QSAR TOOLBOX

The OECD QSAR Toolbox for Grouping Chemicals into Categories

OECD QSAR Toolbox v.4.4.1

Step by step example on how to implement AOP information from Effectopedia Wizard to Toolbox

Outlook

- **Background**
- Specific Aims
- Overview of Effectopedia AOP
- Migrating the Effectopedia AOP to Toolbox
- Building a new AOP-based profiler in Toolbox for predictive purposes
- Application of migrated AOP for collecting weight-of-evidences

Background

- This is a step-by-step presentation designed to take the Toolbox user through the workflow on how to implement and use an already developed Effectopedia AOP within the Toolbox.
- Note:This tutorial only applies to those who run Toolbox in standalone/single user mode.

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Specific aims

This presentation demonstrates:

- Migration of an AOP built in the Effectopedia platform to the Toolbox*;
- configuring of the migrated AOP using functionalities of the Toolbox in order to be used for predictive purposes;
- the capabilities of implemented AOP for collecting weights of evidences – run an example chemical.

*** Note: The AOP used in the current example is just an illustrative example and it is not OECD approved.**

Outlook

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- **Overview of the used Effectopedia AOP**
- Migrating the Effectopedia AOP to Toolbox
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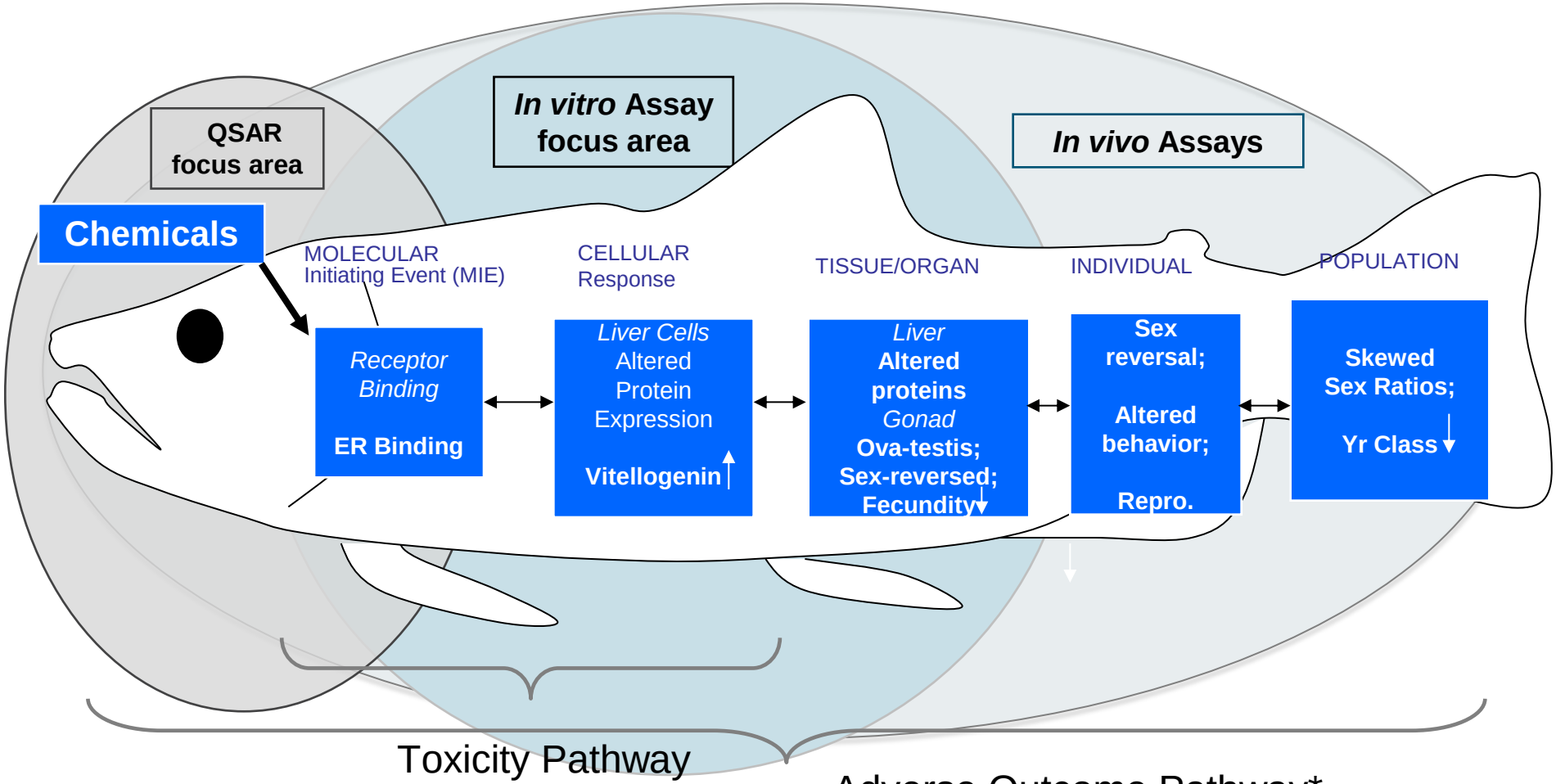
Overview of the used Effectopedia AOP

- An example AOP for assessing endocrine disruption (ED) of chemicals developed in Effectopedia platform will be used;
- The descriptive AOP was implemented based on an AOP cited by P. Schmieder et al, 2004;
- Graphical illustration of the AOP is shown on next slide.

Overview of the used Effectopedia AOP

*Visualization of an example AOP cited by Schmieder**



*An AOP cited by *P. Schmieder* et al, 2004

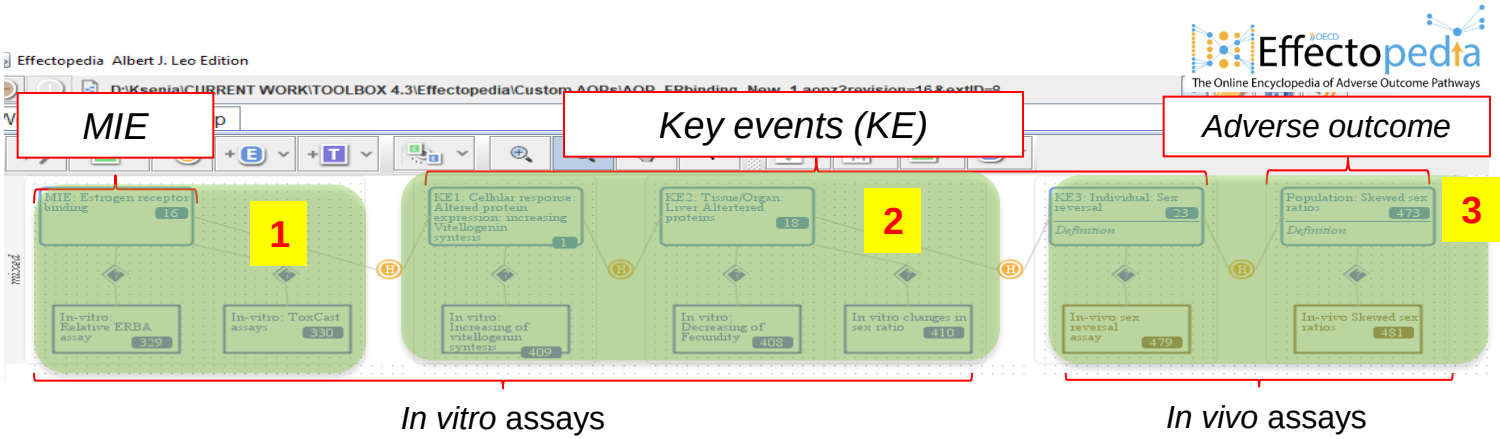
Overview of the used Effectopedia AOP

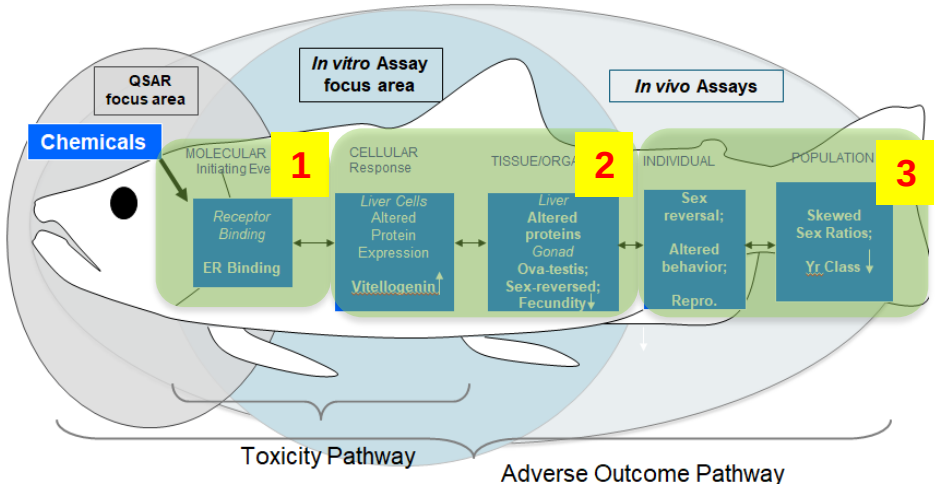
Visualization of AOP built in Effectopedia

- Each AOP node is associated with detailed information for definition and relevant AOP information (such as: test, references, associated pathways etc.)
- Short explanation of the Effectopedia AOP nodes is illustrated on the next few slides

Overview of the used Effectopedia AOP

Visualization of AOP built in Effectopedia

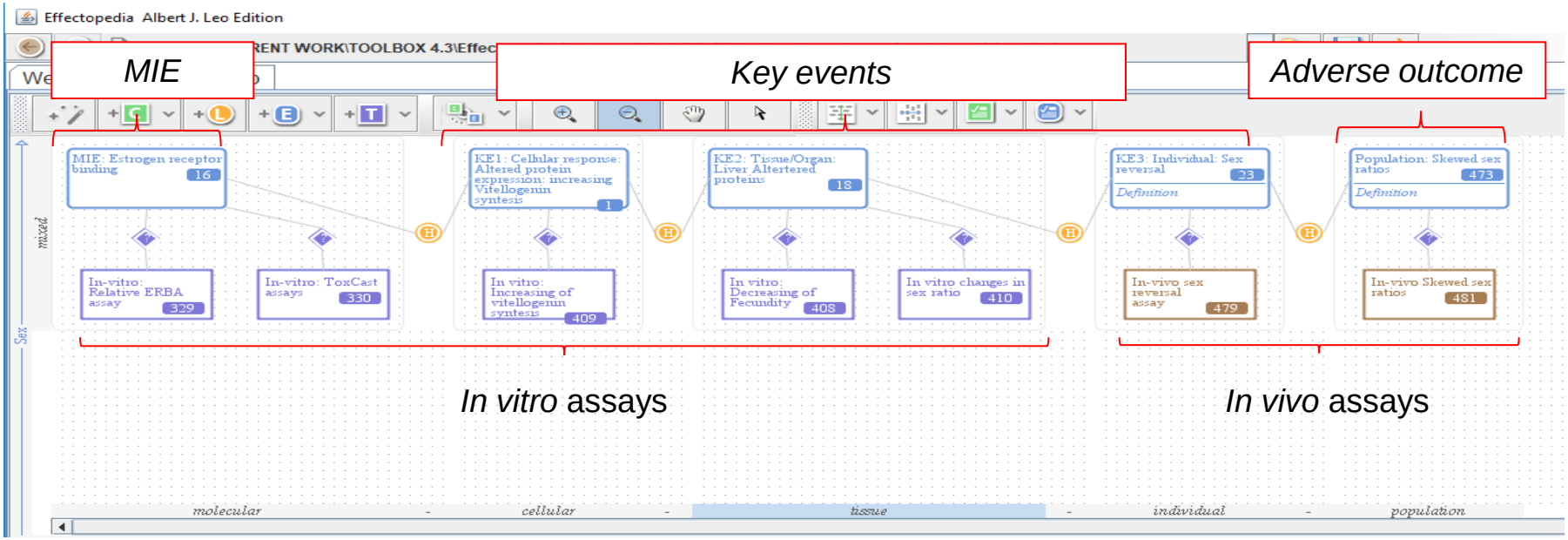




Node correspondence is given in green highlight.

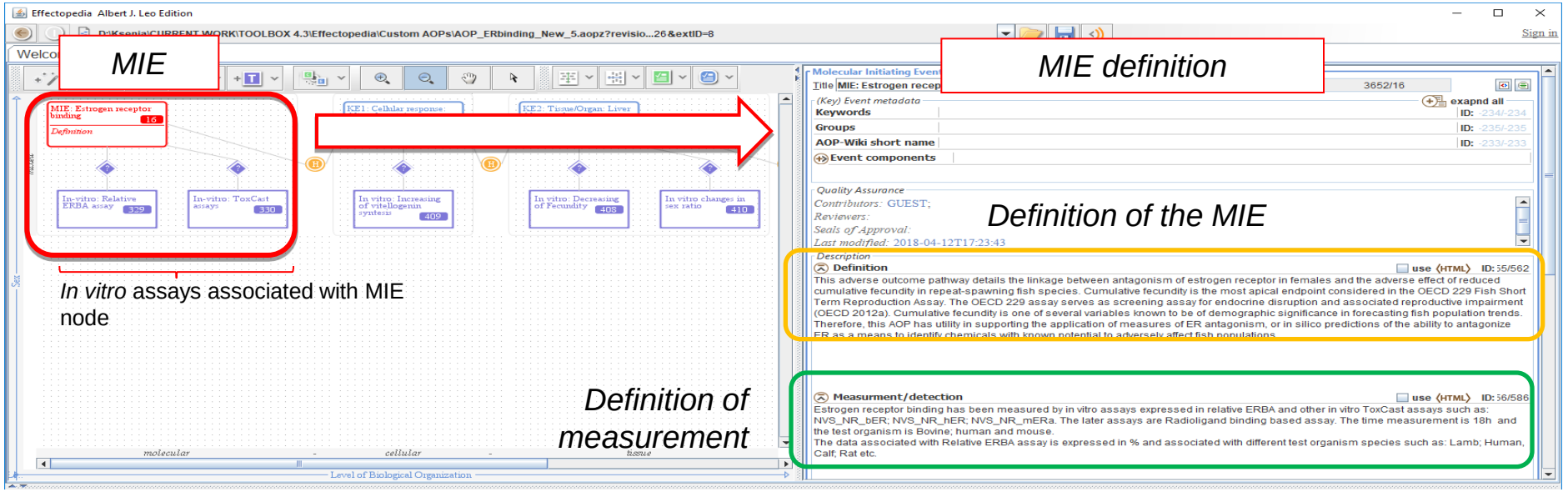
Overview of the used Effectopedia AOP

Visualization of AOP built in Effectopedia



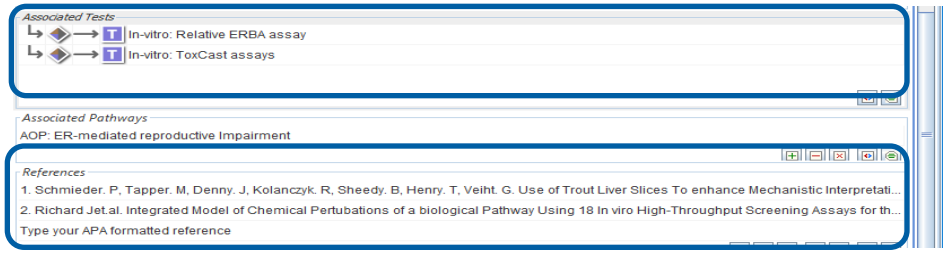
Overview of the used Effectopedia AOP

Visualization of AOP built in Effectopedia



Associated tests

References



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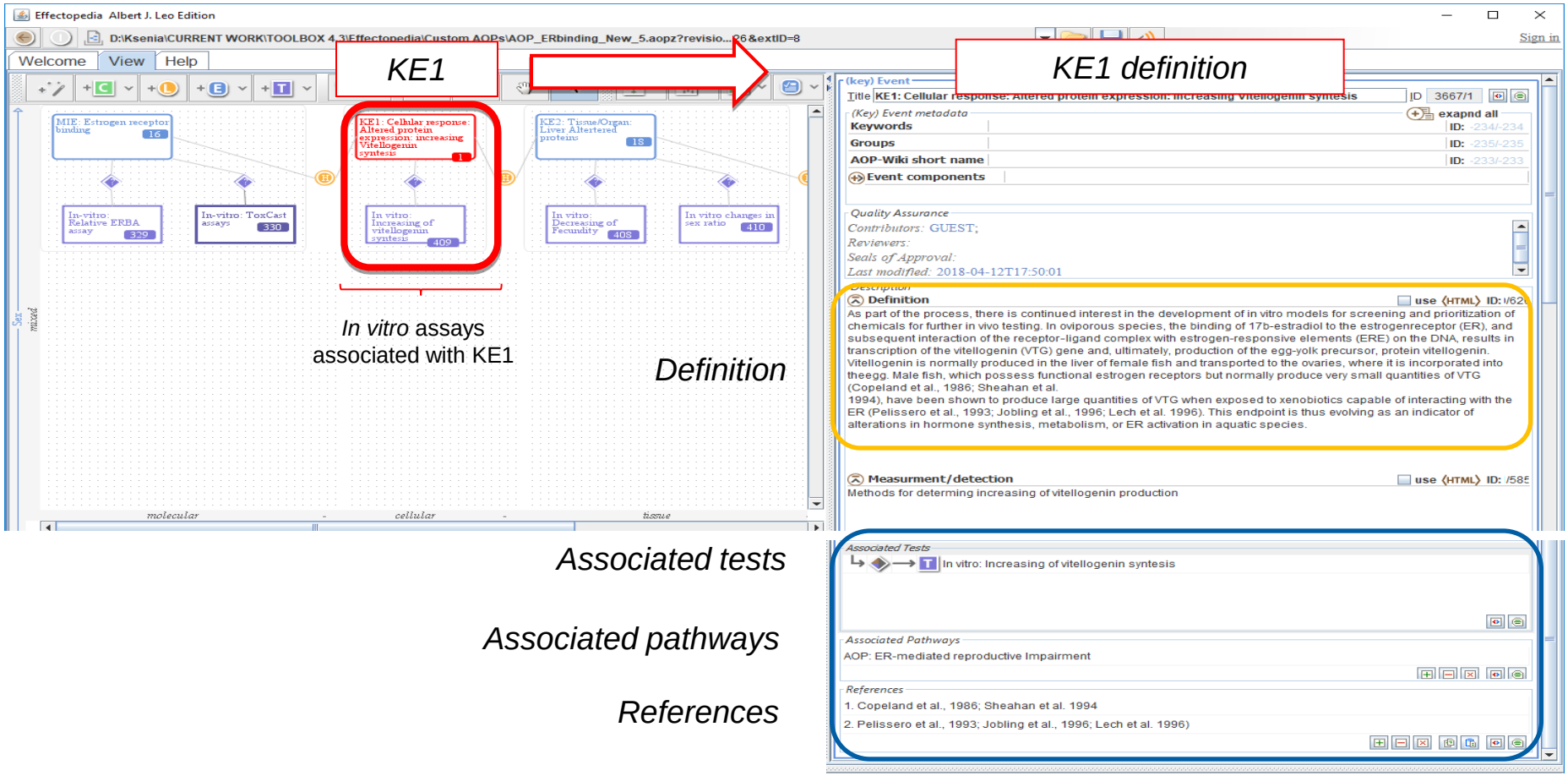
Overview of the used Effectopedia AOP

