

OECD QSAR Toolbox v.3.0

Step-by-step example of how to build and evaluate a category based on mechanism of action with protein and DNA binding

Outlook

- **Background**
- Objectives
- Specific Aims
- The exercise
- Workflow of the exercise

Background

- This is a step-by-step presentation designed to take you through the workflow of the Toolbox in building a category and then performing a preliminary evaluation of the category.
- By now you have experience in using the Toolbox so there will be multiple key strokes between screen shots.

Outlook

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Objectives

- **This presentation demonstrates:**
- Identifying chemicals which could be grouped into a category.
- Conducting a preliminary evaluation of the category.

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Specific Aims

- To examine the workflow of building a category.
- To introduce the user to new functionalities within selected modules.
- To explain the rationale behind each step of the exercise.
- To demonstrate with a practical example how to use the Toolbox to build a category according to the OECD Guidance on Grouping of Chemicals.

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Exercise

- In this exercise we will build a category around the target chemical 3-mercapto-propionic butyl ester.
- We will performed this by first categorizing using Protein binding by OASIS v1.1 and subsequently subcategorize using EcoSAR classification.
- We will perform a preliminary evaluation of the final category for Ames mutagenicity and skin sensitization.
- The predictions will be made by "read-across" analysis.

Exercise

Side-Bar: Developing a Category Based on Mechanism of Action

- First identify the mechanism and mode of action of a representative member of the category, by profiling the chemical.
- If a specific mechanism or mode is identified, then it is recommended to base the category definition on this mechanism or mode.
- Other members of the category can be found by searching for chemicals which have the same mechanism or mode of action.
- The search results can then be refined by eliminating chemicals which are structurally dissimilar.

Exercise

Side-Bar: Developing a Category Based on Mechanism of Action

- If no specific mechanism or mode of action is identified for a representative member of the category, then it is recommended to base the category definition on close structural similarity.
- In this case members of the category can be found by searching for chemicals which are structurally similar to the target chemical.
- The search results can then be refined by eliminating those chemicals which have specific mechanisms or modes of action.

Outlook

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Workflow of the exercise

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

Outlook

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- **Workflow of the exercise**
 - **Chemical Input**

Chemical Input Overview

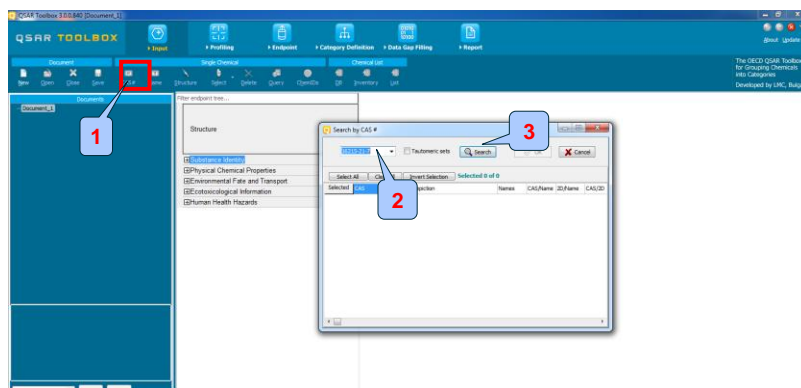
- This module provides the user with several means of entering the chemical of interest or the target chemical.
- Since all subsequent functions are based on chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Chemical Input Ways of entering chemicals

- **Remember there are several ways to enter a target chemical and the most often used are:**
 - CAS#,
 - SMILES (simplified molecular information line entry system) notation, and
 - Drawing the structure.
- **Click** on **CAS #**
- **Enter** **16215-21-7**.
- **Click** **Search**. (see next screen shot).

Chemical Input

Input target chemical by CAS#

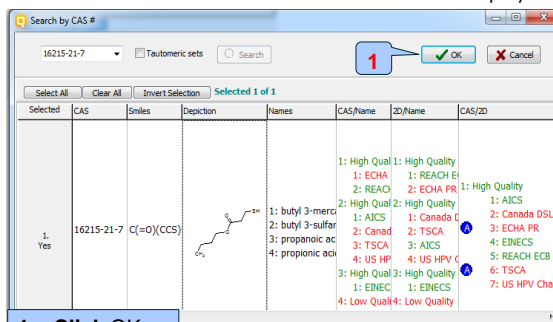


1. Click on CAS#; 2. Enter 16215-21-7; 3. Click Search.

Chemical Input

Target chemical identity

The Toolbox now searches the Toolbox databases and inventories for the presence of the chemical with structure related to the current CAS #. It is displayed as a 2D image.



1. Click OK



In case a structure has several CAS numbers or a structure could be related to more than one substance (e.g. in the case of compounds), more than one chemical identity could be retrieved. In this case the user can decide which substance is to be retained for the subsequent workflow.

