How to derive relative potency factors (RPFs) using the EuroMix toolbox

Exercise 1

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Dose addition

- Exposure of each substance is multiplied with the relative potency factor (RPF)
- Potency scaled exposure added to get the potency scaled exposure expressed as reference substance equivalents

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Relative potency factors

- Point of departure (PoD) = NOAEL or BMD for each substance in assessment group
- Relative potency factor (RPF) = PoD of reference substance / PoD substance

Substance	PoD	RPF
Reference substance	10	10/10 = 1
Substance 1	20	10/20 = 0.5
Substance 2	5	10/ 5 = 2

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Sources for RPFs

- RPF data from literature
- PoD data from literature
- Experimental dose response data
- TTC values when no in vitro or in vivo data is available

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Aim of the exercises

- In these exercises you will learn how to derive relative potency factors (RPFs) based on three types of data:
  - RPF data from literature
  - NOEALs from in vivo studies
  - Dose response data using benchmark dose modelling

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Example

- The example is based on five* substances that have been grouped in an assessment group for liver steatosis
- Substances: Clothianidin, Flusilazole, Difenoconazole, Thiacloprid, Imazalil
- Reference substance: Flusilazole

- Exercise 1: RPFs based on in vivo NOAELs from literature
- Exercise 2: NOAELs based on in vivo studies
- Exercise 3: in vitro dose response data

*Most real assessment group contain many more substances. Only five substances are used in this example to make it easy to follow

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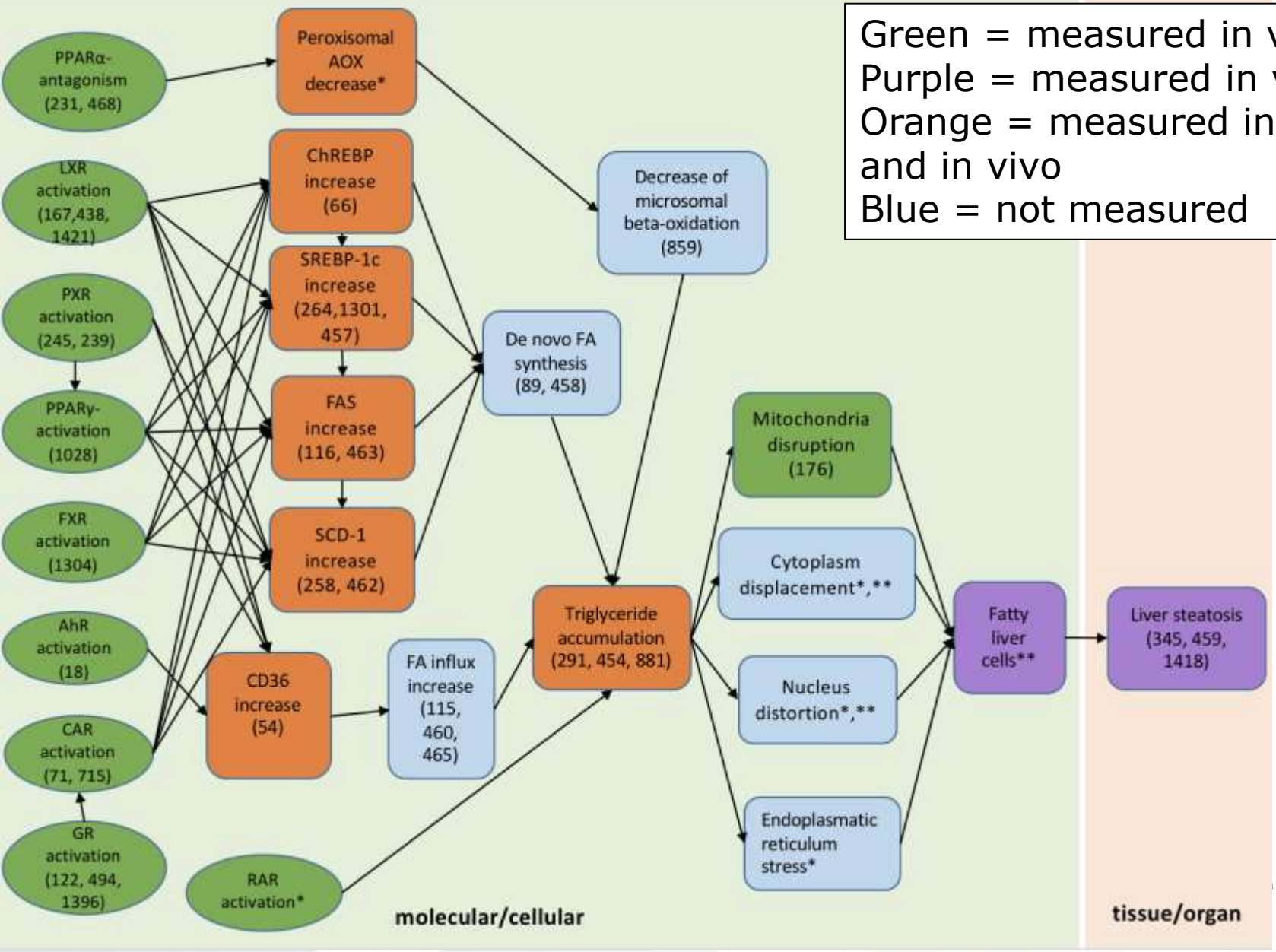
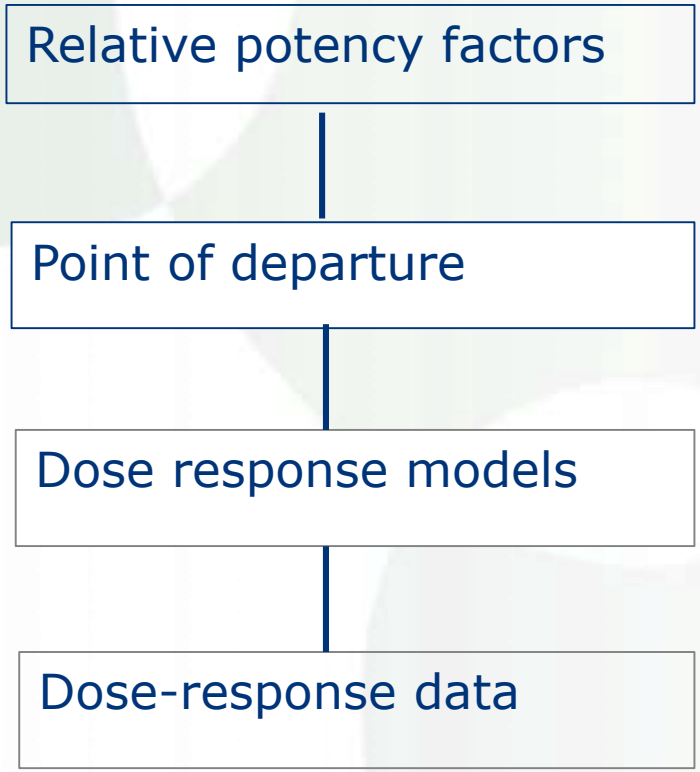
Steatosis AOP network

EuroMix

Green = measured in vitro
Purple = measured in vivo
Orange = measured in vitro and in vivo
Blue = not measured



Modules for hazard data



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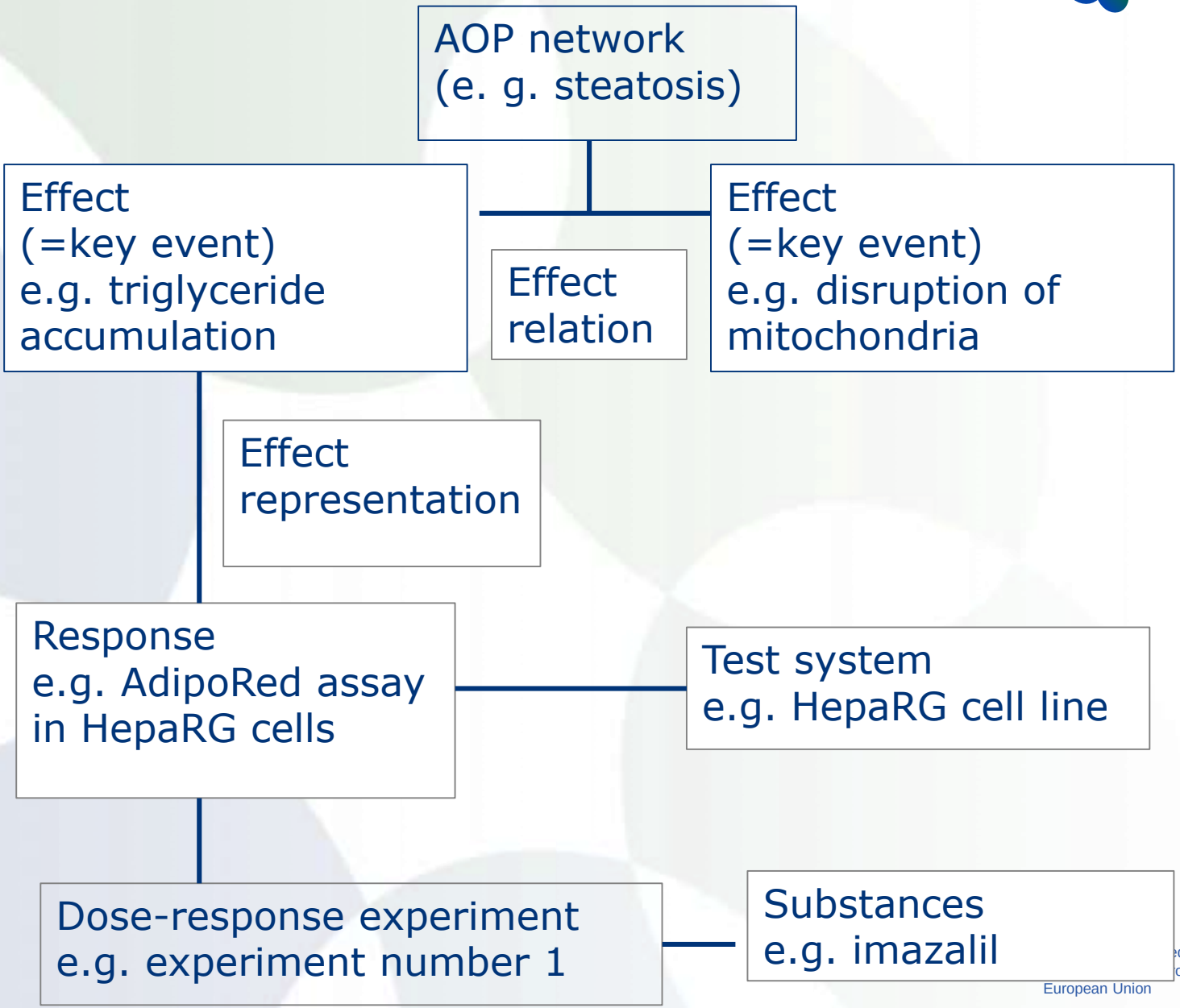
Modules for hazard data



