QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.3.2

How to use the Toolbox AOP workflow for Skin Sensitization

Outlook

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

Background
AOP concept and description

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

*Slide presented on last MG WebEx (April 2013)

Background
AOP concept and description *(contd.)*

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

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Objectives

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

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

**Demonstrated examples are obtained with Toolbox v3.2*

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

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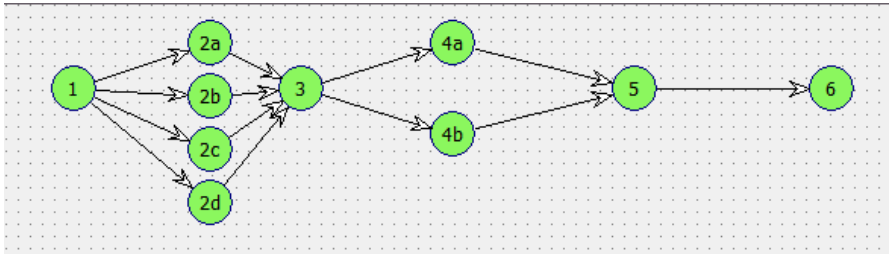
Overview of implemented AOP scheme



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

Outlook

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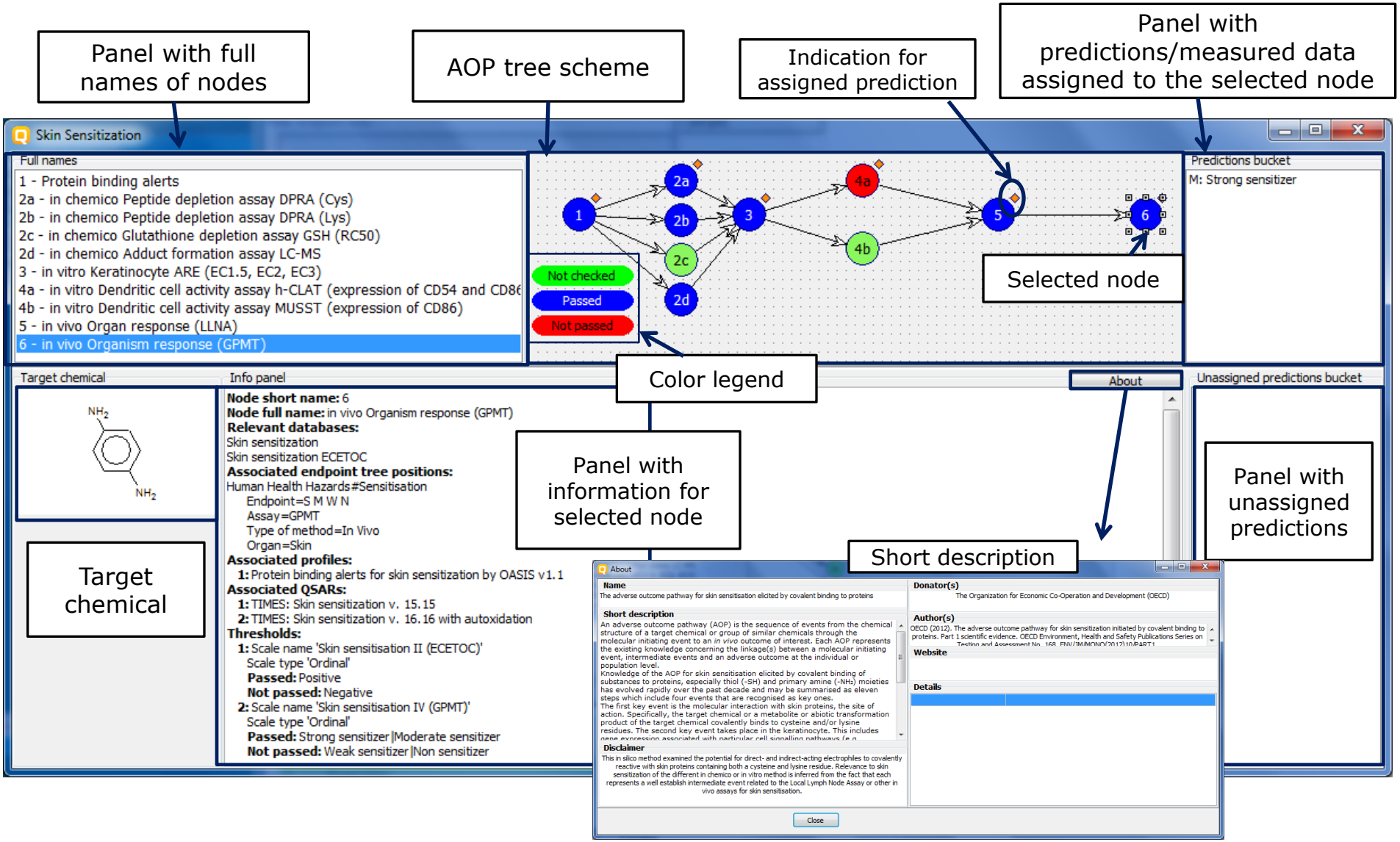
- **Overview of AOP scheme as implemented in the Toolbox**
  - **Details of AOP window**
  - AOP workflow for skin sensitization
  - Thresholds of the node of AOP
- The exercise

Overview of the AOP scheme as implemented in Toolbox

Details of AOP window



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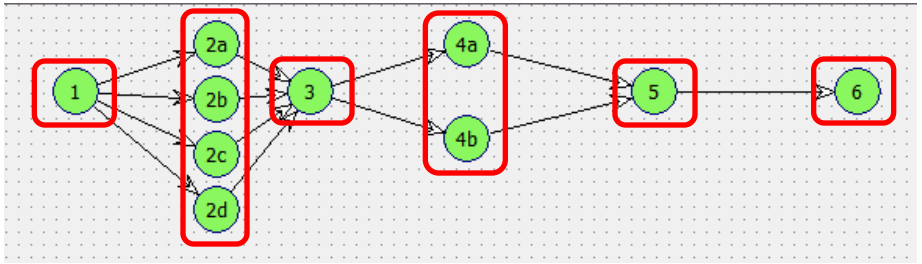
Overview of the AOP scheme as implemented in Toolbox

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 ARE

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

In vivo: Organ response (LLNA)

In vivo: Organism response (GPMT)

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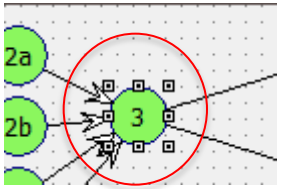
Overview of the AOP scheme as implemented in Toolbox

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:
1: Scale name 'Keratinocytes gene expression EC (ordinal)'
Scale type 'Ordinal'
Passed: Very High|High|Moderate|Low
Not passed: Negative



Overview of the AOP scheme as implemented in Toolbox

Implemented thresholds for the AOP nodes

Node name	Data thresholds	Node status: Pass	Node status: Not pass
1- Protein binding alerts		presence of alert	absence of alert
2a and 2b *in chemico* DPRA Cys and Lys	Peptide depletion, PD (%) > 80 - High 40%≥ PD ≤80% – Moderate 5%≥ PD ≤40% – Low 5%< PD – Not reactive	High |Moderate | Low	Not Reactive
2c - *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
2d - *in chemico* Adduct formation assay LC-MS	Adduct formation (%) ≥ 30% - Positive Adduct formation (%) < 30% - Negative	Positive	Negative
3 - *in vitro* Keratinocyte ARE (EC1.5, EC2, EC3)	EC3 (%) ≤ 20 – Very High 20 > EC3 ≤ 50 – High 50 > EC3 ≤ 100 – Moderate 100 > EC3 ≤ 2000 – Low EC3 > 2000 - Negative	Very High |High| Moderate | Low	Negative
4a and 4b *in vitro* Dendritic cell activity assay h-CLAT and MUSST (expression of CD54 and CD86)	expression of CD54 and CD86 Positive Negative	Positive	Negative
5 - *in vivo* Organ response (LLNA)	0 ≥ EC3 (%) <50 – Positive EC3 > 50 - Negative	Positive	Negative
6 - *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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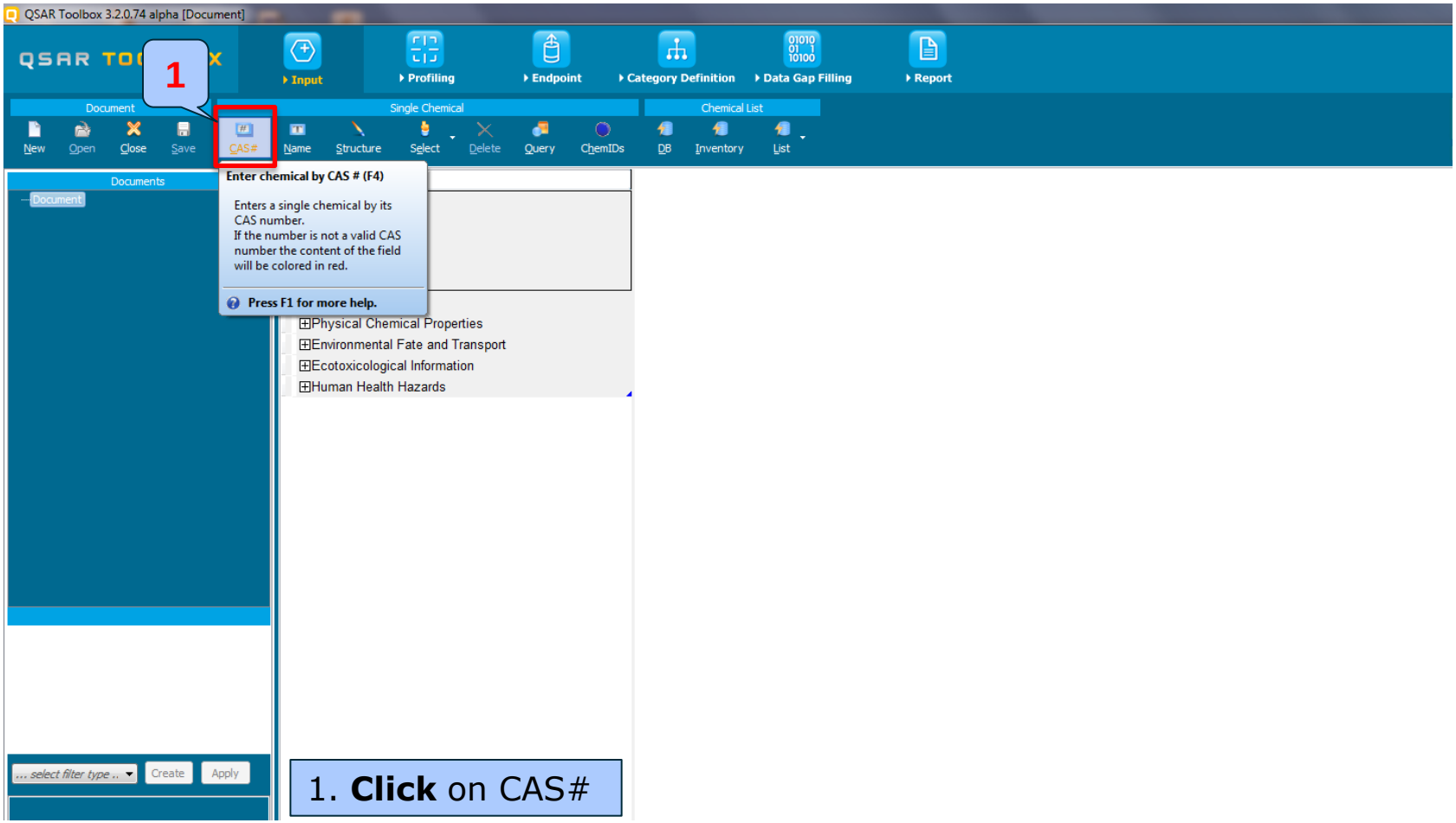
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - **Input**

Chemical Input
Input Screen

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

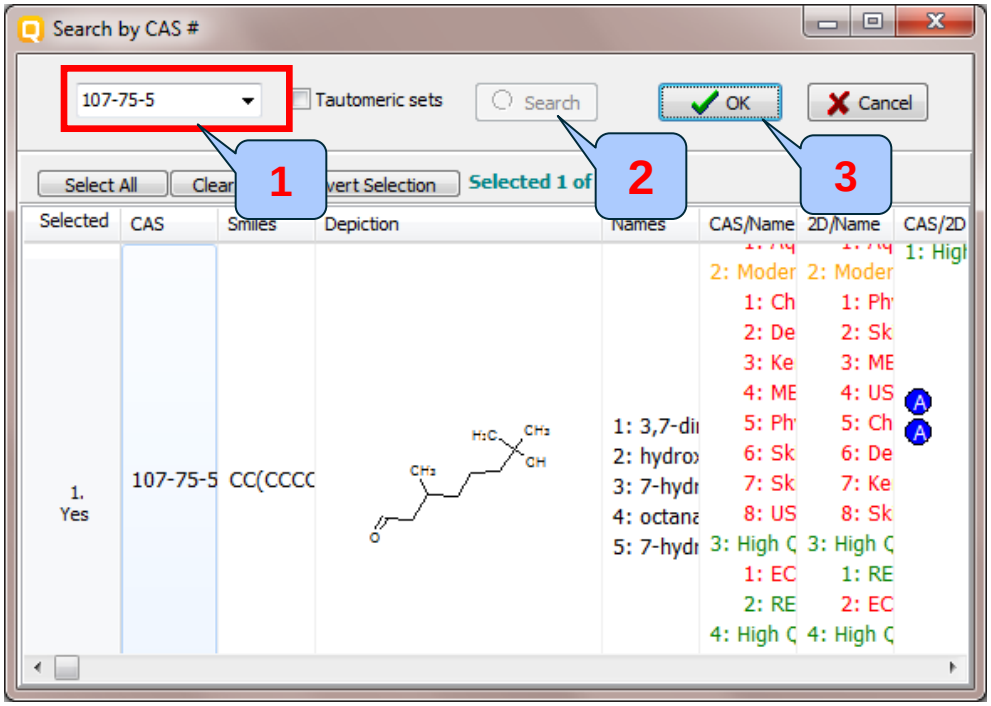
Chemical Input

Input target chemical by CAS#



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

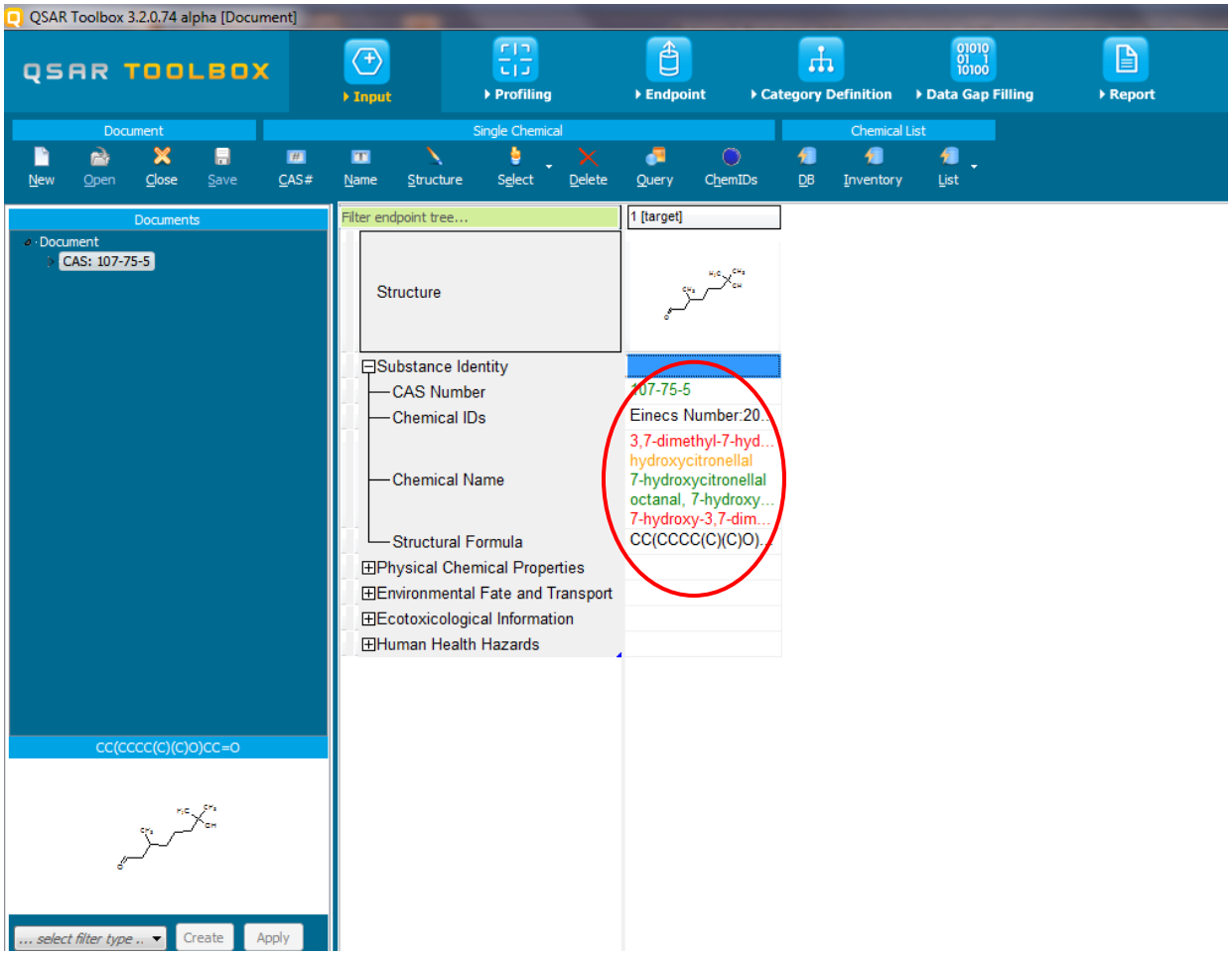


1. **Enter** the CAS# In the blank field; 2. **Click** Search button; 3. **Press** OK

Chemical Input
Target chemical identity

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

Chemical Input
Target chemical identity



Chemical Input
Target chemical identity

The colour code indicates the reliability of the chemical identifier:

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

Outlook

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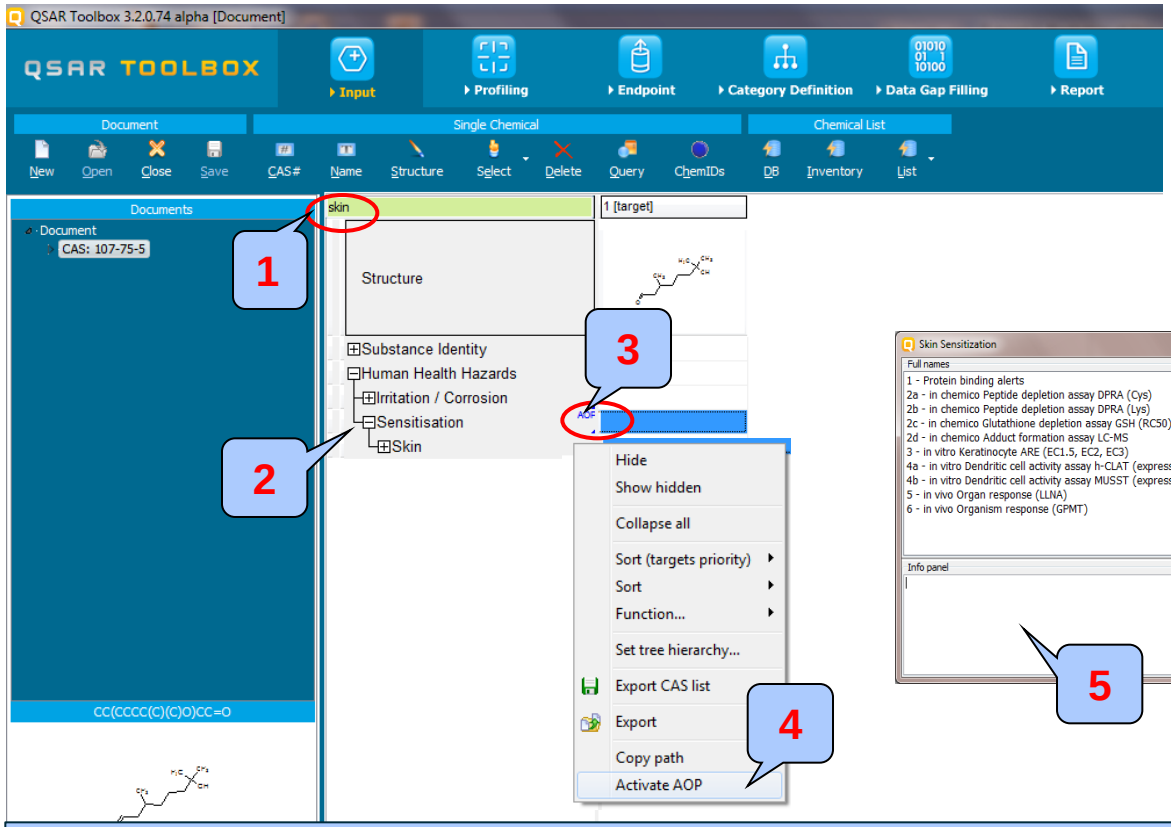
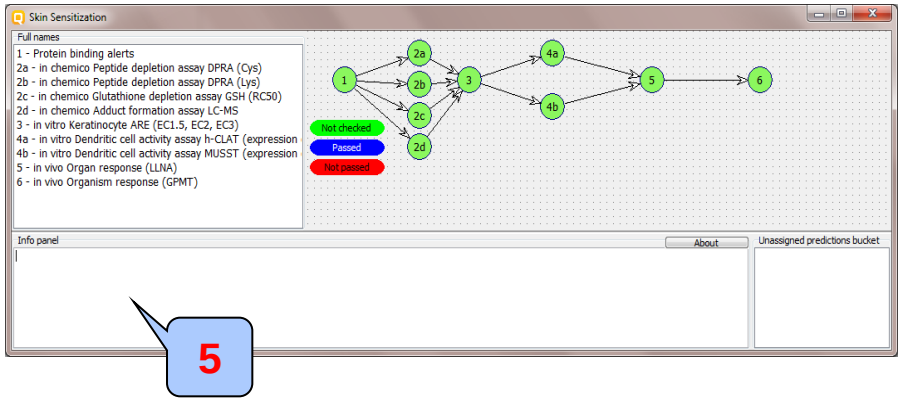
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - Input
    - **Activate AOP and set target**

Activate AOP

Set target chemical for AOP



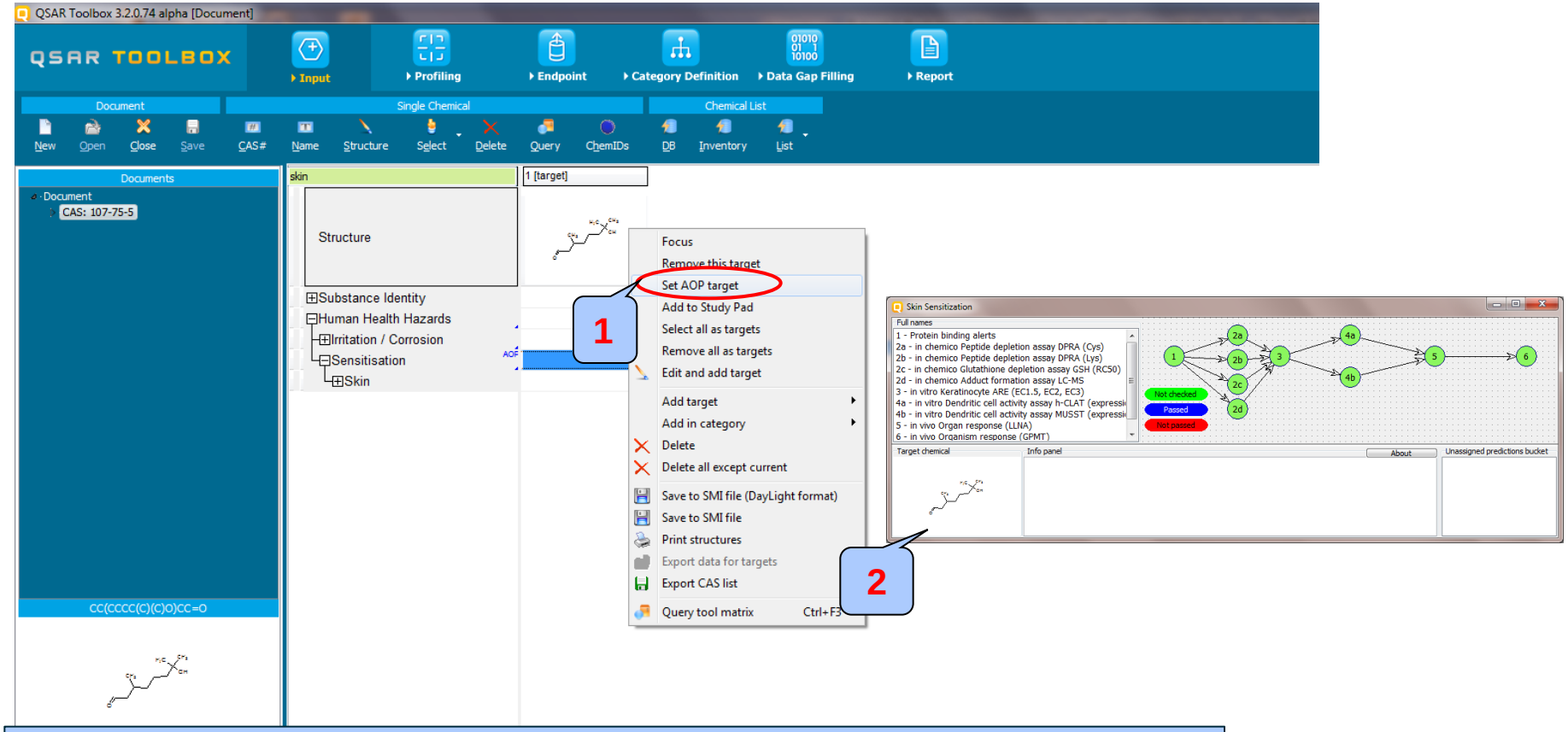


1. Filter endpoint tree – **write skin** in the green filed
2. **Expand the tree –** open the tree to the Sensitization node
3. **Right click** near the AOP label
4. **Select** activate AOP 5. AOP window appears

Continued on the next slide

Activate AOP

Set target chemical for AOP



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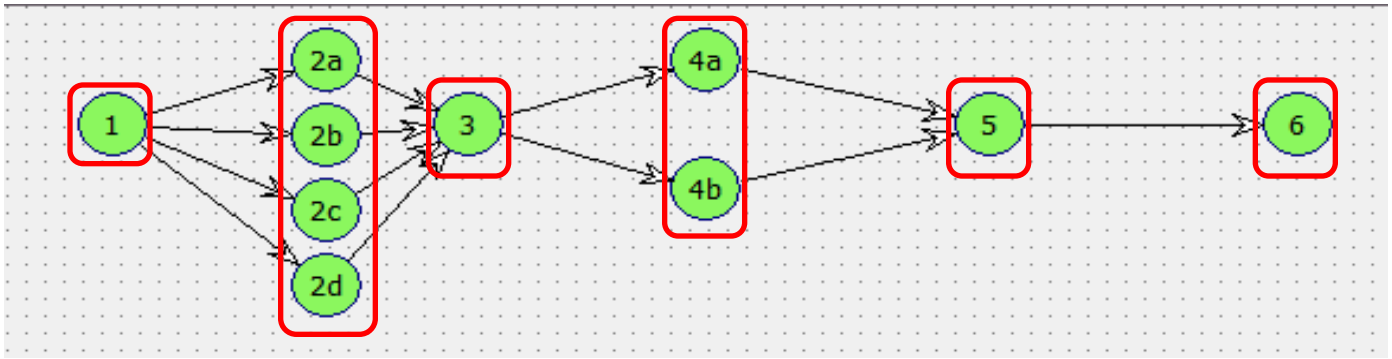
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - Input
    - Activate AOP and set target
    - **Workflow process**

Workflow process

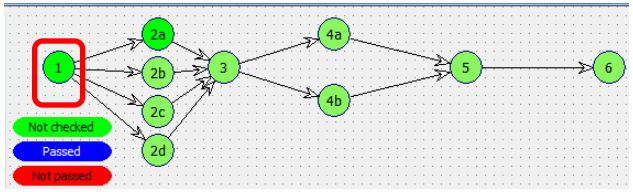
- Workflow process start from molecular initiating event to the *in vivo* organism respond



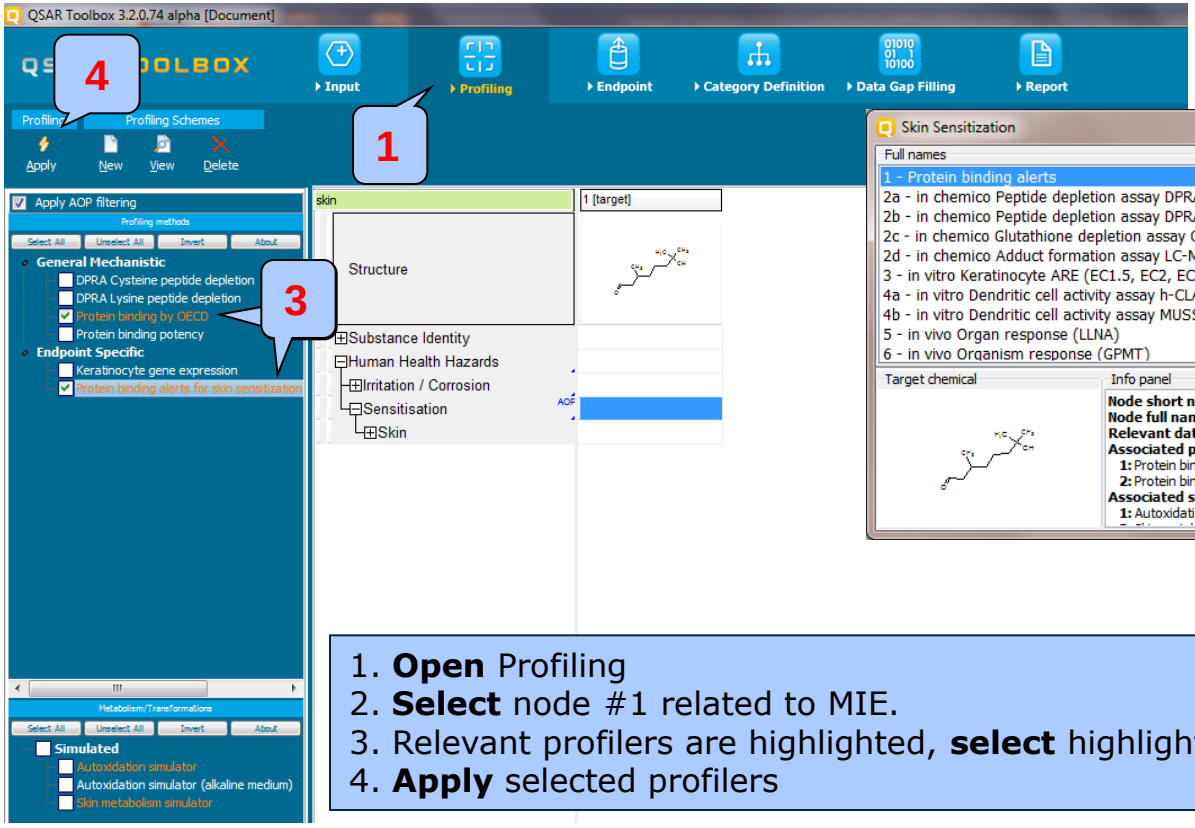
Workflow process

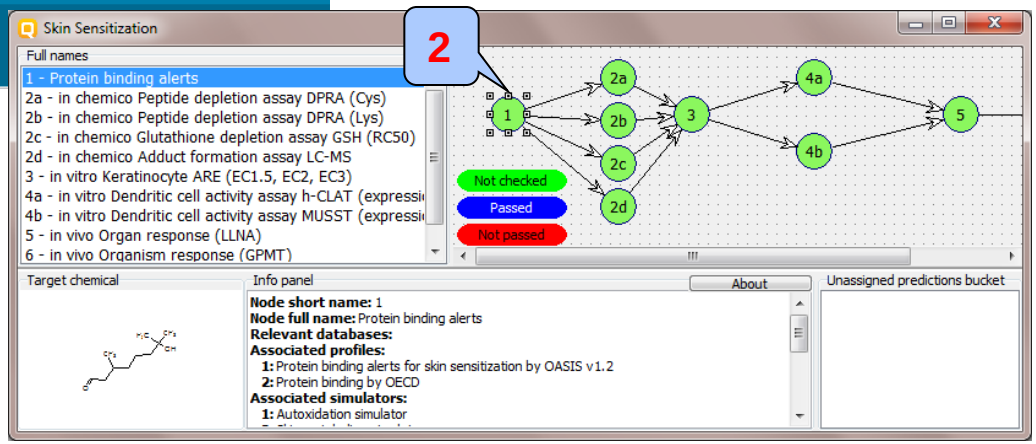
Step 1. MIE: protein binding

Example 1



Start with profiling of target chemical



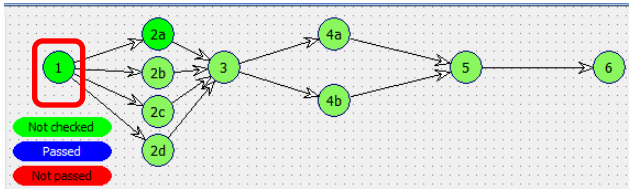


1. **Open** Profiling
2. **Select** node #1 related to MIE.
3. Relevant profilers are highlighted, **select** highlighted profilers
4. **Apply** selected profilers

