# QSAR TOOLEOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

# OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 1 and related assessment elements

### **Outlook**

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

### Background

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

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### **Keywords**

**TARGET CHEMICAL -** chemical of interest

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

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

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

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

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

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

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

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### **Objectives**

# This presentation demonstrates a number of functionalities of the Toolbox:

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

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### **Specific Aims**

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

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### Read Across Assessment Framework (RAAF)

- The example
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### Read Across Assessment Framework (RAAF) Overview

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

### Read Across Assessment Framework (RAAF) RAAF scenarios

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

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

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

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

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

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

\*Read-Across Assessment Framework (RAAF) available at <u>https://echa.europa.eu/documents/10162/13628/raaf\_en.pdf</u>

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### • The example

• Workflow

### **The Example**

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

### **The Example** Sidebar On Repeated dose toxicity (RDT)

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

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### Workflow

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

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

### Workflow

#### Scheme illustrating the Toolbox workflow



### **Input** Overview

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

### **Input Screen** Enter target chemical by CAS#



structure appears, verify the correctness of the chemical. In the current case the relationship CAS-SMILES shows "High" relation for the identifiers. Finally click **OK** (4).

### **Data** Overview

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

### **Data** Gather data



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### **Profiling** Overview

- "Profiling" refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database;
- "Profiling" module contains all the knowledge in the system coded in profiling schemes (profilers);
- "Profilers" are a collection of empirical and mechanism knowledge (expertly derived) which could be used to analyse the structural properties of chemicals;
- The "profilers" identify the affiliation of the target chemical(s) to preliminary defined categories (functional groups/alerts);
- The "Profiling" module contains also observed and simulated metabolisms/transformations, which could be used in combination with the profiling schemes;
- The outcome of the profiling determines the most appropriate way to search for analogues, but they are also useful for preliminary screening or prioritization of substances;
- The "profilers" are not (Q)SARs, i.e. they are not prediction models themselves;
- Based on the "profilers' relevancy" (determined by the defined target endpoint), the most suitable once are getting colour highlighted\*.

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

### Profiling Profiling the target chemical

|   | Profilite<br>Apply View New Delete | bata     Category definition     bata Gap Filling  | Report     |                |   |
|---|------------------------------------|--|------------|----------------|---|
| 3 | Documents                          | Filter endpoint tree  Filter endpoint tree  Structure  Structure info Parameters Physical Chemical Properties Finvironmental Fate and Transport Ecotoxicological Information Human Health Hazards Acute Toxicity Bioaccumulation Carcinogenicity Developmental Toxicity / Teratogenicity Genetic Toxicity Immunotoxicity | 1 [target] |                |   |
| 3 |                                    | Irritation / Corrosion     Neurotoxicity     Blotainducad toxicity     Repeated Dose Toxicity     Sensusation     ToxCast     Toxicity to Reproduction     Toxicokinetics, Metabolism and Distribution   |            | 1.<br>2.<br>3. | Go to <b><u>Profiling</u></b> module;<br>Select the cell related to "Repeated Dose<br>Toxicity";<br>Unselect All. Select <i>Organic functional group</i><br>( <i>OFG</i> ) profiler and Repeated dose toxicity<br>(HESS) and include both <i>Hydrolysis (neutral)</i><br>and in <i>vivo Rat liver simulators</i> ;<br>Click <b>Apply.</b> |

### **Profiling** Profiling results

- 1) No RDT alerts are identified in the target's structure as a parent;
- The chemical is classified as "ester" according to structure-based OFG profiler;
- 3) 2 hydrolysing products\* are obtained as a result of abiotic activation (*Hydrolysis simulator (neutral*);
- 4) 15 metabolites\* are produced as a result of biotic activation (*in vivo Rat metabolism simulator*);

See on the next slide

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

### **Profiling** Profiling results

| QSA  | R TOOLBOX   | Image: Profiling   | ▶ Data     ▶ Category definition     ▶ Data Gap Filling   | ► Report   |                               |
|--|---|--|---|--|-------------------------------|
| Profiling<br>Apply \   | g Custom profile  |  |   |  |                               |
| $\odot$  | Documer   | nts  | Filter endpoint tree Y  | 1 [target]   |                               |
| Coptions a<br>f<br>Sk<br>Coptions a<br>f<br>Sk<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions<br>Coptions | Profiling me<br>Select All<br>kni intration/corrosion Inclusion rul<br>inc<br>hemical elements<br>rogans of elements<br>pinski Rule Oase<br>granic functional groups (nested)<br>rganic functional groups (US EPA)<br>rganic functional groups (US PA)  | thods           2 Selected           Unselect All         Invert           es by BIR         ^           Harder (checkmon) | Structure   | H <sub>3</sub> C/CH <sub>3</sub>                           |                               |
| ✓ ✓ Toxic<br>✓ Toxic<br>✓ Ret<br>Pr<br>Pr  | oucture smarty<br>automers unstable<br>cological<br>epeated dose (HESS)<br>om<br>xample Prioritization Scheme (PBT<br>-benzoquinone precursors<br>recursors of 1,2,4-TCB<br>recursors of primary damines  | )  | Human Health Hazards Acute Toxicity ADME Bioaccumulation Carcinogenicity Developmental Toxicity / Teratogenicity Genetic Toxicity Immunotoxicity Immunotoxicity                       |  |                               |
| Ontions 4  | Metabolism/Trans  | formations<br>2 Selected   | Neurotoxicity   |  |                               |
| Options         Image: Constraint of the second secon  | Select All<br>Jmented<br>bserved Mammalan metabolism<br>bserved Marinalan metabolism<br>bserved Rat In vivo metabolism<br>bserved Rat Liver S9 metabolism<br><b>lated</b><br>utoxidation simulator<br>utoxidation simulator<br>utoxidation simulator<br>vidrolysis simulator (lakaline met<br>ssociation simulator<br>vidrolysis simulator (lakaline met<br>ssociation simulator<br>vidrolysis simulator (lakaline met<br>ssociation simulator<br>vidrolysis simulator (lakaline met<br>vidrolysis simulator (lakalin | Unselect All Invert  | Photoinduced toxicity Repeated Dose Toxicity Sensitisation AW SW AOP ToxCast Toxicity to Reproduction Toxicokinetics, Metabolism and Distribution Profiling Organic functional groups | Alkane, branche<br>Aryl<br>Carboxylic acid es<br>Isopropyl | 2: Categorized as 'ester'     |
| ✓ In<br>Mi<br>Ra<br>Sk   | two kat metabolism simul<br>ticrobal metabolism simul<br>at liver S9 metabolism simulator<br>automerism   | 15 <i>in vivo</i><br>etabolites  | Toxicological     Repeated dose (HESS)     Metabolism/Transformation     Hydrolysis simulator (neutral)     in vivo Rat metabolism simulator  | Not categorized<br>2 metabolite(s)<br>15 metabolite(s)     | 3: two hydrolysis<br>products |

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### Recap

- In module *Input*, you have entered the target chemical. The target has a high relationship CAS-SMILES. So it is considered good quality.
- In the *Data* module, you checked the databases corresponding to the selected target endpoint. No data has been found for the target.
- In the *Profiling* module, you profiled the target chemical with profiling schemes and metabolic simulators, labelled as plausible for the selected target endpoint.
- The target chemical is classified as a "Carboxylic Ester" according to structure-based profilers and does not have repeated-dose alerts responsible for the toxic effect based on the HESS profiler. No effect is expected.
- As seen 2 hydrolysing products and 15 *in vivo* rat liver metabolites are produced after applying (a)biotic simulation (hydrolysis at neutral pH, rat *in vivo* metabolism).
- Based on the fact that esters very easily undergo a chemical or an enzymatic hydrolysis
  [1-3] it is expected that this will be one of the first reactions to which the target chemical
  is exposed.
- Thus, the next actions are focused on investigation of the hydrolysis products of the target chemical.
- Go to *Data* module again to check whether there are any data for the metabolites.

<sup>1.</sup> Flavouring Group Evaluation 4<sup>1</sup>: 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.

<sup>3. 2-</sup>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 42ZCZS of the Therapeutic Goods Regulations 1990 (the Regulations), NICNAS, November **2015**.

