

## 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

# Outlook

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

# 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*

# Outlook

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

# Outlook

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



# Overview of the AOP scheme as implemented in Toolbox

## Details of AOP window

The screenshot displays the AOP window in the QSAR Toolbox software, which is used for grouping chemicals into categories based on their adverse outcome pathways (AOPs). The window is divided into several panels, each with a specific function:

- Panel with full names of nodes:** Located on the left, it lists the full names of the nodes in the AOP scheme. The nodes are organized into three main categories:
  - Molecular initiating event (MIE) – Protein binding alerts:**
    - Key event (KE 1) – Molecular interactions:**
      - 1a - In chemico peptide depletion assay DPRA (Cys)
      - 1b - In chemico peptide depletion assay DPRA (Lys)
      - 1c - In chemico Glutathione depletion assay GHS (RC50)
      - 1d - In chemico Adduct formation assay LC-MS
    - KE 2 – Cellular responses (gene expression):**
      - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
      - 2b - In vitro LuSens (EC1.5, EC2)
    - KE 3 – Cellular responses (activation of dendritic cells):**
      - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
      - 3b - In vitro dendritic cell activity assay MUSST (expression of CD86)
      - 3c - In vitro dendritic cell activity assay mMUSST (expression of CD86)
- AOP tree scheme:** Located in the center, it shows the AOP tree scheme. The nodes are represented by colored circles (blue for 'Not checked', green for 'Not passed', and red for 'Passed'). The nodes are connected by arrows, indicating the flow of the AOP. The nodes are labeled: KE 1 (in chemico), KE 2 (in vitro), KE 3 (in vitro), KE 4 (in vivo), and AO (in vivo).
- Indication for assigned prediction:** Located at the top right, it shows the indication for assigned prediction. The nodes are color-coded: blue for 'Not checked', green for 'Not passed', and red for 'Passed'.
- Panel with predictions/measured data assigned to the selected node:** Located on the right, it shows the predictions bucket. The predictions are listed in a table:
 

Predictions bucket
Protein binding alerts for skin sensitization according to OECD
No alert found
Protein binding alerts for skin sensitization by OASIS
No alert found
Protein binding alerts for skin sensitization according to OECD
6 x No alert found
3 x Skin sensitization Category 1B
2 x Skin sensitization Category 1B >> Aldehydes
1 x Skin sensitization Category 1B >> C-Nitroso compounds
Protein binding alerts for skin sensitization by OASIS
8 x No alert found
1 x Nucleophilic addition
1 x Nucleophilic addition >> Nucleophilic addition
- Color legend:** Located at the bottom center, it shows the color legend for the nodes. The colors are: blue for 'Not checked', green for 'Not passed', and red for 'Passed'.
- Panel with information for selected node:** Located at the bottom left, it shows the information for the selected node. The information includes:
  - Node short name:** MIE
  - Node full name:** Molecular initiating event (MIE)
  - Associated profiles:**
    - Protein binding by OASIS
    - Protein binding alerts for skin sensitization by OASIS
    - Protein binding alerts for skin sensitization according to GHS
    - Protein binding by OECD
  - Associated simulators:**
    - Autoxidation simulator
    - Skin metabolism simulator
  - Thresholds:**
    - Profile 'Protein binding alerts for skin sensitization by OASIS'
    - Passed:** Any of the profiler categories
    - Not passed:** No alert found
- Target chemical:** Located at the bottom left, it shows the chemical structure of the target chemical. The chemical structure is a benzene ring with a methyl group (CH<sub>3</sub>) and an amino group (NH<sub>2</sub>).
- Short description:** Located at the bottom right, it shows the short description of the AOP. The description is:
 

The adverse outcome pathway (AOP) for skin sensitization elicited by covalent binding to proteins

An adverse outcome pathway (AOP) is the sequence of events from the chemical structure of a target chemical or group of similar chemicals through the molecular initiating event to an in vivo outcome of interest. Each AOP represents the existing knowledge concerning the linkage(s) between a molecular initiating event, intermediate events and an adverse outcome at the individual or population level.

Knowledge of the AOP for skin sensitization elicited by covalent binding to proteins, especially thiol (-SH) and primary amine (-NH<sub>2</sub>) moieties has evolved rapidly. The AOP for skin sensitization is summarized as eleven steps which include four events that are recognized in the OECD AOP framework.

The molecular initiating event is the molecular interaction with skin proteins, the site of action. The first key event is related to covalent binding of target or a metabolite/abiotic transformation product of the target chemical to cysteine and/or lysine residues. In addition two in chemico assays associated with Glutathione depletion (GHS (RC50)) and adduct formation (LC-MS) are part of the first key event. The second key event takes place in the keratinocyte. This includes gene expression associated with particular cell signaling pathways (e.g. antioxidant/electrophile response element-dependent pathways). The third key event is activation of dendritic cells which is typically assessed by expression of specific cell surface markers, chemokines and cytokines. The final key event intermediate event is T-cell proliferation, which is indirectly measured in the murine Local Lymph Node Assay.

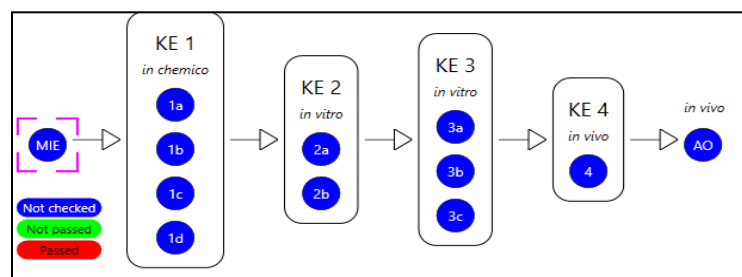
Methods and databases for the quantification of protein-binding are further in development than for other key events. Among the other key events, keratinocyte responses, especially via the Keap1/Nrf2/ARE/EpRE cell signaling path (i.e. KeratinoSens) and dendritic cell activation (i.e. Myleoid U-937 Skin Sensitization Test (MUSST), modified MUSST (mMUSST) and human Cell Line Activation Test (h-CLAT)) have the most robust databases.

The implementation of the Skin Sensitization AOP module in this version of the OECD Toolbox was supported by the 2015 Lush Black Box Prize awarded to Prof. Terry W. Schultz.

# 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

# AOP workflow for skin sensitization



## Key node

**MIE**

Protein binding alerts

**1a**

Peptide depletion assay DPRA (Cys)

**1b**

Peptide depletion assay DPRA (Lys)

**1c**

Glutathione depletion assay GSH (RC50)

**1d**

Adduct formation assay LC-MS

**KE1**

**2a**

KeratinoSens assay (EC1.5, EC2, EC3)

**2b**

LuSens (EC1.5, EC2, EC3)

**3a**

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

**3b**

Dendritic cell activity assay MUSST (expression of CD86)

**3c**

Dendritic cell activity assay mMUSST (expression of CD86)

**KE3**

**4**

Organ response (EC3 LLNA)

**AO**

Organism response (GPMT)

**KE4**

## Key event

Protein binding – in silico/theoretical

Protein binding potency in chemico

Cellular response (gene expression)

Cellular response (activation of dendritic cells)

Organ response

Organism response

# Outlook

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

# Overview of the AOP scheme as implemented in Toolbox

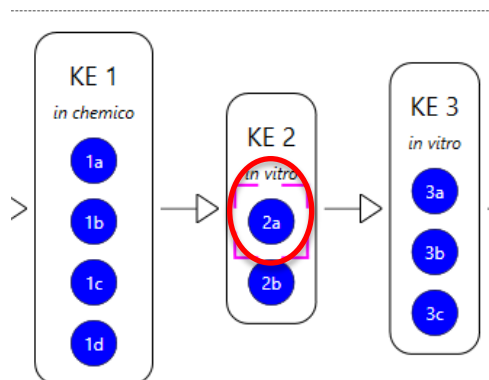
## Implemented thresholds for the AOP nodes

- Thresholds are implemented for each AOP node
- Each threshold is 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 <i>in chemico</i> DPRA Cys and Lys	Peptide depletion, PD (%): PD > 9 - Passed PD ≤ 9% - Not passed	> 9 % - Passed	≤ 9 % - Not passed
1c - <i>in chemico</i> Glutathione depletion assay GSH (RC50)	RC50 (mmol/L) ≤ 0.099 – Extremely reactive 0.1 ≥ RC50 ≤ 0.99 – Highly reactive 1 ≥ RC50 ≤ 15 – Moderately reactive 16 ≥ RC50 ≤ 70 – Slightly reactive 70.1 ≥ RC50 ≤ 135 – Suspect RC50 > 135 – Not reactive	Extremely Reactive   Highly Reactive   Moderately Reactive   Slightly Reactive	Suspect   Not Reactive   Not reactive at saturation
1d - <i>in chemico</i> Adduct formation assay LC-MS	Adduct formation (%) ≥ 30% - Positive Adduct formation (%) < 30% - Negative	Positive	Negative
2a - <i>in vitro</i> Keratinocyte (EC1.5, EC2, EC3)  AND  2b – <i>in vitro</i> LuSens (EC1.5, EC2)	EC3 (%) ≤ 20 – Very High 20 > EC3 ≤ 50 – High 50 > EC3 ≤ 100 – Moderate 100 > EC3 ≤ 2000 – Low EC3 > 2000 - Negative	Very High   High   Moderate   Low	Negative
3a;3b and 3c <i>in vitro</i> Dendritic cell activity assay h-CLAT; MUSST and mMUSST (expression of CD54 and CD86)	expression of CD54 and CD86 Positive Negative	Positive	Negative
4 - <i>in vivo</i> Organ response (LLNA)	0 ≥ EC3 (%) < 50 – Positive EC3 ≥ 50 – Negative  Or	Positive	Negative
AO - <i>in vivo</i> Organism response (GPMT)	Data provided: Strong sensitizer; Moderate sensitizer; Weak sensitizer; Non sensitizer	Strong sensitizer   Moderate sensitizer	Weak sensitizer   Non sensitizer

# Outlook

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

# 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#

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes options like Document, Single Chemical, Chemical List, Search, and Target Endpoint. Below this is a toolbar with various icons. The 'CAS#' icon, which shows a chemical structure with a hash symbol, is highlighted with a red rectangular box. A white box with the number '1' is positioned above the red box, with a line pointing to the callout box. The callout box contains the following text:

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

At the bottom of the interface, a blue box contains the instruction: **1. Click on CAS#**

# Chemical Input

## Enter CAS# 107-75-5

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

Search by CAS #

107755 Search OK Cancel

Select All Unselect All Invert Selection Selected 1 of 3

<input checked="" type="checkbox"/>	1	CAS	107-75-5	SMILES	<chem>CC(CCCC(C)(C)O)CC=O</chem>	CS Relation	High	Substance	Mono constituent	Composition		Name	3,7-Dimethyl-7-hydroxy-octanal, 7-H	Sources	NICNAS Canada DSI	
<input type="checkbox"/>	2	CAS	107-75-5	SMILES	<chem>CC(C)(O)CCCC(C)(C)CC=O</chem>	CS Relation	Low	Substance	Mono constituent	Composition		Name	3,7-Dimethyl-7-hydroxyoctanal	Sources	GSH Experimental RC50	
<input type="checkbox"/>	3	CAS	107-75-5	SMILES	<chem>C[C@H](CCCC(C)(C)O)CC=O</chem>	CS Relation	Low	Substance	Mono constituent	Composition		Name	Hydroxycitronellal	Sources		

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

The screenshot shows the QSAR Toolbox software interface. The 'Target Endpoint' tab is active, displaying a list of chemical identifiers for a target compound. A red circle highlights the 'Structure info' section, which includes the following information:

- EC Number: 2035187
- CAS Number: 107-75-5
- CAS-SMILES relation: High
- Chemical name(s): 3,7-Dimethyl-7-hydroxy-octanal
- Composition: C10H20O2
- Predefined substance type: Mono constituent
- SMILES: CC(C)CC(C)(O)CC=O

The interface also shows a 'Documents' panel on the left with 'Document 1' and its CAS number (107755). The top menu bar includes options like 'Document', 'Single Chemical', 'Chemical List', 'Search', and 'Target Endpoint'.

