

OECD QSAR Toolbox v.4.1

Example for predicting acute aquatic toxicity to fish of mixture with known components

Outlook

- **Background**
- Objectives
- The exercise
- Workflow
- Save the prediction

Background

- This is a step-by-step presentation designed to take the user of the Toolbox through the workflow of prediction acute aquatic toxicity to fish of mixture with known components

Outlook

- Background
- **Objectives**
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Objectives

- **This presentation reviews a number of functionalities of the Toolbox:**
 - The 2D editor for defining Mixture components
 - Filling data gaps by Similar mode approach

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Exercise

- In this exercise we will predict the aquatic toxicity to fish of mixture with defined components, which is the “target” chemical.
- Investigate the mode of action of components of the mixture
- Gather available experimental data for target chemical and its components
- Predict acute aquatic toxicity using Similar mode approach

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Workflow

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

Outlook

- Background
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- **Workflow**
 - **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 a mixture

User alternatives for defining mixtures with known compositions:

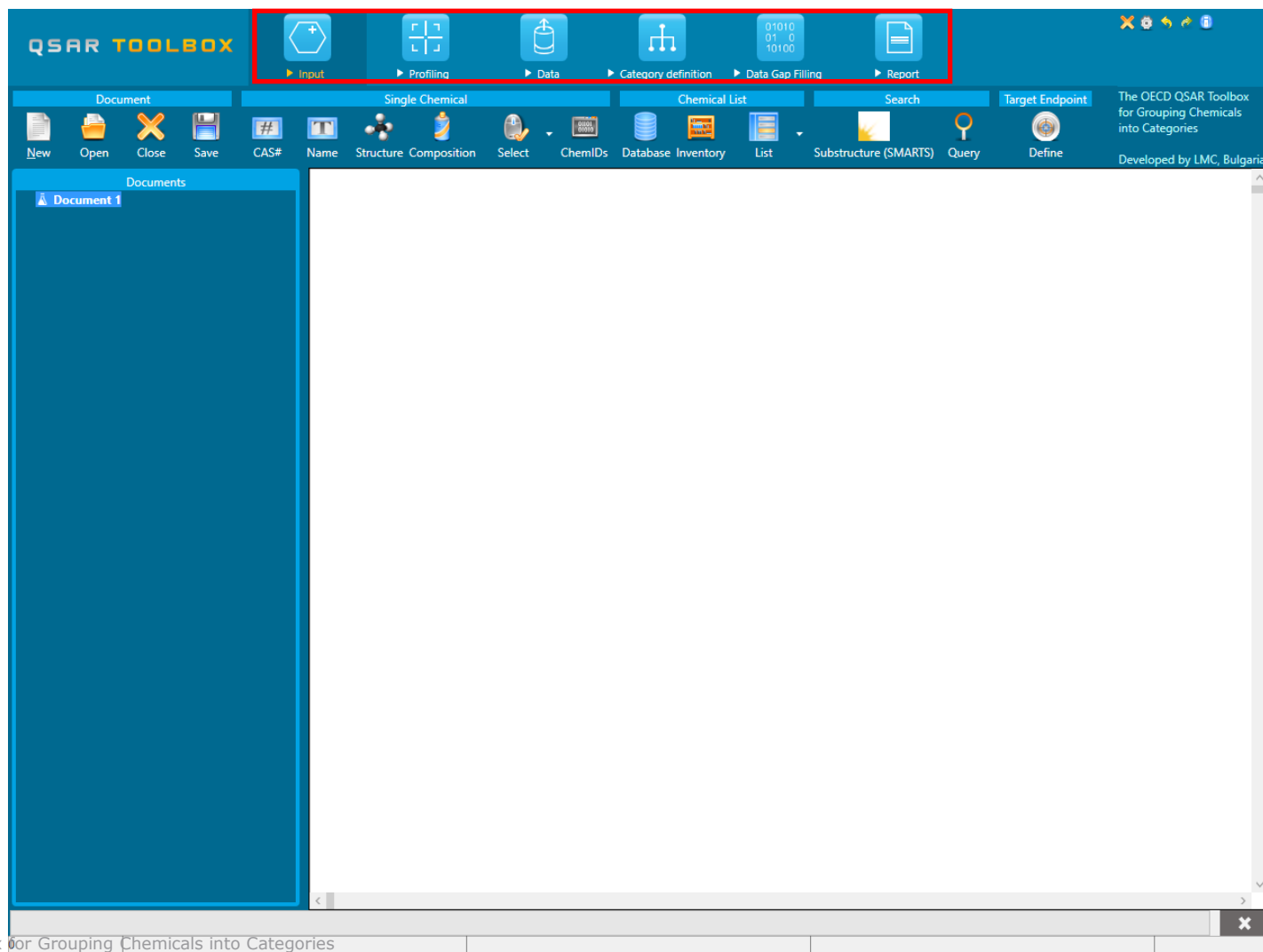
- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing mixture constituents and defining their quantities
- Select from User List/Inventory/Databases
- Chemical IDs such as EC number, Einecs number
- Load file with mixture

Chemical Input

Getting Started

- Open the Toolbox.
- The six modules in the workflow are seen listed next to “QSAR TOOLBOX”.
- **Click** on “Input” (see next screen shot)

Chemical Input Input Screen



Chemical Input by Drawing

- Inputting the target chemical (mixture) by drawing its components within the “Composition” tool
- It is accomplished by a series of operations within the “Composition” tool (see next screen shot).
- The subsequent series of screen shots will take you through the process of drawing constituents of mixture and defining their quantities.

