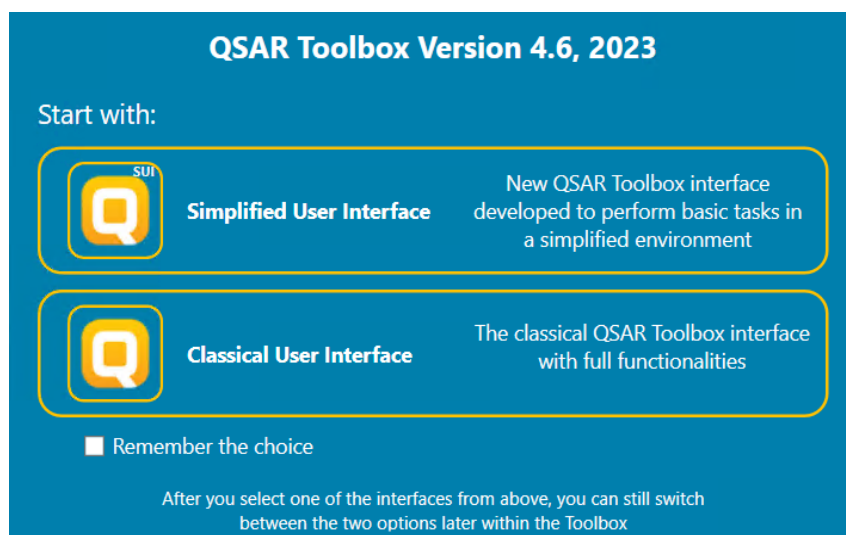



GETTING STARTED: QUICK REFERENCE GUIDE FOR THE CLASSICAL USER INTERFACE

Choose which user interface you want to work with: *Simplified User Interface* or *Classical User Interface*.



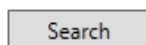
 If you want to use the same user interface everytime when you open the software then check **Remember the choice** checkbox before selecting one of the two interfaces.

Step 1: Input - Define chemical of interest or "target chemical"

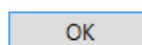
Define your target chemical by Chemical Name, CAS number, SMILES, drawing the molecule or selecting it from a list. To define a chemical by CAS number:



Click **CAS#** → enter the number, and then click



→ the program displays the structure →



The structure is displayed on the data matrix.

The screenshot displays the QSAR TOOLBOX software interface. The top menu bar includes options like Document, Single Chemical, Chemical List, Search, IUCLID search, and Target Endpoint. The left sidebar shows a list of documents, with 'Document 1' selected, displaying its CAS number: 122043. The main window shows the chemical structure of 4-Nitrobenzoyl chloride, which is a benzene ring with a nitro group (-NO2) and a carbonyl group (-C(=O)Cl) in the para position. Below the structure, a table lists various identifiers and properties for the chemical.

Structure info	Value
Additional Ids	EC Number:2045174
CAS Number	122-04-3
CAS-SMILES relation	High
Chemical name(s)	4-Nitrobenzoyl chloride
Identity	Sources:15
Molecular formula	C7H4ClNO3
Predefined substance type	Mono constituent
SMILES	[O-][N+](=O)c1ccc(cc1)C(=O)Cl


Below the structure information, there are expandable sections for Parameters, Physical Chemical Properties, Environmental Fate and Transport, Ecotoxicological Information, and Human Health Hazards.

