

OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 3 and related
assessment elements

Outlook

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

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data gap filling exercise and assessing of the outcome whether read across is scientifically acceptable or not
- The read-across prediction will be justified by fulfilling all information requirements according to the Read Across Assessment Framework (RAAF).

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Keywords

TARGET CHEMICAL - chemical of interest

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

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

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

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

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

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

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

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Define target endpoint;
- Relevancy of profiles and data availability;
- Searching of analogues accounting for metabolism;
- Category consistency check;
- Selection of a RAAF scenario;
- Filling in the report sections related to each read across assessment element.

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Specific Aims

- To familiarize the user with the Read Across Assessment Framework (RAAF) and more specifically with Scenario 3;
- To explain to the user how to search for analogues producing a common metabolite;
- To introduce to the user the read across assessment elements (AE) and to provide examples with possible content of them;
- To introduce to the user the report basket;
- To provide to the Toolbox user the rationale behind each step of the exercise.

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Read Across Assessment Framework (RAAF)

Overview

- RAAF has been developed by ECHA as an internal tool providing a framework for a consistent and structured assessment of grouping and read across approaches under REACH.
- The outcome of the assessment is a conclusion on whether the read across is scientifically acceptable or not.
- The RAAF defines different scenarios for different read-across approaches.
- Each scenario is associated with particular aspects (assessment elements, AEs).
- Total six scenarios are available: two for an analogue approach and four for a category approach

Read Across Assessment Framework (RAAF)

Criteria for the different RAAF scenarios

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

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

Read Across Assessment Framework (RAAF)

Selection of a RAAF scenario

1. Distinguish whether an analogue or a category approach is decided based on the number (N) of analogues*:
 - a) N of analogues ≤ 3 is an Analogue approach (scenario 1-2)
 - b) N of analogues > 3 is a Category approach (scenario 3-6)
2. To identify the basis of the read across hypothesis
 - a) (Bio)transformation to common compound(s) – the read across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed
 - b) Different compounds have the same type of effect(s) – the read across hypothesis is that the organism is not exposed to common compounds but rather, as a result of similarity, that different compounds have similar (eco)toxicological and fate properties. These compounds may be the **source** and **target substances themselves** or **one or more of their (bio)transformation products**.
3. For a category approach (scenario 3-6) there is a need to take further account whether or not a quantitative variations in the properties are observed among the category members:
 - a) There is quantitative variation in the (eco) toxicity when it is more than 1 log units** (scenario 3 and 4)
 - b) A quantitative variation is not expected in the (eco) toxicity when it is less or equal to 1 log unit (scenario 5-6)

* The threshold for the number of analogues which distinguishes an analogue from a category approach is proposed by LMC

**The quantitative variation in the (eco)toxicity of 1 log unit is proposed by LMC due to empirically observations.

Read Across Assessment Framework (RAAF)

Selection of a RAAF scenario

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

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

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The Exercise

- In this exercise we will predict *Skin Sensitization* of Eugenol [CAS# 97-53-0], which will be the “target” chemical;
- The target endpoint will be preliminary defined;
- The category will be defined based on analogues having a common metabolite produced after a skin metabolism;
- A read-across approach will be used for the prediction. The prediction will be based on a category approach relying on a common metabolite generated for the source and the target substances;
- Read across assessment elements will be included to the report.
- Examples for the possible content of each of AEs will be provided.

The Exercise On Skin Sensitization

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

Outlook

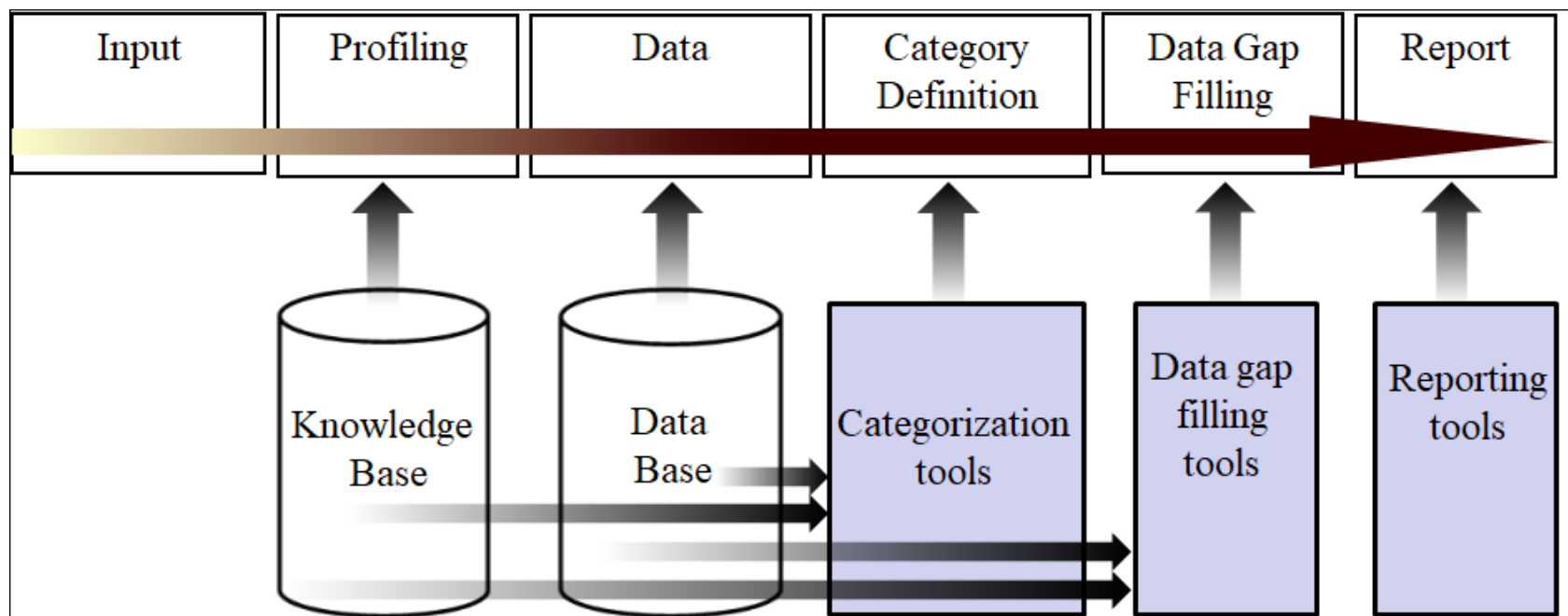
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Workflow

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

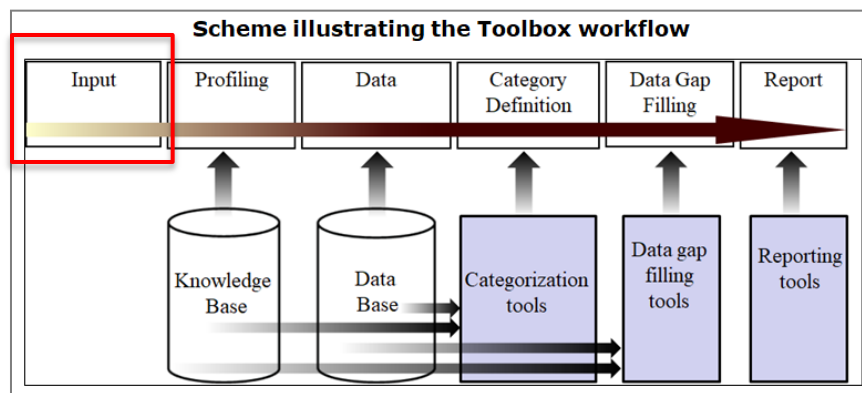
Workflow

Scheme illustrating the Toolbox workflow



Input Overview

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



Input

Input target chemical by CAS#

The screenshot shows the QSAR Toolbox software interface. The 'Input' menu is highlighted in the top toolbar. A search dialog box is open, showing the search results for CAS# 97-53-0. The results table lists the chemical name as 'eugenol (4-allyl-2-methoxyphenol)' and shows its chemical structure. The 'OK' button is highlighted in the dialog box.

1	CAS	97-53-0
	SMILES	COc1cc(CC=C)ccc1O
	CS Relation	High
	Substance	Mono constituent
	Composition	
	Name	eugenol (4-allyl-2-methoxyphenol)
	Sources	NICNAS Canada DSL

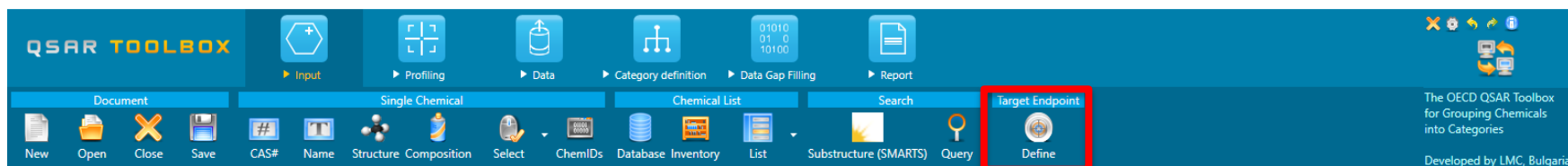
1. Click **CAS#**;
2. Enter the **CAS# 97-53-0** in the blank field;
3. Click **Search**;
4. When the structure with the requested CAS # appears, click **OK**.

Input

Define target endpoint

Defining of the endpoint allows entering the endpoint of interest e.g. EC3, Chromosome aberration, LC50 etc., along with specific metadata information. Based on the metadata, different relevancy scores for profiles could be provided for same endpoint.

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



Input

Define target endpoint

The screenshot shows the QSAR Toolbox software interface. The top toolbar has a 'Target Endpoint' button (1) with a 'Define' sub-button. Below the toolbar, the 'Documents' panel on the left shows 'Document 1' with CAS# 97530. The main workspace displays a chemical structure and a 'Filter endpoint tree...' on the left. The 'Select endpoint' dialog box is open on the right, showing a tree view of endpoints. The 'Human Health Hazards' category is expanded (2), and 'Sensitization' is selected (3). The 'Next' button at the bottom right of the dialog is highlighted (4).