Visualization of AOP build in Effectopedia



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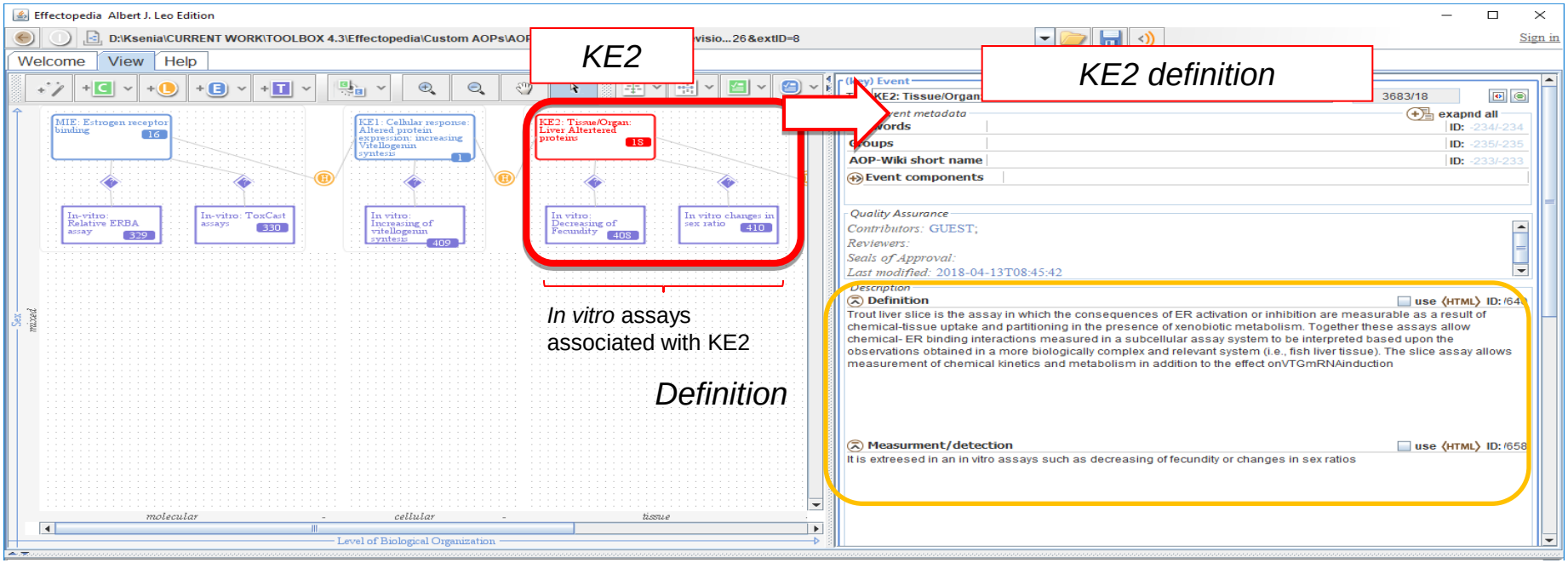
Overview of the used Effectopedia AOP

Visualization of AOP build in Effectopedia



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- Background
- Specific Aims
- Overview of the used Effectopedia AOP
- **Migrating the Effectopedia AOP to Toolbox**
- Building a new AOP-based profiler in Toolbox for predictive purposes
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Migrating the Effectopedia AOP to Toolbox

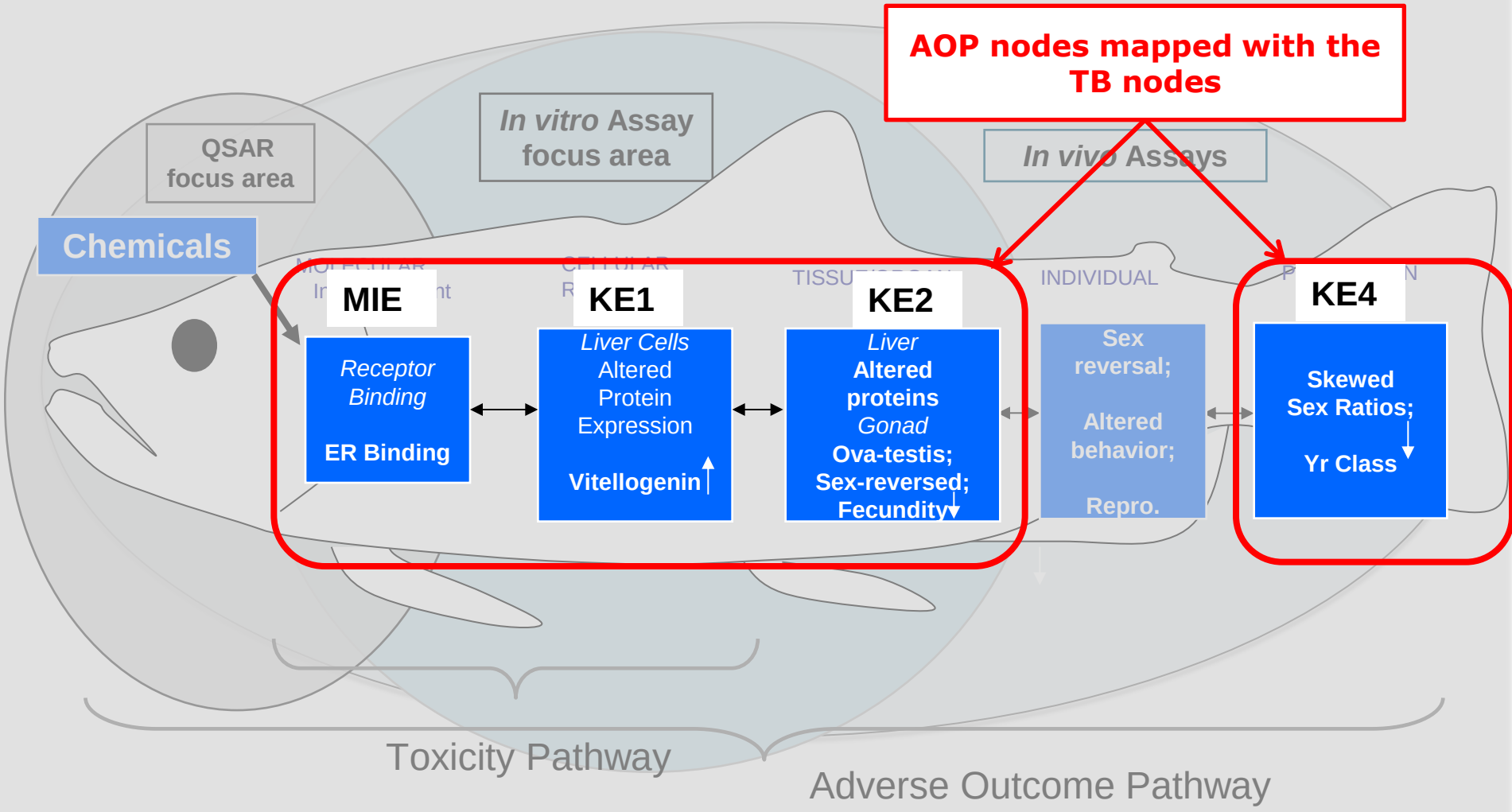
- The basic migration principle is the AOP nodes from Effectopedia AOP to coincide with the AOPs nodes from Toolbox (TB)
- Please note that this migration should be performed with good knowledge of the AOP and the key events in order to select the correct nodes in the Toolbox.

Migrating the Effectopedia AOP to Toolbox

- The most important TB fields needed to establish the correspondence between both type of nodes are:
  - Endpoint (data to be available for the endpoint of interest)
  - Data unit (unit of the endpoint data to coincide with unit of the data in Toolbox)
  - Profilers (profilers related to MIEs to be available)
- Preliminary analysis of the corresponding TB endpoints and profilers is performed for this purpose. Data is not available for all the Effectopedia AOP nodes. The AOP nodes mapped with the Toolbox nodes (data is available for the endpoints) are illustrated on the next few slide.

Migrating the Effectopedia AOP to Toolbox

Correspondence between nodes



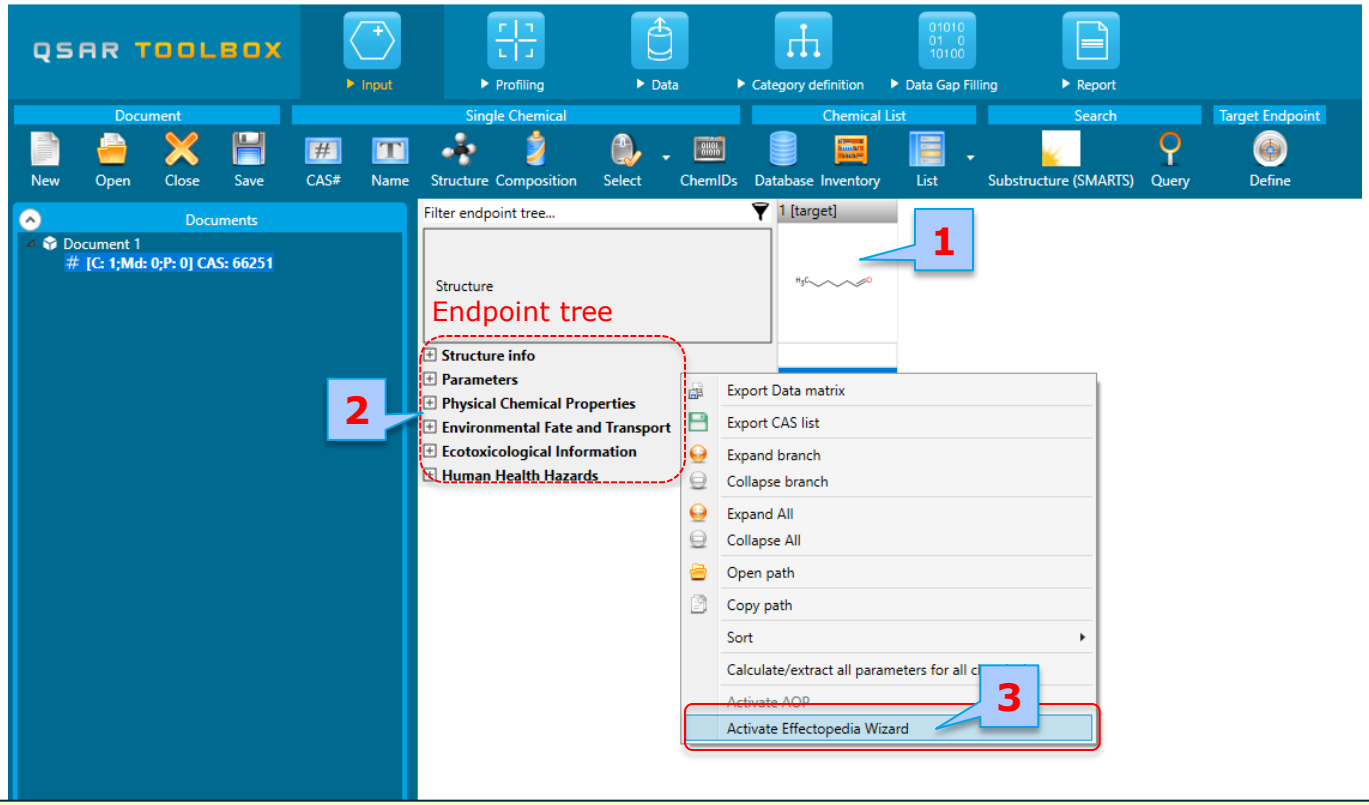
*An AOP cited by *P. Schmieder* et al, 2004

Migrating the Effectopedia AOP to Toolbox

- The migration process is a sequence of two simple steps illustrated on the next two slides

Migrating the Effectopedia AOP to Toolbox

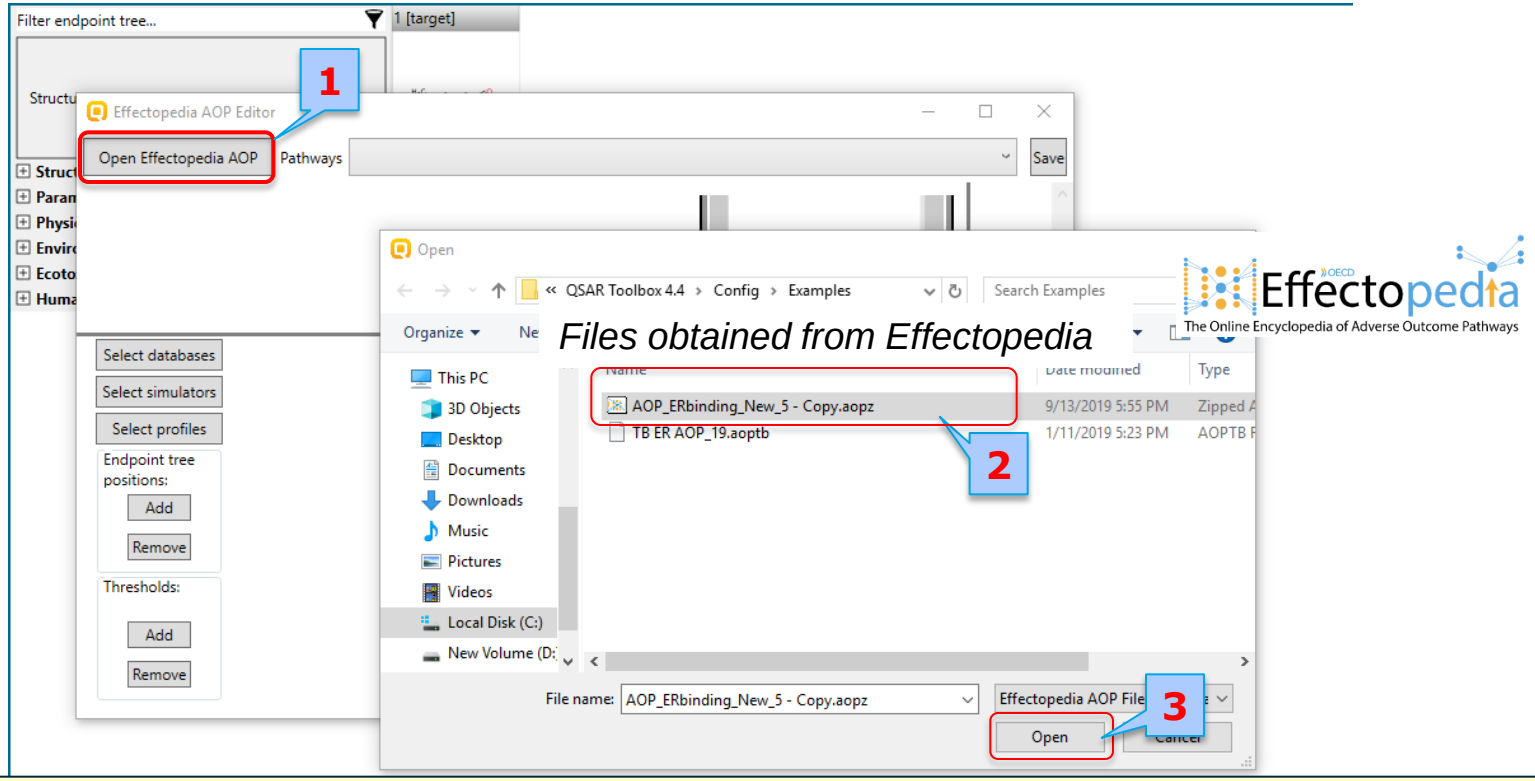
Step1: Activating the Effectopedia wizard



1. Once the Toolbox is opened, enter a chemical in the document (does not matter which one - this will not affect the exercise. The reason for this action is the endpoint tree to be visible on the screen); In this example we enter a chemical with CAS# 66-25-1;
2. Right-click over the endpoint tree;
3. Select **Activate Effectopedia Wizard.**

