QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.4.1

Tutorial illustrating new options for grouping with metabolism

Outlook

- **Background**
- Keywords
- Objectives
- Specific Aims
- The exercise
- Workflow

Background

- Grouping with metabolism is a procedure for finding analogues accounting for metabolism activation of the chemicals;

- This is a step-by-step presentation designed to take the user through the options for grouping with metabolism.

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Keywords

TARGET CHEMICAL - chemical of interest

MODULE – a Toolbox module is a section dedicated to specific actions and options

WORKFLOW – the use, in combination, of the different modules (e.g. prediction workflow: from input to report)

PROFILER - algorithm (rule set) for the identification of specific features of the chemicals. Several types of profilers are available, such as structural (e.g. Organic functional groups), mechanistic (e.g. Protein binding by OECD) and endpoint-specific (e.g. in vitro in vitro mutagenicity (Ames test) alerts by ISS) profilers.

ALERT - the profilers consist of sets of rules or alerts. Each of the rules consists of a set of queries. The queries could be related to the chemical structure, physicochemical properties, experimental data, comparison with the target or list with substances and external queries from other predefined profilers (reference queries).

CATEGORY – "group" of substances sharing same characteristics (e.g. the same functional groups or mode of action). In a typical Toolbox workflow, it consists of the target chemical and its analogues gathered according to the selected profilers

ENDPOINT TREE – Endpoints are structured in a branched scheme, from a broader level (Phys-Chem properties, Environmental Fate and transport, Ecotoxicology, Human health hazard) to a more detailed one (e.g. EC3 in LLNA test under Human health hazard-Skin sensitization)

DATA MATRIX – Table reporting the chemical(s) and data (experimental results, profilers outcomes, predictions). Each chemical is in a different column and each data in a different row

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Objectives

This presentation demonstrates a number of functionalities for searching of analogues accounting for metabolism:

- Identify analogues based on the metabolites with:
  - common specific structure features;
  - common specific profiling results;
  - common specific parameter results.
- Identify analogues based on the parent and metabolites package.

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Specific Aims

- To familiarize the user with the metabolic transformation map similarity options when defining a category with metabolism.

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The Exercise

- In this exercise we will search for suitable analogues of 1,2-Ethanediamine (CAS# 107-15-3) for predicting of skin sensitization potential.
- Even though the chemical has experimental EC3 data in Skin sensitization database its structural features are suitable for the concept of this exercise.
- The target chemical has no protein binding alert for skin sensitization.
- Skin metabolism of target chemical will be accounted for.
- Different metabolic transformation map similarity options will be applied for defining a category.

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Workflow

- As you know the Toolbox has 6 modules which are typically used in sequence:
  - Input
  - Profiling
  - Data
  - Category Definition
  - Data Gap Filling
  - Report
- In this example we will use only the first four modules, tailored to the aims of the example.

Outlook

- Background
- Keywords
- Objectives
- Specific Aims
- The exercise
- **Workflow**
  - **Input**

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.

Input
Ways of Entering a Chemical

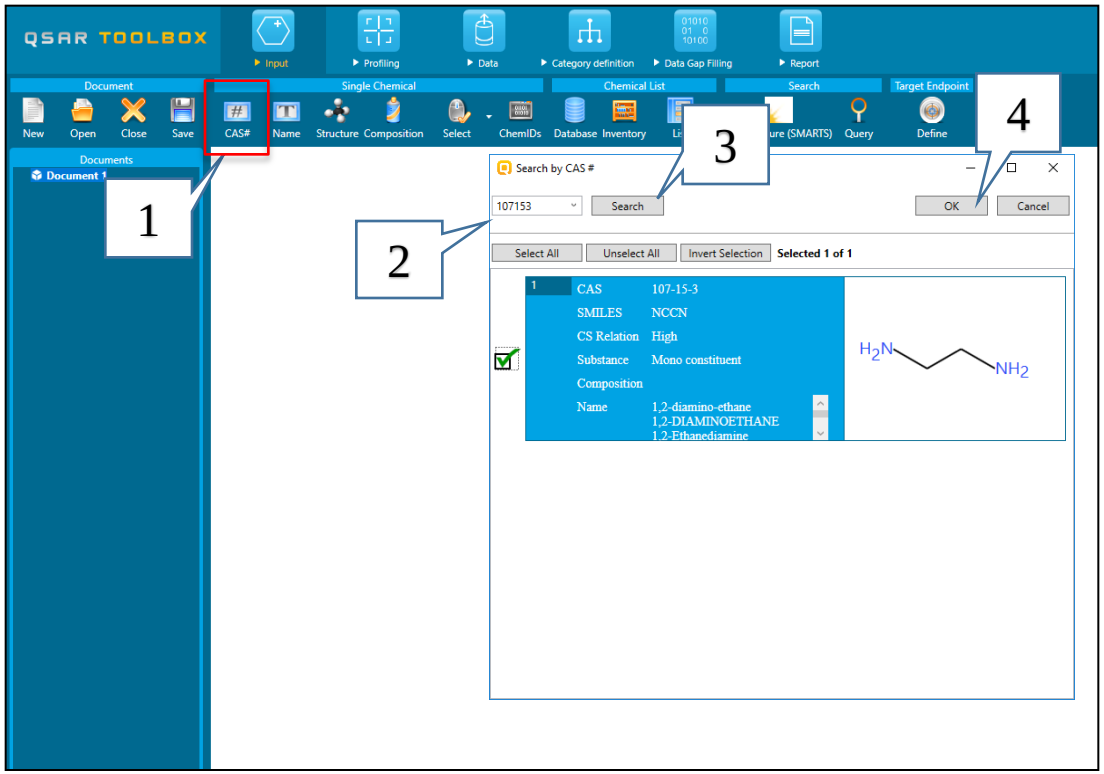
User Alternatives for Chemical ID:

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

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

Input Screen

Input target chemical by CAS#



1. Click **CAS#**;
2. Type **107153** in the blank field;
3. Click **Search**;
4. Confirm by clicking "**OK**".

Outlook

- Background
- Keywords
- Objectives
- Specific Aims
- The exercise
- **Workflow**
  - 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 metabolisms.

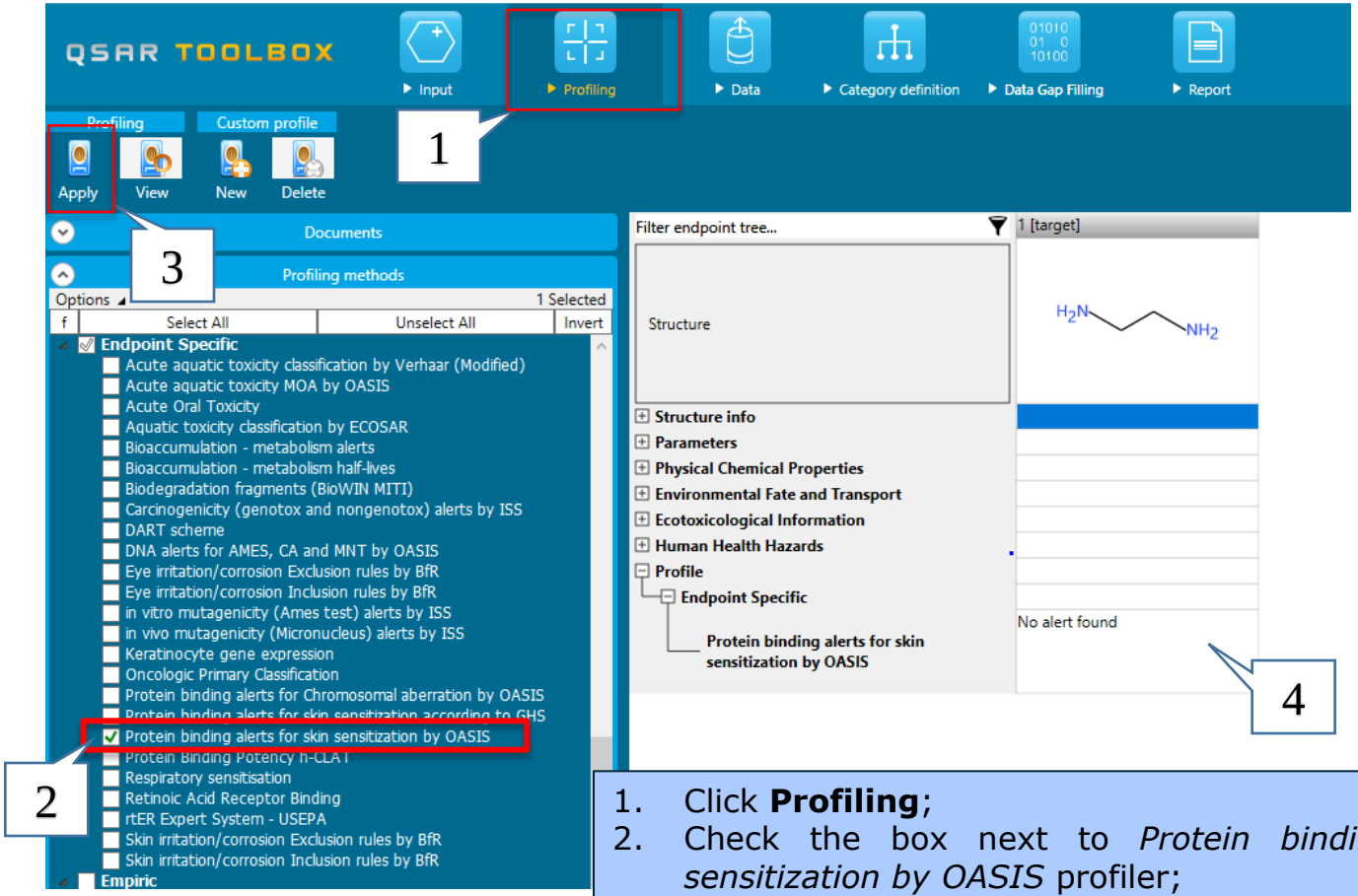
Profiling

Profiling the target chemical

- The actual profiling will take up to several seconds depending on the number and type of profilers selected.
- Including the metabolic simulator is more time consuming.
- The results of profiling automatically appear under the target chemical
- This result will be used to search for suitable analogues in the next steps of the exercise.

Profiling

Profiling the target chemical

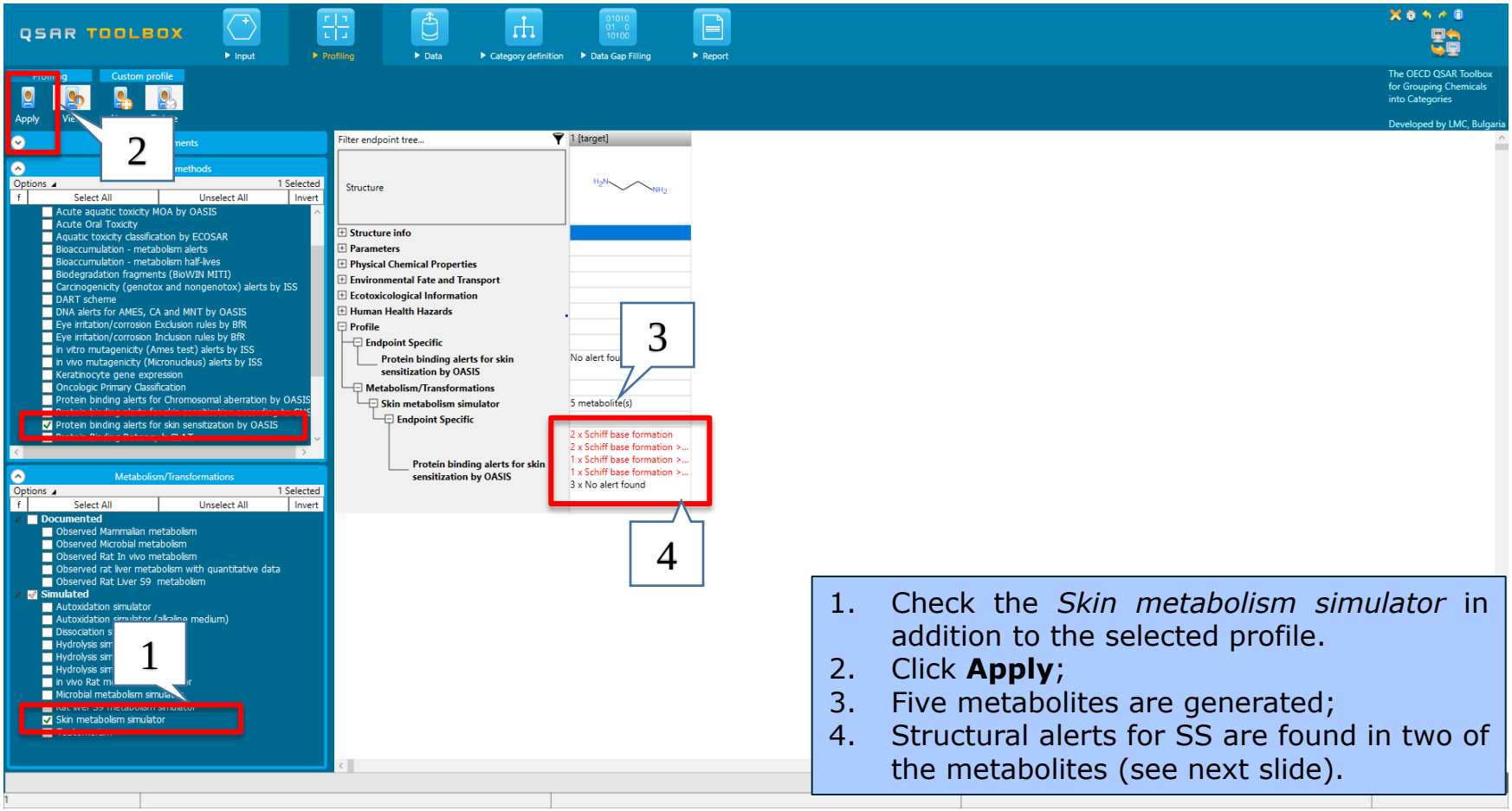


1. Click **Profiling**;
2. Check the box next to *Protein binding alerts for skin sensitization by OASIS* profiler;
3. Click **Apply**;
4. *No alert is found* in the target structure.

