

## 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

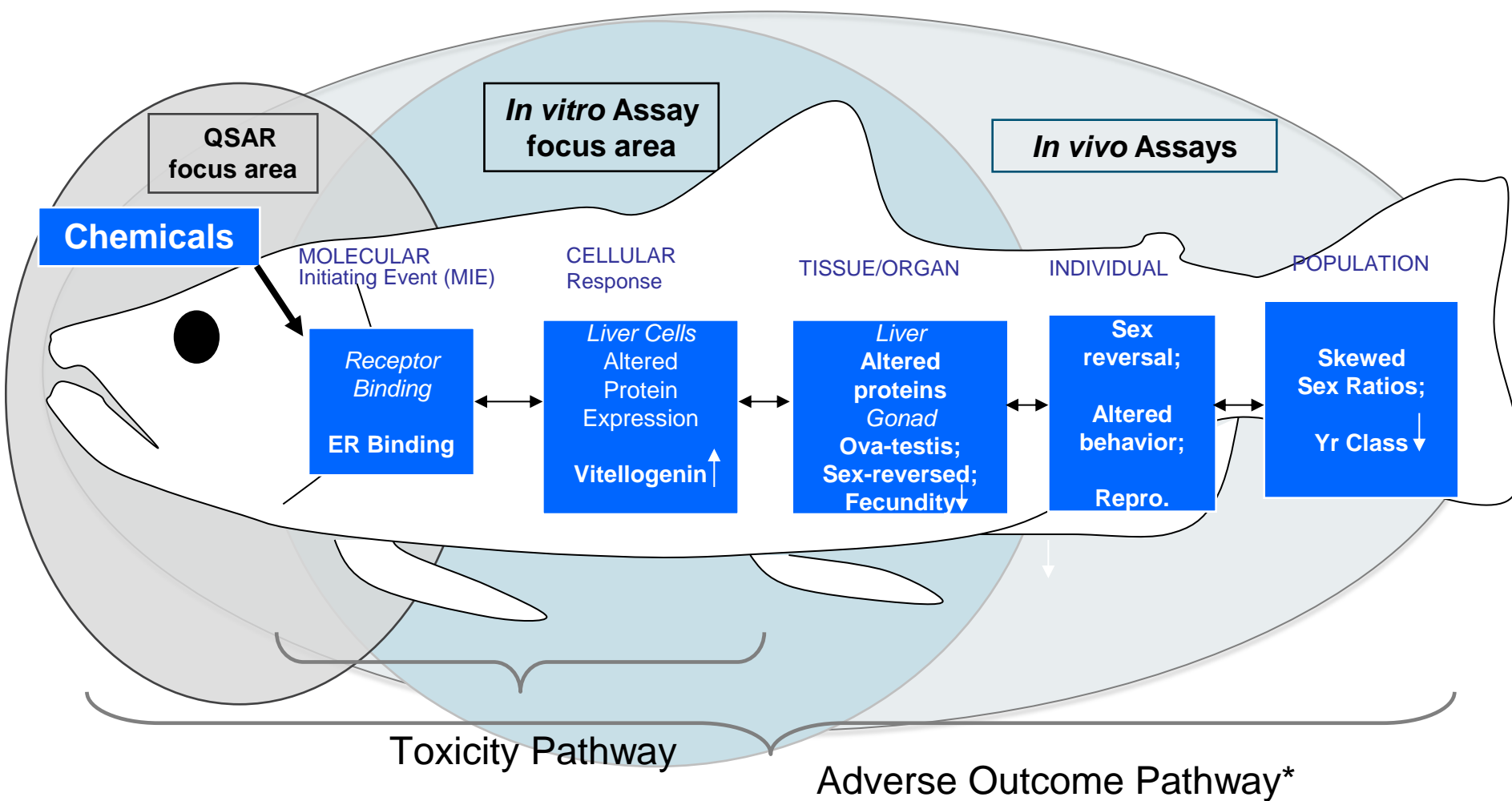
- Background
- Specific Aims
- **Overview of the used Effectopedia AOP**
- Migrating the Effectopedia AOP to Toolbox
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## Overview of the used Effectopedia AOP

- 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



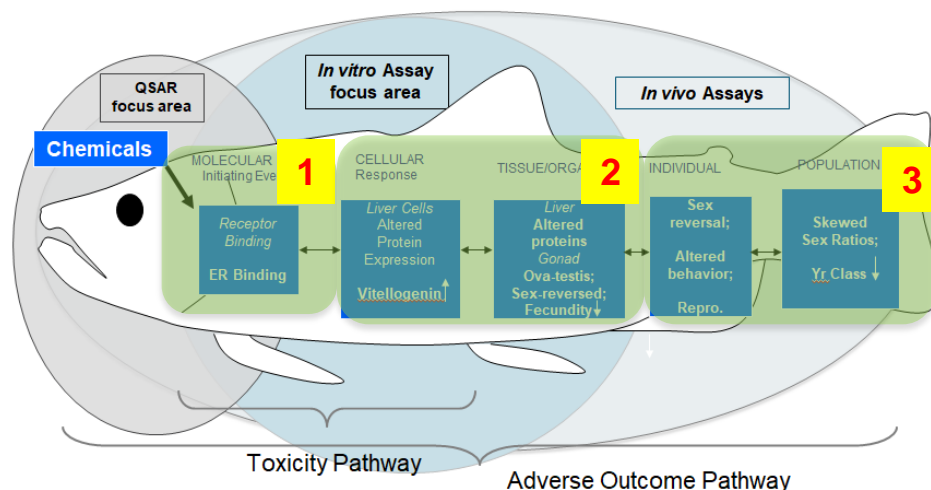
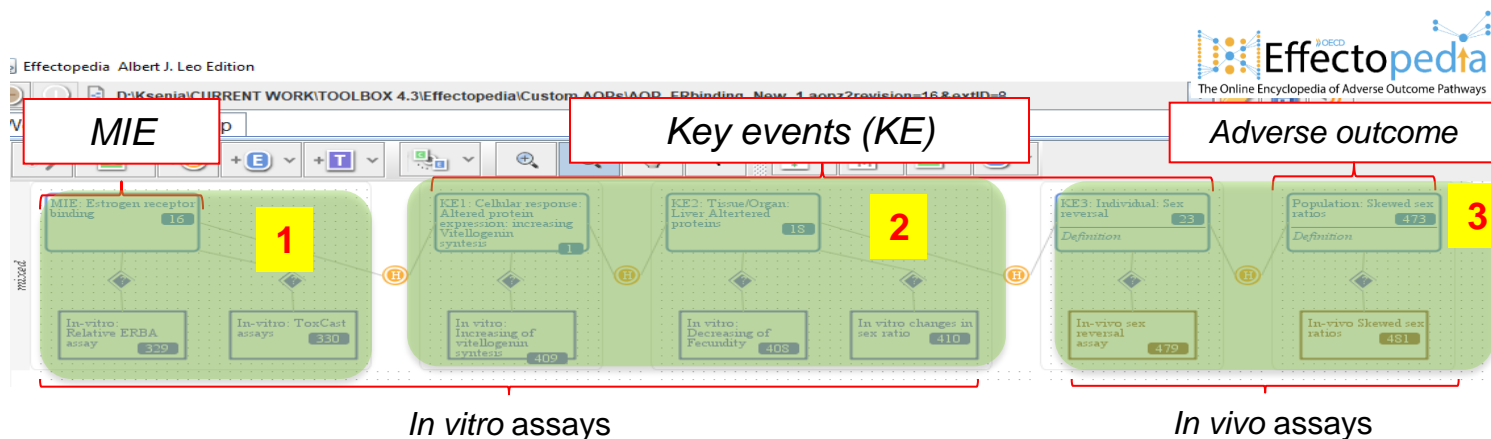
# Overview of the used Effectopedia AOP

## *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

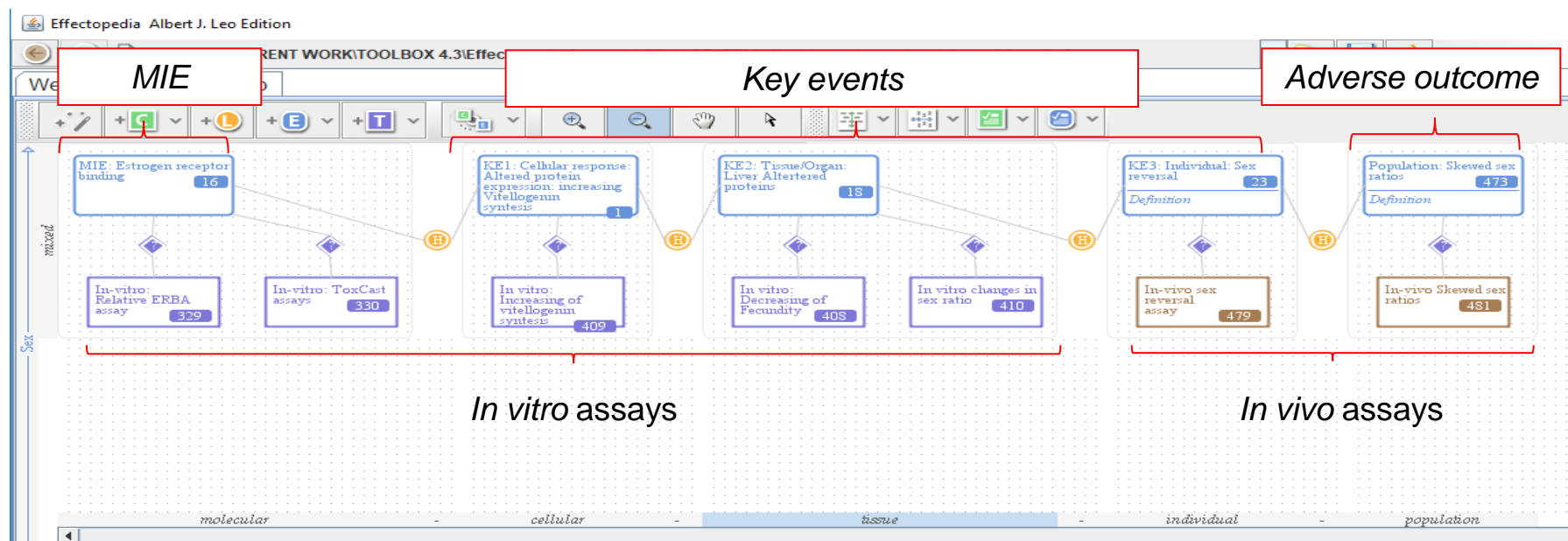
## Visualization of AOP built in Effectopedia



