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 predict the skin sensitisation potential of a chemical by read-across based on an analogue approach

Outlook

- **Background**
- Read across and analogue approach
- Keywords
- Objectives
- Specific Aims
- The exercise
- Workflow
- Save the prediction result

Background

- This is a step-by-step presentation designed to take the first-time user of the Toolbox through the workflow of a data filling exercise by read-across based on an analogue approach.

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- **Read-across and analogue approach**
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Read-across and Analogue Approach
Overview

- A read-across (RA) can be used to estimate missing data from a single or limited number of chemicals using an analogue approach. It is especially appropriate for "qualitative" endpoints for which a limited number of results are possible (e.g. positive, negative, equivocal).
- In the analogue approach, endpoint information for a single or small number of tested chemicals is used to predict the same endpoint for an untested chemical that is considered to be "similar".
- Analogous sets of chemicals are often selected based on the hypothesis that the toxicological effects of each member of the category will show a common behaviour.

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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 of the Toolbox:

- Identify analogues for a target chemical.
- Retrieve experimental results available for those analogues.
- Fill data gaps by read-across.

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Specific Aims

- To introduce to the first-time user the workflow of Toolbox.
- To familiarize the first-time user with the six modules of Toolbox.
- To familiarize the first-time user with the basic functionalities within each module.
- To explain to the first-time user the rationale behind each step of the exercise.

Outlook

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The Exercise

- In this exercise we will predict the skin sensitization potential (EC3 LLNA) for an untested compound, (4-nitrobenzoyl chloride) [CAS # 122-04-3], which will be the "target" chemical.
- This prediction will be accomplished by collecting a small set of test data for chemicals considered to be in the same category as the target molecule.
- The category will be defined by the mechanism of protein binding common to all the chemicals in the category.
- The prediction itself will be made by "read-across".

The Exercise
Theoretical considerations on Skin Sensitization

- Allergic contact dermatitis that results from skin sensitization is a significant health concern.
- Skin sensitization is a toxicological endpoint that is complex and conceptually difficult.
- Many organic chemicals have been shown to induce skin sensitization after covalent binding to skin proteins[1].
- Therefore, the mechanisms by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents.

[1] OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

Outlook

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- **Workflow**
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Workflow

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

Outlook

- Background
- Read across and analogue approach
- Objectives
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- The exercise
- **Workflow**
  - **Chemical Input**

Chemical Input
Overview

- This module provides the user with several means of entering the chemical of interest or the target chemical.
- Since all subsequent functions are based on chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Chemical Input

Ways of Entering a Chemical

User Alternatives for Chemical ID:

A. Single target chemical

- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing chemical structure
- Select from User List/Inventory/Databases
- Chemical IDs such as EC number, custom IDs
- Substructure search by using SMARTs

B. Group of chemicals

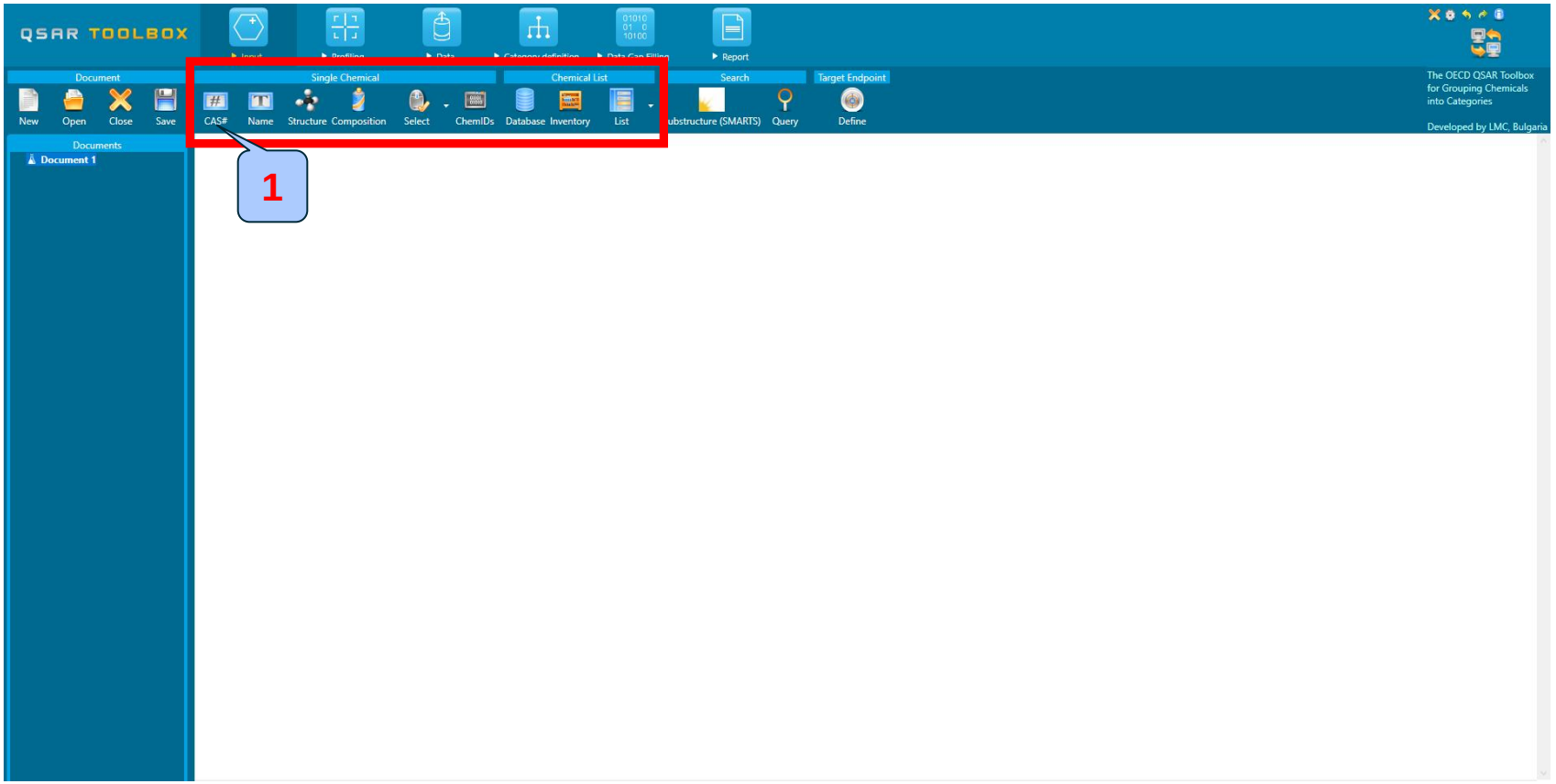
- User List/Inventory
- Specialized Databases

Getting Started

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

Chemical Input Screen

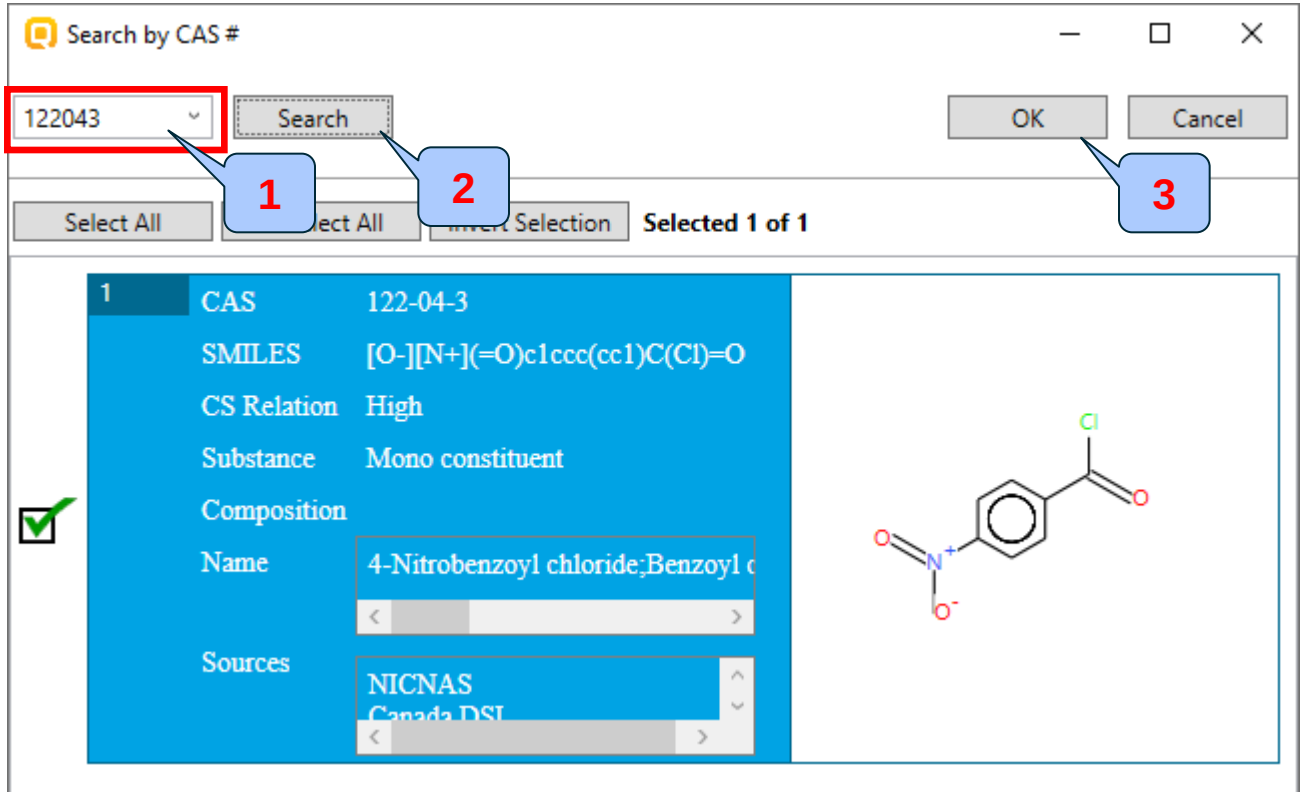
Input screen



1. Click on **CAS#**

Chemical Input Screen

Enter CAS# of 4-nitrobenzoyl chloride



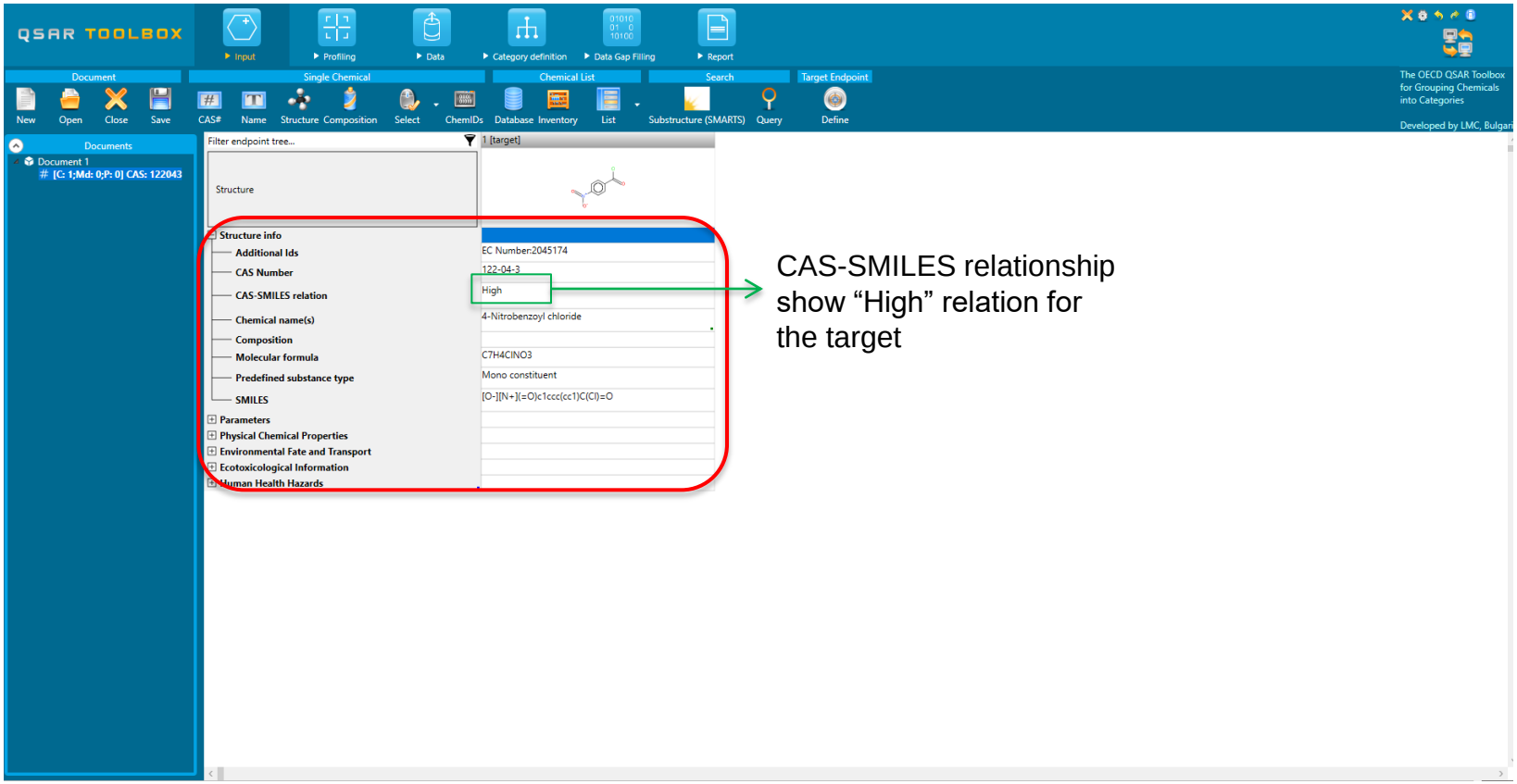
1. Enter the **CAS#** In the field; 2. Click **Search** button; 3. Press **OK**

