QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.3.4

Step by step example how to predict acute aquatic toxicity to Daphnia for the 3-ethyl-5-methyl-3-methoxyphenol by the trend analysis approach

Outlook

- **Background**
- Objectives
- Specific Aims
- Trend analysis
- The exercise
- Workflow of the exercise
- Save the prediction result

Background

- This is a step-by-step presentation designed to take the user of the Toolbox through the workflow of a data filling exercise by the trend analysis approach.

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Objectives

- **This presentation reviews a number of functionalities of the Toolbox:**
  - Identify analogues for a target chemical
  - Retrieve experimental results available for those analogues
  - Fill data gaps by trend-analysis

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Specific Aims

- To review the workflow of the Toolbox.
- To review the six modules of the Toolbox.
- To reacquaint the user with the basic functionalities within each module.
- To explain to the user the rationale behind each step of the exercise.

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Trend Analysis
Overview

- For a given (eco)toxicological endpoint, the members of a category are often related by a trend (e.g. increasing, decreasing or constant). The trend could be related to molecular mass, carbon chain length, or to some other physicochemical property.
- A demonstration of consistent trends in the behaviour of a group of chemicals is one of the desirable attributes of a chemical category and one of the indicators that a common mechanism for all chemicals is involved. When some chemicals in a category have measured values and a consistent trend is observed, missing values can be estimated by simple scaling from the measured values to unmeasured values as a means of filling data gaps.

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Exercise

- In this exercise we will predict the acute toxicity to daphnids for an untested compound, (3-ethyl-5-methyl-4-methoxyphenol), which is the "target" chemical.
- This prediction will be accomplished by collecting a set of test data for chemicals considered to be in the same category as the target molecule.
- The category will be defined using the following categorization schemes:

  - Acute aquatic toxicity classification by ECOSAR – for structural grouping.

  - Acute aquatic toxicity MOA by OASIS – for mechanistic grouping.

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Workflow

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

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- **Workflow of the exercise**
  - **Chemical 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

User Alternatives for Chemical ID:

A. Single target chemical
- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing chemical structure
- Select from User List/Inventory/Databases
- Chemical IDs such as EC number, Einecs number

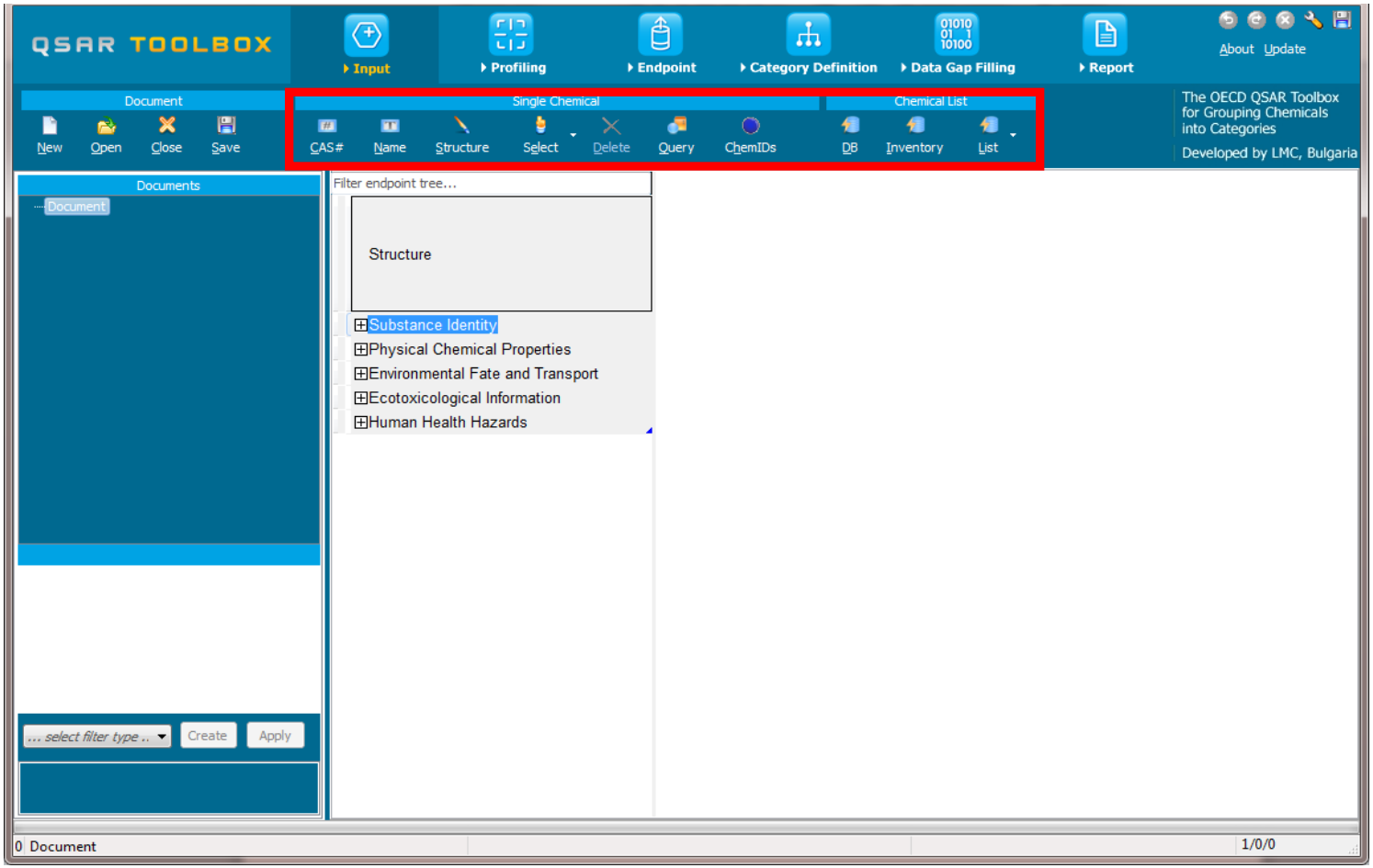
B. Group of chemicals
- User List/Inventory
- Specialized Databases

Getting Started

- Open the Toolbox.
- The six modules in the workflow are seen listed next to "QSAR TOOLBOX".
- Click on "Input" (see next screen shot).

Chemical Input Screen

Input screen

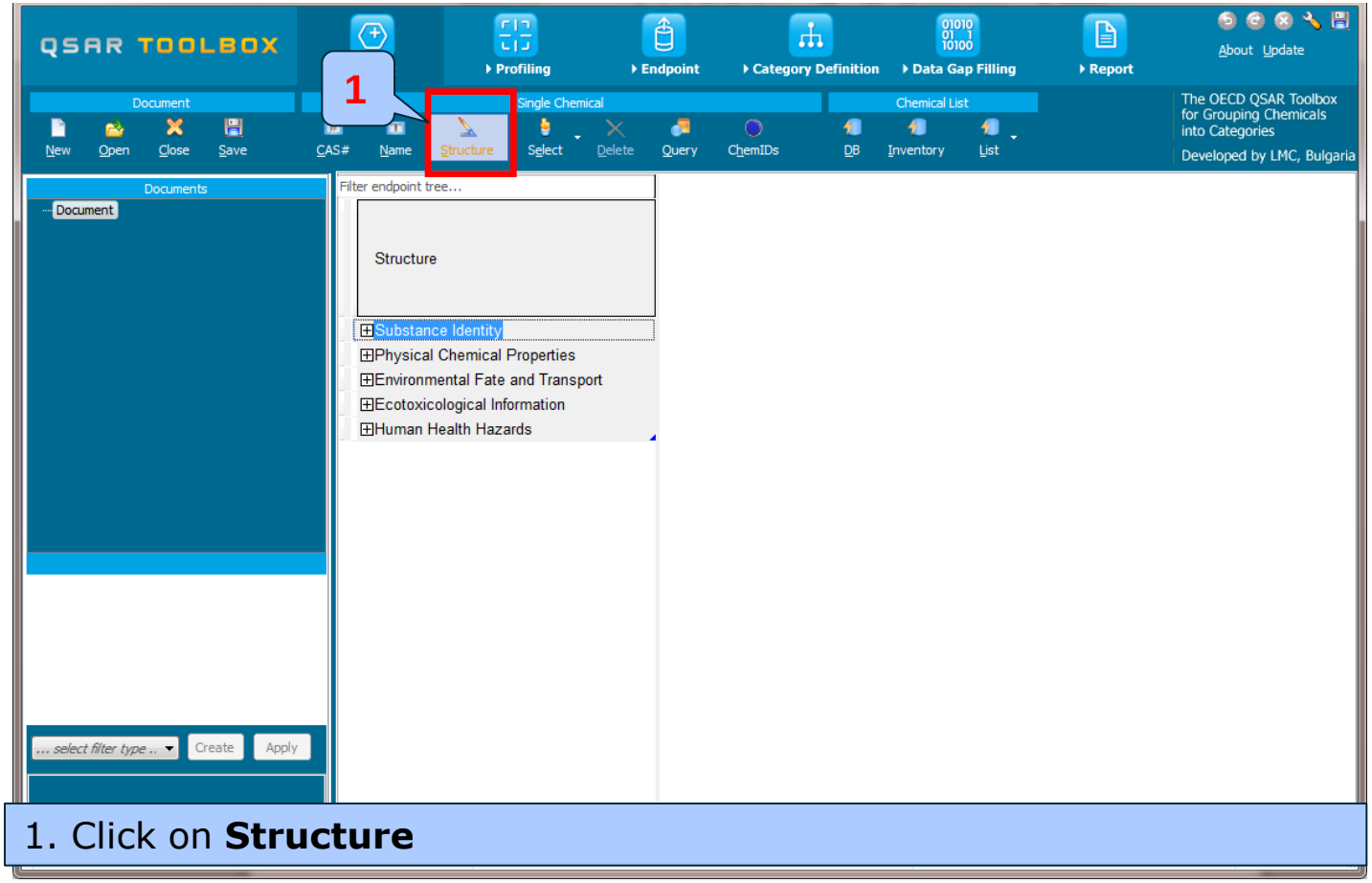


Chemical Input by Drawing

- Inputting the target chemical by drawing varies in difficulty with the structural complexity of the molecule.
- It is accomplished by a series of point-click-move-click operations within the 2D-editor which drops down when you click on "structure" (see next screen shot).
- The subsequent series of screen shots will take you through the process for the target chemical.

Chemical Input Screen

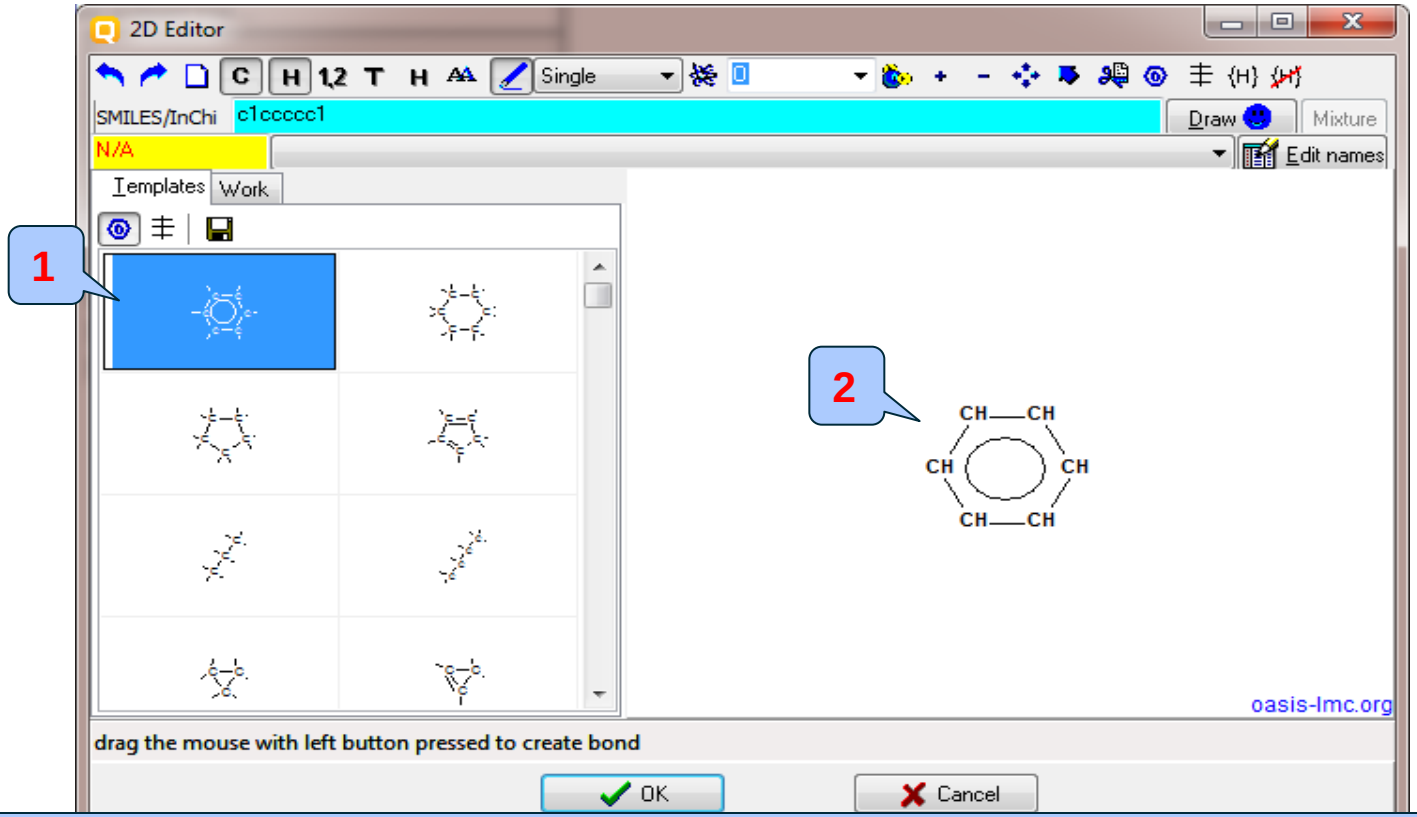
Input target chemical by drawing



1. Click on **Structure**

Chemical Input

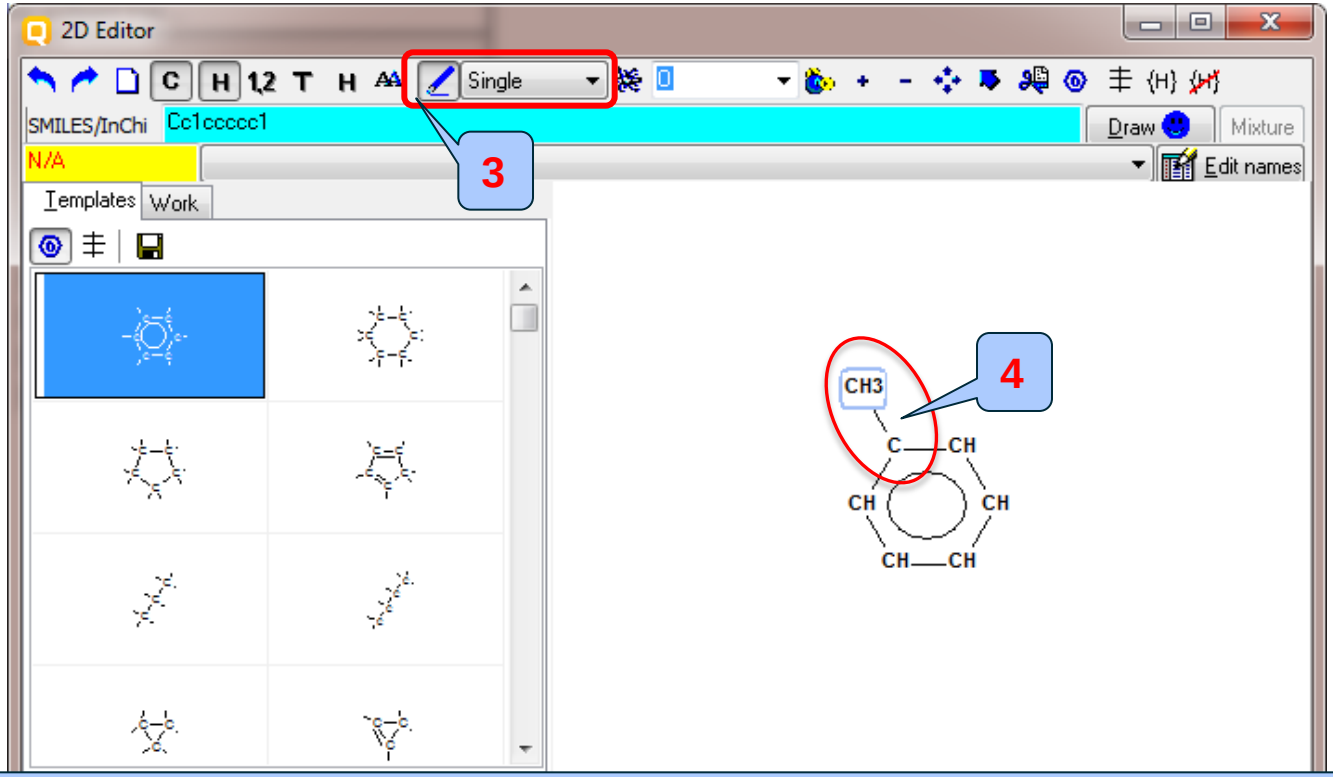
Drawing the target "3-ethyl-5-methyl-4-methoxyphenol" by 2-D editor



1. **Left Click** on the appropriate template form from "templates".
2. Move the curser to the large clear area and **left click** again, this puts the selected template on the plot.

Chemical Input

Drawing the target "3-ethyl-5-methyl-4-methoxyphenol" by 2-D editor



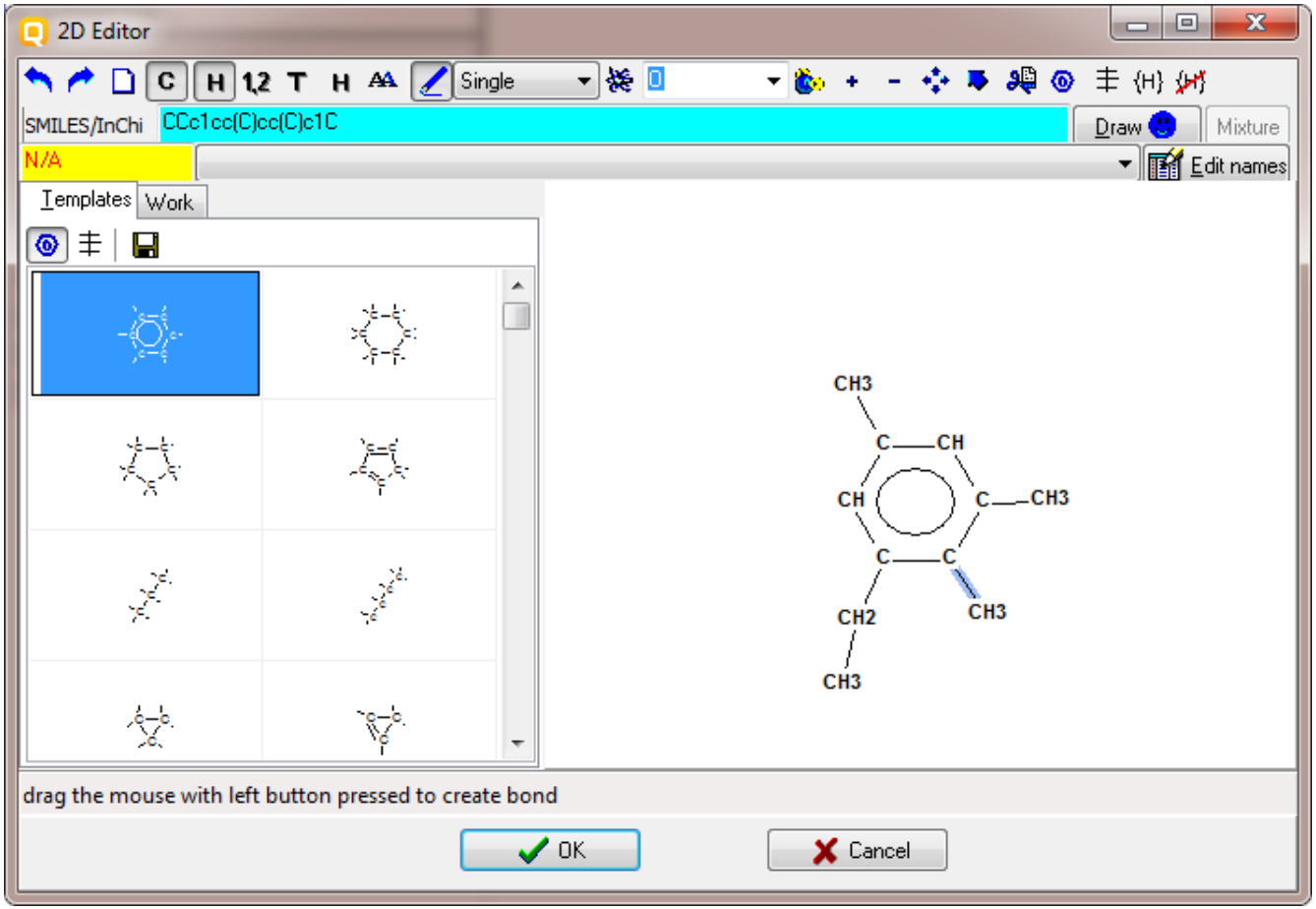
3. Click on button to add a bond of selected type ("Single" in this case).
4. Drag the mouse (pointing finger) to the appropriate atom and **left click** to create a single bond.

Chemical Input by Drawing

- Note the default is addition of a CH_3-group.
- By moving the 'finger' to other C-atoms and left clicking the mouse adds other hydrocarbon fragments.
- If you make an incorrect entry you can click on the 'undo' icon in the upper corner of the screen to remove the addition.
- This process allows you to build the hydrocarbon skeleton of the target molecule (see next screen shot).