# 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

Filter endpoint tree... 1 [target]

Structure

Structure info

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

Parameters

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

EC Number: 2035187

107-75-5

High

3,7-Dimethyl-7-hydroxy-octanal

C10H20O2

Mono constituent

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

107755 / CC(CCCC(C)(C)O)CC=O

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

OK

1. Double click on the **"High"** CAS-SMILES Relation;
2. Click **OK**.

2

# Chemical Input

## Target chemical identity

The code indicates the reliability of the chemical identifier:

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

# Outlook

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

# Activate AOP

The screenshot shows the QSAR Toolbox interface. On the left, the 'Filter endpoint tree...' panel is expanded to 'Sensitisation'. A red circle highlights the 'Sensitisation' node, and a blue circle highlights the 'AOP' label. A right-click context menu is open, with 'Activate AOP' highlighted. On the right, the 'Skin Sensitization' window displays a scheme with four key events (KE 1 to KE 4) and their associated assays. The 'MIE' node is highlighted with a red box. The 'Info panel' on the right shows details for the 'MIE' node, including its full name, associated profiles, simulators, and thresholds.

1. Expand the endpoint tree to the **"Sensitisation"** node

2. Right click near the **AOP** label

3. Select **"Activate AOP"**

4. AOP window appears

Continued on the next slide



# Activate AOP

**1**

**target chemical**

CC(C)CC(=O)C

**Full names**

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

**Scheme**

MIE (Not checked, Not passed, Passed) → KE 1 (in chemico) → KE 2 (in vitro) → KE 3 (in vitro) → KE 4 (in vivo) → AO (in vivo)

**Info panel**

**Node short name:** MIE

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

**Associated profiles:**

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

**Associated simulators:**

- Autoxidation simulator
- Skin metabolism simulator

**Thresholds:**

- Profile 'Protein binding alerts for skin sensitization by OASIS'
- Passed:** Any of the profiler categories

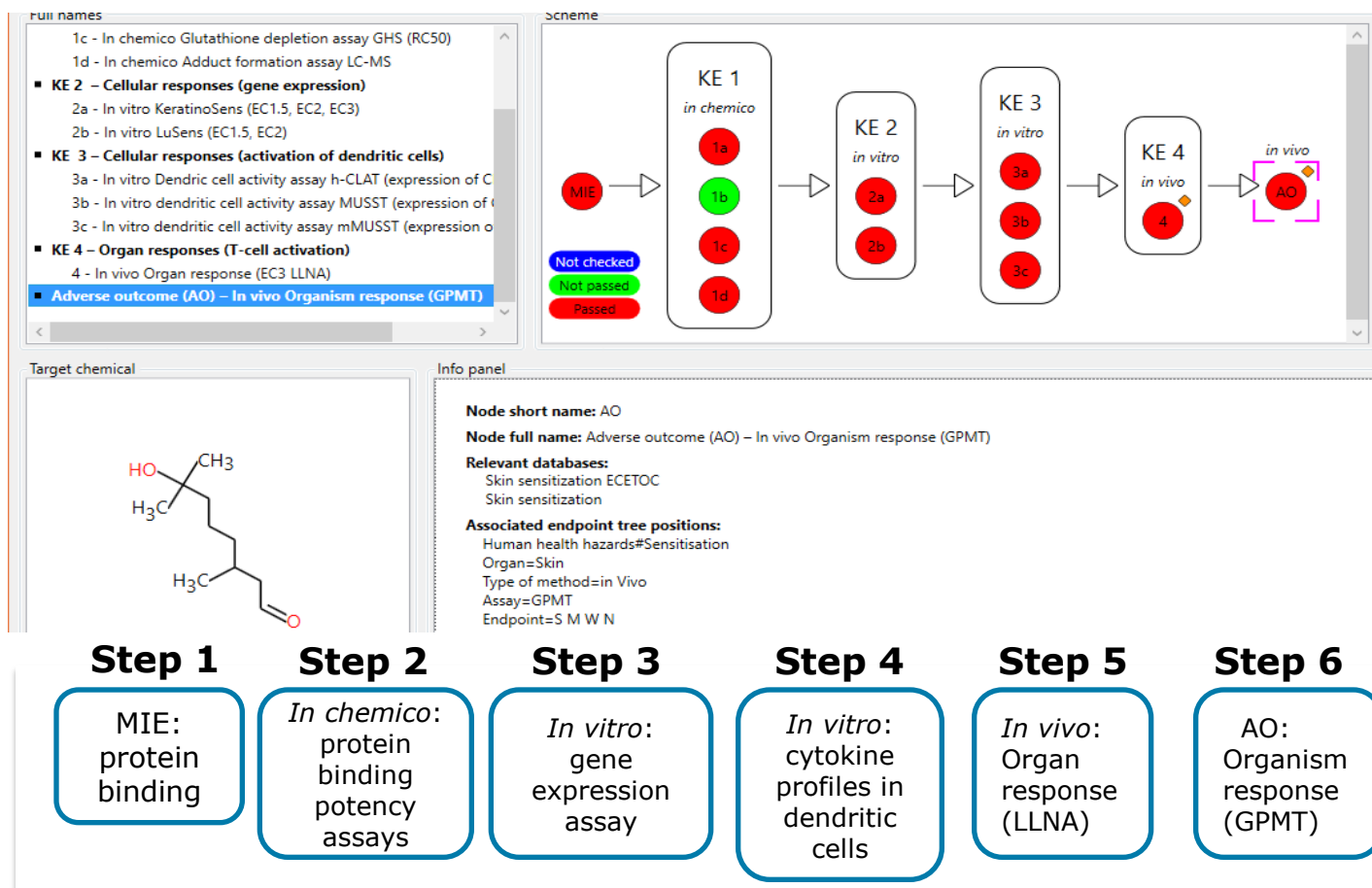
1. The target chemical automatically appears in the AOP window. The AOP is ready to be executed.

# Outlook

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

# Workflow process

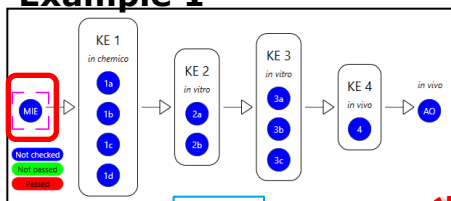
- Workflow process starts from molecular initiating event to the *in vivo* organism response. The forthcoming slides illustrate the sequence of steps for assessment of each of the nodes



# Workflow process

## Step 1. MIE: protein binding

### Example 1



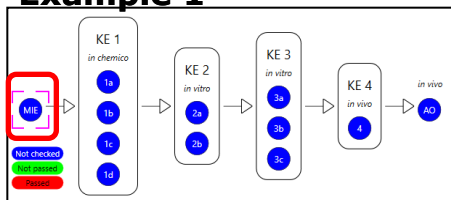
MIE: Start with profiling of target chemical

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

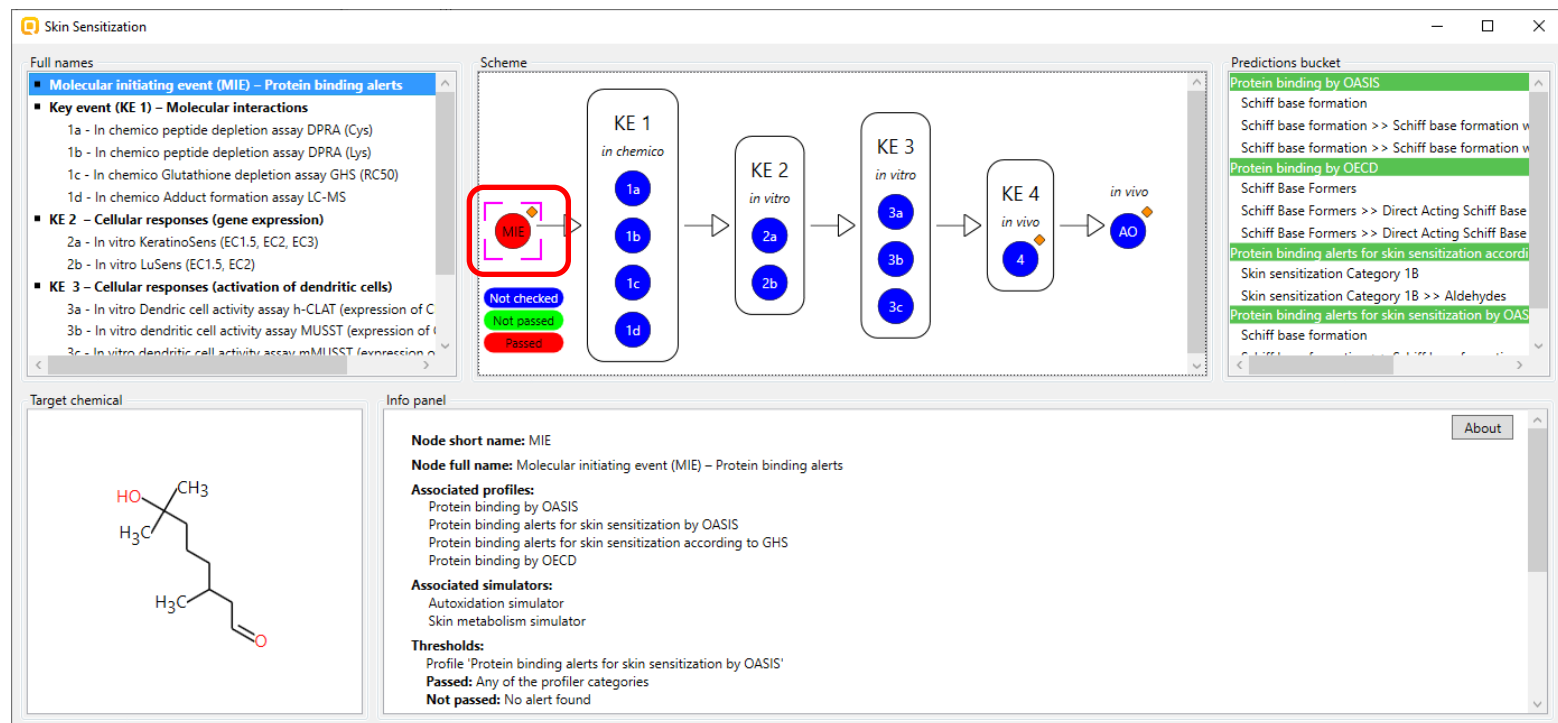
**2** The node MIE is automatically changed to passed based on the profiling outcome results and implemented thresholds (see slide #14).

**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

**5** View node details

**Node info for Node 1a:**

- Node short name:** 1a
- Node full name:** In chemico peptide depletion assay DPRA (Cys)
- Relevant databases:** Chemical Reactivity COLIPA
- Associated endpoint tree positions:** Physical Chemical Properties#Chemical reactivity Assay=DPRA Endpoint=% depletion of Cysteine
- Associated profiles:** Protein binding potency Cys (DPRA 13%)
- Associated simulators:** Autoxidation simulator

**Predictions bucket:**

- Protein binding potency Cys (DPRA 13%)
- DPRA above 21% (DPRA 13%)
- DPRA above 21% (DPRA 13%) >> Non-Conjugated
- DPRA less than 9% (DPRA 13%)
- DPRA less than 9% (DPRA 13%) >> Alcohols
- Grey zone 9-21% (DPRA 13%)
- Grey zone 9-21% (DPRA 13%) >> Non-Conjugated

**Profilers:**

- ☒ Protein binding by OASIS
- ☒ Protein binding potency Cys (DPRA 13%)
- ☒ Protein binding potency GSH
- ☒ Protein binding potency Lys (DPRA 13%)

# Workflow process

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

### Example 1

**KE1, node 1a: Gather data for target chemical**

The screenshot shows the QSAR Toolbox interface with several components highlighted by numbered callouts (1-6):

- 1:** Node 1a in the Scheme diagram is selected.
- 2:** The 'Data' module is selected in the top navigation bar.
- 3:** The 'Physical Chemical Properties' and 'Chemical reactivity COLIPA' databases are selected in the 'Databases' list.
- 4:** The 'Gather' button is clicked in the 'Documents' list.
- 5:** The 'Chemical reactivity' and 'DPRA' endpoints are selected in the 'Filter endpoint tree'.
- 6:** The 'Scheme' diagram shows the workflow from KE1 to KE4, with node 1a highlighted.

