

## OECD QSAR Toolbox v.4.1

Tutorial on how to predict Skin sensitization potential  
taking into account alert performance

# Outlook

- **Background**
- Objectives
- Specific Aims
- Read across and analogue approach
- The exercise
- Workflow

# Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow of a data filling exercise accounting alert performance.

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# Objectives

**This presentation demonstrates a number of functionalities of the Toolbox:**

- Define target endpoint;
- Relevancy of profiles and data availability;
- Calculation of alert performance (AP).

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## Specific Aims

- To introduce to the Toolbox user the workflow of the defining target endpoint;
- To familiarize the user with the new interface of the Toolbox;
- To familiarize the user with the coloring of the profiles and databases;
- To familiarize the user with the calculation of alert performance;
- To explain to the Toolbox user the rationale behind each step of the exercise.

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# Alert performance

## Overview

- Alert performance (AP) is used to define how much relevant to a target endpoint an alert is;
- AP reflects the alerts usability for category formation;
- AP can be calculated for any endpoint and any profile; one should only have preliminary defined target endpoint;
- AP can be calculate for an alert with or without accounting metabolism;

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## The Exercise

- In this exercise we will predict the skin sensitization potential for an untested compound, (*pyridaphenthion*) [CAS# 119-12-0], which will be the “target” chemical.
- We will preliminary define the target endpoint.
- This prediction will be accomplished by calculation of alert performance.
- 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

## Sidebar On 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.
- However, there is growing agreement that most organic chemicals must react covalently with skin proteins in order to behave as skin sensitizers.
- Therefore, mechanisms by which organic chemicals bind with proteins are relevant to grouping chemicals that may be skin sensitizing agents.

# Outlook

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- **Workflow**

# Workflow

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

# Outlook

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  - **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/InChi
- 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#

The screenshot shows the QSAR Toolbox software interface. The top toolbar contains icons for Input, Profiling, Data, Category definition, Data Gap Filling, and Report. Below the toolbar are tabs for Document, Single Chemical, Chemical List, Search, and Target Endpoint. The 'Input' menu is open, showing options like New, Open, Close, Save, and CAS#. A search dialog box is open, showing a search field with '119120' entered, a 'Search' button, and a list of search results. The first result is selected, showing its CAS number, SMILES string, CS Relation, and Name. A chemical structure is also displayed next to the result.

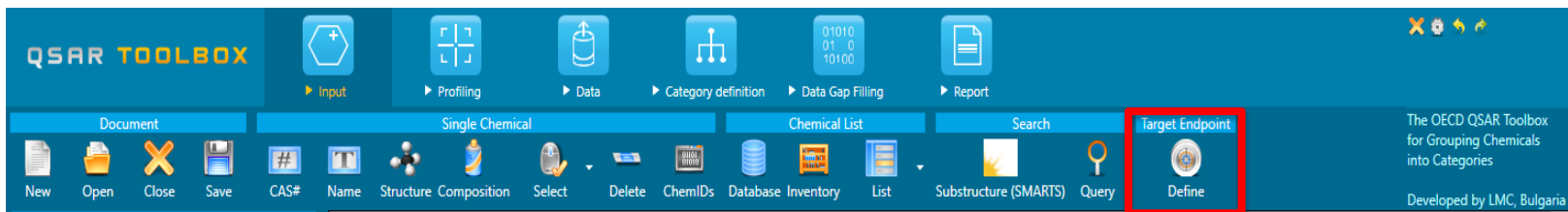
Click on **CAS#** (1); Insert CAS **119-12-0** in the blank field (2) and click on **Search** (3). When the structure appears, click on **OK** (4).

# Input

## Define target endpoint

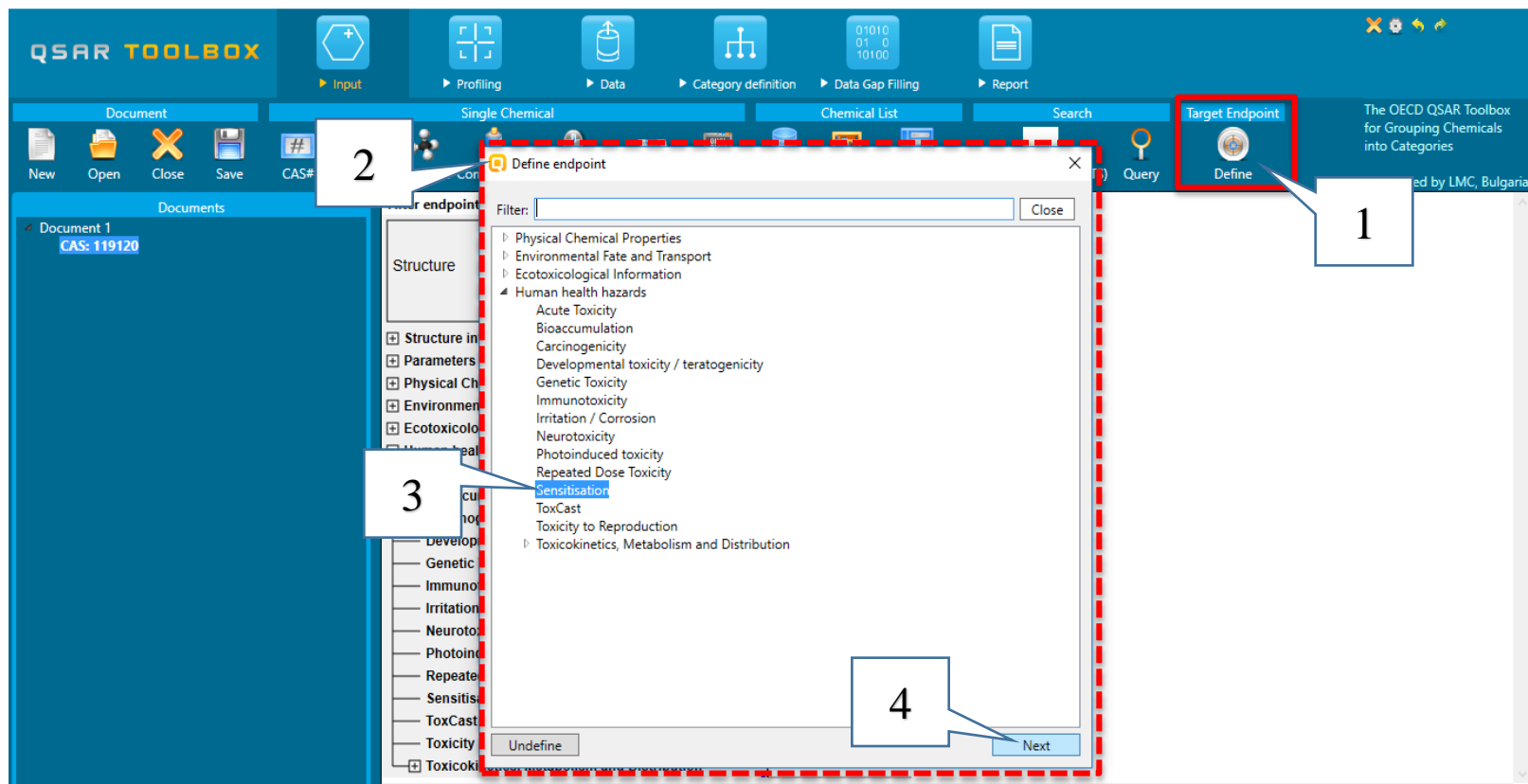
Calculation of alert performance (AP) is only possible if the target endpoint is preliminary selected.

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 metadata, different relevancy scores for profiles could be provided for same endpoint.



# Input

## Define target endpoint



When click on **Define** (1) and the *Define target* window appears (2). Select **Sensitization** in the *Human health hazards* category (3) and click on **Next** (4).

