

## OECD QSAR Toolbox v.3.1

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

# Outlook

- **Background**
- Objectives
- The exercise
- Workflow

# 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**
- The exercise
- Workflow

# 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

# Outlook

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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

- Background
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- **Workflow**



## 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:**

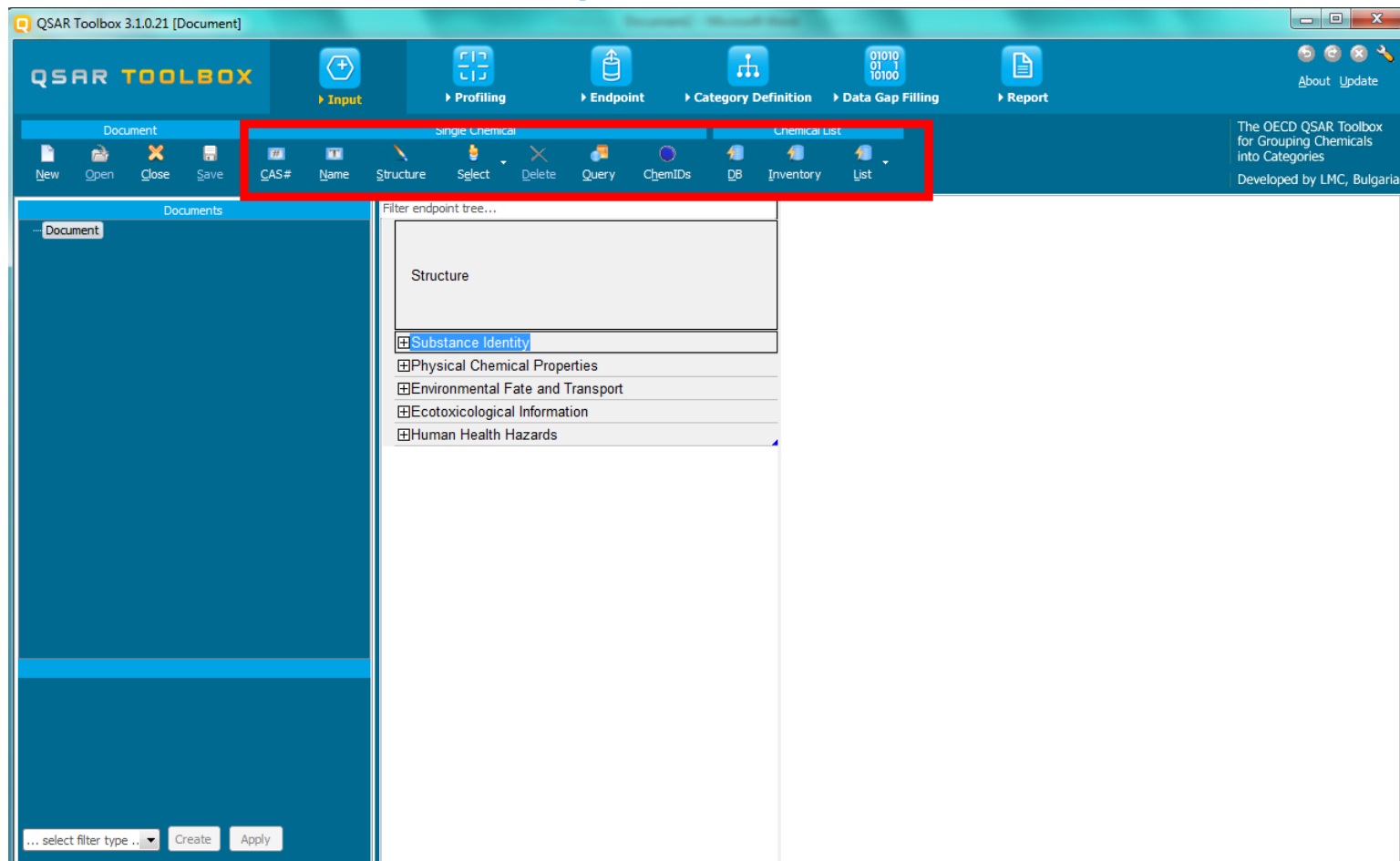
- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing mixture constituents and defining their quantities
- Select from User List/Inventory/Databases
- Chemical IDs such as EC number, Einecs number
- Load file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