1. Click **Define** button;

2. Open *Human health hazards*;

3. Select **Sensitization**;

4. Click **Next**.

Input

Define target endpoint

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

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

Select endpoint

Human Health Hazards
Sensitisation

Organ
Type of method
Assay
Endpoint

Skin
in Vivo
LLNA
EC3

Selection of additional metadata fields:

Add
Up
Down
Clear
Remove

Undefine
Back
Finish

Input

Define target endpoint

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

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Document', 'Single Chemical', 'Chemical List', 'Search', and 'Target Endpoint'. The 'Target Endpoint' menu is currently active, showing options like 'Define'. The main workspace is divided into two panes. The left pane, titled 'Filter endpoint tree...', shows a hierarchical tree of toxicity endpoints. The right pane, titled '1 [target]', shows the chemical structure of the target endpoint, which is a phenol derivative. The 'Define' button in the 'Target Endpoint' menu is highlighted with a red rectangle. The 'EC3' endpoint in the tree is highlighted with a yellow background.

Documents

Document 1
[C: 1;Md: 0;P: 0] CAS: 97530

Filter endpoint tree...

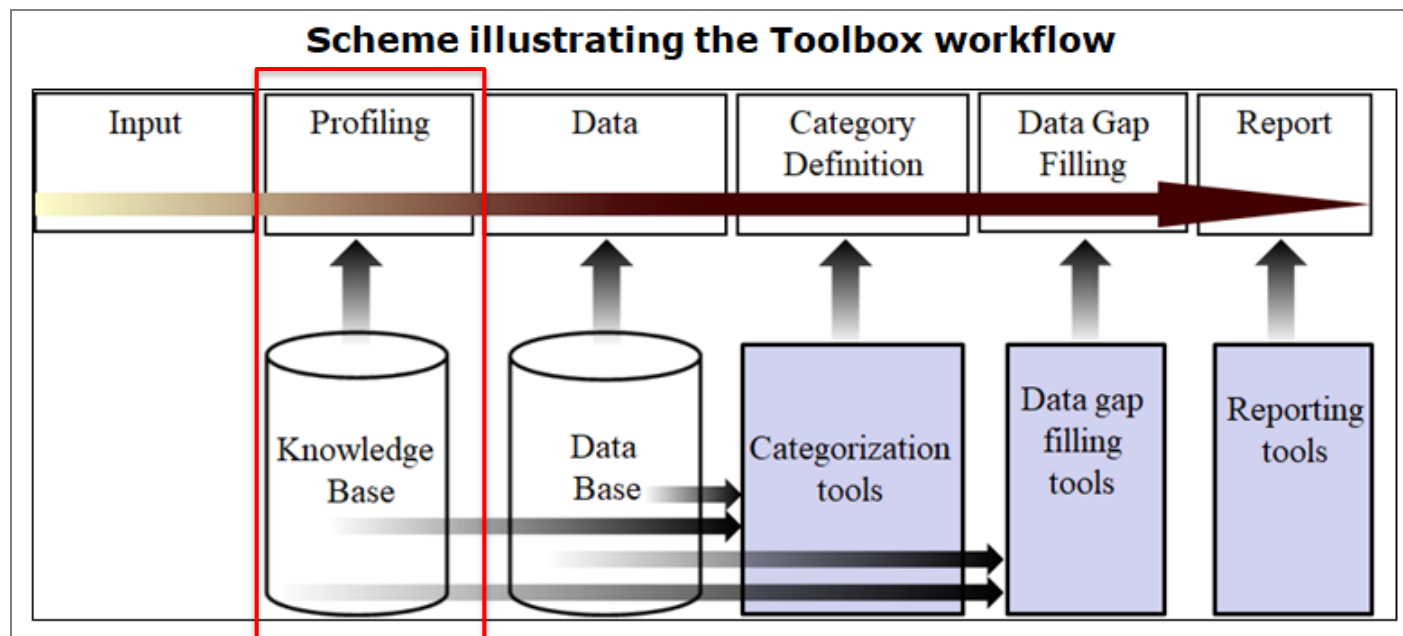
Structure

Genetic Toxicity
Immunotoxicity
Irritation / Corrosion
Neurotoxicity
Photoinduced toxicity
Repeated Dose Toxicity
Sensitisation
Skin
in Vivo
LLNA
EC3

1 [target]

Chemical structure: CC1=CC=C(C=C1)O

Profiling Overview



Profiling Overview

- “Profiling” refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database;
- “Profiling” module contains all the knowledge in the system coded in profiling schemes (profilers);
- “Profilers” are a collection of empirical and mechanism knowledge (expertly derived) which could be used to analyse the structural properties of chemicals;
- The “profilers” identify the affiliation of the target chemical(s) to preliminary defined categories (functional groups/alerts);
- The “Profiling” module contains also observed and simulated metabolisms/transformations, which could be used in combination with the profiling schemes;
- The outcome of the profiling determines the most appropriate way to search for analogues, but they are also useful for preliminary screening or prioritization of substances;
- The “profilers” are not (Q)SARs, i.e. they are not prediction models themselves;
- Based on the “profilers’ relevancy” (determined by the defined target endpoint), the most suitable 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 module. The interface includes a top toolbar with icons for Profiling, Data, Category definition, Data Gap Filling, and Report. Below the toolbar is a sub-menu with 'Apply', 'View', 'New', and 'Delete'. The main workspace is divided into three panels: 'Documents', 'Profiling methods', and 'Metabolism/Transformations'. The 'Documents' panel shows 'Document 1' with chemical information. The 'Profiling methods' panel has a list of methods with checkboxes, including 'Suitable' (checked) and 'Plausible'. The 'Metabolism/Transformations' panel also has a list of methods with checkboxes, including 'Suitable' (checked) and 'Plausible'. The 'Filter endpoint tree...' panel on the right shows a hierarchical tree of endpoints, with 'EC3' highlighted. A chemical structure of a target chemical is displayed in the top right corner.

1. Go to **Profiling** module

2. Select all **suitable** profiling schemes and simulators

3. Click on **Apply**

Profiling

Profiling results

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

See on the next slide

Profiling

Profiling results

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Filling Report

Profiling Custom profile

Apply View New Delete

Documents

Document 1
[C: 1;Md: 0;P: 0] CAS: 97530

Profiling methods

Options 3 Selected

Select All Unselect All Invert

☒ Suitable

- ☒ Protein binding alerts for skin sensitization
- ☒ Protein binding alerts for skin sensitization
- ☒ Protein binding by OASIS

☐ Plausible

- ☐ Aquatic toxicity classification by ECOSAR
- ☐ Chemical elements
- ☐ Groups of elements

Metabolism/Transformations

Options 2 Selected

Select All Unselect All Invert

☒ Suitable

- ☒ Autoxidation simulator
- ☒ Skin metabolism simulator

☐ Plausible

- ☐ Autoxidation simulator (alkaline medium)
- ☐ Dissociation simulator

Filter endpoint tree...

Structure

1 [target]

Endpoint Specific

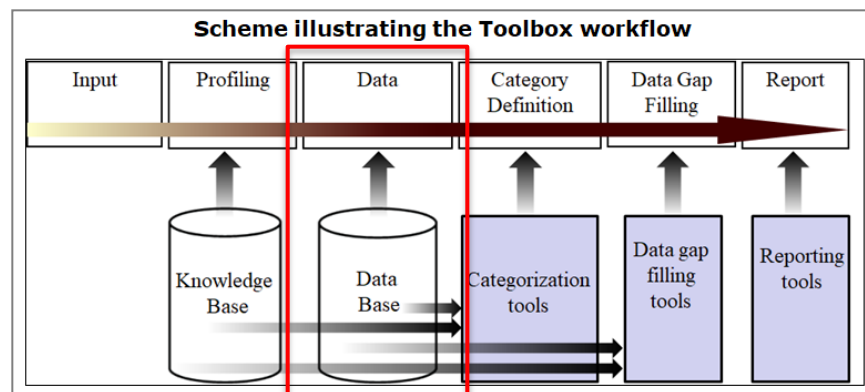
- Protein binding alerts for skin sensitization No alert found
- Protein binding alerts for skin sensitization No alert found

Metabolism/Transformation

- Autoxidation simulator** 5 metabolite(s)
 - General Mechanistic
 - Protein binding by OASIS
 - 1 x Michael addition
 - 1 x Michael addition >> Michael addition on conj...
 - Endpoint Specific
 - Protein binding alerts for skin s... 1 x Skin sensitization Category 1A
 - Protein binding alerts for skin sensitization by OASIS
 - 1 x Michael Addition
 - 1 x Michael Addition >> Michael addition on qin...
- Skin metabolism simulator** 5 metabolite(s)
 - General Mechanistic
 - Protein binding by OASIS
 - 2 x Michael addition
 - Endpoint Specific
 - Protein binding alerts for skin s... 3 x Skin sensitization Category 1A
 - Protein binding alerts for skin sensitization by OASIS
 - 2 x Michael Addition
 - 2 x Michael Addition >> Michael addition on qin...

Data Overview

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



Data

Gather data

The screenshot illustrates the 'Gather data' workflow in the QSAR Toolbox. The process involves navigating through the 'Data' module, selecting specific databases from the 'Databases' list, filtering endpoints to 'Sensitisation', and finally clicking 'OK' to read the data.

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

Data

Gather data

The screenshot shows the QSAR Toolbox interface. The 'Data points' window is open, displaying a table with columns: Datapoints, #, Value, Original value, and Assay. The table contains two rows of data for 'Human Health Hazards;Sensitisation'.

Datapoints	#	Value	Original value	Assay
Human Health Hazards;Sensitisation	1	M: 5.4 % (Skin sensitization EC3 (ratio))	5.4 % (Skin sensitization EC3(ratio))	LLNA
Human Health Hazards;Sensitisation	2	M: 5.4 % (Skin sensitization EC3 (ratio))	5.4 % (Skin sensitization EC3(ratio))	LLNA

A callout box (1) indicates: 36 points added across 1 chemicals. OK

A callout box (2) points to the 'EC3' endpoint in the hierarchical tree, which shows 1/23 data points.

A callout box (3) points to the 'EC3' endpoint in the hierarchical tree, which shows 1/23 data points.

Experimental data for EC3 has been found for the target varying from 5.4 to 41 %

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

Data

Gather data

- Toxicity information on the target chemical is electronically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues is performed only among the chemicals which are listed in the selected database(s). In this example these are only the *ECHA REACH, Skin Sensitization and REACH Skin sensitization database (normalised)*.

