

OECD (Q)SAR Toolbox v.4.4.1

Tutorial of how to predict ecotoxicological endpoint
of chemicals by standardized workflow

Outlook

- **Background**
- Keywords
- Objectives
- Specific Aims
- Standardized workflow for Ecotoxicity
- The exercise
- Standardized workflow execution

Background

This is a step-by-step presentation designed to take the user of Toolbox through the Standardized workflow (SW) for ecotoxicity prediction.

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Keywords

TARGET CHEMICAL - chemical of interest

MODULE – a Toolbox module is a section dedicated to specific actions and options (e.g. Profiling)

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:

- Identify analogues for a target chemical;
- Retrieve experimental results available for those analogues;
- Color the profiling schemes according to their suitability for subcategorization;
- Fill data gaps by standardized workflow;

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Specific Aims

- To introduce to the Toolbox user to the standard workflow for predicting of ecotox endpoint (LC50);
- To familiarize the user with the new Toolbox interface;
- To explain to the user the rationale behind each step of the exercise.

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Standardized workflow for Ecotoxicity endpoints

Overview

Standardized workflow for Ecotox covers the following acute aquatic toxicity endpoints:

- Fish, LC50 (EC50), 96h, mortality

or

- Invertebrates, EC50(LC50), 48h, mortality, immobilization, intoxication

or

- Algae, LC50 (EC50), 72-96h, population or growth

Standardized workflow for Ecotox endpoints

Overview

- The standardized workflow (SW) is designed to apply data gap filling for discrete chemicals only
- The SWs has been developed to be applicable for the same endpoints used for application of the AWs (i.e. LC50, Mortality, 96h, *Pimephales promelas*).
- Once started, the SW follows the implemented logic under the user control.
- As opposite to the automated workflow (AW), the domain of application is expanded in the SWs (including other endpoints, effects, species, durations, etc.) and SWs allow interactions by the user.
- In case more than one further application is possible, the workflow stops and waits for the decision of the user.
- SW can be executed for a single chemical only.

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The Exercise

- In this exercise we will predict the acute aquatic toxicity endpoint (LC50) of N-Octylamine [CAS# 111-47-8], which will be the “target” chemical.
- This prediction will be accomplished by using of the developed standardized workflow for ecotoxicity (LC50, Mortality, 96h, *P.promelas*).

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Standardized workflow execution

- Only three of the general Toolbox modules are used in a sequential workflow:
 - Input
 - Data Gap Filling
 - Report

The rest of the modules – *Profiling, Data* and *Category definition* are included as a part of the algorithm of the standardized workflow. The workflow stops at them and waits for the decision of the user.

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 - Input

Chemical Input Overview

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

Chemical Input

Ways of Entering a Chemical

I. Single target chemical:

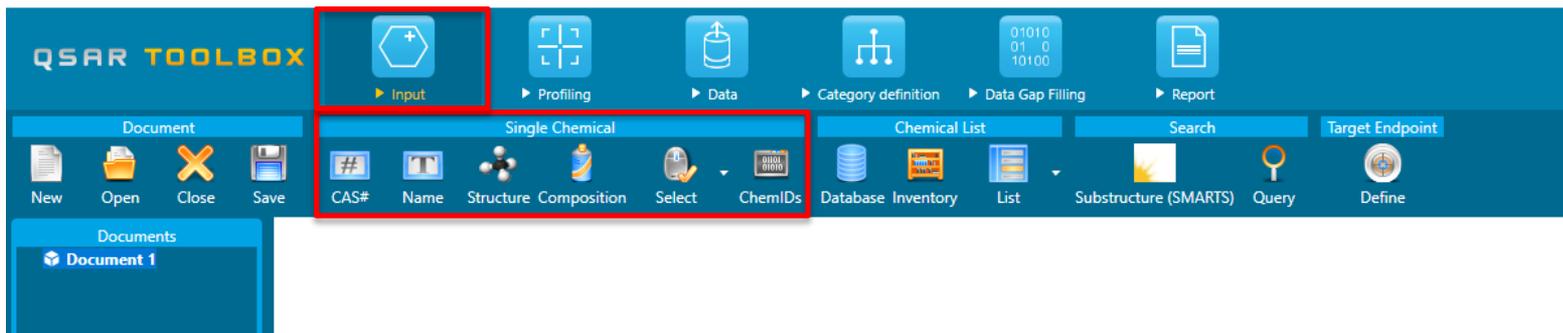
- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation
- Chemical with defined composition
- Drawing chemical structure
- Select from User List/Inventory/Databases

II. Group of chemicals:

- User's List
- Inventory/Database

Chemical Input Single chemical

As mentioned before SW is allowed for single chemical only. In this respect below are provided different ways for entering a single chemical



- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- Drawing chemical structure
- Select from User List/Inventory/Databases

Chemical Input

Single chemical: CAS RN

1. Press **CAS#** (1);

2. Enter the CAS of N-Octylamine # 111-86-4 (2);

3. Click on **Search** (3);

4. Press **OK** (4).

1	CAS	111-86-4
	SMILES	CCCCCCCCN
	CS Relation	High
	Substance	Mono constituent
	Composition	
	Name	1-aminoctane;1-Octanamine;1-oc
	Sources	NICNAS ECHA's DSF

Chemical Input

Target chemical identity

The screenshot shows the QSAR Toolbox interface with the 'Target Endpoint' tab selected. A 'Filter endpoint tree...' dialog is open, displaying the 'Structure info' section for a target molecule. The 'Structure info' section is highlighted with a red box, and a callout bubble with the number '1' points to it. The 'Structure info' section contains the following information:

Structure	<chem>CCCCCCCCN</chem>
Structure info	
Additional ids	EC Number:2039160
CAS Number	111-86-4
CAS-SMILES relation	High
Chemical name(s)	1-amino-octane 1-Octanamine
Composition	
Molecular formula	C ₈ H ₁₉ N
Predefined substance type	Mono constituent
SMILES	CCCCCCCCN
Parameters	
Physical Chemical Properties	
Environmental Fate and Transport	
Ecotoxicological Information	
Human Health Hazards	

1. Open **Structure info** level to see chemical ID of the target molecule

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 - Input
 - **Data Gap Filling**

Data gap filling

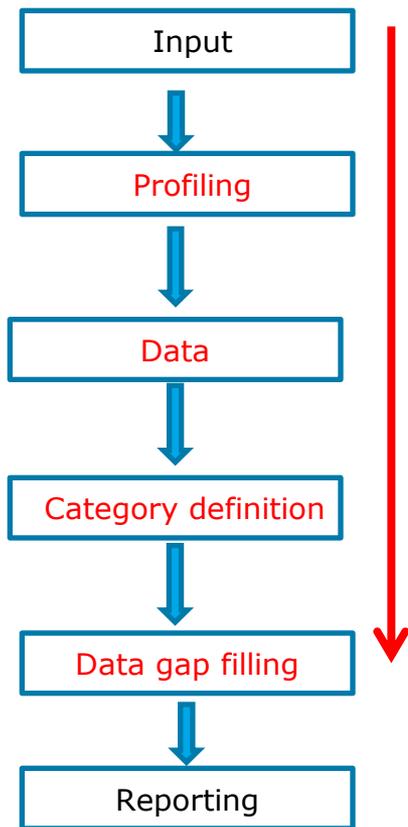
An overview

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

Data gap filling

An overview

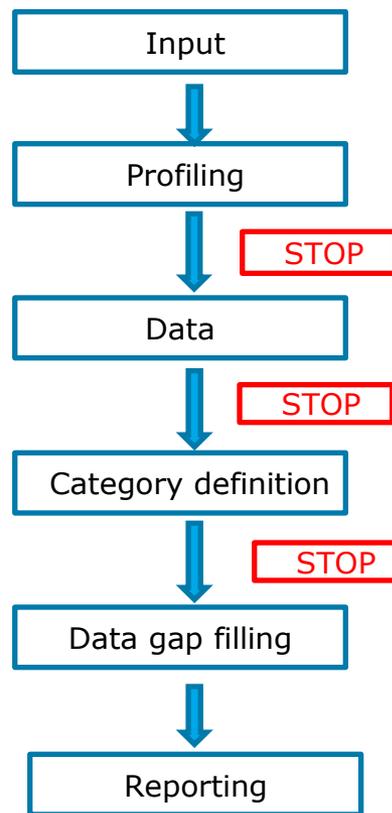
Automated workflow (AW)