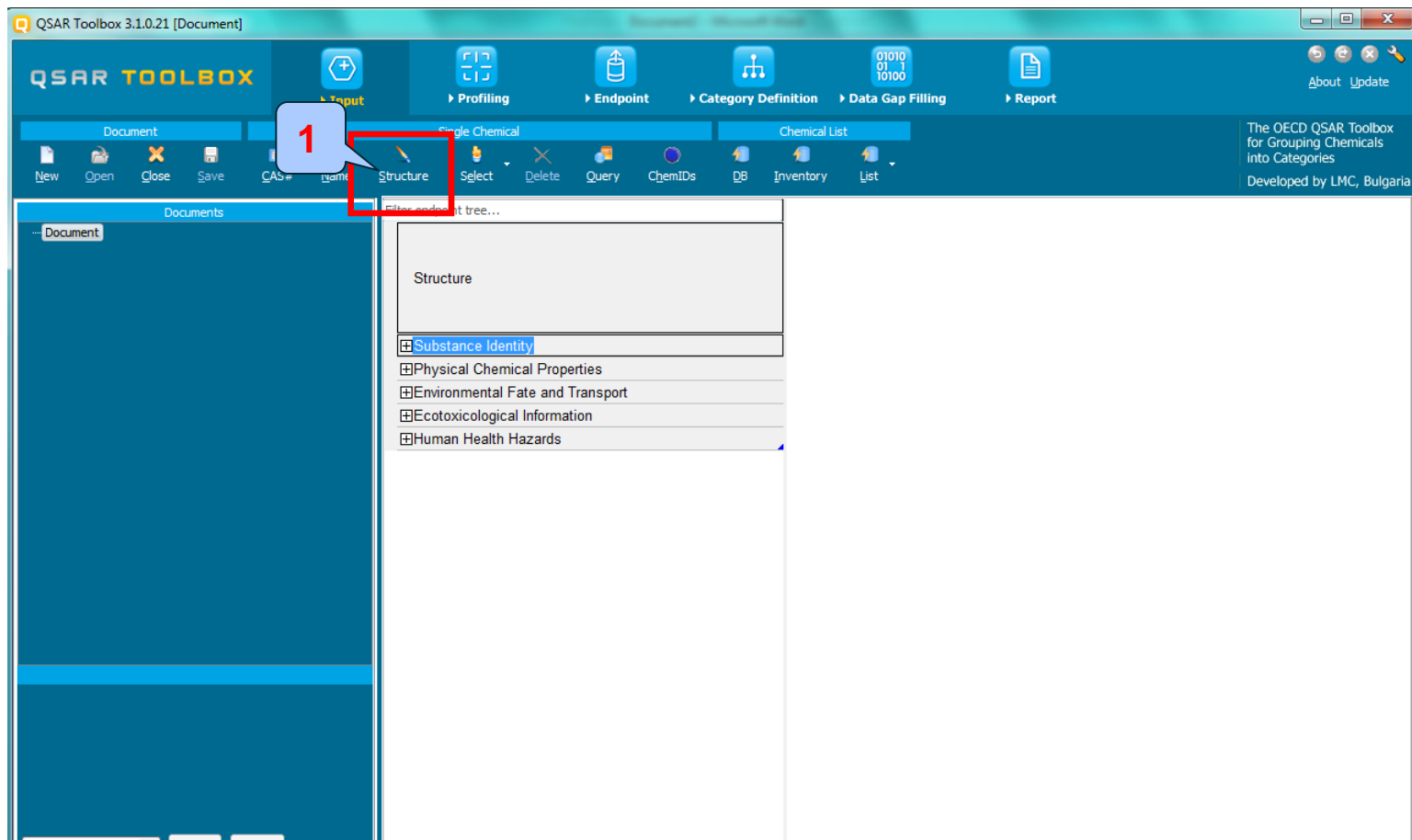


## Chemical Input by Drawing

- 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

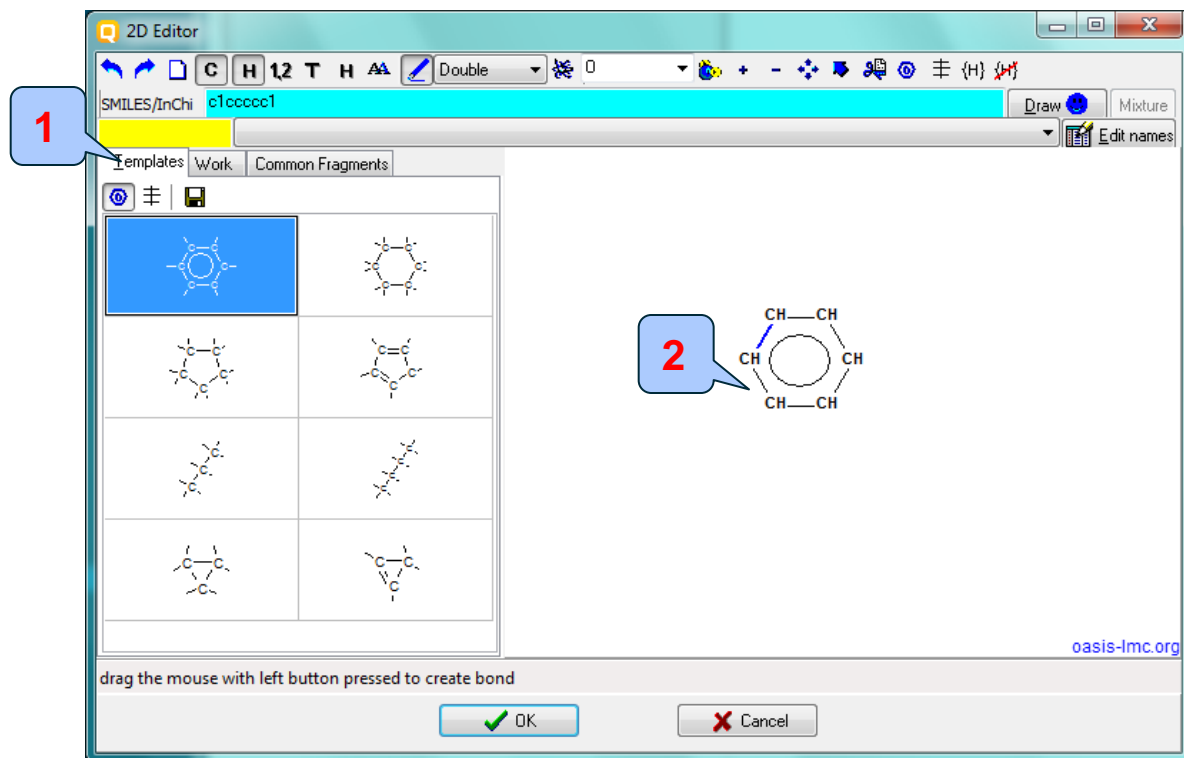


1. **Click** on Structure



# Chemical Input

## Drawing the target mixture by 2D editor



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


2D Editor

SMILES/InChI c1(C)ccccc1

Templates Work Common Fragments

drag the mouse with left button pressed to create bond

oasis-lmc.org

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 SMILES string c1(C)c(C)c(C)c(C)cc1 in the input field. The main canvas displays the chemical structure of 1-(2,3,4-trichlorophenyl)ethan-1-one, which consists of a benzene ring with three chlorine atoms at the 2, 3, and 4 positions, and an ethyl group at the 1 position. The ethyl group is shown as a CH-CH<sub>3</sub> chain attached to the ring. The interface includes a toolbar with various drawing tools, a template gallery on the left, and a status bar at the bottom with 'OK' and 'Cancel' buttons.

# Chemical Input

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


2D Editor

SMILES/InChi c1(C)c(C)c(C)c(C)cc1

Templates Work Common Fragments

drag the mouse with left button pressed to create bond


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

The screenshot shows the 2D Editor window with the SMILES string CC(=O)c1c(Cl)c(Cl)c(Cl)c1 in the input field. The toolbar contains various drawing tools, with the 'O' button highlighted by a red box and a blue callout labeled '3'. The main canvas displays the chemical structure of 1-(2,3,4-trichlorophenyl)ethan-1-one, with a blue callout labeled '4' pointing to the methyl group. The interface includes a 'Templates' panel on the left and a 'Draw' button on the right.

3. **Click** on  button to add a hetero atom (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 c1(C)c(C)c(C)c(C(C)O)cc1 in the input field. A dropdown menu is open over the 'Double' bond option, and a callout '5' points to it. Another callout '6' points to the C-O bond in the structure.

