

OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 2 and related
assessment elements

Outlook

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

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and 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;
- Calculation of alert performance (AP) accounting for metabolism;
- Searching of analogues accounting for metabolism;
- Category consistency check;
- Selection of RAAF scenario;
- Filling in the report sections related to each read-across assessment element.

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Specific Aims

- To familiarize the user with the Read-Across Assessment Framework (RAAF) and more specifically with Scenario 2;
- To introduce to the user the read across assessment elements;
- To introduce to the user the report basket;
- To provide sufficient information to the user 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 was developed by ECHA as an internal tool which provides a framework for a consistent and structured assessment of grouping and read across approaches under REACH.
- The outcome of the assessment is a conclusion on whether the read across is scientifically acceptable or not.
- The RAAF defines different scenarios for different read-across approaches.
- Each scenario is associated with particular aspects (assessment elements, AEs).
- Total six scenarios are available: two for analogue approach and four for category approach.

Read-Across Assessment Framework (RAAF)

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) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

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 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

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The Example

- In this example we will predict the skin sensitization potential of chemical: *1,3-Propanediamine, N-(3-aminopropyl)* - [CAS# 56-18-8], which will be the “target” chemical;
- The category will be defined based on protein binding mechanism identified in the target chemical after skin metabolism is taken into account. The identified protein binding mechanism is common to all the chemicals in the category;
- A read-across approach will be used for the prediction. The read-across will be based on analogue approach expressed as common underlying mechanism for metabolites of source and target substances;
- Read-across assessment elements will be included to the report;
- Examples for the possible content of each of AEs will be provided.

The Example

Sidebar On Skin Sensitization

- Allergic contact dermatitis that results from skin sensitization is a significant health concern.
- Skin sensitization is a toxicological endpoint that is complex and conceptually difficult.
- However, there is growing agreement that most organic chemicals must react covalently with skin proteins in order to behave as skin sensitizers.
- Therefore, mechanisms by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents.

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- **Workflow**

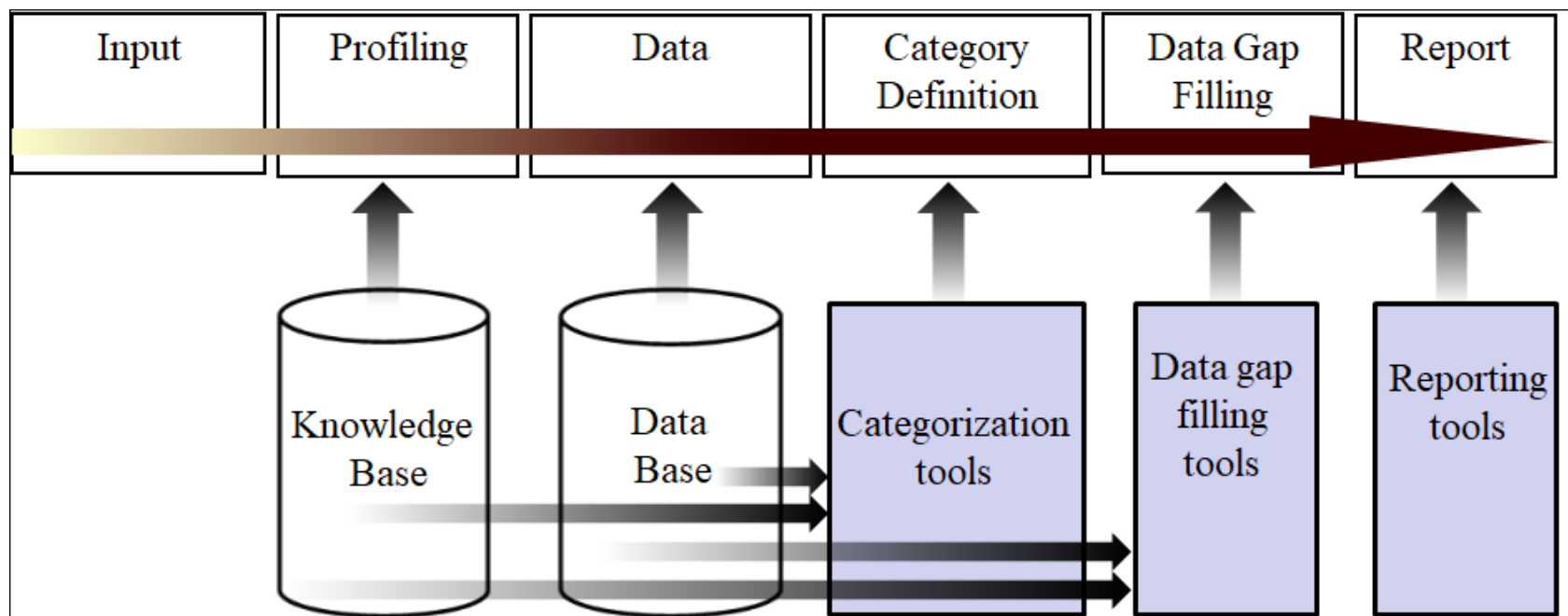
Workflow

- **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.

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 chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Input Screen

Input target chemical by CAS#

The screenshot shows the QSAR TOOLBOX software interface. The 'Input' tab is selected in the top menu. The 'CAS#' button in the toolbar is highlighted with a red box and labeled '1'. A dialog box titled 'Search by CAS #' is open, showing the search field with '56188' and the 'Search' button labeled '3'. The 'OK' button is labeled '4'. The search results show a single entry for CAS 56-18-8, with details like SMILES, CS Relation, Substance, Composition, Name, and Sources. A chemical structure is displayed next to the results. A callout '2' points to the search field.

1	CAS	56-18-8
	SMILES	NCCCNCCCN
	CS Relation	High
	Substance	Mono constituent
	Composition	
	Name	"1,3-propanediamine_n-(3-aminopro
	Sources	NICNAS Canada DSI

Chemical structure: NCCCNCCCN

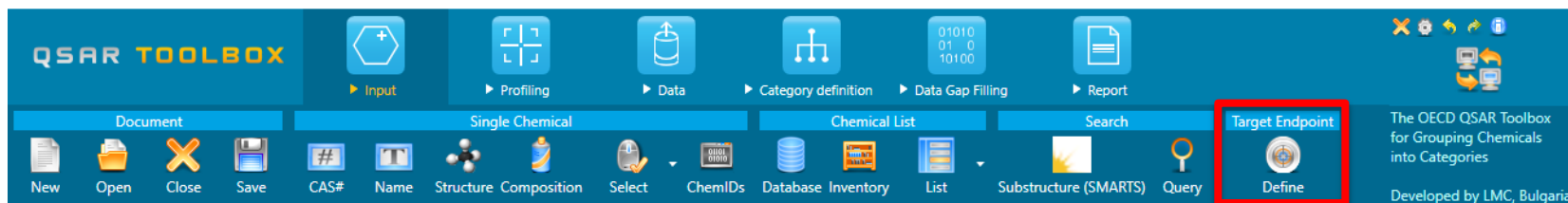
Click **CAS#** button (1); Type CAS **56-18-8** in the blank field (2) and click **Search** (3). When the structure appears, click **OK** (4).

Input

Define target endpoint

Defining of the endpoint allows entering the endpoint of interest e.g. EC3, LC50, gene mutation etc., along with specific metadata information. Based on the metadata, relevancy of the profiles is provided expressed in different highlighting.

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



Input

Define target endpoint

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Filling Report

Document Single Chemical Chemical List Search Target Endpoint

New Open Close Save CAS# Name Structure Composition Select ChemIDs Database Inventory List Substructure (SMARTS) Query Define

The OECD QSAR Toolbox for Grouping Chemicals into Categories

Developed by LMC, Bulgaria

Documents Filter endpoint tree... 1 [target]

Select endpoint

Filter: [] Close

- Physical Chemical Properties
- Environmental Fate and Transport
- Ecotoxicological Information
- Human Health Hazards
 - Acute Toxicity
 - ADME
 - Bioaccumulation
 - Carcinogenicity
 - Developmental Toxicity / Teratogenicity
 - Genetic Toxicity
 - Immunotoxicity
 - Irritation / Corrosion
 - Neurotoxicity
 - Photoinduced toxicity
 - Repeated Dose Toxicity
 - Sensitisation**
 - Toxicity to Reproduction
 - Toxicokinetics, Metabolism and Distribution

Undefined Next

Select endpoint

Human Health Hazards Sensitisation

Organ Skin

Type of method in Vivo

Assay LLNA

Endpoint EC3

Selection of additional metadata fields:

