

OECD QSAR Toolbox v.3.4

How to use the Toolbox AOP workflow for Skin
Sensitization

Outlook

- **Background**
- Objectives
- Overview of AOP scheme as implemented in the Toolbox
- The exercise

Background

AOP concept and description

- The OECD has developed the AOP concept as a means of providing transparent mechanistic justification and weight-of-evidence to reduce uncertainty in the predictions for complex toxicological endpoints and it is considered to be the focal point of the future development of the Toolbox*.



*Slide presented on last MG WebEx (April 2013)

Background

AOP concept and description (*contd.*)

- A proof-of-concept AOP for skin sensitization is implemented in Toolbox
- The AOP scheme is a directed graph including a sequence of roots
- The AOP workflow uses filtered Toolbox functionalities
- New endpoint-specific AOP databases and profilers are implemented in Toolbox
- The implemented AOP scheme is used *only* to demonstrate two examples using AOP functionalities based on data rich chemicals

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Objectives

This presentation demonstrates a number of functionalities of the Toolbox*:

- Simulating skin metabolism for the target chemical
- Identifying analogues of the active metabolite
- Predicting sensitization potential for potentially active metabolites
- Assigning of the prediction for the metabolite to the parent chemical
- Predict skin sensitization potential using implemented AOP

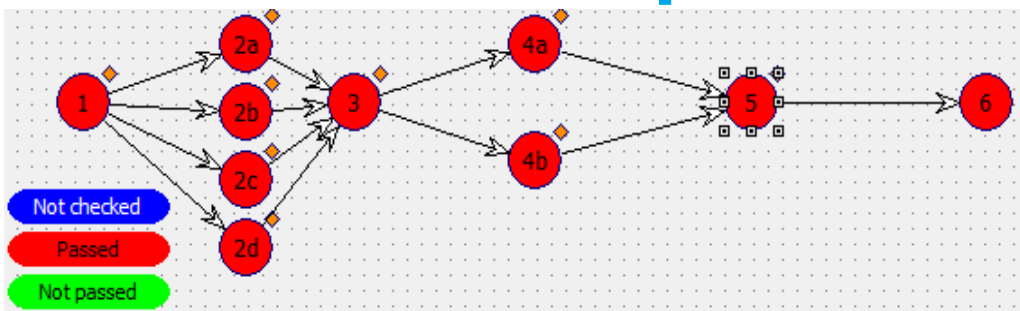
**Demonstrated examples are obtained with Toolbox v3.4*

Disclaimer - for the purposes of the tutorial on the use of the workflow and do not represent a guidance on the prediction for the particular chemicals which are rich in data in each node of the workflow

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Overview of implemented AOP scheme



Key node

1	Protein binding alerts	→	Protein binding – in silico/theoretical
2a	<i>in chemico</i> Peptide depletion assay DPRA (Cys)	} →	Protein binding potency in chemico
2b	<i>in chemico</i> Peptide depletion assay DPRA (Lys)		
2c	<i>in chemico</i> Glutathione depletion assay GSH (RC50)		
2d	<i>in chemico</i> Adduct formation assay LC-MS		
3	<i>in vitro</i> Keratinocyte ARE (EC1.5, EC2, EC3)	} →	Cellular response
4a	<i>in vitro</i> Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)		
4b	<i>in vitro</i> Dendritic cell activity assay MUSST (expression of CD86)		
5	<i>in vivo</i> Organ response (LLNA)	→	Organ response
6	<i>in vivo</i> Organism response (GPMT)	→	Organism response

Outlook

- Background
- Objectives
- **Overview of AOP scheme as implemented in the Toolbox**
 - **Details of AOP window**
 - AOP workflow for skin sensitization
 - Thresholds of the node of AOP
- The exercise

Overview of the AOP scheme as implemented in Toolbox

Details of AOP window

The screenshot shows the 'Skin Sensitization' AOP window. The interface is divided into several panels:

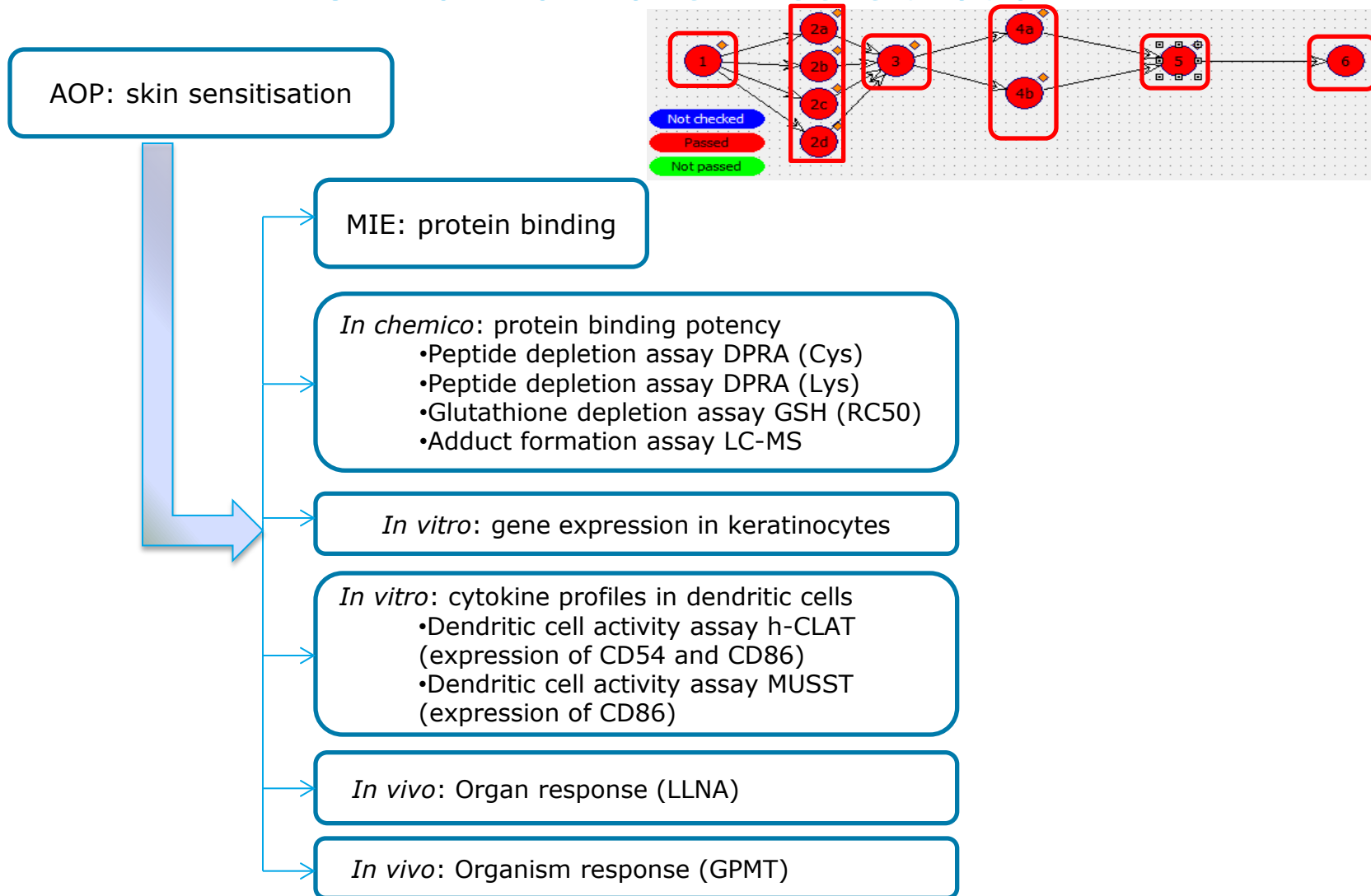
- Panel with full names of nodes:** A list on the left side of the window showing nodes 1 through 6 with their full names, such as '1 - Protein binding alerts' and '2a - in chemico Peptide de'.
- AOP tree scheme:** A central diagram showing a flow of nodes from 1 to 6, with sub-nodes 2a, 2b, 2c, 2d, 4a, and 4b. Node 1 is selected, and arrows indicate the progression to nodes 3, 5, and 6.
- Color legend:** A legend below the tree scheme showing three states: 'Not checked' (blue), 'Passed' (red), and 'Not passed' (green).
- Panel with information for selected node:** An 'Info panel' at the bottom left providing details for node 1, including its short name, full name, relevant databases, associated profiles, simulators, and thresholds.
- Target chemical:** A chemical structure is displayed in a panel at the bottom left, labeled 'Target chemical'.
- Panel with assigned prediction:** A 'Predictions bucket' on the right side showing predicted results for the selected node, such as 'prof. res: "No alert"'. Below it is an 'Unassigned predictions bucket'.
- Short description:** A pop-up 'About' window at the bottom center providing a detailed description of the AOP pathway, its author (OECD), and a disclaimer.