Migrating the Effectopedia AOP to Toolbox

Step2: Open the Effectopedia AOP

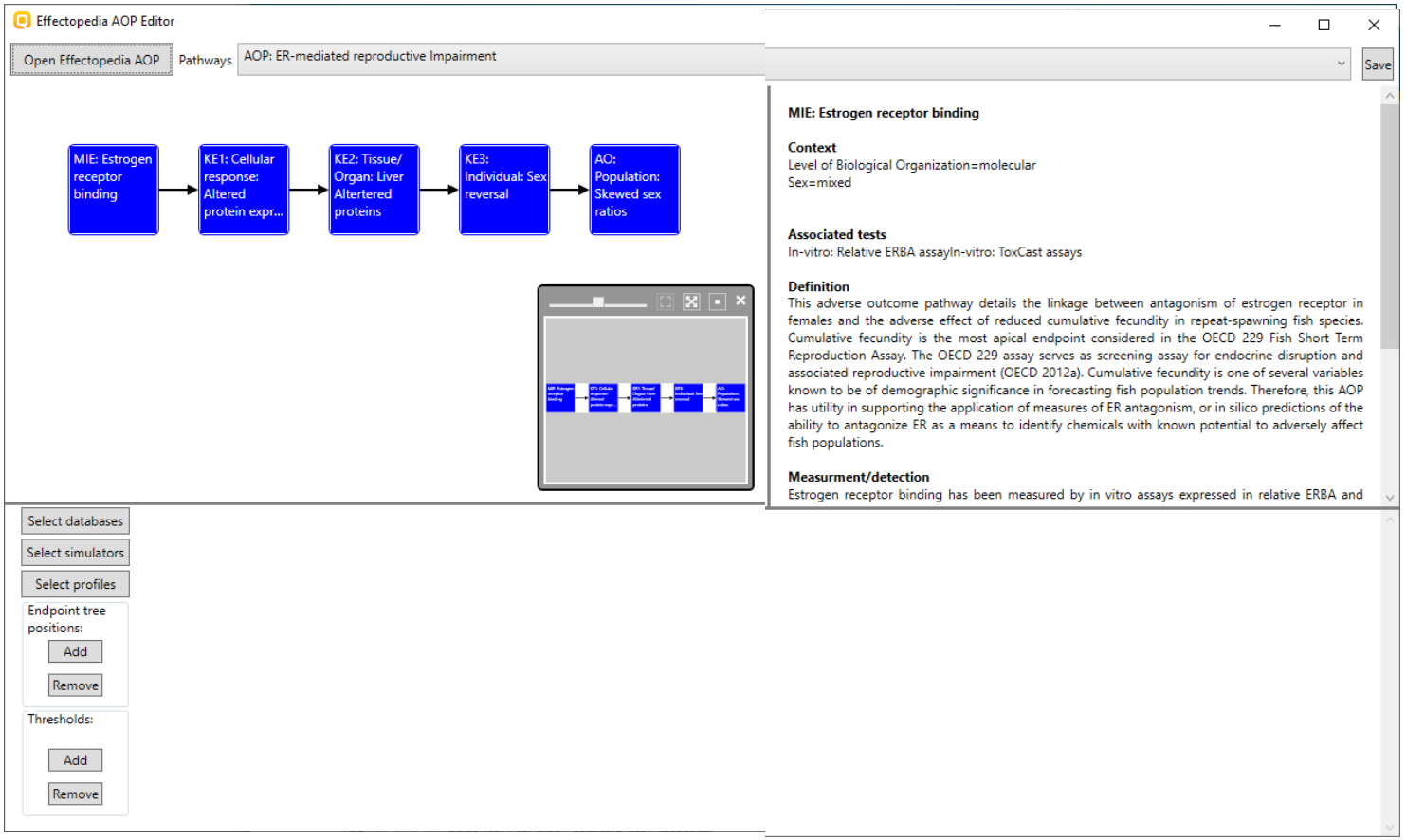


1. Click **Open Effectopedia AOP** and browse to the example folder (by default it is located here: *C:\Program Files (x86)\Common Files\QSAR Toolbox 4.4\Config\Examples*). An example AOP is available.;
2. Select the **"AOP Erbinding_New_5-Copy.aopz"** file;
3. Click **Open**;

The AOP is loaded in the editor (see next slide).

Migrating the Effectopedia AOP to Toolbox

Migrated Effectopedia AOP to the Effectopedia editor



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Configuring the Effectopedia AOP in Toolbox

Objectives

- After migration of the AOP into the TB, the process continues with configuring of the AOP nodes;
- Configuring is a mandatory process in order for the migrated AOP to be used for predictive purposes. Each node must be configured separately.
- AOP node is associated with configuration of the following TB objects:
  - Databases
  - Profilers
  - Simulators
  - Endpoints
  - Thresholds
- Each AOP nodes has three states:
  - Passed – chemical meets the criteria of the node
  - Not passed – chemical does not meet the criteria of the node
  - Not checked – rules are not applied to the chemicals

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Configuring the Effectopedia AOP in Toolbox

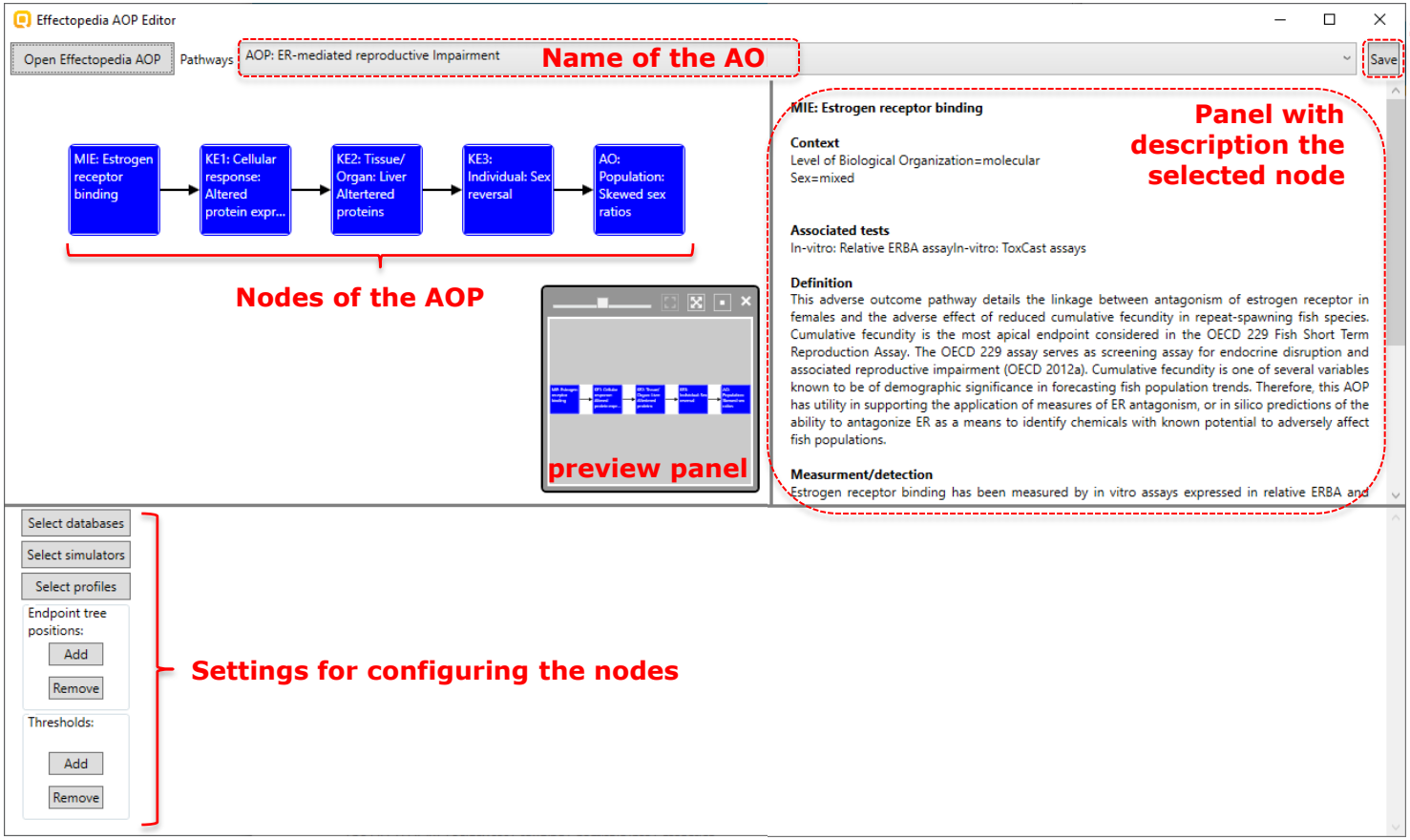
Objectives

- In order for the migrated AOP to be used for predictive purpose (pass/not pass) each node must be configured in terms of:
  - databases/profilers (specify which databases/profilers to be used in AOP)
  - selected endpoints (specify which endpoints from the selected database to be used for pass/not pass state)
  - thresholds for:
    - data (specify data threshold above/less which the node to be pass or not passed)
    - profilers (specify which categories from a selected profiler to be considered for pass or not pass state)
- As mentioned before, preliminary analysis of the profilers and endpoint data corresponding to the nodes of the Effectopedia AOP is done.
- Mapping is possible for four out of five AOP nodes (MIE, KE1, KE2 and AO nodes).
- The user should be aware of the different endpoint data and possible unit conversions in order to be able to arrange the nodes correctly.
- Configuring of each node with respect to the mentioned TB objects will be shown in the next slides.

Configuring the migrated AOP

Effectopedia AOP Editor

Migration of an AOP to Toolbox is possible via the Effectopedia Editor



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Solely for the purposes of the current tutorial it is proposed the following TB objectives to be considered for configuring MIE node as they are related to the Estrogen receptor binding endpoint:

1) Relevant databases:

- Receptor Mediated Effects;
- ToxCastDB;
- Toxicity to reproduction.

2) Relevant endpoints (associated with Toxicity to reproduction):

- Relative ERBA
- EC50 <OR> IC50
- AC50

3) Relevant Profilers:

- Estrogen Receptor Binding
- rtER Expert System – USEPA

4) Relevant thresholds for pass/not pass (positive/negative)

- for profilers:
  - categories associated with activity should be passed
  - categories associated with negative should be not passed
- for data: threshold for endpoints configured in point 2) need to be defined:
  - Data bigger than 0.001% relative ER (or AC50<0.000021 mg/l*) should be passed
  - Data less than 0.001% (or AC50>0.000021 mg/l*) should be not passed

*AC50 <2.10E-05 mg/l – arbitrary taken threshold

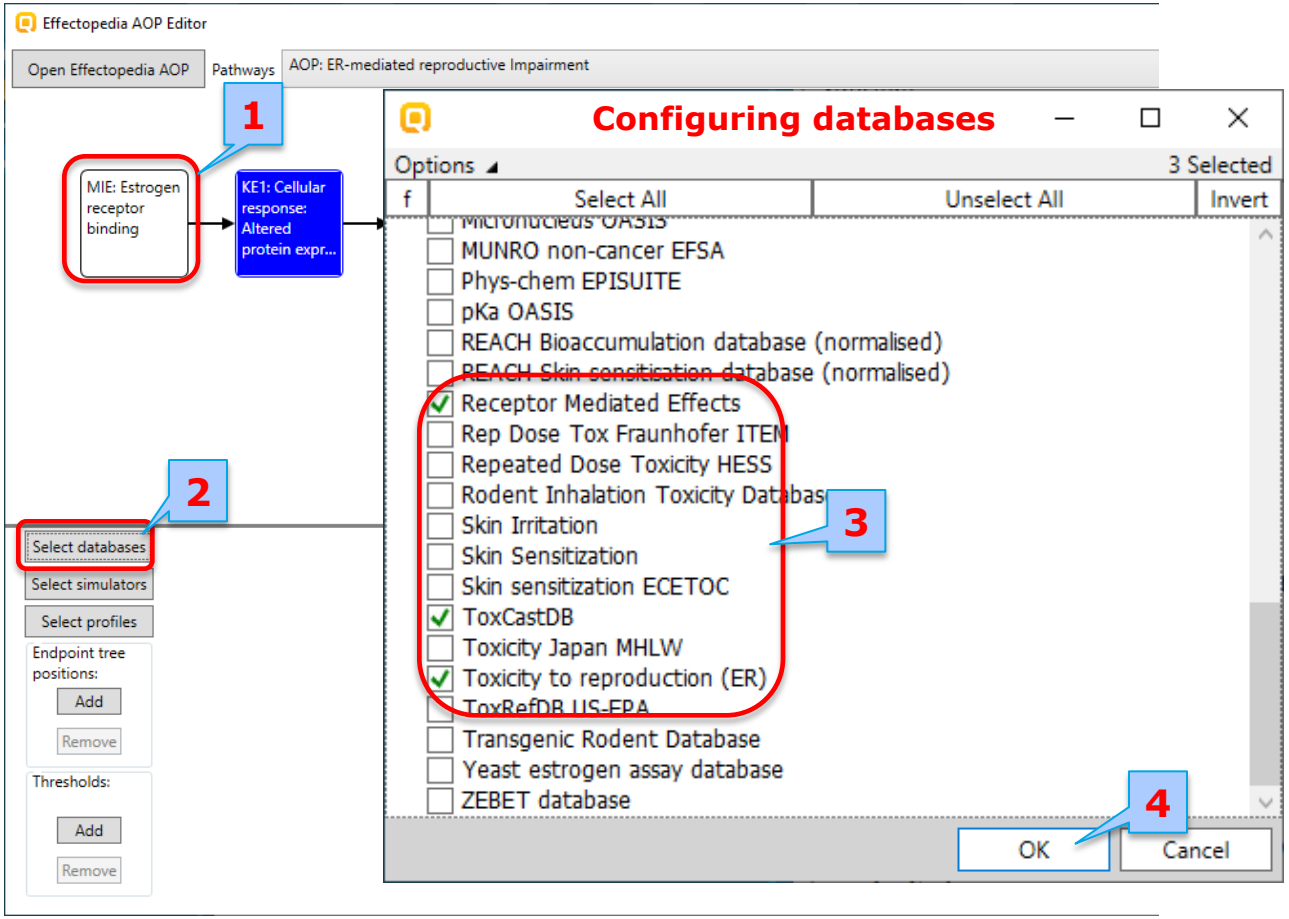
Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

1) Relevant databases:

- Receptor Mediated Effects;
- ToxCastDB;
- Toxicity to reproduction



1. Click the node (**MIE**) ;
2. Click **Select databases** button;
3. Select the following databases from the list: **ToxCast; Receptor Mediated Effects; Toxicity to reproduction;**
4. Click **OK**. Configuring of the databases is done.

Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

2) Relevant endpoints:

- ***Relative ERBA***
- EC50 <OR> IC50
- AC50

For defining the endpoints a specific endpoint path should be added, see the sequence of step illustrated on the right.

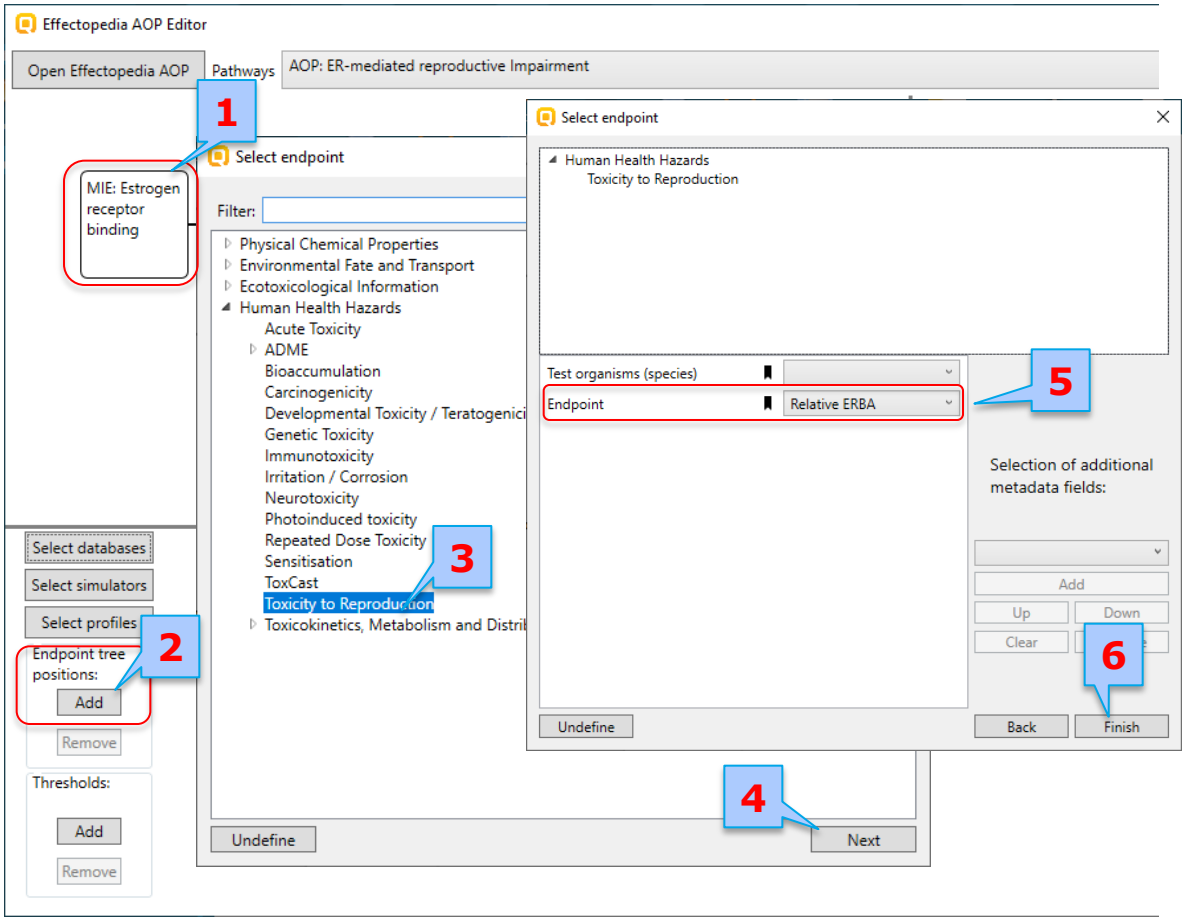
1. Keep the first node (MIE) selected;
2. Click **Add** to define the endpoint tree position;
3. Select **Toxicity to reproduction;**
4. Click **Next;**
5. Select **Relative ERBA** for endpoint;
6. Click **Finish (6).**

The first endpoint is configured, continue with second one.

Note: The AOP window is always minimized during the configuration of the nodes

Continues on next slide

Configuring endpoints: Relative ERBA



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

2) Relevant endpoints:

- Relative ERBA
- ***EC50 <OR> IC50***
- AC50

For defining the endpoints a specific endpoint path should be added, see the sequence of step illustrated on the right.

Repeat steps 2 and 3 from previous slide (the first step is done already);
4. Click button for **defining a list**;
5.Click **Select**;
From the predefined list with endpoints select **EC50 (6,7)** and **IC50 (8,9)**;
10. Click **OK;**
11. Click **Finish (11)**.
The second endpoint is configured, continue with the last one.

