# QSAR TOOLEOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

# OECD (Q)SAR Toolbox v.4.4.1

Example illustrating RAAF Scenario 4 and related assessment elements

#### **Outlook**

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

#### Background

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

#### **Outlook**

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

### **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

#### **Outlook**

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

## **Objectives**

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

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

#### **Outlook**

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

### **Specific Aims**

- To familiarize the user with the Read Across Assessment Framework (RAAF) and specifically with Scenario 4;
- To introduce to the user the read across assessment elements;
- To introduce to the user the report basket;
- To provide sufficient information allowing a scientific assessment of the outcome;
- To explain to the Toolbox user the rationale behind each step of the exercise.

#### **Outlook**

- Background
- Keywords
- Objectives
- Specific Aims

#### Read Across Assessment Framework (RAAF)

- The exercise
- Workflow

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

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

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

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

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

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

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

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

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

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

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

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

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

#### **Outlook**

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

#### **The Exercise**

- In this exercise we will predict *Repeated Dose Toxicity (RDT)* of 3,5dimethyl-aniline [CAS# 108-69-0], which will be the "target" chemical.
- In this exercise the category will be defined based on the aniline functionality identified in the package: parent and metabolites based on Repeated dose toxicity profiler. This chemical class is related with *Hemolytic anemia with a methemoglobinemia* accounting. The effect is considered when *in vivo* Rat metabolism is taken into account;
- The read across approach will be used for the prediction. The read-across will be based on a category approach relying on a common metabolite generated for the source and target substances;
- Read-across assessment elements will be included to the report;
- Examples for the possible content of each of AEs will be provided.

#### **Outlook**

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

### Workflow

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

#### Workflow

#### Scheme illustrating the Toolbox workflow



### **Input** Overview

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

## **Input** Input the target chemical by CAS#



## **Input** Define the target endpoint

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

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



#### **Input** Define the target endpoint



Click **Define** (1), select **Repeated Dose Toxicity** (2) and then click **Next** (3). Select **LOEL** as an endpoint from the drop-down menu and then consecutively the following metadata: *Effect*: **Total**, *Organism(tissue): Whole body*, *Test organism(species):* **Rat**, *Route of administration:* **Oral (gavage)** (4). Finally click **Finish** (5).

# **Input** Define the target endpoint

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



#### **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\*.

<sup>\*</sup>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

	Profiling	01010 01 0 10100 y definition ► Data Gap Filling	► Report	
Profi 3 Apply View New Delete				The OECD QSAR Toolbox for Grouping Chemicals into Categories
	Filter and point tree	1 [target]		Developed by LMC, Bulgaria
Occuments      Profiling methods      Options ▲     f Select All      ✓ Suitable      ✓ Repeated dose (HESS)      ✓	Structure	CH3 H3C NiH2		
Plausible     Aquatic toxicity classification by ECOSAR     Chemical elements     Groups of elements     Lipinski Rule Oasis     OECD HPV Chemical Categories     Organic functional groups     Organic functional groups     Organic functional groups (US EPA)     Organic functional groups. Norbert Haider (checkr	Human Health Hazards Acute Toxicity ADME Bioaccumulation Carcinogenicity Developmental Toxicity / Teratogenicity		<ol> <li>Go to <b>Profiling</b></li> <li>Tick the checkl profile - <i>Repeated</i> <i>in vivo Rat metabo</i></li> <li>Click <b>Apply</b>.</li> </ol>	module; boxes of the suitable dose (HESS) and of plism simulator;
Metabolism/Transformations         Options ▲         f       Select All         J       Plausible         Bissociation simulator         Hydrakysis simulator (neutral)         V       in vivo Rat metabolism simulator         Undescrifted         (Test) ONS-Carbons         Autoxidation simulator         Autoxidation simulator (akaline medium)         Hydrolysis simulator (basic)         Hydrolysis simulator (basic)         Ohserverd Mammalian metabolism	Genetic Toxicity Immunotoxicity Irritation / Corrosion Neurotoxicity Photoinduced toxicity Repeated Dose Toxicity Rat Oral (Gavage) Whole body Total LOEL Sensitisation AW SW AOP ToxCast Toxicity to Reproduction			~

## **Profiling** Profiling the target chemical

QSAR TOOLEOX	Fining → Data → Category	offinition → Data Gap Filling → Report	
Profiling Custom profile			The OECD QSAR Toolbox for Grouping Chemicals into Categories
Apply View New Delete			Developed by LMC, Bulgaria
Documents	Filter endpoint tree 🍸	1 [target]	^
Profiling methods       Options ▲       f     Select All       ✓ Suitable       ✓ Repeated dose (HESS)       ▲     Plausible       ▲     Aquatic toxicity classification by ECOSAR	Structure	H <sub>3</sub> C NH <sub>2</sub>	Anilines (Henolytic anemia with methemoglobinemia) Rank A alert is identified in the target chemical (1)
Chemical elements Groups of elements Lipinski Rule Oasis OECD HPV Chemical Categories Organic functional groups Organic functional groups (nested) Organic functional groups (US EPA) Organic functional groups, Norbert Haider (checkr	Toxic	-	
Metabolism/Transformations       Options ▲       f     Select All       f     Select All       Unselect All     Invert       ▲     Plausible       ■     Dissociation simulator       ■     Hydrolysis simulator (neutral)       ♥     Invert metabolism simulator       ■     Unclassified	Toxicological     Repeated dose (HESS)     Metabolism/Iransformations     in vivo Rat metabolism simulator     Toxicological	Anilines (Hemolytic anemia with methemoglobinemia) Rank 7 metabolite(s) 1 x Anilines (Hemolytic anemia with methemoglobinemia) R 1 x Anilines (Hepatotoxicity) Rank C	ank A
(Test) ONS-Carbons Autoxidation simulator Autoxidation simulator (alkaline medium) Hydrokysis simulator (acidic)	Repeated dose (HESS)	1 x p-Aminophenols (Renal toxicity) Rank B 2 x o-/ p-Aminophenols (Hemolytic anemia with methemog 4 x Not categorized	lobinemia) Rank B

# **Profiling** Profiling the target chemical

QSAR TOOLEOX	Profiling     Data	y definition       Data Gap Filling      Report	
Profiling Custom profile			The OECD QSAR Toolbox for Grouping Chemicals into Categories
Documents     Profiling methods	Filter endpoint tree 🍸	(1 [target]	Same alerts: <i>Anilines</i> (Henolytic anemia with
Options ▲       f     Select All       Unselect All     Invert       ✓     Suitable       ✓     Repeated dose (HESS)       ✓     Plausible       Aquatic toxicity classification by ECOSAR       Chemical elements	Structure	H <sub>3</sub> C NH <sub>2</sub>	<i>methemoglobinemia)</i> Rank A and Anilines (Hepatotoxicity) Rank C are identified in the target chemical as a parent as well as after a metabolic
Groups of elements Lipinski Rule Oasis			activation.
OECD HPV Chemical Categories Organic functional groups Organic functional groups (nested) Organic functional groups (US EPA) Organic functional groups Nothert Haider (checkr	Sensitisation AW SW AOP Toxc Toxi Toxi Toxi Toxi		Rank A label is assigned for the alerts that have a documented mechanism.
< >>	Profile	·	
Metabolism/Transformations	Repeated of the (HESS)	Anilines (Hemolytic anemia with methemoglobinemia) Rank A	
f Select All Unselect All Invert	Metabolicm/Transformations	A similar premovat anoma maninearenogioonterina/ hank A	
	in vivo Rat meta olism simulator	7 metabolite(s)	
<ul> <li>In vivo Rat metabolism simulator</li> <li>✓ in vivo Rat metabolism simulator</li> <li>✓ Unclassified</li> <li>(Test) ONS-Carbons</li> <li>Autoxidation simulator</li> <li>Autoxidation simulator (akaline medium)</li> <li>Hydrolysis simulator (acidic)</li> </ul>	Repeated dose (HESS)	1 x Anilines (Hemolytic anemia with methemoglobinemia) Rank A 1 x Anilines (Hepatotoxicity) Rank C 1 x p-Aminophenols (Renal toxicity) Rank B 2 x o-/ p-Aminophenols (Hemolytic anemia with methemoglobin 4 x Not categorized	a iemia) Rank B