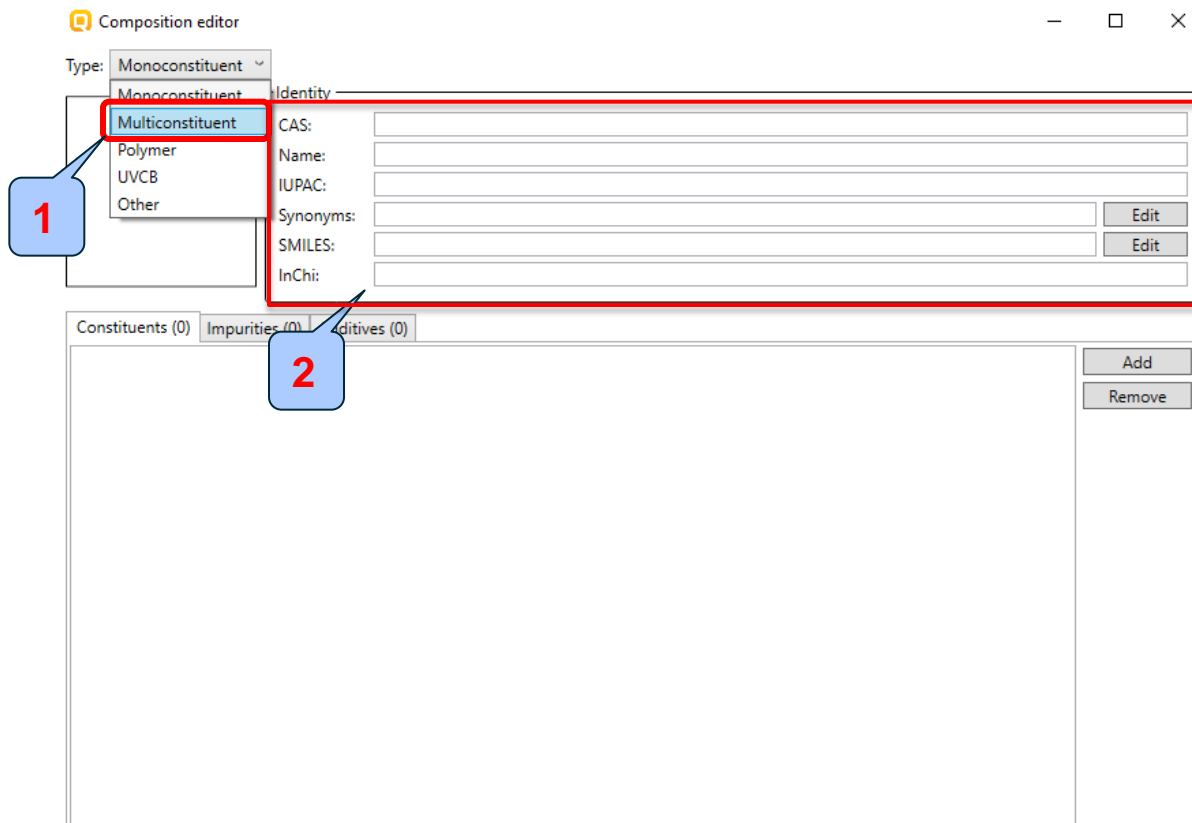
Chemical Input

Input target chemical by drawing

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes options like 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. Below this, a secondary menu bar contains 'Document', 'Single Chemical', 'Chemical List', 'Search', and 'Target Endpoint'. The 'Single Chemical' menu is expanded, showing options: 'New', 'Open', 'Close', 'Save', 'CAS#', 'Name', 'Structure', 'Composition', 'Select', 'ChemIDs', 'Database', 'Inventory', 'List', 'Substructure (SMARTS)', 'Query', and 'Define'. The 'Composition' option is highlighted with a red rectangular box. A white callout bubble with a red border and the number '1' points to this option. Below the software interface, a light blue text box contains the instruction: '1. Click on Composition'. The bottom status bar of the software shows 'The OECD QSAR Toolbox for Grouping Chemicals into Categories', 'July, 2017', and a close button.

Chemical Input

Drawing the target mixture



1. From Drop down menu "Type" select Multiconstituent
2. If there is information for the mixture it could be fill in.

Chemical Input

Drawing the target mixture

1. To define constituents of the mixture click "Add"

2. A form where all data associated with constituents appear.

Chemical Input

Drawing the target mixture

Composition editor

Type: Monoconstituent

Identity

CAS:

Name:

IUPAC:

Synonyms: Edit

SMILES: Edit

InChi:

Constituents (1) Impurities (0) Additives (0)

OH ₂	1	<p>Identity</p> <p>CAS: <input type="text"/></p> <p>Name: <input type="text"/></p> <p>IUPAC: <input type="text"/></p> <p>Synonyms: <input type="text"/> Edit</p> <p>SMILES: <input type="text"/> Edit</p> <p>InChi: <input type="text"/></p> <p>Concentration</p> <p>Typical concentration</p> <p><input type="text"/> Family: Mass Unit: <input type="text"/></p> <p>Concentration range</p> <p><input type="text"/> <input type="text"/> Family: Mass Unit: <input type="text"/></p>
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Add

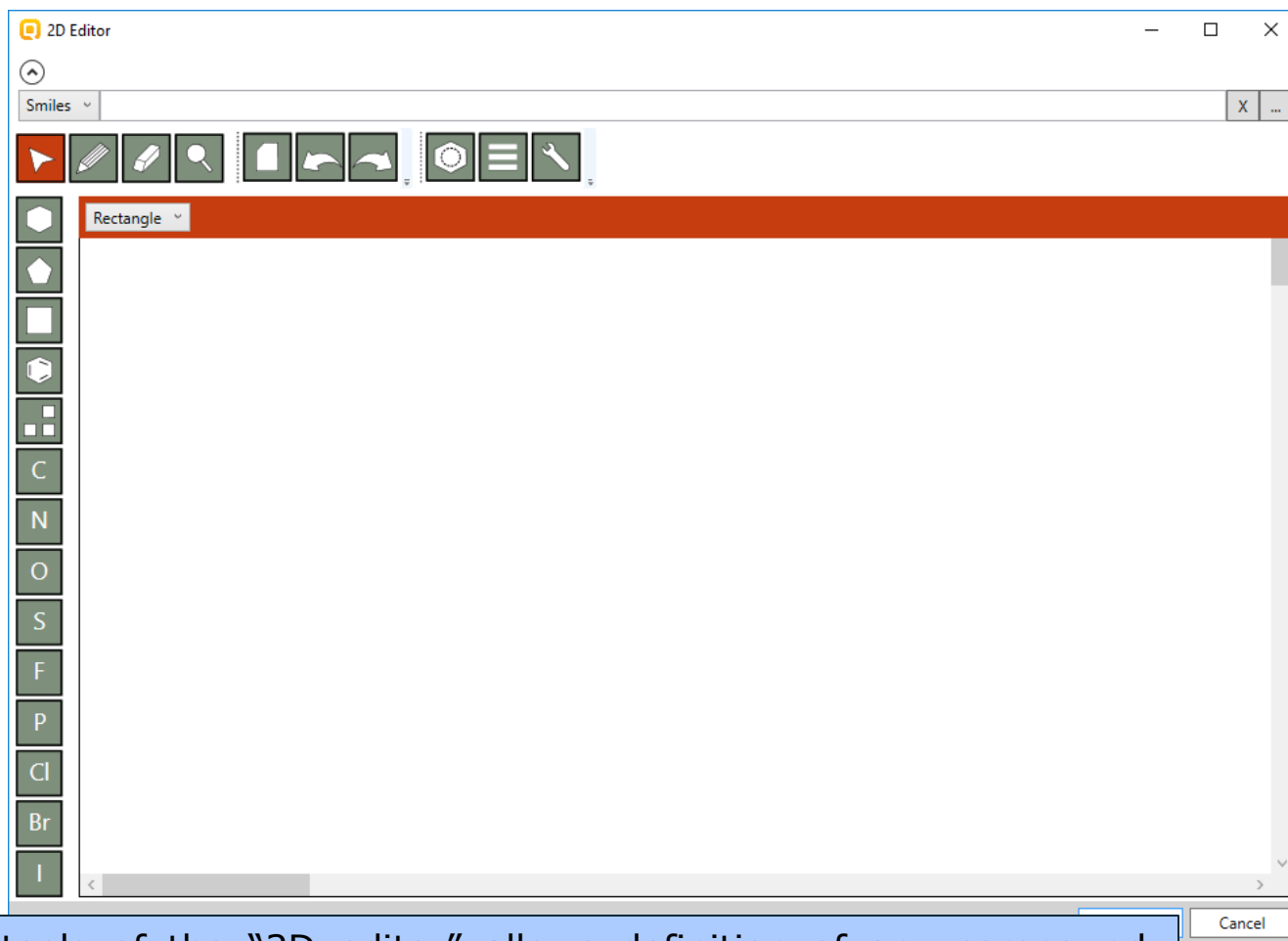
Remove

Cancel

1. Click "Edit" on row SMILES to define the structure of the first constituent

Chemical Input

Drawing the target mixture



1. The tools of the "2D editor" allows definition of any compound. More details for the editor could be found in F1 help.

Chemical Input

Drawing the component of mixture
"Diphenylmethanone" by 2D editor

1. Select Benzene from the template list and
2. Click left mouse button twice on the drawing area.

Chemical Input

Drawing the component of mixture
"Diphenylmethanone" by 2D editor

1. Define the single bonds by using "Drawing tool".

Chemical Input

Drawing the component of mixture
"Diphenylmethanone" by 2D editor

The screenshot shows the 2D Editor window with the following elements:

- Window title: 2D Editor
- SMILES input field: C1=CC=C(C=C1)C(=O)C1=CC=CC=C1
- Toolbar: Contains icons for navigation, drawing, and editing.
- Left sidebar: A vertical menu with chemical symbols: C, N, O, S, F, P, Cl, Br, I.
- Canvas: Displays the chemical structure of Diphenylmethanone. A red box highlights the carbonyl bond, and a blue callout bubble with the number '1' points to it.
- Bottom right: A 'Cancel' button.

1. To change the type of bond hold the pointer on respective bond and click several times to specify the bond.

Chemical Input

Drawing the component of mixture
"Diphenylmethanone" by 2D editor

The screenshot shows the 2D Editor window with the following components:

- Smiles Input:** C1=CC=C(C=C1)C(C1=CC=CC=C1)=C
- Toolbar:** Contains various editing tools. Callout 1 points to the Selection tool (arrow icon).
- Vertical Element Palette:** Lists elements C, N, O, S, F, P, Cl, Br, I.
- Central Drawing Area:** Displays the chemical structure of diphenylmethanone. Callout 2 points to the central carbon atom labeled 'H2C'.
- Object Explorer:** Shows the selected atom's properties. Callout 3 points to the element dropdown menu where 'O' is selected.

