

OECD QSAR Toolbox v.3.4

Example for predicting skin sensitisation potential of (2*E*,6*Z*)-2,6-nonadien-1-ol accounting for skin metabolism

Outlook

- **Background**
- Objectives
- The exercise
- Workflow
- Save the prediction

Background

- This is a step-by-step presentation designed to take the user through the Toolbox workflow for filling data gap for skin sensitization of trans-2,cis-6-nonadienol accounting for its skin metabolism

Outlook

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- **Objectives**
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Objectives

This presentation demonstrates a number of functionalities of the Toolbox:

- Simulating skin metabolism of target chemical
- Identify analogues for a selected active metabolite
- Filling data gaps for active metabolites by read across
- Assign prediction of metabolite to the parent chemical

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The Exercise

- In this exercise we will predict the skin sensitization potential for target chemical (**trans-2,cis-6-nonadienol**) [**CAS # 28069-72-9**].
- Profile the target chemical and identify no Protein binding alert for target chemical.
- Gather available experimental data for the target chemical and identify positive experimental data.
- Skin metabolism of target chemical will be accounted for.
- Read across prediction for active metabolite will be applied.
- The predicted result of metabolite will be assigned to the target chemical.

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Workflow

- **As you know the Toolbox has 6 modules which are typically used in sequence:**
 - Chemical Input
 - Profiling
 - Endpoint
 - Category Definition
 - Data Gap Filling
 - Report
- **In this example we will use the modules in a different order, tailored to the aims of the example.**

Outlook

- Background
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- **Workflow**
 - **Input**

Chemical Input Overview

- This module provides the user with several means of entering the chemical of interest or the target chemical.
- Since all subsequent functions are based on chemical structure, the goal here is to make sure the molecular structure assigned to the target chemical is the correct one.

Chemical Input

Ways of Entering a Chemicals

User Alternatives for input of Chemical:

A. Single target chemical

- Chemical Name
- Chemical Abstract Services (CAS) number (#)
- SMILES (simplified molecular information line entry system) notation/InChi
- Drawing chemical structure
- Select from User List/Inventory/Databases
- Chemical IDs such as EC number, ENECS number

B. Group of chemicals

- User List/Inventory
- Specialized Databases

Chemical Input

Input Screen

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

Chemical Input Input Screen

The screenshot displays the 'Chemical Input' screen of the QSAR Toolbox. The interface is organized into several key sections:

- Top Navigation Bar:** Contains icons and labels for 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'.
- Document Management:** A toolbar on the left side of the top bar includes 'New', 'Open', 'Close', and 'Save' options.
- Central Action Bar (Red Box):** This toolbar is divided into 'Single Chemical' and 'Chemical List' modes. It includes input fields for 'CAS#' and 'Name', a 'Structure' field, and buttons for 'Select', 'Delete', 'Query', 'ChemIDs', 'DB', 'Inventory', and 'List'.
- Main Workspace:**
 - Left Panel:** A sidebar titled 'Documents' showing a list of open documents.
 - Right Panel:** A 'Filter endpoint tree...' view showing a hierarchical list of categories:
 - Substance Identity
 - Physical Chemical Properties
 - Environmental Fate and Transport
 - Ecotoxicological Information
 - Human Health Hazards
- Bottom Control Bar:** Features a dropdown menu for 'select filter type...', and 'Create' and 'Apply' buttons.

Chemical Input

Input target chemical by CAS#

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Input' menu is expanded, showing options like 'New', 'Open', 'Close', 'Save', 'CAS#', 'Name', 'Structure', 'Select', 'Delete', 'Query', 'ChemIDs', 'DB', 'Inventory', and 'List'. A red box highlights the 'CAS#' option, and a callout box with the number '1' points to it. A dialog box titled 'Search by CAS #' is open, showing a search input field, a 'Tautomeric sets' checkbox, and 'Search', 'OK', and 'Cancel' buttons. Below the search field are buttons for 'Select All', 'Clear All', and 'Invert Selection', with a status indicator 'Selected 0 of 0'. The dialog box also contains a table with the following columns: Selected, CAS, Smiles, Depiction, Names, CAS/Name, 2D/Name, and CAS/2D.

1. Click on CAS#

Chemical Input

Enter CAS# of trans-2,cis-6-nonadienol

Search by CAS #

28069729 Tautomeric sets

Select All Clear **1** Invert Selection **2**

| Selected | CAS | Smiles | Depiction | Names | CAS/Name | 2D/Name | CAS/2D |
|-----------|----------|--------|-----------|-------|----------|---------|--------|
| 1. Yes | 28069-72 | CCC=CC | | | | | |

3

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

Chemical Input

Target chemical identity

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

Chemical Input

Target chemical identity

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes options for Document, Single Chemical, and Chemical List. The main workspace is divided into several sections:

- Documents:** Shows a document with CAS: 28069-72-9.
- Structure:** Displays the chemical structure of the target compound.
- Substance Identity:** A tree view showing various identification fields. The 'Chemical IDs' section is expanded, and the following information is listed:
 - CAS Number
 - Chemical IDs
 - Chemical Name
 - Molecular Formula
 - Structural Formula
- Chemical List:** A table showing the identified chemical. The entry for CAS: 28069-72-9 is circled in red. The details for this entry are:
 - 28069-72-9
 - EINECS:2488168
 - trans-2,cis-6-nona...
 - 2,6-nonadien-1-ol, ...
 - (2e,6z)-nona-2,6-di...
 - 2,6-nonadien-1-ol, ...
 - C9H16O
 - CCC=CCCC=CCO

At the bottom of the interface, there is a filter selection dropdown and buttons for 'Create' and 'Apply'.

Chemical Input

Target chemical identity

The colour code indicates the reliability of the chemical identifier:

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

Outlook

- Background
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- The exercise
- **Workflow**
 - Input
 - **Profiling**

Profiling Overview

- “Profiling” refers to the electronic process of retrieving relevant information on the target compound, other than environmental fate, ecotoxicity and toxicity data, which are stored in the Toolbox database.
- Available information includes likely mechanism(s) of action, as well as observed or simulated metabolites.

Profiling

Profiling the target chemical

- The outcome of the profiling determines the most appropriate way to search for analogues (detailed information about profilers could be found in “Manual for Getting started” (Chapter 4) published on the OECD website:
<http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm>
- Table 4 - 1 in chapter 4 (Manual for getting started) lists a selection of profilers and their relevance for different endpoints of regulatory relevance
- The following profiling schemes are relevant to the **Skin sensitization**:
 - Protein binding by OASIS v.1.4 – general mechanistic
 - Protein binding by OECD – general mechanistic
 - Protein Binding Potency – general mechanistic
 - Protein binding alerts for skin sensitization by OASIS v1.4 – endpoint specific

Profiling

Profiling the target chemical

- This selects (a **green** check mark appears) or deselects (**green** check mark disappears) profilers.
- For this example, go through the general and endpoint specific profiling mechanisms and highlight those that apply to skin sensitization(see next screen shot).

