QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.3.0

Step-by-step example of how to categorize an inventory by mechanistic behaviour of the chemicals which it consists

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Outlook

- **Background**
- Objectives
- Specific Aims
- Trend analysis
- The exercise
- Workflow

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Background

- This is a step-by-step presentation designed to take Toolbox users through the workflow of categorizing an inventory using mechanistic similarity.

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Objectives

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

- Clustering chemicals from an imported inventory (chemical list).
- Selecting a cluster and place it into separate data matrix for later analyses.
- And, apply a trend analysis for aquatic toxicity to the selected cluster.

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Specific Aims

- To examine the workflow of clustering an inventory.
- To introduce the user to new functionalities within selected modules.
- To explain the rationale behind each step of the exercise.

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Trend analysis

- For a given toxicological endpoint, the members of a category are often related by a trend (e.g. increasing, decreasing or constant). The trend could be related to structural or chemical property.
- A demonstration of consistent trends in the behavior of a group of chemicals is one of the desirable attributes of a chemical category and an indicator that there is a common mechanism of action. When a consistent trend is observed and some chemicals in the category have measured values, missing values can be estimated by simple scaling from the measured values.

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The Exercise
Part I

- In this part of the exercise we will import a chemical list containing 300 substances, representing a typical inventory of chemicals used by a down stream user.
- The inventory will be loaded into the Toolbox data matrix.
- The inventory will be profiled by the "Aquatic toxicity classification by ECOSAR Classification" profiling scheme.
- This inventory will be clustered by the ECOSAR Classification grouping method.

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The Exercise
Part II

- In this part of the exercise an example cluster or defined category will be examined.
- Data on the chemicals which belong to selected cluster will be gathered.
- A data gap will be filled by trend analysis - In this exercise we will predict the toxicity towards mortality of the *Pimephales promelas* (LC50 for 24h).
- A report of the prediction will be generated.

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The Exercise
Necessary to download the file

- The inventory is stored in the following smi file: OECD Mock Inventory.smi*
- This file contains a list of Chemical Abstract Service (CAS) numbers, which can be opened with Notepad.

*OECD Mock Inventory.smi - file is packed in the zip file with all power point tutorials.

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Workflow

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

  Part I
  - Filling Data Gaps
  - Report

  Part II

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Outlook

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- **Workflow**
  - **Chemical Input**

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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 is the correct one.

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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, Einecs number

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

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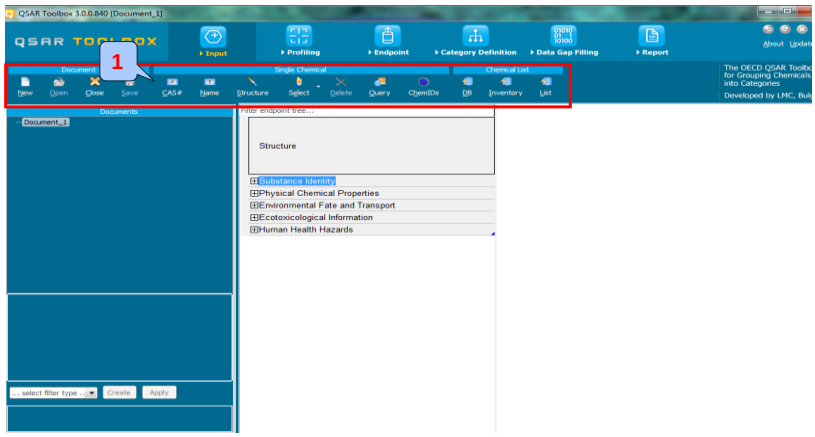
Chemical Input Screen
Input target chemical by CAS#



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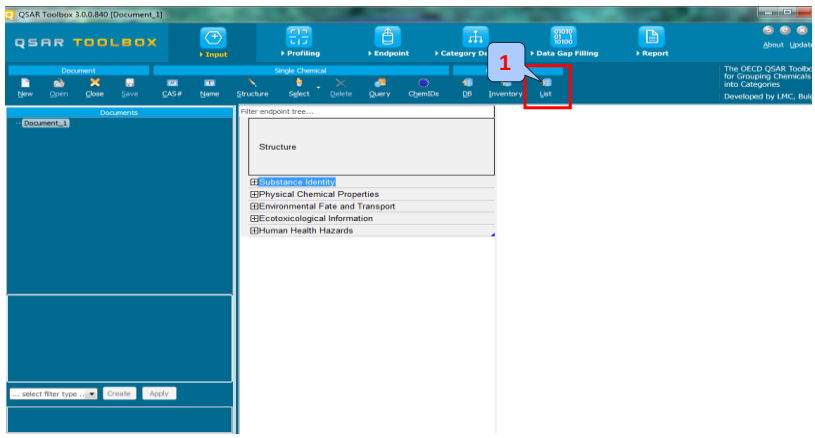
Chemical Input Screen
Input target chemical by CAS#



1. **Click** on List.