Outlook

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 - Details of AOP window
 - **AOP workflow for skin sensitization**
 - Thresholds of the node of AOP
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Overview of the AOP scheme as implemented in Toolbox

AOP workflow for skin sensitization



Outlook

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- **Overview of AOP scheme as implemented in the Toolbox**
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 - AOP workflow for skin sensitization
 - **Thresholds of the AOP nodes**
- The exercise

Overview of the AOP scheme as implemented in Toolbox

Implemented thresholds for the AOP nodes

- Thresholds are implemented for each AOP node
- Each threshold is indicated within description panel of the AOP node
- Threshold are identified based on assay data related to the corresponding node
- The status of the each node (passed/not passed) depends on the implemented thresholds
- Thresholds of the AOP nodes determined by expert group are provided on the slide 15:

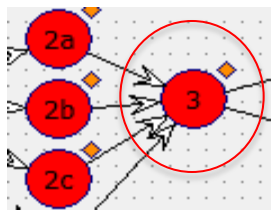
Thresholds:

1: Scale name 'Keratinocytes gene expression EC (ordinal)'

Scale type 'Ordinal'

Passed: Very High|High|Moderate|Low

Not passed: Negative



Overview of the AOP scheme as implemented in Toolbox

Implemented thresholds for the AOP nodes

Node name	Data thresholds	Node status: Pass	Node status: Not pass
1- Protein binding alerts		presence of alert	absence of alert
2a and 2b <i>in chemico</i> DPRA Cys and Lys	Peptide depletion, PD (%) > 80 - High 40% ≥ PD ≤ 80% - Moderate 5% ≥ PD ≤ 40% - Low 5% < PD - Not reactive	High Moderate Low	Not Reactive
2c - <i>in chemico</i> Glutathione depletion assay GSH (RC50)	RC50 (mmol/L) ≤ 0.099 - Extremely reactive 0.1 ≥ RC50 ≤ 0.99 - Highly reactive 1 ≥ RC50 ≤ 15 - Moderately reactive 16 ≥ RC50 ≤ 70 - Slightly reactive 70.1 ≥ RC50 ≤ 135 - Suspect RC50 > 135 - Not reactive	Extremely Reactive Highly Reactive Moderately Reactive Slightly Reactive	Suspect Not Reactive Not reactive at saturation
2d - <i>in chemico</i> Adduct formation assay LC-MS	Adduct formation (%) ≥ 30% - Positive Adduct formation (%) < 30% - Negative	Positive	Negative
3 - <i>in vitro</i> Keratinocyte (EC1.5, EC2, EC3)	EC3 (%) ≤ 20 - Very High 20 > EC3 ≤ 50 - High 50 > EC3 ≤ 100 - Moderate 100 > EC3 ≤ 2000 - Low EC3 > 2000 - Negative	Very High High Moderate Low	Negative
4a and 4b <i>in vitro</i> Dendritic cell activity assay h-CLAT and MUSST (expression of CD54 and CD86)	expression of CD54 and CD86 Positive Negative	Positive	Negative
5 - <i>in vivo</i> Organ response (LLNA)	0 ≥ EC3 (%) < 50 - Positive EC3 ≥ 50 - Negative	Positive	Negative
6 - <i>in vivo</i> Organism response (GPMT)	Data provided: Strong sensitizer; Moderate sensitizer; Weak sensitizer; Non sensitizer	Strong sensitizer Moderate sensitizer	Weak sensitizer Non sensitizer

Outlook

- Background
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- Overview of AOP scheme as implemented in the Toolbox
- The exercise
 - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
 - **Input**

Chemical Input

Input Screen

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

Chemical Input

Input target chemical by CAS#

The screenshot shows the QSAR TOOLBOX software interface. The top menu bar includes options like Document, Input, Profiling, Endpoint, Category Definition, Data Gap Filling, and Report. The 'Input' menu is expanded, showing options for Name, Structure, Select, Delete, Query, ChemIDs, DB, Inventory, and List. The 'CAS#' option is highlighted with a red box and a callout box. The callout box contains the following text:

1

Enter chemical by CAS # (F4)
 Enters a single chemical by its CAS number.
 If the number is not a valid CAS number the content of the field will be colored in red.
 Press F1 for more help.

Below the callout box, there are several expandable sections: Physical Chemical Properties, Environmental Fate and Transport, Ecotoxicological Information, and Human Health Hazards.

At the bottom of the interface, there is a text input field with a dropdown menu labeled "...select filter type..." and buttons for "Create" and "Apply".

1. Click on CAS#

Chemical Input

Enter CAS# 107-75-5

The Toolbox now searches the databases to find out if the CAS# you entered is linked to a molecular structure stored in the Toolbox. It is displayed as a 2-dimensional depiction

Search by CAS #

107-75-5

OK Cancel

Select All Clear All Invert Selection Selected 1 of 2

Selected	CAS	Smiles	Names	CAS/Name	2D/Name	CAS/
1. Yes	107-75-5	CC(CCCC)		1:: Low C 1: 1:: Ac 2: 2:: Mode 3: 1:: Cl 4: 2:: De 5: 3:: Ge	1: 1:: Low C 1: 1:: Ac 2: 2:: Mode 1: 1:: Sl 2: 2:: Pf 3: 3:: Us	Hi
2. No	107-75-5	CC(C)(O)		1: 1:: Low C 1: 1:: G	1: 1:: Low C 1: 1:: G	Lo

1. **Enter** the CAS# In the blank field; 2. **Select** Clear All; 3. **Click** over the first column with label No, then the column become marked with Yes 4. **Click** OK;

Chemical Input

Target chemical identity

- **Double click** "Substance Identity" displays the chemical identification information.
- The user should note that existing names of the target chemical are presented in different colours. This indicates the reliability of relation CAS-Name for the target chemical(see next screen shots).
- The workflow on the first module is now complete, and the user can proceed to the next module.

Chemical Input

Target chemical identity

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

- Top Bar:** Contains the QSAR TOOLBOX logo and navigation buttons for Input, Profiling, Endpoint, Category Definition, Data Gap Filling, and Report.
- Document Bar:** Includes Document, Single Chemical, and Chemical List tabs, along with icons for New, Open, Close, and Save.
- Left Panel (Documents):** Shows a document with CAS: 107-75-5 and the SMILES string CC(CCCC(C)(C)O)CC=O.
- Filter endpoint tree...:** A search box containing "[target]" and a tree view with categories like Substance Identity, Physical Chemical Properties, etc.
- Structure View:** Displays the chemical structure of the target compound.
- Chemical Information Panel:** Lists various identifiers for the target chemical, with a red circle highlighting the following text:
 - 107-75-5
 - EINECS:2035187
 - 3,7-dimethyl-7-hydroxy-octanal
 - hydroxycitronellal
 - octanal, 7-hydroxy-3,7-dimethyl-
 - 7-hydroxycitronellal
 - 7-hydroxy-3,7-dimethyloctanal
 - C10H20O2
 - CC(CCCC(C)(C)O)CC=O

Chemical Input

Target chemical identity

The colour code indicates the reliability of the chemical identifier:

- **Green:** There is a high consistency between the identifier and the structure. This colour is applied if the identifier is the same in several quality assured databases.
- **Yellow:** There is only a moderate consistency between the identifier and the structure. The colour is applied if the identifier is the same in several databases for which the quality assurance could not be established.
- **Red:** There is a poor consistency between the identifier and the structure. The colour is applied if the identifier is allocated to different structures in different databases.

Outlook

- Background
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- Overview of AOP scheme as implemented in the Toolbox
- The exercise
 - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
 - Input
 - **Activate AOP and set target**

Activate AOP

Set target chemical for AOP

1. **Filter** endpoint tree – **write skin** in the green filed
2. **Expand the tree** – open the tree to the Sensitisation node
3. **Right click** near the AOP label
4. **Select** activate AOP
5. AOP window appears

Continued on the next slide

Activate AOP

Set target chemical for AOP

The screenshot shows the QSAR Toolbox interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. Below this is a toolbar with 'Document', 'Single Chemical', and 'Chemical List' tabs. The 'Documents' panel on the left shows a document with CAS: 107-75-5. The main workspace shows a chemical structure and a list of categories: Substance Identity, Human Health Hazards, Irritation / Corrosion, Sensitisation, and Skin. A context menu is open over the structure, with 'Set AOP target' highlighted. A secondary window titled 'Skin Sensitization' is open, showing a flowchart of AOP steps (1-6) and a list of associated assays. The assays are: 1 - Protein binding alerts, 2a - in chemico Peptide depletion assay DPRA (Cys), 2b - in chemico Peptide depletion assay DPRA (Lys), 2c - in chemico Glutathione depletion assay GSH (RC50), 2d - in chemico Adduct formation assay LC-MS, 3 - in vitro KeratinoSens and LuSens (EC1.5, EC2, EC3), 4a - in vitro Dendritic cell activity assay h-CLAT (expression), 4b - in vitro Dendritic cell activity assay MUSST (expression), 5 - in vivo Organ response (LINA), and 6 - in vivo Organism response (GFMT). The flowchart shows step 1 leading to 2a, 2b, 2c, and 2d, which all lead to 3. Step 3 leads to 4a and 4b, which both lead to 5, which leads to 6. A legend indicates 'Not checked' (blue), 'Passed' (red), and 'Not passed' (green).

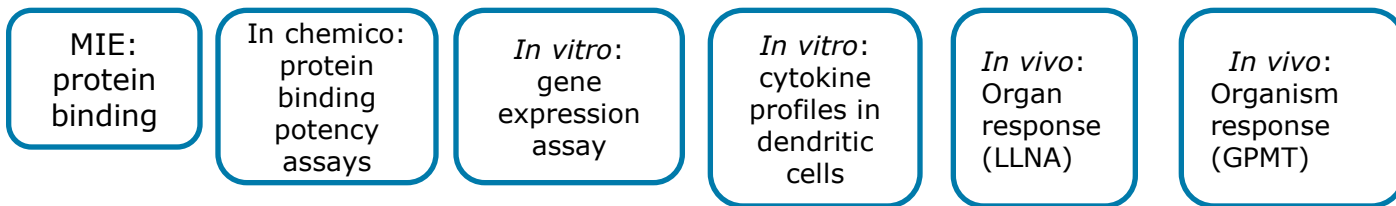
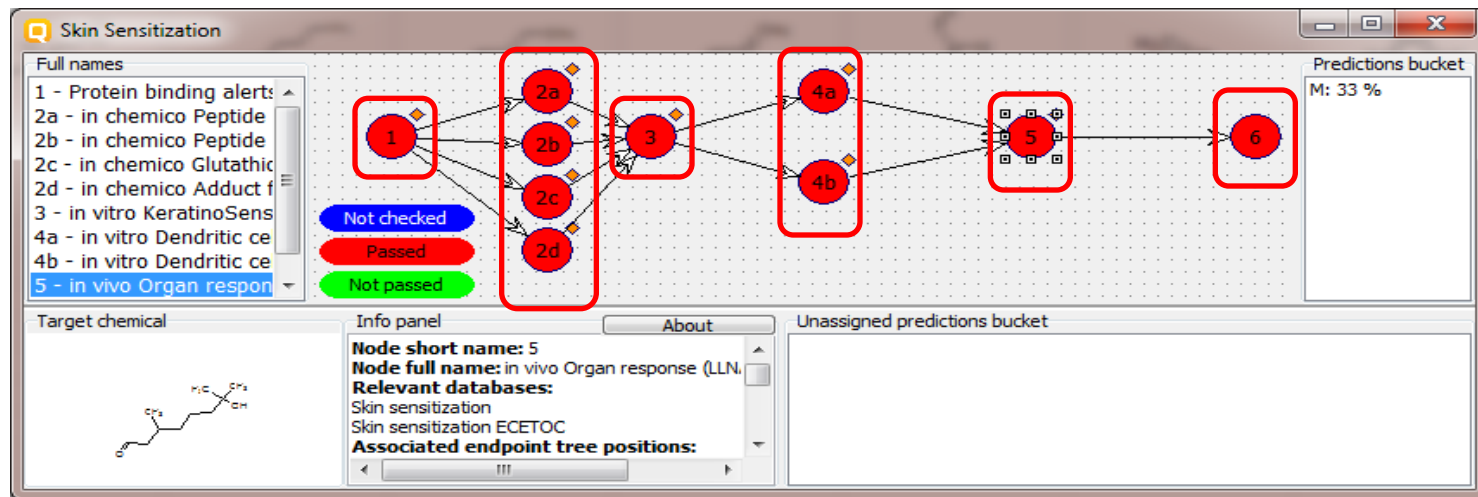
1. **Right click** over the structure and **select** "Set AOP target"
2. The target chemical appears in the AOP window