# Input

## Define target endpoint

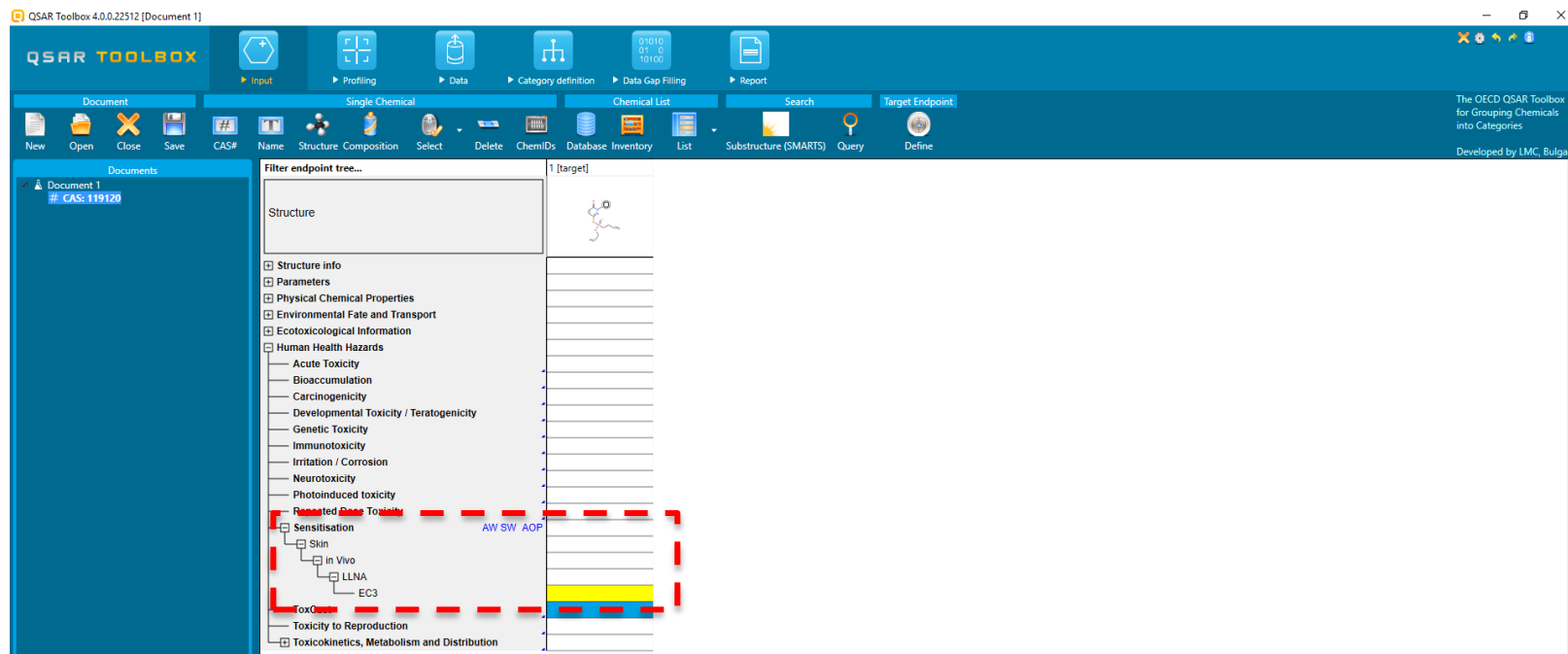
On the next step you have to select the endpoint of interest and additional metadata if needed.

1. Select *Organ*: **Skin**, *Type of method*: **In Vivo**, *Assay*: **LLNA**, *Endpoint*: **EC3**. Selection of additional metadata happens by click on the Add button and selection of the metadata from the drop-down menus; 2. Click on **Finish**

# Input

## Define target endpoint

Once the endpoint is defined along with its metadata, they appear in the endpoint tree and the corresponding row of the data matrix is highlighted.



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  - 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

## Sidebar to profiles` relevancy

Once the endpoint is selected, the relevant profiles and metabolic transformations are highlighted.

The screenshot displays the QSAR Toolbox interface. On the left, the 'Documents' panel shows 'Document 1' with CAS: 119120. Below it, the 'Profiling methods' panel has 'Options' (Select All, Unselect All, Invert) and two categories: 'Suitable' (green) and 'Plausible' (orange). 'Suitable' includes 'Protein binding alerts for skin sensitiza' (checked) and 'Protein binding by OASIS'. 'Plausible' includes 'Aquatic toxicity classification by ECOS', 'Chemical elements', 'Groups of elements', 'Keratinocyte gene expression', 'Lipinski Rule Oasis', and 'OECD HPV Chemical Categories'. The 'Metabolism/Transformations' panel is at the bottom left. The main 'Filter endpoint tree...' sidebar lists endpoints: Structure, Structure info, Parameters, Physical Chemical Properties, Environmental Fate and Transport, Ecotoxicological Information, and Human Health Hazards. Under 'Human Health Hazards', 'Sensitisation' is expanded, showing 'Skin', 'in Vivo', 'LLNA', and 'EC3' highlighted in yellow. Other endpoints like 'Acute Toxicity', 'Bioaccumulation', 'Carcinogenicity', etc., are listed but not highlighted. The right panel shows '1 [target]' with a chemical structure icon.

- **Suitable** (in green) - developed using data/knowledge for the target endpoint;
- **Plausible** (in orange) - structure-based; form broader group of analogues;
- **Unclassified** (no color) – all profilers, which are not classified in any of the categories above.

# Profiling

## Profiling the target chemical

The screenshot displays the QSAR Toolbox interface. At the top, there are navigation icons for Input, Profiling, Data, Category definition, Data Gap Filling, and Report. Below these are tabs for Profiling and Custom profile. In the Profiling tab, the 'Apply' button is highlighted with a red box and a callout '2'. The 'Profiling methods' panel (1) is open, showing a list of methods with checkboxes. The method 'Protein binding alerts for skin sensitization by OASIS' is checked. The main window shows a 'Filter endpoint tree...' with a hierarchical list of endpoints. The 'Sensitisation' category is expanded, showing 'Skin' and 'in Vivo' sub-categories. The 'in Vivo' category is further expanded to show 'LLNA' and 'EC3' endpoints. A chemical structure is visible in the top right corner of the main window.

1. Select *Protein binding alerts for skin sensitization by OASIS*; 2. Click on **Apply**

# Profiling

## Profiling the target chemical

The screenshot displays the QSAR Toolbox interface for profiling a target chemical (CAS: 119120). The 'Filter endpoint tree...' panel shows a hierarchical structure of endpoints, with 'Sensitisation' expanded to show 'Skin' and 'in Vivo' (LLNA, EC3). The 'Profile' section is also expanded to show 'Endpoint Specific' results. The table on the right lists the results, with three rows highlighted in yellow and labeled 'Alert 1', 'Alert 2', and 'Alert 3'. The table content is as follows:

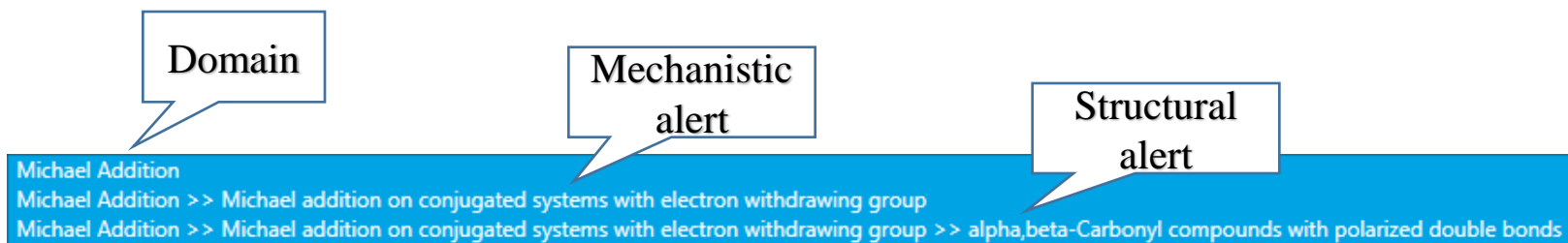
| Alert   | Endpoint              | Description  |
|---------|-----------------------|--|
| Alert 1 | Michael Addition      | Michael Addition >> Michael addition on conjugated systems with electron withdrawing group |
| Alert 2 | Schiff base formation | Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives                       |
| Alert 3 | SN2                   | SN2 >> Nucleophilic substitution at sp3 carbon atom  |

Three protein binding alerts for skin sensitization are found in the target chemical.

# Profiling

## Sidebar on the hierarchical type profiles

*Protein binding alerts for skin sensitization by OASIS* is a hierarchical profile. The organization of the hierarchical profiles includes three levels of information for each category – domain, mechanistic alert and structural alert.



Right click over the structural alert and select on *Explain* opens the profiling scheme, where the user can see more details about the current alert.

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  - 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

## Sidebar on Data availability

Once the endpoint is selected, the databases, which contain such type of data, are highlighted in green.