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Chemical Input Screen
Missing SMILES

- Since in the entered list there are CAS numbers for which there are no SMILES in the Toolbox, the user will be asked if he/she wants the Toolbox to search for in the databases. In this example select **NO**.

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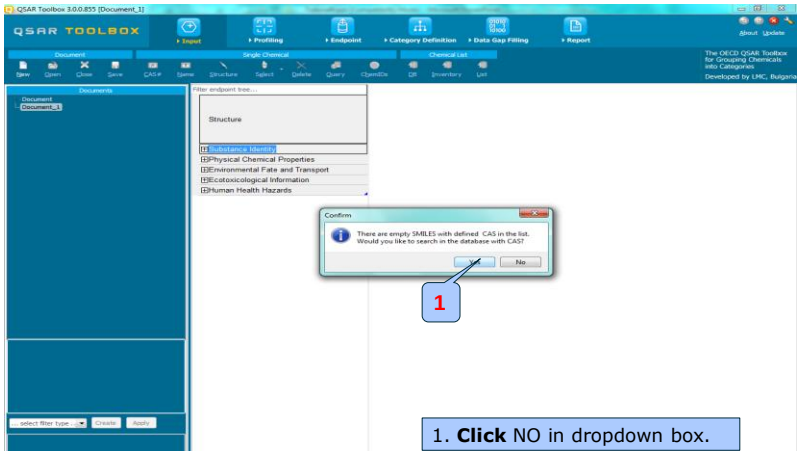
Chemical Input Screen
Missing SMILES



1. **Click** NO in dropdown box.

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Chemical Input Screen
Displaying structures

- The structures of the 300 chemicals are displayed with 2D image. The missing SMILES are displayed with empty window - for example chemical #110 (see next screen shot).
- These empty windows remind the user where are the gaps in the inventory.
- Remember all subsequent functions are based on chemical structure, so the chemicals with blank windows will not be placed in a cluster or category.

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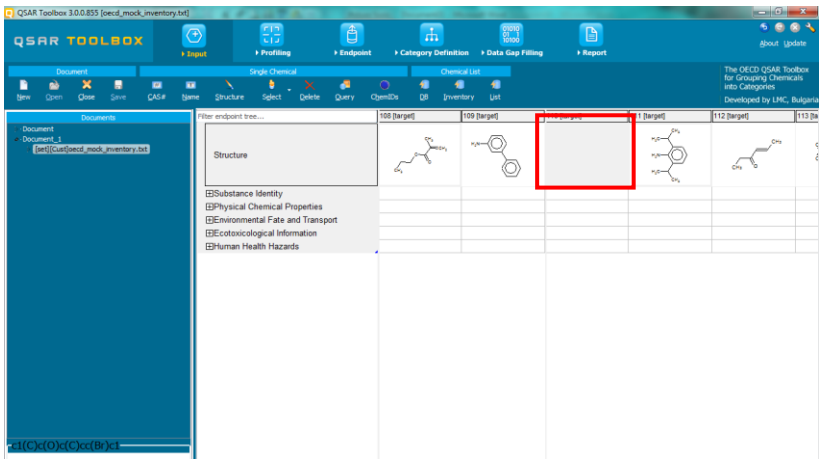
Chemical Input Screen
Displaying structures



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Chemical Input
Chemical Identity

- Double click "Substance Identity"; this displays the chemical identification information.
- Note that existing in the Toolbox names of target chemical are in different colors.
- The workflow on the first module is now complete, and the user can proceed to the next module.

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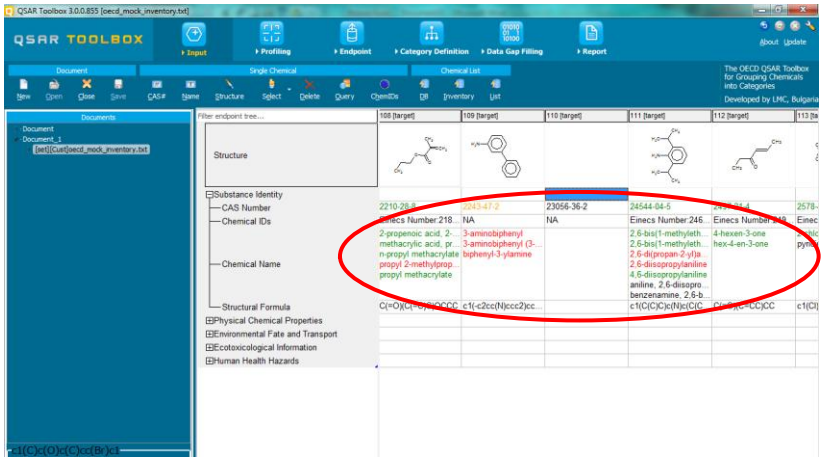
Chemical Input
Chemical Identity



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Chemical Input
Chemical Identity

The color code indicates the reliability of the chemical identifier:

- **Green**: There is a high reliability between the identifier and the structure. This color is attributed if the identifier is the same in several quality assured databases.
- **Yellow**: There is only a moderate reliability between the identifier and the structure. The color is attributed if the identifier is the same in several databases for which the quality assurance could not be established.
- **Red**: There is a poor reliability between the identifier and the structure. The color is attributed if the identifier is allocated to different structures in different databases.
- **Black**: The quality of the structure and its identifier is unknown.

