QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

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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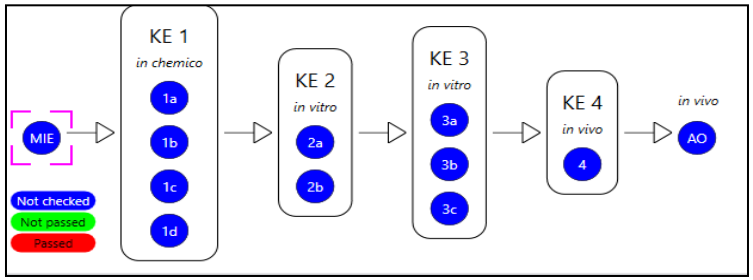
Overview of AOP scheme



Key node

Key event

	MIE	Protein binding alerts	Protein binding – in silico/theoretical
In chemico	KE1	1a Peptide depletion assay DPRA (Cys)	Protein binding potency in chemico
		1b Peptide depletion assay DPRA (Lys)	
		1c Glutathione depletion assay GSH (RC50)	
		1d Adduct formation assay LC-MS	
In vitro	KE2	2a KeratinoSens assay(EC1.5, EC2, EC3)	Cellular response (gene expression)
		2b LuSens (EC1.5, EC2, EC3)	
	KE3	3a Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)	Cellular response (activation of dendritic cells)
		3b Dendritic cell activity assay MUSST (expression of CD86)	
		3c Dendritic cell activity assay mMUSST (expression of CD86)	
In vivo	KE4	4 Organ response (EC3 LLNA)	Organ response
	AO	Organism response (GPMT)	Organism response

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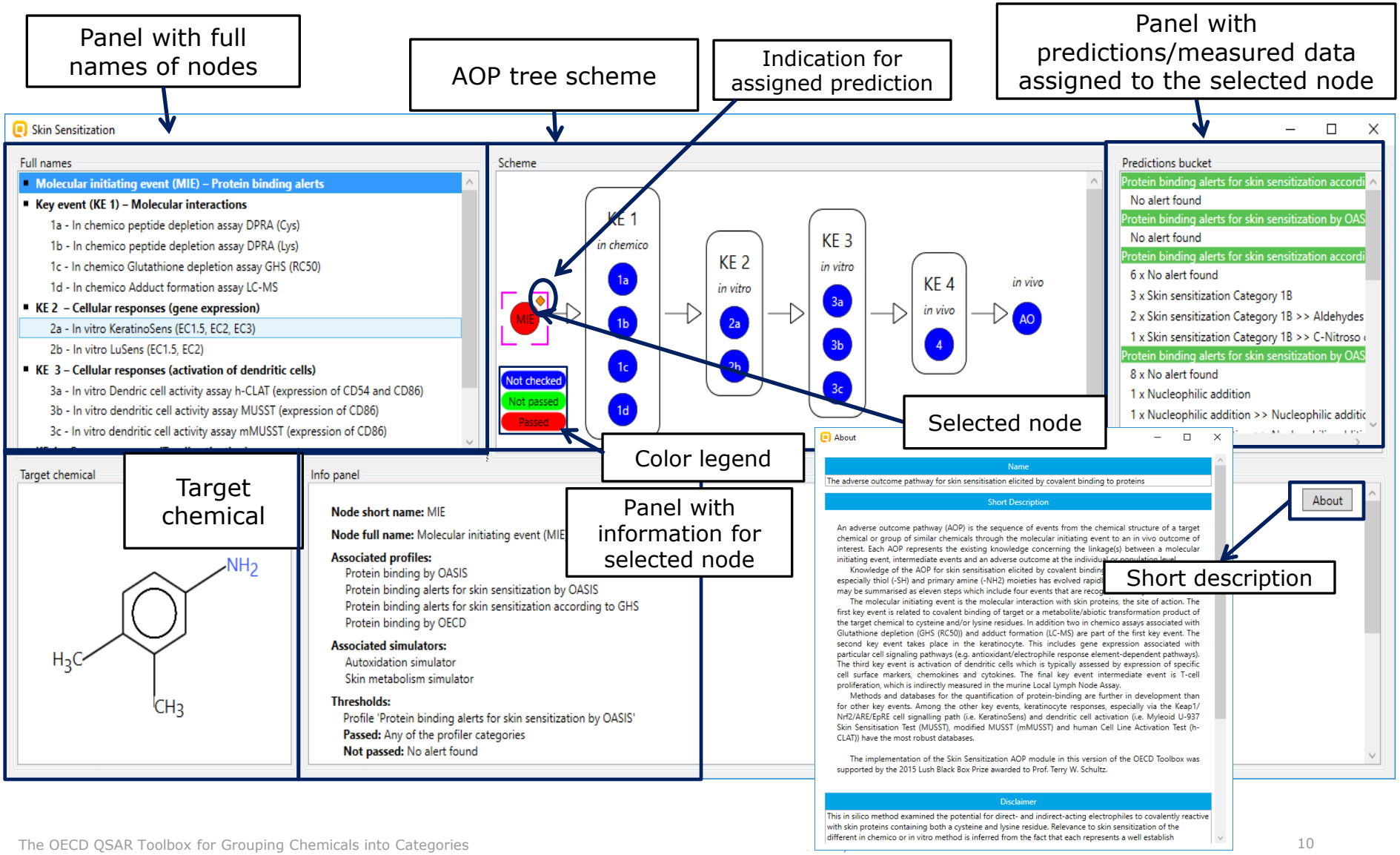
- **Overview of AOP scheme as implemented in the Toolbox**
  - **Details of AOP window**
  - AOP workflow for skin sensitization
  - Thresholds of the node of AOP
- The exercise

Overview of the AOP scheme as implemented in Toolbox

Details of AOP window



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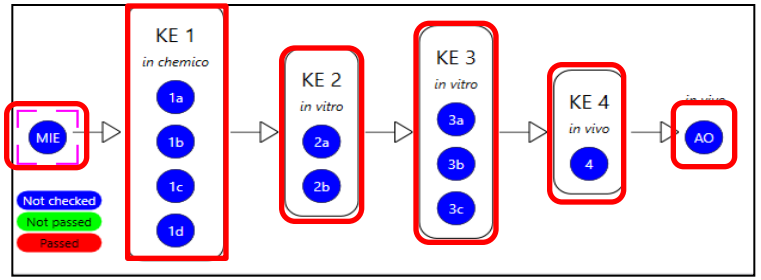
Overview of the AOP scheme as implemented in Toolbox

AOP workflow for skin sensitization

AOP: skin sensitisation



MIE: protein binding

In chemico: protein binding potency
- Peptide depletion assay DPRA (Cys)
- Peptide depletion assay DPRA (Lys)
- Glutathione depletion assay GSH (RC50)
- Adduct formation assay LC-MS

In vitro: gene expression in keratinocytes and Lusens

In vitro: cytokine profiles in dendritic cells
- Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
- Dendritic cell activity assay MUSST and mMUSST (expression of CD86)

In vivo: Organ response (LLNA)

In vivo: Organism response (GPMT)

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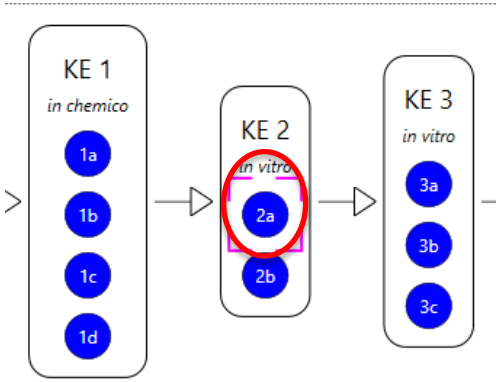
Overview of the AOP scheme as implemented in Toolbox

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 *in chemico* DPRA Cys and Lys	Peptide depletion, PD (%): PD > 9 - Passed PD <=9% - Not passed	> 9 % - Passed	<=9 % - Not passed
1c - *in chemico* 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 - *in chemico* Adduct formation assay LC-MS	Adduct formation (%) ≥ 30% - Positive Adduct formation (%) < 30% - Negative	Positive	Negative
2a - in vitro Keratinocyte (EC1.5, EC2, EC3) AND 2b – *in vitro* 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 *in vitro* Dendritic cell activity assay h-CLAT; MUSST and mMUSST (expression of CD54 and CD86)	expression of CD54 and CD86 Positive Negative	Positive	Negative
4 - in vivo Organ response (LLNA)	0 ≥ EC3 (%) <50 – Positive EC3 ≥ 50 – Negative Or	Positive	Negative
AO - in vivo 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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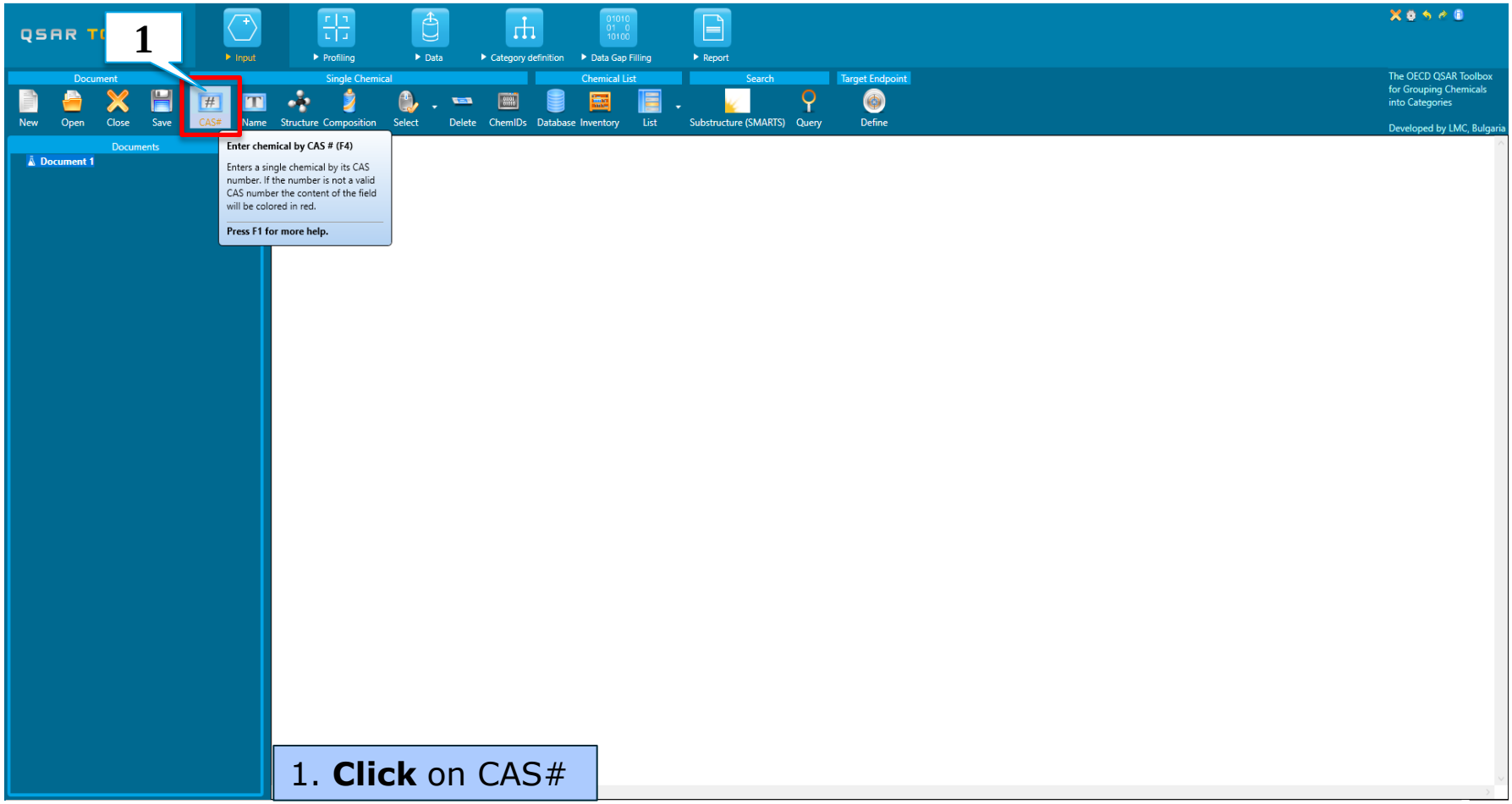
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - **Input**

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)

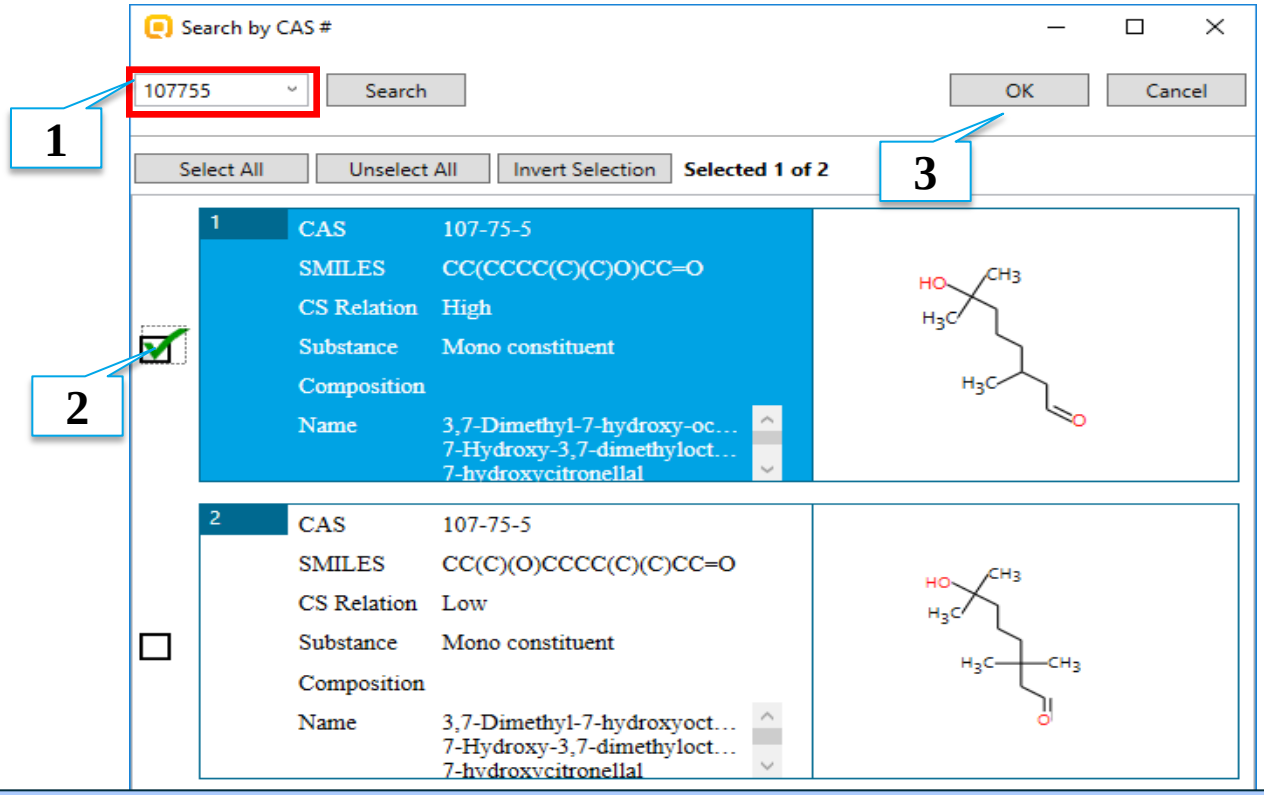
Chemical Input

Input target chemical by CAS#



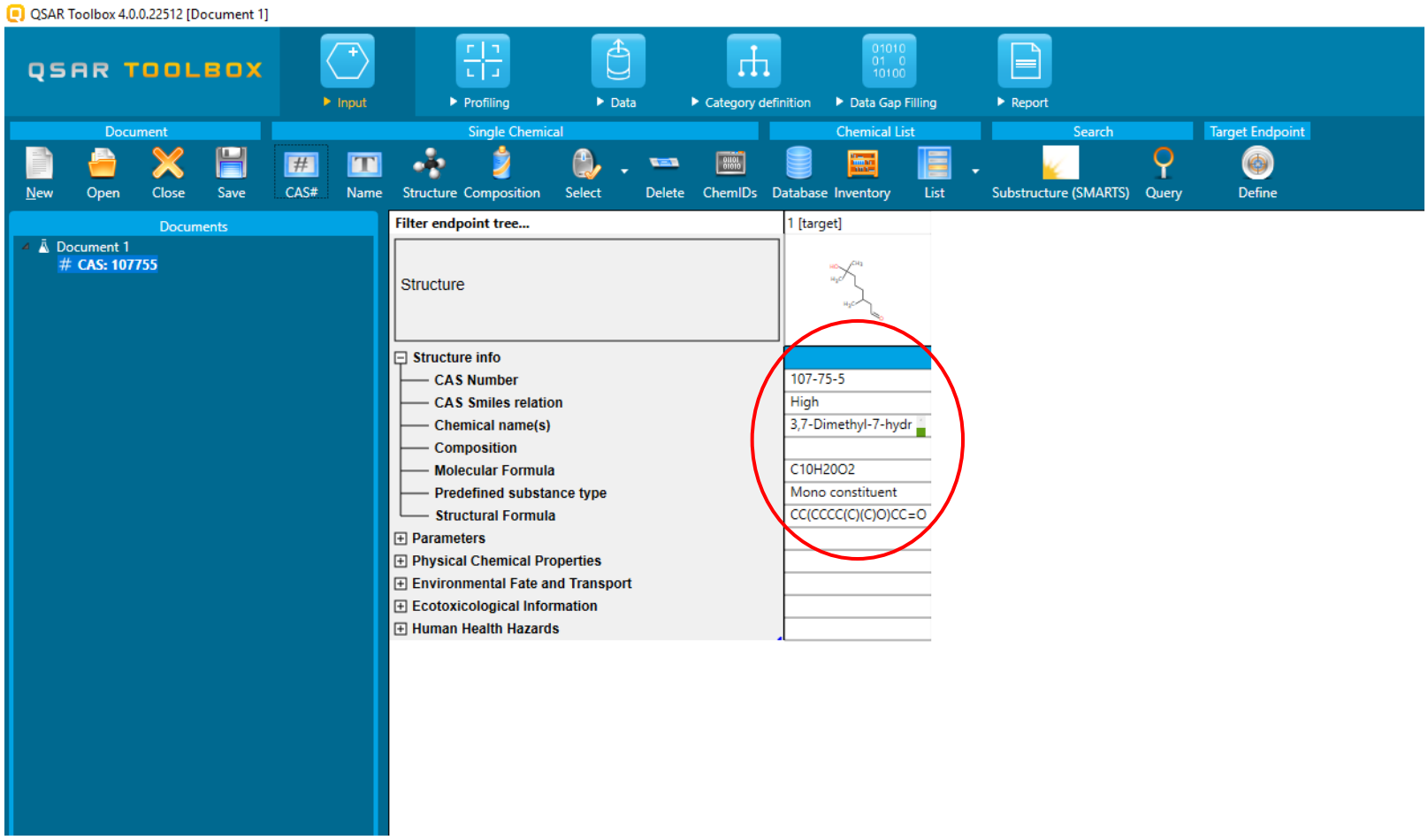
Chemical Input
Enter CAS# 107-75-5

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-demensional depiction



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

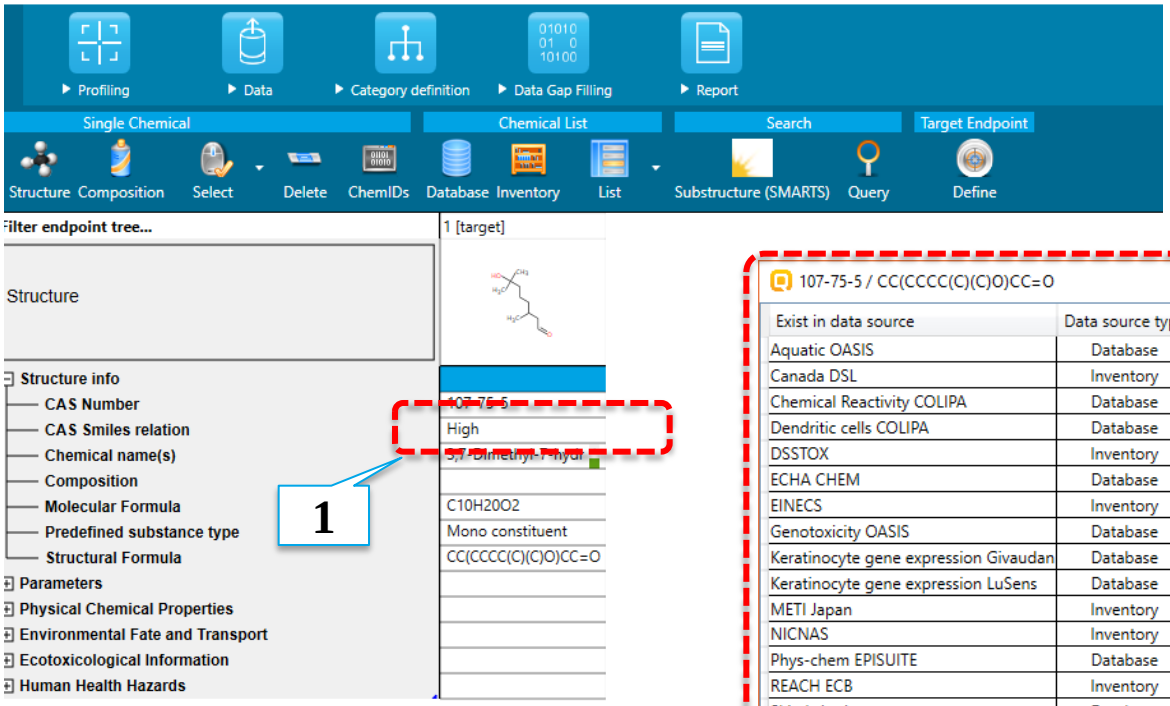
Chemical Input
Target chemical identity

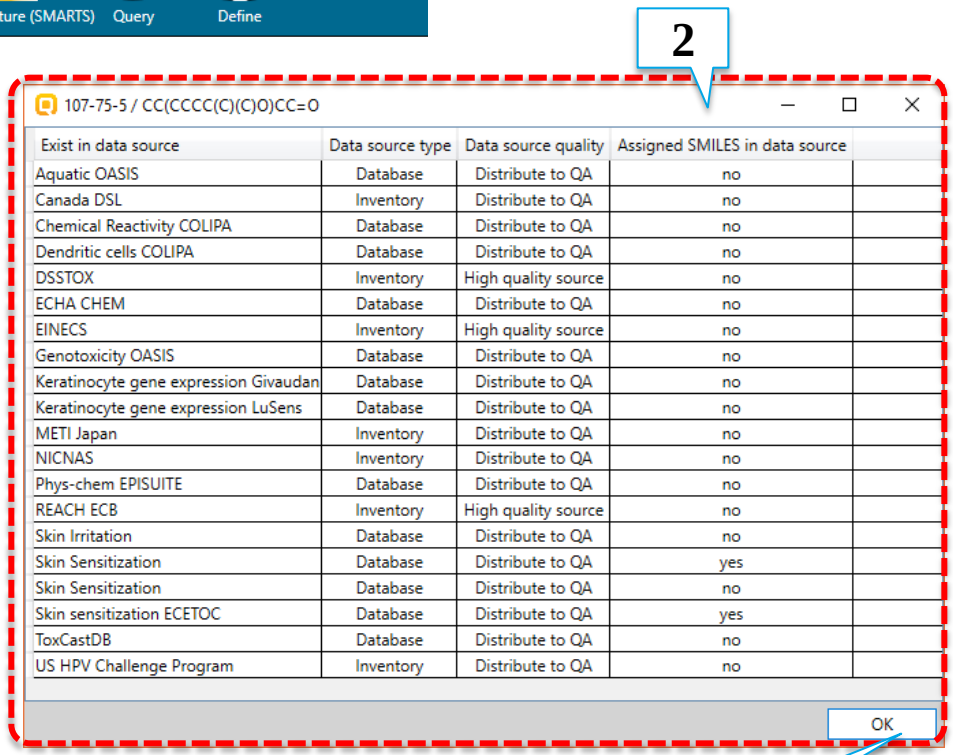


Chemical Input
Target chemical identity

- 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. 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.