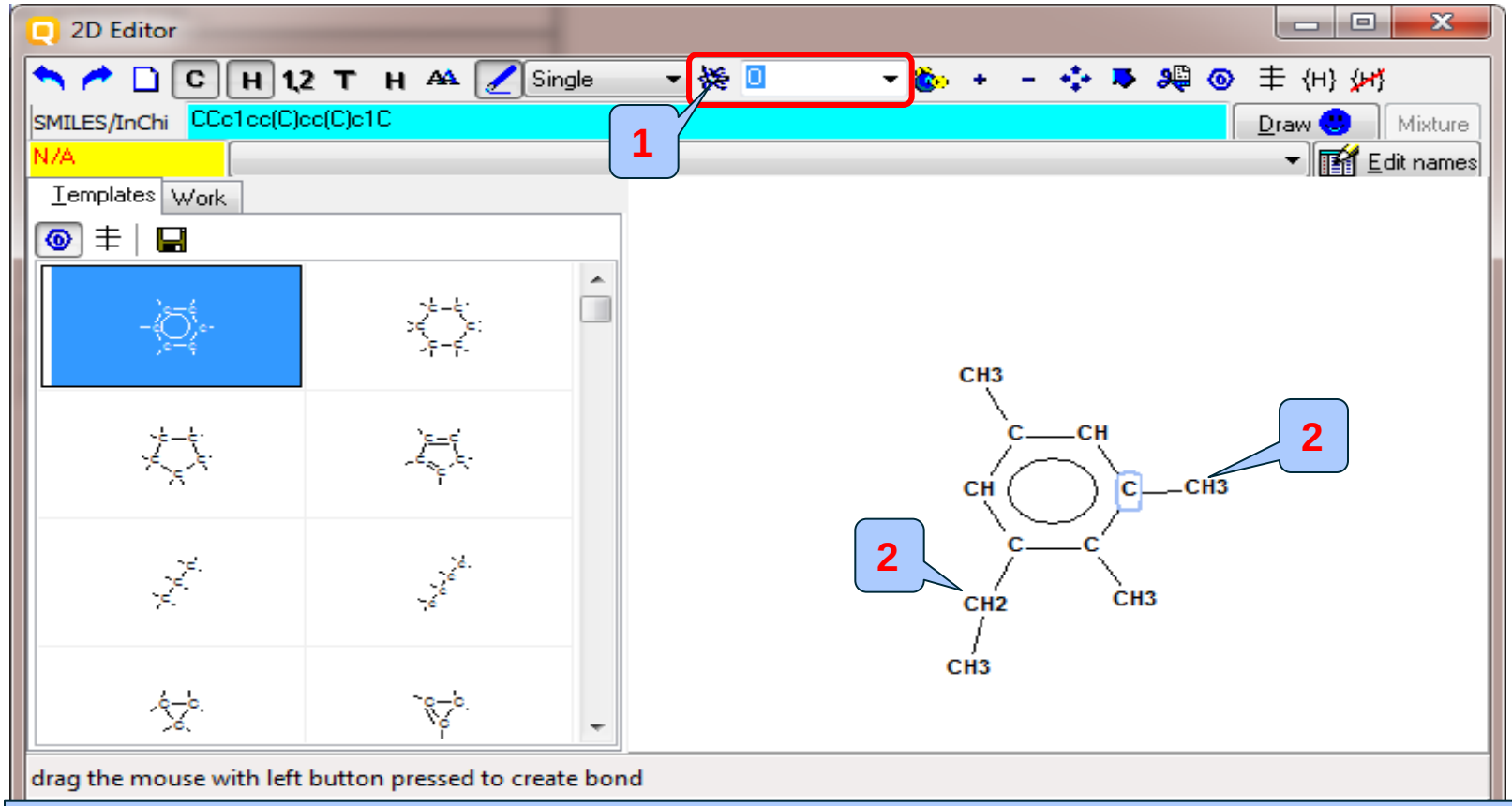
Chemical Input

Drawing the target "3-ethyl-5-methyl-4-methoxyphenol" by 2-D editor



Chemical Input

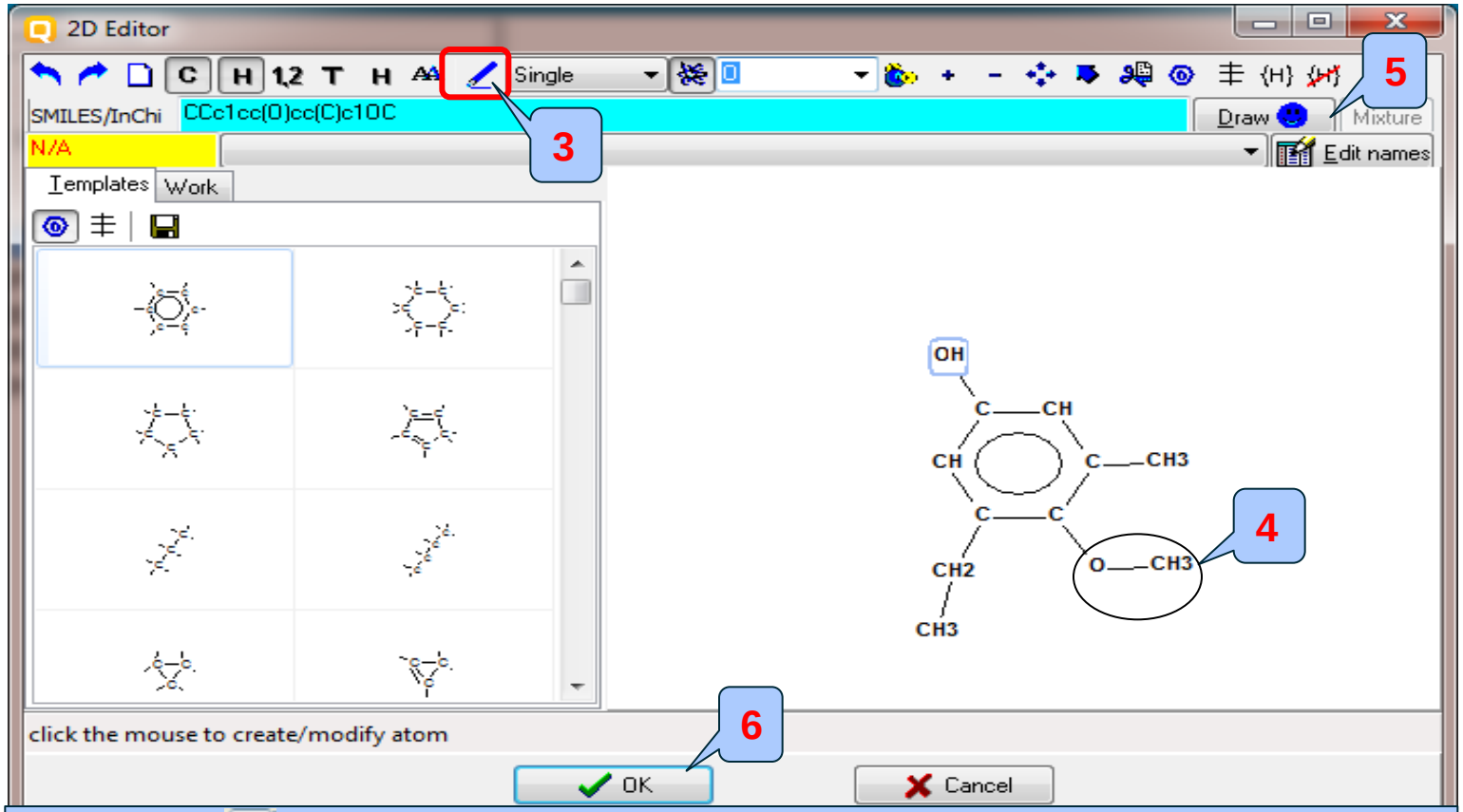
Drawing the target "3-ethyl-5-methyl-4-methoxyphenol" by 2-D editor



1. Click on button to add a hetero atom in this case an oxygen atom.
2. Left click with mouse over the methyl group to insert an oxygen atom.

Chemical Input

Drawing the target "3-ethyl-5-methyl-4-methoxyphenol" by 2-D editor

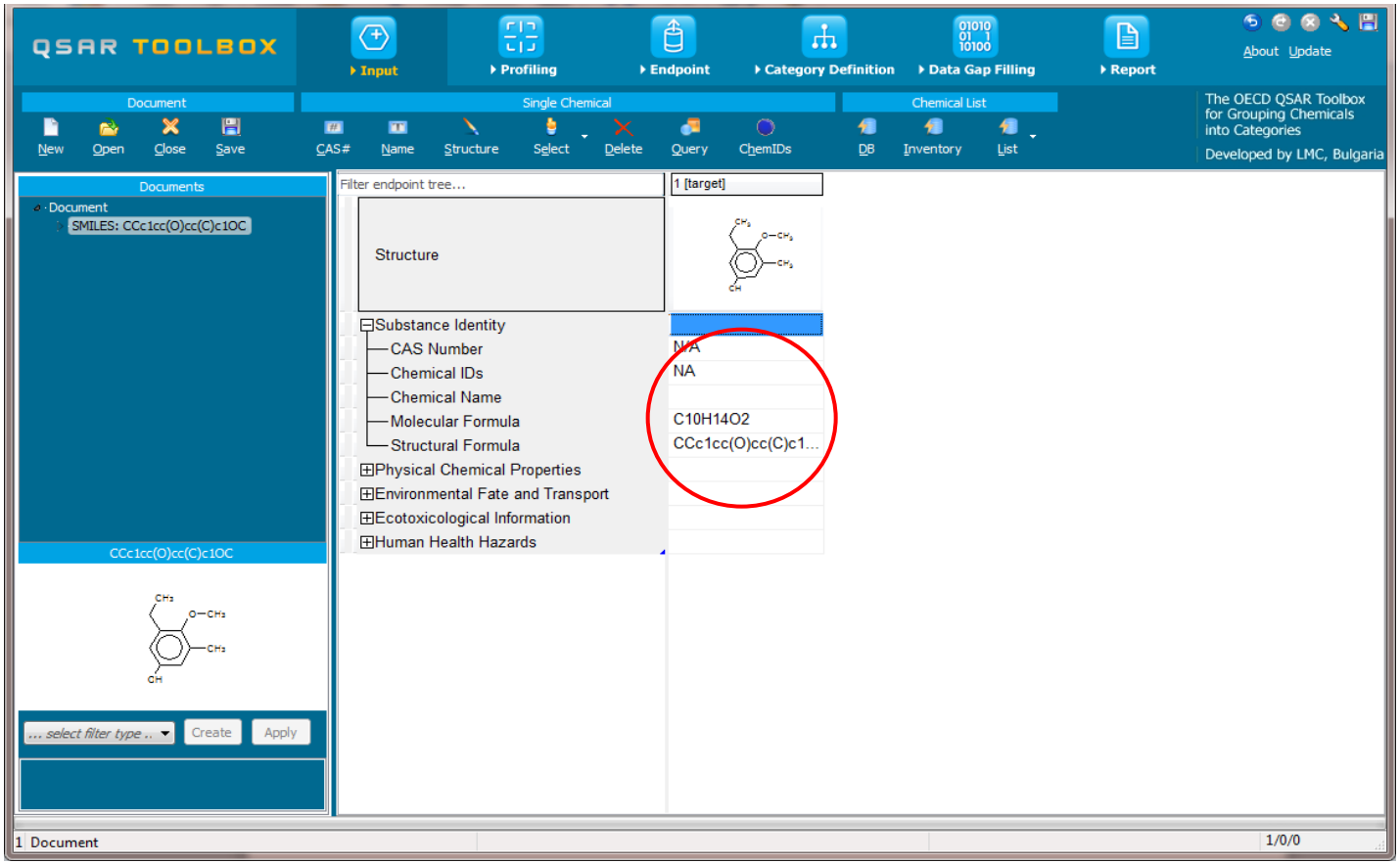


3. Click on button
4. Drag the mouse from the O-atom to create a single bond
5. Click **Draw**
6. Click **OK**

Chemical Input
Target chemical identity

- The already drawn target structure automatically appears on the data matrix
- Note that no CAS number or name is displayed for this chemical. This means the target chemical is not listed in the chemical inventories/databases implemented in the Toolbox (see next slide).

Chemical Input
Target chemical identity



The workflow on the first module is now complete, and the user can proceed to the next module. Click on "Profiling".

Outlook

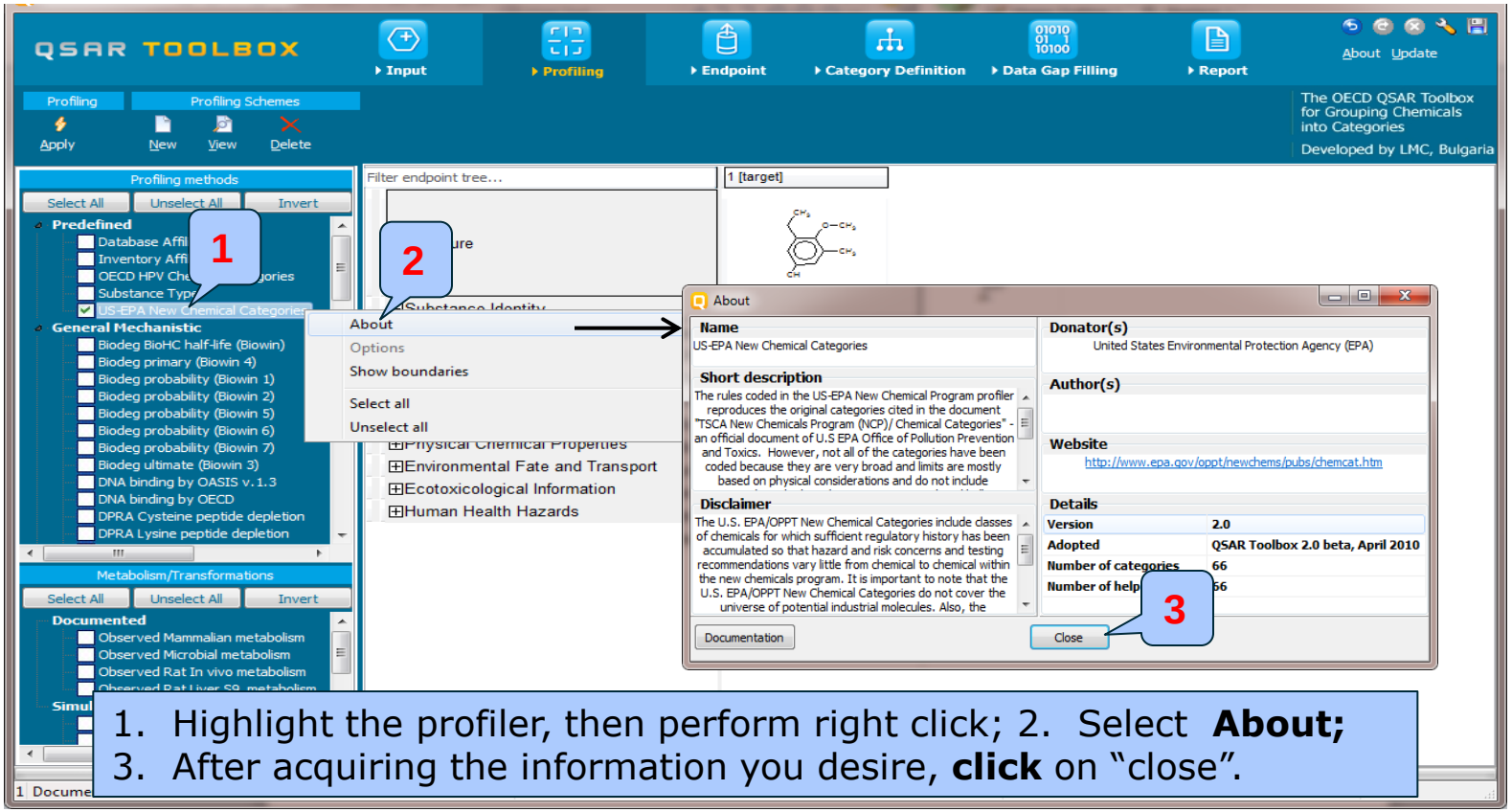
- Background
- Objectives
- Specific Aims
- Trend analysis
- The exercise
- **Workflow of the exercise**
  - Chemical Input
  - **Profiling**

Profiling
Overview

- "Profiling" refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database.
- Available information includes likely mechanism(s) of action, as well as observed or simulated metabolites.

Profiling

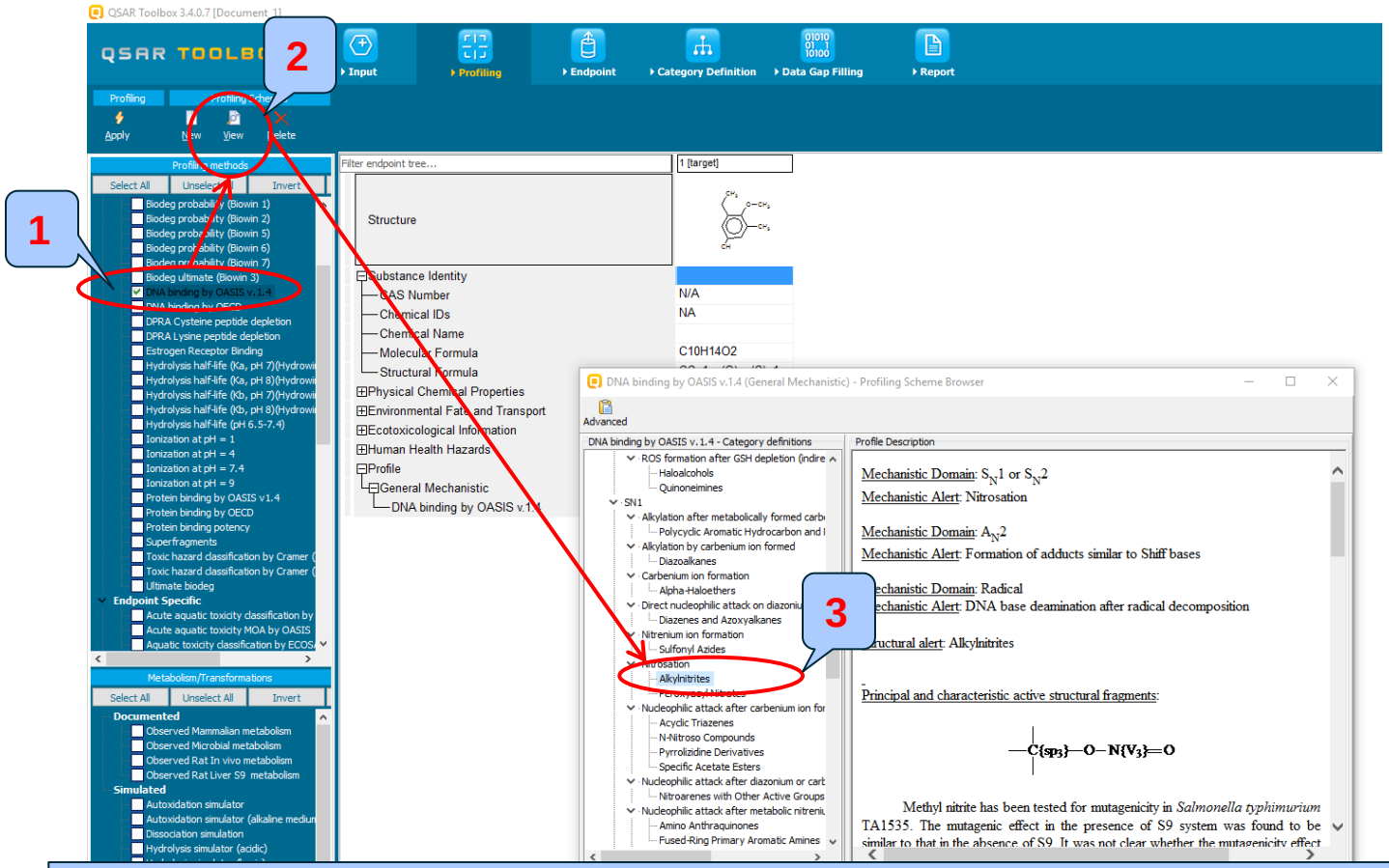
Summary information of the different profilers are provided in the "About".



Profiling

- For most of the profilers, background information can be retrieved by highlighting one of the profilers (for example, DNA binding by OASIS v1.4) and clicking on "View" (see next screenshot).