Chemical Input
Target chemical identity

- Open "substance info" level to see the chemical ID information for the target chemical
- "CAS SMILES relation" displays the chemical identification information. This indicates the reliability of relation CAS-structure for the target chemical(see next 2 slides).
- The workflow on the first module is now complete, and the user can proceed to the next module.*

*For more details about the Input module, press F1 functionality

Chemical Input
Target chemical identity



CAS-SMILES relationship show “High” relation for the target

Chemical Input
Target chemical identity

The code indicates the reliability of the chemical identifier:

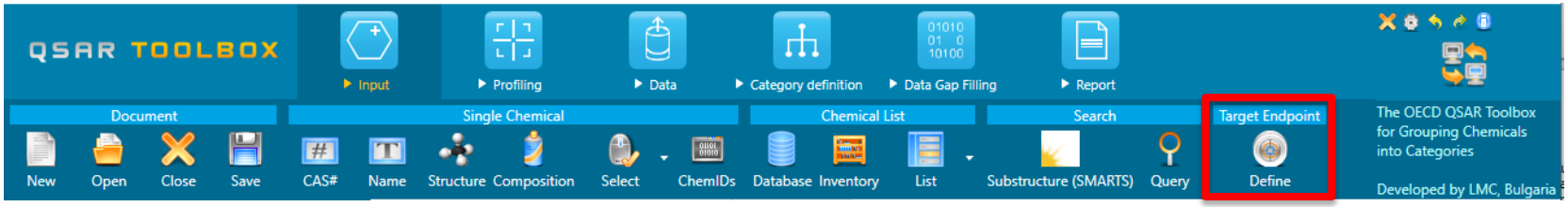
- **High:** This reliability corresponds to high reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to at least one high quality data source (database or inventory)

- **Moderate:** This reliability corresponds to moderate reliability of CAS-SMILES relation. The moderate label is assigned if the chemical belongs to three or more sources with unknown quality (marked with "Distribute to QA").

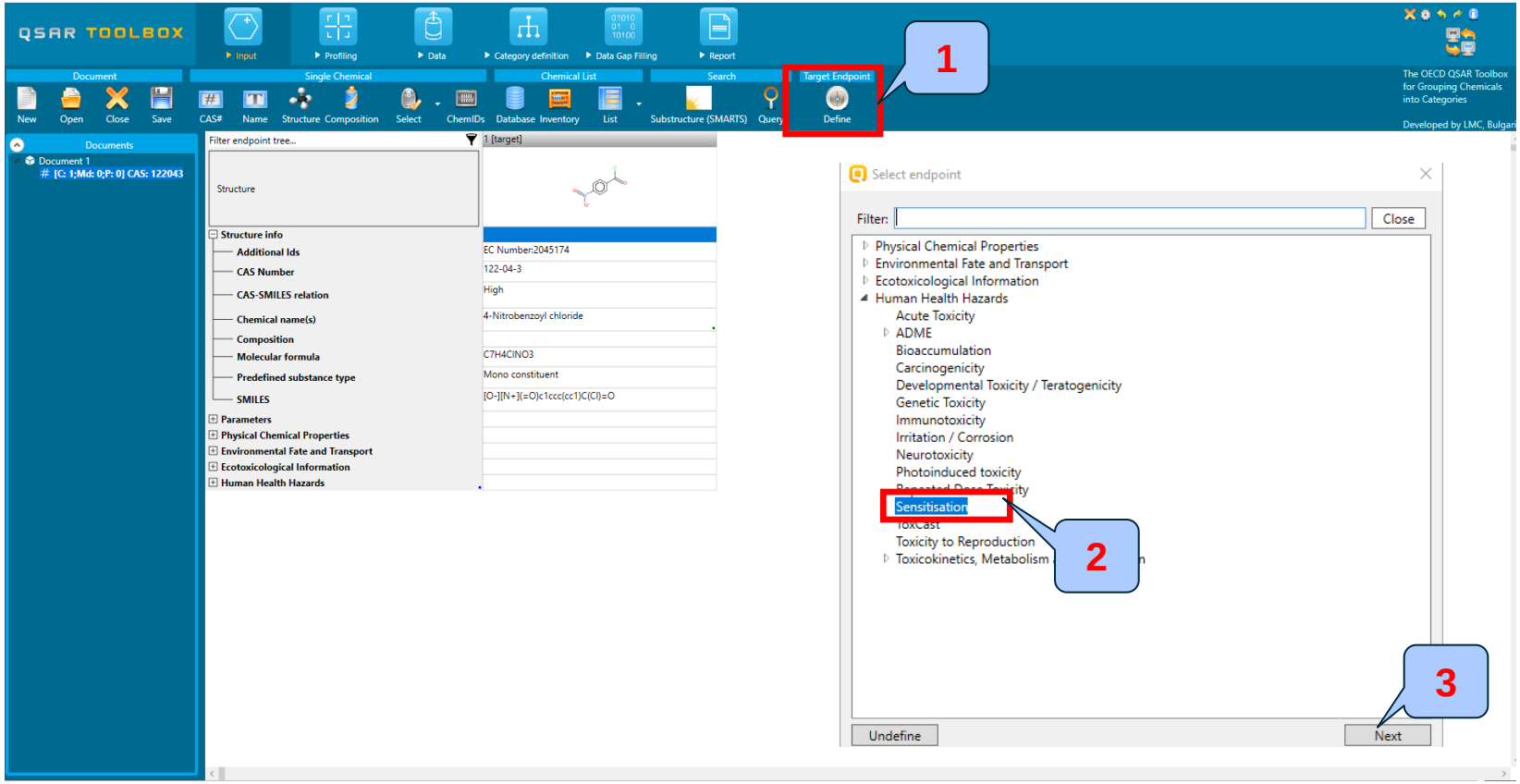
- **Low:** This reliability corresponds to poor reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to less than three, but at least one source with unknown quality ("Distribute to QA").

Chemical Input
Define target endpoint

Defining of the endpoint allows entering the endpoint of interest e.g. EC3, LC50, gene mutation etc., along with specific metadata information. Based on the defined metadata, relevancy of the profiles is provided expressed in different highlighting.

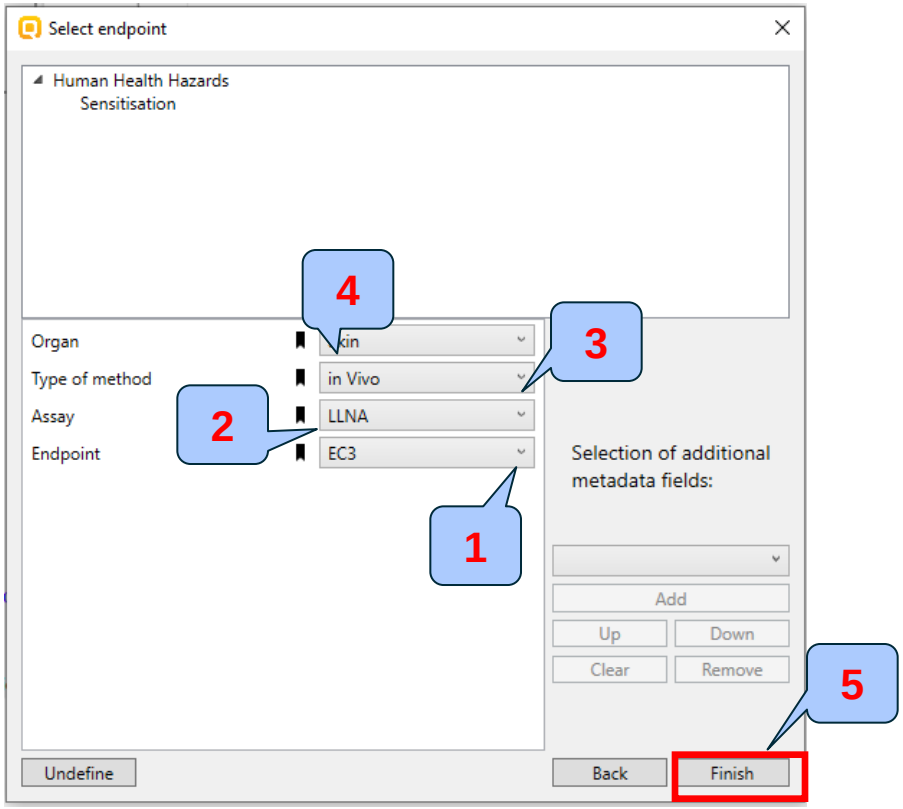


Chemical Input
Define target endpoint



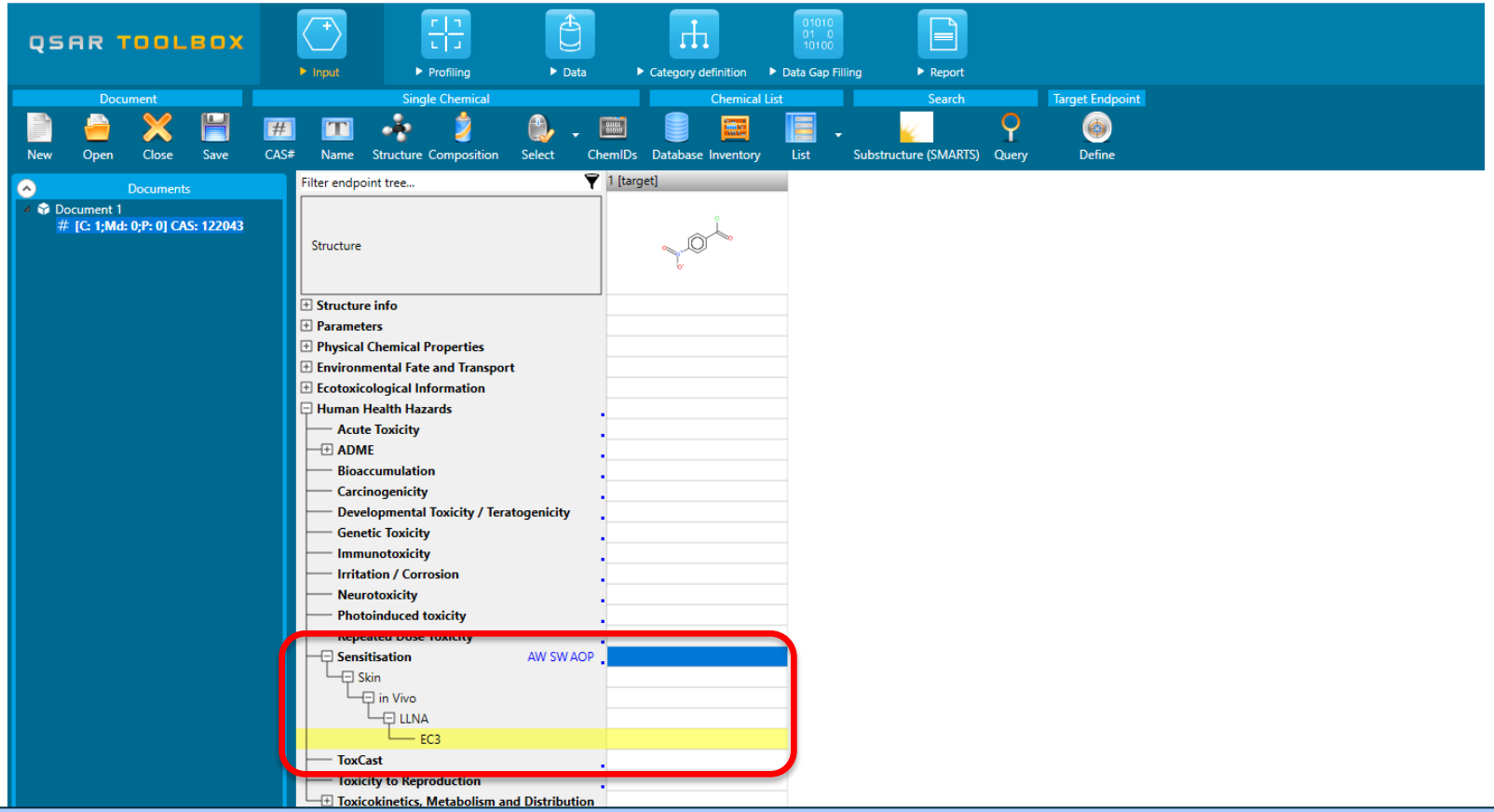
By clicking **Define** (1) you could select the target endpoint. Select **Sensitization** in the *Human health hazards* category (2) and click **Next** (3).

Chemical Input
Define target endpoint



1. First click on **Endpoint** and select EC3 from the drop-down menu, then consecutively select the following metadata:
2. Select **Assay** –LLNA;
3. Next **Type of method** "In Vivo";
4. Select **organ** "Skin".
5. Finally click on **Finish**

Chemical Input
Define target endpoint



The endpoint tree is automatically expanded to the level of the defined endpoint and the row is highlighted in yellow

Chemical Input
Input results

1) In module *Input*, you have entered the target chemical. The target has high relationship CAS-SMILES. So it is considered with good quality.
2) The target endpoint (EC3) is defined using "Define target endpoint" functionality.
3) Based on the defined target endpoint the relevant profiles and databases become highlighted in color (see next slides).