Same components as defined in the AW are used in the SW

The SW pauses at each of the stages and user is able to make different selection than those implemented in the AW

Standardized workflow (SW)



Databases with data for the target endpoint are listed and user select to use all of them or make specific selection

Relevant to the workflow profilers appropriate for DGF are listed and ordered hierarchically based on the population of the group and user is able to select any of them

Additional data filtering could be applied (e.g. different species selection)

In this example, we will use the Standardized workflow approach.

Data Gap Filling

Apply Standardized workflow

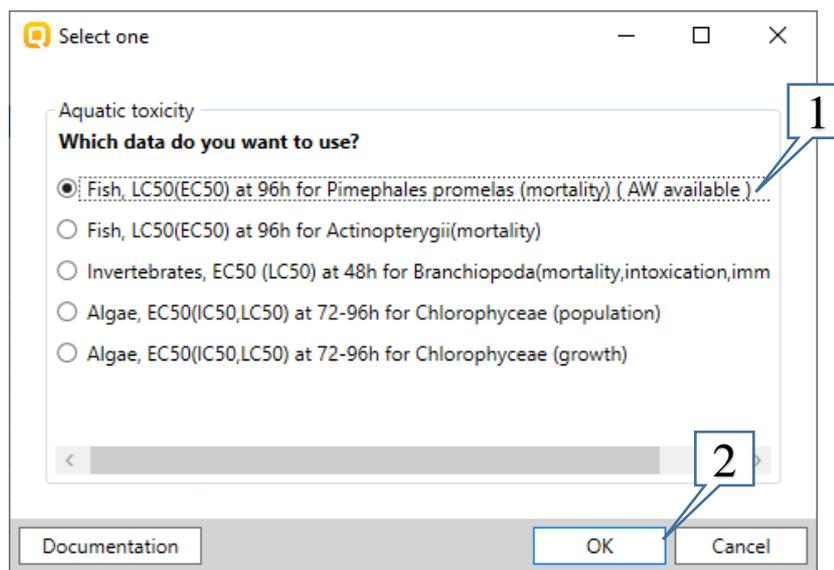
The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Data Gap Filling', which is highlighted with a red box and labeled '1'. Below the menu bar, the 'Workflow' dropdown is open, showing 'Standardized' selected, highlighted with a red box and labeled '2'. A 'Select workflow' dialog box is open, showing two options: 'Ecotoxicological Endpoint' (selected with a radio button) and 'Skin sensitization'. The dialog box is highlighted with a red box and labeled '3'. The 'OK' button in the dialog box is highlighted with a red box and labeled '4'. A blue callout box at the bottom left contains the following instructions:

1. Go to **Data Gap Filling**;
2. Press **Standardized**;
3. Select **Ecotoxicological endpoint**
4. Click **OK**

Data Gap Filling

Apply Standardized workflow

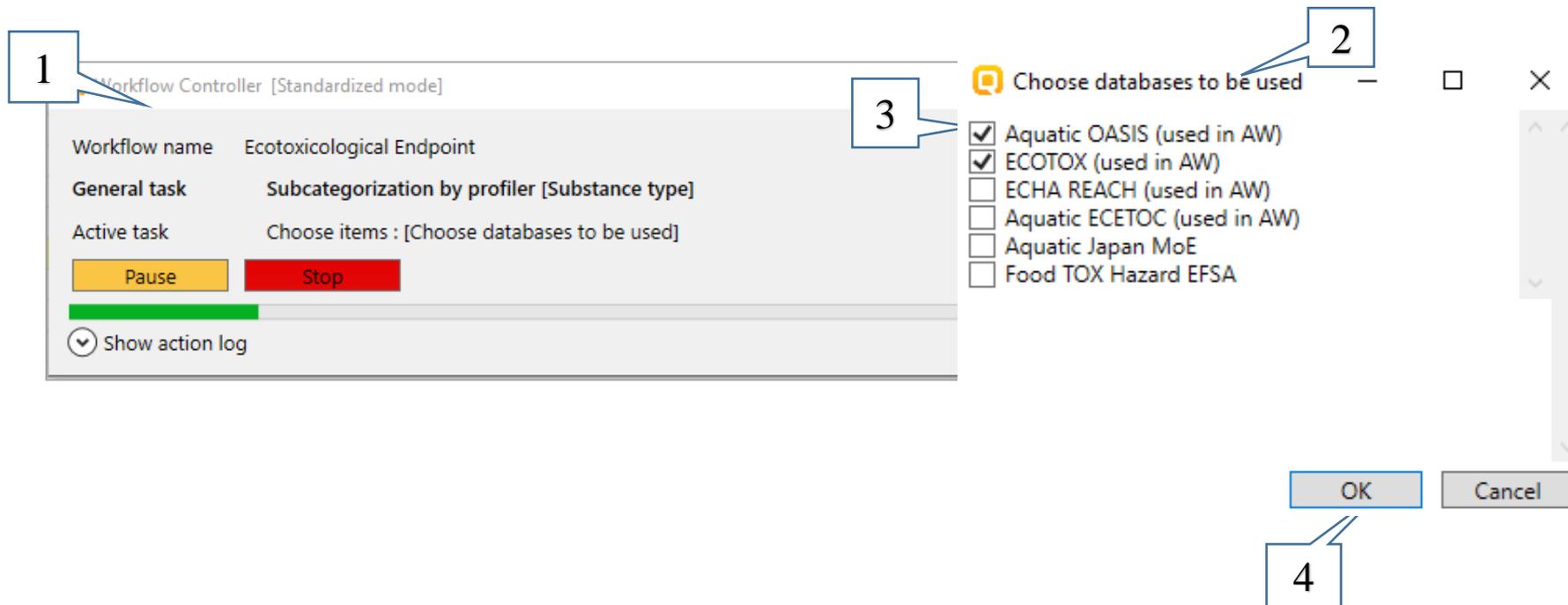
There are several options for selection of endpoint and user should select one of them. The first option covers the same endpoint and metadata as the automated workflow, while the others are associated with different endpoints.