Profiling

Profiling the target chemical

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Profiling Schemes' menu is open, showing various profiling methods. A red circle highlights the 'Apply' button, with a callout '2'. Another red circle highlights the 'Protein binding alerts for skin sensitization by OASIS v1.4' checkbox, with a callout '1'. The 'Filter endpoint tree...' window is also visible, showing the chemical structure and various properties for the target chemical.

1. Check protein binding profiles from **General Mechanistic and **Endpoint specific** group: **Protein binding for skin sensitization by OASIS** profiler**

2. Click Apply

Profiling

Profiling the target chemical

- The actual profiling will take up to several seconds depending on the number and type of profilers selected
- The results of profiling automatically appear as a dropdown box under the target chemical (see next screen shot)
- Please note the specific protein-binding profilers
- No protein binding alert has been found for the test compound (trans-2,cis-6-nonadienol).

Profiling

Profiling the target chemical

The screenshot displays the QSAR Toolbox Profiling interface. On the left, the 'Profiling methods' panel is visible, with 'Protein binding alerts for skin sensitization by OASIS v1.4' checked. The central 'Filter endpoint tree...' panel shows a tree structure where the 'Profile' node is selected. A red circle highlights the 'Profile' node, and a callout box with the number '1' points to it. The right panel shows the results for the selected target chemical, with a red box highlighting the text: 'The target chemical has no protein binding alert. In this respect no skin sensitization effect is expected'. Below this, a table lists the results for various endpoints, with 'Protein binding by OASIS v1.4', 'Protein binding by OECD', and 'Protein binding alerts for skin sensitization by OASIS v1.4' all showing 'No alert found'.

1. Double click on "Profile" node to review the profiling results.

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - **Endpoint**

Endpoint Overview

- “Endpoint” refers to the electronic process of retrieving the environmental fate, ecotoxicity and toxicity data that are stored in the Toolbox.
- Data gathering can be executed in a global fashion (i.e., collecting all data for all endpoints) or on a more narrowly defined basis (e.g., collecting data for a single or limited number of endpoints).

Endpoint

Case study

- In this example, we limit our data gathering to a single toxicity endpoint (skin sensitization).
- In this example, we collect data from the databases containing experimental results for Skin sensitisation (Skin sensitisation and Skin sensitisation ECETOC).
- **Click** on “Endpoint” in the Toolbox workflow.
- **Expand the** “Human Health Hazards” section
- **Click** on the box to select the relevant databases.
- **Click** on “Gather data” (see next screen shot).

Endpoint Gather data

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Endpoint' menu item is highlighted with a red circle and a callout labeled '1'. Below the menu bar, the 'Data' menu is also highlighted with a red circle and a callout labeled '4'. The main window is divided into several panes. On the left, the 'Database' pane shows a list of databases under the 'Human Health Hazards' section, which is expanded with a red circle and callout labeled '2'. Within this section, 'Skin irritation' and 'Skin sensitization' are selected, highlighted with a red circle and callout labeled '3'. The central pane, 'Filter endpoint tree...', shows a tree structure with 'Human Health Hazards' expanded. The right pane, '1 [target]', shows a chemical structure. A blue box at the bottom contains the following instructions:

1. **Click** Endpoint
2. **Expand** the Human Health Hazards section
3. **Select** databases related to the target endpoint
4. **Click** Gather

Endpoint

Gather data

- Toxicity information on the target chemical is electronically collected from the selected dataset(s)
- It should be kept in mind that the search for data and analogues is performed only among the chemicals which are listed in the selected databases, which in this example are **Skin sensitization** and **Skin sensitization ECETOC**
- In this example, there is Positive experimental data for the target chemical(see next screen shots)

Endpoint Gather data

Toxicity information on the target chemical is electronically collected from the selected datasets. A window with "Read data?" appears. Now the user could choose to collect "all" or "endpoint specific" data.

The screenshot displays the QSAR Toolbox software interface. The top navigation bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. Below this, there are tabs for 'Data', 'Import', 'Export', 'Delete', and 'Tautomerize'. The main workspace is divided into several panels:

- Databases:** A list of various toxicity databases, including 'Human Health Hazards', 'Acute Oral Toxicity database (ChemIDPlus)', 'Bacterial mutagenicity ISSSTY', 'Carcinogenic Potency Database (CPDB)', 'Carcinogenicity&mutagenicity ISSCAN', 'Cell Transformation Assay ISSCTA', 'Dendritic cells COLIPA', 'Developmental & Reproductive Toxicity (DART)', 'Developmental toxicity ILSI', 'ECHA CHEM', 'ECOTOX', 'Estrogen Receptor Binding Affinity OASIS', 'Eye Irritation ECETOC', 'Genotoxicity OASIS', 'Human Half-Life', 'Keratinocyte gene expression Givaudan', 'Keratinocyte gene expression LuSens', 'Micronucleus ISSMIC', 'Micronucleus OASIS', 'MUNRO non-cancer EFSA', 'Rep Dose Tox Fraunhofer ITEM', 'Repeated Dose Toxicity HESS', 'Rodent Inhalation Toxicity Database', 'Skin Irritation', 'Skin sensitization', 'Skin sensitization ECETOC', 'ToxCastDB', 'Toxicity Japan MHLW', 'ToxReDB US-EPA', 'Yeast estrogen assay database', and 'ZEBET database'.
- Filter endpoint tree...:** A tree view showing categories like 'Structure', 'Substance Identity', 'Physical Chemical Properties', 'Environmental Fate and Transport', 'Ecotoxicological Information', 'Human Health Hazards', and 'Profile'.
- 1 (target):** A small window showing a chemical structure.
- Read data? dialog:** A modal dialog box with the following options:
 - All endpoints
 - Choose...
 - from Tautomers
 -
 -

A blue callout box with the number '1' points to the 'OK' button in the 'Read data?' dialog. A larger blue callout box at the bottom of the screenshot contains the text: **1. Click OK to read all available data**

Endpoint Gather data

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes options like 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. Below this, there are sub-menus for 'Data', 'Import', 'Export', 'Delete', and 'Tautomerize'. The main workspace is divided into several panels:

- Databases:** A list of databases with checkboxes. 'Human Health Hazards' is checked, and 'Skin Irritation' is also checked under it.
- Filter endpoint tree...:** A tree view showing the hierarchy of endpoints. 'Skin Irritation' is selected, and a callout box with the number '1' points to it.
- Data Matrix:** A table showing the results of the data gathering process. The selected endpoint 'Skin Irritation' is highlighted, and the data matrix shows '(1/1) M: Positive'.

1. Available experimental data appears on data matrix.

Endpoint Gather data

The screenshot shows the QSAR Toolbox software interface. The main window displays a tree view of endpoints under the 'Endpoint' workflow. A 'Data points' window is open, showing a table of data. The table has the following columns: Endpoint, Value, Original value, Organ, Reference source, Phylum (common name), Phylum, Test method / Data source, Type of method, Year, Test organisms (species), Title, Kingdom, Assay, and Author. The first row of data is highlighted in blue, with the 'Endpoint' cell containing 'S M W N'. A blue callout box with the number '1' points to this cell. Another blue callout box with the number '2' points to the 'X' close button in the top right corner of the 'Data points' window.