# **Multiplication of target chemical**

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



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

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

### **Multiplication of target chemical**



### **Collect data for the metabolites**

#### Check for data availability for the generated metabolites



### Recap

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

<sup>1.</sup> GHS Classification. Fourth edition

<sup>2.</sup> 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**

| Data          | Import                               | Export         | Delete             |            |   |          |                             |                    |                |                       |     |
|---------------|--------------------------------------|----------------|--------------------|------------|---|----------|-----------------------------|--------------------|----------------|-----------------------|-----|
| -             | 👝 📷                                  |                |                    |            |   |          |                             |                    |                |                       |     |
| 0-0           |                                      | - <b>-</b> •   |                    |            |   |          |                             |                    |                |                       |     |
| Gather        | Import IUCLID6                       | IUCLID6        | Database Inventory |            |   |          |                             |                    |                |                       |     |
|               |                                      |                |                    |            | Filter endpoint tree                          | Ŷ        | Parent chemical [target]    | metabolite #1      | _              | metabolite #2         |     |
| $\mathbf{>}$  |                                      | Documents      |                    |            |   | <u> </u> |                             |                    |                |                       |     |
| •             |                                      |                |                    |            |   |          |                             |                    |                |                       |     |
| 0.1           |                                      | Databases      |                    | 701.11     |   |          | <u>^</u>                    |                    |                |                       |     |
| Options 4     |                                      |                |                    | / Selected |   |          | ,O                          |                    |                | $\bigcirc$            |     |
| T Ba          | Select All<br>ccenar mucagenicity 15 | 5511           | Unselect All       | Invert     | Structure                                     |          | 5                           | H3C                |                | $\bigcirc$            |     |
| Bic           | cides and plant prote                | ction ISSBIO(  | 2                  | ^          | Structure                                     |          |                             |                    |                |                       |     |
| 🔤 Ca          | rcinogenic Potency Da                | itabase (CPDI  | 3)                 |            |   |          |                             | CH3                | ЮН             |                       |     |
| Ca            | rcinogenicity&mutager                | nicity ISSCAN  |                    |            |   |          | н₃с∽ ∽сн₃                   |                    |                | HO                    |     |
| Ce            | I Transformation Assa                | y ISSCTA       |                    |            |   |          |                             |                    |                |                       |     |
| De            | velopmental & Reproc                 | luctive Toxici | ty (DART)          |            | + Structure info                              |          |                             |                    |                |                       |     |
| De            | velopmental toxicity d               | latabase (CAE  | ESAR)              |            |   |          | Export Data matrix          |                    |                |                       |     |
| De            | velopmental toxicity I               | LSI            |                    |            |   |          | Export CAS list             |                    |                |                       |     |
| V EC          | HA REACH                             |                |                    |            | Physical Chemical Properties                  |          | export ono list             |                    |                |                       |     |
| 🖌 EC          | отох                                 |                |                    |            | Environmental Fate and Transport              |          | Expand branch               |                    |                |                       |     |
| Ey            | e Irritation ECETOC                  |                |                    |            | Ecotoxicological Information                  | 9        | Collapse branch             |                    |                |                       |     |
| GA            | RD Skin sensitization                |                |                    |            | Human Health Hazards                          |          | Expand All                  |                    |                |                       |     |
| Ge            | notoxicity & Carcinoge               | enicity ECVAN  | 1                  |            | Acute Toxicity                                |          |                             |                    |                |                       |     |
| Ge            | notoxicity OASIS                     |                |                    |            | - ± ADME                                      | A        | Collapse All                |                    |                |                       |     |
| Ge            | notoxicity pesticides E              | FSA            |                    |            | Bioaccumulation                               | ۲        | Target endpoint             | 🕨 📄 Cop            | ру             |                       | _   |
| Hu            | iman Half-Life                       |                |                    |            | Carcinogenicity                               | -        | Open path                   | 👌 Defi             | ine            |                       |     |
| Ke            | ratinocyte gene expre                |                | an .               |            | Developmental Toxicity / Teratogenicity       | /        | open paul                   | 👌 Def              | ine here       |                       |     |
| Mie           | cronucleus ISSMIC                    | solori Luberta |                    |            | Genetic Toxicity                              | B        | Copy path                   | & Def              | ine from clink | haard                 |     |
| Mi            | cronucleus OASIS                     |                |                    |            | Immunotoxicity                                |          | Remove chemicals without    | data 🔥             | ine irom cipi  | board                 | _   |
| 🗸 МС          | JNRO non-cancer EFS/                 | A .            |                    |            | Irritation / Corrosion                        | -        |                             | Unc                | define         |                       |     |
| RE            | ACH Skin sensitisation               | database (no   | ormalised)         | _          | Neurotoxicity                                 | 2        | Function                    | •                  |                |                       |     |
| Ke            | ceptor Mediated Effect               | ITEM           |                    | 1          | Photoinduced toxicity                         |          | Sort                        | +                  |                |                       |     |
| v Re<br>√ Re  | peated Dose Toxicity                 | HESS           |                    | -          | Repeated Dose Toxicity                        |          | Activate AOD                |                    |                |                       |     |
| Ro            | dent Inhalation Toxici               | tv Datahase    |                    |            |   |          | Activate ACP                | +03 mg/b           | a bdwt/d       |                       |     |
| 0             |                                      |                |                    |            |   | 177      | Activate Effectopedia Wizar |                    |                | MS: 510 ma/ka bdwt/d  | _   |
|               |                                      | Inventories    |                    | 0.01 1 1   |   | 1/2      |                             | MS: 2 15E+03 mg/k  | a bdwt/d       | mo. 5 to mg/kg buwe a | -   |
| Options 4     | Colore All                           |                | Unerlant All       | 0 Selected |   | 1/1      |                             | MS: 2.15E+03 mg/k  | g buwu/u       |                       |     |
| T Canad       | 5 DSI                                |                | Unselect All       | Invert     |   | 1/1      |                             | WIS: 2.15E+05 mg/k | g bawi/a       |                       |     |
| COSIN         | IG                                   |                |                    |            | Sensitisation AW SW                           | AOP .    | 1 0                         | بمرياه الماريم     |                |                       |     |
| DSST          | DX XC                                |                |                    |            | loxCast                                       | 1.1      | I. K                        | ідпт-сіїск пе      | ext to         | the NOAEL lev         | e   |
| ECHA          | PR                                   |                |                    |            | Ioxicity to Reproduction                      |          | 2. S                        | elect <b>Targe</b> | et end         | point then De         | f   |
| EINEC         | S                                    |                |                    |            | — toxicokinetics, Metabolism and Distribution | Ition    | 2 1                         | hic action         | ic n           | oodod for n           | r,  |
| HPVC          | OECD                                 | Suchern ID-    |                    |            | Profiling                                     |          | 5. 11                       | is action          | 15 11          | eeded for pr          |     |
| Impor<br>METT | t_custom inventory_(<br>Japan        | Luscom IDs     |                    |            |   |          | CC                          | onsistency         | check          | c. The row            |     |
| NICNA         | S                                    |                |                    |            |   |          |                             | ndpoint will       | he col         | lored in vellow       | (   |
| - Michir      |                                      |                |                    |            |   |          | e e                         | iupoint will       | De COI         | loreu in yenow        | (Se |

