QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 3 and related assessment elements

Outlook

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

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and assessing of the outcome whether read across is scientifically acceptable or not

- The read-across prediction will be justified by fulfilling all information requirements according to the Read Across Assessment Framework (RAAF).

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Keywords

TARGET CHEMICAL - chemical of interest

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

WORKFLOW – the use, in combination, of the different modules (e.g. prediction workflow: from input to report)

PROFILER - algorithm (rule set) for the identification of specific features of the chemicals. Several types of profilers are available, such as structural (e.g. Organic functional groups), mechanistic (e.g. Protein binding by OECD) and endpoint-specific (e.g. in vitro in vitro mutagenicity (Ames test) alerts by ISS) profilers.

ALERT - the profilers consist of sets of rules or alerts. Each of the rules consists of a set of queries. The queries could be related to the chemical structure, physicochemical properties, experimental data, comparison with the target or list with substances and external queries from other predefined profilers (reference queries).

CATEGORY – "group" of substances sharing same characteristics (e.g. the same functional groups or mode of action). In a typical Toolbox workflow, it consists of the target chemical and its analogues gathered according to the selected profilers

ENDPOINT TREE – Endpoints are structured in a branched scheme, from a broader level (Phys-Chem properties, Environmental Fate and transport, Ecotoxicology, Human health hazard) to a more detailed one (e.g. EC3 in LLNA test under Human health hazard-Skin sensitization)

DATA MATRIX – Table reporting the chemical(s) and data (experimental results, profilers outcomes, predictions). Each chemical is in a different column and each data in a different row

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

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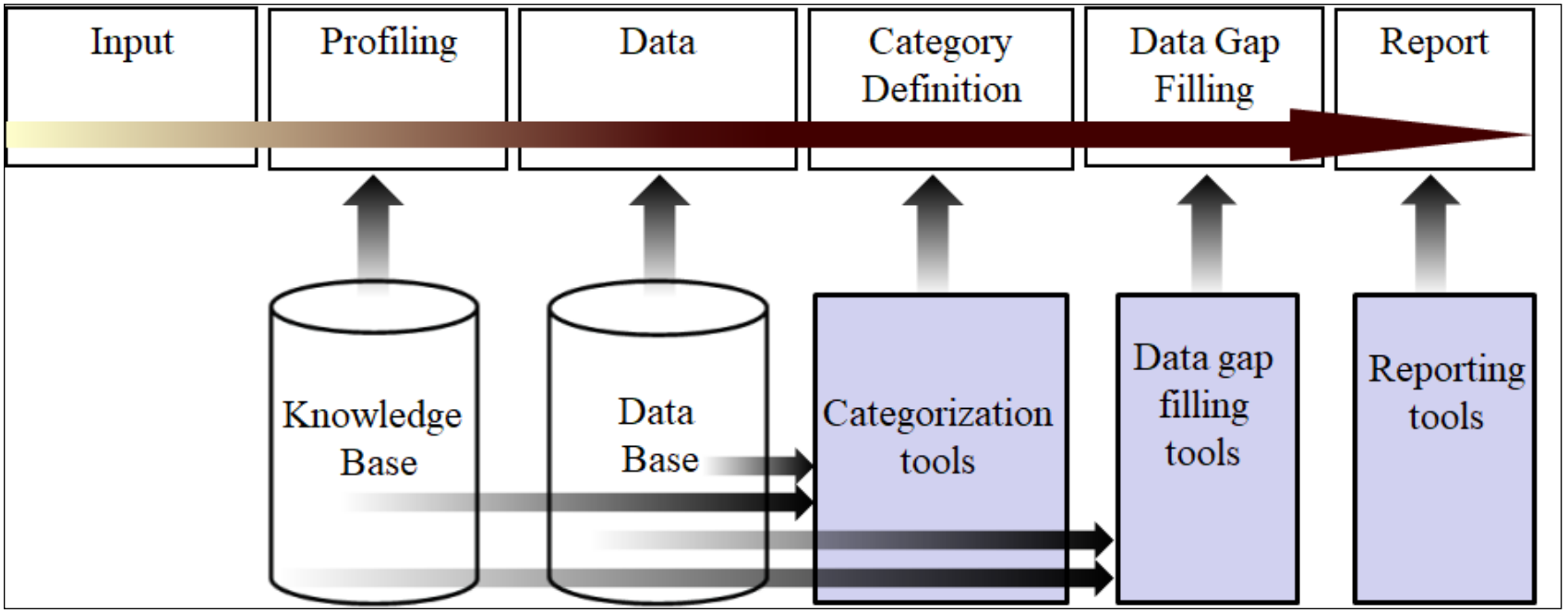
- Read-Across Assessment Framework (RAAF)
- The example
- **Workflow**

Workflow

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

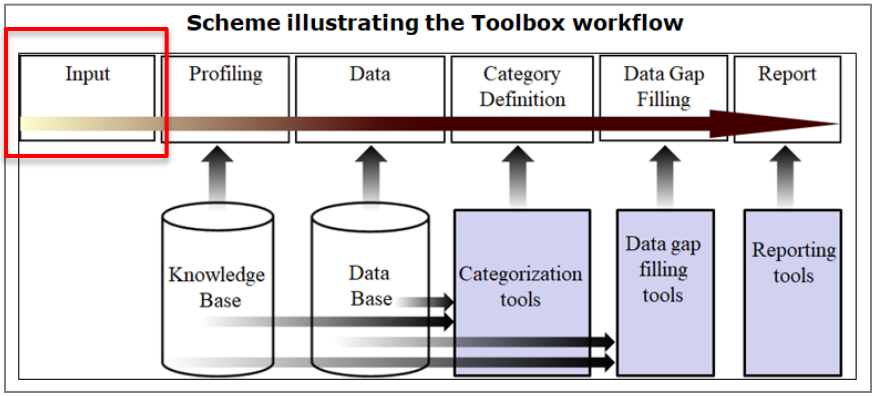
Workflow

Scheme illustrating the Toolbox workflow



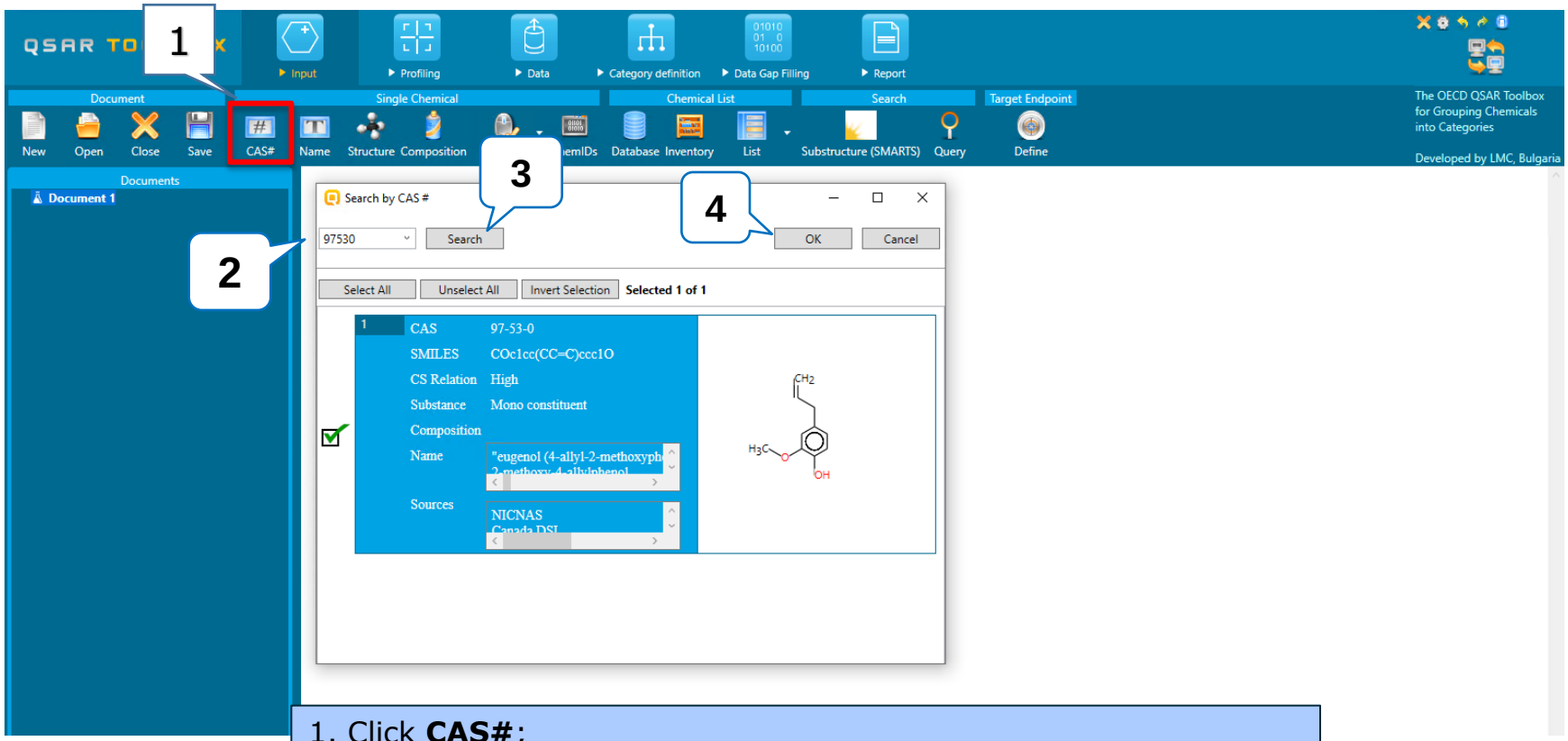
Input
Overview

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



Input

Input target chemical by CAS#

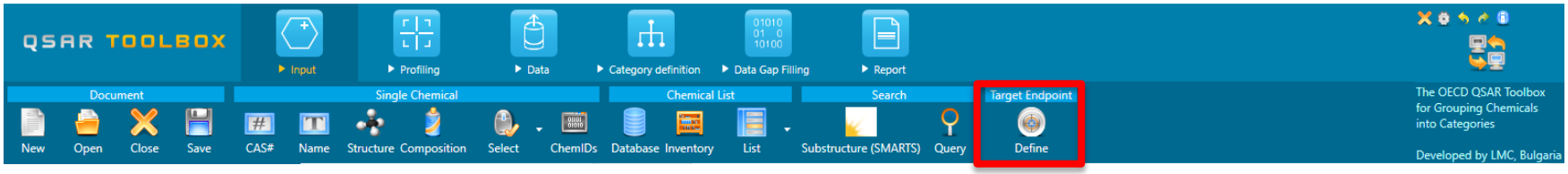


1. Click **CAS#**;
2. Enter the **CAS# 97-53-0** in the blank field;
3. Click ***Search***;
4. When the structure with the requested CAS # appears, click ***OK***.

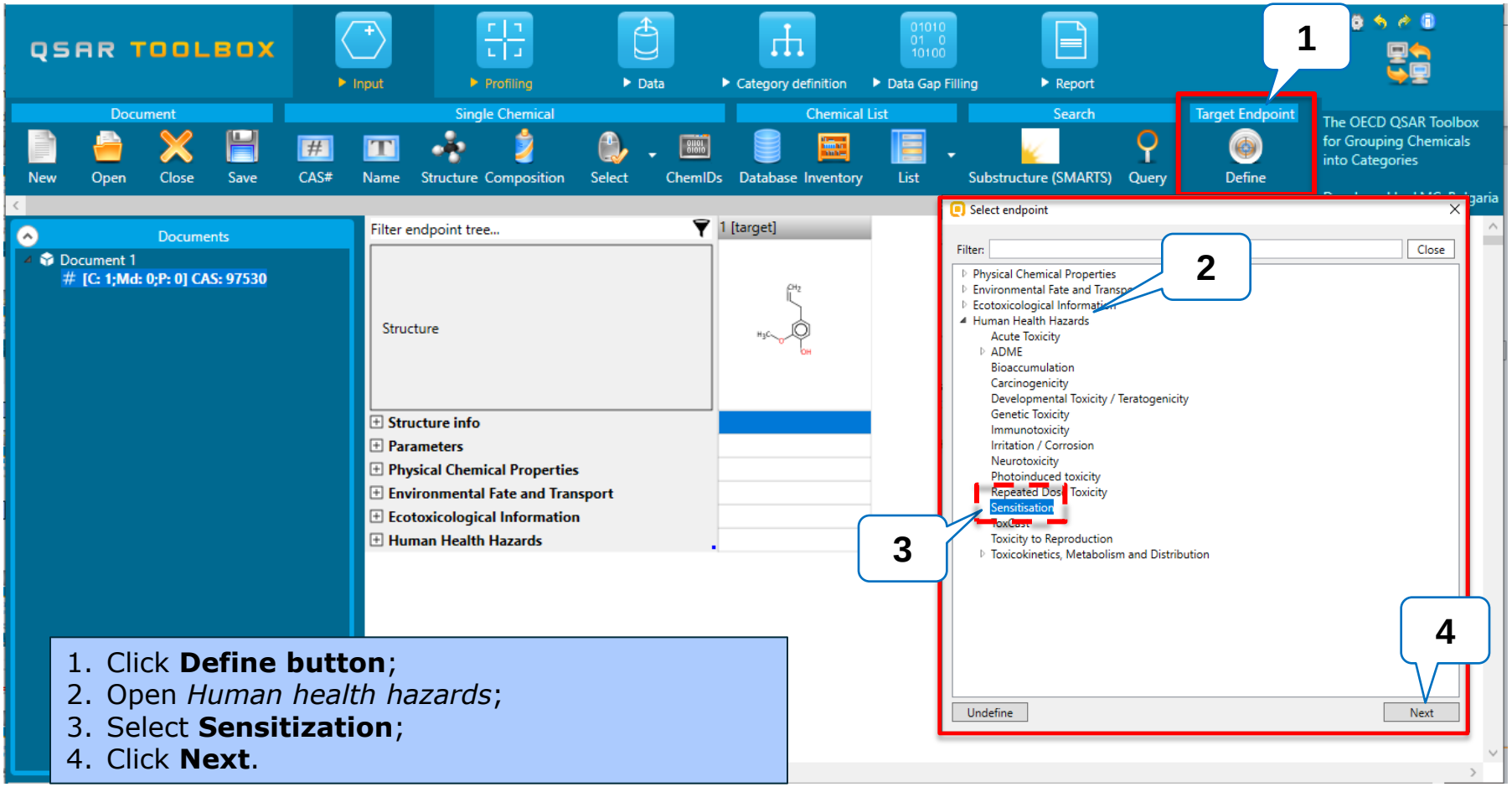
Input
Define target endpoint

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.

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



Input
Define target endpoint

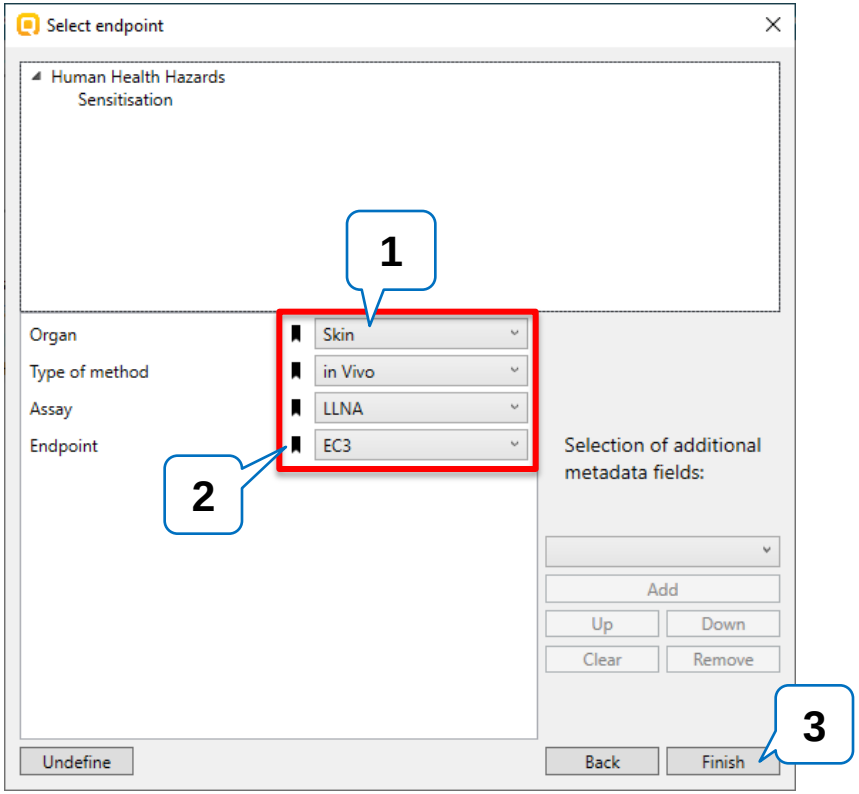


1. Click **Define button**;
2. Open *Human health hazards*;
3. Select **Sensitization**;
4. Click **Next**.

Input
Define target endpoint

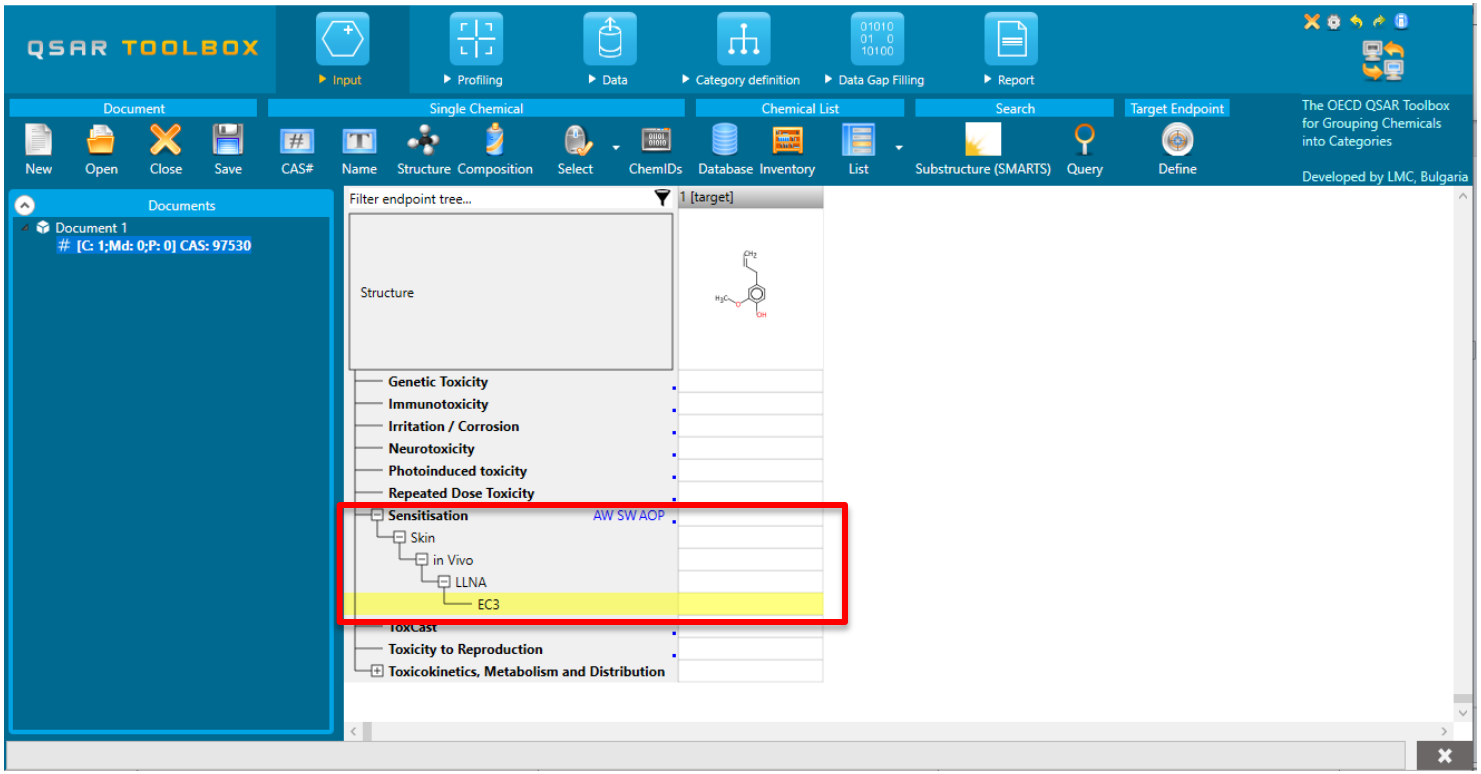
On the next step you have to select the endpoint of interest and additional metadata if needed.