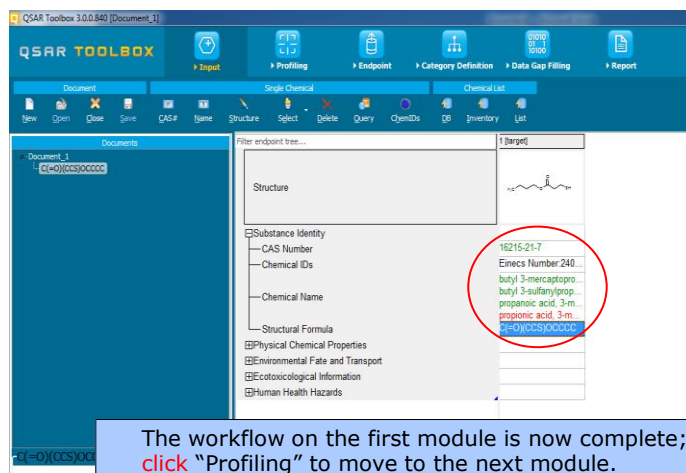
Chemical Input

Target chemical identity

- You have now selected your target chemical and have its structure.
- Remember from here on the workflow will be based on the structure coded in SMILES.
- **Click** on the box next to "Substance Identity"; this displays the chemical identification information. (see next screen shot).

Chemical Input

Target chemical identity



The screenshot shows the QSAR Toolbox 3.0.0.840 interface. The 'Substance Identity' section is expanded, and the 'Eninec Number 240...' entry is circled in red. The text box at the bottom of the screenshot reads: "The workflow on the first module is now complete; click "Profiling" to move to the next module."

Outlook

- Background
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- **Workflow of the exercise**
 - Chemical Input
 - **Profiling**

Profiling Overview

- As you remember "Profiling" refers to the process of retrieving information on the target compound, other than fate and toxicity data.
- Key available information includes likely mechanism(s) of action.
- Background information on a profiler can be viewed by **highlighting** a profiler and **clicking** on "View".

Profiling

Profiling the target chemical

- The outcome of the profiling determines the most appropriate way to search for analogues (detailed information in Manual for getting started (Chapter 4) <http://www.oecd.org/dataoecd/58/56/46210452.pdf>)
- Table 4-1 in chapter 4 (Manual for getting started) lists a selection of profilers and their relevance for different endpoints of regulatory relevance.
- For this example the following mechanistic and endpoint specific profiling methods should be selected :
 - Protein binding by OASIS v1.1 – mechanistic grouping
 - Protein binding by OECD – mechanistic grouping
 - DNA binding by OASIS v.1.1 – mechanistic grouping
 - DNA binding by OECD – mechanistic grouping
 - Organic functional groups
 - Organic functional groups(nested)
 - Acute aquatic toxicity MOA by OASIS
 - Aquatic toxicity classification by ECOSAR
 - Superfragments

Profiling

Profiling the target chemical

- **Select** the “Profiling methods” related to the target endpoint.
- When a profiler is selected a **green** check mark appears (when deselected the **green** check disappears).
- For this example, select the profilers relevant to the genetic toxicity (see next screen shot):
 - Protein binding by OASIS v1.1 – mechanistic grouping
 - Protein binding by OECD – mechanistic grouping
 - DNA binding by OASIS v.1.1 – mechanistic grouping
 - DNA binding by OECD – mechanistic grouping
 - Organic functional group
 - Organic functional groups(nested)
 - Acute aquatic toxicity MOA by OASIS
 - Aquatic toxicity classification by ECOSAR
 - Superfragments

QSAR TOOLBOX

Profiling

Profiling the target chemical

1. Check the profilers related to the target endpoint;
2. Click Apply.

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QSAR TOOLBOX

Profiling

Profiling the target chemical

- The actual profiling will take several seconds depending on the number and type of selected profilers.
- The results of profiling automatically appeared as a dropdown box under the target chemical. (see next screen shot).
- Very specific results are found with the ECOSAR Classification and the Protein Binding profilers.
- These results will be used later in the exercise to build the category.

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Profiling Profiles of "butyl 3-sulfanylpropanoate"

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Profiling Profiles of "butyl 3-sulfanylpropanoate"

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In this case there is structural evidence that the target could interact with DNA and proteins and also has esters and thiol alerts in its structure.

This step is critical for next grouping of analogues.

1. Right click to see why the target is protein binder
2. Select "SN2 -> Interchange reaction with sulphur containing compounds -> Thiols and disulfide compounds". Then click details(see next screen shot).

QSAR TOOLBOX

Profiling Protein binding by OASIS of "butyl 3-sulfanylpropanoate"

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QSAR TOOLBOX

Outlook

- Background
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- **Workflow of the exercise**
 - Chemical Input
 - Profiling
 - **Endpoints**

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Endpoints Overview

- Remember, "Endpoints" refer to the electronic process of retrieving the fate and toxicity data that are stored in the Toolbox.
- Data gathering can be executed in a global fashion or on a more narrowly defined basis.
- Since we are forming a category used further to predict mutagenicity and skin sensitisation, we want to query databases containing mutagenic and skin sensitisation measured data in an effort to gather data for the target chemical.
- Please also remember that when querying for members of the category the Toolbox will search for chemicals which are listed in the selected databases.

