QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.4.1

Step-by-step example on how to build a user-defined profiling scheme accounting for the (a)biotic activation of the chemicals

Outlook

- **Background**
- Objectives
- Profiling
- The exercise

Background

- This is a step-by-step presentation designed to provide guidance to the Toolbox user on how to create their own profiling scheme accounting for the (a)biotic activation of the chemicals.
- The created custom profiler can be used for searching precursors of a given target.
- Two examples will be illustrated:
  - Searching PBT precursors in the Toolbox databases;
  - Searching p-benzoquinone precursors in a specific file.

Note: Please note that building of custom items (such as profilers, (Q)SAR models as well as importing of custom databases) is only enabled in single user mode.
So, if your Toolbox is installed in multiuser mode, you will be not able to follow this tutorial.

Outlook

- Background
- **Objectives**
- Profiling
- The exercise

Objectives

- **This presentation demonstrates how to build and use a new profiling scheme including:**
  - naming of the new scheme;
  - selection of specific (a)biotic activation;
  - searching of specific (a)biotic product;
  - saving the new profiler;
  - defining category by using the custom profiler;
  - profiling a chemical list.

Outlook

- Background
- Objectives
- **Profiling**
- The exercise

Profiling
Overview

- As you know, “Profiling” refers to the electronic process of retrieving relevant information on a compound which is stored in the Toolbox, other than fate and toxicity data.
- The Toolbox has many predefined profilers but it also allows the user to develop new custom profilers.

Outlook

- Background
- Objectives
- Profiling
- **The exercise**

The Exercise
Example 1

- In the first example we will build a profiler that identifies precursors of 1,2,4-trichlorobenzene [CAS 120-82-1] (i.e. we will search for chemicals that are transformed to our target).
- The 1,2,4-trichlorobenzene (1,2,4-TCB) is considered as a substance fulfilling the PBT-criteria[1,2]. Therefore, the potential precursors of 1,2,4-TCB could be prioritized for further PBT-related investigations.
- Precursors of 1,2,4-TCB will be searched in the Toolbox databases containing biodegradation (BOD) 301C data.

[1] European Chemicals Agency (ECHA). PBT/vPvB assessments under the previous EU chemicals legislation.
[2] European Chemicals Agency (ECHA). ECB – Summary fact sheet. PBT working group –PBT list No.4. 2008. TC NES subgroup on identification of PBT and vPvB substances. Results of the evaluation of the PBT/vPvB properties of: 1,2,4-trichlorobenzene.

The Exercise
Start building a new profiler

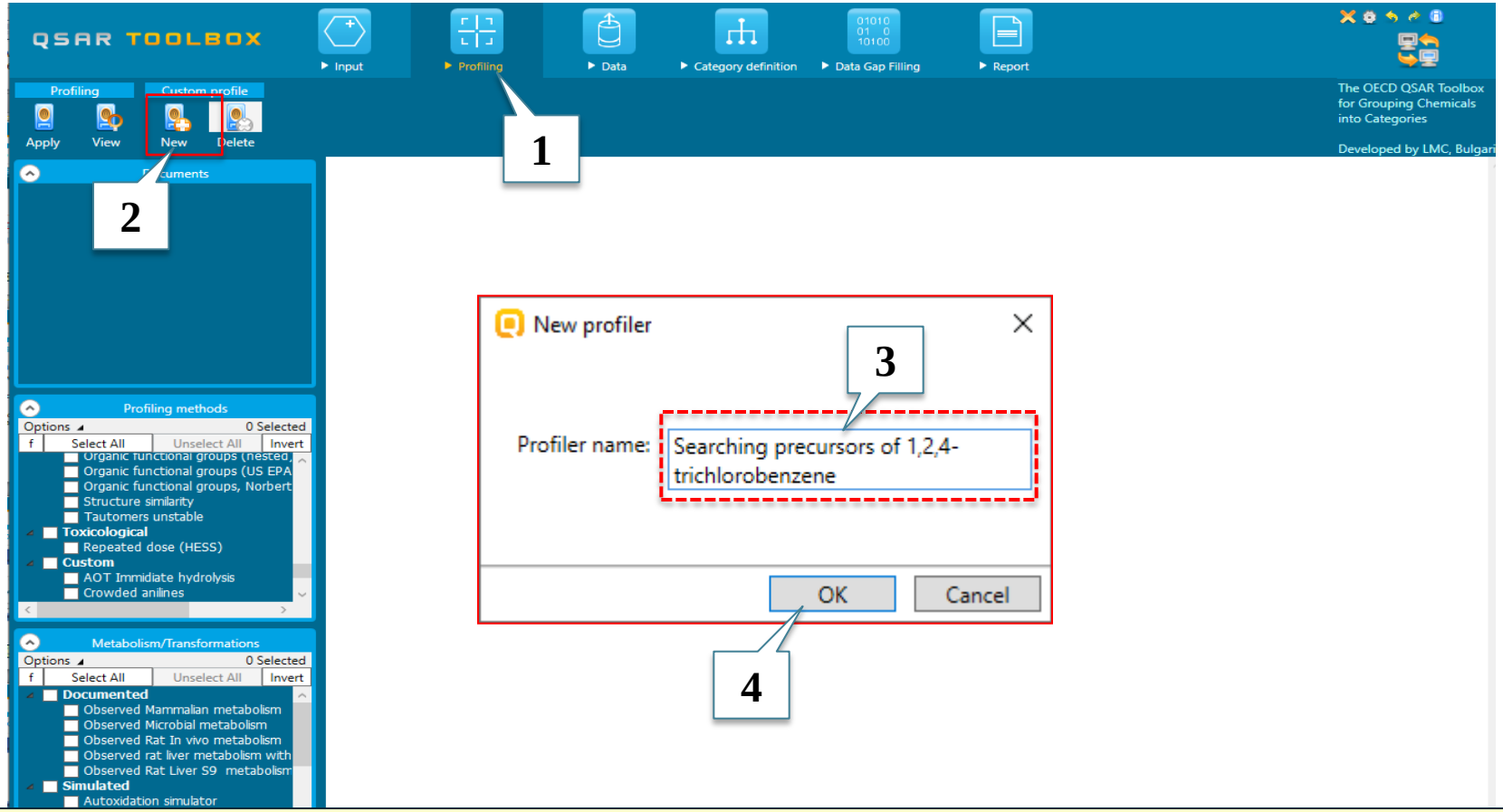
We are going to create a new profiling scheme:

- Open the Toolbox.
- Move to the Profiling module

(see next screen shot).

Building of a new profiler

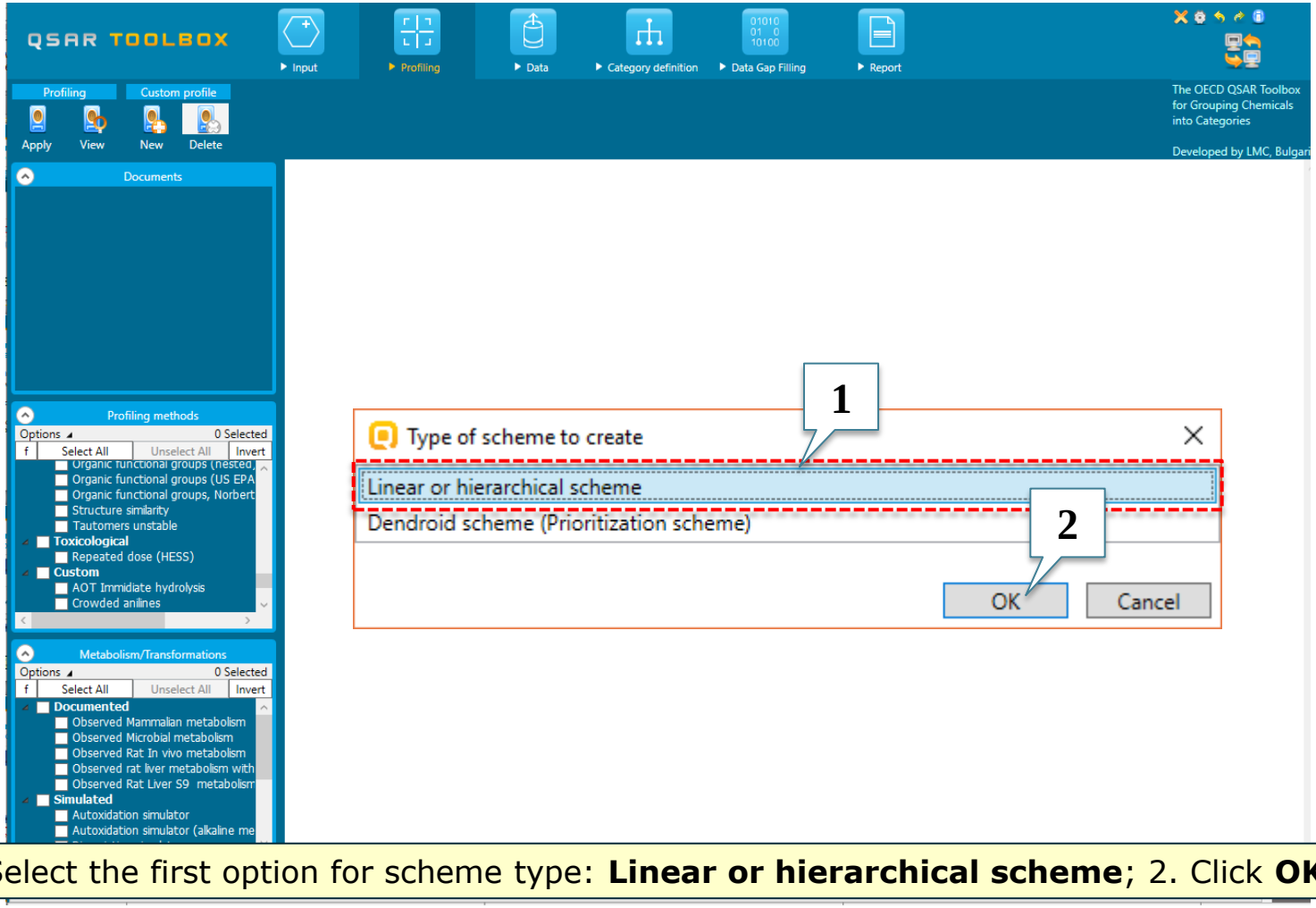
Define the name of the new profiler



1. Go to the ***Profiling*** module; 2. Click **New**; 3. Enter the name of the new scheme, for example ***"Searching precursors of 1,2,4-trichlorobenzene"***; 4. Confirm by click **OK**.

Building of a new profiler

Define type of new profiler



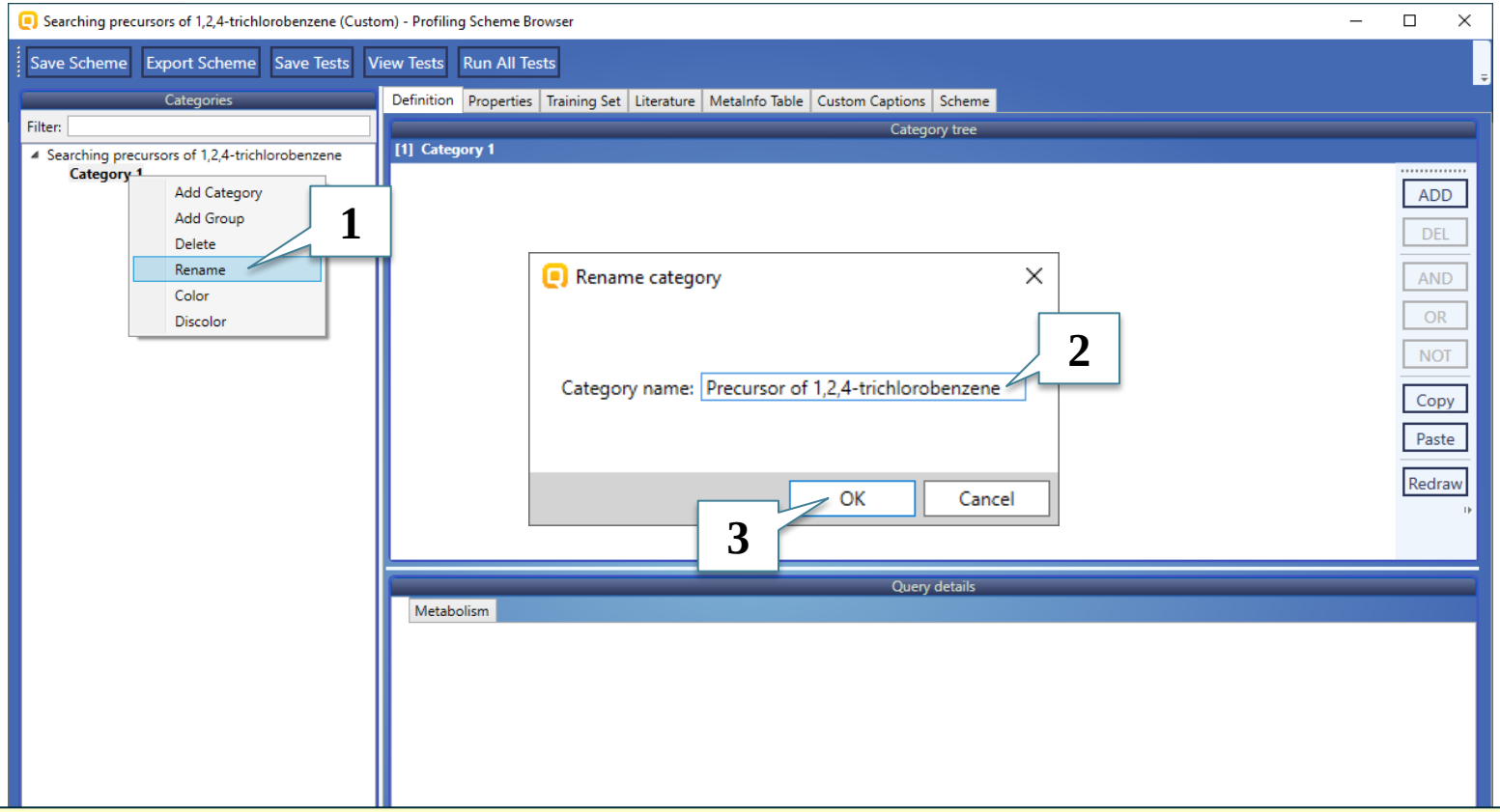
1. Select the first option for scheme type: **Linear or hierarchical scheme**; 2. Click **OK**.

