QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.2

An example illustrating RAAF Scenario 1 and related assessment elements

Outlook

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

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and justification of the outcome.
- The read-across prediction will be justified by fulfilling all information requirements according to the Read Across Assessment Framework (RAAF).

Outlook

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

Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Define target endpoint;
- Multiplication of the target chemical based on metabolism;
- Transferring the experimental data to the target;
- Category consistency check;
- Selection of RAAF scenario;
- Filling in the report sections related to each read-across assessment element.

Outlook

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

Specific Aims

- To familiarize the user with the Read Across Assessment Framework (RAAF) and more specifically with Scenario 1;
- To introduce to the user the read across assessment elements;
- To introduce to the user the report basket;
- To provide sufficient information allowing a scientific assessment of the outcome;
- To explain to the Toolbox user the rationale behind each step of the exercise.

Outlook

- Background
- Objectives
- Specific Aims
- **Read Across Assessment Framework (RAAF)**
- The example
- 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) that are deemed crucial to the assessment
- Total six scenarios are available: two for analogue approach and four for category approach

Read Across Assessment Framework (RAAF)

Selection of a RAAF scenario

SCENARIO	APPROACH	READ-ACROSS HYPOTHESIS BASED ON	QUANTITATIVE VARIATIONS
1	Analogue	(Bio)transformation to common compound(s)	Property of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach.
2	Analogue	Different compounds have qualitatively similar properties	Properties of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach.
3	Category	(Bio)transformation to common compound(s)	Variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach.
4	Category	Different compounds have qualitatively similar properties	Variations in the properties observed among source substances. Prediction based on a regular pattern or on a worst-case approach.
5	Category	(Bio)transformation to common compound(s)	No relevant variations in properties observed among source substances and the same strength predicted for the target substance.
6	Category	Different compounds have qualitatively similar properties	No relevant variations in properties observed among source substances and the same strength predicted for the target substance

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

- Distinguish whether it is an analogue or a category approach
- To identify the basis of the read across hypothesis
  - (Bio)transformation to common compound(s) – the read across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed
  - Different compounds have the same type of effect(s) – the read across hypothesis is that the organism is not exposed to common compounds but rather, as a result of similarity, that different compounds have similar (eco)toxicological and fate properties. These compounds may be the source and target substances themselves or one or more of their (bio)transformation products.
- For a category approach there is a need to take further account whether or not quantitative variations in the properties are observed among the category members

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

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

- Each scenario consists of a pre-defined set of assessment elements (AEs) that, when taken together, cover all of the essential scientific aspects that need to be addressed in the read-across approach for a particular scenario.*
- Each AE reflects a critical scientific aspect of a read-across.
- The AEs could be:
  - **common** for all scenario within one approach - common AEs for Scenario 1 and 2 (analogue approach) and common AEs for Scenario 3, 4, 5 and 6 (category approach)
  - **specific** – addressing specific scenario.

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

Outlook

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

The Example

- In this exercise we will predict the *Repeated dose toxicity* (RDT) of 2-phenylethyl 3-methylbutanoate [CAS# 140-26-1], which will be the "target" chemical;
- Collect experimental data and profiling results for the target;
- Generate hydrolysis products of the target and collect data for them;
- A read-across approach will be used for the prediction. The read-across will be based on an analogue approach relying on the experimental data of generated common product as a result of abiotic simulation (hydrolysis product);
- Category consistency will be checked;
- Read-across assessment elements will be included to the report;
- Examples for the possible content of each of AEs will be provided.

The Example
Sidebar On Repeated dose toxicity (RDT)

- Repeated dose toxicity comprises the adverse general toxicological effects occurring as a result of repeated daily dosing with, or exposure, to a substance for a specified period up to the expected lifespan of the test species.
- The studies yield information on general characteristics of the toxicity, the target organs of toxicity, the dose–response (curve) for each toxicity endpoint, responses to toxic metabolites formed in the organism, delayed responses, cumulative effects, the margin between toxic/non-toxic dose, information on reversibility/irreversibility of the effect, and NOAEL (No Observed Adverse Effect Level), NOEL (No Observed Effect Level) for toxicity.
- The repeated dose study is an integral part of the data package produced to perform a quantitative risk assessment of many type chemicals.
- The point of departure most commonly used for systemic toxicity safety assessment is the NOAEL data
- Therefore, the availability of NO(A)EL endpoint data for the target and its analogues is one of the critical steps in the assessment process along with identifying the toxicity effects to of the target and analogues according to the toxicity-based profilers.

Outlook

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

Workflow

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

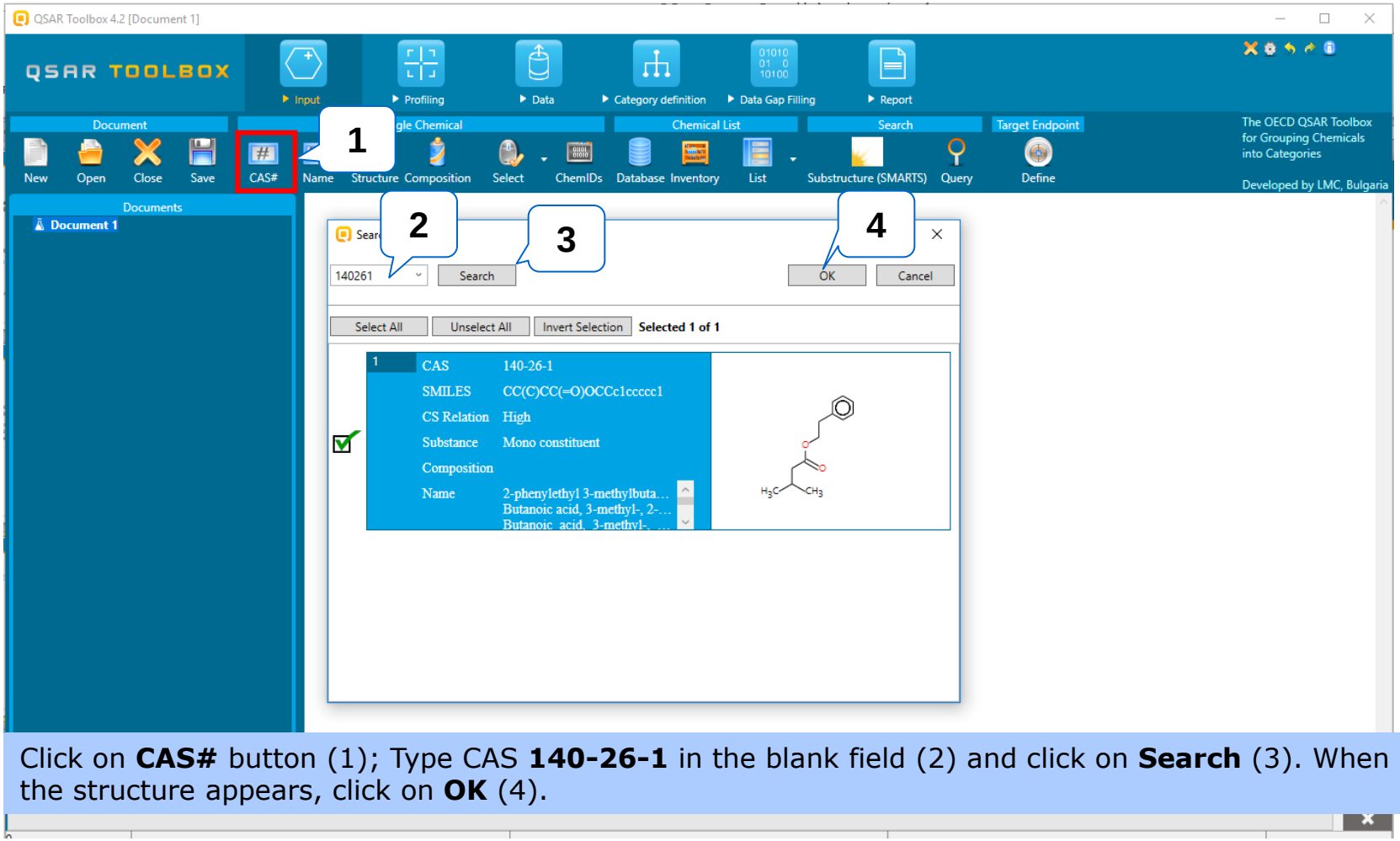
The modules will be presented in different sequence than the one showed above.

Input
Overview

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

Input Screen

Enter target chemical by CAS#

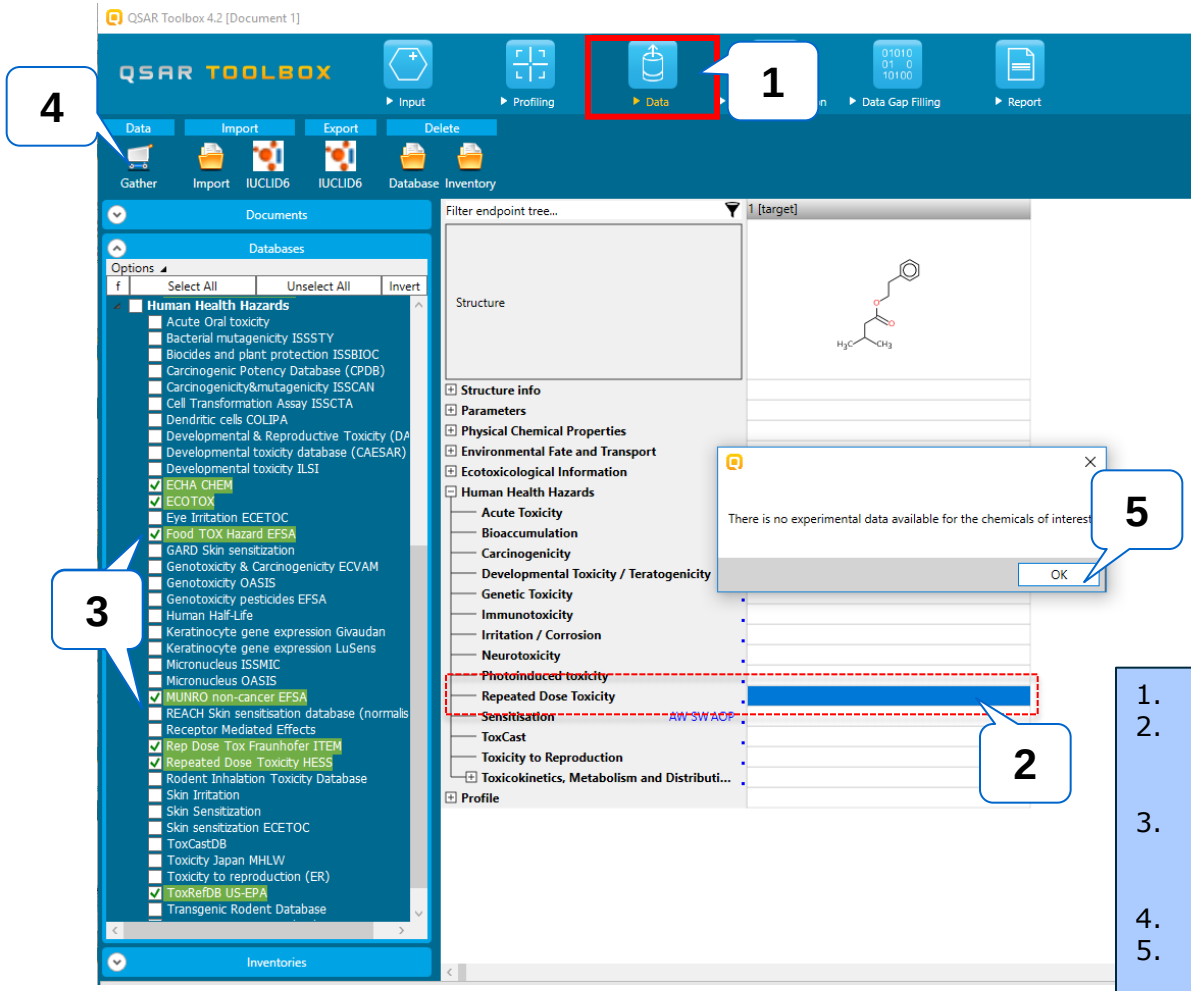


Click on **CAS#** button (1); Type CAS **140-26-1** in the blank field (2) and click on **Search** (3). When the structure appears, click on **OK** (4).

Data
Overview

- "Data" refers to the electronic process of retrieving the environmental fate, ecotoxicity and toxicity data that are stored in the Toolbox.
- Data gathering can be executed in a global fashion (i.e., collecting all data for all endpoints) or on a more narrowly defined basis (e.g., collecting data for a single or limited number of endpoints).
- Once the endpoint is selected (via selecting the data matrix cell corresponding to the endpoint or defined using "target endpoint" functionality), the databases containing such type of data are highlighted in green (see next slide).
- Lets check are there any data for the target chemical.

Data
Gather data



1. Go to ***Data*** module;
2. Expand *Human health hazard* and Click on the cell corresponding to "Repeated dose toxicity" level
3. Select the highlighted databases (these are the databases containing data related to the selected endpoint);
4. Click on **Gather**.
5. A pop-up message informs that there is no data for the target chemical. Click **OK**

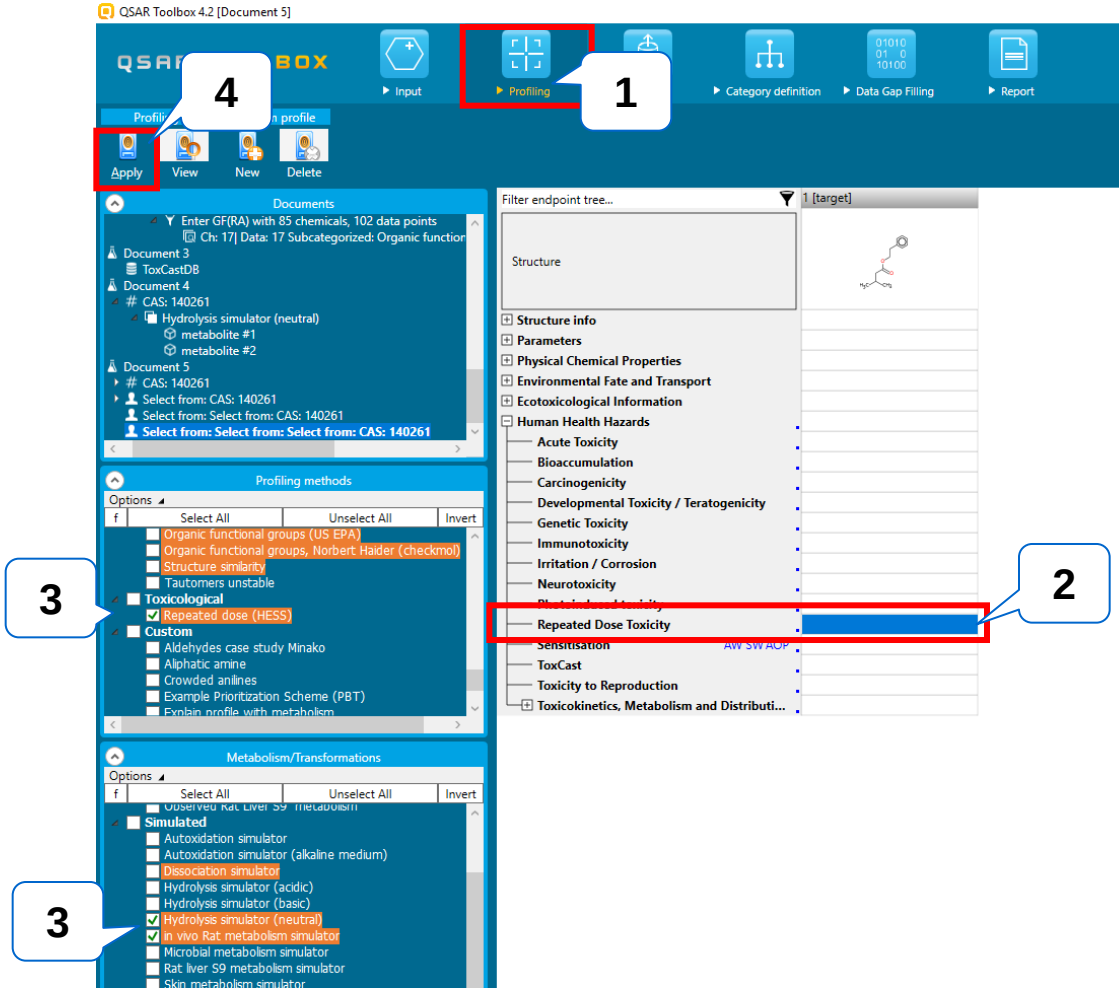
Profiling
Overview

- "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.
- Available information includes likely mechanism(s) of action, as well as observed or simulated metabolites
- Based on the "profilers' relevancy" the most suitable once are getting colour highlighted*
- For the purpose of this example a plausible Repeated dose (HESS) profiler in combination with simulators are used (see next slides)

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

Profiling

Profiling the target chemical



1. Go to ***Profiling*** module
2. Select the cell related to "Repeated Dose Toxicity"
3. Select the *plausible* Repeated dose toxicity (HESS) profiling scheme and both Hydrolysis (neutral) and in vivo Rat liver simulators (orange colored);
4. Click on **Apply**

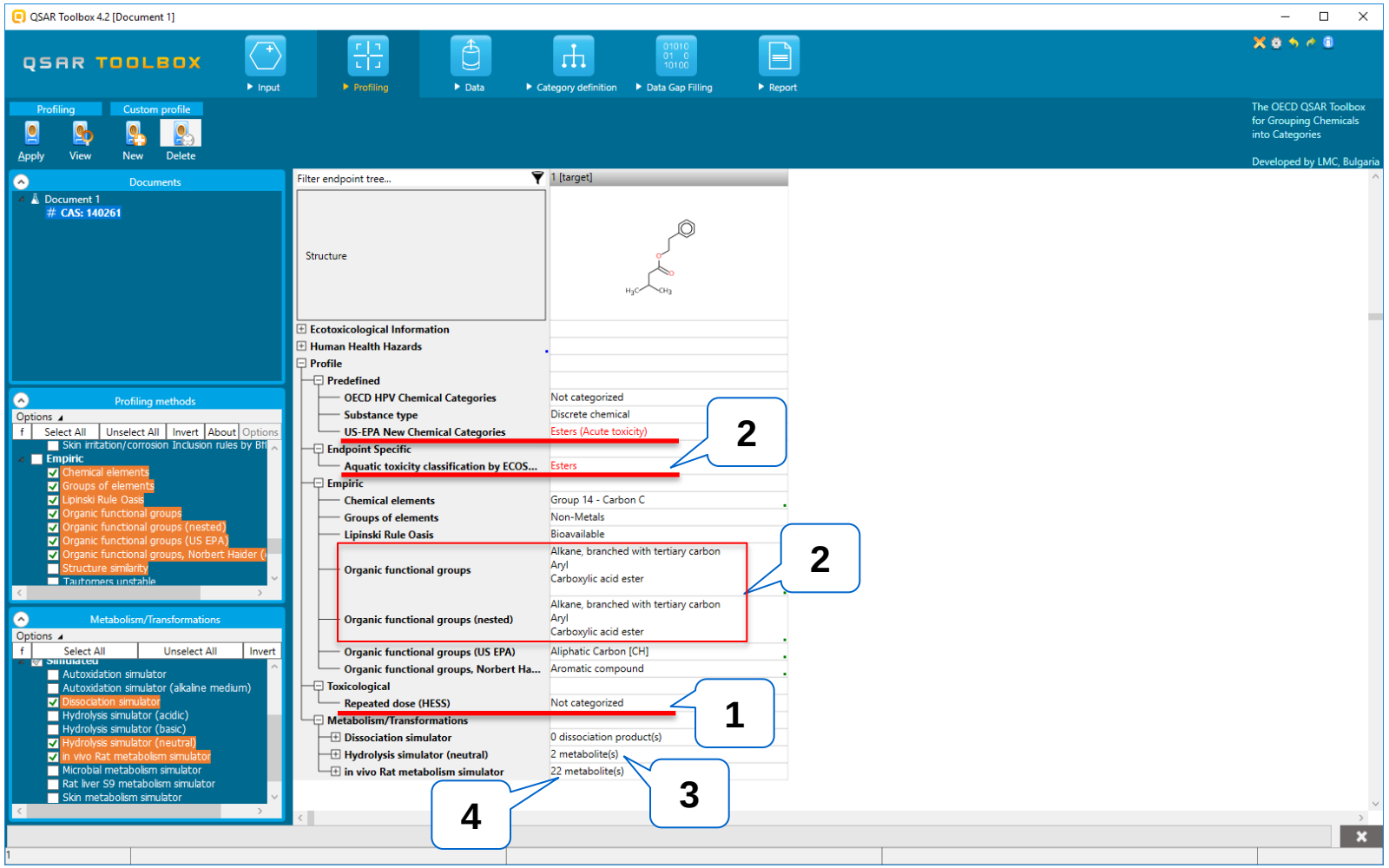
Profiling
Profiling results

1) No RDT alerts are identified in the target's structure as a parent;
2) The chemical is classified as "ester" according to structure-based profilers;
3) 2 hydrolysing products are obtained as a result of abiotic activation (*Hydrolysis simulator (neutral)*;
4) 22 metabolites are produced as a result of biotic activation (*in vivo Rat metabolism simulator*);