Endpoints Expanding the query domain

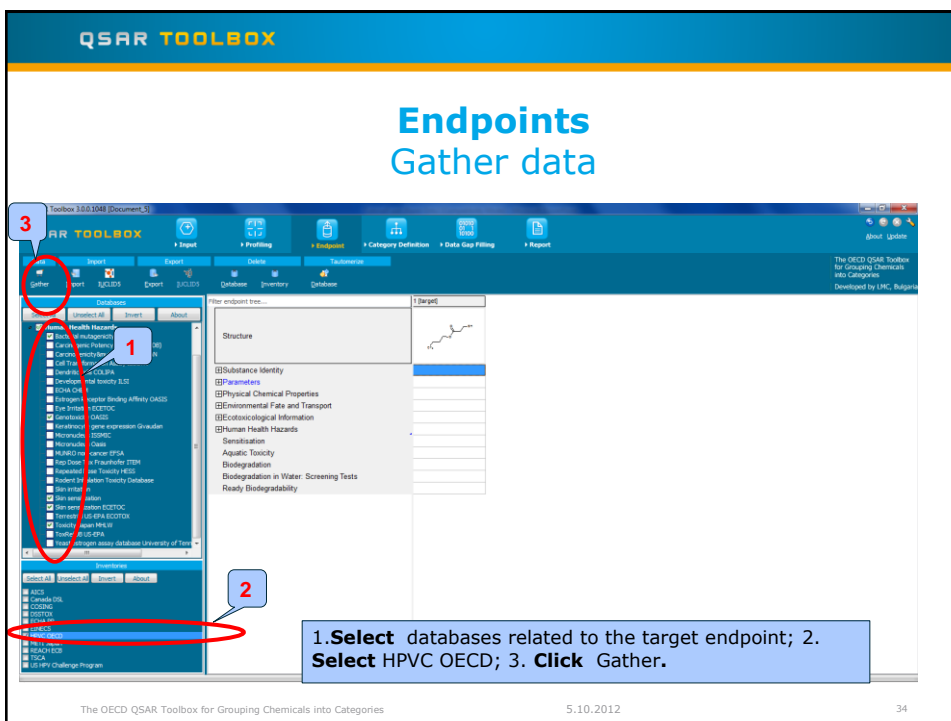
- When building a category, we are also interested in finding chemicals for which no experimental data are available, but which fit into the category, and thereby could be assessed as part of the category.
- We therefore have to define the relevant inventory in which to search for chemicals that could be grouped with the target compound.
- For example, the user can choose to search for chemicals in a national index like the US-TSCA inventory or EU EINECS or in more restricted inventories like the OECD HPV list.
- Remember that the process of searching in inventories is time consuming.

Endpoints Case study

- In this example, we conduct an expanded search for chemicals belonging to a category.
- Among the inventories, select the "OECD HPVC Inventory".
- **Click** "Gather data".
- You will find that no experimental results are available for this chemical.

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Endpoints Gather data



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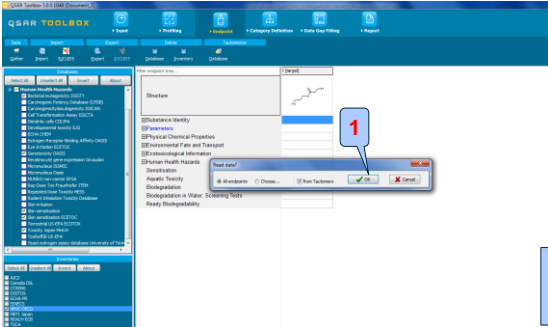
1. Select databases related to the target endpoint; **2. Select** HPVC OECD; **3. Click** Gather.

Q SAR TOOLBOX

Endpoints

Process of collecting data

- Toxicity information on the target chemical is electronically collected from the selected datasets.
- A window with "Read data?" appears. Now the user could choose to collect "all" or "endpoint specific" data.



1. Click OK to read all available data.

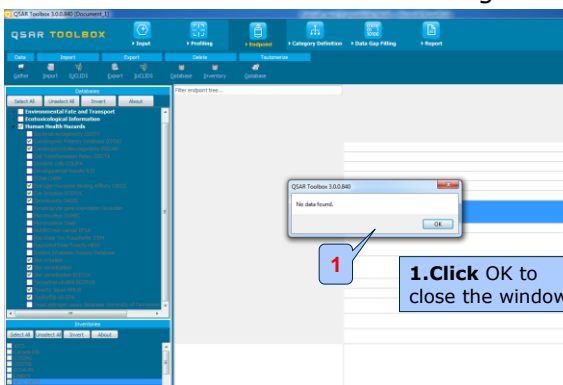
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Q SAR TOOLBOX

Endpoints

Process of collecting data

In this example, an insert window appears stating there was "no data found" for the target chemical.



1. Click OK to close the window.

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Endpoints Recap

- You have entered the target chemical being sure of the correct structure.
- You have profiled the target chemical and found no experimental data is currently available.
- You have checked the databases related to mutagenicity and skin sensitisation experimental data.
- You have defined the inventory in which you want to search for chemicals belonging to the category.