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Outlook

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

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Profiling
Overview

- As you are aware the "profiling" refers to the electronic process of retrieving relevant information on the target compound(s), which are stored in the Toolbox other than fate and toxicity data.
- Key to this exercise is the information on likely mechanism(s) of action.

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Profiling
Side-Bar of Profilers

- Remember detailed explanation of the different profilers is available in the in Manual for getting started (Chapter 4). http://www.oecd.org/dataoecd/58/56/46210452.pdf
- Background information can be retrieved by highlighting a profiler and a click on the button "View".
- For grouping chemicals into meaningful categories for aquatic toxicity the "Aquatic toxicity classification by ECOSAR" is one of the most adequate scheme.

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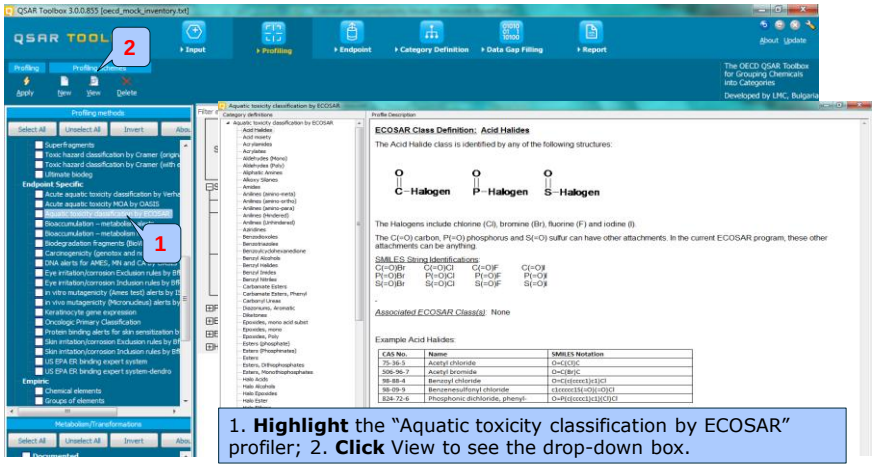
Profiling
Side-Bar of Profilers



1. **Highlight** the "Aquatic toxicity classification by ECOSAR" profiler; 2. **Click** View to see the drop-down box.

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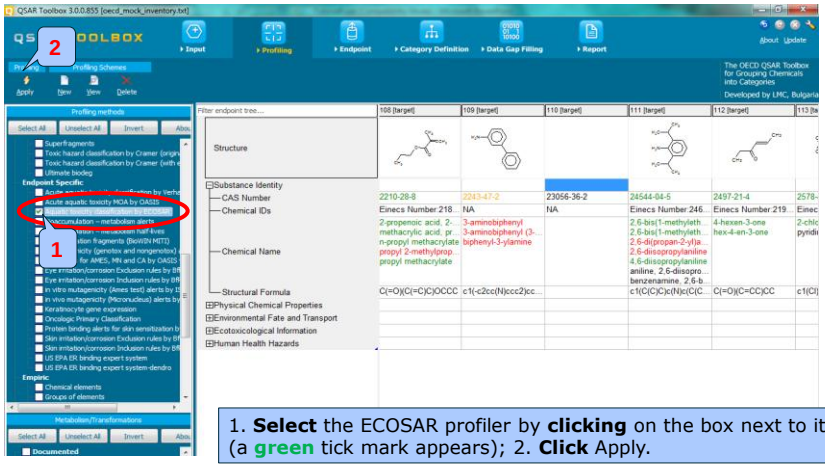
Profiling
Side-Bar of Profilers



1. **Select** the ECOSAR profiler by **clicking** on the box next to it (a **green** tick mark appears); 2. **Click** Apply.

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Profiling
Profiling results

- The actual profiling will take several seconds depending on the number and type of selected profilers.
- The results of profiling automatically appear as a dropdown box (see next screen shot).
- Please note the specific profiling results of Aquatic toxicity classification by ECOSAR.
- These results will be used to division chemicals from the inventory into suitable clusters in the next steps of the exercise.

