QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 1 and related assessment elements

Outlook

- **Background**
- Keywords
- 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).

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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;
- 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.

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Specific Aims

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

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Read Across Assessment Framework (RAAF)
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 outlines various 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 (see next slide)

Read Across Assessment Framework (RAAF)

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)
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 consequently exposed. Here one could also include the cases where target and source chemicals are in metabolic relationship, i.e. target is the parent and the source chemicals are its metabolites or target is a metabolite of the source chemicals.
  - 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) which 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 scenarios 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

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The Example

- In this exercise the *Repeated dose toxicity* (RDT) of 2-phenylethyl 3-methylbutanoate [CAS# 140-26-1] will be assessed. This chemical will be the "target" chemical;
- Experimental data will be collected and profiling results for the target will be retrieved;
- Hydrolysis products of the target will be generated and data will be collected 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.

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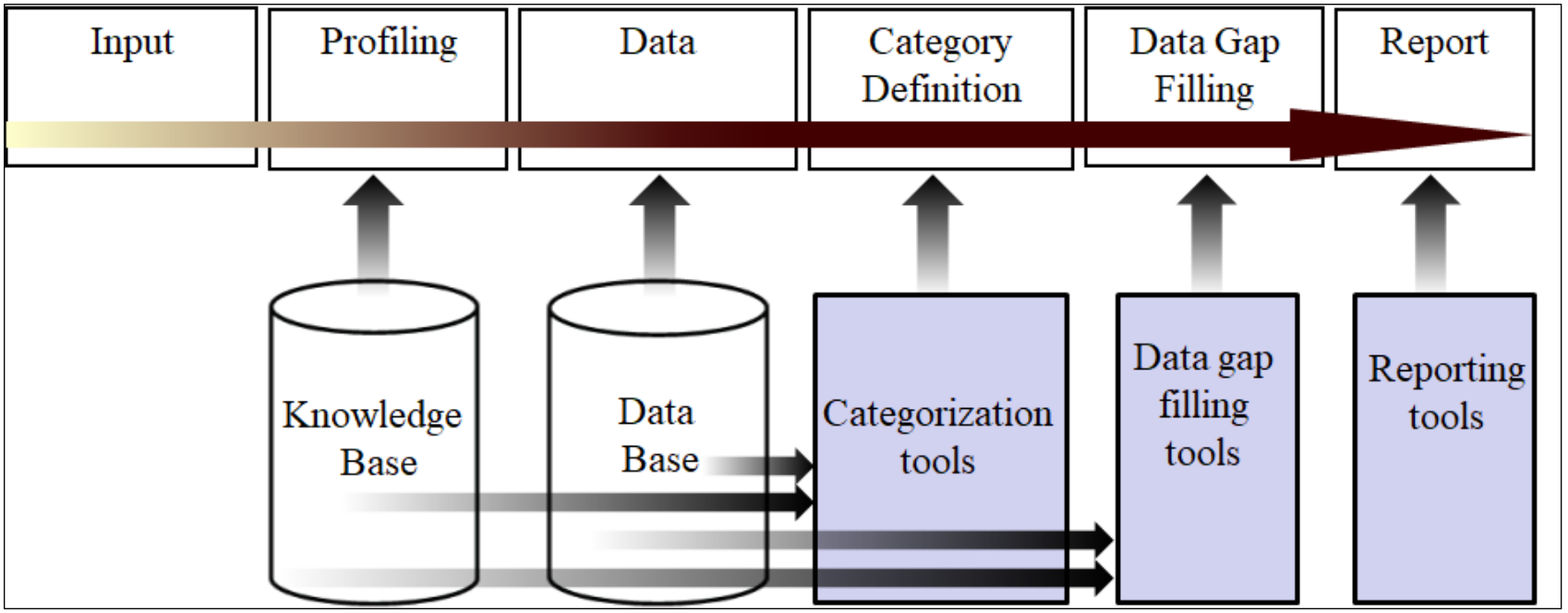
Workflow

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

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

Workflow

Scheme illustrating the Toolbox workflow

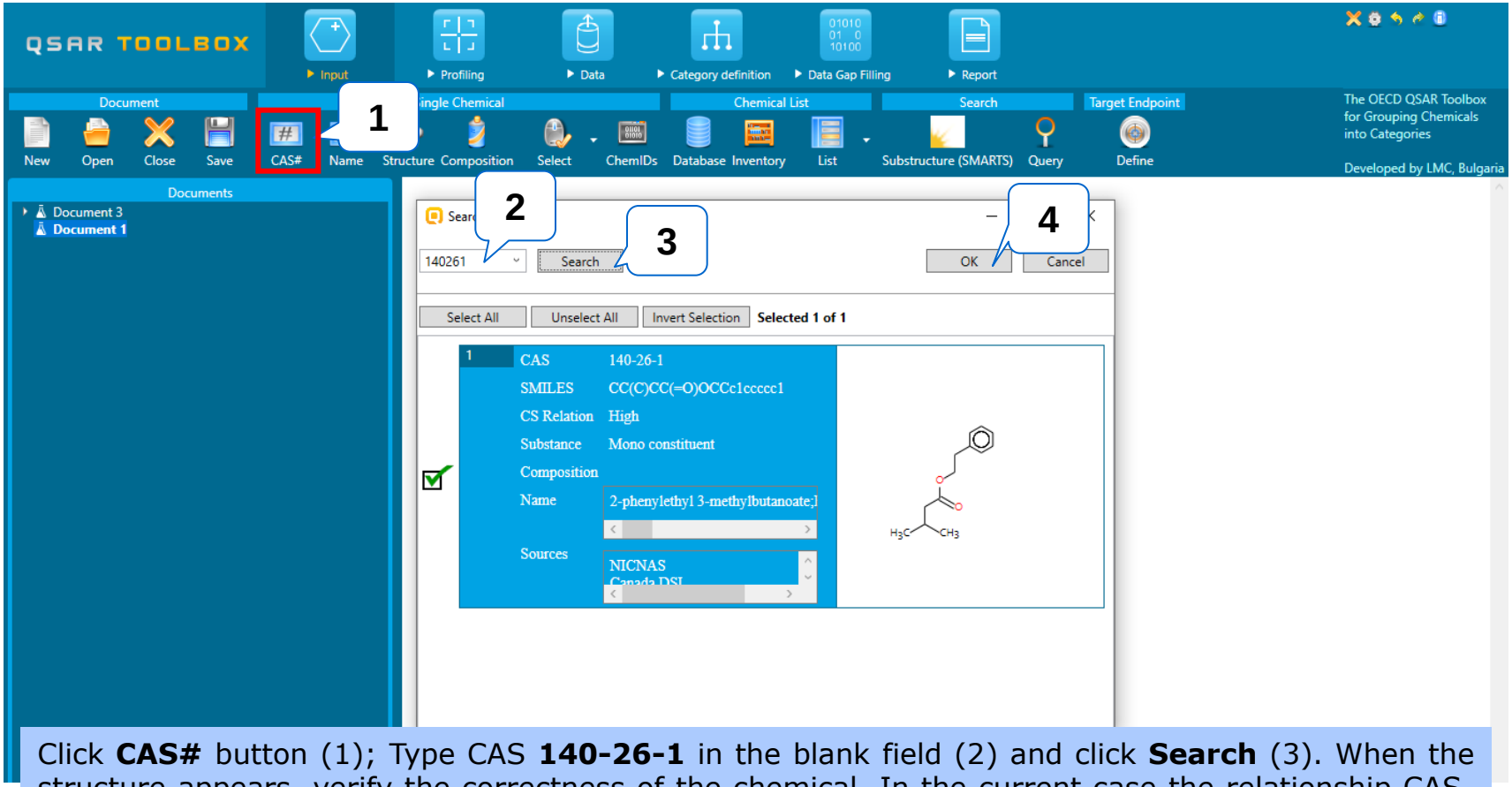


Input
Overview

- 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 that the molecular structure assigned to the target chemical is the correct one.

Input Screen

Enter target chemical by CAS#

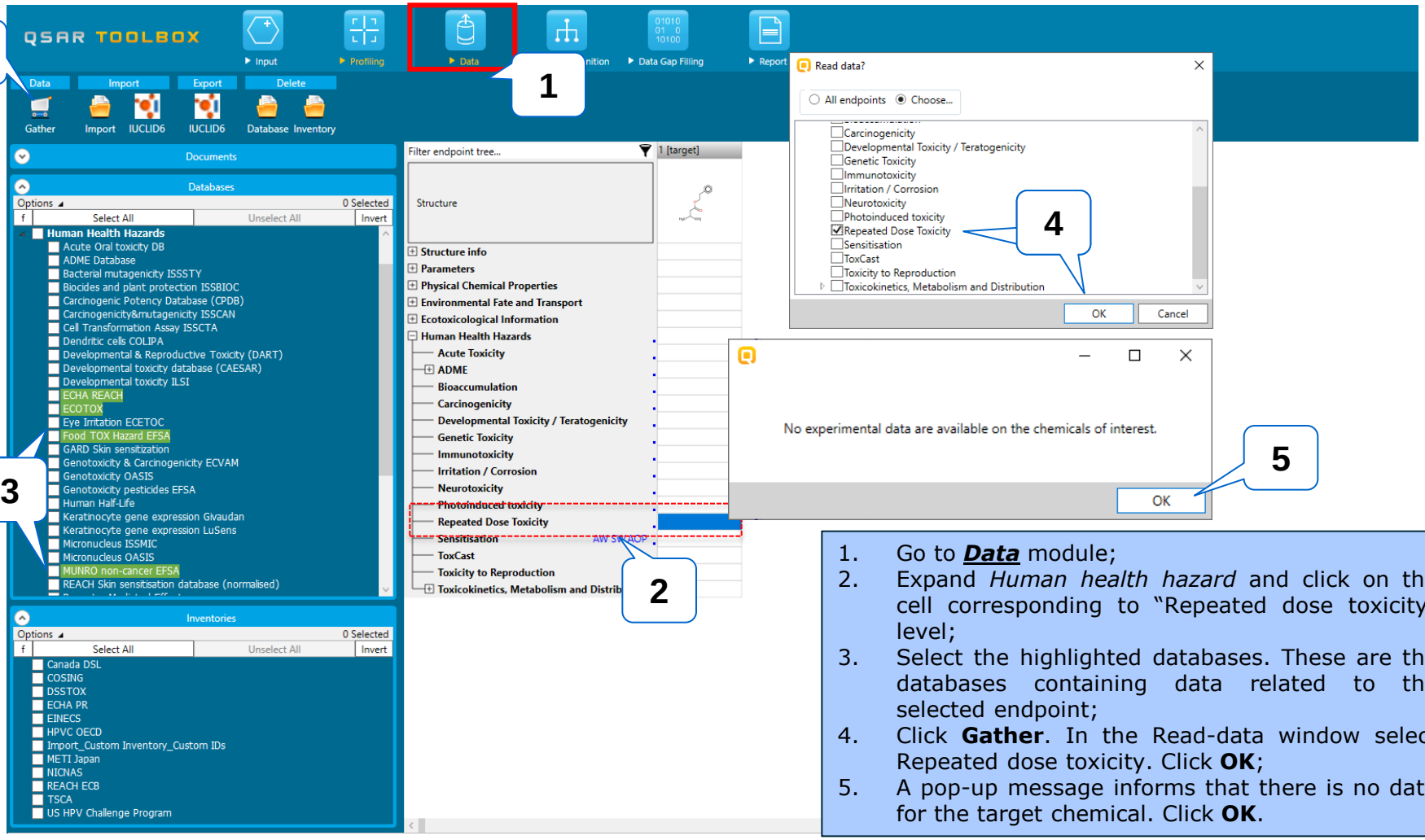


Click **CAS#** button (1); Type CAS **140-26-1** in the blank field (2) and click **Search** (3). When the structure appears, verify the correctness of the chemical. In the current case the relationship CAS-SMILES shows "High" relation for the identifiers. Finally click **OK** (4).

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).
- Let's check if there are 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 **Gather**. In the Read-data window select Repeated dose toxicity. Click **OK**;
5. A pop-up message informs that there is no data for the target chemical. Click **OK**.

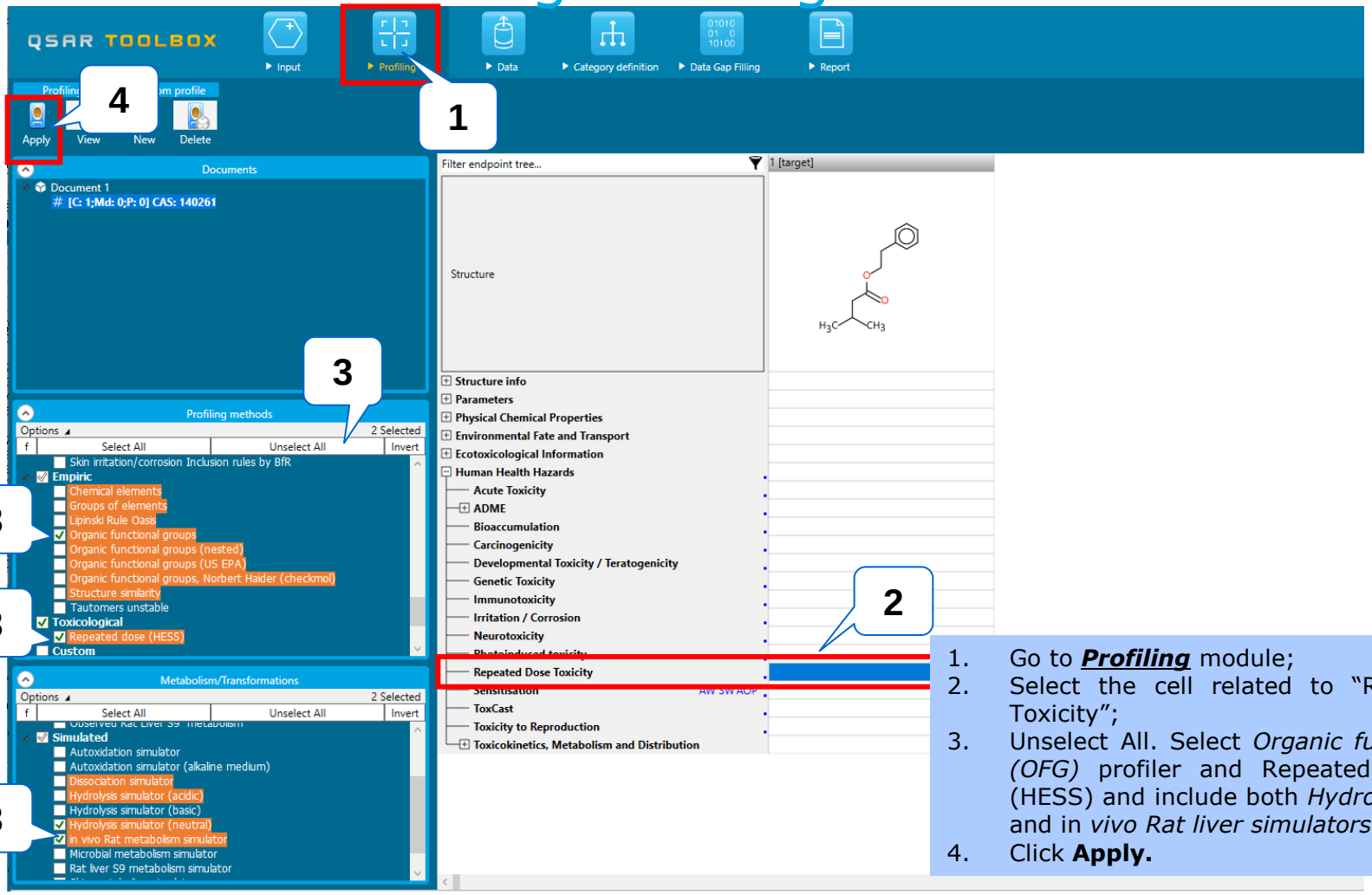
Profiling
Overview

- "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;
- "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 the cell related to "Repeated Dose Toxicity";
3. Unselect All. Select *Organic functional group (OFG)* profiler and Repeated dose toxicity (HESS) and include both *Hydrolysis (neutral)* and in *vivo Rat liver simulators*;
4. Click **Apply.**