# Transfering of observed data of metabolite to the target chemical



\*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**

| QSAR TOOLE   | ■X<br>input                                     | Profiling     > Data   |  |
|--|---|--|--|
| Categories<br>Categories<br>Define Define with metabolism  | gorize  | Category consistency<br>Category elements  |  |
| Docu   | ments   | iker endpoint freeze   |  |
| Organic func<br>Options J<br>f Select All  | tional groups Unselect All Invert               | Structure  |  |
| Plausible<br>Aquatic toxicity classificat  | Category consistency wizard                     | - 🗆 X  |  |
| Chemical elements<br>Groups of elements<br>Lipinski Rule Oasis<br>OECD HPV Chemical Cate<br>Organic functional group:<br>Organic functional groups   | Wizard pages                                    | The physicochemical similarity within a category can be assessed by using calculated parameters and experimental physicochemical data available in the Toolbox.           1. Go to Category definition | odule;   |
| Organic functional group:<br>Organic functional group:<br>Repeated dose (HESS)<br>Structure similarity<br>Substance type   | Physicochemical<br>similarity                   | 2D/3D parameters<br>Parameters<br># 2D<br>Boiling point 2. Click on <b>Category elements</b> ;<br>3. The wizard of Category consis   | tency appeared*.   |
| US-EAA New Chemical Ca<br>Unclassified<br>Acute aquatic toxicity cla<br>Acute aquatic toxicity M(  | Mechanistic similarity<br>(Eco)tox experimental | log Kow<br>Molecular Weight<br>Vapor Pressure (Antoine method)<br>Water Solubility   |  |
| Acute Oral Toxicity<br>Bioaccumulation - metabl<br>Bioaccumulation - metabl  | data<br>Options                                 | Add / Remove M: 510 mg/kg bdwt/d   |  |
| Biodegradation fragment<br>Biodegradation fragment<br>Biodegradation probabilit<br>Biodegradation probabilit<br>Biodegradation probabilit<br>Biodegradation probabilit<br>Biodegradation probabilit                            |   | Physical Chemical Properties     Bolling point     Partition Coefficient:     N-Octanol/Water     Vapour pressure     Water solubility     For the purpose of our exam     Solubility                  | ple not all default  |
| Biodegradation ultimate (<br>Blood brain barrier (beta)<br>Carcinogenictty (genotox<br>Crowded analines<br>DART scheme<br>Database Affiliation<br>DNA alerts for AMES by (<br>DNA alerts for CA and MI<br>DNA birding by OASIS |   | Add / Remove Selections will be preserved.<br>"Structural" and "Mechanistic sim<br>the OFG and RDT profilers will ren<br>similarity the default selections w<br>two slides).                           | ilarity" sections only<br>nain. For phys-chem<br>vere kept (see next |
| DNA binding by OECD<br>Estrogen Receptor Bindir<br>Example Prioritzation Sch<br>Eye irritation/corrosion E<br>Eye irritation/corrosion In<br>Hydrolysis half-life (Ka, ph<br>Hydrolysis half-life (Ka, ph                      |   | Back Next Cancel OK Tutorial_1_TB 4.2. Category consis   | elements see<br>stency   |

### **Category consistency check**

#### Step 1: Physicochemical similarity



### **Category consistency check**

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

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

**Calculated phys-chem properties** 

#### **Experimental phys-chem data**

#### Endpoint data

**Profiling results** 

Ready to move to the "Reporting" and including RAAF scenario

\*For more information on category elements see Tutorial\_27\_TB 4.4. Category elements for assessing Category consistency

### Recap

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

### **Report** Overview

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

### **Report** Selection of RAAF scenario

To select the applicable RAAF scenario for assessment, the following aspect should be identified<sup>\*</sup>:

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



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

The OECD (Q)SAR 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  $\leq 3$  analogues is proposed by LMC for the analogue approach);
- the read-across hypothesis biotransformation to common compound of the target substance;

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



# Read-Across Assessment Framework (RAAF) Scenario 1

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

|        | PARENT SUBSTANCES | (BIO)TRANSFORMATION             | COMMON<br>COMPOUNDS | NON-COMMON<br>COMPOUNDS |  |
|--------|-------------------|---------------------------------|---------------------|-------------------------|--|
| SOURCE | Α                 | A $\rightarrow$ not transformed | A                   | -                       |  |
| TARGET | В                 | B→A                             | A                   | •                       |  |

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

### Report generation according to RAAF-Scenario 1



- 1. Go to the *Report* module and click on the cell with the prediction;
- 2. Click the **Prediction** button;
- 3. Check the box at the top to add RAAF scenario;
- 4. Select **Scenario 1** from the drop-down menu;
- 5. Sections of the report including assessment elements specific to the scenario 1 are highlighted in yellow.

### Report generation according to RAAF-Scenario 1

| Customize report content and approximation | opearance   | - 🗆                             | ×  |               |               |            |               |
|--|---|---------------------------------|--|---------------|---------------|------------|---------------|
| Wizard pages                               |   | Customize report content and a  | ppearance  |               |               | -          |               |
| wizaro pages                               | wizard pages  |                                 |  |               |               |            |               |
| Customization                              | ○ 1.1. Category definition                              |                                 |  |               |               |            |               |
| Prediction                                 |   | Customization                   | ⊙ 2.1. Physicochemical similarity  |               |               |            | <u>^</u>      |
| Target ai                                  | ✓ Covered (target) endpoint(s)                          | Customize report  Prediction    | © 2.2. Structural similarity   |               |               |            |               |
| summary                                    | Category hypothesis                                     | Target and prediction           | Structural similarity  |               |               |            |               |
| Prediction details (I)                     | ○ 1.2. Category members                                 | summary                         | ⊙ Comments on structural similarity  |               |               |            |               |
| Prediction details (II)                    | Information of category members                         | Prediction details (I)          | AE A.2: Link of structural similarity  | / and differe | ences with th | e proposed | prediction    |
| 2 <sup>P</sup> 1                           | Ranges for selected physicochemical properties and cald | Target profiles                 | 2.3. Mechanistic similarity  |               |               |            |               |
| gu I n                                     | Purity / Impurity                                       | Analogues selection             | ⊙ 2.4. Additional endpoints  |               |               |            |               |
| Category                                   | AF A 1: Characterication of source substance            | details                         | ⊙ <mark>2.5. Other AEs</mark>  |               |               |            |               |
| Category definition                        | AE A.1. Characterisation of source substance            | Category<br>Category definition |  | target(s) to  | the common    | n compound | (s)           |
| and members                                |   | and members                     | ○ AE A.4: Bias that influences the property of the propert | ediction      |               |            |               |
| Consistency check                          | 1.3. Profiles/Metabolisms                               | Consistency check               |  |               |               |            |               |
| Options                                    | List of profiles/metabolisms                            | Options                         |  |               |               |            |               |
| Data matrix                                | → AE 1.1: Formation of common (identical) compound(s)   | Data matrix                     |  |               |               |            |               |
| Options                                    |   |                                 |  |               |               |            |               |
|  | $I \odot$ AE 1.4: The impact of parent compounds        | 3                               |  |               |               |            |               |
|  | ♥ → AE 1.5: Formation and impact of non-common compou   | r                               |  |               |               |            |               |
|  |   |                                 |  | <b>`</b>      |               |            | ~             |
| <u>Intro:</u>                              |   |                                 |  | Back          | Next          | Cancel     | Create report |
|  |   |                                 |  |               |               |            |               |

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

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

### Assessment elements of Scenario 1

| Customize report content and approximation  | ppearance  | 0               | Report basket   |   | _  |  | ×  |           |                      |
|---|--|-----------------|---|---|--|--|--|-----------|----------------------|
| Wizard pages  | 3  | Optic<br>f<br>⊿ | Select All Category   |   | Unselect All   | 0 9  | Selected<br>Invert   |           | ~                    |
| Customization<br>Customize report<br>Prediction<br>Target and prediction<br>summary<br>Prediction<br>Prediction<br>Target profiles<br>Analogues selection<br>details<br>Category<br>Category definition | <ul> <li>1.1. Category definition</li> <li>1.2. Category members</li> <li>1.3. Profiles/Metabolisms</li> <li>List of profiles/metabolisms</li> <li>AE 1.1: Formation of common (identical) compound(s)</li> <li>Hint<br/>PURPOSE:<br/>It has to be assessed whether:         <ul> <li>it is explained how the (identical) common product(s) are formed (i.e. the product on the property under consideration); and</li> <li>the provided evidence supports the explanation.</li> </ul> </li> </ul> |                 | 田 Table with profiling results for "     La Table with profiling similarity acc     La Table of category members     La Endpoint data variation (5 selected     La Endpoint data variation (4 selected     La Table with selected 2D/3D para     La Table with calculated structural     La Table with selected endpoint data variation | 'Organic fund<br>counting for<br>counting for<br>'Repeated do<br>counting for<br>counting for<br>cuted: Human<br>d: Boiling poir<br>cted: Physica<br>ameters for<br>d similarity<br>data values | Create new iter<br>Options ▲<br>f Select All<br>▲ Category<br>■Endpoint<br>■Parameter<br>■Chemical p<br>■Mechanist<br>■Table with<br>▲ External conter<br>▲Text prov | data va<br>r variati<br>profile<br>tic simila<br>h calcul<br><b>ent</b><br>ovided by | uns<br>ariation<br>ion<br>arity<br>lated stru<br>by user<br>y user | elect All | ) Selected<br>Invert |
| Consistency check<br>Options<br>Data matrix<br>Options  | AF .2: The biological targets for the common compound<br>4: The impact of parent compounds<br>.5: Formation and impact of non-common compou  | <               | Input<br>Target substance   | Create n  | ew OK  | Car  | OK   | (         | ancel                |