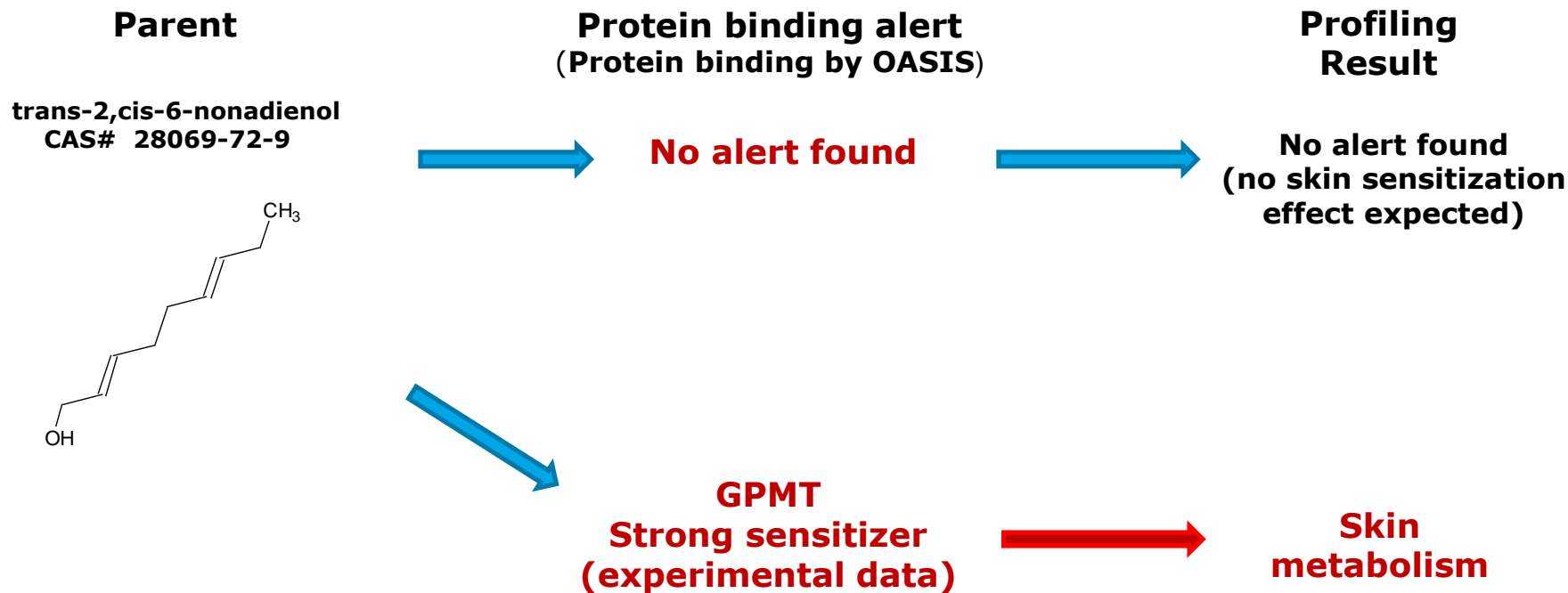
| Endpoint | Value | Original value | Organ | Reference source | Phylum (common name) | Phylum | Test method / Data source | Type of method | Year | Test organisms (species) | Title | Kingdom | Assay | Author |
|----------|---|-------------------|-------|---------------------------------------|----------------------|----------|---------------------------|----------------|------|--------------------------|--|----------|-------|------------------------------|
| S M W N | Positive (Skin sensitisation II (ECETOC)) | Strong sensitizer | Skin | SAR QSAR Environ. Res. 2 (3): 159-179 | Vertebrates | Chordata | GPMT | in Vivo | 1994 | guinea pig | Multivariate QSAR analysis of a skin sensitization database. | Animalia | GPMT | Cronin M. T., Basketter D. A |

1. **Double-click** on the cell displays metadata information for the observed data
2. **Click** on the X to close the window

Recap

- The first module, which introduces the target chemical, ensure correctness of the structure
- The second module shows that there is no protein binding alert for target chemical
- In the third module, you have found that the target chemical has positive skin sensitization data
- The positive experimental data could be due to skin metabolism
- The study continues with accounting for skin metabolism of target chemical(see next slides).

Recap



Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Endpoint
 - **Handling of skin metabolism**

Handling of skin metabolism of target chemical

- Multiplying target chemical by skin metabolism simulator
- Multiplying target chemical by skin metabolism simulator is accomplished in section **Input**
- The generated metabolites appear in tree like form(see next screen shot)

Handling of skin metabolism of target chemical

Multiplication of target chemical

1. **Click** on the SMILES of the target chemical and perform right click on it, then
2. **Select** Multiplication-Metabolism/Transformations
3. **Select** Skin metabolism simulator
4. Generated metabolites appear in tree like form

Handling of skin metabolism of target chemical

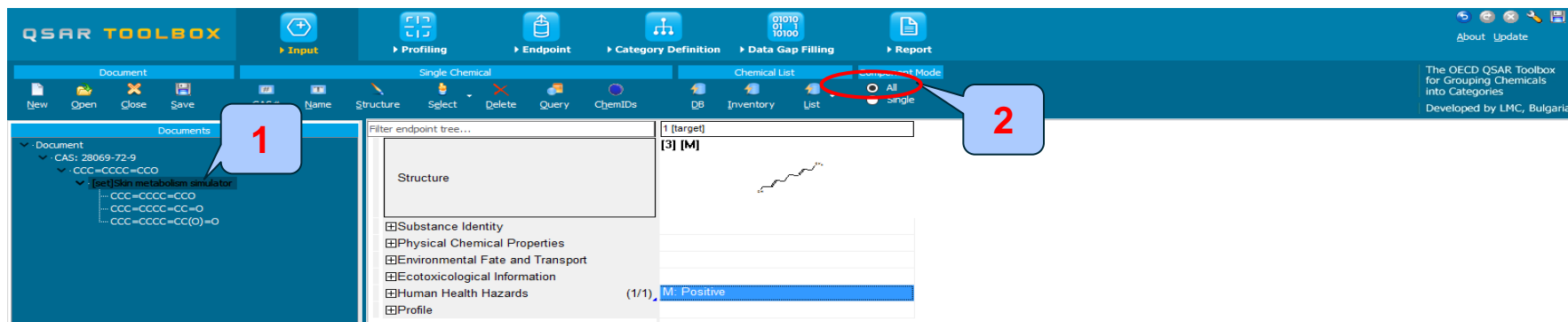
Visualization of modeling modes

- Two component modes are implemented:
 - **Set Mode** - all metabolites are analyzed as a package
 - **Individual Component Mode** - each metabolite is analyzed individually(see next screen shot)