Add Up Down Remove

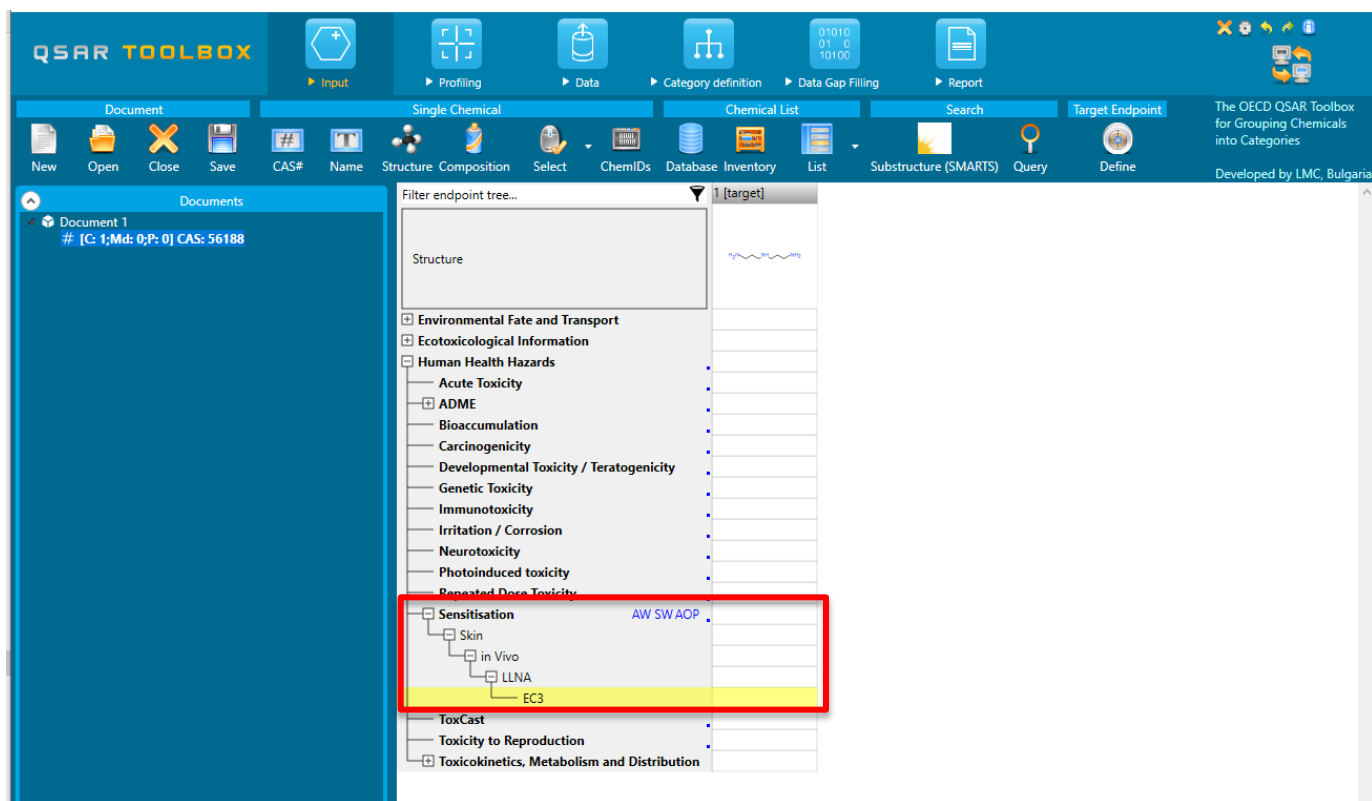
Undefined Back Finish

By clicking **Define** (1) you could select the target endpoint. Select **Sensitisation** in the *Human health hazards* category (2) and click **Next** (3). You need first to select **EC3** endpoint (4) from the drop-down menu and then consecutively the following metadata: Assay: **LLNA**, Organ: **Skin**, Type of method: **In Vivo** (5). Finally click on **Finish** (6).

Input

Define target endpoint

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



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).

Data

Gather data

The screenshot shows the QSAR Toolbox interface. The 'Data' module is selected in the top toolbar (callout 1). In the left sidebar, the 'Databases' list is shown with 'REACH Skin sensitisation database (normalised)' and 'Skin Sensitization' selected (callout 2). The 'Read data?' dialog box is open, showing a list of endpoints with 'Sensitisation' checked (callout 4). A status bar at the bottom indicates '5 points added across 1 chemicals.' (callout 5).

1. Go to **Data** module;
2. Select the highlighted databases without ECHA REACH (these are the databases containing data for the defined target endpoint (slide 24));
3. Click **Gather**;
4. Select only 'Sensitization' from the appeared window and click OK;
5. A pop-up message informs that 5 experimental data points for the target chemical has been found.

Data

Gather data

- Toxicity information on the target chemical is electronically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues (and further calculation of AP) is performed only among the chemicals which are listed in the selected databases. In this example only the *Skin sensitization database and REACH Skin sensitization database (normalized)* are selected.
- In this example, a pop-up window appears stating there are 5 experimental data points found for the target chemical. There are three out of five positive experimental data for the target chemical.
- Go to the *Profiling* module to check for the possible reasons of the positive effect (to check for an alert identified in the target chemical).

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 (i.e. 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 profilers;
- 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 ones 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

The screenshot shows the QSAR Toolbox Profiling interface. The top toolbar has icons for Input, Profiling (highlighted with a red box and callout 1), Data, Category definition, Data Gap Filling, and Report. The left sidebar contains 'Documents' and 'Profiling methods' (with callout 2 pointing to 'Unselect All') and 'Metabolism/Transformations' (with callout 3 pointing to 'Suitable' checkboxes). The main area displays a chemical structure, a 'Filter endpoint tree...' on the left, and a results table on the right. The results table shows categories like 'Human Health Hazards', 'ADME', 'Bioaccumulation', 'Carcinogenicity', 'Developmental Toxicity / Teratogenicity', 'Genetic Toxicity', 'Immunotoxicity', 'Irritation / Corrosion', 'Neurotoxicity', 'Photoinduced toxicity', 'Repeated Dose Toxicity', 'Sensitisation', 'Skin', 'in Vivo', 'GPMT', 'LLNA', 'EC3', and 'Miscellaneous'. The table also shows 'M: Category 1B', 'M: 0.882 %', 'M: 0.9 %', 'M: 0.9 %', and 'M: Category C'.

1. Go to **Profiling** section
2. Click **Unselect All**;
3. Select all *suitable* profiling schemes and simulators (green highlighted);
4. Click **Apply**.

Profiling

Profiling results

- 1) No alerts are identified in the target structure as a parent;
- 2) No metabolites are produced as a result of abiotic activation (*Autoxidation simulator*);
- 3) 5 metabolites are produced as a result of biotic activation (*Skin metabolism simulator*);
- 4) Endpoint specific protein binding alerts are identified in the metabolites produced by the Skin metabolism simulator.

See on the next slide

Profiling

Profiling results

The screenshot displays the QSAR Toolbox Profiling results interface. The sidebar on the left shows the 'Documents' section with two documents: Document 1 (CAS: 140261) and Document 2 (CAS: 56188). The 'Profiling methods' section shows 3 selected methods: Suitable, Plausible, and Aquatic toxicity classification by ECOSAR. The 'Metabolism/Transformations' section shows 2 selected methods: Autoxidation simulator and Skin metabolism simulator. The main panel displays the 'Filter endpoint tree...' and the 'Structure' of the target molecule. The results table shows the following data:

Endpoint	Results
General Mechanistic	No alert found
Endpoint Specific	No alert found
Protein binding alerts for skin sensitiz...	No alert found
Protein binding alerts for skin sensitiz...	No alert found
Metabolism/Transformation	0 metabolite(s)
Autoxidation simulator	0 metabolite(s)
General Mechanistic	4 metabolite(s)
Endpoint Specific	3 x Schiff base formation 3 x Schiff base formation >> Schiff base formation with carbonyl... 2 x Schiff base formation >> Schiff base formation with carbonyl... 1 x Schiff base formation >> Schiff base formation with carbonyl... 1 x No alert found
Skin metabolism simulator	1 x Skin sensitization Category 1A 3 x Schiff base formation 3 x Schiff base formation >> Schiff base formation with carbonyl... 2 x Schiff base formation >> Schiff base formation with carbonyl... 1 x Schiff base formation >> Schiff base formation with carbonyl... 1 x No alert found
General Mechanistic	1 x No alert found
Protein binding by OASIS	1 x No alert found
Endpoint Specific	1 x No alert found
Protein binding alerts for skin s...	1 x No alert found
Protein binding alerts for skin sensitization by OASIS	1 x No alert found

1. No alerts are identified in the target structure as a parent;
2. No metabolites are produced as a result of abiotic activation (Autoxidation simulator);
3. 4 metabolites are produced as a result of biotic activation (Skin metabolism simulator);
4. Endpoint specific protein binding alerts are identified in the metabolites produced by the Skin metabolism simulator.

Recap

- In module *Input*, you have entered the target chemical and defined the target endpoint.
- In the *Data* module, you saw the database corresponding to the defined target endpoint. You also found some experimental data for the target chemical available in the selected databases.
- In the *Profiling* module, you profiled the target chemical with profiling schemes and metabolic simulators, suitable for the selected target endpoint.
- Protein binding alerts for skin sensitization were identified for some of the metabolites produced by simulating of biotic activation (skin metabolism).
- Click “Category Definition” to move to the next module.

Category Definition

Overview

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

Category Definition

Grouping methods

- The different grouping methods allow the user to group chemicals into chemical categories according to different measures of “similarity” so that within a category data gaps can be filled by read-across.
- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind by the same mechanism and for which experimental results are available.
- If no alert is identified in the target structure, but is identified in its metabolites, analogues can be searched accounting for a metabolism (e.g. metabolites of the analogues to have same metabolic pattern as metabolites of the parent chemical). In this way the target chemical and the identified analogues will have similar metabolic pattern.
- When more than one alert is found in the target structure before or after metabolic activation, ‘Alert performance’ functionality could help for defining which of them is the most suitable for primary categorization. The latter is shown on the next few slides.

Category Definition

Searching for analogues accounting for skin metabolism

1. Click **Define with metabolism**;

2. Select *Skin metabolism simulator*;

3. Click **OK**;

4. Target and all metabolites produced by the selected simulator appear.

Chemical	Query	Criteria
Parent <chem>NCCCCCCCCN</chem>	none	No criteria. 4
Metabolite 1 <chem>NCCCCCCCC(=O)O</chem>	none	No criteria.
Metabolite 2 <chem>NCCCCCCCC=O</chem>	none	No criteria.
All chemicals		
Parent & Metabolites	none	No criteria.

Category Definition

Searching for analogues accounting for skin metabolism

1. Define *profile* query for the package – “Parent & Metabolites” and then select *Protein binding alerts for skin sensitization by OASIS*.

