

OECD QSAR Toolbox v.4.2

An example illustrating RAAF Scenario 1 and related assessment elements

Outlook

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

Background

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

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

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

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Specific Aims

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

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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) that are deemed crucial to the assessment
- Total six scenarios are available: two for analogue approach and four for category approach

Read Across Assessment Framework (RAAF)

Selection of a RAAF scenario

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

Read Across Assessment Framework (RAAF)

Selection of a RAAF scenario

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

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

Read Across Assessment Framework (RAAF)

Selection of a RAAF scenario

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

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

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- Read Across Assessment Framework (RAAF)
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- Workflow

The Example

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

The Example

Sidebar On Repeated dose toxicity (RDT)

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

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Workflow

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

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

Input Overview

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

Input Screen

Enter target chemical by CAS#

1

2

3

4

1	CAS	140-26-1
<input checked="" type="checkbox"/>	SMILES	CC(C)CC(=O)OCCc1ccccc1
	CS Relation	High
	Substance	Mono constituent
	Composition	
	Name	2-phenylethyl 3-methylbutanoic acid, 3-methyl-, 2-phenylethyl ester

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

Data Overview

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

Data Gather data

The screenshot shows the QSAR Toolbox 4.2 interface. At the top, the 'Data' module is selected in the toolbar (callout 1). The 'Gather' button is highlighted in the 'Data' menu (callout 4). On the left, a list of databases is shown under 'Human Health Hazards', with several databases selected (callout 3). In the center, the 'Filter endpoint tree...' window is open, showing a tree structure where 'Repeated Dose Toxicity' is selected (callout 2). On the right, a chemical structure is displayed. A pop-up message box states 'There is no experimental data available for the chemicals of interest' (callout 5).

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

Profiling Overview

- “Profiling” refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database.
- Available information includes likely mechanism(s) of action, as well as observed or simulated metabolites
- Based on the “profilers’ relevancy” the most suitable ones are getting colour highlighted*
- For the purpose of this example a plausible Repeated dose (HESS) profiler in combination with simulators are used (see next slides)

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

Profiling

Profiling the target chemical

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

Profiling

Profiling results

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

See on the next slide

Profiling

Profiling results

The screenshot displays the QSAR Toolbox 4.2 interface. The main window shows the chemical structure of a target compound and its profiling results. The results are organized into a tree view under the 'Profile' section. The following table summarizes the highlighted results:

Category	Sub-category	Result
US-EPA New Chemical Categories	Substance type	Discrete chemical
	US-EPA New Chemical Categories	Esters (Acute toxicity)
Endpoint Specific	Aquatic toxicity classification by ECOS...	Esters
	Organic functional groups	Alkane, branched with tertiary carbon Aryl Carboxylic acid ester
Organic functional groups (nested)	Organic functional groups (nested)	Alkane, branched with tertiary carbon Aryl Carboxylic acid ester
	Organic functional groups (US EPA)	Aliphatic Carbon [CH]
Toxicological	Repeated dose (HESS)	Not categorized
	in vivo Rat metabolism simulator	22 metabolite(s)

Callout 1 points to 'Repeated dose (HESS)'. Callout 2 points to 'US-EPA New Chemical Categories' and 'Organic functional groups'. Callout 3 points to 'in vivo Rat metabolism simulator'. Callout 4 points to the 'Metabolism/Transformations' section.

Recap

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

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

Multiplication of target chemical

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

The screenshot shows the QSAR Toolbox 4.2 interface. The top menu bar has 'Input' highlighted with a red box and callout '1'. The toolbar has 'Close' highlighted with a red box and callout '2'. A context menu is open over a chemical structure, with 'Multiplication' highlighted with a red box. A sub-menu is open, with 'Hydrolysis simulator (neutral)' highlighted with a red box and callout '3'. The chemical structure shown is CC(=O)OCC1=CC=CC=C1.

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

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

Multiplication of target chemical

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

The screenshot displays the QSAR Toolbox 4.2 interface. The top menu bar includes options like 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. Below the menu, there are buttons for 'Data', 'Import', 'Export', and 'Delete'. The main workspace is divided into several sections:

- Documents:** A tree view on the left shows a document with CAS: 140261 and a 'Hydrolysis simulator (neutral)' containing 'metabolite #1' and 'metabolite #2'.
- Structure:** The central panel shows the chemical structure of the parent chemical (Izovalerate acid) and its two metabolites (Izovalerate acid and Phenethyl alcohol).
- Structure info:** A list of properties to be simulated, including Physical Chemical Properties, Environmental Fate and Transport, Ecotoxicological Information, Human Health Hazards, and Profile.
- Databases:** A section at the bottom left for selecting hazard databases, with 'Human Health Hazards' expanded to show options like Acute Oral toxicity, Bacterial mutagenicity, etc.

Annotations on the screenshot include:

- A red box labeled 'Parent chemical' pointing to the parent chemical structure.
- A white box labeled 'Hydrolyzing products' pointing to the metabolite structures.
- Blue arrows pointing from the metabolite structures to two boxes below: 'Hydrolyzing product 1: Izovalerate acid' and 'Hydrolyzing product 2: Phenethyl alcohol'.