See on the next slide

Profiling

Profiling results



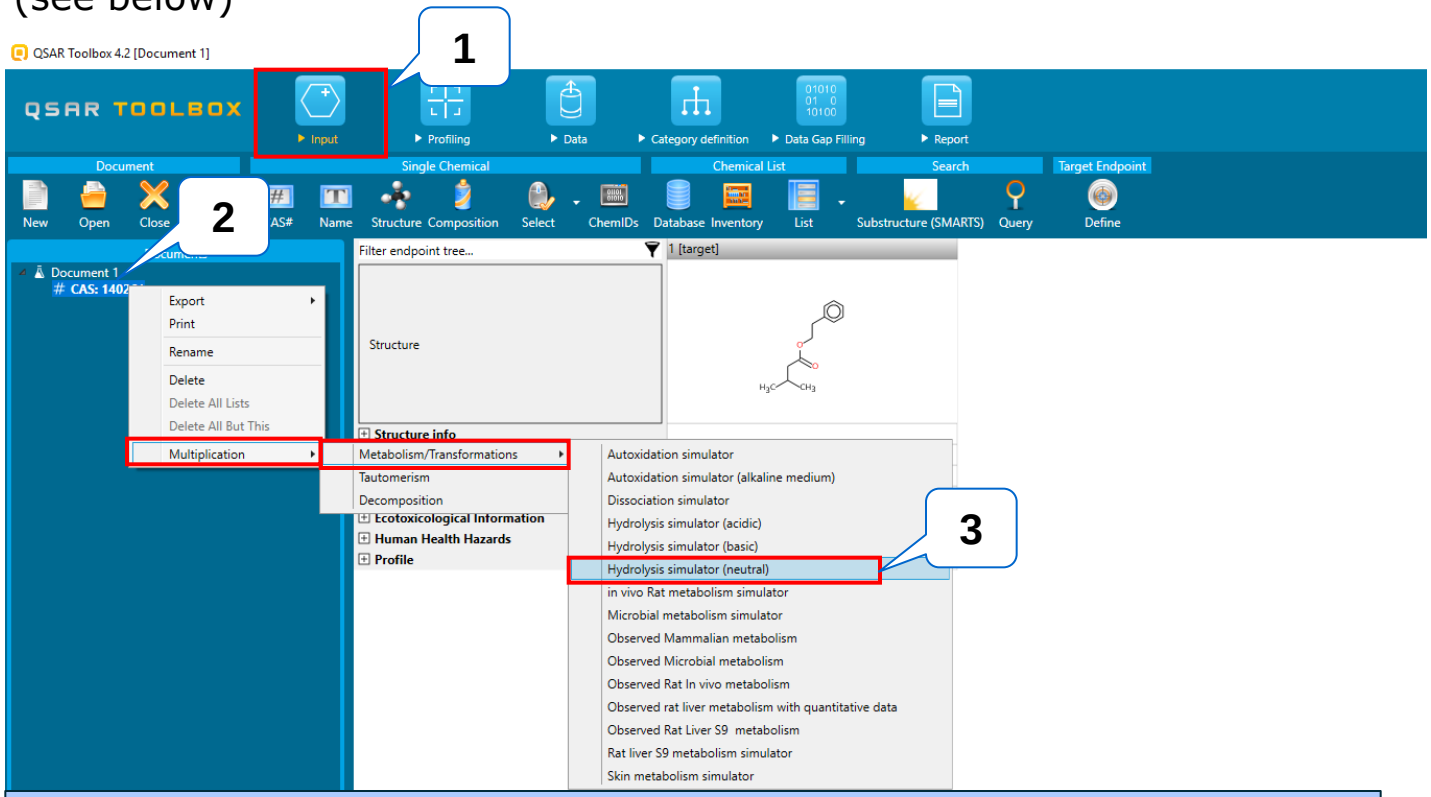
Recap

- In module one, you have entered the target chemical.
- In the *Data* module, you saw the databases corresponding to the selected target endpoint. No data has been found for the target.
- In the *Profiling* module, you profiled the target chemical with profiling schemes and metabolic simulators, plausible for the selected target endpoint.
- The target chemical is classified as a "Carboxylic Ester" according to structure-based profilers and does not have repeated-dose alerts responsible for the toxic effect based on the HESS profiler. No effect is expected.
- As seen 2 hydrolysing products and 22 in vivo rat liver metabolites are produced after accounting for (a)biotic simulation (hydrolysis at neutral pH, rat in vivo metabolism).
- Based on the fact that esters very easily undergo a chemical or an enzymatic hydrolysis [1-3] it is expected that this will be one of the first reactions to which our target chemical is exposed.
- Thus, the next actions are focused on investigation of the hydrolysis products of the target chemical.
- Go to Data module again to check are there any data for the metabolites.

1. Flavouring Group Evaluation 41: 2-Ethylhexyl derivatives from chemical group 2, Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). *EFSA J.*, **2009**, 929, 1-46.
2. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission related to Flavouring Group Evaluation 6 (FGE.06): Straight- and branched-chain aliphatic unsaturated primary alcohols, aldehydes, carboxylic acids, and esters from chemical groups 1 and 4. *EFSA J.*, **2004**, 108, 1-69.
3. 2-Ethylhexanoic acid and its derivatives, Part A – Final decisions on matters referred to an expert advisory committee. *In*: Final decisions and reasons for decisions by delegates of the Secretary to the Department of Health. Notice under subsections 42ZCZS and 42ZCZX of the Therapeutic Goods Regulations 1990 (the Regulations), NICNAS, November **2015**.

Multiplication of target chemical

Before check for data availability for the metabolites, it is need to simulate them upfront (see below)

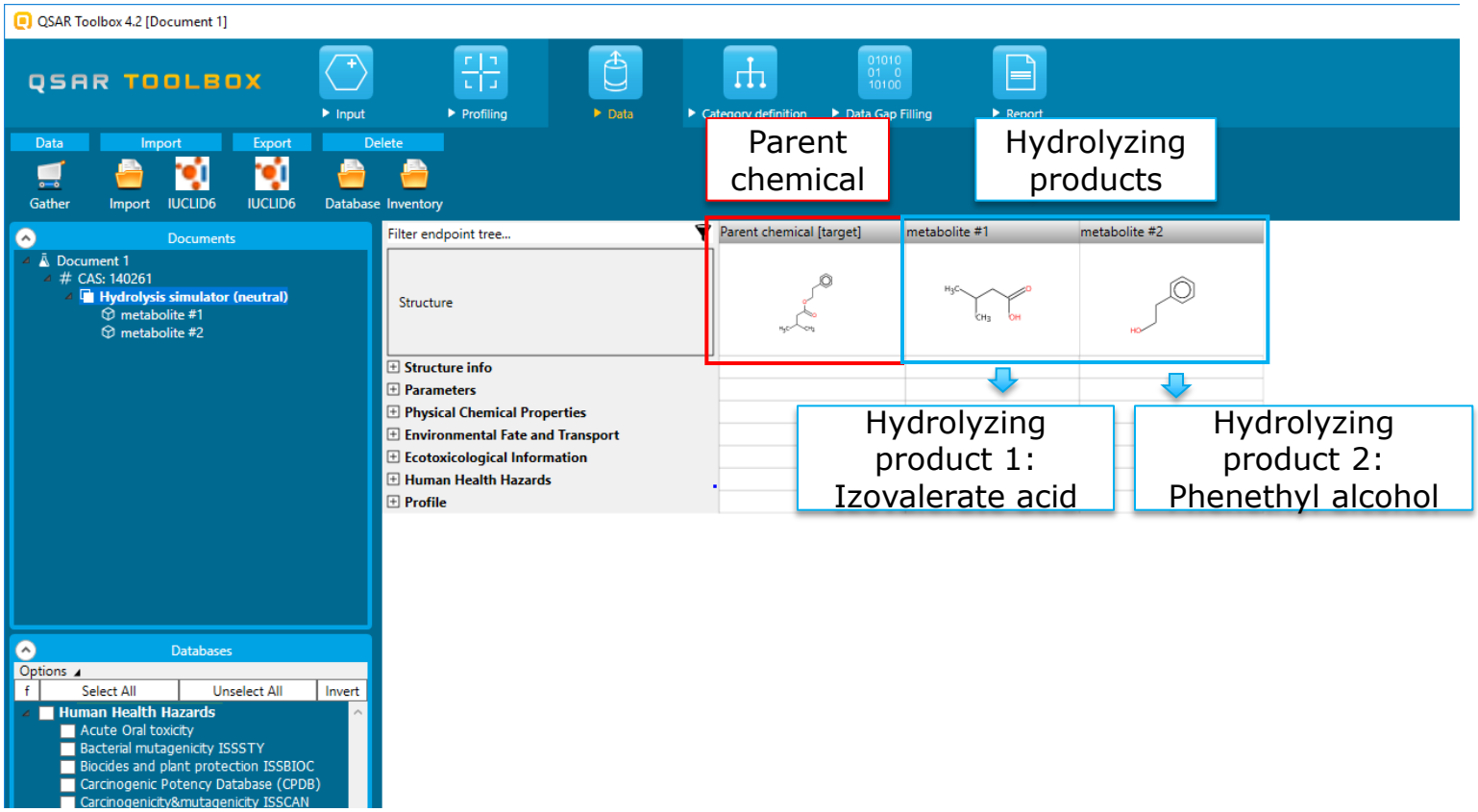


1. Go to ***Input*** module
2. Click on the level with **CAS #** of the target chemical and perform right click on it, then
3. Select **Multiplication-Metabolism/Transformations /Hydrolysis simulator (neutral)**

The product appeared next to the target (see next slide)

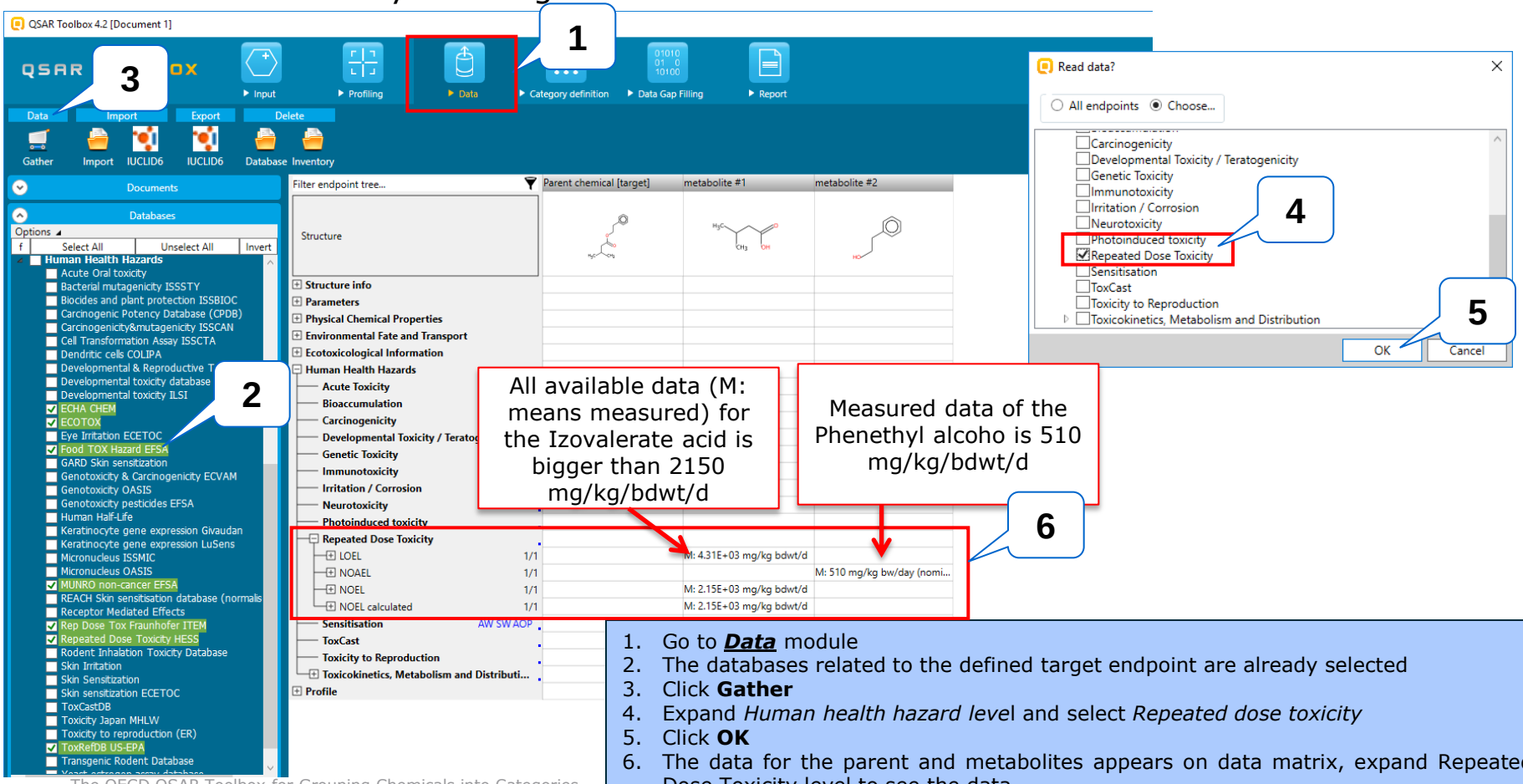
Multiplication of target chemical

Before check for data availability for the metabolites, it is need to simulate them upfront (see below)



Collect data for the metabolites

Check for data availability for the generated metabolites



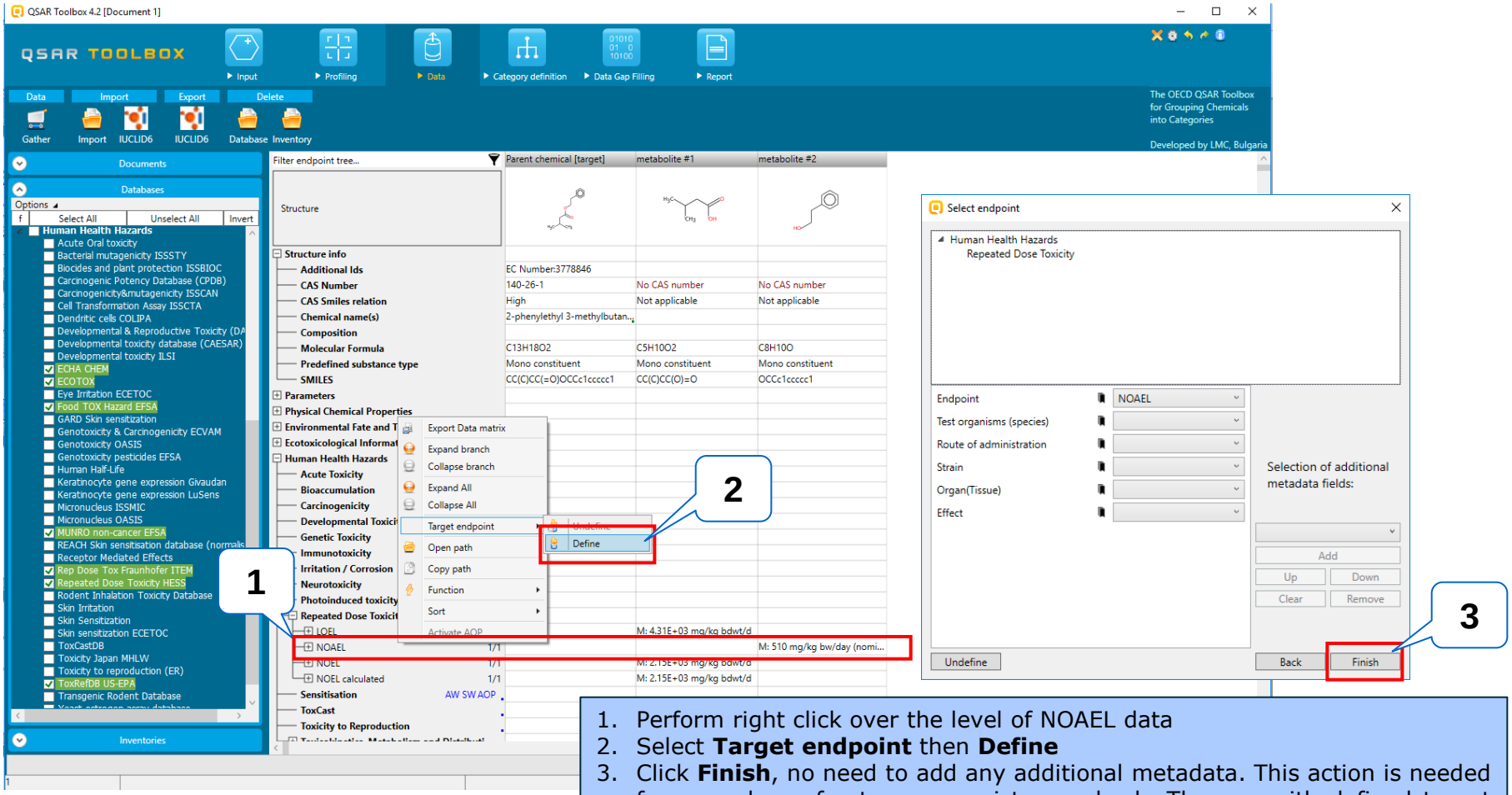
1. Go to ***Data*** module
2. The databases related to the defined target endpoint are already selected
3. Click **Gather**
4. Expand *Human health hazard level* and select *Repeated dose toxicity*
5. Click **OK**
6. The data for the parent and metabolites appears on data matrix, expand Repeated Dose Toxicity level to see the data