Outlook

- Background
- Objectives
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
 - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
 - Input
 - Activate AOP and set target
 - **Workflow process**

Workflow process

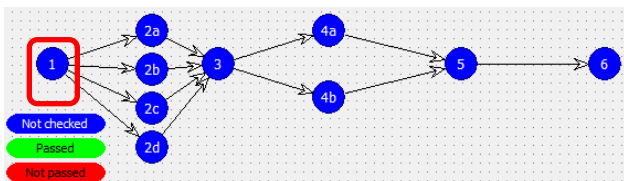
- Workflow process start from molecular initiating event to the *in vivo* organism response



Workflow process

Step 1. MIE: protein binding

Example 1



Start with profiling of target chemical

1. Open Profiling

2. Select node #1 related to MIE.

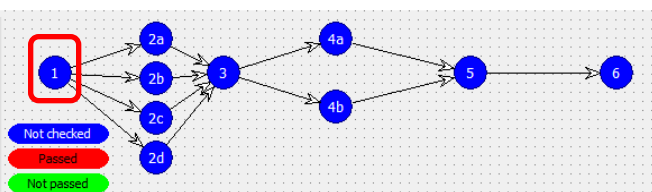
3. Relevant profilers are highlighted, **select** highlighted profilers

4. Apply selected profilers

Workflow process

Step 1. MIE: protein binding

Example 1



Start with profiling of target chemical

Information
Some nodes had their status changed automatically.
OK

1. The target chemical has protein binding alert according to both protein binding profilers
2. The node is automatically changed to passed based on both profiling outcome results and implemented thresholds (see slide #14-15). **Click OK**
3. Prediction assigned to the selected node appears in the panel "prediction bucket"

Workflow process

Molecular initiating events

Example 1

Full names

- 1 - Protein binding alerts
- 2a - in chemico Peptide depletion assay DPRA (Cys)
- 2b - in chemico Peptide depletion assay DPRA (Lys)
- 2c - in chemico Glutathione depletion assay GSH (RC50)
- 2d - in chemico Adduct formation assay LC-MS
- 3 - in vitro KeratinoSens and LuSens (EC1.5, EC2, EC3)
- 4a - in vitro Dendritic cell activity assay h-CLAT (expression)
- 4b - in vitro Dendritic cell activity assay MUSST (expression)
- 5 - in vivo Organ response (LLNA)
- 6 - in vivo Organism response (GPMT)

Info panel

Node short name: 1
Node full name: Protein binding alerts
Relevant databases:
Associated profiles:
 1: Protein binding alerts for skin sensitization by OASIS v1.4
 2: Protein binding by OECD
Associated simulators:
 1: Autoxidation simulator

Predictions bucket

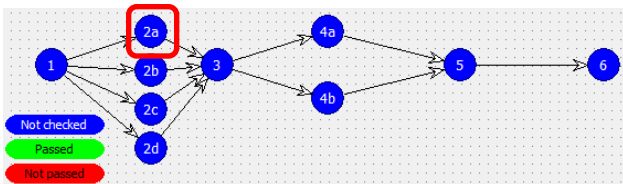
prof. res: "Schiff Base Formers",
 prof. res: "Schiff base formation"

- The node MIE is passed due to the presence of protein binding alert identified for the target chemical by the two protein binding profilers
- The workflow should move further to the *in chemico* assays

Workflow process

Step 2. *In chemico* Protein binding potency (Cysteine depletion) (node 2a)

Example 1



Profiling target chemical

1. Select node 2a related to Cys depletion assay.

2. The row related to the selected node is getting highlighted

3. The profilers related to node 2a are highlighted **Select related Profilers**

4. Click Apply

5. The target chemical has "long chain aliphatic aldehyde" - low reactive alert

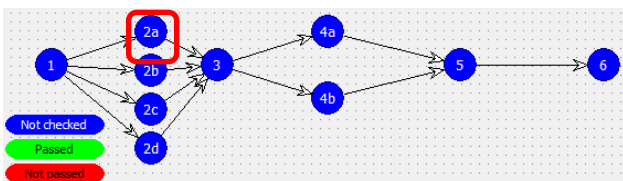
6. Perform **right click and **select** "Use for AOP"**

7. The profiling result appears in the bucket of the node. This last action is not related with change of node status. The node status depends on implemented data thresholds (see slide 14-15)

Workflow process

Step 2. *In chemico* Protein binding potency (Cysteine depletion) (node 2a)

Example 1



Gather data for target chemical

The screenshot shows the QSAR Toolbox interface with several windows and callouts:

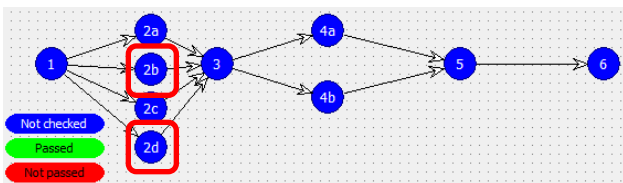
- Callout 1:** Points to the 'Endpoint' button in the top toolbar.
- Callout 2:** Points to the 'Physical Chemical Properties' database selection in the left sidebar.
- Callout 3:** Points to the 'Gather' button in the top toolbar.
- Callout 4:** Points to the highlighted row '% Depletion of Cystine (1/1) M: 17.5 %' in the data matrix.
- Callout 5:** Points to the 'Skin Sensitization' window, which displays a list of endpoints and their status (Not checked, Passed, Not passed).
- Callout 6:** Points to an 'Information' dialog box that says 'Some nodes had their status changed automatically.' with an 'OK' button.

1. **Go** to Endpoint and check are there any experimental data for the node 2a
2. **Select** highlighted database
3. **Click** Gather
4. Data appears on data matrix
5. Based on presence of data for the chemical and implemented thresholds (slide # 14-15) node 2a is getting passed
6. Node 2b and 2d are automatically changed as passed based the implemented thresholds. Click OK

Workflow process

Step 2. *In chemico* Protein binding potency (Lysine depletion) (node 2b) and *in chemico* Adduct formation LC-MS (node 2d)

Example 1



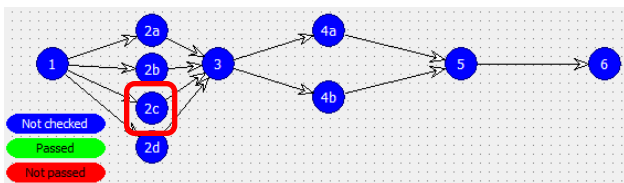
In this case there is available experimental data for the target chemical related to nodes 2b and 2d. In this respect these two nodes are getting passed. The workflow could proceed with next node

1. Select node 2b
2. Select node 2d
 The two experimental data appeared in the bucket.

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 2c)

Example 1



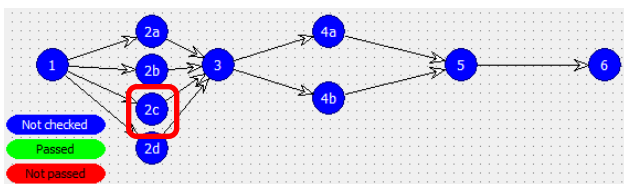
In this case there is no available experimental data for the target chemical related to node 2c, so the next step is to investigate category with similar analogues

1. Select node 2c related to *in chemico* glutathione depletion assay
2. The row related to the selected node is highlighted
3. Select highlighted database
4. Click Gather
5. No data has been found for the target chemical, click OK

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 2c)

Example 1



The category of similar analogue should be investigated.

1. Select node 2c related to *in chemico* glutathione depletion assay

2. The row related to the selected node is highlighted

3. Select highlighted category

4. Click Define

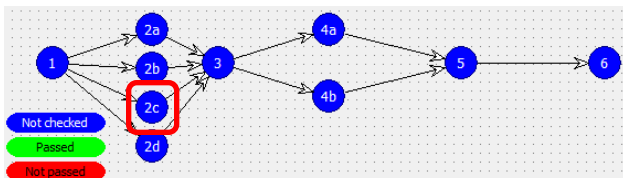
5. There are no structural alerts identified for the target chemical according to this profiler (no mechanistic and structural explanation).