#### **Data** Overview

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

## Data Collecting experimental data

QSRR TOOLBOX P Input Data Gather Import IUCLID6 IUCLID6 Database Inver	Profiling Data Category	definition		
<ul> <li>Documents</li> </ul>	Filter endpoint tree 💙	1 [target]		
A Document 1 # CAS: 108690	Structure	H3 NH2		
Databases	- Dimensional Advances		-1	Coloct the coll corresponding
Options         Image: Constraint of the second	Carcinogenicity		1.	Select the cell corresponding
MUNRO non-cancer EFSA REACH Skin sensitisation database (n	Developmental Toxicity / Teratogenicity     Genetic Toxicity			to the target endpoint;
Receptor Mediated Effects	Immunotoxicity		2.	Go to module <b>Data</b> and
Repeated Dose Toxicity HEC				unselect all databases:
Skin Irritation	Photoinduced toxicity     Repeated Dose Toxicity		2	Check Perceted dece
Inventories	Rat Cral (Gavage)	1	3.	Check <b>Repeated</b> aose
f Select All Unselect All Invert	Whole body			(HESS) database;
			4.	Select <b>Gather</b> .
ECHA PR EINECS	Sensitisation AW SW AOP	·L		
HPVC OECD	Toxicity to Reproduction			
NICNAS	+ Toxicokinetics. Metabolism and Distributi			

#### Data Collecting experimental data

QSAR TOOLEOX	Profiling     Data     Category definition     Data Gap Filling     Report
Gather Import IUCLID6 IUCLID6 Database Inv	entory Control
Documents	Filter endpoint tree 🝸 1 [target]
✓ ▲ Document 1 # CAS: 108690	Structure
	Hydriasis 1/2 M: 360 mg/kg bdwt/d
<ul> <li>Databases</li> </ul>	Cher Findings 1/2 M: 360 mg/kg bdwt/d
Options 🖌	HiPerection 1/2 M: 360 mg/kg bdwt/d
f Select All Unselect All Invert About Options	Tross/palpeoral Cosure 1/2 Mi. Soc mg/kg buw/d
REACH Skin sensitisation database (normalised)	Straub Tail     1/2 W: 360 mo/kg bdwt/d
Receptor Mediated Effects	
Rep Dose Tox Fraunhofer ITEM  Repeated Dose Toxicity HESS  Rodent Inhalation Toxicity Database Skin Irritation Skin Concitization	M: 60 mg/kg bdwt/d M: 60 mg/kg bdwt/d LOEL 1/2
Inventories	
Options 🖌	NOEL 1/2 M: 10 mg/kg bdwt/d
f Select All Unselect All Invert	Tremor/Convulsion 1/2 M: 360 mg/kg bdwt/d
	Uvery Vocalization 1/2 M: 360 mg/kg bdwt/d
DSSTOX	Sensitisation AW SWAUP
ECHA PR EINECS	Toxicity to Reproduction

1. The extracted data for *LOEL Repeated dose toxicity* is displayed on the data matrix; Both experimental data for target chemical are equal (*60 mg/kg bdw/d*).

## Data

#### Collecting experimental data

- Toxicity information on the target chemical is automatically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues is performed only among the chemicals which are listed in the selected database(s), which in this example is *Repeated dose (HESS)*.
- Two experimental data related to the defined target endpoint are found.

# Based on the observed data (60 mg/kg bw/d) the target chemical is classified as Category 2 regarding GHS classification $^{1}$

Route of exposure	Units	Guidance value range (dose/concentration)
Oral (rat)	mg/kg bw/d	10 - 100
Dermal (rat or rabbit)	mg/kg bw/d	20 - 200
Inhalation (rat) gas	ppm/6h/d	50 - 250
Inhalation (rat) vapour	mg/litre/6h/d	0.2 - 1.0
Inhalation (rat) dust/mist/fume	mg/litre/6h/d	0.02 - 0.2

Table 3.9.2: Guidance values to assist in Category 2 classification

#### See on the next slide

<sup>1</sup> Globally Harmonized System of Classification and Labeling of Chemicals (GHS): <u>http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs\_rev04/English/ST-SG-AC10-30-Rev4e.pdf</u>

## Category definition Overview

- This module provides the user with several means of grouping chemicals into a toxicologically meaningful category that includes the target molecule.
- This is the critical step in the workflow.
- Several options are available in the Toolbox to assist the user in refining the category definition.
- As the RDT is a systemic endpoint the metabolism could take place. The primary category in the current example will be defined accounting for an *in vivo rat* metabolism.

# **Category Definition**

# Searching for analogues accounting for an *in vivo* Rat metabolism



# **Category Definition**

# Searching for analogues accounting for an *in vivo* Rat metabolism

Grouping options (in viv	o Rat metabolism s	imulator)		0			_		$\times$
All queries At least of At	one			Target					
Chemical	Query		Criteria	Anilines (Hemolytic	anemia with methemo	globinemia) Ran	k A		
Parent				Anilines (Hepatotox	(icity) Rank C	<u> </u>			
CH <sub>3</sub>		No Martin		Not categorized					
Hack Nets	none ~	No criteria.		o-/ p-Aminophenol	ls (Hemolytic anemia wi	th methemoglo	binemia) Ran	k B	
				p-Aminophenols (R	enal toxicity) Rank B			)	
Metabolite 1	none ~	No criteria.							
Нус Мана				Options					
	1	hicals		Down	Up	Reset		Options	5
Parent & Metabolites	Profile v	Profiler: Repeated dose (HESS)   Options: Edit		Profiles (N/A) 2-Acetylaminofluor	ene (Hepatotoxicity) Al	ert			< >
│└───│ 1		3		2-Amino-4,3-dipne	nyi thiazole (Rehal toxic	ity) Alert			
Alert perform Scales Calculate				Combine profiles	<ul> <li>Invert result</li> <li>Strict</li> </ul>				
					Sort results				
							OK	Car	ncel

- 1. Select a **profile** option for the package "parent & metabolites";
- 2. Select "Repeated dose (HESS)" profile;
- 3. Click the **Edit** button. Remove all categories except *Anilines (Hemolytic anemia with a methemoglobinemia) Rank A*<sup>\*</sup> category by double click or using "Down" button;

\*The categories with *Rank A* are supported with training sets chemicals having reliable experimental data.

# **Category Definition**

# Searching for analogues accounting for an *in vivo* Rat metabolism

Grouping options (in vivo	Rat metabolism s	imulator) — 🗆		×			
All queries At least on	ne			Target			
Chemical	Query	Criteria		Anilines (Hemolytic anemia with methemoglobinemia) Rank A			
Parent	none ~	No criteria.					
Metabolite 1	none v	No criteria.		Options			
		All chemicals		Down Up Reset Options			
Parent & Metabolites	Profile ~	Profiler: Repeated dose (HESS) V Options: Edit		Profiles (N/A) 2-Acetylaminofluorene (Hepatotoxicity) Alert 2-Amino-4,5-diphenyl thiazole (Renal toxicity) Alert Combine profiles	< >		
Alert performance				Invert result			
Scales Calculate		2	Canc	AND OR Strict	el		

- 1. Click **OK** to confirm the defined search criteria.
- 2. Click **OK** in Map similarity options window to execute the search.

#### In this way we will search for analogues that have this alert as a parent or as a metabolite.

#### **Filter data matrix**



All information on data matrix, which is not needed at the current moment could be removed using a filter.

Click the **Advanced filter** icon (1). A window with the endpoint tree organization appears. Select only the nodes which you want to see in the data matrix and confirm by clicking OK (3).