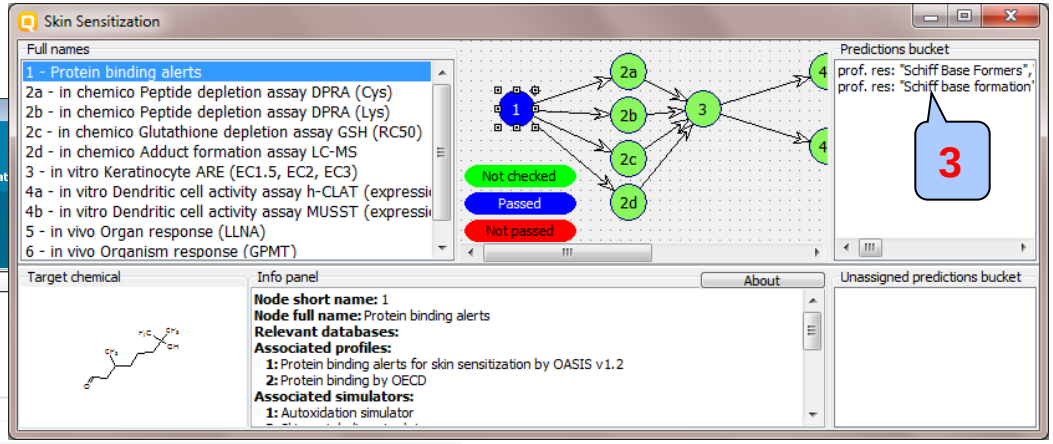
Workflow process

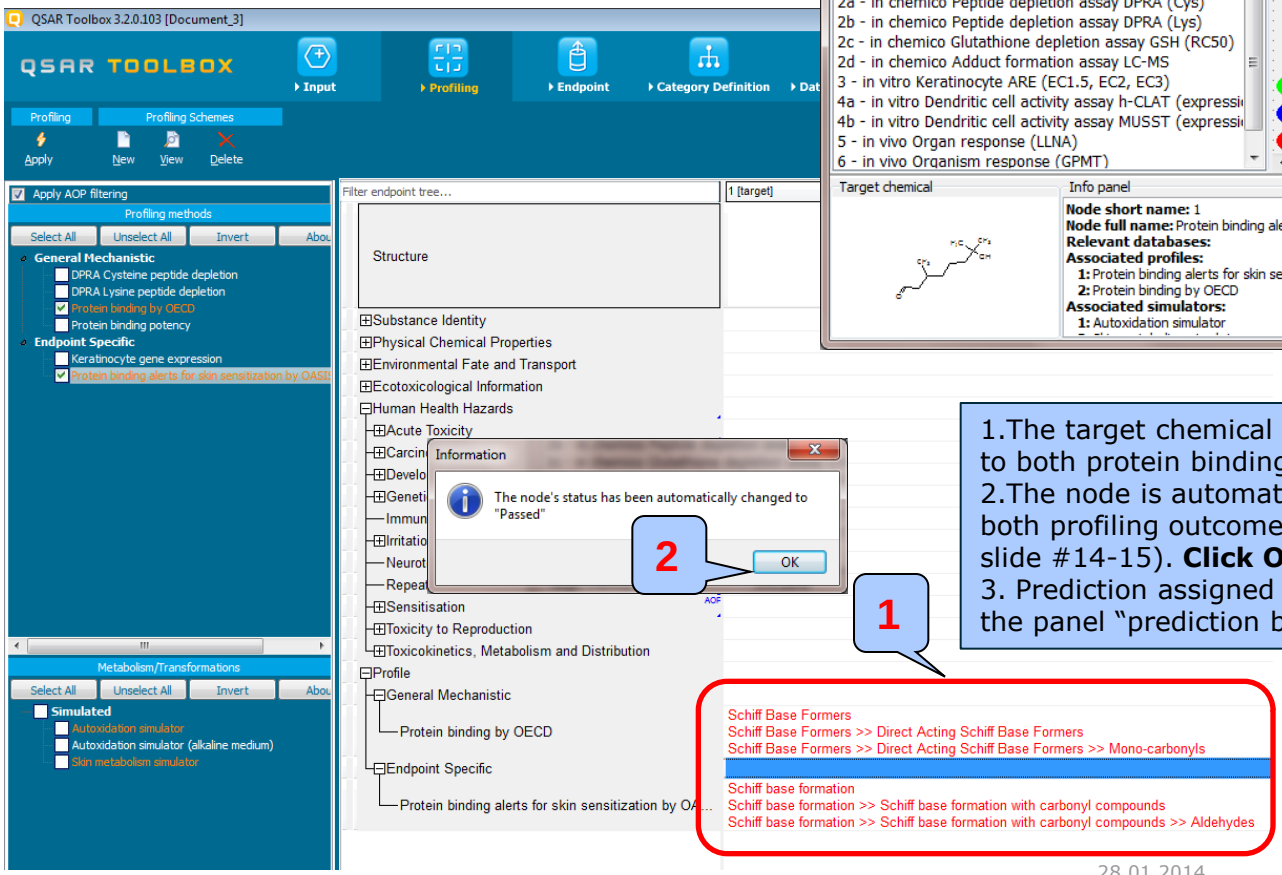
Step 1. MIE: protein binding

Example 1



Start with profiling of target chemical



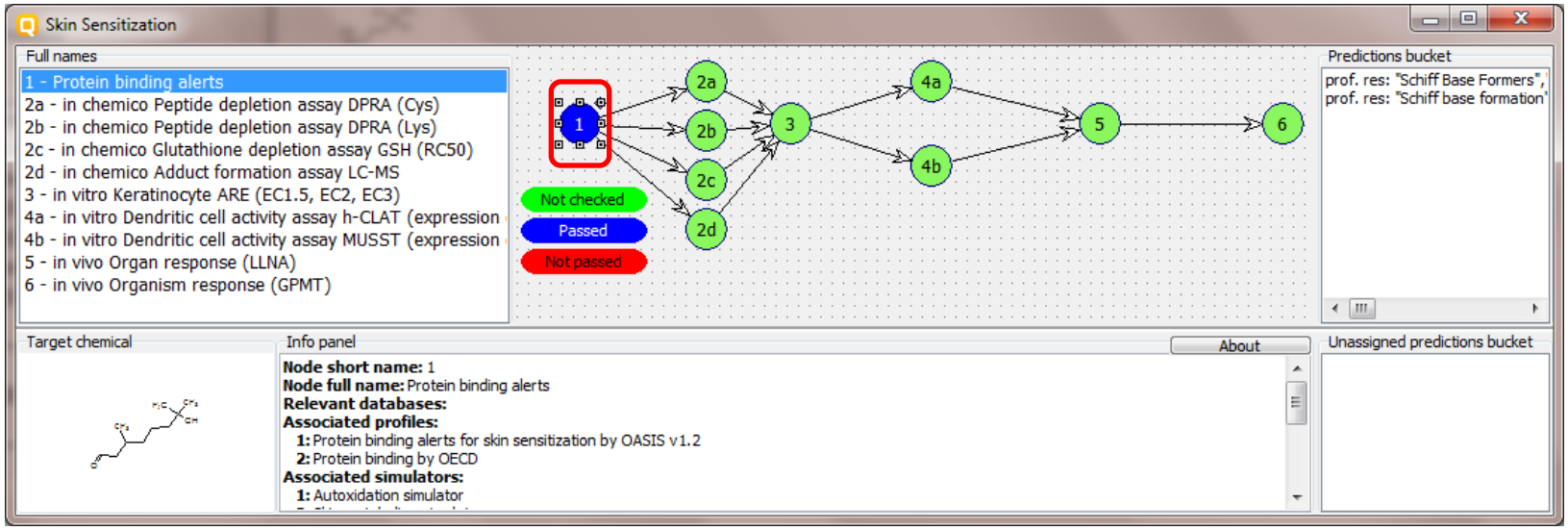


1.The target chemical has protein binding alert according to both protein binding profilers
2.The node is automatically changed to passed based on both profiling outcome results and implemented thresholds (see slide #14-15). **Click OK**
3. Prediction assigned to the selected node appears in the panel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

Workflow process

Molecular initiating events

Example 1

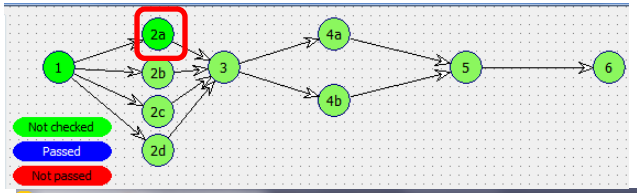


- The node MIE is passed due to the presence of protein binding alert identified for the target chemical by the two protein binding profilers
- The workflow should move further to the *in chemico* assays

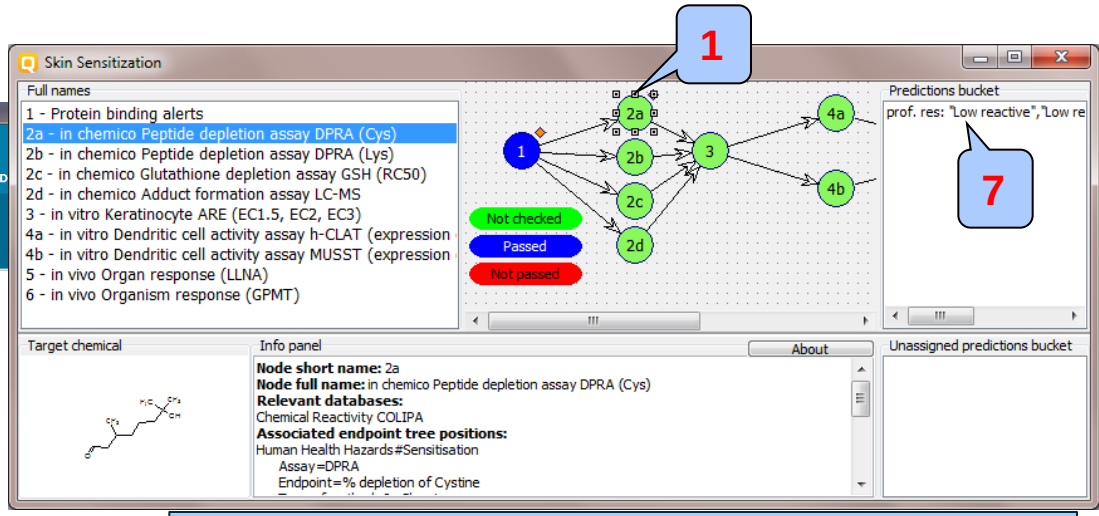
Workflow process

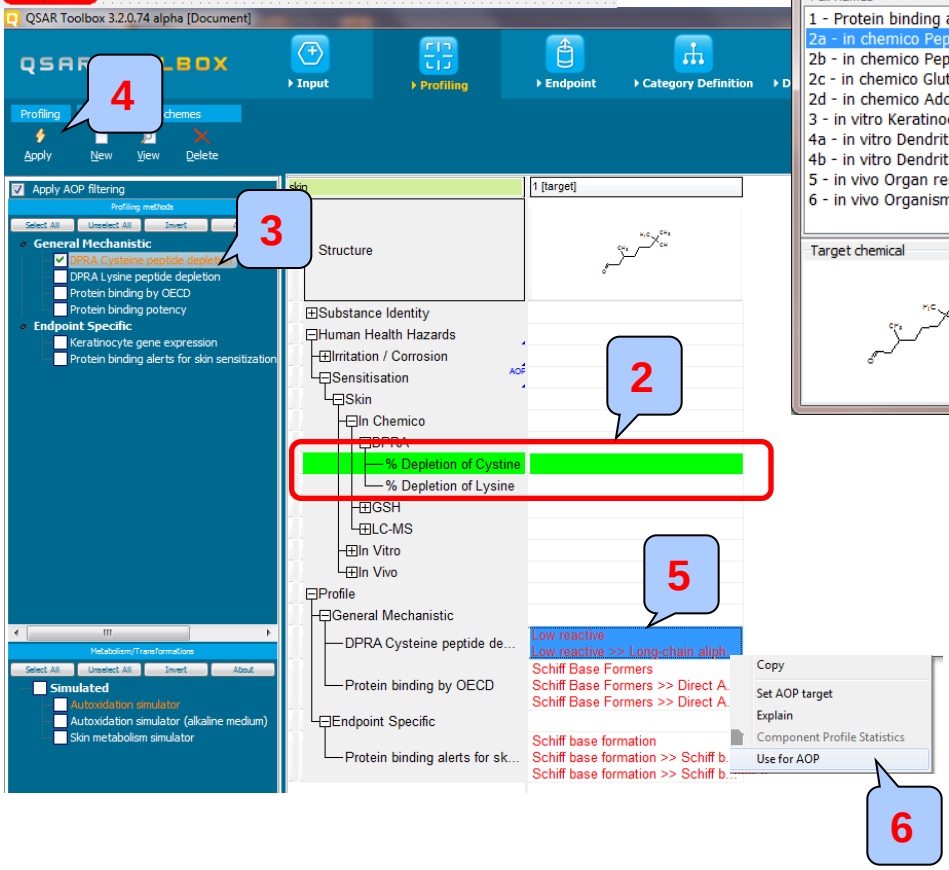
Step 2. *In chemico* Protein binding potency (Cysteine depletion) (node 2a)

Example 1



Profiling target chemical



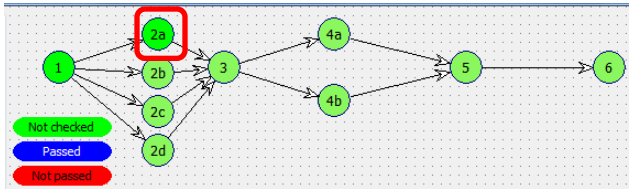


1. Select node 2a related to Cys depletion assay.
2.The row related to the selected node is getting highlighted
3.The profilers related to node 2a are highlighted **Select** related Profilers
4. **Click** Apply
5. The target chemical has "long chain aliphatic aldehyde" – low reactive alert
6. Perform **right click** and **select** "Use for AOP"
7. The profiling result appears in the bucket of the node. This last action is not related with change of node status. The node status depends on implemented data thresholds (see slide 14-15)

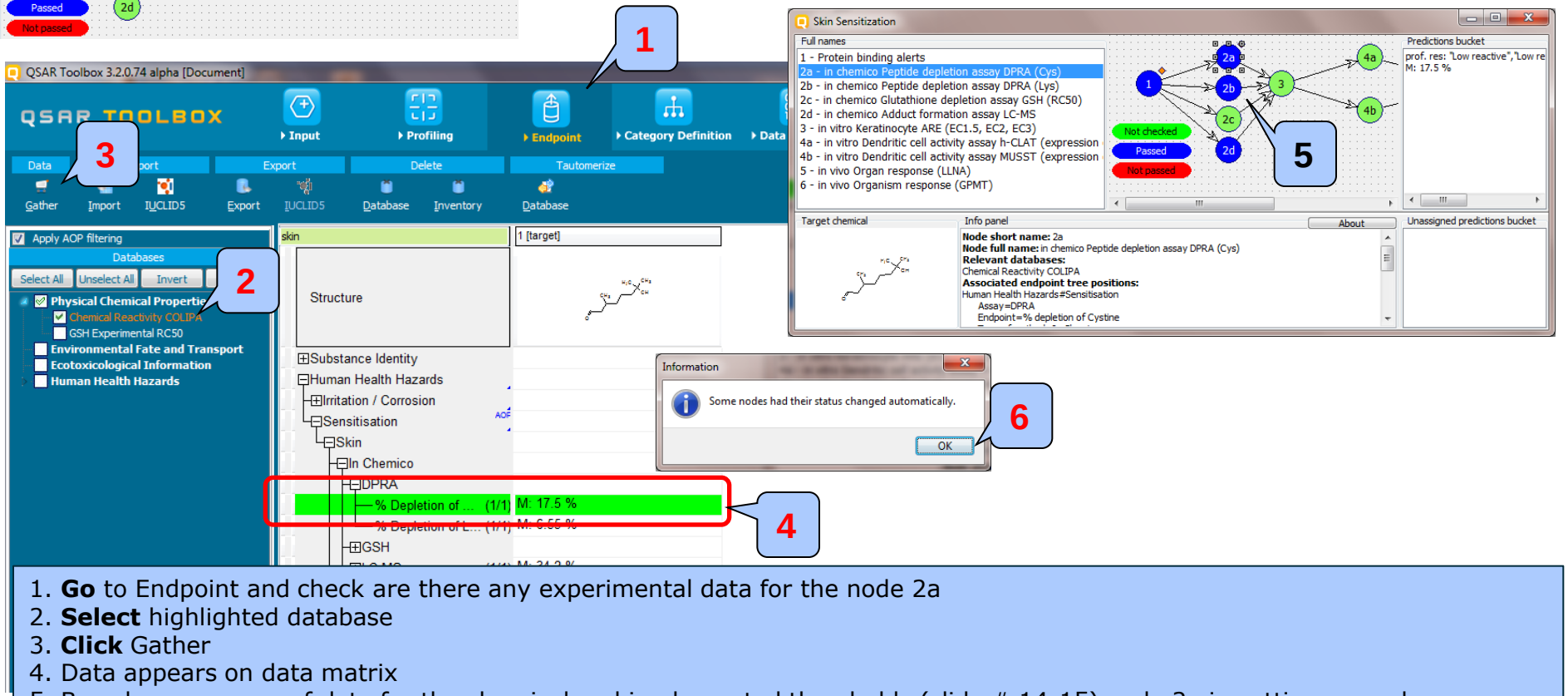
Workflow process

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

Example 1



Gather data for target chemical

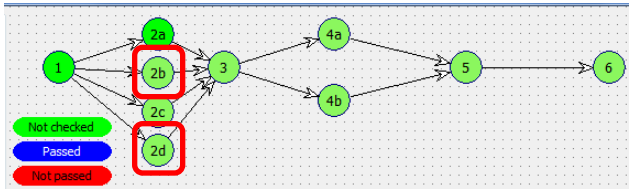


1. **Go** to Endpoint and check are there any experimental data for the node 2a
2. **Select** highlighted database
3. **Click** Gather
4. Data appears on data matrix
5. Based on presence of data for the chemical and implemented thresholds (slide # 14-15) node 2a is getting passed
6. Node 2b and 2d are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

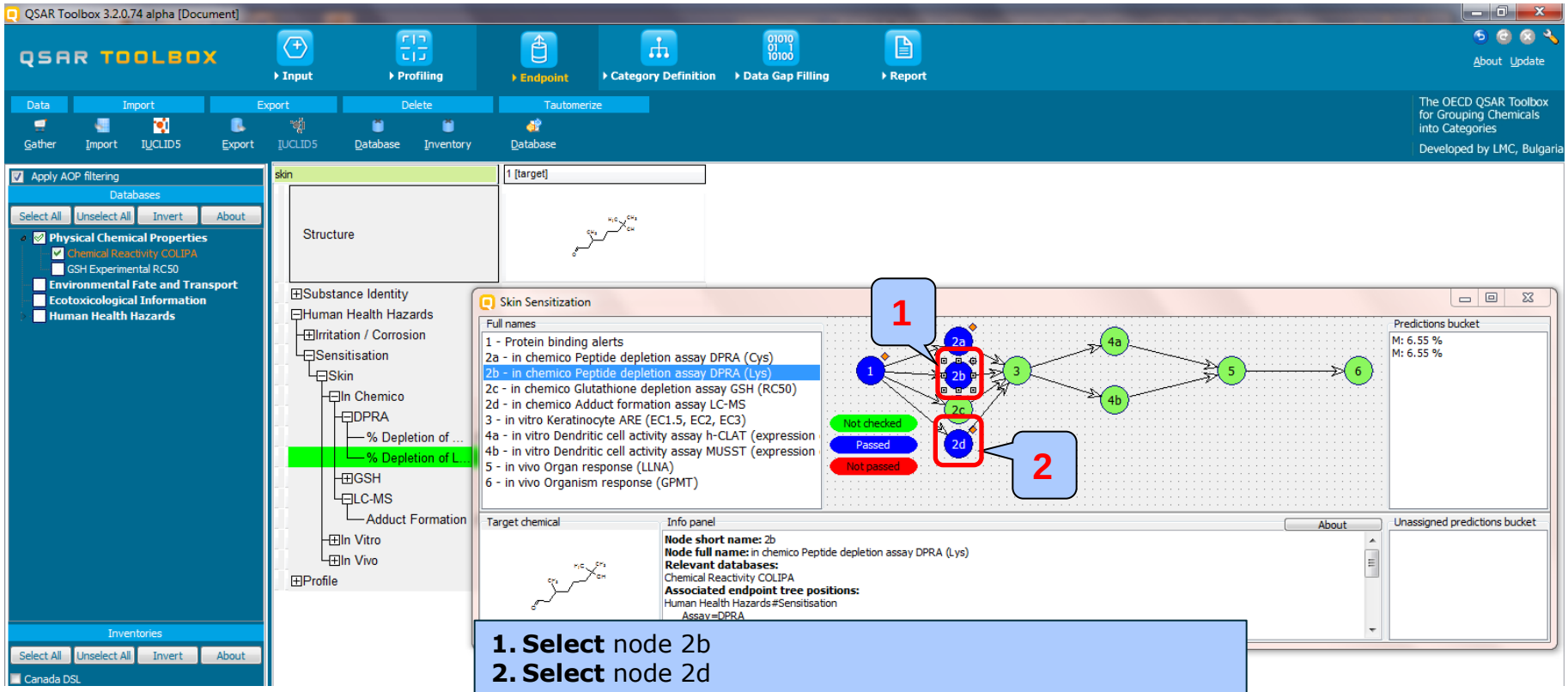
Workflow process

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

Example 1



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



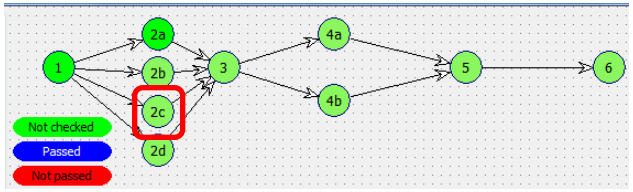
1. **Select** node 2b
2. **Select** node 2d

The two experimental data appeared in the bucket.

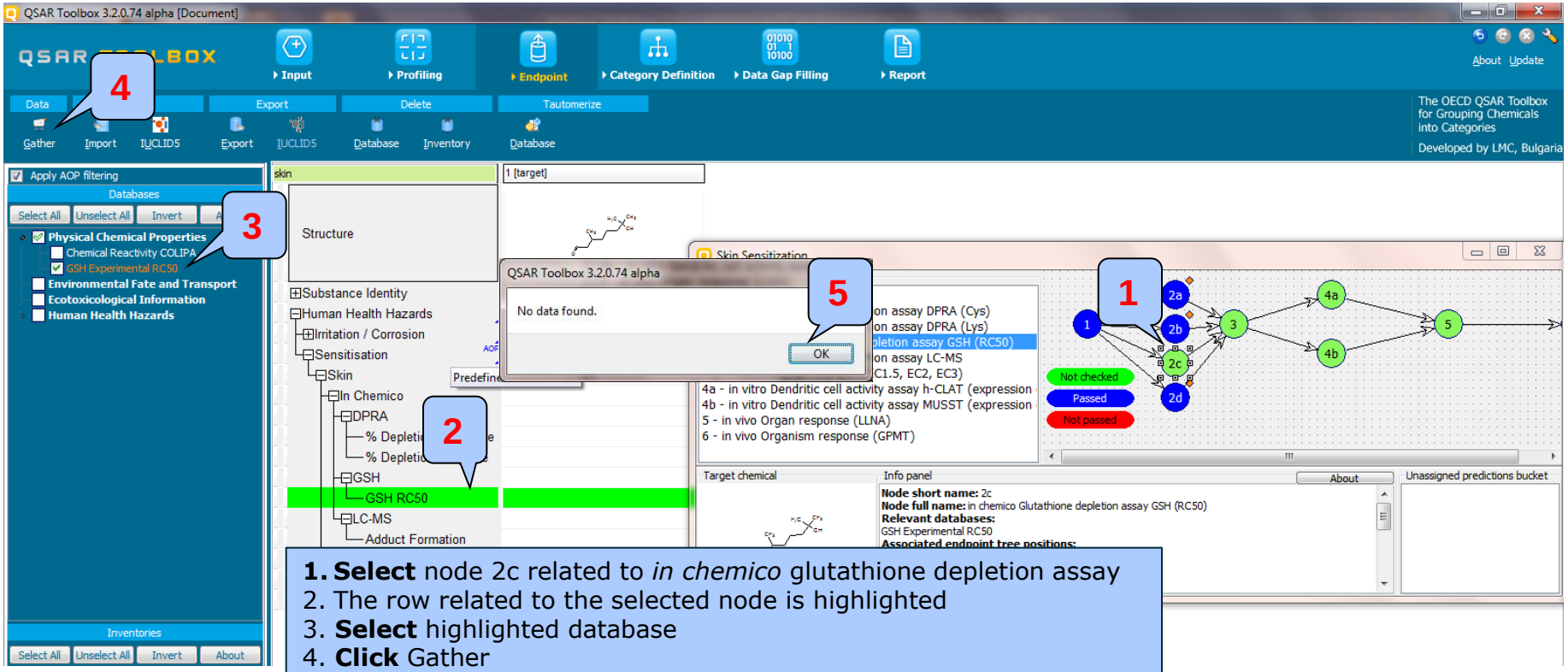
Workflow process

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

Example 1



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

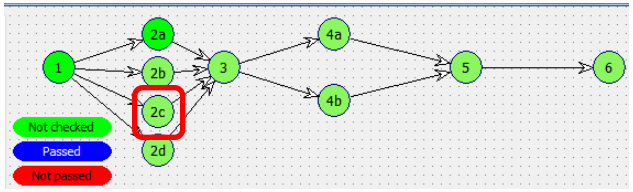


1. **Select** node 2c related to *in chemico* glutathione depletion assay
2. The row related to the selected node is highlighted
3. **Select** highlighted database
4. **Click** Gather
5. No data has been found for the target chemical, **click** OK

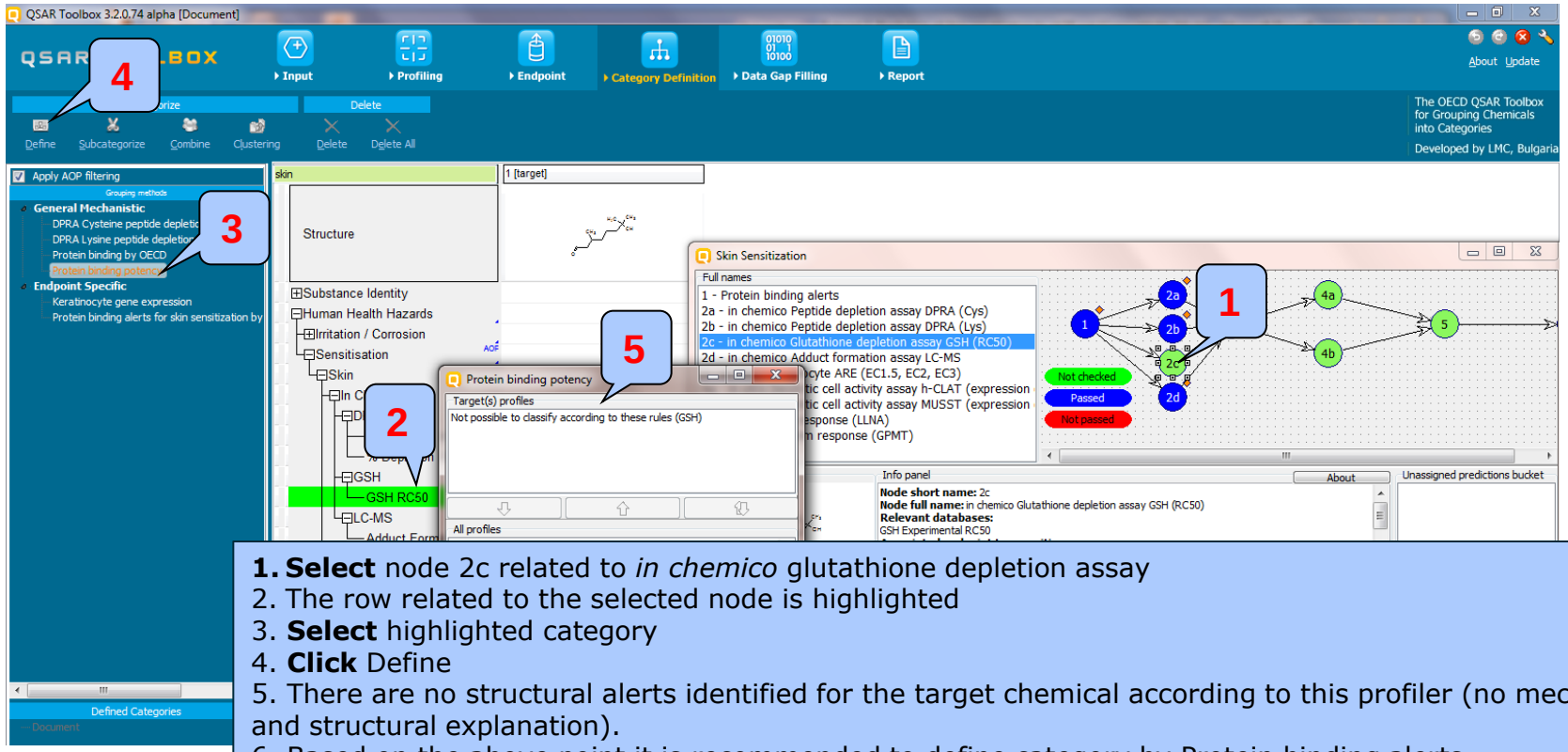
Workflow process

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

Example 1



The category of similar analogue should be investigated.