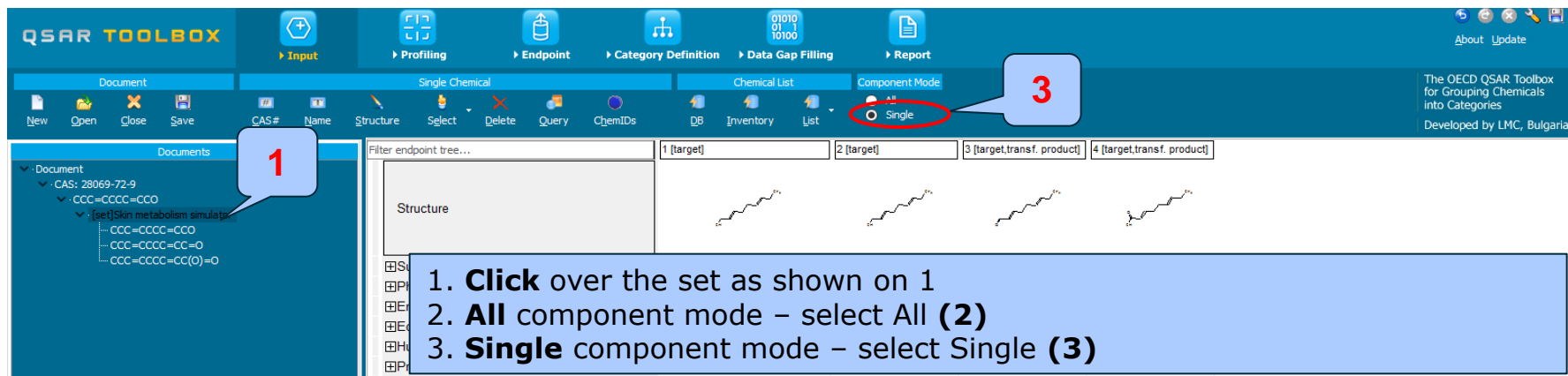
Handling of skin metabolism of target chemical

Visualization of modeling modes

- All Component Mode – all metabolites are analyzed as a package



- Single Mode – each metabolite is analyzed individually



Outlook

- Background
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- **Workflow**
 - Input
 - Profiling
 - Endpoint
 - **Handling of skin metabolism of target chemical**
 - **Profiling set of metabolites**

Handling of skin metabolism of target chemical

Profiling set of metabolites

- This module identifies profilers of target chemical and its metabolites
- Protein binding profiles related to skin sensitization are applied on package of target and metabolites
- Profiling results of target and metabolites are illustrated in Single Component mode
- Click on "**Profiling**" to go to the required module
- Apply **Protein binding profilers** (see next screen shot)

Handling of skin metabolism of target chemical

Protein binding result of parent and metabolites

QSAR Toolbox 3.4.0.17 [Document_6]

The screenshot shows the QSAR Toolbox interface. The 'Profiling' menu is open, and the 'Profiling methods' list is visible. The 'Protein binding alerts for skin sensitization by OASIS v1.4' method is selected. The results table shows the following data:

| Endpoint | Target | Target | Target | Target |
|-------------------------------|------------------------|------------------------|---|---|
| Protein binding by OASIS v1.4 | No alert found | No alert found | AN2 AN2 >> Michael a... AN2 >> Michael a... Michael addition Michael addition >... Schiff base formation Schiff base formati... Schiff base formati... | AN2 AN2 >> Michael a... AN2 >> Michael a... |
| | No alert found | No alert found | Michael addition Michael addition >... Michael addition >... Schiff Base Formers Schiff Base Forme... Schiff Base Forme... | No alert found |
| Protein binding by OECD | Not possible to cla... | Not possible to cla... | Highly reactive (GSH) Highly reactive (G... | Not possible to cla... |
| | No alert found | No alert found | Michael Addition Michael Addition >... Michael Addition >... | No alert found |

The profiling results indicates no protein binding alerts or target chemical. However, two of simulated metabolites exhibit interaction with proteins via two different protein binding mechanisms (Michael and Schiff base)

1. **Go** to Profiling 2. **Check** the profilers related to the target endpoint 3. **Click** Apply

Handling of skin metabolism of target chemical

Recap

- The profiling results indicates no protein binding for target chemical
- Two of simulated skin metabolites have positive protein binding alerts
- One of the reactive metabolites is used for further read across analysis
- The next two parts of the exercise will focus one of the reactive metabolites and find similar analogues of the reactive one (see next screenshot).

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Endpoint
 - **Handling of skin metabolism of target chemical**
 - Profiling set of metabolites
 - **Focus of active metabolite**

Handling of skin metabolism of target chemical

Focus of active metabolite

The screenshot displays the QSAR Toolbox interface with a target chemical structure at the top. Below it, a table lists three targets. The third target, labeled '3 [target transf. product]', is circled in red. A context menu is open over this target, with the 'Focus' option highlighted. A callout box points to the 'Focus' option, and another callout box points to the selected metabolite structure in the table.

1 This metabolite is selected for further read-across prediction

2 Focus

Focus menu options:

- Remove this target
- Set AOP target
- Add to Study Pad
- Select all as targets
- Remove all as targets
- Edit and add target
- Add target
- Add in category
- Delete
- Delete all except current
- Save to SMI file (DayLight format)
- Save to SMI file
- Print structures
- Export data for targets
- Export CAS list
- Query tool matrix Ctrl+F3

Table content (Target 3):

| | | |
|--------------------------------|------------------------|---|
| (2/2) M: Positive | M: Positive | AN2 AN2 >> Michael a... Michael addition Michael addition >... Michael addition >... Schiff base formation Schiff base formati... Schiff base formati... |
| No alert found | No alert found | |
| No alert found | No alert found | |
| Not possible to classify ac... | Not possible to cla... | |
| No alert found | No alert found | Highly reactive (G... Michael Addition Michael Addition >... Michael Addition >... |

1. Right click over the active metabolite
2. Select Focus from the appeared menu

“Focus” functionality allows the selected metabolite to be used as post target representative of the target chemical

Handling of skin metabolism of target chemical

Focus of active metabolite

The screenshot displays the QSAR Toolbox interface. On the left, the 'Documents' tree shows a hierarchy: Document > CAS: 28069-72-9 > CCC=CCCC=CCO > [set]Skin metabolism simulator > CCC=CCCC=CCO > CCC=CCCC=CC=O (circled in red). The main window shows the chemical structure of the selected metabolite, CCC=CCCC=CC=O, which is also circled in red. Below the structure, a 'Filter endpoint tree...' panel lists various endpoints, with 'Protein binding by OASIS v1.4' selected. To the right, a data matrix shows the results for this endpoint, including 'AN2', 'Michael addition', and 'Schiff base formation'.

The selected metabolite appears in a new data matrix.