1. To change the carbon atom chose "Selection tool"
2. Set the focus on the carbon atom by left mouse click
3. Chose the atom from the dropdown menu

Chemical Input

Drawing the component of mixture
"Diphenylmethanone" by 2D editor

The screenshot shows the '2D Editor' window with the SMILES string C1=CC=C(C=C1)C(=O)C1=CC=CC=C1 in the input field. The main drawing area displays the chemical structure of Diphenylmethanone. A blue callout box contains the following instructions:

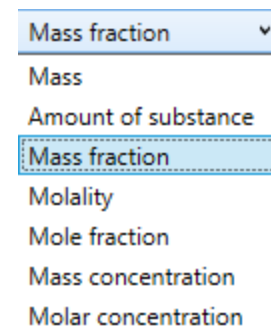
1. The structure of first constituent is defined
2. Click Ok.

The 'OK' button at the bottom right of the window is highlighted with a red box.

Chemical Input

Input quantities of mixture

- Quantities of the constituents should be added manually
- There are several ways to add mixture quantity:
 - Mass fraction
 - Mass
 - Amount of substance
 - Molality
 - Mole fraction
 - Mass concentration
 - Molar concentration
- Select "Mass fraction %" then "Weight %"



Chemical Input

Input quantities of mixture

Composition editor

Type: Monoconstituent

Identity

CAS:

Name:

IUPAC:

Synonyms:

SMILES:

InChi:

Constituents (1) Impurities (0) Additives (0)

1

Identity

CAS:

Name:

IUPAC:

Synonyms:

SMILES:

InChi:

Concentration

Typical concentration

= Family: Mass fraction Unit: weight %

Concentration range

Family: Mass fraction Unit:

2

1

1. Select "Mass fraction %" then "Weight %"
2. Specify the value to be equal to 9.

Chemical Input

Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor

The screenshot shows the 'Composition editor' window. At the top, the 'Type' is set to 'Monoconstituent'. Below this is a list of constituents. The first constituent is 'OH2' with a concentration of '1'. To the right of this list is an information panel for the selected constituent. This panel includes fields for 'Identity' (CAS, Name, IUPAC, Synonyms, SMILES, InChi) and 'Concentration' (Typical concentration and Concentration range). The 'SMILES' field contains 'O'. Three callouts are present: callout 1 points to the 'Add' button, callout 2 points to the information panel, and callout 3 points to the 'Edit' button in the information panel.

1. To add the next constituent click again "Add"
2. The info panel for new constituent appear.
3. Then click "Edit" to activate the 2D editor

Chemical Input

Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor

Smiles C1=CC(=C(C(=C1C(C)=O)Cl)Cl)Cl

Rectangle Make first C

Object explorer

Atom: Cl

1

Element: Cl

Charge: 0

Hybridization: undefined

Valent state: v4

Isotope: 0

Implicit hydrogens: 3

Atom number: 6

Aromatic: False

Parity: None

1. By using drawing tools define the structure of 1-(2,3,4-trichlorophenyl)ethan-1-one

Chemical Input

Drawing the component of mixture "1-(2,3,4-trichlorophenyl)ethan-1-one" by 2D editor

1. Select "Mass fraction %" then "Weight %"
2. Specify the value to be equal to 1.

Chemical Input

Drawing the component of mixture "Butan-1-ol" by 2D editor

1. To add the next constituent click again "Add"
2. The info panel for new constituent appear.
3. Then click "Edit" to activate the 2D editor

Chemical Input

Drawing the component of mixture "Butan-1-ol"
by 2D editor

The screenshot shows the 2D Editor window with the SMILES string CCCCO entered. The main canvas displays the skeletal structure of butan-1-ol, with the methyl group labeled H3C and the hydroxyl group labeled OH. The Object explorer panel on the right shows the following properties for the selected atom (O):

Atom:	O
Element:	O
Charge:	0
Hybridization:	undefined
Valent state:	v4
Isotope:	0
Implicit hydrogens:	3
Atom number:	6
Aromatic:	False
Parity:	None
Radical:	undefined

1. By using drawing tools define the structure of Butan-1-ol

Chemical Input

Drawing the component of mixture "Butan-1-ol" by 2D editor

The screenshot shows the 'Composition editor' window. The 'Type' is set to 'Monoconstituent'. The main area shows a list of constituents, with the first one highlighted in blue. This constituent is 'Butan-1-ol', represented by a chemical structure and the SMILES string 'CCCCO'. The 'Concentration' section for this constituent is highlighted with a red box. A callout bubble with the number '1' points to the 'Family' dropdown menu, which is set to 'Mass fraction'. Another callout bubble with the number '2' points to the 'Typical concentration' input field, which is set to '90'. The 'Unit' dropdown menu is also highlighted with a red box and a callout bubble with the number '1', showing it is set to 'weight %'.

1. Select "Mass fraction %" then "Weight %"
2. Specify the value to be equal to 90.

Chemical Input

Drawing the target mixture

Composition editor

Type: Monoconstituent

Identity

CAS:

Name:

IUPAC:

Synonyms: Edit

SMILES: Edit

InChi:

Constituents (3) Impurities (0) Additives (0)

Identity

CAS:

Name:

IUPAC:

Synonyms: Edit

SMILES: Edit

InChi:

Concentration

Typical concentration

= 90 Family: Mass fraction Unit: weight %

Concentration range

Family: Mass fraction Unit:

1

1

OK

1. Confirm the mixture constituents by click Ok

Chemical Input

Target chemical identity

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes options like 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. Below this, there are sub-menus for 'Document', 'Single Chemical', 'Chemical List', 'Search', and 'Target Endpoint'. The 'Chemical List' sub-menu is active, showing options like 'New', 'Open', 'Close', 'Save', 'CAS#', 'Name', 'Structure', 'Composition', 'Select', 'ChemIDs', 'Database Inventory', 'List', 'Substructure (SMARTS)', 'Query', and 'Define'. The main workspace is divided into a left sidebar with 'Documents' and 'Substance' tabs, and a central area with a 'Filter endpoint tree...' panel. This panel contains a 'Structure' field and a list of categories: 'Structure info', 'Parameters', 'Physical Chemical Properties', 'Environmental Fate and Transport', 'Ecotoxicological Information', and 'Human Health Hazards'. A red circle highlights a chemical structure in the 'Structure' field, which is also visible in the 'Database Inventory' list. A blue text box at the bottom of the screenshot contains the text: 'The already drawn mixture automatically appears on the data matrix'.

