QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD (Q)SAR Toolbox v.4.4.1

Implementation of 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*.

*Slides presented on MG WebEx (April 2013)

Background
AOP concept and description *(contd.)*

- A proof-of-concept AOP for skin sensitization is implemented in the 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 the Toolbox
- The implemented AOP scheme is used *only* to demonstrate an example of 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 v4.4*

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

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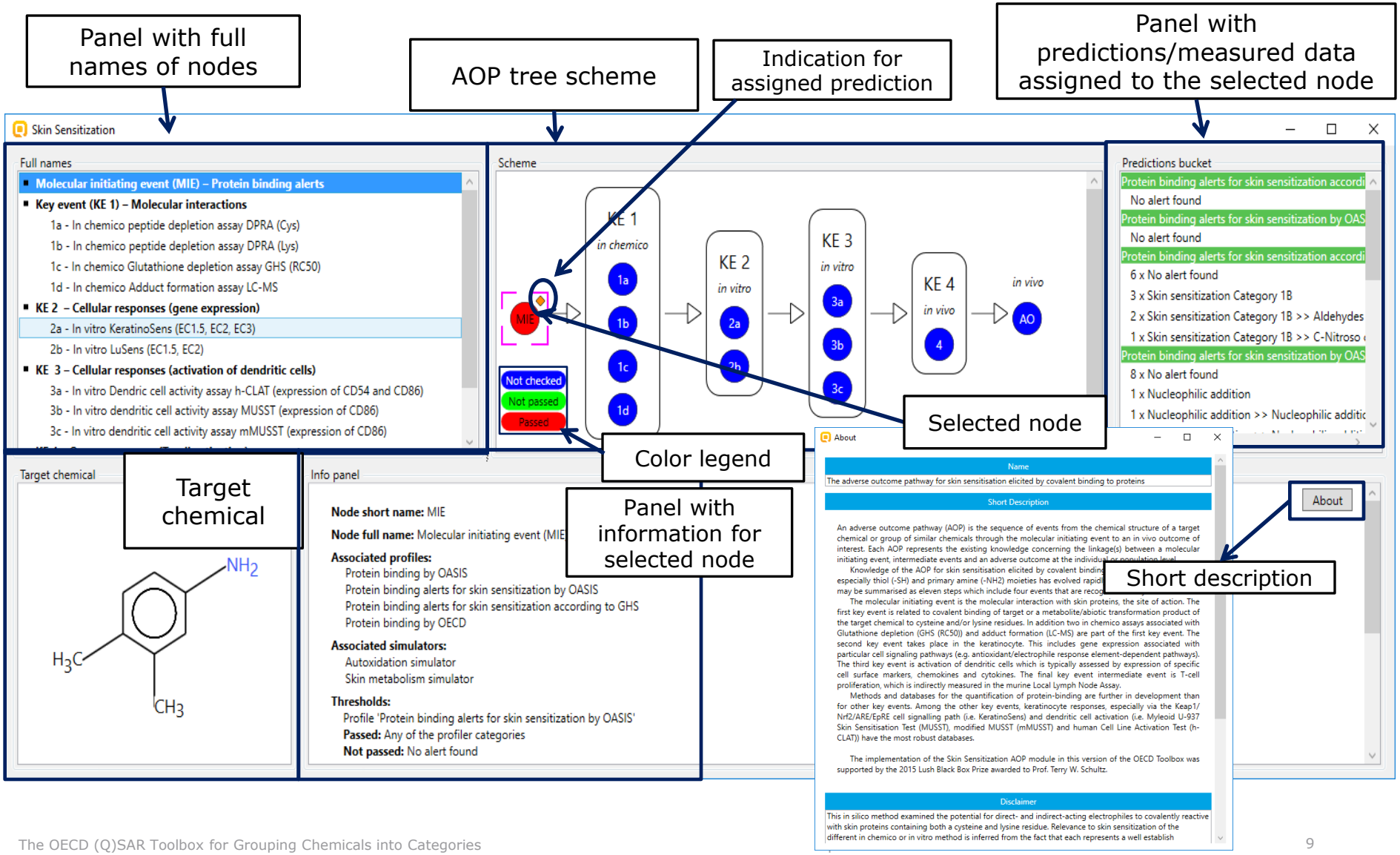
Outlook

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

Overview of the AOP scheme as implemented in Toolbox

Details of AOP window



Outlook

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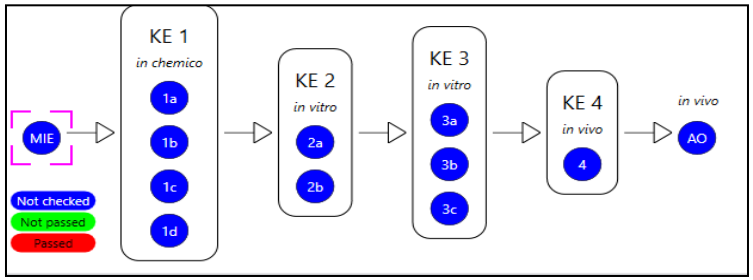
AOP workflow for skin sensitization



	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

Outlook

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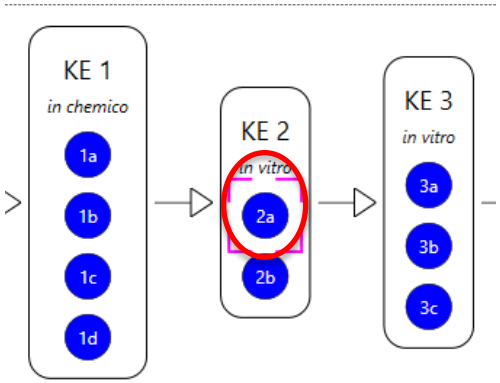
Overview of the AOP scheme as implemented in Toolbox

Implemented thresholds for the AOP nodes

- Thresholds are implemented for each AOP node
- Each threshold is available in the 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 next slide:

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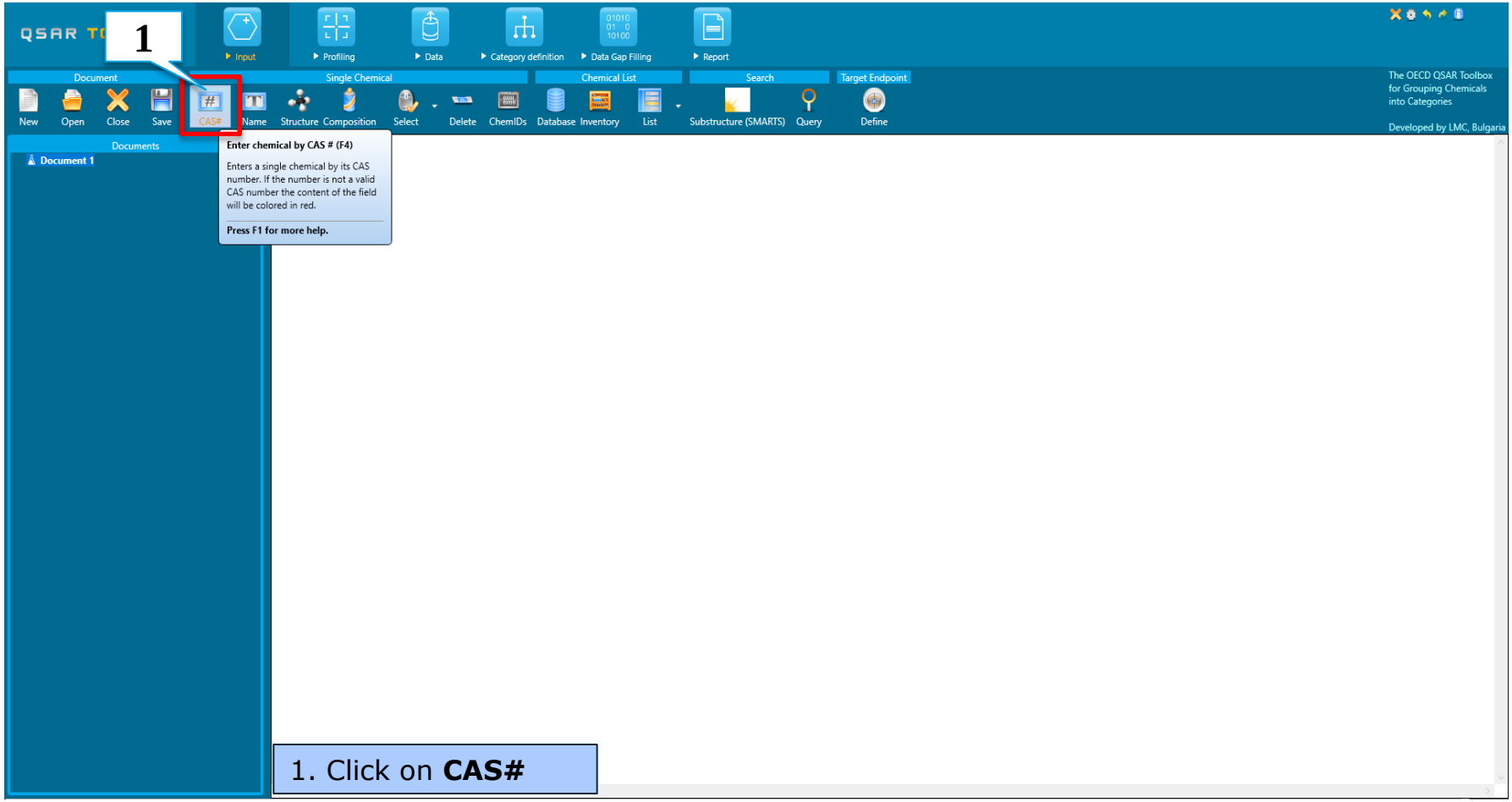
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - **Input**

Chemical Input
Input Screen

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

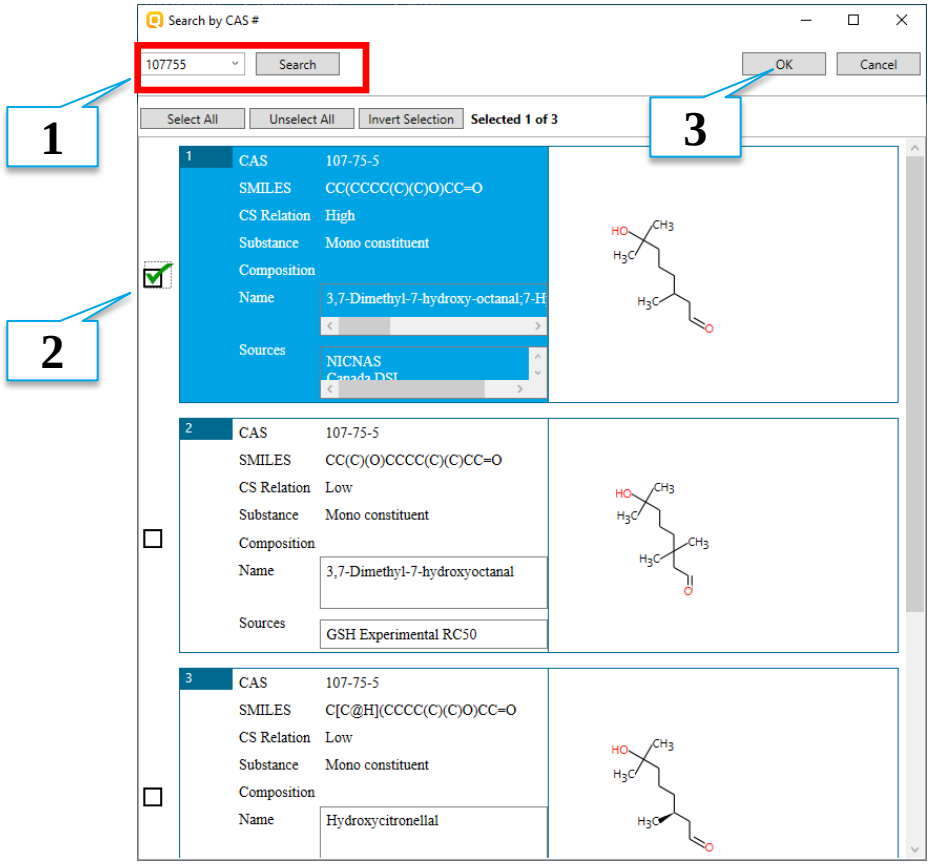
Chemical Input

Input target chemical by CAS#



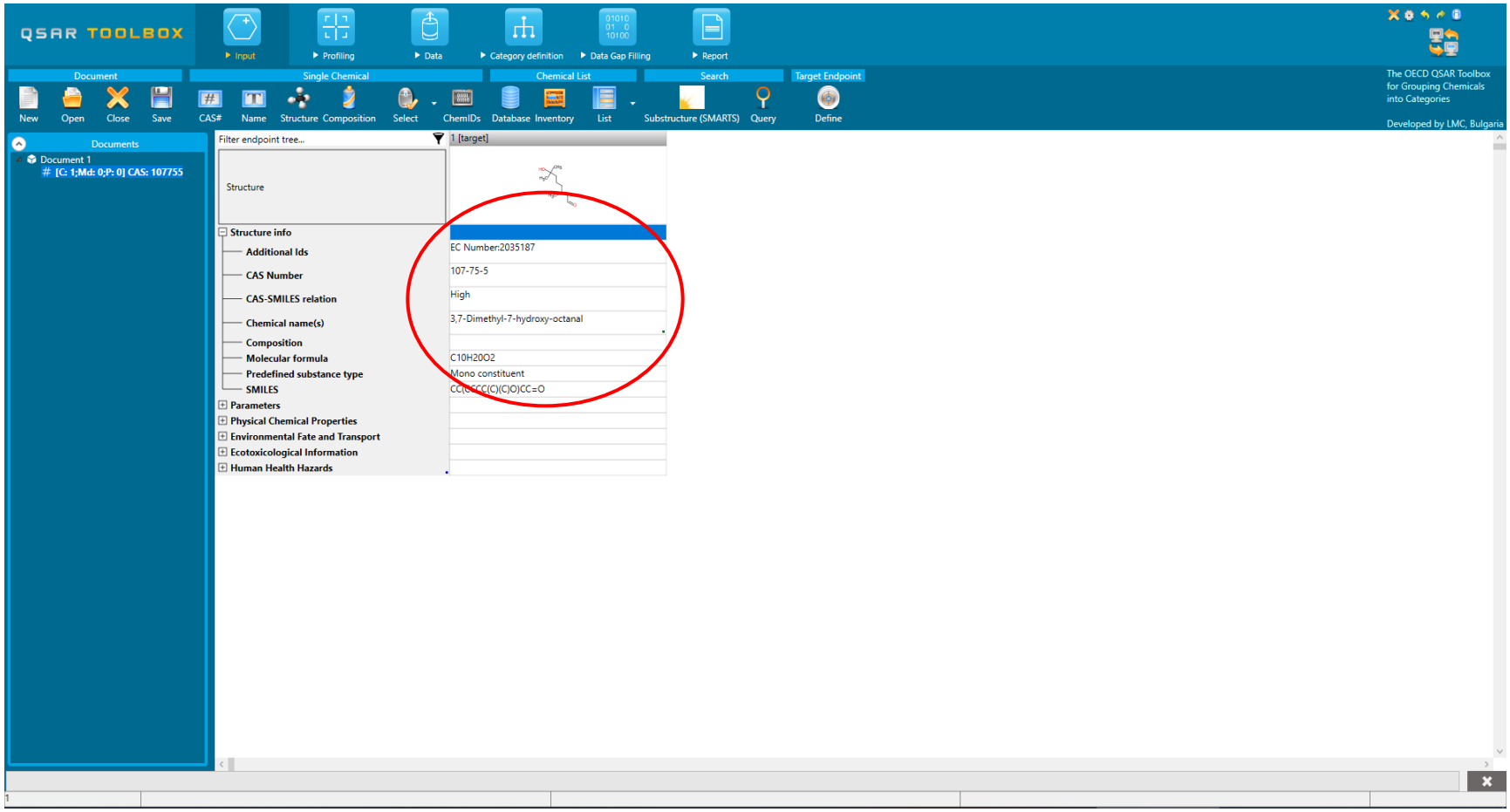
Chemical Input
Enter CAS# 107-75-5

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**

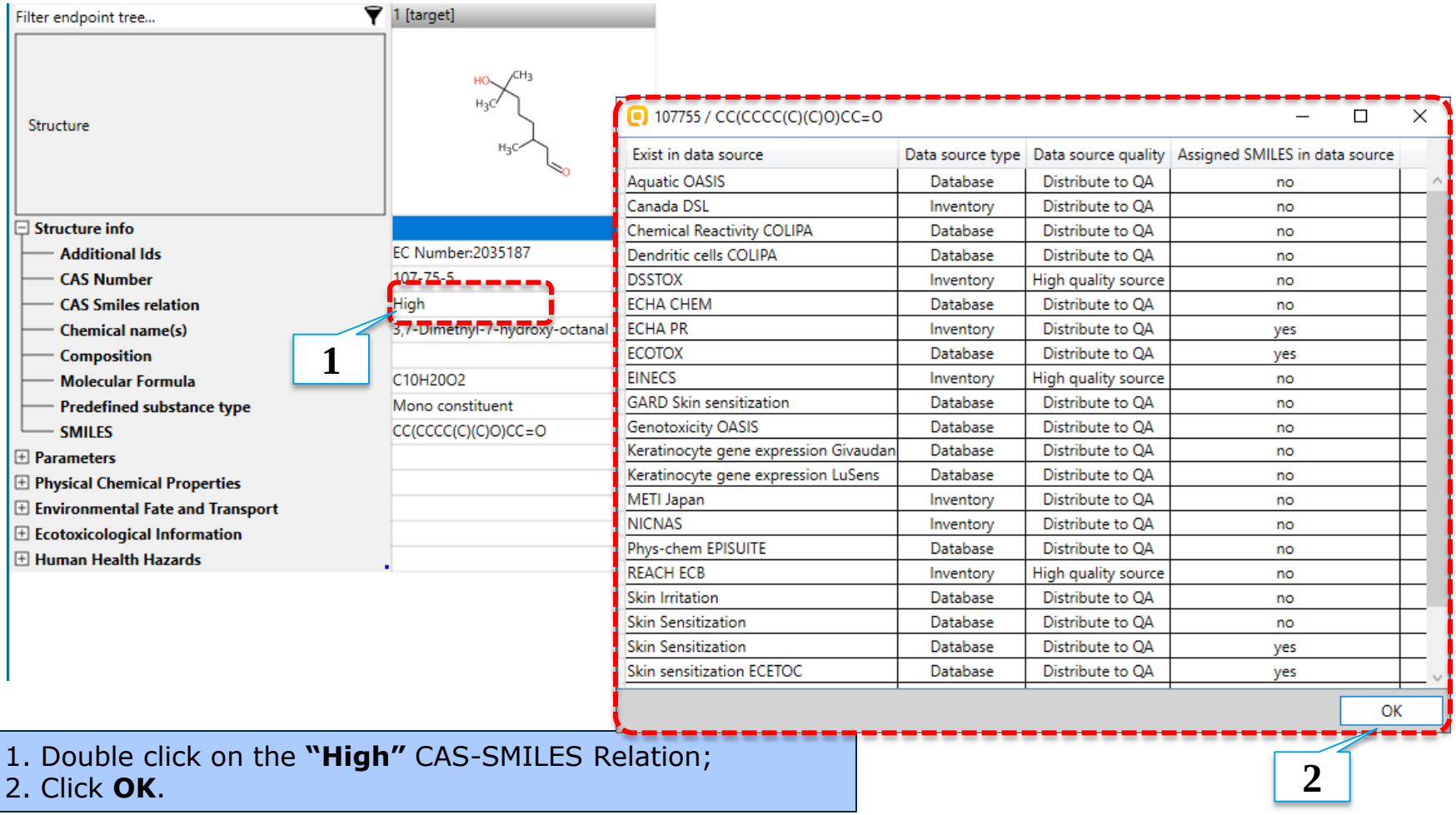
Chemical Input
Target chemical identity



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 on the **"High"** CAS-SMILES Relation;
2. 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
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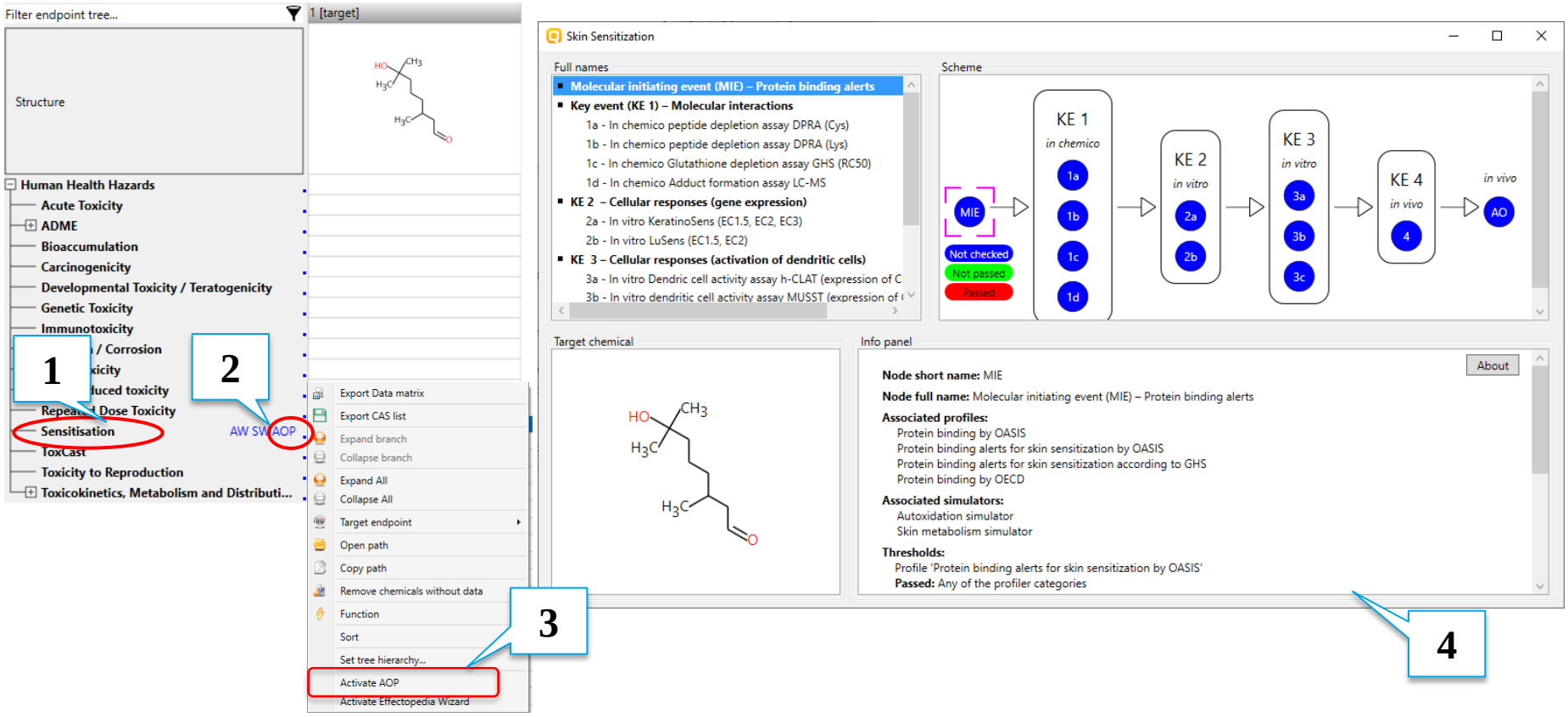
- Overview of AOP scheme as implemented in the Toolbox
- The exercise
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - Input
    - **Activate AOP**