2. Click **Edit** to see all identified alerts in the parent and metabolites.

Category Definition

Alert performance calculation

Grouping options (Skin metabolism simulator)

All queries At least one

Chemical	Query	Criteria
Parent	none	No criteria.

Aggregation options

Categorical scale (ordinal)

Maximal

Skin sensitization II (ECETOC)

Skin sensitization I (Oasis)

Skin Sensitization (Danish EPA)

Skin sensitization GHS (ordinal)

OK Cancel

Alert performance

Scales

Calculate

OK Cancel

1. Click **Scales** button,
2. Select *Skin sensitization II (ECETOC)* scale
3. Click **Calculate** to evaluate the alert performance.

Category Definition

Alert performance calculation

Alert performance results...

Using of "Skin met. simulator" Combined paren products requirer No al found<AND>Schi formation >> Schi formation with ca compounds > Aldehydes<AND>Sc formation >> Schi formation with ca compounds >> Bis a (Protein binding aler sensitization by C	Positive	80.00%	Show chemicals... With data(12)...	Show all(15)...
	Negative	20.00%	Show chemicals... With data(3)...	
Using of "Skin met. simulator" Combined paren products requirer No alert (Protein binding aler sensitization by C	Positive	46.22%	Show chemicals... With data(611)...	Show all(1322)...
	Negative	53.78%	Show chemicals... With data(711)...	
Using of "Skin met. simulator" Combined paren products requirer Schiff I formation >> Schi formation with ca compounds >> Alc (Protein binding aler sensitization by C	Positive	51.09%	Show chemicals... With data(187)...	Show all(366)...
	Negative	48.91%	Show chemicals... With data(179)...	
Using of "Skin met. simulator" Combined paren products requirer Schiff I formation >> Schi formation with ca compounds >> Bis a (Protein binding aler	Positive	82.35%	Show chemicals... With data(14)...	Show all(17)...
	Negative	17.65%	Show chemicals... With data(3)...	

Close

Statistic for **all** alerts identified in the package "Parent & Metabolites"

Statistic for **each** of the alerts identified in the package "Parent & Metabolites"

The alert with the best performance in this case.
"Bis aldehydes" alert will be used for searching for analogues (see next slides).



Keep in mind that the statistic is obtained from the chemicals and data, available in the selected databases

Category Definition

Searching for analogues accounting for skin metabolism

1. Click **Edit**;

2. Remove all alerts except this with the best performance (*Bis aldehydes*);

3. Confirm the change by clicking **OK**;

4. Confirm that you have selected different from the target categories. This is just an informative message

5. Click **OK** in the main window to start the search.

Category Definition

Summary information for Analogues

17 chemicals with 44 experimental results are found across all 28 analogues related to the defined target endpoint (data for skin sensitization is selected only).

The screenshot shows the QSAR Toolbox interface. On the left, the 'Filter endpoint tree...' panel displays a hierarchical tree of endpoints. The 'Sensitisation' endpoint is selected, and its sub-endpoint 'EC3' is highlighted. A red circle and a callout box point to the value '17/44' next to 'EC3', indicating 17 chemicals and 44 experimental results. The main table displays chemical statistics for various endpoints. A red box highlights the row for 'EC3' with a value of 17/44, indicating 17 chemicals and 44 experimental results.

Endpoint	1 [target]	2	3	4	5	6	7	8
Structure								
Photoinduced toxicity								
Repeated Dose Toxicity								
Sensitisation								
Skin								
in Vivo								
GPMT								
HRIPT								
LLNA								
EC3	15/20 1/2	M: Category 1B M: Positive	M: Negative			M: Category 1A		
Miscellaneous	17/44	M: 0.882 %	M: 1.68 %		M: Positive		M: Negative	M: 8.4 %
ToxCast								
Toxicity to Reproduction								
Toxicokinetics, Metabolism and Distribution								
Profiling								
General Mechanistic								
Protein binding by OASIS	No alert found							
Endpoint Specific								
Protein binding alerts for skin sensitiz...	No alert found							
Protein binding alerts for skin sensitiz...	No alert found							



Chemical statistics representing the number of chemicals and the available experimental data for them.

Data Gap Filling Overview

- “Data Gap Filling” module give access to five different data gap filling tools:
 - Read-across
 - Trend analysis
 - (Q)SAR models
 - Standardized workflow
 - Automated workflow
- Depending on the situation, the most relevant data gap mechanism should be chosen, taking into account the following considerations:
 - Read-across is the appropriate data-gap filling method for “qualitative” endpoints like skin sensitisation or mutagenicity for which a limited number of results are possible (e.g. positive, negative, equivocal). Furthermore read-across is recommended for “quantitative endpoints” (e.g., 96h-LC50 for fish) if only a low number of analogues with experimental results are identified.
 - Trend analysis is the appropriate data-gap filling method for “quantitative endpoints” (e.g., 96h-LC50 for fish) if a high number of analogues with experimental results are identified.
 - “(Q)SAR models” can be used to fill a data gap if no adequate analogues are found for a target chemical.

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

Data Gap Filling

Apply Read-across

1. Click on the row with the target endpoint and the cell corresponding to the target chemical;

2. Go to Data gap filling module;

3. Click **Read-across** button;

4. Select *Skin sensitisation II (ECETOC)*;

5. Click **OK**.

Data Gap Filling Apply Read-across

The screenshot displays the OECD QSAR Toolbox software interface. The main window is titled 'Subcategorization' and shows a list of chemical alerts on the left and a list of chemical structures on the right. The 'Profilers' window is also visible, showing a list of chemical elements and a list of chemical structures. The 'Subcategorization' window has a 'Select / filter data' button and a 'Subcategorize' button. The 'Profilers' window has a 'Select / filter data' button and a 'Subcategorize' button. The interface is annotated with numbers 1 through 5 indicating the steps for data gap filling.

1) Protein binding alerts for skin sensitization by OASIS profiler in combination with Autoxidation simulator, 2) Remove the different analogues; 3) Select Structural similarity 4) Select all analogues (3) similar less than 30% to the target chemical, by hold Ctrl button; 5) Click Remove

Data Gap Filling

Apply Category consistency elements

The screenshot displays the QSAR Toolbox software interface. The main window is divided into several sections. On the left, there is a 'Documents' panel showing a list of chemical documents. Below it, the 'Grouping methods' panel is visible. The central area shows a 'Filter endpoint tree...' with a table of results. The right side features a 'Category consistency wizard' dialog box. The wizard has a 'Wizard pages' list on the left and a main content area on the right. The '2D/3D parameters' section is expanded, showing a list of parameters. The 'Physico-chemical data' section is also expanded, showing a list of properties. The 'Read-across prediction for E' section is visible at the bottom of the wizard. The 'Accept prediction' button is highlighted with a green checkmark.

1. After subcategorization process go back to the **Category definition** module and apply category elements* (1).

2. No different selection than the default is needed – click **OK** (2).

3. Once the category elements are applied **accept the prediction** (3).

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

Recap

- In the *Category definition* module you found analogues based on the alert with the best performance accounting for skin metabolism.
- In *Data gap filling* module you applied a read-across approach. Read-across is the appropriate data-gap filling method for “qualitative” endpoints like skin sensitisation. Since the most of the analogues and all five neighbouring tested chemicals in the category were positive, it was easy to accept the prediction of positive for the target chemical.
- Category consistency was checked by applying the category elements.
- You are now ready to complete the final module and to create the report.
- Click “Report” to proceed to the last module.

Report Overview

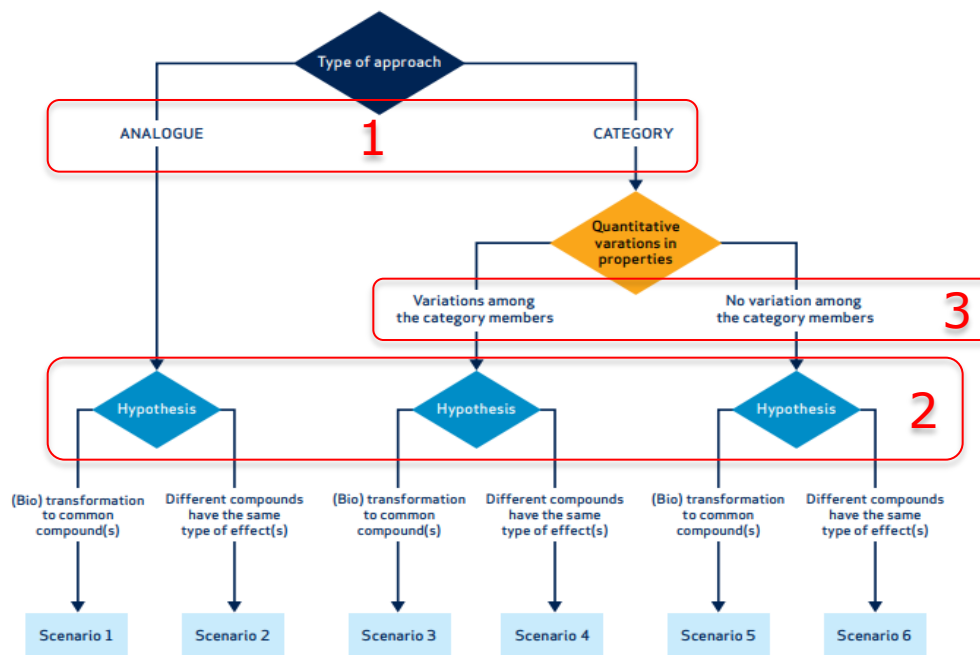
- The report module generates a report for predictions performed within the Toolbox.
- The report module contains a predefined report template which users can customize.
- Additionally a specific RAAF scenario could be chosen. Selection of one of the scenarios will append automatically the related assessment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