Recap

- Two hydrolysing products are generated for the target chemical: *Izovalerate acid* and *Phenethyl alcohol*
- In the *Data* module, you have found that RDT data is available for both products
- As seen the data for both products is bigger than hazard threshold of 100 mg/kg/data according to GHS classification [1],
- However, metabolite phenethyl acohol is more toxic than the acid based on the experimental data
- Moreover it is expected that the acid (*Izovalerate acid*) will be directly excreted and will not contribute towards the toxicity of the target [2]
- Thus, it is expected that the toxicity of the target chemical phenethyl isovalerate will be a result of Phenethyl alcohol
- The forthcoming slides are focused on defining the target endpoint by using the functionality of the TB (this is needed for the category consistency check) and obtaining a read-across prediction for the target based on the data of the *Phenethyl alcohol (*assumed to be an analogue)
- Finally before generating a report, the category will be checked for category consistency

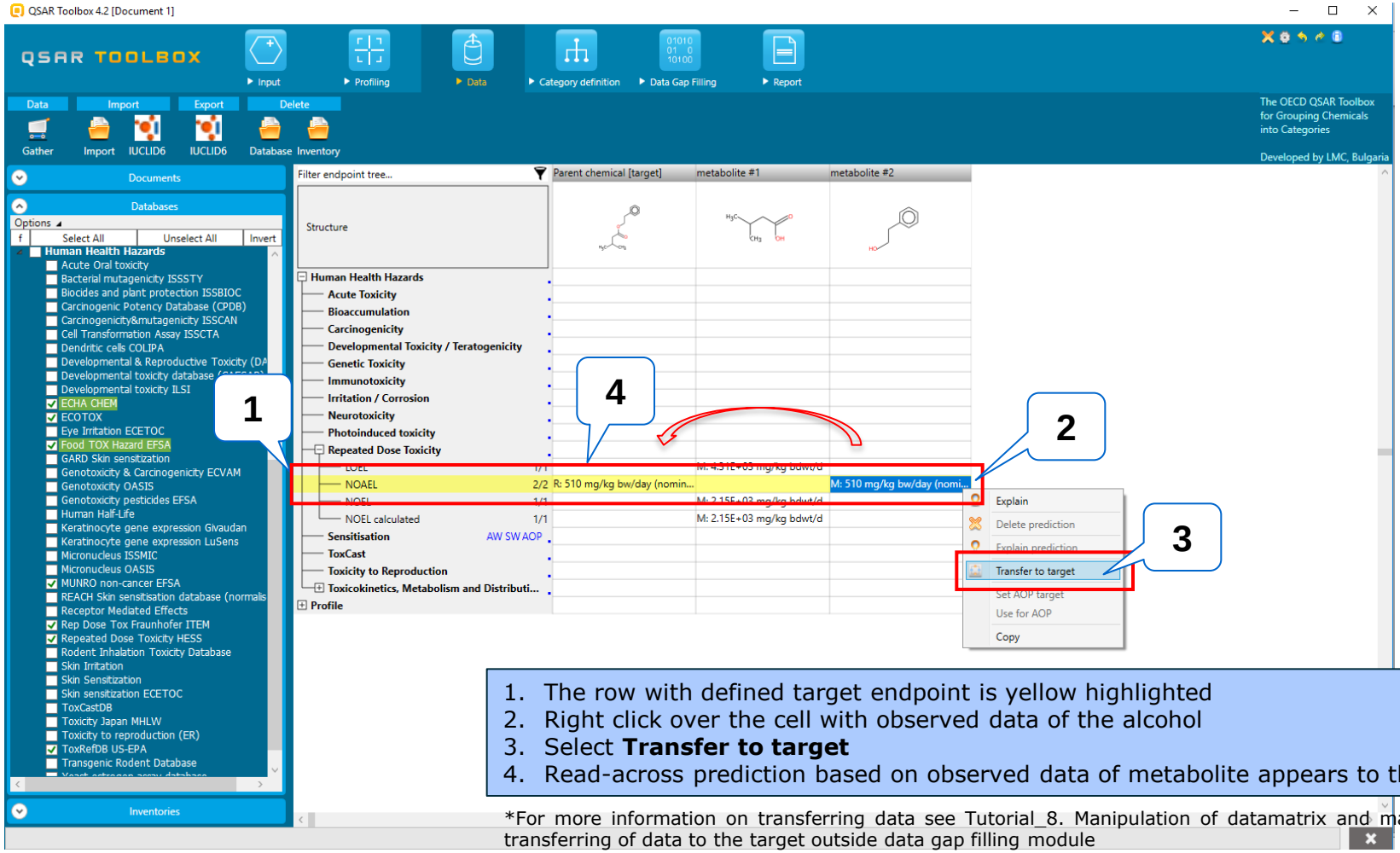
1. GHS Classification. Fourth edition
2. RIFM, 2012. RIFM (Research Institute for Fragrance Materials, Inc), 2012. A Toxicological and Dermatological Assessment of Aryl Alkyl Alcohol Simple Acid Ester Derivatives when Used as Fragrance Ingredients. RIFM report number 65259 (RIFM, Woodcliff Lake, NJ, USA.).

Define target endpoint



1. Perform right click over the level of NOAEL data
2. Select **Target endpoint** then **Define**
3. Click **Finish**, no need to add any additional metadata. This action is needed for procedure of category consistency check. The row with defined target endpoint will become yellow highlighted (see next slide).

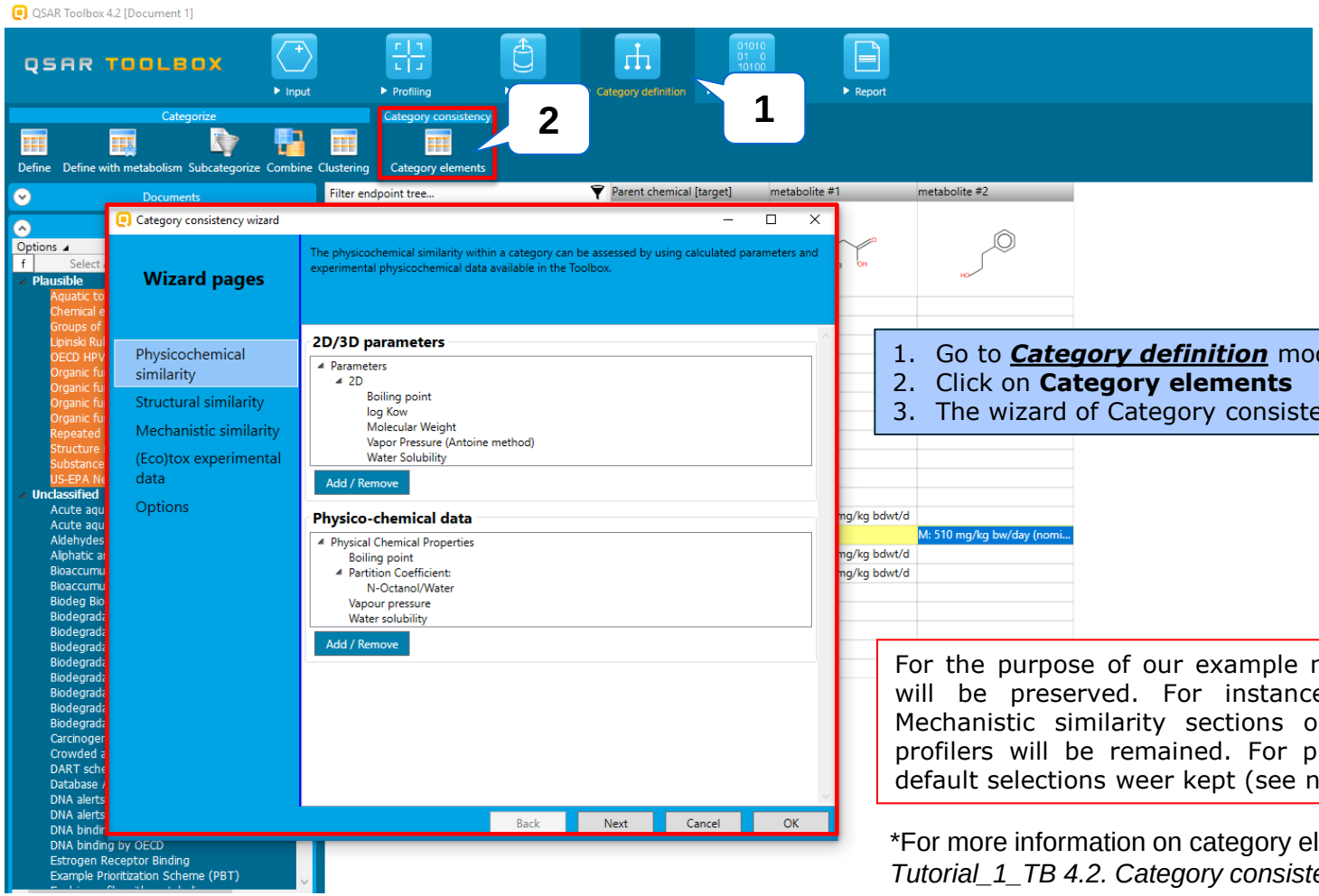
Transfer of observed data of metabolite to the target chemical



1. The row with defined target endpoint is yellow highlighted
2. Right click over the cell with observed data of the alcohol
3. Select **Transfer to target**
4. Read-across prediction based on observed data of metabolite appears to the target*.

*For more information on transferring data see Tutorial_8. Manipulation of datamatrix and manual transferring of data to the target outside data gap filling module

Category consistency check



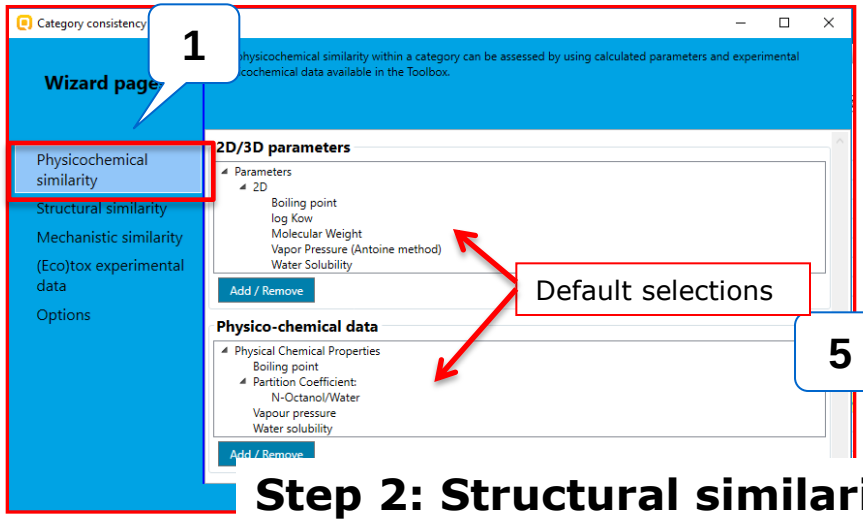
1. Go to ***Category definition*** module
2. Click on **Category elements**
3. The wizard of Category consistency appeared*.

For the purpose of our example not all default selections will be preserved. For instance for Structural and Mechanistic similarity sections only the OFG and RDT profilers will be remained. For phys-chem similarity the default selections weer kept (see next slide)

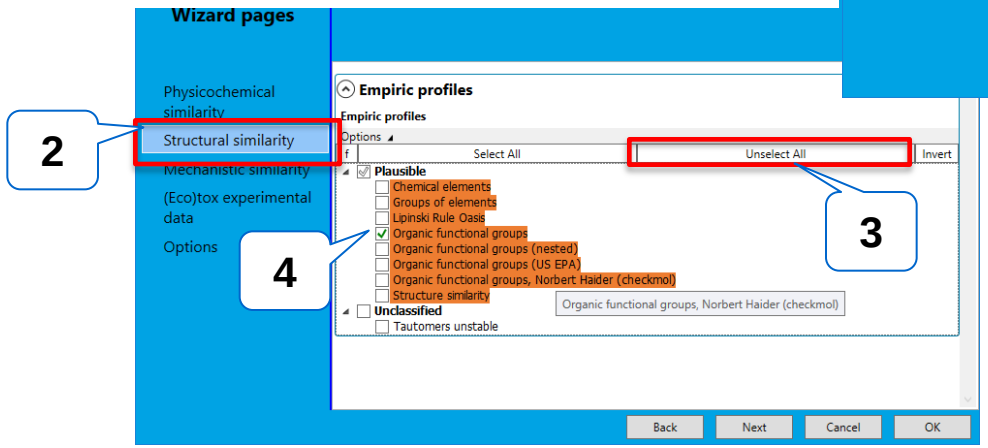
*For more information on category elements see *Tutorial_1_TB 4.2. Category consistency*

Category consistency check

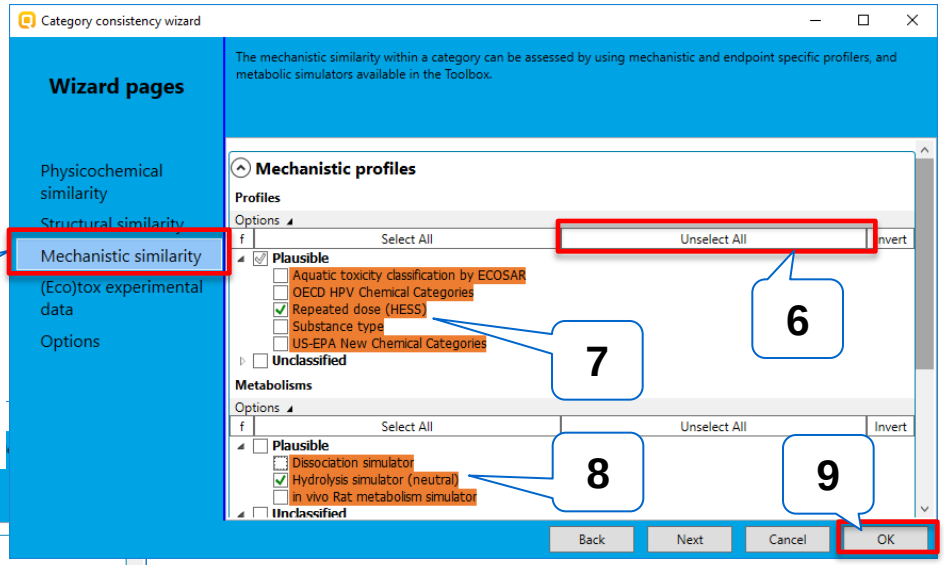
Step 1: Physicochemical similarity



Step 2: Structural similarity



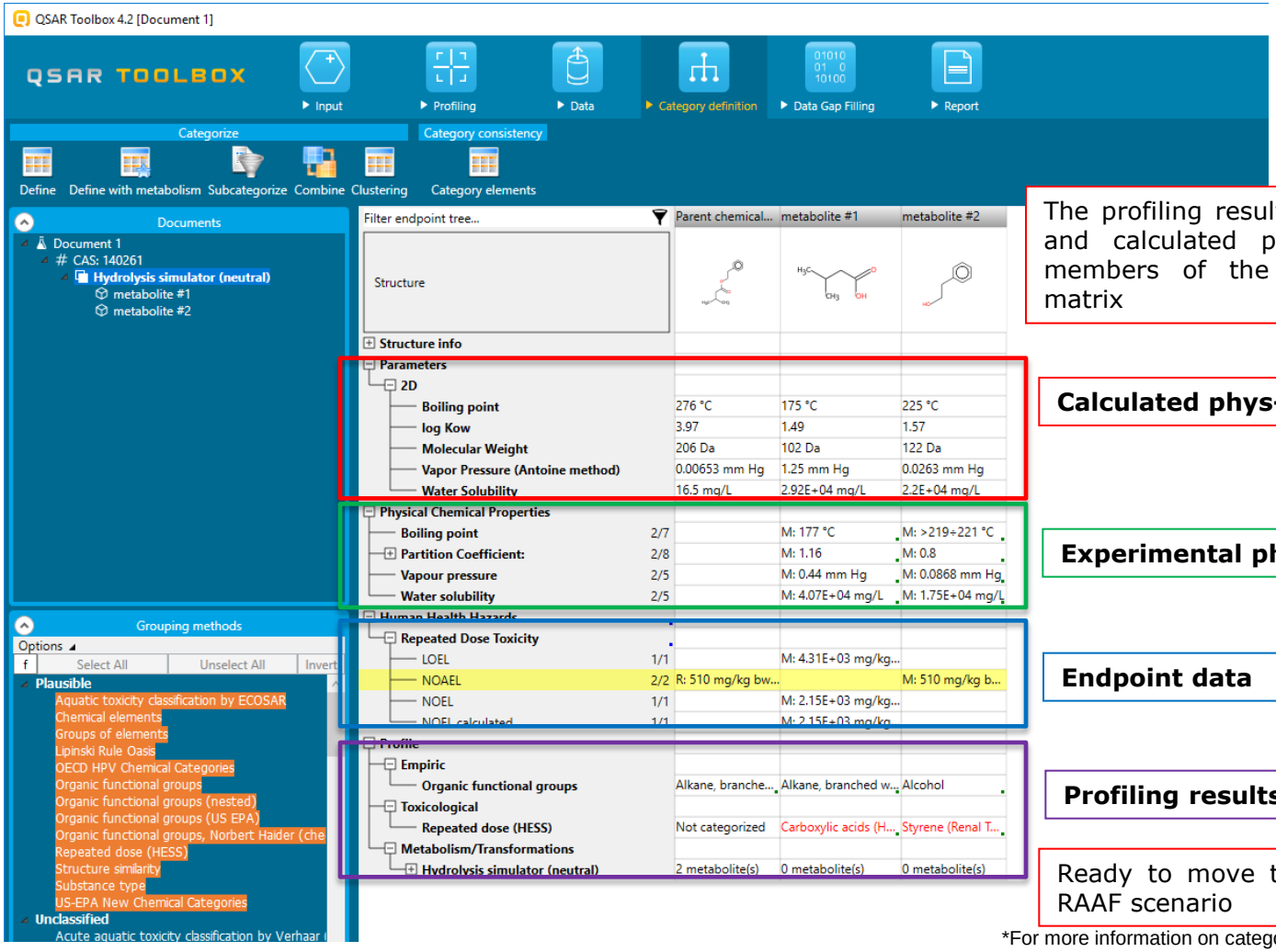
Step 3: Mechanistic similarity



1. Select **Physicochemical similarity**. Keep the default selections here.
2. Select **Str. similarity** section
3. Unselect all default selected profilers first, then
4. Select **OGF** profiler
5. Select **Mechanistic similarity**
6. Unselect all
7. Select **Repeated dose (HESS)** profiler
8. Select **Hydrolysis simulator (neutral)**
9. Click **OK.** The profiling results/data/parameters will appeared on datamatrix (see next slides)

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

Category consistency check



The profiling results, experimental endpoint data and calculated phys-chem properties for the members of the category appeared on data matrix

Calculated phys-chem properties

Experimental phys-chem data

Endpoint data

Profiling results

Ready to move to reporting and including RAAF scenario

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

Recap

- The hydrolysis products are generated and data has been found for them.
- As expected from the literature the toxicity effect of the target will be due to the alcohol product. Less toxicity data has been found for it.
- The toxic data of the alcohol has been transferred to the target chemical by using "Transfer data" functionality
- The hydrolysing products are assumed as members of the category
- In the *Category definition* module category consistency was checked by applying the category elements.
- You are now ready to complete the final module and to create the report.
- Click "Report" to proceed to the last module.