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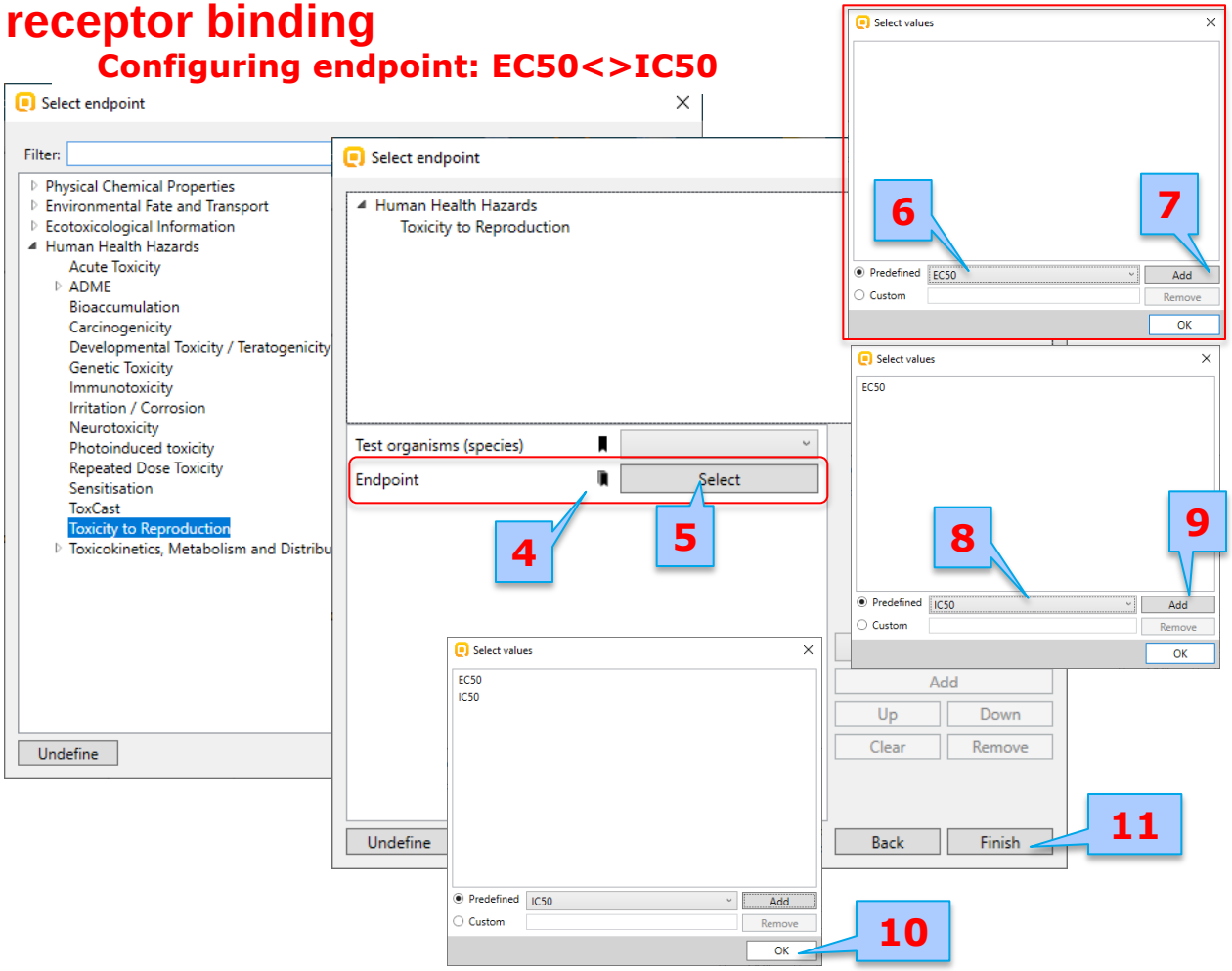
Configuring endpoint: EC50<>IC50



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

2) Relevant endpoints:

- Relative ERBA
- EC50 <OR> IC50
- ***AC50***

AC50 endpoints is associated with specific metadata information such as: assay; provider and species. For more details see the sequence of step illustrated on the right.

Repeat steps 1 and 2 from slide 29;
3. Select **ToxCast;**
4. Select endpoint **AC50;**
5.Consecutively add the following metadata: assay provider: **Novascreen**; assay: **Novascreen Bovine ER**; Test organism (species): **Bos Taurus;**
6. Click **Finish.**
The AC50 endpoint associated with assay "Novascreen Bovine ER" is configured. Moreover two additional assays related to AC50 endpoint need to be defined – see next slide.

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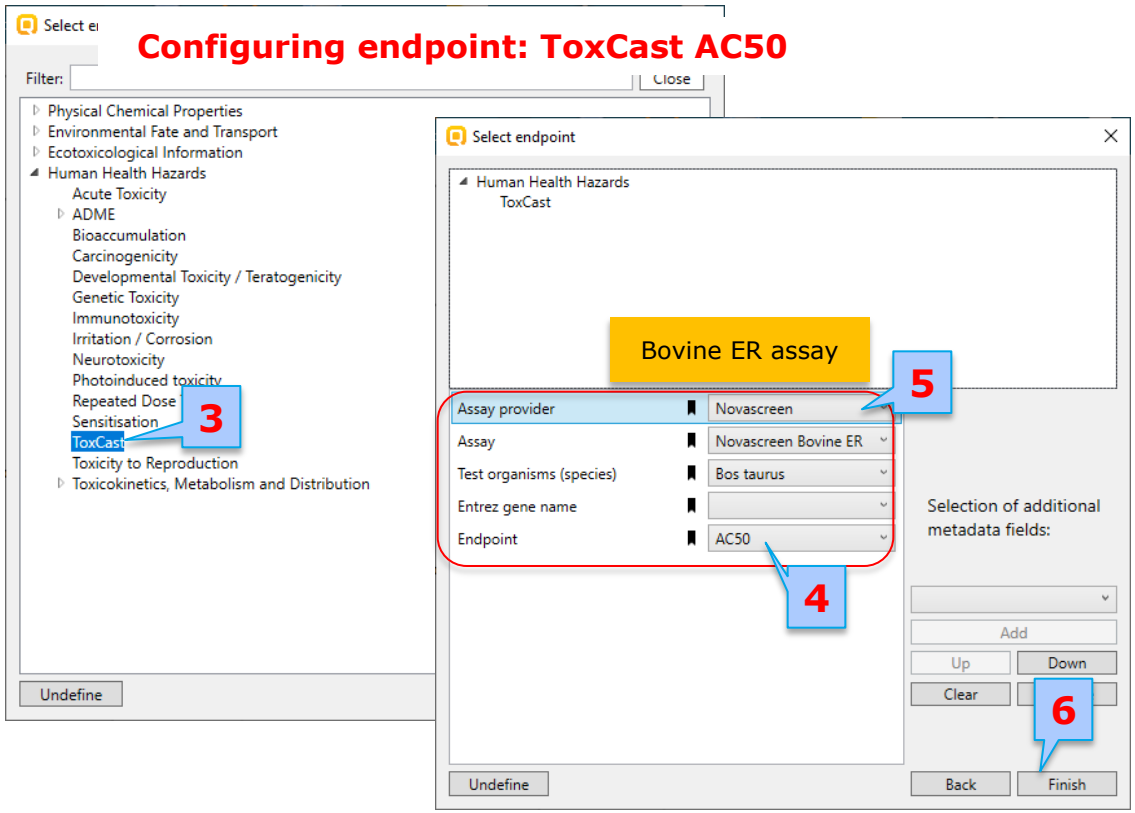
Configuring endpoint: ToxCast AC50



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

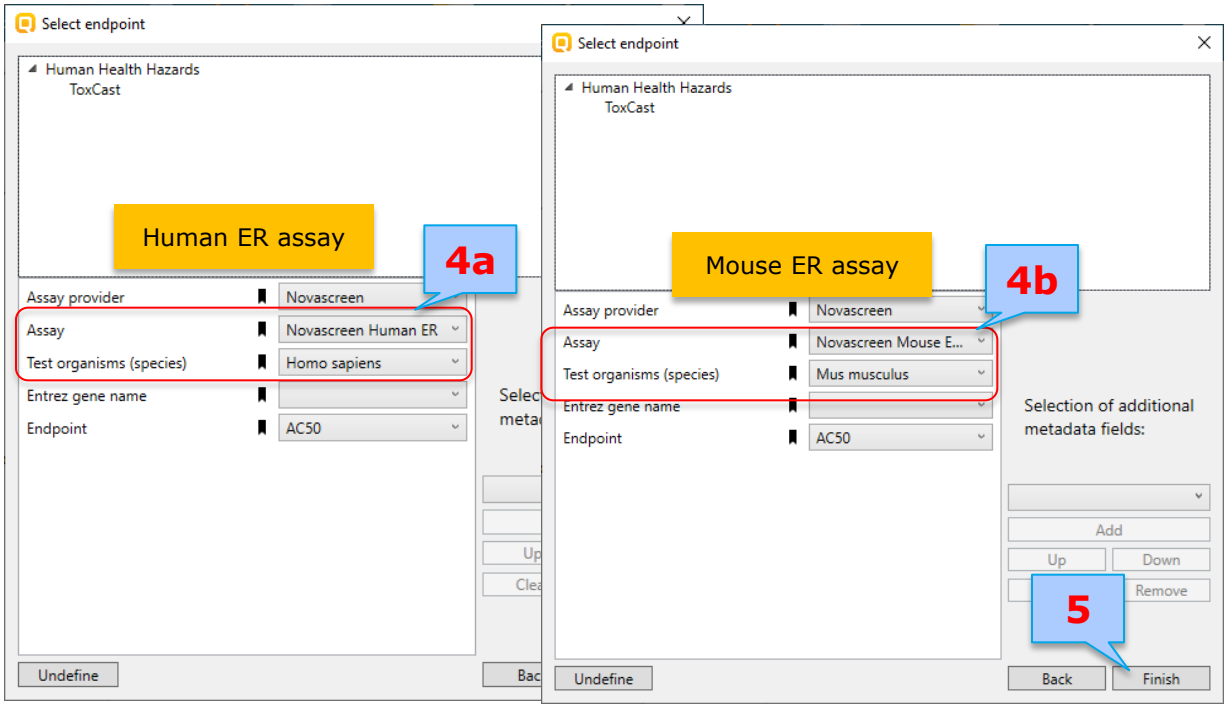
2) Relevant endpoints:

- Relative ERBA
- EC50 <OR> IC50
- ***AC50***

AC50 endpoints is associated with specific metadata information such as: assay; provider and species. For more details see the sequence of step illustrated on the right.

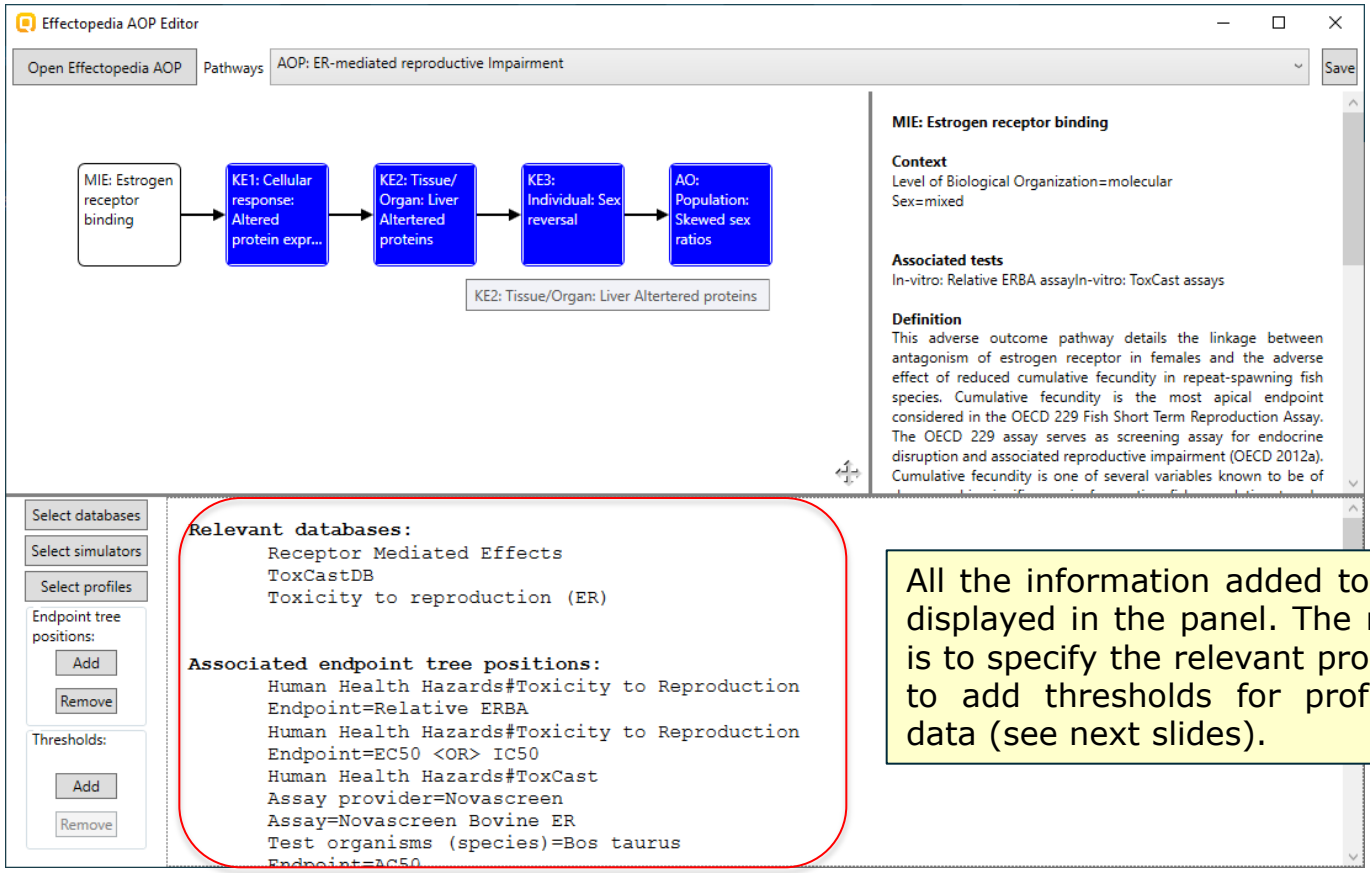
Repeat steps 1 and 2 and 3 from previous few slides including selecting ToxCast level. Once ready with selection of AC50 endpoint add the following metadata:
4a. assay: ***Novascreen Human ER***; species: ***Homo sapiens*** and one more assay:
4b. assay: ***Novascreen Mouse ERα***; species: ***Mus musculus***.
The difference between previous selection is the assay and test organism(species) fields;
5.Click **Finish.**

Configuring endpoint: ToxCast AC50



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding



All the information added to nodes is displayed in the panel. The next step is to specify the relevant profilers and to add thresholds for profilers and data (see next slides).

Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

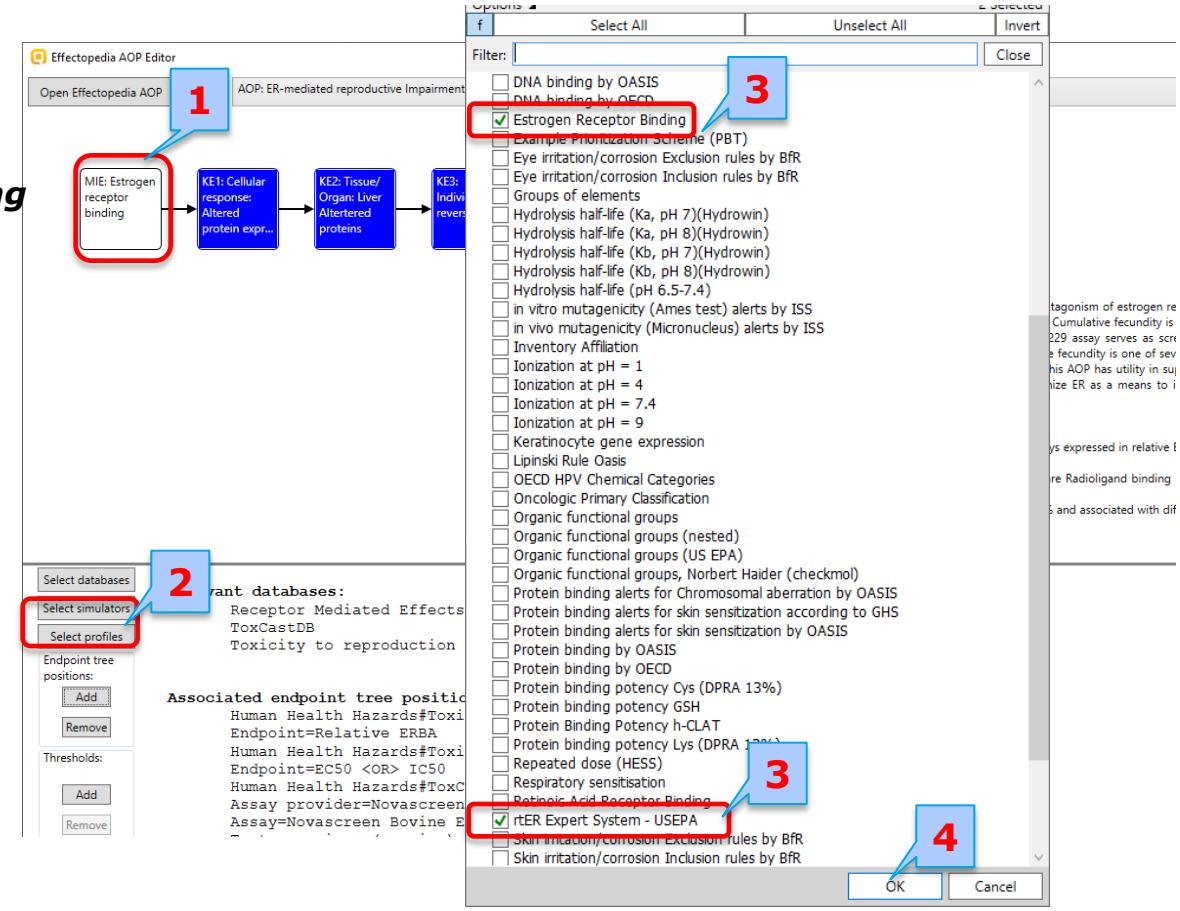
Configuring MIE node:

3) Relevant profilers:

- ***Estrogen Receptor Binding***
- ***rtER Expert System – USEPA***

1. Keep the first node selected;
2. Click **Select profilers**;
3. Select the profilers from the list: **Estrogen Receptor Binding; rtER Expert System – USEPA;**
4. Click **OK.**

Configuring the profilers



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

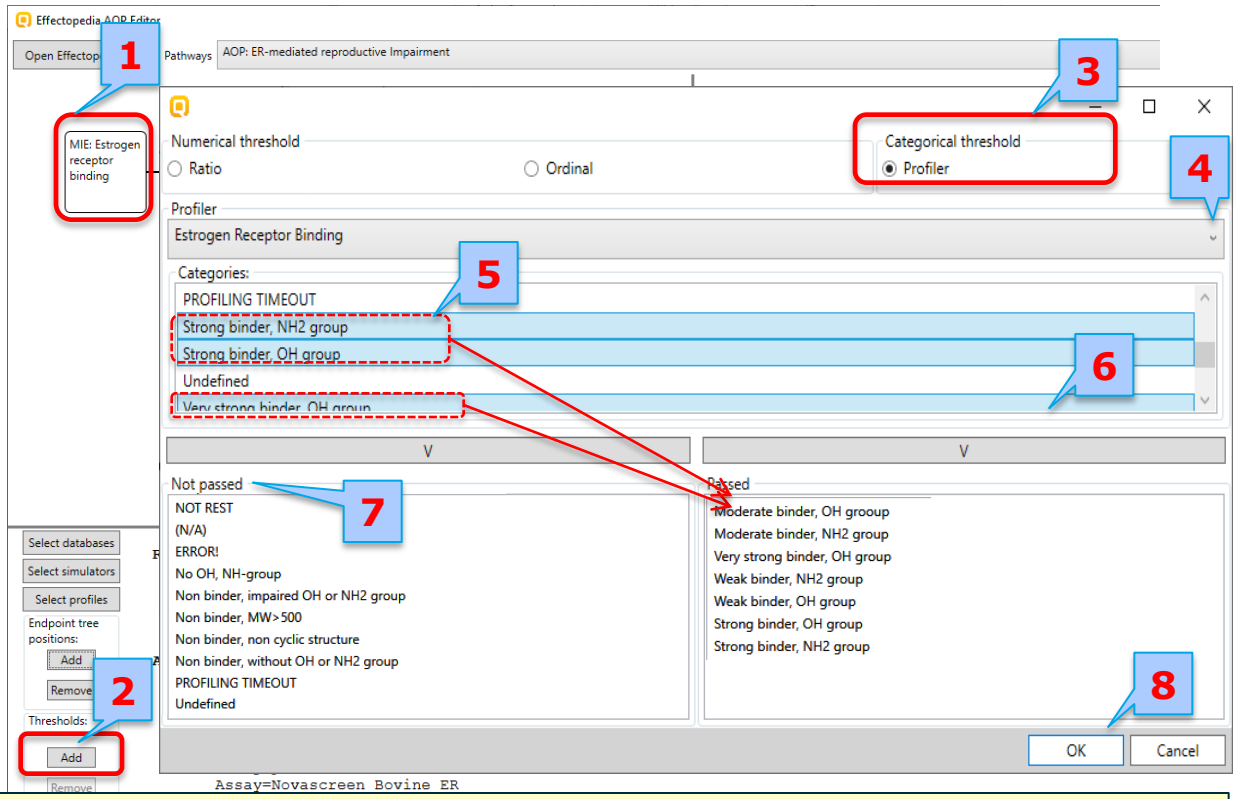
4) Threshold for the relevant profilers:

Profiler: Estrogen Receptor binding

- ***Passed:***
  - Strong binder, NH2 group;
  - Strong binder, OH group;
  - Very strong binder, OH group;
  - Moderate binder, NH2 group;
  - Moderate binder, OH group;
  - Weak binder, NH2 group;
  - Weak binder, OH group

- ***Not passed:***
  - No OH, NH-group
  - Non binder, impaired OH or NH2 group
  - Non binder, MW>500
  - Non binder, non cyclic structure
  - Non binder, without OH or NH2 group
  - Auxiliary categories (e.g. Profiling timeout)

Configuring thresholds for using the profilers



First node **(MIE)** is selected (1); Click **Add (2)** in the threshold section; Click on **Profiler (3)** radio button; Select **"Estrogen Receptor Binding"** profiler from the list (4); Select all categories related to the "pass" condition of the node (usually these are all categories related with activation). Select all by hold **Ctrl button** and click the **arrow button** (6), to move them in the panel **"Passed".** Repeat the same procedure with all categories associated with "not pass" condition of the node. They are moved to the panel **"Not passed"(7)**; Click **OK (8).**
Note: A category can be removed from the list by double click.

Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

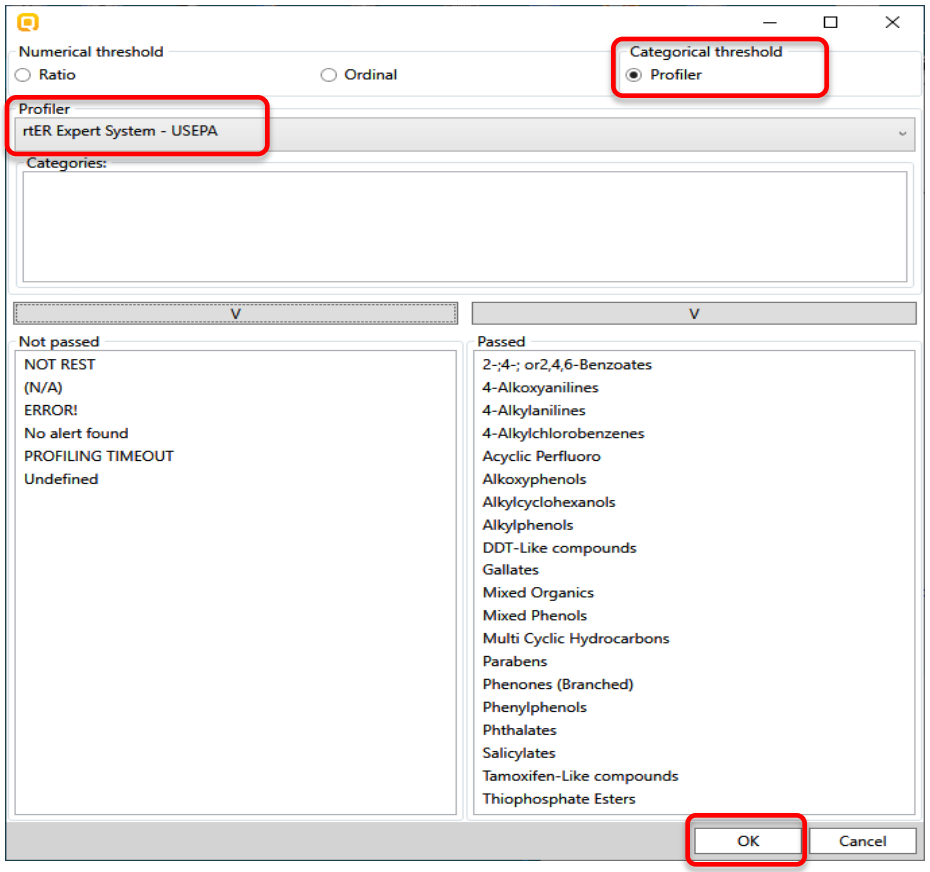
Configuring MIE node:

4) Threshold for the relevant profilers:

Profiler: rtER Expert System – USEPA

-***Passed*:**
- all categories except No alert found and Auxiliary categories (e.g. Profiling timeout)

-***Not passed:***
- No alert found category
- Auxiliary categories (e.g. Profiling timeout)



Repeat the same procedure as described in the previous slide, but related to the profiler **"rtER Expert System – USEPA".** The snapshot illustrates already configured profiler. Keep in mind that all categories except "No alert found" and auxiliary categories (e.g. Profiling timeout) are moved to "Passed" panel. Once ready click **OK**

Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

4) Data thresholds:

ER data is associated with different scales. In this respect configuration is performed for each individual scale:

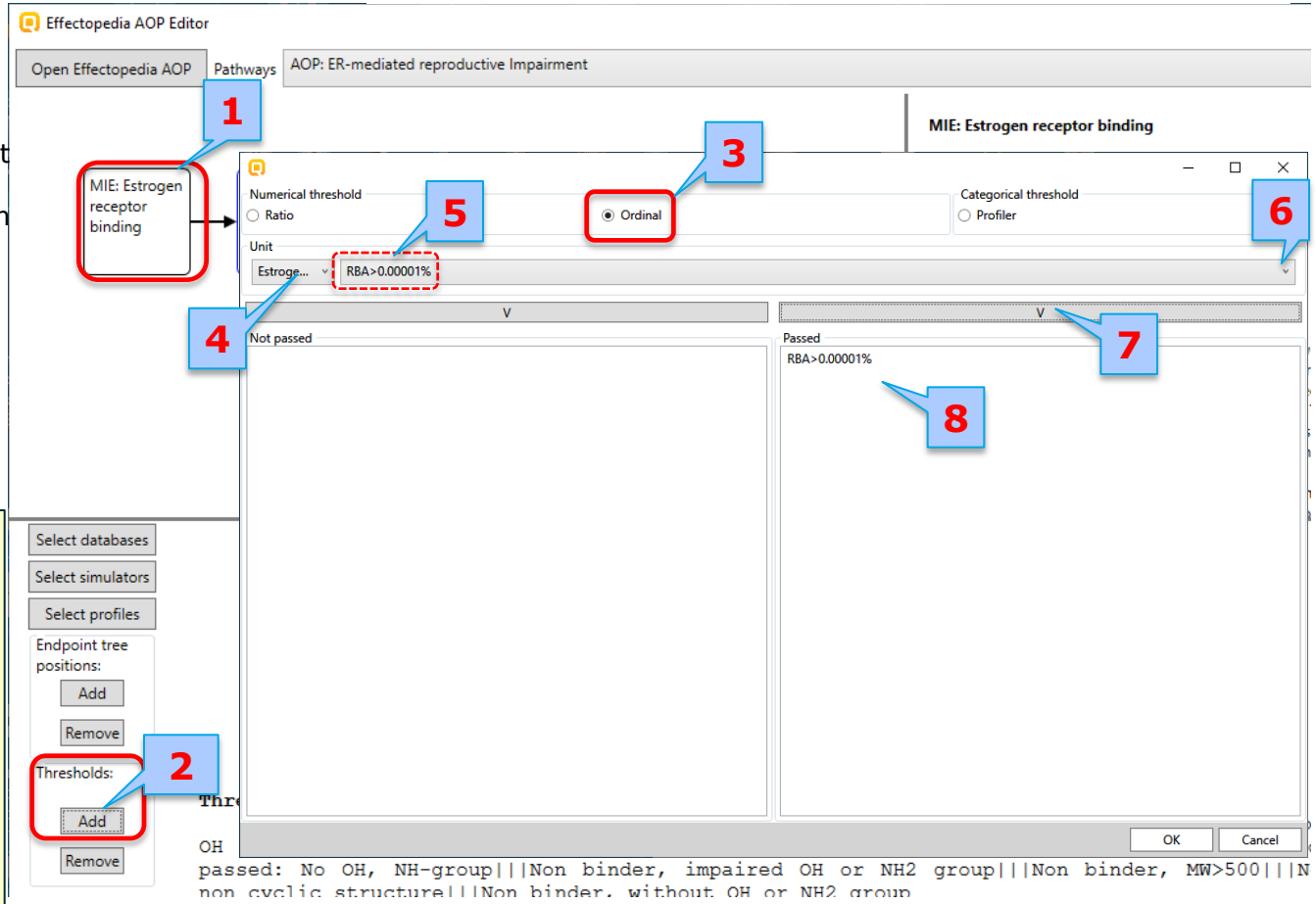
Scale: "Estrogen Binding Affinity(USA)"

- ***Passed:***
  - RBA>0.00001%
- ***Not passed***
  - RBA<0.00001%

1. Select node MIE;
2. Click **Add** in the threshold section;
3. Select radio button **"Ordinal"**.
4. Select scale **"Estrogen Binding Affinity(USA)"** from the list with units.
5. Select **"RBA>0.00001%"** from the drop-down menu (6).

7. Click "V" button to moved it to the **"Passed"** panel (8).

Configuring thresholds for data



Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

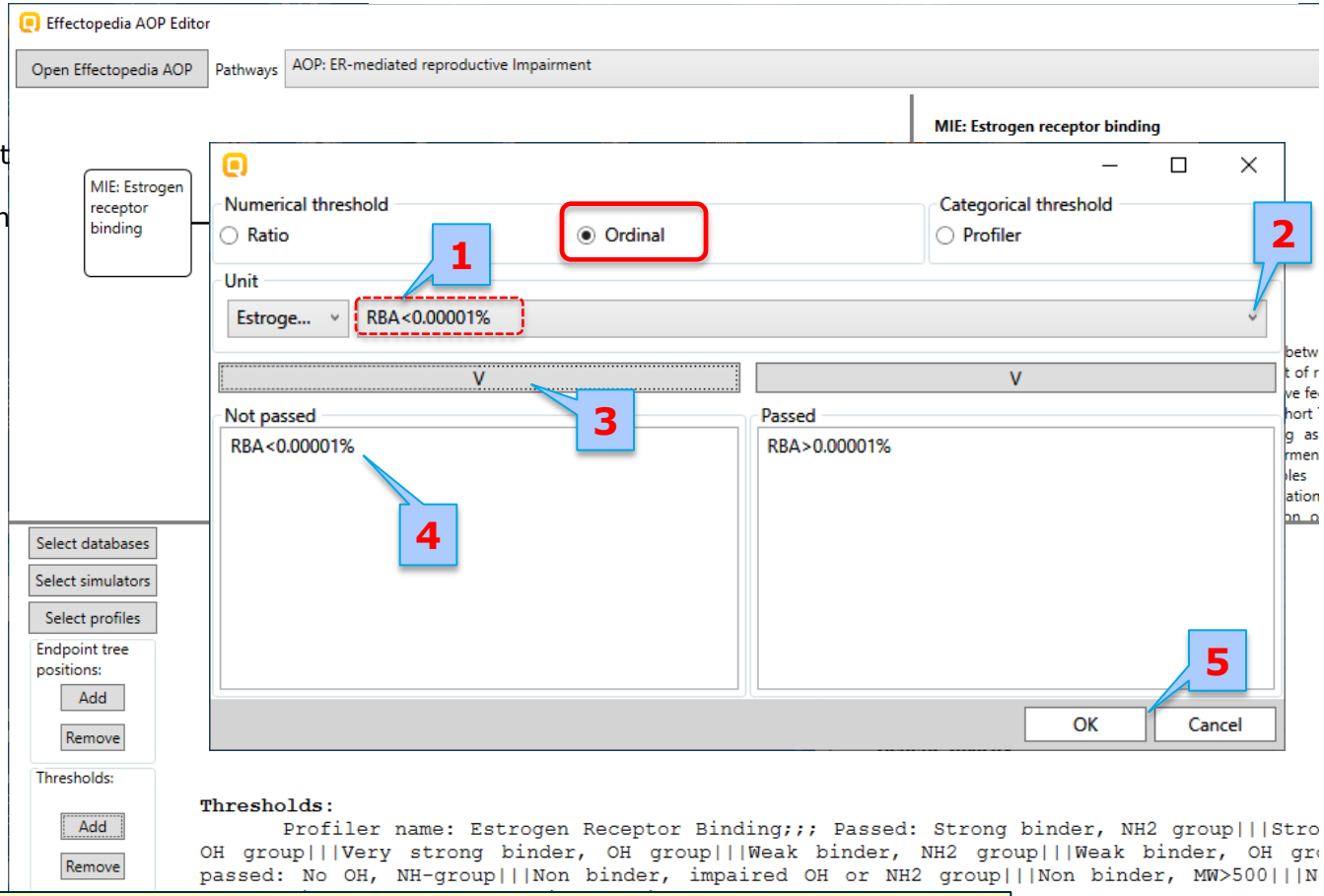
4) Data thresholds:

ER data is associated with different scales. In this respect configuration is performed for each individual scale:

Scale: "Estrogen Binding Affinity(USA)"

- ***Passed:***
  - RBA>0.00001%
- ***Not passed***
  - RBA<0.00001%

Configuring thresholds for data



Select **"RBA<0.00001%"** (1) from the drop-down menu (2).
Click "V" button (3) to moved it to the **"Not Passed"** panel (4).
Finally click **OK** (5).