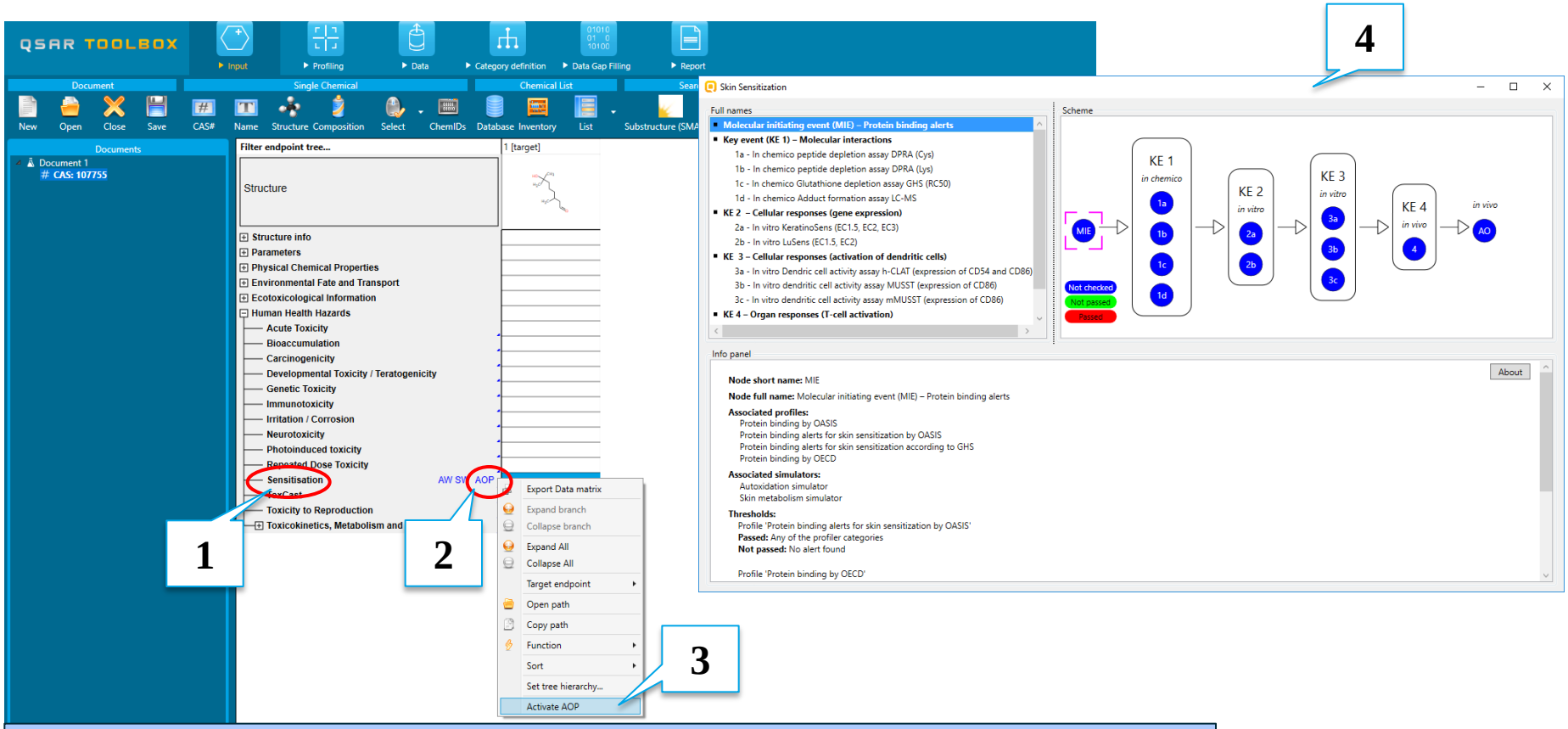
- **Low:** This reliability corresponds to poor reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to less than three, but at least one "Distribute to QA" data sources.

Outlook

- 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**

Activate AOP

Set target chemical for AOP

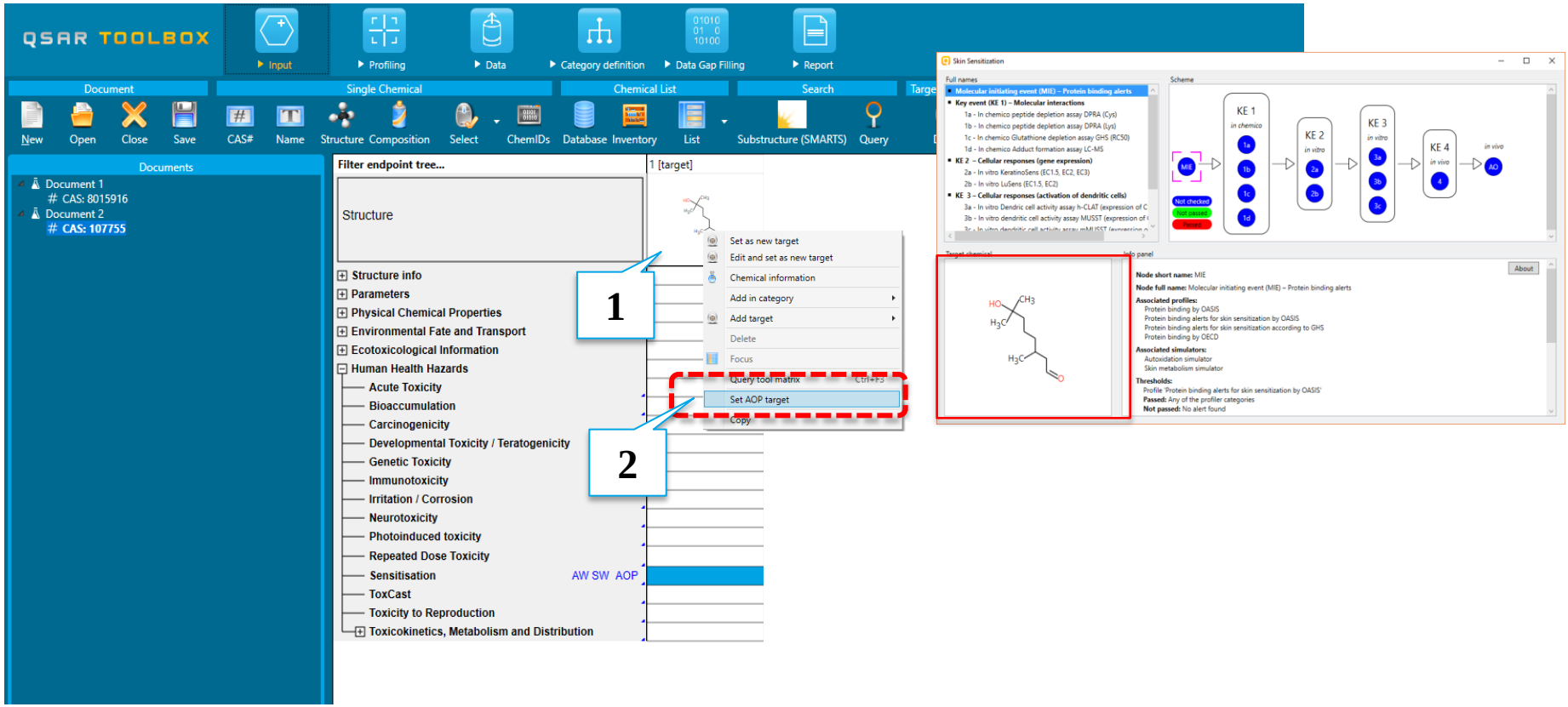


1. **Expand the endpoint tree** to the "Sensitization" node
2. **Right click** near the AOP label
3. **Select** activate AOP 4. AOP window appears

Continued on the next slide

Activate AOP

Set target chemical for AOP



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

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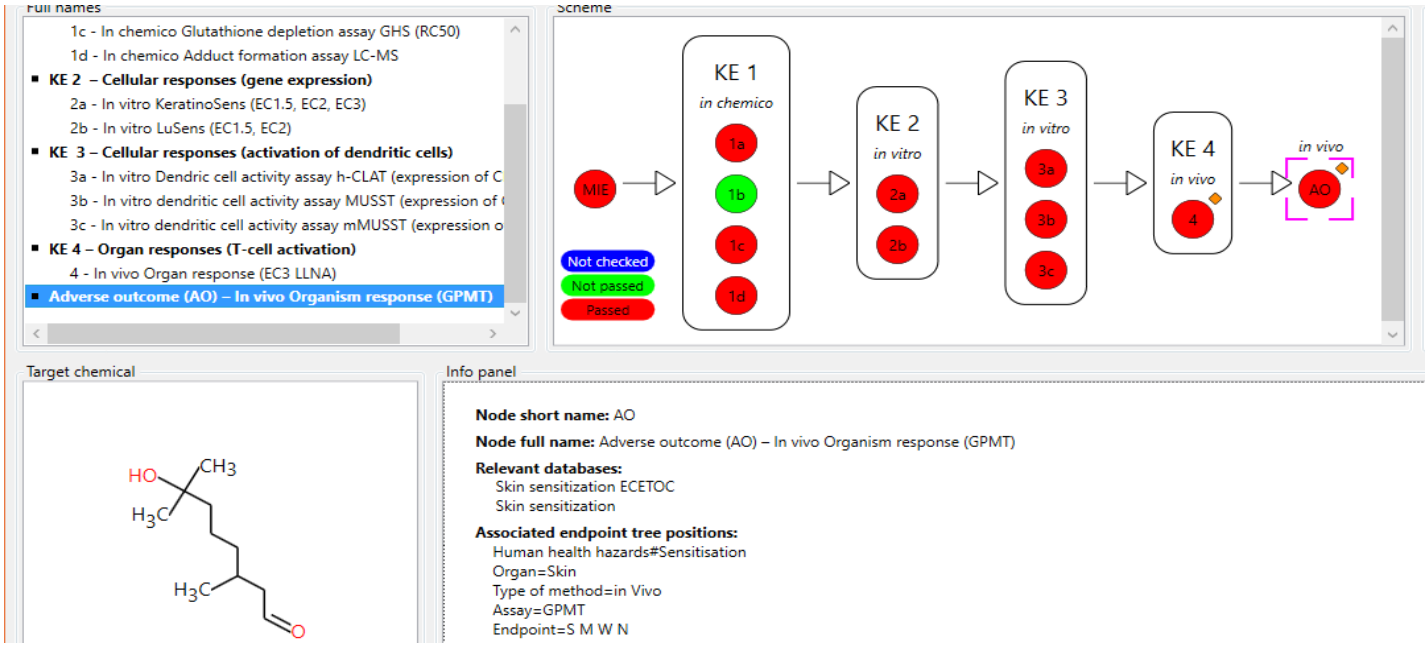
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - Input
    - Activate AOP and set target
    - **Workflow process**

Workflow process

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



MIE: protein binding

In chemico: protein binding potency assays

In vitro: gene expression assay

In vitro: cytokine profiles in dendritic cells

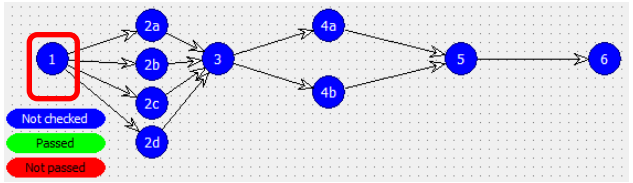
In vivo: Organ response (LLNA)

AO: Organism response (GPMT)

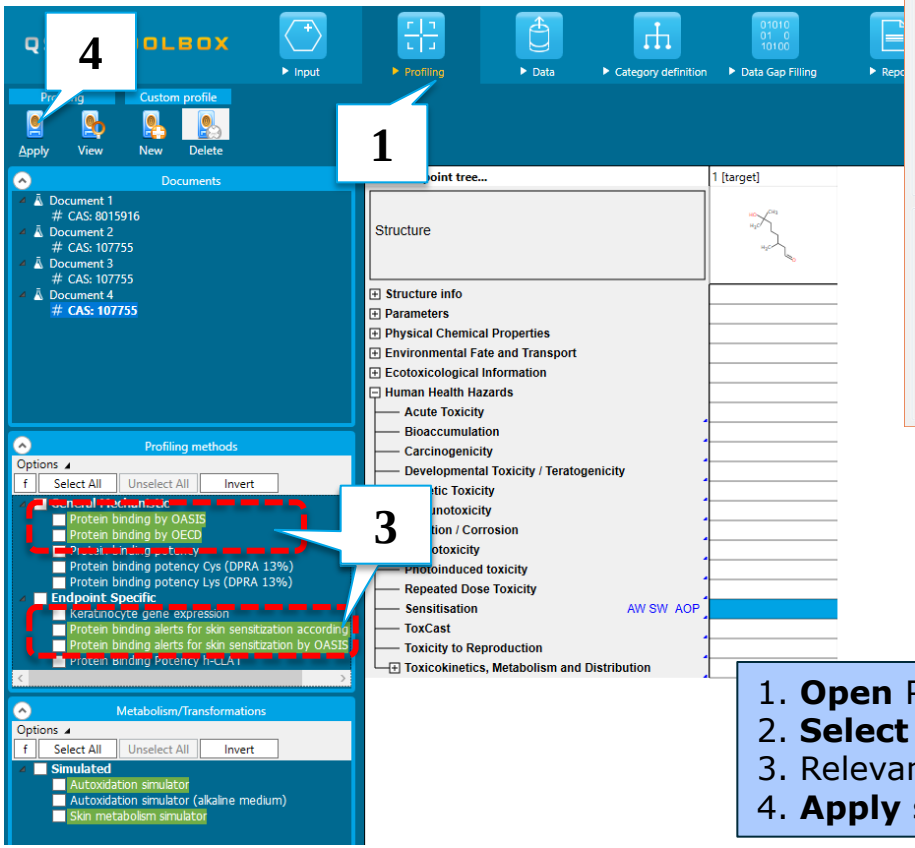
Workflow process

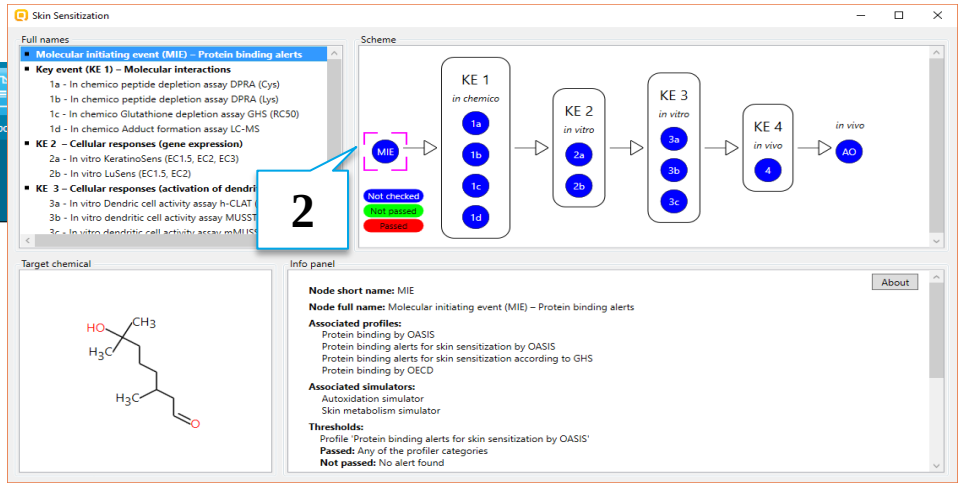
Step 1. MIE: protein binding

Example 1



Start with profiling of target chemical



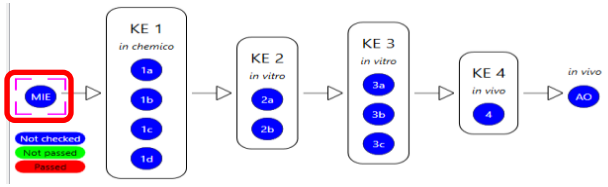


1. **Open** Profiling
2. **Select** node #1 related to MIE.
3. Relevant profilers are highlighted, **select** highlighted profilers
4. **Apply** selected profilers

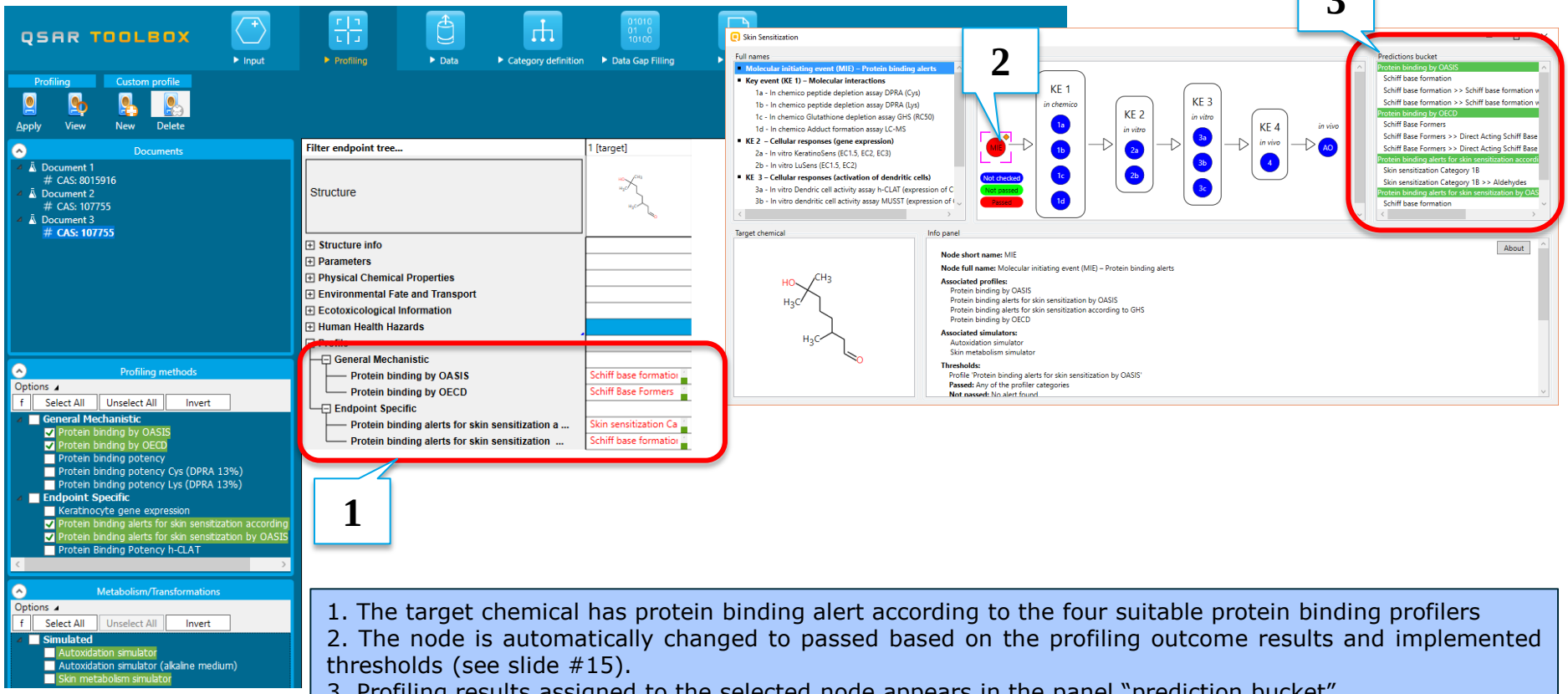
Workflow process

Step 1. MIE: protein binding

Example 1



Start with profiling of target chemical

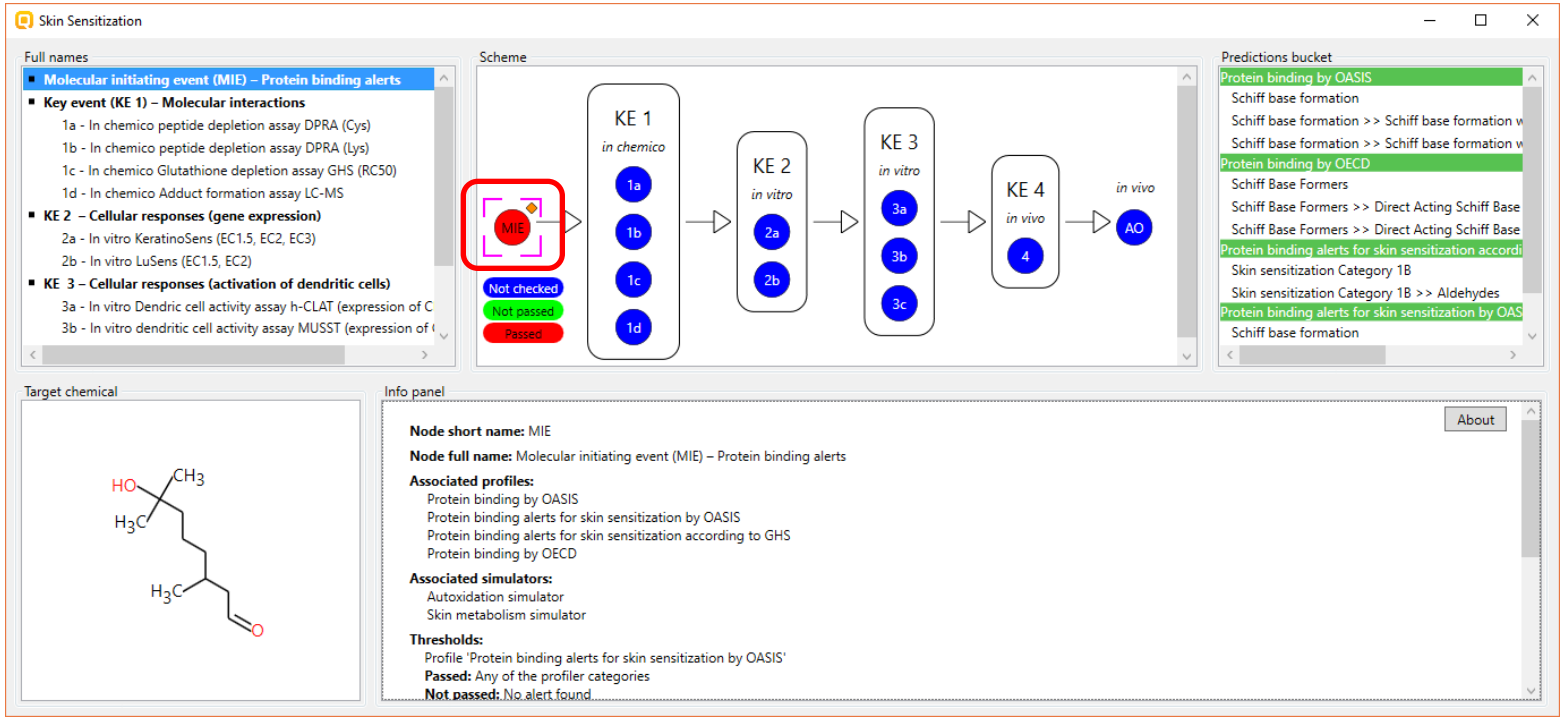


1. The target chemical has protein binding alert according to the four suitable protein binding profilers
2. The node is automatically changed to passed based on the profiling outcome results and implemented thresholds (see slide #15).
3. Profiling results assigned to the selected node appears in the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

