QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.3.4

Predicting acute aquatic toxicity to fish of *Dodecanenitrile (CAS 2437-25-4)* taking into account tautomerism

Outlook

- **Background**
- Objectives
- The exercise
- Workflow
- Save prediction

Background

- This is a step-by-step presentation designed to take the user of the Toolbox through the workflow for filling data gap for acute aquatic toxicity to fish taking into account tautomerism of target chemical.

Outlook

- Background
- **Objectives**
- The exercise
- Workflow
- Save prediction

Objectives

- **This presentation reviews a number of functionalities of the Toolbox:**
  - Providing tautomeric set of target chemical
  - Identify analogues for the active tautomeric form
  - Retrieve experimental results available for those analogues
  - Perform trend analysis for the active tautomeric form
  - Assigning of the prediction for the active tautomer to the target chemical
  - Finally saved the prediction result

Outlook

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- Save prediction

The Exercise

- In this exercise we will predict *LC50* for fish: *P.promelas* for target chemical *Dodecanenitrile (CAS 2437-25-4)*
- Set of simulated tautomers for the target chemical will be provided
- Analyze the profilers of the tautomeric forms within tautomeric set
- Filling data gaps for active tautomer by trend analysis
- Assign prediction for the tautomeric forms to the target chemical

Outlook

- Background
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- **Workflow**
- Save prediction

Workflow

- **As you know the Toolbox has 6 modules which are typically used in sequence:**
  - Chemical Input
  - Profiling
  - Endpoint
  - Category Definition
  - Data Gap Filling
  - Report

Chemical Input
Ways of Entering a Chemicals

User Alternatives for input of Chemical:

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, ENECS number

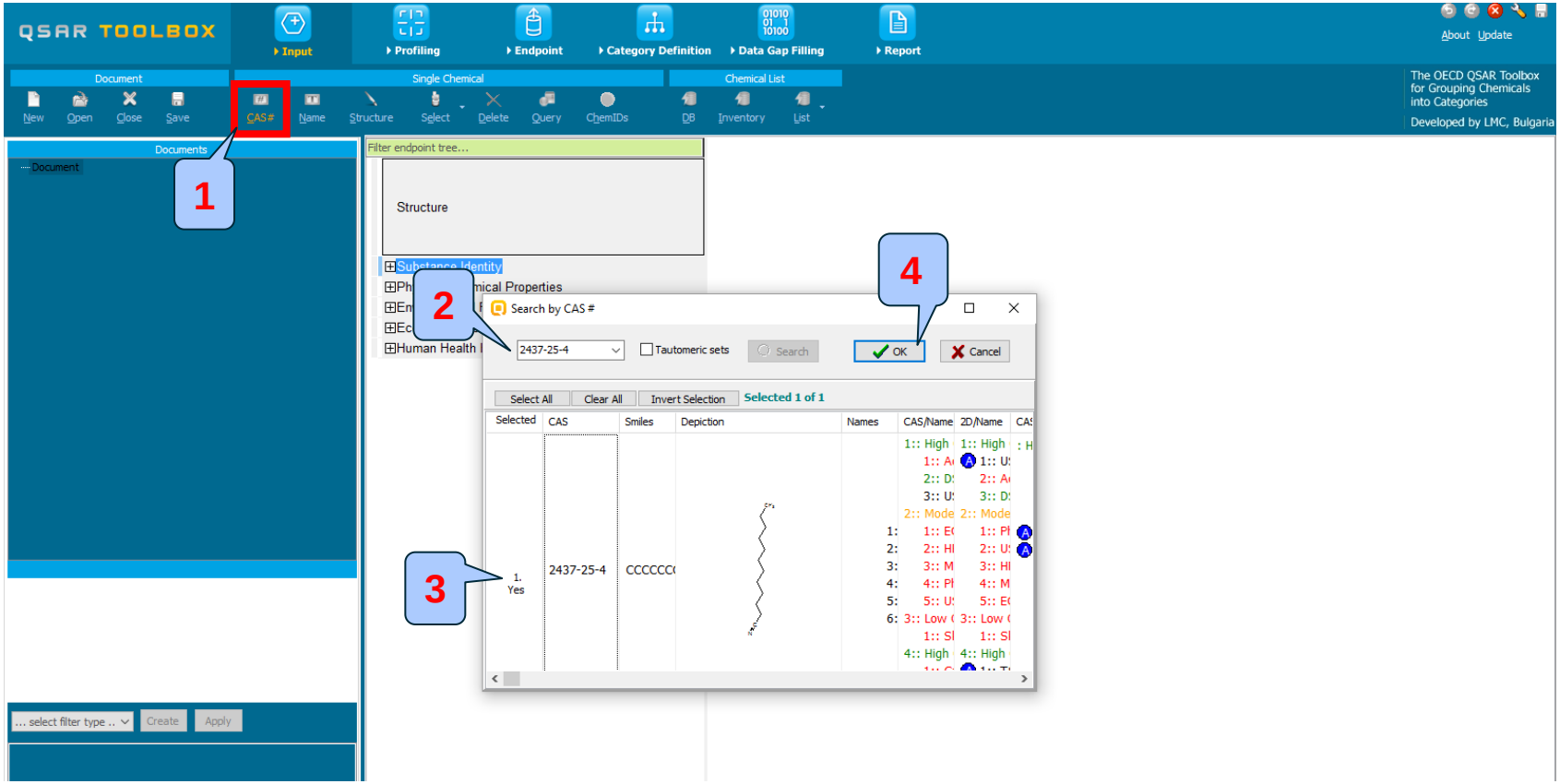
B. Group of chemicals
- User List/Inventory
- Specialized Databases

Chemical Input
Input Screen

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

Chemical Input

Input target chemical by CAS#

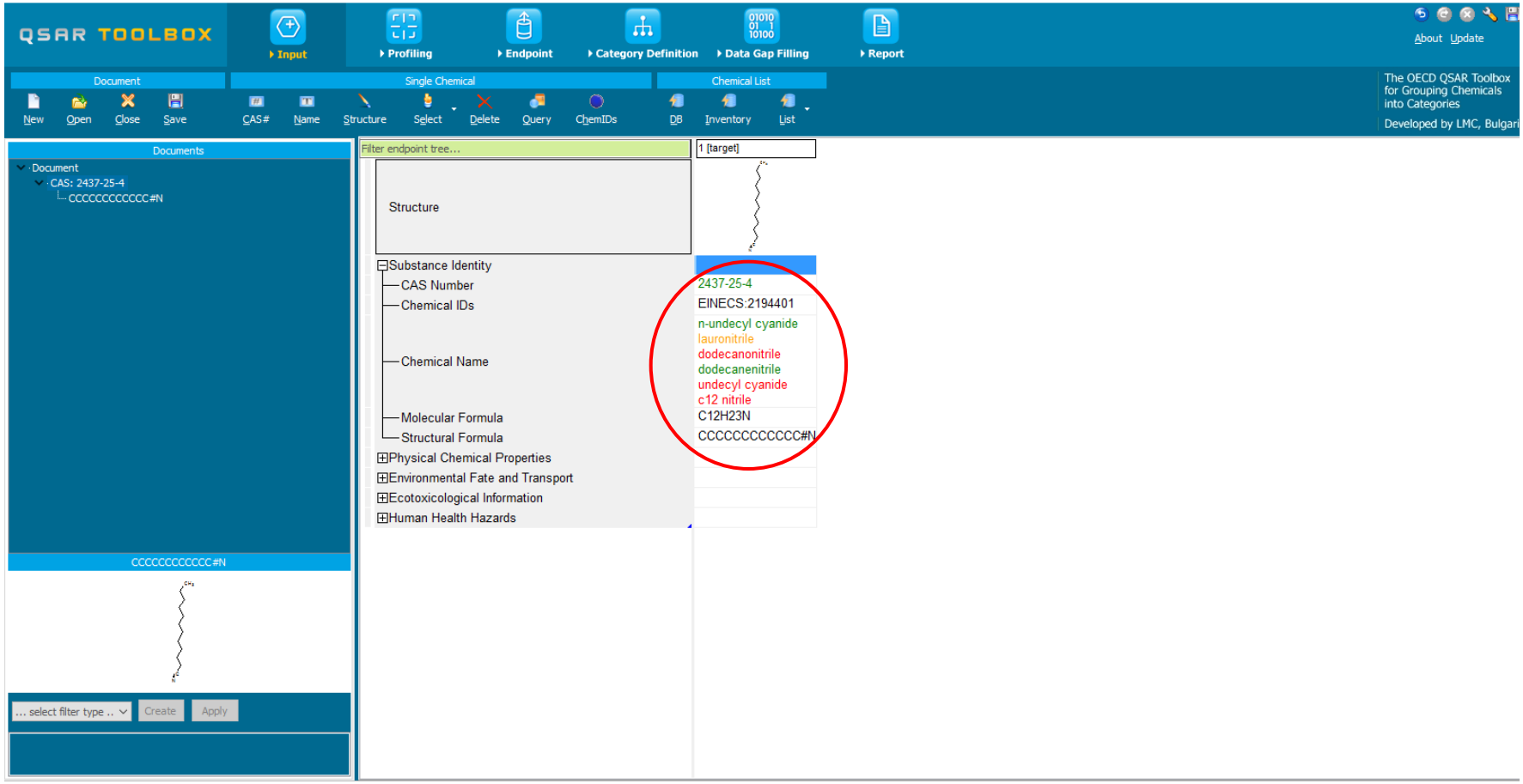


1. Click on CAS#; 2. **Enter** 2437-25-4; 3. The system identify the structure; 4. **OK**

Chemical Input
Target chemical identity

- Double click "Substance Identity" displays the chemical identification information.
- The user should note that existing names of the target chemical are presented in different colours. This indicates the reliability of relation CAS-Name for the target chemical(see next screen shots).
- The workflow on the first module is now complete, and the user can proceed to the next module.

Chemical Input
Target chemical identity



Chemical Input
Target chemical identity

The colour code indicates the reliability of the chemical identifier:

- **Green**: There is a high consistency between the identifier and the structure. This colour is applied if the identifier is the same in several quality assured databases.
- **Yellow:** There is only a moderate reliability between the identifier and the structure. The colour is applied if the identifier is the same in several databases for which the quality assurance could not be established.
- **Red**: There is a poor reliability between the identifier and the structure. The colour is applied if the identifier is allocated to different structures in different databases.

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - **Profiling**

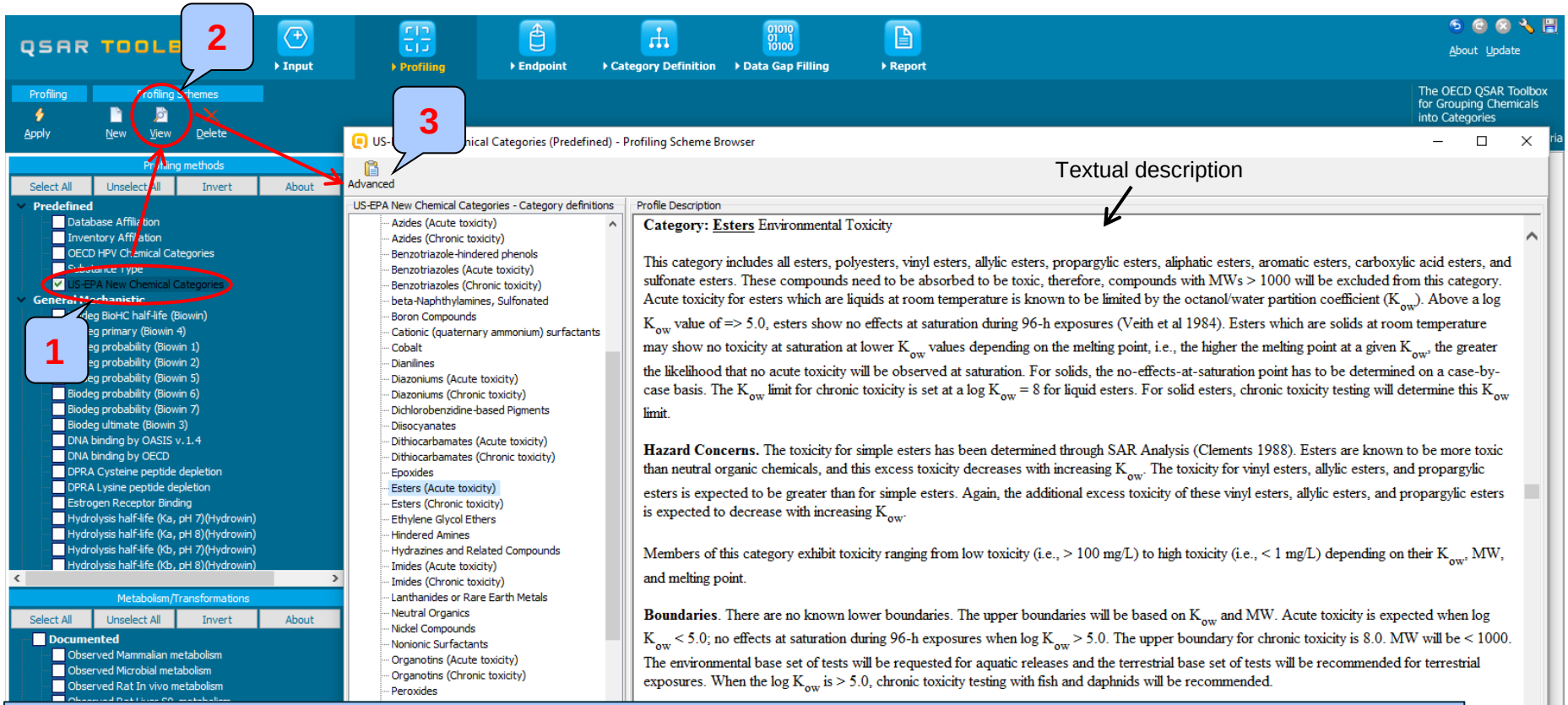
Profiling
Overview

- "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
Side-Bar to Profiling

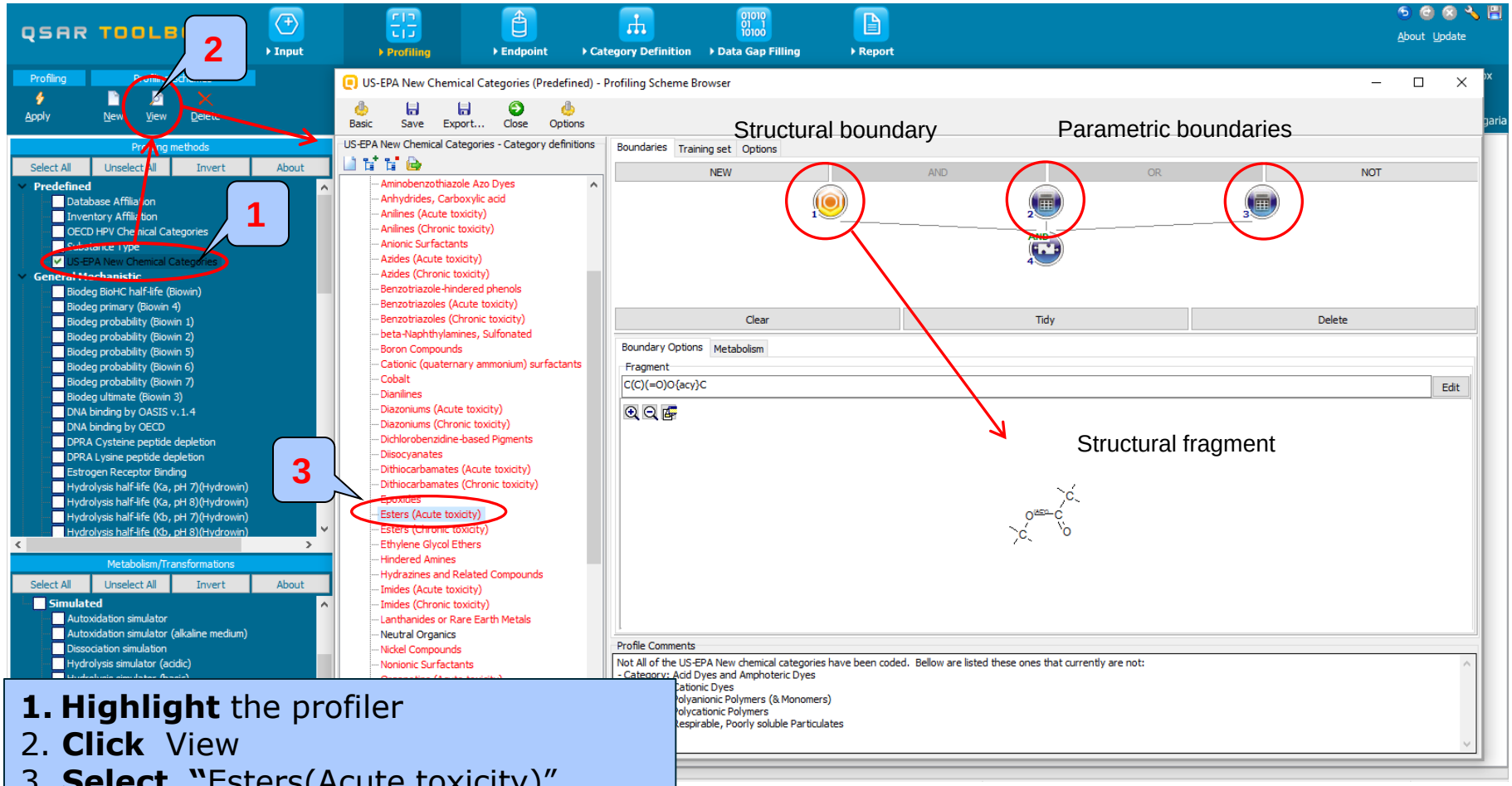
- For most of the profilers, background information can be retrieved by highlighting one of the profilers (for example, US-EPA New Chemical categories and clicking on “View” (see next screen shot).

Profiling
Side-Bar to Profiling



1. **Highlight** the profiler
2. **Click** View
3. **Click** Advance in order to see detailed description of highlighted category (in this case "Esters")

Profiling
Side-Bar to Profiling



1. **Highlight** the profiler
2. **Click** View
3. **Select** "Esters(Acute toxicity)"

Profiling

Profiling the target chemical

- The outcome of the profiling determines the most appropriate way to search for analogues (detailed information about profilers could be found in "Manual for Getting started" (Chapter 4) published on the OECD website:

  http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm

- Table 4 - 1 in chapter 4 (Manual for getting started) lists a selection of profilers and their relevance for different endpoints of regulatory relevance

- The following profiling schemes are relevant to the **Acute aquatic toxicity**:
  - Aquatic toxicity classification by ECOSAR
  - Acute aquatic toxicity MOA by OASIS
  - Acute aquatic toxicity classification by Verhaar
  - Protein binding alerts by OASIS v1.4
  - Protein binding by OECD
  - Organic function groups – all four profilers are used in the assessment
  - Chemical elements
- More details about profiling schemes used for categorization and collection of analogues is provided in stage "Category formation" on slide 50

Profiling
Profiling the target chemical

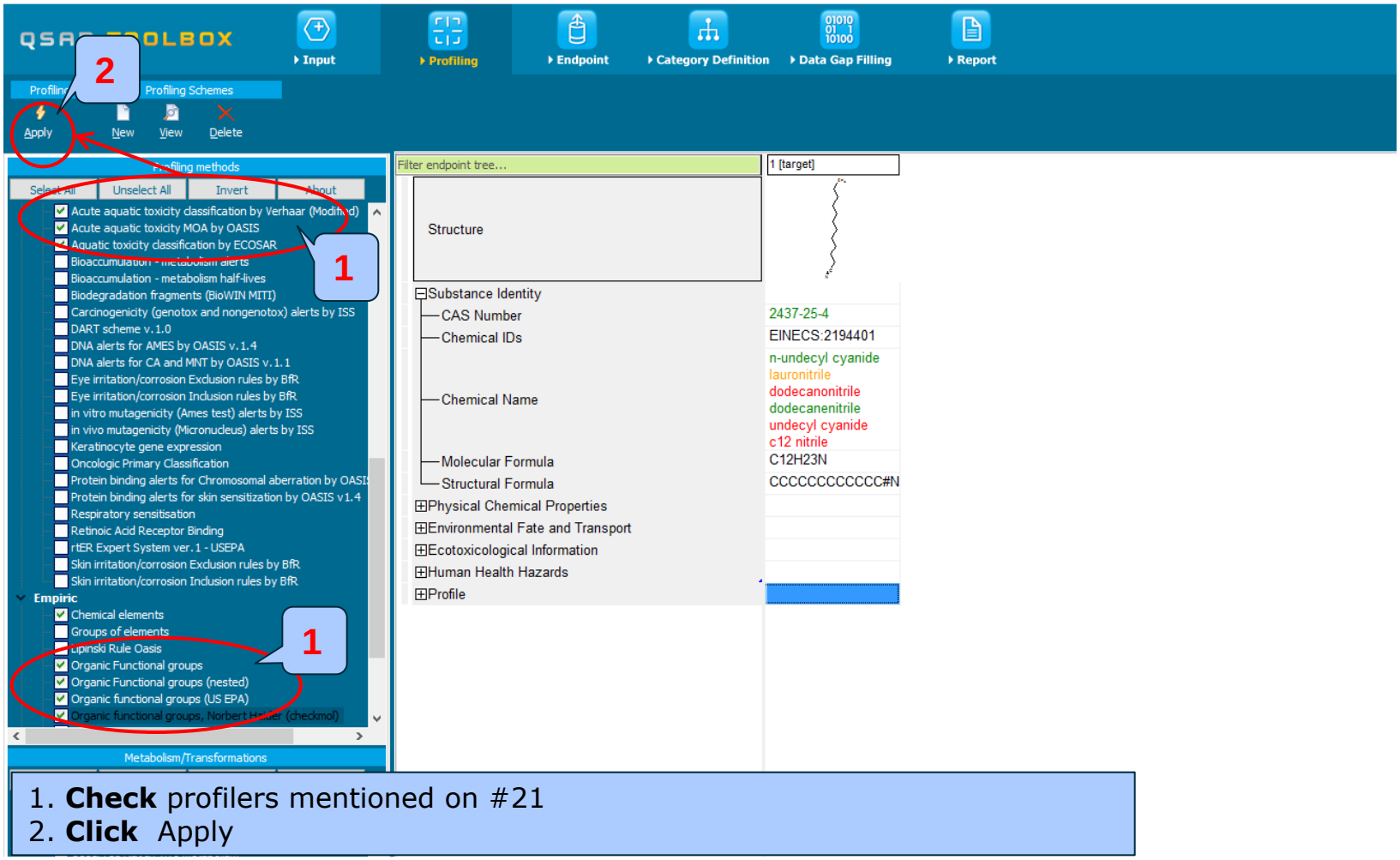
- Select the "Profiling methods" related to the target endpoint by clicking on the box next to the profilers name

- This selects (a **green** check mark appears) or deselects (**green** check mark disappears) profilers.