The 'Predictions bucket' on the right shows the following results:

- Data
- M: 17.5 %
- Protein binding potency Cys (DPRA 13%)
- DPRA above 21% (DPRA 13%)
- DPRA above 21% (DPRA 13%) >> Non-Conjugated
- DPRA less than 9% (DPRA 13%)
- DPRA less than 9% (DPRA 13%) >> Alcohols
- Grey zone 9-21% (DPRA 13%)
- Grey zone 9-21% (DPRA 13%) >> Non-Conjugated

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**".

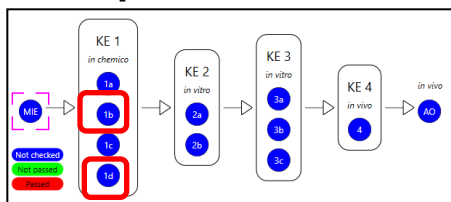
6. Moreover node **1b** and **1d** automatically changed their status based data found for the target and implemented thresholds (see next slide)



# 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

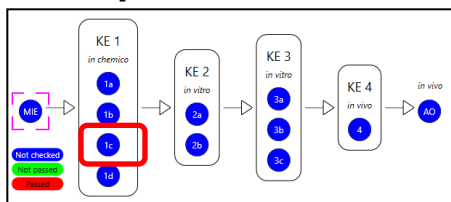
1. Select **node 1b**. Data is found for the target chemical. The node is "not passed" due to implemented data thresholds (see slide 14)
2. Select **node 1d**. Data is found for the target chemical. The node is "passed" due to implemented data thresholds (see slide 14)

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**

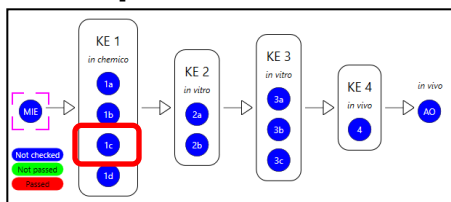
3. Click **Gather**.

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



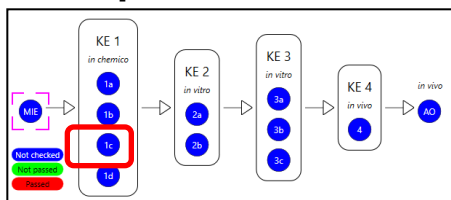
**KE1, node 1c:** The category of similar analogue should be investigated.

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



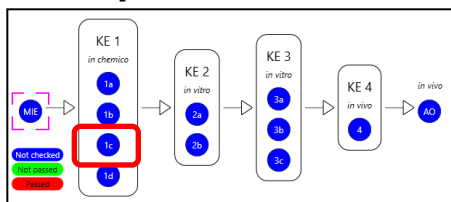
**KE1, node 1c:** In this case we should investigate the category by Protein binding alerts for SS. The reason for this is that GHS RC50 depends on mechanism of protein binding interaction

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. There are 100 data point for 57 chemicals. Click **OK**

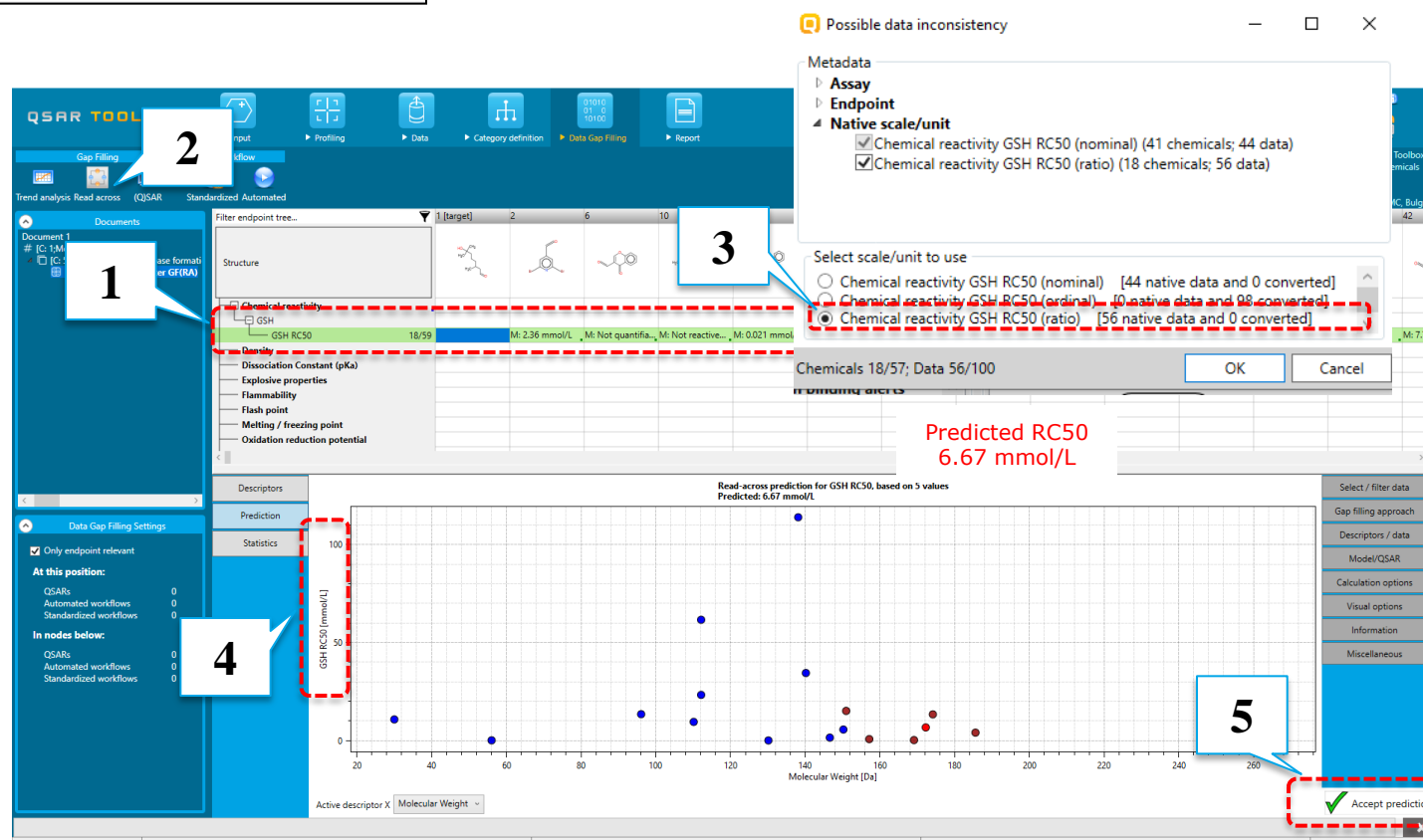
# Workflow process

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)

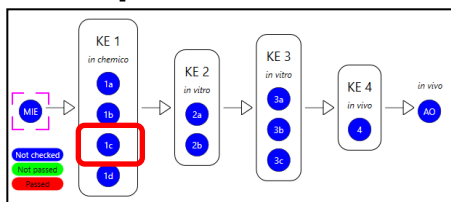
## Data thresholds

- $RC_{50} \text{ (mmol/L)} \leq 0.099$  – Extremely reactive
- $0.1 \geq RC_{50} \leq 0.99$  – Highly reactive
- $1 \geq RC_{50} \leq 15$  – Moderately reactive
- $16 \geq RC_{50} \leq 70$  – Slightly reactive
- $70.1 \geq RC_{50} \leq 135$  – Suspect
- $RC_{50} > 135$  – 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

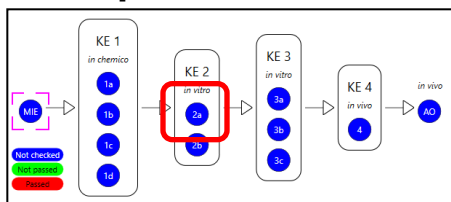


- The nodes related to three of the *in chemico* assays are “Passed” due to positive experimental data found for the target chemical (node 1a and 1d) and the positive read-across prediction for the target based on experimental data found for analogues (node 1c).
- Only one of all *in chemico related* 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

1. Select **node 2a**

The screenshot displays the 'Skin Sensitization' workflow interface. The 'Full names' panel on the left lists various key events (KE) and their associated assays. Node 2a, 'In vitro KeratinoSens (EC1.5, EC2, EC3)', is selected and highlighted in blue. The 'Scheme' panel on the right shows the workflow diagram, with node 2a highlighted in a red box and a callout '1' pointing to it. The 'Info panel' at the bottom right provides detailed information about the selected node, including its short name, full name, relevant databases, and associated endpoint tree positions.

**Full names**

- Molecular initiating event (MIE) – Protein binding alerts
- Key event (KE 1) – Molecular interactions
  - 1a - In chemico peptide depletion assay DPRA (Cys)
  - 1b - In chemico peptide depletion assay DPRA (Lys)
  - 1c - In chemico Glutathione depletion assay GHS (RC50)
  - 1d - In chemico Adduct formation assay LC-MS
- KE 2 – Cellular responses (gene expression)
  - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
  - 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 – Cellular responses (activation of dendritic cells)
  - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of CCR4)
  - 3b - In vitro dendritic cell activity assay MUSST (expression of CD86)
  - 3c - In vitro dendritic cell activity assay mMUSST (expression of CD86)

**Target chemical**

Chemical structure: CC(C)CC(C)(O)C=O

**Info panel**

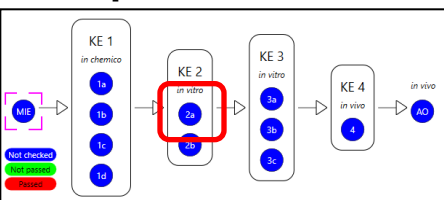
**Node short name:** 2a  
**Node full name:** In vitro KeratinoSens (EC1.5, EC2, EC3)  
**Relevant databases:** Keratinocyte gene expression Givaudan  
**Associated endpoint tree positions:** Human health hazards#Sensitisation  
 Organ=Skin  
 Type of method=in Vitro  
 Assay=KeratinoSens  
 Endpoint=EC1.5



# 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

**6** Gather

**2** Documents

**3** Data

**4** Options

**5** Human Health Hazards

**7** KeratinoSens

**1** KE 2

**8** Node 2a

**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**

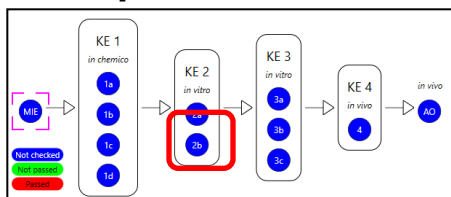
**7.** The experimental data appears on Data matrix

**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

The screenshot shows the QSAR Toolbox interface. The top menu bar includes 'Data', 'Export', 'Delete', 'Input', 'Profiling', and 'Gap Filling'. The 'Data' module is selected, and the 'Gather' button is highlighted with a red box and a callout '5'. The 'Documents' panel on the left shows a list of documents, with 'Document 1' selected. The 'Filter endpoint tree...' panel on the right shows a list of endpoints, with 'LuSens' selected and highlighted with a red box and a callout '6'. The 'Data matrix' panel at the bottom shows a table with columns for 'Structure', 'Parameters', 'Physical Chemical Properties', 'Environmental Fate and Transport', 'Ecotoxicological Information', 'Human Health Hazards', 'Acute Toxicity', 'ADME', 'Bioaccumulation', 'Carcinogenicity', 'Developmental Toxicity / Teratogenicity', 'Genetic Toxicity', 'Immunotoxicity', 'Irritation / Corrosion', 'Neurotoxicity', 'Photoinduced toxicity', 'Repeated Dose Toxicity', 'Sensitisation', 'Skin', 'In vitro', 'LuSens', 'EC1.5', 'EC2', 'ToxCast', 'Toxicity to Reproduction', 'Toxicokinetics, Metabolism and Distribution', and 'Profiling'. The 'LuSens' endpoint is highlighted with a red box and a callout '6'.