Profiling

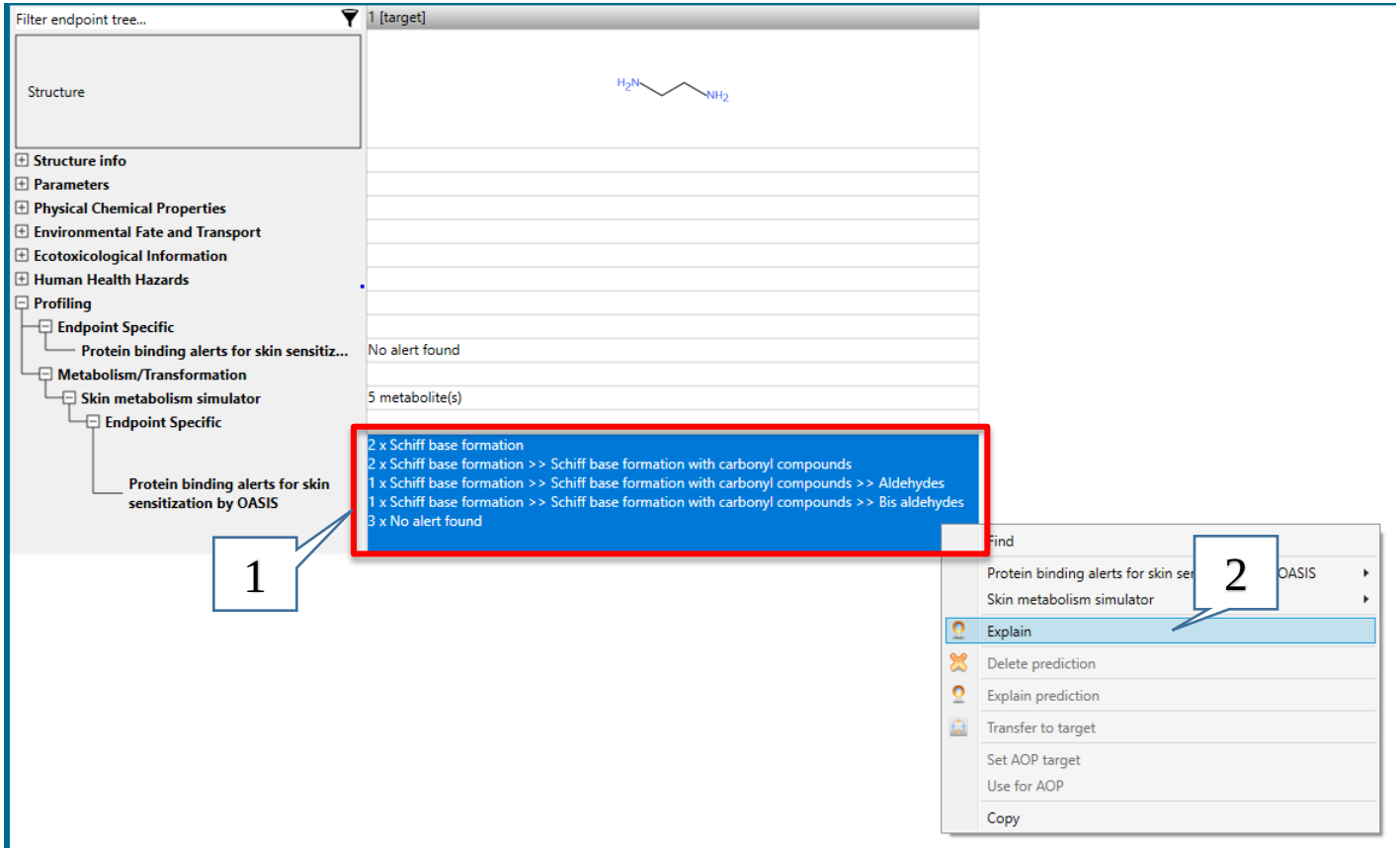
Profiling the target chemical

Profiling results for the target metabolites can be also retrieved.



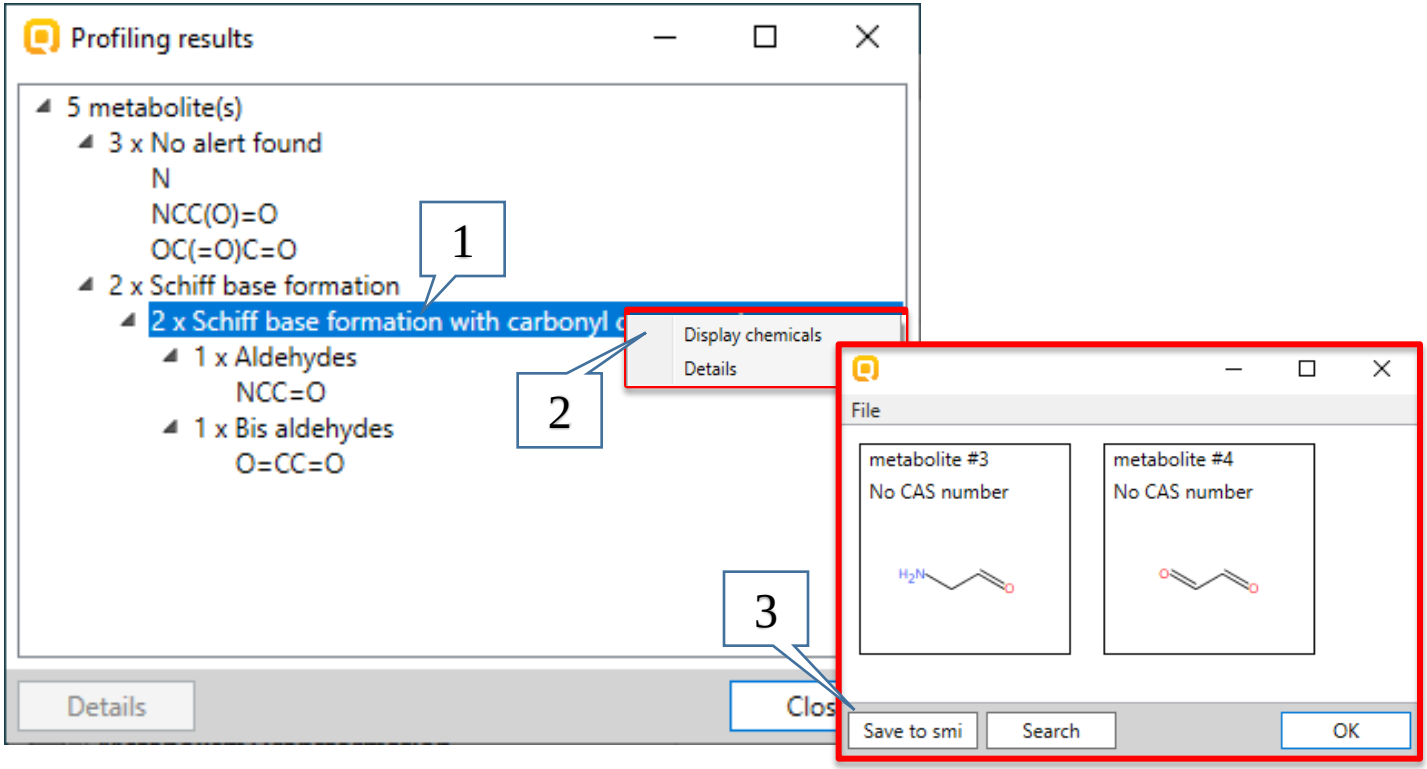
1. Check the *Skin metabolism simulator* in addition to the selected profile.
2. Click **Apply**;
3. Five metabolites are generated;
4. Structural alerts for SS are found in two of the metabolites (see next slide).

Profiling
Explain of profiling results



1. Right-click over the cell containing the results.
2. Select ***Explain*** for more details.

Profiling
Explain of profiling results



1. Right-click on the alert;
2. Select ***Display chemicals*** to see for which structures (3) have alerts;
3. The structures can be saved as *.smi* file.

Outlook

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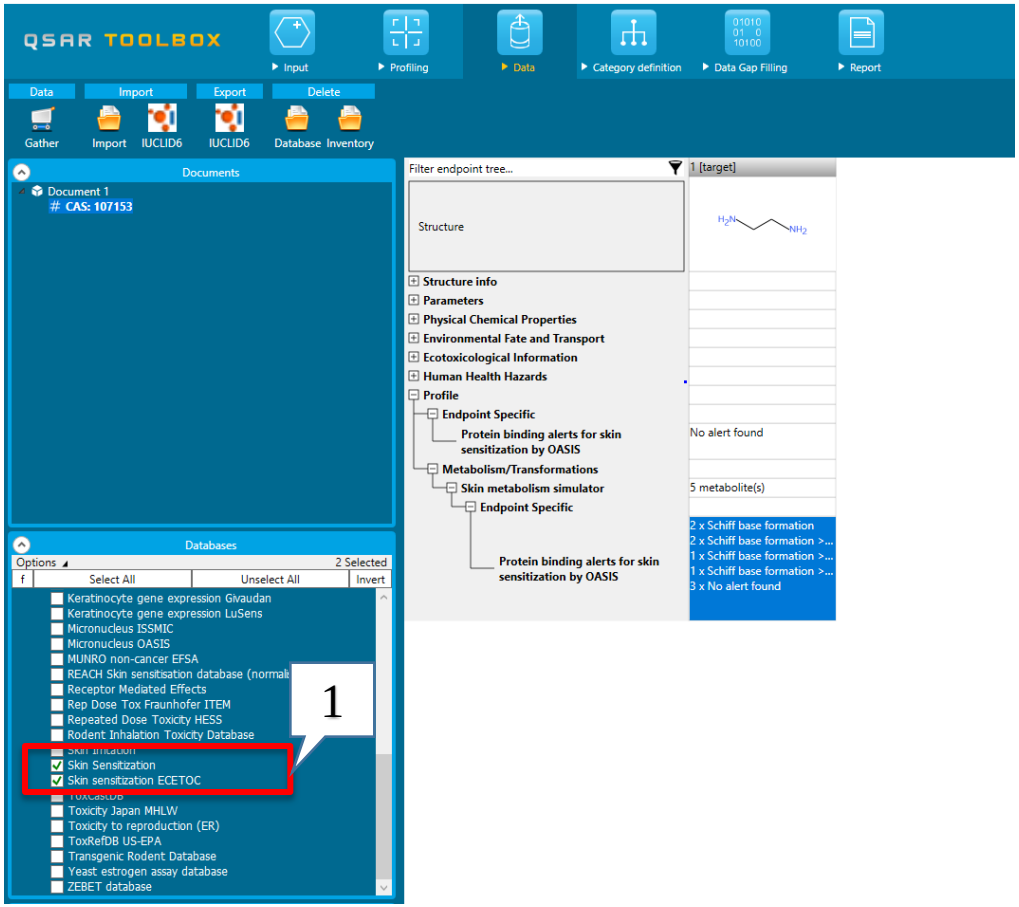
- **Workflow**
  - Input
  - Profiling
  - **Data**

Data
Overview

- "Data" 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).

Data
Selecting databases



1. Select **Skin sensitization** and **Skin sensitization ECETOC** databases

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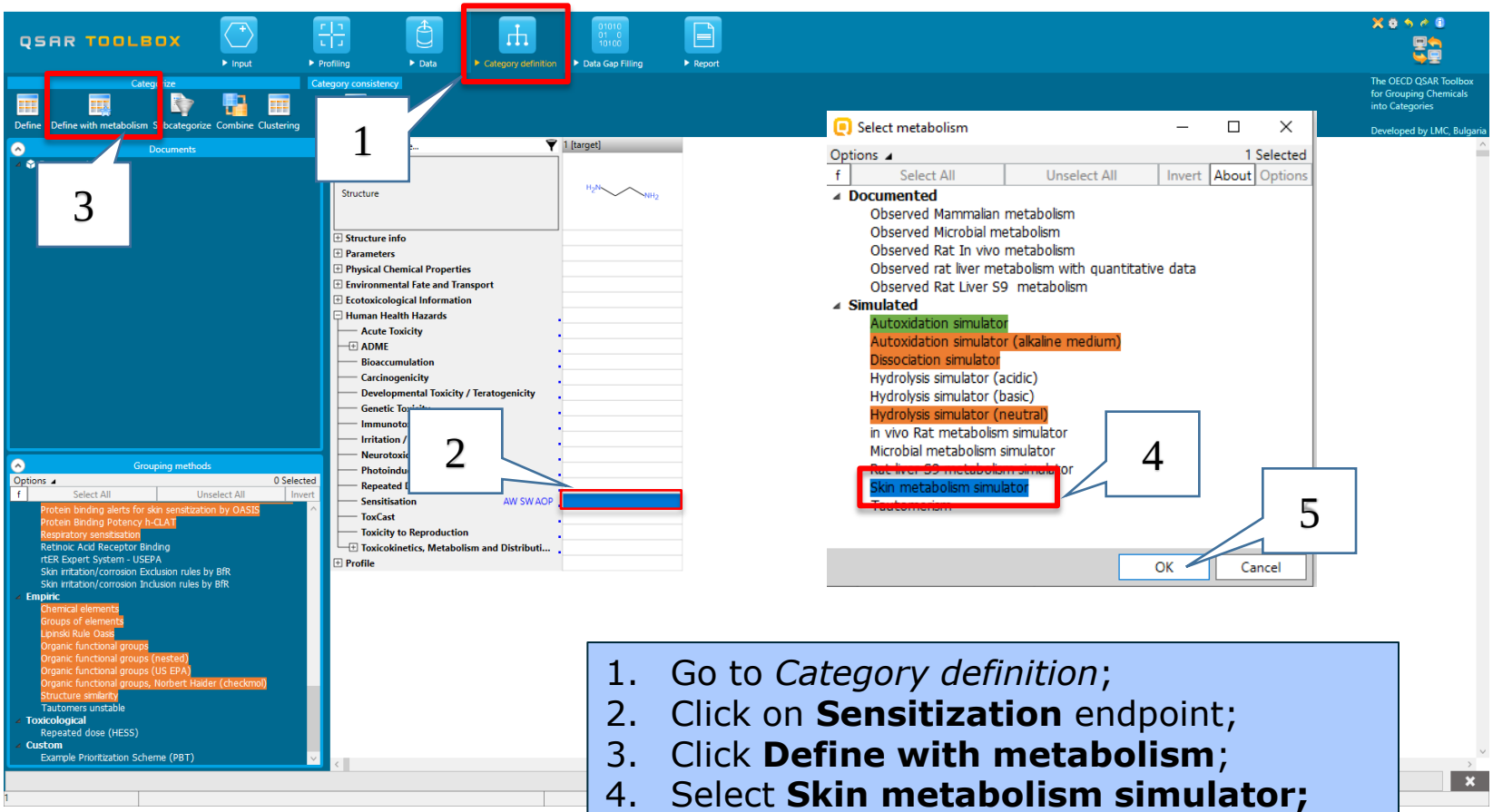
- **Workflow**
  - Input
  - Profiling
  - Data
  - **Category definition**

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 refining the category definition.
- A category can be defined with and without metabolism.
- Grouping with accounting for metabolic transformation is a procedure for finding analogues accounting for metabolism activation of the chemicals.