1. Select *Endpoint*: **EC3,** *Assay*: **LLNA,** *Type of method*: **In Vivo,** *Organ:* **Skin**.
2. In case of definition of multiple metadata then small black button need to be clicked.
3. Once ready click on **Finish.**

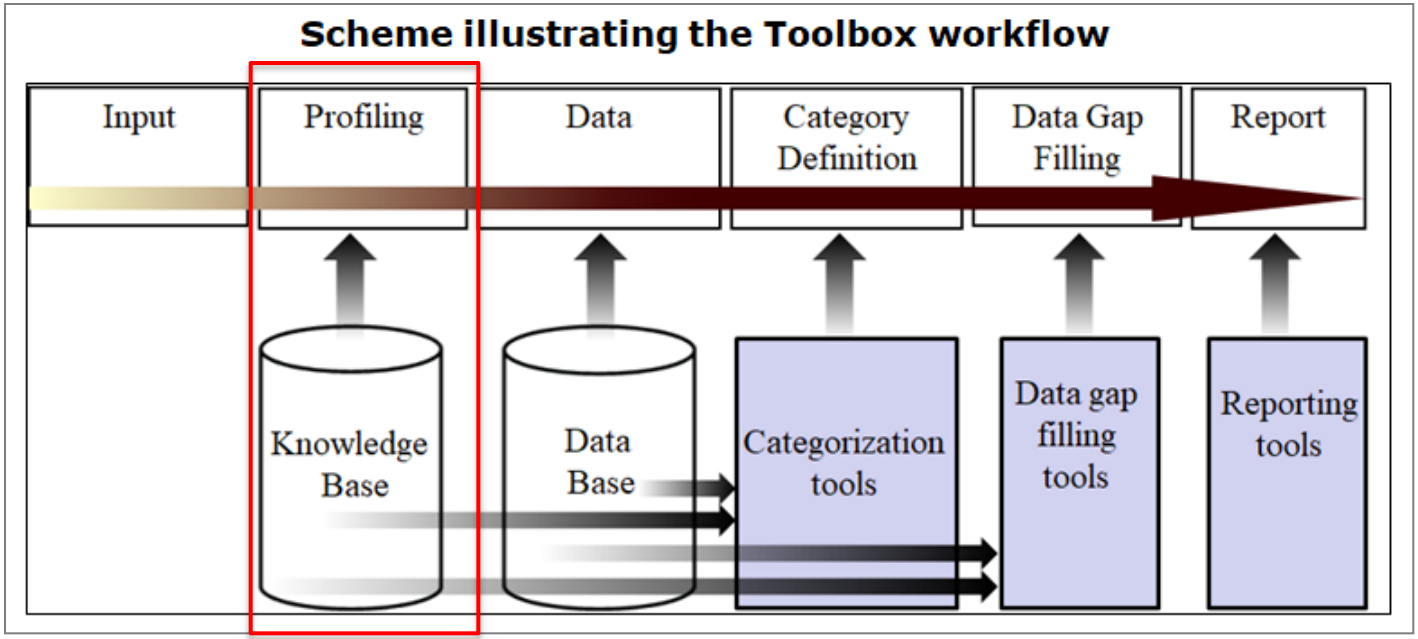


Input
Define target endpoint

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



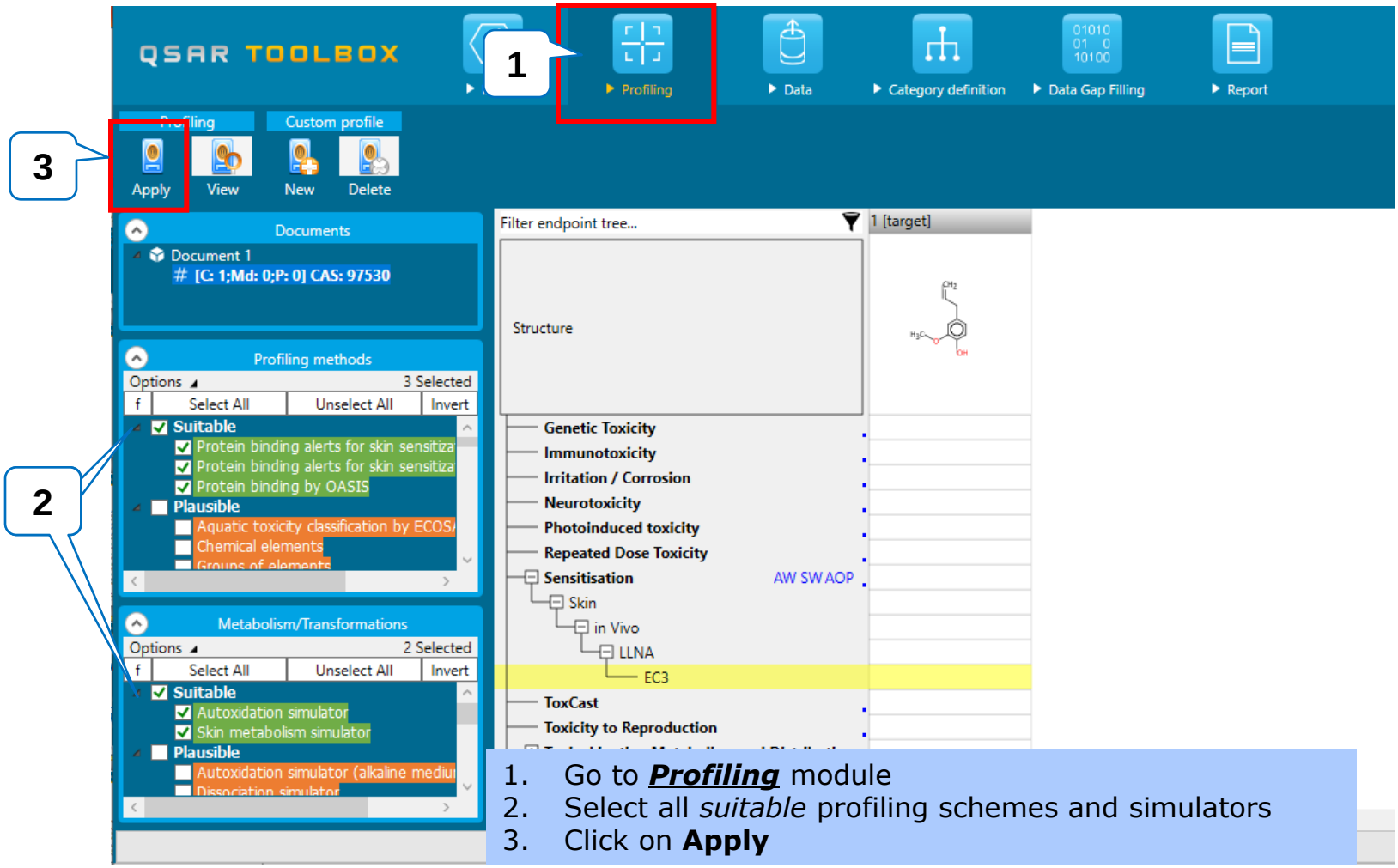
Profiling
Overview

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

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

Profiling

Profiling the target chemical



1. Go to ***Profiling*** module
2. Select all *suitable* profiling schemes and simulators
3. Click on **Apply**

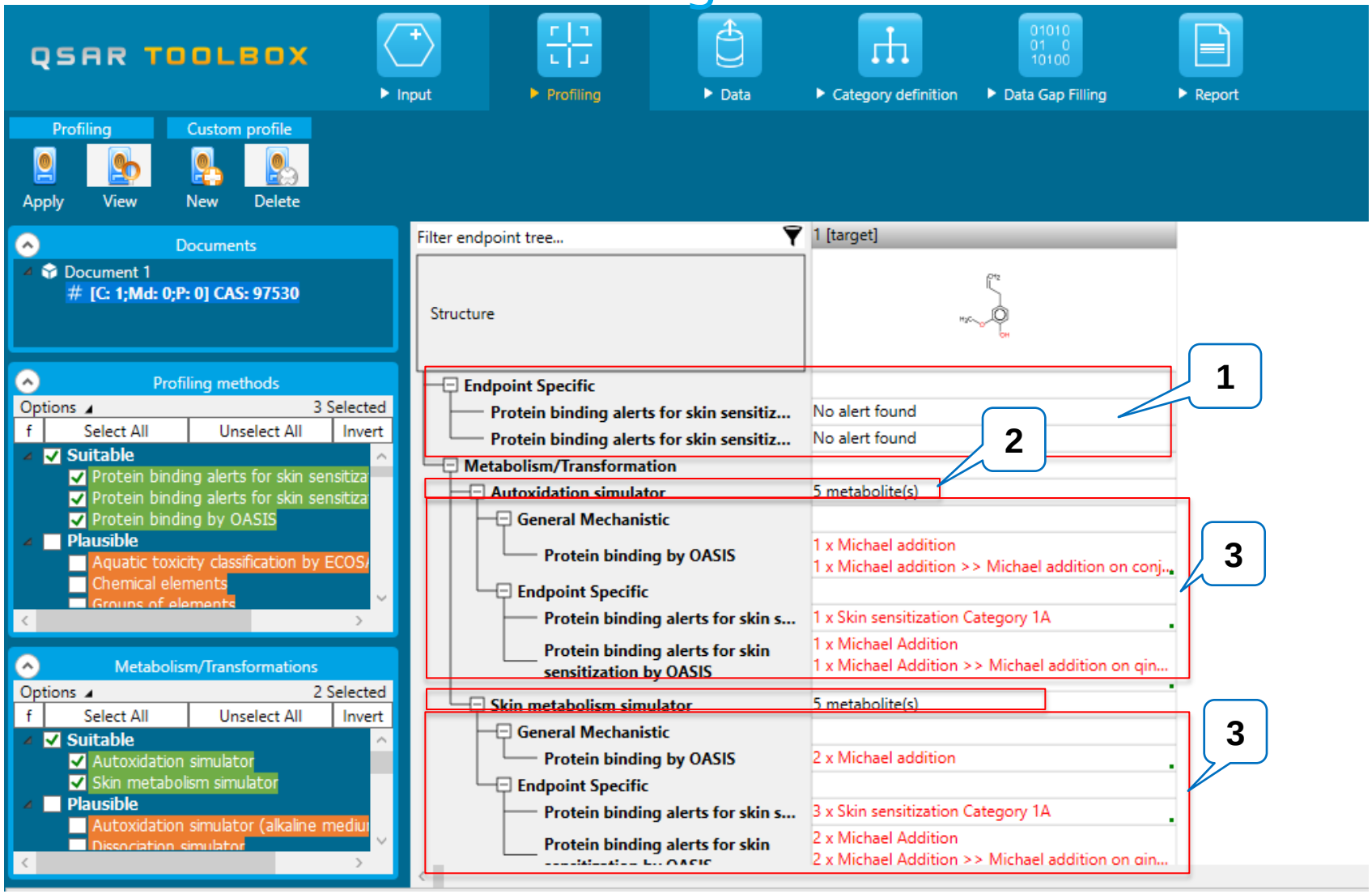
Profiling
Profiling results

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

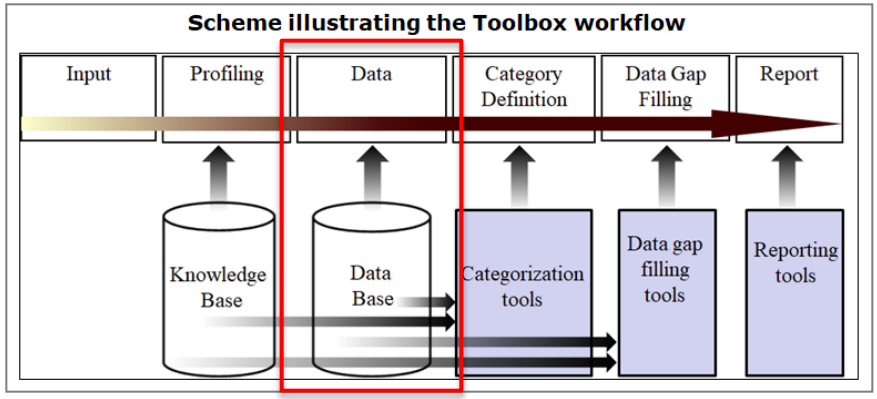
Profiling

Profiling results

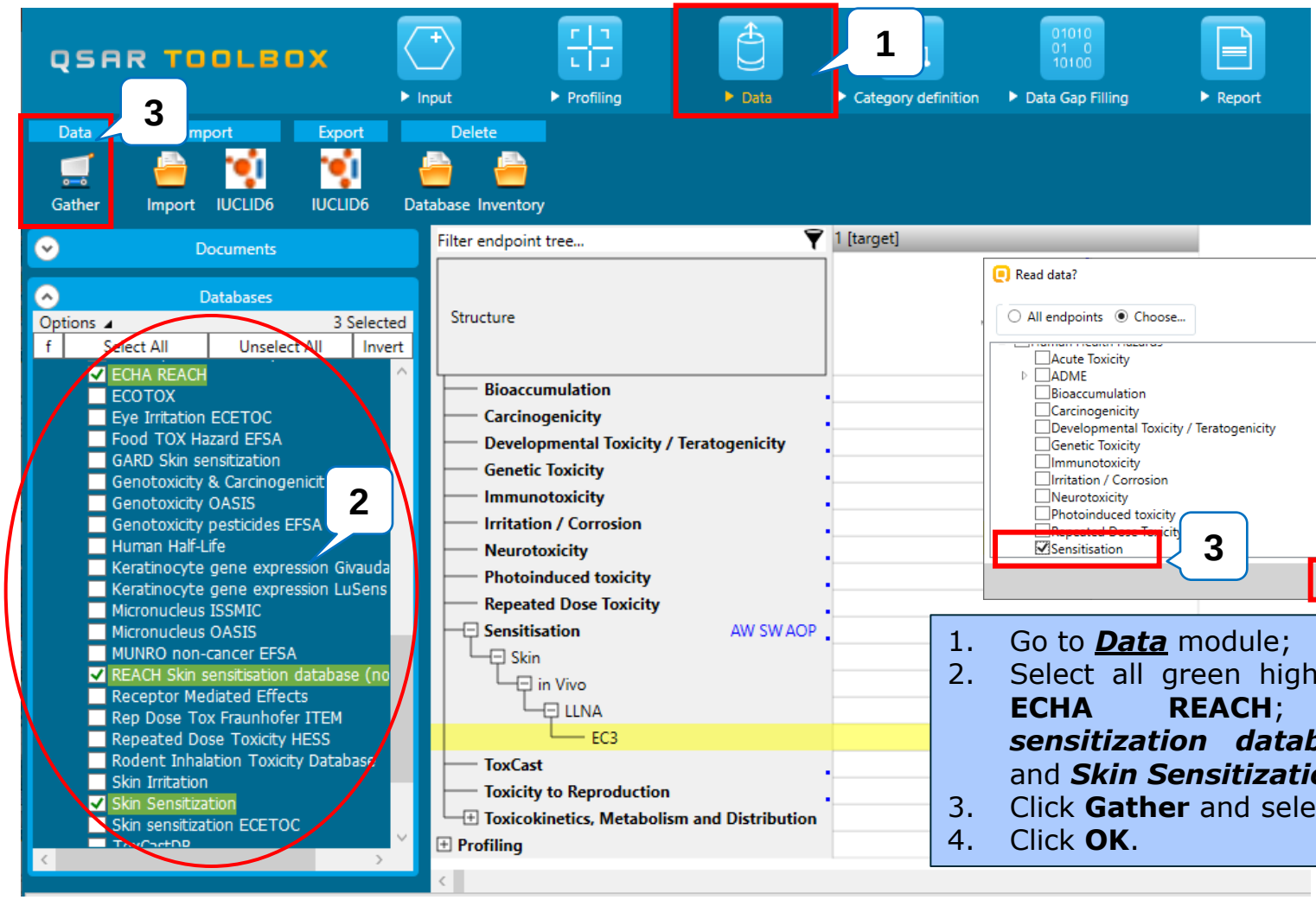


Data
Overview

- "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).

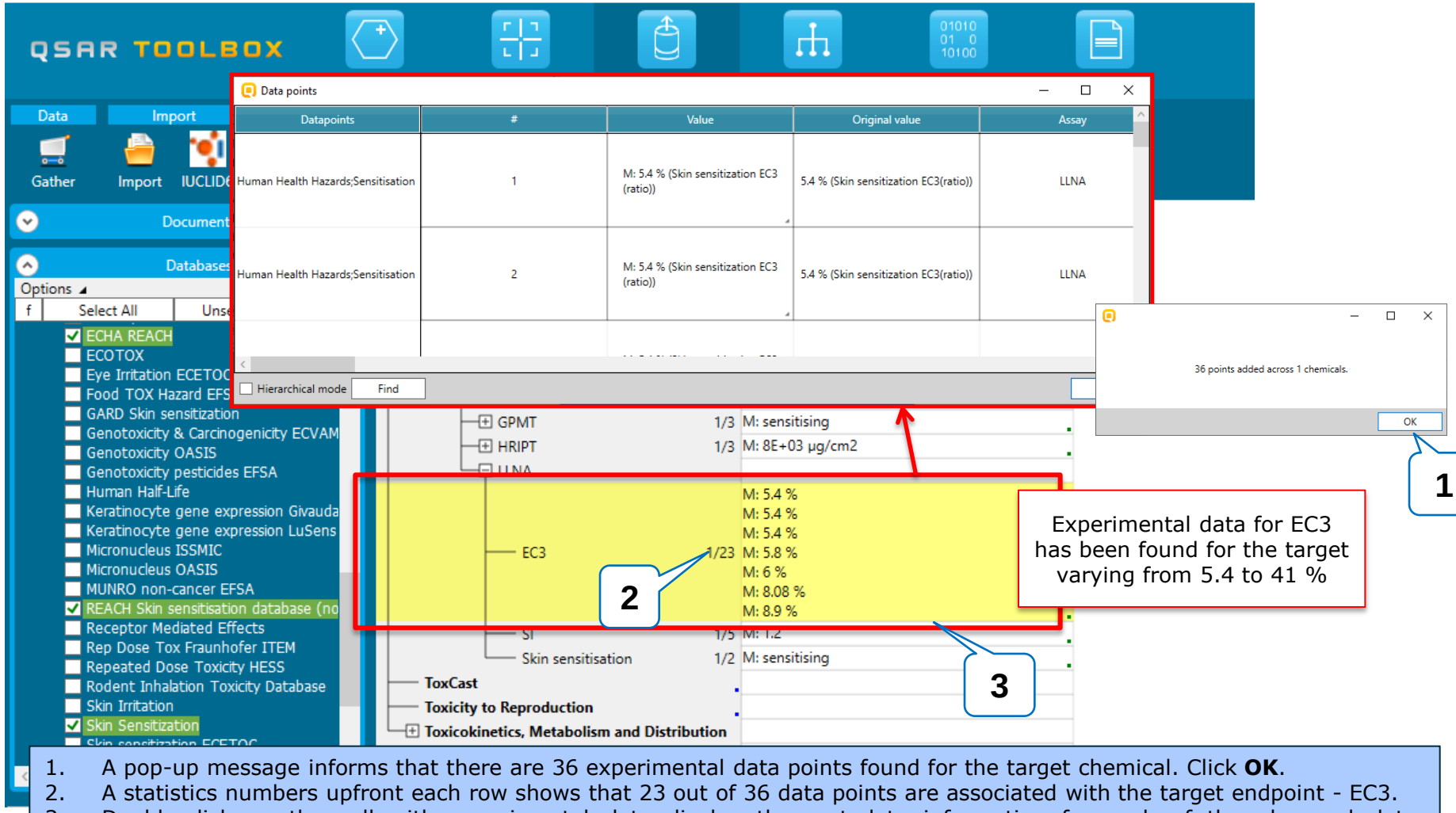


Data
Gather data



1. Go to ***Data*** module;
2. Select all green highlighted databases – **ECHA REACH**; ***REACH Skin sensitization database (normalized)*** and ***Skin Sensitization***;
3. Click **Gather** and select **Sensitization**;
4. Click **OK**.

Data
Gather data



1. A pop-up message informs that there are 36 experimental data points found for the target chemical. Click **OK**.
2. A statistics numbers upfront each row shows that 23 out of 36 data points are associated with the target endpoint - EC3.
3. Double-click on the cell with experimental data display the metadata information for each of the observed data. Experimental data vary from 6 to 41 % which falls in the range of Positive outcome.

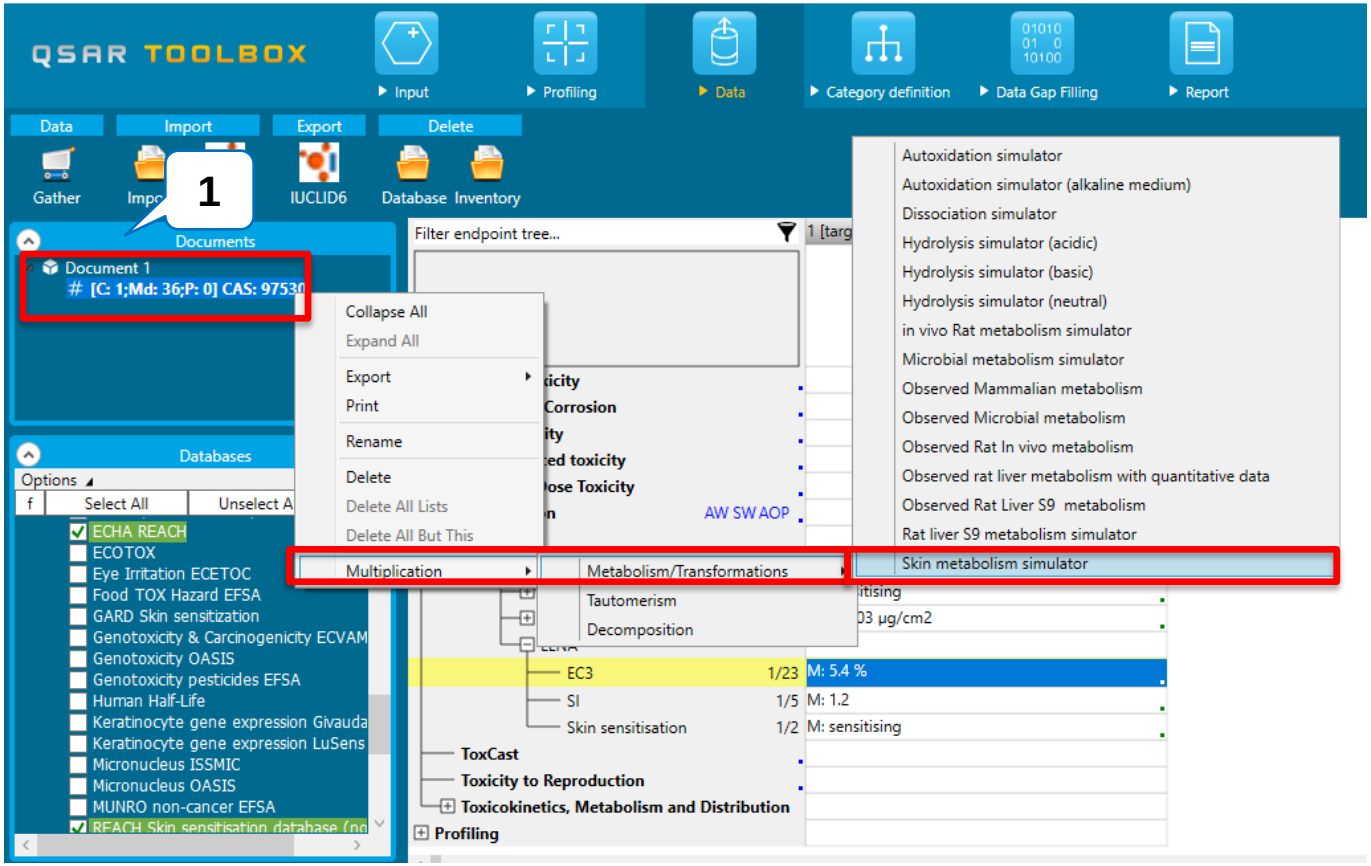
Data
Gather data

- 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 these are only the *ECHA REACH*, *Skin Sensitization and REACH Skin sensitization database (normalised).*

Recap

- In the module *Input*, you have entered the target chemical via a CAS number and define the endpoint of interest (e.g. EC3, Skin sensitization).
- In the *Profiling* module, you have profiled the target chemical with profiling schemes and metabolic simulators, suitable for the selected target endpoint.
- No protein binding alert has been found for the target chemical. However, protein binding alerts were identified for some of the metabolites produced after abiotic and biotic activation of the chemical (autooxidation, skin metabolism).
- In the *Data* module, you saw the databases 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, further steps of the example are focused on investigation the of skin metabolism trying to explain the positive experimental data of the target

Investigation of a skin metabolism for the target chemical



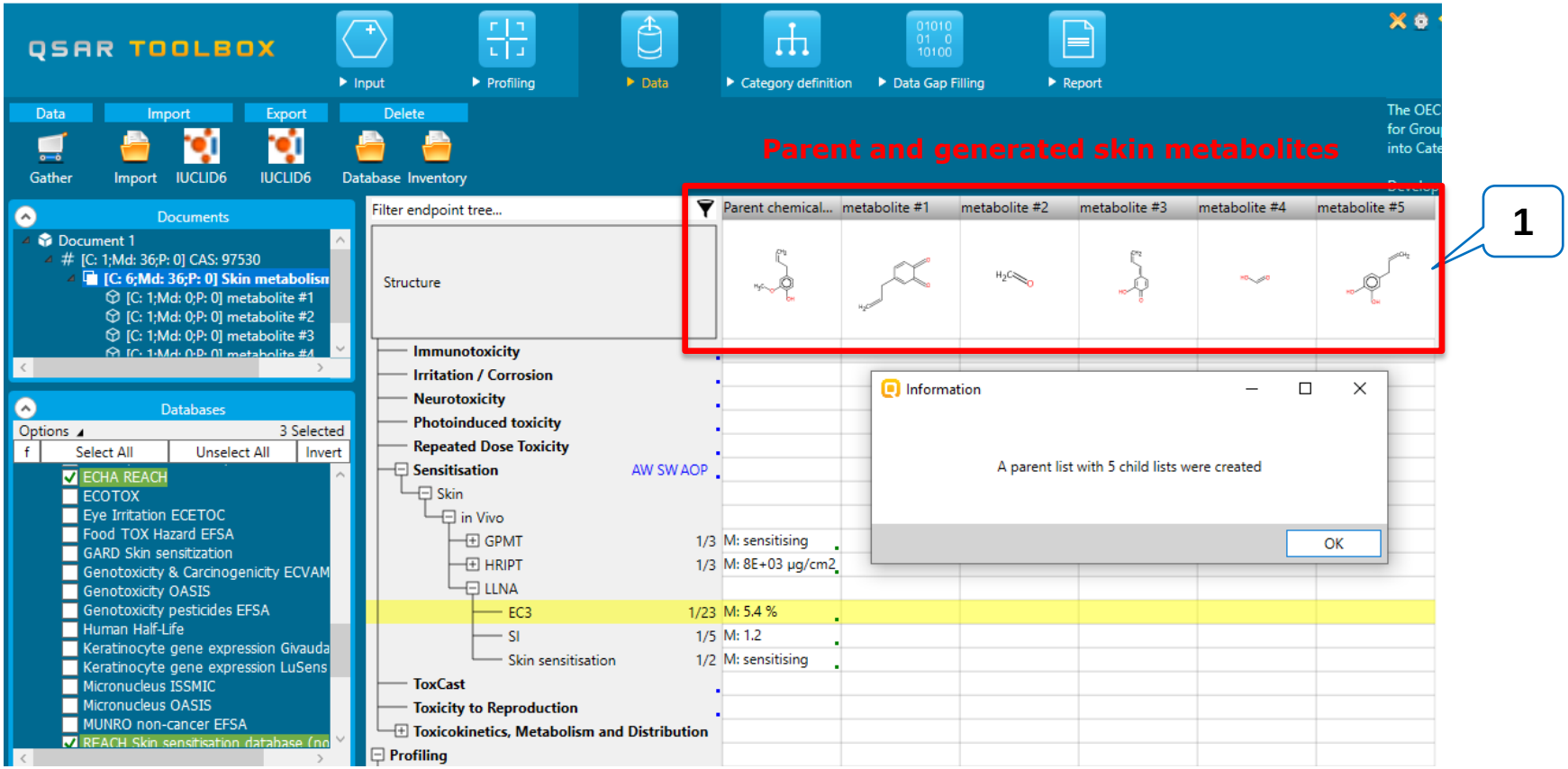
Step 1: For the investigation of metabolism first all skin metabolites need to be produced. Generate skin metabolite upfront gap filling (how to do it see steps shown in the blue box).