- For this example, go through the general and endpoint specific profiling mechanisms and highlight those that apply to acute aquatic toxicity(see next screen shot).

Profiling

Profiling the target chemical



1. **Check** profilers mentioned on #21
2. **Click** Apply

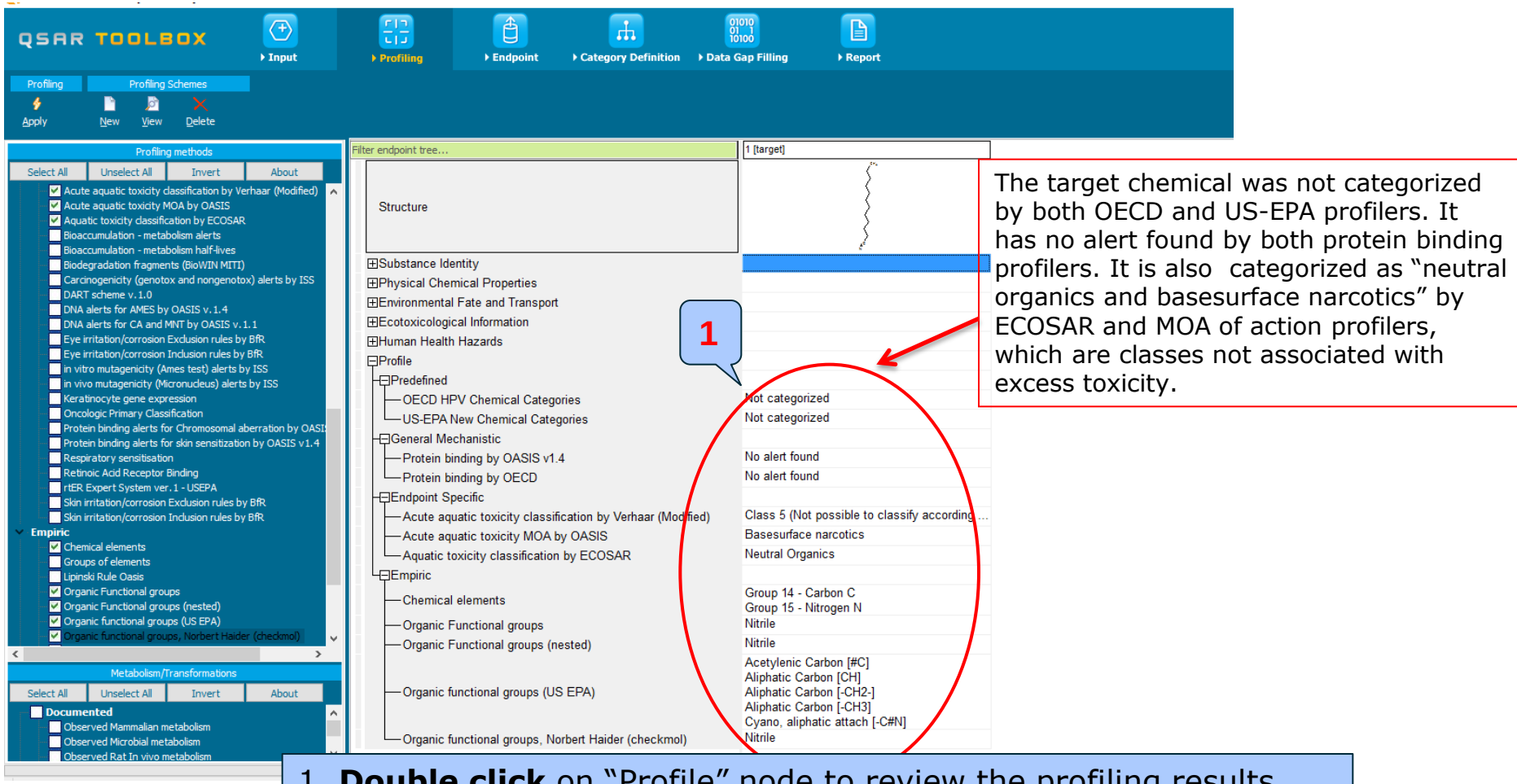
Profiling
Profiling the target chemical

- The actual profiling will take up to several seconds depending on the number and type of profilers selected
- The results of profiling automatically appear as a dropdown box under the target chemical (see next screen shot)
- Please note the endpoint specific profilers and structure based profilers such as US-EPA and ECOSAR
- No structural and endpoint specific alerts have been found for the test compound.

(see next screenshot)

Profiling

Profiling the target chemical



The target chemical was not categorized by both OECD and US-EPA profilers. It has no alert found by both protein binding profilers. It is also categorized as "neutral organics and basesurface narcotics" by ECOSAR and MOA of action profilers, which are classes not associated with excess toxicity.

1. **Double click** on "Profile" node to review the profiling results.

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - **Endpoint**

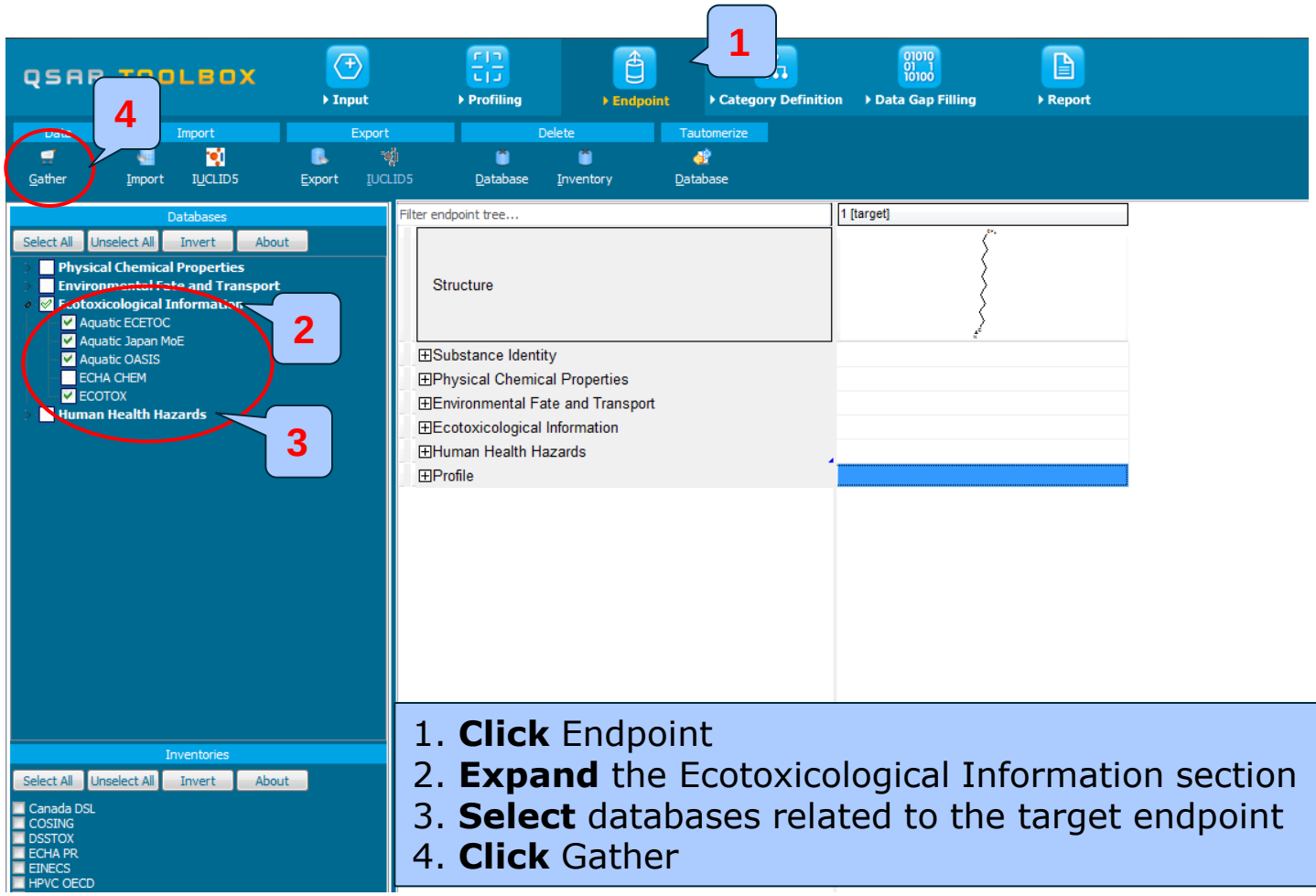
Endpoint
Overview

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

Endpoint
Case study

- In this example, we limit our data gathering to a single toxicity endpoint (acute aquatic toxicity).
- In this example, we collect data from the databases containing experimental results for acute aquatic toxicity (Aquatic toxicity OASIS; Aquatic ECETOC; Aquatic Japan MoE and ECOTOX).
- Click on "Endpoint" in the Toolbox workflow.
- Expand the "Ecotoxicological information" section
- Click on the box to select the relevant databases.
- Click on "Gather data" (see next screen shot).

Endpoint
Gather data



1. **Click** Endpoint
2. **Expand** the Ecotoxicological Information section
3. **Select** databases related to the target endpoint
4. **Click** Gather

Endpoint
Gather data

- Toxicity information on the target chemical is electronically 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 databases, which in this example are Aquatic toxicity OASIS; Aquatic ECETOC; Aquatic Japan MoE and ECOTOX

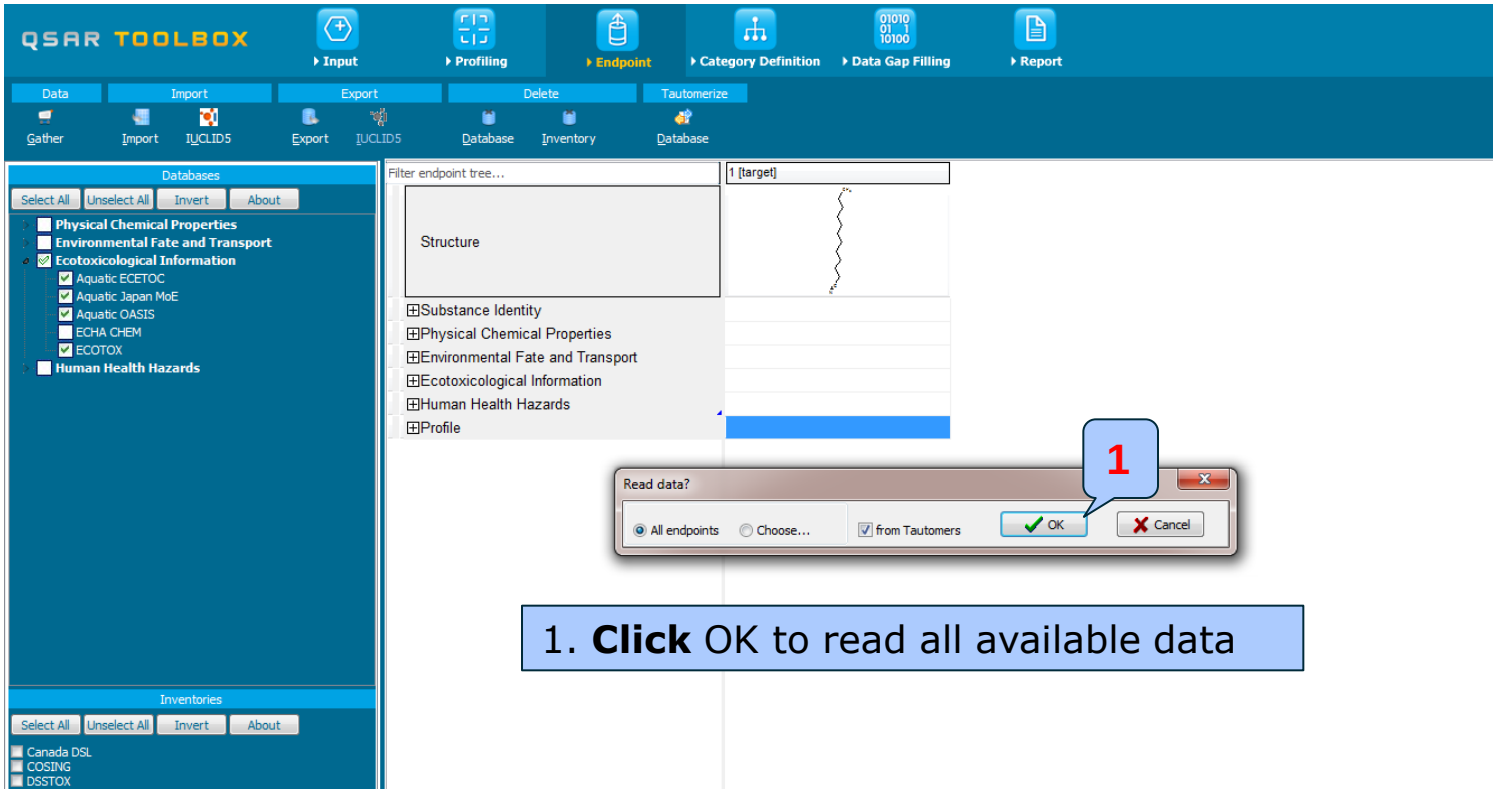
- In this example, there is LC50 experimental data for *P. promelas* (96h) for the target chemical (see next screen shots)

- The experimental data for the investigated endpoint falls within the toxic range (less than 1mg/l[1])

[1] Globally Harmonized System of Classification and Labeling of Chemicals (GHS):
http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev06/English/ST-SG-AC10-30-Rev6e.pdf

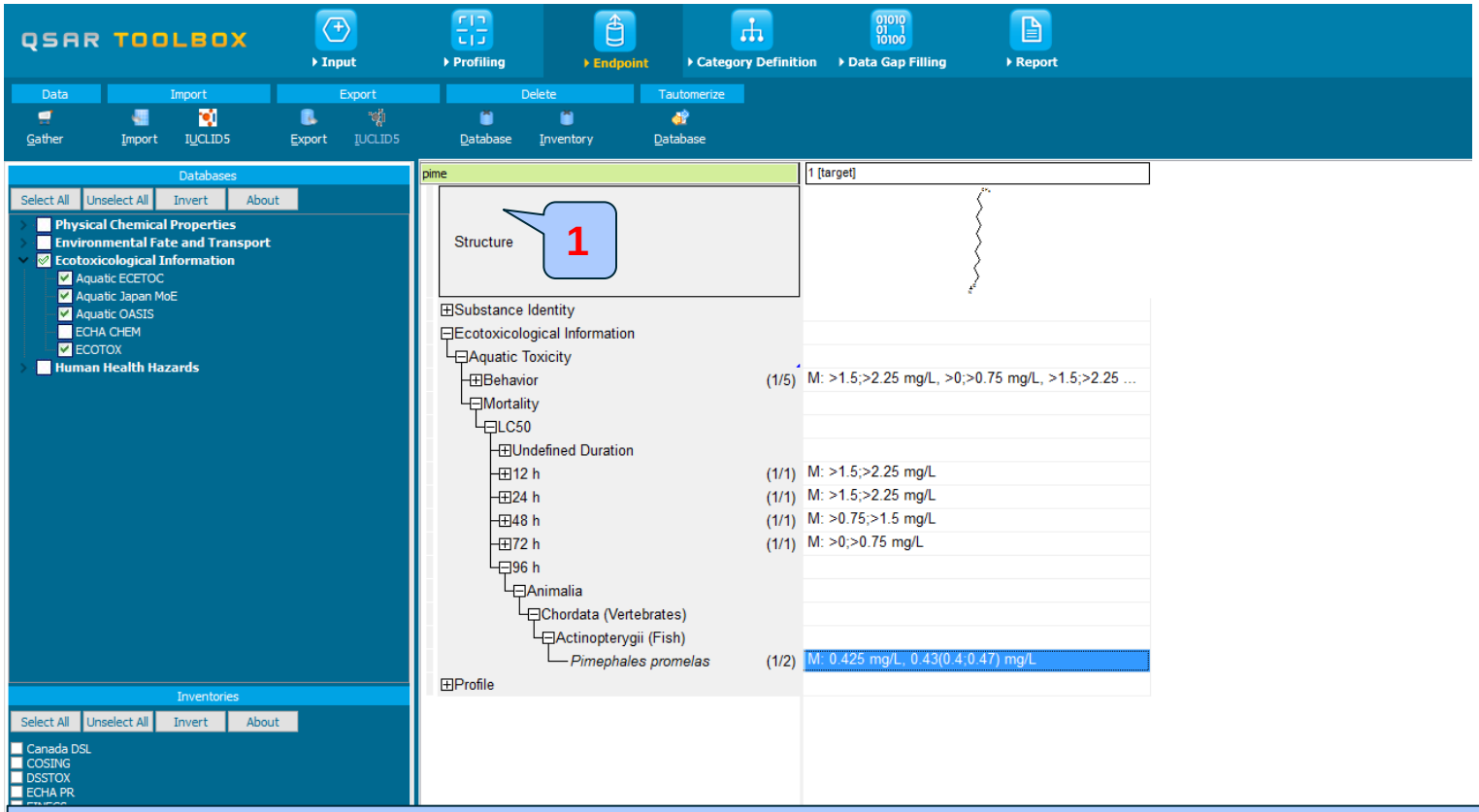
Endpoint
Gather data

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.



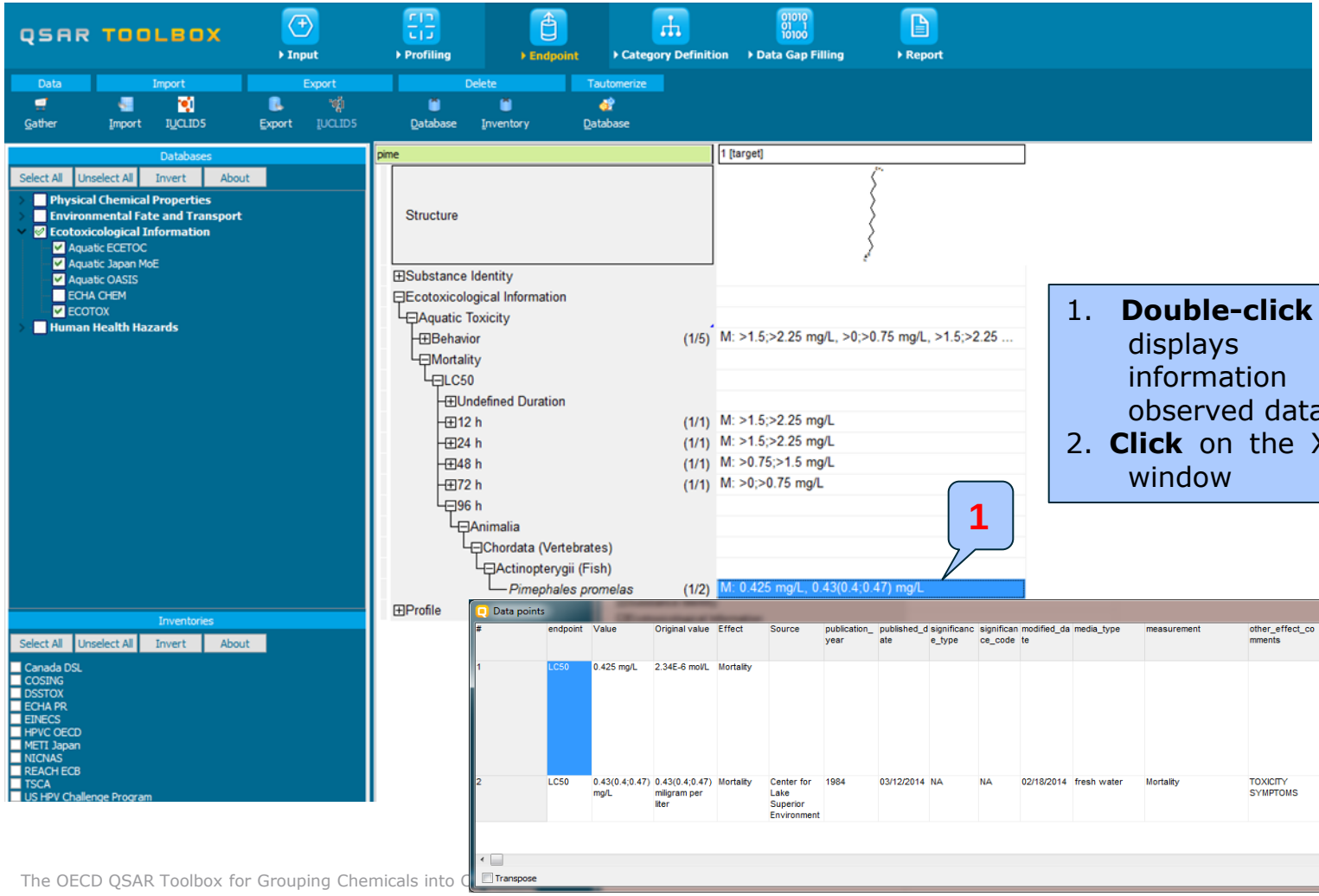
1. **Click** OK to read all available data

Endpoint
Gather data



1. **Type** "Pime" in the filter tree in order to filter the tree to the investigated endpoint
2. Available experimental data appears on datamatrix (LC50 0.425 mg/l species: *P.promelas,* duration: 96h)

Endpoint
Gather data



1. **Double-click** on the cell displays metadata information for the observed data
2. **Click** on the X to close the window

Recap

- The first module, which introduces the target chemical, ensure correctness of the structure
- The second module shows that there is no structural or endpoint specific alerts for target chemical
- In the third module, you have found that the target chemical has toxic experimental data for the investigated endpoint
- The study continues with accounting for tautomersim of target chemical trying to explain toxic experimental data of the target chemical (see next slides).