Outlook

- Background
- Read across and analogue approach
- Keywords
- Objectives
- Specific Aims
- The exercise
- **Workflow**
  - Chemical Input
  - **Profiling**

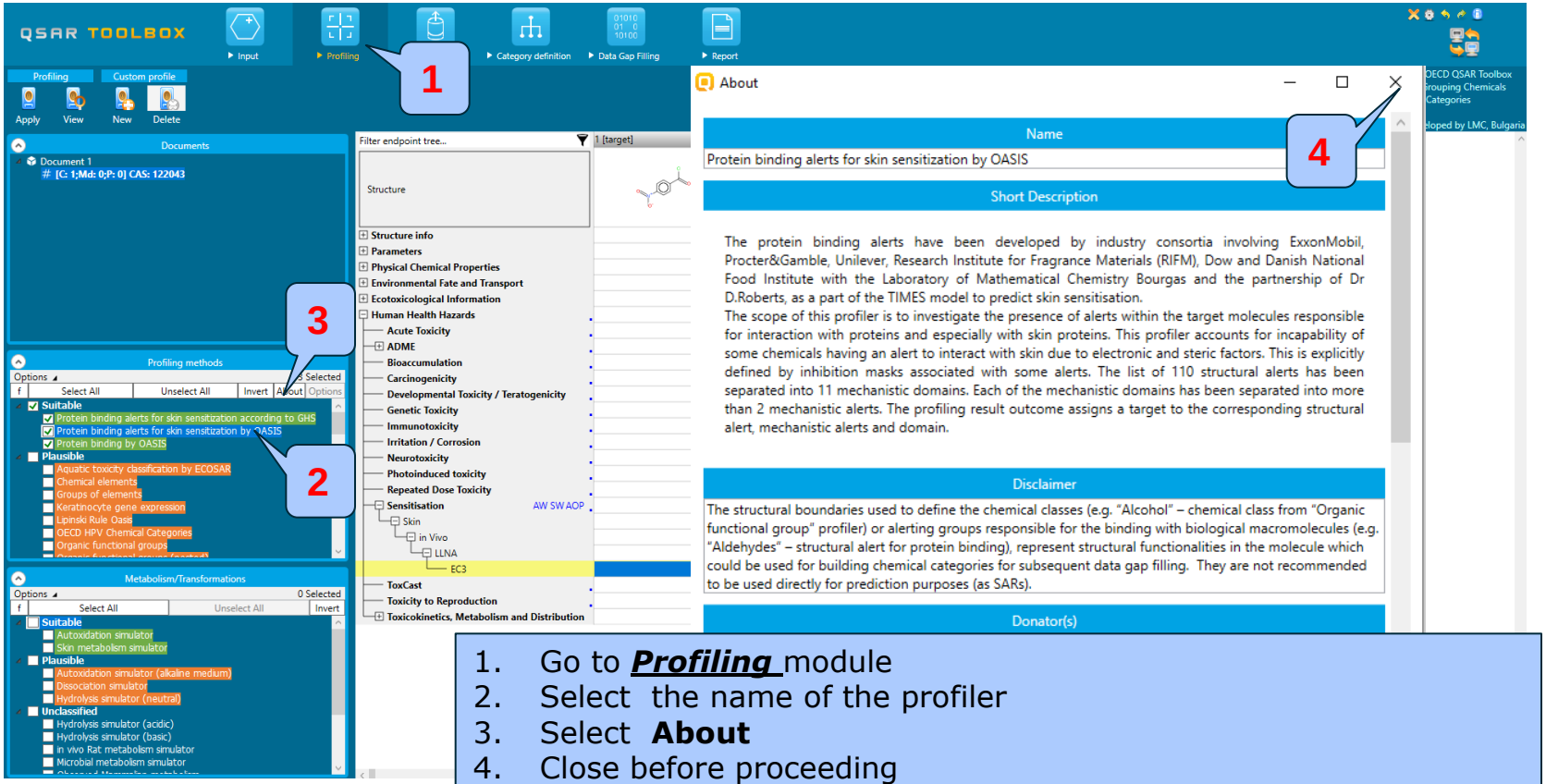
Profiling
Overview

- "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;
- "Profiling" module contains all the knowledge in the system coded in profiling schemes (profilers);
- "Profilers" are a collection of empirical and mechanism knowledge (expertly derived) which could be used to analyse the structural properties of chemicals;
- The "profilers" identify the affiliation of the target chemical(s) to preliminary defined categories (functional groups/alerts);
- The "Profiling" module contains also observed and simulated metabolisms/transformations, which could be used in combination with the profiling schemes;
- The outcome of the profiling determines the most appropriate way to search for analogues, but they are also useful for preliminary screening or prioritization of substances;
- The "profilers" are not (Q)SARs, i.e. they are not prediction models themselves;
- Based on the "profilers' relevancy" (determined by the defined target endpoint), the most suitable ones become highlighted in colour*.

*For more details regarding relevancy of the profilers see ppt: Example for predicting skin sensitization taking into account alert performance

Profiling
Background of profilers

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

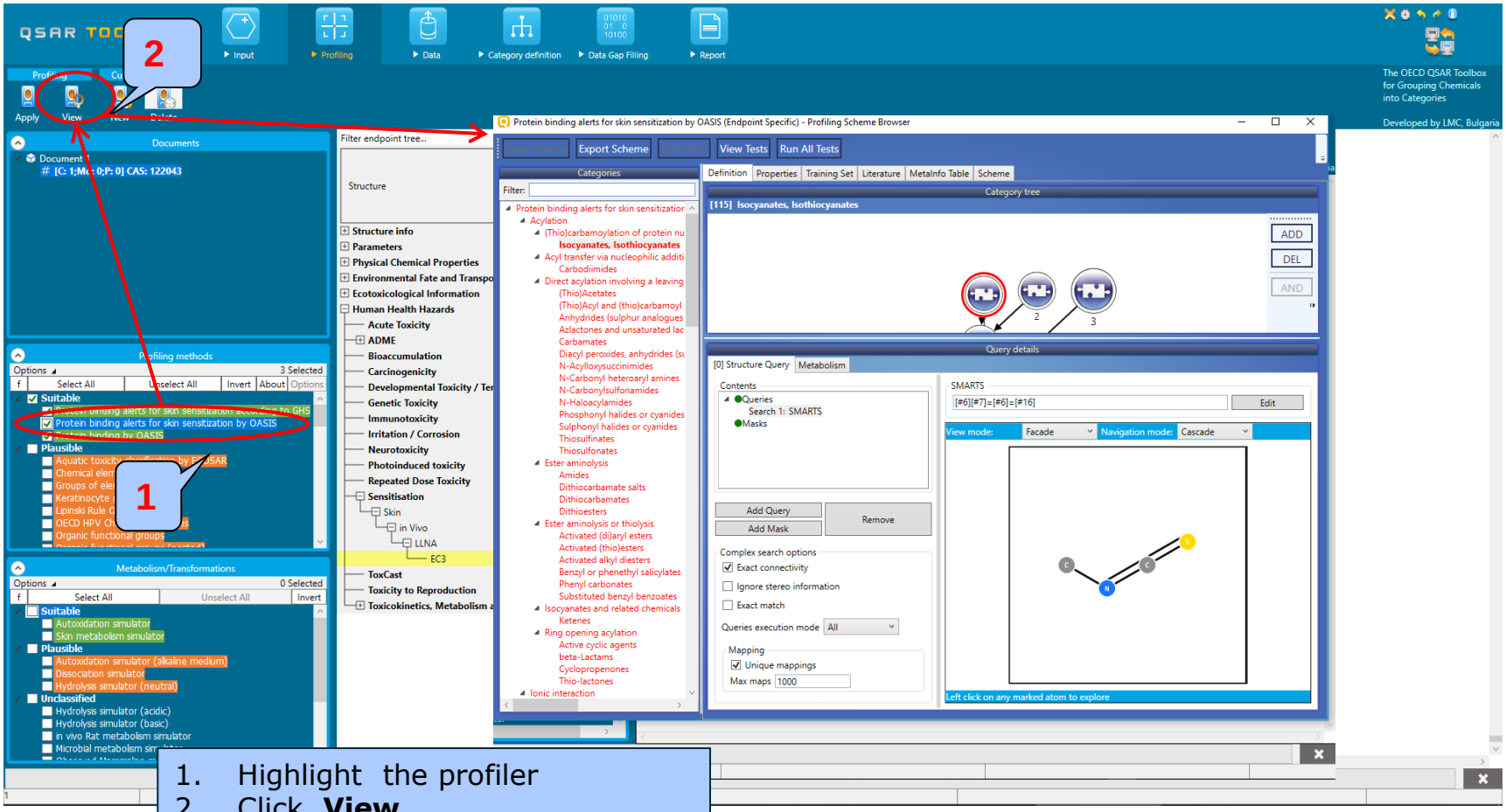


1. Go to ***Profiling*** module
2. Select the name of the profiler
3. Select **About**
4. Close before proceeding

Profiling
Background of profilers

- For most of the profilers, background information can be retrieved by highlighting one of the profilers (for example, Protein binding alerts for skin sensitization by OASIS) and clicking on “View” (see next screen shot).

Profiling
Background of profilers



1. Highlight the profiler
2. Click **View**

Profiling
Background of profilers

- The outcome of the profiling determines the most appropriate way to search for analogues;
- To help the user to choose the most appropriate profiling methods, the profilers are highlighted in different colors:
  - **in green** – the most **suitable** for the target endpoint profilers. These are the profilers developed using data/knowledge associated with the mechanisms conditioning the target endpoint (e.g. Protein binding alerts for skin sensitization by OASIS);
  - **in orange -** are indicated the **plausible profilers**. These are the profilers for which data/knowledge used for building them is known to be somehow related to the target endpoint, i.e. these which are not directly related to the target endpoint, but still could be used (e.g. Organic functional groups),
  - unclassified – these are profilers for which there is no evidence for the relation data/knowledge used for building them and the target endpoint.
- The profilers identifying structural groups are marked as plausible, while the profilers based on mechanistic knowledge are highlighted in green (see next few slides).

Profiling
Background of profilers

- The following profiling schemes are relevant to the Skin sensitization endpoint:
  - **Suitable profilers**
    - Protein binding by OASIS
    - Protein binding alerts for skin sensitization by OASIS
    - Protein binding alerts for skin sensitization according to GHS
  - **Plausible profilers**
    - Aquatic toxicity classification by ECOSAR*
    - Protein binding by OECD
    - Protein Binding Potency
    - OECD HPV Chemical Categories
    - Organic functional group
    - Organic functional group (nested)
    - ……

*ECOSAR is a rule-based profiler, which very well describe the functional groups present in the target molecules (It is a method for identifying chemical classes). Hence this method is one of the most robust of the mechanistic grouping method and it is often the method of choice for different hazards endpoints (e.g. acute aquatic toxicity and skin sensitization).

Profiling
Profiling the target chemical

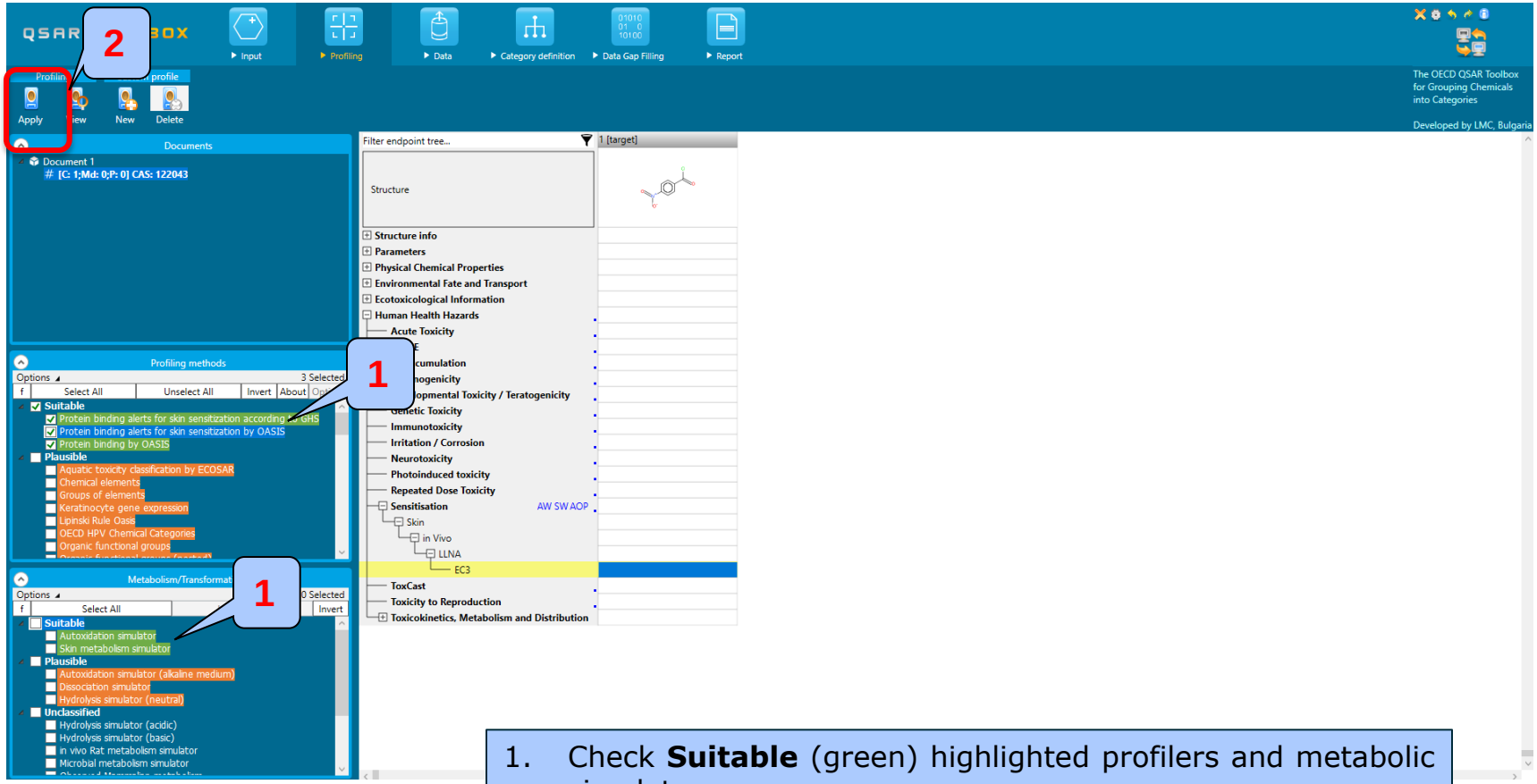
- **Tick the** box of the selected profiling methods related to the target endpoint.

