QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.2

An example illustrating RAAF Scenario 5 and related assessment elements

Outlook

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

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and 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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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Define target endpoint;
- Relevancy of profiles and data availability;
- 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 5;
- To explain to the user how to search for analogues producing common metabolite;
- To introduce to the user the read across assessment elements (AE) and to provide examples with possible content of them;
- To introduce to the user the report basket;
- To provide to the Toolbox user the rationale behind each step of the exercise.

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

Read Across Assessment Framework (RAAF)
Overview

- RAAF 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 particular aspects (assessment elements, AEs).
- Total six scenarios are available: two for analogue approach and four for category approach

Read Across Assessment Framework (RAAF)

Criteria for the different RAAF scenarios

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 RAAF scenario

1. Distinguish whether analogue or category approach is decided based on number (N) of analogues*:
   a) N of analogues ≤ 3 is Analogue approach (scenario 1-2)
   b) N of analogues > 3 is Category approach (scenario 3-6)
2. To identify the basis of the read across hypothesis
   a) (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
   b) 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**.
3. For a category approach (scenario 3-6) there is a need to take further account whether or not quantitative variations in the properties are observed among the category members:
   a) There is quantitative variation in the (eco) toxicity when it is more than 1 log units** (scenario 3 and 4)
   b) 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 number of analogues which distinguishes analogue from category approach is proposed by LMC**
****The quantitative variation in the (eco)toxicity of 1 log unit is proposed by LMC due to empirically observations.**

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

Outlook

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

The Exercise

- In this exercise we will predict *Repeated dose toxicity* of Eugenol [CAS# 97-53-0], which will be the "target" chemical;
- The target endpoint will be preliminary defined;
- The category will be defined based on analogues having common metabolite produced after *in vivo* Rat liver metabolism;
- A read-across approach will be used for the prediction. The prediction will be based on category approach relying on 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.

Outlook

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

Workflow

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

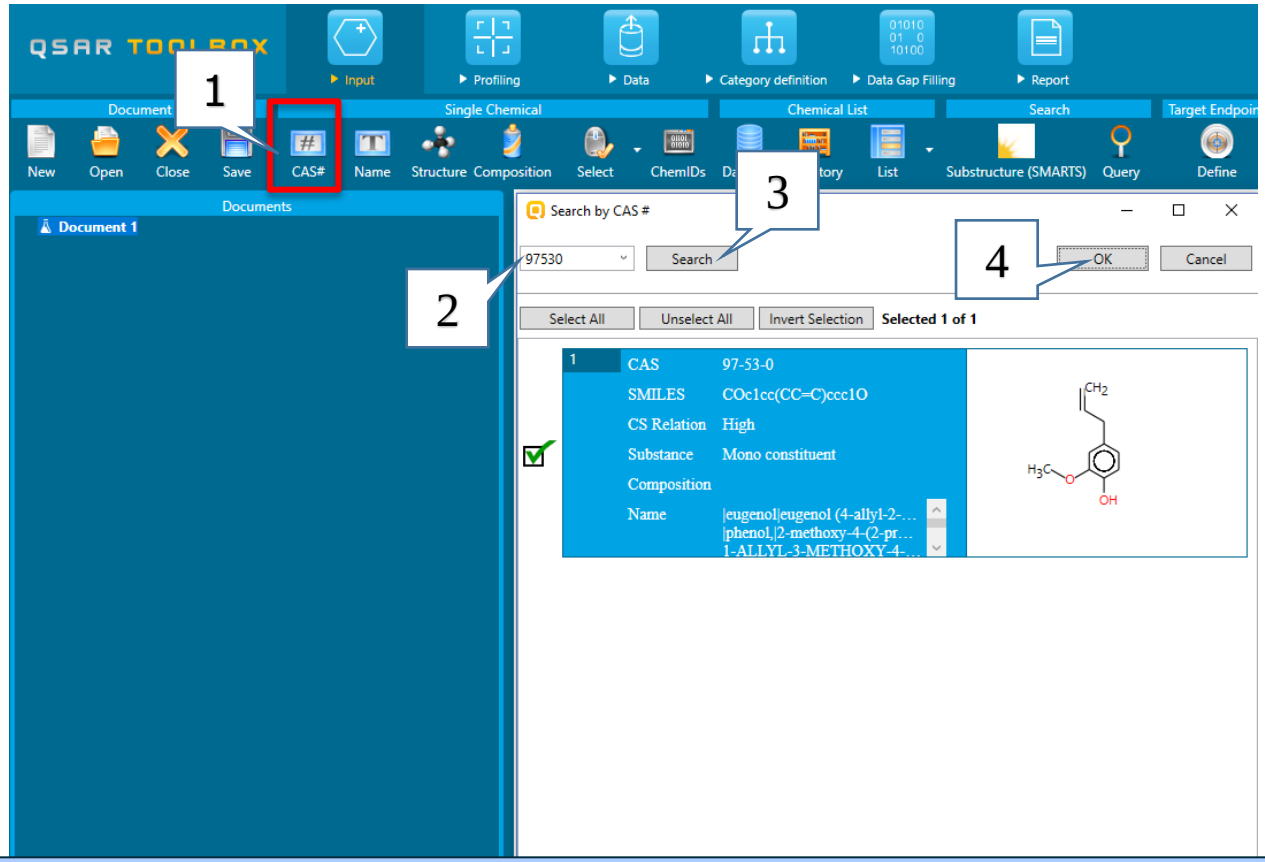
The modules will be presented in different sequence than the showed above.

Input
Overview

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

Input

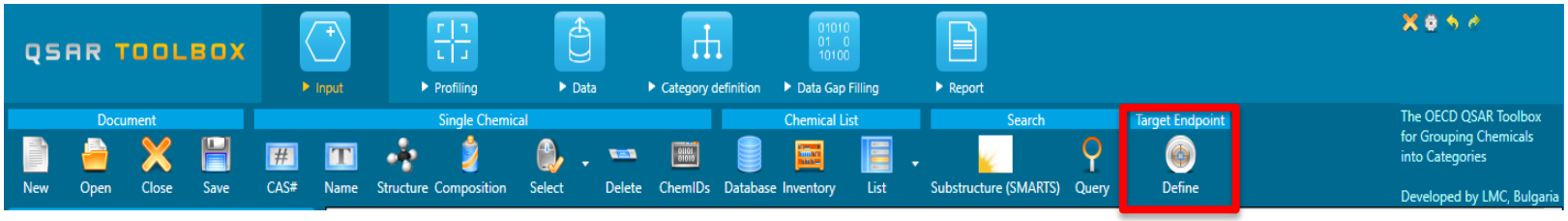
Input target chemical by CAS#



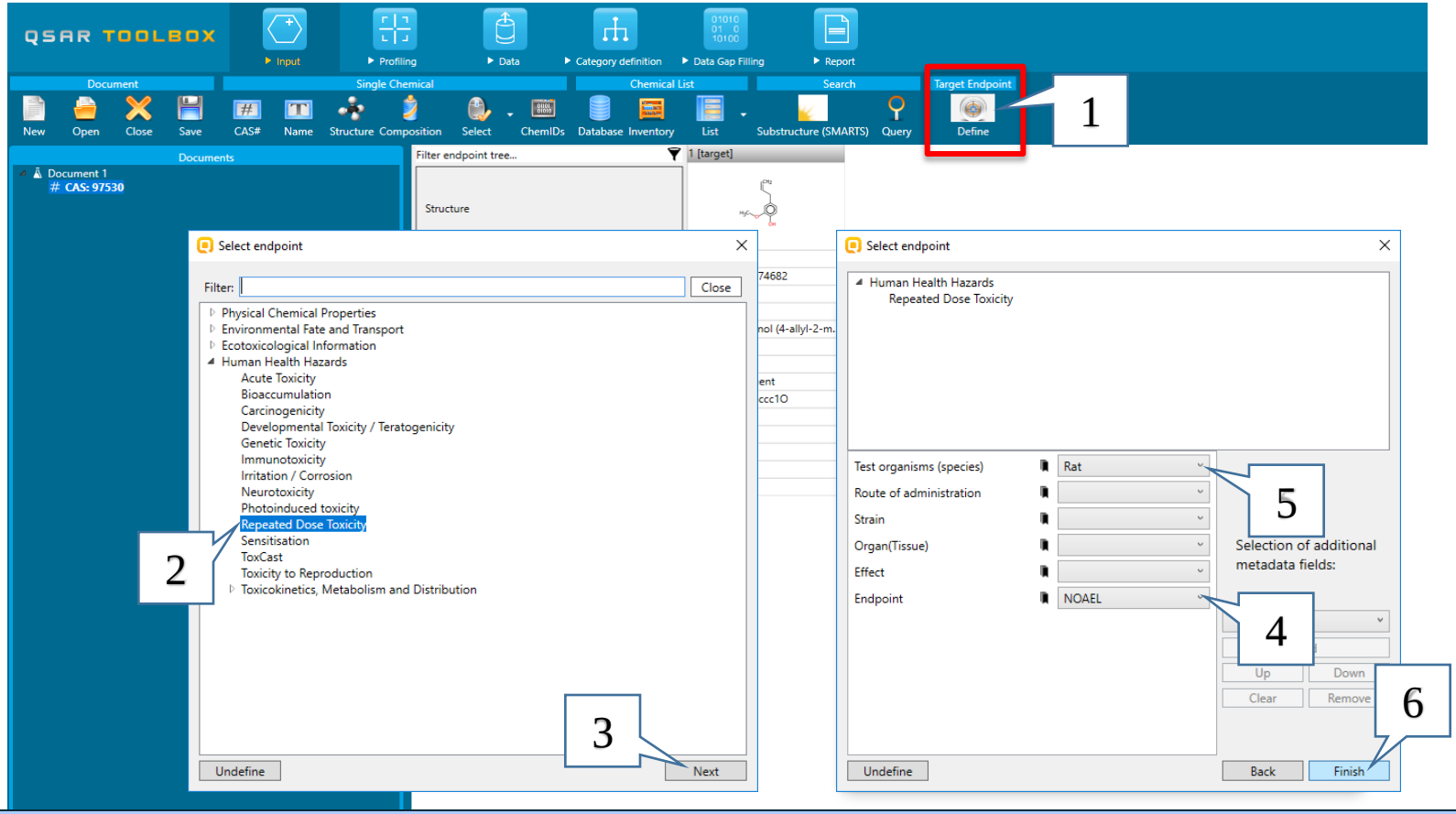
1. Click **CAS#**; 2. Enter the **CAS# 97-53-0** in the blank field; 3. Click ***Search;*** 4. When the structure with the requested CAS # appears, click ***OK.***

Input
Define target endpoint

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



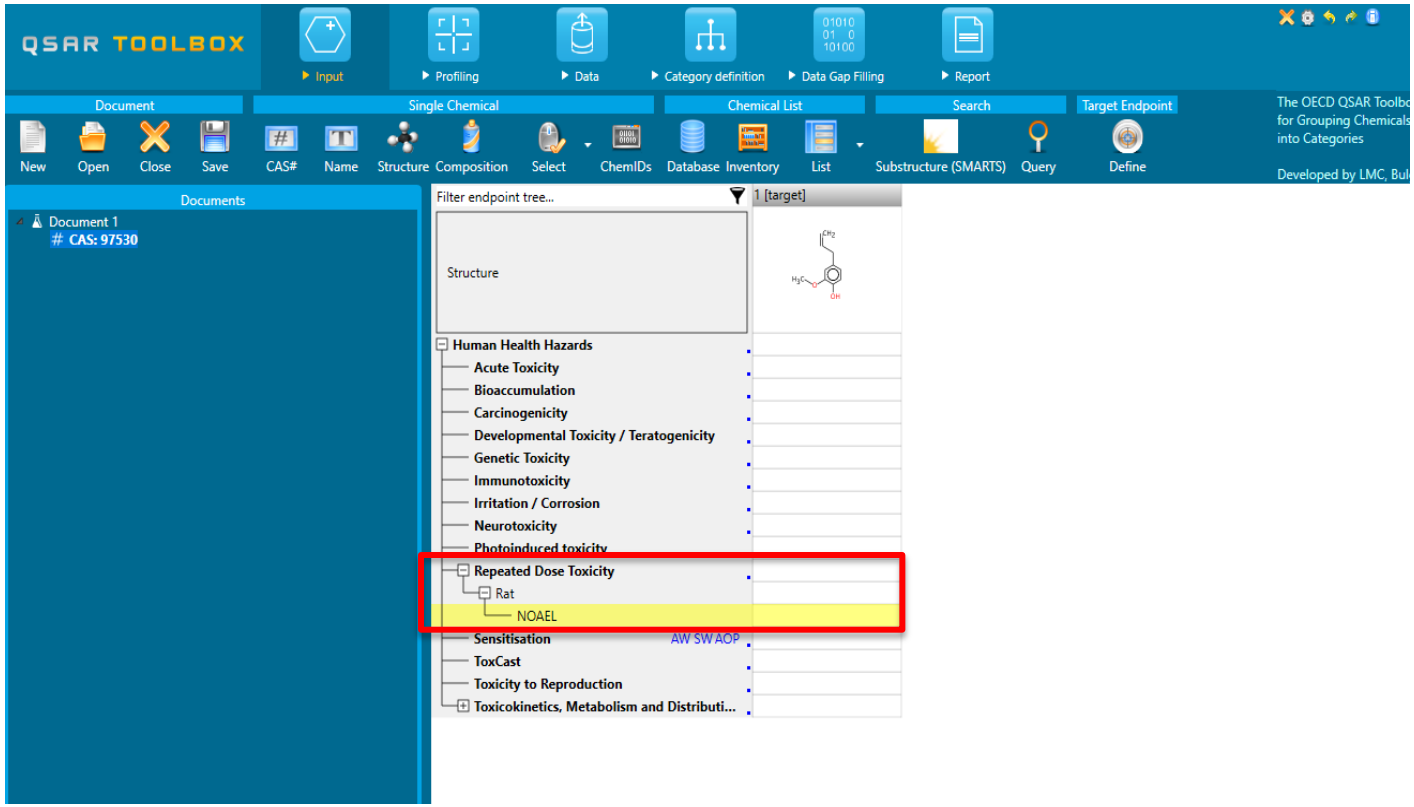
Input
Define target endpoint



When click on **Define** (1) you should select the target endpoint. Select **Repeated Dose Toxicity** in the *Human health hazards level* (2) and click on **Next** (3). Select **NOAEL** endpoint (4) and **Rat** test organism (5) from the drop-down menus. Finally click on **Finish** (6).

Input
Define target endpoint

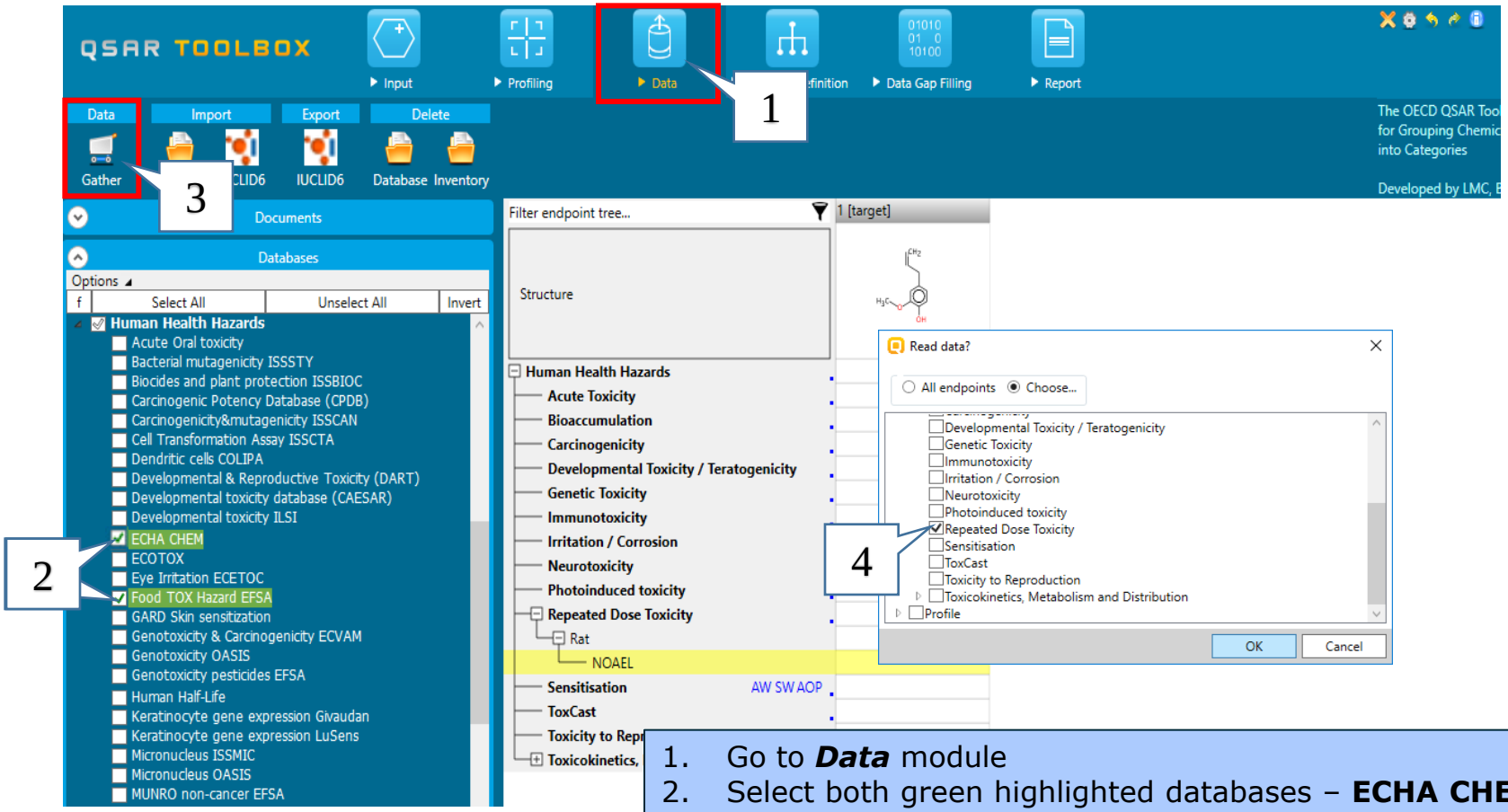
Once the endpoint is defined along with its metadata, they appear in the endpoint tree and the corresponding row of the data matrix is yellow highlighted.



