

OECD QSAR Toolbox v.4.1

Implementation AOP workflow in Toolbox:
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 example 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

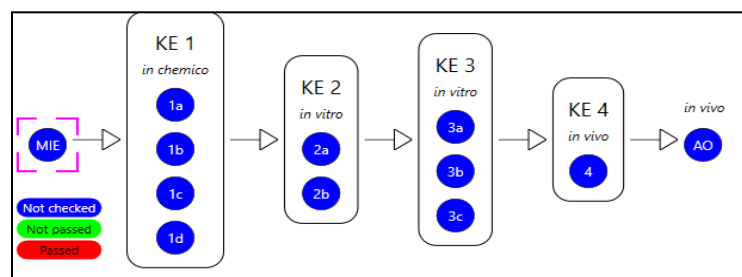
**Demonstrated examples are obtained with Toolbox v4.1*

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 AOP scheme



Key node

MIE

Protein binding alerts

1a

Peptide depletion assay DPRA (Cys)

1b

Peptide depletion assay DPRA (Lys)

1c

Glutathione depletion assay GSH (RC50)

1d

Adduct formation assay LC-MS

2a

KeratinoSens assay (EC1.5, EC2, EC3)

2b

LuSens (EC1.5, EC2, EC3)

3a

Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)

3b

Dendritic cell activity assay MUSST (expression of CD86)

3c

Dendritic cell activity assay mMUSST (expression of CD86)

4

Organ response (EC3 LLNA)

AO

Organism response (GPMT)

Key event

Protein binding – in silico/theoretical

Protein binding potency in chemico

Cellular response (gene expression)

Cellular response (activation of dendritic cells)

Organ response

Organism response

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- **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 displays the AOP window in the QSAR Toolbox software, which is used for assessing the potential of chemicals to cause adverse outcomes based on a sequence of events (AOP).

Panel with full names of nodes: This panel lists the full names of the nodes in the AOP scheme. It includes:

- Molecular initiating event (MIE) – Protein binding alerts**
- Key event (KE 1) – Molecular interactions**
 - 1a - In chemico peptide depletion assay DPRA (Cys)
 - 1b - In chemico peptide depletion assay DPRA (Lys)
 - 1c - In chemico Glutathione depletion assay GHS (RC50)
 - 1d - In chemico Adduct formation assay LC-MS
- KE 2 – Cellular responses (gene expression)**
 - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
 - 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 – Cellular responses (activation of dendritic cells)**
 - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
 - 3b - In vitro dendritic cell activity assay MUSST (expression of CD86)
 - 3c - In vitro dendritic cell activity assay mMUSST (expression of CD86)

AOP tree scheme: This panel shows the AOP tree scheme, which is a flowchart representing the sequence of events from the MIE to the AO. The nodes are color-coded: blue for 'Not checked', green for 'Not passed', and red for 'Passed'. The MIE node is highlighted with a red border.

Indication for assigned prediction: This panel shows the indication for assigned prediction, which is a red 'X' mark next to the MIE node.

Panel with predictions/measured data assigned to the selected node: This panel shows the predictions/measured data assigned to the selected node. It includes:

- Predictions bucket**
 - Protein binding alerts for skin sensitization according to OECD
 - No alert found
 - Protein binding alerts for skin sensitization by OASIS
 - No alert found
 - Protein binding alerts for skin sensitization according to OECD
 - 6 x No alert found
 - 3 x Skin sensitization Category 1B
 - 2 x Skin sensitization Category 1B >> Aldehydes
 - 1 x Skin sensitization Category 1B >> C-Nitroso compounds
 - Protein binding alerts for skin sensitization by OASIS
 - 8 x No alert found
 - 1 x Nucleophilic addition
 - 1 x Nucleophilic addition >> Nucleophilic addition

Target chemical: The target chemical is 2,4-dimethylbenzylamine, shown as a chemical structure with a benzene ring, a methyl group (CH₃), and an amino group (NH₂).

Info panel: This panel provides information for the selected node (MIE). It includes:

- Node short name:** MIE
- Node full name:** Molecular initiating event (MIE)
- Associated profiles:**
 - Protein binding by OASIS
 - Protein binding alerts for skin sensitization by OASIS
 - Protein binding alerts for skin sensitization according to OECD
 - Protein binding by OECD
- Associated simulators:**
 - Autoxidation simulator
 - Skin metabolism simulator
- Thresholds:**
 - Profile 'Protein binding alerts for skin sensitization by OASIS'
 - Passed:** Any of the profiler categories
 - Not passed:** No alert found

Color legend: This panel shows the color legend for the AOP tree scheme, indicating that blue represents 'Not checked', green represents 'Not passed', and red represents 'Passed'.

Panel with information for selected node: This panel shows the information for the selected node (MIE), including the node short name, node full name, associated profiles, associated simulators, and thresholds.

Short description: This panel provides a short description of the AOP, explaining the sequence of events from the MIE to the AO.

About: This panel provides information about the AOP module, including the implementation of the Skin Sensitization AOP module in this version of the OECD Toolbox, which was supported by the 2015 Lush Black Box Prize awarded to Prof. Terry W. Schultz.

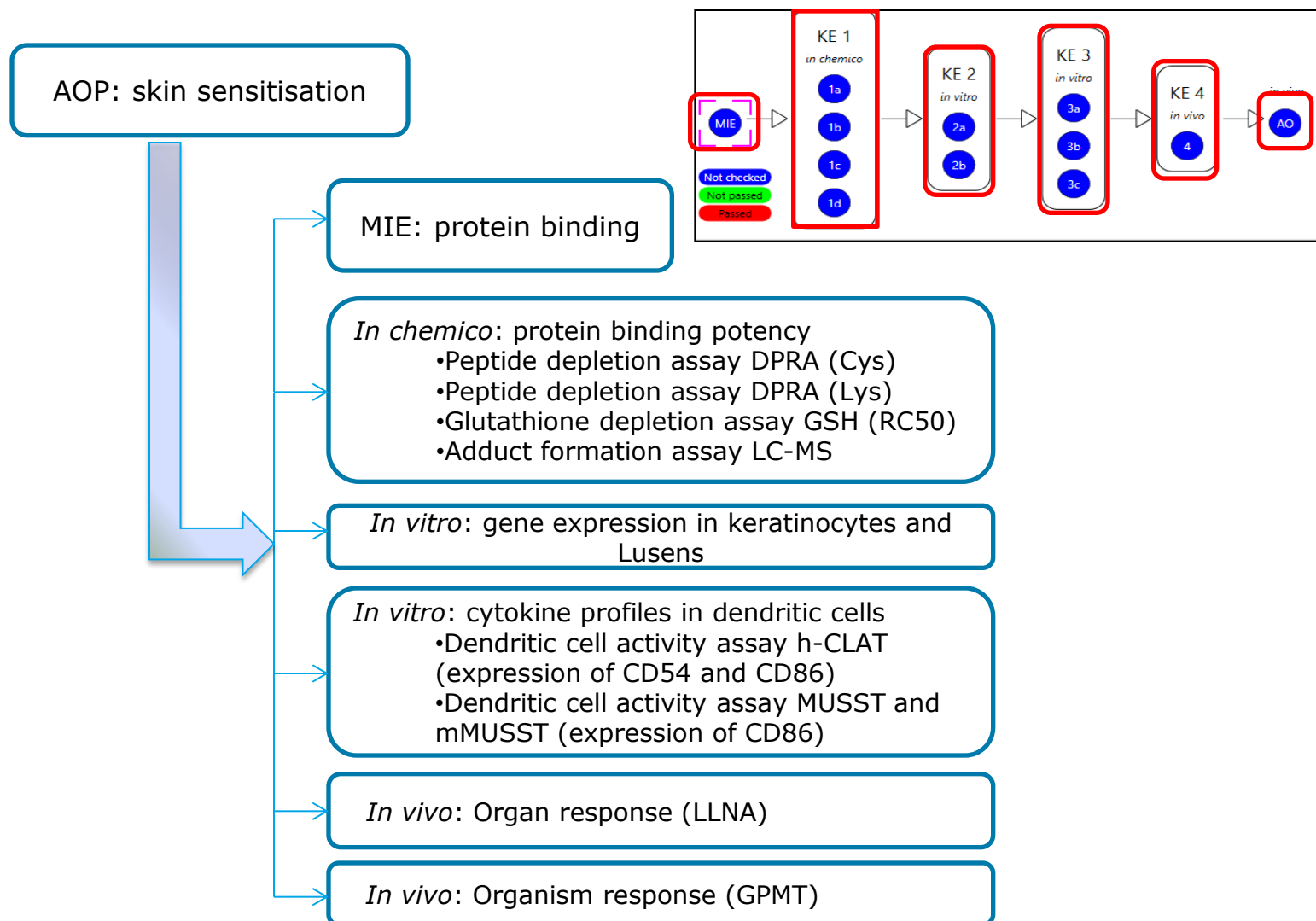
Disclaimer: This panel provides a disclaimer, stating that the in silico method examined the potential for direct- and indirect-acting electrophiles to covalently react with skin proteins containing both a cysteine and lysine residue. Relevance to skin sensitization of the different in chemico or in vitro method is inferred from the fact that each represents a well established method.

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 - **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



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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:

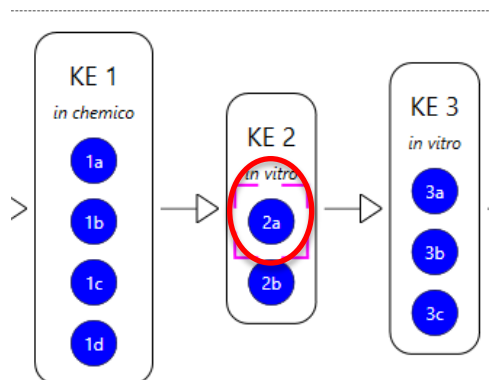
Thresholds:

Scale name 'Gene expression EC (ordinal)'

Scale type 'Ordinal'

Passed: High | Low | Moderate | Very High

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
MIE - Protein binding alerts		presence of alert	absence of alert
1a and 1b <i>in chemico</i> DPRA Cys and Lys	Peptide depletion, PD (%): PD > 9 - Passed PD ≤ 9% - Not passed	> 9 % - Passed	≤ 9 % - Not passed
1c - <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
1d - <i>in chemico</i> Adduct formation assay LC-MS	Adduct formation (%) ≥ 30% - Positive Adduct formation (%) < 30% - Negative	Positive	Negative
2a - <i>in vitro</i> Keratinocyte (EC1.5, EC2, EC3) AND 2b – <i>in vitro</i> LuSens (EC1.5, EC2)	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
3a;3b and 3c <i>in vitro</i> Dendritic cell activity assay h-CLAT; MUSST and mMUSST (expression of CD54 and CD86)	expression of CD54 and CD86 Positive Negative	Positive	Negative
4 - <i>in vivo</i> Organ response (LLNA)	0 ≥ EC3 (%) < 50 – Positive EC3 ≥ 50 – Negative Or	Positive	Negative
AO - <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

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 - 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 'Input' menu is open, and the 'CAS#' option is highlighted with a red box. A tooltip is displayed over the 'CAS#' option, providing instructions on how to enter a chemical by its CAS number. The tooltip text is as follows:

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.

A white box with the number '1' and an arrow points to the 'CAS#' option. At the bottom of the interface, a blue box contains the text: **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 #

107755 Search OK Cancel

Select All Unselect All Invert Selection Selected 1 of 2

1	<p>CAS 107-75-5</p> <p>SMILES <chem>CC(CCCC(C)(C)O)CC=O</chem></p> <p>CS Relation High</p> <p>Substance Mono constituent</p> <p>Composition</p> <p>Name 3,7-Dimethyl-7-hydroxy-oct... 7-Hydroxy-3,7-dimethyloct... 7-hydroxycitronellal</p>	
2	<p>CAS 107-75-5</p> <p>SMILES <chem>CC(C)(O)CCCC(C)(C)CC=O</chem></p> <p>CS Relation Low</p> <p>Substance Mono constituent</p> <p>Composition</p> <p>Name 3,7-Dimethyl-7-hydroxyoct... 7-Hydroxy-3,7-dimethyloct... 7-hydroxycitronellal</p>	

1. **Enter** the CAS# In the blank field; 2. **Click** over the box associated with chemical with high CAS-SMILES Relation (CS Relation) 3. **Click** OK

Chemical Input

Target chemical identity

QSAR Toolbox 4.0.0.22512 [Document 1]

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Filling Report

Document Single Chemical Chemical List Search Target Endpoint

New Open Close Save CAS# Name Structure Composition Select Delete ChemIDs Database Inventory List Substructure (SMARTS) Query Define

Documents

Document 1
CAS: 107755

Filter endpoint tree...