Collect data for the metabolites

Check for data availability for the generated metabolites

1. Click **Data**

2. The databases related to the defined target endpoint are already selected

3. Click **Gather**

4. Expand *Human health hazard level* and select *Repeated dose toxicity*

5. Click **OK**

6. The data for the parent and metabolites appears on data matrix, expand *Repeated Dose Toxicity* level to see the data

All available data (M: means measured) for the Izovalerate acid is bigger than 2150 mg/kg/bdwt/d

Measured data of the Phenethyl alcoho is 510 mg/kg/bdwt/d

Endpoint	Parent chemical [target]	metabolite #1	metabolite #2
Repeated Dose Toxicity	LOEL	M: 4.31E+03 mg/kg bdwt/d	
	NOAEL		M: 510 mg/kg bw/day (nomi...
	NOEL	M: 2.15E+03 mg/kg bdwt/d	
	NOEL calculated	M: 2.15E+03 mg/kg bdwt/d	

Recap

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

1. GHS Classification. Fourth edition

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

Define target endpoint

The screenshot shows the QSAR Toolbox 4.2 interface. On the left, the 'Documents' sidebar shows a tree view of endpoints under 'Human Health Hazards'. A red box labeled '1' highlights the 'NOAEL' endpoint. A context menu is open over this endpoint, with 'Target endpoint' selected and 'Define' highlighted by a red box labeled '2'. The main window displays a table with columns for 'Parent chemical [target]', 'metabolite #1', and 'metabolite #2'. The 'NOAEL' row is highlighted in yellow. A 'Select endpoint' dialog box is open on the right, with 'Finish' highlighted by a red box labeled '3'.

Parent chemical [target]	metabolite #1	metabolite #2
EC Number:3778846 CAS Number: 140-26-1 CAS Smiles relation: High Chemical name(s): 2-phenylethyl 3-methylbutan... Composition: Molecular Formula: C13H18O2 Predefined substance type: Mono constituent SMILES: CC(C)CC(=O)OCCc1cccc1	No CAS number Not applicable C5H10O2 Mono constituent CC(C)CC(O)=O	No CAS number Not applicable C8H10O Mono constituent OCCc1cccc1
<input type="checkbox"/> NOAEL 1/1 <input type="checkbox"/> NOEL 1/1 <input type="checkbox"/> NOEL calculated 1/1 <input type="checkbox"/> Sensitisation AW SW AOP <input type="checkbox"/> ToxCast <input type="checkbox"/> Toxicity to Reproduction	M: 4.31E+03 mg/kg bw/d/d M: 2.15E+05 mg/kg bw/d/d M: 2.15E+03 mg/kg bw/d/d	M: 510 mg/kg bw/day (nomi...

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

Transfer of observed data of metabolite to the target chemical

Documents

Databases

Options

- Select All
- Unselect All
- Invert
- Human Health Hazards
 - Acute Oral toxicity
 - Bacterial mutagenicity ISSSTY
 - Biocides and plant protection ISSBIOC
 - Carcinogenic Potency Database (CPDB)
 - Carcinogenicity&mutagenicity ISSCAN
 - Cell Transformation Assay ISSCTA
 - Dendritic cells COLIPA
 - Developmental & Reproductive Toxicity (DA)
 - Developmental toxicity database (P01010)
 - Developmental toxicity ILSI
 - ECHA CHEM
 - 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 (normals)
 - Receptor Mediated Effects
 - Rep Dose Tox Fraunhofer ITEM
 - Repeated Dose Toxicity HESS
 - Rodent Inhalation Toxicity Database
 - Skin Irritation
 - Skin Sensitization
 - Skin sensitization ECETOC
 - ToxCastDB
 - Toxicity Japan MHLW
 - Toxicity to reproduction (ER)
 - ToxRefDB US-EPA
 - Transgenic Rodent Database
 - Year 2000 rodent assay database

Filter endpoint tree...

Parent chemical [target] metabolite #1 metabolite #2

Structure	Parent chemical [target]	metabolite #1	metabolite #2
Structure	<chem>CC(=O)O</chem>	<chem>CC(O)C</chem>	<chem>CCO</chem>
Human Health Hazards			
Acute Toxicity			
Bioaccumulation			
Carcinogenicity			
Developmental Toxicity / Teratogenicity			
Genetic Toxicity			
Immunotoxicity			
Irritation / Corrosion			
Neurotoxicity			
Photoinduced toxicity			
Repeated Dose Toxicity			
LOEL	1/1	M: 4.31E+05 mg/kg bw/d	
NOAEL	2/2 R: 510 mg/kg bw/day (nomin...	M: 510 mg/kg bw/day (nomin...	
NOEL	1/1	M: 2.15E+03 mg/kg bw/d	
NOEL calculated	1/1	M: 2.15E+03 mg/kg bw/d	
Sensitisation	AW SWAOP	M: 2.15E+03 mg/kg bw/d	
ToxCast			
Toxicity to Reproduction			
Toxicokinetics, Metabolism and Distributi...			
Profile			

1. The row with defined target endpoint is yellow highlighted

2. Right click over the cell with observed data of the alcohol

3. Select **Transfer to target**

4. Read-across prediction based on observed data of metabolite appears to the target*.

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

Category consistency check

1. Go to **Category definition** module

2. Click on **Category elements**

3. The wizard of Category consistency appeared*.

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

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

Category consistency check

Step 1: Physicochemical similarity

Step 2: Structural similarity

Step 3: Mechanistic similarity

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

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

Category consistency check

QSAR Toolbox 4.2 [Document 1]