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 limited number of endpoints).

Data
Gather data



1. Go to ***Data*** module
2. Select both green highlighted databases – **ECHA CHEM** and **Food TOX Hazard EFSA**;
3. Click ***Gather***.
4. Choose to collect repeated dose toxicity data, only

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 is performed only among the chemicals which are listed in the selected databases. In this example ECHA CHEM and *Food TOX Hazard EFSA* databases are selected.
- In this example, an insert window appears stating there are 23 experimental data points for the target chemical. Six data points (varying from 67 mg/kg bdwt/d to 1250 mg/kg bdwt/d) are available for the defined target endpoint. We will try to reproduce the worst case scenario (67 mg/kg bdwt/d).
- Go to the *Profiling* module to check for the reason of the possible effect (to check for an alert identified in the target chemical).

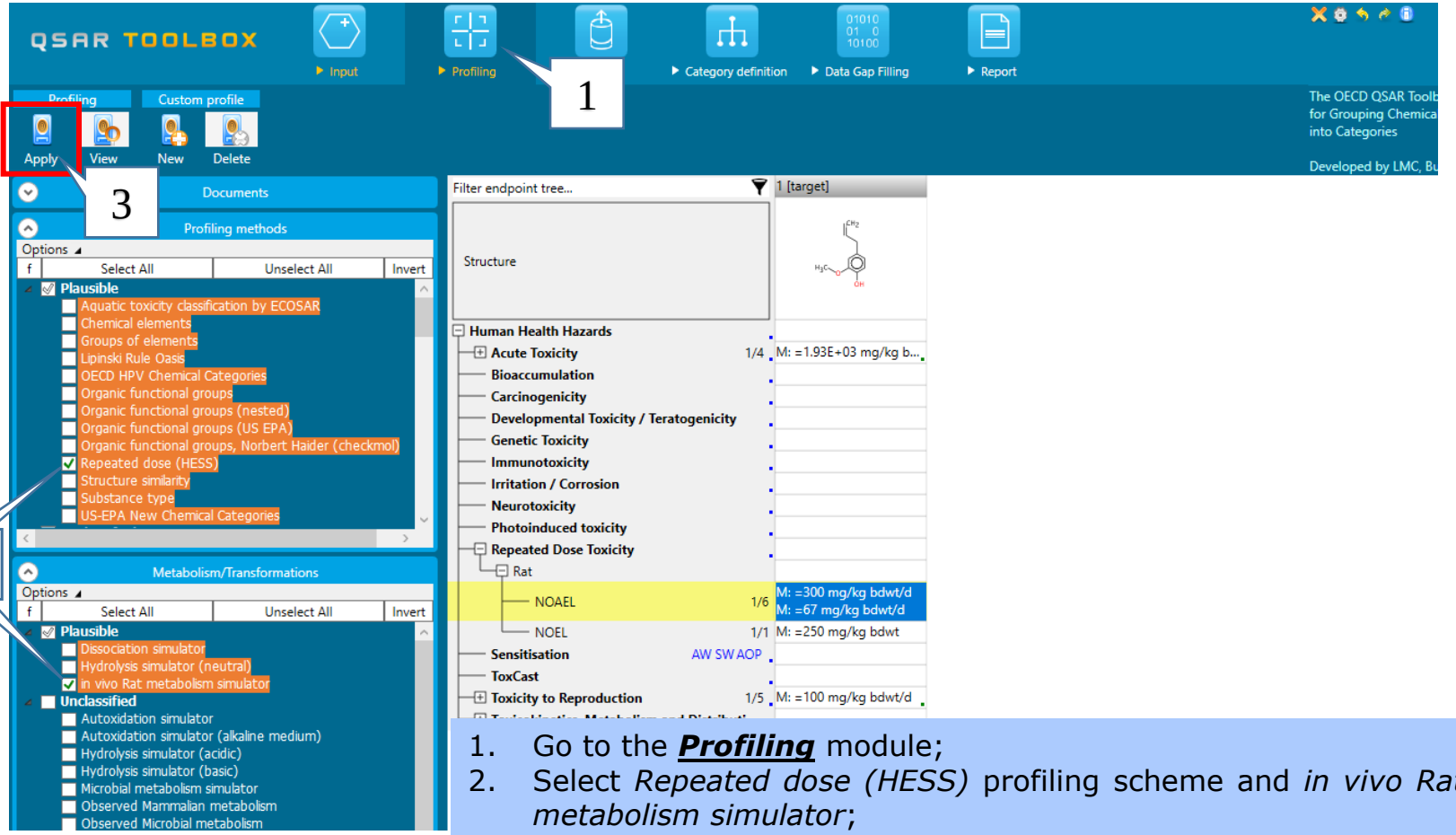
Profiling
Overview

- "Profiling" refers to the electronic process of retrieving relevant information for the target compound, other than its environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox databases.
- Available information includes probable mechanism(s) of action, as well as observed or simulated metabolites.
- Based on the "profilers' relevancy" the most suitable once are getting colour highlighted*
- For the purpose of this example suitable profilers in combination with simulators are used (see next slide)

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

Profiling

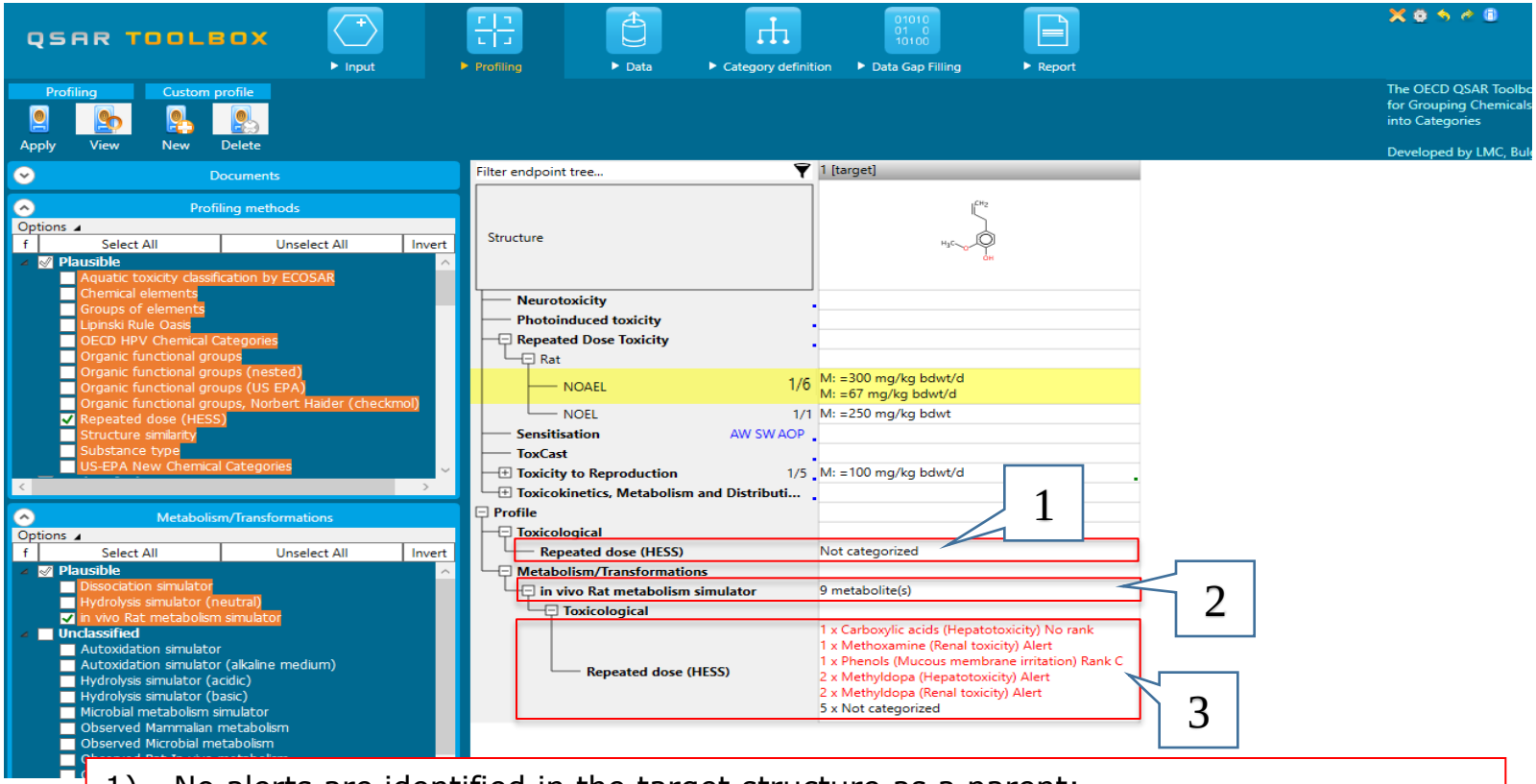
Profiling the target chemical



1. Go to the ***Profiling*** module;
2. Select *Repeated dose (HESS)* profiling scheme and *in vivo Rat metabolism simulator*;
3. Click on **Apply**

Profiling

Profiling results

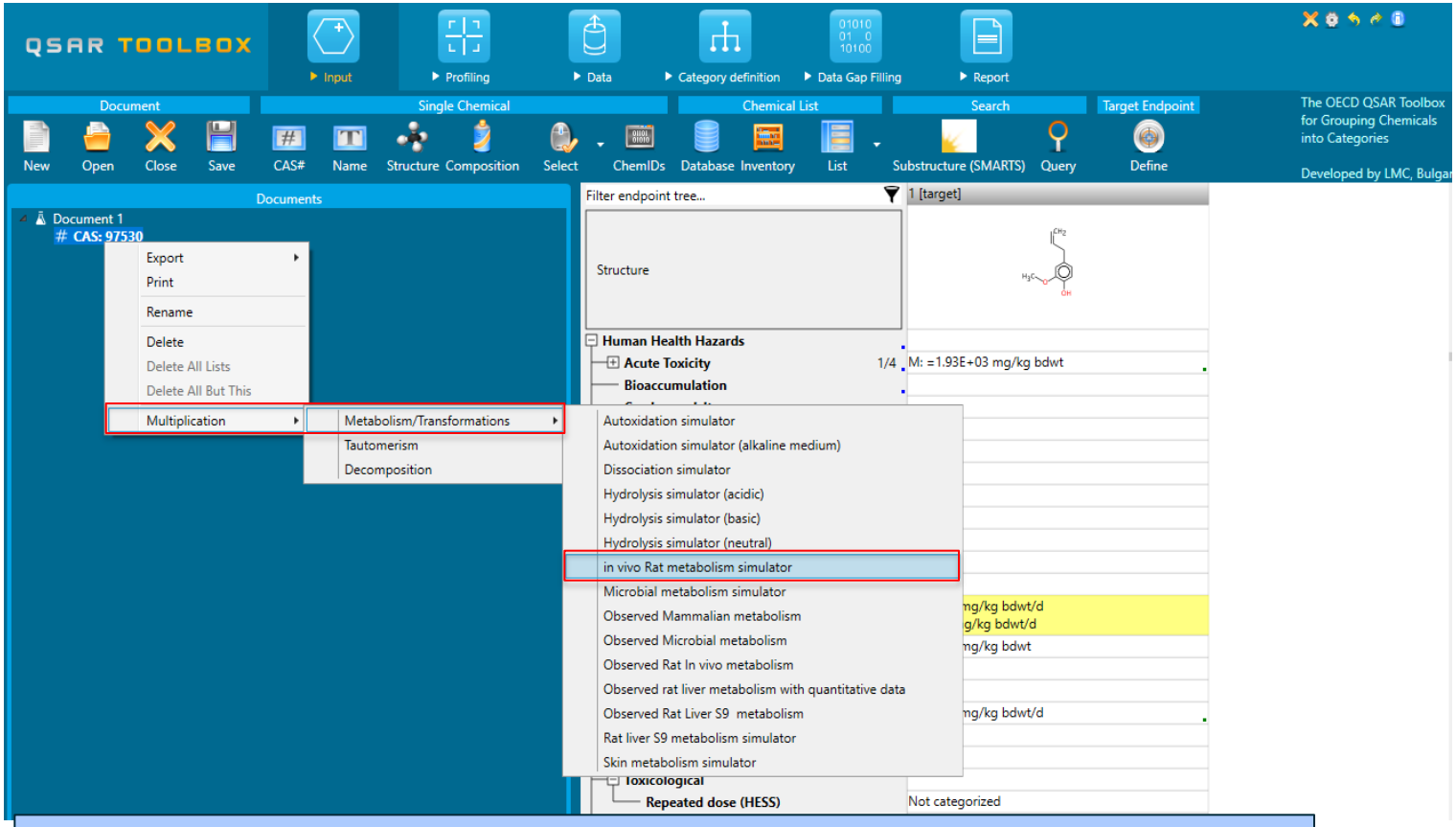


1) No alerts are identified in the target structure as a parent;
2) 9 metabolites are generated as a result of *in vivo Rat metabolism simulator*;
3) Alerts for repeated dose toxicity are identified in four of the generated metabolites.

Recap

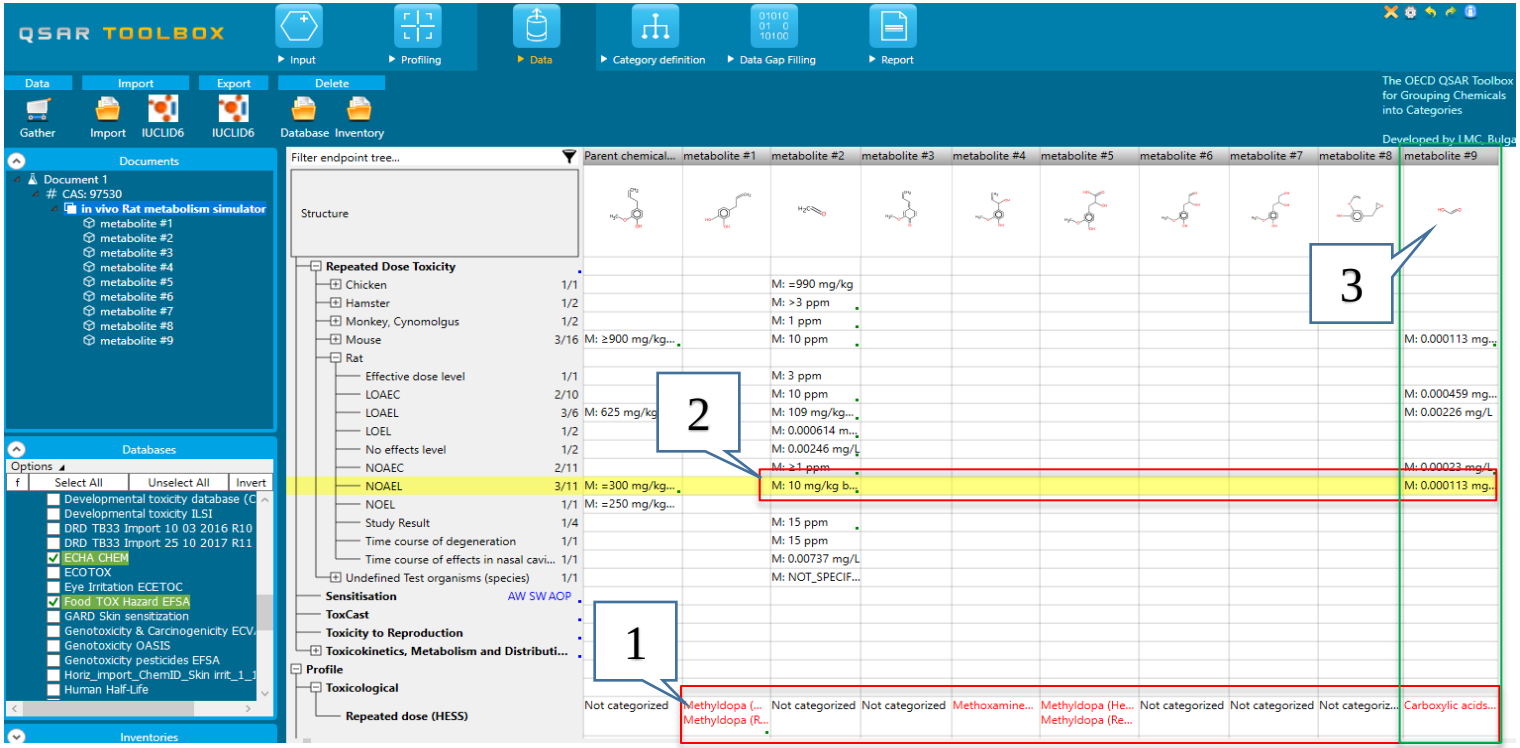
- In module *Input*, you entered the target chemical and defined the target endpoint.
- In the *Data* module, you saw the database corresponding to the defined target endpoint. You also found experimental data for the target available in the selected database.
- In the *Profiling* module, you profiled the target chemical with profiling scheme and metabolic simulator related to the selected target endpoint.
- Alerts for repeated dose toxicity were identified for some of the metabolites produced by simulating of metabolic activation.