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

- For this example, **tick** all the suitable profilers and simulators and **click** on apply (see next screen shot).

Profiling

Profiling the target chemical

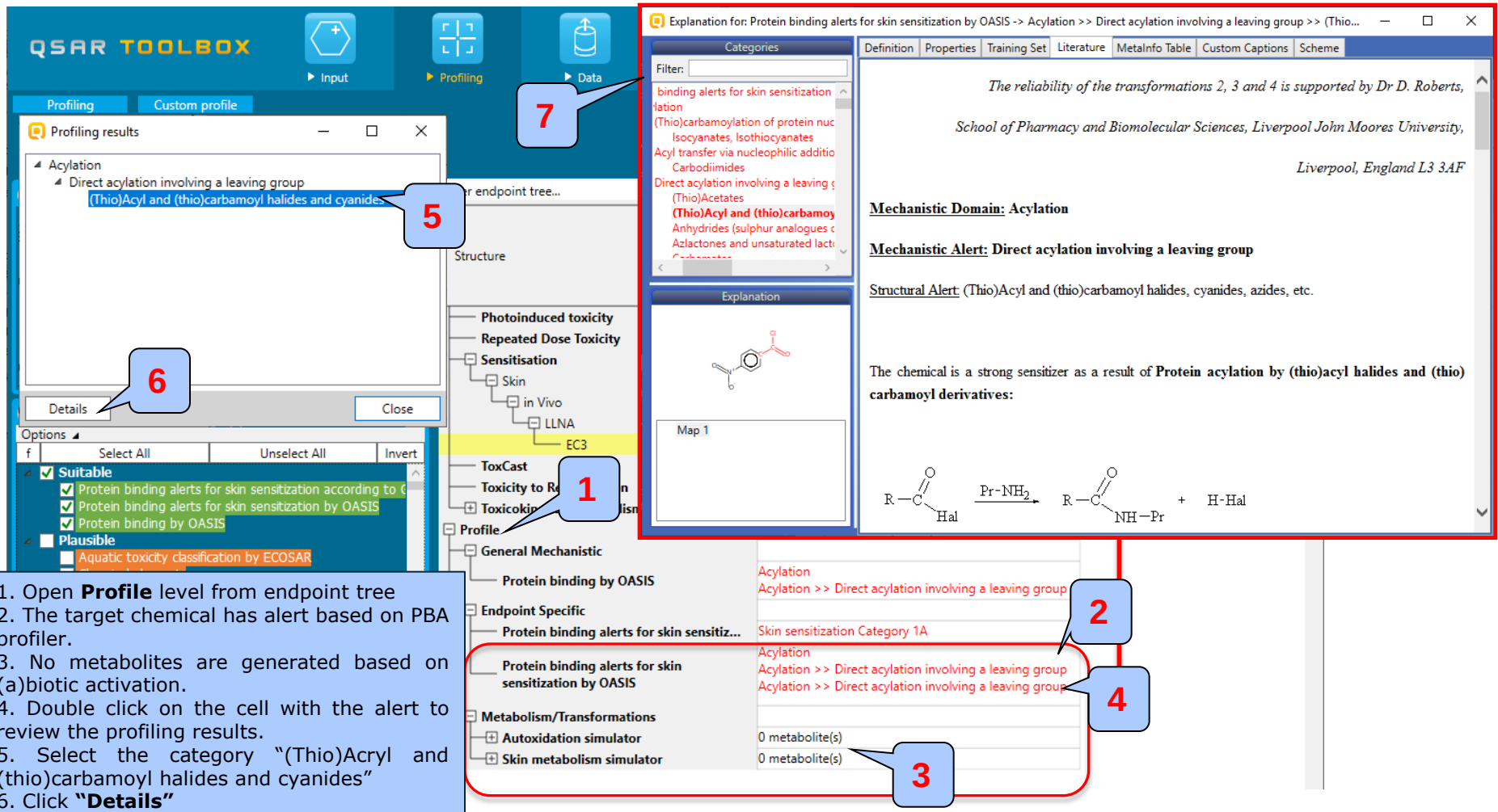


1. Check **Suitable** (green) highlighted profilers and metabolic simulators
2. Click **Apply**

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 protein-binding profiler – Protein binding alerts for SS by OASIS (PBA).
- This result will be used to search for suitable analogues in the next steps of the exercise.

Profiling
Profiling results



1. Open **Profile** level from endpoint tree
2. The target chemical has alert based on PBA profiler.
3. No metabolites are generated based on (a)biotic activation.
4. Double click on the cell with the alert to review the profiling results.
5. Select the category "(Thio)Acryl and (thio)carbamoyl halides and cyanides"
6. Click **"Details"**
7. A new window with literature info appears

Profiling
Profiling results

1) Protein binding alerts are identified in the target's structure as a parent;
2) No metabolites are generated as a a result of (a)biotic activation
3) Skin sensitization effect is expected for the target chemical as parent molecule

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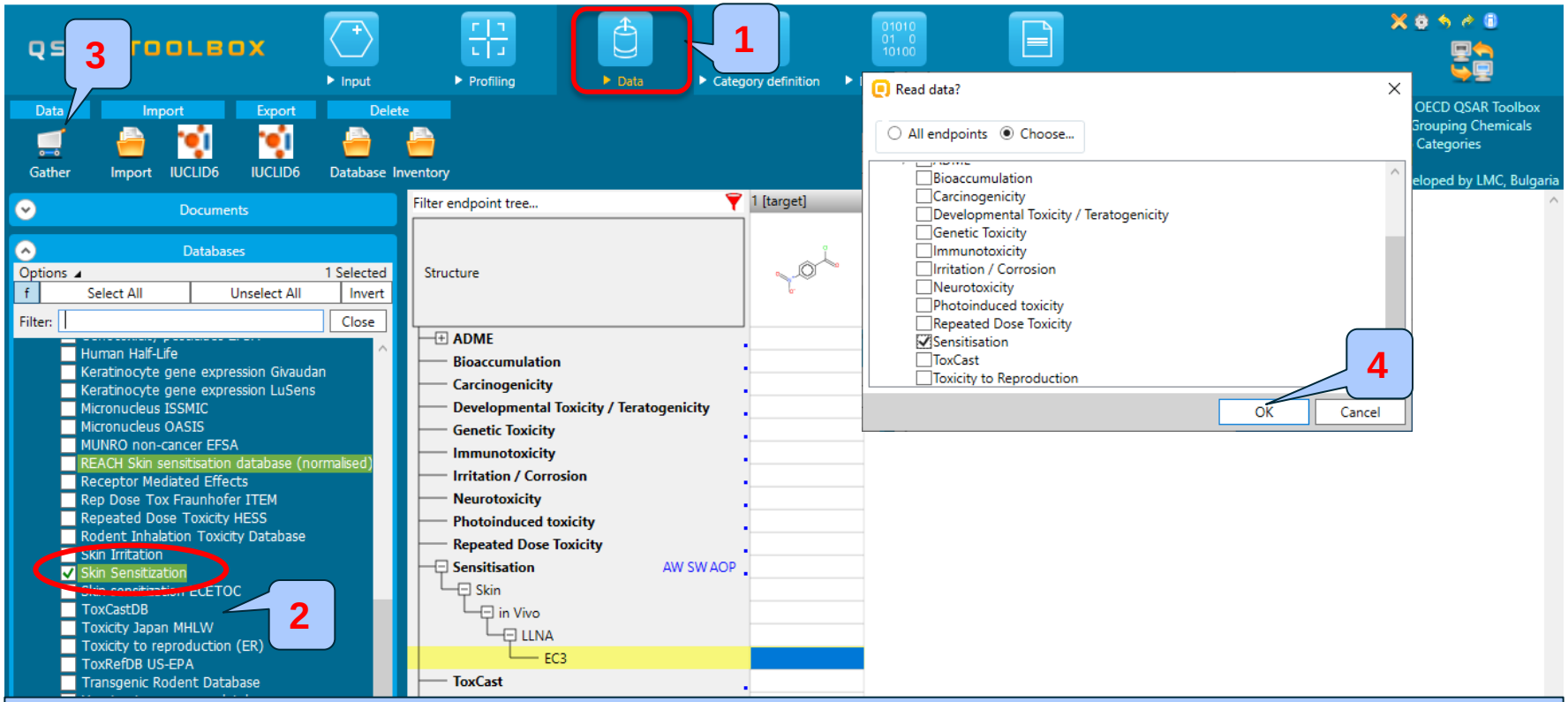
- **Workflow**
  - Chemical Input
  - Define target endpoint
  - Profiling
  - **Data**

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).
- Database "relevancy" is determined based on defined target endpoint (see next slide)

Data

Gather data for the target chemical



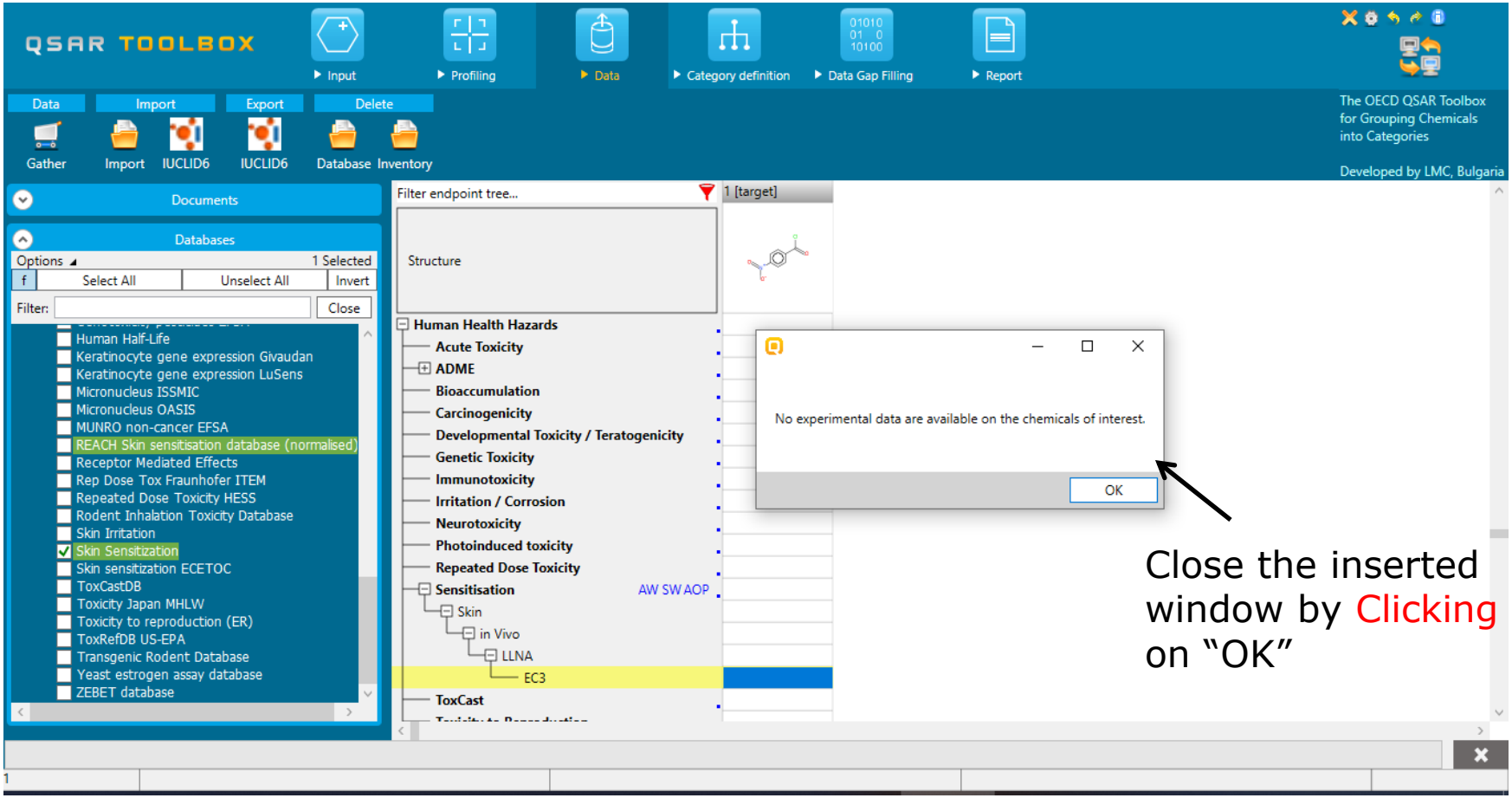
1. Go to ***Data*** module
2. There are three green highlighted databases (these are the databases containing data related to the defined endpoint). For the current case select only **Skin sensitization** database
3. Click **Gather**
4. Select only "Sensitisation" from the appeared window and click **OK**

Data
Gather data - background

- 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 is Skin sensitization.
- In this example, an insert window appears stating there was “no data found” for the target chemical (see next screen shot).

Data

Gather data for the target chemical



Recap

- In module one, you have entered the target chemical CAS RN in order to retrieve the correct structure.
- In the second module, you have profiled the target chemical.
- In the third module, you have found that no experimental data is currently available in the Toolbox for this structure.
- In other words, you have identified a data gap which you would like to fill.
- Click on "Category Definition" to move to the next module.