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 c1(C(=O)c2ccccc2)ccccc1\_C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1\_C(O)CCC. The interface includes a toolbar with drawing tools, a template palette on the left, and a main workspace. In the workspace, two chemical structures are highlighted with callouts: structure 8 is Diphenylmethanone (two benzene rings connected by a carbonyl group), and structure 9 is Butan-1-ol (a four-carbon chain with a hydroxyl group at the end). The template palette shows various ring and chain structures available for drawing.

9

8

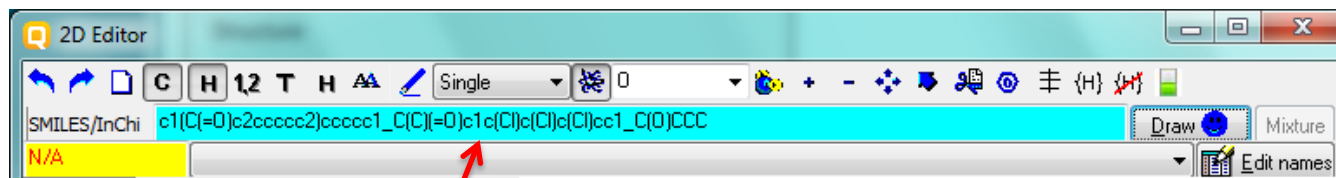
- 8. **Draw** the second mixture component - Diphenylmethanone
- 9. **Draw** the third mixture component - butan-1-ol



# 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

1

2D Editor

SMILES/InChi c1(C(=O)c2ccccc2)cccc1\_C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1\_C(O)CCC

Draw Mixture

Common Fragments

click the mouse to create/modify atom

oasis-lmc.org

1. Click on  button to add mixture quantities

# Chemical Input

## Input quantities of mixture

2D Editor

SMILES/InChi c1(C(=O)c2ccccc2)ccccc1\_C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1\_C(O)CCC

Unit families

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

Units

- weight %
- mol %
- weight %

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

CH<sub>3</sub>—CH<sub>2</sub>—CH<sub>2</sub>—OH

oasis-lmc.org

click the mouse to create/modify atom

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

# Chemical Input

## Input quantities of mixture

1

Unit families

Mass

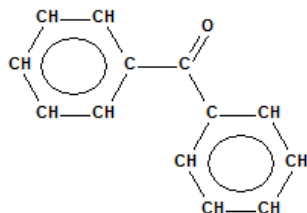
Mass Concentration

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

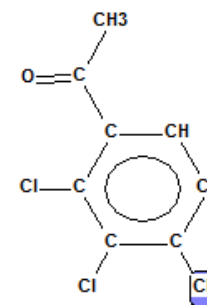
Mass Concentration

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

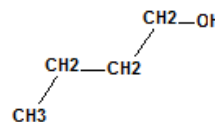
Mass Concentration

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

## 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

The left screenshot shows the software interface with the 'Component Mode' dropdown set to 'All'. A red box highlights the 'Single' radio button, with a callout box containing the number '1'. The right screenshot shows the same interface with 'Component Mode' set to 'Single', displaying a table of three chemical components. The table is circled in red.