#### Intro:

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

Additionally, new items (including items with external content) can be created (4).

| Customize report content and approximation | ppearance                                 | 连 Report basket  | -   |  |       |                        |                             |                          |                         |
|--|---|--|---|--|-------|------------------------|-----------------------------|--------------------------|-------------------------|
| Wizard pages                               |   | Options ▲       f     Select All       ▲     ✓ Category       □     □       Table with profiling s | Unselect All<br>results for "Organic fu<br>similarity accounting fo | 1 Selected<br>Invert<br>Inctional group<br>or metabo |       | Purity / Impurity<br>  | ation of source subst       |                          | manually editable field |
| Customization                              | 🕑 1.1. Category def 🛛 👝                   | Table with profiling s   | similarity accounting f   | or metabo  | 3 🔽 д |                        | Name                        | SMTI ES                  | Structure               |
| Customize report                           | A 1.2 Category may 2                      | Table with profiling s   | similarity accounting for   | or metabo  |       | 140-26-1               | phenethyl isovalerate       | = CC(C)CC(=0)OCCc1ccccc1 |                         |
| Prediction                                 | 1.2. Category me                          | Table with profiling s   | similarity accounting f   | or metabolism  |       |                        |                             |                          |                         |
| Target and prediction                      | Information of category mer               | Table of category m  | embers  |  |       |                        |                             |                          |                         |
| summary                                    | Ranges for selected physicol              | Endpoint data variati  | ion (5 selected: Hum  | an Health Haza                                       | - I.  |                        |                             |                          |                         |
| Prediction details (I)                     | Burity (Impurity)                         |  | ion (4 selected: Bolling p  | ical Chemical Pi                                     |       |                        |                             |                          |                         |
| Prediction details (II)                    | C Purity / Impurity                       | □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □  | 2D/3D parameters fo   | r category me  | 1     |                        |                             |                          | нзс снз                 |
| Tanaat and files                           | AE A.1: Characterisation of s             | 日本 Table with calculated   | d structural similarity   |  | 2     | No CAS number          | r Hydrolysis simulator      | OCCc1ccccc1              |                         |
| larget profiles                            | Hint                                      | Table with selected  | endpoint data values  |  | NI.   |                        | (neutral): metabolite<br>#2 |                          |                         |
| Analogues selection                        | PURPOSE:                                  | A Target substance   |   |  |       |                        |                             |                          |                         |
| details                                    | The substance which is used as the source |  |   |  |       |                        |                             |                          |                         |
| Category                                   | assessed whether:                         |  |   |  |       |                        |                             |                          | HO                      |
| Category definition                        | across; and                               | 3  |   |  |       | AE A 2: Deliability of | nd adequacy of the          |                          |                         |
| and members                                | - the impurity profile is                 | Create ne  | W OK  | Cancel   |       | Not provided by use    | er                          | source study             |                         |
| Consistency check                          | Name, CAS and/or E                        | tructure should be provided.   |   |  |       |                        |                             |                          |                         |
| Ontions                                    |   |  |   |  |       |                        |                             |                          |                         |
| Data matrix                                | Add / Remove                              |  |   |  |       |                        |                             |                          |                         |
|  |   | 6.1 · · ·  |   |  |       |                        |                             |                          |                         |
| Options -                                  | AE A.3: Reliability and adeq              | uacy of the source study   |   |  |       |                        |                             |                          |                         |
|  | Hint                                      |  |   |  |       |                        |                             |                          |                         |
|  |   |  |   |  |       |                        |                             |                          |                         |
|  | Add / Kemove                              |  |   |  |       |                        |                             |                          |                         |
|  | 21.3. Profiles/Metabolismo                |  |   |  |       |                        |                             |                          |                         |
|  |   |  |   | ~  |       |                        |                             |                          |                         |
|  | •   | Back Next  | Cancel  | Create report  |       |                        |                             |                          |                         |

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

AE A.1 Characterization of source substance. The user should manually open the report basket (1) and select the item: Table of category members (2). If impurities/additives of the used analogues are available, they will appear under the AE A.1 in Purity / Impurity. The current analogues have no additives/impurities.

An example of how the AE A.1. will look in the generated report is shown on the right (3).

• AE A.3 Reliability and adequacy of the source study should be filled in manually (4) (see next slide)

| Customize report content and appearance   | e   | 💽 Report basket  | -  |   | Create new items   | _  |   | ×      |
|---|---|--|--|---|--|--|---|--------|
| Customize report content and appearance   | c.  | Options  |  |   |  |  |   |        |
| Wizard pages  |   | f Select All  ✓ Category  □ □ Table with profiling results for  □ □. Table with profiling similarity a  □ □. Table with profiling similarity a   | Unselect All<br>r "Organic functional group<br>accounting for metabolism<br>accounting for metabolism  | ps"<br>("Hydrolysis sin<br>("in vivo Rat m  | Options ▲           f         Select All           Unselect All           ▲ Category           □ Endpoint data variation                                     | Invert   | 1 Se<br>About 0   | ptions |
| Customization<br>Customize report<br>Prediction<br>Target and prediction<br>summary<br>Prediction details (I)<br>Prediction details (II)<br>Prediction details (II)<br>Target profiles<br>Analogues selection<br>details<br>Category definition<br>and members<br>Consistency check | 1. Category definition<br>2. Category members<br>Information of category mem<br>Ranges for selected physicoch<br>Purity / Impurity<br>AE A.1: Characterisation of so<br>AE A.3: Reliability and adequa<br>Hint<br>PUROSE:<br>The source study needs to match the default<br>equested for any other key study. It has to I<br>the study design reported for the source st<br>in read-across:<br>the study design should cover the key para | A Table with profiling similarity a     Table with profiling similarity a     A Table with profiling similarity a     A Table with profiling similarity a     A Table with profiling results for     Table with avariation (5 select     Table with selected 20/30 p     Table with selected 20/30 p | ccounting for metabolism<br>"Repeated dose (HESS)"<br>(ccounting for metabolism<br>(counting for metabolism<br>("Organic functional group<br>"Repeated dose (HESS)"<br>ected: Human Health Haz<br>ed: Bolling point; log Kow;<br>ected: Physical Chemical Farameters for category me<br>ral similarity<br>(data values | ("In vivo Rat m<br>("Hydrolysis sin<br>("In vivo Rat m<br>ps"<br>ards#Repeated<br>Molecular Weis<br>Yoperties#Bolin<br>embers |  | tural simila<br>3<br>OK -<br>-<br>   | rity  |        |
| Options<br>Data matrix<br>Options<br>1  | the study design should cover an exposure<br>eferred to in Article 13(3); and<br>there is adequate and reliable documentat<br>provided. The test material used represents of<br>and impurities.<br>Add / Remove<br>The source substance is tested in<br><b>3. Profiles/Metabolisms</b>  | Cree   | rivpop OK<br>rivpop 6<br>rov Edit Preview  | Cancel  | The source substance is t<br>dermal toxicity:90-Day-sti<br>OECD 411<br>The study is used to pred<br>toxicity study according t<br>411 for the target substar | ested in a su<br>ady test accord<br>ct the repeat<br>o OECD guid<br>ce<br>OK | ub chronic<br>ording to<br>ated dose<br>deline<br>5<br>Cancel |        |

**AE A.3:** Click the **Add/Remove** button (1) and click **"Create new"** item **(2).** From the appeared window called "Report basket" click on item **"Text provided by user"** (3). Click **OK** (4). In the text field paste the following example text and click **OK** (5): *"The source substance is tested in a sub chronic dermal toxicity:90-Day-study test according to OECD 411 The study is used to predict the repeated dose toxicity study according to OECD guideline 411 for the target substance"* 

