QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.2

An example illustrating RAAF Scenario 3 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 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 more specifically with Scenario 3;
- To explain to the user how to search for analogues producing a 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 an analogue approach and four for a 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 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 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 a 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) 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 due to 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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- Specific Aims
- Read Across Assessment Framework (RAAF)
- **The exercise**
- Workflow

The Exercise

- In this exercise we will predict *Skin Sensitization* 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 a common metabolite produced after a skin metabolism;
- A read-across approach will be used for the prediction. The prediction will be based on a category approach relying on a common metabolite generated for the source and the 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 Exercise
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 a 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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- Read-Across Assessment Framework (RAAF)
- The example
- **Workflow**

Workflow

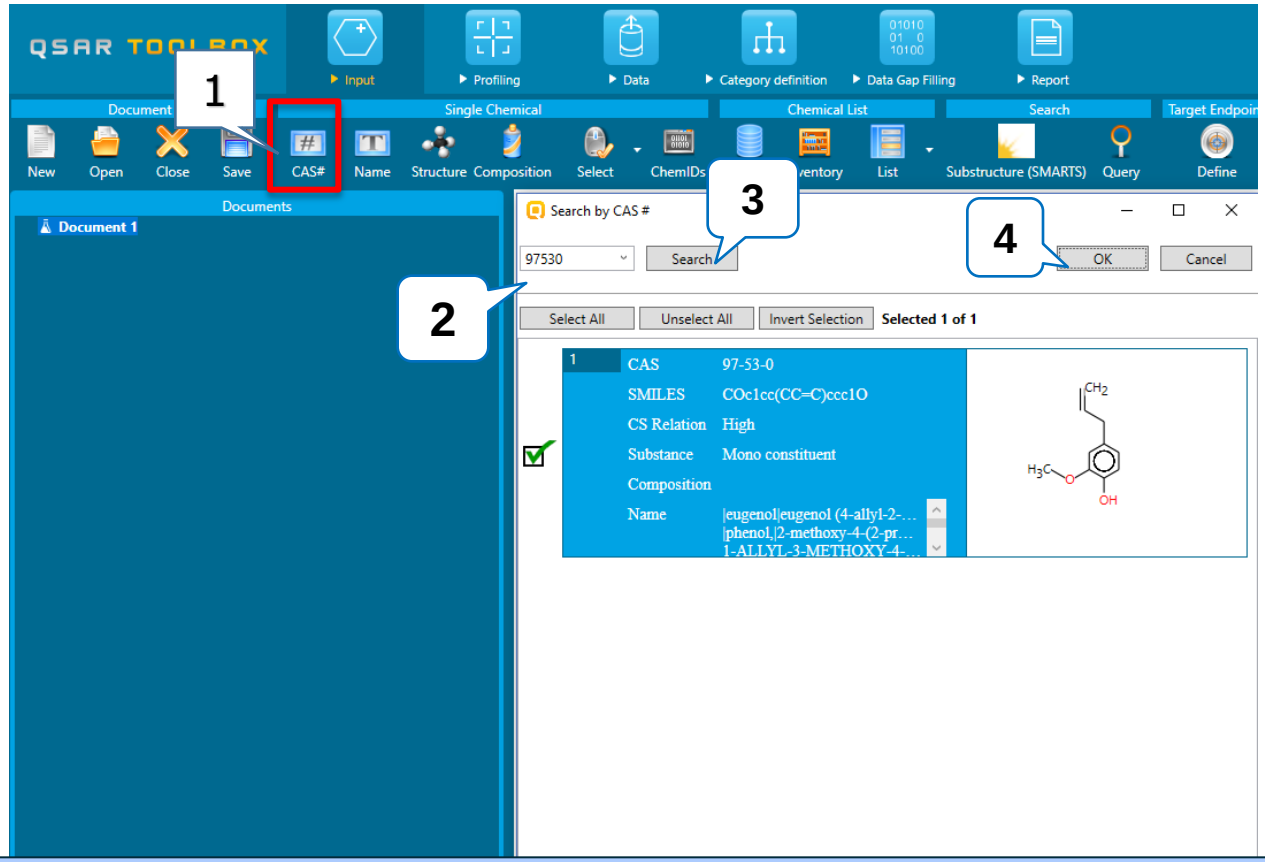
- **The Toolbox has six modules which are used in a sequential workflow:**
  - Input
  - Profiling
  - Data
  - Category Definition
  - Data Gap Filling
  - Report

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

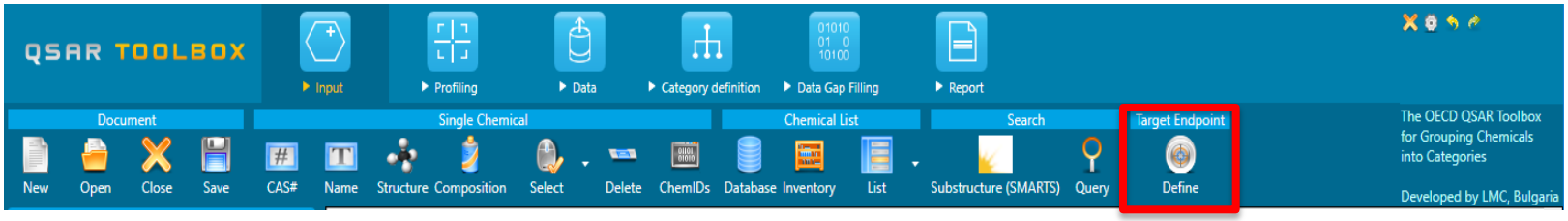
Input target chemical by CAS#



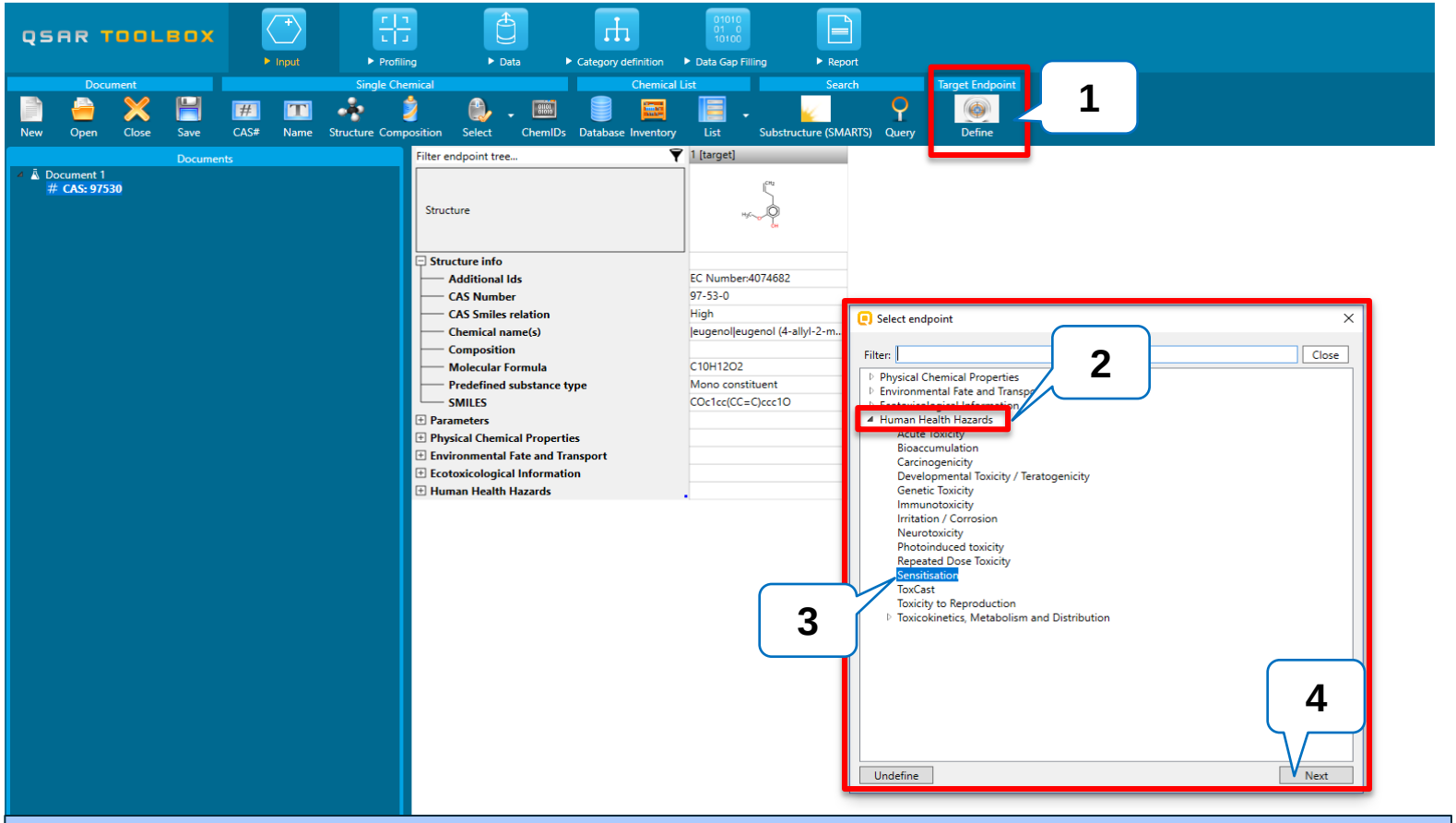
1. Click **CAS#**; 2. Enter the CAS# 97-53-0 in the blank field; 3. Click ***Search;*** 4. When the structure with the requested CAS # appears, click ***OK*.**

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.



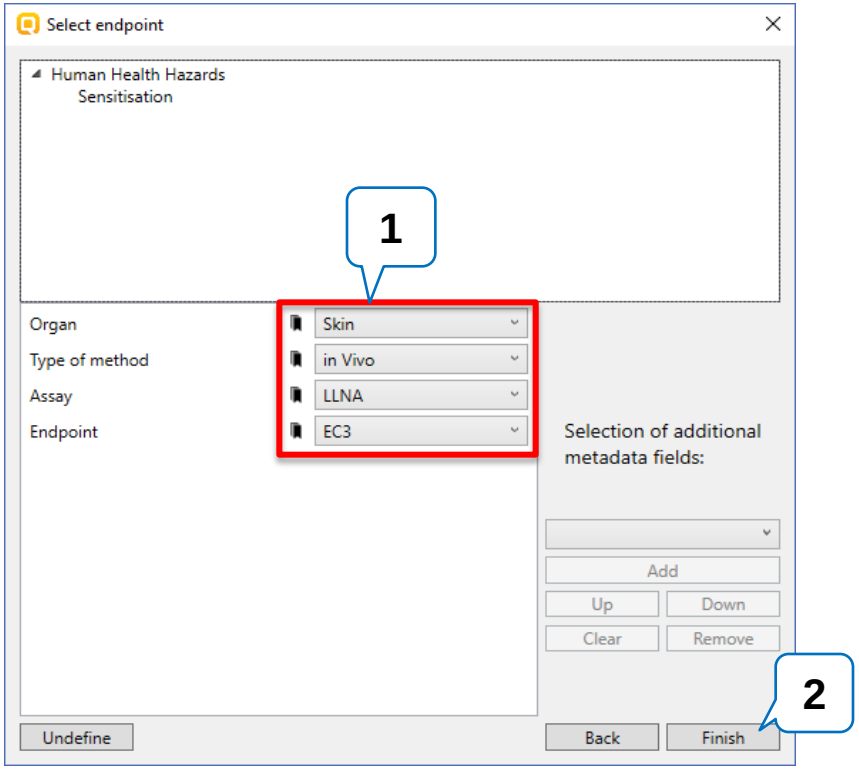
Input
Define target endpoint



Click on the **Define button** (1) open *Human health hazards (2)* and select **Sensitization** (3) then click on **Next** (4).

Input
Define target endpoint

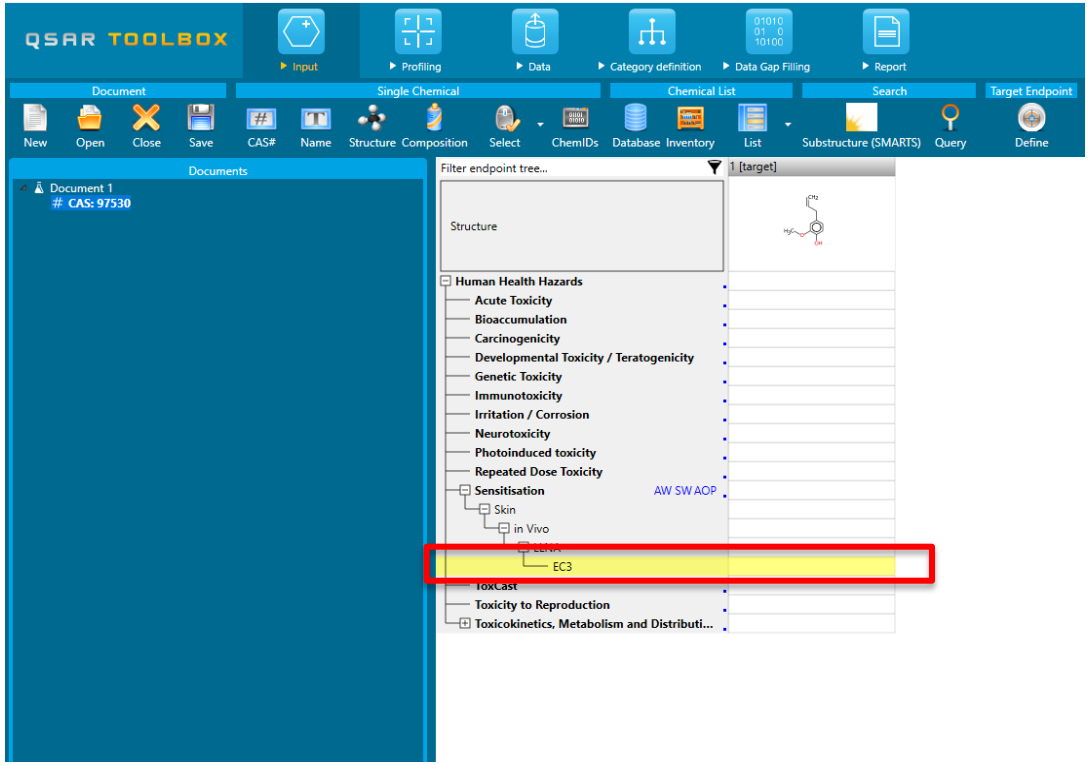
On the next step you have to select the endpoint of interest and additional metadata if needed.



1. Select *Endpoint*: **EC3,** *Assay*: **LLNA,** *Type of method*: **In Vivo,** *Organ:* **Skin**. Click on **Finish**

Input
Define target endpoint

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



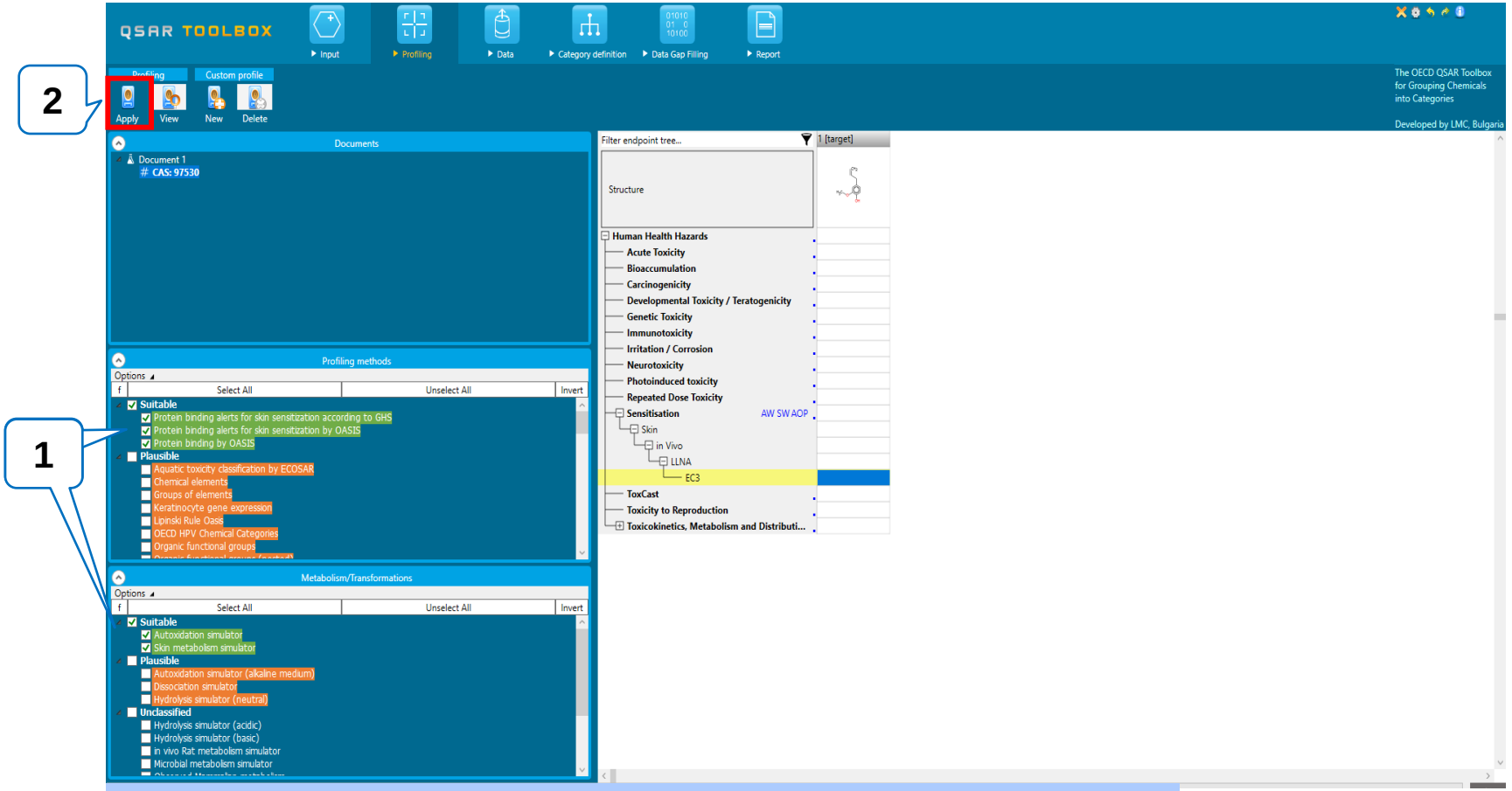
Profiling
Overview

- "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.
- The available information includes probable mechanism(s) of action, as well as observed or simulated metabolites.
- Based on the "profilers' relevancy" the most suitable ones are getting colour highlighted*
- For the purpose of this example the 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. Select all *suitable* profiling schemes and simulators
2. Click on **Apply**

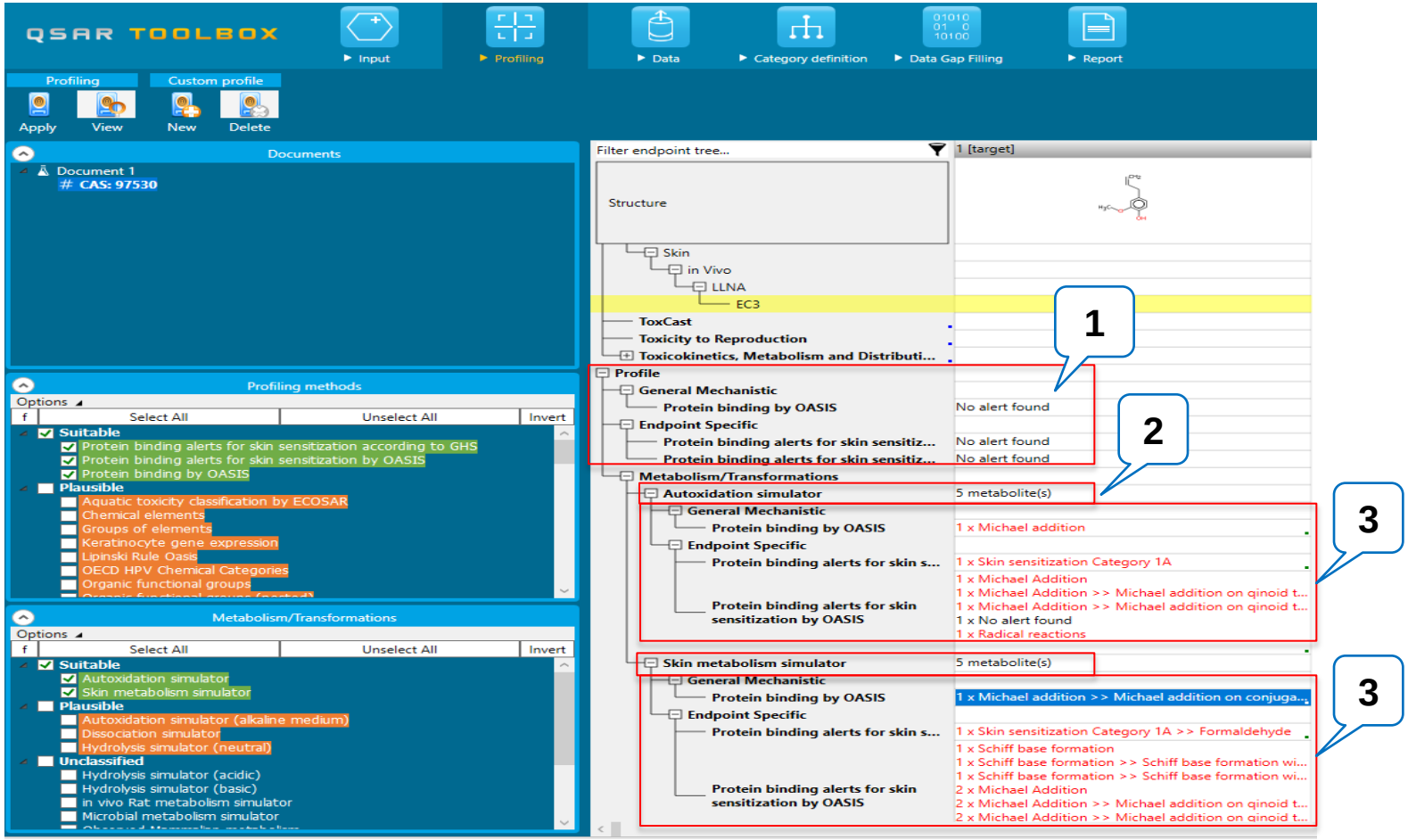
Profiling
Profiling results

1) No alerts are identified in the target's structure as a parent;
2) 5 metabolites are generated as a result of abiotic activation (*Autoxidation simulator*) and biotic activation (*Skin metabolism simulator*);
3) General mechanistic and endpoint specific protein binding alerts are identified in the metabolites produced by Autoxidation simulator and Skin metabolism simulator.