In our case select the first endpoint – **Fish, LC50(EC50) at 96h for Pimephales promelas (1)**. The user could make other selection. *AW available in brackets* means that the endpoint is used in the automated workflow;
2. Click on **OK (2)**

Data Gap Filling

Apply Standardized workflow



A workflow controller window (1) is displayed and then a dialogue window for selection (2) of databases appears. Indication about which databases are used in the AWs is also included in brackets. Select the databases which you would like to be used in SW. In our case the first two databases are selected (3); Click on **OK**(4)

Data Gap Filling

Apply Standardized workflow

Workflow Controller [Standardized mode]

Workflow name: Ecotoxicological Endpoint

General task: Defining category with

Active task: Choose item : [Choose]

Buttons: Pause, Stop

Show action log

Choose primary category profiler

- Aquatic toxicity classification by ECOSAR | 1032 analogues [(103 with data) 210 data points][Used in AW]
- US-EPA New Chemical Categories | 820 analogues [(87 with data) 178 data points]
- Acute aquatic toxicity MOA by OASIS | 315 analogues [(67 with data) 146 data points]
- Organic functional groups | 307 analogues [(36 with data) 73 data points]
- Organic functional groups, Norbert Haider (checkmol) | 298 analogues [(36 with data) 73 data points]
- Organic functional groups (US EPA) | 143 analogues [(28 with data) 55 data points]

Buttons: OK, Cancel

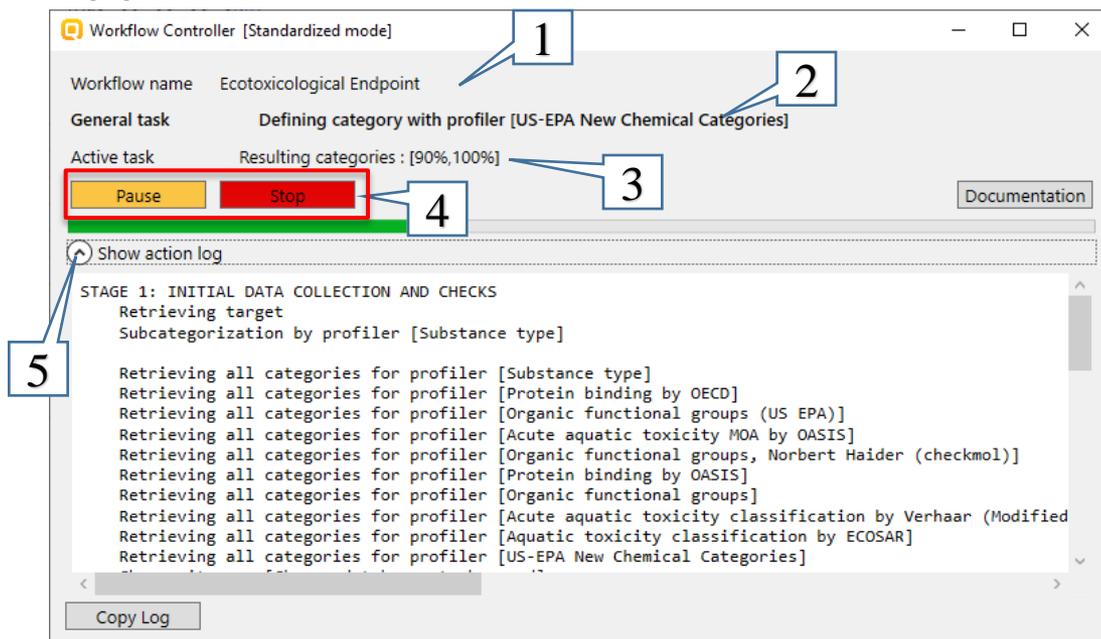
Now the system define groups with analogues and provide a list with the most appropriate for primary grouping profilers starting with the most populated one. Here, we select **Aquatic toxicity classification by ECOSAR** (1) and then click on **OK** (2).

Data Gap Filling

Apply Standardized workflow

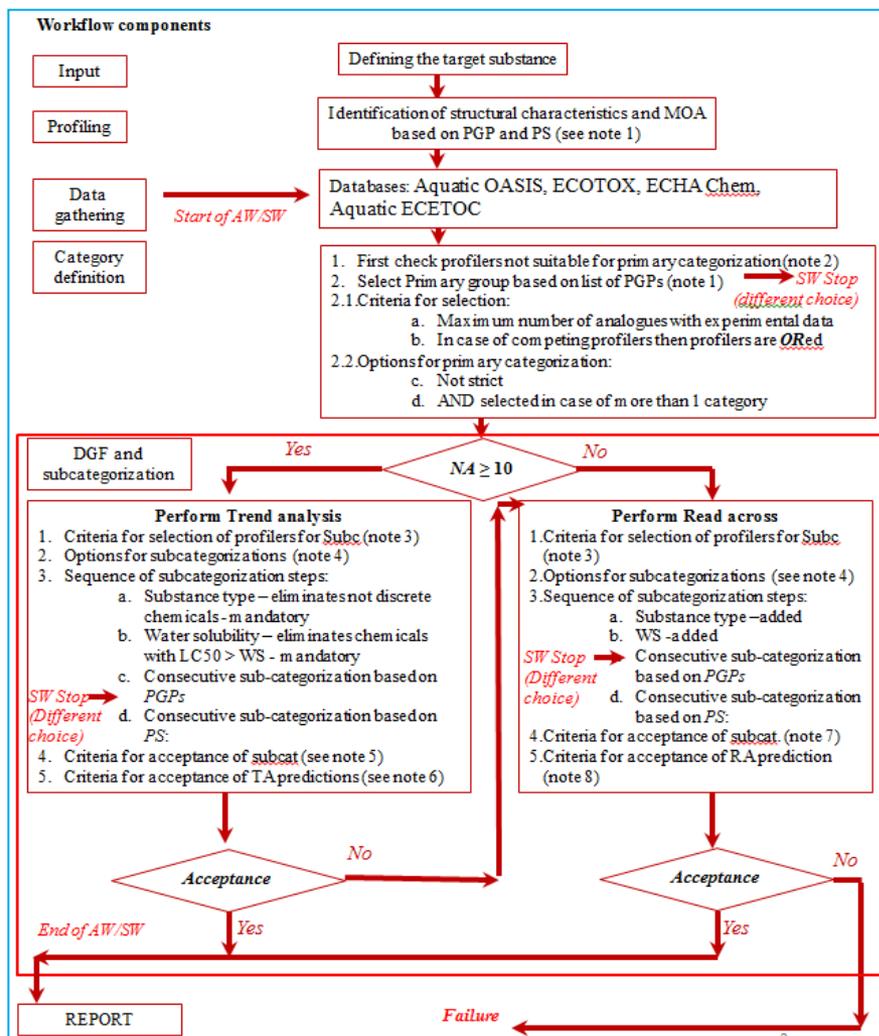
A workflow controller window is displayed throughout the standardized workflow procedure. It includes:

- 1) Workflow name (1)
- 2) General task (2)
- 3) Active task (this is subtask of the general task, which is currently being performed)(3)
- 4) Navigation options (4)
- 5) Activity log (5)



Data gap filling

Algorithm of Ecotoxicological workflow



Data Gap Filling

Apply Standardized workflow

When the profiling scheme for primary categorization is selected, the workflow makes a category and enters in Data Gap Filling, where the next step is subcategorization. Depending on the outcome obtained by the applied subcategorization, the profilers are colored as follows:

- **Green** – application of the profiler will satisfy the criteria for acceptance of the prediction
- **Blue** – application of the profiler increase the confidence of the prediction only
- **Yellow** – application of the profiler does not change the current state
- **Red** – criteria for acceptance the subcategorization will be not reached
- **Grey** – already applied profiler