1 [target]	2 [target,mx.component]	3 [target,mx.component]	4 [target,mx.component]
[3] [Mix]			
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %
	N/A	N/A	N/A
	NA	NA	NA
	Chemical Name		
	Structural Formula	<chem>C(C)=O)c1c(Cl)c(C(=O)c1c(Cl)c(Cl)c1)C(C)=O</chem>	<chem>c1(C(=O)c2ccccc2)c(O)CCC</chem>
	Physical Chemical Properties		
	Environmental Fate and Transport		
	Ecotoxicological Information		
	Human Health Hazards		

1. 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 3.1.0.21 interface. The 'Profiling' side-bar on the left contains a list of profilers under 'Endpoint Specific'. The 'About' dialog box is open for the 'Acute aquatic toxicity MOA by OASIS' profiler, showing its name, short description, disclaimer, donor information, author information, website, and details such as the number of nodes (18) and scheme version (3.0). A 'Close' button is visible at the bottom of the dialog box.

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 Aquate aquatic toxicity MOA

The screenshot displays the QSAR Toolbox 3.1.1 Profiling Scheme Browser. The left sidebar lists various profiling methods under 'Endpoint specific' and 'Metabolism/Transformations'. The 'Endpoint specific' section is highlighted with a red circle (1), and the 'Acute aquatic toxicity MOA by OASIS' method is selected. The 'View' button is circled in red (2). A red arrow points from the 'View' button to a node in the central flowchart (3). The 'Reference' button in the top right of the right-hand pane is circled in red (4). The right-hand pane shows the 'Basesurface narcotics' category and a list of chemical classes. The bottom pane shows a 'Query Tree' and 'Common Fragments' table.

Definition	1	2	3	4
RX <sub>3</sub>	S-	N-	I-	R-R R-R

1. **Highlight** the profiler
2. **Click** View
3. **Click** on one of the nodes
4. **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):
  - Aquatic toxicity classification by ECOSAR – structural grouping
  - Acute aquatic toxicity MOA by OASIS – mechanistic grouping
  - Acute aquatic toxicity classification by Verhaar – grouping by reactivity
  - Protein binding by OASIS v.1.1
  - Protein binding by OECD

# Profiling

## Profiling the target chemical

The screenshot shows the QSAR Toolbox software interface. The 'Profiling' menu is open, and the 'Apply' button is highlighted with a red circle and the number 2. In the 'Profiling methods' list, several checkboxes are checked, including 'Protein binding by OASIS v1.1' and 'Acute aquatic toxicity MOA by OASIS', which are highlighted with a red circle and the number 1. The 'Metabolism/Transformations' section is also visible.

Filter endpoint tree...	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
Structure	[3] [Mix] 	 Qty: 1 weight %	 Qty: 9 weight %	 Qty: 90 weight %
Substance Identity				
CAS Number	N/A	N/A	N/A	N/A
Chemical IDs	NA	NA	NA	NA
Chemical Name				
Structural Formula	{X=1/weight %}C(C)...	C(C)(=O)c1c(Cl)c(C...	c1(C(=O)c2ccccc2)...	C(O)CCC
Physical Chemical Properties				
Environmental Fate and Transport				
Ecotoxicological Information				
Human Health Hazards				
Profile				

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 3.1.0.21 interface. The 'Profiling Schemes' panel on the left lists various methods, with 'Protein binding by OASIS v1.1' and 'Acute aquatic toxicity MOA by OASIS' checked. The 'Filter endpoint tree' in the center shows a tree structure with 'Profile' selected. The table on the right displays four chemical components with their respective profiles. A red circle highlights the 'Profile' node in the tree, and a red box highlights the 'Profile' node in the table. A callout box with the number '1' points to the 'Profile' node in the tree.

1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
[3] [Mix]	<chem>CC1=CC=C(C=C1)C(=O)C2=CC=CC=C2</chem>	<chem>CC1=CC=C(C=C1)C(=O)C2=CC=CC=C2</chem>	<chem>CC1=CC=C(C=C1)C(=O)C2=CC=CC=C2</chem>
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %
Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
No alert found	SNAr SNAr >> Nucleophi...	No alert found	No alert found
SNAr >> Nucleophi...	SNAr >> Nucleophi...	No alert found	No alert found
No alert found	No alert found	No alert found	No alert found
Class 1 (narcosis o...	Class 3 (unspecific ...	Class 3 (unspecific ...	Class 1 (narcosis o...
Class 3 (unspecific ...	Class 3 (unspecific ...	Class 3 (unspecific ...	Class 3 (unspecific ...
Basesurface narcotics	Basesurface narcotics	Basesurface narcotics	Basesurface narcotics
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

- Background
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- **Workflow**
  - Input
  - 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 3.1.0.21 interface. The 'Endpoint' tab is selected. In the 'Databases' panel, the 'Ecotoxicological Information' category is expanded, and several sub-databases are checked with green checkmarks: Aquatic ECETOC, Aquatic Japan MoE, Aquatic OASIS, Aquatic US-EPA ECOTOX, ECHA CHEM, and Terrestrial US-EPA ECOTOX. The 'Gather' button is circled in red. The main window displays a table with chemical structures and their corresponding categories and alerts.