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  - Define target endpoint
  - 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.

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.
- For example, starting from a target chemical for which a specific protein binding mechanism is identified, analogues can be found which can bind to the proteins by the same mechanism and for which experimental results are available.

Category Definition

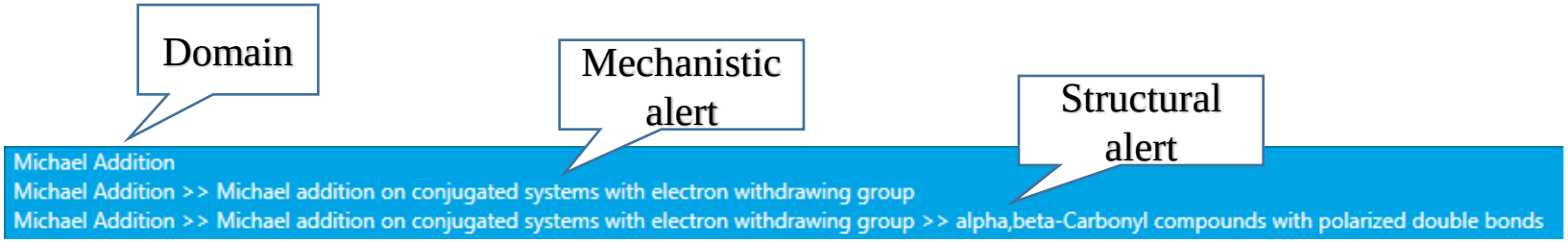
Protein binding alerts for skin sensitization by OASIS grouping method

- This is one of the best grouping methods in the Toolbox. It is built on conventional organic chemical reactions and as such is qualitative in character.
- This method is particularly relevant for respiratory and skin sensitization and acute aquatic toxicity, but also for chromosomal aberration and acute inhalation toxicity.

Category Definition

Background to Protein binding alerts for skin sensitization by OASIS categorization

- This scheme includes 110 categories organized in three level of information:
  - ✓ Level I: Mechanistic Domains (11 categories)
  - ✓ Level II: Mechanistic alerts associated to each mechanistic domain are created on the basis of a common reactive centre being activated by a number of substituents (50 categories)
  - ✓ Level III: A number of structural alerts specifying the substituents to a common reactive centre are made up for each mechanistic alert (112 categories)



Category Definition

Background to Protein binding alerts for skin sensitization by OASIS categorization

- Each category from level III is presented by a defined 2-dimensional structural alerts that is responsible for eliciting the toxic effect, such as skin sensitization which is a result of protein binding.

- The associated chemical reactions are in accordance with existing knowledge on electrophilic interaction mechanisms of various structural functionalities.

Category Definition

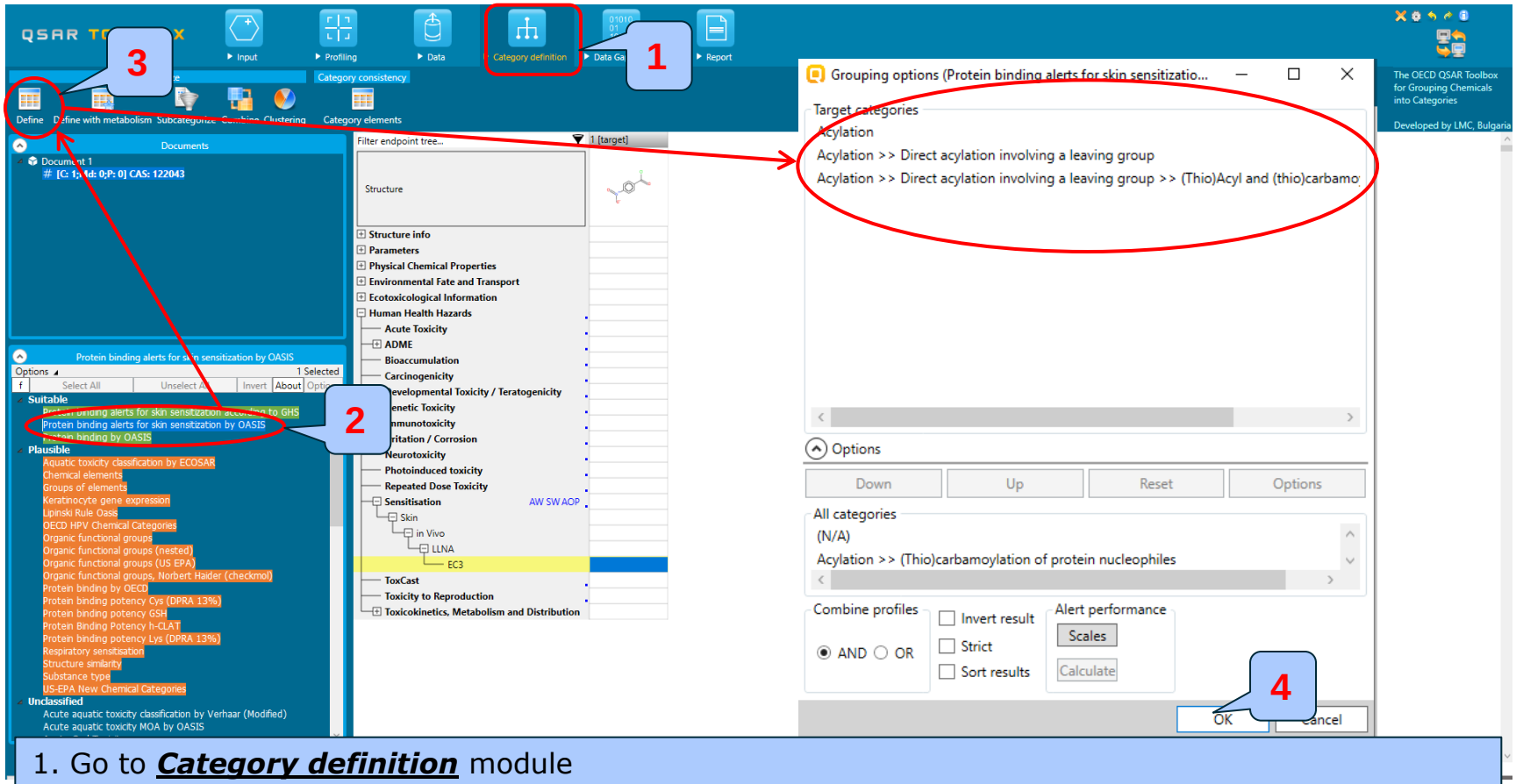
Background to Protein binding alerts for skin sensitization by OASIS categorization

- There is an agreement that many organic chemicals induce skin sensitization after covalent binding to skin proteins[1].
- Therefore, chemical reactions by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents. So you have mechanistic plausibility for defining your category based on similar protein-binding mechanism.

[1] OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

Category Definition

Defining Protein binding alerts for Skin sensitization by OASIS



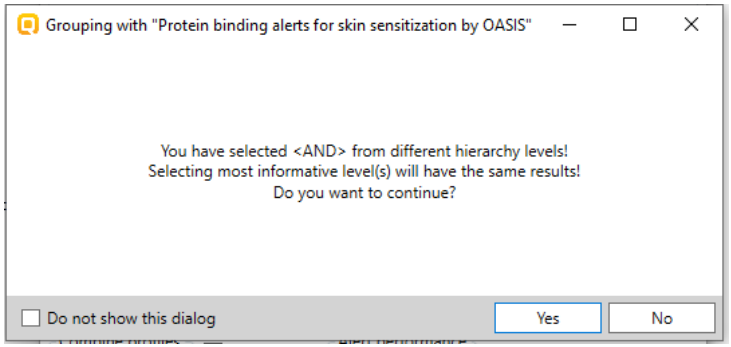
1. Go to ***Category definition*** module
2. Select the **"Protein binding alerts for skin sensitization by OASIS"**;
3. Click **Define**;
4. Click **OK** to confirm the defined categories for the target chemical

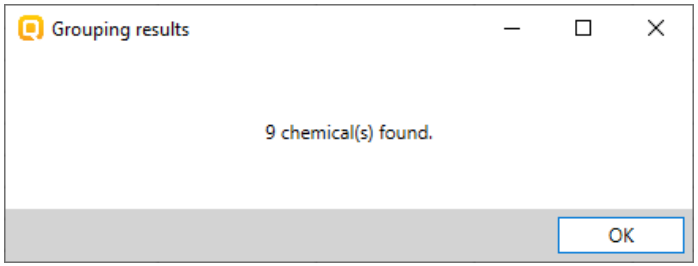
Category Definition
Analogues

- The data is automatically collected.

- Based on the defined category (Acylation<AND>Acylation >> Direct acylation involving a leaving group<AND>Acylation >> Direct acylation involving a leaving group >> (Thio)Acyl and (thio)carbamoyl halides and cyanides) 9 analogues have been identified

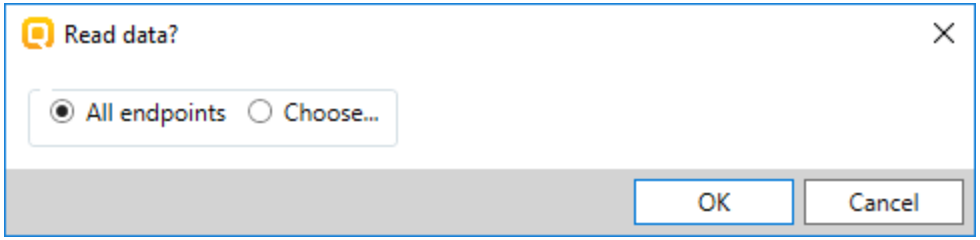
- In other words, these 9 compounds along with the target chemical form a category (see below), which can be used for data filling (see next slides).





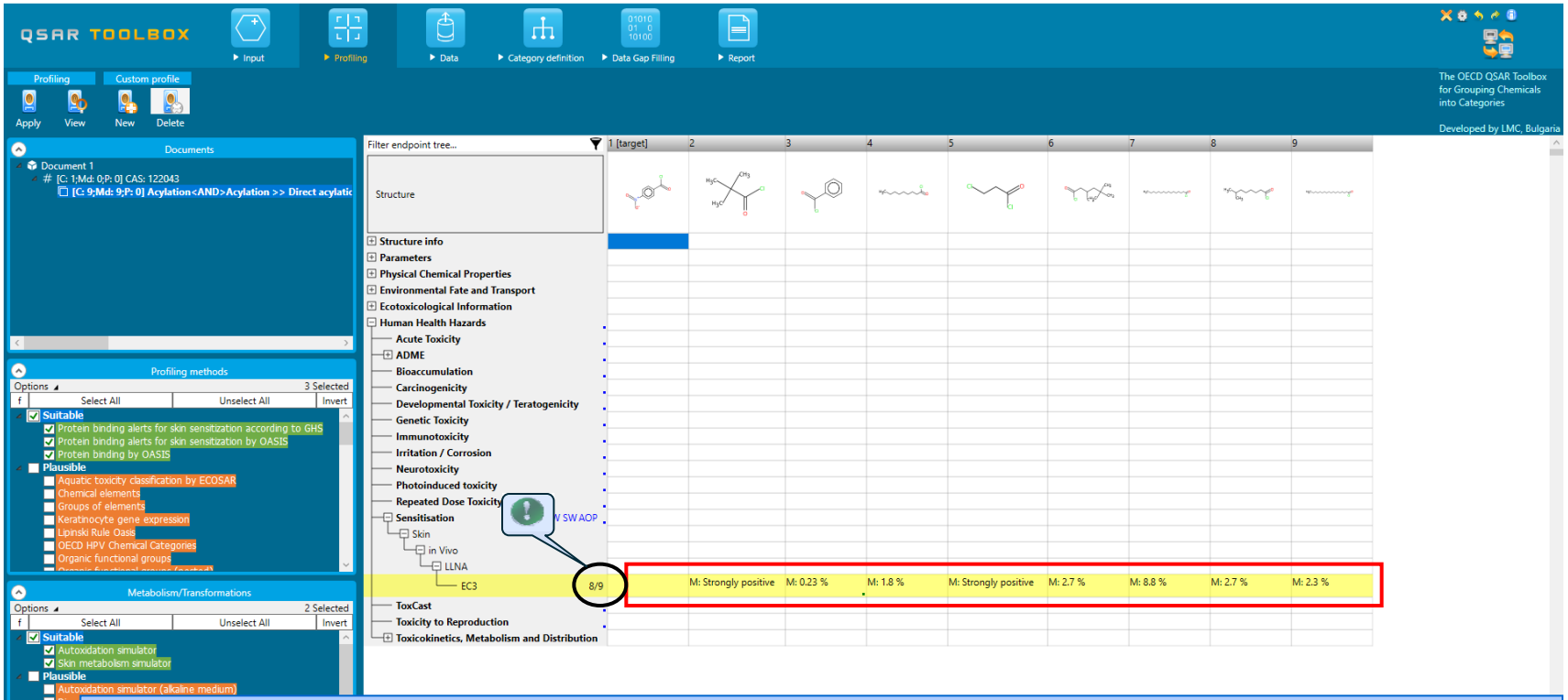
Category Definition
Read data for Analogues

- The Toolbox automatically requests 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, because only databases that contain information for skin sensitization endpoint are selected, both options give the same results.