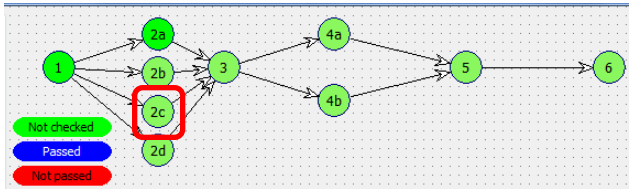


1. **Select** node 2c related to *in chemico* glutathione depletion assay
2. The row related to the selected node is highlighted
3. **Select** highlighted category
4. **Click** Define
5. There are no structural alerts identified for the target chemical according to this profiler (no mechanistic and structural explanation).
6. Based on the above point it is recommended to define category by Protein binding alerts

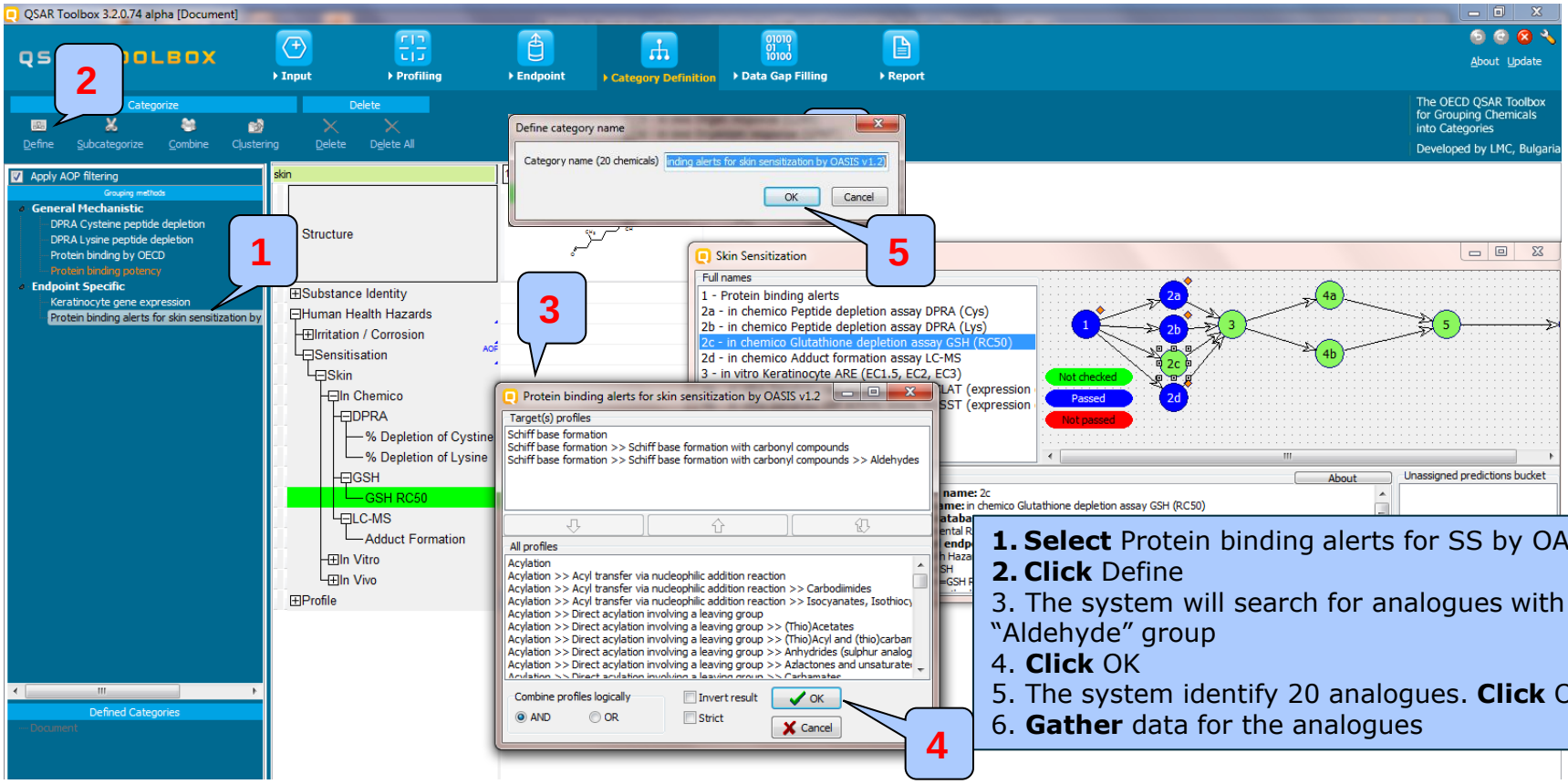
Workflow process

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

Example 1



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

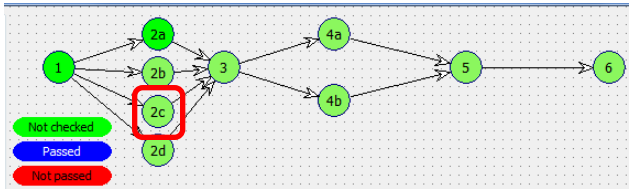


1. **Select** Protein binding alerts for SS by OASIS v1.2
2. **Click** Define
3. The system will search for analogues with "Aldehyde" group
4. **Click** OK
5. The system identify 20 analogues. **Click** OK
6. **Gather** data for the analogues

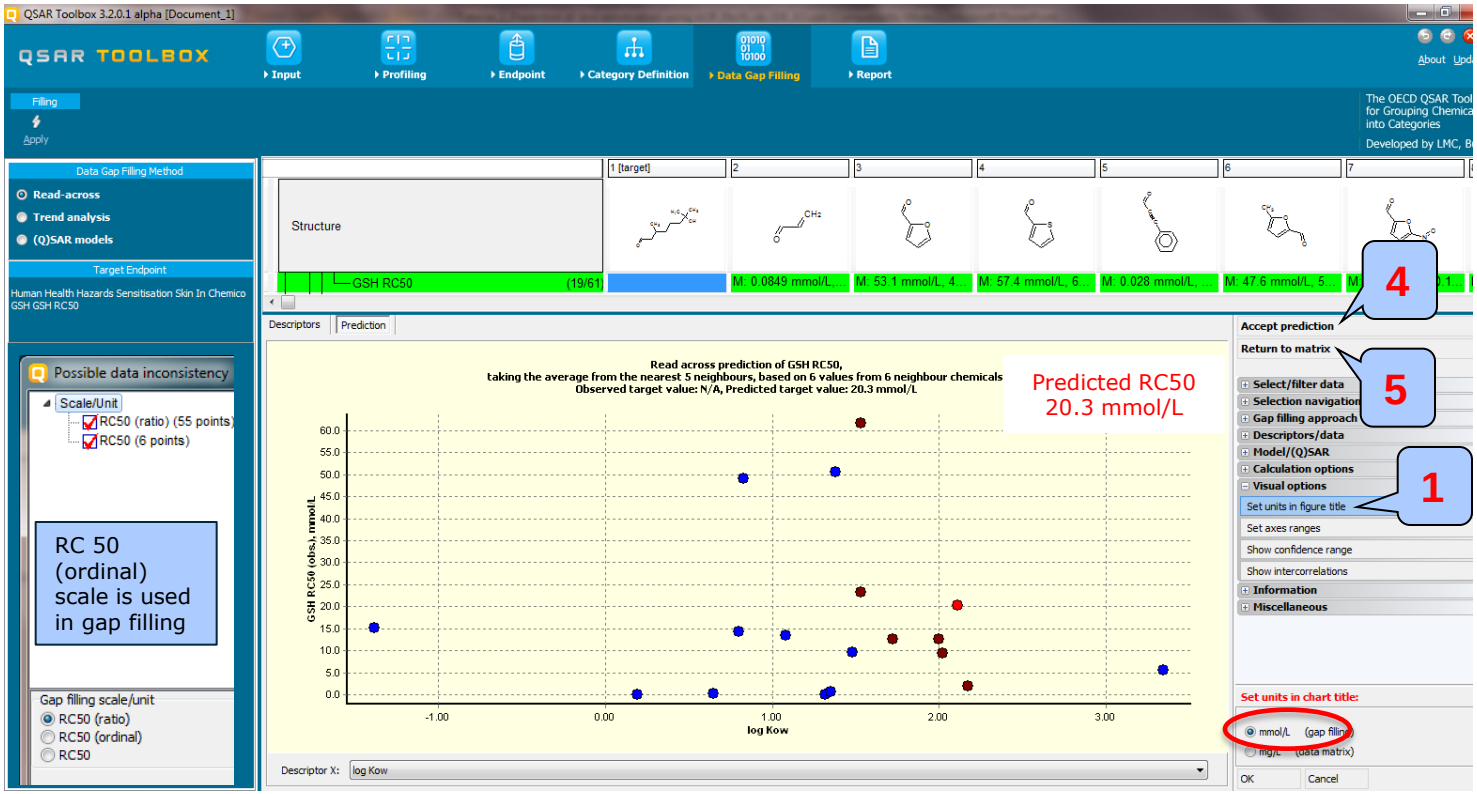
Workflow process

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

Example 1



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



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

Data thresholds

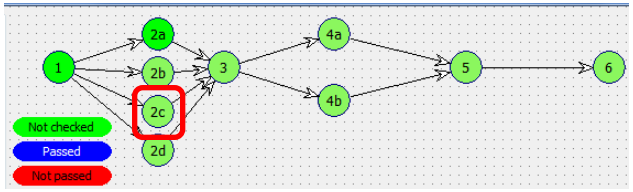
RC50 (mmol/L) ≤ 0.099 – Extremely reactive
0.1 ≥ RC50 ≤ 0.99 – Highly reactive
1 ≥ RC50 ≤ 15 – Moderately reactive
16 ≥ RC50 ≤ 70 – Slightly reactive
70.1 ≥ RC50 ≤ 135 – Suspect
RC50 > 135 – Not reactive

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

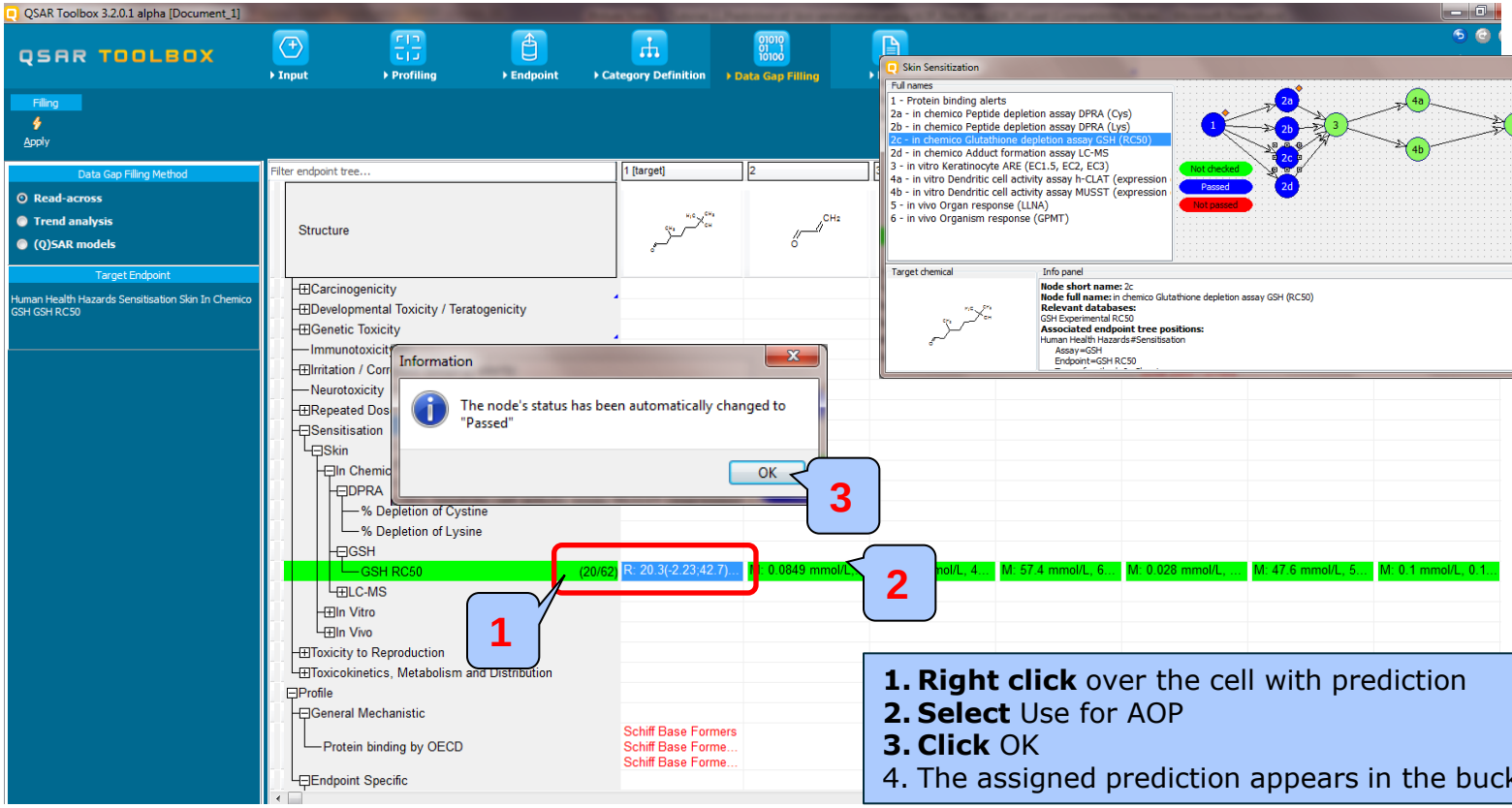
Workflow process

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

Example 1



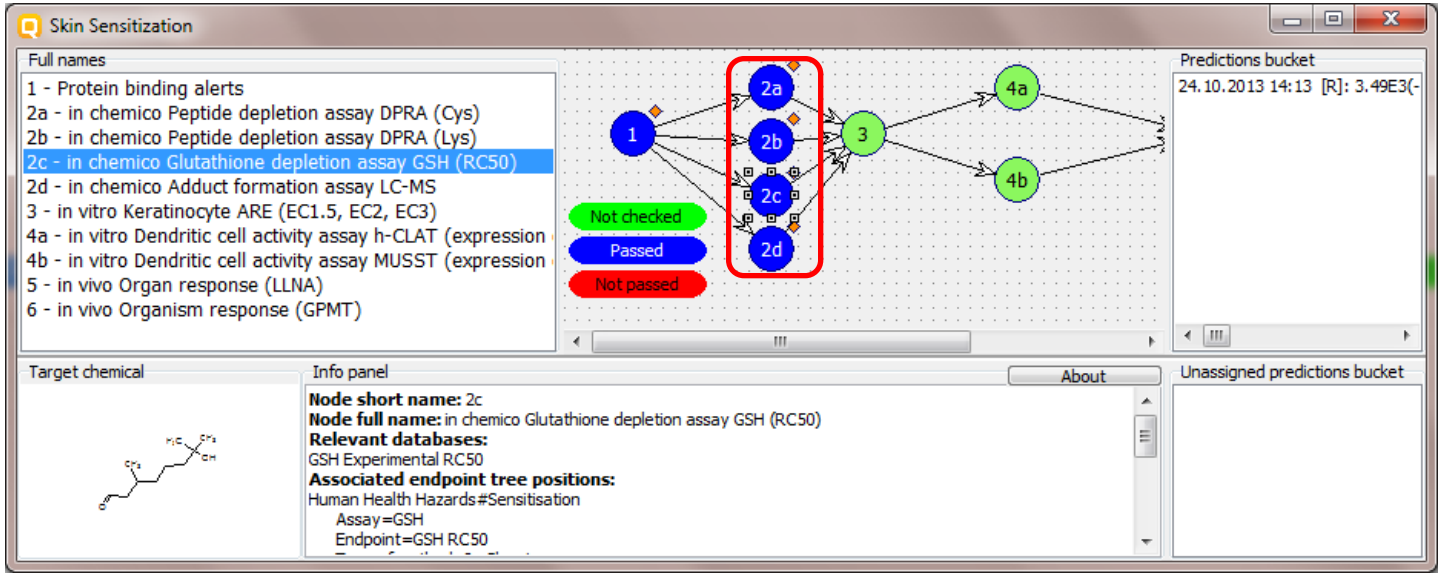
The next step is to use read-across prediction for AOP



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

Workflow process
In chemico assays

Example 1

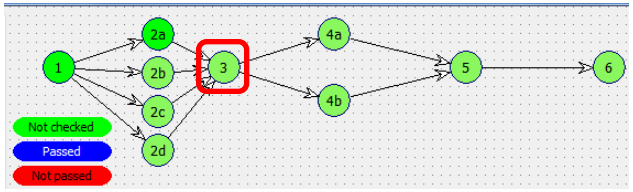


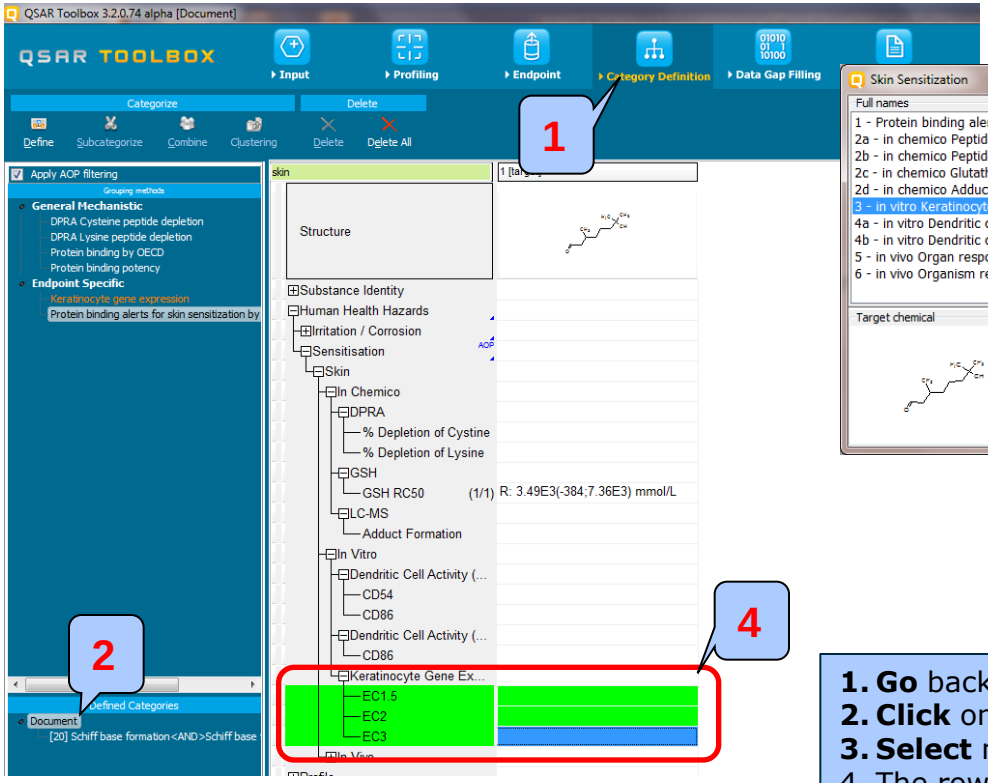
- The nodes related to the *in chemico* assays are passed due to positive experimental data for the target chemical (node 2a, 2b and 2d) and the positive experimental data found for analogues with an "Aldehyde" group(2c)
- The workflow should move further to the *in vitro* assay (node 3)

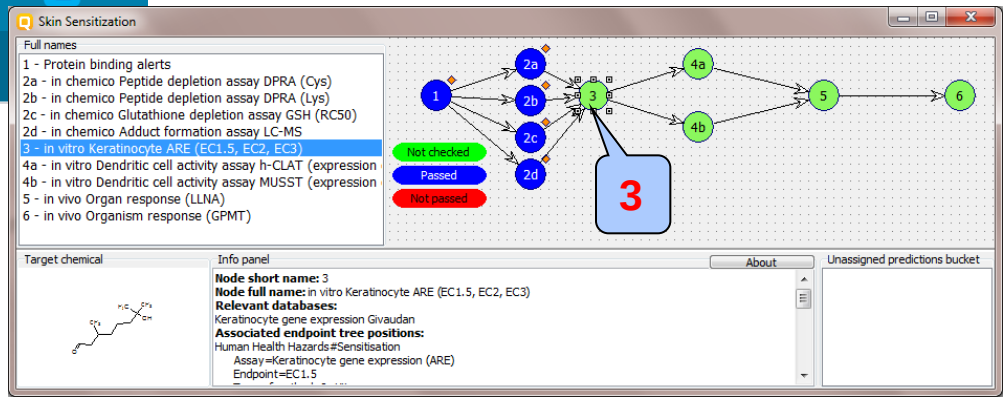
Workflow process

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

Example 1





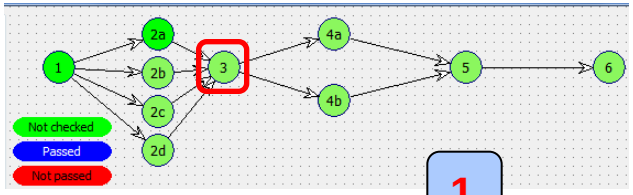


1. **Go** back to Category definition
2. **Click** on Documents in order to return to datamatrix of the target
3. **Select** node 3 related to the in vitro assay
4. The rows related to in vitro assay are getting highlighted

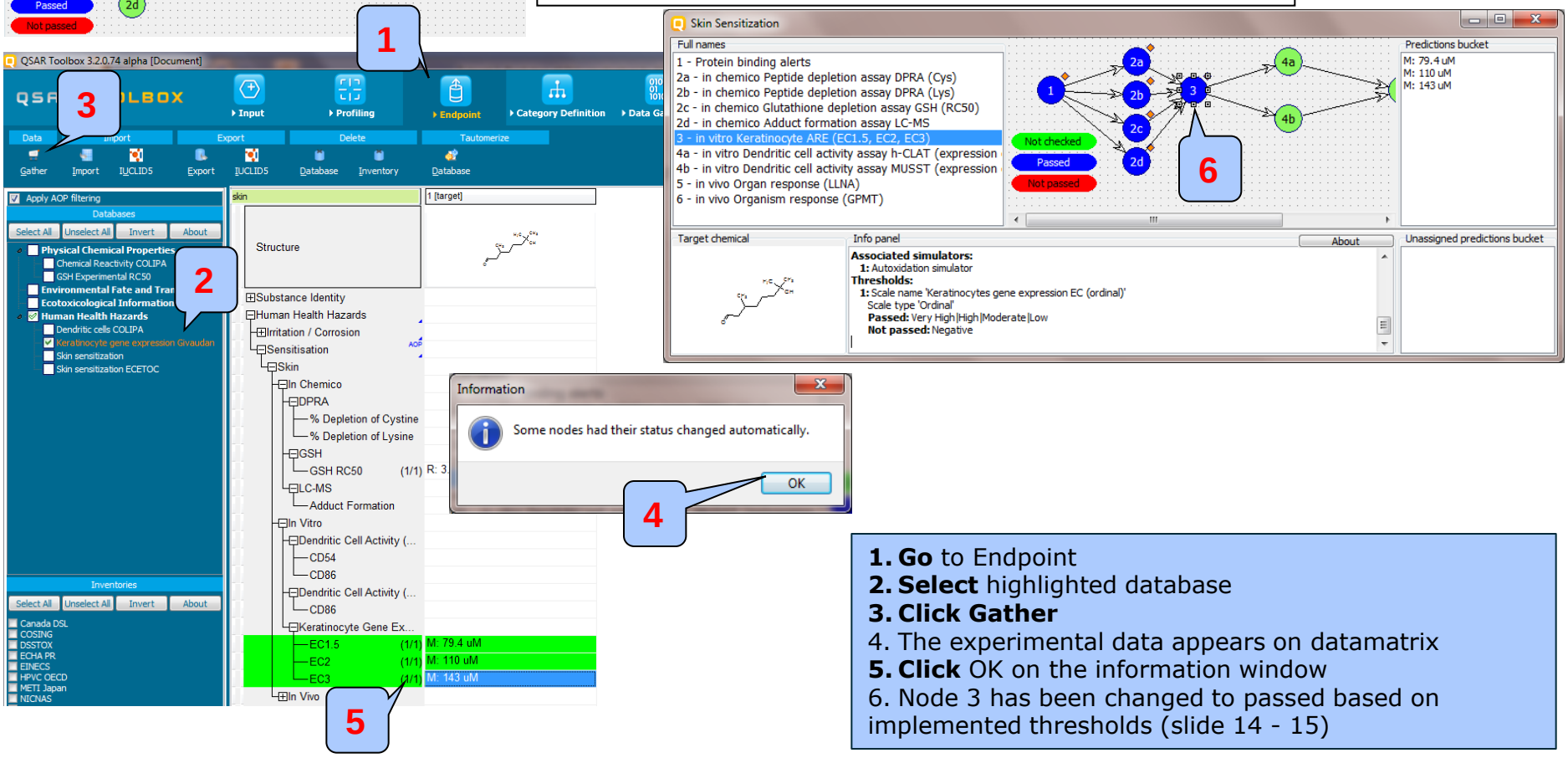
Workflow process

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

Example 1



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

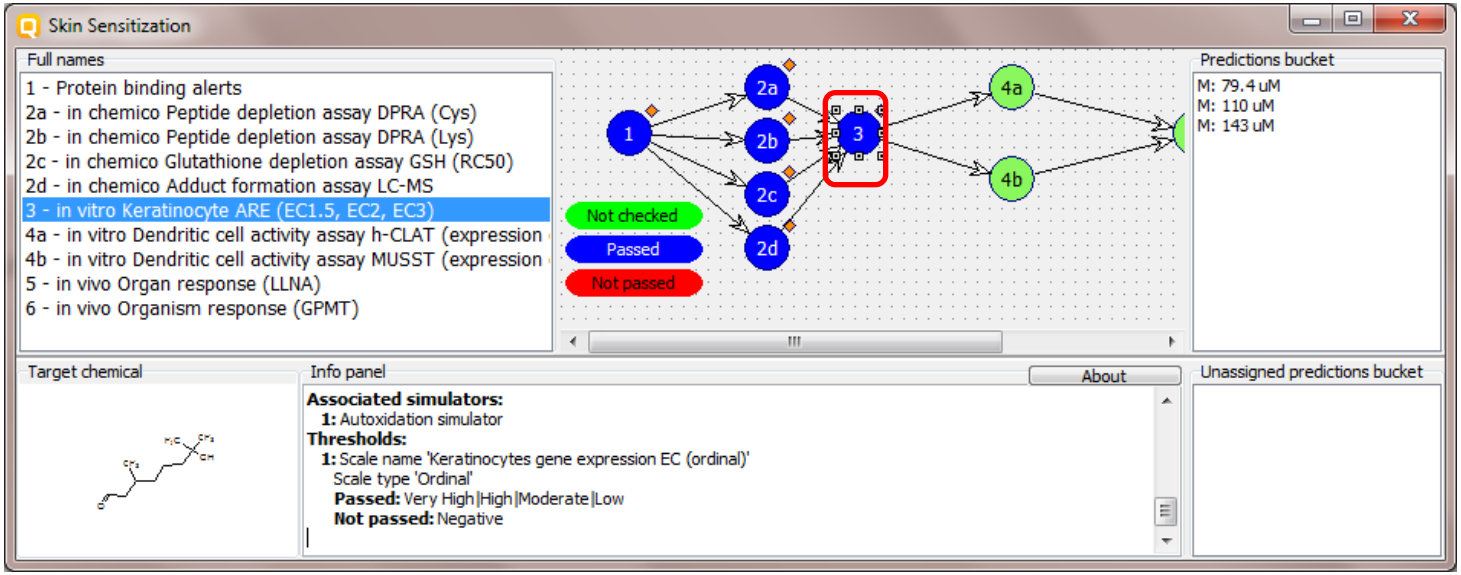


1. **Go** to Endpoint
2. **Select** highlighted database
3. **Click Gather**
4. The experimental data appears on datamatrix
5. **Click** OK on the information window
6. Node 3 has been changed to passed based on implemented thresholds (slide 14 - 15)

Workflow process

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

Example 1

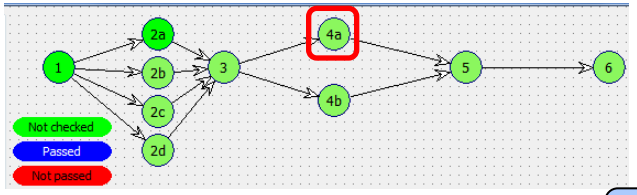


- The node 3 related to the *in vitro* assay is passed due to positive experimental data found for the target chemical and implemented thresholds (slide #14 -15)
- The workflow should move further to the other *in vitro* assays (nodes 4a and 4b)

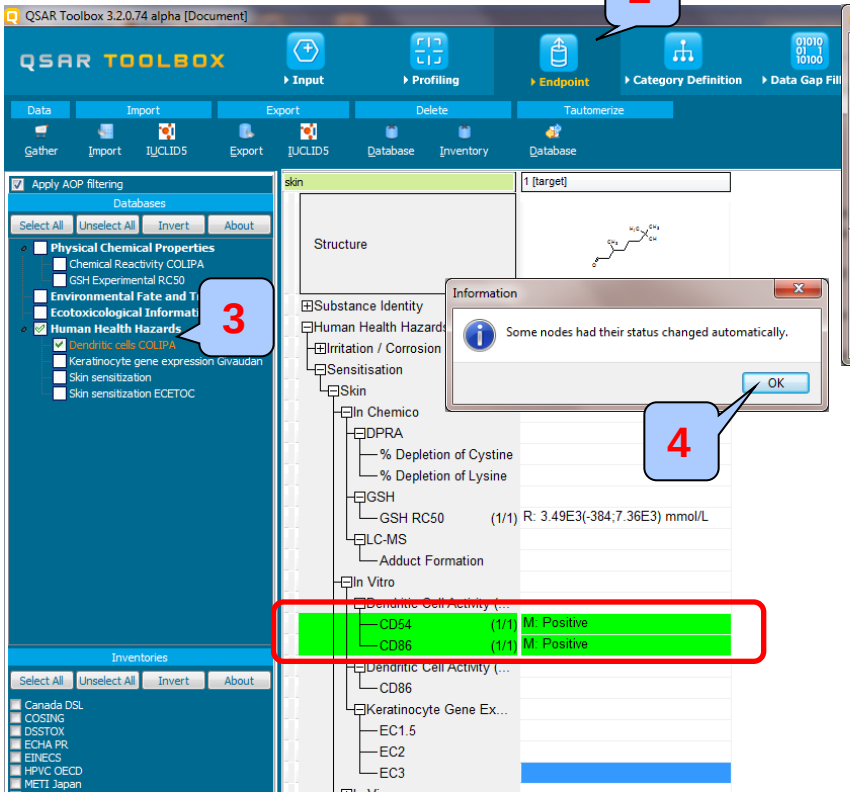
Workflow process

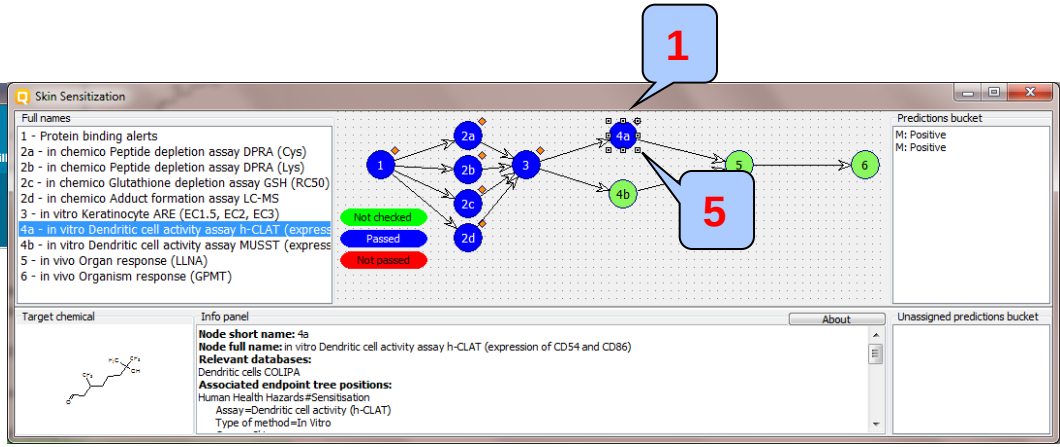
Step 4. *in vitro* Dendritic cell activity assay h-CLAT (expression of CD54 and CD86) (node 4a)

Example 1



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



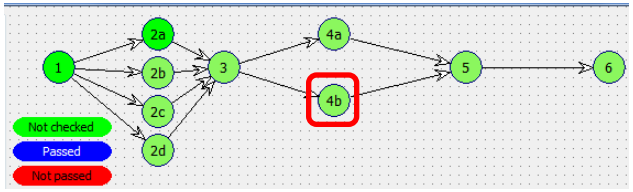


1. **Select** node 4a
2. **Go** to Endpoint
3. **Select** database related to node 4a
4. **Gather** data and click **OK** in the appeared message
5. The status of node 4a was changed to passed

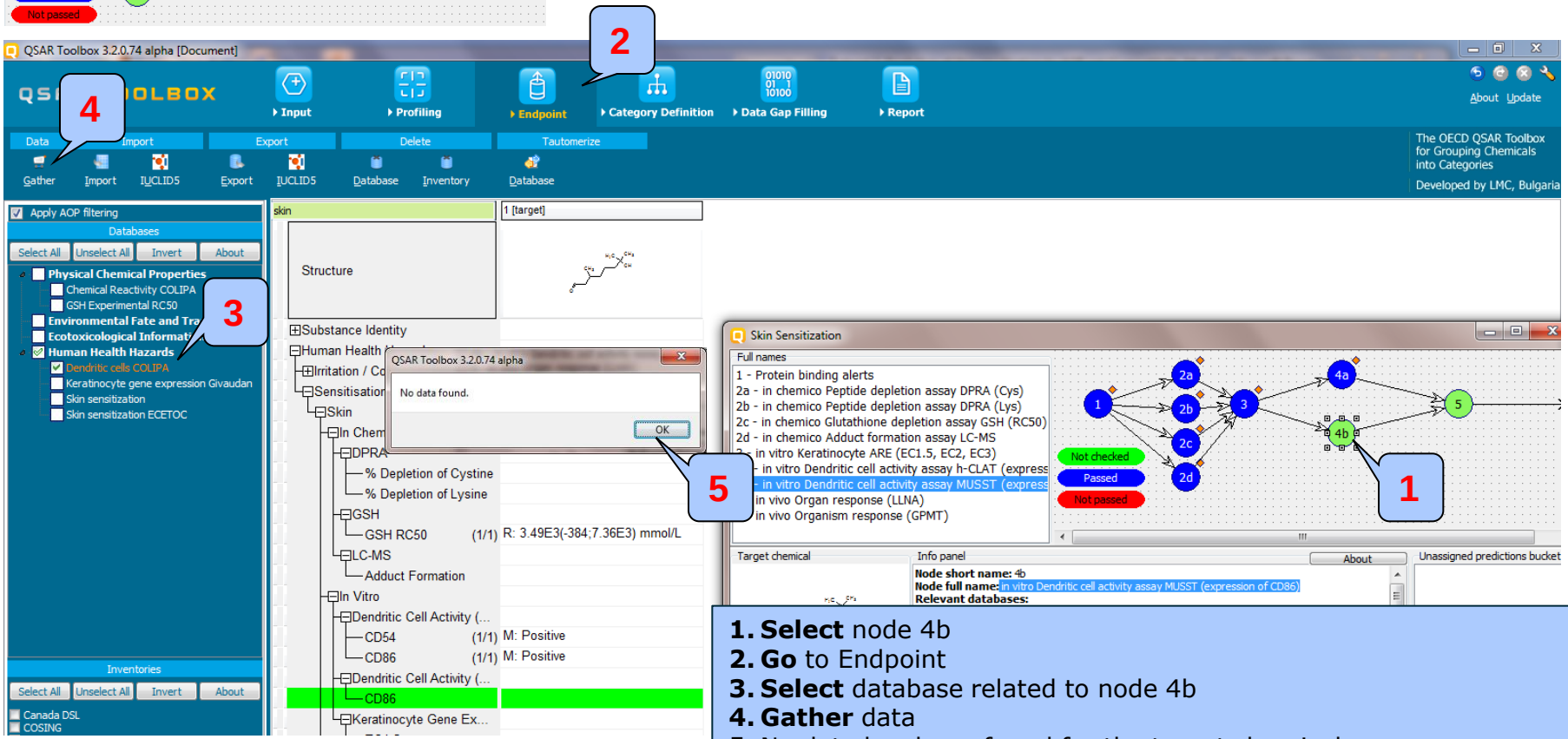
Workflow process

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

Example 1



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

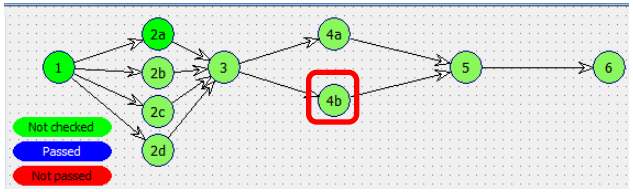


1. **Select** node 4b
2. **Go** to Endpoint
3. **Select** database related to node 4b
4. **Gather** data
5. No data has been found for the target chemical