6. Based on the above point it is recommended to define category by Protein binding alerts

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 2c)

Example 1



In this case we should investigate the category by Protein binding alerts. The reason for this is that GHS RC 50 depends on mechanism of protein binding interaction

1. Select Protein binding alerts for SS by OASIS v1.4

2. Click Define

3. The system will search for analogues with "Aldehyde" group

4. Click OK

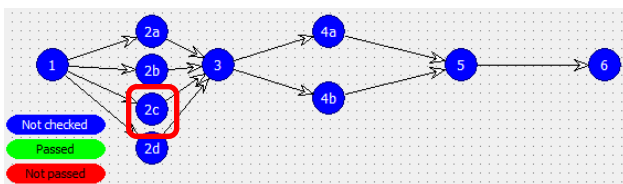
5. The system identify 63 analogues. **Click** OK

6. Gather data for the analogues

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 2c)

Example 1



Performed read-across in node 2c is used *only* to exemplify the workflow

The obtained read-across prediction falls in the range "Slightly reactive" based on the implemented thresholds (see slide 15-16) - the status of the node is changed to pass (see next slide)

Possible data inconsistency

- Scale/Unit
- RC50 (42 points)
- RC50 (ratio) (86 points)

RC 50 (ratio) scale is used in gap filling

Gap filling scale/unit

- RC50
- RC50 (ordinal)
- RC50 (ratio)

Structure	1 [target]	4	5	6	7	12	14
Structure		<chem>CC(=O)O</chem>	<chem>C=O</chem>	<chem>O=C1OC1</chem>	<chem>C=O</chem>	<chem>C1=CC=C(C=C1)C(F)(F)F</chem>	<chem>CCCCC=O</chem>
GSH RC50	(28/94)	M: 4.76 mg/L, 4.82	M: 5.1E3 mg/L, 4	M: 284 mg/L, 361	M: 80.4 mg/L, 104	M: 3	M: 6.4

Read across prediction of GSH RC50
taking the average from the nearest 5 neighbours, based on 5 values from 5 neighbours
Observed target value: N/A, Predicted target value: 5.74 mmol/L

Predicted RC50 5.74 mmol/L

Accept prediction

Return to matrix

- mmol/L (gap filling)
- mg/L (data matrix)

Data thresholds

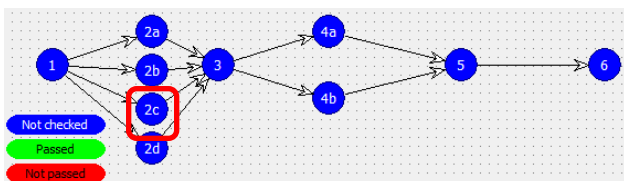
- RC50 (mmol/L) ≤ 0.099 – Extremely reactive
- 0.1 ≥ RC50 ≤ 0.99 – Highly reactive
- 1 ≥ RC50 ≤ 15 – Moderately reactive
- 16 ≥ RC50 ≤ 70 – Slightly reactive
- 70.1 ≥ RC50 ≤ 135 – Suspect
- RC50 > 135 – Not reactive

1. Change units on the title to mmol/l in order read-across to be consistent with data on datamatrix
2. The average (default option) values are used in the prediction
3. The logKow descriptor as the most suitable for predicting skin sensitization effect is used in RA prediction
4. **Accept prediction**
5. **Return to datamatrix**

Workflow process

Step 2. In chemico Glutathione depletion assay GSH (RC50) (node 2c)

Example 1



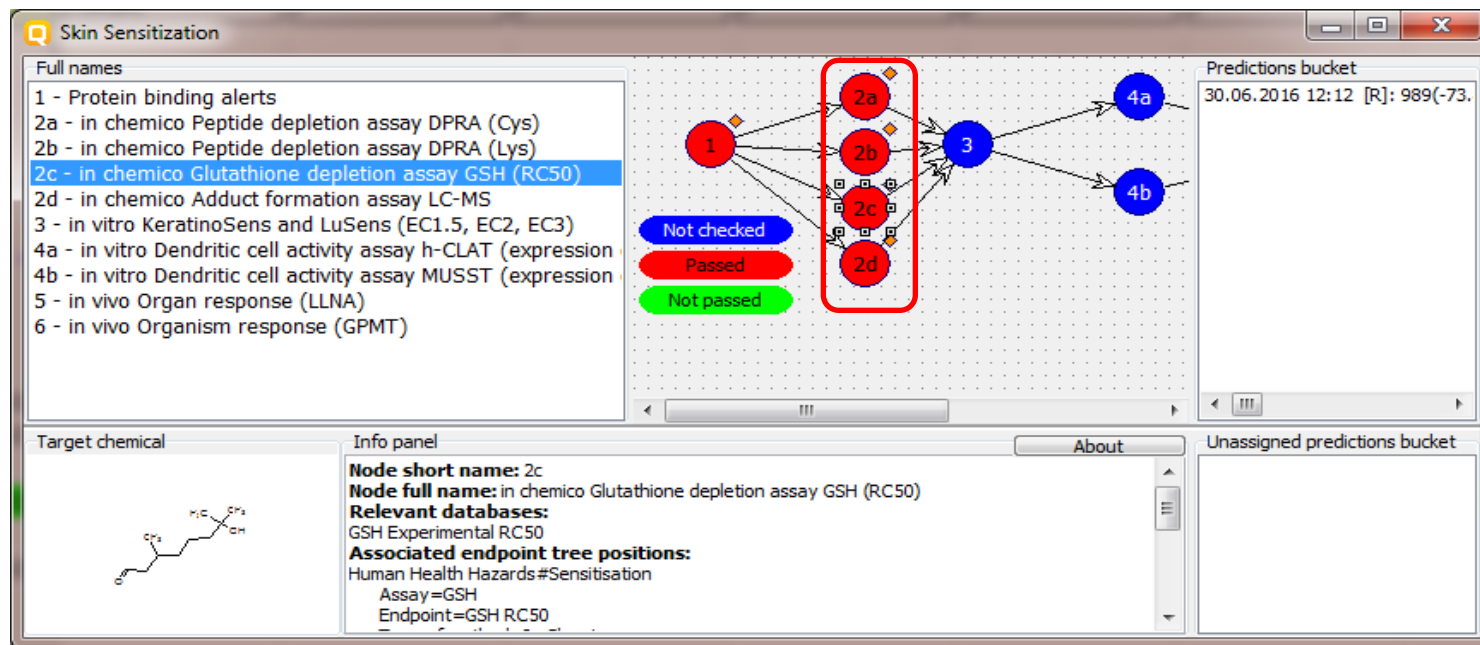
The next step is to use read-across prediction for AOP

1. Right click over the cell with prediction
2. Select Use for AOP
3. Click OK
4. The assigned prediction appears in the bucket of this node

Workflow process

In chemico assays

Example 1

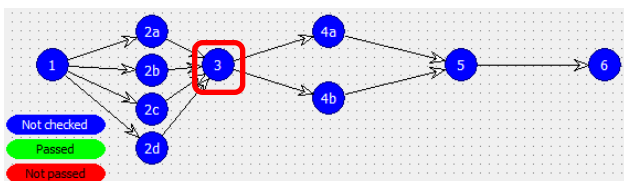


- The nodes related to the *in chemico* assays are passed due to positive experimental data for the target chemical (node 2a, 2b and 2d) and the positive experimental data found for analogues with an "Aldehyde" group(2c)
- The workflow should move further to the *in vitro* assay (node 3)

Workflow process

Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 3)

Example 1



1 Go back to Category definition

2 Click on Documents in order to return to datamatrix of the target

3 Select node 3 related to the *in vitro* assay

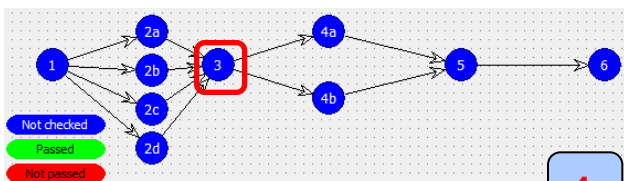
4 The rows related to *in vitro* assay are getting highlighted

Low reactive
 Low reactive >> Lo...
 Schiff Base Formers
 Schiff Base Forme...
 Schiff Base Forme...

Workflow process

Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 3)

Example 1



Check are there are data for the target chemical for the in vitro assay

Endpoint	Count	Metric	Value
KeratinoSens			
EC1.5	(23/24)	M: 13.7 mg/L	
EC2	(25/28)	M: 19 mg/L	
EC3	(25/28)	M: 24.6 mg/L	
LuSens			
EC1.5	(6/6)	M: 32.6 mg/L	
EC2	(6/6)	M: 73.2 mg/L	

1. Go to Endpoint
2. Select highlighted database
3. Click Gather
4. The experimental data appears on datamatrix
5. Click OK on the information window
6. Node 3 has been changed to passed based on implemented thresholds (slide 14 - 15)

Workflow process

Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 3)

Example 1

The screenshot shows the 'Skin Sensitization' workflow in the QSAR Toolbox. The workflow diagram consists of nodes 1, 2a, 2b, 2c, 2d, 3, 4a, and 4b. Node 3 is highlighted with a red box and a 'Passed' status. Node 1 is 'Not checked', and nodes 2a, 2b, 2c, and 2d are 'Not passed'. Nodes 4a and 4b are 'Not checked'. The 'Info panel' for node 3 provides the following details:

- Node short name:** 3
- Node full name:** *in vitro* KeratinoSens and LuSens (EC1.5, EC2, EC3)
- Relevant databases:** Keratinocyte gene expression Givaudan, Keratinocyte gene expression LuSens
- Associated endpoint tree positions:** Human Health Hazards#Sensitisation Assay=KeratinoSens

The 'Predictions bucket' for node 3 shows the following results:

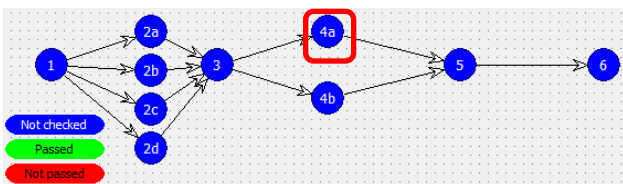
Assay	M (uM)
4a	79.4
4b	110
4c	143
4d	425
4e	189

- The node 3 related to the *in vitro* assay is passed due to positive experimental data found for the target chemical and implemented thresholds (slide #14 -15)
- The workflow should move further to the other *in vitro* assays (nodes 4a and 4b)

Workflow process

Step 4. *in vitro* Dendritic cell activity assay h-CLAT (expression of CD54 and CD86) (node 4a)