Outlook

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 - Input
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 - Endpoint
 - **Handling of skin metabolism of target chemical**
 - Profiling set of metabolites
 - Focus of active metabolite
 - **Defining category for active metabolite**

Category Definition

Grouping methods

- The different grouping methods allow the user to group chemicals into chemical categories according to different measures of “similarity” so that within a category data gaps can be filled by read-across.
- Detailed information about grouping chemical (Chapter 4) could be found in document “Manual for Getting started” published on OECD website:

<http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm>

Basic guidance for category formation and assessment

Suitable categorization phases:

1. Structure-related profilers
2. Endpoint specific profilers (for sub-cat)
3. Additional structure-related profilers, if needed to eliminate dissimilar chemicals (to increase the consistency of category) (e.g. chemical elements)

Performing categorization:

1. The categorization phases should be applied successively
2. The application order of the phases depend on the specificity of the data gap filling
3. More categories of same Phase could be used in forming categories
4. Some of the phases could be skipped if consistency of category members is reached

Graphical illustration of suitable categorization phases is shown on next slide

Suitable Categorization/Assessment Phases

Phase I. Structure based

- US EPA Categorization
- OECD Categorization
- Organic functional group
- Structural similarity
- ECOSAR

**Broad grouping
Endpoint Non-specific**

Repeating Phase I due to Multifunctionality of chemicals

Phase II. Mechanism based

- DNA binding mechanism
- Protein binding mechanism
- Genotoxicity/carcinogenicity
- Cramer rules
- Verhaar rule
- Skin/eye irritation corrosion rules

**Subcategorization
Endpoint Specific**

Metabolism accounted for

Phase III. Eliminating dissimilar chemicals

**Apply Phase I – for structural dissimilarity
Filter by test conditions – for Biological dissimilarity**

**Subcategorization
Endpoint Specific**

Handling of skin metabolism of target chemical

Category definition for active metabolite

- In this exercise, the reactive metabolite is classified as: Aldehyde by US-EPA New chemical category (phase I)
- The identified Protein binding profiler of the reactive metabolite is: Michael addition >> a,b-unsaturated carbonyl compounds >> Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes (phase II)
- In order to expand the initial group of identified analogues the US-EPA New chemical category is used for categorization purposes (phase I).
- Searching for similar analogues is accomplished using the two skin sensitization databases: Skin sensitization and Skin sensitization ECETOC

Handling of skin metabolism of target chemical

Defining US-EPA category

- The category **US-EPA New chemical category (strict)** is used
- **Strict** functionality means that the software will identify analogues having **ONLY** the categories of the target (i.e aldehydes) and will exclude the analogues having any other categories
- **Select** US-EPA New Chemical category
- **Click** Define (see next screen shots)

Handling of skin metabolism of target chemical

Defining US-EPA category

The screenshot shows the QSAR Toolbox interface in the 'Category Definition' step. The 'Predefined' grouping methods list includes 'US-EPA New Chemical Categories', which is highlighted with a red circle and callout 1. The 'Define' button in the top toolbar is circled in red with callout 2. A dialog box titled 'US-EPA New Chemical Categories' is open, showing 'Aldehydes (Acute toxicity)' as the target profile. The 'Strict' checkbox is checked and circled in red with callout 3. The 'OK' button is circled in red with callout 4. The main window shows a chemical structure and a list of endpoint categories.

1. **Highlight** "US-EPA New Chemical Categories" 2. **Click** Define 3. **Select** Strict 4. **Click** OK to confirm the category **Aldehydes (Acute toxicity)** defined by US-EPA category.

Handling of skin metabolism of target chemical

Defining US-EPA category

The screenshot shows the QSAR Toolbox software interface. The main window is titled 'Category Definition' and displays a list of endpoints on the left, a chemical structure in the center, and a list of defined categories on the right. A dialog box titled 'Define category name' is open, showing the category name 'toxicity Strict (US-EPA New Chemical Categories)' for 75 chemicals. A blue callout bubble with the number '1' points to the 'OK' button. A blue box at the bottom contains the instruction: '1. Click OK to confirm the name of the category'.

Handling of skin metabolism of target chemical

Category analogues

- The Toolbox now identifies all chemicals corresponding to *Aldehydes(Acute toxicity)* by US-EPA listed in the skin sensitization databases.
- 75 analogues including the target chemical are identified; they form a mechanistic category named “**Aldehydes (Acute toxicity)**”, which will be used for further data gap filling.
- The experimental data for analogues in the category appears on datamatrix.

Handling of skin metabolism of target chemical

Summary information for Analogues

The experimental results for the analogues appeared on datamatrix

The screenshot shows the QSAR Toolbox interface with a datamatrix table. The table has 8 columns representing different chemical categories. A red box highlights the first row of data, which is annotated with a callout bubble containing the number '1'. The highlighted row shows the following data:

| | | | | | | | |
|---------|-------------|--|--|-------------|--|-----------------------|--|
| (17/17) | M: Negative | | | M: Positive | | M: Positive, 370 μ... | |
|---------|-------------|--|--|-------------|--|-----------------------|--|

The interface also displays a list of predefined and general mechanistic endpoints on the left, and a filter endpoint tree in the center. The datamatrix table below the tree shows the following data for the highlighted row:

| | | | | | | | |
|---------|-------------|-------------|-------------|-------------|------------------------|------------------------|-------------------------|
| (17/17) | M: Negative | | | M: Positive | | M: Positive, 370 μ... | |
| (22/40) | | | | M: Positive | | M: Positive, 370 μ... | |
| (62/66) | M: Positive | | | M: Negative | | M: Positive | M: Negative |
| (16/30) | | M: Negative | M: Positive | | M: Negative, Positi... | M: Negative, Positi... | M: Positive, Positiv... |
| (7/7) | | | | | | M: Positive | |

Chemical statistic (1) presenting number of chemicals and the available experimental data.

Recap

- In this case “US-EPA New-category Aldehydes(strict) is used for categorization purposes.
- The defined category consist of 75 analogues along with the target chemical
- The available experimental data for these 75 analogues have been collected from two skin sensitization databases.
- But before the user can proceed with the “Filling Data Gap” module, he/she should navigate through the endpoint tree and find the specific gap that will be filled in (in this case Human Health Hazards#Sensitisation#Skin#In Vivo).
- In this case we mixed assays and endpoints (see slides #62-64)

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Endpoint
 - **Handling of skin metabolism of target chemical**
 - Profiling set of metabolites
 - Focus of active metabolite
 - Category definition for active metabolite
 - **Data gap filling**

Data gap filling

Apply Read across

The screenshot displays the QSAR Toolbox interface during a data gap filling session. The 'Data Gap Filling' menu is active, and the 'Read-across' method is selected. The target endpoint is 'Human Health Hazards Sensitisation Skin In Vivo'. The main workspace shows a list of chemical structures and a table of data points. A red circle highlights a specific cell in the table, corresponding to the 'Sensitisation / Skin / In vivo' endpoint for a chemical with the ID (74/160). The cell contains the text 'M: Negative, Positive'. A red arrow points from this cell to the 'Read-across' method selection in the sidebar. Another red circle highlights the 'Apply' button in the top-left corner of the software window.