Data Gap Filling

Apply Standardized workflow

Subcategorization 1 | Current state $R^2 = 0.392$ 95% residuals 2.142

Options ▾ Profilers 0 Selected

f Select All Unselect All Invert

Primary grouping

- [Suitable for acceptance]Aquatic toxicity classification by ECOSAR(64 analogues) | $R^2 = [0.777]$, 95% Residuals = [1.38]
- [Suitable for acceptance]Acute aquatic toxicity MOA by OASIS(53 analogues) | $R^2 = [0.866]$, 95% Residuals = [0.9]
- [Suitable for acceptance]Organic functional groups (US EPA)(24 analogues) | $R^2 = [0.841]$, 95% Residuals = [1.22]
- [Suitable for acceptance]Organic functional groups, Norbert Haider (checkmol)(23 analogues) | $R^2 = [0.836]$, 95% Residuals = [1.2]
- [Suitable for acceptance]Organic functional groups(13 analogues) | $R^2 = [0.893]$, 95% Residuals = [1.31]
- ~~US EPA New Chemical Categories(61 analogues) | $R^2 = [0.437]$, 95% Residuals = [1.86]~~

Secondary grouping

- [Suitable for acceptance]Chemical Elements(38 analogues) | $R^2 = [0.844]$, 95% Residuals = [1.16]
- [Suitable for acceptance]Structure Similarity(12 analogues) | $R^2 = [0.97]$, 95% Residuals = [0.624]
- Protein binding by OASIS(71 analogues) | $R^2 = [0.393]$, 95% Residuals = [2.11]
- Protein binding by OECD(74 analogues) | $R^2 = [0.392]$, 95% Residuals = [2.14]

Unclassified

Options ▾ Metabolisms 0 Selected

f Select All Unselect All Invert

Do not account metabolism

Adjust options

Target

Differ from target by

At least one category All categories [STOP]

Analogues

Once the primary group is selected, the implemented logic of Ecotoxicological workflow is applied. As result a list with profilers appears highlighted and ordered appropriately. The profilers are separated into two sections in the subcategorization window (1): *Primary grouping* (2) and *Secondary grouping* (3).

Data Gap Filling

Apply Standardized workflow

6 Subcategorization 1 | Current state $R^2 = 0.392$ 95% residuals 2.142

Options Select All Unselect All Invert About Options 1 Selected

Primary grouping

(Suitable for acceptance)Aquatic toxicity classification by ECOSAR(64 analogues) | $R^2 = [0.777]$, 95% Residuals = [1.38]

(Suitable for acceptance)Acute aquatic toxicity MCA by OASIS(65 analogues) | $R^2 = [0.860]$, 95% Residuals = [1.31]

(Suitable for acceptance)Organic functional groups (US EPA)(24 analogues) | $R^2 = [0.841]$, 95% Residuals = [1.22]

(Suitable for acceptance)Organic functional groups, Norbert Haider (checkmol)(23 analogues) | $R^2 = [0.836]$, 95% Residuals = [1.22]

(Suitable for acceptance)Organic functional groups(13 analogues) | $R^2 = [0.893]$, 95% Residuals = [1.31]

US-EPA New Chemical Categories(61 analogues) | $R^2 = [0.437]$, 95% Residuals = [1.86]

Secondary grouping

(Suitable for acceptance)Chemical Elements(38 analogues) | $R^2 = [0.844]$, 95% Residuals = [1.16]

(Suitable for acceptance)Structure Similarity(12 analogues) | $R^2 = [0.97]$, 95% Residuals = [0.624]

Protein binding by OASIS(71 analogues) | $R^2 = [0.393]$, 95% Residuals = [2.11]

Protein binding by OECD(74 analogues) | $R^2 = [0.392]$, 95% Residuals = [2.14]

Unclassified

Options Select All Unselect All Invert 0 Selected

Metabolisms

Do not account metabolism

Adjust options

Target

Aliphatic Amines

ECOSAR(64 analogues) | $R^2 = [0.777]$, 95% Residuals = [1.38]

Differ from target by

At least one category [STOP]

All categories

Analogues

(5) Acid moiety

(74) Aliphatic Amines

(1) Neonicotinoids

(2) Phenol Amines

(2) Phenols

(1) Triazines, Aliphatic

(1) Triazines, Aromatic

Selected 10 (64/74)

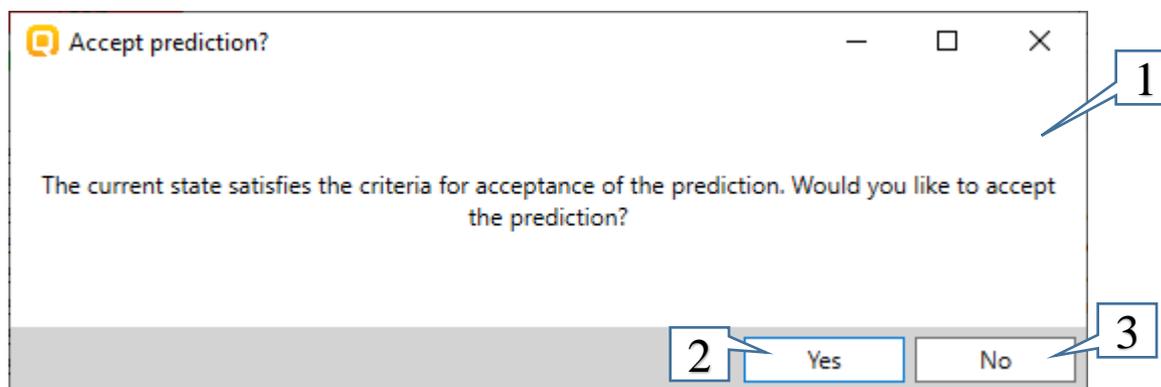
Select different

Remove selected

Clicking on the first profiler (1) shows all categories found in the analogues (2) as the ones colored in blue (3) are the ones that are not applicable to the target chemical (different to the target) and hence can be removed (4). Moreover a statistic coefficients which will be obtained if the profiler is applied are provided after profilers' name (5). The statistics of the current state is available too (6) and user could easily compare them.

Data Gap Filling

Apply Standardized workflow



- If obtained results after applied subcategorization satisfy the criteria for acceptance the prediction, then the message appears:
- *The current state satisfy the criteria for acceptance the prediction. Would you like to accept the prediction ?(1)*
- Press Yes (2) , if you want to accept the prediction (next slide)
- Press No (3) and continue with the workflow if you are not satisfy with the outcome and want to continue

Data Gap Filling

Apply Standardized workflow

The screenshot displays the QSAR Toolbox interface during a data gap filling process. A 'Success' dialog box (1) is overlaid on a 'Workflow Controller' window (2). The background shows a chemical structure tree on the left and a data table with columns for EC numbers and toxicity values.