Workflow process

Molecular initiating events

Example 1

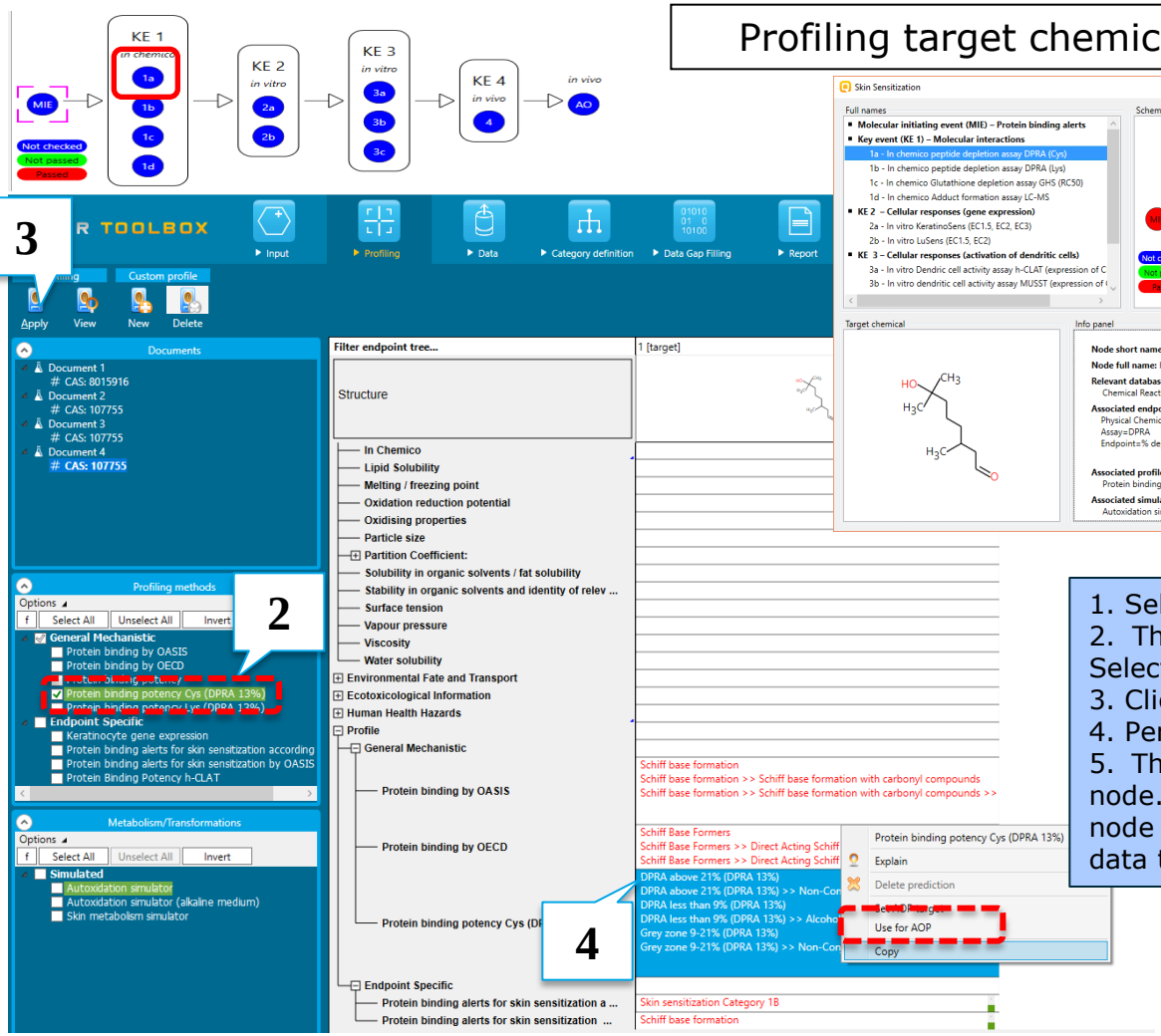


- The node MIE is passed due to the presence of protein binding alert identified for the target chemical by the two protein binding profilers
- The workflow should move further to the *in chemico* assay

Workflow process

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

Example 1

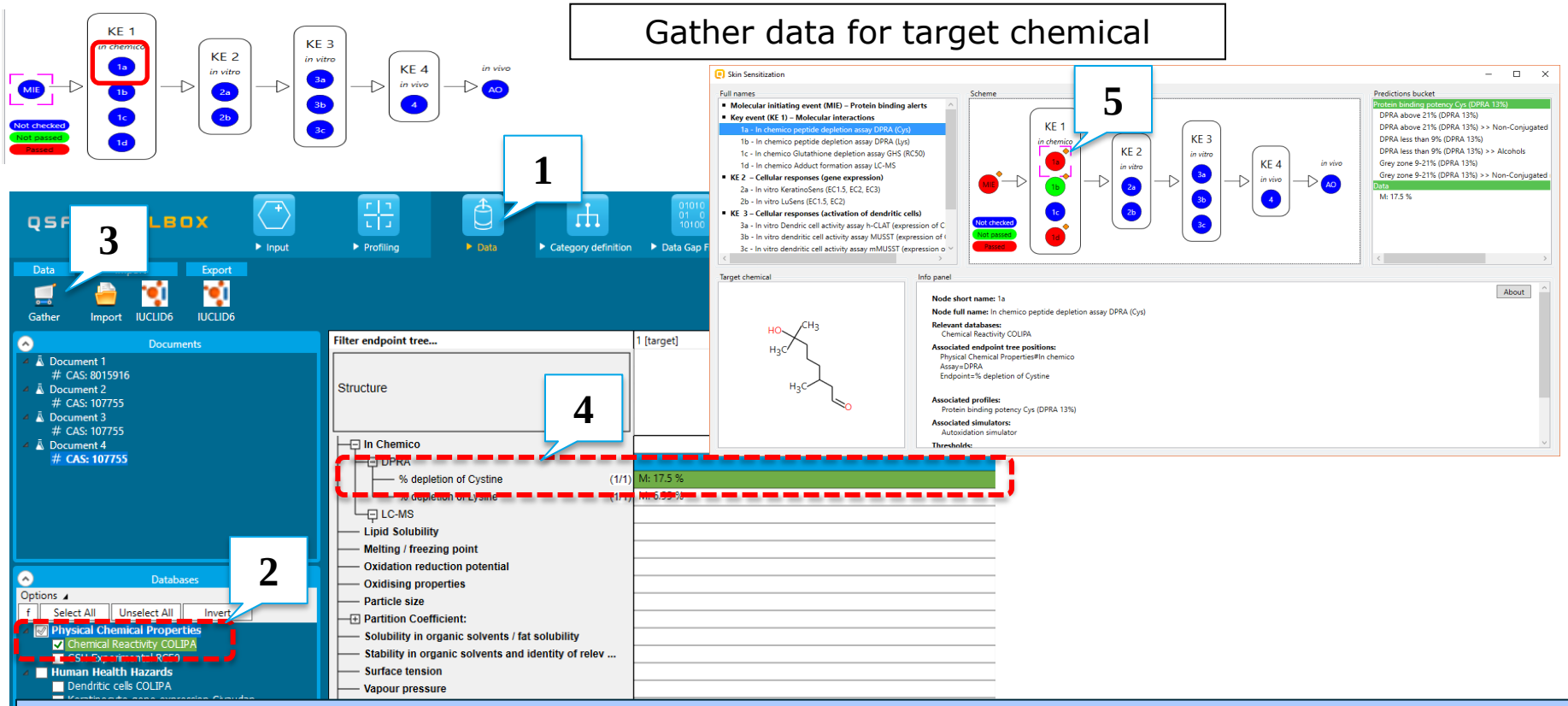


1. Select node **1a** related to Cys depletion assay.
2. The profilers related to node 1a are highlighted. Select **related Profilers**
3. Click **Apply**
4. Perform **right click** and **select** "Use for AOP"
5. The profiling result appears in the bucket of the node. This last action is not related 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

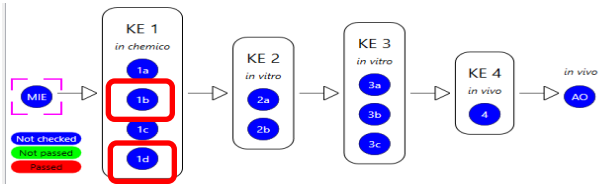


1. **Go** to Data and check are there any experimental data for the node 1a
2. **Select** highlighted database
3. **Click** Gather
4. Data appears on data matrix
5. Based on presence of data for the chemical and implemented thresholds (slide #15) node 1a is getting passed. Node 1b and 1d are automatically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

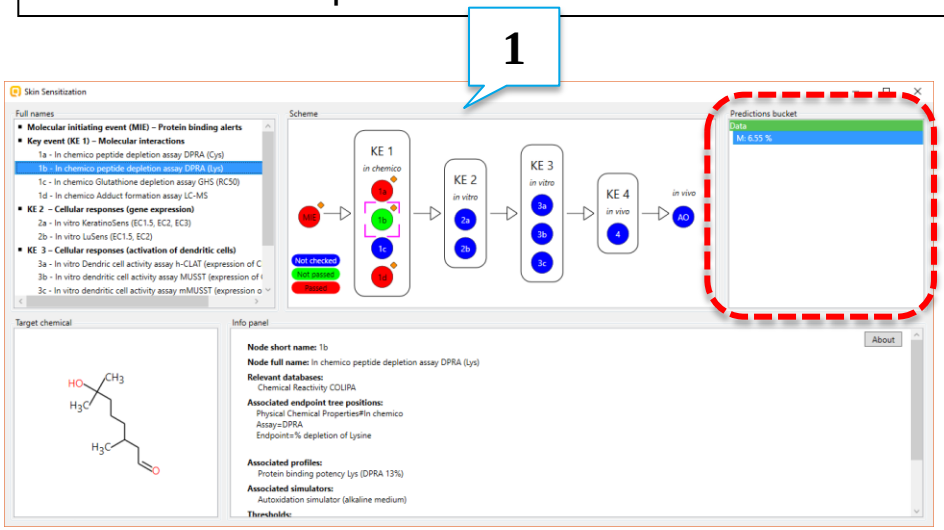
Workflow process

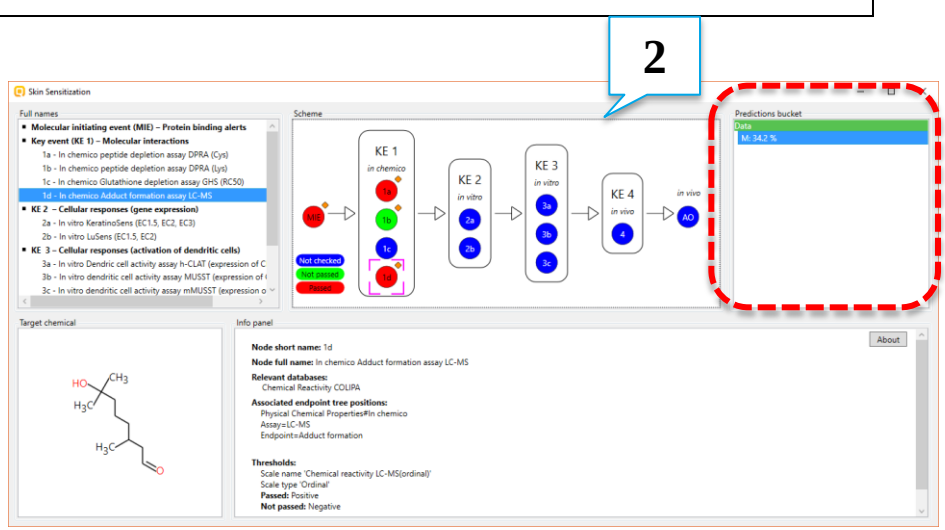
Step 2.*In chemico* peptide depletion assay DPRA (Lys) (node 1b) and *In chemico* Adduct formation assay LC-MS (node 1d)

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





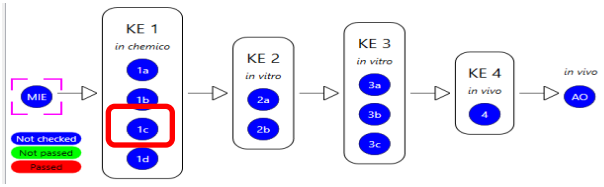
1. Select **node 1b**
2. Select **node 1d**

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

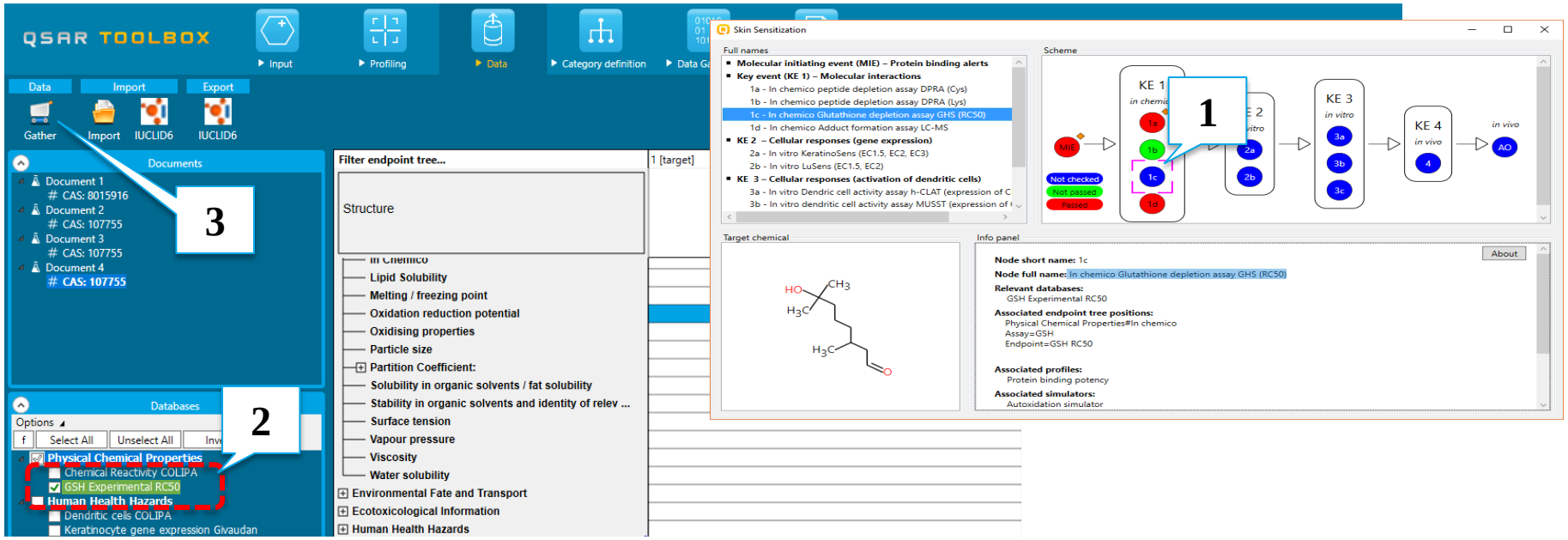
Workflow process

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



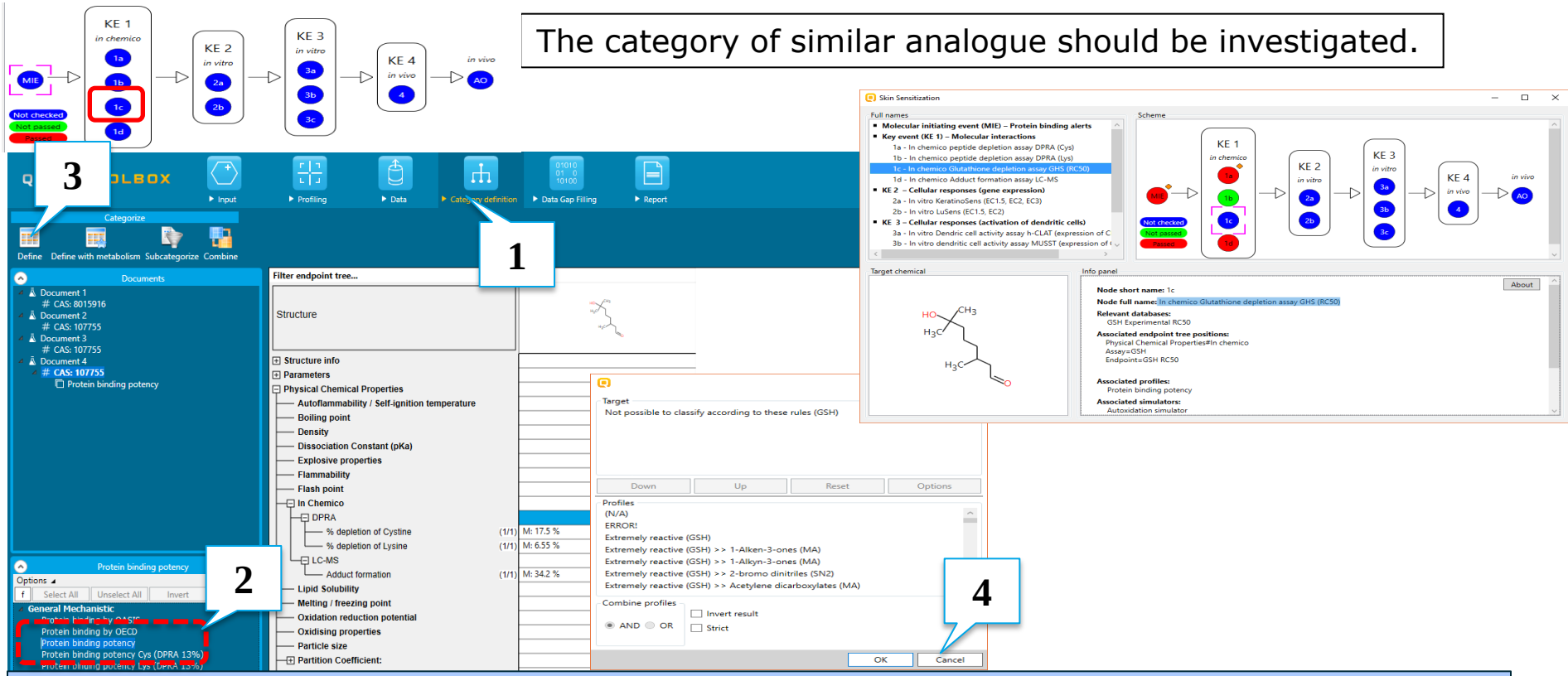
1. Select **node 1c** related to *in chemico* glutathione depletion assay
2. **Select** highlighted database
3. **Click** Gather. No data has been found for the target chemical

Workflow process

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

Example 1

The category of similar analogue should be investigated.