Profiling



1. Highlight the profiler
2. Click **View**
3. Click on one of the Structural alerts (for example Alkylnitriles)

Profiling
Side-Bar to Profiling

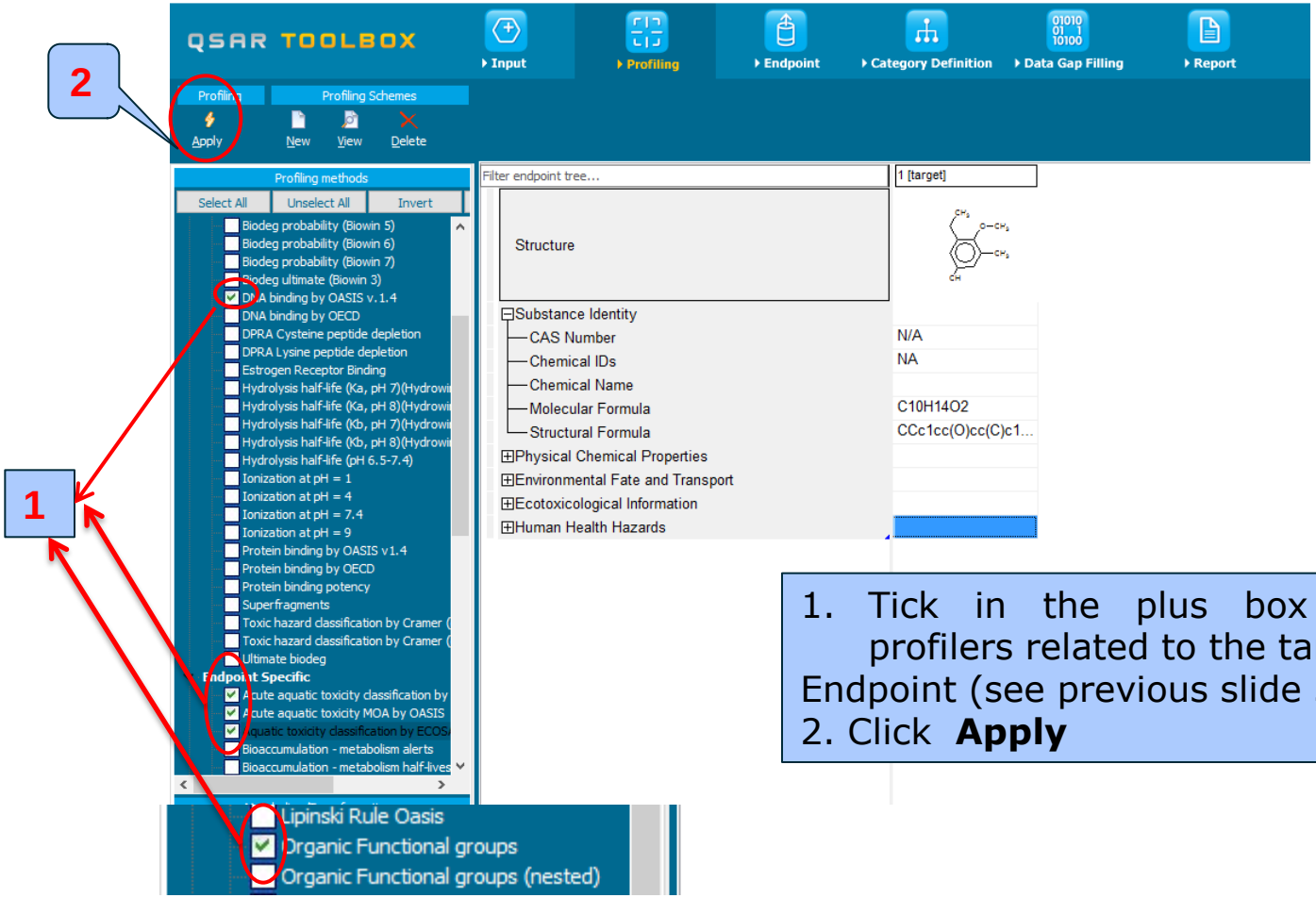
- The outcome of the profiling determines the most appropriate way to search for analogues (detailed information in Manual for getting started– Toolbox 2.0 (Chapter 4). http://www.oecd.org/dataoecd/58/56/46210452.pdf
- Table 4-1 in chapter 4 (Manual for getting started – Toolbox 2.0) lists a selection of profilers and their relevance for different endpoints of regulatory relevance.
- For this example, the following mechanistic profiling methods are relevant to the aquatic toxicity:
  - ECOSAR – for structural grouping
  - Acute aquatic toxicity MOA by OASIS – mechanistic grouping
  - Protein binding by OASIS v.1.4– mechanistic grouping
  - Acute aquatic toxicity classification by Verhaar (Modified) – grouping by reactivity
  - Organic functional groups – empiric knowledge

Profiling
Profiling the target chemical

- Select the "Profiling methods" related to the target endpoint by ticking the box next to the profilers name.
- This selects (a green check mark appears) or deselects(the green check disappears) profilers.
- For this example, select the following profilers which are relevant to the aquatic toxicity (see next screen shot):
  - ECOSAR – for structural grouping
  - Acute aquatic toxicity MOA by OASIS – mechanistic grouping
  - Protein binding by OASIS v.1.4 – mechanistic grouping
  - Acute aquatic toxicity classification by Verhaar(Modified) – grouping by reactivity
  - Organic functional groups – empiric knowledge

Profiling

Profiling the target chemical



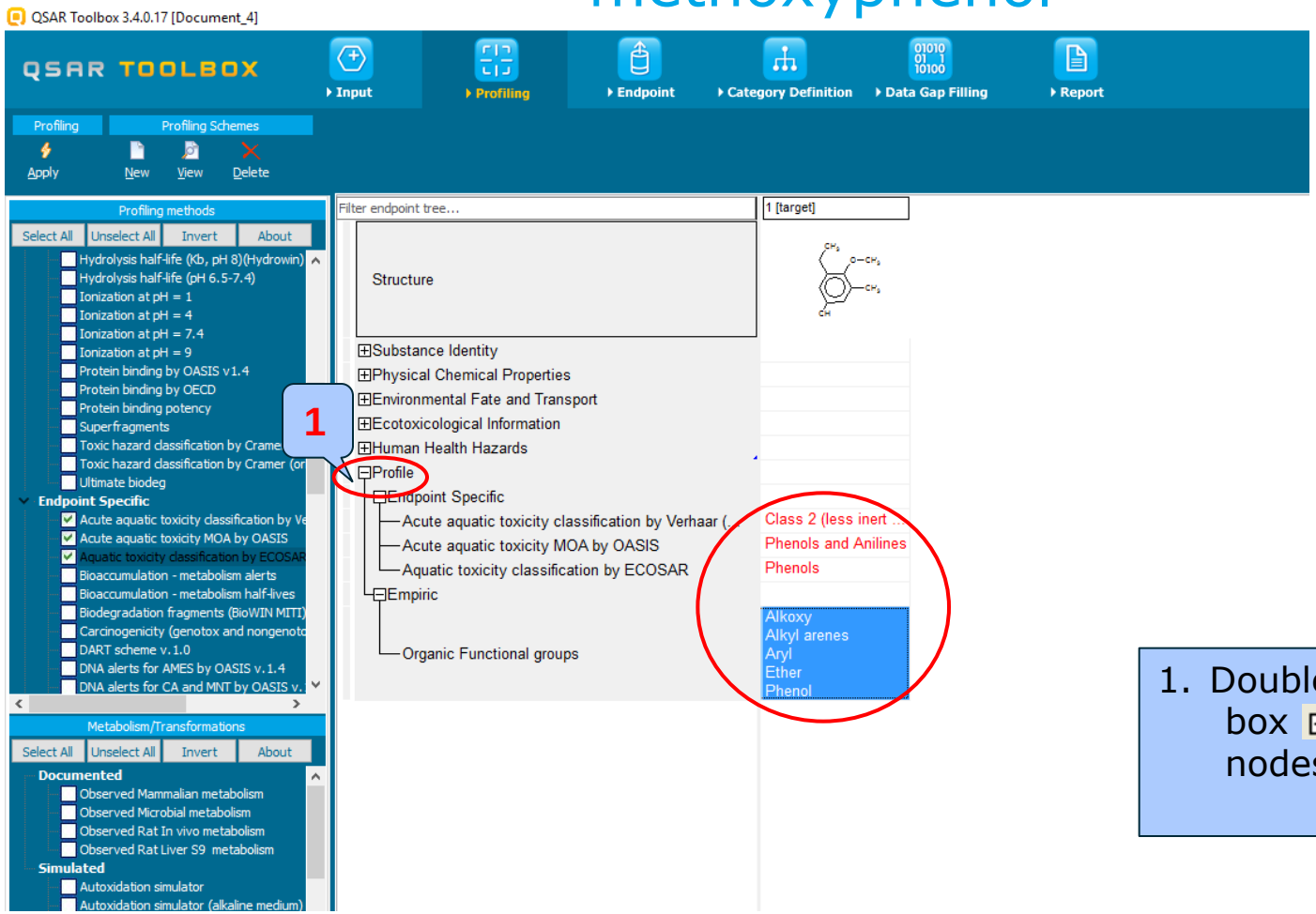
1. Tick in the plus box next to the profilers related to the target Endpoint (see previous slide 35);
2. Click **Apply**

Profiling
Profiling the target chemical

- The actual profiling will take several seconds depending on the number and type of selected profilers.
- The results of profiling automatically appear as a dropdown box under the target chemical.
- Please note the specific profiling results by Classification by ECOSAR and MOA by OASIS (see next slide).
- These results will be used to search for suitable analogues in the next steps of the exercise.

Profiling

Profiles of the target "3-ethyl-5-methyl-4-methoxyphenol



1. Double click on the box ⊞ to open the nodes of the tree

Outlook

- Background
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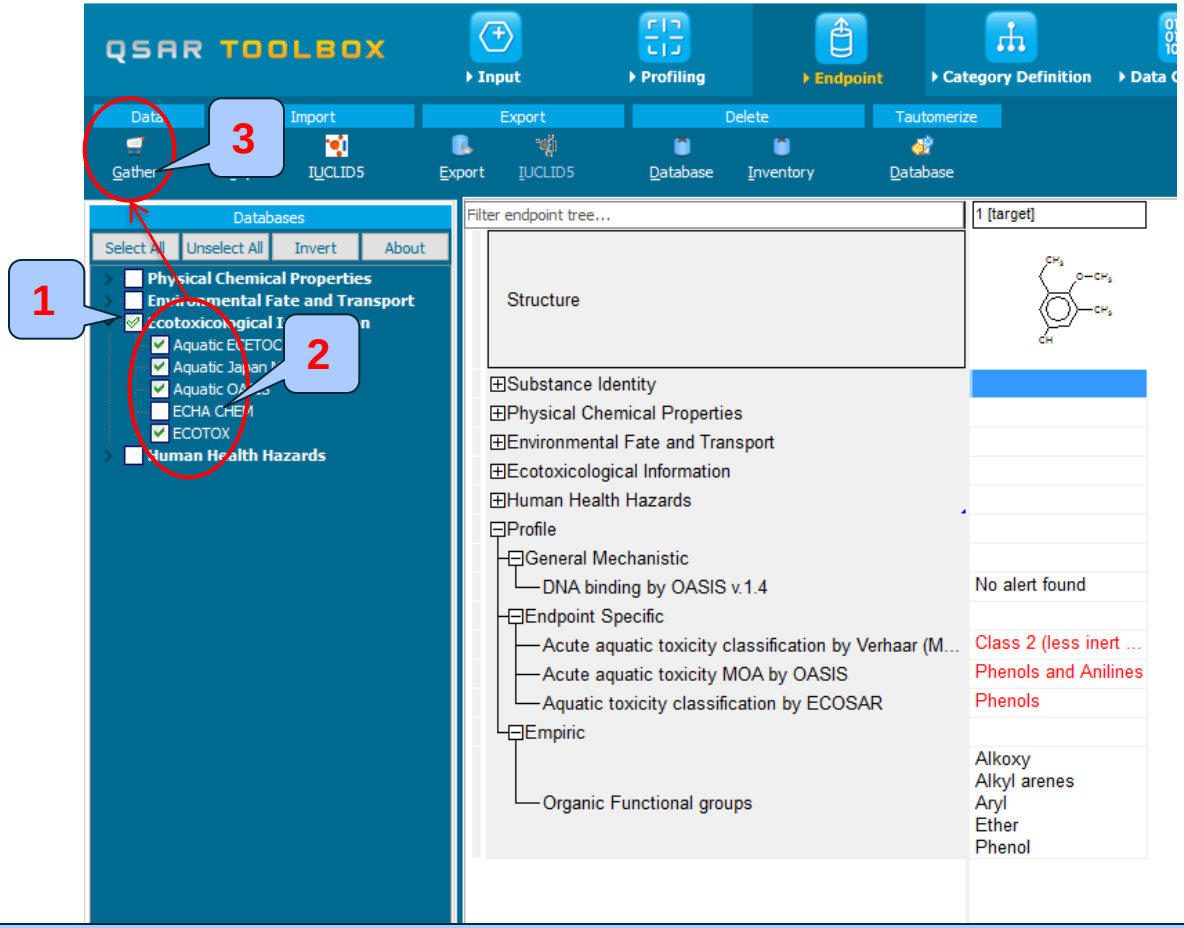
- **Workflow of the exercise**
  - Chemical Input
  - Profiling
  - **Endpoint**

Endpoint

- "Endpoint" refer to the electronic process of retrieving the environmental fate, ecotoxicity and toxicity data that are stored in the Toolbox database.
- Data gathering can be executed in a global fashion (i.e., collecting all data of all endpoints) or on a more narrowly defined basis (e.g., collecting data for a single or limited number of endpoints).
- In this example, we limit our data gathering to the common aquatic toxicity endpoints from databases containing aquatic toxicity data (Aquatic ECETOC, Aquatic Japan MoE, ECOTOX, and Aquatic OASIS).

Endpoint

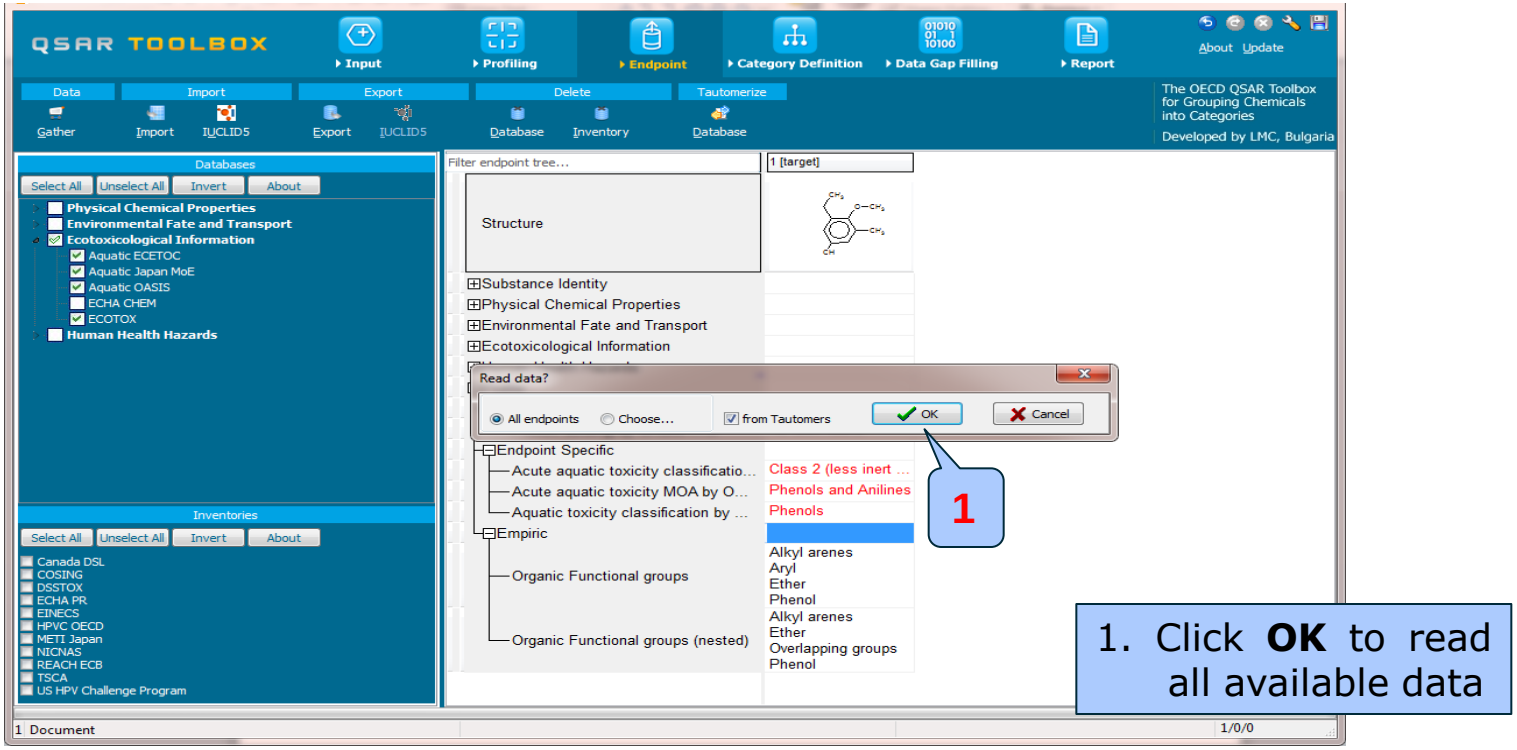


1. Expand the **Ecotoxicological Information** section;
2. Select databases related to the target endpoint by adding a green check in the box before the database name; 3. Click **Gather**

Endpoint
Process of collecting data

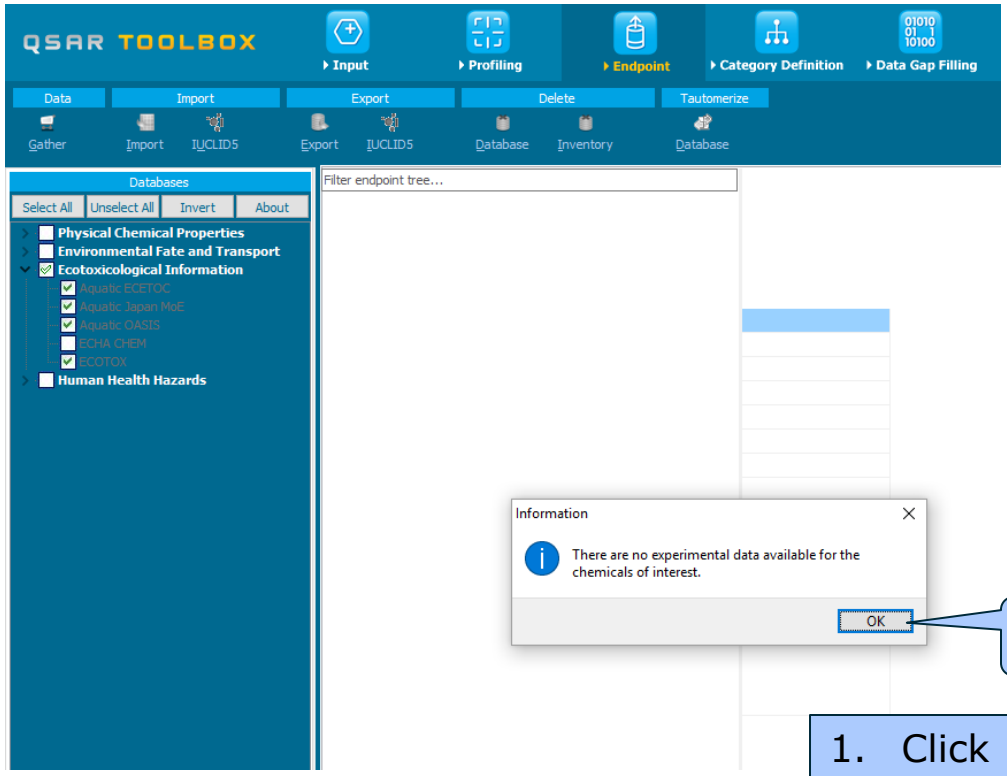
Toxicity information on the target chemical is electronically collected from the selected datasets.

A window with “Read data?” appears. Now the user could choose to collect “all” or “endpoint specific” data.



Endpoint
Process of collecting data

In this example, an insert window appears stating that no experimental data is available for the chemical of interest



1. Click **OK** to close the window

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  - Chemical Input
  - Profiling
  - Endpoint
  - **Category definition**

Recap

- You have entered the target chemical being sure of the correct structure.
- You have profiled the target chemical and found no experimental data is currently available for this structure.
- In other words, you have identified a data gap, which you would like to fill.
- Now you are ready to continue with next step of the workflow “Category Definition”.

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 defining the category definition.

Category Definition
Grouping methods

- The different grouping methods allow the user to group chemicals into chemical categories according to different measures of "similarity" so that within a category data gaps can be filled by trend-analysis.
- Detailed information about grouping chemical (Chapter 4) could be downloaded from:

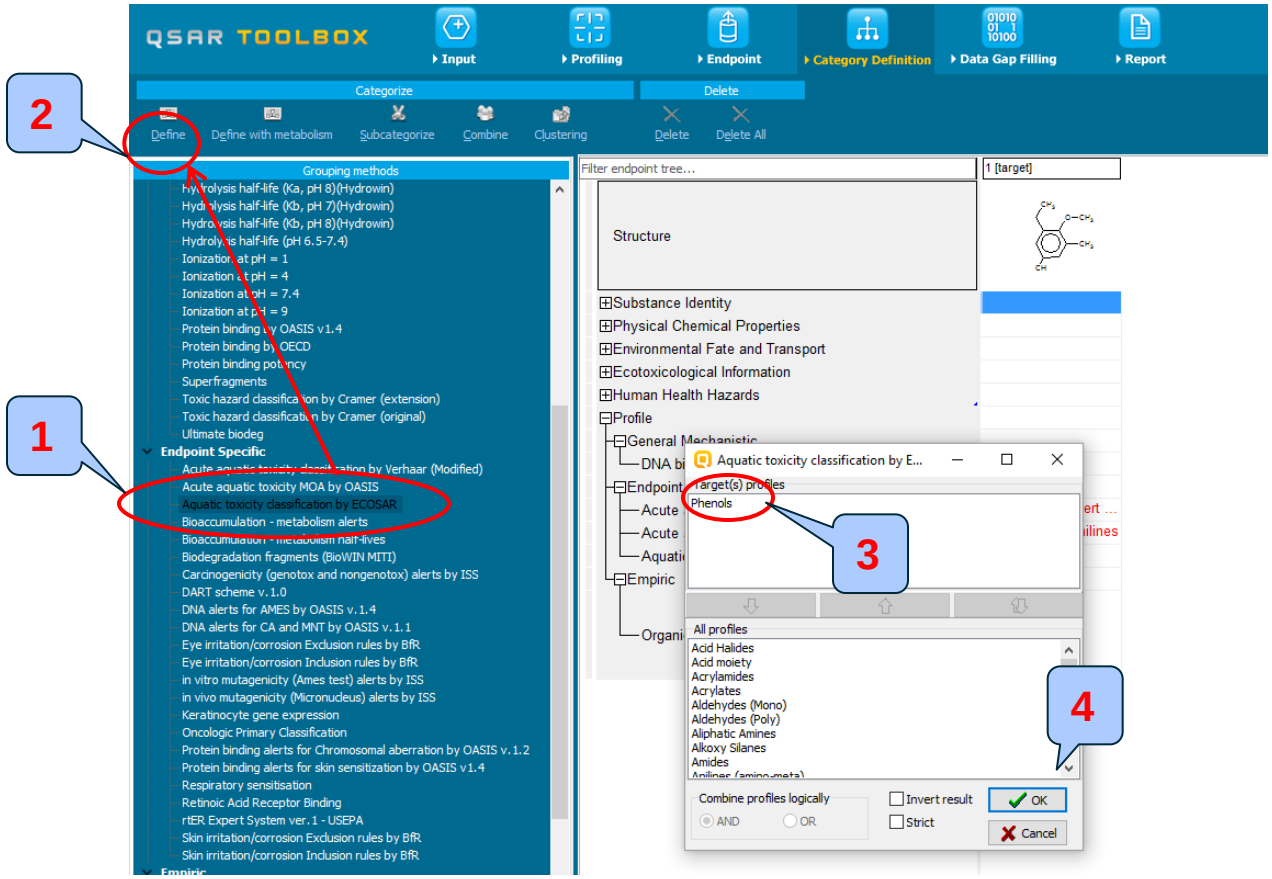
  http://www.oecd.org/dataoecd/58/56/46210452.pdf
- For this example, starting from the target chemical a specific EcoSAR classification is identified, subsequently analogues are found within the same specific classification for which experimental results are available.