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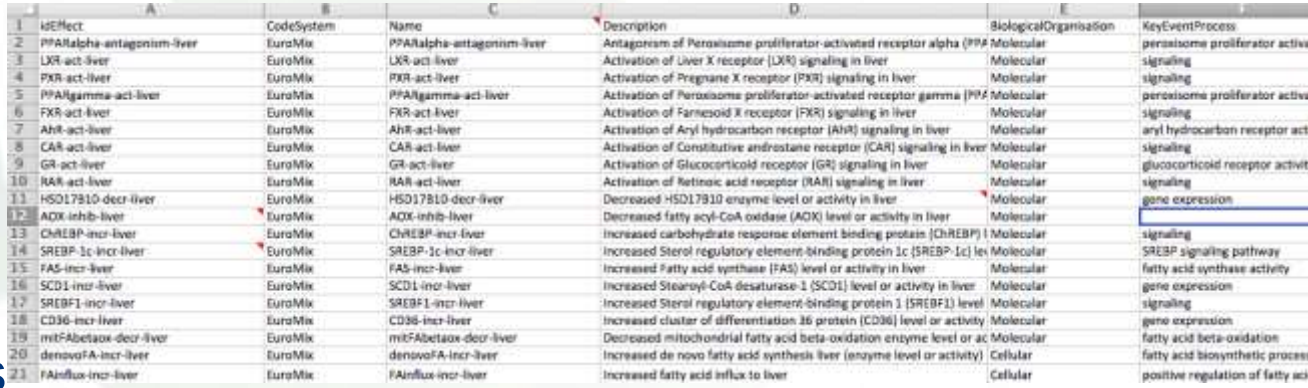
Information in Excel sheets

Excel sheets for

- AOP networks
- Effects
- Effect Relations
- Test systems
- Responses
- Effect Representations
- Substances
- Dose-response experiments
- Relative potency factors
- Point of departure



	A	B	C	D	E	F
1	idEffect	CodeSystem	Name	Description	BiologicalOrganisation	KeyEventProcess
2	PPARalpha-antagonism-liver	EuroMix	PPARalpha-antagonism-liver	Antagonism of Peroxisome proliferator-activated receptor alpha (PPA	Molecular	peroxisome proliferator activa
3	LXR-act-liver	EuroMix	LXR-act-liver	Activation of Liver X receptor (LXR) signaling in liver	Molecular	signaling
4	PXR-act-liver	EuroMix	PXR-act-liver	Activation of Pregnane X receptor (PXR) signaling in liver	Molecular	signaling
5	PPARgamma-act-liver	EuroMix	PPARgamma-act-liver	Activation of Peroxisome proliferator-activated receptor gamma (PPA	Molecular	peroxisome proliferator activa
6	FXR-act-liver	EuroMix	FXR-act-liver	Activation of Farnesoid X receptor (FXR) signaling in liver	Molecular	signaling
7	AhR-act-liver	EuroMix	AhR-act-liver	Activation of Aryl hydrocarbon receptor (AhR) signaling in liver	Molecular	aryl hydrocarbon receptor act
8	CAR-act-liver	EuroMix	CAR-act-liver	Activation of Constitutive androstane receptor (CAR) signaling in liver	Molecular	signaling
9	GR-act-liver	EuroMix	GR-act-liver	Activation of Glucocorticoid receptor (GR) signaling in liver	Molecular	glucocorticoid receptor activit
10	RAR-act-liver	EuroMix	RAR-act-liver	Activation of Retinoic acid receptor (RAR) signaling in liver	Molecular	signaling
11	HSD17B10-decr-liver	EuroMix	HSD17B10-decr-liver	Decreased HSD17B10 enzyme level or activity in liver	Molecular	gene expression
12	AOX-inhib-liver	EuroMix	AOX-inhib-liver	Decreased fatty acyl-CoA oxidase (AOX) level or activity in liver	Molecular	
13	ChREBP-incr-liver	EuroMix	ChREBP-incr-liver	Increased carbohydrate response element binding protein (ChREBP) l	Molecular	signaling
14	SREBP-1c-incr-liver	EuroMix	SREBP-1c-incr-liver	Increased Sterol regulatory element-binding protein 1c (SREBP-1c) le	Molecular	SREBP signaling pathway
15	FAS-incr-liver	EuroMix	FAS-incr-liver	Increased Fatty acid synthase (FAS) level or activity in liver	Molecular	fatty acid synthase activity
16	SCD1-incr-liver	EuroMix	SCD1-incr-liver	Increased Stearoyl-CoA desaturase-1 (SCD1) level or activity in liver	Molecular	gene expression
17	SREBF1-incr-liver	EuroMix	SREBF1-incr-liver	Increased Sterol regulatory element-binding protein 1 (SREBF1) level	Molecular	signaling
18	CD36-incr-liver	EuroMix	CD36-incr-liver	Increased cluster of differentiation 36 protein (CD36) level or activity	Molecular	gene expression
19	mitFAbetaox-decr-liver	EuroMix	mitFAbetaox-decr-liver	Decreased mitochondrial fatty acid beta-oxidation enzyme level or ac	Molecular	fatty acid beta-oxidation
20	denovoFA-incr-liver	EuroMix	denovoFA-incr-liver	Increased de novo fatty acid synthesis liver (enzyme level or activity)	Cellular	fatty acid biosynthetic process
21	FAinflux-incr-liver	EuroMix	FAinflux-incr-liver	Increased fatty acid influx to liver	Cellular	positive regulation of fatty ac

- Include unique codes and additional data needed for modelling or as reference

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Information needed for the exercise 1

- Information on the following modules is needed. The information is in Excel sheets that are uploaded into the toolbox.

- Effects (steatosis-liver)
- Substances (Clothianidin, Flusilazole, Difenoconazole, Thiacloprid, Imazalil)
- Relative potency factors

	A	B	C	D
1	idEffect	active Substance	idSubstance	RPF
2	Steatosis-liver	Clothianidin	RF-0101-001-PPP	0,01962963
3	Steatosis-liver	Difenoconazole	RF-0133-001-PPP	0,112765957
4	Steatosis-liver	Flusilazole	RF-0218-001-PPP	1
5	Steatosis-liver	Imazalil (aka enilconazole)	RF-0246-001-PPP	0,1325
6	Steatosis-liver	Thiacloprid	RF-0417-001-PPP	0,441666667
7				
8				

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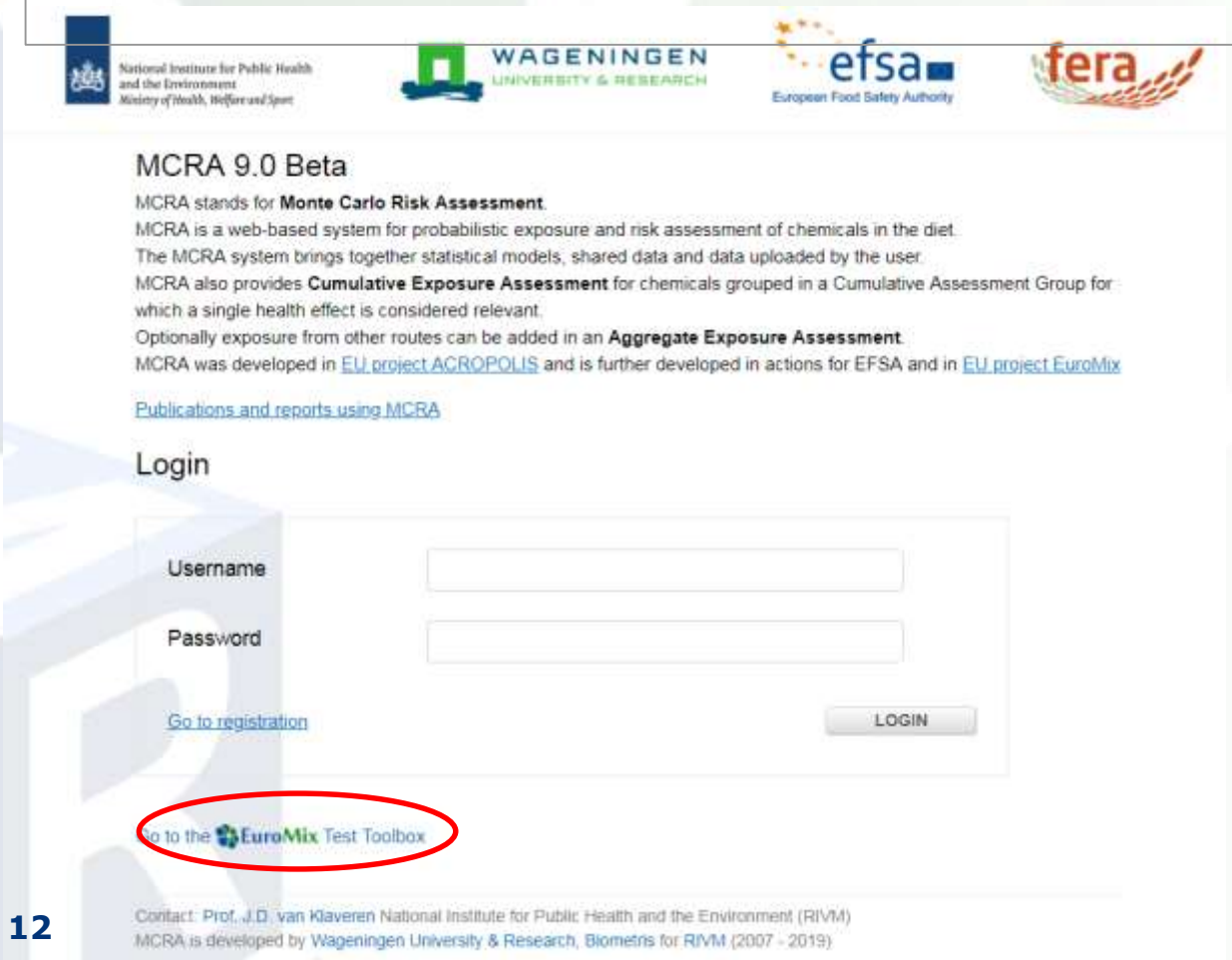
Login via MCRA 9 beta