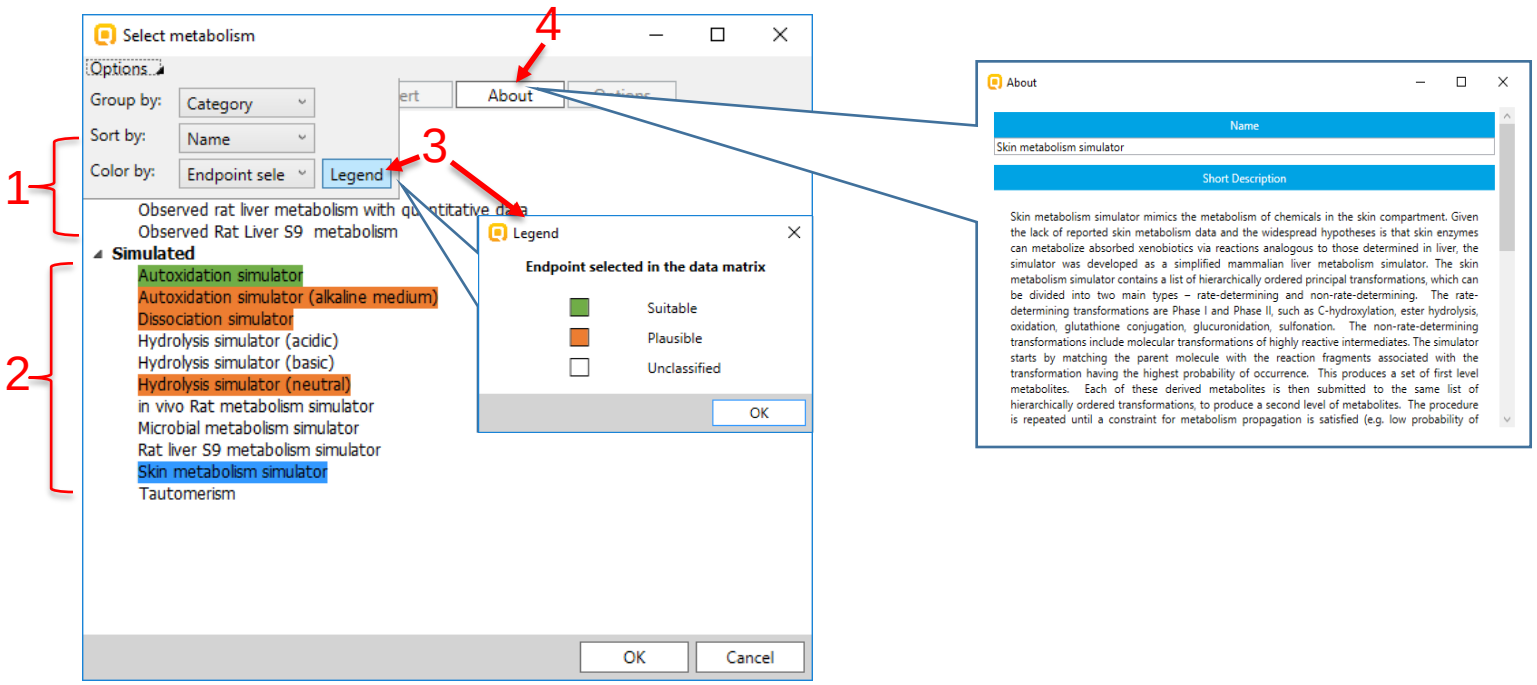
Category definition
Define with metabolism



1. Go to *Category definition*;
2. Click on **Sensitization** endpoint;
3. Click **Define with metabolism**;
4. Select **Skin metabolism simulator;**
5. Click **OK.**

Category definition

On defining with metabolism



All available transformation maps – documented (1) and simulated (2) in Toolbox can be used in the primary grouping. The simulators are coloured if a target endpoint is selected in the data matrix. The meaning of the colours is explained in in the Legend (3). Short description for each of the metabolic transformations can be seen if you click ***About***.

Category definition
Define with metabolism

When the transformation map is selected, the "Map similarity options" (Grouping options) dialogue appears. It shows all the generated metabolites of the target chemical by the simulator that was preliminary selected. The dialogue has two subsections:

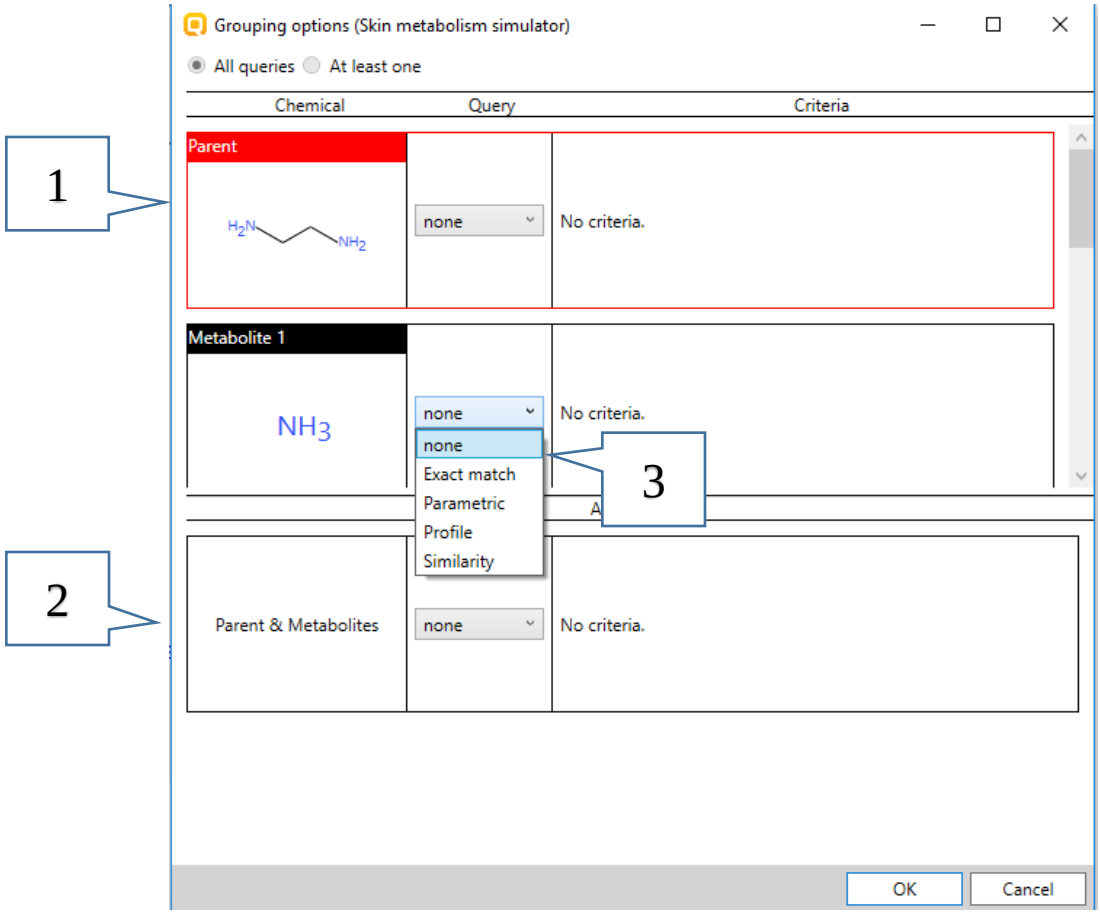
- First subsection (1) shows parent and each of the generated metabolites (by the preliminary selected metabolism simulator) is separate rows. This allows defining of different criteria for each of structures for finding analogues.
- Second subsection (2) is working with whole package "parent + metabolites", i.e. the criteria is provided for the whole package but not for separate metabolite.

A drop down menu (3) is available for each of the structures (in the column "Query") which allow setting the type of criteria for further looking for analogues.

See on the next slide.

Category definition

Define with metabolism



Category definition
Define with metabolism

Explanation of different options from the drop down menu:

- **None** – default options; no criterion is set;
- **Exact** – provides opportunity to search for metabolites in the analogues having exact to the specified metabolite structure; only available for the metabolites and the package "parent + metabolites" but not for the parent chemical;
- **Parametric** – to have specific value or range of variation of defined parameter (a list with all parameters currently available in the Toolbox is provided);
- **Profile** – to have specific category by selected profiler (a list with all profilers is provided);
- **Structural** – to have specific similarity based on the atom centered fragments.

Category definition
Define with metabolism – Case 1

Different map similarity options will be examined.

Case 1: Searching of analogues based on a metabolite with defined profile

As you remember, structural alerts have been found for only two of the generated metabolites (*slide 22*).

The alerts are identical and therefore we can choose only one of the structures.

Category definition
Define with metabolism – Case 1

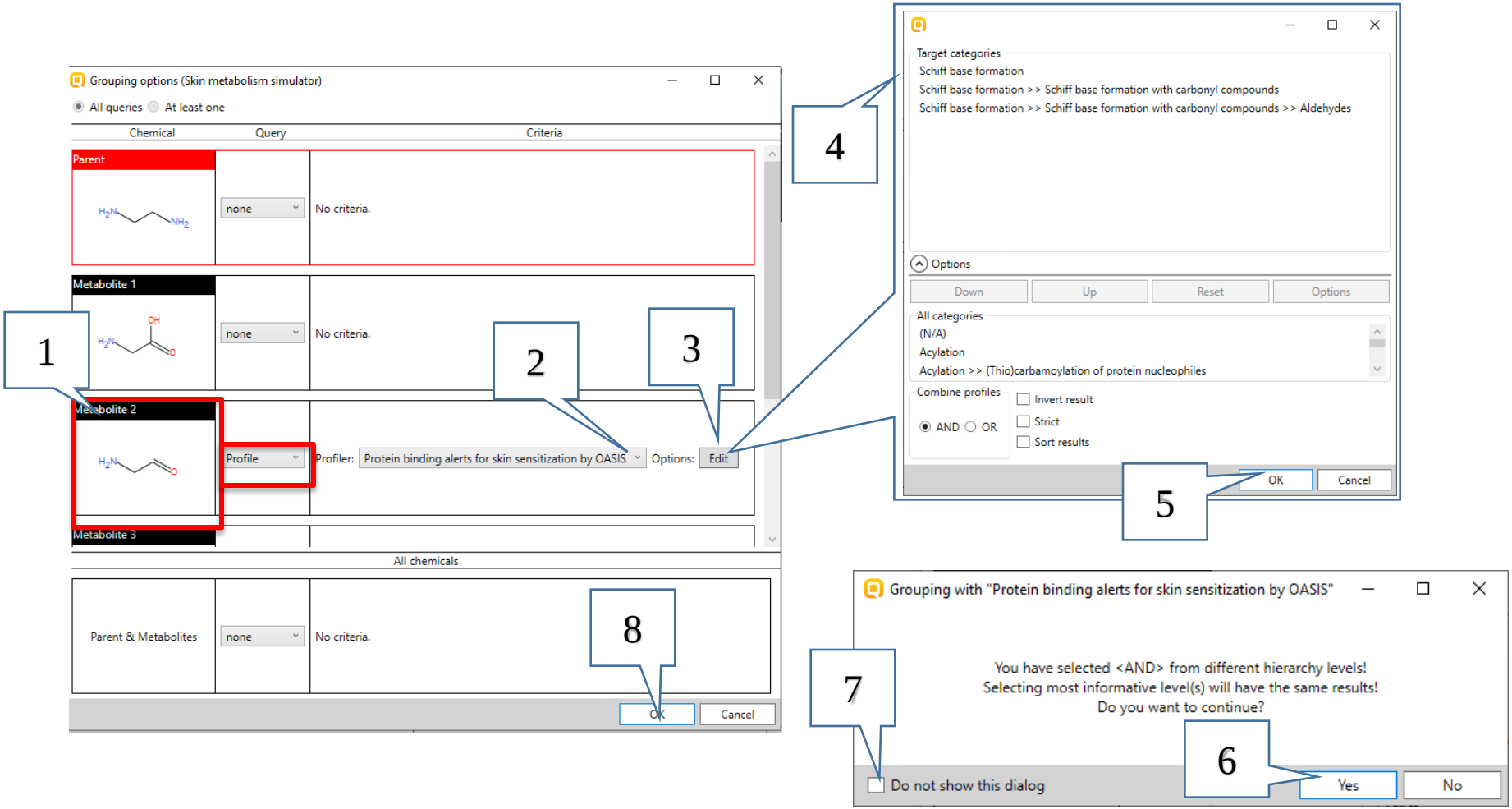
In order to find analogues based on metabolites with common profiling result, you have to follow the described steps below:

1. On the row with the target metabolite select a query which you will use for searching. Select "**profile**" as a criteria for the first metabolite. New drop-down menu with all available profiles appears;
2. Select *Protein binding alerts for skin sensitization by OASIS* from the drop-down menu;
3. You can see the found alerts in the metabolite by click on the **Edit** button. If more than one alert is available, the user can select whether they will search analogues with all or only one of the alerts.
4. In this case there is only one structural alert and you click **OK**.
5. When you are ready click on the **OK** button.
6. Then a system alerts you that the categories from different hierarchy levels are combined. This is just a warning message, which will appear every time when a hierarchical type profiling scheme is used.
7. You can manage whether this message to appear or not by selecting the box "Do not show this dialog".
8. Finally click **OK** in the main form.