Category Definition
ECOSAR categories

- ECOSAR has been used by the U.S. Environmental Protection Agency since 1981 to predict the aquatic toxicity of new industrial chemicals in the absence of test data.
- "Aquatic toxicity classification by ECOSAR" in the Toolbox is used for grouping of chemicals by structural similarity which may or may not have mechanistic meaning. Experience has shown ECOSAR to be a robust profiler which makes it a logical choice in an initial profiling scheme.

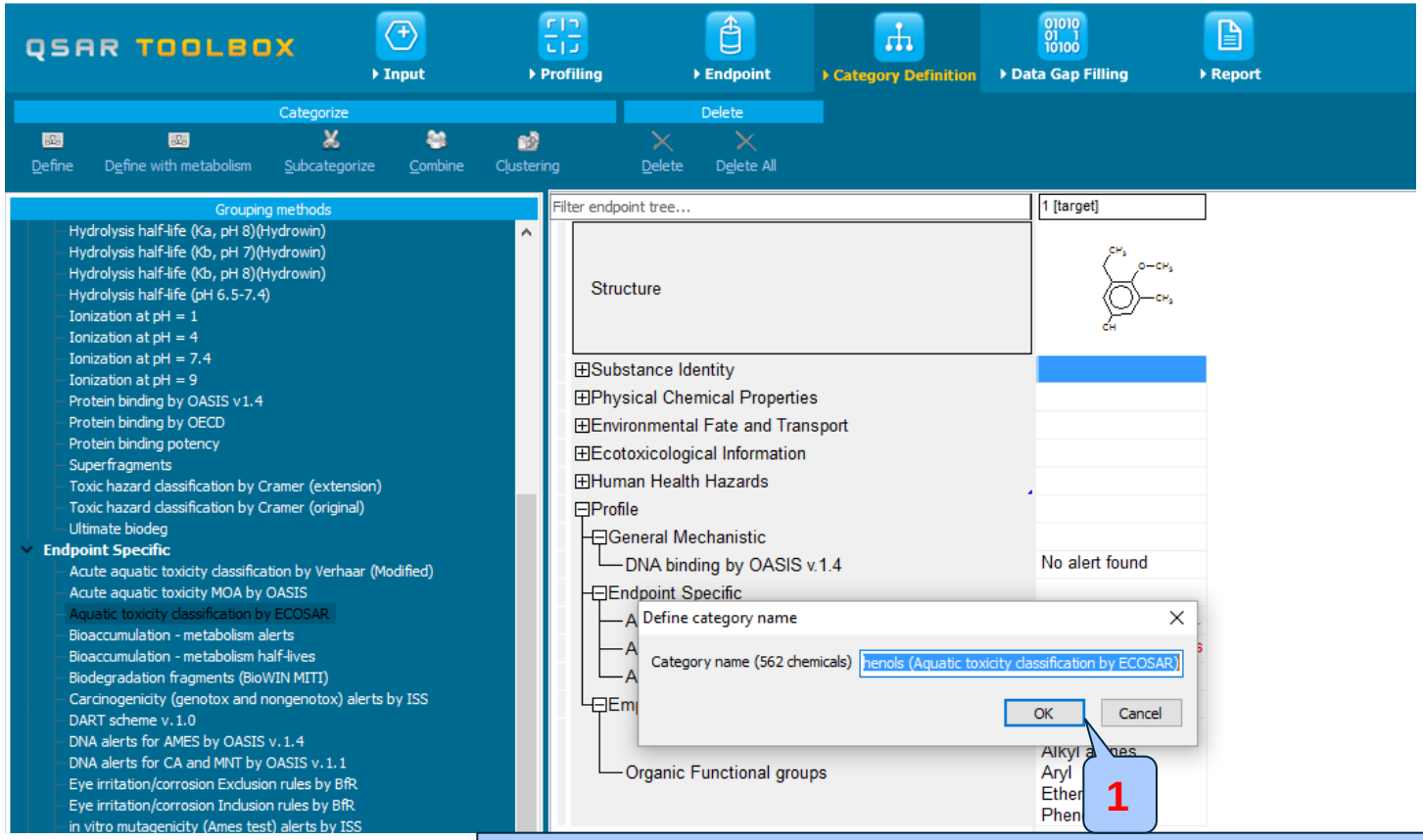
Category Definition

Defining ECOSAR category



1. Highlight **"Aquatic toxicity classification by ECOSAR";**
2. Click **Define;** 3. Confirm the category **Phenols** and 4. Click **OK**

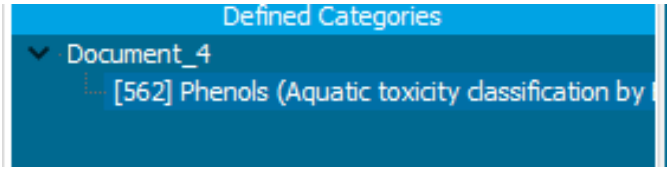
Category Definition
Defining ECOSAR category



1. Click **OK** to confirm the name of the category and to gather experimental data

Category Definition
Analogues

- The Toolbox now identifies all chemicals corresponding to the ECOSAR classification of “phenols” which are listed in the databases selected under “Endpoint”.
- 562 analogues are identified. Along with the target they form a category (Phenols) which can be used for data gap filling.
- The name of the category appears in the “Defined Categories” window, along with the number of substances belonging to the category.



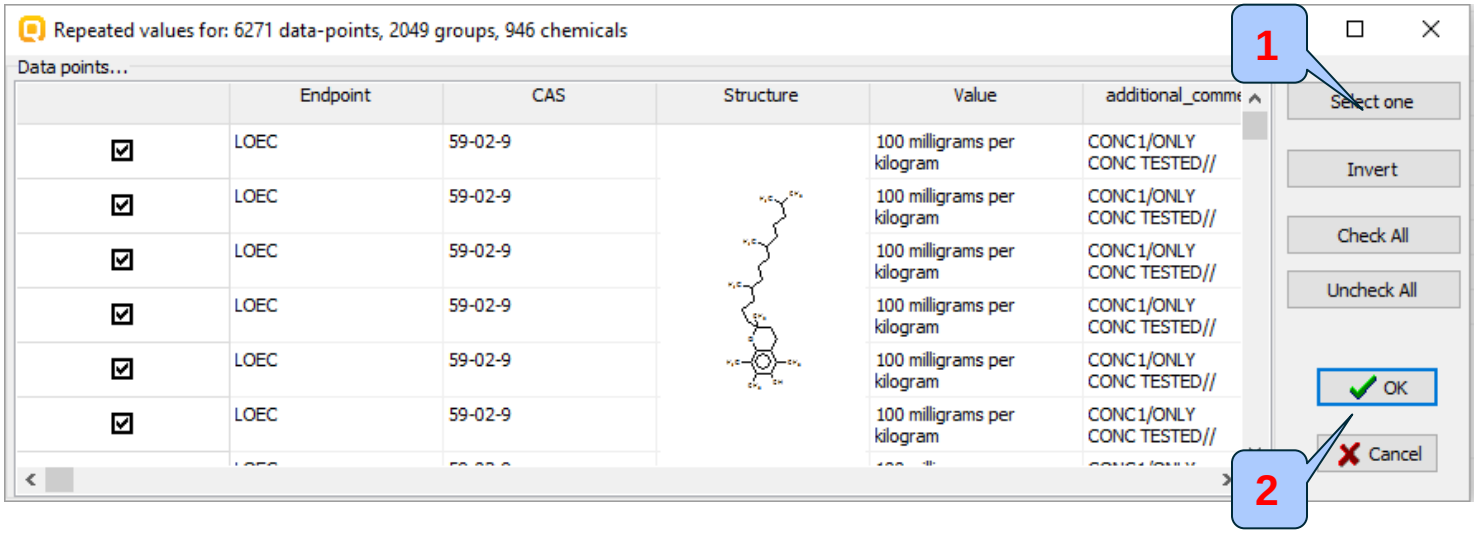
Category Definition
Read data for Analogues

- The Toolbox automatically request the user to select the endpoint that should be retrieved.
- The user can either select the specific endpoint or by default choose to retrieve data on all endpoints (see below).

- In this example, since only databases that contain information for ecotoxicological endpoints are selected, both options give the same results.
- As the Toolbox must search the database, this may take some time.

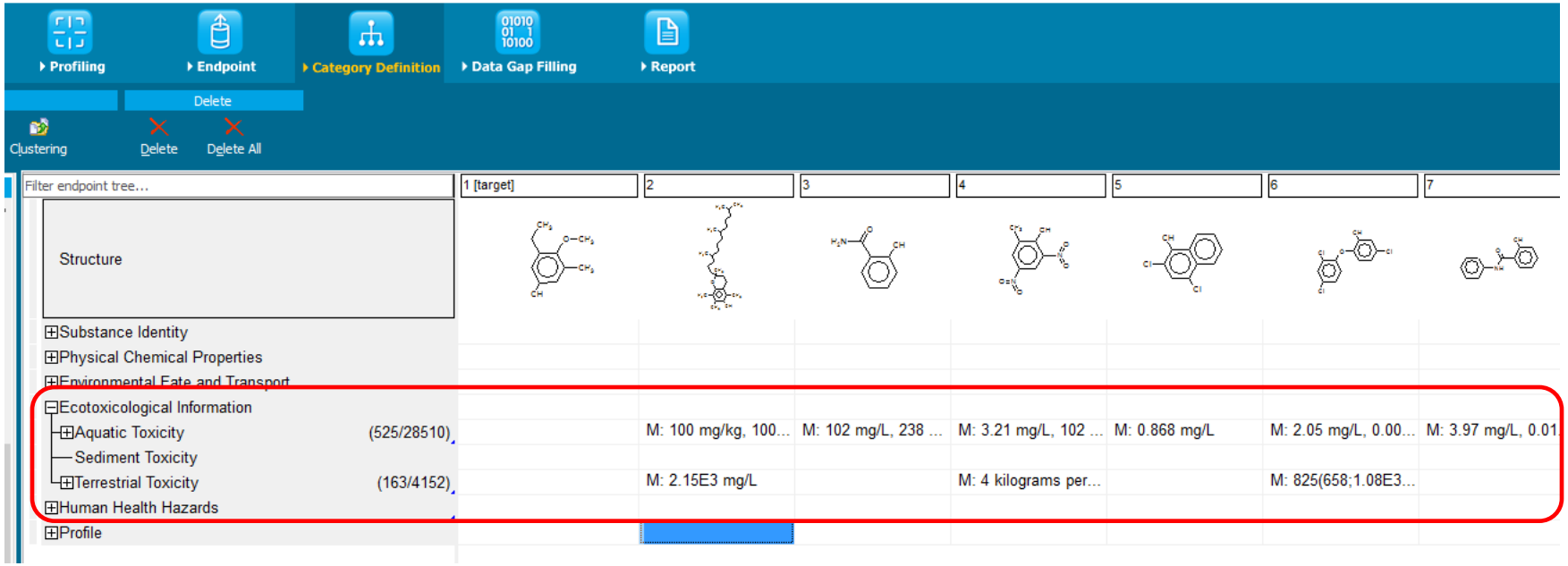
Category Definition
Read data for Analogues

Due to overlap between the Toolbox databases for intersecting chemicals the same data may be found simultaneously. Data redundancies are identified and the user has the opportunity to select either a single data value or all data values.

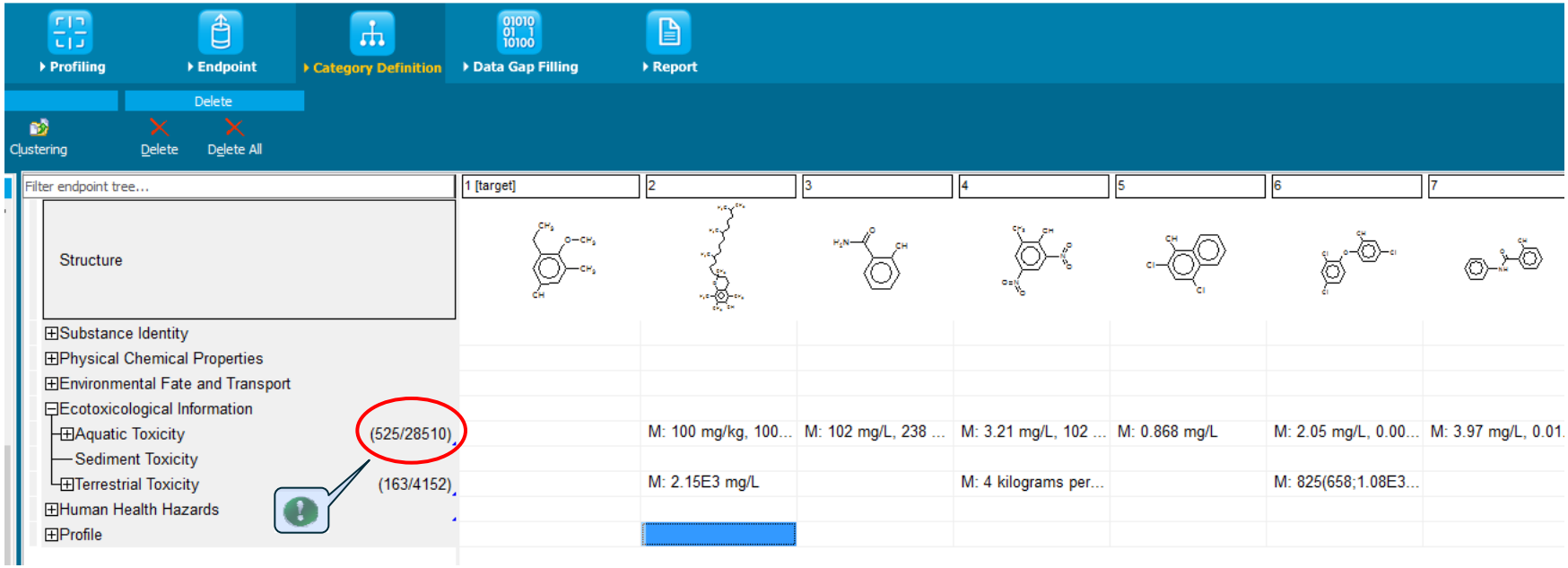


1. Click **Select one** and then
2. Click **OK**

Category Definition
Summary of Analogues

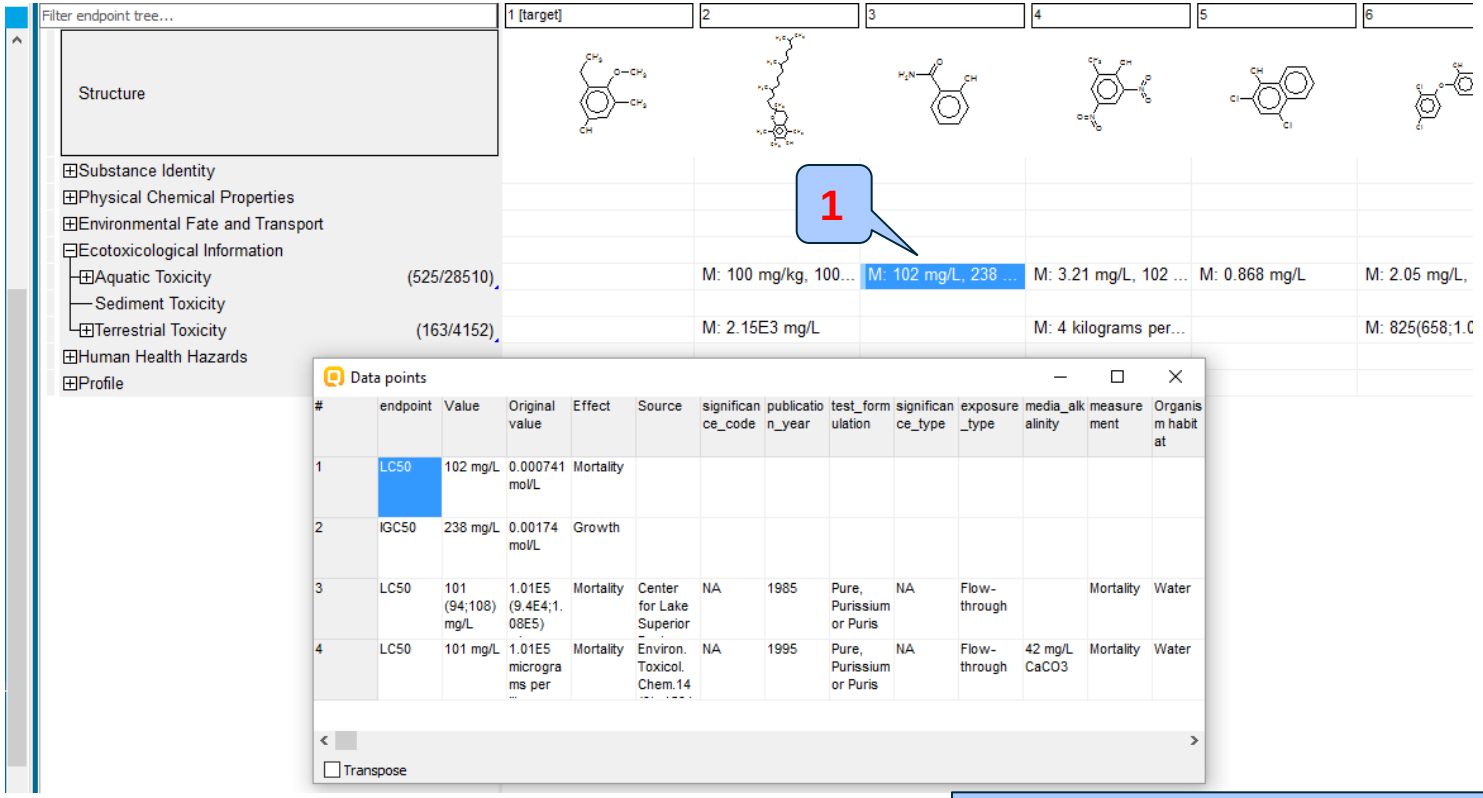


Category Definition
Summary information of Analogues



Chemical statistics presenting the number of chemicals and the available experimental data. This is statistics for the current row on data matrix.

Category Definition
Experimental data



#	endpoint	Value	Original value	Effect	Source	significance_code	publication_year	test_formulation	significance_type	exposure_type	media_alkalinity	measurement	Organism habitat
1	LC50	102 mg/L	0.000741 mol/L	Mortality									
2	IGC50	238 mg/L	0.00174 mol/L	Growth									
3	LC50	101 (94;108) mg/L	1.01E5 (9.4E4;1.08E5)	Mortality	Center for Lake Superior	NA	1985	Pure, Purissium or Puris	NA	Flow-through		Mortality	Water
4	LC50	101 mg/L	1.01E5 micrograms per	Mortality	Environ. Toxicol. Chem.14	NA	1995	Pure, Purissium or Puris	NA	Flow-through	42 mg/L CaCO3	Mortality	Water

1. Double-click on the **cell** with measured data provides a dropdown box ("Data points") which provides detailed information.

Recap

- You have identified a category ("phenols") with the "Aquatic toxicity classification by ECOSAR" profiler for the target chemical 3-ethyl-5-methyl-4-methoxyphenol.
- The available experimental results for these 562 analogues have been collected from the selected databases (Aquatic ECETOC, Aquatic Japan MoE, ECOTOX, and Aquatic OASIS).
- But before the user can proceed with the "Filling Data Gap" module, he/she should navigate through the endpoint tree and find the specific gap that will be filled.