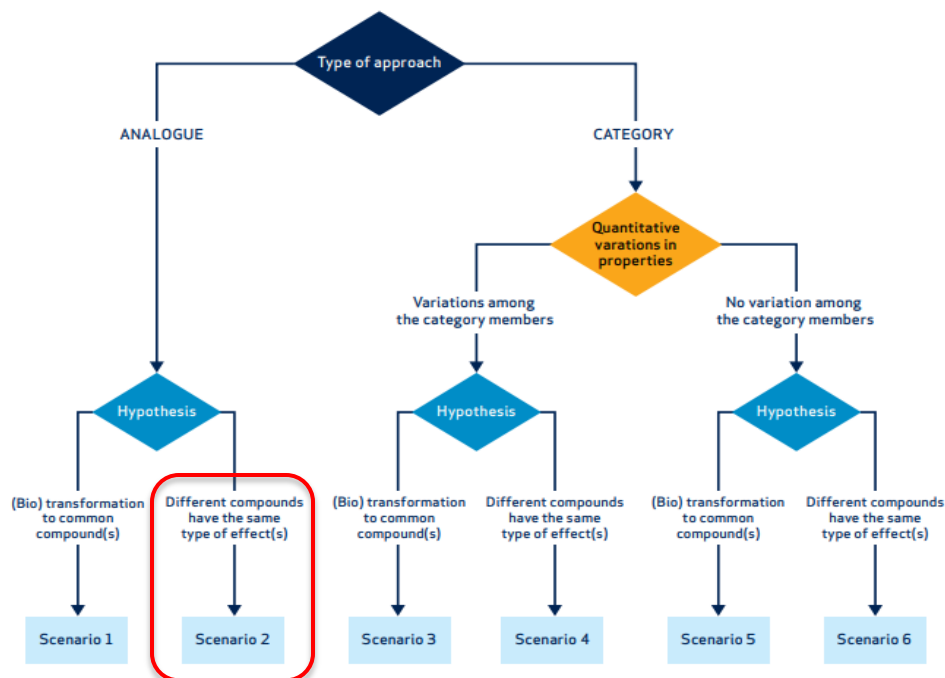
Report

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 – **different compounds with common underlying mechanism for metabolites of source and target substances** ;

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



Read-Across Assessment Framework (RAAF)

Scenario 2

- Scenario 2 covers the analogue approach for which the read-across hypothesis is based on different compounds with qualitatively similar properties.
- 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 2 of the RAAF*. The target (B) and the source chemicals (A) are biotransformed to substances causing the same type of effects through a common mechanism (A1 and B1). The rest of the obtained compounds, non-common for the target and the source substance does not influence the prediction of the property under the consideration.

	PARENT SUBSTANCES	(BIO)TRANSFORMATION	COMMON COMPOUND	NON-COMMON COMPOUNDS
SOURCE	A	A → A1 + A2	A1	A2
TARGET	B	B → B1	B1	-

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

Report

Generation report according to RAAF-Scenario 2

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. The 'Reports' menu is open, showing options like 'Prediction', 'Category', 'QMRF', 'SMI File', 'SDF File', 'CAS List', and 'Data Matrix'. The 'Customize report content and appearance' dialog is open, showing the 'Wizard pages' section with checkboxes for 'Add RAAF scenario', 'Prediction', 'Category', and 'Data matrix'. The 'Filter endpoint tree...' window is also open, showing a tree structure of endpoints with 'EC3' highlighted. Numbered callouts 1 through 4 indicate specific features: 1 points to the 'EC3' endpoint in the filter tree; 2 points to the 'Reports' menu; 3 points to the 'Add RAAF scenario' checkbox in the customization dialog; and 4 points to the 'Wizard pages' section header in the customization dialog.

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 2** from the drop-down menu. Section of the report to which the related AE automatically appeared are getting yellow highlighted.

Report

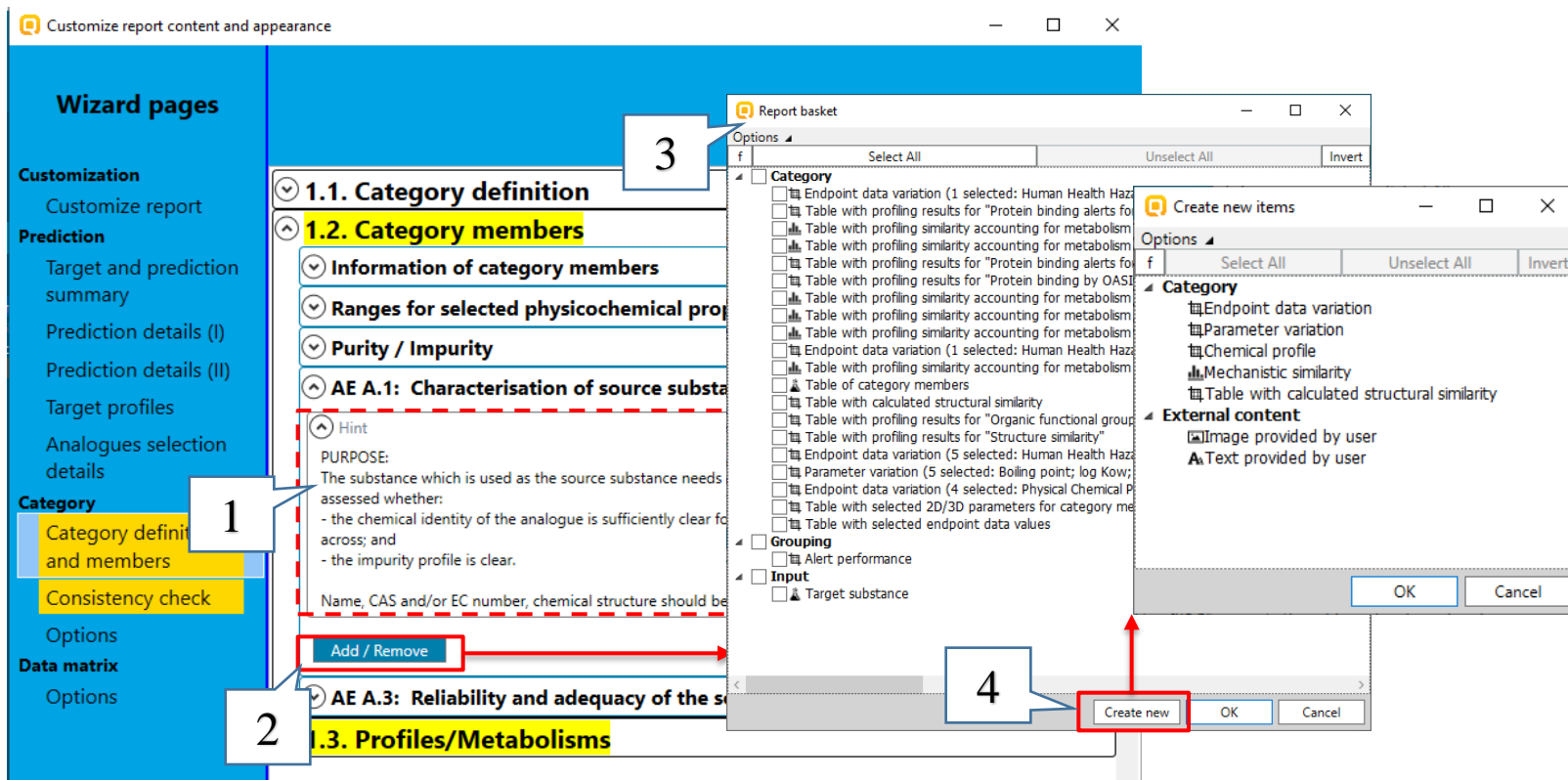
Generation report according to RAAF-Scenario 2

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: **Category definition and members** (2) and **Consistency check** (3).