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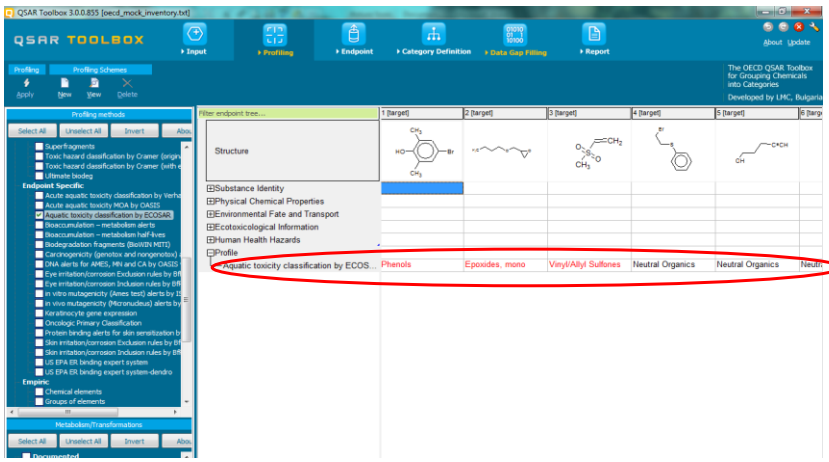
Profiling
Profiling results



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Outlook

- Background
- Objectives
- Specific Aims
- Trend analysis
- The exercise
- **Workflow**
  - Chemical Input
  - Profiling
  - **Endpoint**

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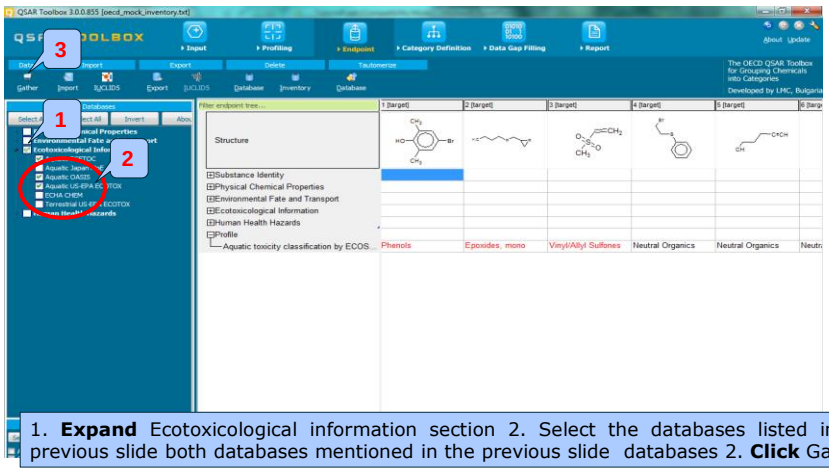
Endpoints
Overview

- "Endpoint" refers to the electronic process of retrieving the environmental fate and toxicity data stored in the Toolbox database.
- Data gathering can be executed in a global fashion (i.e., collecting all data of all endpoints) or on a more narrowly defined basis (e.g., collecting data for a single or limited number of endpoints).

Endpoints
Case study

- In this example, since we categorize an inventory for later analysis we do not need of data gathering.
- In this module we need only to select the Aquatic ECETOC, Aquatic OASIS and Aquatic US-EPA ECETOX databases.
- These databases contain results on aquatic toxicity for various species, and durations.
- Click on the "Category Definition" to move to the next module.

Endpoints
Case study



1. **Expand** Ecotoxicological information section 2. Select the databases listed in the previous slide both databases mentioned in the previous slide databases 2. **Click** Gather

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Outlook

- Background
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- The exercise
- **Workflow**
  - Chemical Input
  - Profiling
  - Endpoint
  - **Category definition**

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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 to define a category.

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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 trend-analysis.
- Detailed information about grouping chemical (Chapter 4) could be downloaded from:

  http://www.oecd.org/dataoecd/58/56/46210452.pdf

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Category Definition
Side bar to ECOSAR category

- ECOSAR has been used by the U.S. Environmental Protection Agency to predict the aquatic toxicity of new industrial chemicals in the absence of test data.
- SARs are developed for chemical classes (e.g. phenols) based on measured test data which have been submitted by industry or they were taken from other sources.
- The ECOSAR classification in the Toolbox is used for grouping chemicals by structural (and in some cases mechanistic) similarity.

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Category Definition
Clustering

The chemicals in the inventory can be clustered in to 55 clusters based on EcoSAR Categories.

- 72 are neutral organics.
- 30 are esters.
- 28 are phenols.
- 23 are anilines (unhindered).
- 14 are aldehydes (mono)
- 10 are aliphatic amines
- Other clusters consist of less than 10 compounds.