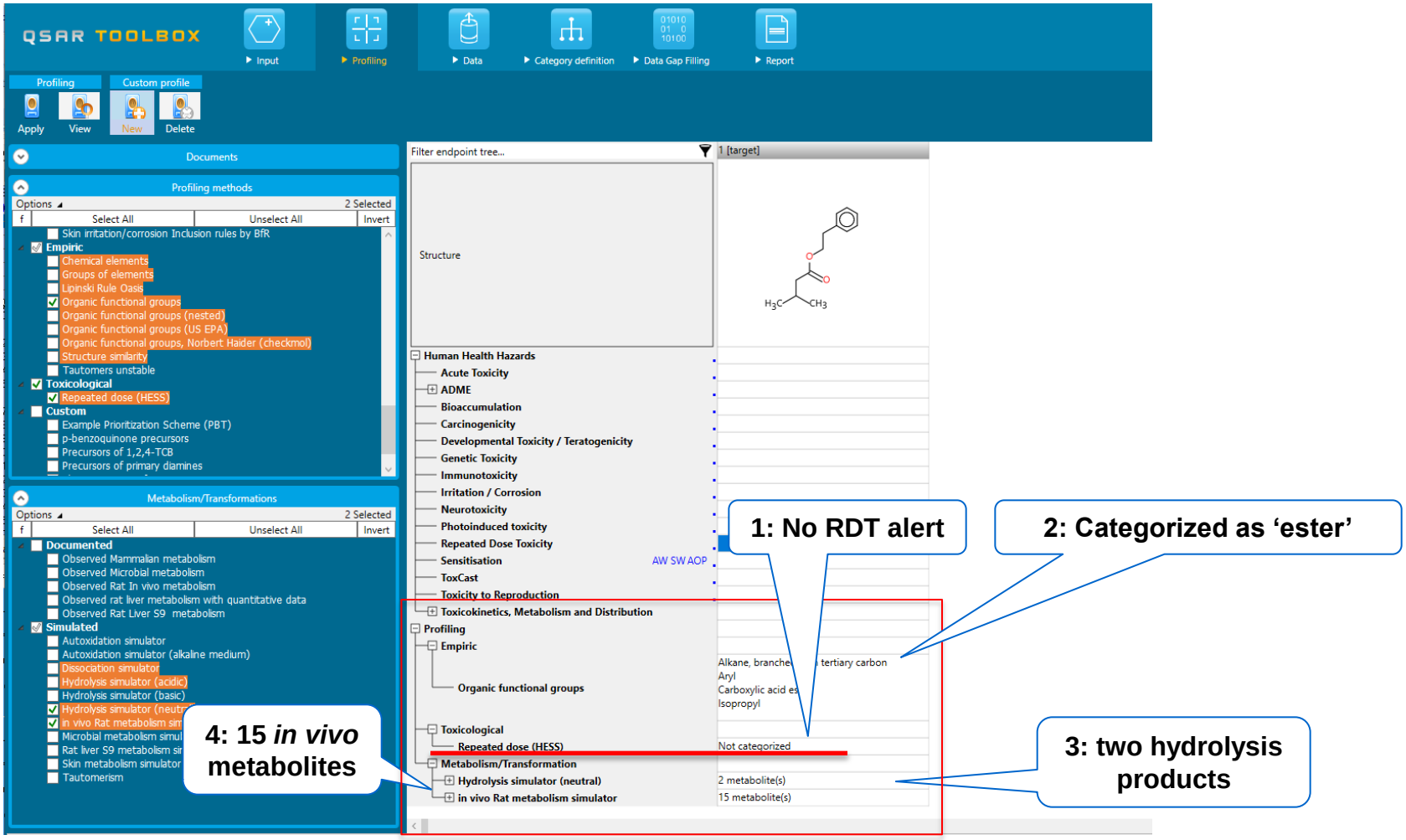
Profiling
Profiling results

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 OFG profiler;
3) 2 hydrolysing products* are obtained as a result of abiotic activation (*Hydrolysis simulator (neutral)*;
4) 15 metabolites* are produced as a result of biotic activation (*in vivo Rat metabolism simulator*);

See on the next slide

*Hydrolyzing products and in vivo rat liver metabolites are obtained as a result of simulation process

Profiling
Profiling results



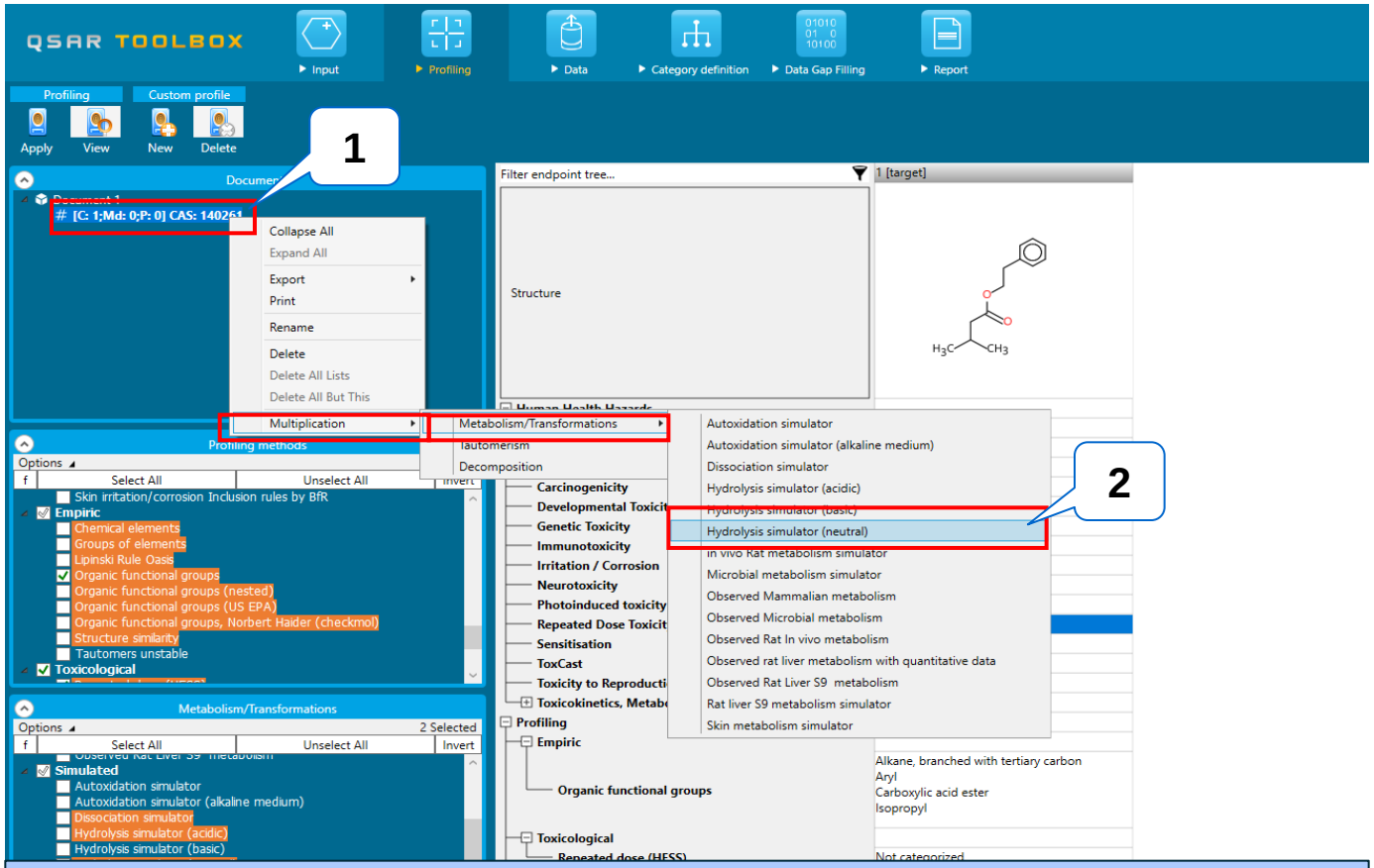
Recap

- In module *Input*, you have entered the target chemical. The target has a high relationship CAS-SMILES. So it is considered good quality.
- In the *Data* module, you checked 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, labelled as 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 15 *in vivo* rat liver metabolites are produced after applying (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 the 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 whether there are any data for the metabolites.

1. Flavouring Group Evaluation 4[1]: 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

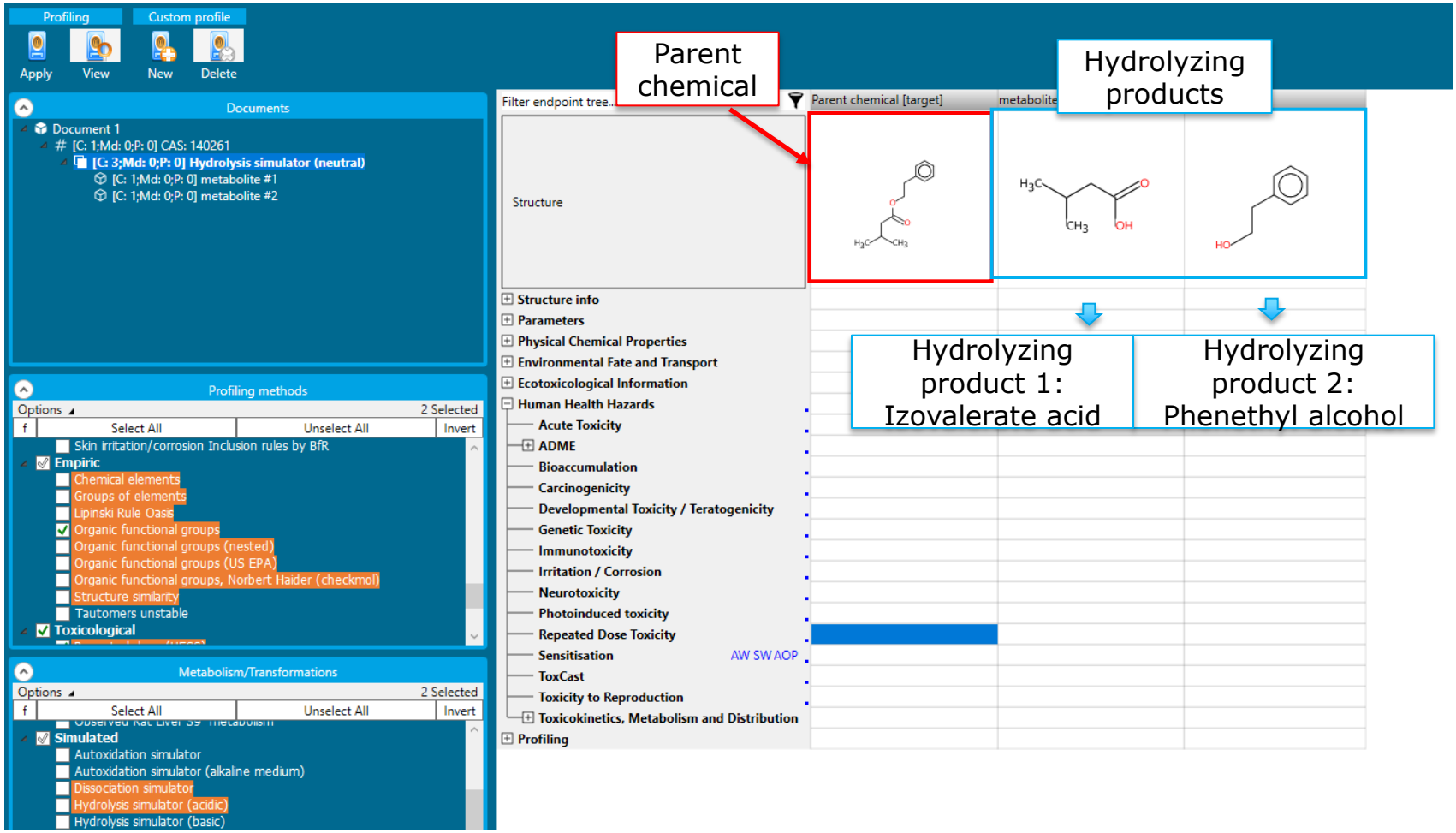
Before checking for data availability for the metabolites, they have to be produced upfront (see below).



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

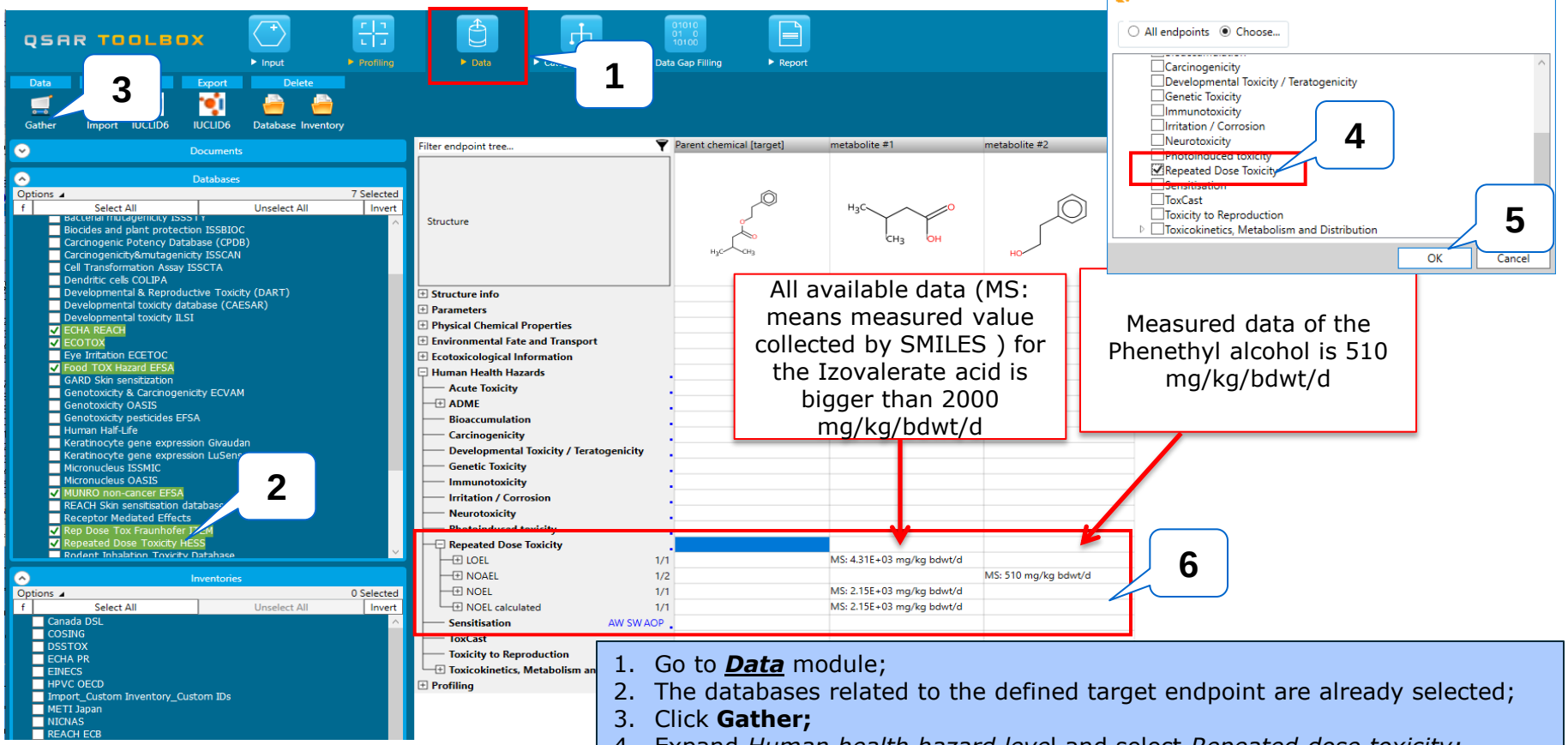
The products appeared next to the target (see next slide)

Multiplication of target chemical



Collect data for the metabolites

Check for data availability for the generated metabolites



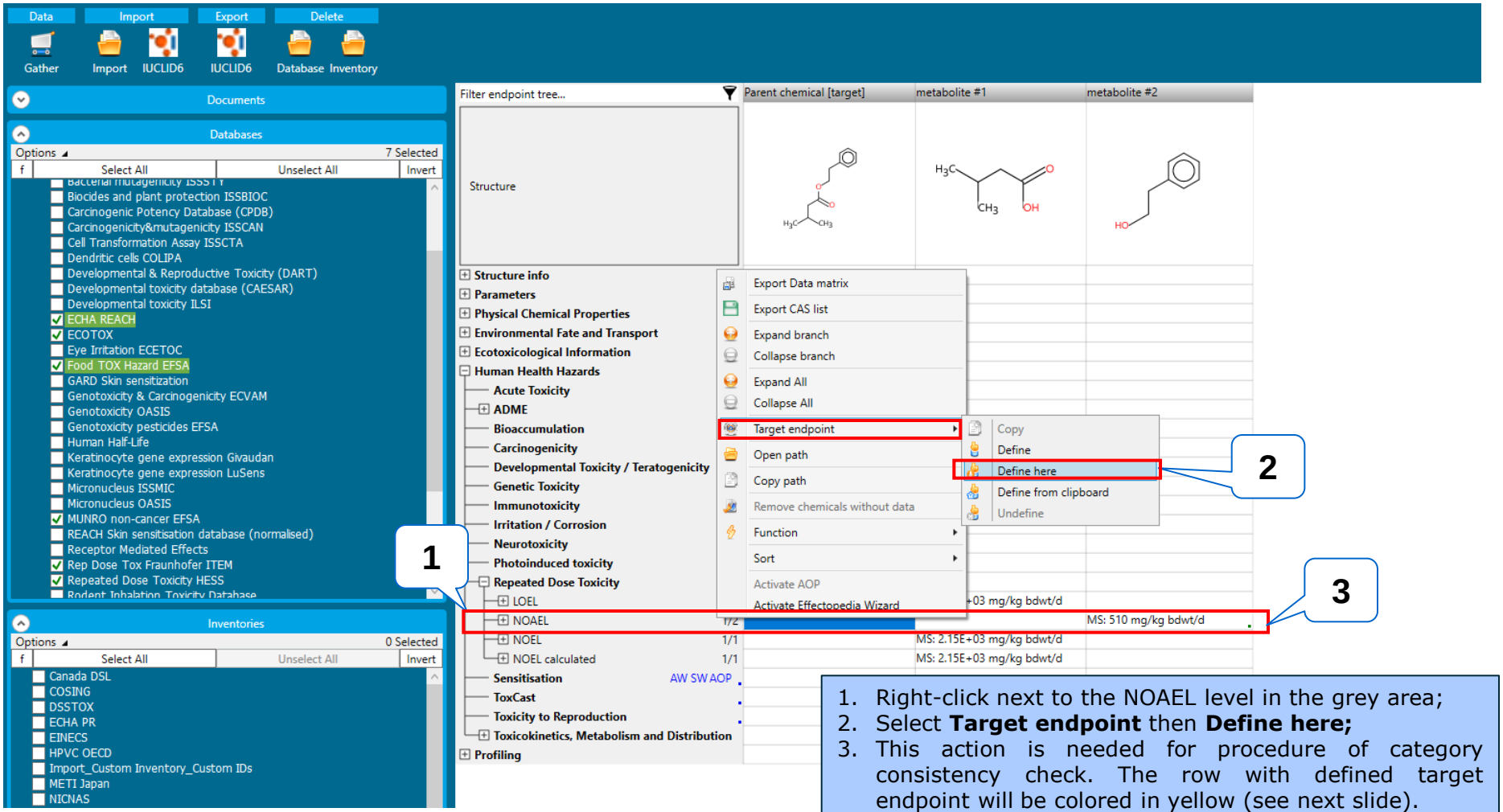
1. Go to ***Data*** module;
2. The databases related to the defined target endpoint are already selected;
3. Click **Gather;**
4. Expand *Human health hazard level* and select *Repeated dose toxicity;*
5. Click **OK**;
6. The data for the parent and metabolites appears on data matrix, expand Repeated 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, the 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 of a result of metabolite *Phenethyl alcohol.*
- The forthcoming slides are focused on applying the worst-case scenario. The latter is possible by assigning the experimental data of the *Phenethyl alcohol* (assumed to be an analogue) to the target chemical using *Transfer to target* functionality.
- For the purpose of the read-across it is needed to define the target endpoint (e.g. RDT) by using the functionality of the TB (this is needed for the category consistency check) and finally before generating a report, the category will be checked for category consistency.