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



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

| Customize report content and a   |   |  |
|--|---|--|
| Wizard pages   |   | AE A.3: Reliability and adequacy of the source study<br>"The source substance is tested in a sub chronic dermal (text provided by user)<br>"The source substance is tested in a sub chronic dermal toxicity:90-Day-study test according to OECD 411<br>The study is used to predict the repeated dose toxicity study according to OECD guideline 411 for the<br>target substance"  |
| Customization<br>Customize report  | ⊙ 1.1. Category definition  | image from clipboard No.1 (image provided by user)   |
| Prediction   | I.2. Category members   |  |
| summary  | Information of category members   |  |
| Prediction details (I)   | Ranges for selected physicochemical properties and calculated parameters  |  |
| Prediction details (II)  | Purity / Impurity   |  |
| Target profiles  | AE A.1: Characterisation of source substance  | QSAR Toolbox 4.4 QSAR TOOLBOX TPRF v4.4  |
| Analogues selection  | (a) AE A.3: Reliability and adequacy of the source study  |  |
| details Category Category definition and members Consistency check Options Data matrix Options | <ul> <li>Hint</li> <li>PURPOSE:</li> <li>The source study needs to match the default REACH requirements in terms of reliability and adequacy as requested for any other key study. It has to be assessed whether:</li> <li>the study design reported for the source study is adequate and reliable for the purpose of the prediction based on read-across:</li> <li>the study design should cover the key parameters in the corresponding test method referred to in Article 13(3);</li> <li>the study design should cover an exposure duration comparable to or longer than the corresponding method referred to in Article 13(3); and</li> <li>there is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided. The test material used represents the source substance as described in the hypothesis in terms of purity and impurities.</li> </ul> | Chemicals category       3/9   |
|  |   | 2 = karbia my kitty o my<br>(− karbia my kitty o my<br>(− karbia my kitty o my<br>− − wisk, tri kitty o my<br>− − wisk, tri kitty o my<br>− − wisk, tri kitty o my<br>tri kitty o my<br>tr |
|  | Lait Preview  | La Maria Maria Maria La Talana - O X<br>Encome<br>Register<br>Register<br>Data and tar. Talana Sarana Sar<br>Sarana Sarana Saran<br>Sarana Sarana Saran  |
|  |   | Auer tent honorhammet euroreau autor 0003/balter 11 balteres er Euror Baute 1000 er en   |
| An example   | of how the AFs part of <b>AE A.3</b> , will look  | And a start of the   |
| in the gene<br>finished with<br>Continues w  | rated report is shown on the right. We<br>the section "1.2. Category members".  |  |

### Assessment elements of Scenario 1

| Customize report content and appearance  | Report basket  | Create new items  | • ×  |
|--|--|---|--|
| Wizard pages   | Options  | Options J<br>f Select All Unselect All I  | Item name<br>Automatically generated<br>Select your image here:  |
| Customization       ○ 1.1.         Customize report       ○ 1.2.         Prediction       ○ 1.3.         Summary       ○ Li         Prediction details (I)       ○ Li         Prediction details (II)       ○ Hill   | Category de Category me Catego | a Category  a Endpoint data variation  a Parameter variation  a Chemical profile  a Mechanistic similarity  a Table with calculated struct  b Content  b Content  b Content  c Image provided by user | Target substance<br>$H_{5} \leftarrow H_{3} \rightarrow H_{9} \leftarrow H_{1} \leftarrow H_{1} \leftarrow H_{1} \leftarrow H_{2} \leftarrow H_$ |
| Target profiles<br>Analogues selec<br>details<br>Category<br>Category definition   | Impose  | a Text provided by user   | Specify how much of the page width is occupied by the image:<br>Image width, % 75<br>OK Cancel   |
| Active of the second se | Image from clipboard No.1 (image pro         Hydrolysis of the e         ■ Input   | vidСк   | Cancel 6   |
| Options $\bigcirc$ A   | E 1.4: The impade <<br>E 1.5: Formation Create new 2   | OK Cancel   | )  |

Open section 1.3. Profiles/metabolism and then sub-section AE1.1. Formation of common (identical) compound(s). Once you are in section AE1.1, click on **Add/Remove** button (1) and then **Create new** (2). Select to create item with external content – *Image provided by user* (3) and click **OK** (4). New window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture is saved\*. Finally confirm by **OK** (6).

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

### Assessment elements of Scenario 1



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

• Source substance B and Target substance A.

THE OLCD (QISAK TOOIDOX TOF GLOUPING CHEMICAIS INTO CATEGORIES

- 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)

| Customize report content and a  | appearanceX   |  |
|---|---|--|
| <ul> <li>Customize report content and a</li> <li>Wizard pages</li> <li>Customization         <ul> <li>Customize report</li> </ul> </li> <li>Prediction         <ul> <li>Target and prediction summary</li> <li>Prediction details (I)</li> <li>Prediction details (II)</li> <li>Target profiles</li> <li>Analogues selection details</li> </ul> </li> <li>Category</li> </ul> | Pypearance  | 1.3. Profiles/Metabolisms         List of profiles/metabolisms         AE 1.1: Formation of common (identical) compound(s)         Hydrolysis of the ester.jpg (image provided by user)         Target substance   |
| Prediction details (I)<br>Prediction details (II)<br>Target profiles<br>Analogues selection<br>details<br>Category<br>Category definition<br>and members<br>Consistency check<br>Options<br>Data matrix<br>Options  | <ul> <li>AE 1.1: Formation of common (identical) compound(s)</li> <li>Hint</li> <li>PURPOSE:</li> <li>It has to be assessed whether:         <ul> <li>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</li> <li>the provided evidence supports the explanation.</li> </ul> </li> <li>Add / Remove         <ul> <li>Hydrolysis of the ester.jpg (image provided by user)</li> <li>Edit Preview</li> <li>Source substance B and Target substance A. A is claimed (text pro Edit Preview)</li> <li>AE 1.2: The biological targets for the common compound(s)</li> <li>AE 1.4: The impact of parent compounds</li> </ul> </li> </ul> | Source substance B and Target substance A. A is claimed (text provided by user)<br>Source substance B and Target substance A.<br>A is claimed to be metabolized to B and that the organism is only systemically exposed to B upon external<br>exposure to A.<br>Therefore it is expected B to be responsible for the toxic effect of the target substance A<br>AE 1.2: The biological targets for the common compound(s)<br>Not provided by user<br>AE 1.4: The impact of parent compounds<br>Not provided by user<br>AE 1.5: Formation and impact of non-common compounds<br>Not provided by user<br>2. Consistency check |
| Both newly c<br>can be edited<br>An example   | ○ AE 1.5: Formation and impact of non-common compounds created items appear under the <b>AE 1.1</b> . (1). Each of the items d (2) or just previewed (3) in a <i>.pdf</i> format or re-ordered of how the AE 1.1. and related description will look in the  | OSAR Toolbox 4.4 OSAR TOOL ROX TPRF v4.4   |

| Customize report content and approximately content and approximatel | opearance – 🗆  | ×   |  |
|---|--|-----|--|
| Wizard pages  |  |     | The following text is used as an example for both assessment elements <b>AE 1.2</b> and <b>AE 1.4</b> . Both text are added as a new text report item (steps are illustrated on slide 45).   |
| Customize report<br>Prediction<br>Target and prediction<br>summary<br>Prediction details (I)<br>Prediction details (I)<br>Target p<br>Analogu<br>details<br>Category<br>Category definition<br>and members  | <ul> <li>♥ 1.1. Category definition</li> <li>♥ 1.2. Category members</li> <li>♥ 1.3. Profiles/Metabolisms</li> <li>♥ List of profiles/metabolisms</li> <li>♥ AE 1.1: Formation of common (identical) compound(s)</li> <li>● AE 1.2: The biological targets for the common compound(s)</li> <li>● AE 1.2: The biological targets for the common compound(s)</li> <li>● Hint<br/>PURPOSE:<br/>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:         <ul> <li>the same biological targets are affected by the common compound(s); and</li> <li>the provided evidence supports the explanation.</li> </ul> </li> <li>Add / Remove</li> </ul>   |     | <ul> <li>An example text for AE1.2. The biological targets for the common compounds (1):</li> <li>Example for differences in distribution pattern leading to different biological targets for the common compound <ul> <li>Substance A is converted to substance B in the liver based on hydrolysis reaction.</li> <li>Oral study with B is used to predict the toxicity of A after oral administration.</li> </ul> </li> <li>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.</li> </ul>  |
| Options<br>Data matrix<br>Options<br>2  | <ul> <li>AE 1.4: The impact of parent compounds</li> <li>Hint</li> <li>PURPOSE:</li> <li>(Bio)transformation of parent compounds, i.e. target and source substances, may not be immediate and/or or target substances. In addition, the impurity profiles associated with the source and target substances may have an impact on the prediction of the property under consideration has been addressed;</li> <li>the parent compound and its impact on the prediction of the property under consideration has been addressed;</li> <li>the greent compound and its impact on the prediction; and</li> <li>the provided evidence supports the explanation.</li> <li>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.</li> <li>Add / Remove</li> <li>A E 1.5: Formation and impact of non-common compounds</li> </ul> | ort | <ul> <li>An example text for AE1.4. The impact of parent compounds (2):</li> <li>Substance A is converted to substance B in the liver</li> <li>Substance B is clamed to derive the effect</li> <li>The parent chemical A is present in significant amounts (its is monoconstituent without any additives or impurities)</li> <li>Substance A is suspected to have toxicity of its own</li> <li>The Substance B is used as a source to predict the effect for Substance A</li> <li>The impact of impurity if available should be addressed here. An example text is provided below:</li> <li>Substance A consist of the main constituent A, there is an impurity X of 5%</li> <li>The substance B consists of the main constituent B, there is the same impurity X of 3% and impurity Y of 2 %</li> </ul> |