Category Definition
Summary information for Analogues

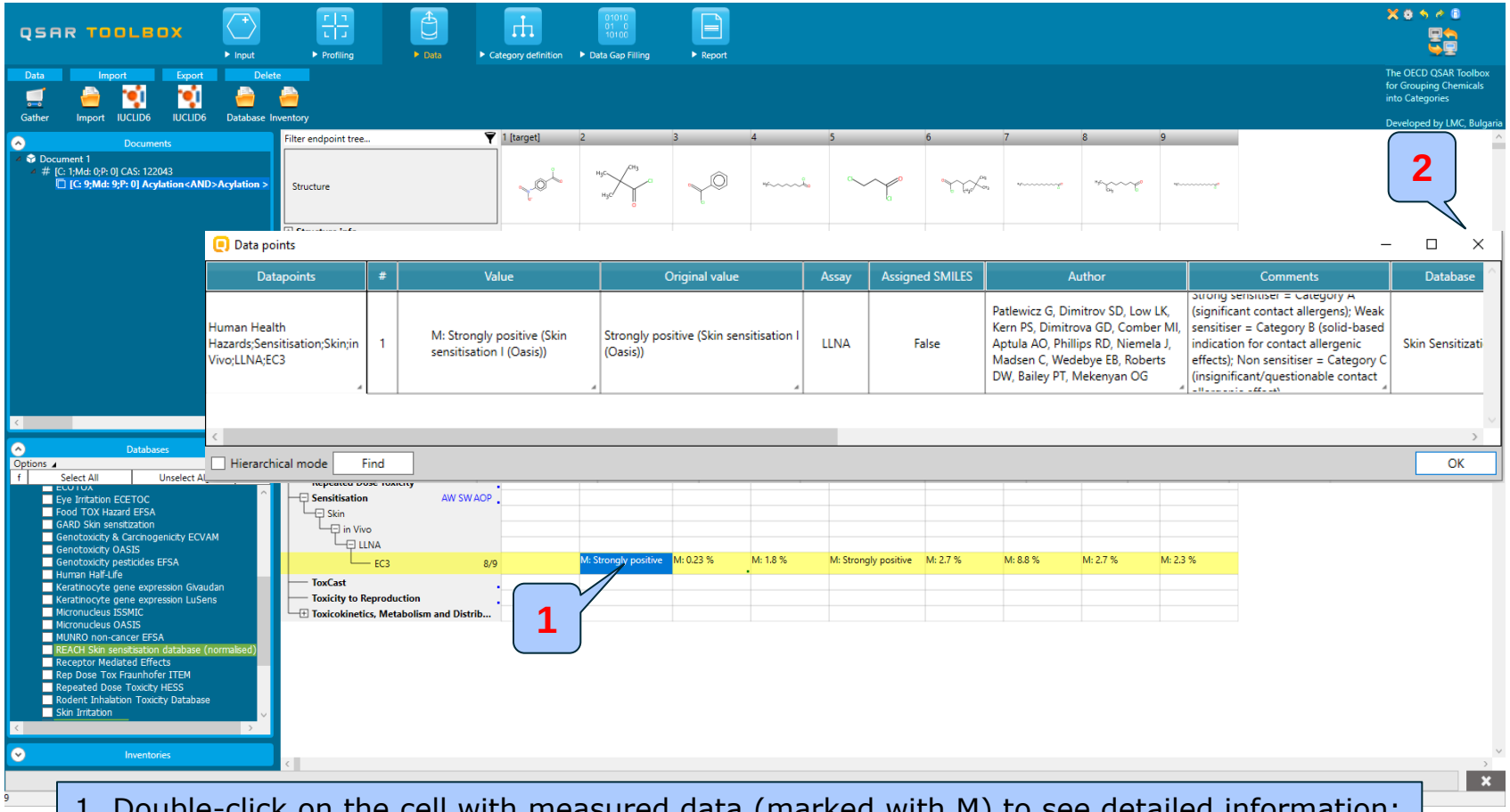
- The experimental results for the analogues are inserted into the matrix



Chemical statistics presenting the number of chemicals and the available experimental data for them. In this case for 8 out of 9 analogues forming the category there are 9 data points, which means that for some chemicals there is more than one data point.

Category Definition

Side bar of experimental data



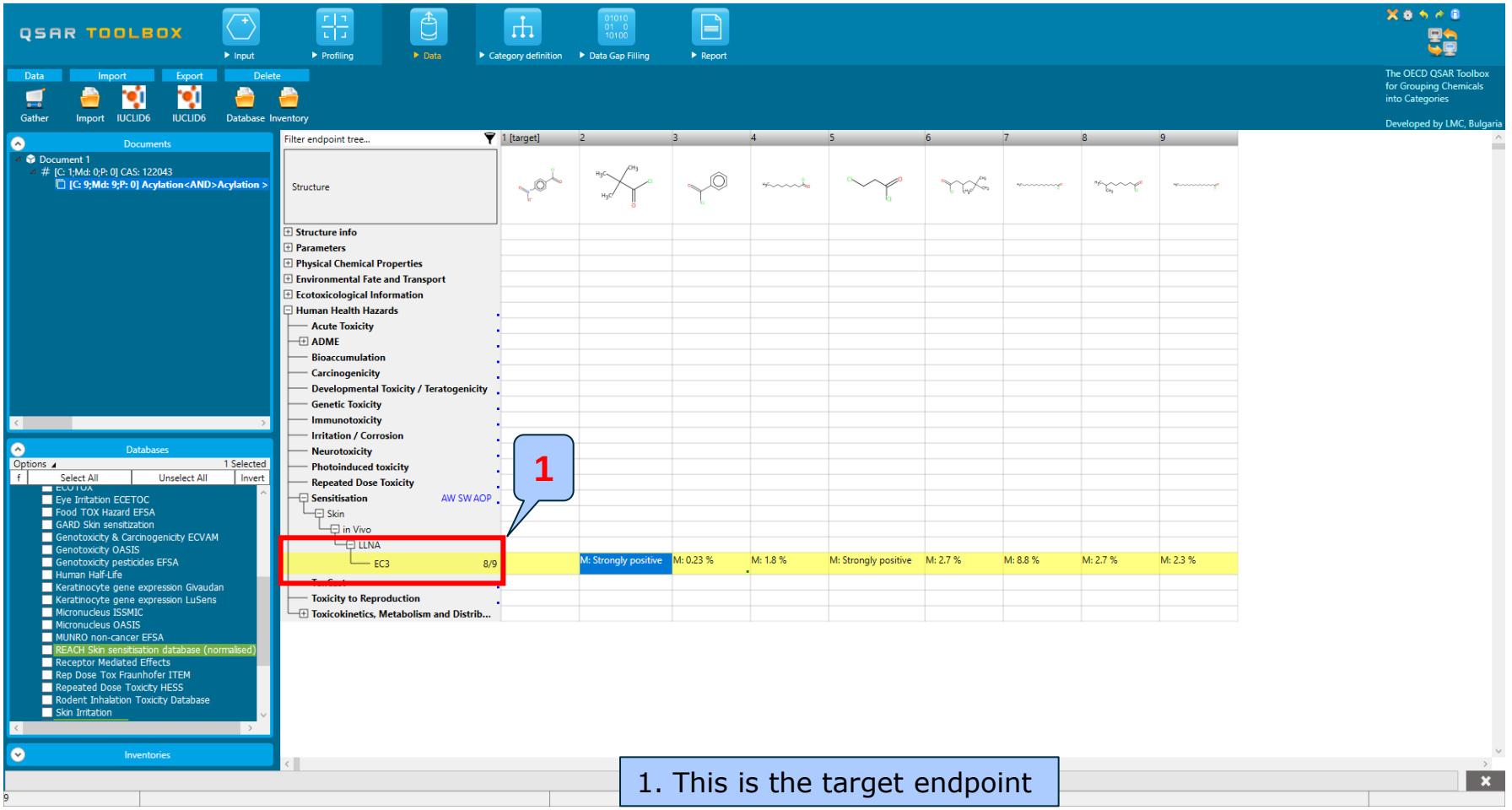
1. Double-click on the cell with measured data (marked with M) to see detailed information;
2. Click on the X to close the appeared window.

Category Definition
Navigation through the endpoint tree

- The user can navigate through the endpoint tree by closing or opening the nodes of the endpoint tree.
- Click on the plus sign next to Human Health Hazards then Sensitisation, followed by Skin, In Vivo and LLNA and finally EC3.
- The local lymph node assay is an *in vivo* method for testing of relative skin sensitization potential of chemicals. The potential is expressed as EC3 values.
- In this example, results from skin sensitisation testing for chemicals reacting via nucleophilic substitution of acyl halides are available (see next screenshot).

Category Definition

Navigation through the endpoint tree



1. This is the target endpoint

Category definition
Recap

- You have identified a mechanistic category (Acylation<AND>Acylation >> Direct acylation involving a leaving group<AND>Acylation >> Direct acylation involving a leaving group >> (Thio)Acyl and (thio)carbamoyl halides and cyanides) for the target chemical (4-nitrobenzoyl chloride);

- You have now retrieved in the available experimental results on skin sensitisation (EC3) values for 8 analogues chemicals with the same mechanism of protein binding as the target compound, which were found in the “Skin Sensitisation” databases;

- The user can now proceed to the next module; click on ”Data Gap Filling”.

Outlook

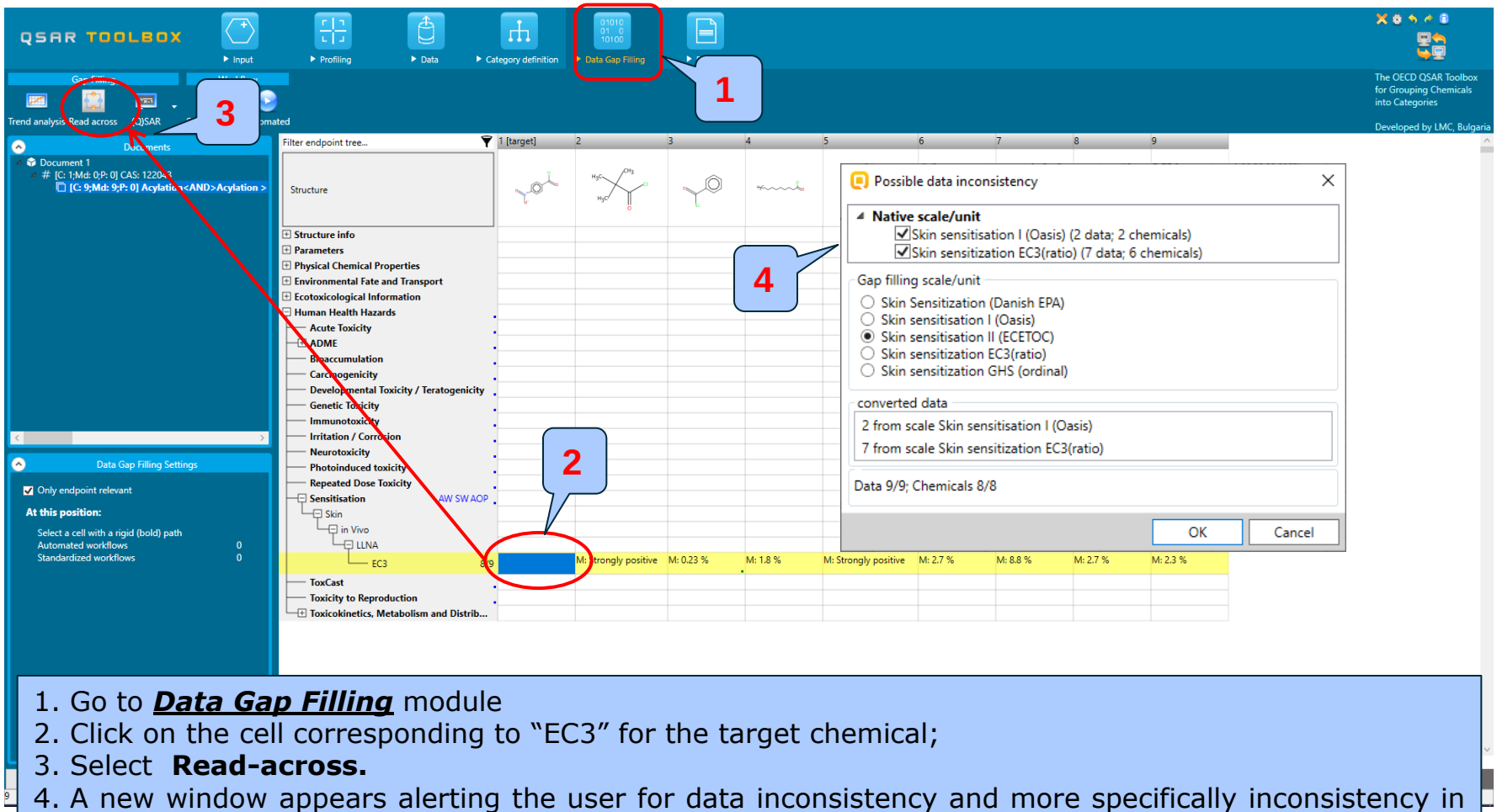
- Background
- Read across and analogue approach
- Keywords
- Objectives
- Specific Aims
- The exercise
- **Workflow**
  - Chemical Input
  - Define target endpoint
  - Profiling
  - Data
  - Category definition
  - **Data Gap Filling**

Data Gap Filling
Overview

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

In this example we will use the read-across approach.