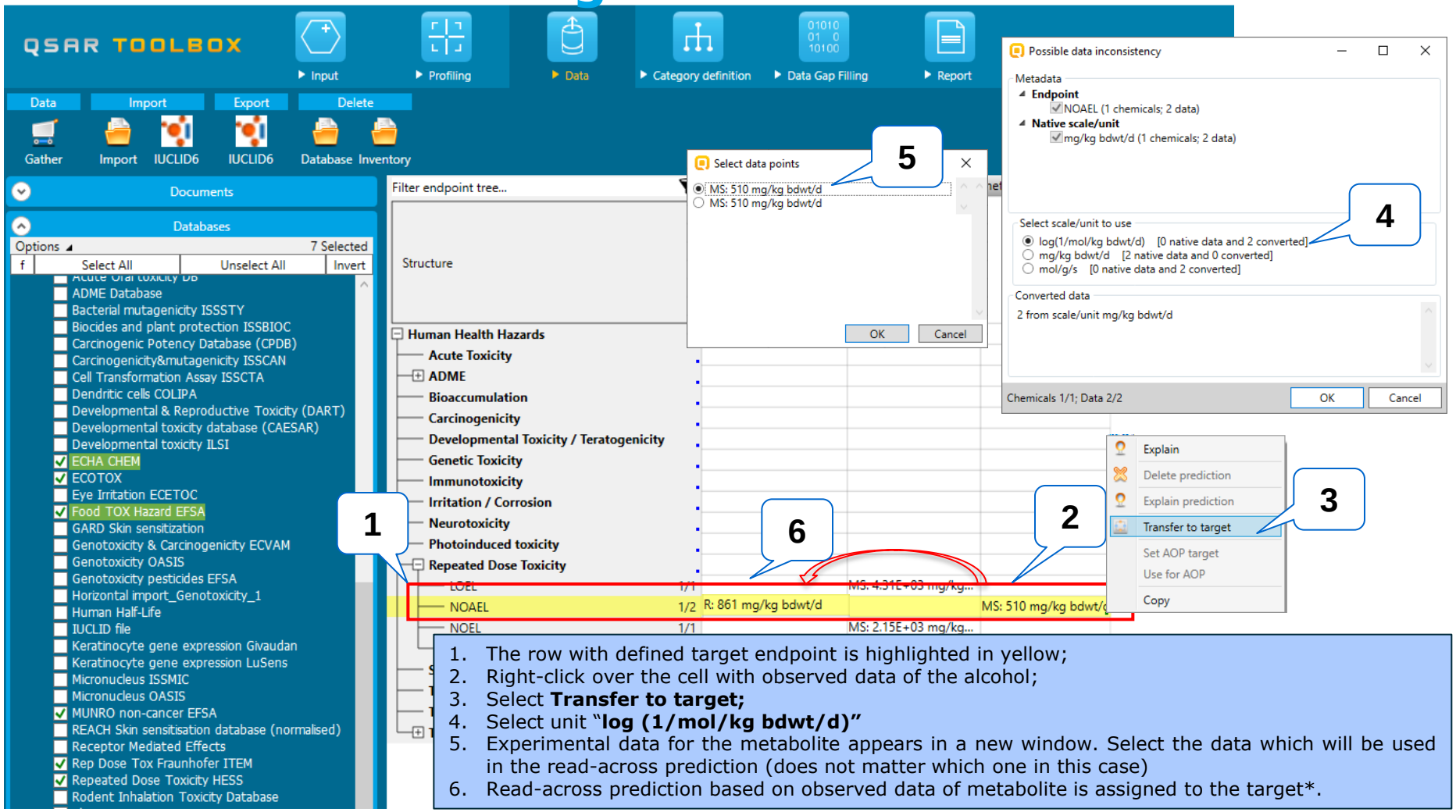
1. GHS Classification. Fourth edition
2. RIFM, 2012. RIFM (Research Institute for Fragrance Materials, Inc), 2012. A Toxicological and Dermatological Assessment of Aryl Alkyl Alcohol Simple Acid Ester Derivatives when Used as Fragrance Ingredients. RIFM report number 65259 (RIFM, Woodcliff Lake, NJ,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

Define target endpoint



1. Right-click next to the NOAEL level in the grey area;
2. Select **Target endpoint** then **Define here;**
3. This action is needed for procedure of category consistency check. The row with defined target endpoint will be colored in yellow (see next slide).

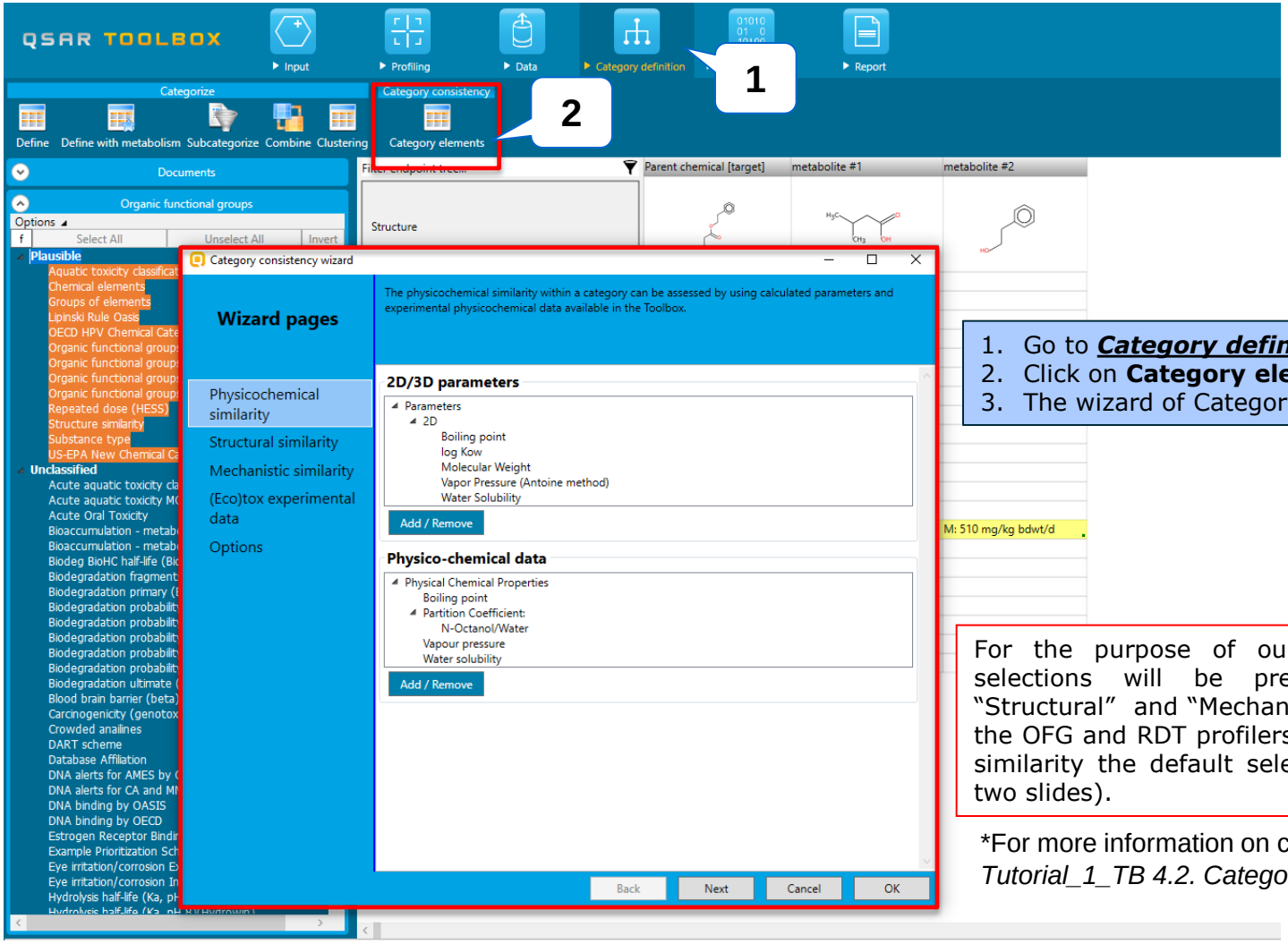
Transfering of observed data of metabolite to the target chemical



1. The row with defined target endpoint is highlighted in yellow;
2. Right-click over the cell with observed data of the alcohol;
3. Select **Transfer to target;**
4. Select unit "**log (1/mol/kg bdwt/d)"**
5. Experimental data for the metabolite appears in a new window. Select the data which will be used in the read-across prediction (does not matter which one in this case)
6. Read-across prediction based on observed data of metabolite is assigned 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



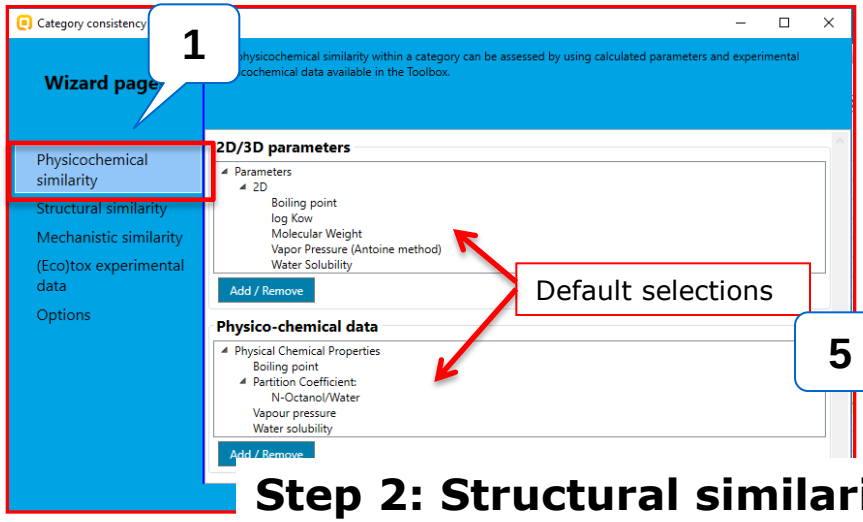
1. Go to ***Category definition*** module;
2. Click on **Category elements**;
3. The wizard of Category consistency appeared*.

For the purpose of our example not all default selections will be preserved. For instance in "Structural" and "Mechanistic similarity" sections only the OFG and RDT profilers will remain. For phys-chem similarity the default selections were kept (see next two slides).

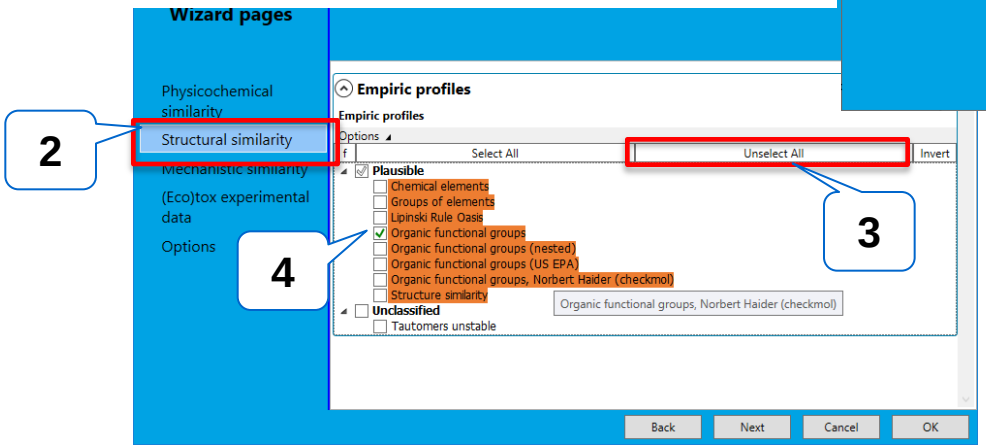
*For more information on category elements see *Tutorial_1_TB 4.2. Category consistency*

Category consistency check

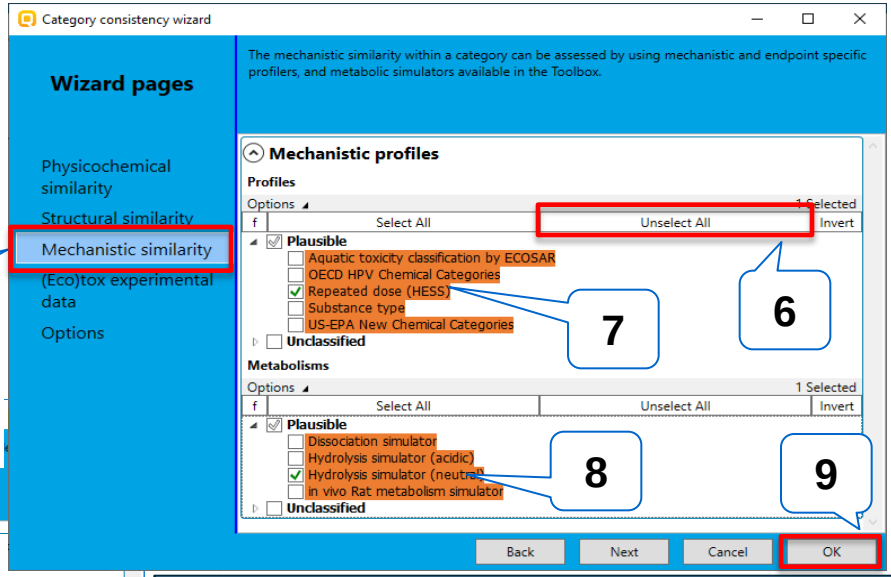
Step 1: Physicochemical similarity



Step 2: Structural similarity



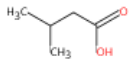
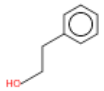
Step 3: Mechanistic similarity



1. Select **Physicochemical similarity**. Keep the default selections;
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)** only;
9. Click **OK.** The profiling results/data/parameters will appeared on datamatrix (see next slides).

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

Category consistency check

Filter endpoint tree...		Parent chemical [target]	metabolite #1	metabolite #2
Structure				
Structure info				
Parameters				
2D				
Boiling point		276 °C	175 °C	225 °C
log Kow		3.97	1.49	1.57
Molecular Weight		206 Da	102 Da	122 Da
Vapor Pressure (Antoine method)		0.00653 mm Hg	1.25 mm Hg	0.0263 mm Hg
Water Solubility		16.5 mg/L	2.92E+04 mg/L	2.2E+04 mg/L
Physical Chemical Properties				
Boiling point	3/11	M: 263 °C	MS: 177 °C	MS: >98÷<100 °C
Partition Coefficient:	2/9		MS: 1.16	MS: 0.8
Vapour pressure	2/6		MS: 0.44 mm Hg	MS: ca.0.0707 Torr
Water solubility	2/5		MS: 4.07E+04 mg/L	MS: 1.75E+04 mg/L
Human Health Hazards				
Repeated Dose Toxicity				
NOAEL	2/3	R: 861 mg/kg bdwt/d		MS: 510 mg/kg bdwt/d
Profiling				
Empiric				
Organic functional groups		Alkane, branched with... Aryl	Alkane, branched with... Carboxylic acid	Alcohol Aryl
Toxicological				
Repeated dose (HESS)		Not categorized	Carboxylic acids (Hep... Glycolic acid (Renal To...	Styrene (Renal Toxicity)... Toluene (Renal toxicity...
Metabolism/Transformation				
Hydrolysis simulator (neutral)		2 metabolite(s)	0 metabolite(s)	0 metabolite(s)

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 the "Reporting" and including RAAF scenario

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

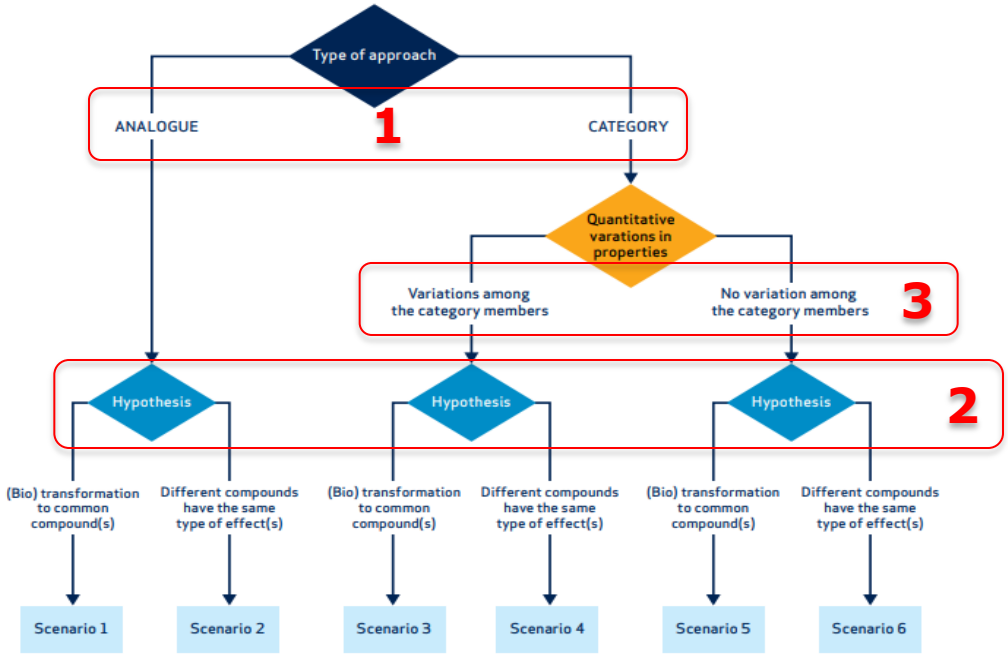
Report
Overview

- Report module allows generating a report for any of the predictions performed within the Toolbox.
- The report module contains 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 RAAF scenario