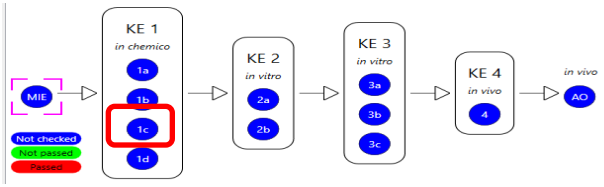


1. Switch to **Category definition**
2. **Select** highlighted category
3. **Click** Define
4. There are no structural alerts identified for the target chemical according to this profiler (no mechanistic and structural explanation). Click **Cancel**
   Based on the above point it is recommended to define category by "Protein binding alerts" profiler

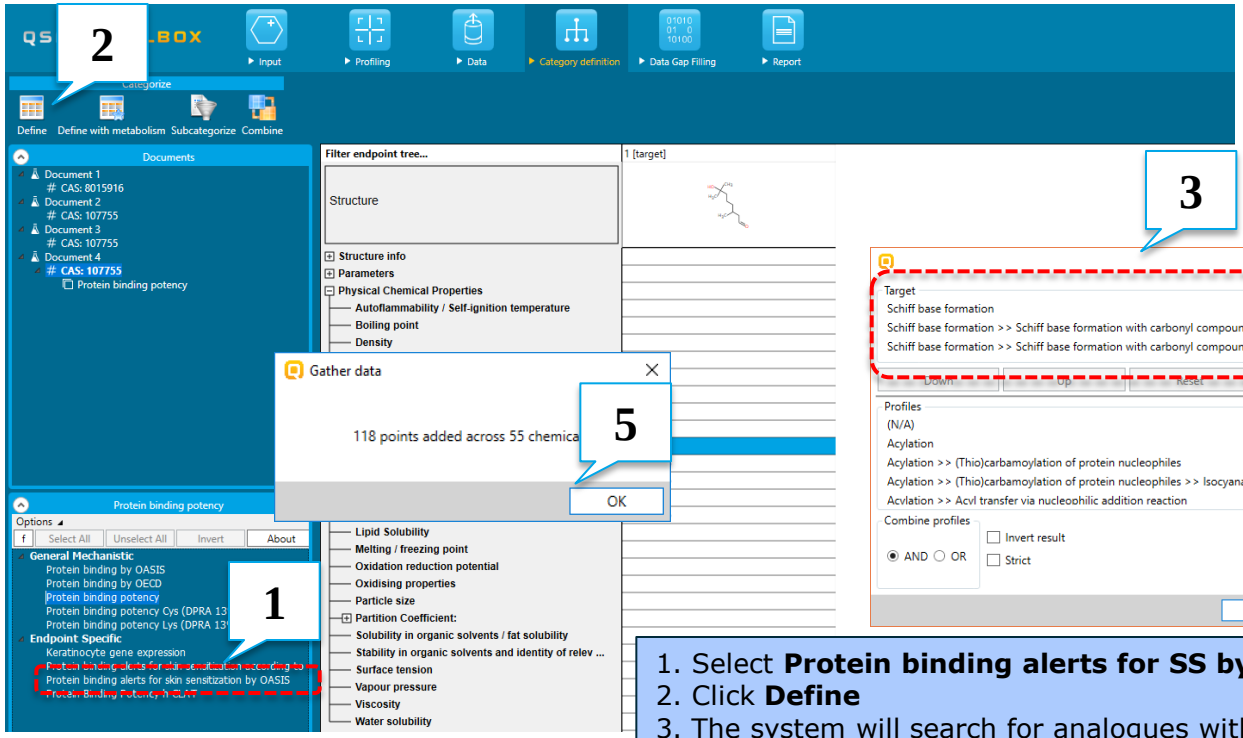
Workflow process

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

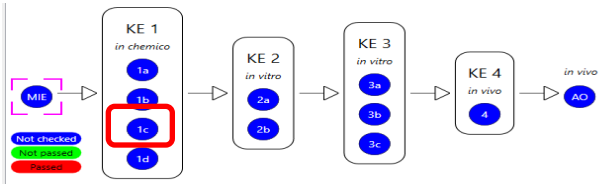


1. Select **Protein binding alerts for SS by OASIS v1.4**
2. Click **Define**
3. The system will search for analogues with "**Aldehyde**" group
4. Click **OK**
5. The system identify 55 analogues with 118 data points. Click **OK**

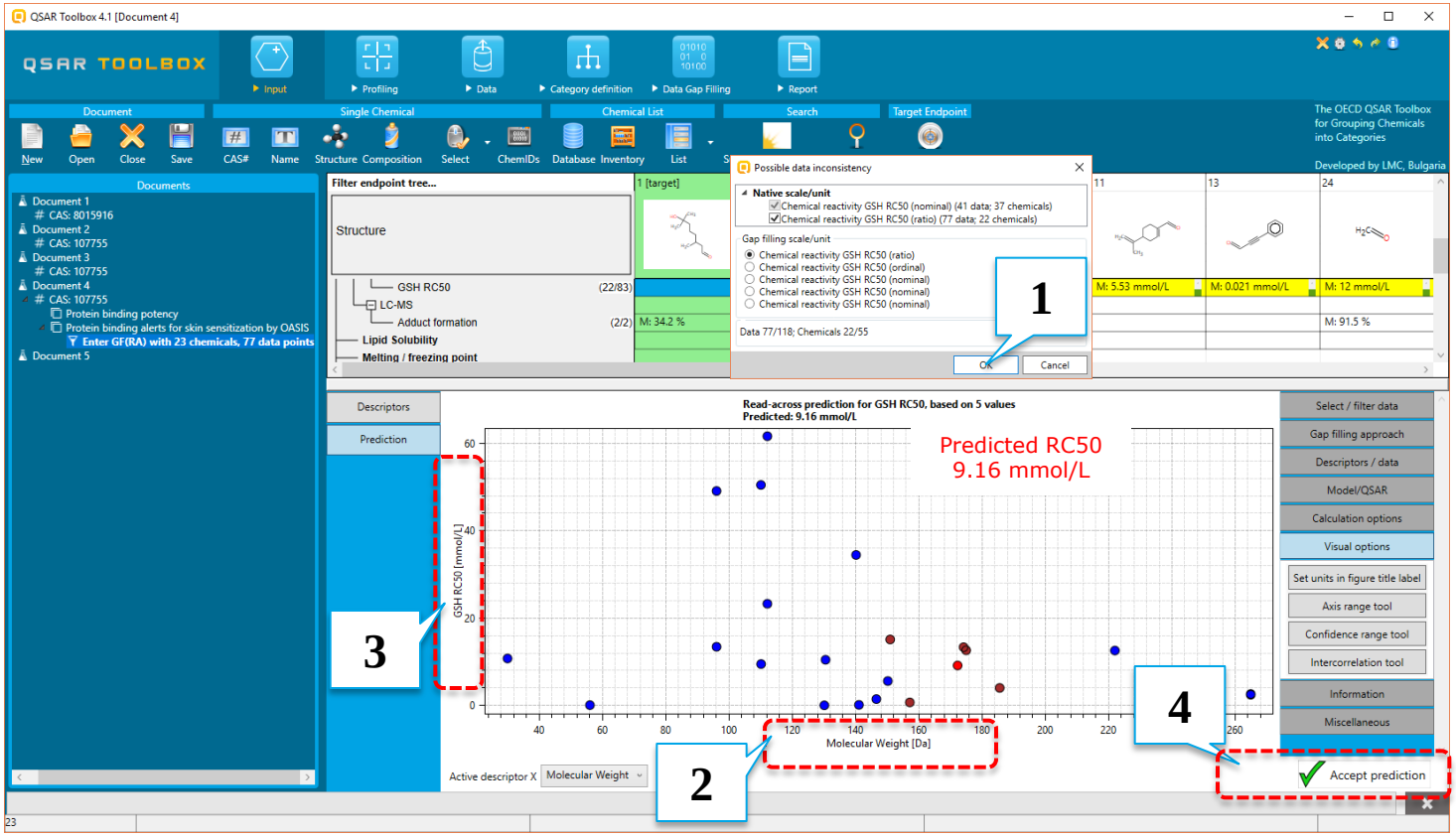
Workflow process

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

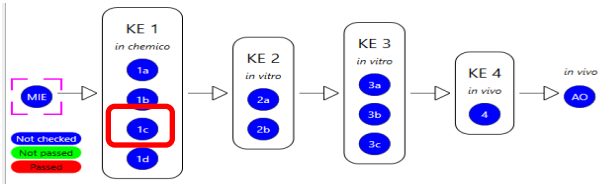
RC50 (mmol/L) ≤ 0.099 – Extremely reactive
0.1 ≥ RC50 ≤ 0.99 – Highly reactive
1 ≥ RC50 ≤ 15 – Moderately reactive
16 ≥ RC50 ≤ 70 – Slightly reactive
70.1 ≥ RC50 ≤ 135 – Suspect
RC50 > 135 – Not reactive

1. 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**.
2. The Molecular weight descriptor as the most suitable for predicting skin sensitization effect is used in RA prediction
3. RC50 values are presented in mmol/L
4. **Accept prediction**

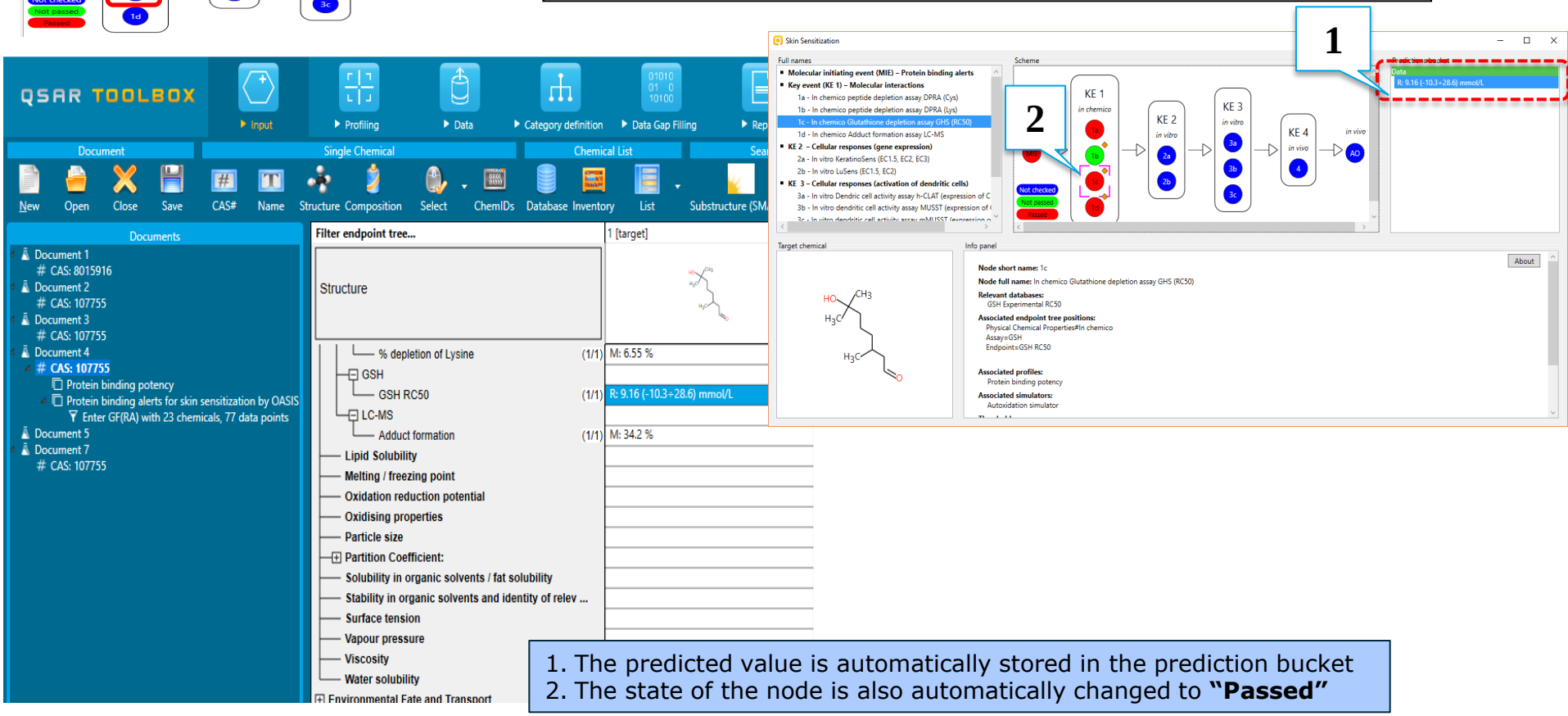
Workflow process

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

Example 1



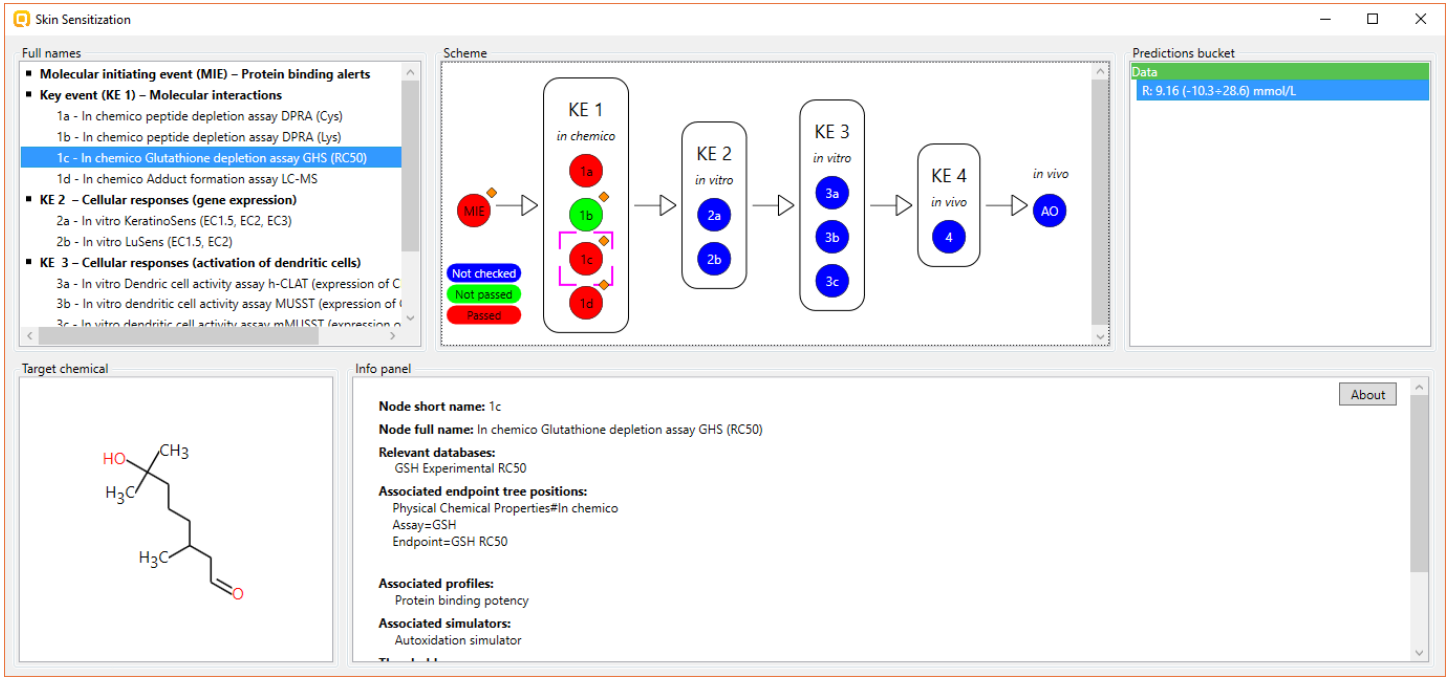
The next step is to use read-across prediction for AOP



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

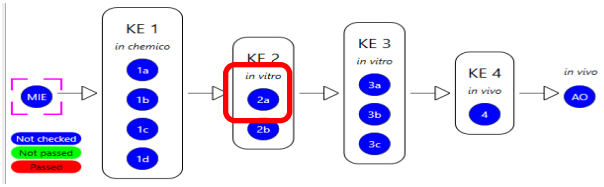


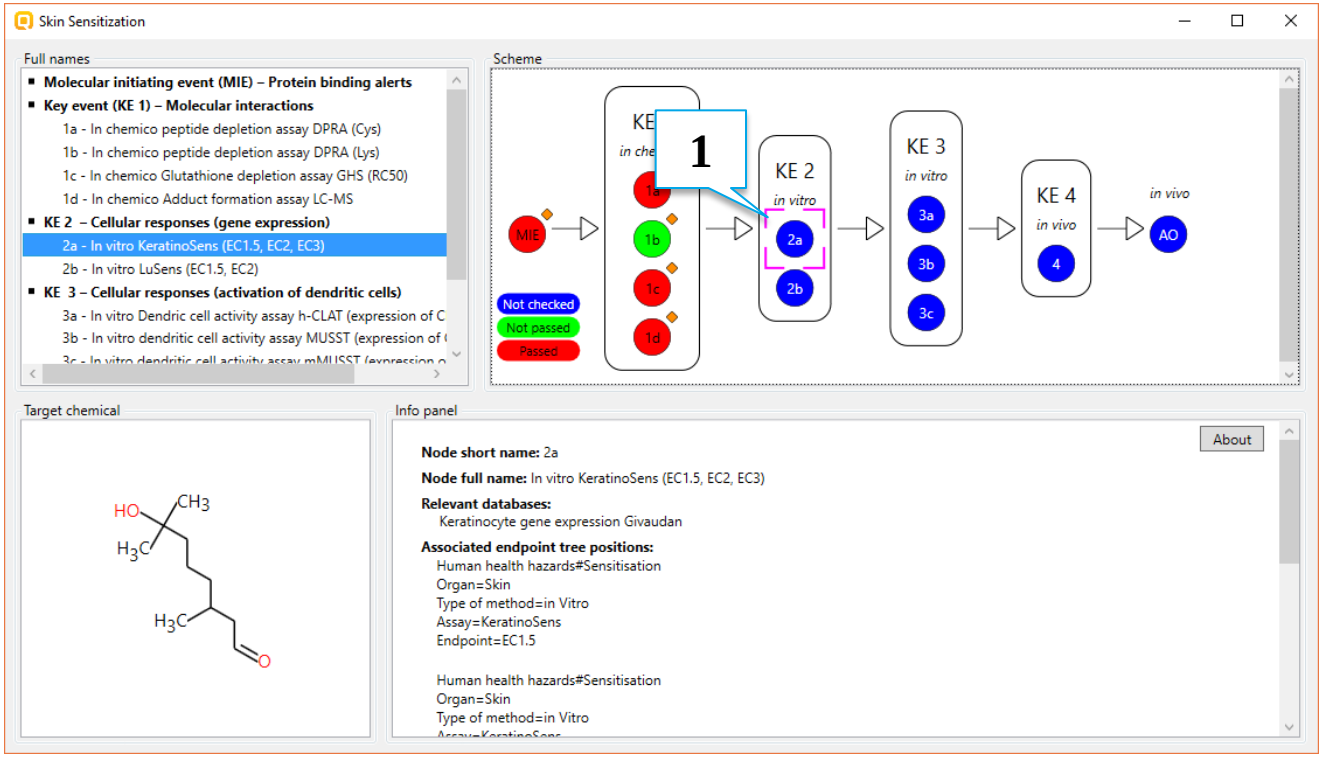
- The nodes related to three of the *in chemico* assays are passed due to positive experimental data for the target chemical (node 1a and 1d) and the positive experimental data found for analogues with an "Aldehyde" group(1c).
- Only one of all *in chemico related* nodes (node 1b) is assigned as "Not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



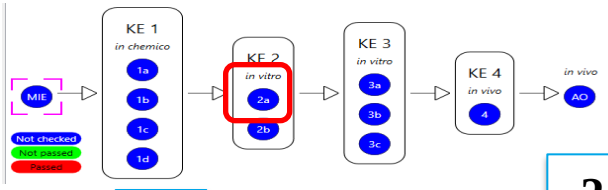


1. Select **node 2a**

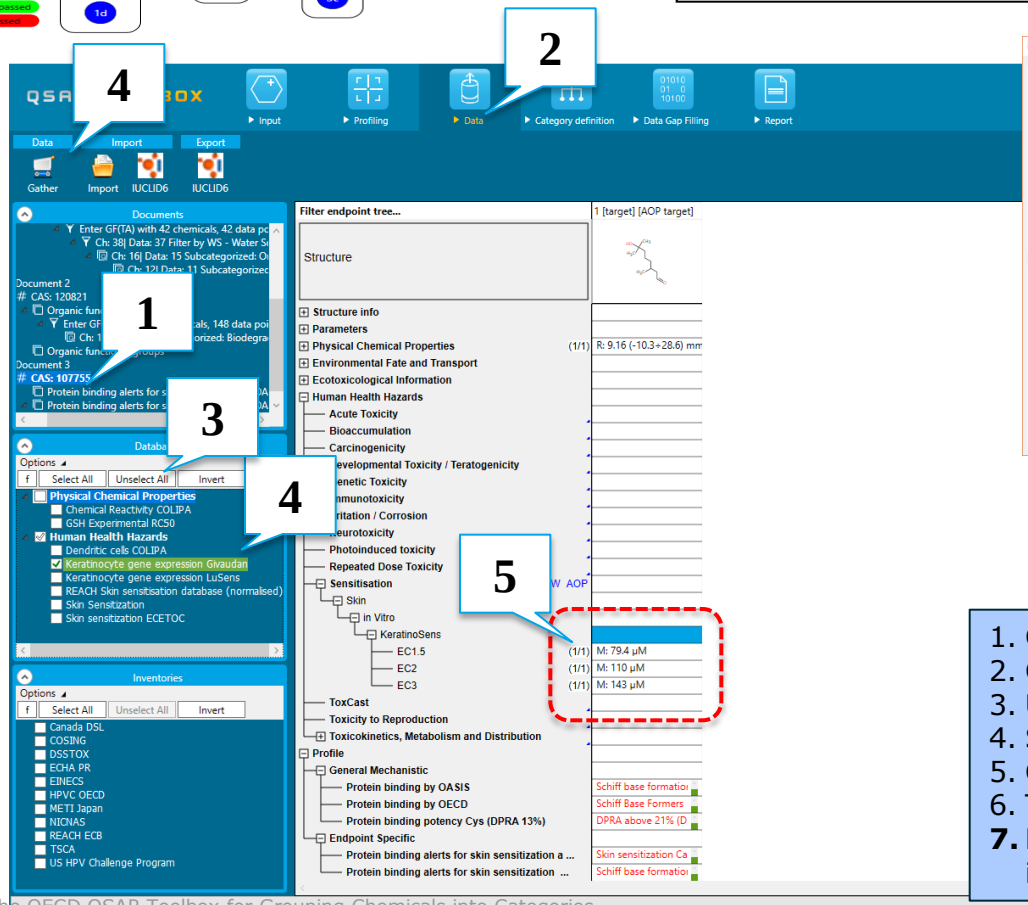
Workflow process

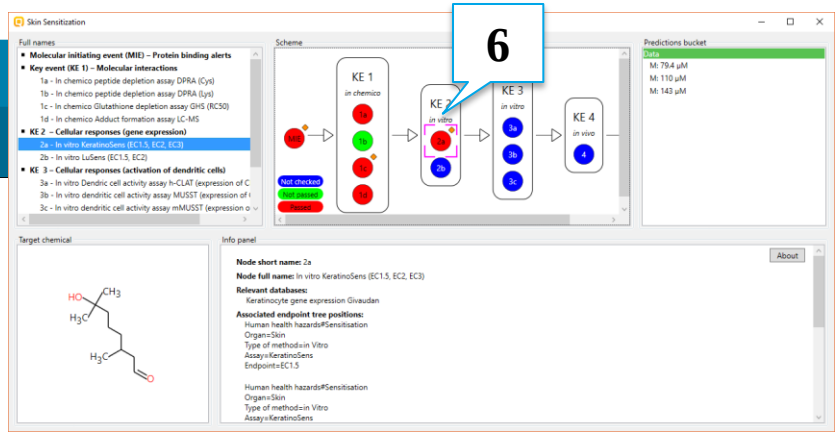
Step 3. *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3) (node 2a)

Example 1



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



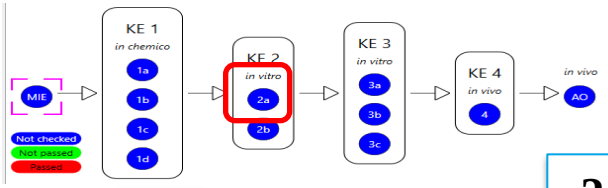


1. Go to level of CAS number from document tree
2. Go to **Data**
3. Unselect **all databases**
4. Select **highlighted database**
5. Click **Gather**
6. The experimental data appears on Data matrix
7. **Node 2a** has been changed to passed based on implemented thresholds (slide #15)