See on the next slide

Profiling

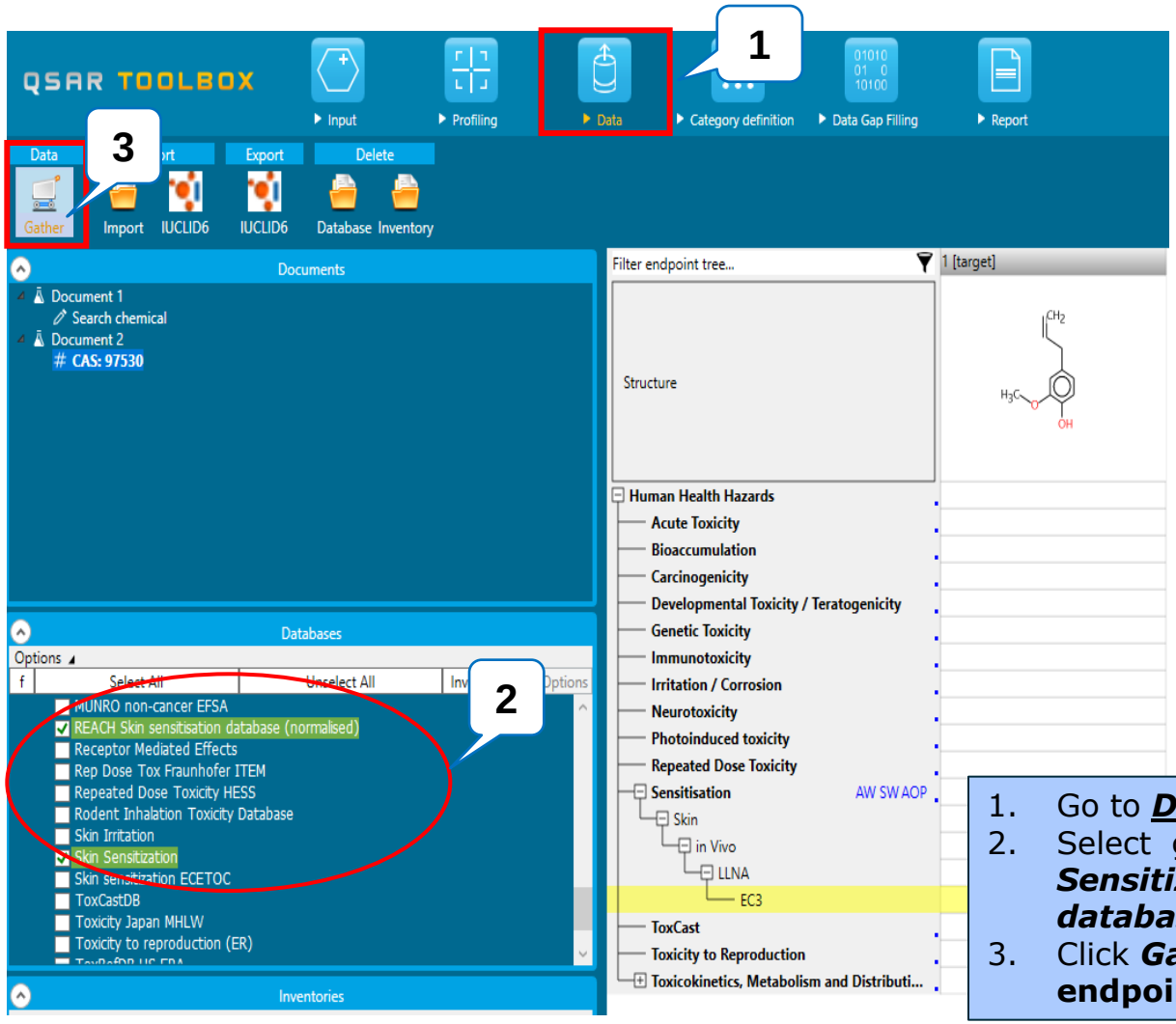
Profiling results



Data
Overview

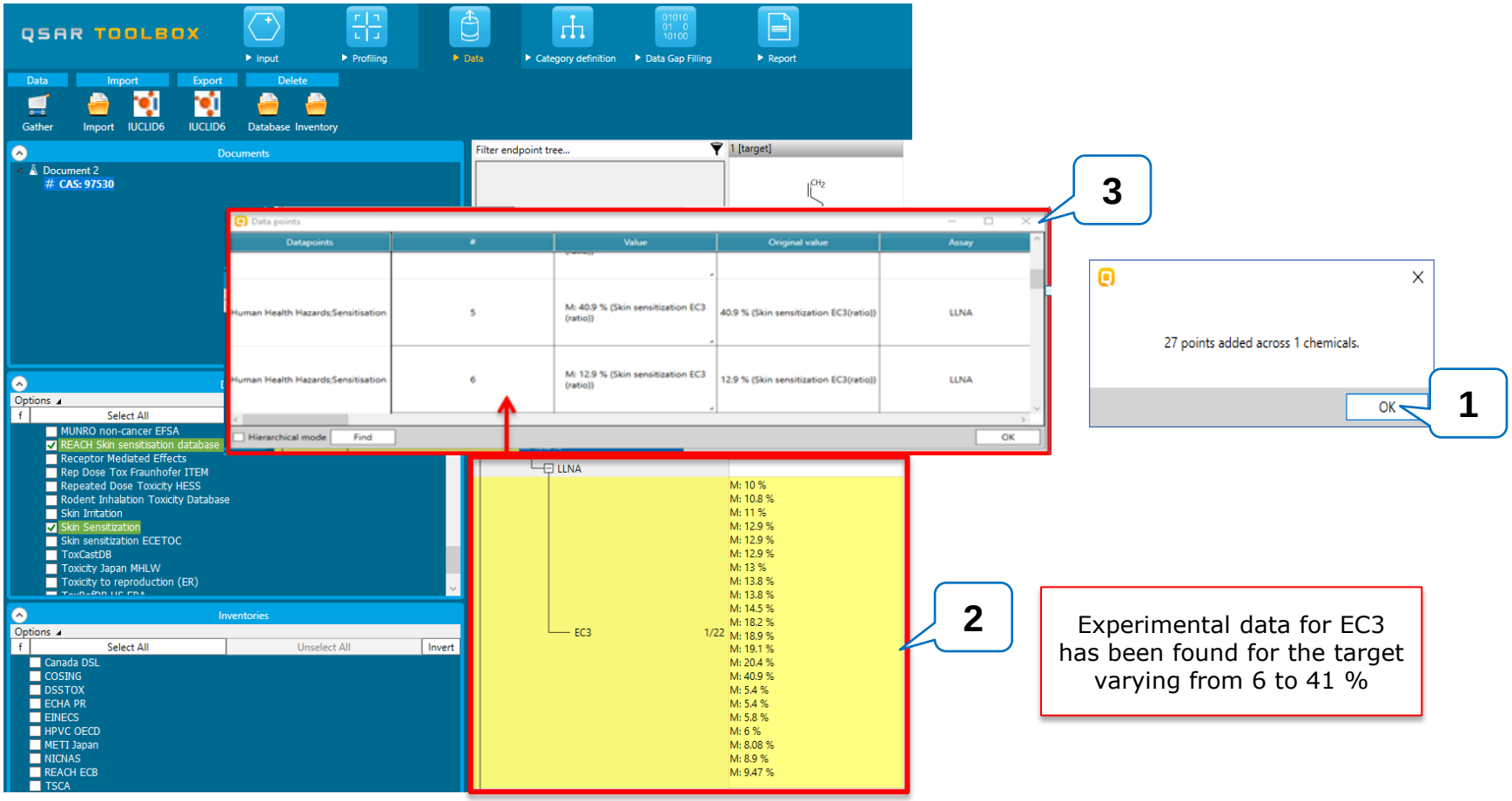
- "Data" refers to the electronic process of retrieving the environmental fate, eco-toxicity and toxicity data that are stored in the Toolbox.
- Data gathering can be executed in a global fashion (i.e., collecting all data for all endpoints) or on a more narrowly defined basis (e.g., collecting data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

Data
Gather data



1. Go to ***Data*** module
2. Select green highlighted databases - ***Skin Sensitization, REACH Skin sensitisation database (normalised)***;
3. Click ***Gather*** **(read data for all endpoints)**

Data
Gather data



1. A pop-up message informs that there are 27 experimental data points for the target chemical. Click **OK**
2. 22 out of 27 data points are associated with the target endpoint EC3 and they vary from 6 to 41 % which falls in the range of Positive data
3. **Double-click** on the cell to display the metadata information for the observed data

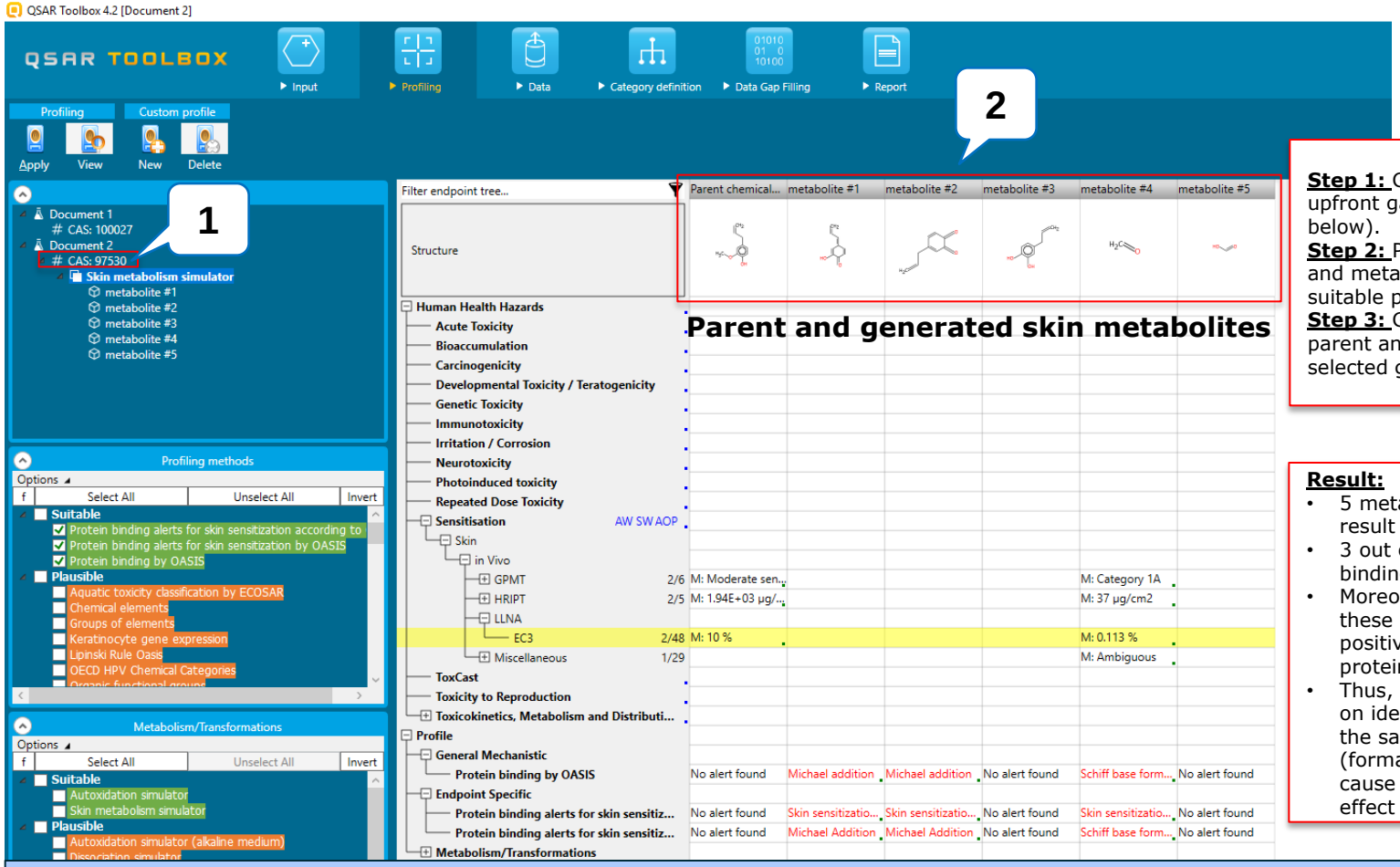
Data
Gather data

- 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 database(s). In this example only the *Skin Sensitization and REACH and Skin sensitisation database (normalised)* are selected.

Recap

- In module *Input*, you entered the target chemical and defined the target endpoint.
- 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 a(n) (a)biotic activation.
- In the *Data* module, you saw the database corresponding to the defined target endpoint. You also found positive experimental data for the target available in the selected databases.
- As skin sensitization is an in vivo effect, the skin generated metabolites are further investigated trying to explain the positive experimental data of the target

Handling of a skin metabolism



Step 1: Generate skin metabolite upfront gap filling (how to do it see below).
Step 2: Profile the package: parent and metabolites according to all suitable profilers
Step 3: Gather data for package: parent and metabolites from the selected green Dbs

Result:

- 5 metabolites are generated as a result of a skin metabolism
- 3 out of 5 have a positive protein binding alert
- Moreover a formaldehyde (out of these 3 metabolites) having a positive alert for interaction with proteins has positive EC3 data
- Thus, next actions are focused on identifying analogues having the same metabolite (formaldehyde), which could cause the skin a sensitization effect

1. Right click over the level with **CAS#** in the document tree and select **Multiplication/Metabolism/Transformation/Skin metabolites** (all steps are not shown here)
2. Metabolites appeared next to the parent

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.

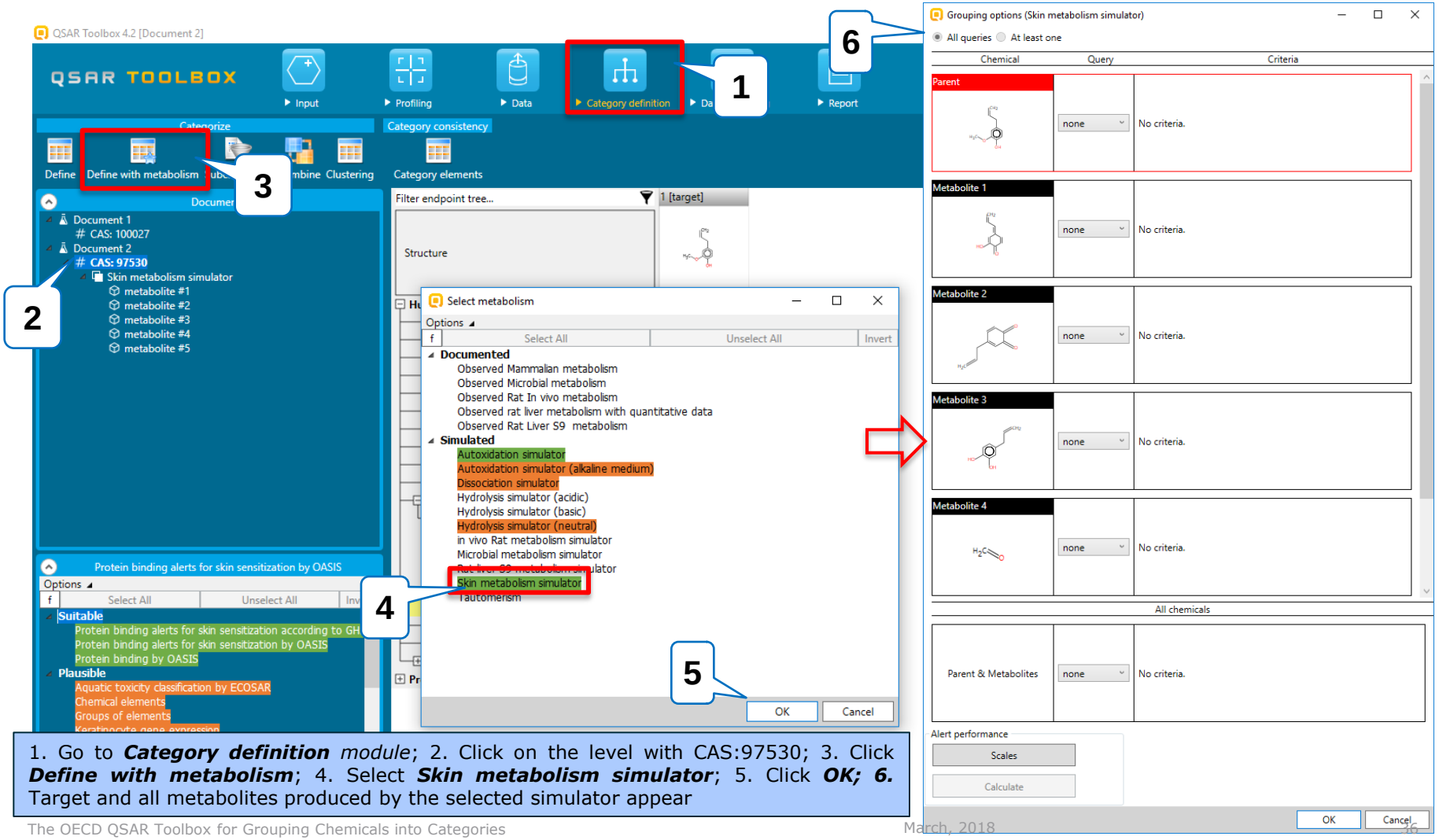
- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind by the same mechanism and for which experimental results are available.