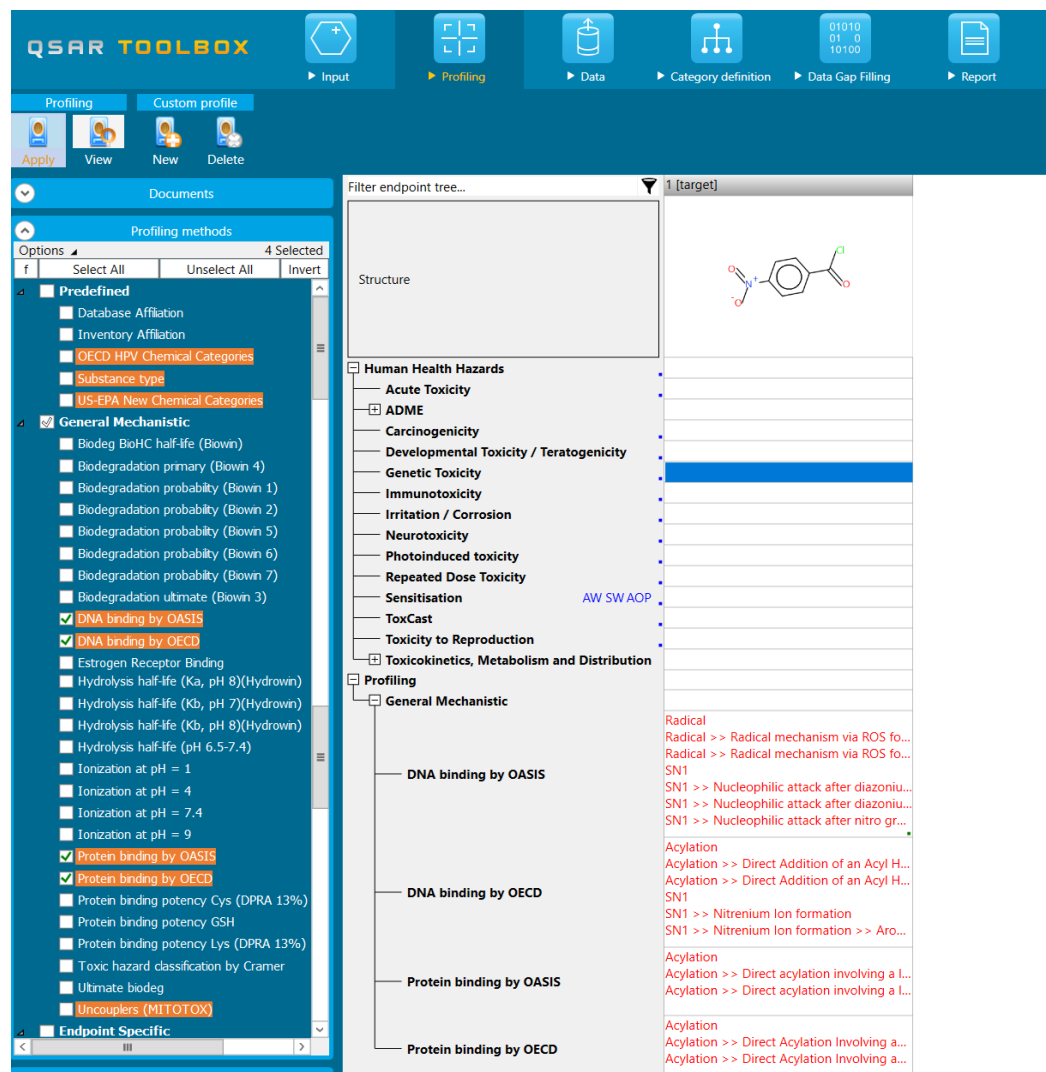


To define the target endpoint, which will be used for the predictions click




Step 2: Profiling - Retrieve information based on the identity of the substance or its structure

Select profilers by ticking the corresponding boxes → . The program establishes a "profile" of the chemical based on its structure.