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - **Handling of tautomerism of target chemical**

Handling of tautomerism of target chemical

Visualization of modeling modes

There are two ways for simulating tautomers of chemicals

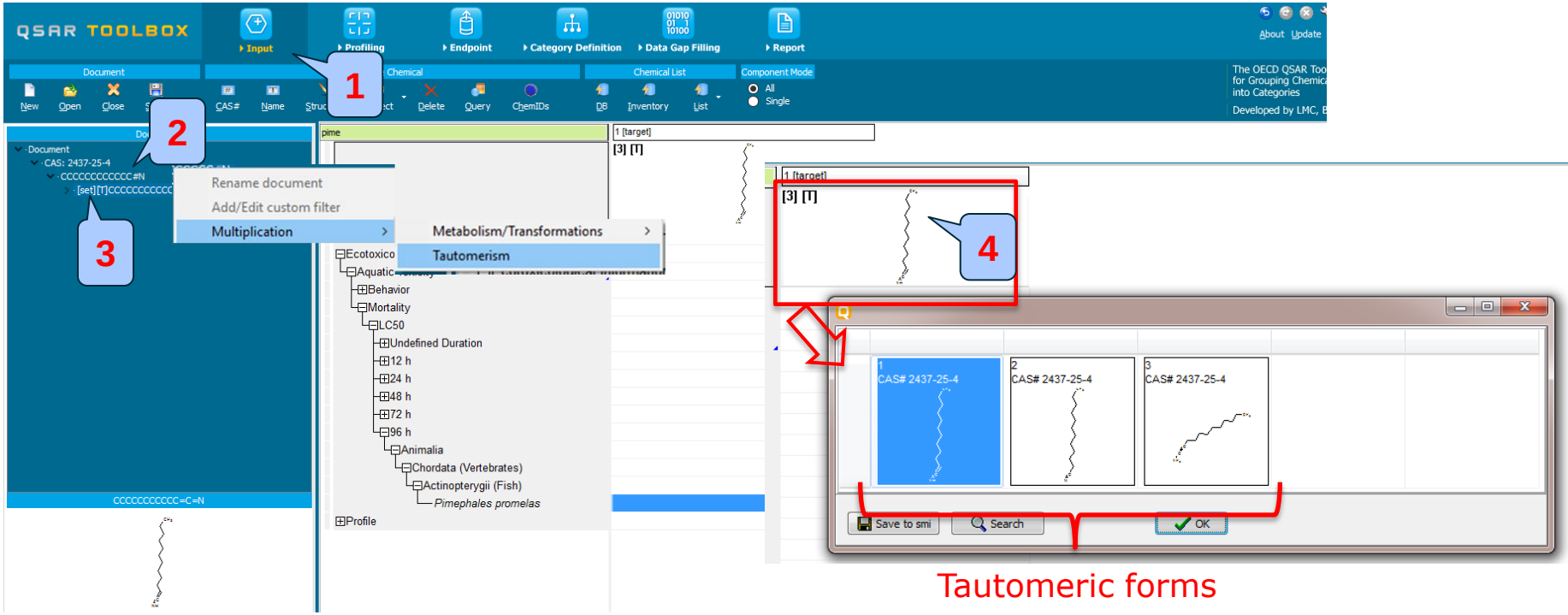
- During the process of entering the structure into the system
- Simulating tautomersim of already entered structure

In order to accelerate the workflow all databases are preliminary tautomerized, calculated and profiled. The results are stored in the database.

See next screen shots

Handling of tautomerism of target chemical

Visualization of modeling modes



Tautomeric forms

1. **Go** to Input
2. **Right click** over the node with SMILES and **select** Multiplication and then **Tautomerism**
3. **Open** the tree to the end leaf wit [set] indication
4. Three tautomeric forms are generated for the target chemical. Double click over the 2D structure with [3][T]

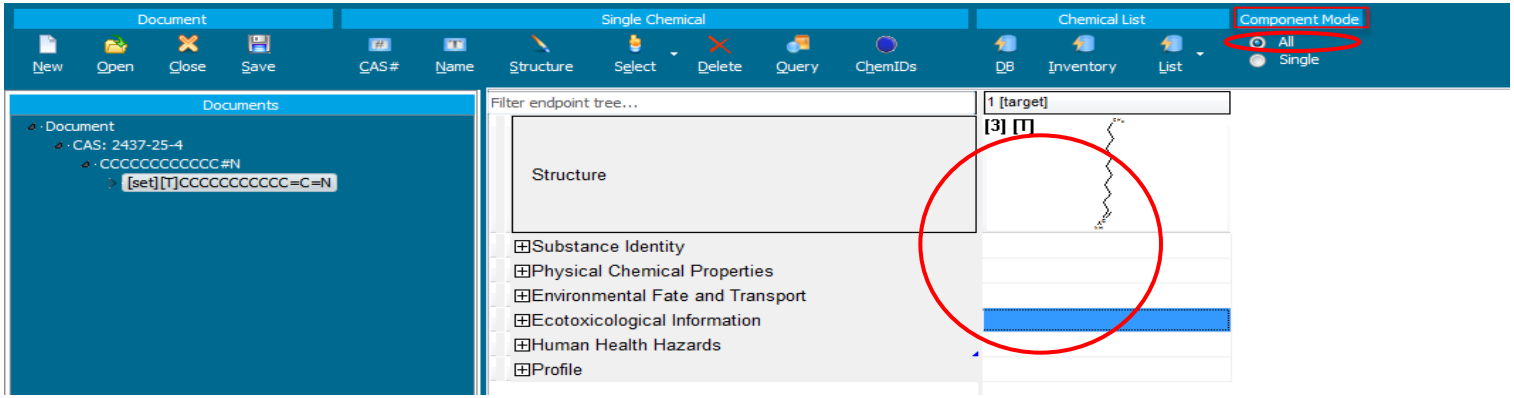
Handling of tautomerism of target chemical
Visualization of modeling modes

- Two component modes are implemented:
  - Set Mode - all tautomers are analyzed as a package
  - Individual Component Mode - each tautomer is analyzed individually

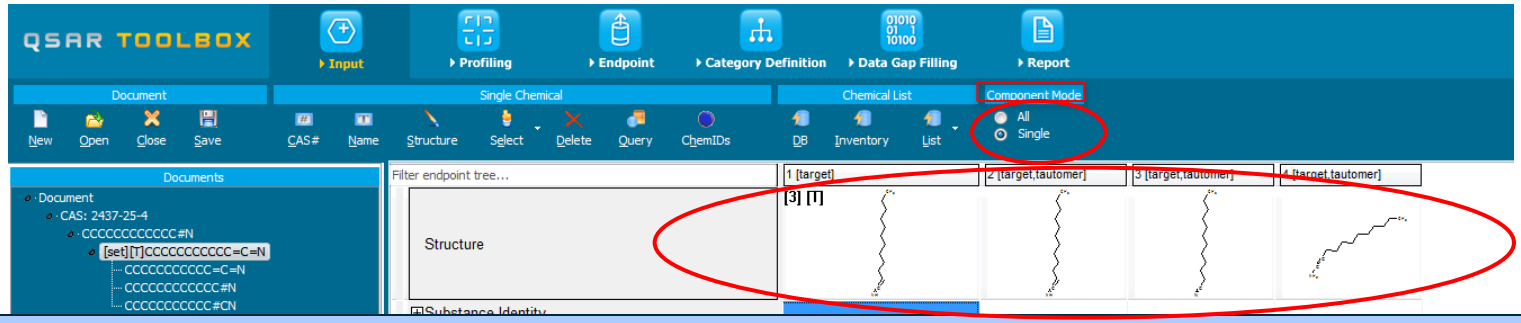
(see next screen shot)

Handling of tautomerism of target chemical
Visualization of modeling modes

- **Component Mode All –** all tautomeric forms are analyzed in a package



- **Component Mode Single** – each tautomeric form is analyzed individually



Different modes for visualization for the set of target and its tautomeric forms is implemented. The latter are visualized when the node with [set] is selected. Single mode is used in further trend analysis.

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
    - **Profiling set of tautomers**

Handling of tautomerism of target chemical
Profiling set of tautomers

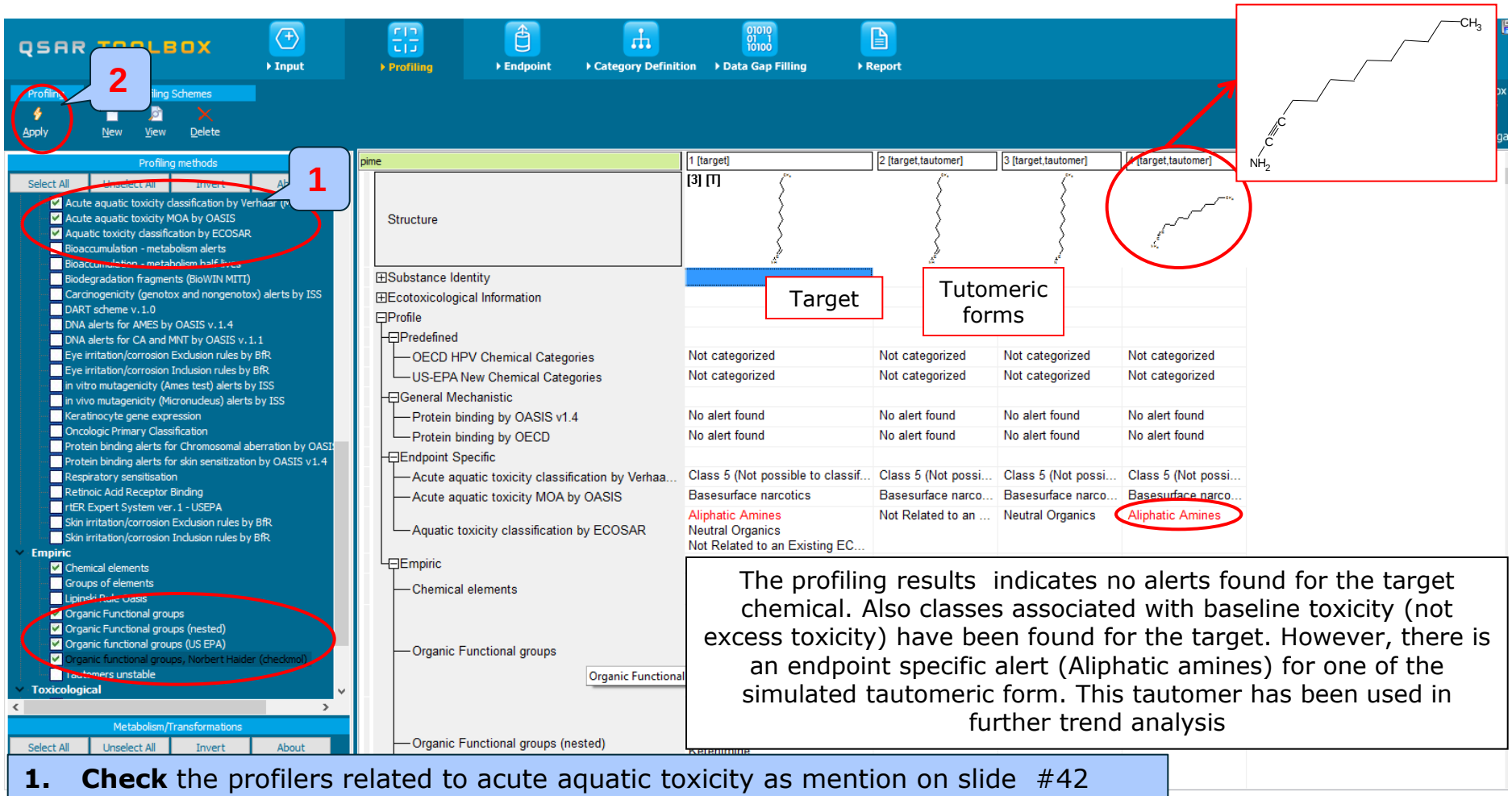
- This module identifies profilers of target chemical and its tautomeric forms
- Endpoint specific and structurally based profiles related to acute aquatic toxicity are applied on the set of tautomers
- Profiling results of tautomers are illustrated in Single Component mode
- Click on "Profiling" to go to the required module (see next screen shots)

Handling of tautomerism of target chemical
Profiling set of tautomers

- The following primary profilers relevant to the aquatic toxicity are used in this example(see next screenshot):
  - OECD HPV Chemical Categories
  - US-EPA New chemical category
  - Aquatic toxicity classification by ECOSAR
  - Acute aquatic toxicity MOA by OASIS
  - Acute aquatic toxicity classification by Verhaar
  - Protein binding by OASIS v1.4
  - Protein binding by OECD
  - Organic function groups – all four profilers are used in the assessment
- Select the "Profiling methods" related to the target endpoint by clicking on the box next to the profilers name.
- This selects (a green check mark appears) or deselects(green check disappears) profilers.

Handling of tautomerism of target chemical

Profiling set of tautomeric forms



The profiling results indicates no alerts found for the target chemical. Also classes associated with baseline toxicity (not excess toxicity) have been found for the target. However, there is an endpoint specific alert (Aliphatic amines) for one of the simulated tautomeric form. This tautomer has been used in further trend analysis

1. **Check** the profilers related to acute aquatic toxicity as mention on slide #42
2. **Click** Apply

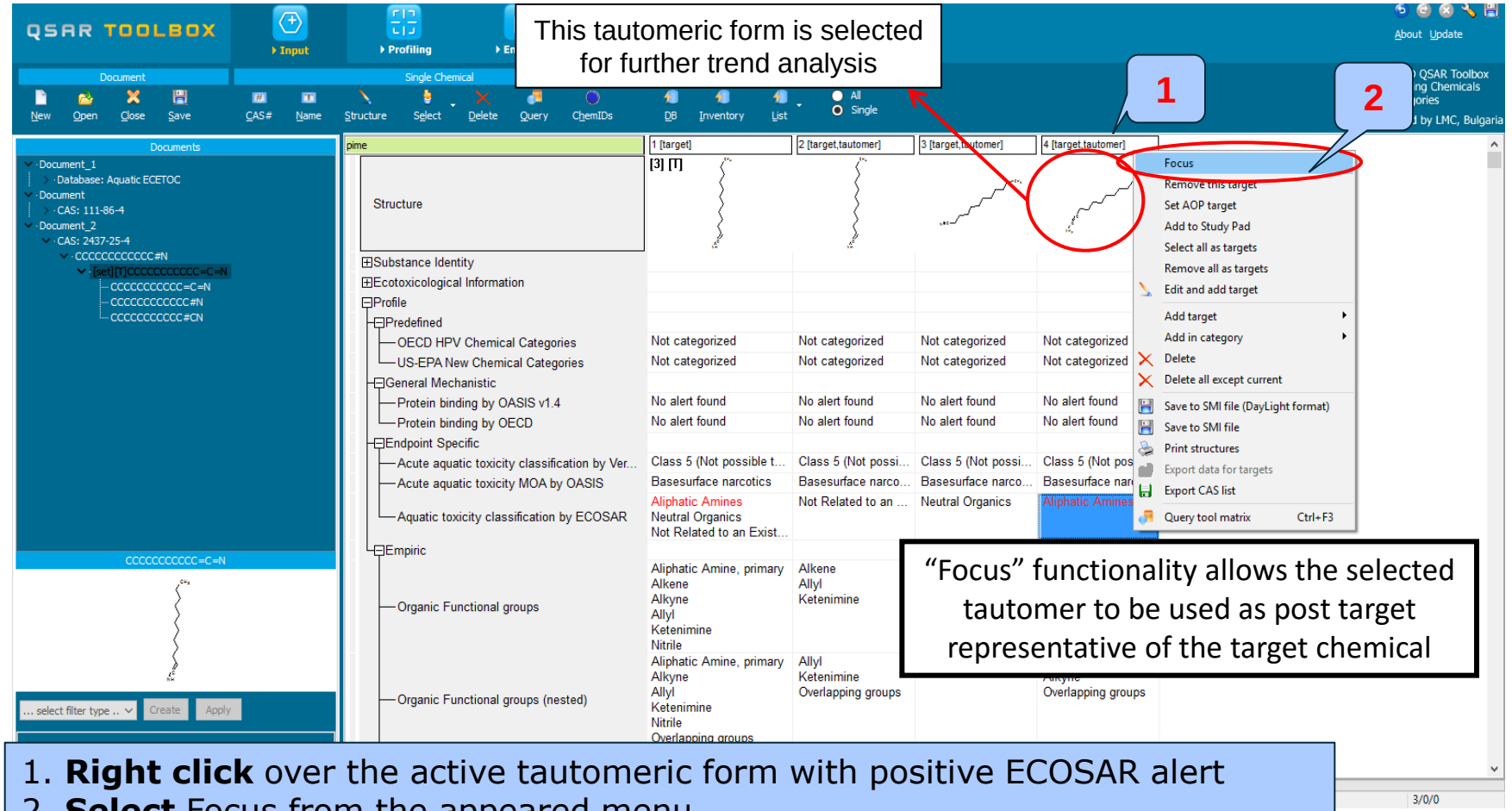
Handling of tautomerism of target chemical
Recap

- The profiling results indicates no endpoint specific or active structural alerts for target chemical
- One of the simulated tautomeric form has positive endpoint specific alert identified by ECOSAR
- The reactive tautomer is used for further trend analysis
- The next two parts of the exercise will focus the reactive tautomer and identify the category of similar analogues (see next screenshots).

Outlook

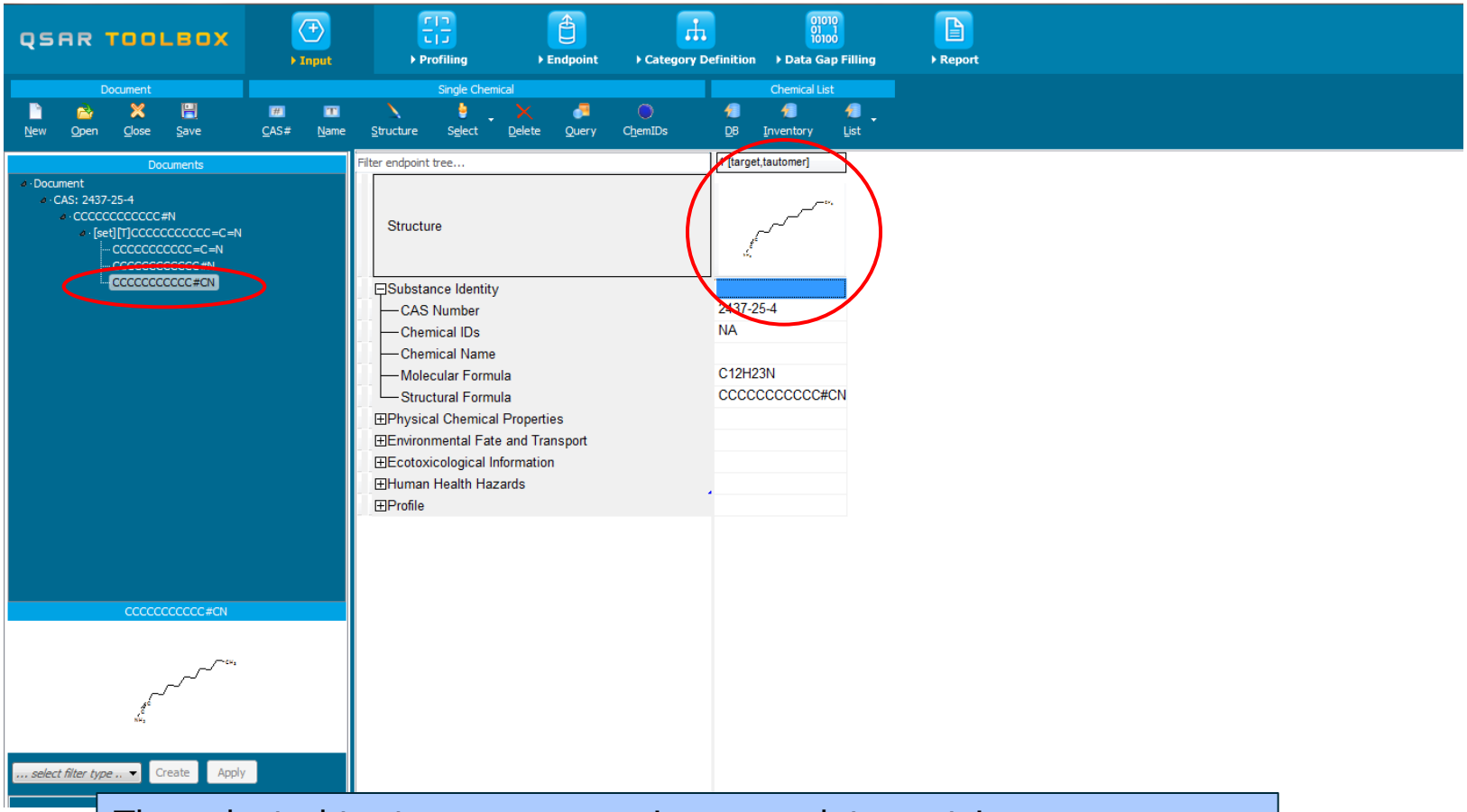
- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
    - Profiling set of tautomers
    - **Focus active tautomer**

Handling of tautomerism of target chemical
Focus of active tautomer



1. **Right click** over the active tautomeric form with positive ECOSAR alert
2. **Select** Focus from the appeared menu

Handling of tautomerism of target chemical
Focus of active tautomer



The selected tautomer appears in a new data matrix.

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
    - Profiling set of tautomers
    - Focus active tautomer
    - **Defining category for active tautomer**

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 read-across/trend analysis.

- Detailed information about grouping chemical (Chapter 4) could be found in document "Manual for Getting started" published on OECD website:

  http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm

Basic guidance for category formation and assessment

Usually, a three stages procedure is recommended for building categories for read-across, in Toolbox. The categorization phases could be organized as follows:

- Stage I: Broad and endpoint non-specific primary categorization of chemicals based on their belonging to common chemical classes, predefined categories or being structurally similar
- Stage II: Subcategorization based on mechanisms conditioning the target endpoint thus coming to endpoint specific subset of chemicals reacting by same interaction mechanisms.
- Stage III: Further narrowing down the category based on elimination of chemicals most dissimilar to target one by using additional structure-related profilers

This sequence of stages is not mandatory and depends on the specificity and number of the chemical analogues and target endpoint. Moreover, some of the stages could be skipped if consistency of category members is reached earlier. It is also recommended only primary categorization to be applied in the Category Definition phase of the Toolbox workflow whereas the subcategorization to be applied at Data gap filling phase; thus, one could follow up the effect of subcategorization on the read-across results (having visualization of the endpoint vs. parameter relationship).

The structural similarity is not recommended to be applied as primary categorization. However, often it is needed to be used in the last stage of the subcategorization – for eliminating most dissimilar chemicals. This holds for read-across implementation for any endpoint.

