

## OECD QSAR Toolbox v.3.4

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

# Outlook

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## Workflow

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

# Outlook

- Background
- Objectives
- The exercise
- **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

The screenshot displays the QSAR Toolbox Chemical Input screen. The top navigation bar includes icons for **Input**, **Profiling**, **Endpoint**, **Category Definition**, **Data Gap Filling**, and **Report**. Below this, a secondary toolbar is highlighted with a red box, containing options for **Document** (New, Open, Close, Save) and **Chemical List** (CAS#, Name, Structure, Select, Delete, Query, ChemIDs, DB, Inventory, List). The main workspace is divided into a left sidebar with a **Documents** panel and a central **Filter endpoint tree...** panel. The **Filter endpoint tree...** panel shows a tree structure with **Structure** at the top, followed by **Substance Identity**, **Physical Chemical Properties**, **Environmental Fate and Transport**, **Ecotoxicological Information**, and **Human Health Hazards**. The **Substance Identity** item is highlighted. At the bottom of the main workspace, there is a dropdown menu for **select filter type ..** and **Create** and **Apply** buttons. The status bar at the bottom shows **0 Document** on the left and **1/0/0** on the right.

## Chemical Input by Drawing

- Inputting the target chemical (mixture) by drawing its components within the 2D-editor
- It is accomplished by a series of point-click operations within the 2D-editor which appears when you click on “structure” (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 'Document', 'Profile', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. Below this is a toolbar with icons for 'New', 'Open', 'Close', 'Save', 'CAS#', 'Name', 'Structure', 'Select', 'Delete', 'Query', 'ChemIDs', 'DB', 'Inventory', and 'List'. The 'Structure' button is highlighted with a red box, and a callout bubble with the number '1' points to it. The main window area shows a 'Filter endpoint tree...' panel with 'Structure' selected. Below the main window, there is a blue box with the text '1. Click on Structure'.

1. Click on Structure



# Chemical Input


## Drawing the target mixture by 2D editor

The screenshot shows the 2D Editor window with the SMILES string c1ccccc1 in the input field. The 'Templates' panel on the left contains a grid of chemical structures, with the first one (benzene) highlighted. A callout '1' points to this template. The main plot area shows a benzene ring being drawn, with a callout '2' pointing to it. The interface includes a toolbar, a status bar with 'drag the mouse with left button pressed to create bond', and 'OK' and 'Cancel' buttons at the bottom.

1. **Left Click** on the appropriate chemical form from the "Templates" panel.
2. **Move** the cursor to the large blank area and **left click** again, this puts the selected template on the plot.

# Chemical Input

## Drawing the target mixture by 2D editor

3. **Click** on  button to add a bond of selected type ("Single" in this case).
4. **Drag** the mouse to the appropriate atom and **left click** to create a single bond.

## Chemical Input by Drawing

- CH<sub>3</sub>-group is added by default when you perform left click over the atoms.
- If you make an incorrect entry you can click on the 'undo' icon in the upper corner of the screen to remove the last action
- This process allows you to build the hydrocarbon skeleton of the target molecule (see next screenshot).
- More details about how to use the 2D editor for drawing chemical compounds click F1 help: section D.2.1.3.4.1. Details of 2D Editor

# Chemical Input

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

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


- Title Bar:** 2D Editor
- Toolbar:** Includes icons for undo, redo, copy, paste, and various drawing tools. A dropdown menu is set to "Single".
- SMILES/InChi:** CC(C)c1ccc(C)cc1C
- Buttons:** "Draw" (with a globe icon) and "Mixture" are visible on the right.
- Templates:** A grid of chemical structures is shown on the left. The top-left template, representing a benzene ring, is highlighted in blue.
- Work Area:** The main drawing area shows a benzene ring with three chlorine atoms (Cl) at the 2, 3, and 4 positions. A methyl group (CH<sub>3</sub>) is attached to the 1-position, and a carbonyl group (C=O) is also attached to the 1-position. The carbonyl oxygen is highlighted with a blue box.
- Status Bar:** "drag the mouse with left button pressed to create bond"
- Buttons:** "OK" (with a green checkmark) and "Cancel" (with a red X) are at the bottom.

# Chemical Input

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


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

- Toolbar:** Contains various drawing tools. A red box highlights the 'Add Hetero Atom' button (a globe icon).
- SMILES/InChI:** The text 'CC(C)c1ccc(C)c(C)c1C' is displayed in the top bar.
- Chemical Structure:** A 2D skeletal structure of 1-(2,3,4-trichlorophenyl)ethan-1-one is shown. Three callout boxes labeled '2' point to the methyl groups on the phenyl ring.
- Callout Box '1':** Points to the 'Add Hetero Atom' button in the toolbar.
- Callout Box '2':** Points to the methyl groups on the phenyl ring.
- Left Panel:** Shows a grid of chemical templates under the 'Templates' tab.
- Bottom Bar:** Contains the text 'click the mouse to create/modify atom' and the URL 'oasis-lmc.org'.

1. **Click** on  button to add a hetero atom (in this case chlorine atom).
2. **Left click** with mouse over the methyl group to insert the selected chlorine atoms.

# Chemical Input

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

3. Use the arrow and select O atom **Click** on  button to select it (in this case an oxygen atom).
4. **Left click** with mouse over the methyl group to insert an oxygen atom.