Handling of in vivo rat liver metabolism



Step 1: Generate in vivo metabolites upfront gap filling
Right click over the level with CAS# in the document tree and select in vivo Rat metabolism simulator.
9 metabolites are produced. The metabolites appeared next to the parent (see next slide).

Handling of in vivo rat liver metabolism



Step 2: Profile the package: parent and metabolites according to Repeated dose (HESS) profiler only (uncheck the metabolic simulator)
Alerts are identified in four out of nine generated metabolites (1).

Step 3: Gather data for package: parent and metabolites from the selected database (gather only repeated dose toxicity data)
Experimental data for the defined target endpoint is found for two of the metabolites (2).

The metabolite having an alert and available experimental data will be used for searching of analogues (3) (see next slide).

Category Definition
Overview

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

Category Definition
Grouping methods

- The different grouping methods allow the user to group chemicals into chemical categories according to different measures of "similarity" so that within a category data gaps can be filled by read-across.

- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind by the same mechanism and for which experimental results are available.

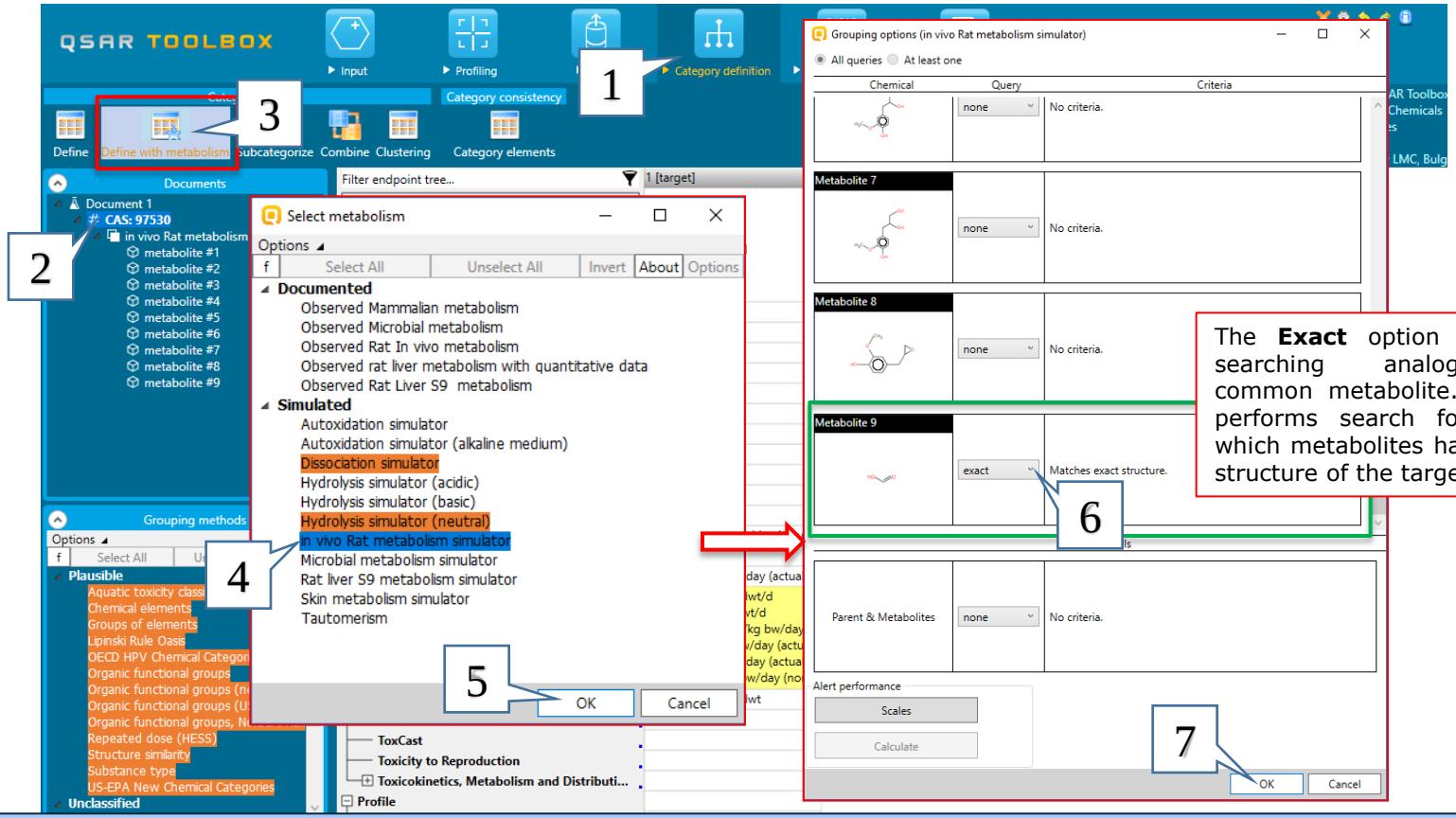
- If no alert is identified in the target structure, but is identified in its metabolites, analogues can be searched accounting for metabolism. In this way the target chemical and the identified analogues will have similar metabolic pattern.

- In our case we will use *Food TOX Hazard EFSA* database only in order to accelerate the work (before going to the Category definition module uncheck ECHA CHEM database). *ECHA CHEM* is not cached in advance and its metabolising will take some time.

- Searching for analogues will be based on a common metabolite (formic acid) generated as a result of *in vivo* Rat metabolism (see next slide)

Category Definition

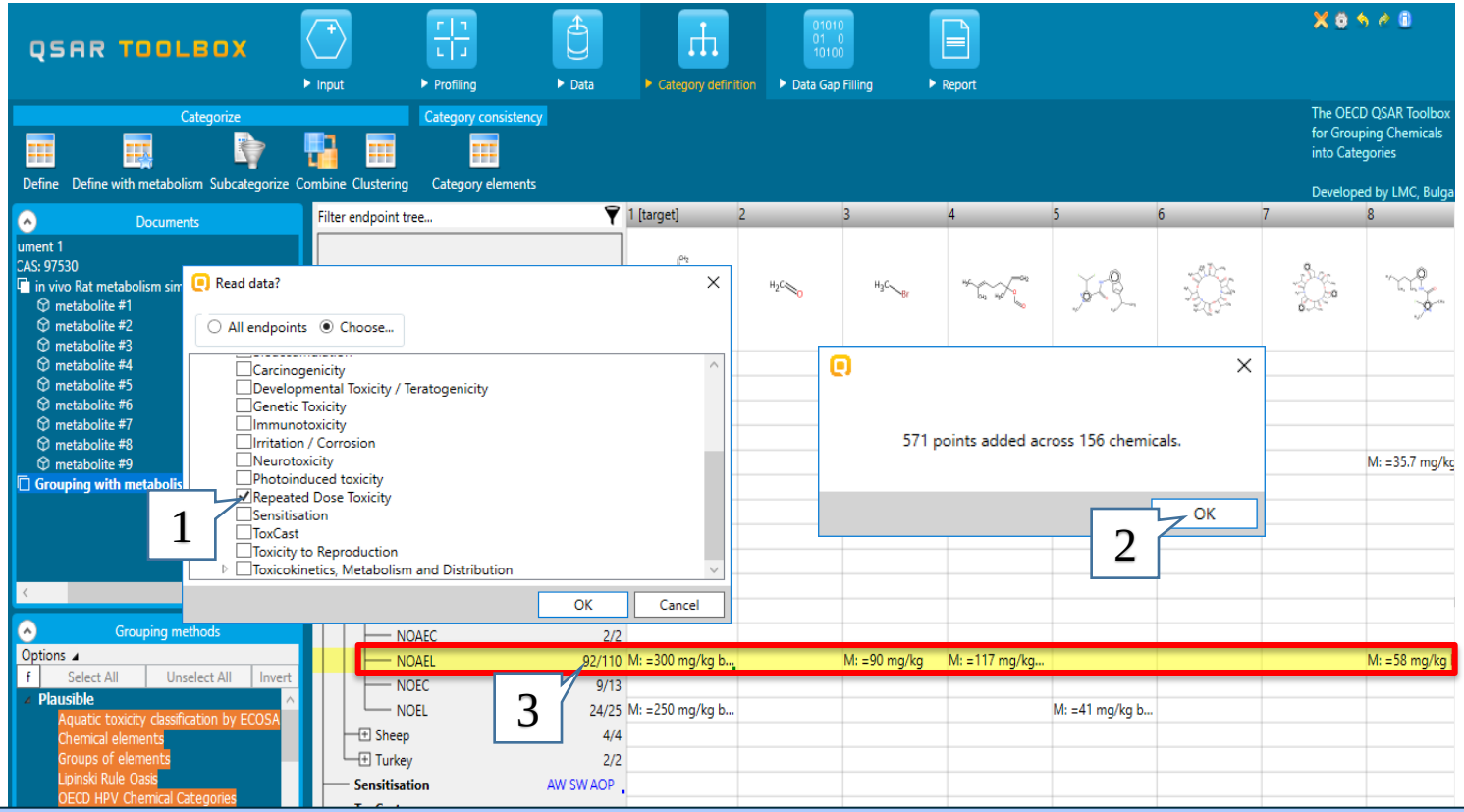
Searching for analogues accounting for in vivo rat liver metabolism



1. Go to ***Category definition*** *module*; 2. Click on the level with CAS:97530; 3. Click ***Define with metabolism***; 4. Select ***in vivo Rat metabolism simulator***; 5. Click ***OK***; 6. Target and all metabolites produced by the selected simulator appear. Find the formic acid structure (Metabolite #9) and specify "**Exact**" query; 7. Execute the search by click **OK** (The selected databases are not cached. Therefore, first running of this example will take a few minutes).

Category Definition

Searching for analogues accounting for in vivo rat liver metabolism



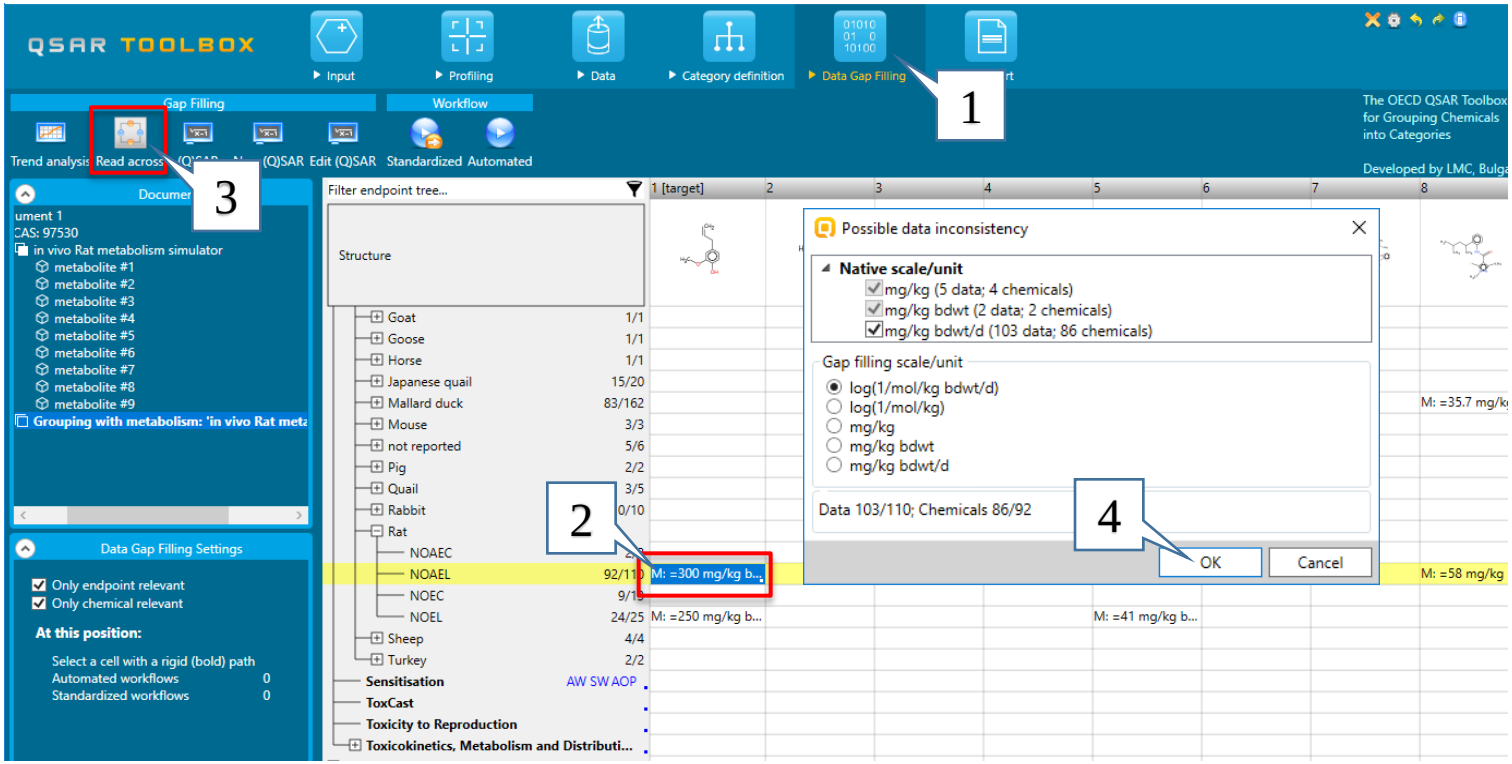
1. Click ***Choose...*** and select Repeated Dose Toxicity data to be collected only; 2. An information window appears informing about the number of experimental data collected and the number of chemicals in the category, click **OK**; 3. 92 chemicals with 110 experimental data has been found related to the target endpoint.

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.

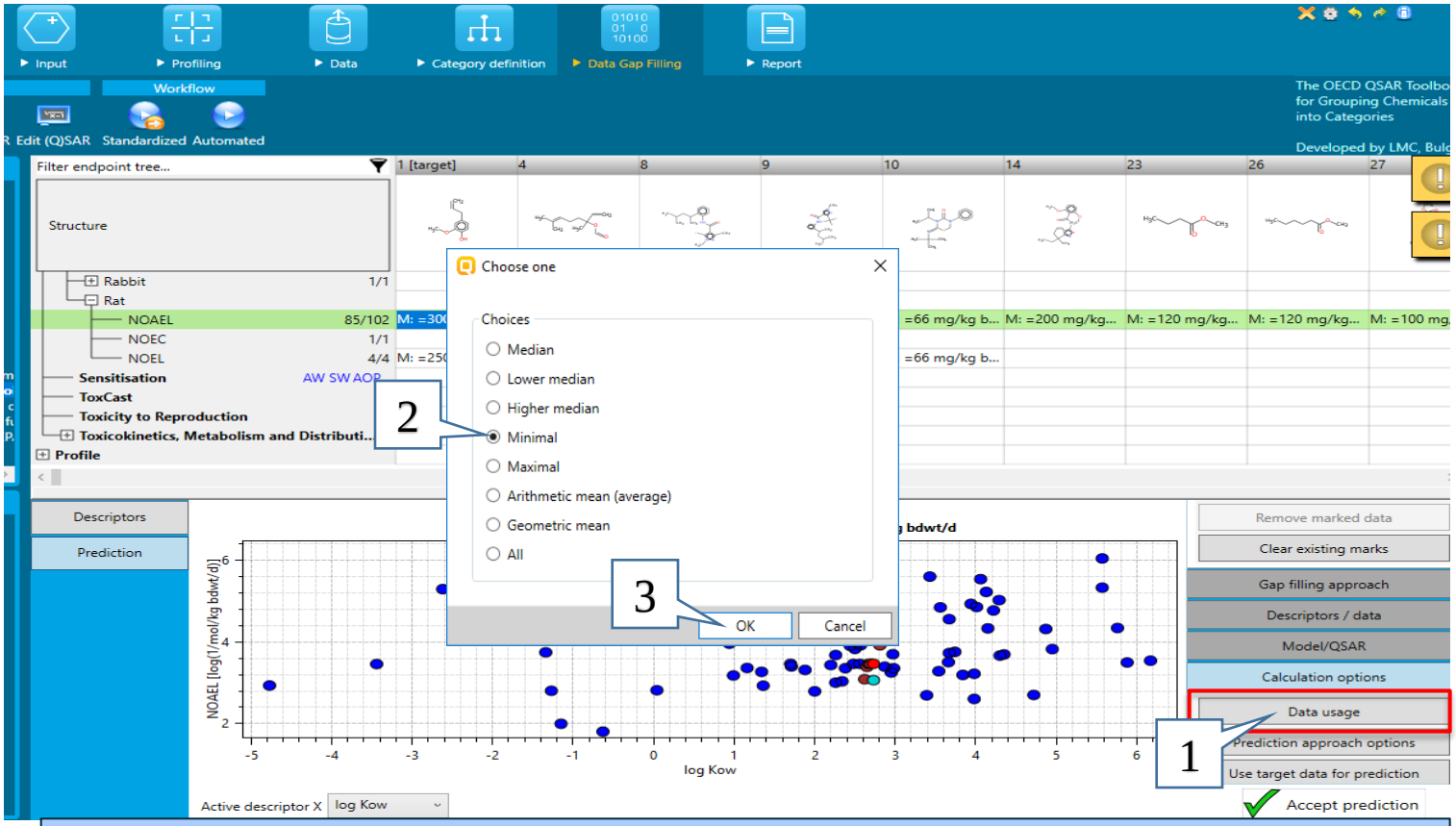
In this example we will use the read-across approach.

