QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.3.4

Example for predicting acute aquatic toxicity to fish of mixture with known components

Outlook

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

Background

- This is a step-by-step presentation designed to take the user of the Toolbox through the workflow of prediction acute aquatic toxicity to fish of mixture with known components

Outlook

- Background
- **Objectives**
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Objectives

- **This presentation reviews a number of functionalities of the Toolbox:**
  - The 2D editor for defining Mixture components
  - Filling data gaps by Similar mode approach

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Exercise

- In this exercise we will predict the aquatic toxicity to fish of mixture with defined components, which is the "target" chemical.
- Investigate the mode of action of components of the mixture
- Gather available experimental data for target chemical and its components
- Predict acute aquatic toxicity using Similar mode approach

Outlook

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- **Workflow**
- Save the prediction

Workflow

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

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - **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 mixture

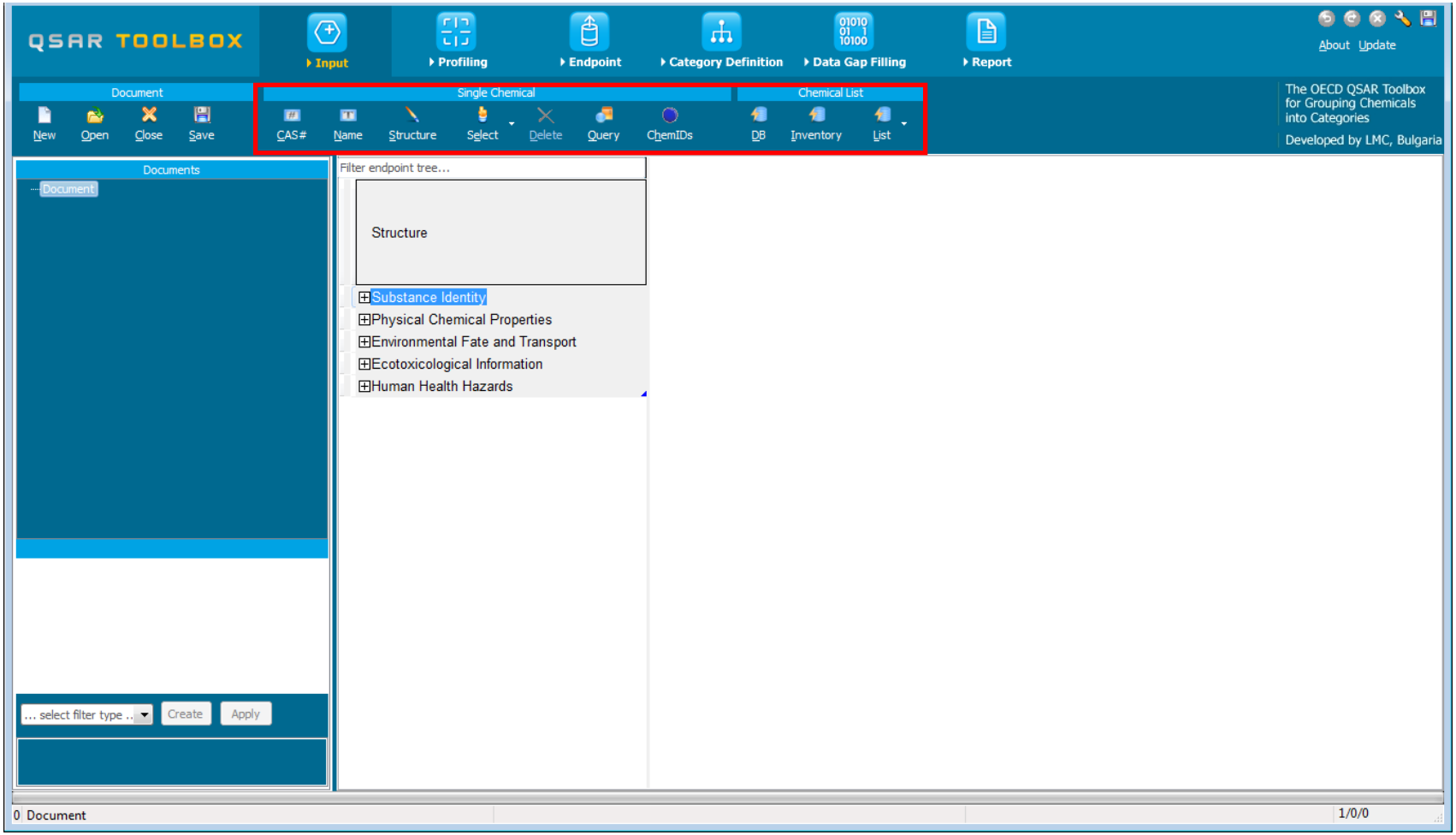
User alternatives for defining mixtures with known compositions:

- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing mixture constituents and defining their quantities
- Select from User List/Inventory/Databases
- Chemical IDs such as EC number, Einecs number
- Load file with mixture

Chemical Input
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
Input Screen



Chemical Input by Drawing

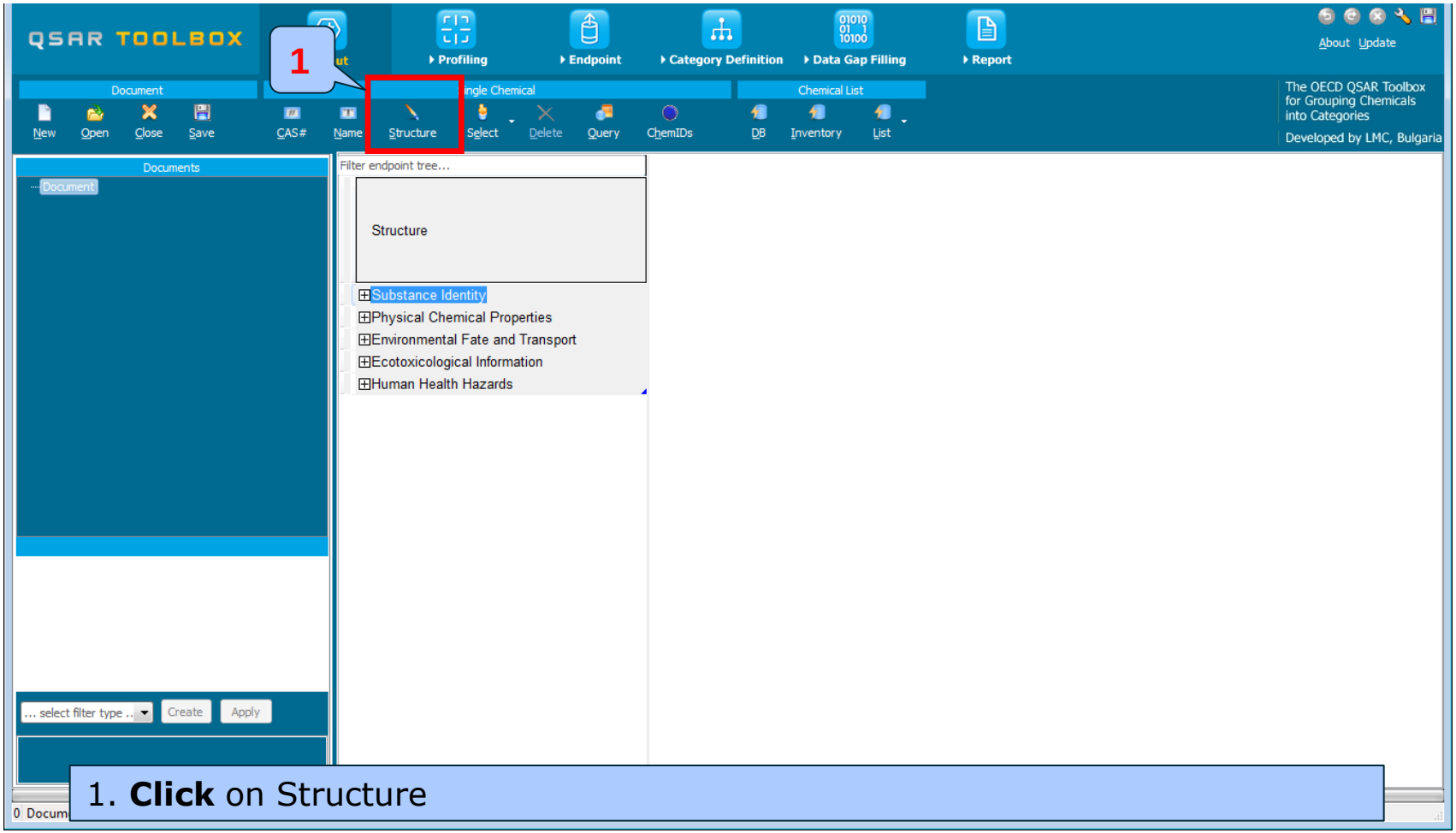
- Inputting the target chemical (mixture) by drawing its components within the 2D-editor

- It is accomplished by a series of point-click operations within the 2D-editor which appears when you click on “structure” (see next screen shot).

- The subsequent series of screen shots will take you through the process of drawing constituents of mixture and defining their quantities.

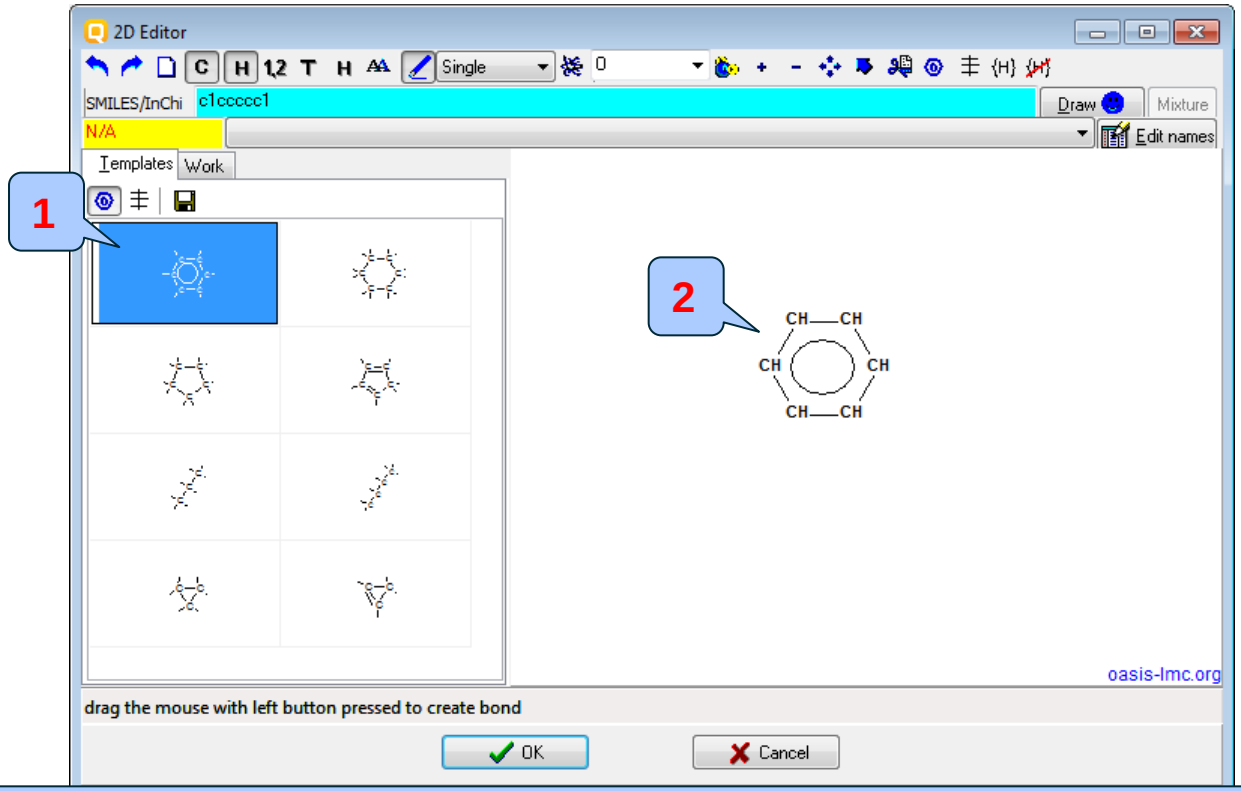
Chemical Input

Input target chemical by drawing



Chemical Input

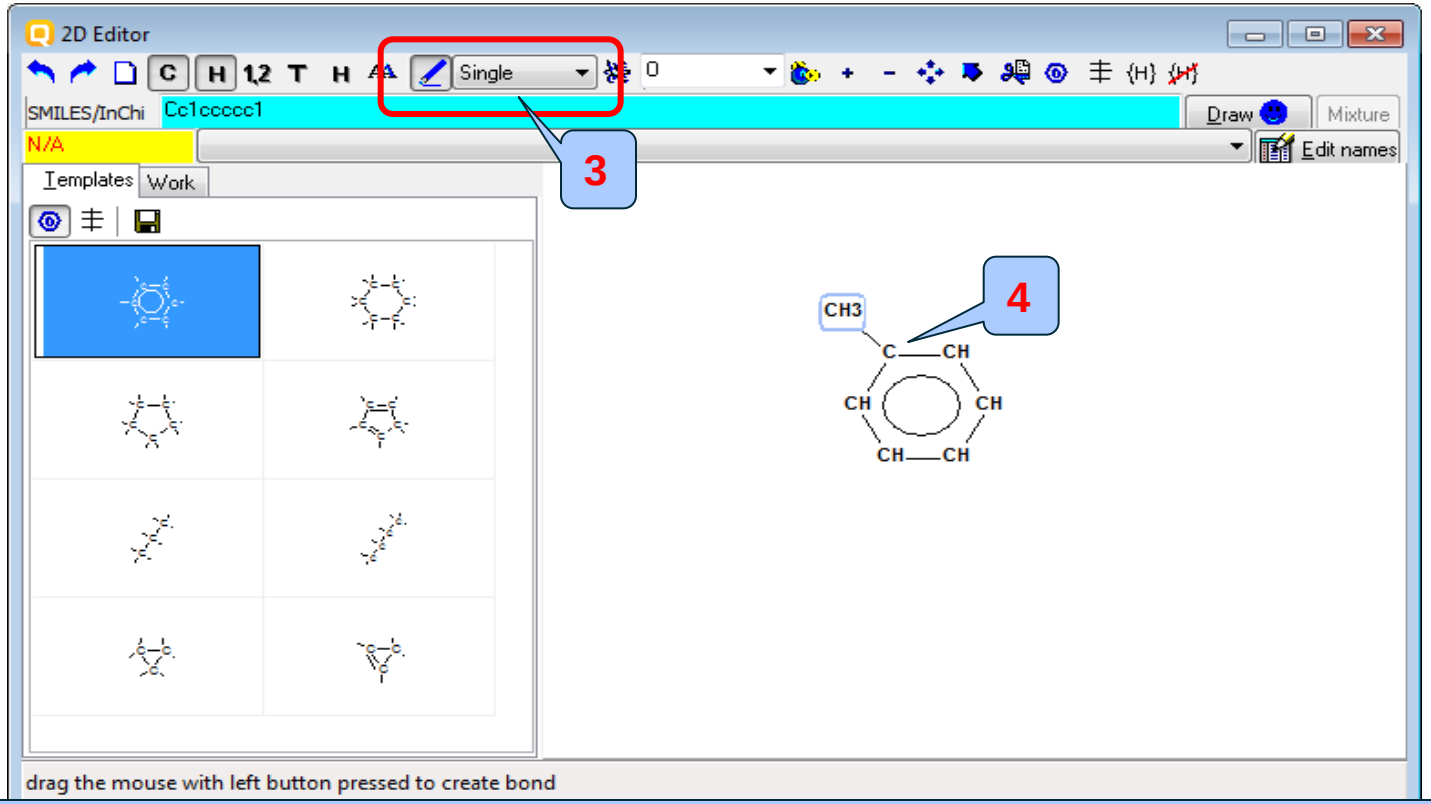
Drawing the target mixture by 2D editor



1. **Left Click** on the appropriate chemical form from the "Templates" panel.
2. **Move** the cursor to the large blank area and **left click** again, this puts the selected template on the plot.

Chemical Input

Drawing the target mixture by 2D editor



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

Chemical Input by Drawing

- CH_3-group is added by default when you perform left click over the atoms.