Workflow Controller [Standardized mode]

Workflow name: Ecotoxicological Endpoint
 General task: Retrieving different analogues for profiler [Structure Similarity]
 Active task: [Progress bar]
 Documentation: [Button]
 Show action log: [Button]
 Step [1] Subcategorization [Structure Similarity] R2 [0.970] 95% Residuals [0.624]
 Selecting chemical list
 Performing visual subcategorization
 Selecting chemical list
 List satisfies subcategorization acceptance criteria : [Suitable for acceptance] R² = Question : [The current state satisfies the criteria for acceptance of the prediction.]
 Accepting prediction
 Copy Log: [Button]

Success

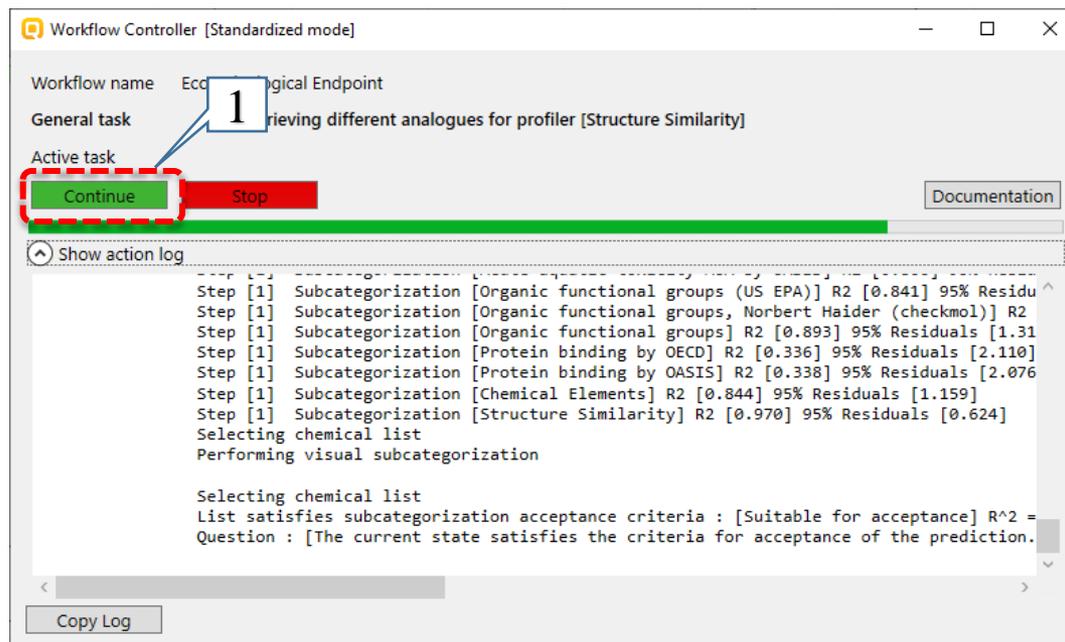
Prediction accepted successfully
 OK: [Button]

- A pop-up window (1) is displayed informing that the prediction is accepted
- Press **OK** (2)

Data Gap Filling

Apply Standardized workflow

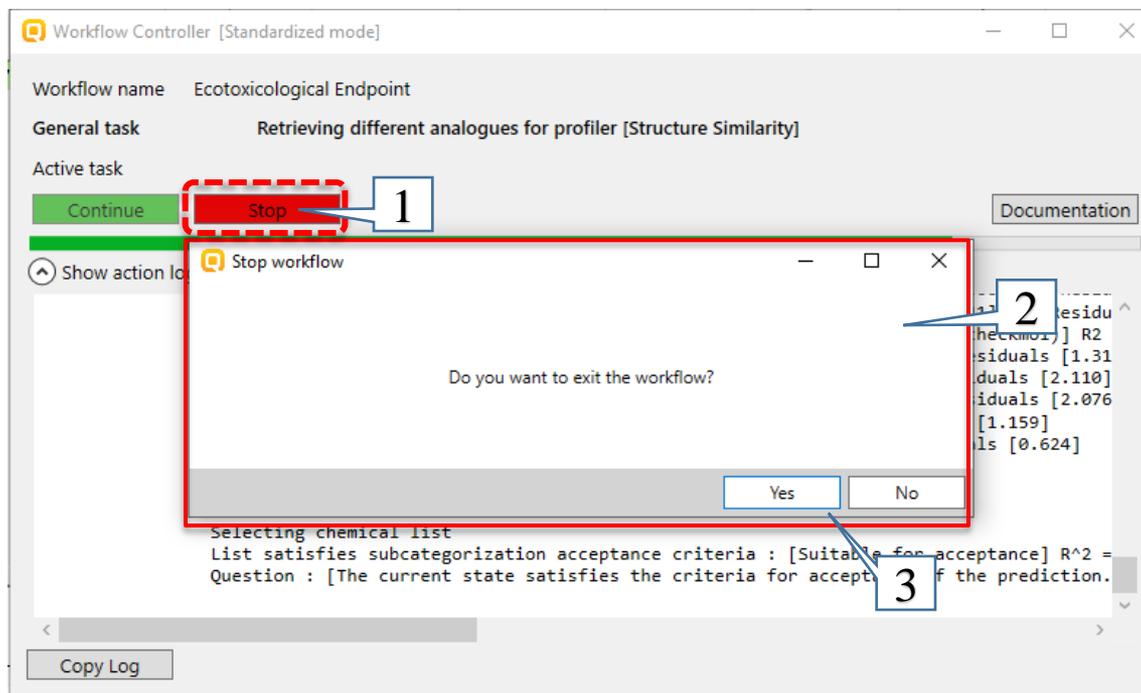
- Continue the workflow if you are not satisfied with the outcome.



- Press Continue button (1) in the workflow controller
- Then repeat the steps described on slide 34

Data Gap Filling

Apply Standardized workflow

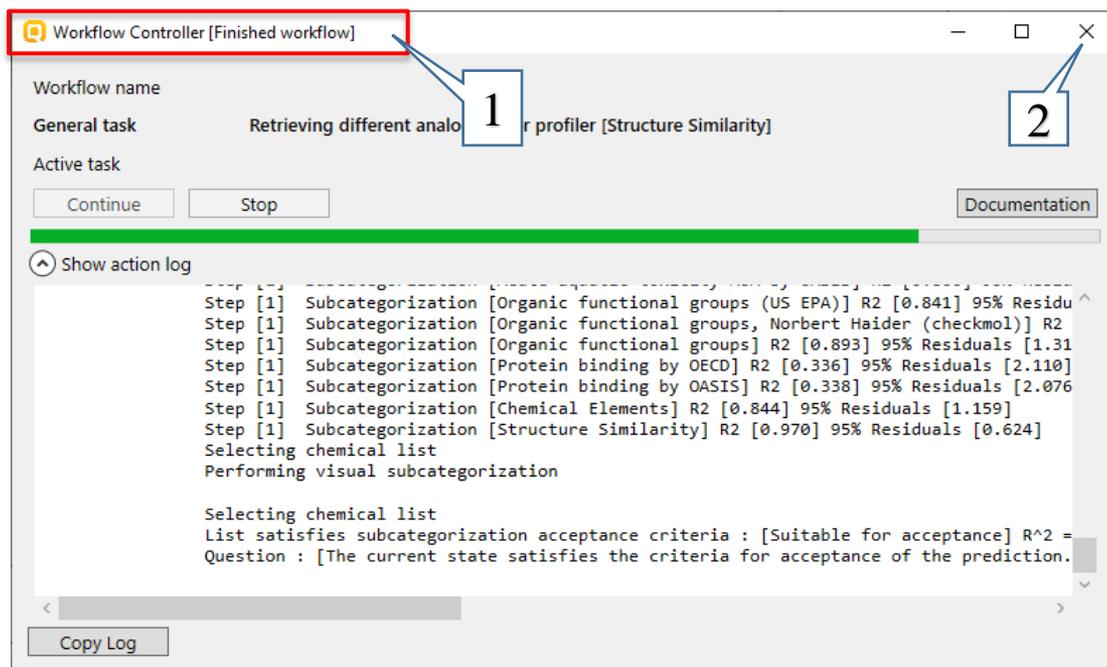


- Press Stop button (1) on the Workflow controller, once you are satisfied with the prediction
- A pop-up window (2) asks to confirm the exit of the workflow
- Press Yes button(3)

Data Gap Filling

Apply Standardized workflow

- A message is displayed that the workflow has finished (1)
- Press **X** button (2)



Data Gap Filling

Apply Standardized workflow

If the subcategorization window is closed by pressing **X** button (1) without performing any subcategorization, a dialog window is displayed (2):

- Press Yes if you want to exit the trend analysis and continue with read-across analysis.
- Press No if you want to finish the workflow.