Structure

Structure info

- CAS Number
- CAS Smiles relation
- Chemical name(s)
- Composition
- Molecular Formula
- Predefined substance type
- Structural Formula

Parameters

- Physical Chemical Properties
- Environmental Fate and Transport
- Ecotoxicological Information
- Human Health Hazards

1 [target]

107-75-5

High

3,7-Dimethyl-7-hydr

C10H20O2

Mono constituent

CC(CCCC(C)(C)O)CC=O

Chemical Input

Target chemical identity

- Double click “CAS Smiles relation” displays the chemical identification information.
- This indicates the reliability of relation CAS-Name for the target chemical (see next screen shots).

Chemical Input

Target chemical identity

1

2

Exist in data source	Data source type	Data source quality	Assigned SMILES in data source
Aquatic OASIS	Database	Distribute to QA	no
Canada DSL	Inventory	Distribute to QA	no
Chemical Reactivity COLIPA	Database	Distribute to QA	no
Dendritic cells COLIPA	Database	Distribute to QA	no
DSSTOX	Inventory	High quality source	no
ECHA CHEM	Database	Distribute to QA	no
EINECS	Inventory	High quality source	no
Genotoxicity OASIS	Database	Distribute to QA	no
Keratinocyte gene expression Givaudan	Database	Distribute to QA	no
Keratinocyte gene expression LuSens	Database	Distribute to QA	no
METI Japan	Inventory	Distribute to QA	no
NICNAS	Inventory	Distribute to QA	no
Phys-chem EPISUITE	Database	Distribute to QA	no
REACH ECB	Inventory	High quality source	no
Skin Irritation	Database	Distribute to QA	no
Skin Sensitization	Database	Distribute to QA	yes
Skin Sensitization	Database	Distribute to QA	no
Skin sensitization ECETOC	Database	Distribute to QA	yes
ToxCastDB	Database	Distribute to QA	no
US HPV Challenge Program	Inventory	Distribute to QA	no

OK

3

1. Double **Click** 2. Relationships CAS-SMILE; 3. Click **OK**.

Chemical Input

Target chemical identity

The code indicates the reliability of the chemical identifier:

- **High:** This reliability corresponds to high reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to at least one high quality data source (database or inventory)
- **Moderate:** This reliability corresponds to moderate reliability of CAS-SMILES relation. The moderate label is assigned if the chemical belongs to three "Distribute to QA" data sources.
- **Low:** This reliability corresponds to poor reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to less than three, but at least one "Distribute to QA" data sources.

Outlook

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- 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

The screenshot shows the QSAR Toolbox software interface. The 'Filter endpoint tree...' on the left lists various endpoints, with 'Sensitisation' highlighted by a red circle and labeled '1'. A right-click context menu is open over the 'Sensitisation' node, with 'Activate AOP' at the bottom, labeled '3'. The 'AOP' label next to 'Sensitisation' is circled in red and labeled '2'. The 'Skin Sensitization' AOP window is open on the right, showing a flowchart of the AOP process (MIE to KE1 to KE2 to KE3 to KE4 to AO) and associated information, labeled '4'.

1. **Expand the endpoint tree** to the "Sensitization" node
2. **Right click** near the AOP label
3. **Select** activate AOP
4. AOP window appears

Continued on the next slide

Activate AOP

Set target chemical for AOP

The screenshot illustrates the steps to activate an Adverse Outcome Pathway (AOP) in the QSAR Toolbox. The main interface shows the 'Filter endpoint tree...' on the left, with a list of endpoints on the right. A chemical structure is displayed in the center. A context menu is open over the structure, with 'Set AOP target' highlighted. A red dashed box and a blue callout '2' point to this option. Another blue callout '1' points to the chemical structure. To the right, a 'Skin Sensitization' window is open, showing a flowchart of key events (KE 1 to KE 4) and a list of associated profiles and simulators. A red box highlights the chemical structure in the 'Target chemical' panel of this window.

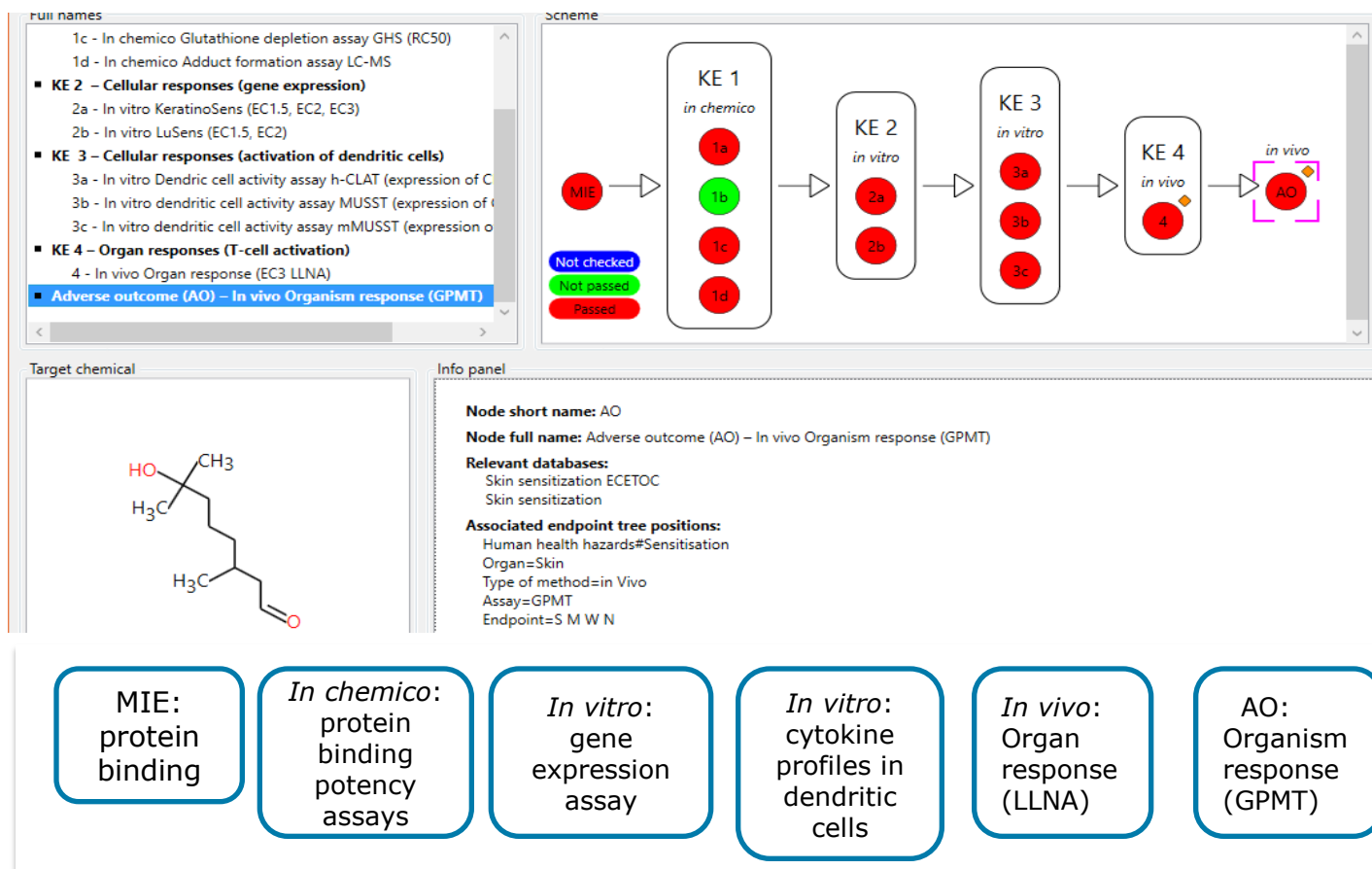
1. **Right click** over the structure and **select** "Set AOP target"
2. The target chemical appears in the AOP window

Outlook

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 - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
 - Input
 - Activate AOP and set target
 - **Workflow process**

Workflow process

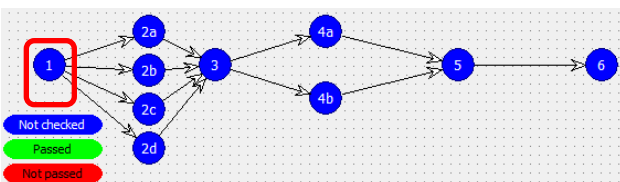
- Workflow process start from molecular initiating event to the *in vivo* organism respond



Workflow process

Step 1. MIE: protein binding

Example 1



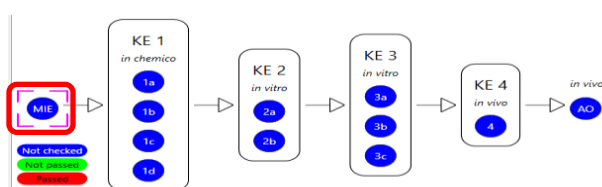
Start with profiling of target chemical

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

1

2

3

1. The target chemical has protein binding alert according to the four suitable protein binding profilers

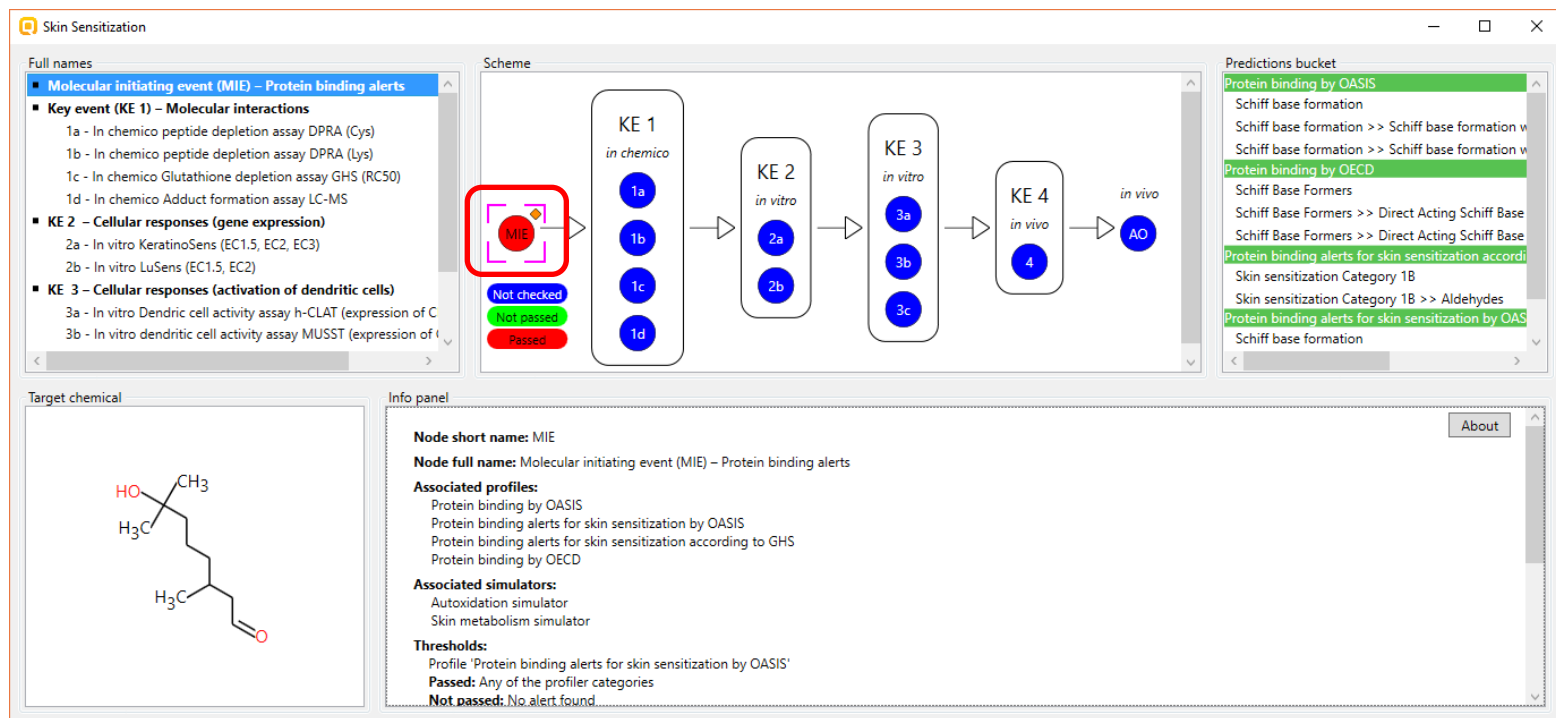
2. The node is automatically changed to passed based on the profiling outcome results and implemented thresholds (see slide #15).