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Filling Report

Categorize Category consistency

Define Define with metabolism Subcategorize Combine Clustering Category elements

Documents

- Document 1
 - # CAS: 140261
 - Hydrolysis simulator (neutral)
 - metabolite #1
 - metabolite #2

Filter endpoint tree... Parent chemical... metabolite #1 metabolite #2

Structure

Structure info

Parameters

2D	Parent chemical...	metabolite #1	metabolite #2
Boiling point	276 °C	175 °C	225 °C
log Kow	3.97	1.49	1.57
Molecular Weight	206 Da	102 Da	122 Da
Vapor Pressure (Antoine method)	0.00653 mm Hg	1.25 mm Hg	0.0263 mm Hg
Water Solubility	16.5 mg/L	2.92E+04 mg/L	2.2E+04 mg/L

Physical Chemical Properties

	Parent chemical...	metabolite #1	metabolite #2
Boiling point	2/7	M: 177 °C	M: >219+221 °C
Partition Coefficient:	2/8	M: 1.16	M: 0.8
Vapour pressure	2/5	M: 0.44 mm Hg	M: 0.0868 mm Hg
Water solubility	2/5	M: 4.07E+04 mg/L	M: 1.75E+04 mg/L

Human Health Hazards

Repeated Dose Toxicity

	Parent chemical...	metabolite #1	metabolite #2
LOEL	1/1	M: 4.31E+03 mg/kg...	
NOAEL	2/2	R: 510 mg/kg bw...	M: 510 mg/kg b...
NOEL	1/1	M: 2.15E+03 mg/kg...	
NOEL calculated	1/1	M: 2.15E+03 mg/kg...	

Profile

Empiric

Organic functional groups

	Parent chemical...	metabolite #1	metabolite #2
Organic functional groups	Alkane, branche...	Alkane, branched w...	Alcohol

Toxicological

Repeated dose (HESS)

	Parent chemical...	metabolite #1	metabolite #2
Repeated dose (HESS)	Not categorized	Carboxylic acids (H...	Styrene (Renal T...

Metabolism/Transformations

Hydrolysis simulator (neutral)

	Parent chemical...	metabolite #1	metabolite #2
Hydrolysis simulator (neutral)	2 metabolite(s)	0 metabolite(s)	0 metabolite(s)

Grouping methods

Options

Select All Unselect All Invert

Plausible

- Aquatic toxicity classification by ECOSAR
- Chemical elements
- Groups of elements
- Lipinski Rule Oasis
- OECD HPV Chemical Categories
- Organic functional groups
- Organic functional groups (nested)
- Organic functional groups (US EPA)
- Organic functional groups, Norbert Haider (che
- Repeated dose (HESS)
- Structure similarity
- Substance type
- US-EPA New Chemical Categories

Unclassified

- Acute aquatic toxicity classification by Verhaar

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

Calculated phys-chem properties

Experimental phys-chem data

Endpoint data

Profiling results

Ready to move to reporting and including RAAF scenario

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

Recap

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

Report Overview

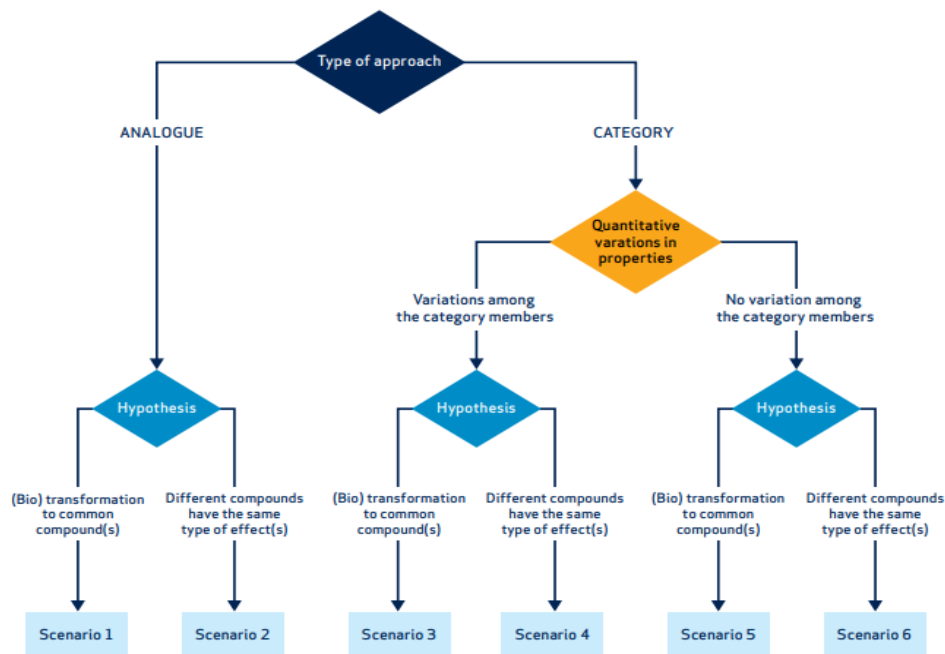
- Report module allows generating a report for any of the predictions performed within the Toolbox.
- The report module contains predefined report template which users can customize.
- Additionally specific RAAF scenario could be chosen. Selection of one of the scenarios will append automatically the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