Outlook

- Background
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- The exercise
- **Workflow of the exercise**
 - Chemical Input
 - Profiling
 - Endpoints
 - **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 defining 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.
- Detailed information about grouping chemical (Chapter 4) could be downloaded from:
<http://www.oecd.org/dataoecd/58/56/46210452.pdf>

Category definition Case study

- For this example, the user could first select the Protein binding by OASIS v1.1 mechanism of the target chemical and query for all the chemicals with the same mechanism in the selected inventory and databases (see next screen shot).
- The user has first to query according to one profiler and then subcategorise the results step-by-step according to other profilers.

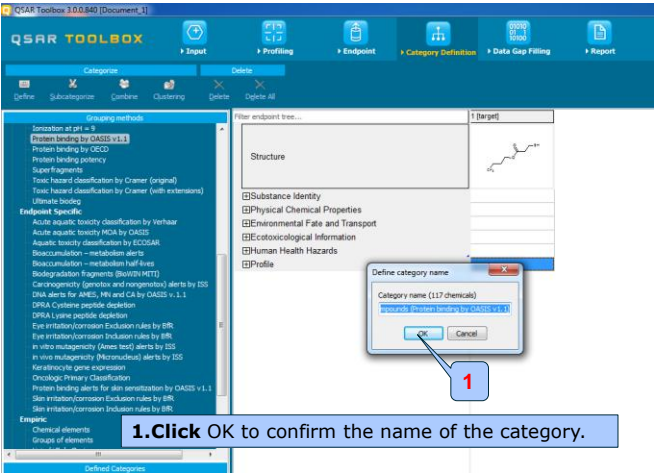
Category definition Defining Protein binding by OASIS v1.1 category

1. **Highlight** "Protein binding by OASIS v1.1"; 2. **Click** Define, the message that grouping could be slow due to selected inventories appears; 3. **Click** Yes; 4. **Confirm** the category of the target and **click** OK.

Q SAR TOOLBOX

Category definition

Defining Protein binding by OASISv1.1 category



1. Click OK to confirm the name of the category.

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Q SAR TOOLBOX

Category definition

Defining Protein binding by OASIS category

- The Toolbox now identifies all chemicals with structural fragment **"Thiols and disulfide compounds"** corresponding to mechanism **"Interchange reaction with sulphur containing compounds"** and domain **"SN2"** by Protein binding by OASIS v1.1 listed in the databases selected under "Endpoints".
- 117 analogues are identified. Along with the target they form a mechanistic category, used for gap filling.
- The name of the category appear in the "Defined Categories" window, indicating the number of substances belonging to the category.

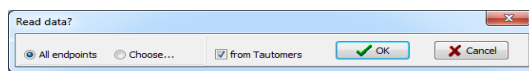
[117] SN2<AND>SN2 >> Interchange reaction with sulphur containing compounds<AND>SN2 >> Interchange reaction with sulphur containing compounds >> Thiols and disulfide compounds [Pr

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Category definition

Reading data for Analogues

- The Toolbox will now retrieve those chemicals that have the same protein binding mechanism (disulfide formation) as the target compound.
- The Toolbox automatically request 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).

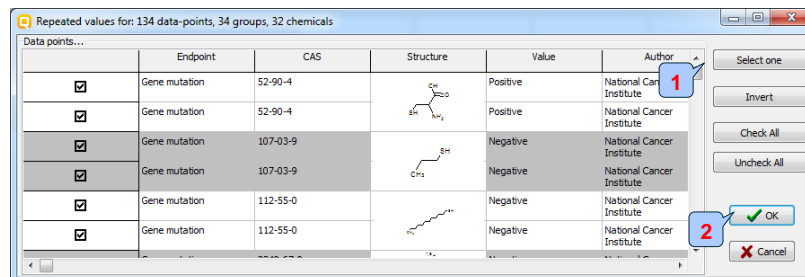


- Note that in this example, as only databases are selected that contain information for genetic toxicity endpoint, both options give the same results.

Category definition

Reading data for Analogues

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

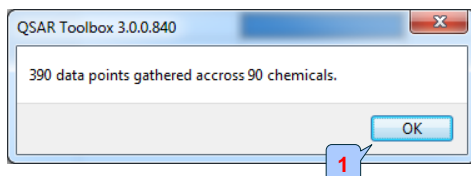


1. Click Select one and then 2. Click OK

Category Definition

Read data for Analogues

The system automatically gives indication for the number of gather experimental data points



1. Click OK

Category definition

Summary information for Analogues

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

Endpoint	1	2	3	4	5	6	7	8
Structure	<chem>CCCCO</chem>	<chem>CC(O)C</chem>	<chem>O=C1C=CC(=O)N1</chem>	<chem>CC1=CC=CC=C1</chem>	<chem>CC1=CC=C(C=C1)O</chem>	<chem>CC1=CC=C(C=C1)N</chem>	<chem>CC1=CC=C(C=C1)S</chem>	<chem>CC1=CC=C(C=C1)F</chem>
EPSubstance Identity								
EPPhysical Chemical Properties								
EPEnvironmental Fate and Transport								
EPToxicological Information								
EPHuman Health Hazards								
EPHazard	(0/0/0/0)	M. Negative, Positiv...	M. Negative, Negati...	M. Positive, Negati...	M. Negative, Negati...	M. Negative	M. Negative, Negati...	M. Negative, Negati...

