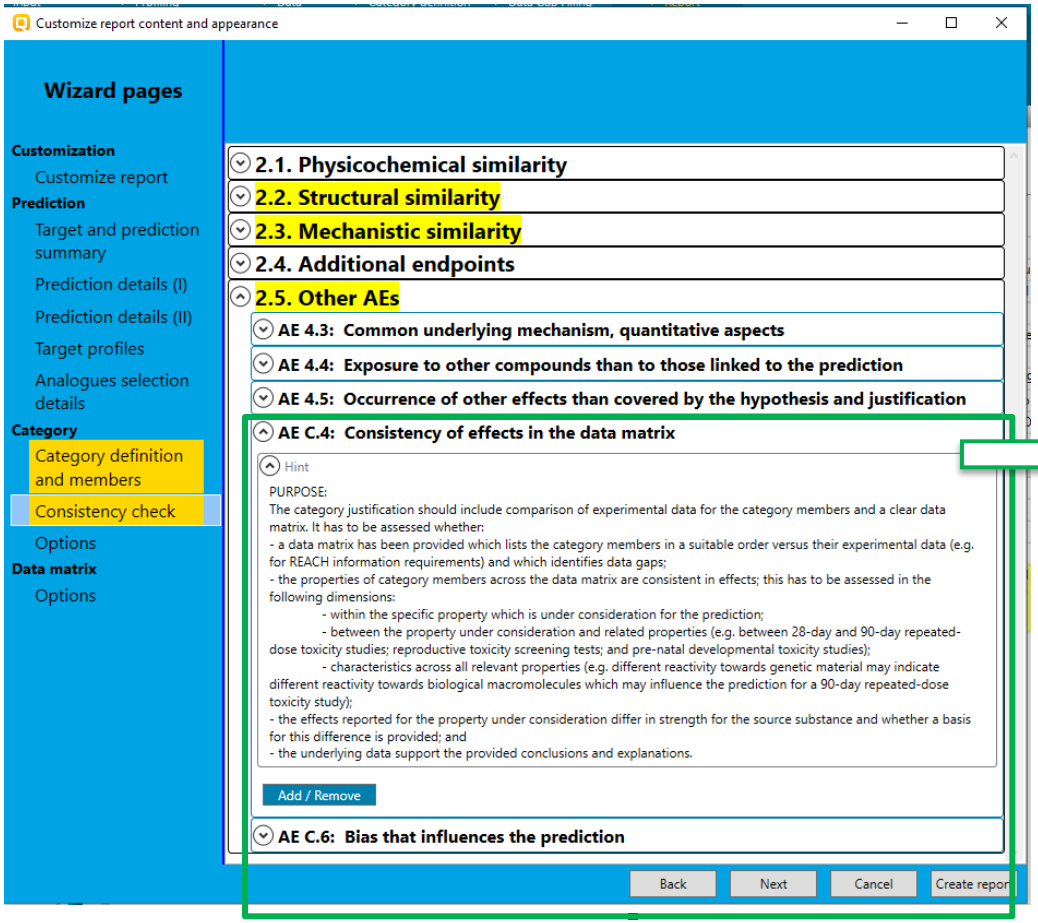


Table summarizing number of metabolites including parent with specific alerts

Repeated dose (HESS)	P1 108-69-0	P2 95-78-3	P3 95-68-1	P4 95-64-7	P5 87-62-7	P6 87-59-2
Anilines (Hemolytic anemia with methemoglobinemia) Rank A	2	3	3	3	2	3
Anilines (Hepatotoxicity) Rank C	2	3	3	3	2	3
Bromfenac (Hepatotoxicity) Alert	0	0	0	0	1	0
Mefenamic Acid (Hepatotoxicity) Alert	0	0	0	3	1	6
Menadione (Hepatotoxicity) Alert	0	0	0	0	1	1
Not categorized	4	6	6	5	3	2
o-/ p-Aminophenols (Hemolytic anemia with methemoglobinemia) Rank B	2	2	1	2	1	2
p-Aminophenols (Renal toxicity) Rank B	1	1	0	0	1	1
Toluene (Renal toxicity) Alert	0	1	1	0	1	1

Additionally to the entered text, the profiling similarity could be also included. To do this click **Add/Remove** button and check the box of ***Profiling similarity*** (2). This item stored in the report basket, is triggered by the used simulators and the profiling scheme for the primary grouping. Right-click and preview the item (3). Tables with generated metabolites for each parent along with the profiling result will be provided. A table summarizing all profiling results for each of the packages "parent and metabolites" is provided at the end (4) .