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

**MIE**

**MIE definition**

**Definition of the MIE**

**In vitro assays associated with MIE node**

**Definition of measurement**

**Associated tests**

**References**

**Continues on next slide**

# Overview of the used Effectopedia AOP

## Visualization of AOP build in Effectopedia

The screenshot displays the Effectopedia software interface. On the left, a hierarchical diagram shows the relationship between molecular, cellular, and tissue levels. A red box highlights the 'KE1' event, which is defined as 'Cellular response: Altered protein expression, increasing vitellogenin synthesis'. Below this, a red box highlights the 'In vitro assays associated with KE1', which include 'In vitro: Increasing of vitellogenin synthesis'. A red arrow points from the 'KE1' box to the 'KE1 definition' box on the right. The 'KE1 definition' box contains the following text:

**KE1 definition**

(Key) Event metadata  
**Title** KE1: Cellular response: Altered protein expression, increasing vitellogenin synthesis  
**ID** 3667/1  
**Keywords**  
**Groups**  
**AOP-Wiki short name**  
**Event components**

**Quality Assurance**  
**Contributors:** GUEST;  
**Reviewers:**  
**Seals of Approval:**  
**Last modified:** 2018-04-12T17:50:01

**Description**  
**Definition** ID: /62  
 As part of the process, there is continued interest in the development of in vitro models for screening and prioritization of chemicals for further in vivo testing. In oviparous species, the binding of 17 $\beta$ -estradiol to the estrogenreceptor (ER), and subsequent interaction of the receptor-ligand complex with estrogen-responsive elements (ERE) on the DNA, results in transcription of the vitellogenin (VTG) gene and, ultimately, production of the egg-yolk precursor, protein vitellogenin. Vitellogenin is normally produced in the liver of female fish and transported to the ovaries, where it is incorporated into the egg. Male fish, which possess functional estrogen receptors but normally produce very small quantities of VTG (Copeland et al., 1986; Sheahan et al. 1994), have been shown to produce large quantities of VTG when exposed to xenobiotics capable of interacting with the ER (Pelissiero et al., 1993; Jobling et al., 1996; Lech et al. 1996). This endpoint is thus evolving as an indicator of alterations in hormone synthesis, metabolism, or ER activation in aquatic species.

**Measurement/detection** ID: /58  
 Methods for determining increasing of vitellogenin production

**Associated Tests**  
 In vitro: Increasing of vitellogenin synthesis

**Associated Pathways**  
 AOP: ER-mediated reproductive impairment

**References**  
 1. Copeland et al., 1986; Sheahan et al. 1994  
 2. Pelissiero et al., 1993; Jobling et al., 1996; Lech et al. 1996)

Labels on the diagram:

- KE1** (red box)
- KE1 definition** (red box)
- In vitro assays associated with KE1** (red box)
- Definition** (black text)
- Associated tests** (black text)
- Associated pathways** (black text)
- References** (black text)

Continues on next slide

# Overview of the used Effectopedia AOP

## Visualization of AOP build in Effectopedia

The screenshot displays the Effectopedia software interface. On the left, a hierarchical diagram shows the relationship between various AOPs. A red box highlights the KE2 AOP, which is defined as 'Tissue/Organ: Liver Altered protein'. Below this, a red box highlights the 'In vitro assays associated with KE2', which include 'In vitro: Decreasing of Fecundity' and 'In vitro changes in sex ratio'. A red arrow points from the KE2 AOP to the right-hand panel, which shows the 'KE2 definition'. This panel includes a table of 'Event metadata', a 'Quality Assurance' section with contributors and reviewers, a 'Description' section with a definition of the assay, and a 'Measurement/detection' section. Below the definition panel, a blue box highlights the 'Associated Tests' section, which lists the two in vitro assays. At the bottom, a blue box highlights the 'References' section, which lists three scientific references.

**KE2**

**KE2 definition**

**In vitro assays associated with KE2**

**Definition**

**Associated tests and pathways**

**References**

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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.

