OECD QSAR Toolbox v.4.4.1

Step-by-step example on how to build a user-defined profiling scheme accounting for the (a)biotic activation of the chemicals
Outlook

• Background
• Objectives
• Profiling
• The exercise
Background

• This is a step-by-step presentation designed to provide guidance to the Toolbox user on how to create their own profiling scheme accounting for the (a)biotic activation of the chemicals.

• The created custom profiler can be used for searching precursors of a given target.

• Two examples will be illustrated:
  o Searching PBT precursors in the Toolbox databases;
  o Searching p-benzoquinone precursors in a specific file.

Note: Please note that building of custom items (such as profilers, (Q)SAR models as well as importing of custom databases) is only enabled in single user mode. So, if your Toolbox is installed in multiuser mode, you will be not able to follow this tutorial.
Outlook

• Background

• Objectives

• Profiling

• The exercise
Objectives

- This presentation demonstrates how to build and use a new profiling scheme including:
  - naming of the new scheme;
  - selection of specific (a)biotic activation;
  - searching of specific (a)biotic product;
  - saving the new profiler;
  - defining category by using the custom profiler;
  - profiling a chemical list.
Outlook

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• The exercise
Profiling
Overview

• As you know, “Profiling” refers to the electronic process of retrieving relevant information on a compound which is stored in the Toolbox, other than fate and toxicity data.

• The Toolbox has many predefined profilers but it also allows the user to develop new custom profilers.
Outlook

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- Profiling
- The exercise
The Exercise
Example 1

• In the first example we will build a profiler that identifies precursors of 1,2,4-trichlorobenzene [CAS 120-82-1] (i.e. we will search for chemicals that are transformed to our target).

• The 1,2,4-trichlorobenzene (1,2,4-TCB) is considered as a substance fulfilling the PBT-criteria\(^1,2\). Therefore, the potential precursors of 1,2,4-TCB could be prioritized for further PBT-related investigations.

• Precursors of 1,2,4-TCB will be searched in the Toolbox databases containing biodegradation (BOD) 301C data.

The Exercise
Start building a new profiler

We are going to create a new profiling scheme:
• Open the Toolbox.
• Move to the Profiling module
(see next screen shot).
Building of a new profiler
Define the name of the new profiler

1. Go to the Profiling module; 2. Click New; 3. Enter the name of the new scheme, for example “Searching precursors of 1,2,4-trichlorobenzene”; 4. Confirm by click OK.
Building of a new profiler
Define type of new profiler

1. Select the first option for scheme type: **Linear or hierarchical scheme**; 2. Click **OK**.
Building of a new profiler
Creating a profiling category

Once the scheme is created, it will be defined:

• the name of the profiling category, which will appear on the data matrix;
• the structure which we will be searching for (i.e. 1,2,4-TCB);
• the simulator, which will be used to simulate the transformations of the chemicals (i.e. Microbial degradation simulator).
1. Right click on “Category 1” and select to **Rename** it; 2. Put a name that will appear on the data matrix when profile, e.g. **Precursor of 1,2,4-trichlorobenzene**; 3. Confirm by **OK**.
First we need to define the structural criteria, i.e. the structure of the metabolite which we will searching for. The structural criteria will be defined using the SMARTS language.

1. Click on the **ADD** button; 2. Select **Structure query**.
Building of a new profiler
Define the structural criteria

1. Click on the **Add Query** button; 2. Select to add SMARTS; 3. Confirm by **OK**.
Building of a new profiler
Define the structural criteria

1. Click on the created SMARTS query in order to define it. The SMARTS editor will appear;
2. Click **Edit** to define the structure.
Building of a new profiler
Define the structural criteria

Once the SMART editor is opened, we need to draw the structure which we will searching for (i.e. 1,2,4-TCB)

Click on the drawing tool (1a) and draw three bonds to the benzene scaffold (1b). Then click the chlorine symbol (i.e. Cl) (2a) and click on the bonds` ends (2b). Finally click OK (3)
Building of a new profiler
Define the structural criteria

The structural criteria is defined. Now we need to specify the metabolic simulator, which will be used to simulate the microbial degradation of the chemicals.