Configuring the migrated AOP

Node: MIE – Estrogen receptor binding

Configuring MIE node:

4) Data thresholds:
ER data is associated with different scales. In this respect configuration is performed for each individual scale

Scale: Estrogen Binding Affinity(OASIS II)":

Passed:
- 0.001<RBA<0.1%
- 0.1<RBA<10%
  0<RBA<0.001%
- RBA>10%

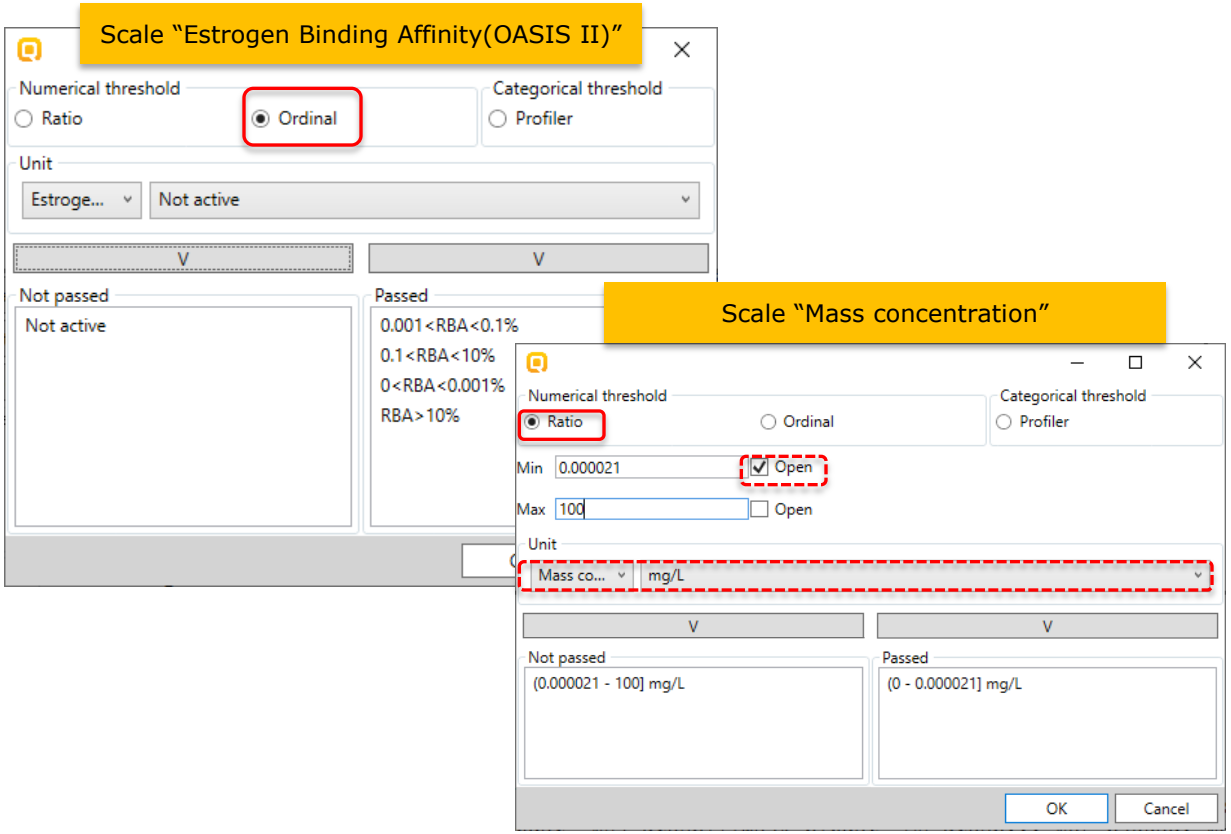
Not passed:
- Not active

Scale: "Mass concentration":

Passed:
- (0 - 0.000021] mg/L

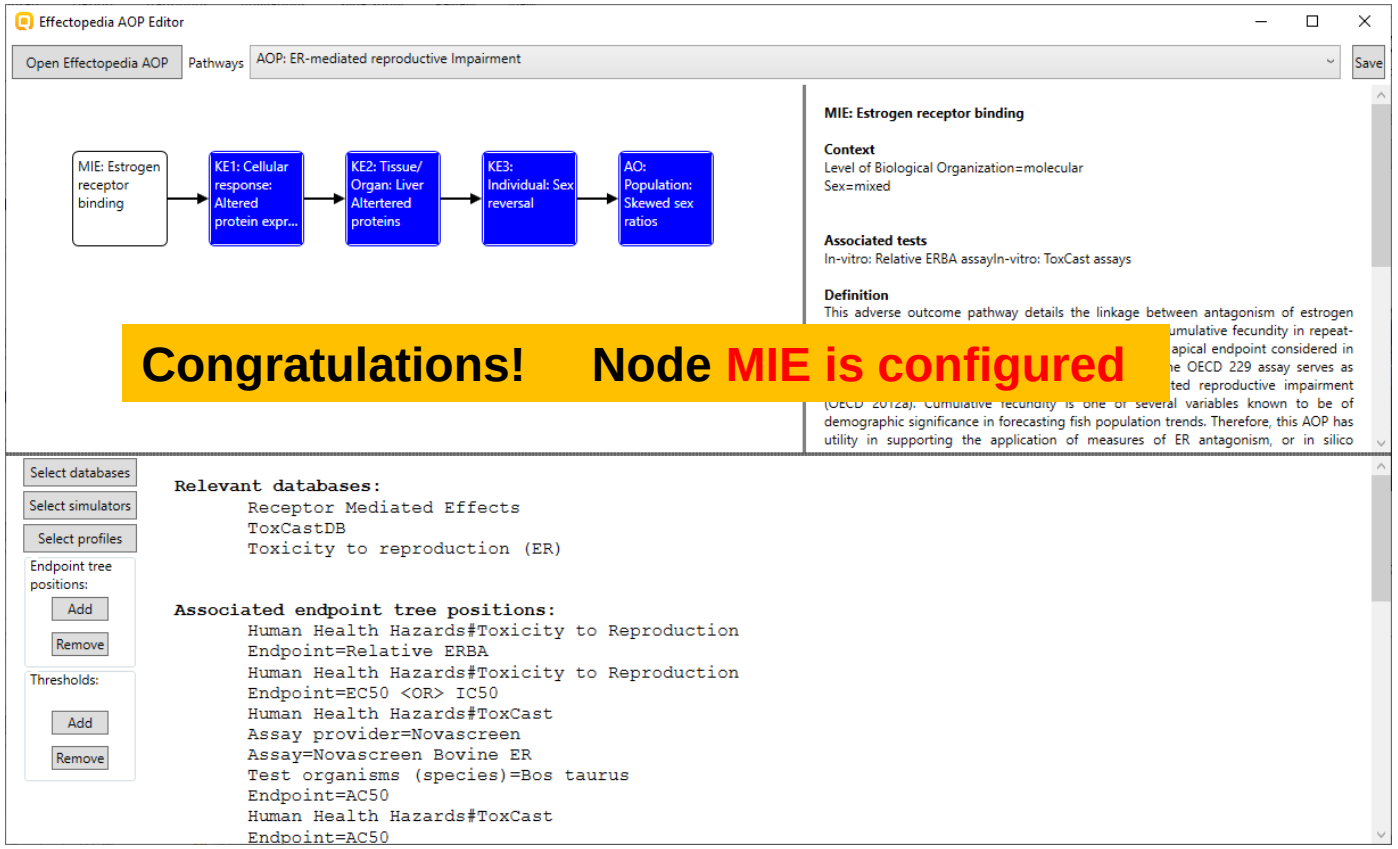
Not passed:
- (0.000021 - 100] mg/L

Configuring thresholds for data



Repeat steps from 1-2 from previous slide. The **"Ordinal"** radio button should be selected, when scale **"Estrogen Binding Affinity(OASIS II)"** is configured and **"Ratio"** when scale **"Mass concentration"** is used. Then add the members of the scale to passed and not passed panels as shown on the picture. Keep in mind for sign of the ranges when define members of the "Mass concentration scale" (the box "Open" should be checked when the interval is open ")" and not checked when the interval is closed "]"). Data in "Mass concentration" use "mg/l" unit. Once ready click **OK.** Configuring the node with the thresholds is ready.

Configuring the migrated AOP



Configuring the migrated AOP

Node KE1 - Cellular response: Altered protein expression: increasing Vitellogenin syntesis

The following TB objectives need to be considered for configuring KE1 node in order to work correctly:

1) Relevant databases:
- ECOTOX;

2) Relevant endpoints (related to Acute aquatic toxicity endpoint tree):
- LOEC:
  - Effect: Biochemistry
  - Measurement: Vitellogenin
  - Trend: Increasing
- NOEC:
  - Effect: Biochemistry
  - Measurement: Vitellogenin
  - Trend: Increasing

3) Relevant Profilers:
- No relevant profilers

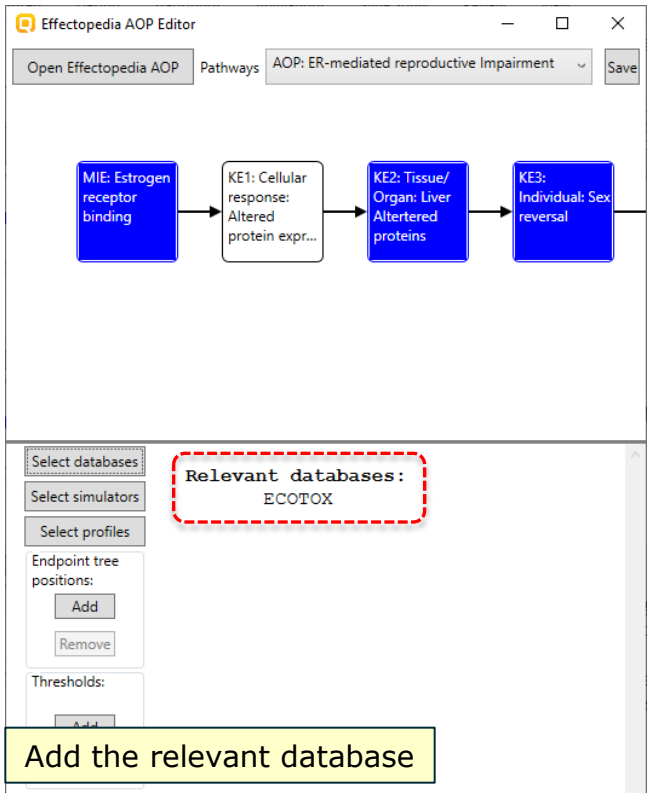
4) Relevant thresholds for pass/not pass (positive/negative)
- Chemicals with data in the range [0 - 1) mg/L should be passed
- Chemicals with data in the range [1 - 1000] mg/l should be not passed

Note: The final outcome of the configuring the nodes is illustrated in the forthcoming slides. For configuring the nodes please use the steps illustrated on the previous slides.

Configuring the migrated AOP

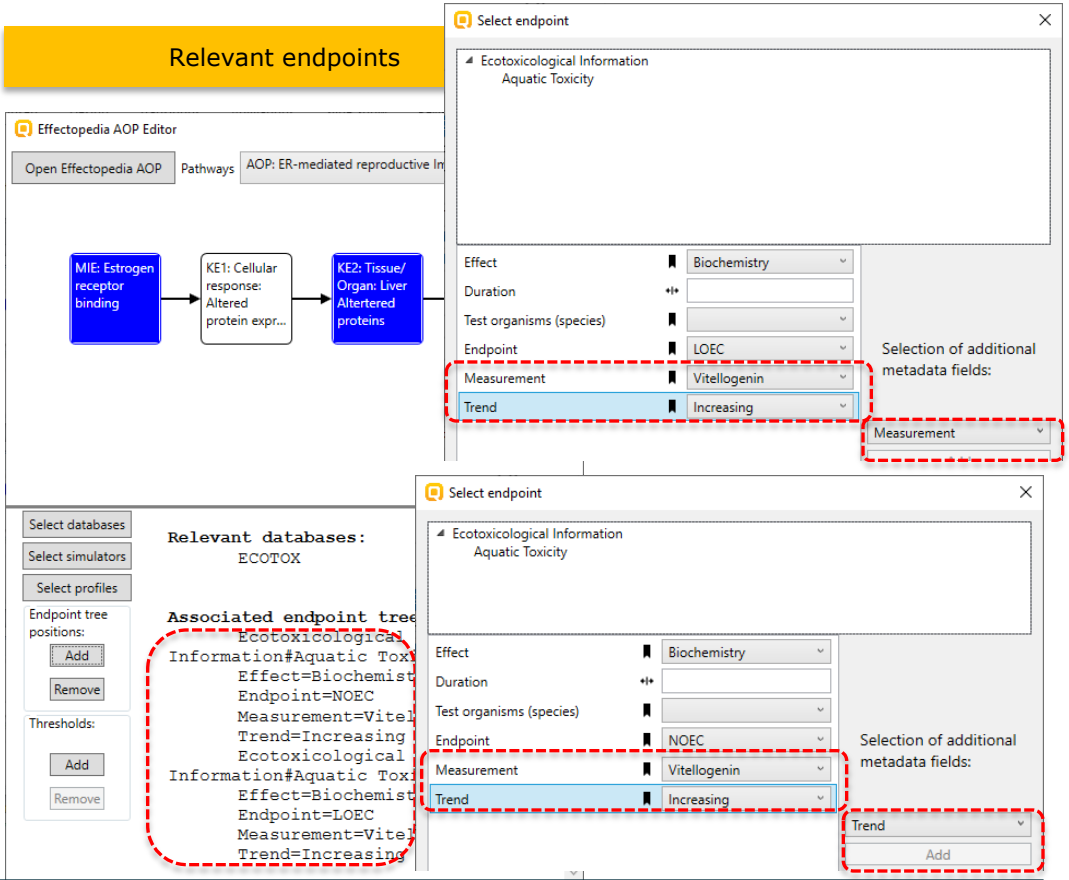
Node KE1 - Cellular response: Altered protein expression: increasing Vitellogenin syntesis

Relevant database "ECOTOX"



Add the relevant database

Relevant endpoints

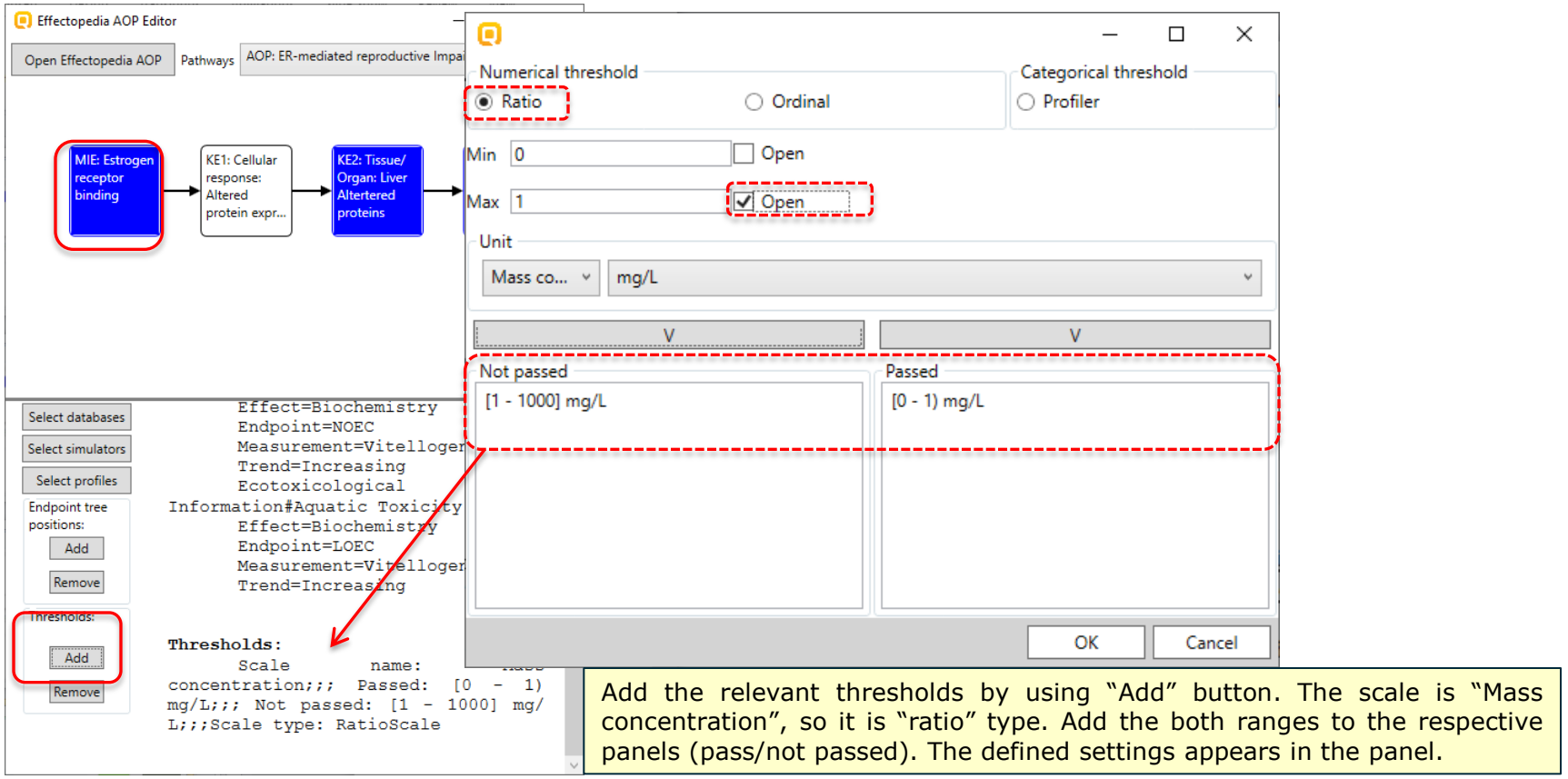


Add the relevant endpoint tree positions. Keep in mind two additional metadata fields called "Measurement" and "Trend" are added to the list with default fields.

Configuring the migrated AOP

Node KE1 - Cellular response: Altered protein expression: increasing Vitellogenin syntesis

Relevant thresholds



Add the relevant thresholds by using "Add" button. The scale is "Mass concentration", so it is "ratio" type. Add the both ranges to the respective panels (pass/not passed). The defined settings appears in the panel.