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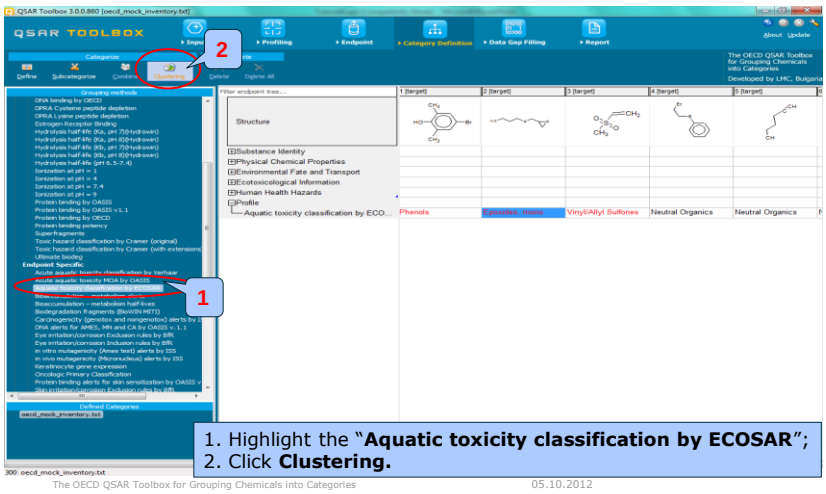
Category Definition
Clustering



1. Highlight the "**Aquatic toxicity classification by ECOSAR**";
2. Click **Clustering.**

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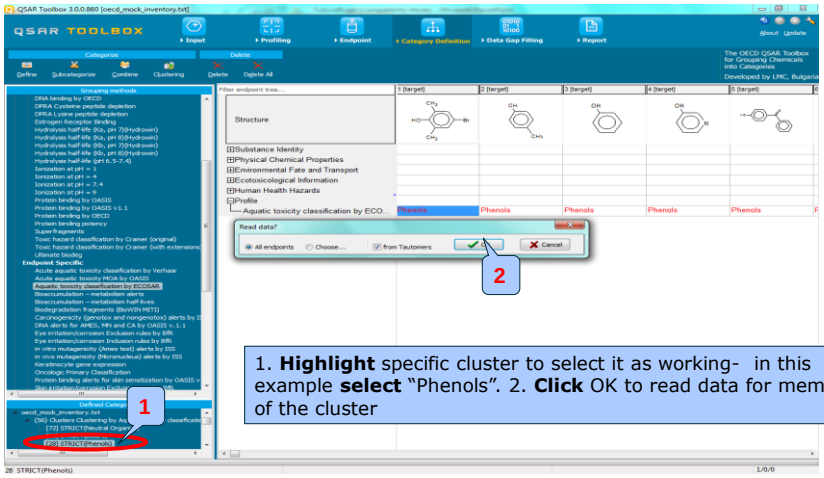
Category Definition
Select working cluster



1. **Highlight** specific cluster to select it as working- in this example **select** "Phenols". 2. **Click** OK to read data for members of the cluster

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Category Definition
Read data for the members of the cluster

- The chemicals which belong to the selected cluster are immediately loaded in a separate data matrix.
- The Toolbox automatically starts the process of data gathering. The user is asked to select the endpoint that should be retrieved.
- In " Read data?", the user can either "Choose" a specific endpoint or by default retrieve data on "All endpoints" which are stored in previously selected data bases (see below).

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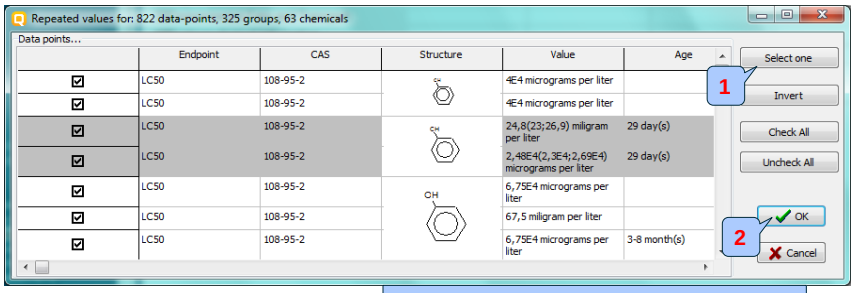
Category Definition
Read data for the members of the cluster

- Due to the overlap between the Toolbox databases same data for intersecting chemicals could be found simultaneously in more than one database. The data redundancy is identified and the user has the opportunity to select either a single data value or all data.



1. **Click** Select one and then; 2. **Click** OK.