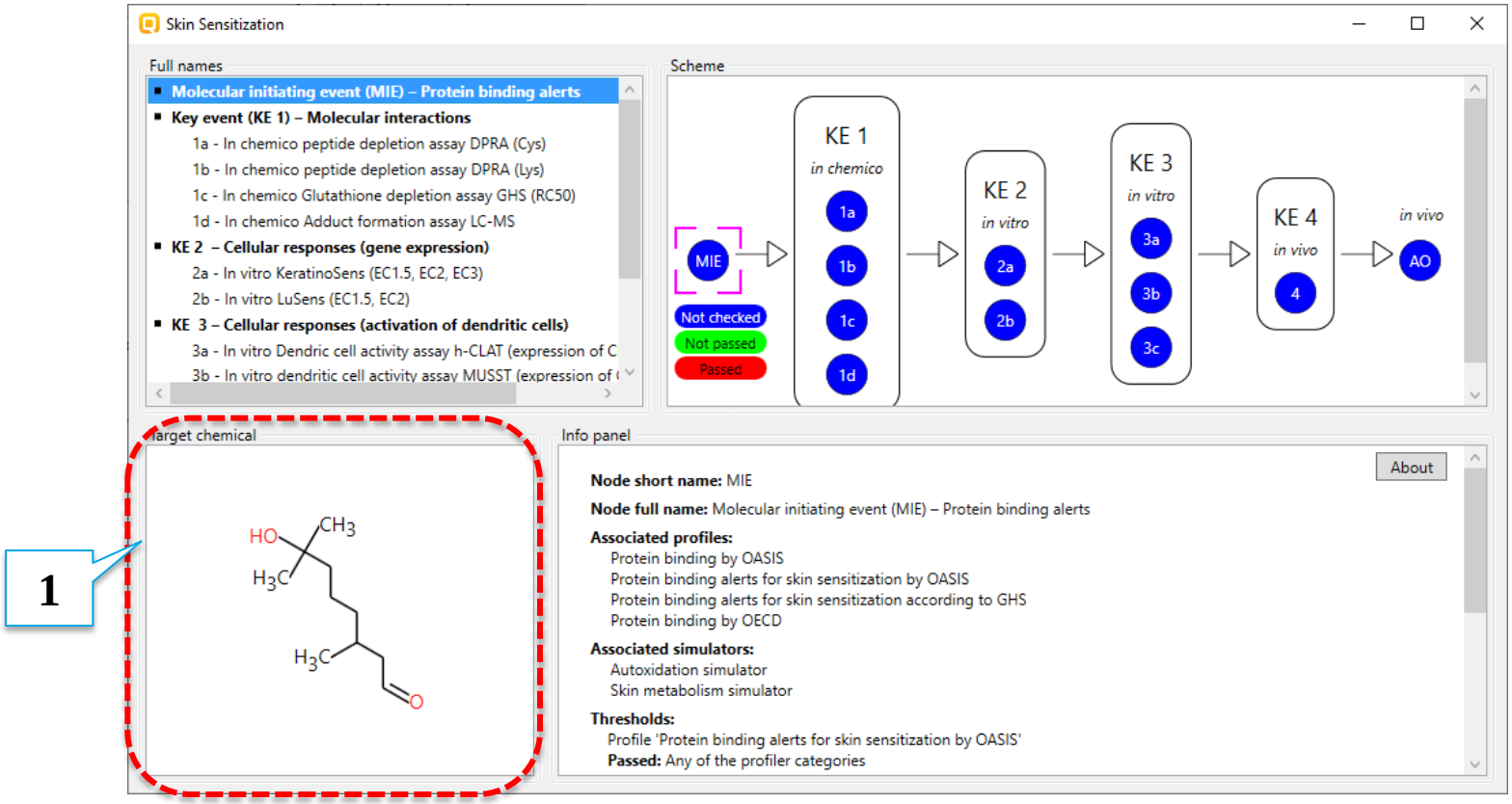
Activate AOP



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



1. The target chemical automatically appears in the AOP window. The AOP is ready to be executed.

Outlook

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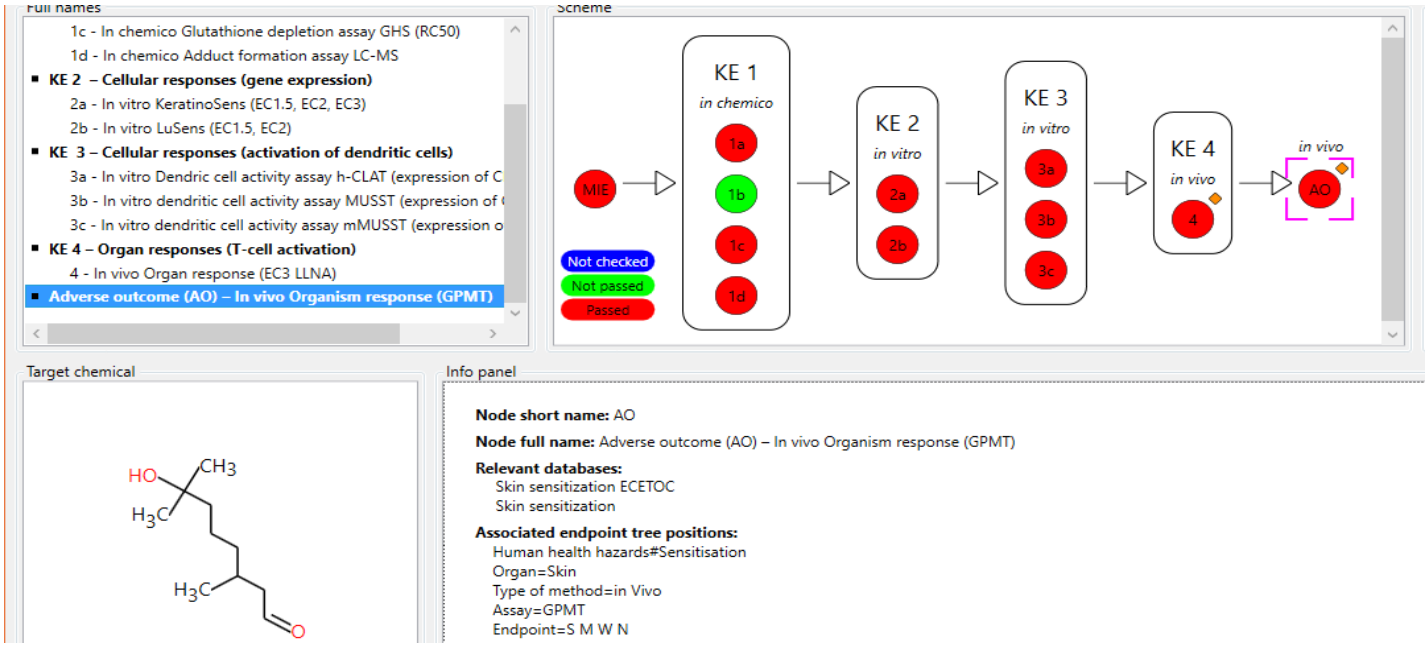
  - Example 1: 3,7-dimethyl-7-hydroxy-octanal (CAS 107-75-5)
    - Input
    - Activate AOP
    - **Workflow process**

Workflow process

- Workflow process starts from molecular initiating event to the *in vivo* organism response. The forthcoming slides illustrate the sequence of steps for assessment of each of the nodes

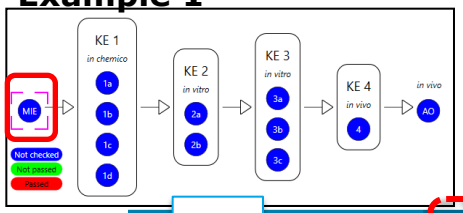


Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
MIE: protein binding	*In chemico*: protein binding potency assays	*In vitro*: gene expression assay	*In vitro*: cytokine profiles in dendritic cells	*In vivo*: Organ response (LLNA)	AO: Organism response (GPMT)

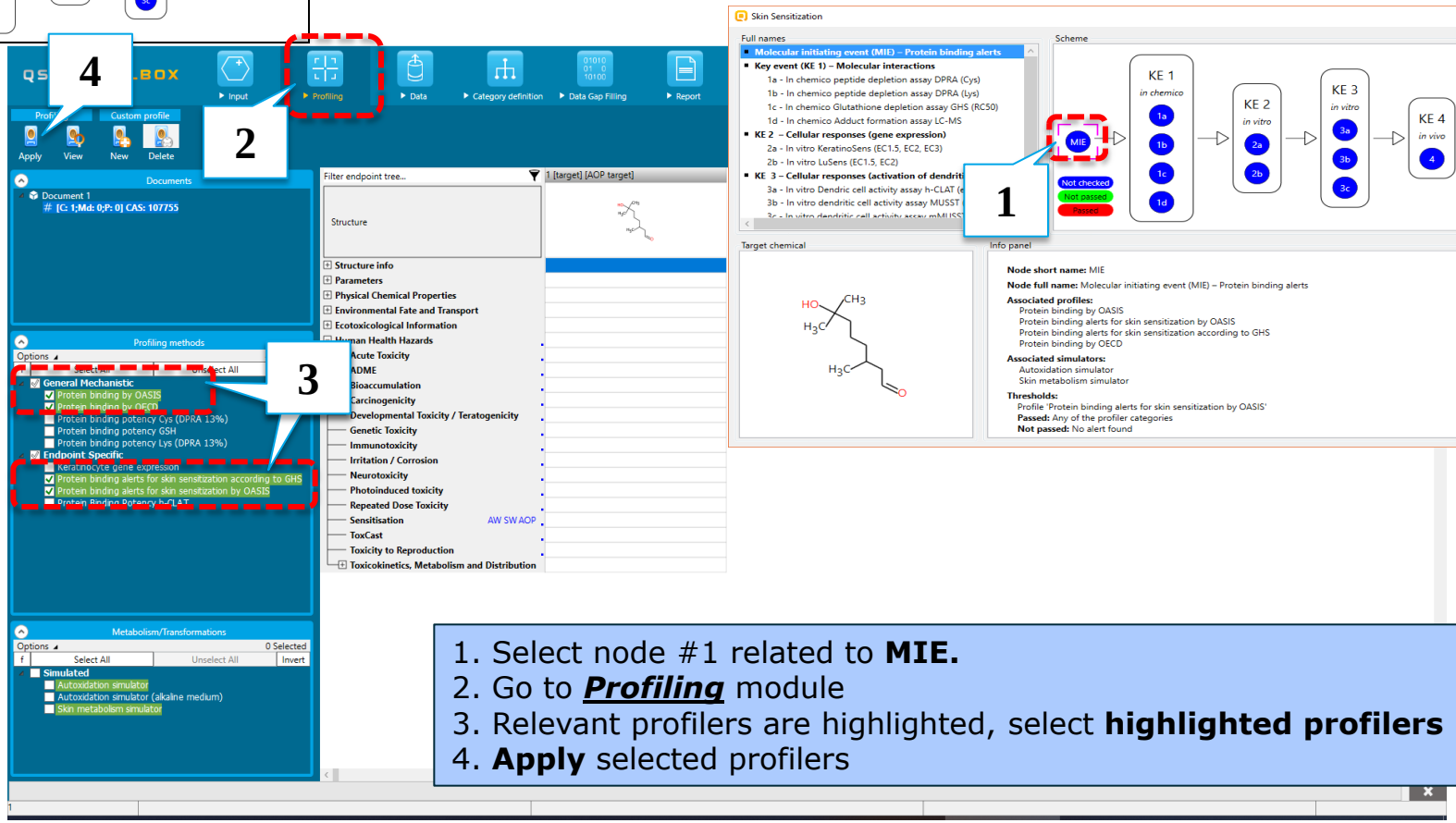
Workflow process

Step 1. MIE: protein binding

Example 1



MIE: Start with profiling of target chemical

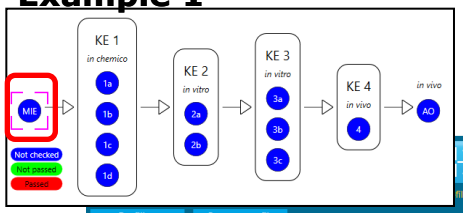


1. Select node #1 related to **MIE.**
2. Go to ***Profiling*** module
3. Relevant profilers are highlighted, select **highlighted profilers**
4. **Apply** selected profilers

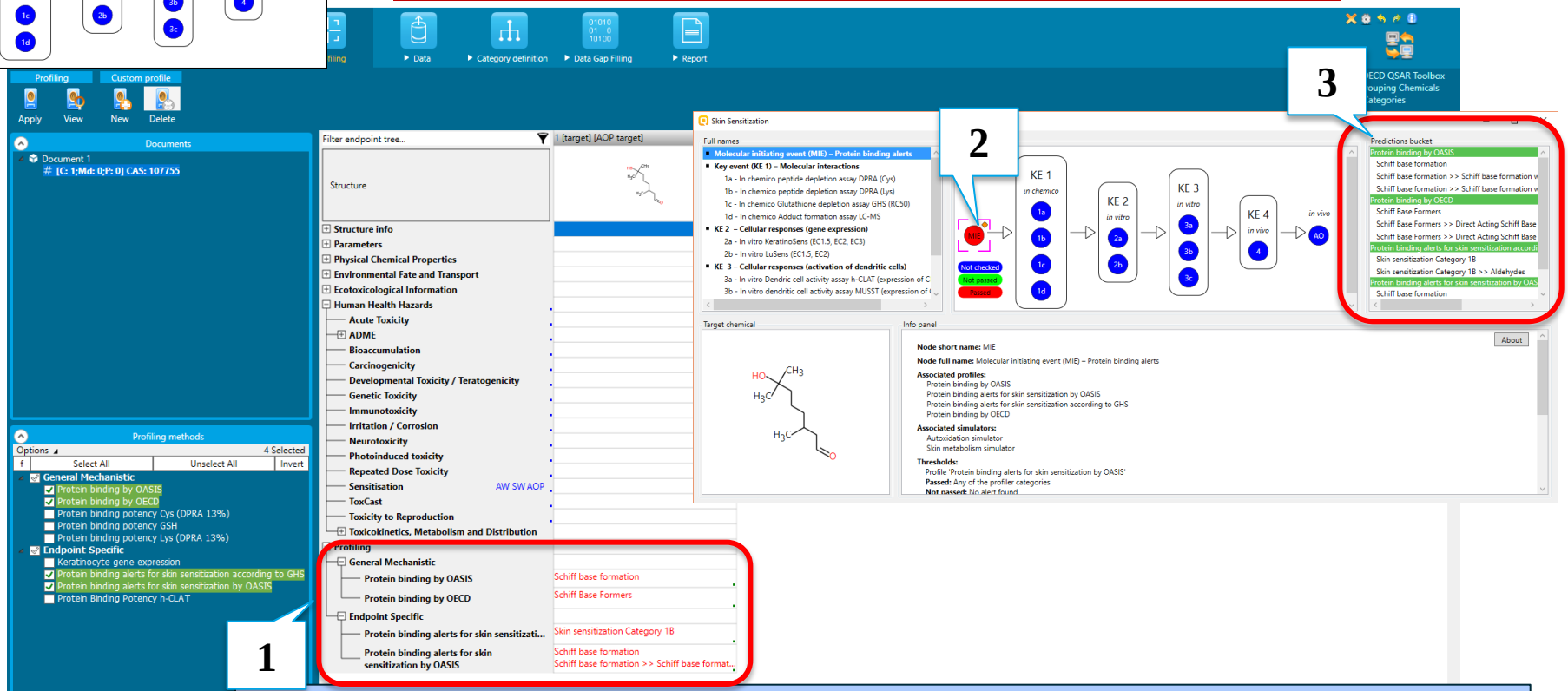
Workflow process

Step 1. MIE: protein binding

Example 1



MIE node: Start with profiling of target chemical

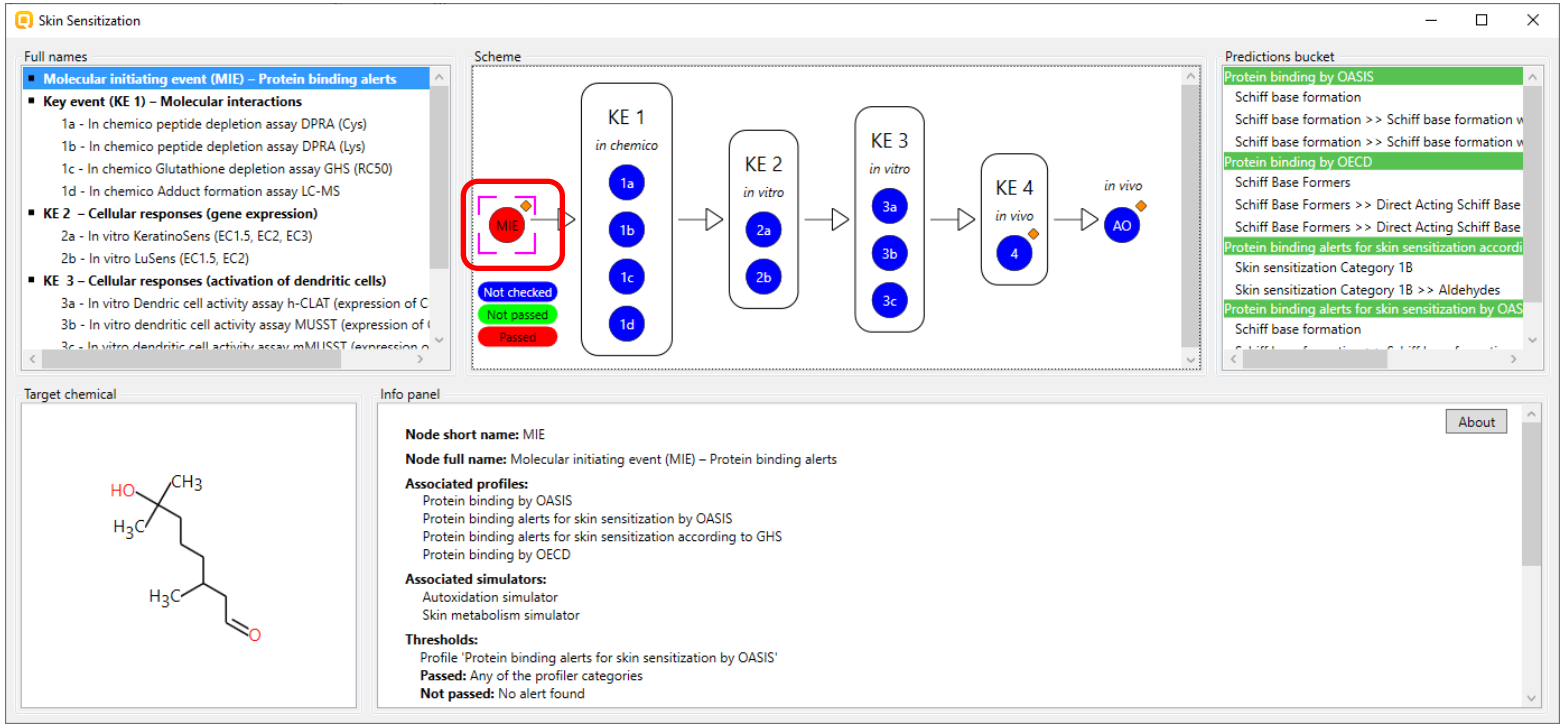


1. The target chemical has protein binding alert according to the four suitable protein binding profilers
2. The node MIE is automatically changed to passed based on the profiling outcome results and implemented thresholds (see slide #14).
3. Profiling results assigned to the selected node appears in the panel "prediction bucket"

Workflow process

Status of node Molecular initiating event

Example 1



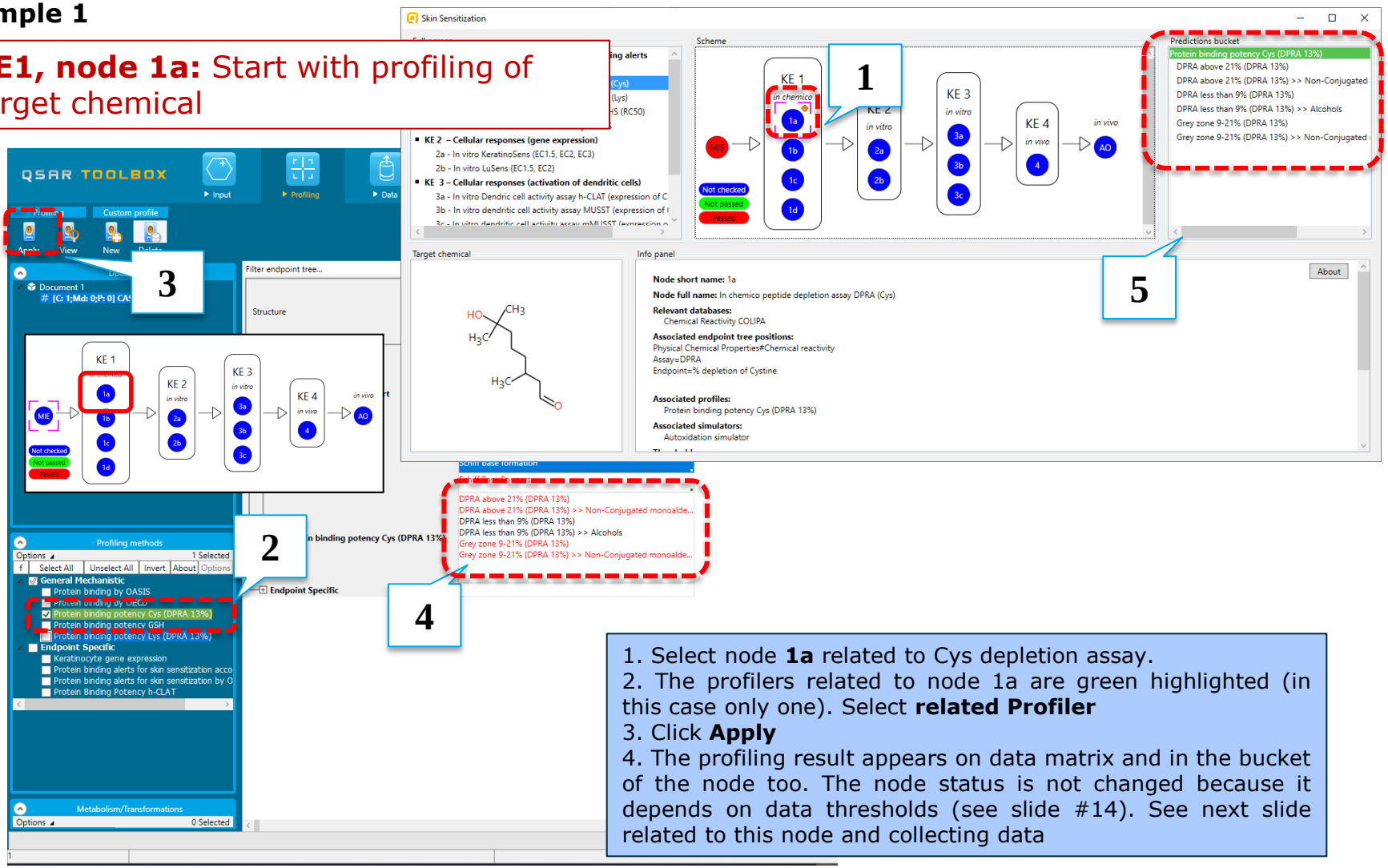
- The node MIE is "passed" due to the presence of protein binding alert identified for the target chemical by the relevant protein binding profilers
- The workflow should move further to the *in chemico* assay (KE1 – node 1a)

Workflow process

Step 2. *In chemico* peptide depletion assay DPRA (Cys) (KE1,1a)

Example 1

KE1, node 1a: Start with profiling of target chemical



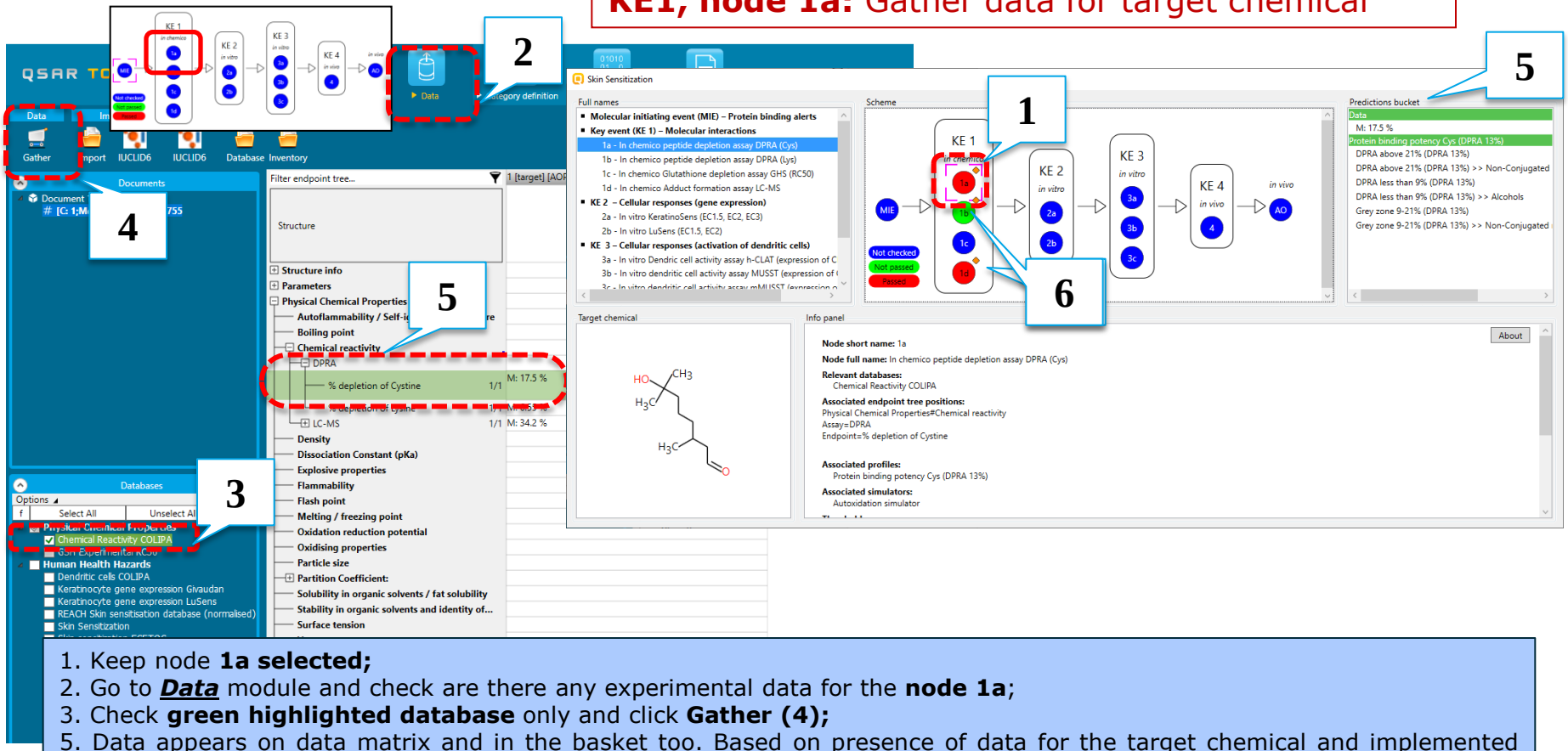
1. Select node **1a** related to Cys depletion assay.
2. The profilers related to node 1a are green highlighted (in this case only one). Select **related Profiler**
3. Click **Apply**
4. The profiling result appears on data matrix and in the bucket of the node too. The node status is not changed because it depends on data thresholds (see slide #14). See next slide related to this node and collecting data