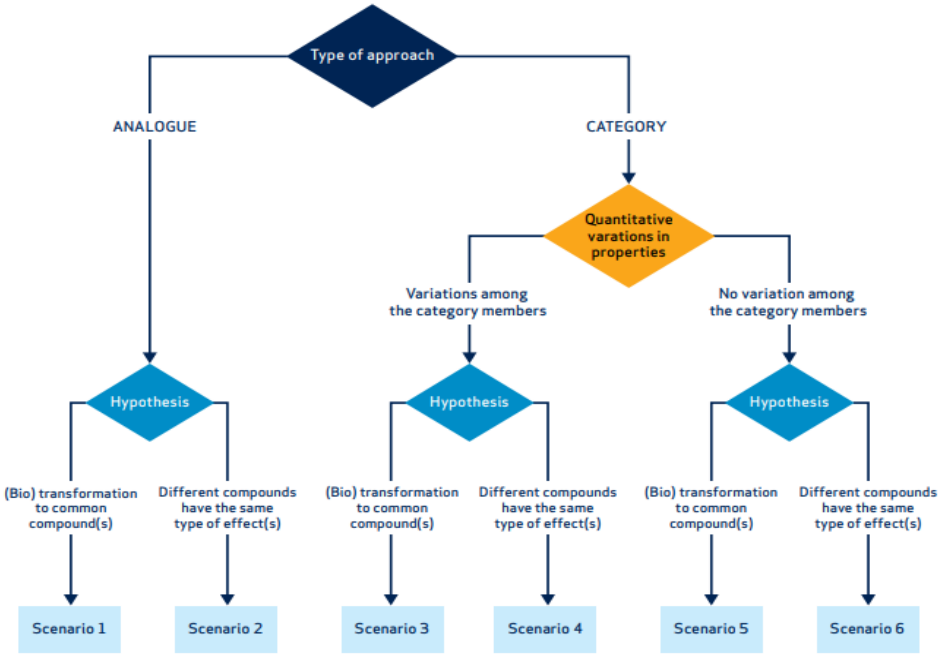
Report
Overview

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

Report
Selection of RAAF scenario

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

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



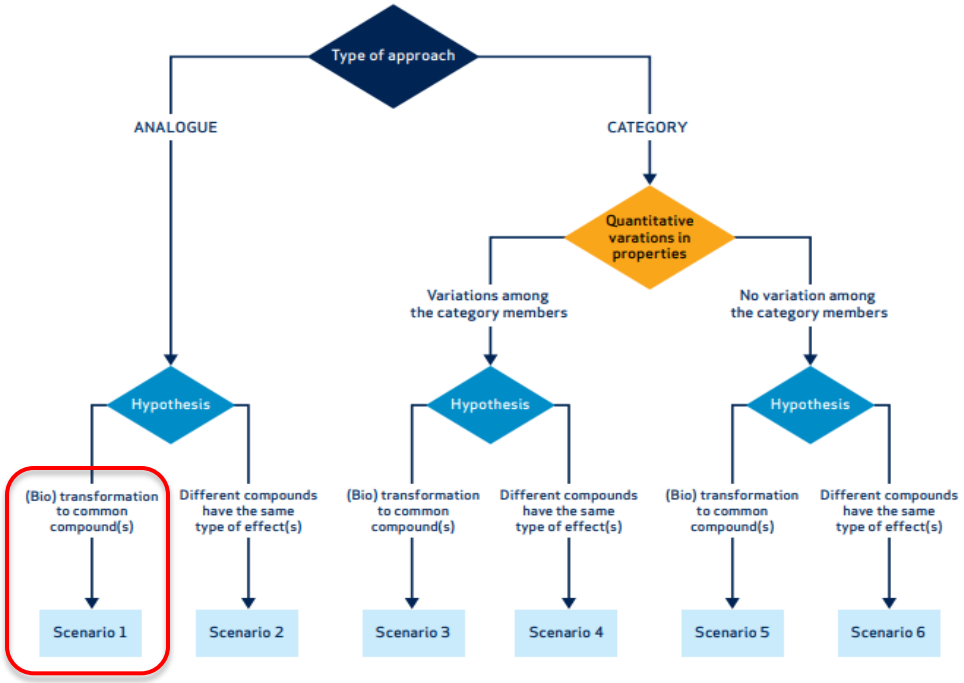
*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Report
Selection of RAAF scenario

For the current example:

- the type of approach applied - analogue approach is used (threshold of ≤3 analogues is proposed by LMC for the analogue approach) ;
- the read-across hypothesis – biotransformation to common compound of the target substance;

Based on that Scenario I was identified as appropriated for the current example.



Read-Across Assessment Framework (RAAF)

Scenario 1

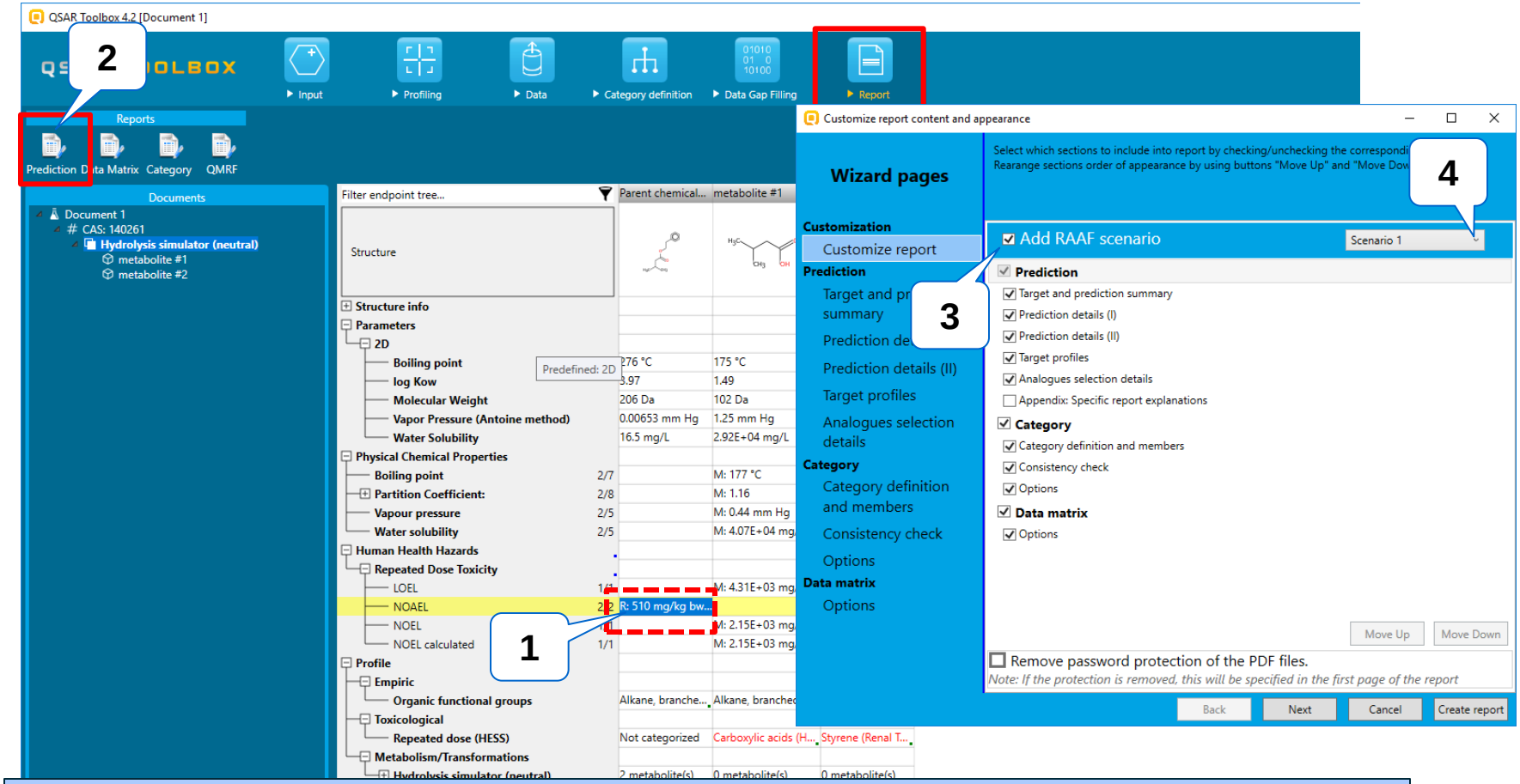
- Scenario 1 covers the analogue approach for which the read-across hypothesis is based on (bio) transformation to a common compound
- For the REACH information requirement under consideration, the property investigated in a study conducted with one source substance is used to predict properties that would be observed in a study with the target substance if it were to be conducted.
- The current case corresponds to Example 2 for Scenario 1 of the RAAF*. The target (B) and the source chemicals (A) are structurally similar substances, which are rapidly and extensively absorbed (bio)transformed to the substance A and therefore no/negligible systemic exposure to the substance B occurs. The source substance A is the common compound in this analogue approach. The common compound A is solely responsible for the (absence of) effects. The effects of the target substance B are predicted to be equal to the effects of the source substance A for the property under consideration.

	PARENT SUBSTANCES	(BIO)TRANSFORMATION	COMMON COMPOUNDS	NON-COMMON COMPOUNDS
SOURCE	A	A → not transformed	A	-
TARGET	B	B → A	A	-

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

Report

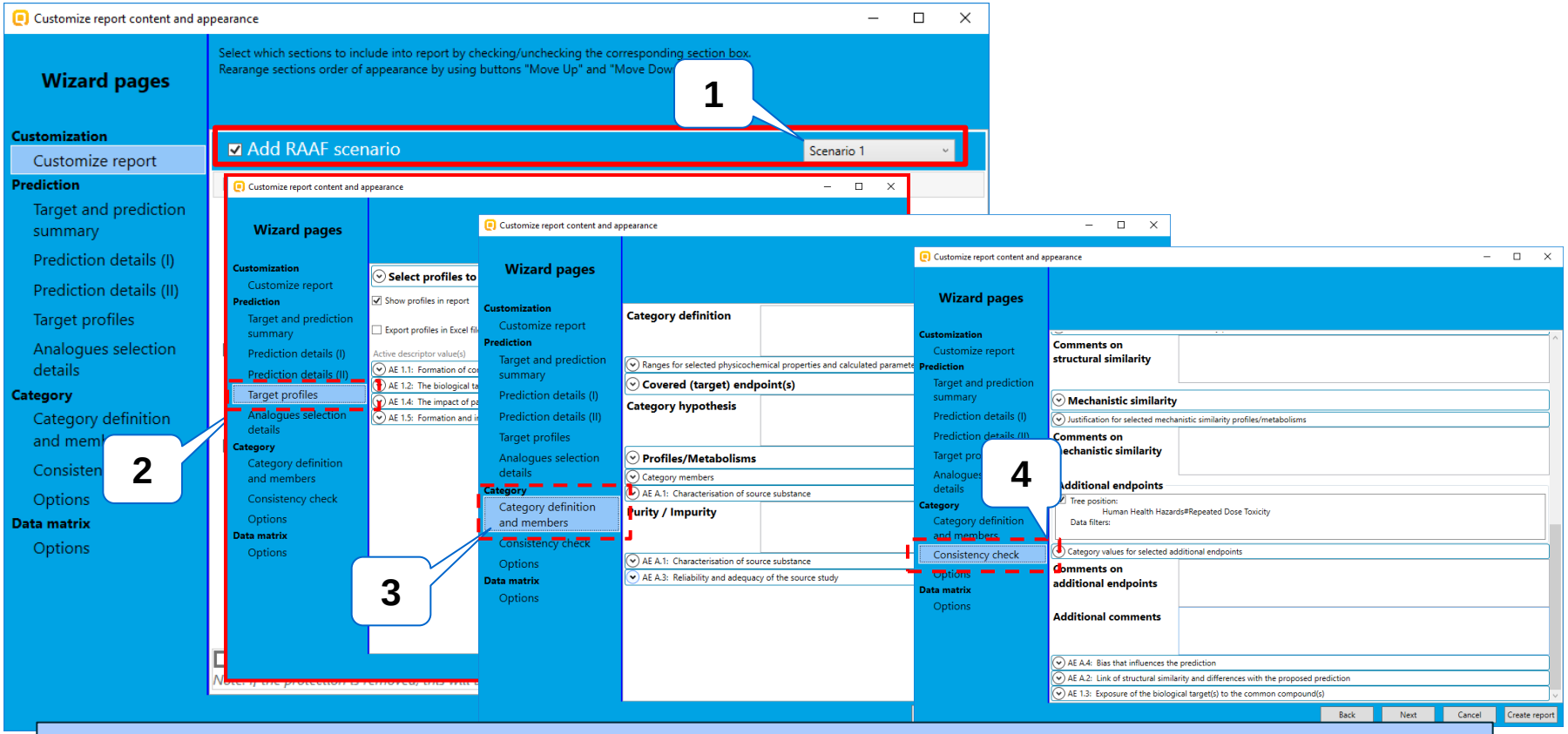
Report generation according to RAAF-Scenario 1



1. Go to the ***Report*** module and click on the cell with the prediction; 2. Click the **Prediction** button; 3. Check the box at the top to add RAAF scenario; 4. Select **Scenario 1** from the drop-down menu.

Report

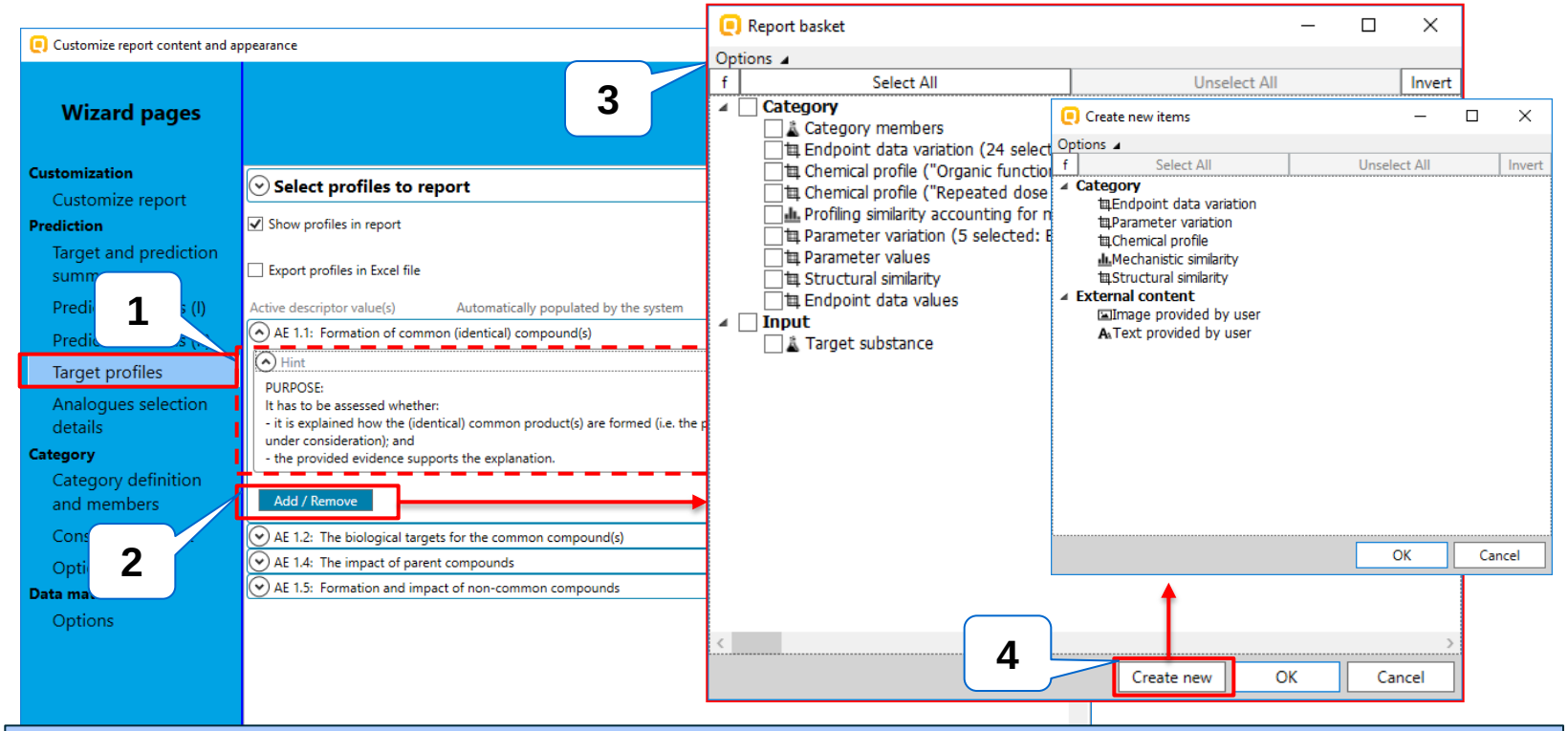
Report generation according to RAAF-Scenario 1



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

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

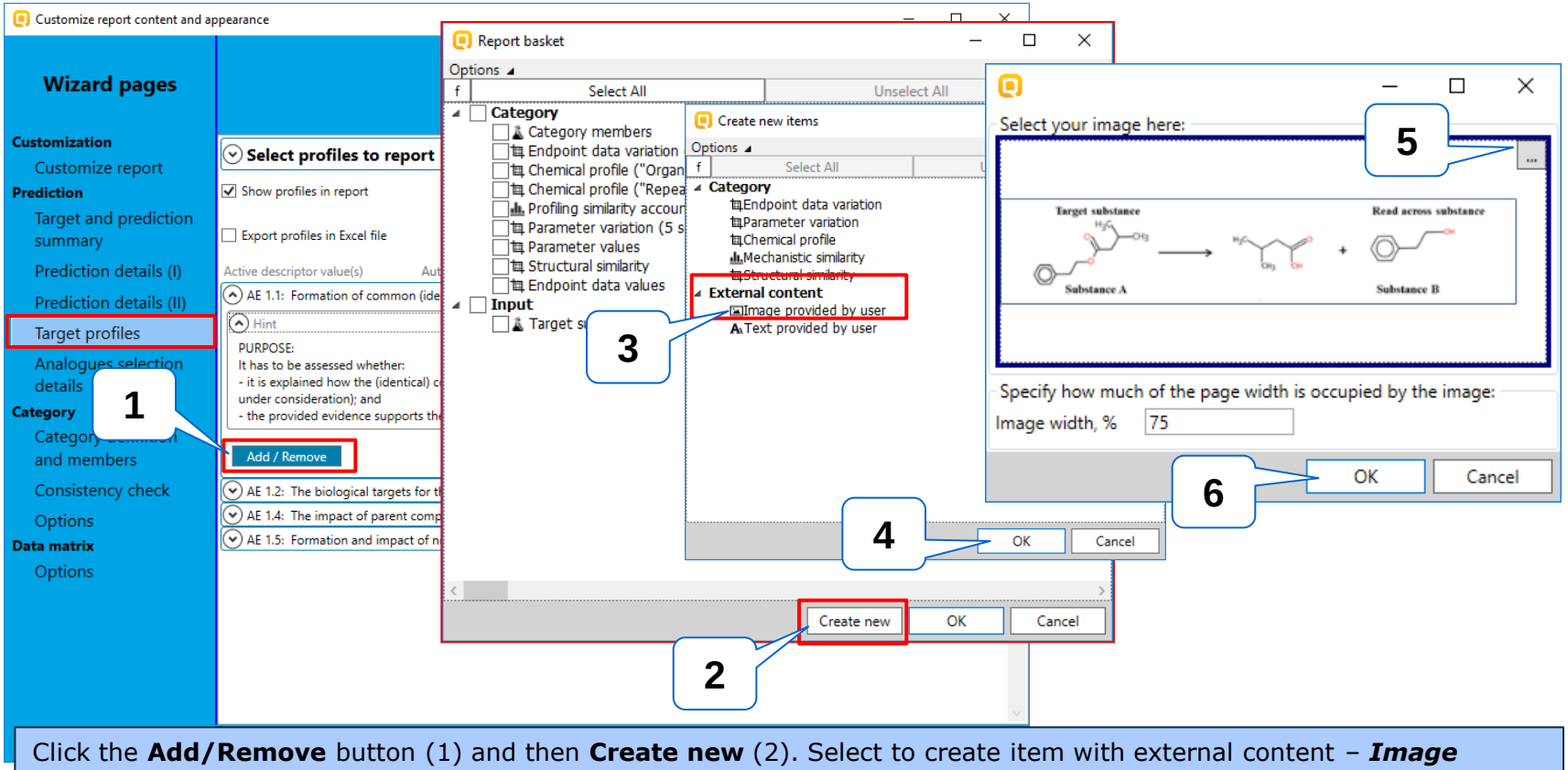
Report
Assessment elements of Scenario 1



Hint for each of the assessment elements is available (1). Information can be included by clicking the **Add/Remove** button (2) located below the corresponding AE. The *Add/Remove* button invokes the so-called "***Report basket***" (3). The latter contain different items triggered by the actions of the user during the workflow (e.g. Alert performance calculation, applying of category elements, etc.).
Additionally, new items (including items with external content) can be created (4).