# Chemical Input

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

The screenshot shows the 2D Editor window with the SMILES string CC(=O)c1ccc(Cl)c(Cl)c1Cl in the top bar. A dropdown menu is open over the 'Double' bond button, with 'Double' selected. A callout '5' points to this menu. On the right, a chemical structure of 1-(2,3,4-trichlorophenyl)ethan-1-one is shown, with a callout '6' pointing to the C=O double bond being formed.

5. Click on  and select  bond.

6. Drag the mouse from the C-atom to O-atom to create a double bond

# Chemical Input

Drawing the components of mixture  
 "Diphenylmethanone" and "Butan-1-ol"

The screenshot shows the 2D Editor software interface. At the top, the SMILES/InChI string is CCCCO\_CC(=O)c1ccc(Cl)c(Cl)c1Cl\_c1ccccc1C(=O)c1ccccc1. Below the string is a template palette with various chemical structures. In the main workspace, two chemical structures are shown. Structure 8 is Diphenylmethanone, which is circled in black. Structure 9 is Butan-1-ol, which is also circled in black. A blue callout box with the number 8 points to the Diphenylmethanone structure, and a blue callout box with the number 9 points to the Butan-1-ol structure. The text "oasis-lmc.org" is visible in the bottom right corner of the software window.

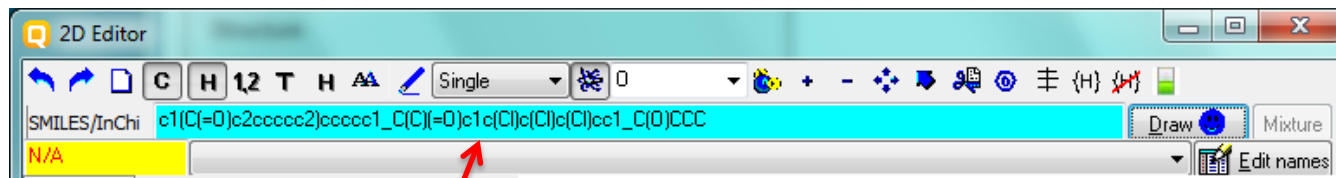
- 8. **Draw** the second mixture component - Diphenylmethanone
- 9. **Draw** the third mixture component - butan-1-ol



# Chemical Input

## Alternatives for defining components of mixture

- The other alternative of drawing mixture is to:
  - Drawn the SMILES of each component
  - Link the SMILES of the components with underscore character
  - Copy the linked SMILES and Paste it in the SMILES/InChi filed of 2D editor window



Paste the linked SMILES of the components of the mixture in the SMILES/InChi field

# Chemical Input

## Input quantities of mixture

- Once the constituents of the mixtures are pasted or drawn in the 2D editor window, a specific button for defining quantities appears (see next screenshot)
- Quantities of the constituents should be added manually
- There are several ways to add mixture quantity:
  - Mass
  - Mass Concentration
  - Volume Concentration
  - Fraction %
- Select "Fraction %" then "Weight %"



# Chemical Input

## Input quantities of mixture

Q2D Editor

SMILES/InChi X=0/weight %)c1cccc1C(=O)c1cccc1\_X=0/weight %)CC(=O)c1ccc(Cl)c(Cl)c1Cl\_X=0/weight %)CCCCO

Unit families

- Mass
- Mass Concentration
- Volume Concentration
- Fraction, %

Units

	Units	
	weight %	
	mol %	
	weight %	
1	0	CCCCCO
2	0	CC(=O)c1ccc(Cl)c(Cl)c1Cl
3	0	c1cccc1C(=O)c1cccc1

click/drag with: left button to select; right button to move

1. **Select** radio button "Fraction %"
2. **Select** "Wight %" from the appeared pop-up menu

# Chemical Input

## Input quantities of mixture

1

Unit families

Mass

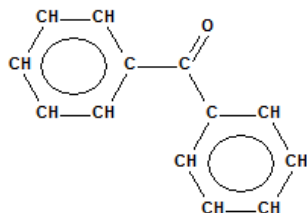
Mass Concentration

Volume Concentration

Fraction, %

Units weight %

1	9	c1(C(=O)c2ccccc2)ccccc1
2	0	C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1
3	0	C(O)CCC



2

Unit families

Mass

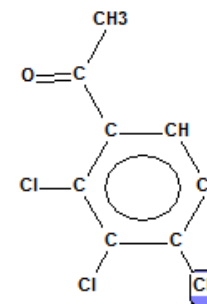
Mass Concentration

Volume Concentration

Fraction, %

Units weight %

1	9	c1(C(=O)c2ccccc2)ccccc1
2	1	C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1
3	0	C(O)CCC



3

Unit families

Mass

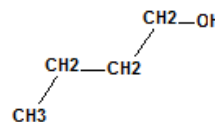
Mass Concentration

Volume Concentration

Fraction, %

Units weight %

1	9	c1(C(=O)c2ccccc2)ccccc1
2	1	C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1
3	90	C(O)CCC



1. Type 9 for mixture component #1
2. Type 1 for mixture component #2
3. Type 90 for mixture component #3

# Chemical Input

## Input quantities of mixture

The screenshot shows the QSAR Toolbox software interface. The main window is the '2D Editor', which displays a chemical list table. The table has columns for 'Units', 'weight %', and 'SMILES/InChI'. The SMILES string in the background is C=C(C=C)C(=O)C1=CC=CC=C1. The chemical structure shown is a benzene ring with a vinyl group (-CH=CH2) and a carbonyl group (-C(=O)-) attached to the same carbon atom.