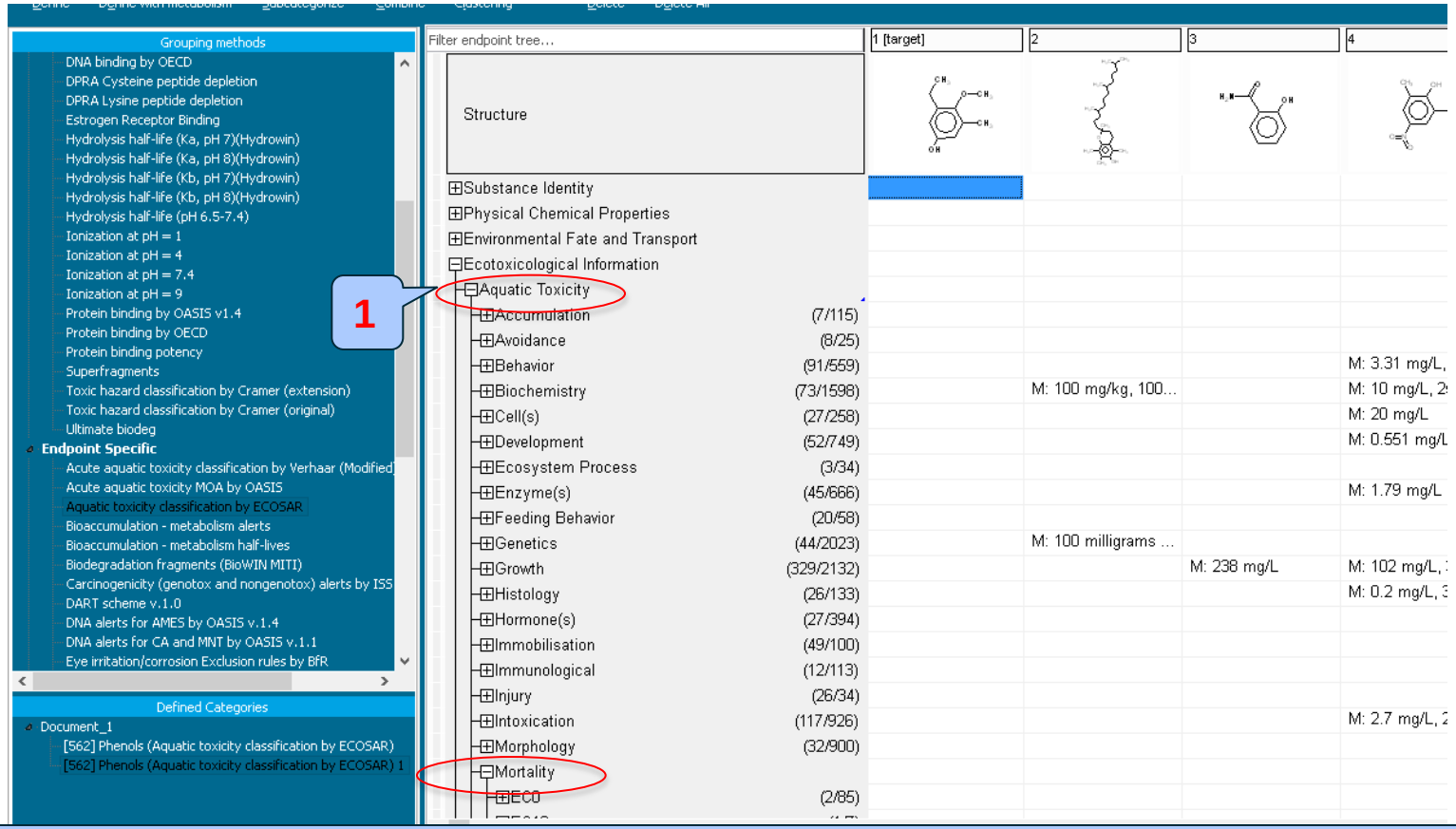
Category Definition

Navigation through the endpoint tree

- The user can navigate through the data tree by opening (or closing) the nodes of the tree.
- The data tree is extensive but logically constructed; it can be mastered with a practice.
- In this example, the "48 h LC50 Mortality for *Daphnia magna*" is the target endpoint.
- You can navigate through the endpoint tree by typing the species "Daphnia magna" in the "Filter endpoint tree…" box and clicking (Aquatic Toxicity, Mortality, LC50, 48 h, Animalia, etc to *Daphnia magna*- the specific endpoint (see next two screen shots)

Category Definition

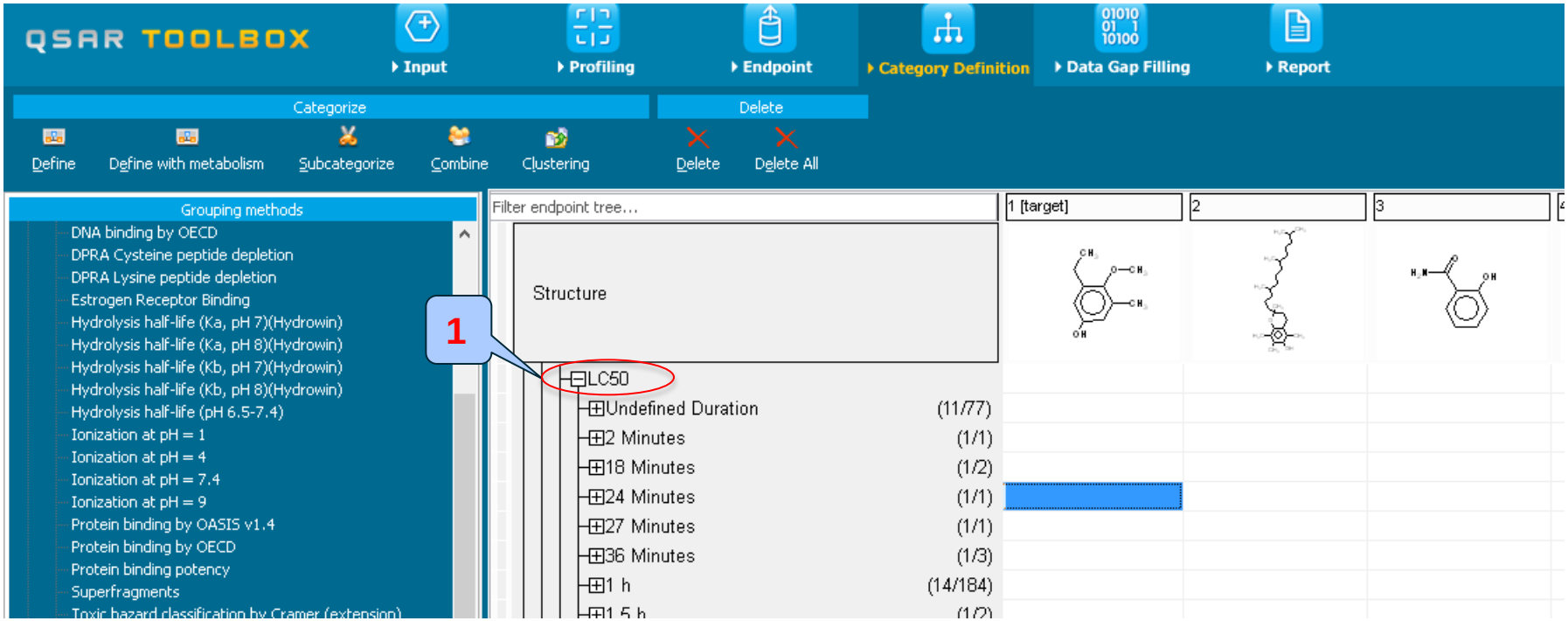
Navigation through the endpoint tree



1. Expand the following nodes: **Aquatic toxicity**; Mortality; LC50; Animalia; Arthropodata (Invertebrates); Branchiopoda (branchiopodos)
2. Find *Daphia magna* - this is the species related to target endpoint

Category Definition

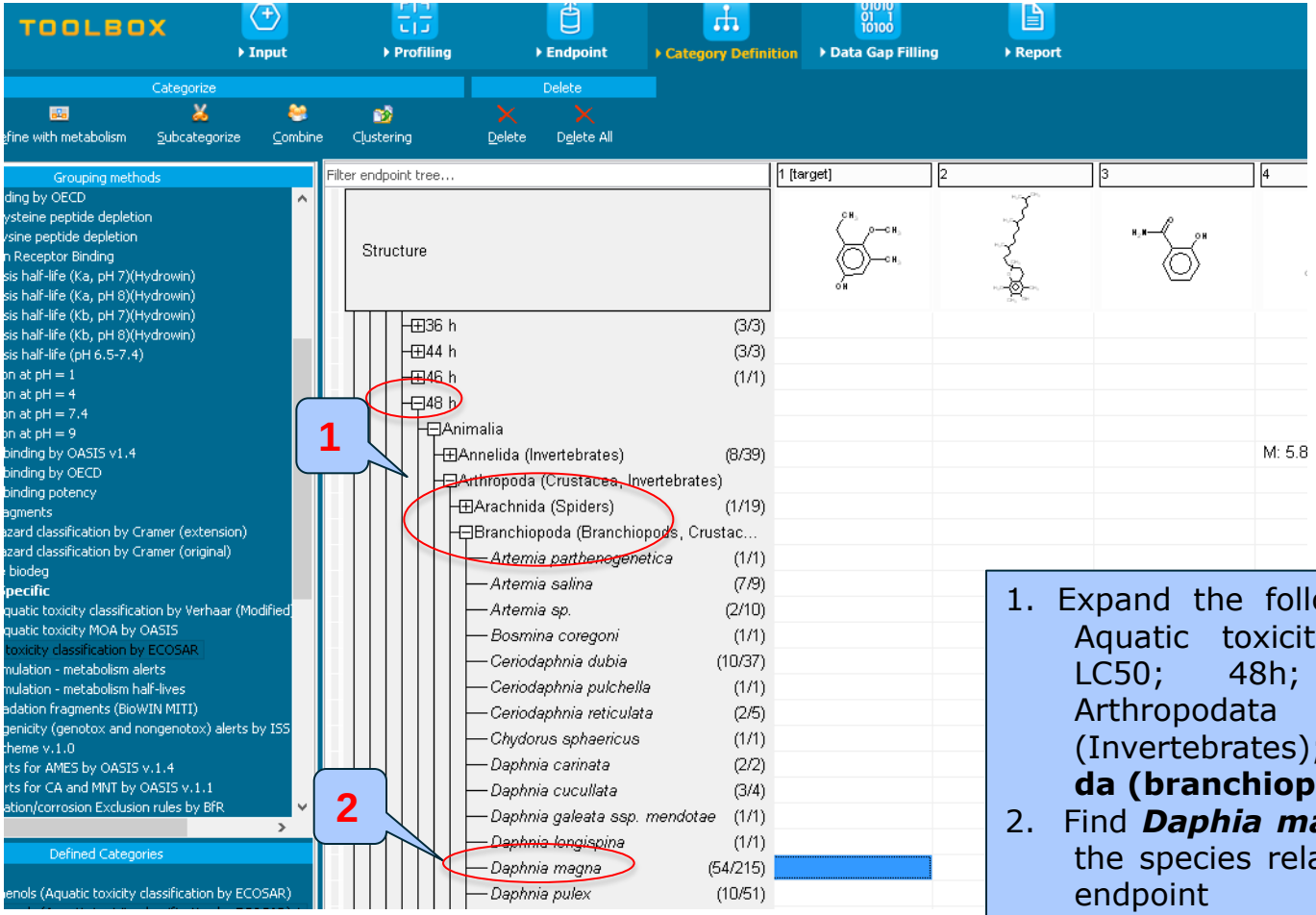
Navigation through the endpoint tree



1. Expand the following nodes: Aquatic toxicity; **Mortality**; LC50; 48h; Animalia; Arthropodata (Invertebrates); Branchiopoda (branchiopodos)
2. Find *Daphia magna* - this is the species related to target endpoint

Category Definition

Navigation through the endpoint tree

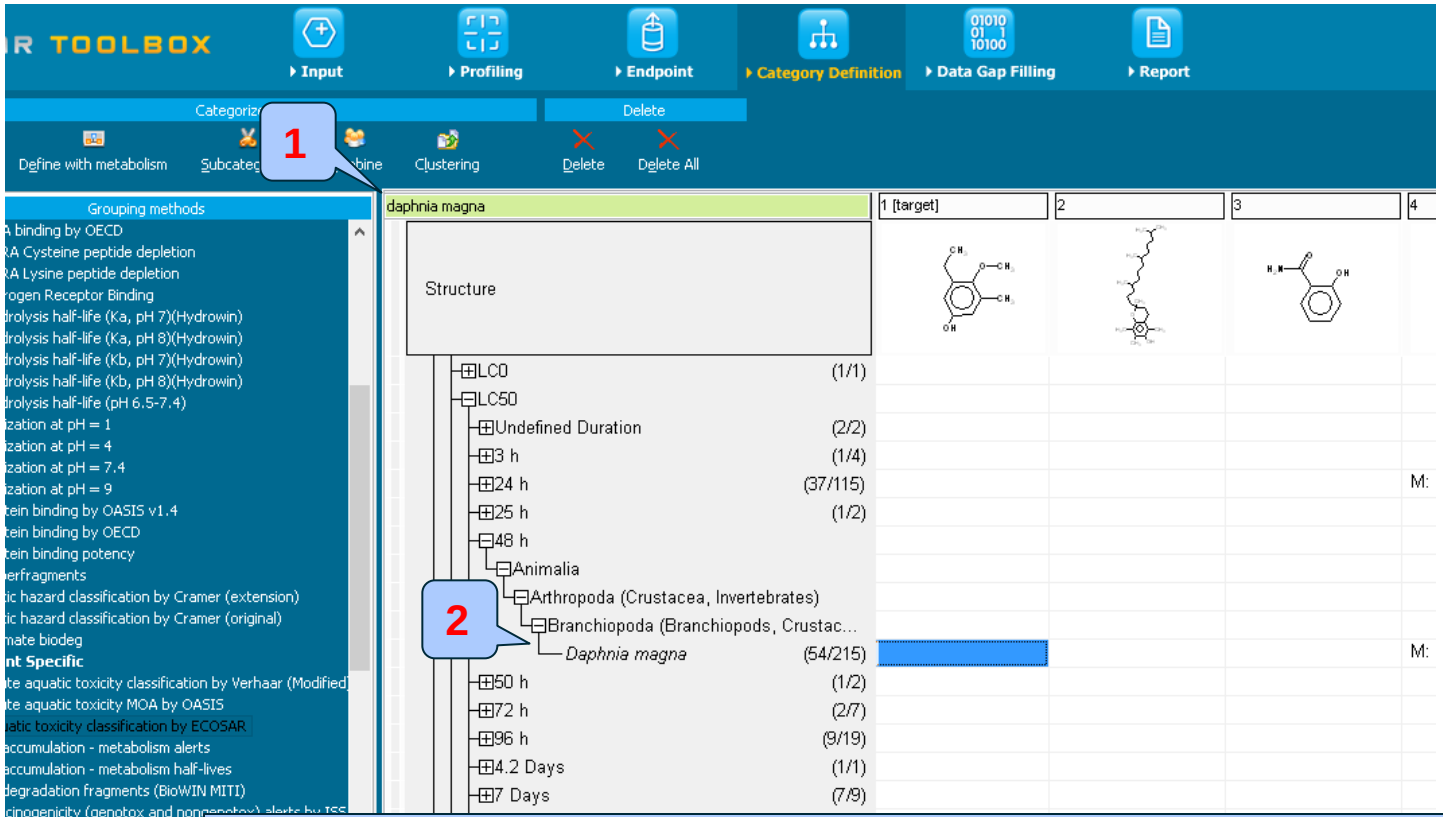


1. Expand the following nodes: Aquatic toxicity; Mortality; LC50; 48h; **Animalia**; Arthropodata (Invertebrates);**Branchiopoda (branchiopodos**)
2. Find ***Daphia magna*** - this is the species related to target endpoint

Category Definition

Navigation through the endpoint tree

- Navigation throw the tree by “Filtering”



1. Insert type “**Daphnia magna**” in the filter box, then press **Enter;**
2. Open the tree to the target endpoint by double left clicking.

Recap

- You have now retrieved the available experimental data on aquatic toxicity for 562 chemicals classified as "phenols" by the "Aquatic toxicity classification by ECOSAR" profiler found in the databases Aquatic ECETOC, Aquatic Japan MoE, ECOTOX, and Aquatic OASIS.
- You have identified the target endpoint of "48 h LC50 Mortality for *Daphnia magna*".
- You are ready to fill in the data gap so click on "Data Gap Filling" (see next screen shot).

Outlook

- Background
- Objectives
- Specific Aims
- Trend analysis
- The exercise
- **Workflow of the exercise**
  - Chemical Input
  - Profiling
  - Endpoint
  - Category definition
  - **Data Gap Filling**

Data Gap Filling

Overview

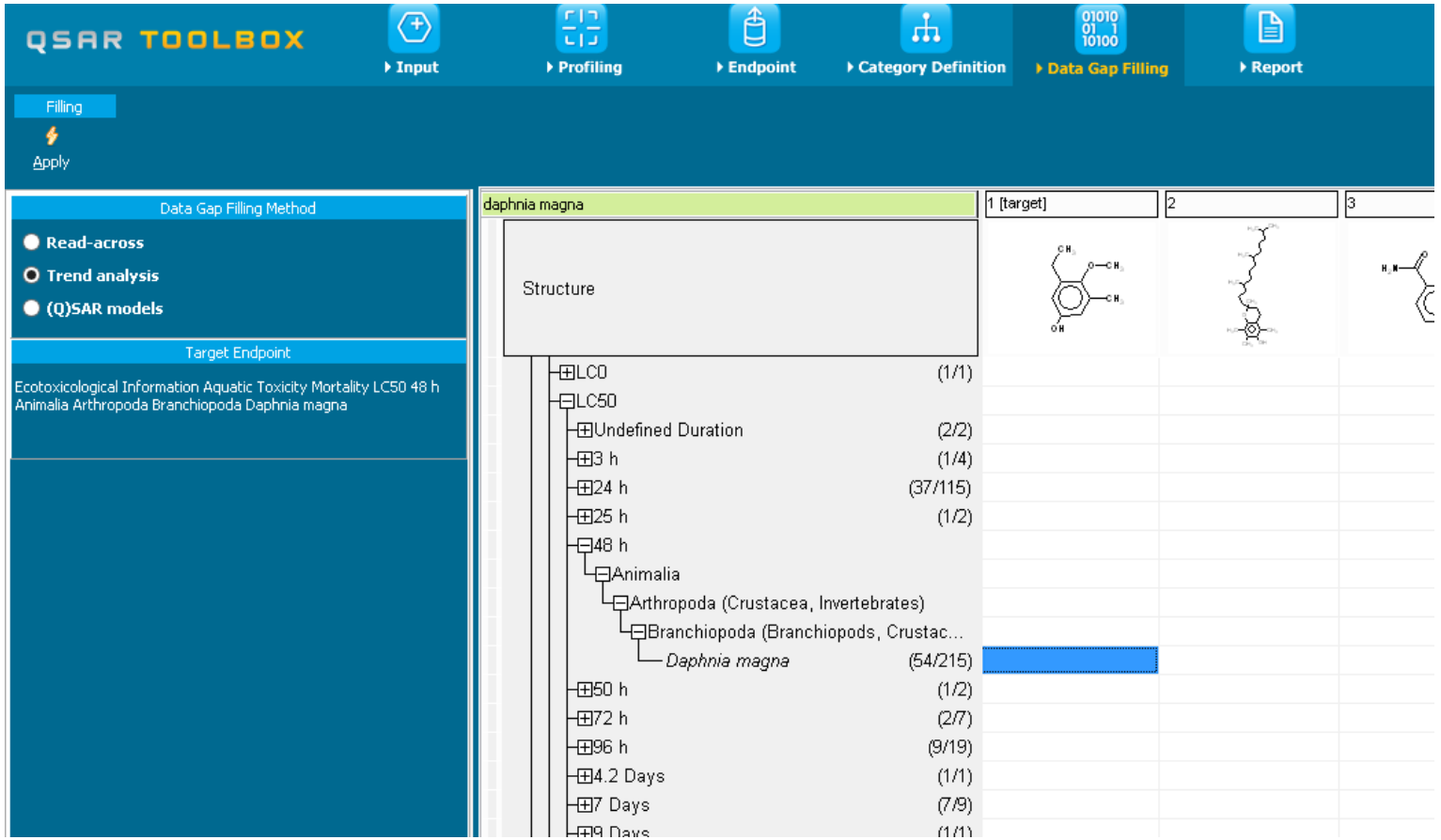
- "Data Gap Filling" module gives access to three different data gap filling tools:
  - Read-across
  - Trend analysis
  - (Q)SAR models

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

- In this example, we use trend analysis.