3. Profiling results assigned to the selected node appears in the panel "prediction bucket"

Workflow process

Molecular initiating events

Example 1



- 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* assay

Workflow process

Step 2. *In chemico* peptide depletion assay DPRA (Cys) (node 1a)

Example 1

Profiling target chemical

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

2. The profilers related to node 1a are highlighted. Select **related Profilers**

3. Click **Apply**

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

5. 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 #15)

Workflow process

Step 2. *In chemico* peptide depletion assay DPRA (Cys) (node 1a)

Example 1

Gather data for target chemical

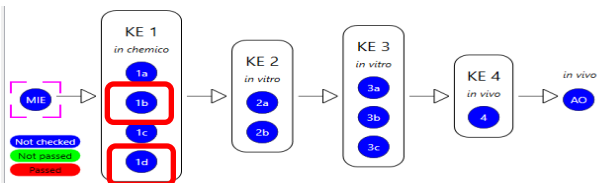
The screenshot illustrates the workflow for gathering data for a target chemical. The interface shows the 'Data' tab with 'Gather', 'Import', 'IUCLID6', and 'IUCLID6' buttons. A 'Filter endpoint tree...' window shows a tree structure with 'In Chemico' expanded, and 'DPRA' selected. A 'Predictions bucket' window shows the results of the DPRA assay, including 'Protein binding potency Cys (DPRA 13%)' and 'Data: Mt: 17.5 %'.

1. **Go** to Data and check are there any experimental data for the node 1a
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 #15) node 1a is getting passed. Node 1b and 1d are automatically changed as passed based the implemented thresholds.

Workflow process

Step 2. *In chemico* peptide depletion assay DPRA (Lys) (node 1b) and *In chemico* Adduct formation assay LC-MS (node 1d)

Example 1



In this case there is available experimental data for the target chemical related to nodes 1b and 1d. In this respect these two nodes changed their status to passed and not passed. The workflow could proceed with next node

1

2

Full names

- Molecular initiating event (MIE) - Protein binding alerts
- Key event (KE 1) - Molecular interactions
 - 1a - In chemico peptide depletion assay DPRA (Cys)
 - 1b - In chemico peptide depletion assay DPRA (Lys)
 - 1c - In chemico Glutathione depletion assay GHS (RCSO)
 - 1d - In chemico Adduct formation assay LC-MS
- KE 2 - Cellular responses (gene expression)
 - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
 - 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 - Cellular responses (activation of dendritic cells)
 - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of C)
 - 3b - In vitro dendritic cell activity assay mMUSST (expression of I)
 - 3c - In vitro dendritic cell activity assay mMUSST (expression of o)

Target chemical

Node short name: 1b
Node full name: In chemico peptide depletion assay DPRA (Lys)
Relevant databases: Chemical Reactivity COLIPA
Associated endpoint tree positions: Physical Chemical Properties/In chemico Assay=DPRA Endpoints=% depletion of lysine
Associated profiles: Protein binding potency Lys (DPRA 13%)
Associated simulators: Autocatalysis simulator (alkaline medium)
Thresholds:

Full names

- Molecular initiating event (MIE) - Protein binding alerts
- Key event (KE 1) - Molecular interactions
 - 1a - In chemico peptide depletion assay DPRA (Cys)
 - 1b - In chemico peptide depletion assay DPRA (Lys)
 - 1c - In chemico Glutathione depletion assay GHS (RCSO)
 - 1d - In chemico Adduct formation assay LC-MS
- KE 2 - Cellular responses (gene expression)
 - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
 - 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 - Cellular responses (activation of dendritic cells)
 - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of C)
 - 3b - In vitro dendritic cell activity assay mMUSST (expression of I)
 - 3c - In vitro dendritic cell activity assay mMUSST (expression of o)

Target chemical

Node short name: 1d
Node full name: In chemico Adduct formation assay LC-MS
Relevant databases: Chemical Reactivity COLIPA
Associated endpoint tree positions: Physical Chemical Properties/In chemico Assay=LC-MS Endpoints=Adduct formation
Thresholds: Scale name: Chemical reactivity LC-MS(ordinal) Scale type: Ordinal Passed: Positive Not passed: Negative

1. Select **node 1b**

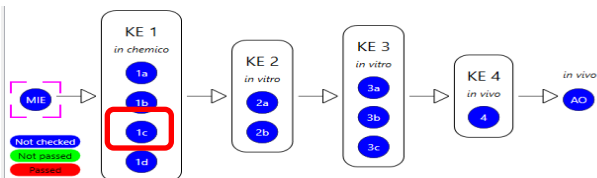
2. Select **node 1d**

The prediction buckets of both nodes were filled with experimental data.

Workflow process

Step 2. *In chemico* Glutathione depletion assay GHS (RC50) (node 1c)

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 1c** related to *in chemico* glutathione depletion assay
2. **Select** highlighted database
3. **Click** Gather. No data has been found for the target chemical

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 1c)

Example 1

The category of similar analogue should be investigated.

3

1

2

4

Target chemical

Target

Not possible to classify according to these rules (GSH)

Profiles

(N/A)

ERROR!

Extremely reactive (GSH)

Extremely reactive (GSH) >> 1-Alken-3-ones (MA)

Extremely reactive (GSH) >> 1-Alkyn-3-ones (MA)

Extremely reactive (GSH) >> 2-bromo dinitriles (SN2)

Extremely reactive (GSH) >> Acetylene dicarboxylates (MA)

Combine profiles

☒ AND ☐ OR

☐ Invert result

☐ Strict

OK Cancel

Info panel

Node short name: 1c

Node full name: *In chemico* Glutathione depletion assay GSH (RC50)

Relevant databases:

GSH Experimental RC50

Associated endpoint tree positions:

Physical Chemical Properties#*In chemico* Assay=GSH

Endpoints=GSH RC50

Associated profiles:

Protein binding potency

Associated simulators:

Autoxidation simulator

1. Switch to **Category definition**

2. **Select** highlighted category

3. **Click** Define

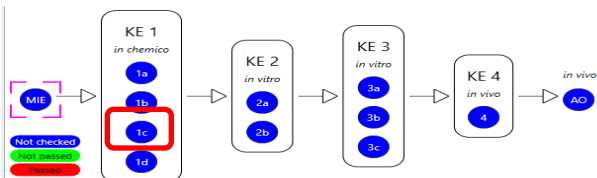
4. There are no structural alerts identified for the target chemical according to this profiler (no mechanistic and structural explanation). Click **Cancel**

Based on the above point it is recommended to define category by "Protein binding alerts" profiler

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 1c)

Example 1



In this case we should investigate the category by Protein binding alerts. The reason for this is that GHS RC50 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

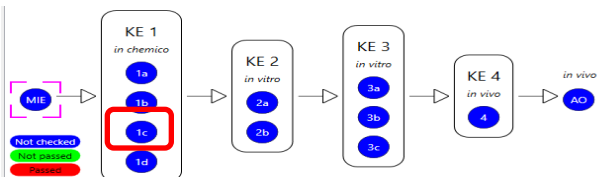
4. Click **OK**

5. The system identify 55 analogues with 118 data points. Click **OK**

Workflow process

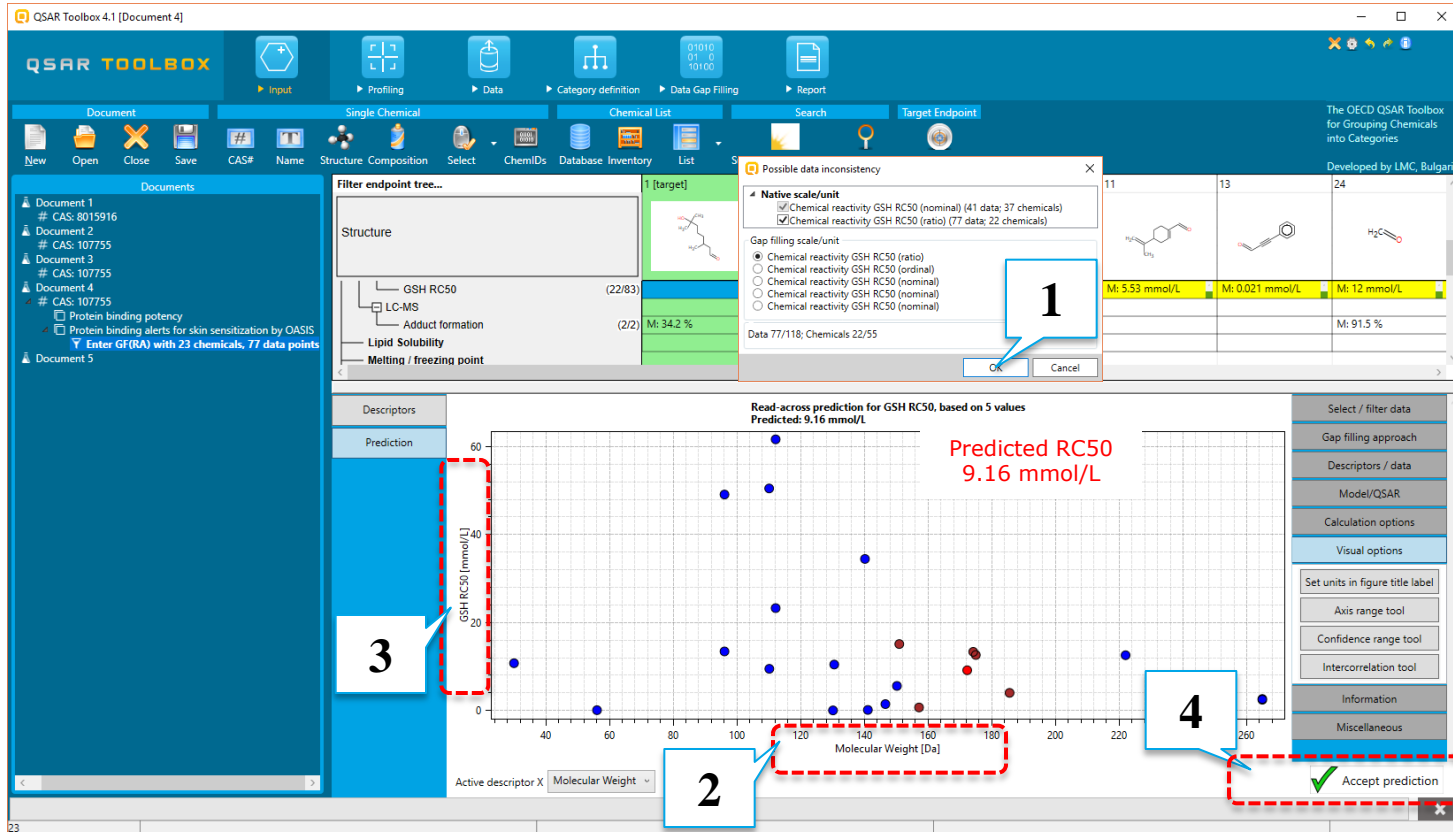
Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 1c)

Example 1



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

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



Data thresholds

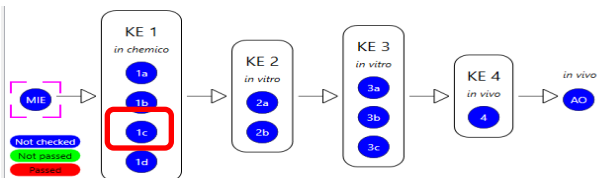
RC50 (mmol/L) ≤ 0.099 - Extremely reactive
 $0.1 \geq \text{RC50} \leq 0.99$ - Highly reactive
 $1 \geq \text{RC50} \leq 15$ - Moderately reactive
 $16 \geq \text{RC50} \leq 70$ - Slightly reactive
 $70.1 \geq \text{RC50} \leq 135$ - Suspect
 $\text{RC50} > 135$ - Not reactive

- Before enter in RA the user is asked to select In possible data inconsistency window a scale/unit. By default RC50 ratio scale is selected. Click **OK**.
- The Molecular weight descriptor as the most suitable for predicting skin sensitization effect is used in RA prediction
- RC50 values are presented in mmol/L
- Accept prediction**

Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (node 1c)