Q SAR TOOLBOX

Category definition

Side-bar of experimental data

The screenshot shows the Q SAR Toolbox interface with the 'Category Definition' window open. The 'Side-bar of experimental data' is visible, showing a list of chemical structures and their corresponding experimental data points. A red circle highlights a cell in the 'Data points' table, and a callout box with the number '1' points to it. The callout text reads: '1. Double-click on the cell with measured data to see detailed information for the data points.'

The 'Data points' table is as follows:

#	Endpoint	Value	Original value	Strain	Type of method	Source of metabolic system	Title
1	Gene mutation	Negative (Gene mutation 0)	Negative (Gene mutation 0)	TA 100	In Vitro		Short-Term Testing Program in the National
2	Gene mutation	Positive (Gene mutation 0)	Positive (Gene mutation 0)	TA 100	In Vitro	Source of metabolic system	Testing Program in the National
3	Gene mutation	Negative (Gene mutation 0)	Negative (Gene mutation 0)	TA 98	In Vitro		Short-Term Testing Program in the National
4	Gene mutation	Negative (Gene mutation 0)	Negative (Gene mutation 0)	TA 98	In Vitro	Source of metabolic system	Short-Term Testing Program in the National
5	Gene mutation	Positive (Gene mutation 0)	Positive (Gene mutation 0)	No strain info	In Vitro	- rat	Derivation and validation of isocaproates for

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Q SAR TOOLBOX

Category definition

Side-bar of experimental data

- You have identified a mechanistic category consisting of 117 analogous (**Protein thiol-disulphide interchange**) by Protein binding by OASIS v1.1 classification.
- The available experimental data for these 117 similar chemicals are collected from the previously selected databases under Endpoint section.
- The user can proceed with subcategorisation process.

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Category definition

Categorization by ECOSAR

- After the available data has been retrieved, the user can then further subcategorize the results according to "EcoSAR Classification".
- These steps are summarized in the next screen shot.

Category definition

Subcategorization by ECOSAR by "All categories"

1. Select current category; 2. Click Subcategorise ; 3. Select ECOSAR profiler;

4. Select "All categories"; 5. Remove; 6. Confirm the new category by clicking OK.

Category Definition

The result of subcategorization by ECOSAR by "All categories"

- In this example, the retrieved chemicals have identical mechanistic profiles.
- The number of chemicals retrieved is therefore low.
- One could consider building the category allowing for two subcategories to remain.
- For example, the user could decide to build a category with the same protein binding mechanism but allowing chemicals belonging to either one of the two EcoSAR classes.
- This is done by selecting the radio-button "At least one category" and "pruning" all others (see next screen shot).

Category Definition

The result of subcategorization by ECOSAR by "At least one"

1. Click on first category 2. Click Subcategorise; 3. Select ECOSAR profiler; 4. Select "At least one category"; 5. Remove; 6. Confirm name of new category and click OK.

Category Definition

The result of subcategorization by ECOSAR by "At least one"

- The result of the second subcategorisation is a chemical category with 35 members along with the target (see next screen shot).
- After identifying category members according to a specific mechanism or mode of action it is always necessary to verify whether any of the selected chemicals have additional mechanisms or modes of action, which would make them unsuitable for the category. This can be done by using the "Subcategorisation" procedures.
- For example, there could be chemicals that have specific DNA binding mechanisms, due to additional functional groups in the molecule (this is demonstrated in the next screen shot).

Category Definition

Verifying of identified category

1. Select current category; 2. Click Subcategorise; 3. Select DNA binding by OASIS v1.1; 4. Remove dissimilar to the target chemicals; 5. Confirm name of new category by clicking OK.

QSAR TOOLBOX

Category Definition

The results of eliminating dissimilar chemicals

The screenshot shows the QSAR Toolbox interface with the 'Category Definition' step selected. The left sidebar lists various grouping methods, including 'Hydroxyl half-life (log₁₀ pH (hydroxyl))', 'Hydroxyl half-life (log₁₀ pH (hydroxyl))', 'Hydroxyl half-life (log₁₀ pH (hydroxyl))', 'Ionization at pH = 1', 'Ionization at pH = 4', 'Ionization at pH = 7.4', 'Ionization at pH = 11', 'Protein binding by CAS2 v1.1', 'Protein binding by CAS2', 'Protein binding by CAS2', 'Superfragments', 'Toxic hazard identification by Cancer (original)', 'Toxic hazard identification by Cancer (with-endpoints)', 'Alkylene binding', 'Endpoint Specific', 'Acute aquatic toxicity classification by hazard', 'Acute aquatic toxicity MOA by CAS2', 'Aquatic toxicity classification by ECOSAR', 'Bioaccumulation - metabolic alerts', 'Bioaccumulation - metabolic alerts', 'Biodegradation: Fragment (BioBiot-NTD)', 'Carcinogenicity: Genotoxic and nongenotoxic alerts by QSAR', 'Data alerts for Ames, Ames and CA by CAS2 v1.1', 'DNA Cytosine methylase disruption', 'DNA Cytosine methylase disruption', 'Default Categories', '1117 [2]-[2]-[2] >> Interchange reaction with sulfur content', '[2] Subcategory: Aquatic toxicity classification by ECOSAR', '[2] Subcategory: Aquatic toxicity classification by ECOSAR', '[2] Subcategory: DNA binding by CAS2 v1.1'.