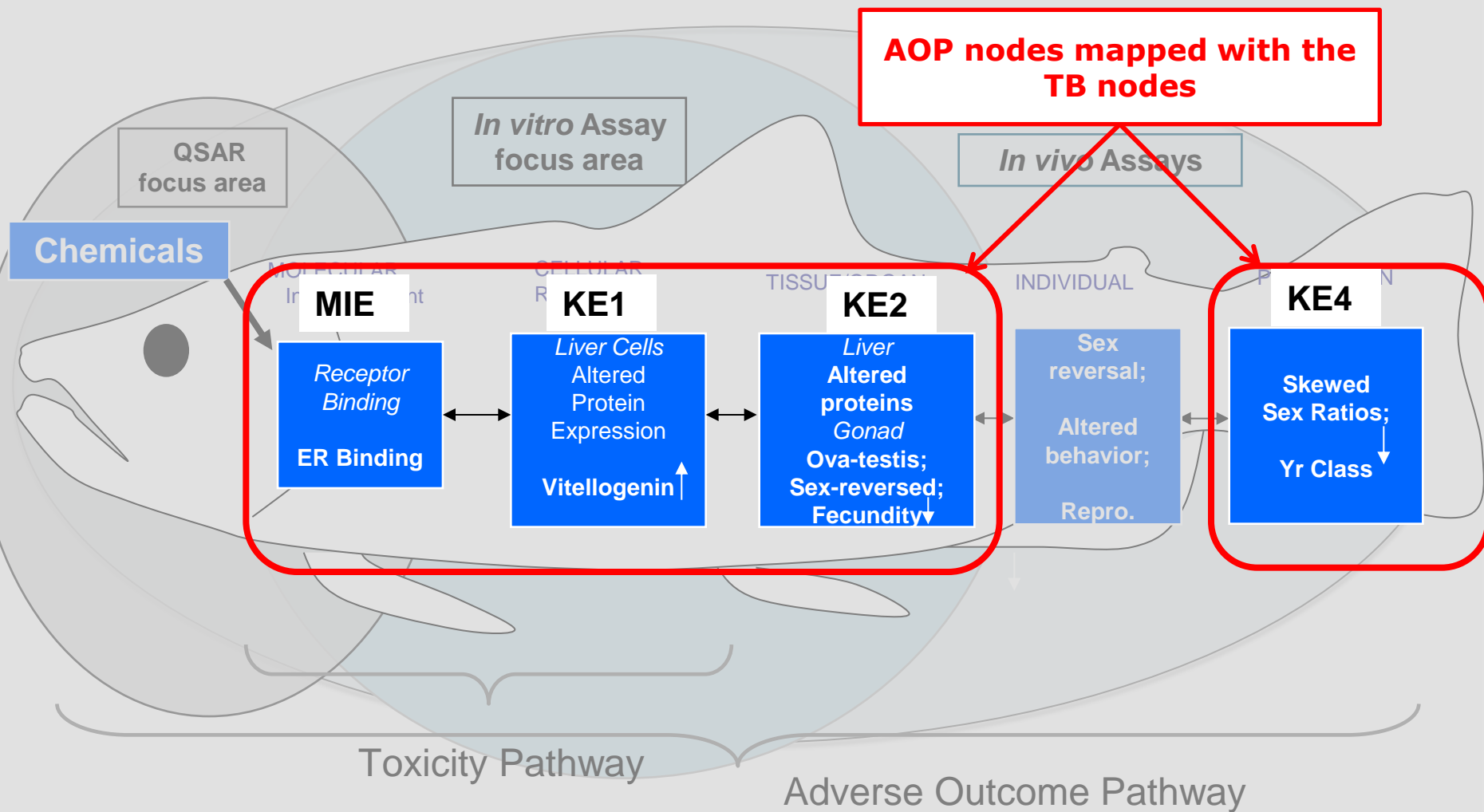


# Migrating the Effectopedia AOP to Toolbox

- 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

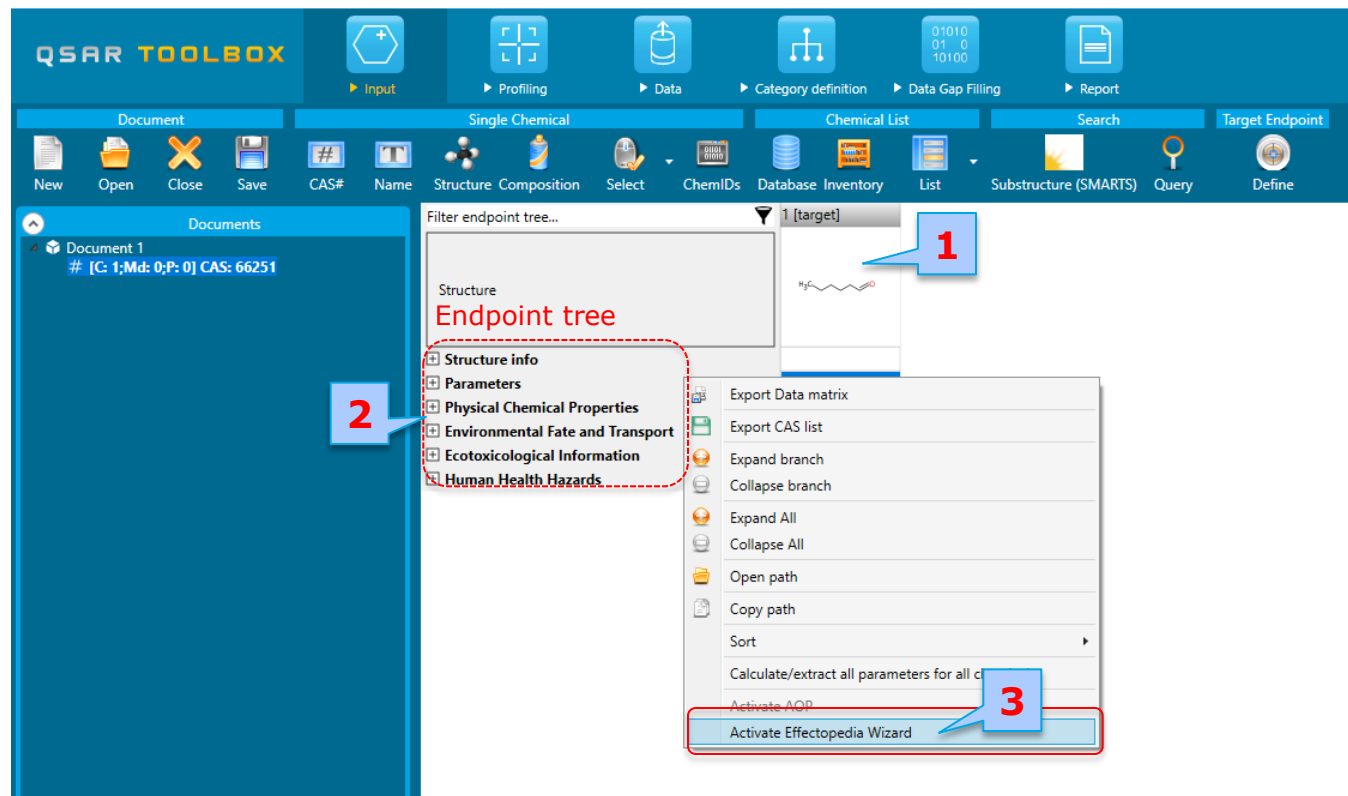


# Migrating the Effectopedia AOP to Toolbox

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

# Migrating the Effectopedia AOP to Toolbox

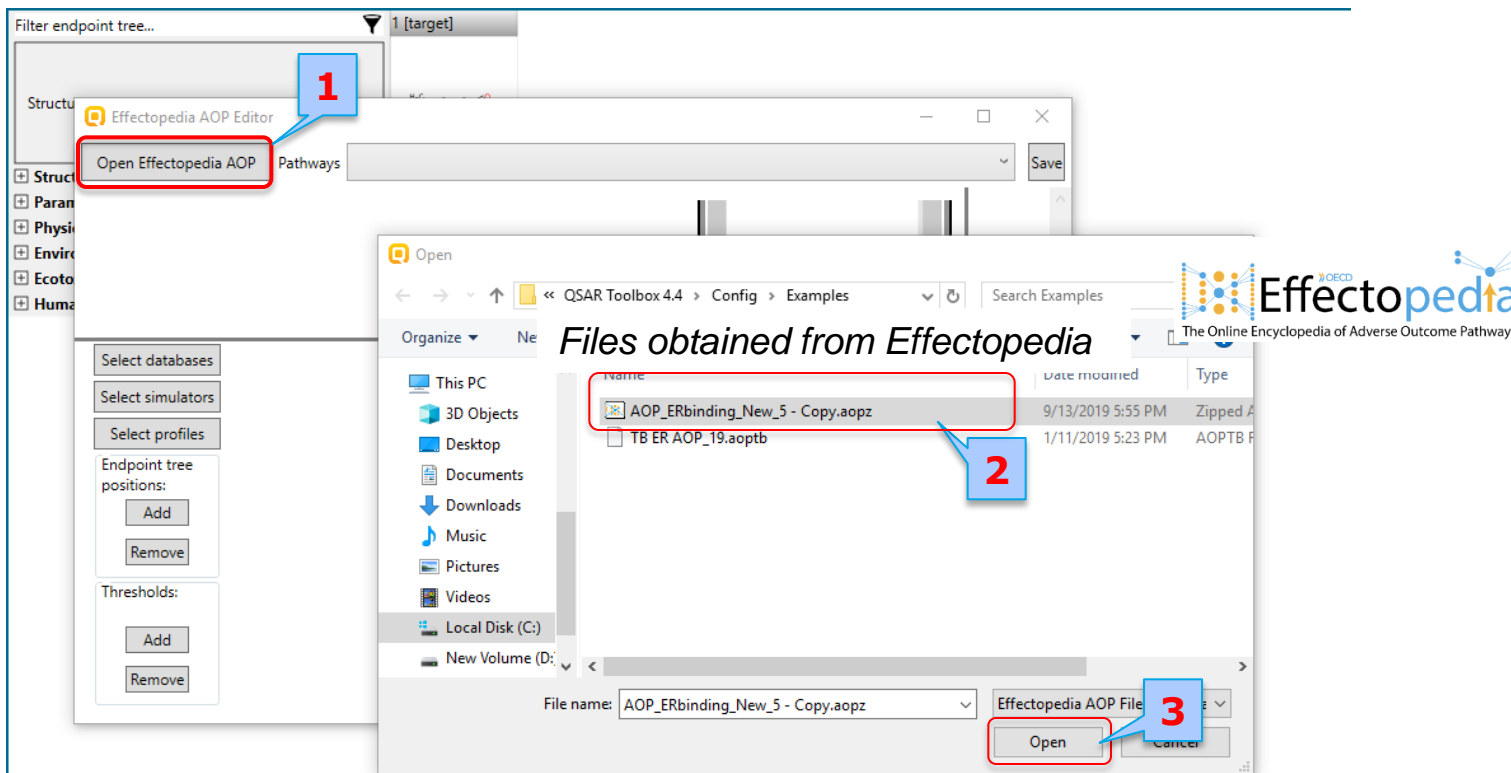
## Step1: Activating the Effectopedia wizard



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

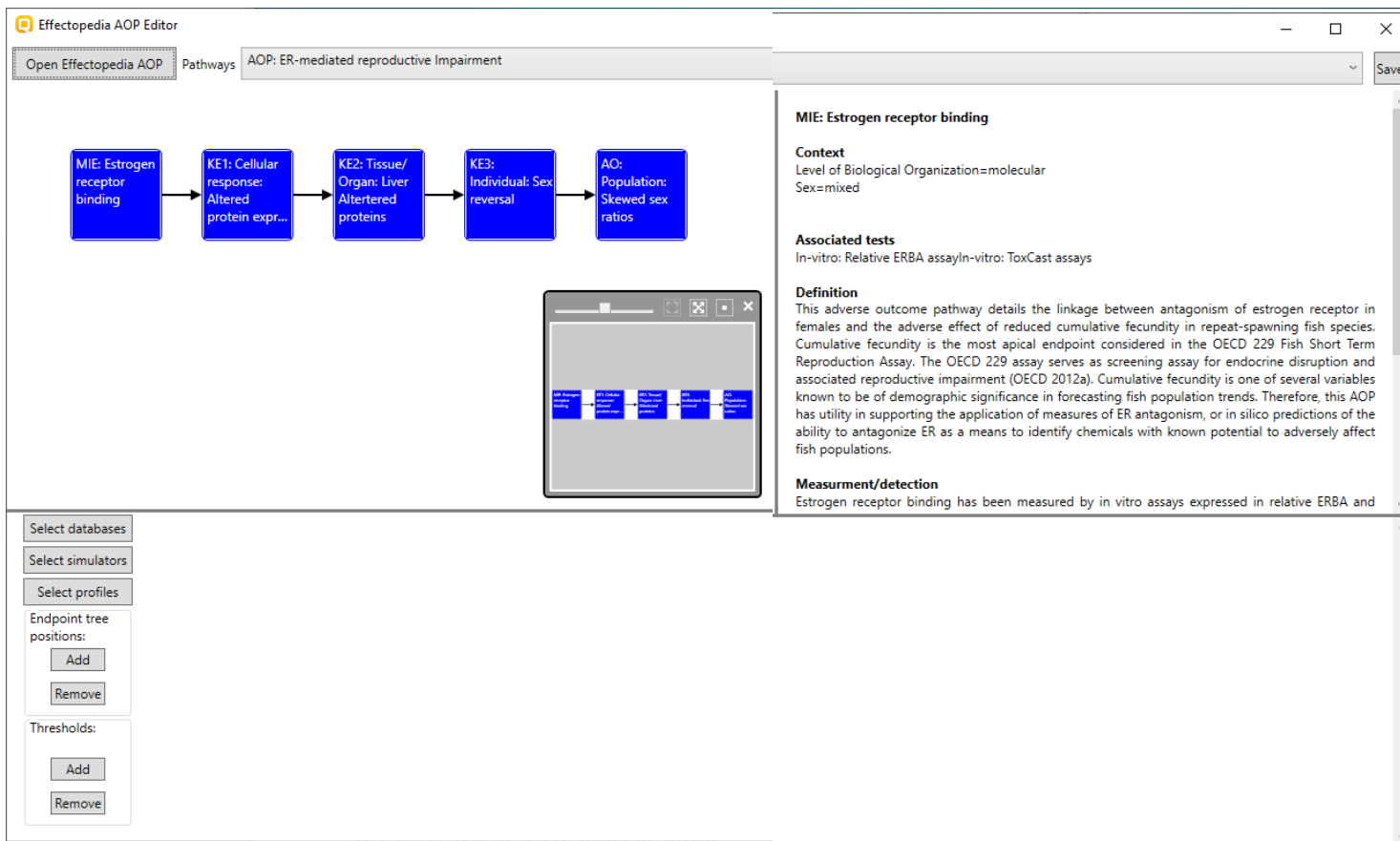
## Step2: Open the Effectopedia AOP



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

*Continues on next slide*



# 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

The screenshot displays the 'Effectopedia AOP Editor' window. At the top, a dropdown menu shows 'AOP: ER-mediated reproductive Impairment' with a red dashed box around it labeled 'Name of the AO'. To the right of this menu is a 'Save' button, highlighted with a red dashed box and labeled 'Save button to save the configured AOP'. Below the menu, a workflow diagram shows a sequence of nodes: 'MIE: Estrogen receptor binding' → 'KE1: Cellular response: Altered protein expr...' → 'KE2: Tissue/Organ: Liver Altered proteins' → 'KE3: Individual: Sex reversal' → 'AO: Population: Skewed sex ratios'. A red bracket underlines the first four nodes, labeled 'Nodes of the AOP'. Below the diagram is a 'preview panel' showing a smaller version of the workflow. On the right side, a panel with a red dashed border contains details for the selected node 'MIE: Estrogen receptor binding', including 'Context', 'Associated tests', 'Definition', and 'Measurement/detection'. At the bottom left, a sidebar contains configuration options: 'Select databases', 'Select simulators', 'Select profiles', 'Endpoint tree positions' (with 'Add' and 'Remove' buttons), and 'Thresholds' (with 'Add' and 'Remove' buttons). A red bracket groups these options, labeled 'Settings for configuring the nodes'.