1. Make sure you are using Google Chrome
2. Copy https://mcra-test.rivm.nl in Google Chrome
3. Click on EuroMix toolbox to get access to the EuroMix model test toolbox



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Login EuroMix toolbox

1. Log in: click on
2. Then log in

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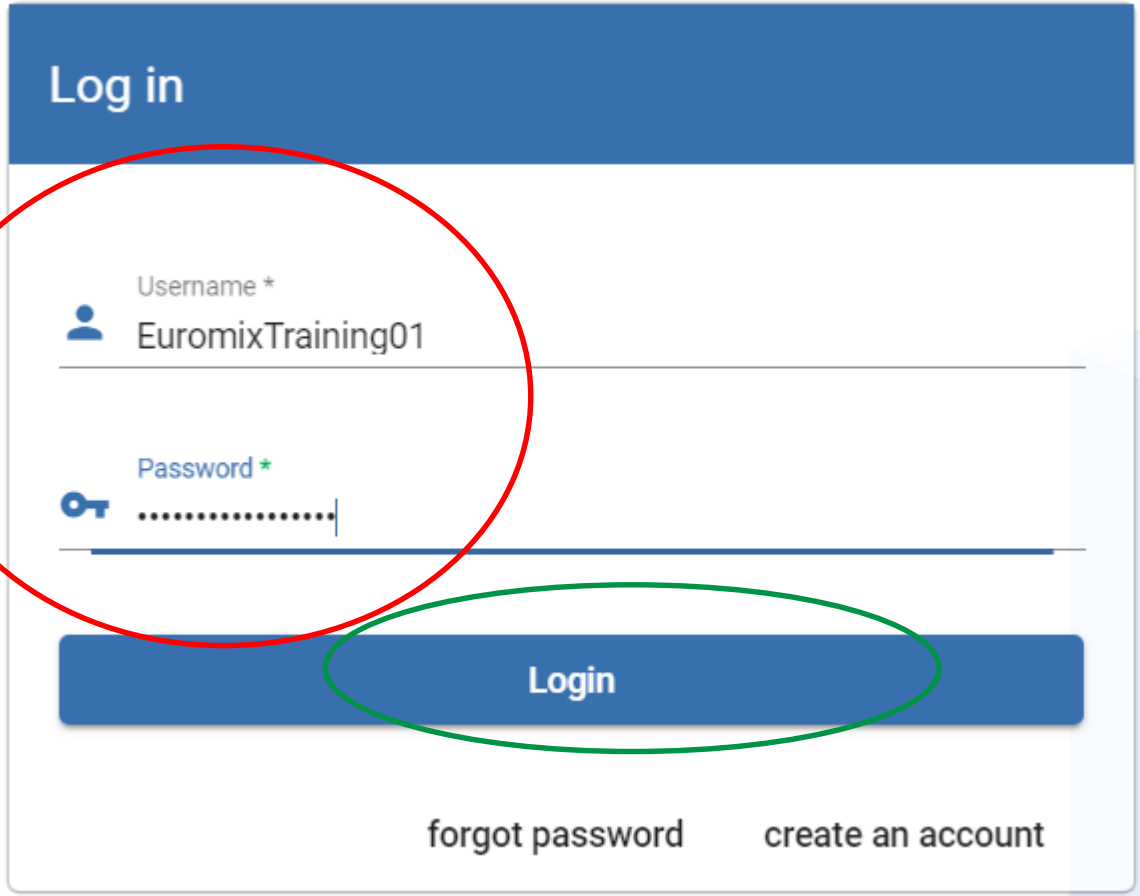
Insert username password



1. Login with the username and password provided by the tutor
2. Login

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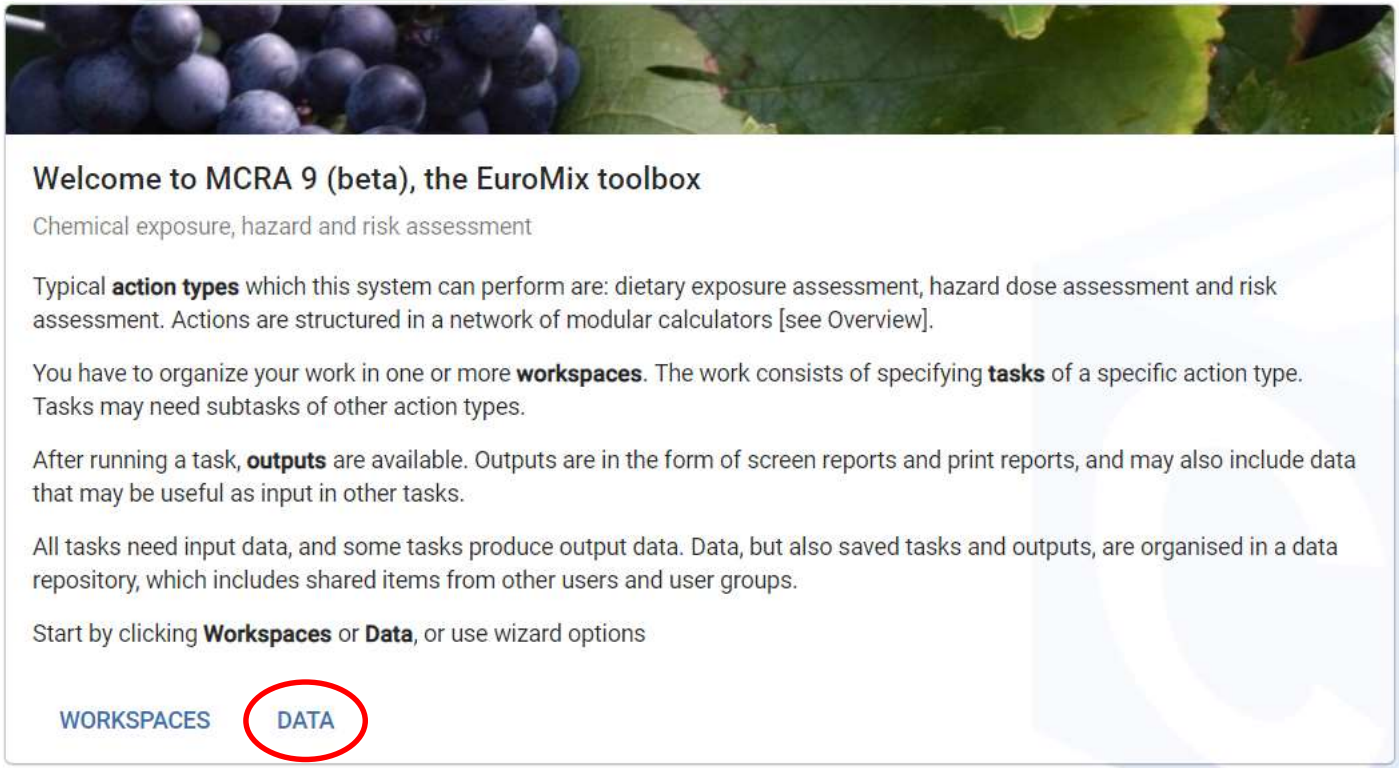
Data folders

1. Click on Data



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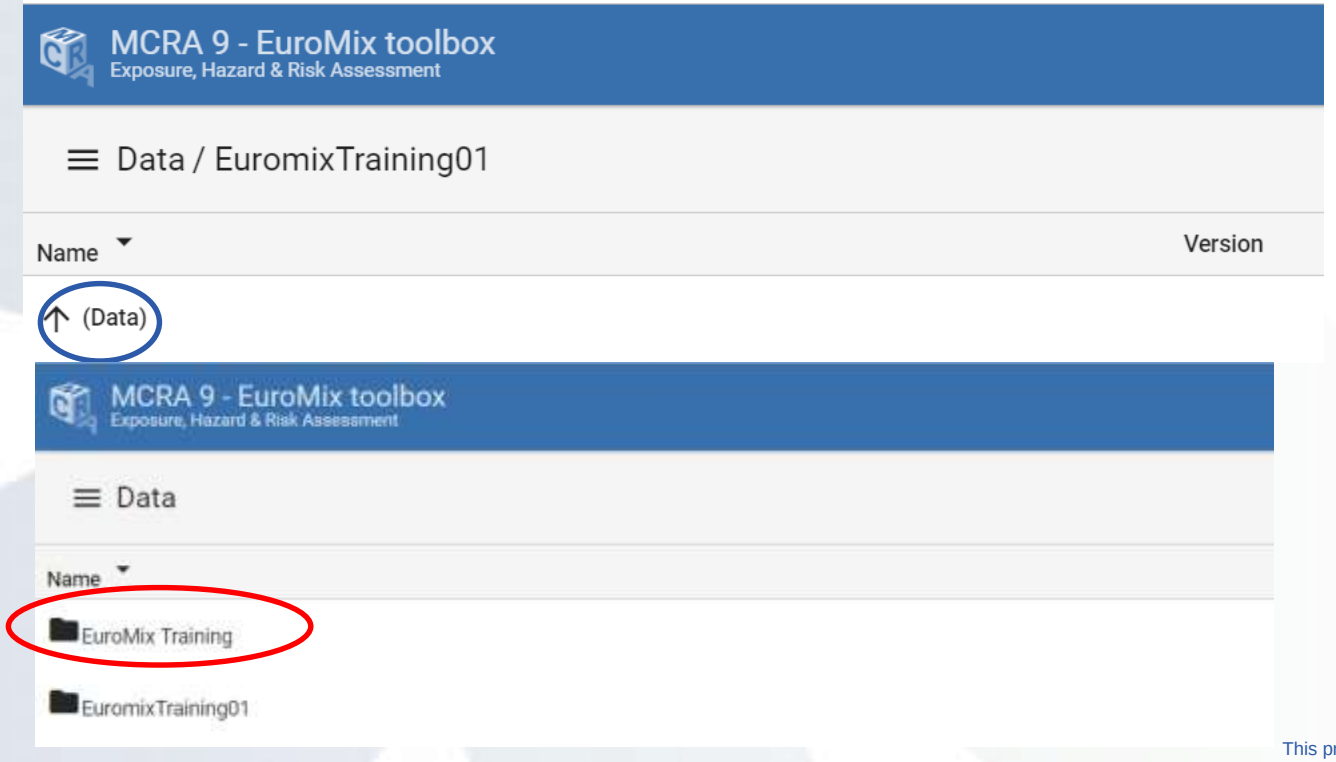
Data folders