Example 1



Check if there are any data for the target chemical for the *in vitro* h-CLAT assay (node 4a)

1 Skin Sensitization window details:

- Full names:
 - 1 - Protein binding alerts
 - 2a - in chemico Peptide depletion assay D
 - 2b - in chemico Peptide depletion assay D
 - 2c - in chemico Glutathione depletion assay D
 - 2d - in chemico Adduct formation assay L
 - 3 - in vitro KeratinoSens and LuSens (EC1)
 - 4a - *in vitro* Dendritic cell activity assay h-CLAT
 - 4b - *in vitro* Dendritic cell activity assay MIEC3
- Legend: Not checked (blue), Passed (green)
- Node short name: 4a
- Node full name: *in vitro* Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
- Relevant databases: Dendritic cells COLIPA
- Associated endpoint tree positions: Human Health Hazards#Sensitisation Assay=Dendritic cell activity (h-CLAT)

2 Endpoint menu

3 Human Health Hazards database selection:

- Physical Chemical Properties
- Environmental Fate and Transport
- Ecotoxicological Information
- Human Health Hazards
 - Dendritic cells COLIPA
 - Keratinocyte gene expression Givaudan
 - Keratinocyte gene expression LuSens
 - Skin sensitization
 - Skin sensitization ECETOC

4 Gather button

5 Information message: Some nodes had their status changed automatically.

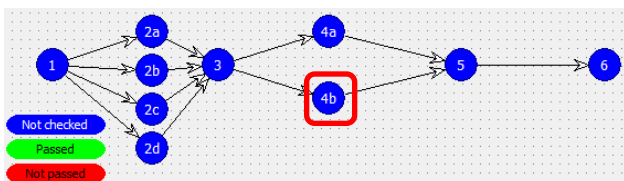
Endpoint	Count	Status
CD54	(7/13)	M: Positive, Positive
CD86	(7/13)	M: Positive, Positive
Dendritic Cell Activity (h-CLAT)	(3/3)	M: Positive
Dendritic Cell Activity (mMUSST)	(7/8)	M: Positive

1. Select node 4a
2. Go to Endpoint
3. Select database related to node 4a
4. Gather data and click **OK** in the appeared message
5. The status of node 4a and 4b was changed to passed

Workflow process

Step 4. *in vitro* Dendritic cell activity assay MUSST (expression of CD86) (node 4b)

Example 1



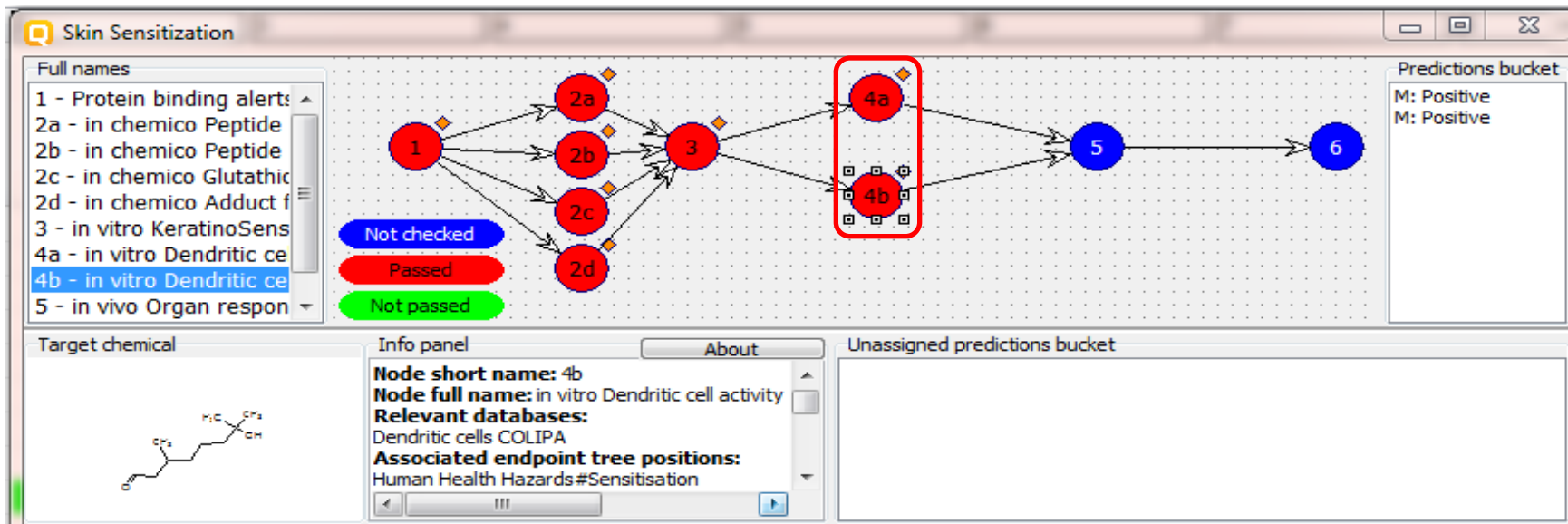
Check if there are any data for the target chemical for the *in vitro* MUSST assay (node 4b)

1. Select node 4b
The experimental data appeared in the bucket

Workflow process

Step 4. *in vitro* Dendritic cell activity assay (node 4a and 4b)

Example 1

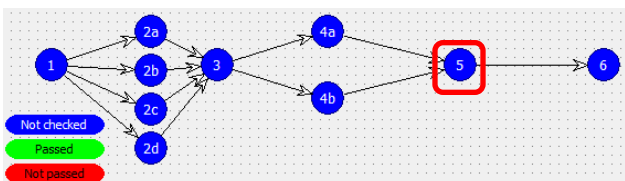


- The nodes 4a and 4b related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to positive experimental data found for the target chemical
- The workflow moves further to the *in vivo* LLNA assay (node 5)

Workflow process

Step 5. *In vivo* Organ response (LLNA)(node 5)

Example 1



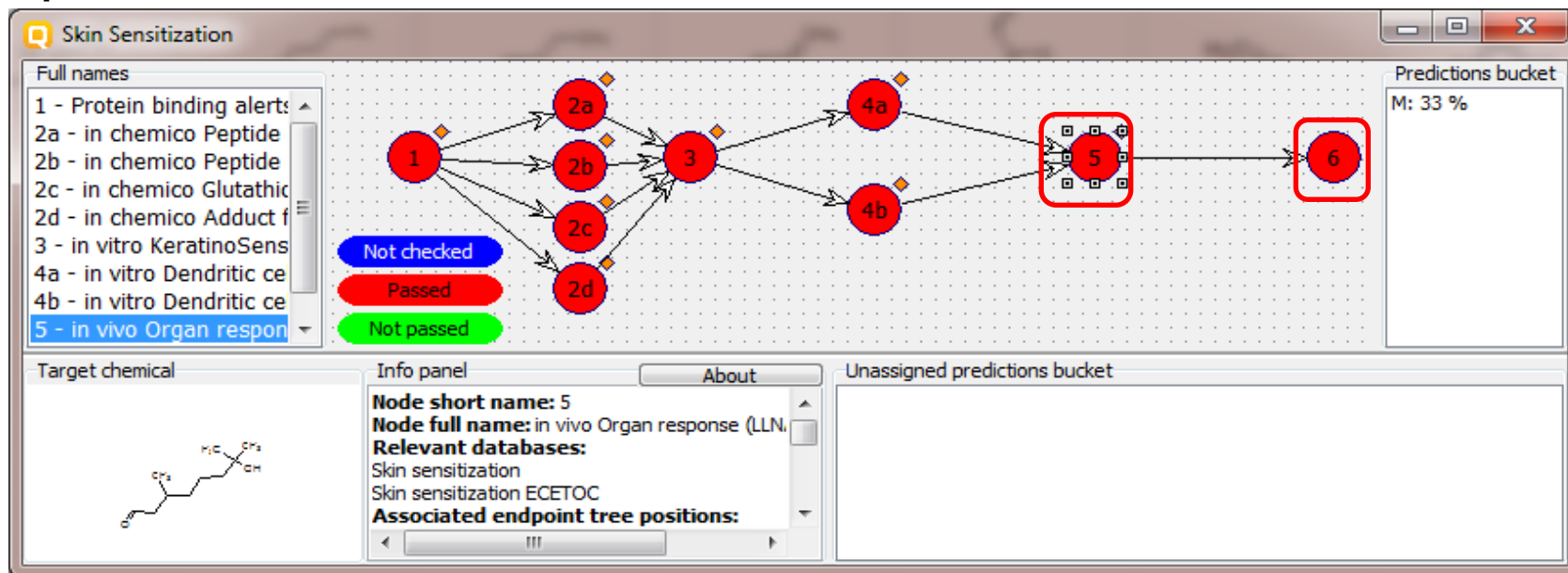
Check are there any data for the target chemical for the *in vivo* Organ response (LLNA)(node 5)

1. Select node 5
2. Go to Endpoint
3. Select database related to the node 5
4. Click Gather
5. Click OK
6. The data appears in the bucket of the node
7. The node 5 and 6 are automatically changed to passed, based on experimental data for the target chemical and the implemented thresholds (see slide #14 -15)

Workflow process

Step 5. *in vivo* Organ and Organism assays (node 5 and 6)

Example 1



- Both nodes related to the two *in vivo* assays (LLNA and GPMT) are passed based on the positive experimental data for the target chemical according to the implemented thresholds

Outlook

- Background
- Objectives
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
 - Example 2: Eugenol (CAS 97-53-0)
 - **Input target**

Chemical Input

Enter CAS# 97-53-0

The Toolbox now searches the databases to find out if the CAS# you entered is linked to a molecular structure stored in the Toolbox. It is displayed as a 2-dimensional depiction

The screenshot shows the 'Search by CAS #' dialog box. The search input field contains '97530' (highlighted with a red box and labeled '1'). The 'Search' button is highlighted with a blue box and labeled '2'. The 'OK' button is highlighted with a blue box and labeled '3'. Below the search area, a table displays the search results. The first result is selected and highlighted in blue. The table columns are: Selected, CAS, Smiles, Depiction, Names, CAS/Name, 2D/Name, and CAS/2D. The 'Depiction' column shows a 2D chemical structure of 4-methoxy-2-propenylphenol. The 'CAS/Name' column shows the CAS number and the name of the compound.