Recap

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

Investigation of a skin metabolism for the target chemical

The screenshot shows the QSAR Toolbox interface. In the 'Documents' panel, a right-click context menu is open over 'Document 1' (CAS: 97530). The menu path 'Multiplication > Metabolism/Transformations > Skin metabolism simulator' is highlighted. The 'Databases' panel on the left shows various toxicity endpoints, with 'ECHA REACH' and 'REACH Skin sensitisation database' checked. The 'Filter endpoint tree...' window is also visible, showing a list of endpoints including 'Skin sensitisation'.

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



Result:

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

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

Investigation of a skin metabolism for the target chemical

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Filling Report

Data Import Export Delete

Gather Import IUCLID6 IUCLID6 Database Inventory

Documents

Document 1

- [C: 1;Md: 36;P: 0] CAS: 97530
- [C: 6;Md: 36;P: 0] Skin metabolism
 - [C: 1;Md: 0;P: 0] metabolite #1
 - [C: 1;Md: 0;P: 0] metabolite #2
 - [C: 1;Md: 0;P: 0] metabolite #3
 - [C: 1;Md: 0;P: 0] metabolite #4

Databases

Options 3 Selected

Select All Unselect All Invert

- ☒ ECHA REACH
- ☐ ECOTOX
- ☐ Eye Irritation ECETOC
- ☐ Food TOX Hazard EFSA
- ☐ GARD Skin sensitization
- ☐ Genotoxicity & Carcinogenicity ECVAM
- ☐ Genotoxicity OASIS
- ☐ Genotoxicity pesticides EFSA
- ☐ Human Half-Life
- ☐ Keratinocyte gene expression Givaudan
- ☐ Keratinocyte gene expression LuSens
- ☐ Micronucleus ISSMIC
- ☐ Micronucleus OASIS
- ☐ MUNRO non-cancer EFSA
- ☒ REACH Skin sensitisation database (no)

Filter endpoint tree...

Structure

- Immunotoxicity
- Irritation / Corrosion
- Neurotoxicity
- Photoinduced toxicity
- Repeated Dose Toxicity
- Sensitisation
 - in Vivo
 - GPMT 1/3 M: sensitising
 - HRIP 1/3 M: 8E+03 µg/cm2
 - LLNA
 - EC3 1/23 M: 5.4 %
 - SI 1/5 M: 1.2
 - Skin sensitisation 1/2 M: sensitising
- ToxCast
- Toxicity to Reproduction
- Toxicokinetics, Metabolism and Distribution

Parent chemical... metabolite #1 metabolite #2 metabolite #3 metabolite #4 metabolite #5

Chemical structures are displayed for the parent chemical and its five metabolites.

Information

A parent list with 5 child lists were created

OK

1. Metabolites appeared next to the parent.

Investigation of a skin metabolism for the target chemical

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

Result:

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

1. Go to **Profiling** module;

2. Select **suitable profilers** (green highlighted);

3. Unselect all metabolism simulators

4. Click **Apply**.

Profile of the generated skin metabolites

Parent chemical...	metabolite #1	metabolite #2	metabolite #3	metabolite #4	metabolite #5
<chem>O=C1C=CC(=O)C=C1</chem>	<chem>O=C1C=CC(=O)C=C1</chem>	<chem>O=C1C=CC(=O)C=C1</chem>	<chem>O=C1C=CC(=O)C=C1</chem>	<chem>O=C1C=CC(=O)C=C1</chem>	<chem>O=C1C=CC(=O)C=C1</chem>
1/36 M: sensitising					
No alert found	Michael addition	Schiff base form...	Michael addition	No alert found	No alert found
No alert found	Skin sensitization...	Skin sensitization...	Skin sensitization...	No alert found	No alert found
No alert found	Michael Addition	Schiff base form...	Michael Addition	No alert found	No alert found

Investigation of a skin metabolism for the target chemical

The screenshot shows the QSAR Toolbox software interface. The 'Data' module is selected in the top toolbar (labeled 1). The 'Gather' button is highlighted in the bottom left (labeled 3). A dialog box 'Read data?' is open, showing a list of endpoints with 'Sensitisation' selected (labeled 4). The 'Databases' list on the left shows several databases highlighted in green, including 'ECHA REACH', 'ECOTOX', 'Eye Irritation ECETOC', 'Food TOX Hazard EFSA', 'GARD Skin sensitization', 'Genotoxicity & Carcinogenicity ECVAM', 'Genotoxicity OASIS', 'Genotoxicity pesticides EFSA', 'Human Half-Life', 'Keratinocyte gene expression Givaudan', 'Keratinocyte gene expression HESS', 'Micronucleus ISSMIC', 'Micronucleus OASIS', 'MUNRO non-cancer EFSA', 'REACH Skin sensitisation database (no data)', 'Receptor Mediated Effects', 'Rep Dose Tox Fraunhofer ITEM', 'Repeated Dose Toxicity HESS', 'Rodent Inhalation Toxicity Database', 'Skin Irritation', 'Skin Sensitization', and 'Skin sensitization ECETOC' (labeled 2). The main table displays results for the parent chemical and five metabolites. The table has columns for 'Parent chemical...', 'metabolite #1', 'metabolite #2', 'metabolite #3', 'metabolite #4', and 'metabolite #5'. The table shows chemical structures and associated data for each metabolite.

Parent chemical...	metabolite #1	metabolite #2	metabolite #3	metabolite #4	metabolite #5
2 M: sensitising		MS: sensitising			
5 M: 8E+03 µg/cm2		MS: 370 µg/cm2			
EC3 2/63 M: 5.4 %		MS: 0.113 %			
SI 2/12 M: 1.2		MS: 1			
Skin sensitisation 2/9 M: sensitising		MS: Category 1A...			
Miscellaneous 1/29		MS: Ambiguous			
Undefined Type of Method 1/1				MS: not sensiti...	
No alert found	Michael addition	Schiff base form...	Michael addition	No alert found	
No alert found	Skin sensitization	Skin sensitization	Skin sensitization	No alert found	
No alert found	Michael Addition	Schiff base form...	Michael Addition	No alert found	

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

Result:

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

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

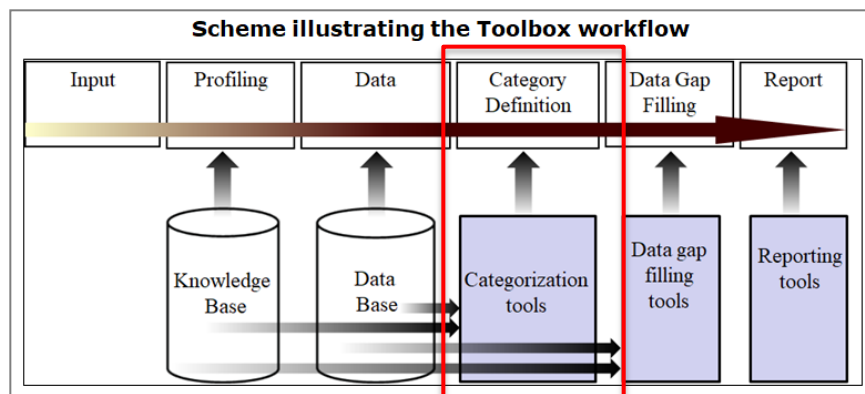
Handling skin metabolism

Recap

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

Category Definition Overview

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



Category Definition

Grouping methods

- The different grouping methods allow the user to group chemicals into chemical categories according to different measures of “similarity” so that within a category data gaps can be filled by read-across.
- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind by the same mechanism and for which experimental results are available.
- If no alert is identified in the target structure, but is identified in its metabolites, analogues can be searched accounting for metabolism. In this way the target chemical and the identified analogues will have similar metabolic pattern.
- In our case searching for the analogues is based on a common metabolite (formaldehyde) generated as a result of skin metabolism. In other words we will search for the analogues having the same metabolite (i.e. formaldehyde) as the target chemical (see next slide).

Category Definition

Searching for analogues accounting for skin metabolism

1. Go to **Category definition** module;

2. Click on the level with #CAS:97530;

3. Click **Define with metabolism**;

4. Select **Skin metabolism simulator**;

5. Click **OK**;






6. Target and all metabolites produced by the selected simulator (SS in this case) appear.

Category Definition

Searching for analogues accounting for skin metabolism

Grouping options (Skin metabolism simulator)

☒ All queries ☐ At least one

Chemical	Query	Criteria
Parent 	none	No criteria.
Metabolite 1 	none	No criteria.
Metabolite 2 	none	No criteria.
Metabolite 3 	<div> <div>none</div> <div>none</div> <div>Exact match</div> <div>Parametric</div> <div>Profile</div> <div>Similarity</div> <div>none</div> </div>	No criteria.
Metabolite 4 	none	No criteria.
All chemicals		
Parent & Metabolites	none	No criteria.

Alert performance

Scales

Calculate

OK Cancel

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

1. Scroll down to **Metabolite # 2** (*Formaldehyde*) and select **Exact match** option from the drop-down menu;
2. Click **OK** in Grouping options window to execute the search.

More details for grouping with metabolism could be found in the following tutorial:
Tutorial_20_TB_4.4_New options for grouping with metabolism.pdf

Category Definition

Searching for analogues accounting for a skin metabolism

The screenshot displays the QSAR Toolbox software interface. The 'Category definition' workflow is active. A 'Read data?' dialog box is open, showing a list of endpoints with 'Sensitization' selected. A 'Gather data' dialog box is also open, indicating '1573 points added across 518 chemicals'. The main data matrix shows a yellow row for 'EC3' with 174/297 data points. A text box states: 'A category of 174 chemicals with 297 experimental EC3 data has been defined.'

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

Data Gap Filling Overview

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

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

Data Gap Filling

Apply Read-across

The screenshot shows the Q SAR Toolbox interface. The 'Data Gap Filling' workflow is selected. The 'Read across' button is highlighted with a red circle and a '2' callout. A 'Possible data inconsistency' dialog box is open, showing a list of assays and endpoints. The 'Skin sensitization EC3 (ratio)' option is selected and highlighted with a red circle and a '3' callout. A yellow row in the 'Filter endpoint tree...' table is highlighted with a red circle and a '1' callout.