Units	weight %	SMILES/InChI
90	CCCCO	
1	CC(=O)c1cc(C)c(C)c(C)c1Cl	
9	c1ccccc1C(=O)c1ccccc1	

Callouts in the image indicate the following actions:

- 1: Type 9 for mixture component #1
- 2: Type 1 for mixture component #2
- 3: Type 90 for mixture component #3

1. Type 9 for mixture component #1
2. Type 1 for mixture component #2
3. Type 90 for mixture component #3

# Chemical Input

## Target chemical identity

- The already drawn mixture automatically appears on the data matrix
- 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).
- Visualization of components of the mixture is possible when user select Single Component Mode (see next slide)

# Chemical Input

## Target chemical identity

1

Structure

Substance Identity

- CAS Number
- Chemical IDs
- Chemical Name
- Structural Formula

Physical Chemical Prop...

Environmental Fate and ...

Ecotoxicological Informa...

Human Health Hazards

1 [target]			
[3] [Mix]			
CAS Number	N/A		
Chemical IDs	NA		
Chemical Name			
Structural Formula	<chem>[X=1/weight %]CC(=O)c1ccc(Cl)c(Cl)c1Cl_[X=9/...</chem>		

2

Structure

Substance Identity

- CAS Number
- Chemical IDs
- Chemical Name
- Structural Formula

Physical Chemical Prop...

Environmental Fate and ...

Ecotoxicological Informa...

1 [target]	2 [target, mix component]	3 [target, mix component]	4 [target, mix component]
[3] [Mix]			
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %
CAS Number	N/A	N/A	N/A
Chemical IDs	NA	NA	NA
Chemical Name			
Structural Formula	<chem>[X=1/weight %]CC(=O)c1ccc(Cl)c(Cl)c1Cl_[X=9/...</chem>	<chem>CC(=O)c1ccc(Cl)c(Cl)c1Cl</chem>	<chem>O=C(c1ccccc1)c1ccccc1</chem>

1. Select "All" radio button to see all components
2. Select "Single" radio button to see all individual components



# 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 displays the QSAR Toolbox software interface. The top navigation bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Profiling' section is active, showing a sidebar with 'Profiling methods' and 'Metabolism/Transformations' sections. A callout '1' points to the 'About' button in the 'Profiling methods' sidebar. The main window shows a 'Filter endpoint tree...' with a 'Structure' section containing chemical structures and labels like '1 [target]', '2 [target,mix.component]', '3 [target,mix.component]', and '4 [target,mix.component]'. A callout '2' points to the 'About' button in the 'Structure' section. An 'About' dialog box is open, displaying details for the 'Acute aquatic toxicity MOA by OASIS' profiler. A callout '3' points to the 'Close' button in the dialog box. The 'About' dialog box contains the following information:

<b>Name</b>	Acute aquatic toxicity MOA by OASIS
<b>Donator(s)</b>	Laboratory of Mathematical Chemistry (LMC), Bourgas, Bulgaria
<b>Author(s)</b>	Laboratory of Mathematical Chemistry (LMC), Bourgas, Bulgaria
<b>Website</b>	<a href="http://www.oasis-lmc.org">http://www.oasis-lmc.org</a>
<b>Details</b>	
<b>Number of nodes</b>	18
<b>Number of help files</b>	16
<b>Scheme version</b>	3.0
<b>Adopted</b>	2010 x 2.0 beta, April 2010

**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 Aquatic toxicity MOA

QSAR Toolbox 3.4.0.17 [Document\_6]

Acute aquatic toxicity MOA by OASIS (Endpoint Specific) - Profiling Scheme Browser

1. Highlight the profiler

2. Click View

3. Click on one of the nodes

4. Boundaries defined the rules

5. Click Reference to see detailed information. (Base surface narcotics)

# 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 v.1.4
  - Protein binding by OECD

# Profiling

## Profiling the target chemical

The screenshot shows the QSAR Toolbox Profiling interface. The 'Profiling' menu is open, and the 'Apply' button is circled in red, labeled with a red '2'. In the 'Profiling methods' list on the left, three methods are checked with green checkmarks, labeled with a red '1': 'Protein binding by OASIS v1.4', 'Protein binding by OECD', and 'Acute aquatic toxicity classification by ECOSAR'. The main window displays a table of results for four endpoints: '1 [target]', '2 [target,mix.component]', '3 [target,mix.component]', and '4 [target,mix.component]'. The table includes columns for 'Structure', 'Substance Identity', 'Physical Chemical Properties', 'Environmental Fate and Transport', 'Ecotoxicological Information', 'Human Health Hazards', and 'Profile'. Chemical structures are shown for each endpoint, and numerical values are provided for the 'Ecotoxicological Information' and 'Human Health Hazards' columns.

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

The screenshot shows the QSAR Toolbox software interface. The top navigation bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Profiling' menu is open, showing various profiling methods. A red circle highlights the 'Profile' button in the 'Profile' section of the menu. Below the menu, a table displays the results of profiling for four target components (1 [target], 2 [target,mix.component], 3 [target,mix.component], 4 [target,mix.component]). The table is organized into sections: Substance Identity, Physical Chemical Properties, Environmental Fate and Transport, Ecotoxicological Information, Human Health Hazards, and Profile. The 'Profile' section is expanded, showing results for 'Predefined', 'General Mechanistic', and 'Endpoint Specific' categories. Red boxes highlight specific rows in the 'Profile' section, indicating that components 1, 2, 3, and 4 share the same mode of action for several categories: US-EPA New Chemical Categories (Neutral Organics), Protein binding by OASIS v1.4 (No alert found), Protein binding by OECD (No alert found), Acute aquatic toxicity classification by Verhaar (MOA), Acute aquatic toxicity MOA by OASIS (Basesurface narco...), and Acute toxicity classification by ECOSAR (Neutral Organics).