Selected	CAS	Smiles	Depiction	Names	CAS/Name	2D/Name	CAS/2D
1. Yes	97-53-0	COc1cc(C=C)cc1			1:: Low (: High 1:: A 2:: High 1: 1:: Ba 1:: U: 2: 2:: C A 2:: E: 3: 3:: C 3:: R: 4: 4:: C 4:: C: 5: 5:: Cl 5:: C: 6: 6:: D 6:: C: 7: 7:: D 7:: K: 8: 8:: E 8:: M: 9: 9:: E 9:: M A 10: 10:: E 10:: f A 11: 11:: C 11:: C		

1. **Enter** the CAS# In the blank field; 2. **Click** Search button; 3. **Press** OK

Chemical Input

Target chemical identity

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes options for Document, Single Chemical, and Chemical List. The main workspace is divided into several sections:

- Documents:** A tree view on the left shows a document with CAS: 97-53-0 selected.
- Structure:** A chemical structure of eugenol is shown in the top right.
- Substance Identity:** A list of identifiers is displayed, with the following items circled in red:
 - 97-53-0
 - EINECS:2025891
 - eugenol (4-allyl-2-methoxyphenol)
 - eugenol
 - 4-allyl-2-methoxy-phenol
 - phenol, 2-methoxy-4-(2-propenyl)-
 - 2-methoxy-4-(prop-2-en-1-yl)phenol
 - phenol, 4-allyl-2-methoxy-
 - 1-allyl-3-methoxy-4-hydroxybenzene
 - 2-methoxy-4-(2-propenyl)phenol
 - 4-allyl-2-methoxyphenol
 - p-allylguaiacol
 - eugenol (4-allyl-2-methoxyphenol)e...
- Molecular Formula:** C₁₀H₁₂O₂
- Structural Formula:** COc1cc(CC=C)ccc1O

At the bottom, there is a filter endpoint tree and a 'Create' button.

Outlook

- Background
- Objectives
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
 - Example 2: Eugenol (CAS 97-53-0)
 - Input target
 - **Set AOP target**

Activate AOP

Set target chemical for AOP

The screenshot illustrates the QSAR Toolbox interface during the activation of an AOP. Key elements include:

- Documents Panel (1):** The endpoint tree is filtered to show 'skin' in a green file.
- Structure Panel (2):** The tree is expanded to the 'Sensitization' node, with 'Skin' selected.
- Context Menu (3, 4):** A right-click action is performed near the 'AOP' label, and the 'Activate AOP' option is selected from the menu.
- Skin Sensitization Window (5):** The window opens, displaying a flowchart and a list of full names for the endpoints.
- Target Setting (6):** The target chemical is set for the AOP, as indicated by the callout box.

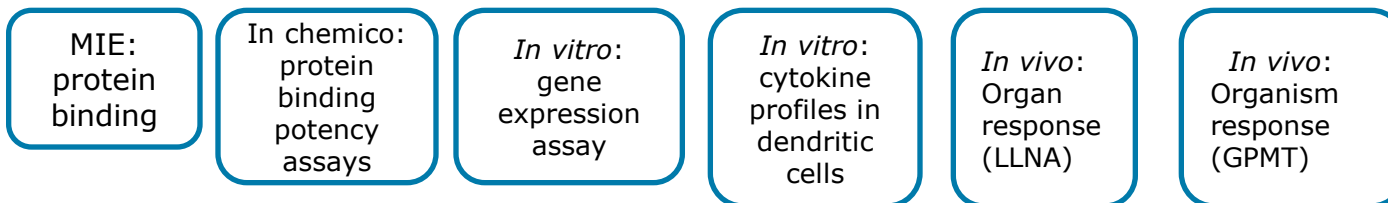
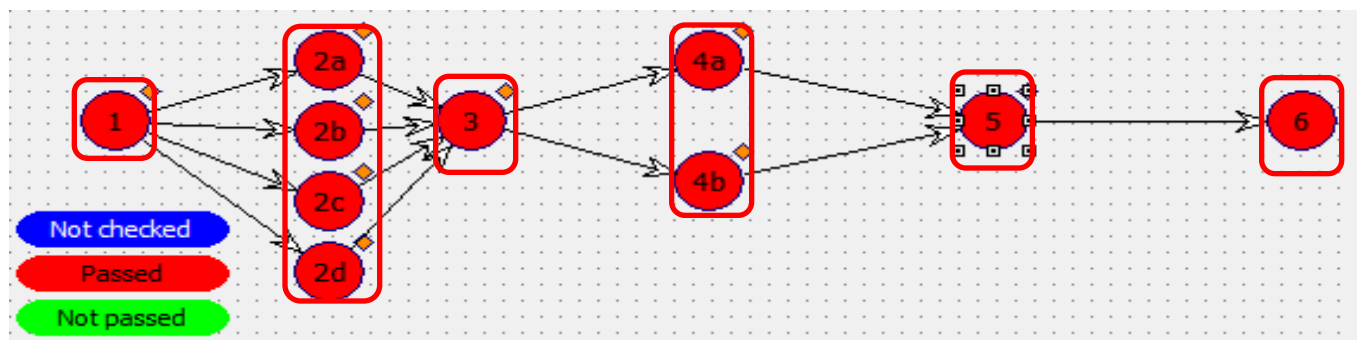
1. Filter endpoint tree – **write skin** in the green filed
2. **Expand the tree** – open the tree to the Sensitization node
3. **Right click** near the AOP label
4. **Select** activate AOP
5. AOP window appears
6. Set target for AOP (see slide 25)

Outlook

- Background
- Objectives
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
 - Example 2: Eugenol (CAS 97-53-0)
 - Input
 - Activate AOP and set target
 - **Workflow process**

Workflow process

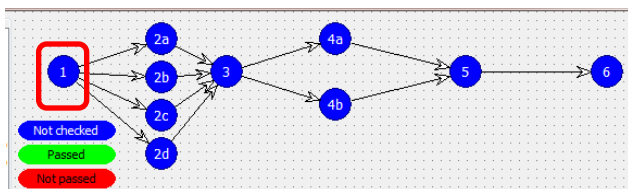
- Workflow process start from molecular initiating event to the *in vivo* organism response



Workflow process

Step 1. MIE: protein binding

Example 2



Start with profiling of target chemical

1. Open Profiling

2. Select node #1 related to MIE.

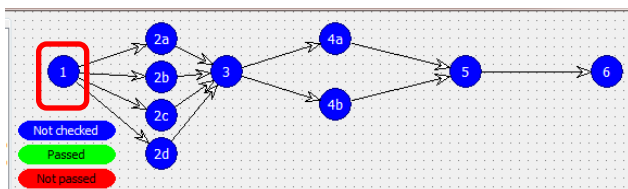
3. Relevant profilers are highlighted, select highlighted profilers

4. Apply selected profilers to the target chemical

Workflow process

Step 1. MIE: protein binding

Example 2



Start with profiling of target chemical

Information

Some nodes had their status changed automatically.

OK

1. The target chemical has no protein binding alert

2. The node is automatically changed to not passed based on absence of alert. **Click OK**

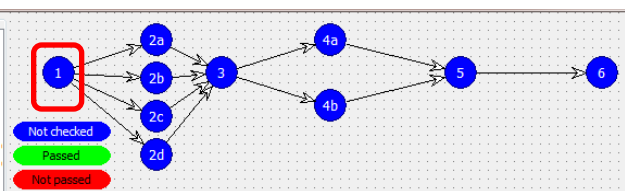
3. The next step is to investigate whether the substance has skin sensitization potential via autoxidation

Workflow process

Step 1. MIE: protein binding

Example 2

Simulate Autoxidation products of the target chemical

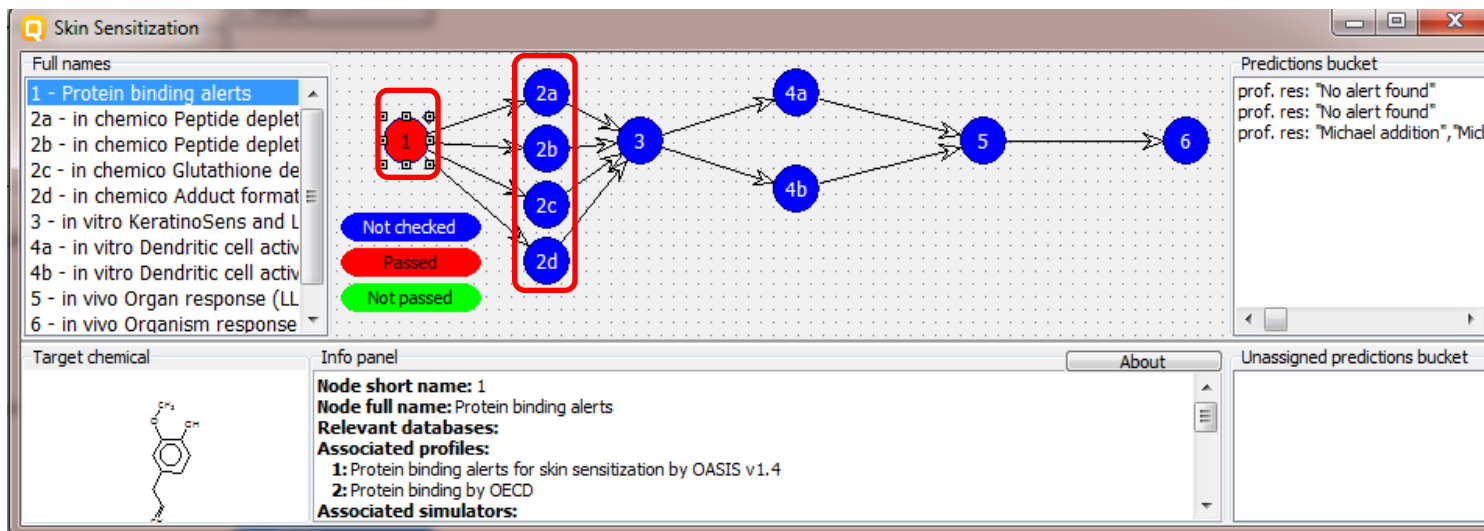


1. **Select** Autoxidation simulator
2. **Select** highlighted profilers relevant to the MIE
3. **Click** Apply
4. The profiling results appeared on data matrix
5. **Right click** over the node 1 and perform "Not checked"
6. Right click over the cell with profiling results and select "Use for the AOP"
7. Status of node 1 is changed to "Passed" based on the implemented thresholds (slide #14-15)