Configuring the migrated AOP

Node KE2 - Tissue/Organ: Liver Altertered proteins

The following TB objectives need to be considered for configuring KE2 node in order to work correctly:

1) Relevant databases:
- ECOTOX

2) Relevant endpoints (related to Acute aquatic toxicity endpoint tree):
- LOEC:
  - Effect: Reproduction
  - Measurement: Fecundity
  - Trend: Decreasing
- NOEC:
  - Effect: Reproduction
  - Measurement: Fecundity
  - Trend: Decreasing

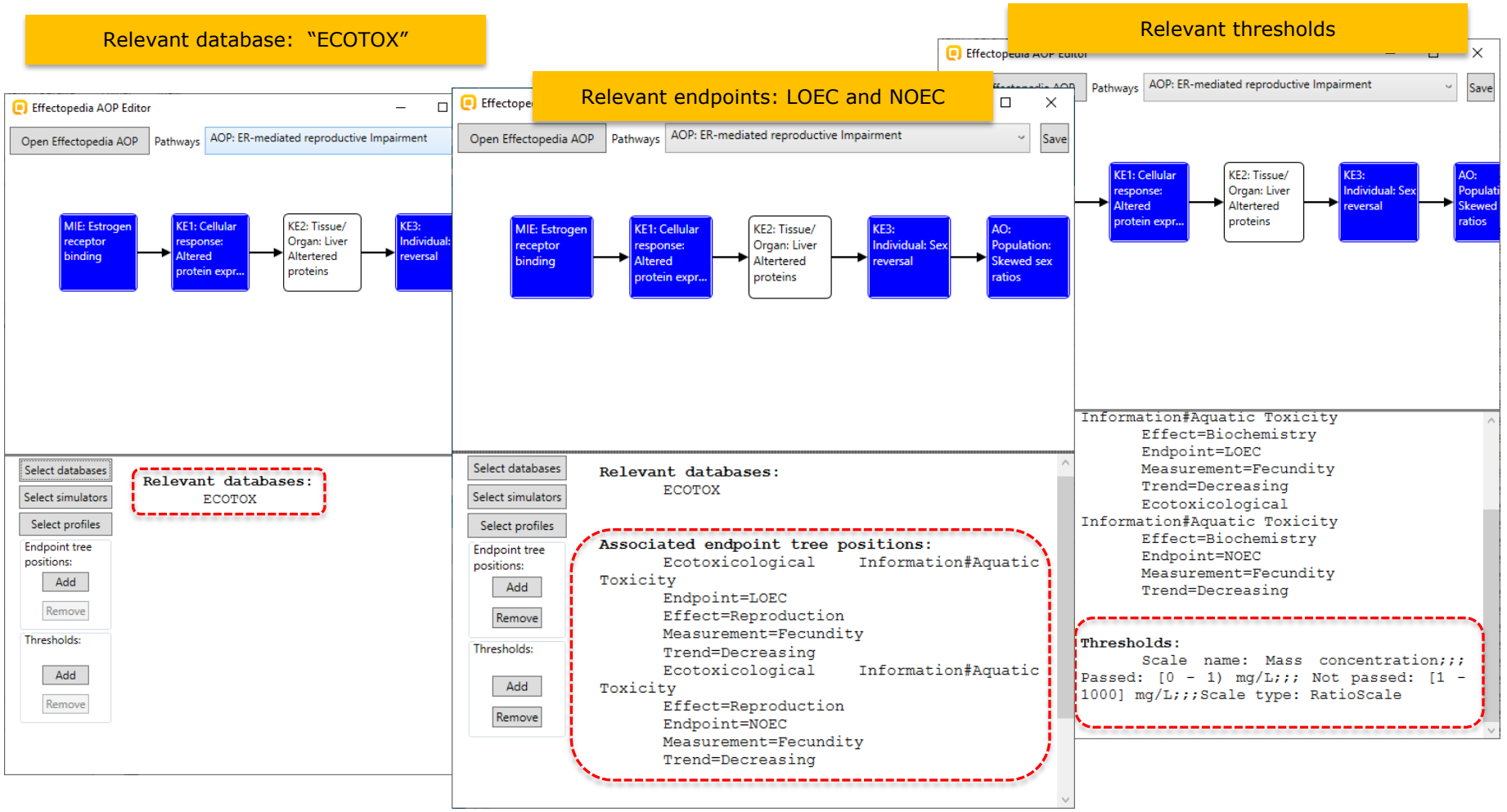
3) Relevant Profilers:
- No relevant profilers

4) Relevant thresholds for pass/not pass (positive/negative) (same as in KE1)
- Chemicals with data in the range [0 - 1) mg/L should be passed
- Chemicals with data in the range [1 - 1000] mg/l should be not passed

Note: The final outcome of the configuring the nodes is illustrated in the forthcoming slides.

Configuring the migrated AOP

Node KE2 - Tissue/Organ: Liver Altertered proteins



Configuring the migrated AOP

Node KE3 - Individual: Sex reversal

- This Effectopedia AOP node is not configured, because there are no Toolbox data avaialble and relevant profilers.
- Node: keep it as it is.

Configuring the migrated AOP

Node AO - Population: Skewed sex ratios

The following TB objectives need to be considered for configuring AO node in order to work correctly:

1) Relevant databases:
- ECOTOX

2) Relevant endpoints (related to Acute aquatic toxicity endpoint tree):
- LOEC:
  - Effect: Population
  - Measurement: Sex ratio
  - Trend: Change
- NOEC:
  - Effect: Population
  - Measurement: Sex ratio
  - Trend: Change

3) Relevant Profilers:
- No relevant profilers

4) Relevant thresholds for pass/not pass (positive/negative) (same as in KE1 and KE2)
- Chemicals with data in the range [0 - 1) mg/L should be passed
- Chemicals with data in the range [1 - 1000] mg/l should be not passed

Note: The final outcome of the configuring the nodes is illustrated in the forthcoming slides.

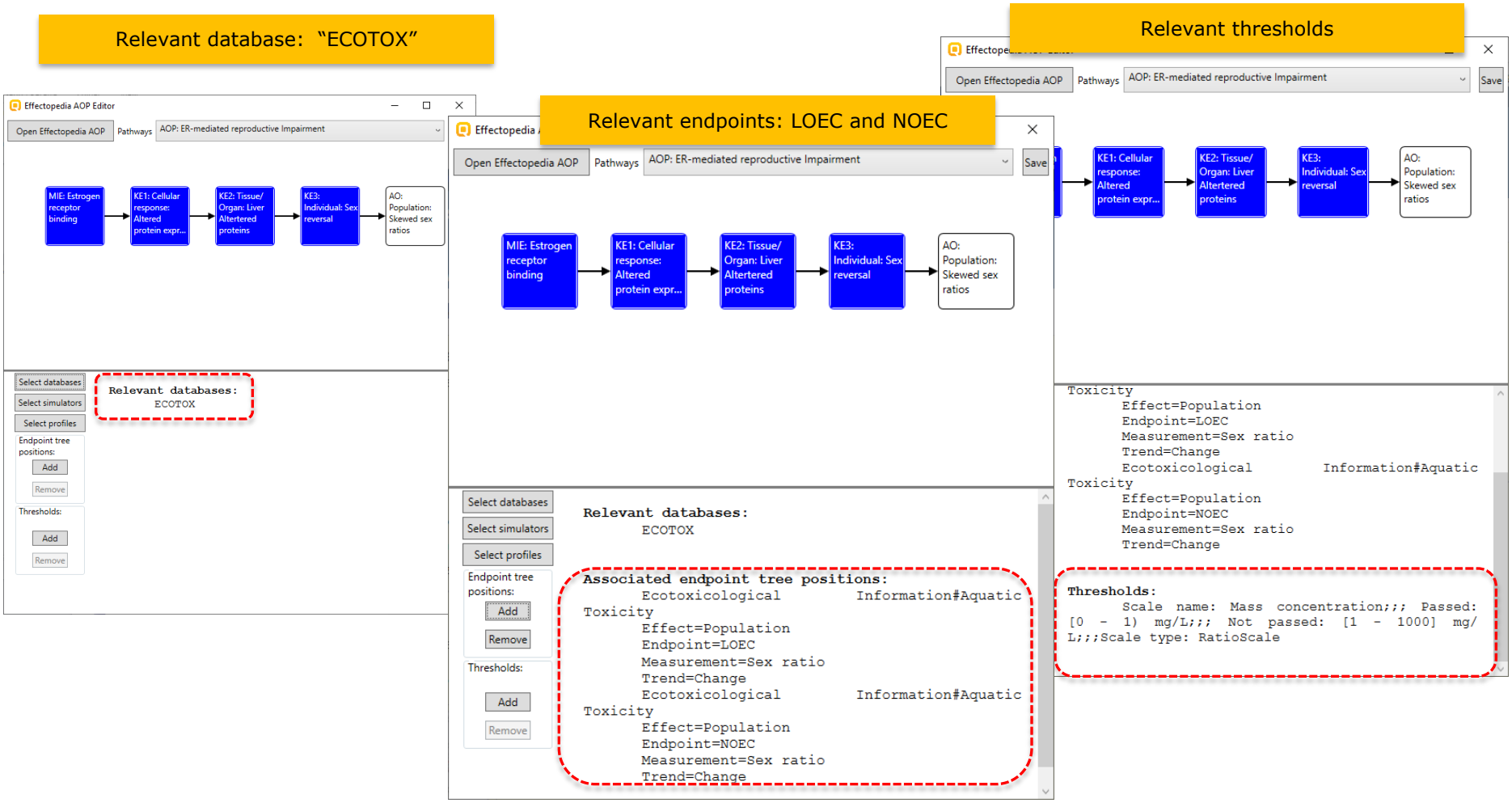
Configuring the migrated AOP

Node AO - Population: Skewed sex ratios

Relevant database: "ECOTOX"

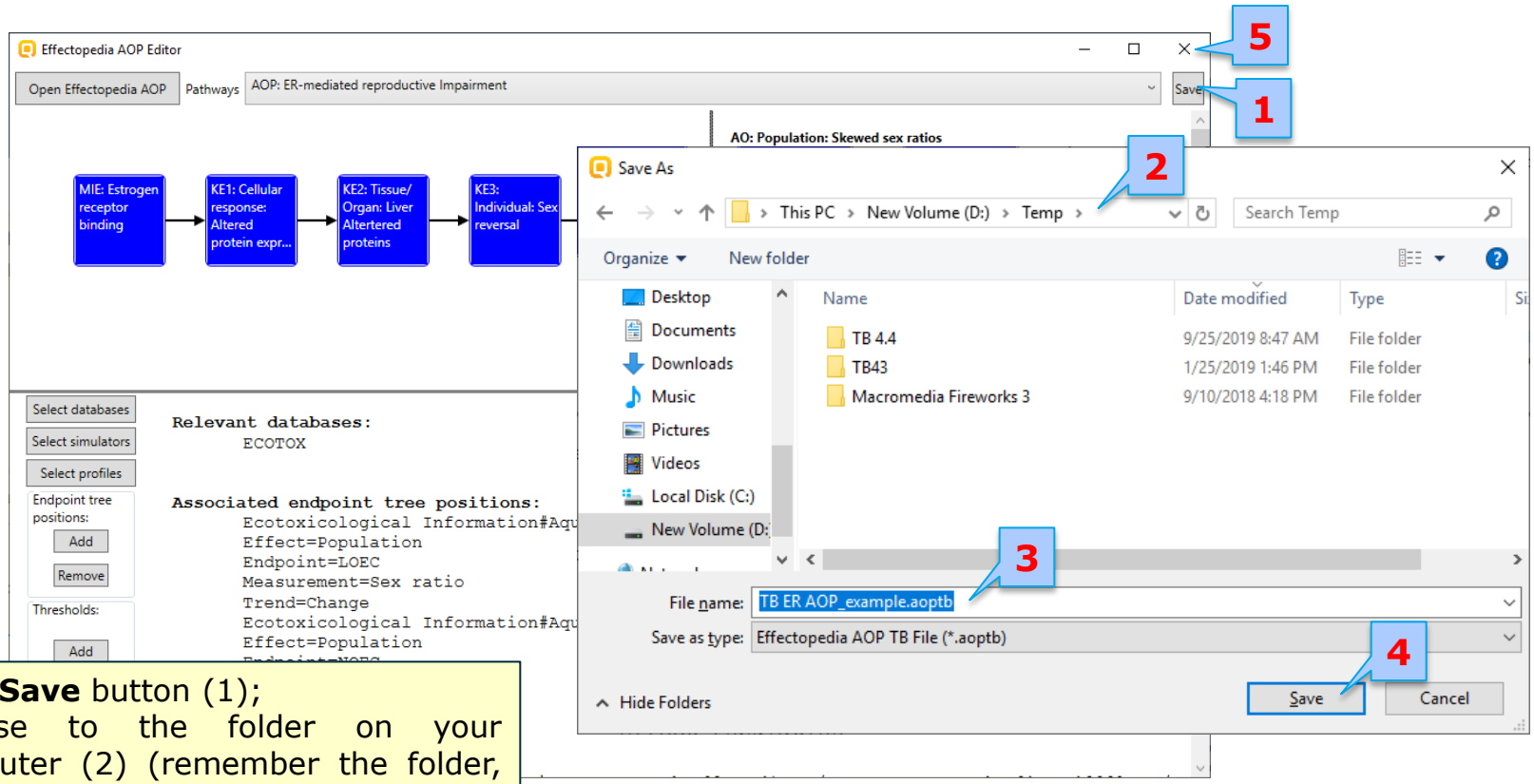
Relevant endpoints: LOEC and NOEC

Relevant thresholds



Configuring the migrated AOP

All AOP nodes are configured in Toolbox and ready to be used for predictive purposes. Finish the process by saving the AOP



Click **Save** button (1);
Browse to the folder on your computer (2) (remember the folder, you will need it (see next slide)), gave **name** of the custom AOP (3);
Click **Save** (4);
Close the window of the AOP editor (5).

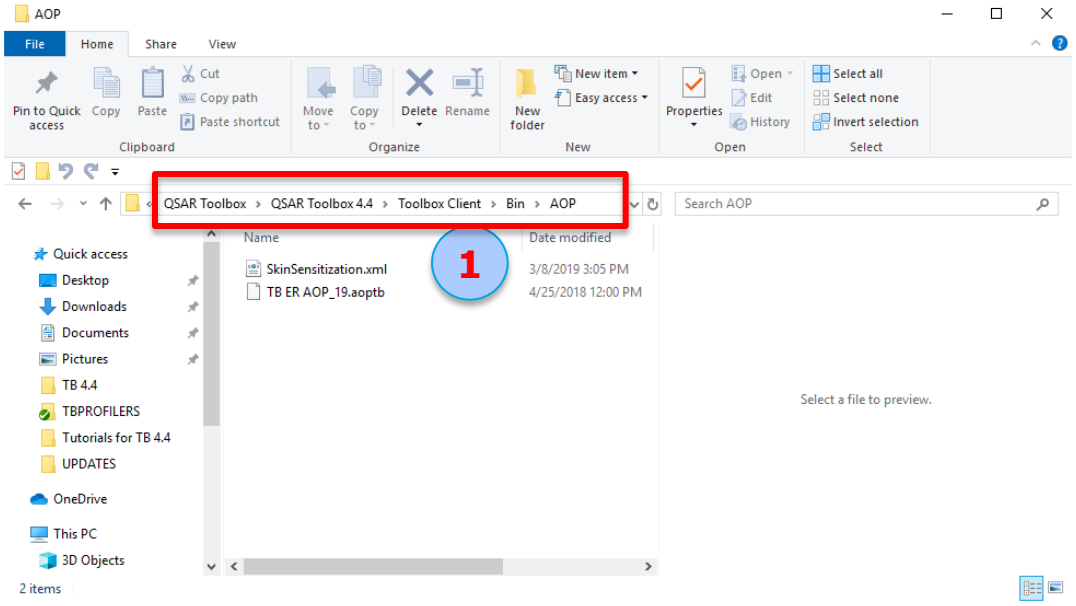
Configuring the migrated AOP

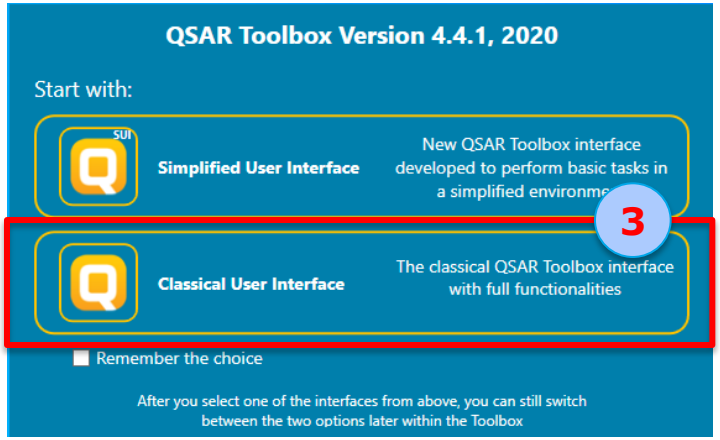
In order to use already configured AOP you need to:

1) paste the saved AOP file (*.aoptb) in the following installation folder: *C:\Program Files (x86)\QSAR Toolbox\QSAR Toolbox 4.4\Toolbox Client\Bin\AOP* (by default)

2) restart the Toolbox client (TB 4.4.1)

3) start the Classical User Interface (3)





Outlook

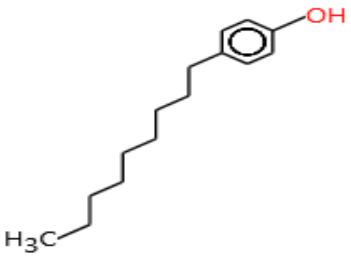
- Background
- Objectives
- Specific Aims
- Overview of the used Effectopedia AOP
- Migrating the Effectopedia AOP to Toolbox
- Building a new AOP-based profiler in Toolbox for predictive purposes
- **Application of migrated AOP for collecting weight-of evidence**