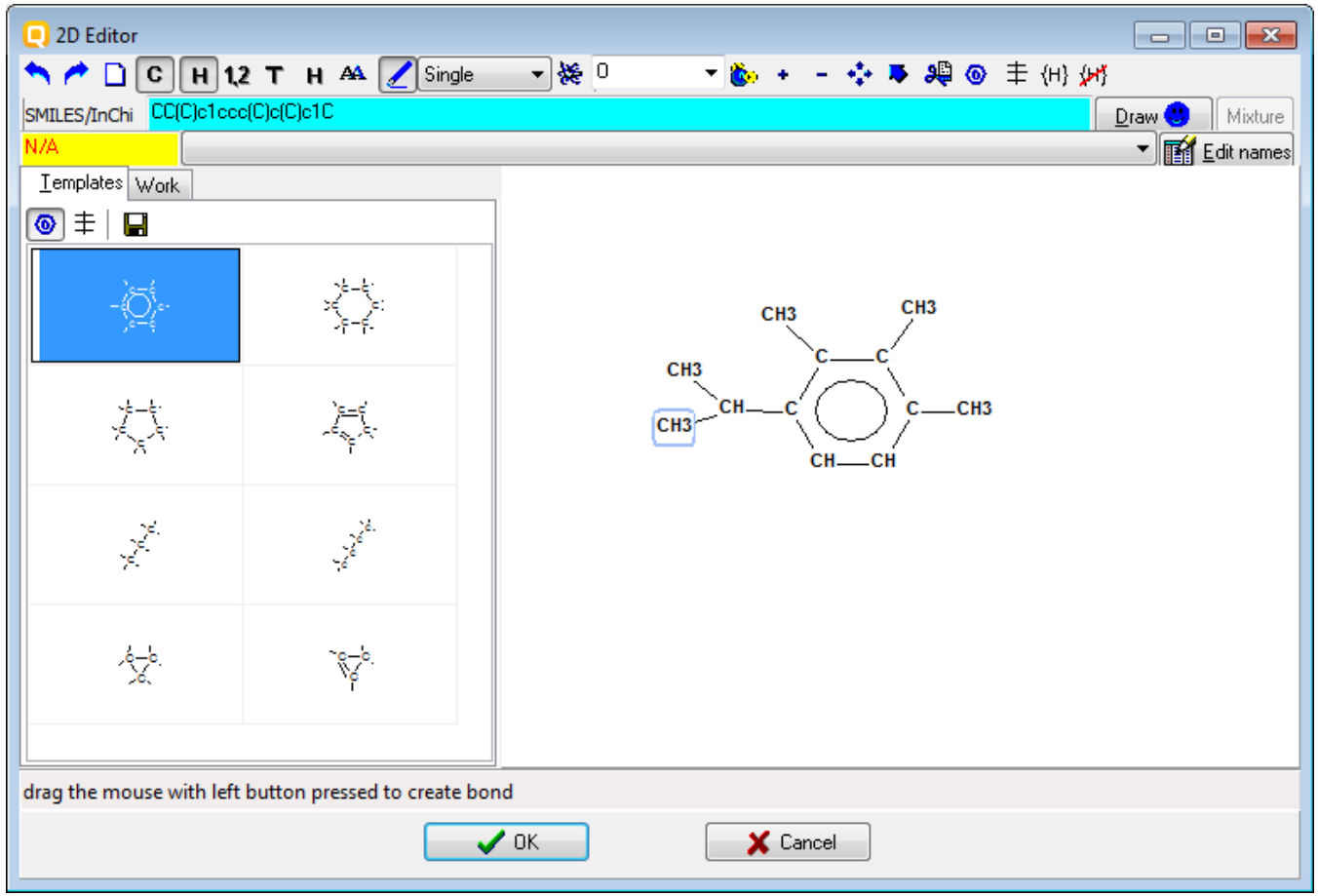
- If you make an incorrect entry you can click on the ‘undo’ icon in the upper corner of the screen to remove the last action

- This process allows you to build the hydrocarbon skeleton of the target molecule (see next screenshot).

- More details about how to use the 2D editor for drawing chemical compounds click F1 help: section D.2.1.3.4.1. Details of 2D Editor

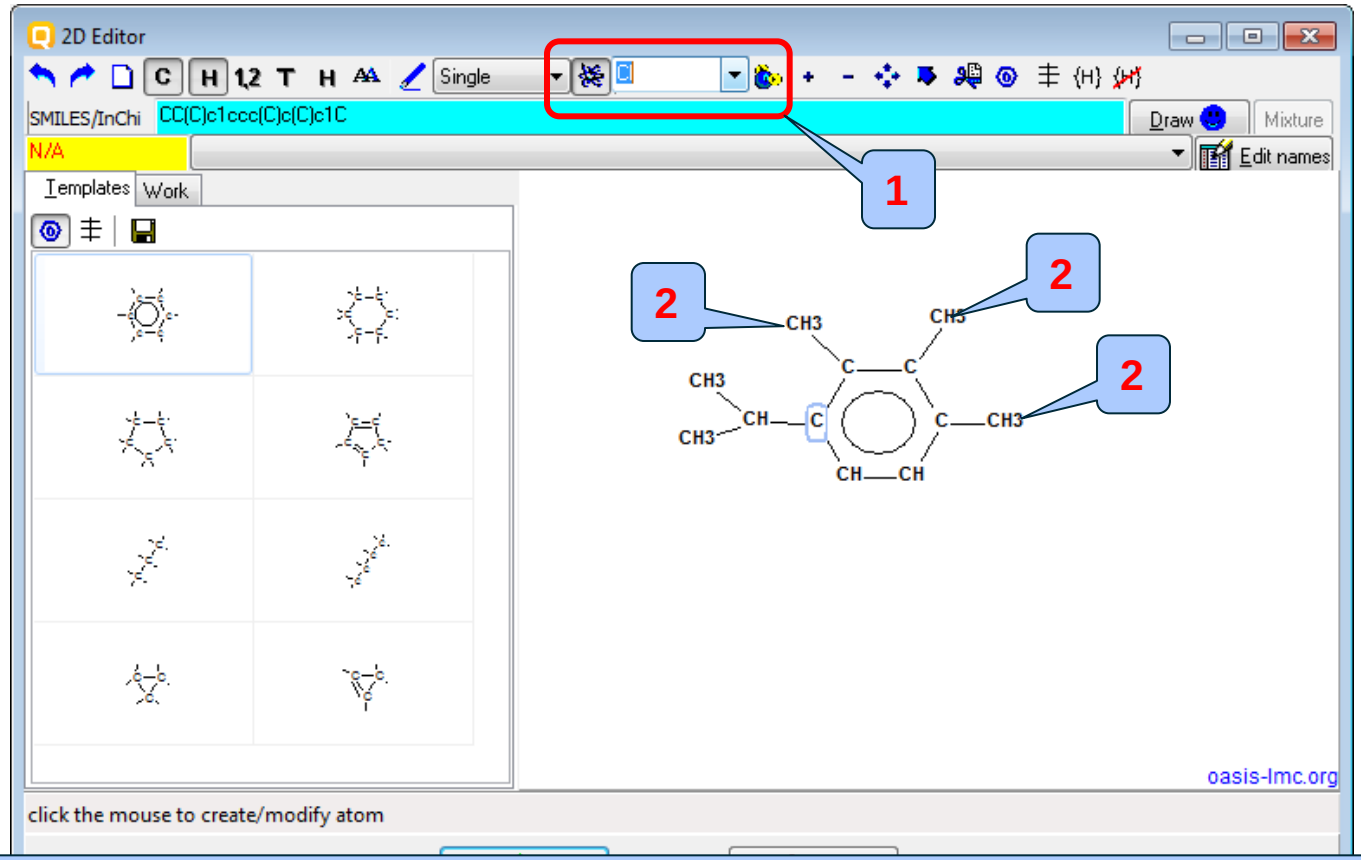
Chemical Input

Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor



Chemical Input

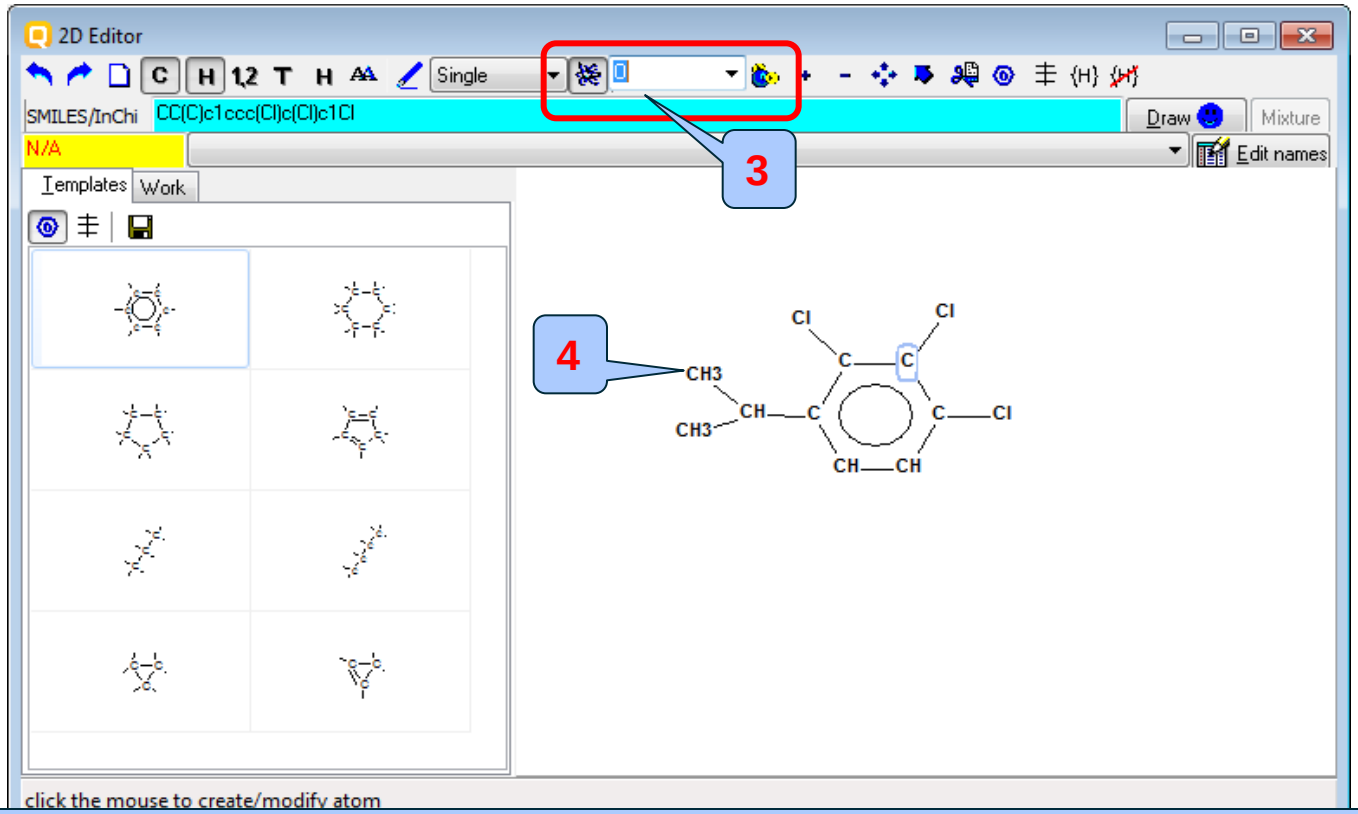
Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor



1. **Click** on button to add a hetero atom (in this case chlorine atom).
2. **Left click** with mouse over the methyl group to insert the selected chlorine atoms.

Chemical Input

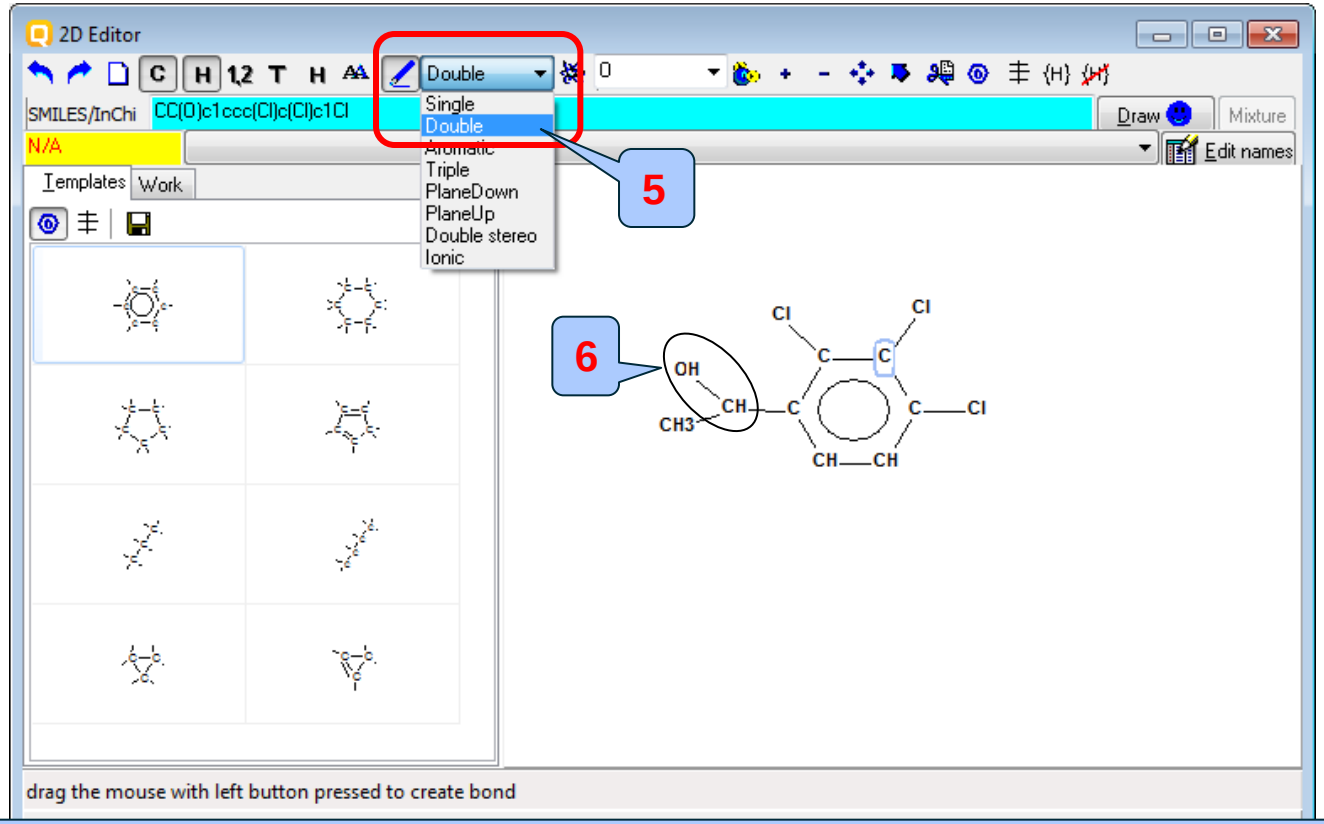
Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor



3. Use the arrow and select O atom **Click** on  button to select it (in this case an oxygen atom).
4. **Left click** with mouse over the methyl group to insert an oxygen atom.