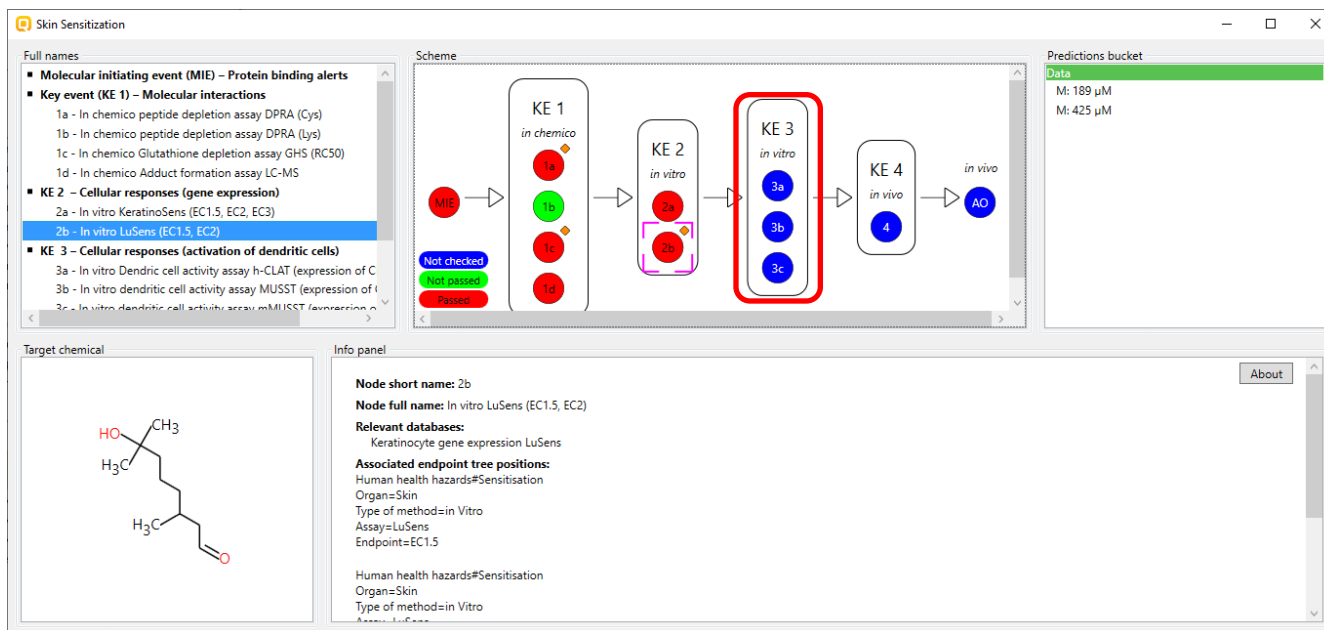
The screenshot shows the 'Skin Sensitization' module. The 'Full names' panel on the left lists the workflow process, with 'KE 2 - Cellular responses (gene expression)' selected and highlighted with a red box and a callout '7'. The 'Scheme' panel on the right shows a diagram of the workflow process, with node 2b highlighted with a red box and a callout '7'. The 'Predictions bucket' panel on the far right shows a list of predictions, with 'M: 189 µM' and 'M: 425 µM' listed.

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

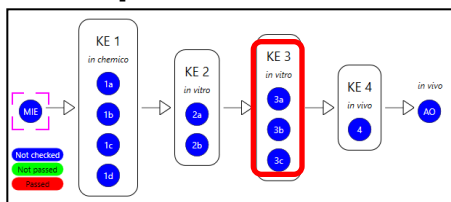
## Status of nodes in vitro KeratinoSens and LuSens assays

### Example 1

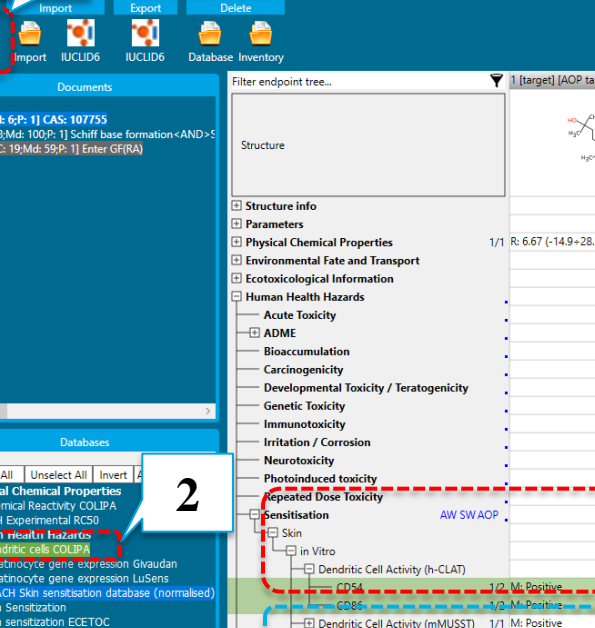


- The both nodes related to *in vitro* assays are “Passed” due to positive experimental data found for the target chemical and implemented thresholds (slide #14)
- The workflow should move further to the other *in vitro* assays (KE3, nodes 3a, 3b and 3c)

### 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



QSA BOX

1

Input Profiling Data Category definition Data Gap Filling

Gather Import IUCLID6 IUCLID6 Database Inventory

Documents

Document 1

# [C: 1-Md: 6-P: 1] CAS: 107755

[C: 58-Md: 100-P: 1] Schiff base formation<AND>S

[C: 19-Md: 59-P: 1] Enter GF(RA)

Filter endpoint tree...

1 [target] [AOP target]

Structure

Structure info

Parameters

Physical Chemical Properties

Environmental Fate and Transport

Ecotoxicological Information

Human Health Hazards

Acute Toxicity

ADME

Bioaccumulation

Carcinogenicity

Developmental Toxicity / Teratogenicity

Genetic Toxicity

Immunotoxicity

Irritation / Corrosion

Neurotoxicity

Photoinduced toxicity

Repeated Dose Toxicity

Sensitisation

Skin

in Vitro

Dendritic Cell Activity (h-CLAT)

CD54

CD85

Dendritic Cell Activity (mMUSST)

Dendritic Cell Activity (MUSST)

ToxCast

Toxicity to Reproduction

Toxicokinetics, Metabolism and Distribution

Profiling

1/1 R: 6.67 (-14.9+28.2) mmol/L

AW SW AOP

J2: M: Positive

J3: M: Positive

1/1 M: Positive

1/1 M: Positive

**Full names**

- 1b - In chemico peptide depletion assay DPRA (lys)
- 1c - In chemico Glutathione depletion assay GHS (RC50)
- 1d - In chemico Adduct formation assay LC-MS
- **KE 2 – Cellular responses (gene expression)**
  - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
  - 2b - In vitro LuSens (EC1.5, EC2)
- **KE 3 – Cellular responses (activation of dendritic cells)**
  - 3a - In vitro Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)
  - 3b - In vitro dendritic cell activity assay mMUSST (expression of CD54 and CD86)
  - 3c - In vitro dendritic cell activity assay mMUSST (expression of CD54 and CD86)
- **KE 4 – Organ responses (T-cell activation)**
  - 4 - In vivo Organ response (EC3 LLNA)
- **Adverse outcome (AO) - In vivo Organ response (GDMT)**

**Scheme**

Flowchart illustrating the assay process:

- Node 1: In chemico (KE 1) - Not checked
- Node 2: In vitro (KE 2) - Not checked
- Node 3: In vitro (KE 3) - Not checked
- Node 4: In vitro (KE 4) - Not checked
- Node 5: In vitro (KE 3) - Not checked
- Node 6: In vitro (KE 3) - Not checked
- Node 7: In vitro (KE 3) - Not checked
- Node 8: In vivo (AO) - Not checked

**Predictions bucket**

Predictions
M: Positive
M: Positive
M: Positive
M: Positive

**Target chemical**

Chemical structure: CC(C)(C)CC(C)CC(=O)O

**Info panel**

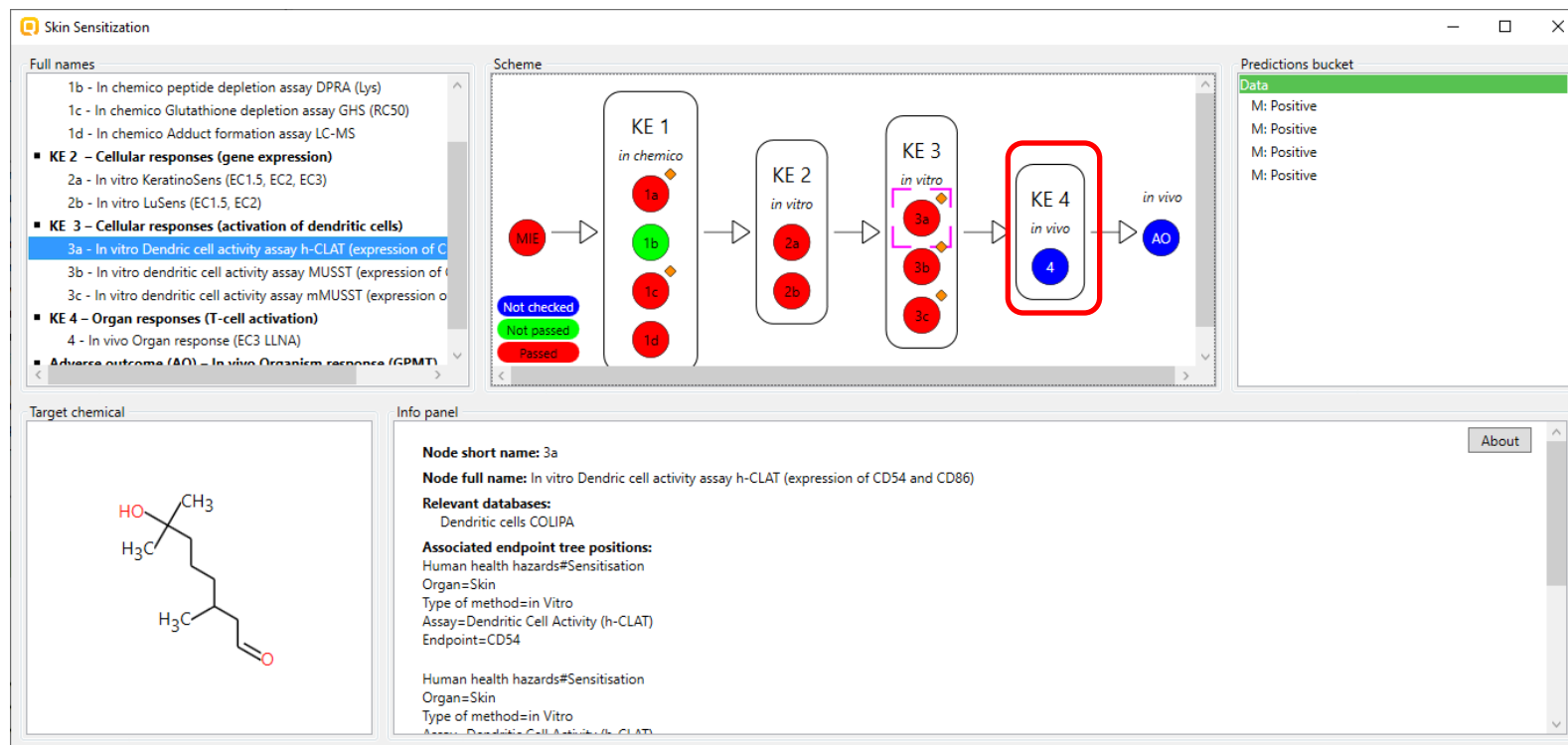
**Node short name:** 3a  
**Node full name:** In vitro Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)  
**Relevant databases:** Dendritic cells COLIPA  
**Associated endpoint tree positions:** Human health hazards#Sensitisation Organ#Skin Type of method#in Vitro Assays#Dendritic Cell Activity (h-CLAT) Endpoint#CD54  
 Human health hazards#Sensitisation Organ#Skin Type of method#in Vitro Assays#Dendritic Cell Activity (h-CLAT) Endpoint#CD54