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

Report

Assessment elements of Scenario 2

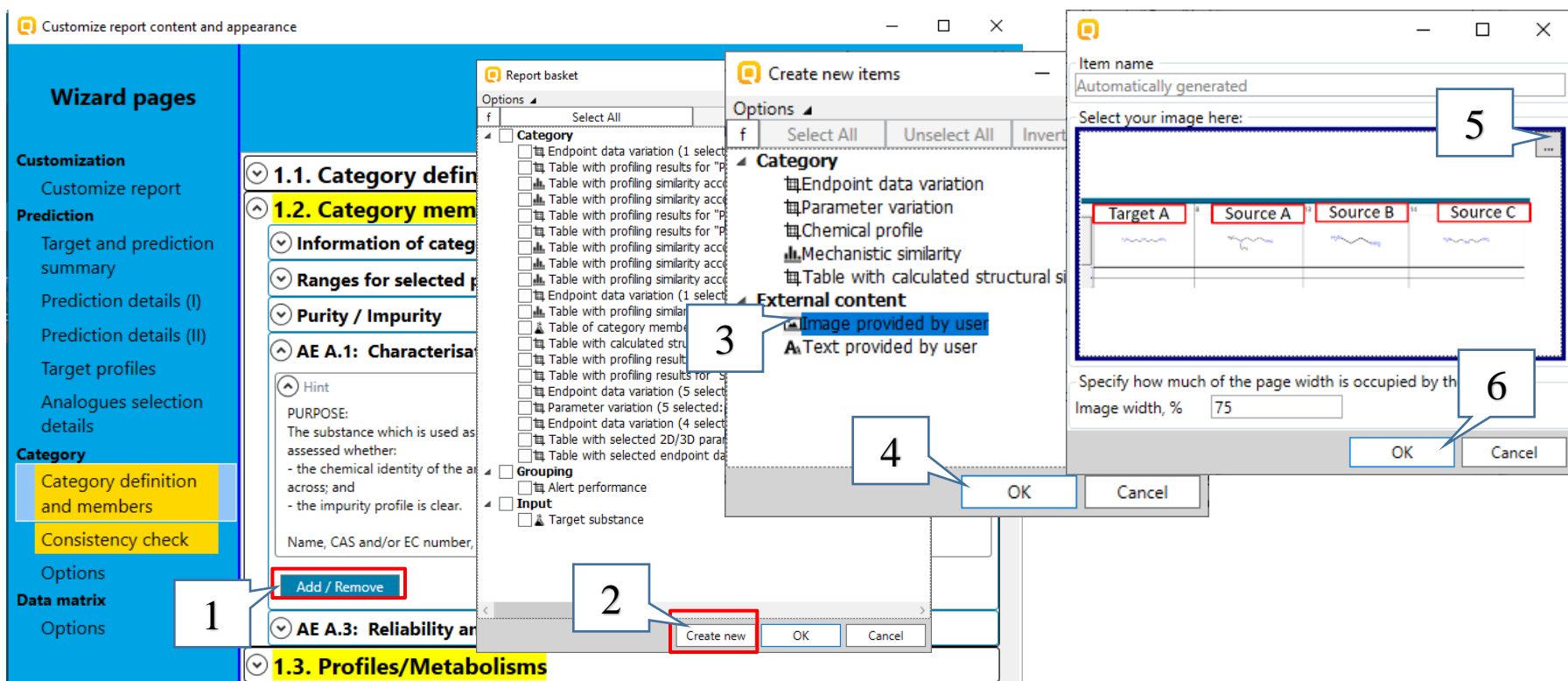


Hint for each of the assessment elements is available (1). Information can be included by clicking the **Add/Remove** button (2) located below the corresponding AE. The *Add/Remove* button invokes the so-called "**Report basket**" (3). The latter contain different items triggered by the actions of the 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 2.1. Compounds the test organism is exposed to**

Report

Assessment elements of Scenario 2

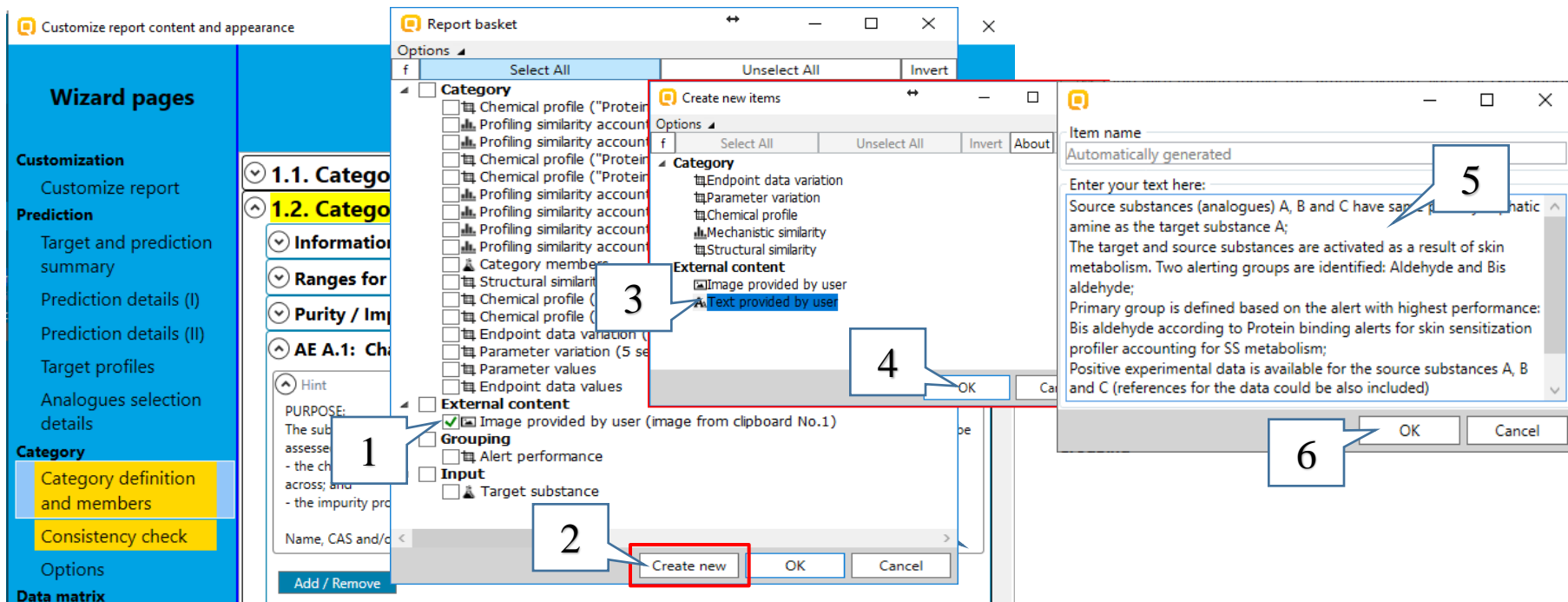


Click the **Add/Remove** button (1) and then **Create new** (2). Select to create an item with external content – **Image provided by user** (3) and click **OK** (4). New window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture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

*In the current example a picture illustrating the target chemical marked as **Target A** and source chemicals marked as **Source A, B** and **C** was prepared in advance.

Report

Assessment elements of Scenario 2



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 substances (analogues) A, B and C have same primary aliphatic amine as the target substance A;
- The target and source substances are activated as a result of skin metabolism. Two alerting groups are identified: Aldehyde and Bis aldehyde;
- Primary group is defined based on the alert with highest performance: Bis aldehyde according to Protein binding alerts for skin sensitization profiler accounting for SS metabolism;
- Positive experimental data is available for the source substances A, B and C (references for the data could be also included)
- Substances A, B and C are used to predict the toxic effect of target A.

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

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

1

2

3

4

1.1. Category definition

1.2. Category members

Information of category members

Ranges for selected physicochemical properties and calculated parameters

Purity / Impurity

AE A.1: Characterisation of source substance

Hint

Add / Remove

image from clipboard No.1 (image provided by user)

Source substances (analogues) A, B and C have same primary ... (text provided by user)

Edit

Edit

Preview

AE A.3: Reliability and adequacy of the source study

1.3. Profiles/Metabolisms

Purity / Impurity

Not provided by the user

manually editable field

AE A.1: Characterisation of source substance

Image from clipboard No.1 (image provided by user)

Target A	Source A	Source B	Source C

Source substances (analogues) A, B and C have same primary ... (text provided by user)

Source substances (analogues) A, B and C have same primary aliphatic amine as the target substance A; The target and source substances are activated as a result of skin metabolism. Two alerting groups are identified: Aldehyde and Bis aldehyde;

Primary group is defined based on the alert with highest performance: Bis aldehyde according to Protein binding alerts for skin sensitization profiler accounting for SS metabolism;

Positive experimental data is available for the source substances A, B and C (references for the data could be also included)

Substances A, B and C are used to predict the toxic effect of target A.

AE A.3: Reliability and adequacy of the source study

Not provided by user

1.3. Profiles/Metabolisms

List of profiles/metabolisms

Profiles used for grouping/subcategorization:

- Using of "Skin metabolism simulator" Combined parent and products requirements: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes (Protein binding alerts for skin sensitization by OASIS) (primary grouping)
- Protein binding alerts for skin sensitization by OASIS with Skin metabolism simulator (subcategorization)

Both newly created items appear under the **AE 2.1.** (1). Each of the items can be edited (2) or just previewed (3) in a .pdf format. The order of the appearance in the report could also be changed

Example of how the AE 2.1. and related description will look in the generated report is shown on the right (4).

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

Report basket

Options

f

Select All

Unselect All

Invert

Category

Endpoint data variation (1 selected: Human Health Hazards#Sensitisation)

Table with profiling results for "Protein binding alerts for skin sensitization acco

Table with profiling similarity accounting for metabolism ("Autooxidation simulat

Table with profiling results for "Protein binding by OASIS"

Table with profiling similarity accounting for metabolism ("Autooxidation simulat

Table with profiling similarity accounting for metabolism ("Skin metabolism sim

Endpoint data variation (1 selected: Human Health Hazards#Sensitisation)

Table with profiling similarity accounting for metabolism ("Autooxidation simulat

Table with category members

Table with calculated structural similarity

Table with profiling results for "Organic functional groups"

Table with profiling results for "Structure similarity"

Endpoint data variation (5 selected: Human Health Hazards#Sensitisation#(s)

Parameter variation (5 selected: Boiling point; log Kow; Molecular Weight; Va

Endpoint data variation (4 selected: Physical Chemical Properties#Boiling point

Table with selected 2D/3D parameters for category members

Table with selected endpoint data values

External content

Image from clipboard No.3 (image provided by user)

Source substances (analogues) A, B and C have same primary ... (text provid

Grouping

Alert performance

Input

Target substance

Create new

OK

Cancel

1.1. Category definition

1.2. Category members

Information of category

Ranges for selected physicochemical properties

Purity / Impurity

AE A.1: Characterisation of source substance

Hint

PURPOSE:

The substance which is used as the source substance assessed whether:

- the chemical identity of the analogue is clear; and
- the impurity profile is clear.

Name, CAS and/or EC number, chemical structure should be provided.