The main window displays a table of chemical structures and their associated hazard endpoints. The table has columns for 'Structure', 'Target', and 'Endpoint'. The 'Structure' column shows chemical structures for various thiol compounds. The 'Target' column shows 'A' and 'B'. The 'Endpoint' column shows 'M. Positive, Ne', 'M. Negative', 'M. Negative, Negati.', 'M. Negative, Negati.', 'M. Negative'.

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QSAR TOOLBOX

Category Definition

Recap

- In this example, no outliers in terms of mechanism of action are identified and no chemicals will be eliminated from the category.
- The result is a group of chemicals that can bind to protein by the same mechanism (disulfide formation) and that belong to either the EcoSAR class(es) of "Thiols (mercaptans)" or "Esters AND Thiols (mercaptans)".
- Chemicals with other specific mechanisms or modes of actions have been eliminated so it is expected that the remaining chemicals have similar behaviour for many regulatory endpoints.
- Note that for aquatic toxicity, it is expected that differences in trends could be observed between chemicals belonging to the EcoSAR class(es) of "Thiols (mercaptans)" or "Esters AND Thiols (mercaptans)" and therefore these should be considered as two subcategories.

The OECD QSAR Toolbox for Grouping Chemicals into Categories
19.07.2011
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QSAR TOOLBOX

Category Definition

Limited number of experimental data

- While the Toolbox has identified 34 chemicals which belong to the category, the amount of available experimental results is very limited for this category.
- The statistics if category consisting of 34 analogues (indicated on the left of the data matrix) shows more limited experimental data in comparison with category of 35 analogues. (see next screen shot).

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19.07.2011
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QSAR TOOLBOX

Category Definition

Limited number of experimental data

The statistics shows differences in two fields: Genetic toxicity and Skin sensitisation .

The OECD QSAR Toolbox for Grouping Chemicals into Categories
19.07.2011
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Category Definition

Preliminary evaluation of the category

- In this particular example, insufficient data is available to fill data gaps and further testing may be necessary.
- Nevertheless, for Ames mutagenicity and sensitisation, the coherence and consistency of the available data can be assessed.
- Regarding point mutation according to the Ames test, the Toolbox has identified 15 chemicals across category consisting of 38 analogues for which results are available. (see next screen shot).
- As point mutation is a “qualitative” endpoint, the data gap can be filled by read-across.

Category Definition

Selecting Data Point for AMES mutagenicity

- In this example **navigate** through the endpoint tree by opening the nodes of tree.
- **Highlight** the blank space for “AMES mutagenicity” under the target chemical.
- In this case we miss all experimental results with different metabolic activation(see next screen shot).

Outlook

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- The exercise
- **Workflow of the exercise**
 - Chemical Input
 - Profiling
 - Endpoints
 - Category definition
 - **Data gap filling**
 - Ames

Data Gap Filling(Ames)

Apply read-across

The screenshot shows the QSAR Toolbox software interface. The main window displays a data matrix for Ames test results. The columns are labeled 'Structure', 'Target', and various Ames test endpoints. The rows represent different chemical structures. A red circle highlights the 'Data Gap Filling' button in the menu bar (labeled '3'). A red circle highlights a cell in the data matrix corresponding to the Ames test endpoint for a specific chemical (labeled '2'). A red circle highlights the 'Ames test' endpoint in the sidebar (labeled '1').

Structure	Target	Ames test	Ames test (S9)	Ames test (S9) (1000)	Ames test (S9) (10000)	Ames test (S9) (100000)	Ames test (S9) (1000000)	Ames test (S9) (10000000)	Ames test (S9) (100000000)	Ames test (S9) (1000000000)
<chem>CCCC</chem>	A	M. Positive, No.								
<chem>CC1=CC=CC=C1</chem>	B									
<chem>SCC</chem>	C									
<chem>C1CCOCC1</chem>	D									
<chem>C1=CC=C(C=C1)S</chem>	E									
<chem>CCCCS</chem>	F									
<chem>CCS</chem>	G									

1. In order to use more qualitative category highlight the category with bigger number of experimental data [38]. 2. **Highlight** the cell in data matrix corresponding to mixed Ames under the target chemical. It will be empty. 3. **Go** to data gap filling.

Data Gap Filling(Ames)

Apply read-across

1. Select Read-across; 2. Click Apply; An insert window alerting you to possible data inconsistencies appears, 3. Click OK.

Data Gap Filling(Ames)

Results of Read-across

Read across prediction of Human Health Hazard/Genetic Toxicity, taking the maximal from the nearest 5 neighbours, based on 5 values from 5 neighbours, observed target value N/A, Predicted Target value: Positive.