- 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 searching for analogues is based on a common metabolite (formaldehyde) generated as a result of skin metabolism (see next slide)

Category Definition

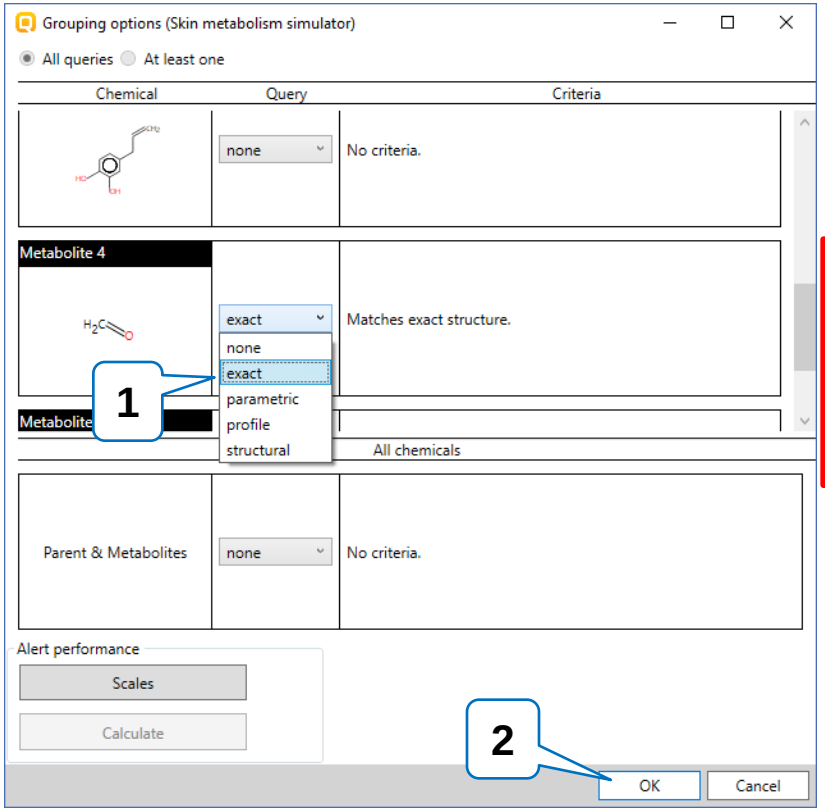
Searching for analogues accounting for skin metabolism



1. Go to ***Category definition*** *module*; 2. Click on the level with CAS:97530; 3. Click ***Define with metabolism***; 4. Select ***Skin metabolism simulator***; 5. Click ***OK; 6.*** Target and all metabolites produced by the selected simulator appear

Category Definition

Searching for analogues accounting for skin metabolism



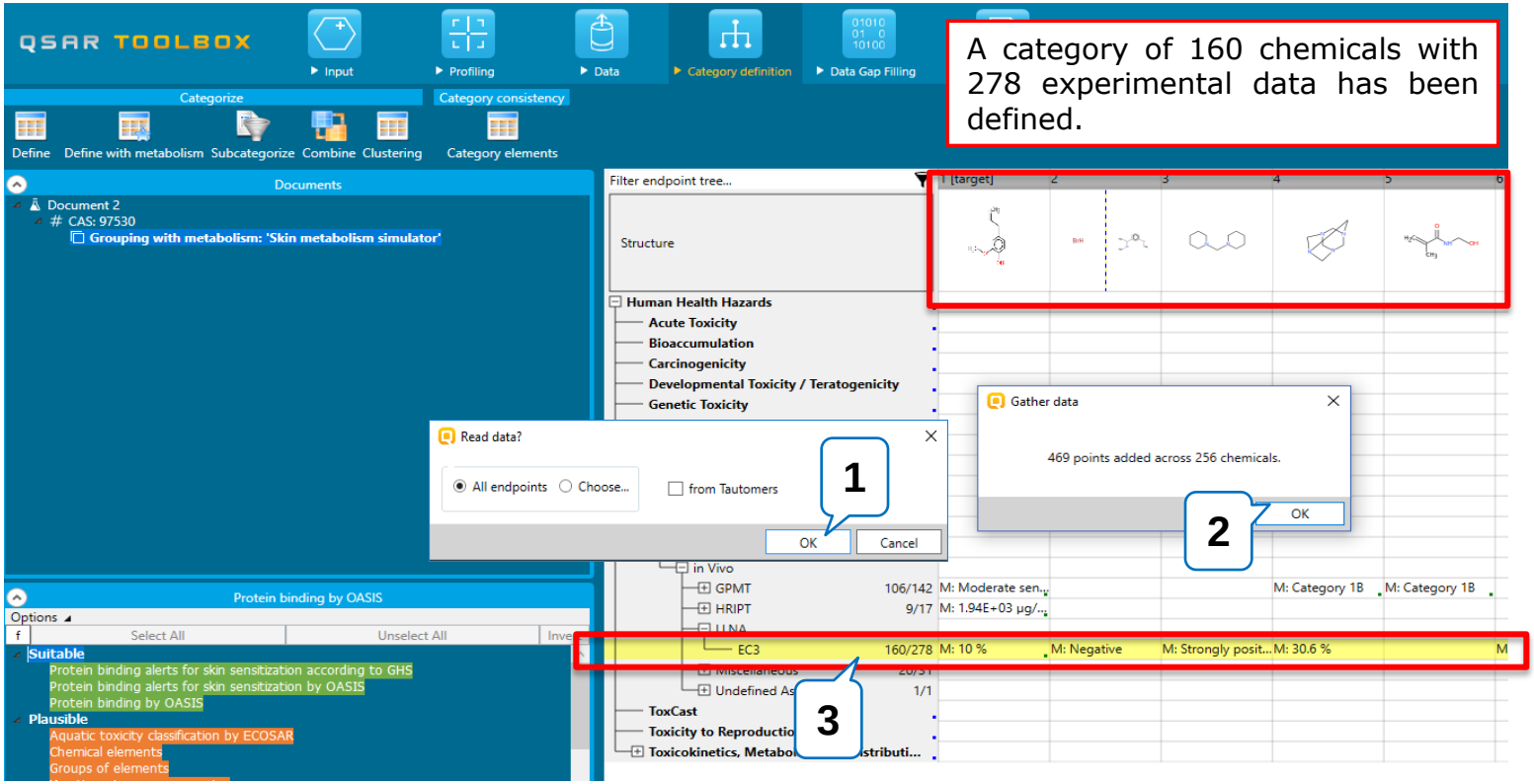
The **Exact** option is used for searching analogues with for common metabolite. This option performs search for analogues which metabolites have the exact structure of the target metabolite

1. Select ***Metabolite # 4*** *(Formaldehyde)* and click on ***Exact*** option from drop down menu;
2. Click ***OK*** in Grouping options window to execute the search.

More details for grouping with metabolism could be found in the following tutorial:
http://oasis-lmc.org/media/74762/Tutorial_20_TB_4.1_New%20options%20for%20grouping%20with%20metabolism.pdf

Category Definition

Searching for analogues accounting for a skin metabolism



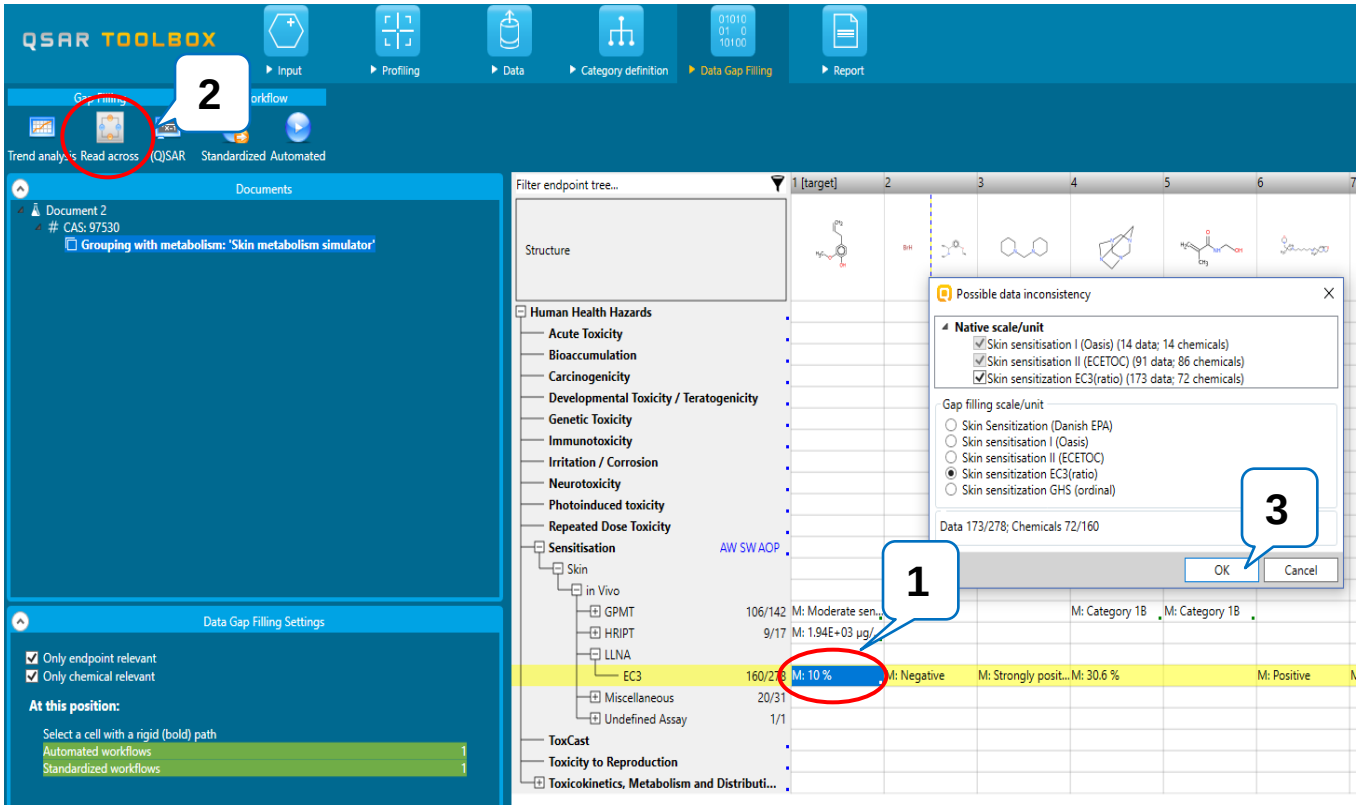
1. Click ***OK*** to read all data; 2. An information window appears informing about the number of experimental data collected and the number of chemicals in the category, click ***OK***.
3. The experimental data of analogues are displayed on data matrix in a yellow colored row.

Data Gap Filling
Overview

- "Data Gap Filling" module gives 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.

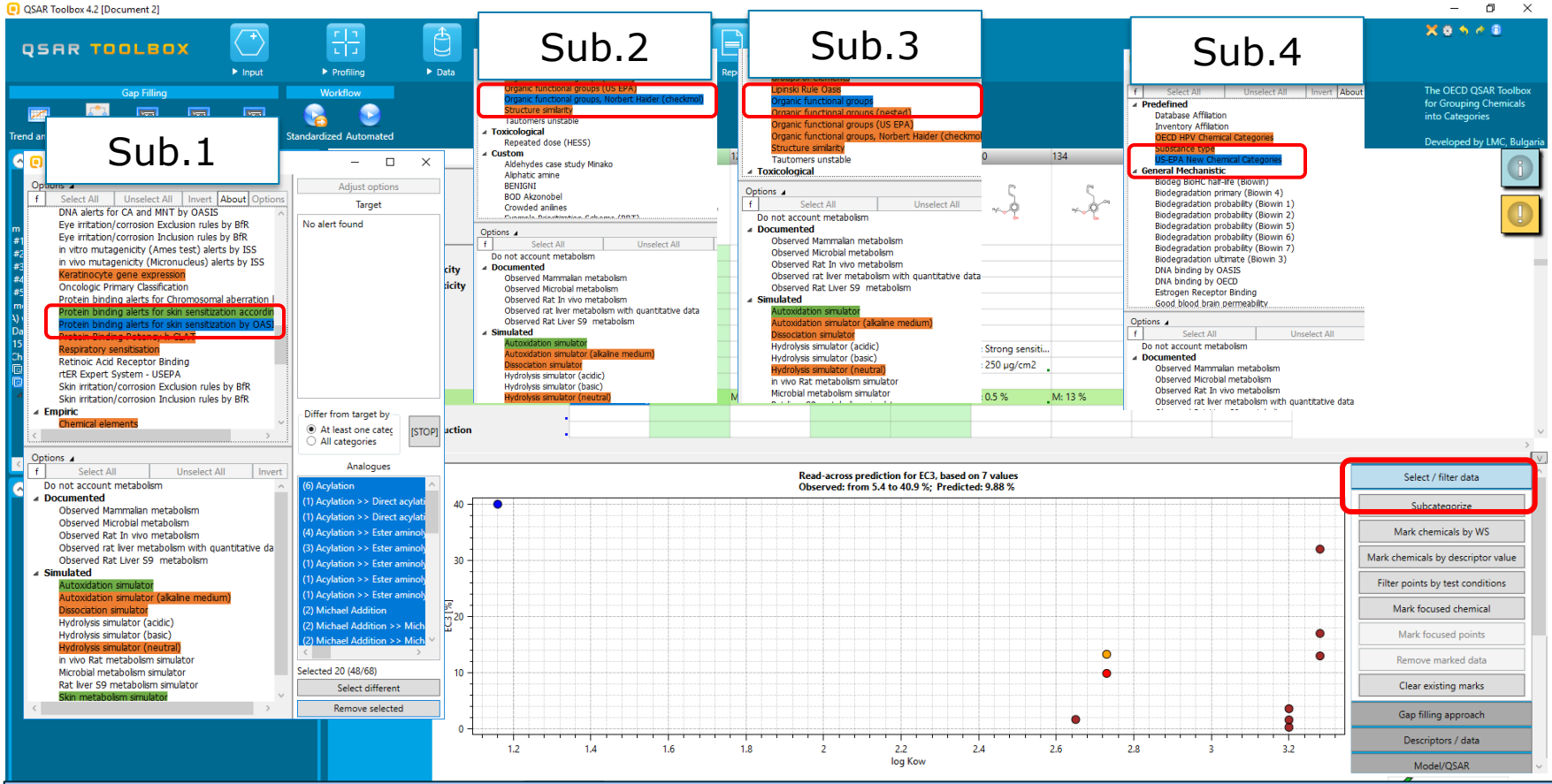
In this example we will use the read-across approach.

Data Gap Filling
Apply Read-across



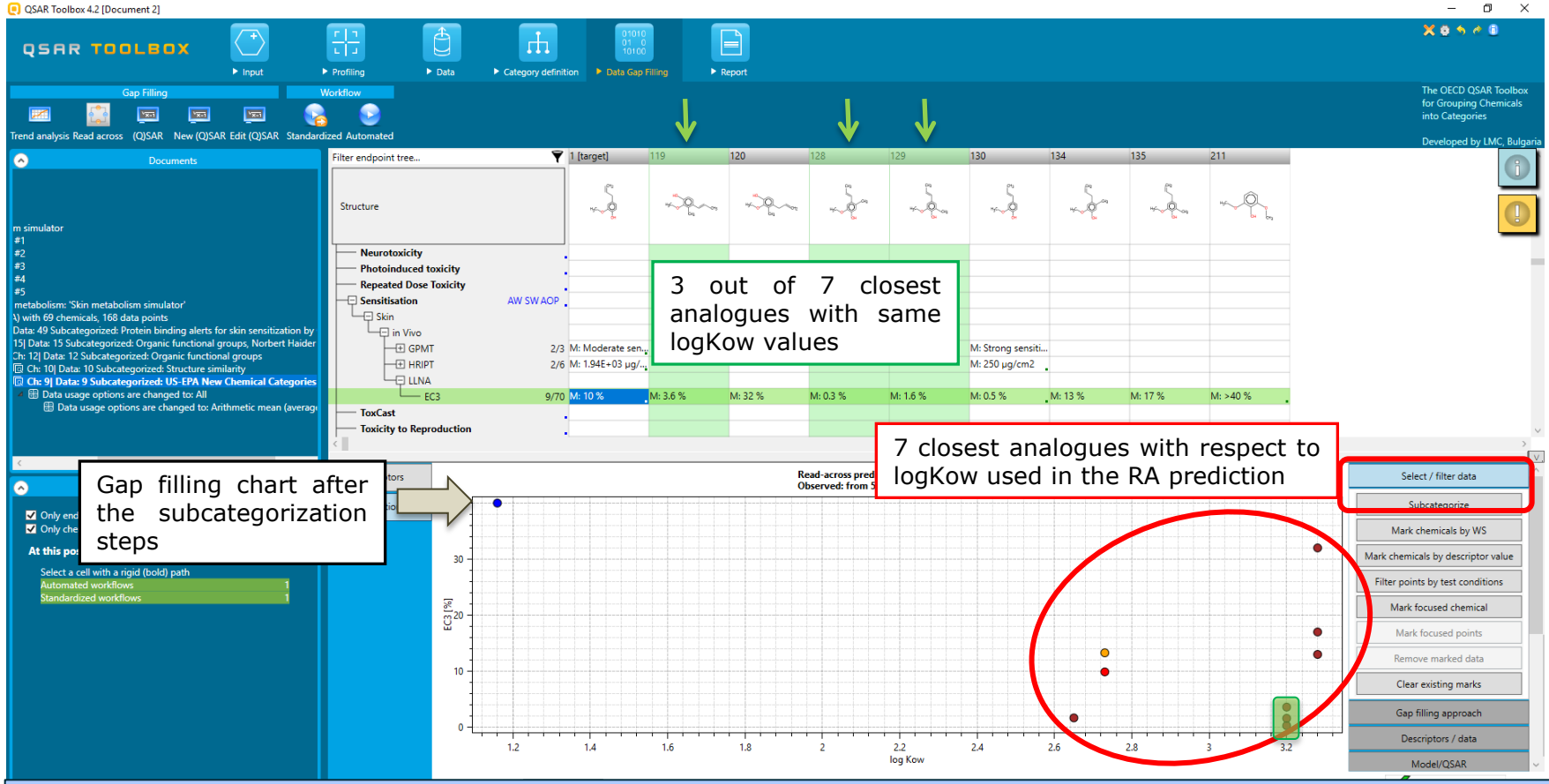
1. Click the cell corresponding to ***Human Health Hazards#Sensitisation#Skin#in Vivo#LLNA#EC3*** for the target chemical; 2. Click ***Read-across***; 3. A pop-up window informing about possible data inconsistency appears, select ***Skin sensitization EC3 (ratio) and OK***

Data Gap Filling
Apply Read-across



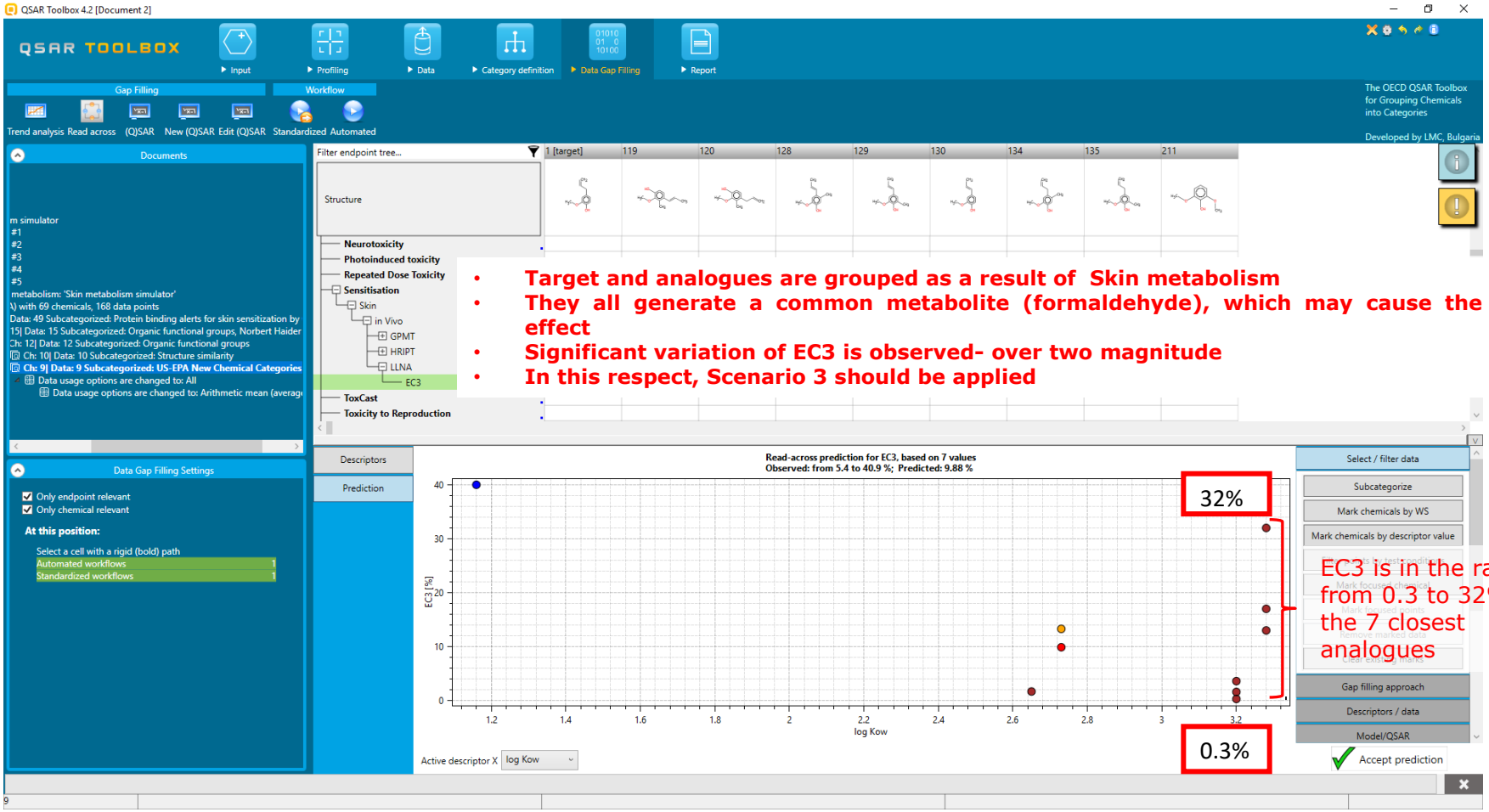
Open **Select/Filter data** and **Subcategorize** by: 1) Protein binding alerts for skin sensitization by OASIS; 2) Organic functional groups, Norbert Haider (checkmol); 3) Organic functional groups; 4) US-EPA New Chemical Categories. After each appalied subcategorization, remove dissimilar analogues using "Remove selected" button

Data Gap Filling
Apply Read-across



Aa a result the predicted EC3 value for the target is 9.88% based on 7 closest analogues with respect to logKow. The prediction is obtained on 7 instead of 5 analogues because 3 of the analogues have same logKow values.