## **Data Gap Filling** Apply Read across

QSAR TOOLBOX	r     n       L     J       ▶ Profiling     ▶ Data	gory definition	ap Filling > Rep	Sort			Xos	
Gan tilling 2 Workflow							The OECD C for Groupin into Catego	ISAR Toolbox 9 Chemicals ries
Irend analysis Read across QISAR Standardized Automa	ted	2					Developed	oy LMC, Bulgaria
O Documents	Filter endpoint tree	1 [target]	2 3	4 5 6 7	8	9	10	11 ^
<ul> <li>Document 1</li> <li># [C: 1;Md: 784;P: 0] CAS: 108690</li> <li>[C: 60;Md: 84;P: 0] Grouping with metabolism:</li> </ul>	Structure	Hgc Htg		Possible data inconsistency — □ ×  Metadata     ▲ Effect     ☑ Total (45 chemicals; 84 data)     ▲ Endopint	, <sup>0</sup>		nys Q	ng Sang
	Structure info	-		LOEL (45 chemicals; 84 data)				_
	Additional Ids	EC Number:2036070	EC Number:2022 EC	A Native scale/unit Imp/ka hdut/d (45 chemicals: 84 data)	EC Number:2031.	EC Number:2058.	EC Number:2094	EC Number:
	CAS Number	108-69-0	93-68-5 10	Organ(Tissue)	103-69-5	156-43-4	578-54-1	579-10-2
	CAS-SMILES relation	High	High Hi	⊈ Whole body (45 chemicals; 84 data)	High	High	High	High
	—— Chemical name(s)	"3,5-xylidine"	2'-methylacetoa 1,4	4 Route of administration	Aniline, N-ethyl-	4-ethoxy-aniline	2-ethyl-aniline	Acetamide,
	Composition							
	Molecular formula	C8H11N	C11H13NO2 C1	Rat (45 chemicals; 84 data)	C8H11N	C8H11NO	C8H11N	C9H11NO
	Predefined substance type	Mono constituent	Mono constituent Me	c .	Mono constituent	t Mono constituent	Mono constituer	t Mono const
		Cc1cc(C)cc(N)c1	CC(=O)CC(=O)N CC		CCNc1ccccc1	CCOc1ccc(N)cc1	CCc1ccccc1N	CN(C(C)=O)
	Human Health Hazards	•						
	Repeated Dose Toxicity	·		Select scale/unit to use				
< >	Rat		1	Iog(1/mol/kg bdwt/d) [0 native data and 84 converted]				
	Gavage)			mg/kg bdwt/d [84 native data and 0 converted]				
Data Gap Filling Settings				<ul> <li>mol/g/s [0 native data and 84 converted]</li> </ul>				
Only endpoint relevant	lotal	ht 60 mg/kg bdwt/d	M: 90 mg/kg bd M		M: 5 mg/kg bdw	M: 40 mg/kg hd	M: 125 mg/kg b	M: 20 mg/k
At this position:	LOEL 45/	84 M: 60 mg/kg bdwt/d	M: 00 mg/kg bd M: M:		M: 5 mg/kg bdw.	M: 40 mg/kg bd	. m. 12.5 mg/kg b	M: 20 mg/k
Select a cell with a rigid (bold) path				Converted data				
Automated workflows 0 Standardized workflows 0				84 from scale/unit mg/kg bdwt/d				
				Chemicals 45/45; Data 84/84 OK Cancel				

After using of the *Advanced filter* only selected information appears on the data matrix.

- 1. Click the cell corresponding to the target chemical in the row with the defined endpoint;
- 2. Click Read-across;
- 3. Possible data inconsistency window appears, keep the default selection. Click OK.

#### **Data Gap Filling** Subcategorizations



- 2) Chemical elements;
- 3) OECD HPV Chemical Categories.

O)SAR Toolbox for Grouping Chemicals into Categories

## **Data Gap Filling** Subcategorizations



QSAR TOOLEOX

#### Data Gap Filling RA prediction for CAS 108-69-0 Results

#### **Observed data:**

Total LOEL - 60 mg/kg bdw/day

**Prediction:** 

Total LOEL – 25.6 mg/kg bdw/day

Based on the predicted data (for Total LOEL) the target chemical is classified as *Category* 2 regarding GHS classification <sup>1</sup>. The RA results is in accordance with the observed data.

Route of exposure	Units	Guidance value range (dose/concentration)
Oral (rat)	mg/kg bw/d	10 - 100
Dermal (rat or rabbit)	mg/kg bw/d	20 - 200
Inhalation (rat) gas	ppm/6h/d	50 - 250
Inhalation (rat) vapour	mg/litre/6h/d	0.2 - 1.0
Inhalation (rat) dust/mist/fume	mg/litre/6h/d	0.02 - 0.2

Table 3.9.2: Guidance values to assist in Category 2 classification

<sup>1</sup> Globally Harmonized System of Classification and Labeling of Chemicals (GHS): <u>http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs\_rev04/English/ST-SG-AC10-30-Rev4e.pdf</u>

#### **Report** Overview

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

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

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

- 1) the type of approach applied an analogue approach or a category approach;
- 2) the read-across hypothesis;
- 3) For 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

April, 2020

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

For the current example:

- the type of approach applied a category approach is used (a threshold of >3 analogues is proposed by LMC for the category approach);
- the read-across hypothesis different compounds with a common underlying mechanism for metabolites of source and target substances;
- For a category approach The observed quantitative variation of *Total LOEL* among the category members is more than 1 log unit<sup>\*</sup>.

#### Scenario 4 was selected for the current example based on the RAAF selection criteria.



\*The range of quantitative variation in the (eco)toxicity among the category members of 1 log unit is proposed by LMC based on empirically observations.

#### Report Generation according to RAAF-Scenario 4



- 1. Go to the **<u>Report module</u>** and click on the cell with the prediction (**R:25.6 mg/kg bdwt/d**);
- 2. Click the Prediction button;
- 3. Check the box at the top to add a RAAF scenario;
- 4. Select Scenario 4 from the drop-down menu.

#### **Report Generation according to RAAF-Scenario 4**

		Customize report content and ap	pearance	– 🗆 X
Customize report content and ap     Wizard pages	ppearance	Wizard pages		
Customization Customize report Prediction Target and prediction summary Prediction details (I) Predicti Target p Analogue perection details Consistency check Options	<ul> <li>1.1. Category definition</li> <li>1.2. Category members</li> <li>Information of category members</li> <li>Ranges for selected physicochemical properties ar</li> <li>Purity / Impurity</li> <li>AE C.1: Substance characterization</li> <li>AE C.5: Reliability and adequacy of the source stu</li> <li>1.3. Profiles/Metabolisms</li> <li>List of profiles/metabolisms</li> <li>AE 4.1: Compounds the test organism s exposed</li> </ul>	Customization Customize report Prediction Target and prediction summary Prediction details (I) Prediction details (II) Target rofiles 2 is selection Category Categoly definition and men bers	<ul> <li>2.1. Physicochemical similarity</li> <li>2.2. Structural similarity</li> <li>Structural similarity</li> <li>Comments on structural similarity</li> <li>AE C.2: Structural similarity and structure</li> <li>AE C.3: Link of structural similarity and</li> <li>2.3. Mechanistic similarity</li> <li>Mechanistic similarity</li> <li>Mechanistic similarity</li> <li>AE comments on mechanistic similarity</li> <li>AE 4.2: Common underlying mechanism</li> </ul>	All AEs of <b>Scenario 4</b> are distributed as follows: hree AEs associated with <i>Category definition and</i> <i>members</i> (1), and eight AEs are associated with he <i>Consistency check</i> 2).
Options	Back	Options Data matrix Options	<ul> <li>◇ 2 5. Other AFs</li> <li>◇ AE 4.3: Common underlying mechanism, qu</li> <li>◇ AE 4.4: Exposure to other compounds than</li> <li>◇ AE 4.5: Occurrence of other effects than con</li> <li>◇ AE C.4: Consistency of effects in the data m</li> <li>◇ AE C.6: Bias that influences the prediction</li> </ul>	aantitative aspects to those linked to the prediction vered by the hypothesis and justificat atrix

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

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

#### Report Generation according to RAAF-Scenario 4

#### • Category definition and members section



One AE (1) related to the characterization of the category members (target and source substances) is included in the *Category definition and members* section. The **AE C.1** should be manually populated with items available in the *Report basket*. Click Add/Remove (2) button and select item Table of category members (3). Click **OK (4)**; If impurities/additives of the used analogues are available, they will be also included. The current analogues have no additives/impurities. The selected report item appears under the Add/remove button. Click **Preview** button in order to see how the AE C.1. will look in the generated report (5).