1. Click on the cell corresponding to "Sensitisation/Skin/In vivo" for the target chemical(active metabolite) **2. Select** Read-across **3. Click** Apply

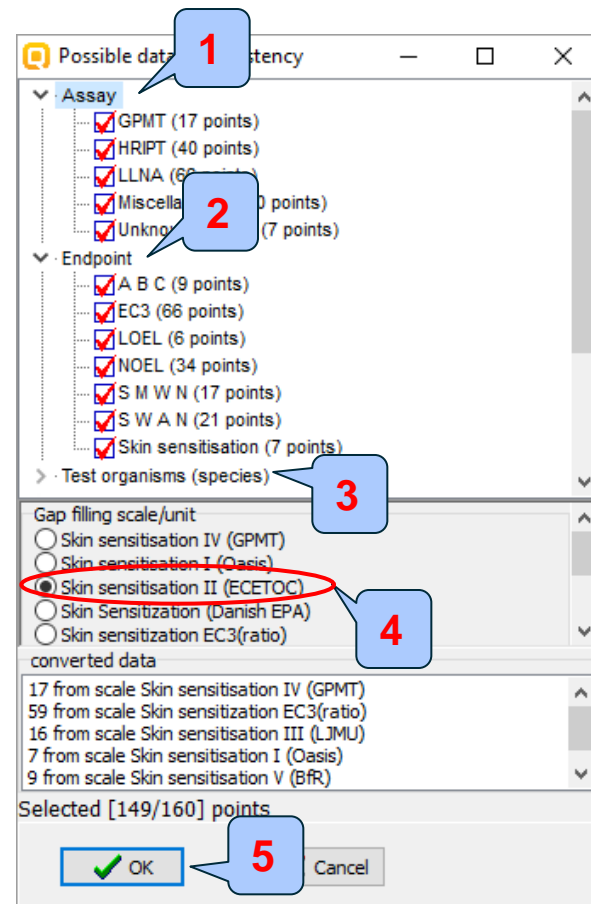
Data gap filling for active metabolite

Scale definition

- Skin sensitisation is “qualitative” endpoint for which the results are presented with categorical data (for example: positive; negative; weak sensitizer; strong sensitizer ,etc).
- Skin sensitisation potential of the chemicals came from different authors coded with different names (for example: data from John Moores University of Liverpool are: *Strongly sensitizing, Moderately sensitizing etc.*; data from European centre for Ecotoxicology and Toxicology of chemicals are: *Positive, Negative, and Equivocal*).
- The main purpose of the scales is to unify all data available in the Toolbox databases for a certain endpoint.
- The default scale for Skin Sensitisation is “Skin Sensitisation ECETOC”. It converts all skin data into: Positive, Negative, and Equivocal.

Data gap filling for active metabolite

Scale definition



1. In this case all assays (1), endpoints (2) and Test organisms (3) related to skin sensitization are taken into account

4. Default scale is Skin sensitization II (ECETOC)

5. **Click** OK to enter data gap filling

Data gap filling for active metabolite

Read-across

- The resulting plot is experimental results of all analogues (Y axis) according to a descriptor (X axis) with the default descriptor of log Kow (see next screen shot).
- The **RED** dot represents predicted results for the target chemical .
- The **BROWN** dots represent the experimental results available for the analogues that are used for the read-across.
- The **BLUE** dot represent the experimental results available for the analogues but not used for read-across.

Data gap filling for active metabolite Read-across

QSAR TOOLBOX

Input Profiling Endpoint Category Definition Data Gap Filling Report

The OECD QSAR Toolbox for Grouping Chemicals into Categories
Developed by LMC, Bulgaria

Filing
Apply

Data Gap Filling Method

- Read-across
- Trend analysis
- (Q)SAR models

Target Endpoint

Human Health Hazards Sensitisation Skin In Vivo

Structure

1 [target,transf. product] 2 3 4 5 6 7 8

In Vivo (73/159) M: Negative, Positive M: Negative M: Positive M: Negative, Negat... M: Negative, Positi... M: Positive, Positiv... M: Negative

Descriptors Prediction

Read across prediction of A B C, EC3, NOEL, S M W N, S W A N, Skin sensitisation, taking the highest mode from the nearest 5 neighbours, based on 6 values from 5 neighbour chemicals, Observed target value: N/A, Predicted target value: 'Positive'

Descriptor X: log Kow

Accept prediction
Return to matrix

- Select/filter data
- Selection navigation
- Gap filling approach
- Descriptors/data
- Model/(Q)SAR
- Calculation options
- Visual options
- Information
- Miscellaneous

75 Aldehydes (Acute toxicity) Strict (US-EPA New Chemical Categories) Create prediction by gap filling 0/1 1/1/0

Data Gap Filling

Selecting the Data Type

The screenshot displays the QSAR Toolbox interface during a data gap filling operation. The 'Data Gap Filling' tab is active, showing a list of chemical structures and their predicted values. The 'Prediction' plot shows a scatter plot of observed vs. predicted values for skin sensitisation. The 'Accept prediction' panel on the right is open, showing the 'Data usage' section where 'Maximal' is selected. The 'Calculation options' section is also visible, with 'Data usage' highlighted. The 'Miscellaneous' section shows 'Set usage of data per chemical' with 'Maximal' selected.

1. **Open** Calculation options; 2. **Select** Data usage
3. **Select** type of data to use (in this case we use Maximal value in order to apply worst case scenario);
4. **Click** OK

Data gap filling for active metabolite Subcategorization: Protein binding potency

1. Select filter data/subcategorize

2. Select Protein binding potency.

3. Eliminate dissimilar analogues.

75 Aldehydes (Acute toxicity) Strict (US-EPA New Chemical Categories)

Create prediction by gap filling

Data gap filling for active metabolite

Select: Protein binding alerts for skin sensitization by OASIS v1.4

The screenshot shows the 'Subcategorization' window in the QSAR Toolbox. On the left, under 'Endpoint Specific', the option 'Protein binding alerts for skin sensitization by OASIS v1.4' is highlighted with a red circle and a callout '1'. The central 'Target' section shows 'Michael Addition' with a callout '2'. Below it, the 'Analogues' section lists several entries related to Michael Addition. The right panel displays a chemical structure and a label 'M: Positive, Positive'. The bottom right panel contains 'Accept prediction' and 'Return to matrix' options.

1. Select Protein binding alerts for skin sensitization by OASIS v1.4. All the chemicals belong to Michael addition (MA) domain. In this case we are fine with the positive prediction of the metabolite and the obtained analogues of it. We will not eliminate dissimilar analogues due to the fact that they all belong to the same mechanistic domain (MA) **2.** Closed the window

Data gap filling for active metabolite

Results after subcategorization

The screenshot shows the QSAR Toolbox interface during a data gap filling process. The main workspace displays a table of predicted values for a target endpoint (Skin sensitisation) based on 4 values from 5 neighbour chemicals. The predicted value is 'Positive'. A red circle highlights this value, and a red arrow points to the 'Accept prediction' button in the right sidebar. A blue callout box '1' points to the predicted value, '2' points to the 'Accept prediction' button, '3' points to the 'OK' button in an information dialog box, and '4' points to the 'Return to matrix' button. An information dialog box is open in the center, displaying 'The current prediction was accepted'.