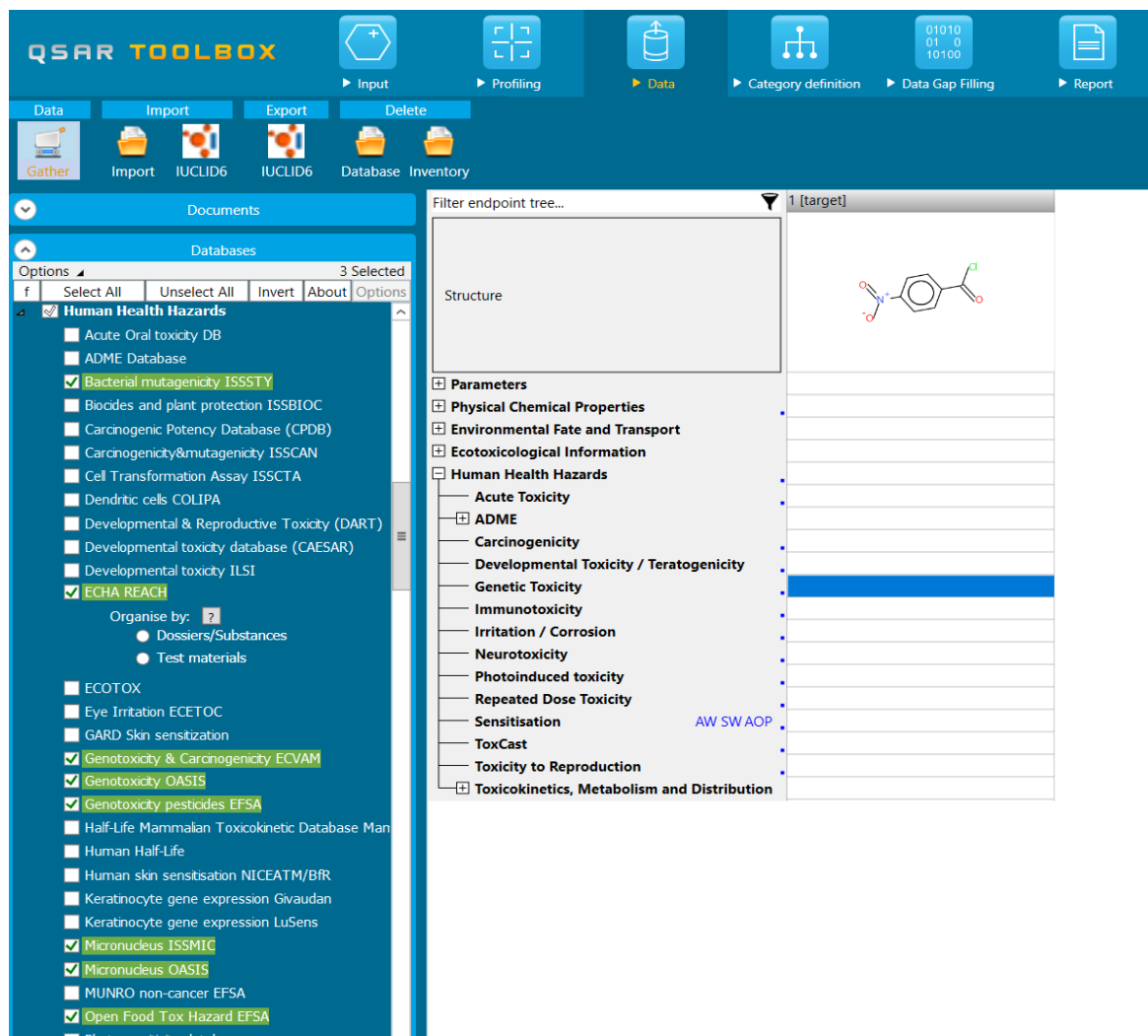
! To obtain the general background information on any profiler, right click on it and select **About**. To obtain the scientific information used to build the profiler,

select it and click .

! The highlighted profiles correspond to the selected endpoint in the data matrix or to the previously defined endpoint if any.

Step 3: Endpoint - Retrieve experimental results from the resident databases

Select databases by ticking ☒ the corresponding databases → . The retrieved information is displayed according to four subsections in the endpoint tree:



The screenshot displays the QSAR TOOLBOX software interface. The top toolbar includes icons for Input, Profiling, Data, Category definition, Data Gap Filling, and Report. Below this, a menu bar contains Data, Import, Export, and Delete. The main workspace is divided into three panels. The left panel, titled 'Documents', shows a list of databases with checkboxes for selection. The middle panel, titled 'Filter endpoint tree...', displays a hierarchical tree of endpoints. The right panel shows the chemical structure of the target molecule, 1-chloro-4-nitrobenzene.