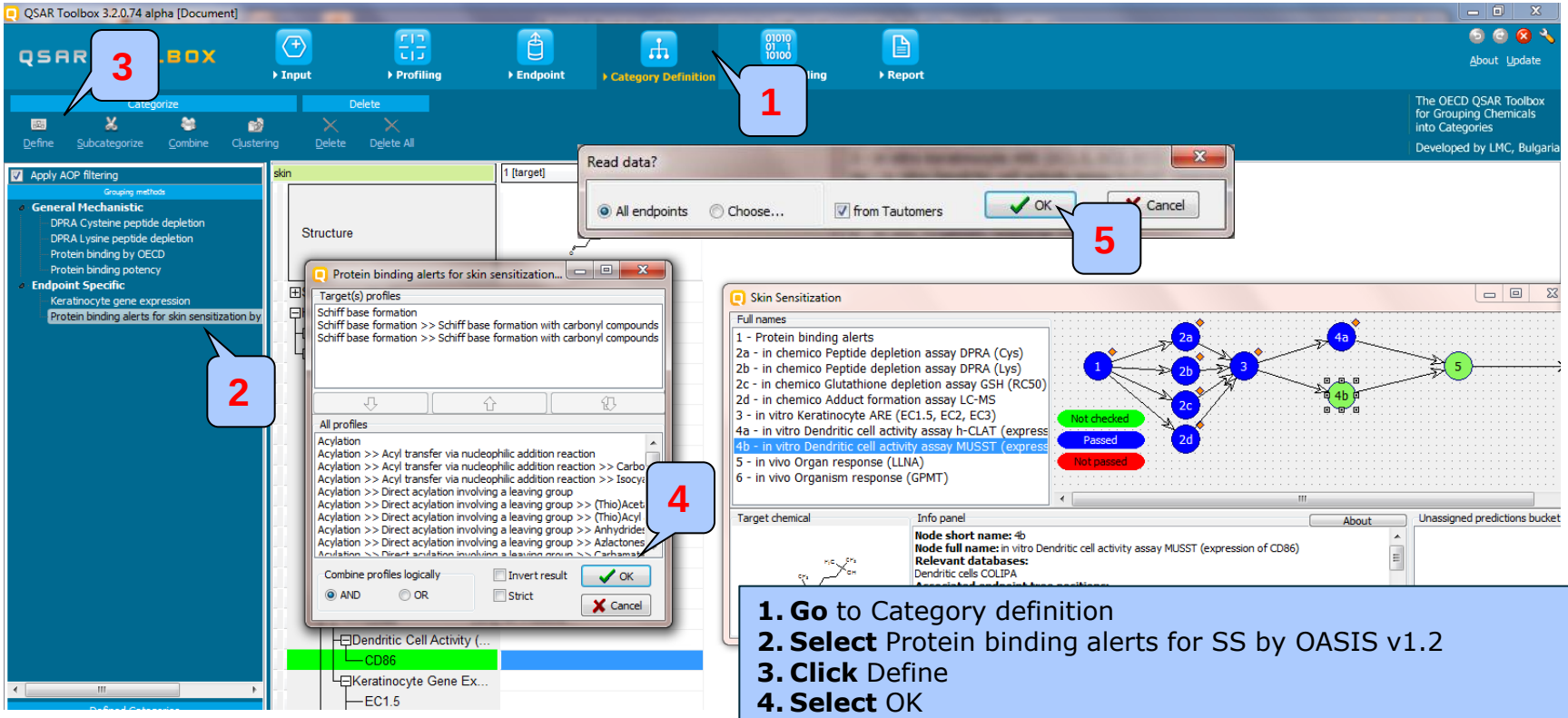
Workflow process

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

Example 1



Investigate category of similar analogue

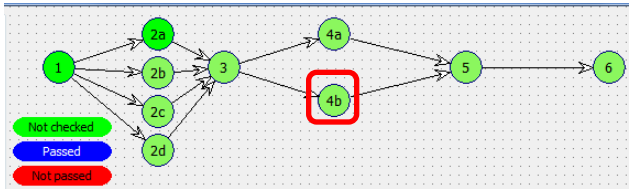


1. **Go** to Category definition
2. **Select** Protein binding alerts for SS by OASIS v1.2
3. **Click** Define
4. **Select** OK
5. **Read** data for the analogues

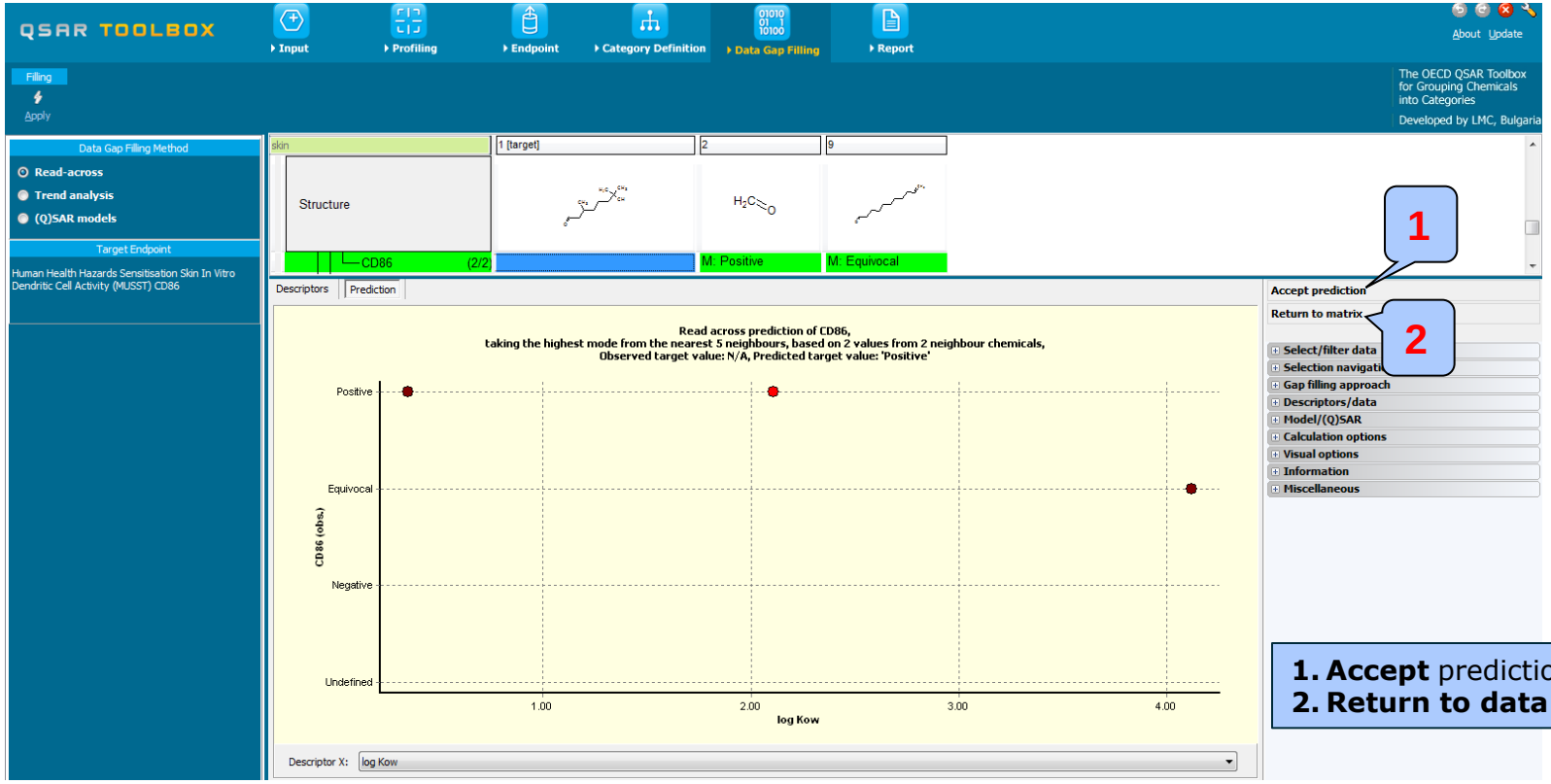
Workflow process

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

Example 1



Performed RA prediction is used *only* to exemplify the workflow

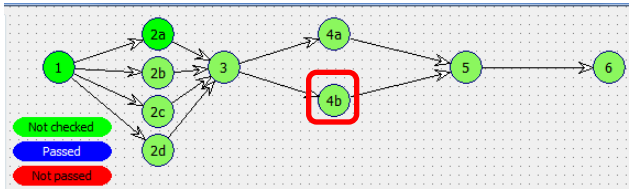


1. **Accept** prediction
2. **Return to data matrix**

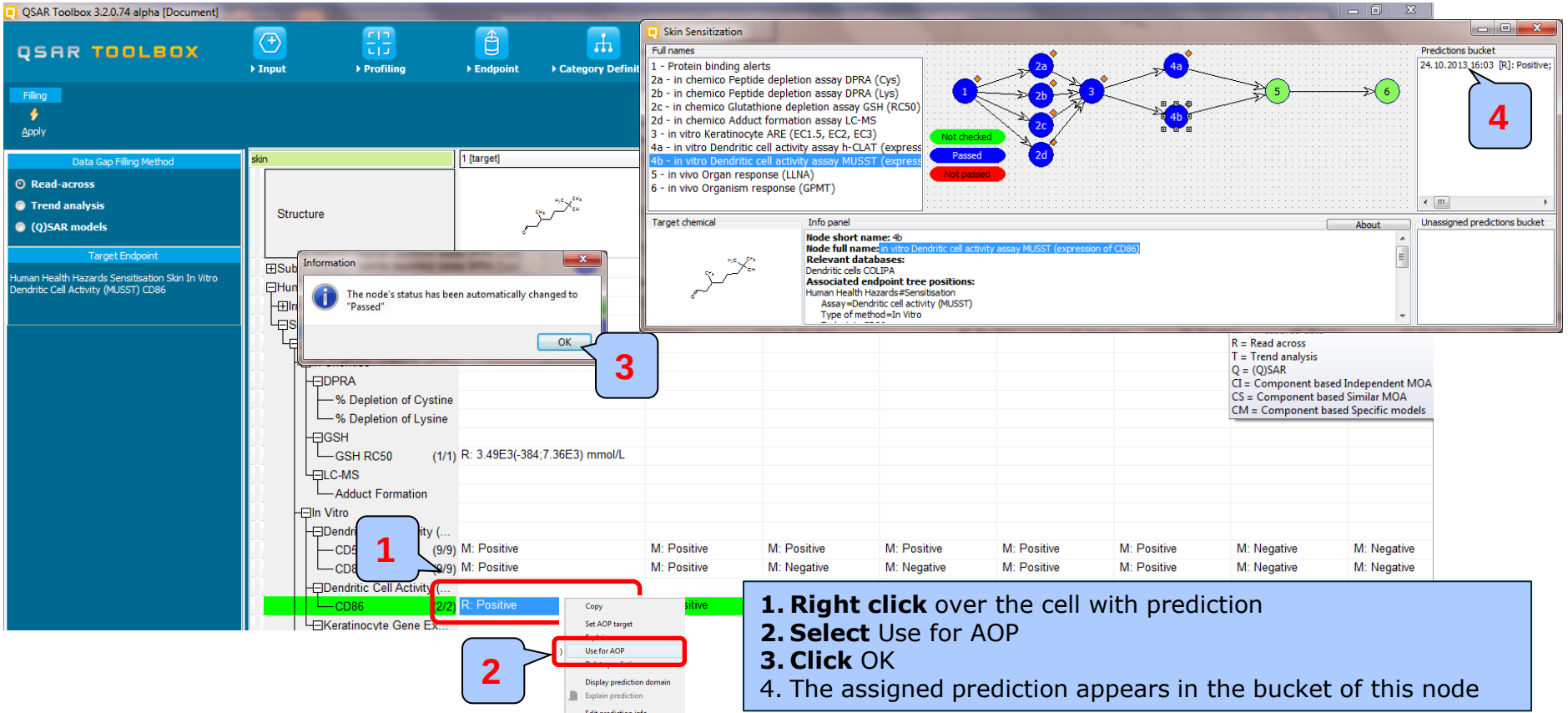
Workflow process

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

Example 1



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

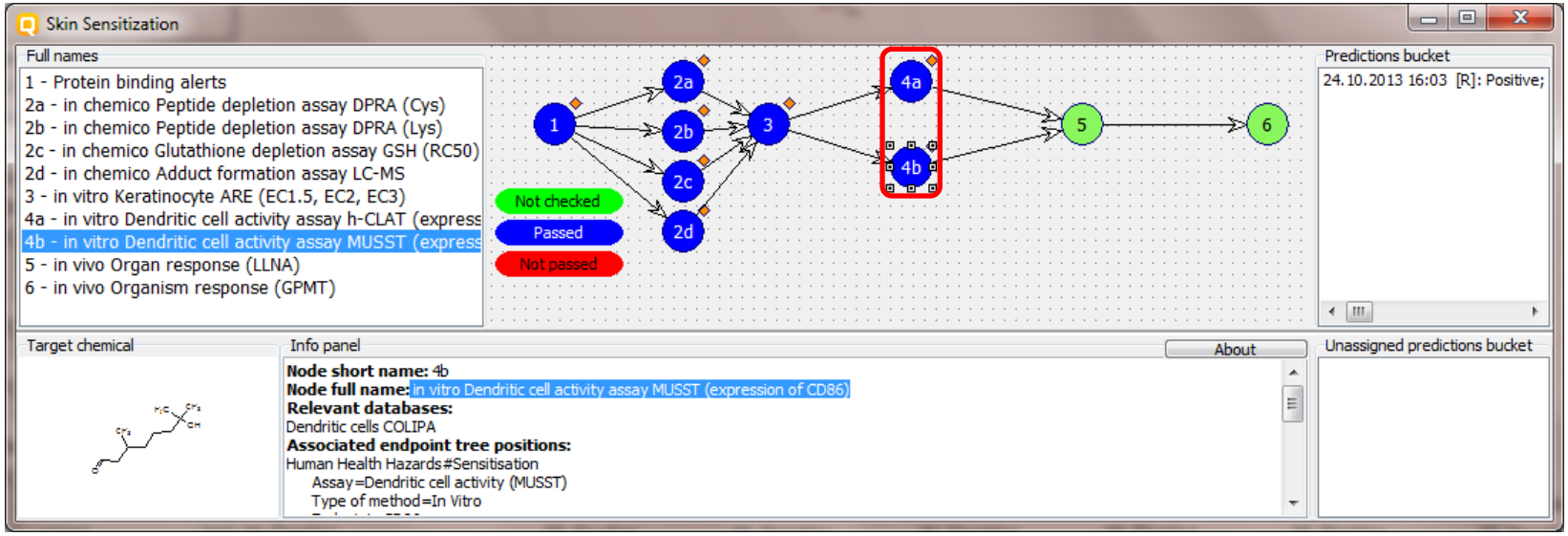


1. **Right click** over the cell with prediction
2. **Select** Use for AOP
3. **Click** OK
4. The assigned prediction appears in the bucket of this node

Workflow process

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

Example 1

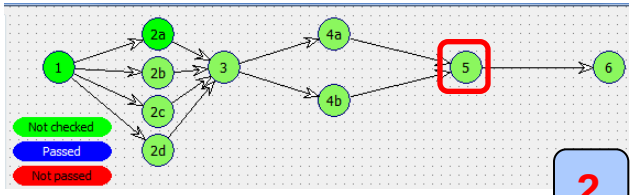


- The node 4a related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to positive experimental data found for the target chemical
- The node 4b related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to the positive experimental data found for analogues present in the category of Schiff base formers.
- The workflow moves further to the *in vivo LLNA* assay (node 5)

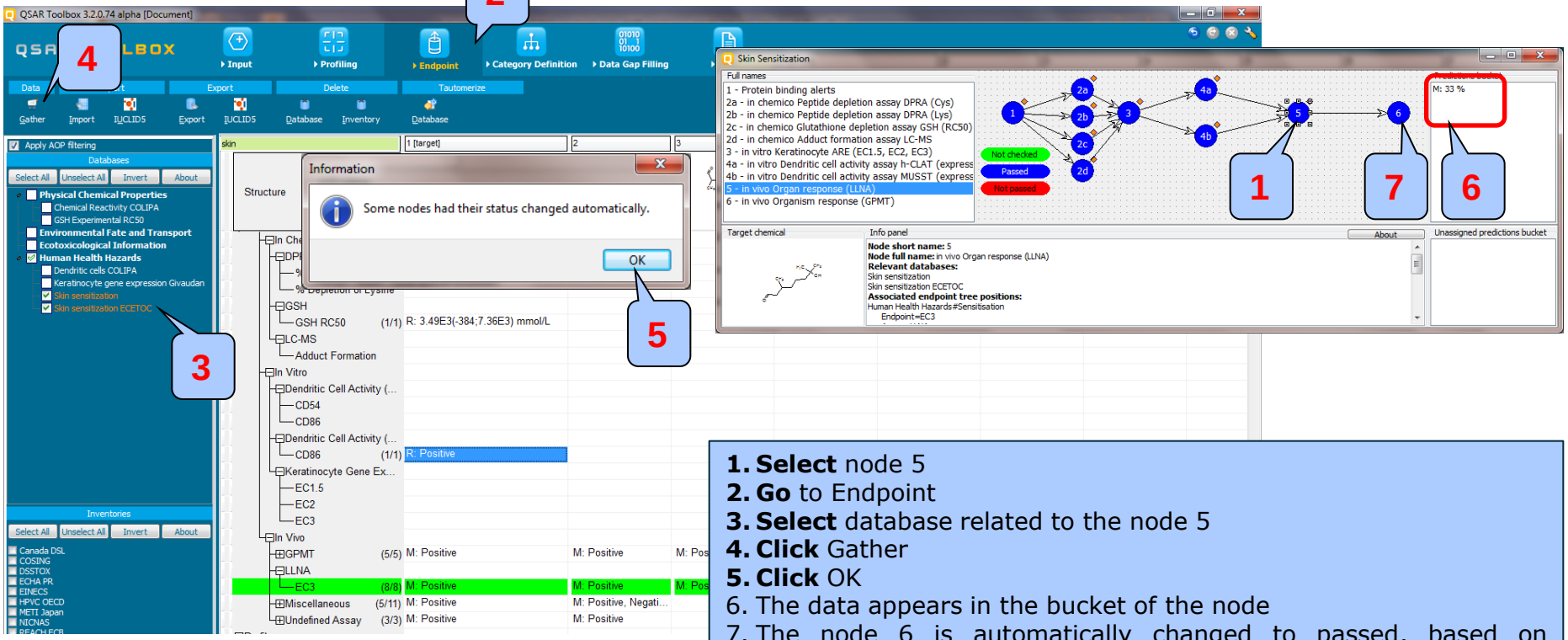
Workflow process

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

Example 1



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

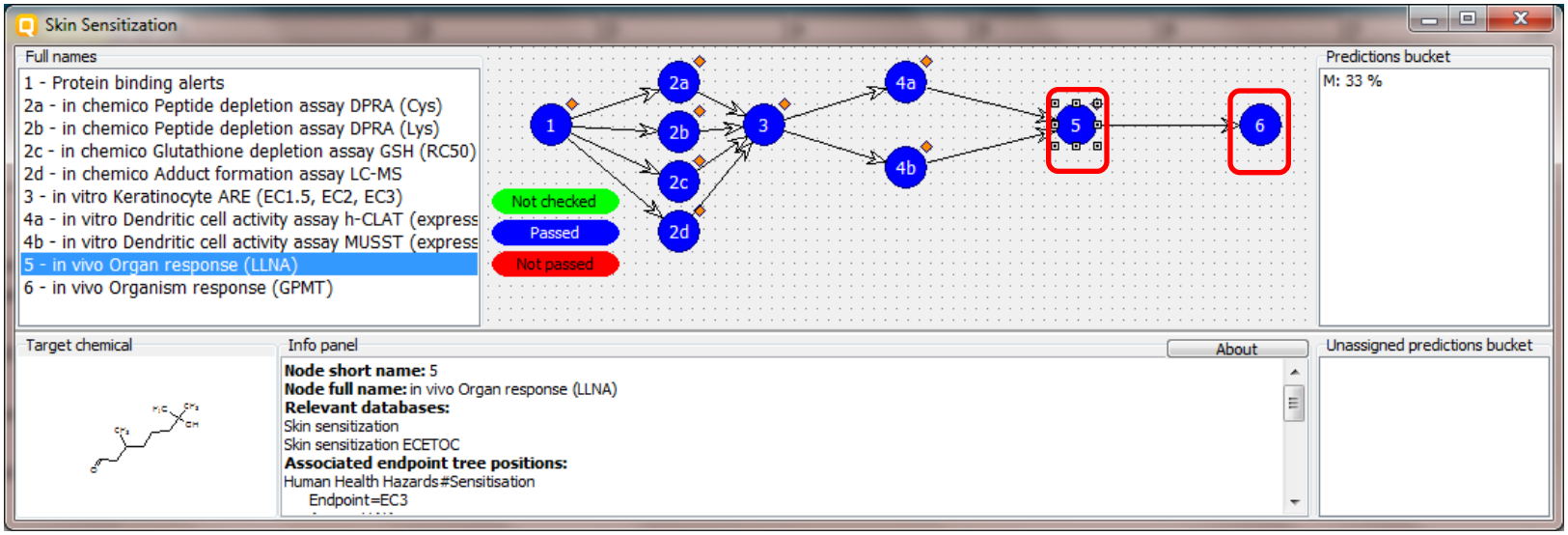


1. **Select** node 5
2. **Go** to Endpoint
3. **Select** database related to the node 5
4. **Click** Gather
5. **Click** OK
6. The data appears in the bucket of the node
7. The node 6 is automatically changed to passed, based on experimental data for the target chemical and the implemented thresholds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

Workflow process

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

Example 1



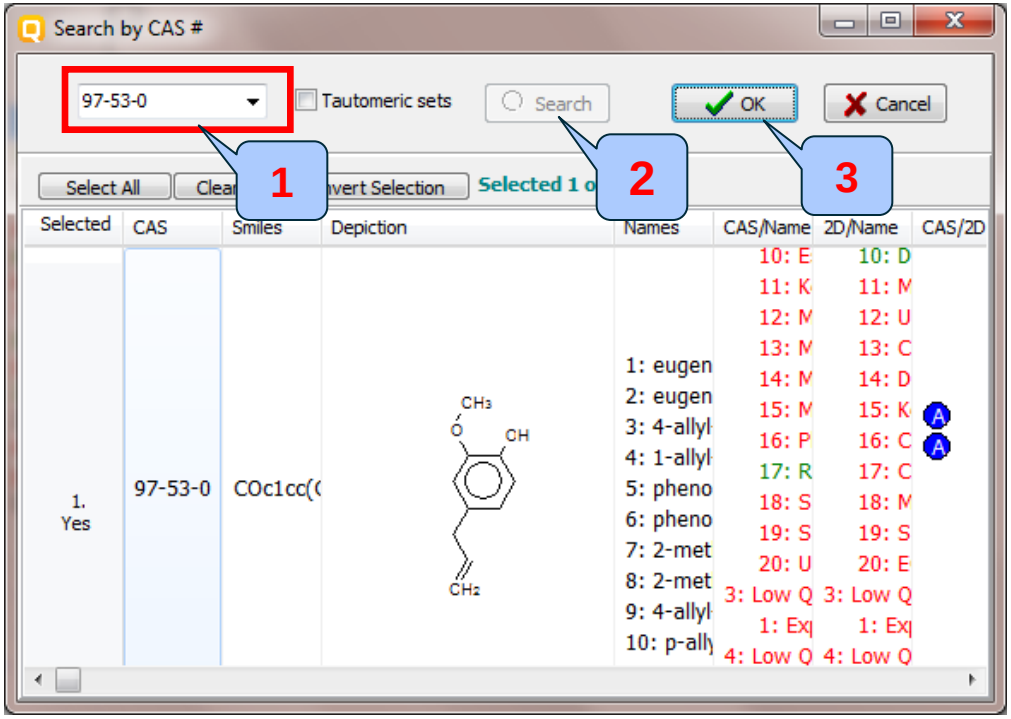
- Both nodes related to the two in vivo assays (LLNA and GPMT) are passed based on the positive experimental data for the target chemical according to the implemented thresholds

Outlook

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

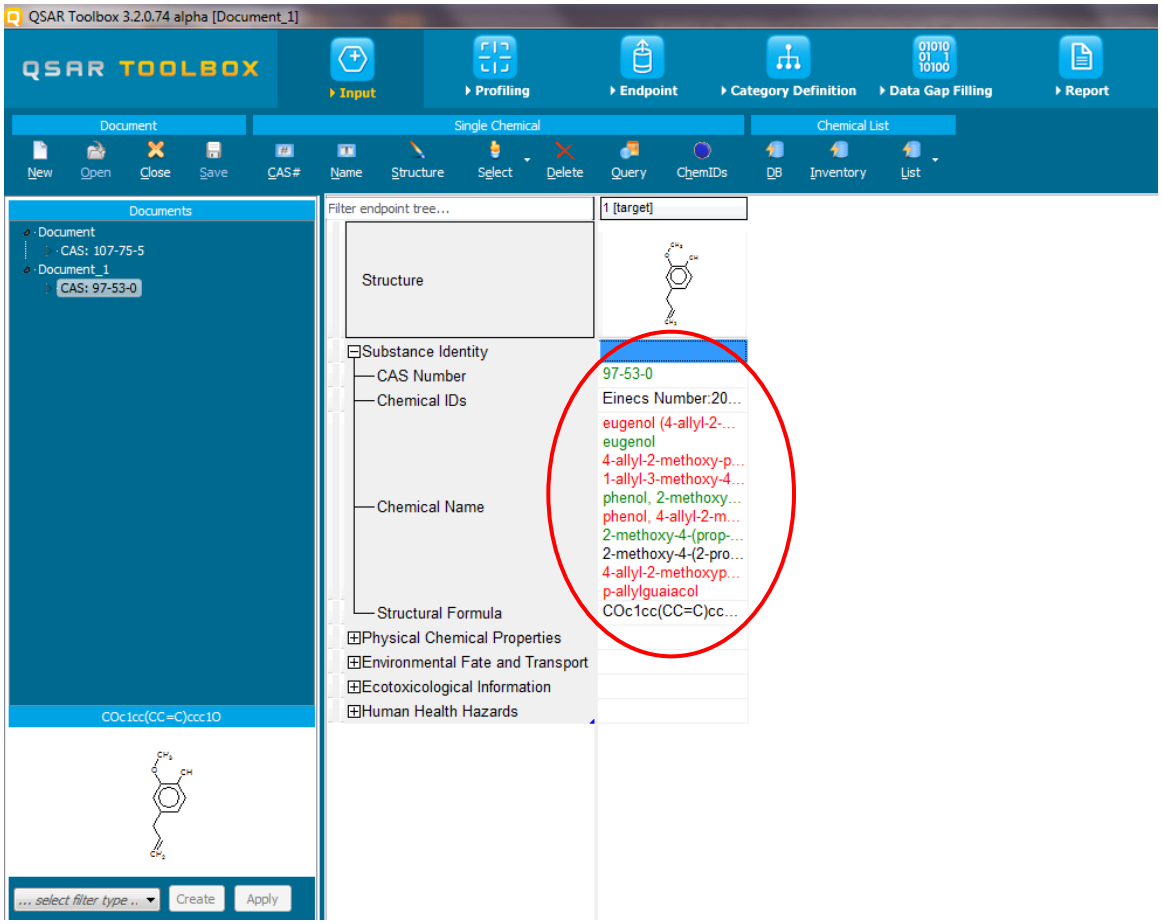
Chemical Input
Enter CAS# 97-53-0

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



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

Chemical Input
Target chemical identity

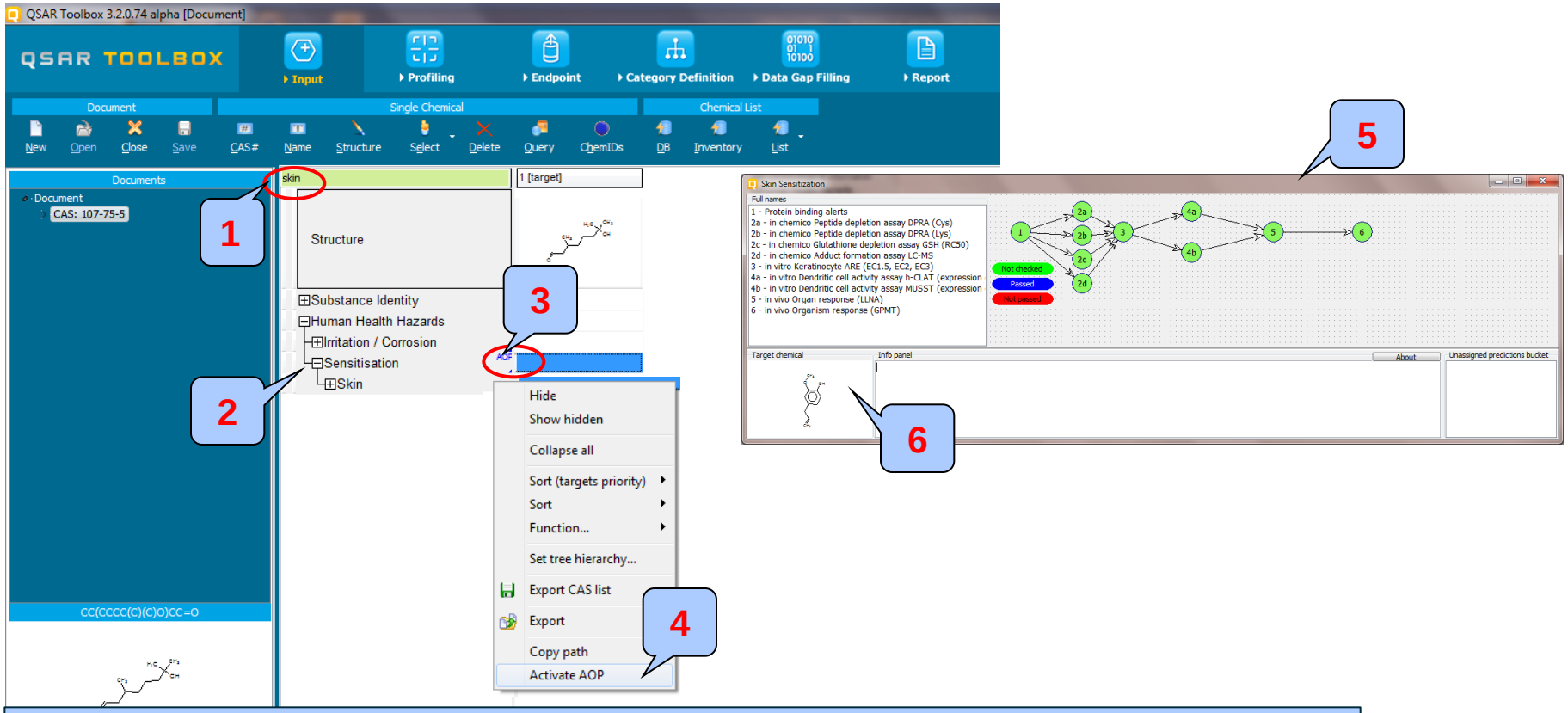


Outlook

- 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



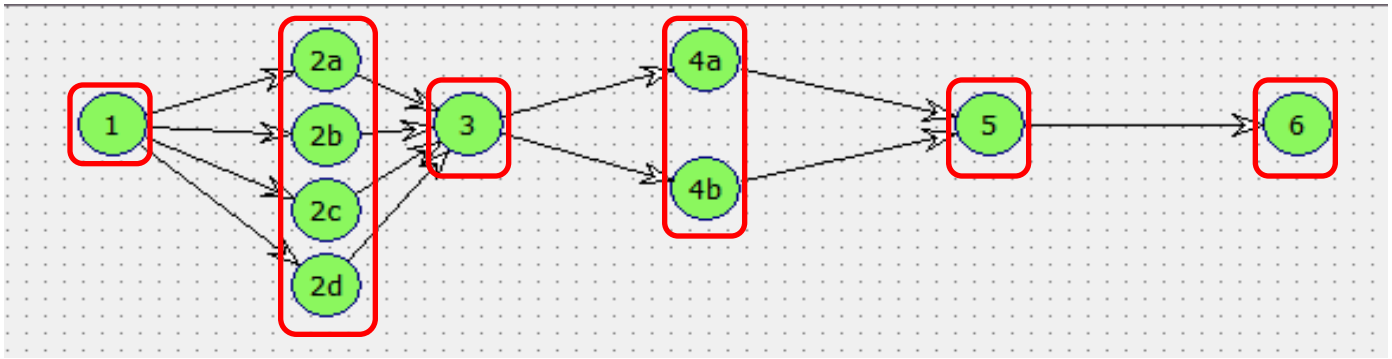
1. Filter endpoint tree – **write skin** in the green filed
2. **Expand the tree –** open the tree to the Sensitization node
3. **Right click** near the AOP label
4. **Select** activate AOP 5. AOP window appears 6. Set target for AOP (see slide 25)