Data Gap Filling(Ames) Interpretation of Read-across

- The all 15 analogues are non-mutagenic in the Ames assay, except for one chemical.
- The same non-mutagenic potential (Negative) is, therefore, predicted with confidence for the target chemical.
- Before data gap filling it is recommended to check the similarity of the analogues used in the prediction (see next screen shot).
- This is performed in order to build a group of mechanistically and structurally similar analogues. However, structurally similar analogues interact to DNA at same mechanism.
- Perform subcategorisation by DNA binding by OASIS v.1.1.

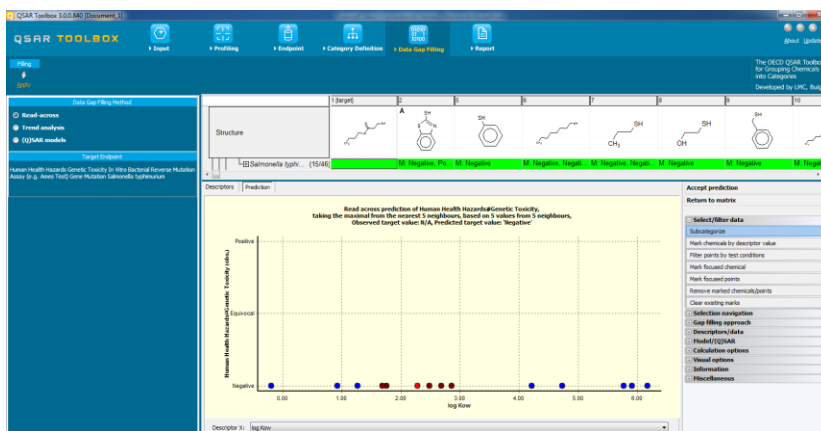
Data Gap Filling(Ames) Subcategorization by DNA by OASIS

The screenshot displays the QSAR Toolbox interface. On the left, the 'Subcategory generation' window is open, showing a list of methods. A red circle with the number '1' highlights the 'DNA binding by OASIS' method. In the center, the 'Read across prediction' plot is shown, with a red dot indicating the target chemical. A legend below the plot shows the predicted values for each chemical: M. Negative, P.0, M. Negative, M. Negative, Negat., M. Negative, Negat., M. Negative, M. Negative, M. Negative, M. Negative, M. Negative, M. Negative, M. Negative, M. Negative. A red circle with the number '2' highlights the 'Remove' button at the bottom of the plot.

1. Select DNA binding by OASIS v.1.1; 2. Click Remove to eliminate dissimilar chemical.

Data Gap Filling(Ames)

Results after Subcategorization by DNA by OASIS v.1.1



Data Gap Filling(Ames)

Interpretation of Read-across

- All results of the category members are consistent. They all are negative in the Ames test. The available results for point mutation therefore appear to confirm the adequacy of the category.
- The same exercise can be performed for skin sensitisation (see next screen shot).

Outlook

- Background
- Objectives
- Specific Aims
- The exercise
- **Workflow of the exercise**
 - Chemical Input
 - Profiling
 - Endpoints
 - Category definition
 - **Data gap filling**
 - Ames
 - **Skin Sensitization**

Data Gap Filling (Skin sensitization) Apply Read-across

The screenshot displays the QSAR Toolbox software interface. On the left, a sidebar contains a tree view of endpoints. A red circle labeled '1' highlights the 'Skin Sensitization' endpoint under the 'Human Health Hazards' category. Another red circle labeled '2' highlights the 'Read-across' option in the sidebar. At the top, a red circle labeled '3' highlights the 'Apply' button in the 'Data Gap Filling' menu. In the center, a dialog box titled 'Possible data inconsistency' is open, with a red circle labeled '4' highlighting the 'OK' button. The dialog box lists three options: 'Skin Sensitization (EC50s) (2 points)', 'Skin Sensitization (Drazen) (2 points)', and 'Skin Sensitization (BES/TC)'. The 'Skin Sensitization (Drazen) (2 points)' option is selected. The background shows a data table with columns for 'Structure', 'Target', and 'M. Positive'. A blue box with the text 'Starting gap filling...' is overlaid on the table.

1. **Highlight** the data endpoint box corresponding to Skin sensitisation under the target chemical, note it will be empty; 2. **Select** Read-across; 3. **Click** Apply, an insert window alerting you to possible data inconsistencies appears (this issue is related to Scales, see more details on next screen shot); 4. **Click** OK.

Data Gap Filling(Skin sensitization) Apply Read-across Scales

- This window shows all available scales corresponding to skin experimental data.
- The checked scale is the default one. This means that all other are converted into the default one.
- To see scale details go to: **Options**; Click on **Edit scale definitions** button. Edit scale definitions...
- This conversion is performed in Toolbox in order of standardize skin sensitisation experimental data.