Building of a new profiler
Creating a profiling category

Once the scheme is created, it will be defined:

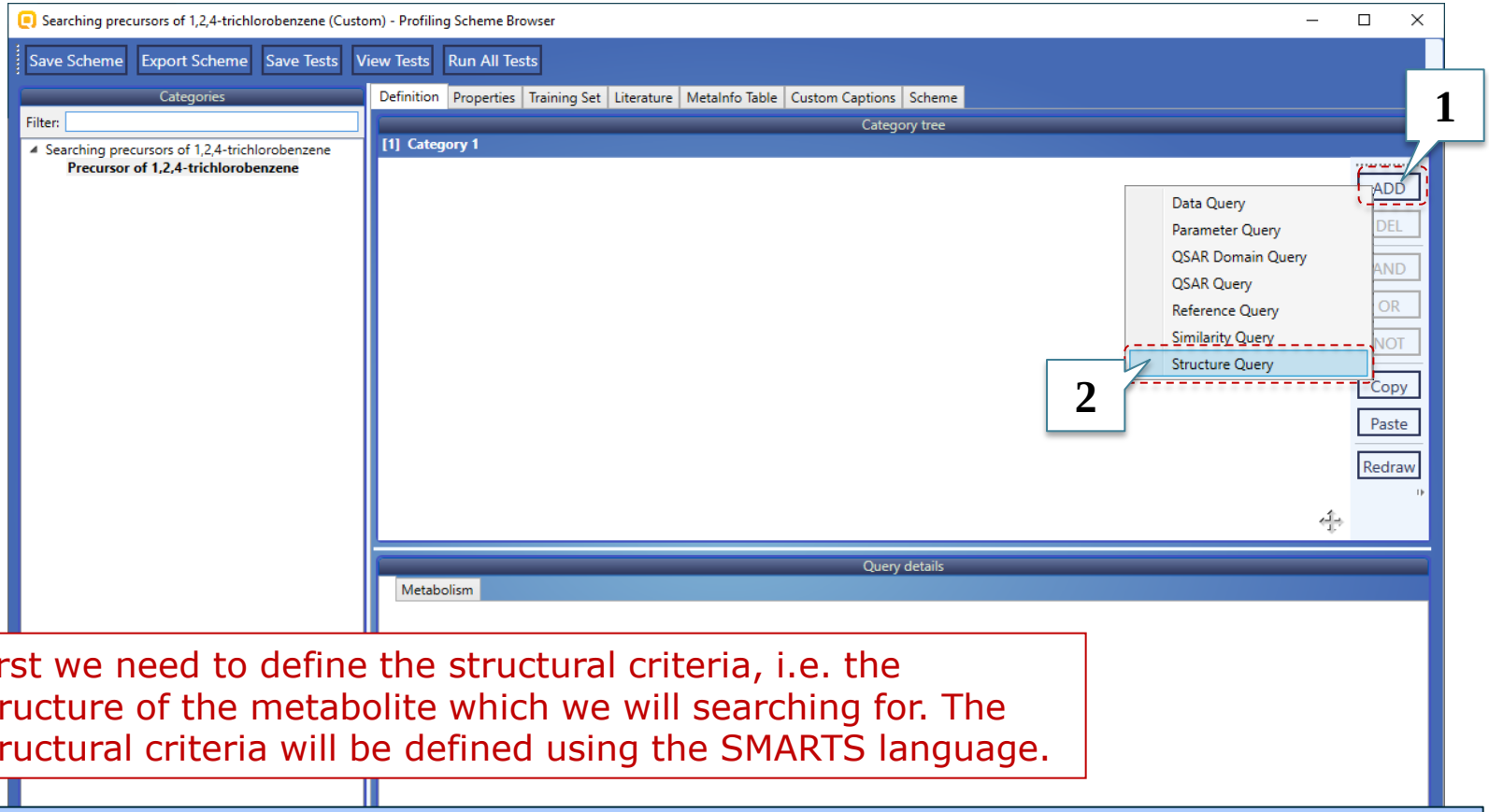
- the name of the profiling category, which will appear on the data matrix;
- the structure which we will be searching for (i.e. *1,2,4-TCB*);
- the simulator, which will be used to simulate the transformations of the chemicals (i.e. *Microbial degradation simulator*).

Building of a new profiler
Name a profiling category



1. Right click on "Category 1" and select to **Rename** it; 2. Put a name that will appear on the data matrix when profile, e.g. *Precursor of 1,2,4-trichlorobenzene*; 3. Confirm by **OK**.

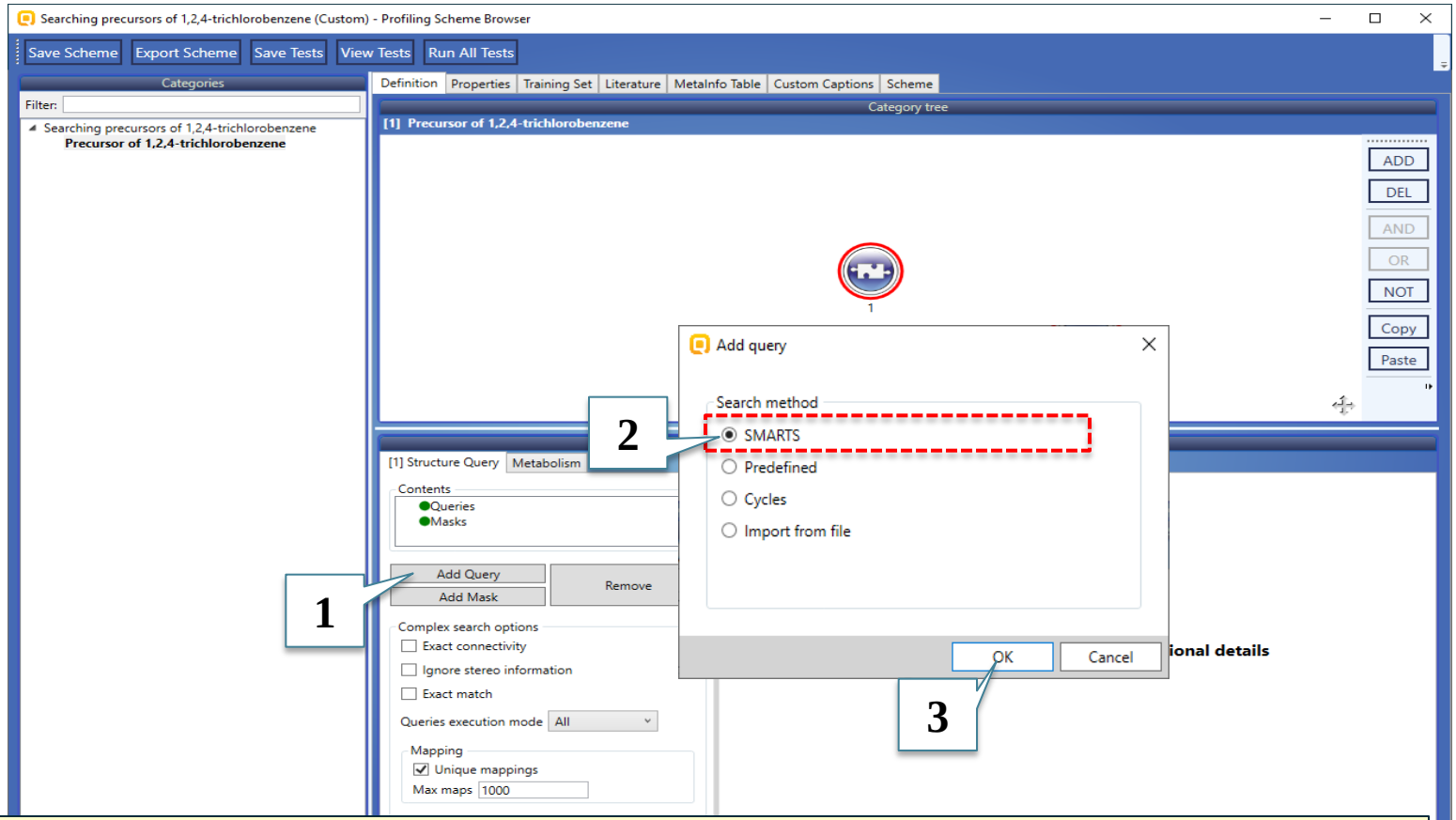
Building of a new profiler
Define the structural criteria



First we need to define the structural criteria, i.e. the structure of the metabolite which we will searching for. The structural criteria will be defined using the SMARTS language.

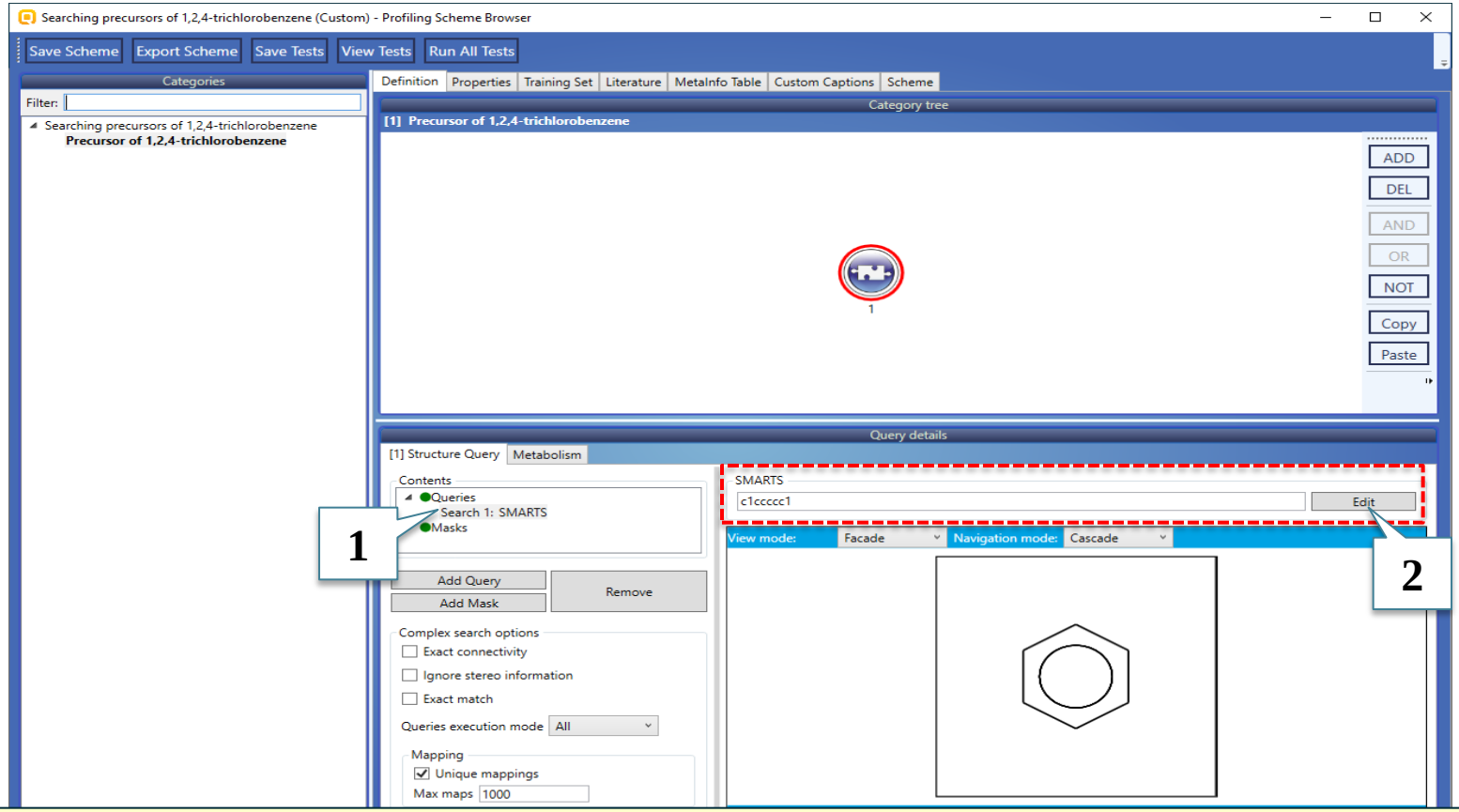
1. Click on the **ADD** button; 2. Select **Structure query**.

Building of a new profiler
Define the structural criteria



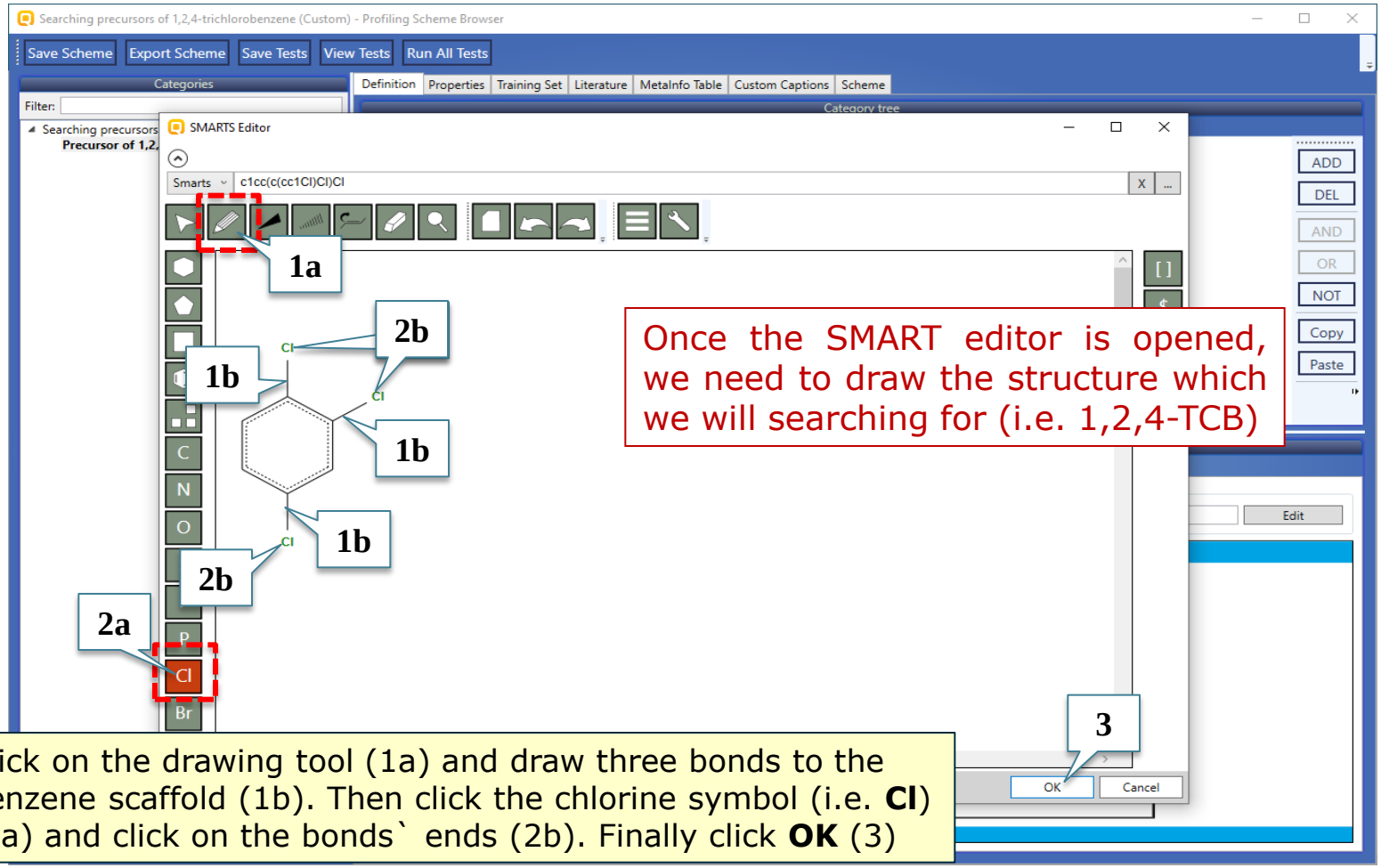
1. Click on the **Add Query** button; 2. Select to add SMARTS; 3. Confirm by **OK**.