### Report Generation according to RAAF-Scenario 4

Customize report content and a	ppearance – 🗆 X
Wizard pages	
Customization	
Customize report	⊙ 1.1. Category definition
Prediction	⊙ 1.2. Category members
Target and prediction	✓ Information of category members
summary	Ranges for selected physicochemical properties and calculated parameters
Prediction details (I)	
Prediction details (II)	W AF C 1. Substance characterization
Target profiles	
Analogues selection details	AE C.5: Reliability and adequacy of the source study(ies)     Aint
Category Category definition and members	PURPOSE: The source study needs to match the default REACH requirements in terms of reliability and adequacy as requested for any other key study. It has to be assessed whether: - the study design reported for the source study is adequate and reliable for the purpose of the prediction based on re-
Consistency check	- the study design should cover the key parameters in the corresponding test method referred to in Article 13(3);     - the study design should cover an exposure duration comparable to or longer than the corresponding method referred to
Data matrix	in Article 13(3); and - there is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided.
Options	The test material used represents the source substance as described in the hypothesis in terms of purity and impurities.
	Add / Remove
	(21.3. Profiles/Metabolisms)
	Bark Next Cancel Create report
	back ivext Cancel Create report

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

- The target substance has been tested according to test guideline 407
- All of the five source substances with one exception (substance E) has been tested based on test guideline 407: Repeated does 28day oral toxicity study in Rodents
- For the source substances E and B the study was based on report NTP Long term and OECD: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

A snapshot from *Filter points by test conditions* could be provided.

#### Report Generation according to RAAF-Scenario 4

Customize report content and appearance ↔ Report basket × Options 🖌 3 f Select All Unselect All Invert Wizard pages Category 1 Chemical profile ("Protein binding alerts for skin sensitization according to GHS" L Profiling similarity accounting for Customization Create new items × ① 1.1. Category definition Profiling similarity accounting for Customize report 1 Chemical profile ("Protein binding Options 🖌 1.2. Category members 1 Chemical profile ("Protein binding Prediction Select All Unselect All Invert Profiling similarity accounting for 1.3. Profiles/Metabolisms Target and prediction ▲ Category In Profiling similarity accounting for Indepoint data variation summary In Profiling similarity accounting for 🕑 List of profiles/metabolisms L Profiling similarity accounting for Parameter variation Prediction details (I) Category members ta Chemical profile AE 4.1: Compounds the test organism is exposed to 1 Structural similarity .Mechanistic similarity Prediction details (II) ኳ Chemical profile ("Organic functi Hint Structural similarity 🔁 Chemical profile ("Structure simi Target profiles ∡ External content PURPOSE: 🛱 Endpoint data variation (5 selec Image provided by user In this scenario, it is claimed that different compounds have the same effects for the p Analogues sele Parameter variation (5 selected: different compounds may be the source and target substances themselves and/or the A Text provided by user a Parameter values details to be assessed whether: a Endpoint data values - the compounds to which the test organism is exposed (after administration of the Category Grouping been established in the documentation; and Category definition - the provided evidence supports the explanation Input and members 🗌 👗 Target substance Add / Remove OK Cancel Consistency che 2 Options Data matrix Δ Options Create new OK Cancel

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

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

Items with an external content (picture and text) will be added for **AE 4.1**. **Compounds the test organism is exposed to** 

#### Report Generation according to RAAF-Scenario 4

Customize report content and ap	opearance — 🗆	×						
Wizard pages								
Customization Customize report Prediction	<ul> <li>⊙ 1.1. Category definition</li> <li>⊙ 1.2. Category members</li> </ul>	]^	Possibl the tes	le cont t orga	tent of nism is	AE 4.1 s expo	: Com sed to	pounds
Target and prediction	⊙ 1.3. Profiles/Metabolisms		Target A	Source B	Source C	Source D	Source E	Source F
summary Prediction details (I)	<ul> <li>List of profiles/metabolisms</li> <li>AE 4.1: Compounds the test organism is exposed to</li> </ul>		Hyperson	ngt Cong	ngc O Cong	mac Cana	Hyc Cong	NyC- Ung
Prediction details (II) Target profiles Analogues selection details Category Category definition and members Consistency check Options Data matrix Options Items with for AE 4.1	Hint     PURPOSE:     In this scenario, it is claimed that different compounds have the same effects for the property under consideration. Such different compounds may be the source and target substances themselves and/or their (bio)transformation products. It has to be assessed whether:         - the compounds to which the test organism is exposed (after administration of the source and the target substances) nave-         established in the documentation; and         - the provided evidence supports the explanation.     Add / Remove	<ul> <li>There are target substance A a source substances (B, C, D, E and</li> <li>The source substances (B, C, D, E and</li> <li>The source substances (analogue D, E and F have the same functionality as the target substance ba aniline functionality identified e the parent itself or as met according to RDT profiler account the rat in vivo metabolism.</li> </ul>					87-62-7 Ccleccc(C)cIN ce A ar alogues same substanc ned bas ified eit s meta account	a7-59-2 Cc1cccc(N)c1C F) s) B, C, aniline ce sed on ther in abolites ting for
	Back Next Cancel Create rep	port						

See how to add the textual content of AE 4.1 along with an illustrative picture of the target and source substances in the next slides.

#### Report Generation according to RAAF-Scenario 4

Customize report content and approximation	ppearance	– 🗆 ×		
Customize report content and ap Wizard pages Customization Customize report Prediction Target and pu Summary Prediction details (I) Prediction details (I) Prediction details (II) Target profiles Analogues selection details Category Category definition and members	Image: Separance         Image: Separaconce	Create new items Options  f Select All Unse Category  Category  Chemical pro Mechanistics  Chemical pro Mechanistics  F Structural sin Calmage provided by user  A.Text provided by user  OK Cancel	Invert About Opt	X tions - C X source substances (B, C, D, E and F) les) B, C, D, E and F have the same rget substance A lesed on aniline functionality titself or as metabolites according r rat in vivo metabolitsm
Consistency check Options Data matrix Options	2 2			6 OK Cancel
	In order to add text information to the button (2), click <b>Create new</b> (3) in R <b>user</b> (4), write in or paste the text in the	report: expand eport basket wi e empty field (5)	the AE (1), clion ndow, click <b>Te</b> , click <b>OK</b> (6).	ck Add/Remove ext provided by

# **Reporting** Report Generation according to RAAF-Scenario 4



The entered text is listed in the **Report basket** under *External content* section and the check box is ticked (1). Click **OK** (2). The new item is added under the corresponding AE (3). There are two options for each of the report items - **edit** (4) (if you want to change the content) and **preview** (5) (if you want to see the information provided by this item).

#### Report Generation according to RAAF-Scenario 4

Customize report content and approximation	pearance	- 0	×					
Wizard pages								
Customization	1 1 Catagory definition	rt basket	– 🗆 X				-	
Customize report	Options	4		(I)		_	5	×
Prediction	Category members	Select All    Create new	v items	Select your image h	ere:			
Target and prediction	🔿 1.3. Profiles/Metabolisms 👘 🗌	Profiling similarity accour Options      Findpoint data variation						<b></b>
summary	✓ List of profiles/metabolisms	Category members Category members Category	ect All Unselect All	Target A Source B	Source C Source	D Source E	Source F	
Prediction details (I)	AF 4.1: Compounds the test or	車 Scruccural similarity 車 Endpoint data values 車Param	oint data variation eter variation	5		Ô	Ô	
Prediction details (II)		A Text provided by user (	ical profile	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		*		
Target profiles		Category purity/impurit) ↓ External co	cural similarity 3					
Analogues selection	In this scenario, it is claimed that different cor	Target substance	provided by user	Celec(C)ce(N)e1 Celece(C)e(N)e	I Cc1ccc(N)c(C)c1 Cc1ccc(N)cc1	C Celecce(C)e1N	Celecce(N)e1C	
details	property under consideration. Such different ( substances themselves and/or their (bio)trans	ALTEXC	Stovided by user					
Category	whether:			Specify how much o	f the page width is o	ccupied by the	e image: —	
Category definition	<ul> <li>the compounds to which the test organism ( and the target substances) have been establis</li> </ul>			Image width, % 7	5			
and members	- the provided evidence supports the explana			L	Г	01		-
Consistency check	Add / Remove					UK	Cancel	
Options					6			
Data matrix	A There are targete A and				$\bigcup$			
Options			4					
	4	2		OK Cancel				
		Create new	OK Cancel					

In order to also add an image: click **Add/Remove** button again (1), *create a new* item (2) and select **Image provided by user** (3) and click **OK** (4). A new window appears where you can add your custom picture by Copy/Paste or browsing (5) to the directory in your PC where the desired picture is saved. Finally confirm by clicking **OK** (6).