1. The predicted result is **positive** 2. **Accept prediction** 3. **Click OK** 4. **Return to matrix**

Data gap filling for active metabolite

Read-across prediction

The screenshot shows the QSAR Toolbox interface during a data gap filling process. The 'Data Gap Filling Method' is set to 'Read-across'. The 'Filter endpoint tree...' sidebar shows a tree structure of endpoints, with 'Sensitisation' expanded to 'Skin' and 'In Vivo'. The 'In Vivo' endpoint is circled in red. The main data matrix shows chemical structures in the first row and prediction results in the last row. The prediction for the circled 'In Vivo' endpoint is 'R: Positive', which is highlighted in blue. A text box at the bottom states: 'The read-across prediction result for metabolite appears on data matrix'.

The read-across prediction result for metabolite appears on data matrix

Data gap filling for active metabolite

Interpreting Read-across

- In this example, all analogues have same protein binding alerts
- All analogues exhibit positive skin sensitization
- The same positive sensitising potential is therefore predicted for the target (i.e. active metabolite).
- The prediction of metabolite is further transferred to the parent chemical using Independent MOA (see next screen shots)

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Endpoint
 - **Handling of skin metabolism of target chemical**
 - Profiling set of metabolites
 - Focus of active metabolite
 - Category definition for active metabolite
 - Data gap filling
 - **Assigning prediction of active metabolite to parent**

Handling skin metabolism of target chemical

Assigning data to parent chemical

The screenshot shows the QSAR Toolbox interface with the following components:

- Top Bar:** Includes the 'QSAR TOOLBOX' logo and navigation tabs for 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'.
- Document Panel (Left):** Shows a list of documents. The selected document is 'CAS: 28069-72-9'. A red circle highlights the '[set] Skin metabolism simulator' node, with a callout '2' pointing to it.
- Structure Panel (Middle-Left):** Displays the chemical structure of the target chemical.
- Filter Endpoint Tree (Middle-Left):** A hierarchical tree of endpoints. The 'Skin' endpoint is expanded, and the 'R: Positive' prediction result is highlighted with a red circle and callout '1'.
- Data Matrix (Right):** A table with columns representing different chemicals and rows representing different endpoints. The 'R: Positive' prediction is visible in the matrix.

1. The read-across prediction for the metabolite is positive then 2. **Select** data matrix of the target chemical: click over the node "[set] Skin metabolism simulator"

Handling skin metabolism of target chemical

Assigning data to parent chemical

The screenshot shows the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Data Gap Filling' menu item is highlighted with a callout '2'. The left sidebar shows a document tree with a selected chemical structure. The center panel shows a filter endpoint tree with 'Skin' selected. The right panel shows a data table with four columns representing different target types. The cell containing 'R: Positive' is circled in red and labeled with a callout '1'.

| | 1 [target] | 2 [target] | 3 [target,transf. product] | 4 [target,transf. product] |
|--------------------------------------|-------------------|-------------|----------------------------|----------------------------|
| Structure | | | | |
| Substance Identity | | | | |
| Physical Chemical Properties | | | | |
| Environmental Fate and Transport | | | | |
| Ecotoxicological Information | | | | |
| Human Health Hazards | | | | |
| Acute Toxicity | | | | |
| Bioaccumulation | | | | |
| Carcinogenicity | | | | |
| Developmental Toxicity / Teratoge... | | | | |
| Genetic Toxicity | | | | |
| Immunotoxicity | | | | |
| Irritation / Corrosion | | | | |
| Neurotoxicity | | | | |
| Photoinduced Toxicity | | | | |
| Repeated Dose Toxicity | | | | |
| Sensitisation | | | | |
| Skin | | | | |
| In Chemico | | | | |
| In Vitro | | | | |
| In Vivo | (3/3) M: Positive | M: Positive | R: Positive | |
| ToxCast | | | | |
| Toxicity to Reproduction | | | | |
| Toxicokinetics, Metabolism and Di... | | | | |
| Profile | | | | |

1. **Select** cell corresponding to skin sensitization 2. **Select** Data Gap Filling

Handling skin metabolism of target chemical

Assigning data to parent chemical

The screenshot shows the QSAR Toolbox interface during the 'Data Gap Filling' process. The 'Independent MOA' option is selected in the left sidebar. The main table displays chemical structures and their associated data. A red circle highlights the 'M: Positive' cell in the table. A blue callout box with the number '1' points to the 'Independent MOA' option, and another blue callout box with the number '2' points to the 'Apply' button in the top left.

| Filter endpoint tree... | 1 [target] | 2 [target] | 3 [target,transf. product] | 4 [target,transf. product] |
|--------------------------------------|------------|-------------|----------------------------|----------------------------|
| Structure | | | | |
| Substance Identity | | | | |
| Physical Chemical Properties | | | | |
| Environmental Fate and Transport | | | | |
| Ecotoxicological Information | | | | |
| Human Health Hazards | | | | |
| Acute Toxicity | | | | |
| Bioaccumulation | | | | |
| Carcinogenicity | | | | |
| Developmental Toxicity / Teratoge... | | | | |
| Genetic Toxicity | | | | |
| Immunotoxicity | | | | |
| Irritation / Corrosion | | | | |
| Neurotoxicity | | | | |
| Photoinduced Toxicity | | | | |
| Repeated Dose Toxicity | | | | |
| Sensitisation | | | | |
| Skin | | | | |
| In Chemico | | | | |
| In Vitro | | | | |
| In Vivo | (3/3) | M: Positive | M: Positive | R: Positive |
| ToxCast | | | | |
| Toxicity to Reproduction | | | | |
| Toxicokinetics, Metabolism and Di... | | | | |

1. Check Independent MOA **2. Click Apply**

Handling skin metabolism of target chemical

Assigning data to parent chemical

- The following actions (steps) are used for assigning data to parent chemical:
 - Accept prediction
 - Return to matrix
- Final prediction for the parent compound labeled as CI (Component based Independent mode) is **positive** (see next screen shot)

Handling skin metabolism of target chemical

Assigning data to parent chemical

The screenshot displays the QSAR Toolbox interface during a 'Data Gap Filling' operation. The left sidebar shows the 'Filter endpoint tree...' with 'Human Health Hazards Sensitisation Skin In Vivo' selected. The main workspace shows a table with four columns representing different chemical entities: '1 [target]', '2 [target]', '3 [target.transf. product]', and '4 [target.transf. product]'. The 'Structure' row contains chemical structures for each. The 'Human Health Hazards' section is expanded to 'Skin', which is further expanded to 'In Vivo'. The 'In Vivo' row shows '(3/4) M: Positive' and 'R: Positive'. A blue box highlights the 'M: Positive' prediction, and a red circle highlights the 'R: Positive' prediction. A curved arrow points from the 'R: Positive' prediction back to the 'M: Positive' prediction. A blue text box at the bottom states: 'The prediction for the metabolite is assigned to the parent chemical'.