Data Gap Filling
Apply Read-across



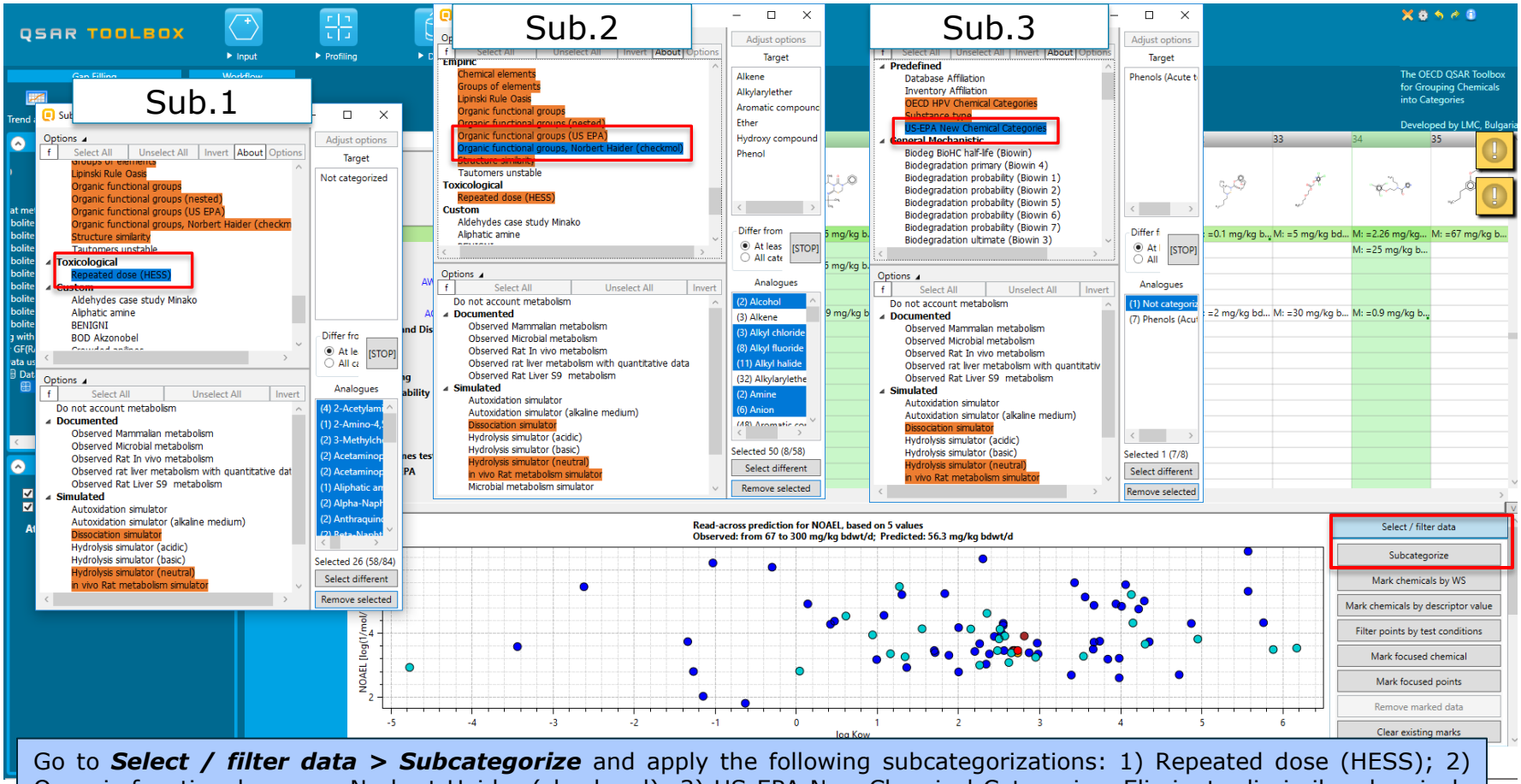
1. Go to ***Data Gap Filling*** module; 2. Click the cell corresponding to the target chemical and defined endpoint.; 3. Apply **Read across**; 4. A pop-up window informing about possible data inconsistency appears click **OK.**

Data Gap Filling
Apply worst-case scenario



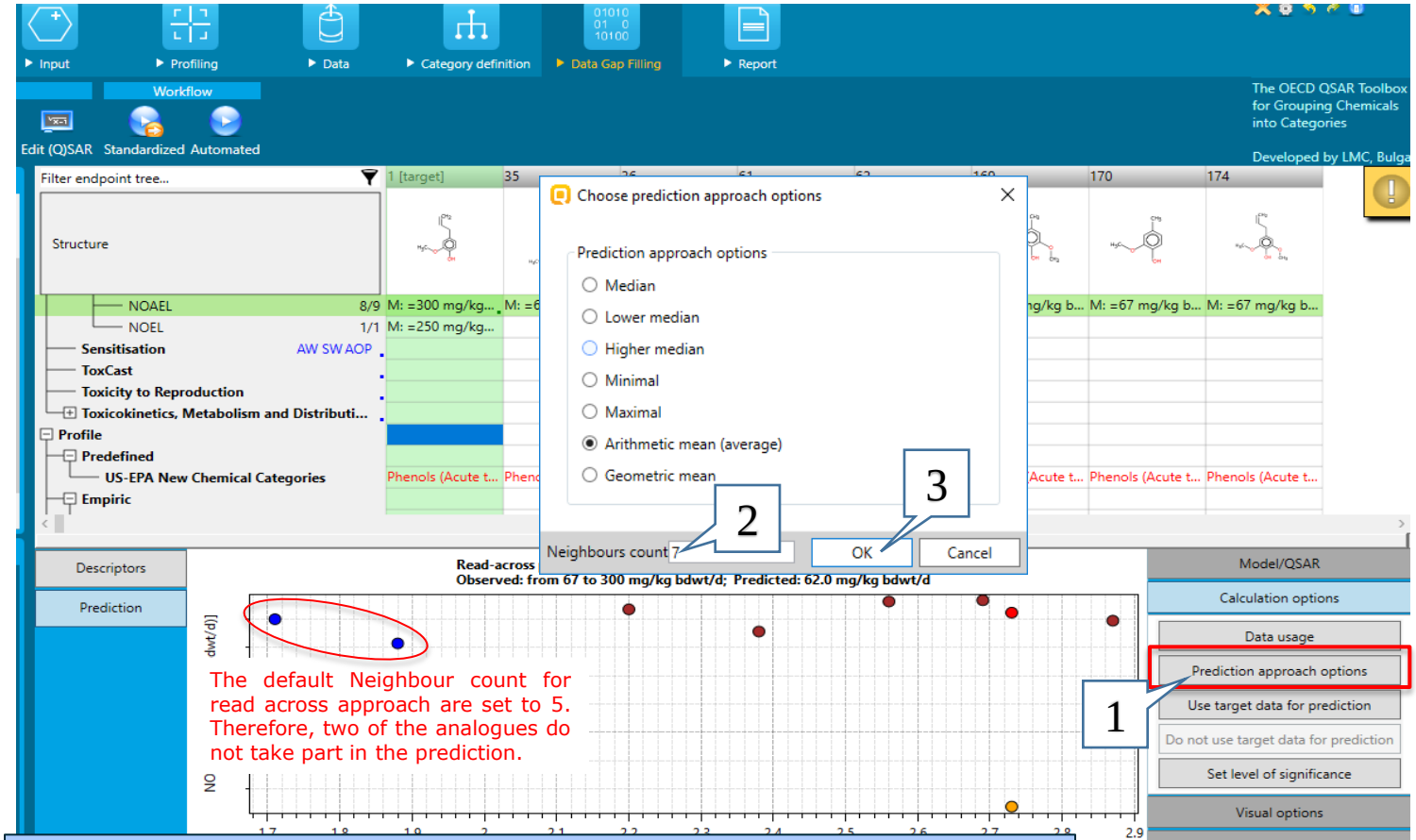
Apply the worst case scenario: 1) Go to ***Calculation options>Data usage***; 2) Click ***Minimal*** radio button; 3. Confirm with **OK**.

Data Gap Filling
Subcategorize



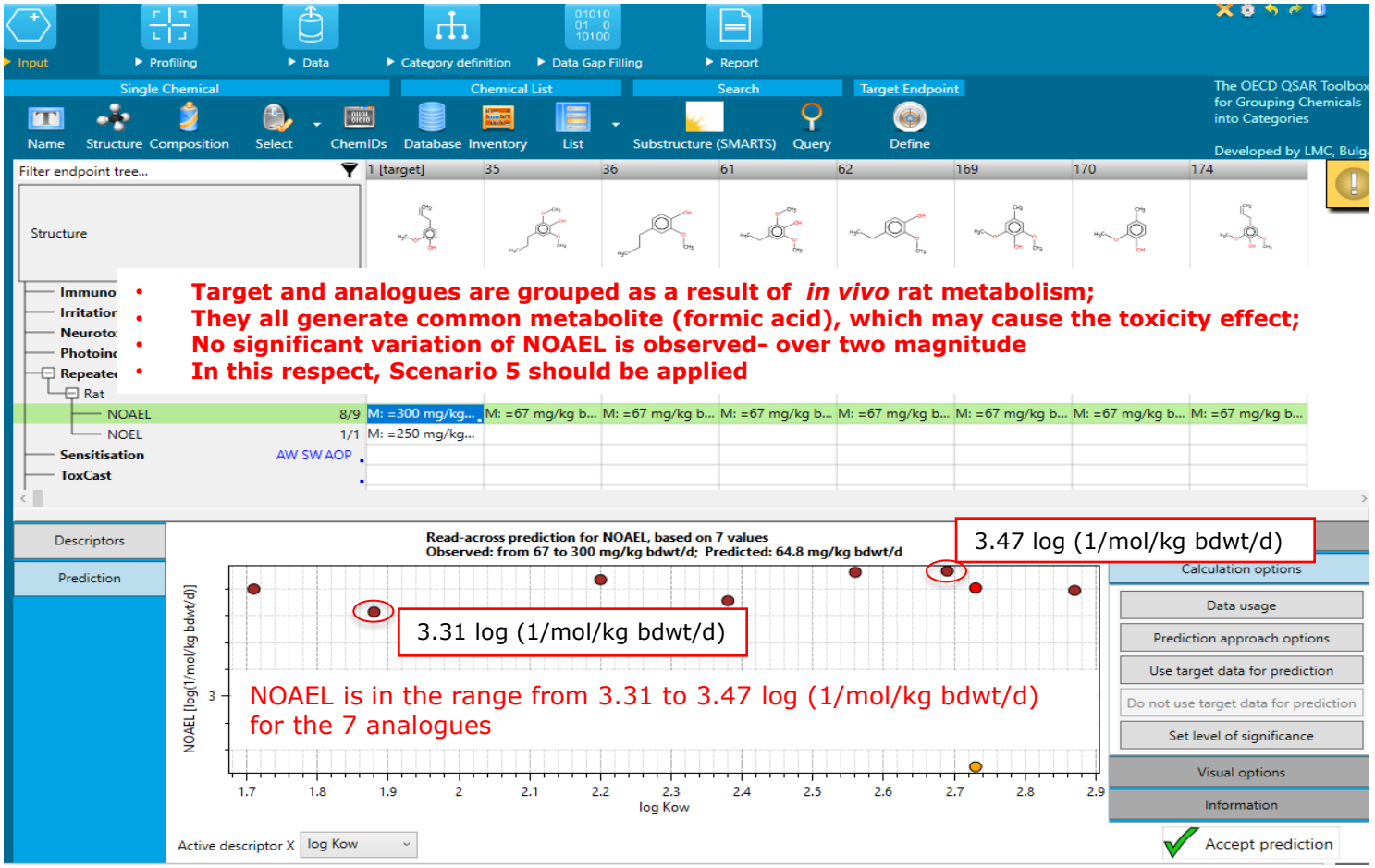
Go to ***Select / filter data > Subcategorize*** and apply the following subcategorizations: 1) Repeated dose (HESS); 2) Organic functional groups, Norbert Haider (checkmol); 3) US-EPA New Chemical Categories. Eliminate dissimilar chemicals after each of the applied subcategorizations using the "Remove selected" button

Data Gap Filling
Approach options

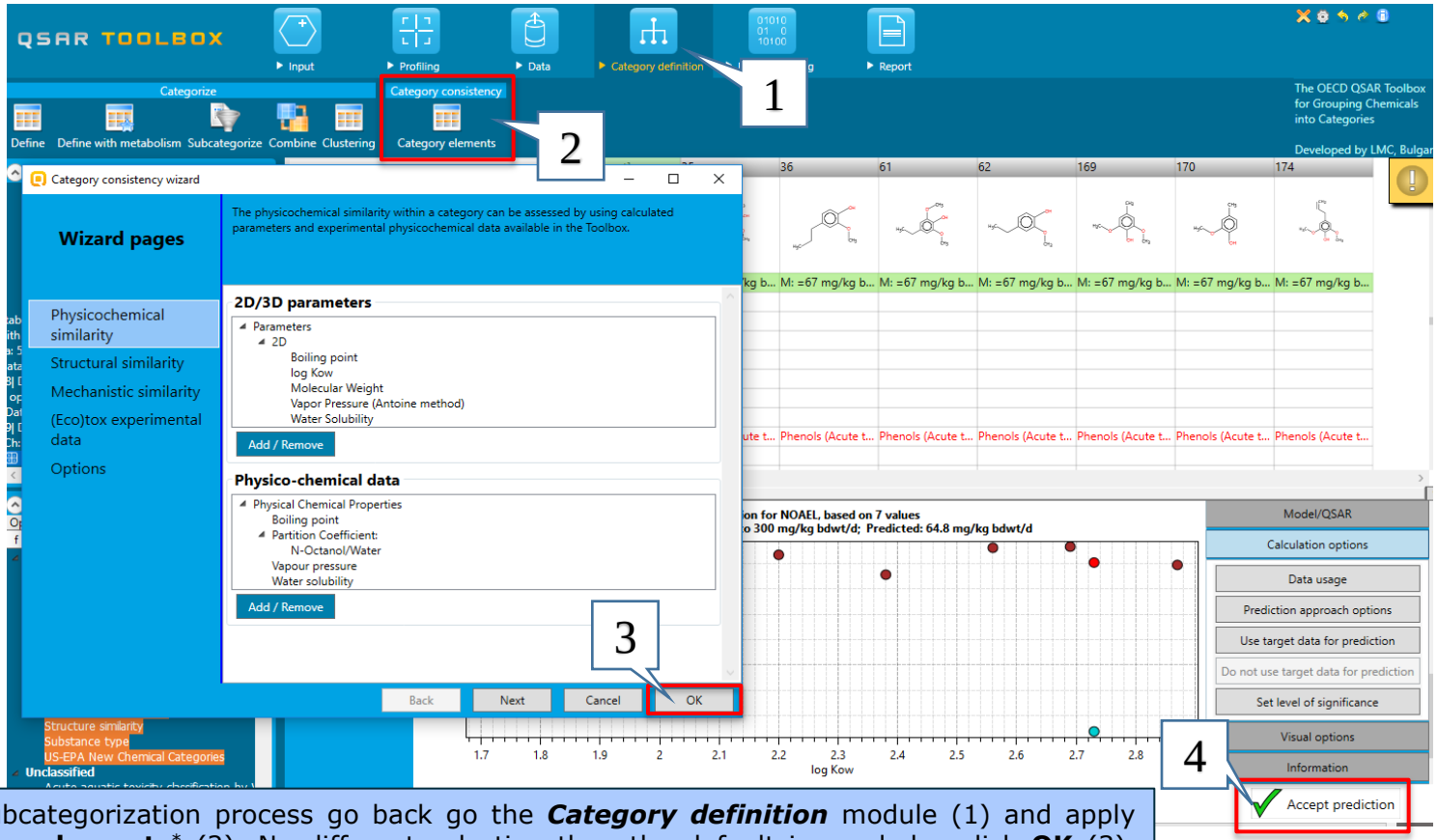


1. Go to ***Calculation options > Prediction approach options***; 2. Define the *Neighbours count* to be **7** instead of 5; 3. Confirm by OK.

Data Gap Filling
Data variation



Data Gap Filling
Category consistency check



After subcategorization process go back go the ***Category definition*** module (1) and apply **Category elements*** (2). No different selection than the default is needed – click **OK** (3). Once the category elements are **applied accept** the prediction (4).

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

Recap

- In the *Category definition* module you found 246 chemicals having common metabolite (formic acid) as a result of in vivo rat metabolism.
- 92 out of all 246 chemicals have data for the defined endpoint.
- In *Data gap filling* module you applied a read-across approach. As a result of subcategorization the number of analogues was reduced to 7.
- Prediction approach options were changed in order the data for all seven analogues to be used for the prediction.
- No significant variation of NOAEL data was observed for the closest analogues.
- Category consistency was checked by applying the category elements.
- You are now ready to complete the final module and to create the report.
- Click “Report” to proceed to the last module.