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

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



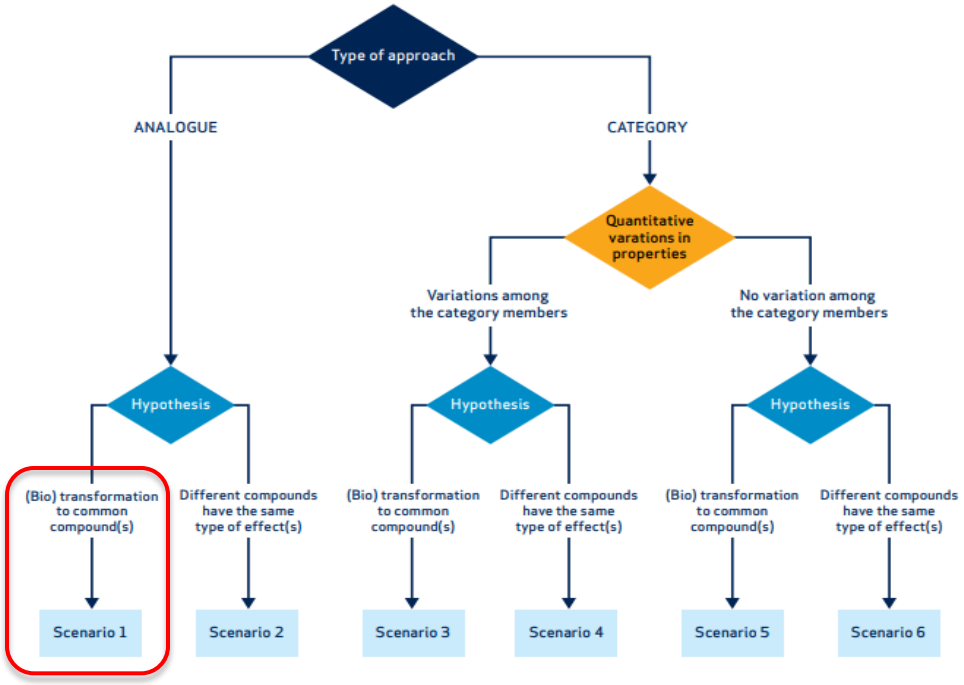
*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Report
Selection of 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

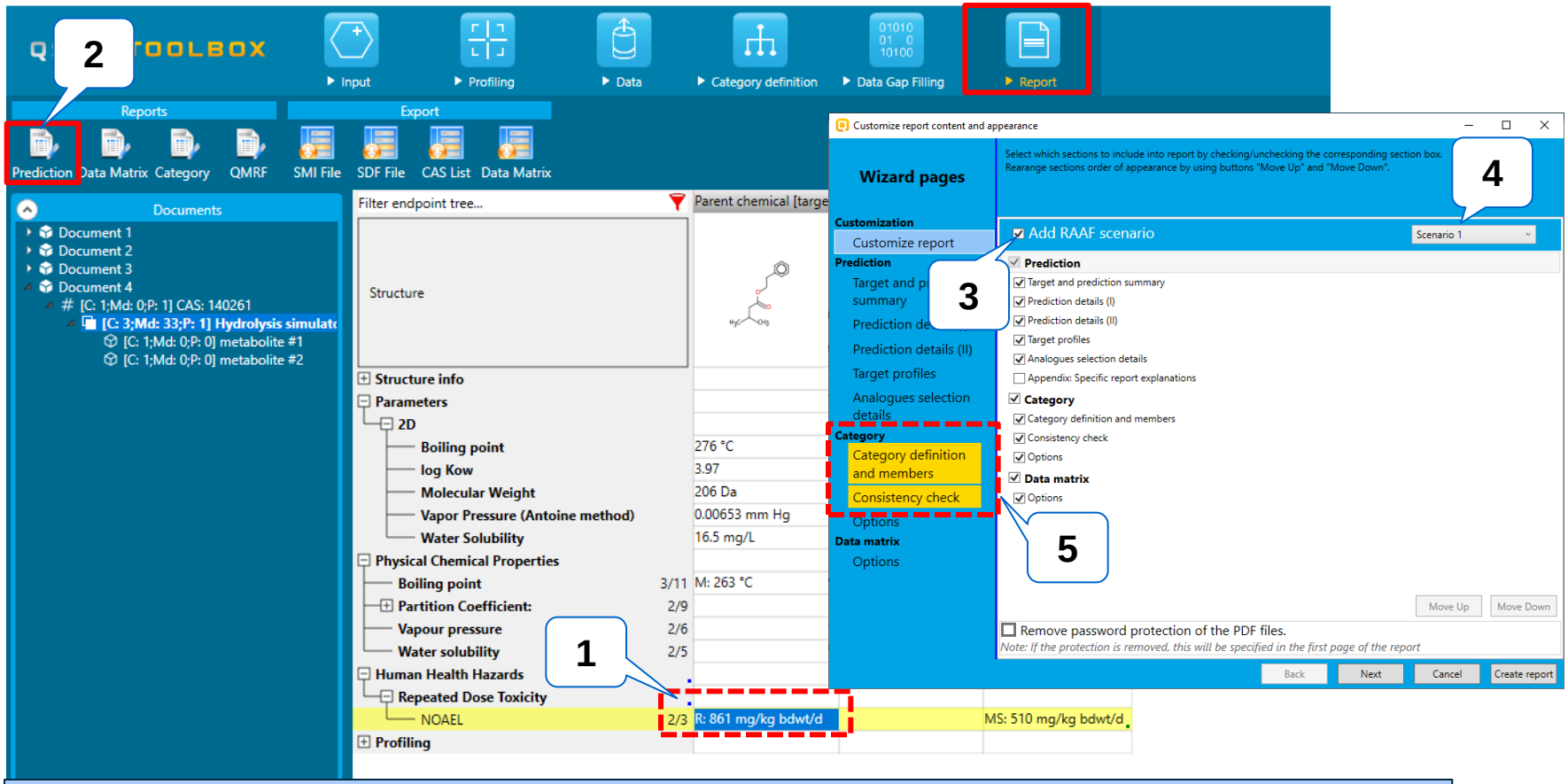
- Scenario 1 covers the analogue approach for which the read-across hypothesis is based on (bio) transformation to a common compound
- For the REACH information requirement under consideration, the property investigated in a study conducted with one source substance is used to predict properties that 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 (or so called analogue in our case) 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

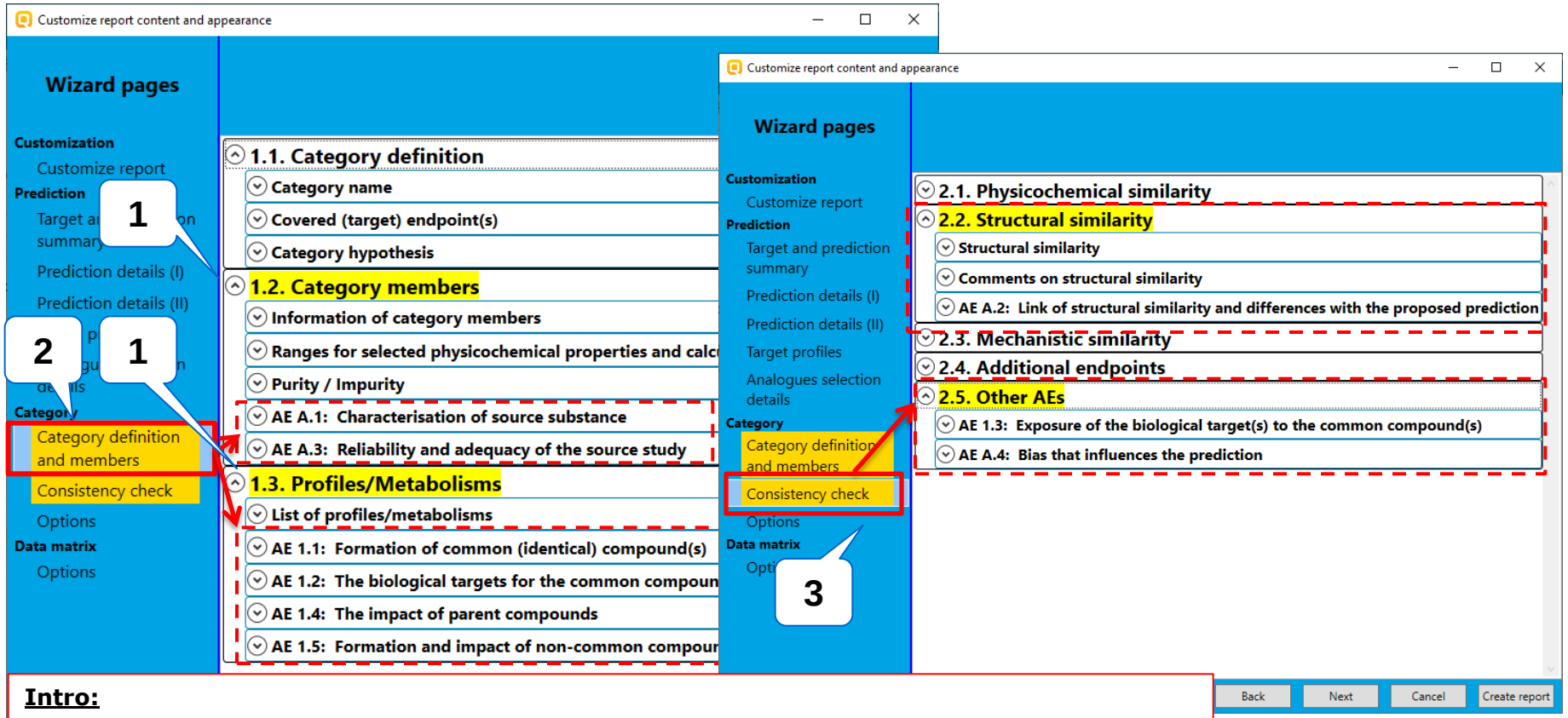
Report generation according to RAAF-Scenario 1



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;
5. Sections of the report including assessment elements specific to the scenario 1 are highlighted in yellow.

Report

Report generation according to RAAF-Scenario 1

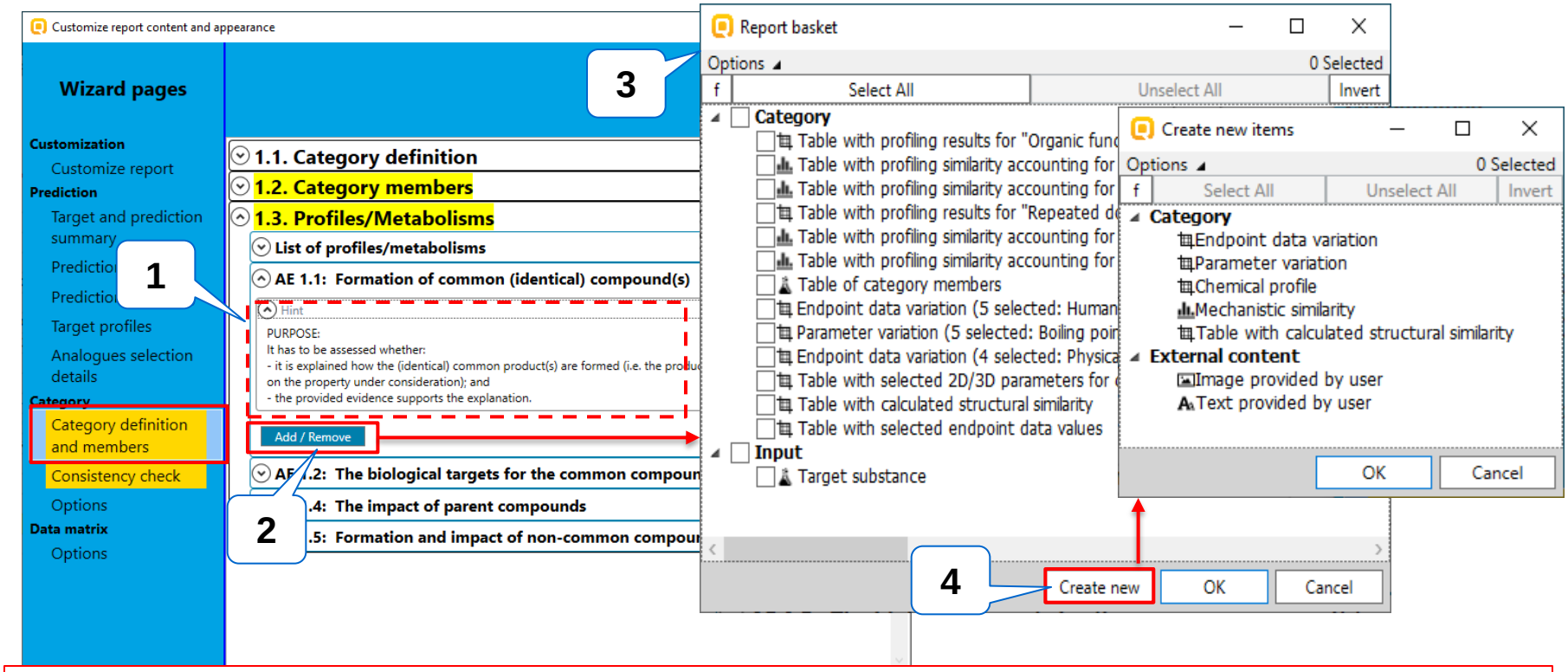


Intro:

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

Each of the AEs will be explained further in the next few slides.

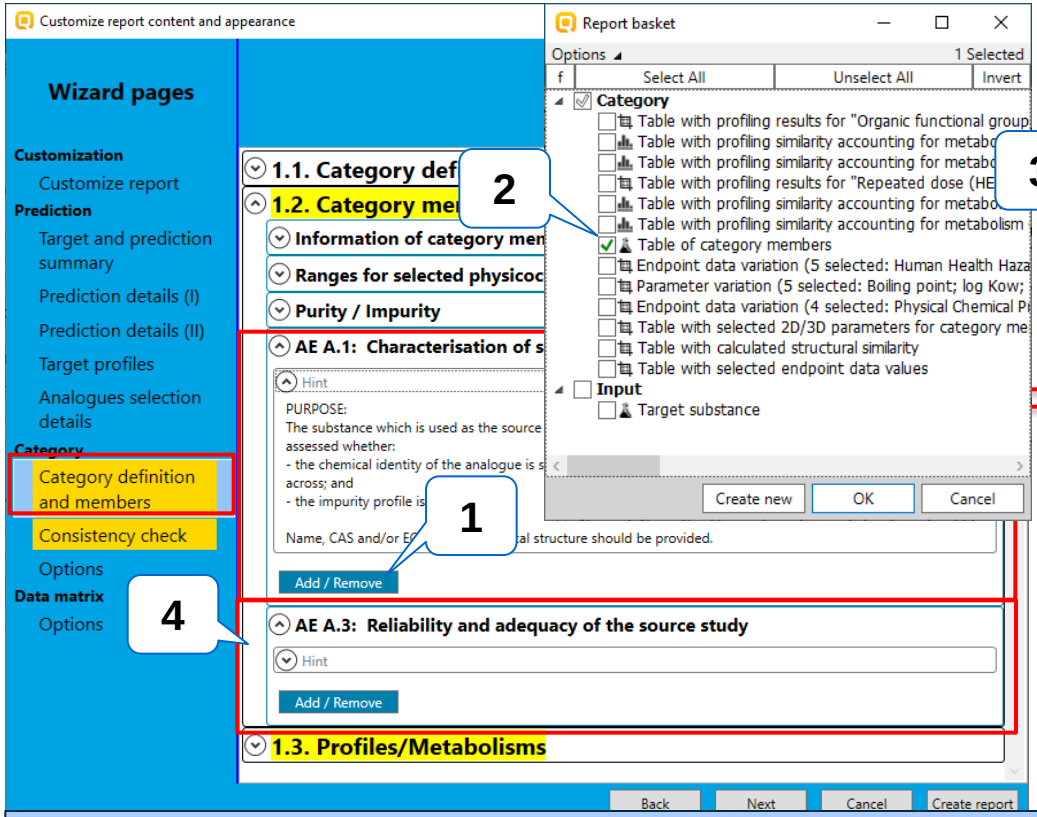
Report
Assessment elements of Scenario 1

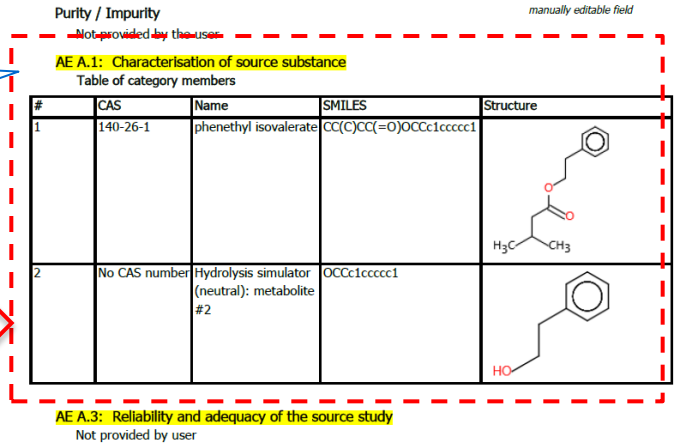


Intro:

Hint for each of the assessment elements is available (1). Information can be added to each AE 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).