Example 1

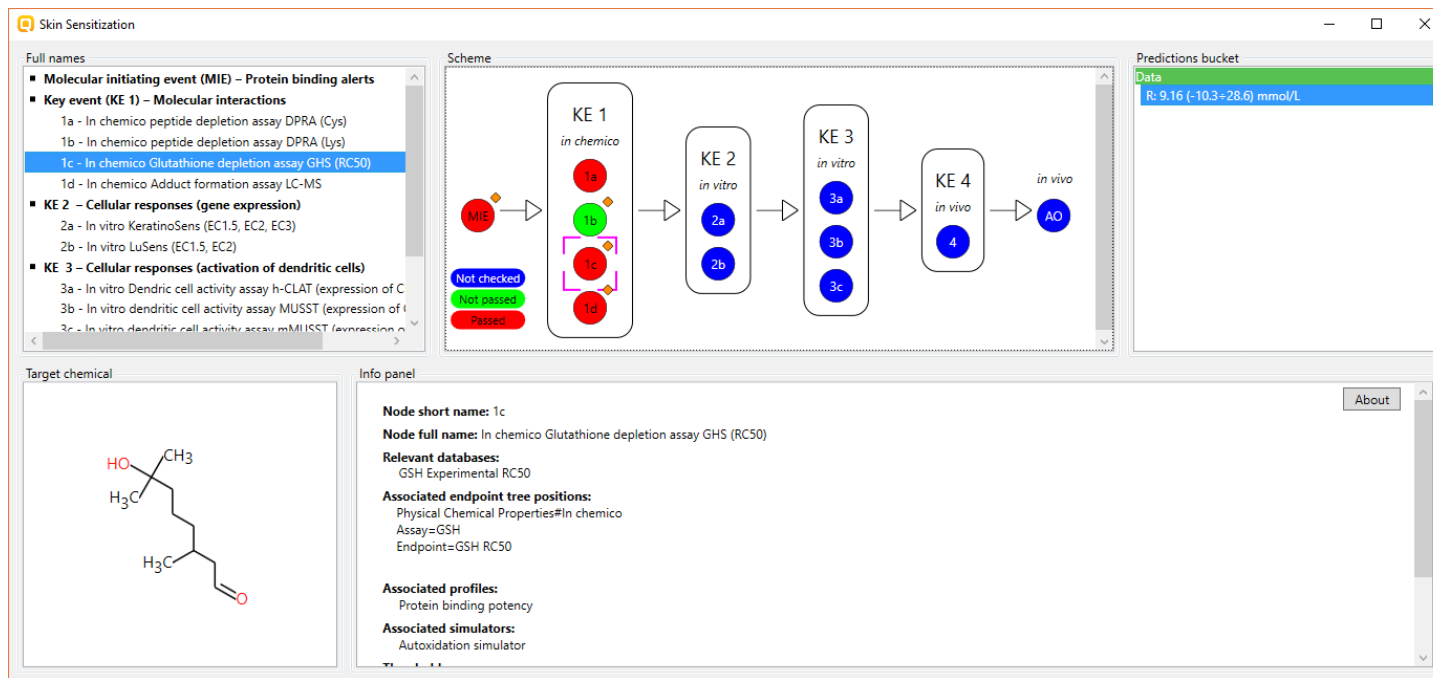


The next step is to use read-across prediction for AOP

1. The predicted value is automatically stored in the prediction bucket
2. The state of the node is also automatically changed to "Passed"

Workflow process *In chemico* assays

Example 1

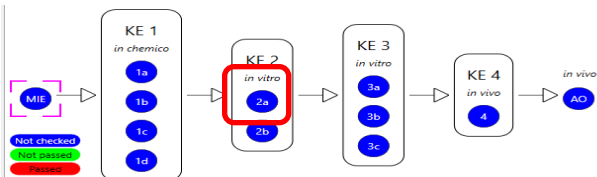


- The nodes related to three of the *in chemico* assays are passed due to positive experimental data for the target chemical (node 1a and 1d) and the positive experimental data found for analogues with an "Aldehyde" group(1c).
- Only one of all *in chemico related* nodes (node 1b) is assigned as "Not passed" due to negative experimental data for Lysine depletion
- The workflow should move further to the *in vitro* assay (nodes 2a and 2b)

Workflow process

Step 3. *In vitro* KeratinoSens (EC1.5, EC2, EC3) (node 2a)

Example 1



Skin Sensitization

Full names

- Molecular initiating event (MIE) – Protein binding alerts
- Key event (KE 1) – Molecular interactions
 - 1a - In chemico peptide depletion assay DPRA (Cys)
 - 1b - In chemico peptide depletion assay DPRA (Lys)
 - 1c - In chemico Glutathione depletion assay GHS (RC50)
 - 1d - In chemico Adduct formation assay LC-MS
- KE 2 – Cellular responses (gene expression)
 - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
 - 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 – Cellular responses (activation of dendritic cells)
 - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of C)
 - 3b - In vitro dendritic cell activity assay MUSST (expression of C)
 - 3c - In vitro dendritic cell activity assay mMUSST (expression of C)

Target chemical

Info panel

Node short name: 2a

Node full name: In vitro KeratinoSens (EC1.5, EC2, EC3)

Relevant databases:
Keratinocyte gene expression Givaudan

Associated endpoint tree positions:
Human health hazards#Sensitisation
Organ=Skin
Type of method=in Vitro
Assay=KeratinoSens
Endpoint=EC1.5

Human health hazards#Sensitisation
Organ=Skin
Type of method=in Vitro
Assay=KeratinoSens

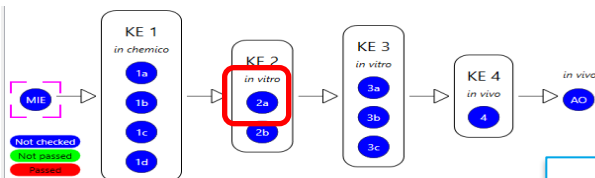
About

1. Select **node 2a**

Workflow process

Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 2a)

Example 1



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

Screenshot of the QSAR Toolbox software interface. The 'Data' tab is selected, showing a list of documents. The 'Filter endpoint tree...' panel is open, showing a tree structure of endpoints. The 'Data' tab is highlighted with a red box.

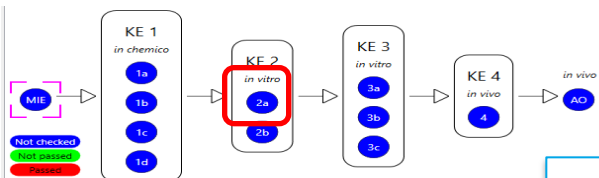
Screenshot of the 'Skin Sensitization' window. The 'Full names' tab is selected, showing a list of full names. The 'Scheme' tab is also visible, showing a diagram of the workflow process. The 'Scheme' tab is highlighted with a red box.

1. Go to level of CAS number from document tree
2. Go to **Data**
3. Unselect **all** databases
4. Select **highlighted** database
5. Click **Gather**
6. The experimental data appears on Data matrix
7. **Node 2a** has been changed to passed based on implemented thresholds (slide #15)

Workflow process

Step 3. *In vitro* LuSens (EC1.5, EC2) (node 2b)

Example 1



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

The screenshot shows the QSAR Toolbox software interface. The workflow process is displayed in the top right, with node 2b highlighted. The main window shows the 'Data' tab, where the 'Filter endpoint tree...' is visible. The 'Structure' tab shows the chemical structure of the target chemical. The 'Parameters' tab shows the 'Physical Chemical Properties' and 'Environmental Fate and Transport' sections. The 'Human Health Hazards' section is expanded, showing 'Acute Toxicity', 'Bioaccumulation', 'Carcinogenicity', 'Developmental Toxicity / Teratogenicity', 'Genetic Toxicity', 'Immunotoxicity', 'Irritation / Corrosion', 'Neurotoxicity', 'Photoinduced toxicity', 'Repeated Dose Toxicity', 'Sensitisation', and 'Skin'. The 'Skin' section is expanded, showing 'in Vitro' and 'LuSens'. The 'LuSens' section is expanded, showing 'EC1.5' and 'EC2'. The 'EC1.5' section is expanded, showing 'M: 189 µM' and 'M: 425 µM'. The 'EC2' section is expanded, showing 'M: 189 µM' and 'M: 425 µM'. The 'Data' tab shows the 'Data matrix' with the following data:

Target chemical	EC1.5	EC2
1 [target]	M: 189 µM	M: 425 µM

The 'Data' tab also shows the 'Predictions bucket' with the following data:

Target chemical	EC1.5	EC2
1 [target]	M: 189 µM	M: 425 µM

The 'Data' tab also shows the 'Options' panel with the following settings:

- Options: Select All, Unselect All, Invert, About
- Database: LuSens
- Endpoint: EC1.5
- Method: LuSens
- Endpoint: EC1.5

The 'Data' tab also shows the 'Inventories' panel with the following settings:

- Options: Select All, Unselect All, Invert, About
- Database: LuSens
- Endpoint: EC1.5
- Method: LuSens
- Endpoint: EC1.5

The 'Data' tab also shows the 'Inventories' panel with the following settings:

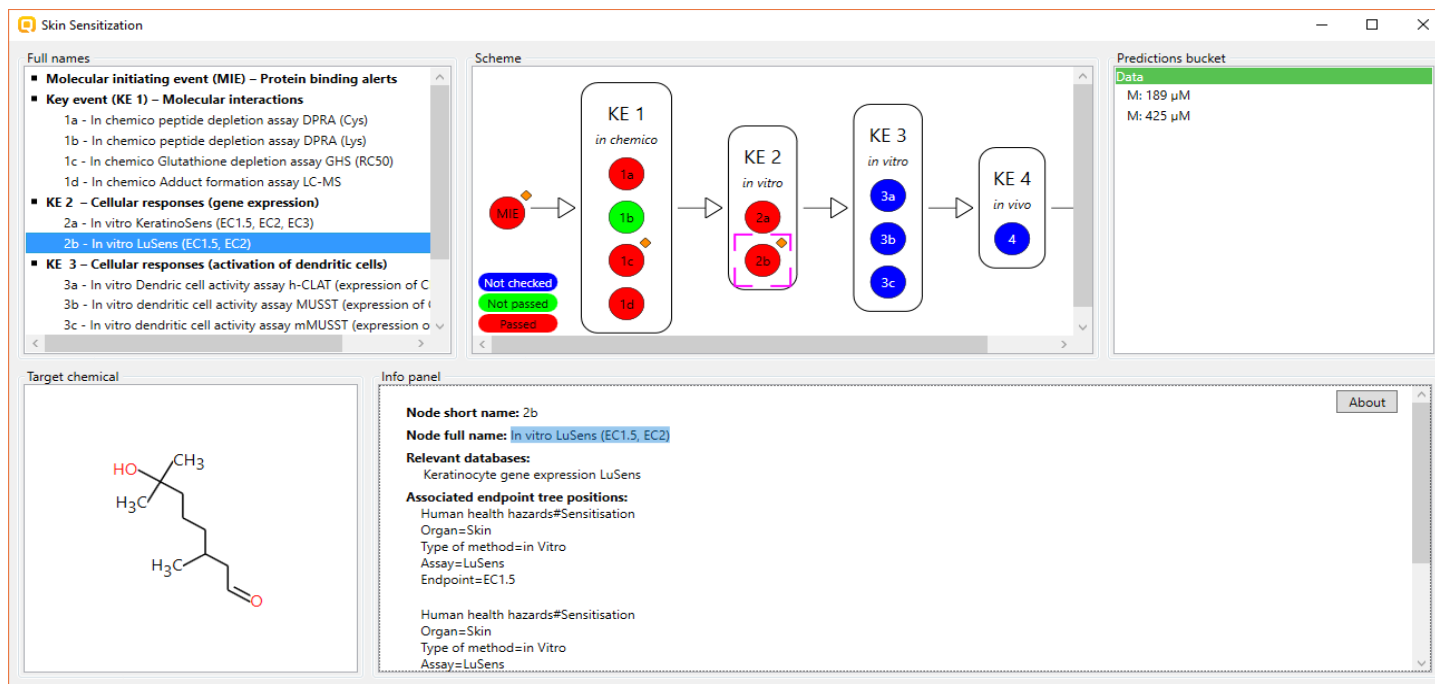
- Options: Select All, Unselect All, Invert, About
- Database: LuSens
- Endpoint: EC1.5
- Method: LuSens
- Endpoint: EC1.5

1. Select node 2b
2. Go to **Data**
3. Unselect **all** databases
4. Select **highlighted** database
5. Click **Gather**
6. The experimental data appears on Data matrix
7. Node 2b has been changed to passed based on implemented thresholds (slide #15)

Workflow process

Step 3. *in vitro* Keratinocyte ARE and *In vitro* LuSens (nodes 2a&2b)

Example 1

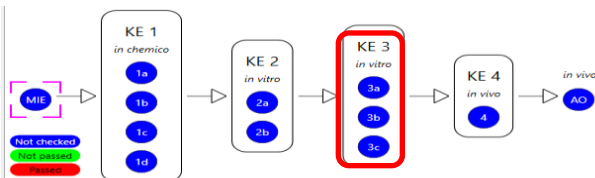


- The both nodes related to *in vitro* assays are passed due to positive experimental data found for the target chemical and implemented thresholds (slide #15)
- The workflow should move further to the other *in vitro* assays (nodes 3a, 3b and 3c)

Workflow process

Step 4. *In vitro* Dendritic cell activity assays h-CLAT, MUSST and mMUSST (nodes 3a, 3b and 3c)