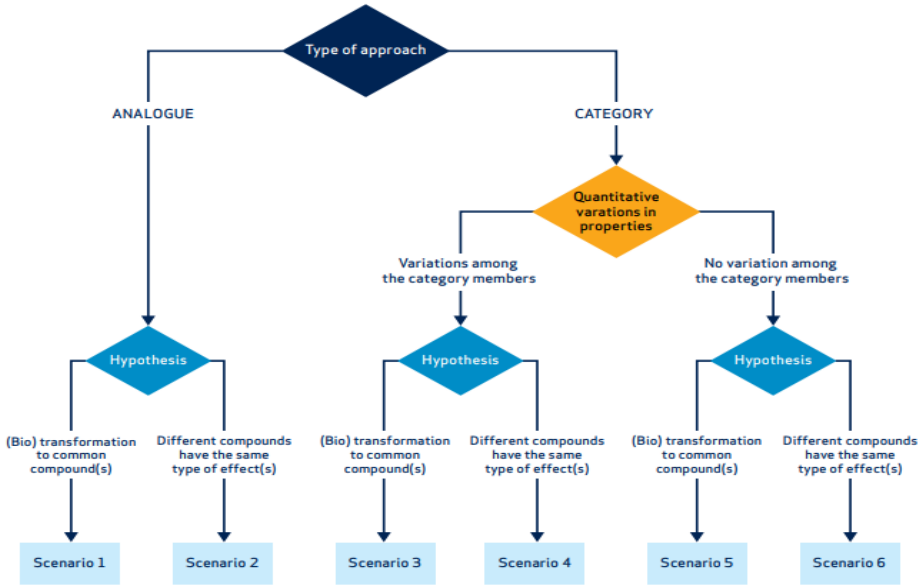
Report
Overview

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

Report
Selection of RAAF scenario

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

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



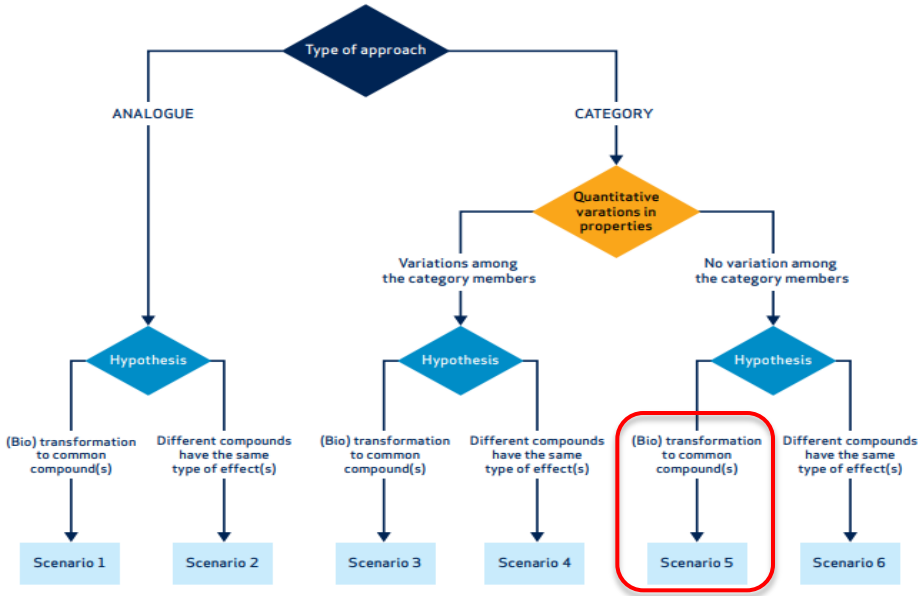
*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Report
Selection of RAAF scenario

For this example the following criteria are met :

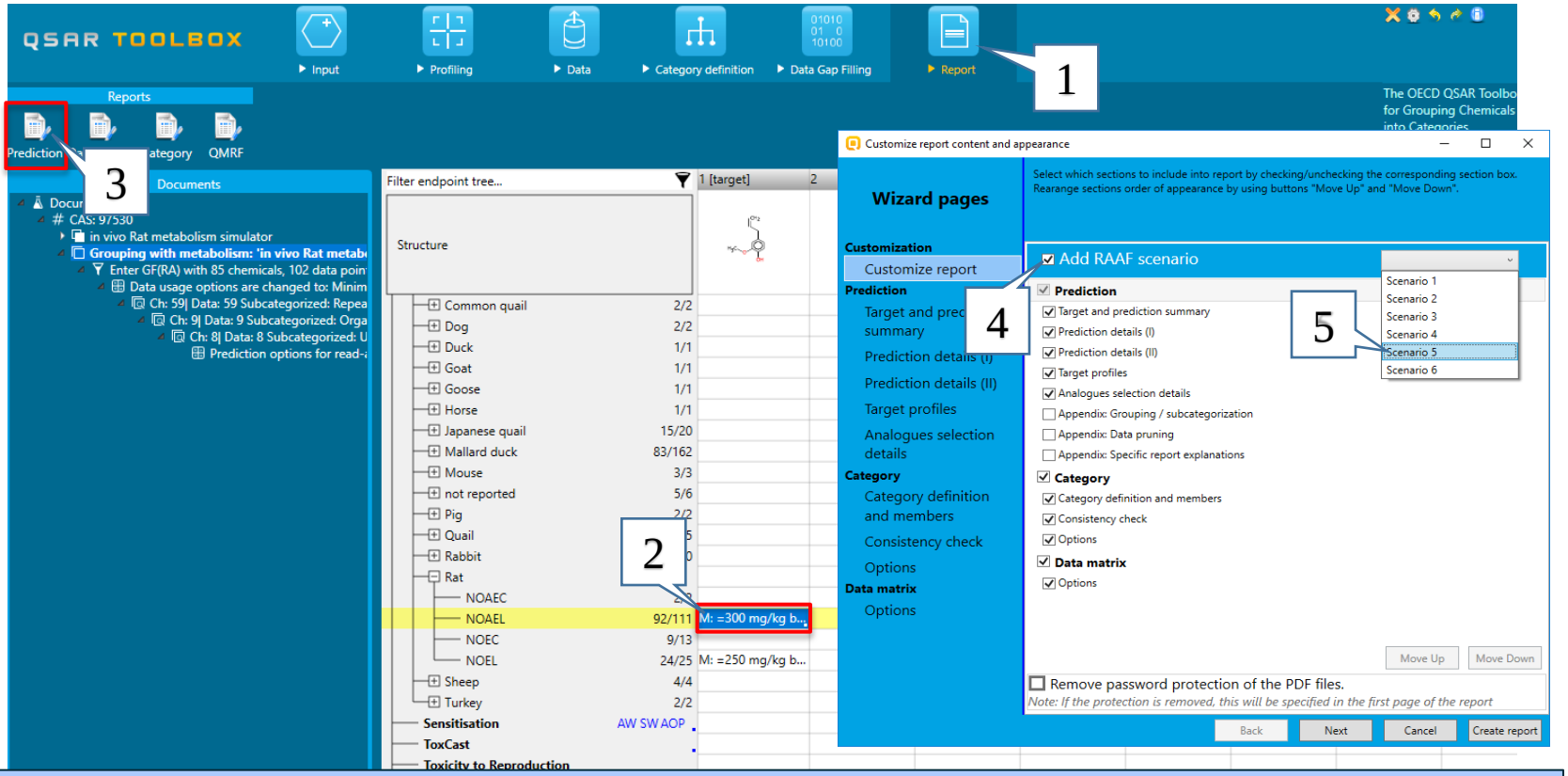
- the type of approach applied - category approach is used (threshold of > 3 analogues is proposed by LMC for the category approach);
- the read-across hypothesis – different compounds (bio)transformed to the common compound;
- There is no significant variation in the property under investigation (NOAEL) among the category members

Based on that RAAF scenario 5 was identified as the most appropriate for the current example.



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

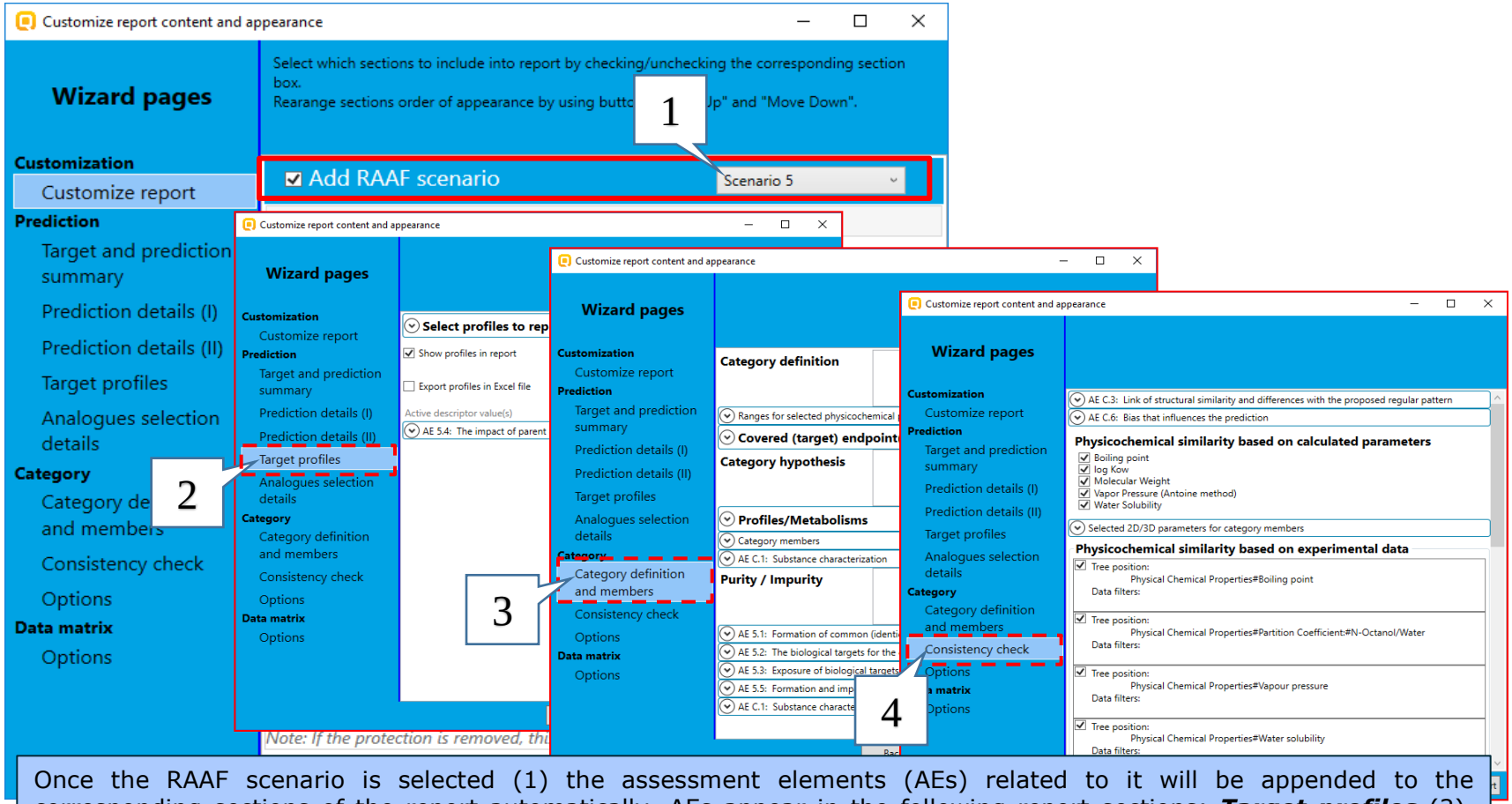
Report
Generation report according to RAAF-Scenario 5



1. Go to ***Report*** section; 2.Select a cell with prediction; 3.Click ***Prediction***; 4. Check the box at the top to add RAAF scenario; 5. Select **Scenario 5** from the drop-down menu.

Report

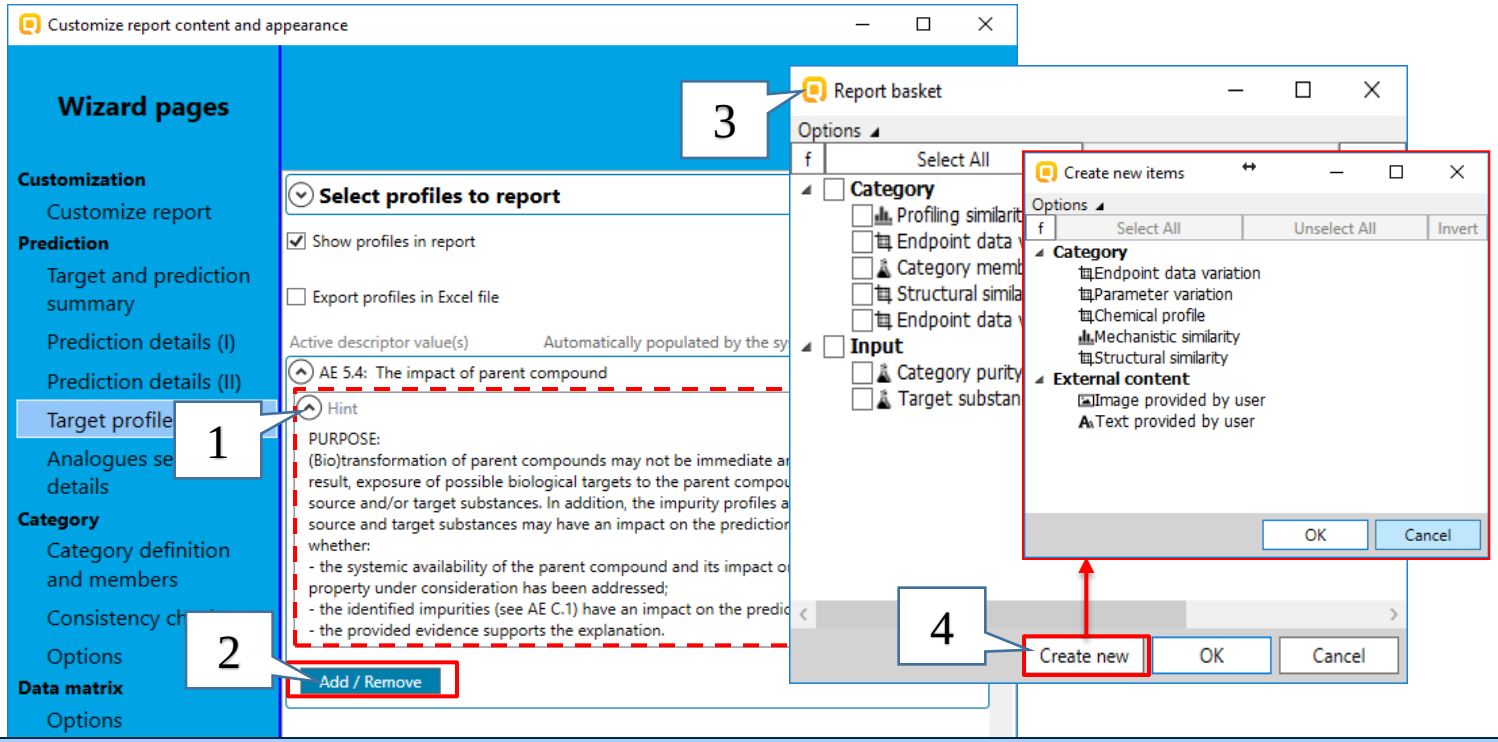
Generation report according to RAAF-Scenario 5



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

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

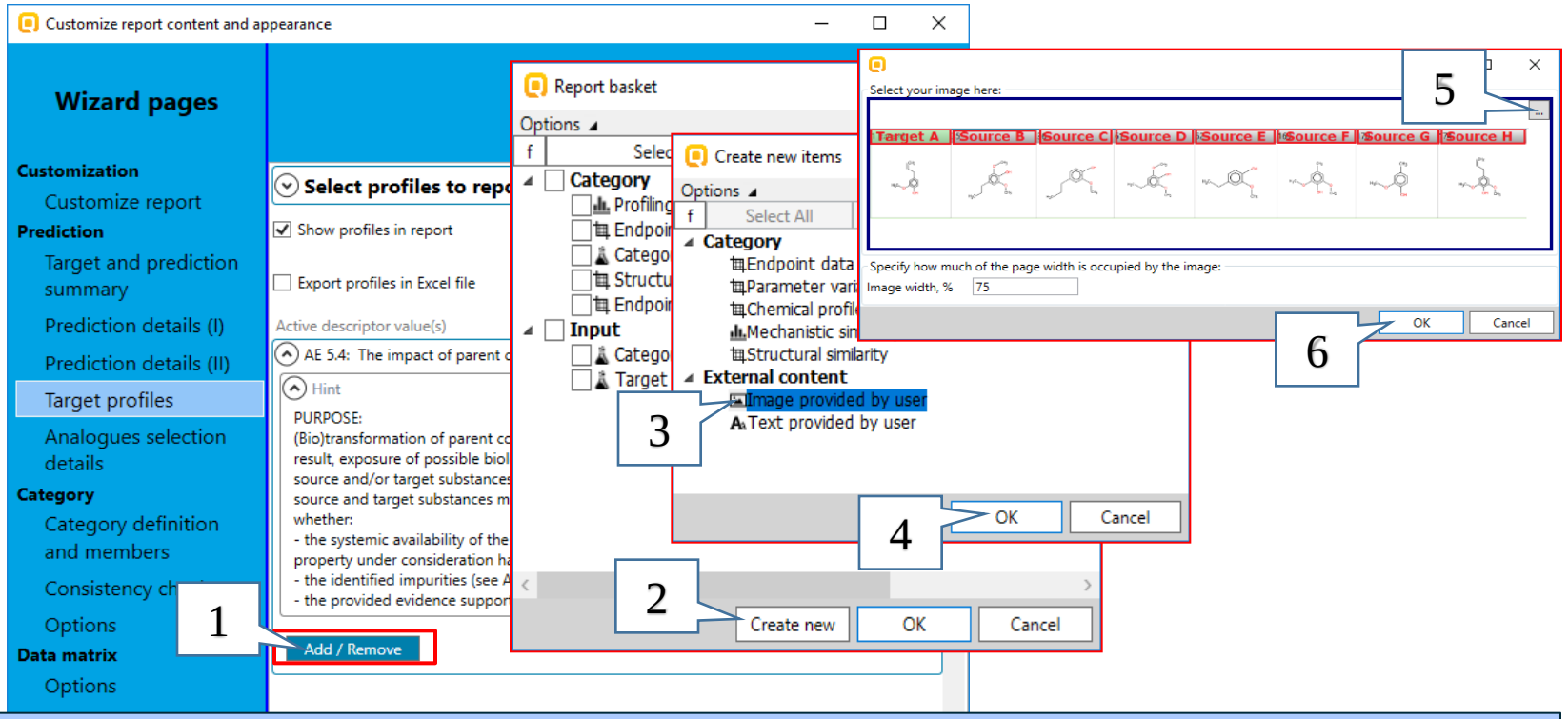
Report
Assessment elements of Scenario 5