Graphical illustration of suitable categorization phases is shown on next slide

Suitable Categorization/Assessment Phases

Phase I. Structure based

- US EPA Categorization
- OECD Categorization
- Organic functional group
- Structural similarity
- ECOSAR

Repeating Phase I due to Multifunctionality of chemicals

Broad grouping
Endpoint Non-specific

Phase II. Mechanism based*

- DNA binding mechanism
- Protein binding mechanism
- Genotoxicity/carcinogenicity
- Cramer rules
- Verhaar rule
- Skin/eye irritation corrosion rules

Metabolism accounted for

Subcategorization
Endpoint Specific

Phase III. Eliminating dissimilar chemicals

Apply Phase I – for structural dissimilarity
Filter by test conditions – for Biological dissimilarity

Subcategorization
Endpoint Specific

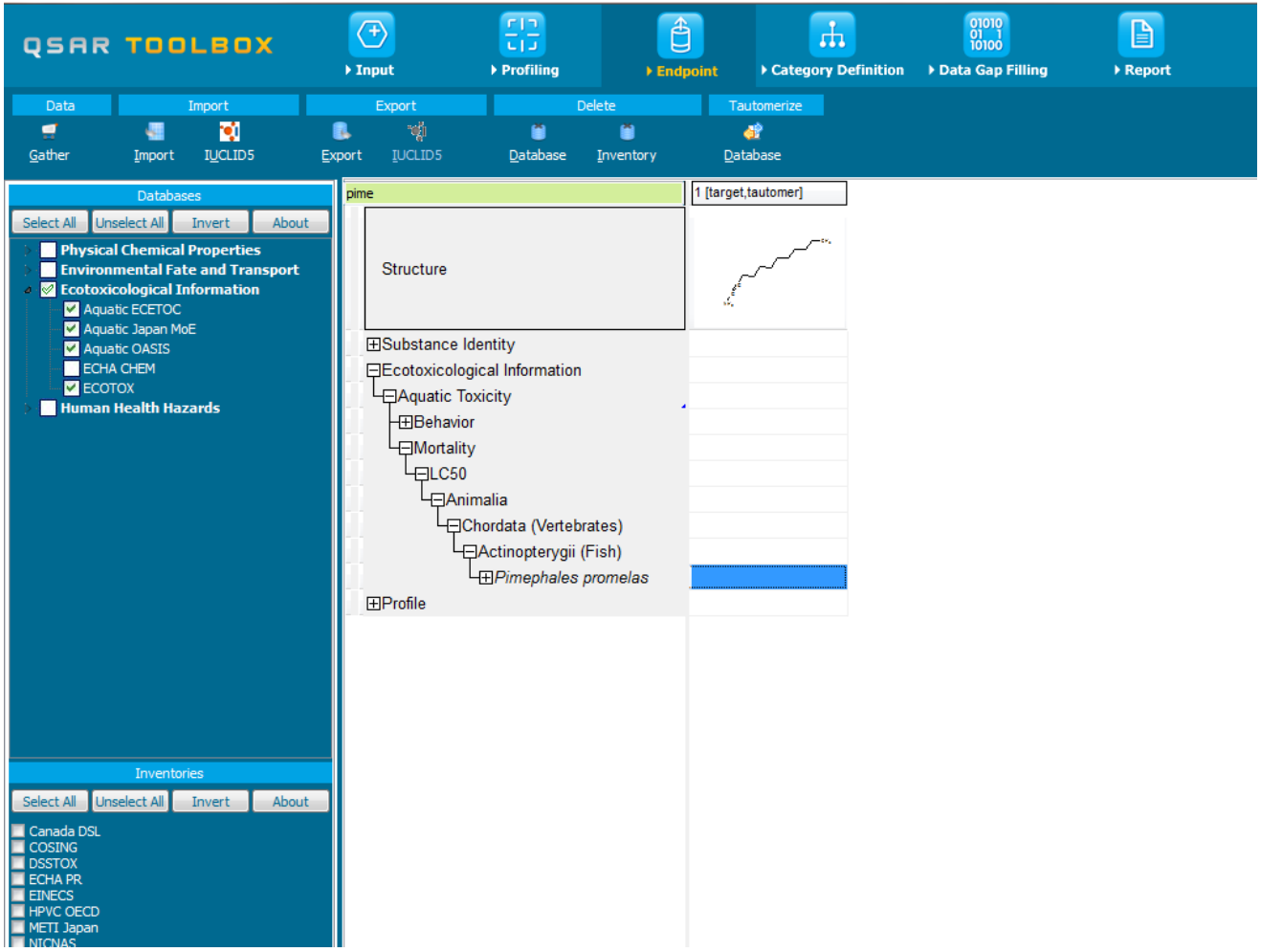
*General strategy not specific to the investigated acute aquatic tox endpoint

Handling of tautomerism of target chemical
Category definition for active tautomeric form

- In this exercise, the active tautomer is classified as: Aliphatic amine by ECOSAR category (phase I)
- Searching for similar analogues of the selected active tautomeric form is accomplished using ECOSAR category
- Searching for similar analogues is accomplished using four acute aquatic toxicity databases: Aquatic toxicity OASIS; Aquatic ECETOC; Aquatic Japan MoE and ECOTOX
- Before defining the category make sure that four aquatic aquatic databases have been selected (see next screenshot)

Handling of tautomerism of target chemical

Check databases

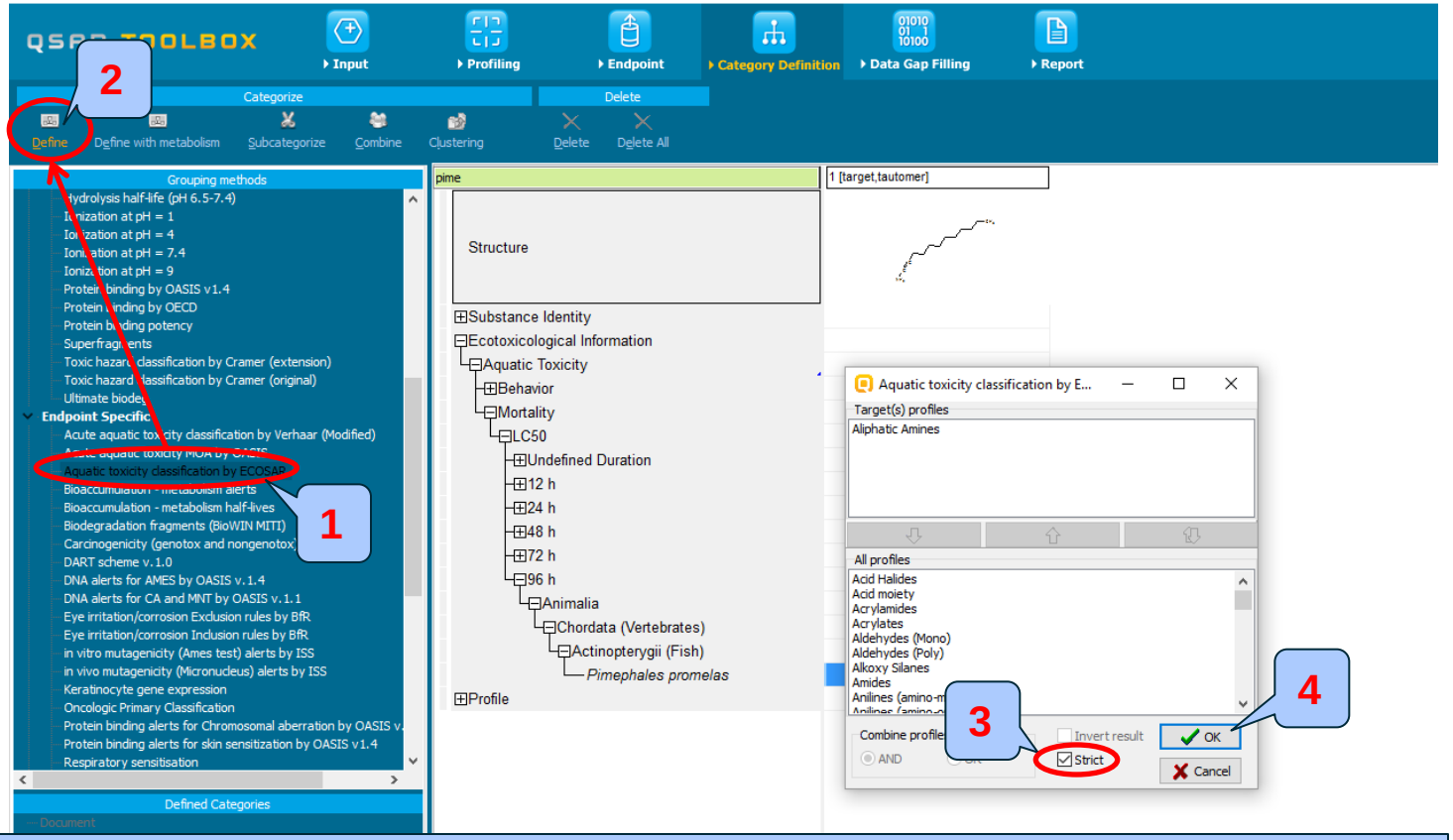


Handling of tautomerism of target chemical
Defining ECOSAR category

- The category ECOSAR (strict) is used
- **Strict** functionality means that the software will identify analogues having ONLY the categories of the target (e.g. aliphatic amines) and will exclude the analogues having any other categories
- Select Aquatic toxicity classification by ECOSAR category
- Click Define (see next screen shots)

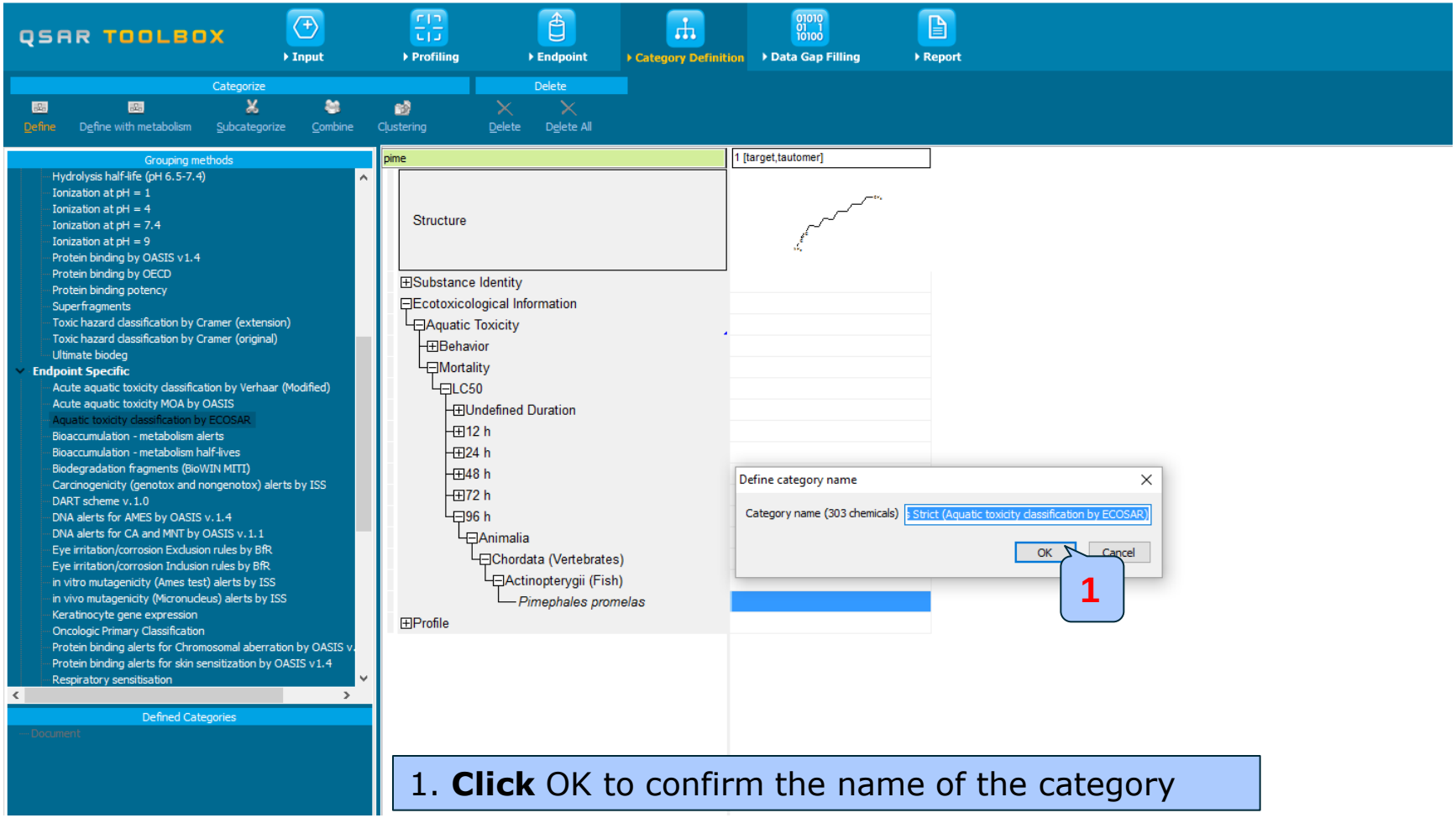
Handling of tautomerism of target chemical

Defining ECOSAR category



1. Highlight "Aquatic toxicity classification by ECOSAR" 2. **Click** Define **3. Select** Strict 4. **Click** OK to confirm the category **Aliphatic amines** defined by ECOSAR.

Handling of tautomerism of target chemical
Defining ECOSAR category



1. **Click** OK to confirm the name of the category

Handling of tautomerism of target chemical
Category analogues

- The Toolbox now identifies all chemicals corresponding to *Aliphatic amines* by ECOSAR listed in the four aquatic databases.

- 303 analogues including the target chemical are identified; they form a mechanistic category named "**Aliphatic amines**", which will be used for further data gap filling.

- The experimental data for analogues in the category appears on datamatrix

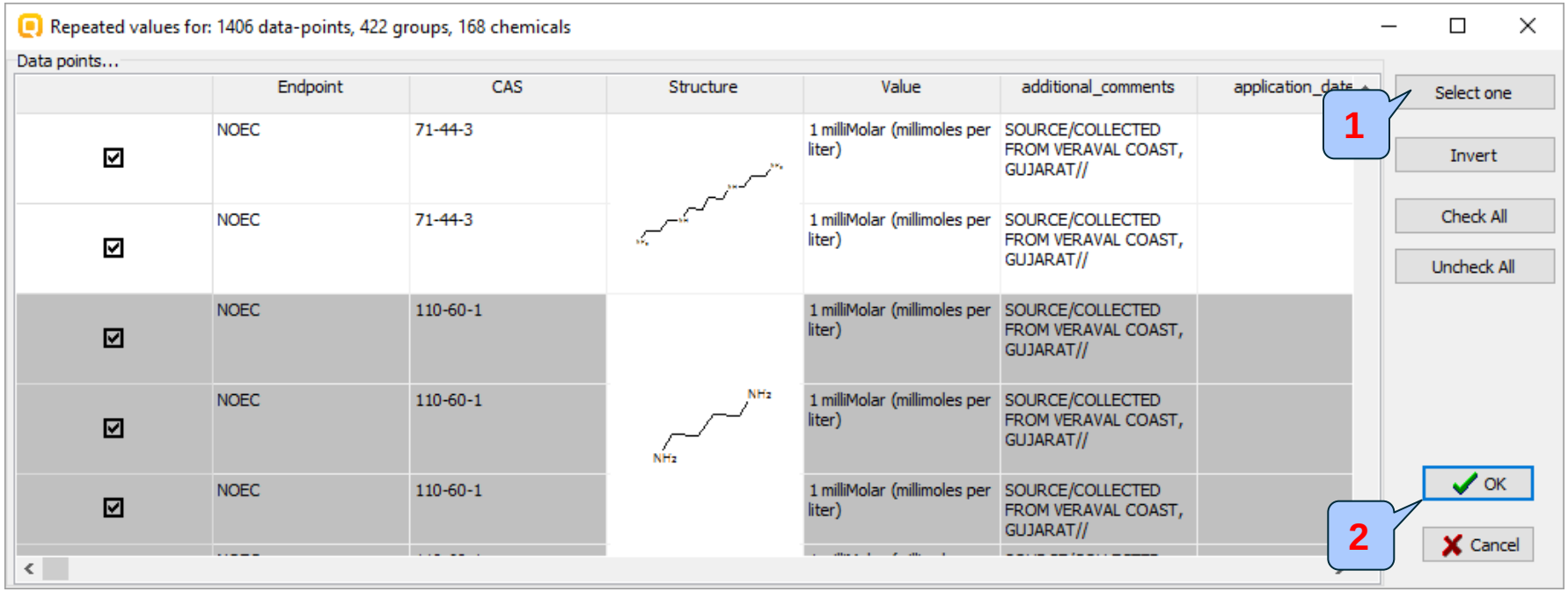
Handling of tautomerism of target chemical
Read data for Analogues

- 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.

Handling of tautomerism of target chemical
Read data for Analogues

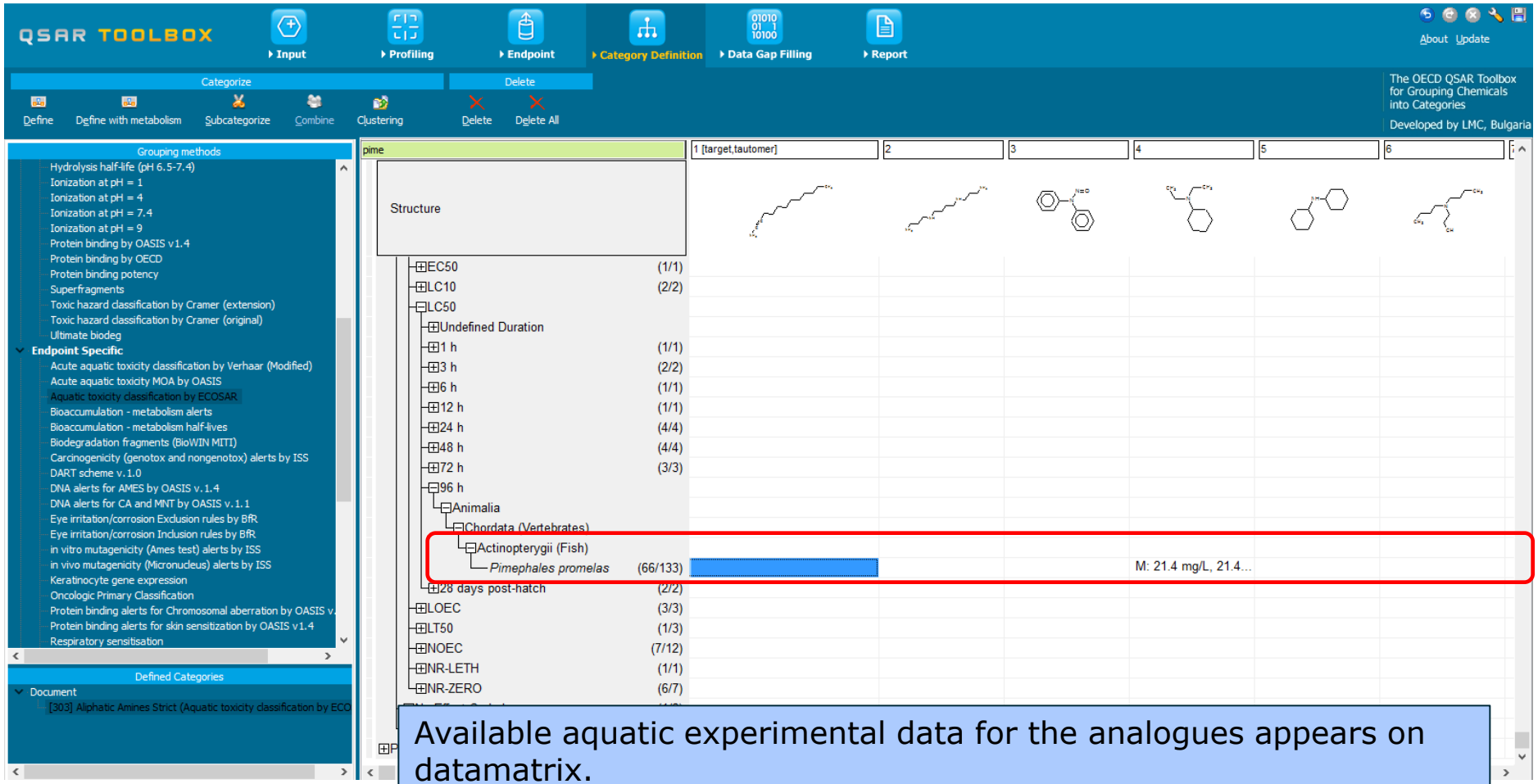
Due to overlap between the Toolbox databases for intersecting chemicals the same data may be found simultaneously. Data redundancies are identified and the user has the opportunity to select either a single data value or all data values.



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

Handling of tautomerism of target chemical

Summary information for Analogues



Available aquatic experimental data for the analogues appears on datamatrix.

Recap

- You have identified a category (“Aliphatic amines”) with the “Acute aquatic toxicity classification by ECOSAR” profiler for the target chemical *Dodecanenitrile (CAS 2437-25-4)*

- The available experimental results for these 303 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.

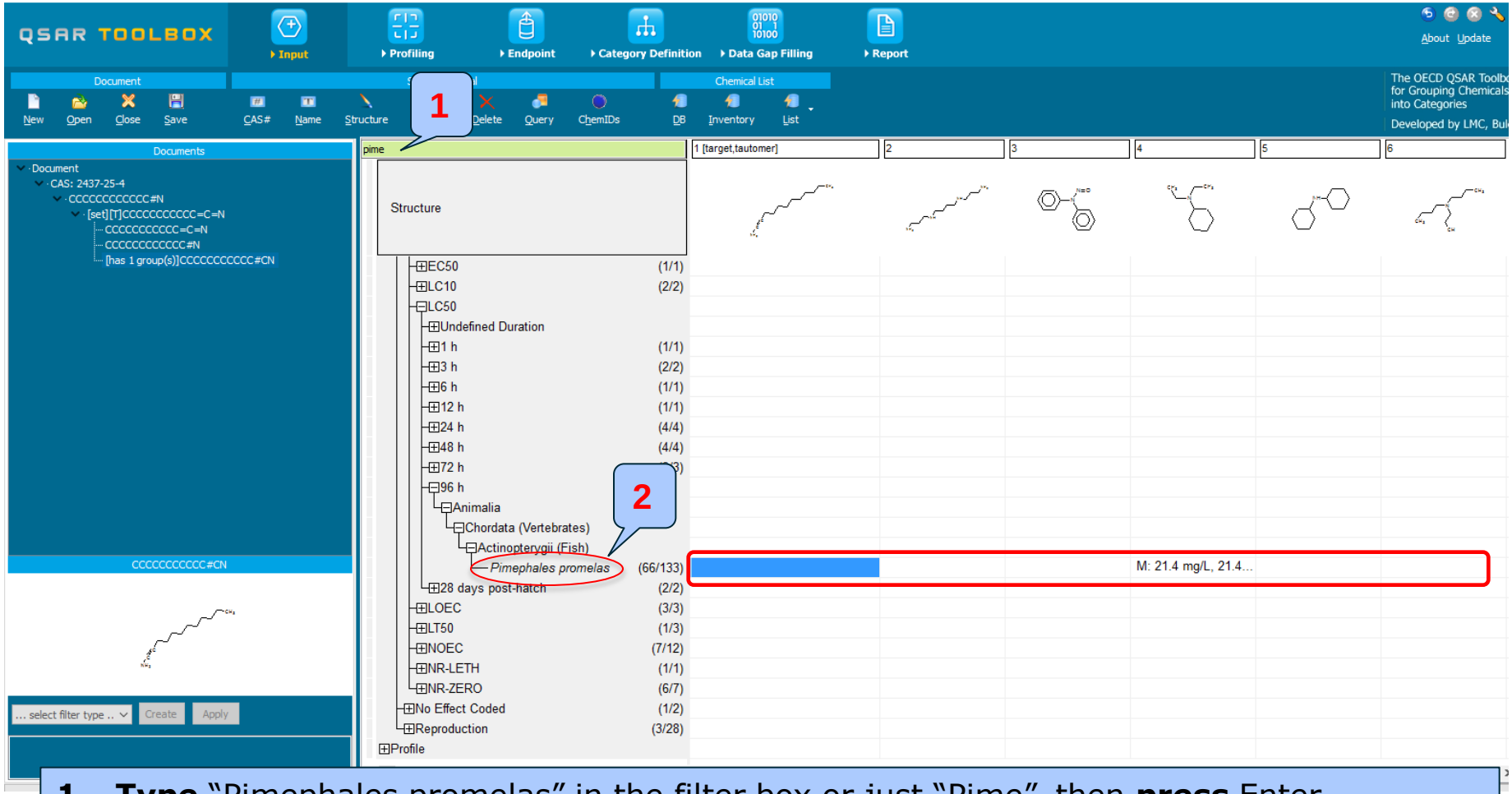
Handling of tautomerism of target chemical

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 "96 h LC50 Mortality for *Pimephales promelas*" is the target endpoint.
- You can navigate through the endpoint tree by typing the species "*Pimephales promelas*" in the "Filter endpoint tree…" box and double click on Aquatic Toxicity, Mortality, LC50, 96 h, Animalia, etc to *Pimephales promelas* - the specific endpoint (see next screenshot)

Handling of tautomerism of target chemical

Navigation through the endpoint tree



1. **Type** "Pimephales promelas" in the filter box or just "Pime", then **press** Enter
2. **Open** the tree to the target endpoint by **single left** click on the ⊞ sign

Recap

- You have now retrieved the available experimental data on aquatic toxicity for 303 analogue chemicals of focused tautomeric form classified as “Aliphatic amines” by the “ECOSAR” profiler.