Example 1



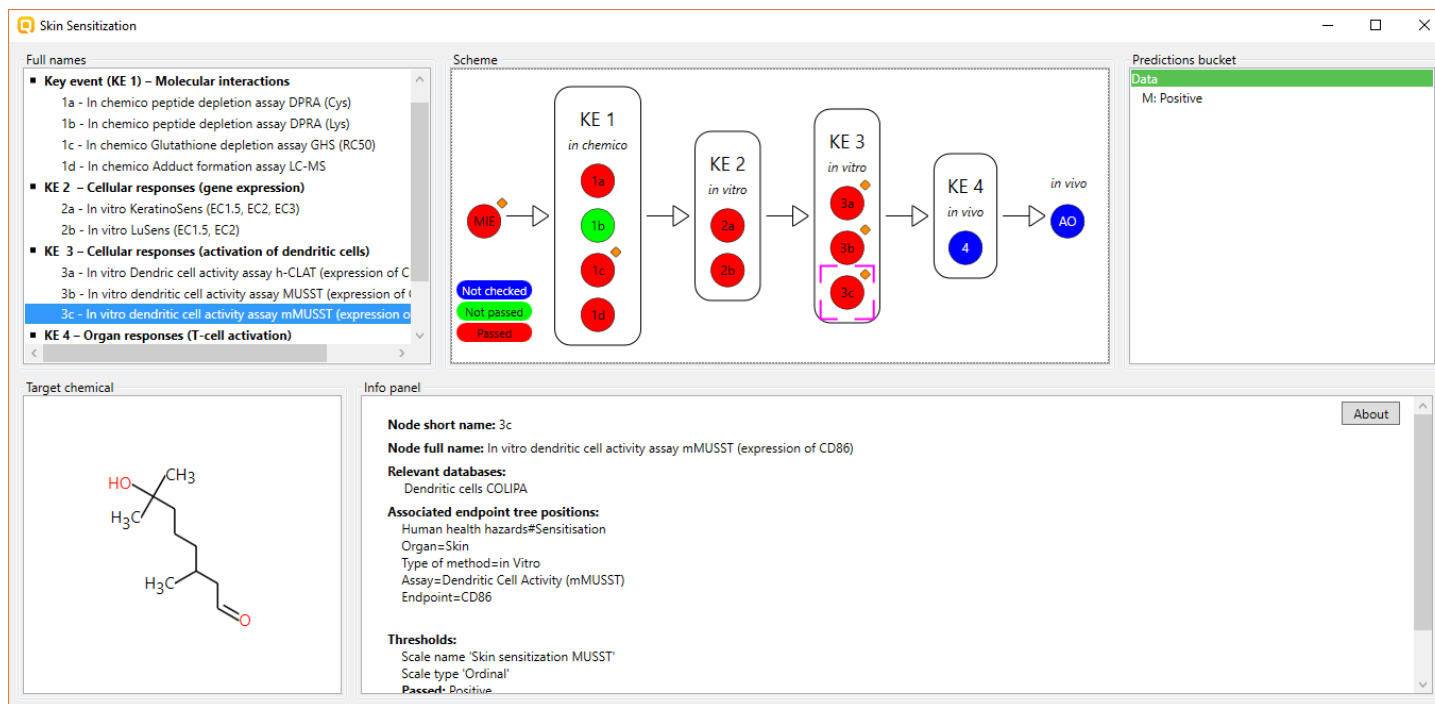
Check if there are any data for the target chemical for the *in vitro* Dendritic cell activity data

1. Select **node 3a**
2. Select database related to node 3a as before thata unselect all others
3. **Gather** data and click **OK** in the appeared message
4. The experimental data appears on Data matrix and the status of nodes 3a, 3b and 3c was changed to **Passed**

Workflow process

Step 4. *in vitro* Dendritic cell activity assay (nodes 3a, 3b and 3c)

Example 1

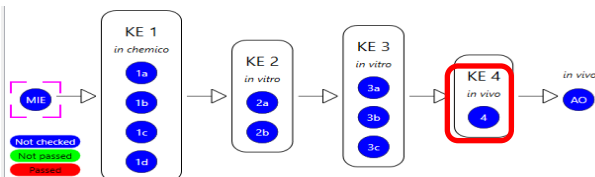


- The nodes 4a and 4b related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to positive experimental data found for the target chemical
- The workflow moves further to the *in vivo* LLNA assay (node 4)

Workflow process

Step 5. *In vivo* Organ response (LLNA)(node 4)

Example 1



Check are there any data for the target chemical for the *in vivo* Organ response (LLNA) (node 4)

The screenshot displays the QSAR Toolbox software interface. The main window shows the workflow process with nodes KE 1, KE 2, KE 3, KE 4, and AO. Node 4 is highlighted with a red box. The left sidebar shows the 'Documents' and 'Databases' panels. The 'Databases' panel is open, showing a list of databases with 'Skin sensitization ECETOC' selected. The 'Options' panel is also open, showing the 'Physical Chemical' and 'Human Health Hazards' sections. The 'Databases' panel is open, showing a list of databases with 'Skin sensitization ECETOC' selected. The 'Options' panel is also open, showing the 'Physical Chemical' and 'Human Health Hazards' sections. The 'Databases' panel is open, showing a list of databases with 'Skin sensitization ECETOC' selected. The 'Options' panel is also open, showing the 'Physical Chemical' and 'Human Health Hazards' sections.

1. Select **node 4**

2. Select database related to the node 4, as before that unselect all others

3. Gather data and click **OK** in the appeared message

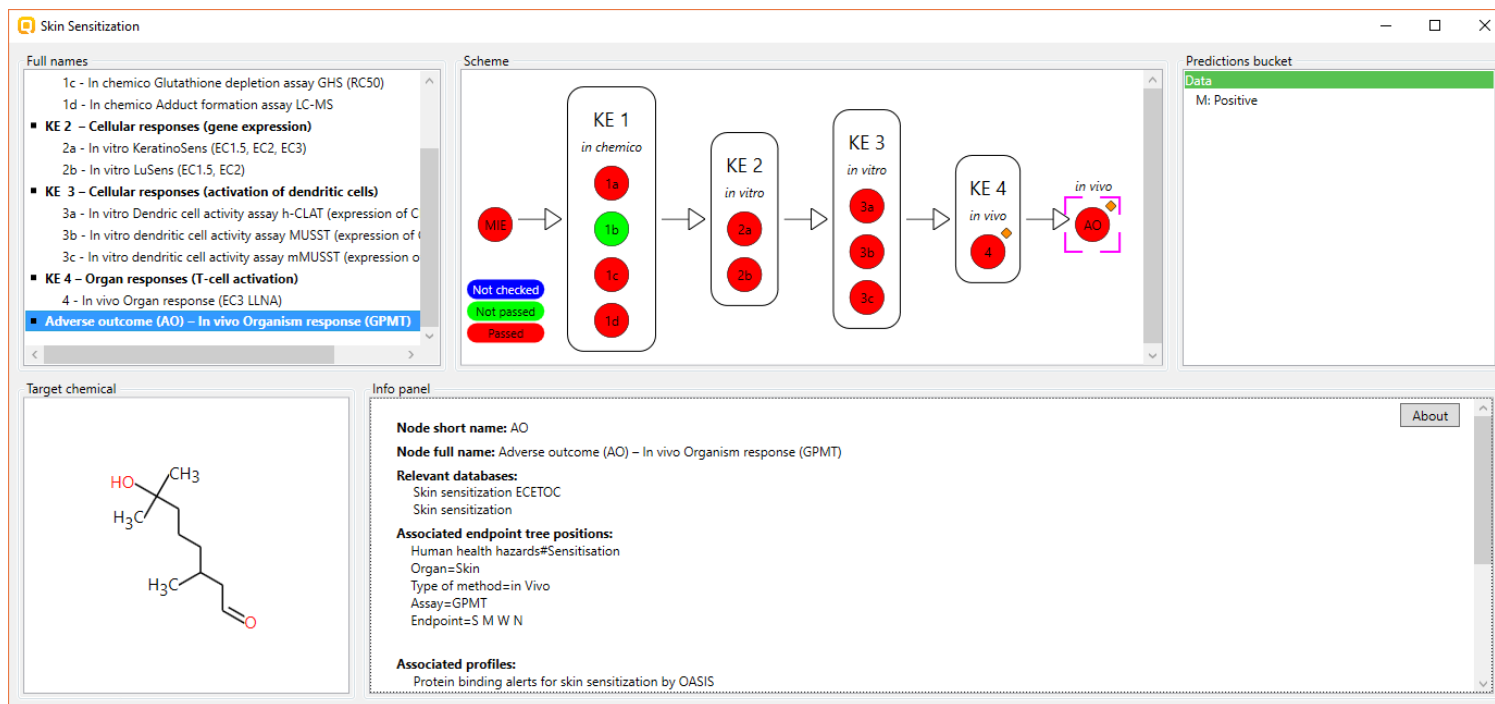
4. The data appears in the datamatrix

5. The **node 4** and **node Adverse outcome (AO)** are automatically changed to **passed**, based on experimental data for the target chemical and the implemented thresholds (see slide #15)

Workflow process

Step 5. *in vivo* Organ and Organism assays (node 4 and AO)

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

Search by CAS

1 2 3

97530 Search OK Cancel

Select All Unselect All Invert Selection Selected 1 of 1

1	CAS	97-53-0
	SMILES	COc1cc(CC=C)ccc1O
	CS Relation	High
	Substance	Mono constituent
	Composition	
	Name	1-ALLYL-3-METHOXY-4-HYDROXY-2-PROPENYL... 1-allyl-3-methoxy-4-hydroxy-2-propenyl... 2-methoxy-4-(2-propenyl)p...

1. Create new document and Enter the **CAS# 97-53-0** In the blank field; 2. Click **Search button**;
3. Press **OK**

Chemical Input

Target chemical identity

- Double click “CAS Smiles relation” displays the chemical identification information.
- 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 software interface. The top menu bar includes 'Data', 'Import', and 'Export'. Below it, there are icons for 'Gather', 'Import', 'IUCLID', and 'IUCLID6'. The main workspace is divided into several panels. On the left, there are 'Documents' and 'Databases' panels. The 'Documents' panel shows a list of documents, with 'Chemical names containing 'trans-2,cis-6-nonadi'' selected. The 'Databases' panel shows a list of databases, with 'ToxCastDB' selected. The 'Inventories' panel shows a list of inventories, with 'Canada DSL' selected. The 'Filter endpoint tree...' dialog is open in the center, showing a tree structure with 'Structure' selected. A red box highlights the 'High' value in the 'Structure' column. A callout bubble with the number '1' points to this box. To the right of the dialog, a 'Relationships CAS-SMILES' window is open, showing a table with columns: 'Exist in data source', 'Data source type', 'Data source quality', and 'Assigned SMILES in data source'. A callout bubble with the number '2' points to this window. The table contains data for various data sources, including Canada DSL, DSSTOX, ECHA PR, EINECS, NICNAS, REACH ECB, Skin Sensitization, and TSCA. The 'Assigned SMILES in data source' column shows 'yes' for most sources and 'no' for Canada DSL. The 'Relationships CAS-SMILES' window has an 'OK' button at the bottom right.

1. Double **Click** 2. Relationships CAS-SMILES

Chemical Input

Target chemical identity

The code indicates the reliability of the chemical identifier:

- **High:** This reliability corresponds to high reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to at least one high quality data source (database or inventory)
- **Moderate:** This reliability corresponds to moderate reliability of CAS-SMILES relation. The moderate label is assigned if the chemical belongs to three "Distribute to QA" data sources.
- **Low:** This reliability corresponds to poor reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to less than three, but at least one "Distribute to QA" data sources.

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

QSAR Toolbox 4.1 [Document 10]

The screenshot displays the QSAR Toolbox 4.1 interface with several callouts indicating the steps to activate AOP and set a target chemical:

- 1**: Points to the 'Documents' list on the left sidebar, specifically to 'Document 10' with CAS: 97530.
- 2**: Points to the 'Human Health Hazards' section in the 'Filter endpoint tree...' panel, specifically to the 'AOP' label.
- 3**: Points to the 'Activate AOP' option in the context menu that appears when right-clicking on the 'AOP' label.
- 4**: Points to the 'Structure' field in the 'Filter endpoint tree...' panel, where a target chemical structure is being set.

On the right side of the interface, a 'Skin Sensitization' window is open, showing a 'Scheme' diagram with key events (KE 1 to KE 4) and a 'Target chemical' section displaying the chemical structure of 4-allylphenol (C=CC1=CC=C(C=C1)O).