The screenshot displays the QSAR Toolbox interface. At the top, there is a navigation bar with icons for Input, Profiling, Data, Category definition, Data Gap Filling, and Report. Below this is a secondary bar with 'Data', 'Import', and 'Export' tabs, and sub-buttons for 'Gather', 'Import', 'IUCLID6', and 'IUCLID6'. The main interface is divided into several panels:

- Documents:** Shows a tree view with 'Document 1' containing '# CAS: 119120'.
- Databases:** A list of databases with checkboxes. The 'REACH Skin sensitisation database (no)' and 'Skin Sensitization' are highlighted in green. Other databases include Receptor Mediated Effects, Repeated Dose Toxicity HESS, Rodent Inhalation Toxicity Database, Skin Irritation, Skin sensitization ECETOC, test Iu6, ToxCastDB, Toxicity Japan MHLW, and Toxicity to reproduction (FR).
- Filter endpoint tree...:** A hierarchical tree of endpoints. The 'Sensitisation' endpoint is selected, and its sub-endpoints 'Skin', 'in Vivo', 'LLNA', and 'EC3' are visible. The 'Sensitisation' node is highlighted in blue.
- 1 [target]:** A panel showing a chemical structure diagram.

# Data Gather data

The screenshot shows the QSAR Toolbox interface. The top menu bar includes 'Data', 'Definition', 'Data Gap Filling', and 'Report'. The 'Data' menu is open, showing 'Gather', 'Import', and 'IUCLID6'. The 'Gather' button is highlighted with a red box and labeled '3'. The 'Databases' list on the left includes 'Skin Sensitization', which is selected and highlighted in green, labeled '2'. A 'Filter endpoint tree...' window is open, showing a tree structure with 'Skin Sensitization' selected. A pop-up message with a red dashed border states: 'There is no experimental data available for the chemicals of interest.' with an 'OK' button. A white box with the number '1' points to the 'Data' menu item.

1. Go to **Data**; 2. Select **Skin sensitization** database;  
3. Click on **Gather**.  
A pop-up message informs that there is no experimental data for the target chemical.



# Data

## Gather data

- Toxicity information on the target chemical is electronically collected from the selected dataset(s).
- It should be kept in mind that the search for data and analogues (and therefore calculation of AP) is performed only among the chemicals which are listed in the selected databases. In this example only the Skin sensitization database is selected.
- In this example, an insert window appears stating there was “no data found” for the target chemical.

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

## Recap

- In module one, you have entered the target chemical and define the target endpoint.
- In the second module, you have profiled the target chemical with profiling scheme, which is suitable for the selected target endpoint.
- In the third module, you have seen the database corresponding to the defined target endpoint. You have found that no experimental data is currently available in the database for the 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.

# 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 by the same mechanism and for which experimental results are available.

# Category Definition

## Protein binding 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

## Sidebar to Protein binding by OASIS categorization

- This scheme includes 112 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 each mechanistic alert (112 categories)

# Category Definition

## Sidebar to Protein binding by OASIS categorization

- Each category from level III is presented by defined 2-dimensional structural alerts that is responsible for the eliciting toxic effects, such as skin sensitization which are 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

## Sidebar to Protein binding by OASIS categorization

- There is an agreement that most organic chemicals must react covalently with skin proteins in order to behave as skin sensitizers.
- 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.

## Category Definition

- When more than one alert is found in the target structure before or after metabolic activation, Alert performance could be used to define which of them is the most suitable for primary categorization

# Alert performance

## Overview

The performance of an alert represents the number of chemicals with data related to the predefined scale across all chemicals from the selected databases, which have the same alert. It provides and distribution of data according to a given effect (e.g. positive, negative) in percentages.

# Category Definition

## Calculation of Alert performance

The screenshot displays the QSAR Toolbox interface during the 'Category definition' phase. The top toolbar includes buttons for 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. The 'Category definition' button is highlighted in yellow. Below the toolbar, the 'Categorize' menu is open, showing options: 'Define', 'Define with metabolism', 'Subcategorize', and 'Combine'. The 'Define' button is highlighted with a red box and labeled with a callout '2'. The 'Documents' panel on the left shows a list of alerts, with 'Protein binding alerts for skin sensitization by OASIS' selected and labeled with a callout '1'. The 'Filter endpoint tree...' panel on the right shows a hierarchical tree of endpoints, with 'Sensitisation' expanded to show 'Skin' and 'In Vivo' sub-categories. A yellow highlight is visible on the 'Sensitisation' row in the table on the right.

1. Select **Protein binding alerts for skin sensitization by OASIS**; 2. Click on **Define**

# Category Definition

## Calculation of Alert performance

After the click on Define, the Categorization dialog appears. It consists of all protein binding alerts for SS found in the target structure.

The most suitable alert for category formation is determined by comparison of their alert performance.

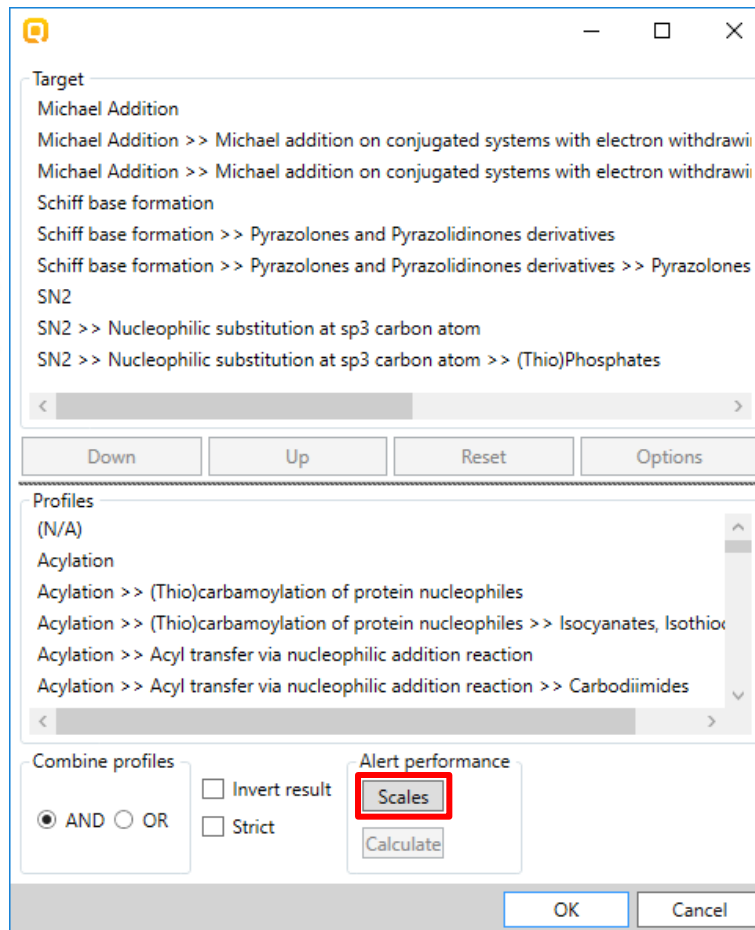
Additional section for calculating "Alert performance" is designed in this dialogue, when the endpoint is preliminary defined. (see on the next slide).

Alert performance can be calculate for only one, for combination of alerts or for all found alerts.