# 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

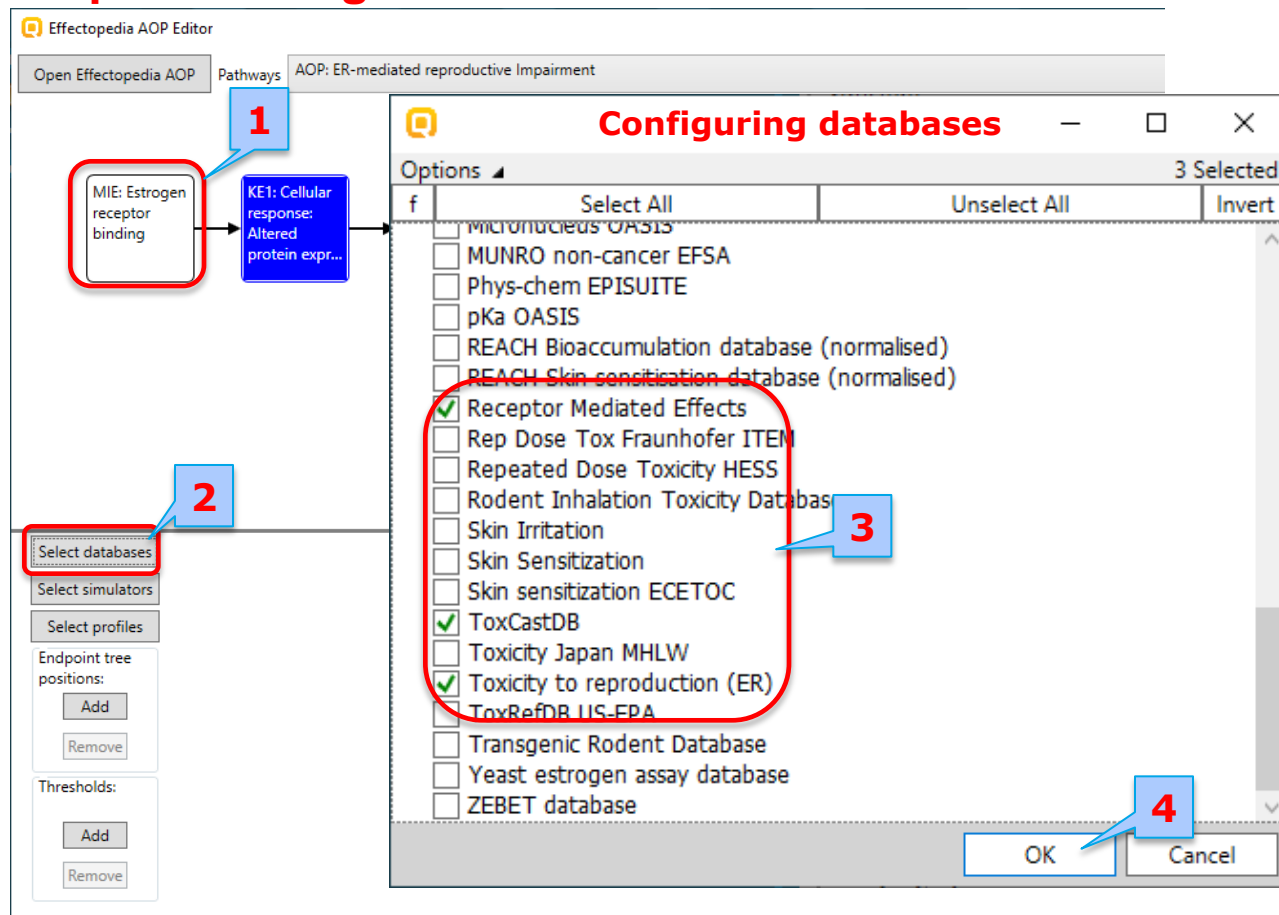
# Configuring the migrated AOP

## 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 endpoints: Relative ERBA

Configuring MIE node:

### 2) Relevant endpoints:

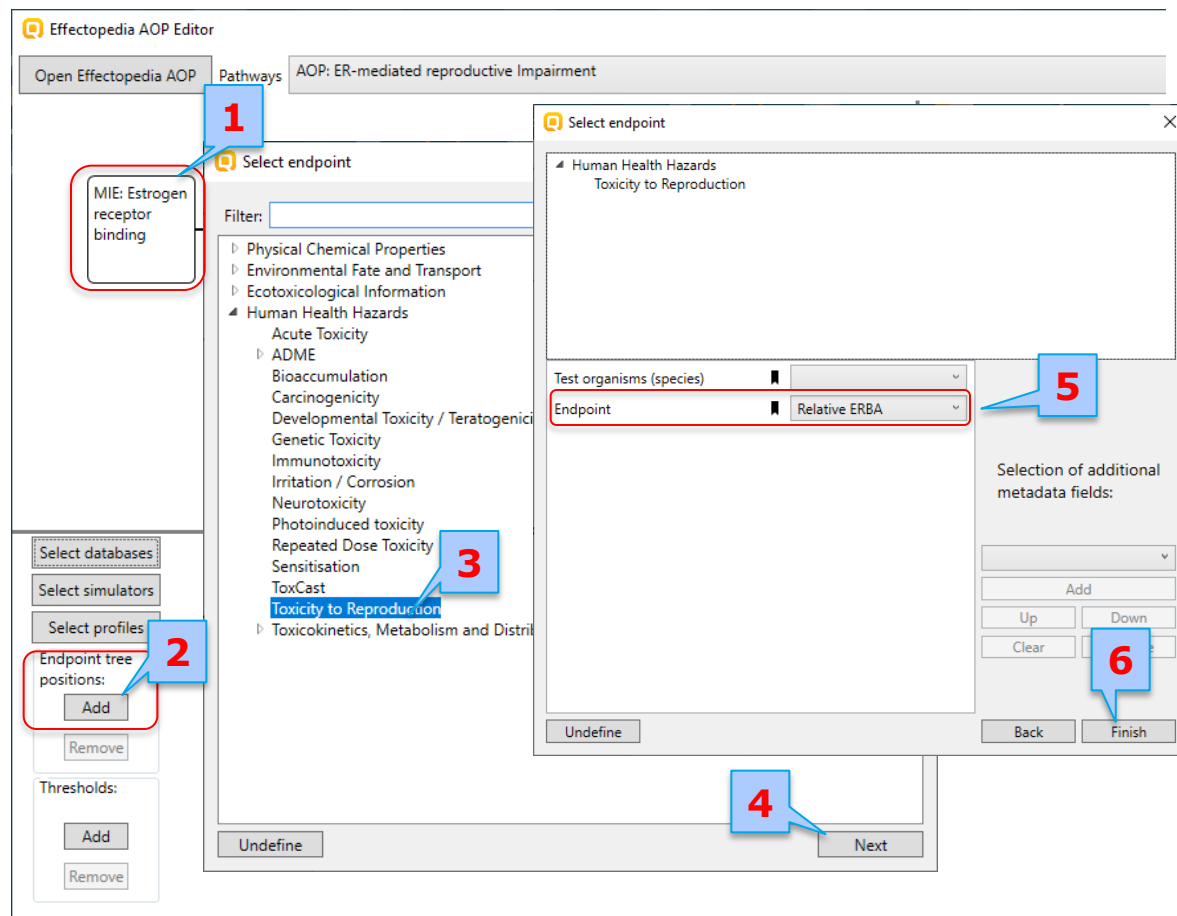
- **Relative ERBA**
- EC50 <OR> IC50
- AC50

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 the migrated AOP

## Node: MIE – Estrogen receptor binding

Configuring MIE node:

Configuring endpoint: EC50<>IC50

### 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**;

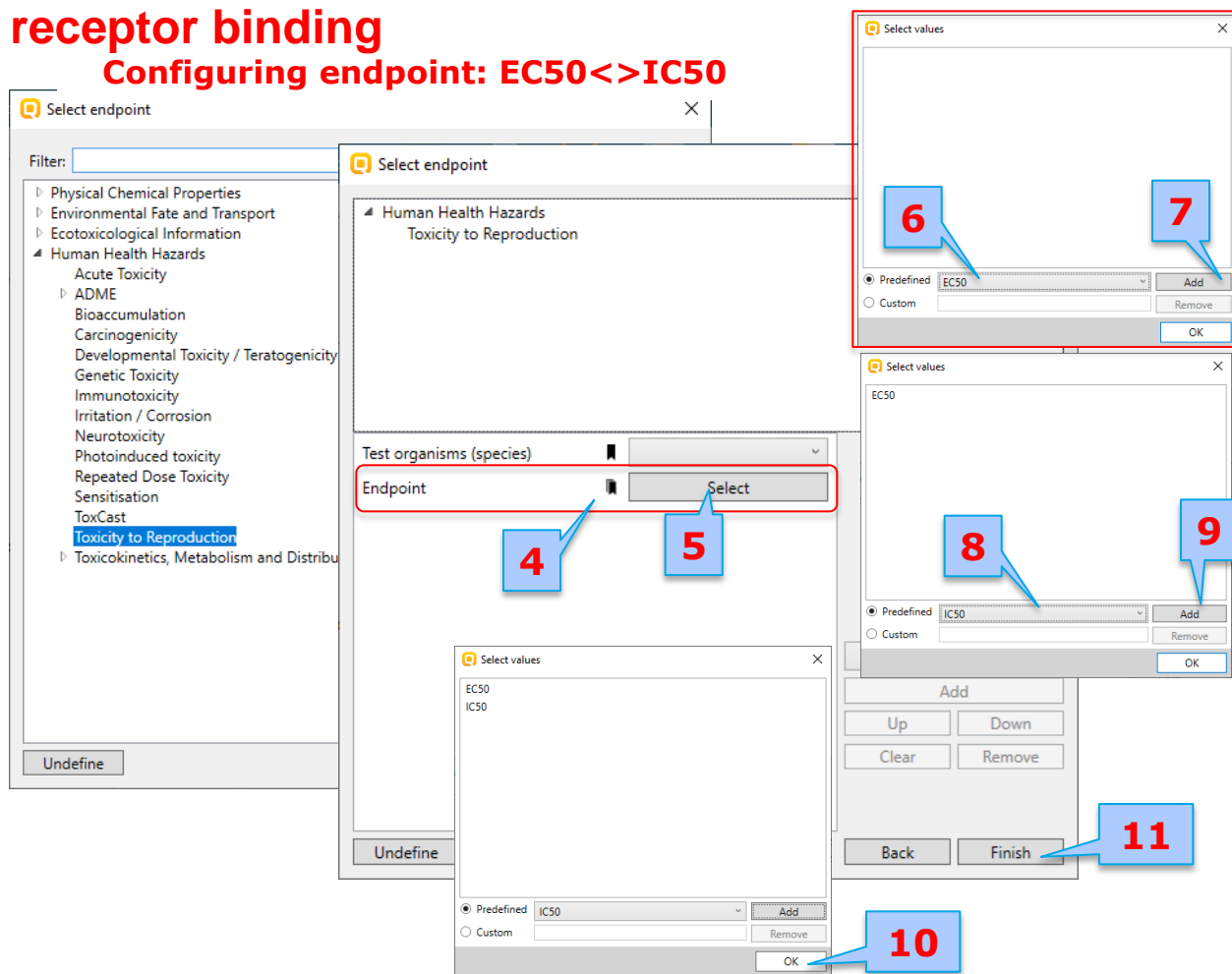
From the predefined list with endpoints select **EC50 (6,7)** and **IC50 (8,9)**;

**10.** Click **OK**;

**11.** Click **Finish (11)**.

The second endpoint is configured, continue with the last one.

*Continues on next slide*



# 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**;

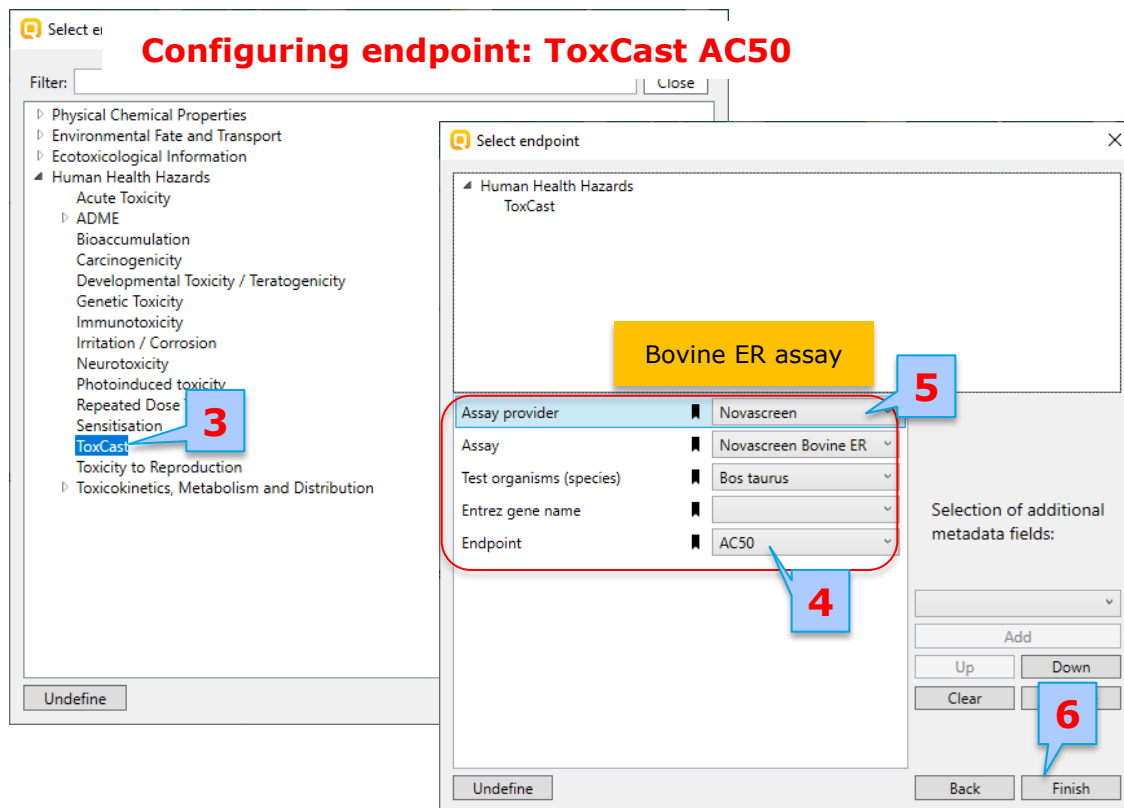
### 4. Select endpoint **AC50**;

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.

*Continues on next slide*



# 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 ER $\alpha$** ; species: **Mus musculus**.

The difference between previous selection is the assay and test organism(species) fields;

**5.** Click **Finish**.

### Configuring endpoint: ToxCast AC50

The image displays two side-by-side screenshots of the 'Select endpoint' dialog box in the QSAR Toolbox software. Both windows show the 'Human Health Hazards' category selected, with 'ToxCast' as the chosen level. The left window, labeled '4a', is configuring the 'Human ER assay'. It shows 'Novascreen' as the assay provider, 'Novascreen Human ER' as the assay, and 'Homo sapiens' as the test organism species. The endpoint is set to 'AC50'. The right window, labeled '4b', is configuring the 'Mouse ER assay'. It shows 'Novascreen' as the assay provider, 'Novascreen Mouse ER' as the assay, and 'Mus musculus' as the test organism species. The endpoint is also set to 'AC50'. In both windows, a red box highlights the 'Assay' and 'Test organisms (species)' fields. A blue callout '4a' points to the 'Assay' field in the left window, and a blue callout '4b' points to the 'Assay' field in the right window. A blue callout '5' points to the 'Finish' button in the right window. The right window also shows a 'Selection of additional metadata fields' section with 'Add', 'Up', 'Down', and 'Remove' buttons.



# Configuring the migrated AOP

## Node: MIE – Estrogen receptor binding

Effectopedia AOP Editor

Open Effectopedia AOP Pathways: AOP: ER-mediated reproductive Impairment Save

```

graph LR
    MIE[MIE: Estrogen receptor binding] --> KE1[KE1: Cellular response: Altered protein expression]
    KE1 --> KE2[KE2: Tissue/Organ: Liver Altered proteins]
    KE2 --> KE3[KE3: Individual: Sex reversal]
    KE3 --> AO[AO: Population: Skewed sex ratios]
  
```

**MIE: Estrogen receptor binding**

**Context**  
Level of Biological Organization=molecular  
Sex=mixed

**Associated tests**  
In-vitro: Relative ERBA assayIn-vitro: ToxCast assays

**Definition**  
This adverse outcome pathway details the linkage between antagonism of estrogen receptor in females and the adverse effect of reduced cumulative fecundity in repeat-spawning fish species. Cumulative fecundity is the most apical endpoint considered in the OECD 229 Fish Short Term Reproduction Assay. The OECD 229 assay serves as screening assay for endocrine disruption and associated reproductive impairment (OECD 2012a). Cumulative fecundity is one of several variables known to be of

Select databases  
Select simulators  
Select profiles  
Endpoint tree positions:  
Add  
Remove  
Thresholds:  
Add  
Remove

**Relevant databases:**  
Receptor Mediated Effects  
ToxCastDB  
Toxicity to reproduction (ER)

**Associated endpoint tree positions:**  
Human Health Hazards#Toxicity to Reproduction  
Endpoint=Relative ERBA  
Human Health Hazards#Toxicity to Reproduction  
Endpoint=EC50 <OR> IC50  
Human Health Hazards#ToxCast  
Assay provider=Novascreen  
Assay=Novascreen Bovine ER  
Test organisms (species)=Bos taurus  
Endpoint=EC50

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

The screenshot shows the 'Effectopedia AOP Editor' interface. At the top, the AOP is titled 'AOP: ER-mediated reproductive Impairment'. The diagram shows a flow from 'MIE: Estrogen receptor binding' to 'KE1: Cellular response: Altered protein expr...' to 'KE2: Tissue/ Organ: Liver Altered proteins' to 'KE3: Individual response'. The 'Select profilers' dialog is open, showing a list of profilers. The 'Estrogen Receptor Binding' and 'rtER Expert System - USEPA' profilers are selected. The 'OK' button is highlighted.