Select the options for **Exact connectivity** and **Exact match** (1) in order to search for exactly the same structure, without any other substituents. Then go to the **Metabolism** tab (2).
Building of a new profiler
Define the metabolic criteria

In the current example we search for chemicals that are potential PBT substances based on their PBT degradation product. Therefore, the *Microbial metabolism simulator* will be used.

Select the *Microbial metabolism simulator* from the drop-down menu (1). This simulator will be applied on the chemicals as parents (i.e. *Input* >> *Parents* (2)) and we will search within the whole package – parent and produced metabolites (i.e. *Output* >> *All* (3)). The process mode should be *Any* (parent and produced metabolites) (4). Finally, click on *Save Scheme* (5).
Building of a new profiler

Custom profiler location

The newly created profiling scheme appears under the Custom section. The custom schemes can be used in the same way as the rest of profiling schemes within the Toolbox installation – for profiling and category definition.
Precursors of 1,2,4-TCB will be searched in the databases containing BOD data. Therefore, we need first to define the target endpoint, which will highlight the relevant databases.

1) Enter the target by CAS (CAS 120-82-1); 2) Click on the Define button in order to define the target endpoint; 3) Select Environmental Fate and Transport >> Biodegradation >> Biodegradation in Water: Screening Tests >> Ready biodegradable and then specify the BOD endpoint for 28d according to OECD 301C guideline; 4) Click Finish to finalize the endpoint definition.
Building of a new profiler

Custom profiler usage

1) Go to the Data module; 2) Select the highlighted database.
Building of a new profiler
Custom profiler usage

1) Go to the **Category definition** module; 2) Select the custom profiler; 3) Click on the **Define** button; 4) Click **OK** button to initialize the search of 1,2,4-TCB precursors.
Building of a new profiler
Custom profiler usage

Six chemicals are found (the target plus five analogues. Confirm by OK (1). The system offers you to check for available experimental data for the found analogues in the selected databases. Click OK (2).

Note: The tutorial is consistent with Toolbox v.4.4 (and TB 4.4.1), where the Biodegradation NITE database is preliminary cached with the microbial metabolism. If older version of Toolbox is used, the search will take up to several minutes if you make this example for first time.
Building of a new profiler
Custom profiler usage

Experimental data for BOD 301C is available for all analogues identified in the selected databases. The analogues are not biodegradable having data <60%\(^1\).

\(^1\) OECD guideline for testing of chemicals 301. 1992. Ready biodegradability.
The Exercise
Example 2: Background

• Micronucleus positive results for p-benzoquinone [CAS 106-51-4] are reported in the literature\textsuperscript{[1-3]} and are also available in the Toolbox.

• Therefore, it could be assumed that the chemicals producing p-benzoquinone (p-BQ) as a metabolite would also elicit positive effects in the micronucleus test.

• The hydroquinone/quinone molecular transformations are reproduced by the \textit{in vivo} rat simulator available in the Toolbox.

The Exercise
Example 2

• In the current example we will build a profiler that identifies chemicals producing p-BQ (i.e. precursors of p-BQ) as a result of \textit{in vivo} rat metabolism.

• It will be illustrated how to search precursors in a specific database. The same could be done using a custom file.
Building of a new profiler
Define the name of the new profiler

1. Go to the Profiling module; 2. Click New; 3. Enter the name of the new scheme, for example "Searching precursors of p-benzoquinone"; 4. Confirm by click OK.
Building of a new profiler
Define type of new profiler

1. Select the first option for scheme type: **Linear or hierarchical scheme**; 2. Click **OK**.
Building of a new profiler
Creating a profiling category

Once the scheme is created, it will be defined:

- the name of the profiling category, which will appear on the data matrix;
- the structure which we will searching for (i.e. p-BQ);
- the simulator, which will be used to simulate the transformations of the chemicals (i.e. *in vivo* Rat metabolism simulator).
1. Right click on “Category 1” and select to Rename it; 2. Put a name that will appear on the data matrix when profile, e.g. Precursor of p-benzoquinone; 3. Confirm by OK.
First we need to define the structural criteria, i.e. the structure of the metabolite which we will searching for. The structural criteria will be defined using the SMARTS language.