# Category Definition

## Calculation of Alert performance

alert 1 {  
 alert 2 {  
 alert 3 {



# Category Definition

## Calculation of Alert performance

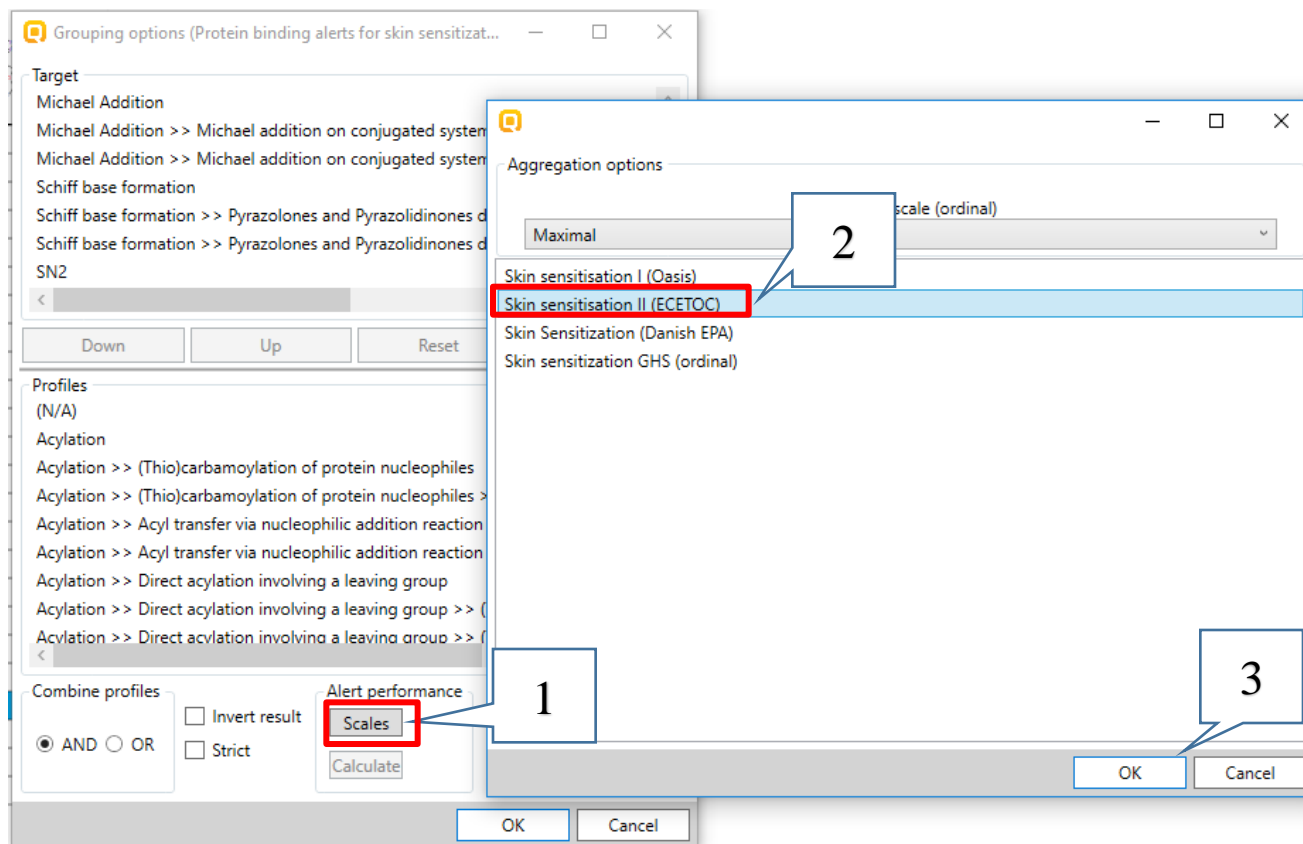
In order to calculate the performance of an alert, first of all you have to click on Scales.

The main purpose of the scales is to unify all data available in the Toolbox databases for a certain endpoint. Therefore, the most appropriate scale is "Skin Sensitisation II (ECETOC)". It is a dichotomous scale that converts all skin data into: Positive and Negative.

Additional option for applying different weight of the data that is available is also provided. Worst case scenario have been taken into account, i.e. "Maximal" data is set as default.

# Category Definition

## Calculation of Alert performance

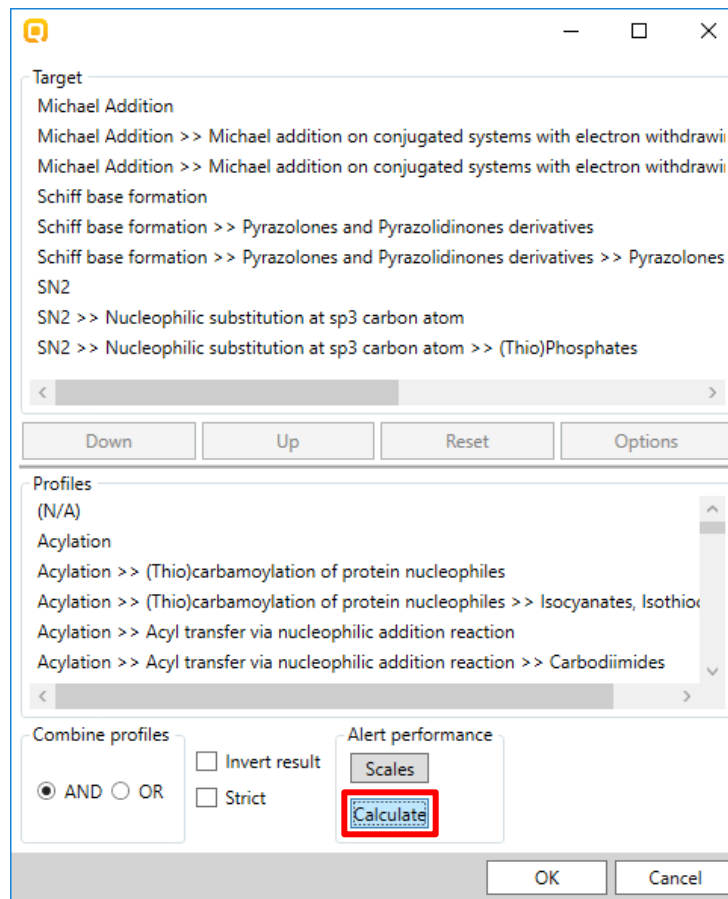


1. Click on **Scales**; 2. Select **Skin sensitization II (ECETOC)** scale; 3. Confirm with “OK”



# Category Definition

## Calculation of Alert performance



# Category Definition

## Calculation of Alert performance

Information for the calculated AP for each of the alerts appears in the following window:

| Category  | Alert Type | Percentage | Show chemicals...                     | Show all...     |
|---|------------|------------|---------------------------------------|-----------------|
| SN2 >> Nucleophilic substitution at sp3 carbon atom >> (Thio)Phosphates<AND>Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives >> Pyrazolones and Pyrazolidinones<AND>Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds | Positive   | 100.00%    | Show chemicals...<br>With data(1)...  | Show all(3)...  |
|   | Negative   | 0.00%      | Show chemicals...<br>With data(0)...  |                 |
| SN2 >> Nucleophilic substitution at sp3 carbon atom >> (Thio)Phosphates   | Positive   | 100.00%    | Show chemicals...<br>With data(2)...  | Show all(2)...  |
|   | Negative   | 0.00%      | Show chemicals...<br>With data(0)...  |                 |
| Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives >> Pyrazolones and Pyrazolidinones   | Positive   | 100.00%    | Show chemicals...<br>With data(47)... | Show all(82)... |
|   | Negative   | 6.00%      | Show chemicals...<br>With data(3)...  |                 |

Close

# Category Definition

## Calculation of Alert performance for one alert

Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds

|          |        |                                       |                 |
|----------|--------|---------------------------------------|-----------------|
| Positive | 94.00% | Show chemicals...<br>With data(47)... | Show all(82)... |
| Negative | 6.00%  | Show chemicals...<br>With data(3)...  |                 |

The system informs that 82 analogues with the searched alert have been found. Out of these 82 analogues data have been found for 50 substances. Of them:

- 47 out of 50 chemicals have positive data (94%)
- 3 out of 50 chemicals have negative data (6%).



Keep in mind that the statistic is obtained from the chemicals and data, available in the selected databases

# Category Definition

## Calculation of Alert performance for one alert

In summary we see that the first alert is the most suitable to define a category.

Target

- Michael Addition
- Michael Addition >> Michael addition on conjugated systems with electron withdrawing group <AND> Michael Addition >> Michael addition on conjugated systems with electron withdrawing Schiff base formation
- Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives
- Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives >> Pyrazolones SN2
- SN2 >> Nucleophilic substitution at sp3 carbon atom
- SN2 >> Nucleophilic substitution at sp3 carbon atom >> (Thio)Phosphates

Profiles (N/A)

Acylation

- Acylation >> (Thio)carbamylation of protein nucleophiles
- Acylation >> (Thio)carbamylation of protein nucleophiles >> Isocyanates, Isothiocyanates
- Acylation >> Acyl transfer via nucleophilic addition reaction
- Acylation >> Acyl transfer via nucleophilic addition reaction >> Carbodiimides

Combine profiles:  AND  OR

Alert performance:  Invert result,  Strict

Buttons: Down, Up, Reset, Options, Scales, Calculate, OK, Cancel

Michael Addition <AND> Michael Addition >> Michael addition on conjugated systems with electron withdrawing group <AND> Michael Addition >> Michael addition on conjugated systems with electron withdrawing Schiff base formation >> alpha,beta-Carbonyl compounds with polarized double bonds

Positive 94.00%

Negative 6.00%

Buttons: Show chemicals... With data(47)..., Show chemicals... With data(3)..., Show all(82)...