**1** MIE: Estrogen receptor binding

**2** Select profilers

**3** Estrogen Receptor Binding

**3** rtER Expert System - USEPA

**4** OK

# Configuring the migrated AOP

## Node: MIE – Estrogen receptor binding

Configuring MIE node:

### 4) Threshold for the relevant profilers:

#### Profiler: Estrogen Receptor binding

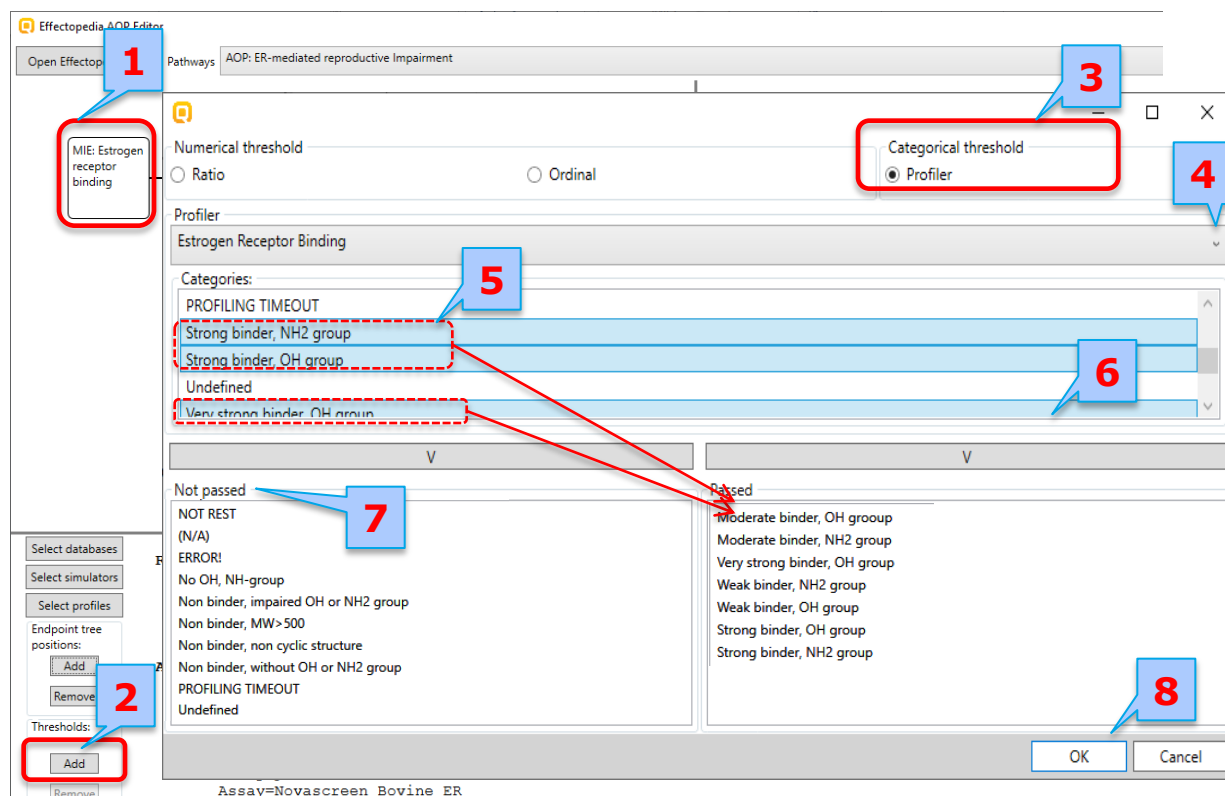
##### - **Passed:**

- 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:

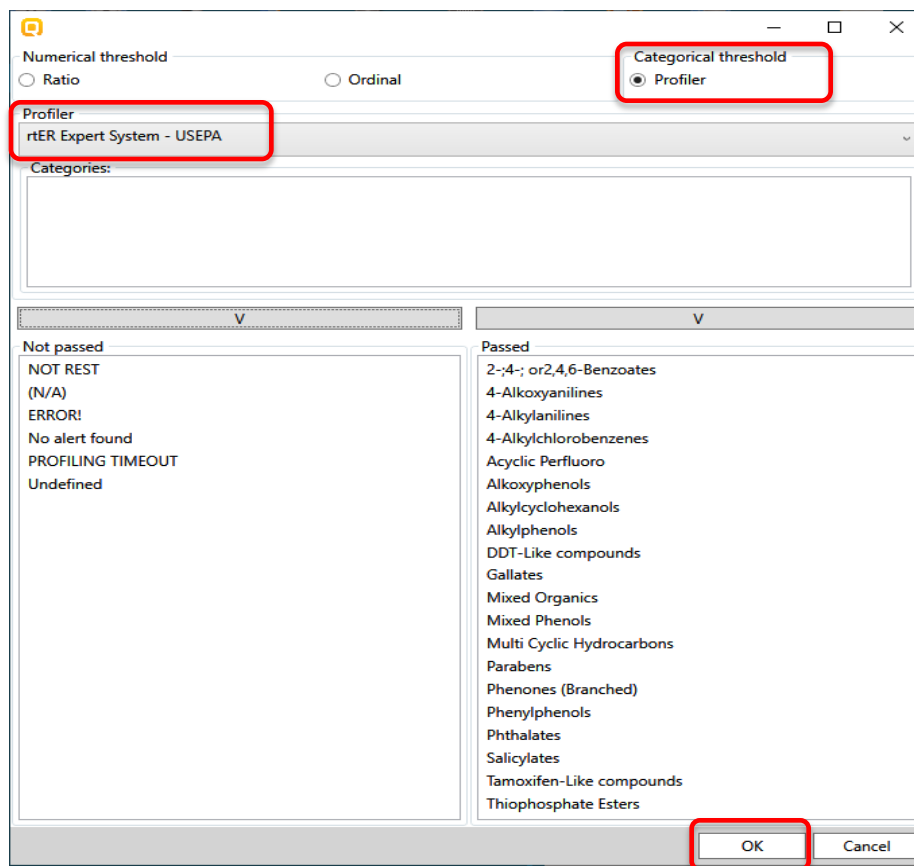
**Profiler: rtER Expert System – USEPA**

#### -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 thresholds for data

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**
  - $RBA < 0.00001\%$

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impairment

MIE: Estrogen receptor binding

Numerical threshold  
☐ Ratio  
☒ Ordinal  
 Unit: Estrogen...  
 RBA > 0.00001%

Categorical threshold  
☐ Profiler

Not passed

Passed  
 RBA > 0.00001%

OH passed: No OH, NH-group|||Non binder, impaired OH or NH2 group|||Non binder, MW>500|||N non cyclic structure|||Non binder, without OH or NH2 group

OK Cancel

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 move it to the **"Passed"** panel (8).

# Configuring the migrated AOP

## Node: MIE – Estrogen receptor binding

## Configuring thresholds for data

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%

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impairment

MIE: Estrogen receptor binding

Numerical threshold

☐ Ratio ☒ Ordinal ☐ Profiler

Unit

Estroge... RBA<0.00001%

V

Not passed

RBA<0.00001%

Passed

RBA>0.00001%

OK Cancel

Thresholds:

Profiler name: Estrogen Receptor Binding;;; Passed: Strong binder, NH2 group|||Stro  
OH group|||Very strong binder, OH group|||Weak binder, NH2 group|||Weak binder, OH gr  
passed: No OH, NH-group|||Non binder, impaired OH or NH2 group|||Non binder, MW>500||N

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 thresholds for data

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:**

- $0.001 < \text{RBA} < 0.1\%$
- $0.1 < \text{RBA} < 10\%$
- $0 < \text{RBA} < 0.001\%$
- $\text{RBA} > 10\%$

##### **Not passed:**

- Not active

#### Scale: “Mass concentration”:

##### **Passed:**

- $(0 - 0.000021] \text{ mg/L}$

##### **Not passed:**

- $(0.000021 - 100] \text{ mg/L}$

The image shows two configuration windows from the QSAR Toolbox. The top window, titled "Scale "Estrogen Binding Affinity(OASIS II)"", has the "Ordinal" radio button selected under the "Numerical threshold" section. The "Unit" is set to "Estroge..." and "Not active". The "Not passed" panel contains "Not active". The "Passed" panel lists four ranges: "0.001 < RBA < 0.1%", "0.1 < RBA < 10%", "0 < RBA < 0.001%", and "RBA > 10%". The bottom window, titled "Scale "Mass concentration"", has the "Ratio" radio button selected. The "Min" value is 0.000021 and the "Max" value is 100. The "Open" checkbox is checked. The "Unit" is set to "Mass co..." and "mg/L". The "Not passed" panel contains the range "(0.000021 - 100] mg/L". The "Passed" panel contains the range "(0 - 0.000021] mg/L".

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

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impairment Save

```

graph LR
    MIE[MIE: Estrogen receptor binding] --> KE1[KE1: Cellular response: Altered protein expr...]
    KE1 --> KE2[KE2: Tissue/ Organ: Liver Altered proteins]
    KE2 --> KE3[KE3: Individual: Sex reversal]
    KE3 --> AO[AO: Population: Skewed sex ratios]
    
```

**MIE: Estrogen receptor binding**

**Context**  
Level of Biological Organization=molecular  
Sex=mixed

**Associated tests**  
In-vitro: Relative ERBA assayIn-vitro: ToxCast assays

**Definition**  
This adverse outcome pathway details the linkage between antagonism of estrogen receptor and the adverse outcome of skewed sex ratios. Cumulative fecundity in repeat testing is a critical endpoint considered in the OECD 229 assay serves as a surrogate for adverse reproductive impairment (OECD 2012a). Cumulative fecundity is one of several variables known to be of demographic significance in forecasting fish population trends. Therefore, this AOP has utility in supporting the application of measures of ER antagonism, or in silico

**Congratulations! Node MIE is configured**

Select databases  
Select simulators  
Select profiles  
Endpoint tree positions:  
Add  
Remove  
Thresholds:  
Add  
Remove

**Relevant databases:**  
Receptor Mediated Effects  
ToxCastDB  
Toxicity to reproduction (ER)

**Associated endpoint tree positions:**  
Human Health Hazards#Toxicity to Reproduction  
Endpoint=Relative ERBA  
Human Health Hazards#Toxicity to Reproduction  
Endpoint=EC50 <OR> IC50  
Human Health Hazards#ToxCast  
Assay provider=Novascreen  
Assay=Novascreen Bovine ER  
Test organisms (species)=Bos taurus  
Endpoint=AC50  
Human Health Hazards#ToxCast  
Endpoint=AC50



# Configuring the migrated AOP

## Node **KE1 - Cellular response: Altered protein expression: increasing Vitellogenin synthesis**

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 synthesis**

Relevant database "ECOTOX"

The screenshot shows the 'Effectopedia AOP Editor' window. The 'Pathways' dropdown is set to 'AOP: ER-mediated reproductive Impairment'. The main area displays a sequence of four nodes: 'MIE: Estrogen receptor binding' (blue), 'KE1: Cellular response: Altered protein expr...' (white), 'KE2: Tissue/ Organ: Liver Altered proteins' (blue), and 'KE3: Individual: Sex reversal' (blue). Below the pathway, there are buttons for 'Select databases', 'Select simulators', and 'Select profiles'. The 'Relevant databases:' section shows 'ECOTOX' selected and highlighted with a red dashed box. At the bottom, a yellow box contains the text 'Add the relevant database'.