1. Click on data

2. The data folder will be unfolded (see second screenshot)
   You will see EuroMix Training01 which is your own folder. You can upload your own files on this folder. For the training you click on EuroMix Training



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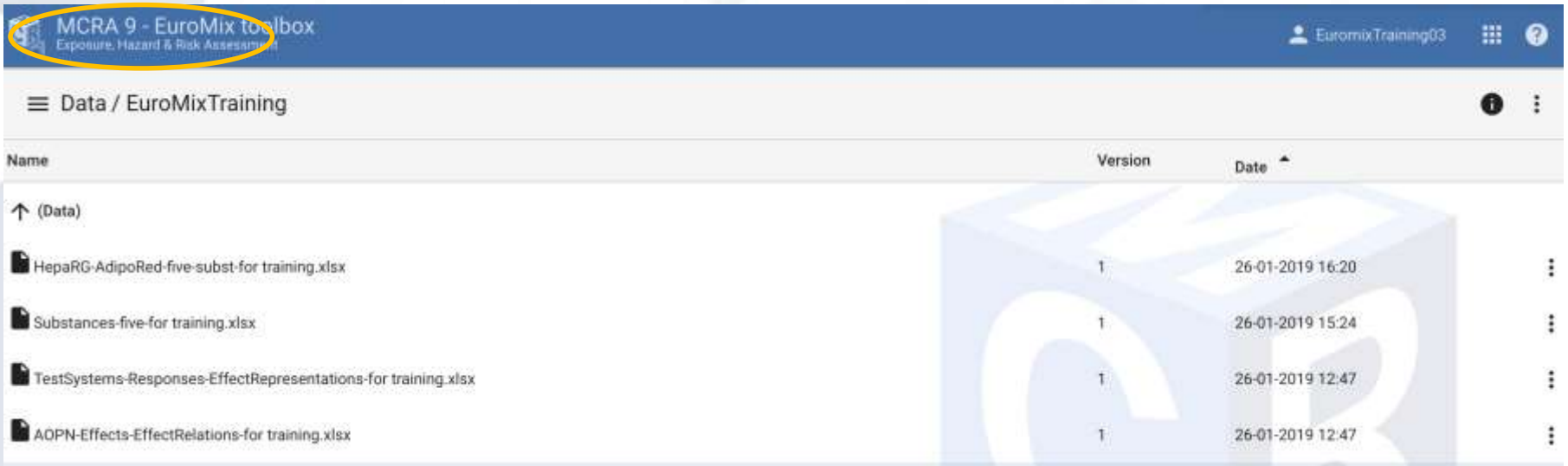
Data folders

The shared folder contains Excel files with:

1. Description of Effects
2. Description of Substances
3. Relative potency factors
4. Go to EuroMix toolbox main page by clicking on MCRA 9 – EuroMix toolbox



Actions needs to be done in workspaces

1. Click on workspaces and create a new workspace

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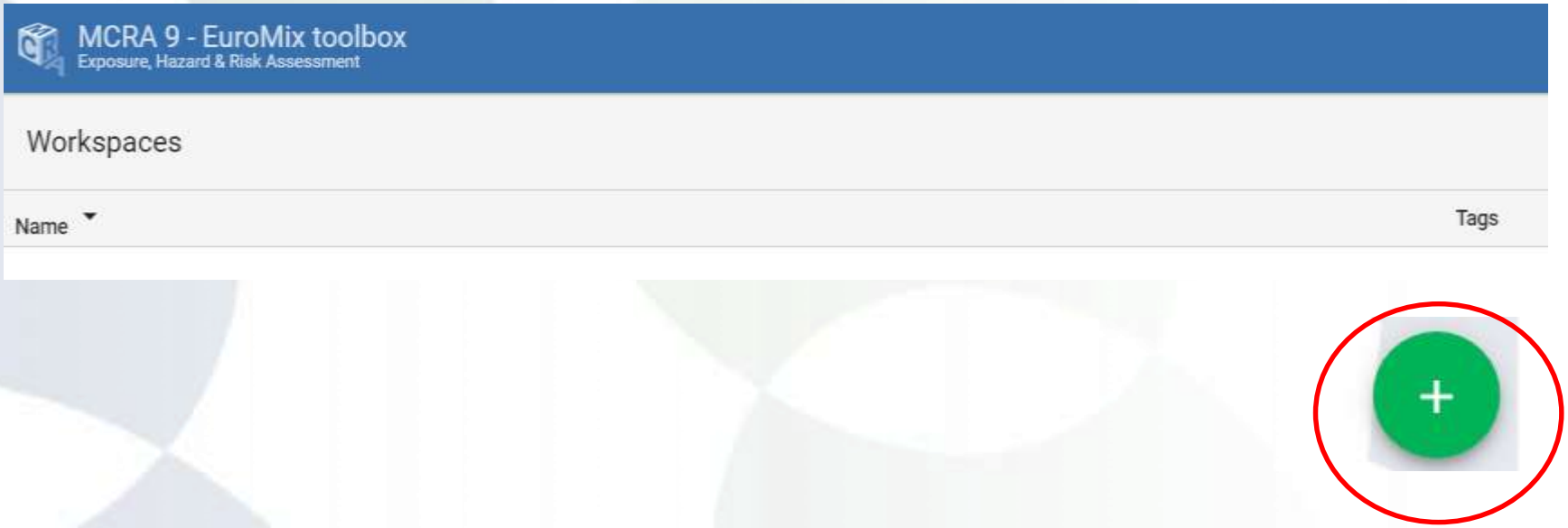
Create a new workspace (1)

1. Click on + and a new window pops up



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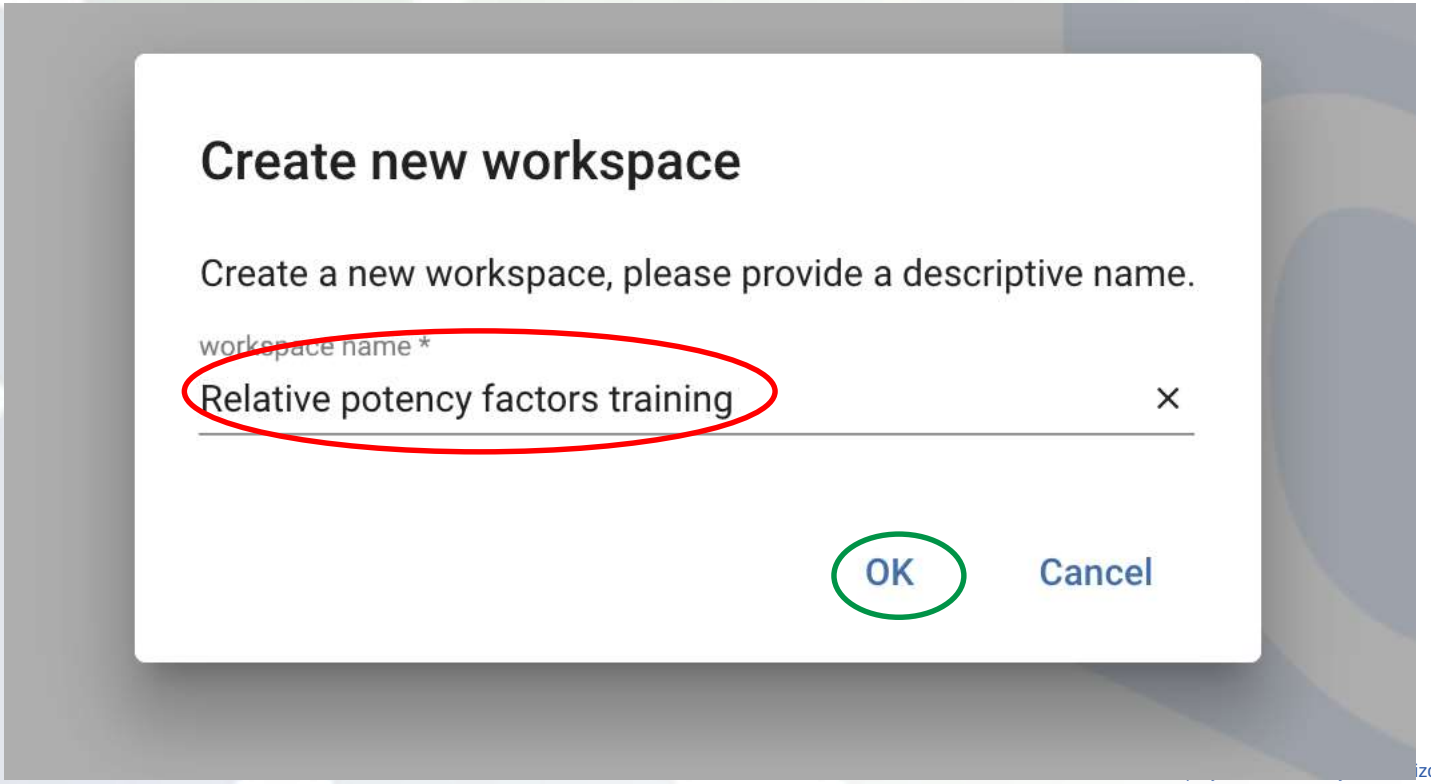
Create a new workspace (2)