Building of a new profiler
Define the structural criteria



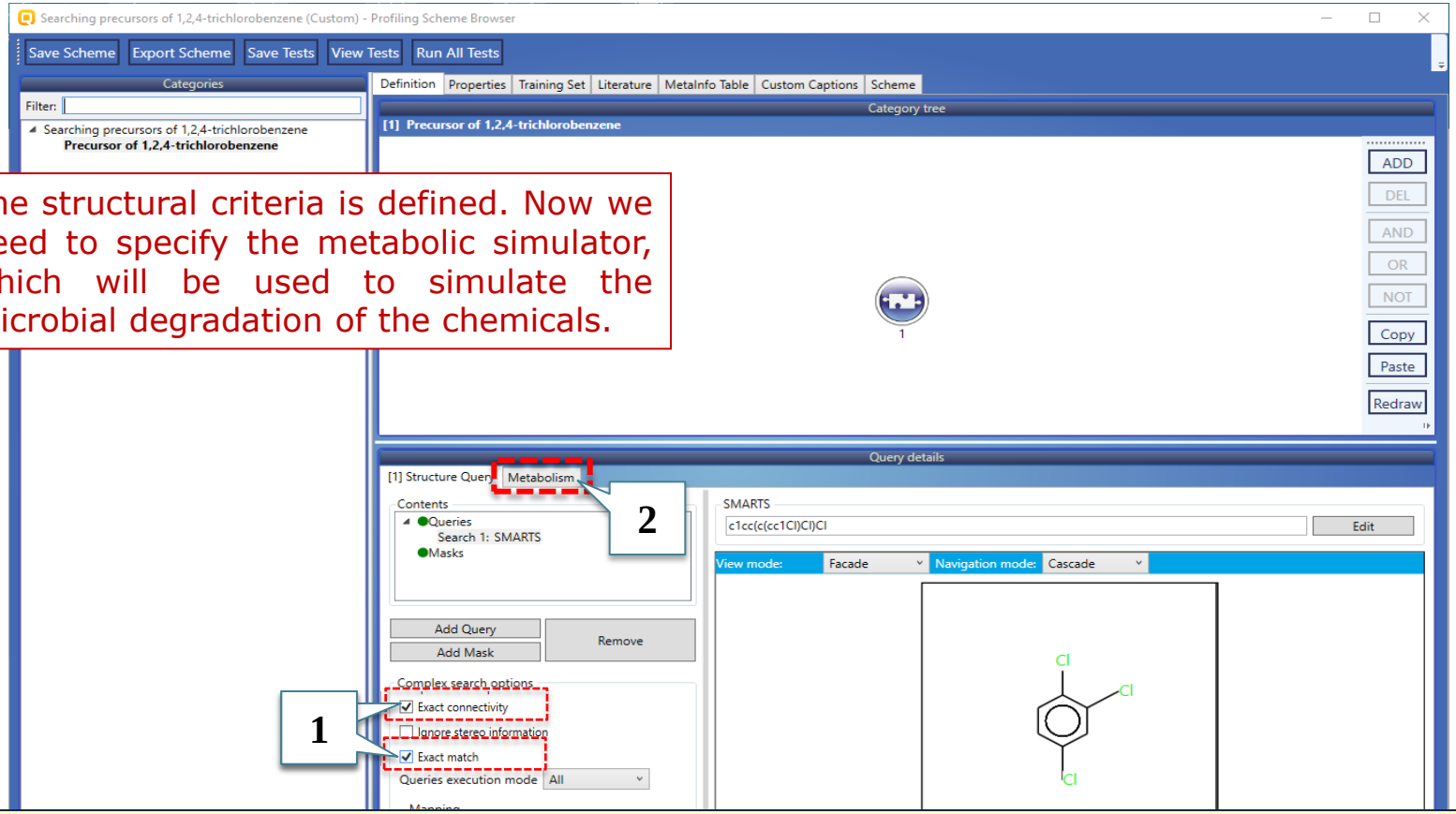
1. Click on the created SMARTS query in order to define it. The SMARTS editor will appear;
2. Click **Edit** to define the structure.

Building of a new profiler
Define the structural criteria



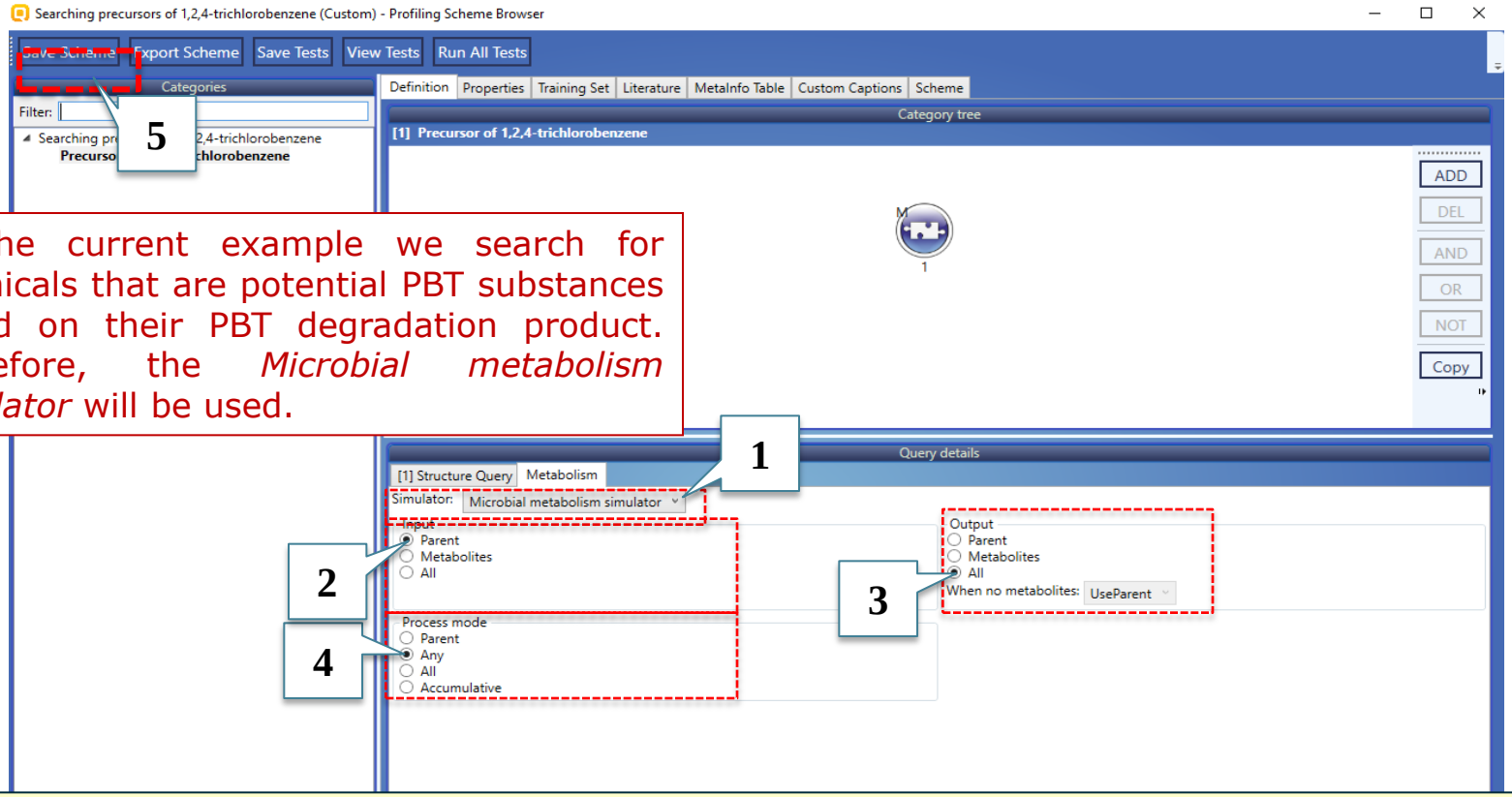
Building of a new profiler
Define the structural criteria

The structural criteria is defined. Now we need to specify the metabolic simulator, which will be used to simulate the microbial degradation of the chemicals.



Select the options for **Exact connectivity** and **Exact match** (1) in order to search for exactly the same structure, without any other substituents. Then go to the **Metabolism** tab (2).

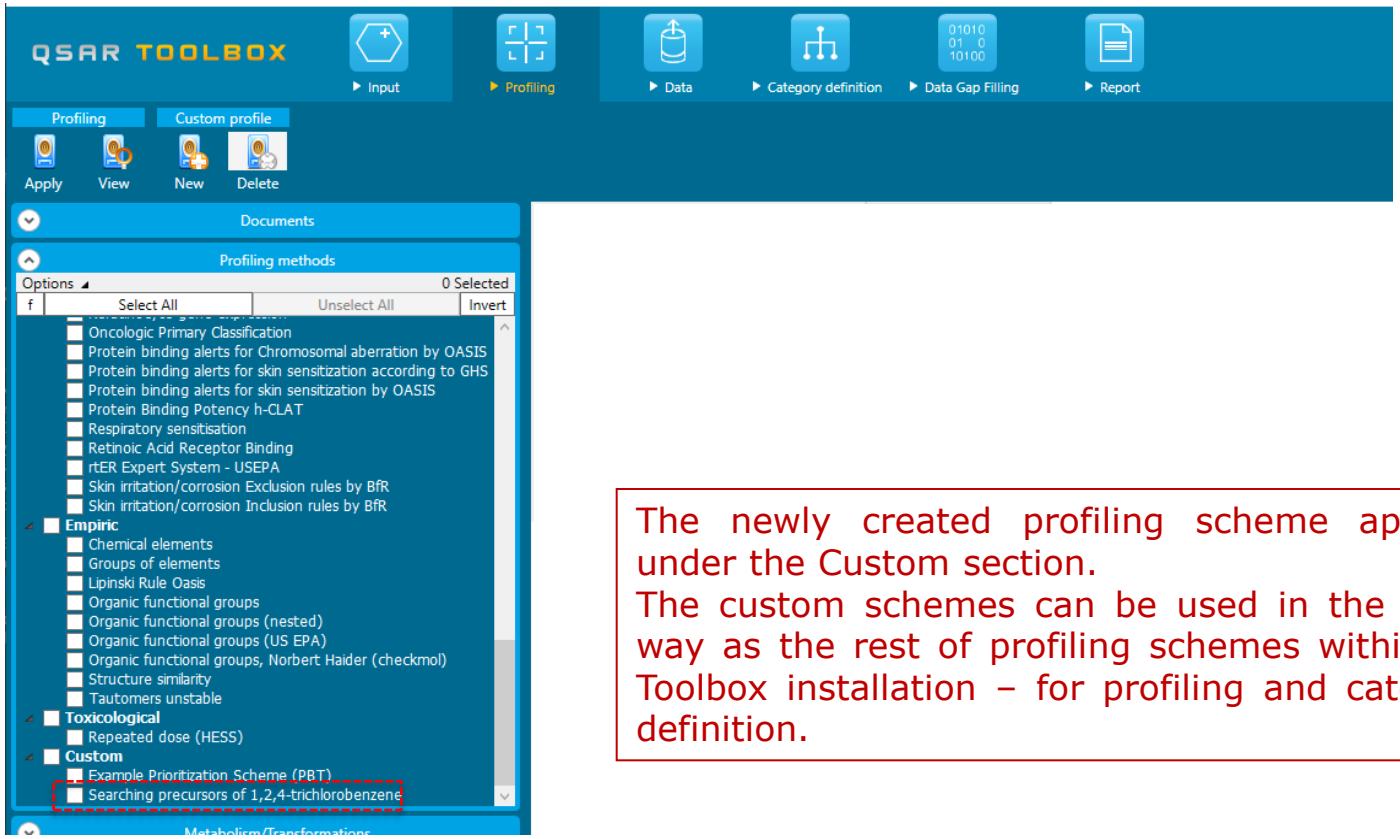
Building of a new profiler
Define the metabolic criteria



In the current example we search for chemicals that are potential PBT substances based on their PBT degradation product. Therefore, the *Microbial metabolism simulator* will be used.

Select the ***Microbial metabolism simulator*** from the drop-down menu (1). This simulator will be applied on the chemicals as parents (i.e. *Input* >> *Parents* (2)) and we will search within the whole package – parent and produced metabolites (i.e. *Output* >> *All* (3)). The process mode should be *Any* (parent and produced metabolites) (4). Finally, click on **Save Scheme** (5).

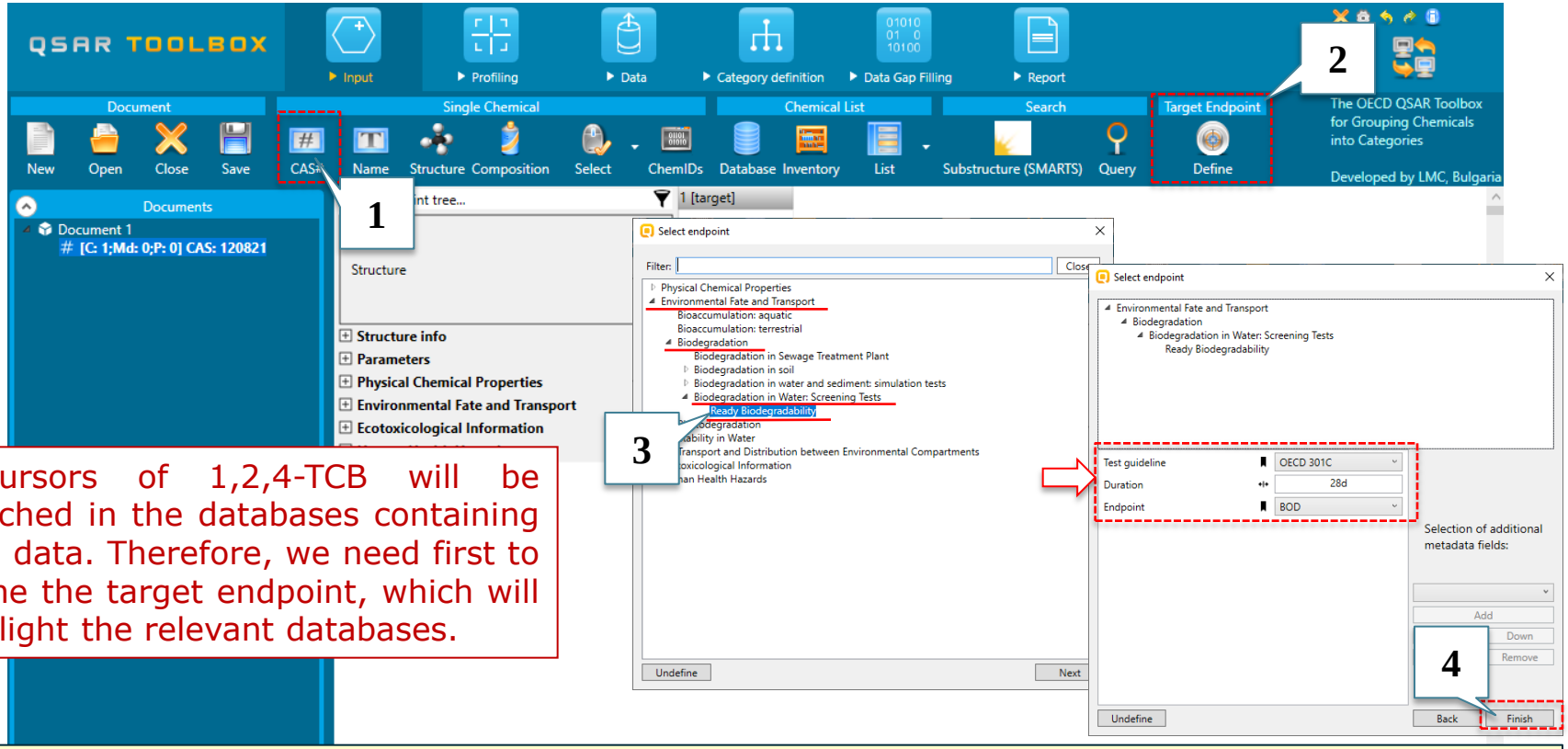
Building of a new profiler
Custom profiler location



The newly created profiling scheme appears under the Custom section.
The custom schemes can be used in the same way as the rest of profiling schemes within the Toolbox installation – for profiling and category definition.