Relevant endpoints

The screenshot shows the 'Effectopedia AOP Editor' window with the 'Pathways' dropdown set to 'AOP: ER-mediated reproductive Impairment'. The main area displays the same sequence of four nodes as the previous screenshot. Below the pathway, there are buttons for 'Select databases', 'Select simulators', and 'Select profiles'. The 'Relevant databases:' section shows 'ECOTOX' selected. The 'Associated endpoint tree' section shows a list of endpoints, with 'Ecotoxicological Information#Aquatic Toxicity' selected and highlighted with a red dashed box. To the right, there are two 'Select endpoint' dialog boxes. The top dialog shows the 'Effect' dropdown set to 'Biochemistry', 'Duration' set to '+', 'Test organisms (species)' set to 'LOEC', and 'Endpoint' set to 'Vitellogenin'. The 'Measurement' dropdown is set to 'Increasing'. The bottom dialog shows the 'Effect' dropdown set to 'Biochemistry', 'Duration' set to '+', 'Test organisms (species)' set to 'NOEC', and 'Endpoint' set to 'Vitellogenin'. The 'Measurement' dropdown is set to 'Increasing'. Both dialog boxes have a 'Trend' dropdown set to 'Increasing' and a 'Measurement' dropdown set to 'Vitellogenin'. A yellow box at the bottom contains the text '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 synthesis**

Relevant thresholds

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impa

MIE: Estrogen receptor binding → KE1: Cellular response: Altered protein expr... → KE2: Tissue/Organ: Liver Altered proteins

Select databases  
Select simulators  
Select profiles  
Endpoint tree positions:  
Add  
Remove  
Thresholds:  
Add  
Remove

Effect=Biochemistry  
Endpoint=NOEC  
Measurement=Vitellogenin  
Trend=Increasing  
Ecotoxicological  
Information#Aquatic Toxicity  
Effect=Biochemistry  
Endpoint=LOEC  
Measurement=Vitellogenin  
Trend=Increasing

Thresholds:  
Scale name: mass concentration  
concentration;;; Passed: [0 - 1] mg/L;;; Not passed: [1 - 1000] mg/L;;;Scale type: RatioScale

Numerical threshold  
☒ Ratio ☐ Ordinal  
Categorical threshold  
☐ Profiler

Min 0 Max 1  
☐ Open ☒ Open

Unit  
Mass co... mg/L

Not passed [1 - 1000] mg/L  
Passed [0 - 1] mg/L

OK Cancel

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 Altered 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 Altered proteins

Relevant database: "ECOTOX"

Relevant thresholds

**Relevant databases:**  
ECOTOX

**Relevant endpoints: LOEC and NOEC**

**Relevant thresholds**

**Relevant databases:**  
ECOTOX

**Associated endpoint tree positions:**

Ecotoxicological      Information#Aquatic

Toxicity

Endpoint=LOEC

Effect=Reproduction

Measurement=Fecundity

Trend=Decreasing

Ecotoxicological      Information#Aquatic

Toxicity

Effect=Reproduction

Endpoint=NOEC

Measurement=Fecundity

Trend=Decreasing

**Thresholds:**

Scale name: Mass concentration;;

Passed: [0 - 1) mg/L;; Not passed: [1 - 1000] mg/L;; Scale type: RatioScale

# Configuring the migrated AOP

## Node **KE3 - Individual: Sex reversal**

- This Effectopedia AOP node is not configured, because there are no Toolbox data available 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 thresholds

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impairment

MIE: Estrogen receptor binding → KE1: Cellular response: Altered protein expr... → KE2: Tissue/Organ: Liver Altered proteins → KE3: Individual: Sex reversal → AO: Population: Skewed sex ratios

Select databases  
Select simulators  
Select profiles  
Endpoint tree positions:  
Add  
Remove  
Thresholds:  
Add  
Remove

Relevant databases:  
ECOTOX

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impairment

MIE: Estrogen receptor binding → KE1: Cellular response: Altered protein expr... → KE2: Tissue/Organ: Liver Altered proteins → KE3: Individual: Sex reversal → AO: Population: Skewed sex ratios

Select databases  
Select simulators  
Select profiles  
Endpoint tree positions:  
Add  
Remove  
Thresholds:  
Add  
Remove

Relevant databases:  
ECOTOX

Associated endpoint tree positions:  
Ecotoxicological Information#Aquatic  
Toxicity  
Effect=Population  
Endpoint=LOEC  
Measurement=Sex ratio  
Trend=Change  
Ecotoxicological Information#Aquatic  
Toxicity  
Effect=Population  
Endpoint=NOEC  
Measurement=Sex ratio  
Trend=Change

Effectopedia AOP Editor

Open Effectopedia AOP Pathways AOP: ER-mediated reproductive Impairment

KE1: Cellular response: Altered protein expr... → KE2: Tissue/Organ: Liver Altered proteins → KE3: Individual: Sex reversal → AO: Population: Skewed sex ratios

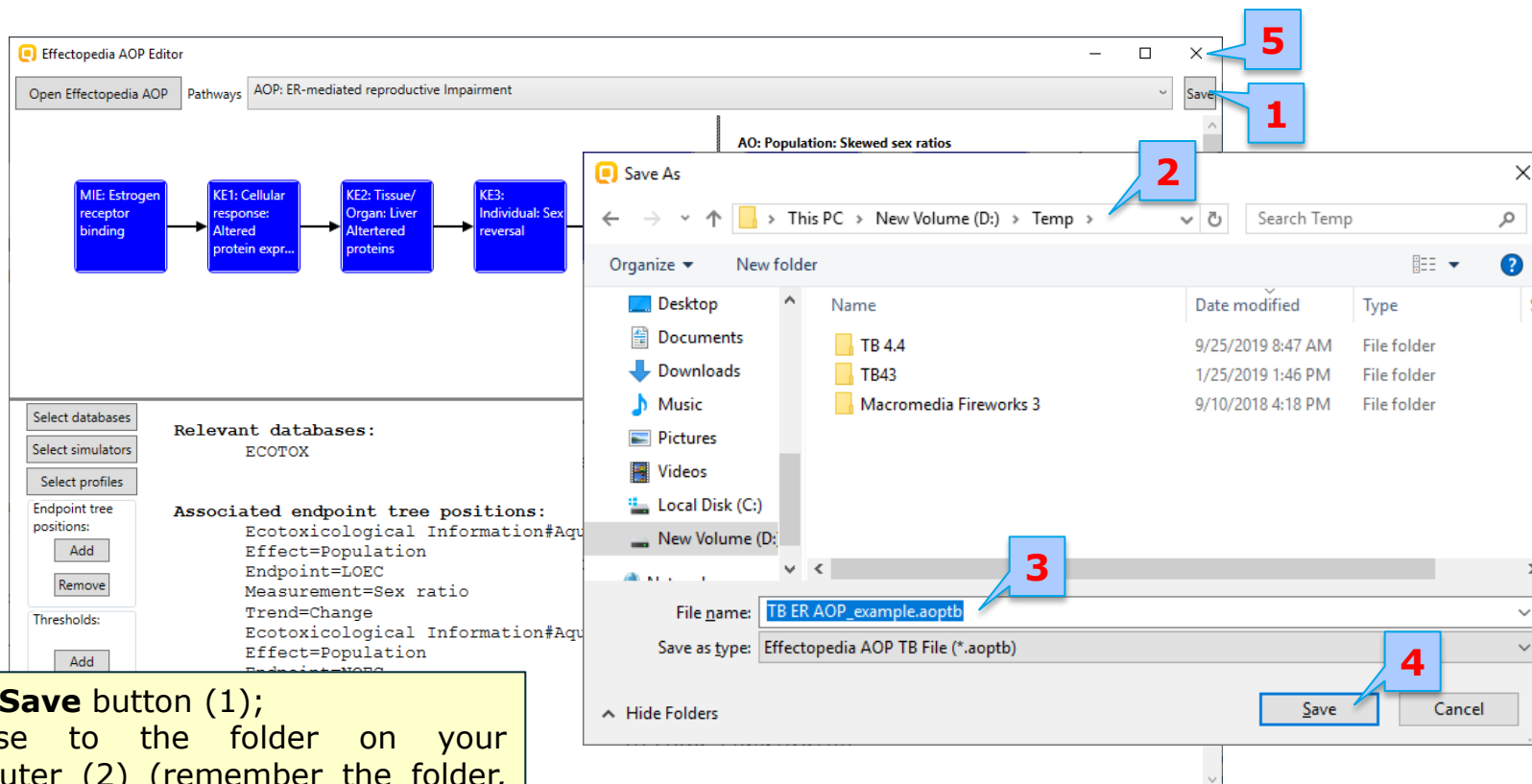
Toxicity  
Effect=Population  
Endpoint=LOEC  
Measurement=Sex ratio  
Trend=Change  
Ecotoxicological Information#Aquatic  
Toxicity  
Effect=Population  
Endpoint=NOEC  
Measurement=Sex ratio  
Trend=Change