Structure	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
[Chemical Structure]	[3] [Mix]	[Chemical Structure]	[Chemical Structure]	[Chemical Structure]
		Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %
Substance Identity				
Physical Chemical Properties				
Environmental Fate and Transport				
Ecotoxicological Information				
Human Health Hazards				
Profile				
Predefined				
US-EPA New Chemical Categories	Neutral Organics	Neutral Organics	Neutral Organics	Neutral Organics
General Mechanistic				
Protein binding by OASIS v1.1	No alert found SNAr SNAr >> Nucleophi... SNAr >> Nucleophi...	SNAr SNAr >> Nucleophi... SNAr >> Nucleophi...	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	Class 1 (narcosis o... Class 3 (unspecific ...	Class 3 (unspecific ...	Class 3 (unspecific ...	Class 1 (narcosis o...
Acute aquatic toxicity MOA by OASIS	Basesurface narcotics	Basesurface narcotics	Basesurface narcotics	Basesurface narcotics
Aquatic toxicity classification by ECOSAR	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

# 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

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 3.1.0.21 interface. The main window displays a table of experimental data for the endpoint LC50; *P.promelas*; 96h. The table has 4 columns representing different components of a mixture. A red circle highlights a row with 10 experimental data points for the investigated endpoint.

	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
Structure	[3] [Mix]	A	A	A
	<chem>C1=CC=C(C=C1)C(=O)O</chem>	<chem>C1=CC=C(C=C1)C(=O)O</chem>	<chem>C1=CC=C(C=C1)C(=O)O</chem>	<chem>C1=CC=C(C=C1)C(=O)O</chem>
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %	
Substance Identity				
Ecotoxicological Information				
Aquatic Toxicity				
Behavior	(2/5)	M: 2 mg/L	M: 14.9 mg/L, 15.2 ...	
Biochemistry	(1/1)		M: 5.15 mg/L	
Development	(1/2)		M: 3.31 mg/L, 6.38 ...	
Growth	(1/13)		M: 0.54;0.57 mg/L, ...	
Mortality				
EC50	(3/3)	M: 2 mg/L	M: 15.3 mg/L	M: 1.73E3 mg/L
LC50				
1 h	(1/2)			M: 1.95E3 mg/L, 1....
24 h	(2/4)		M: 14.8 mg/L, 15.2 ...	M: 1.95E3 mg/L, 1....
48 h	(2/4)		M: 15.2 mg/L, 14.5 ...	M: 1.95E3 mg/L, 1....
72 h	(1/2)			M: 1.95E3 mg/L, 1....
96 h				
Animalia				
Chordata(Vertebrates)				
Actinopterygii(Fish)				
<i>Pimephales promelas</i>	(3/10)	M: 1.99 mg/L, 2 mg/L	M: 14.8 mg/L, 15.3(...	M: 1.74E3 mg/L, 1....
7 Days	(1/1)		M: 6.66(5.98;7.41) ...	
LOEC	(1/1)		M: 9.24 mg/L	
MATC	(1/1)		M: 7.36 mg/L	
NOEC	(1/1)		M: 5.86 mg/L	
Undefined Endpoint	(1/8)		M: 0.991;62.4 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
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- **Workflow**
  - 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 #49-54
- 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)**

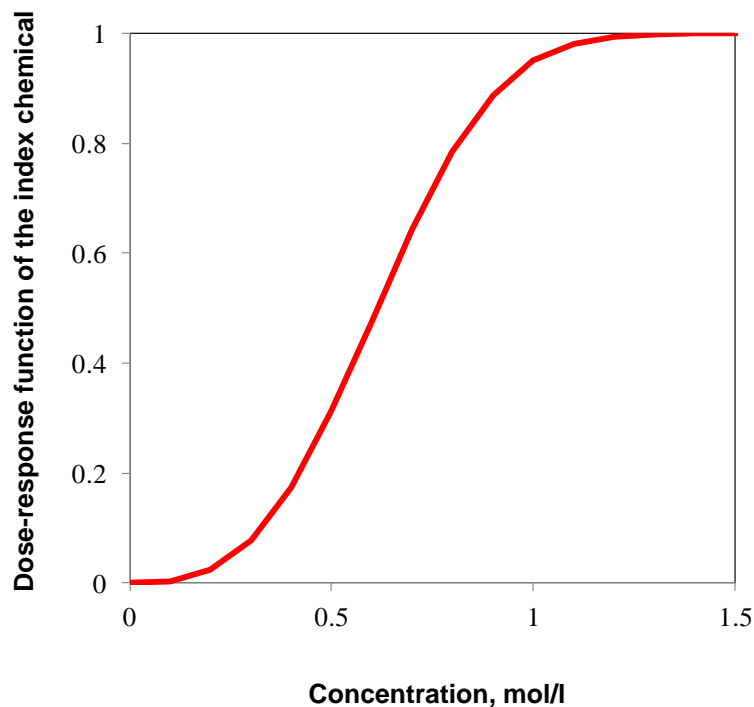
$$ICED = \sum_{j=1}^J CED_j^{(i)} = \sum_{j=1}^J RPF_j^{(i)} d_j$$