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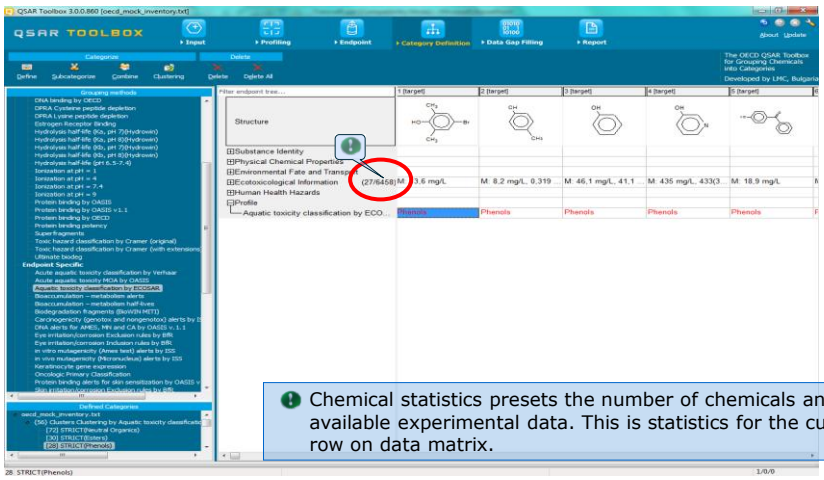
Category Definition
Select working cluster



Chemical statistics presets the number of chemicals and the available experimental data. This is statistics for the current row on data matrix.

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Category Definition
Navigation through the endpoint tree

- The user can navigate through the data tree by closing or opening the nodes of the tree.
- In this example, the LC50-24h Mortality for *Pimephales promelas* is the target endpoint.

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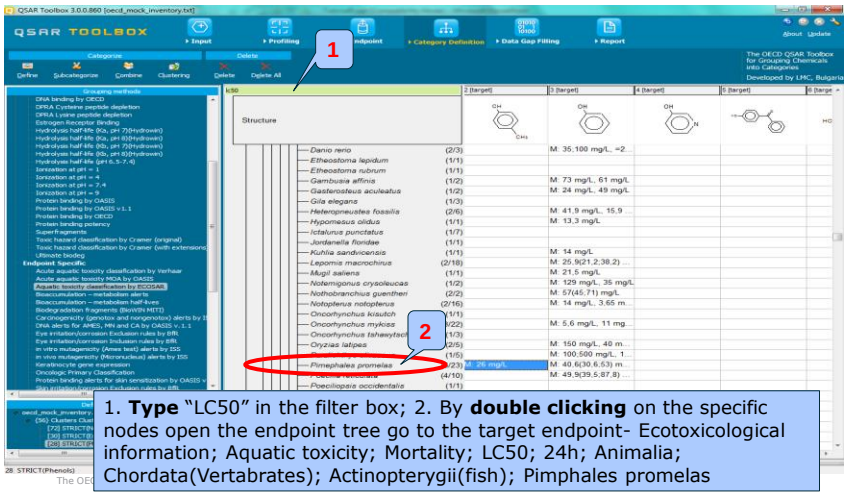
Category Definition
Navigation through the endpoint tree-use filter



1. **Type** "LC50" in the filter box; 2. By **double clicking** on the specific nodes open the endpoint tree go to the target endpoint- Ecotoxicological information; Aquatic toxicity; Mortality; LC50; 24h; Animalia; Chordata(Vertabrates); Actinopterygii(fish); Pimphales promelas

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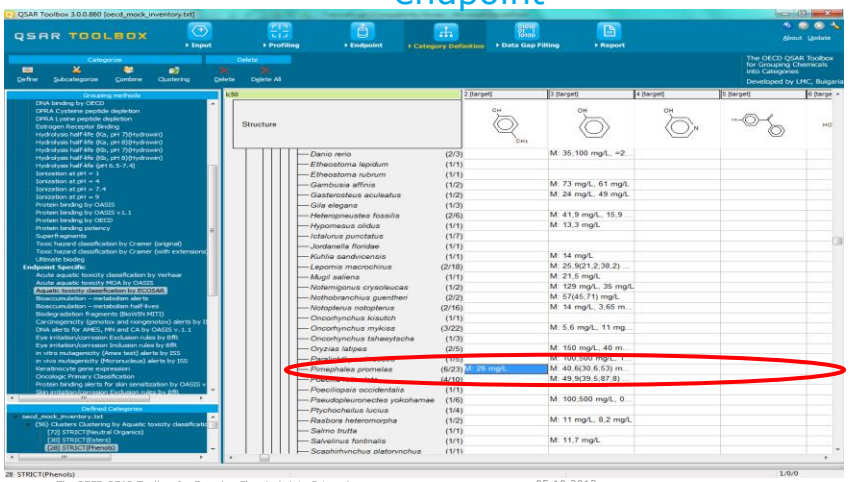
Category Definition

Navigation through the endpoint tree-target endpoint



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The Exercise - Part I

Recap

- We have loaded in the Toolbox data matrix a user predefined list of 300 chemicals.
- The inventory has been profiled by the "Aquatic toxicity classification by ECOSAR" scheme.
- The inventory has been clustered by the ECOSAR categorization method.
- The "Phenols" cluster has been selected as the working cluster and experimental data have been gathered for all its members.
- Now we are ready to proceed with the Part II of the exercise.