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

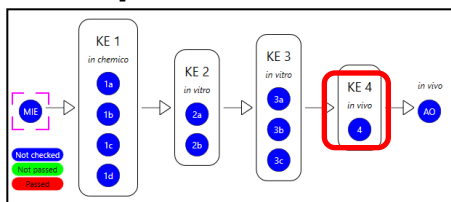


- The status of nodes 3a, 3b and 3c related to the *in vitro* Dendritic cell activity assay (h-CLAT, mMUSST and MUSST) are “passed” due to positive experimental data found for the target chemical
- The workflow moves further to the *in vivo* LLNA assay (KE4, node 4)

# 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)

3

2

4

1

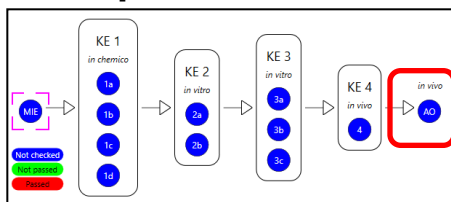
5

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).

The screenshot shows the QSAR Toolbox software interface. The 'Documents' panel on the left shows a document titled 'Document 1' with a list of endpoints. The 'Databases' panel on the right shows a list of databases, with 'Skin Sensitization ECETOC' highlighted. The 'Endpoints' panel on the right shows a list of endpoints, with 'Adverse outcome (AO) - In vivo Organism response (GPMT)' highlighted. The 'Structure' panel on the right shows the chemical structure of the target chemical, which is a branched alkane with a hydroxyl group and a carbonyl group.

The screenshot shows the 'Skin Sensitization' window. The 'Full names' panel on the left lists the endpoints, with 'Adverse outcome (AO) - In vivo Organism response (GPMT)' highlighted. The 'Scheme' panel on the right shows the workflow process, with the final node 'AO' highlighted. The 'Info panel' on the right shows the details of the selected node, including the node name, full name, relevant databases, and associated endpoint tree positions.

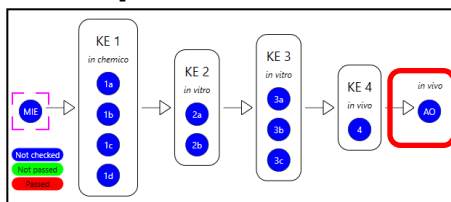
1. Select **node AO**
2. Click **Unselect all** and select **highlighted database (they are same as in the previous step 5: node 4)**
3. Click **Gather**
4. No data for the target endpoint (GPMT assay) is found. Data is available but for other endpoints. Therefore next step is to make read-across for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

The screenshot shows the QSAR Toolbox software interface during the 'Category definition' module. The interface includes a 'Full names' list, a 'Scheme' diagram, a 'Target chemical' structure, and an 'Info panel'.

**Full names:**

- 1c - In chemico Glutathione depletion assay GHS
- 1d - In chemico Adduct formation assay LC-MS
- KE 2 - Cellular responses (gene expression)
  - 2a - In vitro KeratinoSens (EC1.5, EC2, EC3)
  - 2b - In vitro LuSens (EC1.5, EC2)
- KE 3 - Cellular responses (activation of dendritic)
  - 3a - In vitro Dendritic cell activity assay h-CLAT (ex)
  - 3b - In vitro dendritic cell activity assay MUSST (ex)
  - 3c - In vitro dendritic cell activity assay mMUSST (ex)
- KE 4 - Organ responses (T-cell activation)
  - 4 - In vivo Organ response (EC3 LLNA)
- Adverse outcome (AO) - In vivo Organism response (GPMT)

**Scheme:**

The scheme diagram shows a sequence of nodes: KE 1 (in chemico), KE 2 (in vitro), KE 3 (in vitro), KE 4 (in vivo), and AO (in vivo). The AO node is highlighted with a red box.

**Target chemical:**

The target chemical structure is shown as a chemical structure diagram.

**Info panel:**

**Node short name:** AO  
**Node full name:** Adverse outcome (AO) - In vivo Organism response (GPMT)  
**Relevant databases:**  
 Skin sensitization ECETOC  
 Skin sensitization  
 REACH Skin sensitisation database (normalised)  
**Associated endpoint tree positions:**  
 Human health hazards#Sensitisation  
 Organ=Skin  
 Type of method=In Vivo  
 Assay=GPMT  
 Endpoint=S M W N  
 Human health hazards#Sensitisation

**Options:**

**Target categories:**  
 Schiff base formation  
 Schiff base formation >> Schiff base formation with carbonyl compounds  
 Schiff base formation >> Schiff base formation with carbonyl compounds >> A

**Options:**  
 Down Up Reset Opt

**All categories (N/A)**

**Combine profiles:**  
☐ AND ☐ OR  
☐ Invert result  
☐ Strict  
☐ Sort results

**OK**

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)

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

### Example 1

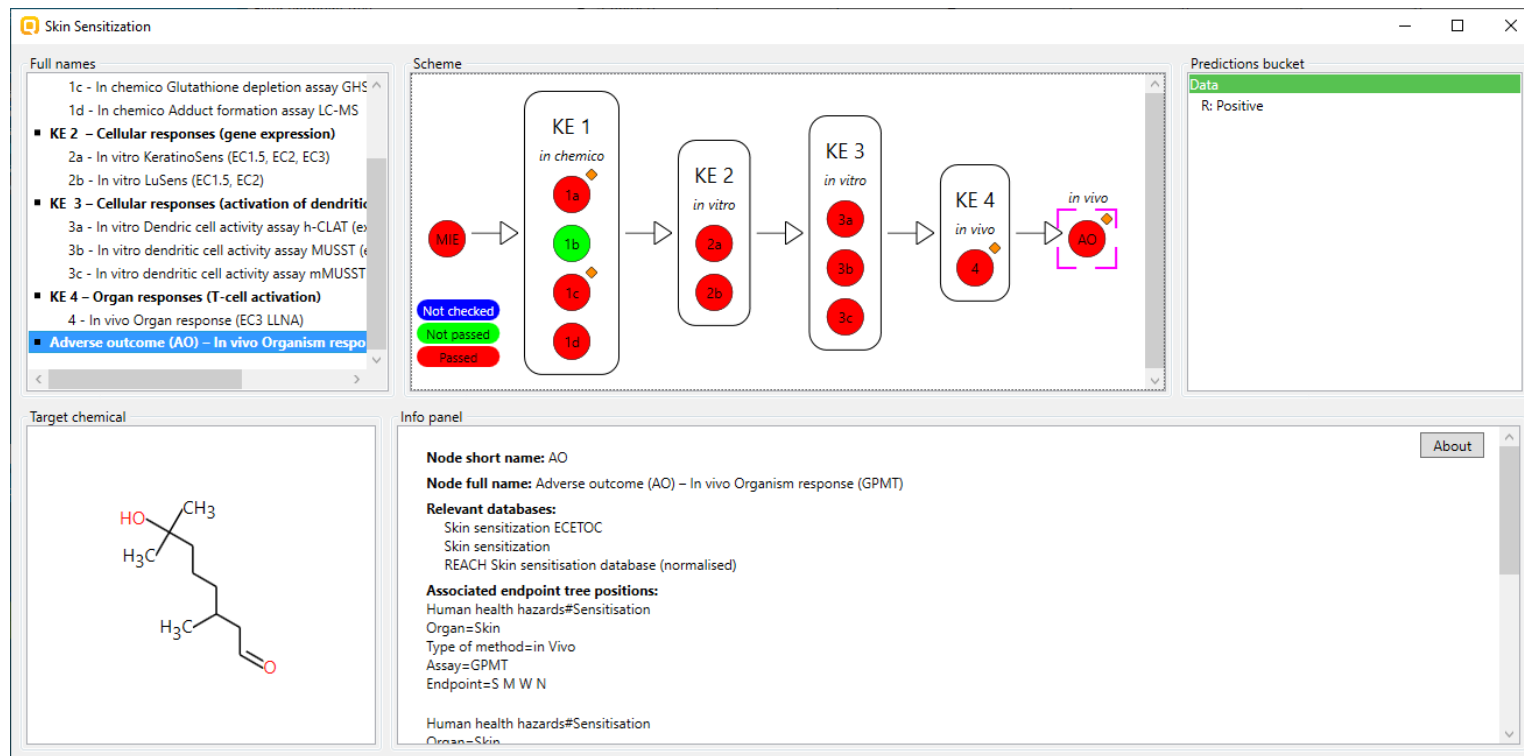
The screenshot displays the QSAR Toolbox interface during the 'Data Gap Filling' step. The 'Subcategorization' panel on the left shows two subcategorizations: 5a (Protein binding alerts for SS + skin metabolism) and 5b (Str. similarity - remove analogues with similarity less than 30%). The central workspace shows a chemical structure and a 'Read-across prediction' plot with a positive prediction. The right sidebar shows a 'Possible data inconsistency' dialog and a 'Select / filter data' panel. Numbered callouts (1-7) highlight key steps in the workflow.

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-dimensional depiction

The screenshot shows the 'Search by CAS#' dialog box. A red box highlights the input field containing '97530' (labeled 1). A blue box highlights the 'Search' button (labeled 2). Another blue box highlights the 'OK' button (labeled 3). Below the search bar, there are buttons for 'Select All', 'Unselect All', and 'Invert Selection', with a status 'Selected 1 of 1'. The search results table shows one entry with a green checkmark in the first column:

	CAS	97-53-0
	SMILES	<chem>COc1cc(CC=C)ccc1O</chem>
	CS Relation	High
	Substance	Mono constituent
	Composition	
	Name	"eugenol (4-allyl-2-methoxyphenol)"
	Sources	NICNAS Canada DSI

To the right of the table is a 2D chemical structure of Eugenol, which is 4-allyl-2-methoxyphenol. The structure shows a benzene ring with a methoxy group (H<sub>3</sub>C-O-) at position 2, an allyl group (-CH<sub>2</sub>-CH=CH<sub>2</sub>) at position 4, and a hydroxyl group (-OH) at position 1.