Data Gap Filling
Read-across recap

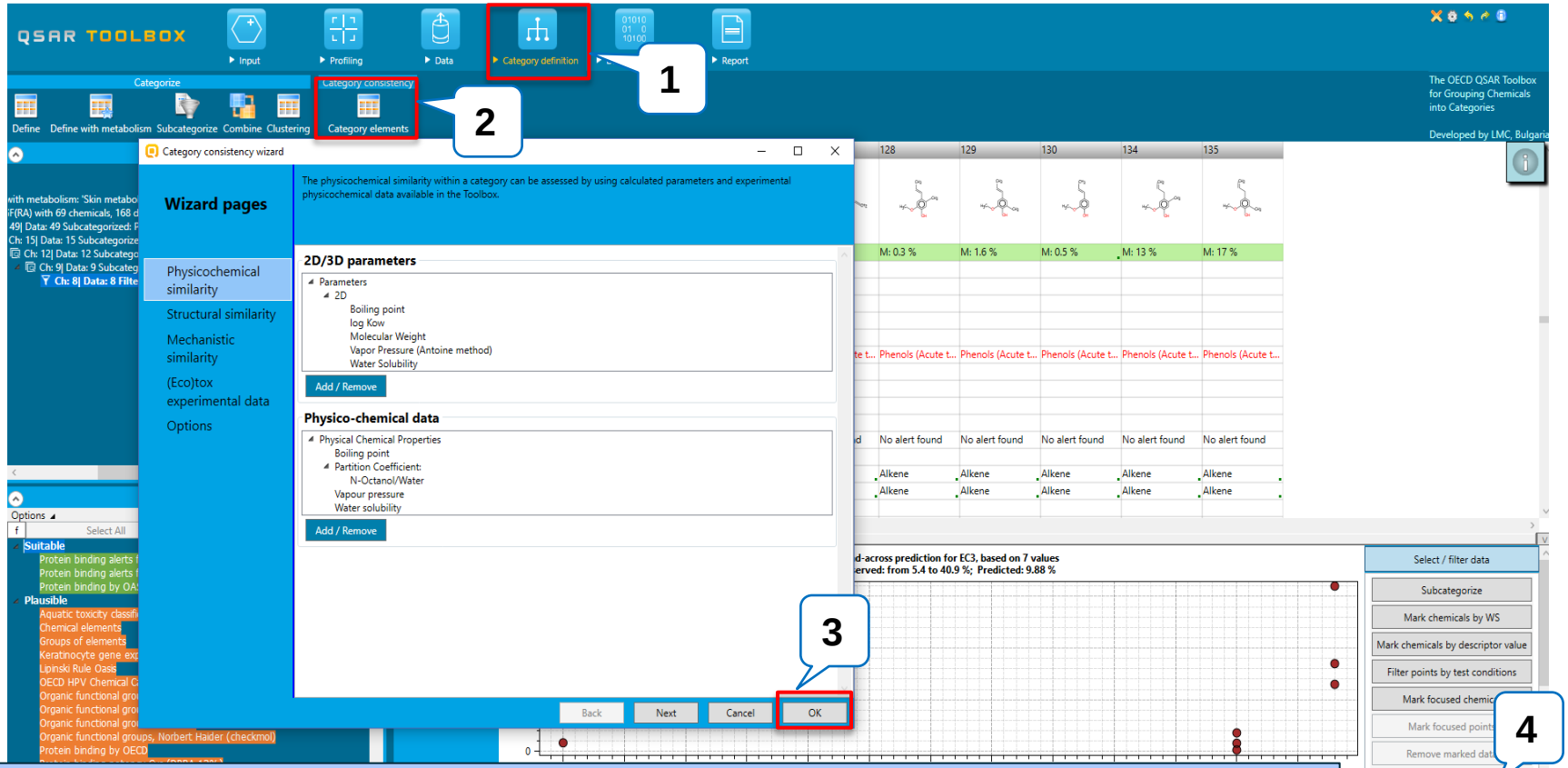


- **Target and analogues are grouped as a result of Skin metabolism**
- **They all generate a common metabolite (formaldehyde), which may cause the toxicity effect**
- **Significant variation of EC3 is observed- over two magnitude**
- **In this respect, Scenario 3 should be applied**

EC3 is in the range from 0.3 to 32% for the 7 closest analogues

Data Gap Filling

Apply Category consistency elements



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 72 chemicals having common metabolites (formaldehyde) as a result of skin metabolism.
- In *Data gap filling* module you applied a read-across approach. Read-across is the appropriate data-gap filling method for a "qualitative" endpoints like skin sensitisation. Three subcategorizations based on a protein binding mechanism and structural features are applied. As a result read-across prediction is based on 7 closest analogues.
- Significant variation of EC3 data is 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.

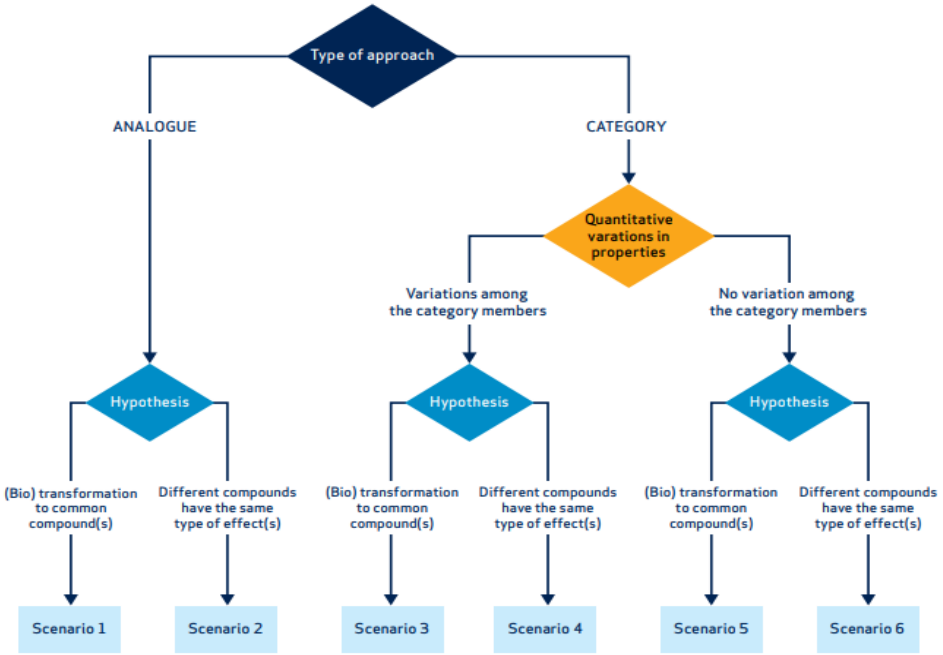
Report
Overview

- 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 a RAAF scenario

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

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



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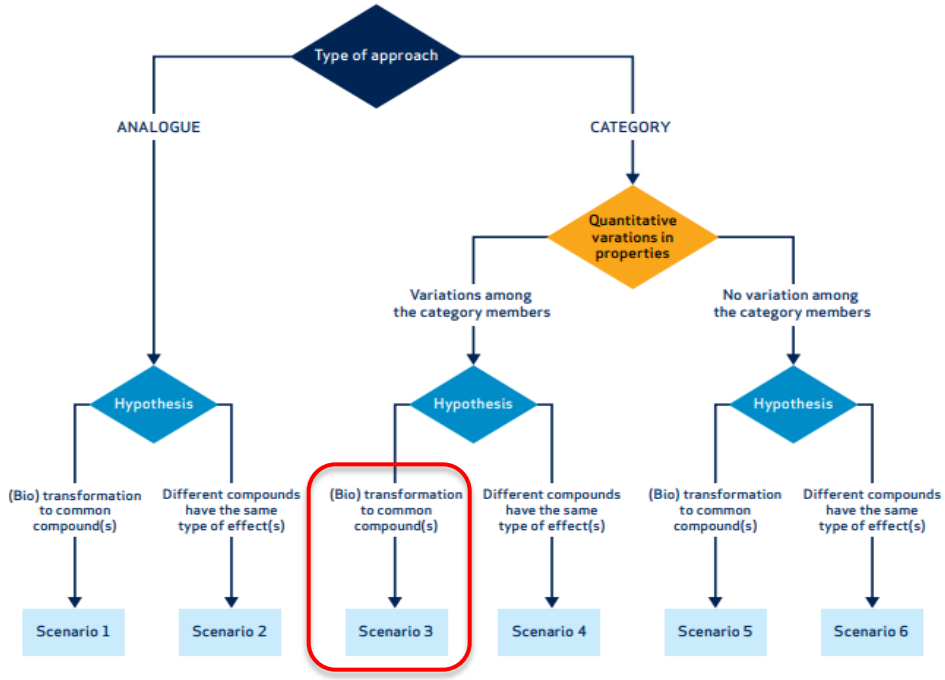
Report
Selection of a RAAF scenario



For this example the following criteria are met :

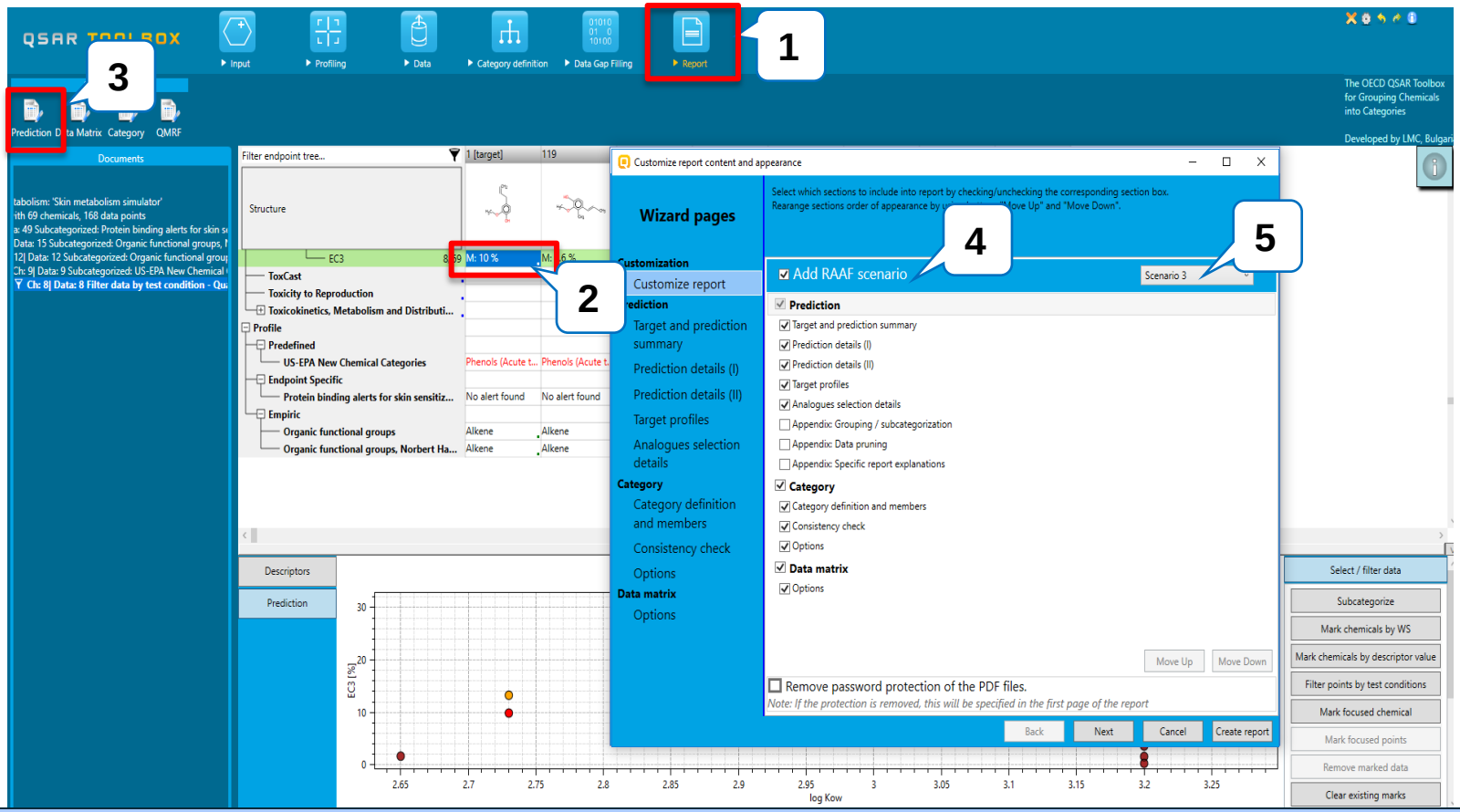
- the type of approach applied - category approach is used (threshold of > 3 analogues is proposed by LMC for the category approach);
- the read-across hypothesis – different compounds (bio)transformed to the common compound;
- There is a significant variation of the toxic effect (EC3) among the category members

Based on that a RAAF scenario 3 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

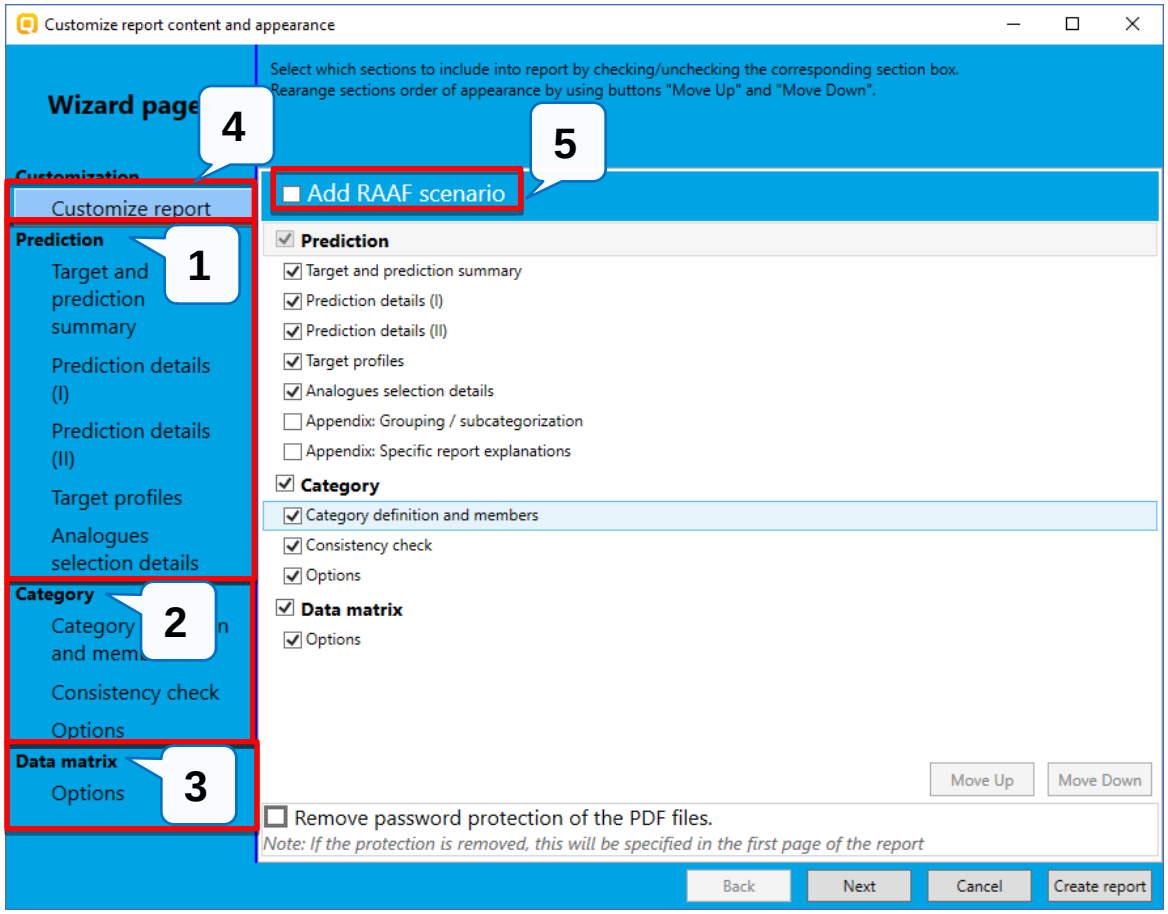
Report

Report Generation according to RAAF-Scenario 3



1. Go to the ***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 3** from the drop-down menu.

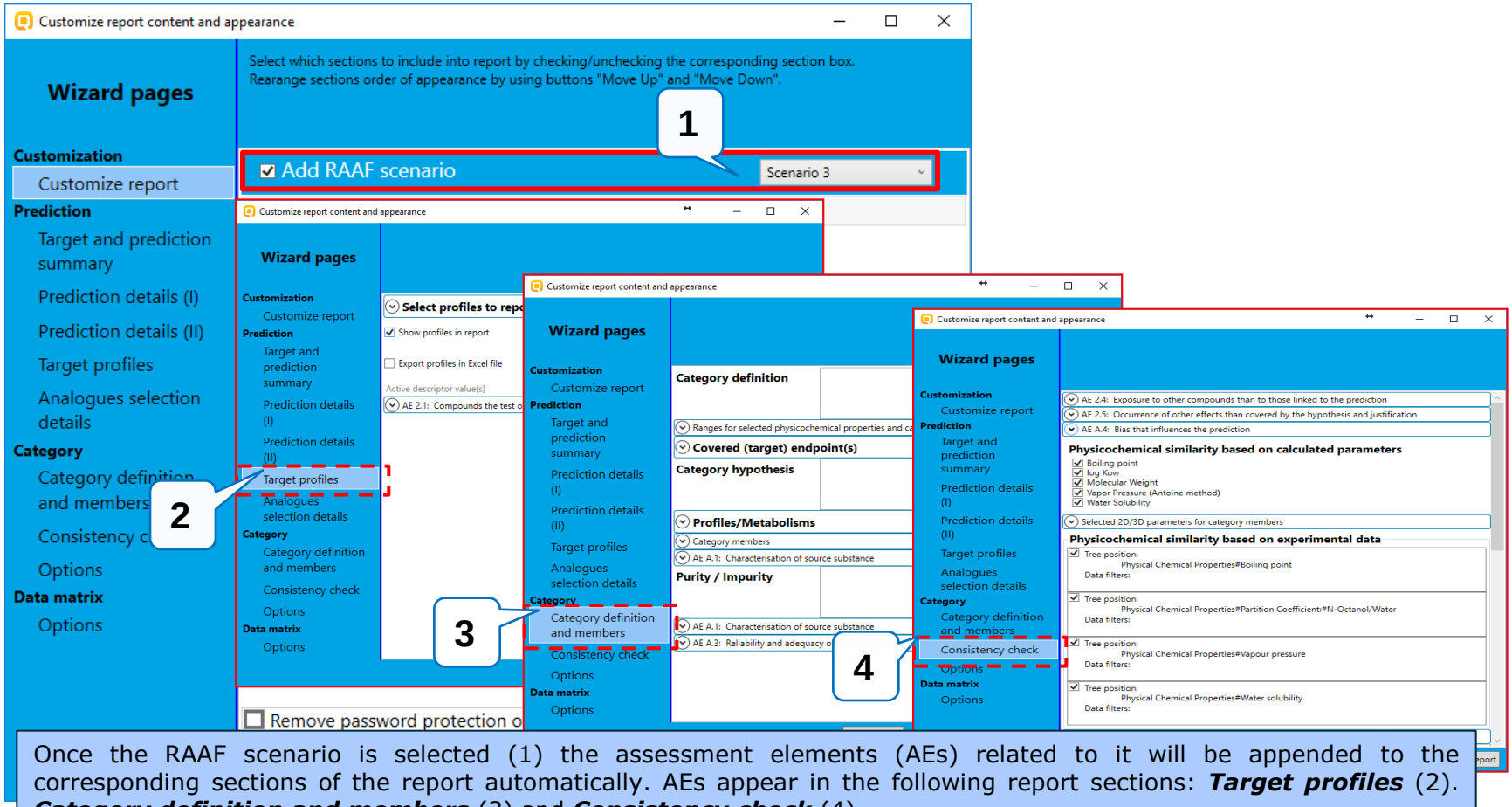
Report
Report Generation according to RAAF-Scenario 3