Database Selection (Left Panel):

- ☒ Human Health Hazards
 - ☐ Acute Oral toxicity DB
 - ☐ ADME Database
 - ☒ Bacterial mutagenicity ISSSTY
 - ☐ Biocides and plant protection ISSBIOC
 - ☐ Carcinogenic Potency Database (CPDB)
 - ☐ Carcinogenicity&mutagenicity ISSCAN
 - ☐ Cell Transformation Assay ISSCTA
 - ☐ Dendritic cells COLIPA
 - ☐ Developmental & Reproductive Toxicity (DART)
 - ☐ Developmental toxicity database (CAESAR)
 - ☐ Developmental toxicity ILSI
 - ☒ ECHA REACH
 - Organise by: ?
 - ☐ Dossiers/Substances
 - ☐ Test materials
 - ☐ ECOTOX
 - ☐ Eye Irritation ECETOC
 - ☐ GARD Skin sensitization
 - ☒ Genotoxicity & Carcinogenicity ECVAM
 - ☒ Genotoxicity OASIS
 - ☒ Genotoxicity pesticides EFSA
 - ☐ Half-Life Mammalian Toxicokinetic Database Man
 - ☐ Human Half-Life
 - ☐ Human skin sensitisation NICEATM/BIR
 - ☐ Keratinocyte gene expression Givaudan
 - ☐ Keratinocyte gene expression LuSens
 - ☒ Micronucleus ISSMIC
 - ☒ Micronucleus OASIS
 - ☐ MUNRO non-cancer EFSA
 - ☒ Open Food Tox Hazard EFSA
 - ☐ Photosensitivity database

Endpoint Tree (Middle Panel):

- ☒ Parameters
- ☒ Physical Chemical Properties
- ☒ Environmental Fate and Transport
- ☒ Ecotoxicological Information
- ☒ Human Health Hazards
 - ☐ Acute Toxicity
 - ☒ ADME
 - ☒ Carcinogenicity
 - ☒ Developmental Toxicity / Teratogenicity
 - ☒ Genetic Toxicity
 - ☒ Immunotoxicity
 - ☒ Irritation / Corrosion
 - ☒ Neurotoxicity
 - ☒ Photoinduced toxicity
 - ☒ Repeated Dose Toxicity
 - ☒ Sensitisation
 - ☒ ToxCast
 - ☒ Toxicity to Reproduction
 - ☒ Toxicokinetics, Metabolism and Distribution

Chemical Structure (Right Panel):


O=[N+]([O-])c1ccc(Cl)cc1

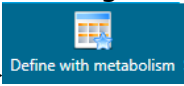
! To open the data tree: left-click on the nodes. To access detailed information on the experimental results: double-click on the result in the matrix.

! The highlighted databases correspond to the selected endpoint in the data matrix or to the previously defined endpoint if any.

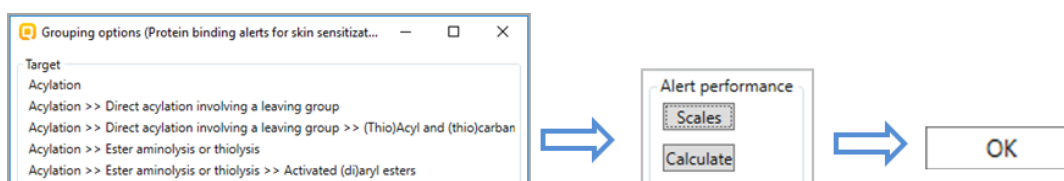
Step 4: Category definition - Identify chemicals which could form a category with the "target" chemical


Select one grouping method according to the profile of your target chemical in the

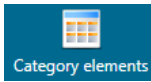
window **Grouping methods** →  **Define**.

To identify the analogues of the target accounting for metabolic activation of the chemicals based on specific criteria select →  **Define with metabolism**

To check how much relevant to a target endpoint an alert is, once the **Define (with metabolism) button** is selected then calculate the Alert performance:



To identify the subgroups within an existing category that are determined by the definitions of the currently selected profiling method →  **Clustering**

To assess the consistency of the defined category →  **Category elements**


! The category consistency is endpoint specific. Four layers of information are considered important: Physicochemical similarity; Structural similarity and Mechanistic similarity and ADME similarity.