Thresholds:  
Scale name: Mass concentration;;; Passed: [0 - 1) mg/L;;; Not passed: [1 - 1000] mg/L;;;Scale type: RatioScale



# 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

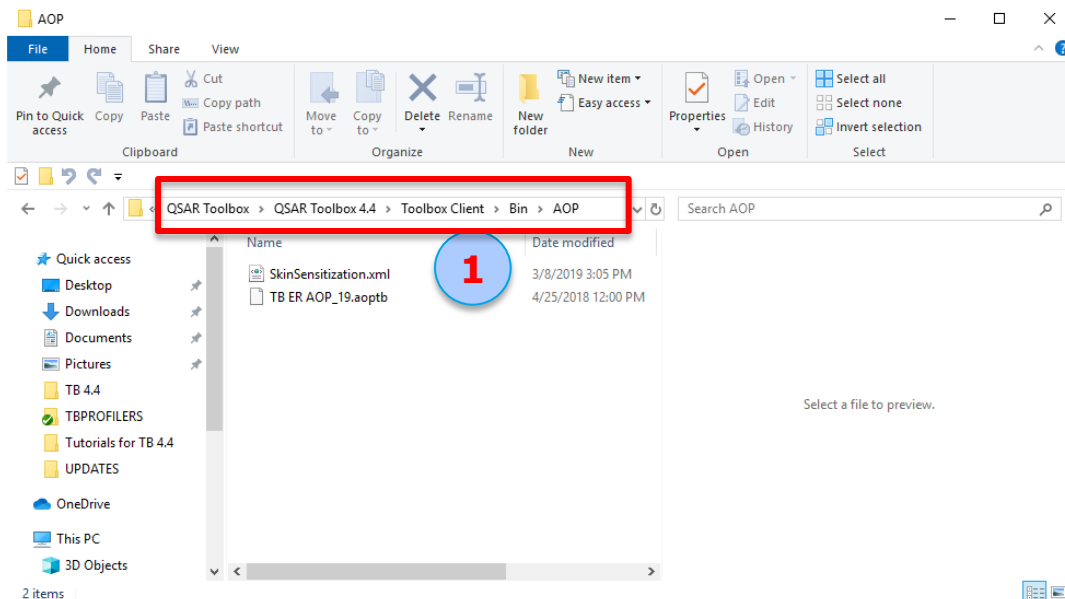


Click **Save** button (1);  
Browse to the folder on your computer (2) (remember the folder, you will need it (see next slide)), gave **name** of the custom AOP (3);  
Click **Save** (4);  
Close the window of the AOP editor (5).

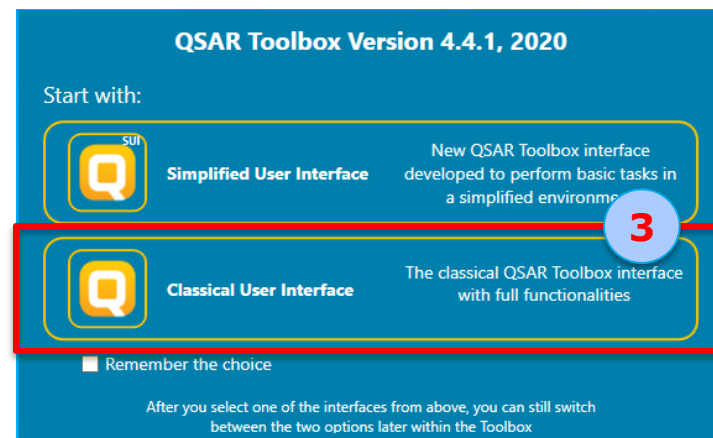
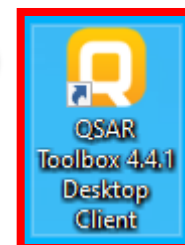
# Configuring the migrated AOP

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)**



2



# Outlook

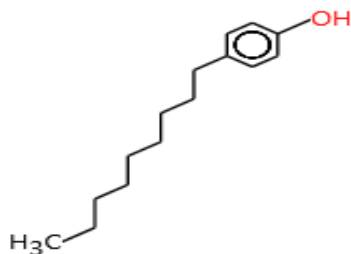
- Background
- Objectives
- Specific Aims
- Overview of the used Effectopedia AOP
- Migrating the Effectopedia AOP to Toolbox
- Building a new AOP-based profiler in Toolbox for predictive purposes
- **Application of migrated AOP for collecting weight-of evidence**

# 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

The screenshot illustrates the application of a migrated AOP in the QSAR Toolbox. The interface is divided into several panels:

- Top Menu Bar:** Includes 'Input', 'Profiling' (highlighted with a red box and number 2), 'Data', 'Category definition', 'Data Gap Filling', and 'Report'.
- Left Panel:** Contains 'Filter endpoint tree...' and 'Profiling methods'. The 'Profiling methods' section shows 'Estrogen Receptor Binding' selected (highlighted with a red box and number 3).
- Center Panel:** Displays the chemical structure of the target chemical, 17β-estradiol, and a list of endpoints. The 'MIE: Estrogen receptor binding' endpoint is highlighted with a red box and number 1.
- Right Panel:** Shows the 'Scheme' window, which displays a flowchart of the AOP. The flowchart starts with 'MIE: Estrogen receptor binding' (highlighted with a red box and number 1), followed by 'KE1: Cellular response: Altered protein expression: increasing Vit', 'KE2: Tissue/Organ: Liver Altered proteins', 'KE3: Individual: Sex reversal', and finally 'AO: Population: Skewed sex ratios'.
- Bottom Panel:** Contains 'Settings' and 'Documentation' windows. The 'Settings' window shows the 'Node short name: MIE' and 'Node full name: MIE: Estrogen receptor binding'. The 'Documentation' window provides context and associated tests for the MIE.

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

The screenshot displays the QSAR Toolbox software interface. The top menu bar includes 'Data', 'Category definition', 'Data Gap Filling', and 'Report'. The 'Data' menu is highlighted with a red box and a blue callout '1'. The left sidebar shows 'Databases' and 'Inventories' sections. In the 'Databases' section, 'Human Health Hazards' is selected, and 'Receptor Mediated Effects', 'ToxCastDB', and 'Toxicity to reproduction (ER)' are highlighted in green. A blue callout '2' points to this selection. The 'Gather' button in the top menu is highlighted with a red box and a blue callout '3'. The main workspace shows a 'Scheme' diagram with a red box around 'MIE: Estrogen receptor binding', a blue callout '4' pointing to the 'OK' button in the 'Read data?' dialog, and a blue callout '5' pointing to the 'OK' button in the 'Toxicity to reproduction (ER)' pop-up message. The 'Toxicity to reproduction (ER)' message states '121 points added across 1 chemicals.'.

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

The screenshot illustrates the application of a migrated AOP in the QSAR Toolbox. The interface is divided into several sections:

- Top Menu Bar:** Contains icons for 'Input', 'Profiling', 'Data' (highlighted with a red box and number 2), 'Category definition', 'Data Gap Filling', and 'Report'.
- Left Sidebar:**
  - Documents:** Shows a document named 'Document 1' with a file icon (highlighted with a red box and number 4) and a list of documents.
  - Databases:** Lists various databases, including 'Environmental Fate and Transport', 'Ecotoxicological Information', and 'Human Health Hazards' (highlighted with a red box and number 3).
  - Inventories:** Shows a list of inventories with checkboxes for 'Canada DSL' and 'COSING'.
- Main Window:**
  - Filter endpoint tree...** displays a list of endpoints, including 'KE1: Cellular response: Altered protein expression: increasing Vt' (highlighted with a red box and number 1).
  - Structure:** Shows the chemical structure of the target chemical.
  - Target chemical:** Displays the chemical structure of the target chemical.
- Pop-up Windows:**
  - Read data?:** A dialog box with 'All endpoints' selected and 'OK' button (highlighted with a red box and number 5).
  - Read data?:** A second dialog box showing '2887 points added across 1 chemicals.' and 'OK' button (highlighted with a red box and number 6).

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")

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

- Full names:** A list of AOP nodes including MIE: Estrogen receptor binding, KE1: Cellular response: Altered protein expression, KE2: Tissue/Organ: Liver Altered proteins, KE3: Individual: Sex reversal, and AO: Population: Skewed sex ratios. The AO node is highlighted in blue.
- Scheme:** A flowchart showing the sequence of AOP nodes: MIE (red) → KE1 (red) → KE2 (red) → KE3 (blue) → AO (red). The AO node is highlighted with a pink border. A legend below indicates: Not checked (blue), Not passed (green), and Passed (red).
- Predictions bucket:** A table showing data for the target chemical:
 

Data
M: 0.01 mg/L
M: 0.01 mg/L
- Target chemical:** A chemical structure of 1-octanol is shown, with the hydroxyl group highlighted in red.
- Settings:**
  - Node short name:** AO:
  - Node full name:** AO: Population: Skewed sex ratios
  - Relevant databases:** ECOTOX
  - Associated endpoint tree positions:** Ecotoxicological Information#Aquatic Toxicity, Effect=Population, Endpoint=LOEC, Measurement=Sex ratio, Trend=Change
  - Ecotoxicological Information#Aquatic Toxicity:** Effect=Population, Endpoint=NOEC
- Documentation:**
  - AO: Population: Skewed sex ratios** (About button)
  - Context:** Level of Biological Organization=population, Sex=mixed
  - Associated tests:** In-vivo Skewed sex ratios
  - Definition:** Provide brief description of the (key) event
  - Measurement/detection:** Provide a summary of the available measurement and detection methods.



# 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.