Report

Selection of RAAF scenario

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

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



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

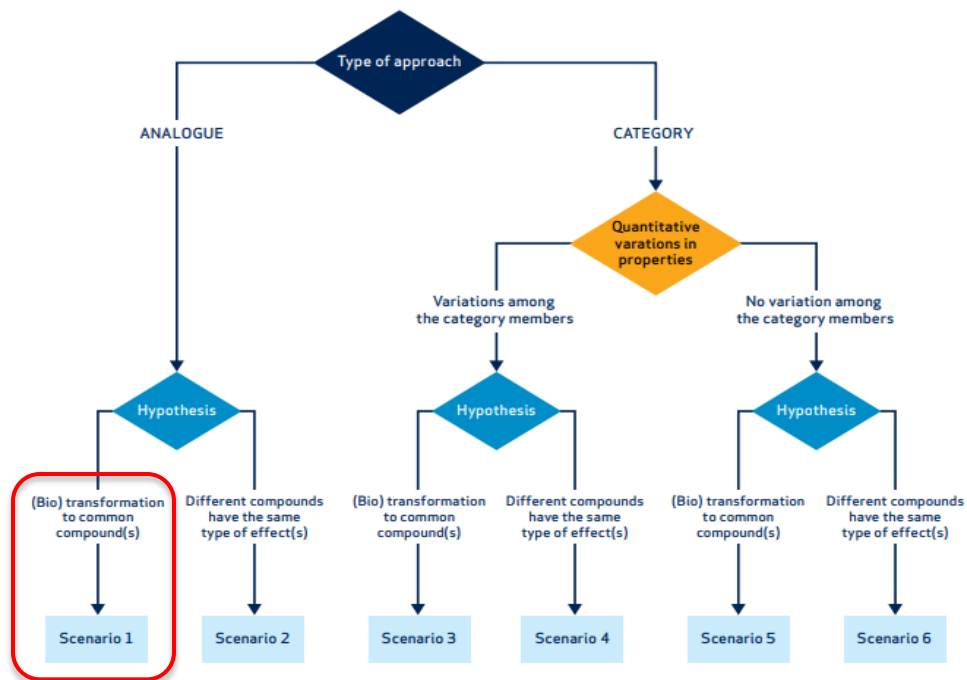
Report

Selection of RAAF scenario

For the current example:

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

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



Read-Across Assessment Framework (RAAF) Scenario 1

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

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

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

Report

Report generation according to RAAF-Scenario 1

The screenshot displays the QSAR Toolbox 4.2 interface. The main window shows a hierarchical tree of endpoints on the left and a data table on the right. The table has columns for 'Parent chemical...', 'metabolite #1', and 'metabolite #2'. A red dashed box labeled '1' highlights a cell in the 'NOEL' row under 'Repeated Dose Toxicity'. The top toolbar contains icons for 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. A red box labeled '2' highlights the 'Report' icon. A 'Reports' menu is open, showing 'Prediction', 'Data Matrix', 'Category', and 'QMRf'. A red box labeled '3' highlights the 'Customize report' button in the 'Wizard pages' dialog. The dialog has a 'Customization' section with 'Customize report' selected. Under 'Prediction', several options are checked. Under 'Category', 'Category definition and members' is checked. Under 'Data matrix', 'Options' is checked. A dropdown menu for 'Add RAAF scenario' is open, showing 'Scenario 1' selected. A red box labeled '4' highlights this selection. At the bottom of the dialog, there are 'Move Up', 'Move Down', 'Remove password protection of the PDF files.', 'Back', 'Next', 'Cancel', and 'Create report' buttons.

Endpoint	Parent chemical...	metabolite #1	metabolite #2
Structure			
Structure info			
Parameters			
2D			
Boiling point	276 °C	175 °C	
log Kow	3.97	1.49	
Molecular Weight	206 Da	102 Da	
Vapor Pressure (Antoine method)	0.00653 mm Hg	1.25 mm Hg	
Water Solubility	16.5 mg/L	2.92E+04 mg/L	
Physical Chemical Properties			
Boiling point	2/7	M: 177 °C	
Partition Coefficient:	2/8	M: 1.16	
Vapour pressure	2/5	M: 0.44 mm Hg	
Water solubility	2/5	M: 4.07E+04 mg	
Human Health Hazards			
Repeated Dose Toxicity			
LOEL	1/4	M: 4.31E+03 mg	
NOEL	2/2	R: 510 mg/kg bw...	
NOEL		M: 2.15E+03 mg	
NOEL calculated	1/1	M: 2.15E+03 mg	
Profile			
Empiric			
Organic functional groups	Alkane, branche...	Alkane, branched	
Toxicological			
Repeated dose (HESS)	Not categorized	Carboxylic acids (H...	Styrene (Renal T...
Metabolism/Transformations			
Hydrolysis simulator (neutral)	2 metabolite(s)	0 metabolite(s)	0 metabolite(s)

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

Report

Report generation according to RAAF-Scenario 1

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

Report

Assessment elements of Scenario 1

The image displays the 'Customize report content and appearance' wizard page on the left, the 'Report basket' dialog in the center, and the 'Create new items' dialog on the right. Callout 1 points to the 'Target profiles' section in the wizard. Callout 2 points to the 'Add / Remove' button below the first assessment element (AE 1.1). Callout 3 points to the 'Report basket' dialog, which lists various report items under 'Category' and 'Input'. Callout 4 points to the 'Create new' button in the 'Create new items' dialog, which is used to add new items to the report basket.