#### Report Generation according to RAAF-Scenario 4

Wizard pages		
Possible content of AE C.2: Structu	Pos	sible content of AE C.2: Structural
Customization © 2.1. Physicochemical similarity and structural difference with in the category	© 2.1. Physicochemical similarity Sim	hilarity and structural differences
Prediction © 2.2. Structural similarity	O 2.2. Structural similarity	
Target and prediction Structural similarity		ne structural similarity between the
Summary Comments on structural similarity Comments on structural similarit	Comments on structural similarity	ubstances (R. C. D. E. and E) according
Prediction details (i)	AE C.2: Structural similarity and structural differences within the category	o Str. similarity profiler is in the range
Target profiles	⊘ Hint	of [33-78%]
Analogues selection details       PURPOSE:         Category       The aim of this AE is to verify that all category members indeed meet the criteria for structural similarities and allowed structural differences used for the category description. It has to be assessed whether:         • Target A and substances B, F have 1 same reactivity pattern with respect         • There are structural differences which are allowed within the category.	PURPOSE:       The aim of this AE is to verify that all category members indeed meet the criteria for structural similarities and allowed       • Ta         structural differences used for the category description. It has to be assessed whether:       • the structural similarities identified apply to all category members; and       • Sa         • there are structural differences which are allowed within the category.       • there are structural differences which are allowed within the category.       • the	arget A and substances B, F have the ame reactivity pattern with respect to
Category definition and members Add / Remove - The source substances C and D ha	Add / Remove • T	The source substances C and D have
Consistency check C.3: Link of structural similarity and differences with the proposed regular pattern	O AE C.3: Link of structural similarity and differences with the proposed regular pattern	ne same reactivity pattern as source E,
Determine 2.3. Mechanistic similarity	© 2.3. Mechanistic similarity	win one additional group. precursor
Options	© 2.4. Additional endpoints	
© 2.5. Other AEs	🕑 <mark>2.5. Other AEs</mark>	
	v	

Cancel

Create report

Back

Next

#### Report Generation according to RAAF-Scenario 4

Customize report content and ap	pearance – $\Box$ X	
Customize report content and ap     Wizard pages     Customization     Customize report     Prediction     Target and prediction     summary     Prediction details (I)     Prediction details (II)     Target profiles     Analogues selection     details	→ Bearance     -     ×            • Continues →         • Continues →         • Category         • Category         • Category         • Continues insulation (1 selected: Human Health Hazards#Repeated Dose         • Category members         • Category members:         • Comments on structural         • Structural similarity         • Comments on structural         • Mint         PuproSte:         Te aim of this AE is to verify that I         structural differences use users use to serve the structural similarities identified apply to all category members; and         • Hint         • Structural similarities identified apply to all category members; and         • Hint         • Structural similarities identified apply to all category members; and         • Structural similarities identified apply to all category members; and         • Structural similarities identified apply to all category members; and         • Structural similarities         • Structural similarities identified apply to all category members; and         • Structural similarities         • Structural similarities	Structural similarity Options Mode: Hologram, CombineAllFeatures Measure: -Dice Molecular features: -AtomCenteredFragments Atom characteristics: -AtomType -CountHAttached -Hybridization Calculated structure similarity Chemical 1 [Chemical 2] Chemical 3 [Chemical 4] Chemical 5 [Chemical 6]
details	- the structural similarities identified apply to all category members; and	Chemical 1 Chemical 2 Chemical 3 Chemical 4 Chemical 5 Chemical 6
Category	there are structural differences which are allowed within the category.	Chemical 1 100% 55.6 % 55.6 % 55.6 % 33.3 % 33.3 %
Category definition	Add / Remove	Chemical 2 55.6 % 100% 100 % 77.8 % 66.7 % 77.8 %
and members		Chemical 3 55.6 % 100 % 100% 77.8 % 66.7 % 77.8 %
Consistency check	⊘ AE C.3: Link of structural similarity and differences with the proposed regular pattern	Chemical 4 55.6 % 77.8 % 77.8 % 100% 77.8 % 66.7 %
Options	⊙2.3. Mechanistic similarity	Chemical 5 33.3 % 66.7 % 66.7 % 77.8 % 100% 77.8 %
Data matrix	⊙ 2.4. Additional endpoints	Chemical 6[3.3.3 % 77.8 % 77.8 % 66.7 % 77.8 % 100%
Options	⊙ <mark>2.5. Other AEs</mark>	

Two additional items have to be added in order to support the textual information: A *structural similarity* item and an item with the results of the OFG profiler.

Click **Add/Remove** button (1) and check the *Structural similarity* item (2) which is stored in the *Report basket*. Right click and preview the item (3). A table providing structural similarity between each of the category members is shown (4).

#### **Report Generation according to RAAF-Scenario 4**

Wizard pages         Customization Customize report Prediction summary         Prediction Prediction details (I) Prediction	Customize report content and ap	appearance — 🗆	×				
Analogues selection details Category Category Category definition and members Consistency check Options Option	Customize report content and ap     Wizard pages     Customization     Customize report     Prediction     Target and prediction     summary     Prediction details (I)     Prediction details (II)     Target profiles	appearance	X - X All Inv	- X Profiler Organic function Organic function Organic function Organic function Protein binding a	I groups I groups (nested) I groups (US EPA) I groups, Norbert Haider (checkmol) I groups, Norbert Jacob (Checkmol) I groups, Norbert Jacob (Checkmol)	4	×
In order to create an item with the OFG profiling results click the <b>Create new</b> button (1), se <b>Chemical profile</b> (2) and click <b>OK</b> (3). Select <b>Organic functional groups</b> profiler from the dr down menu (4) and confirm by <b>OK</b> *	Prediction details (II) Target profiles Analogues selection details Category Category definition and members Consistency check Options Options Options Options Options	<ul> <li>AL C.2. Structural similarity and differences with the proposed regular patter</li> <li>○ A.E.C.3: Link of structural similarity</li> <li>○ 2.3. Mechanistic similarity</li> <li>○ 2.4. Additional endpoints</li> <li>○ 2.5. Other A.E.</li> </ul>	Cancel	Organic function. Organic function. Protein binding a Protein binding a Protein binding b Protein binding b Protein binding p Protein binding p Protein binding p Protein binding p Protein binding p Protein binding p Repeated dose (H Respiratory sensi Retinoic Acid Rec rtER Expert Systet Skin irritation/con	Il groups (US EPA) Il groups, Norbert Haider (checkmol) lerts for Chromosomal aberration by OASIS lerts for skin sensitization according to GHS lerts for skin sensitization by OASIS y OASIS y OECD otency CSC totency GSH otency GSH otency LOERA 13%) ESS) isstion septor Binding n - USEPA rosion Exclusion rules by BfR		~
		<sup>®</sup> In order to create an item with the OFG profi <b>Chemical profile</b> (2) and click <b>OK</b> (3). Select down menu (4) and confirm by <b>OK</b> .*	ling re <b>Orga</b> i	esults clio <b>nic func</b>	k the <b>Create new</b> tional groups pro	✔ button (1), ofiler from the	select drop-

Back Next Cancel Create report

\*In the current example the category elements are not applied. If the consistency of the category is checked then this item will be automatically generated by the system. April, 202