The already drawn mixture automatically appears on the data matrix

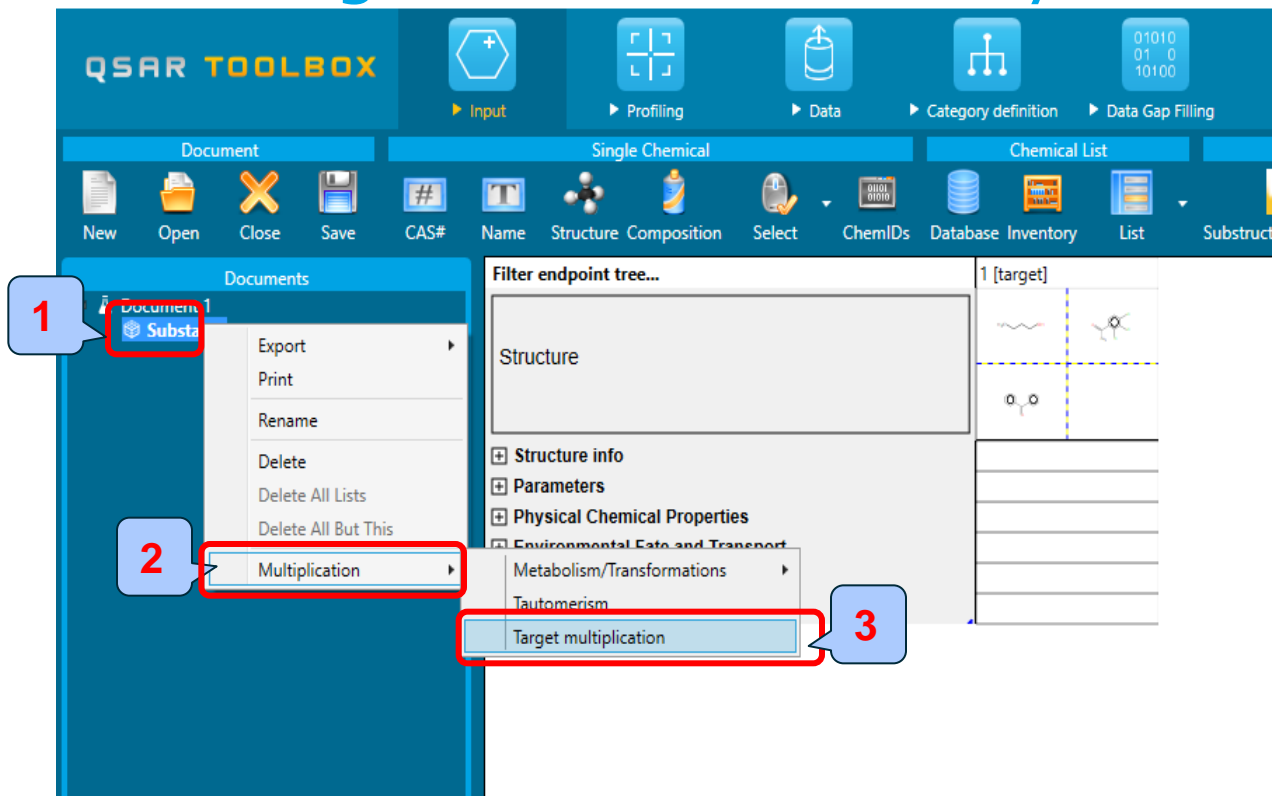
Chemical Input

Target chemical identity

- Note that no CAS number or name is displayed for this chemical. This means the target chemical is not listed in the chemical inventories/databases implemented in the Toolbox(see next slide).
- See next slide how to visualize separately the mixture components for further analysis.

Chemical Input

Target chemical identity



1. Select "Substance"
2. By right mouse click select "Multiplication/Target multiplication"

Chemical Input

Target chemical identity

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes options like Document, Single Chemical, Chemical List, Search, and Target Endpoint. Below the menu bar, there are icons for various functions such as New, Open, Close, Save, CAS#, Name, Structure, Composition, Select, ChemIDs, Database, Inventory, List, Substructure (SMARTS), Query, and Define. On the left, a 'Documents' panel shows a tree view with 'Document 1' containing a 'Substance' which has a 'Composition list' with three constituents. The main workspace shows a 'Filter endpoint tree...' on the left and a table on the right. The table has columns for 'Parent chemical', 'Constituent #1', 'Constituent #2', and 'Constituent #3'. The 'Parent chemical' column shows a mixture of three chemical structures. The 'Constituent #1' column shows the chemical structure of 1-butanol (CCCCO). The 'Constituent #2' column shows the chemical structure of 2,4-dichloroacetophenone (CC(=O)c1ccc(Cl)cc1Cl). The 'Constituent #3' column shows the chemical structure of benzophenone (O=C(c1ccccc1)c2ccccc2). A red box highlights the 'Parent chemical' and 'Constituent #1' columns. A blue text box at the bottom of the table area contains the text: 'The mixture and all components are shown'.

Parent chemical	Constituent #1	Constituent #2	Constituent #3
	<chem>H3C-CH2-CH2-CH2-OH</chem>		

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - 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 Side-Bar to Profiling

Summary information of the different profilers are provided in the "About".

The screenshot shows the QSAR Toolbox interface with the Profiling sidebar on the left. In the 'Profiling methods' list, 'Acute aquatic toxicity MOA by OASIS' is highlighted (callout 1). A context menu is open over it, with 'About' selected (callout 2). An 'About' dialog box is open in the center, showing details for the selected profiler (callout 3). The dialog includes a name, short description, disclaimer, donor/author information, website, and a details table.

Details	
Name	Value
Scheme type	Dendroid
Scheme nature	EndpointSpecific
Version	3.0
Number of categories	7

1. **Highlight** the profiler
2. **Select** About
3. **Click** Close

Profiling

Side-Bar to Profiling

- For most of the profilers, background information can be retrieved by highlighting one of the profilers (for example, Acute aquatic toxicity MOA by OASIS and clicking on “View” button(see next screen shot).

Profiling

Side-Bar to Profiling for Aqute aquatic toxicity MOA

The screenshot displays the QSAR Toolbox interface. On the left, the 'Profiling' sidebar is visible, with the 'Acute aquatic toxicity MOA' profiler selected and highlighted by a red box and callout 1. A right-click context menu is open over this selection, with 'View scheme' highlighted by a red box and callout 2. In the center, a decision tree diagram shows a path leading to a node labeled 'Unexplained (Active O and N)', which is highlighted by a red box and callout 3. On the right, the 'Query details' window is shown, containing a SMARTS query and search options, enclosed in a red circle and callout 4. A red circle and callout 5 also highlight the 'View scheme' button in the sidebar.

1. **Highlight** the profiler
2. Right mouse click and select **"View scheme"**
3. **Click** on one of the nodes
4. Boundaries defined the rules

Profiling

Profiling the target chemical

- Select the “Profiling methods” related to the target endpoint by clicking on the box next to the profilers name.
- This selects (a green check mark appears) or deselects (green check disappears) profilers.
- For this example, the following primary profilers relevant to the aquatic toxicity are selected (see next screenshot):
 - US-EPA New Chemical Categories
 - Aquatic toxicity classification by ECOSAR – structural grouping
 - Acute aquatic toxicity MOA by OASIS – mechanistic grouping
 - Acute aquatic toxicity classification by Verhaar (Modified) – grouping by reactivity
 - Protein binding by OASIS
 - Protein binding by OECD

Profiling

Profiling the target chemical

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. The 'Profiling' menu is open, showing options: 'Apply', 'View', 'New', and 'Delete'. The 'Apply' button is circled in red, with a red callout box containing the number '2'. Below the menu, the 'Profiling methods' panel is visible, with a blue callout box containing the number '1' pointing to several checked checkboxes. The 'Endpoint Specific' section includes:

- Ionization at pH = 9
- Protein binding by OASIS
- Protein binding by OECD
- Protein binding potency
- Protein binding potency Cys (DPRA 13%)
- Protein binding potency Lys (DPRA 13%)
- Toxic hazard classification by Cramer
- Toxic hazard classification by Cramer (extended)
- Endpoint Specific
 - Acute aquatic toxicity classification by Verhaar (Modified)
 - Acute aquatic toxicity MOA by OASIS
 - Acute aquatic toxicity classification by ECOSAR
 - Bioaccumulation - metabolism alerts
 - Bioaccumulation - metabolism half-lives
 - Biodegradation fragments (BioWIN MITI)
 - Carcinogenicity (genotox and nongenotox) alerts by IS
 - DART scheme

 The 'Metabolism/Transformations' section includes:

- Documented
 - Observed Mammalian metabolism
 - Observed Microbial metabolism
 - Observed Rat In vivo metabolism
 - Observed rat liver metabolism with quantitative data
 - Observed Rat Liver S9 metabolism
- Simulated
 - Autoxidation simulator
 - Autoxidation simulator (alkaline medium)
 - Dissociation simulator
 - Hydrolysis simulator (acidic)
 - Hydrolysis simulator (basic)

 The right side of the interface shows a 'Filter endpoint tree...' panel with a 'Structure' input field and a table with columns for 'Parent chemical', 'Constituent #1', 'Constituent #2', and 'Constituent #3'. The table contains chemical structures and SMILES strings, such as CCCCO for Constituent #1.