Data Gap Filling
Apply Read across

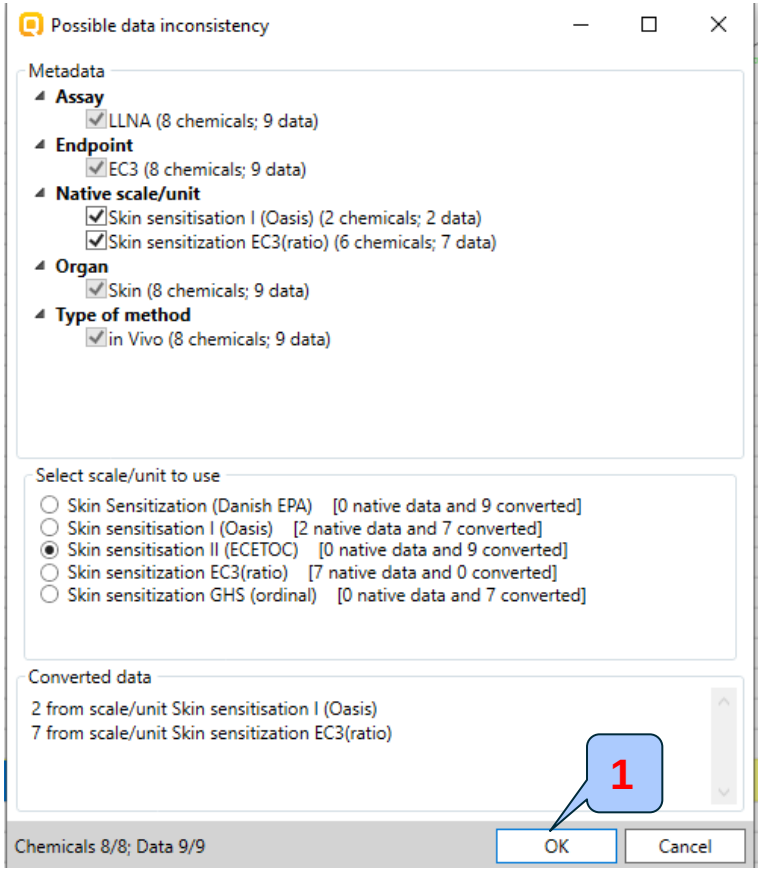


1. Go to ***Data Gap Filling*** module
2. Click on the cell corresponding to "EC3" for the target chemical;
3. Select **Read-across.**
4. A new window appears alerting the user for data inconsistency and more specifically inconsistency in the scales. See next few slides.

Data Gap Filling
Scale definition - background

- Skin sensitisation is a "qualitative" endpoint for which the results are presented with categorical data (for example: positive; negative; weak sensitizer; strong sensitizer, etc).
- Data for the skin sensitisation potential of the chemicals came from different authors and were coded with different names (for example: data from John Moores University of Liverpool are: *Strongly sensitizing, Moderately sensitizing etc*.; data from European centre for Ecotoxicology and Toxicology of chemicals are: *Positive, Negative, and Equivocal*).
- The main purpose of the scales is to unify all data available in the Toolbox databases for a certain endpoint.
- The default scale for Skin Sensitisation is "Skin Sensitisation ECETOC". It converts all skin data into: Positive and Negative (see next slide).

Data Gap Filling
Scale definition



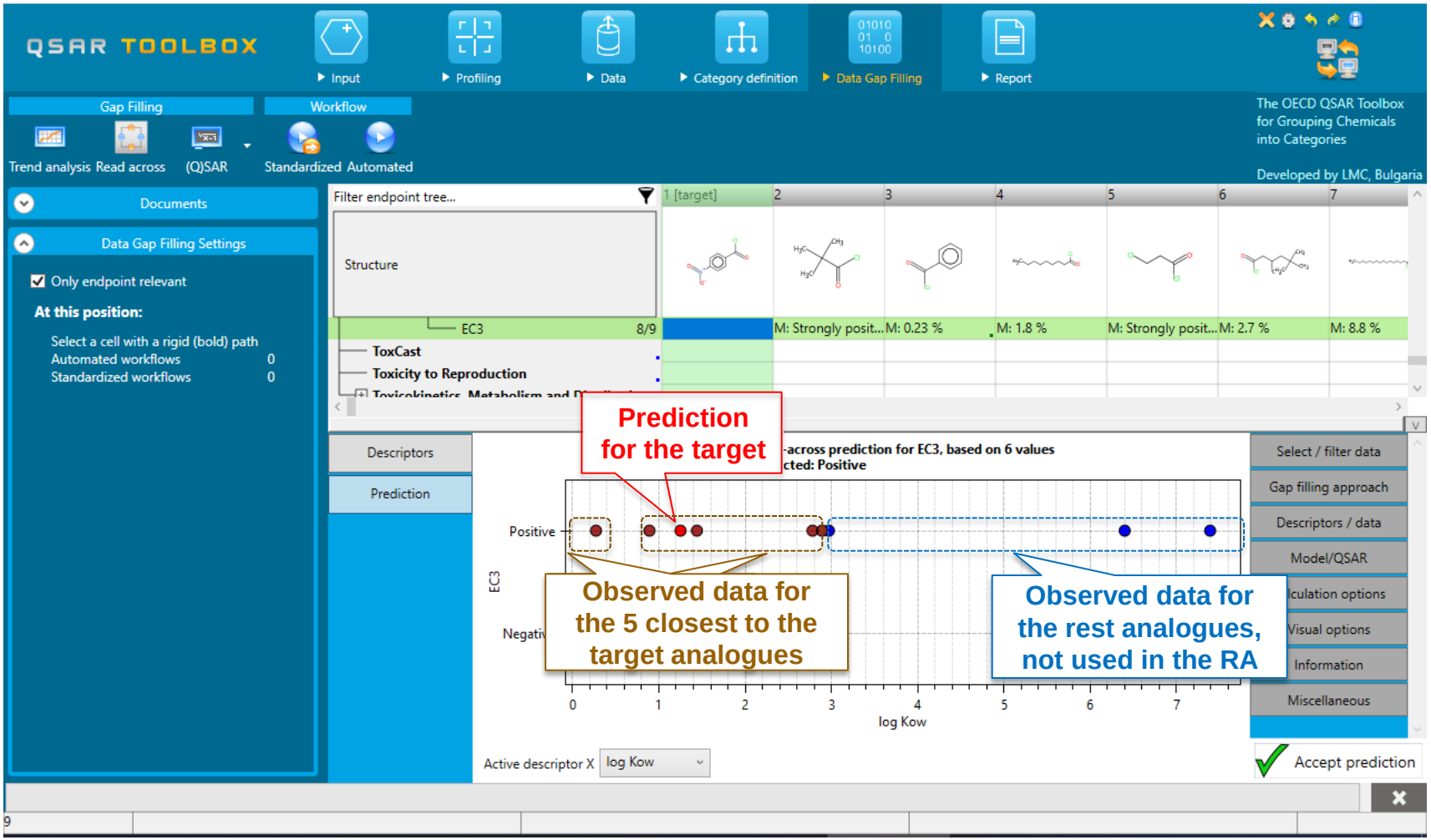
1. Click OK

In the current case the potency scale "Skin sensitization II (ECETOC) is used for filling the data gap"

Data Gap Filling
Read-across

- The resulting plot is experimental results of all analogues (Y axis) according to a descriptor (X axis) with the default descriptor of log Kow (see next screen shot).
- The **RED** dot represents predicted results for the target chemical.
- The **BROWN** dots represent the experimental results available for the analogues that are used for the read-across.
- The **BLUE** dots represent the experimental results available for the analogues but not used for read-across.

Data Gap Filling
Read-across

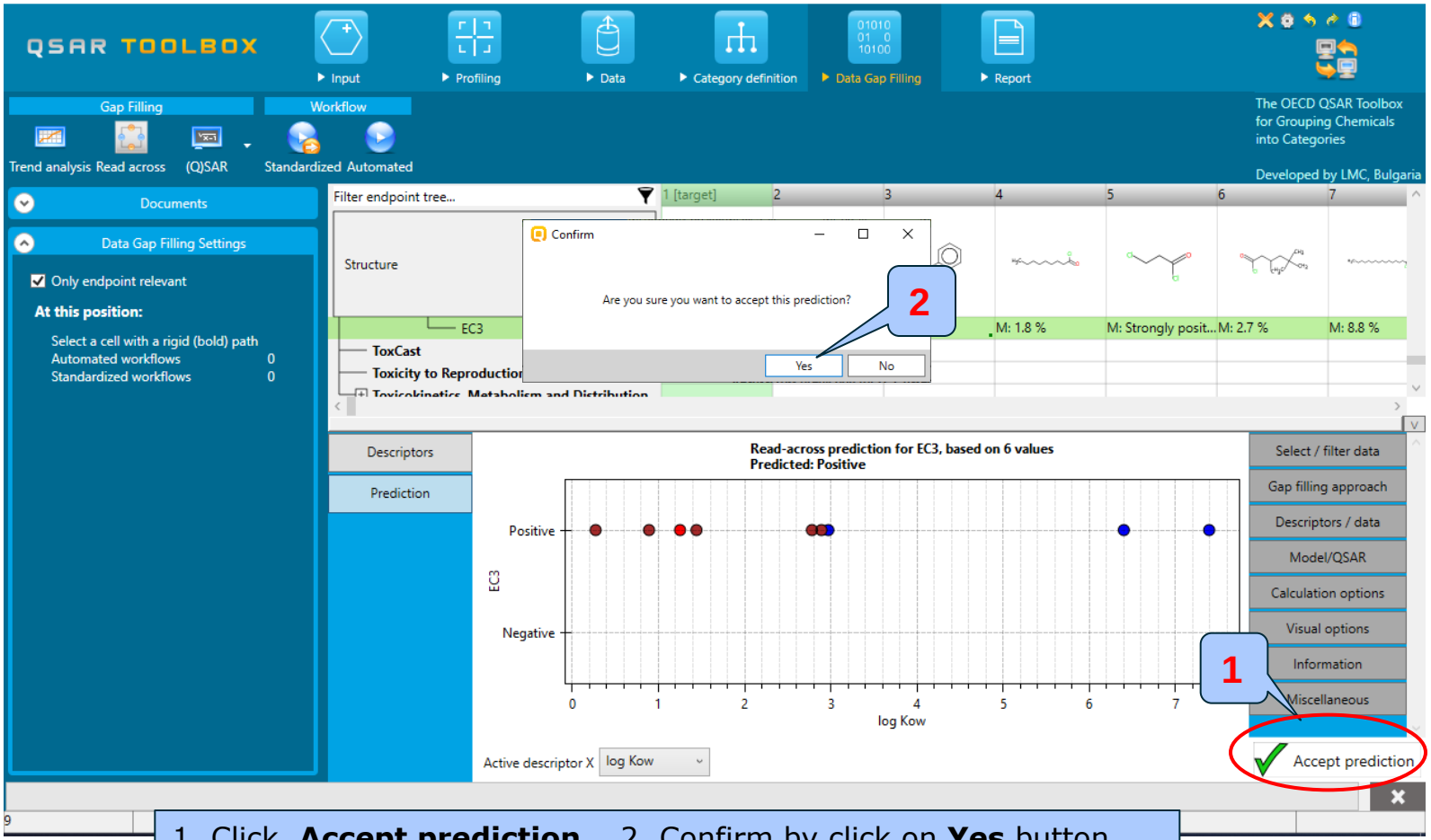


Data Gap Filling
Interpreting Read-across

- In this example, all results of the analogues are consistent; all the analogues are Skin sensitizers.
- All the analogues have protein-binding alerts based on Protein binding alerts for SS profiler.
- The same positive sensitising potential is therefore predicted for the target chemical.
- Accept the prediction by clicking "Accept prediction" (see next screen shot).

Data Gap Filling

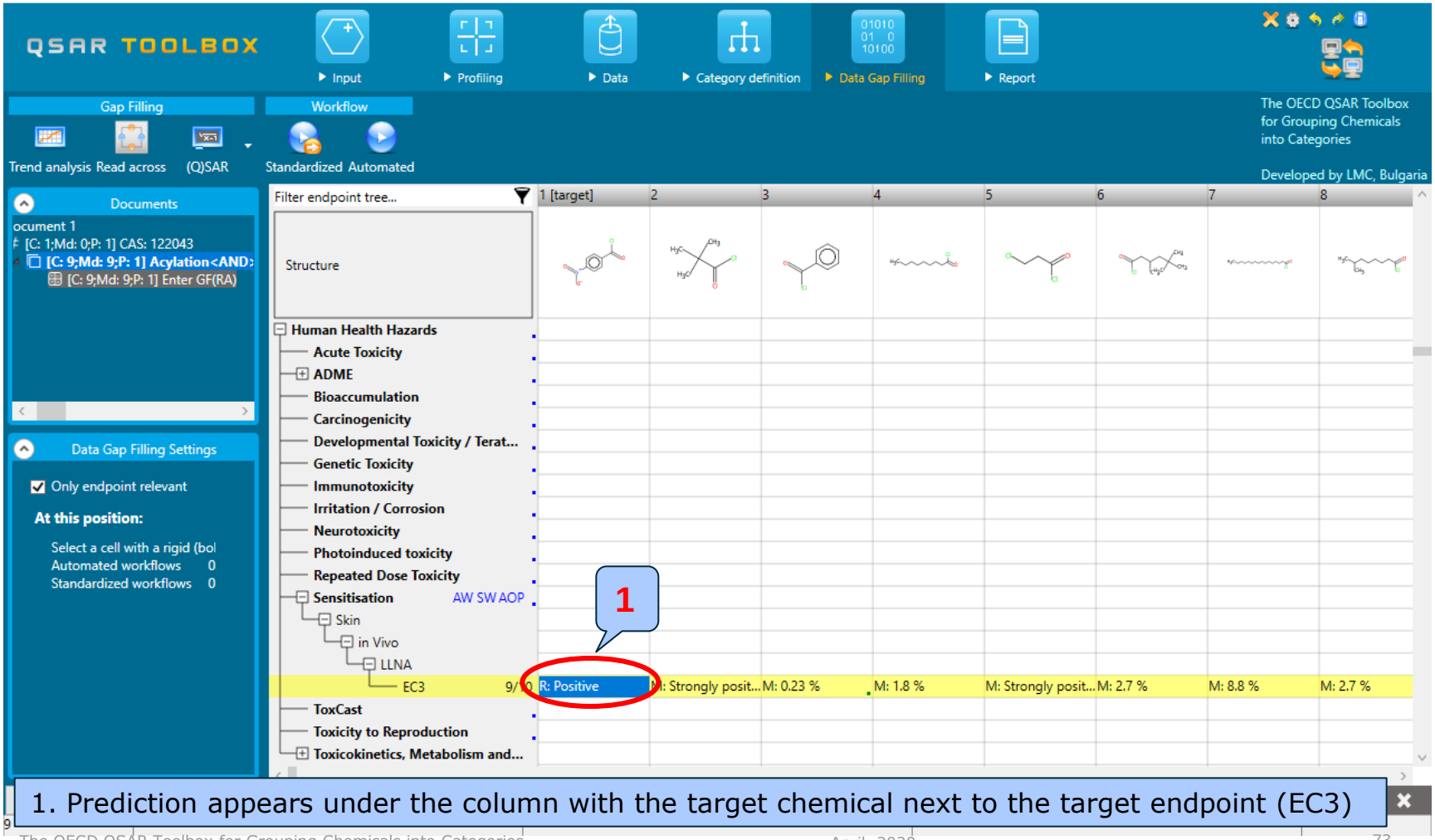
Accepting the predicted result



1. Click **Accept prediction** 2. Confirm by click on **Yes** button.

Data Gap Filling

Accepting the predicted result



1. Prediction appears under the column with the target chemical next to the target endpoint (EC3)

Recap

- Read-across is the appropriate data-gap filling method for "qualitative" endpoints like skin sensitisation. Since all the tested chemicals in the category were positive, it was easy to accept the positive predictions for the target chemical.
- You are now ready to complete the final module and to download the report.