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

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

Report Assessment elements of Scenario 1

The screenshot illustrates the steps to add a custom image to a report. It shows the 'Customize report content and appearance' wizard with 'Target profiles' selected. The 'Add / Remove' button is used to manage the report basket. A new item is created with 'External content' and 'Image provided by user'. A dialog box prompts the user to select an image, showing a chemical reaction scheme. The process concludes with clicking 'OK' in the dialog and 'OK' in the 'Report basket' window.

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

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

Report Assessment elements of Scenario 1

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

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

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

Report

Assessment elements of Scenario 1

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 details
 - Conductivity check
 - Options
- Data matrix
 - Options

Select profiles to report

Show profiles in report

Export profiles in Excel file

Active descriptor value(s) Automatically populated by the system

AE 1.1: Formation of common (identical) compound(s)

Hint

PURPOSE:
It has to be assessed whether:
- it is explained how the (identical) common product(s) are formed (i.e. the product(s) claimed to drive the impact on the property under consideration); and
- the provided evidence supports the explanation.

Add / Remove

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

Text provided by user (• Source substance B and Target substance A. •) Edit Preview

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

AE 1.4: The impact of parent compounds

AE 1.5: Formation and impact of non-common compounds

Back Next Cancel Create report

Prediction of NOAEL for phenethyl isovalerate 4 / 6

Target profiles
(OECD principle 5 - Chemical and biological mechanisms)
Profiles used for grouping/subcategorization
log Kow (calculated): 3.97

AE 1.1: Formation of common (identical) compound(s)

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

Target substance CC(C)C(=O)OCCc1ccccc1 → CC(C)C(=O)O + OCCc1ccccc1
Substance A Read across substance Substance B

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

AE 1.2: The biological targets for the common compound(s)
Not provided by user

AE 1.4: The impact of parent compounds
Not provided by user

AE 1.5: Formation and impact of non-common compounds
Not provided by user

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

Report

Assessment elements of Scenario 1

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 mapping
 - Consistency
 - Options
- Data matrix
 - Options

Select profiles to report

Show profiles in report

Export profiles in Excel file

Active descriptor value(s) Automatically populated by the system

- AE 1.1: Formation of common (identical) compound(s)
- AE 1.2: The biological targets for the common compound(s)
- AE 1.4: The impact of parent compounds

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

Hint

PURPOSE:
The hypothesis claims that the common compound(s) have the same biological target(s) (and hence cause the same effects). It has to be assessed whether:

- the same biological targets are affected by the common compound(s); and
- the provided evidence supports the explanation.

AE 1.4: The impact of parent compounds

Hint

PURPOSE:
(Bio)transformation of parent compounds, i.e. target and source substances, 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 parent compound and its impact on the prediction of the property under consideration has been addressed;
- the identified impurities have an impact on the prediction; and
- the provided evidence supports the explanation.

Under this scenario, the parent compound(s) should not significantly influence the predictions. This means that the (bio)transformation of the parent compounds should be rapid and complete or it is known that the parent compound is toxicologically silent.

Buttons: Back, Next, Cancel, Create report

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

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

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

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

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

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

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

Report Assessment elements of Scenario 1

Customize report content and appearance

Wizard pages

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

Select profiles to report

Show profiles in report

Export profiles in Excel file

Active descriptor value(s) Automatically populated by the system

AE 1.1: Formation of common (identical) compound(s)

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

Hint

Add / Remove

1

Text provided by user (Example for differences in distribution pattern) Edit Preview

AE 1.4: The impact of parent compounds

Hint

Add / Remove

2

Text provided by user (Substance A is converted to substance B in the Edit Preview

AE 1.5: Formation and impact of non-common compounds

Back Next Cancel Create report

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

Substance A \rightarrow Substance B

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

AE 1.2: The biological targets for the common compound(s)
 Example for differences in distribution pattern leading to different biological targets for the common compound
 Substance A is converted to substance B in the liver based on hydrolysis reaction.
 Oral study with B is used to predict the toxicity of A after oral administration.
 Differences in the exposure of organ/tissues to the common compound B have to be expected when exposures are compared between B administered directly or when formed from A.

AE 1.4: The impact of parent compounds
 Substance A is converted to substance B in the liver
 Substance B is claimed to derive the effect
 The parent chemical A is present in significant amounts (Its is monoconstituent without any additives or impurities)
 Substance A is suspected to have toxicity of its own
 The Substance B is used as a source to predict the effect for Substance A

AE 1.5: Formation and Impact of non-common compounds
 Not provided by user

QSAR Toolbox 4.2
 Database version: 4.2

QSAR TOOLBOX

TPRF v4.2

Report Assessment elements of Scenario 1

The screenshot displays the 'Customize report content and appearance' wizard. The left sidebar shows 'Wizard pages' with sections for 'Customization', 'Prediction', and 'Category'. The 'Target profiles' page is selected, and 'AE 1.5: Form' is highlighted with callout 1. Below it, the 'Add / Remove' button is highlighted with callout 2. The 'Report basket' window shows a list of items, with 'Endpoint data values' highlighted by callout 4. The 'Create new items' dialog has 'Image provided by user' selected under 'External content', highlighted by callout 5. The 'Image provided by user' dialog shows a grid of chemical structures, with callout 6 pointing to the grid and callout 7 pointing to the 'Select your image here:' text. The 'Image width, %' field is set to 75, and the 'OK' button is highlighted by callout 8.