Structure	1 [target]	2	3
in Chemico	24/67		
in Vitro	33/218		
in Vivo			
Buehler Test	34/38		
Draize Test	5/7		
Freund's Complete Adjuvant T...	3/3		
GPMT	180/294	M: sensitising	
HR IPT	11/19	M: 8E+03 µg/cm ²	
Intracutaneous Test	2/2		
LLNA			
EC3	174/297	M: 5.4 %	
Other Endpoint	16/39		
SI	83/288	M: 1.2	M: 0.94
Skin sensitisation	187/212	M: sensitising	M: not ser
Maurer Optimisation Test	2/2		
Miscellaneous	20/31		
Mouse Ear Swelling Test	1/1		
Mouse Local Lymph Node Ass...	8/33		
Not Specified	1/1		
Open Epicutaneous Test	4/4		
Other Assay	10/10		
Split Adjuvant Test	3/3		

Possible data inconsistency

Metadata

- Assay
 - LLNA (174 chemicals; 297 data)
- Endpoint
 - EC3 (174 chemicals; 297 data)
- Native scale/unit
 - Skin sensitisation I (Oasis) (14 chemicals; 14 data)
 - Skin sensitisation II (ECETOC) (82 chemicals; 87 data)
 - Skin sensitization EC3(ratio) (88 chemicals; 196 data)
- Organ
 - Skin (174 chemicals; 297 data)
- Type of method
 - in Vivo (174 chemicals; 297 data)

Select scale/unit to use

- ☐ Skin Sensitization (Danish EPA) [0 native data and 297 converted]
- ☐ Skin sensitisation I (Oasis) [14 native data and 278 converted]
- ☐ Skin sensitisation II (ECETOC) [87 native data and 210 converted]
- ☒ Skin sensitization EC3(ratio) [196 native data and 0 converted]
- ☐ Skin sensitization GHS (ordinal) [0 native data and 286 converted]

Chemicals 88/174; Data 196/297

OK Cancel

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

Data Gap Filling Apply Read-across

The screenshot displays the QSAR Toolbox interface with five subcategory windows (Sub.1 to Sub.5) and a read-across prediction plot.

Sub.1: Shows the 'Oncologic Primary Classification' and 'Protein binding alerts for Chromosomal ab'.

Sub.2: Shows 'Organic functional groups, Norbert Haider (checkmol)'.

Sub.3: Shows 'Organic functional groups'.

Sub.4: Shows 'US-EPA New Chemical Categories'.

Sub.5: Shows 'Structure similarity'.

The read-across prediction plot shows the relationship between EC_{50} [%] (Y-axis) and $\log K_{ow}$ (X-axis). The plot title is 'Read-across prediction for EC3, based on 6 values' and the text below the plot is 'Observed: from 5.4 to 40.9 %; Predicted: 11.1 %'.

The plot shows a single data point at $\log K_{ow} \approx 1.2$ and $EC_{50} \approx 40$ %.

The right sidebar contains a 'Select / filter data' button, a 'Gap filling approach' button, and a list of categories: Descriptors / data, Model/QSAR, Calculation options, Visual options, Information, and Miscellaneous.

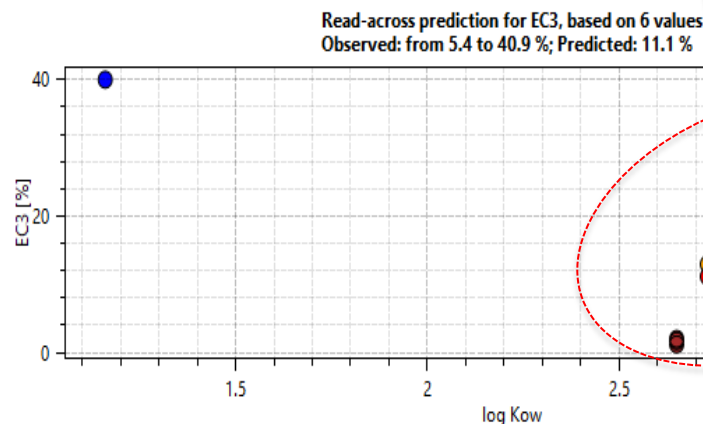
Open **Select/Filter data** and **Subcategorize** by: 1) Protein binding alerts for skin sensitization by OASIS; 2) Organic functional groups, Norbert Haider (checkmol); 3) Organic functional groups; 4) US-EPA New Chemical Categories. 5) Str.similarity – remove analogues with similarity less than 50%. After each applied subcategorization, remove dissimilar analogues using "Remove selected" button.

Data Gap Filling Read-across recap

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

Analogues used in RA prediction

[target]	373	394	395	421	424	425	595
M: 5.4 %	M: 32 %	M: 13 %	M: 17 %	M: 1.2 %	M: <2 %	M: 0.5 %	M: Weakly p



EC3 is in the range from 1.7 to 32% for the 6 closest analogues

✓ Accept prediction

Data Gap Filling

Apply Category consistency elements

The screenshot shows the QSAR Toolbox software interface. The top toolbar includes buttons for Input, Profiling, Data, **Category definition** (1), and Report. Below the toolbar is a 'Categorize' section with buttons for Define, Define with metabolism, Subcategorize, Combine, and Clustering. The 'Category consistency' button (2) is highlighted. The main window displays the 'Category consistency wizard' with a 'Wizard pages' sidebar on the left. The 'Physicochemical similarity' page is active, showing '2D/3D parameters' and 'Physico-chemical data'. A 'Read-across prediction for EC3, based on 6 values' plot is shown, with 'Observed: from 5.4 to 40.9 %; Predicted: 11.1 %'. The 'OK' button (3) is highlighted. On the right, a 'Select / filter data' panel contains buttons for Subcategorize, Mark chemicals by WS, Mark chemicals by descriptor value, Filter points by test conditions, Mark focused chemical, Mark focused points, Remove marked data, and Clear existing marks. The 'Accept prediction' button (4) is highlighted with a green checkmark.

After subcategorization process go back go the **Category definition** module (1) and apply **Category elements*** (2). No different selection than the default is needed – click **OK** (3). Once the category elements are **applied** **accept** the prediction (4).

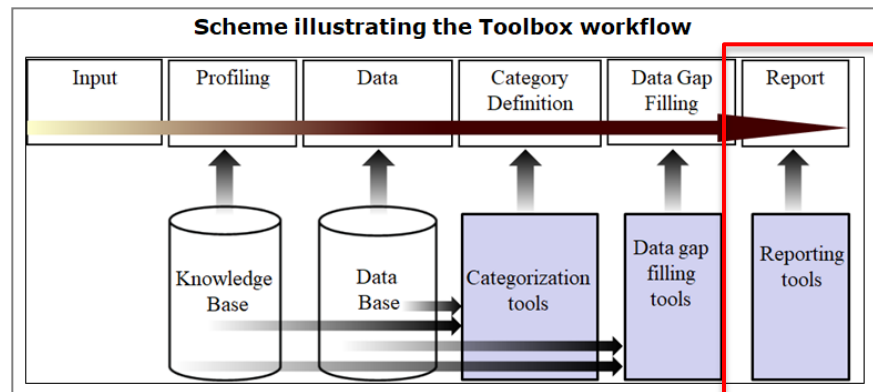
*For more information on category elements see [Tutorial_27_TB 4.4. Category elements for assessing Category consistency](#)

Recap

- In the *Category definition* module you found 173 chemicals with EC3 data having common metabolites (formaldehyde) as a result of skin metabolism.
- In *Data gap filling* module you applied a read-across approach. Read-across is the appropriate data-gap filling method for a “qualitative” endpoints like skin sensitisation. Five subcategorizations based on a protein binding mechanism and structural features are applied. As a result read-across prediction is based on the 6 closest analogues.
- Significant variation of EC3 data is observed for the closest analogues.
- Category consistency was checked by applying the category elements.
- You are now ready to complete the final module and to create the report.
- Click “Report” to proceed to the last module.