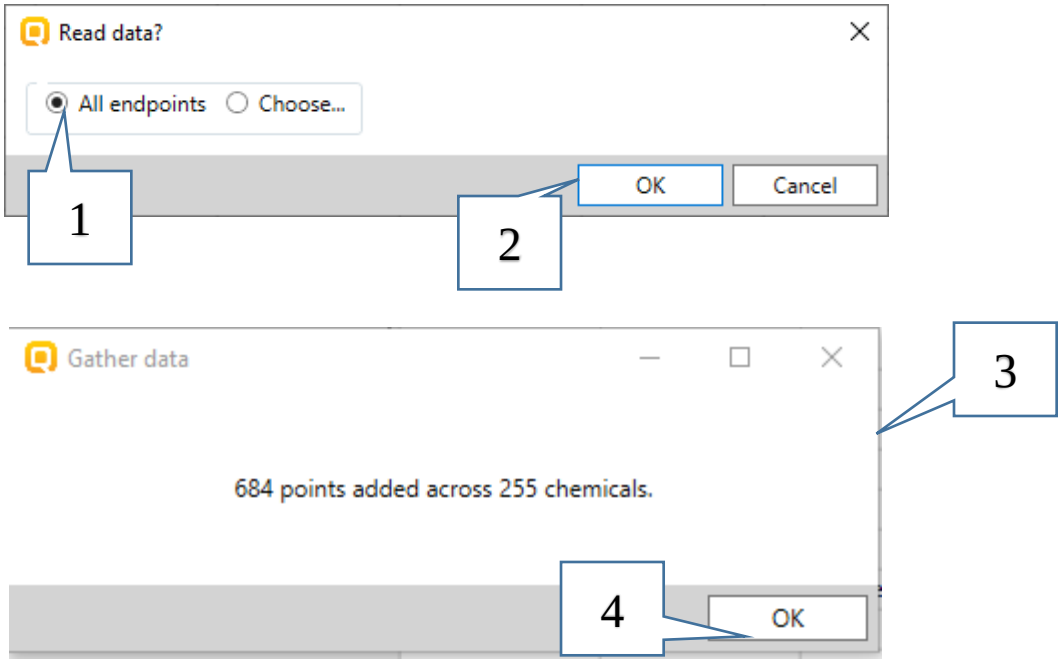
See the illustrated steps on the next slide.

Category definition

Define with metabolism – Case 1



Category definition
Define with metabolism – Case 1

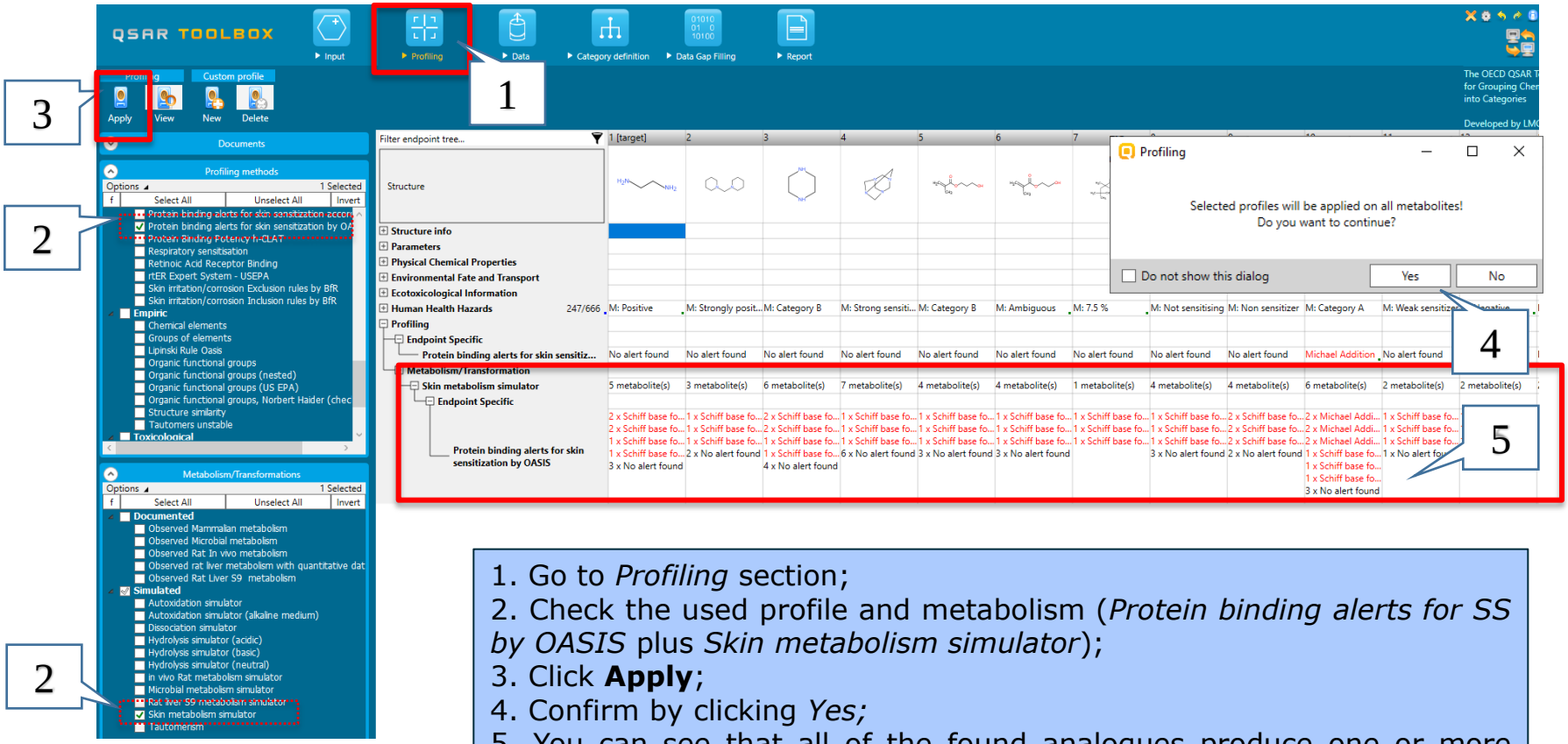


1. Keep **All endpoints** data checked;
2. Click **OK**;
3. A pop-up message is displayed where the number of chemicals (target chemical + analogues) and the total number of data points is shown;
Note: The numbers of chemicals and data points depend on the database version you are working with.
4. Click **OK** .

Category definition

Define with metabolism – Case 1

246 analogues are found. You can return to the profiling section and to check whether the found structures correspond to the defined query.



1. Go to *Profiling* section;
2. Check the used profile and metabolism (*Protein binding alerts for SS by OASIS* plus *Skin metabolism simulator*);
3. Click **Apply**;
4. Confirm by clicking *Yes;*
5. You can see that all of the found analogues produce one or more metabolites with the searched alert.

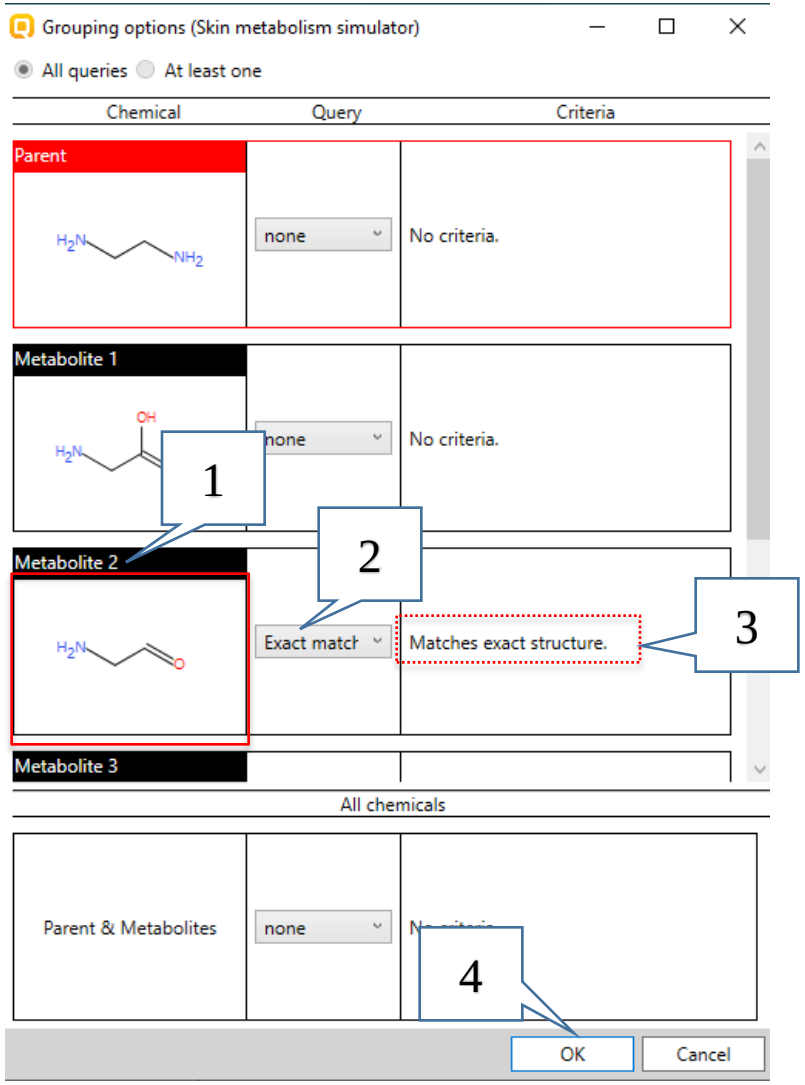
Category definition
Define with metabolism – Case 2

Case 2: Searching for analogues with a common metabolite

With the "**Exact**" option the user can search for analogues, which have a metabolite exactly the same as the selected metabolite generated for the target chemical.

Category definition

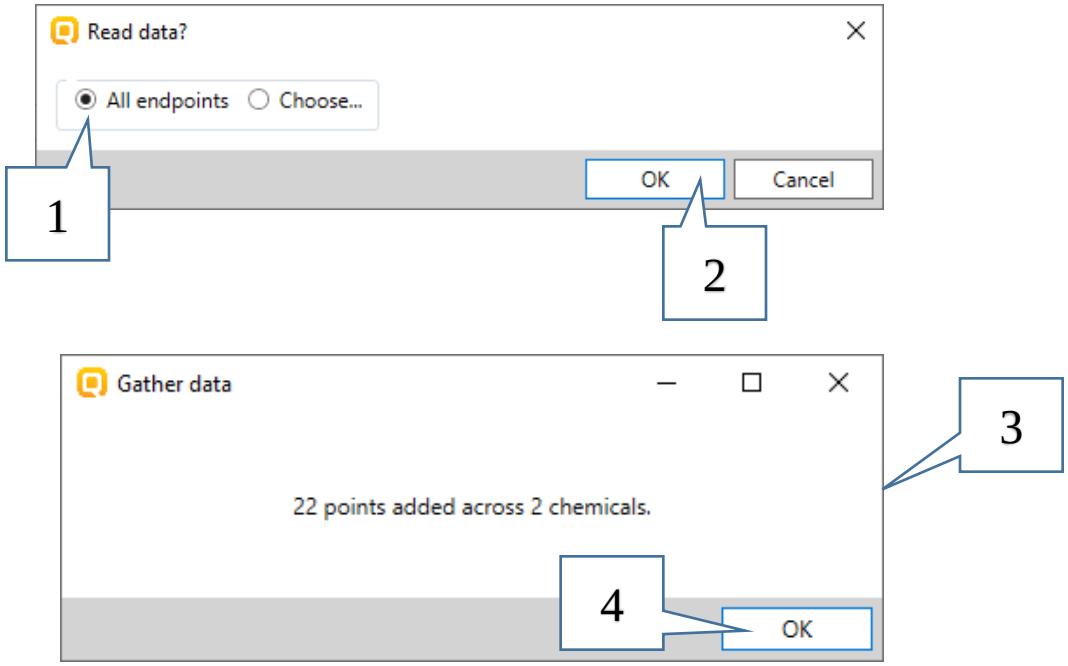
Define with metabolism – Case 2



Before repeating the steps illustrated below you need to follow once again the steps showed on slides # 30-31.

1. Go to the metabolite of interest
2. Select **Exact match** option from the drop-down menu (1);
3. "***Matches exact structure***" is displayed in the criteria;
4. Click ***OK.***

Category definition
Define with metabolism – Case 2

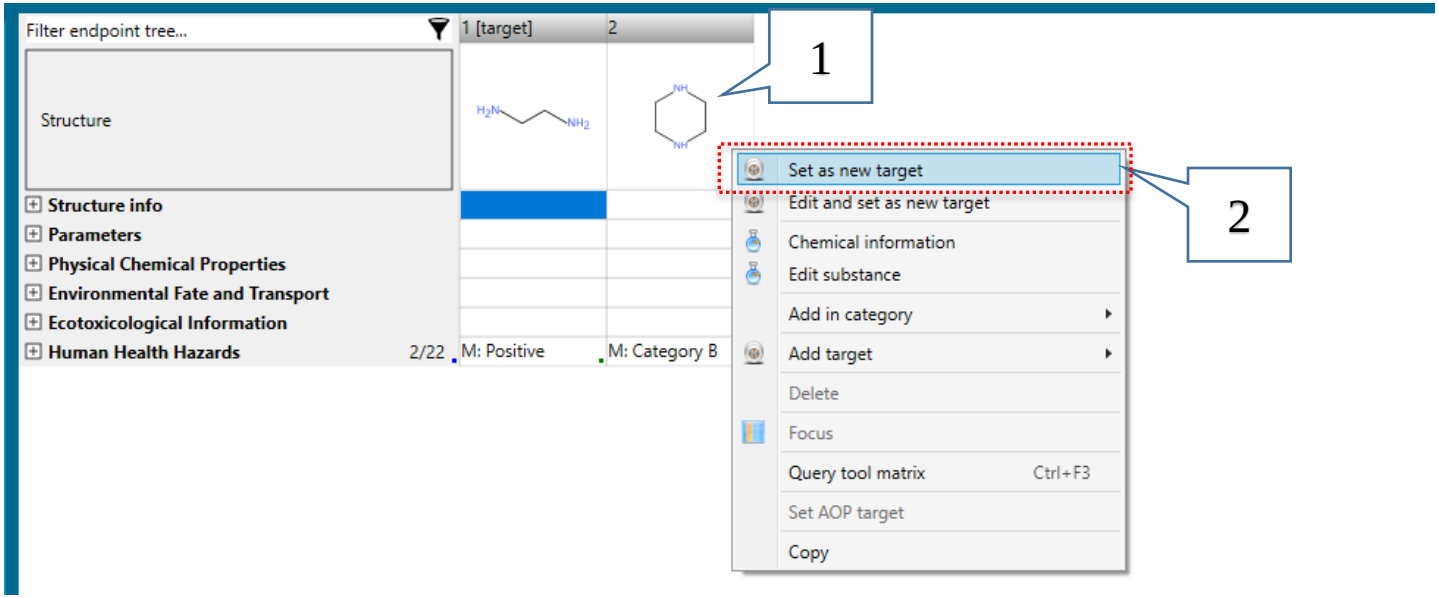


1. Keep **All endpoints** data checked;
2. Click **OK**;
3. A pop-up message is displayed where the number of chemicals (target chemical + analogues) and the total number of data points are shown;
Note: The numbers of chemicals and data points depend on the database version you are working with;
4. Click **OK** .