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

2

Exist in data source	Data source type	Data source quality	Assigned SMILES in data
Acute Oral toxicity DB	Database	Distribute to QA	no
ADME Database	Database	Distribute to QA	no
Aquatic OASIS	Database	Distribute to QA	no
Bacterial mutagenicity ISSSTY	Database	Distribute to QA	no
Canada DSL	Inventory	Distribute to QA	no
Carcinogenic Potency Database (CPE)	Database	Distribute to QA	no
Carcinogenicity&mutagenicity ISSCA	Database	Distribute to QA	no
Cell Transformation Assay ISSCTA	Database	Distribute to QA	no
Chemical Reactivity COLIPA	Database	Distribute to QA	no
Dendritic cells COLIPA	Database	Distribute to QA	no
DSSTOX	Inventory	High quality source	no
ECHA CHEM	Database	Distribute to QA	no
ECHA PR	Inventory	Distribute to QA	yes
ECOTOX	Database	Distribute to QA	yes
EINECS	Inventory	High quality source	no
Experimental pKa	Database	Distribute to QA	no
Food TOX Hazard EFSA	Database	Distribute to QA	no
GARD Skin sensitization	Database	Distribute to QA	no
Genotoxicity OASIS	Database	Distribute to QA	no
Genotoxicity pesticides EFSA	Database	Distribute to QA	no
GSH Experimental RC50	Database	Distribute to QA	no

OK

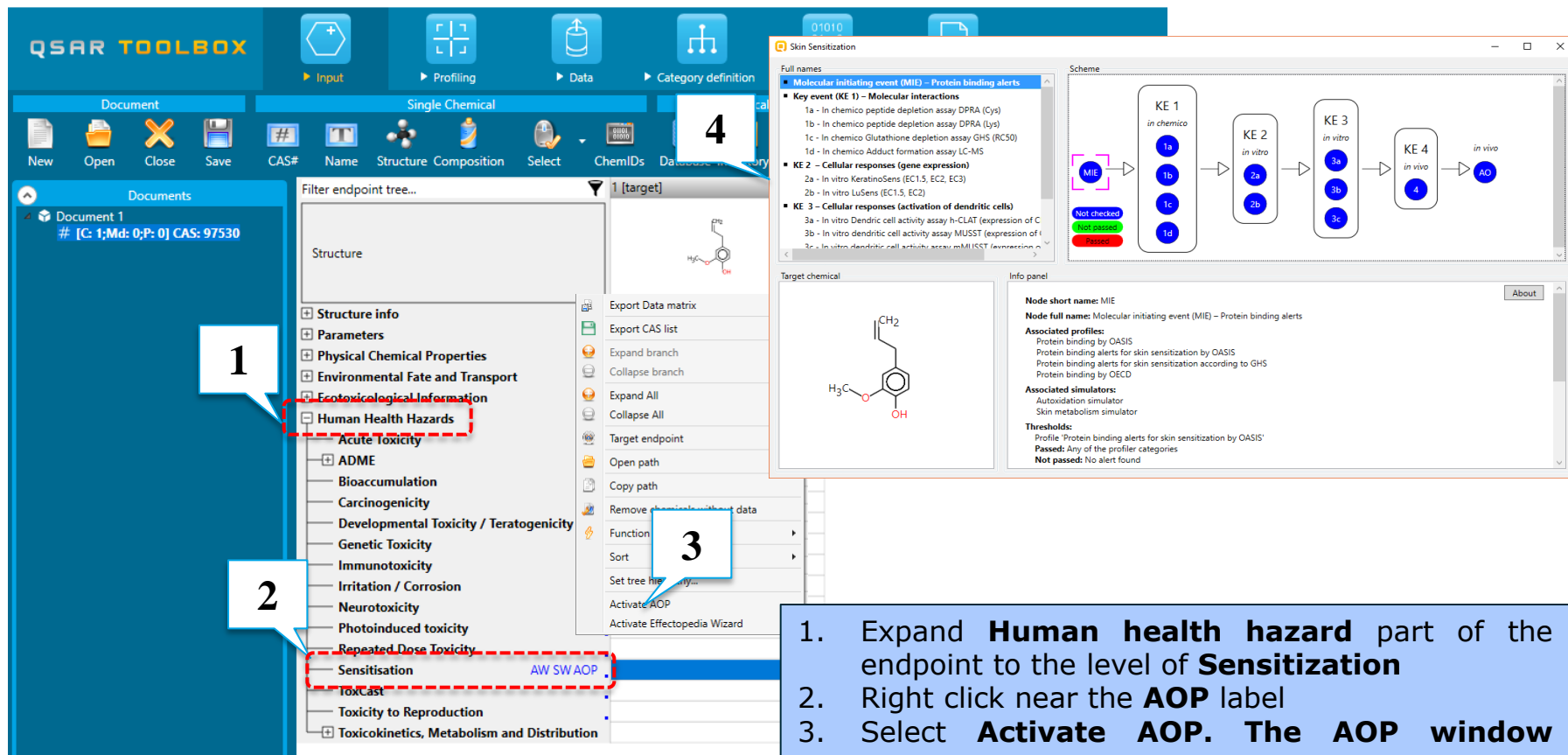
1. Double **Click**

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 **Sensitisation**

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.

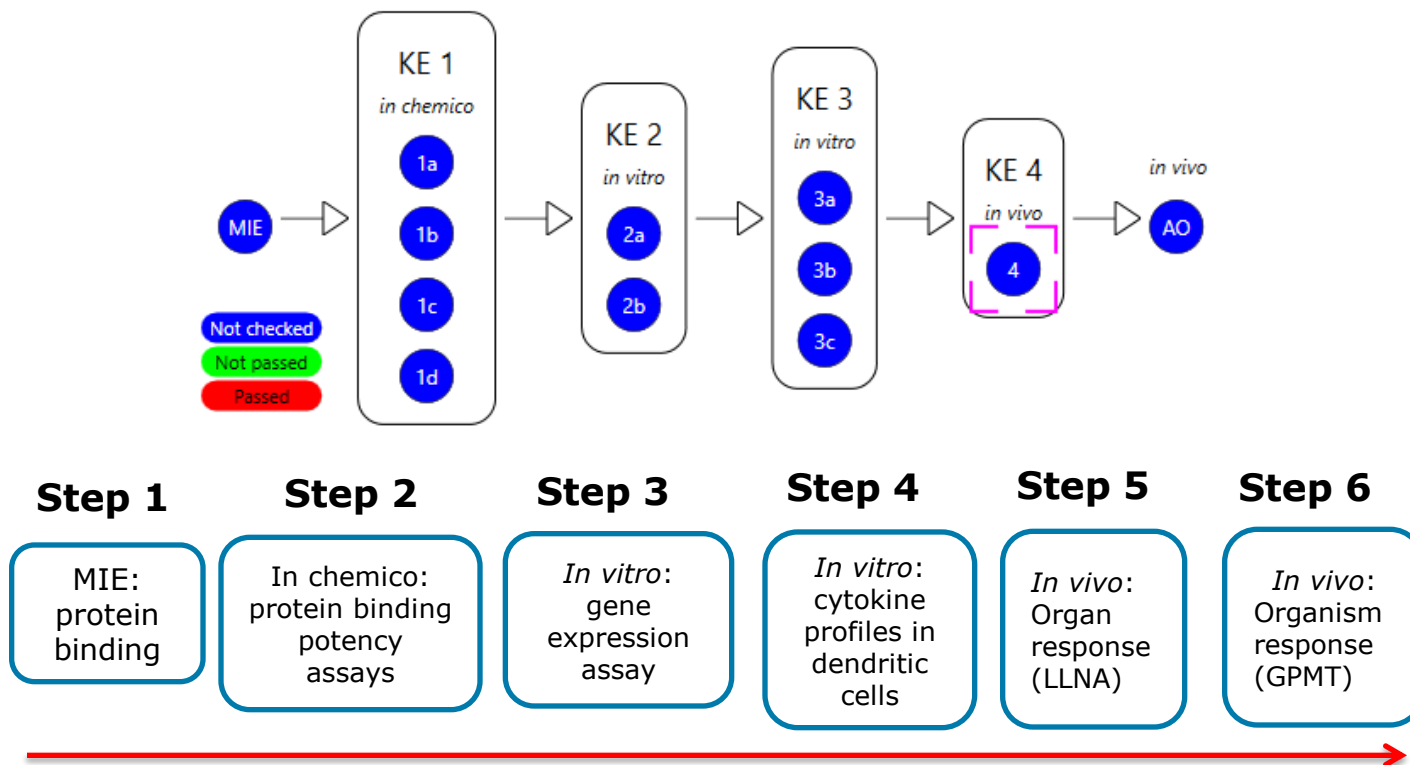


# Outlook

- 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

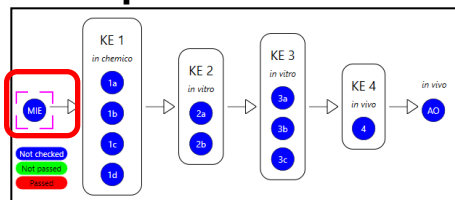
- Workflow process start from molecular initiating event to the *in vivo* organism respond



# Workflow process

## Step 1. MIE: protein binding

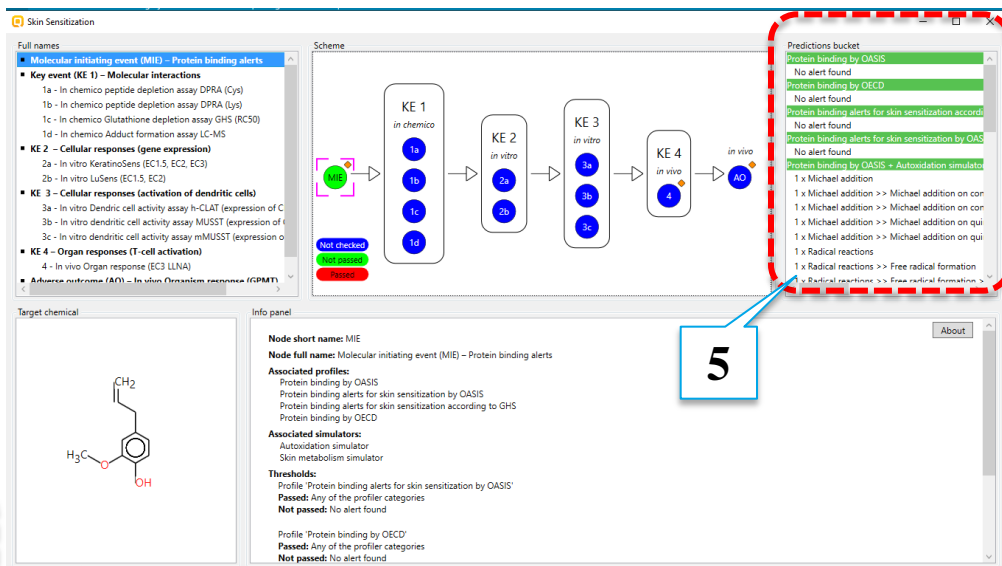
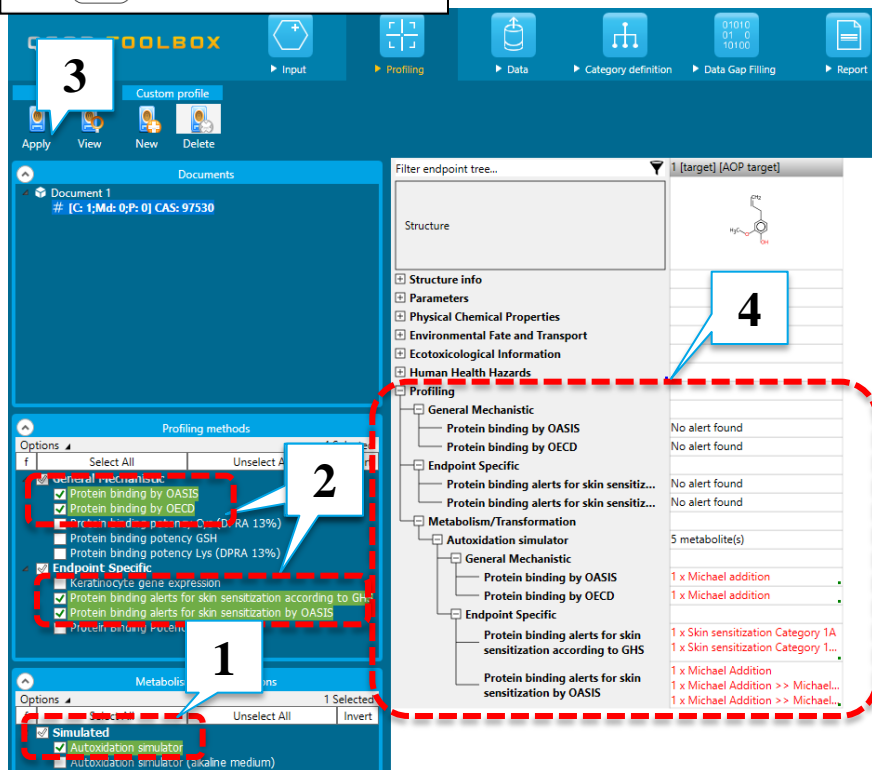
### Example 2