Result:

- 5 metabolites are generated as a result of a skin metabolism (see next slide)

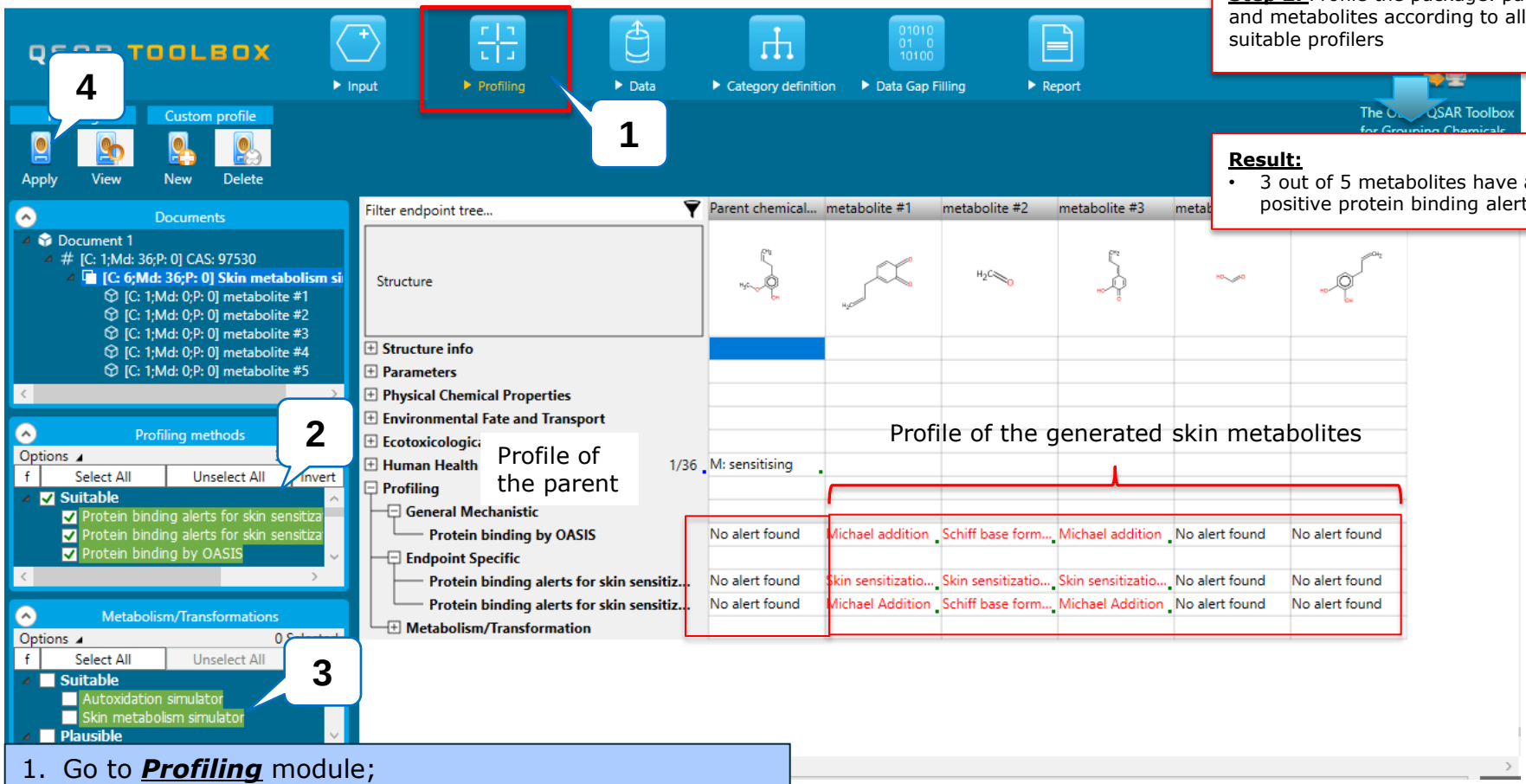
1. Right-click on the **CAS#** in the document tree and select **Multiplication/Metabolism/Transformation/Skin metabolites**;
2. Metabolites appeared next to the parent (see next slide)

Investigation of a skin metabolism for the target chemical



1. Metabolites appeared next to the parent.

Investigation of a skin metabolism for the target chemical



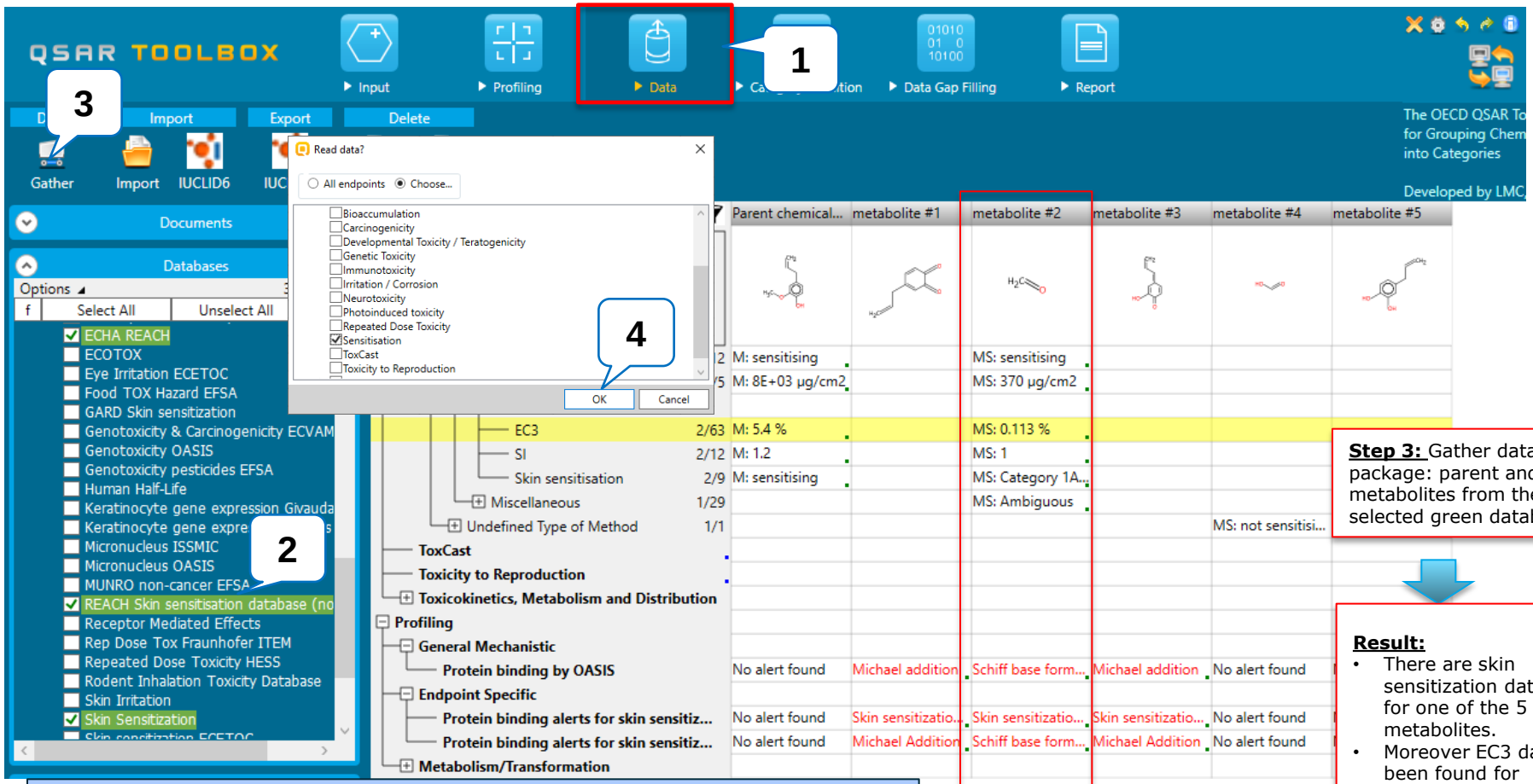
Step 2: Profile the package: parent and metabolites according to all suitable profilers

Result:

- 3 out of 5 metabolites have a positive protein binding alert

1. Go to ***Profiling*** module;
2. Select **suitable profilers** (green highlighted);
3. Unselect all metabolism simulators
4. Click **Apply.**

Investigation of a skin metabolism for the target chemical



1. Go to ***Data*** module;
2. Green databases are already highlighted;
3. Click **Gather**. Select Sensitization only. Click **OK (4)**.

Step 3: Gather data for the package: parent and metabolites from the selected green databases

Result:

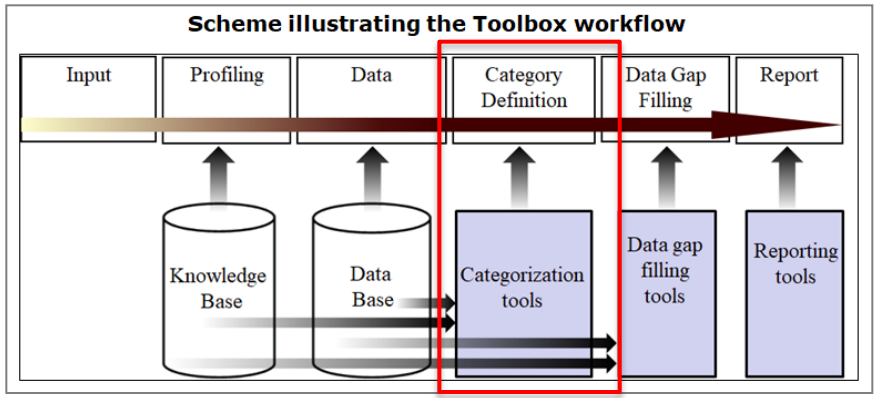
- There are skin sensitization data found for one of the 5 skin metabolites.
- Moreover EC3 data has been found for formaldehyde (having a positive alert for interaction with proteins

Handling skin metabolism
Recap

- In step 1 skin metabolites (simulated) have been generated for the parent chemical;
- In step 2 a package of the parent and metabolites has been profiled by the list of profilers suitable to the target endpoint. Positive protein binding alerts have been found for three out of 5 skin metabolites;
- In step 3 experimental data have been collected for the package parent and generated metabolites;
- Moreover a formaldehyde having a positive protein binding alert has also positive skin sensitization (EC3) data;
- Thus, next actions are focused on identifying analogues producing the same metabolite (formaldehyde), which could cause the skin sensitization effect for the target chemical.

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.

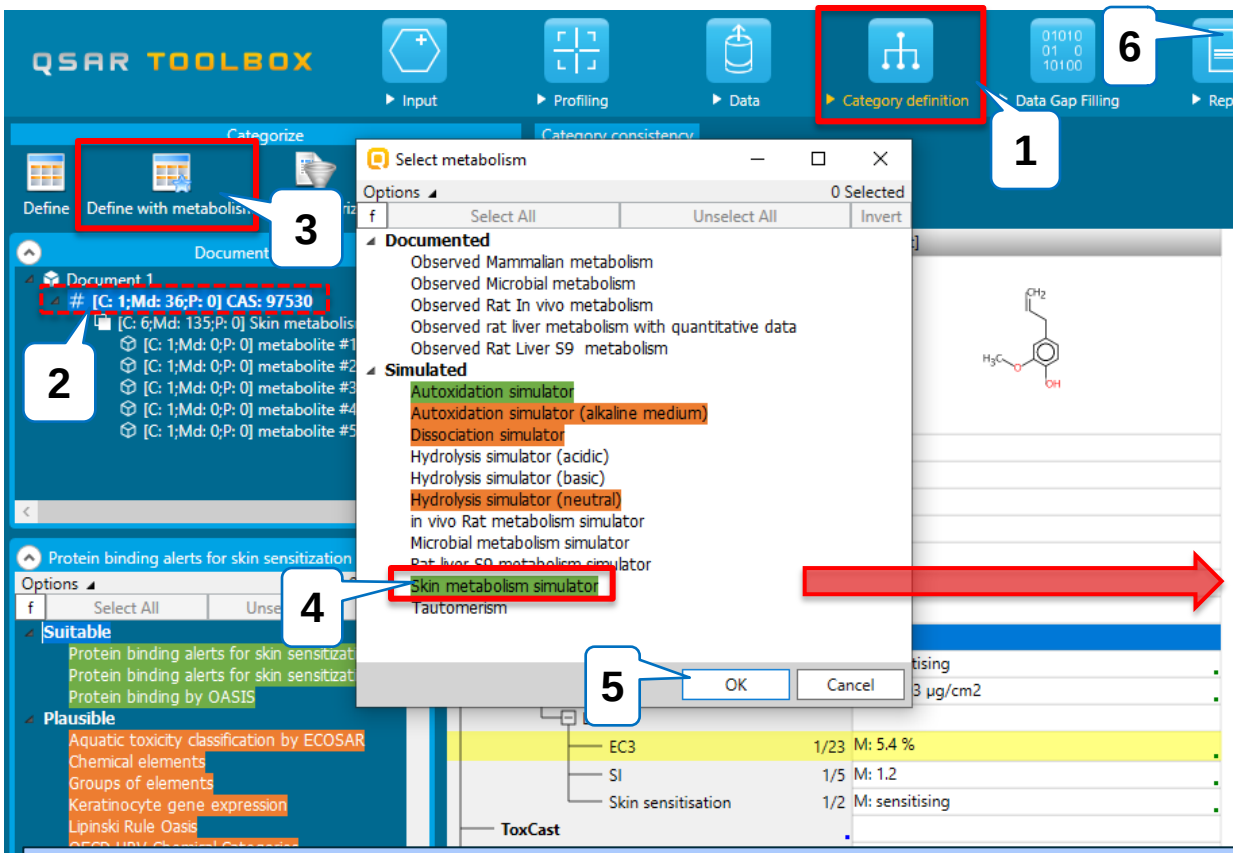
- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind by the same mechanism and for which experimental results are available.

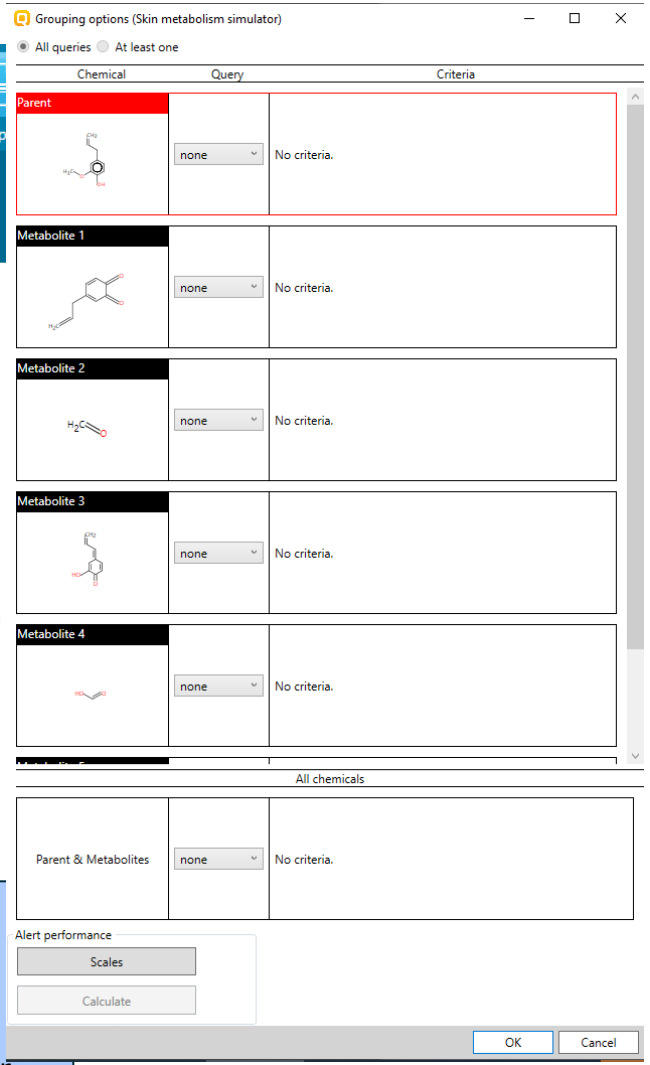
- If no alert is identified in the target structure, but is identified in its metabolites, analogues can be searched accounting for metabolism. In this way the target chemical and the identified analogues will have similar metabolic pattern.

- In our case searching for the analogues is based on a common metabolite (formaldehyde) generated as a result of skin metabolism. In other words we will search for the analogues having the same metabolite (i.e. formaldehyde) as the target chemical (see next slide).

Category Definition

Searching for analogues accounting for skin metabolism

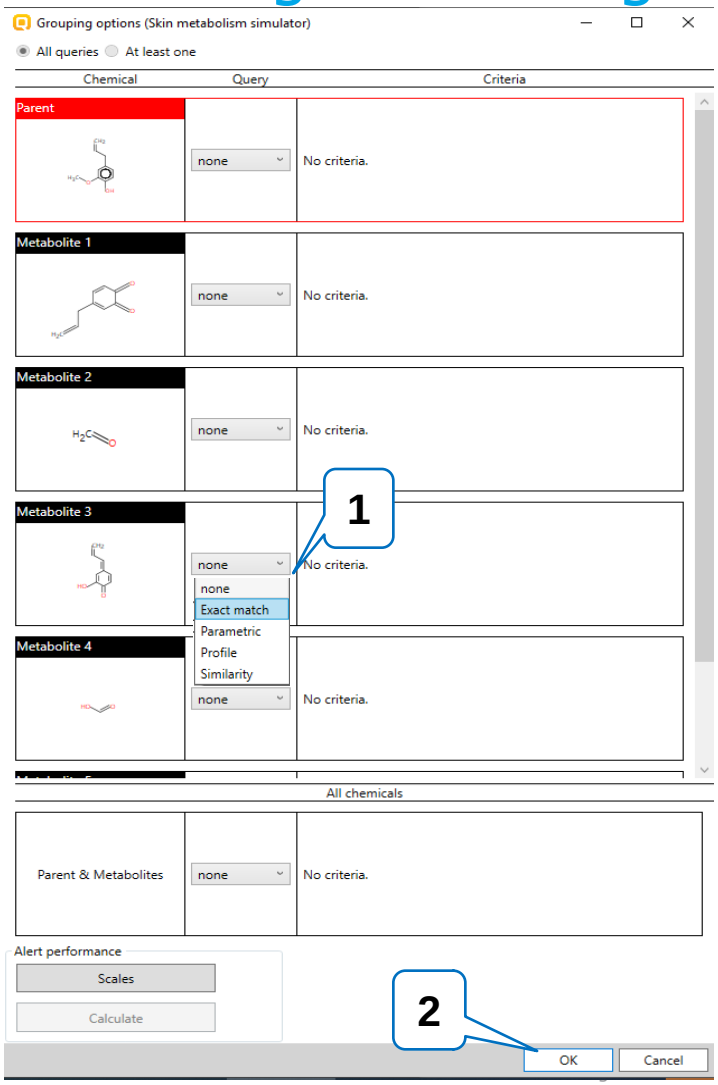




1. Go *to* ***Category definition*** *module*;
2. Click on the level with #CAS:97530;
3. Click ***Define with metabolism***;
4. Select ***Skin metabolism simulator***;
5. Click ***OK;***
6. Target and all metabolites produced by the selected simulator (SS in this case)appear.