Building of a new profiler

Custom profiler usage

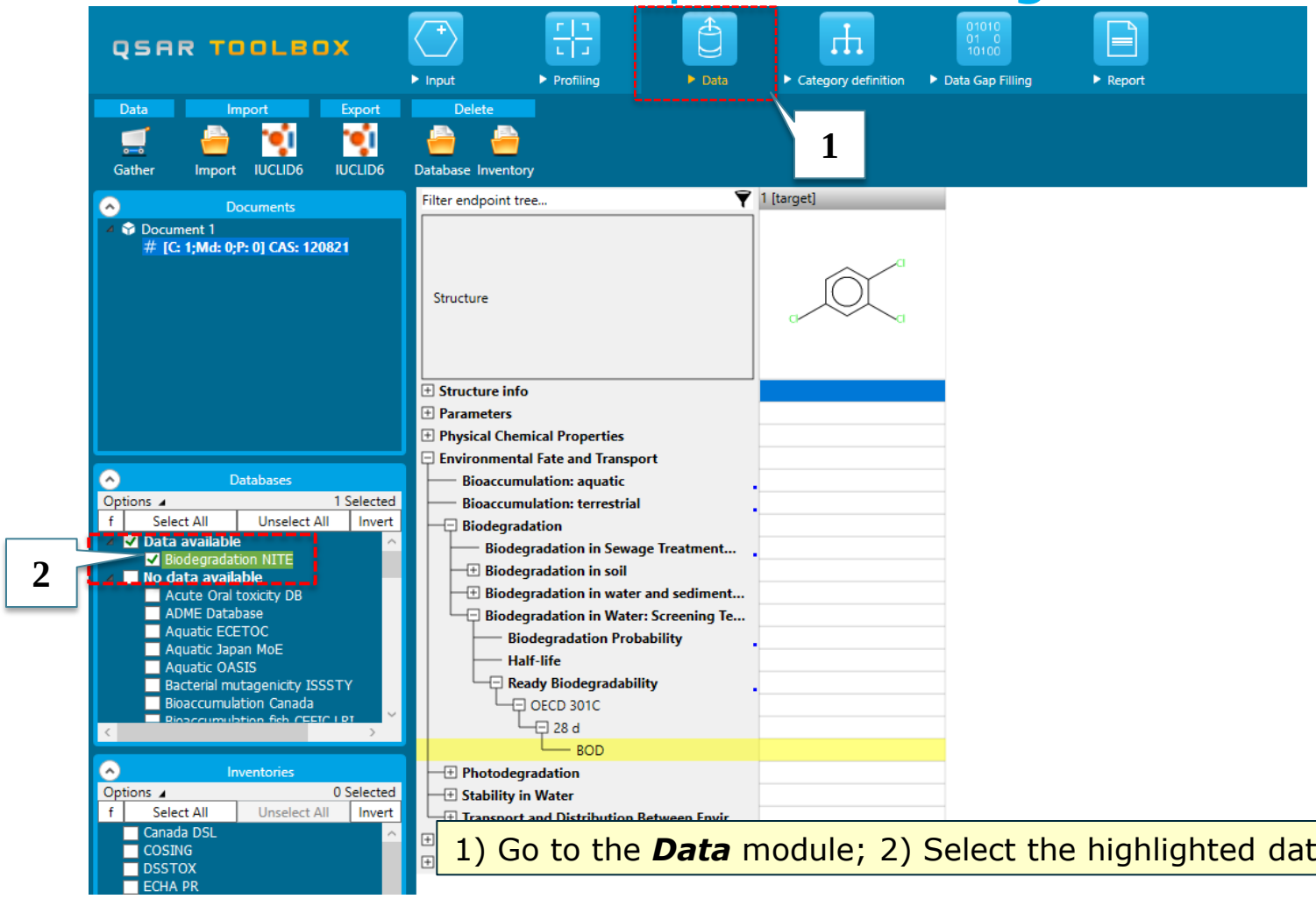


Precursors of 1,2,4-TCB will be searched in the databases containing BOD data. Therefore, we need first to define the target endpoint, which will highlight the relevant databases.

1) Enter the target by *CAS* (CAS 120-82-1); 2) Click on the **Define** button in order to define the target endpoint; 3) Select *Environmental Fate and Transport >> Biodegradation >> Biodegradation in Water: Screening Tests >> Ready biodegradability* and then specify the *BOD* endpoint for *28d* according to *OECD 301C* guideline; 4) Click **Finish** to finalize the endpoint definition.

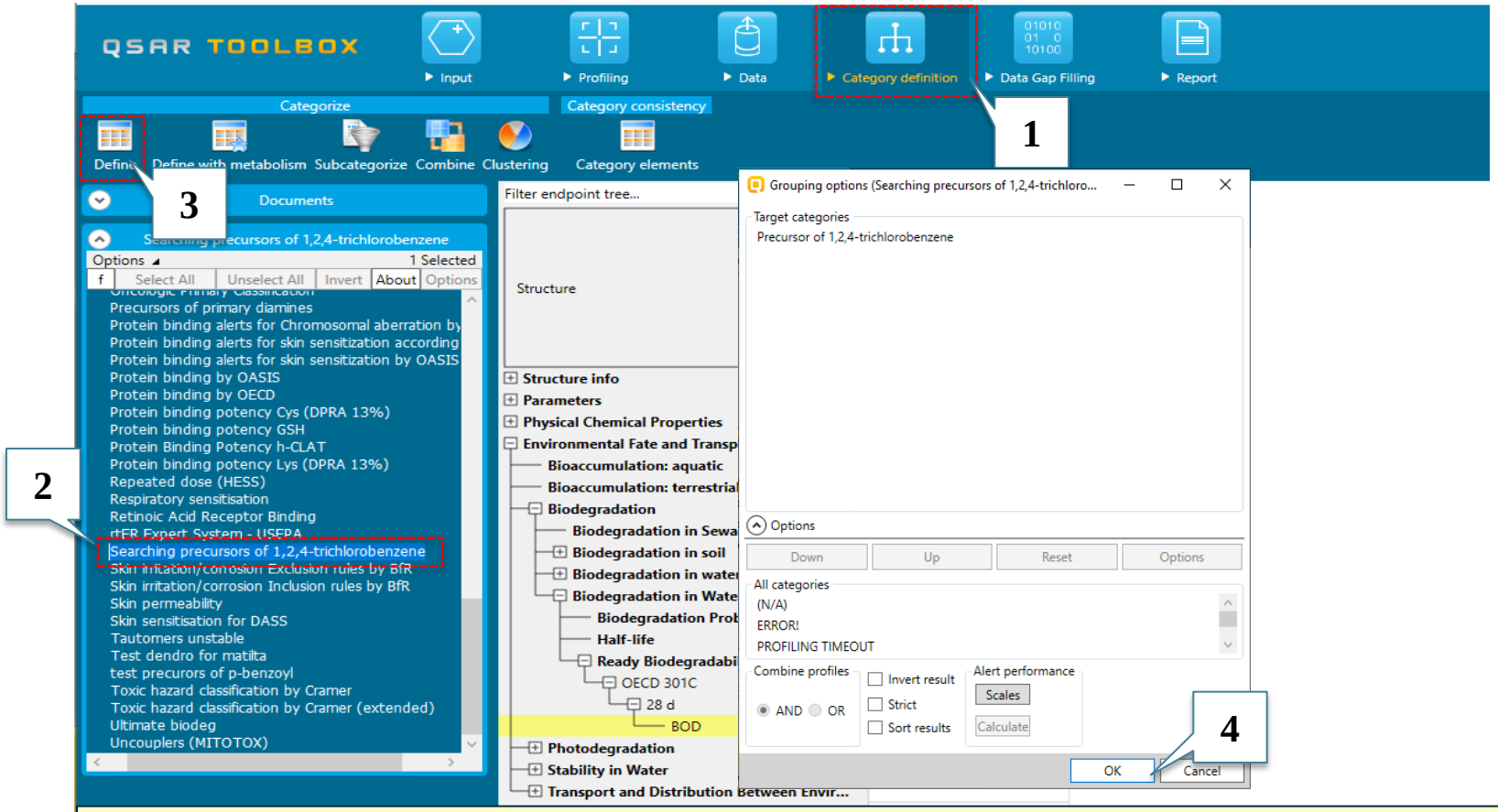
Building of a new profiler

Custom profiler usage



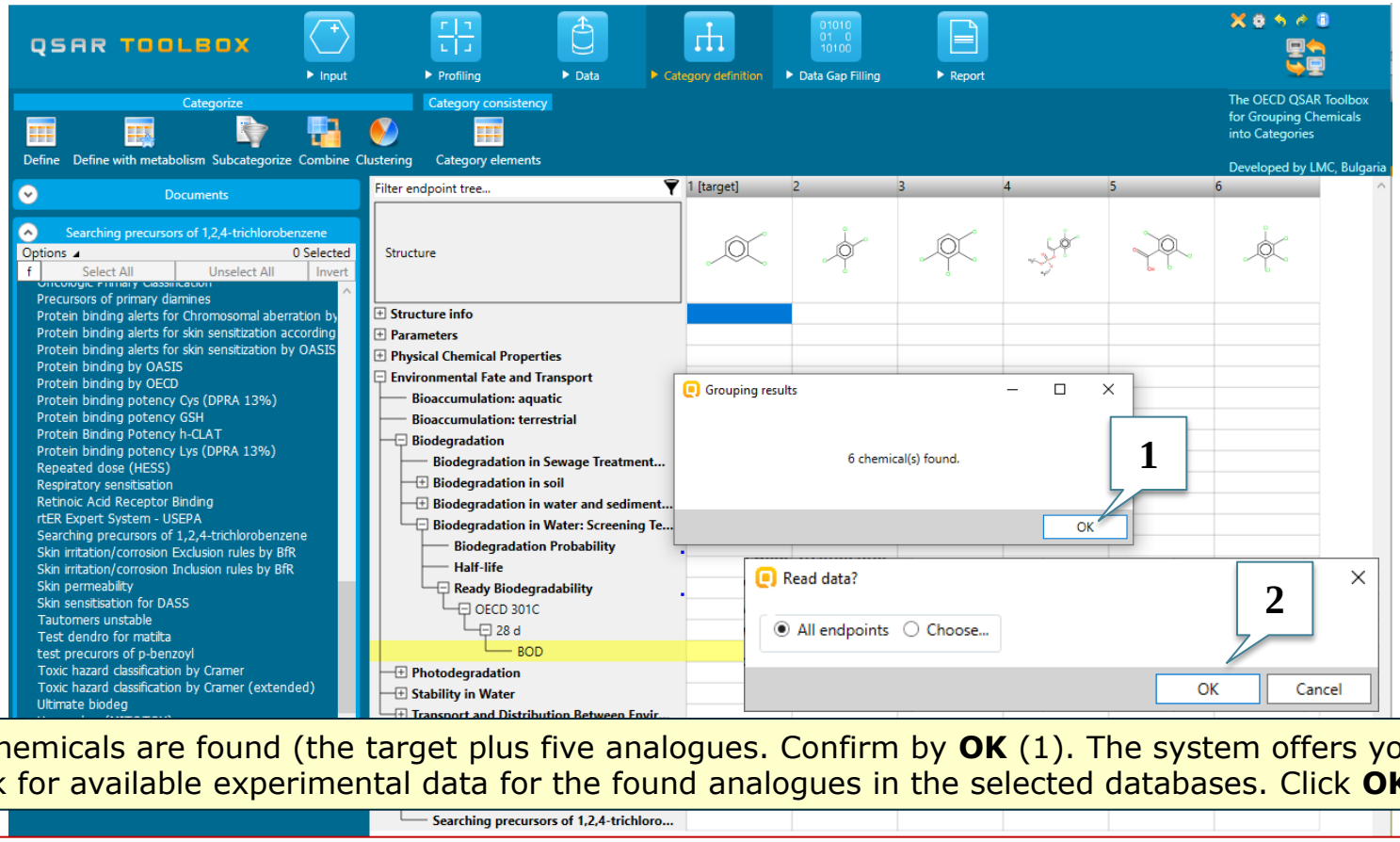
1) Go to the ***Data*** module; 2) Select the highlighted database.

Building of a new profiler
Custom profiler usage



1) Go to the ***Category definition*** module; 2) Select the custom profiler; 3) Click on the **Define** button; 4) Click **OK** button to initialize the search of 1,2,4-TCB precursors.

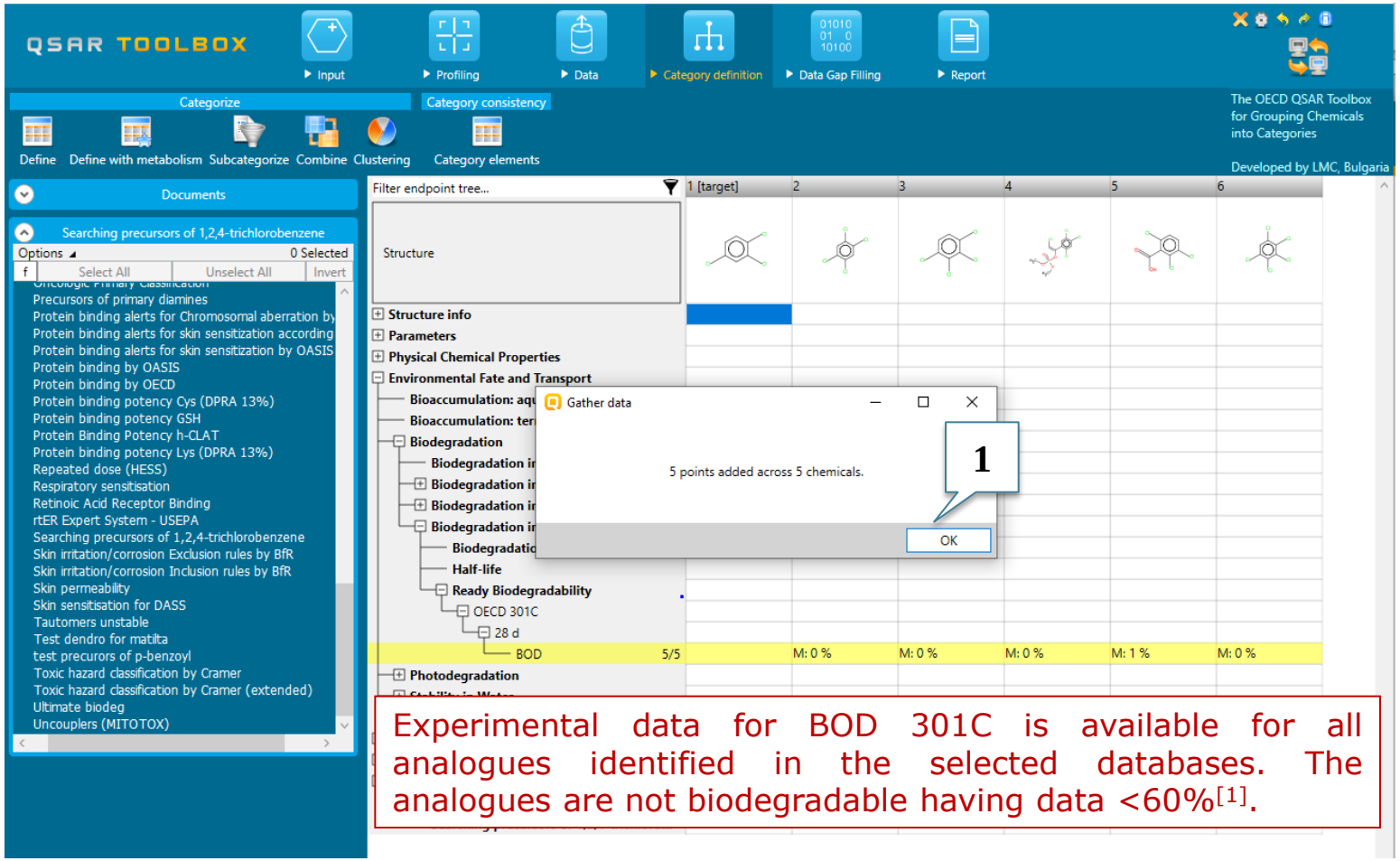
Building of a new profiler
Custom profiler usage



Six chemicals are found (the target plus five analogues. Confirm by **OK** (1). The system offers you to check for available experimental data for the found analogues in the selected databases. Click **OK** (2).

Note: The tutorial is consistent with Toolbox v.4.4 (and TB 4.4.1), where the *Biodegradation NITE* database is preliminary cached with the microbial metabolism.
If older version of Toolbox is used, the search will take up to several minutes if you make this example for first time.