Outlook

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

Workflow process

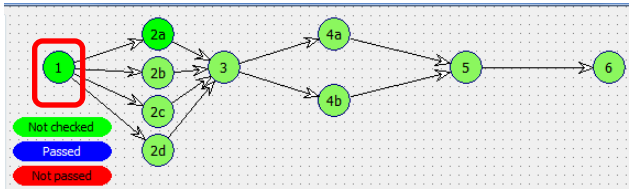
- Workflow process start from molecular initiating event to the *in vivo* organism respond



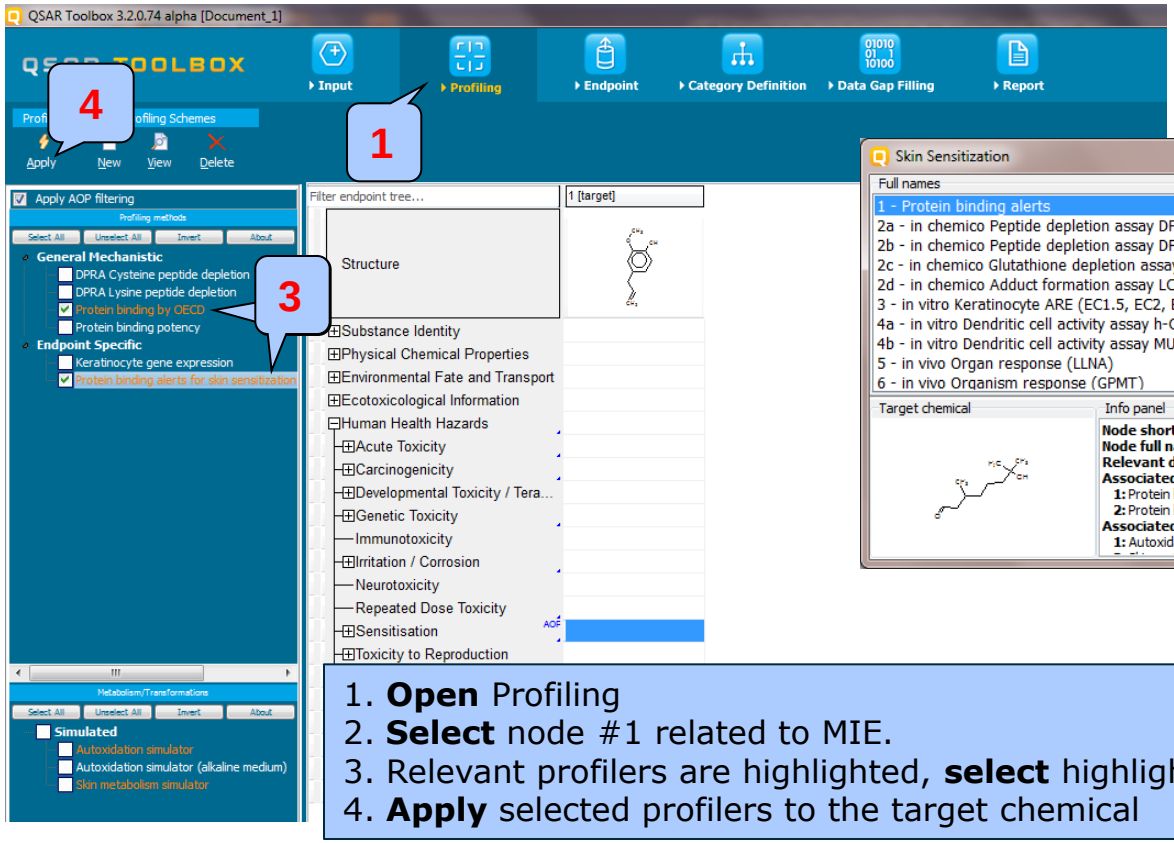
Workflow process

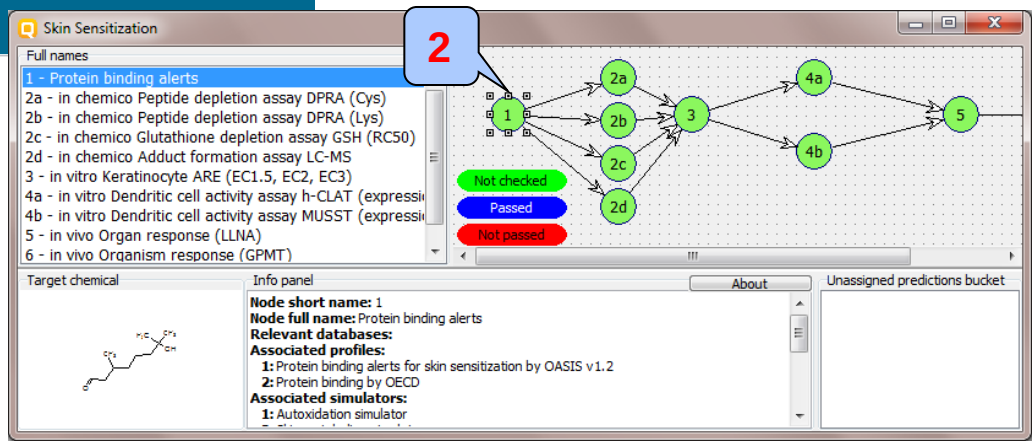
Step 1. MIE: protein binding

Example 2



Start with profiling of target chemical



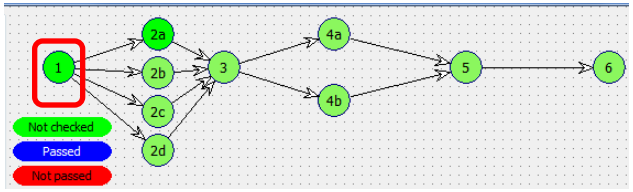


1. **Open** Profiling
2. **Select** node #1 related to MIE.
3. Relevant profilers are highlighted, **select** highlighted profilers
4. **Apply** selected profilers to the target chemical

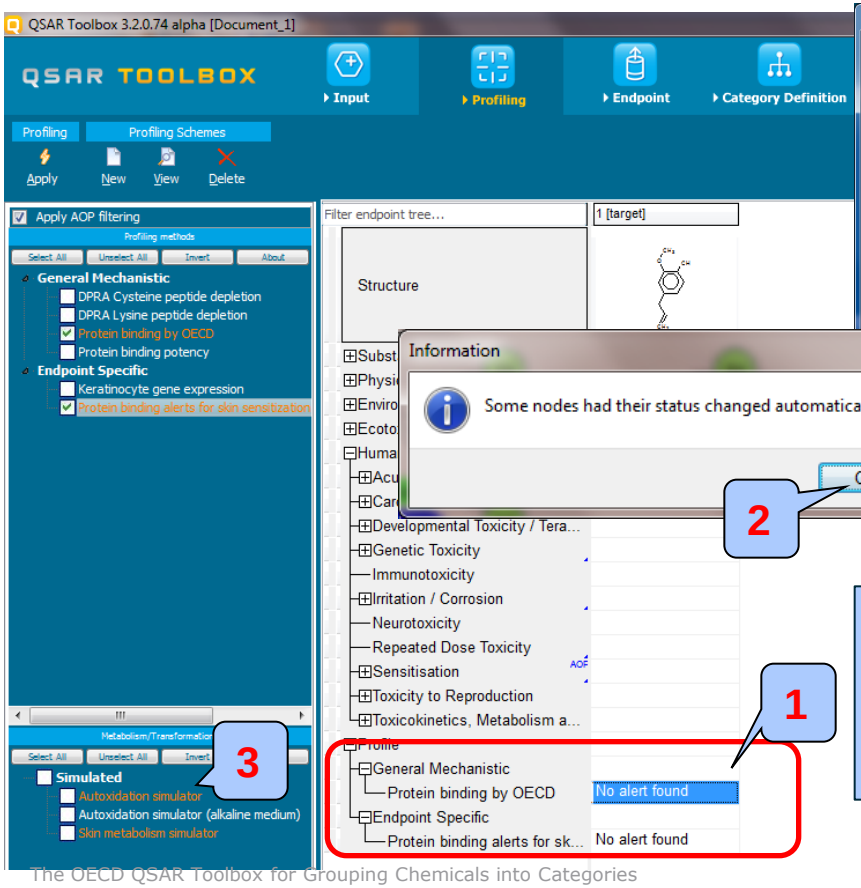
Workflow process

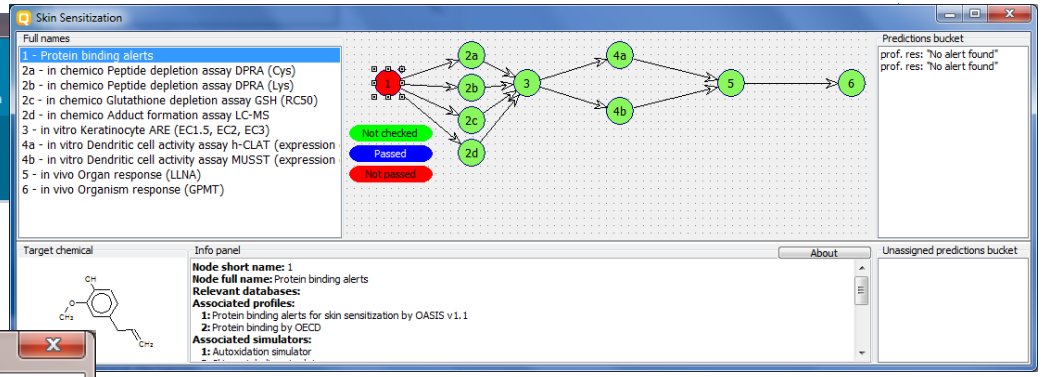
Step 1. MIE: protein binding

Example 2



Start with profiling of target chemical



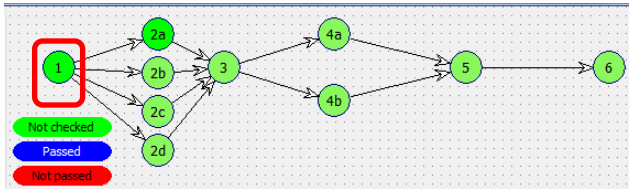


1.The target chemical has no protein binding alert
2.The node is automatically changed to not passed based on absence of alert. **Click OK**
3. The next step is to investigate whether the substance has skin sensitization potential via autoxidation

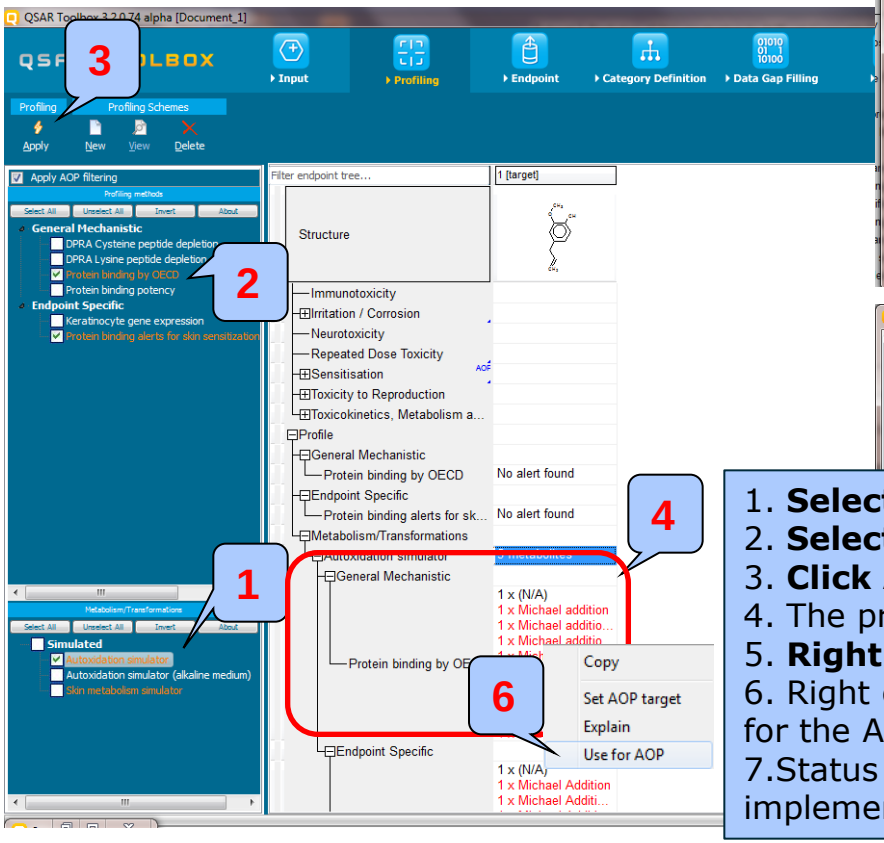
Workflow process

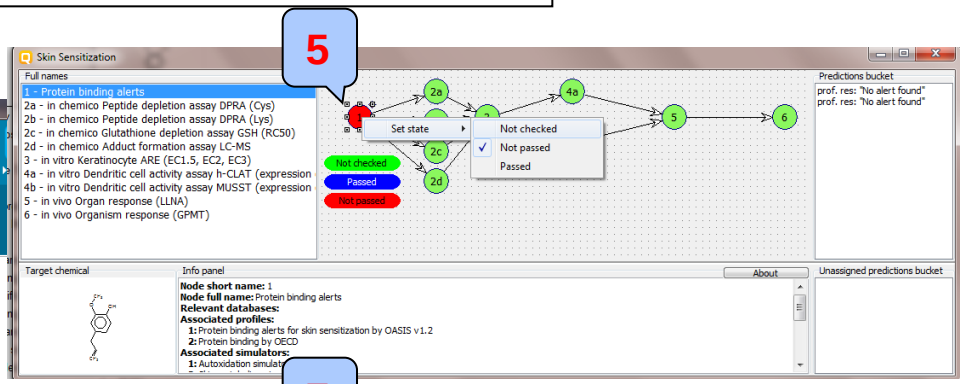
Step 1. MIE: protein binding

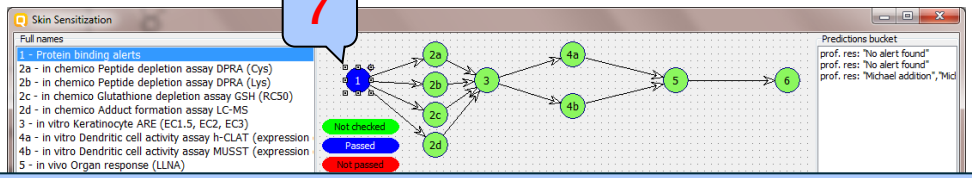
Example 2



Simulate Autoxidation products of the target chemical





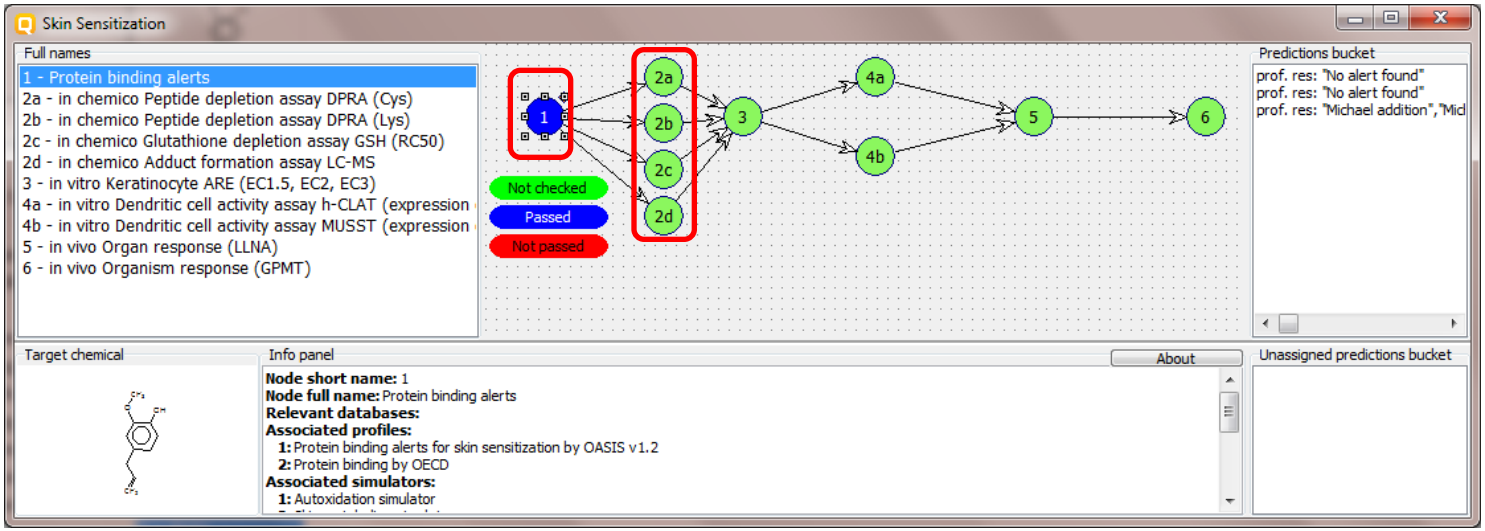


1. **Select** Autoxidation simulator
2. **Select** highlighted profilers relevant to the MIE
3. **Click** Apply
4. The profiling results appeared on data matrix
5. **Right click** over the node 1 and perform “Not checked”
6. Right click over the cell with profiling results and select “Use for the AOP”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

Workflow process

Molecular initiating events

Example 2

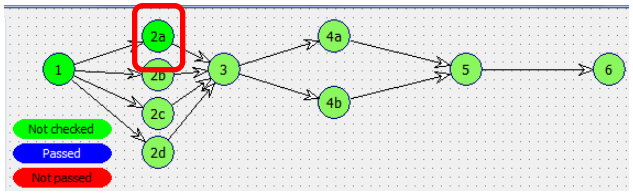


- The node MIE is passed due to the presence of positive protein binding alert identified for the Autoxidation products of the target chemical
- The workflow should move further to the *in chemico* assays

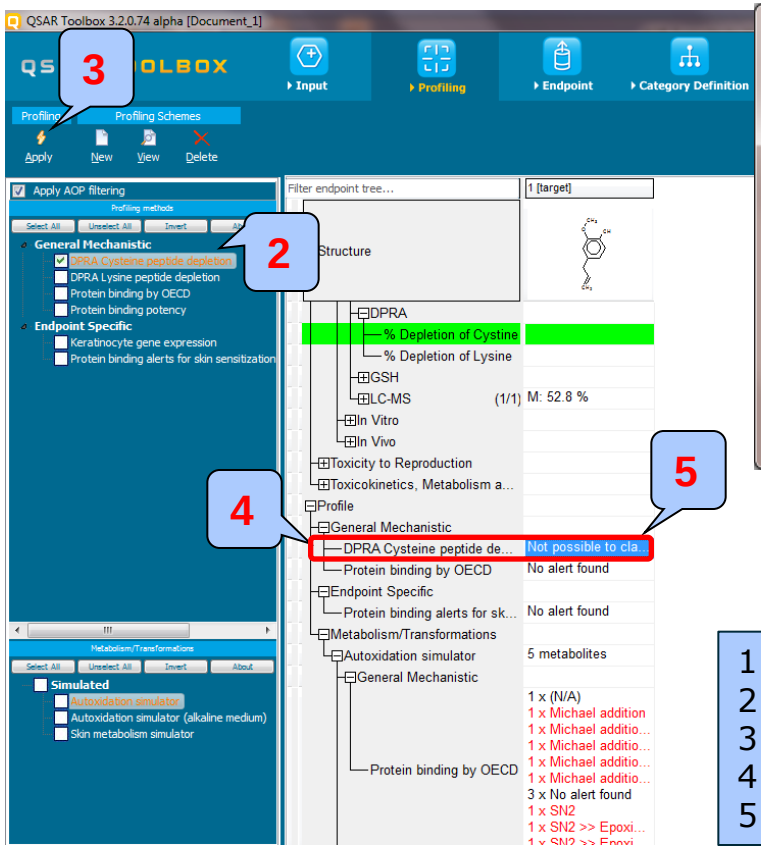
Workflow process

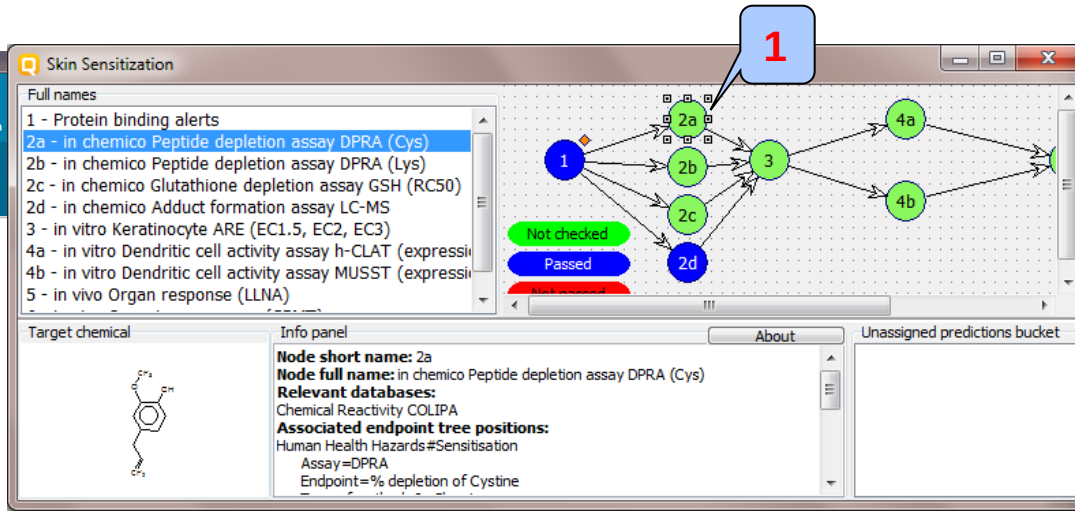
Step2. *In chemico* Peptide depletion assay DPRA (Cys) (node 2a)

Example 2



Profiling target chemical



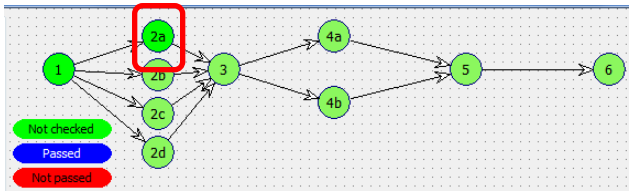


1. **Select** node 2a
2. **Select** highlighted profilers relevant to node 2a
3. **Click** Apply
4. The profiling results appeared on data matrix
5. There is no alert found for the target

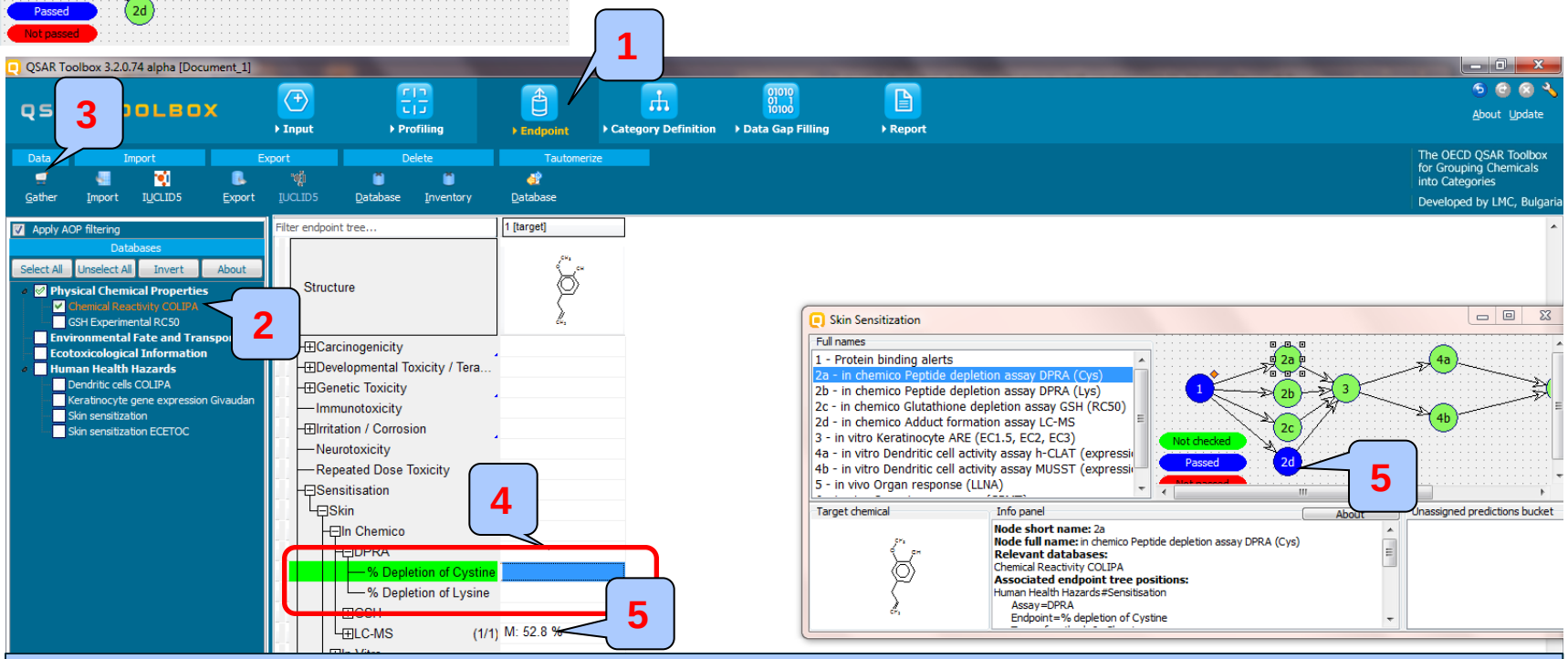
Workflow process

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

Example 2



Check for experimental data

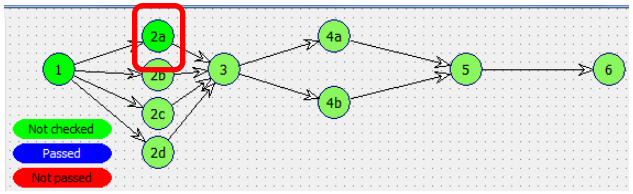


1. **Go to Endpoint**
2. **Select** highlighted database
3. **Click** Gather
4. There is no data for the target chemical
5. There is data for node 2d and node is getting passed.

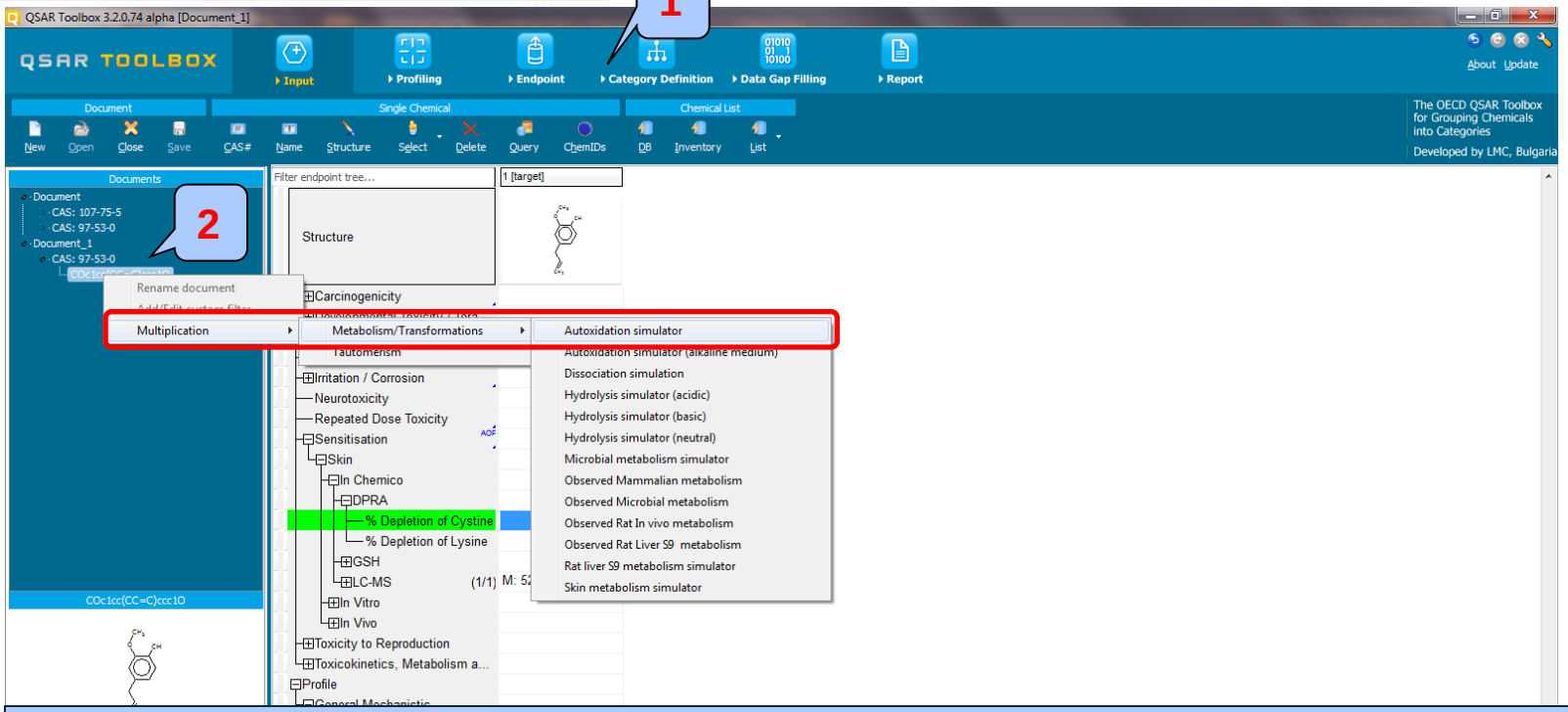
Workflow process

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

Example 2



In view of positive protein binding alert identified for the autoxidation products of the target chemical, these products will be further investigated.