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



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

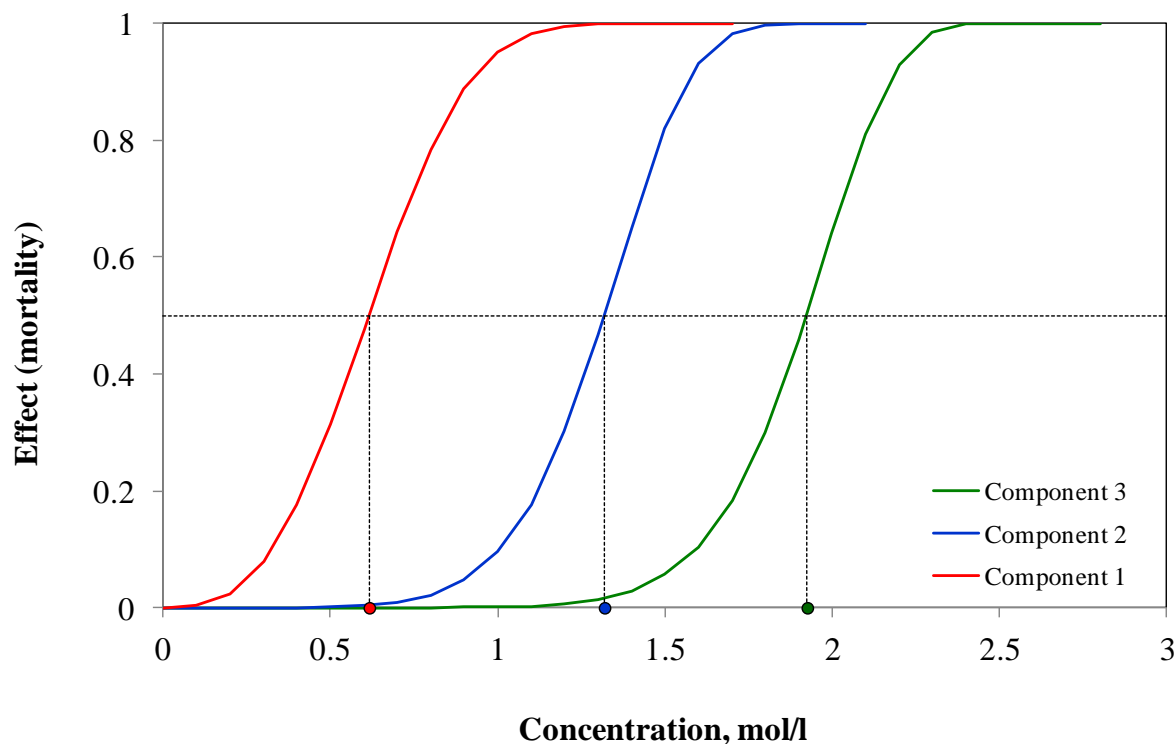
$f_i$  - dose-response function of the index chemical