#### Report Generation according to RAAF-Scenario 4

Customize report content and	appearance — $\Box$ X	_
Wizard pages		Possible content of AE 4.2. A common
Customization Customize report  Prediction Target and prediction summary Prediction details (I) Prediction details (II) Target profiles Analogues selection details Category Category definition and members Consistency check Options Data matrix Octions	<ul> <li> <b>2.1. Physicochemical similarity</b> </li> <li> <b>2.2. Structural similarity</b> </li> <li> <b>2.3. Mechanistic similarity</b> </li> <li> <b>Mechanistic similarity</b> </li> <li> <b>Mechanistic similarity</b> </li> <li> <b>Comments on mechanistic similarity</b> </li> <li> <b>AE 4.2: Common underlying mechanism, qualitative aspects</b> </li> <li> <b>Hint</b> </li> <li> <b>PURPOSE:</b> </li> <li>             The hypothesis/justification has to explain how the compounds the test organism is exposed to lead to the same type of effects/absence of effects. It has to be assessed whether:         <ul> <b>•</b> the documentation has established a common underlying mechanism;</ul></li> <b>•</b> this mechanism links the structures of these compounds under consideration with the possibility to predict qualitatively similar type of effects for the target substance for the property under consideration; and             <b>•</b> the provided evidence supports the explanation.  </ul> <li>         Add / Remove         <ul> <li> <b>2.4. Additional endpoints</b> </li> </ul></li>	<ul> <li>aspects</li> <li>The Target substance A and the source substances B, C, D, E and F all react via a common underling mechanism according to the RDT profiler</li> <li>They all have anilines functionality either as a parent or after a metabolic activation</li> <li>The similarity with respect to the metabolic pattern could be seen in AE 4.5. above.</li> </ul>
	© 2.5. Other AEs Back Next Cancel Create report	Additionally, metabolic maps (for each of the analogues), produced by external software or found in the literature, could be included to this AE in order to support the mechanistic similarity of the

### Report Generation according to RAAF-Scenario 4

Customize report content and a	pearance	-		Х			
	💽 Report basket		_		×		
Minud an area	Options 🖌						
wizard pages	f Select All	Unselect Al			Invert		
	口口口 Chemical profile ("Repeated dose	(HESS)")					
Customization	Figure 2	netabolism (تنه o Rat metabolism) ed: Human Health Hazards#Rep	olism simi oeated D	ulator" : ose To:	and "Rep kicity)		
Customize report	Category members						
Prediction	O 2.2. Structural s □ II Structural similarity						
Target and prediction	A Machanictic      A      External content	nal groups")					
summary	Z.J. IVIECTIAIIISCI	om clipboard No.1)	etan co A	and	、		
	2.4. Additional A District Input	I Similarity between Target Sub	stance A	anu	)		
Prediction details (I)	2.5. Other AEs						
Prediction details (II)	AE 4 3: Common						
Target profiles	All 4.5. Common				>		
Analogues selection	Hint	Create new	ОК	Ca	incel		
details	PURPOSE: Under this scenario, guantitative differences for the same type of effects are e	pected caused by the underlying r	nechanisr	n.	}		
Category	It has to be assessed whether:	+,			þ		
Category definition	<ul> <li>the documentation established that the strengths of the same type of effect</li> <li>the prediction is derived from the relation between an observed property ar</li> </ul>	; vary in predictable manner; d the independent variable which c	letermine				
and members	the order within the category (prediction model);	a the macpendent variable when t	i coci i i i i i i i i i i i i i i i i i				
Consistancy shack	<ul> <li>the prediction model is consistent with the common mechanism; and the prediction of the prediction</li> </ul>						
Consistency check	- the provided evidence supports the explanation.						
Options	Add / Remove						
Data matrix				-			
Options	○ AE 4.4: Exposure to other compounds than to those I	nked to the prediction					
	$\odot$ AE 4.5: Occurrence of other effects than covered by t	he hypothesis and justifi	cation				
AFC 4: Consistency of effects in the data matrix							
AE C.6: Bias that influences the prediction							
				$\checkmark$			
	Back	Next Cancel	Create	report			

Possible content of AE 4.3. A common underlying mechanism, quantitative aspects

- The target substance A and the five source substances have a common reactivity pattern.
- They all formed aniline functionality either as parents or as metabolites responsible for the toxicity effects
- Similar toxic effects observed in sources substances support the prediction for the target
- Toxic effects of all source substances and target are supported by the identified additional RDT data (references could be included).
- The range of variation of the LOEL experimental data for all category members is shown below:

After the last bullet include the Endpoint data variation item stored in the report basket (1).

# Report Generation according to RAAF-Scenario 4

Customize report content and a	ppearance – 🗆	<	
Wizard pages			
Customization Customize report Prediction Target and prediction summary Prediction details (I) Prediction details (II) Target profiles Analogues selection details Category Category definition and members Consistency check Options Data matrix Options	<ul> <li>2.1. Physicochemical similarity</li> <li>2.2. Structural similarity</li> <li>2.3. Mechanistic similarity</li> <li>2.4. Additional endpoints</li> <li>2.4. Additional endpoints</li> <li>2.5. Other AEs</li> <li>A E 4.3: Common underlying mechanism, quantitative aspects</li> <li>A E 4.4: Exposure to other compounds than to those linked to the prediction</li> <li>A Hint PUROSE: Other compounds than those linked in the hypothesis to the prediction may be formed via other (bio)transformation pathways or may be intermediates/metabolites of the identified pathway. In addition, the impurity profiles associated with the source and target substances may have an impact on the prediction. The other compounds has been identified by the assessing expert. It has to be assessed whether: <ul> <li>other compounds that those linked to the prediction may be formed (e.g. via another (bio)transformation pathway or as inducting the substances may have an impact on the prediction of the property under consideration.</li> </ul> </li> <li>Add / Remove <ul> <li>A E 4.5: Occurrence of other effects than covered by the hypothesis and justification</li> <li>A E C.4: Consistency of effects in the data matrix</li> <li>A E C.6: Bias that influences the prediction</li> </ul> </li> </ul>		<ul> <li>Possible content of AE 4.4: Exposure to other compounds than to those linked to the prediction</li> <li>Target substance A and source substances B, C, D, E and F all have common reactivity pattern. They all formed anilines responsible for the toxicity effects (refer to Appendix Metabolites/Profiling):</li> <li>The metabolism in vivo shows formation of other reactive groups (reference to Appendix Metabolites/Profiling) such as: <ul> <li>p-Aminophenols (Rank B)</li> <li>o/p-Aminophenols (Rank B)</li> <li>Acetaminophen (Alert)</li> </ul> </li> <li>Being less reliable (Rank B, C or Alert only) these categories gave enough prove that they do not have significant impact over the influence of the toxic effect</li> <li>Our assumption is that Anilines is the functionality responsible for the toxic effect.</li> </ul>
	Back Next Cancel Create repo	ort	

# **Reporting** Report Generation according to RAAF-Scenario 4

Customize report content and a	appearance — 🗆 🗙
Wizard pages	
Customization Customize report	
Prediction	⊙ <mark>2.2. Structural similarity</mark>
Target and prediction	
summary	⊙ 2.4. Additional endpoints
Prediction details (I)	2.5. Other AEs
Prediction details (II)	AF 4 3: Common underlying mechanism guantitative aspects
Target profiles	
Analogues selection details	<ul> <li>AE 4.4: Exposure to other compounds than to those linked to the prediction</li> <li>AE 4.5: Occurrence of other effects than covered by the hypothesis and justification</li> </ul>
ategory Category definition and members Consistency check Options	Hint     PURPOSE:     It has to be assessed whether:     - additional mechanisms than those identified in the hypothesis may be acting on the basis of mechanistic insights or     derived from information in the data matrix; and     - these additional mechanisms affect the prediction for the property under consideration.
)ata matrix	Add / Remove
Options	✓ AE C.4: Consistency of effects in the data matrix
	○ AE C.6: Bias that influences the prediction
	v
	Back Next Cancel Create report

Possible content of AE 4.5: Occurrence of other effects than covered by the hypothesis and justification

- The target substance A and the source substances B,C, D, E and F have a common reactivity pattern based on Anilines functionality
- Additional alerts for repeated dose toxicity have been identified in the parents and their metabolites. The additional mechanisms are with Rank B and Rank C.
- Rank A is assigned only to the used Anilines (Hemolytic anemia with methemoglobinemia) category. The categories with Rank A are supported with training sets chemicals having reliable experimental data.
- It is assumed that the additional mechanism will not affect the prediction for the property under consideration.