Workflow process

Step 2. *In chemico* peptide depletion assay DPRA (Cys) (KE1,1a)

Example 1

KE1, node 1a: Gather data for target chemical

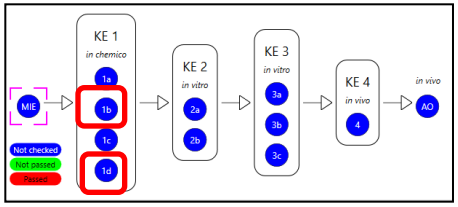


1. Keep node **1a selected;**
2. Go to ***Data*** module and check are there any experimental data for the **node 1a**;
3. Check **green highlighted database** only and click **Gather (4);**
5. Data appears on data matrix and in the basket too. Based on presence of data for the target chemical and implemented thresholds (slide #14) node **1a** is **"passed".**
6. Moreover node **1b** and **1d** automatically changed their status based data found for the target and implemented thresholds (see next slide)

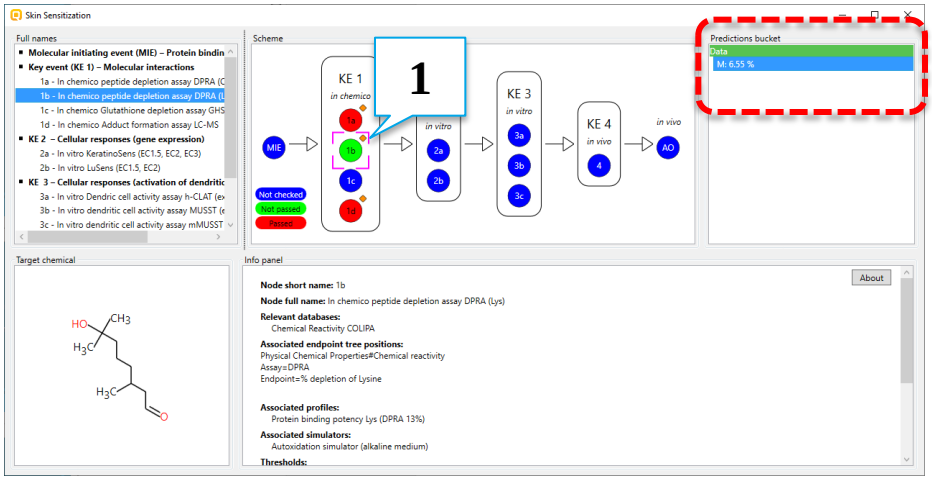
Workflow process

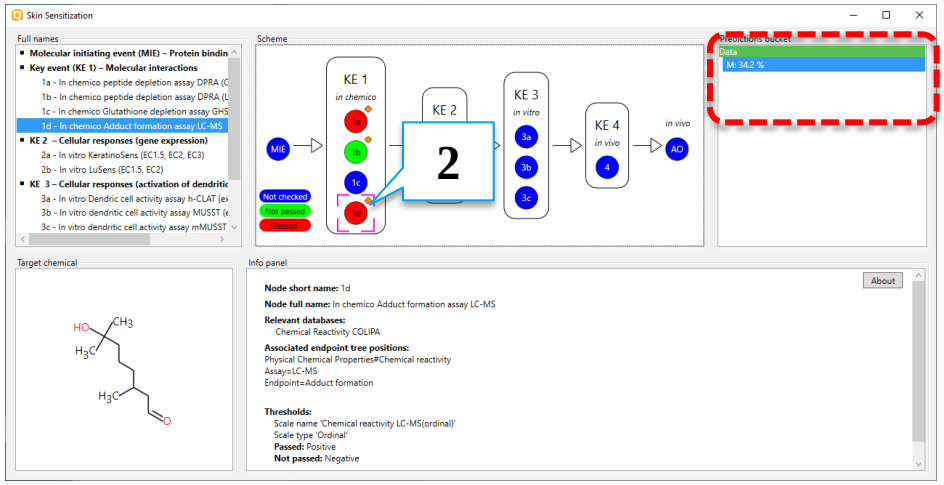
Step 2. *In chemico* peptide depletion assay DPRA (Lys) (KE1, 1b) and *In chemico* Adduct formation assay LC-MS (KE1, 1d)

Example 1



KE1, node 1b and 1d: data is found for the target chemical. Hence, these two nodes changed their status to passed and not passed. The workflow could proceed with next node





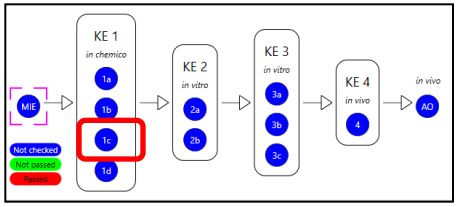
1. Select **node 1b.** Data is found for the target chemical. The node is "not passed" due to implemented data thresholds (see slide 14)
2. Select **node 1d.** Data is found for the target chemical. The node is "passed" due to implemented data thresholds (see slide 14)

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

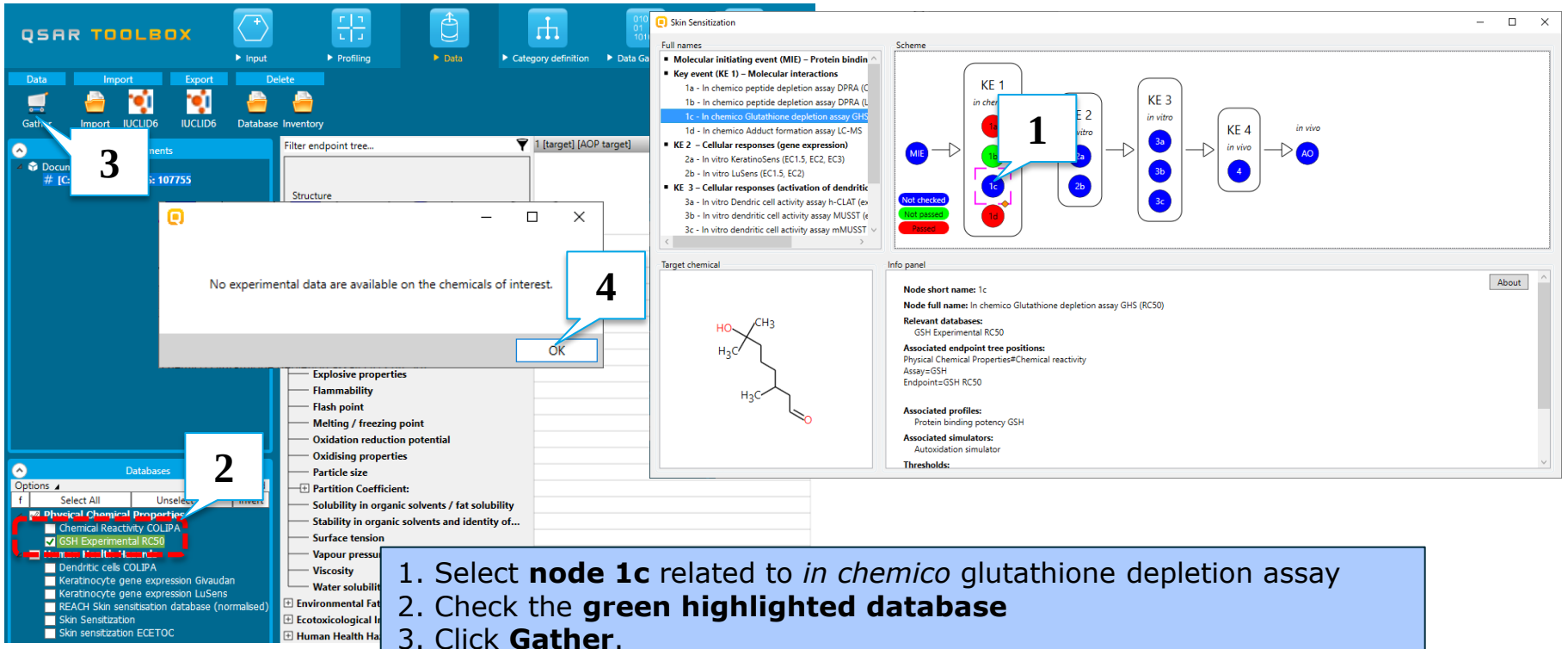
Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (KE1, 1c)

Example 1



KE1, node 1c: 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

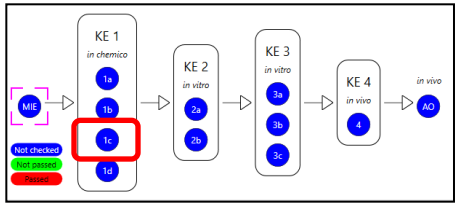


1. Select **node 1c** related to *in chemico* glutathione depletion assay
2. Check the **green highlighted database**
3. Click **Gather**.
4. No data has been found for the target chemical, click **OK.**

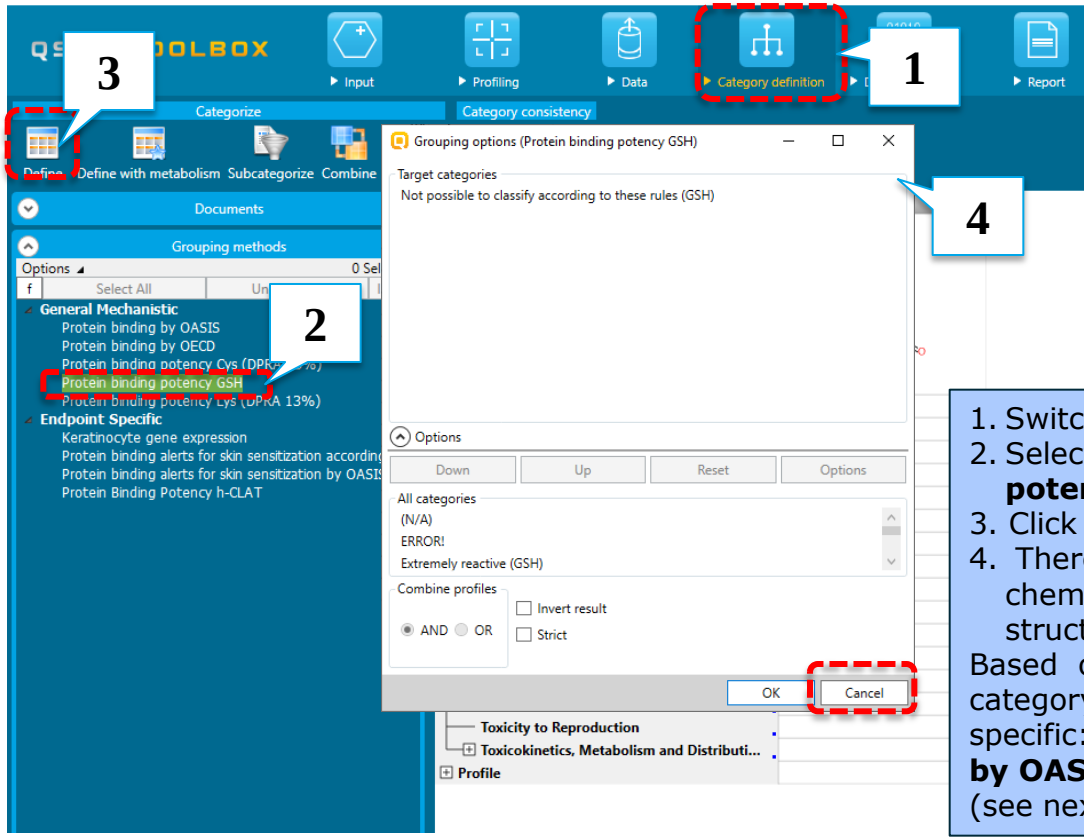
Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (KE1, 1c)

Example 1



KE1, node 1c: The category of similar analogue should be investigated.



1. Switch to **Category definition**
2. Select green highlighted profiler **"Protein binding potency GSH"**
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.**

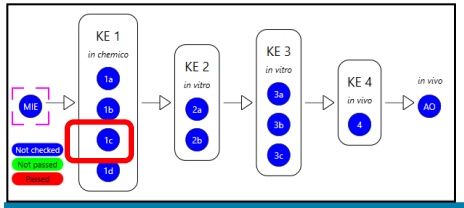
Based on the above point it is recommended to define category by other profiler. In this case use endpoint-specific: **"Protein binding alerts for skin sensitization by OASIS"** profiler (PBA SS)

(see next slide)

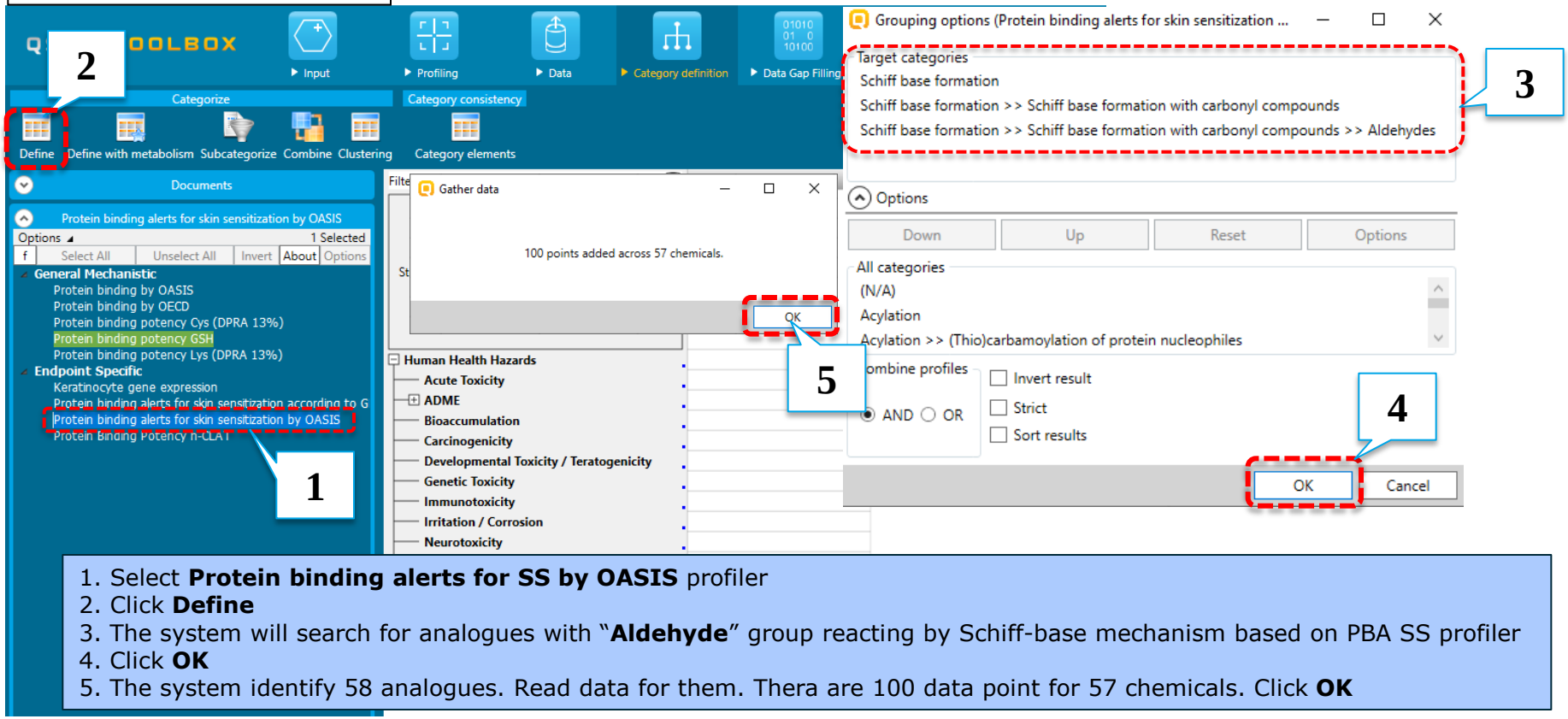
Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (KE1, 1c)

Example 1



KE1, node 1c: In this case we should investigate the category by Protein binding alerts for SS. The reason for this is that GHS RC50 depends on mechanism of protein binding interaction

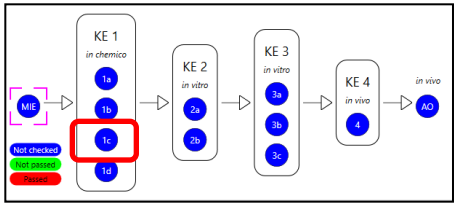


1. Select **Protein binding alerts for SS by OASIS** profiler
2. Click **Define**
3. The system will search for analogues with "**Aldehyde**" group reacting by Schiff-base mechanism based on PBA SS profiler
4. Click **OK**
5. The system identify 58 analogues. Read data for them. Thera are 100 data point for 57 chemicals. Click **OK**

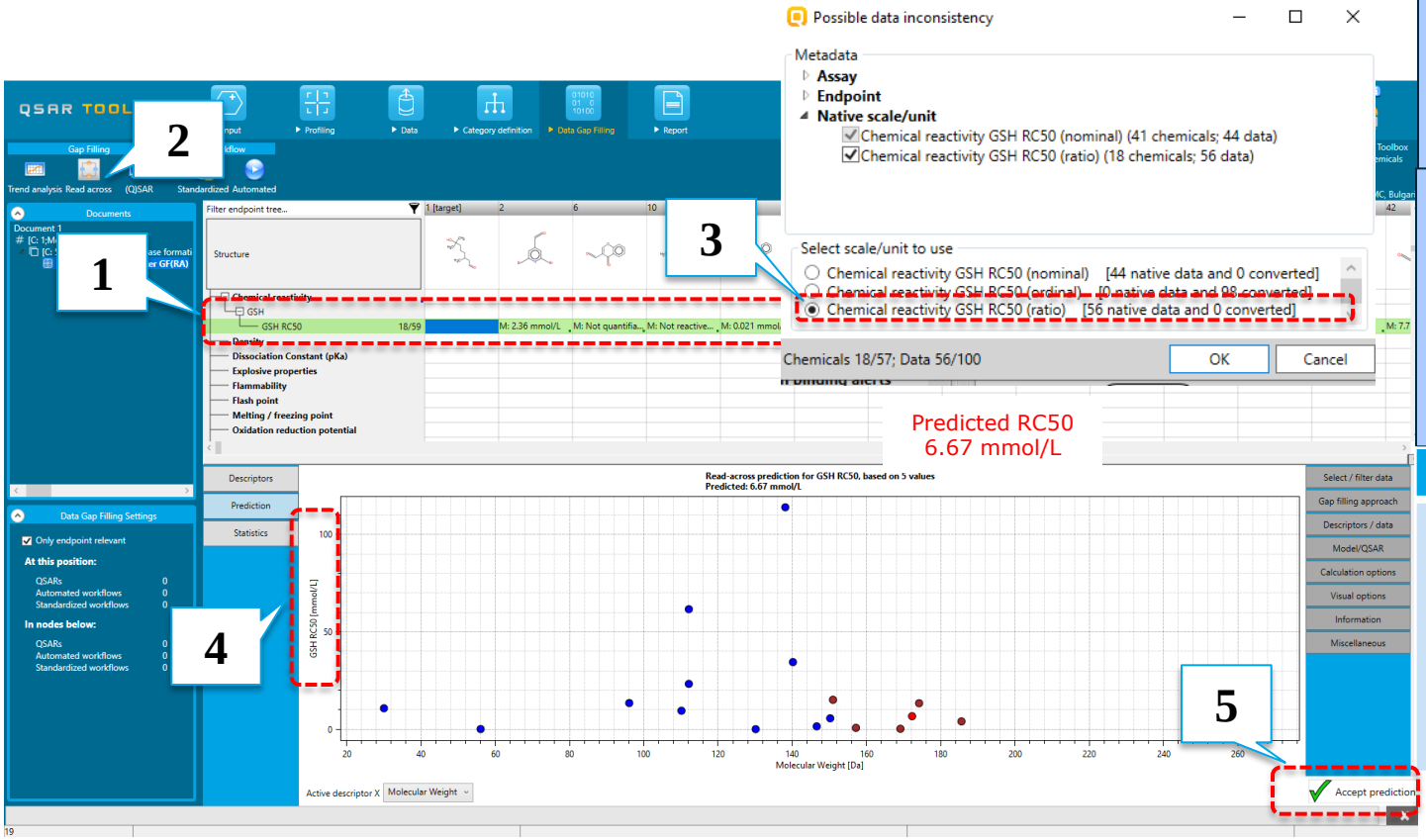
Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (KE1, 1c)

Example 1



KE1, node 1c: Read-across is performed in this case. The read-across is used only to exemplify the workflow.