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

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

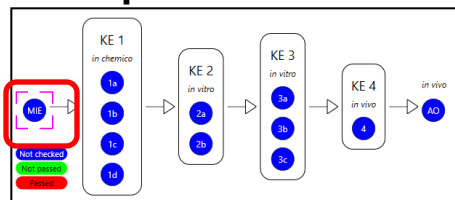


1. **Select** Autooxidation simulator.
2. **Select** highlighted profilers relevant to the MIE (they should be already checked)
3. **Click** Apply
4. The profiling results appeared on data matrix. As seen protein binding alerts are identified for the AU products
5. The profiling results for the package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

The screenshots illustrate the steps to change the MIE node status:

- Right click on the MIE node:** The first screenshot shows the 'MIE' node highlighted with a red box and a right-click context menu open. The 'Set state' option is selected.
- Select Set state:** The second screenshot shows the 'Set state' option selected in the context menu.
- Change the state from Not Passed to Passed:** The third screenshot shows the MIE node status changed from 'Not checked' (green) to 'Passed' (red).

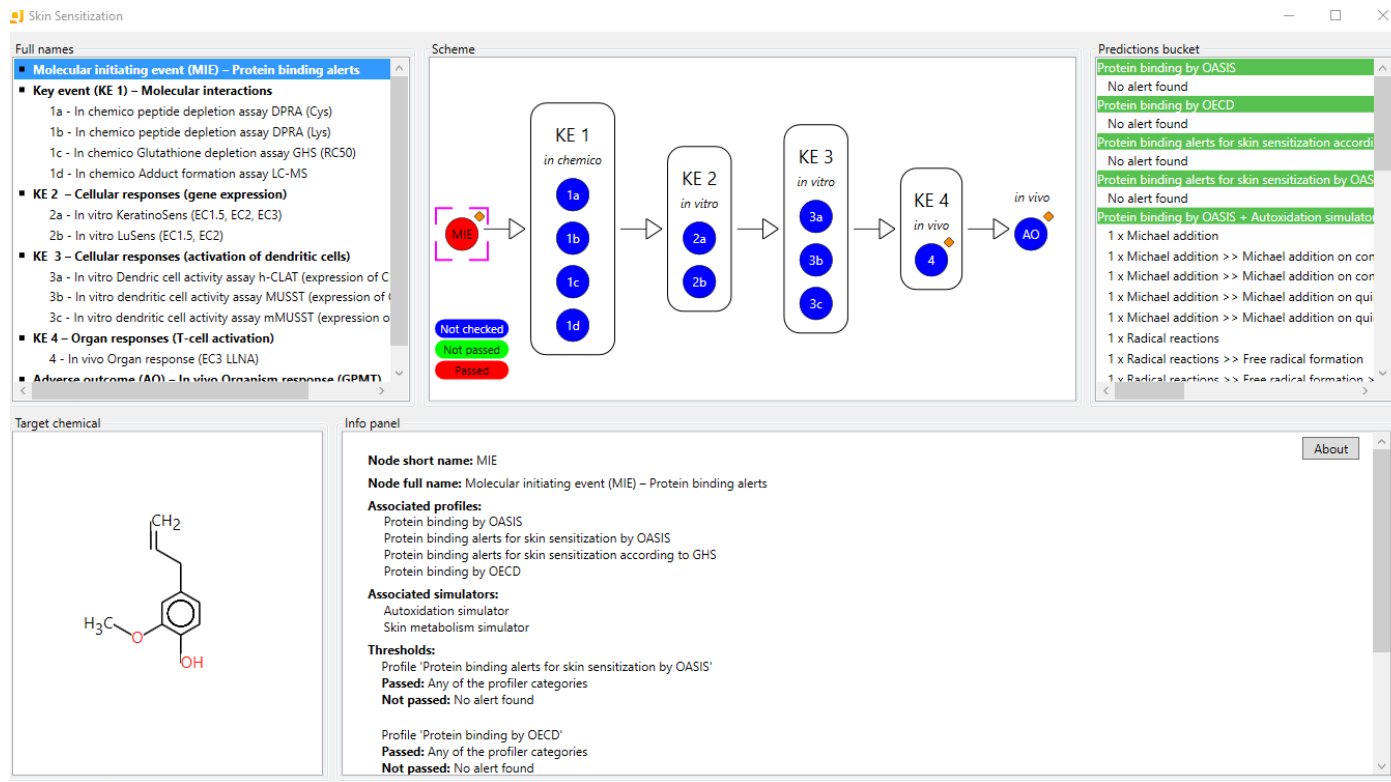
The interface also displays the 'Predictions bucket' and 'Scheme' panels, showing the overall workflow and the chemical structure of the target chemical (4-methylbenzyl alcohol).

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

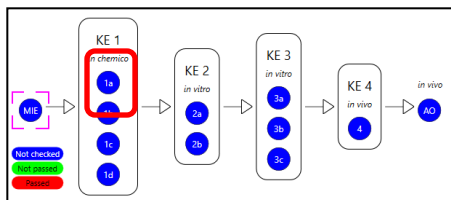


- The node MIE is passed due to the presence of positive protein binding alert identified for the Autoxidation products of the target chemical
- The workflow should move further to the *in chemico* assays

# Workflow process

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

### Example 2



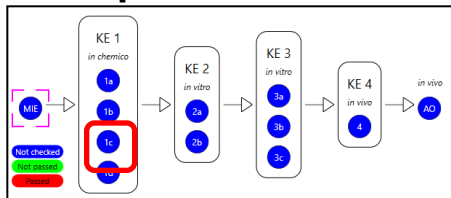
KE1, node 1a: Check for experimental data

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

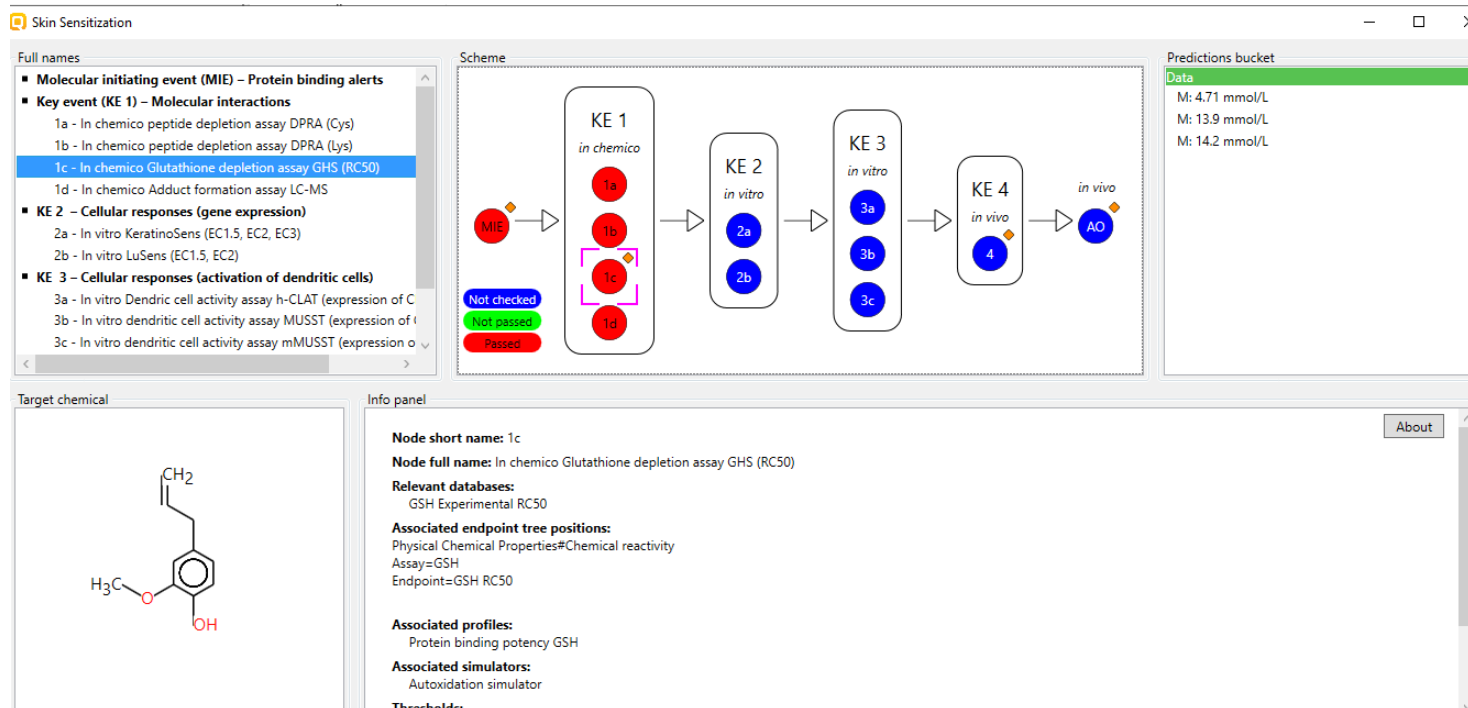
1. **Select** node 1c
2. **Select highlighted database** (unselect all the rest)
3. Click **Gather**
4. **Node 1c** is getting passed based on the thresholds for experimental data



# Workflow process

## Status of *In chemico* assays

### Example 2

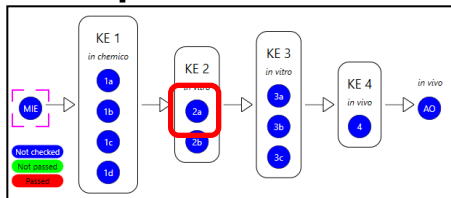


- The nodes related to the *in chemico* assays are passed due to positive experimental data found for the target chemical (node 1a, 1b, 1c and 1d) The workflow should move further to the *in vitro* assay (node 2a and 2b)

# Workflow process

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

### Example 2



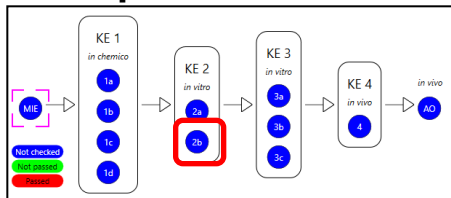
**KE2, node 2a:** Check are there experimental data for the parent chemical for node 2a