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

Report Assessment elements of Scenario 1

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

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

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

Report Assessment elements of Scenario 1

1 Target profiles

2 Add / Remove

3 Create new

4 Endpoint data variation

5 OK

6 Repeated Dose Toxicity

7 LOEL, NOAEL, NOEL

8 Endpoint data variation (3 selected: Human Health Hazards#Repeater)

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

Report Assessment elements of Scenario 1

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

Select profiles to report

Show profiles in report
 Export profiles in Excel file

Active descriptor value(s) Automatically populated by the system

- AE 1.1: Formation of common (identical) compound(s)
- AE 1.2: The biological targets for the common compound(s)
- AE 1.4: The impact of parent compounds
- AE 1.5: Formation and impact of non-common compounds

Hint

Add / Remove

- Endpoint data variation (3 selected: Human Health Hazards#Repeate... **Edit** **Preview**
- Image provided by user (image from clipboard No.2) **Edit** **Preview**
- Text provided by user (Target substance A is an ester which is know... **Edit** **Preview**

Cancel **Create report**

monocoustituent without any additives or impurities
Substance A is suspected to have toxicity of its own
The Substance B is used as a source to predict the effect for Substance A

AE 1.5: Formation and impact of non-common compounds

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

QSAR Toolbox 4.2 Database version: 4.2 **QSAR TOOLBOX** TPRF v4.2

Prediction of NOAEL for phenethyl isovalerate 5 / 7

Human Health Hazards data variation

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

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

Parent chemical (target)	metabolite #1	metabolite #2

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

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

Report Assessment elements of Scenario 1

1 Category definition and members

2 Purity / Impurity

3 Profiles/Metabolisms

4 Category members

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

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

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

Report Assessment elements of Scenario 1

The image shows two windows from the QSAR Toolbox software. The left window is titled 'Customize report content and appearance' and has a sidebar with 'Wizard pages' including 'Customization', 'Prediction', 'Category', and 'Data matrix'. Callout 1 points to 'Category definition and members', callout 2 points to 'Text provided by user', and callout 3 points to the 'Data Gap Filing' section. The right window is the main application interface showing a 'Filter endpoint tree' with a table of results. A red box highlights the 'test guideline' column in the 'Data points' table, which contains 'OECD Guideline 411 (Subchronic Dermal Toxicity: 90-Day Study)'. Below this, a 'Data points' dialog box is open, showing a table with columns for 'Datapoints', 'Study result type', 'Test guideline', and 'Test material equivalent'. The 'Test guideline' column contains 'OECD Guideline 411 (Subchronic Dermal Toxicity: 90-Day Study)'.

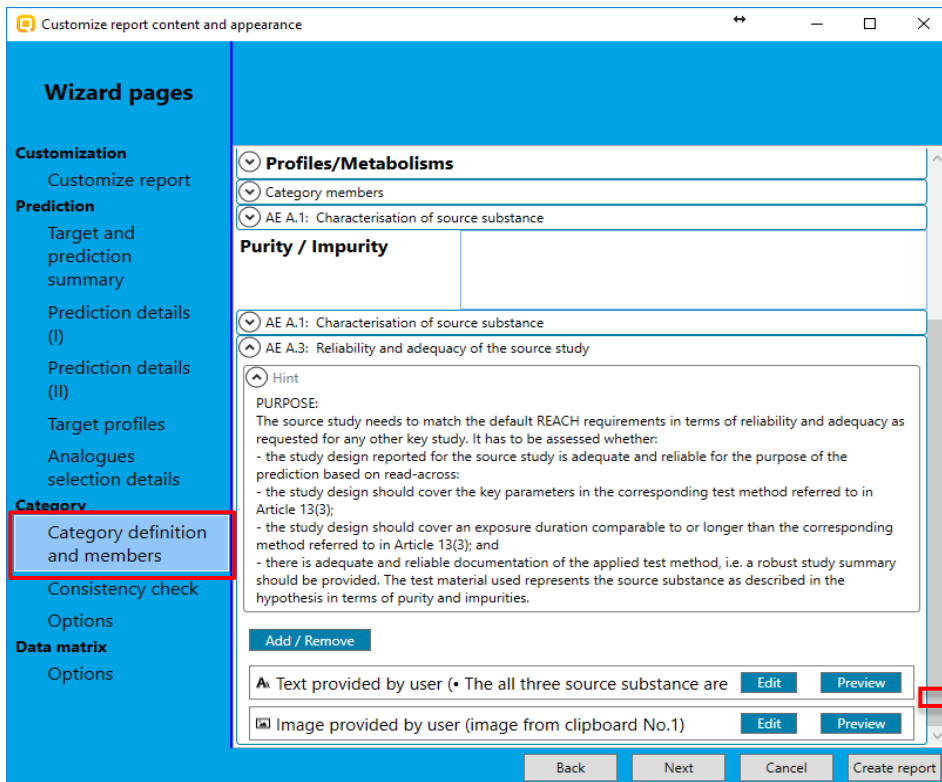
AE A.3: Click the **Add/Remove** button (1) and **create new item** with textual content (2) (see slide 45).