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

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

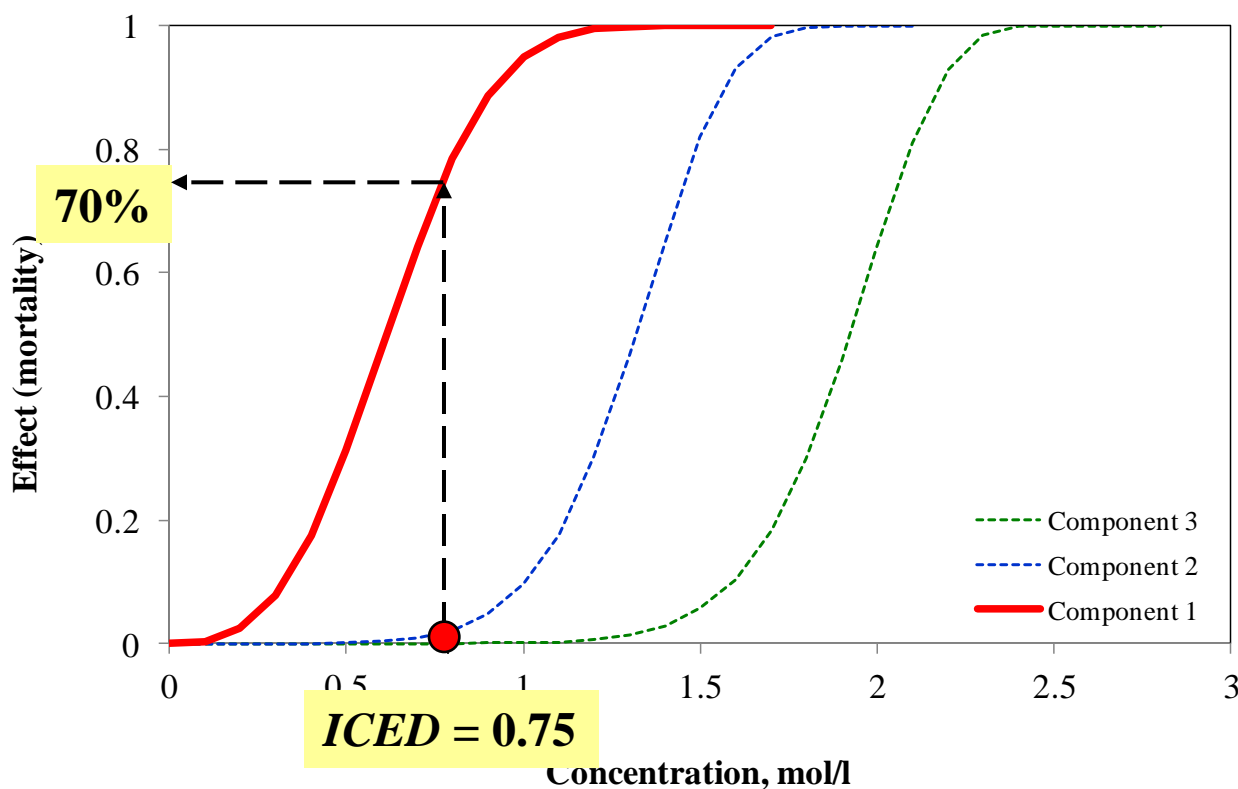
Index Chemical Equivalent Concentration

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

*ICED*  $\longrightarrow$  *Effect*<sup>Mixture</sup> = *f*<sub>i</sub>(*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

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

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

QSAR Toolbox 3.1.0.21 [Document]

Input Profiling Endpoint Category Definition Data Gap Filling Report

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

Structure	1 [target]	2 [target,mix.component]	3 [target,mix.component]	4 [target,mix.component]
	[3] [Mix]	A	A	A
	<chem>CC1=CC=C(C=C1)C(=O)C</chem>	<chem>CC1=CC=C(C=C1)C(=O)C</chem>	<chem>CC1=CC=C(C=C1)C(=O)C</chem>	<chem>CC1=CC=C(C=C1)C(=O)C</chem>
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %	
Substance Identity				
Ecotoxicological Information				
Aquatic Toxicity				
Behavior	(2/5)	M: 2 mg/L	M: 14.9 mg/L, 15.2 ...	
Biochemistry	(1/1)		M: 5.15 mg/L	
Development	(1/2)		M: 3.31 mg/L, 6.38 ...	
Growth	(1/13)		M: 0.54;0.57 mg/L, ...	
Mortality				
LC50	(3/3)	M: 2 mg/L	M: 15.3 mg/L	M: 1.73E3 mg/L
LC50				
1 h	(1/2)			M: 1.95E3 mg/L, 1...
24 h	(2/4)		M: 14.8 mg/L, 15.2 ...	M: 1.95E3 mg/L, 1...
48 h	(2/4)		M: 15.2 mg/L, 14.5 ...	M: 1.95E3 mg/L, 1...
72 h	(1/2)			M: 1.95E3 mg/L, 1...
96 h				
Animalia				
Chordata(Vertebrates)				
Actinopterygii(Fish)				
Pimephales promelas	(3/10)	M: 1.99 mg/L, 2 mg/L	M: 14.8 mg/L, 15.3(...	M: 1.74E3 mg/L, 1...
7 Days	(1/1)		M: 6.65(5.96;7.41) ...	
LOEC	(1/1)		M: 9.24 mg/L	
MATC	(1/1)		M: 7.36 mg/L	

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



# Data Gap Filling Results of Similar MOA

QSAR Toolbox 3.1.0.21 [Document]

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(Vertebrates) Actinopterygii(Fish) Pimephales promelas

**Structure**

Pimephales

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: 1.99 mg/L, 2 mg/L	M: 14.8 mg/L, 15.3	M: 1.74E3 mg/L, 1...	

Pimephales promelas (3/10)

**Prediction**

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

LC50 (obs., log1(mg/L))

log Kow

Descriptor X: log Kow

**Accept prediction**

Return to matrix

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

4 Document Data gap filling 0/100 2/1/0

# 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

QSAR Toolbox 3.1.0.21 [Document]

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(Vertebrates) Actinopterygii(Fish) Pimephales promelas

1 [target]	2 [target, mix. component]	3 [target, mix. component]	4 [target, mix. component]
[3] [Mix]	A	A	A
Structure	<chem>CC(=O)c1ccc(Cl)cc1</chem>	<chem>O=C(c1ccccc1)c2ccccc2</chem>	<chem>CCCCO</chem>
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %
	M: 1.99 mg/L, 2 mg/L	M: 14.8 mg/L, 15.3	M: 1.74E3 mg/L, 1

Pimephales promelas (4/11) CS: 82.8 mg/L

Prediction

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

Information

The current prediction was accepted

OK

Accept prediction

Return to matrix

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

Descriptor X: log Kow

1. **Click** Accept prediction  
3. **Click** Return to matrix

2. **Click** OK

# Data Gap Filling

## Predicted value for LC50

QSAR Toolbox 3.1.0.21 [Document]

QSAR TOOLBOX

Input Profiling Endpoint Category Definition Data Gap Filling Report

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

Apply

Data Gap Filling Method

- Independent MOA
- Similar MOA
- Specific models

Target Endpoint

Ecotoxicological Information Aquatic Toxicity Mortality LC50 96 h  
Animalia Chordata(Vertebrates) Actinopterygii(Fish) Pimephales promelas