1. Insert a name for the new workspace
2. Click on ok



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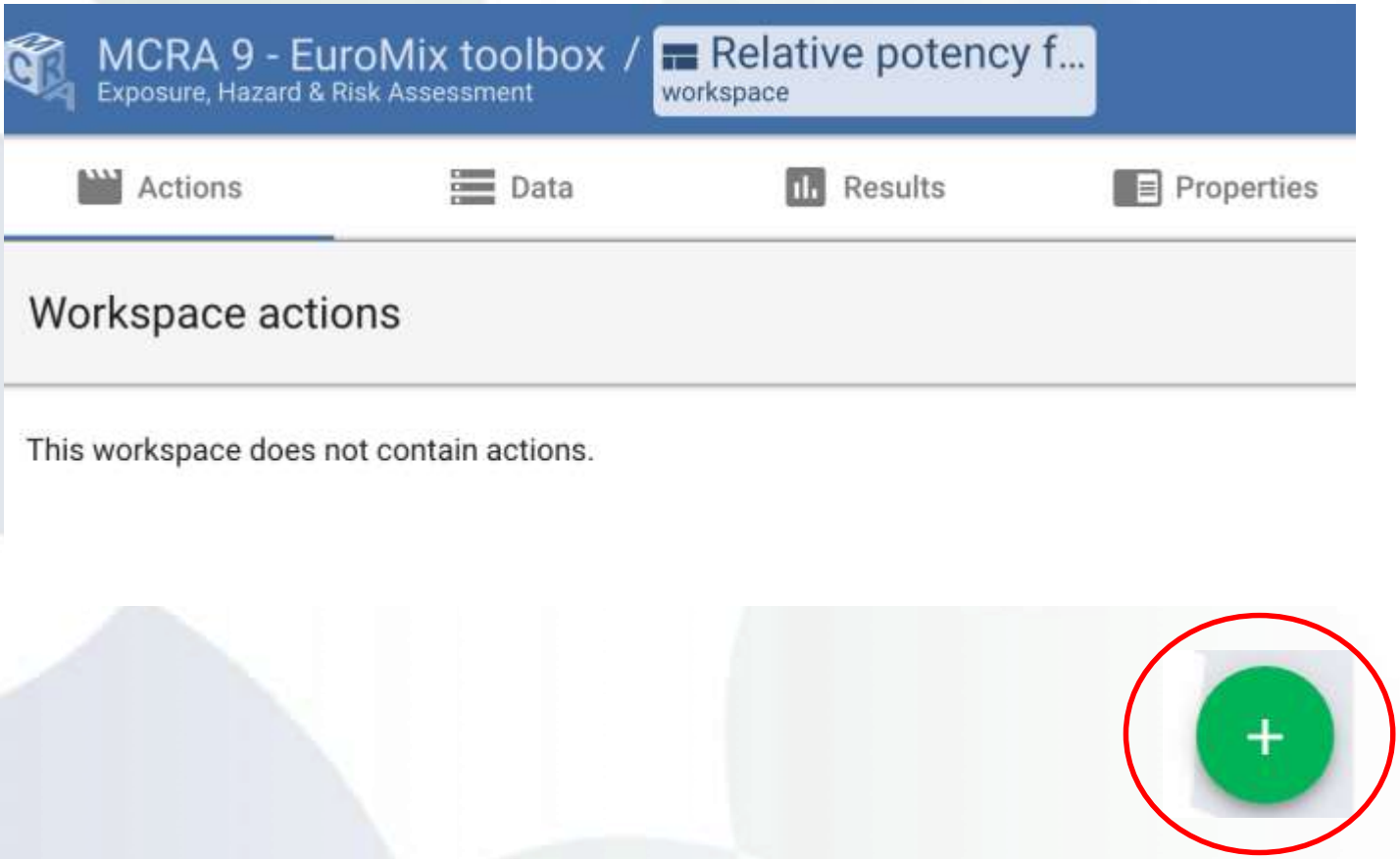
Create an action in the workspace (1)

1. Click on + and a new window pops up



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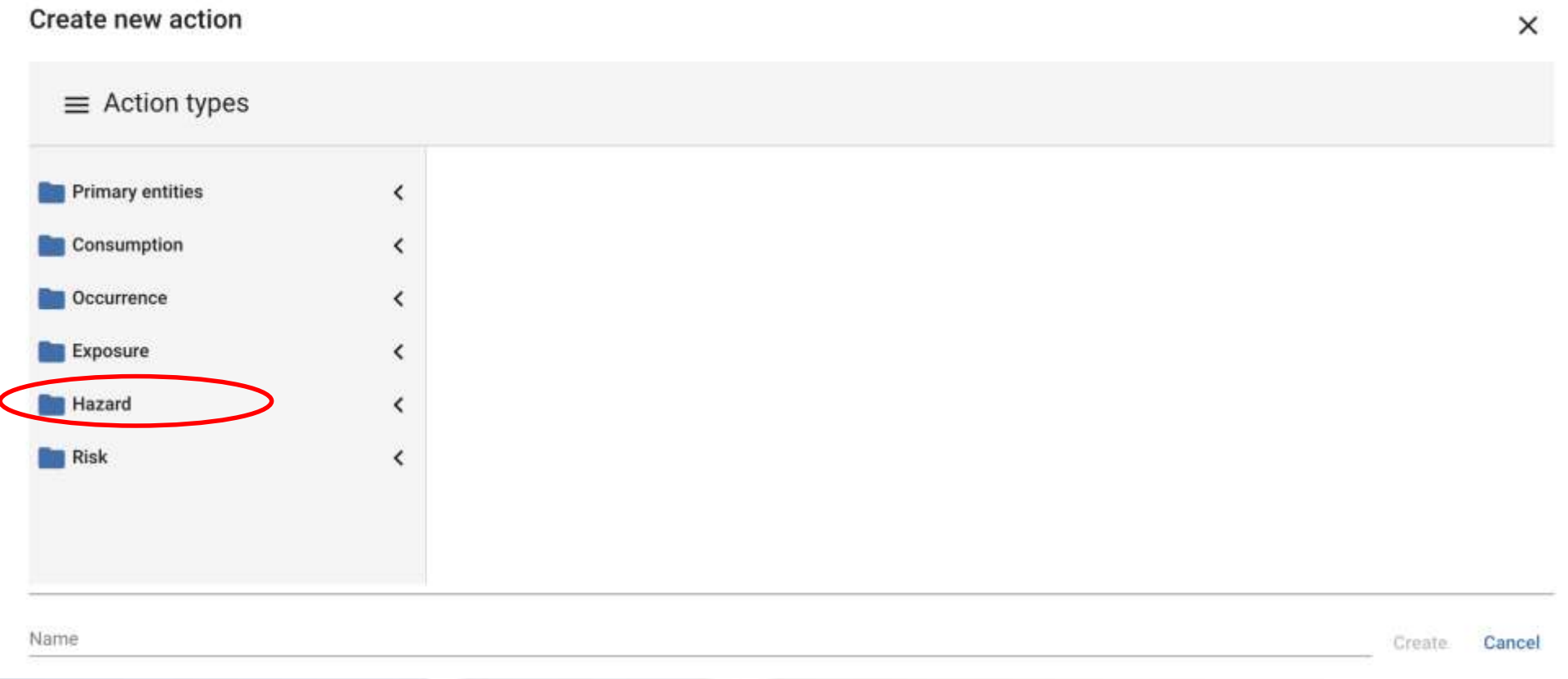
Create an action in the workspace (2)

1. Create a new action Hazard



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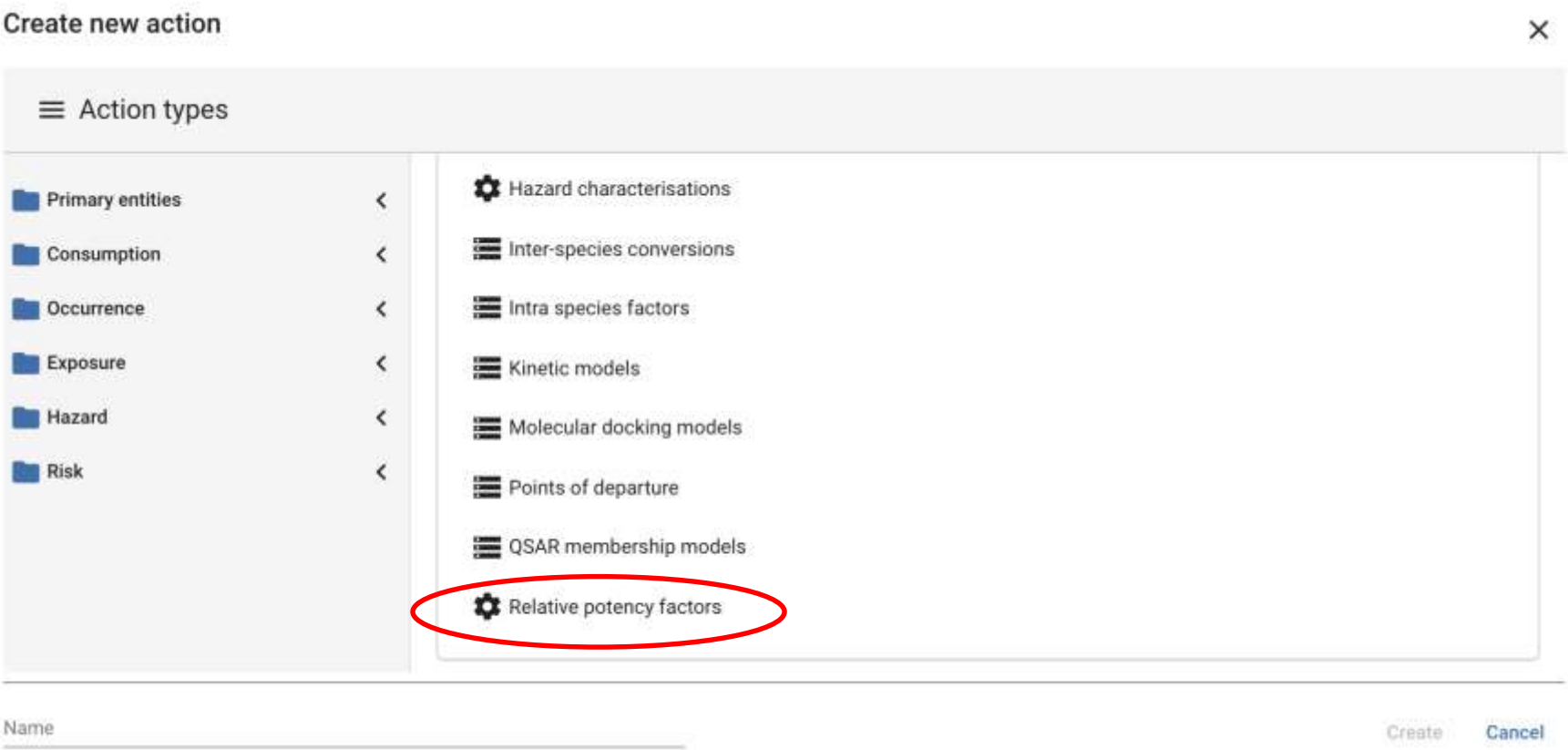
Create an action in the workspace (3)