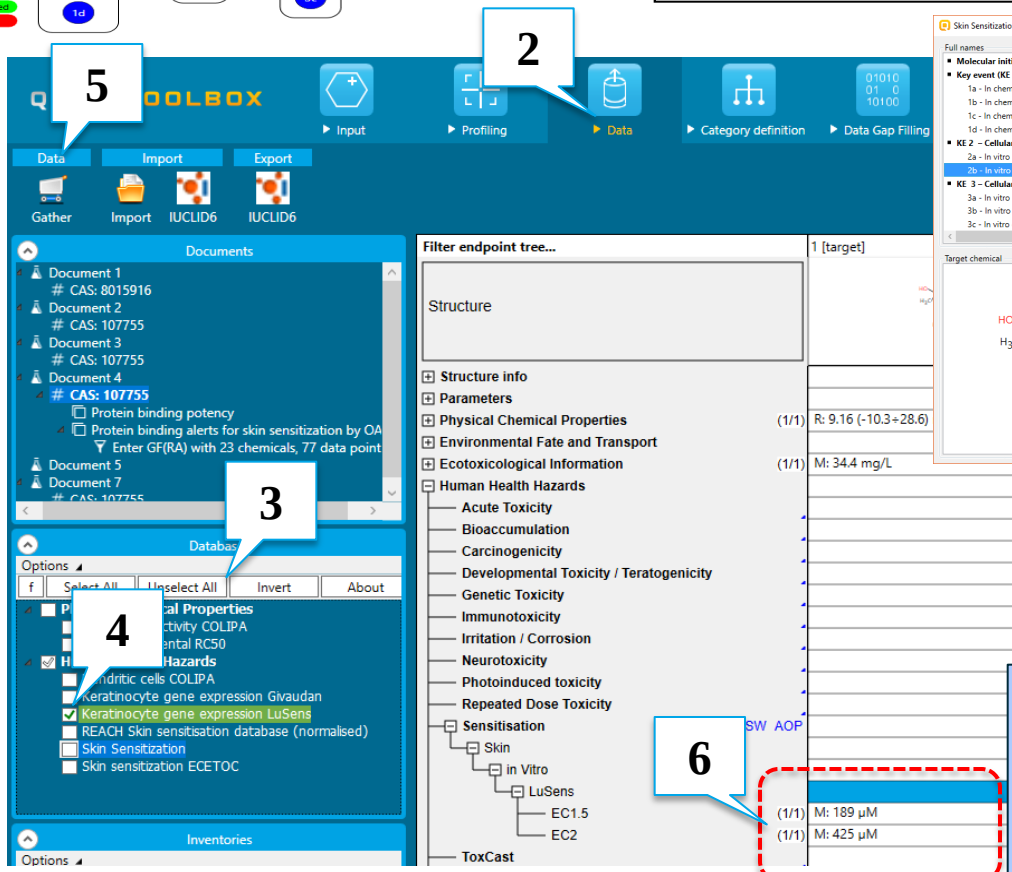
Workflow process

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

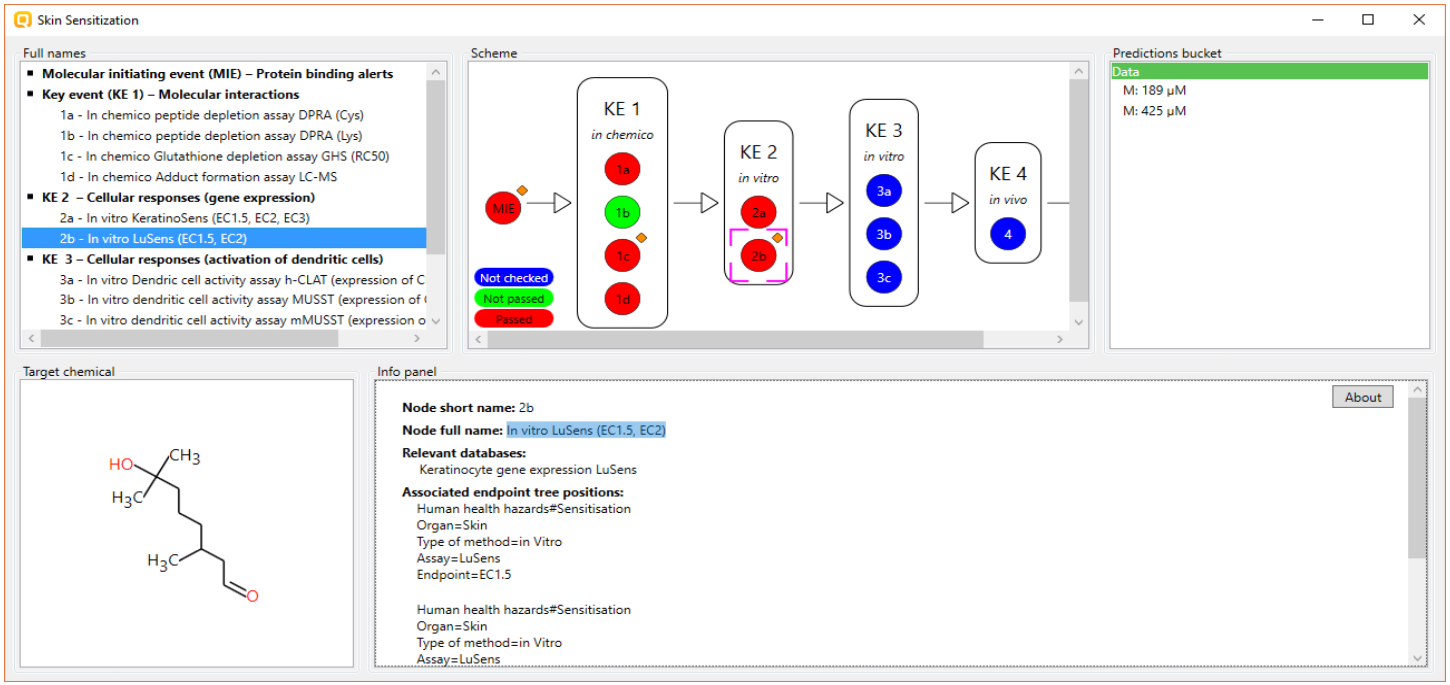


1. Select node **2b**
2. Go to **Data**
3. Unselect **all databases**
4. Select **highlighted database**
5. Click **Gather**
6. The experimental data appears on Data matrix
7. Node 2b has been changed to passed based on implemented thresholds (slide #15)

Workflow process

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

Example 1

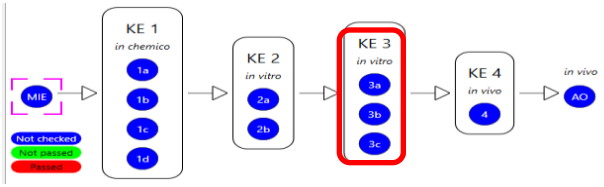


- The both nodes related to *in vitro* assays are passed due to positive experimental data found for the target chemical and implemented thresholds (slide #15)
- The workflow should move further to the other *in vitro* assays (nodes 3a, 3b and 3c)

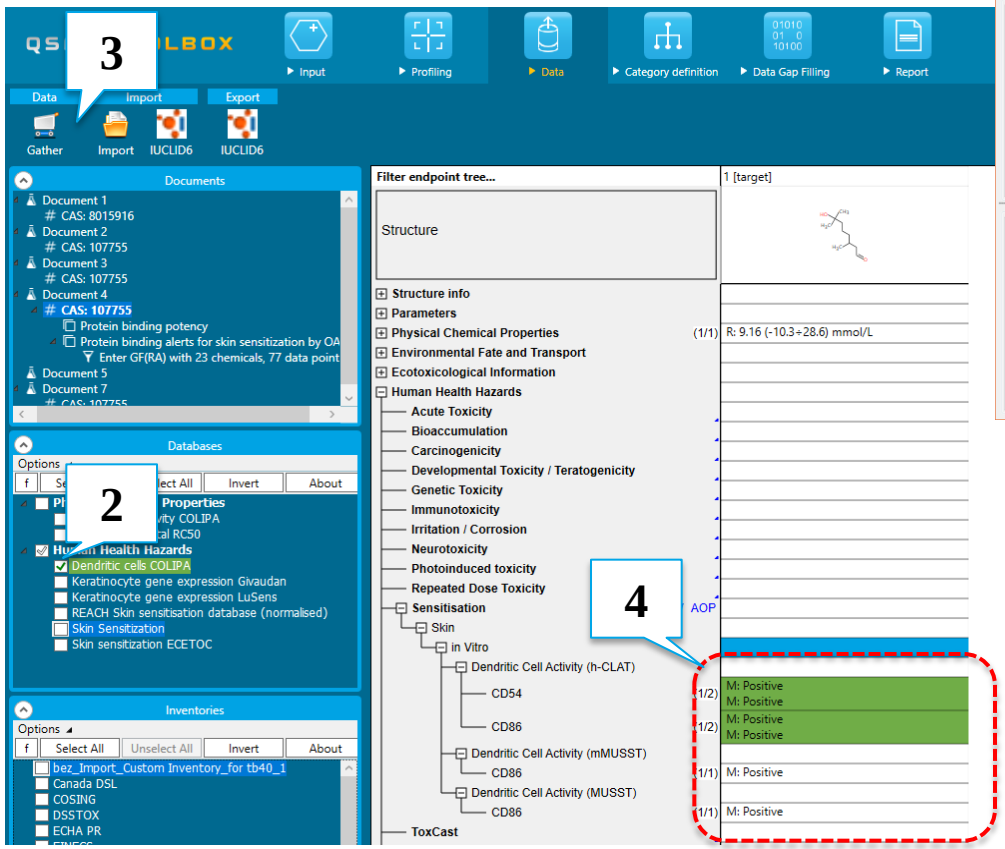
Workflow process

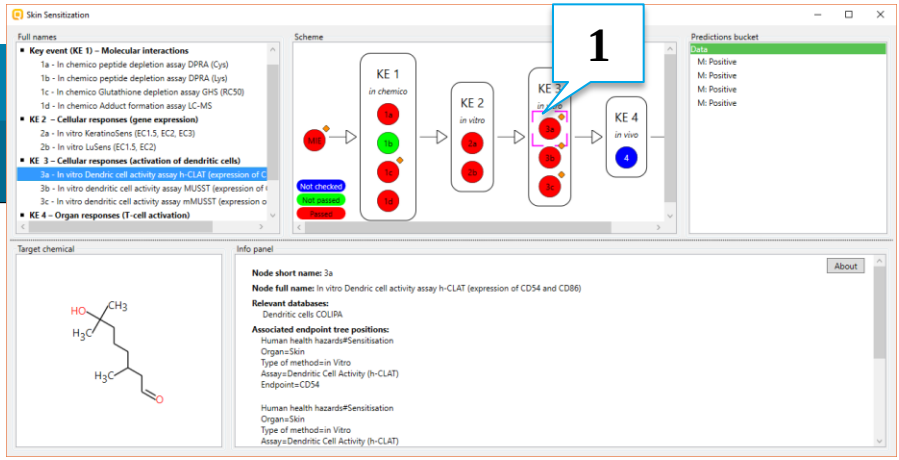
Step 4. *In vitro* Dendric cell activity assays h-CLAT, MUSST and mMUSST (nodes 3a, 3b and 3c)

Example 1



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



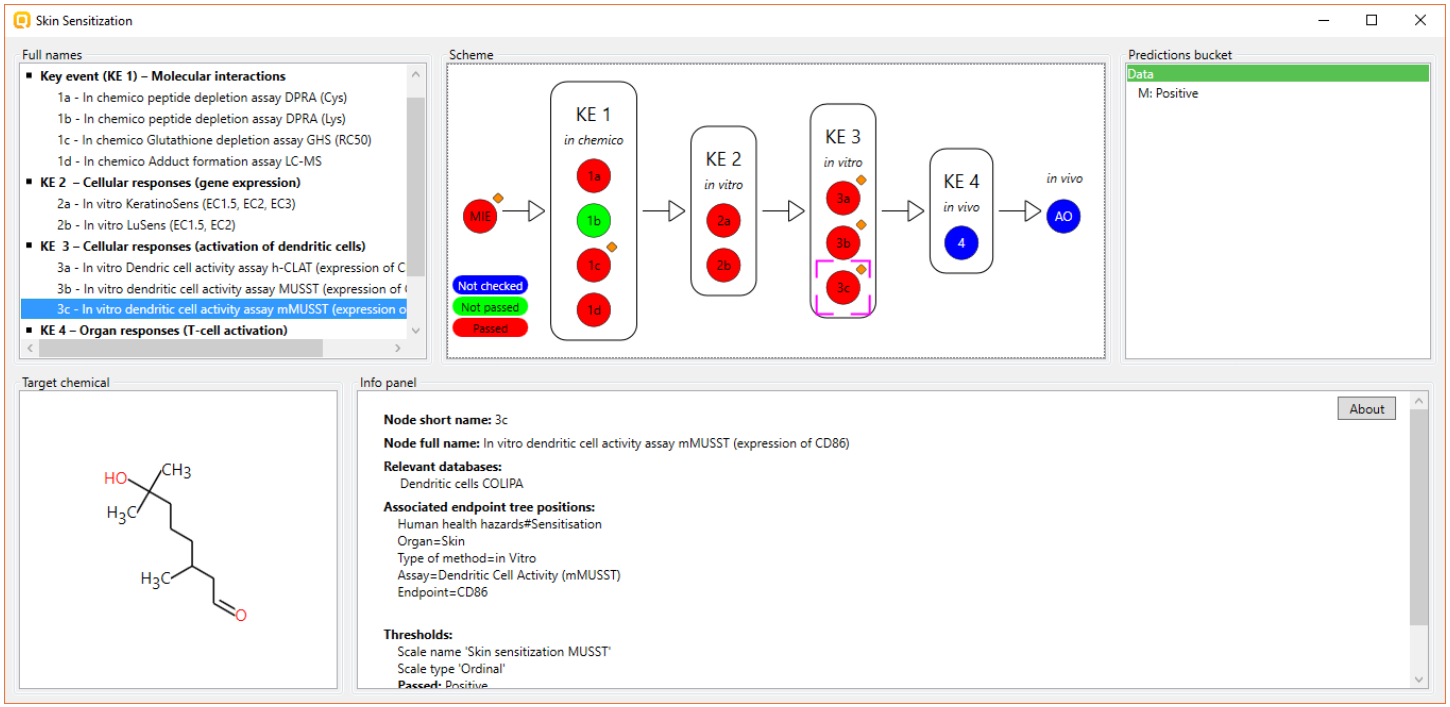


1. Select **node 3a**
2. Select database related to node 3a as before thata unselect all others
3. **Gather** data and click **OK** in the appeared message
4. The experimental data appears on Data matrix and the status of nodes 3a, 3b and 3c was changed to **Passed**

Workflow process

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

Example 1

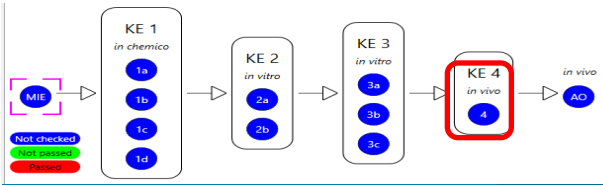


- The nodes 4a and 4b related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to positive experimental data found for the target chemical
- The workflow moves further to the *in vivo LLNA* assay (node 4)

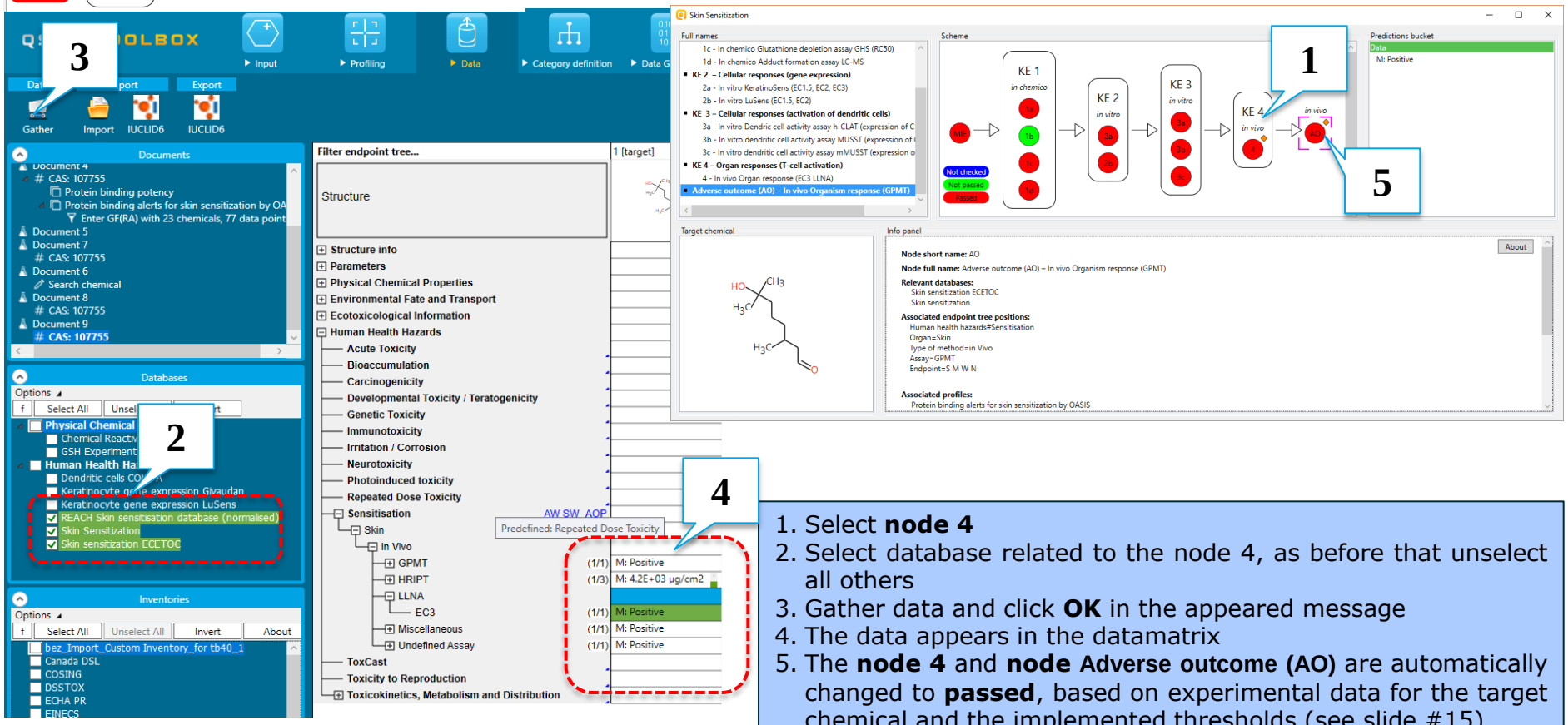
Workflow process

Step 5. I*n 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)

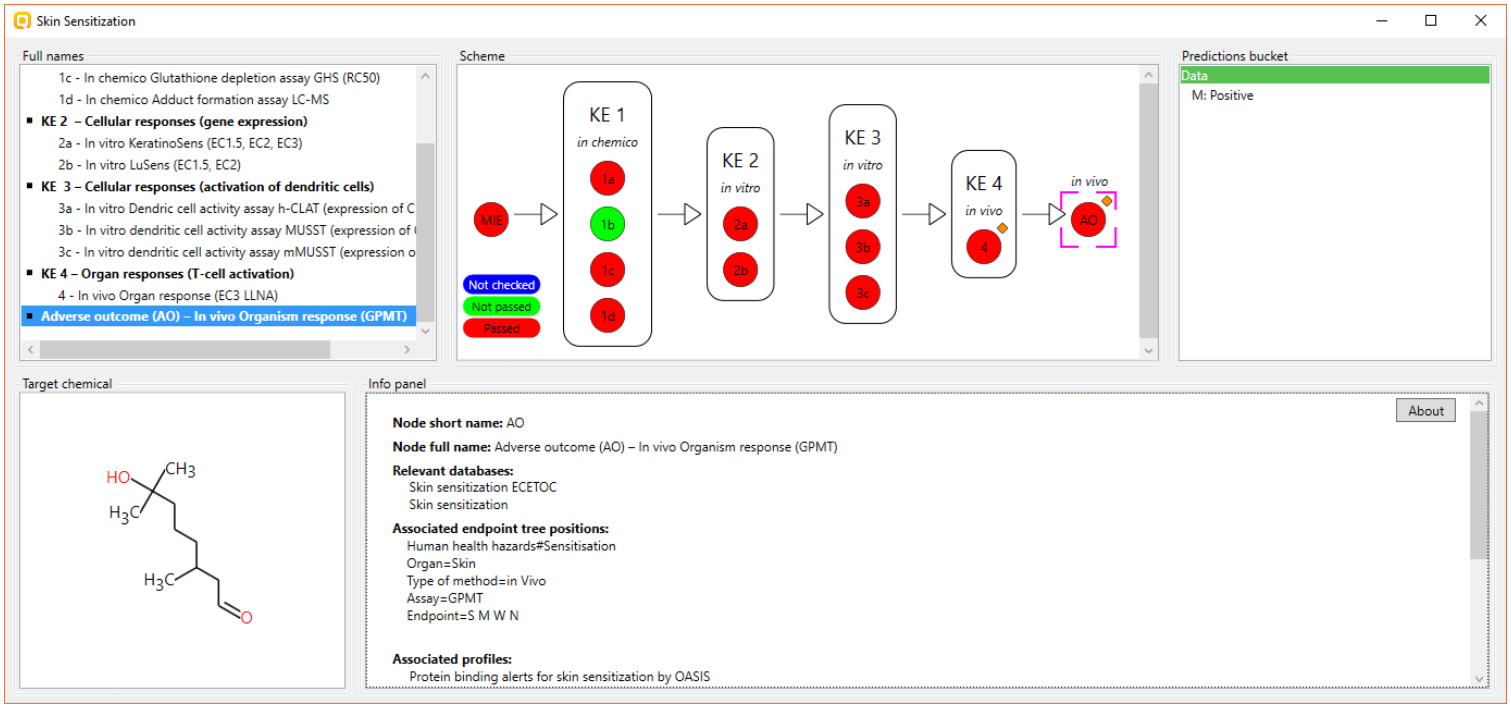


1. Select **node 4**
2. Select database related to the node 4, as before that unselect all others
3. Gather data and click **OK** in the appeared message
4. The data appears in the datamatrix
5. The **node 4** and **node Adverse outcome (AO)** are automatically changed to **passed**, based on experimental 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