| Customize report content and a  | ppearance – 🗆 >  |        |  |
|---|--|--------|--|
| Wizard pages  | Q12 Category members   | ^      | The report items associated with <b>AE 1.2</b> (1) and <b>AE 1.4</b> (2) appeared under the respective sections of the report. How they look in the generated report is shown on the right.  |
| Customize report<br>Prediction<br>Target and prediction<br>summary<br>Prediction details (I)<br>Prediction details (II)<br>Target profiles<br>Analogues selection<br>details<br>Catego<br>and me. | <ul> <li>A.3. Profiles/Metabolisms</li> <li>List of profiles/metabolisms</li> <li>AE 1.1: Formation of common (identical) compound(s)</li> <li>AE 1.2: The biological targets for the common compound(s)</li> <li>Hint<br/>PURPOSE:<br/>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:         <ul> <li>the same biological targets are affected by the common compound(s); and</li> <li>the provided evidence supports the explanation.</li> </ul> </li> </ul>   |        | AE 1.2: The biological targets for the common compound(s)     Example for differences in distribution pattern leading (text provided by user)     Example for differences in distribution pattern leading (text provided by user)     Example for differences in distribution pattern leading (text provided by user)     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 or compared between B administered directly or when formed from A.     E 1.4: The impact of parent compoundB     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user)     Substance A is converted to substance B in the liver (text provided by user) |
| Consistency check<br>Options<br>Data matrix<br>Options  | <ul> <li>▲ Example for differences in distribution pattern leading (text provi Edit Preview )</li> <li>AE 1.4: The impact of parent compounds</li> <li>→ Hint</li> <li>PURPOSE:</li> <li>(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.</li> <li>It has to be assessed whether:</li> <li>the parent compound and its impact on the prediction of the property under consideration has been addressed;</li> <li>the provided evidence supports the explanation.</li> <li>Under this scenario, the parent compounds) 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.</li> </ul> |        | Chemicals category       4/9         Substance A is converted to substance B in the liver         Substance B is clamed to derive the effect         The parent chemical A is present in significant amounts (its is monoconstituent without any additives or impurities)         Substance A is suspected to have toxicity of its own         The Substance B is used as a source to predict the effect for Substance A         The impact of impurity if available should be addressed here. An example text is provided below:         Substance A consist of the main constituent A, there is an impurity X of 5%         The substance B consists of the main constituent B, there is the same impurity X of 3% and impurity Y of 2% <b>AE 1.5: Formation and impact of non-common compounds</b> Not provided by user   |
|   | A: Substance A is converted to substance B in the liver (text provide Edit Preview      A: Substance A is converted to substance B in the liver (text provide Edit Preview     A: Substance A is converted to substance B in the liver (text provide Edit Preview     Back Next Cancel Create reac   | v<br>t |  |



| Customize report content and   | appearance – 🗆 X   |
|--|--|
| Wizard pages   |  |
| istomization   | © 1.1. Category definition   |
| Customize report   |  |
| ediction   | O 1.2. Category members  |
| Target and prediction  | O 1.3. Profiles/Metabolisms  |
| Dradiatian dataila (I)   | ✓ List of profiles/metabolisms   |
| Prediction details (I)   |  |
| Predictio (II)   | ○ AE 1.2: The biological targets for the common compound(s)  |
| Target p   | ○ AE 1.4: The impact of parent compounds   |
| Analogues selection  | A E 1 E: Formation and impact of non-common compounds  |
| Category<br>Category definition<br>and members<br>Consistency check<br>Options<br>ta matrix<br>Options | Hint     PURPOSE:     The formation of common compound(s) often goes together with the formation of non-common compound     (s) and/or potential intermediates during the formation of the common compound(s). Source and/or target     substances can also be (bio)transformed by other pathways than the one involved in the formation of the     common compound(s), leading to 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 validity of the explanation.  Add / Remove |
| 2  | Figure7_Firew.jpg (image provided by user)   |
|  | A Target substance A is an ester which is known that hydrolyzes . Edit Preview   |
|  | 3 4  |
|  | Back Next Cancel Create report   |

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

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

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

|   | 1     |
|---|-------|
|   |       |
| Target substance A is an ester which is known that hydrolyzes (text provided by | user) |
| Target substance A is an ester which is known that hydrolyzes (a)blotically     |       |
| After a laboration of the second substance 2)                                   |       |
| 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   |       |



The OECD (Q)SAR Toolbox for Grouping Chemicals into Categories

| Customize report content and ap                   | opearance – 🗆 X   |  |   |   |   |                                  |
|---|---|--|---|---|---|----------------------------------|
| Wizard pages                                      |   |  |   |   |   |                                  |
| Customization<br>Customize report                 | © 1.1. Category definition  | AE 1.5: Formation and imp<br>Table with Human Hea  | <mark>act of non-common</mark><br>Ith Hazards data varia  | compounds<br>tion   | 3   |                                  |
| Prediction<br>Target and prediction<br>summary    | © 1.2. Category members   | Position   | Variation   |   | unit (family)   | Number of<br>chemicals           |
| Prediction details (I)<br>Prediction details (II) | AE 1.1: Formation of common (identical) compound(s)   | uata   |   |   |   |                                  |
| Target profiles<br>Analogues selection            | <ul> <li>◇ AE 1.2: The biological targets for the common compound(s)</li> <li>◇ AE 1.4: The impact of parent compounds</li> </ul> | Figure7_Firew,jpg (image   | Provided by user)   | metabolite #1   | metabolite #2   |                                  |
| details<br>Category                               | AE 1.5: Formation and impact of non-common compounds  | ucture   | Hoc Cons  | H3C OH  |   |                                  |
| and members Consistency check                     | Add / Remove  | Structure info     Structure info     Parameters     Physical Chemical Properties     Environmental Fate and Transport   |   |   |   |                                  |
| Options<br>Data matrix                            | Figure7_Firew.jpg (image provided by user)     Edit     Preview   | Ecotoxicological Information     Human Health Hazards  |   |   |   |                                  |
| Options   | A Target substance A is an ester which is known that hydrolyzes (te Edit Preview  | Target substance A is an e<br>Target substance A is an es<br>acid (Substance Z)<br>After oral absorption, subst<br>The substance responsible<br>It is also known that the Su | ster which is known ti<br>ster which is known th<br>tance A hydrolyzed to<br>for the effect is subst<br>ubstance Z (acid) is le | hat hydrolyzes .<br>nat hydrolyzes (<br>the B and Z<br>cance B (alcohol<br>ss toxic than th | (text provided by<br>a)biotically to alcol<br>)<br>e substance alcoho | r user)<br>nol (substance B) and |
| An example of how in the generated re             | v the AE 1.5 and related description will look<br>port is shown on the right.   |  |   |   |   |                                  |

| Customize report content and ap | pearance – 🗆 X   | 7 |
|---------------------------------|--|---|
| Wizard pages                    |  |   |
| ustomization                    | ⊙ 2.1. Physicochemical similarity  |   |
| ediction                        | O 2.2. Structural similarity   |   |
| Target and prediction           | Structural similarity  |   |
| summary                         | Structure similarity profilers   |   |
| Prediction details (I)          | Options  |   |
| Prediction details (II)         | <ul> <li>✓ Plausible</li> </ul>  |   |
| Target profiles                 | Chemical elements  |   |
| Analogues selection             | Lipinski Rule Oasis  |   |
| details                         | ✓ Organic functional groups ☐ Organic functional groups (nested)                     |   |
| tegory                          | Organic functional groups (US EPA)   |   |
| Category definition             | Structure similarity   |   |
| and members                     |  |   |
| Consistency check               | Add / Remove   |   |
| Options                         | Table with calculated structural similarity  | 1 |
| Options                         | Table with profiling results for "Organic functional groups"                         |   |
|                                 | ✓ Comments on structural similarity  | Г |
| 1                               | ♦ AE A.2: Link of structural similarity and differences with the proposed prediction |   |
|                                 | Hint   |   |
|                                 | Add / Remove   |   |
|                                 | © 2.3. Mochanistic similarity  |   |
|                                 |  |   |
|                                 | v 2.4. Additional endpoints  |   |
|                                 | C 2.5. Other AEs   |   |
|                                 |  |   |
|                                 | Back Next Cancel Create report   |   |
|                                 | Conter Conter Conter Conter  |   |