	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
Structure	[Chemical Structure]	[Chemical Structure]	[Chemical Structure]	[Chemical Structure]
Substance Identity				
Physical Chemical Properties				
Environmental Fate and Transport				
Ecotoxicological Information (3/174)		M: 1.99 mg/L, 2 m...	M: 2.6E3 mg/L, 1...	M: 27 mg/L, 14.8 ...
Human Health Hazards				
Profile				
Predefined				
US-EPA New Chemical Categories	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
General Mechanistic				
Protein binding by OASIS v1.4	No alert found SNAr SNAr >> Nucleoph...	SNAr SNAr >> Nucleoph...	No alert found	No alert found
Protein binding by OECD	No alert found	No alert found	No alert found	No alert found
Endpoint Specific				
Acute aquatic toxicity classification by Verhaar (MOA)	Class 1 (narcosis ... Class 5 (Not possi...	Class 1 (narcosis ...	Class 1 (narcosis ...	Class 5 (Not possi...
Acute aquatic toxicity MOA by OASIS	Basesurface narco...	Basesurface narco...	Basesurface narco...	Basesurface narco...
Acute toxicity classification by ECOSAR	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics

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

1. **Single click** on the box (or double click on Profile) to open the nodes of the tree

# Outlook

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  - Profiling
  - **Endpoint**

## Endpoint

- “Endpoint” 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; Aquatic US-EPA ECOTOX.**

# Endpoint

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The toolbar below has 'Data', 'Import', 'Export', 'Delete', and 'Tautomerize'. The left sidebar shows a tree view of 'Ecotoxicological Information' with checkboxes for various databases. The main window displays a 'Filter endpoint tree...' table with columns for different target endpoints and their associated chemical structures and data.

Filter endpoint tree...	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
Structure	[3] [Mix]	A	A	A
Substance Identity				
Physical Chemical Properties				
Environmental Fate and Transport				
Ecotoxicological Information (3/174)		M: 1.99 mg/L, 2 m...	M: 2.6E3 mg/L, 1...	M: 27 mg/L, 14.8 ...
Human Health Hazards				
Profile				

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

# Endpoint 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. The 'Endpoint' tab is active, displaying a 'Filter endpoint tree...' window. In the left sidebar, under 'Databases', the 'Human Health Hazards' section is expanded, and the 'Aquatic ECETOC' checkbox is circled in red. A 'Read data?' dialog box is open in the foreground, with the 'All endpoints' radio button selected. The 'OK' button in the dialog is highlighted with a blue callout box containing the number '1'.

**1. Click OK to read all available aquatic tox data**

# Endpoint Process of collecting data

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

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

- Navigation:** Input, Profiling, Endpoint, Category Definition, Data Gap Filling, Report.
- Databases:** Physical Chemical Properties, Environmental Fate and Transport, Ecotoxicological Information (checked), Human Health Hazards.
- Inventories:** Canada DSL, COSING, DSSTOX, ECHA PR, EINECS, HPVC OECD, METI Japan, NICNAS, REACH ECB.
- Tree View:** Ecotoxicological Information > Aquatic Toxicity > Mortality > LC50 > 96 h.
- Table:**

	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
[3] [Mix]				
Behavior (2/5)		M: 2 mg/L		M: 13.7 mg/L, 14.5...
Development (1/5)				M: 2.43 mg/L, 6.38...
Growth (1/15)				M: 0.54,0.57 mg/L,...
Mortality (3/3)		M: 2 mg/L	M: 1.73E3 mg/L	M: 15.3 mg/L
EC50 (1/2)			M: 1.95E3 mg/L, 1...	
24 h (2/4)			M: 1.94E3 mg/L, 1...	M: 14.8 mg/L, 15.2...
48 h (2/4)			M: 1.94E3 mg/L, 1...	M: 14.5 mg/L, 15.2...
72 h (1/2)			M: 1.95E3 mg/L, 1...	
96 h (3/10)		M: 1.99 mg/L, 2 mg/L	M: 1.74E3 mg/L, 1...	M: 14.8 mg/L, 15.2...
Actinopterygii (Fish)				
Pimephales promelas (1/1)				M: 6.65(5.96,7.41) ...
7 Days (1/3)				M: 9.24 mg/L, 6.38...
LOEC (1/2)				M: 7.36 mg/L, 4.58...
NOEC (1/4)				M: 5.86 mg/L, 8.66...