The screenshot displays the 'Subcategorization 2' window with the following details:

- Title Bar:** Subcategorization 2 | Current state [Suitable for acceptance] R² = 0.793 95% residuals 1.372
- Options:** Select All, Unselect All, Invert, About, Options (1 Selected)
- Primary grouping:**
 - [Suitable for acceptance]US-EPA New Chemical Categories(54 analogues) | R² = [0.84], 95% Residuals = [1.23]
 - [Suitable for acceptance]Acute aquatic toxicity MOA by OASIS(52 analogues) | R² = [0.686], 95% Residuals = [1.853]
 - [Suitable for acceptance]Organic functional groups (US EPA)(22 analogues) | R² = [0.877], 95% Residuals = [0.833]
 - [Suitable for acceptance]Organic functional groups (Norbert Haider (checkmol))(22 analogues) | R² = [0.902], 95% Residuals = [0.828]
 - [Suitable for acceptance]Organic functional groups(12 analogues) | R² = [0.962], 95% Residuals = [0.608]
 - Aquatic toxicity classification by ECOSAR
- Secondary grouping:**
 - [Suitable for acceptance]Chemical Elements(36 analogues) | R² = [0.874], 95% Residuals = [0.799]
 - [Suitable for acceptance]Structure Similarity(12 analogues) | R² = [0.97], 95% Residuals = [0.624]
 - [Suitable for acceptance]Protein binding by OECD(63 analogues) | R² = [0.793], 95% Residuals = [1.37]
 - [Suitable for acceptance]Protein binding by OASIS(60 analogues) | R² = [0.793], 95% Residuals = [1.39]
- Unclassified:** (empty)

The right-hand panel shows a list of categories with a '1' pointing to the 'Aliphatic Amines' entry. Below it, a 'Differ from target by' section has radio buttons for 'At least one category' (selected) and 'All categories', with a '[STOP]' button. An 'Analogues' list shows: (54) Aliphatic Amines, (2) Hydrazines and Related Compounds, and (7) Not categorized.

A 'Continue?' dialog box is overlaid on the bottom, with a '2' pointing to it. The dialog text reads: 'You cancelled the current subcategorization, would you like to continue with a read-across?'. It features 'Yes' and 'No' buttons, which are highlighted with a red box.

Data Gap Filling

Apply Standardized workflow

The screenshot shows the QSAR Toolbox interface. The top navigation bar includes 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. Below this, there are 'Gap Filling' and 'Workflow' tabs. The 'Documents' list on the left shows a document with the following entries:

- [C: 1;Md: 0;P: 1] CAS: 111864
- [C: 820;Md: 178;P: 1] Aliphatic Amines (US-EPA New Chemical Categories)
- [C: 1032;Md: 210;P: 1] Aliphatic Amines (Aquatic toxicity classification by ECOSAR)
- [C: 84;Md: 184;P: 1] Enter GF (SW by trend analysis)
- [C: 75;Md: 171;P: 1] Subcategorized: Substance type
 - [C: 78;Md: 170;P: 1] Filter by WS - Exp Water Solubility
 - [C: 76;Md: 160;P: 0] Filter by WS - Water Solubility
 - [C: 75;Md: 155;P: 0] Filter by WS - Water Solubility (fragments)
 - [C: 76;Md: 160;P: 1] Filter by WS - Water Solubility
 - [C: 75;Md: 155;P: 0] Filter by WS - Water Solubility (fragments)
 - [C: 75;Md: 155;P: 1] Filter by WS - Water Solubility (fragments)
 - [C: 65;Md: 137;P: 1] Subcategorized: Aquatic toxicity classification by E
 - [C: 56;Md: 125;P: 1] Subcategorized: US-EPA New Chemical Catego
- [C: 307;Md: 73;P: 1] Amine, primary<AND>Aliphatic amine, primary (Organic functional group)
- [C: 143;Md: 55;P: 1] Aliphatic Carbon [-CH3]<AND>Aliphatic Carbon [-CH2-]<AND>Aliphatic
- [C: 298;Md: 73;P: 1] Amine<AND>Primary amine<AND>Primary aliphatic amine (Organic functional group)
- [C: 315;Md: 146;P: 1] Narcotic Amine (Acute aquatic toxicity MOA by OASIS)

The 'Filter endpoint tree' on the right shows a hierarchy of endpoints. The 'Aquatic Toxicity' section is expanded, showing 'Mortality' and 'EC50 <OR> LC50'. The 'Mortality' section is further expanded, showing 'Animalia (animals)', 'Chordata (chordata)', 'Actinopterygii...', and 'Pimphale...'. The 'Pimphale...' section is highlighted in yellow, and a callout box '1' points to it.

The data matrix on the right shows the results of the data gap filling. The matrix has 6 columns and 1 row. The first column is labeled '1 [target]'. The data matrix is as follows:

1 [target]	2	3	4	5	6
M: 5.15 mg/L M: 5.19 (4.73+5.64) T: 8.64 (0.547+1.14)	M: 25 (22.6+27.6)	M: 102 (97.9+106.1)			

A callout box '2' points to the document entry in the 'Documents' list.

- The result is displayed on the data matrix (1) marked with "T" ("M" stands for measured data)
- The workflow finishes on the document level of the primary grouping(2)

Data Gap Filling

Apply Standardized workflow

```

Document 1
├── [C: 1;Md: 0;P: 1] CAS: 111864
│   └── [C: 820;Md: 178;P: 1] Aliphatic Amines (US-EPA New Chemical Categories)
│       └── [C: 1032;Md: 210;P: 1] Aliphatic Amines (Aquatic toxicity classification by ECOSAR)
│           ├── [C: 84;Md: 184;P: 1] Enter GF (SW by trend analysis)
│           │   └── [C: 79;Md: 171;P: 1] Subcategorized: Substance type
│           │       ├── [C: 78;Md: 170;P: 1] Filter by WS - Exp Water Solubility
│           │       ├── [C: 76;Md: 160;P: 1] Filter by WS - Water Solubility
│           │       └── [C: 75;Md: 155;P: 1] Filter by WS - Water Solubility (fragments)
│           │           └── [C: 65;Md: 137;P: 1] Subcategorized: Aquatic toxicity classification by ECOSAR
│           └── [C: 307;Md: 73;P: 1] Amine<AND>Aliphatic amine, primary (Organic functional groups)
│               └── [C: 143;Md: 55;P: 1] Aliphatic Carbon [-CH3]<AND>Aliphatic Carbon [-CH2-]<AND>Aliphatic Carbon [CH]<AND>Amino, aliphatic attach [-NH2]
│                   └── [C: 298;Md: 73;P: 1] Amine<AND>Primary amine<AND>Primary aliphatic amine (Organic functional groups, Norbert Haider (checkmol))
│                       └── [C: 315;Md: 146;P: 1] Narcotic Amine (Acute aquatic toxicity MOA by OASIS)

```

All the steps executed in the SW are listed in the Document's panel.
The grey highlighted level(s) of documented tree indicates that a prediction is accepted at this level.