1. Place a green check in the box before profilers related to the target endpoint.
 2. Click Apply

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 appear as a dropdown box under the target chemical.
- Please note the specific profiling results by Classification by ECOSAR; MOA by OASIS; US-EPA; Protein binding by OECD(see next slide).
- The results of profiling shows same mode of action for the three components of the mixture

Profiling

Profiling the target chemical

1

Apply View New Delete

Documents

Profiling methods

Options

- Ionization at pH = 9
- Protein binding by OASIS
- Protein binding by OECD
- Protein binding potency
- Protein binding potency Cys (DPRA 13%)
- Protein binding potency Lys (DPRA 13%)
- Toxic hazard classification by Cramer
- Toxic hazard classification by Cramer (extended)
- Endpoint Specific**
 - Acute aquatic toxicity classification by Verhaar (Modified)
 - Acute aquatic toxicity MOA by OASIS
 - Aquatic toxicity classification by ECOSAR
 - Bioaccumulation - metabolism alerts
 - Bioaccumulation - metabolism half-lives
 - Biodegradation fragments (BioWIN MITI)
 - Carcinogenicity (genotox and nongenotox) alerts by IARC
 - DART scheme

Metabolism/Transformations

Options

- Documented
 - Observed Mammalian metabolism
 - Observed Microbial metabolism

Filter endpoint tree...

Structure

Structure info

Parameters

Physical Chemical Properties

Environmental Fate and Transport

Ecotoxicological Information

Human Health Hazards

Profile

Predefined

- US-EPA New Chemical Categories

General Mechanistic

- Protein binding by OASIS
- Protein binding by OECD

Endpoint Specific

- Acute aquatic toxicity classification by Verhaar (Modified)
- Acute aquatic toxicity MOA by OASIS
- Aquatic toxicity classification by ECOSAR

Parent chemical	Constituent #1	Constituent #2	Constituent #3
Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
No alert found	No alert found	Schiff base formation	No alert found
No alert found	No alert found	No alert found	No alert found
Class 1 (narcosis or base surface narcotics)	Class 1 (narcosis or base surface narcotics)	Class 3 (unspecific reaction)	Class 5 (Not possible to classify)
Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics

Visualization the nodes of the tree

Components of the mixture have same mode of action according to ECOSAR; US-EPA; MOA and Protein binding by OECD profilers

Outlook

- Background
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- **Workflow**
 - Input
 - Profiling
 - **Data**

Data

- “Data” refer to the electronic process of retrieving the environmental fate, ecotoxicity and toxicity data that are 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).
- In this example, we limit our data gathering to the common aquatic toxicity endpoints from four aquatic databases containing aquatic toxicity data – **Aquatic ECETOC; Aquatic Japan MoE; Aquatic OASIS; ECOTOX.**

Data

The screenshot shows the QSAR Toolbox interface. The 'Data' menu is open, and the 'Gather' button is highlighted with a red circle and the number '3'. In the 'Databases' section, 'Ecotoxicological Information' is selected with a red box and the number '1'. Underneath it, several databases are checked with green boxes and the number '2': 'Aquatic ECE 100', 'Aquatic Japan MoE', 'Aquatic OASIS', 'ECHA CHEM', 'ECOTOX', and 'Human Health Hazards'. The 'Filter endpoint tree...' panel is visible on the right, and a table below it shows chemical structures and their classifications.

Parent Chemical	Constituent #1	Constituent #2	Constituent #3
	<chem>H3C-CH2-CH2-CH2-OH</chem>		
Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
No alert found	No alert found	Schiff base formation	No alert found
No alert found	No alert found	No alert found	No alert found
Class 1 (narcosis or base)	Class 1 (narcosis or base)	Class 3 (unspecific react)	Class 5 (Not possible to)
Basesurface narcotics	Basesurface narcotics	Basesurface narcotics	Basesurface narcotics
Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics

1. **Expand** the Ecotoxicological Information
2. **Select** databases related to the target endpoint by adding a green check in the box before the database name.
3. **Click** Gather

Data

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

The screenshot shows the QSAR Toolbox software interface. On the left, there are panels for 'Documents' and 'Databases'. The 'Databases' panel is expanded to show 'Environmental Fate and Transport' and 'Ecotoxicological Information'. The 'Ecotoxicological Information' section is checked, and sub-sections like 'Aquatic ECETOC', 'Aquatic Japan MoE', 'Aquatic OASIS', 'ECHA CHEM', 'ECOTOX', and 'Human Health Hazards' are also checked. A 'Read data?' dialog box is open in the center, with the 'All endpoints' radio button selected. A blue callout box with the number '1' points to the 'OK' button. The background shows a table with columns for 'Parent Chemical', 'Constituent #1', 'Constituent #2', and 'Constituent #3', and rows for various toxicity endpoints like 'Protein binding by OASIS', 'Acute aquatic toxicity classification by Verha...', etc.

1. Click OK to read all available aquatic tox data

Data

Process of collecting data

Target endpoint: LC50; *P.promelas*; 96h

The screenshot displays the QSAR Toolbox interface with the 'Data' menu selected. The 'Filter endpoint tree...' panel is expanded to show 'Ecotoxicological Information' > 'Aquatic Toxicity' > 'Mortality' > 'LC50' > '96 h'. The 'Parent Chemical' section shows a chemical structure. The table below lists experimental data for three constituents.

Endpoint	Constituent #1	Constituent #2	Constituent #3
Accumulation (1/2)	M: 25+45 mg/L		
Avoidance (1/1)	M: 185+1.48E+03 mg/L		
Behavior (3/8)	M: 1.41E+03 mg/L	M: 2 mg/L	M: 13.7 mg/L
Biochemistry (1/2)	M: 1 mg/L		
Development (2/7)	M: 823 mg/L		M: 1.78 mg/L
Growth (2/27)	M: > 1E+03 mg/L		M: 0.46 mg/L
Growth Inhibition (2/4)	M: > 1E+03 mg/L		M: 1 mg/L
Immobilisation (2/2)	M: > 1E+03 mg/L		M: > 10 mg/L
Intoxication (2/8)	M: 1.86E+03 mg/L		M: 0.28 (0.21-0.37) mg
Mortality			
EC50 (3/5)	M: 1.73E+03 mg/L	M: 2 mg/L	M: 15.3 mg/L
LC0 (1/2)	M: 1.17E+03 mg/L		
LC100 (1/2)	M: 1.22E+03 mg/L		
LC50			
1 h (1/2)	M: 1.94E+03 mg/L		
4 h (1/1)	M: 0.45 % v/v		
24 h (2/13)	M: > 1E+03 mg/L		M: 14.8 mg/L
48 h (2/13)	M: > 1E+03 mg/L		M: 14.5 mg/L
72 h (2/3)	M: 1.94E+03 mg/L		M: 5 mg/L
96 h			
Animalia (animals)			
Arthropoda (arthropods) (1/1)	M: 661 mg/L		
Chordata (chordates)			
Actinopterygii (ray-finned fishes ...)			
Alburnus alburnus (1/2)	M: 2.25E+03-2.4E+03 mg/L		
Lepomis macrochirus (1/1)	M: 100 (100-500) mg/L		
Leuciscus idus (1/1)	M: 1E+03 mg/L		
Oryzias latipes (2/2)	M: > 100 mg/L		M: > 10 mg/L
Pimephales promelas (3/11)	M: 1.73E+03 (1.63E+03-1.83E+03) mg/L	M: 1.99 mg/L	M: 10.9 (9.64-12.3) mg/L

10 experimental data for the investigated endpoint: LC 50; 96h; *P.promelas* have been found for the components of the mixture

Recap

- You have entered the chemical mixture with defined components
- The results of profiling shows same mode of action for the three components of the mixture
- You have gather available experimental data for the target chemical mixture and found no experimental data for mixture. However experimental data for the components has been found
- You are ready to predict Acute aquatic toxicity to fish of mixture: Endpoint: LC50, Duration:96h; Effect: mortality; species: *Pimephales promelas*
- Now you are ready to continue with next step of the workflow "Data Gap Filling".