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

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  - Input
  - Profiling
  - Endpoint
  - **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 #50-55
- 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)**

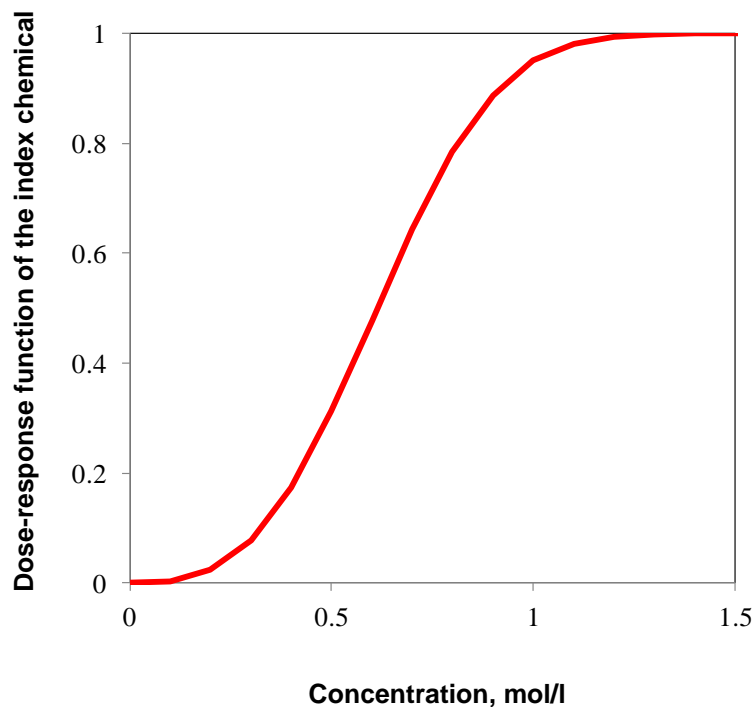
$$ICED = \sum_{j=1}^J CED_j^{(i)} = \sum_{j=1}^J RPF_j^{(i)} d_j$$