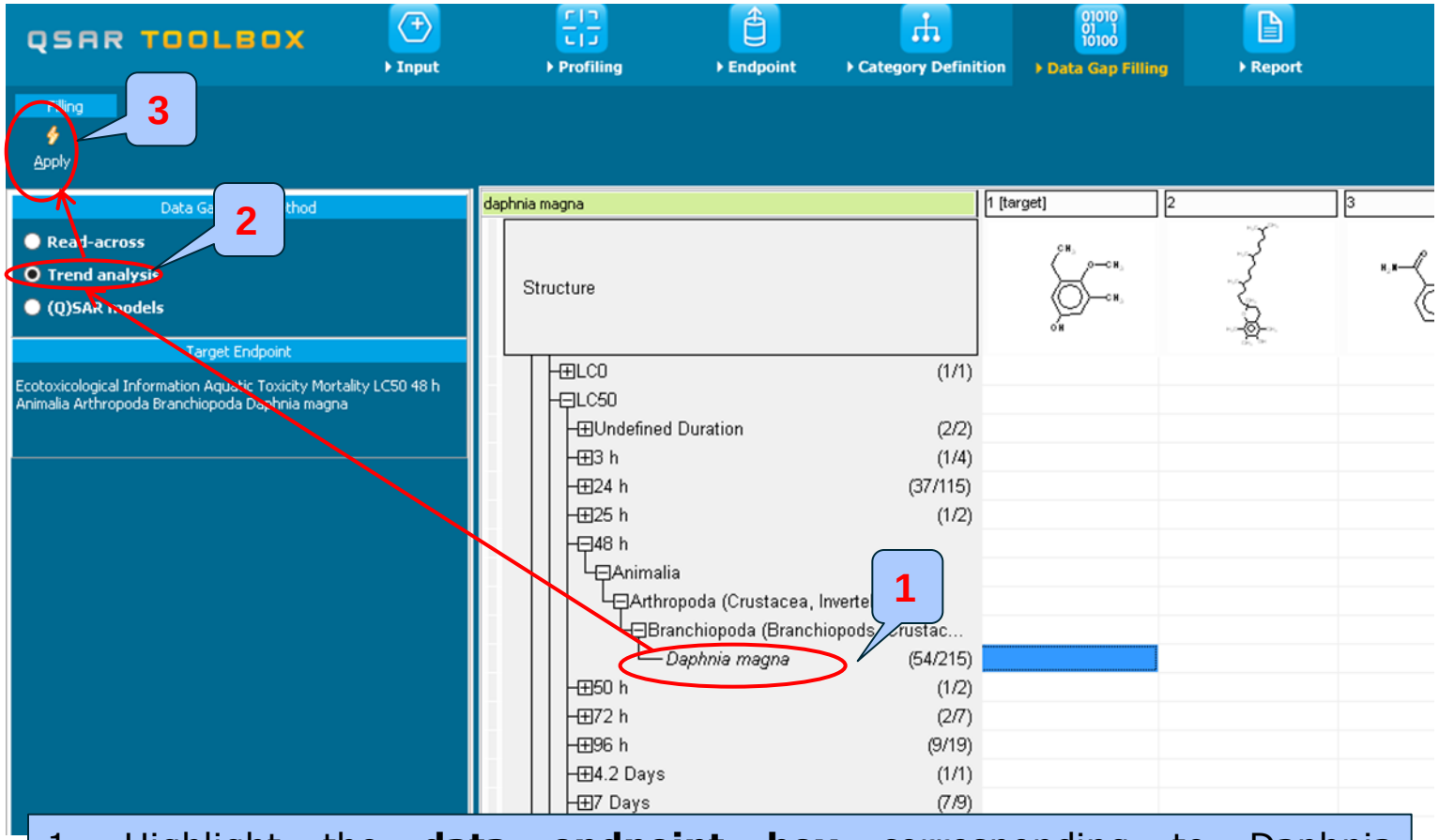
Data Gap Filling

Data Gap window



Data Gap Filling

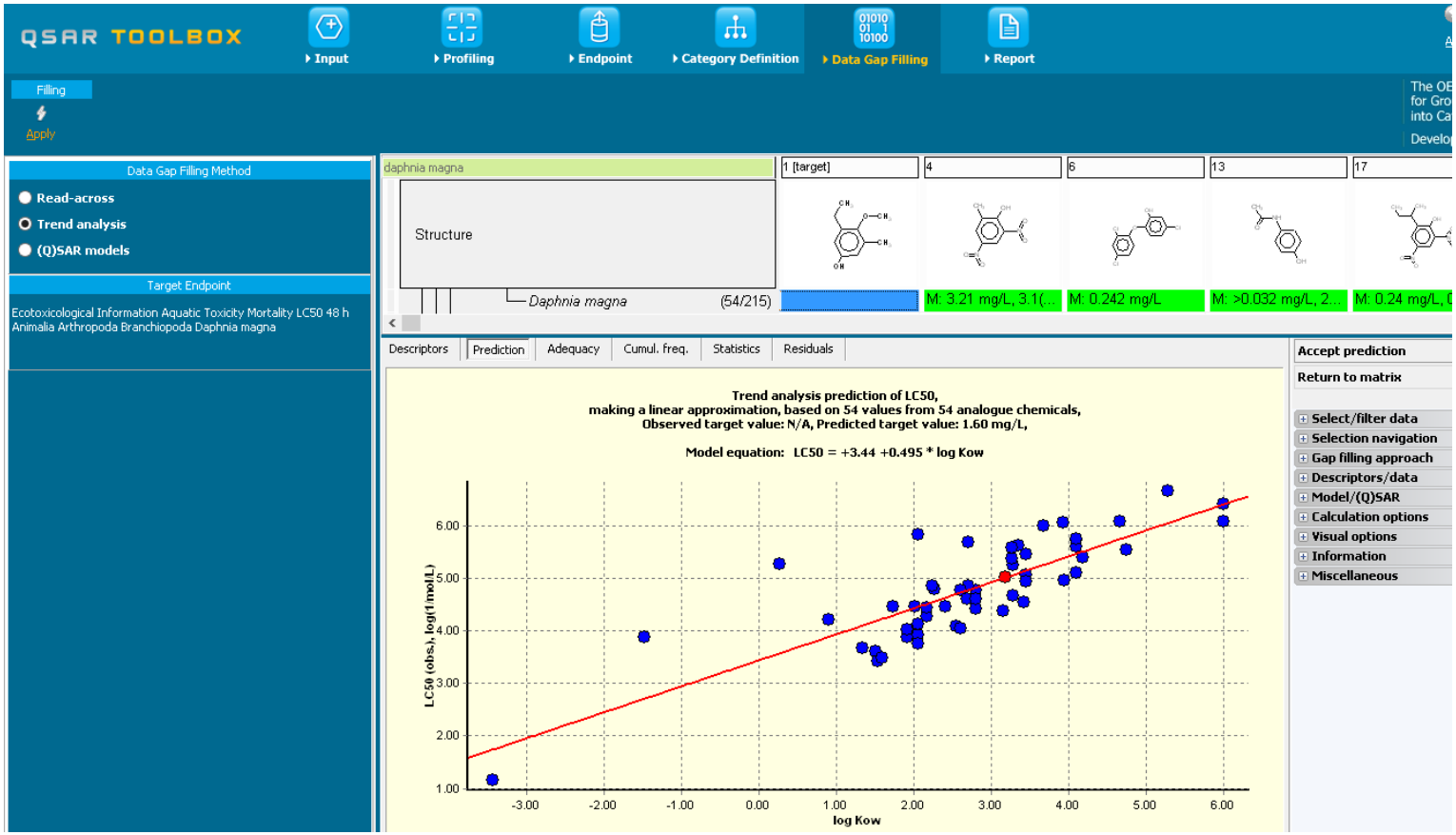
Apply Trend analysis



1. Highlight the **data endpoint box** corresponding to Daphnia magna/LC50/48h under the target chemical. It will be empty;
2. Select **Trend analysis;** 3. Click **Apply**

Data Gap Filling

Results of Trend analysis



Data Gap Filling

Interpreting Trend analysis

- The resulting plot outlines the log of the experimental LC50 results of all analogues (Y axis) according to a descriptor (X axis) with Log *Kow* being the default descriptor (see next slide).
- The **RED** dot represents the predicted value for the target chemical.
- The **BLUE** dots represent the experimental results available for the analogues used in the trend analysis.
- Before accepting the estimated result for the target chemical, the trend analysis should be further refined by subcategorisation (see following slides).

Data Gap Filling

Side-Bar of Subcategorisation

- Remember in the Toolbox, a category refers to a group of chemicals which have the same profiling result according to one of the profilers listed in the module "Profiling".

- Subcategorisation refers to the process of applying additional profilers to the previously defined category; subcaregorisation identifies chemicals which have differing profiling results and eventually eliminating these chemicals from the final category.

Data Gap Filling

Side-Bar of Subcategorisation

In this example, subcategorisation allows for the elimination of analogues which are dissimilar to the target chemical with respect to:

- Substance type (mixtures and hydrolizing chemicals)

  The categorisation based on substance type allows keeping among the analogues only those that are of the same chemical type: discrete chemicals, mixtures, polymers, inorganics, organometalics. The current target is a discrete chemical hence the analogues should also be discrete chemicals.

- OASIS Mode of action (all except phenols and anilines)

  The categorization based on mode of action identifies analogues having the same mode of action as the target which is in the group of phenols and anilines.

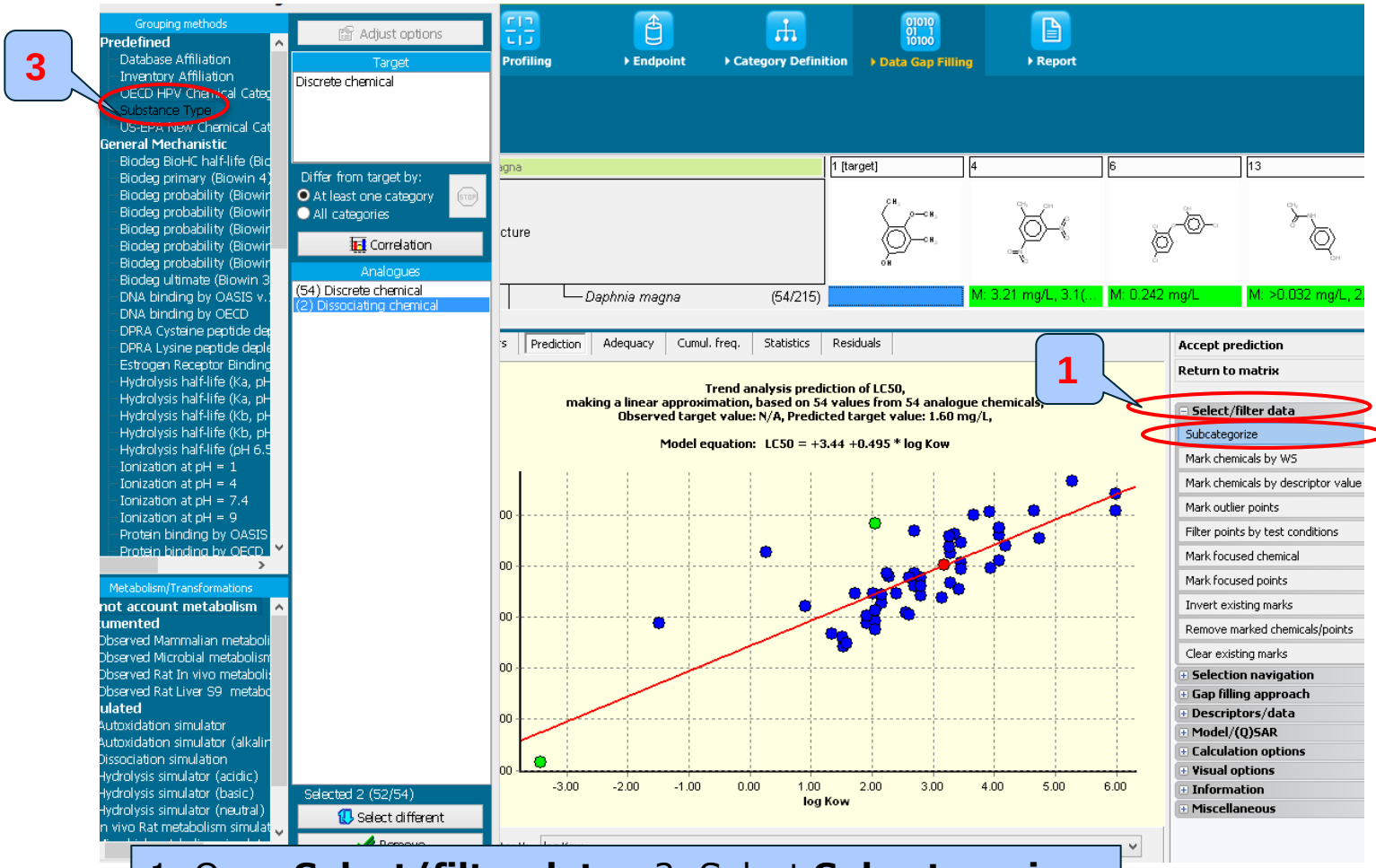
- Chemical elements

  The profiler aimed to identify analogues consisting of same elements as those presented in the target chemical

Subcategorisation is demonstrated in the next 4 screen shots.

Data Gap Filling

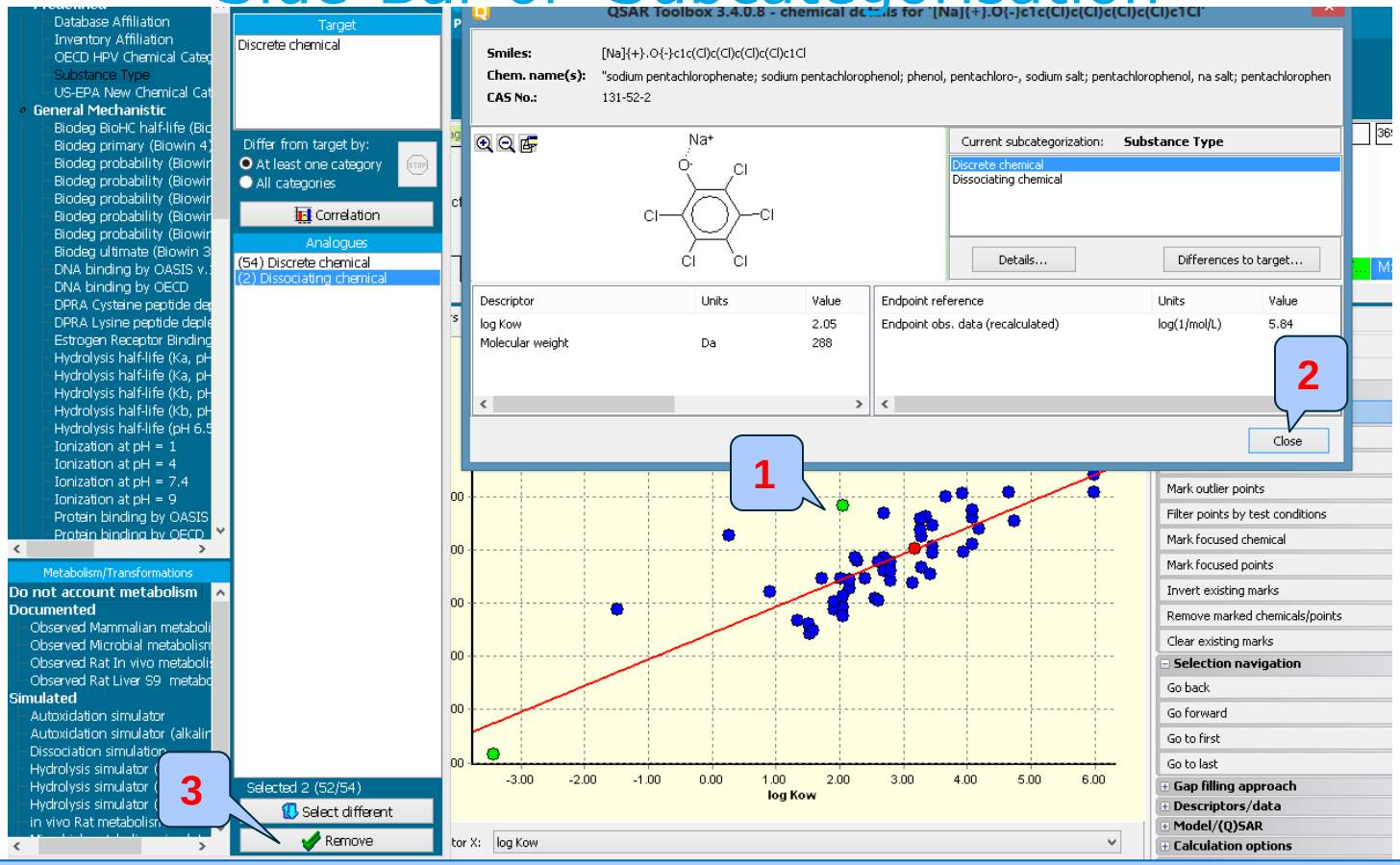
Side-Bar of Subcategorisation



1. Open **Select/filter data;** 2. Select **Subcategorize;**
3. Select **Substance type**

Data Gap Filling

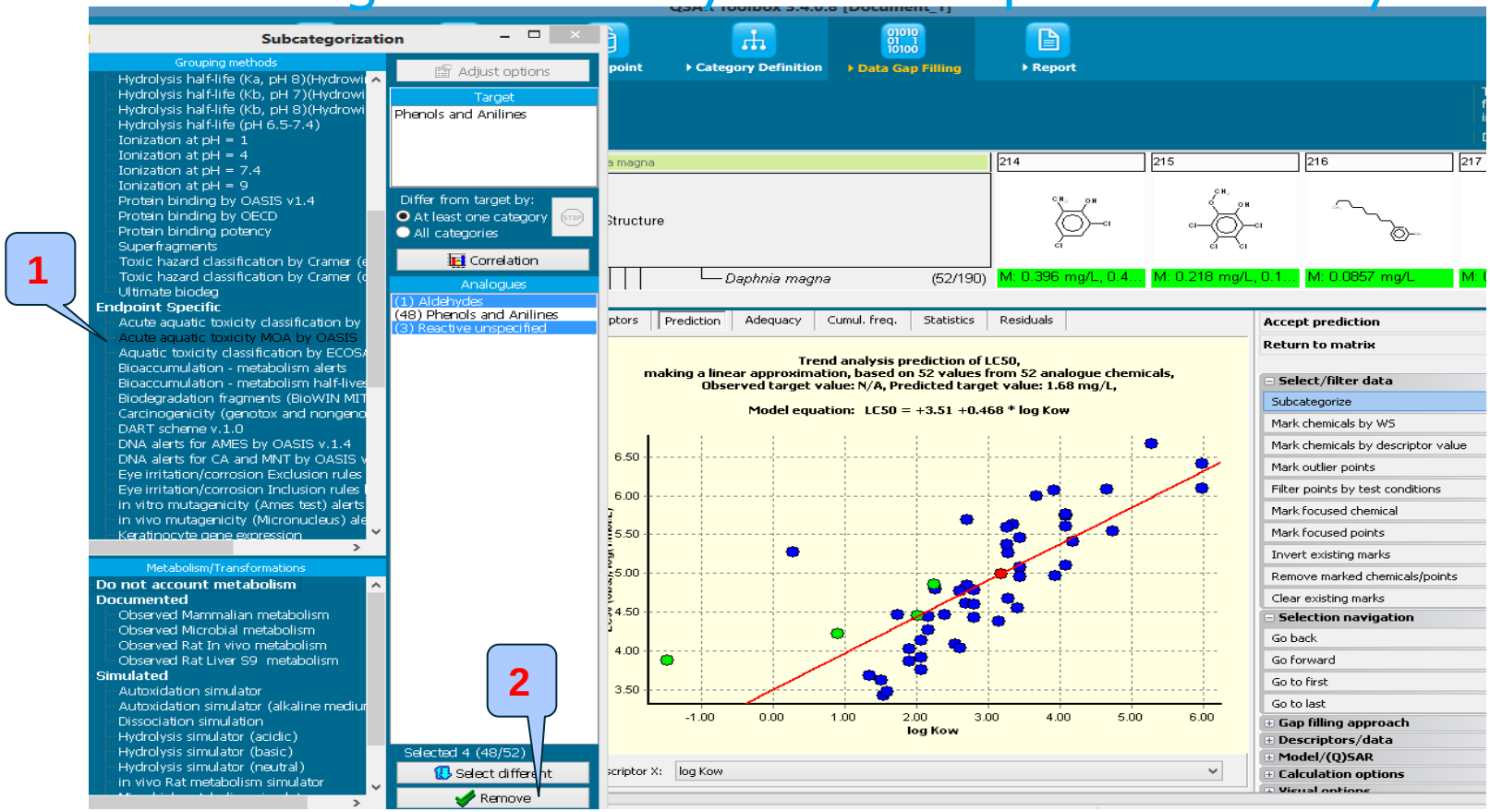
Side-Bar of Subcategorisation



1. **Double click** above the outlier to see why this chemical is different to the target
   The chemical is dissociating chemical and has to be eliminated being different substance type compared to the target, which is a discrete chemical.
2. **Close**; 3. Click **Remove** to eliminate dissimilar chemical

Data Gap Filling

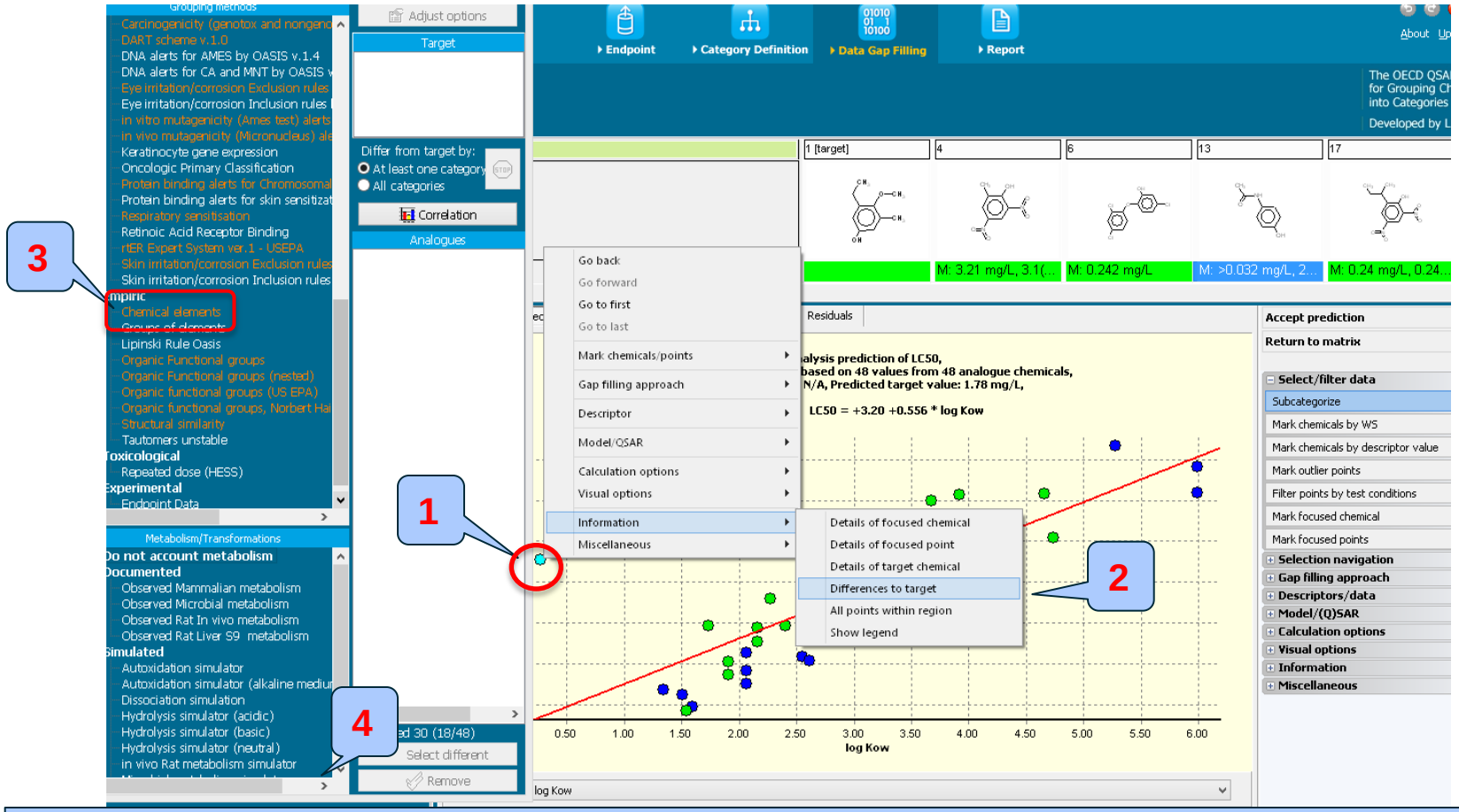
Subcategorisation by Acute-aquatic toxicity MOA