1. Create a new action type Relative potency factors



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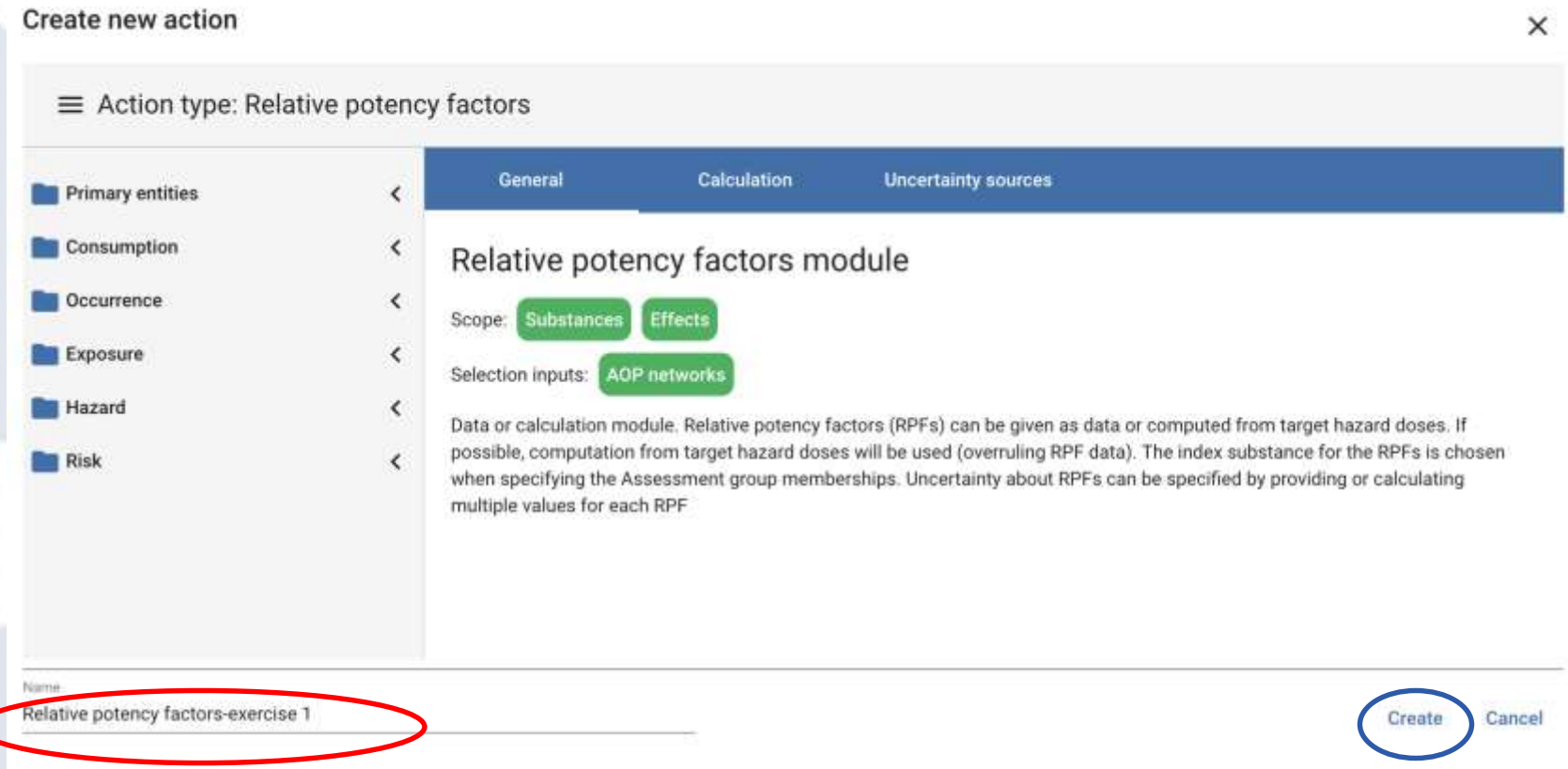
Create an action in the workspace (4)

1. Insert a name for the action
2. Click on create



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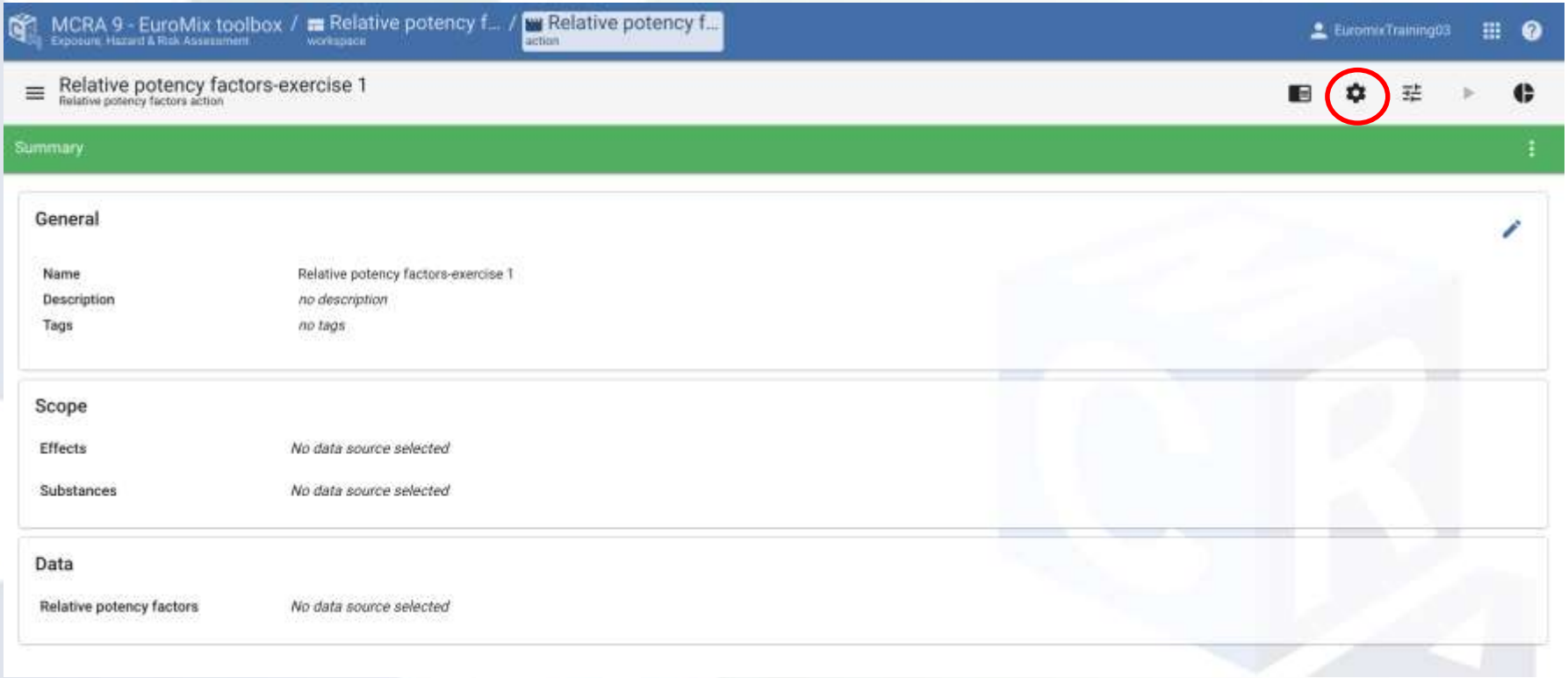
Action settings

1. You entered the summary overview.
2. Go to action settings



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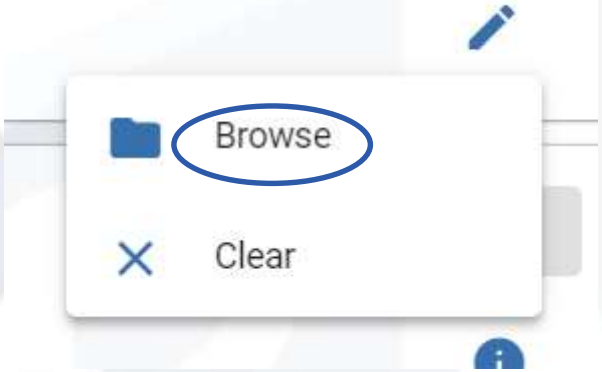
Specify Substances (1)

1. Since you will use data for Relative potency factors in the first exercise, click on Use data
2. ⚠ indicates that something needs to be done
3. If you are using the toolbox for the first time, we advise you to work from top of the screen.
4. Start with inserting data for Substances. Click on Substances.