Reporting
Report Generation according to RAAF-Scenario 4

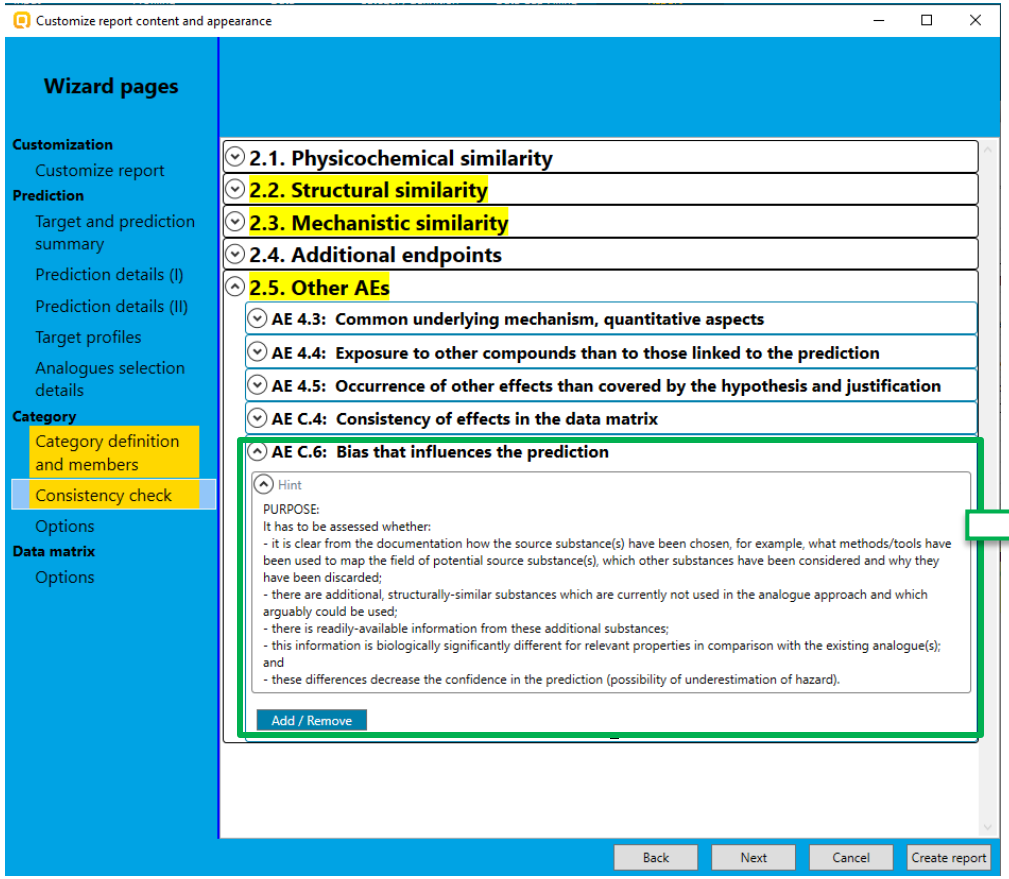


Possible content of AE C.4: Consistency of effects in the data matrix

- The target substance A and the five source substances (B, C, D, E and F) show indication for a repeated dose effect especially for reduce red blood cell. The Total LOEL read-across prediction in this case is around 30 mg/kg bdw/day which classify the target chemical in the range of Category 2 according to GHS classification
- The latter is supported by the experimental data found for all of used source substances for the investigated endpoint and other similar properties

Here should be provided the data matrix snapshot or reference to the *Data matrix report*.

Reporting
Report Generation according to RAAF-Scenario 4



Possible content of AE C.6: Bias that influences the prediction

- The used source chemicals have been found based on a common underlying mechanism for repeated dose toxicity accounting for *in vivo* Rat metabolism;
- The most reliable category was selected (with Rank A);
- The primary group was refined by applying of the following subcategorizations: 1) US-EPA New Chemical Categories, 2) Chemical elements; 3) OECD HPV Chemical Categories.

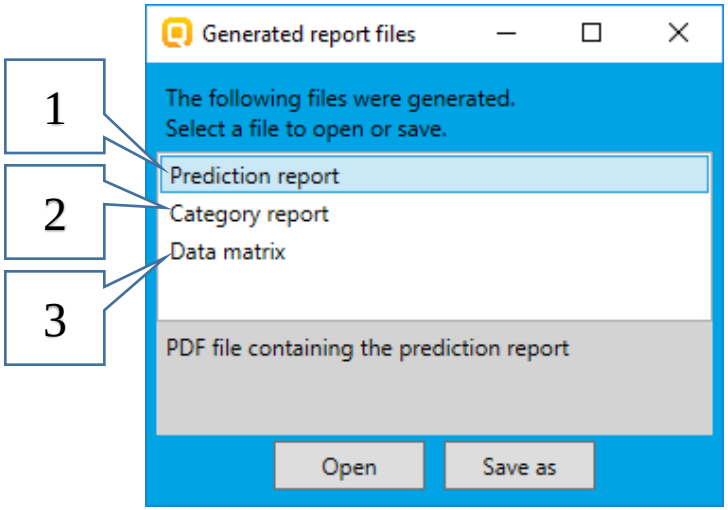
A chemical expert can provide additional literature search of similar analogues with similar effects

Report
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 first two files.
All generated files should be provided when submitting a prediction.