Items with external content (picture and text) will be added for **AE 1.1**. **Formation of common (identical) compound(s)**

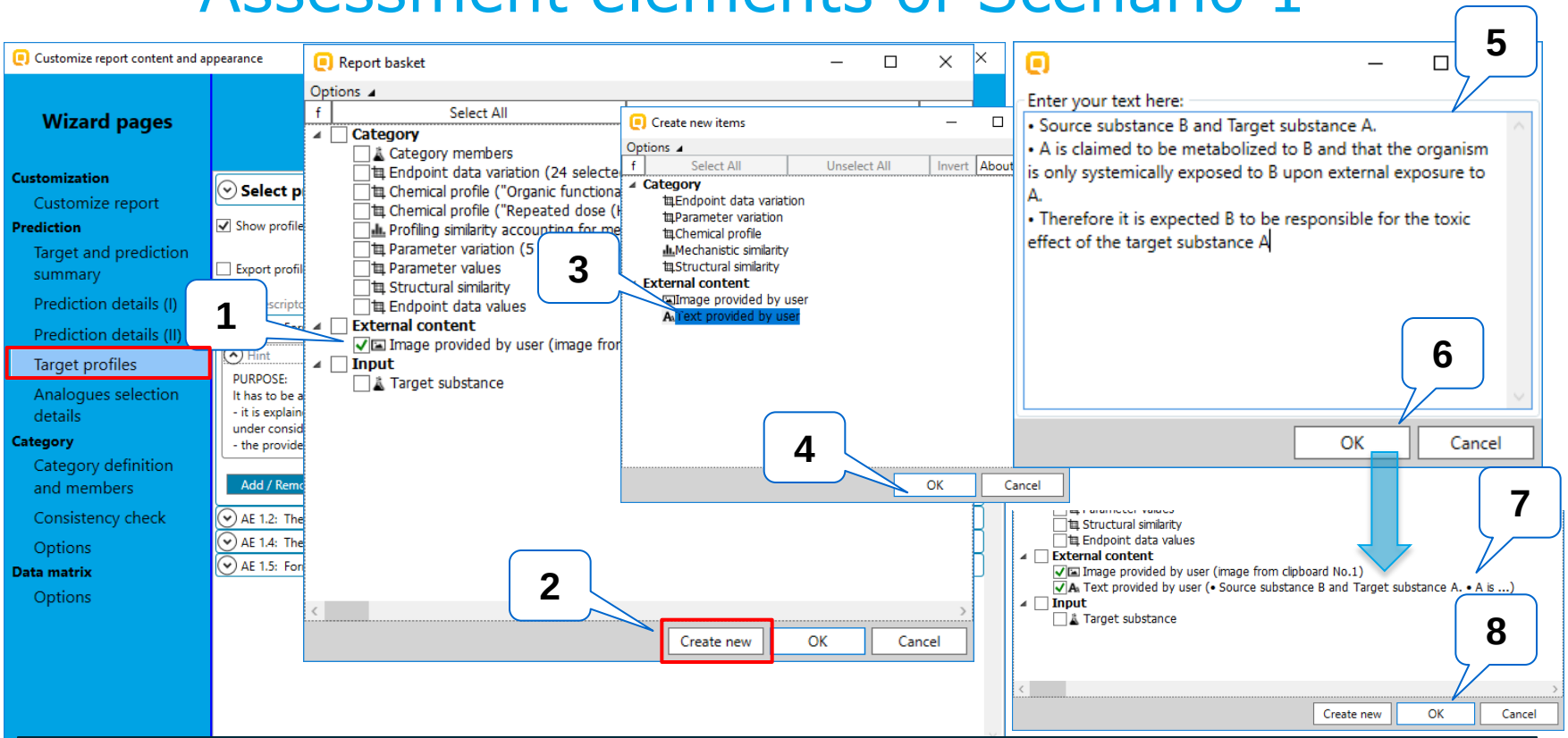
Report
Assessment elements of Scenario 1



Click the **Add/Remove** button (1) and then **Create new** (2). Select to create item with external content – ***Image provided by user*** (3) and click **OK** (4). New window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture is saved*. Finally confirm by **OK** (6).

*In the current example a picture illustrating the target chemical marked as **Target A** and source chemicals marked as **Substance B** and how the A is transformed to B was prepared in advance.

Report
Assessment elements of Scenario 1

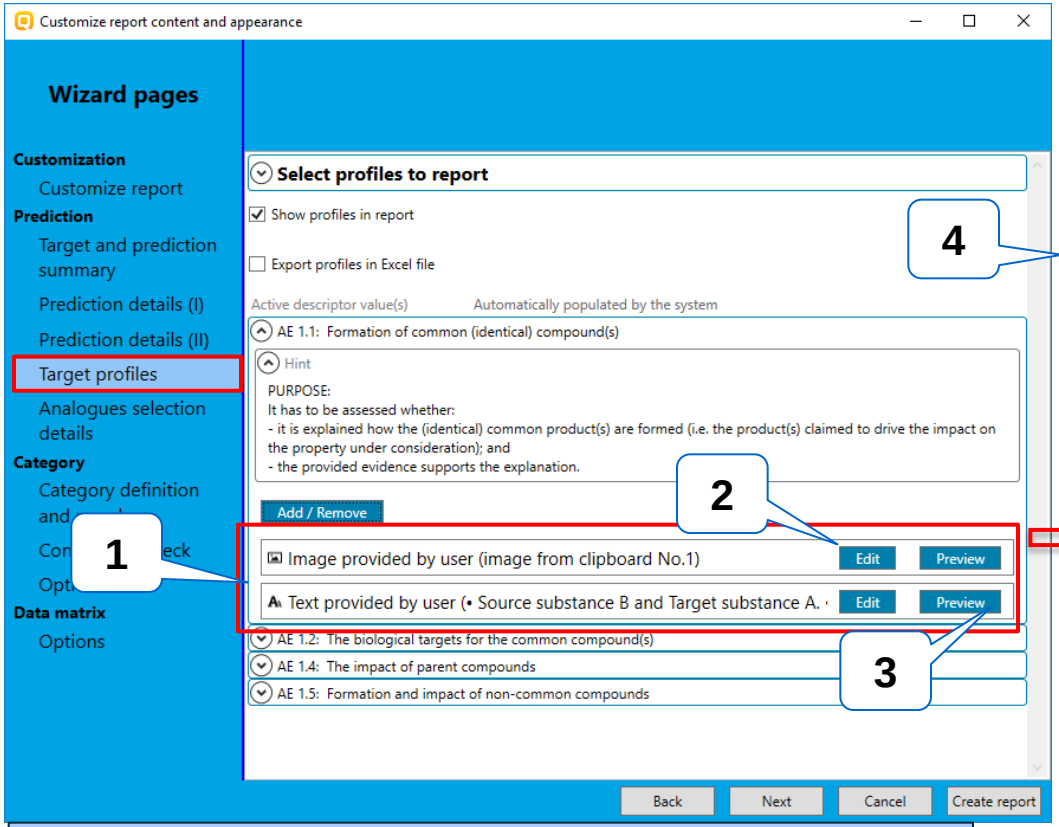


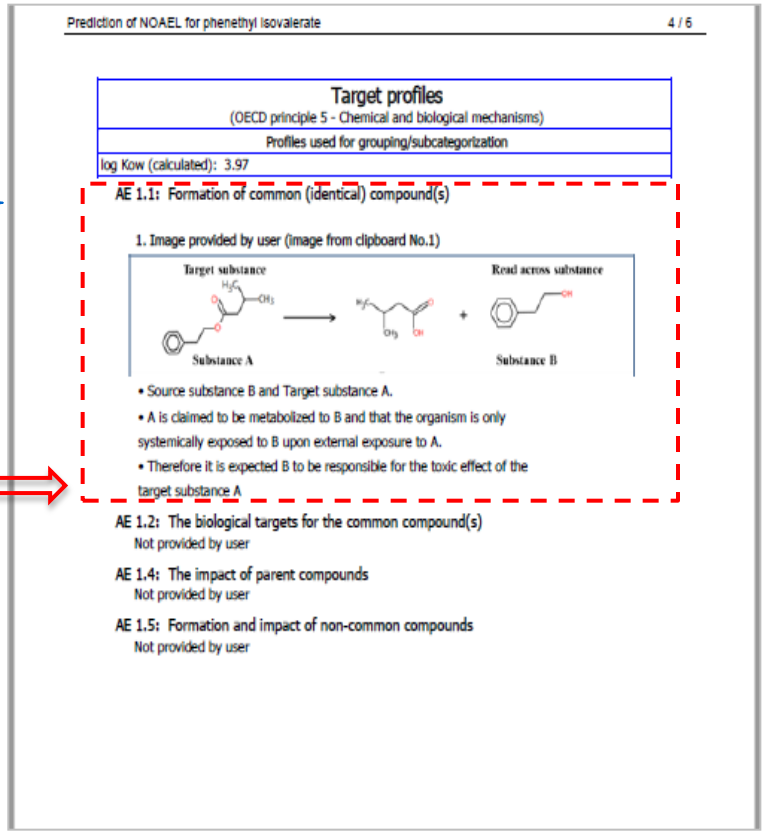
The newly created item appears in the ***Report basket*** (1). Now text will be also included. Click **Create new** (2), select ***Text provided by user*** (3) and click **OK** (4). Copy the following example text:

- *Source substance B and Target substance A.*
- *A is claimed to be metabolized to B and that the organism is only systemically exposed to B upon external exposure to A.*
- *Therefore it is expected B to be responsible for the toxic effect of the target substance A*

and paste it in the new window (5). Finally confirm by **OK** (6).
The newly created report item appears in the **"Report basket"** (7). Click **OK (8)**

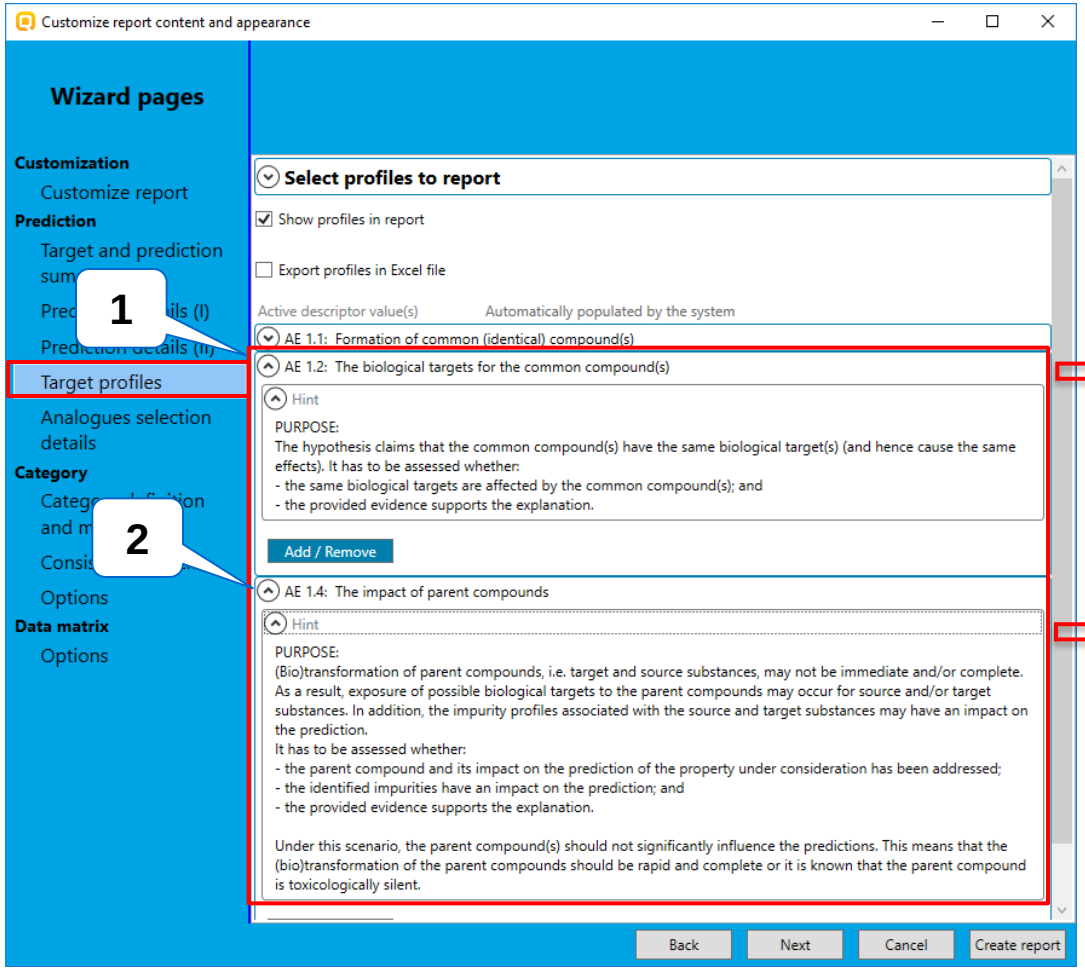
Report
Assessment elements of Scenario 1





Both newly created items appear under the **AE 1.1**. (1). Each of the items can be edited (2) or just previewed (3) in a *.pdf* format.
An example of how the AE 1.1. and related description will look in the generated report is shown on the right(4).

Report
Assessment elements of Scenario 1



The following text is used as an example for both assessment elements **AE 1.2** and **AE 1.4**. Both text are added as a new text report item (steps are illustrated on slide 45).

An example text for **AE1.2. The biological targets for the common compounds (1)**:

- *Example for differences in distribution pattern leading to different biological targets for the common compound*
  - *Substance A is converted to substance B in the liver based on hydrolysis reaction.*
  - *Oral study with B is used to predict the toxicity of A after oral administration.*
- *Differences in the exposure of organ/tissues to the common compound B have to be expected when exposures are compared between B administered directly or when formed from A.*

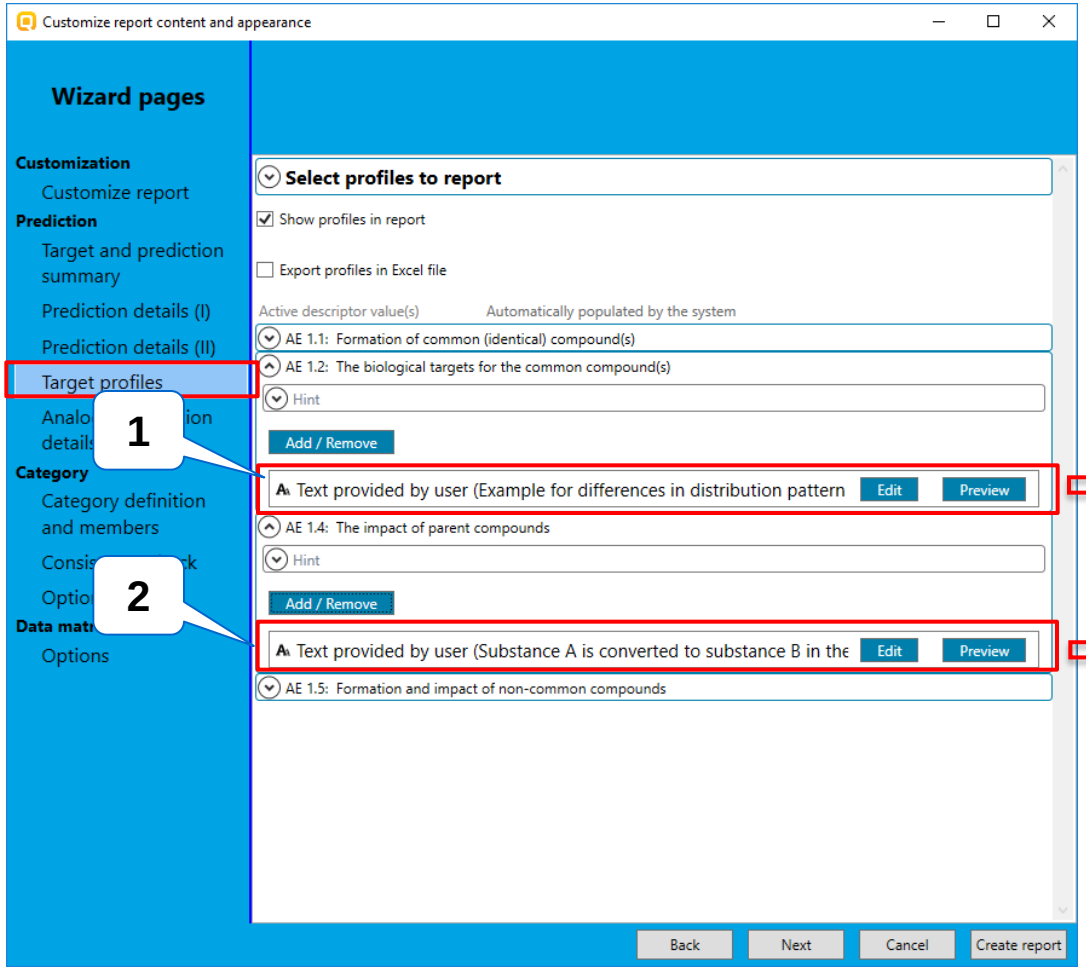
An example text for **AE1.4. The impact of parent compounds (2):**

- *Substance A is converted to substance B in the liver*
- *Substance B is clamed to derive the effect*
- *The parent chemical A is present in significant amounts (its is monoconstituent without any additives or impurities)*
- *Substance A is suspected to have toxicity of its own*
- *The Substance B is used as a source to predict the effect for Substance A*