Chemical Input

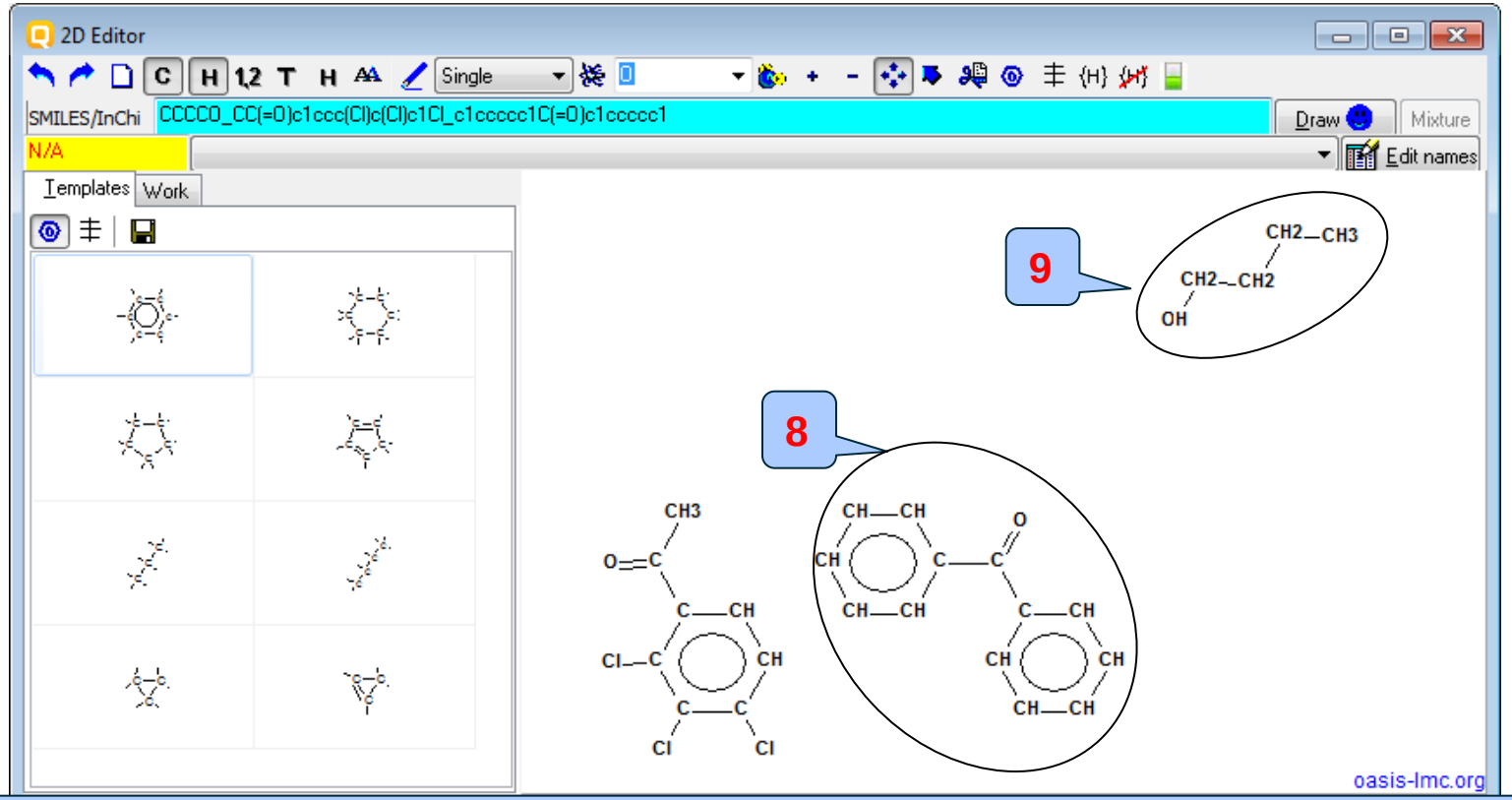
Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor



5. **Click** on Single and **select** Double bond.
6. **Drag** the mouse from the C-atom to O-atom to create a double bond

Chemical Input

Drawing the components of mixture "Diphenylmethanone" and "Butan-1-ol"

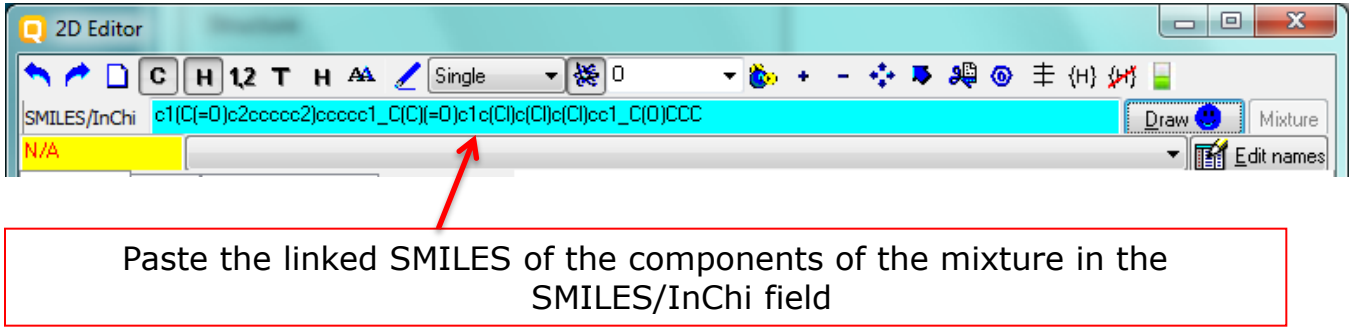


8. **Draw** the second mixture component - Diphenylmethanone
9. **Draw** the third mixture component - butan-1-ol

Chemical Input

Alternatives for defining components of mixture

- The other alternative of drawing mixture is to:
  - Drawn the SMILES of each component
  - Link the SMILES of the components with underscore character
  - Copy the linked SMILES and Paste it in the SMILES/InChi filed of 2D editor window

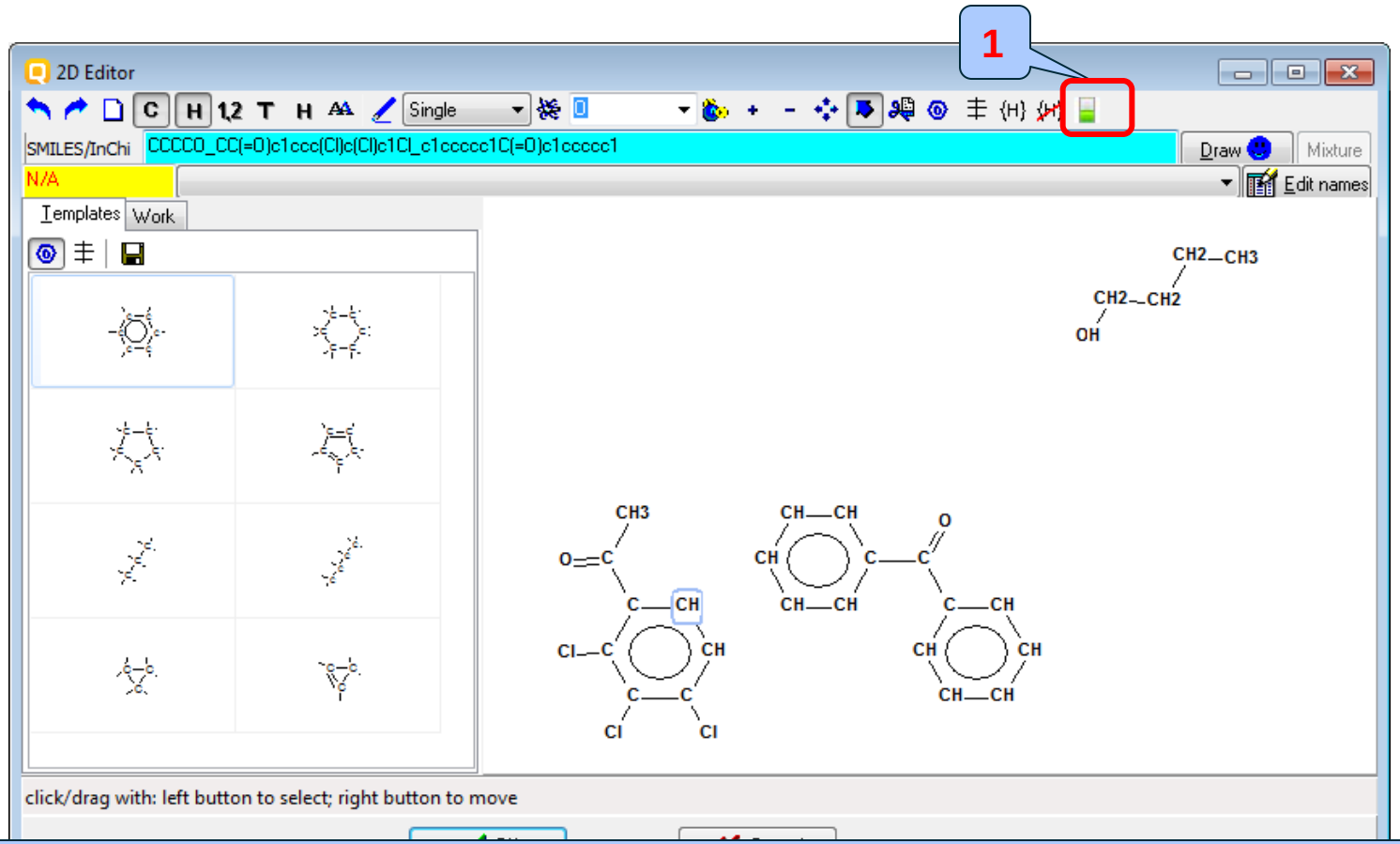


Chemical Input
Input quantities of mixture

- Once the constituents of the mixtures are pasted or drawn in the 2D editor window, a specific button for defining quantities appears (see next screenshot)
- Quantities of the constituents should be added manually
- There are several ways to add mixture quantity:
  - Mass
  - Mass Concentration
  - Volume Concentration
  - Fraction %
- Select “Fraction %” then “Weight %”

Chemical Input

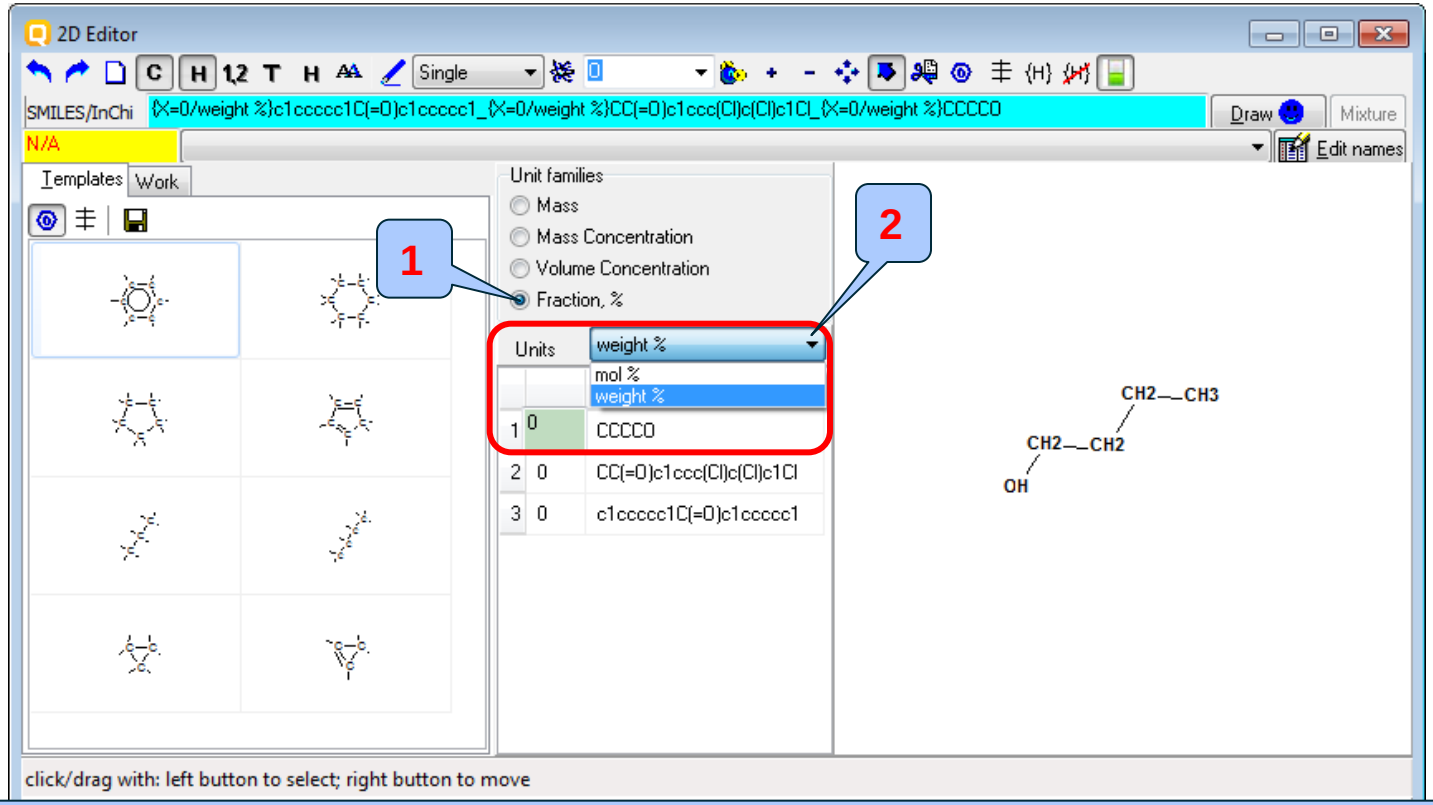
Input quantities of mixture



1. **Click** on button to add mixture quantities

Chemical Input

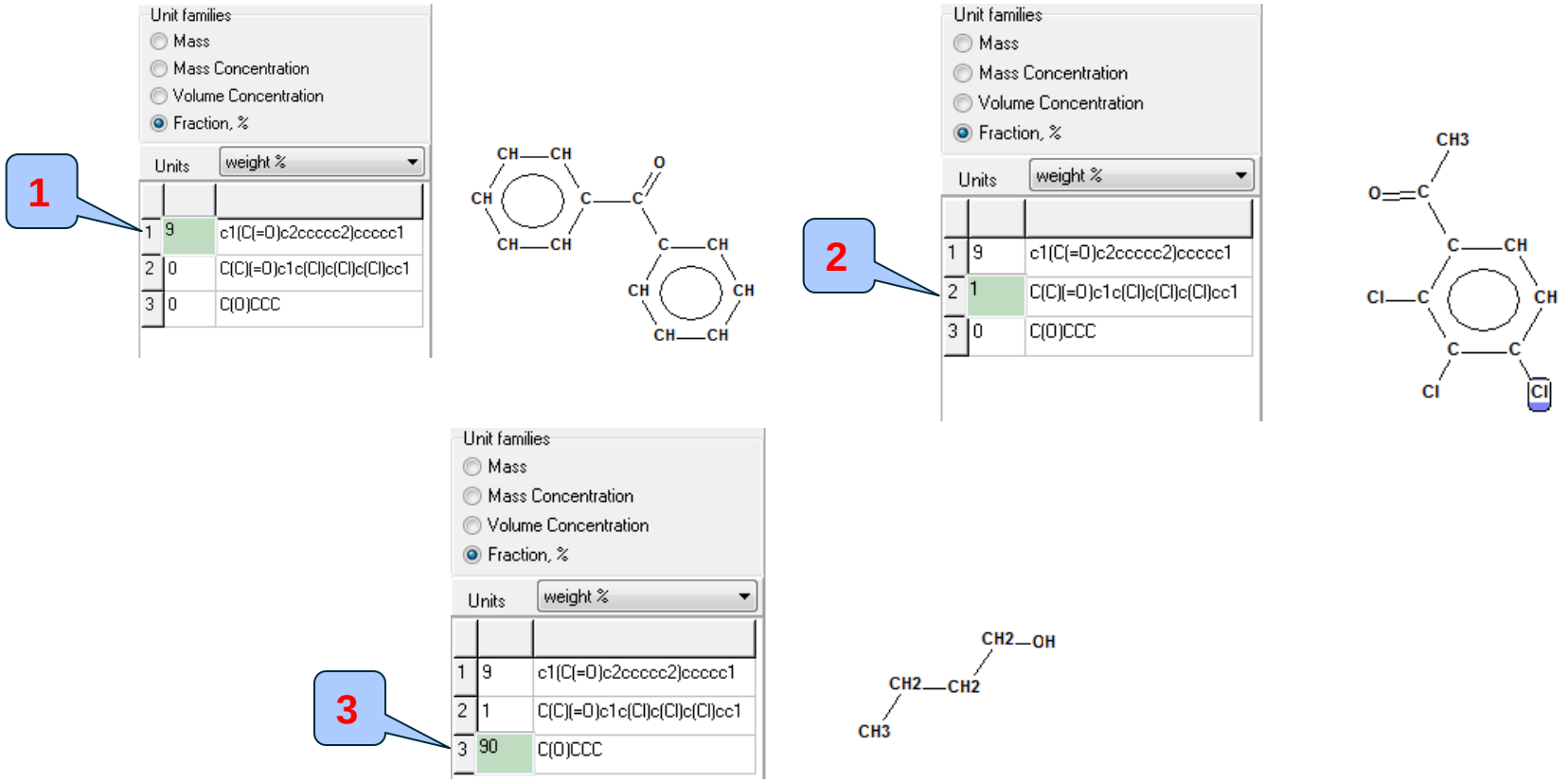
Input quantities of mixture



1. **Select** radio button "Fraction %"
2. **Select** "Wight %" from the appeared pop-up menu