Building of a new profiler
Custom profiler usage



[1] OECD guideline for testing of chemicals 301. 1992. Ready biodegradability.

The Exercise
Example 2: Background

- Micronucleus positive results for p-benzoquinone [CAS 106-51-4] are reported in the literature[1-3] and are also available in the Toolbox.
- Therefore, it could be assumed that the chemicals producing p-benzoquinone (p-BQ) as a metabolite would also elicit positive effects in the micronucleus test.
- The hydroquinone/quinone molecular transformations are reproduced by the *in vivo* rat simulator available in the Toolbox.

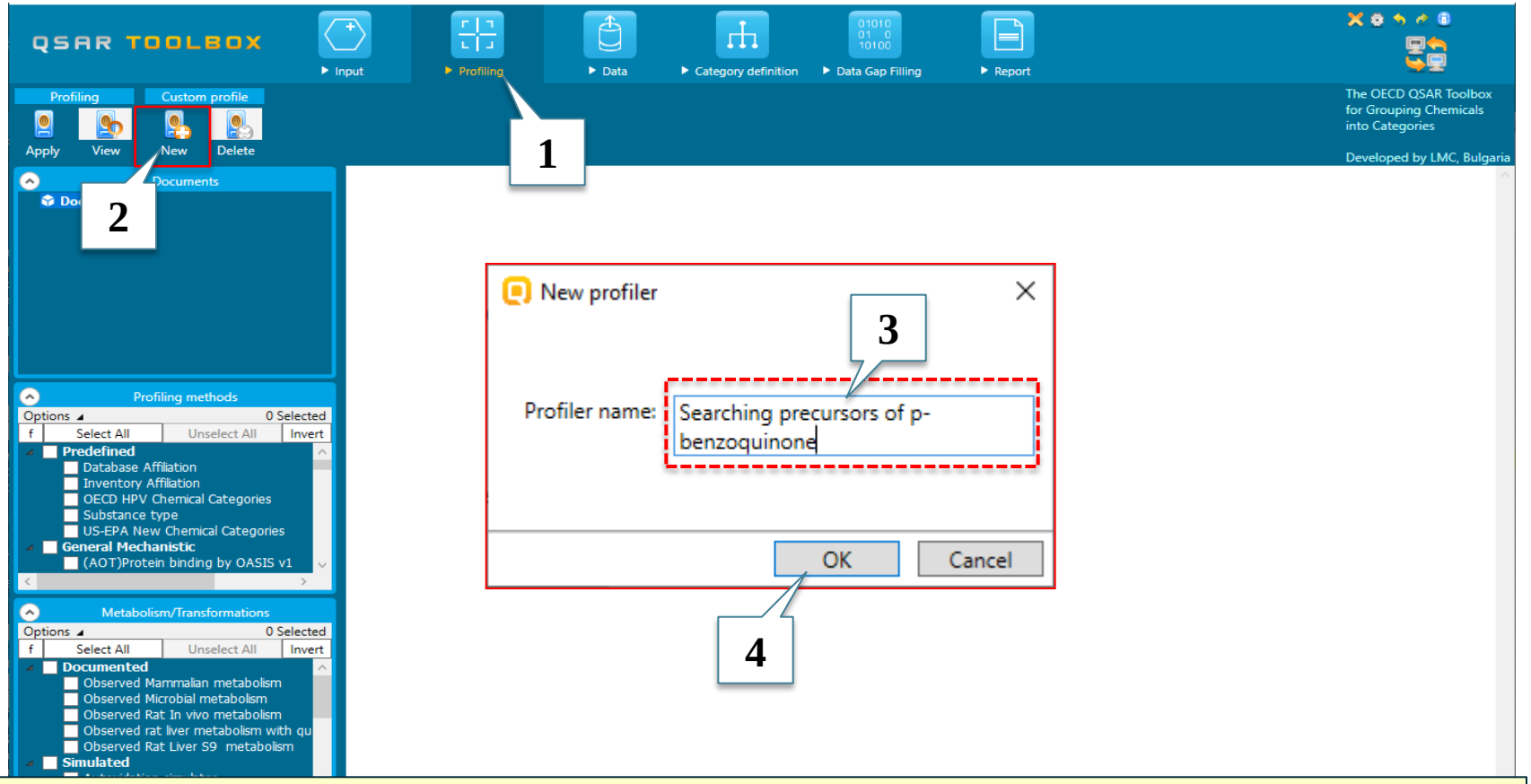
[1] J.L. Bolton, M.A. Trush, T.M. Penning, G. Dryhurst, T.J. Monks. Role of quinones in toxicology. Chem. Res. Toxicol., 13 (2000), pp. 135-160
[2] J.L. Bolton, J.A. Thompson. Formation, biological targets, and resulting toxicity of quinone methides. S.E. Rokita (Ed.), Reactive intermediates in chemistry and biology: Quinone methides, Wiley (2009), pp. 329-356
[3] R. Ciranni, R. Barale, A. Marrazzini, N. Loprieno. Benzene and the genotoxicity of its metabolites. I. Transplacental activity in mouse fetuses and in their dams. Mutat. Res., 208 (1988), pp. 61-67

The Exercise
Example 2

- In the current example we will build a profiler that identifies chemicals producing p-BQ (i.e. precursors of p-BQ) as a result of *in vivo* rat metabolism.
- It will be illustrated how to search precursors in a specific database. The same could be done using a custom file.

Building of a new profiler

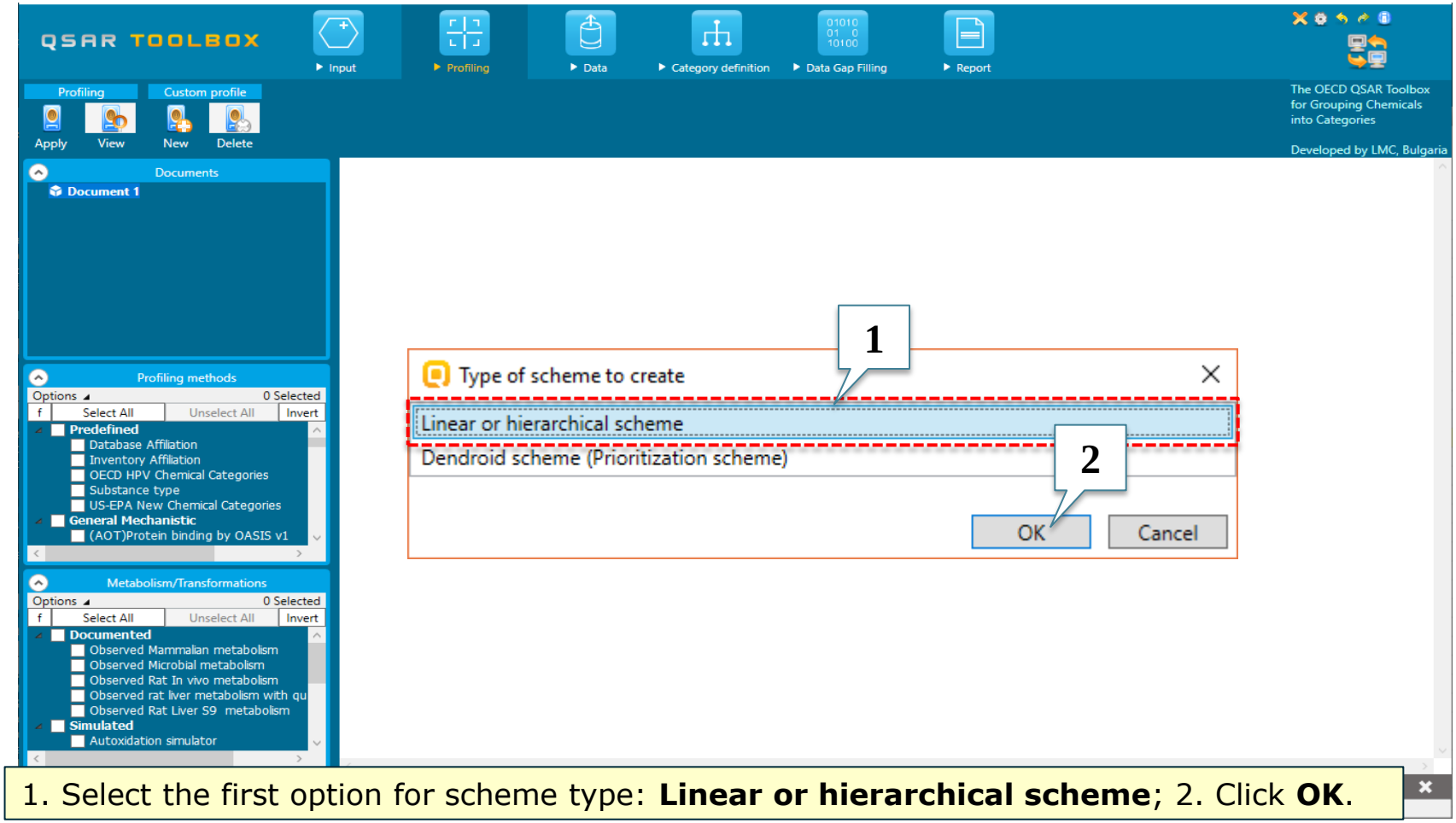
Define the name of the new profiler



1. Go to the ***Profiling*** module; 2. Click **New**; 3. Enter the name of the new scheme, for example ***"Searching precursors of p-benzoquinone"***; 4. Confirm by click **OK**.

Building of a new profiler

Define type of new profiler



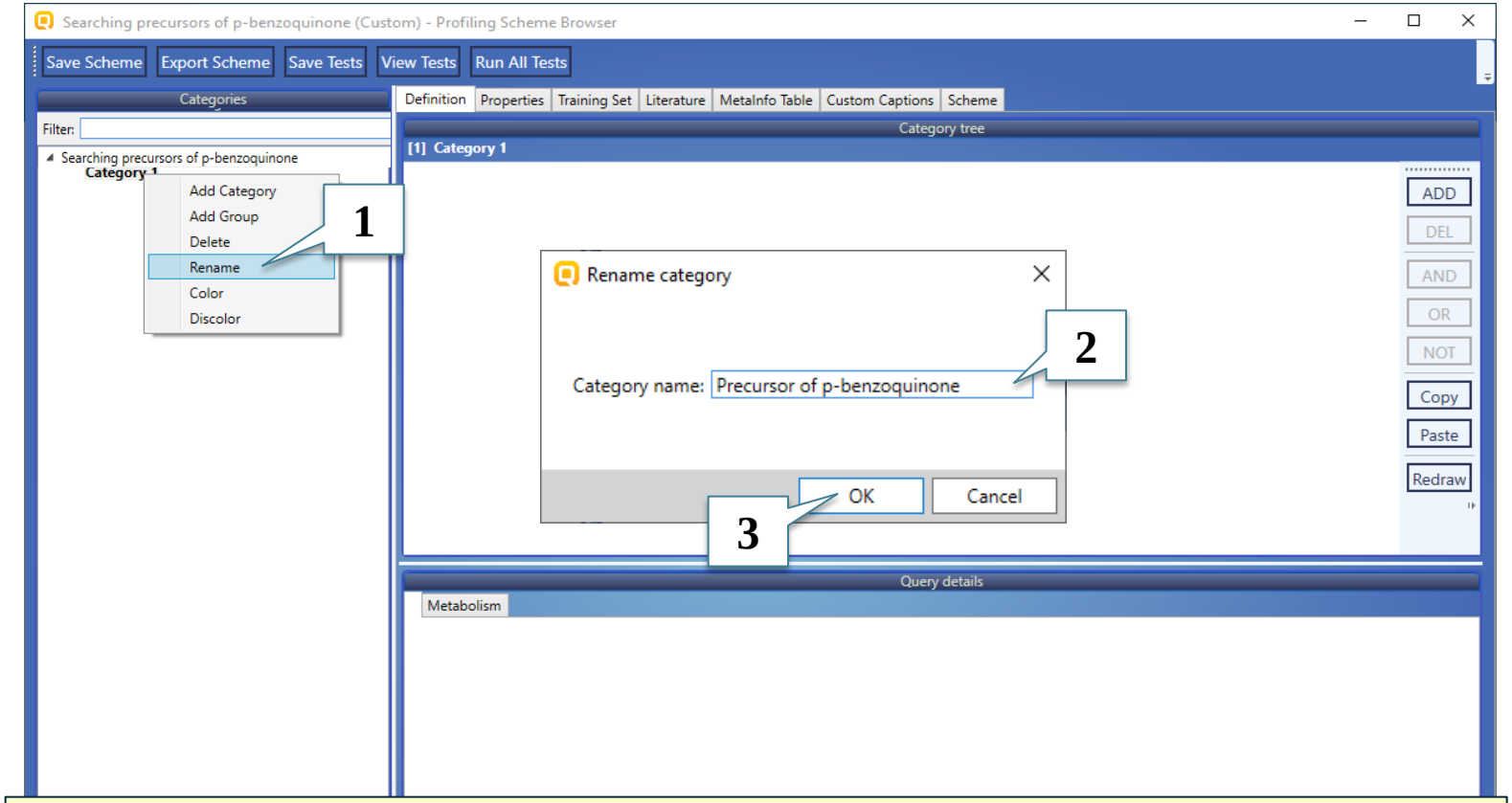
1. Select the first option for scheme type: **Linear or hierarchical scheme**; 2. Click **OK**.

Building of a new profiler
Creating a profiling category

Once the scheme is created, it will be defined:

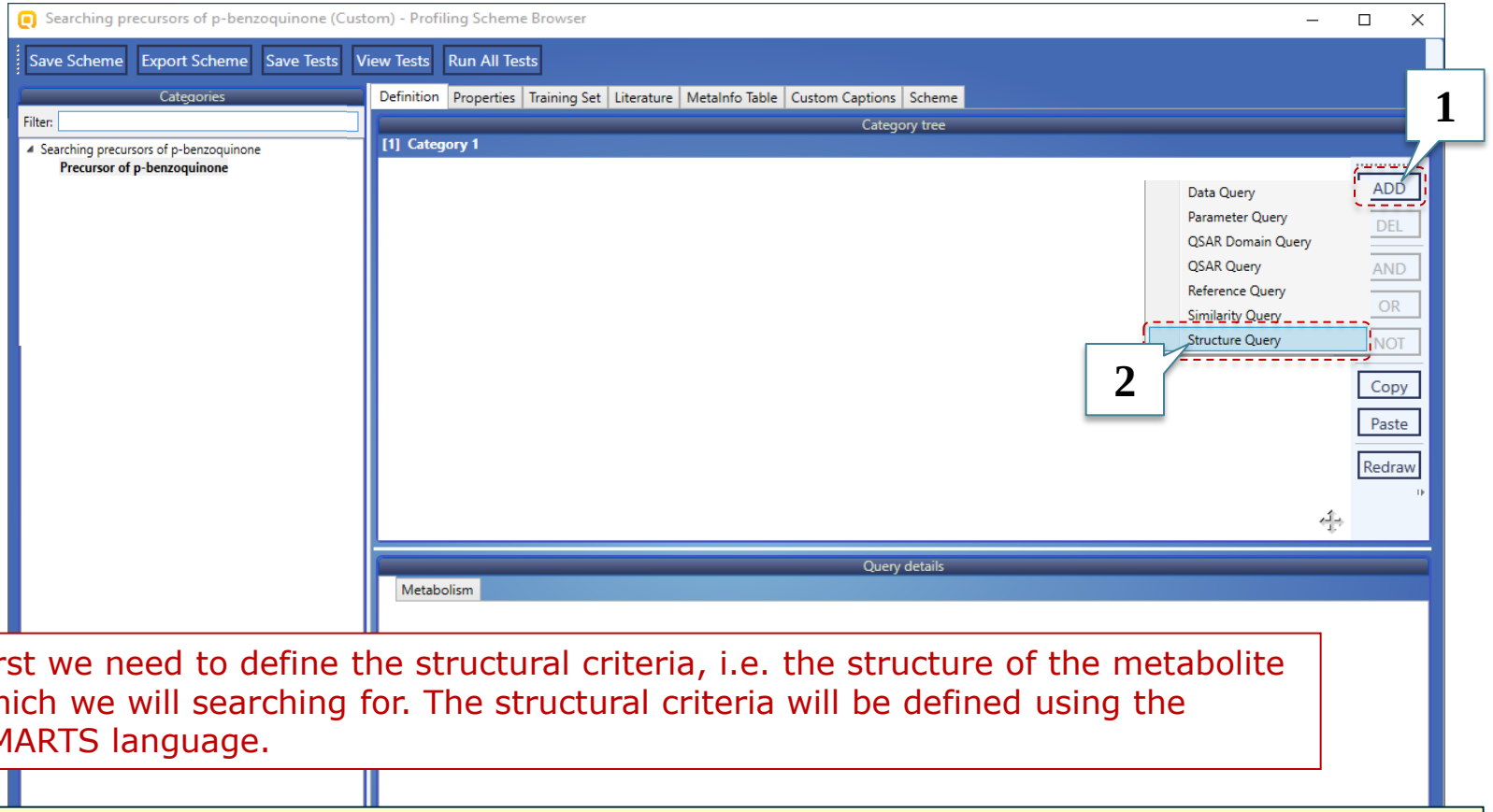
- the name of the profiling category, which will appear on the data matrix;
- the structure which we will searching for (i.e. p-BQ);
- the simulator, which will be used to simulate the transformations of the chemicals (i.e. *in vivo Rat metabolism simulator*).