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Data
 - **Data Gap filling**

Data Gap Filling Overview

- “Data Gap Filling” module give access to two different data gap filling tools:
 - **Independent MOA-** all components are with different mode of action
 - **Similar MOA-** all components are with similar mode of action
- More details about different MOA is given on next six slides #56-61
- In this particular case all components of the current mixture are with similar mode of action. In this respect Similar MOA is applied

Data Gap Filling

Independent MOA

Assumption – combined effect can be calculated from the effects caused by the individual mixture components by following the statistical concept of independent random events

Mixture response:
$$E(C_{Mix}) = 1 - \prod_{i=1}^N [1 - E(C_i)]$$

$E(C_{Mix})$ - the effect provoked by the total mixture

$E(C_i)$ - the effects that the individual components would cause if applied singly at that concentration at which they are present in the mixture

Problem - dose-response relationships are practically unknown

Data Gap Filling

Similar MOA

Assumption – components in a mixture contribute to the joint effect, in proportion to their prevalence and individual potency

- **Components act at the same target site**
- **Components act by the same mechanism**
- **Components have similar effect (rather than mechanism)**

Method for calculation toxic effect of mixture with components acting by same mechanisms is given on next slide

Data Gap Filling Similar MOA

Relative potency factor

$$RPF_j^{(i)} = \frac{ED_{resp}^{(i)}}{ED_{resp}^{(j)}}$$

i – index (reference) chemical

ED_{resp} – dose (concentration) of a chemical that cause a specified response (fraction of animals that respond, fractional change in a measured physiological value, etc.)

Chemical Equivalent Dose (Concentration)

$$CED_j^{(i)} = RPF_j^{(i)} d_j$$

Dose (concentration) of the reference chemical i that will cause the same effect as chemical j at dose (concentration) d_j

Index Chemical Equivalent Dose (Concentration)

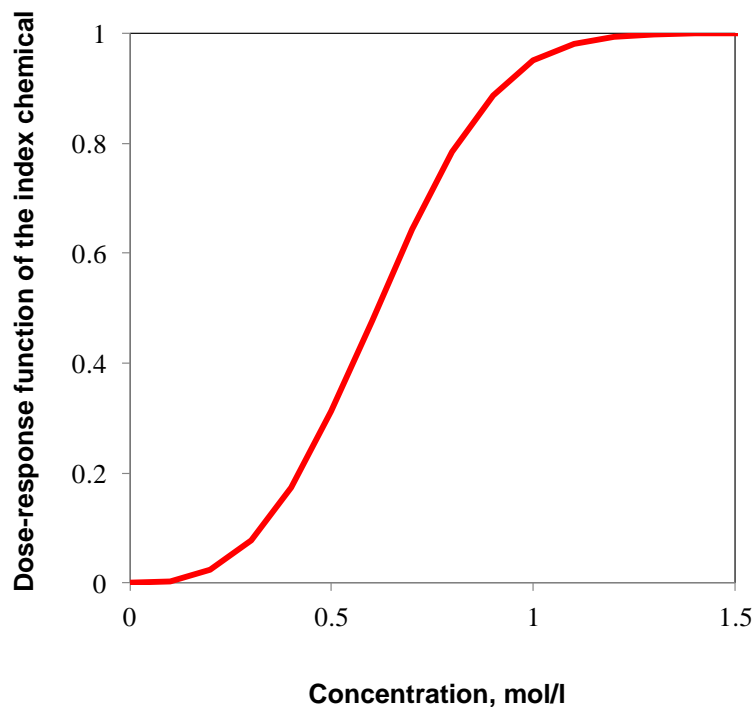
$$ICED = \sum_{j=1}^J CED_j^{(i)} = \sum_{j=1}^J RPF_j^{(i)} d_j$$

Equivalent dose (concentration) of the reference chemical i that will cause the same effect as the mixture

Data Gap Filling

Similar MOA

Toxic effect of mixture - response (fraction of animals that respond, fractional change in a measured physiological value, etc.) as a result of exposure to mixture



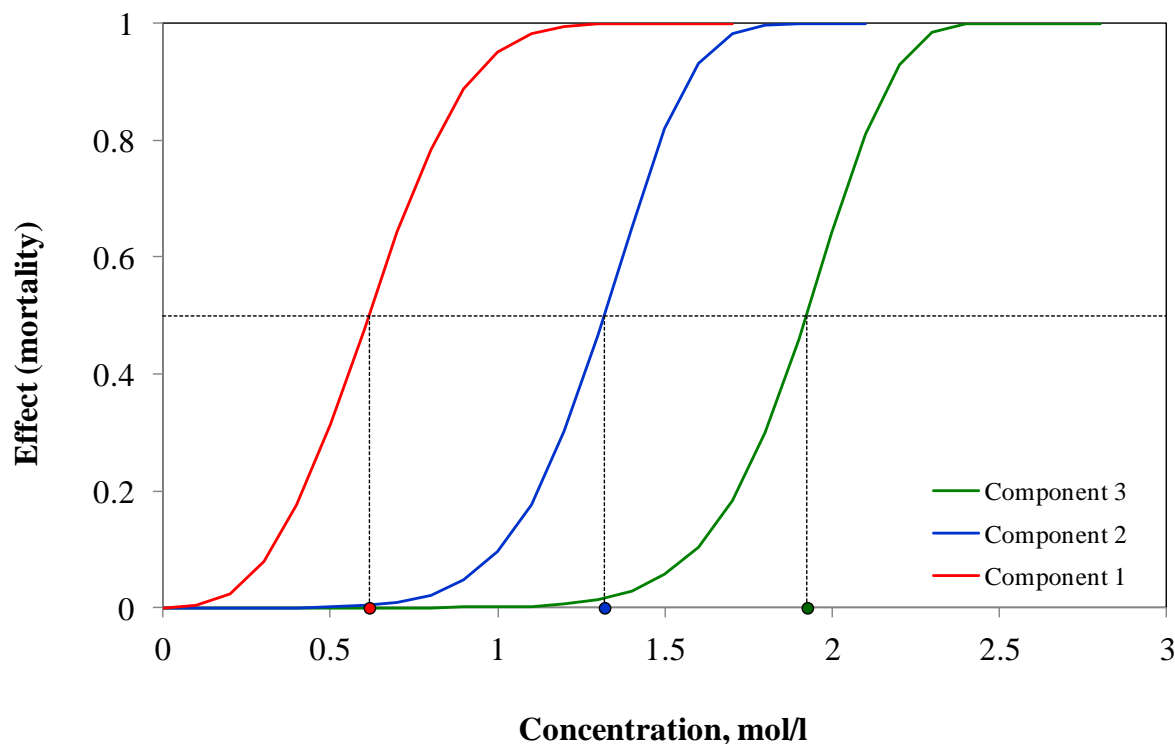
$$Effect^{Mixture} = f_i(ICED)$$

f_i - dose-response function of the index chemical

Illustration of calculating effect of mixture is given on next two slides

Data Gap Filling Similar MOA (Illustration)