Chemical Input

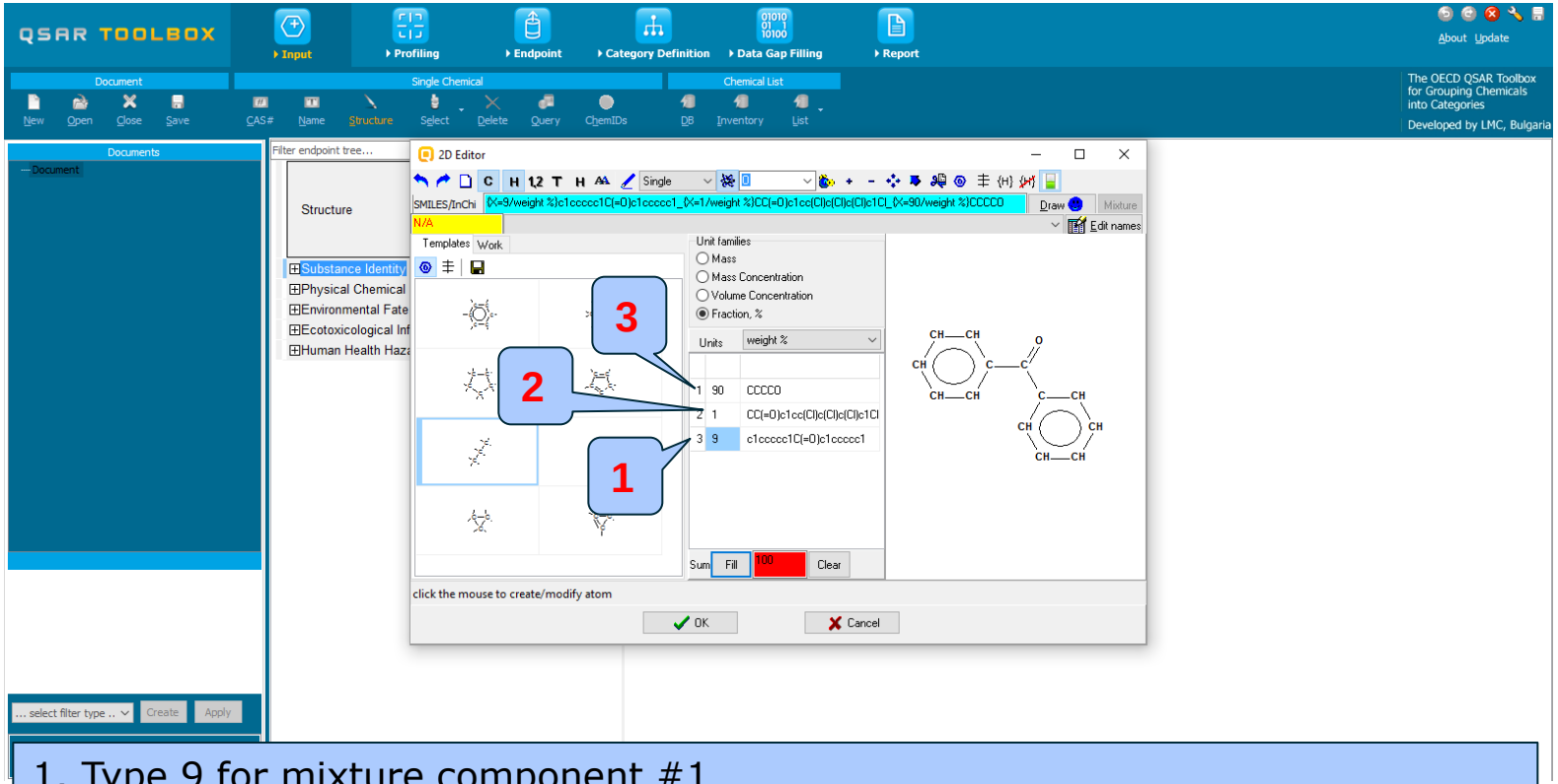
Input quantities of mixture



1. Type 9 for mixture component #1
2. Type 1 for mixture component #2
3. Type 90 for mixture component #3

Chemical Input

Input quantities of mixture

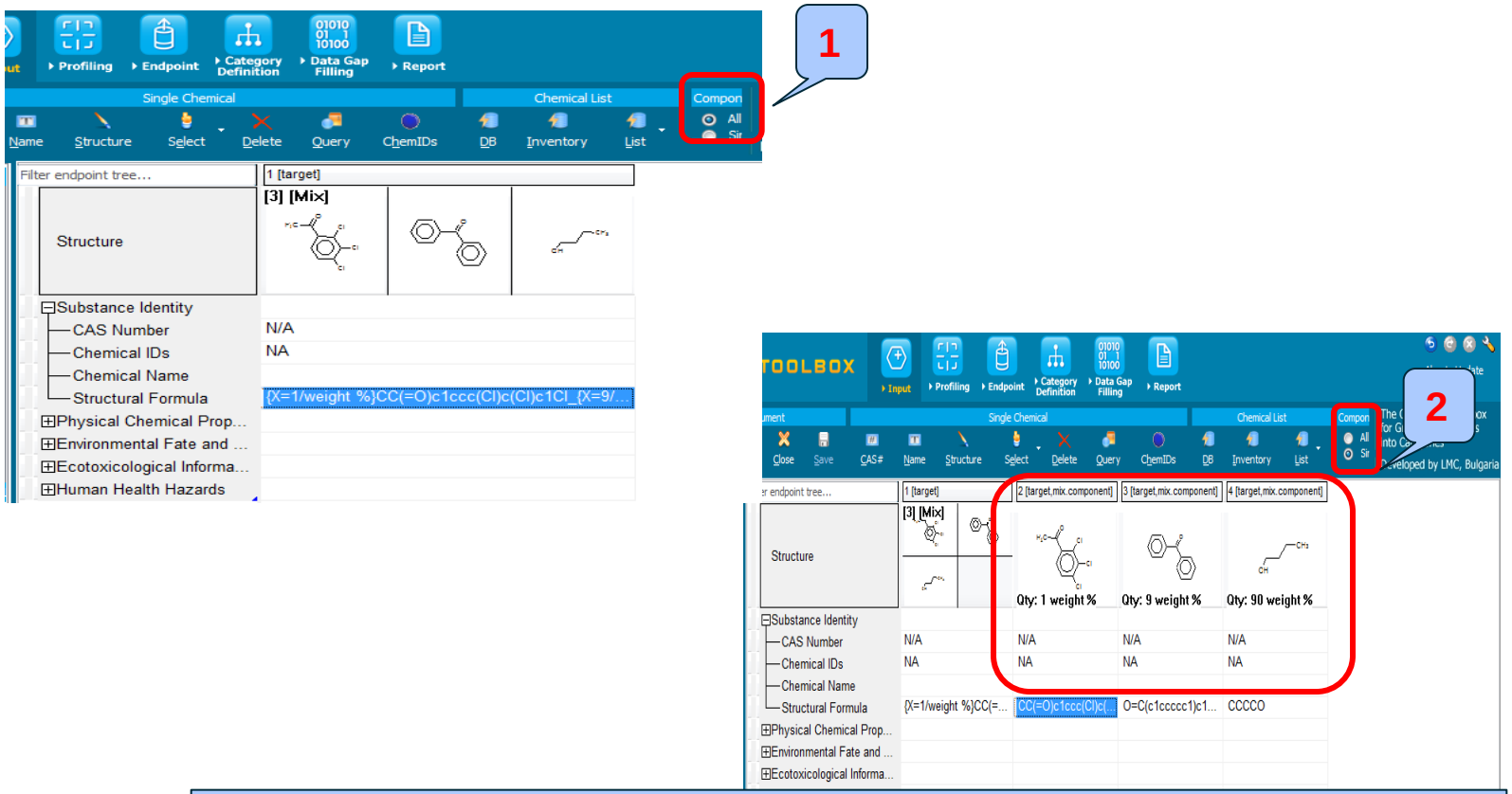


1. Type 9 for mixture component #1
2. Type 1 for mixture component #2
3. Type 90 for mixture component #3

Chemical Input
Target chemical identity

- The already drawn mixture 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).
- Visualization of components of the mixture is possible when user select Single Component Mode (see next slide)

Chemical Input
Target chemical identity



1. Select “All” radio button to see all components
2. Select “Single” radio button to see all individual components

Outlook

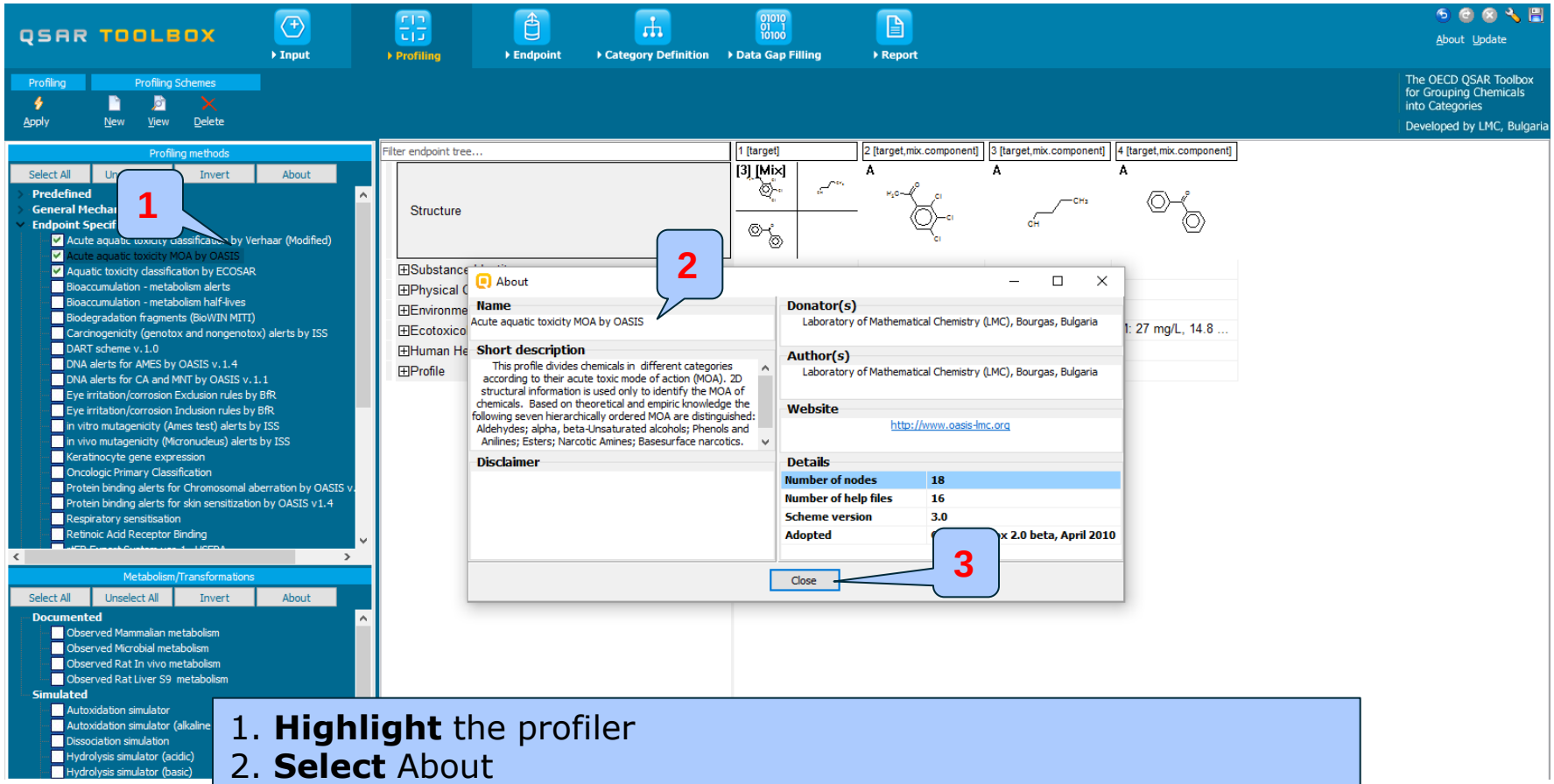
- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - **Profiling**

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

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



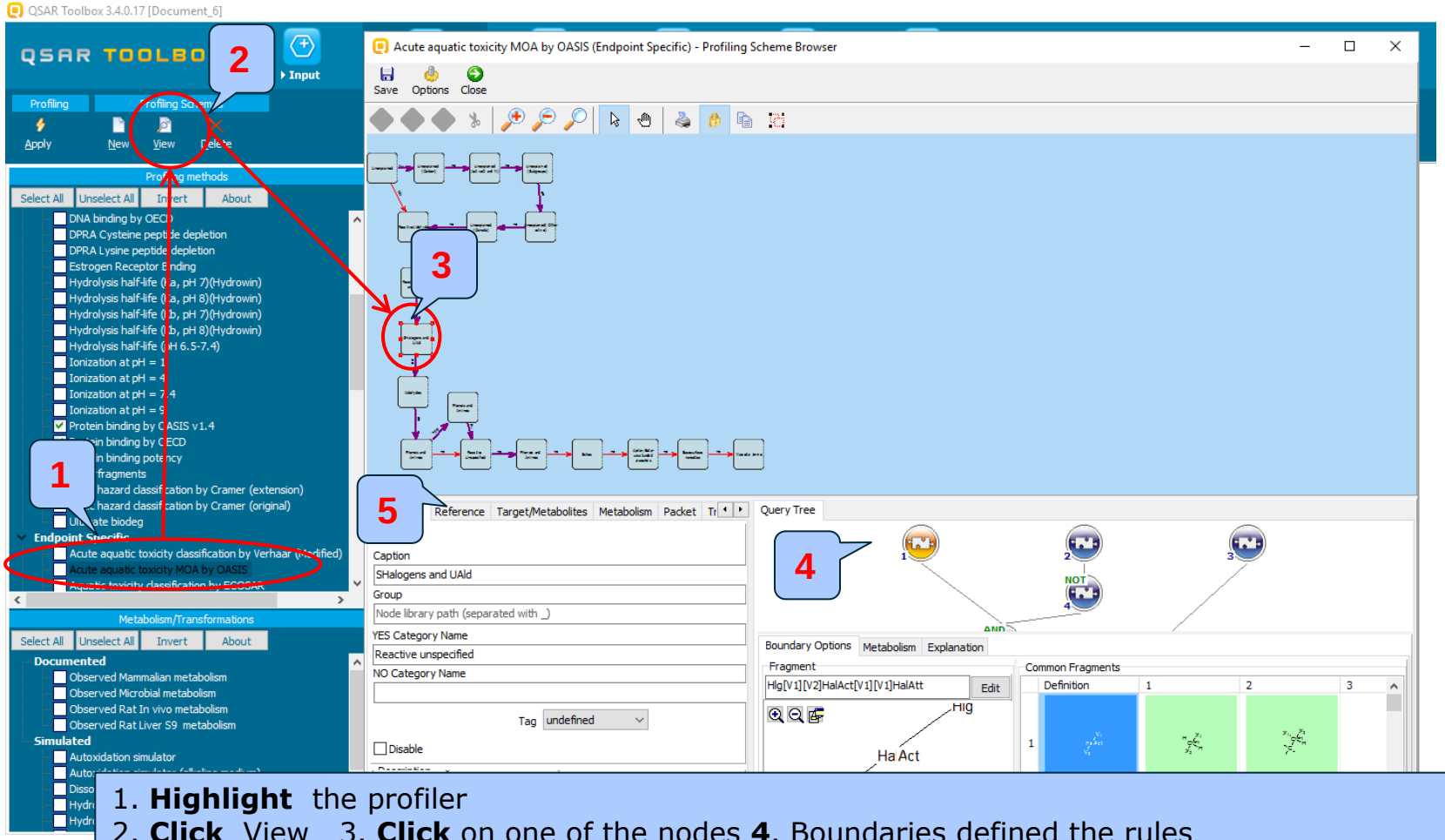
1. **Highlight** the profiler
2. **Select** About
3. **Click** Close

Profiling
Side-Bar to Profiling

- For most of the profilers, background information can be retrieved by highlighting one of the profilers (for example, Acute aquatic toxicity MOA by OASIS and clicking on "View" button(see next screen shot).