Building of a new profiler
Name a profiling category



1. Right click on "Category 1" and select to **Rename** it; 2. Put a name that will appear on the data matrix when profile, e.g. *Precursor of p-benzoquinone; 3.* Confirm by **OK**.

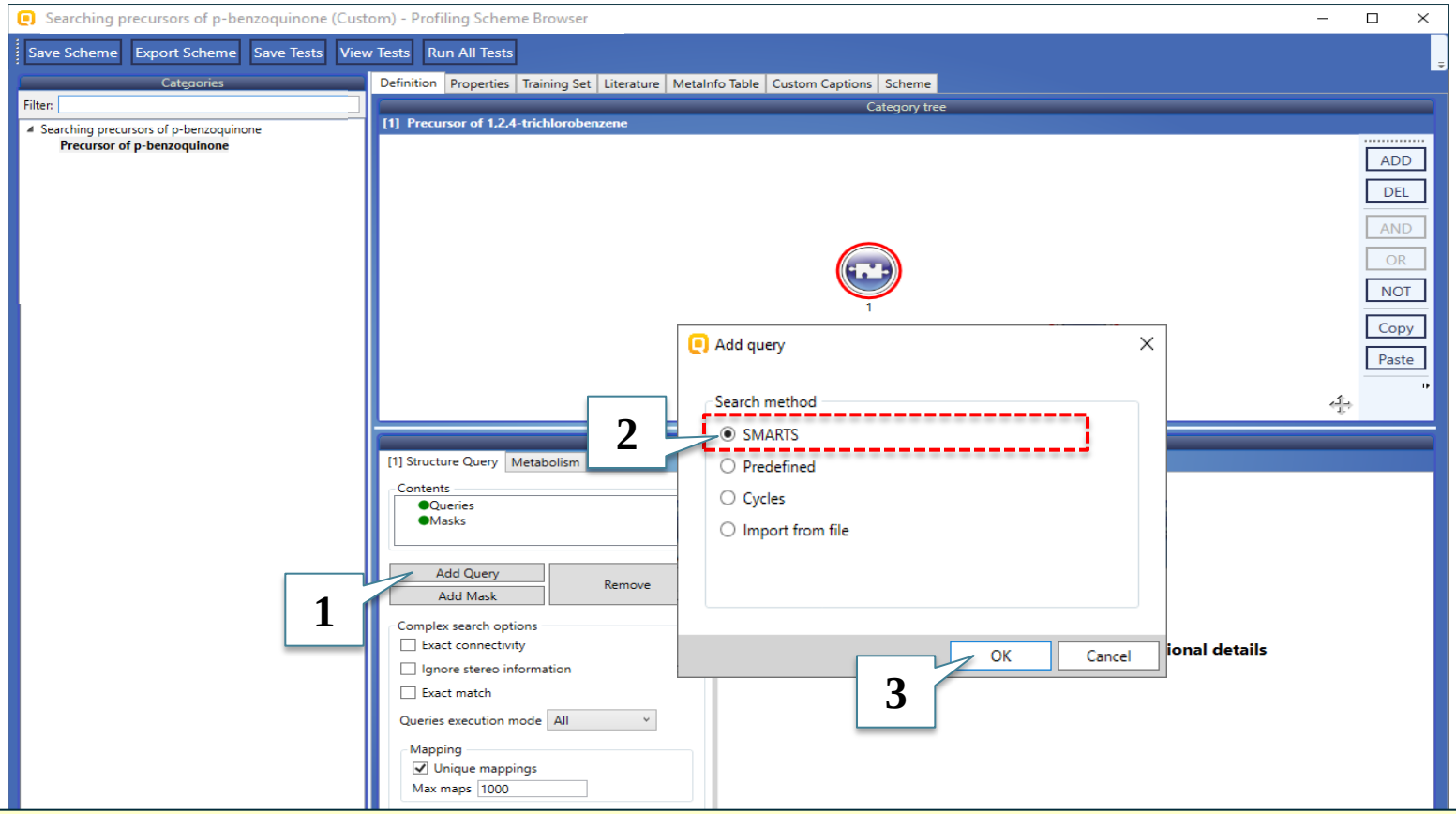
Building of a new profiler
Define the structural criteria



First we need to define the structural criteria, i.e. the structure of the metabolite which we will searching for. The structural criteria will be defined using the SMARTS language.

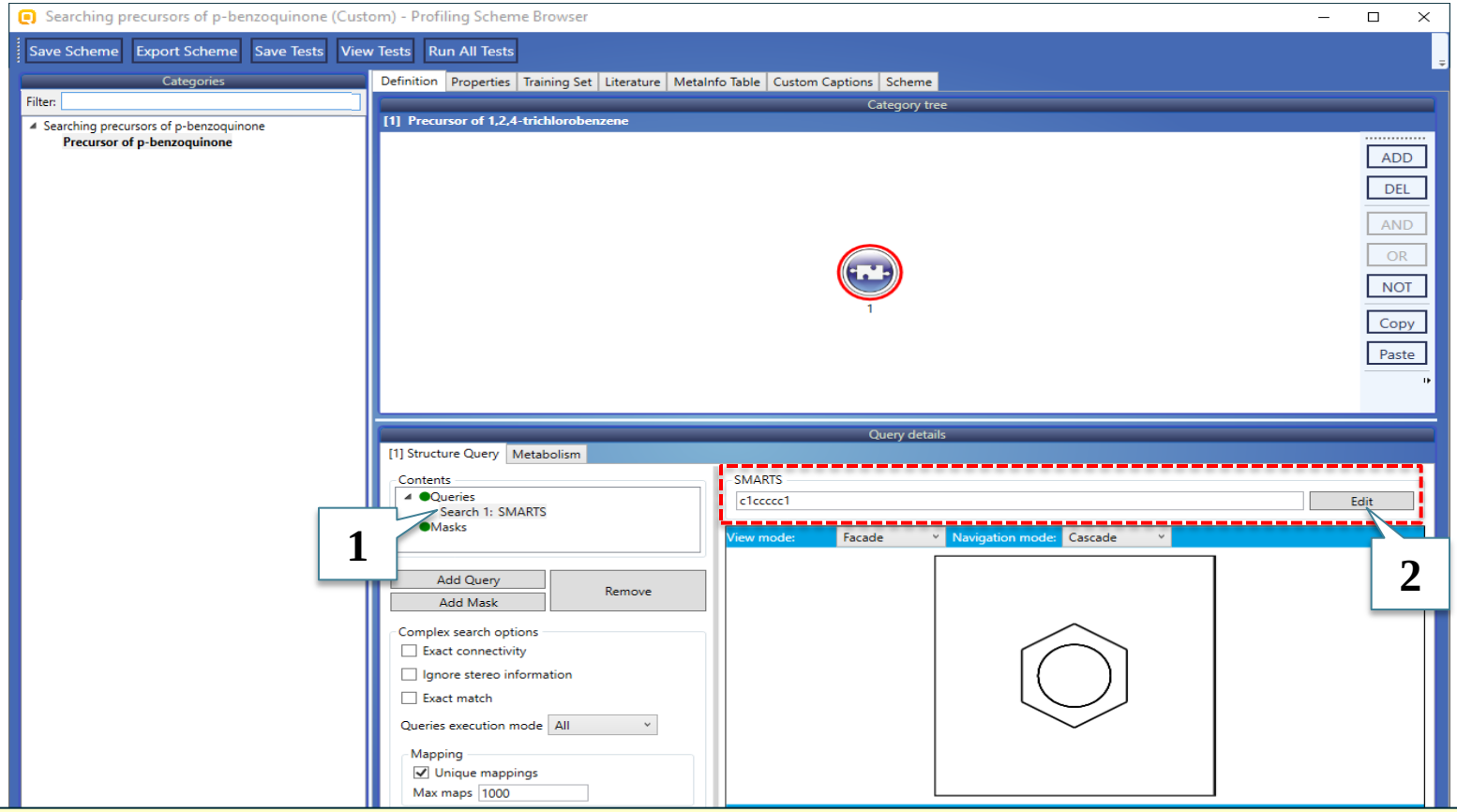
1. Click on the **ADD** button; 2. Select **Structure query**.

Building of a new profiler
Define the structural criteria



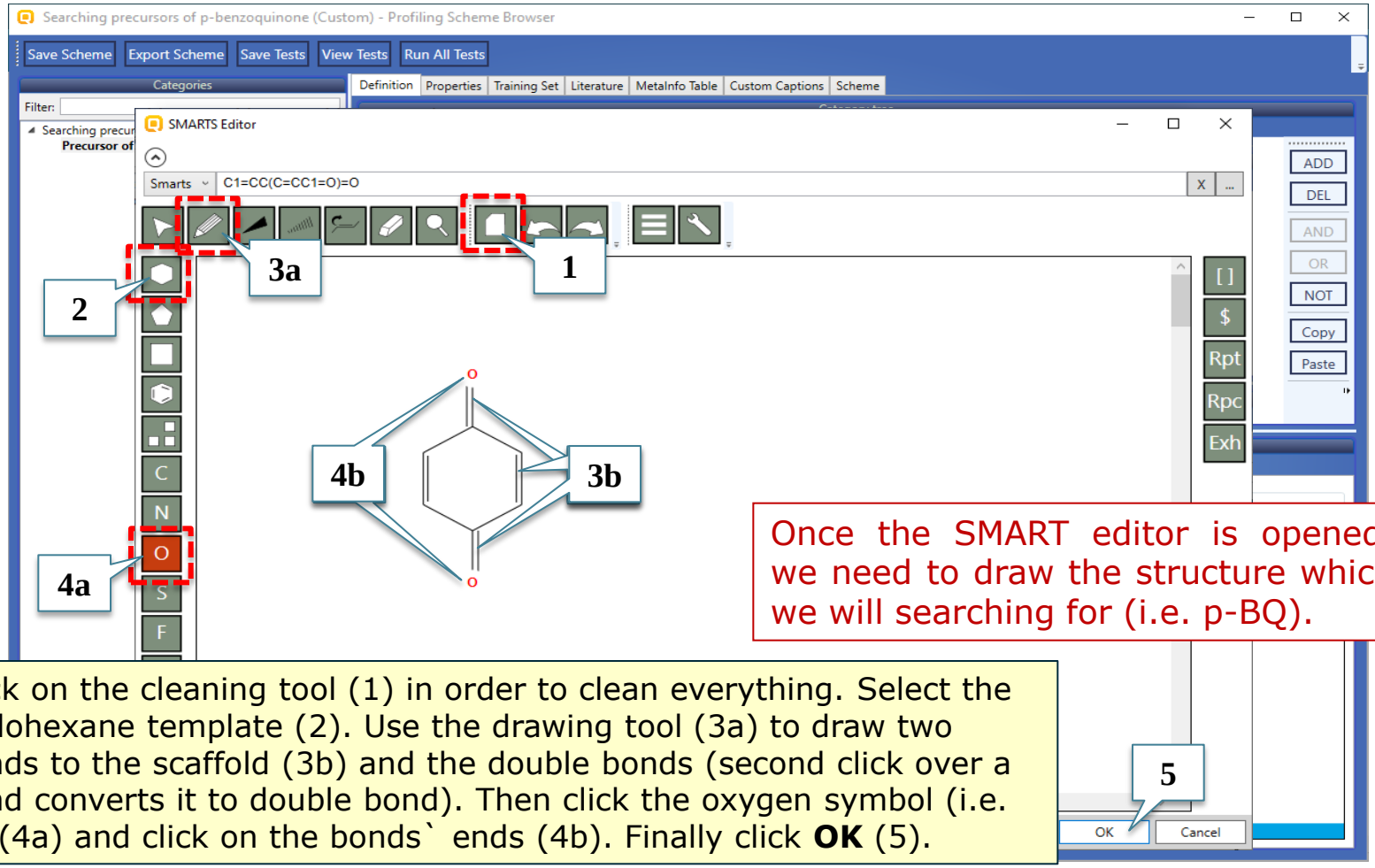
1. Click on the **Add Query** button; 2. Select to add SMARTS; 3. Confirm by **OK**.

Building of a new profiler
Define the structural criteria



1. Click on the created SMARTS query in order to define it. The SMARTS editor will appear;
2. Click **Edit** to define the structure.