Add / Remove

AE A.3: Reliability and adequacy of the source study

1.3. Profiles/Metabolisms

Purity / Impurity

Not provided by the user

AE A.1: Characterisation of source substance

Table of category members

#	CAS	Name	SMILES	Structure
1	56-18-8	Iminobis-3-propylamine	NCCCNCCCN	
2	109-55-7	DMAPA	CN(C)CCCN	
3	107-15-3	Ethylenediamine	NCCN	
4	111-40-0	DETA	NCCNCCN	

Image from clipboard No.1 (image provided by user)

Target A	Source A	Source B	Source C

Source substances (analogues) A, B and C have same primary ... (text provided by user)

Source substances (analogues) A, B and C have same primary aliphatic amine as the target substance A: The target and source substances are activated as a result of skin metabolism. Two alerting groups are identified: Aldehyde and Bis aldehyde;

Primary group is defined based on the alert with highest performance: Bis aldehyde according to Protein binding alerts for skin sensitization profiler accounting for SS metabolism; Positive experimental data is available for the source substances A, B and C (references for the data could be also included)

Two AE (AE A.1 and A.3) related to Scenario 2 are included in the *Category definition and members* section.

- **AE A.1 Characterization of source substance.** The user should open the *Report basket* by clicking the *Add/Remove* button (1) and manually select the item *Table with category members* (2). Click *OK* button (3). 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.

Example of how the AE A.1. will look in the generated report is shown on the right(4).

- **AE A.3 Reliability and adequacy of the source study** should be filled in manually (5) (see on the next slide)

Report

Assessment elements of Scenario 2

Report basket

Options: Select All, Unselect All, Invert

Category

- ☐ Endpoint data variation (1 selected: Human Health)
- ☐ Table with profiling similarity accounting for meta
- ☐ Table of category members
- ☐ Table with calculated structural similarity
- ☐ Table with profiling results for "Organic functiona
- ☐ Table with profiling results for "Struc
- ☐ Table with profiling results for "Prote
- ☐ Table with profiling results for "Prote
- ☐ Table with profiling similarity account
- ☐ Table with profiling similarity accounting for meta
- ☐ Table with profiling similarity accounting for meta
- ☐ Table with profiling similarity accounting for meta
- ☐ Endpoint data variation (5 selected: Human Health Hazards#Sensitizat
- ☐ Parameter variation (5 selected: Boiling point; log Kow; Molecular We
- ☐ Endpoint data variation (4 selected: Physical Chemical Properties#Boil
- ☐ Table with selected 2D/3D parameters for category members
- ☐ Table with selected endpoint data values

External content

- ☐ Image from clipboard No.1 (image provided by user)
- ☐ A Source substances (analogues) A, B and C have same primary ... (text)

Input

- ☐ Target substance

1.1. Category definition

1.2. Category member

Information of category r

Ranges for selected physi

Purity / Impurity

AE A.1: Characterisation

AE A.3: Reliability and ad

Hint

PURPOSE:
The source study needs to match the requested for any other key study. It should cover the following aspects:
- the study design reported for the source study should be referred to in Article 13(3); and
- the study design should cover an exposure scenario referred to in Article 13(3); and
- there is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided. The test material used represents the source substance as described in the hypothesis in terms of purity and impurities.

1.3. Profiles/Metabolisms

2

3

Data filter

Use subcategorization

Combine categories

Unique

Continued by AND

Target

Source

Author

Reference source

Title

Year

Materials/Methods

Endpoint

Qualifier of guideline

Test guideline

Test method / Data source

Type of method

Species/Organism/Tissue

Strain

Test organisms (species)

Results

Open

Study result type

Data referenced

Assigned SMILES

GLP compliance

Qualifier

Reliability

Other

Bibliographic source

Comment EC3

Comments

Compound(s)

Confounders

Database

Identity in file

Institution and country

Principles of method if cat

Purpose of study

Substance type

Test material equivalent to

Test material identity (Cat)

Test material identity (Test)

Test material identity (ID)

Test material identity (URL)

Select different

Remove

3

Read-across prediction for EC3 based on 13 values

Observed: Positive (x13); Predicted: Positive

Positive

Negative

log Kow

AE A.3: Click the **Add/Remove** button (1) and create new item with textual content (2) (see slide 55).

In the text field paste the following example text:

*"The all three source substance are tested according to the Local lymph node assay (LLNA)
The study is used to predict the skin sensitization effect concerning LLNA study for the target substance"*

Additionally a snapshot of the filter by test conditions window (3) could be added to confirm the consistency regarding the assay (create new image item).

Report Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization
Customize report

Prediction
Target and prediction summary
Prediction details (I)
Prediction details (II)
Target profiles
Analogues selection details

Category
Category definition and members
Consistency check
Options

Data matrix
Options

Information of category members

Ranges for selected physicochemical properties and calculated parameters

Purity / Impurity

AE A.1: Characterisation of source substance

AE A.3: Reliability and adequacy of the source study

Hint
PURPOSE:
The source study needs to match the default REACH requirements in terms of reliability and adequacy as requested for any other key study. It has to be assessed whether:
- the study design reported for the source study is adequate and reliable for the purpose of the prediction based on read-across;
- the study design should cover the key parameters in the corresponding test method referred to in Article 13(3);
- the study design should cover an exposure duration comparable to or longer than the corresponding method referred to in Article 13(3); and
- there is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided. The test material used represents the source substance as described in the hypothesis in terms of purity and impurities.

Add / Remove

image from clipboard No.2 (image provided by user) **Edit** **Preview**

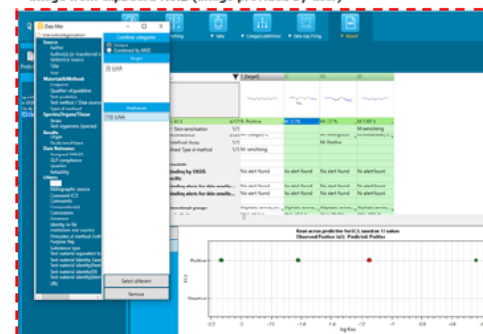
A. The all three source substance are tested according ... (text provide **Edit** **Preview**

1.3. Profiles/Metabolisms

Back **Next** **Cancel** **Create report**

AE A.3: Reliability and adequacy of the source study

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



The all three source substance are tested according ... (text provided by user)

The all three source substance are tested according to the Local lymph node assay (LLNA)

The study is used to predict the skin sensitization effect concerning LLNA study for the target substance

1.3. Profiles/Metabolisms

List of profiles/metabolisms

Profiles used for grouping/subcategorization:

- Using of "Skin metabolism simulator" Combined parent and products requirements: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes (Protein binding alerts for skin sensitization by OASIS) (primary grouping)
- Protein binding alerts for skin sensitization by OASIS with Skin metabolism simulator (subcategorization)
- Structure similarity (subcategorization)

AE 2.1: Compounds the test organism is exposed to

Not provided by user

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

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

1.1. Category definition

1.2. Category members

1.3. Profiles/Metabolisms

List of profiles/metabolisms

AE 2.1: Compounds the test organism is exposed to

Hint

PURPOSE:

In this scenario, it is claimed that different compounds have the same effects for the property under consideration. Such different compounds may be the source and target substances themselves and/or their (bio)transformation products. It has to be assessed whether:

- the compounds to which the test organism is exposed (after administration of the source and the target substances) have been established in the documentation; and
- the provided evidence supports the explanation.

Add / Remove

Target substance (A) and the source substances are all ... (text provided by user) Edit Preview

image from clipboard No.6 (image provided by user) Edit Preview

Back Next Cancel Create report

AE 2.1: Compounds the test organism is exposed to

Target substance (A) and the source substances are all ... (text provided by user)

Target substance (A) and the source substances are all aliphatic amines. • None of them has protein binding alert identified in the parent structure • None of them undergo autoxidation transformation and respectively do not activate as a result of abiotic oxidation • All of the substances (target and sources) undergo skin metabolism transformations resulting in activation. In other words, the substances are activated enzymatically in the skin by producing aldehyde and bis aldehyde metabolites • A schematic illustration of the skin metabolism is provided here:

image from clipboard No.6 (image provided by user)

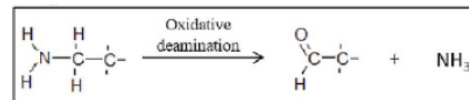
QSAR Toolbox 4.4
Database version: 4.4

QSAR TOOLBOX

TPRF v4.4

Chemicals category

3 / 13



An example text and illustration related to **AE 2.1: Compounds the test organism is exposed to** is shown above.

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization
Customize report

Prediction
Target and prediction summary
Prediction details (I)
Prediction details (II)
Target profiles
Analogues selection details

Category
Category definition and members
Consistency check

Data matrix
Options

2.1. Physicochemical similarity

2.2. Structural similarity
Structural similarity
Comments on structural similarity
AE A.2: Link of structural similarity and differences with the proposed prediction

2.3. Mechanistic similarity
Mechanistic similarity
Comments on mechanistic similarity
AE 2.2: Common underlying mechanism, qualitative aspects

2.4. Additional endpoints

2.5. Other AEs
AE 2.3: Common underlying mechanism, quantitative aspects
AE 2.4: Exposure to other compounds than to those linked to the prediction
AE 2.5: Occurrence of other effects than covered by the hypothesis and justification
AE A.4: Bias that influences the prediction

Back Next Cancel Create report

AEs included to the *Consistency check* section are six.