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- **Workflow**
  - Chemical Input
  - Profiling
  - Endpoint
  - Category definition
  - **Data Gap Filling**

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Data gap filling
Overview

- "Data Gap Filling" module give access to three different data gap filling tools:
  - Read-across
  - Trend analysis
  - (Q)SAR models
- Select the most relevant data gap method:
  - Read-across is the appropriate data-gap filling method for "qualitative" endpoints where possible results are positive, negative or equivocal. Read-across is also recommended for "quantitative endpoints" (e.g., 96h-LC50 for fish) when there are few analogues with experimental results.
  - Trend analysis is the appropriate data-gap filling method for "quantitative endpoints" (e.g., 96h-LC50 for fish) when a high number of analogues with experimental results.
  - "(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 trend analysis.

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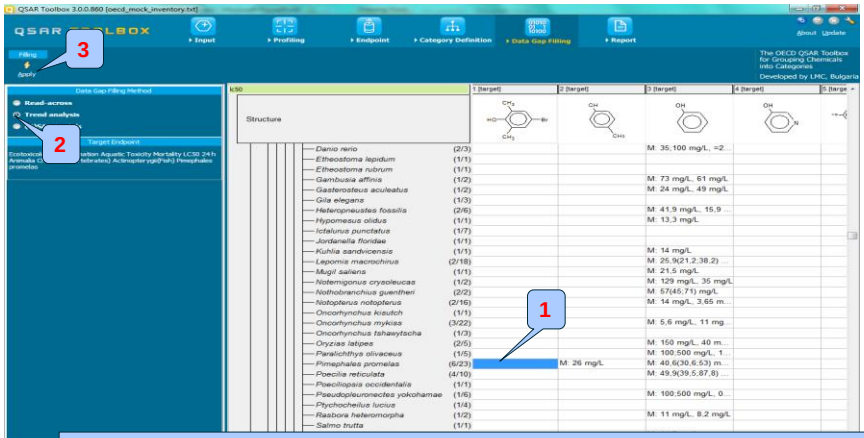
Data gap filling
Apply Trend analysis



1. **Highlight** the data endpoint box corresponding to the target endpoint under 4-bromo-2,6-dimethylphenol; 2. **Select** Trend analysis**; 3. Click** Apply**.**

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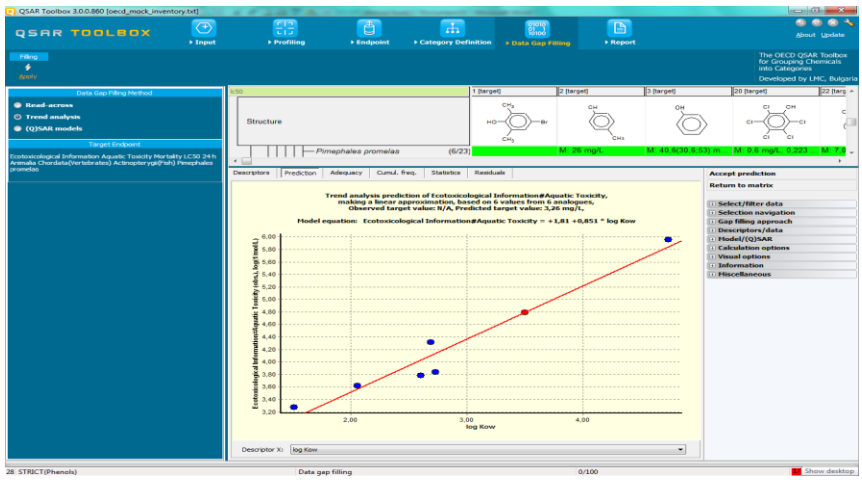
Data gap filling
Trend analysis



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Data gap filling
Interpreting Trend analysis

- The resulting plot outlines the experimental LC50 results of all analogues (Y axis) according to a descriptor (X axis) with Log Kow being the default descriptor.
- The **RED** dot represents the estimated result for the target chemical.
- The **BLUE** dots represent the experimental results available for the analogues.

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Data gap filling
Analysis of the results

- The resulting trend analysis reveals a consistent trend in results with toxicity increase linearly with log Kow.
- It can be used to fill the data gap.
- By extension it can be used to fill data gaps for this endpoint for all chemicals in this cluster (i.e., category).
- See also the presentation "How to build user defined QSAR model" available at the OECD website.
- Accept the prediction (see next screen shot).