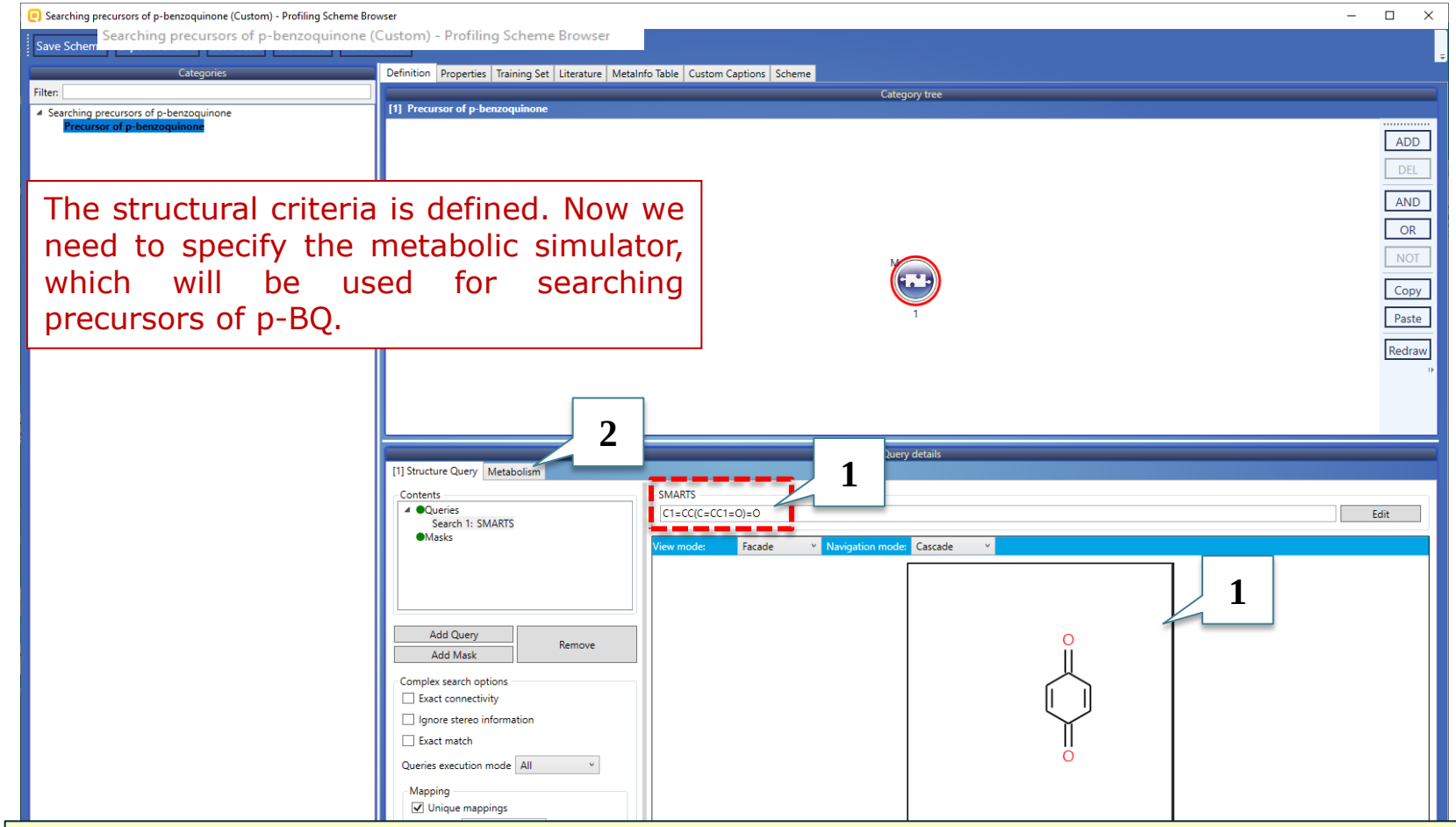
Building of a new profiler
Define the structural criteria



Once the SMART editor is opened, we need to draw the structure which we will searching for (i.e. p-BQ).

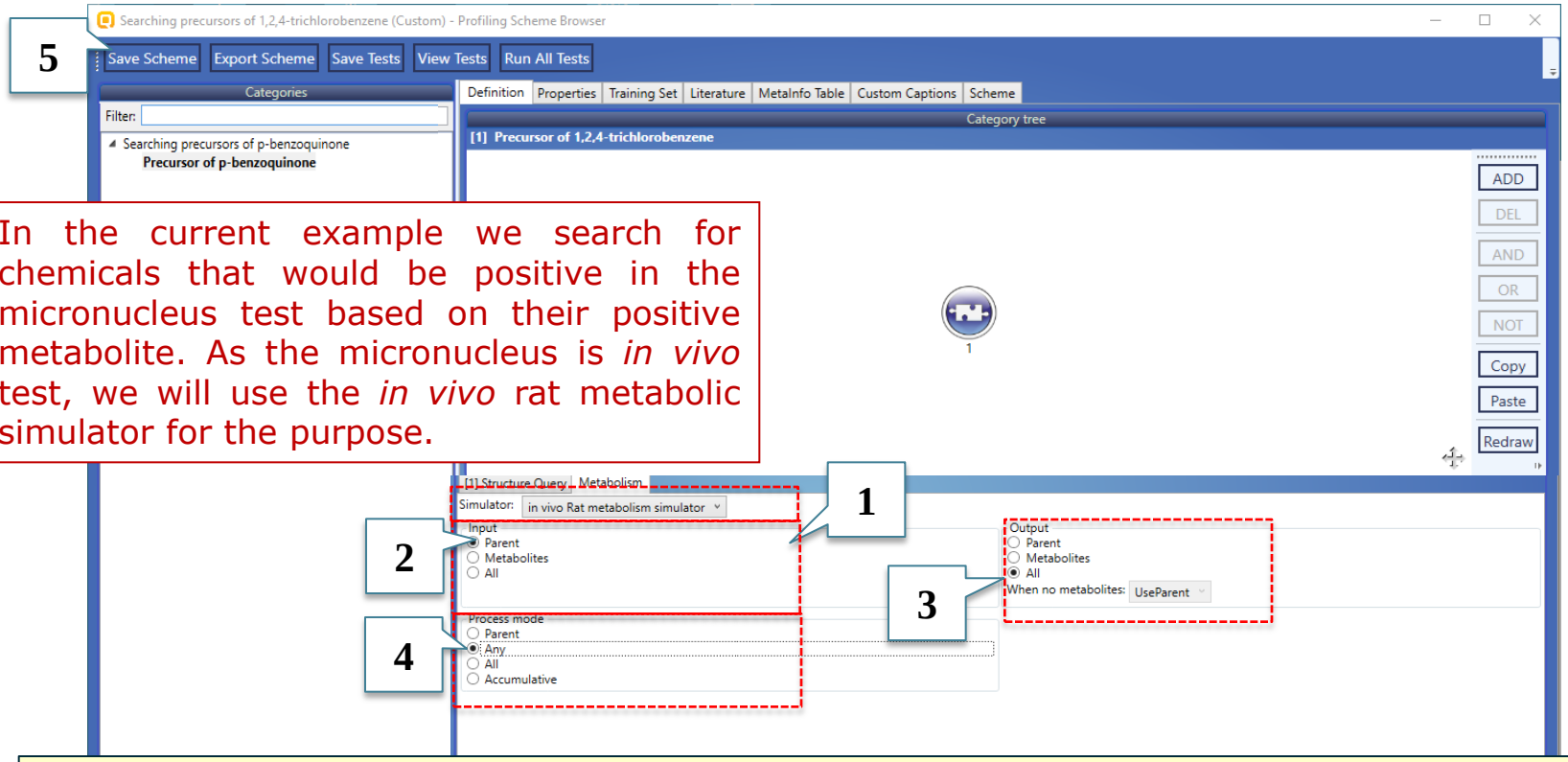
Click on the cleaning tool (1) in order to clean everything. Select the cyclohexane template (2). Use the drawing tool (3a) to draw two bonds to the scaffold (3b) and the double bonds (second click over a bond converts it to double bond). Then click the oxygen symbol (i.e. **O**) (4a) and click on the bonds` ends (4b). Finally click **OK** (5).

Building of a new profiler
Define the structural criteria



The structures of p-benzoquinone is defined(1) Then go to the **Metabolism** tab (2).

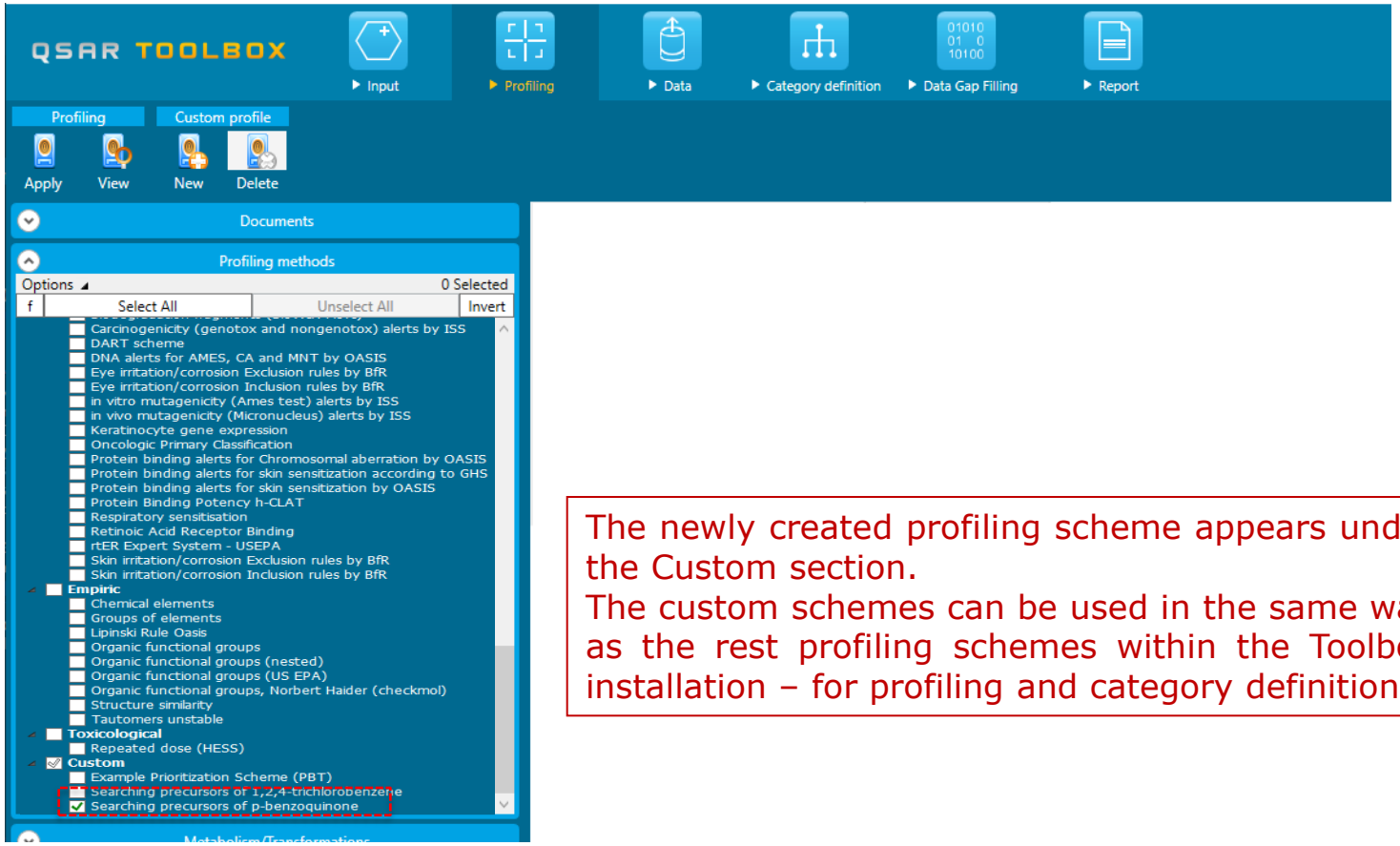
Building of a new profiler
Define the metabolic criteria



In the current example we search for chemicals that would be positive in the micronucleus test based on their positive metabolite. As the micronucleus is *in vivo* test, we will use the *in vivo* rat metabolic simulator for the purpose.

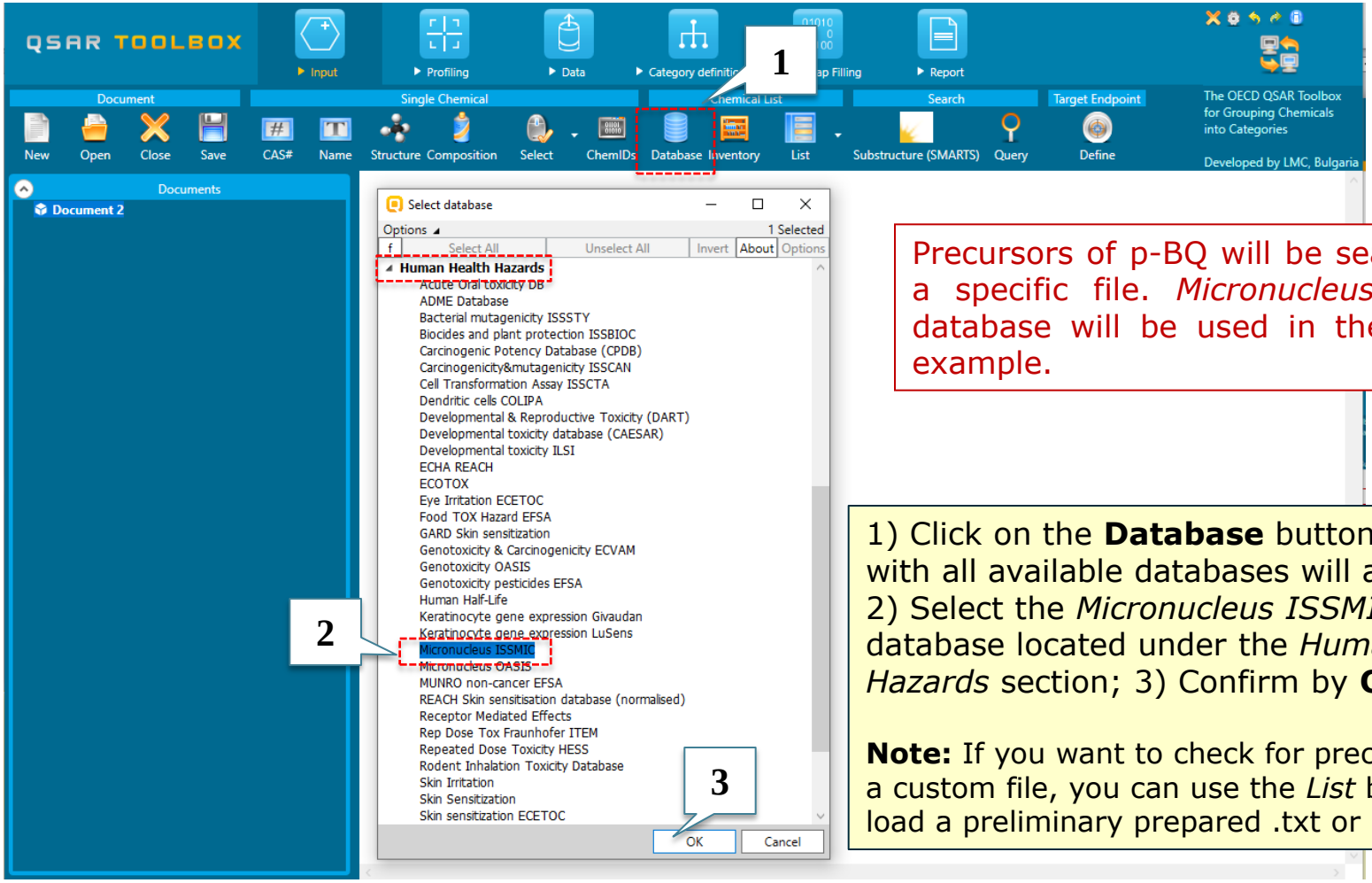
Select the ***in vivo Rat metabolism simulator*** from the drop-down menu (1). This simulator will be applied on the chemicals as parents (i.e. *Input* >> *Parents* (2)) and we will search within the whole package – parent and produced metabolites (i.e. *Output* >> *All* (3)); select Any process mode (4). Finally, click on **Save Scheme** (5).

Building of a new profiler
Custom profiler location



The newly created profiling scheme appears under the Custom section.
The custom schemes can be used in the same way as the rest profiling schemes within the Toolbox installation – for profiling and category definition.