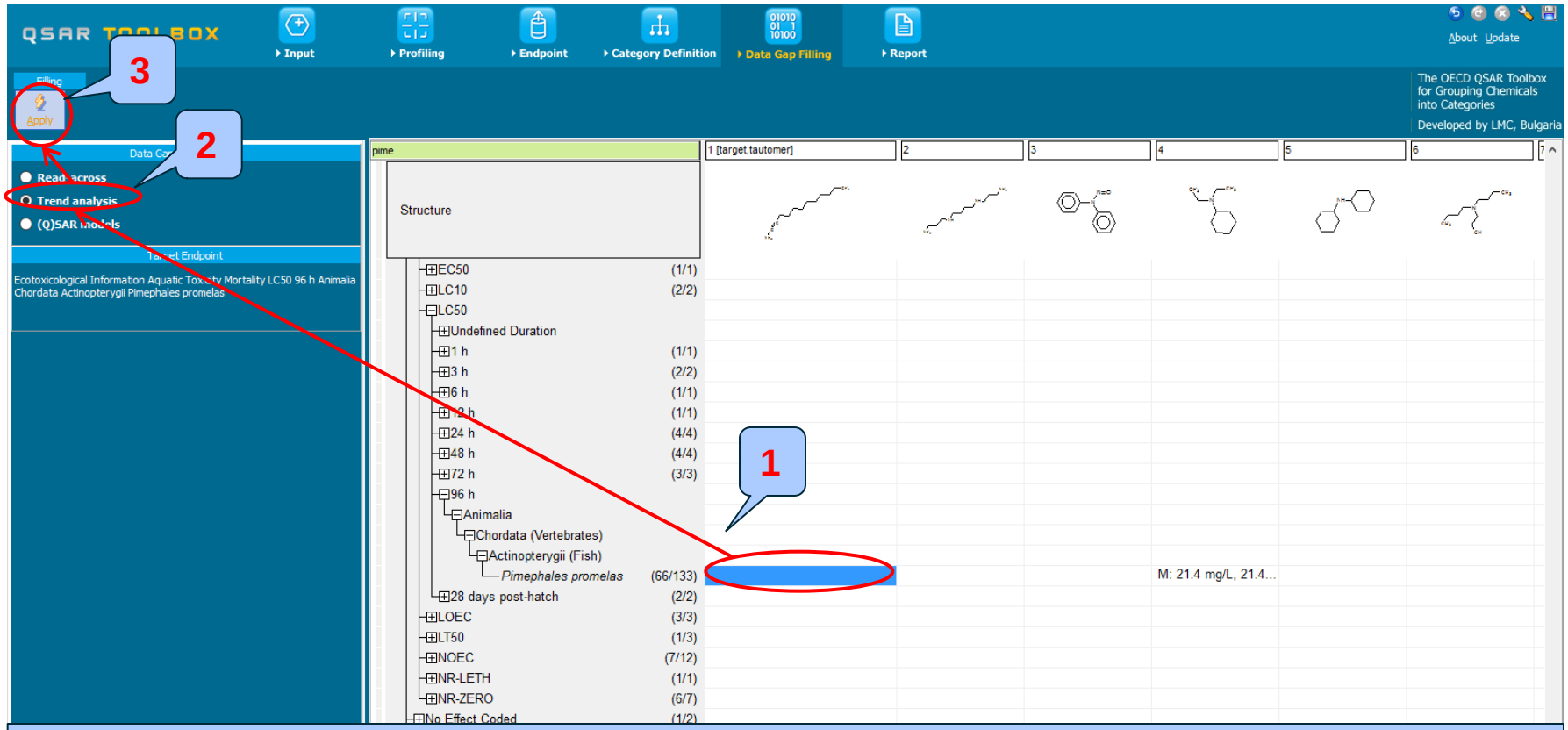
- You have identified the target endpoint of “96 h LC50 Mortality for *Pimephales promelas*”.

- You are ready to fill in the data gap so click on “Data Gap Filling” (see next screen shots).

Outlook

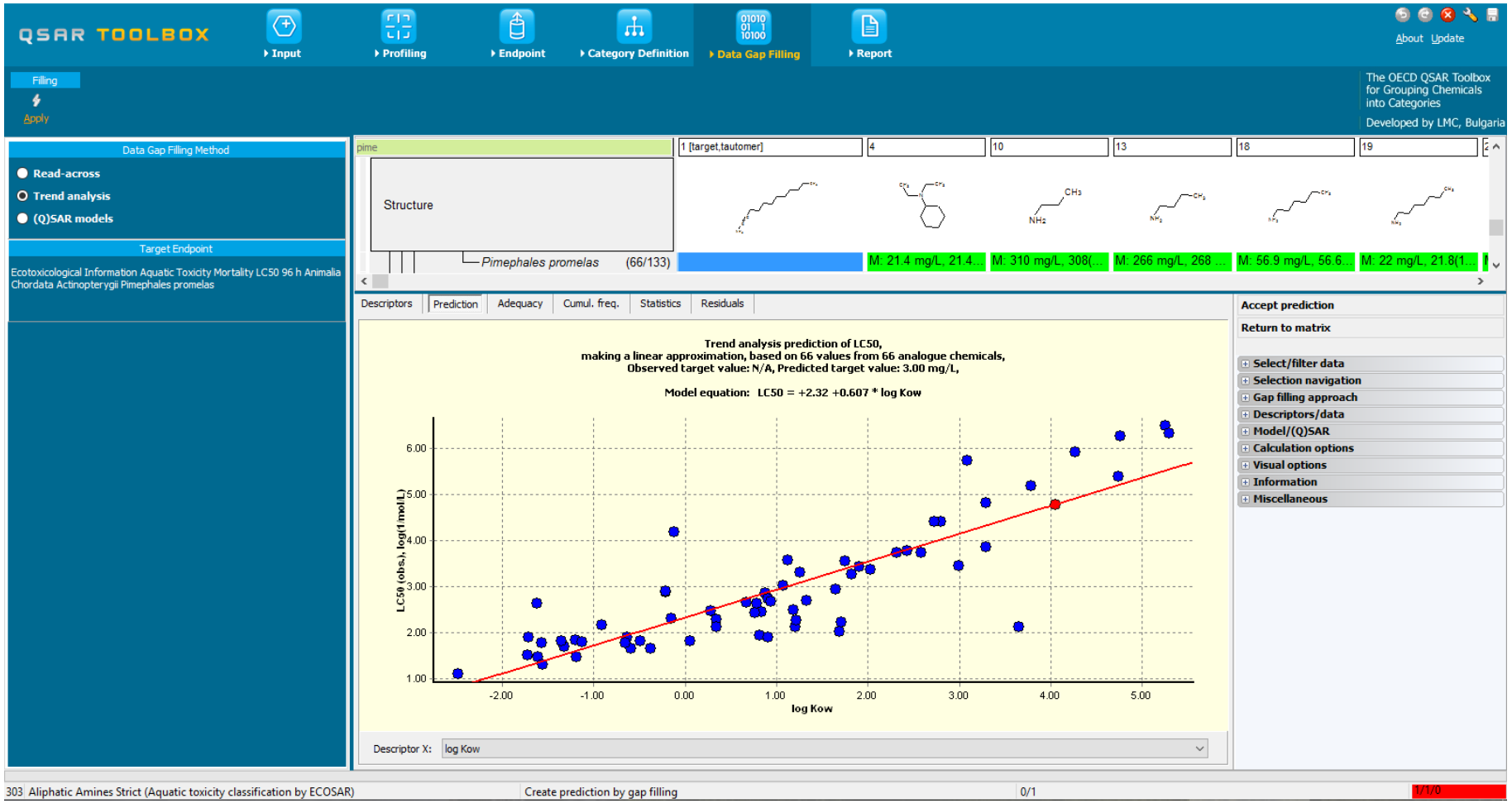
- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
    - Profiling set of tautomers
    - Focus active tautomer
    - Defining category for active tautomer
    - **Trend analysis of the focused tautomer**

Data Gap Filling
Apply Trend analysis



1. **Highlight** the endpoint box corresponding to *Pimephales promelas*/LC50/96h under the target chemical.
2. **Select** Trend analysis
3. **Click** Apply

Data Gap Filling
Results of Trend analysis



Data Gap Filling
Side-Bar of Subcategorisation

- In this example, the following subcategorizations are applied in order to eliminate dissimilar analogues (phase II):

  - Chemical elements

    The categorisation based on Chemical elements allows keeping among the analogues only those that have same chemical elements as the target chemical (target tautomeric form).

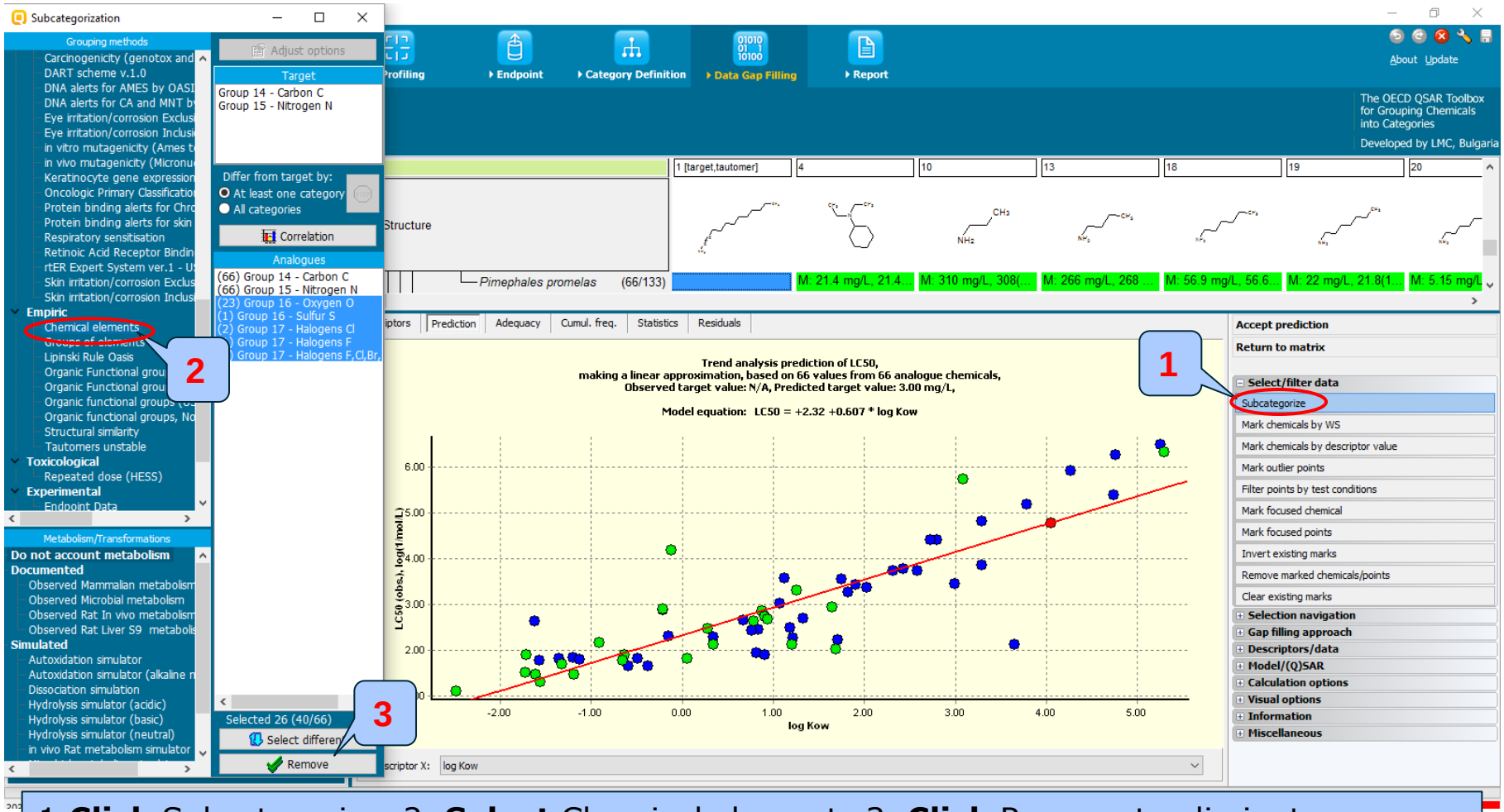
  - Organic functional groups (nested)

    Subcategorization by OFG (nested) eliminates dissimilar analogues with respect to structural functionalities. This subcategorization will eliminate structurally dissimilar analogues such as aromatic amines.

    Subcategorisation steps are demonstrated on the next screen shots.

Data Gap Filling

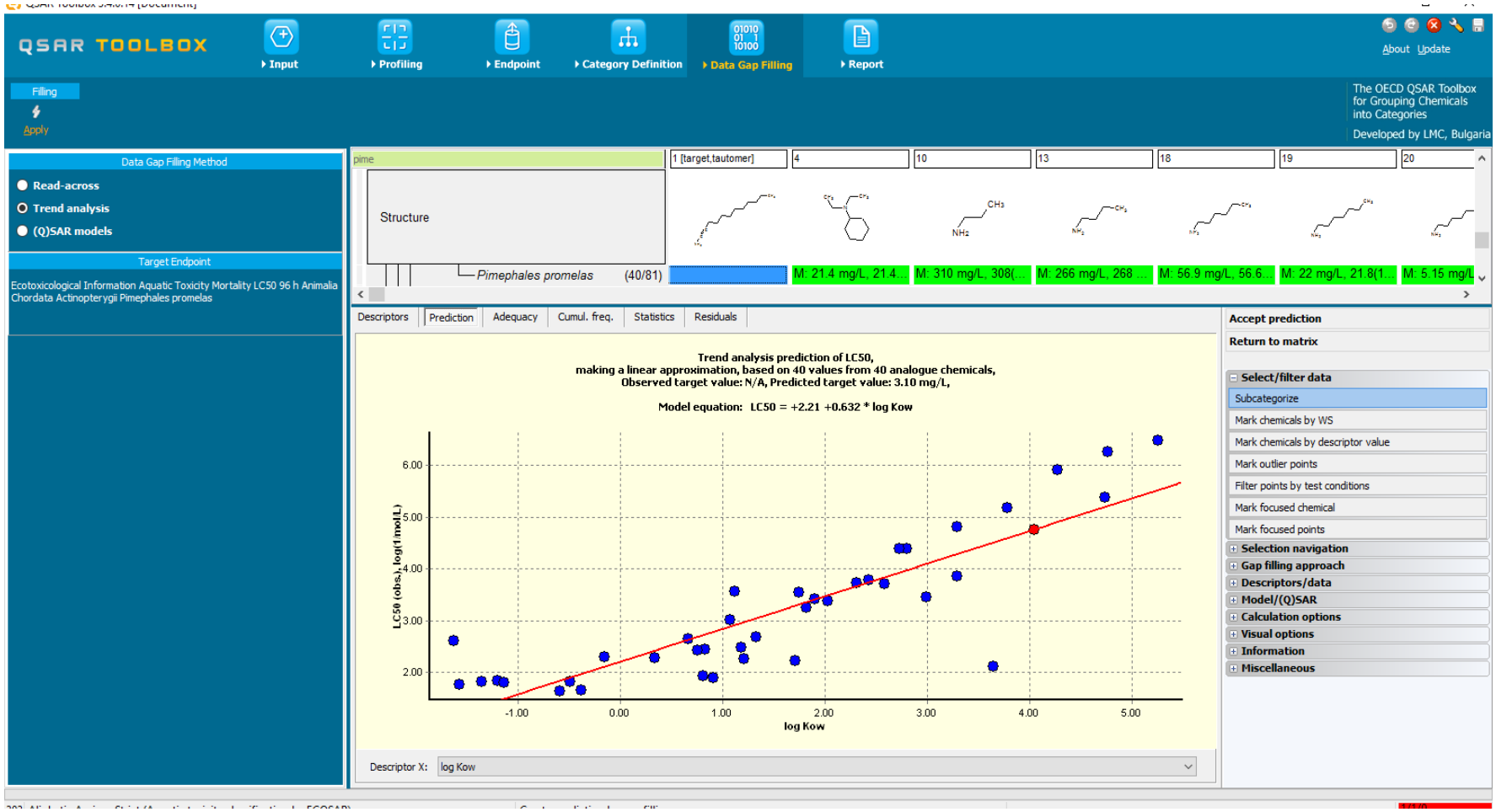
Subcategorisation by Chemical elements



1.**Click** Subcategorize 2. **Select** Chemical elements 3. **Click** Remove to eliminate dissimilar analogues