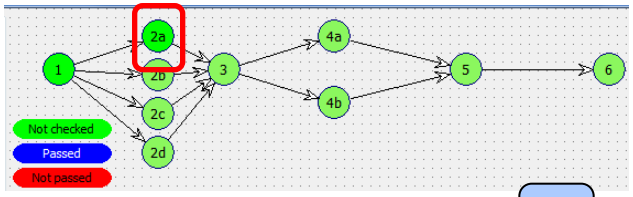


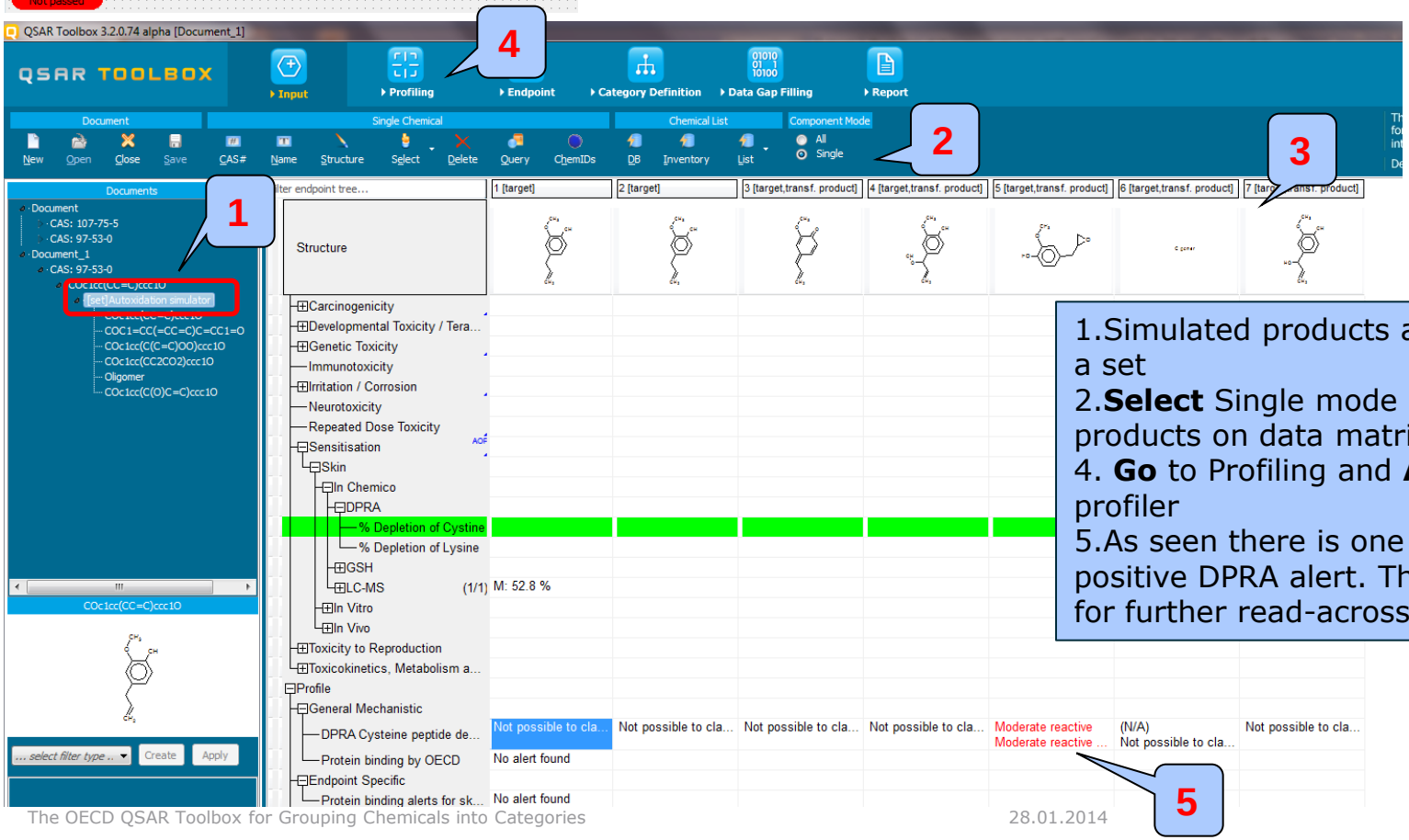
1. **Go** to Input
2. **Open** nodes of the tree and **Right click** over the node with SMILES and select Multiplication by Autoxidation simulator

Workflow process

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

Example 2



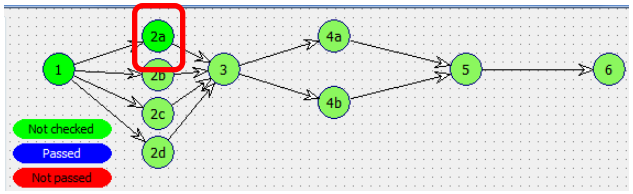


1.Simulated products are presented as a set
2.**Select** Single mode in order to display all products on data matrix (3)
4. **Go** to Profiling and **Apply** highlighted profiler
5.As seen there is one product with positive DPRA alert. This metabolite is used for further read-across analysis

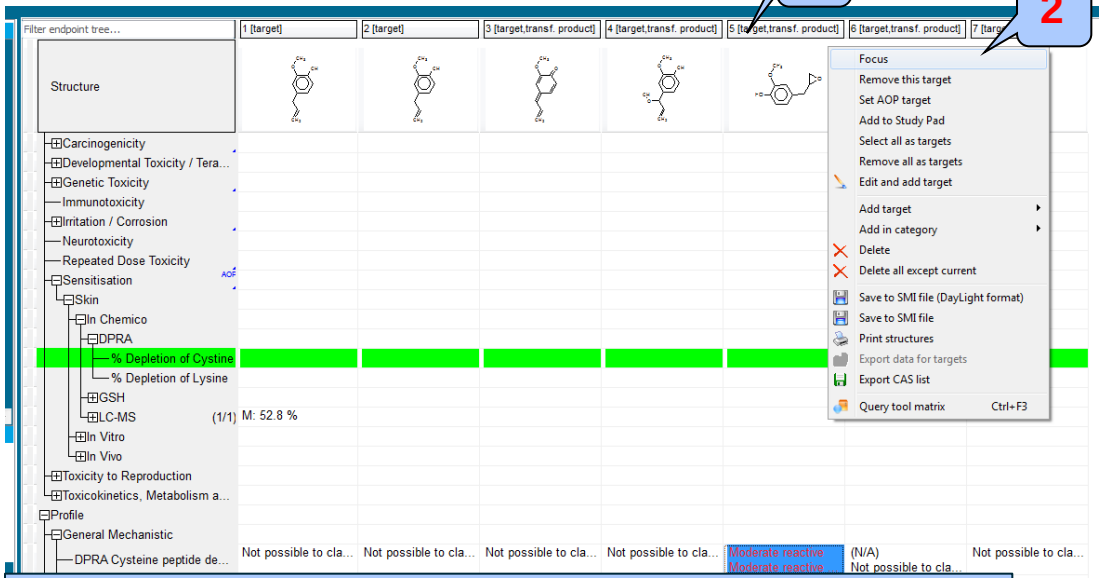
Workflow process

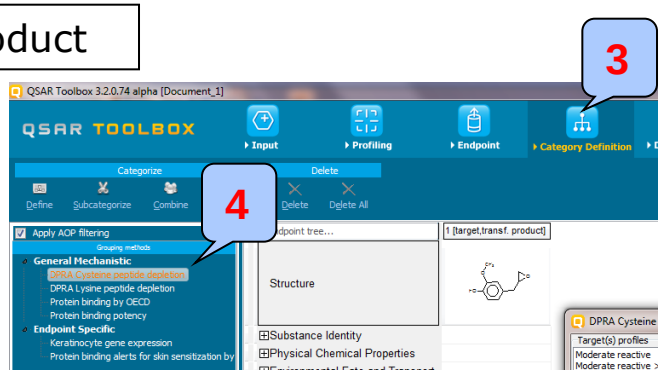
Step2. *In chemico* Peptide depletion assay DPRA (Cys) (node 2a)

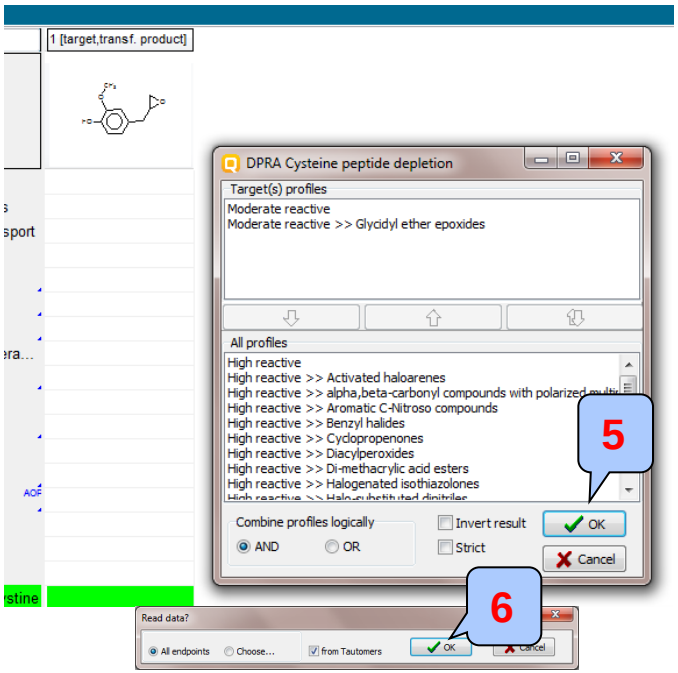
Example 2



RA analysis of the AO product





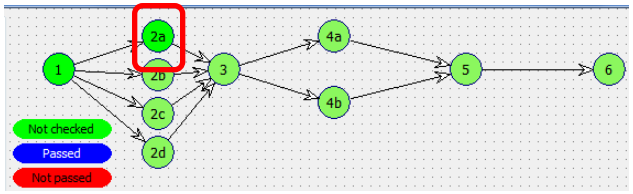


1.Right click over the metabolite #5
2.**Select** Focus, chemical appears in a new data matrix
3.**Go** to Category definition and
4. **Define category** by the highlighted category.
5. **Click** OK
6. **Read data** for analogues

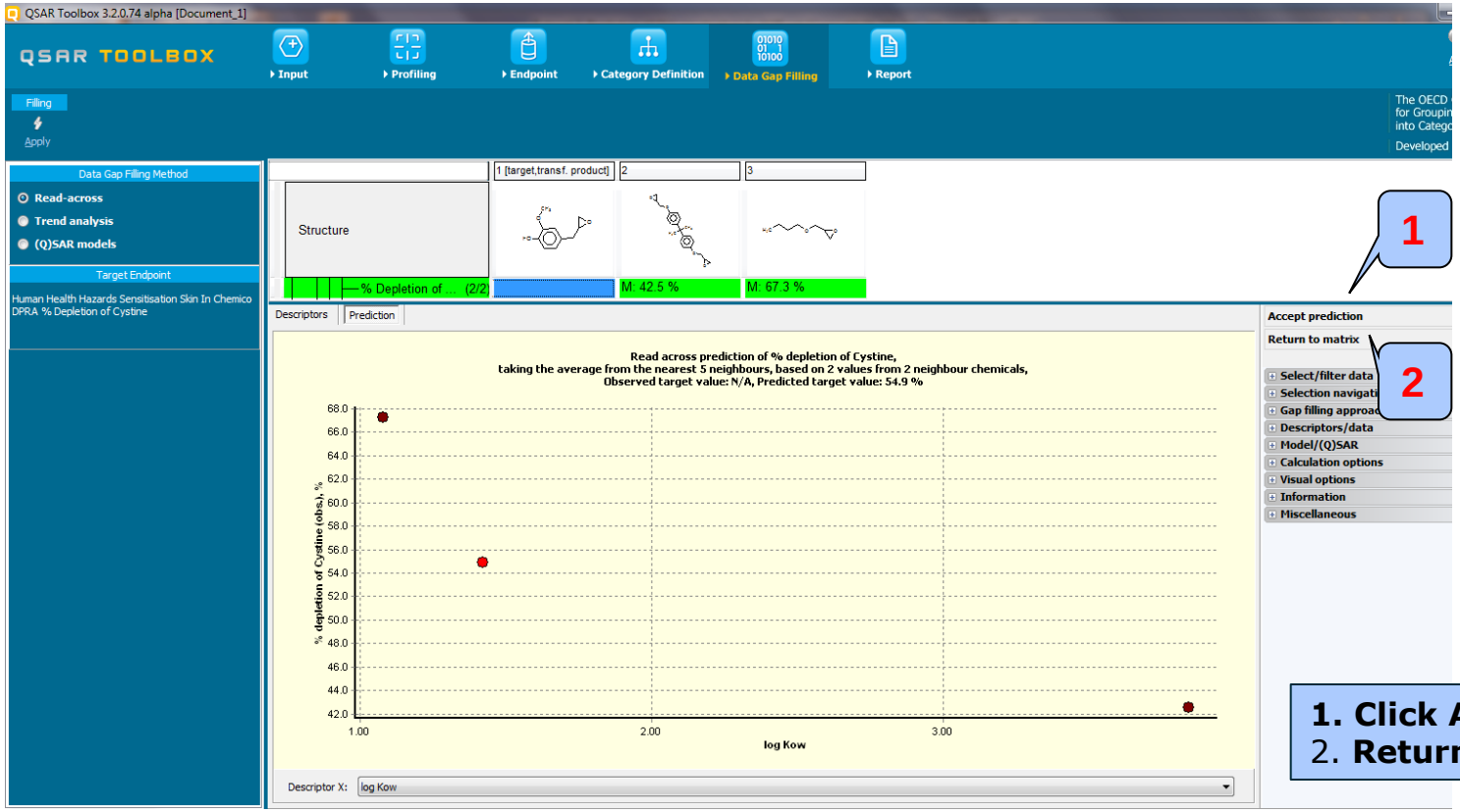
Workflow process

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

Example 2



Perform RA prediction for Autoxidation product of the target chemical

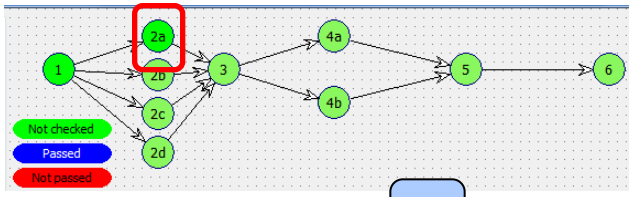


1. Click Accept
2. **Return to matrix**

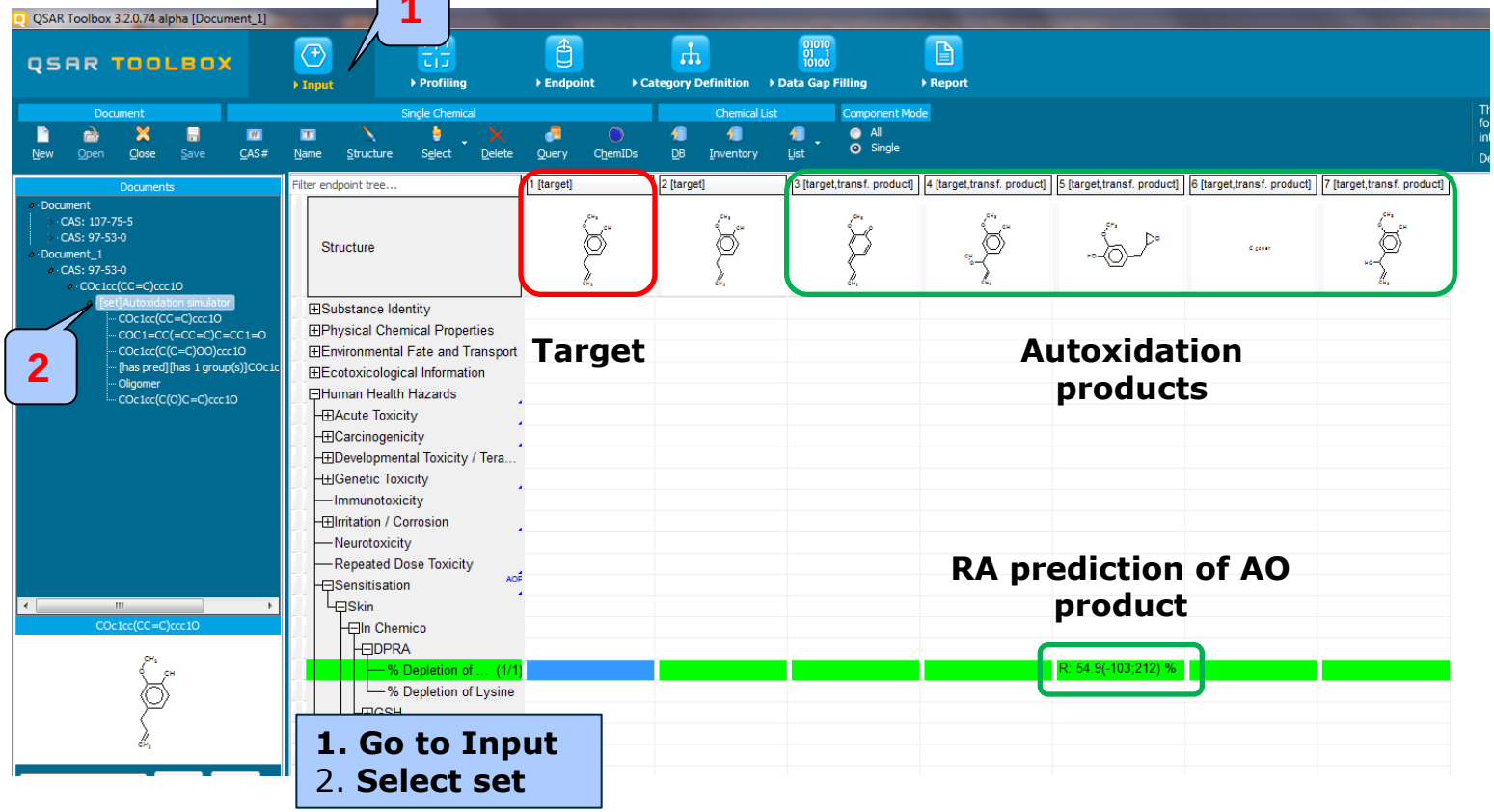
Workflow process

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

Example 2



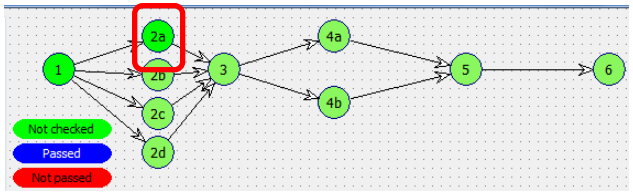
Assign prediction of AO product to the parent chemical



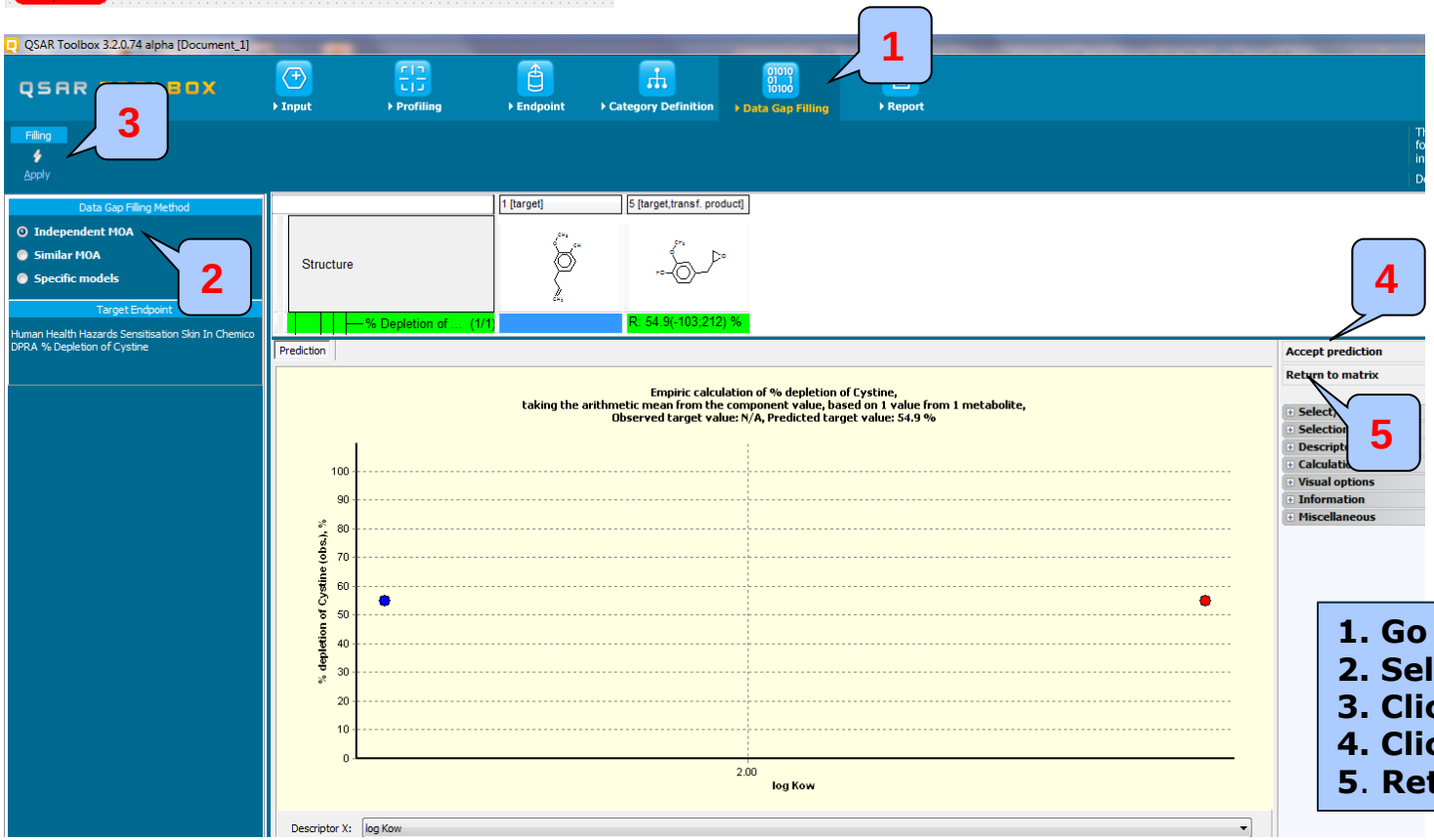
Workflow process

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

Example 2



Assign prediction of AO product to the parent chemical

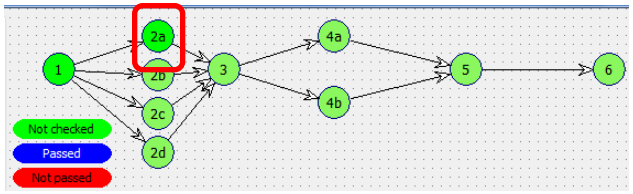


1. **Go** to Gap filling
2. **Select** Independent mode
3. **Click** Apply
4. **Click** Accept
5. **Return to matrix**

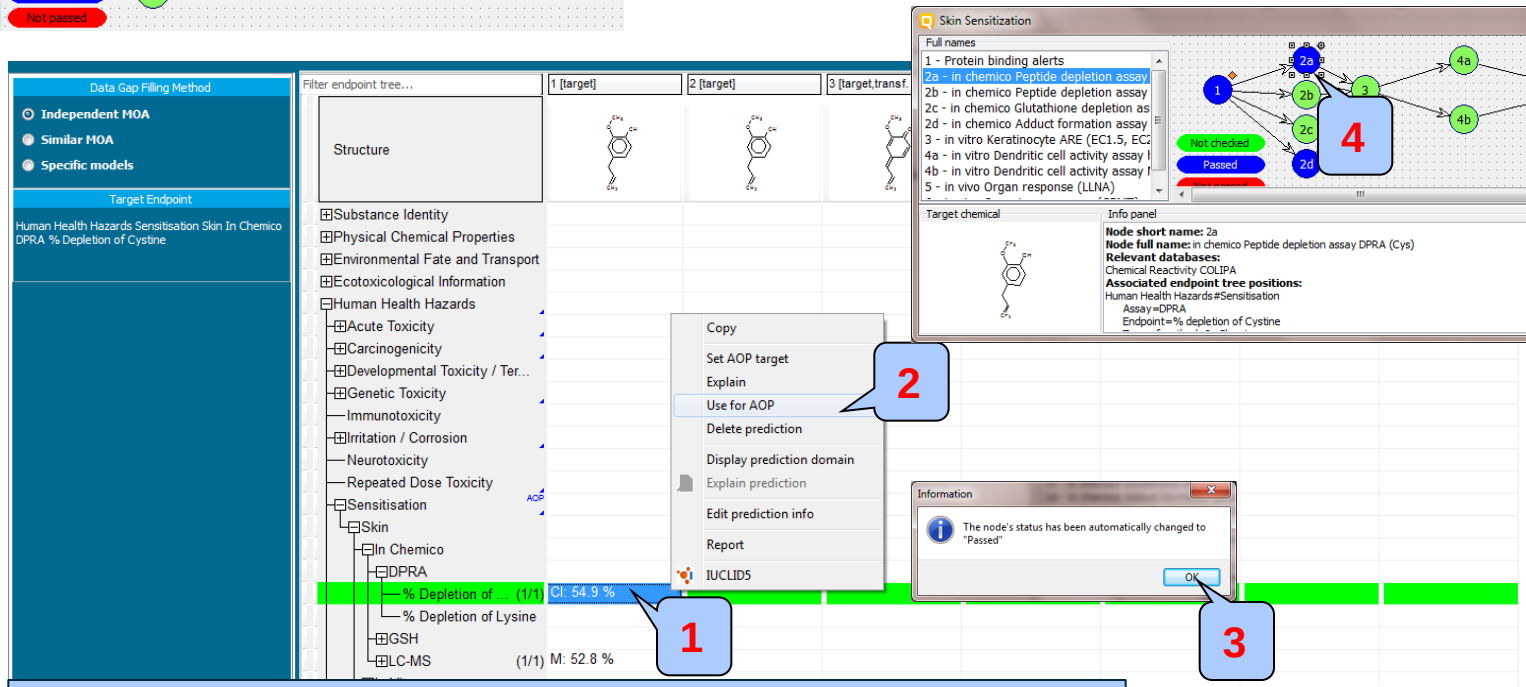
Workflow process

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

Example 2



Use read-across prediction for AOP



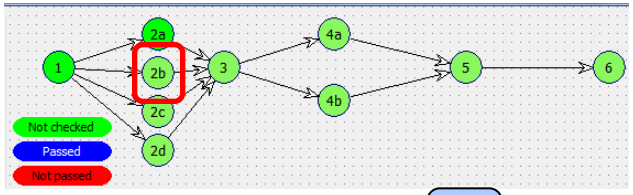
1. Prediction assigned to the target chemical is marked as CI (additional info provided on the right box)
2. **Right click** over the cell with prediction and click Use for AOP
3. **Click** OK
4. The status of the node was changed to passed
5. The prediction appears in the bucket

CI – component Independent mode (prediction based on Independent mode of action)
CS – component Similar mode (prediction based on Similar mode of action)

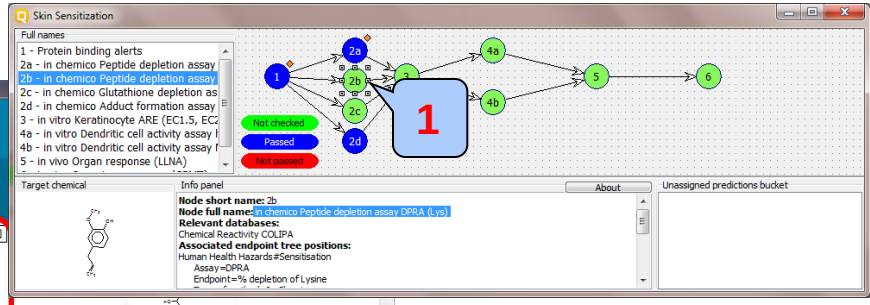
Workflow process

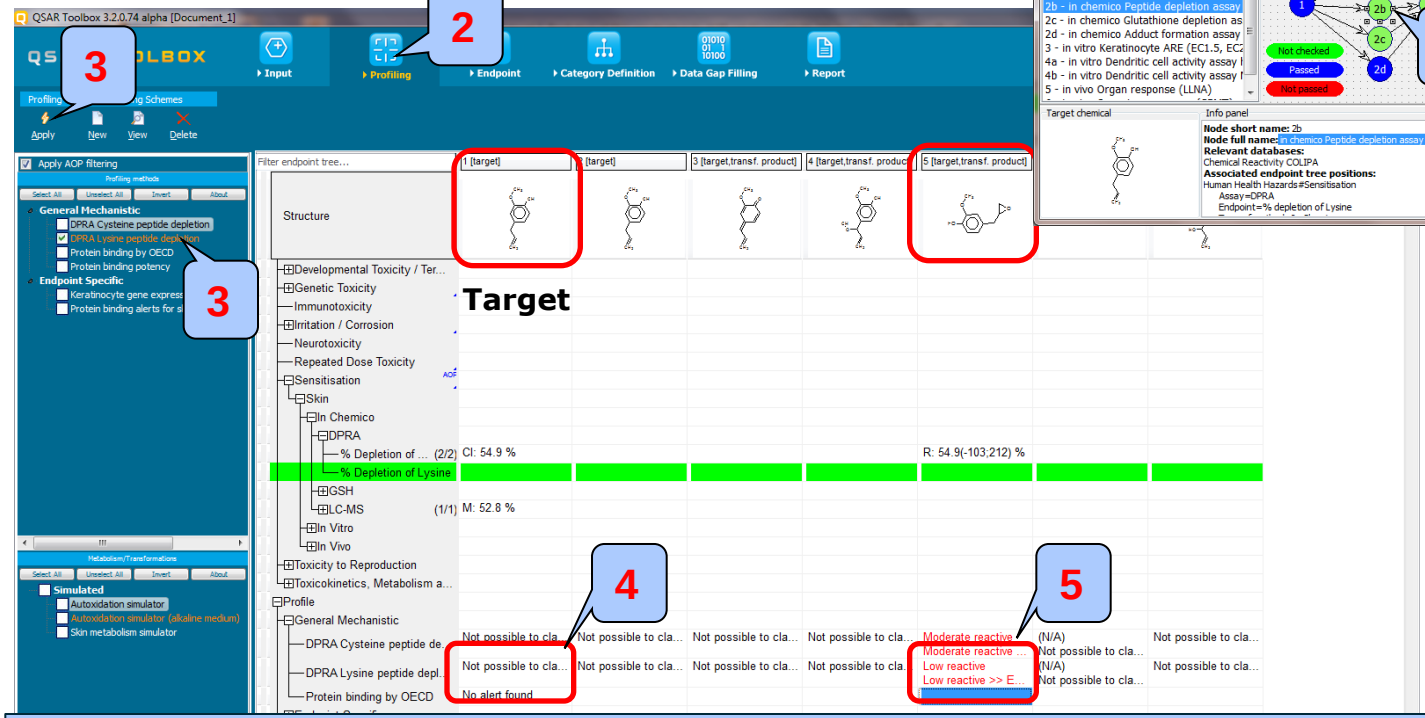
Step2. *In chemico* Peptide depletion assay DPRA (Lys) (node 2b)

Example 2



Investigate node 2b – Lysine depletion assay



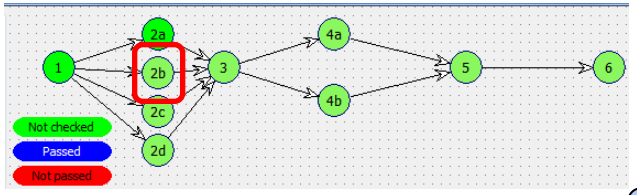


1. Select node 2b related to Lysine peptide depletion assay
2. **Go** to Profiling
3. **Select** relevant profiler and **Apply** to the parent and AO products
4. As seen there is no DPRA alert for the parent. However a positive Lys alert is identified for the metabolite #5
5. In this respect RA across was obtained for the AO product based on the fact that no alert was found for the parent chemical

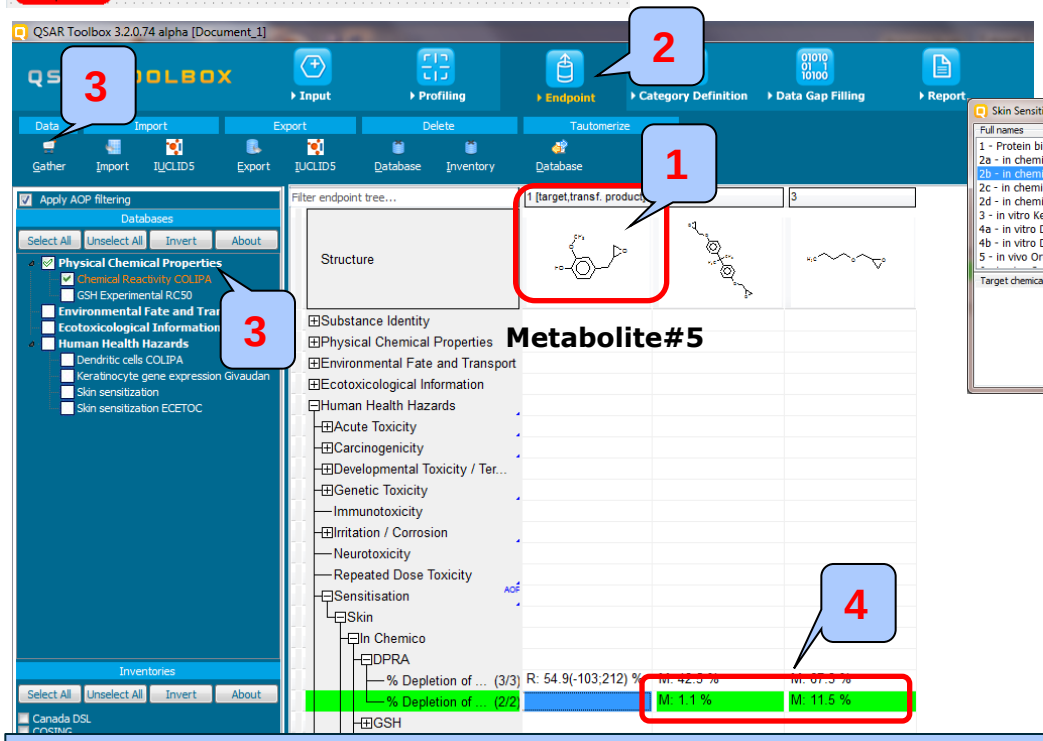
Workflow process

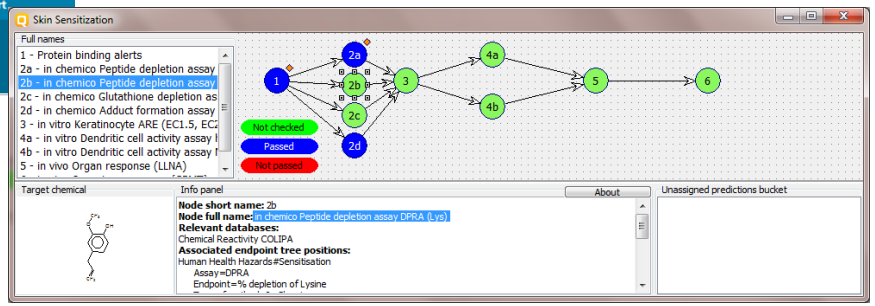
Step2. *In chemico* Peptide depletion assay DPRA (Lys) (node 2b)

Example 2



Collect experimental data for metabolite and analogues within category



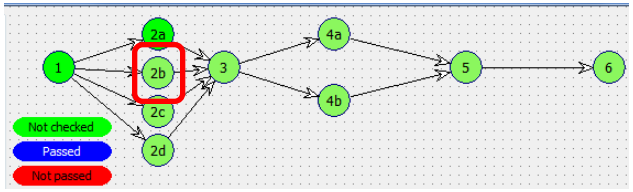


1. Focus metabolite #5. It appears in a new datamatrix
2. **Go to Endpoint** and check if there are any data for the metabolite and analogues within category
3. **Select** relevant database and click **Gather**
4. As seen there is Lysine data for the two analogues. The next step is to perform RA analysis