In the text field paste the following example text:

*"The source substance is tested in a sub chronic dermal toxicity:90-Day-study test according to OECD 411
The study is used to predict the repeated dose toxicity study according to OECD guideline 411 for the target substance"*

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

Report Assessment elements of Scenario 1



- AE A.3: Reliability and adequacy of the source study**
- The source substance is tested in a sub chronic dermal toxicity:90-Day-study test according to OECD 411
 - The study is used to predict the repeated dose toxicity study according to OECD guideline 411 for the target substance

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

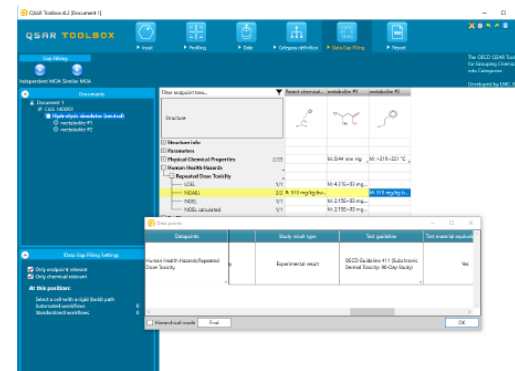
QSAR Toolbox 4.2
Database version: 4.2

QSAR TOOLBOX

TPRF v4.2

Chemicals category

3 / 8



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

Report Assessment elements of Scenario 1

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

AE A.4: 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).

Add / Remove

Report basket

Options

Select All Unselect All Invert

- Category**
 - Category members
 - Endpoint data variation (24 selected: Human Health Hazards#Sensit...
 - Chemical profile ("Organic functional groups")
 - Chemical profile ("Repeated dose (HESS)")
 - Profiling similarity accounting for metabolism ("Hydrolysis simulator (ne...
 - Parameter variation (5 selected: Boiling point; log Kow; Molecular We...
 - Parameter values
 - Structural similarity
 - Endpoint data values
 - Endpoint data variation (3 selected: Human Health Hazards#Repeated...
- External content**
 - Image provided by user (image from clipboard No.1)
 - Text provided by user (• Source substance B and Target substance A
 - Text provided by user (• Example for differences in distribution patter
 - Text provided by user (• Substance A is converted to substance B in
 - Image provided by user (image from clipboard No.2)
 - Text provided by user (• Target substance A is an ester which is kno
 - Text provided by user (• The source substance is tested in a sub chr
 - Image provided by user (image from clipboard No.3)
 - Text provided by user (• Two hydrolysing products are generated for
 - Text provided by user (• Structural similarity between Substance A and
- Input**
 - Target substance

Create new OK Cancel

1

2

3

4

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

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

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

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

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

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

AEs regarding Consistency check section continues on next slide

Report Assessment elements of Scenario 1

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

AE A.4: 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).

Add / Remove

Text provided by user (• Two hydrolysing products are generated for : Edit Preview

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

Hint

PURPOSE:
The aim of this AE is to verify that the source and target substances are covered by the read-across hypothesis. It has to be assessed whether:

- the scientific hypothesis establishes the structural similarities and differences of source and target;
- structural similarities and differences are linked with the possibility to predict similar properties; and
- the provided evidence supports the proposed link between structural similarities and the possibility to predict.

Add / Remove

Structural similarity Edit Preview

Text provided by user (o Structural similarity between Substance A an Edit Preview

AE 1.3: Exposure of the biological target(s) to the common compound(s)

Hint

PURPOSE:
Under this scenario, it is normally expected that the exposure of the biological targets to the common compound(s) is similar (thereby causing similar strength of effects). It has to be assessed whether:

- the documentation has explained why the exposure of the biological targets to the common compound(s) is similar; and
- the provided evidence supports the explanation.

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

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

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

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

Report

Assessment elements of Scenario 1

Customize report content and appearance

Wizard pages

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

1

Selected physicochemical properties for category members

Comments on physicochemical similarity

Structural similarity

Justification for selected structure similarity profiles

Add / Remove

Structural similarity Edit Preview

Chemical profile ("Organic functional groups") Edit Preview

Comments on structural similarity

Mechanistic similarity

Justification for selected mechanistic similarity profiles/metabolisms

Add / Remove

Chemical profile ("Repeated dose (HESS)") Edit Preview

Profiling similarity accounting for metabolism ("Hydrolysis simulator (") Edit Preview

Comments on mechanistic similarity

Additional endpoints

Tree position: Human Health Hazards#Repeated Dose Toxicity

Data filters:

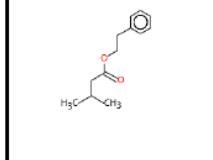
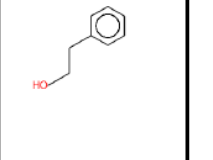
Category values for selected additional endpoints

Comments on

Calculated structure similarity

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

Chemical profile ("Organic functional groups")

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

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

Report Assessment elements of Scenario 1

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

Comments on physicochemical similarity

Selected physicochemical properties for category members

Structural similarity

Justification for selected structure similarity profilers

Add / Remove

Structural similarity Edit Preview

Chemical profile ("Organic functional groups") Edit Preview

Comments on structural similarity

Mechanistic similarity

Justification for selected mechanistic similarity profiles/metabolisms

Add / Remove

Chemical profile ("Repeated dose (HESS)") Edit Preview

Profiling similarity accounting for metabolism ("Hydrolysis simulator (") Edit Preview

Comments on mechanistic similarity

Additional endpoints

Tree position: Human Health Hazards#Repeated Dose Toxicity

Data filters:

Category values for selected additional endpoints

Comments on additional endpoints

Chemical profile ("Repeated dose (HESS)")