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



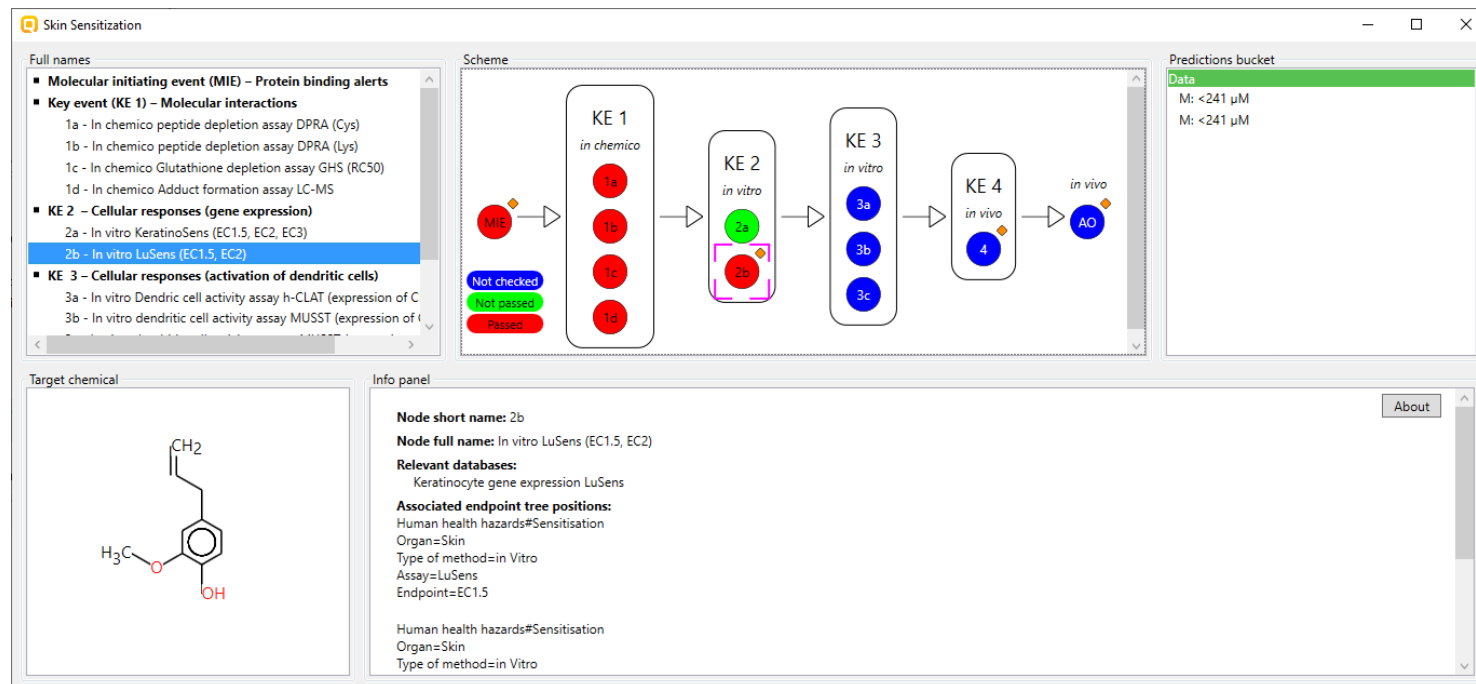
**KE2, node 2b:** Check are there experimental data for the parent chemical for node 2b

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

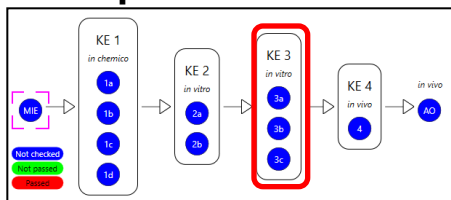


- The node 2a related to the Keratinocyte ARE assay is “not passed” based on negative data found for the target chemical. The node 2b related to the LuSens assay is “passed” based on the positive experimental data found for the target chemical (threshold is specified on slide # 14).
- The workflow should move 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* Dendritic cell activity assay

Screenshot of the QSAR Toolbox software interface. The interface shows the workflow process and the data matrix. The 'Data' tab is selected, and the 'Import' button is highlighted. The 'Data matrix' table is visible, showing the results of the assay for the target chemical.

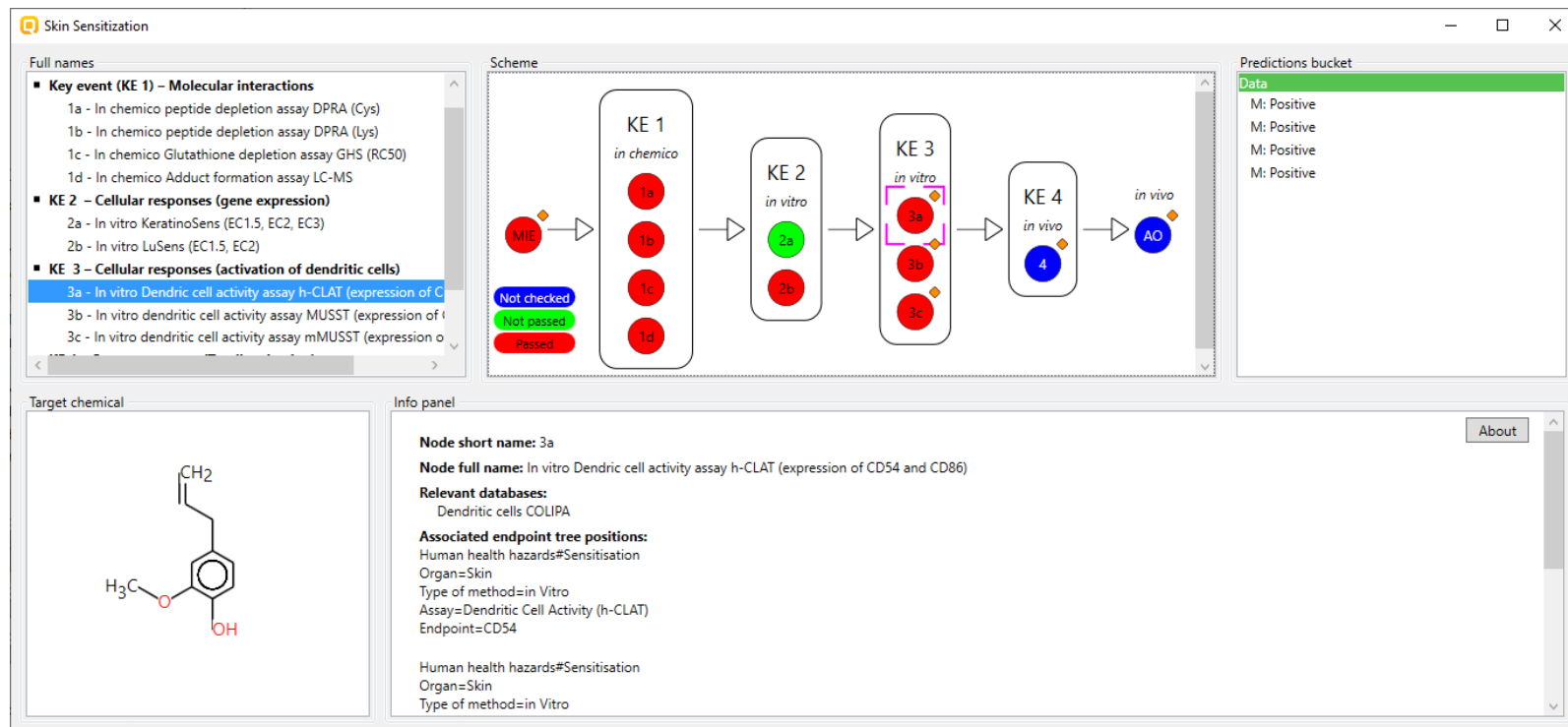
Screenshot of the 'Skin Sensitization' window in the QSAR Toolbox software. The 'Scheme' tab is selected, showing the workflow process. The 'Predictions bucket' is visible, showing the results of the assay for the target chemical.

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

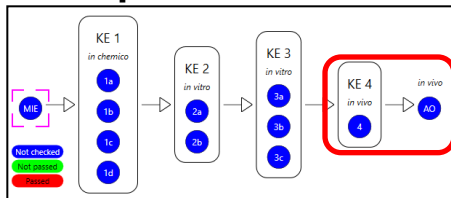


- The node 3a, 3b and 3c related to the *in vitro* Dendritic cell activity assays (h-CLAT, MUSST and mMUSST) are "passed" due to positive experimental data found for the target chemical
- The workflow could further move to the *in vivo* LLNA assay (nodes 4)

# Workflow process

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

### Example 2



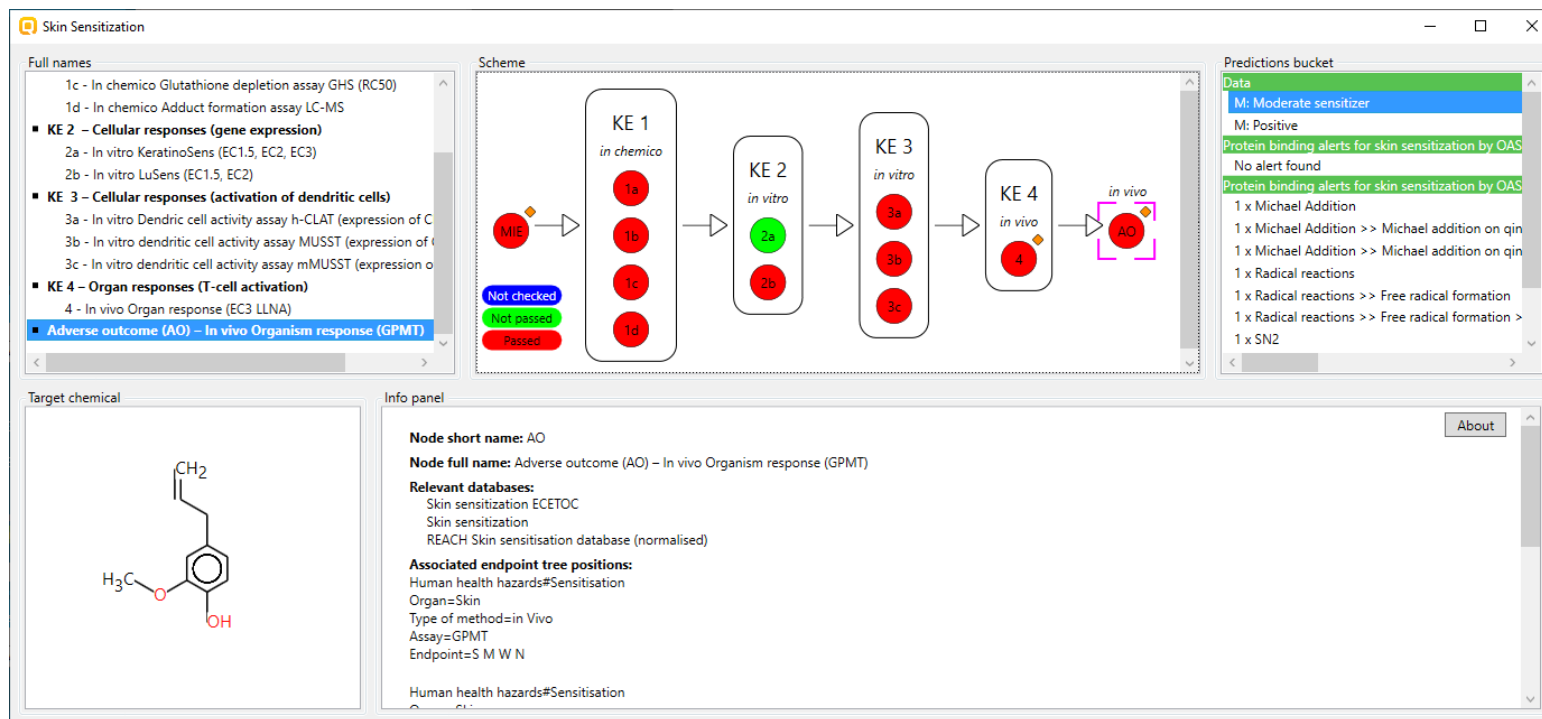
**KE4, node 4:** Check are there any data for the target chemical for the *in vivo* Organ response (LLNA) assay

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.