Outlook

- Background
- Keywords
- Objectives
- Specific Aims
- Standardized workflow for Ecotoxicity
- The exercise
- **Standardized workflow execution**
 - Input
 - Data Gap Filling
 - **Report**

Report Overview

- The report module can generate reports on predictions performed with the Toolbox.
- The report module contains a predefined report template which users can customized.
- Three types of report files are generated:
 - Prediction report – containing information for the target
 - Category report – containing information for the analogues in the category
 - Data matrix – containing information for the analogues used for the prediction

Report Generation report

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. The 'Report' module is selected, and the 'Prediction' option is highlighted in the 'Export' menu. A 'Customize report content and appearance' wizard window is open, showing various sections to be included in the report, such as Prediction, Category, and Data matrix. The wizard window has a 'Wizard pages' sidebar with options like 'Customize report', 'Prediction', 'Category', and 'Data matrix'. The main window shows a document tree on the left, a chemical structure in the center, and a data table on the right.

1. Go to **Report** module (1)

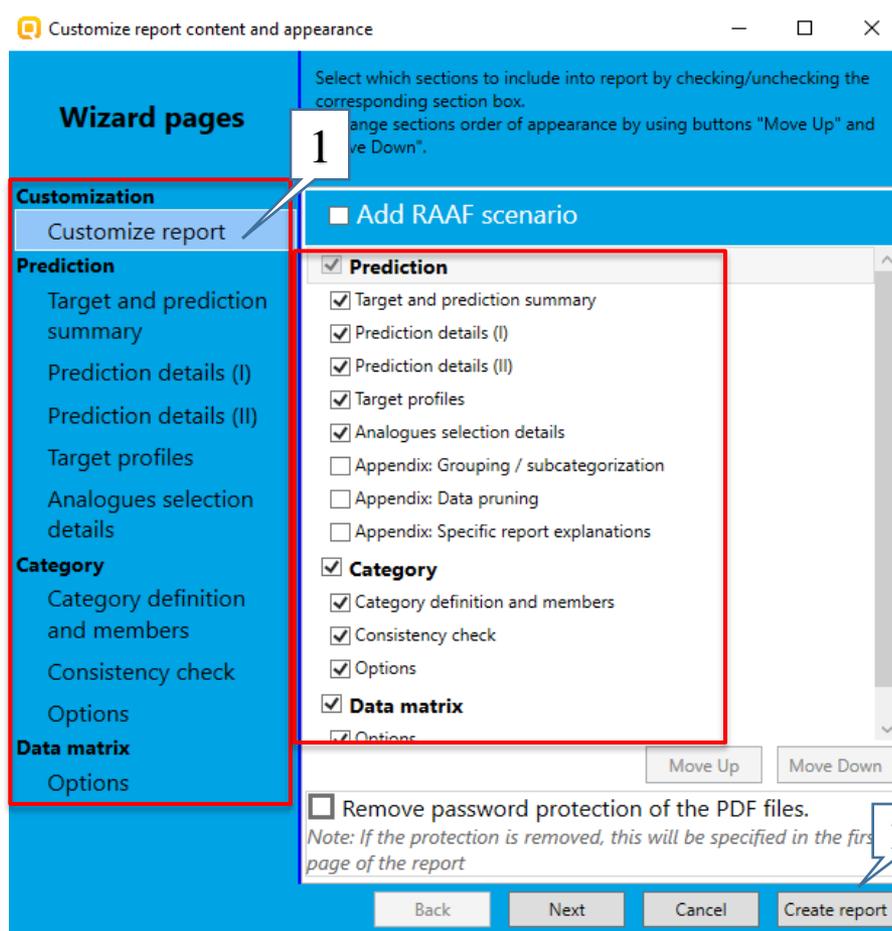
2. Select **Prediction** (2)

3. A Wizard pages window is displayed (3)

Report Generation report

The user can customize the report content (1) and appearance.

Generation of the reports happens by click on the **Create report** button (2).



Report Wizard pages

I. Customized report - the user is able to include or exclude the sections in the report;

II. Prediction report:

- **Target and prediction summary** – This section includes substance ID of the target chemical and the prediction outcome. Fields which are automatically populated by the system are indicated. Here the user could add information for the author, contact details and summary information;
- **Prediction details** and **Prediction details (II)** – section prediction details provides details about the prediction and its reliability. Prediction details (II) is optional it provides specific information about the prediction depending on the gap filling approach;
- **Target profiles** – this section summarize profiles used for the prediction. Additional profiles could be also included by the user;
- **Analogues selection details** – This section illustrates how analogues were selected. It displays selected databases, category boundaries and applicability domain.

Report Wizard pages

III. Category report:

- **Category definition and members** – This part includes sections related to list of category members, basic definition of the target endpoint and category hypothesis. Also information for calculated physico-chemical parameters for the category members are provided. Some of the sections are automatically populated while for the others a report items from Report basket could be added manually.
- **Consistency check** – This part includes sections related to the layers of the consistency check: physicochemical similarity; structural similarity, mechanistic similarity and additional endpoint data. Similarly to the previous section some of the sections are automatically populated and for the others items from the Report basket could be added.;
- **Options** – in this section number of the category members used for reporting could be changed;

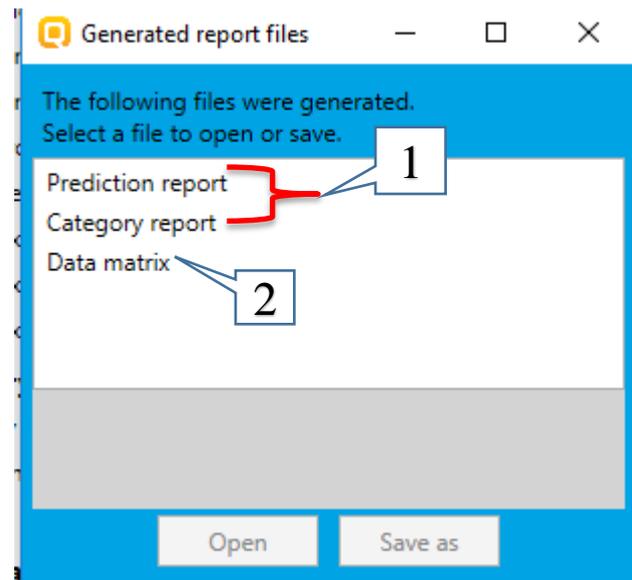
IV. Data matrix report

- Data matrix report gives the possibility to export information for the chemicals in the data matrix including parameters, profilers and experimental data.

Report Generation report

After clicking on the Create report button, *Generated report files* window appears. It contains two 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.



Report

Prediction report

Prediction of LC50 for octylamine

1 / 6

Prediction report

QSAR Toolbox prediction for single chemical

Date: 14 Apr 2020

Author(s):

Contact details:

Target information		
Structural information	Numerical identifiers	Chemical names
SMILES: CCCCCCCCN	CAS#: 111-86-4 Other: EC Number:2039160	1-aminooctane 1-Octanamine 1-octylamine
Structure 		

Prediction summary
Predicted endpoint: LC50; Mortality; Pimephales promelas; 96 h; No guideline specified
Predicted value: 8.64 (from 0.547 to 136)
Unit/scale: mg/L
Data gap filling method: Trend analysis
Summary: manually editable field
Not provided by the user

Using of a standardized workflow for predicting of ecotoxicological endpoint is noted in the *Prediction report*.

Report

Category report

Category report

QSAR Toolbox report for category

Information for the members of the category obtained as a result of SW application is included in the *Category report*.