1. Expand the endpoint tree to the level of "Physical Chemical Properties#Chemical reactivity#GSH#GSH RC50"
2. Apply **Read-across**
3. Select scale **Chemical reactivity GSH RC50 (ratio).** Click **OK**.
4. RC50 values are presented in mmol/L
5. **Accept prediction**

The obtained read-across prediction falls in the range "Moderately reactive" based on the implemented thresholds (given below). Based on the latter the status of the node will be changed to passed (see next slide)

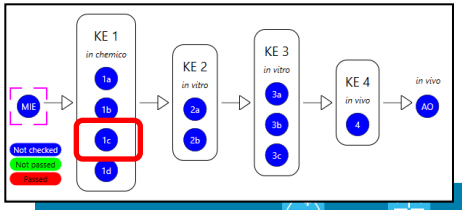
Data thresholds

- 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

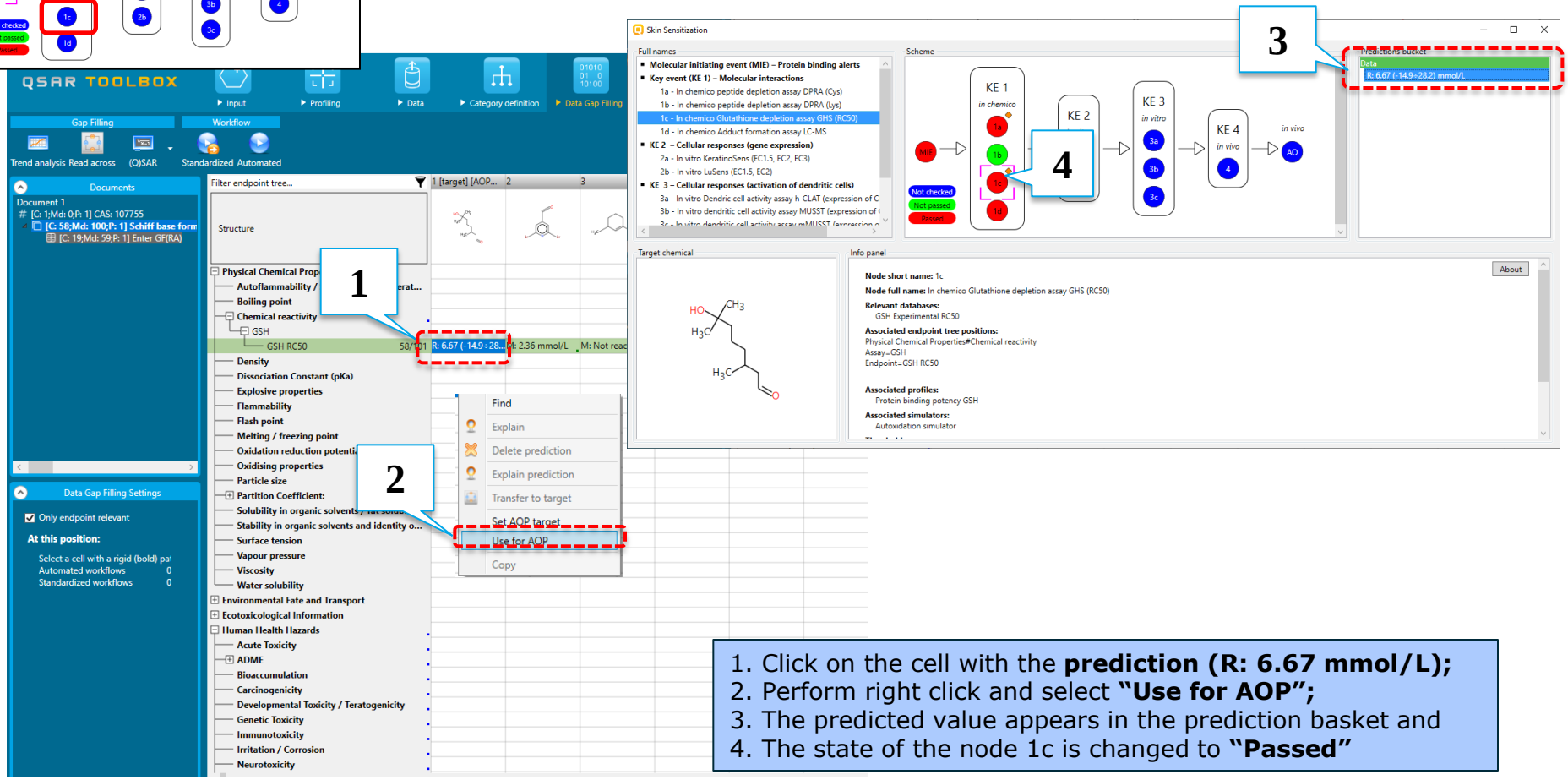
Workflow process

Step 2. *In chemico* Glutathione depletion assay GSH (RC50) (KE1, 1c)

Example 1



KE1, node 1c: The next step is to use read-across prediction for AOP

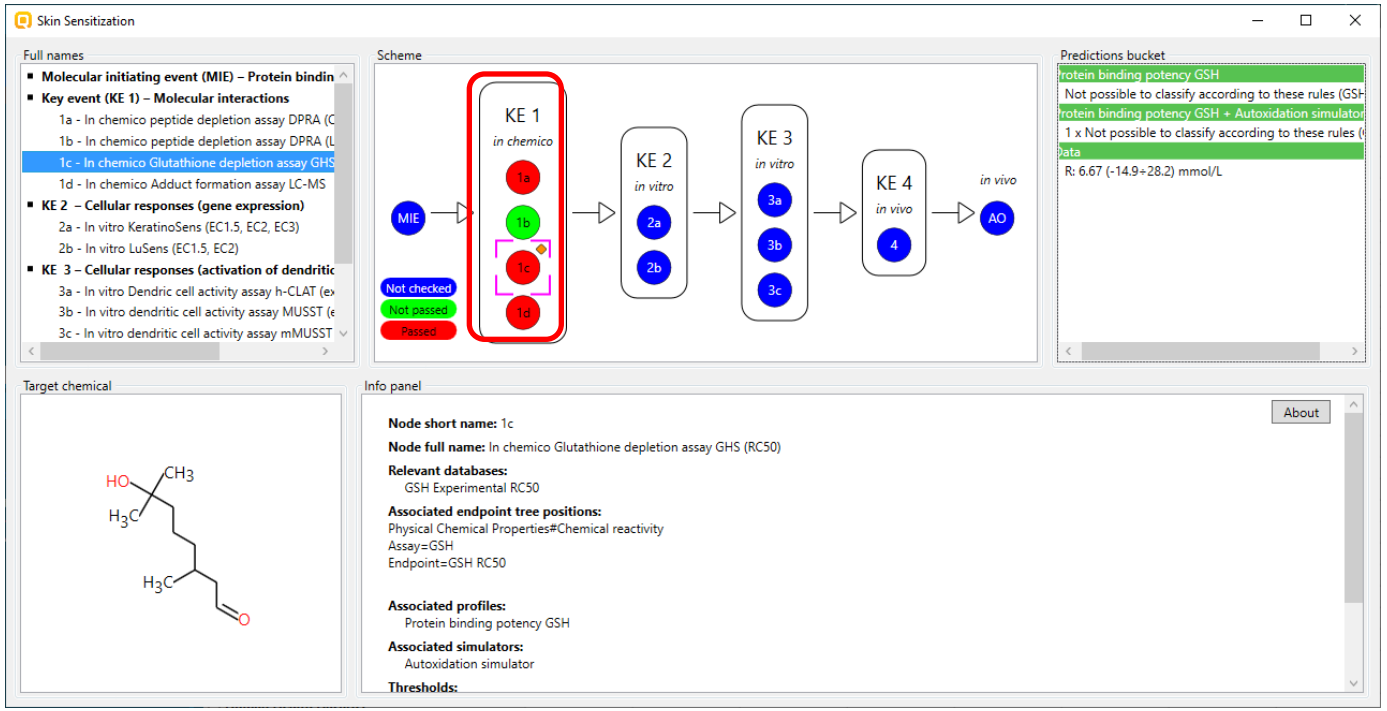


1. Click on the cell with the **prediction (R: 6.67 mmol/L);**
2. Perform right click and select **"Use for AOP";**
3. The predicted value appears in the prediction basket and
4. The state of the node 1c is changed to **"Passed"**

Workflow process

Status of nodes related to In chemico assays

Example 1

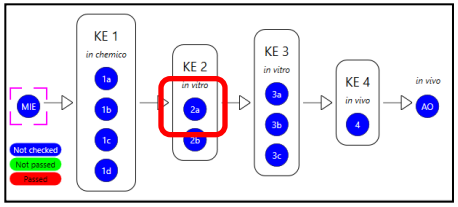


- The nodes related to three of the *in chemico* assays are "Passed" due to positive experimental data found for the target chemical (node 1a and 1d) and the positive read-across prediction for the target based on experimental data found for analogues (node 1c).
- Only one of all *in chemico related*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

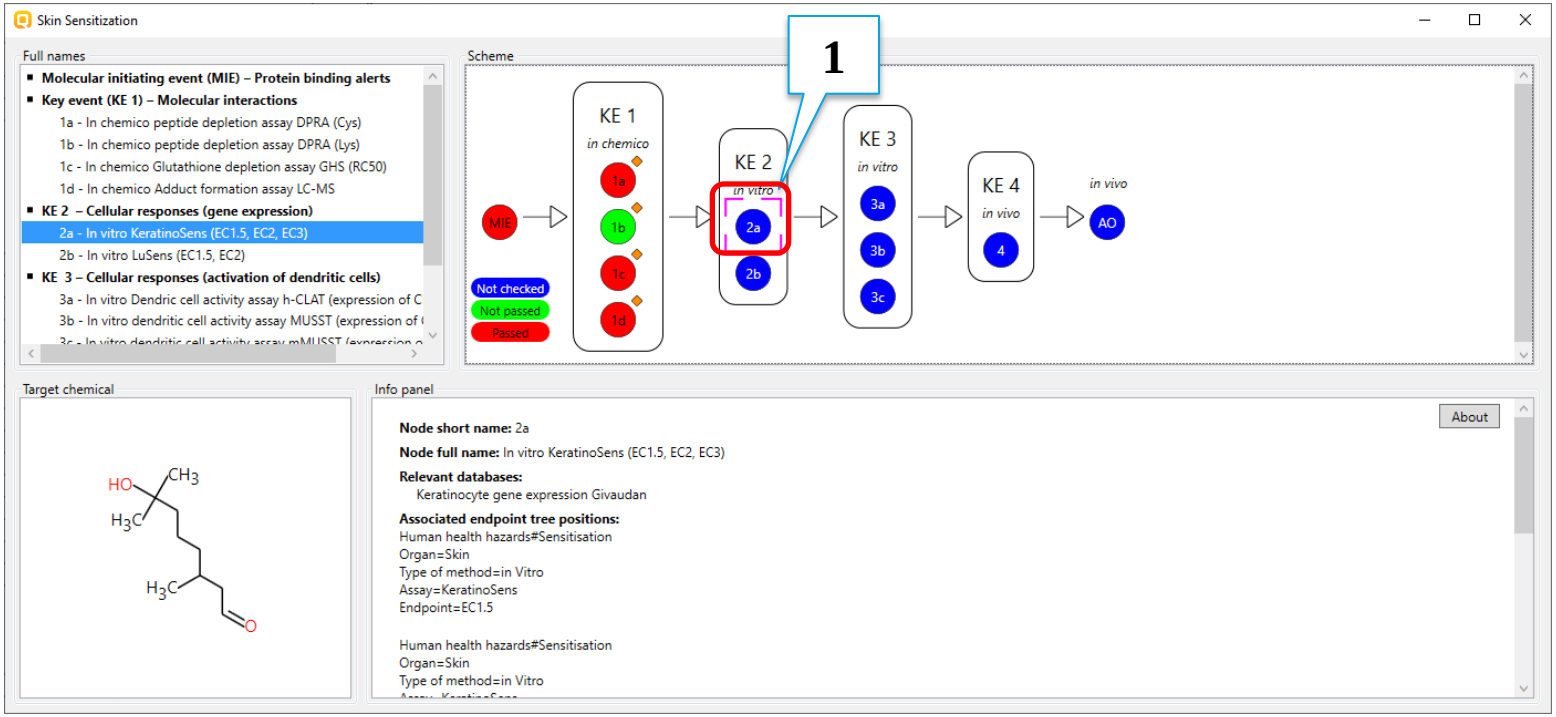
Workflow process

Step 3. *In vitro* KeratinoSens (KE2, node 2a)

Example 1



KE2, node 2a: The next step of the workflow is node 2a related to the in vitro KeratinoSens assay

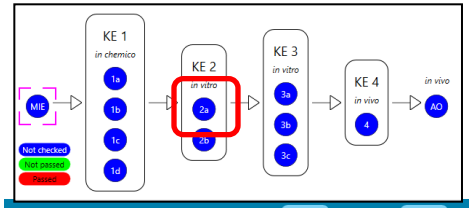


1. Select **node 2a**

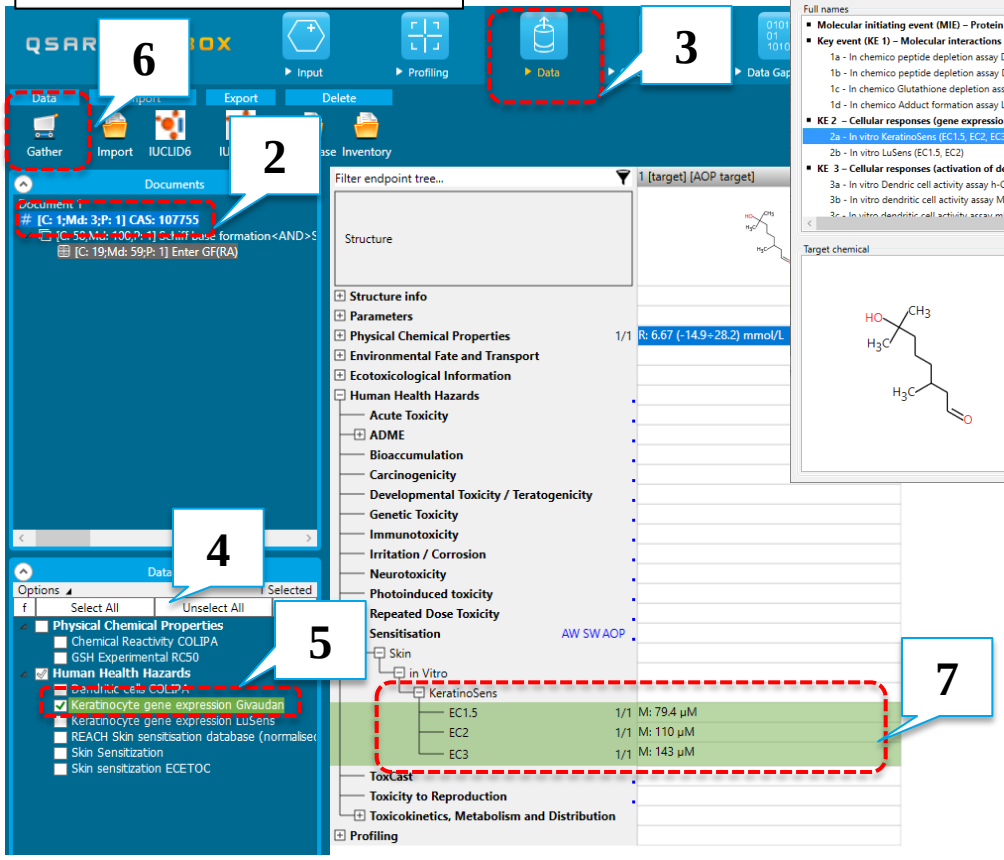
Workflow process

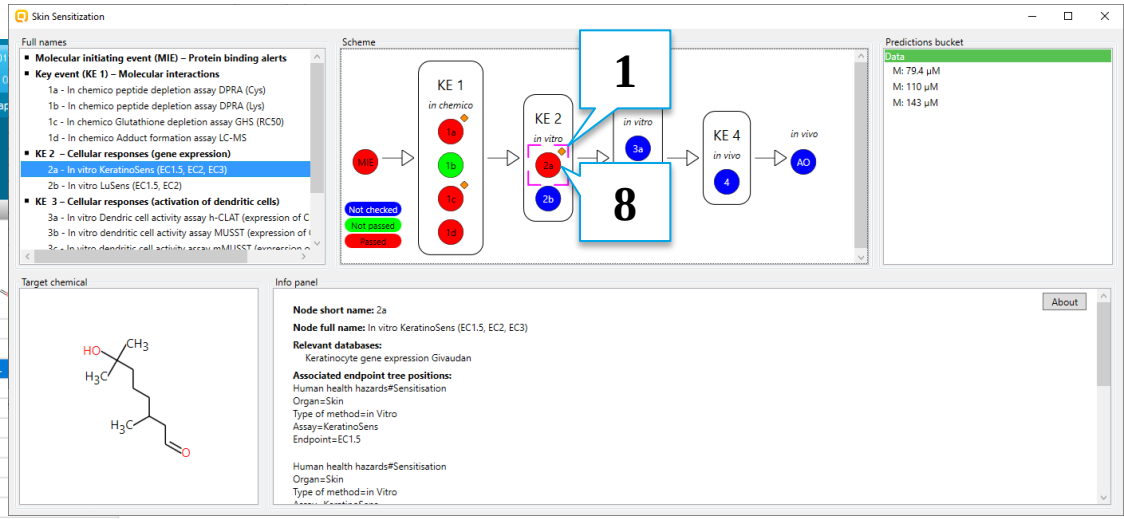
Step 3. *In vitro* KeratinoSens (KE2, node 2a)

Example 1



KE2, node 2a: In this step we will check are there any data for the target chemical for the *in vitro KeratinoSens* assay



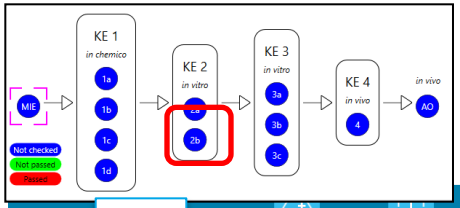


1. Keep the **node 2a** selected
2. Go to level of CAS number from document tree
3. Go to **Data**
4. Unselect **all databases**
5. Select **highlighted database**
6. Click **Gather**
7. The experimental data appears on Data matrix
8. **Node 2a** has been changed to passed based on implemented thresholds (slide #14)

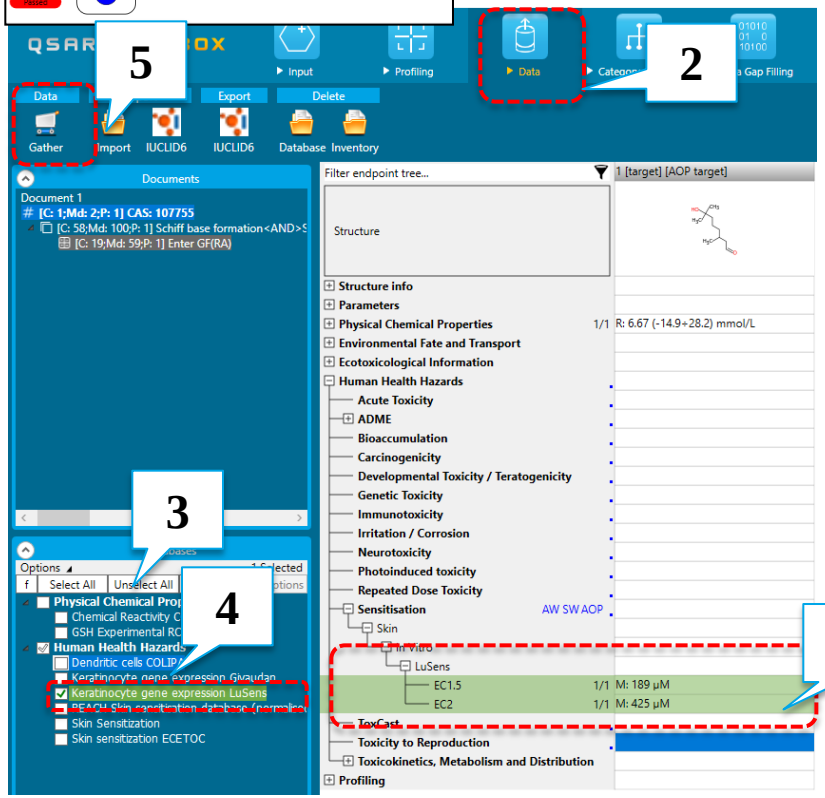
Workflow process

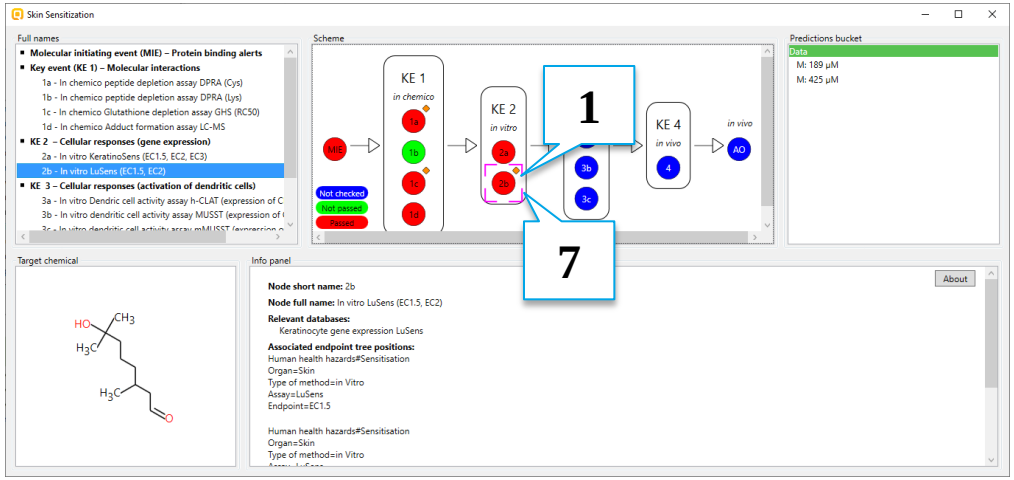
Step 3. *In vitro* LuSens (KE2, node 2b)

Example 1



KE2, node 2b: In this step we will check are there are data for the target chemical for the *in vitro LuSens* assay





1. Select node **2b**
2. Go to ***Data*** module
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 #14)

Workflow process

Status of nodes in vitro KeratinoSens and LuSens assays

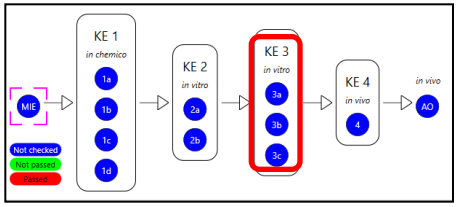
Example 1

- The both nodes related to *in vitro* assays are "Passed" due to positive experimental data found for the target chemical and implemented thresholds (slide #14)
- The workflow should move further to the other *in vitro* assays (KE3, nodes 3a, 3b and 3c)

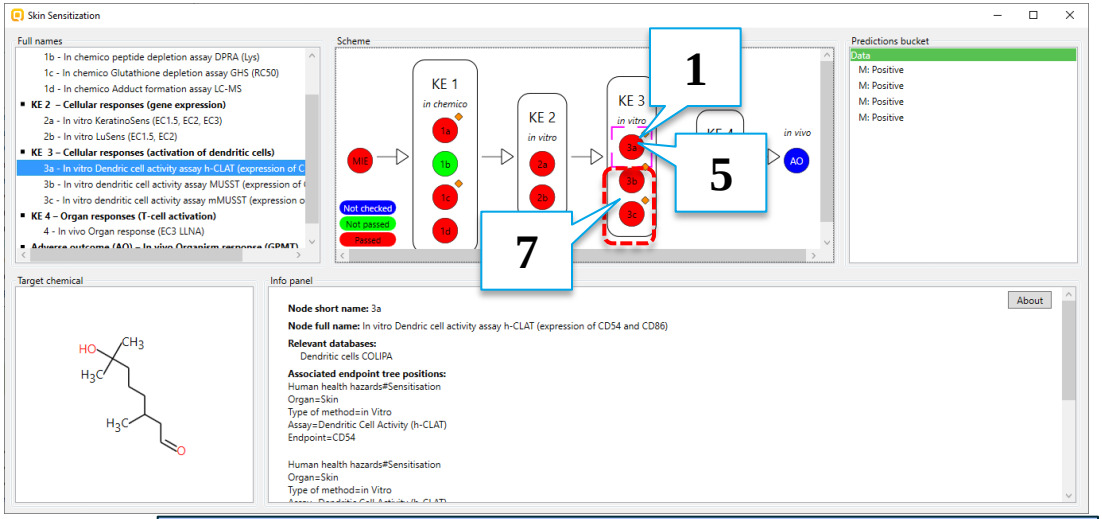
Workflow process

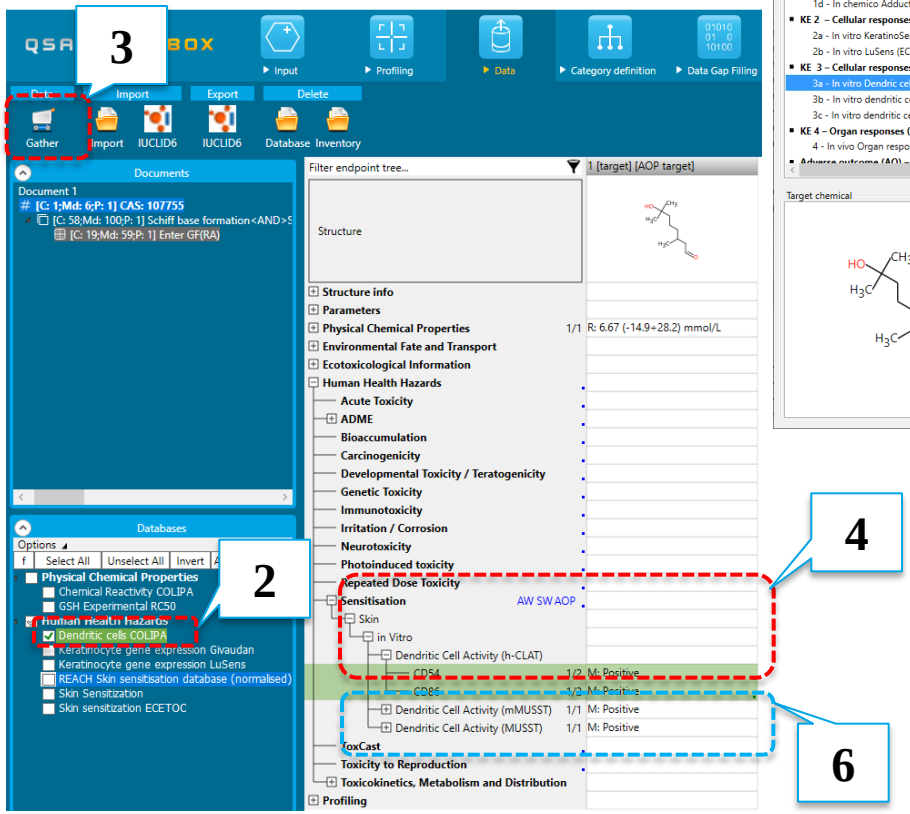
Step 4. *In vitro* Dendric cell activity assays h-CLAT, MUSST and mMUSST (nodes 3a, 3b and 3c)

Example 1



KE3, node 3a: In this step we will check are there are data for the target chemical for the *in vitro Dendritic cell activity (h-CLAT)* assay