Schiff base formation <AND> Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives <AND> Schiff base formation >> Pyrazolones and Pyrazolidinones derivatives >> Pyrazolones and Pyrazolidinones

Positive 100.00%

Negative 0.00%

Buttons: Show chemicals... With data(2)..., Show chemicals... With data(0)..., Show all(2)...

SN2 <AND> SN2 >> Nucleophilic substitution at sp3 carbon atom <AND> SN2 >> Nucleophilic substitution at sp3 carbon atom >> (Thio)Phosphates

Positive 100.00%

Negative 0.00%

Buttons: Show chemicals... With data(1)..., Show chemicals... With data(0)..., Show all(3)...

# Category Definition

## Sidebar on Alert performance accounting metabolism

AP can be also calculated for alert(s) identified after AU or SM activation.

Example: CAS 90-05-1

The screenshot shows the QSAR Toolbox 4.0.0.22512 interface. The top navigation bar includes 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. The sidebar on the left has 'Documents' (with a callout '3' pointing to it), 'Profiling methods', and 'Metabolism/Transformations'. In the 'Metabolism/Transformations' section, 'Skin metabolism simulator' is selected (callout '2'). The main area shows a tree view of endpoints, with 'Skin metabolism simulator' selected under 'Metabolism/Transformations' (callout '1'). The right panel shows the chemical structure of the target and a table of results. The table lists '4 metabolites' and includes the following entries:

|   |
|---|
| 1 x Schiff base formation   |
| 1 x Schiff base formation >> Schiff base formation with carbonyl compounds              |
| 1 x Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes |
| 1 x Michael Addition  |

# Category Definition

## Sidebar on Alert performance accounting metabolism

AP can be also calculated for alert(s) identified after AU or SM activation.

Example: CAS 90-05-1

The screenshot displays the QSAR Toolbox interface for CAS 90-05-1. The 'Filter endpoint tree...' sidebar is expanded to show 'Human Health Hazards' > 'Sensitisation' > 'Skin' > 'Skin metabolism simulator' > 'Endpoint Specific' > 'Protein binding alerts for skin sensitization ...'. The 'Metabolism/Transformations' section is active, with 'Skin metabolism simulator' checked. The 'Endpoint Specific' section shows 'Protein binding alerts for skin sensitization ...' with a 'No alert found' message. A red dashed box highlights the 'Metabolism/Transformations' section, showing a list of structural alerts: '1 x Schiff base formation', '1 x Schiff base formation >> Schiff base formation with carbonyl compounds', '1 x Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes', and '1 x Michael Addition'.

No protein binding alerts are found in the parent structure

Structural alerts are found after applying of Skin metabolism simulator

# Category Definition

## Sidebar on Alert performance accounting metabolism

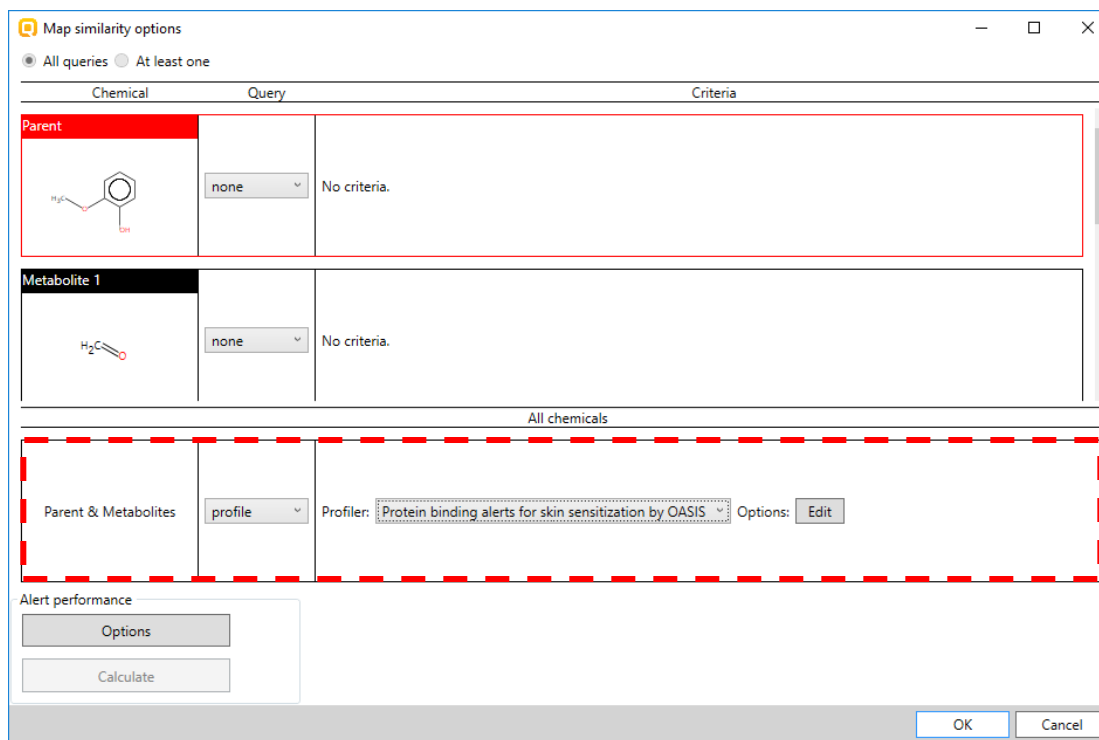
The target chemical has no alert for protein binding as parent but it is activated as a result of skin metabolism. In this respect, the primary category will be defined with accounting for the metabolic activation.

The screenshot displays the QSAR Toolbox interface. The top toolbar includes buttons for 'Input', 'Profiling', 'Data', 'Category definition' (highlighted with a red box), 'Data Gap Filling', and 'Report'. Below the toolbar, the sidebar contains 'Define', 'Define with metabolism' (highlighted with a red box), 'Subcategorize', and 'Combine'. The main window shows a 'Filter endpoint tree...' on the left and a 'Select metabolism' dialog box on the right. The dialog box lists various metabolic simulators, with 'Skin metabolism simulator' highlighted by a red box. The background shows a list of alerts, including 'Keratinocyte gene expression' and 'Protein binding alerts for skin sensitization according to GHS'.

# Category Definition

## Sidebar on Alert performance accounting metabolism

The system will search for chemicals which have similar distribution of the protein binding alerts as identified in the generated package parent and metabolites, accounting for the skin metabolism activation for the analogues.





# Category Definition

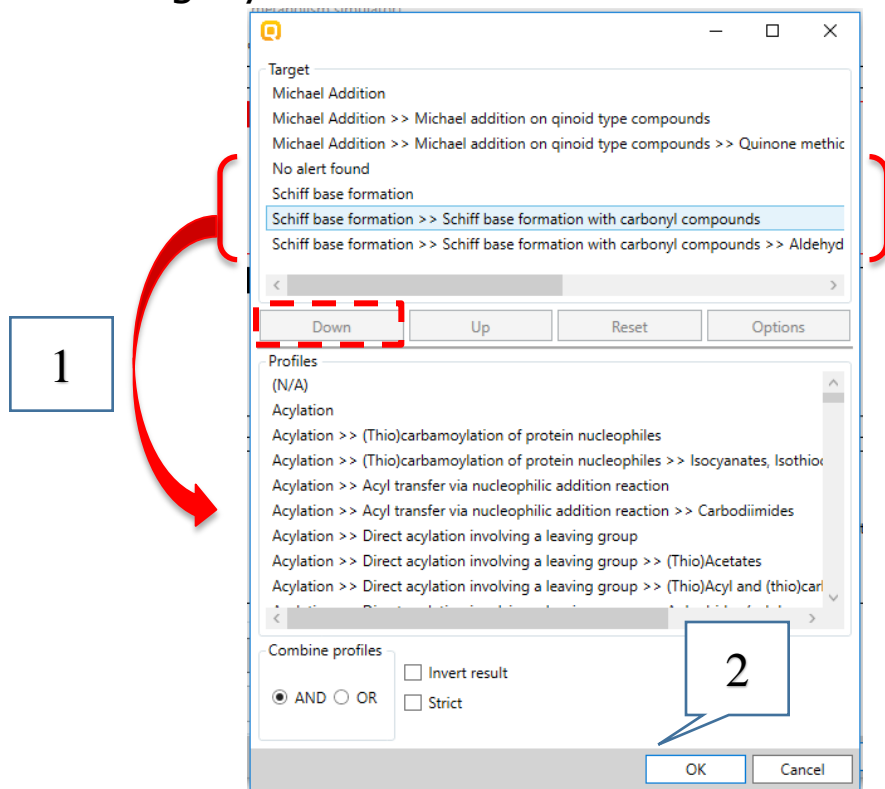
## Sidebar on Alert performance accounting metabolism

The found alerts could be seen by click on the **Edit** button. To calculate AP for one alert – remove all alerts except the alert for which AP will be calculated (three levels of mechanistic information are required – domain, mechanistic and structural alert), select a scale in the **Options** and click on **Calculate**.