After selection the button ***Prediction*** the ***Report wizard*** appears. It consists of three sections related to the types of report - ***Prediction*** (1), ***Category*** (2) and ***Data matrix*** (3).The content of each of these three files could be customized in the first page of the ***Wizard pages*** *(4)*
Here you could select ***Scenario*** through *Add RAAF scenario (5)*

Report

Report Generation according to RAAF-Scenario 3

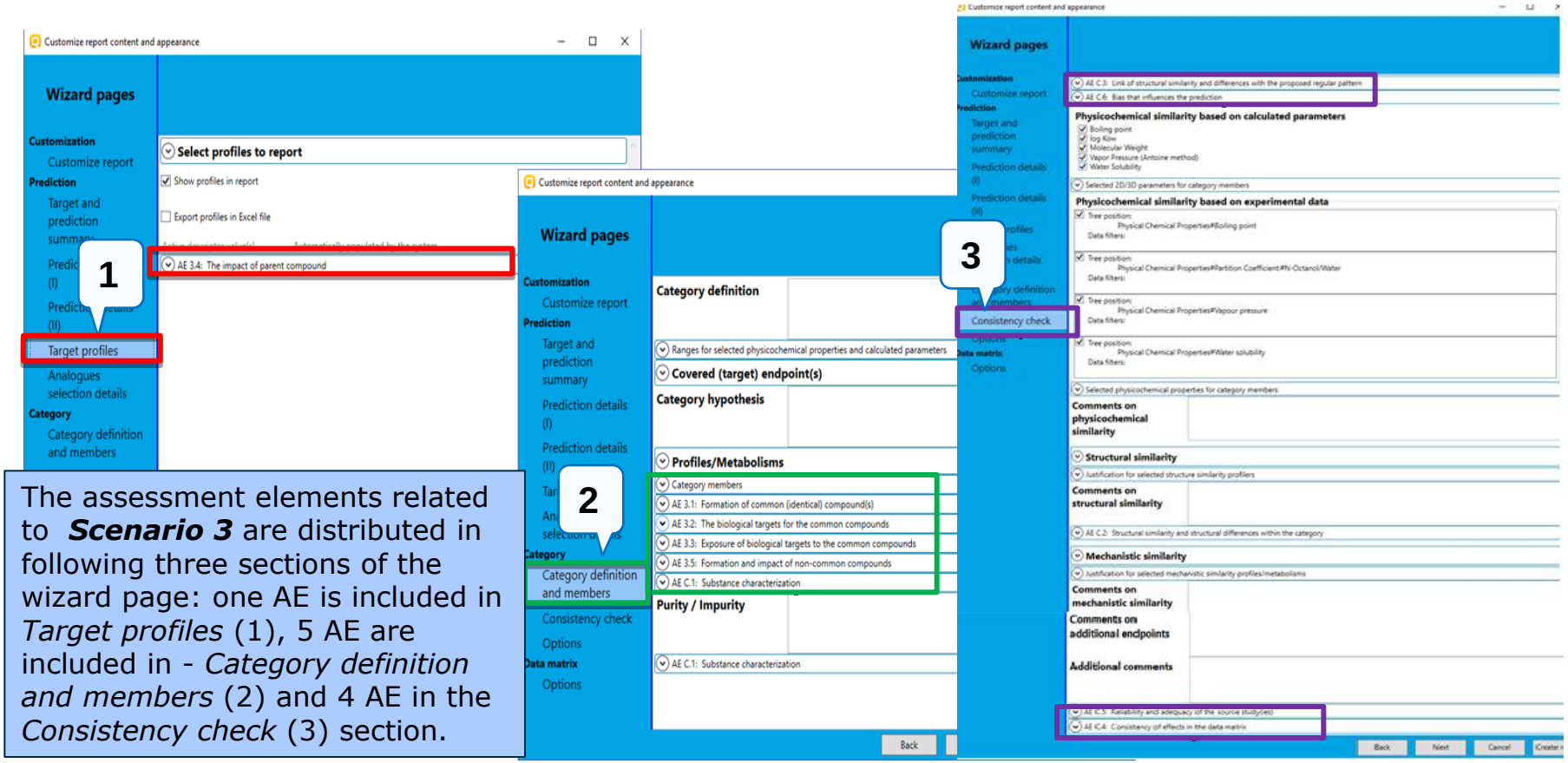


Once the RAAF scenario is selected (1) the assessment elements (AEs) related to it will be appended to the corresponding sections of the report automatically. AEs appear in the following report sections: ***Target profiles*** (2). ***Category definition and members*** (3) and ***Consistency check***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

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

Report
Report Generation according to RAAF-Scenario 3

AEs related to each scenario have been associated to corresponding report section



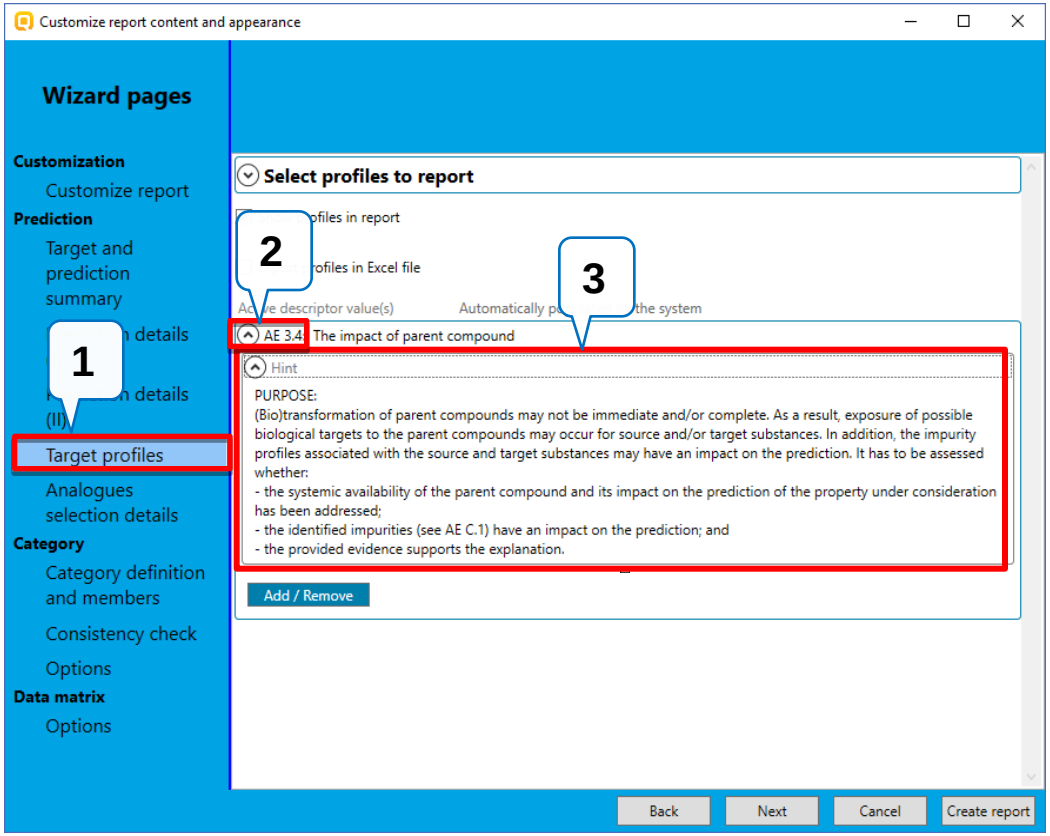
The assessment elements related to ***Scenario 3*** are distributed in following three sections of the wizard page: one AE is included in *Target profiles* (1), 5 AE are included in - *Category definition and members* (2) and 4 AE in the *Consistency check* (3) section.

Report
Report Generation according to RAAF-Scenario 3

Section Prediction

Subsection: Target profilers

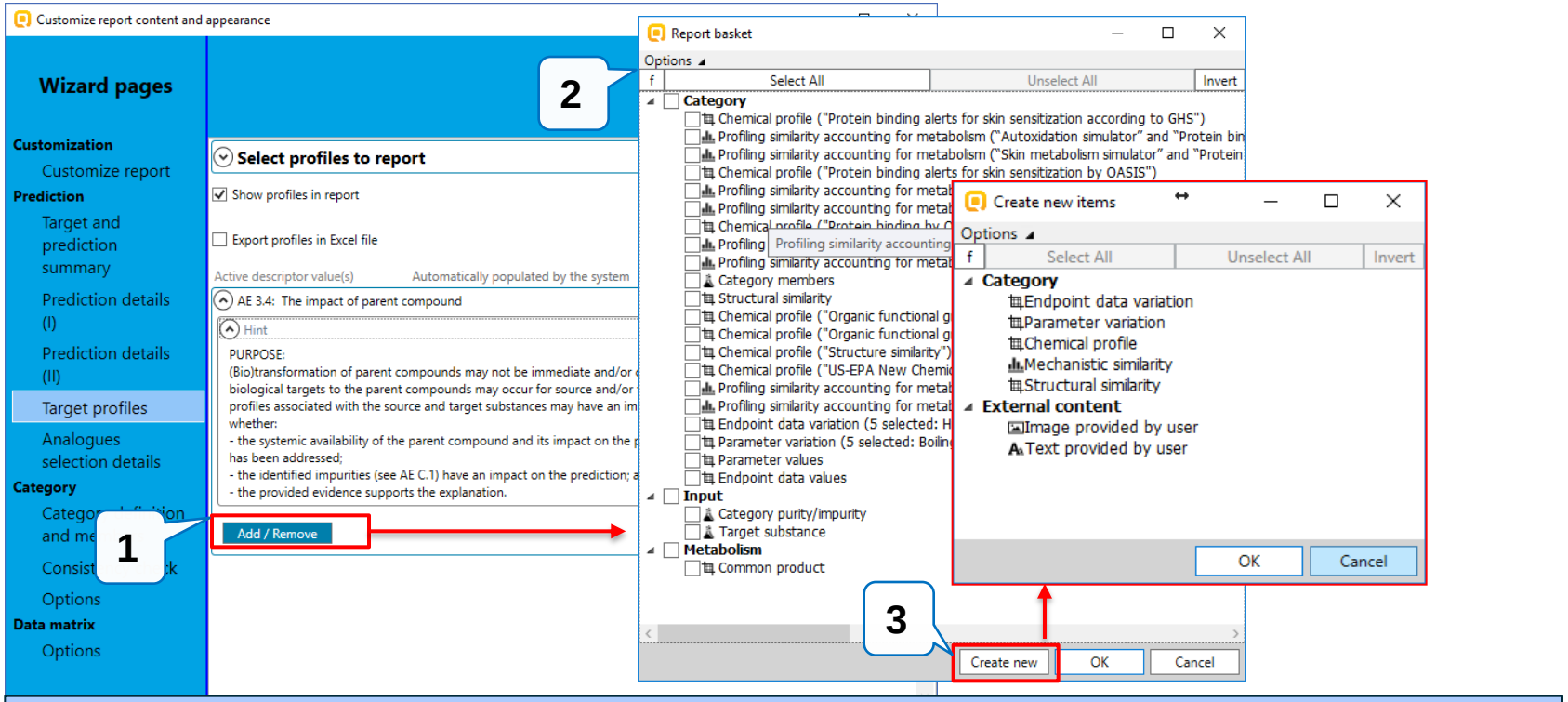
AE 3.4.The impact of a parent compound: manual including to the Target profiles



1. Select ***Target profiles;***
2. Expand the **AE 3.4**
3. Hint showing the purpose of each AE is available.

Report

Report Generation according to RAAF-Scenario 3



Information can be included by clicking the **Add/Remove** button (1) located below the corresponding AE. The *Add/Remove* button invokes the so-called "***Report basket***" (2). 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 (3).

Items with external content (text and picture) will be added for **AE 3.4.The impact of a parent compound: manual including to the Target profiles (see next two slides)**

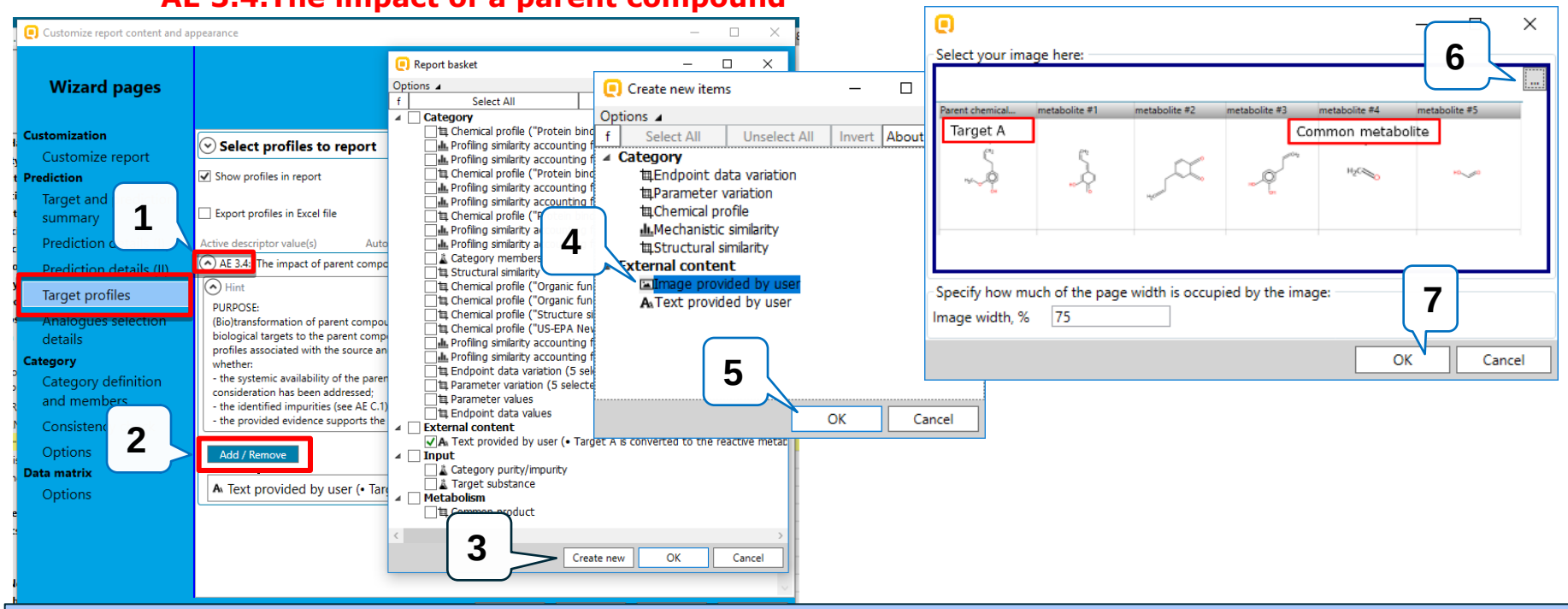
Report

Report Generation according to RAAF-Scenario 3

Section Prediction

Subsection: Target profilers

AE 3.4.The impact of a parent compound



In order to add picture to the report: expand the AE (1), click ***Add/Remove*** (2), click ***Create new*** (3) in Report basket window, then click ***Image provided by user*** (4) and click **OK** (5). A new window appears where you can add your custom picture by Copy/Paste or browsing (6) to the directory in your PC where the desired picture is saved*. Finally confirm by **OK** (7).

*In the current example a picture illustrating the target chemical marked as **Target A** and formaldehyde marked as **Common metabolite** was prepared in advance.

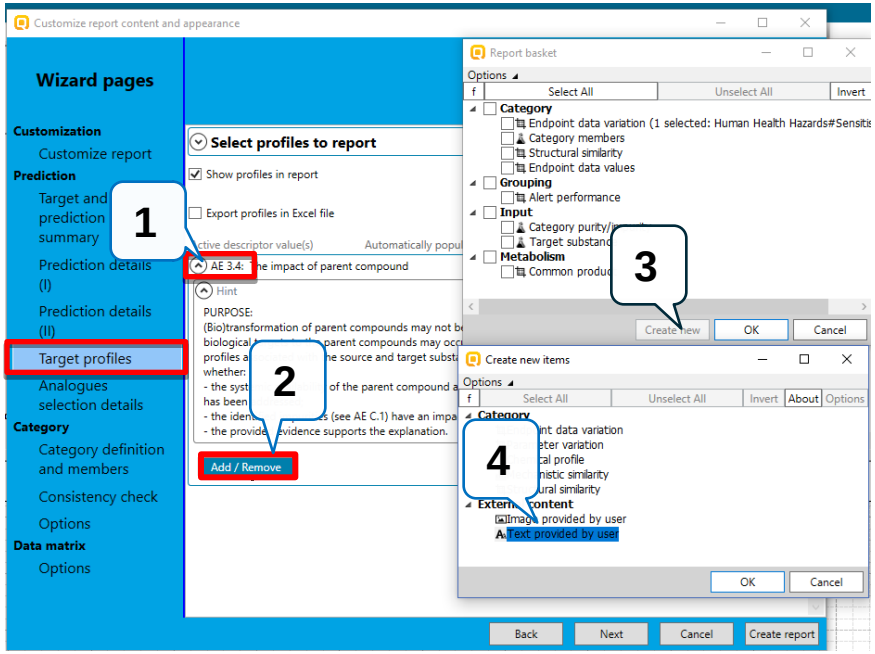
Report

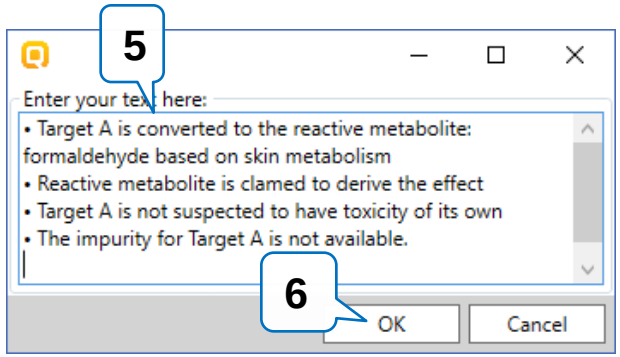
Report Generation according to RAAF-Scenario 3

Section Prediction

Subsection: Target profilers

AE 3.4.The impact of a parent compound





In order to add text information to the report: expand the AE (1), click ***Add/Remove*** (2), click ***Create new*** (3) in the Report basket window, click ***Text provided by user*** (4). Copy the following example text:

- *Target A is converted to the reactive metabolite: formaldehyde based on a skin metabolism*
- *Reactive metabolite is clamed to derive the effect*
- *Target A is not suspected to have toxicity of its own*
- *Impurity for the Target A is not available.*

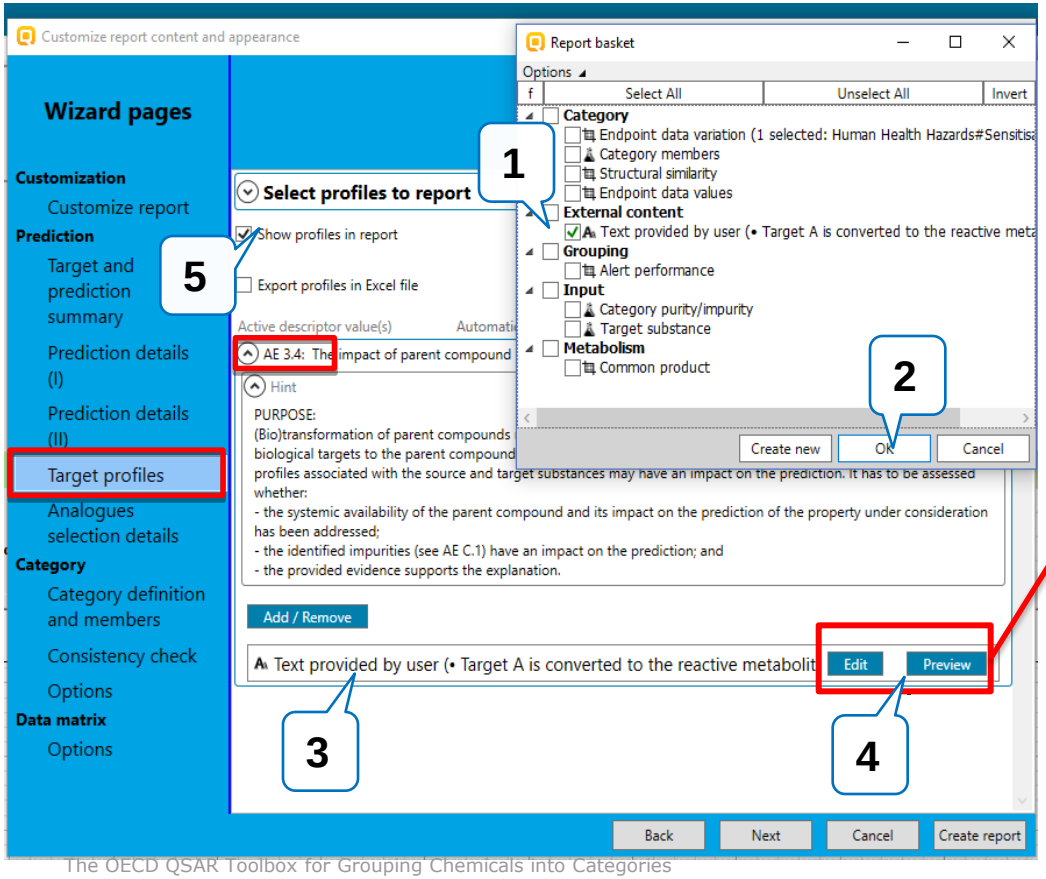
And paste it in the new window(5). Finally confirm by ***OK*** (6).