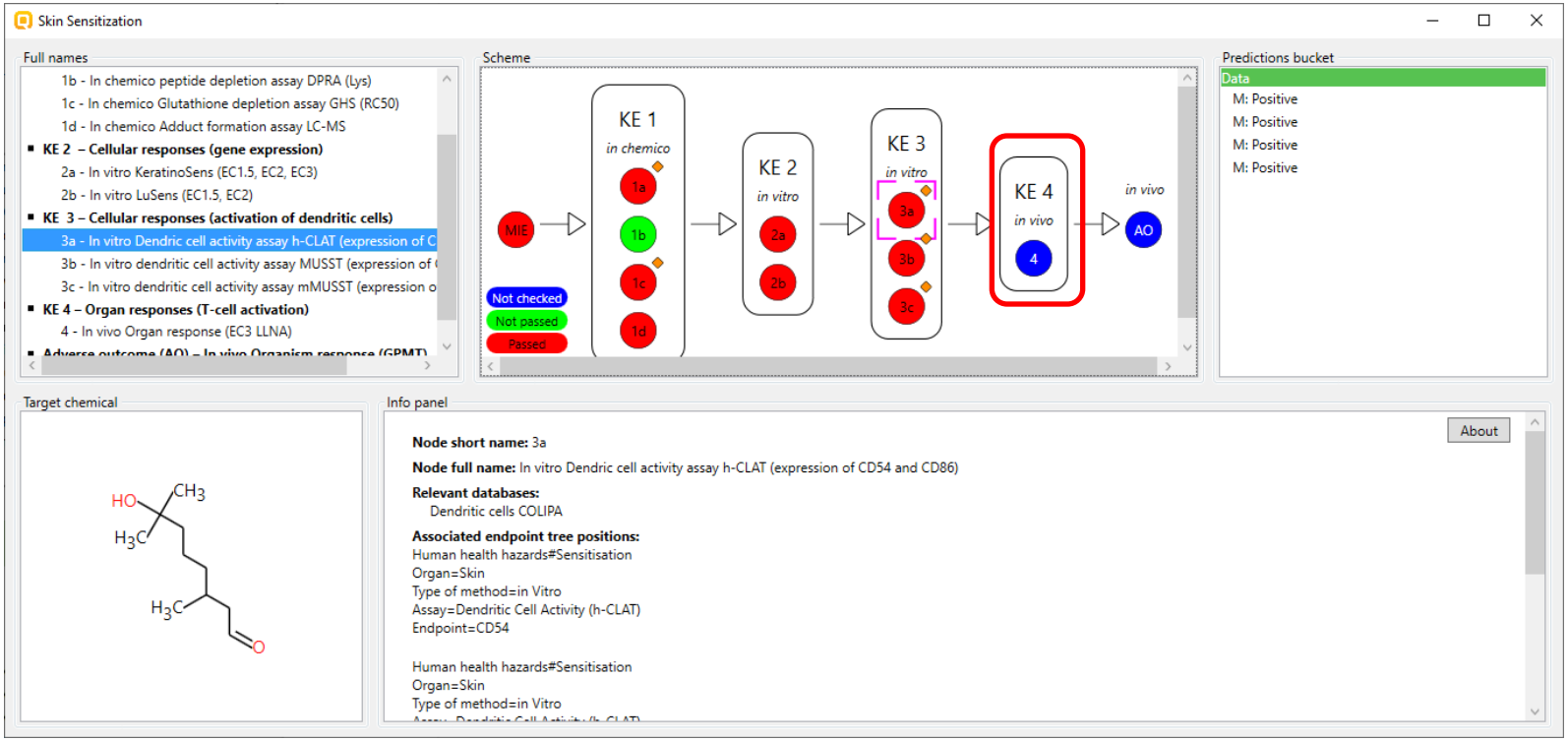


1. Select **node 3a**
2. Select database related to node 3a (**Dendritic cell COLIPA**) but before that unselect all others
3. Click **Gather** and click **OK** in the appeared message
4. The experimental data appears on Data matrix. The endpoint row related to the target assay (Dendritic cell activity (h-CLAT) is getting green highlighted.
5. Based on the implemented threshold status of **node 3a** is changed to **"Passed".**
6. Moreover data is found for the target chemical for the other 2 Dendritic cell assays: mMUSST and MUSST (node **3b** and **3c**).
7. Hence, their status is changed to "**Passed**" based on the implemented data thresholds (slide 14).

Workflow process

Status of nodes in vitro Dendritic cell activity assays)

Example 1

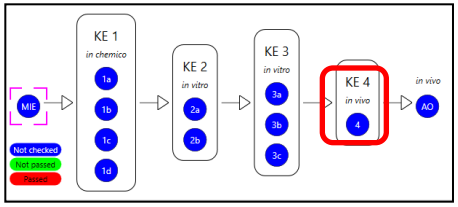


- The status of nodes 3a, 3b and 3c related to the *in vitro* Dendritic cell activity assay (h-CLAT, mMUSST and MUSST) are "passed" due to positive experimental data found for the target chemical
- The workflow moves further to the *in vivo LLNA* assay (KE4, node 4)

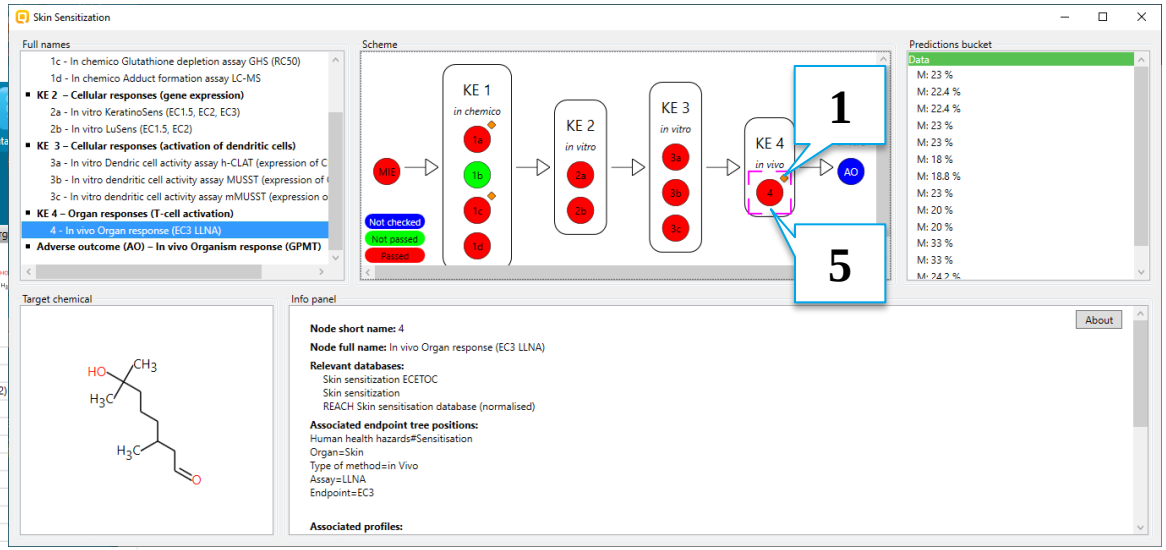
Workflow process

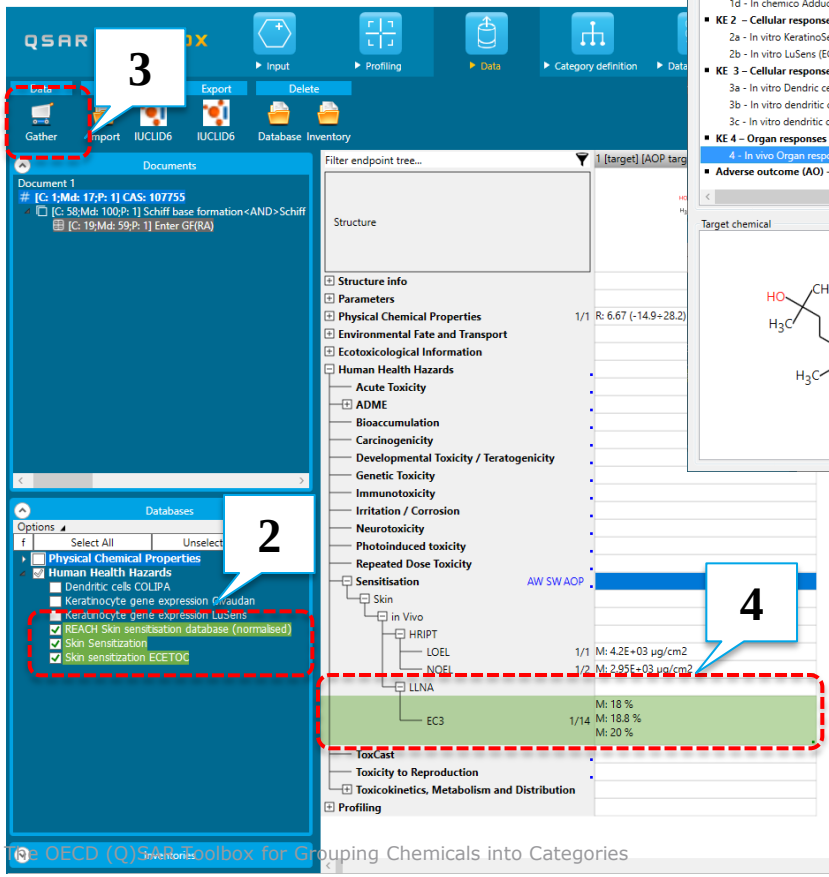
Step 5. *In vivo* Organ response (LLNA)(KE4, node 4)

Example 1



KE4, node 4: In this step we will check are there are data for the target chemical for the *in vivo Organ response (LLNA)*



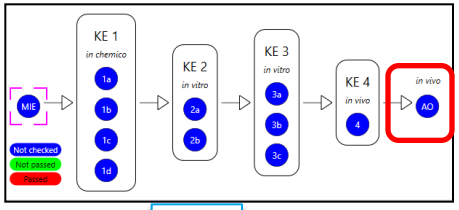


1. Select **node 4**
2. Click **Unselect all** and select **highlighted database**
3. Click **Gather** and click **OK** in the appeared message
4. The data appears in the datamatrix
5. The **node 4** is automatically changed to **passed**, based on experimental data found for the target chemical and the implemented thresholds (see slide #14).

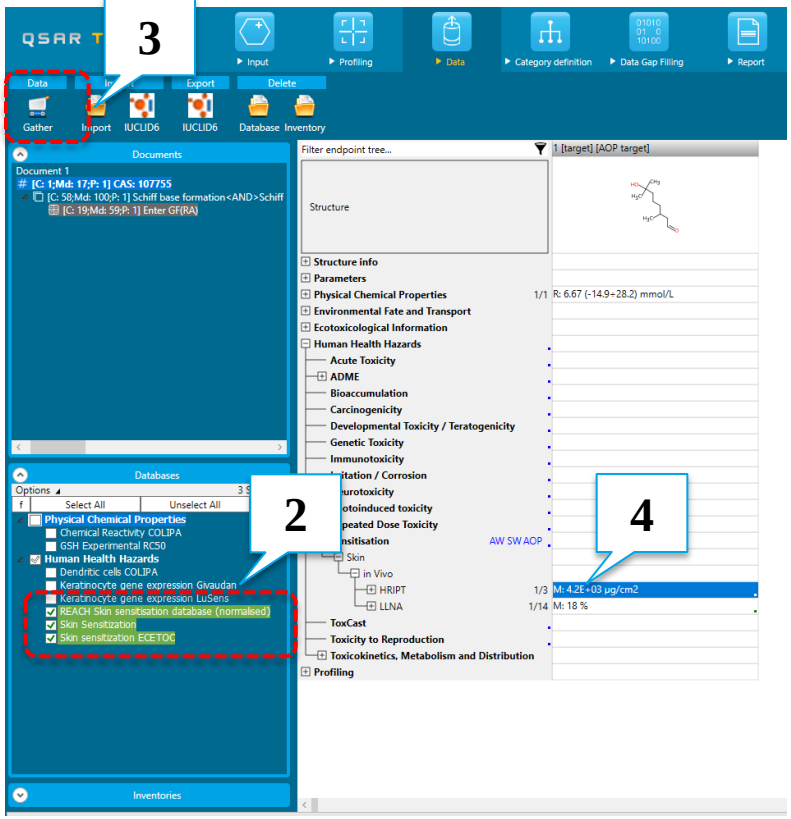
Workflow process

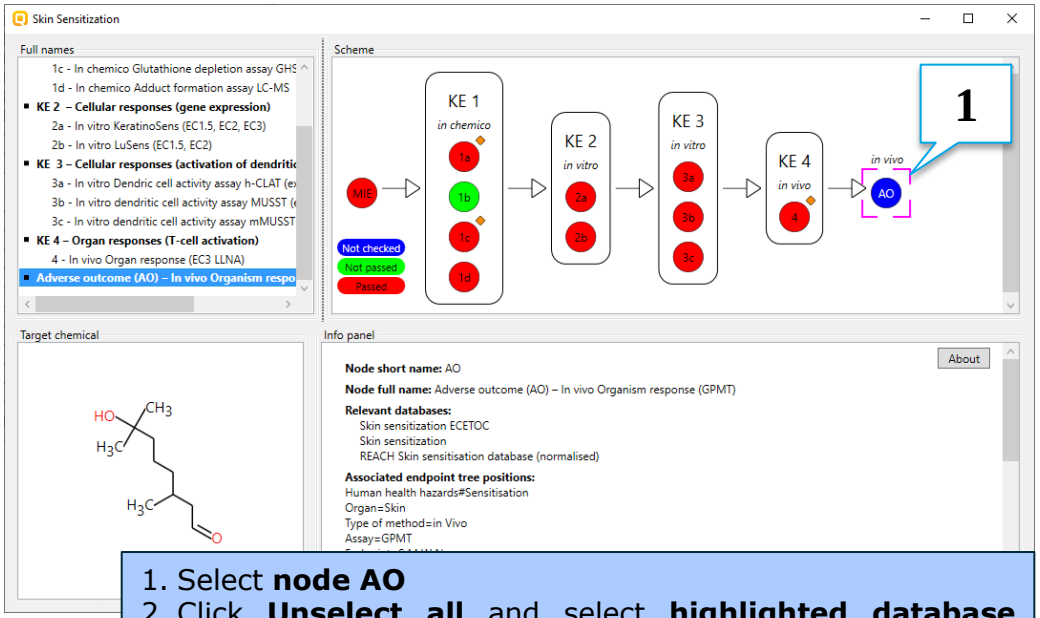
Step 6. *In vivo* Organism response (GPMT)(node AO)

Example 1



Node AO: In this step we will check are there are data for the target chemical for the *in vivo Organism response (GPMT).*





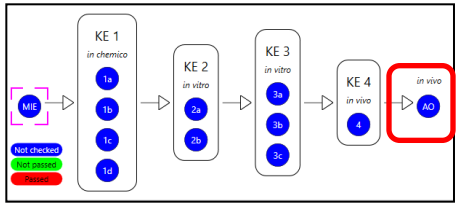
1. Select **node AO**
2. Click **Unselect all** and select **highlighted database (they are same as in the previous step 5: node 4)**
3. Click **Gather**
4. No data for the target endpoint (GPMT assay) is found. Data is available but for other endpoints.

Therefore next step is to make read-across for the target taken into account the most relevant profilers for collecting analogues (see next slide)

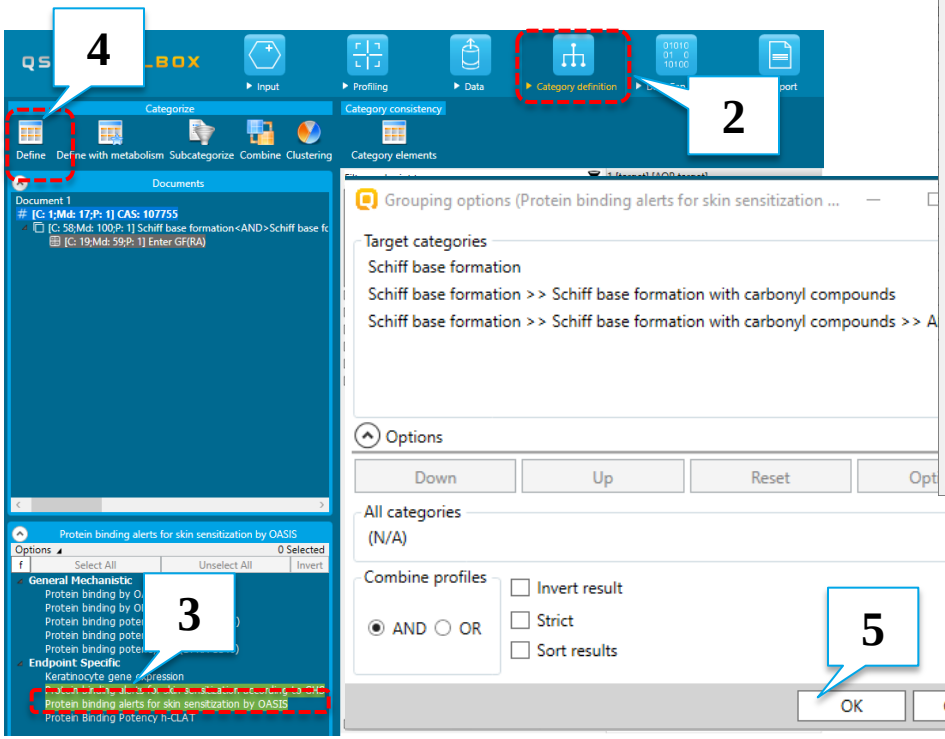
Workflow process

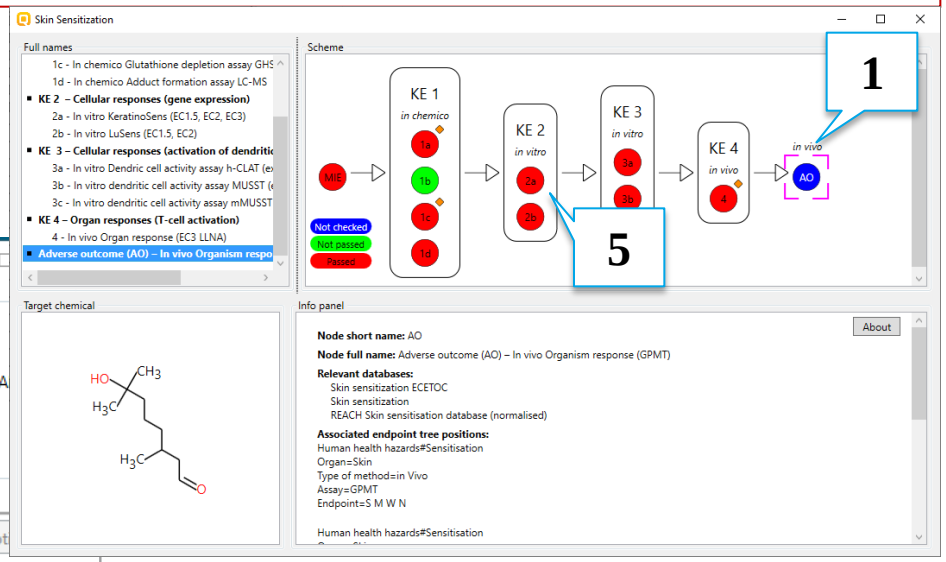
Step 6. *In vivo* Organism response (GPMT)(node AO)

Example 1



Node AO: In this step we will define a category with analogues having same protein binding alerts as the target based on relevant protein binding profiler.





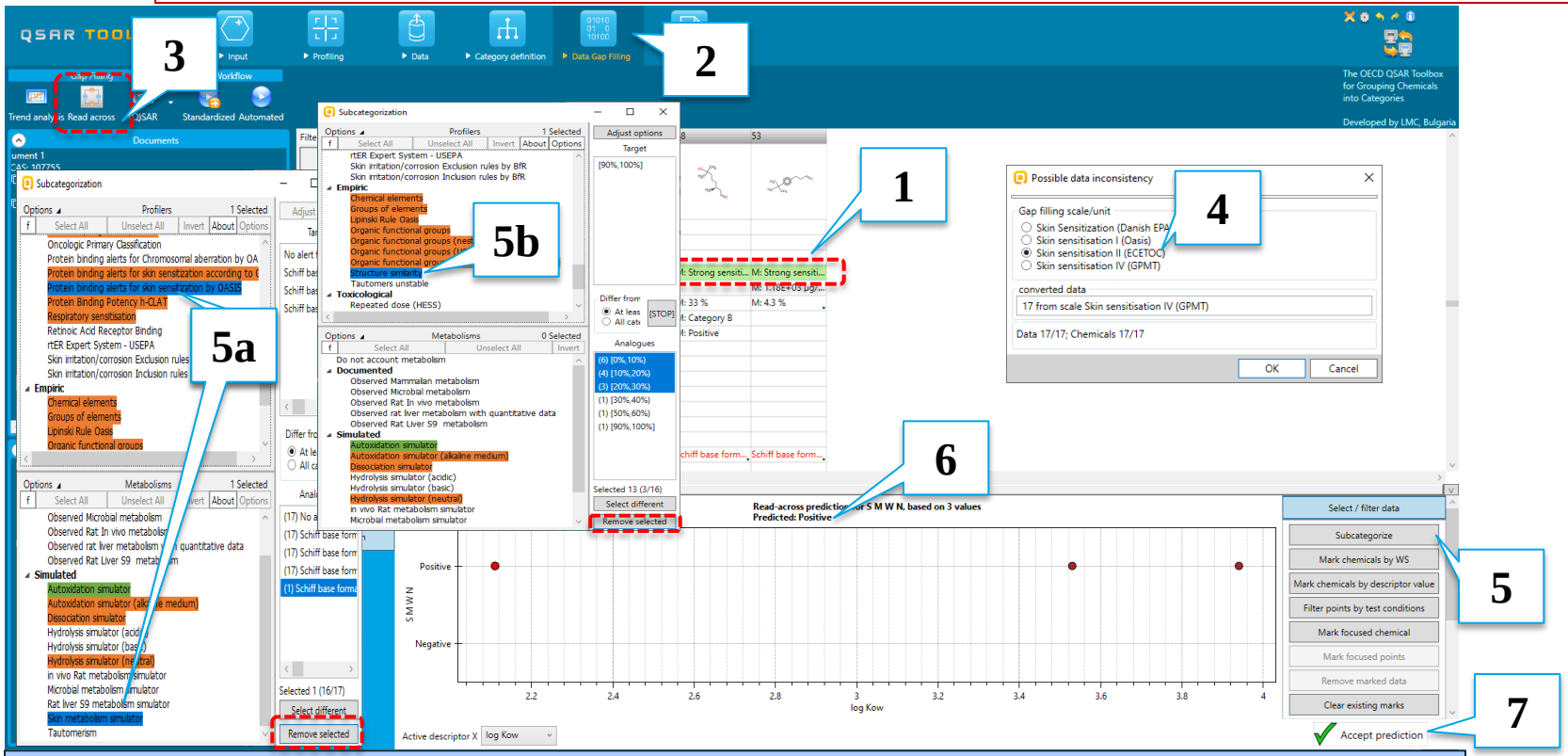
1. Keep **AO node selected**
2. Go to **Category definition** module
3. Select the most relevant profiler **(Protein binding alerts for SS by OASIS)**
4. Click **Define**
5. Confirm the identified categories in the target (aldehydes), which will be used for searching analogues. Click **OK.** 75 analogues are identified. Read-data for the analogues (not shown)

Workflow process

Step 6. *In vivo* Organism response (GPMT)(node AO)

Example 1

Node AO: In this step we will perform read-across for the target for the target endpoint (GPMT) based on the identified analogues.