Reference chemical: **Component 1 ($i = 1$)**



Relative potency factors

$$RPF_j^{(1)} = \frac{LC_{50}^{(1)}}{LC_{50}^{(j)}}$$

Equivalent concentrations

$$CED_j^{(1)} = RPF_j^{(1)} C_j$$

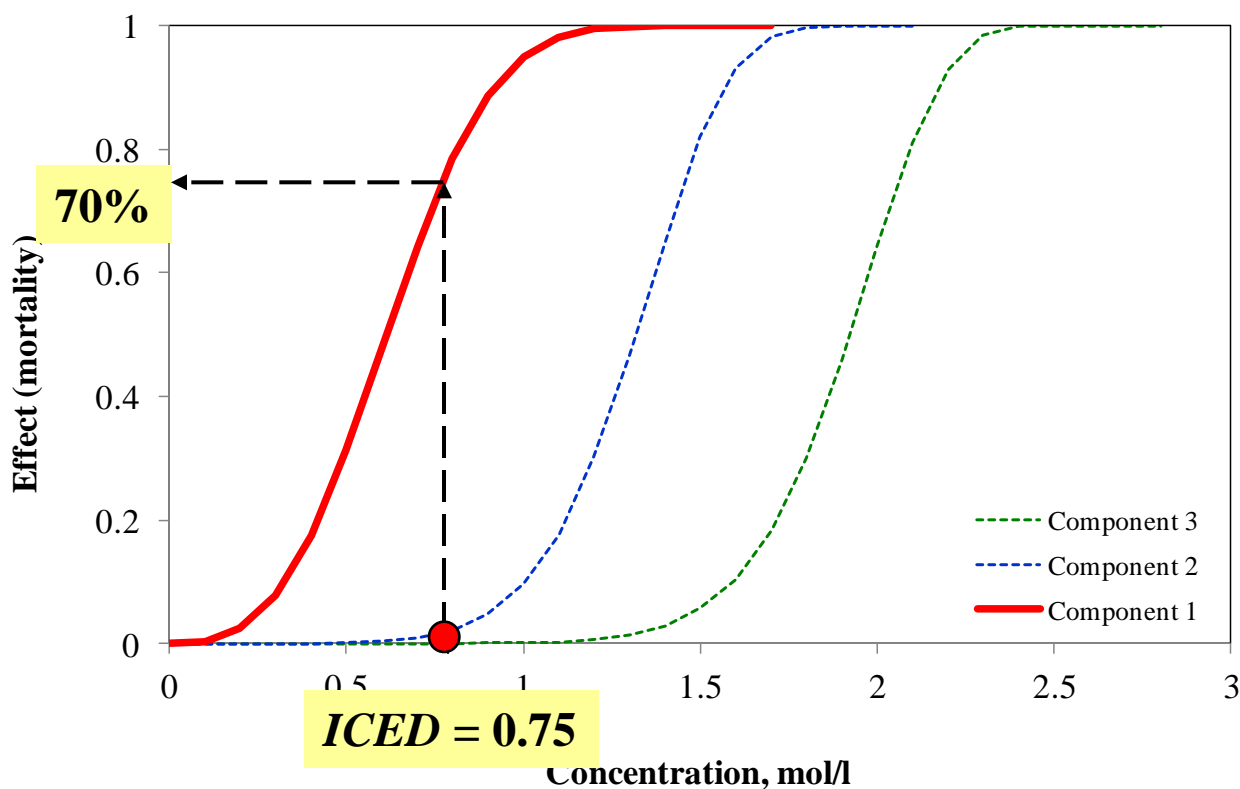
Index Chemical Equivalent Concentration

$$ICED = \sum_{j=1}^J CED_j^{(1)}$$

$ICED$ \longrightarrow $Effect^{Mixture} = f_i(ICED)$

Data Gap Filling Similar MOA (Illustration)

Reference chemical: **Component 1 (i = 1)**



Relative potency factors

$$RPF_j^{(1)} = \frac{LC_{50}^{(1)}}{LC_{50}^{(j)}}$$

Equivalent concentrations

$$CED_j^{(1)} = RPF_j^{(1)} C_j$$

Index Chemical Equivalent Concentration

$$ICED = \sum_{j=1}^J CED_j^{(1)}$$

$$ICED = 0.75 \longrightarrow Effect^{Mixture} = f_i(ICED) \approx 70\% \text{ mortality}$$

Data Gap Filling

Case study

- In this particular case all components of the current mixture are with similar mode of action. In this respect Similar MOA is applied
- Application of Similar MOA for our case study is illustrated on next slides

Data Gap Filling

Apply Similar MOA

The screenshot shows the QSAR Toolbox interface with the following components:

- Top Bar:** QSAR TOOLBOX logo and navigation icons for Input, Profiling, Data, Category definition, Data Gap Filling, and Report.
- Left Panel:**
 - Gap Filling:** A dropdown menu with 'Independent MOA' and 'Similar MOA' (the latter is selected and circled in red with callout '2').
 - Data Gap Filling Settings:**
 - Only endpoint relevant
 - Only chemical relevant
 - At this position:**
 - Select a cell with a rigid (bold) path
 - Automated workflows
 - Standardized workflows
- Filter endpoint tree...:** A tree view showing a hierarchy of endpoints. The endpoint 'Pimephales promelas LC50/96h' is highlighted in blue and circled in red with callout '1'.
- Main Table:** A table with columns for 'Constituent #1', 'Constituent #2', and 'Constituent #3'. The table contains data for various endpoints, including 'Pimephales promelas LC50/96h'.

Endpoint	Constituent #1	Constituent #2	Constituent #3
Growth (2/27)	M: >1E+03 mg/L		M: 0.46 mg/L
Growth Inhibition (2/4)	M: >1E+03 mg/L		M: 1 mg/L
Immobilisation (2/2)	M: >1E+03 mg/L		M: >10 mg/L
Intoxication (2/8)	M: 1.86E+03 mg/L		M: 0.28 (0.21=0.37) mg/L
Mortality (3/5)	M: 1.73E+03 mg/L	M: 2 mg/L	M: 15.3 mg/L
EC50 (1/2)	M: 1.17E+03 mg/L		
LC0 (1/2)	M: 1.22E+03 mg/L		
LC50 (1/2)	M: 1.94E+03 mg/L		
1 h (1/1)	M: 0.45 % v/v		
24 h (2/13)	M: >1E+03 mg/L		M: 14.8 mg/L
48 h (2/13)	M: >1E+03 mg/L		M: 14.5 mg/L
72 h (2/3)	M: 1.94E+03 mg/L		M: 5 mg/L
96 h (2/3)	M: 1.94E+03 mg/L		M: 5 mg/L
Animalia (animals) (1/1)	M: 661 mg/L		
Arthropoda (arthropods) (1/1)	M: 661 mg/L		
Chordata (chordates) (1/1)	M: 661 mg/L		
Actinopterygii (ray-finned fishes) (1/1)	M: 661 mg/L		
Alburnus alburnus (1/2)	M: 25E+03 ± 2.4E+4 mg/L		
Lepomis macrochirus (1/1)	M: 100 (100-500) mg/L		
Leuciscus idus (1/1)	M: 5E+03 mg/L		
Oryzias latipes (2/2)	M: >100 mg/L		M: >10 mg/L
Pimephales promelas (3/11)	M: 1.73E+03 (1.63E+03-1.83E+03) mg/L	M: 1.99 mg/L	M: 10.9 (9.64+12.3) mg/L
Poecilia reticulata (2/2)	M: 1.74E+03 mg/L		M: 15.5 mg/L
Undefined Kingdom (2/3)	M: 2.1E+03 (1.9E+03-2.3E+03) mg/L		M: 5 mg/L

1. Highlight the data endpoint box corresponding to *Pimephales promelas*/LC50/96h under the target chemical. **2. Select** Similar MOA

Data Gap Filling

Apply Similar MOA

The screenshot shows the QSAR Toolbox interface with the 'Data Gap Filling' workflow selected. A dialog box titled 'Possible data inconsistency' is open, allowing the user to choose between different scales and units for data handling. The background displays a hierarchical tree of chemical categories and a table of experimental data for various species.

Possible data inconsistency dialog box options:

- Native scale/unit:**
 - mg/L (2 data; 1 chemicals)
 - mol/L (3 data; 3 chemicals)
 - µg/L (6 data; 3 chemicals)
- Gap filling scale/unit:**
 - log(1/mol/L)
 - mol/L
 - µg/L
 - mg/L

Table of experimental data (from screenshot):

Species	Concentration	Concentration	Concentration
Lepomis macrochirus (1/1)	M: 100 (100=500) mg/L		
Leuciscus idus (1/1)	M: 1E+03 mg/L		
Oryzias latipes (2/2)	M: >100 mg/L		M: >10 mg/L
Pimephales promelas (3/11)	M: 1.73E+03 (1.63E+03)	M: 1.99 mg/L	M: 10.9 (9.64+12.3) mg/L
Poecilia reticulata (2/2)	M: 1.74E+03 mg/L		M: 15.5 mg/L
Undefined Kingdom (2/3)	M: 2.1E+03 (1.9E+03)		M: 5 mg/L

The user will be informed if there is different experimental data. Click Ok.

Data Gap Filling Results of Similar MOA

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Filling Report

Gap Filling Workflow

Trend analysis Read across (Q)SAR Standardized Automated

The OECD QSAR Toolbox for Grouping Chemicals into Categories
Developed by LMC, Bulgaria

Document 1
Substance
Composition list
Constituent #1
Constituent #2
Constituent #3
Enter GF(SimilarMOA) with 4 chemicals, 11 data points
Enter GF(SimilarMOA) with 4 chemicals, 11 data points
Enter GF(SimilarMOA) with 4 chemicals, 11 data points

Filter endpoint tree...