#### Report Generation according to RAAF-Scenario 4

🦲 Customize report content and a	ppearance – 🗆 🗙								
Wizard pages			4						
Customize report	오 2.1. Physicocher 🕛 Report basket –		•						
Prediction	Options ▲		Table summarizing	number of me	tabolites in	ncluding p	arent with :	specific ale	ts
Target and prediction	T         Select All         Unselect All         Invert           Image: Select All         Image: Select	t About Options	Repeated dose (HESS)	P1 P2 108-69-0 95	2 P. 5-78-3 9	93 95-68-1	P4 95-64-7	P5 87-62-7	P6 87-59-2
summary Prediction details (I)	2.4. Addit     4      4	d dose (HESS)")	Anilines (Hemolytic anemia with methemoglobinemia) Rank A	2 3	3	3	3	2	3
Prediction details (II)	∑.3. Other AES	t All	Anilines (Hepatotoxicity) Rank C	2 3	3	3	3	2	3
larget profiles			Bromfenac (Hepatotoxicity) Alert	0 0	0	)	0	1	0
Analogues selection details	AE 4.5: Occurren		Mefenamic Acid (Hepatotoxicity) Alert	0 0	0	)	3	1	6
Category	Hint Lategory pulky input		Menadione (Hepatotoxicity) Alert	0 0	0	)	0	1	1
and members	It has to be assessed wher	Cancel	Not categorized	4 6	6	5	5	3	2
Consistency check	ditional mechanisms 1 ived from information in the data matrix; and tese additional mechanisms affect the prediction for the property under consideration.	Current	o-/ p-Aminophenois (Hemolytic anemia with methemoglobinemia) Rank B	2 2	1	L	2	1	2
Options			p-Aminophenols (Renal	1 1	0	)	0	1	1
Data matrix Options	Add / Remove		Toluene (Renal toxicity) Alert	0 1	1	L	0	1	1

Additionally to the entered text, the profiling similarity could be also included. To do this click **Add/Remove** button and check the box of **Profiling similarity** (2). This item stored in the report basket, is triggered by the used simulators and the profiling scheme for the primary grouping. Right-click and preview the item (3). Tables with generated metabolites for each parent along with the profiling result will be provided. A table summarizing all profiling results for each of the packages "parent and metabolites" is provided at the end (4).

#### Report Generation according to RAAF-Scenario 4

Customize report content and	appearance — $\Box$ >
Wizard pages	
Customization	
Prediction	
Target and prediction	
summary	2.4. Additional endpoints
Prediction details (I)	2.5. Other AEs
Prediction details (II)	✓ AE 4.3; Common underlying mechanism, guantitative aspects
Target profiles	• AF 4.4: Exposure to other compounds than to those linked to the prediction
Analogues selection details	<ul> <li>○ AE 4.5: Occurrence of other effects than covered by the hypothesis and justification</li> </ul>
ategory Category definition and members	AE C.4: Consistency of effects in the data matrix     Hint     PURPOSE:
Consistency check Options ata matrix	The category justification should include comparison of experimental data for the category members and a clear data matrix. It has to be assessed whether: - a data matrix has been provided which lists the category members in a suitable order versus their experimental data (e.g. for REACH information requirements) and which identifies data gaps;
Options	- the properties of category members across the data matrix are consistent in effects; this has to be assessed in the following dimensions:         - within the specific property which is under consideration for the prediction;         - between the property under consideration and related properties (e.g. between 28-day and 90-day repeated-dose toxicity studies; reproductive toxicity screening tests; and pre-natal developmental toxicity studies);         - characteristics across all relevant properties (e.g. different reactivity towards genetic material may indicate different reactivity towards biological macromolecules which may influence the prediction for a 90-day repeated-dose toxicity study;         - the effects reported for the property under consideration differ in strength for the source substance and whether a basis for this difference is provided; and         - the underlying data support the provided conclusions and explanations.
	Add / Remove
	Pack Next Casel Crate res

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

- The target substance A and the five source substances (B, C, D, E and F) show indication for a repeated dose effect especially for reduce red blood cell. The Total LOEL readacross prediction in this case is around 30 mg/kg bdw/day which classify the target chemical in the range of Category 2 according to GHS classification
- The latter is supported by the experimental data found for all of used source substances for the investigated endpoint and other similar properties

Here should be provided the data matrix snapshot or reference to the *Data matrix report*.

#### Report Generation according to RAAF-Scenario 4

Wizard pages       2.1. Physicochemical similarity         Customize report       2.2. Structural similarity         Target and prediction summary       2.3. Mechanistic similarity         Prediction details (I)       2.4. Additional endpoints         Prediction details (I)       2.5. Other AEs         Part 4.4: Exposure to other compounds than to those linked to the prediction         Pace 4.5: Occurrence of other effects than covered by the hypothesis and justification         Pace 4.5: Occurrence of other effects in the data matrix         Pace 2.6: Bias that influences the prediction	
ustomization       © 2.1. Physicochemical similarity         rediction       © 2.2. Structural similarity         Target and prediction summary       © 2.3. Mechanistic similarity         Prediction details (I)       © 2.4. Additional endpoints         Prediction details (I)       © 2.5. Other AEs         Prediction details (II)       © AE 4.3: Common underlying mechanism, quantitative aspects         Analogues selection details       © AE 4.4: Exposure to other compounds than to those linked to the prediction         © AE 4.5: Occurrence of other effects than covered by the hypothesis and justification         © AE C.4: Consistency of effects in the data matrix         Category definition and members	
rediction <sup>©</sup> 2.2. Structural similarity          Target and prediction summary <sup>©</sup> 2.3. Mechanistic similarity          Prediction details (I) <sup>©</sup> 2.4. Additional endpoints          Prediction details (I) <sup>©</sup> 2.5. Other AEs          Prediction details (II) <sup>©</sup> AE 4.3: Common underlying mechanism, quantitative aspects          Analogues selection details <sup>©</sup> AE 4.4: Exposure to other compounds than to those linked to the prediction          Actions <sup>©</sup> AE 4.5: Occurrence of other effects than covered by the hypothesis and justification          Category definition and members <sup>©</sup> AE C.6: Bias that influences the prediction	
Target and prediction summary	
summary       © 2.4. Additional endpoints         Prediction details (I)       © 2.5. Other AEs         Prediction details (II)       © AE 4.3: Common underlying mechanism, quantitative aspects         Target profiles       © AE 4.4: Exposure to other compounds than to those linked to the prediction         Analogues selection details       © AE 4.5: Occurrence of other effects than covered by the hypothesis and justification         © AE 4.4: Exposure to other compounds than to those linked to the prediction         © AE 4.5: Occurrence of other effects than covered by the hypothesis and justification         © AE C.4: Consistency of effects in the data matrix         © AE C.6: Bias that influences the prediction	
Prediction details (I)         Prediction details (II)         Target profiles         Analogues selection details         O AE 4.4: Exposure to other compounds than to those linked to the prediction         O AE 4.4: Exposure to other effects than covered by the hypothesis and justification         O AE C.4: Consistency of effects in the data matrix         Category definition and members	
Prediction details (II) <ul> <li>Aralogues selection details</li> <li>Analogues selection details</li> <li>At 4.3: Common underlying mechanism, quantitative aspects</li> <li>A A 4.4: Exposure to other compounds than to those linked to the prediction</li> <li>A E 4.4: Exposure to other effects than covered by the hypothesis and justification</li> <li>A E C.4: Consistency of effects in the data matrix</li> </ul> <li>A E C.6: Bias that influences the prediction</li>	
Target profiles	
Analogues selection details       Image: Analogue selection details         ategory       Image: Analogue selection details         Category definition and members       Image: Analogue selection details	
Category       Image: All C.4: Consistency of effects in the data matrix         Category definition and members       Image: All C.6: Bias that influences the prediction	
Category definition and members	
Consistency check	
Options         It has to be assessed whether:           - it is clear from the documentation how the source substance(s) have been chosen, for example, what methods/tools have been used to map the field of potential source substance(s), which other substances have been considered and why they have been discarded;           - there are additional, structurally-similar substances which are currently not used in the analogue approach and which	ſ
arguably could be used; - there is readily-available information from these additional substances; - this information is biologically significantly different for relevant properties in comparison with the existing analogue(s); and - these differences decrease the confidence in the prediction (possibility of underestimation of hazard).	
Add / Remove	
	_
Back Next Cancel Create	

#### **Possible content of AE C.6: Bias that influences the prediction**

- The used source chemicals have been found based on a common underlying mechanism for repeated dose toxicity accounting for *in vivo* Rat metabolism;
- The most reliable category was selected (with Rank A);
- The primary group was refined by applying of the following subcategorizations: 1) US-EPA New Chemical Categories, 2) Chemical elements; 3) OECD HPV Chemical Categories.