Category Definition

Searching for analogues accounting for skin metabolism



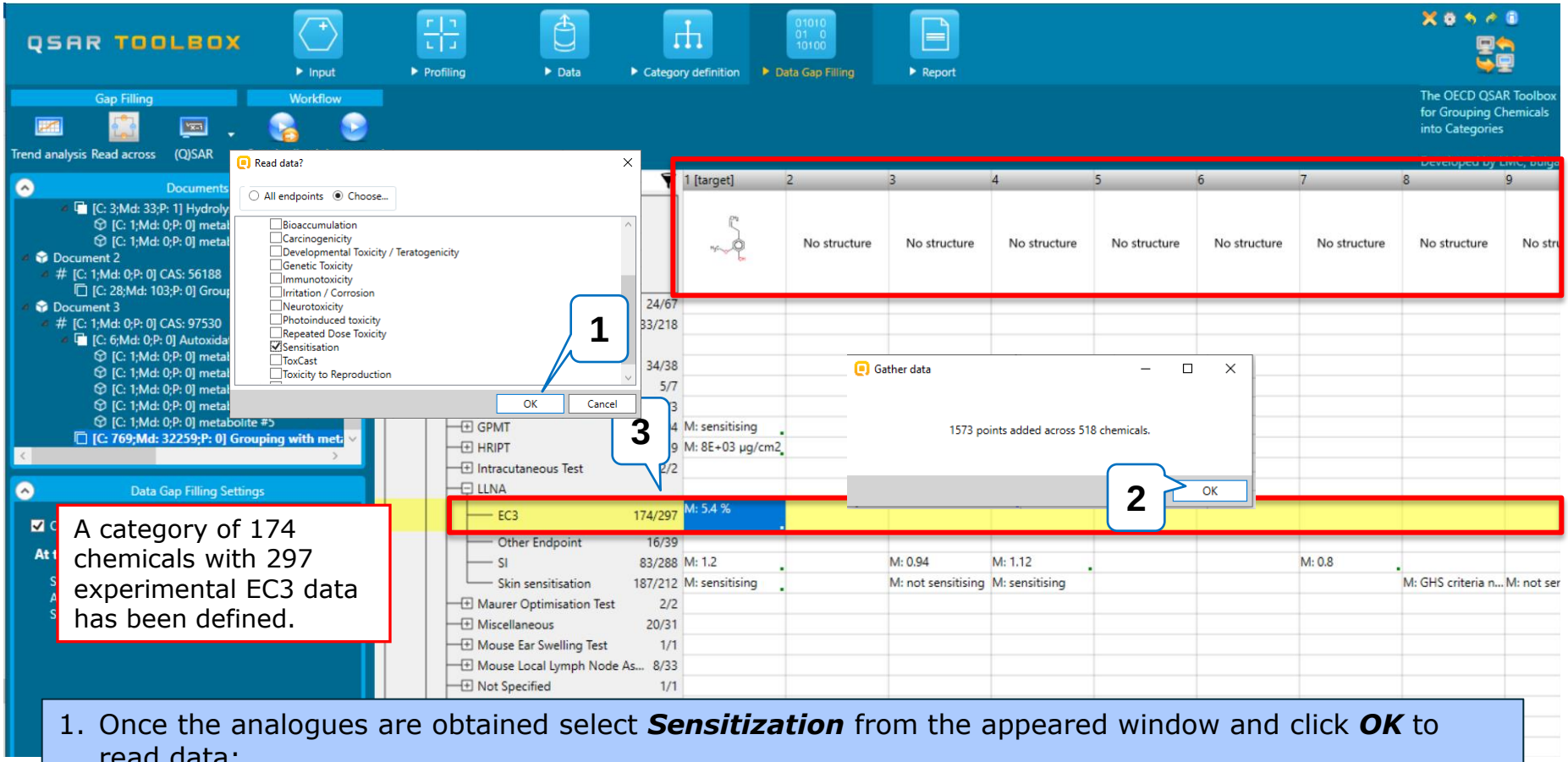
The **Exact** option is used for searching analogues with common metabolite. This option performs search for analogues which metabolites have the exact structure of the target metabolite

1. Scroll down to ***Metabolite # 2*** *(Formaldehyde)* and select ***Exact match*** option from the 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:
Tutorial_20_TB_4.4_New options for grouping with metabolism.pdf

Category Definition

Searching for analogues accounting for a skin metabolism



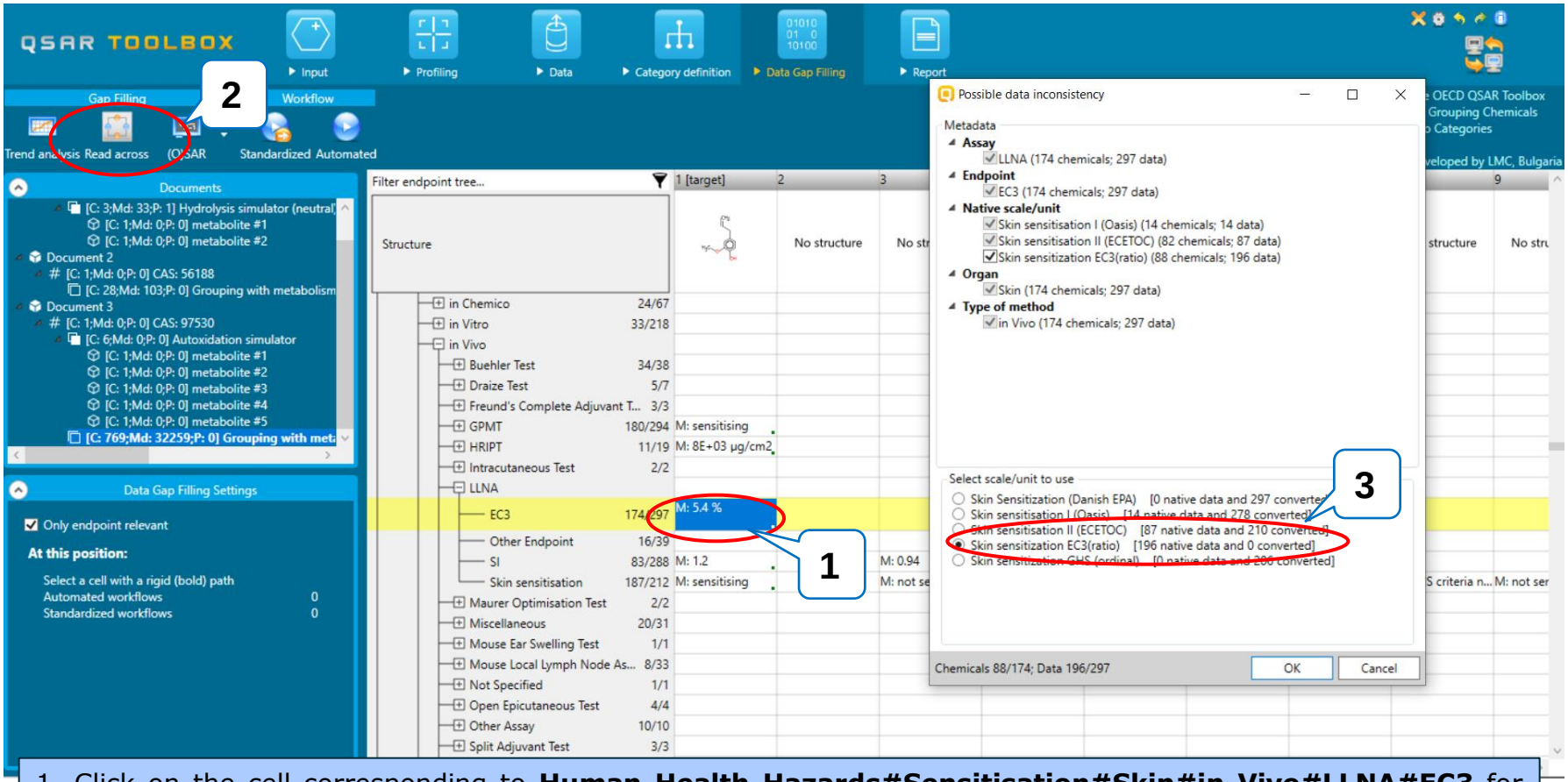
1. Once the analogues are obtained select ***Sensitization*** from the appeared window and click ***OK*** to read data;
2. Additional window appears informing about the number of collected experimental data and the number of chemicals in the category, click ***OK***.
3. The experimental data of the analogues 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.

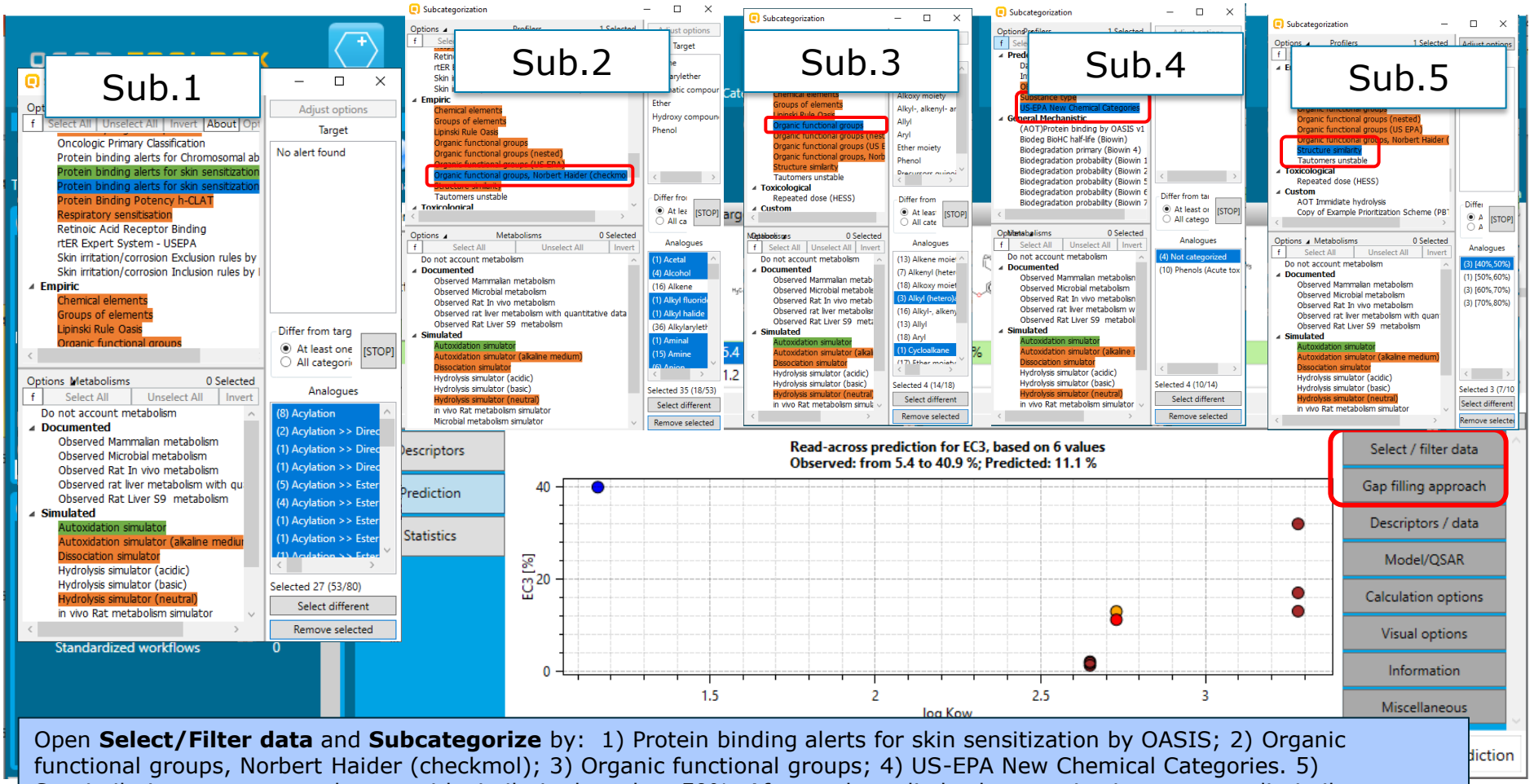
In this example we will use the read-across approach.

Data Gap Filling
Apply Read-across



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

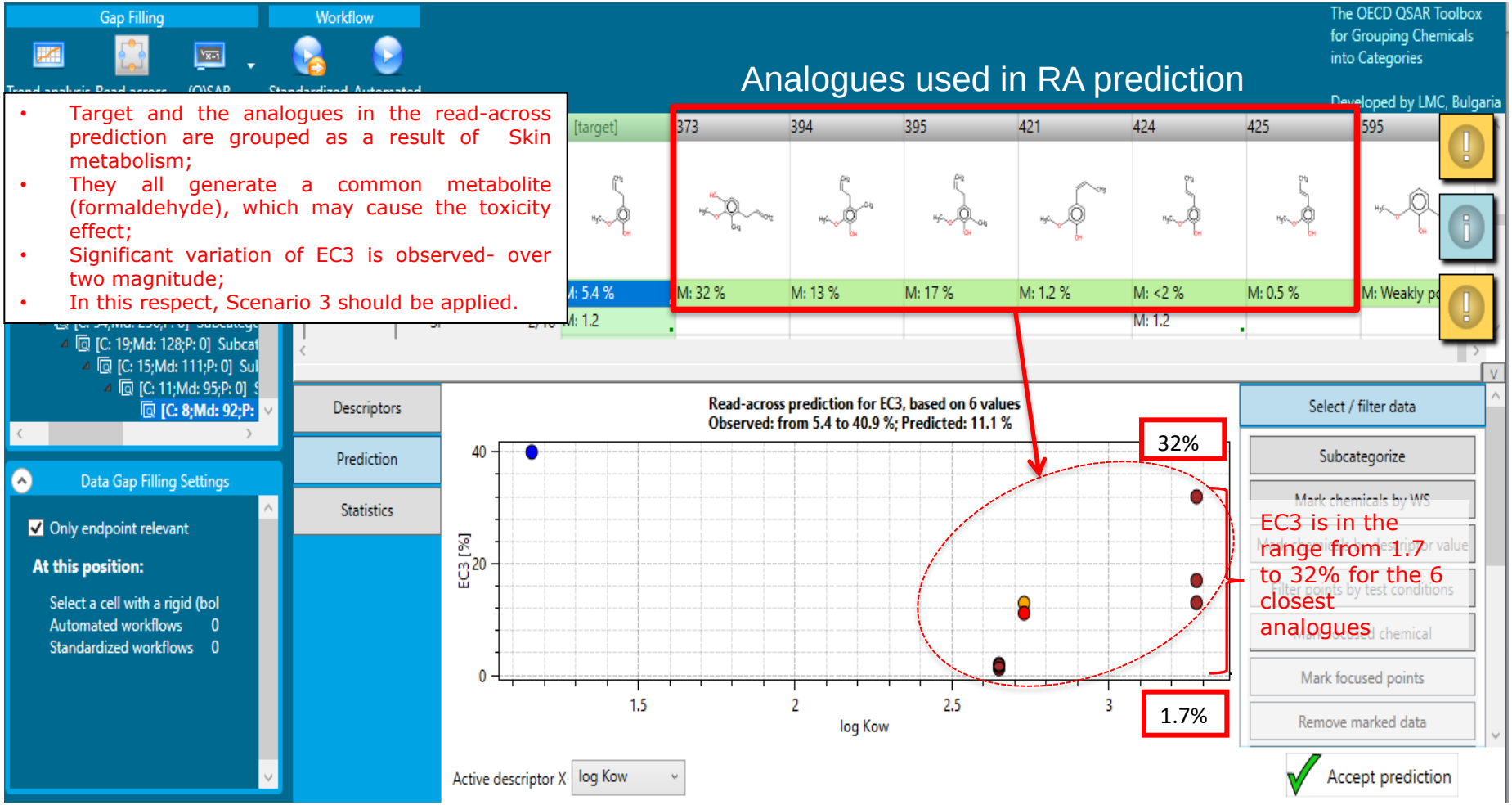
Data Gap Filling
Apply Read-across



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. 5) Str.similarity – remove analogues with similarity less than 50%. After each applied subcategorization, remove dissimilar analogues using "Remove selected" button.

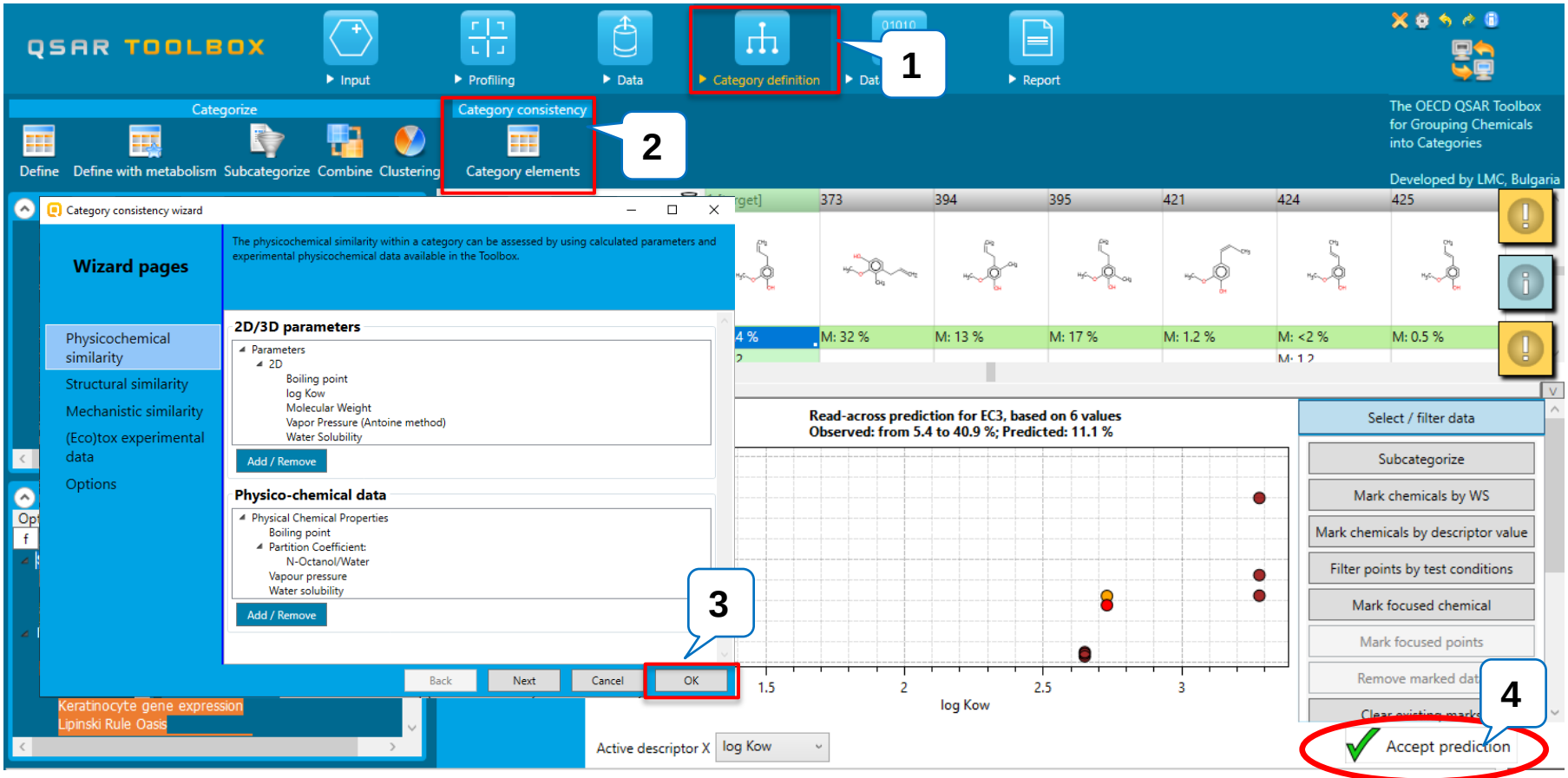
Data Gap Filling
Read-across recap

- Target and the analogues in the read-across prediction 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.



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).

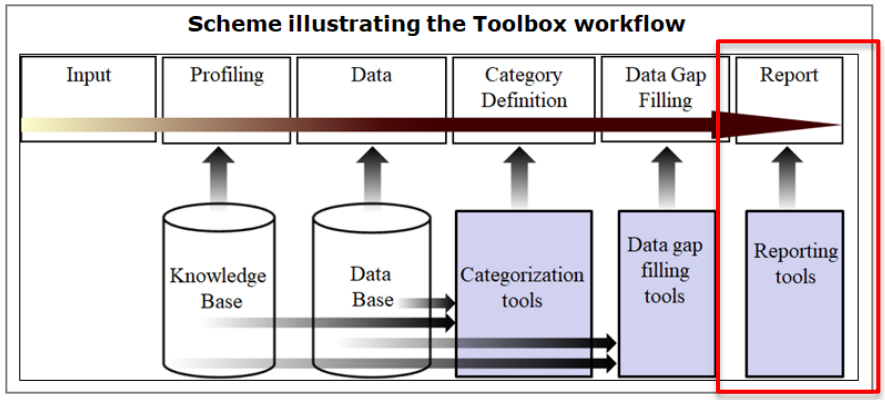
*For more information on category elements see *Tutorial_27_TB 4.4. Category elements for assessing Category consistency*

Recap

- In the *Category definition* module you found 173 chemicals with EC3 data 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. Five subcategorizations based on a protein binding mechanism and structural features are applied. As a result read-across prediction is based on the 6 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

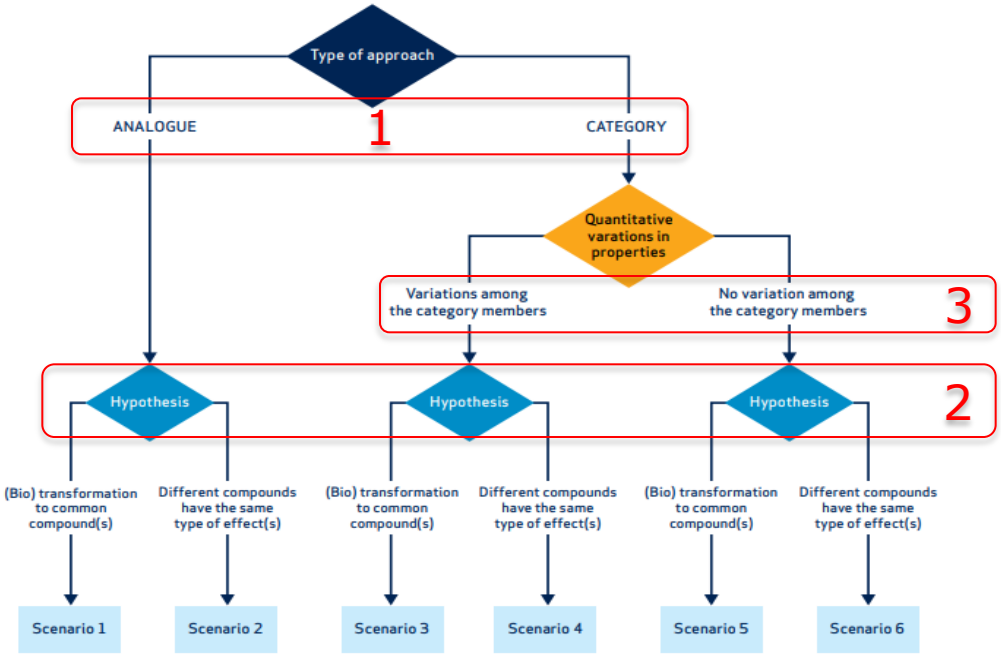
- The report module allows generating a report for predictions performed within the Toolbox.
- The report module contains a predefined report template which users can customize.
- Additionally specific RAAF scenario could be chosen. Selection of one of the scenarios will append automatically the related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



Report
Selection of a RAAF scenario

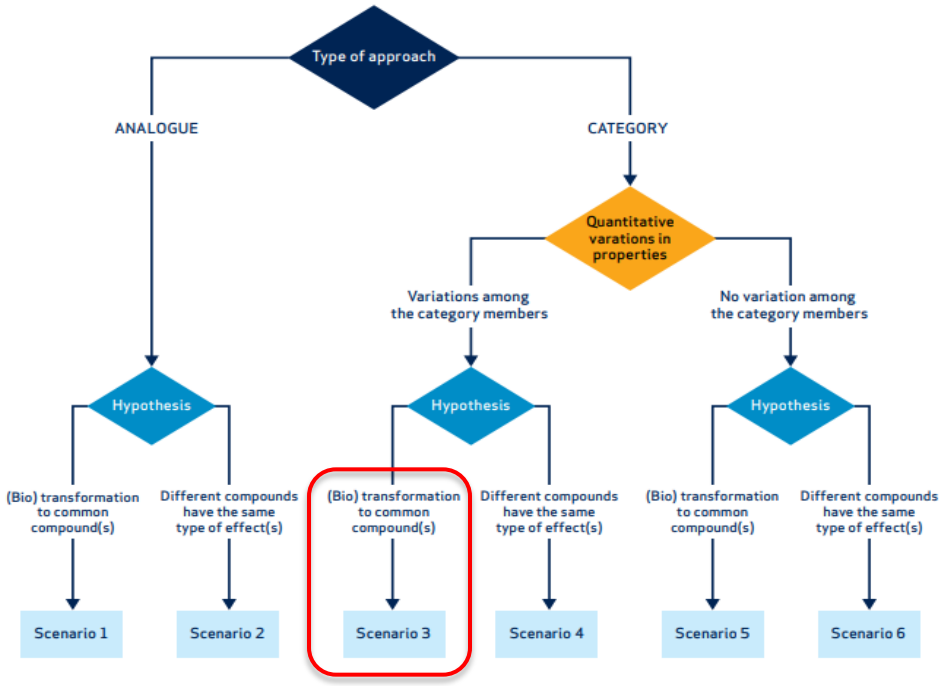
To select the applicable RAAF scenario for assessment, the following aspect should be identified*:

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



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

Report
Selection of a RAAF scenario



For this example the following criteria are met:

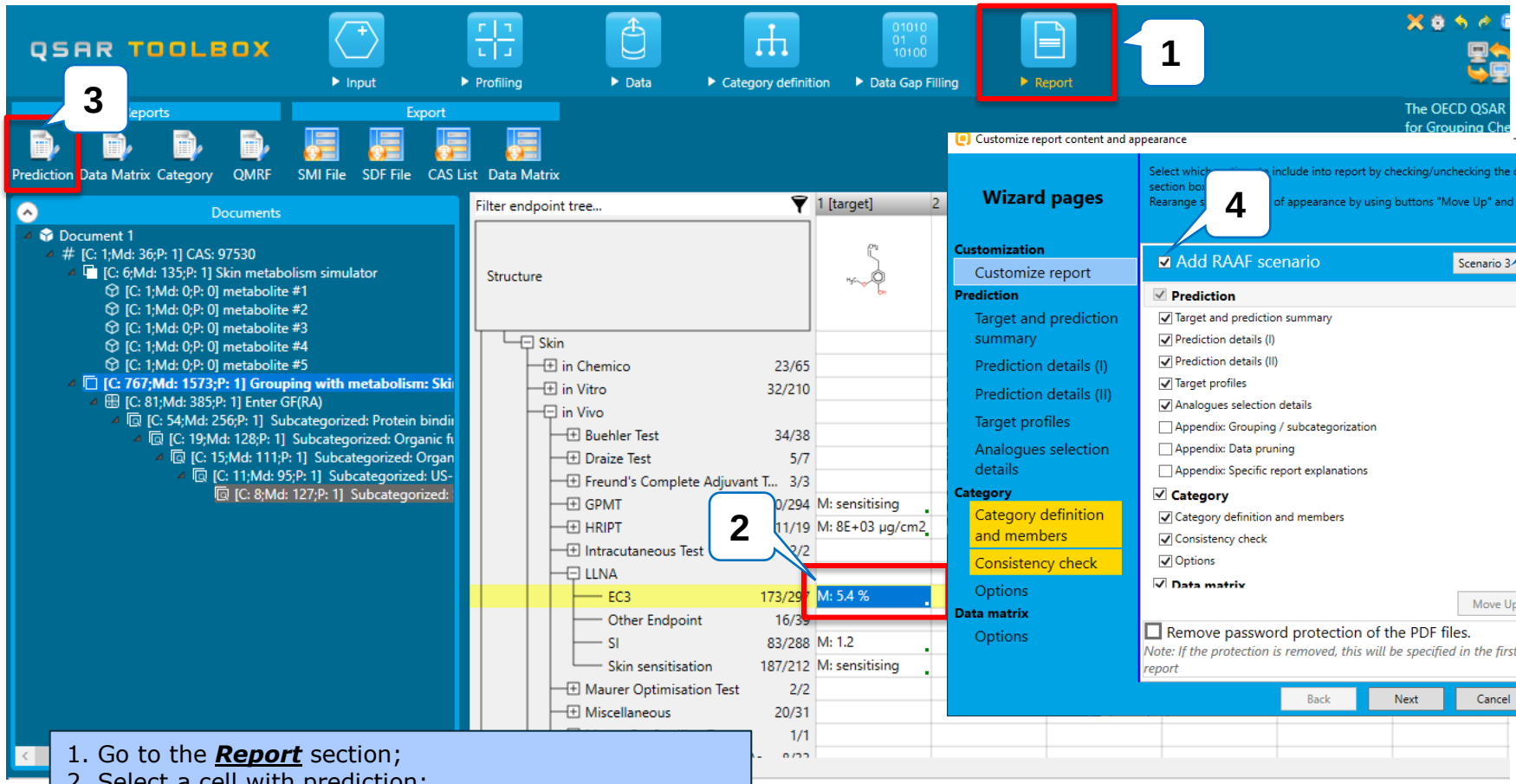
- the type of approach applied - category approach is used (threshold of > 3 analogues is proposed by LMC for the category approach);
- the read-across hypothesis – different compounds (bio)transformed to the common compound;
- There is a significant variation of the toxic effect (EC3) among the category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

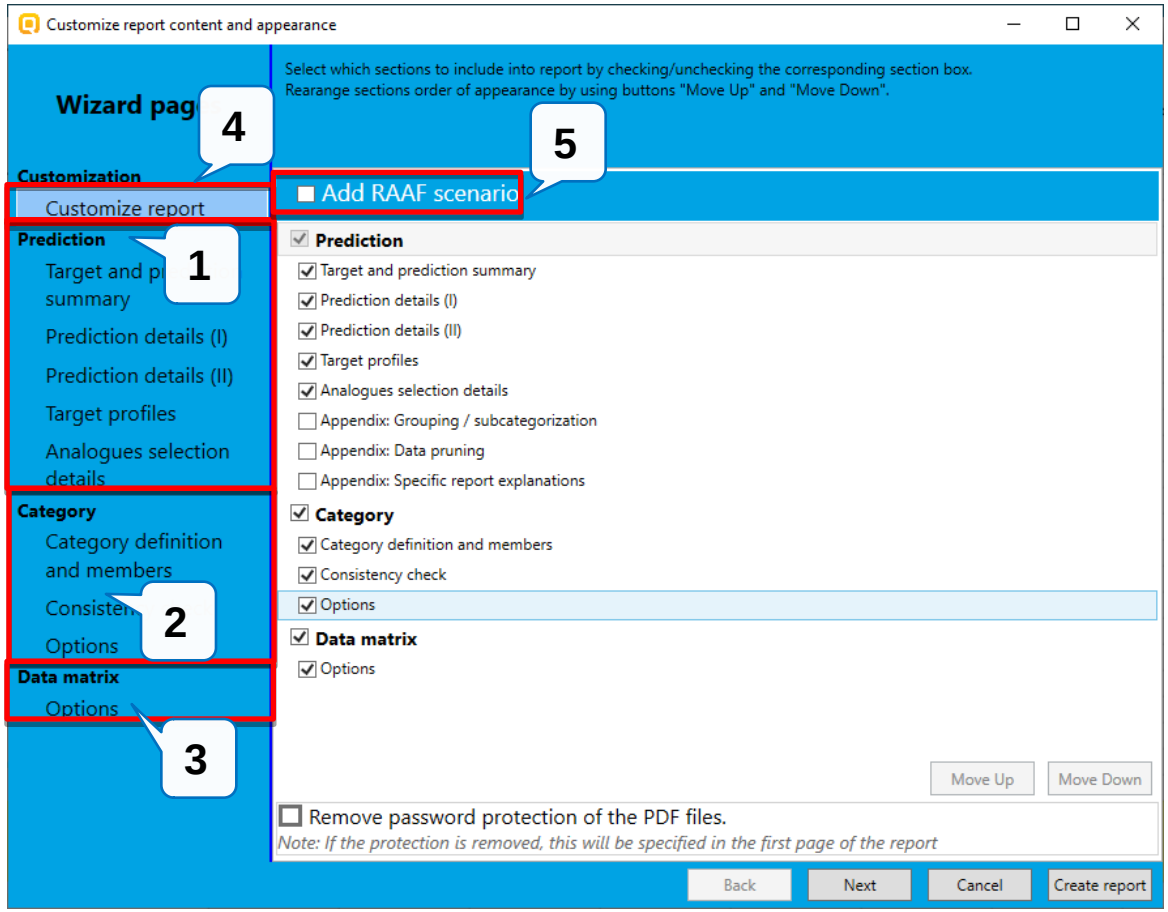
Report

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.

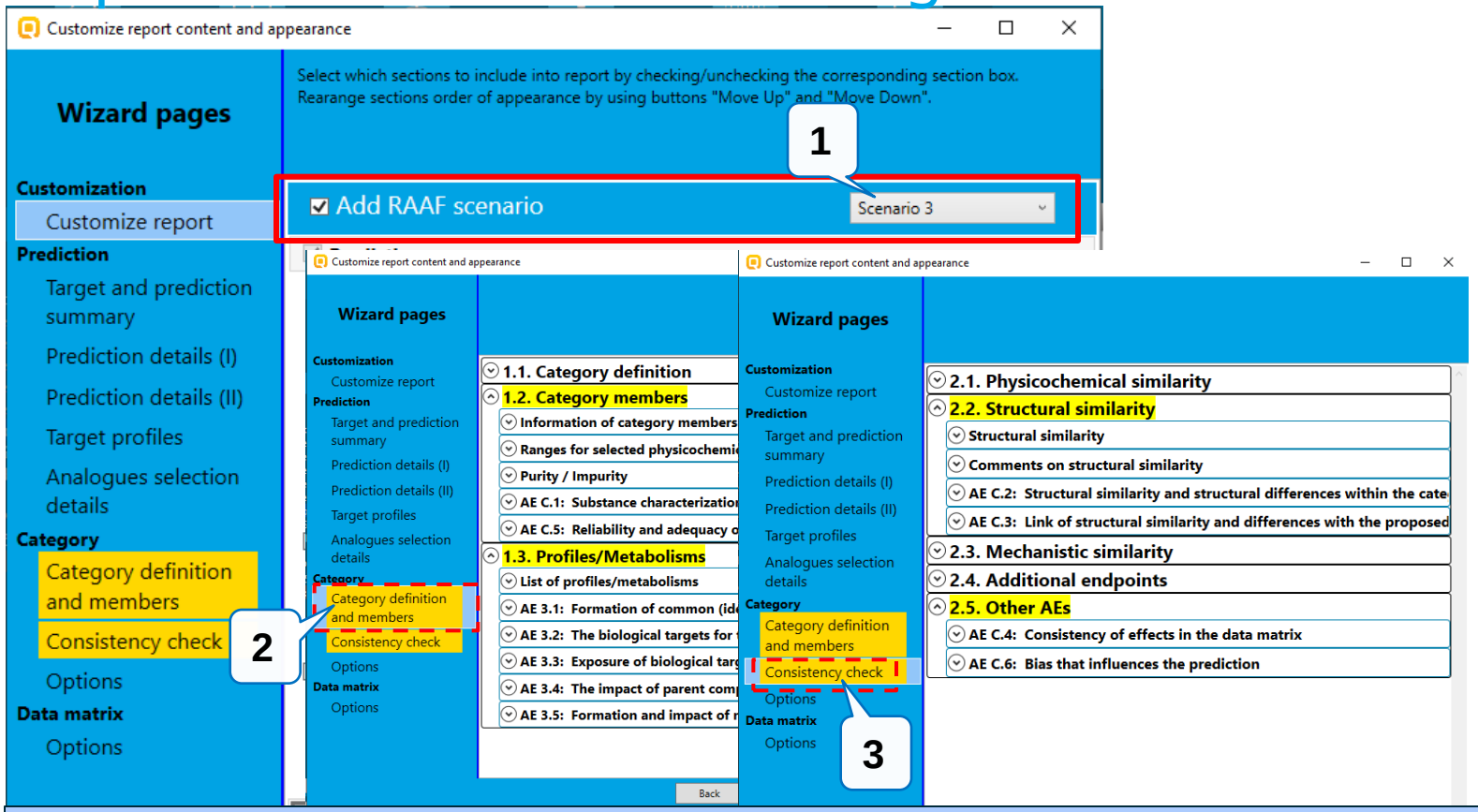
Report
Report Generation according to RAAF-Scenario 3



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 box* (5).

Report

Report Generation according to RAAF-Scenario 3

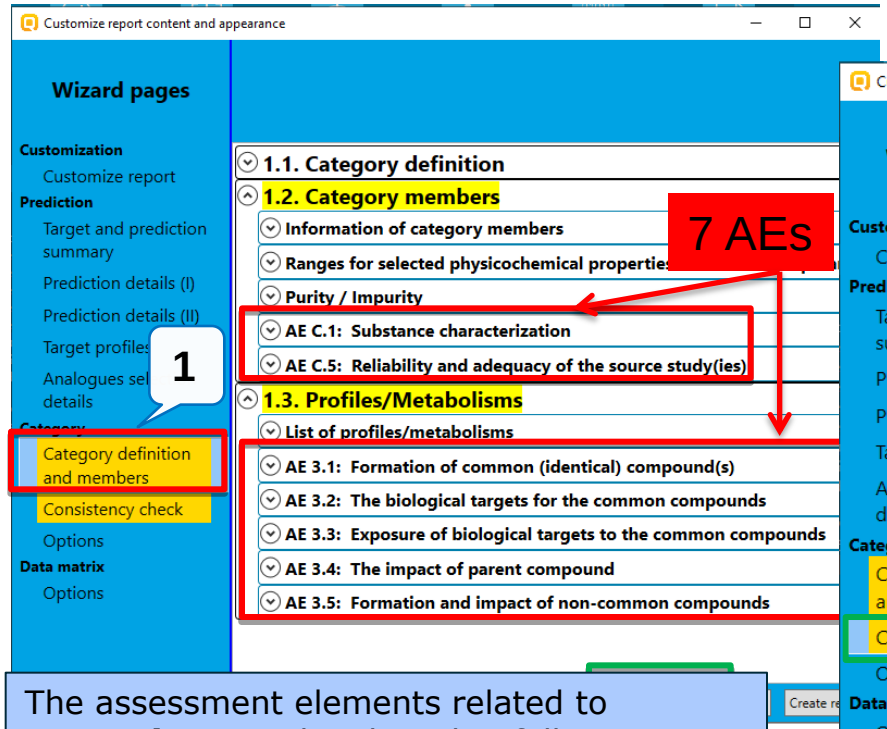


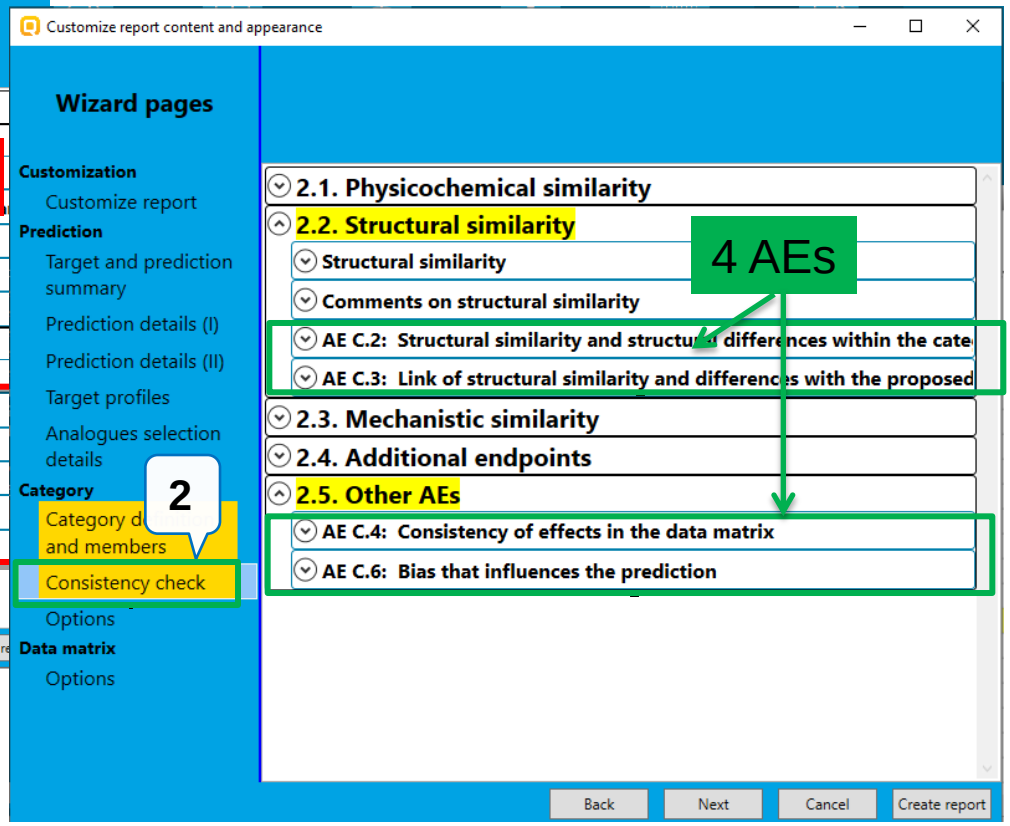
Once the RAAF scenario is selected (1) the related assessment elements (AEs) appeared automatically to the category part of the report. Sections for which the related AEs appear are getting yellow highlighted. They appear in the following category report sections: ***Category definition and members*** (2) and ***Consistency check*** (3).

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





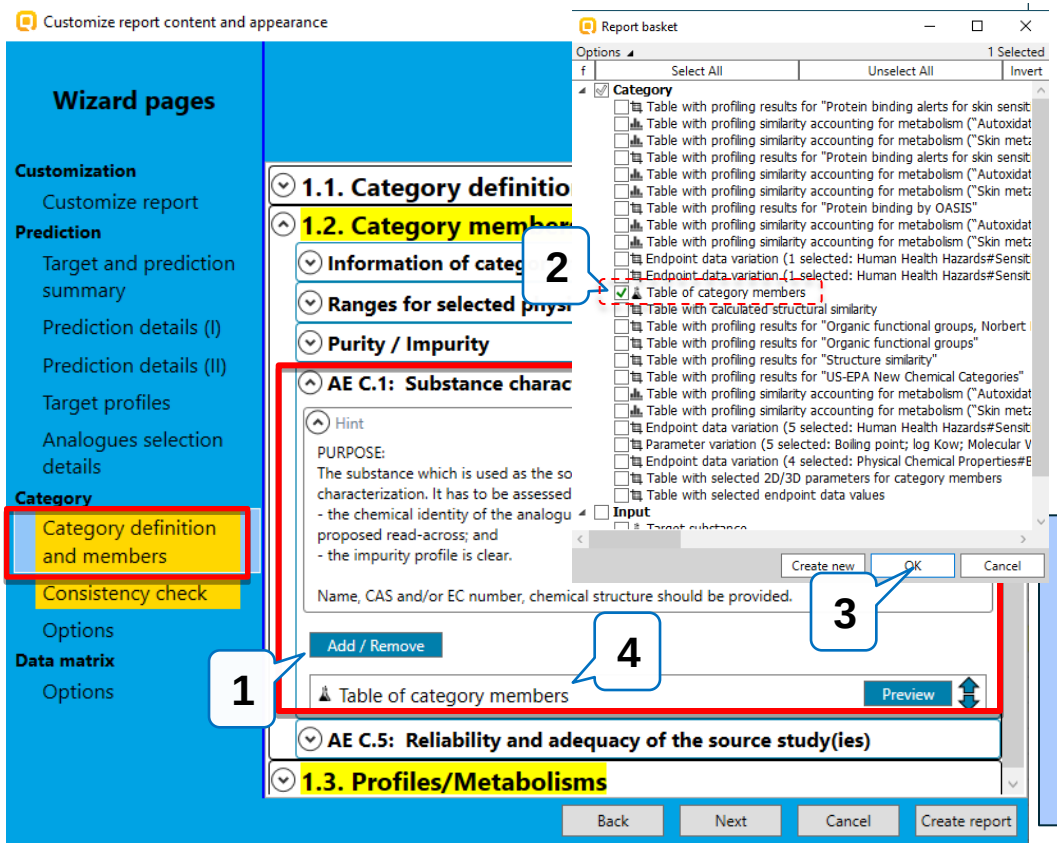
The assessment elements related to ***Scenario 3*** are distributed in following two sections of the wizard page: seven AEs are included in *Category definition and members* (1) and four AEs included in the *Consistency check* (2) section.

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE C.1 Substance characterization



5

AE C.1: Substance characterization

Table of 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	5932-68-3	(E)-Isoeugenol	COc1cc(C=CC)ccc1O	
4	186743-26-0	3-METHYL_EUGENOL	COc1c(O)ccc(CC=C)c1C	

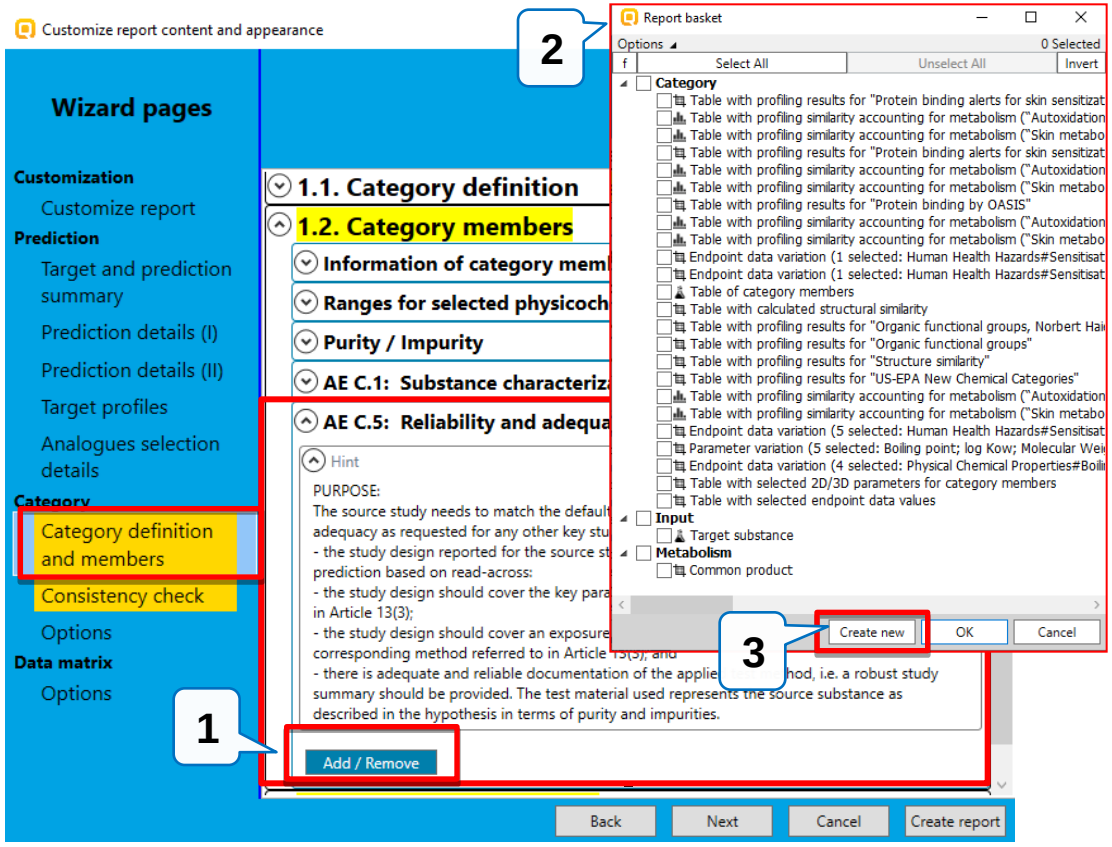
Category elements should be added manually to the **AE C.1** by click on ***Add/ Remove*** (1) button then check already available item **Table of Category members** (2) and click **OK** (3). The item then will appeared in the wizard (4). If impurities/additives of the used analogues are available, they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

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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



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).