Report Overview

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

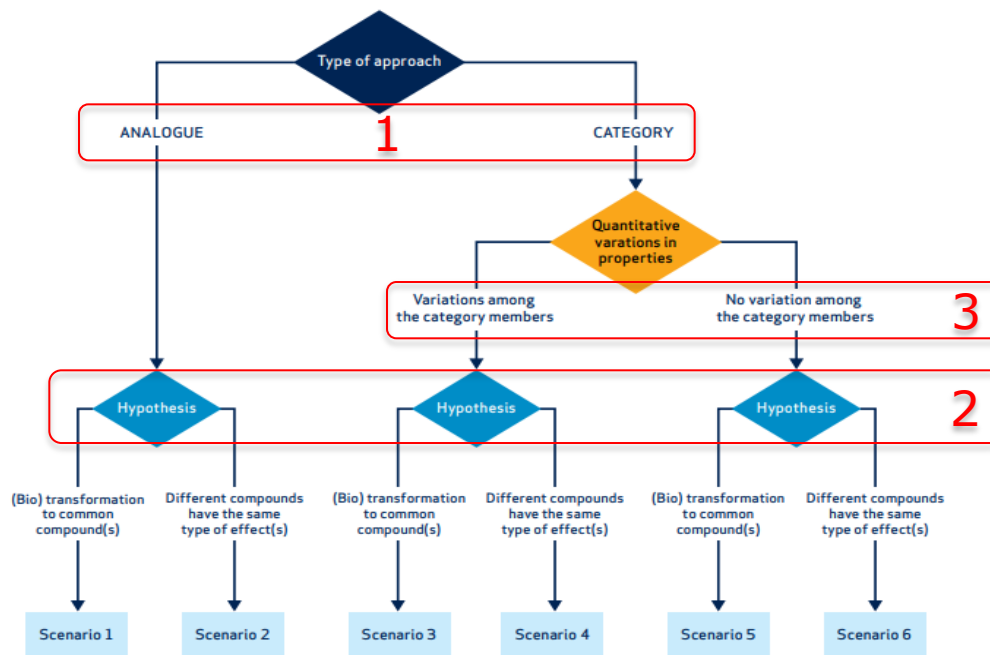


Report

Selection of a RAAF scenario

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

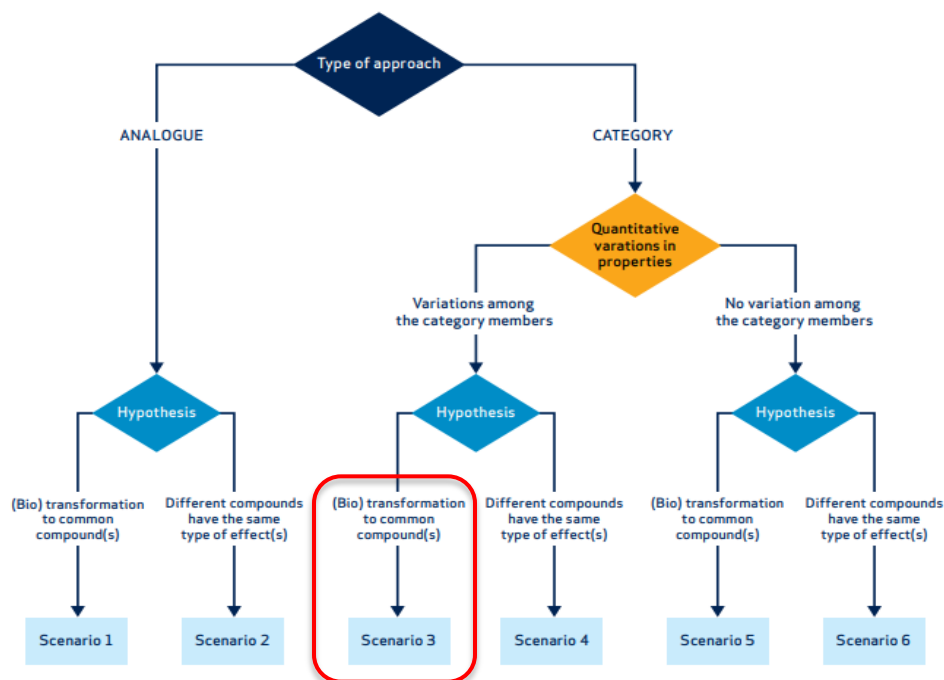
- 1) the type of approach applied - an analogue approach or a category approach;
- 2) the read-across hypothesis;
- 3) For a category approach - whether quantitative variations in the properties are observed among the category members must be considered.



*Read-Across Assessment Framework (RAAF) available at https://echa.europa.eu/documents/10162/13628/raaf_en.pdf
The OECD (Q)SAR Toolbox for Grouping Chemicals into Categories

Report

Selection of a RAAF scenario



For this example the following criteria are met:

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

Based on that a RAAF scenario 3 was identified as the most appropriate for the current example.

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

Report

Report Generation according to RAAF-Scenario 3

1. Go to the **Report** section;

2. Select a cell with prediction;

3. Click **Prediction**;

4. Check the box at the top to add RAAF scenario;

5. Select **Scenario 3** from the drop-down menu.

Report

Report Generation according to RAAF-Scenario 3

Customize report content and appearance

Select which sections to include into report by checking/unchecking the corresponding section box. Rearrange sections order of appearance by using buttons "Move Up" and "Move Down".

Wizard page 4

Customization

Customize report

Prediction 1

Target and prediction summary

Prediction details (I)

Prediction details (II)

Target profiles

Analogues selection details

Category 2

Category definition and members

Consistency check

Options

Data matrix 3

Options

Add RAAF scenario 5

☒ **Prediction**

☒ Target and prediction summary

☒ Prediction details (I)

☒ Prediction details (II)

☒ Target profiles

☒ Analogues selection details

☐ Appendix: Grouping / subcategorization

☐ Appendix: Data pruning

☐ Appendix: Specific report explanations

☒ **Category**

☒ Category definition and members

☒ Consistency check

☒ Options

☒ **Data matrix**

☒ Options

☐ Remove password protection of the PDF files.

Note: If the protection is removed, this will be specified in the first page of the report

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

Report

Report Generation according to RAAF-Scenario 3

The figure consists of three screenshots of the 'Customize report content and appearance' window in the QSAR Toolbox, illustrating the process of generating a report according to RAAF-Scenario 3.

Screenshot 1 (Top): Shows the 'Customization' section where the 'Add RAAF scenario' checkbox is checked. A dropdown menu next to it shows 'Scenario 3' selected. A red box highlights this area, with a callout '1' pointing to the dropdown.

Screenshot 2 (Middle): Shows the 'Prediction' section. The 'Category' part of the report is highlighted in yellow. The 'Category definition and members' and 'Consistency check' sections are also highlighted in yellow. A red box highlights these sections, with a callout '2' pointing to the 'Consistency check' section.

Screenshot 3 (Bottom): Shows the 'Prediction' section. The 'Category' part of the report is highlighted in yellow. The 'Category definition and members' and 'Consistency check' sections are also highlighted in yellow. A red box highlights these sections, with a callout '3' pointing to the 'Consistency check' section.

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

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

Report

Report Generation according to RAAF-Scenario 3

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

Wizard pages

Customization
Customize report

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

Category
1
Category definition and members
Consistency check
Options

Data matrix
Options

1.1. Category definition
1.2. **Category members**
Information of category members
Ranges for selected physicochemical properties
Purity / Impurity
7 AEs
AE C.1: Substance characterization
AE C.5: Reliability and adequacy of the source study(ies)
1.3. **Profiles/Metabolisms**
List of profiles/metabolisms
AE 3.1: Formation of common (identical) compound(s)
AE 3.2: The biological targets for the common compounds
AE 3.3: Exposure of biological targets to the common compounds
AE 3.4: The impact of parent compound
AE 3.5: Formation and impact of non-common compounds

2
Category definition and members
Consistency check
Options

2.1. Physicochemical similarity
2.2. **Structural similarity**
Structural similarity
Comments on structural similarity
4 AEs
AE C.2: Structural similarity and structural differences within the category
AE C.3: Link of structural similarity and differences with the proposed category
2.3. Mechanistic similarity
2.4. Additional endpoints
2.5. **Other AEs**
AE C.4: Consistency of effects in the data matrix
AE C.6: Bias that influences the prediction

Back Next Cancel Create report

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE C.1 Substance characterization

Customize report content and appearance

Report basket

Options: Select All, Unselect All, 1 Selected, Invert

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

Information of category

Ranges for selected physical properties

Purity / Impurity

AE C.1: Substance characterization

Hint

PURPOSE:

The substance which is used as the so characterization. It has to be assessed

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

Input

☐ Target substance

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

Add / Remove

Table of category members

Preview

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

1.3. Profiles/Metabolisms

Back Next Cancel Create report

5

AE C.1: Substance characterization

Table of category members

#	CAS	Name	SMILES	Structure
1	97-53-0	Eugenol	<chem>COc1cc(C=C)ccc1O</chem>	
2	97-54-1	Isoeugenol	<chem>COc1cc(C=C)ccc1O</chem>	
3	5932-68-3	(E)-Isoeugenol	<chem>COc1cc(C=C)ccc1O</chem>	
4	186743-26-0	3-METHYL_EUGENOL	<chem>COc1cc(O)ccc(C)cc1C</chem>	

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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

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

- Information of category members
- Ranges for selected physicochemical properties
- Purity / Impurity
- AE C.1: Substance characterization
- AE C.5: Reliability and adequacy of the source study(ies)**

Hint

PURPOSE:

The source study needs to match the default requirements for the reliability and adequacy as requested for any other key study. The study design reported for the source study should be based on read-across:

- the study design should cover the key parameters in Article 13(3);
- the study design should cover an exposure scenario corresponding method referred to in Article 13(3); and
- there is adequate and reliable documentation of the applied 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.

2

Report basket

Options: Select All, Unselect All, Invert, 0 Selected

- Category**
 - Table with profiling results for "Protein binding alerts for skin sensitization"
 - Table with profiling similarity accounting for metabolism ("Autooxidation")
 - Table with profiling similarity accounting for metabolism ("Skin metabolism")
 - Table with profiling results for "Protein binding alerts for skin sensitization"
 - Table with profiling similarity accounting for metabolism ("Autooxidation")
 - Table with profiling similarity accounting for metabolism ("Skin metabolism")
 - Table with profiling results for "Protein binding by OASIS"
 - Table with profiling similarity accounting for metabolism ("Autooxidation")
 - Table with profiling similarity accounting for metabolism ("Skin metabolism")
 - Endpoint data variation (1 selected: Human Health Hazards#Sensitization)
 - Endpoint data variation (1 selected: Human Health Hazards#Sensitization)
 - Table of category members
 - Table with calculated structural similarity
 - Table with profiling results for "Organic functional groups, Norbert Hain"
 - Table with profiling results for "Organic functional groups"
 - Table with profiling results for "Structure similarity"
 - Table with profiling results for "US-EPA New Chemical Categories"
 - Table with profiling similarity accounting for metabolism ("Autooxidation")
 - Table with profiling similarity accounting for metabolism ("Skin metabolism")
 - Endpoint data variation (5 selected: Human Health Hazards#Sensitization)
 - Parameter variation (5 selected: Boiling point; log Kow; Molecular Weight)
 - 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
- Input**
 - Target substance
- Metabolism**
 - Common product