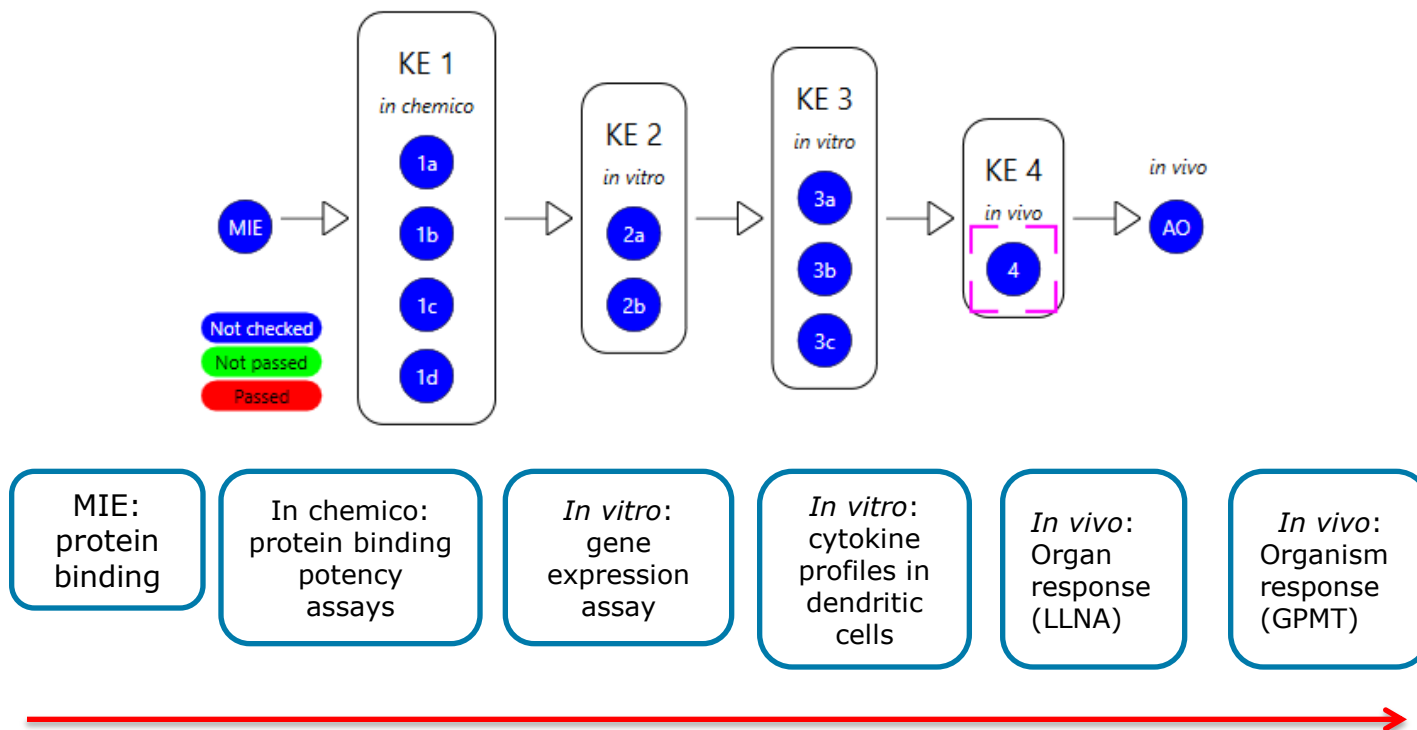
1. Expand **Human health hazard** part of the endpoint
2. Right click near the **AOP** label
3. Select **Activate AOP**
4. Set target for AOP (see slide 26)

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

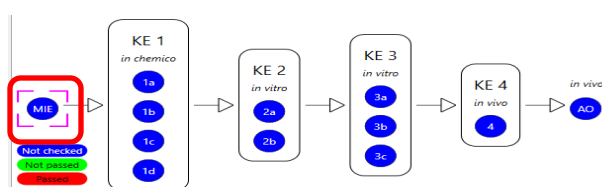
- Workflow process start from molecular initiating event to the *in vivo* organism respond



Workflow process

Step 1. MIE: protein binding

Example 2



Start with profiling of target chemical

1. Open **Profiling**
2. Select node #**MIE**
3. Relevant profilers are highlighted, **select** the profilers
4. **Apply** selected profilers. The node is automatically changed to not passed based on absence of alert. 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

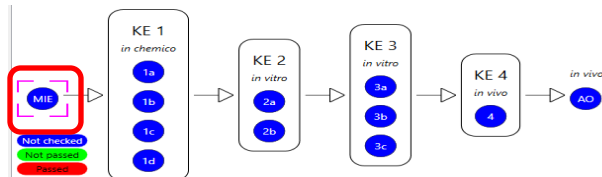
5 The profiling results are also stored at the prediction bucket

The QSAR Toolbox interface displays a workflow for simulating autoxidation products. The top navigation bar includes sections for Input, Profiling, Data, and Category definition. The left sidebar shows a list of documents and a 'Profiling methods' panel where various profilers are selected. The central workspace features a 'Filter endpoint tree' on the left and a 'Scheme' diagram on the right. The 'Scheme' diagram illustrates a workflow starting with a 'MIE' (Molecular Initiating Event) node, followed by four key events (KE 1 to KE 4) and an 'AO' (Adverse Outcome) node. The 'MIE' node is highlighted with a red box. The 'Scheme' diagram also shows a 'Predictions bucket' on the right, which lists the results of the simulation. The 'Predictions bucket' includes a list of 'Predictions' and a 'Predictions bucket' section. The 'Predictions' section lists various alerts, such as 'Protein binding by OASIS', 'Protein binding by OECD', and 'Protein binding alerts for skin sensitization according to OECD'. The 'Predictions bucket' section lists the results of the simulation, including '1 x Michael addition', '1 x Michael addition >> Michael addition on con', '1 x Michael addition >> Michael addition on con', '1 x Michael addition >> Michael addition on qui', '1 x Michael addition >> Michael addition on qui', '1 x Radical reactions', '1 x Radical reactions >> Free radical formation', '1 x Radical reactions >> Free radical formation >', '2 x No alert found', '1 x SN2', and '1 x SN2 >> Ring opening SN2 reaction'.

Workflow process

Step 1. MIE: protein binding

Example 2



Simulate Autoxidation products of the target chemical – manually change the node status

1

2

3

Target chemical: CC(=O)Oc1ccc(C=C)cc1

Info panel:

Node short name: MIE

Node full name: Molecular initiating event (MIE) – Protein binding alerts

Associated profiles:

- Protein binding by OASIS
- Protein binding alerts for skin sensitization by OASIS
- Protein binding alerts for skin sensitization according to GHS
- Protein binding by OECD

Associated simulators:

- Autoxidation simulator
- Skin metabolism simulator

Thresholds:

- Profile: 'Protein binding alerts for skin sensitization by OASIS'
- Passed: Any of the profiler categories
- Not passed: No alert found

Target chemical: CC(=O)Oc1ccc(C=C)cc1

Info panel:

Node short name: MIE

Node full name: Molecular initiating event (MIE) – Protein binding alerts

Associated profiles:

- Protein binding by OASIS
- Protein binding alerts for skin sensitization by OASIS
- Protein binding alerts for skin sensitization according to GHS
- Protein binding by OECD

Associated simulators:

- Autoxidation simulator
- Skin metabolism simulator

Thresholds:

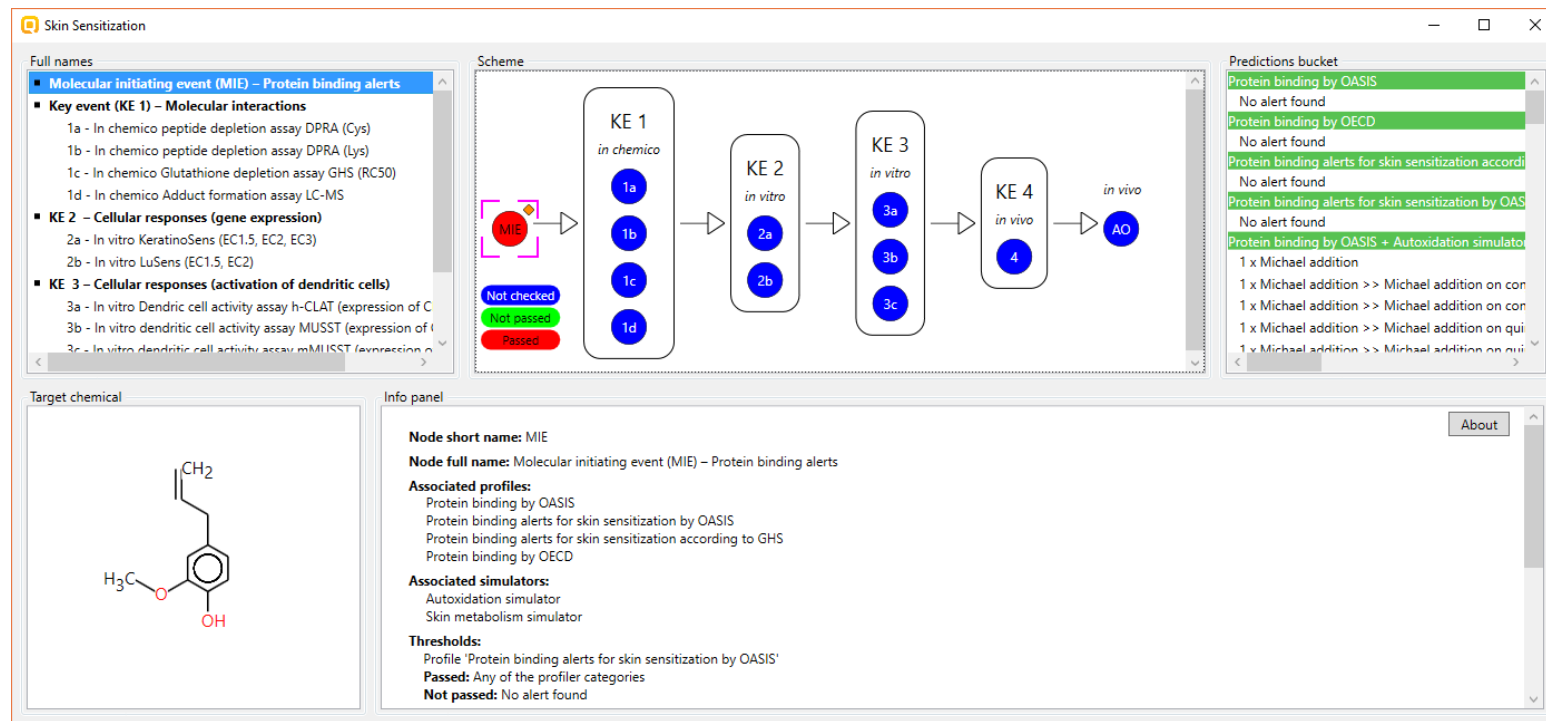
- Profile: 'Protein binding alerts for skin sensitization by OASIS'
- Passed: Any of the profiler categories
- Not passed: No alert found

1. **Right** click on the **MIE** node
2. Select **Set state**
3. Change the state from **Not Passed** to **Passed**

Workflow process

Molecular initiating events

Example 2

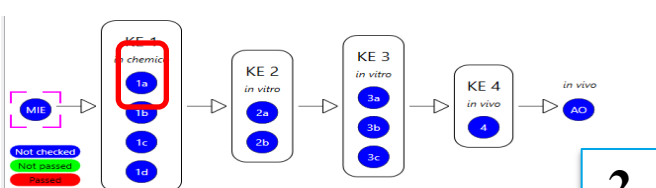


- The node MIE is passed due to the presence of positive protein binding alert identified for the Autoxidation products of the target chemical
- The workflow should move further to the *in chemico* assays

Workflow process

Step2. *In chemico* peptide depletion assay DPRA (Cys) (node 1a)

Example 2



Check for experimental data

QSAR TOOLBOX interface showing the 'Data' tab. The 'Filter endpoint tree...' panel is open, displaying a list of endpoints. The 'Physical Chemical Properties' section is expanded, showing endpoints like 'Autofluorescence / Self-ignition temperature', 'Boiling point', 'Density', 'Dissociation Constant (pKa)', 'Explosive properties', 'Flammability', 'Flash point', 'LC-MS', 'Lipid Solubility', 'Melting / freezing point', 'Oxidation reduction potential', 'Oxidising properties', and 'Particle size'. The 'In Chemico' section is also expanded, showing endpoints like 'DPRA', '% depletion of Cysteine', '% depletion of Lysine', and 'LC-MS'. The 'DPRA' endpoint is highlighted with a red dashed box, and its associated data is shown in a table:

Endpoint	Value	Unit
% depletion of Cysteine	(1/2)	M: 38.3 %
% depletion of Lysine	(1/2)	M: 19.2 %
LC-MS	(1/1)	M: 52.8 %

Diagram illustrating the AOP (Adverse Outcome Pathway) process. The sequence starts with MIE (Molecular Initiating Event), followed by KE 1 (Key Event 1) which includes nodes 1a, 1b, 1c, and 1d. This is followed by KE 2 (in vitro), KE 3 (in vitro), KE 4 (in vivo), and finally the AO (Adverse Outcome). Node 1a is highlighted with a red box and a callout '2' pointing to it.