Category definition
Define with metabolism – Case 2

One analogue is found in the selected databases.
To check whether the found structure fulfills the defined criteria, i.e. whether it produces the exact metabolite, you have to follow the steps:

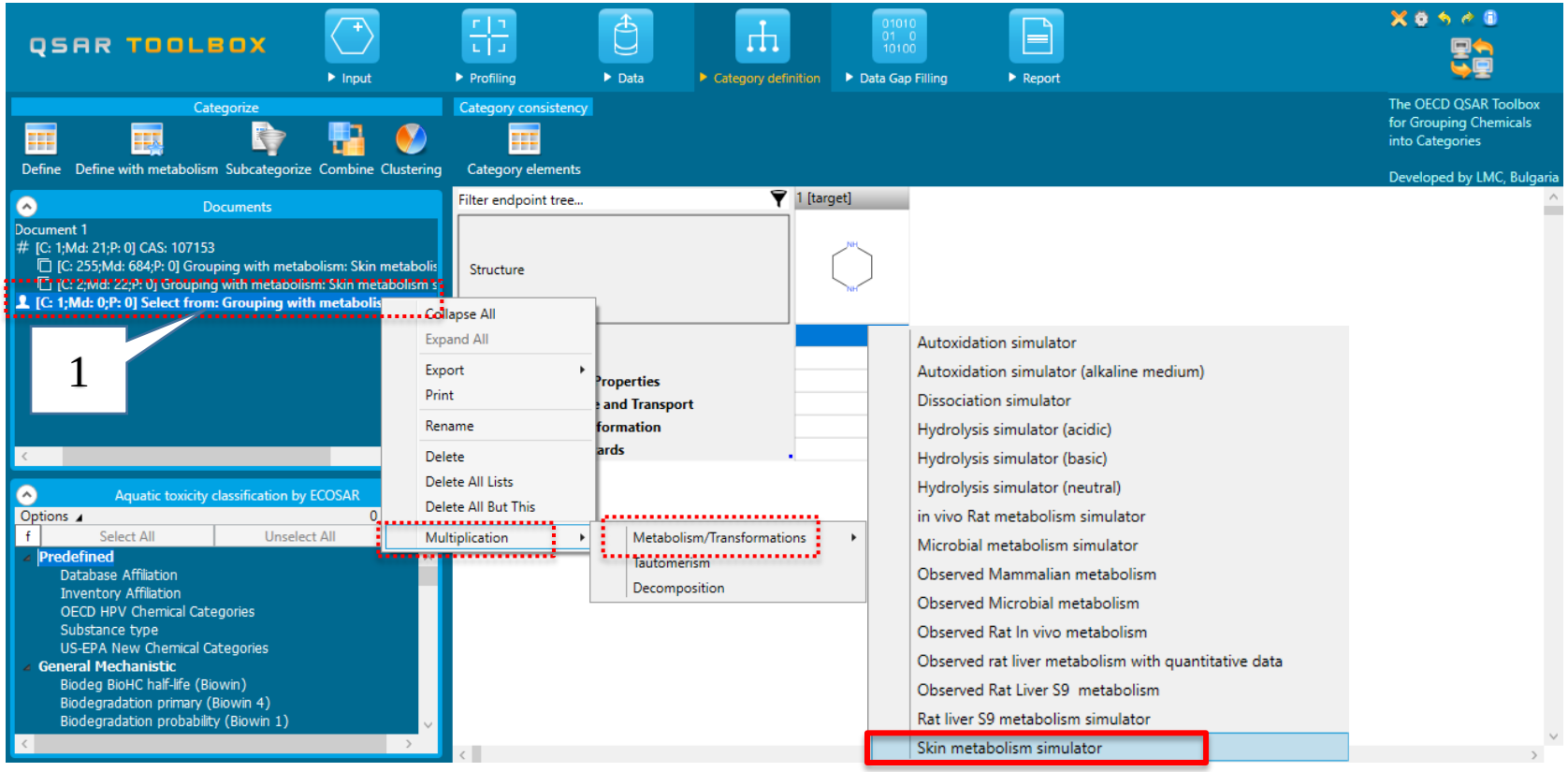
1. Right-click over the found structure; 2 . Select "**Set as new target**".



Category definition

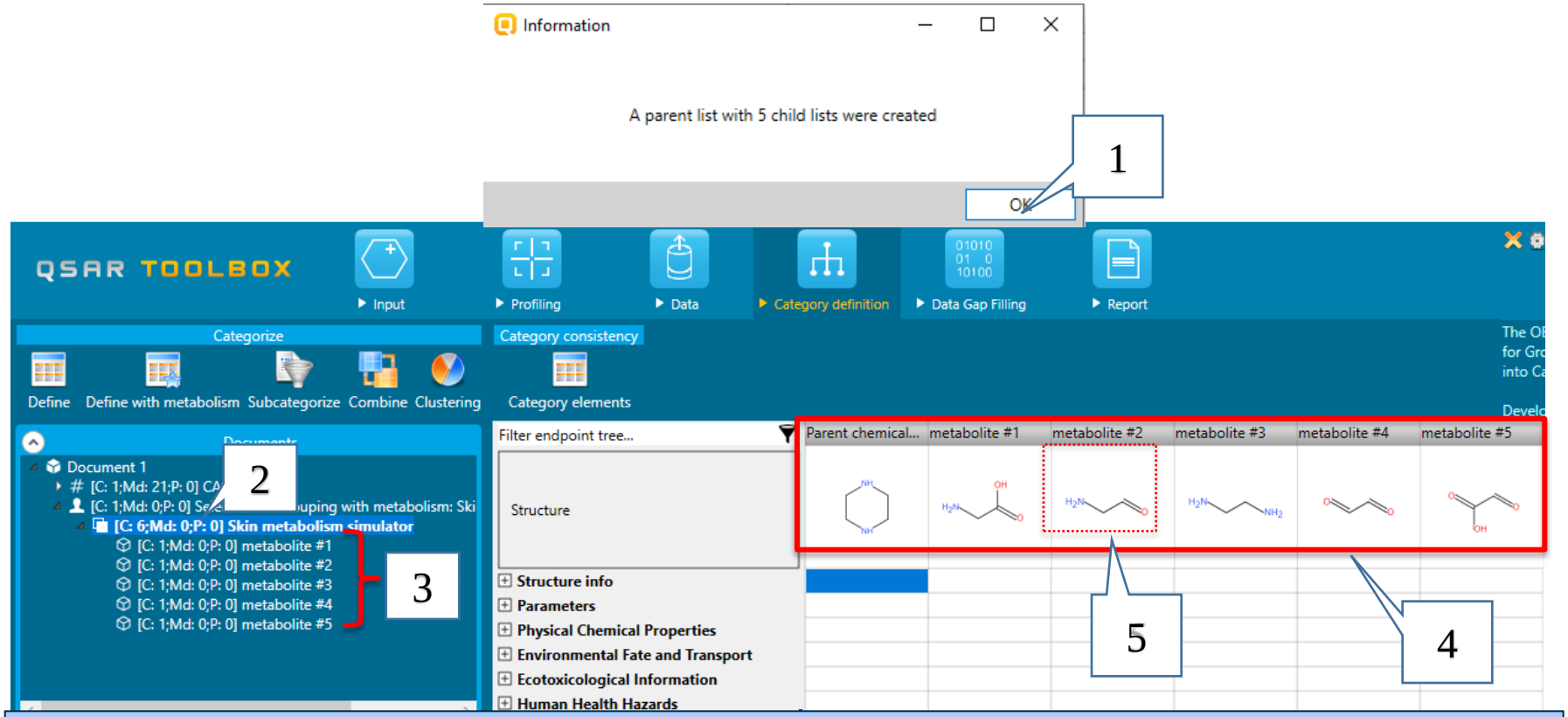
Define with metabolism – Case 2

1. Right-click over the newly appeared branch in the document tree:
Multiplication >> Metabolism/Transformations >> Skin metabolism simulator



Category definition

Define with metabolism – Case 2



1. Click **OK** in the pop-up window; A parent list (**2**) (consists of the target structure and all generated metabolites) with six child lists (**3**) (for each of the metabolites) are created, which are also displayed in the data matrix (**4**). The chemical has the same metabolite (**5**) as the target chemical (CAS#107-15-3)

Category definition
Define with metabolism – Case 3

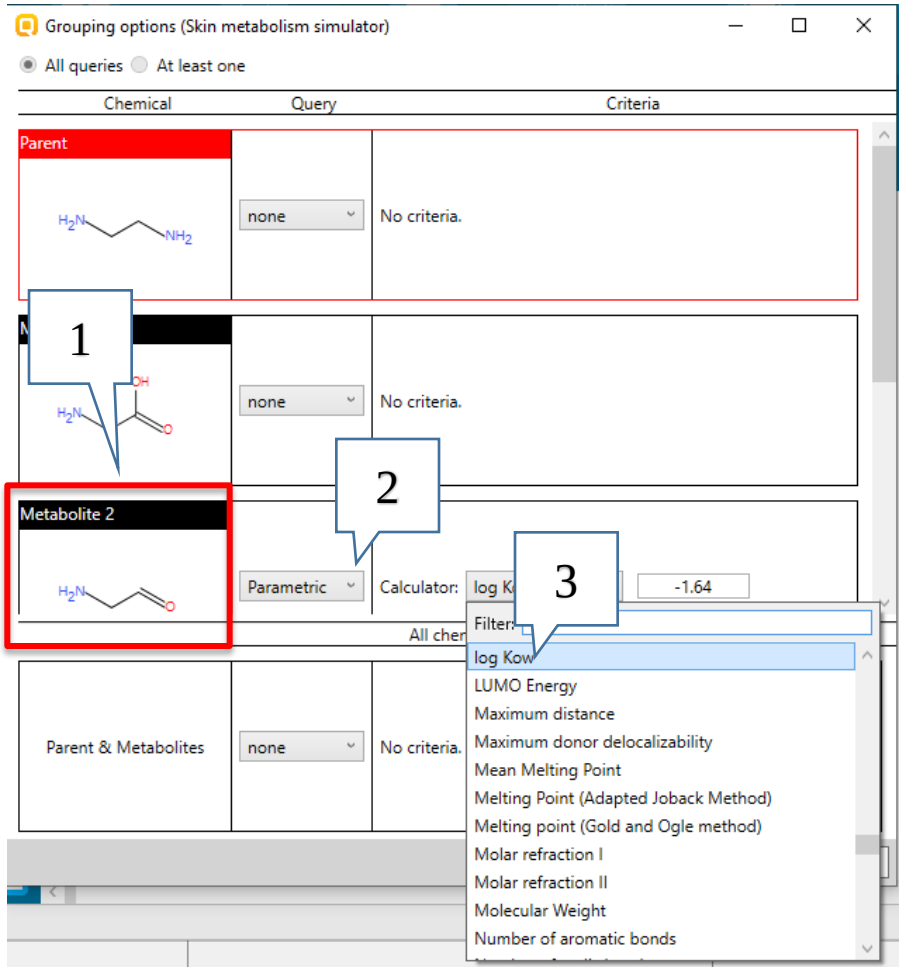
Case 3: Searching of analogues based on a metabolite with defined parameter value

With this option you can search analogues of the target chemical, which have metabolite(s) with defined parameter value.

When the parameter of interest is selected, it is automatically calculated for the current metabolite.

Category definition

Define with metabolism – Case 3

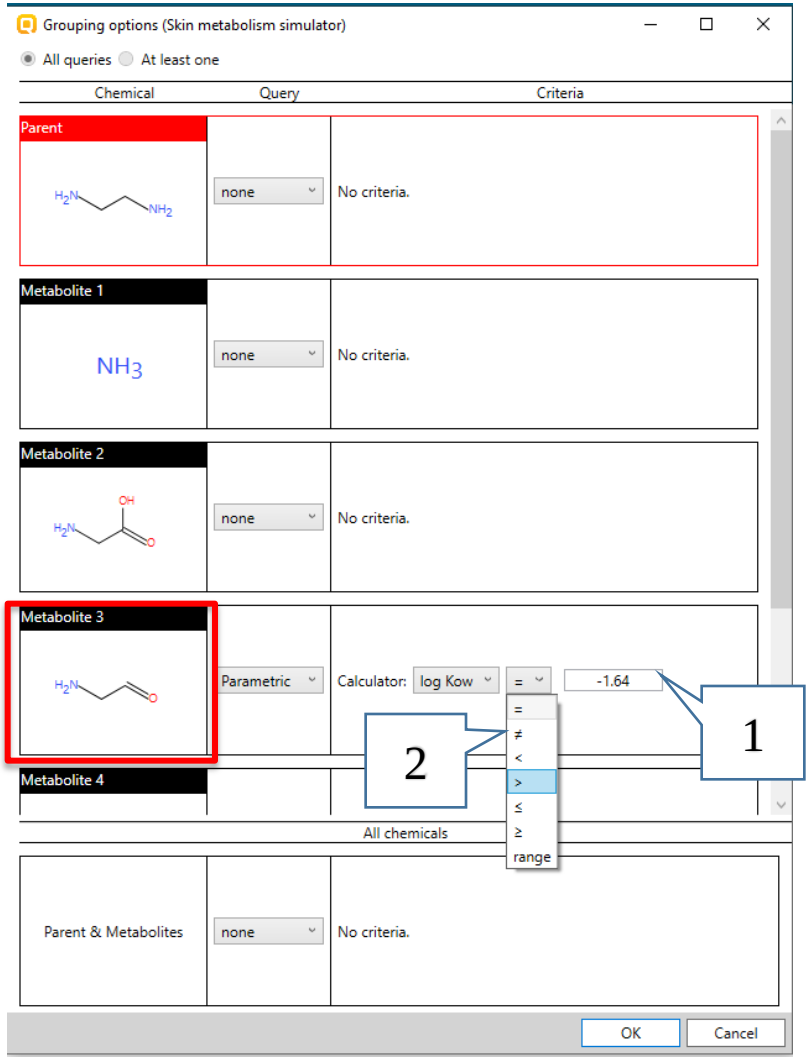


As explained already before repeating the steps illustrated below you need to follow once again the steps showed on slides # 30-31.

Go to the metabolite of interest (1);
Select the **parametric** option from the drop-down menu (2). New calculator drop-down menu appears. Select ***log Kow*** (3). You can use and the filter option to find your parameter more quickly.