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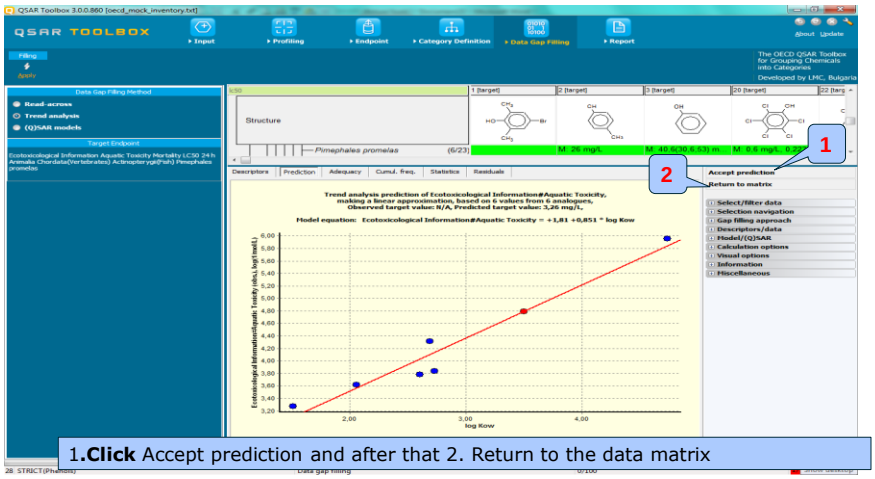
Data gap filling
Accepting the prediction result



1.**Click** Accept prediction and after that 2. Return to the data matrix

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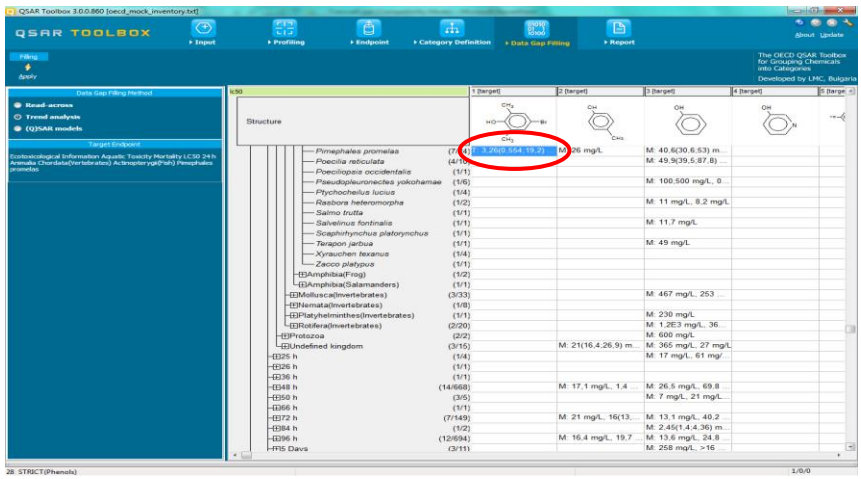
Data gap filling
Accepting the prediction result



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Filled data gap

- By accepting the prediction the data gap was filled (see next screen shot).
- You are now ready to complete the final module and to download the report.
- Click on "Report" to proceed to next module.

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

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Report
Overview

- The Report module can generate a report on any of predictions performed with the Toolbox.
- This module contains predefined report templates as well as a template editor which allows for development of user defined templates.
- The report can be printed or saved in different formats.

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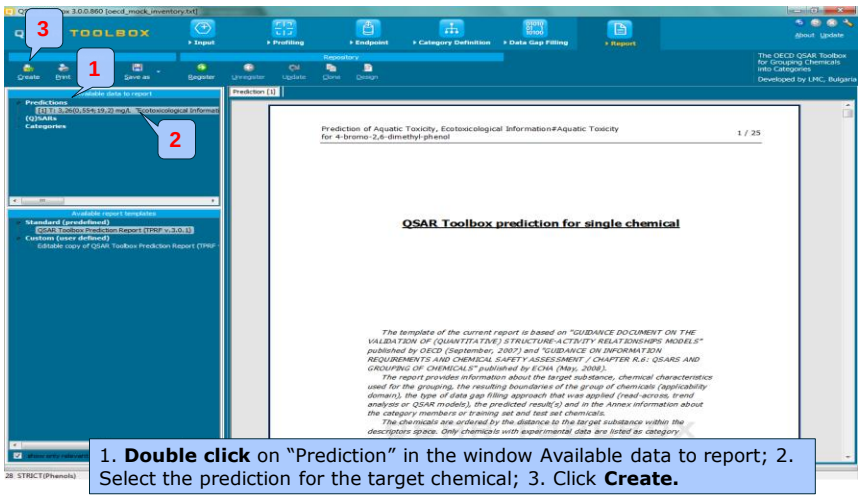
Report
Generate report.



1. **Double click** on "Prediction" in the window Available data to report; 2. Select the prediction for the target chemical; 3. Click **Create.**

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Congratulation

- You have used the Toolbox to categorize an inventory by mechanistic behavior.
- You now know another useful tool in the Toolbox.
- Continue to practice with this and other tool and soon you will be comfortable dealing with many situations where the Toolbox is useful.