Report
Generated report files

Prediction of LOEL for 3,5-xylidine 1 / 6

Prediction report

QSAR Toolbox prediction for single chemical

(In accordance with RAAF scenario 4)

The selected RAAF scenario is specified in the first page

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

Target information

Structural information	Numerical identifiers	Chemical names
SMILES: Cc1cc(C)cc(N)c1 Structure	CAS#: 108-69-0 Other: EC Number:2036070	"3,5-xylidine" 3,5-dimethyl-aniline 3,5-Dimethylaniline

Chemicals category 1 / 32

Category report

QSAR Toolbox report for category

(In accordance with RAAF scenario 4)

1. Category definition

1.1. Category definition *manually editable field*
Not provided by the user

Ranges for selected physicochemical properties and calculated parameters
Not provided by user

1.2. Covered (target) endpoint(s) *manually editable field*
- Human Health Hazards/Repeated Dose Toxicity: LOEL, Total, Whole body, Oral (gavage), Rat

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

1.4. Profiles/Metabolisms *manually editable field*

ping/subcategorization:
"in vivo Rat metabolism simulator" Combined parent and products requirements: Anilines
c anemia with methemoglobinemia) Rank A (Repeated dose (HESS)) (primary grouping)
New Chemical Categories (subcategorization)
l elements (subcategorization)
PV Chemical Categories (subcategorization)

embers *manually editable field*
nce charactertzation

nbers

	Name	SMILES	Structure
	3,5-xylidine	Cc1cc(C)cc(N)c1	

QSAR TOOLBOX TPRF v4.2

Data matrix report

	Neighbour #2	Neighbour #3	Neighbour #4	Neighbour #5
Substance identity				
Structure				
CAS number	95-78-3	95-68-1	95-64-7	87-62-7	87-59-2
Chemical name	2,5-Xylidene	2,4-xylidine	3,4-xylidine	2,6-xylidine	118
Other identifier					
SMILES	Cc1ccc(C)c(N)c1	Cc1ccc(N)c(C)c1	Cc1ccc(N)cc1C	Cc1cccc(C)c1N	Cc1cccc(N)c1C
Parameters unit					
Profilers					
Profiles used for grouping/subcategorization					
Using of "in vivo Rat metabolism simulator" Combined parent and products requirements: Anilines (Hemolytic anemia with methemoglobinemia) Rank A (Repeated dose (HESS)) (primary grouping)	Parent and 7 metabolites.; Has all of the required categories: Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Has the following additional	Parent and 10 metabolites.; Has all of the required categories: Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Has the following additional	Parent and 9 metabolites.; Has all of the required categories: Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Has the following additional	Parent and 11 metabolites.; Has all of the required categories: Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Has the following additional	Parent and 6 metabolites.; Has all of the required categories: Anilines (Hemolytic anemia with methemoglobinemia) Rank A;	Parent and 10 metabolites.; Has all of the required categories: Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Has the following additional
US-EPA New Chemical Categories	Anilines (Acute toxicity)	Anilines (Acute toxicity)	Anilines (Acute toxicity)	Anilines (Acute toxicity)	Anilines (Acute toxicity)	Anilines (Acute toxicity)
Chemical elements (subcategorization)	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N
OECD HPV Chemical Categories	Dimethylaniline	Dimethylaniline	Dimethylaniline	Dimethylaniline	Dimethylaniline	Dimethylaniline
Toxicological						
Repeated dose (HESS)	Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Anilines (Hepatotoxicity) Rank C	Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Anilines (Hepatotoxicity) Rank C; Toluene (Renal toxicity) Alert	Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Anilines (Hepatotoxicity) Rank C; Toluene (Renal toxicity) Alert	Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Anilines (Hepatotoxicity) Rank C	Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Anilines (Hepatotoxicity) Rank	Anilines (Hemolytic anemia with methemoglobinemia) Rank A; Anilines (Hepatotoxicity) Rank C; Mefenamic Acid (Hepatotoxicity)
Measured and predicted data						
Data used for prediction						
environment / endpoint	value unit species, duration, test type, type of method, assay, strain, test guideline	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,	value unit species, duration, test type, type of method,	value unit species, duration, test type, type of method, assay,
Repeated Dose Toxicity LOEL	60 mg/kg bdwt/d Rat 28 d	10 mg/kg bdwt/d Rat 28 d	50 mg/kg bdwt/d Rat 28 d	160 mg/kg bdwt/d Rat 91 d	60 mg/kg bdwt/d Rat 28 d
Repeated Dose Toxicity LOEL	12 mg/kg bdwt/d Rat 28 d	2 mg/kg bdwt/d Rat 28 d	50 mg/kg bdwt/d Rat 28 d	50 mg/kg bdwt/d Rat 42 d	12 mg/kg bdwt/d Rat 28 d

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 4.
- Note, proficiency comes with practice!