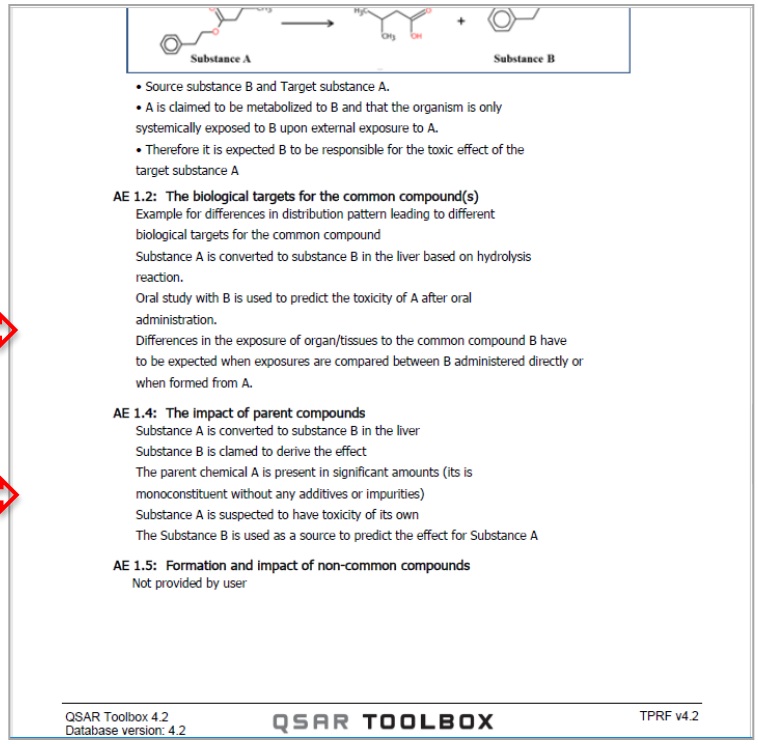
The impact of impurity if available should be addressed here. An example text is provided below:

- *Substance A consist of the main constituent A, there is an impurity X of 5%*
- *The substance B consists of the main constituent B, there is the same impurity X of 3% and impurity Y of 2 %*

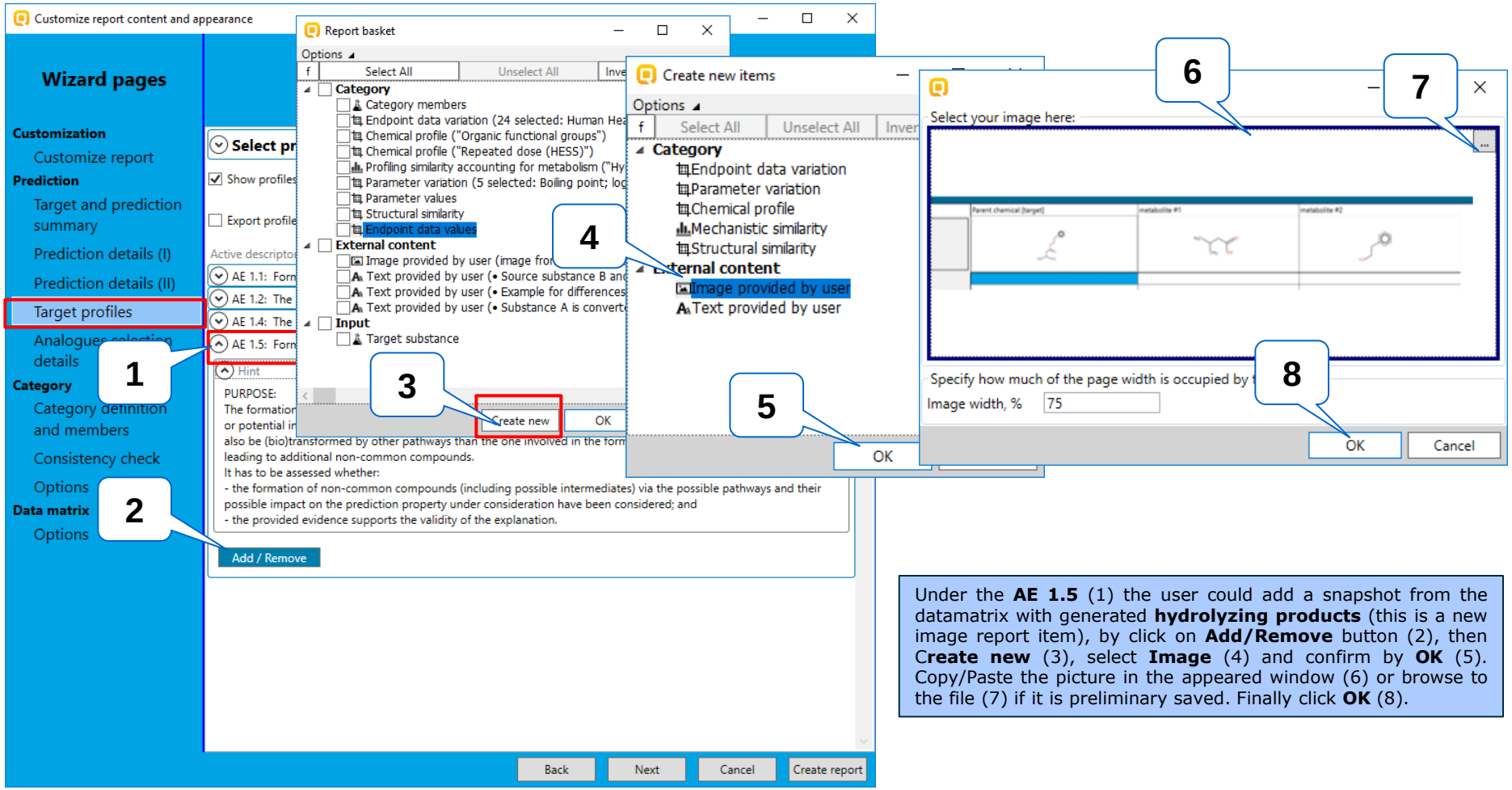
Report
Assessment elements of Scenario 1



The report items associated with **AE 1.2** (1) and **AE 1.4** (2) appeared under the respective sections of the report. How they look in the generated report is shown on the right.

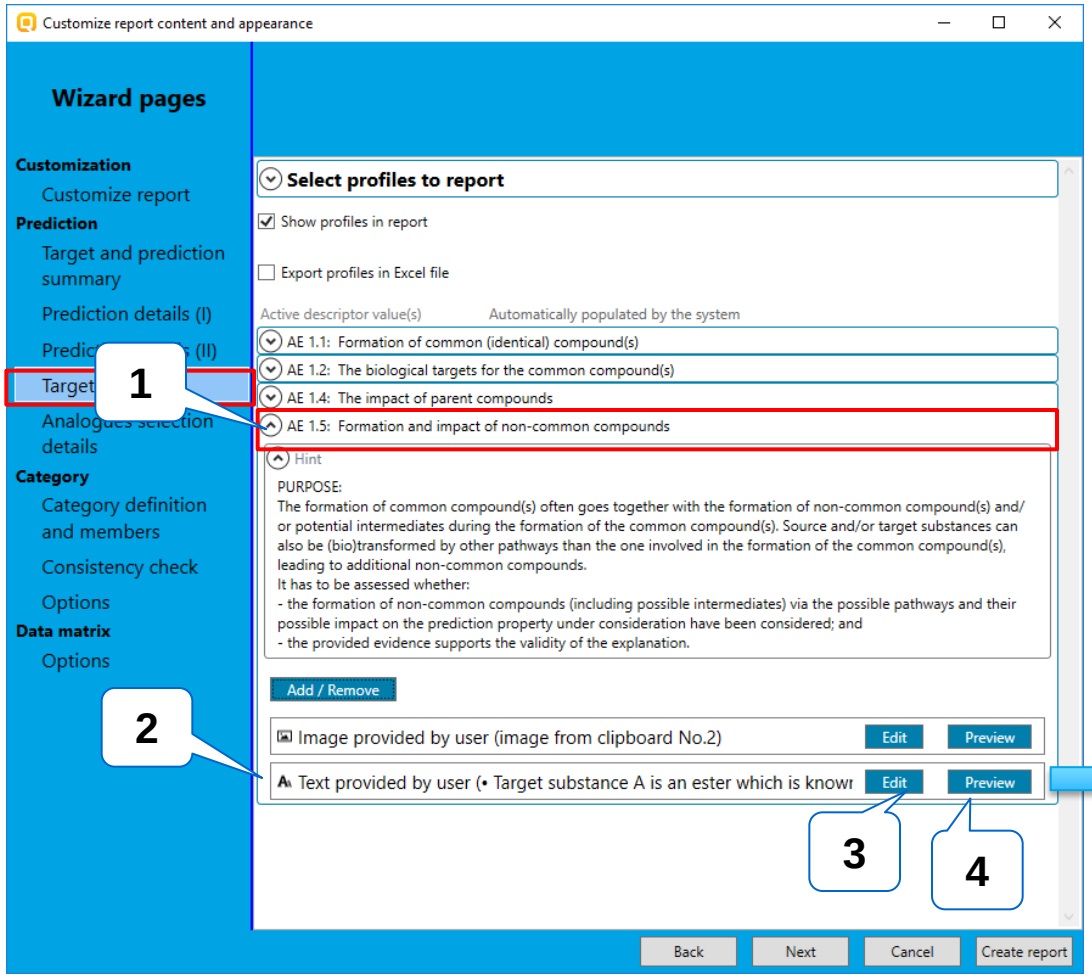


Report
Assessment elements of Scenario 1



Under the **AE 1.5** (1) the user could add a snapshot from the datamatrix with generated **hydrolyzing products** (this is a new image report item), by click on **Add/Remove** button (2), then **Create new** (3), select **Image** (4) and confirm by **OK** (5). Copy/Paste the picture in the appeared window (6) or browse to the file (7) if it is preliminary saved. Finally click **OK** (8).

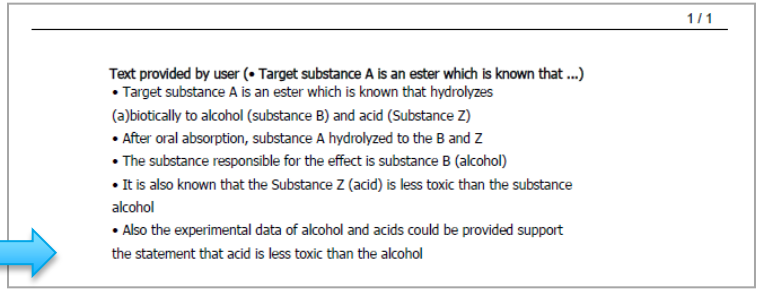
Report
Assessment elements of Scenario 1



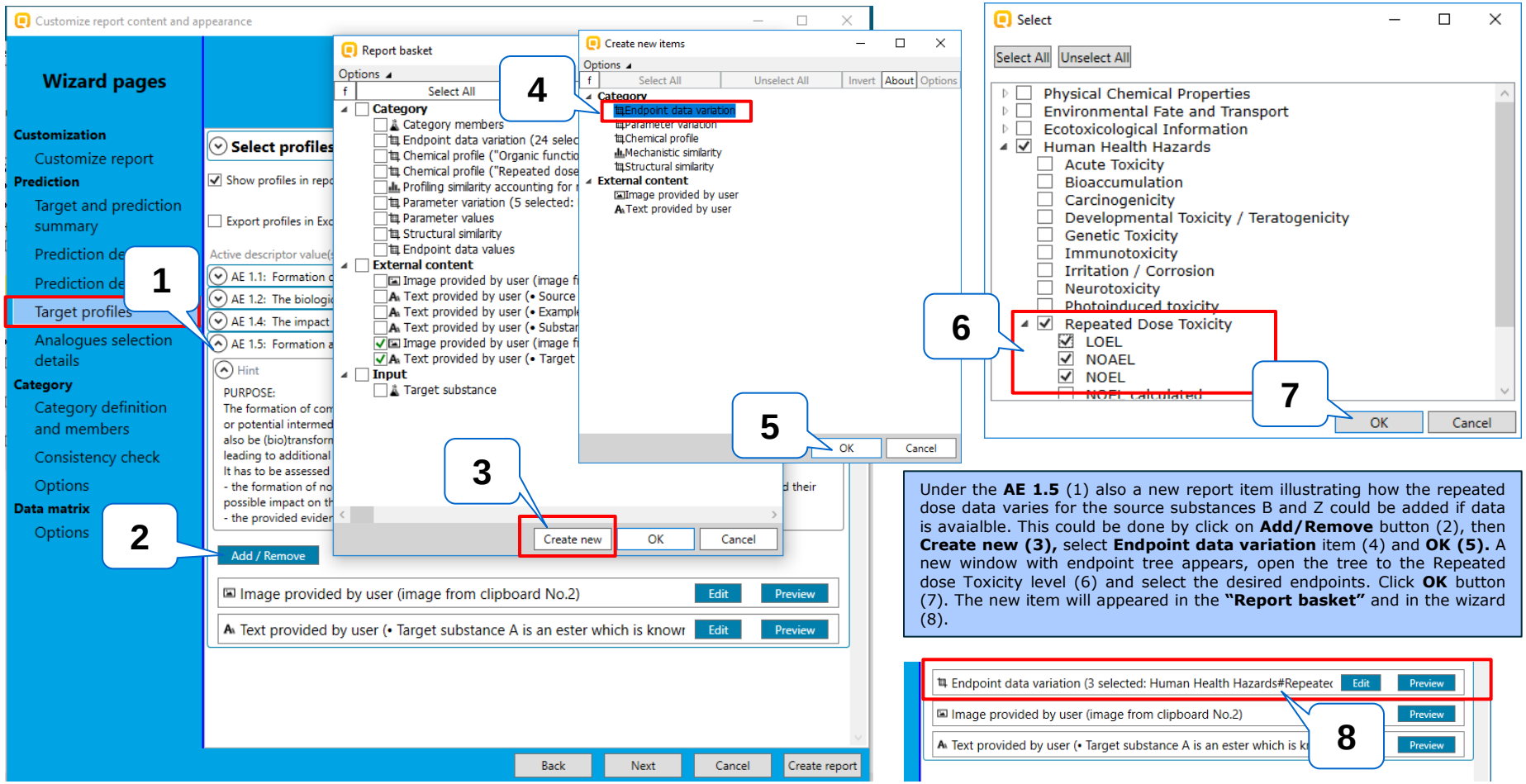
Along with the image a text could be added under **AE 1.5** (1) with the following content. An example text for **AE 1.5. Formation and impact of non-common compounds: manually editable** (copy the text and paste it in the text box, steps are already shown on slide 45)**:**

- *Target substance A is an ester which is known that hydrolyzes (a)biotically to alcohol (substance B) and acid (Substance Z)*
- *After oral absorption, substance A hydrolyzed to the B and Z*
- *The substance responsible for the effect is substance B (alcohol)*
- *It is also known that the Substance Z (acid) is less toxic than the substance alcohol*

Once added the text item appeared in the report wizard (2). It cold be **edited** (3) or just **previewed** (4) as a *.pdf.

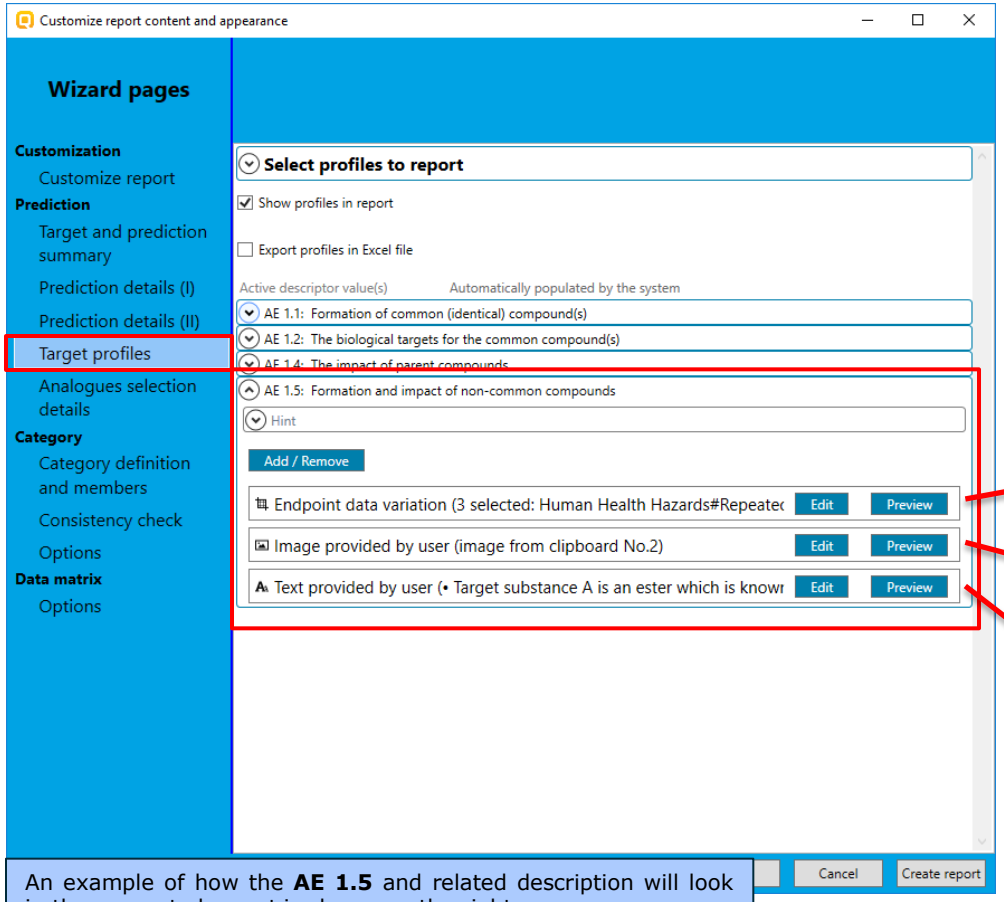


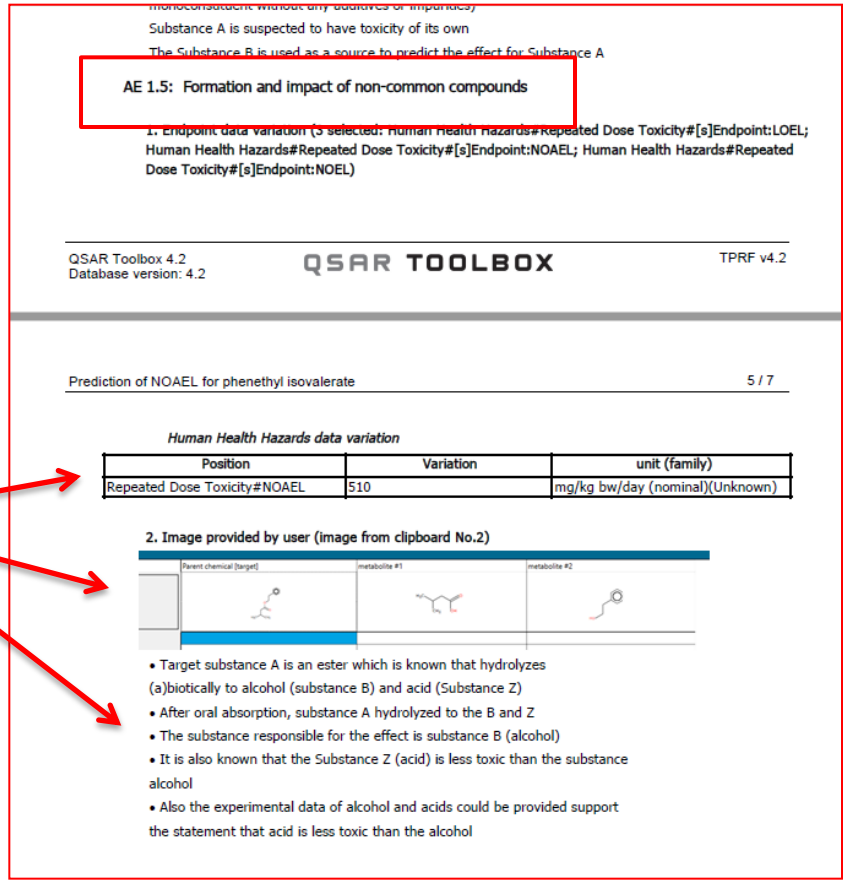
Report
Assessment elements of Scenario 1



Under the **AE 1.5** (1) also a new report item illustrating how the repeated dose data varies for the source substances B and Z could be added if data is avaialble. This could be done by click on **Add/Remove** button (2), then **Create new (3),** select **Endpoint data variation** item (4) and **OK (5).** A new window with endpoint tree appears, open the tree to the Repeated dose Toxicity level (6) and select the desired endpoints. Click **OK** button (7). The new item will appeared in the **"Report basket"** and in the wizard (8).