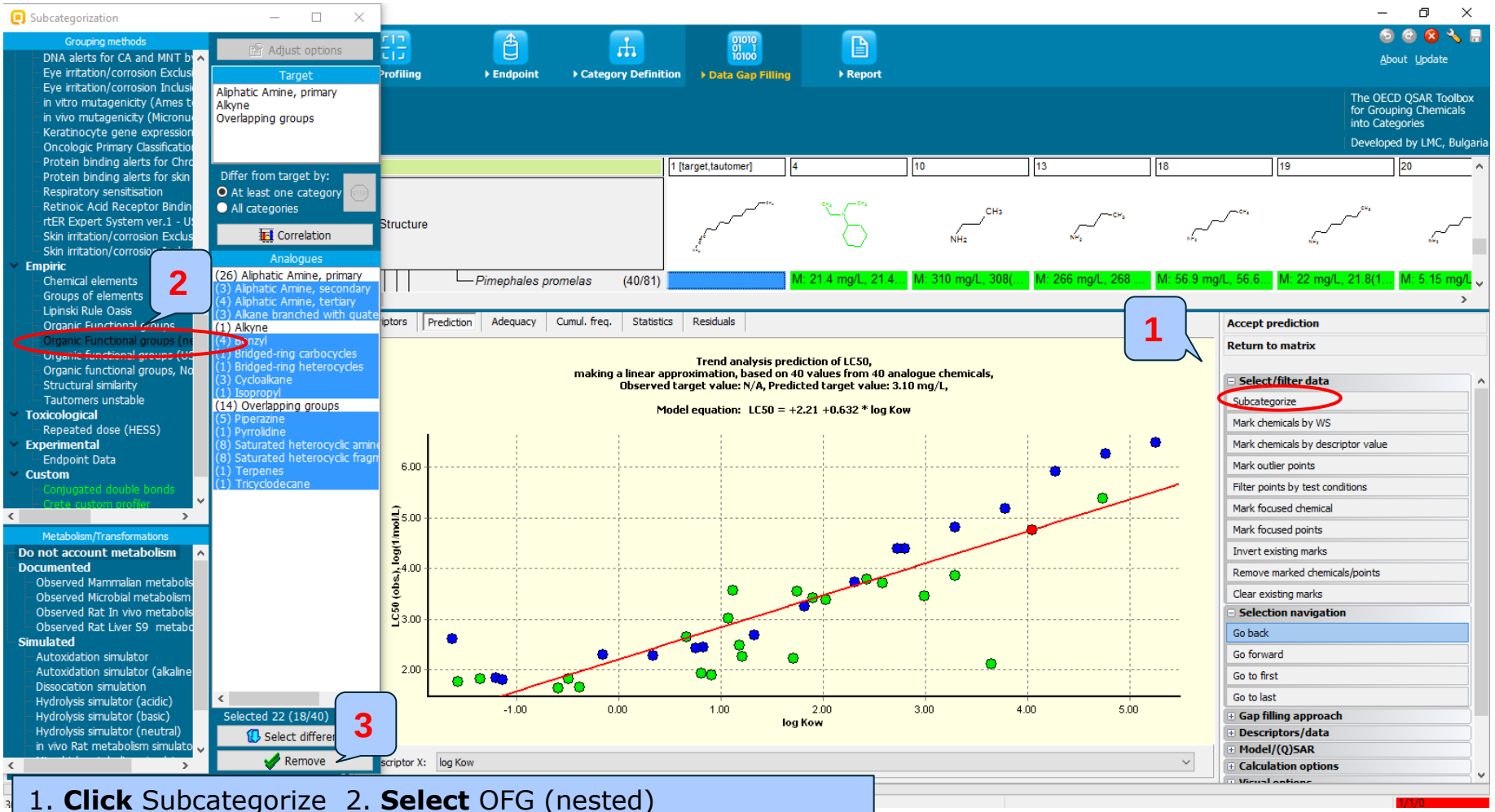
Data Gap Filling

Result of Subcategorisation by Chemical elements



Data Gap Filling

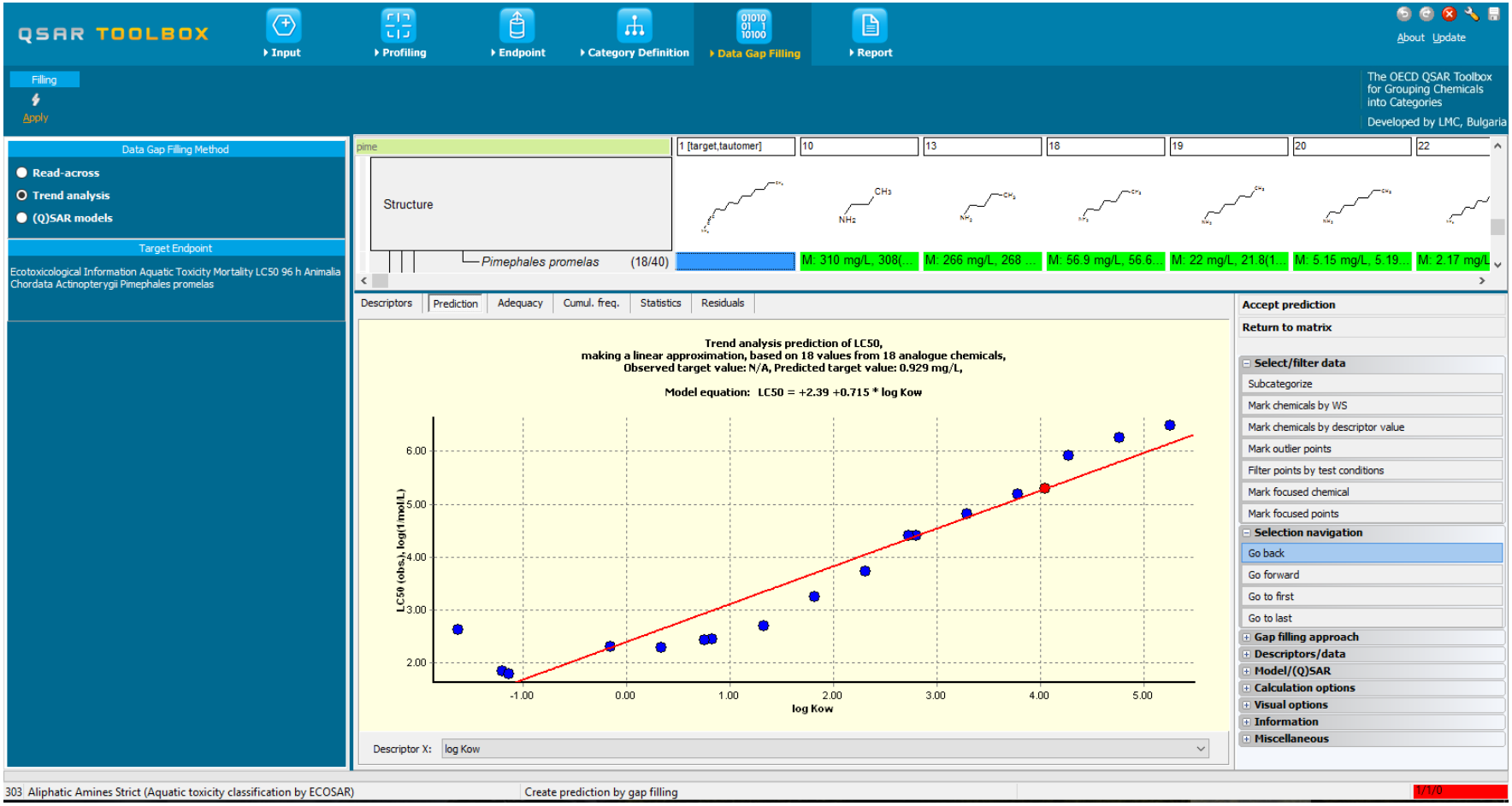
Subcategorisation by OFG (nested)



1. **Click** Subcategorize 2. **Select** OFG (nested)
3. **Click** Remove to eliminate dissimilar analogues

Data Gap Filling

Result of Subcategorisation by OFG (nested)



Data Gap Filling
Side-Bar of Subcategorisation

The last subcategorisation procedure aimed to check and eliminate structurally dissimilar chemicals based on structural similarity

- Structural similarity

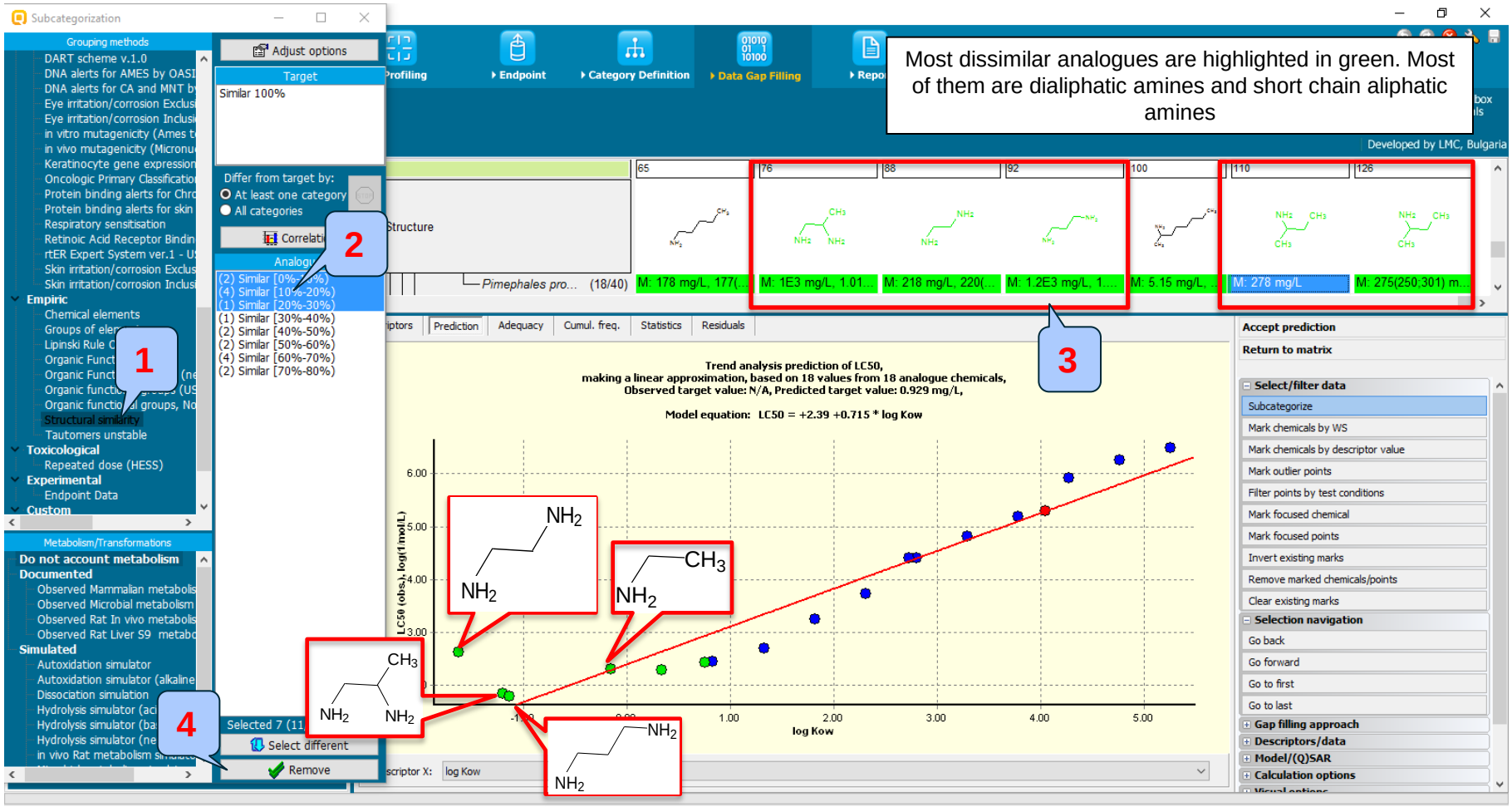
  The options of structural similarity used in the last subcategorization step are as follows: Dice, Atom centred fragments(ACF), atom features: Atom type; Count H attached; Hybridizations;

Analogues with similarity less than 30 % have been eliminated

See next two slide

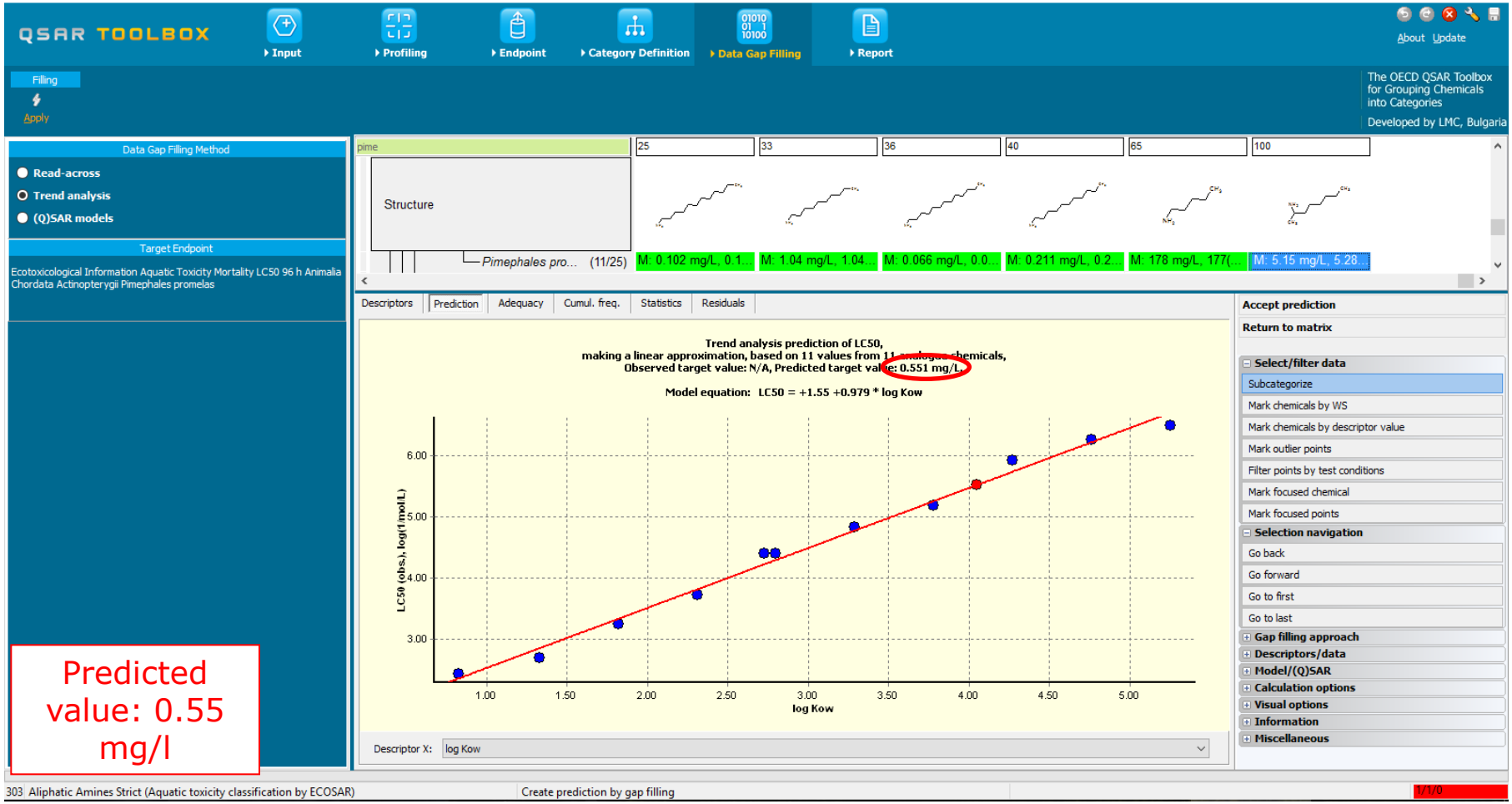
Data Gap Filling

Subcategorisation by Structural similarity



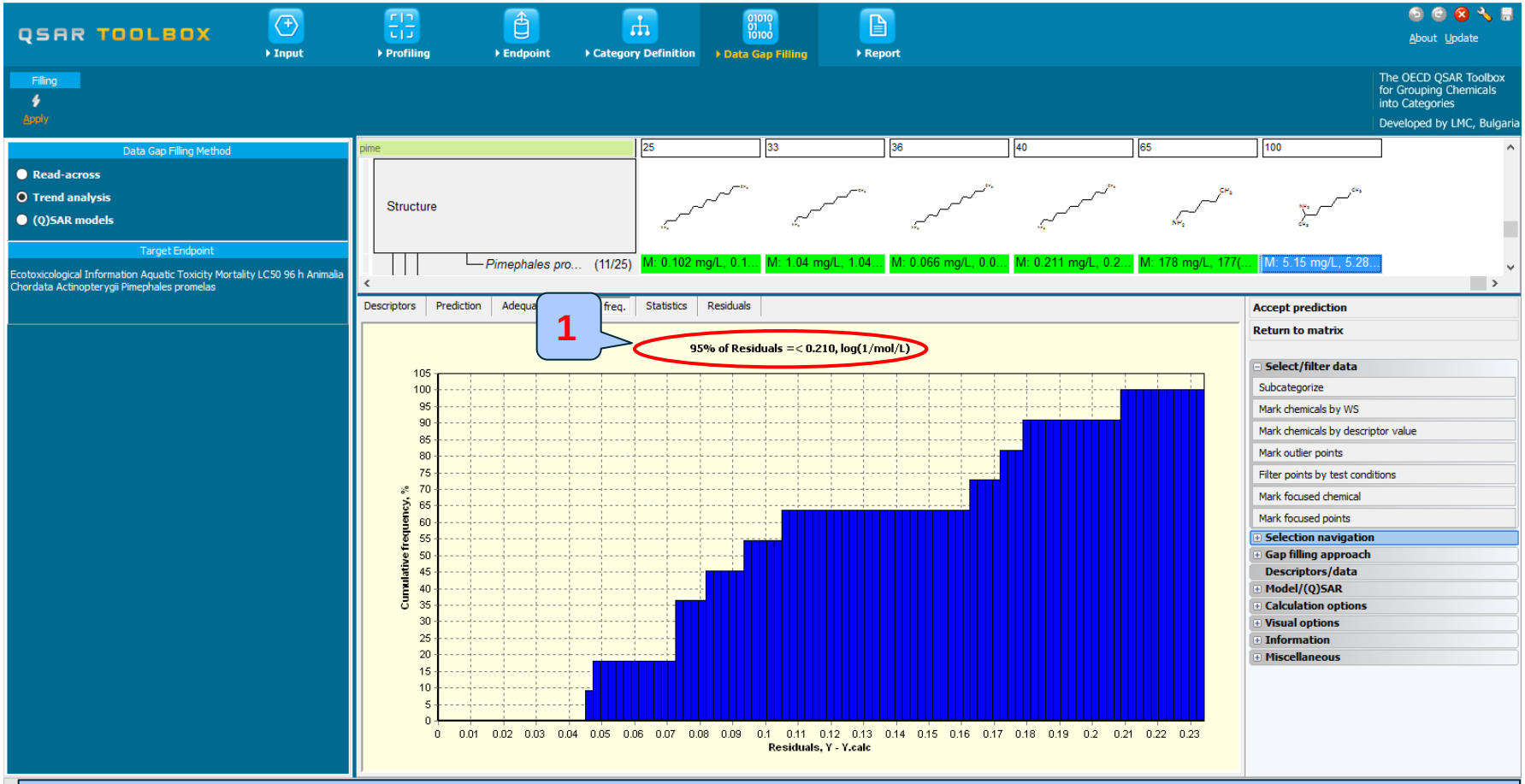
1. **Select** Structure similarity; 2. Manually select categories between 0 and 30% (hold Ctrl button and select categories); 3. Dissimilar analogues are highlighted in green; 4. **Click** Remove to eliminate dissimilar analogues