# Category Definition

## Calculation of Alert performance accounting metabolism for one alert

Now we will calculate AP for each of the alerts in order to see which of them is the most suitable for category formation.



1. Remove No alert found and the second alert by double click or using “Down” button; 2. Click on OK button. Select again scale Skin sensitization (ECETOC)

# Category Definition

## Calculation of Alert performance accounting metabolism for one alert

Performance of the first alert appears in the following window:

The screenshot shows a window with a title bar containing a yellow 'Q' icon and standard window controls (minimize, maximize, close). The main content area is divided into three sections:

- Left Panel:** Contains the category definition text: "Combinded parent and products requirements: Michael Addition<AND>Michael Addition >> Michael addition on qinoid type compounds<AND>Michael Addition >> Michael addition on qinoid type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines (Protein binding alerts for skin sensitization by OASIS)".
- Table:** A table with two rows:
 

|          |        |
|----------|--------|
| Positive | 98.48% |
| Negative | 1.52%  |
- Right Panel:** Contains two "Show chemicals..." buttons. The first button is labeled "With data(65)..." and the second is labeled "With data(1)...".

At the bottom of the window is a "Close" button. On the far right edge of the window, there is a vertical button labeled "Show all(142)...".

The system informs that 142 analogues with the searched alert accounting for skin metabolism have been found. Out of these 142 analogues data have been found for 66 structures. Of them:

- 65 out of 66 chemicals have positive data (98.48%)
- 1 out of 66 chemicals have negative data (1.52%).

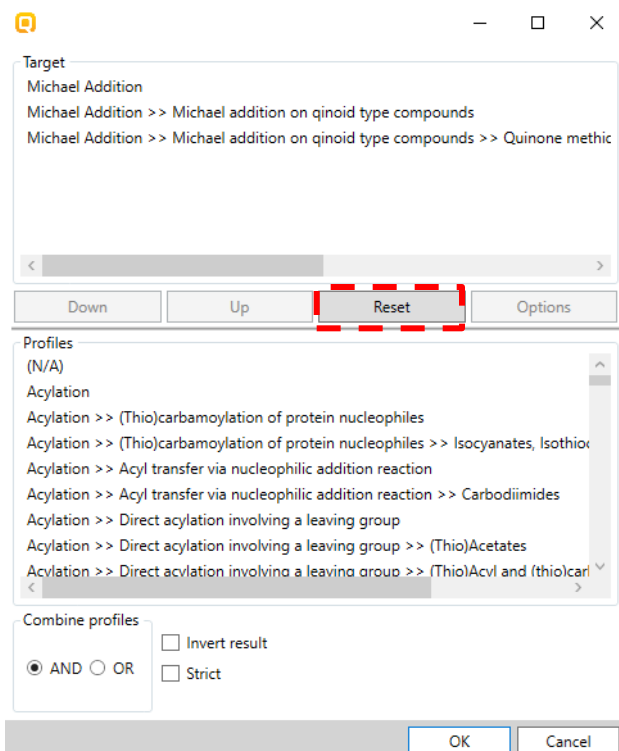


Keep in mind that the statistic is obtained from the chemicals and data, available in the selected databases

# Category Definition

## Calculation of Alert performance accounting metabolism for one alert

Click on the **Reset** button and repeat the alert performance calculation steps for the second alert.



# Category Definition

## Calculation of Alert performance accounting metabolism for one alert

In summary we see that the first alert is the most suitable to define a category.

alert 1 {

Michael Addition

Michael Addition >> Michael addition on qinoid type compounds

Michael Addition >> Michael addition on qinoid type compounds >> Quinone methic

No alert found

alert 2 {

Schiff base formation

Schiff base formation >> Schiff base formation with carbonyl compounds

Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehyd

Down Up Reset Options

Profiles

(N/A)

Acylation

Acylation >> (Thio)carbamoylation of protein nucleophiles

Acylation >> (Thio)carbamoylation of protein nucleophiles >> Isocyanates, Isothio

Acylation >> Acyl transfer via nucleophilic addition reaction

Acylation >> Acyl transfer via nucleophilic addition reaction >> Carbodiimides

Acylation >> Direct acylation involving a leaving group

Acylation >> Direct acylation involving a leaving group >> (Thio)Acetates

Acylation >> Direct acylation involving a leaving group >> (Thio)Acyl and (thio)car

Combine profiles

AND  OR

Invert result

Strict

OK Cancel

Combinded parent and products requirements: Michael Addition <AND> Michael addition on qinoid type compounds <AND> Michael addition on qinoid type compounds <AND> Michael addition on qinoid type compounds >> Quinone methide(s)/ imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines (Protein binding alerts for skin sensitization by OASIS)

|          |        |                                       |
|----------|--------|---------------------------------------|
| Positive | 98.48% | Show chemicals...<br>With data(65)... |
| Negative | 1.52%  | Show chemicals...<br>With data(1)...  |

alert 1

Show all(142)...

Combinded parent and products requirements: Schiff base formation <AND> Schiff base formation >> Schiff base formation with carbonyl compounds <AND> Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes (Protein binding alerts for skin sensitization by OASIS)

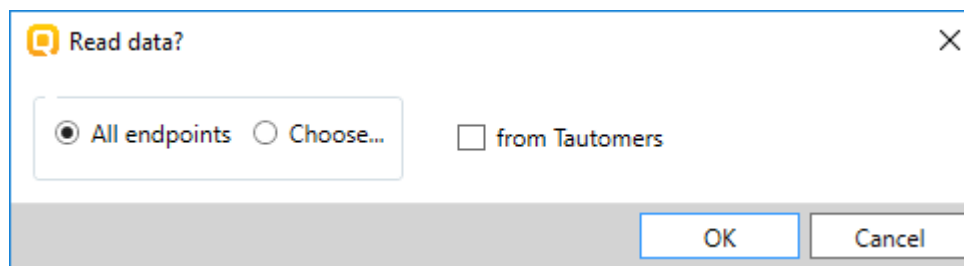
|          |        |  |
|----------|--------|--|
| Positive | 76.55% | Show chemicals...<br>With data(111)... |
| Negative | 23.45% | Show chemicals...<br>With data(34)...  |

alert 2

Show all(265)...

## Category Definition Analogues

- Based on the defined category (*Michael Addition* << *Michael addition on conjugated systems with electron withdrawing group* <<  *$\alpha,\beta$ -Carbonyl compounds with polarized double bonds*) 136 analogues have been identified (including the target chemical CAS: 90-05-1).
- 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).

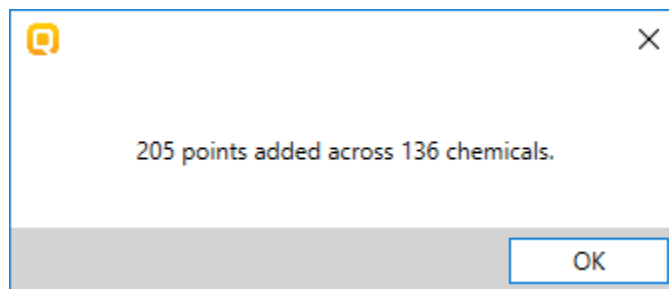


Only Skin sensitization database is selected now and we click on **OK**.

# Category Definition

## Read data for Analogues

- The Toolbox automatically informs the user for the number of gathered data points across the chemicals in the category



- Click **OK** to confirm the appeared message

# Category Definition

## Summary information for Analogues

66 chemicals with 74 experimental results related to the defined target endpoint are found.