Report
Assessment elements of Scenario 1




Substance A is suspected to have toxicity of its own

The Substance B is used as a source to predict the effect for Substance A

AE 1.5: Formation and impact of non-common compounds

1. Endpoint data variation (3 selected: Human Health Hazards#Repeated Dose Toxicity#[s]Endpoint:LOEL; Human Health Hazards#Repeated Dose Toxicity#[s]Endpoint:NOAEL; Human Health Hazards#Repeated Dose Toxicity#[s]Endpoint:NOEL)

QSAR Toolbox 4.2
Database version: 4.2

QSAR TOOLBOX

TPRF v4.2

Prediction of NOAEL for phenethyl isovalerate 5 / 7

Human Health Hazards data variation

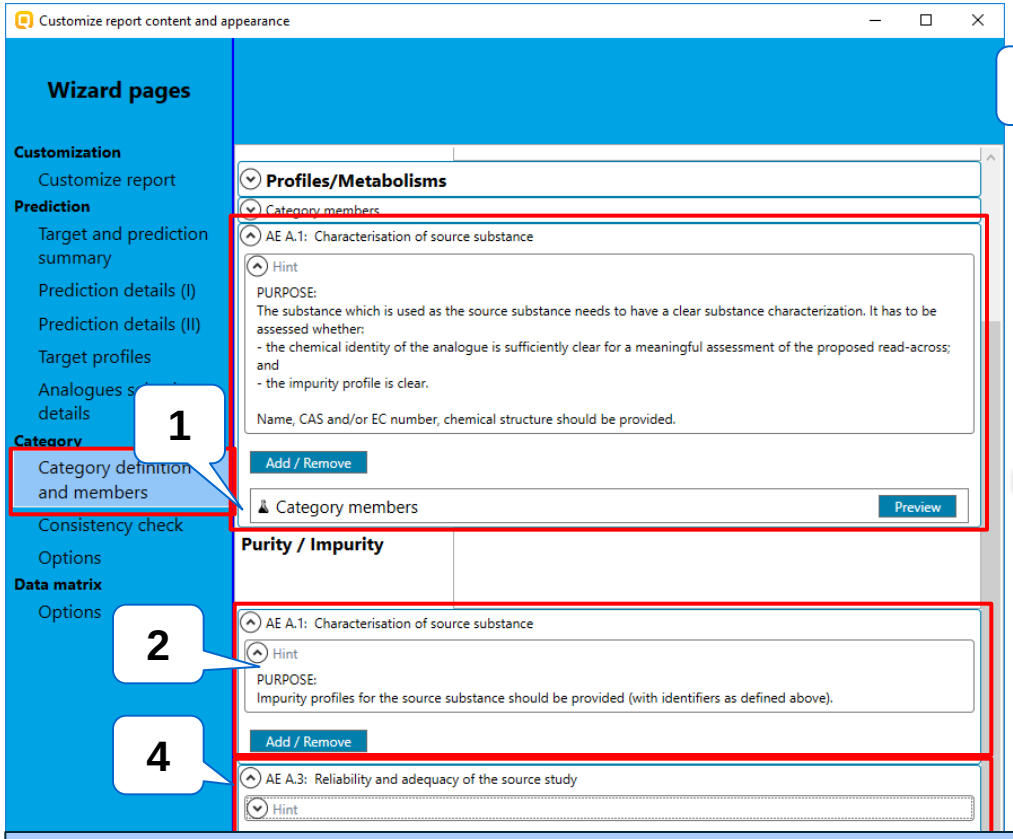
Position	Variation	unit (family)
Repeated Dose Toxicity#NOAEL	510	mg/kg bw/day (nominal)(Unknown)

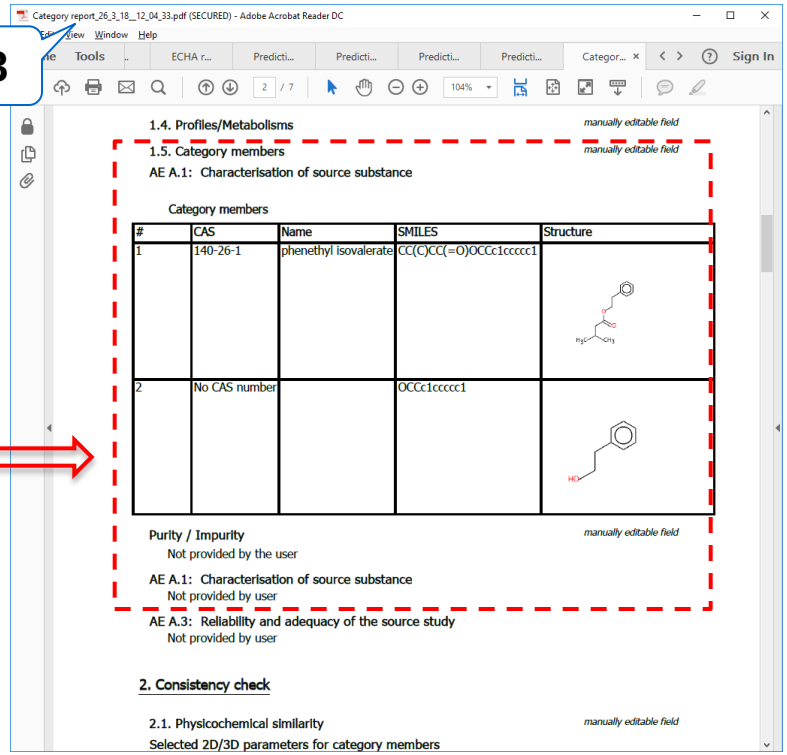
2. Image provided by user (image from clipboard No.2)

- Target substance A is an ester which is known that hydrolyzes (a)biotically to alcohol (substance B) and acid (Substance Z)
- After oral absorption, substance A hydrolyzed to the B and Z
- The substance responsible for the effect is substance B (alcohol)
- It is also known that the Substance Z (acid) is less toxic than the substance alcohol
- Also the experimental data of alcohol and acids could be provided support the statement that acid is less toxic than the alcohol

An example of how the **AE 1.5** and related description will look in the generated report is shown on the right.

Report
Assessment elements of Scenario 1

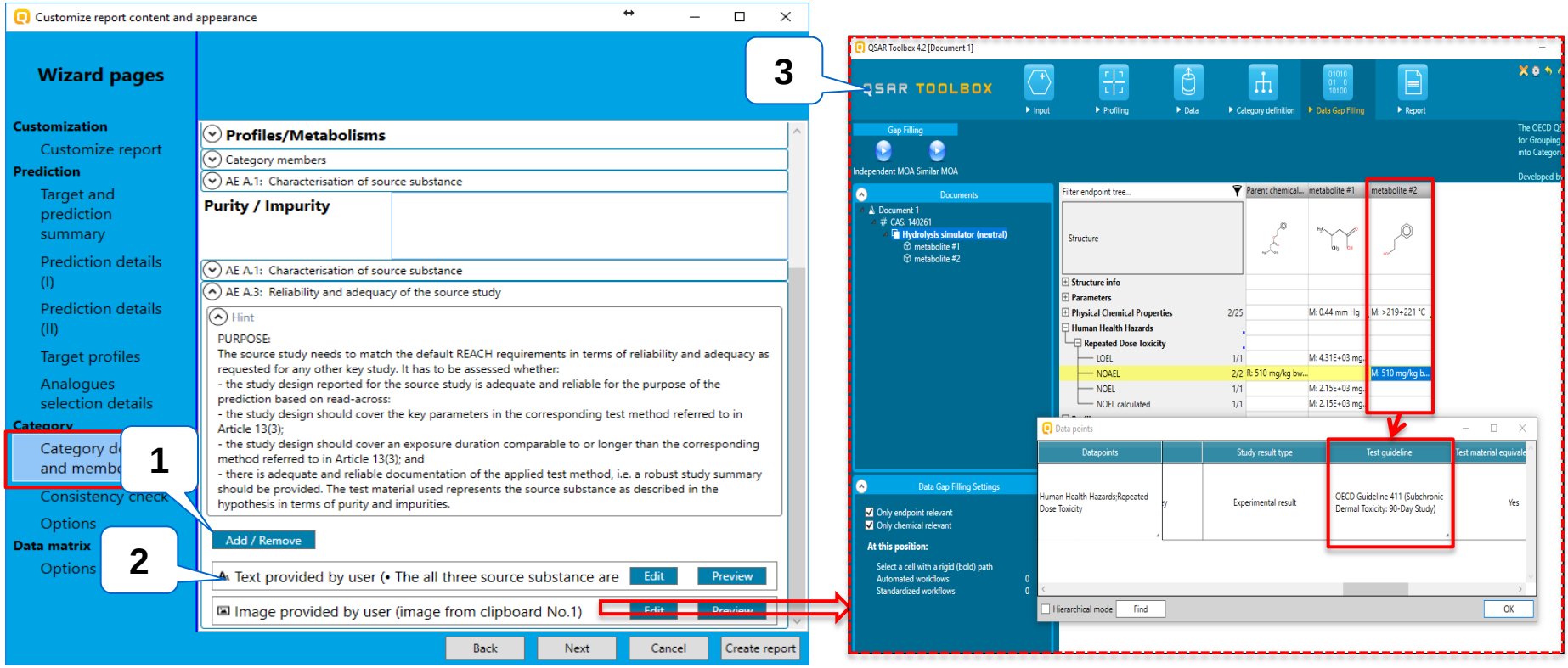




Two AE (AE *A.1* and *A.3*) related to Scenario 1 are included in the *Category definition and members* section.

- **AE A.1 Characterization of source substance** is automatically filled by the system using the available items in the *Report basket*. In this example *Category members* item (1) is appended, only. If impurities/additives of the used analogues are available, they will appear under the ***AE A.1*** in *Purity / Impurity* (2). The current analogues have no additives/impurities.
  An example of how the AE A.1. will look in the generated report is shown on the right (3).
- **AE A.3 Reliability and adequacy of the source study** should be filled in manually (4) *(see on the next slide)*

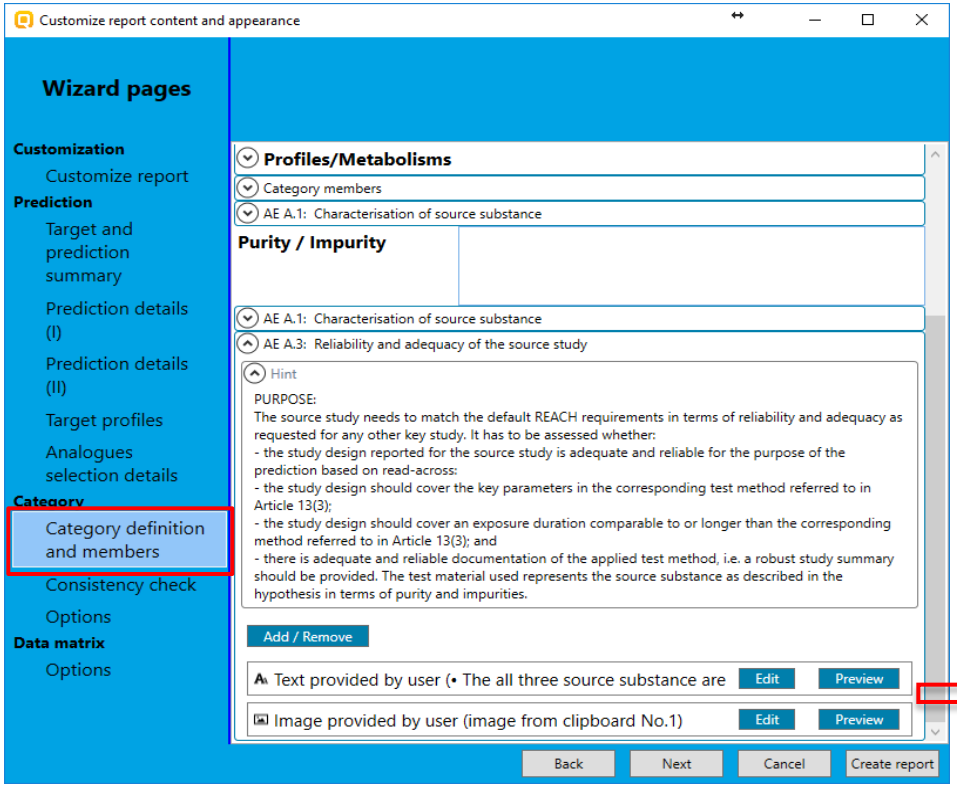
Report
Assessment elements of Scenario 1

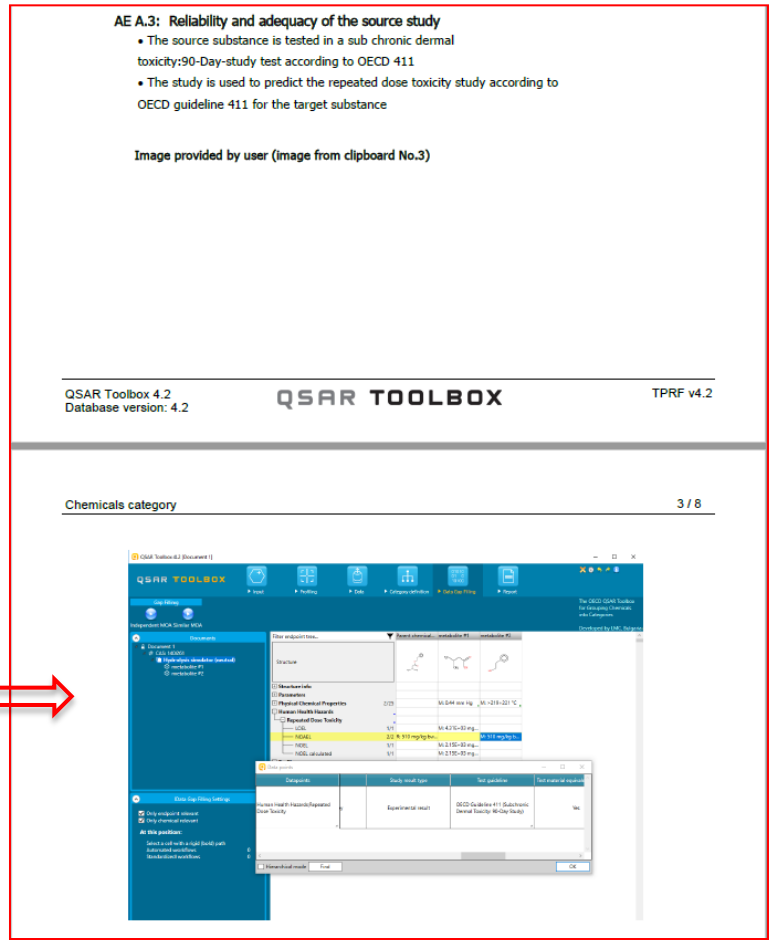


AE A.3: Click the **Add/Remove** button (1) and **create new item** with textual content (2) (see slide 45).
In the text field paste the following example text:
"The source substance is tested in a sub chronic dermal toxicity:90-Day-study test according to OECD 411
The study is used to predict the repeated dose toxicity study according to OECD guideline 411 for the target substance"

Additionally a snapshot of the metadata of the alcohol (3) showing that the data are prepared by for 90-days under OECD Guideline 411 could be added to confirm the consistency regarding the assay.

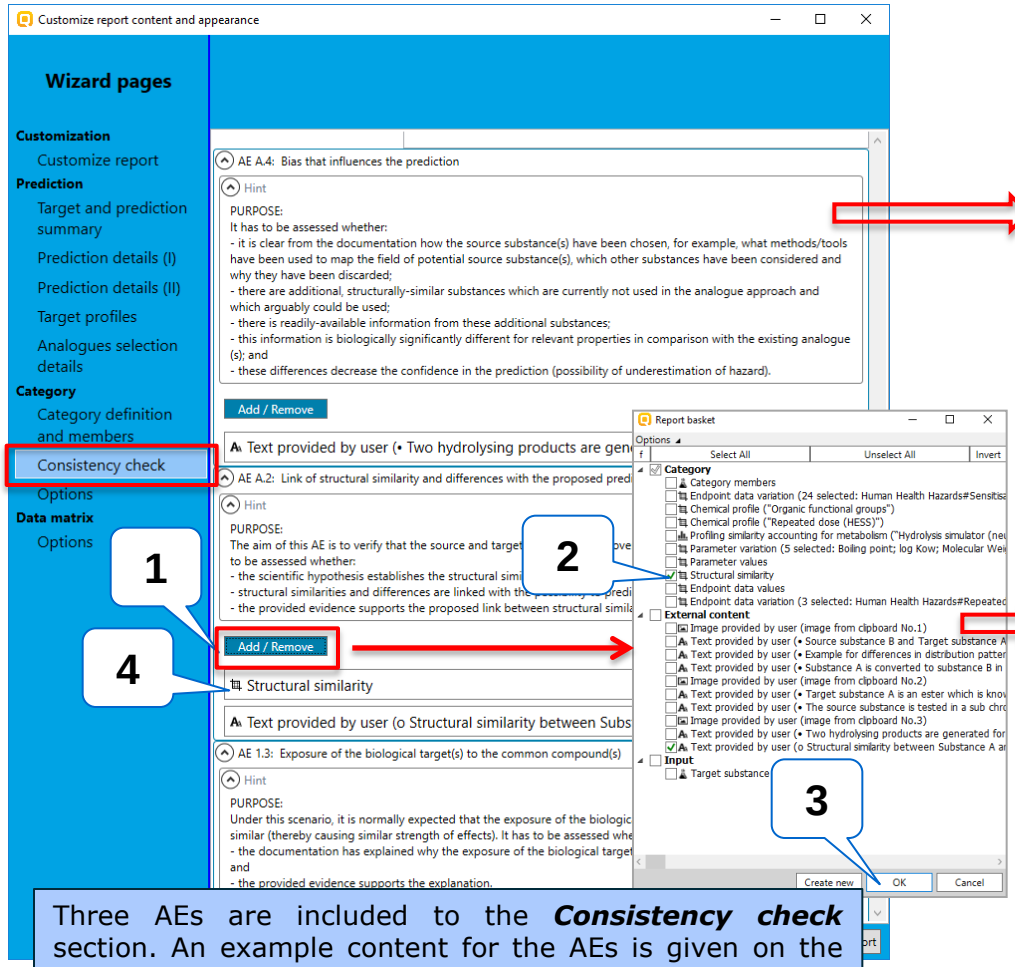
Report
Assessment elements of Scenario 1