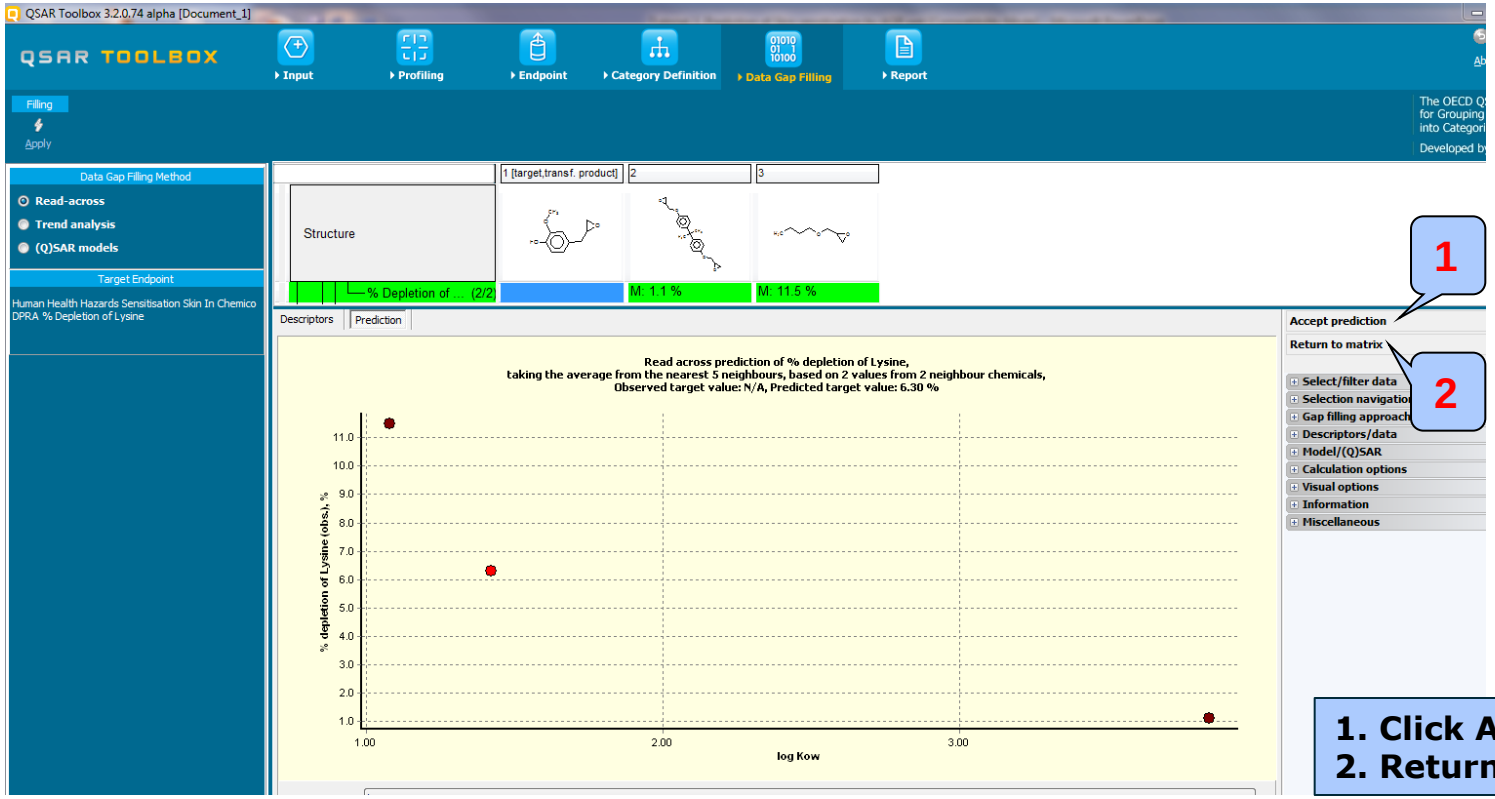
Workflow process

Step2. *In chemico* Peptide depletion assay DPRA (Lys) (node 2b)

Example 2



Perform read-across analysis for the metabolite

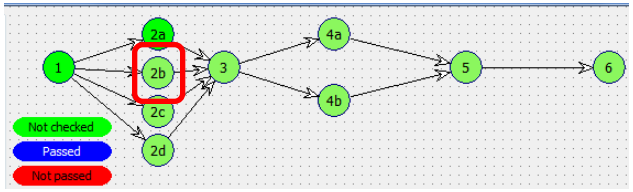


1. Click Accept
2. Return to matrix

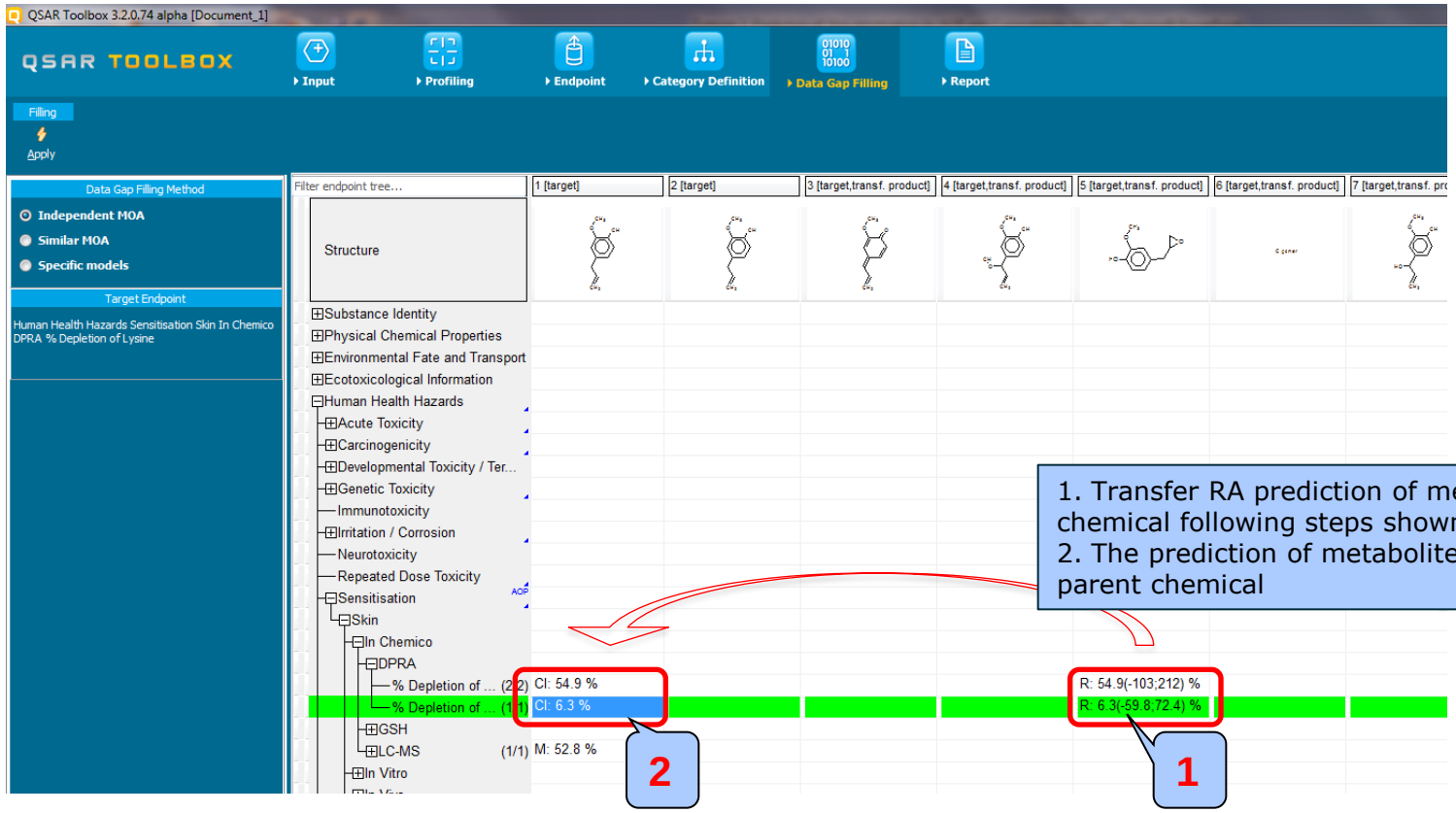
Workflow process

Step2. *In chemico* Peptide depletion assay DPRA (Lys) (node 2b)

Example 2



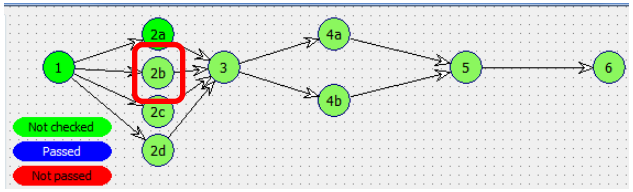
Transferring RA prediction of metabolite to the target chemical



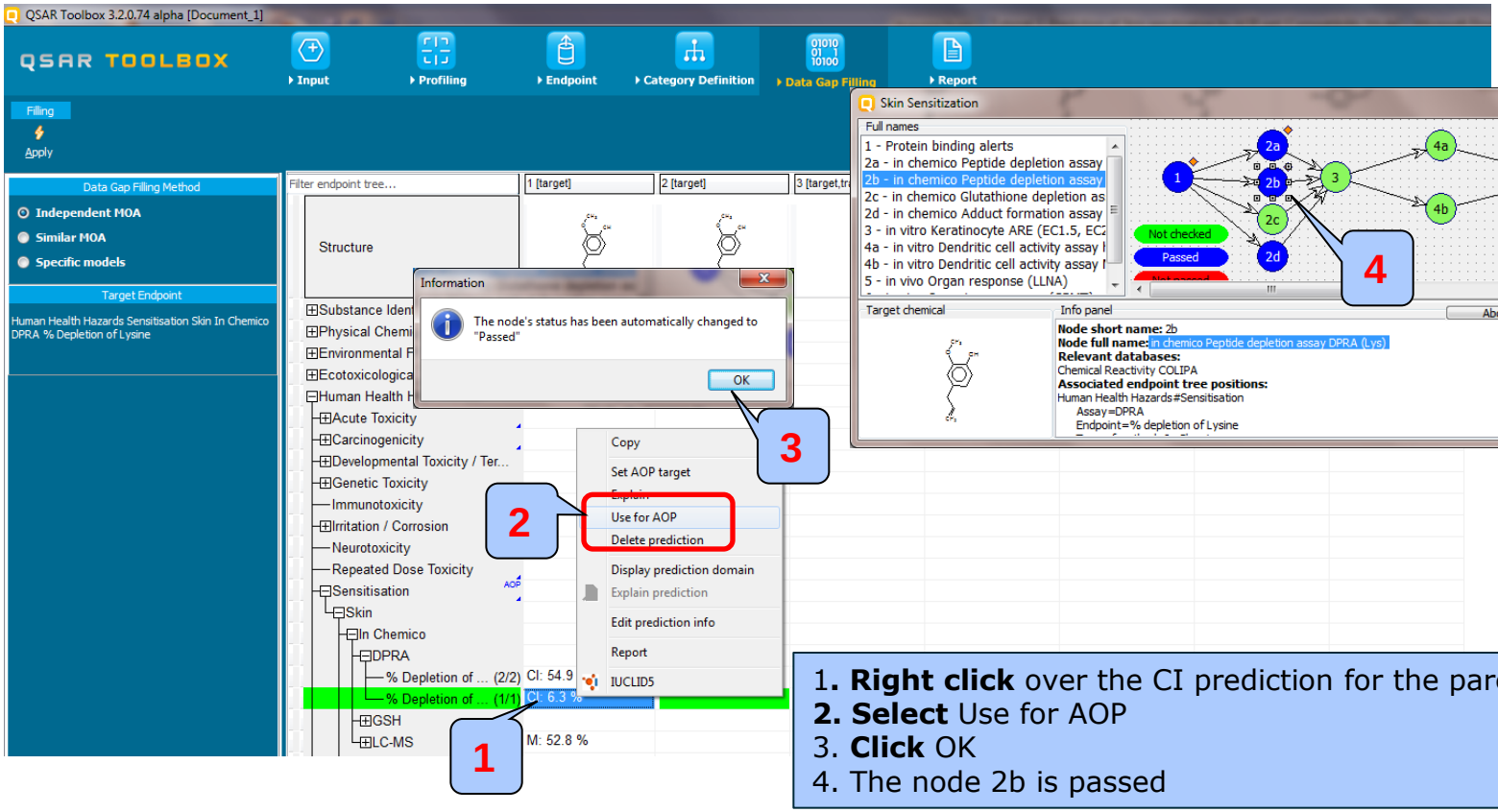
Workflow process

Step2. *In chemico* Peptide depletion assay DPRA (Lys) (node 2b)

Example 2



Use read-across prediction of the parent for AOP

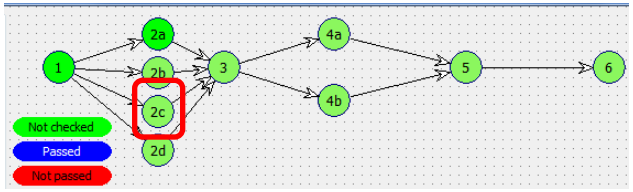


1. **Right click** over the CI prediction for the parent chemical
2. **Select** Use for AOP
3. **Click** OK
4. The node 2b is passed

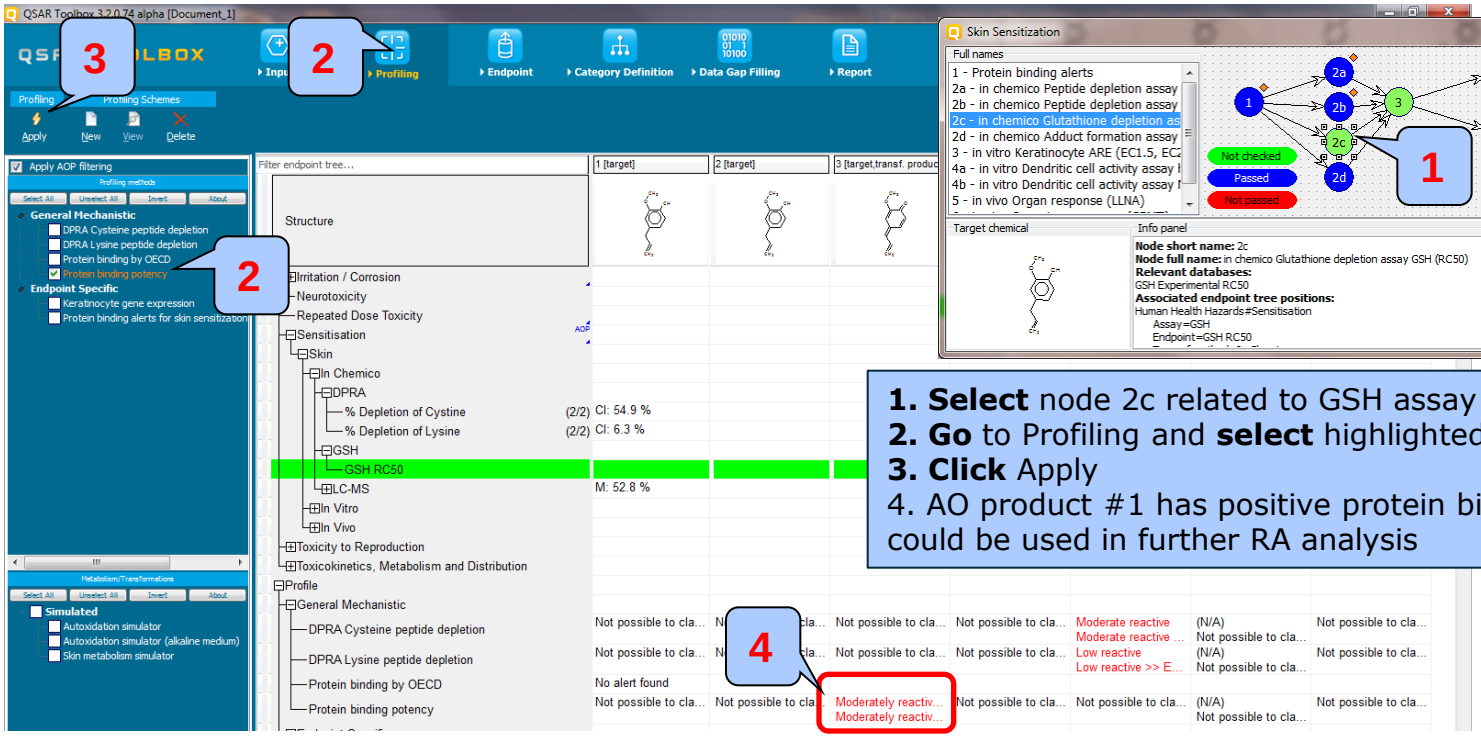
Workflow process

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

Example 2



Analyze node 2c related to GSH depletion assay.
Profile the set of parent and AO products

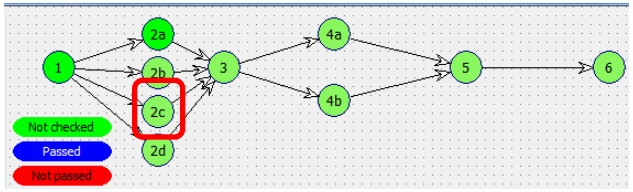


1. **Select** node 2c related to GSH assay
2. **Go** to Profiling and **select** highlighted profile
3. **Click** Apply
4. AO product #1 has positive protein binding potency alert. This could be used in further RA analysis

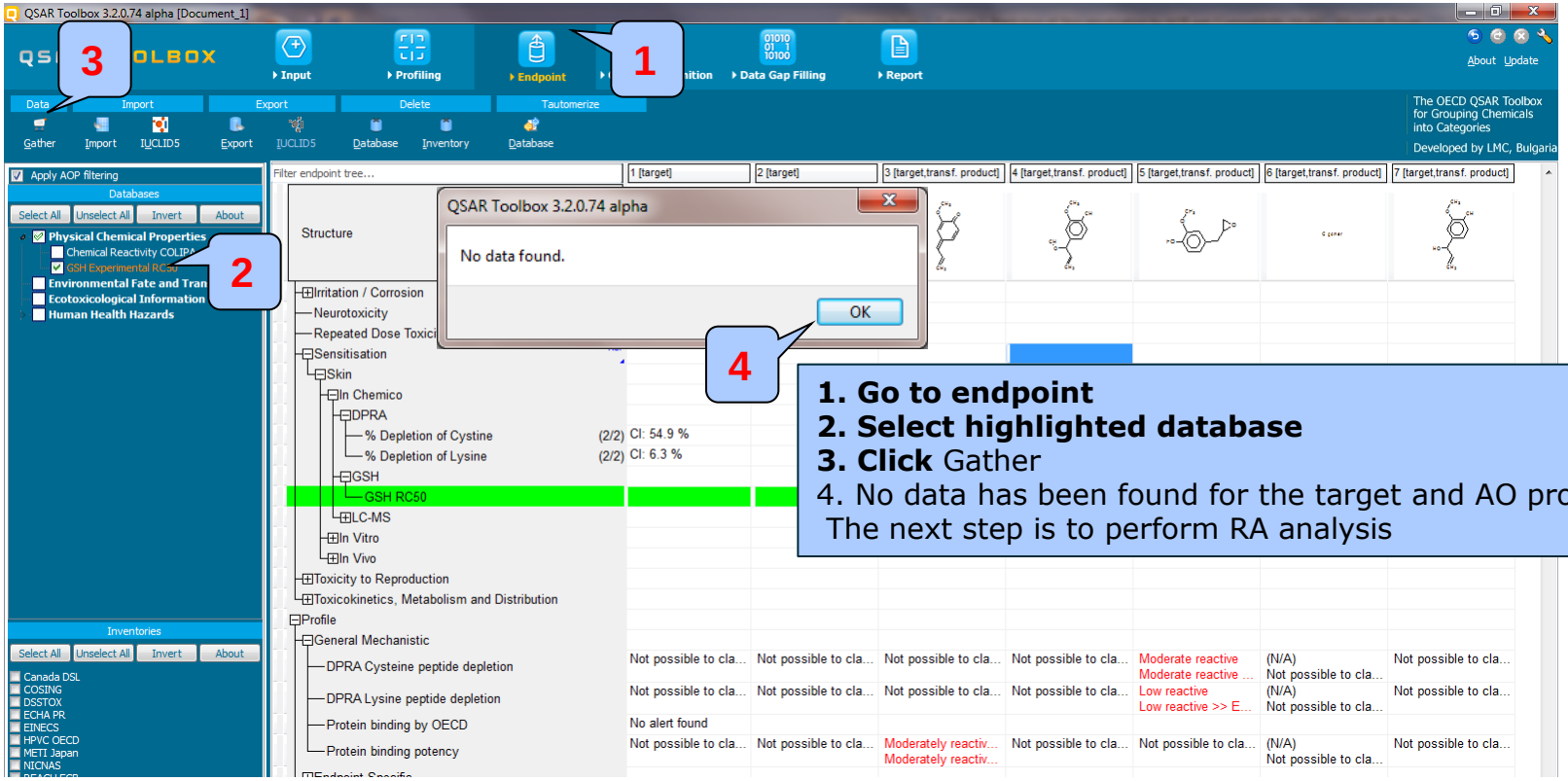
Workflow process

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

Example 2



Check are there any data for the target chemical

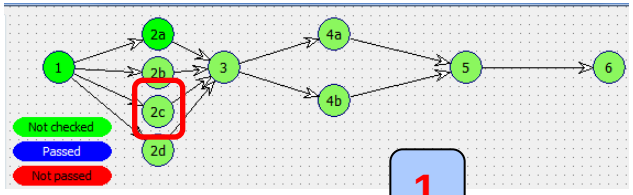


1. **Go to endpoint**
2. **Select highlighted database**
3. **Click** Gather
4. No data has been found for the target and AO products. **Click OK**
   The next step is to perform RA analysis

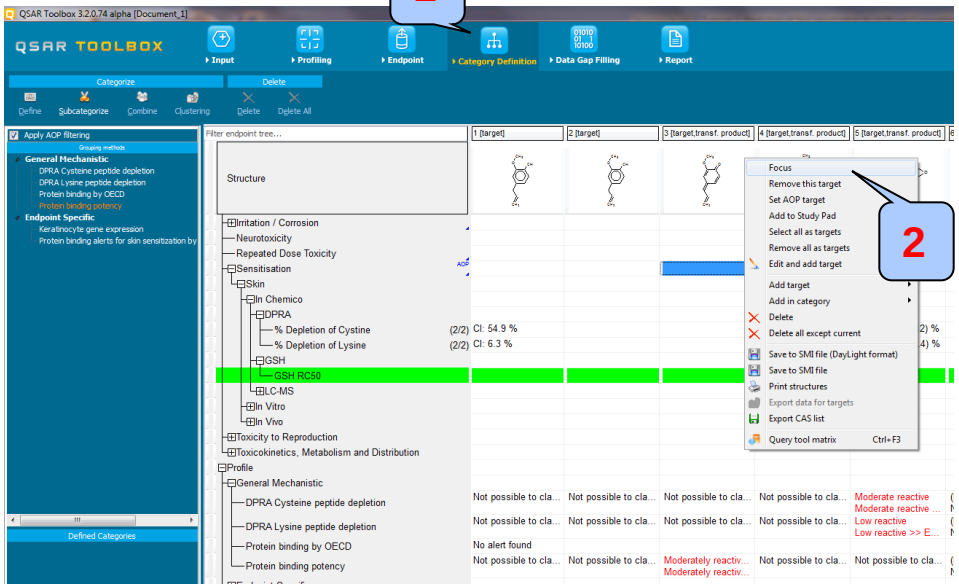
Workflow process

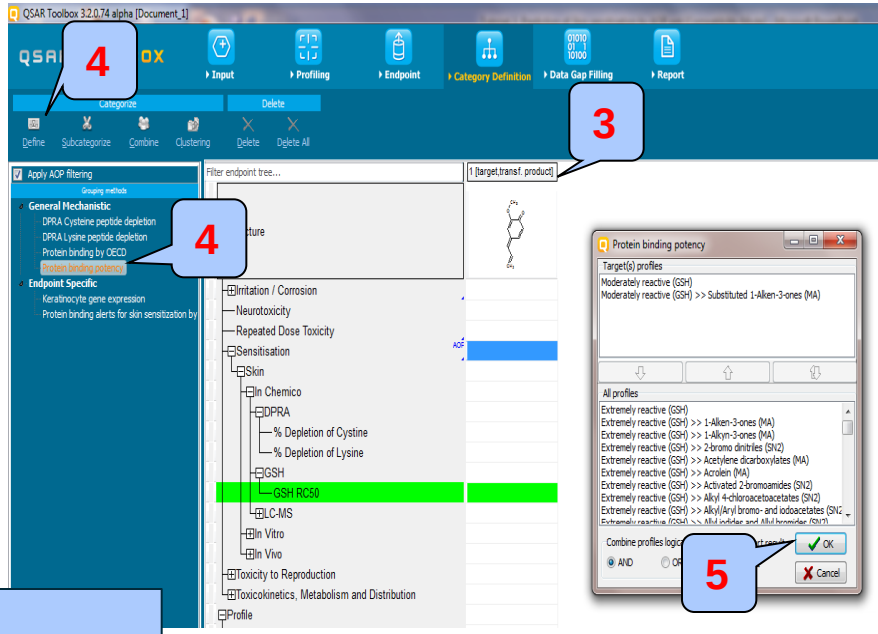
Step2. *In chemico* Glutathione depletion assay GSH (RC50)(node 2c)

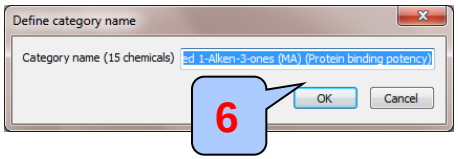
Example 2



Identify category with similar analogues of the AO product #1







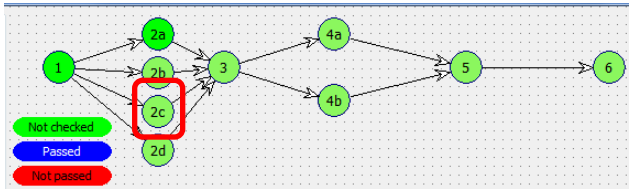
1. **Go** to Category definition
2. **Right** click over the structure and **Select** Focus
3. The focused metabolite appears in a new data matrix
4. **Selec**t highlighted category and **click** Define category
5. **Click** OK
6. Confirm category of 15 analogues. **Click OK** and read-data for the analogues

The next step is to perform RA analysis

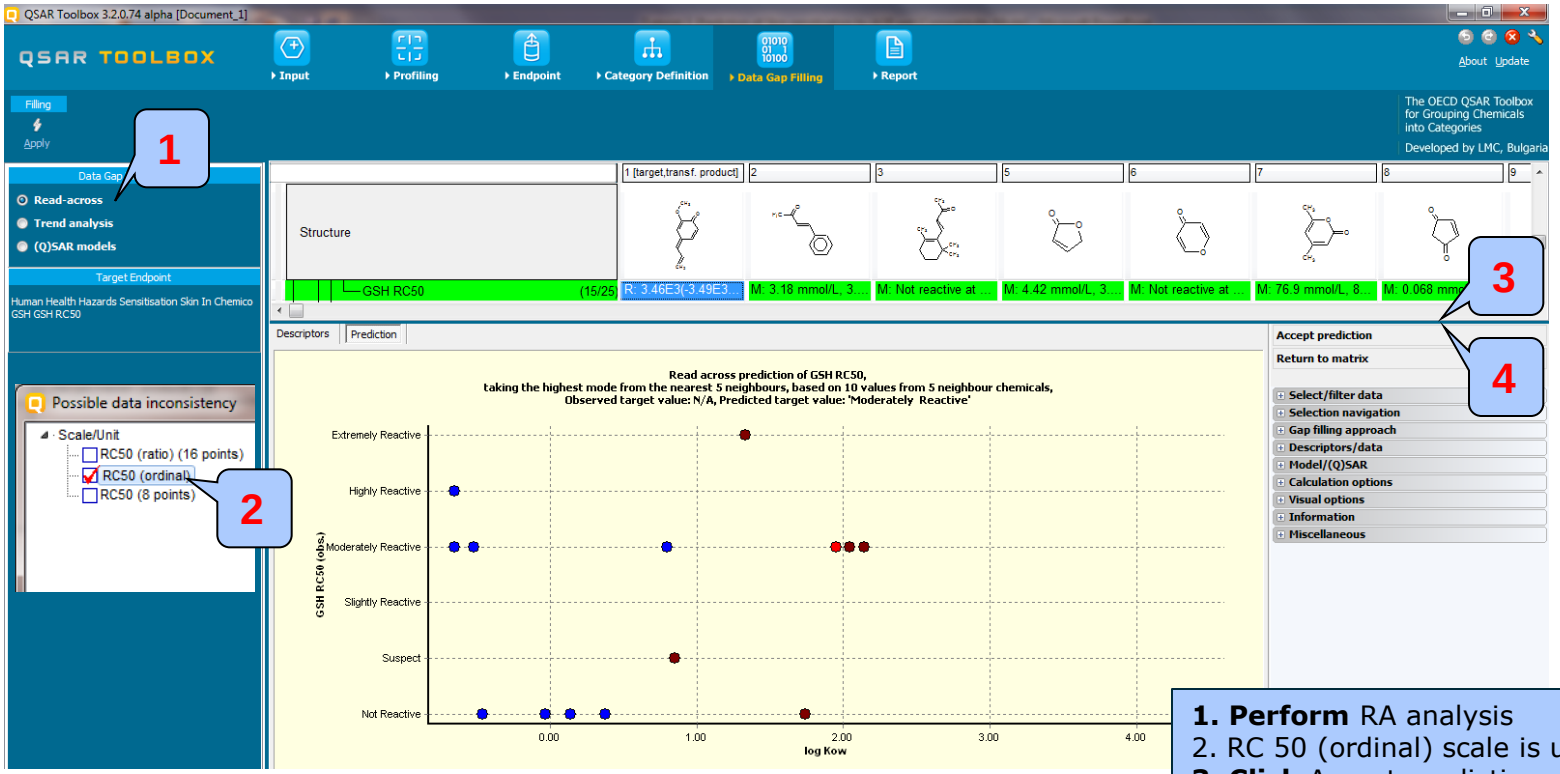
Workflow process

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

Example 2



Perform read-across analysis for the AO product

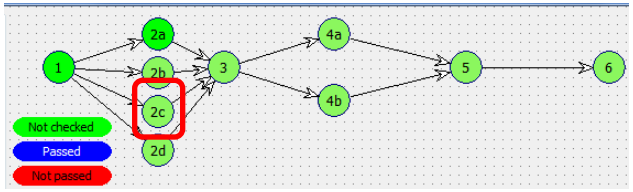


1. Perform RA analysis
2. RC 50 (ordinal) scale is used in RA prediction
3. Click Accept prediction
4. **Return to matrix**

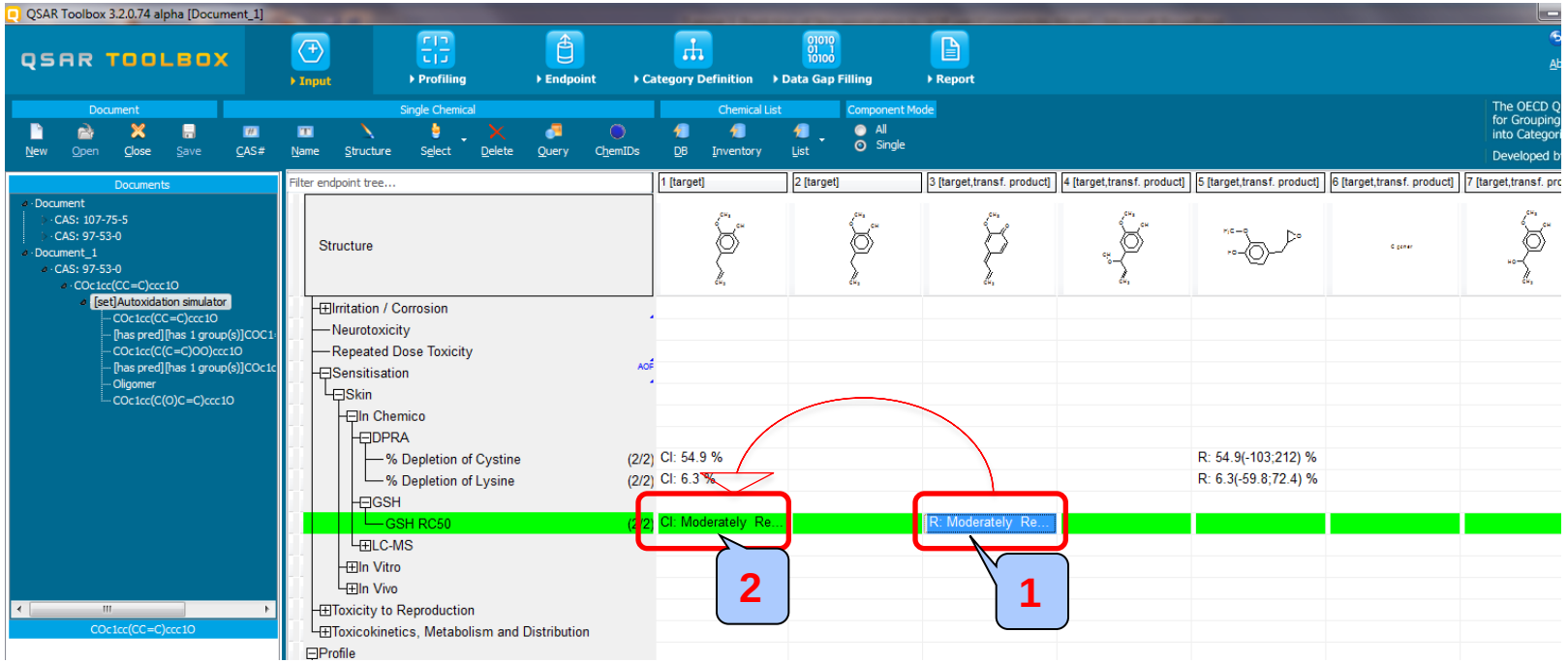
Workflow process

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

Example 2



Transferring RA prediction of metabolite to the target chemical

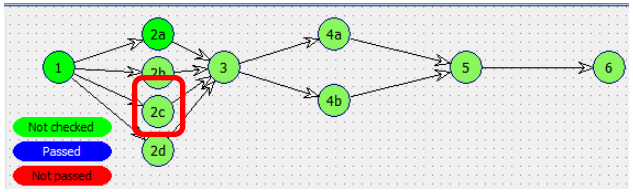


1. Transfer RA prediction for RC50 of metabolite to the parent chemical following steps shown on slide # 68
2. The prediction of metabolite is transferred to the parent chemical