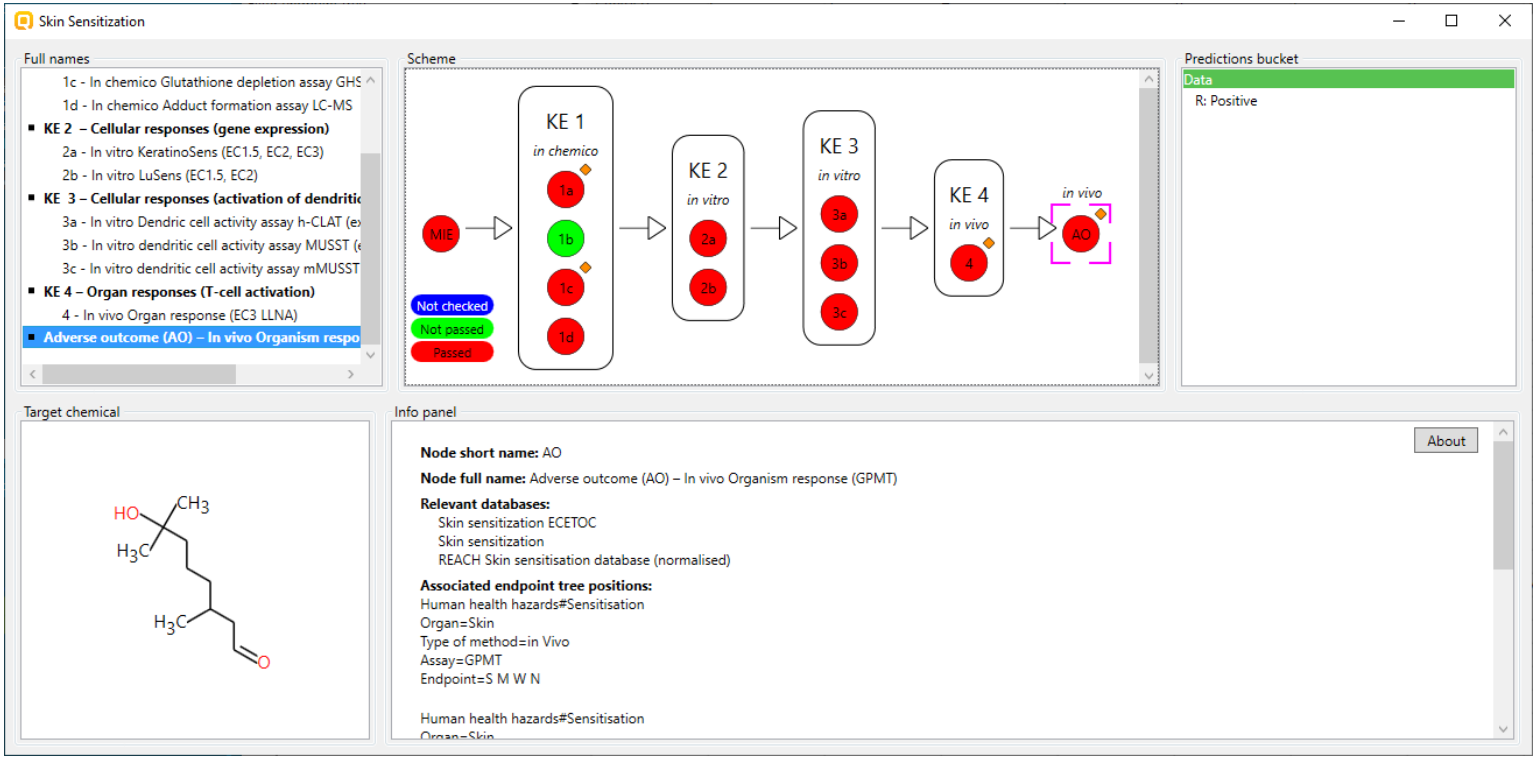


1. Open endpoint tree to the level of Human Health Hazards#Sensitisation#Skin#in Vivo#GPMT#S M W N;
2. Go to **Data Gap filling;**
3. Apply **read-across;**
4. Select the default scale (Skin sensitization II (ECETOC));
5. Apply two consecutive subcategorizations: 5a: Protein binding alerts for SS + skin metabolism; 5b: Str. similarity – remove analogues with similarity less than 30%;
6. The final prediction is positive based on three analogues with positive GPMT data;
7. Click **Accept prediction.** The prediction should be used for AO (steps are shown on slide 38)

Workflow process

Status of nodes in vivo Organ and Organism assays (node 4 and AO)

Example 1



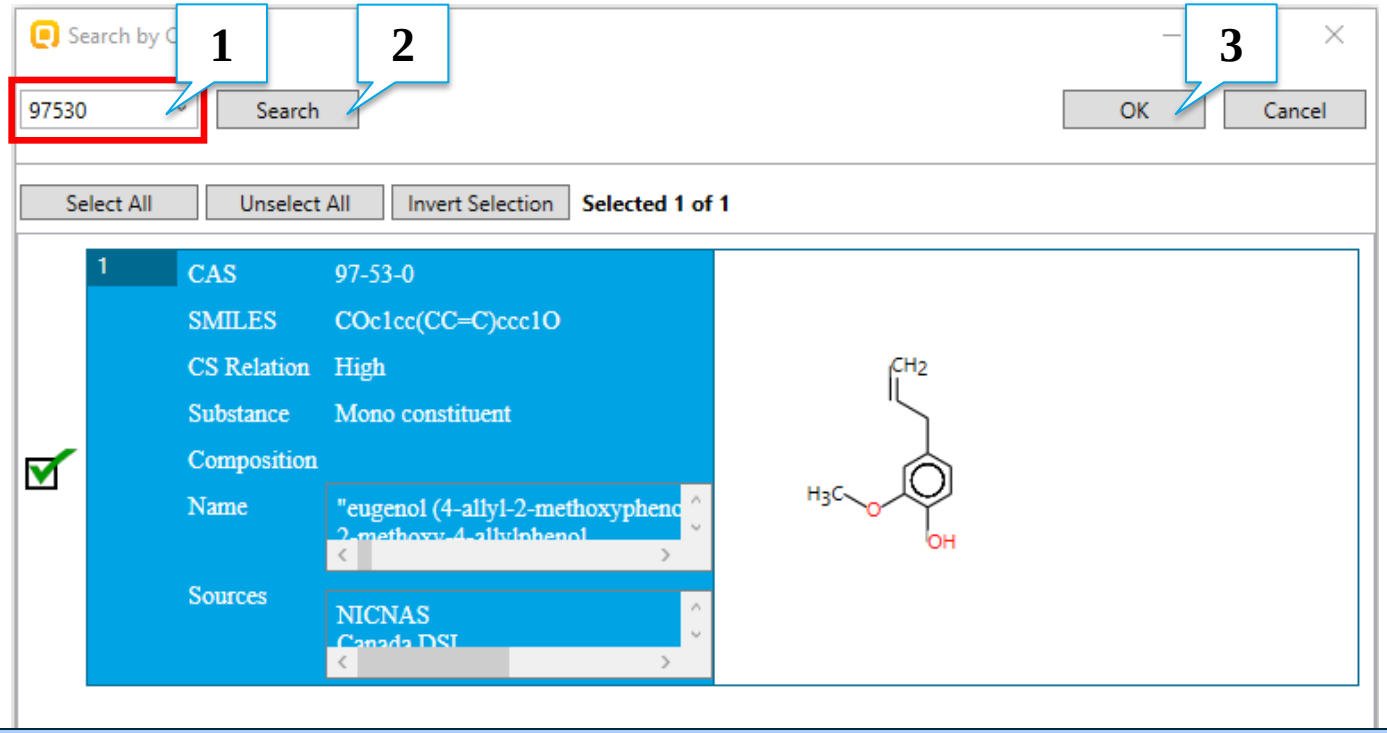
- Both nodes related to the two *in vivo* assays (LLNA and GPMT) are "passed" based on the positive experimental (LLNA) data found for the target chemical and positive read-across prediction (for GPMT assay).

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

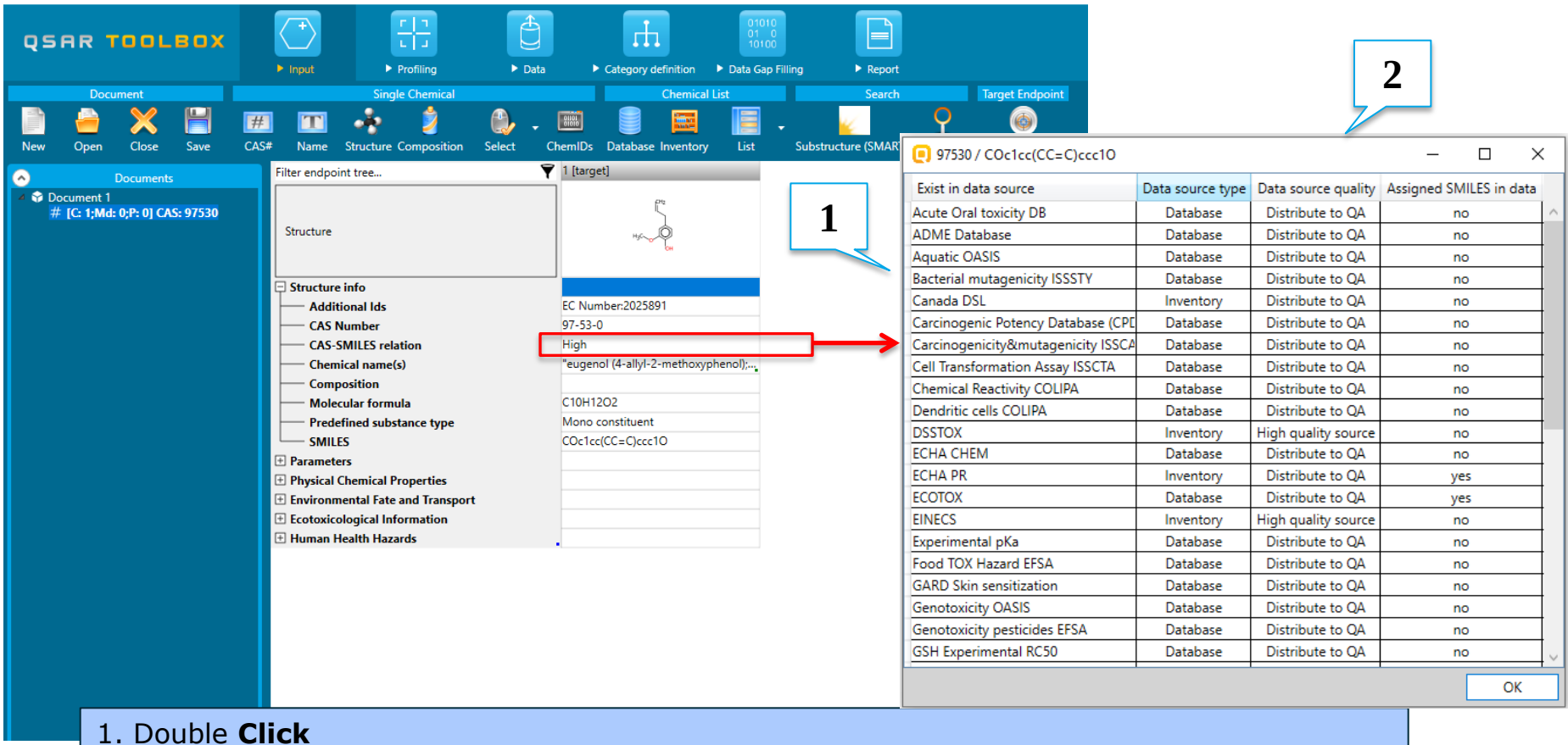


1. Create new document and Enter the **CAS# 97-53-0** In the blank field;
2. Click **Search** button;
3. Press **OK**

Chemical Input
Target chemical identity

- Double clicking "CAS Smiles relation" displays the chemical identification information.
- This indicates the reliability of relation CAS-Name for the target chemical(see next screen shots).
- The workflow on the first module is now complete, and the user can proceed to the next module.

Chemical Input
Target chemical identity

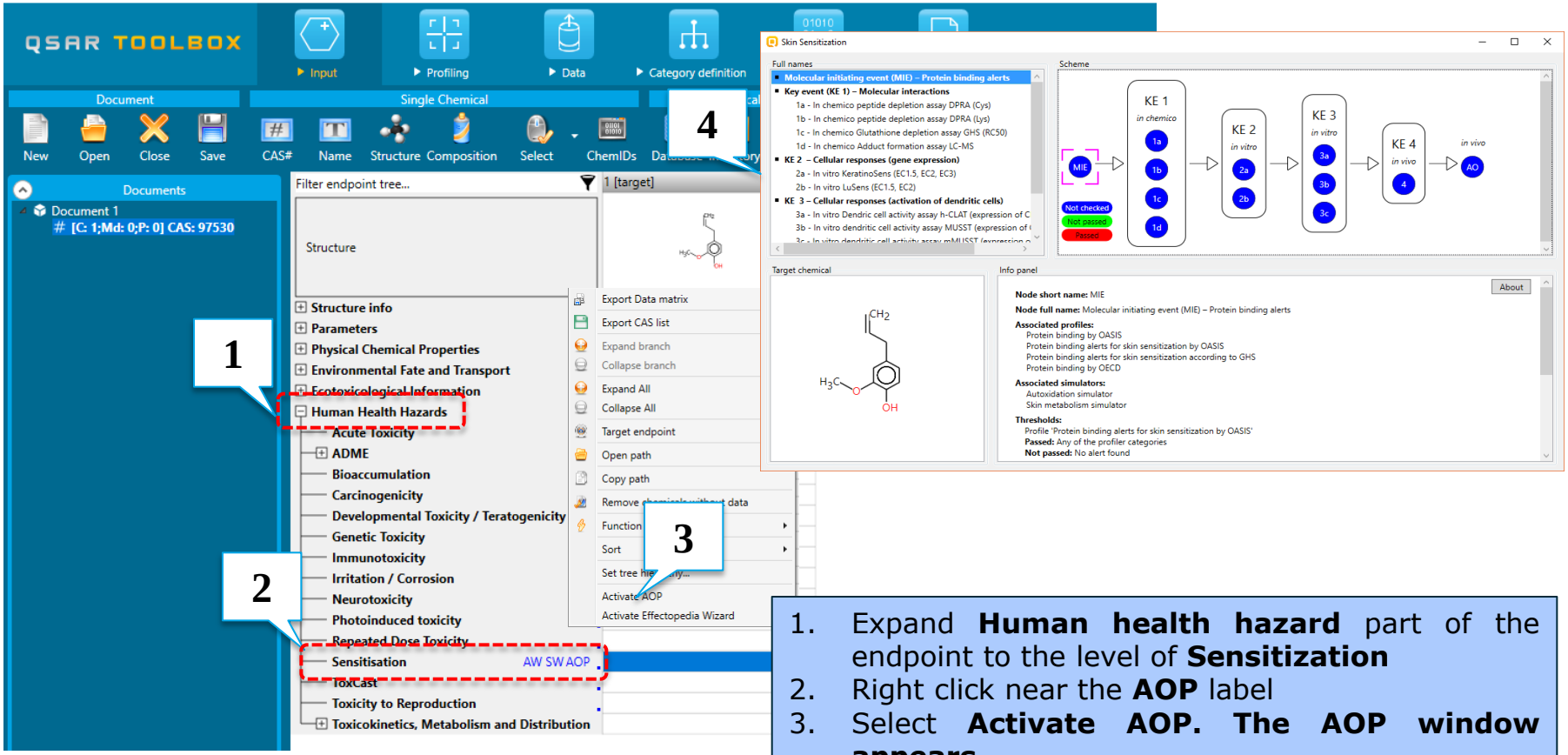


1. Double **Click**
2. Relationships CAS-SMILES. More information for the relation CAS-SMILES could be found on slide 22

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



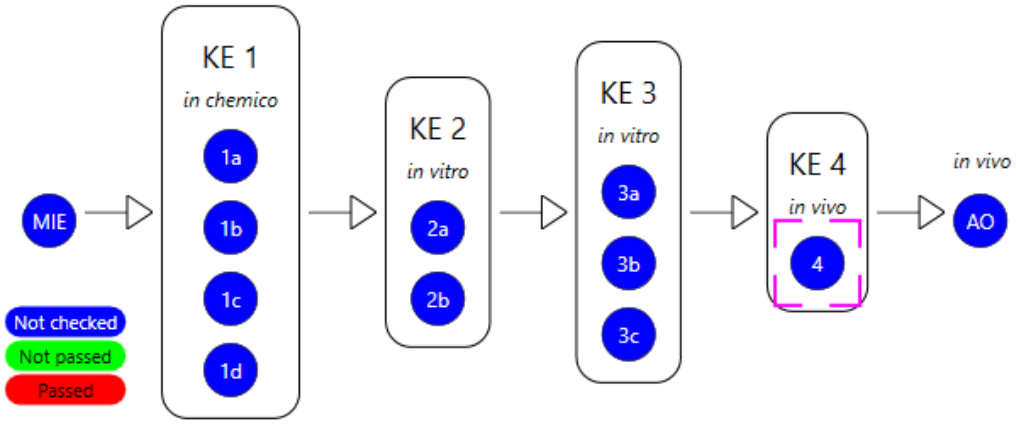
1. Expand **Human health hazard** part of the endpoint to the level of **Sensitization**
2. Right click near the **AOP** label
3. Select **Activate AOP. The AOP window appears**
4. The target chemical automatically appears in the AOP window.

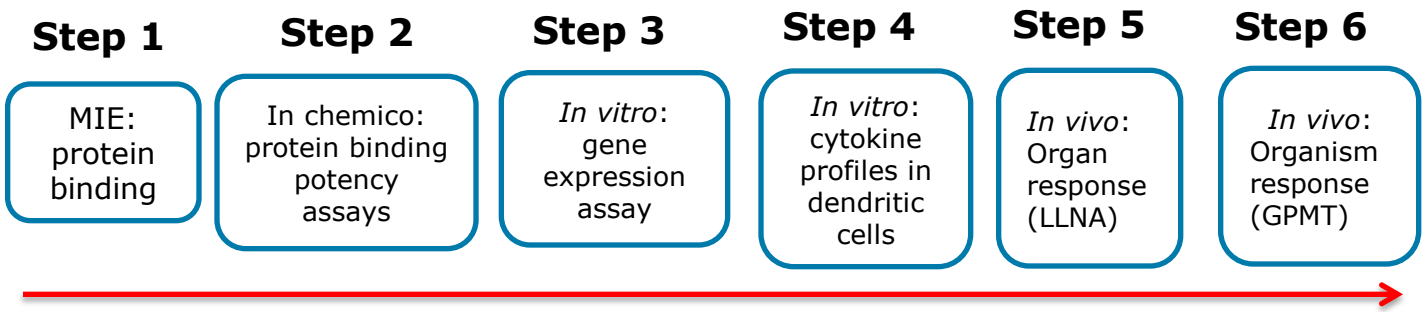
Outlook

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

Workflow process

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

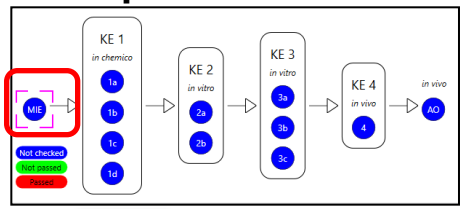




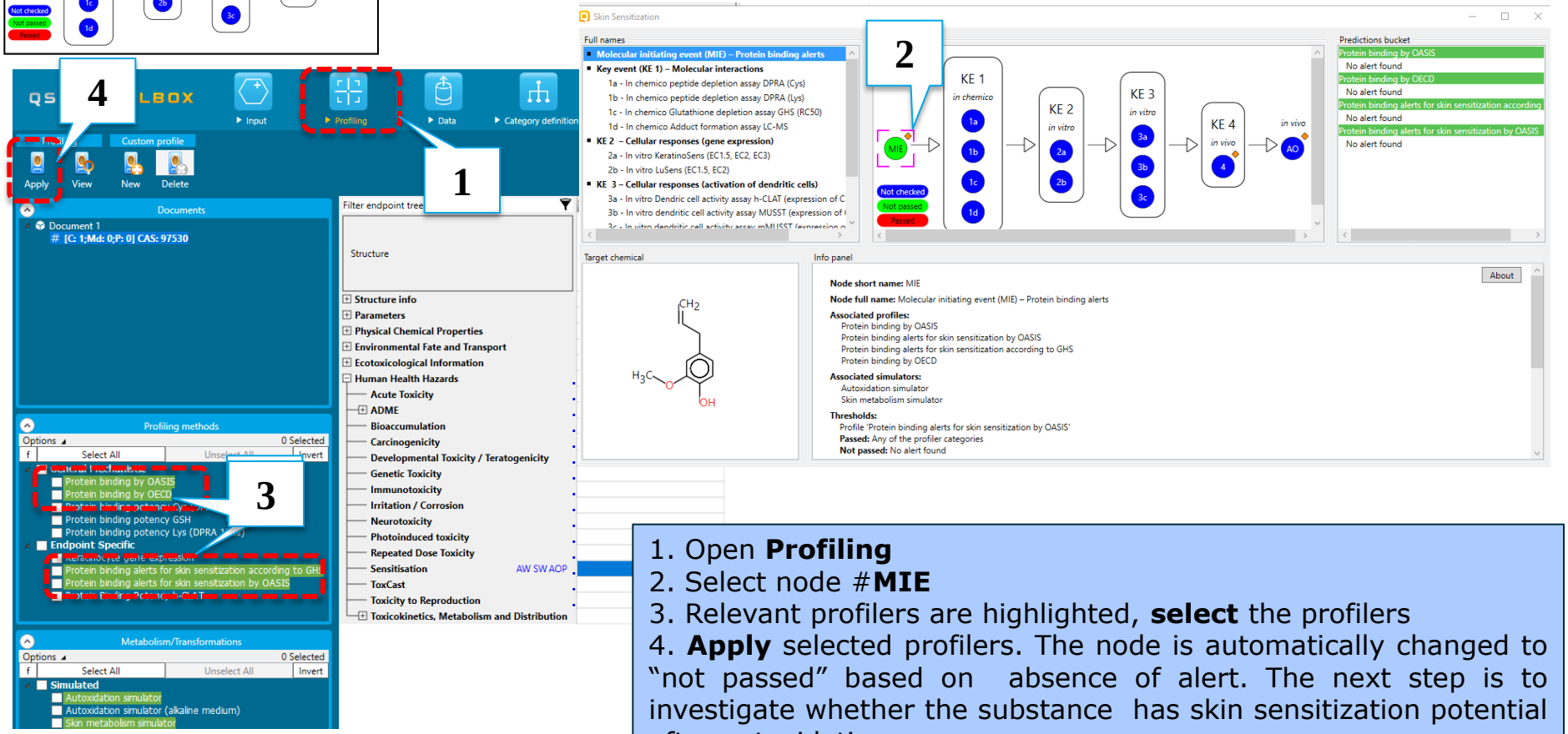
Workflow process

Step 1. MIE: protein binding

Example 2



MIE: Start with profiling of target chemical

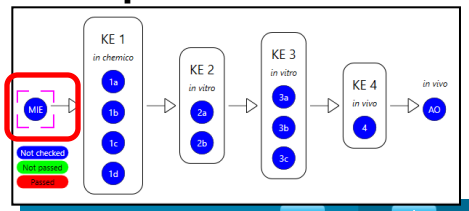


1. Open **Profiling**
2. Select node #**MIE**
3. Relevant profilers are highlighted, **select** the profilers
4. **Apply** selected profilers. The node is automatically changed to "not passed" based on absence of alert. The next step is to investigate whether the substance has skin sensitization potential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

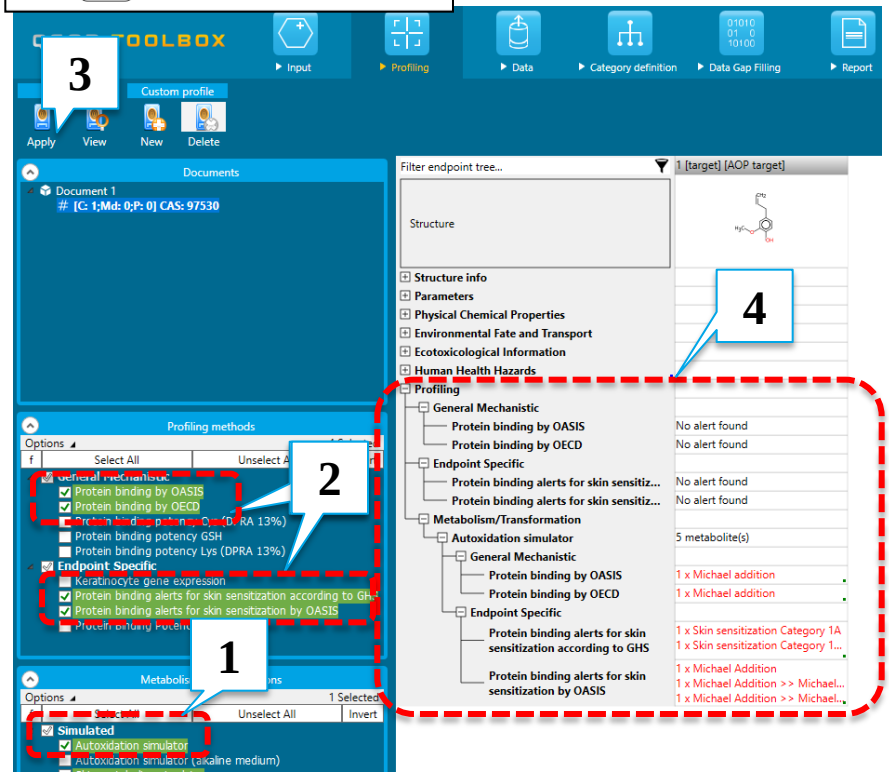
Workflow process

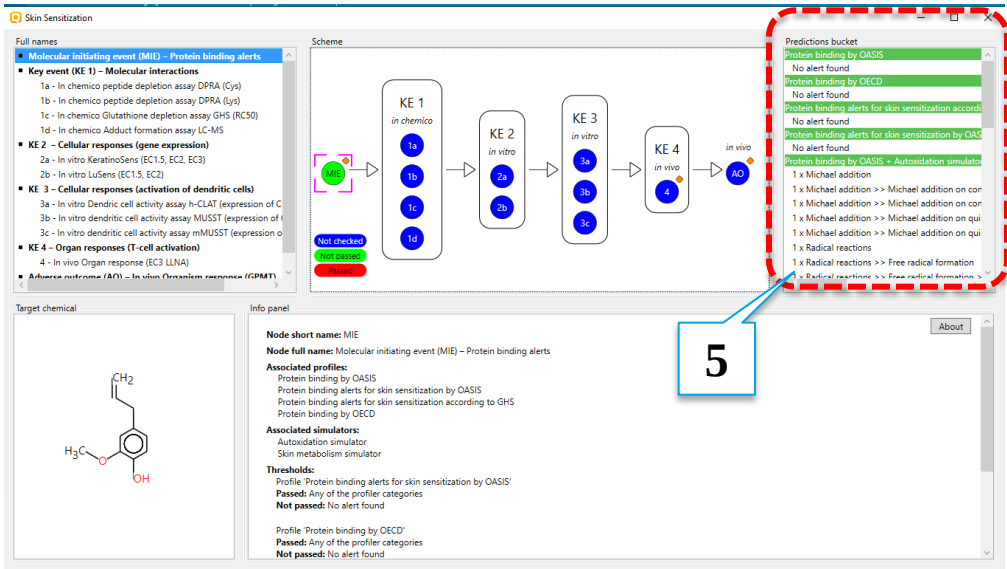
Step 1. MIE: protein binding

Example 2



MIE: Simulate Autoxidation (AU) products of the target chemical



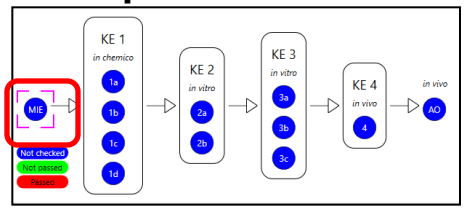


1. **Select** Autoxidation simulator.
2. **Select** highlighted profilers relevant to the MIE (they should be already checked)
3. **Click** Apply
4. The profiling results appeared on data matrix. As seen protein binding alerts are identified for the AU products
5. The profiling results for the package parent & AU products are also stored at the prediction bucket