The screenshot displays the QSAR Toolbox software interface. The 'Filter endpoint tree...' panel on the left shows a tree structure with 'Sensitisation' selected. The main area shows a table with 10 columns, each representing a chemical. The table includes columns for CAS Number, CAS Smiles relation, Chemical name(s), Composition, Molecular Formula, and Predefined substance type. The 'Human Health Hazards' section is expanded, showing various toxicity endpoints. A red box highlights a row in the table with the following data:

|             |             |             |             |             |             |             |             |             |             |             |             |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| (65/73)     | (47/57)     | (54/54)     | (6/12)      | (65/73)     | (47/57)     | (54/54)     | (6/12)      | (65/73)     | (47/57)     | (54/54)     | (6/12)      |
| M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive | M: Positive |

A blue callout box with a green exclamation mark icon points to the highlighted row, containing the text: "Chemical statistics presenting the number of chemicals and the available experimental data."



# Outlook

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

## Recap

- You have identified three protein binding alerts for the target chemical (pyridaphenthion).
- You have calculated and compare the alert performance for each of the alerts.
- You have now retrieved in the available experimental results on skin sensitisation (EC3) values for 65 chemicals with the same mechanism of protein binding as the target compound, which were found in the “Skin Sensitisation” database.
- The user can now proceed to the next module; click on *“Data Gap Filling”*.

# Data Gap Filling Overview

- “Data Gap Filling” module give 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.
  - Standardized and Automated workflows are developed to facilitate the users work. Once started, they follow the implemented logic and finish with prediction. The general differences between the two type of workflows are represented on the next slide.

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

# Data Gap Filling Apply Read across

**1** Click on the row with the target endpoint and the cell corresponding to the target chemical; **2**. Go to **Data gap Filling**; **3**. Select **Read-across**; **4**. Select **Skin sensitisation II (ECETOC)**; **5**. Click on **OK**

| 1 [target]                | 2                             | 3                             | 4                             | 5                             | 6                             | 7                             | 8                             | 9                             | 10                            |
|---------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <chem>Oc1ccc(O)cc1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> | <chem>O=C1C=CC(=O)C=C1</chem> |
| 90-05-1<br>High           | 2832-40-8<br>High             | 98-29-3<br>High               | 3568-90-9<br>Low              | 39703-09-8<br>Low             | 100884-13-7<br>Low            | Invalid CAS number: 0-0       | 55066-56-3<br>High            | 793-24-8<br>High              | 101-72-4<br>High              |
| 2-Methoxyphenol           | 4'-((6-HYDROXY-M-...)         | 1,2-Benzenediol, 4-(          | Deoxylapachol                 | Geranylgeranylhydroch         | 1,1-Dimethylalkylkafee        | Propolis                      | 4-Methylphenyl 3-m            | 1,4-Benzenediamine            | 1,4-Benzen                    |
| C7H8O2                    | C15H15N3O2                    | C10H14O2                      | C15H14O2                      | C26H38O2                      | C14H16O4                      | C13H14O4                      | C12H16O2                      | C18H24N2                      | C15H18N2                      |
| Mono constituent          | Mono constituent              | Mono constituent              | Mono constituent              | Mono constituent              | Mono constituent              | Mono constituent              | Mono constituent              | Mono constituent              | Mono constituent              |
| CO...                     | CO...                         | CO...                         | CO...                         | CO...                         | CO...                         | CO...                         | CO...                         | CO...                         | CO...                         |

1. Click on the row with the target endpoint and the cell corresponding to the target chemical; 2. Go to **Data gap Filling**; 3. Select **Read-across**; 4. Select **Skin sensitisation II (ECETOC)**; 5. Click on **OK**

# Data Gap Filling

## Apply Read across

The screenshot shows the QSAR Toolbox interface during the Data Gap Filling process. The main table displays chemical data across various endpoints. An information dialog box is open, stating: "4 observed values for 4 chemicals were excluded due to missing X descriptor value(s)". A callout box labeled '1' points to the information dialog box, and another callout box labeled '2' points to the 'OK' button on the dialog box.

| Target                                  | 8                | 10                 | 11                 | 24                | 25                     | 28                           | 29                     | 30                   | 31                    |                  |
|---|------------------|--------------------|--------------------|-------------------|------------------------|------------------------------|------------------------|----------------------|-----------------------|------------------|
| Structure                               |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| CAS Number                              | 90-05-1          | 55066-56-3         | 101-72-4           | 1205-17-0         | 1166-52-5              | 1034-01-1                    | 121-79-9               | 186743-30-6          | 54393-89-4            | 2785-87-7        |
| CAS Smiles relation                     | High             | High               | High               | High              | High                   | High                         | High                   | Low                  | High                  | High             |
| Chemical name(s)                        | 2-Methoxyphenol  | 4-Methylphenyl 3-m | 1,4-Benzenediamine | ?-Methyl-1 3-benz | Benzoic acid, 3,4,5-tr | Benzoic acid, 3,4,5-tr       | 3,4,5-trihydroxybenz   | ISOPROPYL_ISO Eugen  | 2-nitro-4-(propylsulf | 2-Methoxy-4-     |
| Composition                             | C7H8O2           | C12H16O2           | C15H18N2           | C11H12O3          | C19H30O5               | C15H22O5                     | C10H12O5               | C12H16O2             | C9H12N2O2S            | C10H14O2         |
| Molecular Formula                       | C7H8O2           | C12H16O2           | C15H18N2           | C11H12O3          | C19H30O5               | C15H22O5                     | C10H12O5               | C12H16O2             | C9H12N2O2S            | C10H14O2         |
| Predefined substance type               | Mono constituent | Mono constituent   | Mono constituent   | Mono constituent  | Mono constituent       | Mono constituent             | Mono constituent       | Mono constituent     | Mono constituent      | Mono constituent |
| Structural Formula                      | CCOC1=CC=CC=C1   | CC(C)CC(=O)N       | NC1=CC=CC=C1N      | CC(C)C1=CC=CC=C1  | CCCCCCCCCCCCOC(=O)C    | CCCCCCCCOC(=O)C1=CC=C(C)C=C1 | CCOC(=O)C1=CC(O)=CC=C1 | CCOC1=CC(=CC=C1)C=C1 | CCCS1=CC(N)C(C1)N     | CCOC1=CC=CC=C1   |
| Parameters                              |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Physical Chemical Properties            |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Environmental Fate and Transport        |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Ecotoxicological Information            |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Human Health Hazards                    |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Acute Toxicity                          |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Bioaccumulation                         |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Carcinogenicity                         |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Developmental Toxicity / Teratogenicity |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Genetic Toxicity                        |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Immunotoxicity                          |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Irritation / Corrosion                  |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Neurotoxicity                           |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Photoinduced toxicity                   |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Repeated Dose Toxicity                  |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Sensitisation                           |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| Skin                                    |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| In Vivo                                 |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| GPMT                                    |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| HRIPT                                   |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| LLNA                                    |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| AW SW AOP                               |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| (18/18)                                 |                  |                    |                    |                   |                        |                              |                        |                      |                       |                  |
| (5/11)                                  | M: Positive      |                    |                    |                   | M: Positive            | M: Positive                  |                        |                      |                       | M: Positive      |
|   |                  |                    |                    |                   |                        |                              |                        |                      |                       | M: Positive      |

1. The Toolbox informs the user that 4 observed values for 4 chemicals were excluded due to missing X descriptor values; 2. Click on **OK**

# Data Gap Filling Apply Read across

The screenshot displays the QSAR Toolbox software interface during a Data Gap Filling workflow. The main window shows a table of chemical data with columns for CAS numbers, SMILES relations, and various descriptors. A 'Filter endpoint tree...' panel is visible on the left, and a 'Read-across prediction for EC3' plot is shown at the bottom. A 'Choose one' dialog box is open, allowing the user to select a prediction mode. Three callout boxes (1, 2, 3) highlight specific actions: 1 points to the 'Data usage' section in the settings, 2 points to the 'Maximal' option in the dialog, and 3 points to the 'OK' button.