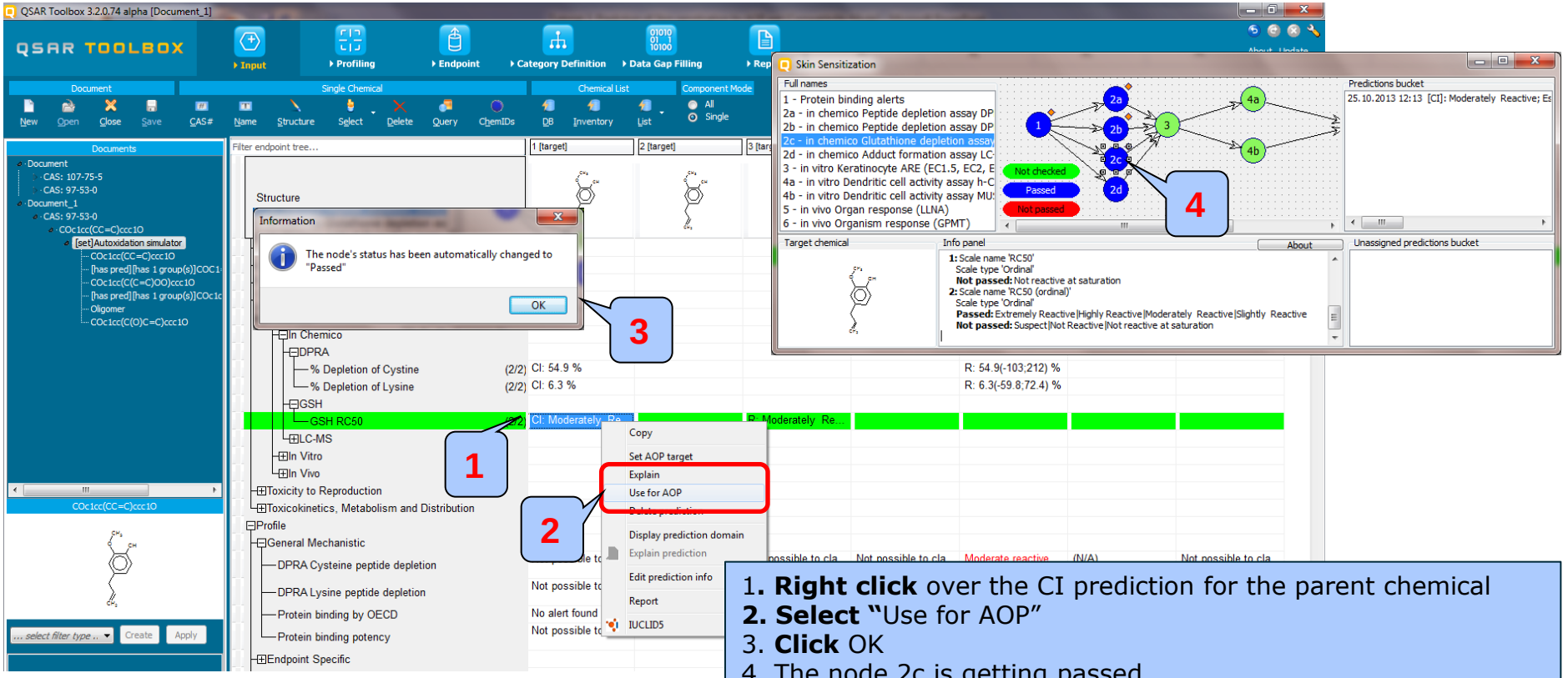
Workflow process

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

Example 2



Use read-across prediction of the parent for AOP

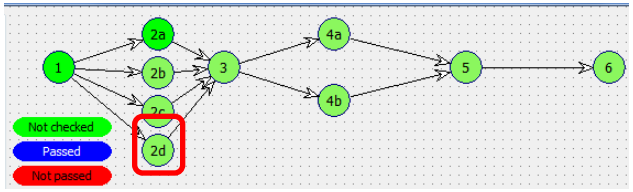


1. **Right click** over the CI prediction for the parent chemical
2. **Select** "Use for AOP"
3. **Click** OK
4. The node 2c is getting passed

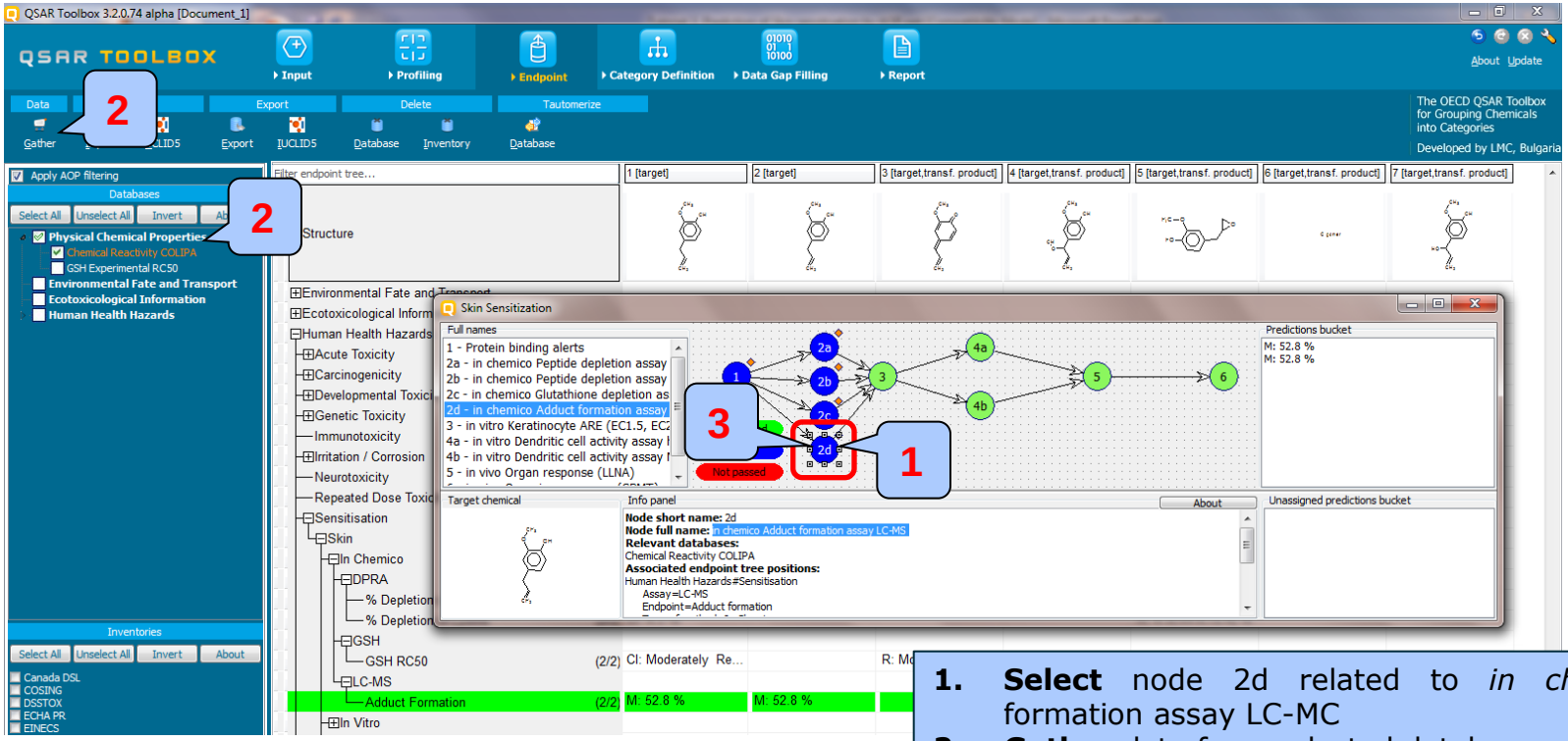
Workflow process

Step2. *In chemico* Adduct formation assay LC-MS (node 2d)

Example 2



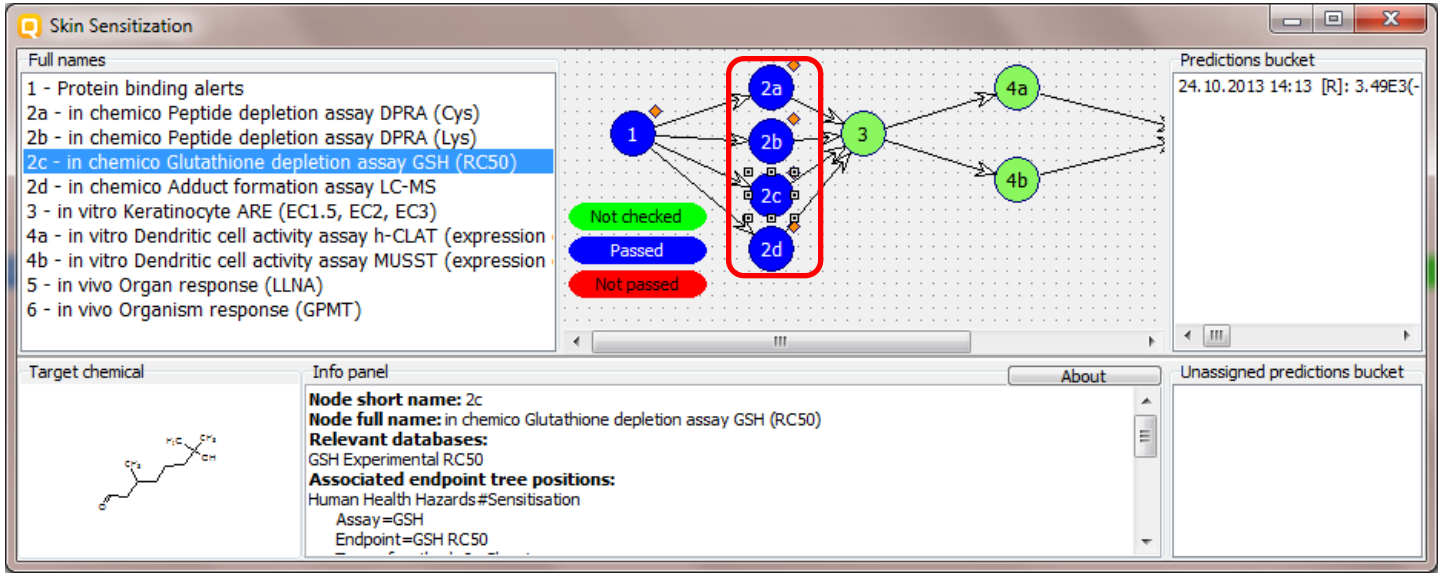
Node 2d *In chemico* adduct formation assay is passed based on measured data



1. **Select** node 2d related to *in chemico* Adduct formation assay LC-MC
2. **Gather** data from selected database.
3. **Node 2d** is getting passed

Workflow process
In chemico assays

Example 2

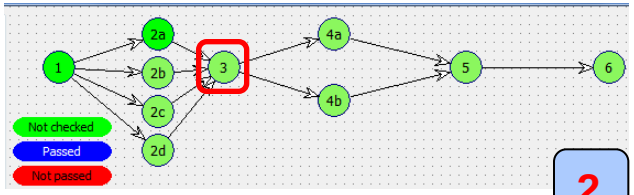


- The nodes related to the *in chemico* assays are passed due to positive experimental data for the target chemical (node 2d) and positive RA predictions for the potential metabolite (node 2a,2b).
- The workflow should move further to the *in vitro* assay (node 3)

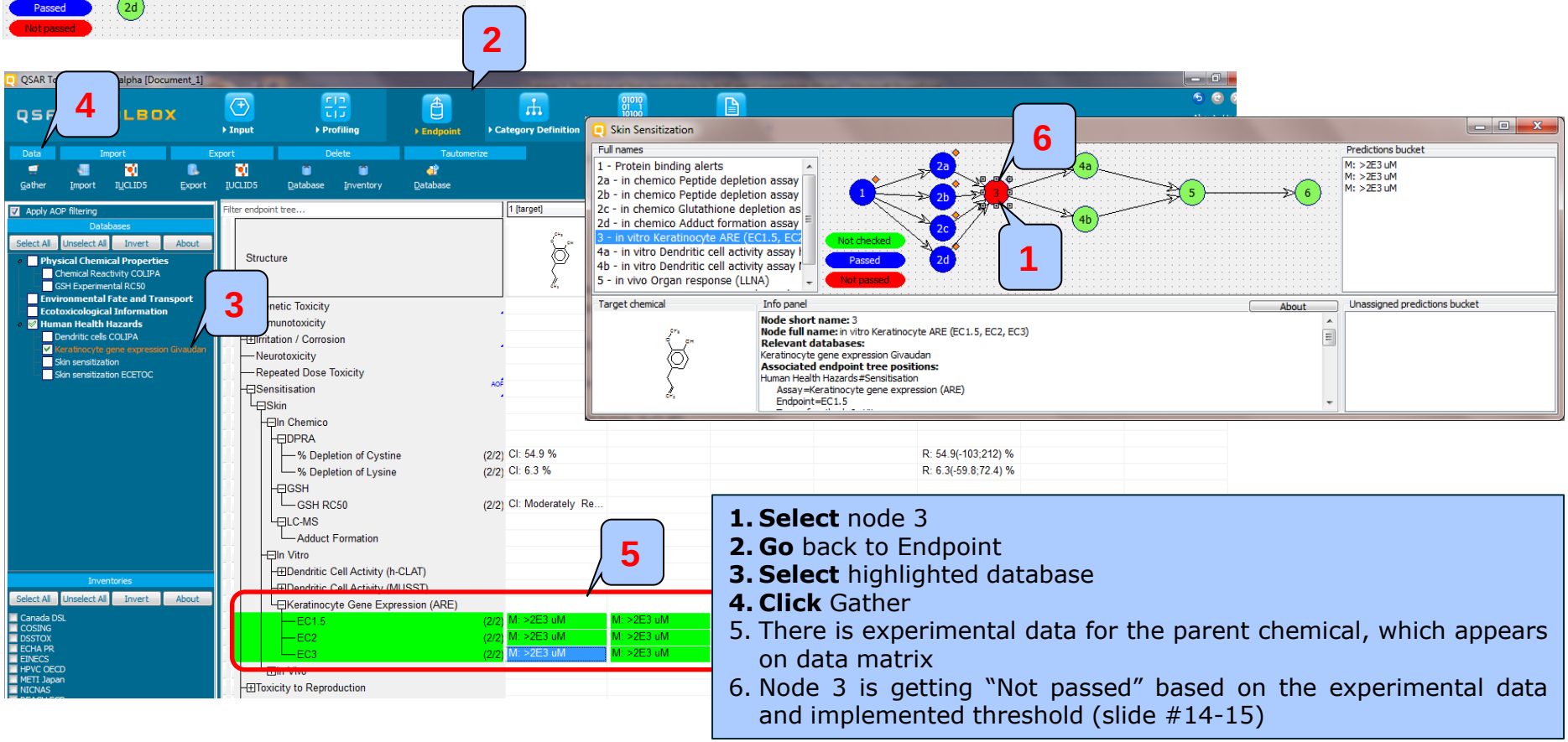
Workflow process

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

Example 2



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

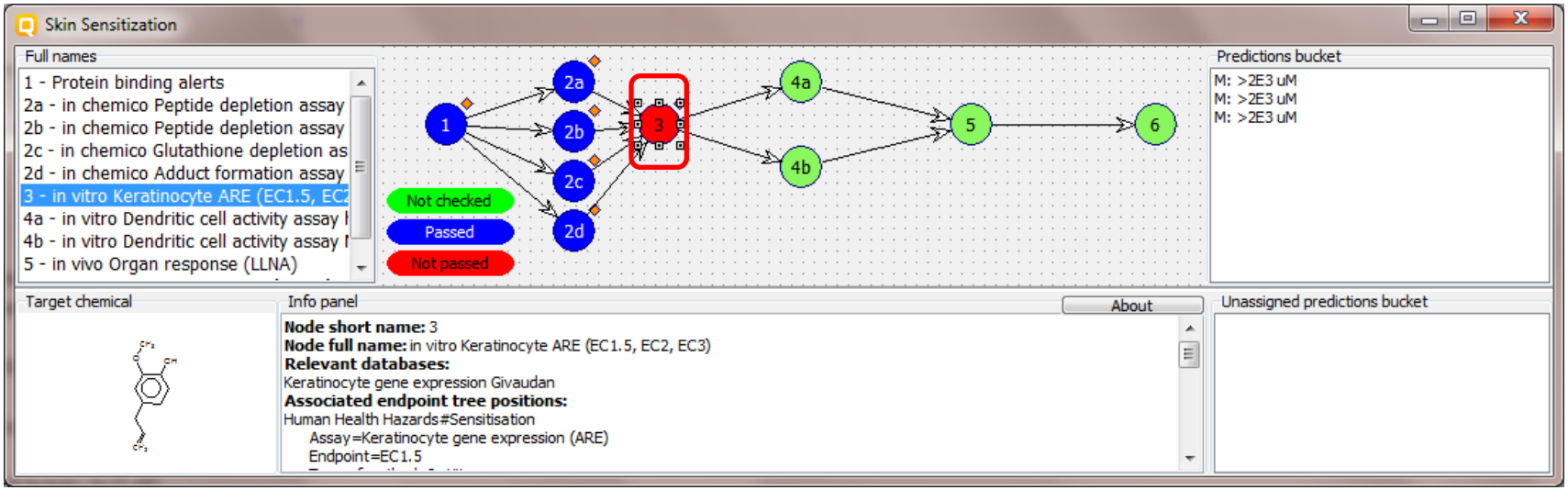


1. **Select** node 3
2. **Go** back to Endpoint
3. **Select** highlighted database
4. **Click** Gather
5. There is experimental data for the parent chemical, which appears on data matrix
6. Node 3 is getting "Not passed" based on the experimental data and implemented threshold (slide #14-15)

Workflow process

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

Example 2

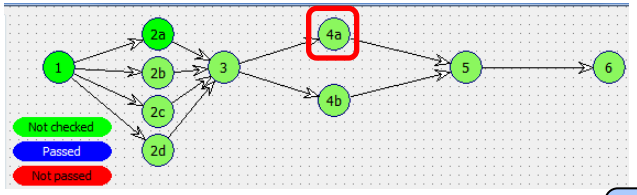


- The node 3 related to the Keratinocyte ARE (EC1.5, EC2, EC3) is Not passed based on the experimental data found for the target chemical (threshold are specified on slide # 15) indicating data below threshold of EC3
- The workflow moves further to the *in vitro* Dendritic cell assay (nodes 4)

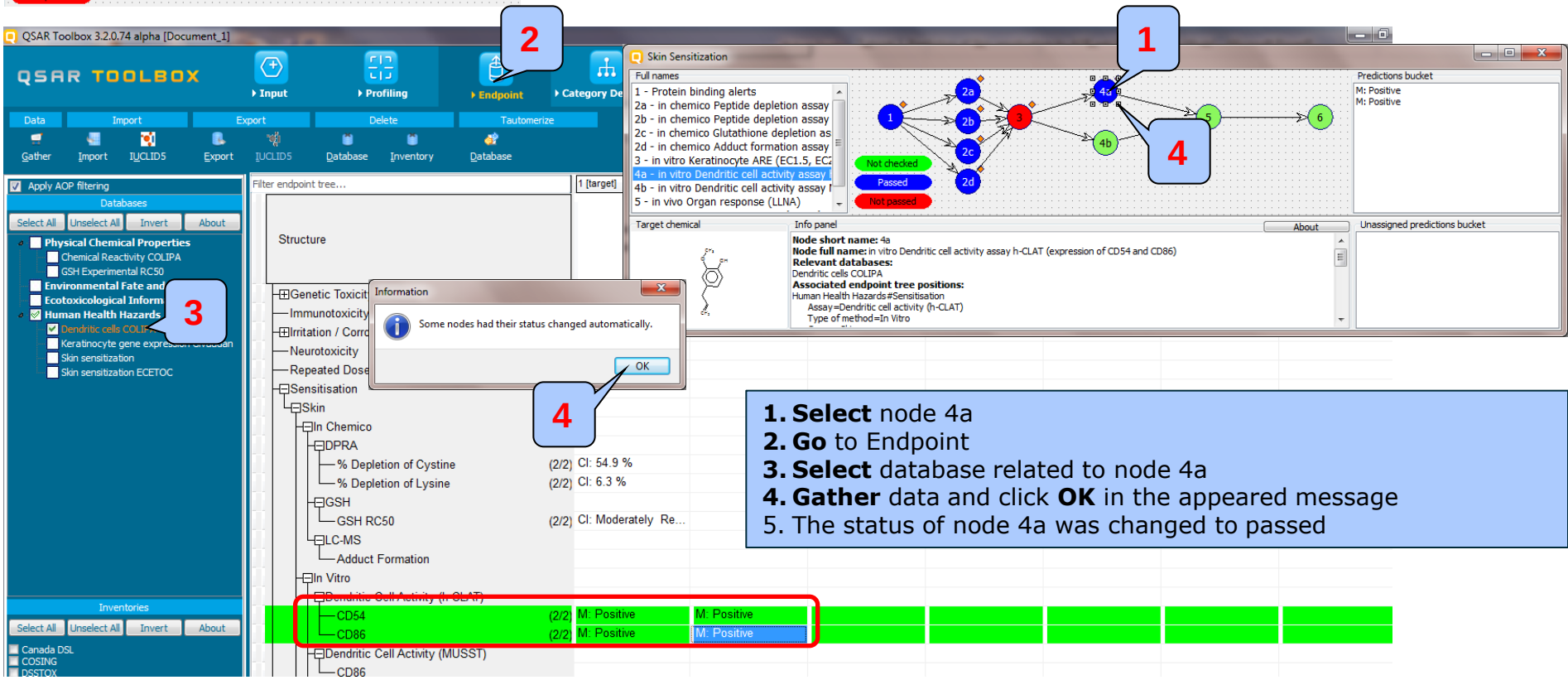
Workflow process

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

Example 2



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

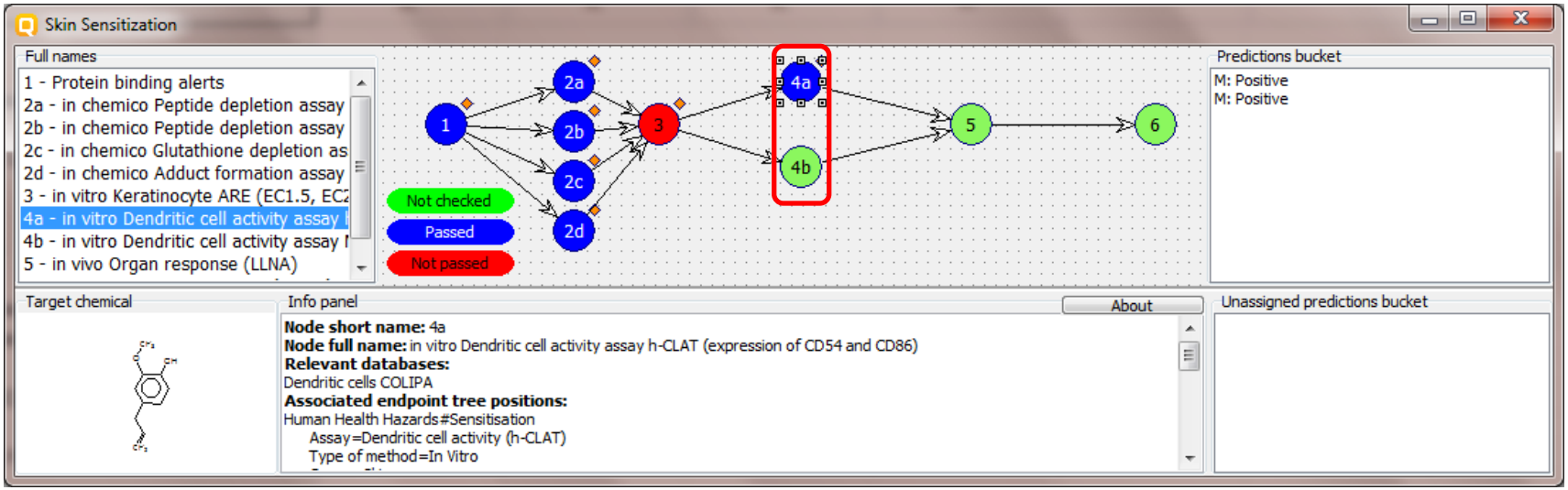


1. **Select** node 4a
2. **Go** to Endpoint
3. **Select** database related to node 4a
4. **Gather** data and click **OK** in the appeared message
5. The status of node 4a was changed to passed

Workflow process

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

Example 2

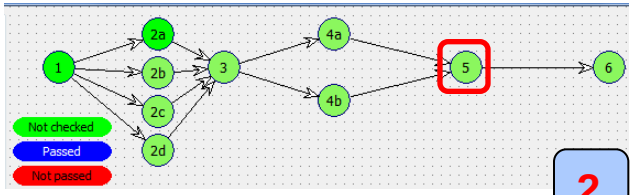


- The node 4a related to the *in vitro* Dendritic cell activity assay (h-CLAT) is passed due to positive experimental data found for the target chemical
- The workflow could further move to the *in vivo LLNA* assay (nodes 5)

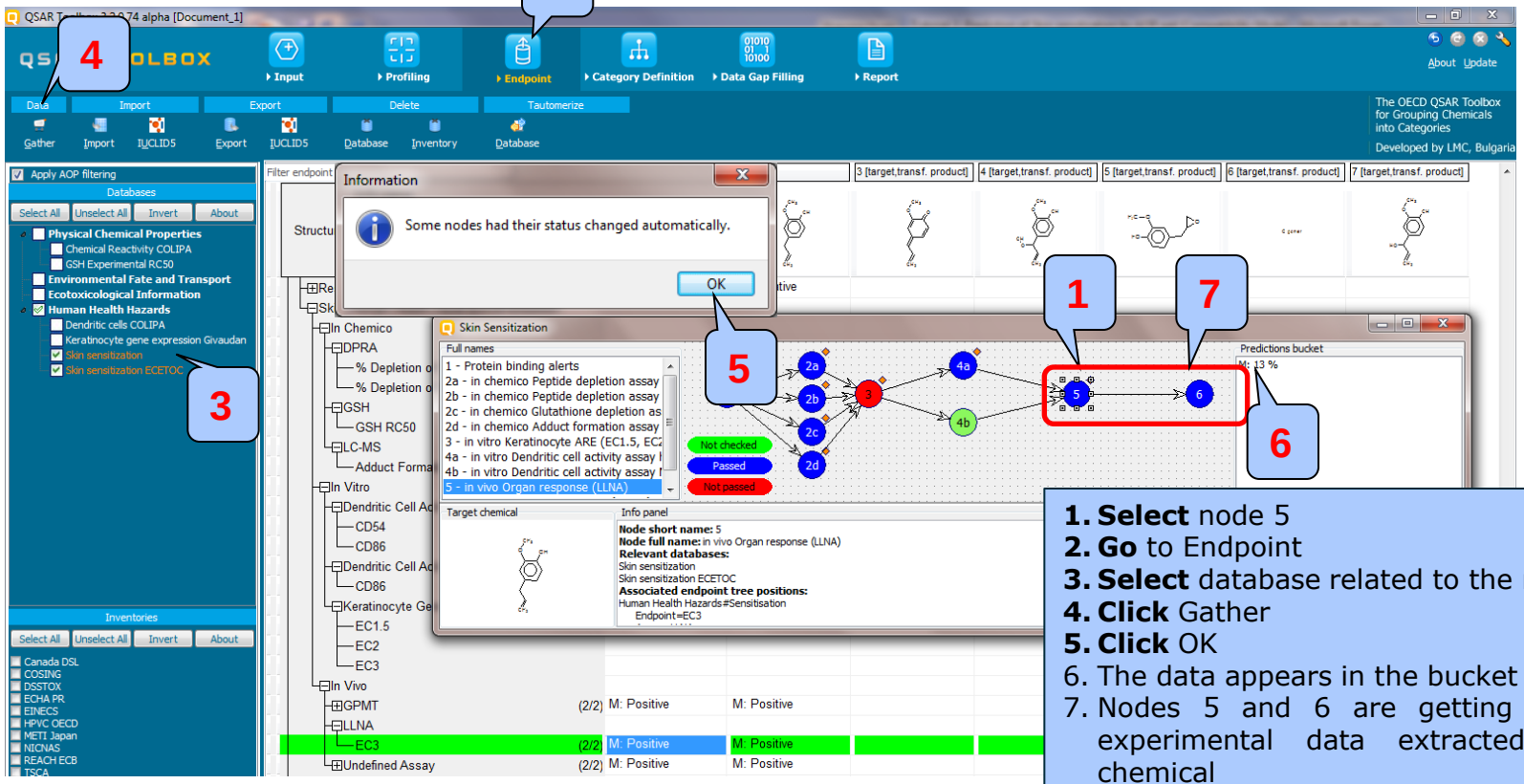
Workflow process

Step 5. 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 5)

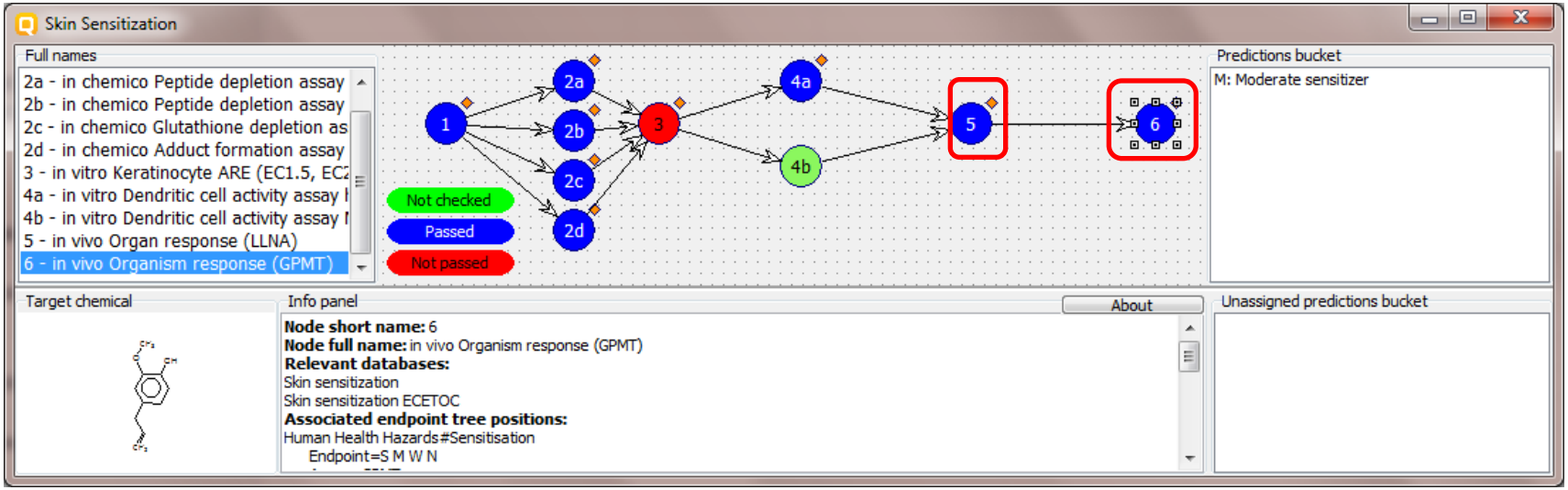


1. **Select** node 5
2. **Go** to Endpoint
3. **Select** database related to the node 5
4. **Click** Gather
5. **Click** OK
6. The data appears in the bucket of the node
7. Nodes 5 and 6 are getting passed based on experimental data extracted for the target chemical

Workflow process

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

Example 1



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

Conclusions

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