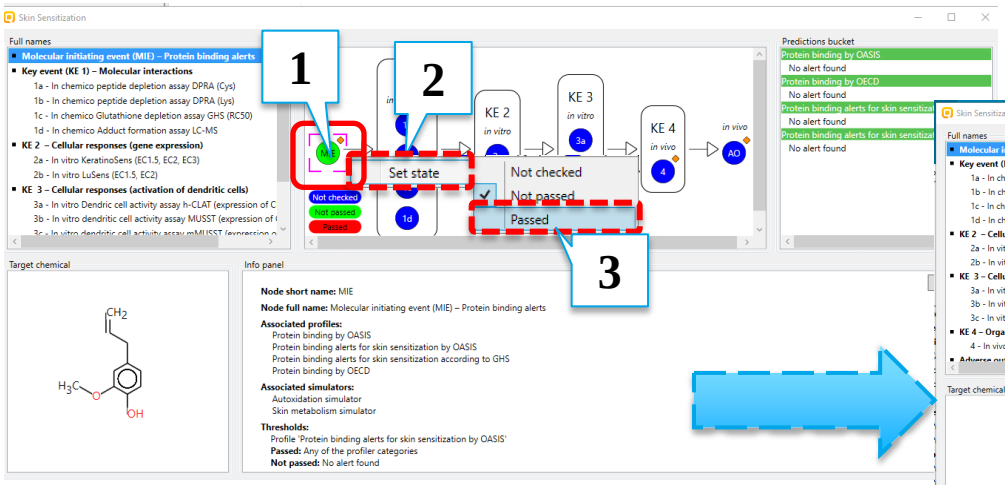
Workflow process

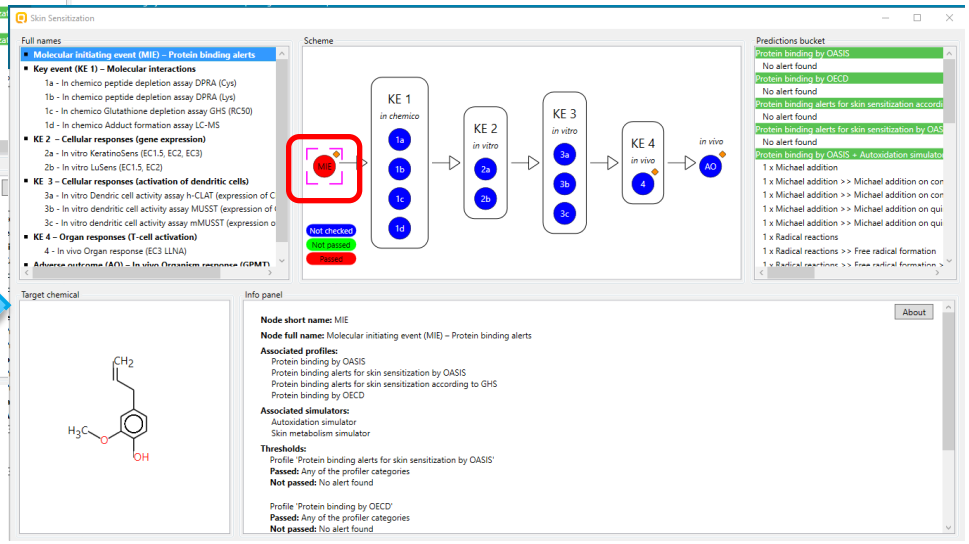
Step 1. MIE: protein binding

Example 2



MIE: Simulate Autoxidation products of the target chemical – in this step we need to change manually the status of the node



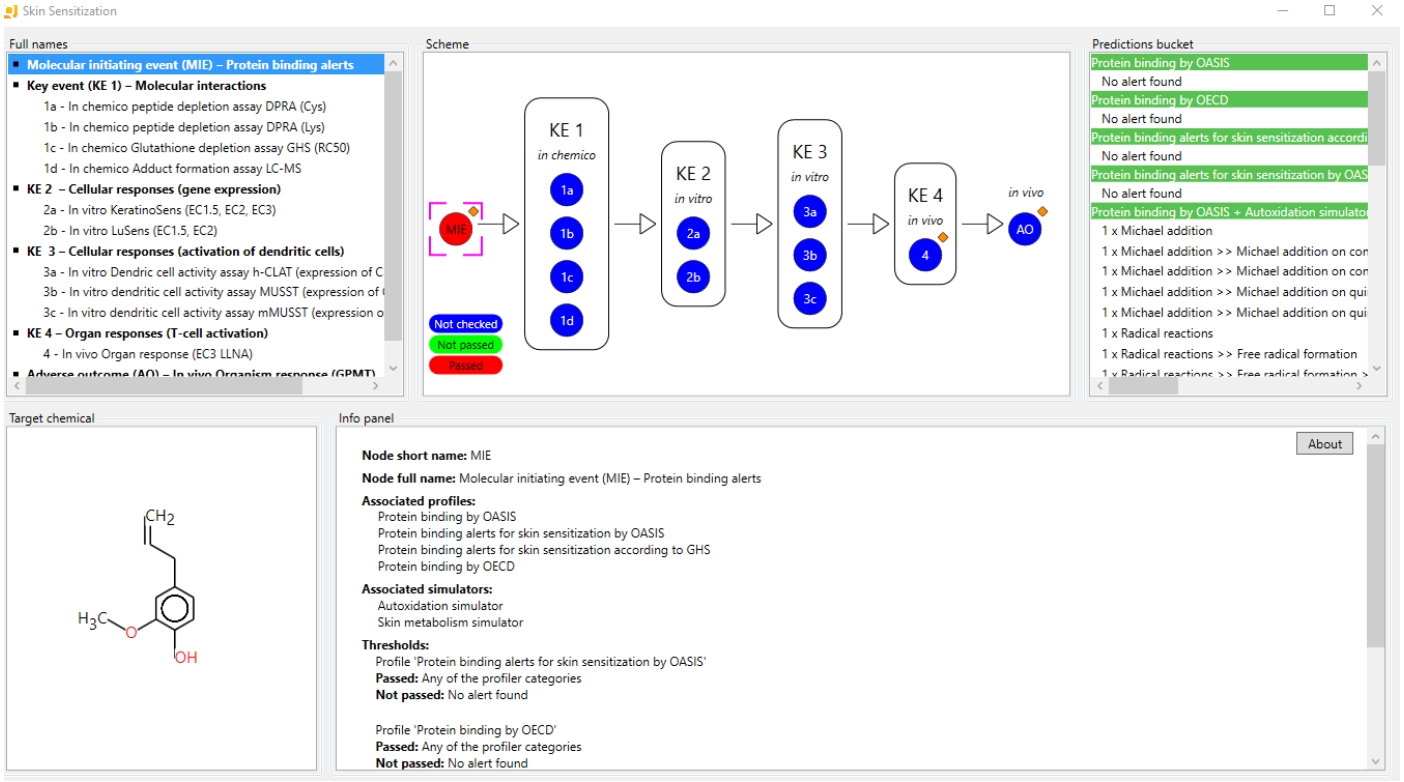


1. **Right** click on the **MIE node**
2. Select **Set state**
3. Change the state from **Not Passed** to **Passed** (the node change its color from green to red)

Workflow process

Status of node Molecular initiating event

Example 2

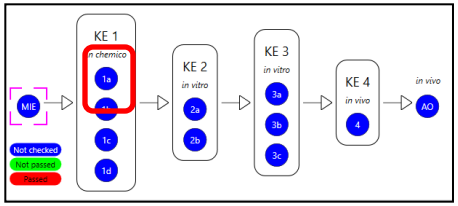


- The node MIE is passed due to the presence of positive protein binding alert identified for the Autoxidation products of the target chemical
- The workflow should move further to the *in chemico* assays

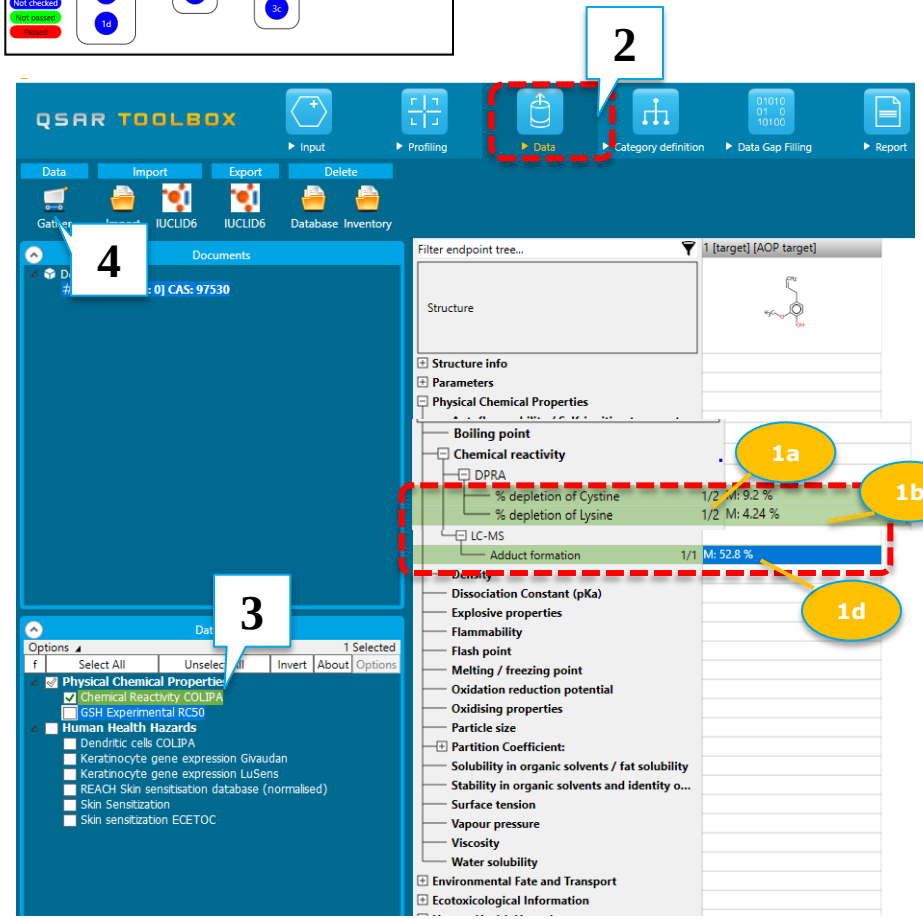
Workflow process

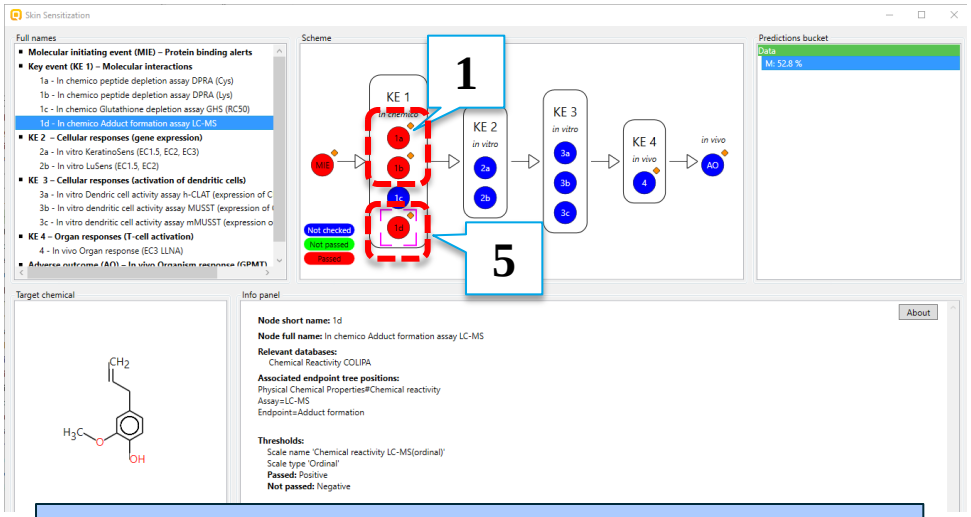
Step2. *In chemico* peptide depletion assay DPRA (Cys) (KE1, node 1a)

Example 2



KE1, node 1a: Check for experimental data



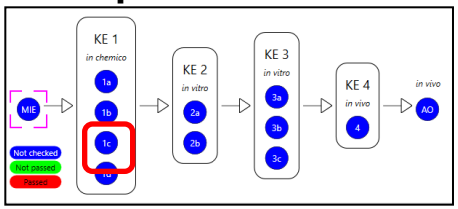


1. Select node **1a**
2. Go to **Data**
3. **Select** highlighted database
4. **Click** Gather
5. The selected database (Chemical reactivity COLIPA) is related to three AOP nodes - 1a, 1b and 1d (i.e. data is available for the endpoints related to these nodes). In our case data is found for the target chemical for all the 3 nodes. Hence, the three nodes are marked as **Passed** (based on the implemented thresholds, see slide 14). The workflow could move to the next KE1 node -1c.

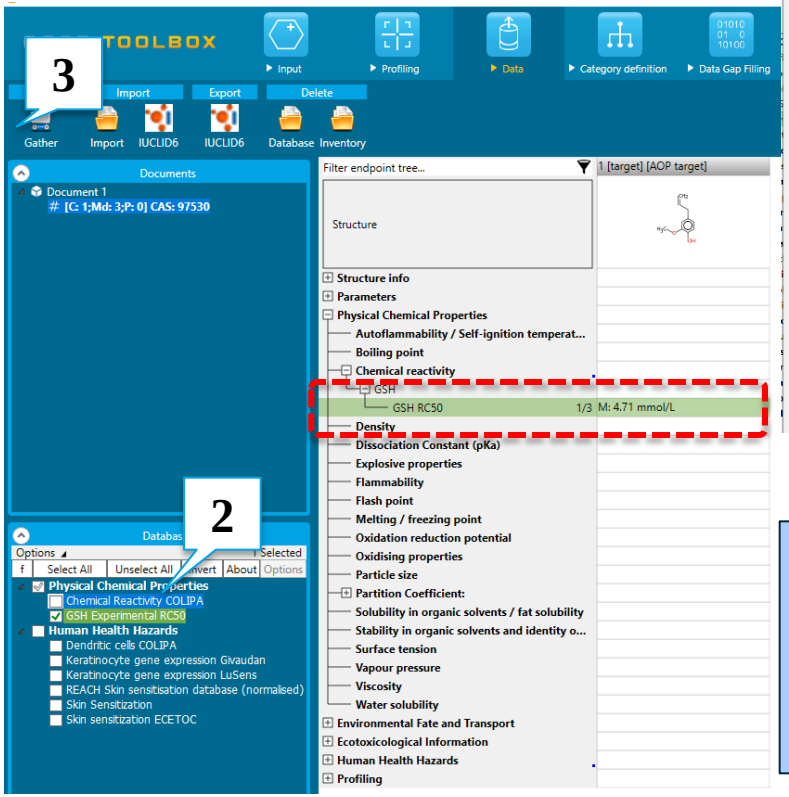
Workflow process

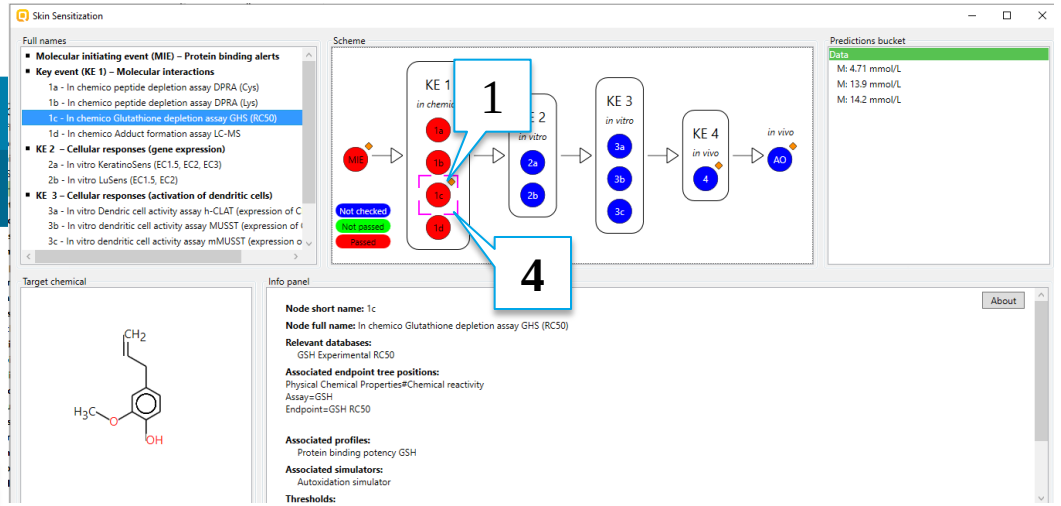
Step2. *In chemico* Glutathione depletion assay GHS (RC50) (KE1, node 1c)

Example 2



KE1, node 1c: Check for experimental data





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

Status of In chemico assays

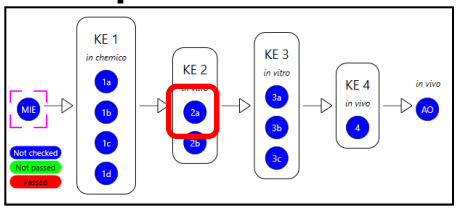
Example 2

- The nodes related to the *in chemico* assays are passed due to positive experimental data found for the target chemical (node 1a, 1b, 1c and 1d) The workflow should move further to the *in vitro* assay (node 2a and 2b)

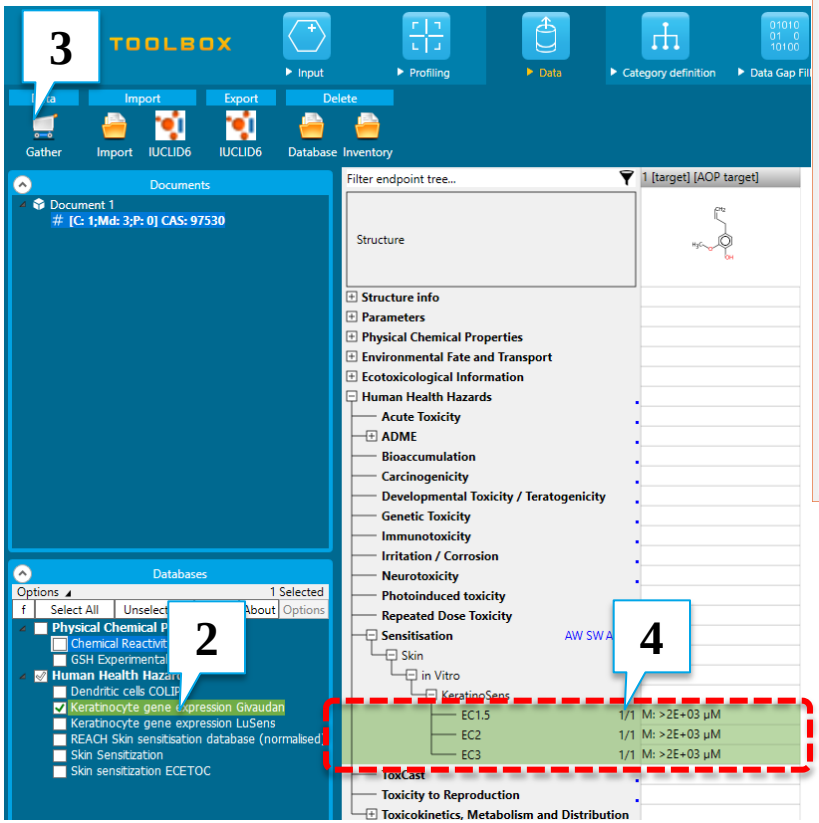
Workflow process

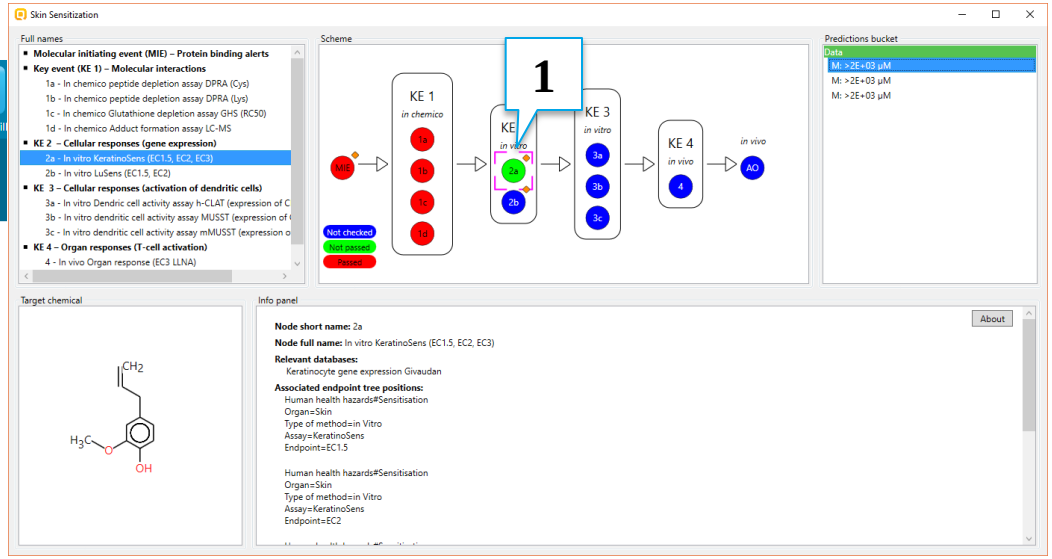
Step 3. *In vitro* KeratinoSens (KE2, node 2a)

Example 2



KE2, node 2a: Check are there experimental data for the parent chemical for node 2a



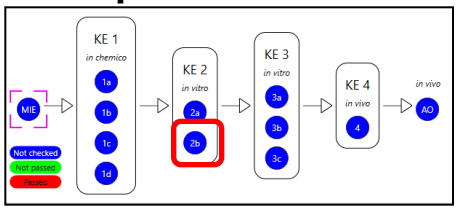


1. **Select** node 2a
2. **Select** highlighted database, unselect all other
3. **Click** Gather
4. There is experimental data for the parent chemical, which appears on data matrix. Node 2a is getting "Not Passed" based on the experimental data and implemented thresholds (see slide #14)