Report
Assessment elements of Scenario 1

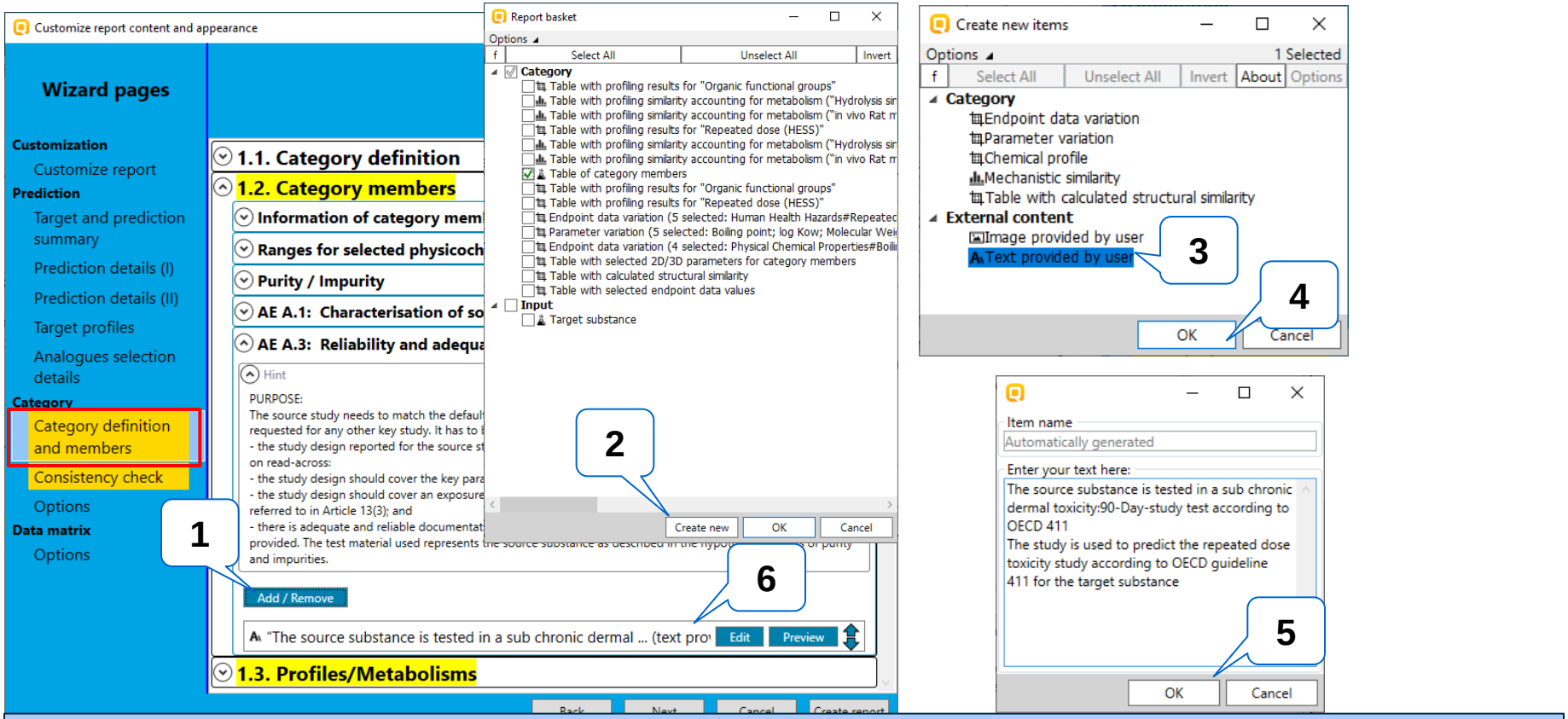




Two AEs (AE *A.1* and *A.3*) related to Scenario 1 are included in the section *Category definition and members*, sub-section *1.2. Category members (no AEs are available in sub-section 1.1. category definition):*

- **AE A.1 Characterization of source substance.** The user should manually open the report basket (1) and select the item: **Table of category members (2).** If impurities/additives of the used analogues are available, they will appear under the ***AE A.1*** in *Purity / Impurity*. 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 next slide)*

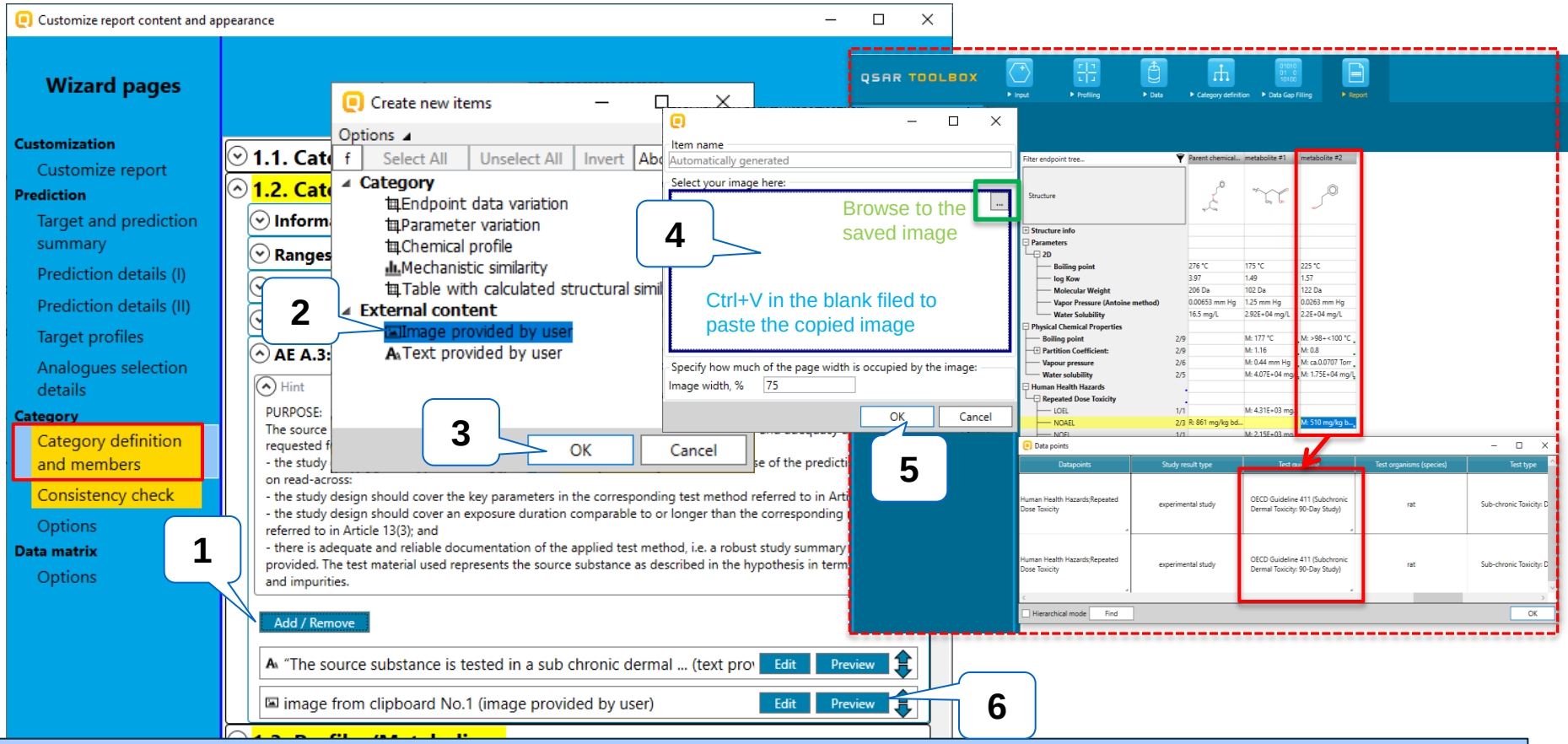
Report
Assessment elements of Scenario 1



AE A.3: Click the **Add/Remove** button (1) and click **"Create new"** item **(2).** From the appeared window called "Report basket" click on item **"Text provided by user"** (3). Click **OK** (4). In the text field paste the following example text and click **OK** (5):
"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"

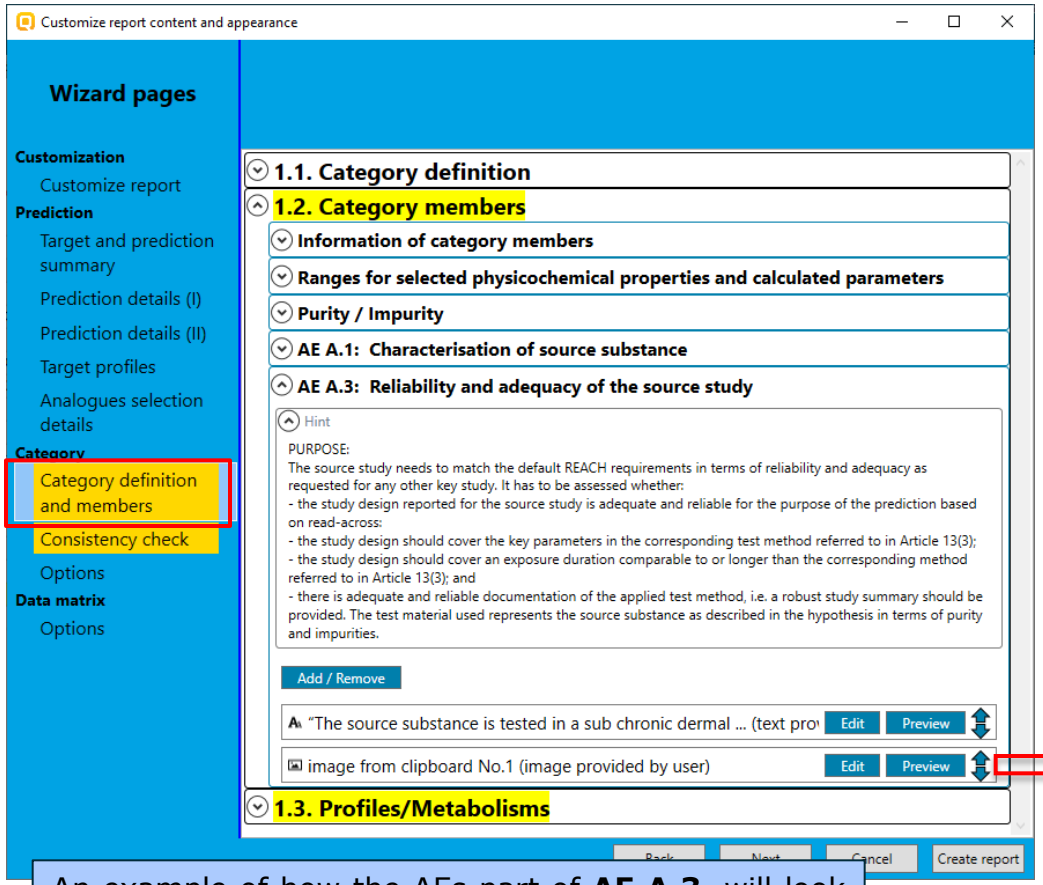
The newly created item appears below the "Add/Remove" button (6).

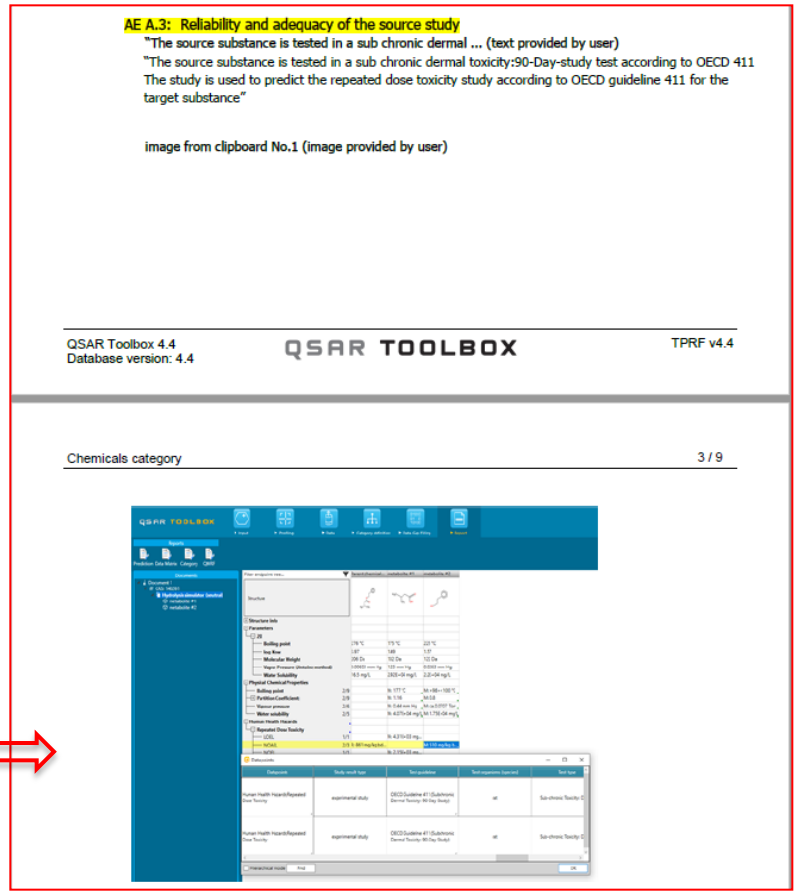
Report
Assessment elements of Scenario 1



AE A.3: Additionally a snapshot with metadata (on the right part of the screen) showing the data of the alcohol prepared by for 90-days under OECD Guideline 411 could be added in order to confirm the consistency regarding the assay. For this purpose click again on **Add/Remove** button (1). From the appeared report basket click on **"Create New"** (for more details see previous slide). Select item **"Image provided by user"** by click on it (2). Click **OK (3).** In the appeared window browse or paste the copied/saved image (4). Finally click OK (5). Already added image could be previewed by "Preview" button (6).

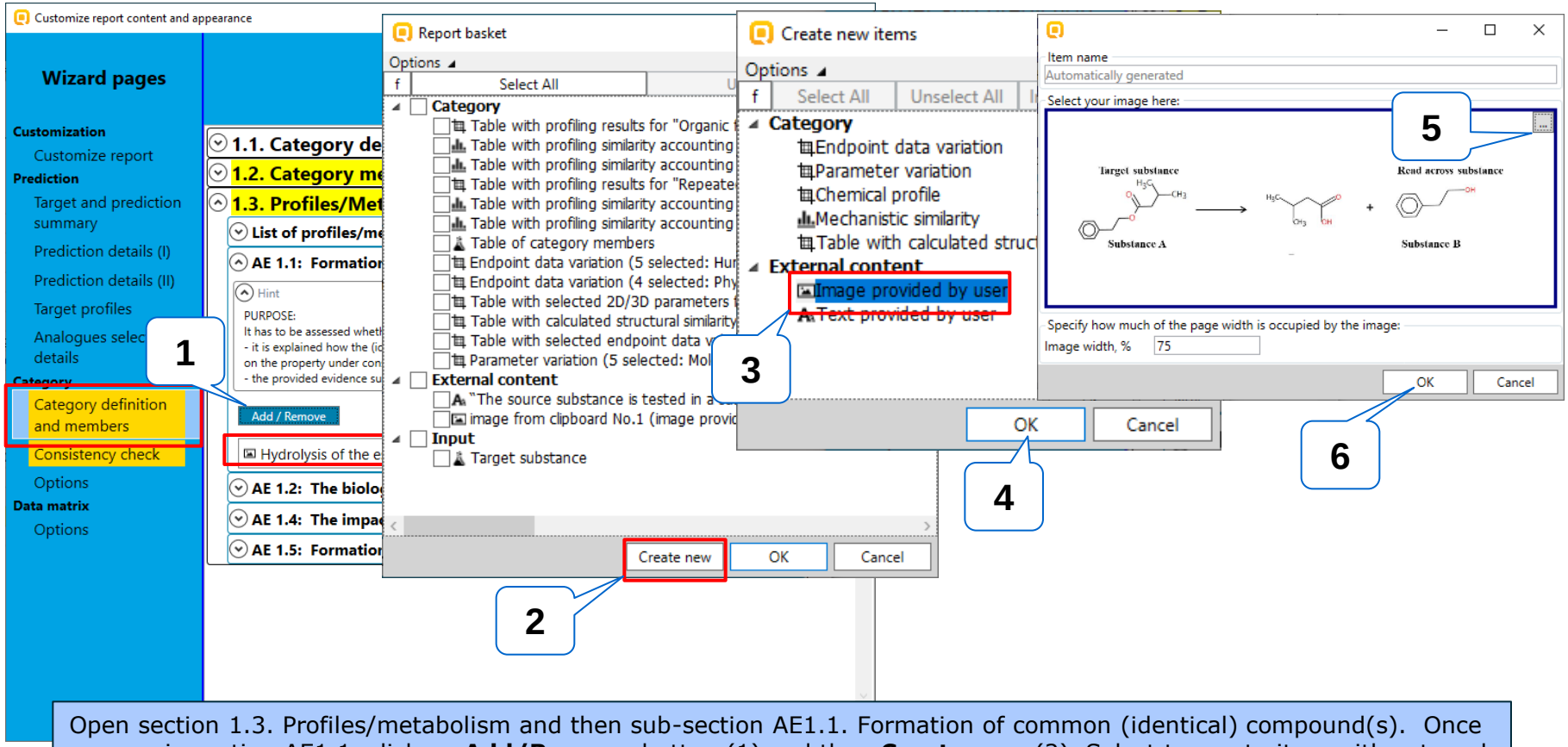
Report
Assessment elements of Scenario 1





An example of how the AEs part of **AE A.3**. will look in the generated report is shown on the right. We finished with section "1.2. Category members". Continues with section "1.3. Profiles/Metabolism"