1. Category definition

1.1. Category definition

Category name

manually editable field

Not provided by the user

Covered (target) endpoint(s)

- Ecotoxicological Information/Aquatic Toxicity: Pimephales promelas, Actinopterygii (ray-finned fishes, spiny rayed fishes), Chordata (chordates), Animalia (animals), EC50 <OR> LC50, Mortality, Duration=96 h

Category hypothesis

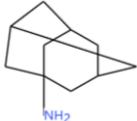
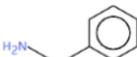
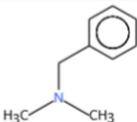
manually editable field

Not provided by the user

1.2. Category members

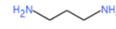
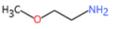
Information of category members

Table of category members

#	CAS	Name	SMILES	Structure
1	111-86-4	octylamine	CCCCCCCCN	
2	768-94-5	Amantadine	NC12CC3CC(C(C3)C1)C2	
3	100-46-9	Benzylamine	NCc1ccccc1	
4	109-76-2	1,3-Diaminopropane	NCCCN	
5	109-85-3	2-methoxyethanamine	COCCN	
6	103-83-3	Benzylidimethyl	CN(C)Cc1ccccc1	

Report data matrix report

Data matrix report

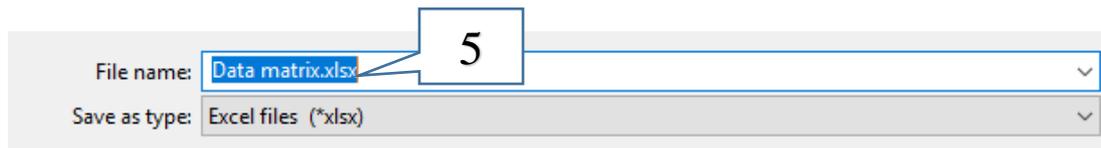
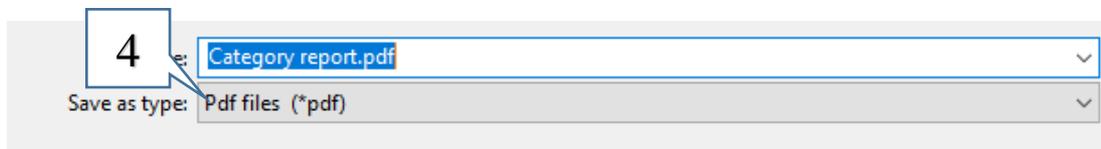
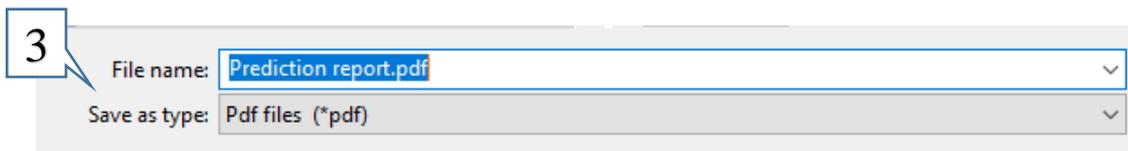
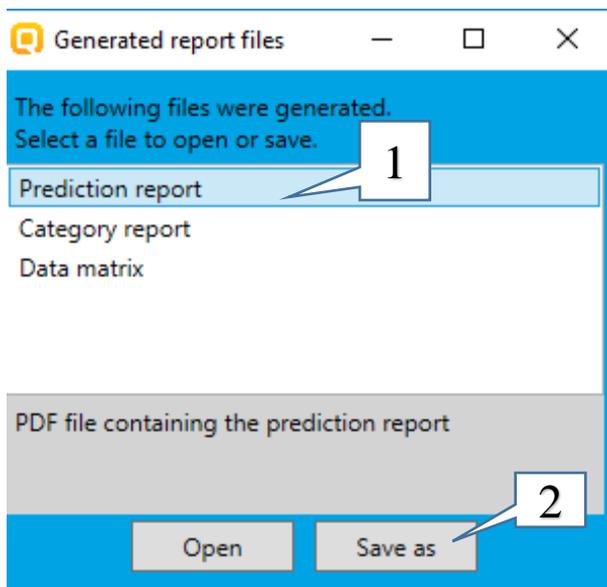
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	
Substance Identity		Target chemical	Analogue #1		Analogue #2		Analogue #3		Analogue #4		Analogue #5		Analogue #6										
Structure																							
CAS number		111-86-4	768-94-5	100-46-9	109-76-2	109-85-3	103-83-3	15673-00-4															
Chemical name		octylamine	Amantadine	Benzylamine	1,3-Diaminopropane	2-methoxyethanamine	Benzyl dimethylamine	3,3-Dimethylbutylamine															
Other Identifier																							
SMILES		CCCCCCCCN	NC12CC3CC(C(C3)C1)C2	NCc1ccccc1	NCCCN	COCCN	CN(C)Cc1ccccc1	CC(C)(C)CCN															
Parameters	unit																						
Profilers																							
<i>Profilers used for grouping/subcategorization</i>																							
Aliphatic Amines (Aquatic toxicity)	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	Aliphatic Amines Discrete chemical;	
Substance type (subcategorization)	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	Mono constituent (predefined); Organic	
Aquatic toxicity classification by ECOSAR	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	
US-EPA New Chemical Categories	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	Aliphatic Amines	
<i>General Mechanistic</i>																							
Protein binding by OASIS	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
Protein binding by OECD	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found	No alert found
<i>Endpoint Specific</i>																							
Acute aquatic toxicity MOA by OASIS	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine	Narcotic Amine
<i>Empiric</i>																							
Organic functional groups (US EPA)	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]	Fused Aliphatic ring unit; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [C]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Tertiary Carbon	Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Amino, aliphatic attach [-N<]	Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Amino, aliphatic attach [-N<]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Oxygen, aliphatic attach [-O-]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]	Aliphatic Carbon [-CH3]; Aliphatic Carbon [-CH2-]; Aliphatic Carbon [CH]; Aliphatic Carbon [CH]; Amino, aliphatic attach [-NH2]; Amino, aliphatic attach [-N<]; Aromatic Carbon [C]
Organic functional groups	Amine, primary; Aliphatic amine, primary	Amine, primary; Tricyclocane; Cycloalkane; Bridged-ring carbocycles; Aliphatic amine, primary	Amine, primary; Aryl; Aliphatic amine, primary	Amine, primary; Aliphatic amine, primary	Amine, primary; Ether; Aliphatic amine, primary	Amine, tertiary; Benzyl; Aryl; Aliphatic amine, tertiary	Alkane, branched with quaternary Amine, primary; tert-Butyl; Aliphatic amine, primary																
Structure similarity	[90% 100%]	[0% 10%]	[10% 20%]	[40% 50%]	[20% 30%]	[0% 10%]	[20% 30%]																
Chemical elements	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N; Group 16 - Oxygen O	Group 14 - Carbon C; Group 15 - Nitrogen N	Group 14 - Carbon C; Group 15 - Nitrogen N																

Analogue used for the target prediction can be seen the **Data matrix** report. Their selected profiling results, experimental data and/or parameters are also shown.

Report

Saving the report files

To save any of the reports, select the **report** (1) and then click on **Save as** (2); The prediction and category reports are saved as a **pdf file** (3, 4) while the **data matrix** is saved as an **.xlsx file** (5)



Congratulations!

- You have completed the tutorial on the standardized workflow for ecotoxicological endpoint.
- You have now been introduced to the consecutive steps of the standardized workflow of the (Q)SAR Toolbox and the rationale behind each step.
- Note, proficiency comes with practice!