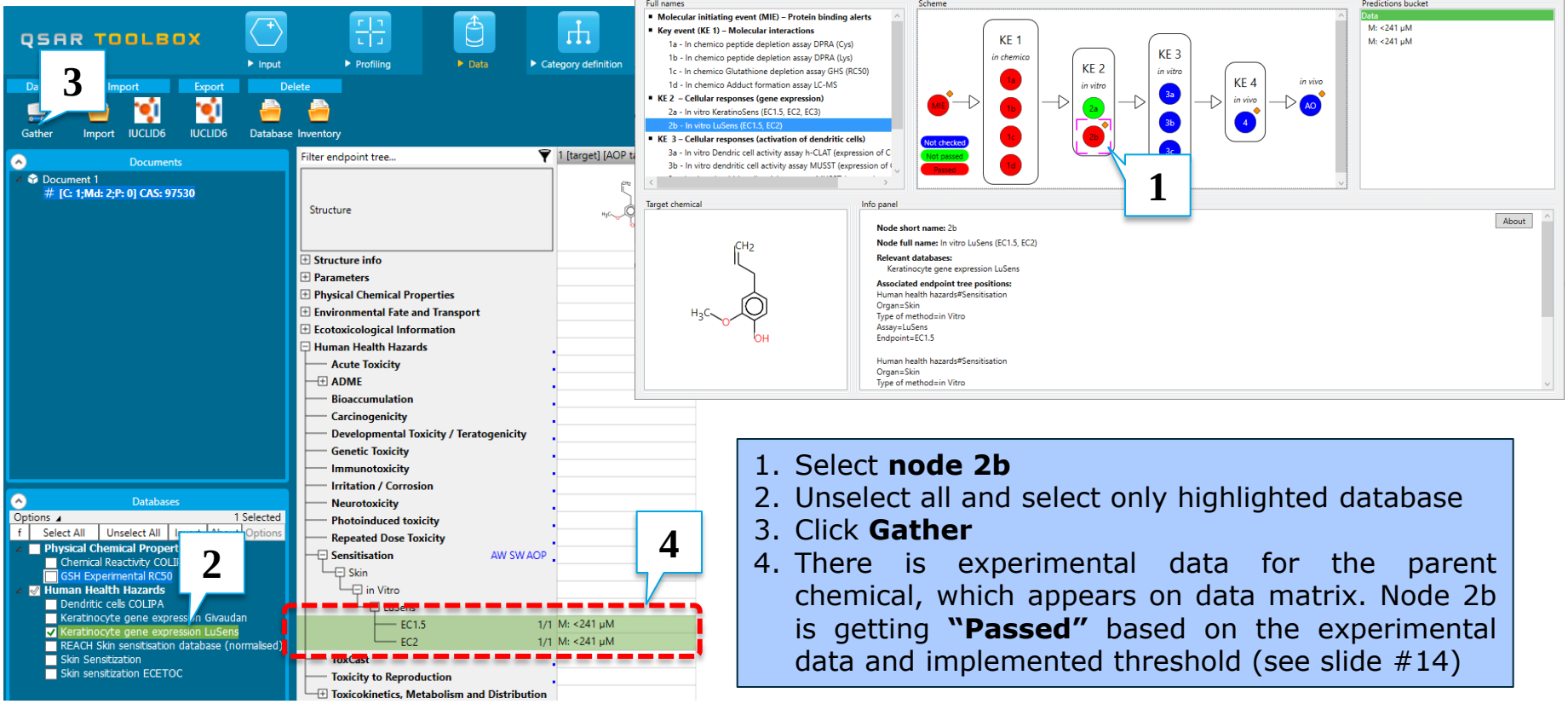
Workflow process

Step 3. *In vitro* LuSens (KE2, node 2b)

Example 2



KE2, node 2b: Check are there experimental data for the parent chemical for node 2b

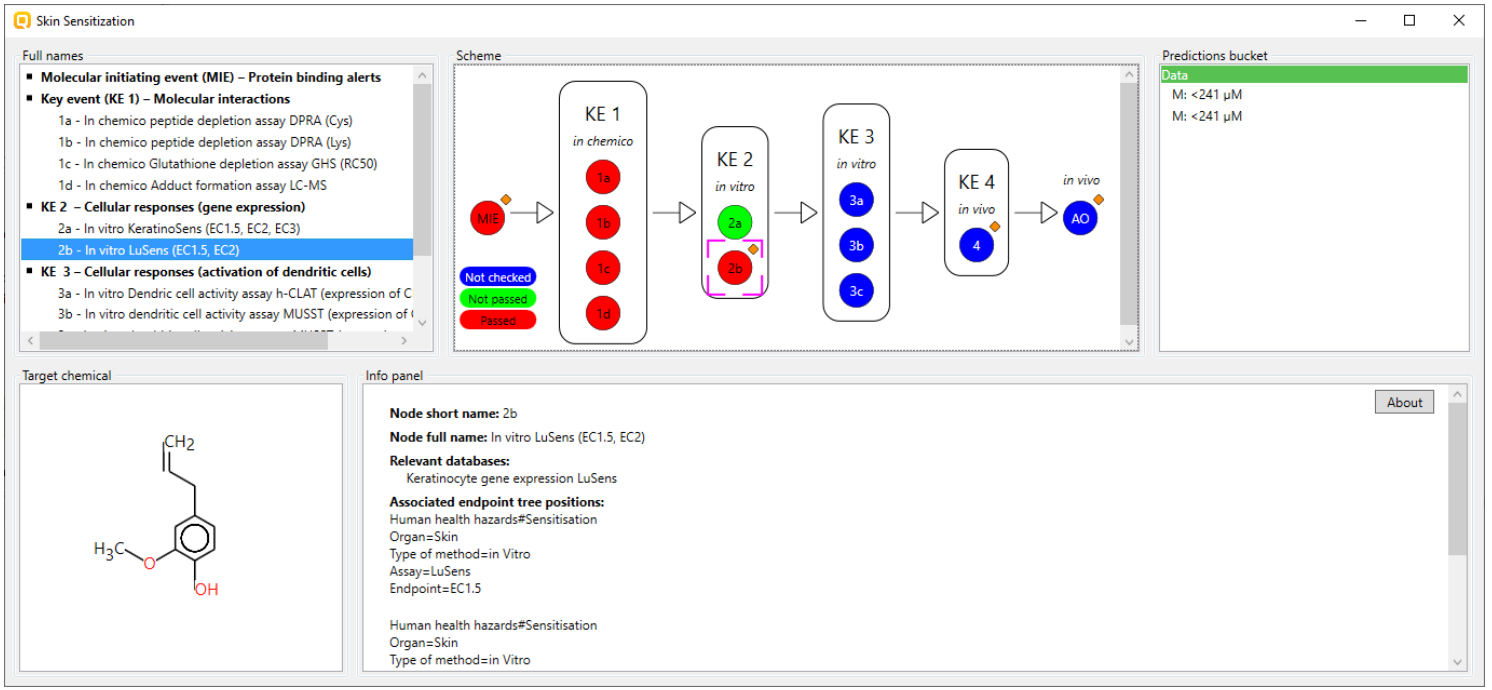


1. Select **node 2b**
2. Unselect all and select only highlighted database
3. Click **Gather**
4. There is experimental data for the parent chemical, which appears on data matrix. Node 2b is getting **"Passed"** based on the experimental data and implemented threshold (see slide #14)

Workflow process

Status of in vitro Keratinocyte ARE and LuSens assays

Example 2

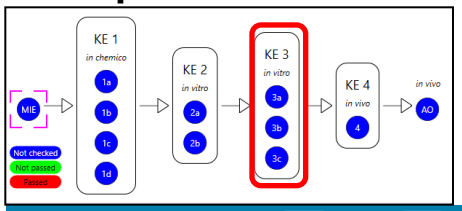


- The node 2a related to the Keratinocyte ARE assay is "not passed" based on negative data found for the target chemical. The node 2b related to the LuSens assay is "passed" based on the positive experimental data found for the target chemical (threshold is specified on slide # 14).
- The workflow should moves further to the *in vitro* Dendritic cell assay (nodes 3a, 3b and 3c)

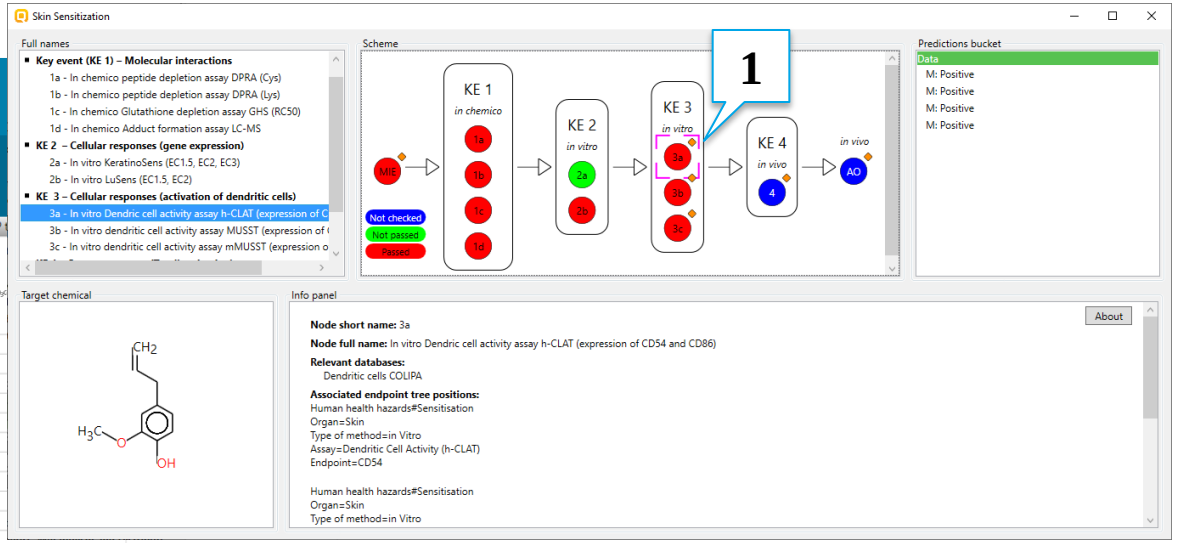
Workflow process

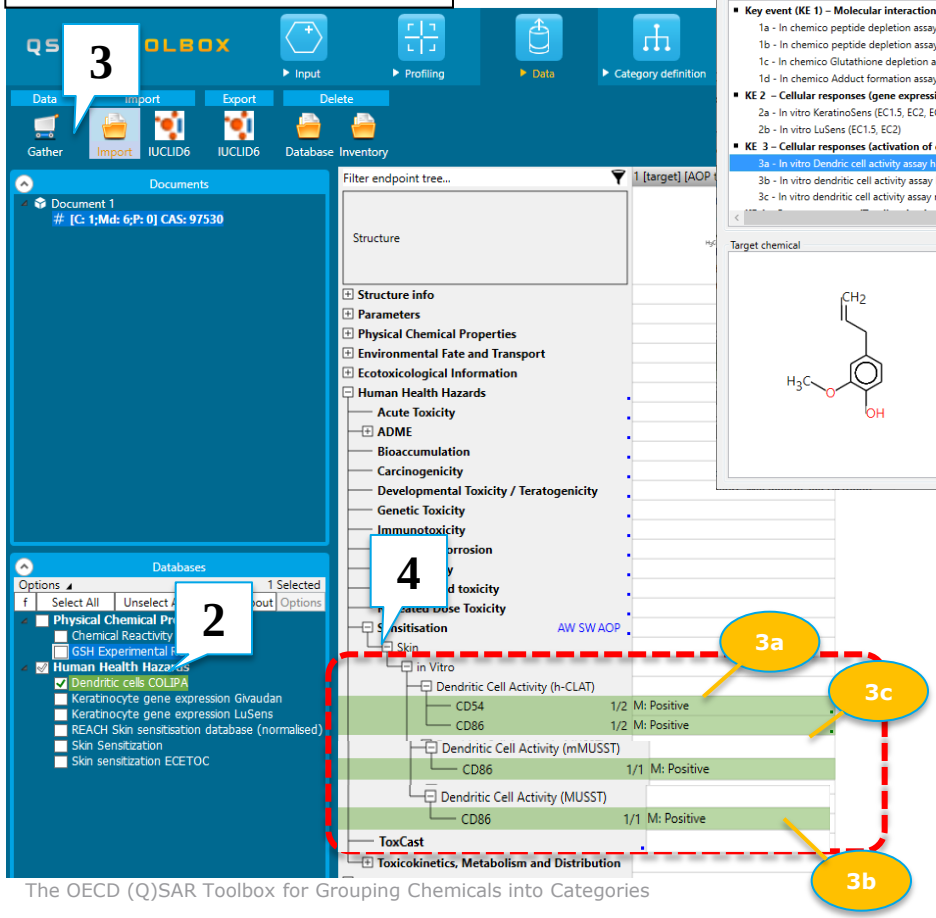
Step 4. *in vitro* Dendritic cell activity assay (KE3, nodes 3a, 3b and 3c)

Example 2



KE3, node 3a: Check if there are any data for the target chemical for the *in vitro Dendric cell activity assay*



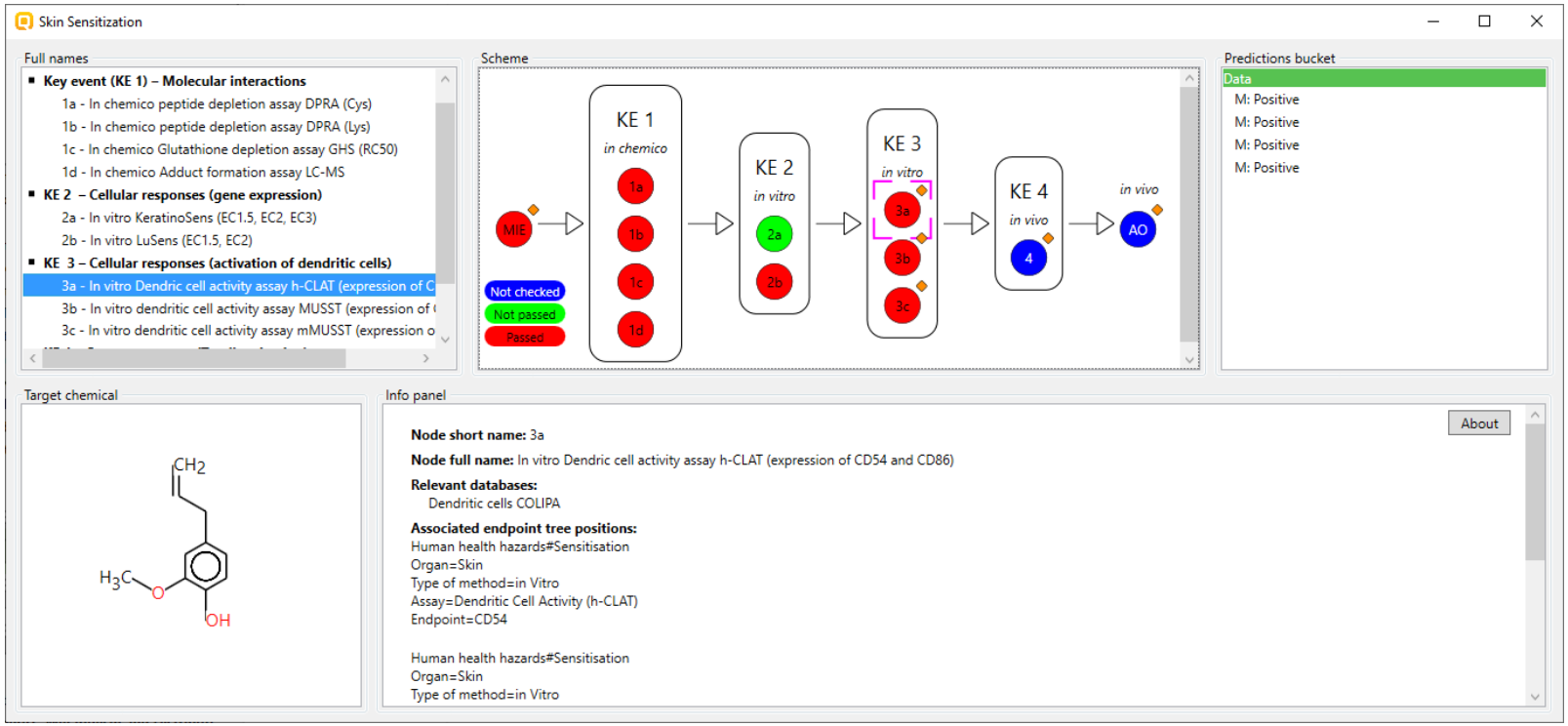


1. Select **node 3a**
2. Select database related to node 3a
3. **Gather** data and click **OK** in the appeared message
4. The experimental data appears on Data matrix. Based on data found for the target chemicals status of nodes 3a, 3b and 3c was changed to **Passed.**

Workflow process

Status of nodes for in vitro Dendritic cell activity assay

Example 2

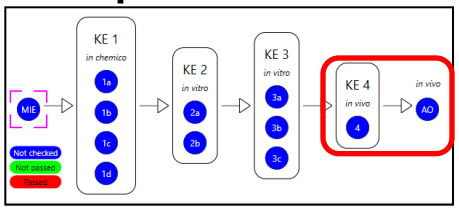


- The node 3a, 3b and 3c related to the *in vitro* Dendritic cell activity assays (h-CLAT, MUSST and mMUSST) are "passed" due to positive experimental data found for the target chemical
- The workflow could further move to the *in vivo LLNA* assay (nodes 4)

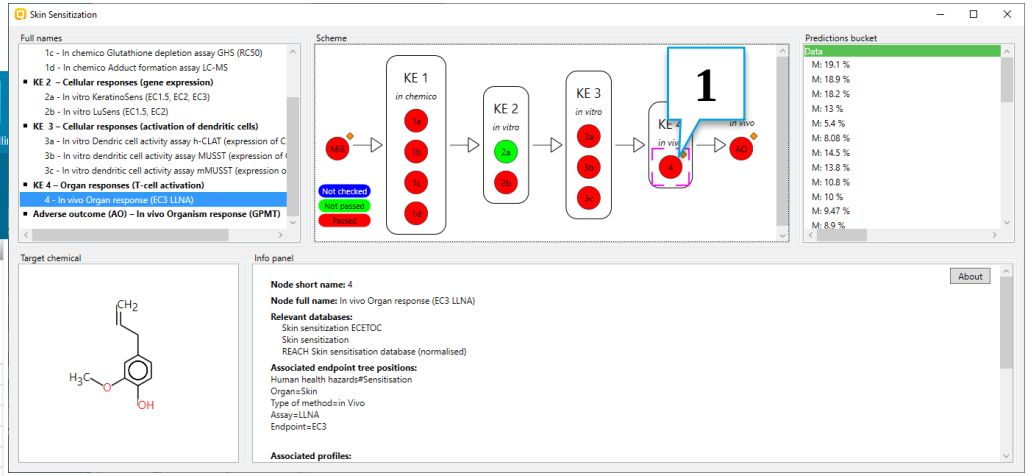
Workflow process

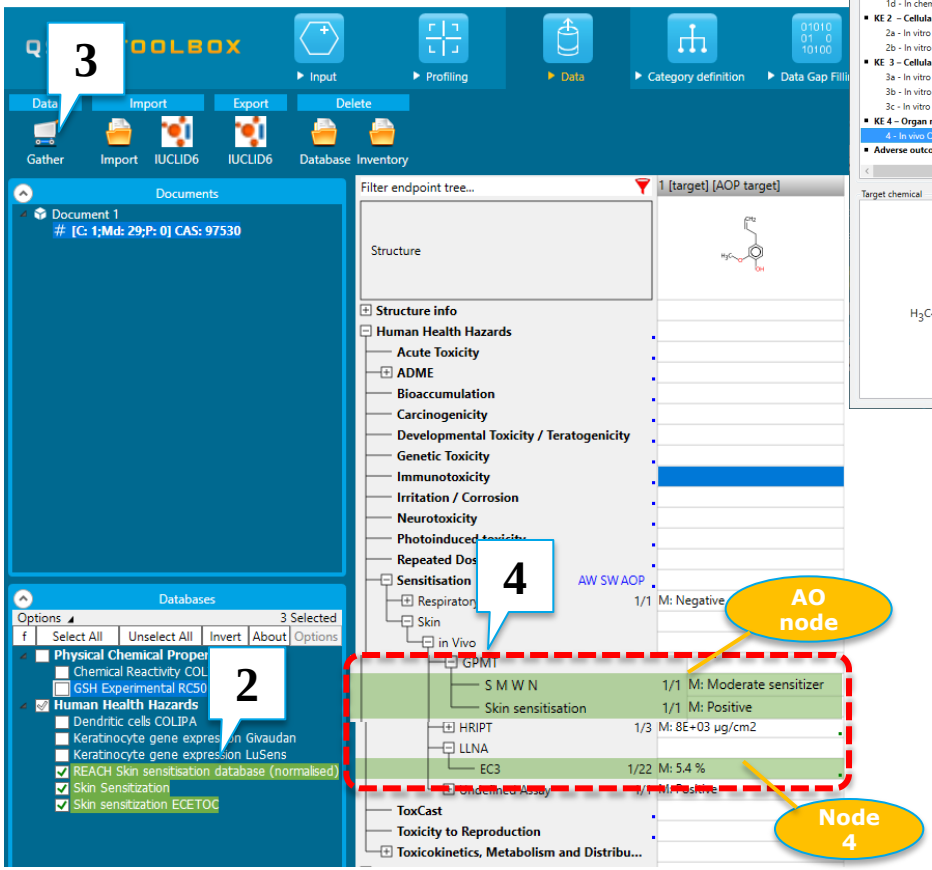
Step 5. *In vivo* Organ response (LLNA)(node 4)

Example 2



KE4, node 4: Check are there any data for the target chemical for the in vivo Organ response (LLNA) assay



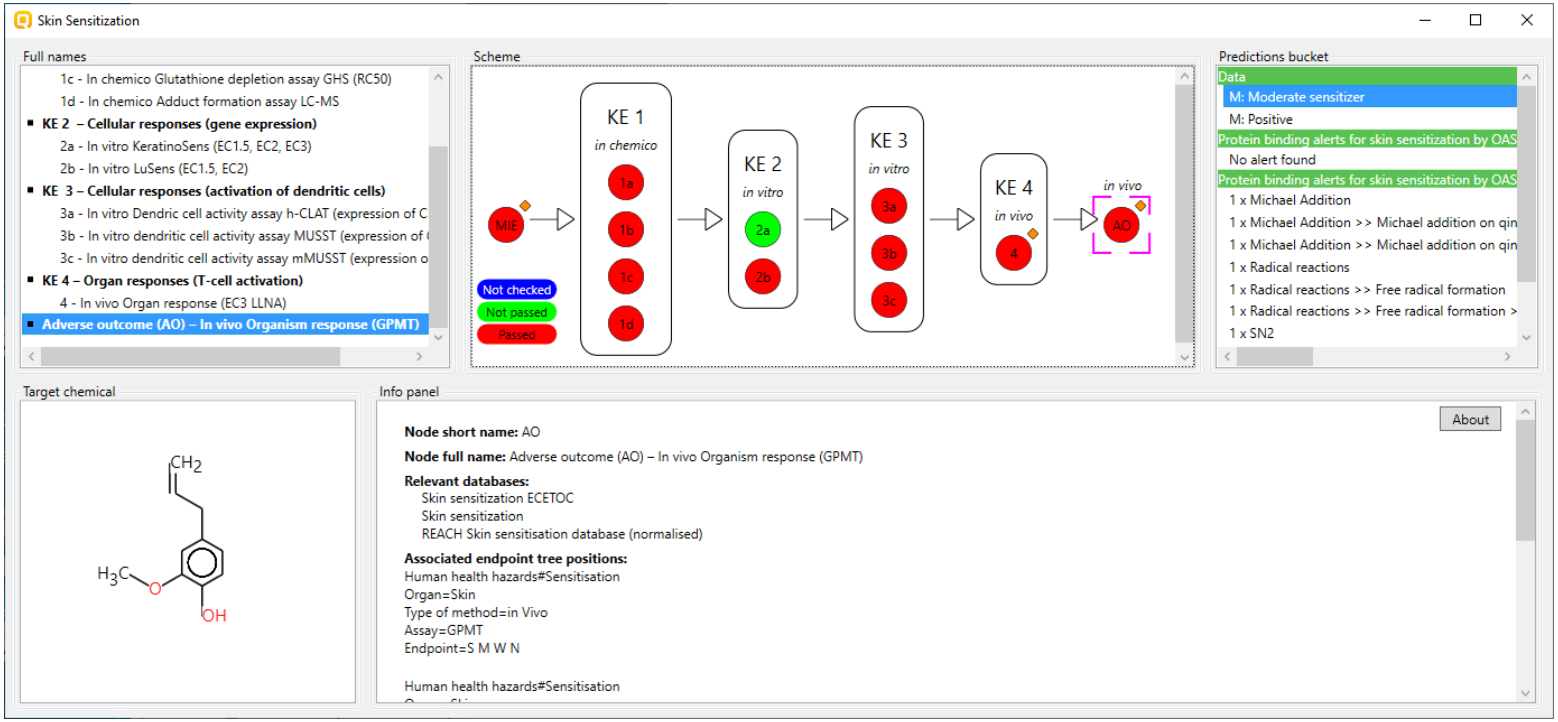


1. **Select** node 4
2. **Select** databases related to the node 4
3. **Click** Gather
4. Nodes 4 and AO are getting "passed" based on experimental data extracted for the target chemical

Workflow process

Status of nodes 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 positive experimental data found 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.