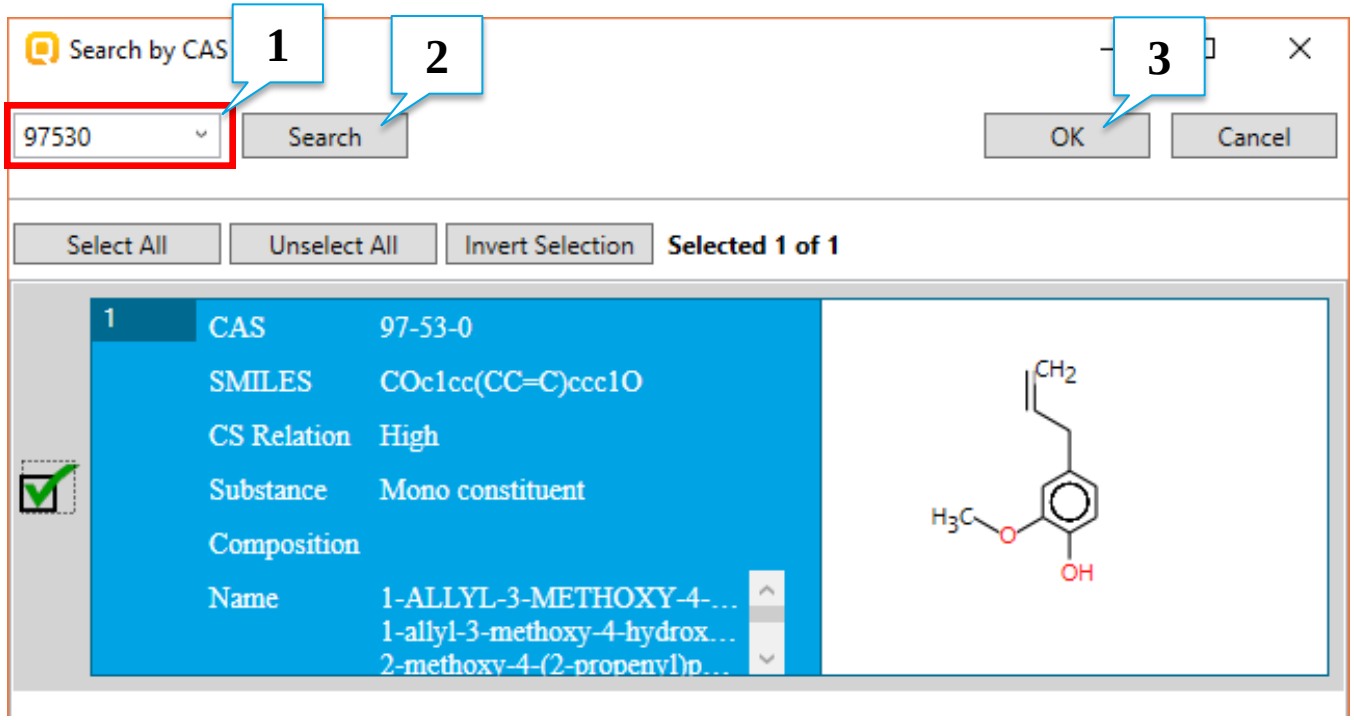
- Both nodes related to the two *in vivo* assays (LLNA and GPMT) are passed based on the positive experimental data for the target chemical according to the implemented thresholds

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-demensional depiction

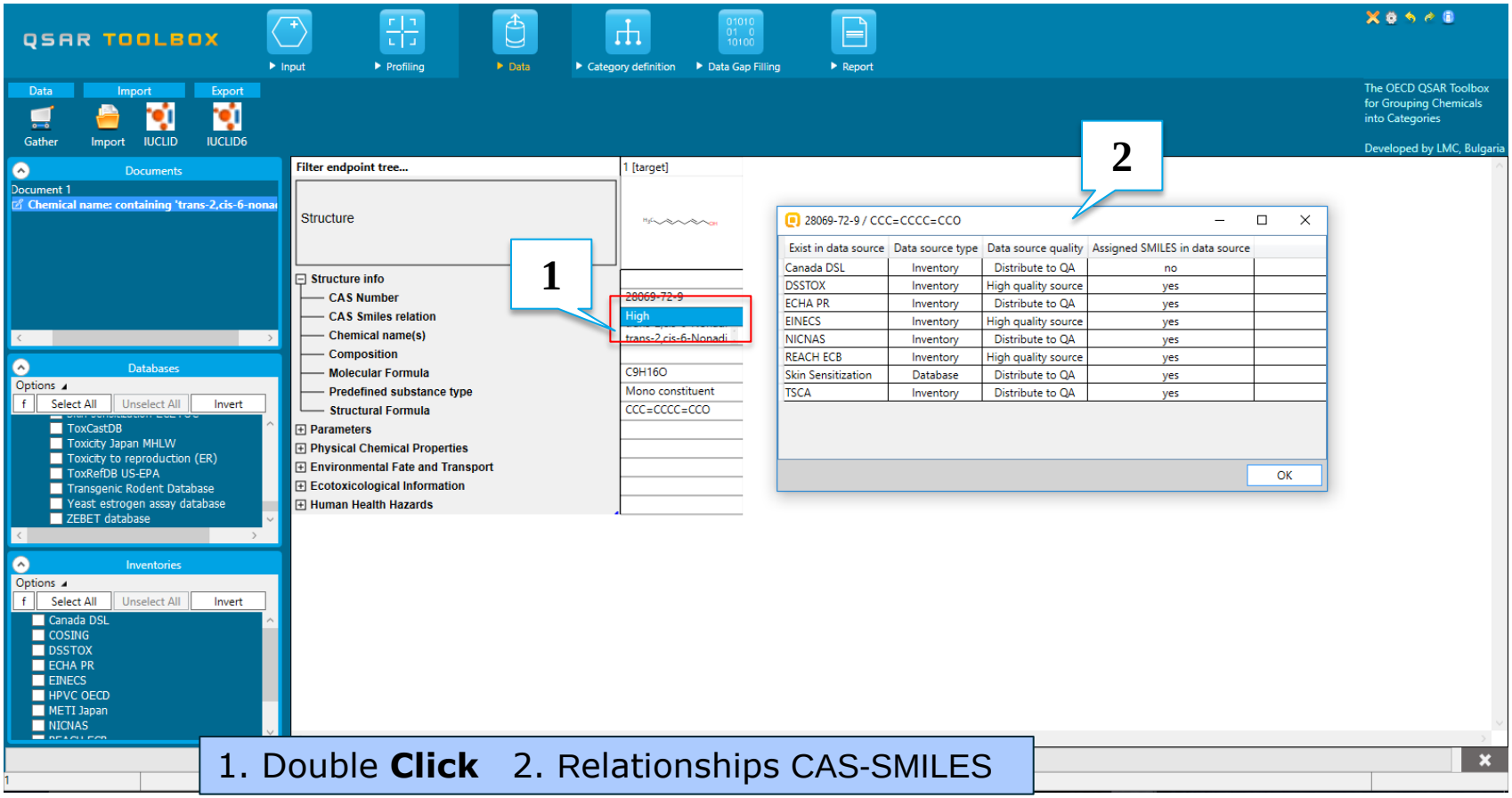


1. Create new document and Enter the **CAS# 97-53-0** In the blank field; 2. Click **Search button**; 3. Press **OK**

Chemical Input
Target chemical identity

- Double click “CAS Smiles ralation” 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



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.

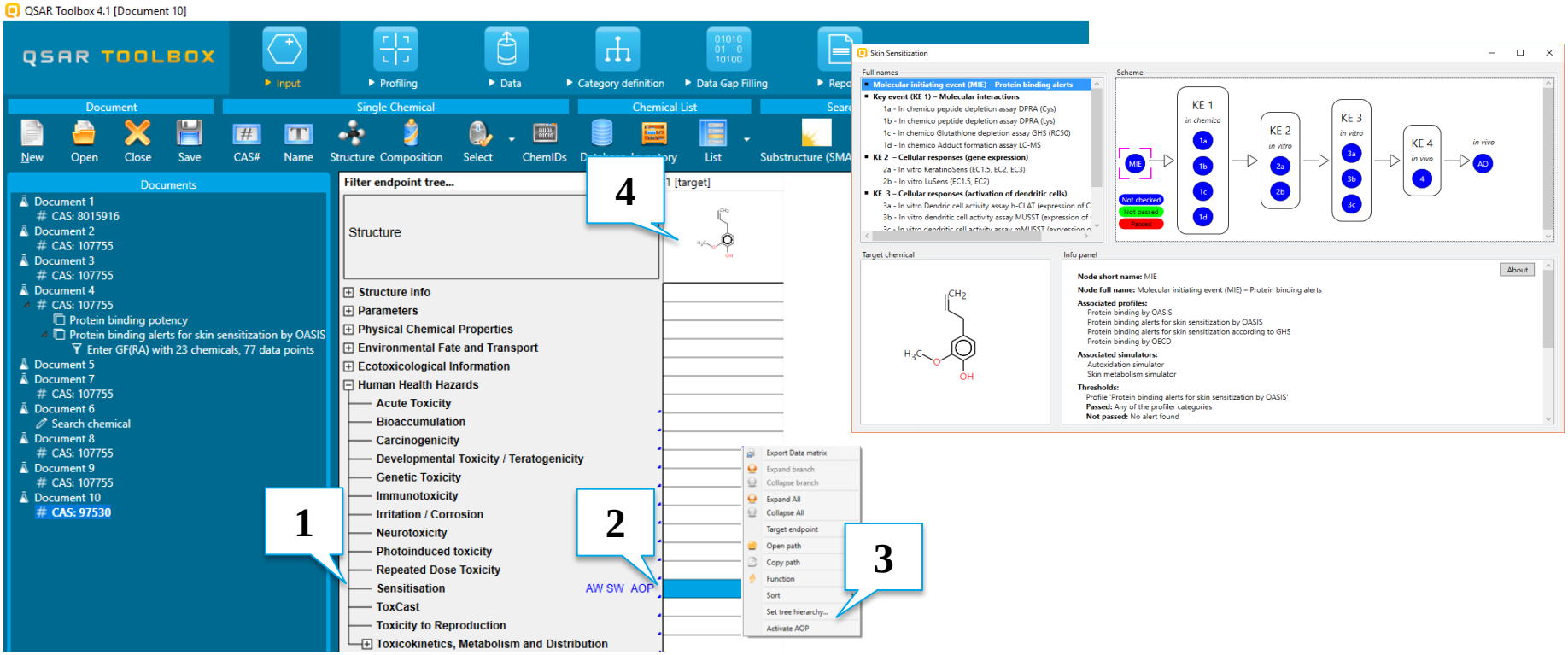
- **Low:** This reliability corresponds to poor reliability of CAS-SMILES relation. This label is assigned if the chemical belongs to less than three, but at least one "Distribute to QA" data sources.

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



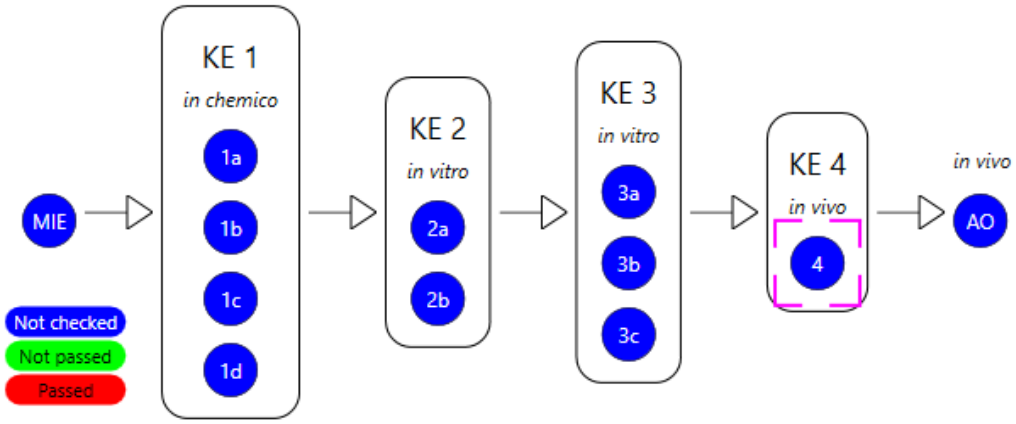
1. Expand **Human health hazard** part of the endpoint
2. Right click near the **AOP** label
3. Select **Activate AOP**
4. Set target for AOP (see slide 26)

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



MIE: protein binding

In chemico: protein binding potency assays

In vitro: gene expression assay

In vitro: cytokine profiles in dendritic cells

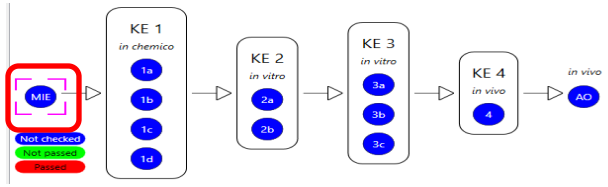
In vivo: Organ response (LLNA)

In vivo: Organism response (GPMT)

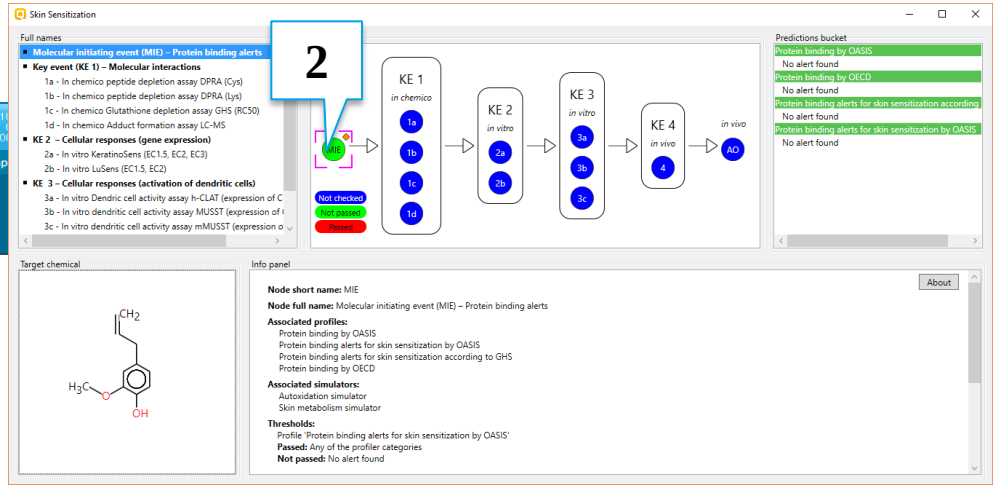
Workflow process

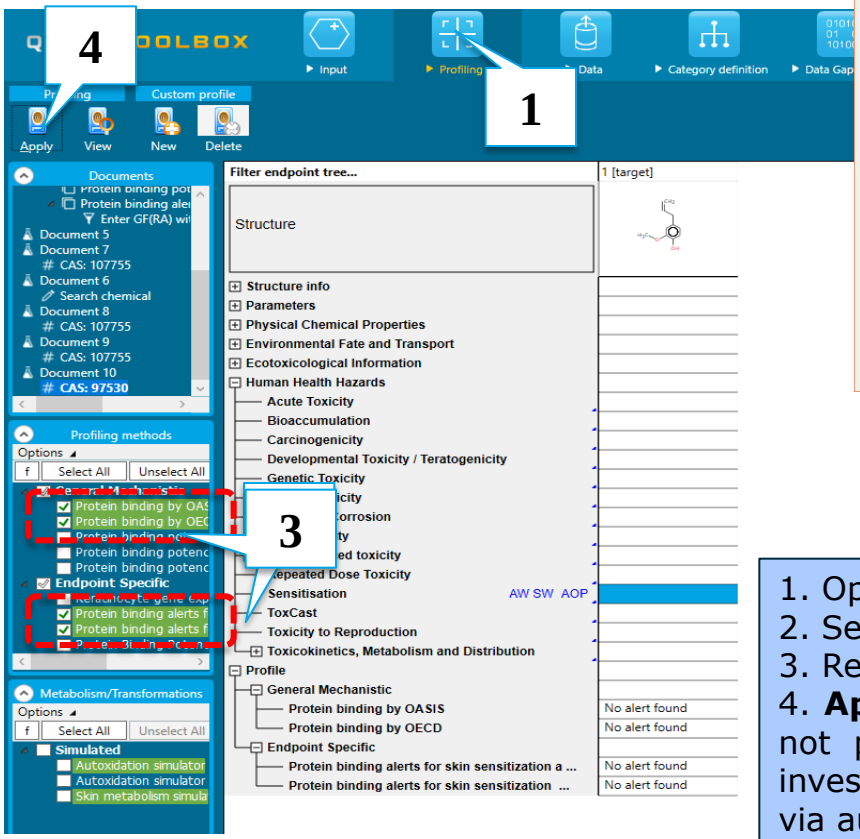
Step 1. MIE: protein binding

Example 2



Start with profiling of target chemical





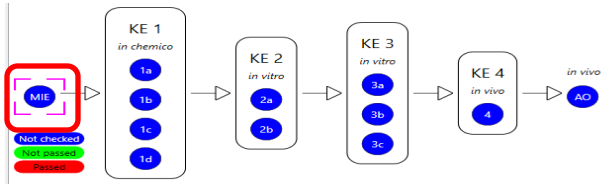
1. Open **Profiling**
2. Select node #**MIE**
3. Relevant profilers are highlighted, **select** the profilers
4. **Apply** selected profilers. The node is automatically changed to not passed based on absence of alert. The next step is to investigate whether the substance has skin sensitization potential via autoxidation

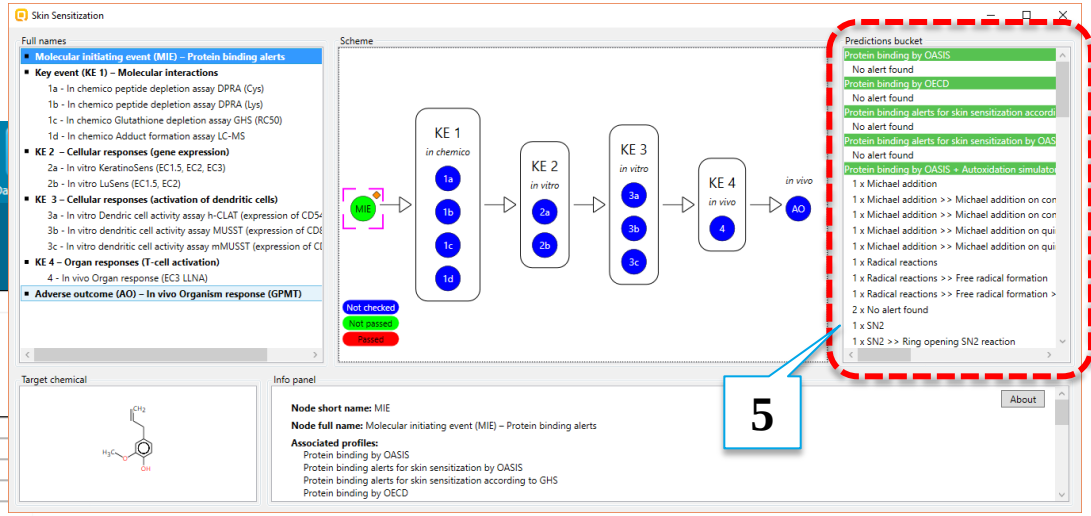
Workflow process

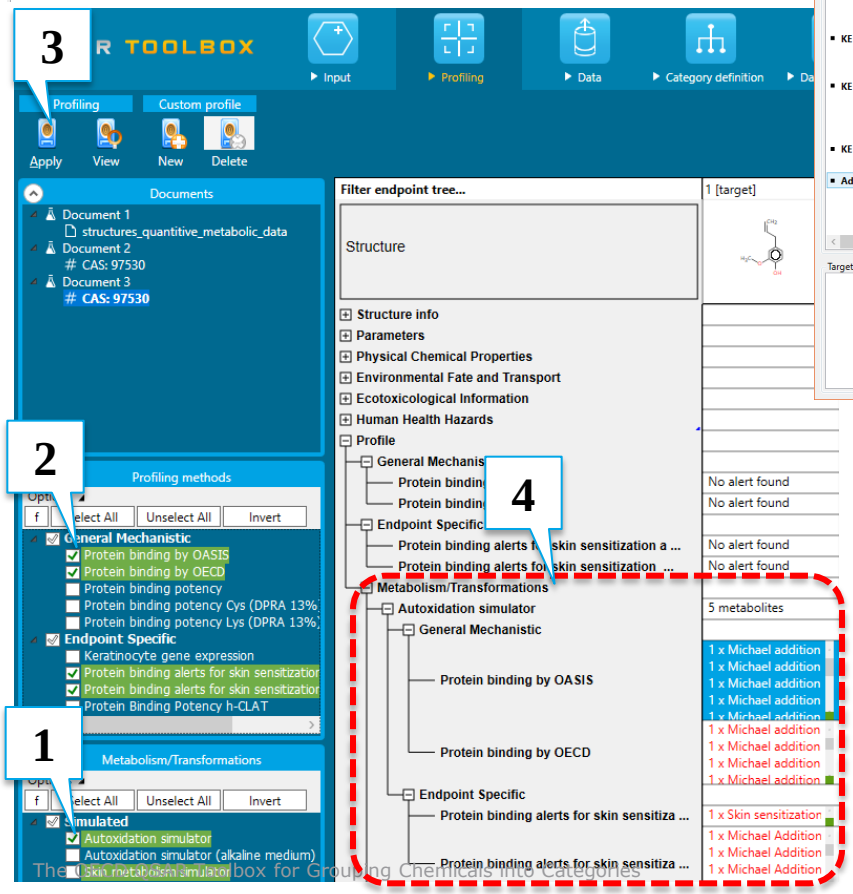
Step 1. MIE: protein binding

Example 2

Simulate Autoxidation products of the target chemical





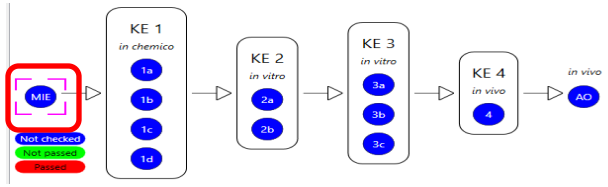


1. **Select** Autoxidation simulator.
2. **Select** highlighted profilers relevant to the MIE
3. **Click** Apply
4. The profiling results appeared on data matrix
5. The profiling results are also stored at the prediction bucket