A chemical expert can provide additional literature search of similar analogues with similar effects

### **Report** Report Generation

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

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





Open

Save as

## **Report** Generated report files

Prediction of LOEL for 3	3,5-xylid	line									1/6		Г																
Predic	tio	SAF	₹ To		Cedi	ction	for single	e chem	ical					Chem	icals ca	ategory			Q	SAR TO	olbo	Са	ate Int for ca	<b>go</b> ategory	ry	rep	ort		
Date: 15 Apr 2020 Author(s): Contact details:	)		(in a	accordance	ew	rith RA	AF scenar	io 4)	]•	The s is sp	selec ecifie	ted F ed in	RAA the	F s firs	cen st pa	age	defi	inition	<b>▶</b> [(	In accord	dance	e with R	AAF scen	nario 4)					
				Target	t in	form	ation				1																a field		
Structural information				Numerica	L III	ntifiere	ation	•				1.1.	Catego	ded I	by the	on user						'	nanually editab	e held					
SMILES: Cc1cc(C)cc(N)c1 Structure C	SMILES: Cc1cc(C)cc(N)c1 Structure			CA5#: 108-69-0 Other: EC Number:2036070				"3,5-xylidine" 3,5-dimethyl-aniline 3,5-Dimethylaniline						Ran 1.2. - 1.3.	Ges for a Not provid Human I Categor	sele ded I d (tz Heal ry h	elected physicochemical properties and ca ied by user ((target) endpoint(s) Health Hazards/Repeated Dose Toxicity: LOEL, y hypothesis					nd calculat	<b>ted para</b> I, Whole t	meters , pody, Ora	nanuaily editable field l (gavage), Rat nanuaily editable field				
* : X ✓ j	fx														1.4.	Not provid	ded /Me	by the taboli: ping/su	user isms ubcate	gorization:					,	nanually editab	le field		
Substance identity Structure CAS number Chemical name Other identifier SMILES	Data m betarce identity uture 5 number emcial name ter identifier		*** ***	challing CH2 CH2 CH2 CH2 CH2 CH2 CH2 CH2	report     science			1 Ne	K ighbour #2 95-68-1 4-xylidine ccc(N)c(C)c1	L M Neig 99 3,4- Cc1ci	N hbour #3	0 р Neigh н <sub>ус</sub> 87 2,6-х Сс1сс	0 P Q Neighbour 84 #C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-		R 5 T Neighbour #5			c anem New Cl I eleme PV Che nembe ince cl	nia wit hemica ents (s emical ers haract	with methemoglo mical Categories ( ts (subcategorizat ical Categories (su racterization		lobinemia) Rank A (Repeated do: ( subcategorization) ation) subcategorization)			se (HESS	)) (primary (	rouping) e field		
Parameters	unit																-18		Nam	e		SMILES	5		Structur	e			
softers offer size (for grouping/hobsergenization onlined parent and products requirements: millines (hermalytic anemia with millines) (hermalytic anemia with hermalgiohimes) (hash (Alegoreted dose HSI) (inclusive remained) hermalia (hermst subcategorization) ECD HPC Chemical Categories university (CD HPC Chemical Categories university)(CD HPC Chemical Categories) with the comparison of the comparison of the comparison of the comparison appealed dose (HESS)		Has Anil M H	Parent and all of the ines (Hem ethemogle as the foll Anilines   Group 1 Dime lines (Hem ethemogle lines (Hem	47 metabolites; required categories; solytic anemia with obienenia Rank A; owing additional (Acute toxicity) 44 - Carbon C; 5 - Nitrogen N thylaniline wolytic anemia with obienenia] Rank A;	metabolites; juired categories: H nemial Rank A; inz additional ute taxicity) - Carbon C; Nitrogen N (aniline ytic anemia with premial Rank A; taxicity Rank A;		10 metaboiltes; required categories; objtic anemia with binemia) Rank &; owing additional Acute toxicity) di - Carbon C; 5 - Nitrogen N thylaniline hobinemia) Rank &; atotoxicity) Rank C;	Parent and 9 metabolites; Parent and 9 metabolites; Parent and 9 metabolites; Parent Search (1996); Parent Sea			11 metabolites; equired categories optica anemia with optical anni Agi wing additional cute toxicity) - Nitrogen N hylaniline olytic anemia with optical Rank Agi	Parent and I Has all of categories: An aner methemosink Anilines (A Group 14 Group 15 Dimeth Anilines (Her with methemog	Parent and 6 metabolites; Has all of the required categories: Anilines (Henolytic ametimong) and the second Anilines (Lotroban C); Group 14 - Carbon C; Group 13 - Nitrogen N Dimethylenalline Anilines (Henolytic anemia with methemoglobinemia) Rani Anilines (Henolytic anemia		Parent and 10 metabolites; Has all of the required categories Anlines (Hemolytic anemia with methemoglobismis) Bank & Arong 14 - Carbon C, Group 15 - Nitrogen N Dimethylaniline Anlines (Hemolytic anemia with te methemoglobismis) Bank & Anlines (Hemolytic anemia with		Parent and 10 metabolites; hail of the required categories; hailines (Hemolytic anemia with methemoglobicmia) Bank & Anillines (Hemolytic bacich) Group 15 - Nathone N Dimethylaniline (Hollies (Hemolytic anemia with methemoglobinemia) Bank & Anilles (Hemolytic anemia with			)	3,5->	,5-xylidine		Cc1cc(	(C)cc(N)c1		н <sub>3</sub> с~	CH3	H2
Measured and predicted data Data used for prediction		Ani	lines (Hep	atotoxicity) kank C		Toluene (Re	nal toxicity) Alert	Toluene (i	enal toxicity) Alert	Aniines (Hepa	itotoxicity) kank c	Anilines (Hepa	atotoxicity) Rank	Mefenan	nic Acid (Hep	patotoxicity)			(	Q S A	R	тос		х			TPRF v4.2		
environment Repeated Dose Taxicity Repeated Dose Taxicity	endpoint LOEL LOEL	value	unit	species, auraun, test type, type of method, assay, strain, test guidelin	ie, ve	60 mg/kg bdwt/c 12 bdwt/c	species, auraion, test type, type of method, assay, strain, test d 28 d t Rat d 28 d	value un 10 mg 2 mg bdw	species, aurouon test type, type a ti method, assay, strain, test kg Rat t/d 28 d kg Rat t/d 28 d	value unit so mg/kq bdwt/ so mg/kq bdwt/ i <	species, duration, test type, type of method, assay, Rat d 28 d g Rat d 28 d	value unit 160 mg/k 50 mg/k bdwt/	species, duration, tesi type, type of method, g Rat 'd 91 d g Rat 'd 42 d	value 60 12	unit ty me mg/kg bdwt/d mg/kg bdwt/d	species, vration, test vpe, type of thod, assay, Rat 28 d Rat 28 d	×												

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

#### 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 4.
- Note, proficiency comes with practice!