Profiling

Side-Bar to Profiling for Aqute aquatic toxicity MOA



1. **Highlight** the profiler
2. **Click** View 3. **Click** on one of the nodes **4**. Boundaries defined the rules
5. **Click** Reference to see detailed information. (Base surface narcotics)

Profiling
Profiling the target chemical

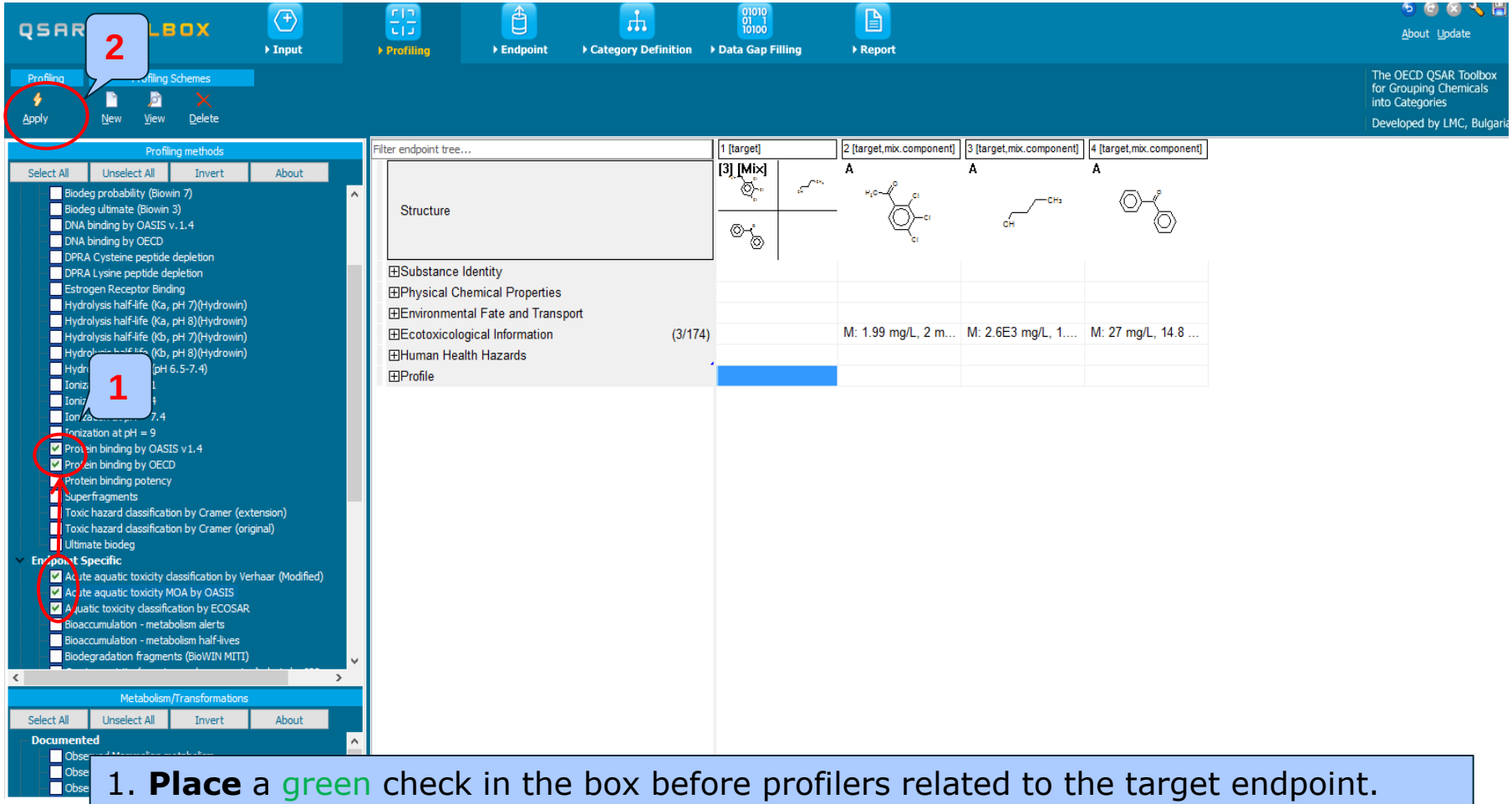
- Select the "Profiling methods" related to the target endpoint by clicking on the box next to the profilers name.

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

- For this example, the following primary profilers relevant to the aquatic toxicity are selected(see next screenshot):
  - US-EPA New Chemical Categories
  - Aquatic toxicity classification by ECOSAR – structural grouping
  - Acute aquatic toxicity MOA by OASIS – mechanistic grouping
  - Acute aquatic toxicity classification by Verhaar (Modified) – grouping by reactivity
  - Protein binding by OASIS v.1.4
  - Protein binding by OECD

Profiling

Profiling the target chemical



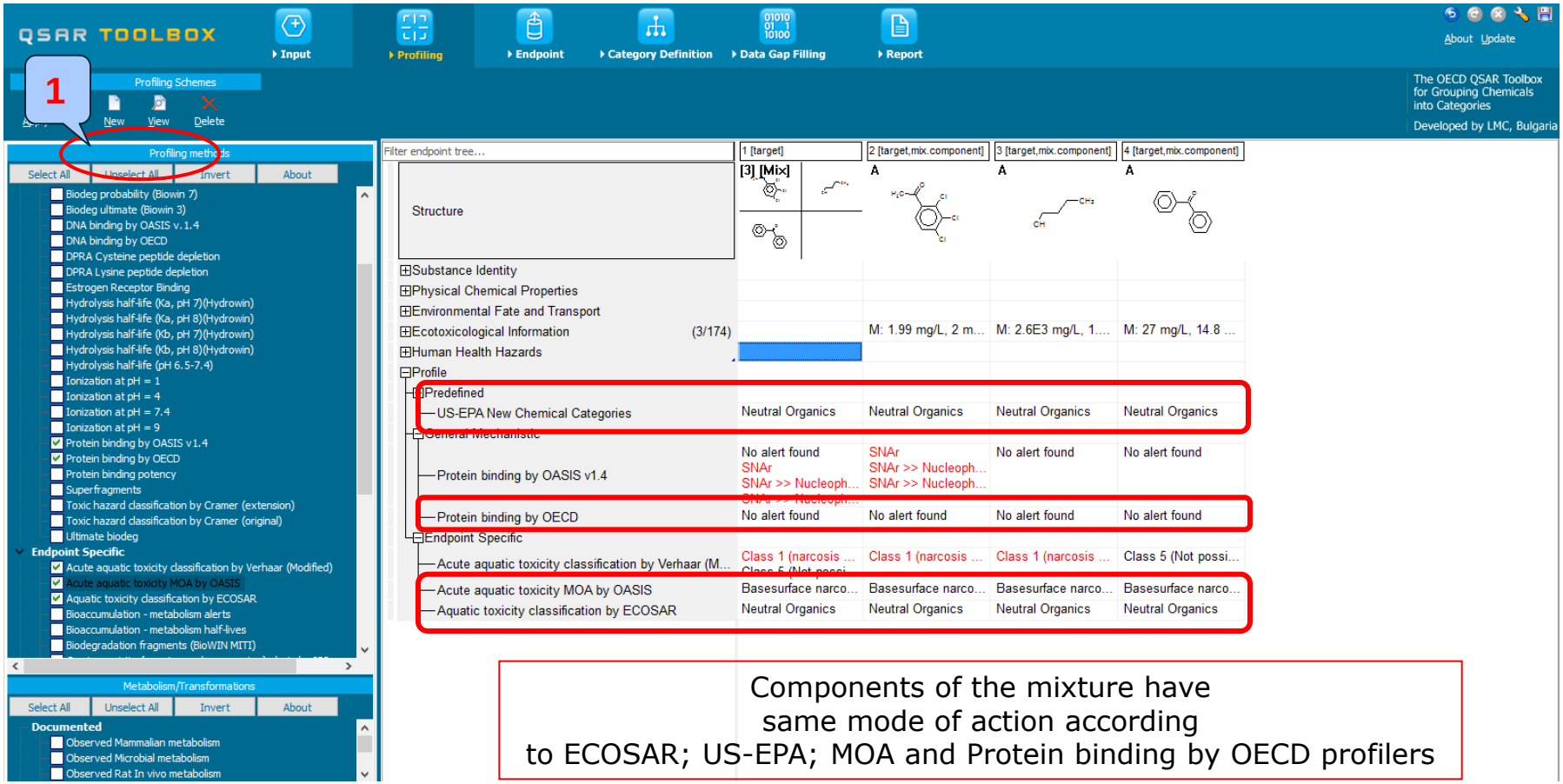
1. **Place** a green check in the box before profilers related to the target endpoint.
2. **Click** Apply

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; MOA by OASIS; US-EPA; Protein binding by OECD(see next slide).
- The results of profiling shows same mode of action for the three components of the mixture

Profiling

Profiling the target chemical



1. **Single click** on the box ⊞ (or double click on Profile) to open the nodes of the tree

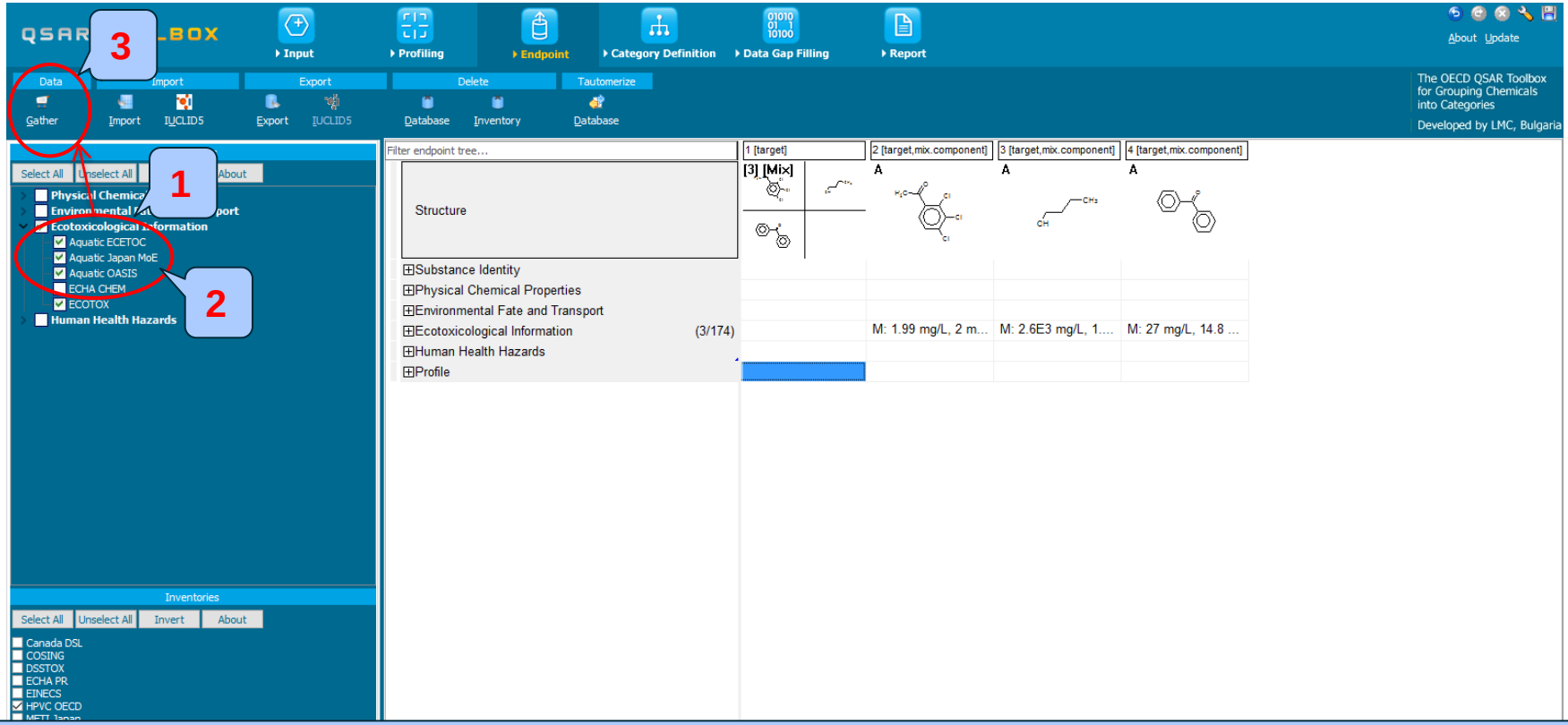
Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - 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 four aquatic databases containing aquatic toxicity data – Aquatic ECETOC; Aquatic Japan MoE; Aquatic OASIS; Aquatic US-EPA ECOTOX.

Endpoint

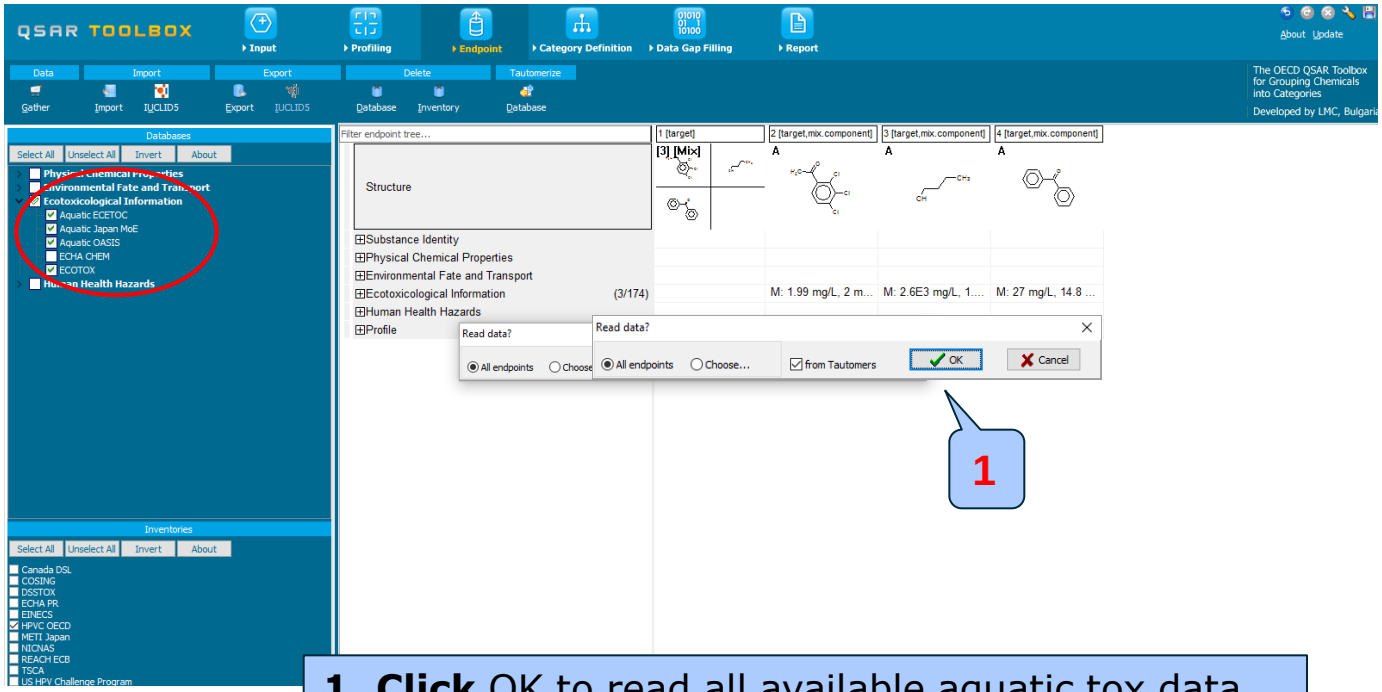


1. **Expand** the Ecotoxicological Information
2. **Select** databases related to the target endpoint by adding a green check in the box before the database name.
3. **Click** Gather