3

Create new, OK, Cancel

Add / Remove

Back, Next, Cancel, Create report

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

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

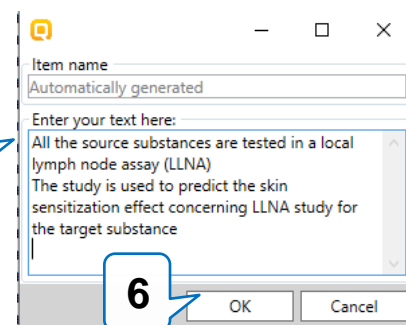
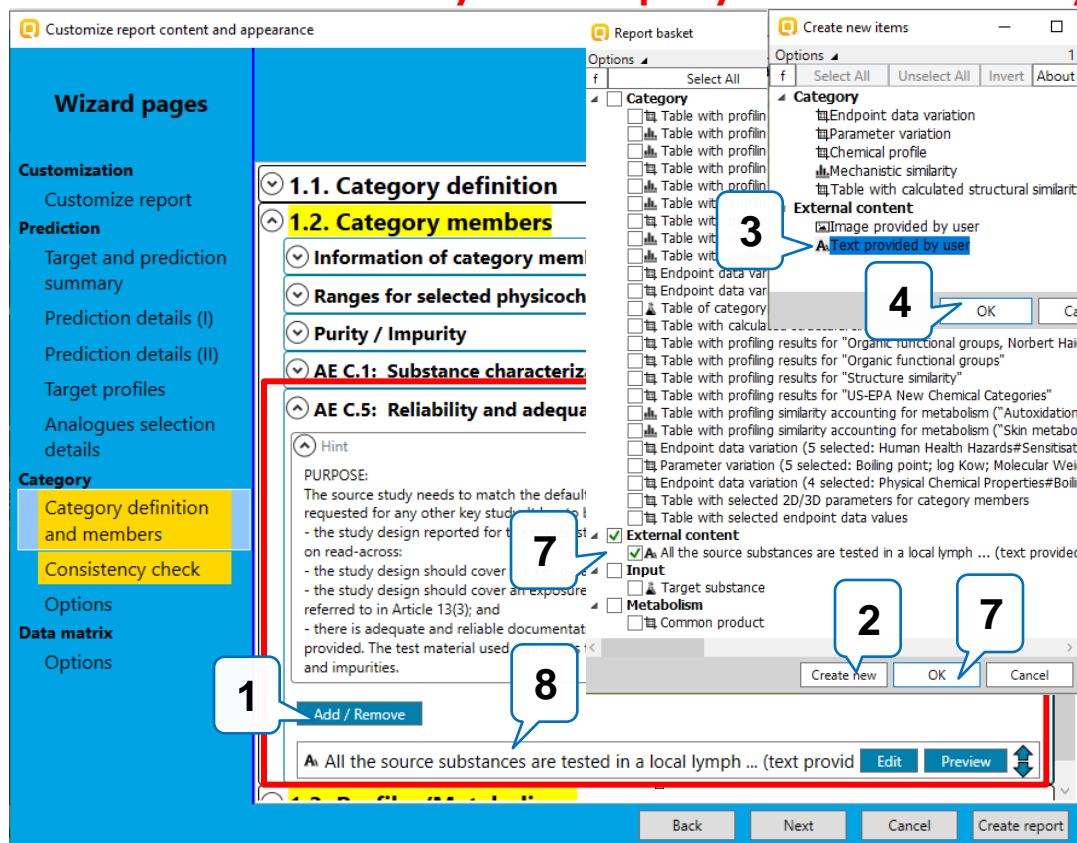
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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



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

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

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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

1. Click on **Add/Remove** button (1).

2. Click **Create new** (2) and then select "Image provided by user" (3).

3. Browse and find the saved image or just paste the copied image (4). Confirm by **OK**.

4. The new item appears in the report basket (5).

5. Click again **OK** button (6). The newly added item appears under AE C.5

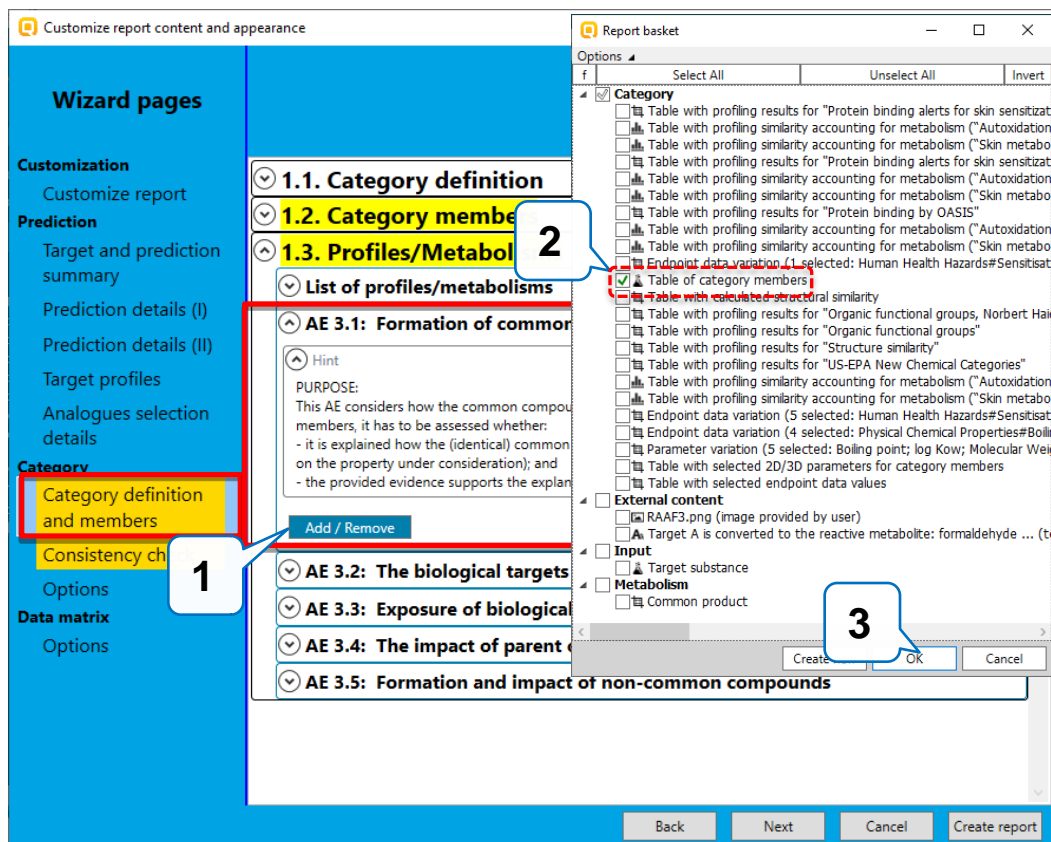
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.1 Formation of (a) common (identical) compound(s)



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

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

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.2 The biological targets for the common compounds

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 3.1: Formation of common (identical) compound(s)
- AE 3.2: The biological targets for the common compounds**
 - Hint
 - PURPOSE:
The hypothesis claims that the common compound(s) have the same biological target(s) (and hence cause the same type of effects). It has to be assessed whether the same biological targets are affected in a consistent manner throughout the category, and by the common compounds; and provided evidence supports the explanation.
 - Add / Remove
 - As a result of grouping accounting for a skin metabolism ... (text p) Edit Preview
- AE 3.3: Exposure of biological targets to the common compounds
- AE 3.4: The impact of parent compound
- AE 3.5: Formation and impact of non-common compounds

Back Next Cancel Create report

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

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

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

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.2 The biological targets for the common compounds

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 3.1: Formation of common (identical) compound(s)

AE 3.2: The biological targets for the common compounds

Hint

Add / Remove

As a result of grouping accounting for a skin metabolism ... (text provided by user) Edit

RAAF3.png (image provided by user) Edit Preview

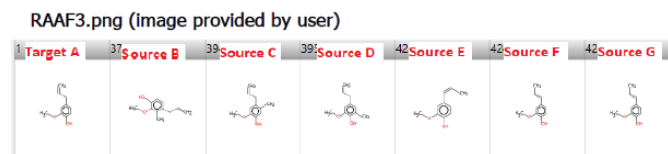
AE 3.3: Exposure of biological targets to the common compounds

AE 3.4: The impact of parent compound

AE 3.5: Formation and impact of non-common compounds

Back Next Cancel Create report

The possible image added to in AE 3.2 (see slide 63 how to create an image report item):



How the newly added item will look like in the report could be seen by preview button

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.3 Exposure of biological targets to the common compounds

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 3.1: Formation of common (identical) compound(s)

AE 3.2: The biological targets for the common compounds

AE 3.3: Exposure of biological targets to the common compounds

Hint

PURPOSE:

Under this scenario, it is proposed that the exposure of the biological targets to the common compound(s) vary in a predictable manner. It has to be assessed whether:

- the documentation established that the exposure of the biological targets to the common compound(s) is varying in a predictable manner;
- the prediction is derived from the relation between an observed property and the independent variable which determines the order within the category (prediction model); and
- the provided evidence supports the explanation. As a default, a prediction based on a regular pattern without a mechanistic explanation is not acceptable.

Add / Remove

1

2

3

A The target chemical A and all the source substances ... (text provided)

Edit Preview

AE 3.4: The impact of parent compound

AE 3.5: Formation and impact of new common compounds

Back Next Cancel Create report

Click on the **Add/Remove** button (1) and **create new item** with possible example text (2)
The new item appears under the AE 3.3 (3)

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

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.4.The impact of a parent compound

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 3.4: The impact of parent compound

Hint

PURPOSE:
(Bio)transformation of parent compounds may not be immediate and/or complete. As a result, exposure of possible biological targets to the parent compounds may occur for source and/or target substances. In addition, the impurity profiles associated with the source and target substances may have an impact on the prediction. It has to be assessed whether:

- the systemic availability of the parent compound and its impact on the prediction of the property under consideration has been addressed;
- the identified impurities (see AE C.1) have an impact on the prediction; and
- the provided evidence supports the explanation.