**1.** Go to *Calculation options* and click on *Data usage*; **2.** Select the worst case scenario, i.e. **“Maximal”** data; **3.** Click on **OK**

# Data Gap Filling

## Apply Read across

The screenshot displays the 'Subcategorization' window in the OECD QSAR Toolbox. The left sidebar shows a tree view of categories, with 'US-EPA New Chemical Categories' selected (callout 2). The main area shows a table of chemical structures and their predicted EC3 values. A 'Read-across prediction for EC3, based on 6 values' plot is shown at the bottom, with a 'Predicted: Positive' result (callout 1). A 'Remove selected' button is highlighted (callout 3).

| Chemical ID | Chemical Structure        | EC3 Prediction |
|-------------|---------------------------|----------------|
| 62          | <chem>Oc1ccc(O)cc1</chem> | M: Positive    |
| 63          | <chem>Oc1ccc(O)cc1</chem> | M: Positive    |
| 69          | <chem>Oc1ccc(O)cc1</chem> | M: Positive    |
| 71          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |
| 72          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |
| 73          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |
| 75          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |
| 76          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |
| 77          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |
| 78          | <chem>Nc1ccc(O)cc1</chem> | M: Positive    |

**1.** Go to *Select/filter data* and click on **Subcategorize**; **2.** Select the **US-EPA New Chemical Categories** profiler; **3.** Click on **Remove selected**

# Data Gap Filling

## Accepting the predicted result

The screenshot displays the QSAR Toolbox interface during the Data Gap Filling process. The main window shows a table of chemical structures (numbered 1 to 51) with their predicted values for various endpoints. A 'Confirm' dialog box is open, asking for confirmation to accept a prediction. A '1' callout points to the 'Accept prediction' button at the bottom right, and a '2' callout points to the 'Yes' button in the dialog.

**1. Click on **Accept prediction**; 2. Confirm with "Yes"**



## Recap

- Read-across is the appropriate data-gap filling method for “qualitative” endpoints like skin sensitisation. Since the most of the analogues and all five neighbouring tested chemicals in the category were positive, it was easy to accepting the prediction of positive for the target chemical.
- You are now ready to complete the final module and to create the report.
- Click on “Report” to proceed to the last module.

# Outlook

- Background
- Objectives
- Specific Aims
- Read across and analogue approach
- The exercise
- **Workflow**
  - Chemical Input
  - Profiling
  - Endpoint
  - Category definition
  - Data Gap Filling
  - **Report**

# Report Overview

- Report module could generate report on any of predictions performed with the Toolbox.
- Report module contains predefined report template which users can customize.
- Two type of report files are generated:
  - *Prediction report* – containing information for the target
  - *Data matrix* – containing information for the analogues used for the prediction.

# Report Generation report

The screenshot displays the QSAR Toolbox interface. At the top, the 'Report' module is selected in the main menu, indicated by callout 1. On the left, the 'Documents' panel shows a tree structure with 'Prediction' highlighted, indicated by callout 2. A central dialog box titled 'Customize report content and appearance' is open, showing a 'Wizard pages' list and a list of report sections with checkboxes. The 'Create report' button at the bottom of the dialog is highlighted with a red box and callout 3. The background shows a data table with columns for target, prediction, and various assay results.

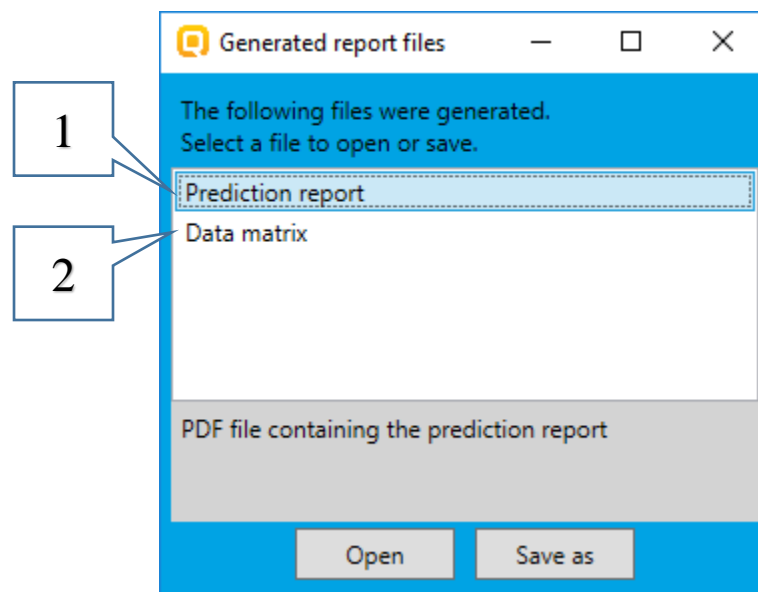
1. Go to the **Report** module; 2. Click on **Prediction**; 3. Click on **Create report**

# Report

## Generation report

After the click on the Create report button, *Generated report files* window appears. It contains two type of files:

- 1) **Prediction report** - a PDF file containing the prediction information related to the target.
- 2) **Data matrix** - a MS Excel file containing chemicals used for prediction along with their data for selected parameters, profiles and endpoint tree positions.



# Report Generated report files

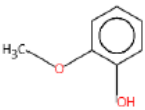
## Prediction report

Prediction of EC3 for guaiaicol 1 / 7

**QSAR Toolbox prediction for single chemical**

Date: 29 Jul 2017  
 Author(s):  
 Contact details:

**Target information**

| Structural information   | Numerical identifiers                   |
|--|---|
| SMILES:<br><chem>COc1ccccc1O</chem>  | EC#: N/A<br>CAS#: 90-05-1<br>Other: N/A |
| Structure<br> |   |

**Prediction summary**

**Predicted endpoint:** EC3; No effect specified; No species specified; No duration guideline specified

**Predicted value:** Positive

**Unit/scale:** Skin sensitisation II (ECETOC)

**Data gap filling method:** Read-across analysis

**Summary:** manually editable field  
 Not provided by the user

## Data matrix report

| Data matrix_29_7_17_15_33_09_(1).xlsx |  |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
|---------------------------------------|--|--|--|--|--|--|--|--|---|--------------|---|--------------|---|---|---|---|---|---|---|---|---|--|
|                                       | A  | B  | C  | D  | E  | F  | G  | H  | I | J            | K | L            | M | N | O | P | Q | R | S | T | U |  |
|                                       | Target chemical  |  |  | Neighbour #1   |  | Neighbour #2   |  | Neighbour #3   |   | Neighbour #4 |   | Neighbour #5 |   |   |   |   |   |   |   |   |   |  |
| 1                                     |  |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 2                                     | Substance identity   |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 3                                     | Structure  |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 4                                     | CAS number   | 90-05-1  | 91-10-1  | 452-86-8   | 488-17-5   | 123-31-9   | 108-46-3   |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 5                                     | Chemical name  | guaiaicol  | 2,6-Dimethoxyphenol  | 4-Methylcatechol   | 3-methylcatechol   | hydroquinone   | resorcin   |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 6                                     | Other identifier   |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 7                                     | SMILES   | <chem>COc1ccccc1O</chem>   | <chem>COc1ccc(O)c(O)c1</chem>  | <chem>Cc1ccc(O)c(O)c1</chem>   | <chem>Cc1ccc(O)c1O</chem>  | <chem>Oc1ccc(O)c1</chem>   | <chem>Oc1ccc(O)c1</chem>   |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 8                                     | Parameters   | unit   |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 9                                     | Profiles   |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 10                                    | Profiles used for grouping/subcategorization                             |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 11                                    | Grouping with metabolism: 'Skin metabolism simulator' (primary grouping) | Parent and 4 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes | Parent and 4 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found, Schiff base formation, Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes | Parent and 2 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found | Parent and 2 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found | Parent and 1 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found | Parent and 1 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found | Parent and 9 metabolites; Has all of the required categories: Michael Addition, Michael Addition >> Michael addition on quinoid type compounds, Michael Addition >> Michael addition on quinoid type compounds >> Quinone methide(s)/imines; Quinoid oxime structure; Nitroquinones, Naphthoquinone(s)/imines; Has the following additional categories: No alert found |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 12                                    |  | Phenols (Acute toxicity)   | Phenols (Acute toxicity)   | Phenols (Acute toxicity)   | Phenols (Acute toxicity)   | Phenols (Acute toxicity)   | Phenols (Acute toxicity)   |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 13                                    |  |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |
| 14                                    | US-EPA New Chemical Categories   |  |  |  |  |  |  |  |   |              |   |              |   |   |   |   |   |   |   |   |   |  |

# Congratulation

- You have now been introduced to the defining of target endpoint;
- You have now been introduced to the coloring of profilers and databases.
- You have now been introduced to the consecutive steps of the calculation of alert performance.
- Note proficiency comes with practice.