1. Select **Acute aquatic toxicity MOA by OASIS;** 2. Click **Remove** to eliminate dissimilar chemical

Data Gap Filling

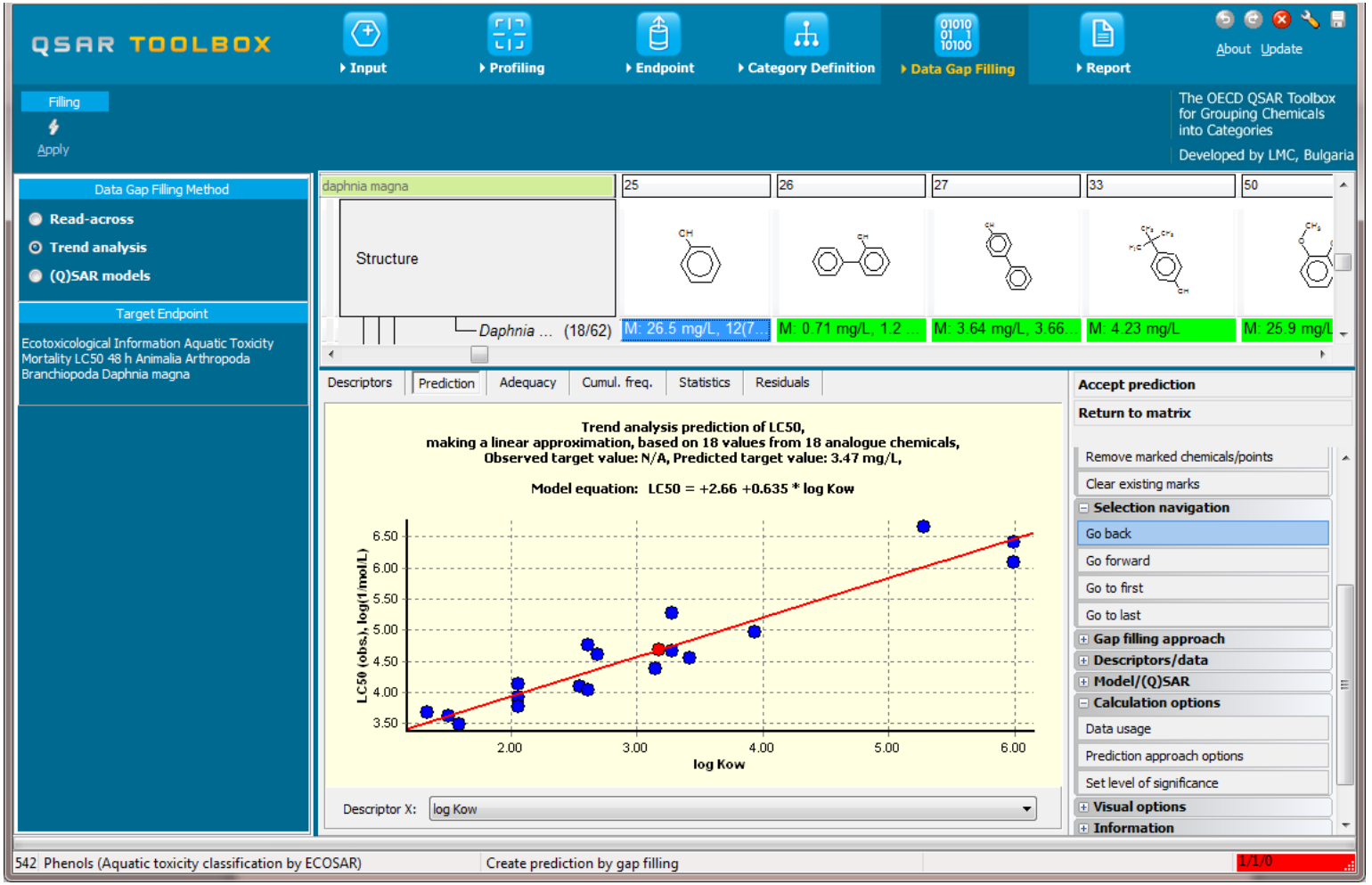
Subcategorisation by Chemical elements



1. Right click over the outlier**;** 2. Select **information** and select **Different to target;** 3. Select **Chemical elements; 4. Click Remove** to eliminate dissimilar

Data Gap Filling

Results



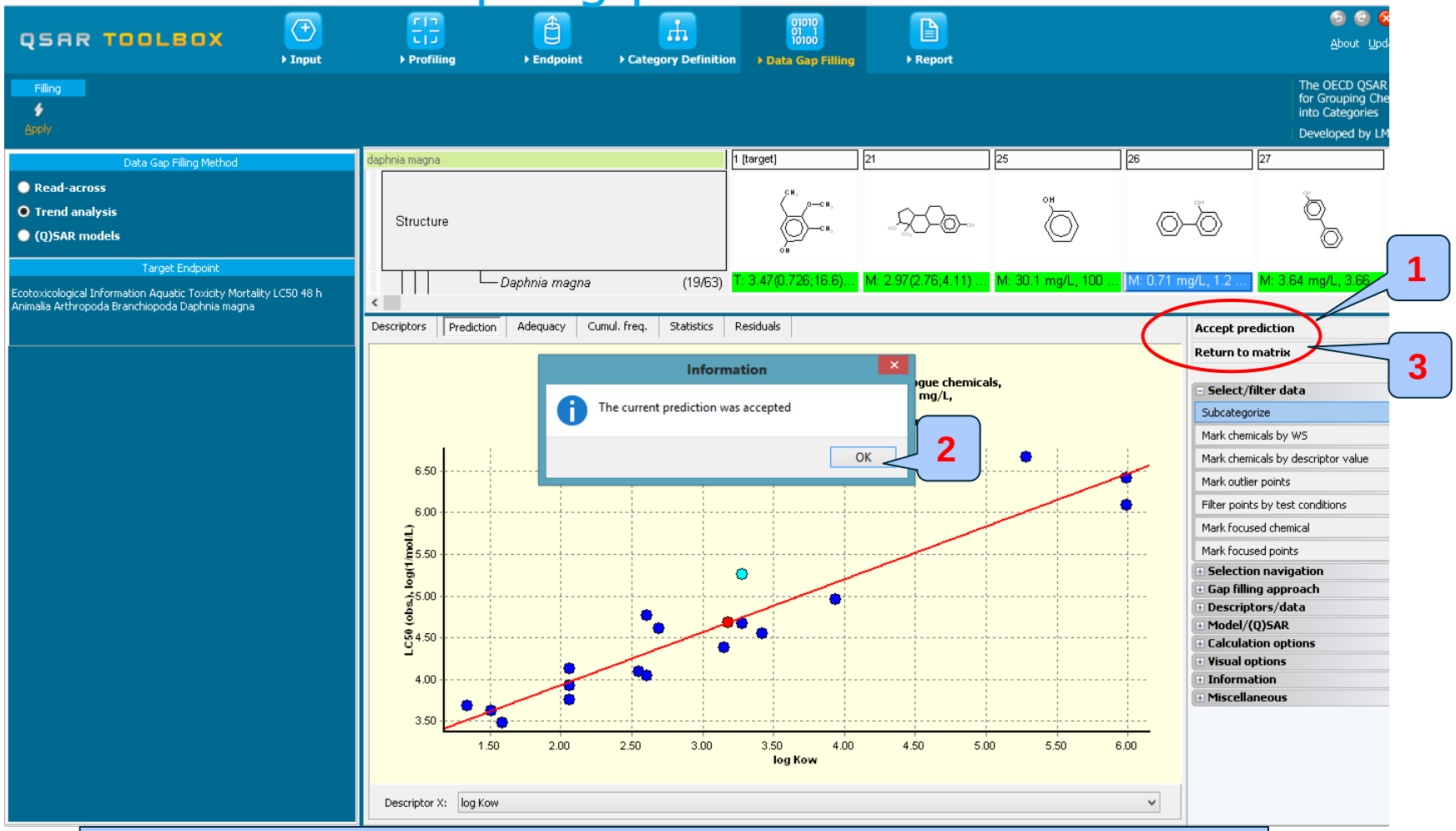
Data Gap Filling

Results

- The remaining chemicals in the graph now all have a consistent profile relevant for aquatic toxicity (i.e. substance type, Classification by ECOSAR, MOA by OASIS and Chemical elements).
- By accepting the prediction the data gap is filled (see next screen shot).
- By clicking on Return to Matrix, the user can close the read-across and proceed with the workflow (see next screen shot).

Data Gap Filling

Accepting prediction results



1. Click **Accept prediction; 2.** Click **OK**; 3. Click **Return to matrix**

Outlook

- Background
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- **Workflow of the exercise**
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  - Data Gap Filling
  - **Export a prediction to IUCLID5**

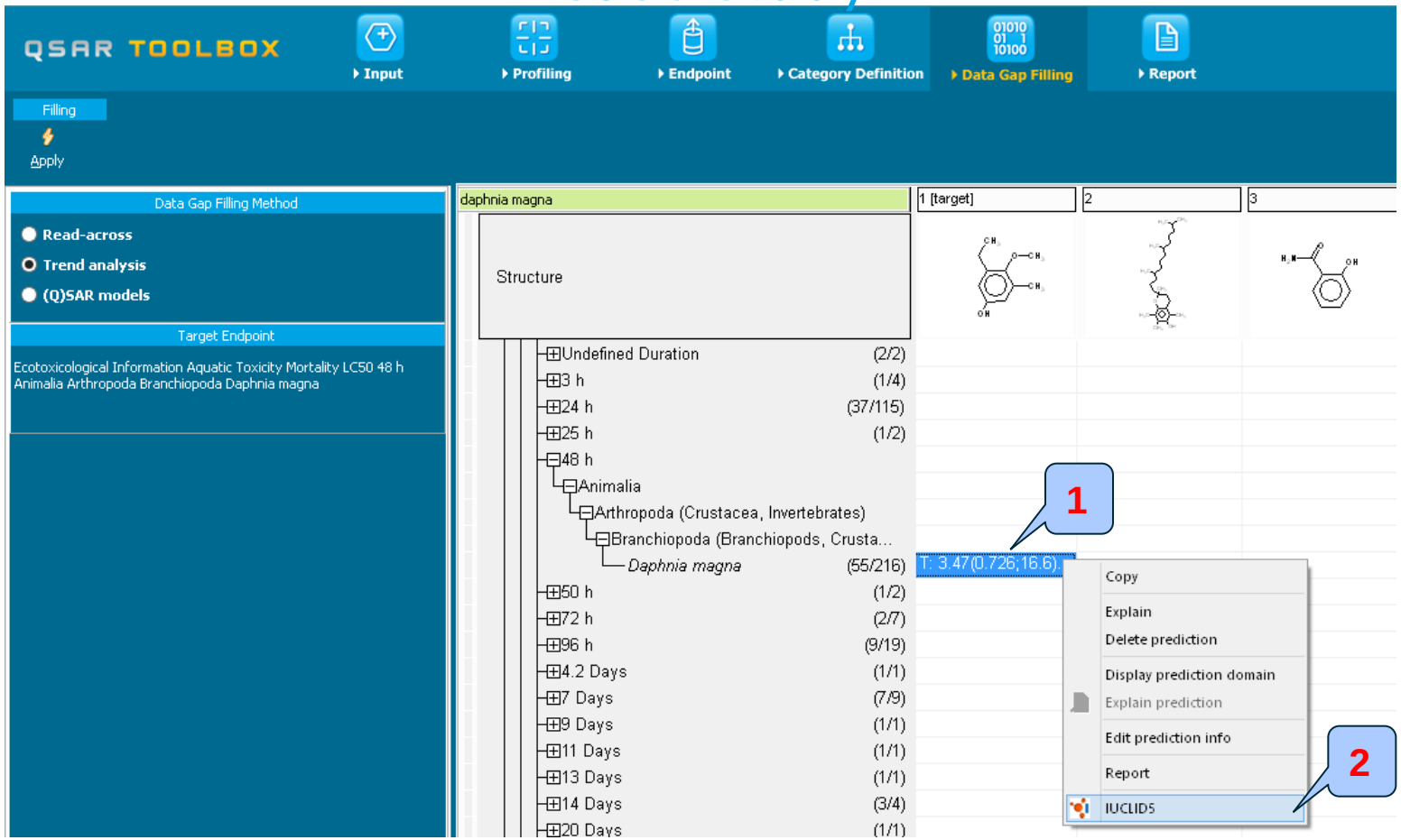
Export prediction to the IUCLID5

Overview

- The OECD QSAR Toolbox allows the users to export predicted data (by means of the Filling Data Gap tools) to IUCLID 5.
- There are two ways of exporting:
  - create an *.i5z file which can then be imported into an IUCLID 5 database.
  - connect to an IUCLID 5 server (via WebServices) and assigning the predicted endpoint data to a selected substance.
- A wizard will guide the user through the different steps of exporting (see next screen shot).
- More detailed information could be found in the following link: http://www.oecd.org/dataoecd/54/27/47136326.pdf