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and membership

Consistency

Options

Data matrix

Options

Structural similarity

Structure similarity profilers

Options 2 Selected

Select All Unselect All Invert

☒ Plausible

- ☐ Chemical elements
- ☐ Groups of elements
- ☐ Lipinski Rule Oasis
- ☒ Organic functional groups
- ☐ Organic functional groups (nested)
- ☐ Organic functional groups (US EPA)
- ☐ Organic functional groups, Norbert Haider (checkmol)
- ☒ Structure similarity

☐ Unclassified

Add / Remove

Table with calculated structural similarity Edit Preview

Table with profiling results for "Organic functional groups" Edit Preview

Table with profiling results for "Structure similarity" Edit Preview

Comments on structural similarity

AE A.2: Link of structural similarity and differences with the proposed prediction

Hint

Add / Remove

Structural similarity between Target substance A and ... (text provided) Edit Preview

Table with calculated structural similarity

Options

Mode: Hologram, CombineAllFeatures

Measure: Dice

Molecular features: AtomCenteredFragments

Atom characteristics: AtomType, CountHAttached, Hybridization

Calculated structure similarity

	1 CAS 56-18-8	11 CAS 109-55-7	18 CAS 107-15-3	19 CAS 111-40-0
1 CAS 56-18-8	100%	37.5 %	61.5 %	87.5 %
11 CAS 109-55-7	37.5 %	100%	36.4 %	28.6 %
18 CAS 107-15-3	61.5 %	36.4 %	100%	72.7 %
19 CAS 111-40-0	87.5 %	28.6 %	72.7 %	100%

Table with profiling results for "Organic functional groups"

1 CAS# 56-18-8	2 CAS# 109-55-7	3 CAS# 107-15-3
<chem>NCCNCCN</chem>	<chem>CC(C)NCCN</chem>	<chem>NCCN</chem>
Amine, primary Amine, secondary Aliphatic amine, primary Aliphatic amine, secondary	Amine, primary Amine, tertiary Aliphatic amine, primary Aliphatic amine, tertiary	Amine, primary Aliphatic amine, primary

4 CAS# 111-40-0
<chem>NCCNCCN</chem>
Amine, primary Amine, secondary Aliphatic amine, primary Aliphatic amine, secondary

AE A.2. Link of structural similarity and differences with the proposed prediction is related to the structural similarity of the final category.

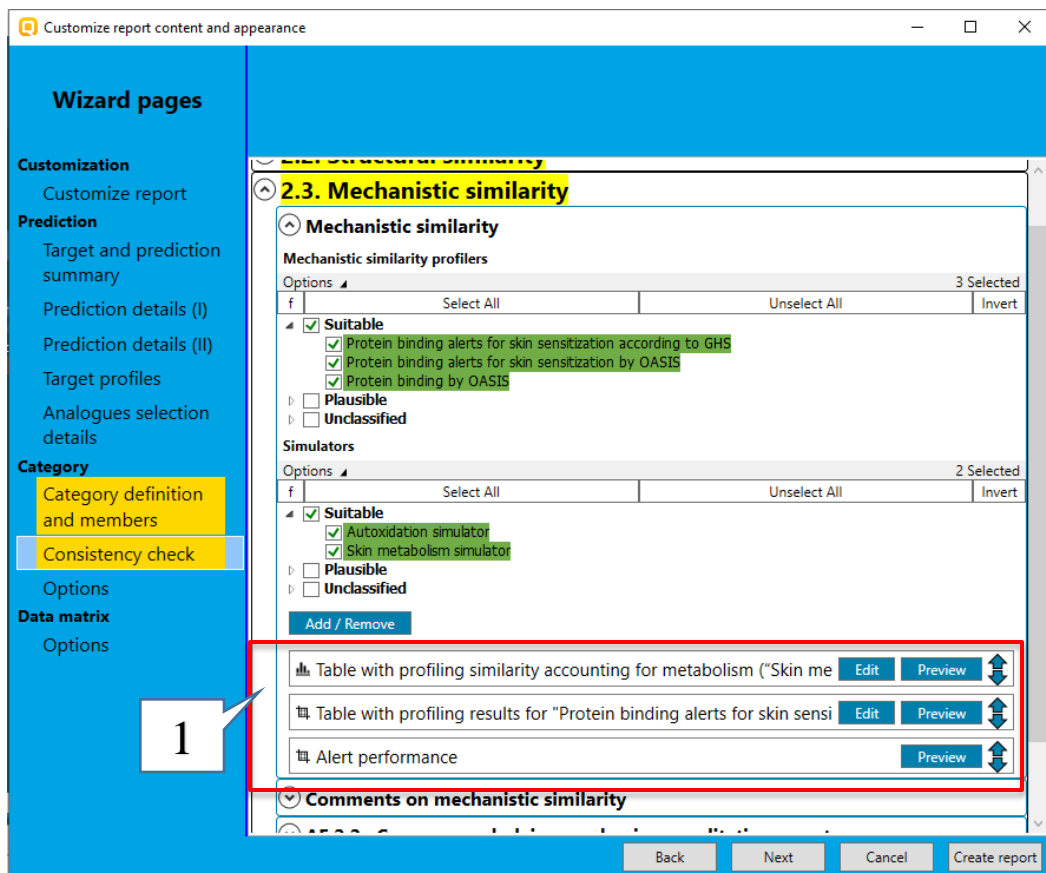
All items in the report basket related to the structural consistency of the category (1) are added automatically.

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

- Structural similarity between Target substance A and 3 source substances A, B and C according to Str.similarity profiler is in the range of [29-88%]
- They all have primary aliphatic amine based on the OFG profiler, while the target substance A and source substance B have additional secondary aliphatic amine and the source substance A has additional tertiary amine functional group.

Report

Assessment elements of Scenario 2



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

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

3

2.1. Physicochemical

2.2. Structural similarity

2.3. Mechanistic similarity

Mechanistic similarity

Comments on mechanism

AE 2.2: Common underlying mechanism, qualitative aspects

Hint

PURPOSE:

The hypothesis/justification has to explain how the compounds the test organism is exposed to lead to the same type of effects/absence of effects. It has to be assessed whether:

- the documentation has established a common underlying mechanism;
- this mechanism links the structures of these compounds under consideration with the possibility to predict qualitatively similar type of effects for the target substance for the property under consideration; and
- the provided evidence supports the explanation.

Add / Remove

Target substance A and source substances A, B and C ... (text provided by user)

The common mechanism of interaction of "Bis aldehydes" with skin proteins is summarized here: (image provided by user)

2.4. Additional endpoints

2.5. Other AEs

2

1

AE 2.2: Common underlying mechanism, qualitative aspects

Target substance A and source substances A, B and C ... (text provided by user)

Target substance A and source substances A, B and C are all metabolized to Bis aldehydes

Bis aldehydes is taken as alerting groups responsible for the toxic effect based on the expert knowledge. Bis aldehydes are taken as alerting group supported by the higher alert performance (82 %) as compared with aldehyde group (45%)

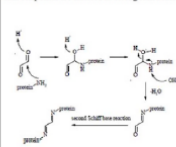
The common mechanism of interaction of "Bis aldehydes" with skin proteins is summarized here: (image provided by user)

Mechanistic Domain: Schiff base formation

Mechanistic Alert: Schiff base formation with carbonyl compounds

Structural Alert: Bis Aldehydes

A chemical with more than one reaction centre with equal reactivity is a potential cross-linking agent. Bis-aldehydes could cross link proteins and as a result have higher skin sensitization potency compared to mono aldehydes. In TMES SS model such compounds are assessed as Strong skin sensitizers.



References:

1. Roberts, D., Apl, A.M., Safford, R., Lako, J. Respiratory Toxicology and Pharmacology 72 (2015) 683–693.

AE 2.2. Common underlying mechanism, qualitative aspects is related to the mechanistic similarity of the final category.

The following example text summarizing the results of the provided mechanistic similarity items can be added (1):

- Target substance A and source substances A, B and C all produce metabolite that is recognized as "Bis aldehyde" according to the "Protein binding alerts for skin sensitization by OASIS" profiler
- As a result of skin metabolism, along with the Bisaldehyde metabolite there are also generated simple aldehydes.
- By applying evaluation of alert performance the results showed that the "Bis aldehyde" has higher positive performance (82%) as compared with the simple aldehyde group (45%).

Additionally, metabolic maps (for each of the analogues), produced by external software or found in the literature, could be included to AE in order to support the mechanistic similarity of the category. In the current case mechanism of interaction of "Bis aldehyde" with skin proteins is added as external report item (2). How the AE looks like in the report is given too (3)

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

2.1. Physicochemical similarity

2.2. Structural similarity

2.3. Mechanistic similarity

2.4. Additional endpoints

2.5. Other AEs

AE 2.3: Common underlying mechanism, quantitative aspects

Hint

PURPOSE:
Under this scenario, there should be no biologically significant quantitative differences for the same type of effects caused by the underlying mechanism or the differences should be used in a conservative prediction (i.e. effects for the target substance are not likely to be under-predicted, worst case approach). It has to be assessed whether:

- the documentation has provided an explanation why a common underlying mechanism leads to the same quantitative outcome (for source and target) with regard to the prediction of the property under consideration; and
- the provided evidence supports the explanation.

Add / Remove

Target substance A is metabolized to Aldehydes and Bis ... (text prc) Edit Preview

AE 2.4: Exposure to other compounds than to those linked to the prediction

AE 2.5: Occurrence of other effects than covered by the hypothesis and justificati

AE A.4: Bias that influences the prediction

Back Next Cancel Create report

AE 2.3. Common underlying mechanism, quantitative aspects is also related to the mechanistic similarity of the final category. The following information could be included here:

1) textual or illustrated explanation why the common underlying mechanism leads to the same quantitative outcome (for source and target) with regard to the prediction of the property under consideration; and

Example text:

- Target substance A is metabolized to Aldehydes and Bis aldehydes
- It is expected that Bis-aldehydes as the alert with higher alert performance is responsible for the toxic effect
- Source substances A, B and C are metabolized to aldehydes and bis-aldehydes, too
- The available experimental EC3 values for the source substances corresponds to the positive effect.
- Similar toxic effects observed in sources substances supports the prediction for the target
- Toxic effects of all source substances and target are supported by the identified additional SS data

2) evidences supporting the explanation.

Include all available SS EC3 data for the target chemical and the source substances in all Toolbox database. See how to do this on the next two slides.