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 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 5.4**. **The impact of parent compound**

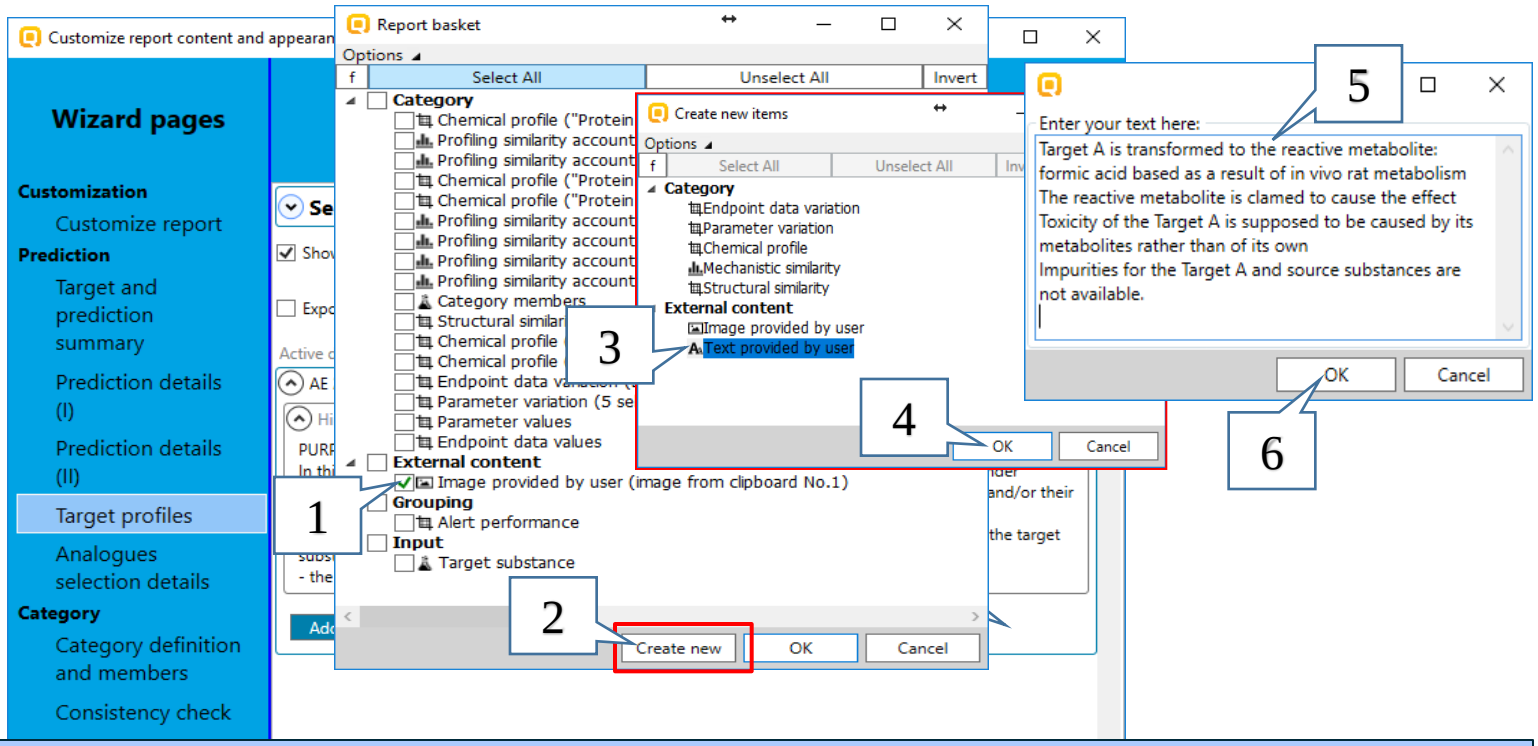
Report
Assessment elements of Scenario 5



Click on the **Add/Remove** button (1) and then **Create new** (2). Select to create 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 B**, **C, D, E, F, G** and **H** was prepared in advance.

Report
Assessment elements of Scenario 5

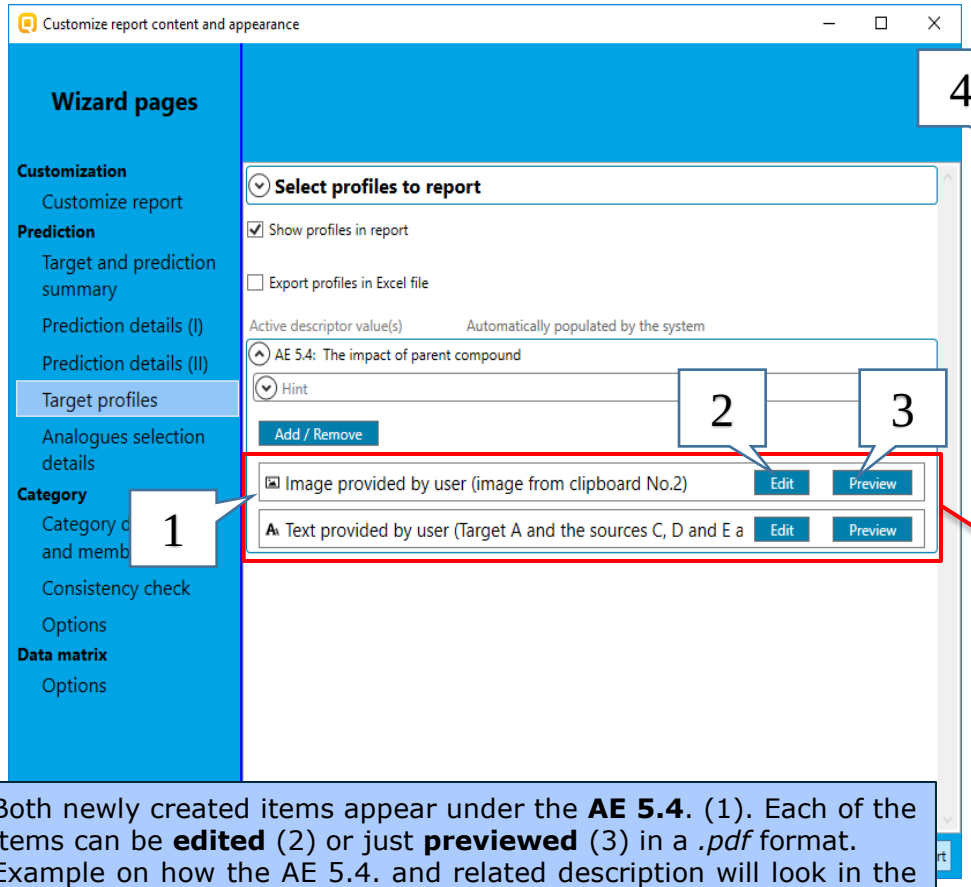


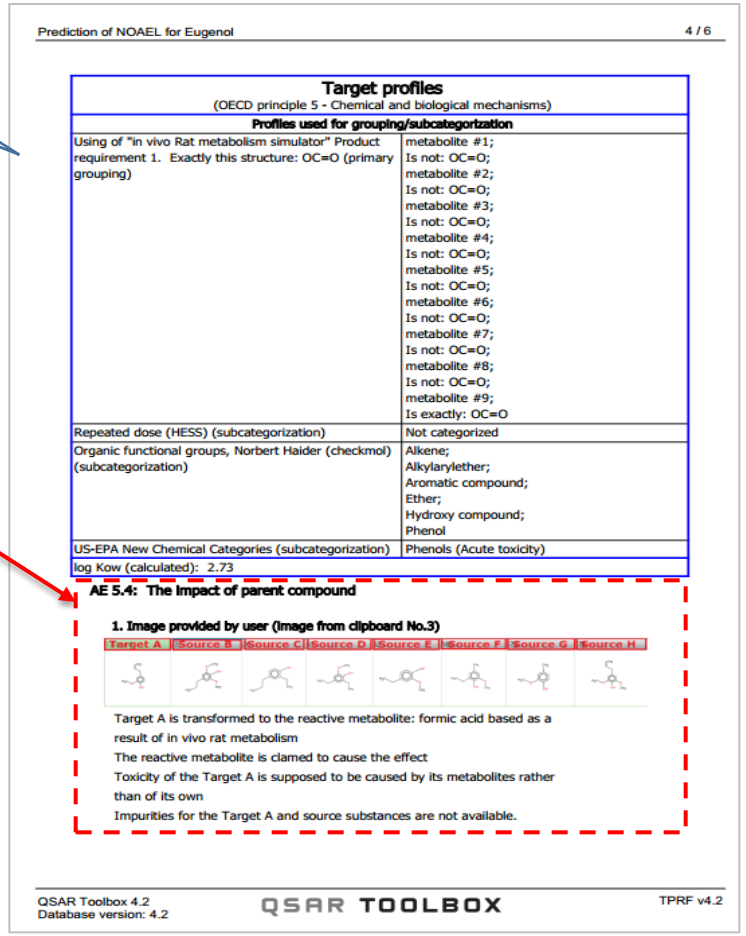
The newly created item appears in the *Report basket* (1). Now text will be also included. Click **Create new** (2), select ***Text provided by user*** (3) and click **OK** (4). Copy the following example text:

- *Target A is transformed to the reactive metabolite: formic acid based as a result of in vivo rat metabolism*
- *The reactive metabolite is clamed to cause the effect*
- *Toxicity of the Target A is supposed to be caused by its metabolites rather than of its own*
- *Impurities for the Target A and source substances are not available.*

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

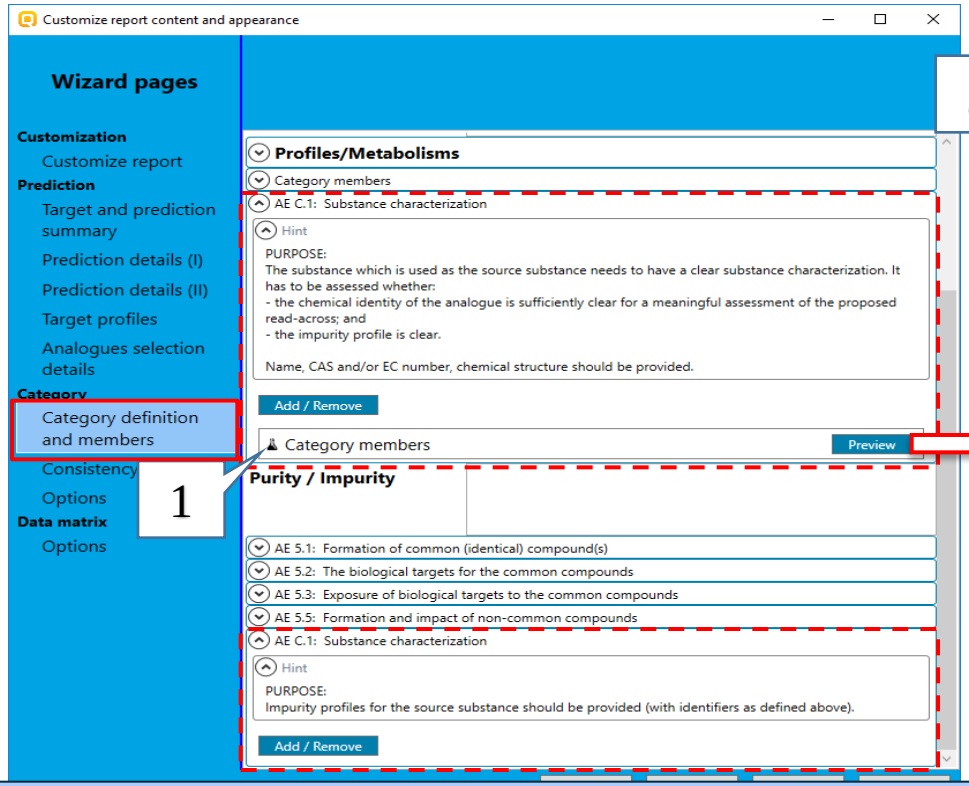
Report
Assessment elements of Scenario 5



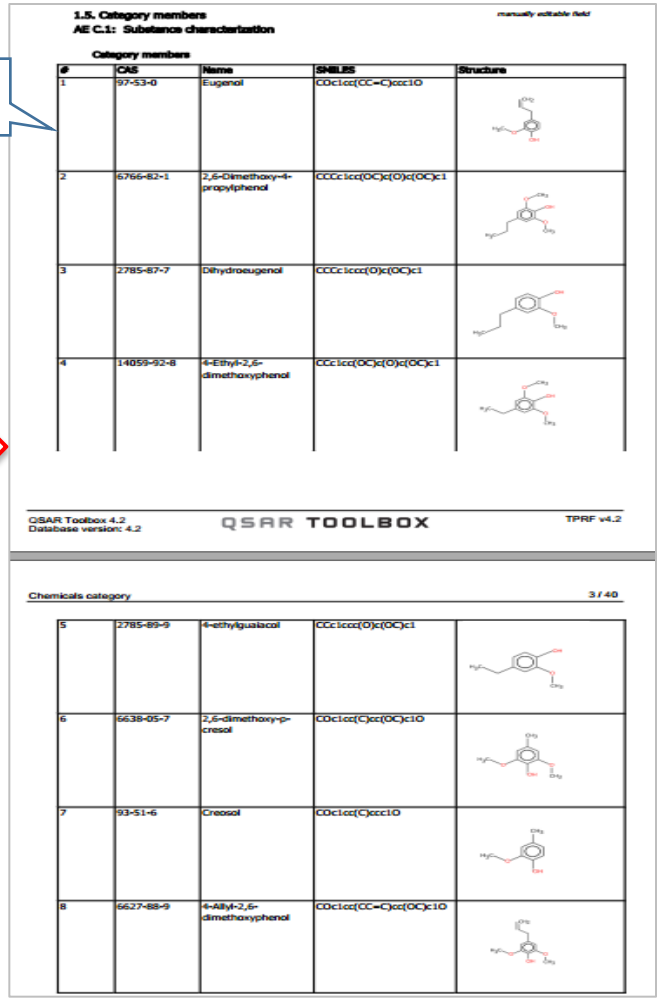


Both newly created items appear under the **AE 5.4**. (1). Each of the items can be **edited** (2) or just **previewed** (3) in a *.pdf* format. Example on how the AE 5.4. and related description will look in the generated report is shown in right (4).