#### Table with calculated structural similarity

Options

Mode: Hologram, CombineAllFeatures

Measure: Dice

Molecular features: AtomCenteredFragments

Atom characteristics: AtomType, CountHAttached, Hybridization

Calculated structure similarity
Parent chemical

|                                    | Parent chemical<br>CAS 140-26-1 | metabolite #2<br>CAS No CAS number |
|------------------------------------|---------------------------------|------------------------------------|
| Parent chemical<br>CAS 140-26-1    | 100%                            | 58.3 %                             |
| metabolite #2<br>CAS No CAS number | 58.3 %                          | 100%                               |



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

| Customize report content and ap         | ppearan    | nce  |   |   |   |  | -                                 | D X           |   |
|---|------------|--|---|---|---|--|-----------------------------------|---------------|---|
| Wizard pages                            |            |  |   |   |   |  |                                   |               |   |
| <b>istomization</b><br>Customize report | ⊙ 2        | 2.1. Physic  | ochemical                                   | similarity                                |   |  |                                   |               | ^ |
| ediction                                |            | Structural   | ural similar<br>similarity                  | <mark>ity</mark>                          |   |  |                                   |               |   |
| summary                                 |            |  | on structura                                | l similarity                              |   |  |                                   |               |   |
| Prediction details (I)                  |            | ΔF Δ.2: 1 ir   | nk of structur                              | al similarity                             | and differer                              | ices with the  | e proposed                        | prediction    |   |
| Prediction details (II)                 | C.         | Hint   |   |   |   |  |                                   |               |   |
| Target profiles                         |            | PURPOSE:   |   |   |   |  |                                   |               |   |
| Analogues selection                     |            | The aim of this A<br>has to be assess                                | AE is to verify that t<br>ed whether:       | he source and tar                         | get substances a                          | re covered by the  | e read-across hy                  | pothesis. It  |   |
| itegory                                 |            | <ul> <li>the scientific hy</li> <li>structural similation</li> </ul> | ypothesis establish<br>arities and differen | es the structural s<br>ces are linked wit | imilarities and di<br>h the possibility t | fferences of sources of sources of sources of sources of the sourc | ce and target;<br>properties; and |               |   |
| Category definition                     |            | - the provided e   | vidence supports tl                         | he proposed link                          | between structur                          | al similarities and  | I the possibility t               | o predict.    |   |
| and members                             |            | Add / Remove   |   |   |   |  |                                   |               |   |
| Consistency check                       |            | A Structural   | similarity betwe                            | en Substance                              | A and B acco                              | rding (text  | Edit Pr                           | eview 😫       |   |
| ata matrix                              | 2          | 2.3. Mecha   | nistic simi                                 | larity                                    |   |  |                                   |               |   |
| Options                                 | 2          | 2.4. Additi  | onal endpo                                  | oints                                     |   |  |                                   |               |   |
|   | <b>⊘</b> 2 | 2.5. Other   | AEs   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               |   |
|   |            |  |   |   |   |  |                                   |               | ~ |
|   |            |  |   |   | Back                                      | Next   | Cancel                            | Create report | t |

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

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

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

| 🖲 R  | eport basket   |                   | -                 |           | $\times$   |
|--|--|-------------------|-------------------|-----------|--|
| Optio  | ns 🖌   |                   |                   | 1         | Selected   |
| f  | Select All   |                   | Unselect All      |           | Invert   |
| 4  | Category   |                   |                   |           |  |
|  | Table with profiling resul   | ts for "Organic   | functional gro    | ups"      |  |
|  | Table with profiling simila  | rity accountin    | g for metabolisi  | m ("Hyd   | rolysis sin  |
|  | Table with profiling simila  | rity accounting   | g for             | n profili | na similari  |
|  | Table with profiling result  | ts for "Repeat    | ed d              | (Num      | lig sinnin   |
|  | L Table with profiling similar   | rity accounting   | g for metabolisi  | m (Hyd    | irolysis sin   |
|  | Table of category memb   | incy accounting   | g for metabolisi  | 11(11)    | NO KAU II  |
|  | The Endpoint data variation (  | 5 selected: Hi    | iman Health Ha    | zarde#    | Reneated   |
|  | 1 Endpoint data variation (  | 4 selected: Ph    | vsical Chemical   | Proper    | ties#Boilir  |
|  | Table with selected 2D/  | 3D parameters     | for category n    | nember    | 5  |
|  | Table with calculated str  | uctural similarit | y                 |           |  |
|  | Table with selected end  | point data valu   | ies               |           |  |
|  | the second | 1 selected: Hu    | uman Health Ha    | zards#    | Repeated   |
| _  | □ □ Parameter variation (5 se  | elected: Molec    | ular Weight; log  | g Kow;    | Boiling po   |
| 4  | External content   |                   |                   |           |  |
|  | A The source substance i   | s tested in a si  | ub chronic dern   | nal (1    | text prov  |
|  | Intege from cipboard No  | .1 (image prov    | ided by user)     |           |  |
|  | A Source substance B and   | Tarnet substa     | ince A A is clair | med       | (text pro  |
|  | A Example for differences  | in distribution   | pattern leading   | (te)      | t provide  |
|  | A Substance A is converte  | d to substance    | B in the liver    | (text     | provided   |
|  | Figure7_Firew.jpg (image   | e provided by     | user)             |           |  |
|  | A Target substance A is an   | rester which i    | s known that h    | yurolyz   | es (te,  |
| ▲ A Structural similarity between Substance A and B according (text pr |  |                   |                   |           |  |
|  | Input  |                   |                   |           |  |
| 🔄 👗 Target substance   |  |                   |                   |           |  |
|  |  |                   |                   |           |  |
|  |  |                   |                   |           |  |
| <  |  |                   |                   |           | >  |
|  |  | Create new        | OK                | 6         | ncel   |
|  |  | create new        | UK                |           | and the second s |