Data Gap Filling
Result



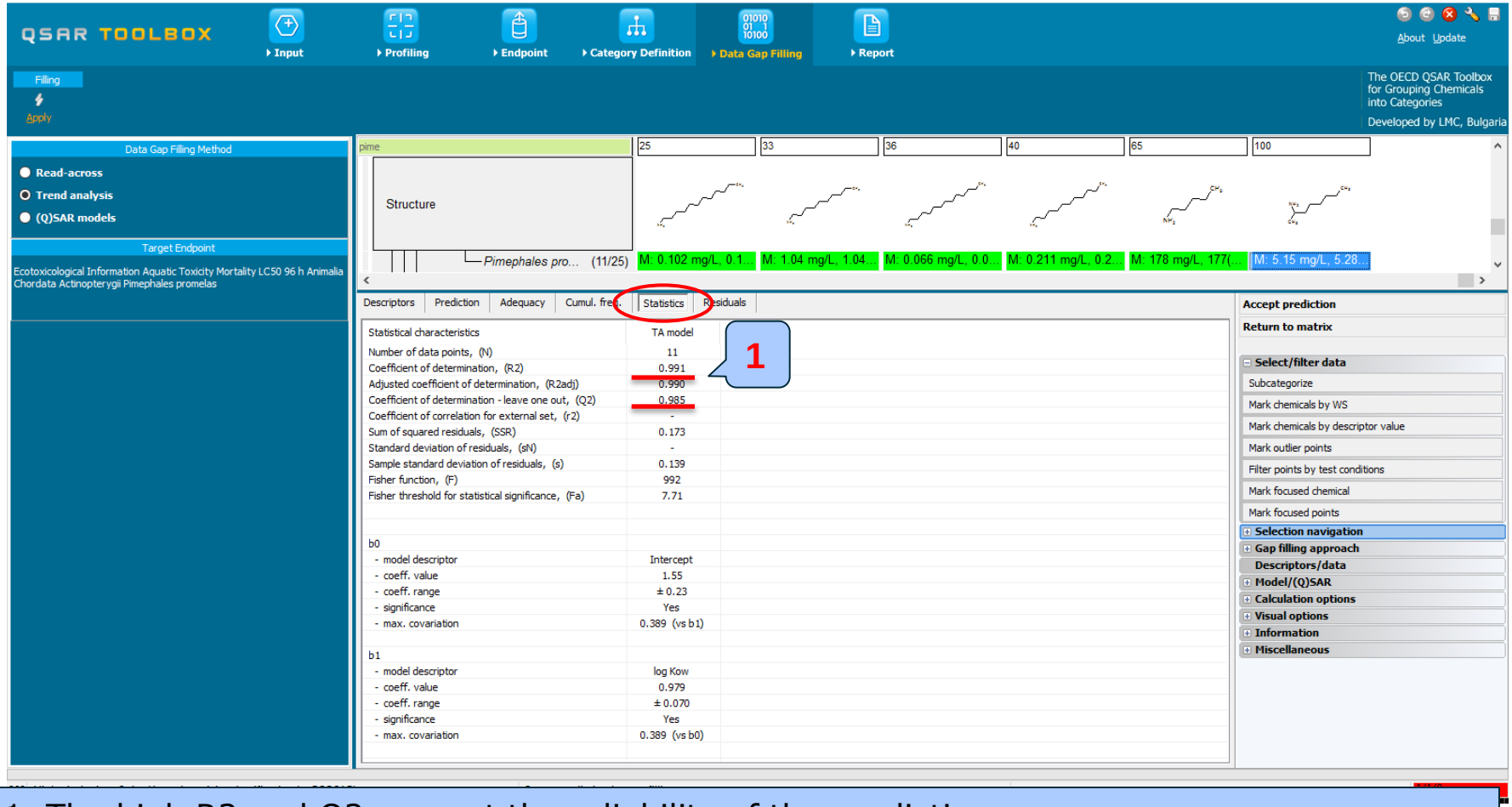
Predicted value: 0.55 mg/l

Data Gap Filling
Cumulated frequency



1. 95% of residuals are in the range of experimental error

Data Gap Filling
Statistics

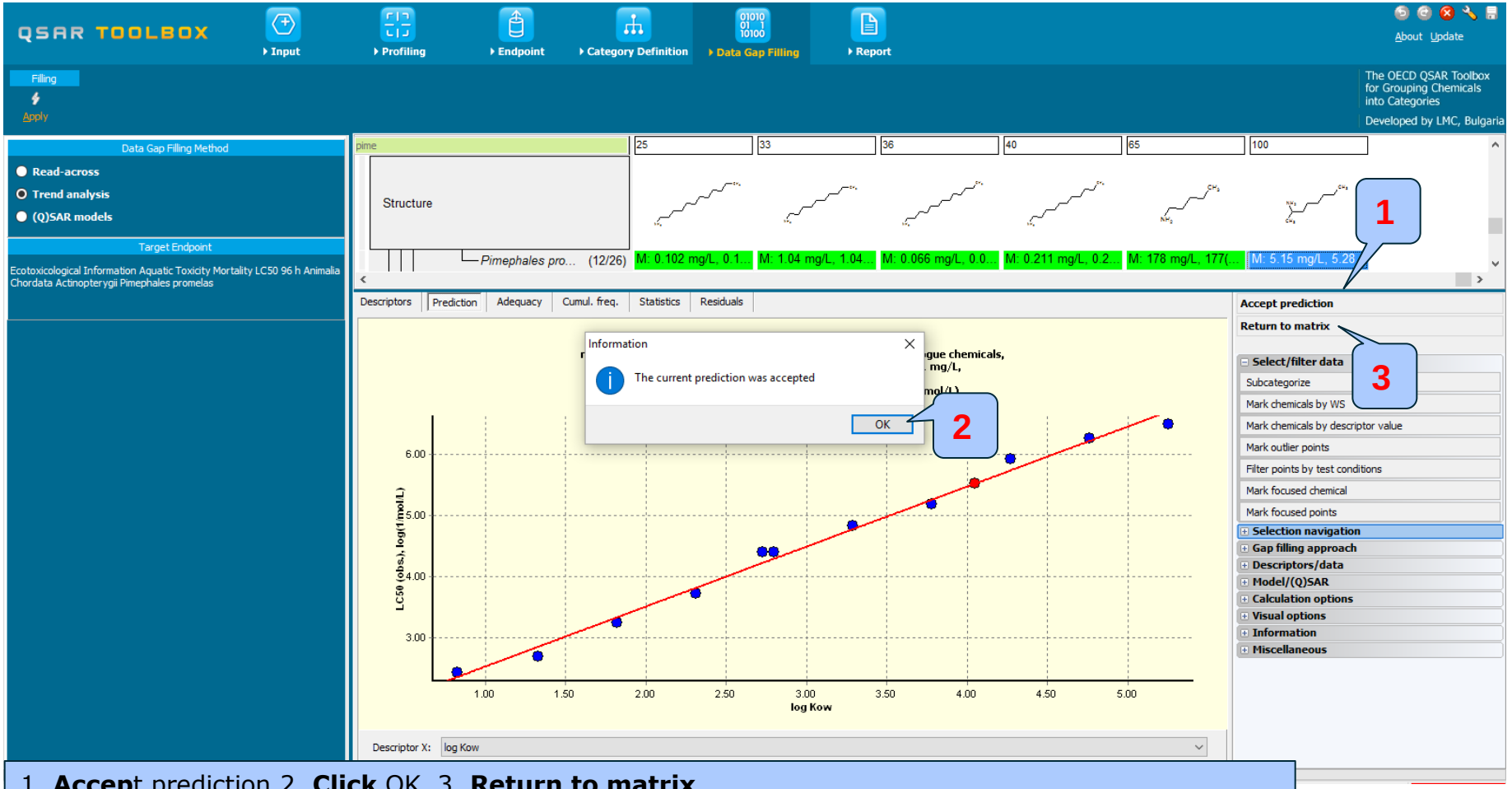


1. The high R2 and Q2 support the reliability of the prediction

Data Gap Filling
Result of trend analysis

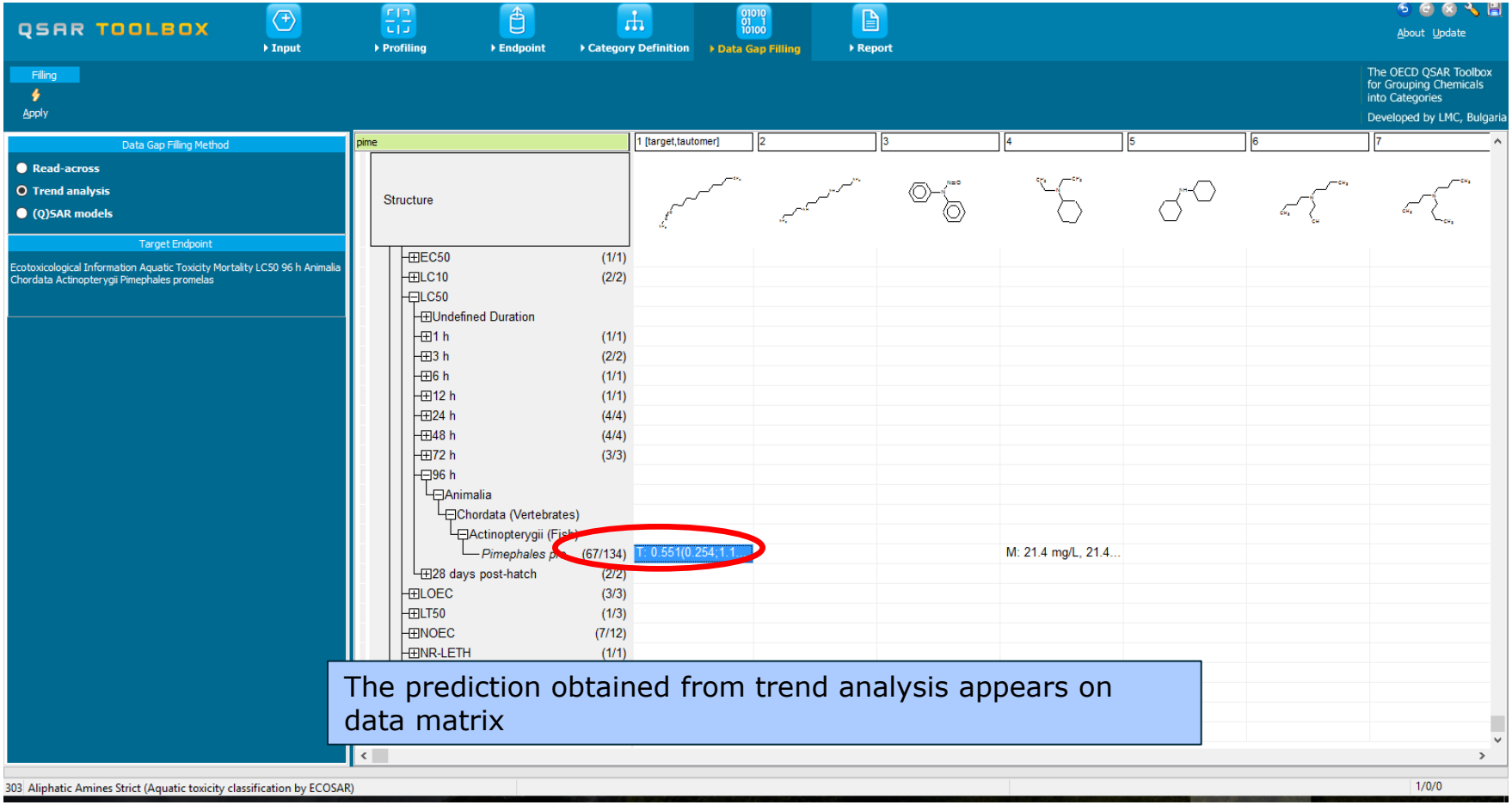
- The analysis of trend analysis shows:
  - The predicted acute aquatic toxicity value is 0.55 mg/l
  - The remaining analogues form robust category of structurally similar analogues (aliphatic amines)
  - The 95% of residuals are in the range of experimental error
  - The high R2 and Q2 coefficient values support the reliability of the prediction

Data Gap Filling
Accept the prediction



1. **Accep**t prediction 2. **Click** OK 3. **Return to matrix**

Data gap filling for focused tautomer
Trend analysis



The prediction obtained from trend analysis appears on data matrix

Data gap filling for focused tautomer
Interpreting Read-across

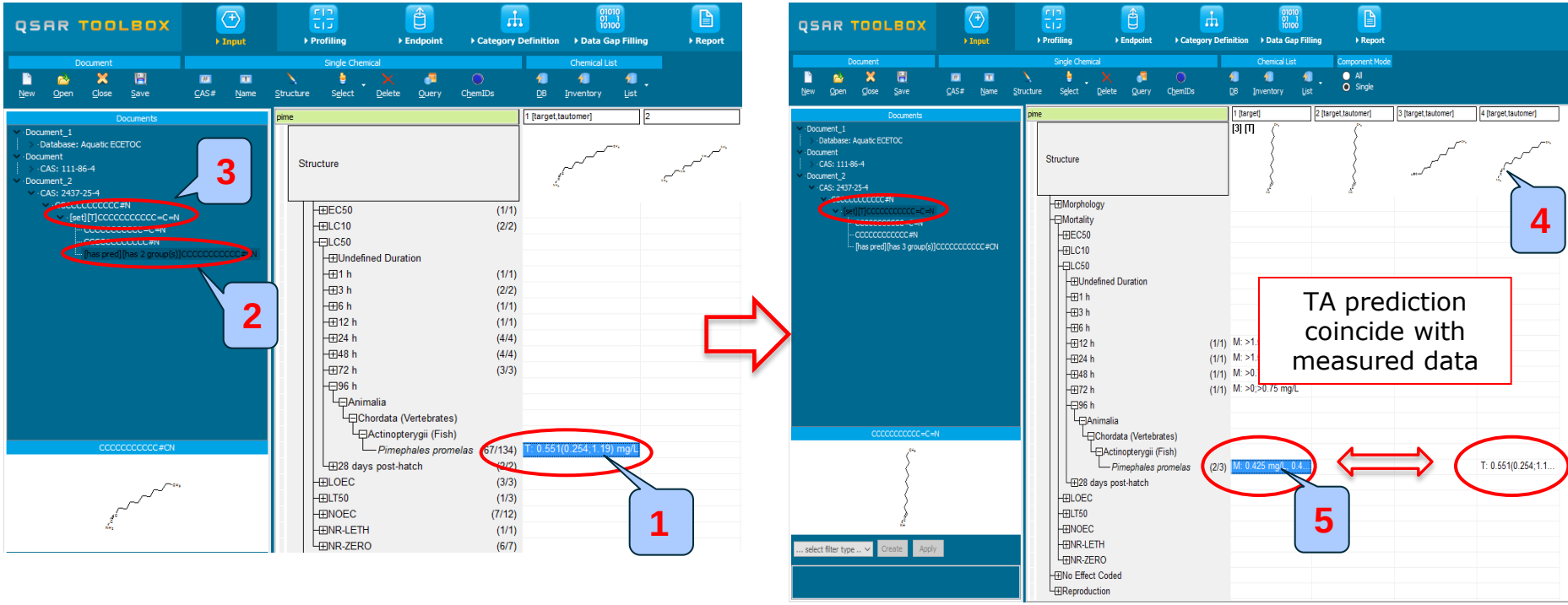
- In this example, all analogues are aliphatic amines
- All analogues exhibit toxic effect to fish (*P.promelas*)
- The same toxic effect is therefore predicted for the target (i.e. focused tautomer).
- The prediction of tautomer is further transferred to the parent chemical using Independent MOA (see next screen shots)

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
    - Profiling set of tautomers
    - Focus active tautomer
    - Defining category for active tautomer
    - Trend analysis of the focused tautomer
  - **Assigning prediction of tautomer to parent**

Handling tautomerism of target chemical

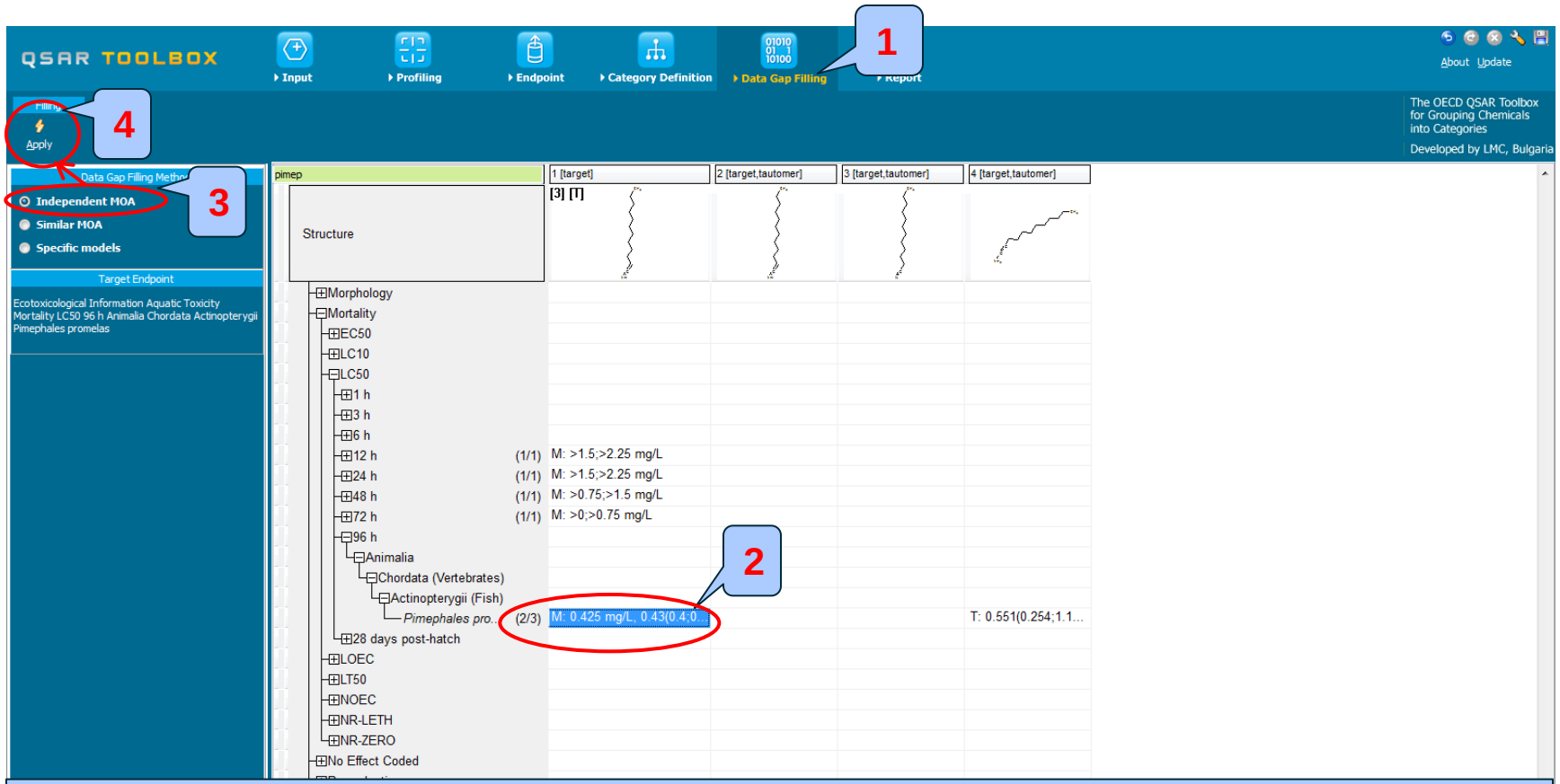
Assigning data to parent chemical



1. The trend analysis prediction appears on datamatrix; 2. The prediction of the tautomeric form is assigned to the last SMILES within the set; 3.**Click** on the first SMILES in order to go back to the set; 4. All tautomeric forms within the set are visualized on data matrix
The TA prediction coincide with experimental data.; 5; **Click** on the cell related to the parent chemical

Handling tautomerism of target chemical

Assigning data to parent chemical



1. **Go** to Data Gap filling 2. **Select** the cell of the parent; The independent MOA is used to transfer the prediction to the parent chemical 3. **Select** Independent mode; 4. **Click** Apply

Handling tautomerism of target chemical
Assigning data to parent chemical

- The following actions (steps) are used for assigning data to parent chemical:
  - Accept prediction
  - Return to matrix

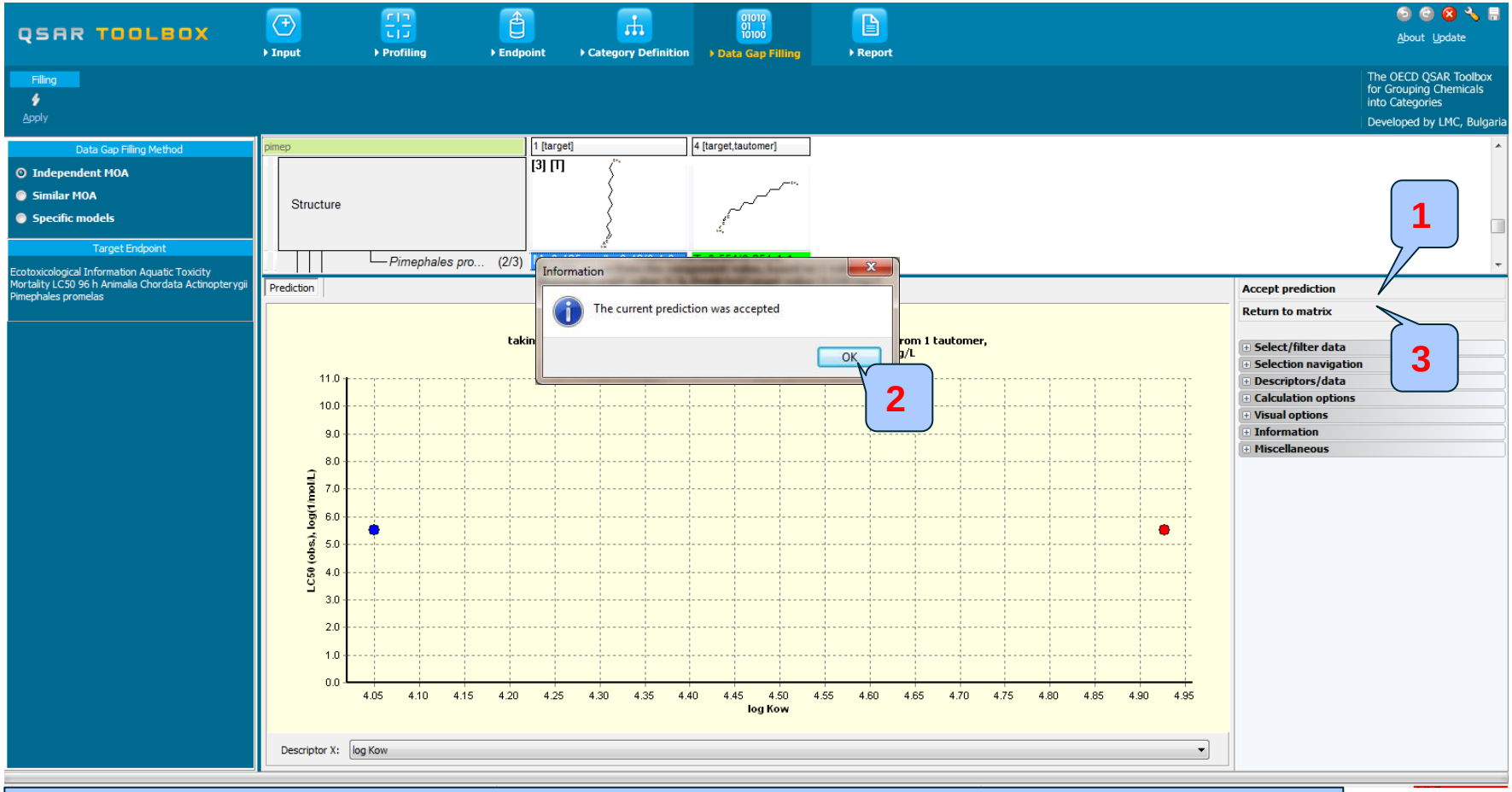
- Independent mode of action is formally used for transferring the value from metabolite to the target chemical.
  -Independent MOA- all components are with different mode of action
  -Similar MOA- all components are with similar mode of action. The quantities of the components are taken into account*

- Final prediction for the parent compound labeled as CI (Component based Independent mode) (see next screen shot)

*Additional information for both MOA could be found in "Tutorial 2 Prediction of Acute fish for mixtures" posted on OECD and LMC website: http://www.oecd.org/chemicalsafety/risk-assessment/Tutorial_12_TB%203.2.pdf

Handling tautomersim of target chemical

Assigning data to parent chemical



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

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
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    - Focus active tautomer
    - Defining category for active tautomer
    - Trend analysis of the focused tautomer
  - Assigning prediction of tautomer to parent
  - **Report**

Report

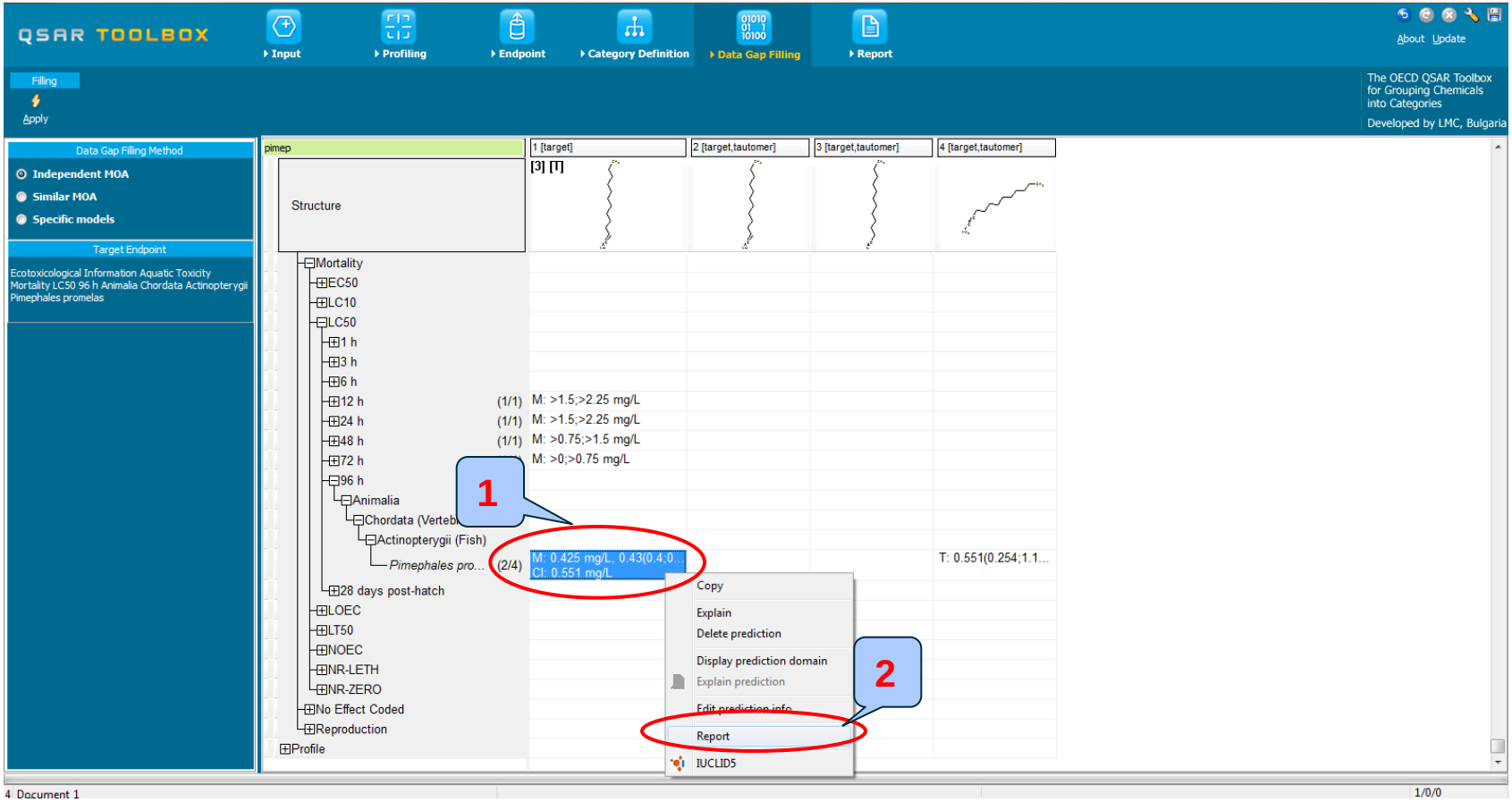
- Remember the report module allows you to generate a report on the predictions performed with the Toolbox. This module contains predefined report templates as well as a template editor with which users can define their own user defined templates. The report can then be printed or saved in different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

- The report consist of two sections:

  - Summary report for the whole tautomeric set
  - Report for the individual prediction obtained for the active tautomeric form