Endpoint
Process of collecting data

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

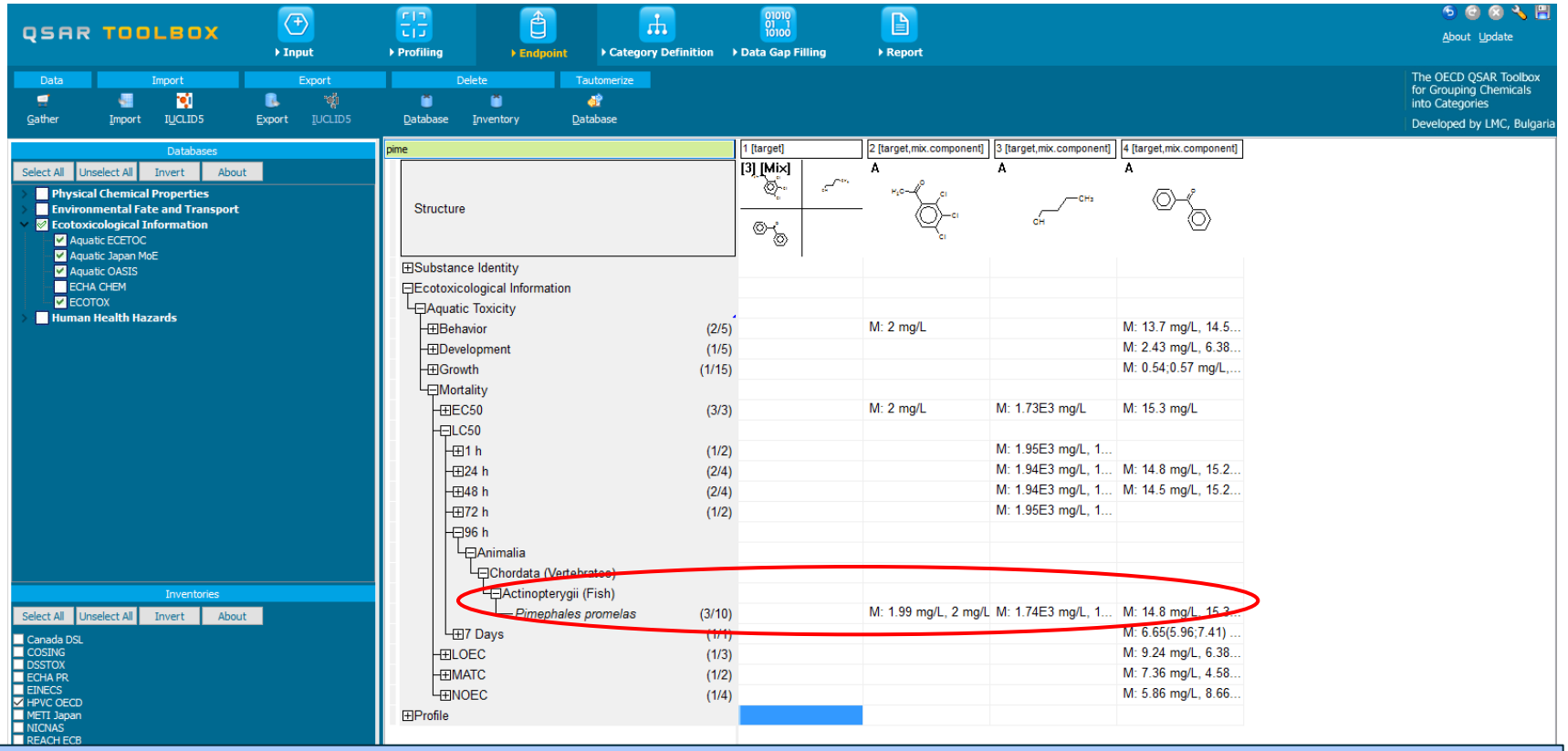
A window with "Read data?" appears. Now the user could choose to collect "all" or "endpoint specific" data



1. Click OK to read all available aquatic tox data

Endpoint
Process of collecting data

Target endpoint: LC50; *P.promelas*; 96h



10 experimental data for the investigated endpoint: LC 50;96h; *P.promelas* have been found for the components of the mixture

Recap

- You have entered the chemical mixture with defined components
- The results of profiling shows same mode of action for the three components of the mixture
- You have gather available experimental data for the target chemical mixture and found no experimental data for mixture. However experimental data for the components has been found
- You are ready to predict Acute aquatic toxicity to fish of mixture: Endpoint: LC50, Duration:96h; Effect: mortality; species: *Pimephales promelas*
- Now you are ready to continue with next step of the workflow "Data Gap Filling".

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - **Data Gap filling**

Data Gap Filling
Overview

- "Data Gap Filling" module give access to two different data gap filling tools:
  - **Independent MOA-** all components are with different mode of action
  - **Similar MOA-** all components are with similar mode of action
- More details about different MOA is given on next six slides #50-55
- In this particular case all components of the current mixture are with similar mode of action. In this respect Similar MOA is applied

Data Gap Filling
Independent MOA

Assumption – combined effect can be calculated from the effects caused by the individual mixture components by following the statistical concept of independent random events

Mixture response: $$E(C_{Mix}) = 1 - \prod_{i=1}^{N} [1 - E(C_i)]$$

$E(C_{Mix})$ - the effect provoked by the total mixture

$E(C_i)$ - the effects that the individual components would cause if applied singly at that concentration at which they are present in the mixture

Problem - dose-response relationships are practically unknown

Data Gap Filling
Similar MOA

Assumption – components in a mixture contribute to the joint effect, in proportion to their prevalence and individual potency

- **Components act at the same target site**
- **Components act by the same mechanism**
- **Components have similar effect (rather than mechanism)**

Method for calculation toxic effect of mixture with components acting by same mechanisms is given on next slide

Data Gap Filling
Similar MOA

Relative potency factor

$$RPF_j^{(i)} = \frac{ED_{resp}^{(i)}}{ED_{resp}^{(j)}}$$

i – index (reference) chemical
ED_{resp} – dose (concentration) of a chemical that cause a specified response (fraction of animals that respond, fractional change in a measured physiological value, etc.)

Chemical Equivalent Dose (Concentration)

$$CED_j^{(i)} = RPF_j^{(i)} d_j$$

Dose (concentration) of the reference chemical *i* that will cause the same effect as chemical *j* at dose (concentration) d_j

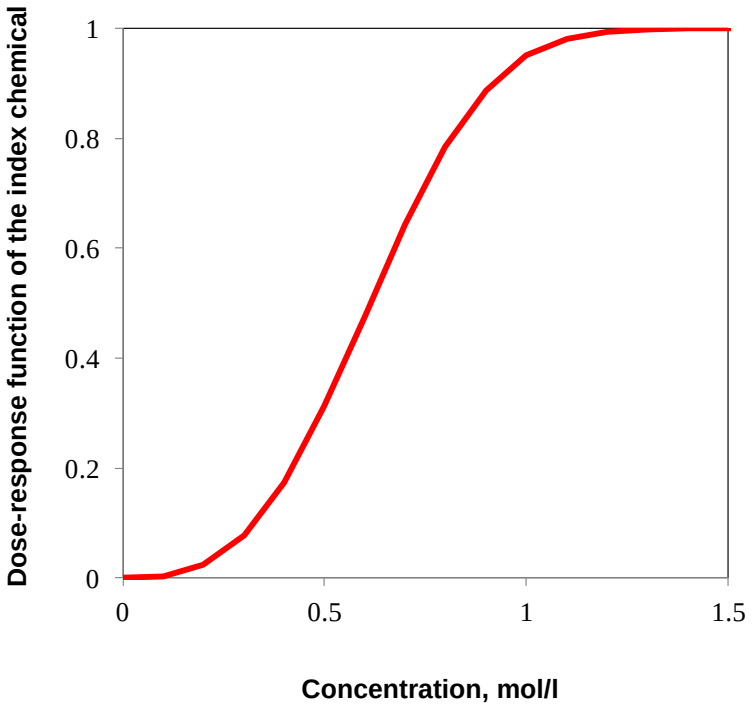
Index Chemical Equivalent Dose (Concentration)

$$ICED = \sum_{j=1}^{J} CED_j^{(i)} = \sum_{j=1}^{J} RPF_j^{(i)} d_j$$

Equivalent dose (concentration) of the reference chemical *i* that will cause the same effect as the mixture

Data Gap Filling
Similar MOA

Toxic effect of mixture - response (fraction of animals that respond, fractional change in a measured physiological value, etc.) as a result of exposure to mixture



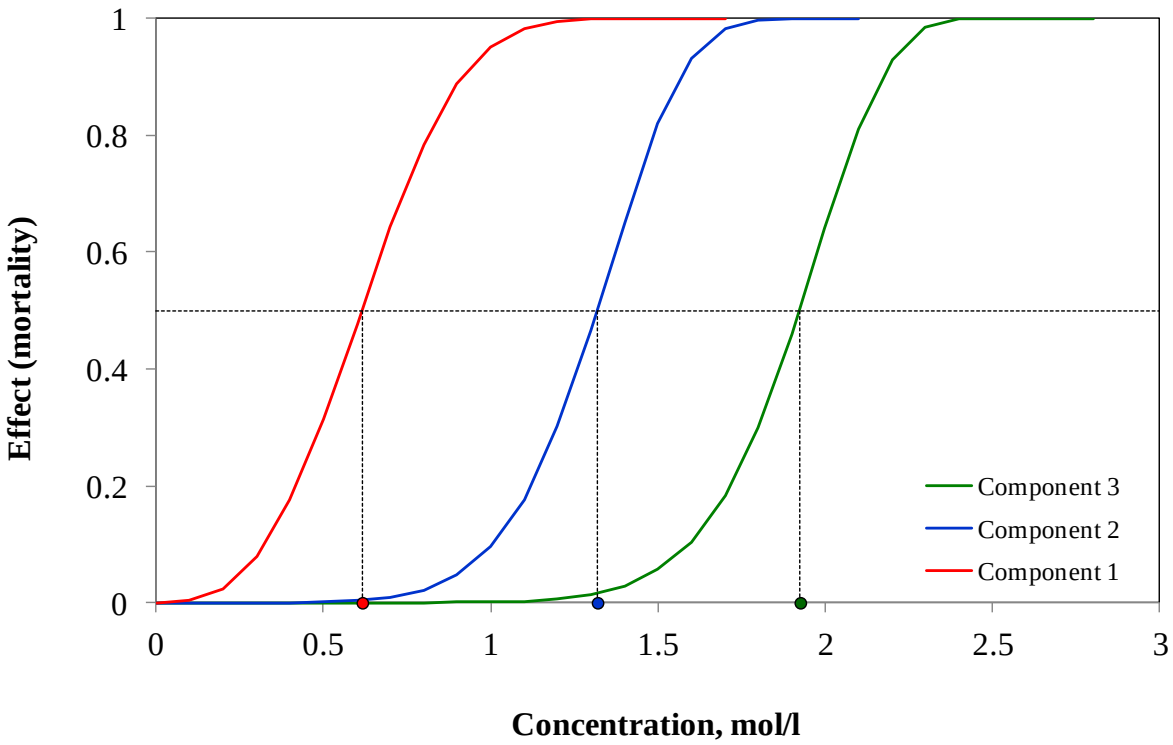
$$Effect^{Mixture} = f_i(ICED)$$

f_i - dose-response function of the index chemical

Illustration of calculating effect of mixture is given on next two slides

Data Gap Filling
Similar MOA (Illustration)

Reference chemical: Component 1 (*i* = 1)



Relative potency factors

$$RPF_j^{(1)} = \frac{LC_{50}^{(1)}}{LC_{50}^{(j)}}$$

Equivalent concentrations

$$CED_j^{(1)} = RPF_j^{(1)} C_j$$

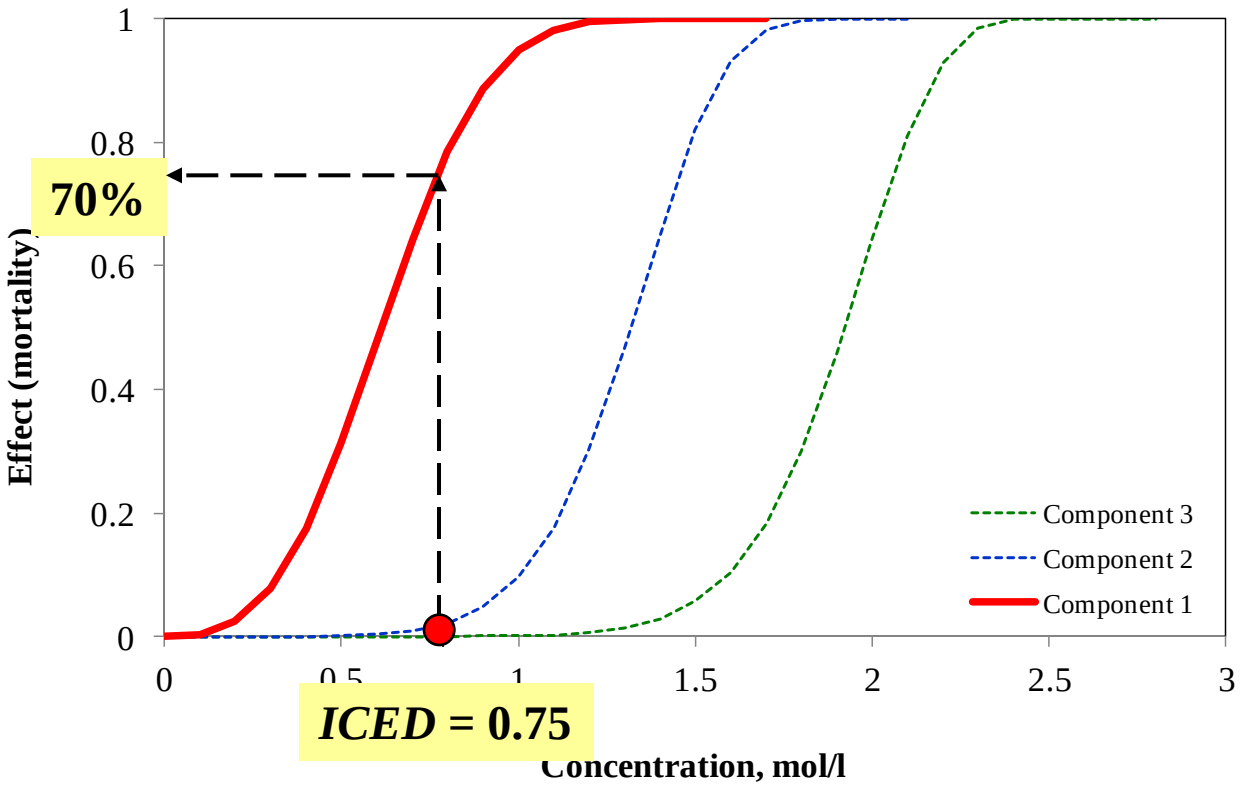
Index Chemical Equivalent Concentration

$$ICED = \sum_{j=1}^{J} CED_j^{(1)}$$

$$ICED \rightarrow Effect^{Mixture} = f_i(ICED)$$

Data Gap Filling
Similar MOA (Illustration)

Reference chemical: Component 1 (*i* = 1)



Relative potency factors

$$RPF_j^{(1)} = \frac{LC_{50}^{(1)}}{LC_{50}^{(j)}}$$

Equivalent concentrations

$$CED_j^{(1)} = RPF_j^{(1)} C_j$$

Index Chemical Equivalent Concentration

$$ICED = \sum_{j=1}^{J} CED_j^{(1)}$$

$$ICED = 0.75 \Rightarrow Effect^{Mixture} = f_i(ICED) \approx 70\% \text{ mortality}$$

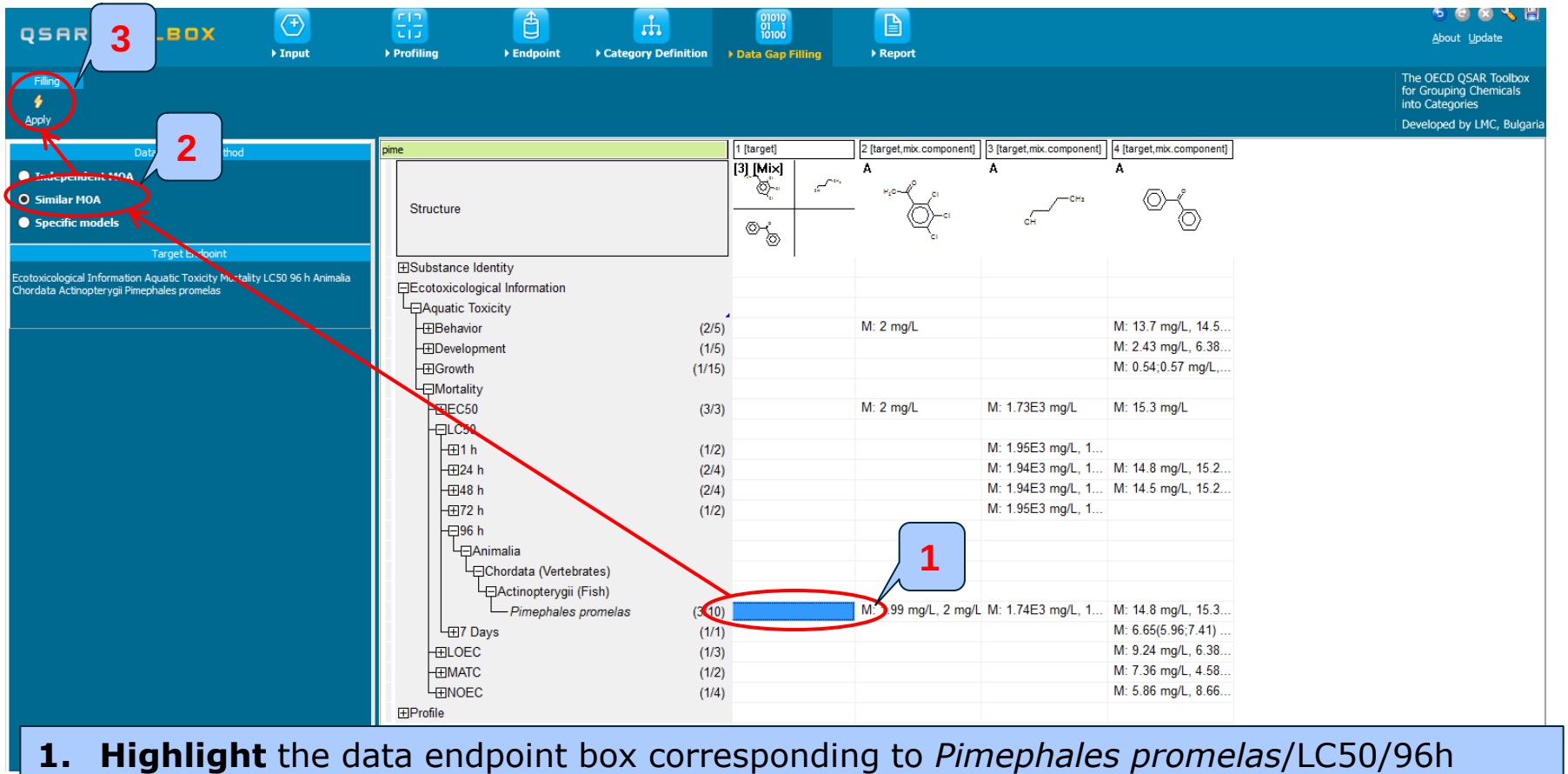
Data Gap Filling
Case study

- In this particular case all components of the current mixture are with similar mode of action. In this respect Similar MOA is applied