1. Select node 1a

2. Go to **Data**

3. **Select** highlighted database

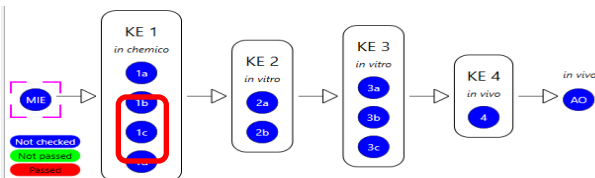
4. **Click** Gather

5. The selected database (Chemical reactivity COLIPA) is related to three AOP nodes - 1a, 1b and 1d. Data for the target for all 3 endpoints are found and the three nodes are marked as Passed (based on the implemented thresholds, see slide 15)

Workflow process

Step2. *In chemico* Glutathione depletion assay GHS (RC50) (node 1c)

Example 2



Check are there any data for the target chemical

1. Select node 1c

2. Select highlighted database (unselect all the rest)

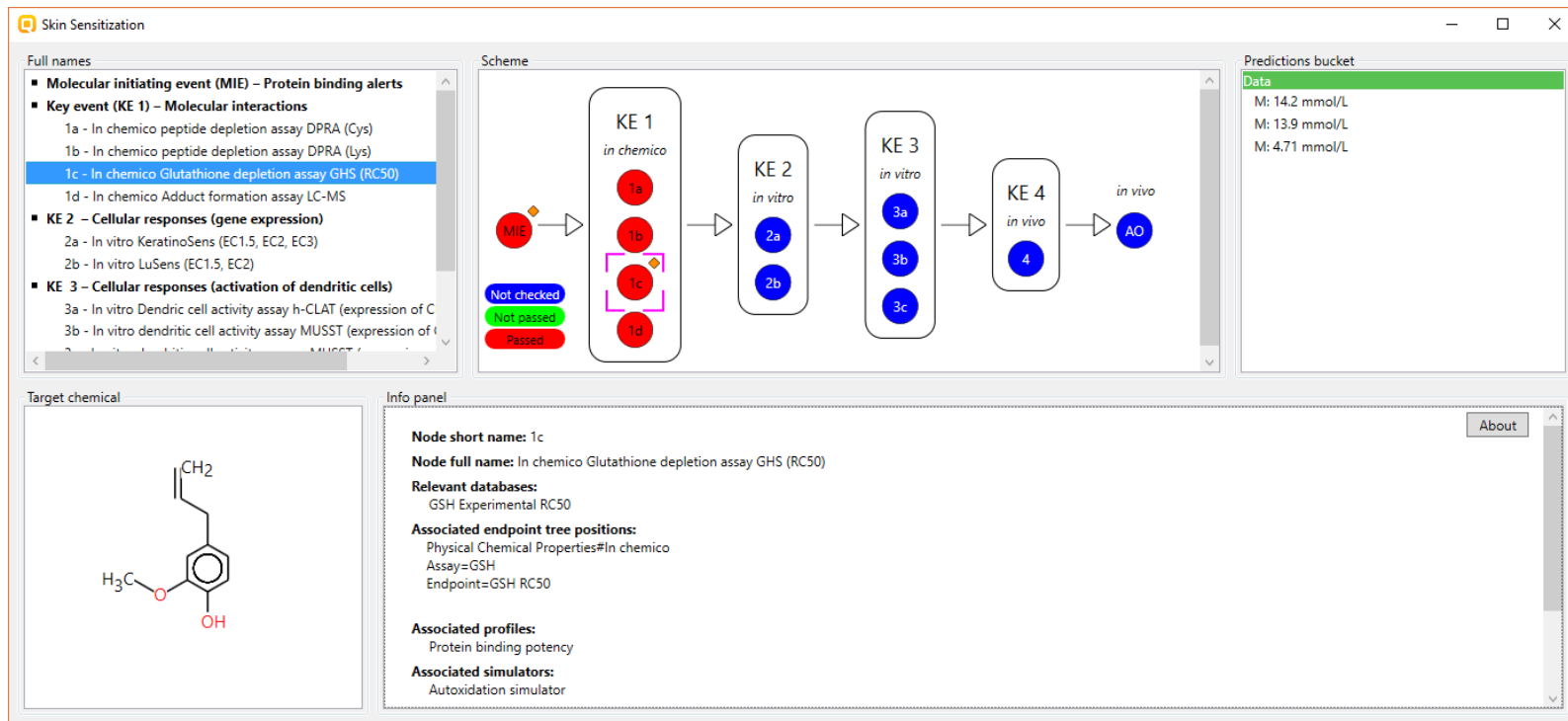
3. Click Gather

4. Node 1c is getting passed based on the thresholds for experimental data

Workflow process

In chemico assays

Example 2

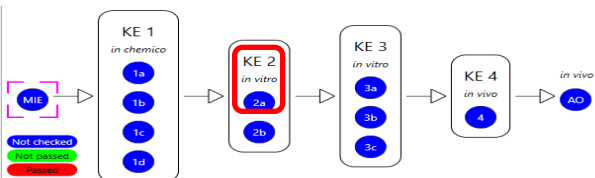


- The nodes related to the *in chemico* assays are passed due to positive experimental data for the target chemical (node 2a, 2b, 2c and 2d) The workflow should move further to the *in vitro* assay (node 2a and 2b)

Workflow process

Step 3. *In vitro* KeratinoSens (EC1.5, EC2, EC3) (node 2a)

Example 2



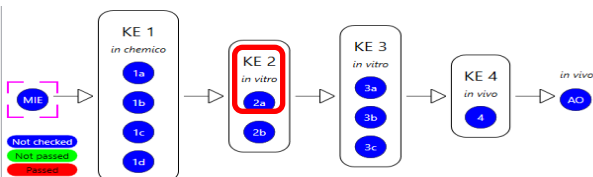
Check are there experimental data for the parent chemical for node 2a

1. Select node 2a
2. Select highlighted database, unselect all other
3. Click Gather
4. There is experimental data for the parent chemical, which appears on data matrix. Node 2a is getting "Not Passed" based on the experimental data and implemented thresholds (see slide #15)

Workflow process

Step 3. *In vitro* LuSens (EC1.5, EC2) (node 2b)

Example 2



Check are there experimental data for the parent chemical for node 2b

The screenshot displays the ToxPi software interface with several key components and annotations:

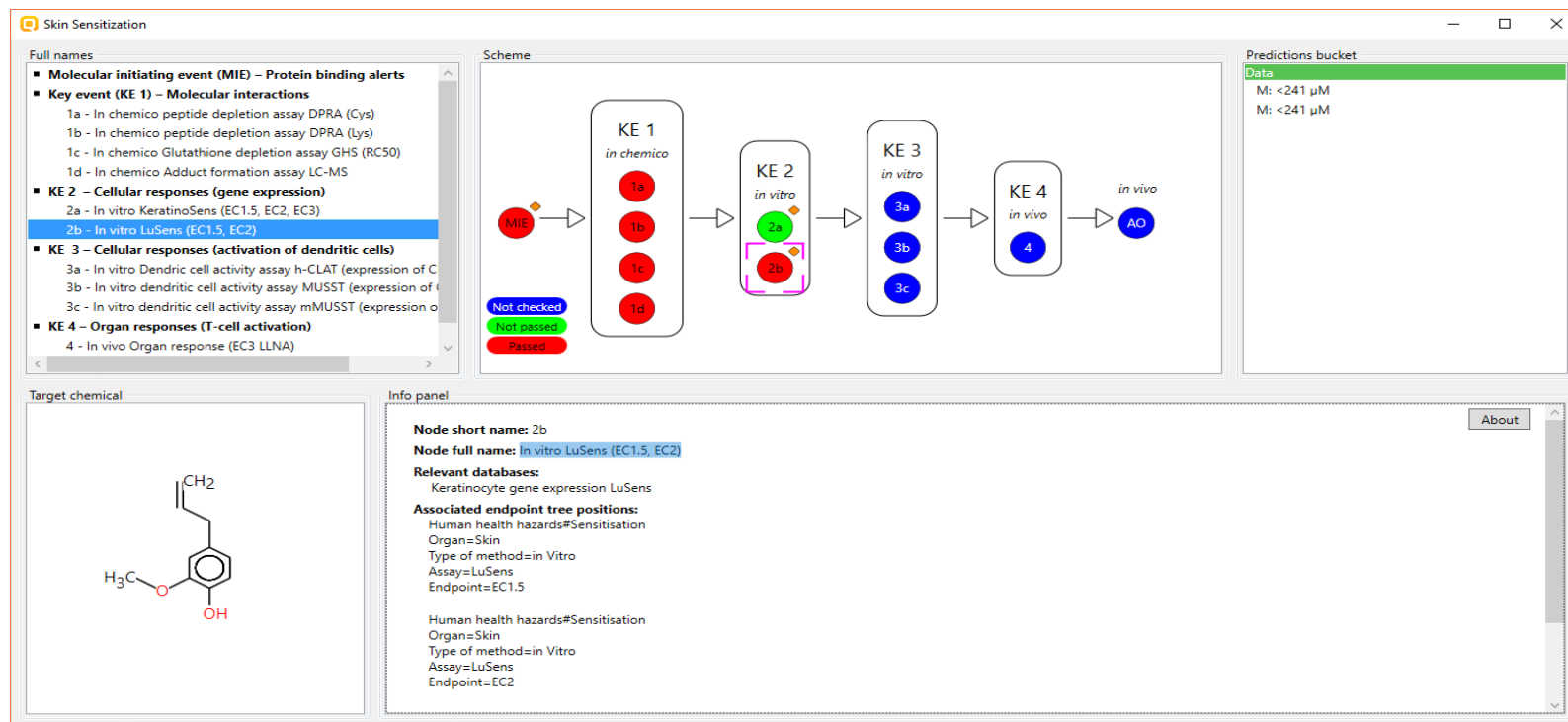
- Top Bar:** Contains icons for Input, Profiling, Data, and Category definition.
- Left Panel:**
 - Documents:** Lists several documents, with "Document 3" (CAS: 97530) highlighted.
 - Databases:** Shows a list of databases under "Human Health Hazards", with "Keratinocyte gene expression LuSens" selected.
 - Inventories:** Displays a list of inventories, with "Keratinocyte gene expression LuSens" selected.
- Central Panel:**
 - Filter endpoint tree...**: A tree view showing the hierarchy of endpoints. The "Skin" category is expanded, and "in Vitro" is selected.
 - Structure**: Shows the chemical structure of the target chemical, 4-methoxybenzoic acid.
 - Target chemical**: Displays the chemical structure and name.
- Right Panel:**
 - Key event (KE) diagram**: A flowchart showing the progression from KE 1 (in vitro) to KE 2 (in vitro) to KE 3 (in vitro) to KE 4 (in vivo). Node 2b is highlighted in green.
 - Info panel**: Provides details for the selected node (Node short name: 2b, Node full name: in vitro LuSens (EC1.5, EC2)).
- Annotations:**
 - 1**: Points to the "Keratinocyte gene expression LuSens" database in the Databases list.
 - 2**: Points to the "Unselect All" button in the Databases panel.
 - 3**: Points to the "Gather" button in the top bar.
 - 4**: Points to the "Skin" category in the Filter endpoint tree.

1. Select **node 2b**
2. Unselect all and select highlighted database
3. Click **Gather**
4. There is experimental data for the parent chemical, which appears on data matrix. Node 2b is getting "Passed" based on the experimental data and implemented threshold (see slide #15)

Workflow process

Step 3. *in vitro* Keratinocyte ARE and *In vitro* LuSens

Example 2

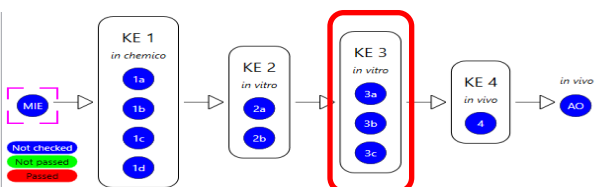


- The nodes 2a and 2b related to the Keratinocyte ARE (EC1.5, EC2, EC3) is passed based on the experimental data found for the target chemical (threshold are specified on slide # 15).
- The workflow moves further to the *in vitro* Dendritic cell assay (nodes 3a, 3b and 3c)

Workflow process

Step 4. *in vitro* Dendritic cell activity assay (nodes 3a, 3b and 3c)

Example 2



Check if there are any data for the target chemical for the *in vitro* Dendritic cell activity data

3

QSAR TOOLBOX

Input Profiling Data Category definition Data

Documents

- Document 1
- Document 2
- Document 3
- Document 4
- Document 5
- Document 6

Databases

Options

- Physical Properties
- Genotoxicity
- Human health Hazards
- Dendritic cells COLIPA
- Keratinocyte gene expression Givaudan
- Keratinocyte gene expression LuSens
- REACH Skin sensitisation database (norm)
- Skin Sensitization
- Skin sensitization ECETOC

Inventories

Options

- Canada DSL
- COSING
- DSSTOX
- ECETOC

Filter endpoint tree...