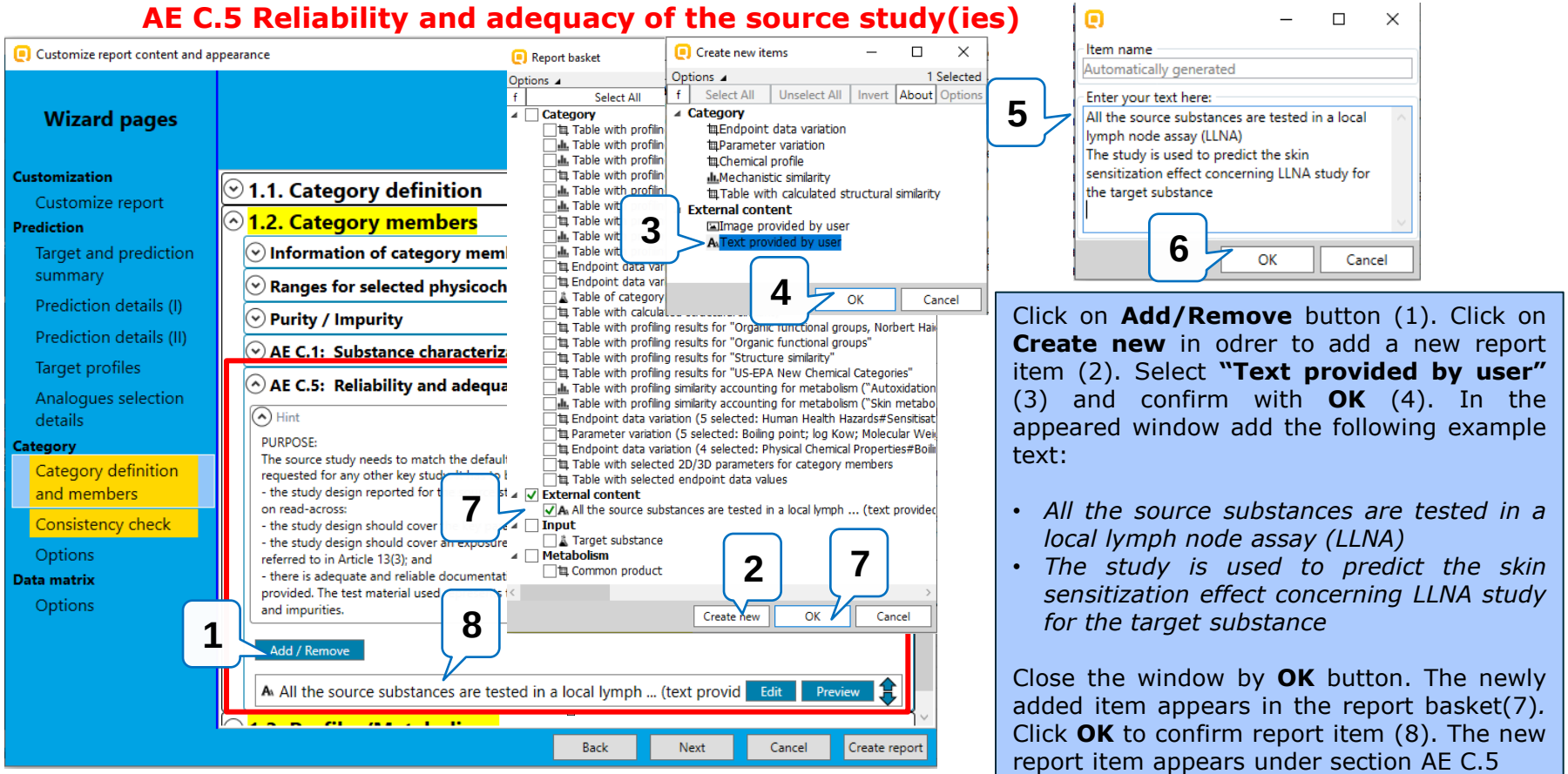
Items with external content (text and picture) will be added for **C.5 Reliability and adequacy of the source study(ies)** (see next two slides)

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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



Click on **Add/Remove** button (1). Click on **Create new** in odrer to add a new report item (2). Select **"Text provided by user"** (3) and confirm with **OK** (4). In the appeared window add the following example text:

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

Close the window by **OK** button. The newly added item appears in the report basket(7). Click **OK** to confirm report item (8). The new report item appears under section AE C.5

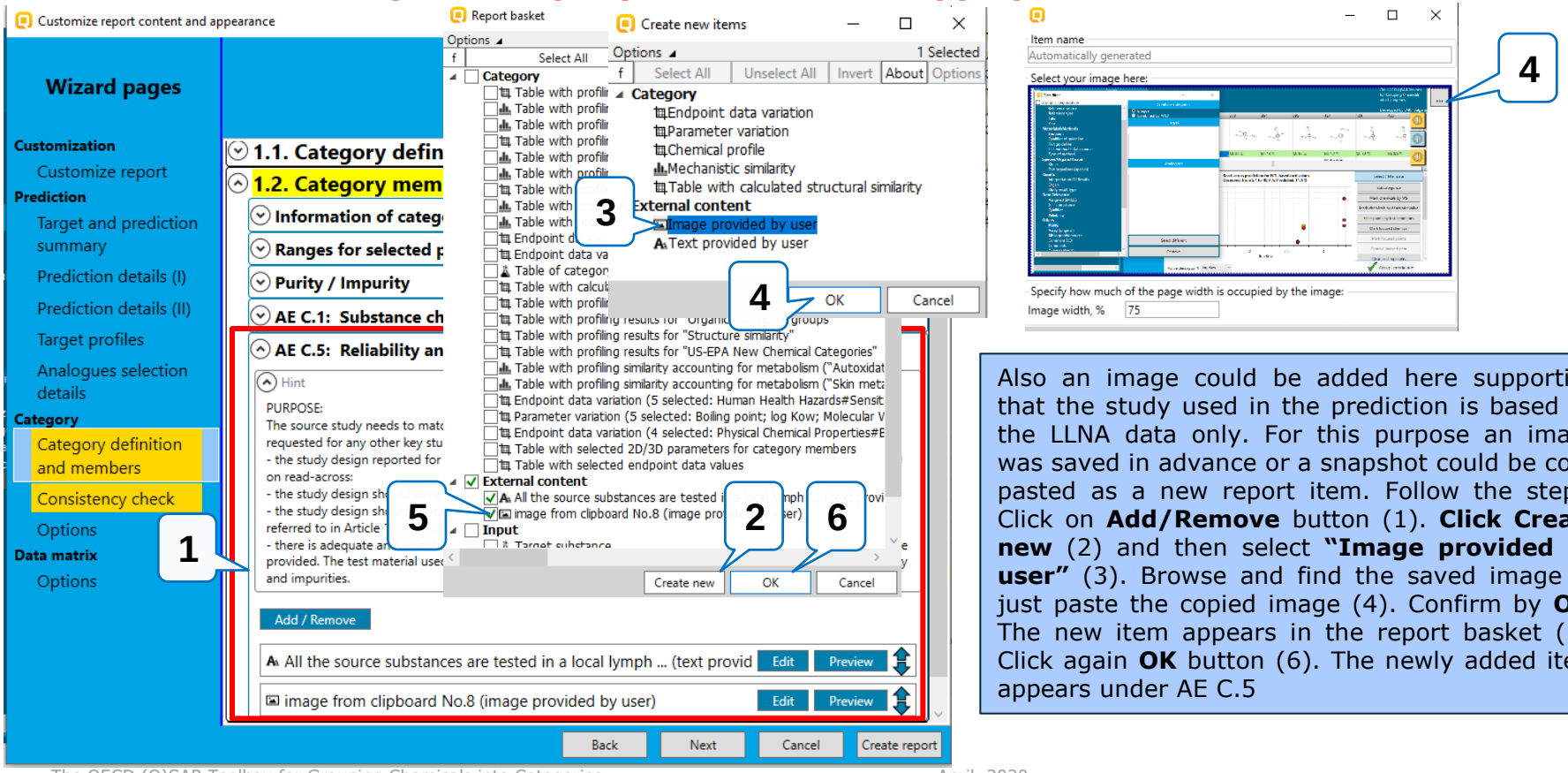
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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



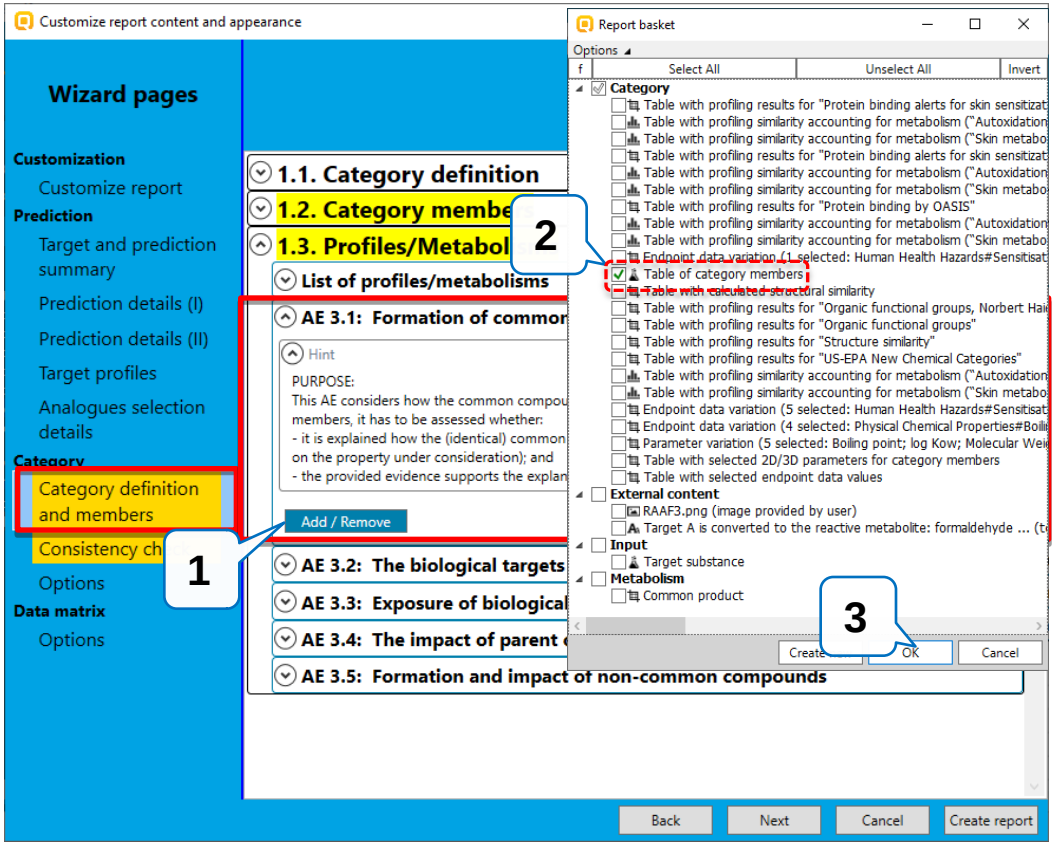
Also an image could be added here supporting that the study used in the prediction is based on the LLNA data only. For this purpose an image was saved in advance or a snapshot could be copy pasted as a new report item. Follow the steps: Click on **Add/Remove** button (1). **Click Create new** (2) and then select **"Image provided by user"** (3). Browse and find the saved image or just paste the copied image (4). Confirm by **OK.** The new item appears in the report basket (5). Click again **OK** button (6). The newly added item appears under AE C.5

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)



Move to the section **1.3. Profiles/Metabolites**. The first AE is **AE 3.1. Formation of common compound.** Here a table with category members could be added manually and also a text. In order to add a table open the *Report basket* (Add/Remove button) (1) and tick the box with "Table with category members" (2), then click **OK (3)**. Additional text could be added by click on the **Add/Remove** button (1) and **create new item** with a textual content (see slide 62, how to add the item)

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

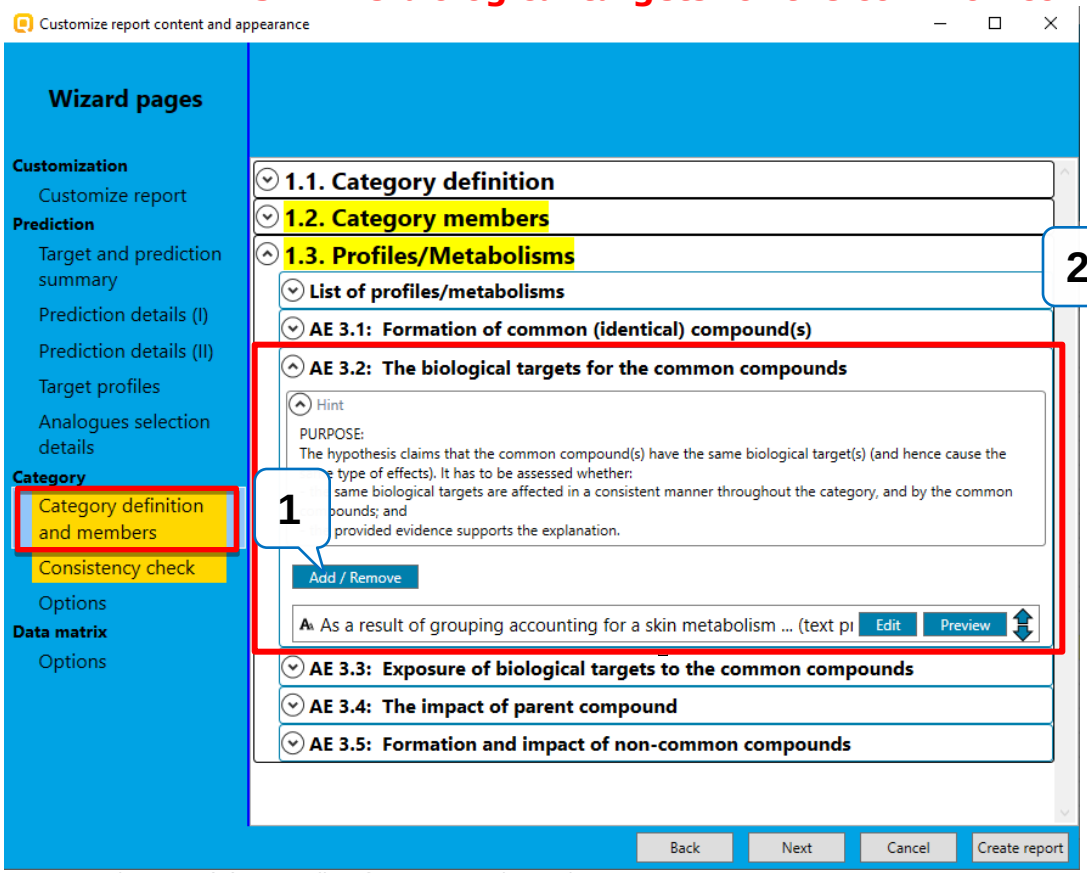
- Target chemical A is claimed to be metabolized to formaldehyde and that the organism is only systemically exposed to formaldehyde upon an external exposure to Target A.
- The five source substances (analogues) as a result of a Skin metabolism have generated the common metabolite - formaldehyde
- Therefore, it is expected for the formaldehyde to be responsible for the toxic effect
- The five 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** item with the following example text (2):

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 six source substances (B, C, D, E, F, G) 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 six source substances are used to predict the toxic effect of substance A

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

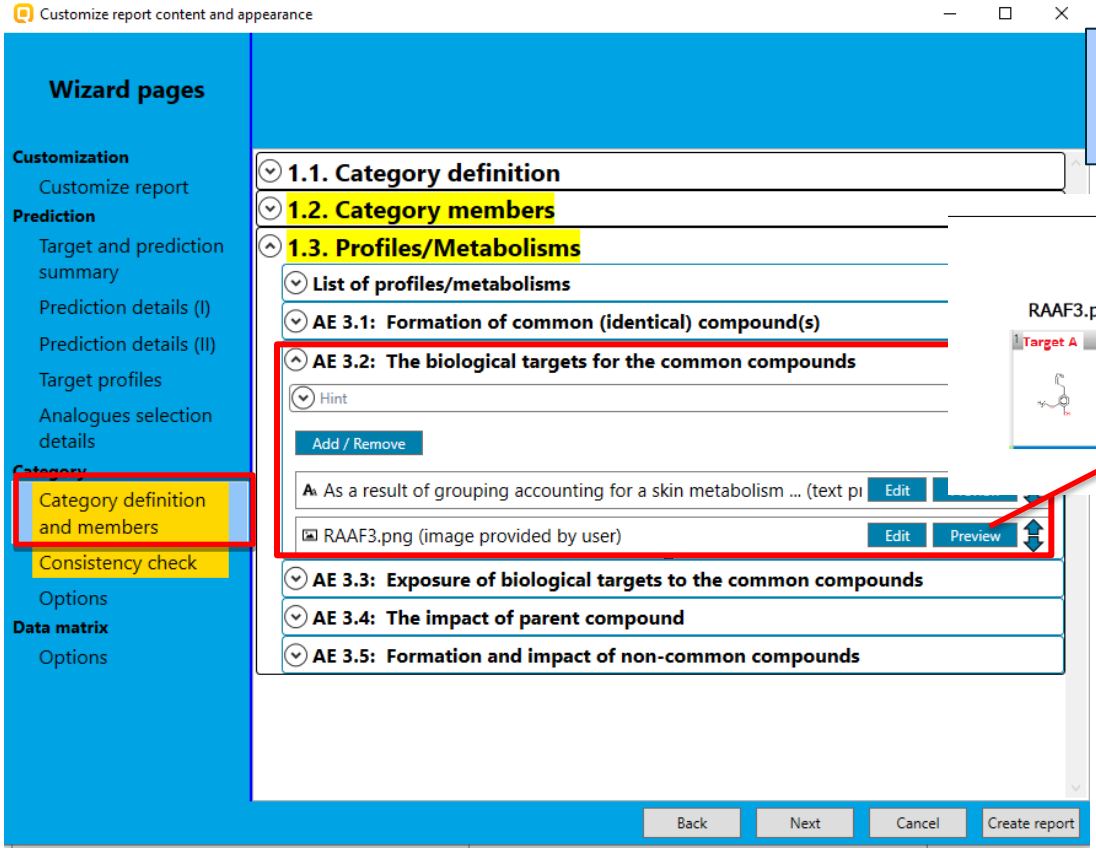
Report

Report Generation according to RAAF-Scenario 3

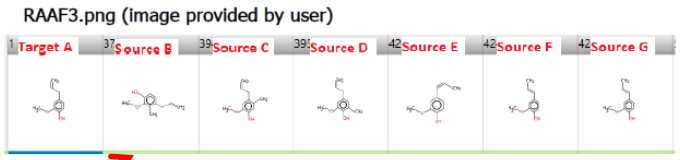
Section Category

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 63 how to create an image report item):



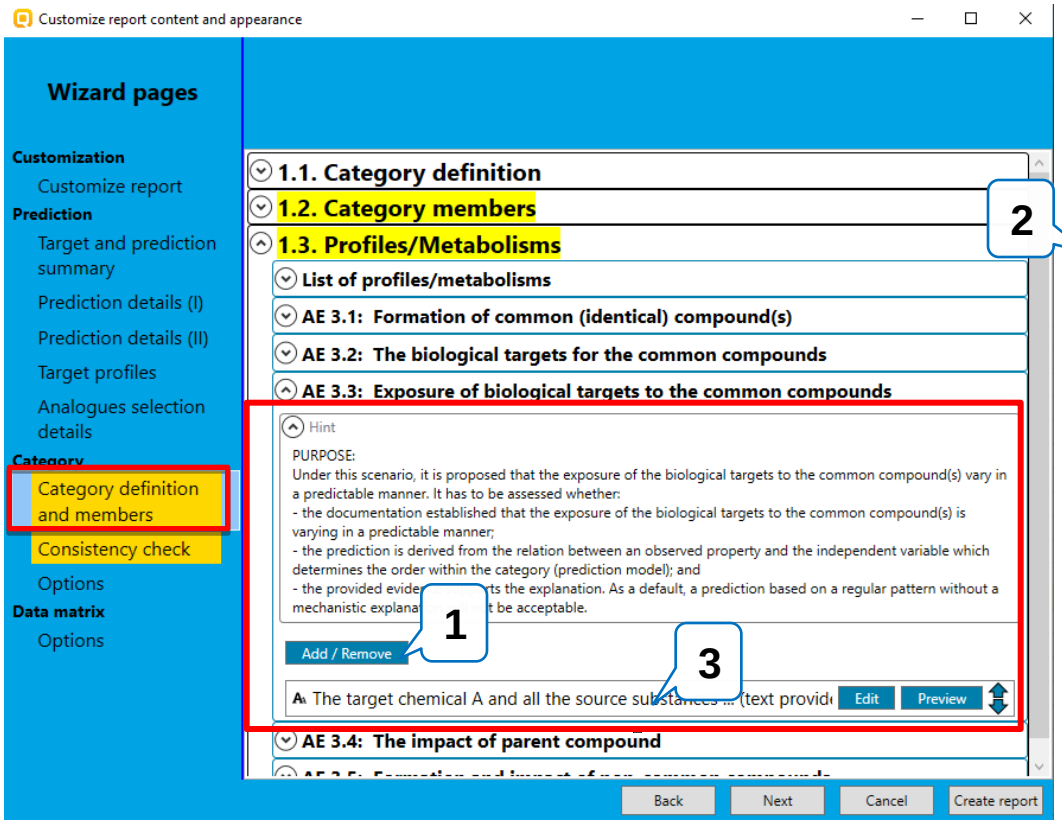
How the newly added item will looks like in the report could be seen by preview btton

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)
The new item appears under the AE 3.3 (3)

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

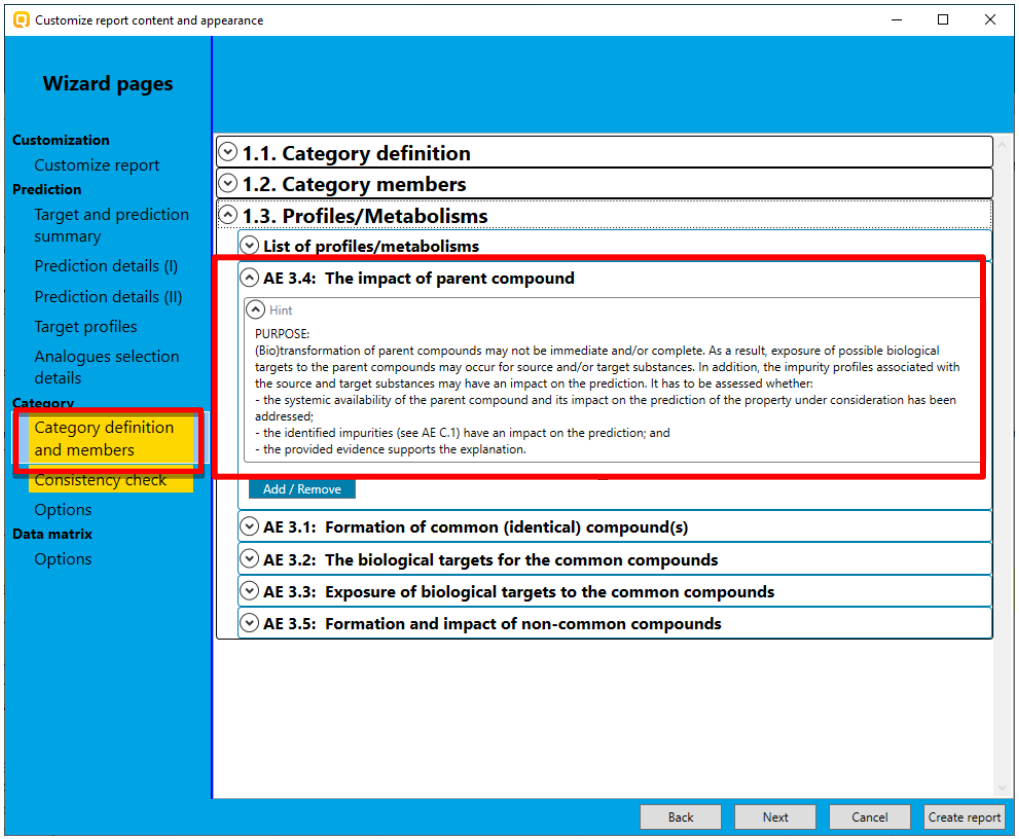
- The target chemical A and all the source substances are metabolized to the common reactive metabolite: formaldehyde
- It well known from the literature (Ref. cited) that all aliphatic aldehydes can potentially undergo a **Schiff base formation** with a primary amine. The generated formaldehyde reacts 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 3.4.The impact of a parent compound



The *AE 3.4.The impact of a parent compound* is associated with the effect of the target chemical to the assessed toxic effect. In this respect a text and image are added to address the issue.