Report
Report Generation according to RAAF-Scenario 3

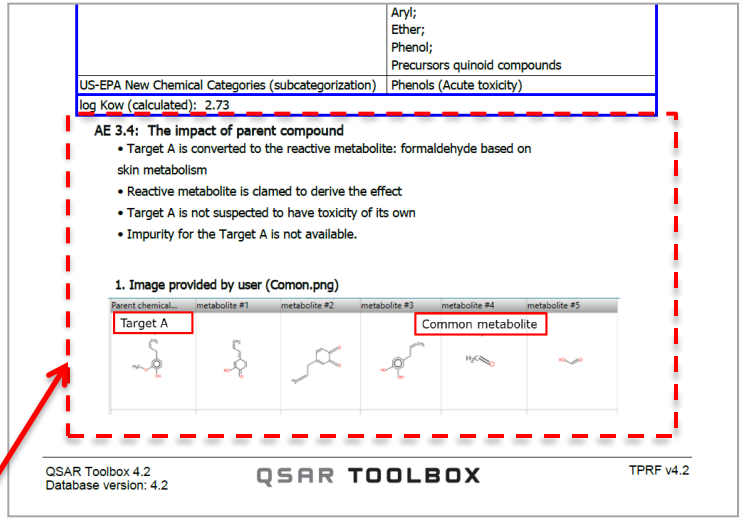
Section Prediction

Subsection: Target profilers

AE 3.4.The impact of a parent compound



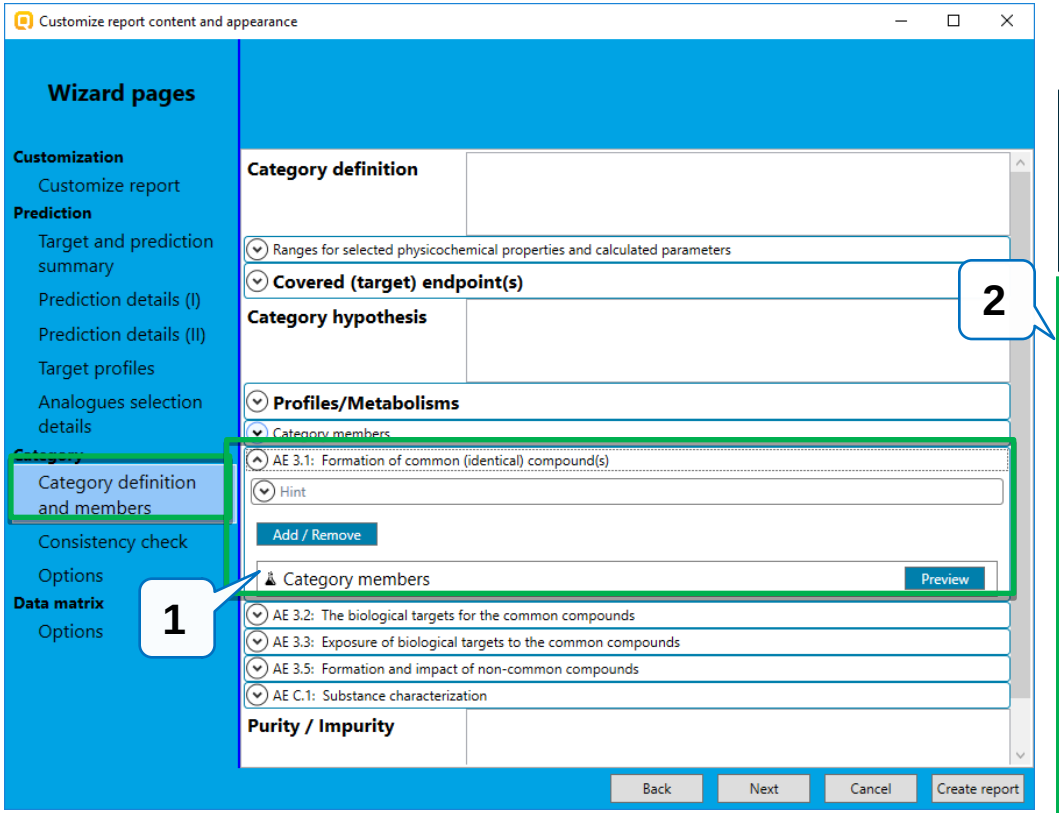
Example how the **AE 3.4** will look in the generated report is shown below.



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 (4) for **previewing** and **editing**.

Report
Report Generation according to RAAF-Scenario 3

Section Category
Subsection: Category definition and members
AE 3.1 Formation of (a) common (identical) compound(s)



The members of the category (the 7 closest analogues) automatically appeared in the **AE 3.1** (1). Additionally text could be added by click on the **Add/Remove** button (1) and **create new item** with a textual content (2) (see slide 55, how to add the item)

An example text for **AE 3.1: Formation of (a) common (identical) compound(s)**

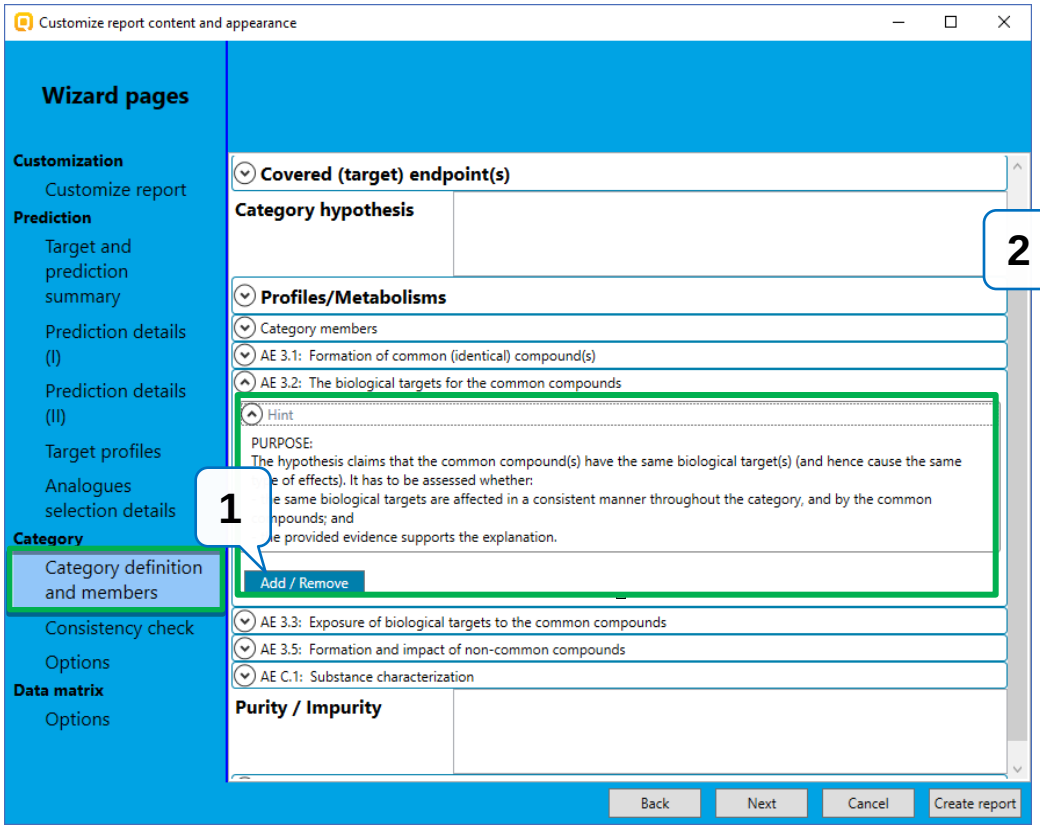
- Target chemical A is claimed to be metabolized to formaldehyde and that the organism is only systemically exposed to formaldehyde upon an external exposure to Target A.
- The seven source substances (analogues) as a result of a Skin metabolism have generated the common metabolite - formaldehyde
- Therefore, it is expected for the formaldehyde to be responsible for the toxic effect
- The seven substances with LLNA assay are used to predict the Skin sensitization effect for the target substance A

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.2 The biological targets for the common compounds



Click on the **Add/Remove** button (1) and **create new** with the following example text (2):

An example text for **AE 3.2. The biological targets for the common compounds**

- As a result of grouping accounting for a skin metabolism the seven source substances (B, C, D, E, F, G and K*) are obtained.
- Both - target and source substances, are activated as a result of a skin metabolism. They all formed a common metabolite: formaldehyde
- The common metabolite is responsible for the binding with proteins via Schiff base mechanism and may cause the toxic effect
- The seven source substances are used to predict the toxic effect of substance A

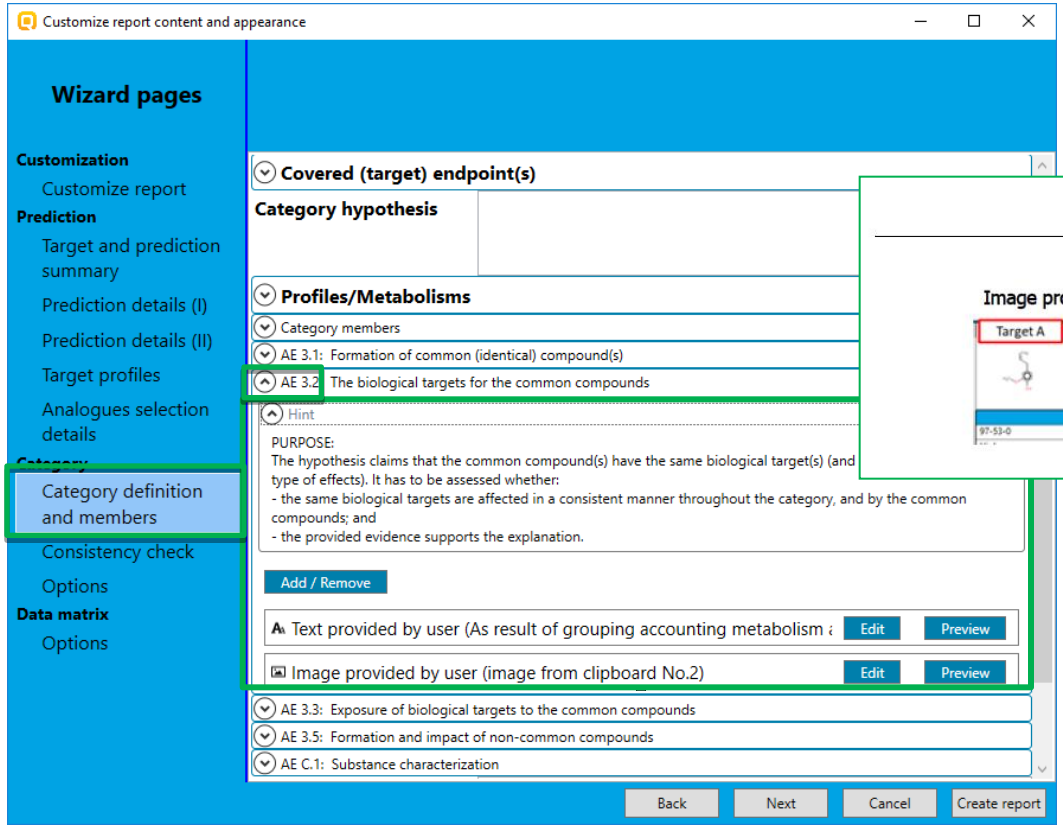
The picture showed the parent and the seven source substances could be added in this AE (see next slide)

Report
Report Generation according to RAAF-Scenario 3

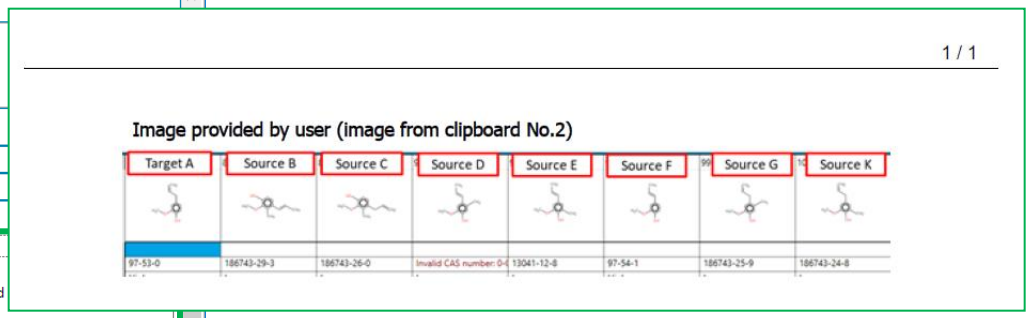
Section Category

Subsection: Category definition and members

AE 3.2 The biological targets for the common compounds



The possible image added to in AE 3.2 (see slide 54 how to create new image report item):

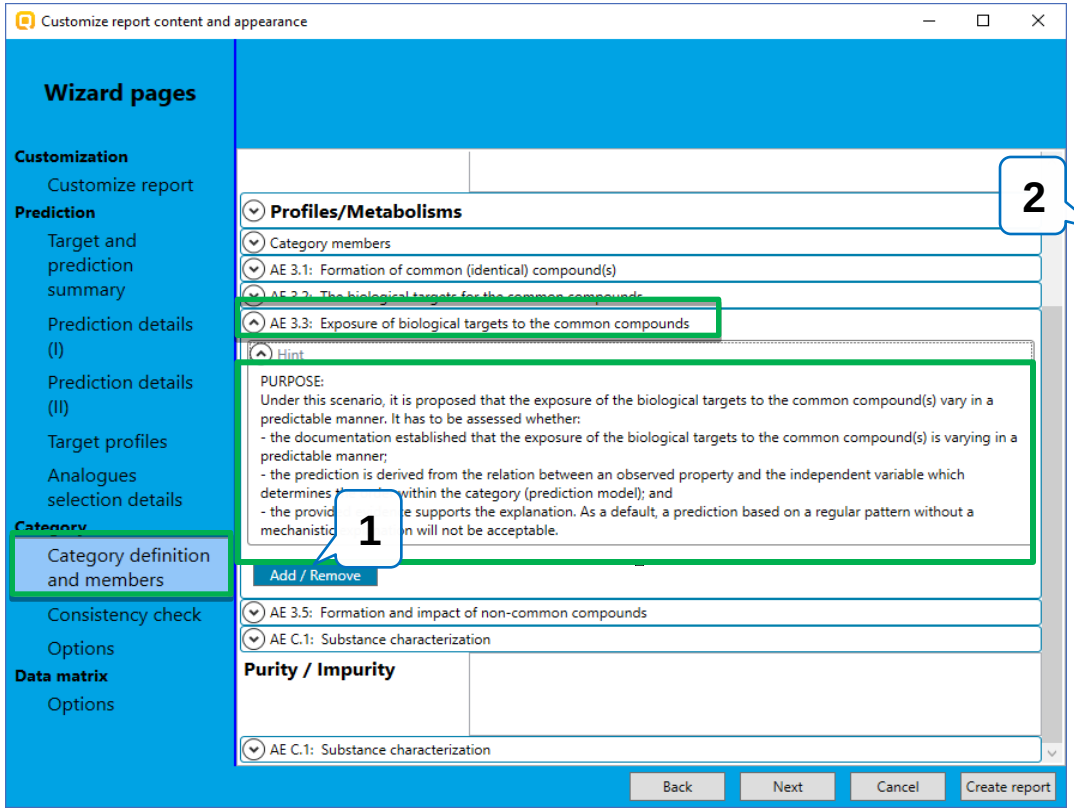


Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.3 Exposure of biological targets to the common compounds



Click on the **Add/Remove** button (1) and **create new item** with possible example text (2) added to the AE 3.3 :

An example text for **AE 3.3 Exposure of biological targets to the common compounds**

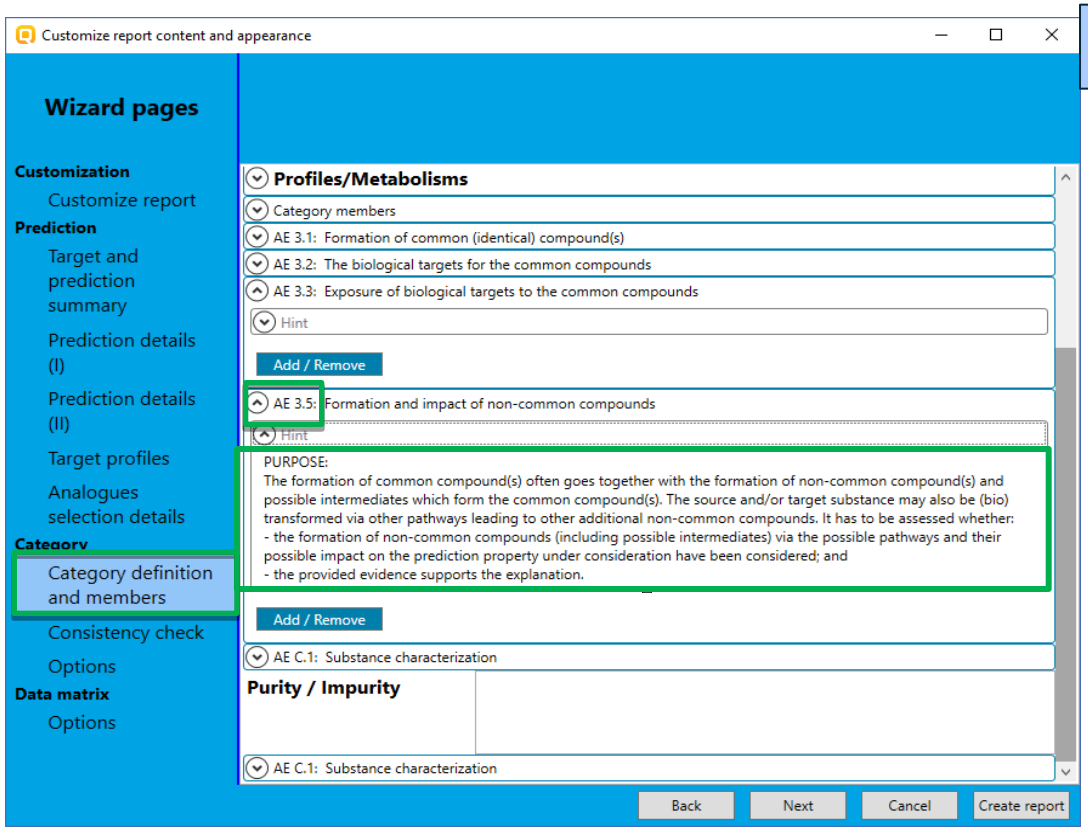
- The target chemical A and the source substances from B to K are metabolized to the common reactive metabolite: formaldehyde
- It well known from the literature (Ref. cited) that all aliphatic aldehydes can potentially undergo a **Schiff base formation** with a primary amine. The generated formaldehyde reacts with proteins via Schiff-base formation mechanism (see profiling results of the generated metabolites)
- It is expected that both the target and the set of source substances have the same metabolism pattern based on the common metabolite

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.5 Formation and impact of non-common compounds



The possible example text could be added to the **AE 3.5**:

An example text for **AE 3.5:**

- The target substance A and the seven source substances (analogues) are metabolized to the common- formaldehyde and non-common compounds (including possible intermediates)
- The positive effect might be due to the common compound (formaldehyde) reacting with proteins via a Schiff-base formation mechanism
- Also a positive effect of formaldehyde is supported by the positive EC3 data found for the target
- Some of the non-common compounds react with proteins by other mechanisms such as: Michael addition on quinoid type compounds, but they are not supported by the experimental data. Therefore:
    - The substance responsible for the skin sensitization effect might be due to the formed common compound
    - Also some of the non-common metabolites react with protein via other protein binding mechanisms. Thus they could cause effect too.