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



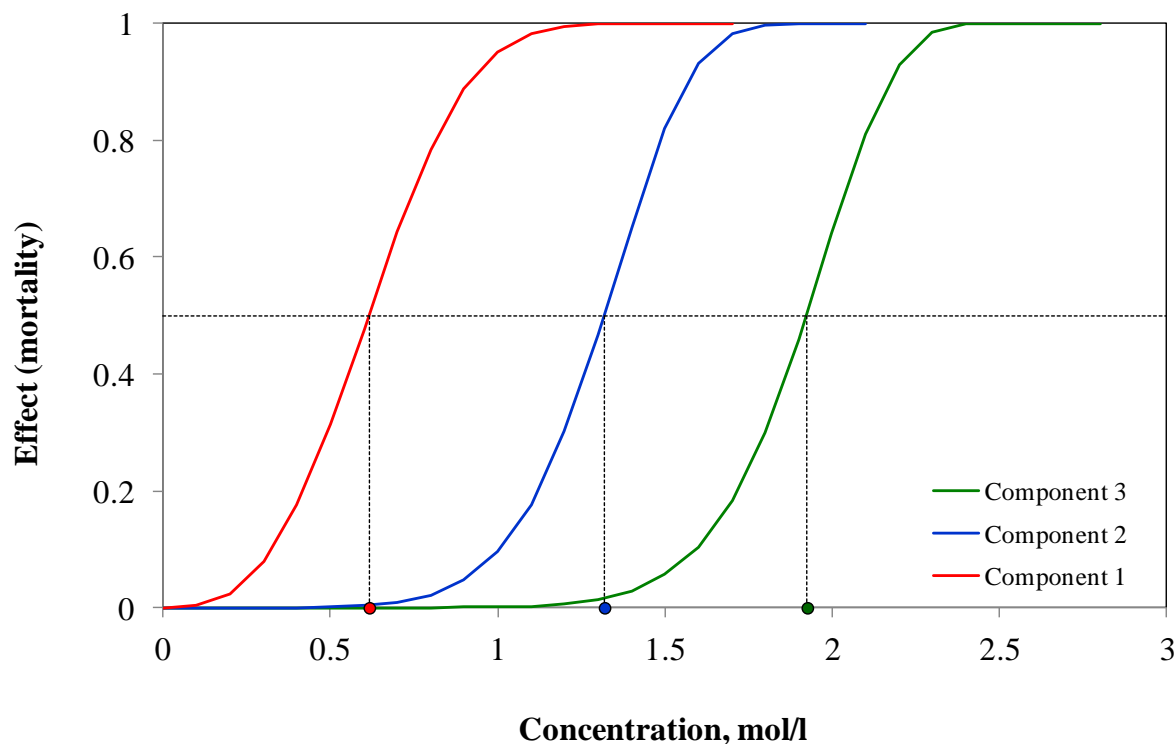
$$Effect^{Mixture} = f_i(ICED)$$

$f_i$  - 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

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

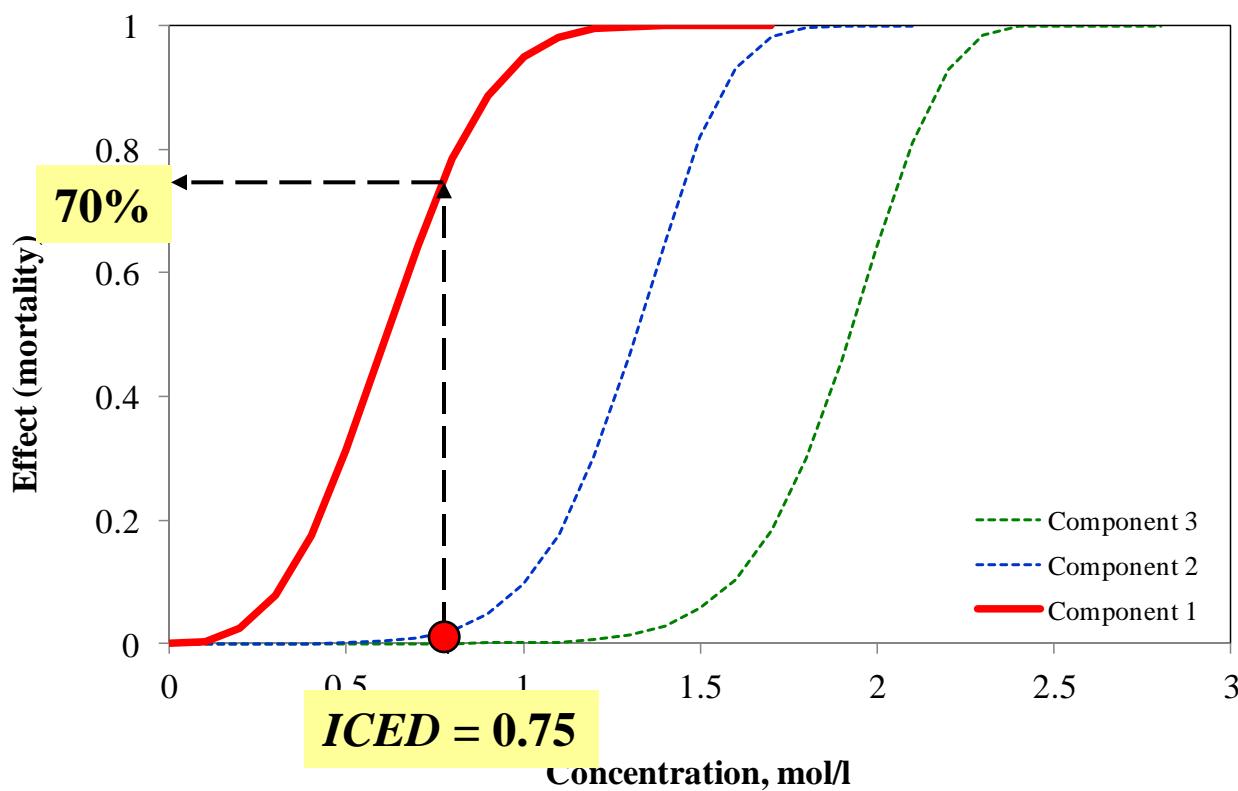
Index Chemical Equivalent Concentration

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

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

# 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 Results of Similar MOA

QSAR TOOLBOX

Input Profiling Endpoint Category Definition Data Gap Filling Report

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

**Data Gap Filling Method**

- Independent MOA
- Similar MOA
- Specific models

**Target Endpoint**

Ecotoxicological Information Aquatic Toxicity Mortality LC50 96 h Animalia Chordata Actinopterygii Pimephales promelas

Structure

1 [target] 2 [target,mix.component] 3 [target,mix.component] 4 [target,mix.component]

[3] [Mix]

CS: 82.8 mg/L

Qty: 1 weight % M: 1.99 mg/L, 2 mg/L

Qty: 9 weight % M: 14.8 mg/L, 15.3...

Qty: 90 weight % M: 1.74E3 mg/L, 1...

Pimephales promelas (4/11)

Prediction

Accept prediction

Return to matrix

- Select/filter data
- Selection navigation
- Descriptors/data
- Calculation options
- Visual options
- Information
- Miscellaneous

Information

The current prediction was accepted

OK

Prediction of LC50, making a dose/concentration addition, based on 3 values from 3 target components, Observed target value: N/A, Predicted target value: 82.8 mg/L

log Kow

LC50 (obs.), log<sub>1</sub>(mmol)

Descriptor X: log Kow

# Data Gap Filling

## Interpreting Similar mode

- The resulting plot outlines the log of the experimental LC50 results of all analogues (Y axis) according to a descriptor (X axis) with Log Kow being the default descriptor (see next slide).
- The **RED** dot represents the predicted value for the target chemical (i.e. mixture).
- The **BLUE** dots represent the experimental results available for the analogues(i.e. components of the mixture) used in the analysis.

## 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).
- By **clicking** on Return to Matrix, the user can close the Similar mode and proceed with the workflow (see next screen shot).

# Data Gap Filling

## Accept prediction results

The screenshot shows the QSAR Toolbox interface during the Data Gap Filling process. The top navigation bar includes tabs for Input, Profiling, Endpoint, Category Definition, Data Gap Filling, and Report. The main workspace displays the chemical structure of *Pimephales promelas* and a table of target components. The table lists four target components with their respective quantities and predicted values:

Target Component	Quantity	Observed Value	Predicted Value
1 [target]	1 weight %	CS: 82.8 mg/L	82.8 mg/L
2 [target,mix.component]	9 weight %	M: 1.99 mg/L, 2 mg/L	1.99 mg/L, 2 mg/L
3 [target,mix.component]	14.8 weight %	M: 14.8 mg/L, 15.3	14.8 mg/L, 15.3
4 [target,mix.component]	90 weight %	M: 1.74E3 mg/L, 1...	1.74E3 mg/L, 1...

The graph shows the prediction of LC50 (log(1 mol/L)) versus log Kow. The predicted target value is 82.8 mg/L. An information dialog box indicates that the current prediction was accepted. The right-hand sidebar contains a 'Return to matrix' button and a list of options: Select/filter data, Selection navigation, Descriptors/data, Calculation options, Visual options, Information, and Miscellaneous. Three numbered callouts (1, 2, 3) highlight the 'Accept prediction', 'OK', and 'Return to matrix' buttons respectively. A bottom banner contains the instructions: 1. Click Accept prediction, 2. Click OK, 3. Click Return to matrix.