Structure

Endpoint	1 [target]	2	3	4
Pimephales promelas (3/11)	M: 1.73E+03 (1.63E+03)	M: 1.99 mg/L	M: 10.9 (9.64+12.3)	
Poecilia reticulata (2/2)	M: 1.74E+03 mg/L		M: 15.5 mg/L	
Undefined Kingdom (2/3)	M: 2.1E+03 (1.9E+03)		M: 5 mg/L	
7 d (1/1)			M: 6.65 (5.96+7.41) mg	
14 d (1/1)	M: 85 mg/L			
LC50/ (1/1)	M: 2.95E+03 mg/L			
LD50 (1/1)	M: 5.49E+03 mg/L			
LOEC (1/3)			M: 6.33 mg/L	
MATC (1/2)			M: 4.58 mg/L	
MRC50 (1/1)	M: 9.33E+03 mg/L			
NOEC (2/5)	M: 46 mg/L		M: 3.31 mg/L	
NR-LETH (1/1)	M: 1.4E+03 ppm			
NR-ZERO (1/1)	M: 1E+03 ppm			

Descriptors

Prediction

Dose/concentration addition for LC50, based on 3 values
Predicted: 83.1 mg/L

log Kow

log(mol/L)

Active descriptor X log Kow

Select / filter data
Descriptors / data
Calculation options
Visual options
Information
Miscellaneous

Accept prediction

Data Gap Filling Results

- The components of the mixture have same mode of action.
- By **accepting the prediction** the data gap is filled (see next screen shot).

Data Gap Filling

Accept prediction results

The screenshot shows the QSAR Toolbox interface during the Data Gap Filling process. A 'Confirm' dialog box is open, asking for confirmation to accept a prediction. A blue callout box with the number '2' points to the 'Yes' button in the dialog. Another blue callout box with the number '1' points to the 'Accept prediction' button at the bottom right of the interface. In the background, a table displays predicted values for various endpoints.

Endpoint	1 [target]	2	3	4
M:	1.73E+03 (1.63E+03)	M: 1.99 mg/L	M: 10.9 (9.64+12.3)	M: 15.5 mg/L
M:	1.74E+03 mg/L		M: 5 mg/L	M: 6.65 (5.96+7.41) mg
M:	2.1E+03 (1.9E+03)			
M:	85 mg/L			
M:	2.95E+03 mg/L			
M:	5.49E+03 mg/L			
M:				M: 6.33 mg/L
M:				M: 4.58 mg/L
M:				M: 3.31 mg/L
M:				M: 1E+03 ppm

1. Click Accept prediction 2. Click OK

Data Gap Filling

Predicted value for LC50

The screenshot displays the QSAR Toolbox software interface. The top navigation bar includes icons for Input, Profiling, Data, Category definition, Data Gap Filling, and Report. The main window is divided into several panels:

- Documents:** Shows a tree view of 'Substance' and 'Composition list' with options to enter GF(SimilarMOA) with 4 chemicals and 11 data points.
- Filter endpoint tree...:** Shows a hierarchical tree of endpoints, including 'Structure', 'LC100', 'LC50', and various time points (1 h, 4 h, 24 h, 48 h, 72 h, 96 h, 7 d, 14 d, LC50/).
- Data Table:** Shows predicted values for various endpoints. The table has columns for 'Parent chemical', 'Constituent #1', 'Constituent #2', and 'Constituent #3'. The predicted value for LC50 is highlighted in yellow and circled in red, with a callout box containing the number '1'.

Endpoint	Parent chemical	Constituent #1	Constituent #2	Constituent #3
LC100 (1/2)		M: 1.17E+03 mg/L		
LC50 (1/2)		M: 1.22E+03 mg/L		
1 h (1/2)		M: 1.94E+03 mg/L		
4 h (1/1)		M: 0.45 % v/v		
24 h (2/13)		M: >1E+03 mg/L		M: 14.8 mg/L
48 h (2/13)		M: >1E+03 mg/L		M: 14.5 mg/L
72 h (2/3)		M: 1.94E+03 mg/L		M: 5 mg/L
96 h (1/1)		M: 661 mg/L		
Actinopterygii (ray-finned fishes)				
Alburnus alburnus (1/2)		M: 2.25E+03 mg/L		
Lepomis macrochirus (1/1)		M: 100 mg/L		
Leuciscus idus (1/1)		M: 1E+03 mg/L		
Oryzias latipes (2/2)		M: >100 mg/L		M: >10 mg/L
Pimephales promelas (4/12)		SMOA: 83.1 mg/L	M: 1.99 mg/L	M: 10.9 (9.64+12.3)
Poecilia reticulata (2/2)		M: 1.74E+03 mg/L		M: 15.5 mg/L
Undefined Kingdom (2/3)		M: 2.1E+03 (1.9E+03)		M: 5 mg/L
7 d (1/1)		M: 85 mg/L		M: 6.65 (5.96+7.41) mg/L
14 d (1/1)		M: 85 mg/L		
LC50/ (1/1)		M: 2.95E+03 mg/L		

1. Predicted value for LC50 of the mixture based on the experimental data of its components is **83.1 mg/l**

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Data
 - Data Gap filling
 - **Report**

Report

- Remember the report module allows you to generate a report on the predictions performed with the Toolbox. This module contains predefined report templates as well as a template editor with which users can define their own user defined templates. The report obtained for mixture includes specific information related to mixture prediction. The report can then be printed or saved in different formats.
- Generating the report is shown on next screenshots

Report

The screenshot displays the QSAR Toolbox software interface. At the top, the 'Report' button in the main menu is circled in red and labeled with a callout '1'. On the left sidebar, the 'Prediction Data Matrix' button is circled in red and labeled with a callout '2'. The central window is titled 'Customize report content and appearance' and features a 'Wizard pages' list on the left, where 'Prediction for mixture' is selected and labeled with a callout '3'. The main area of the dialog box contains the text: 'Highlighted is an individual report (report for a mixture, or report for one of its components). Expand the tree on the left and customize the appearance of the selected individual report.' Below this, it says 'This is an individual report for prediction for mixture' and 'To customize the report appearance navigate through report section using [Back] and [Next] buttons'. At the bottom of the dialog box, there are four buttons: 'Back', 'Next', 'Cancel', and 'Create report'. On the right side of the interface, there is a table with columns 'Constituent #2' and 'Constituent #3', showing chemical structures and predicted values like 'M: 14.8 mg/L', 'M: 14.5 mg/L', 'M: 5 mg/L', and 'M: >10 mg/L'.

3. The user could select the appropriate sections to create the report

1. Select **Report section**
2. Click **Prediction**

Outlook

- Background
- Objectives
- The exercise
- Workflow
 - Input
 - Profiling
 - Data
 - Data Gap filling
 - Report
- **Save the prediction result**

Saving the prediction result

- This functionality allow storing/restoring the current state of Toolbox documents including loaded chemicals, experimental data, profiles, predictions etc, on the same computer. The functionality is implemented based on saving the sequence of actions that led to the current state of the Toolbox document and later executing these actions in the same sequence in order to get the same result(s).
- Saving/Loading the file with TB prediction is shown on next screenshots

Saving the prediction result

The screenshot displays the QSAR Toolbox interface. The top toolbar contains several icons, with the 'Save' icon (a floppy disk) circled in red and labeled with a red '1'. A 'Save as' dialog box is open, showing the file explorer view. The 'File name' field is labeled with a red '2' and contains the text 'mixtures.tb4'. The 'Save as type' dropdown is labeled with a red '3'. The background shows a 'Composition list' table with columns for 'Constituent #1', 'Constituent #2', and 'Constituent #3'. The table contains chemical structures and predicted values in mg/L.

Constituent #1	Constituent #2	Constituent #3
<chem>C1=CC=C(C=C1)C2=CC=CC=C2</chem>	<chem>C1=CC=C(C=C1)C2=CC=CC=C2</chem>	
		M: 13.7 mg/L
		M: 1 mg/L
		M: 1.78 mg/L
		M: 0.46 mg/L
		M: 1 mg/L
		M: >10 mg/L
		M: 0.28 (0.21+0.37) mg/L
		M: 15.3 mg/L
		M: 14.8 mg/L
		M: 14.5 mg/L
		M: 5 mg/L

1. Click on Save button; 2. Define name of the file; 3. Click Save button

Open saved file

Once the file has been saved **1. Go to Input**; **2. Click Open**; **3. Find and select file**; **4. Click Open**