Building of a new profiler
Custom profiler usage



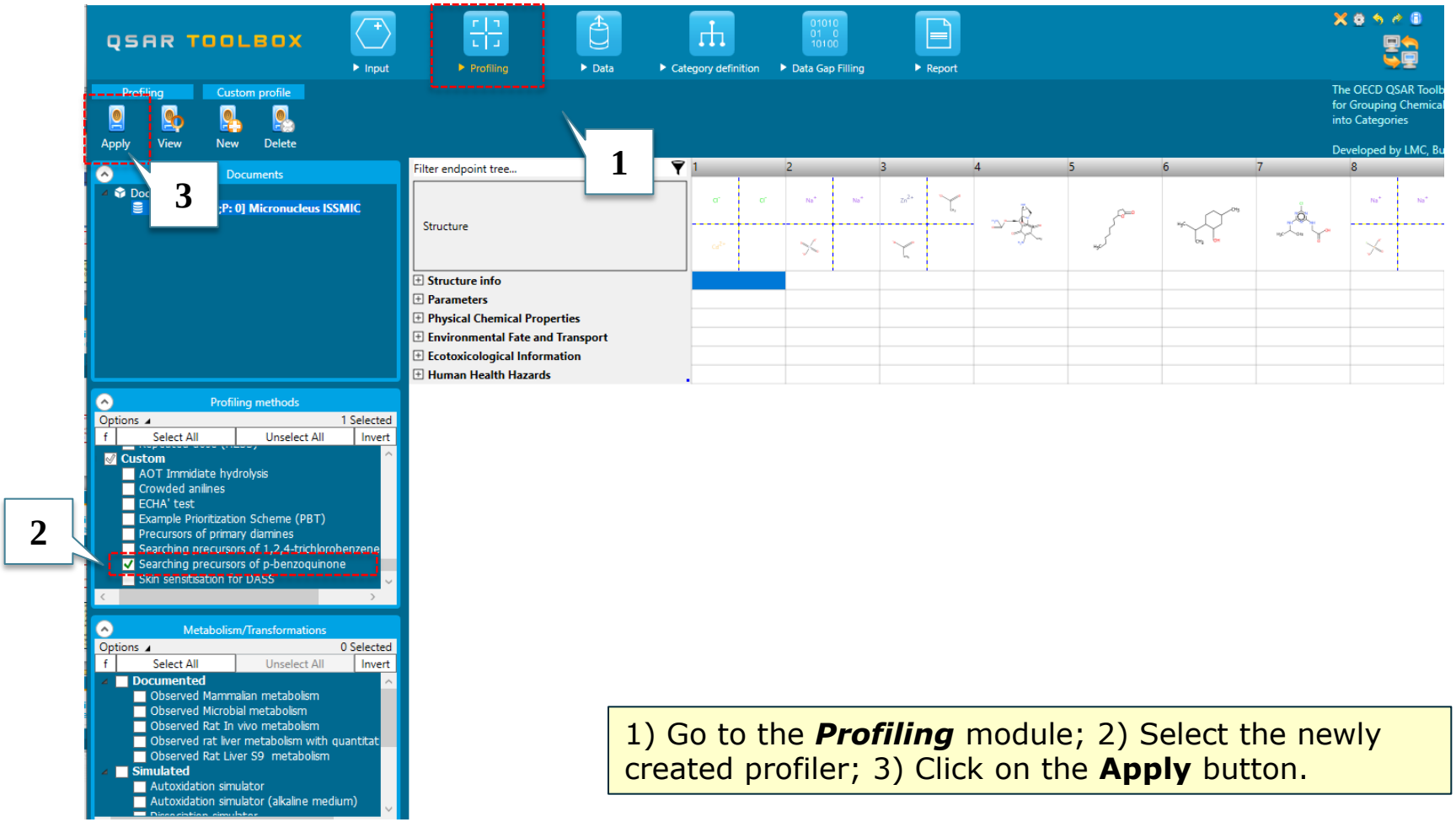
Precursors of p-BQ will be searched in a specific file. *Micronucleus ISSMIC* database will be used in the current example.

1) Click on the **Database** button*. List with all available databases will appear; 2) Select the *Micronucleus ISSMIC* database located under the *Human Health Hazards* section; 3) Confirm by **OK**.

Note: If you want to check for precursors in a custom file, you can use the *List* button to load a preliminary prepared .txt or .smi file

Building of a new profiler

Custom profiler usage



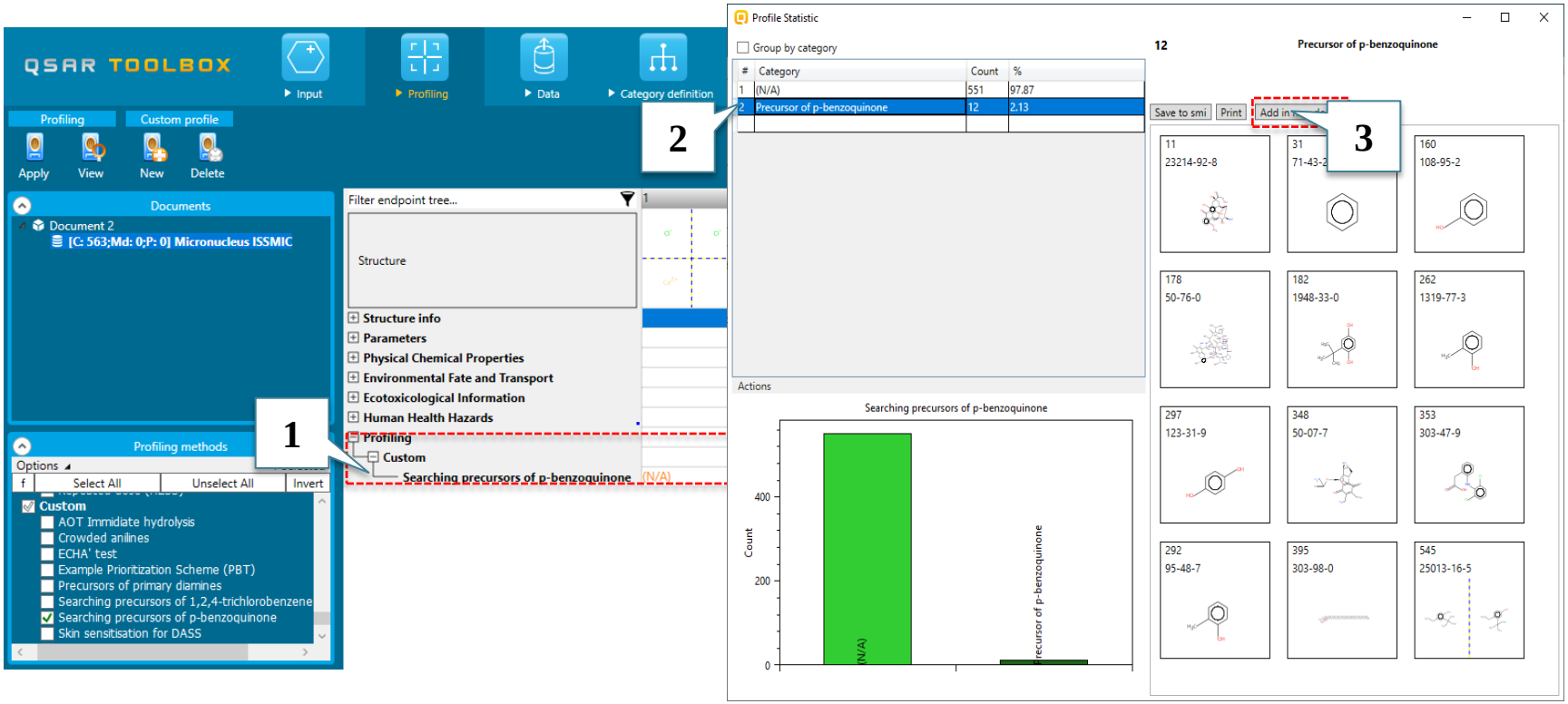
1) Go to the ***Profiling*** module; 2) Select the newly created profiler; 3) Click on the **Apply** button.

Building of a new profiler
Custom profiler usage

Once the chemicals are profiled by the created custom profiler, the results appear on the data matrix:

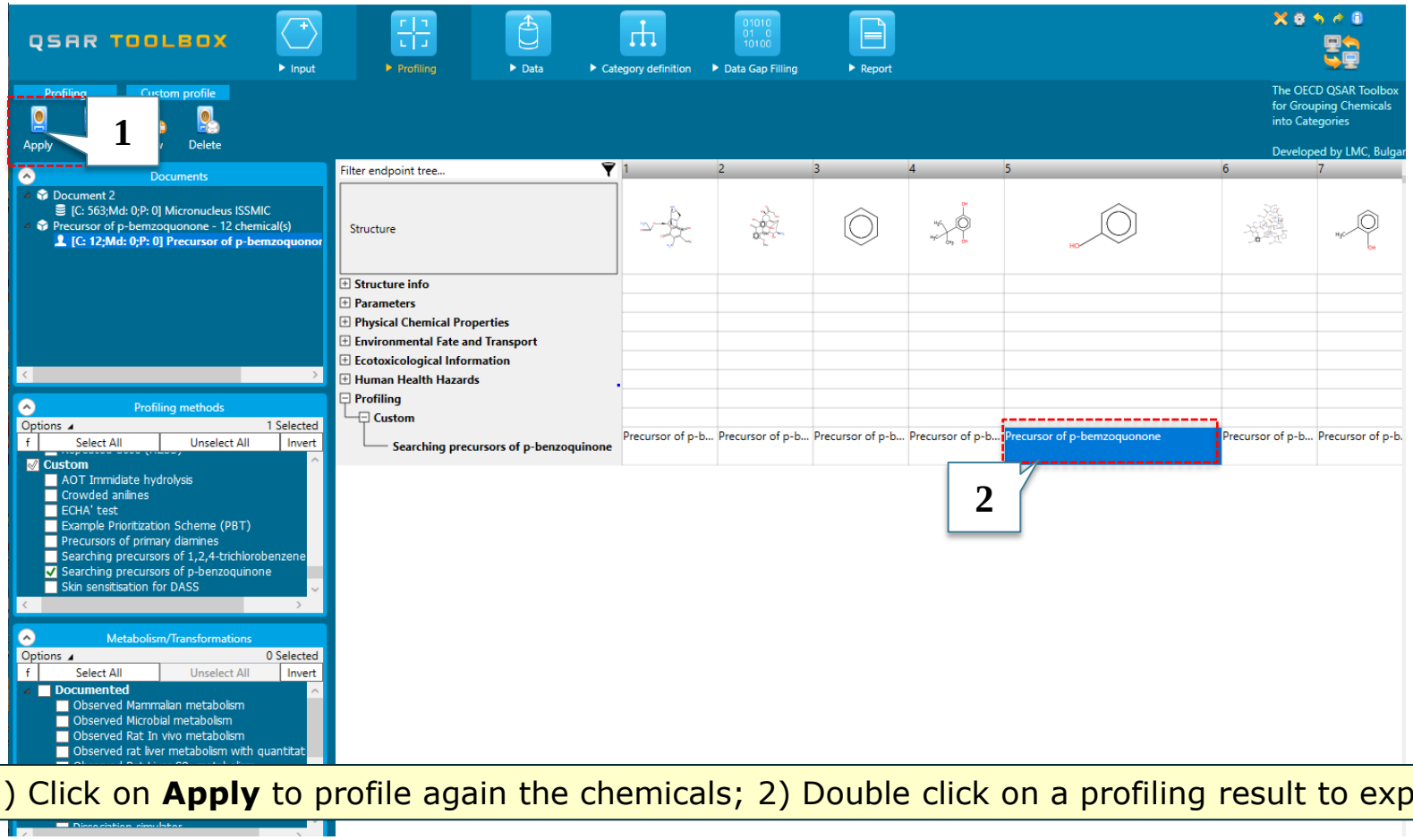
- *"N/A"* result for the chemicals that do not give p-BQ after in vivo metabolic activation
- *"Precursor of p-benzoquinone"* results for the chemicals that metabolize to p-BQ.

Building of a new profiler
Custom profiler usage



1) Apply right click over the name of the custom profiler that appears in the grey field and select **Profile statistic**. It could be seen that the profiler identifies 12 chemicals among the chemicals in the data matrix; 2) Click on *the Precursor of p-benzoquinone* row; 3) Add the chemicals in a new document.

Building of a new profiler
Custom profiler usage



1) Click on **Apply** to profile again the chemicals; 2) Double click on a profiling result to explain it.

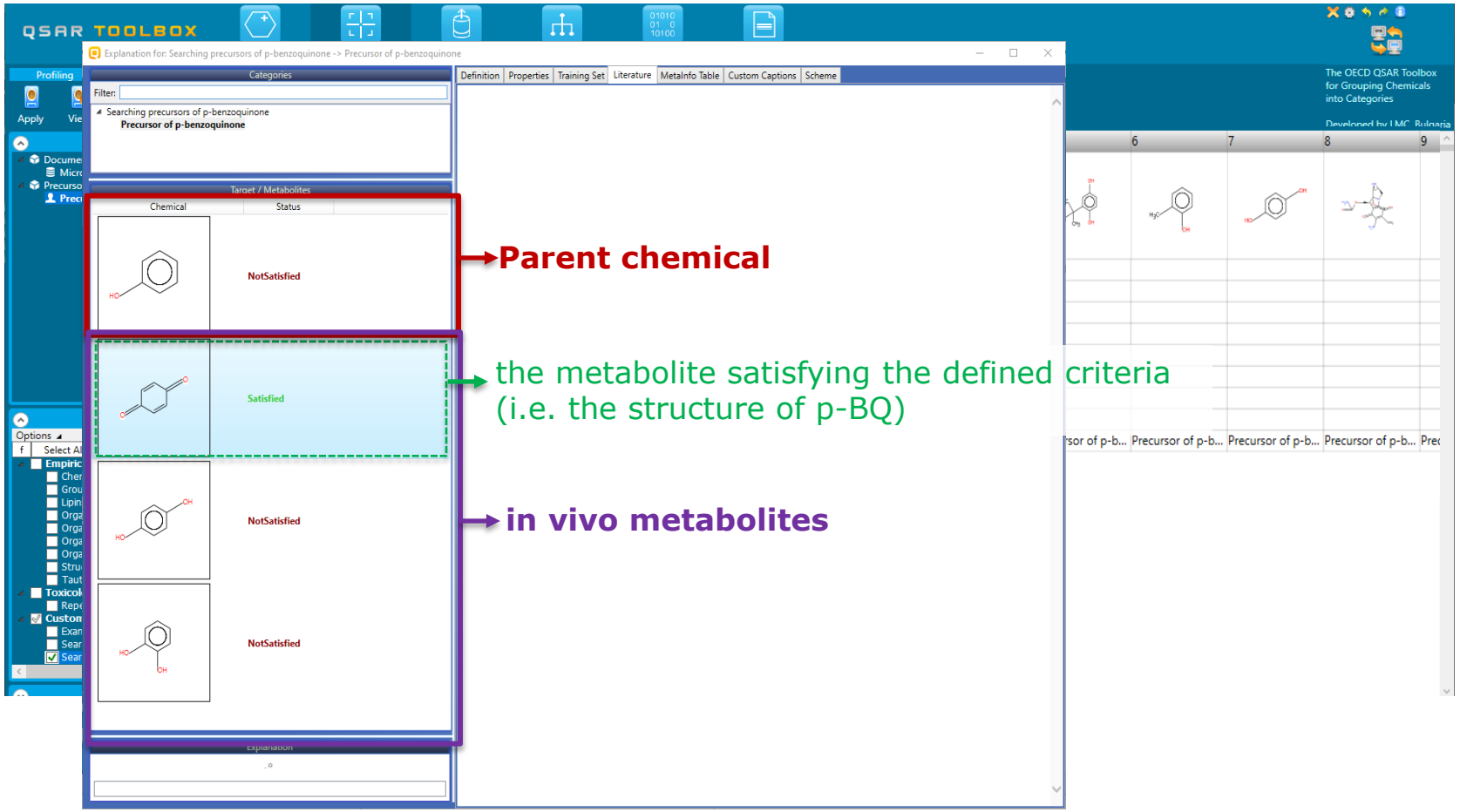
Building of a new profiler
Explaining profiling results

In the new opened window it could be seen the target and all metabolites generated by the selected metabolic simulator (i.e. in vivo rat metabolic simulator in this case).

The status for each molecule is also shown:

- *Satisfied* – means that the corresponding chemical meet the criteria defined in the profiler;
- *Not Satisfied* – means that the corresponding chemical does NOT meet the criteria defined in the profiler.

Building of a new profiler
Explaining profiling results



Congratulations

- Now you know how to:
  - create a new profiler taking into account the (a)biotic activation of the chemicals;
  - use the custom profiler for searching precursors of a target in selected databases;
  - use the custom profiler for searching precursors of a target in a specific file (database);
  - explain the results of the custom profiler.
- Continual use of the Toolbox will increase your skills.