Workflow process

Molecular initiating events

Example 2

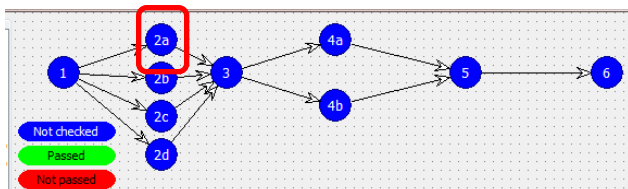


- The node MIE is passed due to the presence of positive protein binding alert identified for the Autoxidation products of the target chemical
- The workflow should move further to the *in chemico* assays

Workflow process

Step2. *In chemico* Peptide depletion assay DPRA (Cys) (node 2a)

Example 2

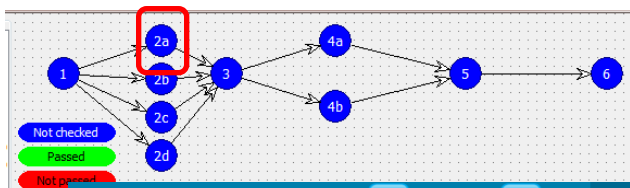


Profiling target chemical

Workflow process

Step2. *In chemico* Peptide depletion assay DPRA (Cys) (node 2a)

Example 2



Check for experimental data

Full names	Node short name	Node full name	Relevant databases
1 - Protein binding alerts	1	Protein binding alerts	Chemical Reactivity COLIPA
2a - in chemico Peptide depletion	2a	in chemico Peptide depletion assay DPRA (Cys)	Human Health Hazards#Sensitisation
2b - in chemico Peptide depletion	2b	in chemico Peptide depletion	Assay=DPRA
2c - in chemico Glutathione deple	2c	in chemico Glutathione depletion	
2d - in chemico Adduct formation	2d	in chemico Adduct formation	
3 - in vitro KeratinoSens and LuS	3	in vitro KeratinoSens and LuS	
4a - in vitro Dendritic cell activity	4a	in vitro Dendritic cell activity	
4b - in vitro Dendritic cell activity	4b	in vitro Dendritic cell activity	
5 - in vivo Organ response (LLNA)	5	in vivo Organ response (LLNA)	
6 - in vivo Organism response (G	6	in vivo Organism response (G)	

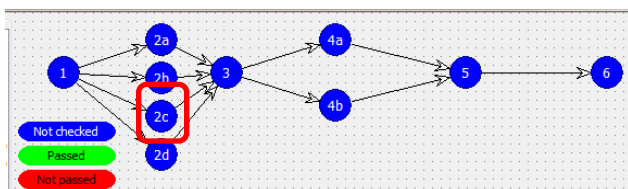
Endpoint	Node	M	Pass	Fail
% Depletion of Cystine	(1/2)	M: 38.3 %	9.2 %	
% Depletion of Lysine	(1/2)	M: 19.2 %	4.24 %	
GSH				
LC-MS	(1/1)	M: 52.8 %		

- Go to Endpoint
- Select highlighted database
- Click Gather
- There are data for node 2a, 2b and 2d and nodes are getting passed.

Workflow process

Step2. *In chemico* Glutathione depletion assay GSH (RC50)(node 2c)

Example 2



Check are there any data for the target chemical

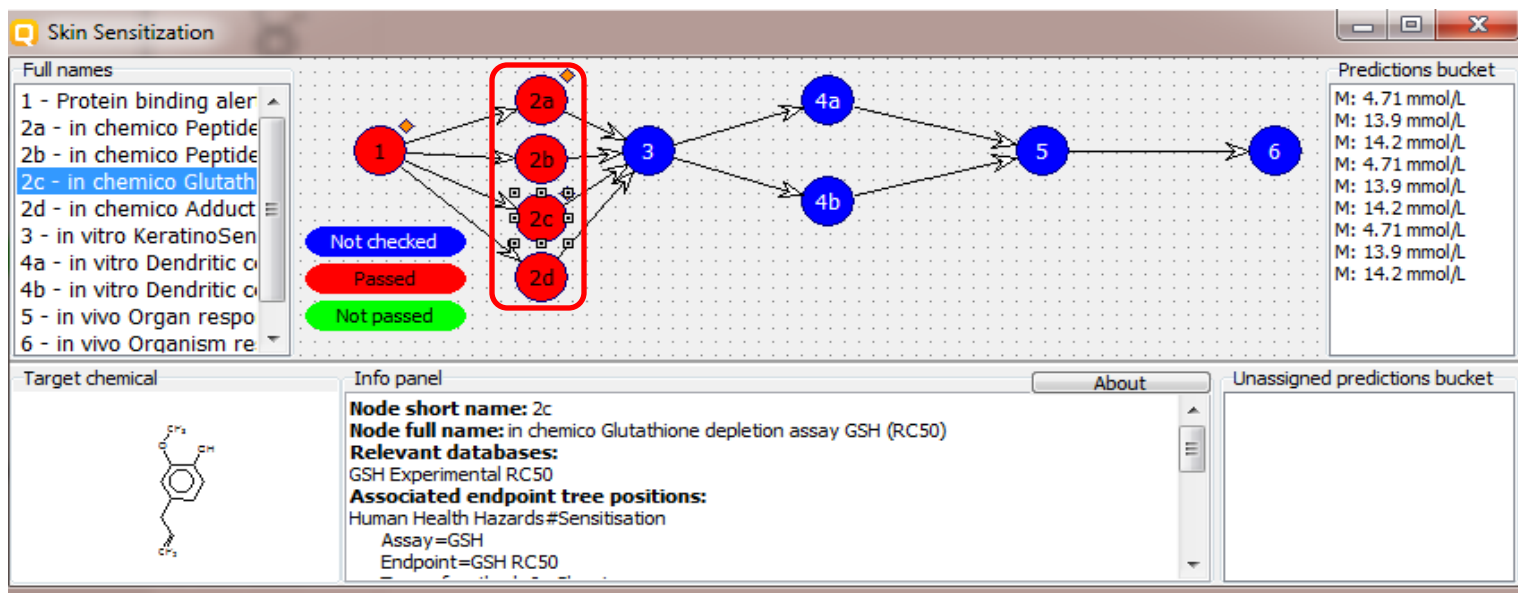
Node	Value
1	M: 4.71 mmol/L
2a	M: 13.9 mmol/L
2b	M: 14.2 mmol/L
2c	M: 4.71 mmol/L
2d	M: 13.9 mmol/L
3	M: 14.2 mmol/L
4a	M: 4.71 mmol/L
4b	M: 13.9 mmol/L
5	M: 14.2 mmol/L
6	M: 4.71 mmol/L

1. Go to endpoint
2. Select highlighted database
3. Click Gather
4. Node 2c is getting passed

Workflow process

In chemico assays

Example 2

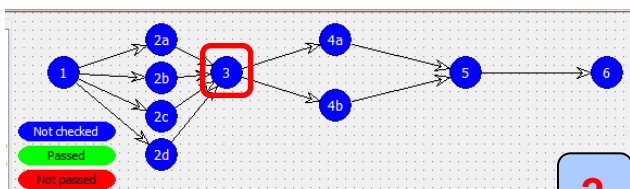


- The nodes related to the *in chemico* assays are passed due to positive experimental data for the target chemical (node 2a, 2b, 2c and 2d) The workflow should move further to the *in vitro* assay (node 3)

Workflow process

Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 3)

Example 2



Check are there experimental data for the parent chemical for node 3

Endpoint	Filter	Cl	R
In Chemo			
DPRA			
% Depletion of Cystine	(2/2)	Cl: 54.9 %	R: 54.9(-103;212) %
% Depletion of Lysine	(2/2)	Cl: 6.3 %	R: 6.3(-59.8;72.4) %
GSH			
GSH RC50	(2/2)	Cl: Moderately Reactive	R: Moderately Re...
LC-MS			
Adduct Formation			
In Vitro			
Dendritic Cell Activity (h-CLAT)			
CD54			
CD86			
Dendritic Cell Activity (MUSST)			
CD86			
Keratinocyte Gene Expression (ARE)			
EC1.5	(2/2)	M: >328 mg/L	M: >328 mg/L
EC2	(2/2)	M: >328 mg/L	M: >328 mg/L
EC3	(2/2)	M: >328 mg/L	M: >328 mg/L

Full names

- 1 - Protein binding alerts
- 2a - in chemo Peptide depletion assay DPRA (Cys)
- 2b - in chemo Peptide depletion assay DPRA (Lys)
- 2c - in chemo Glutathione depletion assay GSH (RC50)
- 2d - in chemo Adduct formation assay LC-MS
- 3 - *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3)
- 4a - *in vitro* Dendritic cell activity assay h-CLAT (express...
- 4b - *in vitro* Dendritic cell activity assay MUSST (express...
- 5 - *in vivo* Organ response (LLNA)
- 6 - *in vivo* Organism response (GPMT)

Target chemical

Info panel

Node short name: 3
Node full name: *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3)
Relevant databases: Keratinocyte gene expression Gvaudan
Associated endpoint tree positions: Human Health Hazards#Sensitisation Assay=Keratinocyte gene expression (ARE) Endpoint=EC1.5

1. Select node 3
2. Go back to Endpoint
3. Select highlighted database
4. Click Gather
5. There is experimental data for the parent chemical, which appears on data matrix
6. Node 3 is getting "Passed" based on the experimental data and implemented threshold (slide #14-15)

Workflow process

Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 3)