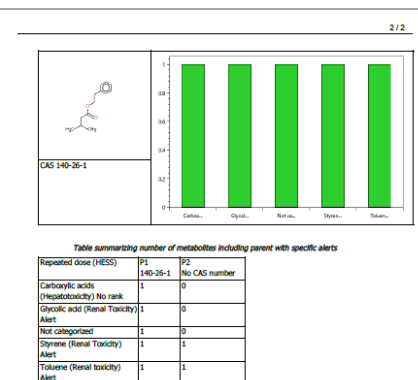
1	2
Not categorized	Styrene (Renal Toxicity) Alert Toluene (Renal toxicity) Alert

1 / 2

Profiling similarity accounting for metabolism ("Hydrolysis simulator (neutral)" and "Repeated dose (HESS)")
Metabolism: Hydrolysis simulator (neutral)
Profiler: Repeated dose (HESS)

Tables with generated metabolites for each analogue with profiling result

P1	M1 P1	M2 P1
Not categorized	Carboxylic acids (Hepatotoxicity) No rank Glycolic acid (Renal Toxicity) Alert	Styrene (Renal Toxicity) Alert Toluene (Renal toxicity) Alert
P2	No metabolites	
	Styrene (Renal Toxicity) Alert Toluene (Renal toxicity) Alert	



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

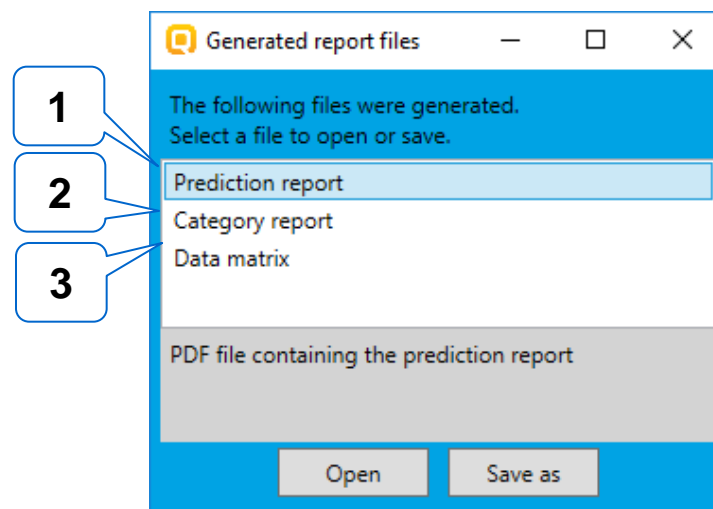
Report Generation

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

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

RAAF AEs are included in the first two files.

All generated files should be provided when submitting a prediction.



Report Generated report files

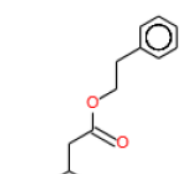
Prediction report

Category report

QSAR Toolbox prediction for single chemical

(in accordance with RAAF scenario 1) ← The selected RAAF scenario is specified in the first page →

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

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

Structure CC(C)CC(=O)OCCc1ccccc1

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

QSAR Toolbox report for category

(in accordance with RAAF scenario 1)

1. Category definition

1.1. Category definition *manually editable field*

Not provided by the user

Ranges for selected physicochemical properties and calculated parameters

Parameter variation (5 selected: Boiling point; log Kow; Molecular Weight; Vapor Pressure (Antoine method); Water Solubility)

2D parameters data variation

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



selected: Physical Chemical Properties#Boiling point; Physical Chemical Properties#Water solubility; Physical Chemical Properties#N-Octanol/Water

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

Point(s) *manually editable field*

Repeated Dose Toxicity: NOAEL

Data matrix report

	A	B	C	D	E	F	G	H	I	J	K	
1												
2	Substance identity		Target chemical				Neighbour #1					
3	Structure											
4	CAS number		140-26-1				No CAS number					
5	Chemical name		phenethyl isovalerate									
6	Other identifier											
7	SMILES		CC(C)CC(=O)OCCc1ccccc1				OCCc1ccccc1					
8	Parameters		unit									
9	vapor Pressure (Antoine method)		mm Hg		0.00653		0.0263					
10	log Kow				5.97		1.57					
11	Molecular Weight		Da		206		122					
12	Boiling point		°C		276		225					
13	Water solubility		mg/L		16.5		2.2E+04					
14												
15												
16	Prefixes											
17	empire											
18	Organic functional groups		Alkane, branched with tertiary carbon; Aryl; Carboxylic acid ester; Isopropyl				Alcohol; Aryl					
19	Toxicological											
20	Repeated dose (HES)		Not categorized				Styrene (Renal Toxicity) Alert; Toluene (Renal Toxicity) Alert					
21	Repeated dose (HES), with Hydrolysis simulator (neutral)		Not categorized; Carboxylic acids (Hepatotoxicity) No rank; Glycolic acid (Renal Toxicity) Alert; Styrene (Renal Toxicity) Alert; Toluene (Renal Toxicity) Alert				Styrene (Renal Toxicity) Alert; Toluene (Renal Toxicity) Alert					
22												
23	Measured and predicted data											
24	Data used for prediction											
25	environment	endpoint	value	unit	species, duration, test type, type of method, assay, strain, test guideline, year, reference	value	unit	species, duration, test type, type of method, assay, strain, test guideline, year, reference				
26	Repeated Dose Toxicity	NOAEL										
27	Physical Chemical Properties#Boiling point											

Congratulation

- You have now been introduced to the RAAF scenario;
- You have now been introduced to the *Report basket*.
- You have now been introduced to the AEs related to Scenario 1.
- Note proficiency comes with practice.