Specify Substances (2)

1. A indicates that you can insert data
2. Click on pencil
3. After you clicked on pencil a window pop-up, select Browse and you will be connected to your data folders



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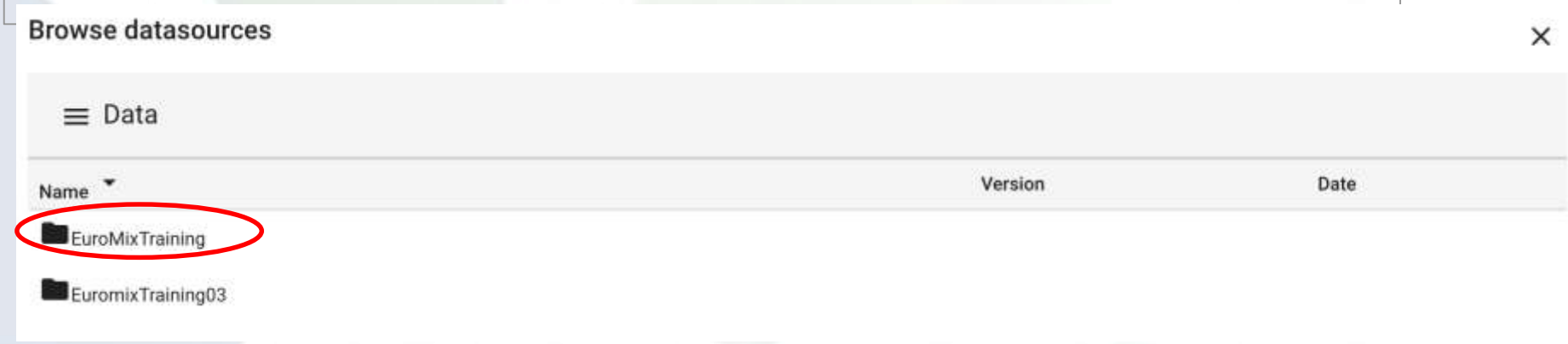
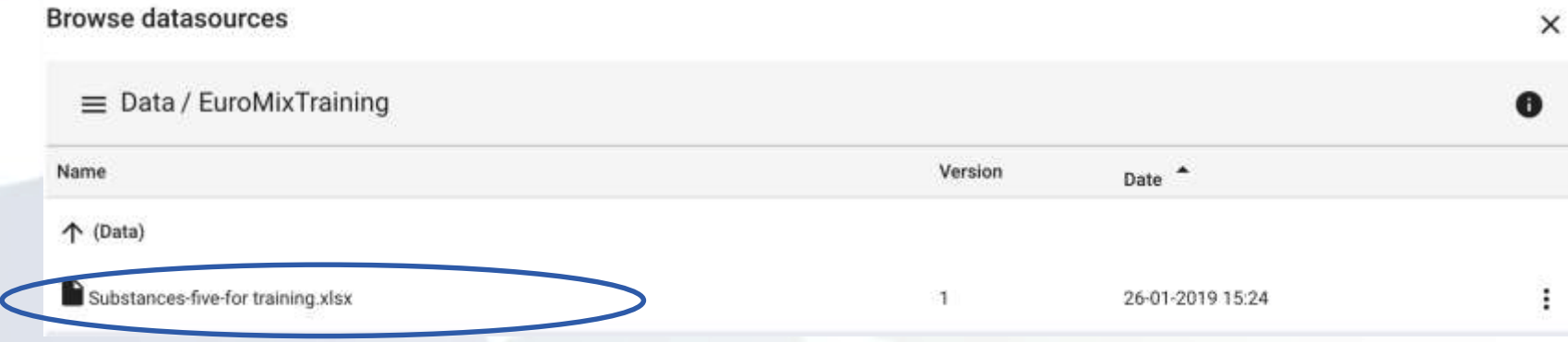
Specify Substances (3)

1. Click on EuroMixTraining
2. A second window will open. Click on Substances-five-for-training.xlsx. The file contains information on Substances.





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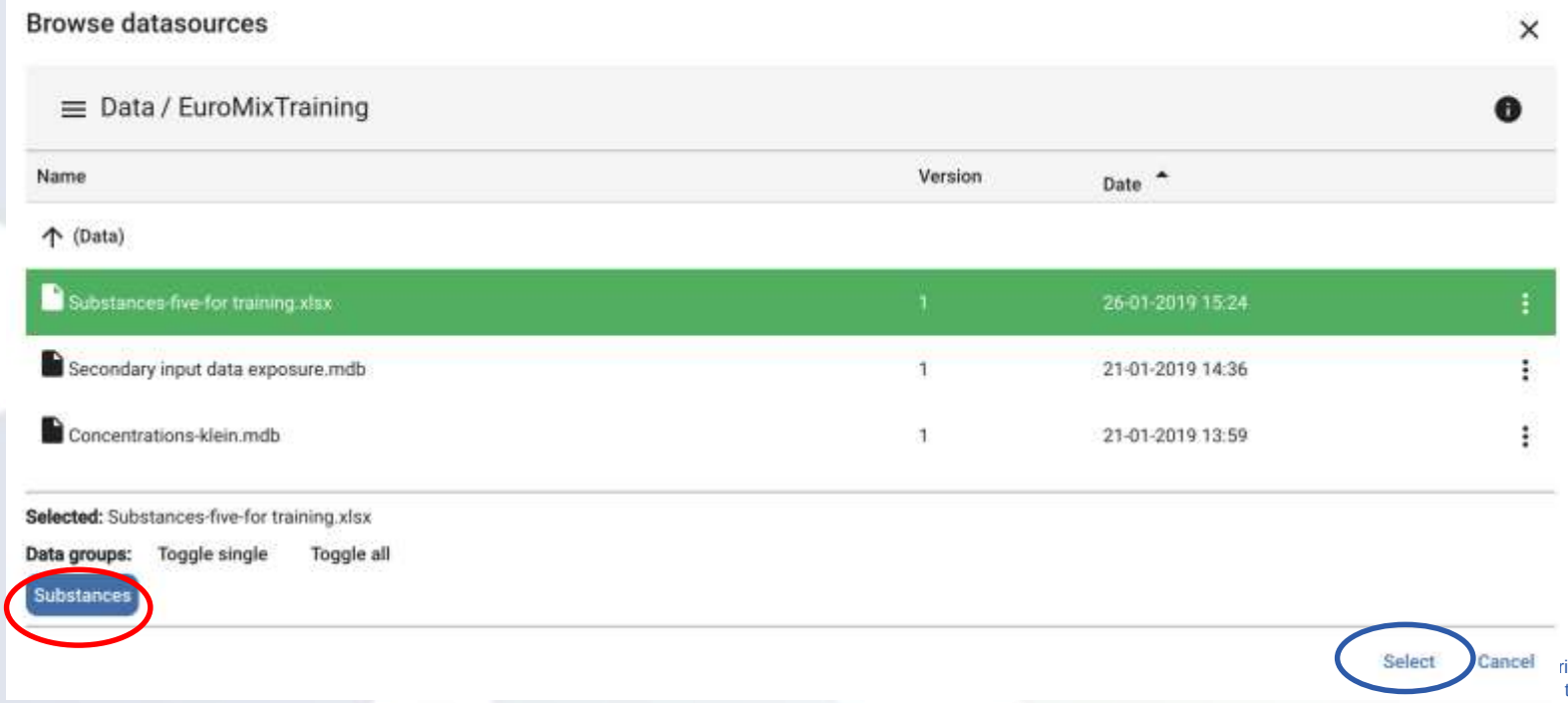
Specify Substances (4)

1. The file Substances-five-for-training.xlsx contains information on Substances.
2. Check that Substances is selected and blue.
3. Click on Select.



Specify Substances (5)

1. Substances data source: The green tick means that the data connection is ok
2. Substances selection: Substances: 5 in scope means that your file contains 5 Substances.
3. Optional: You can click on the pencil to see the code and name of the Substances.
4. Substance settings: Click on Index substance to choose Flusilazole

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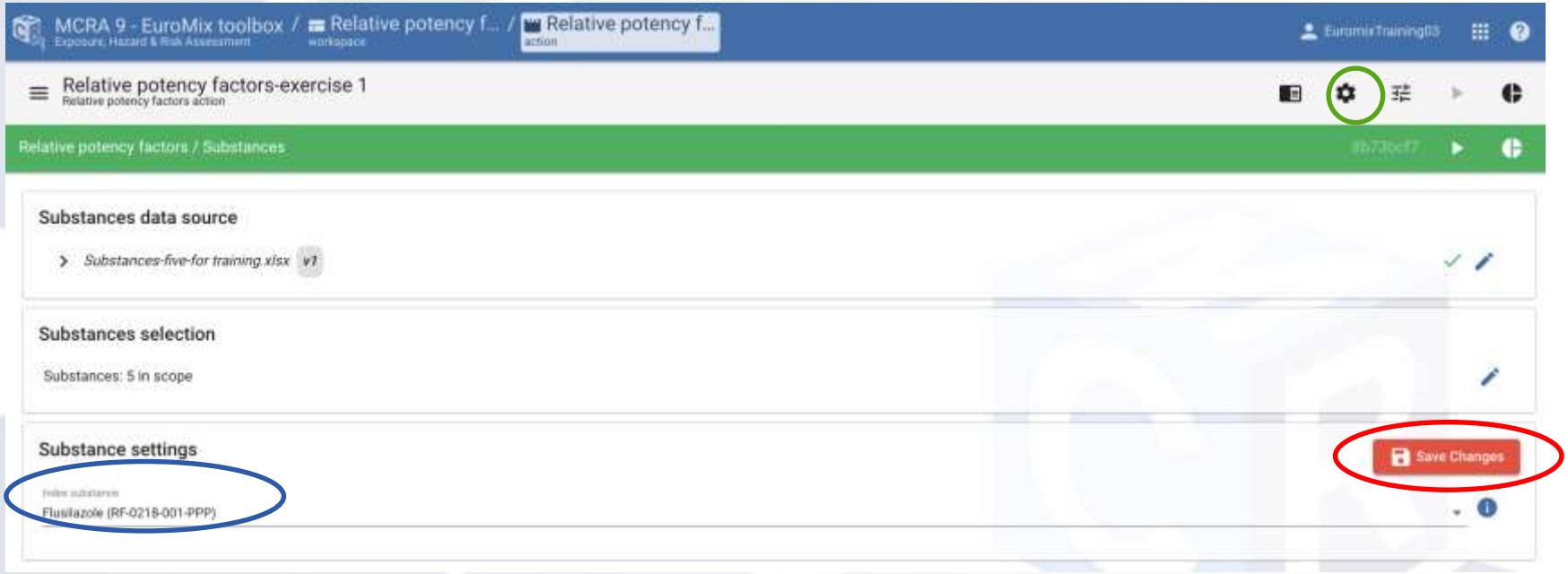
Specify Substances (6)