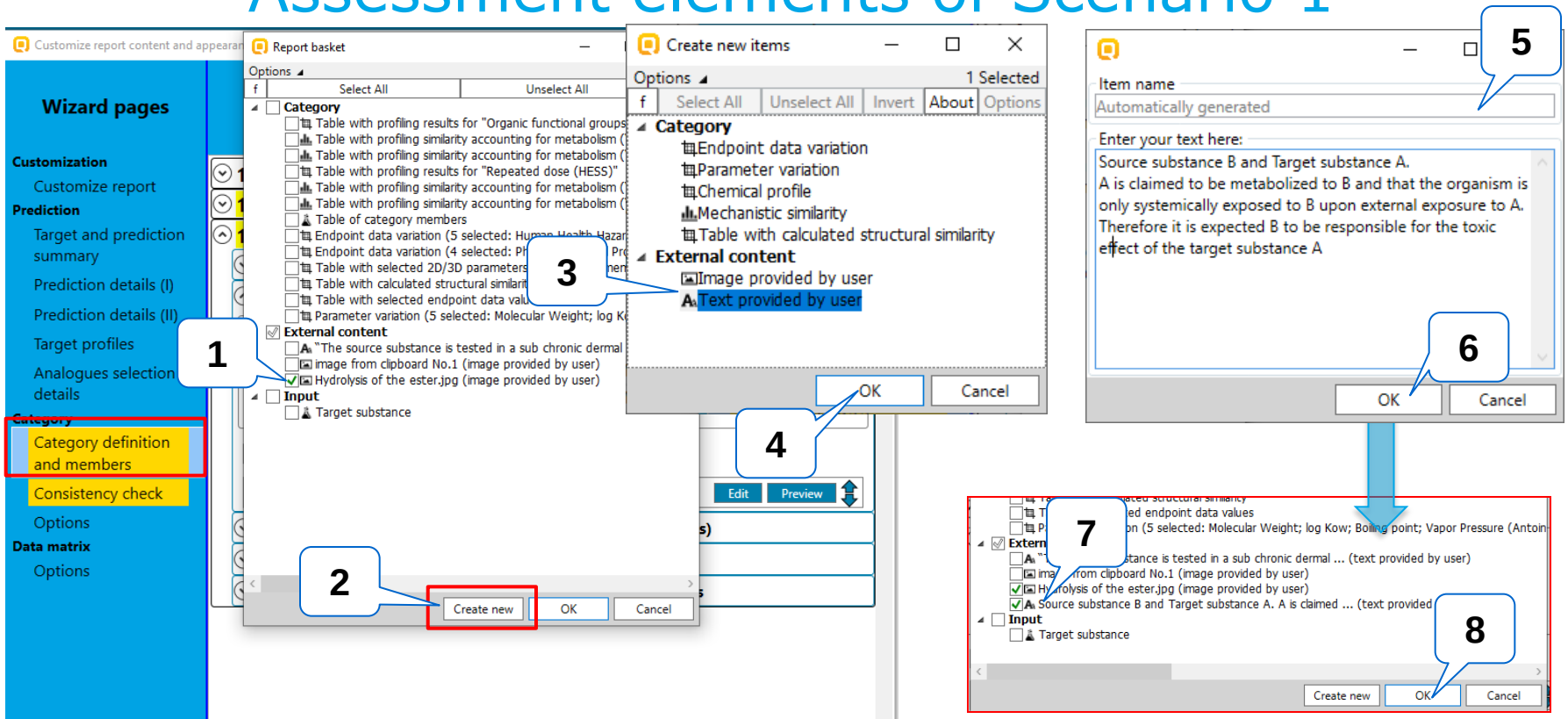
Report
Assessment elements of Scenario 1



Open section 1.3. Profiles/metabolism and then sub-section AE1.1. Formation of common (identical) compound(s). Once you are in section AE1.1, click on **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

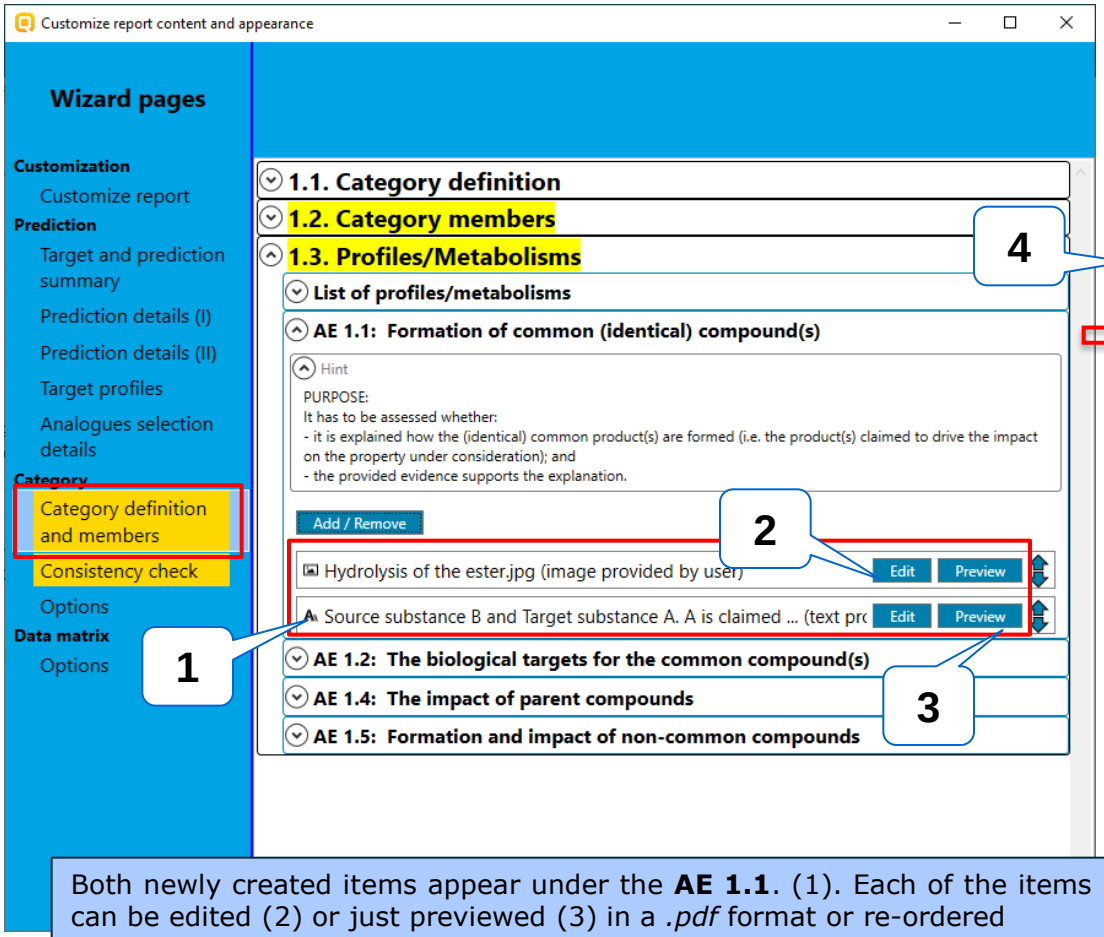


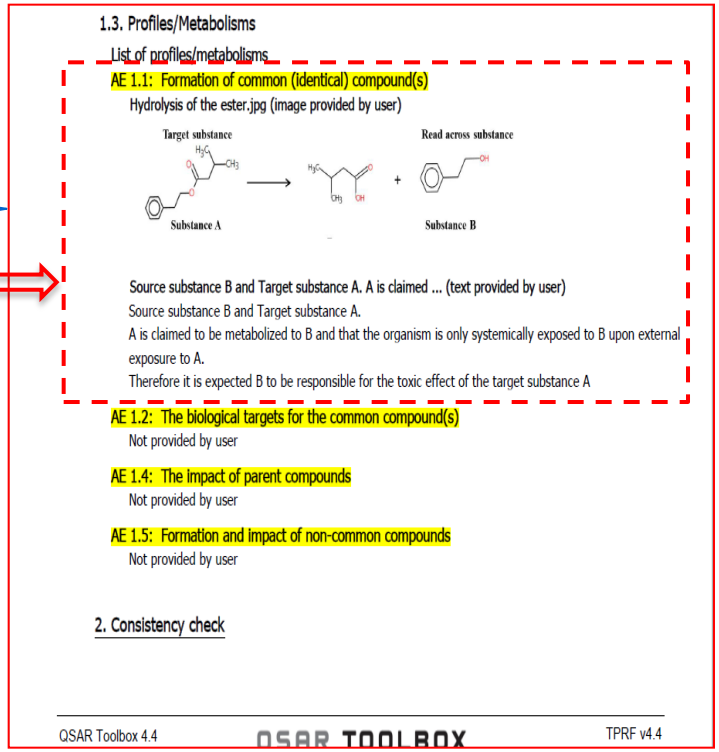
The newly created item appears in the ***Report basket*** (1). Now text could be also included. Click **Create new** (2), select ***Text provided by user*** (3) and click **OK** (4). Copy the following example text:

- *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)**

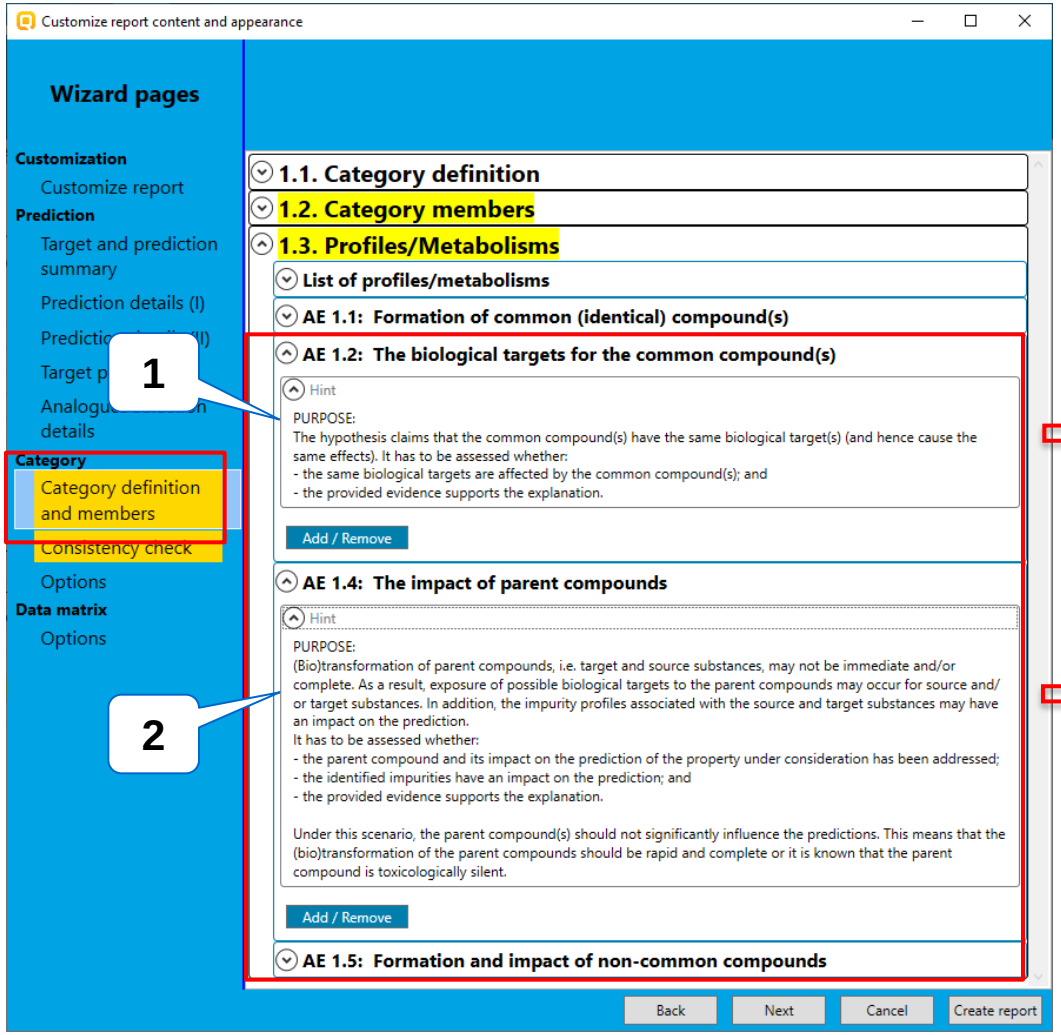
Report
Assessment elements of Scenario 1





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 or re-ordered
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.*

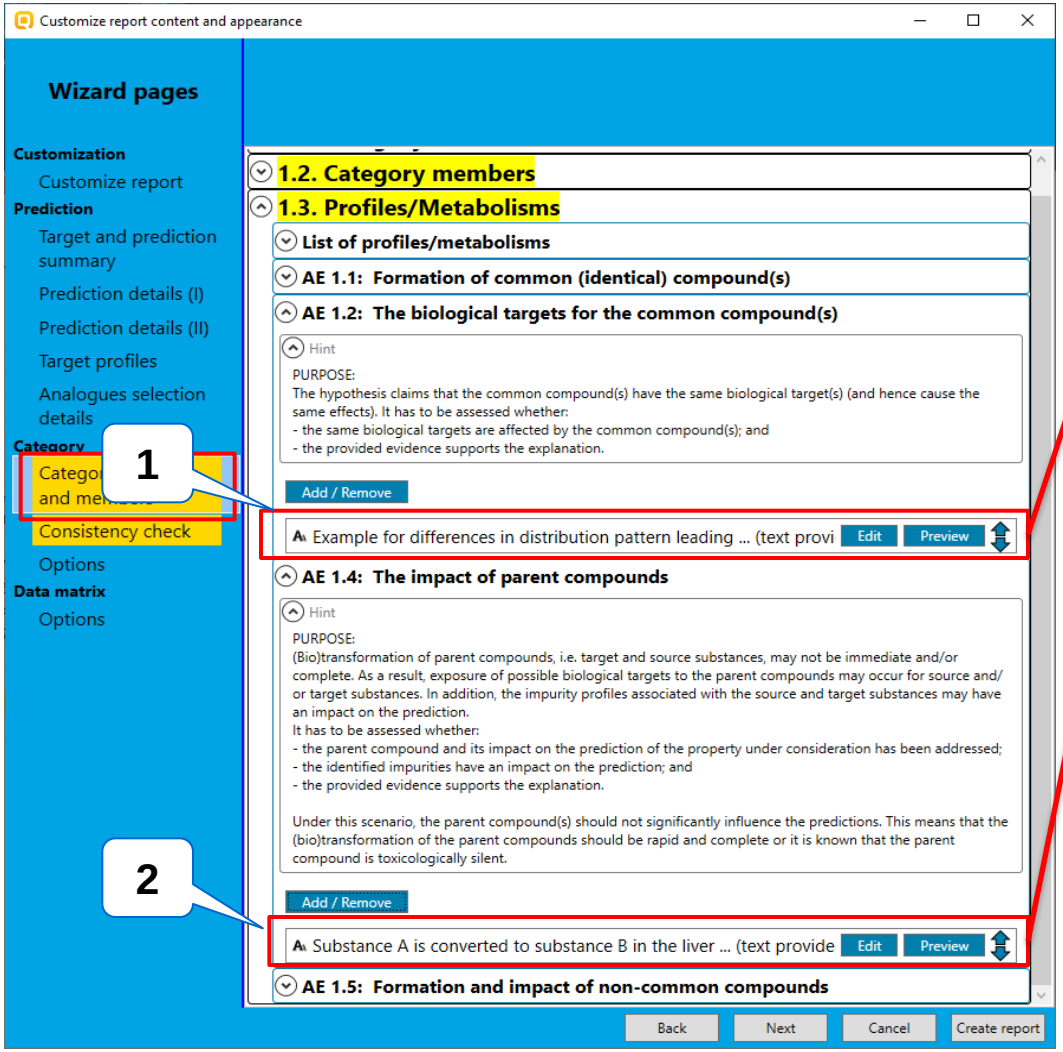
An example text for **AE1.4. The impact of parent compounds (2):**

- *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*

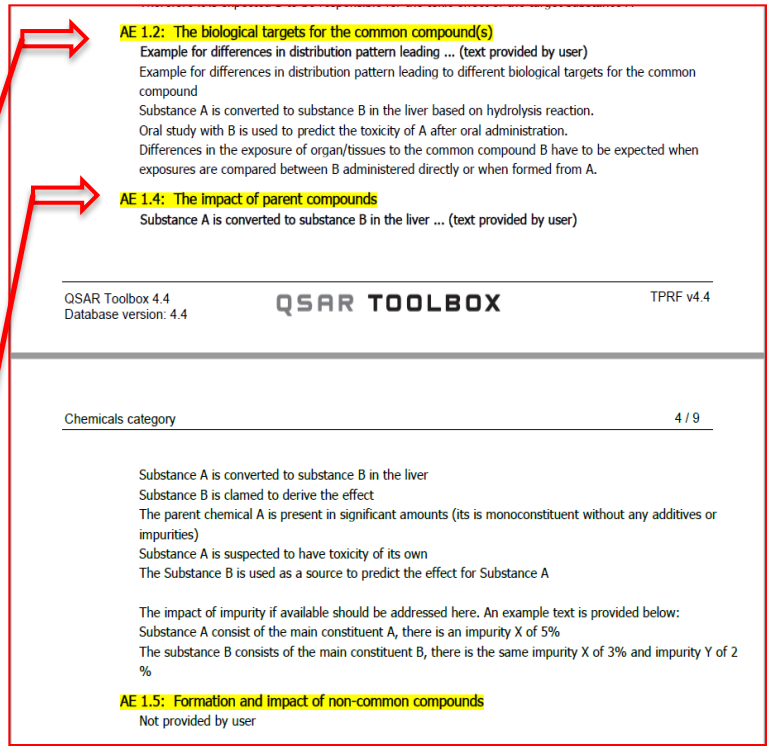
The impact of impurity if available should be addressed here. An example text is provided below:

- *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.