- Application of Similar MOA for our case study is illustrated on next slides

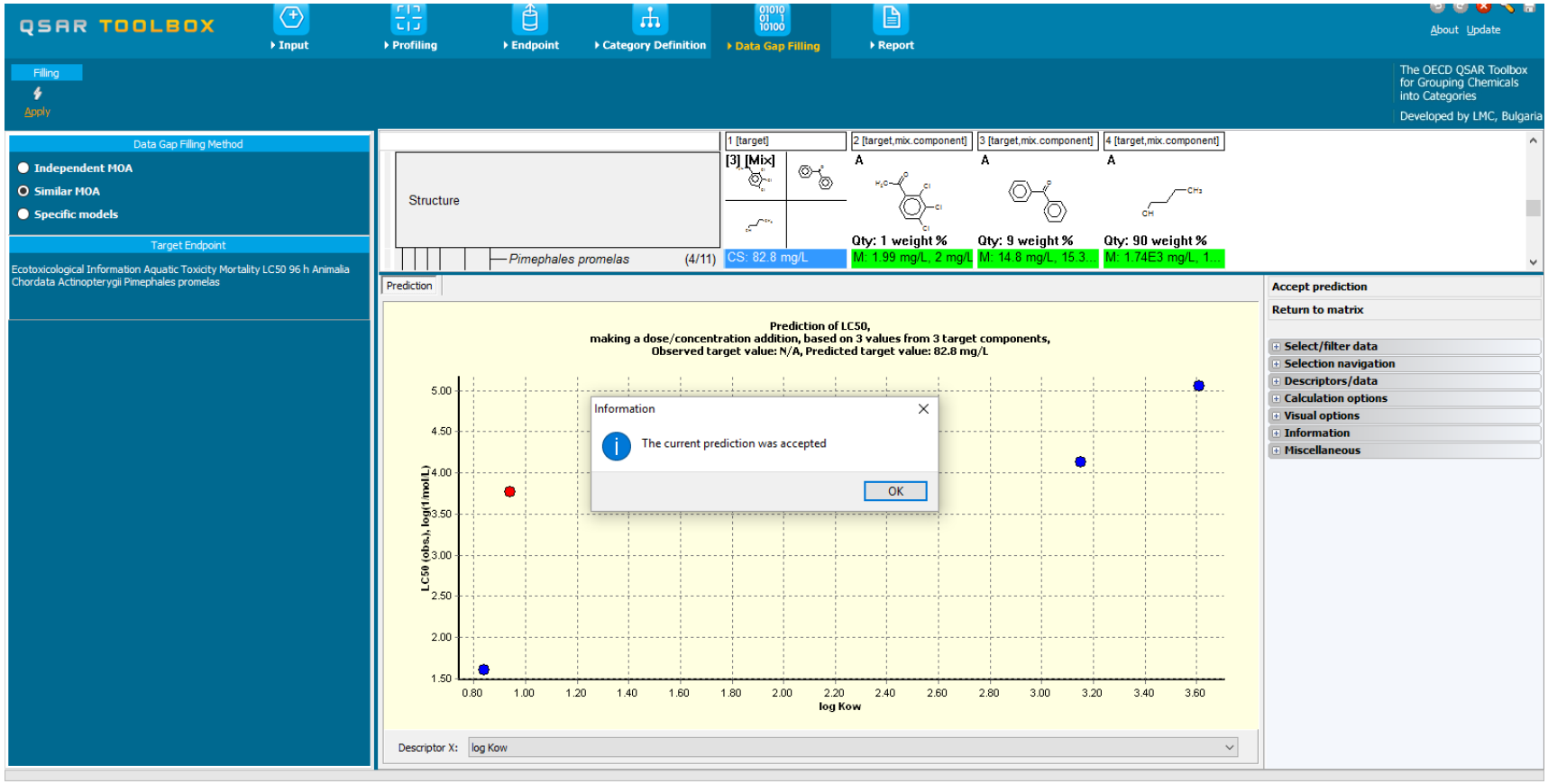
Data Gap Filling

Apply Similar MOA



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

Data Gap Filling
Results of Similar MOA



Data Gap Filling
Interpreting Similar mode

- 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 (i.e. mixture).

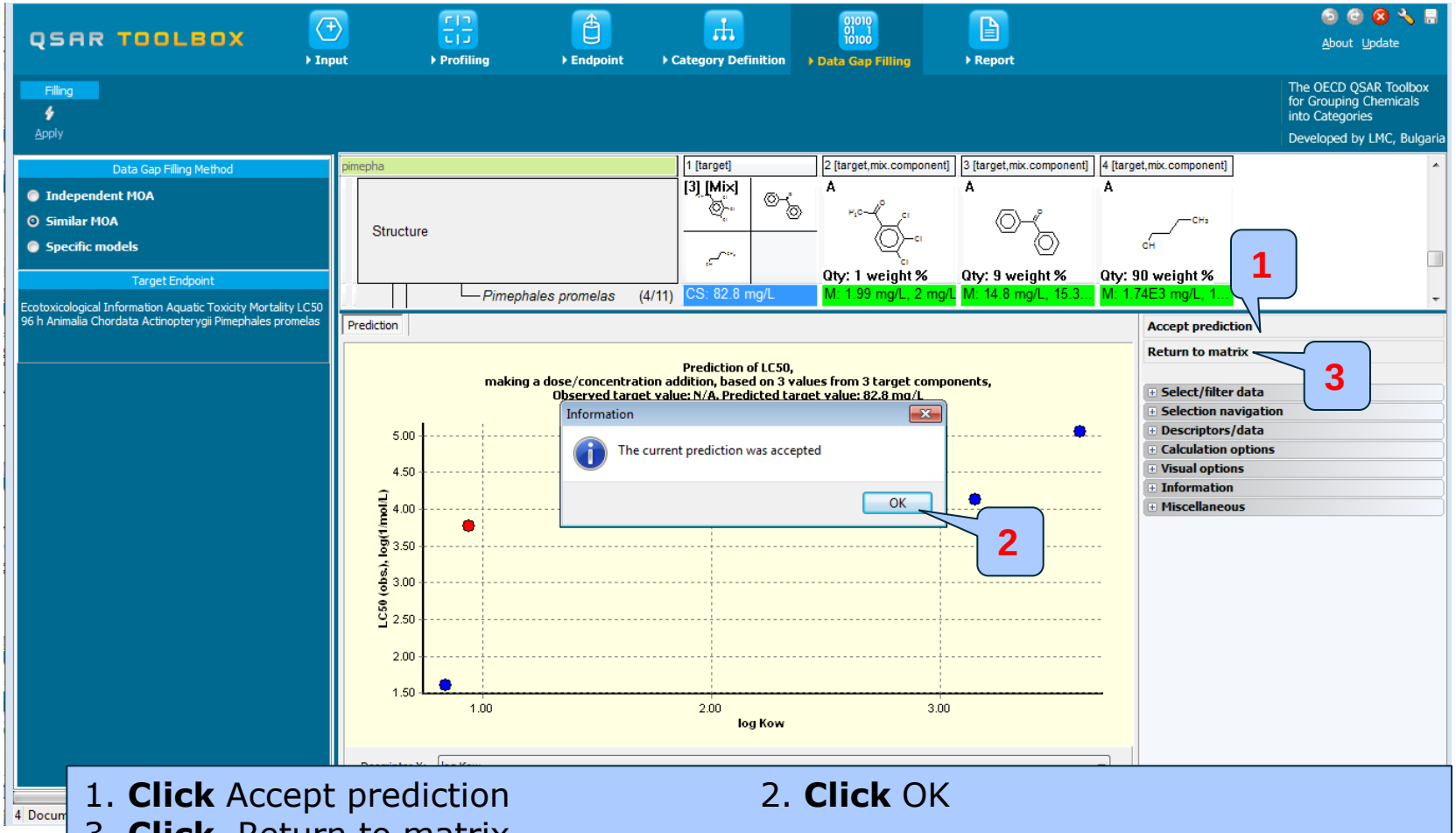
- The **BLUE** dots represent the experimental results available for the analogues(i.e. components of the mixture) used in the analysis.

Data Gap Filling
Results

- The components of the mixture have same mode of action.
- By accepting the prediction the data gap is filled (see next screen shot).
- By clicking on Return to Matrix, the user can close the Similar mode and proceed with the workflow (see next screen shot).

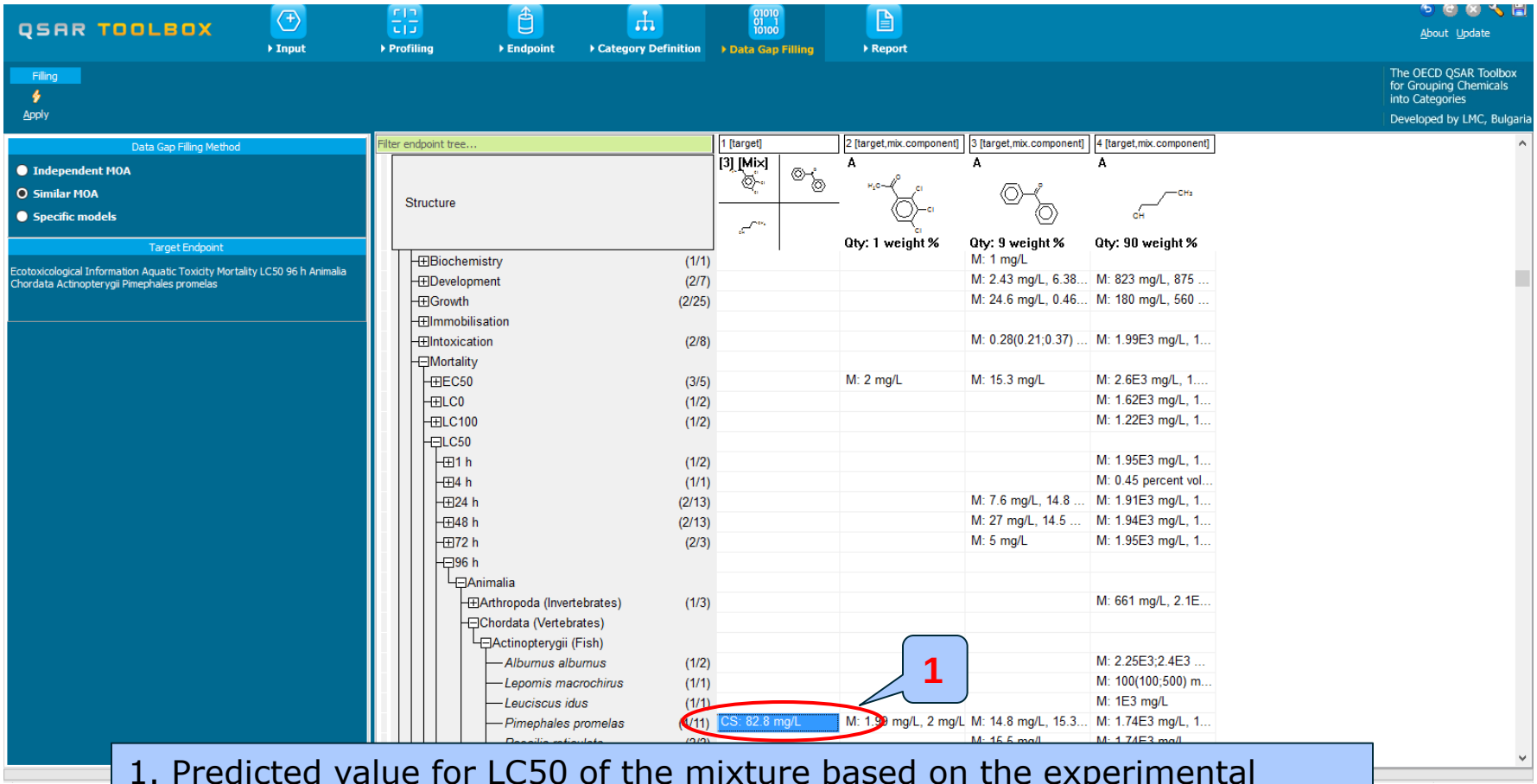
Data Gap Filling

Accept prediction results



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

Data Gap Filling
Predicted value for LC50



1. Predicted value for LC50 of the mixture based on the experimental data of its components is **82.8 mg/l**

Outlook

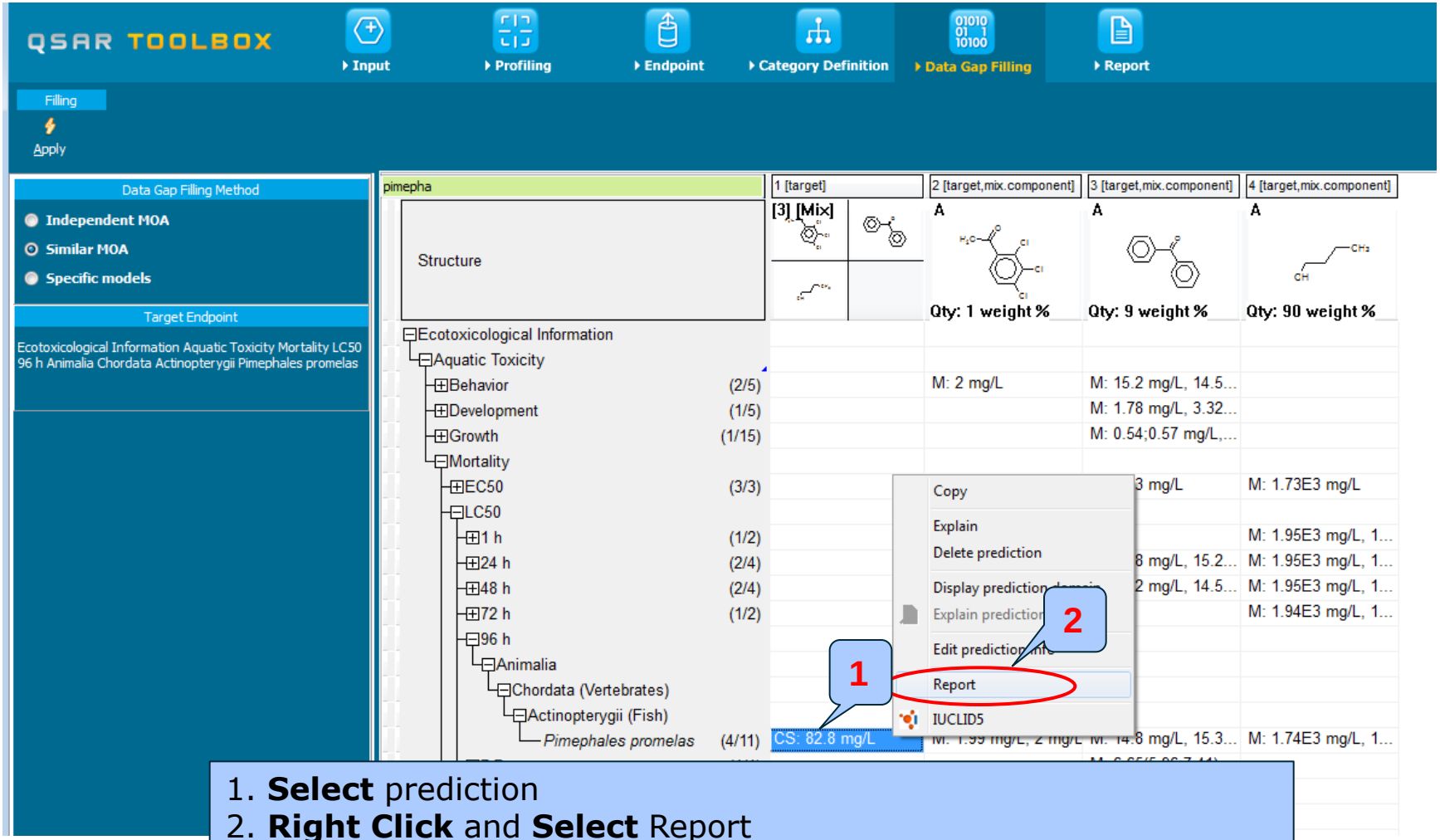
- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - Data Gap filling
  - **Report**

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 obtained for mixture includes specific information related to mixture prediction. The report can then be printed or saved in different formats.