Category definition

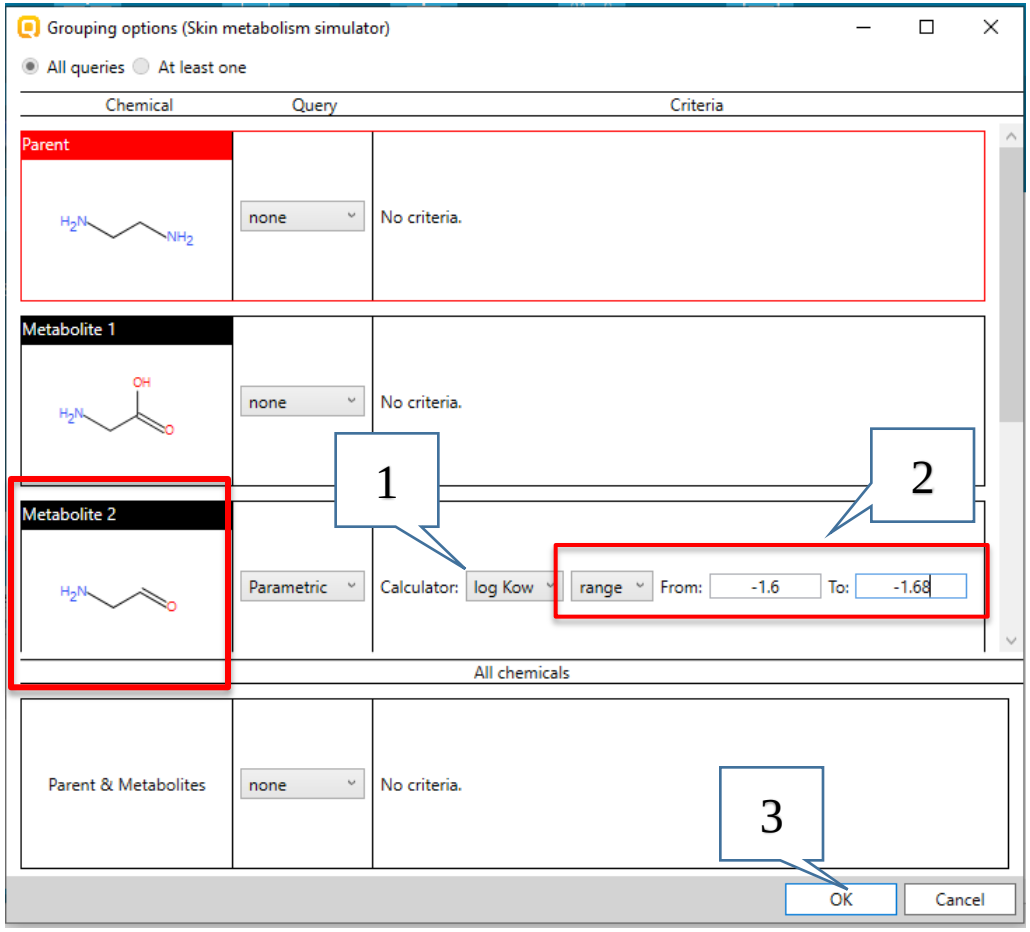
Define with metabolism – Case 3



The parameter value is calculated automatically for the target metabolite (1). Then the user can decide to search with the exact value, to use any of the mathematical symbols or to search parameter values in a range (2).

Category definition

Define with metabolism – Case 3

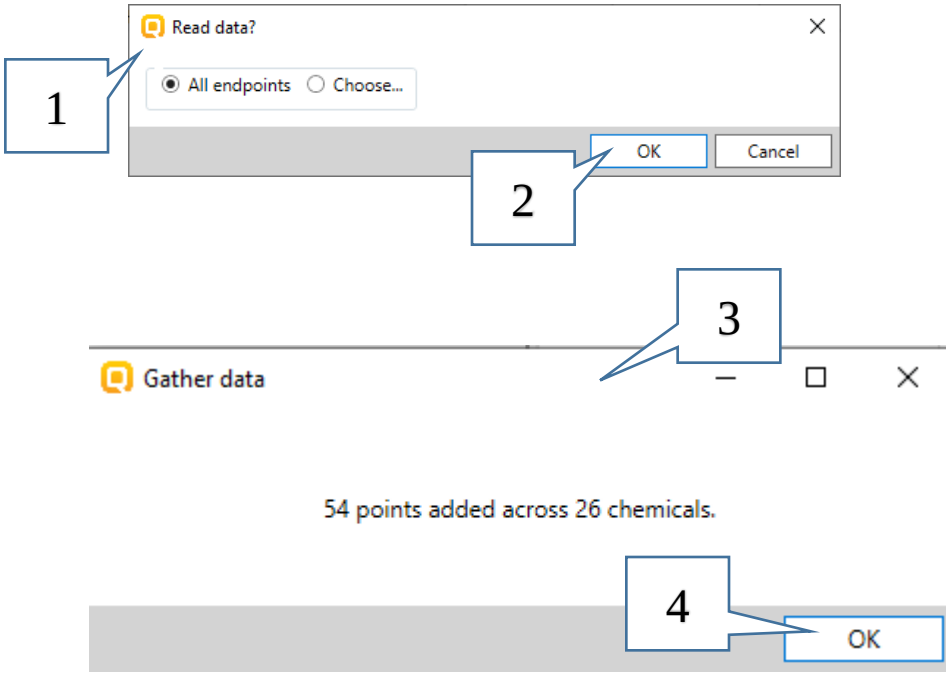


We will select to search in a range (1).
As you remember the calculated *logKow* value for the target metabolite is **-1.64**.
Therefore, we will search analogues, which have metabolite(s) with similar *logKow* values.
In this example we define a range from **-1.6** to **-1.68** (2).
Click **OK** to execute the search (3).

Note: It will take up to several minutes if you make this example for first time.

Category definition

Define with metabolism – Case 3



1. Keep **All endpoints** data checked;
2. Click **OK**;
3. A pop-up message is displayed where the number of chemicals (target chemical + analogues) and the total number of data points is shown;

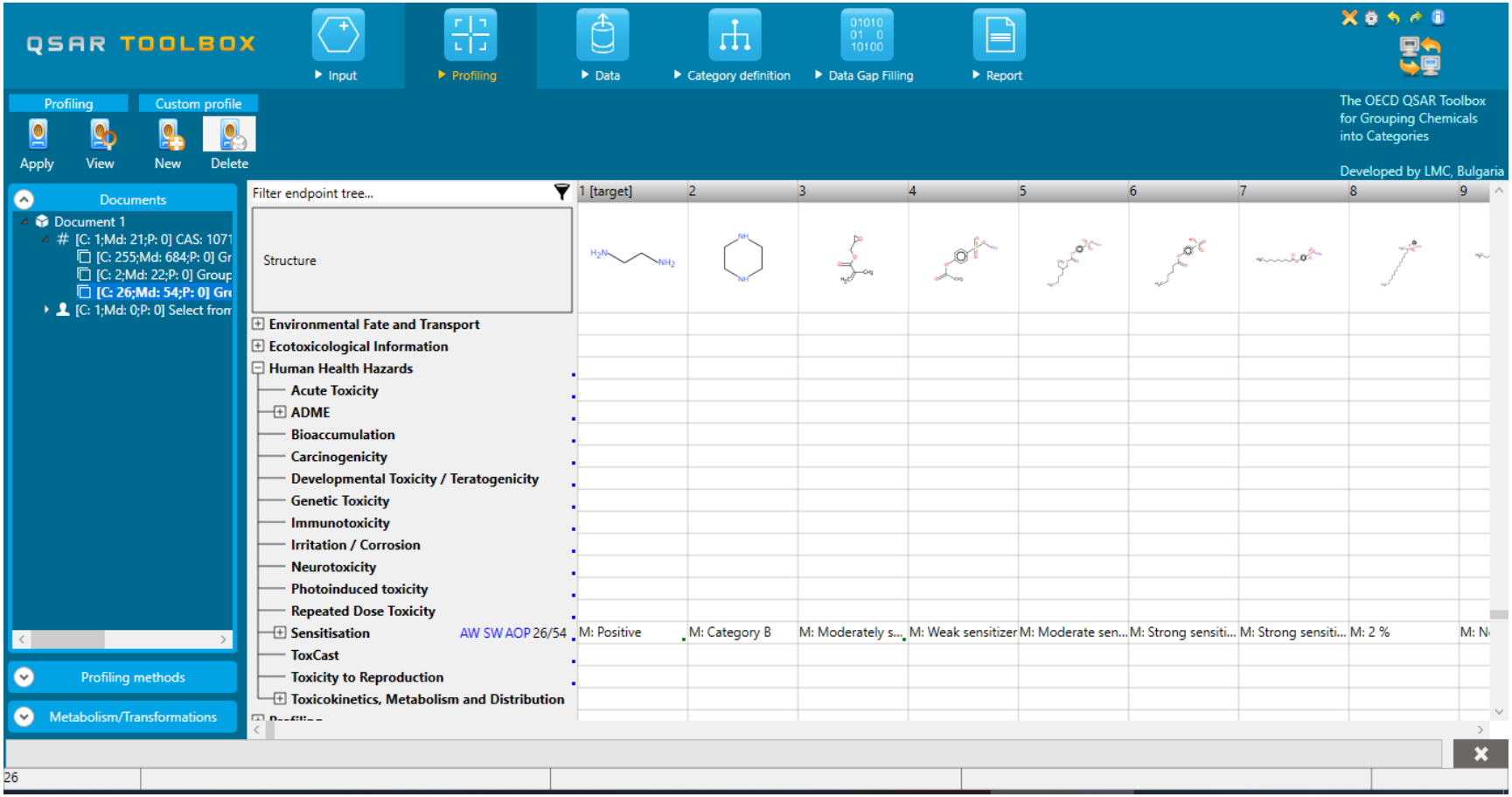
Note: The numbers of chemicals and data points depend on the database version you are working with

4. Click **OK**.

Category definition

Define with metabolism – Case 3

25 analogues are found in the selected database. Each of these structures posses metabolite(s) with logKow value in the previously defined range.



Category definition
Define with metabolism – Case 4

Case 4: Searching of analogues based on similar metabolites

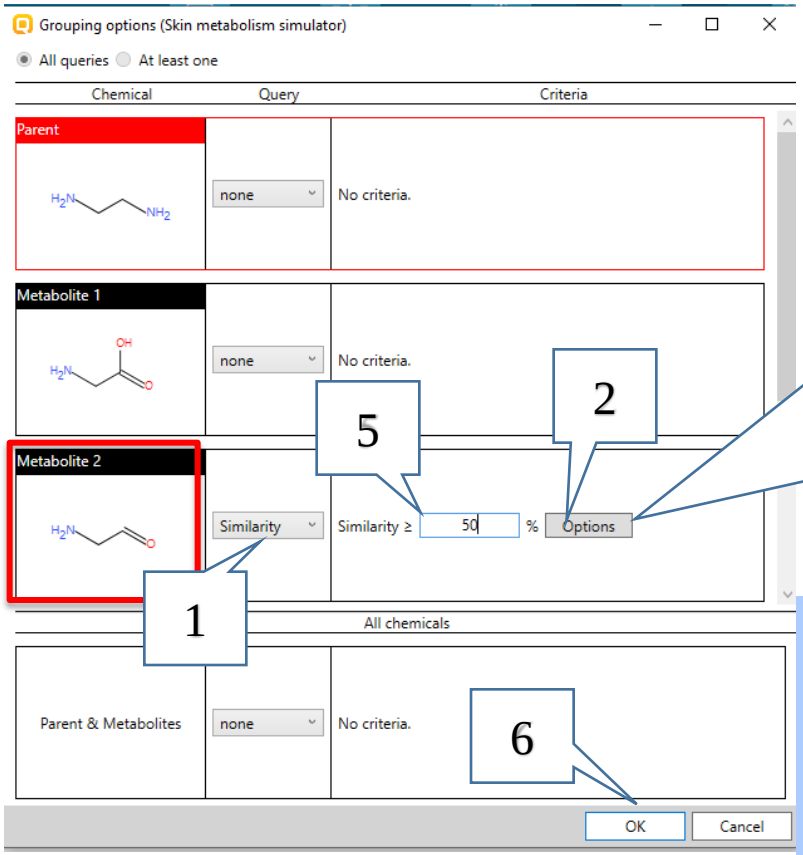
We can search for analogues of our target chemical, which have metabolites structurally similar to the target chemical metabolite(s).

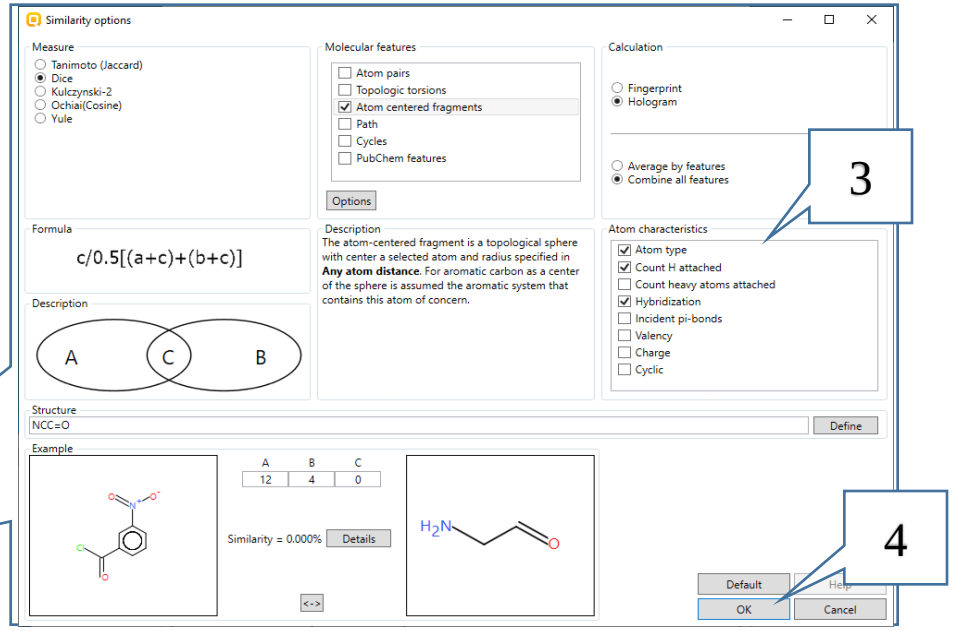
1. Select **structural** option from the drop-down menu for the target metabolite;
2. The default settings could be seen by click on the **Options** button.
3. Close the window by **X** button;
4. Define the similarity threshold.

See the illustrated steps on the next slide.