See next few slides

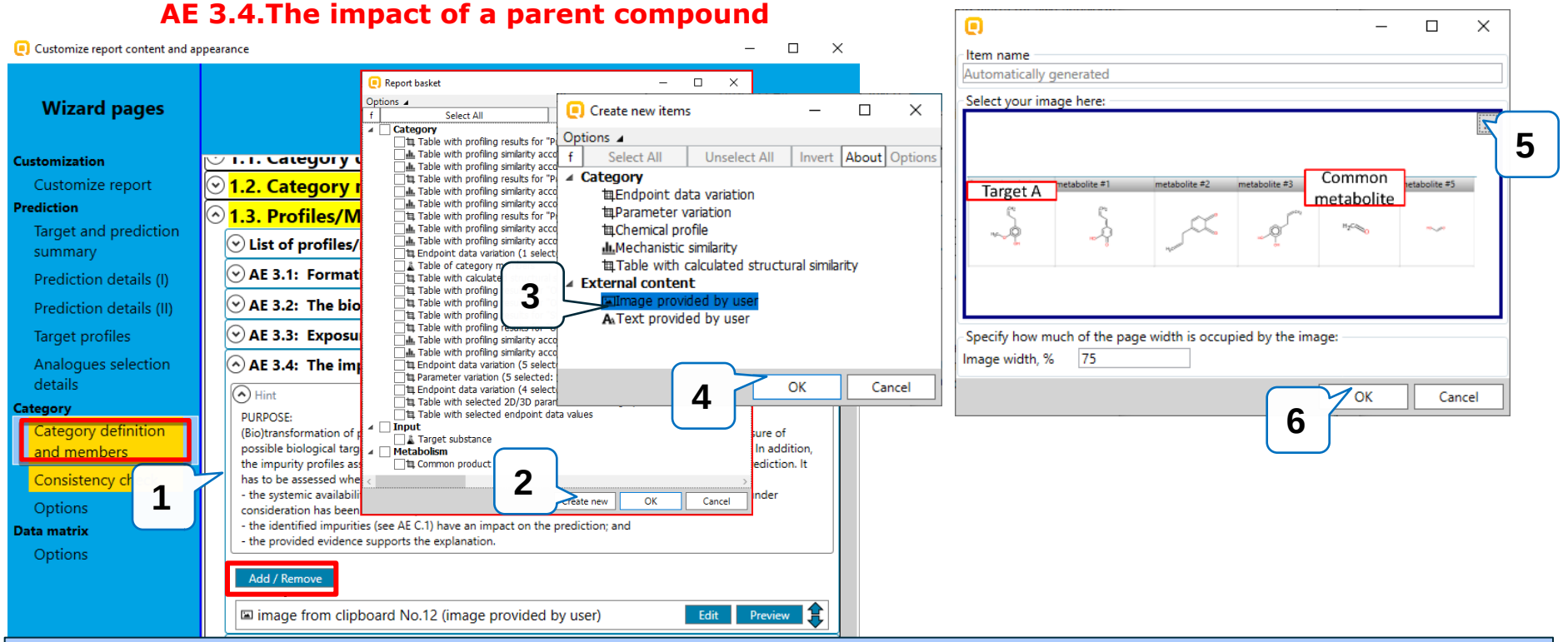
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.4.The impact of a parent compound



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

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

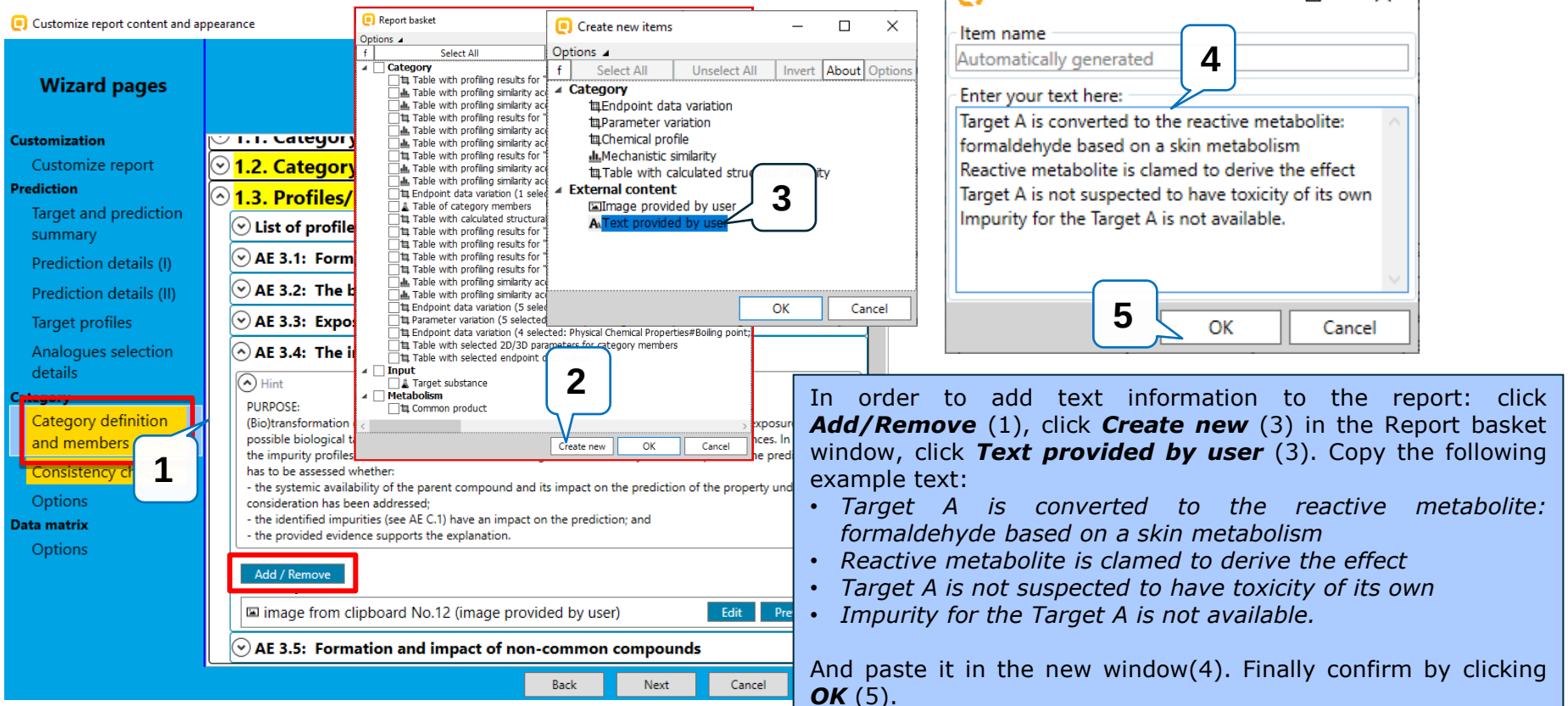
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.4.The impact of a parent compound



In order to add text information to the report: click ***Add/Remove*** (1), click ***Create new*** (3) in the Report basket window, click ***Text provided by user*** (3). 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(4). Finally confirm by clicking ***OK*** (5).

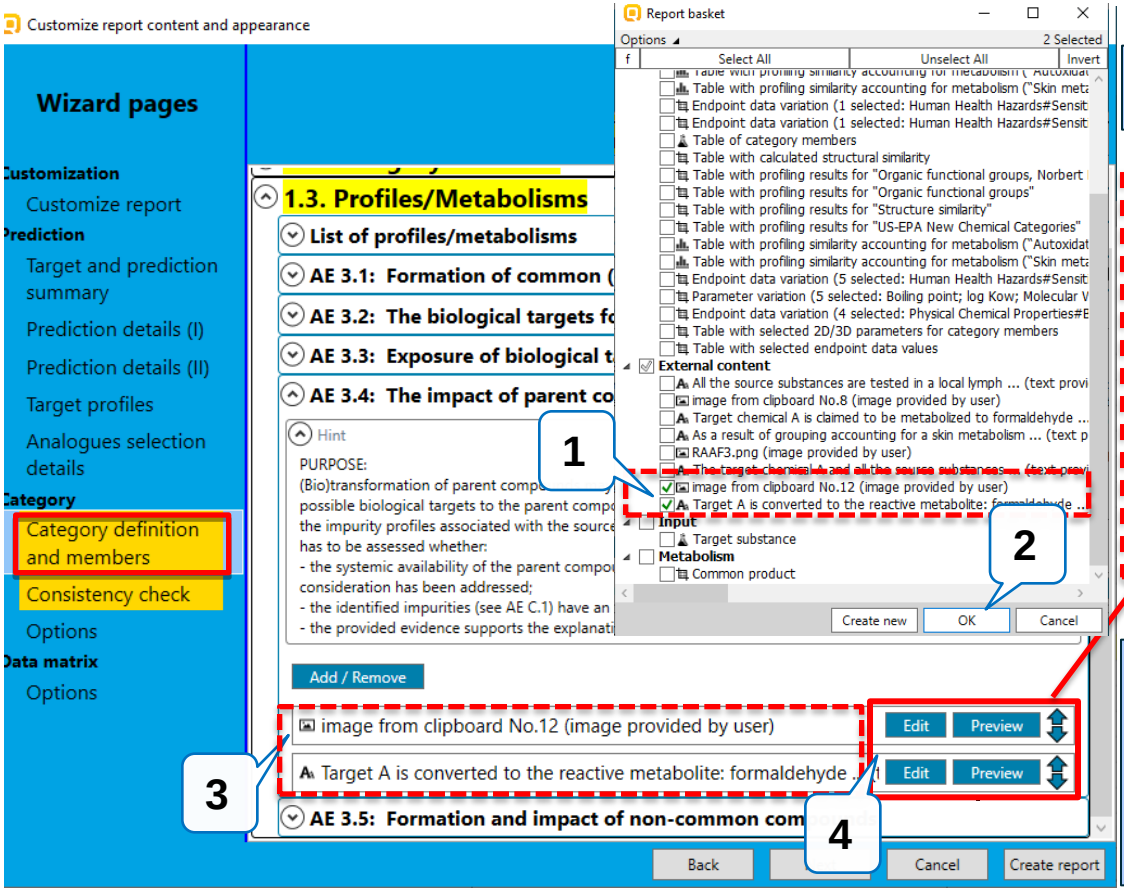
Report

Report Generation according to RAAF-Scenario 3

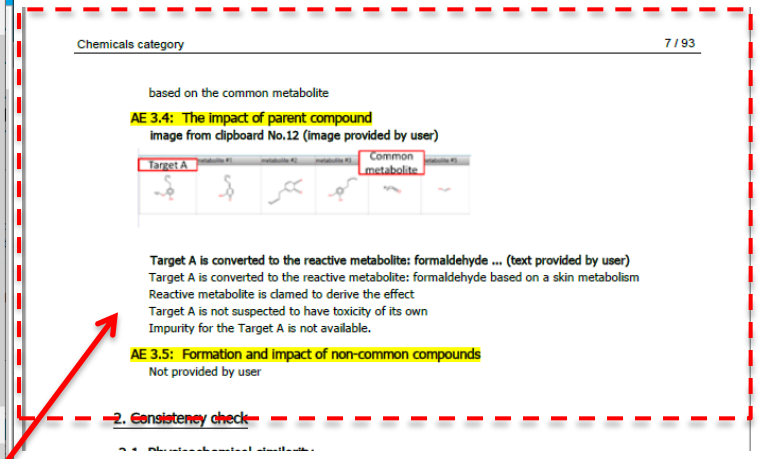
Section Category

Subsection: Category definition and members

AE 3.4.The impact of a parent compound



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



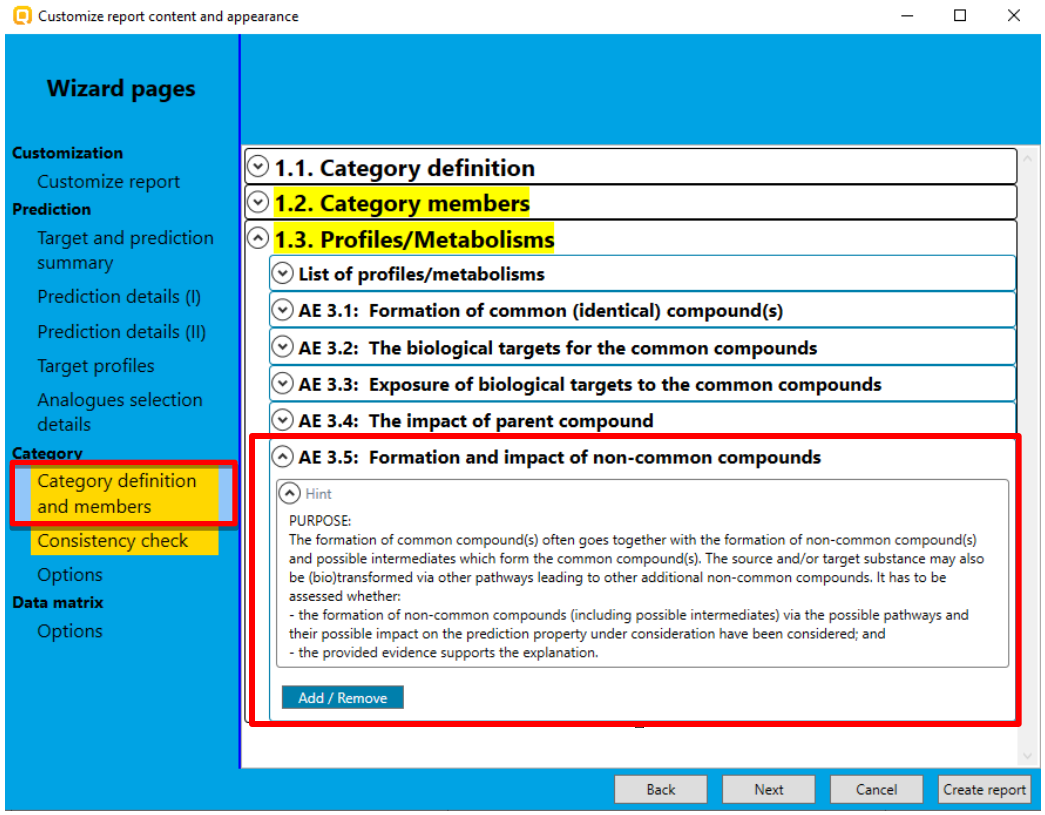
The entered text along with picture added before are listed in the ***Report basket*** under *External content* section and the check box is ticked (1). Click **OK** (2). The new items is added under the corresponding AE 3.4 (3). There are three options (4) for **previewing**, **editing or change the positions** of the items.

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:**

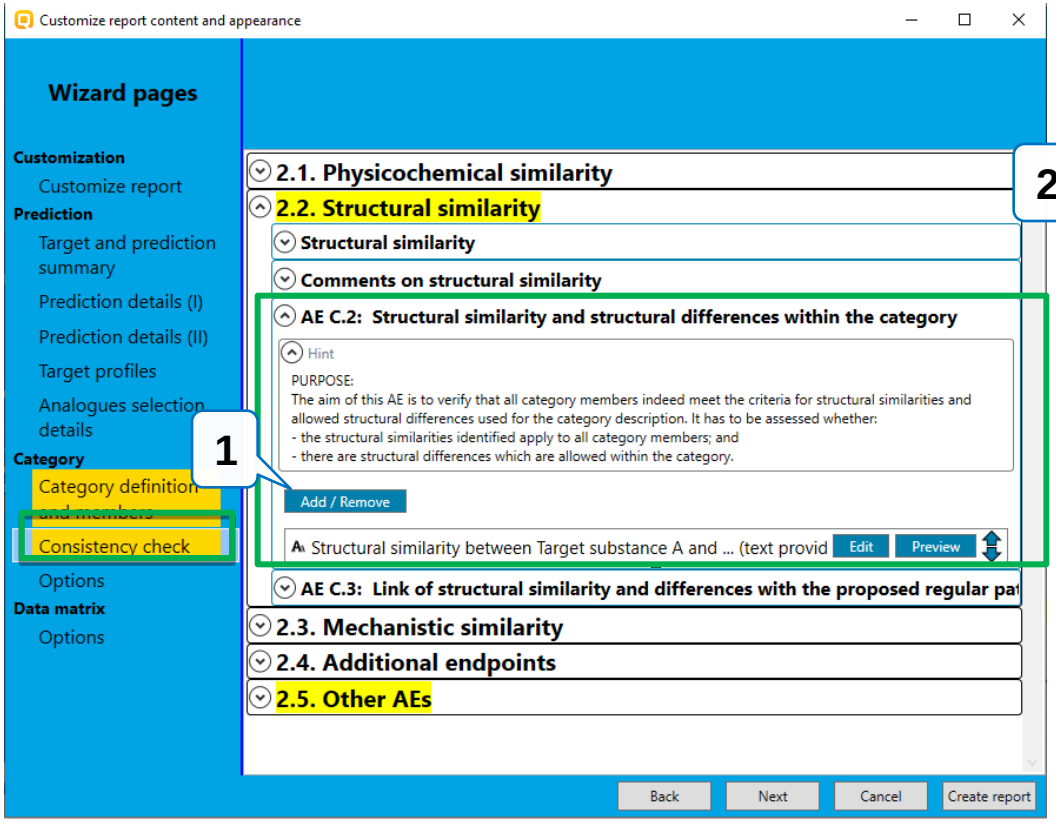
- The target substance A and the six source substances (analogues) are metabolized to the common- formaldehyde and non-common compounds (including possible intermediates)
- The positive effect might be due to the common compound (formaldehyde) reacting with proteins via a Schiff-base formation mechanism
- 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: formaldehyde
    - 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: Consistency check

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



Move to section Consistency check
Click on the **Add/Remove** button (1) **and create new item** with a possible example text (2). The item appeared after that in the wizard under **AE C.2 (3)**

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

- Structural similarity between Target substance A and the six source substances (B, C, D, E, F, G) according to Str.similarity profiler is in the range of [40-85%]
- All the 6 source substances have a selection of Alkene, Ether, Alkoxy, Aryl, Allyl, Alkyl,-alkenyl and alkynyl (hetero)arenes and Phenol groups based on OFG profiler
- 3 out 6 have additional Precursors quinoid compounds (B, C and D)
- While the target substance A and the source substance E, F and G have additional structural fragment "Alkenyl (hetero)arenes"

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

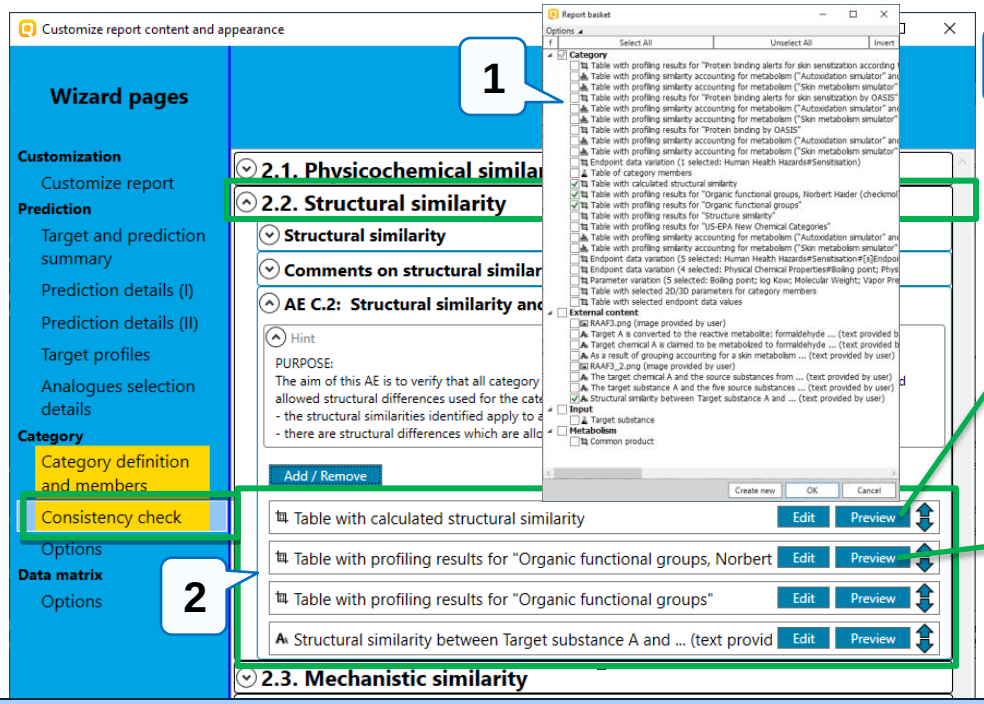
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

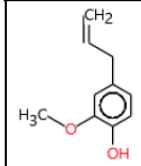
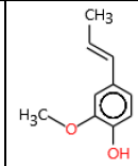
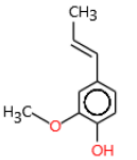
AE C.2 The Structural similarity and structural differences within the category

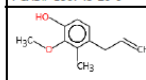
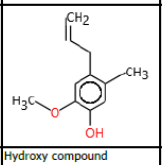
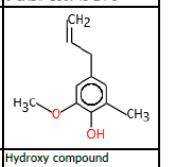


Calculated structure similarity

	1 CAS 97-53-0	354 CAS 97-54-1	355 CAS 5932-68-3	339 CAS 186743-26-0
1 CAS 97-53-0	100%	66.7 %	66.7 %	72 %
354 CAS 97-54-1	66.7 %	100%	100 %	48 %
355 CAS 5932-68-3				
339 CAS 186743-26-0				
363 CAS 186743-25-9				
364 CAS 186743-24-8				

Table with profiling results for "Organic functional groups, Norbert Haider (checkmol)"

1 CAS# 97-53-0	2 CAS# 97-54-1	3 CAS# 5932-68-3
		
Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound	Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound	Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound

4 CAS# 186743-26-0	5 CAS# 186743-25-9	6 CAS# 186743-24-8
		
Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound	Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound	Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound

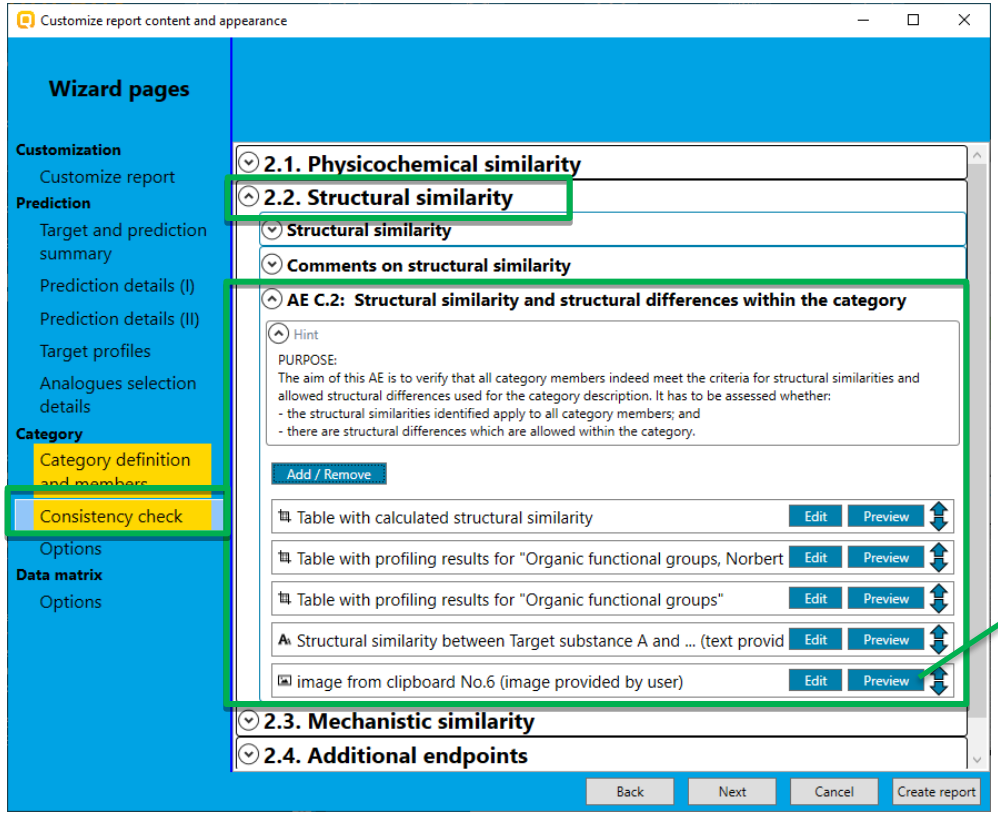
Structural similarity and **Chemical profile** report items with respect to the both empirical profilers (OFG and OFG, Norbert Haider) were stored in the Report basket. They are automatically created during process of applying category consistency elements (in the gap filling stage). They could be added manually to the AE (just tick them). Once selected and confirmed by OK, the items appeared under 2.2 Structutal similarity section (2). How they look in the generated report is shown on the right (3).