AE 1.2: The biological targets for the common compound(s)

Example for differences in distribution pattern leading ... (text provided by user)

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.

AE 1.4: The impact of parent compounds

Substance A is converted to substance B in the liver ... (text provided by user)

QSAR Toolbox 4.4
Database version: 4.4

QSAR TOOLBOX

TPRF v4.4

Chemicals category 4 / 9

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%

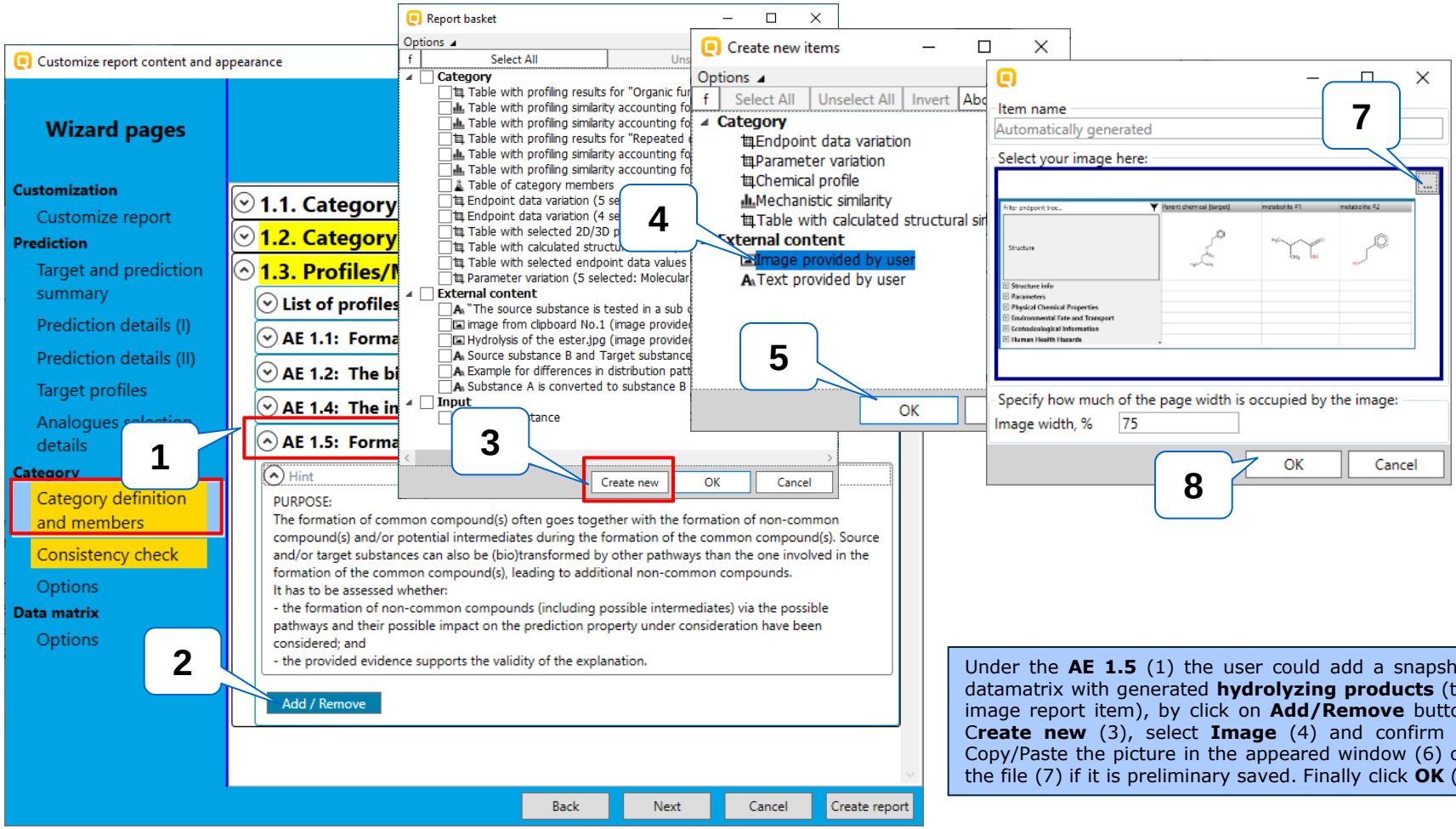
The substance B consists of the main constituent B, there is the same impurity X of 3% and impurity Y of 2 %

AE 1.5: Formation and impact of non-common compounds

Not provided by user

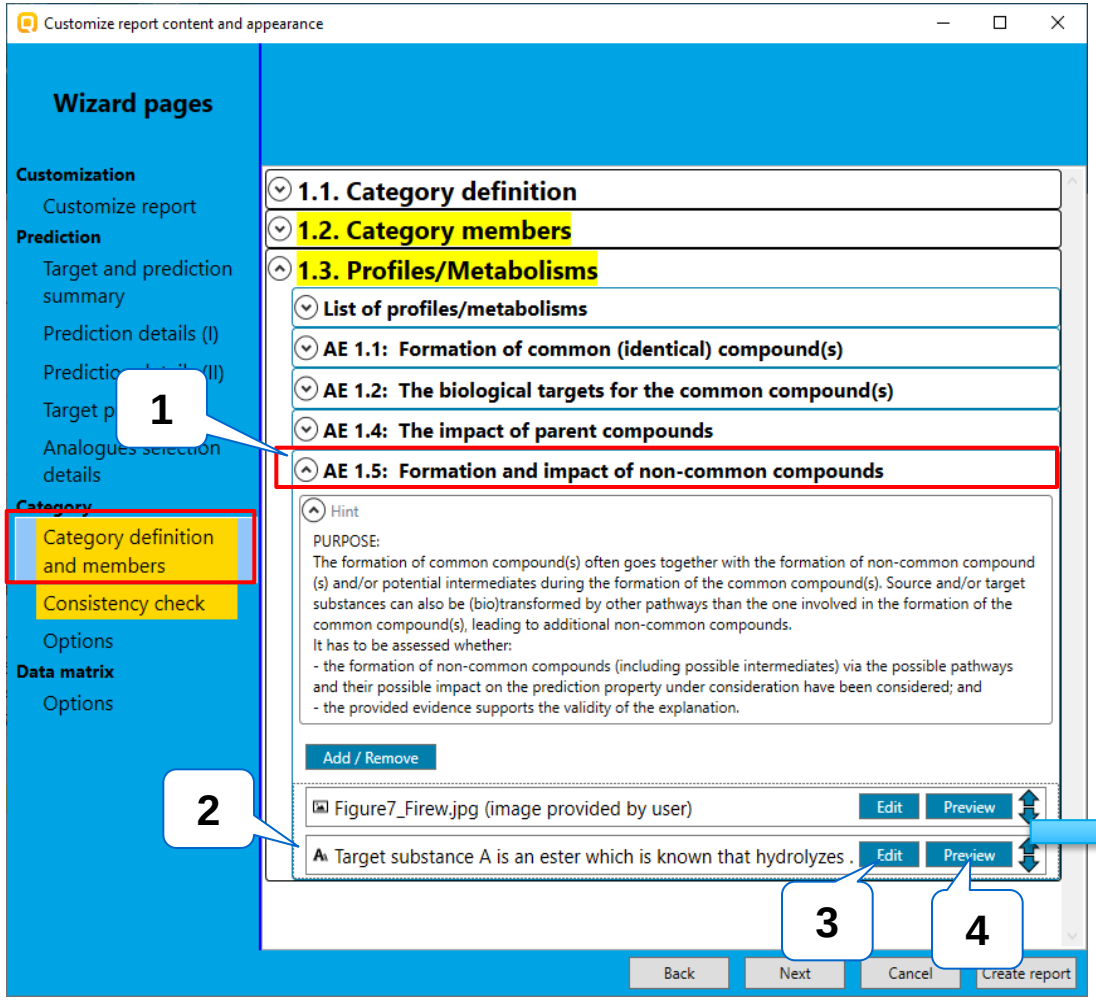
Report

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).

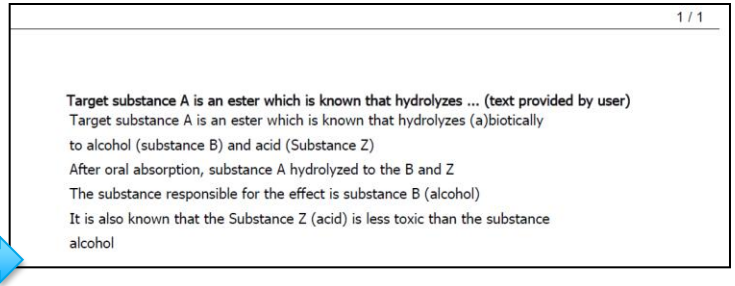
Report
Assessment elements of Scenario 1



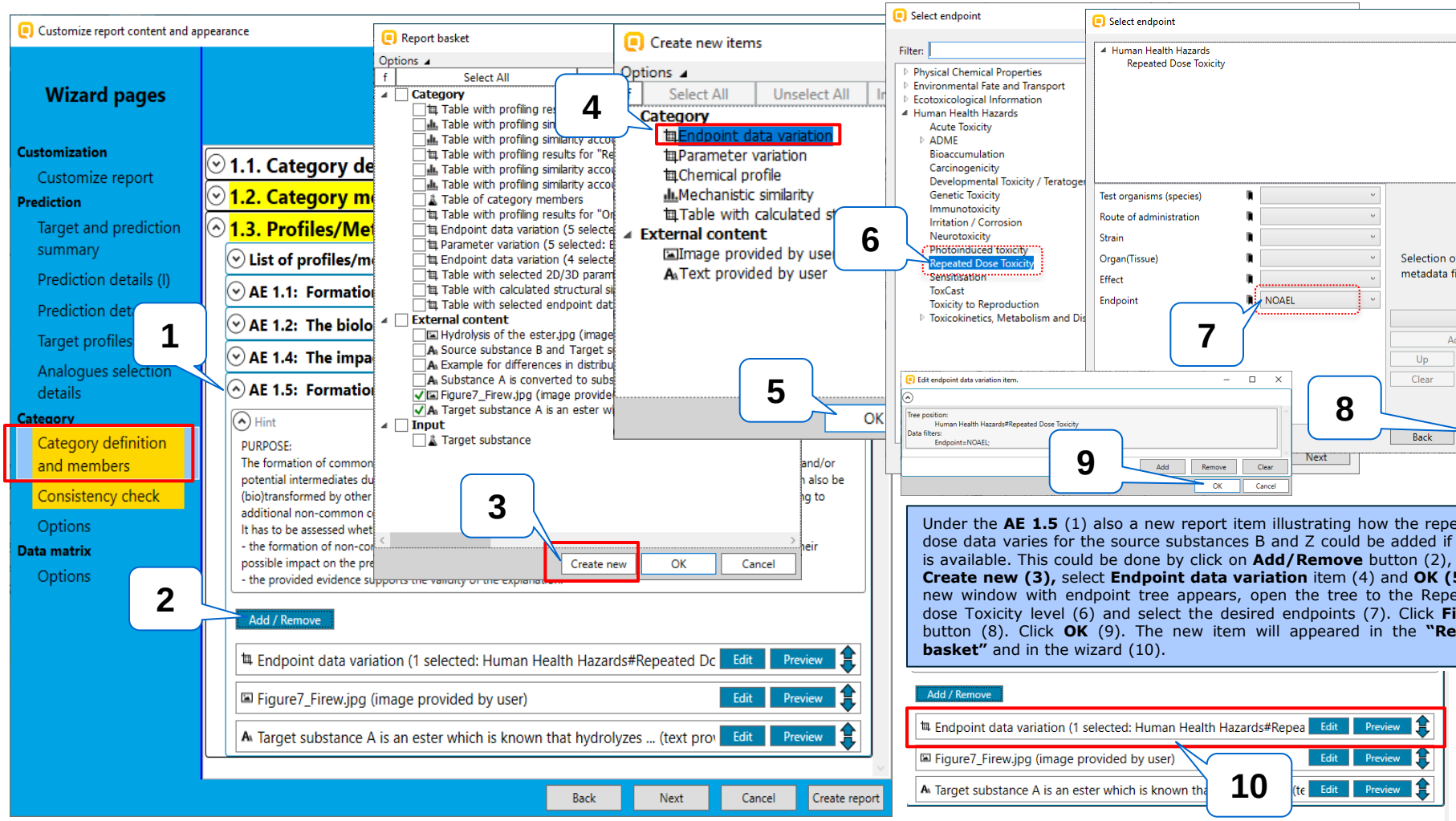
Along with the image a text could be added under **AE 1.5** (1) with the following content. An example text for **AE 1.5. Formation and impact of non-common compounds: manually editable** (copy the text and paste it in the text box, steps are already shown on slide 45)**:**

- *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.

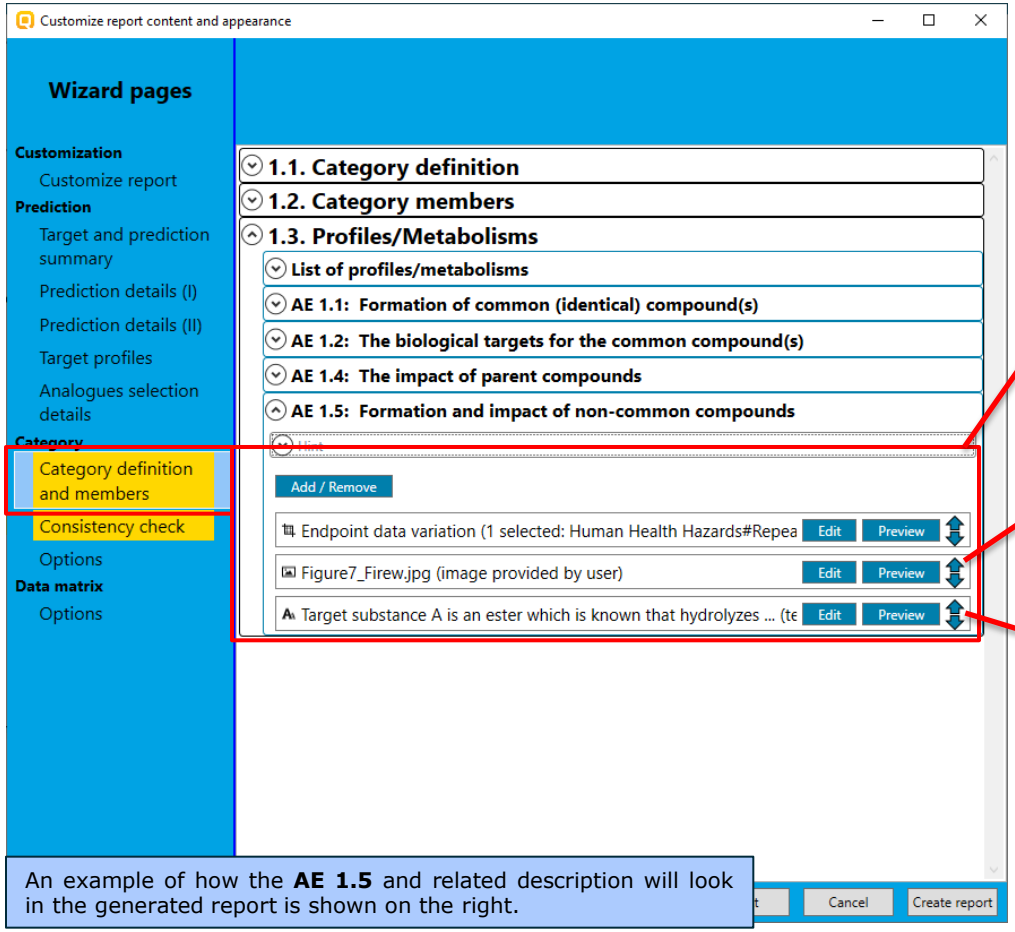


Report
Assessment elements of Scenario 1



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 available. 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 (7). Click **Finish** button (8). Click **OK** (9). The new item will appeared in the **"Report basket"** and in the wizard (10).

Report
Assessment elements of Scenario 1



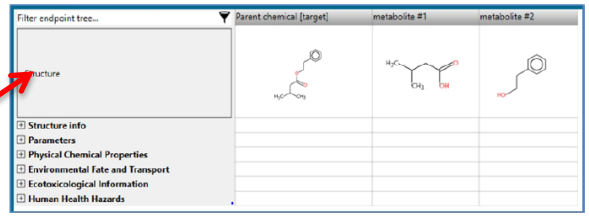
An example of how the **AE 1.5** and related description will look in the generated report is shown on the right.