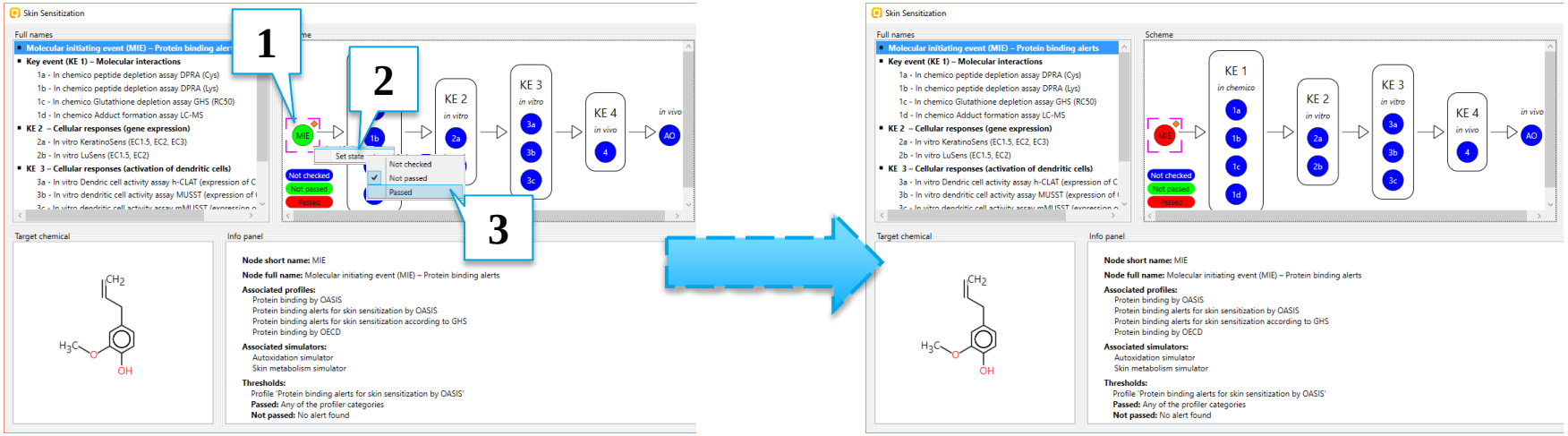
Workflow process

Step 1. MIE: protein binding

Example 2



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

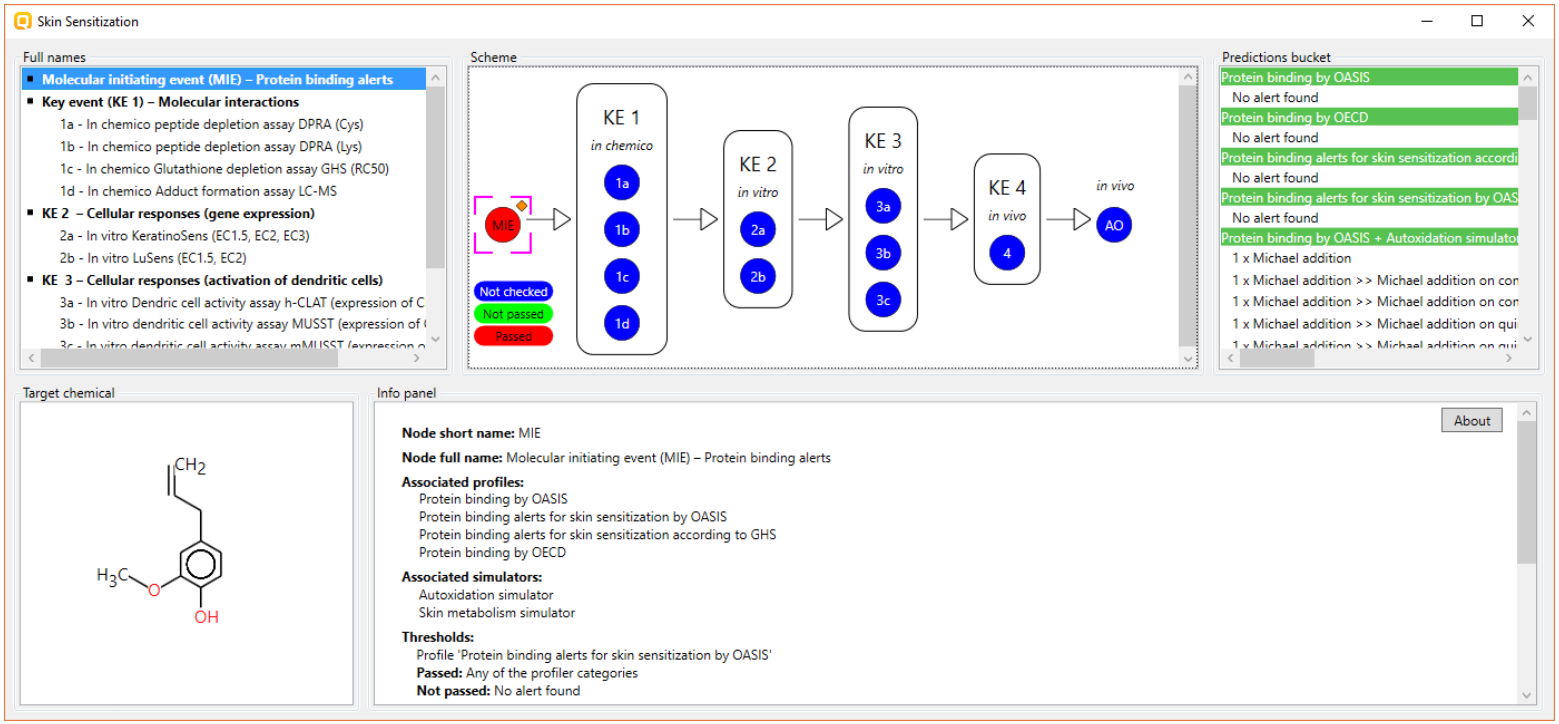


1. **Right** click on the **MIE node**
2. Select **Set state**
3. Change the state from **Not Passed** to **Passed**

Workflow process

Molecular initiating events

Example 2

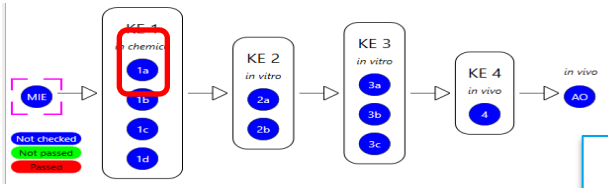


- The node MIE is passed due to the presence of positive protein binding alert identified for the Autoxidation products of the target chemical
- The workflow should move further to the *in chemico* assays

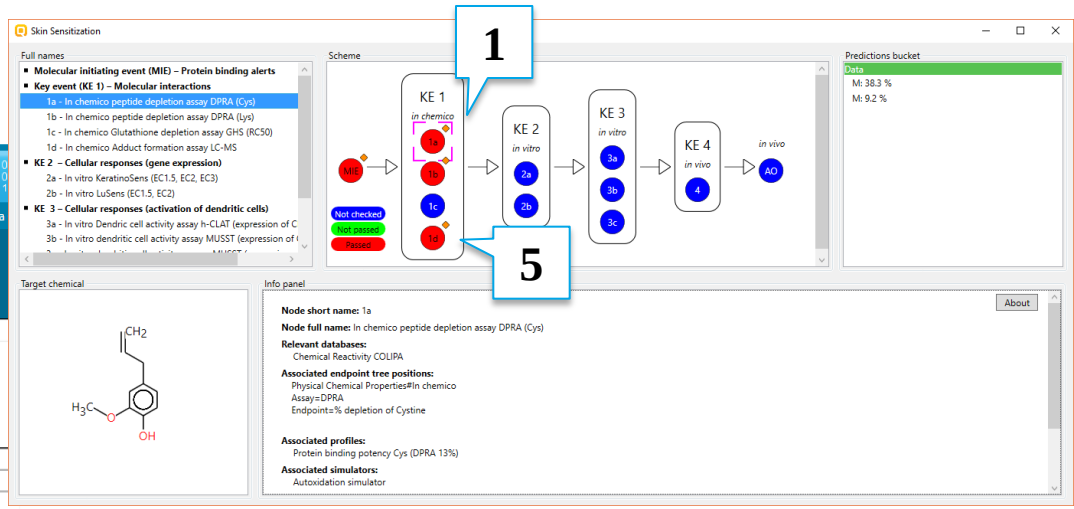
Workflow process

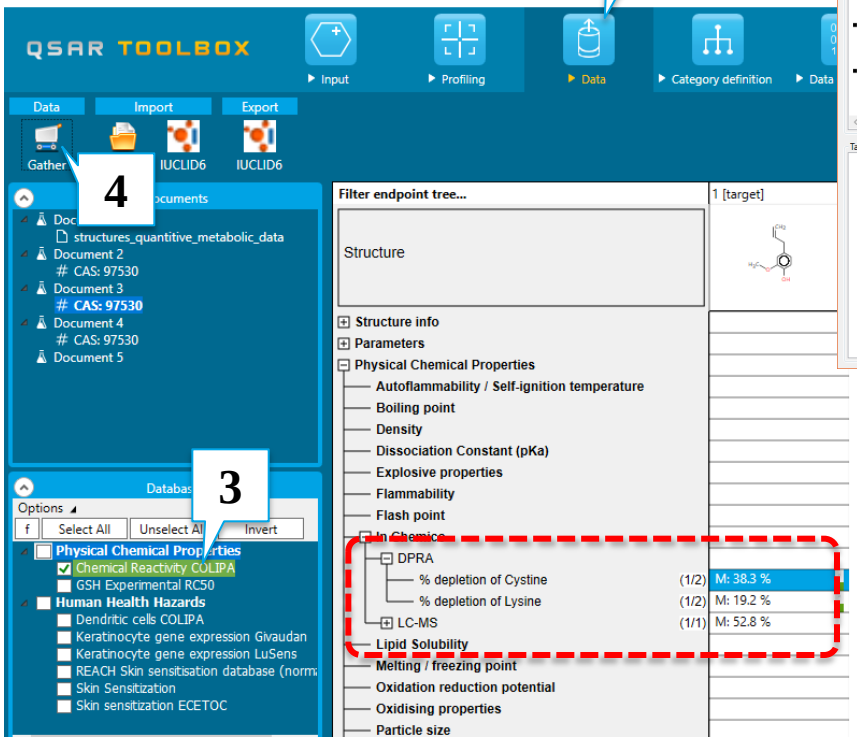
Step2. *In chemico* peptide depletion assay DPRA (Cys) (node 1a)

Example 2



Check for experimental data



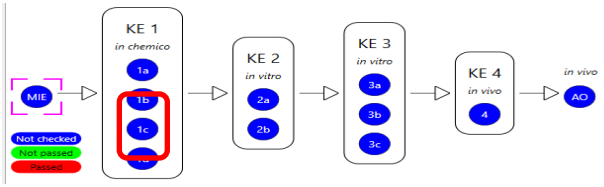


1. Select node **1a**
2. Go to **Data**
3. **Select** highlighted database
4. **Click** Gather
5. The selected database (Chemical reactivity COLIPA) is related to three AOP nodes - 1a, 1b and 1d. Data for the target for all 3 endpoints are found and the three nodes are marked as Passed (based on the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

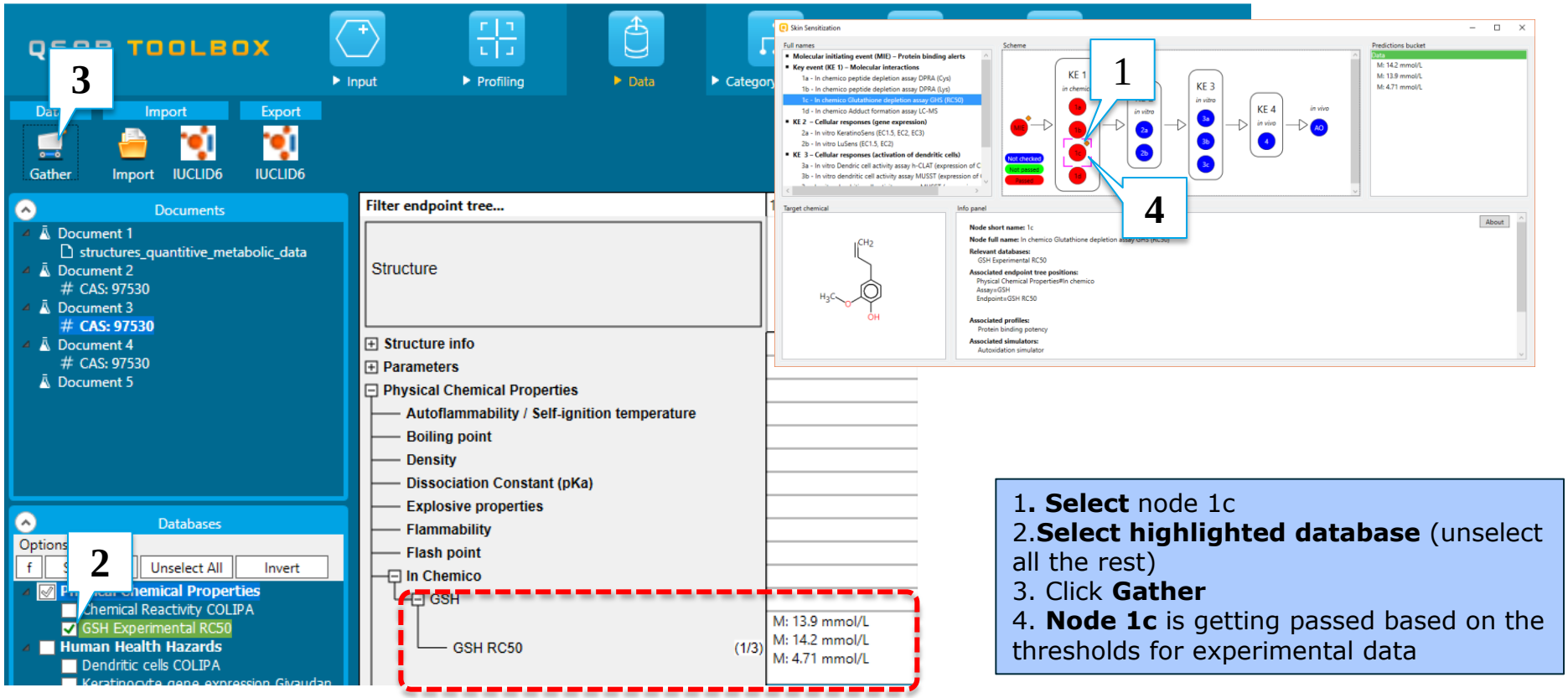
Workflow process

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

Example 2



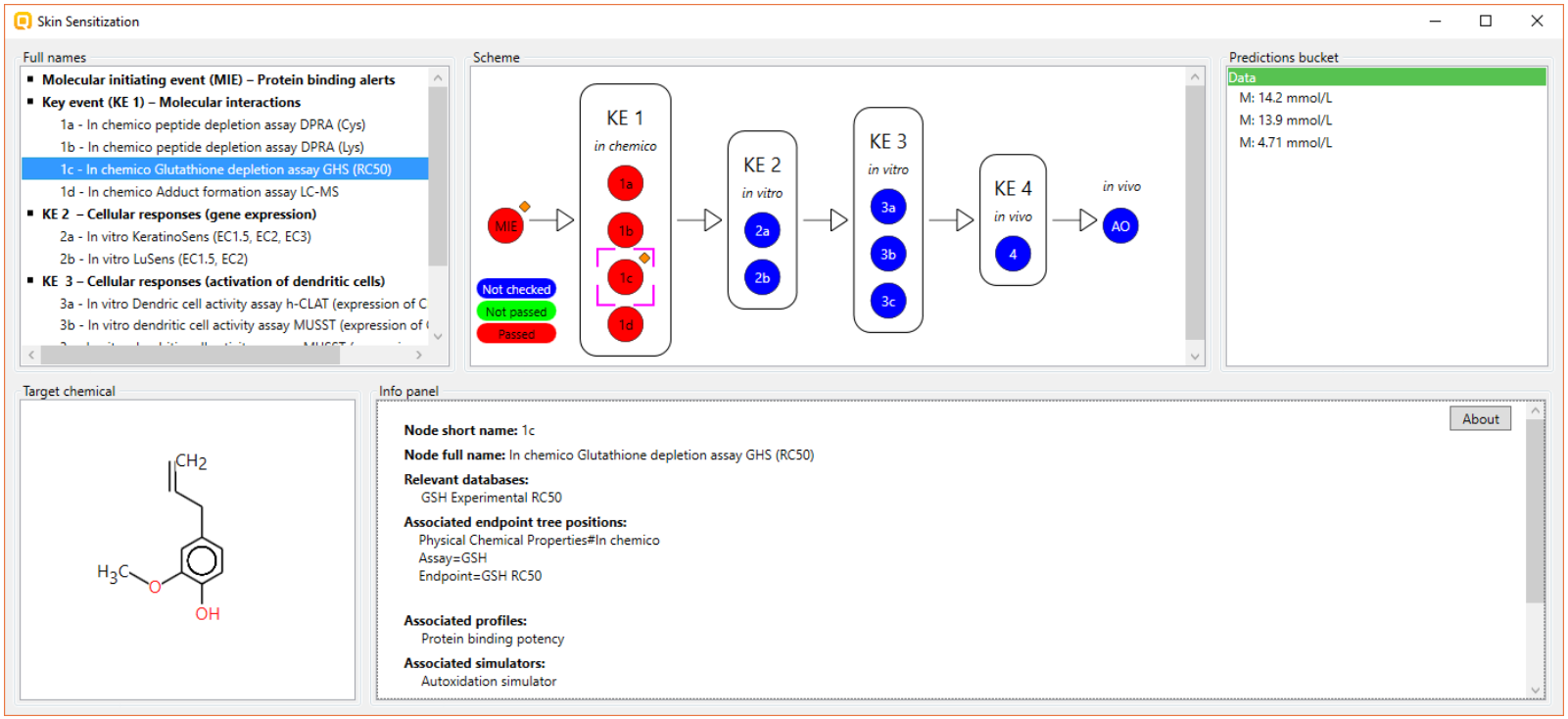
Check are there any data for the target chemical



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

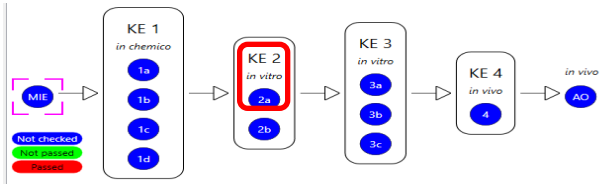


- The nodes related to the *in chemico* assays are passed due to positive experimental data for the target chemical (node 2a, 2b, 2c and 2d) The workflow should move further to the *in vitro* assay (node 2a and 2b)

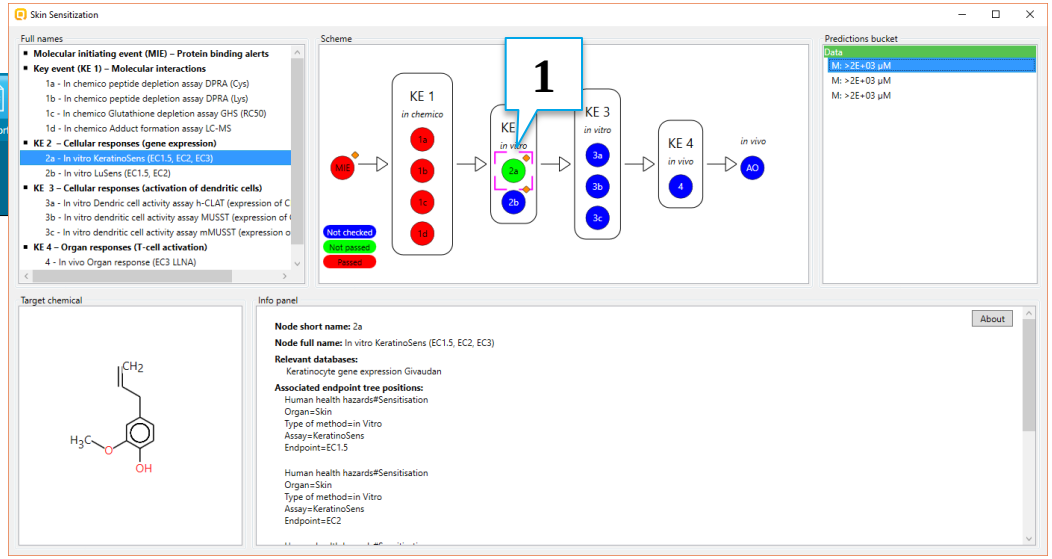
Workflow process

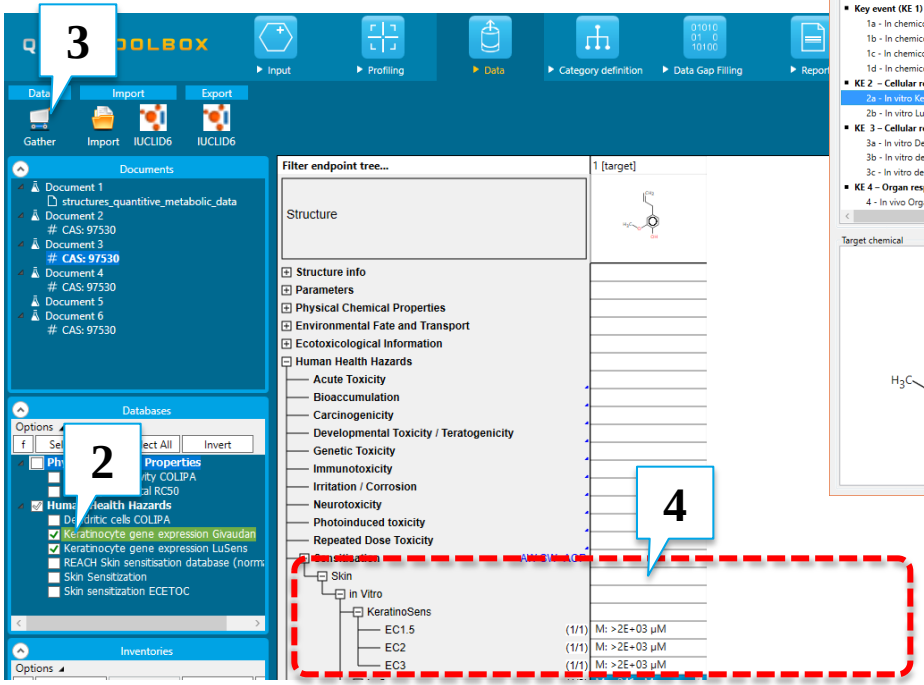
Step 3. *In vitro* KeratinoSens (EC1.5, EC2, EC3) (node 2a)

Example 2



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



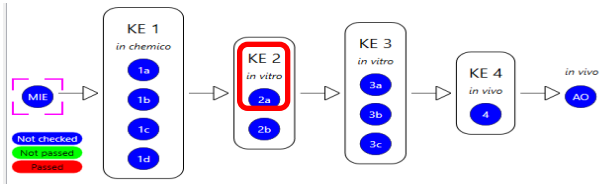


1. **Select** node 2a
2. **Select** highlighted database, unselect all other
3. **Click** Gather
4. There is experimental data for the parent chemical, which appears on data matrix. Node 2a is getting "Not Passed" based on the experimental data and implemented thresholds (see slide #15)