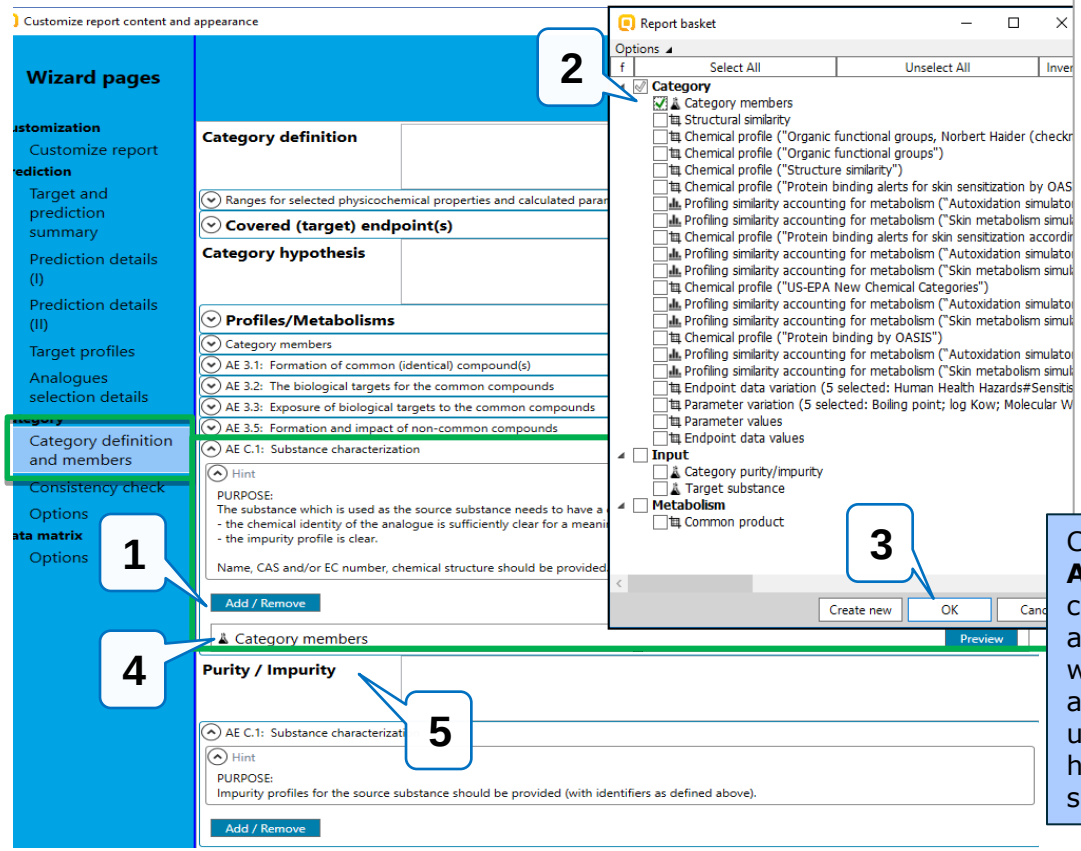
Report

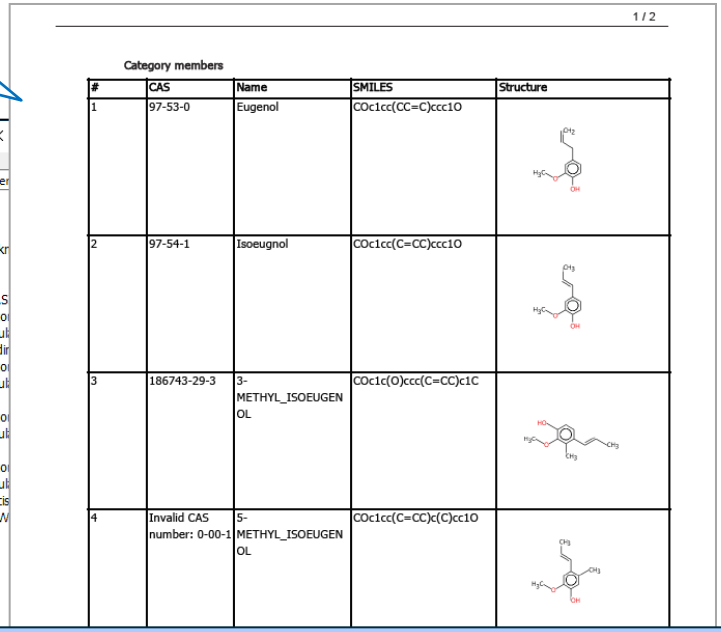
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE C.1 Substance characterization





1 / 2

Category members

#	CAS	Name	SMILES	Structure
1	97-53-0	Eugenol	COc1cc(CC=C)ccc1O	
2	97-54-1	Isoeugnol	COc1cc(C=CC)ccc1O	
3	186743-29-3	3-METHYL_ISOEUGENOL	COc1c(O)ccc(C=CC)c1C	
4	Invalid CAS number: 0-00-1	5-METHYL_ISOEUGENOL	COc1cc(C=CC)c(C)cc1O	

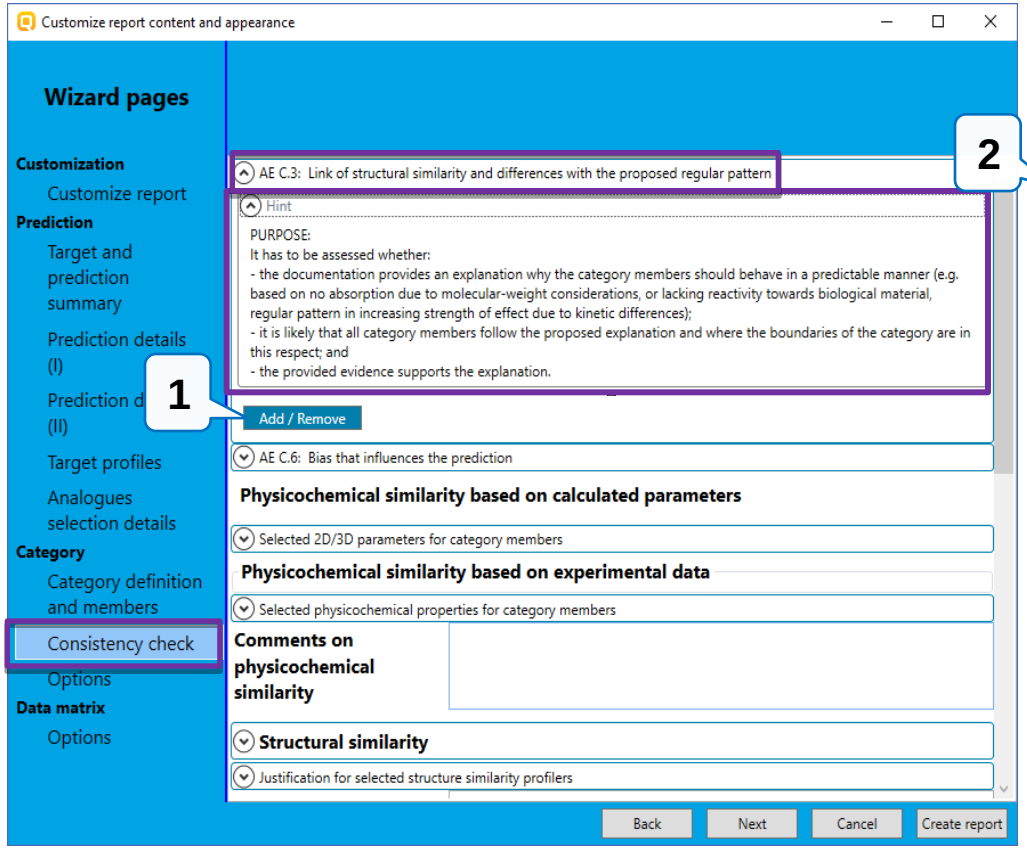
Category elements should be added manually to the **AE C.1** by click on ***Add/ Remove (1)*** button then check already available **Category members** item (2) and click **OK** (3). The item then will appeared in the wizard (4). If impurities/additives of the used analogues are available, they will appear automatically under the ***AE C.1*** in *Purity / Impurity* (5). Example on how the AE A.1. will look in the generated report is shown on the right (6).

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

The AE C.3 Link of structural similarity and differences with the proposed regular pattern



Click on the **Add/Remove** button (1) and **create new item** with the following example text (2) added to the **AE C.3**:

Example text for **AE C.3**

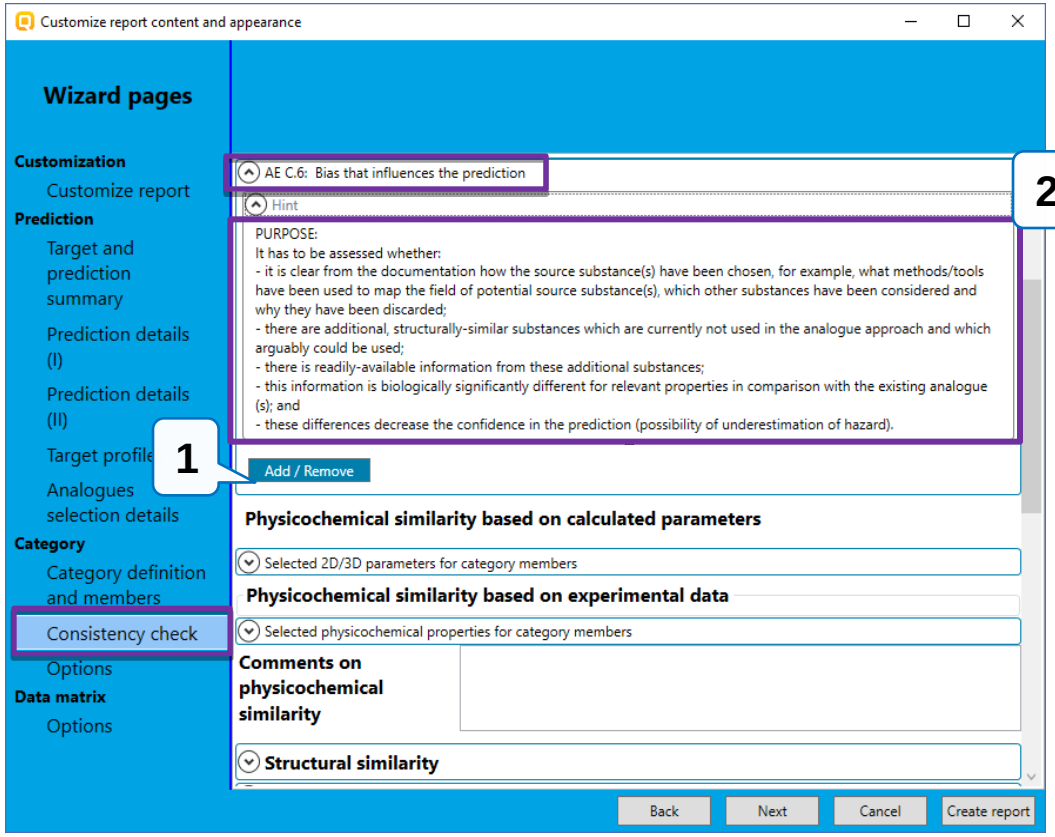
- The category is structurally defined as target (A) and the seven source substances (B,C, D, E, F, G, K) all form a common metabolite - formaldehyde responsible for the toxic effect
- They all consist of a common reactivity pattern responsible for the formation of reactive metabolites

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

The AE C.6 Bias that influences the prediction



Click on the **Add/Remove** button (1) and **create new item** with possible content of example text (2) added to the **AE C.6**:

2

An example text for **AE C.6**

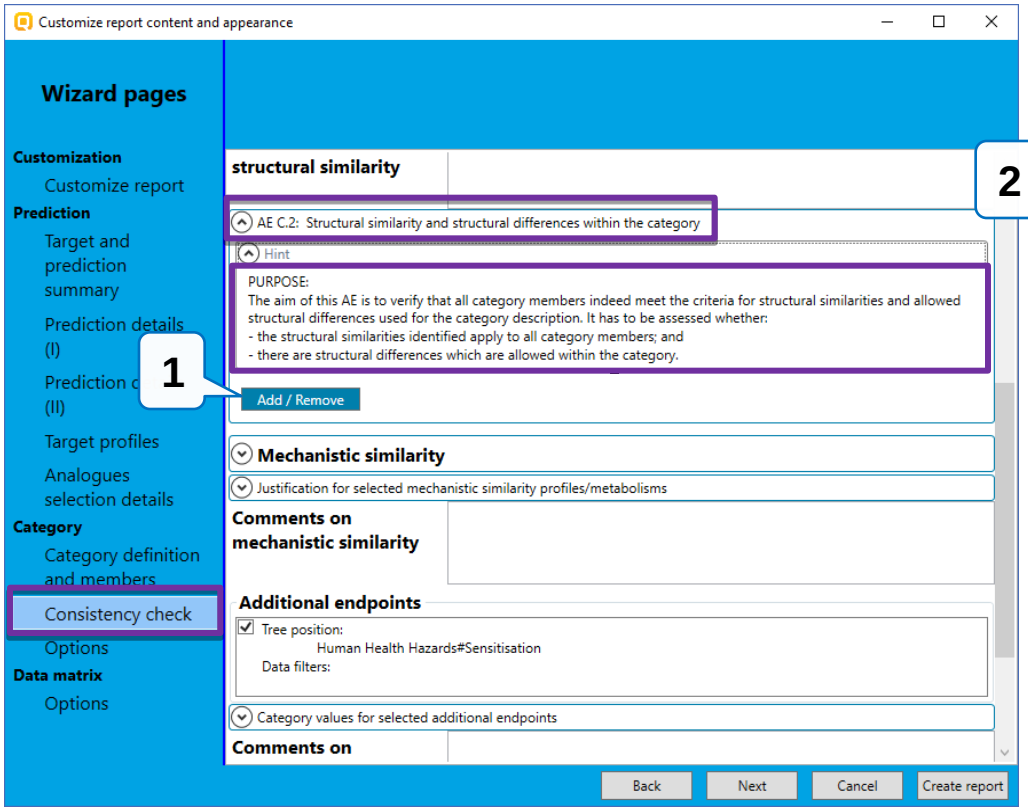
- When there are multiple possible analogues with equivalent structural similarity; or
- The assessing expert has knowledge of such additional structurally-similar analogue(s)
- Expert provides additional literature search of similar analogues with similar to the produced common compounds (formaldehyde) toxic effect

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE C.2 **The Structural similarity and structural differences within the category**



Click on the **Add/Remove** button (1) **and create new item** with a possible example text (2) added to the **AE C.2**:

2

An example text for **AE C.2:**

- Structural similarity between Target substance A and the 7 source substances (B, C, D, E, F, G, K) according to Str.similarity profiler is in the range of [48-72%]
- 3 out of the 7 source substances (B, D and E) have a selection of Alkene, Alkoxy, Alkenyl, Aryl, Ally, Ether and Phenol groups based on OFG profiler
- The other 3 have in addition to the above selection an additional "Precursors quinoid compound" group (C, K, G)
- While the target substance A and the source substance F have additional structural fragments

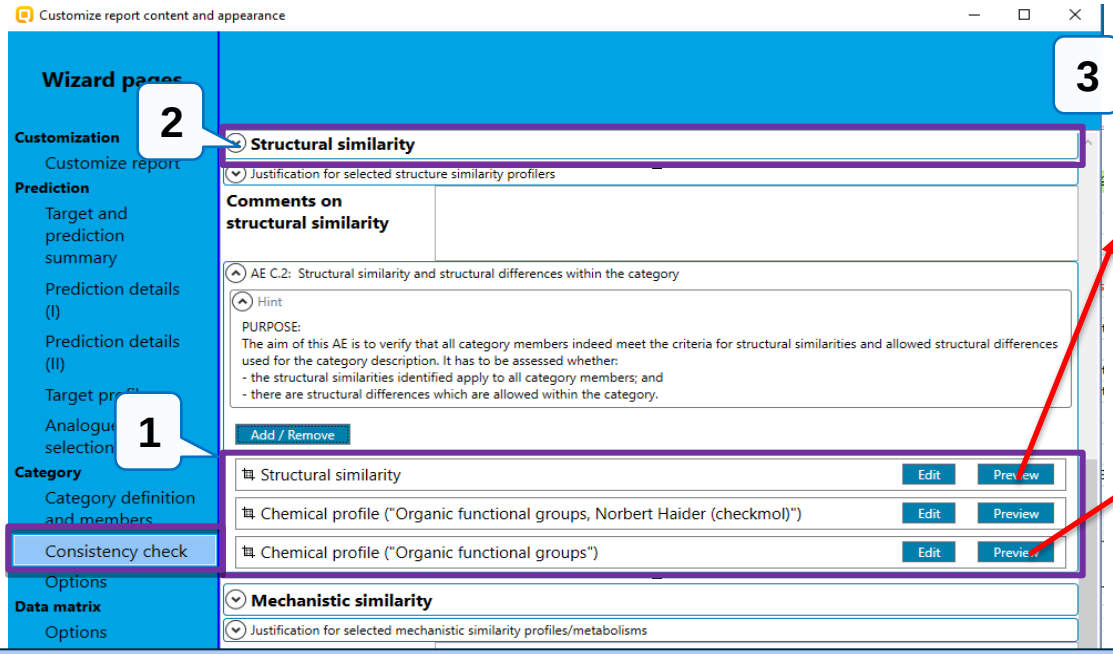
The AE C.2 is focused on the structural similarity, in this respect there are two report items already created and stored in the *Report basket* during the workflow that the user could refer to them. See next slide

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE C.2 The Structural similarity and the structural differences within the category

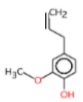
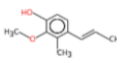


3

Calculated structure similarity

	Target A	Source B	Source C	Source D	Source E	Source F	Source K	Source G
Target A	100%	66.7 %	48 %	48 %	40 %	72 %	72 %	72 %
Source B	66.7 %	100%	72 %	72 %	72 %	48 %	48 %	40 %
Source C	48 %	72 %	100%	69.2 %	53.8 %	69.2 %	38.5 %	30.8 %
Source D	48 %	72 %	69.2 %	100%	84.6 %	38.5 %	69.2 %	61.5 %
Source E	40 %	72 %	53.8 %	84.6 %	100%	30.8 %	61.5 %	69.2 %
Source F	72 %	48 %	69.2 %	38.5 %	30.8 %	100%	69.2 %	53.8 %
Source K	72 %	48 %	38.5 %	69.2 %	61.5 %	69.2 %	100%	84.6 %
Source G	72 %	40 %	30.8 %	61.5 %	69.2 %	53.8 %	84.6 %	100%

Chemical profile ("Organic functional groups")