Example 2

Full names

- 1 - Protein binding alerts
- 2a - in chemico Peptide depletion assay DPRA (Cys)
- 2b - in chemico Peptide depletion assay DPRA (Lys)
- 2c - in chemico Glutathione depletion assay GSH (RC50)
- 2d - in chemico Adduct formation assay LC-MS
- 3 - in vitro KeratinoSens and LuSens (EC1.5, EC2, EC3)
- 4a - in vitro Dendritic cell activity assay h-CLAT (expression)
- 4b - in vitro Dendritic cell activity assay MUSST (expression)
- 5 - in vivo Organ response (LLNA)
- 6 - in vivo Organism response (GPMT)

Target chemical

Info panel

Node short name: 2c
Node full name: in chemico Glutathione depletion assay GSH (RC50)
Relevant databases:
 GSH Experimental RC50
Associated endpoint tree positions:
 Human Health Hazards#Sensitisation
 Assay=GSH
 Endpoint=GSH RC50

Predictions bucket

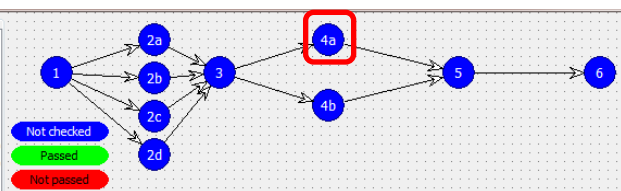
- M: 4.71 mmol/L
- M: 13.9 mmol/L
- M: 14.2 mmol/L

- The node 3 related to the Keratinocyte ARE (EC1.5, EC2, EC3) is passed based on the experimental data found for the target chemical (threshold are specified on slide # 15).
- The workflow moves further to the *in vitro* Dendritic cell assay (nodes 4)

Workflow process

Step 4. *in vitro* Dendritic cell activity assay h-CLAT (expression of CD54 and CD86) (node 4a)

Example 2



Check if there are any data for the target chemical for the *in vitro* h-CLAT assay (node 4a)

2

1 - Protein binding alerts
 2a - in chemico Peptide depletion ass
 2b - in chemico Peptide depletion ass
 2c - in chemico Glutathione depletion
 2d - in chemico Adduct formation ass
 3 - in vitro KeratinoSens and LuSens
 4a - **in vitro Dendritic cell activity ass**
 4b - in vitro Dendritic cell activity ass
 5 - in vivo Organ response (LLNA)

Node short name: 4a
 Node full name: in vitro Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
 Relevant databases:
 Dendritic cells COLIPA
 Associated endpoint tree positions:
 Human Health Hazards#Sensitisation
 Assay=Dendritic cell activity (h-CLAT)

Some nodes had their status changed automatically.

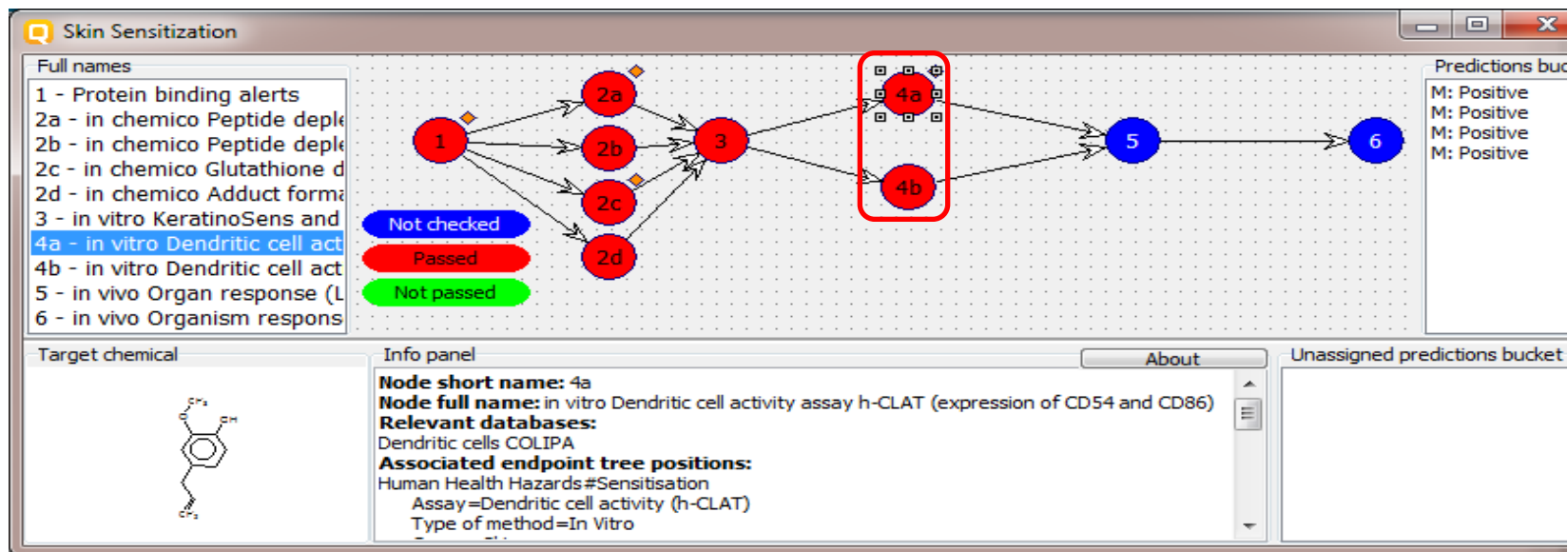
CD54	(1/2)	M: Positive	Positive
CD86	(1/2)	M: Positive	Positive
Dendritic Cell Activity (mMUSST)	(1/1)	M: Positive	
Dendritic Cell Activity (MUSST)	(1/1)	M: Positive	

1. Select node 4a
2. Go to Endpoint
3. Select database related to node 4a
4. Gather data and click OK in the appeared message
5. The status of node 4a and 4b were changed to passed

Workflow process

Step 4. *in vitro* Dendritic cell activity assay (node 4a and 4b)

Example 2



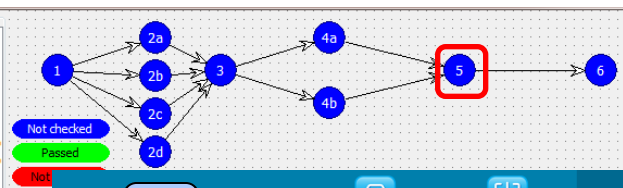
- The node 4a and 4b related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to positive experimental data found for the target chemical
- The workflow could further move to the *in vivo* LLNA assay (nodes 5)

Workflow process

Step 5. *In vivo* Organ response (LLNA)(node 5)

Example 2

Check are there any data for the target chemical for the *in vivo* Organ response (LLNA)(node 5)

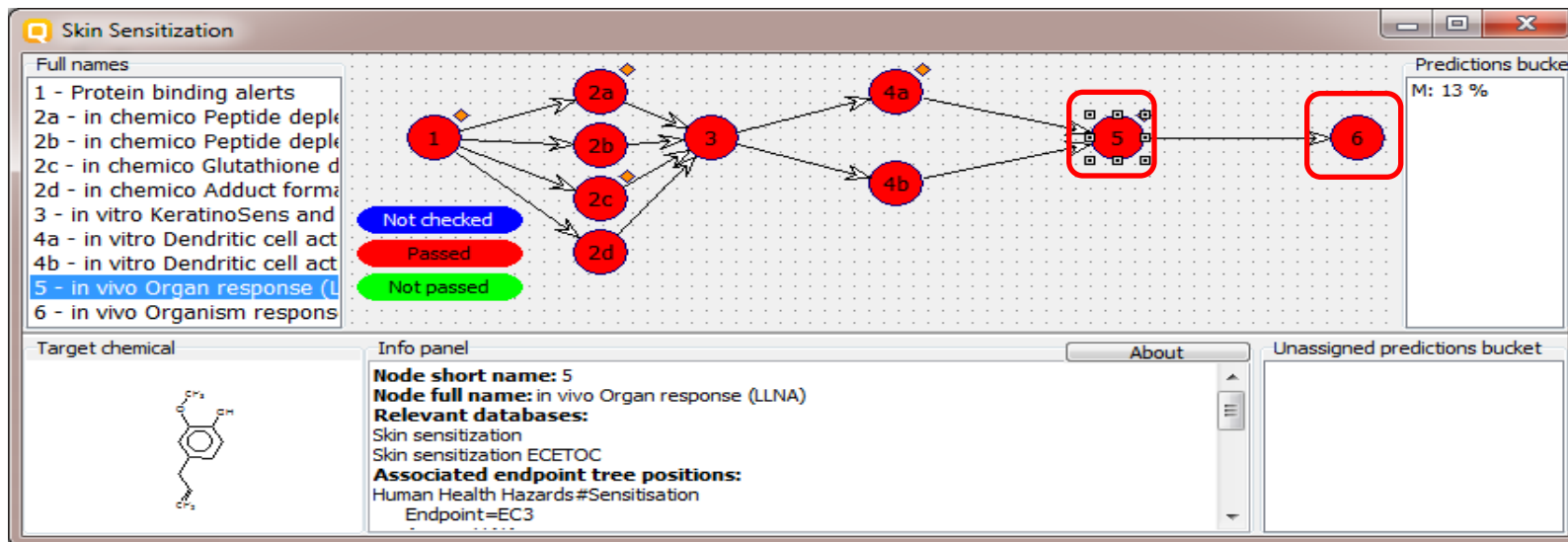


1. Select node 5
2. Go to Endpoint
3. Select database related to the node 5
4. Click Gather
5. Click OK
6. The data appears in the bucket of the node
7. Nodes 5 and 6 are getting passed based on experimental data extracted for the target chemical

Workflow process

Step 5. *in vivo* Organ and Organism assays (node 5 and 6)

Example 1



- Both nodes related to the two *in vivo* assays (LLNA and GPMT) are passed based on the identified positive experimental data for the target chemical

Conclusions

- This tutorial illustrates how implemented proof-of-concept AOP scheme can be used in assessment of skin sensitization of chemicals using different combinations of data and grouping methods related to nodes of the AOP.