Category definition

Define with metabolism – Case 4



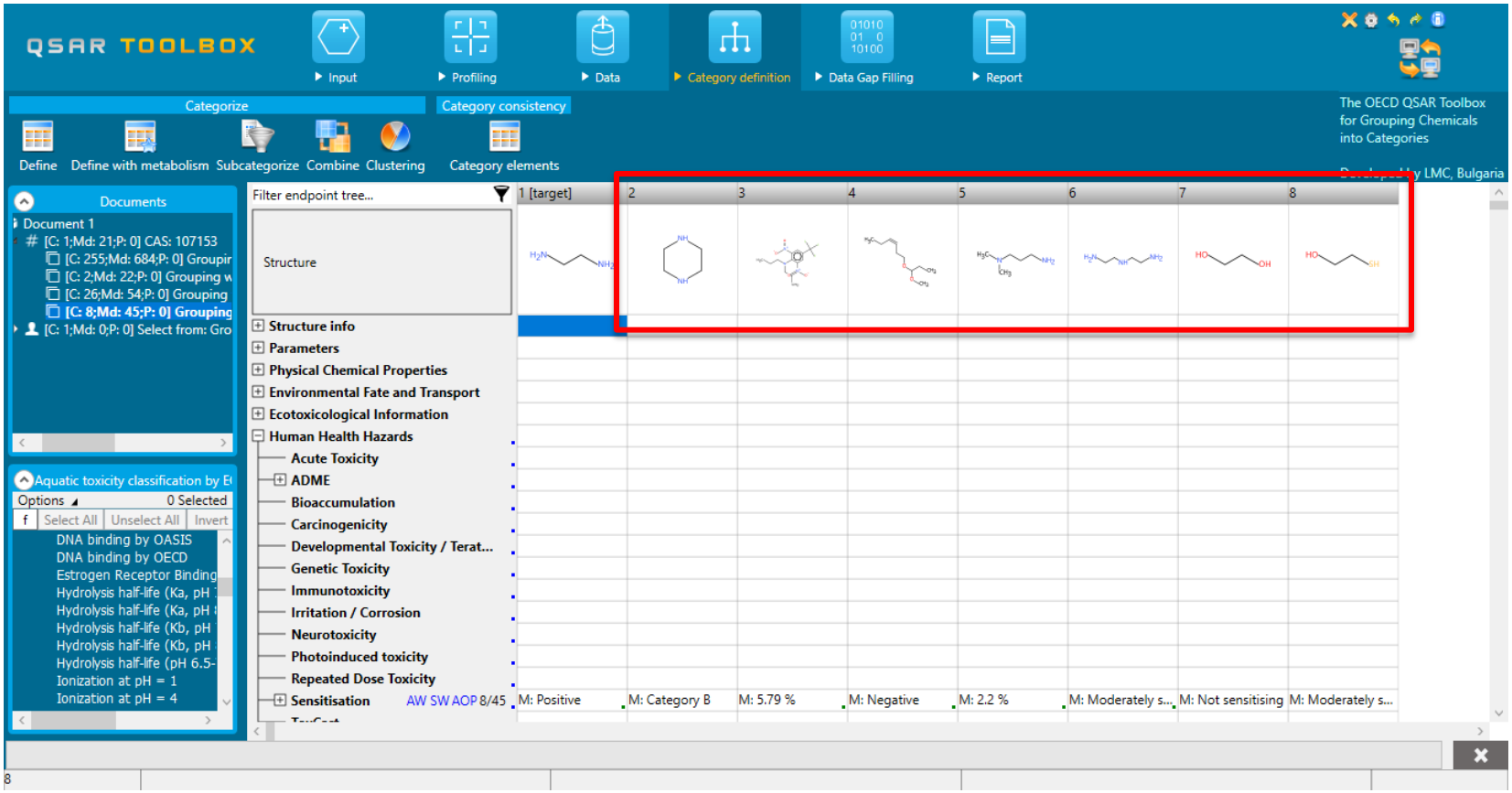


1. Select **Similarity** option from the drop-down menu next to the target metabolite;
2. The default settings could be seen by clicking **Options** button;
3. The settings can be modified by the user;
4. Click ***OK***;
5. Define the similarity threshold.
6. Click ***OK***.

Category definition

Define with metabolism – Case 4

7 analogues are found in the selected databases. Each of them posses metabolite(s) with a similarity of 50% or more to the structure of the previously selected metabolite.



Category definition
Define with metabolism – Case 5

Case 5: Searching for analogues based on defined criteria for the package "target and metabolites"

The user can select a profiling, parametric or structural query for both the target and its metabolites.

In this example we will use a defined profile to search for analogues.

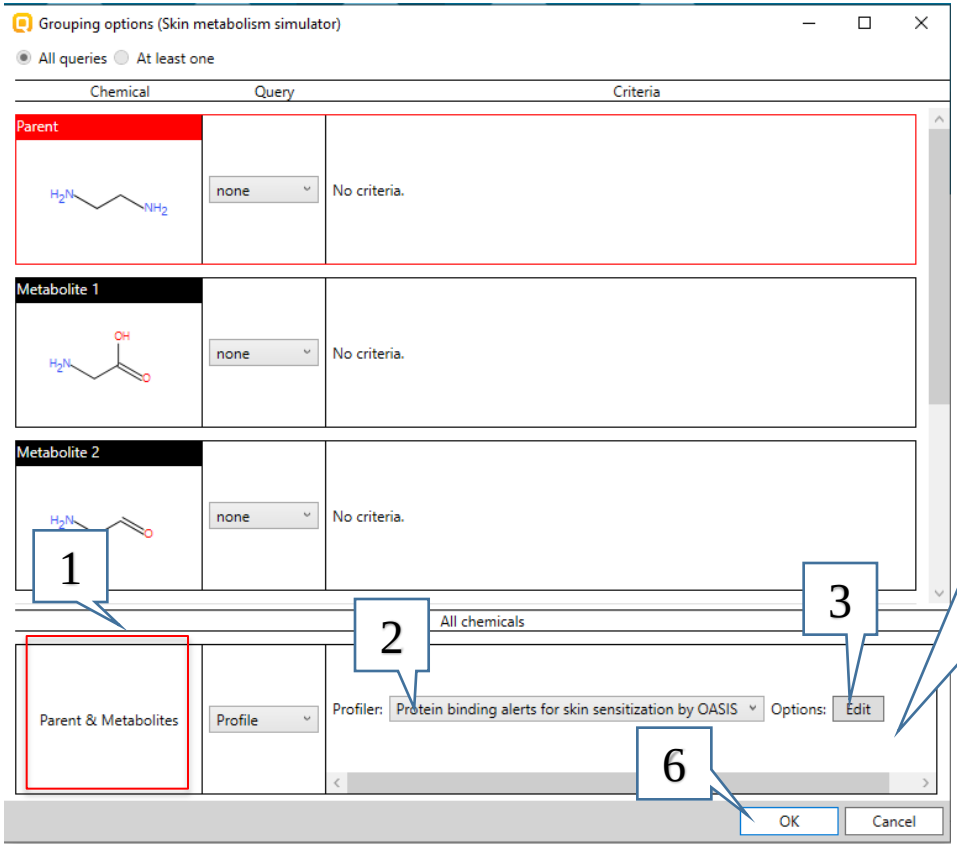
Category definition
Define with metabolism – Case 5

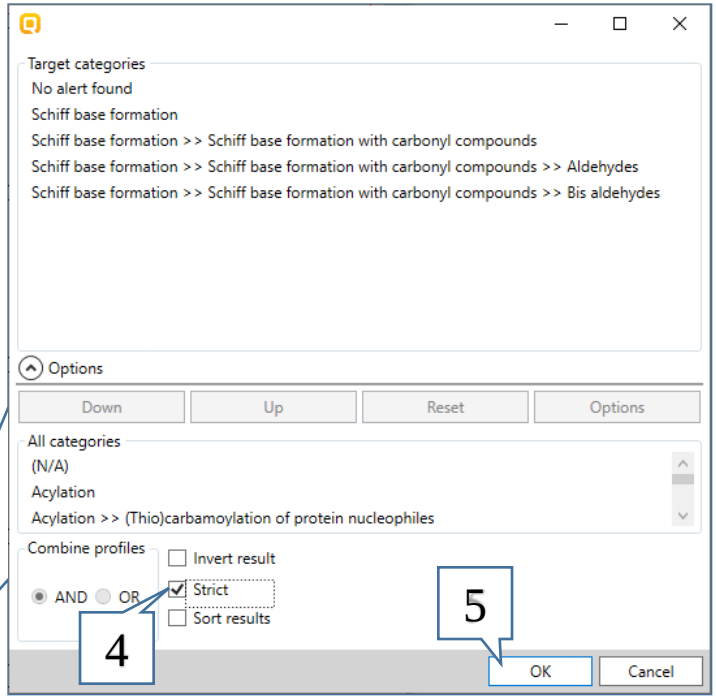
1. Select a profile option for the package "parent & metabolites";
2. Select *Protein binding alerts for SS by OASIS* profile;
3. Click on the **Edit** button. All found alerts in the parent structure and its metabolites are shown.
4. Check "**strict**" option to search only analogues with exact match these alerts.
5. Click on "**OK**" button to confirm the defined searching criteria.
6. Click on "**OK**" button in the general *Grouping options* window to execute the search.

See the illustrated steps on the next slide.

Category definition

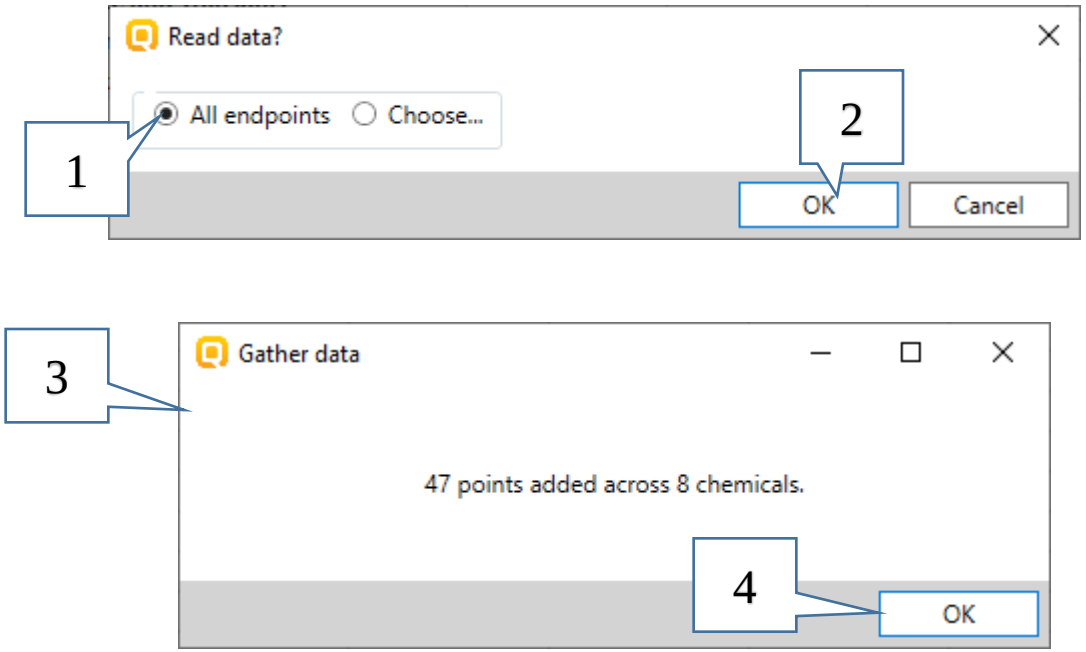
Define with metabolism – Case 5





Category definition

Define with metabolism – Case 5

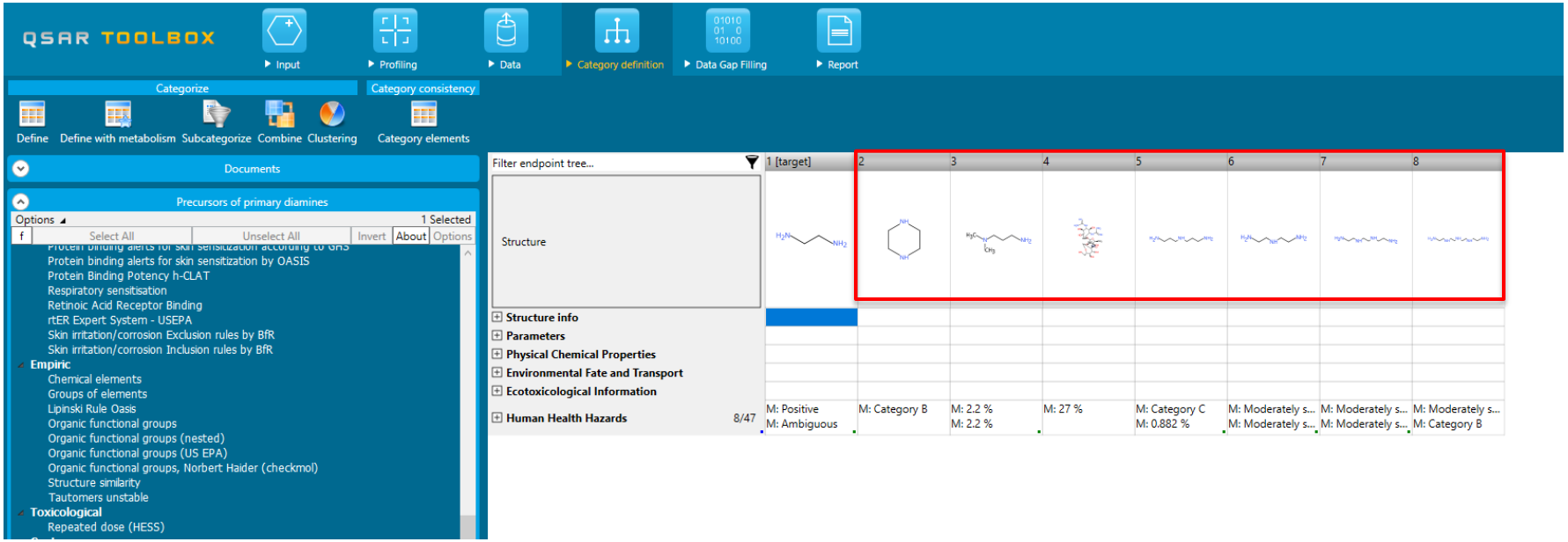


1. Keep **All endpoints** data checked;
2. Click **OK**;
3. A pop-up message is displayed where the number of chemicals (target chemical + analogues) and the total number of data points is shown;
Note: The numbers of chemicals and data points depend on the database version you are working with;
4. Click **OK** .