AE 1.5: Formation and impact of non-common compounds

Table with Human Health Hazards data variation

Position	Variation	unit (family)	Number of chemicals
No data			

Figure7_Firew.jpg (image provided by user)



Target substance A is an ester which is known that hydrolyzes ... (text provided by user)

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

Report
Assessment elements of Scenario 1

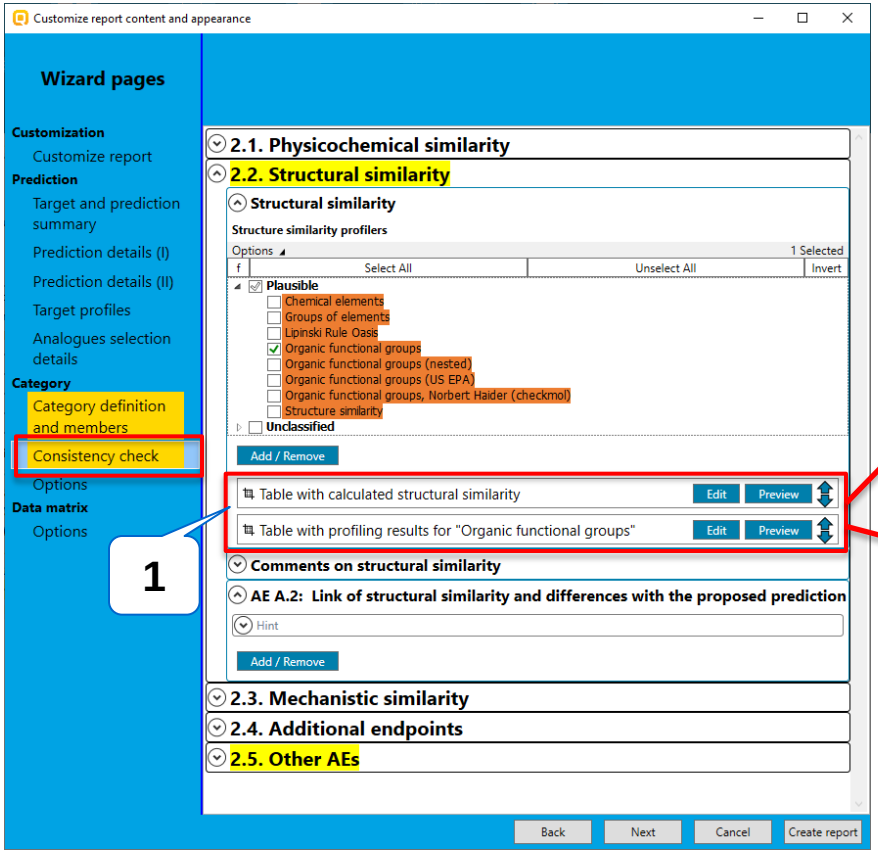


Table with calculated structural similarity

Options

Mode: Hologram, CombineAllFeatures

Measure: Dice

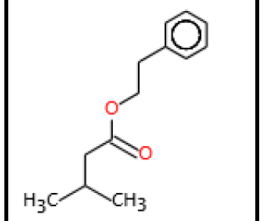
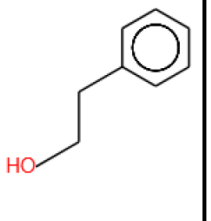
Molecular features: AtomCenteredFragments

Atom characteristics: AtomType, CountHAttached, Hybridization

Calculated structure similarity

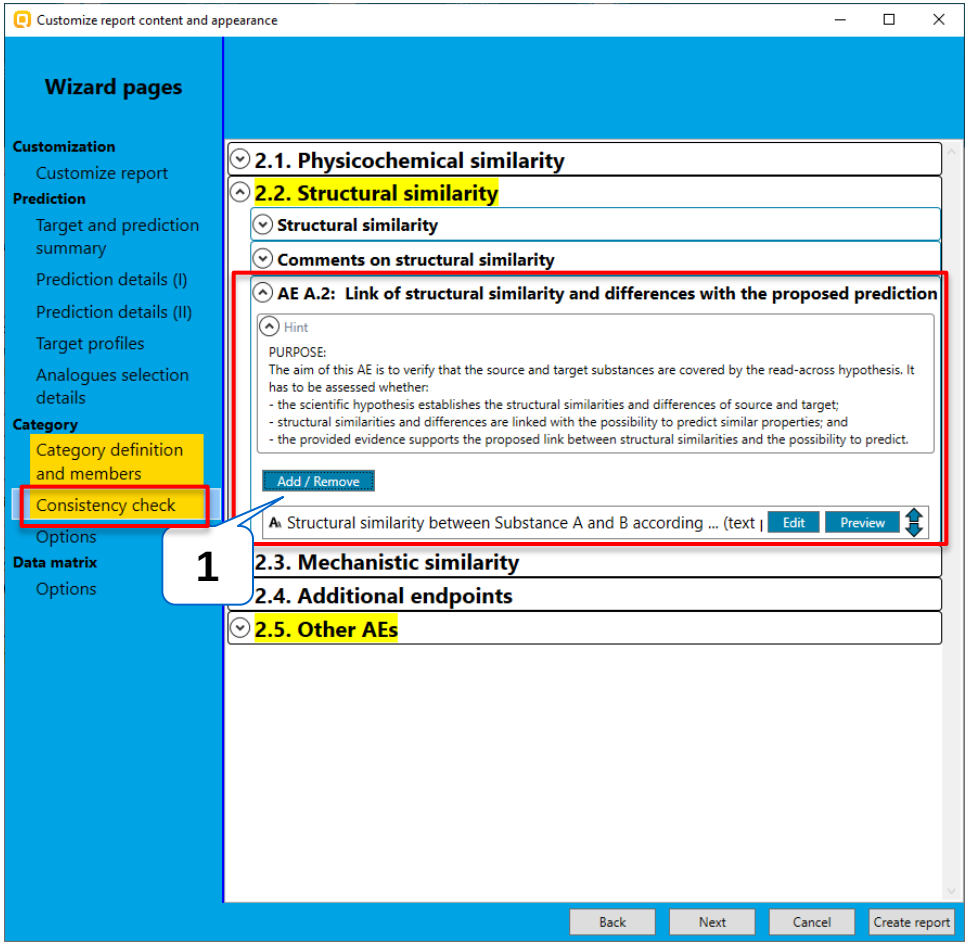
	Parent chemical CAS 140-26-1	metabolite #2 CAS No CAS number
Parent chemical CAS 140-26-1	100%	58.3 %
metabolite #2 CAS No CAS number	58.3 %	100%

Table with profiling results for "Organic functional groups"

1 CAS# 140-26-1	2 CAS# No CAS number
	
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.

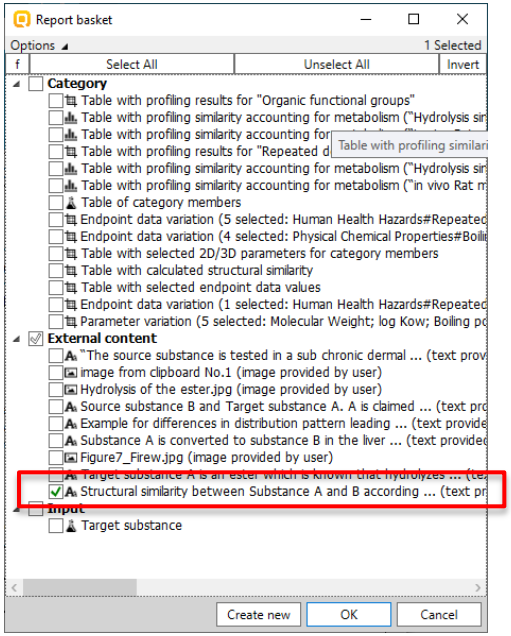
Report
Assessment elements of Scenario 1



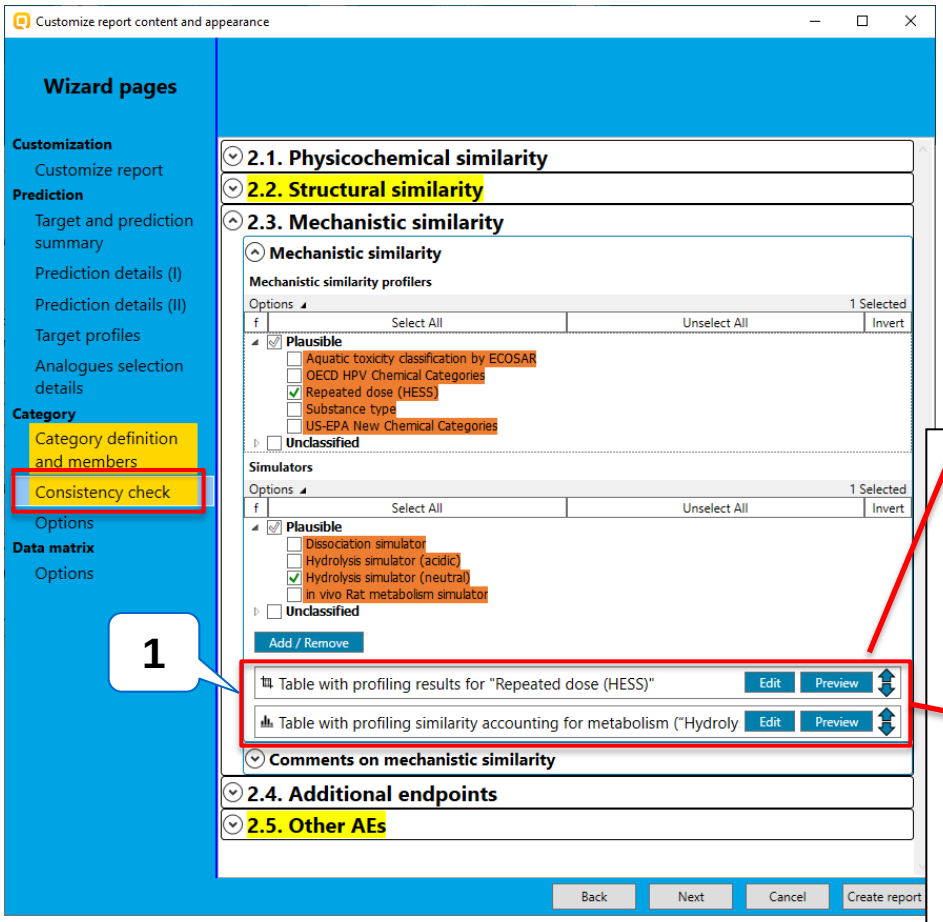
An example text for **AE A.2. Link of structural similarity and differences with the proposed prediction:**

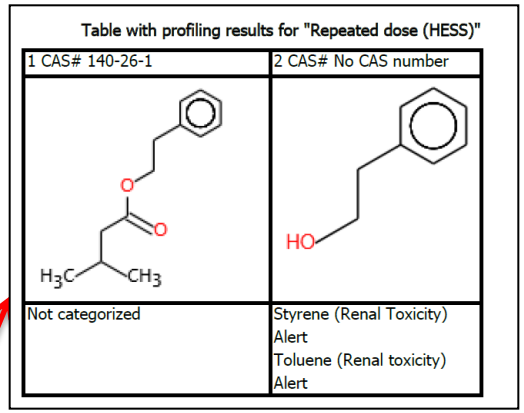
- *Structural similarity between Substance A and B according to Str.similarity profiler is in the range of [50-70%]*
- *Both chemicals has aromatic ring based on OFG profiler*

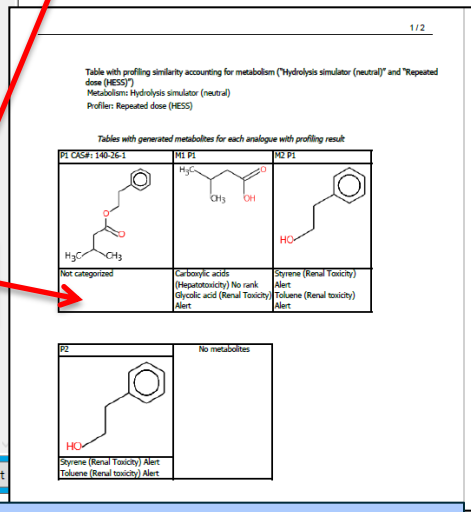
The item is text type added by **Add/Remove** button

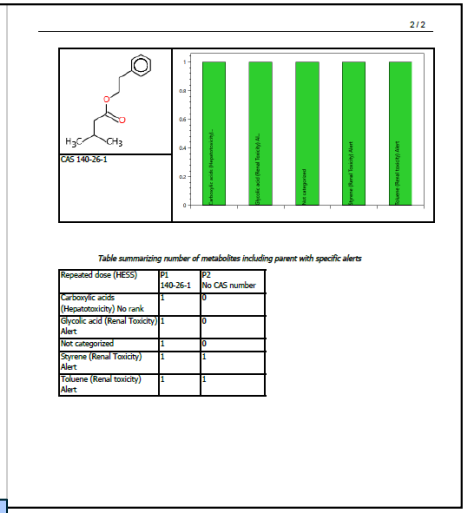


Report
Assessment elements of Scenario 1



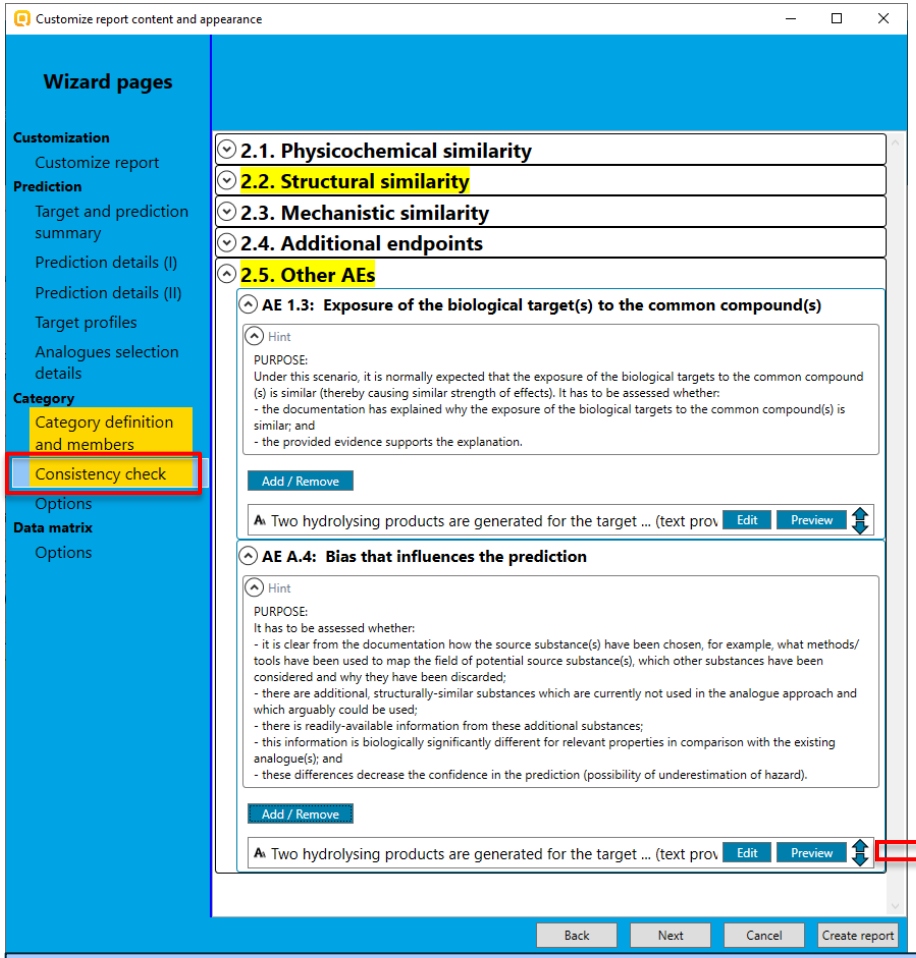






All items in the report basket related to the consistency of the category with respect to Mechanistic similarity (1) are added automatically.

Report
Assessment elements of Scenario 1



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.

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*

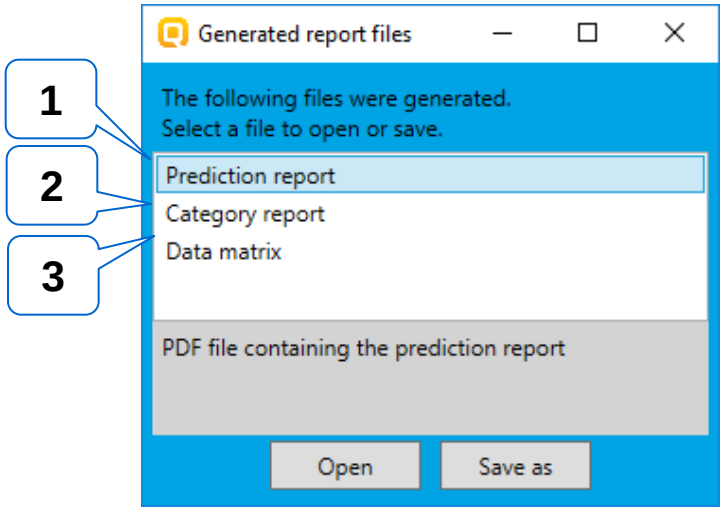
Two additional AEs (2.5. Other AEs) are included to the ***Consistency check*** section. An example content for the AEs is given.

Report Generation

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

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

Prediction of NOAEL for phenethyl isovalerate 1 / 6

QSAR Toolbox prediction for single chemical

(in accordance with RAAF scenario 1)

The selected RAAF scenario is specified in the first page

Date: 1 May 2019
Author(s):
Contact details:

Target information

Structural information	Numerical identifiers	Chemical names
SMILES: CC(C)CC(=O)OCCc1cccc c1	CAS#: 140-26-1 Other: EC Number:2054063	2-phenylethyl 3-meth ylbutanoate Butanoic acid, 3-meth hyl-, 2-phenylethyl

Structure

Prediction summa

Predicted endpoint: NOAEL; No effect specified; No species spe
guideline specified
Predicted value: 861
Unit/scale: mg/kg bdwt/d
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	**unit**		
Boiling point	°C	276	225
log Kow		3.97	1.57
Molecular Weight	Da	206	122
Vapor Pressure (Antoine method)	mm Hg	0.00653	0.0263
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), 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		*species, duration, test*	*species, duration, test*

Category report

QSAR Toolbox report for category

(in accordance with RAAF scenario 1)

1. Category definition

1.1. Category definition

Category name *manually editable field*
Not provided by the user

Covered (target) endpoint(s)
- Human Health Hazards/Repeated Dose Toxicity: NOAEL

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

1.2. Category members

Information of category members
Table of category members

#	CAS	Name	SMILES	Structure
1	140-26-1	phenethyl isovalerate	CC(C)CC(=O)OCCc1ccccc1	
2	No CAS number	Hydrolysis simulator (neutral): metabolite #2	OCCc1ccccc1	

Ranges for selected physicochemical properties and calculated parameters

Table with 2D parameters data variation

Parameter name	Variation	unit (family)
Boiling point	225 ÷ 276	°C(Temperature)
log Kow	1.57 ÷ 3.97	

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!