Add / Remove

AE 3.1: Formation of common (identical) compound(s)

AE 3.2: The biological targets for the common compounds

AE 3.3: Exposure of biological targets to the common compounds

AE 3.5: Formation and impact of non-common compounds

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

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

See next few slides

Report

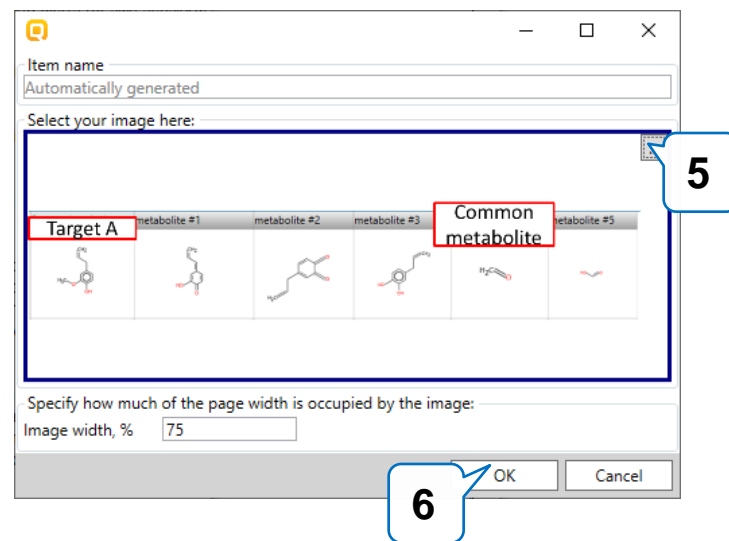
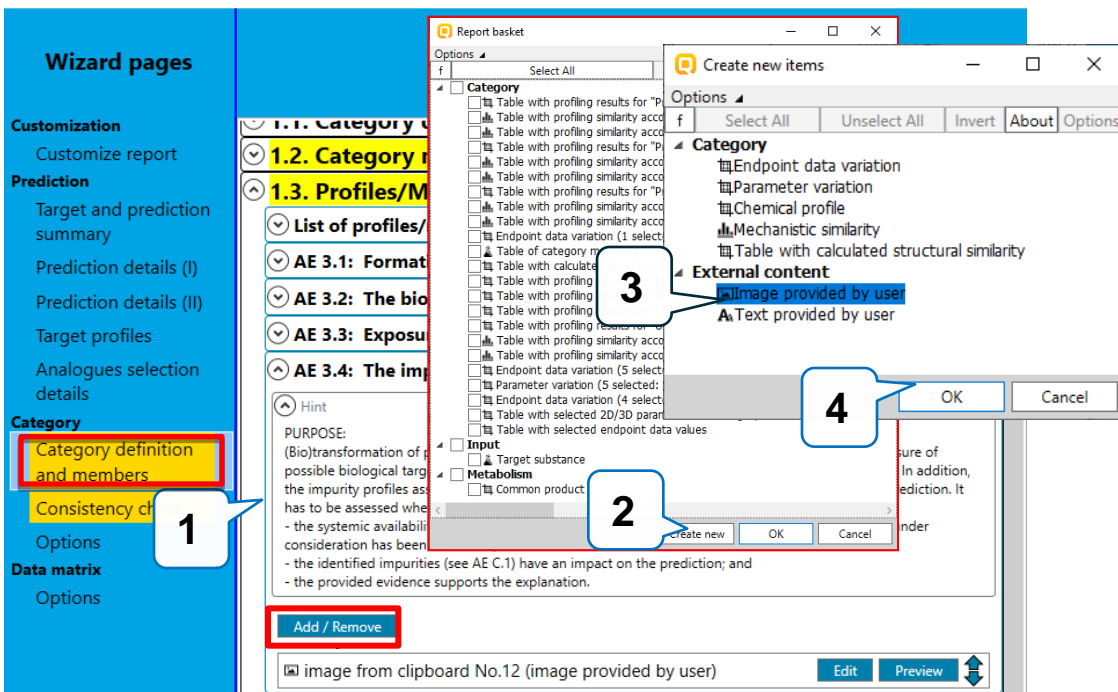
Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.4. The impact of a parent compound

Customize report content and appearance



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

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

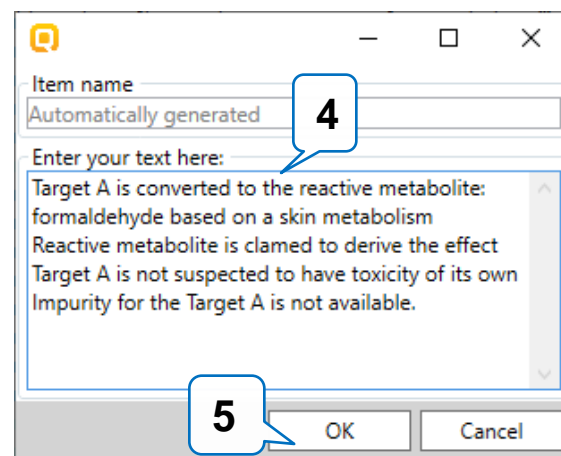
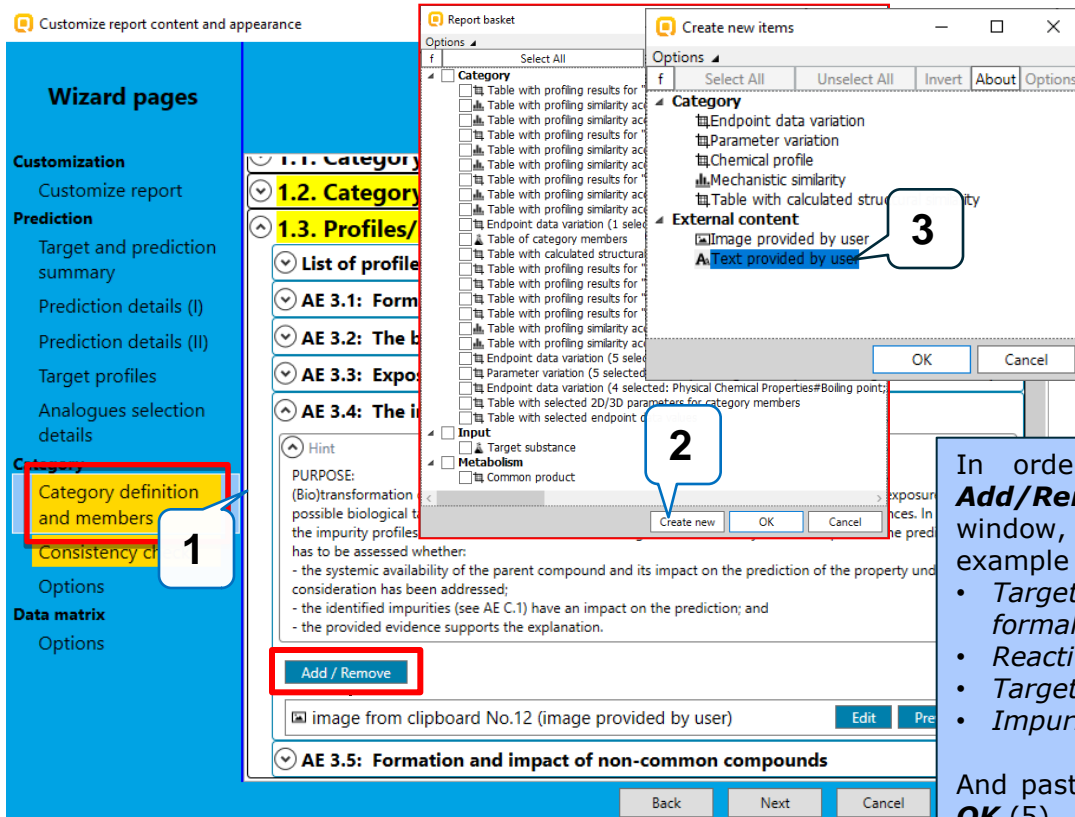
Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.4. The impact of a parent compound



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

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

And paste it in the new window(4). Finally confirm by clicking **OK** (5).

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.4. The impact of a parent compound

Customize report content and appearance

Wizard pages

- Customization
 - Customize report
- Prediction
 - Target and prediction summary
 - Prediction details (I)
 - Prediction details (II)
 - Target profiles
 - Analogue selection details
- Category
 - Category definition and members**
 - Consistency check
 - Options
 - Data matrix
 - Options

1.3. Profiles/Metabolisms

- List of profiles/metabolisms
- AE 3.1: Formation of common (
- AE 3.2: The biological targets fo
- AE 3.3: Exposure of biological t
- AE 3.4: The impact of parent co**
 - Hint

PURPOSE:
(Bio)transformation of parent comp...
possible biological targets to the parent comp...
the impurity profiles associated with the source...
has to be assessed whether:
- the systemic availability of the parent compo...
consideration has been addressed;
- the identified impurities (see AE C.1) have an...
- the provided evidence supports the explanati...
 - External content
 - ☐ All the source substances are tested in a local lymph ... (text provi
 - ☐ image from clipboard No.8 (image provided by user)
 - ☐ Target chemical A is claimed to be metabolized to formaldehyde ...
 - ☐ As a result of grouping accounting for a skin metabolism ... (text p
 - ☐ RAAF3.png (image provided by user)
 - ☒ Target chemical A and all the source substances ... (text provi
 - ☒ image from clipboard No.12 (image provided by user)
 - ☒ Target A is converted to the reactive metabolite: formaldehyde ...
 - ☐ Input
 - ☐ Target substance
 - ☐ Metabolism
 - ☐ Common product
- AE 3.5: Formation and impact of non-common com

3 Add / Remove

4 Edit Preview

Back Cancel Create report

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

Chemicals category 7 / 93

based on the common metabolite

AE 3.4: The impact of parent compound
image from clipboard No.12 (image provided by user)

Target A

Target A is converted to the reactive metabolite: formaldehyde ... (text provided by user)
Target A is converted to the reactive metabolite: formaldehyde based on a skin metabolism
Reactive metabolite is claimed to derive the effect
Target A is not suspected to have toxicity of its own
Impurity for the Target A is not available.

AE 3.5: Formation and impact of non-common compounds
Not provided by user

2. Consistency check

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

AE 3.5 Formation and impact of non-common compounds

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 3.1: Formation of common (identical) compound(s)
- AE 3.2: The biological targets for the common compounds
- AE 3.3: Exposure of biological targets to the common compounds
- AE 3.4: The impact of parent compound
- AE 3.5: Formation and impact of non-common compounds**

Hint

PURPOSE:

The formation of common compound(s) often goes together with the formation of non-common compound(s) and possible intermediates which form the common compound(s). The source and/or target substance may also be (bio)transformed via other pathways leading to other additional non-common compounds. It has to be assessed whether:

- the formation of non-common compounds (including possible intermediates) via the possible pathways and their possible impact on the prediction property under consideration have been considered; and
- the provided evidence supports the explanation.

Add / Remove

Back Next Cancel Create report

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

An example text for **AE 3.5:**

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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

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

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

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

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

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

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 **2.2. Structural similarity**

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

3 **Calculated structure similarity**

	1 CAS 97-53-0	354 CAS 97-54-1	355 CAS 5932-68-3	339 CAS 186743-26-0
1 CAS 97-53-0	100%	66.7 %	66.7 %	72 %
354 CAS 97-54-1	66.7 %	100%	100 %	48 %

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

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

4 CAS# 186743-26-0 **5 CAS# 186743-25-9** **6 CAS# 186743-24-8**

Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound	Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound	Hydroxy compound Phenol Ether Alkylarylether Alkene Aromatic compound

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Category definition and members

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

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

Structural similarity

Comments on structural similarity

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

Hint

PURPOSE:
The aim of this AE is to verify that all category members indeed meet the criteria for structural similarities and allowed structural differences used for the category description. It has to be assessed whether:

- the structural similarities identified apply to all category members; and
- there are structural differences which are allowed within the category.