# Data Gap Filling

## Predicted value for LC50

The screenshot shows the QSAR Toolbox interface with the 'Data Gap Filling' tab selected. The left sidebar shows the 'Data Gap Filling Method' set to 'Independent MOA' and the 'Target Endpoint' set to 'Ecotoxicological Information Aquatic Toxicity Mortality LC50 96 h Animalia Chordata Actinopterygii Pimephales promelas'. The main area displays a table of predicted values for various endpoints, with the predicted LC50 value for the mixture highlighted in red and circled. A callout box with the number '1' points to this value.

Endpoint	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
Structure	[3] [Mix]	A	A	A
Biochemistry (1/1)				
Development (2/7)				
Growth (2/25)				
Immobilisation				
Intoxication (2/8)			M: 0.28(0.21;0.37) ...	M: 1.99E3 mg/L, 1...
Mortality				
EC50 (3/5)		M: 2 mg/L	M: 15.3 mg/L	M: 2.6E3 mg/L, 1...
LC0 (1/2)				M: 1.62E3 mg/L, 1...
LC100 (1/2)				M: 1.22E3 mg/L, 1...
LC50				
1 h (1/2)				M: 1.95E3 mg/L, 1...
4 h (1/1)				M: 0.45 percent vol...
24 h (2/13)			M: 7.6 mg/L, 14.8 ...	M: 1.91E3 mg/L, 1...
48 h (2/13)			M: 27 mg/L, 14.5 ...	M: 1.94E3 mg/L, 1...
72 h (2/3)			M: 5 mg/L	M: 1.95E3 mg/L, 1...
96 h				
Animalia				
Arthropoda (Invertebrates) (1/3)				M: 661 mg/L, 2.1E...
Chordata (Vertebrates)				
Actinopterygii (Fish)				
Alburnus alburnus (1/2)				M: 2.25E3;2.4E3 ...
Lepomis macrochirus (1/1)				M: 100(100;500) m...
Leuciscus idus (1/1)				M: 1E3 mg/L
Pimephales promelas (1/1)				M: 1.74E3 mg/L, 1...
Pimephales promelas (1/1)	CS: 82.8 mg/L	M: 1.99 mg/L, 2 mg/L	M: 14.8 mg/L, 15.3...	M: 1.74E3 mg/L

1. Predicted value for LC50 of the mixture based on the experimental data of its components is **82.8 mg/l**

# Outlook

- Background
- Objectives
- The exercise
- **Workflow**
  - Input
  - Profiling
  - Endpoint
  - 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

Available data to report

- Predictions
- (Q)SARs
- Categories

Available report templates

- Standard (predefined)
  - QSAR Model Reporting Format (QMRF v.3.3)
  - QSAR Toolbox Category Report (CCRF v.3.3)
  - QSAR Toolbox Prediction Report (TPRF v.3.3)
- Custom (user defined)
  - Editable copy of QSAR Model Reporting Format (Q)
  - Editable copy of QSAR Toolbox Category Report (C
  - Editable copy of QSAR Toolbox Prediction Report (P

show only relevant templates

Prediction [1]

Prediction of LC50 for {X=1/weight  
 %)CC(=O)c1ccc(Cl)c(Cl)c1Cl\_(X=9/weight  
 %)O=C(c1cccc1)c1cccc1\_(X=90/weight %)CCCCO

1 / 16

**QSAR Toolbox prediction for multicomponent substance**  
 (uses single component mode for handling of target mixture and its components)

The template of the current report is based on "GUIDANCE DOCUMENT ON THE VALIDATION OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIPS MODELS"

4 Document

## 1. Generated report

# Report

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The main window displays a report for a mixture prediction. The 'Summary' section is circled in red and labeled with a blue callout box containing the number '1'. The table below is also labeled with a blue callout box containing the number '2'. A legend at the bottom explains the callouts.

**Summary**

Toxicity of the target mixture (82.8 mg/L) is predicted from its components using estimation based on 3 values within the range 2.00 - 1.83E+03 mg/L from 3 components having similar mode of action. Both experimental and predicted values for mixture components are used in predicting the target toxicity. The components of a mixture are handled with the functionality for category. The same approach can be applied for mixtures, but also for mono-constituent substances with impurities, multi-constituent substances and UVCBs with identified constituents.

The target mixture FALLS within applicability domain of the prediction (see Section 4.3 for details).

The data used for calculating the current prediction is taken from 10 experimental values selected from the following database(s):

1. Aquatic OASIS
2. ECOTOX

Below is a summary table for endpoint & descriptor values for the target mixture and the mixture components. Experimental values from data matrix are presented in bold font. Recalculated endpoint values (if required by selected data usage option in Gap Filling) are presented in italic font. Recalculated endpoint values based on experimental data only are presented in bold and italic font.