Report
Report Generation according to RAAF-Scenario 3

Section Category

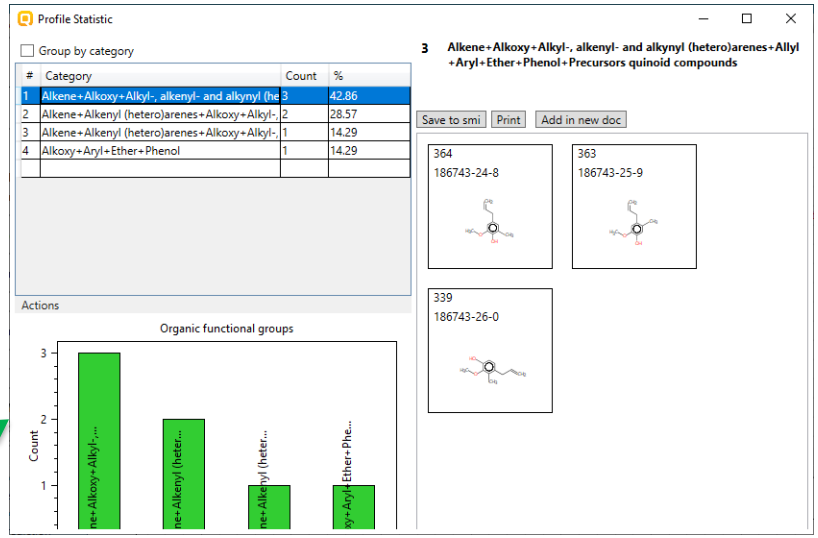
Subsection: Category definition and members

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 61):

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

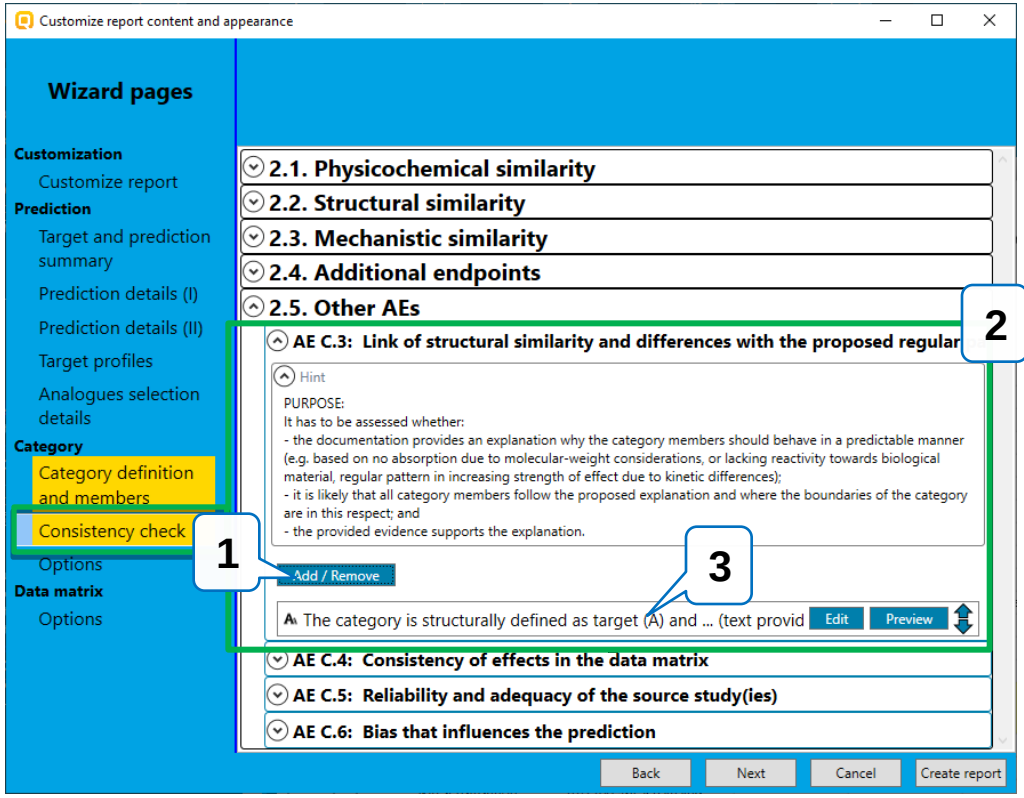


Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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). The item appear under the section AE C.3

2

Example text for **AE C.3**

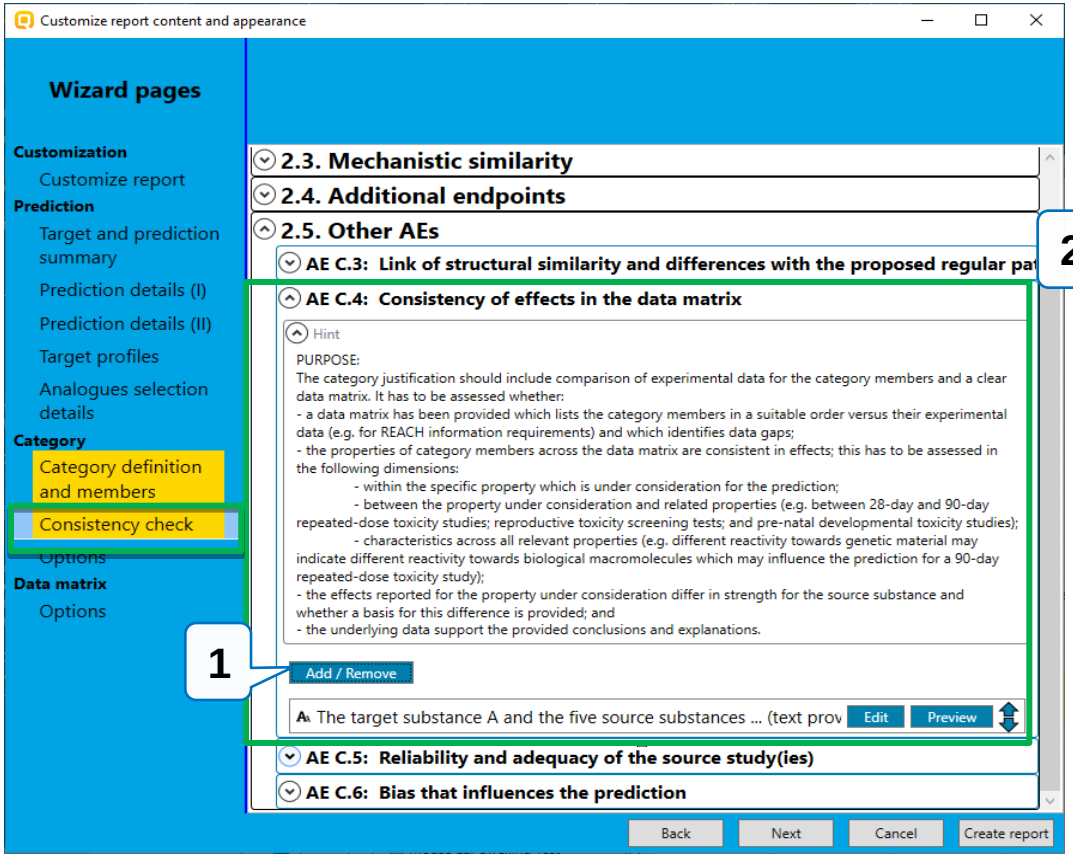
- The category is structurally defined as target (A) and the five source substances (B,C, D, E and F). They all form a common metabolite (formaldehyde) responsible for the toxic effect
- They all consist of a common reactivity pattern responsible for the formation of reactive metabolites

Report
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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):

2

- The target substance A and the five 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

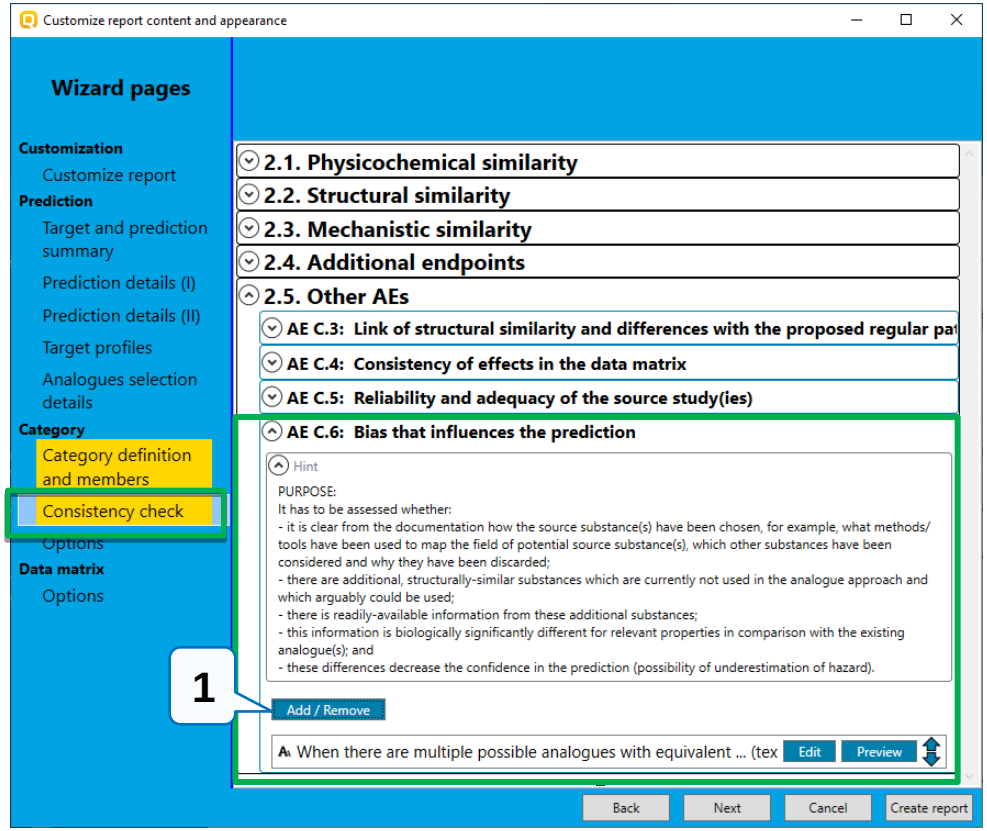
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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):

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

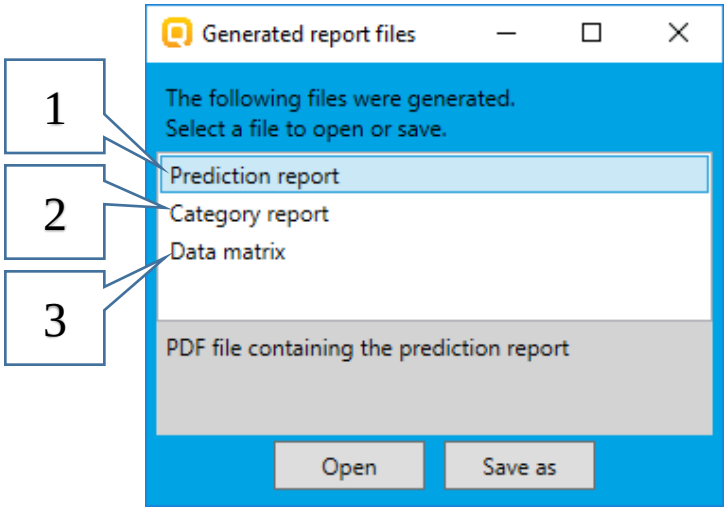
Report
Report Generation

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

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

RAAF AEs are included in the second file.

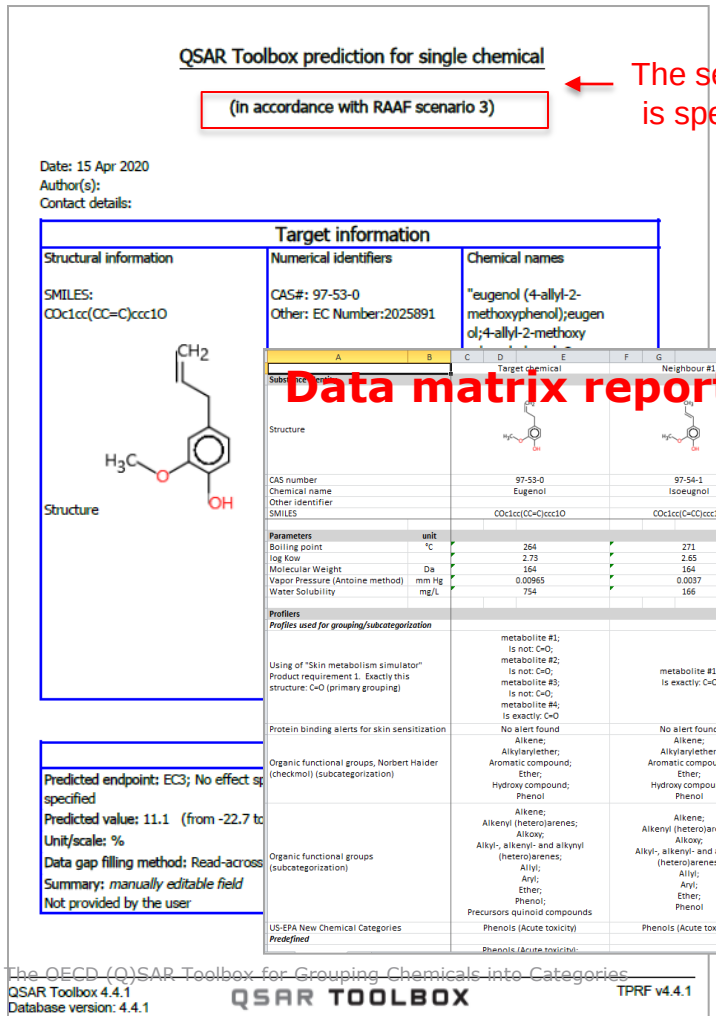
All generated files should be provided when submitting a prediction.



Report
Generated report files

Prediction report

Category report


QSAR Toolbox prediction for single chemical

(in accordance with RAAF scenario 3)

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

Target information

Structural information

SMILES:
COc1cc(CC=C)ccc1O

Structure

Numerical identifiers

CAS#: 97-53-0
Other: EC Number:2025891

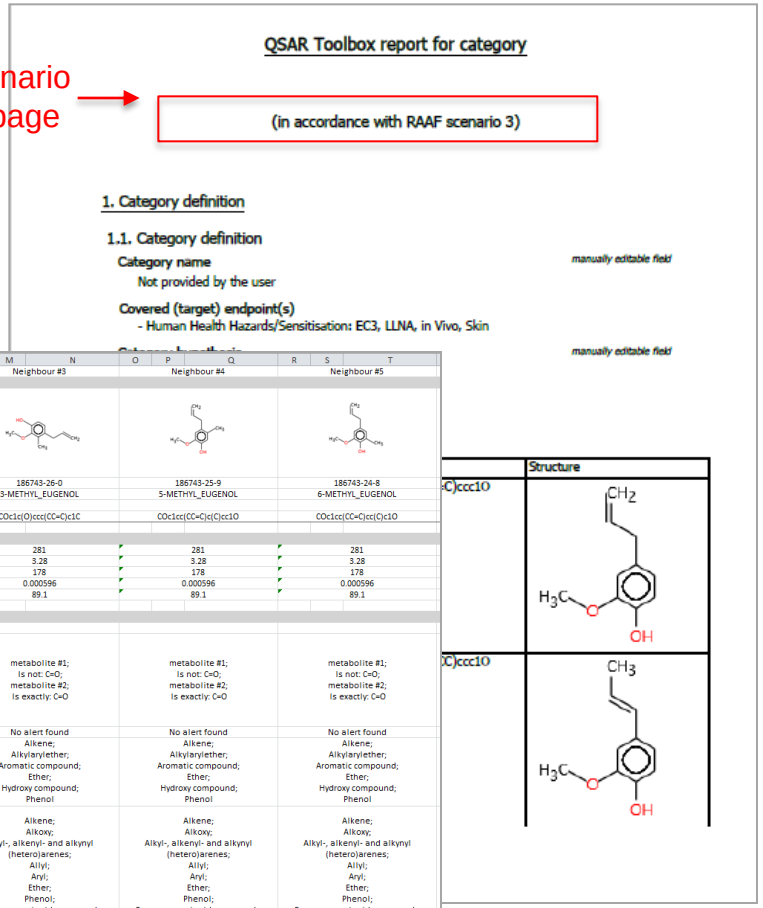
Chemical names

"eugenol (4-allyl-2-methoxyphenol);eugenol;4-allyl-2-methoxy

Predicted endpoint: EC3; No effect sp specified
Predicted value: 11.1 (from -22.7 to
Unit/scale: %
Data gap filling method: Read-across
Summary: *manually editable field*
Not provided by the user

QSAR Toolbox 4.4.1
Database version: 4.4.1
QSAR TOOLBOX
TPRF v4.4.1

The selected RAAF scenario is specified in the first page


QSAR Toolbox report for category

(in accordance with RAAF scenario 3)

1. Category definition

1.1. Category definition

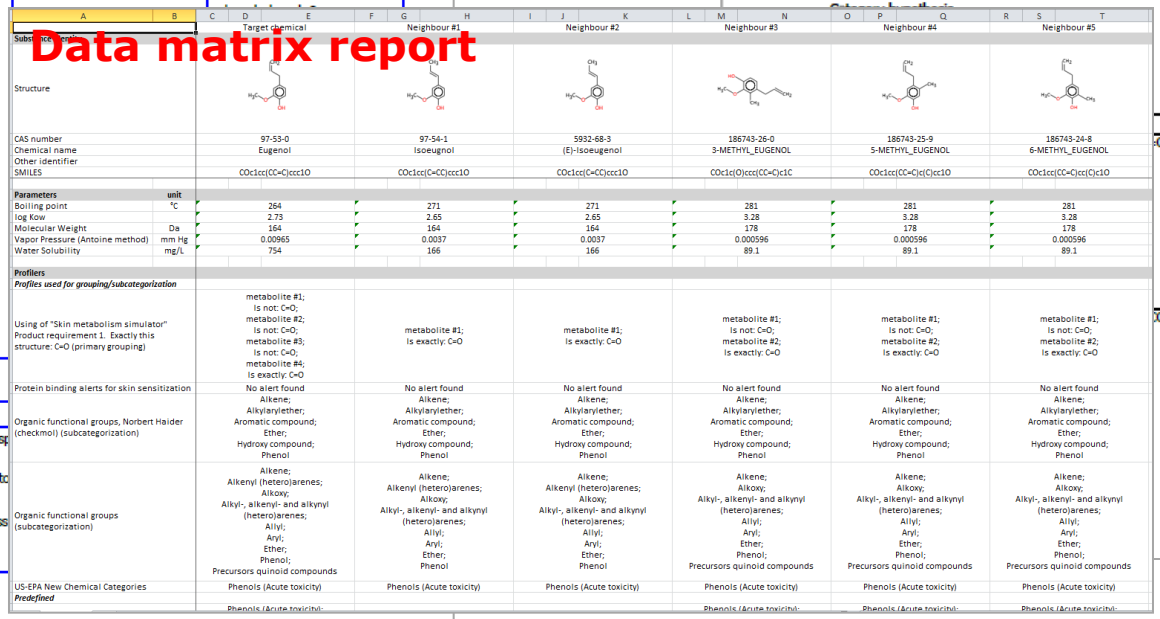
Category name
Not provided by the user

manually editable field

Covered (target) endpoint(s)
- Human Health Hazards/Sensitisation: EC3, LLNA, in Vivo, Skin

manually editable field

Structure


Data matrix report

		Target chemical	Neighbour #1	Neighbour #2	Neighbour #3	Neighbour #4	Neighbour #5
Structure							
CAS number		97-53-0	97-54-1	5932-68-3	186743-26-0	186743-25-9	186743-24-8
Chemical name		Eugenol	Isoeugnol	(E)-Isoeugenol	3-METHYL_EUGENOL	5-METHYL_EUGENOL	6-METHYL_EUGENOL
Other identifier							
SMILES		COc1cc(CC=C)ccc1O	COc1cc(C=CC)ccc1O	COc1cc(C=CC)ccc1O	COc1c(O)ccc(CC=C)c1C	COc1cc(CC=C)c(C)cc1O	COc1cc(CC=C)cc(C)c1O
Parameters	**unit**						
Boiling point	°C	264	271	271	281	281	281
log Kow		2.73	2.65	2.65	3.28	3.28	3.28
Molecular Weight	Da	164	164	164	178	178	178
Vapor Pressure (Antoine method)	mm Hg	0.00965	0.0037	0.0037	0.000596	0.000596	0.000596
Water Solubility	mg/L	754	166	166	89.1	89.1	89.1
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 not: C=O; metabolite #2; Is exactly: C=O	metabolite #1; Is not: C=O; metabolite #2; Is exactly: C=O	metabolite #1; Is not: C=O; metabolite #2; Is exactly: C=O
Protein binding alerts for skin sensitization		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
Organic functional groups (subcategorization)		Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol	Alkene; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds
US-EPA New Chemical Categories		Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)
Predefined							

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!