| Customize report content and approximation | ppearance – 🗆 X   | ] [                           | Table with profiling results for "Repeated  | d dose (HESS)"   |
|--|---|-------------------------------|---|--|
| Wizard pages                               |   |                               | 1 CAS# 140-26-1 2 CAS# No CA  | AS number  |
| Customize report                           | ⊙ 2.1. Physicochemical similarity   |                               |   |  |
| Prediction                                 |   |                               | •   |  |
| Target and prediction                      | 📀 2.3. Mechanistic similarity   |                               |   |  |
| summary                                    | O Mechanistic similarity  |                               | HO~   |  |
| Prediction details (I)                     | Mechanistic similarity profilers  |                               | H <sub>3</sub> C/ \CH <sub>3</sub>  |  |
| Prediction details (II)                    | f Select All Unselect All Invert  | 1                             | Not categorized Styrene (Rena   | al Toxicity)   |
| Analogues selection                        | Plausible     Aquatic toxicity classification by ECOSAR                               |                               | Toluene (Rena   | al toxicity)   |
| details                                    | ☐ OECD HPV Chemical Categories ✓ Repeated dose (HESS)                                 |                               | Alert   |  |
| Category                                   | US-EPA New Chemical Categories  |                               |   |  |
| and members                                | ▷ L     Unclassified       Simulators   | <u> </u>                      | 1/2   | 2/2  |
| Consistency check                          | Options   | Table                         | with profiling similarity accounting for metabolism ("Hydrolysis simulator (neutral)" and "Repeated |  |
| Options                                    | f Select All Unselect All Invert  | Acse<br>Meta<br>Profi         | (HESS)<br>bolism: Hydrolysis simulator (neutral)<br>ler: Repeated dose (HESS)                       |  |
| Data matrix                                | Dissociation simulator<br>Hydrolysis simulator (acidic)                               |                               | ables with generated metabolites for each analogue with profiling result                            |  |
| Options                                    | V Hydrolysis simulator (neutral)  | P1 CAS#: 14                   | 0-26-1 M1 P1 M2 P1  | C/S 140-26-1   |
|  | ▶ Unclassified  |                               | OH3 OH  |  |
| 1  | Add / Kemove  | ĺ                             | HO  |  |
|  | Table with profiling results for "Repeated dose (HESS)"                               | H <sub>3</sub> C/Not categori | CH3<br>zed Carboxylic acids Styrene (Renal Toxicity)<br>(Herostotevicity) No cark Alext             | Table summarizing number of metabolites including parent with specific alerts Repeated dose (HESS) P1 P2 |
|  | 🗄 Table with profiling similarity accounting for metabolism ("Hydroly. Edit Preview 💲 |                               | Blycolic acid (Renal Toxicity)<br>Alert   | 140-zb-1 No CK5 number<br>Carbonylic acids I 0<br>(Hepatotoxicity) No rank   |
|  | ○ Comments on mechanistic similarity  | P2                            | No metabolites  | (Glycolic acid (Renal Toxicity)) 1 0<br>Alert<br>Not categorized 1 0   |
|  | ⊙ 2.4. Additional endpoints   |                               | $\bigcirc$  | Styrene (Renal Toxicity) 1 1<br>Alet<br>Toluene (Renal toxicity) 1 1   |
|  | ⊙ <mark>2.5. Other AEs</mark>   |                               |   | Alet   |
|  |   | HO                            | J   |  |
|  | Back Next Cancel Create report  | Styrene (Rer<br>Toluene (Re   | nal Toxichy) Alert<br>Inal toxichy) Alert   |  |
|  |   |                               |   |  |

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

| nize report                             | © 2.1. Physicochemical similarity  | An example text for AE 1.3. Exposure of the biologic  |
|---|--|---|
| and prediction                          | ♥ 2.2. Structural similarity   | target(s) to the common compound(s):  |
| ry                                      | $\odot$ 2.4. Additional endpoints  | Substance A is transformed to B   |
| ion details (I)                         | © 2.5. Other AEs   | <ul> <li>Also similar target chemicals having ester functionality a<br/>transformed to B too [cite literature here]</li> </ul>  |
| ion details (II)<br>profiles            | ♦ AE 1.3: Exposure of the biological target(s) to the common compound(s)   | Similar reactivity pattern is obtained for all targets transformed the common compound B  |
| ues selection<br>ry definition<br>mbers | 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.  | An expert can provide additional literature search of simi analogues with similar effects.  |
| ency check                              | Add / Remove A Two hydrolysing products are generated for the target (text prov Edit Preview   | An example text for <b>AE A.4. Bias that influences t</b><br>prediction:  |
| J                                       | <ul> <li>A E A.4: bias that influences the prediction</li> <li>Hint</li> <li>PURPOSE:</li> <li>It has to be assessed whether:         <ul> <li>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;</li> <li>there are additional, structurally-similar substances which are currently not used in the analogue approach and which arguably could be used;</li> <li>there is readily-available information from these additional substances;</li> <li>this information is biologically significantly different for relevant properties in comparison with the existing analogue(s); and</li> <li>these differences decrease the confidence in the prediction (possibility of underestimation of hazard).</li> </ul> </li> </ul> | <ul> <li>Two hydrolysing products are generated for the target chemical:<br/>Izovalerate acid and Phenethyl alcohol</li> <li>In the Data module, you have found that RDT data is available fo<br/>both products</li> <li>The data for both products are bigger than hazard threshold of 10<br/>mg/kg/data according to GHS classification [GSH classification],</li> <li>However, metabolite phenethyl acohol is more toxic than the acid<br/>based on the experimental data</li> <li>Moreover it is expected that the acid (Izovalerate acid ) will be<br/>directly excreted and will not contribute towards the toxicity of th<br/>target [RIFM, 2012]</li> </ul> |
|   | Add / Remove A Two hydrolysing products are generated for the target (text prov Edit Preview   | It is expected that the toxicity of the target chemical phenethyl isovalerate will be result of Phenethyl alcohol   |

### **Report** Generation

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

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

RAAF AEs are included in the second file. All generated files should be provided when submitting a prediction.



### **Report** Generated report files

| rediction r   |  | Category repor  |   |   |   |  |                                  |  |
|---|--|---|---|---|---|--|----------------------------------|--|
| ction of NOAEL for phenethyl isovalerat   | 1/6  |   | QSAR Toolbox report for category  |   |   |  |                                  |  |
| QSAR Tool<br>(in acc<br>Date: 1 May 2019<br>uuthor(s):<br>contact details:<br>Structural information<br>SMILES:<br>CC(C)CC(=0)OCCc1cccc<br>c1 | Cordance with RAAF s<br>Target informatio<br>Numerical identifiers<br>CAS#: 140-26-1<br>Other: EC Number:20540 | single chemical The<br>scenario 1) S<br>Scenario 1 | ne selected R<br>specified in   | AAF scenar<br>the first pag                                       | Category definition     1. Category definition     1.1. Category definiti     Category name     Not provided by the     Covered (target) end         - Human Health Ha     Category hypothesis     Not provided by the     1.2. Category member | (In accordance<br>ion<br>e user<br>lpoint(s)<br>zards/Repeated Dose 1<br>e user<br>ers | with RAAF scenario 1)            | manually editable fi<br>manually editable fi |
|   |  | A B<br>Substance identity<br>Structure  | C D E<br>Target chemical  | F G H<br>Neighbour #1   | Information of categ<br>Table of category n<br>CAS<br>L 140-26-1  | ory members<br>nembers<br>Name<br>phenethyl isovalerate                                | SMILES<br>CC(C)CC(=0)OCCc1ccccc1 | Structure                                    |
| Structure H <sub>3</sub> C CH <sub>3</sub>  |  | CAS number<br>Chemical name<br>Other identifier<br>SMILES   | 140-26-1<br>phenethyl isovalerate<br>CC(C)CC(=0)OCCc1ccccc1   | No CAS number OCCc1ccccc1   |   |  |                                  | H <sub>3</sub> C/CH <sub>3</sub>             |
| Prediction summa<br>Predicted endpoint: NOAEL; No effect specified; No species spe<br>guideline specified<br>Predicted value: 861             |  | Parameters         unit           Boiling point         *C           log Kow         Da           Molecular Weight         Da           Water Solubility         mm Hg/L           Profilers         Females  | 276<br>3.97<br>206<br>0.00653<br>16.5   | 225<br>1.57<br>122<br>0.0263<br>2.2£404                           | 2 No CAS number   | r Hydrolysis simulator<br>(neutral): metabolite<br>#2                                  | 0CCc1cccc1                       | Ĵ  |
| Unit/scale: mg/kg bdwt/d<br>Data gap filling method: Read-across analysis<br>Summary: manually editable field<br>Not provided by the user     |  | Organic functional groups Toxicological   | Alkane, branched with tertiary carbon;<br>Aryl;<br>Carboxylic acid ester;<br>Isopropyl  | Alcohol;<br>Aryl  | Ranges for selected physicochemical properties and calculated parameters  |  |                                  | HO   |
|   |  | Repeated dose (HESS), with Hydrolysis<br>simulator (neutral)  | Not categorizeo;<br>Carboxylic acids (Hepatotoxicity) No<br>rank;<br>Glycolic acid (Renal Toxicity) Alert;<br>Styrene (Renal Toxicity) Alert;<br>Toluene (Renal toxicity) Alert | Styrene (Renal Toxicity) Alert;<br>Toluene (Renal toxicity) Alert | Table with 2D p<br>Parameter name<br>Boiling point  | e 225 ÷ 276  | Variation C(1                    | unit (family)<br>Temperature)                |
|   |  | Measured and predicted data<br>Data used for prediction   | species duration text   | species duration that   | og Kow  | 1.57 ÷ 3.9   | )7                               |  |

April, 2020

### 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!