Structure	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 %
Substance Identity				
Ecotoxicological Information				
Aquatic Toxicity				
Behavior	(2/5)	M: 2 mg/L	M: 14.9 mg/L, 15.2 ...	
Biochemistry	(1/1)		M: 5.15 mg/L	
Development	(1/2)		M: 3.31 mg/L, 6.38 ...	
Growth	(1/13)		M: 0.54;0.57 mg/L, ...	
Mortality				
EC50	(3/3)	M: 2 mg/L	M: 15.3 mg/L	M: 1.73E3 mg/L
LC50				
1 h	(1/2)			M: 1.95E3 mg/L, 1....
24 h	(2/4)		M: 14.8 mg/L, 15.2 ...	M: 1.95E3 mg/L, 1....
48 h	(2/4)		M: 15.2 mg/L, 14.5 ...	M: 1.95E3 mg/L, 1....
72 h	(1/2)			M: 1.95E3 mg/L, 1....
96 h				
Animalia				
Chordata(Vertebrates)				
Actinopterygii(Fish)				
Pimephales promelas	(4/11)	CS: 82.8 mg/L	M: 1.99 mg/L, 2 mg/L	M: 14.8 mg/L, 15.3(...)
7 Days	(1/1)		M: 6.65(5.96;7.41) ...	
LOEC	(1/1)		M: 9.24 mg/L	
MATC	(1/1)		M: 7.36 mg/L	
NOEC	(1/1)		M: 5.86 mg/L	
Undefined Endpoint	(1/8)		M: 0.991;62.4 mg/L...	
Profile				

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

The screenshot shows the QSAR Toolbox 3.1.0.21 interface. The 'Report' tab is active, displaying a table of predictions for the substance 'Pimephales'. The table has four columns representing different target mixtures. A context menu is open over the prediction 'CS: 82.8 mg/L' in the first column, with callouts '1' and '2' indicating the steps: '1. Select prediction' and '2. Right Click and Select Report'.

	1 [target]	2 [target, mix. component]	3 [target, mix. component]	4 [target, mix. component]
Structure	[3] [Mix] <chem>C1=CC=C(C=C1)C(=O)O</chem>	A <chem>C1=CC=C(C=C1)C(=O)O</chem>	A <chem>C1=CC=C(C=C1)C(=O)O</chem>	A <chem>C1=CC=C(C=C1)C(=O)O</chem>
	Qty: 1 weight %	Qty: 9 weight %	Qty: 90 weight %	
Substance Identity				
Ecotoxicological Information				
Aquatic Toxicity				
Behavior	(2/5)	M: 2 mg/L	M: 14.9 mg/L, 15.2 ...	
Biochemistry	(1/1)		M: 5.15 mg/L	
Development	(1/2)		M: 3.31 mg/L, 6.38 ...	
Growth	(1/13)		M: 0.54;0.57 mg/L, ...	
Mortality				
EC50	(3/3)		mg/L	M: 1.73E3 mg/L
LC50				
1 h	(1/2)			M: 1.95E3 mg/L, 1...
24 h	(2/4)			mg/L, 15.2 ...
48 h	(2/4)			M: 1.95E3 mg/L, 1...
72 h	(1/2)			M: 1.95E3 mg/L, 1...
96 h				M: 1.95E3 mg/L, 1...
Animalia				
Chordata(Vertebrates)				
Actinopterygii(Fish)				
Pimephales promelas				
7 Days	(4/11)	CS: 82.8 mg/L	M: 1.73E3 mg/L, 1...	M: 1.74E3 mg/L, 1...
LOEC	(1/1)		M: 6.65(5.96;7.41) ...	
MATC	(1/1)		M: 9.24 mg/L	
NOEC	(1/1)		M: 7.36 mg/L	
Undefined Endpoint	(1/1)		M: 5.86 mg/L	
Profile	(1/8)		M: 0.991;62.4 mg/L...	

1. Select prediction
2. Right Click and Select Report



# Report

The screenshot shows the QSAR Toolbox 3.1.0.21 interface. The main window displays a report titled "Prediction [1]". The report content is as follows:

Prediction of LC50 for (X=1/weight  
%)C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1\_{X=9/weight 1 / 16  
%)c1(C(=O)c2cccc2)cccc1\_{X=90/weight %}C(O)CCC

**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" published by OECD (September, 2007) and "GUIDANCE ON INFORMATION REQUIREMENTS AND CHEMICAL SAFETY ASSESSMENT / CHAPTER R.6: QSARS AND GROUPING OF CHEMICALS" published by ECHA (May, 2008).  
 The report provides information about the target substance, chemical characteristics*

## 1. Generated report

# Report

**Prediction of LC50 for {X=1/weight %}C(C)(=O)c1c(Cl)c(Cl)c(Cl)cc1\_{X=9/weight %}c1(C(=O)c2cccc2)cccc1\_{X=90/weight %}C(O)CCC**

**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 endpoint data is selected from the following database(s):

1. Aquatic ECETOC
2. Aquatic Japan MoE
3. Aquatic OASIS
4. Aquatic US-EPA 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) Aquatic Toxicity mg/L
Target mixture	-	-
Mix. comp. No. 1	1.00	<b>2.00</b>
Mix. comp. No. 2	9.00	<b>13.7</b>
Mix. comp. No. 3	90.0	<b>1.83E+03</b>

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