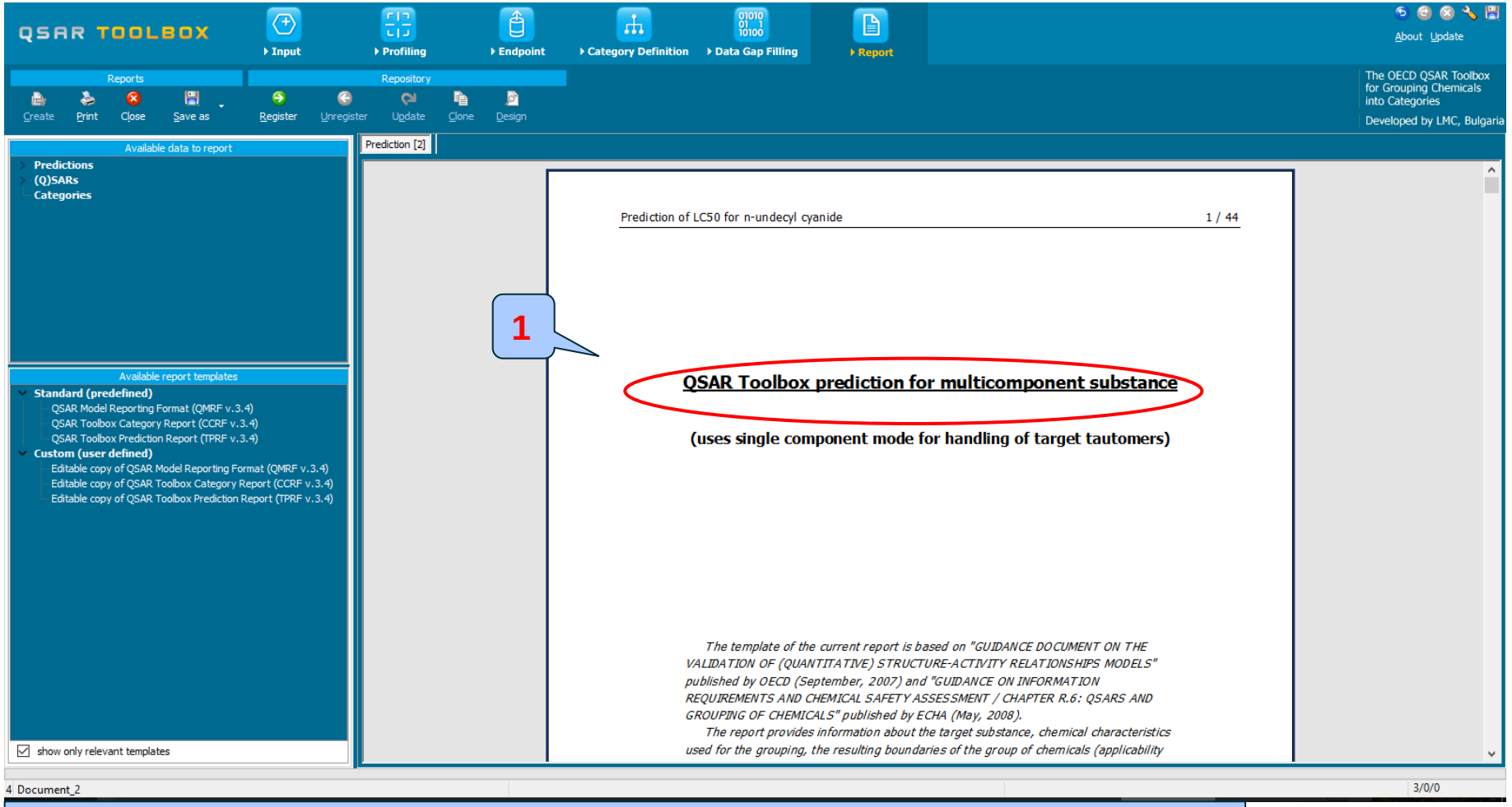
- Generating the report is shown on next screenshots

Report



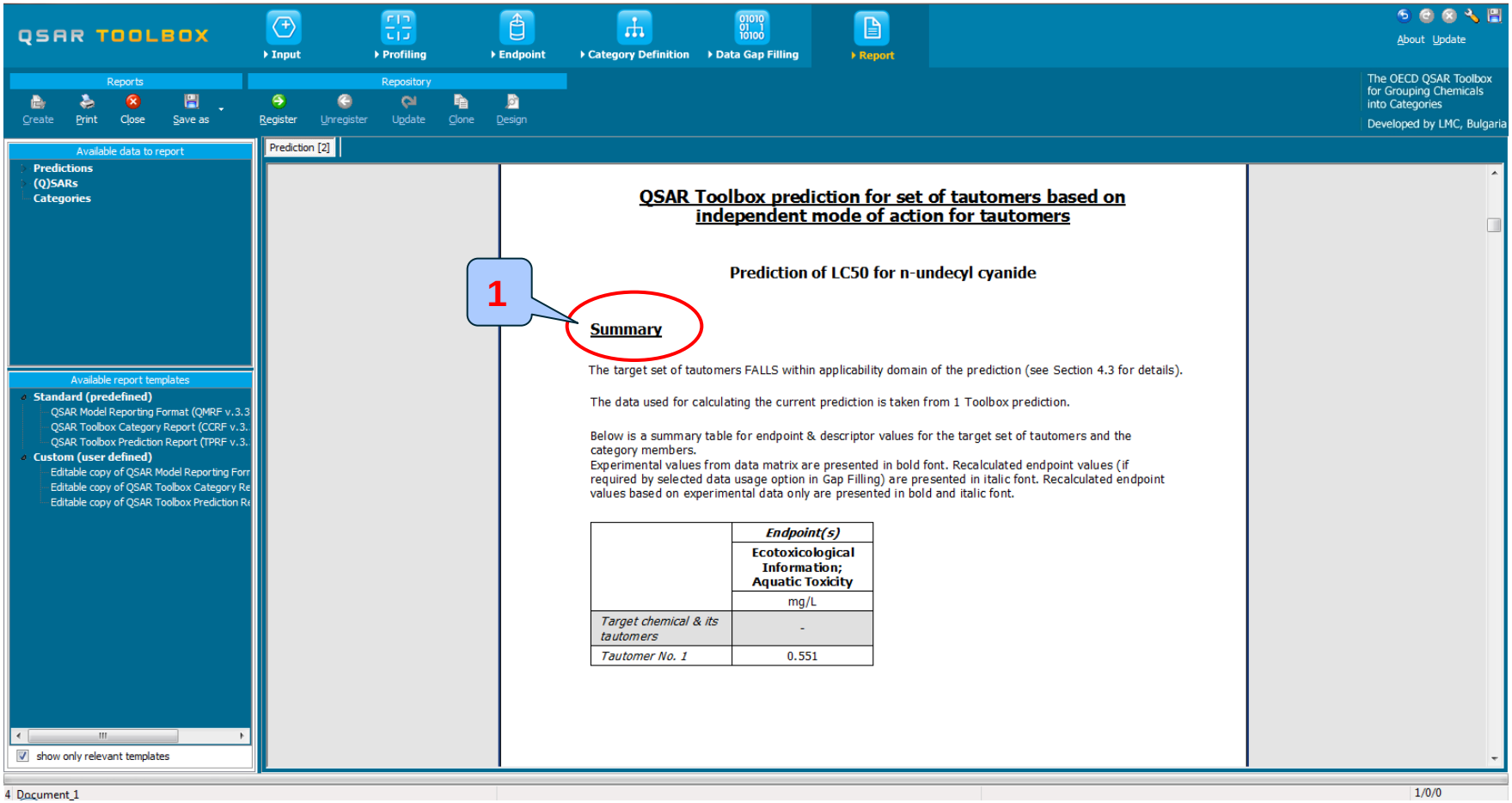
1. **Click** on the cell with prediction
2. Perform **Right click** and **Select** Report

Report



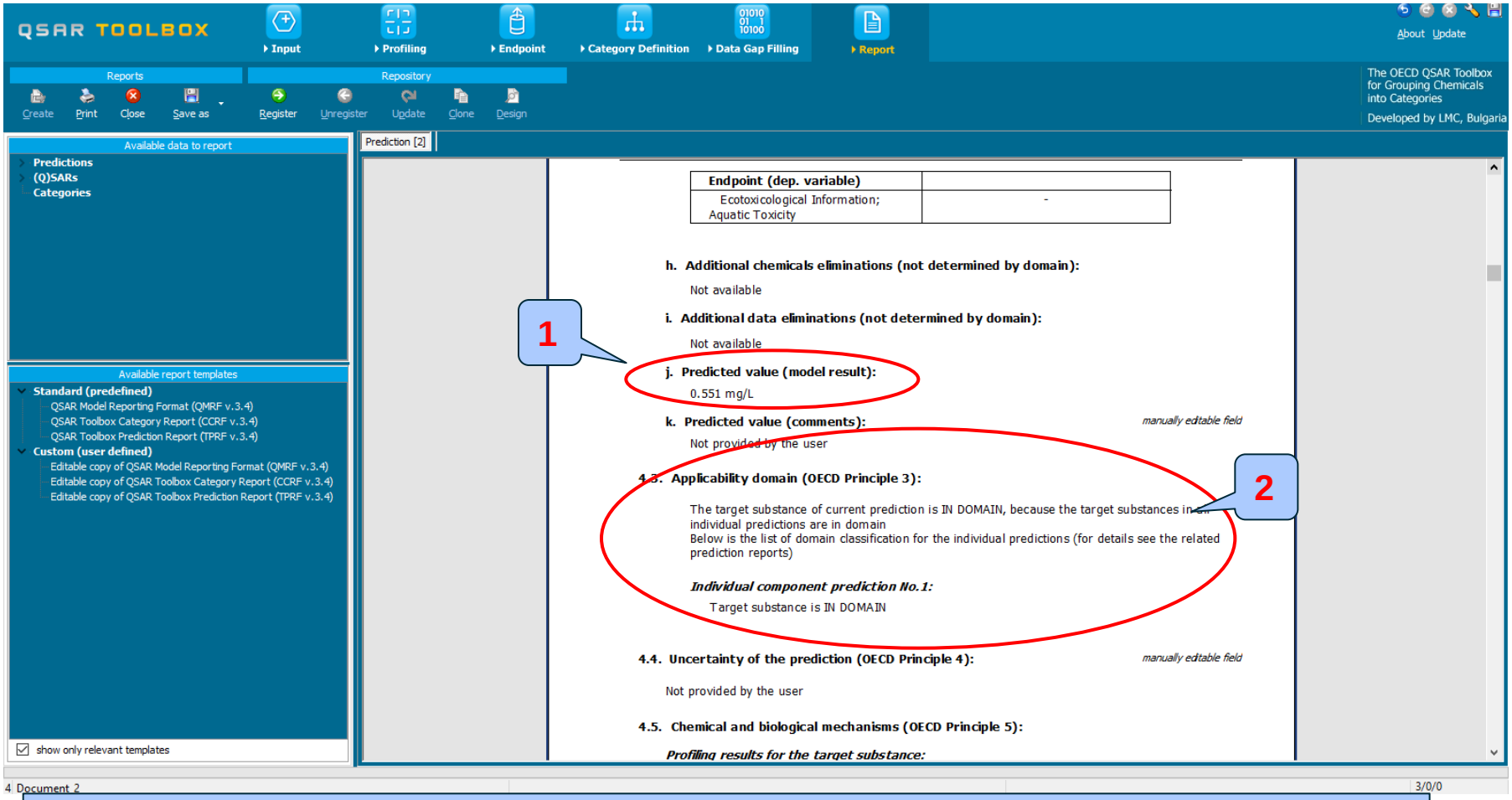
1. TB report for multicomponent substance

Report



1. Summary information for prediction

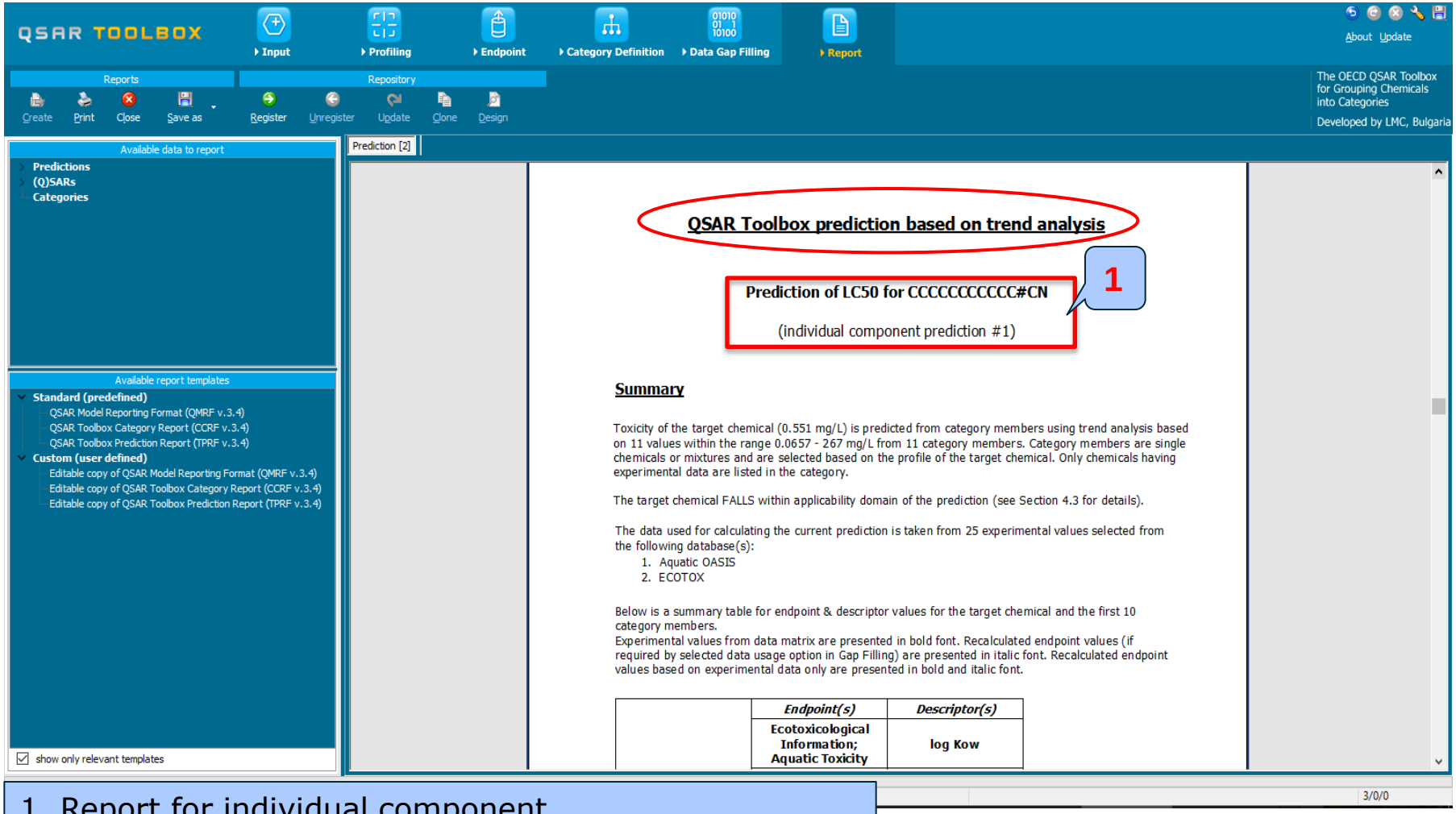
Report



1. Predicted value
2. Applicability domain

The target chemical is "In domain", because the prediction of the single tautomer is "In domain".

Report



1. Report for individual component

Outlook

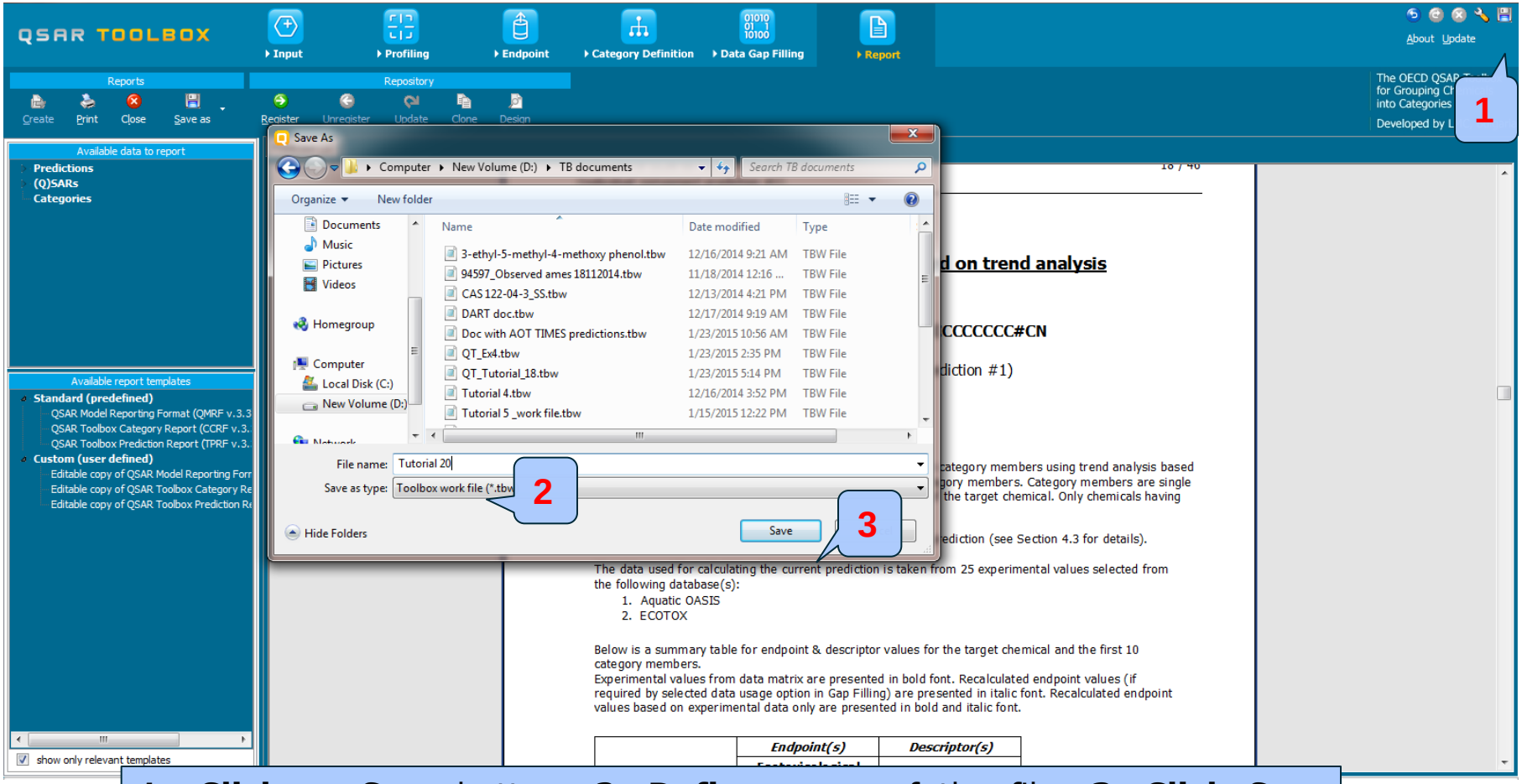
- Background
- Objectives
- The exercise
- Workflow
  - Input
  - Profiling
  - Endpoint
  - Handling of tautomerism of target chemical
  - Assigning prediction of tautomer to parent
  - Report
- **Save prediction**

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).

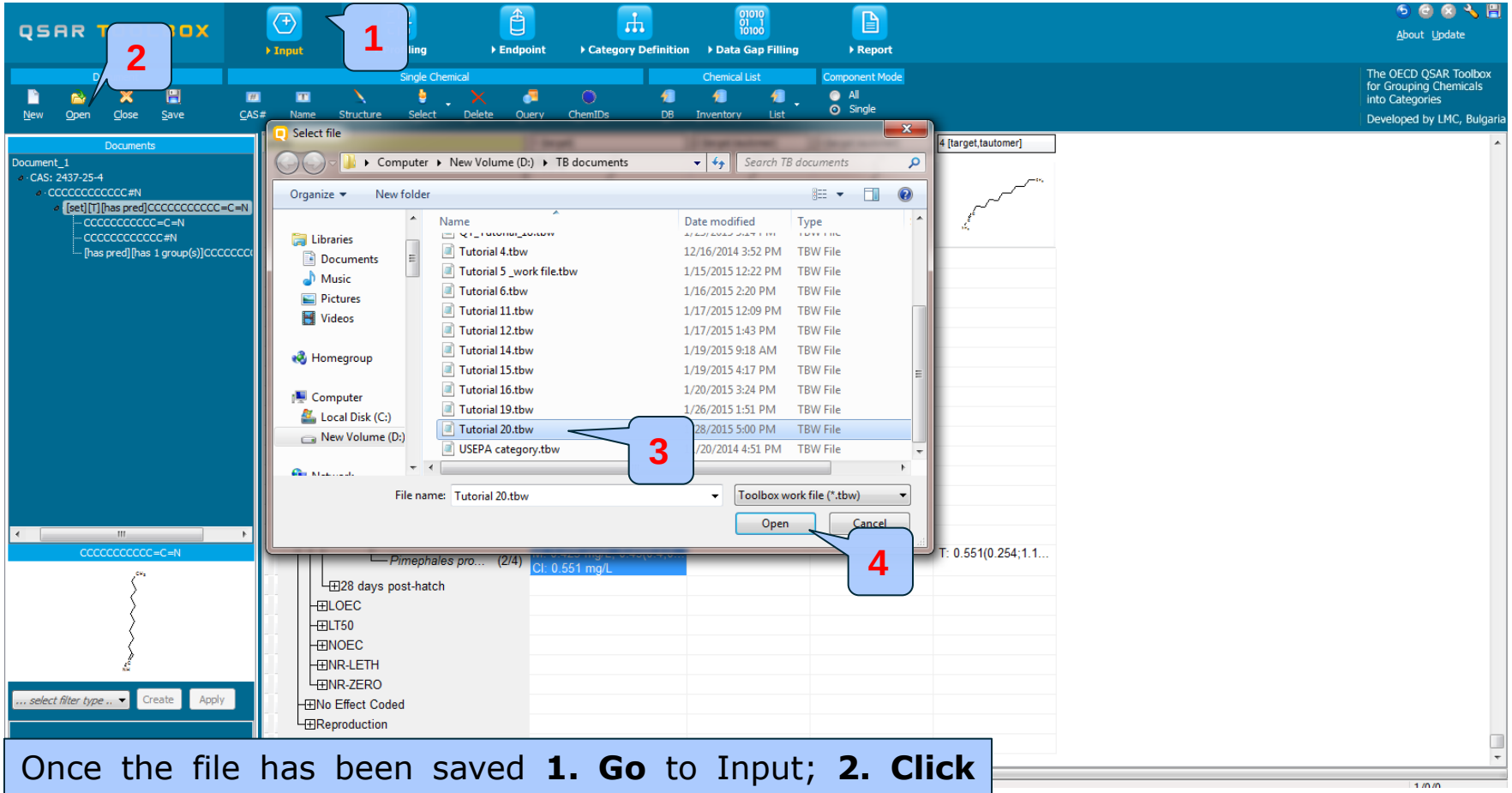
- Saving/Loading the file with TB prediction is shown on next screenshots

Saving the prediction



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