	Qty, weight %	Endpoint(s)
		Ecotoxicological Information; Aquatic Toxicity
		mg/L
<i>Target mixture</i>	-	-
<i>Mix. comp. No. 1</i>	1.00	<b>2.00</b>
<i>Mix. comp. No. 2</i>	9.00	<b>13.7</b>
<i>Mix. comp. No. 3</i>	90.0	<b>1.83E+03</b>

1. Summary information for mixture prediction  
2. Quantity and experimental data for components of the mixture

## Outlook

- Background
- Objectives
- The exercise
- Workflow
  - Input
  - Profiling
  - Endpoint
  - 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

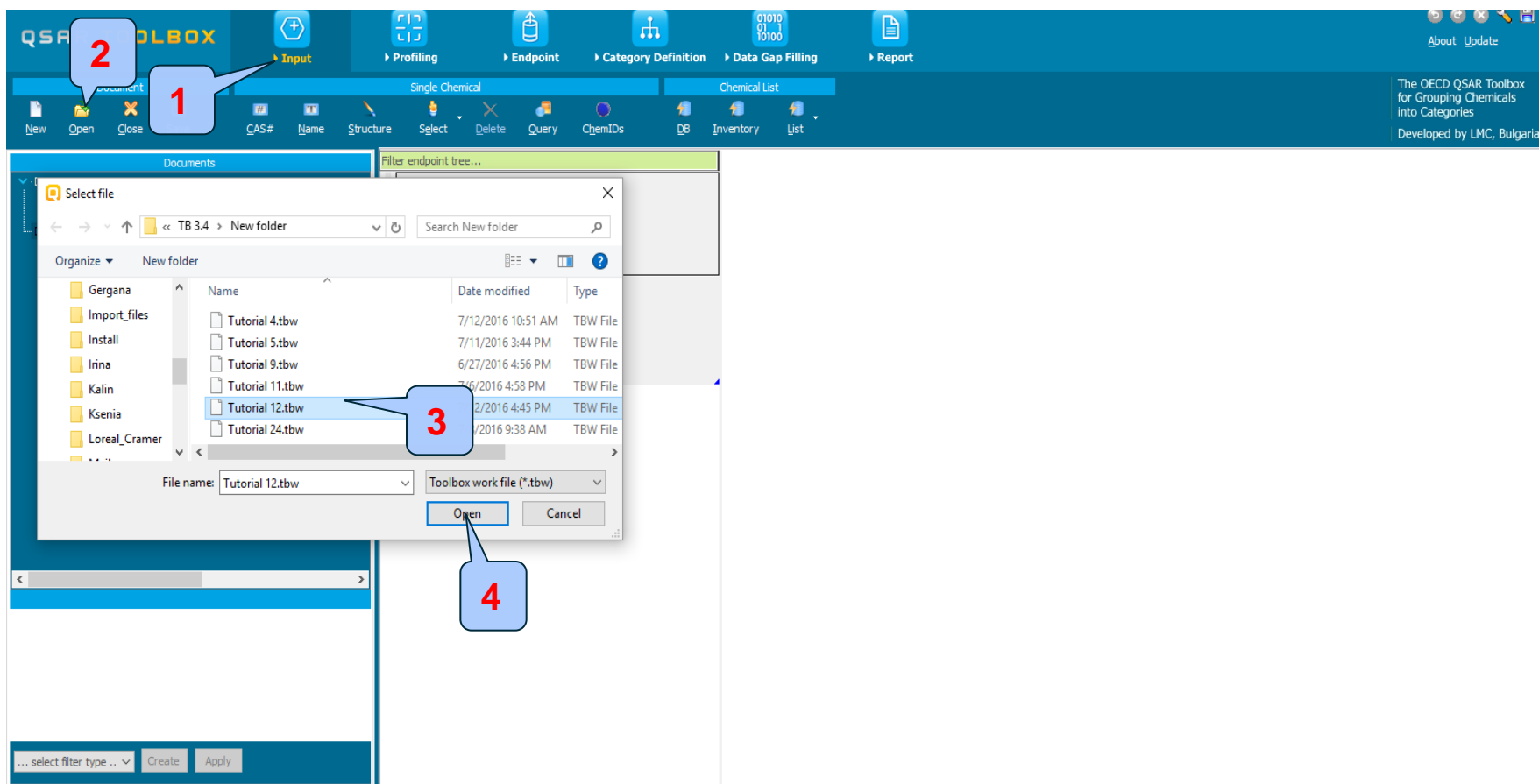
# Saving the prediction result

The screenshot displays the QSAR Toolbox software interface. A 'Save As' dialog box is open, showing the file name 'Tutorial 12.tbw' and the 'Save' button highlighted. Three numbered callouts (1, 2, 3) indicate the steps: 1. Click on Save button; 2. Define name of the file; 3. Click Save button. The background shows a table with columns for target, mix, and component, and rows for various chemical structures and their predicted values.

1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
[3] [Mix]	A	A	A
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %
	M: 1 mg/L	M: 2.43 mg/L, 6.38 ...	M: 823 mg/L, 875 ...
		M: 24.6 mg/L, 0.46...	M: 180 mg/L, 560 ...
		M: 0.28(0.21;0.37) ...	M: 1.99E3 mg/L, 1...
	M: 2 mg/L	M: 15.3 mg/L	M: 2.6E3 mg/L, 1...
			M: 1.62E3 mg/L, 1...
			M: 1.22E mg/L, 1...
			M: 1.95E3 mg/L, 1...
			M: 0.45 percent vol...
		M: 7.6 mg/L, 14.8 ...	M: 1.91E3 mg/L, 1...
		M: 27 mg/L, 14.5 ...	M: 1.94E3 mg/L, 1...
		M: 5 mg/L	M: 1.95E3 mg/L, 1...
			M: 661 mg/L, 2.1E...
			M: 2.25E3;2.4E3 ...
			M: 100(100-500) ...

**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