Recap

- The target chemical **trans-2,cis-6-nonadienol** has been entered into the system.
- It has been profiled by Protein binding profilers; no protein binding has been found for target chemical.
- Positive experimental data has been retrieved for target chemical.
- Skin metabolism of target chemical is investigated. Two of simulated skin metabolites have positive protein binding alerts.
- These metabolites have similar protein binding alert: **α,β -unsaturated aldehydes**. One of the reactive metabolites is used for further read across analysis.
- No experimental data for the selected metabolite has been found, so the category of similar analogues has been defined.
- The initial group of analogues is defined by US-EPA New Chemical categories.
- 75 analogues including the target chemical are identified; they form a mechanistic category "**Aldehydes (Acute toxicity)**", which will be used for gap filling.
- Read-across is used for data gap filling.
- Protein potency categories have been used for refining the initial category.
- Positive skin sensitization has been predicted for the active metabolite.
- Positive prediction for reactive metabolite has been transferred to the parent chemical using Independent MOA.

Outlook

- Background
- Objectives
- The exercise
- **Workflow**
 - Input
 - Profiling
 - Endpoint
 - Handling of skin metabolism of target chemical
- **Report**

Report

- Remember the report module allows you to generate a report on the predictions performed with the Toolbox. This module contains predefined report templates as well as a template editor with which users can define their own user defined templates. The report can then be printed or saved in different formats.
- Generating the report is shown on next screenshots

Report

The screenshot displays the QSAR Toolbox interface during the 'Report' phase. The top navigation bar includes icons for Input, Profiling, Endpoint, Category Definition, Data Gap Filling, and Report. The left sidebar is set to 'Data Gap Filling Method' (Independent MOA, Similar MOA, Specific models) and 'Target Endpoint' (Human Health Hazards Sensitisation Skin In Vivo). The central 'Filter endpoint tree...' pane shows a hierarchical list of hazard categories, with 'Skin' expanded to show 'In Chemico', 'In Vitro', and 'In Vivo'. A context menu is open over the 'In Chemico' prediction, with 'Report' highlighted. A table on the right shows four columns for different target endpoints, each containing a chemical structure and a prediction result (e.g., 'M: Positive', 'C: Positive').

1. **Select** prediction
2. **Right Click** and **Select Report**

Report

The screenshot displays the QSAR Toolbox software interface during report generation. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Report' menu is active, showing options like 'Create', 'Print', 'Close', 'Save as', 'Register', 'Unregister', 'Update', 'Clone', and 'Design'. The left sidebar shows 'Available data to report' (Predictions, QSARs, Categories) and 'Available report templates' (Standard and Custom). The main report preview area shows the title 'QSAR Toolbox prediction for single chemical using metabolism' and a subtitle '(uses single component mode for handling of target chemical and its metabolites/transformation products)'. The report content includes a prediction for trans-2,cis-6-nonadienol and references to OECD and ECHA guidance documents.

Report

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes options like 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. Below the menu, there are sub-menus for 'Reports' and 'Repository'. The main window shows a report titled 'Prediction of S M W N; EC3; NOEL for trans-2,cis-6-nonadienol'. The report content includes a title, a subtitle, and a 'Summary' section. A callout box with the number '1' points to the 'Summary' section.

1

Summary

Toxicity of the target chemical (Positive) is predicted from its metabolites/transformation products using estimation based on 2 values (Positive x2) from 2 metabolites/transformation products having independent mode of action. Both experimental and predicted values for metabolites/transformation products are used in predicting the target toxicity.

Note that the information provided on the metabolites/transformation products is not linked to the possibility of their formation.

The target chemical FALLS within applicability domain of the prediction (see Section 4.3 for details).

The data used for calculating the current prediction is taken from 1 Toolbox prediction and 1 experimental value selected from the following database(s):

1. Skin sensitization

Below is a summary table for endpoint & descriptor values for the target chemical and the metabolites/transformation products.
Experimental values from data matrix are presented in bold font. Recalculated endpoint values (if required by selected data usage option in Gap Filling) are presented in italic font. Recalculated endpoint

1. Summary information for prediction

Report

Not provided by the user

4.2. Applicability domain (OECD Principle 3):

The target substance of current prediction is IN DOMAIN, because the target substances in all individual predictions are in domain
Below is the list of domain classification for the individual predictions (for details see the related prediction reports)

Individual component prediction No. 1:
Target substance is IN DOMAIN

4.4. Uncertainty of the prediction (OECD Principle 4): *manually editable field*

Not provided by the user

4.5. Chemical and biological mechanisms (OECD Principle 5):

QSAR Toolbox 3.4.0.17 QSAR TOOLBOX TPRF v.3.4.1.34101
Database version: 3.8.8/3.1.2

Prediction of S M W N; EC3; NOEL for trans-2,cis-6-nonadienol 9 / 33

Profiling results for the target substance:

DNA binding by OECD

No alert found

1. Applicability domain
The target chemical is "In domain", because the prediction of active metabolite is "In domain".

Outlook

- Background
- Objectives
- The exercise
- Workflow
 - Input
 - Profiling
 - Endpoint
 - Handling of skin metabolism of target chemical
 - Report
- **Save the prediction result**

Saving the prediction result

- This functionality allow storing/restoring the current state of Toolbox documents including loaded chemicals, experimental data, profiles, predictions etc, on the same computer. The functionality is implemented based on saving the sequence of actions that led to the current state of the Toolbox document and later executing these actions in the same sequence in order to get the same result(s).
- Saving/Loading the file with TB prediction is shown on next screenshots

Saving the prediction result

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Input', 'Profiling', 'Endpoint', 'Category Definition', 'Data Gap Filling', and 'Report'. The 'Report' menu is active, showing options like 'Create', 'Print', 'Close', 'Save as', 'Register', 'Unregister', 'Update', 'Clone', and 'Design'. A 'Save As' dialog box is open, showing the file name 'Tutorial 11.tbw' and the file type 'Toolbox work file (*.tbw)'. The background shows a prediction report for 'trans-2,cis-6-nonadienol' with a prediction of 'S M W N; EC3; NOEL'. A 'Save' button is highlighted in the dialog box.

1. Click on Save button; 2. Define name of the file; 3. Click Save button

Open saved file

The screenshot illustrates the steps to open a saved file in the QSAR Toolbox. The interface is divided into several panels: a menu bar at the top, a toolbar below it, a 'Documents' panel on the left, a 'Structure' panel in the center, and a 'Filter endpoint tree...' panel on the right. A 'Select file' dialog box is open, showing a file named 'Tutorial 11.tbw' selected. The 'Open' button in the dialog is highlighted.

1. Go to Input; 2. Click Open; 3. Find and select file; 4. Click Open

Once the file has been saved **1. Go** to Input; **2. Click** Open; **3. Find** and **select** file; **4. Click** Open