Category definition

Define with metabolism – Case 5

8 structures (along with the target) are retrieved. Only the searched structural alerts are found either in the parent structure or in the structures of its metabolites.

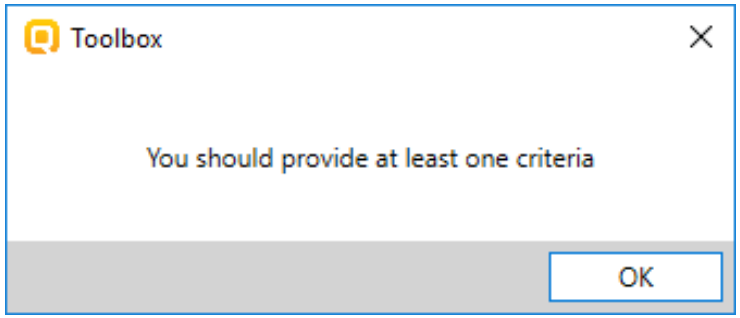


Category definition
Define with metabolism – Case 6

Case 6: Searching of analogues based on combination of queries

The user can search for analogues with combination of different criteria for each of the metabolites as well as the parent structure.

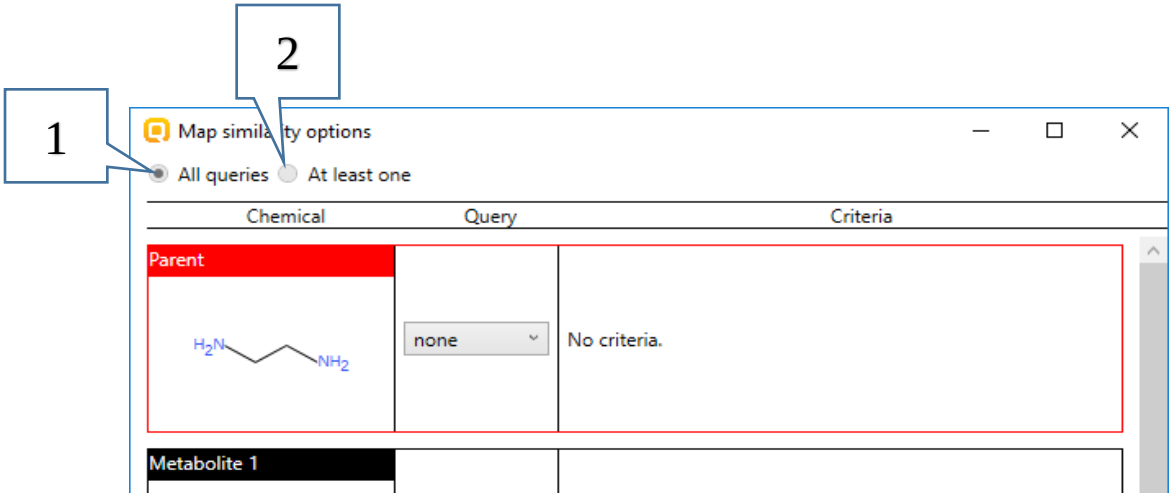
Note: The user can search for analogues based on any of the target characteristics (profiling result, parameter value or structural similarity) and at least one other criteria for a metabolite.
If a criteria is set for the parent structure only, information message will appear:



Category definition
Define with metabolism – Case 6

Case 6: Searching of analogues based on combination of queries

In addition the Toolbox user can choose whether the found analogues must meet all defined queries criteria (1) or at least one of them (2).



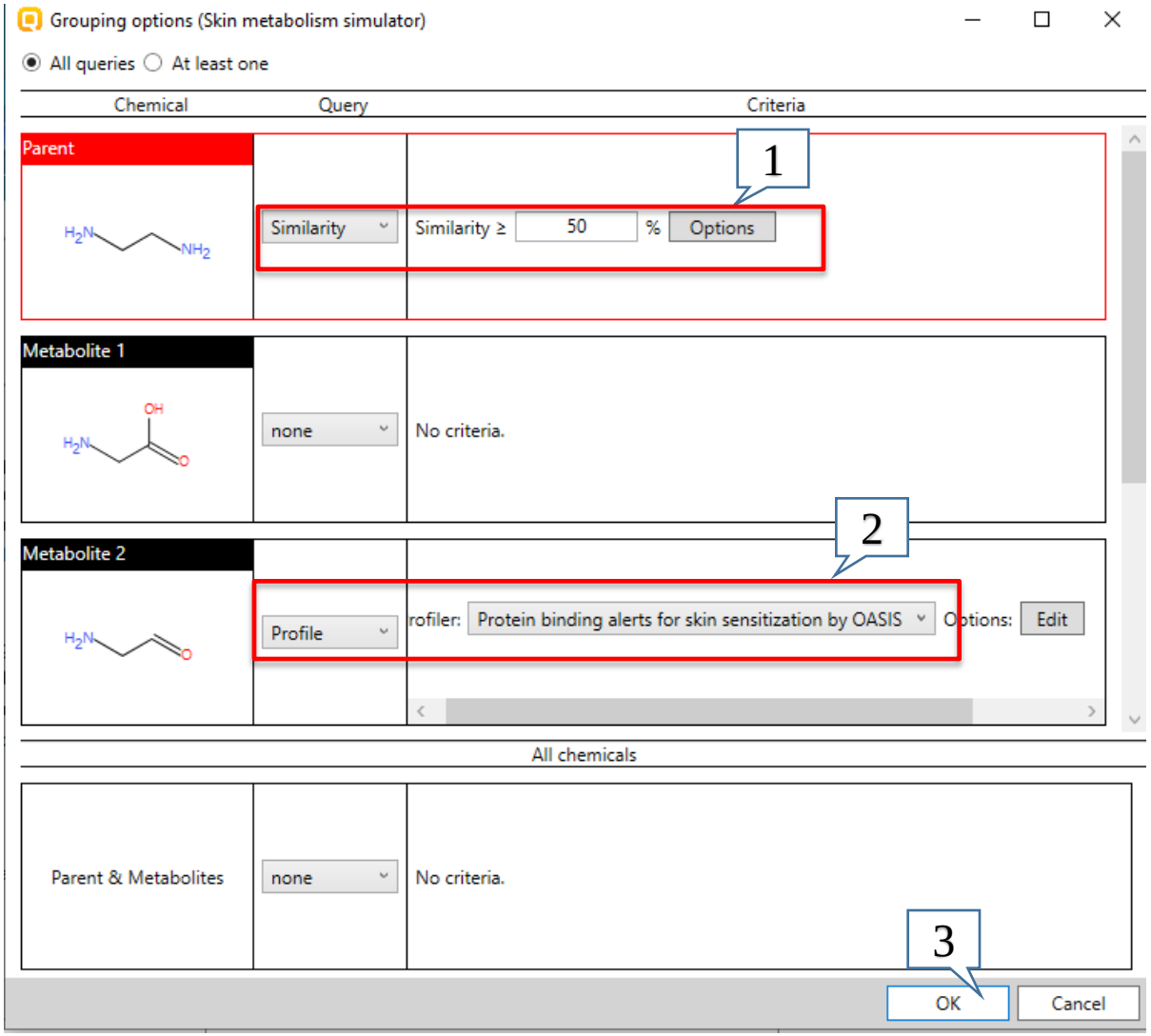
Category definition
Define with metabolism – Case 6

Now we will search for structures which have simultaneously:

- Parent structure similar to the target structure;
- Exact metabolite structure;
- Metabolite with defined profile.

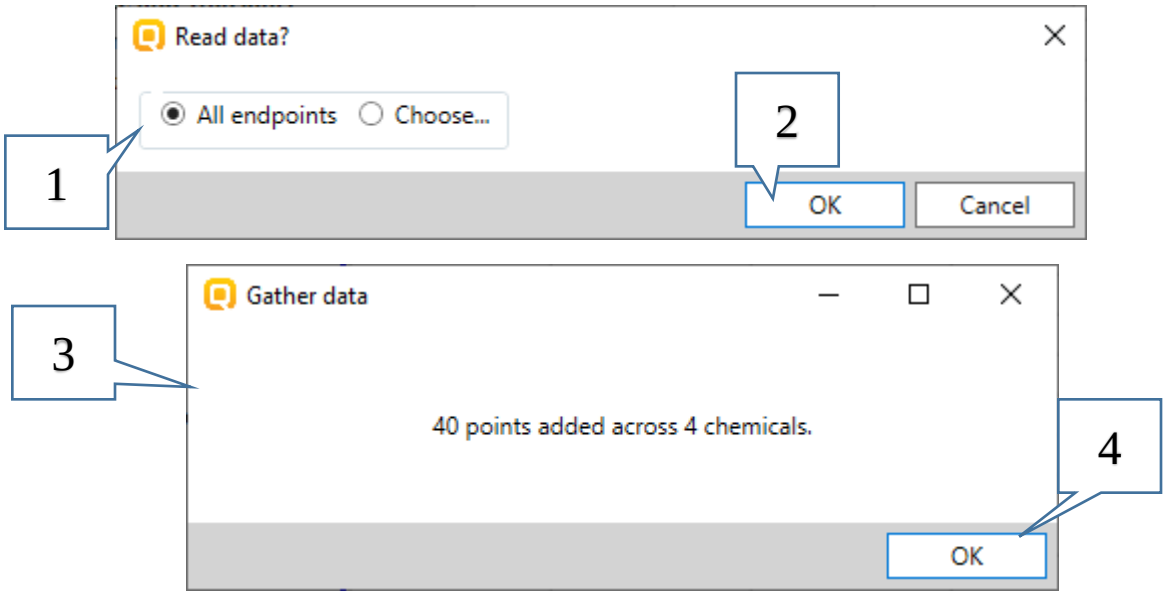
Category definition

Define with metabolism – Case 6



1. Select **structural** option from the drop-down menu for the parent; Set **50%** threshold with default options;
2. Select **profile** option and *Protein binding alerts for skin sensitization by OASIS profile* from the drop-down menus for the first metabolite.
3. Click **OK** to execute the search.

Category definition
Define with metabolism – Case 6

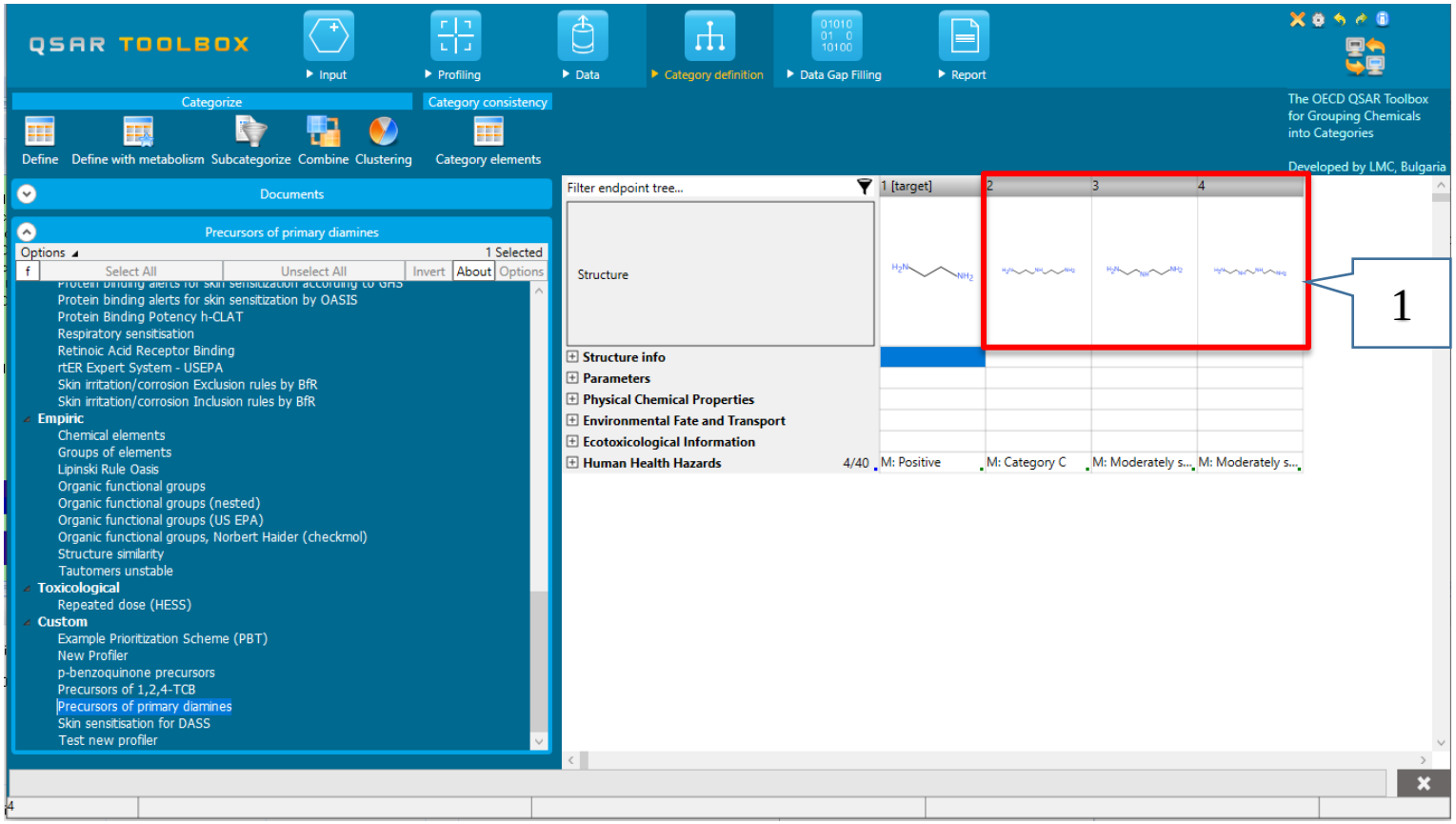


1. Keep **All endpoints** data checked;
2. Click **OK**;
3. A pop-up message is displayed where the number of chemicals (target chemical + analogues) and the total number of data points is shown;
Note: The numbers of chemicals and data points depend on the database version you are working with
4. Click **OK** .

Category definition

Define with metabolism – Case 6

Three analogues which fulfill all requirements are found (1).



Recap

In short, grouping with metabolism in Toolbox 4.4 allows finding analogues that have:

- metabolite categorized with a defined profiler
- the same metabolite as the target chemical
- metabolite with defined parameter value
- metabolite similar to a defined metabolite of the target chemical
- parent and its metabolites with defined profile, parameter value or structural similar
- combination of the above.

Congratulations!

- You have now been familiarized with different map similarity options for grouping with metabolism.

- Note, proficiency comes with practice!