! The highlighted profiles correspond to the selected endpoint in the data matrix or to the previously defined endpoint if any.


Step 5: Data gap filling - Predict missing data by read-across, trend analysis, QSAR models or automated/standardized workflows

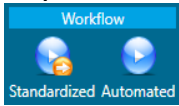
Select data gap filling by clicking in the corresponding cell in the data matrix, and then select one of the data gap filling methods:

- Read-across: for "qualitative" endpoints (skin sensitization or mutagenicity e.g. positive, negative, equivocal) or for "quantitative endpoints" (e.g., 96h-LC50 for fish) if only very few analogues with experimental results are

identified. →  **Read across**

- Trend analysis: for “quantitative” endpoints if many analogues with experimental results are identified. →  Trend analysis

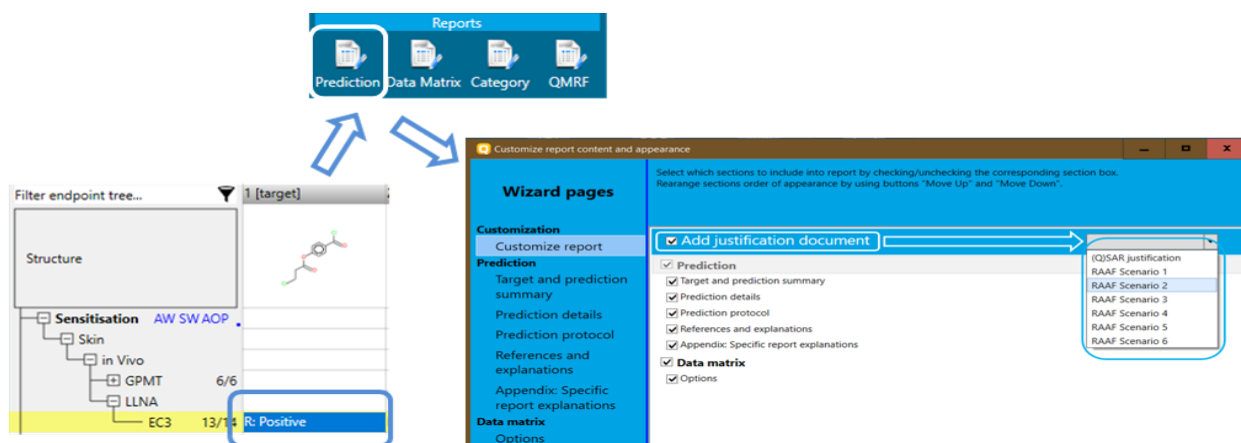
- (Q)SAR models: if no analogue with experimental results is identified or to build a weight of evidence case. →  (Q)SAR

- Standardized and Automated workflows: once started, they follow the implemented logic and finish with prediction. They include read-across or trend analysis method depending on the endpoint →  Workflow
Standardized Automated

Step 6: Report – Obtain a detailed report for your prediction or category

Prediction is needed to generate a *Prediction* report.

Read across Assessment Elements could be included in a separate justification document in case of regulatory interest, by selection of a **RAAF scenario**.



The screenshot illustrates the process of generating a report. On the left, the 'Filter endpoint tree...' window shows a hierarchical structure with 'Sensitisation' selected, and 'EC3' highlighted with a 'Positive' result. Above this, the 'Reports' section contains icons for 'Prediction', 'Data Matrix', 'Category', and 'QMRF'. To the right, the 'Customize report content and appearance' window is open, showing 'Wizard pages' for 'Customization'. Under the 'Prediction' section, various report components are listed with checkboxes, including 'Add justification document', 'Target and prediction summary', 'Prediction details', 'Prediction protocol', 'References and explanations', 'Appendix: Specific report explanations', 'Data matrix', and 'Options'. A list of 'RAAF Scenario' options (1 through 6) is visible on the right side of the customization window.

Four export types are also available in TB 4.6. →  Export

The Export allows exporting of various information from the current data matrix.



Each of the above illustrated functionalities is explained in details in the F1 help file available with installation (press F1).