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

Report
Assessment elements of Scenario 1



Three AEs are included to the ***Consistency check*** section. An example content for the AEs is given on the right

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

- *Two hydrolysing products are generated for the target chemical: Izovalerate acid and Phenethyl alcohol*
- *In the Data module, you have found that RDT data is available for both products*
- *The data for both products are bigger than hazard threshold of 100 mg/kg/data according to GHS classification [GSH classification],*
- *However, metabolite phenethyl acohol is more toxic than the acid based on the experimental data*
- *Moreover it is expected that the acid (Izovalerate acid) will be directly excreted and will not contribute towards the toxicity of the target [RIFM, 2012]*
- *It is expected that the toxicity of the target chemical phenethyl isovalerate will be result of Phenethyl alcohol*

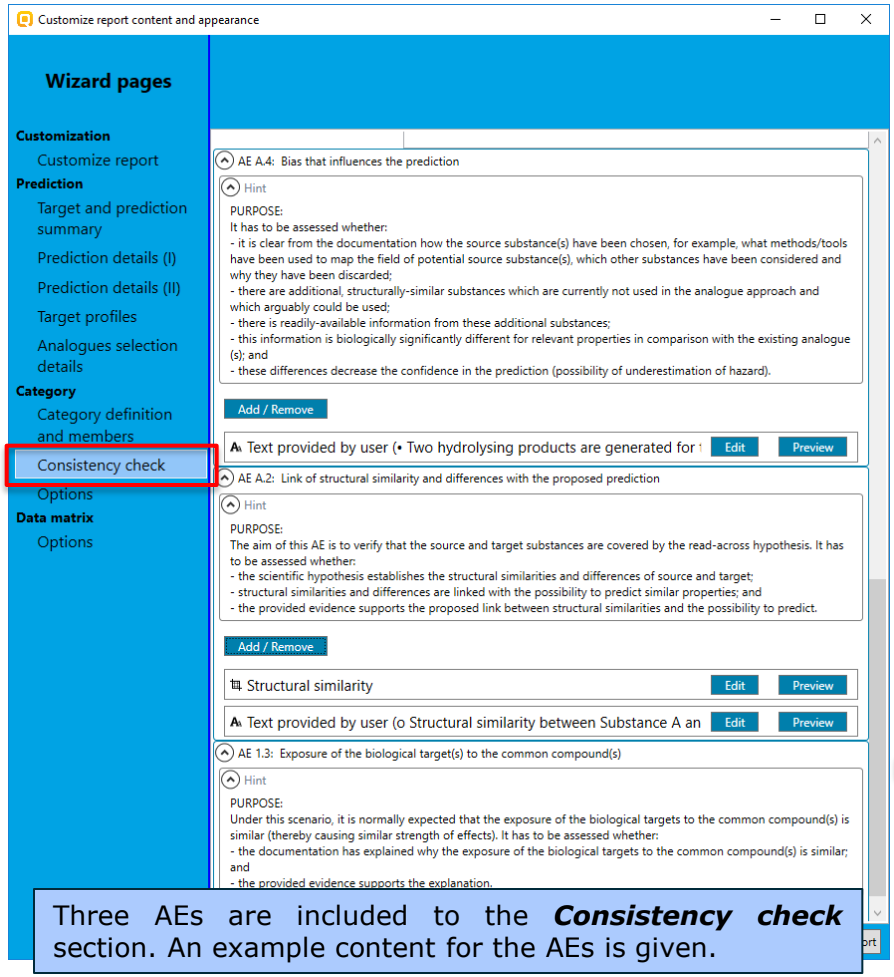
An example text for **AE A.2. Link of structural similarity and differences with the proposed prediction:**

- *Structural similarity between Substance A and B according to Str.similarity profiler is in the range of [50-70%]*
- *Both chemicals has aromatic ring based on OFG profiler*

Also the Str. similarity item could be added here. It is automatically generated during the workflow and stored in the Report basket. You should click on **Add/Remove** button (1) and select **Str.similarity** item (2), then **Click OK** (3) and it will appeared in the wizard (4)

AEs regarding Consistency check section continues on next slide

Report
Assessment elements of Scenario 1



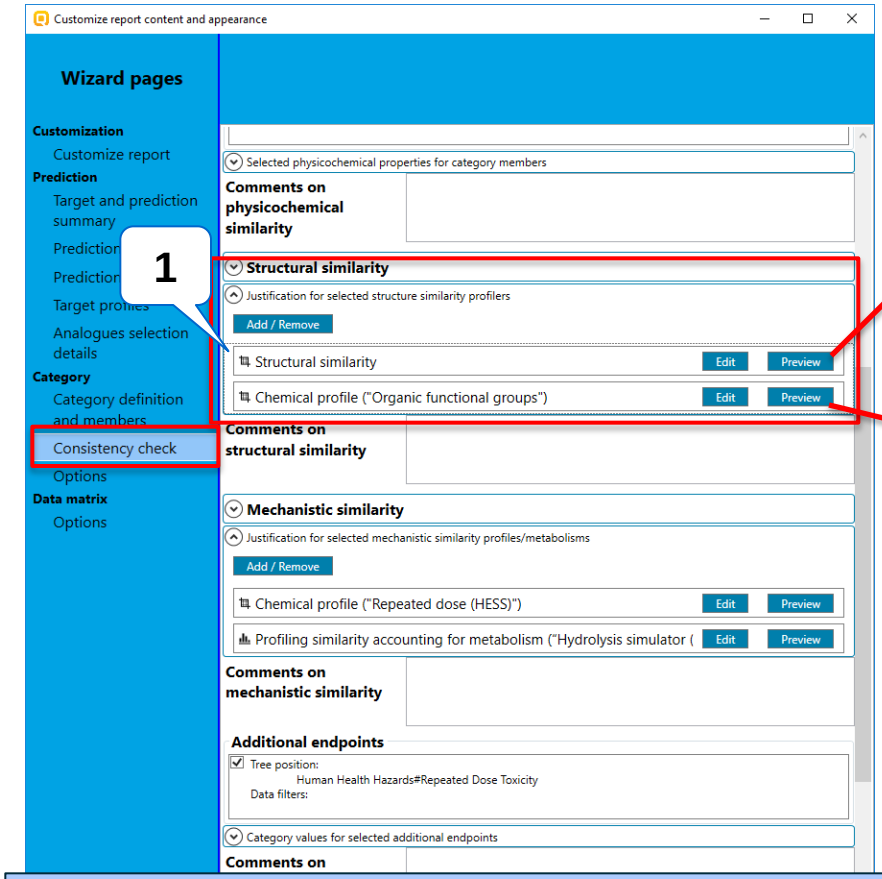
Three AEs are included to the ***Consistency check*** section. An example content for the AEs is given.

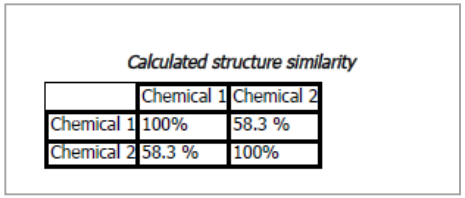
An example text for **AE 1.3. Exposure of the biological target(s) to the common compound(s):**

- *Substance A is transformed to B*
- *Also similar target chemicals having ester functionality are transformed to B too [cite literature here]*
- *Similar reactivity pattern is obtained for all targets transformed to the common compound B*

An expert can provide additional literature search of similar analogues with similar effects.

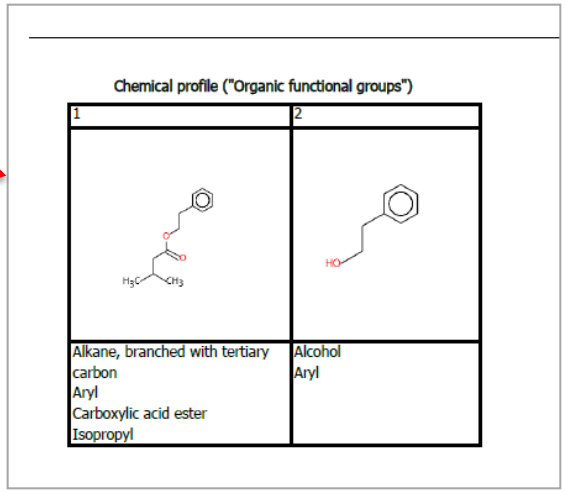
Report
Assessment elements of Scenario 1





Calculated structure similarity

	Chemical 1	Chemical 2
Chemical 1	100%	58.3 %
Chemical 2	58.3 %	100%

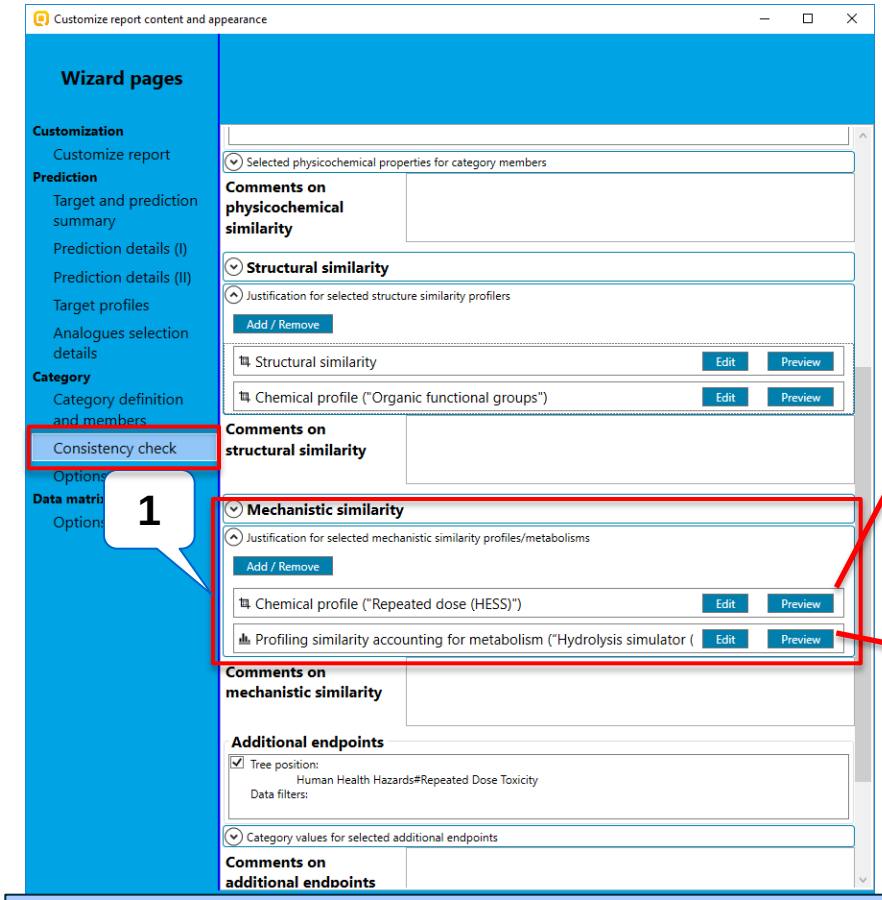


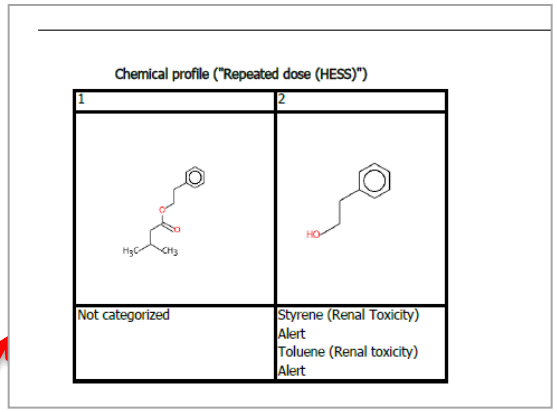
Chemical profile ("Organic functional groups")

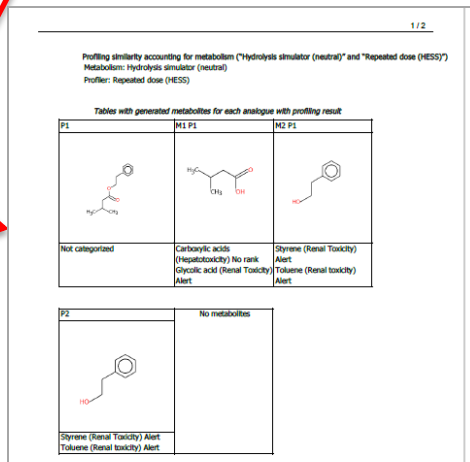
1	2
Alkane, branched with tertiary carbon Aryl Carboxylic acid ester Isopropyl	Alcohol Aryl

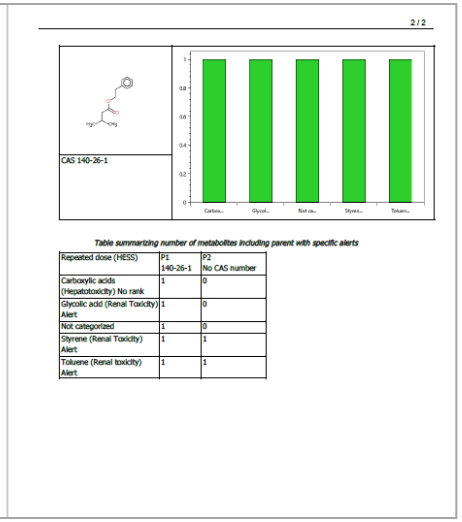
All items in the report basket related to the structural consistency of the category (1) are added automatically.

Report
Assessment elements of Scenario 1









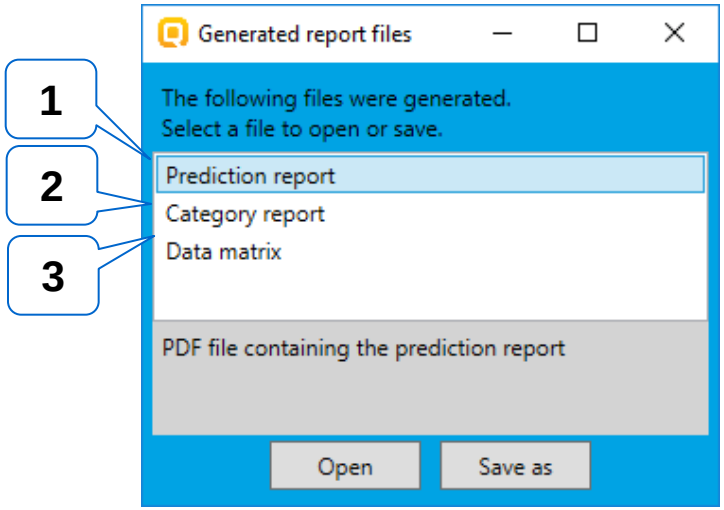
All items in the report basket related to the consistency of the category with respect to Mechanistic similarity (1) are added automatically.

Report Generation

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

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

The selected RAAF scenario is specified in the first page

Date: 26 Mar 2018
Author(s):
Contact details:

Target information

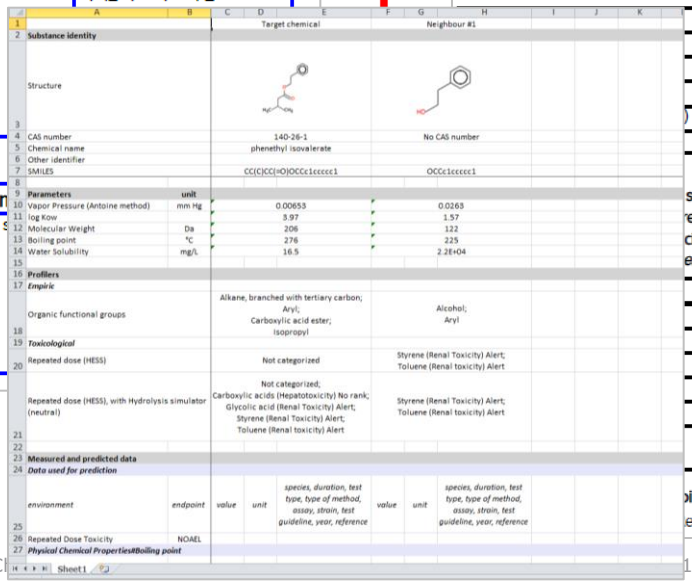
Structural information	Numerical identifiers	Chemical names
SMILES: CC(C)CC(=O)OCCc1ccccc1	CAS#: 140-26-1 Other: EC Number:3778846	2-phenylethyl 3-methylbutanoate Butanoic acid, 3-methyl-, 2-phenylethyl ester Butanoic acid, 3-methyl-, 2-phenylethyl

Structure

Prediction summ

Predicted endpoint: NOAEL; No effect specified; No species guideline specified
Predicted value: 510
Unit/scale: mg/kg bw/day (nominal)
Data gap filling method: Read-across analysis
Summary: *manually editable field*
Not provided by the user

Data matrix report



	Target chemical	Neighbour #1
Substance identity		
Structure		
CAS number	140-26-1	No CAS number
Chemical name	phenethyl isovalerate	
Other identifier		
SMILES	CC(C)CC(=O)OCCc1ccccc1	OCCc1ccccc1
Parameters		
Vapor Pressure (Antoine method) [mm Hg]	0.00653	0.0263
log Kow	3.97	1.57
Molecular Weight [Da]	206	122
Boiling point [°C]	276	225
Water Solubility [mg/L]	16.5	2.2E+04
Profilers		
Empiric		
Organic functional groups	Alkane, branched with tertiary carbon; Aryl; Carboxylic acid ester; Isopropyl	Alcohol; Aryl
Toxicological		
Repeated dose (HESS)	Not categorized	Styrene (Renal Toxicity) Alert; Toluene (Renal toxicity) Alert
Repeated dose (HESS), with Hydrolysis simulator (neutral)	Not categorized; Carboxylic acids (Hepatotoxicity) No rank; Glycolic acid (Renal Toxicity) Alert; Styrene (Renal Toxicity) Alert; Toluene (Renal toxicity) Alert	Styrene (Renal Toxicity) Alert; Toluene (Renal toxicity) Alert
Measured and predicted data		
Data used for prediction		
environment / endpoint / value / unit / *species, duration, test type, type of method, assay, strain, test guideline, year, reference* / value / unit / *species, duration, test type, type of method, assay, strain, test guideline, year, reference*		
Repeated Dose Toxicity: NOAEL		
Physical Chemical Properties#Boiling point		

Category report

QSAR Toolbox report for category

(in accordance with RAAF scenario 1)

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

	Variation	unit (family)
	225 ÷ 276	°C
	1.57 ÷ 3.97	<no units>
	122 ÷ 206	Da
)	0.00653 ÷ 0.0263	mm Hg
	16.5 ÷ 2.2E+04	mg/L

selected: Physical Chemical Properties#Boiling point; Physical Chemical
e; Physical Chemical Properties#Water solubility; Physical Chemical
cient:#N-Octanol/Water)
erties data variation

	Variation	unit (family)
	99 ÷ 220	°C(Temperature)
	0.0868	mm Hg(Pressure)
	10 ÷ 100	Pa(Pressure)
	1.75E+04 ÷ 2E+04	mg/L(Mass concentration)
	2.22E+04	mg/L(Mass concentration)
	0.8 ÷ 1.36	

oint(s) *manually editable field*
epeated Dose Toxicity: NOAEL

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 1.
- Note proficiency comes with practice.