1	2	3
		
Alkene Ether Allyl Aryl Phenol Alkoxy Precursors quinoid compounds Alkenyl (hetero)arenes Alkyl-, alkenyl- and alkynyl (hetero)arenes	Alkene Ether Allyl Aryl Phenol Alkoxy Alkenyl (hetero)arenes Alkyl-, alkenyl- and alkynyl (hetero)arenes	Alkene Ether Allyl Aryl Phenol Alkoxy Alkyl-, alkenyl- and alkynyl (hetero)arenes

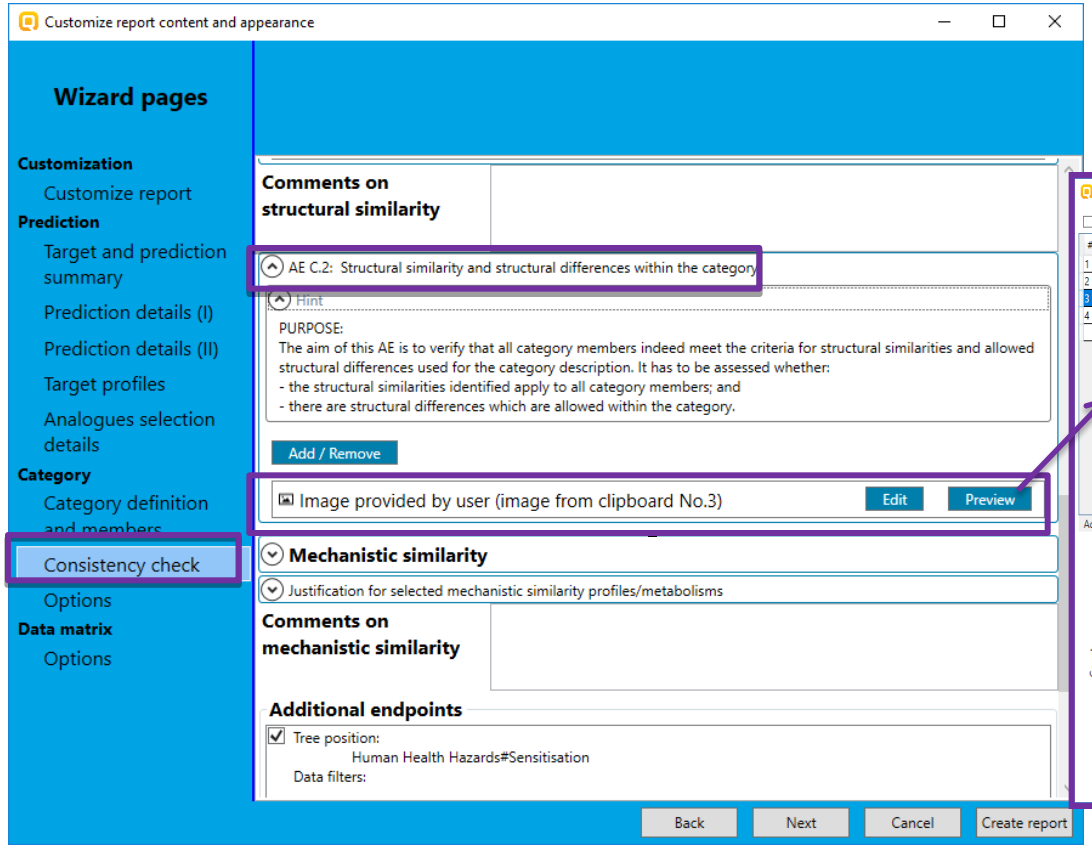
The Str. similarity and the **Chemical profile** with respect to the empirical profilers (OFG, Norbert Haider and OFG) were stored in the Report basket and automatically created in the report wizard (1) due to the application of the elements of the Category consistency during the workflow (remember the elements were applied at the final stage of the gap filling module). Based on that **AE C.2**. could refer to these two items. Both items appeared under the str.similarity section (2) and how they look in the generated report is shown on the right (3).

Report
Report Generation according to RAAF-Scenario 3

Section Category

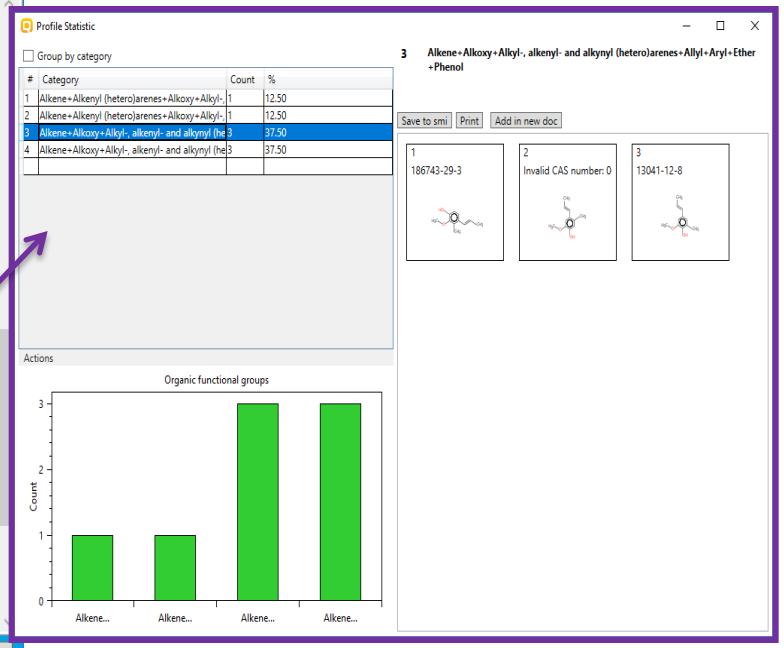
Subsection: Category definition and members

AE C.2 The Structural similarity and structural differences within the category



An additional image (saved in advance) could be added to the **AE C.2** (already explained on slide 54):

Appendix with profiling statistics based on OFG profiler could be added:

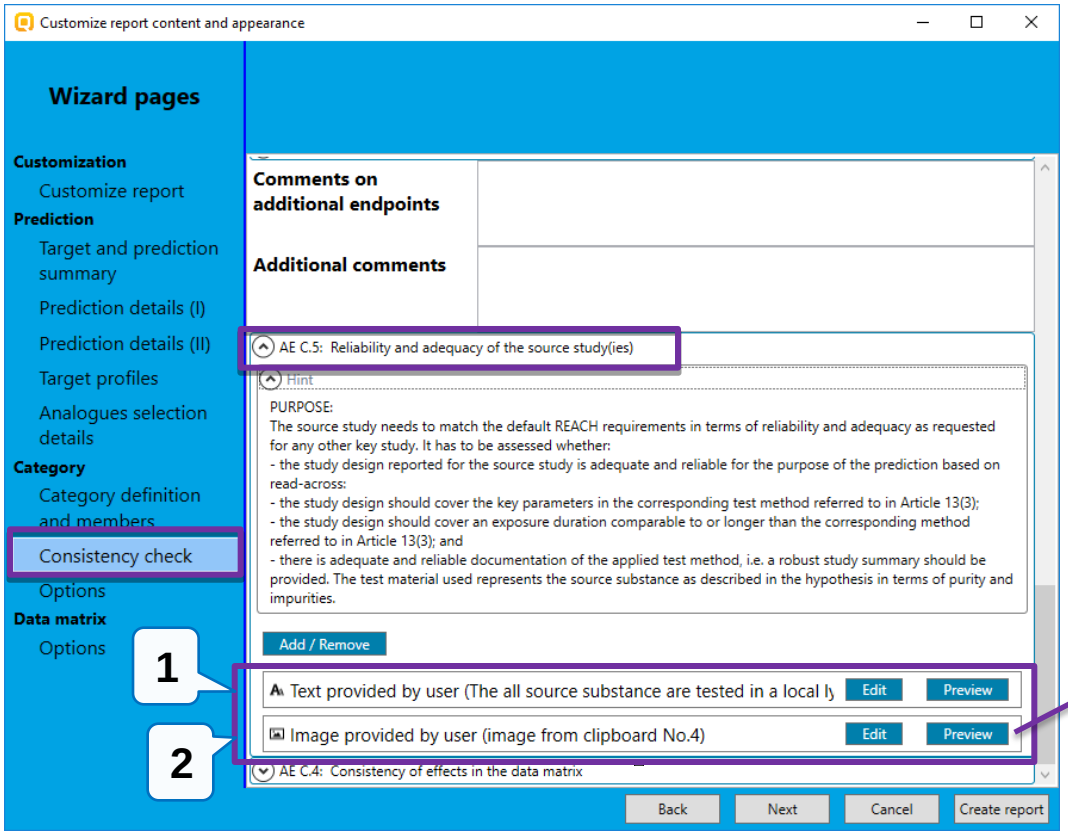


Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

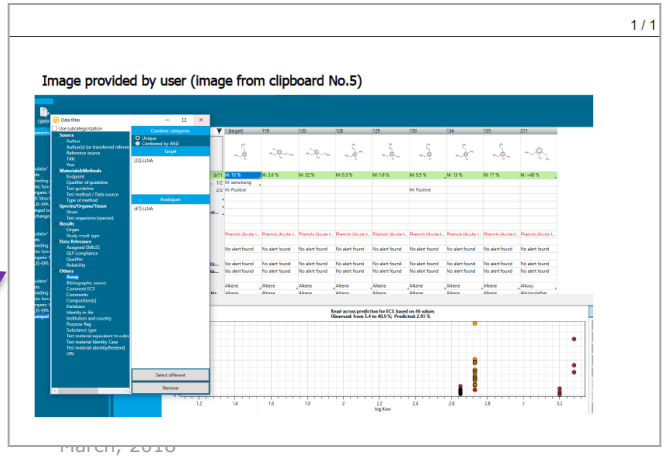
AE C.5 Reliability and adequacy of the source study(ies)



The possible content the added text to **AE C.5** is (1):

- The all source substances aretested in a local lymph node assay (LLNA)
- The study is used to predict the skin sensitization effect concerning LLNA study for the target substance

Also an image could be added here supporting that the study used in the prediction is based on the LLNA data only (2):

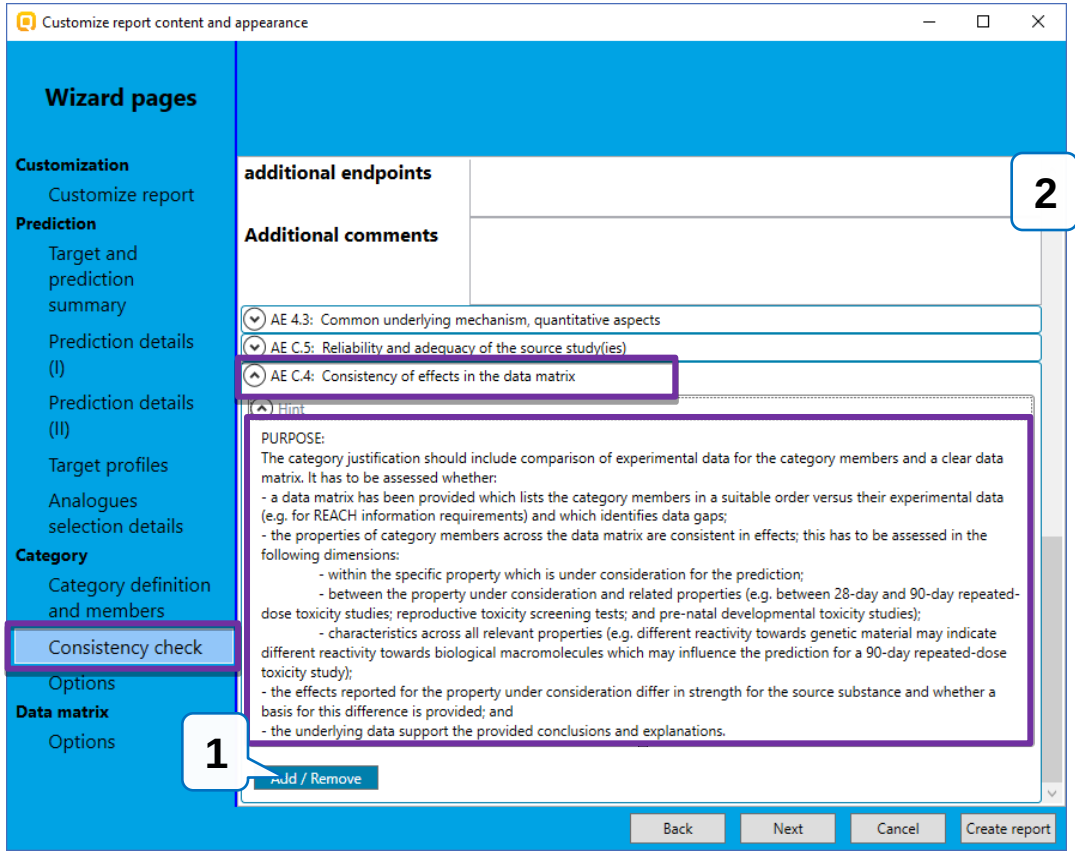


Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE C.4 Consistency of effects in the data matrix



Click on the **Add/Remove** button (1) and **create new** with this possible example text (2):

- The target substance A and the seven source substances show clear indication for a skin sensitization effect
- The latter are supported by the experimental data in accordance to the LLNA test, found for all of them
- All of them are not volatile chemicals and with molecular weight is less than 500 Da
- All experimental data for the target and the source substances are supported with literature references

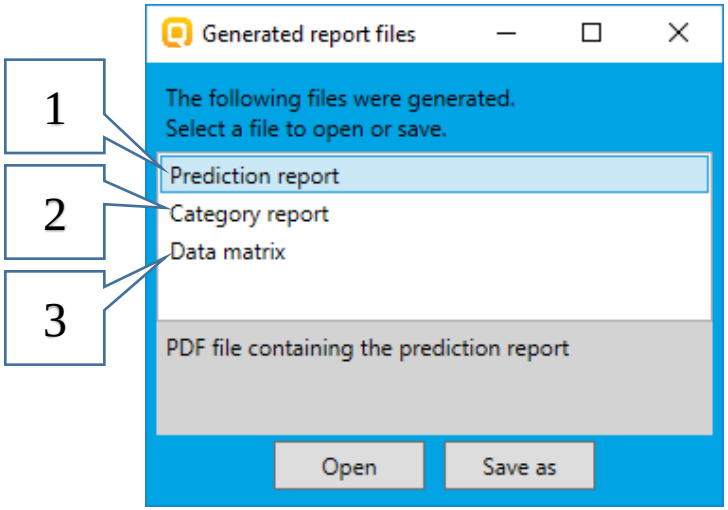
Report
Report Generation

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

1) **A Prediction report** - a PDF file containing the prediction information related to the target.
2) **A Category report** - a PDF file containing information for the consistency of the final category (target plus used analogues)
3) **A Data matrix** - a MS Excel file containing the chemicals used for the 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 report

QSAR Toolbox prediction for single chemical

(in accordance with RAAF scenario 3)

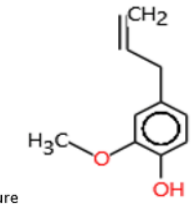
The selected RAAF scenario is specified in the first page

Date: 24 Mar 2018
Author(s):
Contact details:

Target information

Structural information	Numerical identifiers	Chemical names
SMILES: COc1cc(CC=C)ccc1O	CAS#: 97-53-0 Other: EC Number:4074682	|eugenol|eugenol (4-allyl-2-methoxyphenol)|4-allyl-2-methoxy

Structure



Predicted endpoint: EC3; No effect s specified
Predicted value: 9.88 (from -20.6 t
Unit/scale: %
Data gap filling method: Read-acros
Summary: *manually editable field*
Not provided by the user

Category report

QSAR Toolbox report for category

(in accordance with RAAF scenario 3)

1. Category definition

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

Ranges for selected physicochemical properties and calculated parameters

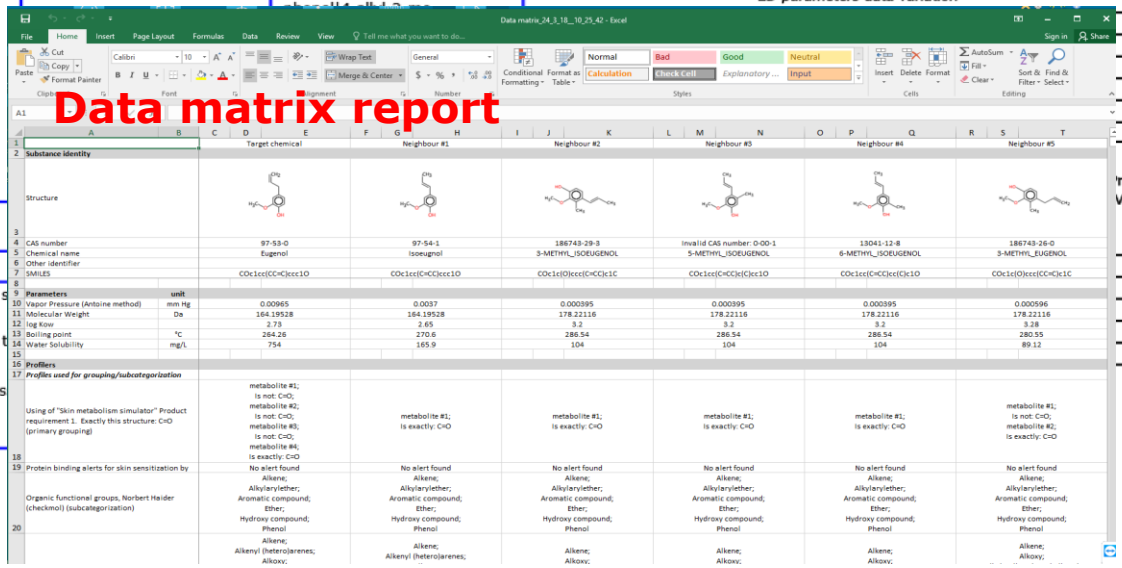
Parameter variation (5 selected: Boiling point; log Kow; Molecular Weight; Vapor Pressure (Antoine method); Water Solubility)
2D parameters data variation

	unit (family)
	°C
	<no units>
	Da
	mm Hg
	mg/L

roperties#Water solubility; Physical Chemical
Vapour pressure; Physical Chemical

	unit (family)
	mg/L(Mass concentration)
	°C(Temperature)
	Pa(Pressure)
	mm Hg(Pressure)

Data matrix report



		Target chemical	Neighbour #1	Neighbour #2	Neighbour #3	Neighbour #4	Neighbour #5
Substance identity							
Structure							
CAS number		97-53-0	97-54-1	186743-29-3	Invalid CAS number: 0-00-1	13041-12-8	186743-26-0
Chemical name		Eugenol	Isoeugnol	3-METHYL_ISOEUGENOL	5-METHYL_ISOEUGENOL	6-METHYL_ISOEUGENOL	3-METHYL_EUGENOL
Other identifier							
SMILES		COc1cc(CC=C)ccc1O	COc1cc(C=CC)ccc1O	COc1c(I)ccc(C=CC)c1C	COc1cc(C=CC)c(C)c1O	COc1cc(C=CC)cc(C)c1O	COc1c(I)ccc(CC=C)c1C
Parameters	unit						
Vapor Pressure (Antoine method)	mm Hg	0.00965	0.0037	0.000395	0.000395	0.000395	0.000596
Molecular Weight	Da	164.19528	164.19528	178.22116	178.22116	178.22116	178.22116
log Kow		2.73	2.65	3.2	3.2	3.2	3.28
Boiling point	°C	264.26	270.6	286.54	286.54	286.54	280.55
Water Solubility	mg/L	754	165.9	104	104	104	89.12
Profilers							
Profiles used for grouping/subcategorization							
Using of "Skin metabolism simulator" Product requirement 1. Exactly this structure: C=O (primary grouping)		metabolite #1; Is not: C=O; metabolite #2; Is not: C=O; metabolite #3; Is not: C=O; metabolite #4; Is exactly: C=O	metabolite #1; Is exactly: C=O	metabolite #1; Is exactly: C=O	metabolite #1; Is exactly: C=O	metabolite #1; Is exactly: C=O	metabolite #1; Is not: C=O; metabolite #2; Is exactly: C=O
Protein binding alerts for skin sensitization by		No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Organic functional groups, Norbert Haider (checkmol) (subcategorization)		Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol
		Alkene; Alkenyl (hetero)arenes; Alkoxy;	Alkene; Alkenyl (hetero)arenes;	Alkene; Alkoxy;	Alkene; Alkoxy;	Alkene; Alkoxy;	Alkene; Alkoxy;

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 3.
- Note proficiency comes with practice.