Data Gap Filling(Skin sensitization) Result Read-across

The screenshot displays the QSAR Toolbox interface with the 'Data Gap Filling' window open. The window is divided into several panes:

- Options:** Contains radio buttons for 'Units' and 'Edit scale definitions...'. The 'Units' button is circled in red and labeled '2'. The 'Edit scale definitions...' button is circled in blue and labeled '3'.
- Data matrix:** A table showing the data for various chemical categories. The table has columns for 'Data matrix' and 'Scale Concentration'. The 'Data matrix' column contains a list of chemical categories, and the 'Scale Concentration' column contains numerical values.
- Conversion:** A table showing the mapping of units to the default unit. The table has columns for 'Conversion' and 'Preferred units'. The 'Conversion' column contains a list of units, and the 'Preferred units' column contains the default unit.
- Result:** A table showing the final read-across results. The table has columns for 'Result' and 'M. Positive'. The 'Result' column contains numerical values, and the 'M. Positive' column contains the final result.

Numbered callouts indicate the steps to follow:

1. Click Options;
2. Select Options and Click Units;
3. Click Edit scale definitions inconsistencies
4. See Skin.

QSAR TOOLBOX

Data Gap Filling(Skin sensitization) Result Read-across

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19.07.2011

QSAR TOOLBOX

Data Gap Filling(Skin sensitization) Result Read-across

In this case there are two chemicals with negative experimental data,
1. Double click on a dot to see detailed information
2. Click difference to target

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Data Gap Filling (Skin sensitization) Result Read-across

The red colour profilers are indication for analogues having categories different to the target.

- In this case, the subcategorization by **Chemical elements** is applied
- Remove dissimilar analogues

Data Gap Filling (Skin sensitization) Interpretation of Read-across Scales

In this case all available skin sensitization results are positive, except for one where a negative result has been found for dodecyl mercaptan [$C_{12}H_{26}S$].

The possible explanation is that the bioavailability of this molecule is too low (Log Kow > 6) to induce sensitization. Overall the limited available experimental results seem to confirm the adequacy of the category.

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Data Gap Filling (Skin sensitization)

Accept the prediction

1. **Click** Accept the prediction; 2. If you want to save the model **click** Yes, otherwise **click** NO; 3. **Click** Return to the matrix.

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Data Gap Filling Recap

- Based on the profiling results of a target chemical, you have built a category with two subcategories.
- You have gathered available experimental results for the members of the category.
- You have performed a preliminary evaluation of the category based on the available experimental data.

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Outlook

- Background
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- The exercise
- **Workflow of the exercise**
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 - **Data gap filling**
 - Ames
 - Skin Sensitization
 - **Evaluation by QSAR model**

Data Gap Filling

Preliminary evaluation using a QSAR model

- The robustness of the category could be further evaluated with the help of external QSARs from the Toolbox library of QSARs.
- To access the available models for a given endpoint, **highlight** a cell in the matrix for a given endpoint (e.g. Sensitisation_skin) and **click** on "(Q)SAR models"
- The list of available QSAR models related to the given endpoint appear in the box "QSAR models" (see next screen shot).
- In this example, only one model "DB Danish EPA Skin sensitisation" is available.

QSAR TOOLBOX

Data Gap Filling

Background information on the external QSAR model

The screenshot shows the 'Models ranking' window with the following data row:

	Predicted value (15)	Title (15)	Source (14)	Endpoint (13)	Species (14)	QMRP	Parameters	Training set (6)	Test set	Domain (1)	Unambiguous Algorithm (16)	Mechanistic Interpretation (17)	Date	R2 (2)	s (3)
1	Positive		Skin sensitisa	Data from GF	Skin sensitisa	YES	Undefined	79	0	In domain			N/A	<no data>	<no data>

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QSAR TOOLBOX

Data Gap Filling

Applying of the model to the members of the category

- The model can be used to evaluate the category by applying it to all the chemicals in the category and analysing the results.
- To apply the model simultaneously to all the chemicals:
 - in the category, **select** the model, **right-click** upon it and select "Predict Endpoint" and "All chemicals".
 - in the domain of the model, **select** the model, **right-click** upon it and select "Predict Endpoint" and "All chemicals in domain" (see next screen shot).

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 - **Report**

Report Overview

- Report module could generate report on any of predictions performed with the Toolbox.
- Report module contains predefined report templates as well as a template editor with which users can define their own user defined templates.
- The report can then be printed or saved in different formats.

Report
Generate Report

The screenshot shows the QSAR Toolbox software interface. The main window displays a report titled "Prediction of Skin sensitization for butyl 3-mercaptopropionate". The report content includes the title "QSAR Toolbox prediction for single chemical" and a reference to the OECD guidance document on the validation of quantitative structure-activity relationships models. The interface includes a menu bar with options like Report, Profiling, Endpoint, Category Definition, Data Gap Filling, and Report. A toolbar at the bottom contains buttons for Delete, Print, Close, Save as, Report, Update, Print, and Design. The 'Available data to report' window is open, showing a list of predictions for skin sensitization. The 'Create' button in the toolbar is highlighted with a red circle and a '2' callout. The 'Available data to report' window is highlighted with a red circle and a '1' callout.

1. Select the prediction in the window Available data to report, then **2. Click** Create.

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