April, 2020

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

2.1. Physicochemical similarity

2.2. Structural similarity

2.3. Mechanistic similarity

2.4. Additional endpoints

2.5. Other AEs

AE 2.3: Common underlying mechanism, quantitative aspects

Hint

PURPOSE:

Under this scenario, there should be no biologically significant quantitative differences for the same type of effects caused by the underlying mechanism or the differences should be used in a conservative prediction (i.e. effects for the target substance are not likely to be under-predicted, worst case approach). It has to be assessed whether:

- the documentation has provided an explanation why a common underlying mechanism leads to the same quantitative outcome (for source and target) with regard to the prediction of the property under consideration and
- the provided evidence supports the explanation.

Add / Remove

Endpoint data variation (1 selected: Human Health Hazards#Sensitisation#Skin#in Vivo#LLNA#EC3) Edit Preview

Target substance A is metabolized to Aldehydes and Bis ... (text provided by user) Edit Preview

AE 2.4: Exposure to other compounds than to those linked to the prediction

AE 2.5: Occurrence of other effects than covered by the hypothesis and justification

AE A.4: Bias that influences the prediction

2.5. Other AEs

AE 2.3: Common underlying mechanism, quantitative aspects

Table with Human Health Hazards data variation

Position	Variation	unit (family)	Number of chemicals
Sensitisation#Skin#in Vivo#LLNA#EC3	0.882 ÷ 5.8	% (Skin sensitization EC3(ratio))	4

Target substance A is metabolized to Aldehydes and Bis ... (text provided by user)

Target substance A is metabolized to Aldehydes and Bis aldehydes

It is expected that Bis-aldehydes as the alert with higher alert performance could be responsible for the toxic effect

Source substances A, B and C are metabolized to aldehydes and bis-aldehydes, too

The available experimental EC3 values for the source substances corresponds to a positive effect.

Similar toxic effects observed in source substances supports the prediction for the target

Toxic effects of all source substances and target are supported by the identified additional SS data

AE 2.4: Exposure to other compounds than to those linked to the prediction

Not provided by user

AE 2.5: Occurrence of other effects than covered by the hypothesis and justification

Not provided by user

QSAR Toolbox 4.4
Database version: 4.4

QSAR TOOLBOX

TPRF v4.4

After creating of the new item, it appears below the AE 2.3. along with the created text item (1). Example on how the AE 2.3. will look in the generated report is shown in right (2).

Report

Assessment elements of Scenario 2

Customize report content and appearance

Wizard pages

Customization

Customize report

Prediction

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category

Category definition and members

Consistency check

Options

Data matrix

Options

2.1. Physicochemical similarity

2.2. Structural similarity

2.3. Mechanistic similarity

2.4. Additional endpoints

2.5. Other AEs

AE 2.3: Common underlying mechanism, quantitative aspects

AE 2.4: Exposure to other compounds than to those linked to the prediction

Hint

Add / Remove

AE 2.5: Occurrence of other effects than covered by the hypothesis and justification

Hint

Add / Remove

AE A.4: Bias that influences the prediction

Hint

Add / Remove

report

Example text for **AE 2.4. Exposure to other compounds than to those linked to the prediction:**

- No impurities are available for the Source substances.
- The target substance A and the source substances A,B and C are enzymatically transformed to the reactive species "bisaldehyde" and simple "aldehyde".
- Aldehydes are not expected to cause skin sensitization effect by the expert knowledge;
- No other reactive metabolites are produced based on the enzymatic transformations.

Example text for **AE 2.5. Occurrence of other effects than covered by the hypothesis and justification:**

- Target substance A and source substances B, C and D metabolize to "Bis aldehyde".
- "Bis aldehyde" is responsible for the skin sensitization effect of the source substances.
- No PBA for chromosomal aberration are identified in the target and source substances, nor in the structures of their metabolites.
- In general, the "aldehyde" moiety is well known alerting group for protein binding. In this respect, all toxicity effects which are based on covalent interaction with protein molecules could be considered as relevant.

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

- Target chemical is activated as result of skin metabolism by producing reactive metabolites – Bisaldehyde and simple aldehyde.
- The source chemicals have been selected based on the same reactivity pattern discovered for the target chemical, i.e. all source chemicals are activated as a result of skin metabolism producing same reactive metabolites – Bis aldehyde and simple aldehyde.
- The mechanism of interaction with skin proteins is Schiff base formation.
- Illustration of the enzymatic transformation is provided to AE 2.1.
- Illustration of the mechanism of interaction of Bis aldehyde to skin proteins is available in AE 2.2.

Example content of the rest AEs which are part of *Consistency check* section is provided on the right. The text is added by creating of new textual report item already explained in the previous slides.

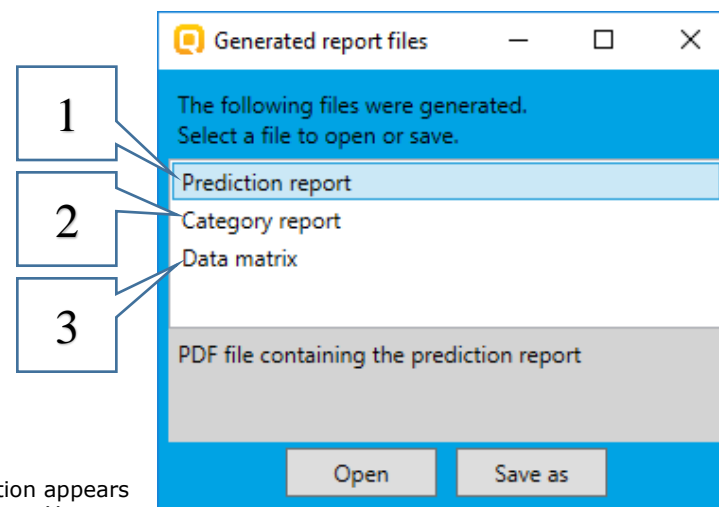
Report Generation

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 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.



*Before appearing of the window with the report files additional window with information appears including how many chemicals used in the prediction belongs to the restricted databases. You can close it.

Report Generated report files

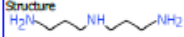
Prediction report

Category report

QSAR Toolbox prediction for single chemical

(in accordance with RAAF scenario 2)

Date: 24 Mar 2020
Author(s):
Contact details:

Target information		
Structural information	Numerical identifiers	Chemical names
SMILES: NCCNCCCN	CAS#: 56-18-8 Other: EC Number:2002612	1,3-Propanediamine, N-(3-aminopropyl)-
Structure 		1,3-Propanediamine, N1-(3-aminopropyl)- 1,3-propanediamine,... n-(3-aminopropyl)-

The selected RAAF scenario is specified in the first page

QSAR Toolbox report for category

(in accordance with RAAF scenario 2)

1. Category definition

1.1. Category definition

Category name

manually editable field

Not provided by the user

Covered (target) endpoint(s)

- Human Health Hazards/Sensitisation: EC3, LLNA, In Vivo, Skin

Category hypothesis

manually editable field

Not provided by the user

1.2. Category members

Location of category members
Table of category members

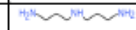



CAS	Name	SMILES	Structure
56-18-8	Iminobis-3-propylamine	NCCNCCCN	
109-55-7	DMAPA	CN(C)CCCN	
107-15-3	Ethylenediamine	NCCN	
111-40-0	DETA	NCCNCCN	

Table for selected physicochemical properties and calculated parameters





Table with 2D parameters data variation

Parameter name	Variation	unit (family)
Boiling point	103 + 228	°C(Temperature)
	-2.13 + -0.45	
Weight	60.1 + 131	Da(Mass)
Vapor Pressure (Antoine method)	0.0913 + 19	mm Hg(Pressure)
Water Solubility	1E+06	mg/L(Mass concentration)

Table with Physical Chemical Properties data variation

Parameter name	Variation	unit (family)
Boiling point	103 + 228	°C(Temperature)
	-2.13 + -0.45	
Weight	60.1 + 131	Da(Mass)
Vapor Pressure (Antoine method)	0.0913 + 19	mm Hg(Pressure)
Water Solubility	1E+06	mg/L(Mass concentration)

Data matrix report

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Target chemical													
2	Neighbour #1													
3	Neighbour #2													
4	Neighbour #3													
5	Structure													
6	CAS number	56-18-8	109-55-7	107-15-3	111-40-0									
7	Chemical name	Iminobis-3-propylamine	DMAPA	Ethylenediamine	NCCNCCN									
8	Other identifier													
9	SMILES	NCCNCCCN	CN(C)CCCN	NCCN	NCCNCCN									
10	Parameters	unit												
11	Boiling point	°C	228	134	103	189								
12	log Kow		-1.15	-0.45	-1.62	-2.13								
13	Molecular Weight	Da	131	102	60.1	103								
14	Vapor Pressure (Antoine method)	mm Hg	0.0913	9.41	19	0.274								
15	Water Solubility	mg/L	1E+06	1E+06	1E+06	1E+06								
16	Profiles													
17	Profiles used for grouping/subcategorization													
18	Using of "skin metabolism simulator" Combined parent and products requirements: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes	Parent and 5 metabolite(s); Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes	Parent and 12 metabolite(s); Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes	Parent and 5 metabolite(s); Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes	Parent and 5 metabolite(s); Has all of the required categories: Schiff base formation >> Schiff base formation with carbonyl compounds >> Bis aldehydes; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes									
19	Protein binding alerts for skin sensitization by	No alert found	No alert found	No alert found	No alert found									
20	Structure similarity (subcategorization)	[90%,100%]	[30%,40%]	[60%,70%]	[80%,90%]									
21	General Mechanistic													
22	Protein binding by OASIS, with Autoxidation	No alert found;	No alert found;	No alert found;	No alert found;									
23		Schiff base formation >> Schiff base	Schiff base formation >> Schiff base	Schiff base formation >> Schiff base	Schiff base formation >> Schiff base									

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 2.
- Note, proficiency comes with practice!