Exporting the prediction to IUCLID5

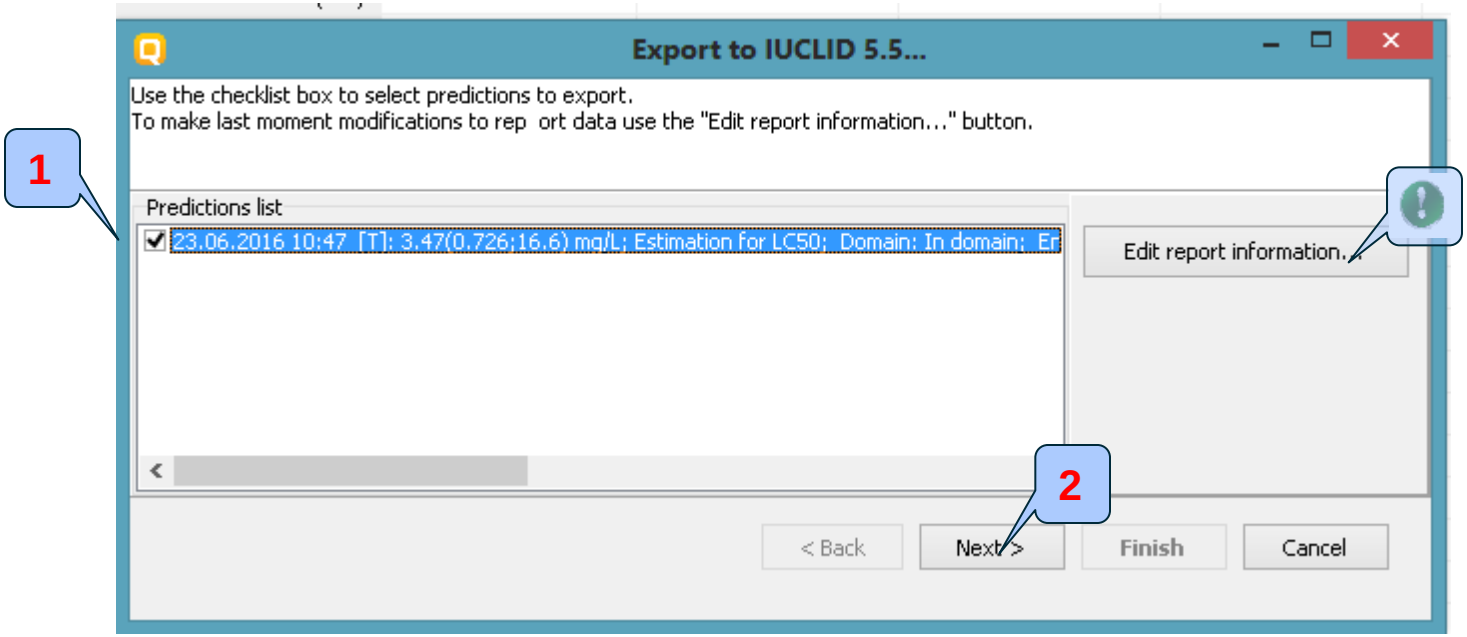
Case study



1. Move the mouse in the column of the target substance and click the **right mouse** button; 2. Select **IUCLUD**

Exporting the prediction to IUCLID5

Case study

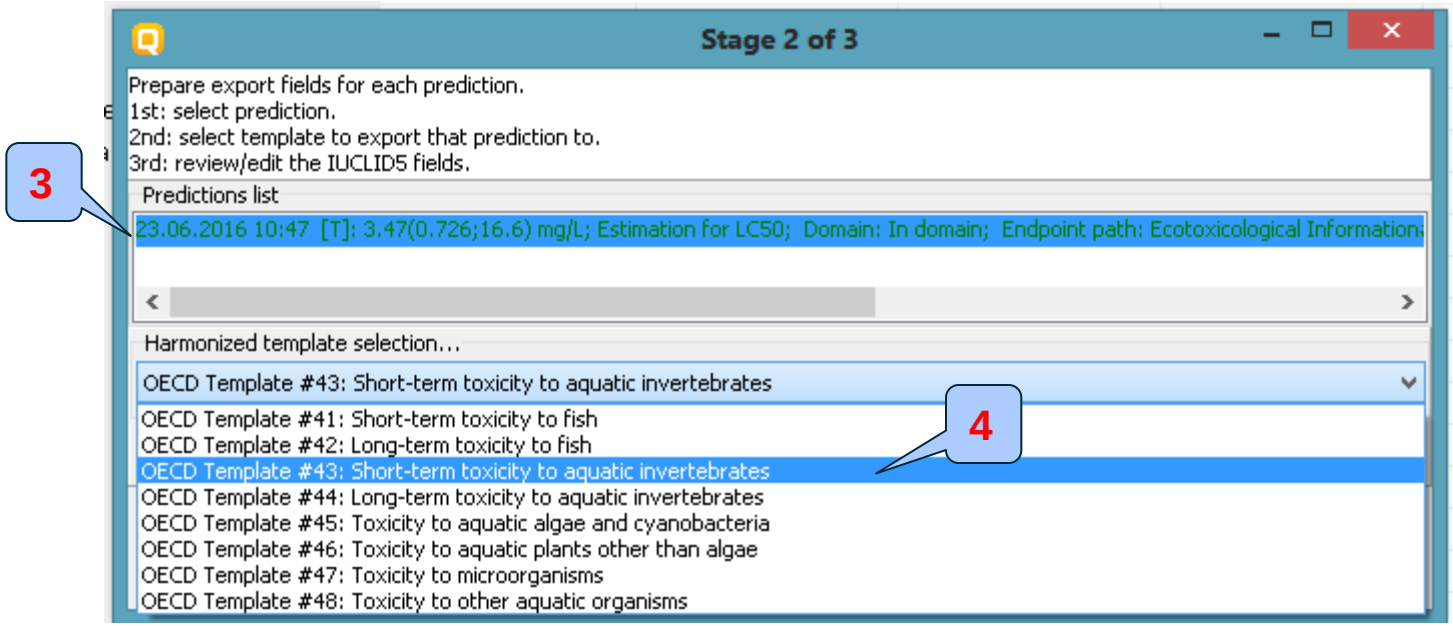


1. Select the **prediction** to export;
2. Click **Next** to move to the next step of the export.

The user could also edit the report information

Exporting the prediction to IUCLID5

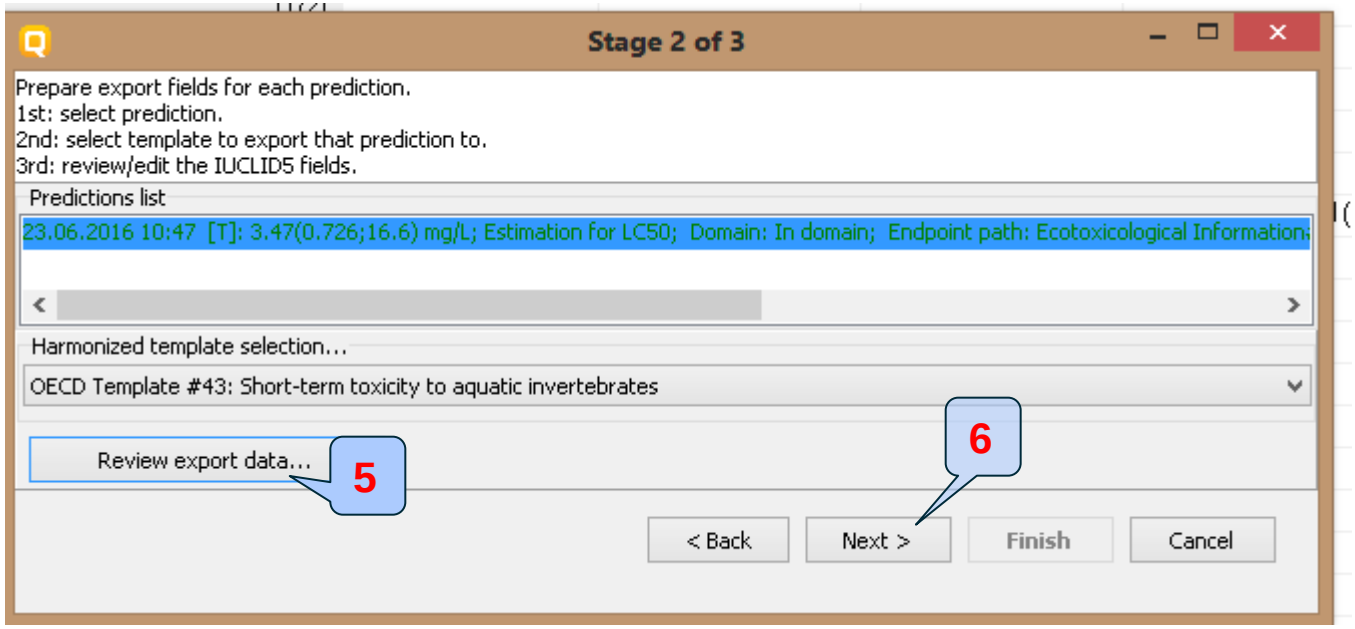
Case study



3. Select **prediction;** 4. Select **template** to export the prediction

Exporting the prediction to IUCLID5

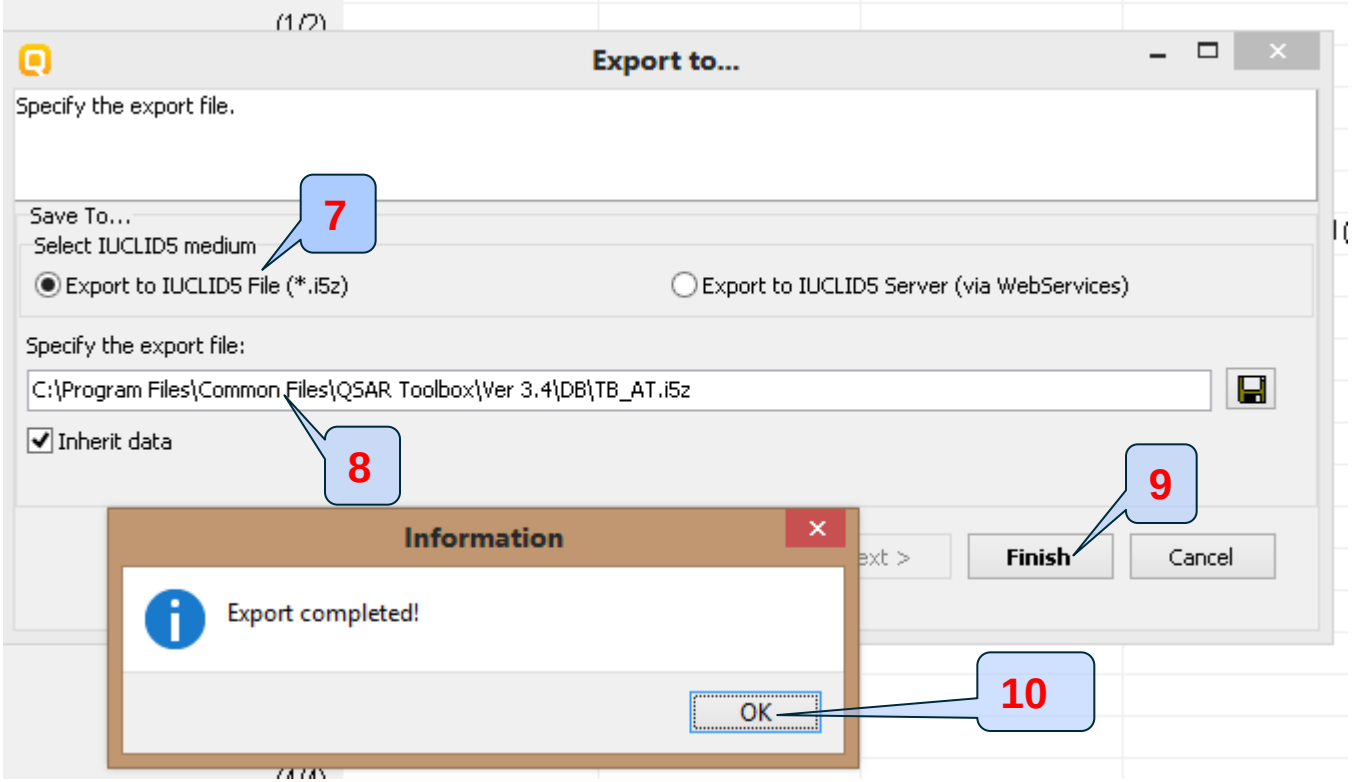
Case study



5. Review/edit the IUCLID5 fields 6. Click **Next**

Exporting the prediction to IUCLID5

Case study



7. Select **medium** to export, i5z file or export via WebServices;
8. Specify the export file; 9. Click **Finish;** 10. Click **OK**

Outlook

- Background
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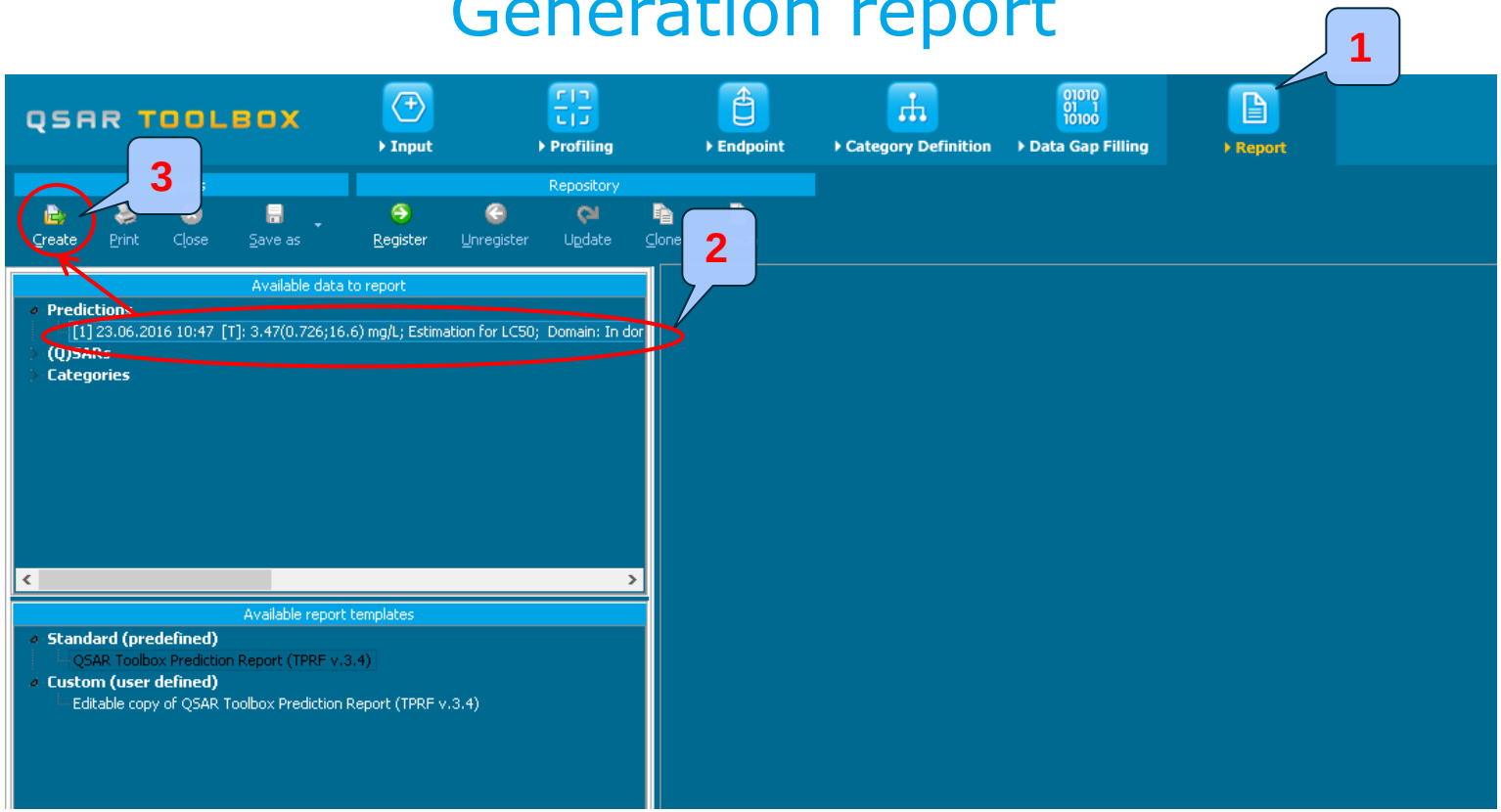
  - Data Gap Filling
  - Export a prediction to IUCLID5
  - **Report**

Report
Overview

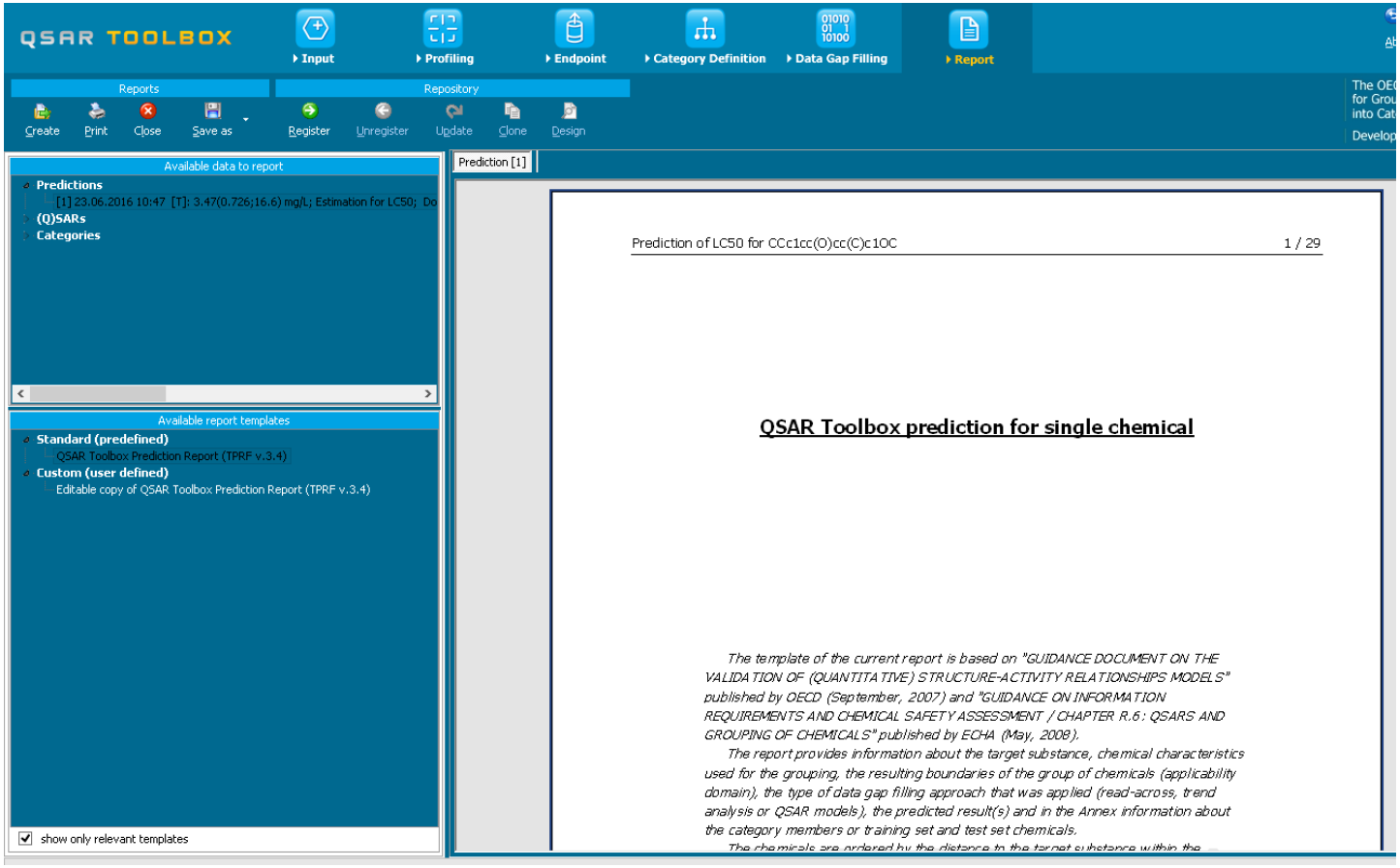
- Report module could generate report on any of predictions performed with the Toolbox.
- Report module contains predefined report templates as well as a template editor with which users can define their own user defined templates.
- The report can then be printed or saved in different formats.

Report
Generation report



1. Go to **Report** section; 2. **Select** prediction for the target chemical from the "Available data to report" window; 3. **Click** Create

Report
Overview



Outlook

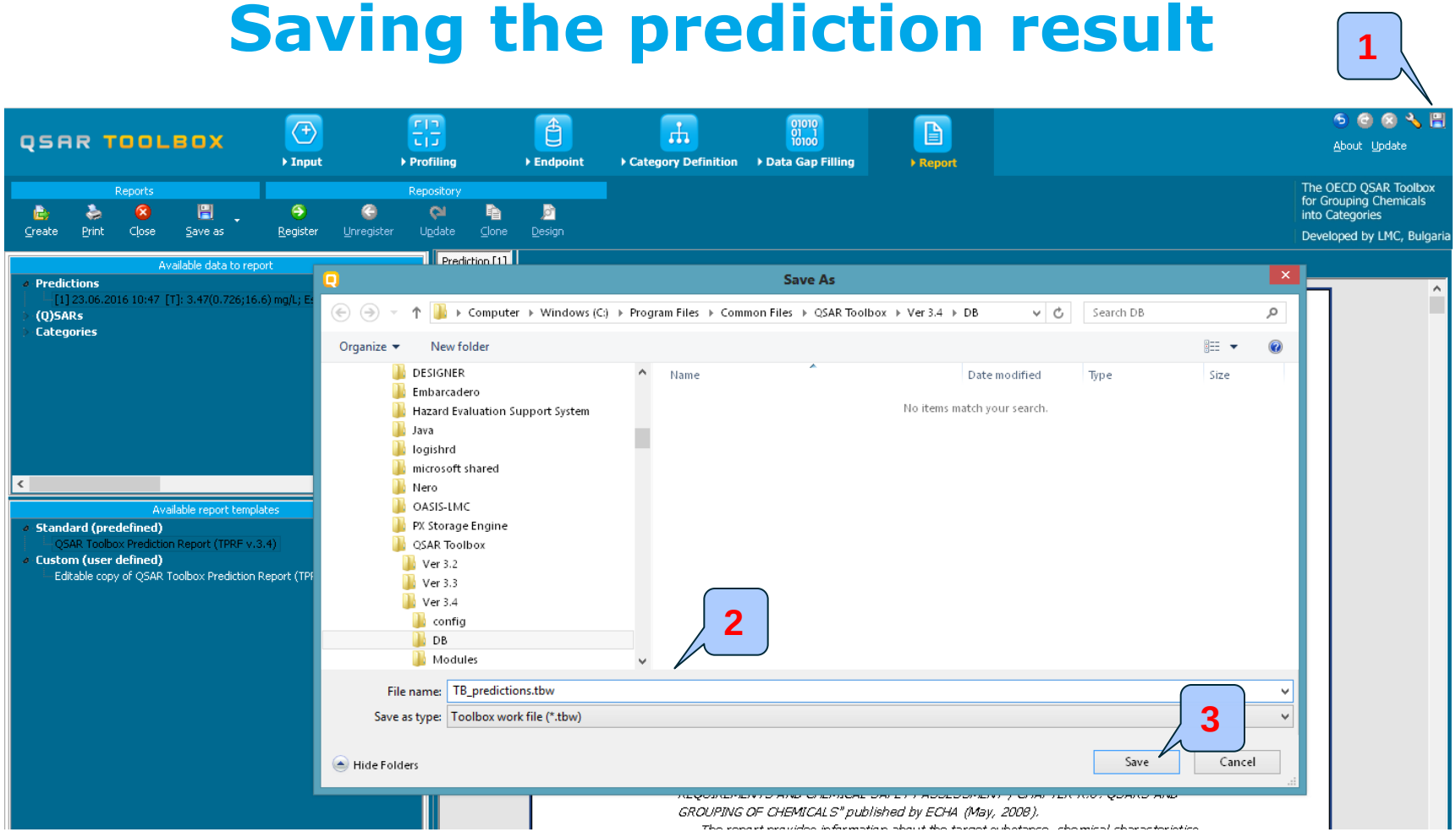
- Background
- Objectives
- Specific Aims
- Trend analysis
- The exercise
- Workflow of the exercise
- **Save the prediction result**

Saving the prediction result

- This functionality allow storing/restoring the current state of Toolbox documents including loaded chemicals, experimental data, profiles, predictions etc., on the same computer. The functionality is implemented based on saving the sequence of actions that led to the current state of the Toolbox document and later executing these actions in the same sequence in order to get the same result(s).

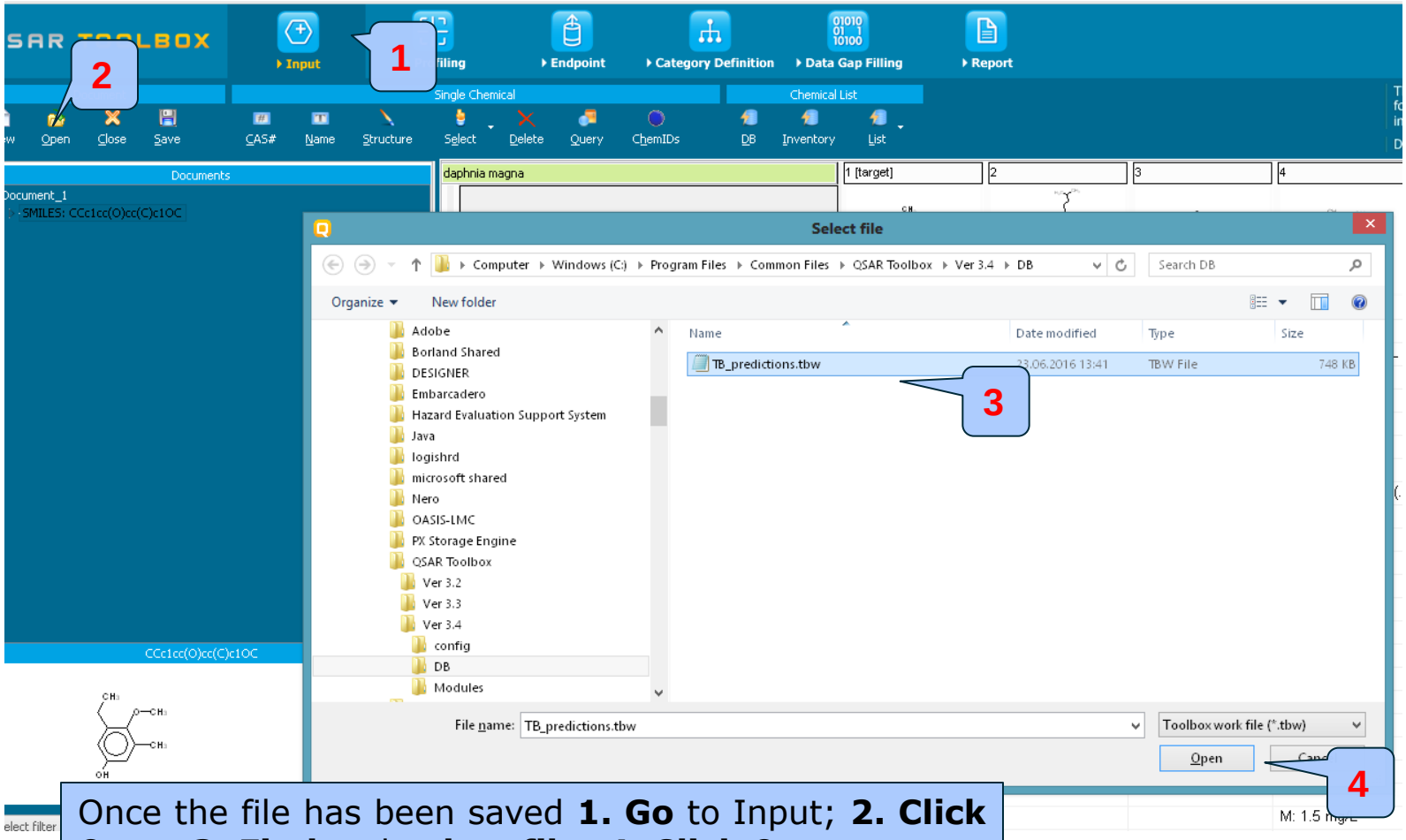
- Saving/Loading the file with TB prediction is shown on next screenshots

Saving the prediction result



1. **Click** on Save button; **2.** **Define** name of the file; **3.** **Click** Save button

Open saved file



Once the file has been saved **1. Go** to Input; **2. Click** Open; **3. Find** and **select file**; **4. Click** Open

Congratulations

- You have now been introduced to the work flow of the Toolbox and completed the tutorial on data gap filling by trend analysis and exported the prediction to IUCLID 5
- You have been introduced to the six modules of the Toolbox, the basic functionalities within each module and the rationale behind each module.
- Remember proficiency comes with practice.