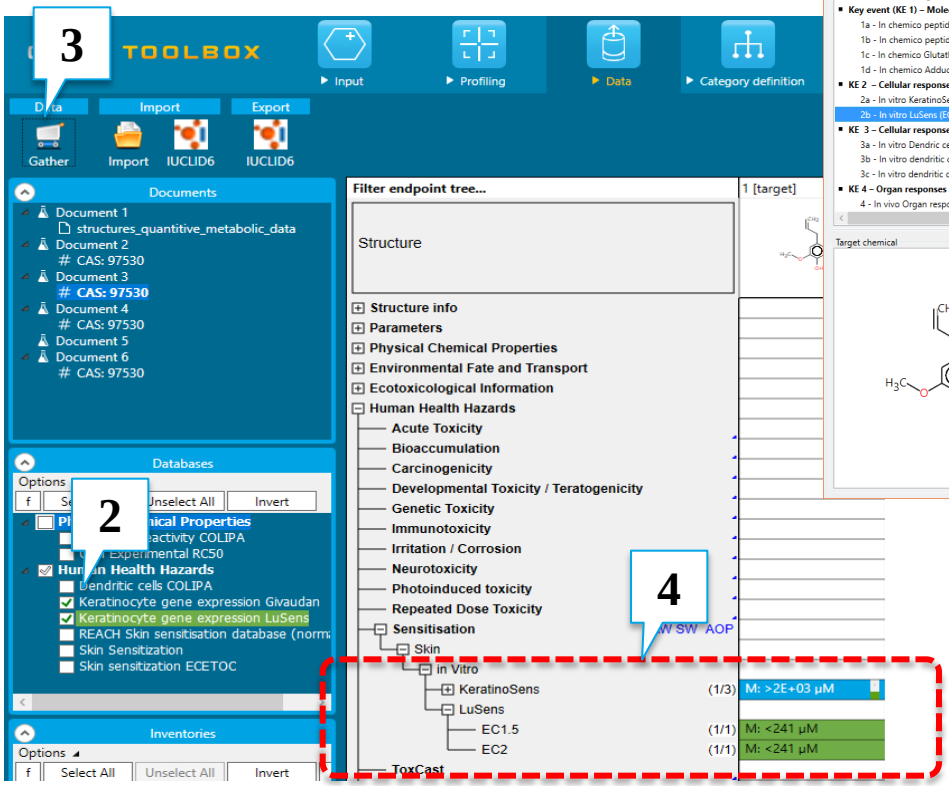
Workflow process

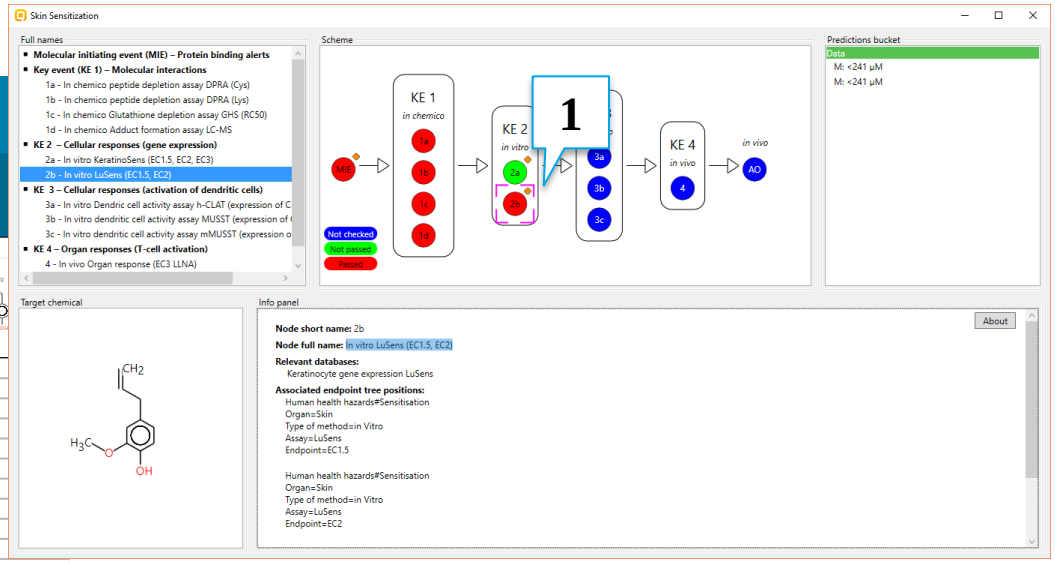
Step 3. *In vitro* LuSens (EC1.5, EC2) (node 2b)

Example 2



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



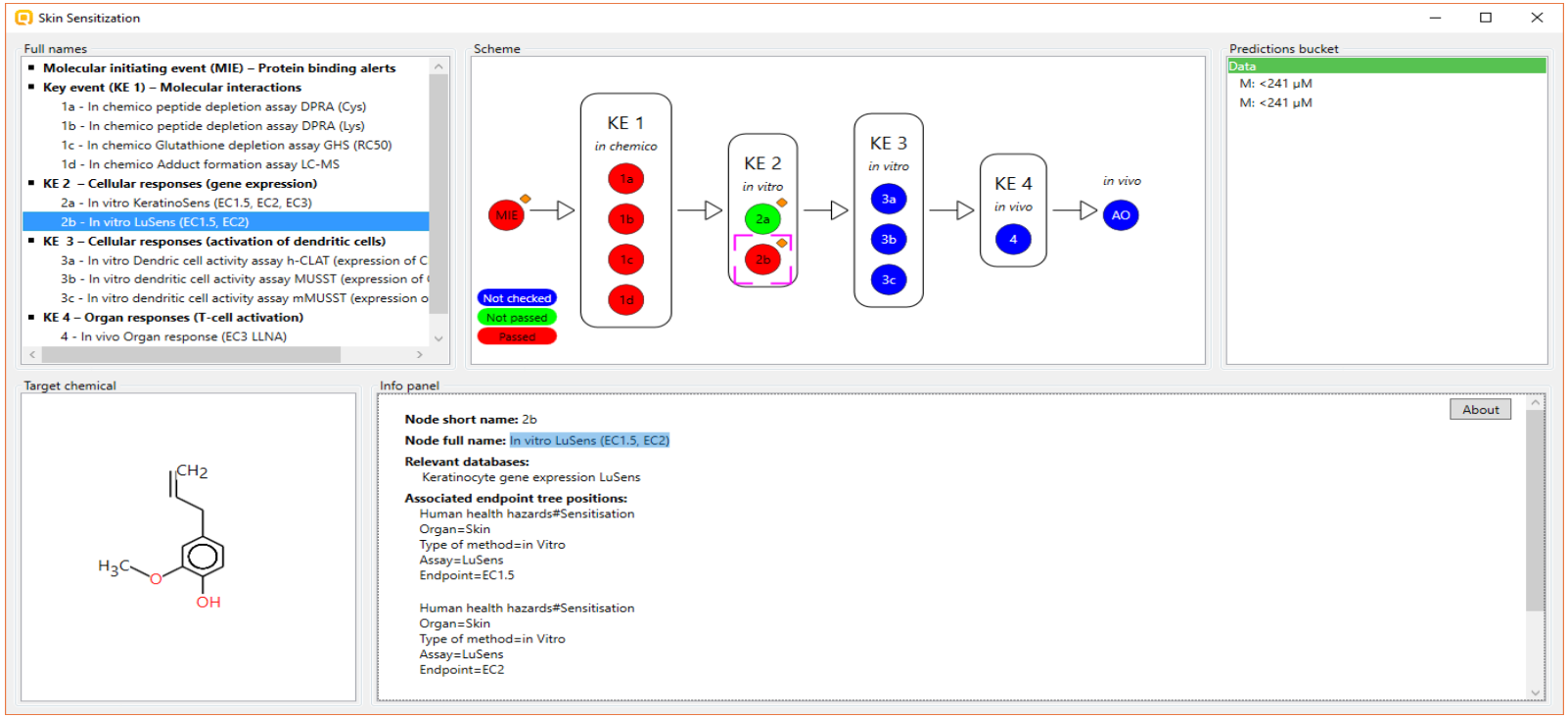


1. Select **node 2b**
2. Unselect all and select highlighted database
3. Click **Gather**
4. There is experimental data for the parent chemical, which appears on data matrix. Node 2b is getting "Passed" based on the experimental data and implemented threshold (see slide #15)

Workflow process

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

Example 2

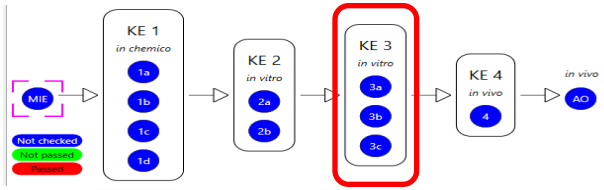


- The nodes 2a and 2b related to the Keratinocyte ARE (EC1.5, EC2, EC3) is passed based on the experimental data found for the target chemical (threshold are specified on slide # 15).
- The workflow moves further to the *in vitro* Dendritic cell assay (nodes 3a, 3b and 3c)

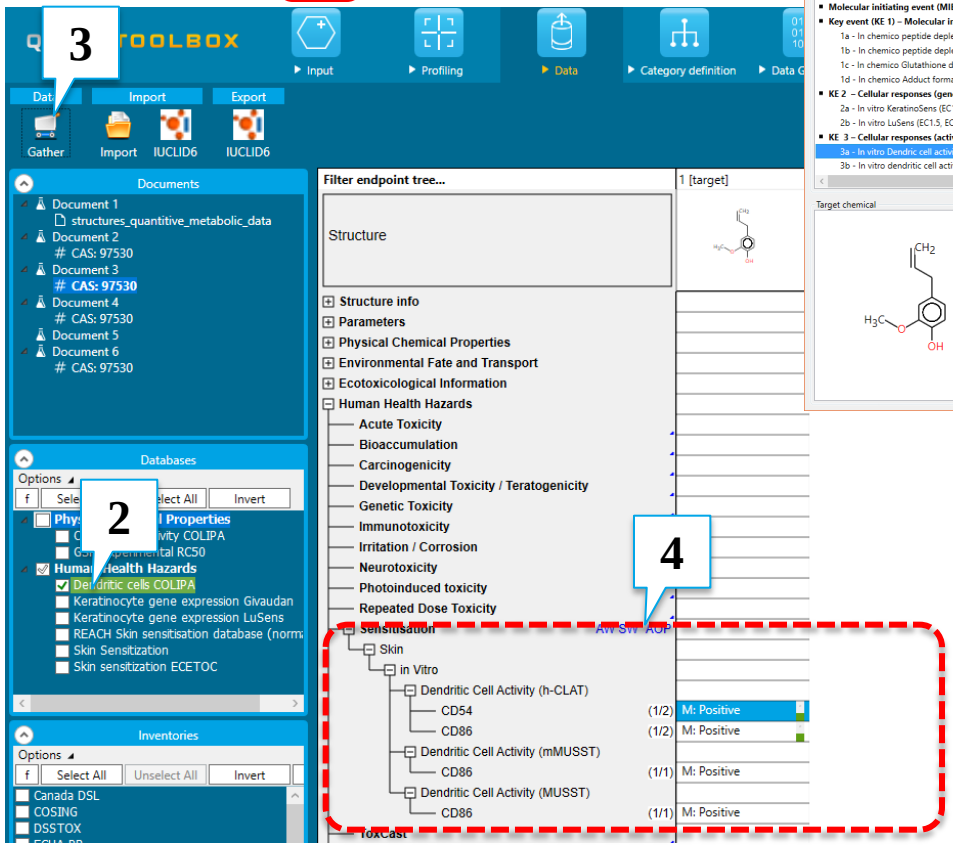
Workflow process

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* Dendric cell activity data

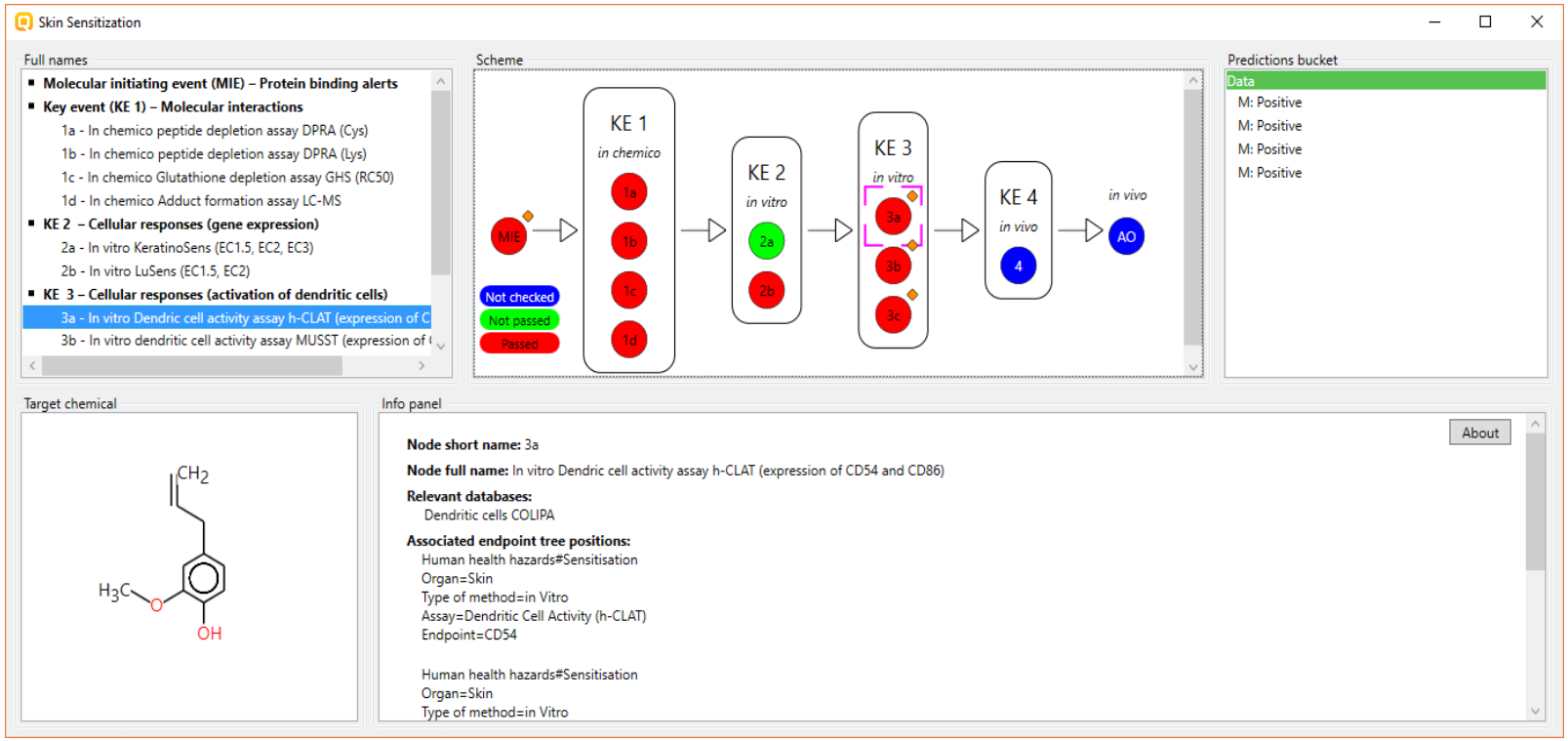


1. Select **node 3a**
2. Select database related to node 3a
3. **Gather** data and click **OK** in the appeared message
4. The experimental data appears on Data matrix and the status of nodes 3a, 3b and 3c was changed to **Passed**

Workflow process

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

Example 2

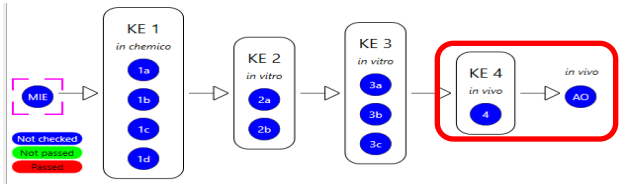


- The node 3a, 3b and 3c related to the *in vitro* Dendritic cell activity assays (h-CLAT, MUSST and mMUSST) are getting passed due to positive experimental data found for the target chemical
- The workflow could further move to the *in vivo LLNA* assay (nodes 4)

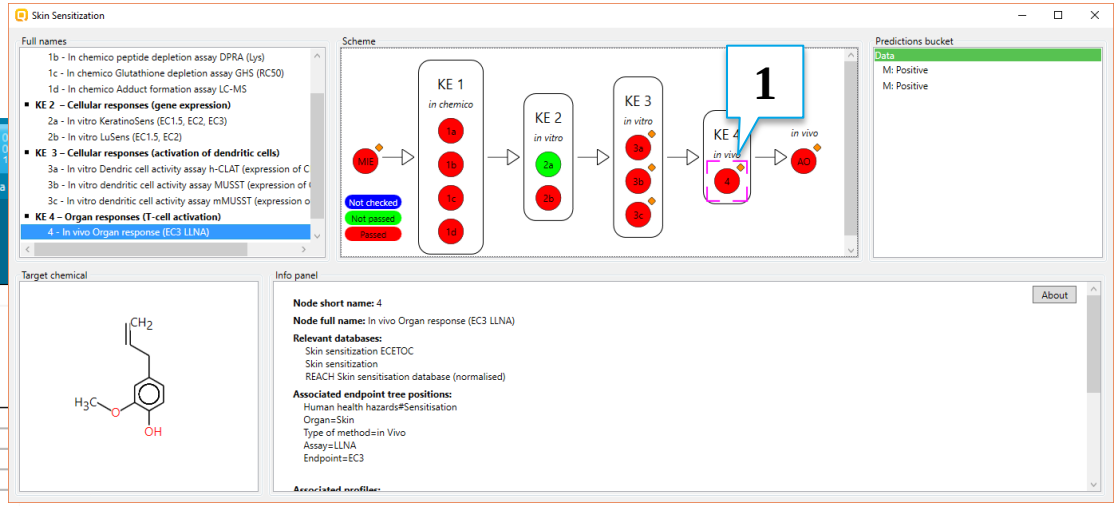
Workflow process

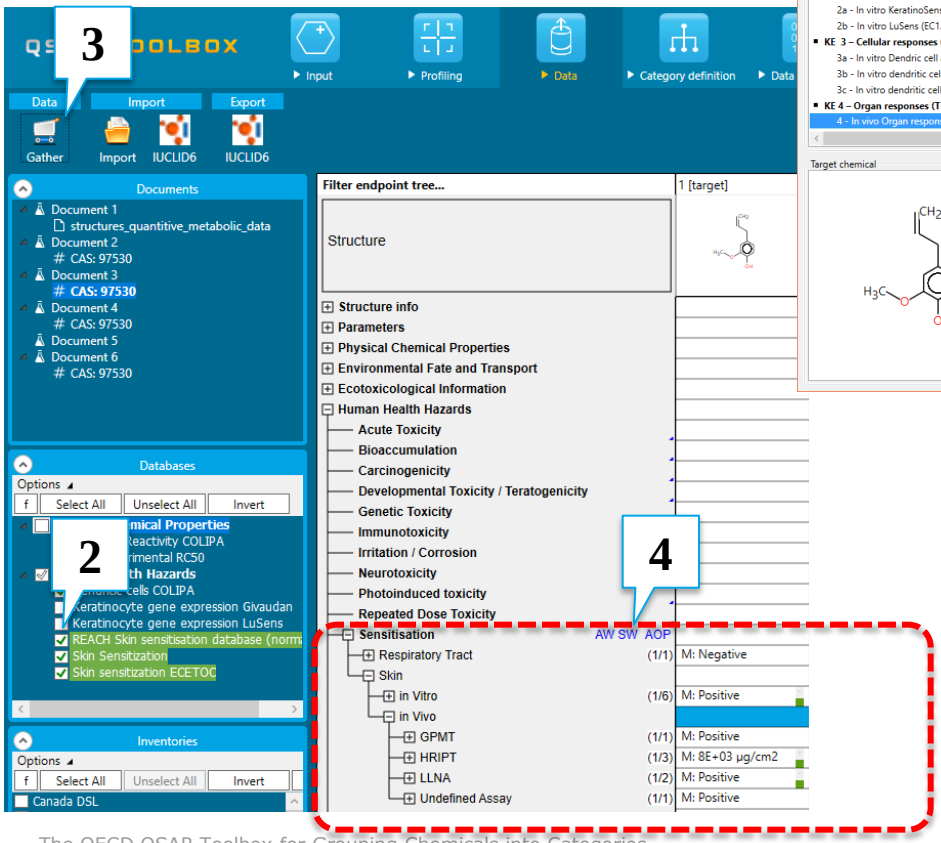
Step 5. I*n vivo* Organ response (LLNA)(node 5)

Example 2



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



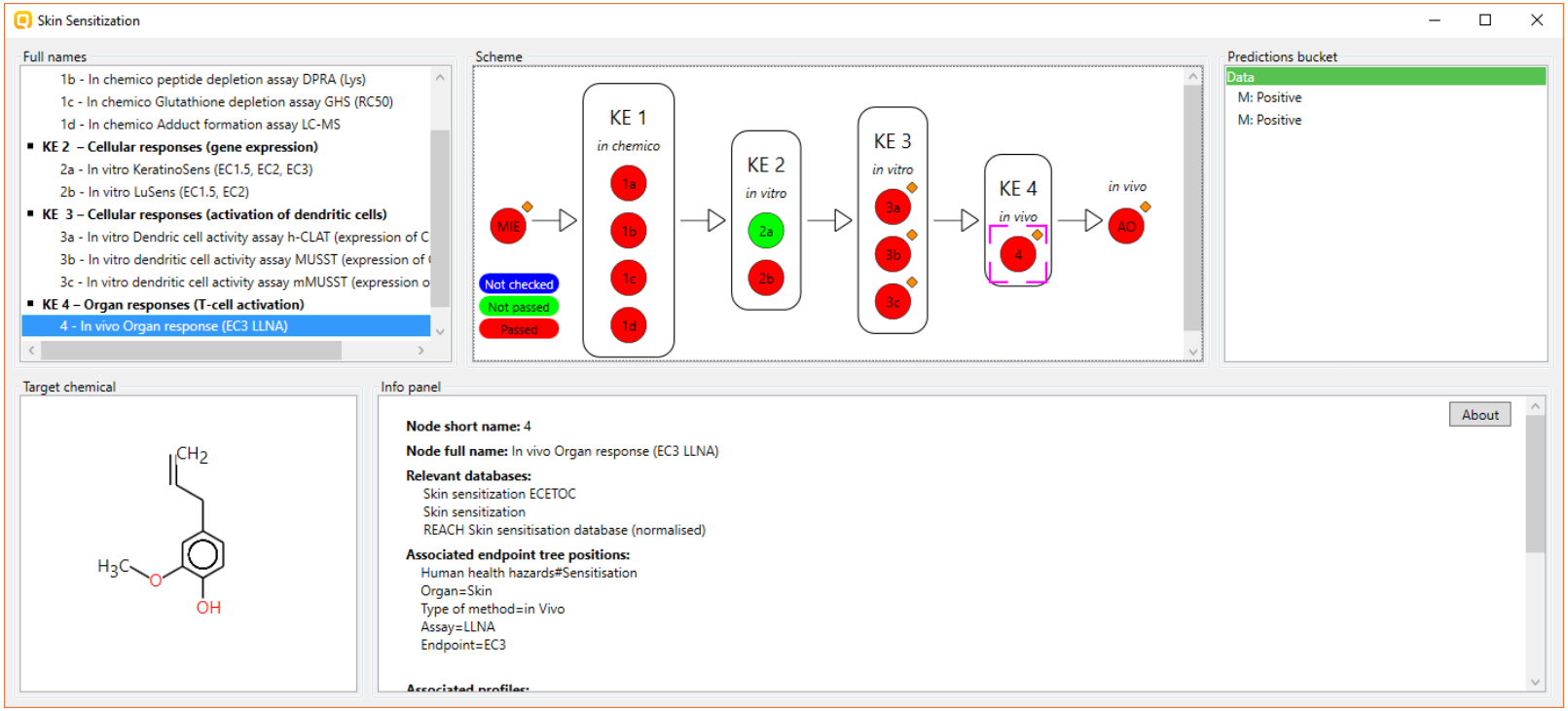


1. **Select** node 4
2. **Select** database related to the node 4
3. **Click** Gather
4. Nodes 4 and AO is getting passed based on experimental data extracted for the target chemical

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.