Report
Assessment elements of Scenario 5



2

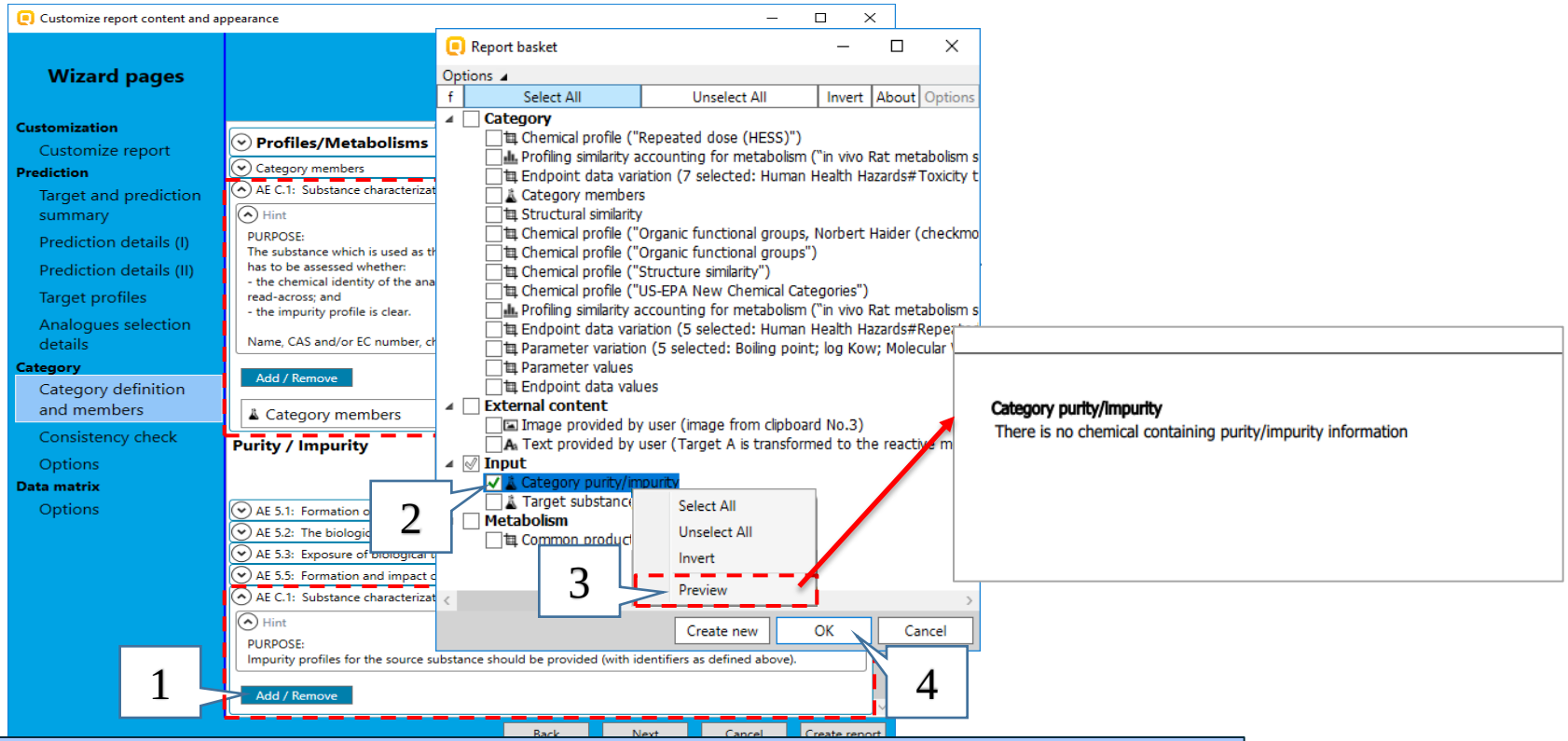


Five AE (AE C.1, 5.1, 5.2, 5.3 and 5.5) related to Scenario 5 are included in the *Category definition and members* section .

- **AE C.1 Substance characterization** is separated into two parts. The first part is automatically filled (1) by the system using the available items in the *Report basket*.

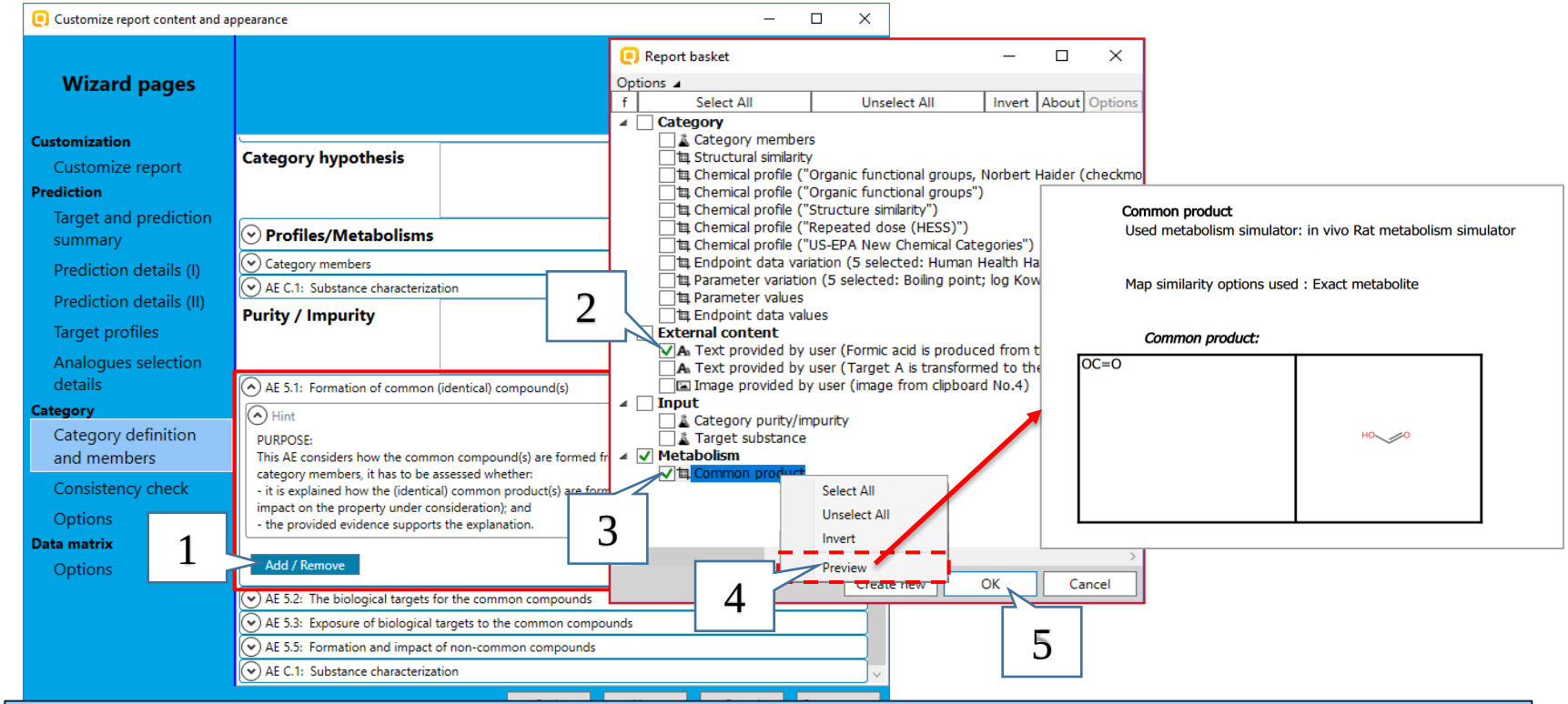
Example on how the AE C.1. will look in the generated report is shown in right (2).

Report
Assessment elements of Scenario 5



- **AE C.1 Substance characterization**: Click **Add/Remove** button in the second part of AE C.1. Check the box next to *Category purity/impurity* item (2). Right click over the item and select **preview** to see the content (3). Finally confirm by **OK** (4).

Report
Assessment elements of Scenario 5



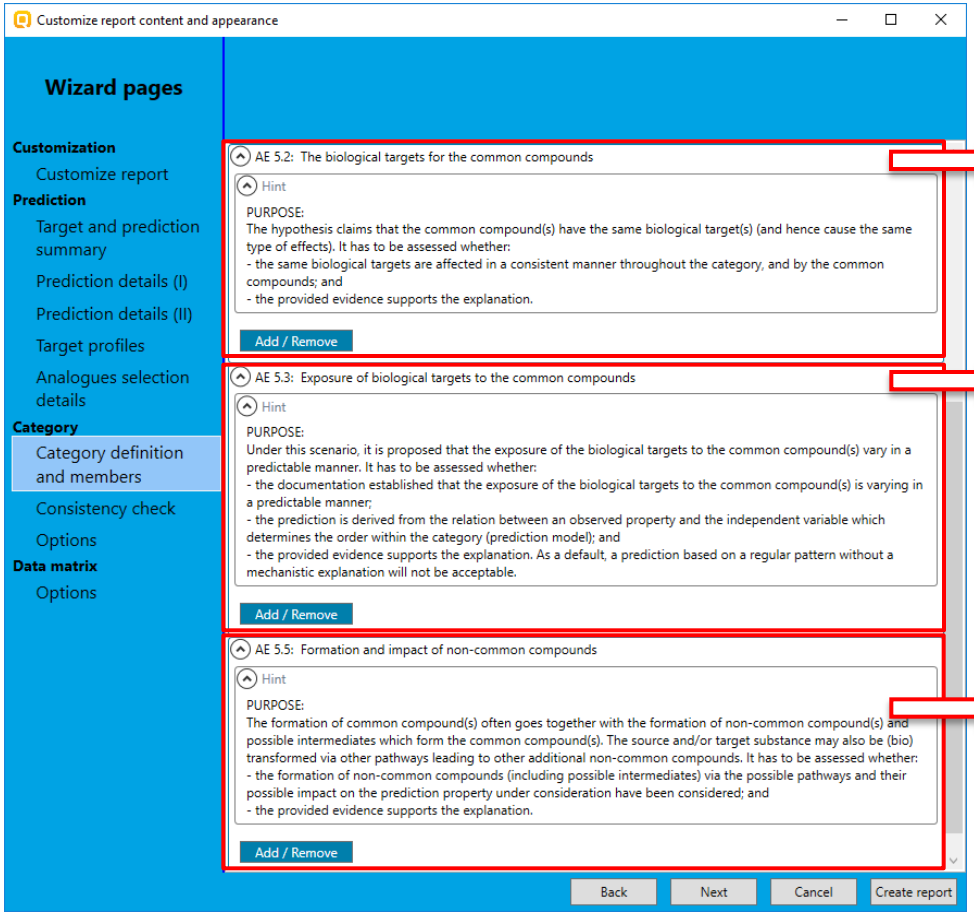
AE 5.1: Formation of common (identical) compound(s) - Click on the **Add/Remove** button (1) and create new item with textual content (see slide 45).

In the text field you paste the following example text:

- *Formic acid is produced from the target substance A and the source substances B-H by in vivo rat metabolism simulator*
- *Alert for repeated dose toxicity and experimental data for the property under consideration are found for the common metabolite.*

Once the text item is created (2), check the box next to the *Common product* tem (3). Right click over the item and select **preview** to see the content (4). Finally confirm by **OK** (5).

Report
Assessment elements of Scenario 5



Example text for **AE 5.2: The biological targets for the common compounds**

- *The target and source substances form a common metabolite: formic acid.*
- *No alerts are identified in the structures of the Target A and Source substances B-H.*
- *The common compound is supposed that may cause the toxic effect.*

Example text for **AE 5.3: Exposure of biological targets to the common compounds**

- *Target chemical A and source substances from B to H are metabolized to the common reactive product: formic acid;*
- *It well known from the literature [1-3] that some carboxylic acids induce adverse effects in the liver. It is expected that the exposure of the biological targets to the common product vary in a predictable manner.*

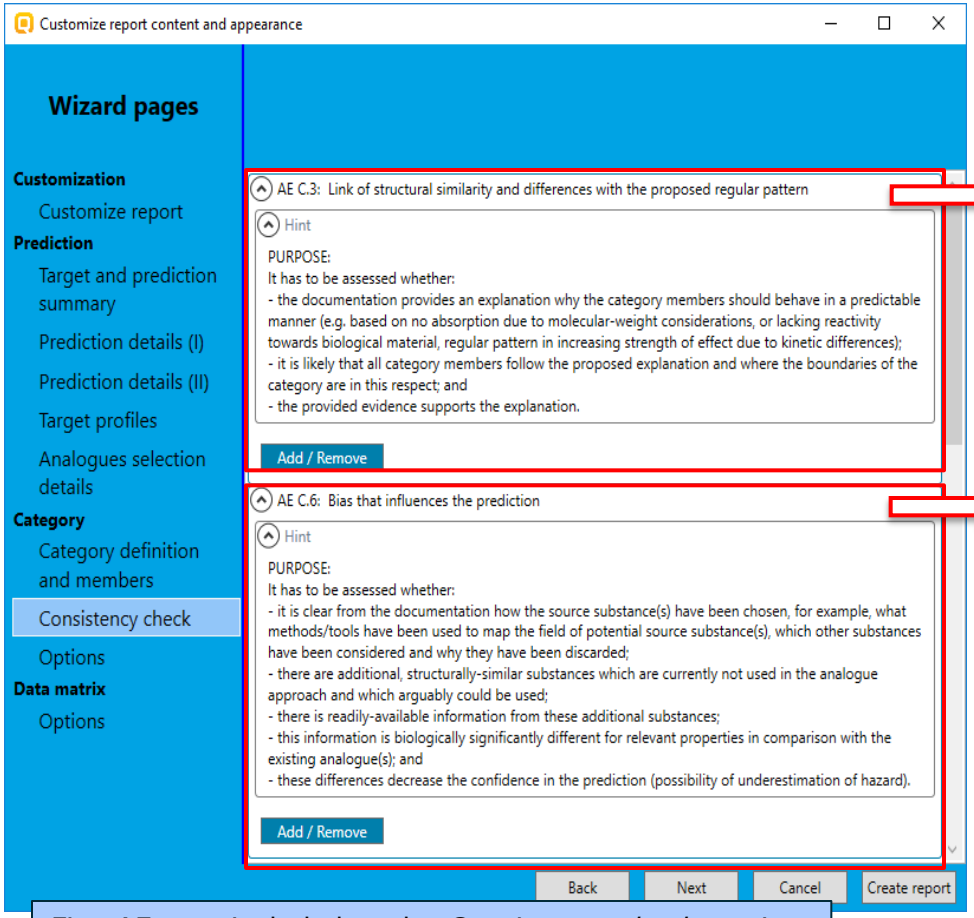
References:

1.Casarett & Doull's Toxicology, Curtis D,Klaassen-7th ed, McGraw-Hill companies, USA(2007)
2. New edition Toxicology, edit by Japanese society of Toxicology, Japan(2009)
3.Toxicology in Medicine, Tetsuo Sato-4th ed, Nankodo, Japan (2010)

Example text for **AE 5.5: Formation and impact of non-common compounds**

- *The target substance A and the seven source substances (analogues) are metabolized to the common- formic acid and non-common compounds (including possible intermediates)*
- *Hepatotoxicity might be caused due to the common compound*
- *Also the positive effect of formic acid is supported by experimental NOAEL data.*
- *Another alerts related to Hepatotoxicity and Renal toxicity are identified in some of the produced non-common compounds.*
- *The lowest experimental NOAEL value was found for the common metabolite.*
- *The common compound is supposed to be responsible for the repeated dose toxicity effect.*

Report
Assessment elements of Scenario 5



Example text for **AE C.3: Link of structural similarity and differences with the proposed regular patterh**

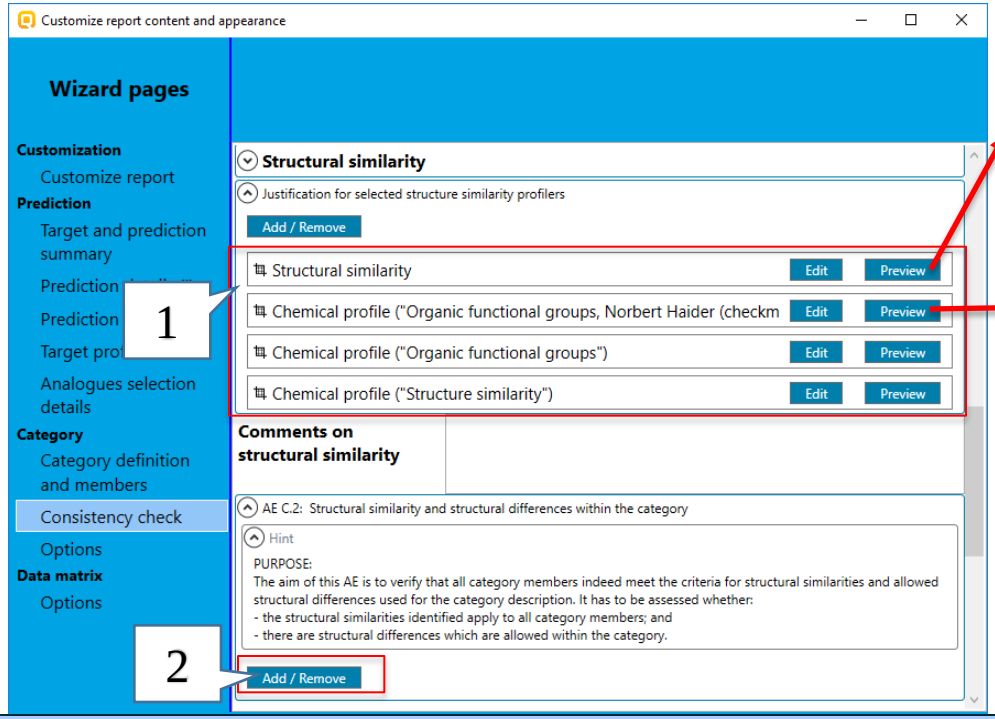
- *The category is structurally defined as target (A) and seven source substances (B, C, D, E, F, G, H) all form a common product – formic acid*
- *They all consist of common reactivity pattern responsible for the formation of reactive metabolites*

Example text for **C.6: Bias that influences the prediiction**

- *Source substances for the target chemical A have been searched based on formation of a specific metabolite as a result of in vivo rat metabolism;*
- *All found analogues that have repeated dose toxicity alert as a parent have been removed in order to be consistent with the target.*
- *On the next level all analogues that differ from the Target A according to OFG, Norbert Haider and US-EPA New Chemical Categories profiling schemes have been removed.*
- *Seven source substances with no significant variation in the property under consideration were used for the prediction.*

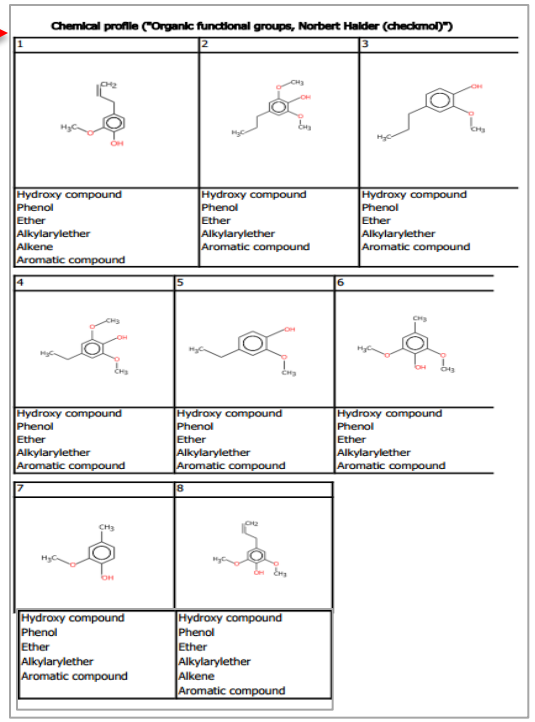
Five AEs are included to the *Consistency check* section. Example content for the first three AEs is given.