Structure

1 [target]

Structure info

Parameters

Physical Chemical Properties

Environmental Fate and Transport

Ecotoxicological Information

Human Health Hazards

- Acute Toxicity
- Bioaccumulation
- Carcinogenicity
- Developmental Toxicity / Teratogenicity
- Genetic Toxicity
- Immunotoxicity
- Irritation / Corrosion
- Neurotoxicity
- Photoinduced toxicity
- Repeated Dose Toxicity
- Sensitization
- Skin
- in Vitro
- Dendritic Cell Activity (h-CLAT)
- CD54
- CD86
- Dendritic Cell Activity (mMUSST)
- CD86
- Dendritic Cell Activity (MUSST)
- CD86

1 (1/2) M: Positive

1 (1/2) M: Positive

1 (1/1) M: Positive

1 (1/1) M: Positive

1

Skin Sensitization

Full names

- Molecular Initiating event (MIE) - Protein binding alerts
- Key event (KE 1) - Molecular interactions
- 1a - In chemico peptide depletion assay DPRA (Cys)
- 1b - In chemico peptide depletion assay DPRA (Lys)
- 1c - In chemico Glutathione depletion assay GHS (RC50)
- 1d - In chemico Adduct formation assay LC-MS
- KE 2 - Cellular responses (gene expression)
- 2a - In vitro KeratinSens (EC1.5, EC2, EC3)
- 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 - Cellular responses (activation of dendritic cells)
- 3a - In vitro Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
- 3b - In vitro dendritic cell activity assay MUSST (expression of CD86)

Scheme

KE 1 in chemico

KE 2 in vitro

KE 3 in vitro

KE 4 in vivo

in vivo

Predictions bucket

Data

M: Positive

M: Positive

M: Positive

M: Positive

Target chemical

Info panel

Node short name: 3a

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

Organs#Skin

Type of methods#in Vitro

Assay=Dendritic Cell Activity (h-CLAT)

Endpoints#CD54

Human health hazards#Sensitisation

Organs#Skin

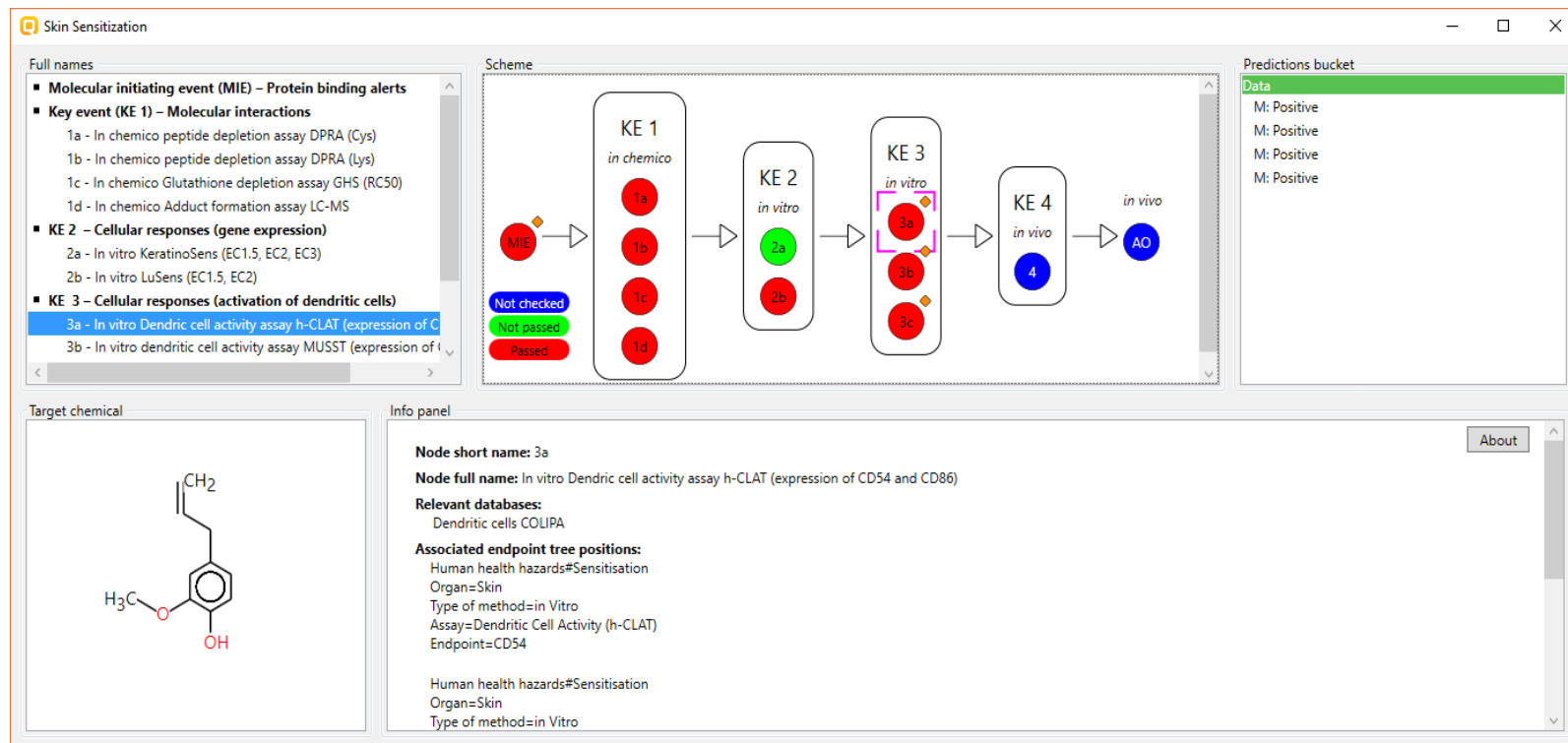
Type of methods#in Vitro

1. Select **node 3a**
2. Select database related to node 3a
3. **Gather** data and click **OK** in the appeared message
4. The experimental data appears on Data matrix and the status of nodes 3a, 3b and 3c was changed to **Passed**

Workflow process

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

Example 2



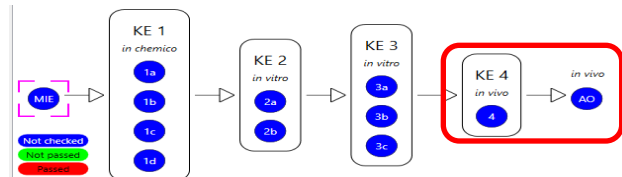
- The node 3a, 3b and 3c related to the *in vitro* Dendritic cell activity assays (h-CLAT, MUSST and mMUSST) are getting passed due to positive experimental data found for the target chemical
- The workflow could further move to the *in vivo* LLNA assay (nodes 4)

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 4)



Documents

- Document 1
- Document 2
- Document 3
- Document 4
- Document 5
- Document 6

Databases

- Chemical Properties
- Physical Chemical Properties
- Environmental Fate and Transport
- Ecotoxicological Information
- Human Health Hazards
- Skin Sensitization
- Skin sensitization ECETOC

Filter endpoint tree...

- Structure
- Parameters
- Physical Chemical Properties
- Environmental Fate and Transport
- Ecotoxicological Information
- Human Health Hazards
- Skin Sensitization
- Skin
- in Vitro
- in Vivo
- GPMT
- HRPT
- LLNA
- Undefined Assay

Assays

Assay	Count	Result
Respiratory Tract	(1/1)	M: Negative
Skin	(1/6)	M: Positive
in Vitro	(1/1)	M: Positive
in Vivo	(1/3)	M: 8E+03 µg/cm2
GPMT	(1/2)	M: Positive
HRPT	(1/1)	M: Positive
LLNA	(1/1)	M: Positive
Undefined Assay	(1/1)	M: Positive

Full names

- 1b - In chemico peptide depletion assay DPRA (Lys)
- 1c - In chemico Glutathione depletion assay GHS (RC50)
- 1d - In chemico Adduct formation assay LC-MS
- KE 2 - Cellular responses (gene expression)
- 2a - In vitro Keratinocyte gene expression EC1.5, EC2, EC3
- 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 - Cellular responses (activation of dendritic cells)
- 3a - In vitro Dendritic cell activity assay h-CLAT (expression of C)
- 3b - In vitro dendritic cell activity assay mMUST (expression of C)
- 3c - In vitro dendritic cell activity assay mMUST (expression of C)
- KE 4 - Organ responses (T-cell activation)
- 4 - In vivo Organ response (EC3 LLNA)

Target chemical

Chemical structure: COc1ccc(cc1)C(=O)O

Info panel

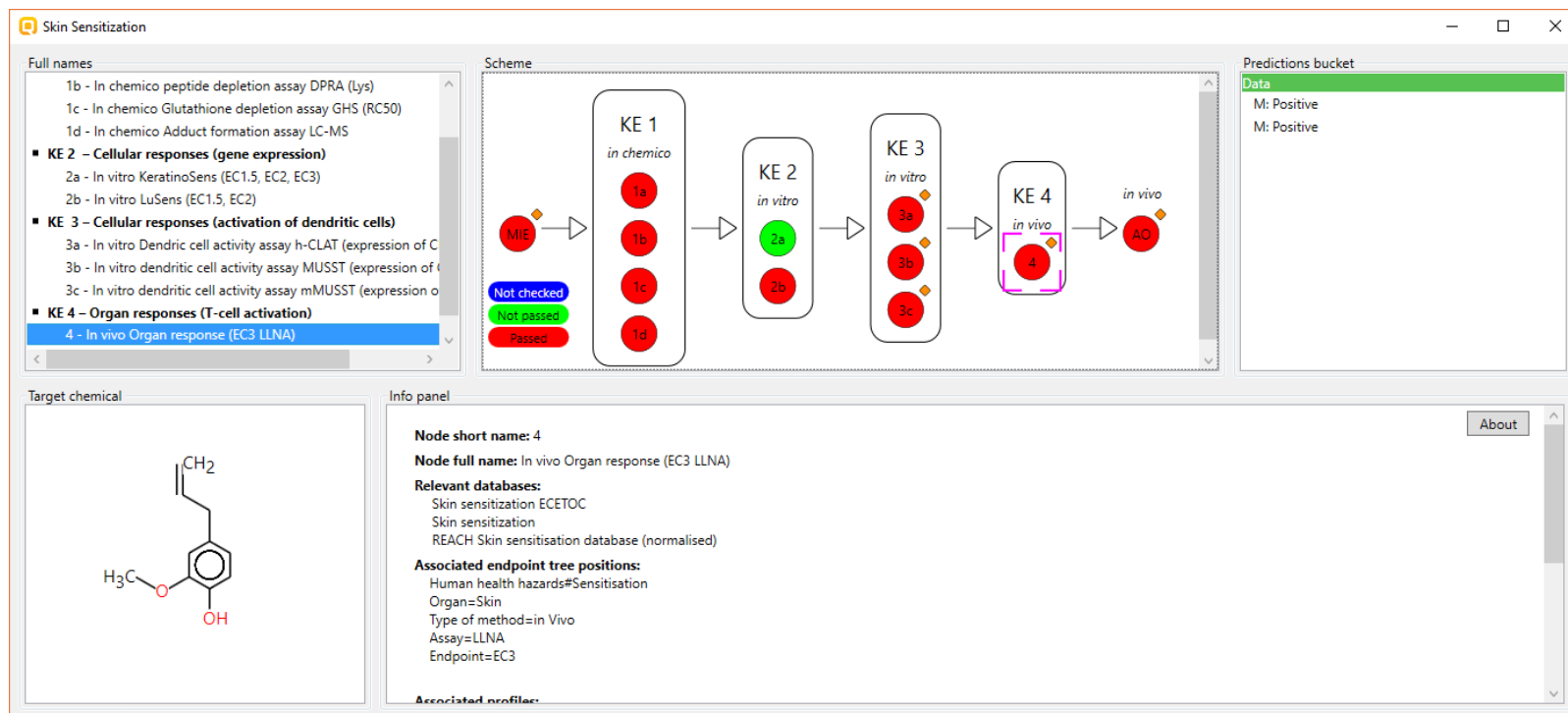
Node short name: 4
Node full name: In vivo Organ response (EC3 LLNA)
Relevant databases: Skin sensitization ECETOC, Skin sensitization, REACH Skin sensitisation database (normalised)
Associated endpoint tree positions: Human health hazards#Sensitisation, Organ=Skin, Type of method=In Vivo, Assay=LLNA, Endpoints=EC3

1. Select node 4
2. Select database related to the node 4
3. Click Gather
4. Nodes 4 and AO is getting passed based on experimental data extracted for the target chemical

Workflow process

Step 5. *in vivo* Organ and Organism assays (node 4 and AO)

Example 2



- 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.