Application of the migrated AOP

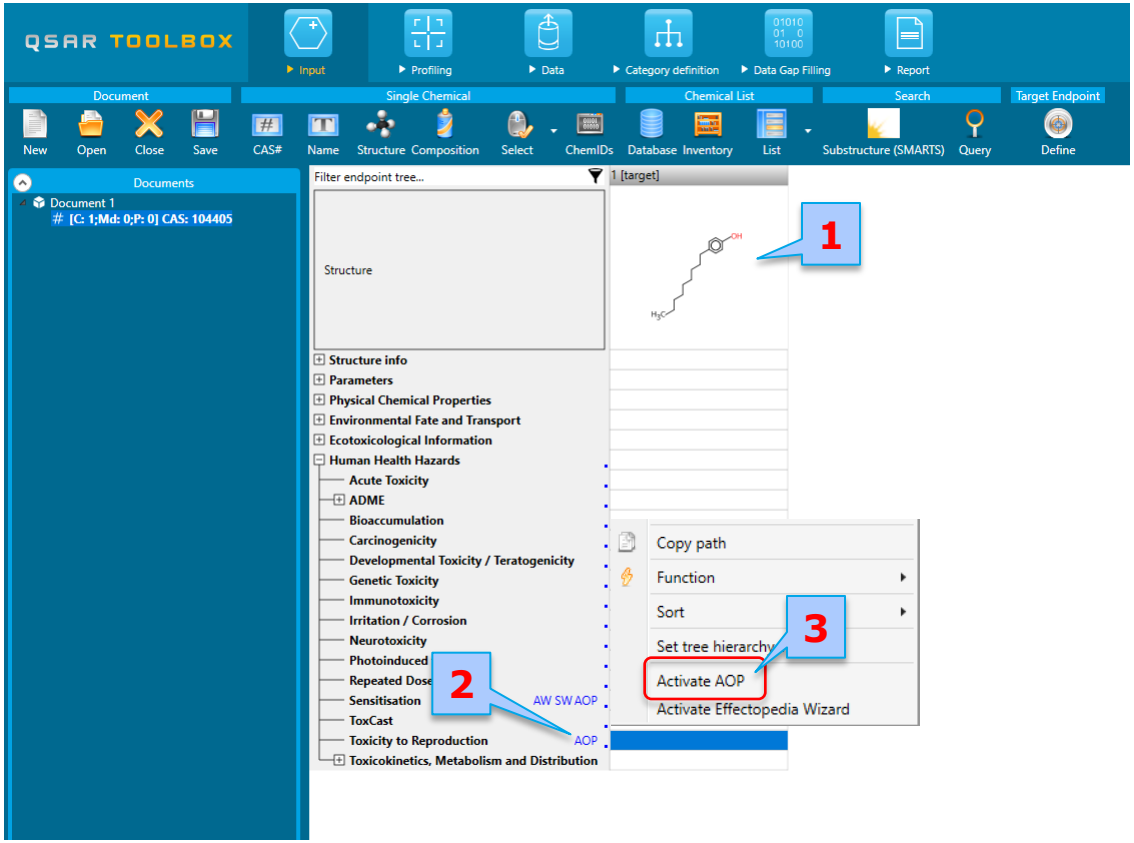
Target chemical:

CAS: 104-40-5

Name: 4-Nonylphenol

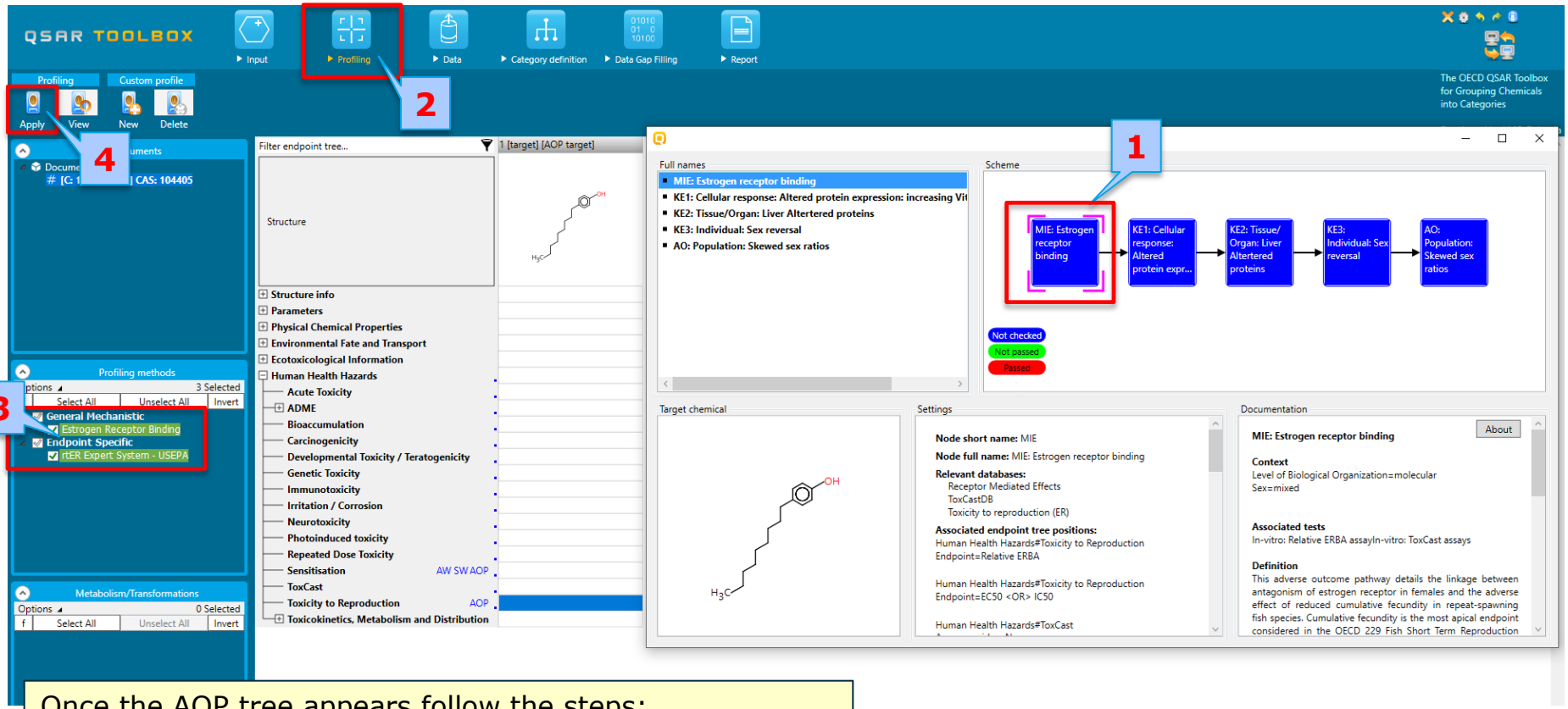


Usage: laundry and dish detergents, pesticides, care body products and plastics



1. Enter the target by **CAS**;
2. Blue AOP sign appears on the **"Toxicity to reproduction"** level and right-click on the sign
3. Select **Activate AOP** (3) in the pop-up menu.

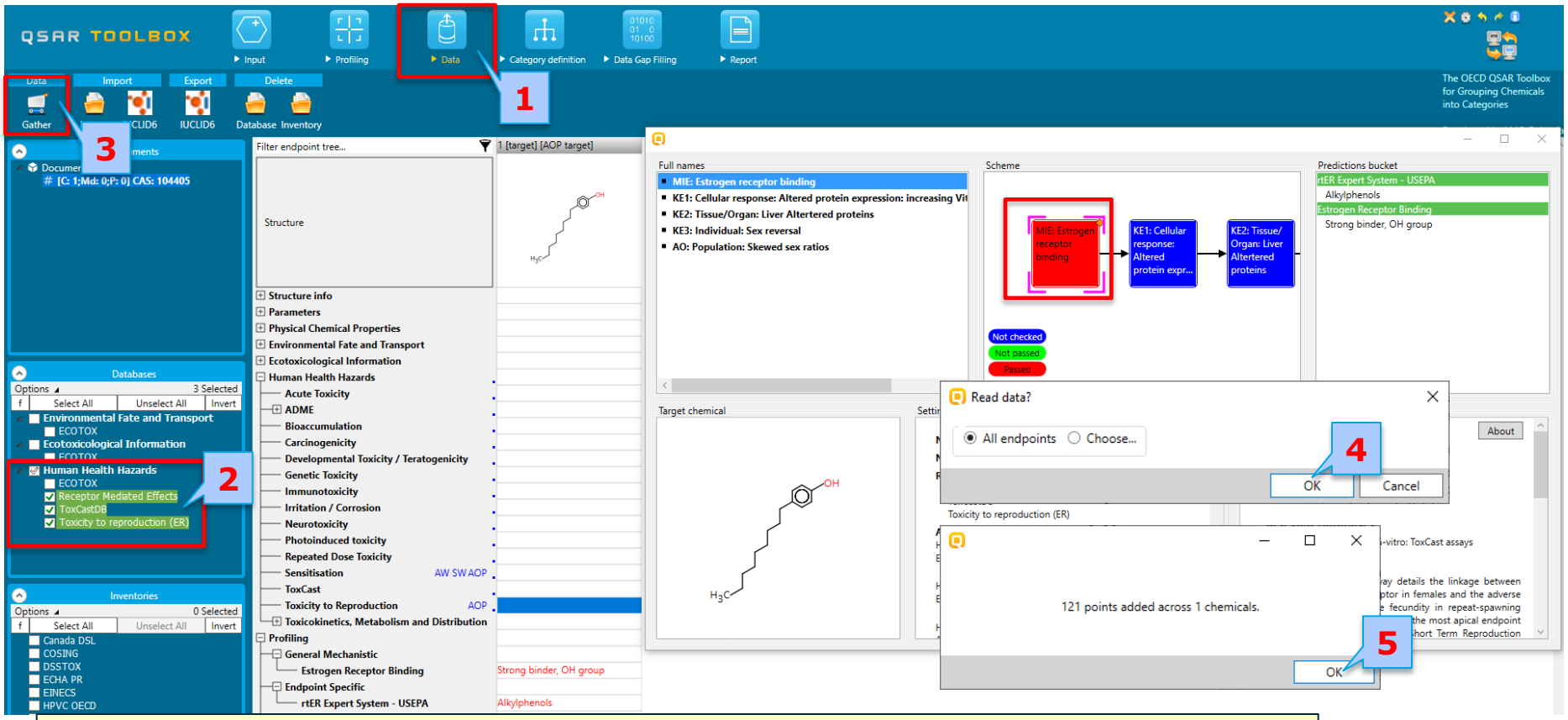
Application of the migrated AOP



Once the AOP tree appears follow the steps:

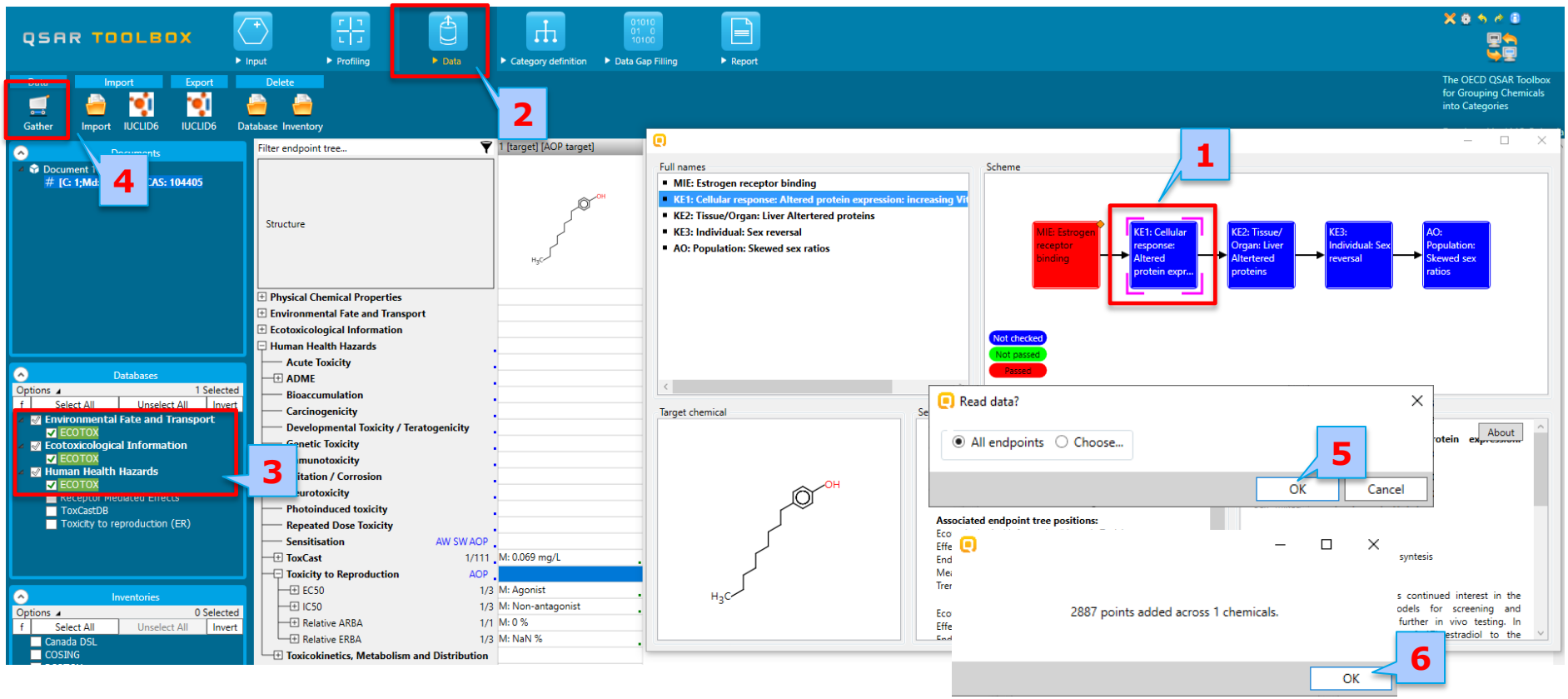
1. Click on node **MIE;**
2. Go to ***Profiling*** module;
3. Select both green profilers;
4. Click **Apply.**

Application of the migrated AOP



1. Go to ***Data*** module;
2. Select databases highlighted in green;
3. Click **Gather;**
4. Click **OK;**
5. A pop-up message informs about the number of collected data. Click **OK.**

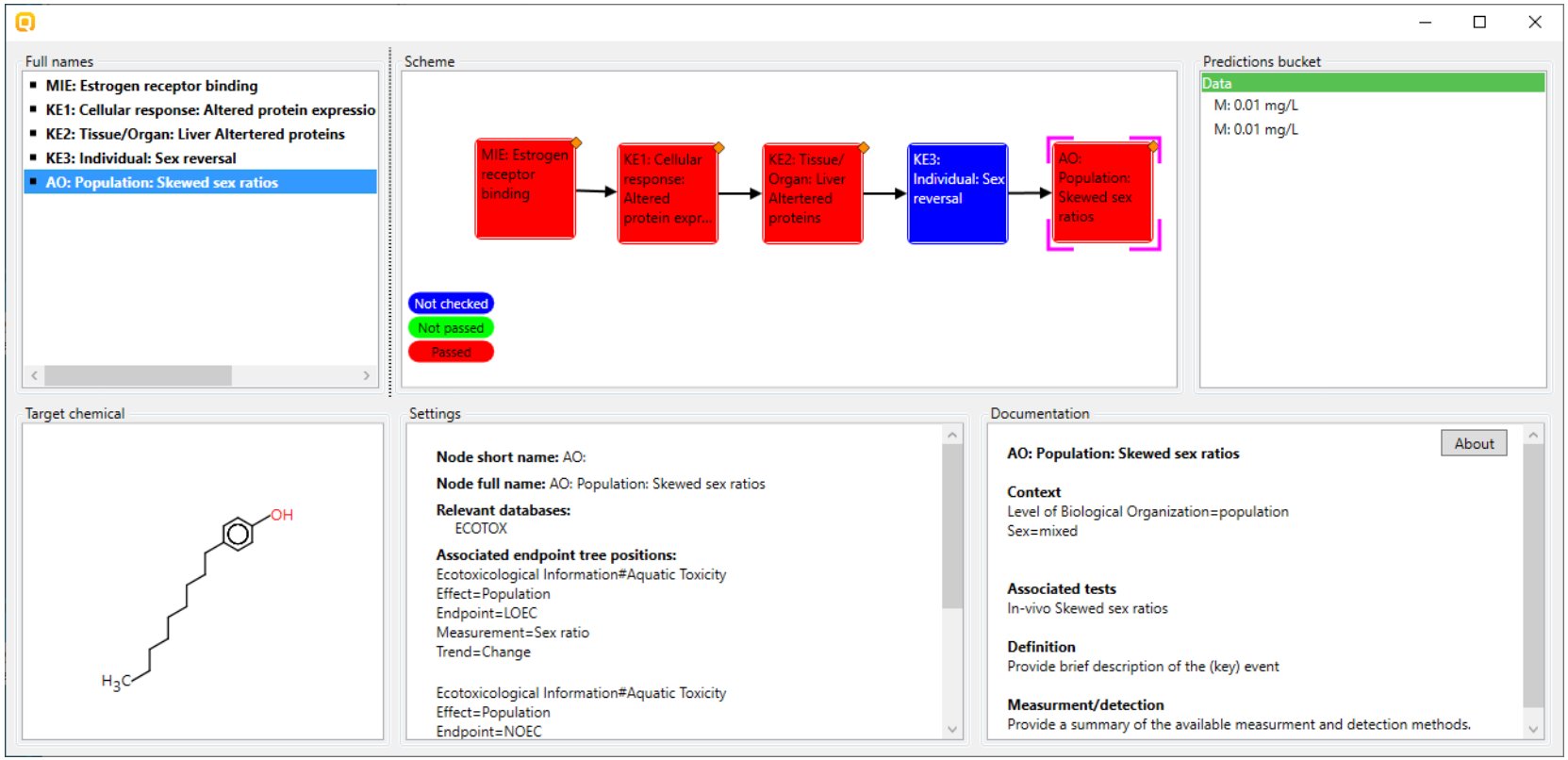
Application of the migrated AOP



1. Click node **KE1: Cellular response: Altered proteins**
2. Go to ***Data*** module;
3. Select the database highlighted in green;
4. Click **Gather;**
5. Click **OK;**
6. A pop-up message informs about the number of collected data. Click **OK.**

Application of the migrated AOP

- Nodes KE1 is colored in red because experimental data is found for the target chemical in ECOTOX;
- KE2 and AO are also colored in red, because experimental data is found for the target chemical in the same database (ECOTOX)
- As a result four AOP nodes (MIE; KE1; KE2 and AO) are passed (e.g. the boundary rules implemented in the nodes are "passed")



Application of the migrated AOP

Outcome of AOP application

- Migrated AOP nodes have been applied to the target chemical. Each node has been executed individually.
- As a result four out of five AOP nodes are passed (MIE; KE1; KE2 and AO).
- In other words, an ER binding alerts related to MIE and experimental data for almost all KEs has been found for the target chemical.
- Based on the outcome of the AOP nodes, it could be concluded that the target may elicit positive estrogen receptor binding effect.