Report
Assessment elements of Scenario 5



Calculated structure similarity

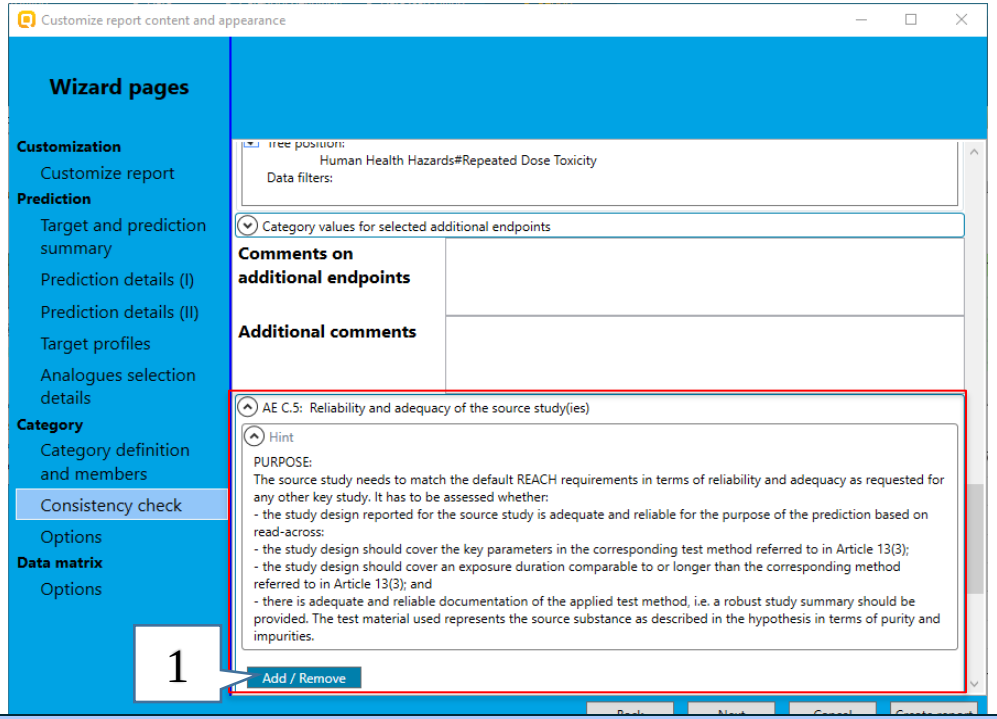
	Chemical 1	Chemical 2	Chemical 3	Chemical 4	Chemical 5	Chemical 6	Chemical 7	Chemical 8
Chemical 1	100%	46.2 %	75 %	48 %	78.3 %	41.7 %	72.7 %	69.2 %
Chemical 2	46.2 %	100%	69.2 %	88.9 %	56 %	76.9 %	41.7 %	78.6 %
Chemical 3	75 %	69.2 %	100%	56 %	87 %	41.7 %	72.7 %	46.2 %
Chemical 4	48 %	88.9 %	56 %	100%	66.7 %	80 %	43.5 %	81.5 %
Chemical 5	78.3 %	56 %	87 %	66.7 %	100%	43.5 %	76.2 %	48 %
Chemical 6	41.7 %	76.9 %	41.7 %	80 %	43.5 %	100%	63.6 %	76.9 %
Chemical 7	72.7 %	41.7 %	72.7 %	43.5 %	76.2 %	63.6 %	100%	41.7 %
Chemical 8	69.2 %	78.6 %	46.2 %	81.5 %	48 %	76.9 %	41.7 %	100%

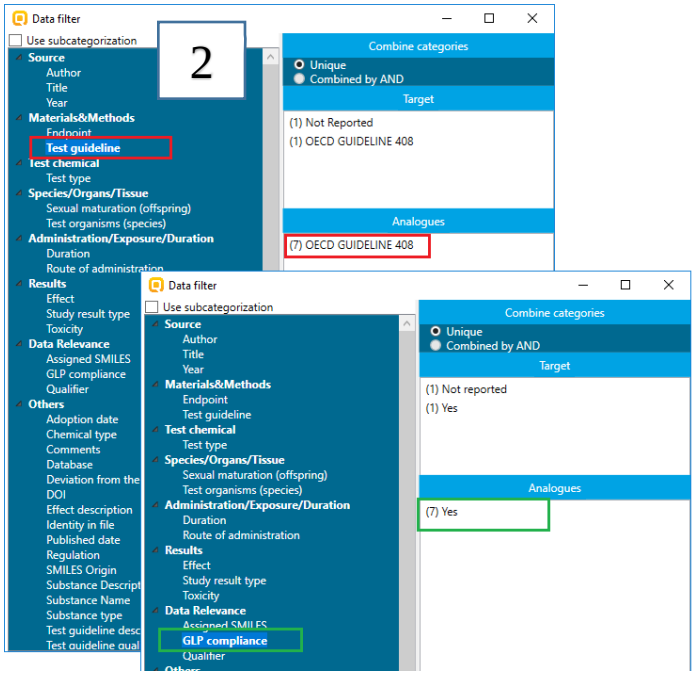


AE C.2. Link of structural similarity and structural differences within the category is related to the structural similarity of the final category.
All items in the report basket related to the structural consistency of the category (1) are added automatically.
The following example text can be added for AE C.2. (2) by analyzing the structural similarity items:

- *Structural similarity between Target substance A and the seven source substances B - H is in the range of [42-89%] according to the default settings of the Structure similarity profiler*
- *"Hydroxy compound", "Phenol", "Ether" , "Alkylarylether" and "Aromatic compound" categories of the OFG, Norber Haider profiler are identified in all category members.*

Report
Assessment elements of Scenario 5



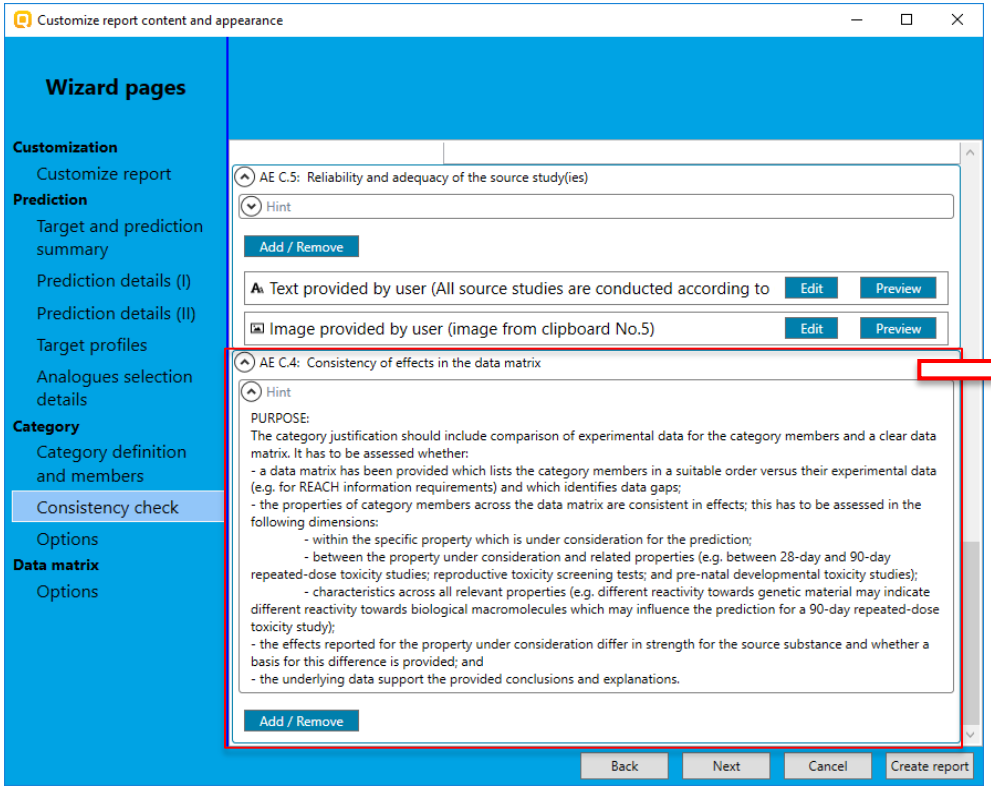


AE C.5: **Reliability and adequacy of the source study(ies)**
The following example text can be added for AE C.5. (2):

- *All source studies are conducted according to OECD Test Guideline 408: Repeated Dose 90-day Oral Toxicity Study in Rodents.*
- *All cource studies are in compliance with the principles of Good Laboratory Practice.*

Additionally snapshots of the filter by test conditions window (2) could be added to confirm the consistency regarding the guideline and GLP compliance.

Report
Assessment elements of Scenario 5



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

The following example text can be added for AE C.4. (2) by analyzing the structural similarity items:

- *Physico-chemical properties, identified alerts and experimental data along with the characteristics of the studies (species, duration, test type, references, etc.) are provided in the generated Data matrix file.*

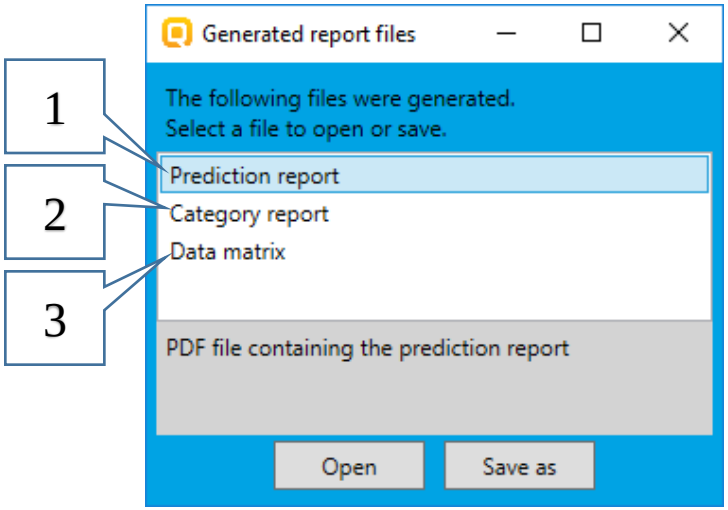
Report
Generation report

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

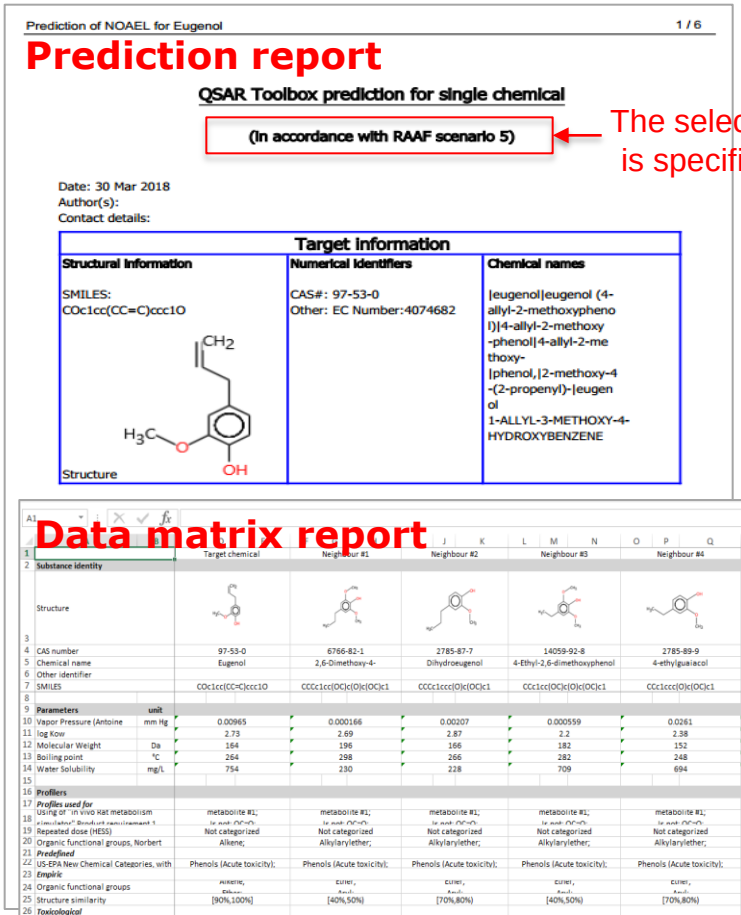
1) **Prediction report** - a PDF file containing the prediction information related to the target.
2) **Category report** - a PDF file containing information for the consistency of the final category (target plus used analogues)
3) **Data matrix** - a MS Excel file containing chemicals used for prediction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

RAAF AEs are included in the first two files.

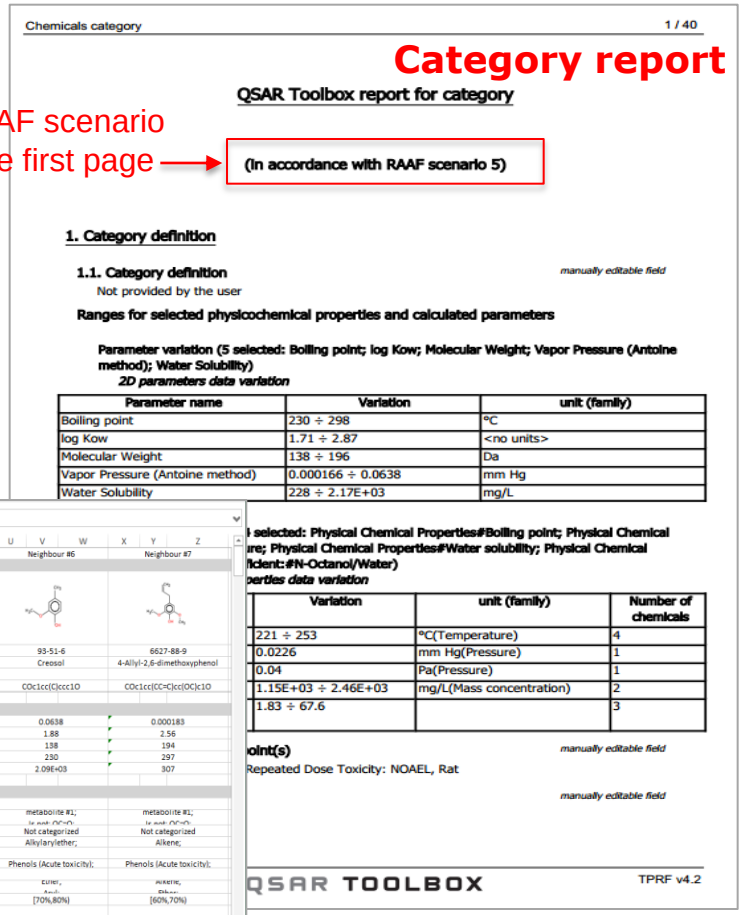
All generated files should be provided when submit a prediction.

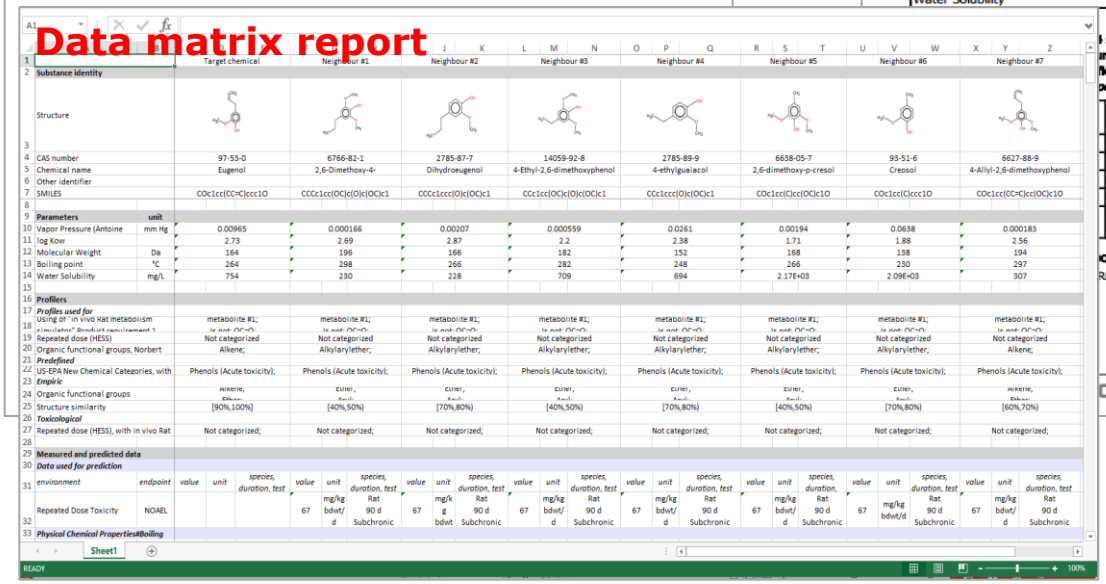


Report
Generated report files



The selected RAAF scenario is specified in the first page





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 5.
- Note proficiency comes with practice.