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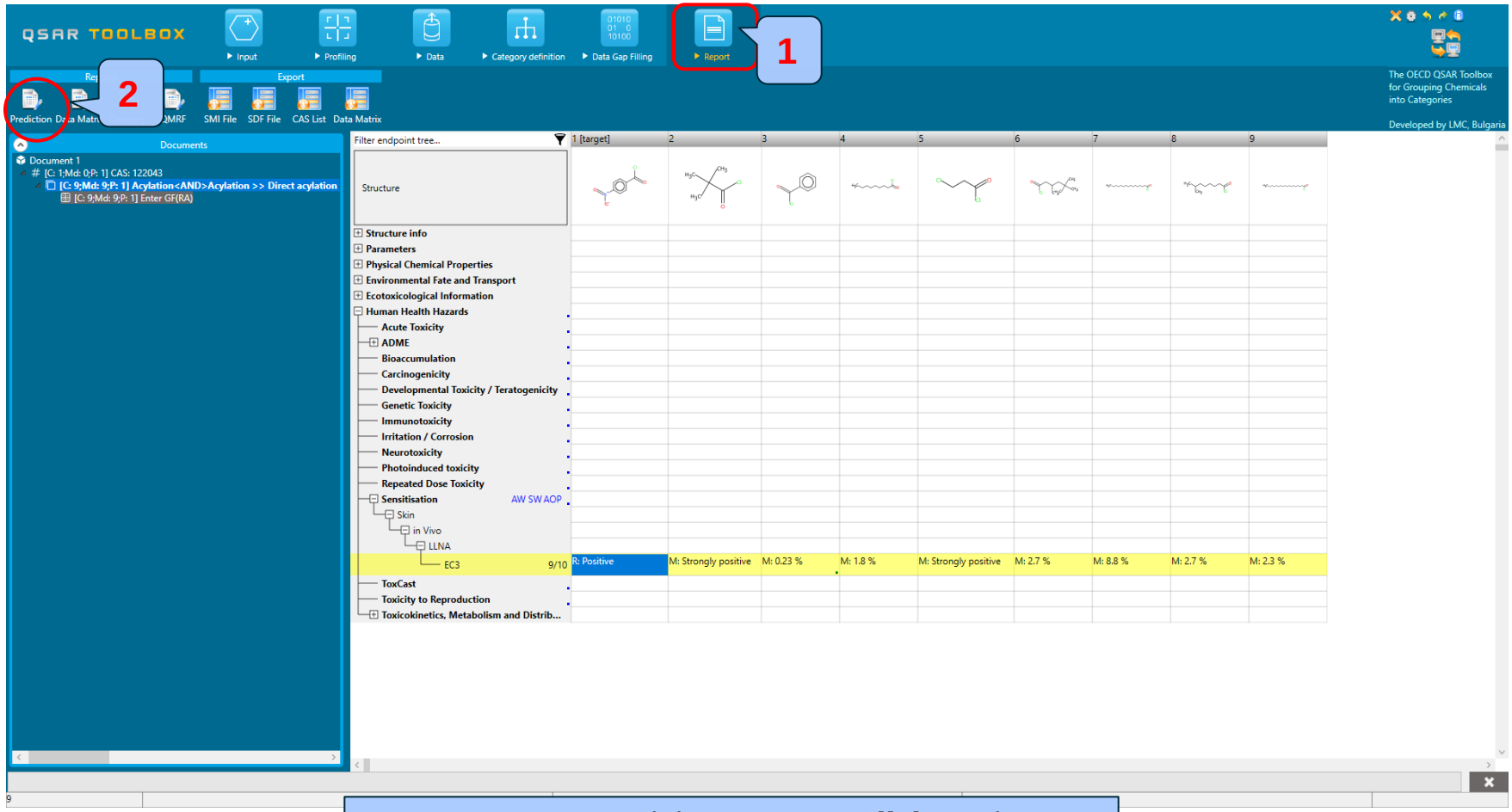
  - Category definition
  - Data Gap Filling
  - **Report**

Report
Overview

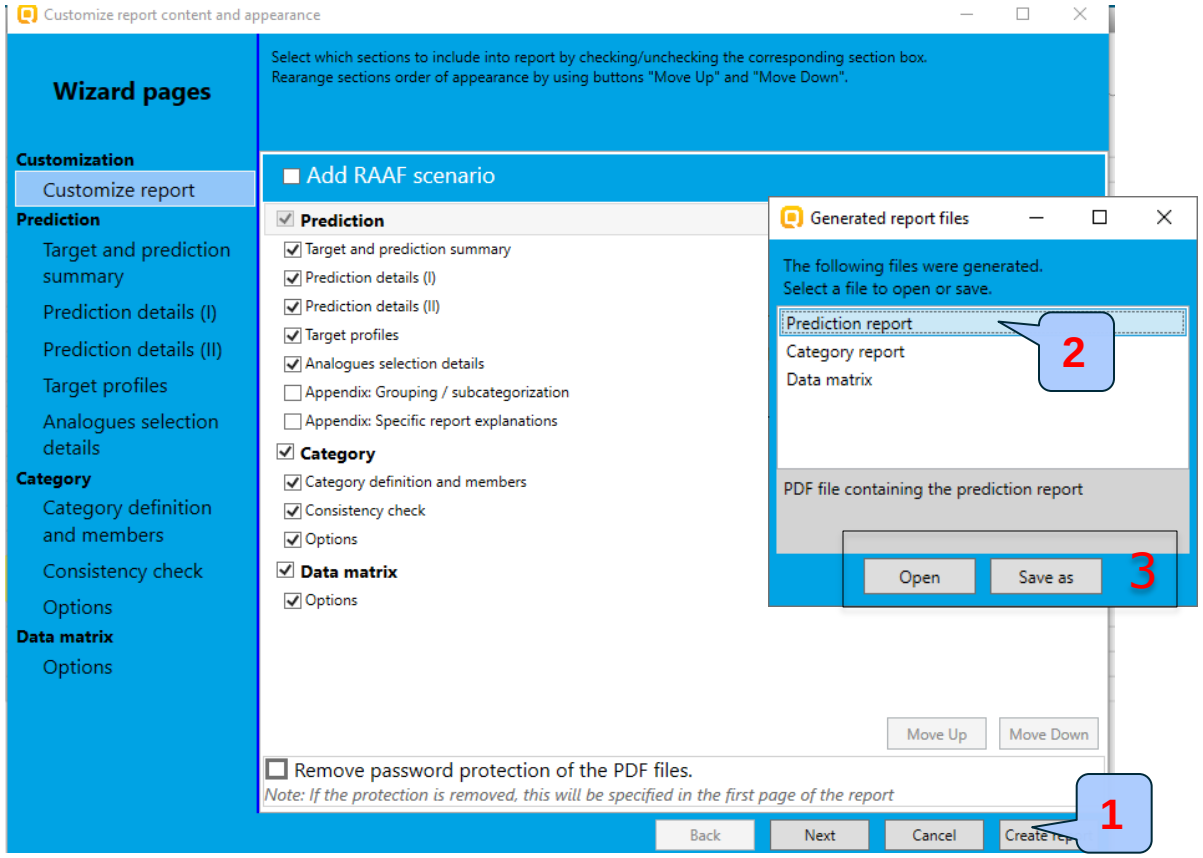
- The report module can generate a report on any of the predictions performed with the Toolbox.
- The report module contains predefined report templates which users can customize.
- The report can then be open and saved in pdf format.

Report
Generation report



1. Go to ***Report*** module; 2. **Click** Prediction

Report
Generation report



The user could select the appropriate sections to create the report. Once ready click **Create report (1).** A new window with three files supporting the report appears (2). Select one of the report files and click one of the option to view it and saved it(3).

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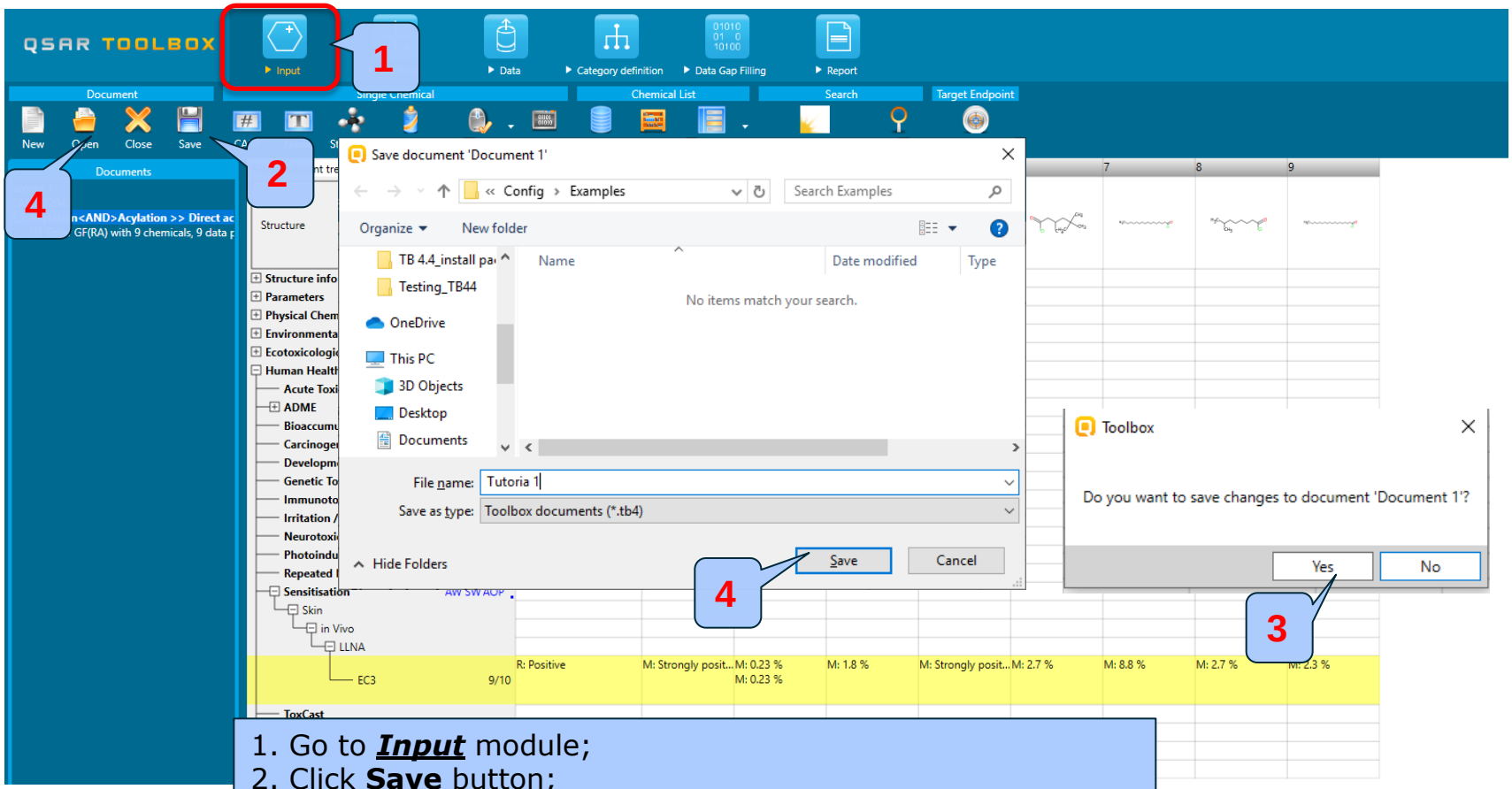
- **Workflow**
  - **Save the TB workflow**

Saving the workflow

- This functionality allows 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 Toolbox prediction is shown on next screenshots.

Saving the prediction result



1. Go to ***Input*** module;
2. Click **Save** button;
3. Click **Yes**;
4. Give name of the "*.tb4 file" and click **Save;**
5. Later on you could open the file by click **Open** button.

Congratulations!

- You have now been introduced to the workflow of the Toolbox and completed the tutorial on data gap filling by read-across based on an analogue approach.
- You have been introduced to the six modules of the Toolbox, the basic functionalities within each module and the rationale behind each module.
- Note, proficiency comes with practice!