1. Click on the **ADD** button; 2. Select **Structure query**.
Building of a new profiler
Define the structural criteria

1. Click on the **Add Query** button; 2. Select to add SMARTS; 3. Confirm by **OK**.
Building of a new profiler
Define the structural criteria

1. Click on the created SMARTS query in order to define it. The SMARTS editor will appear;
2. Click **Edit** to define the structure.
Click on the cleaning tool (1) in order to clean everything. Select the cyclohexane template (2). Use the drawing tool (3a) to draw two bonds to the scaffold (3b) and the double bonds (second click over a bond converts it to double bond). Then click the oxygen symbol (i.e. O) (4a) and click on the bonds` ends (4b). Finally click OK (5).

Once the SMART editor is opened, we need to draw the structure which we will searching for (i.e. p-BQ).
Building of a new profiler
Define the structural criteria

The structural criteria is defined. Now we need to specify the metabolic simulator, which will be used for searching precursors of p-BQ.

The structures of p-benzoquinone is defined(1) Then go to the **Metabolism** tab (2).
Building of a new profiler
Define the metabolic criteria

In the current example we search for chemicals that would be positive in the micronucleus test based on their positive metabolite. As the micronucleus is *in vivo* test, we will use the *in vivo* rat metabolic simulator for the purpose.

Select the **in vivo Rat metabolism simulator** from the drop-down menu (1). This simulator will be applied on the chemicals as parents (i.e. *Input* >> *Parents* (2)) and we will search within the whole package – parent and produced metabolites (i.e. *Output* >> *All* (3)); select Any process mode (4). Finally, click on **Save Scheme** (5).
Building of a new profiler
Custom profiler location

The newly created profiling scheme appears under the Custom section. The custom schemes can be used in the same way as the rest profiling schemes within the Toolbox installation – for profiling and category definition.
Building of a new profiler
Custom profiler usage

1) Click on the Database button*. List with all available databases will appear;
2) Select the Micronucleus ISSMIC database located under the Human Health Hazards section; 3) Confirm by OK.

Note: If you want to check for precursors in a custom file, you can use the List button to load a preliminary prepared .txt or .smi file.

Precursors of p-BQ will be searched in a specific file. Micronucleus ISSMIC database will be used in the current example.
Building of a new profiler

Custom profiler usage

1) Go to the **Profiling** module; 2) Select the newly created profiler; 3) Click on the **Apply** button.
Building of a new profiler
Custom profiler usage

Once the chemicals are profiled by the created custom profiler, the results appear on the data matrix:

- “N/A” result for the chemicals that do not give p-BQ after in vivo metabolic activation
- “Precursor of p-benzoquinone” results for the chemicals that metabolize to p-BQ.
1) Apply right click over the name of the custom profiler that appears in the grey field and select **Profile statistic**. It could be seen that the profiler identifies 12 chemicals among the chemicals in the data matrix; 2) Click on the **Precursor of p-benzoquinone** row; 3) Add the chemicals in a new document.
Building of a new profiler
Custom profiler usage

1) Click on **Apply** to profile again the chemicals; 2) Double click on a profiling result to explain it.
Building of a new profiler
Explaining profiling results

In the new opened window it could be seen the target and all metabolites generated by the selected metabolic simulator (i.e. in vivo rat metabolic simulator in this case). The status for each molecule is also shown:

- *Satisfied* – means that the corresponding chemical meet the criteria defined in the profiler;
- *Not Satisfied* – means that the corresponding chemical does NOT meet the criteria defined in the profiler.
Building of a new profiler
Explaining profiling results

Parent chemical

the metabolite satisfying the defined criteria (i.e. the structure of p-BQ)

in vivo metabolites
Congratulations

• Now you know how to:
  o create a new profiler taking into account the (a)biotic activation of the chemicals;
  o use the custom profiler for searching precursors of a target in selected databases;
  o use the custom profiler for searching precursors of a target in a specific file (database);
  o explain the results of the custom profiler.

• Continual use of the Toolbox will increase your skills.