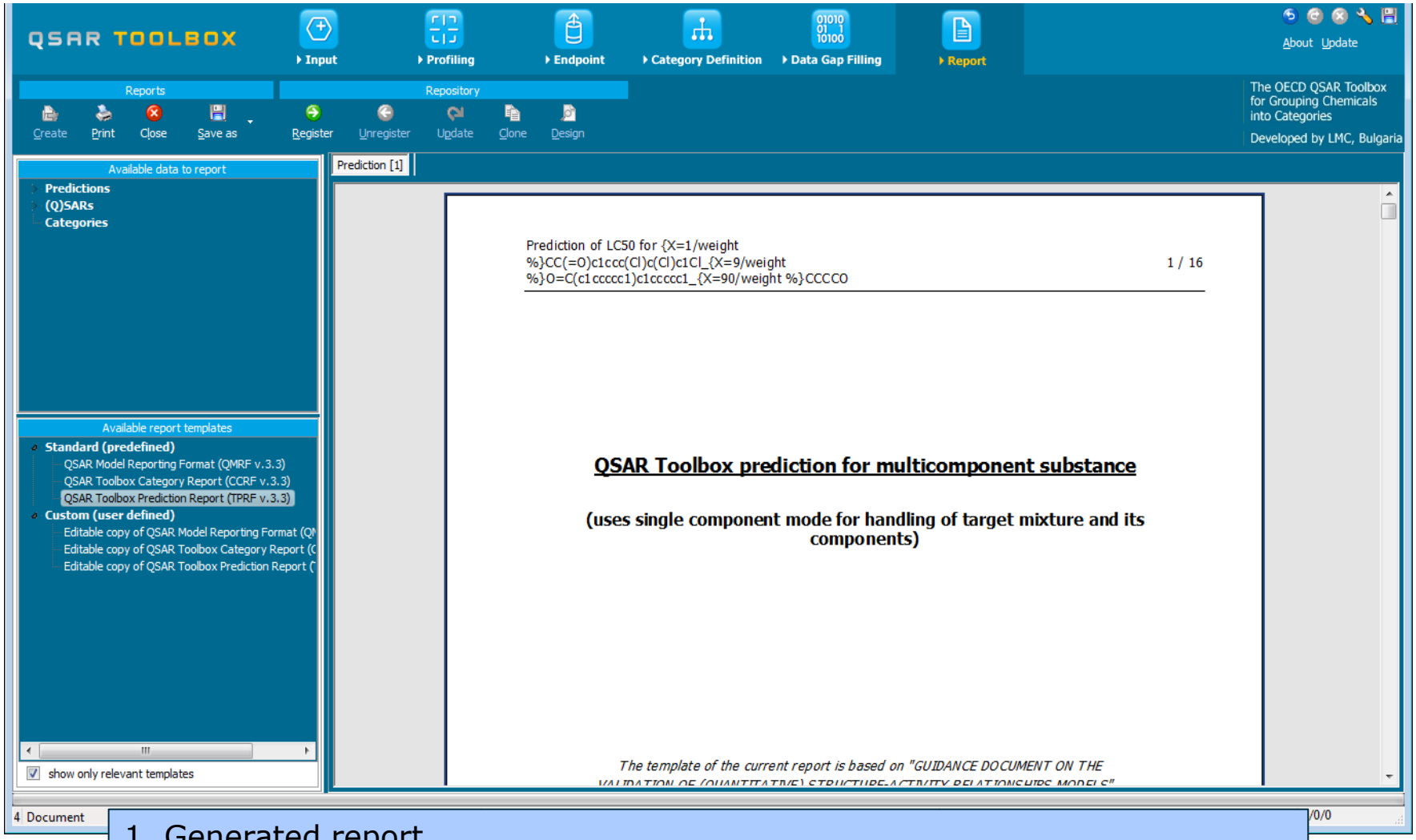
- Generating the report is shown on next screenshots

Report



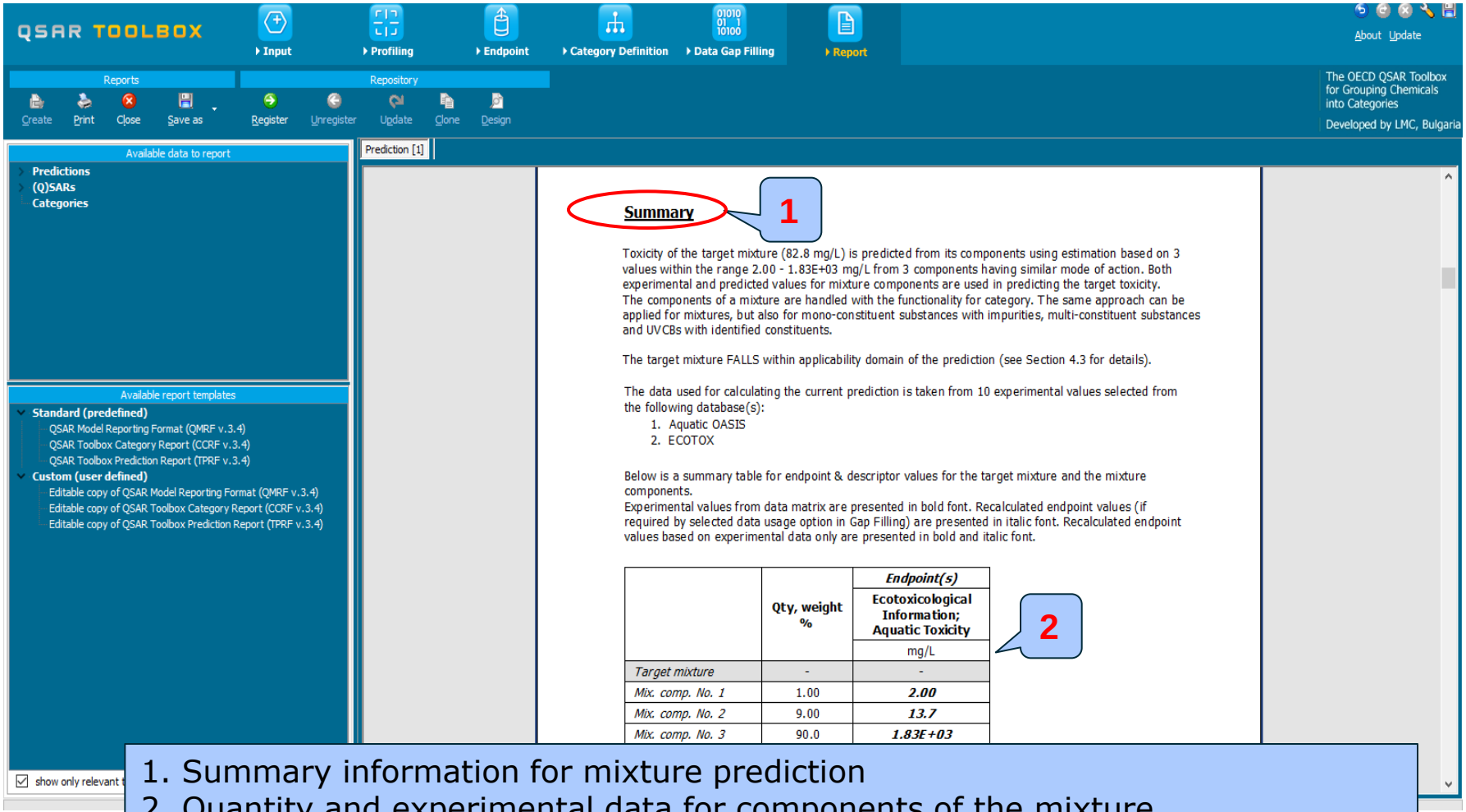
1. **Select** prediction
2. **Right Click** and **Select** Report

Report



1. Generated report

Report



1. Summary information for mixture prediction
2. Quantity and experimental data for components of the mixture

Outlook

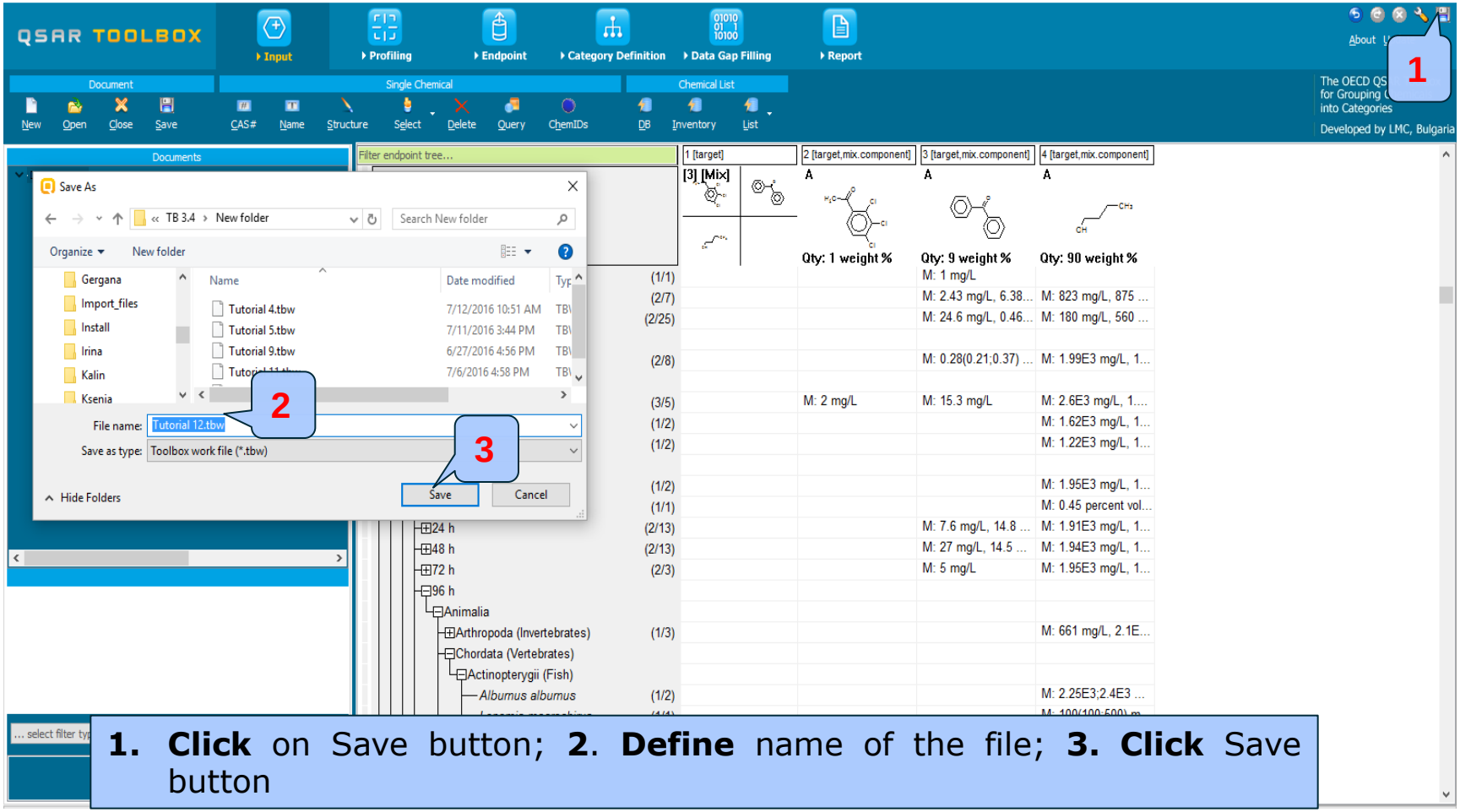
- Background
- Objectives
- The exercise
- Workflow
  - Input
  - Profiling
  - Endpoint
  - Data Gap filling
  - Report
- **Save the prediction result**

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).

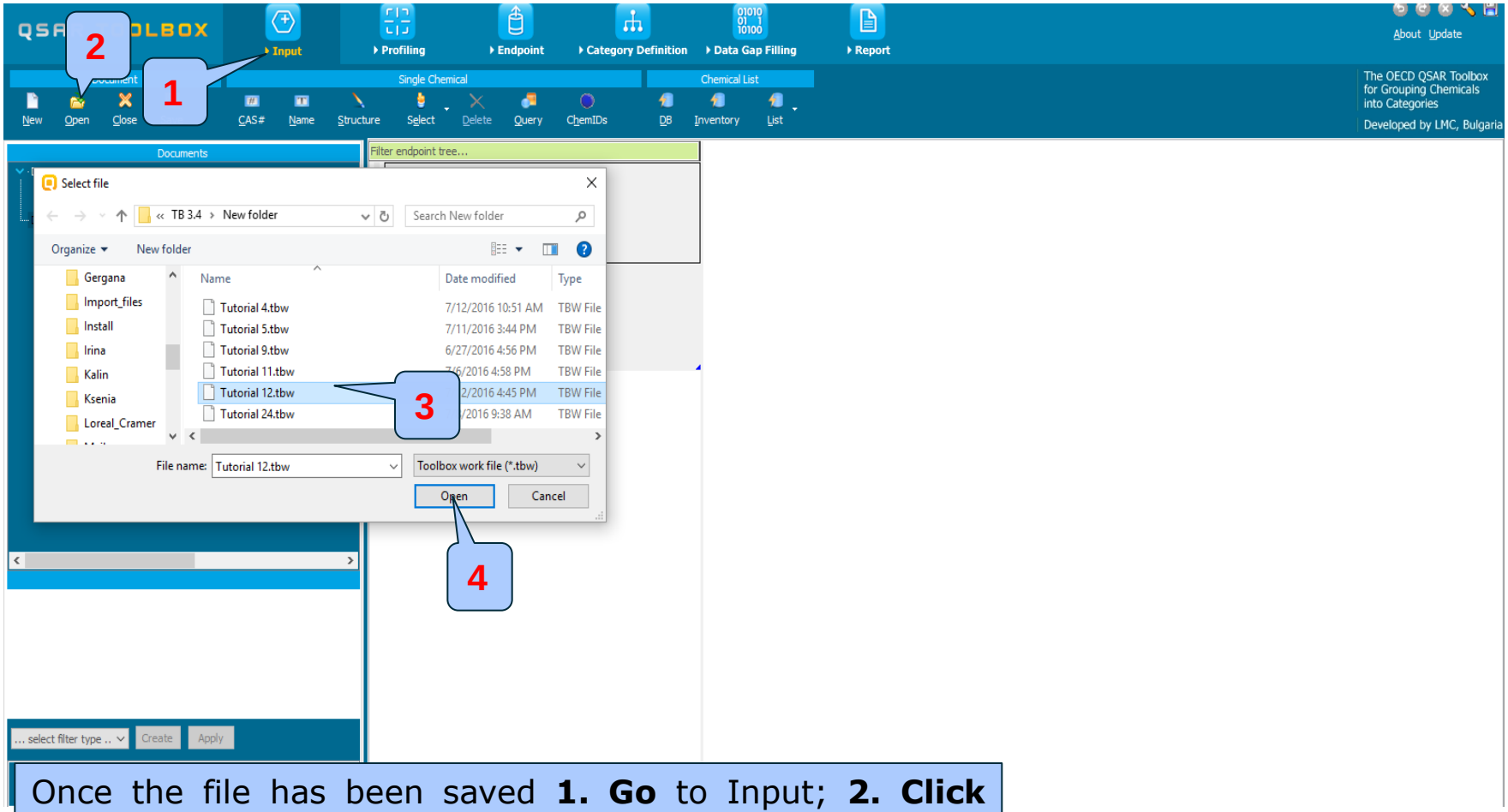
- Saving/Loading the file with TB prediction is shown on next screenshots

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