Add / Remove

Table with calculated structural similarity **Edit** **Preview**

Table with profiling results for "Organic functional groups, Norbert" **Edit** **Preview**

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

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

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

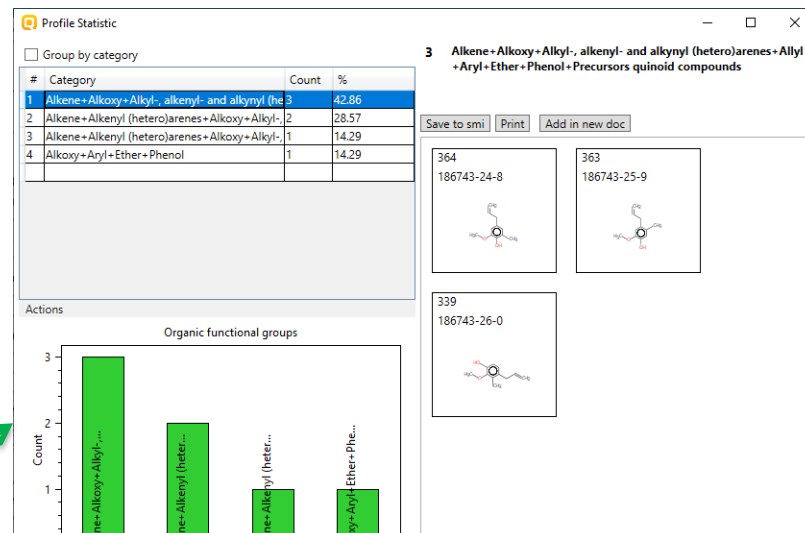
2.3. Mechanistic similarity

2.4. Additional endpoints

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

An additional image (saved in advance) could be added to the **AE C.2** (already explained on slide 61):

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



Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

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

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

2.3. Mechanistic similarity

2.4. Additional endpoints

2.5. Other AEs

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

Hint

PURPOSE:
It has to be assessed whether:

- the documentation provides an explanation why the category members should behave in a predictable manner (e.g. based on no absorption due to molecular-weight considerations, or lacking reactivity towards biological material, regular pattern in increasing strength of effect due to kinetic differences);
- it is likely that all category members follow the proposed explanation and where the boundaries of the category are in this respect; and
- the provided evidence supports the explanation.

1 Add / Remove

2 The category is structurally defined as target (A) and ... (text provid

3 Edit Preview

AE C.4: Consistency of effects in the data matrix

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

AE C.6: Bias that influences the prediction

Back Next Cancel Create report

Click on the **Add/Remove** button (1) and **create new item** with the following example text (2). The item appear under the section AE C.3

Example text for AE C.3

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

AE C.4 Consistency of effects in the data matrix

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
 - Options

2.3. Mechanistic similarity

2.4. Additional endpoints

2.5. Other AEs

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

AE C.4: Consistency of effects in the data matrix

Hint

PURPOSE:
The category justification should include comparison of experimental data for the category members and a clear data matrix. It has to be assessed whether:

- a data matrix has been provided which lists the category members in a suitable order versus their experimental data (e.g. for REACH information requirements) and which identifies data gaps;
- the properties of category members across the data matrix are consistent in effects; this has to be assessed in the following dimensions:
 - within the specific property which is under consideration for the prediction;
 - between the property under consideration and related properties (e.g. between 28-day and 90-day repeated-dose toxicity studies; reproductive toxicity screening tests; and pre-natal developmental toxicity studies);
 - characteristics across all relevant properties (e.g. different reactivity towards genetic material may indicate different reactivity towards biological macromolecules which may influence the prediction for a 90-day repeated-dose toxicity study);
 - the effects reported for the property under consideration differ in strength for the source substance and whether a basis for this difference is provided; and
 - the underlying data support the provided conclusions and explanations.

Add / Remove

1

2

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

AE C.6: Bias that influences the prediction

Back Next Cancel Create report

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

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

Report

Report Generation according to RAAF-Scenario 3

Section Category

Subsection: Consistency check

AE C.6 Bias that influences the prediction

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 C.3: Link of structural similarity and differences with the proposed regular pa**
- AE C.4: Consistency of effects in the data matrix**
- AE C.5: Reliability and adequacy of the source study(ies)**
- AE C.6: Bias that influences the prediction**
 - Hint
 - PURPOSE:
It has to be assessed whether:
- it is clear from the documentation how the source substance(s) have been chosen, for example, what methods/ tools have been used to map the field of potential source substance(s), which other substances have been considered and why they have been discarded;
- there are additional, structurally-similar substances which are currently not used in the analogue approach and which arguably could be used;
- there is readily-available information from these additional substances;
- this information is biologically significantly different for relevant properties in comparison with the existing analogue(s); and
- these differences decrease the confidence in the prediction (possibility of underestimation of hazard).

1 Add / Remove

When there are multiple possible analogues with equivalent ... (tex Edit Preview

Back Next Cancel Create report

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

2

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

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

Report

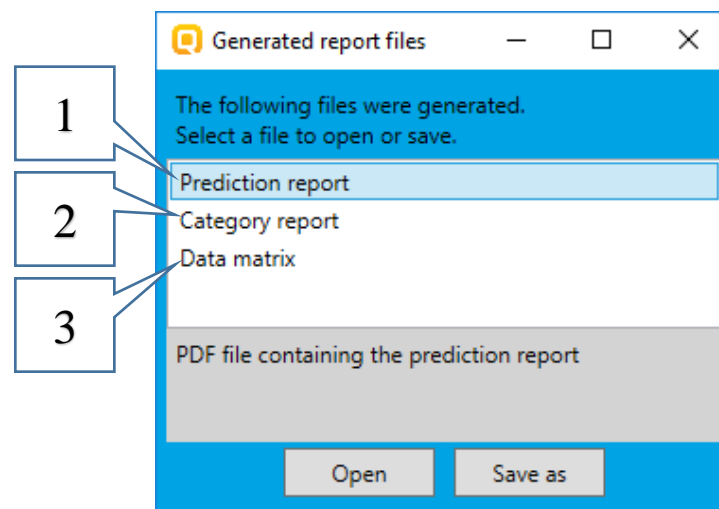
Report Generation

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

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

RAAF AEs are included in the second file.

All generated files should be provided when submitting a prediction.



Report

Generated report files

Prediction report

Category report

QSAR Toolbox prediction for single chemical
(in accordance with RAAF scenario 3)

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

Target information

Structural information
SMILES: COc1cc(C=C)ccc1O

Structure

Chemical names
CAS#: 97-53-0
Other: EC Number: 2025891
*eugenol (4-allyl-2-methoxyphenol); eugenol; 4-allyl-2-methoxy

Parameters

Parameter	Unit	Value
Boiling point	°C	254
log flow		2.73
Molecular Weight	Da	164
Vapor Pressure (Antoine method)	mm Hg	0.00965
Water Solubility	mg/L	754

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

QSAR Toolbox report for category
(in accordance with RAAF scenario 3)

1. Category definition
1.1. Category definition
Category name
Not provided by the user
Covered (target) endpoint(s)
- Human Health Hazards/Sensitisation: EC3, LLNA, in Vivo, Skin

Data matrix report

Substructure	Target chemical	Neighbour #1	Neighbour #2	Neighbour #3	Neighbour #4	Neighbour #5
Structure						
CAS number	97-53-0	97-54-1	5932-68-3	186743-26-0	186743-25-9	186743-24-8
Chemical name	Eugenol	Isoeugenol	(E)-Isoeugenol	5-METHYL_EUGENOL	5-METHYL_EUGENOL	6-METHYL_EUGENOL
Other identifier	COc1cc(C=C)ccc1O	COc1cc(C=C)ccc1O	COc1cc(C=C)ccc1O	COc1cc(C=C)ccc1O	COc1cc(C=C)ccc1O	COc1cc(C=C)ccc1O
Parameters						
Boiling point	254	271	271	281	281	281
log flow	2.73	2.65	2.65	3.28	3.28	3.28
Molecular Weight	164	164	178	178	178	178
Vapor Pressure (Antoine method)	0.00965	0.0037	0.0037	0.000596	0.000596	0.000596
Water Solubility	754	166	166	89.1	89.1	89.1
Profiles						
Profiles used for grouping/subcategorization						
Using of "Skin metabolism simulator"	metabolite #1; Is not: C=O; metabolite #2; Is not: C=O; metabolite #3; Is not: C=O; metabolite #4; Is exactly: C=O	metabolite #1; Is exactly: C=O	metabolite #1; Is exactly: C=O	metabolite #1; Is not: C=O; metabolite #2; Is exactly: C=O	metabolite #1; Is not: C=O; metabolite #2; Is exactly: C=O	metabolite #1; Is not: C=O; metabolite #2; Is exactly: C=O
Protein binding alerts for skin sensitization	No alert found Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	No alert found Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	No alert found Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	No alert found Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	No alert found Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol	No alert found Alkene; Alkylarylether; Aromatic compound; Ether; Hydroxy compound; Phenol
Organic functional groups, Norbert Halder (Chemo) (subcategorization)	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol	Alkene; Alkoxy; Alkenyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkoxy; Alkenyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkoxy; Alkenyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds
Organic functional groups (subcategorization)	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol	Alkene; Alkenyl (hetero)arenes; Alkoxy; Alkyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol	Alkene; Alkoxy; Alkenyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkoxy; Alkenyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds	Alkene; Alkoxy; Alkenyl-, alkenyl- and alkynyl (hetero)arenes; Allyl; Aryl; Ether; Phenol; Precursors quinoid compounds
US-EPA New Chemical Categories	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)
Predefined	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)	Phenols (Acute toxicity)

The selected RAAF scenario is specified in the first page

Congratulation

- You have now been introduced to the RAAF scenario;
- You have now been introduced to the *Report basket*.
- You have now been introduced to the AEs related to Scenario 3.
- Note, proficiency comes with practice!