1. Substance settings: Index substance is Flusilazole
2. Click on Save changes
3. Go back to action settings in the navigation



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Specify Effects (1)

1. Substances: The green tick means that the data connection is ok.
2. Now the specific Effect must be selected.
3. Click on Effects.

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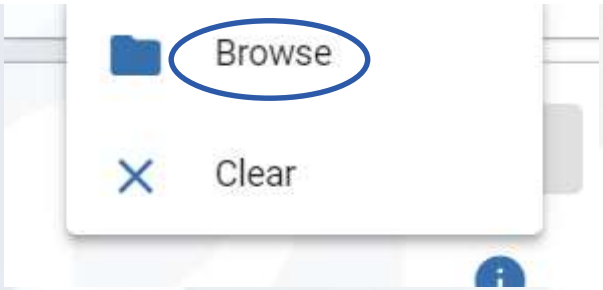
Specify Effects (2)

1. A indicates that you can insert data
2. Click on pencil
3. After you clicked on pencil a window pop-up, select Browse and you will be connected to your data folders



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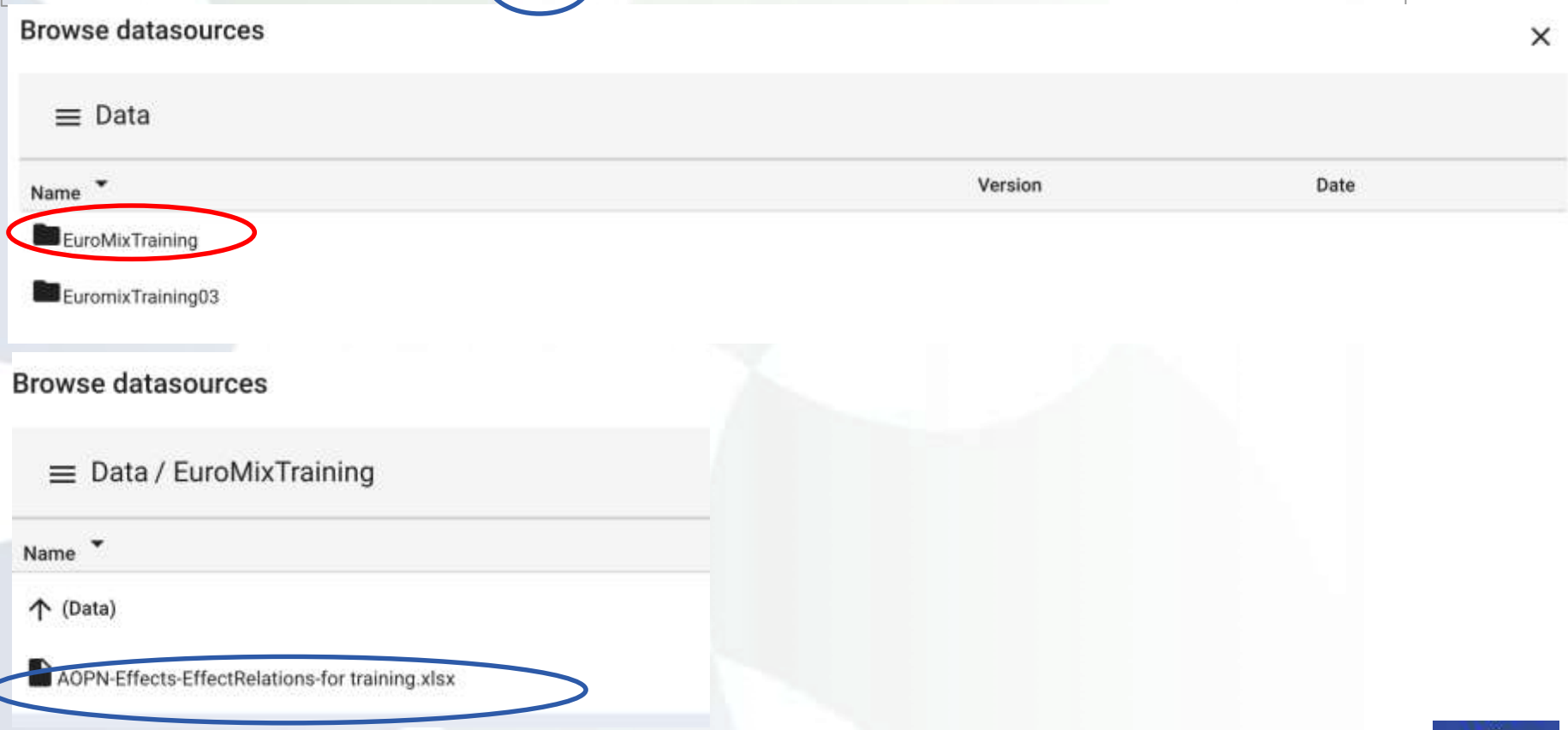
Specify Effects (3)

1. Click on EuroMixTraining
2. A second window will open. Click on AOPN-Effects-EffectRelations-for-training.xlsx. The file contains Effects data.



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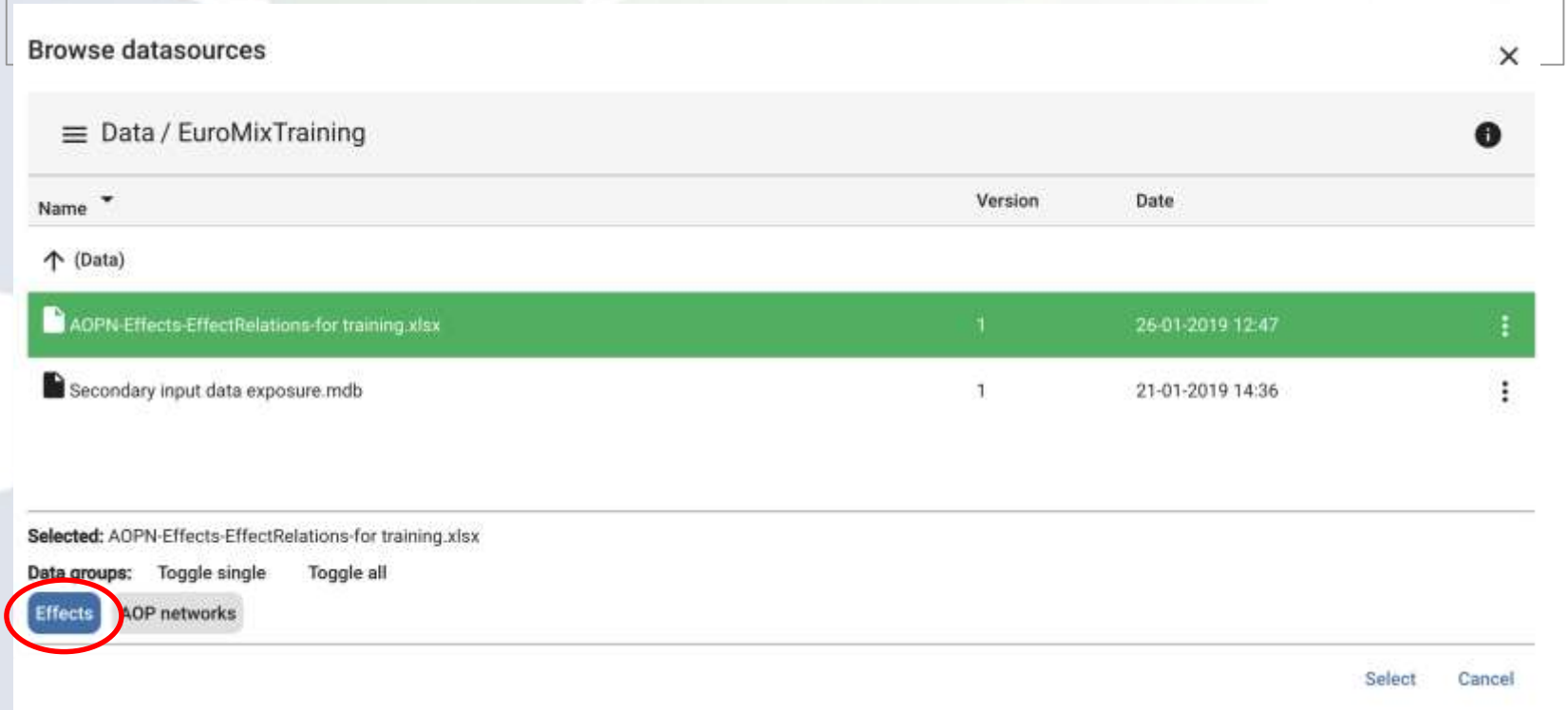
Specify Effects (4)

1. The file AOPN-Effects-EffectRelations-for-training.xlsx contains Effects data.
2. Check that Effects is selected and blue.
3. Click on Select.



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Specify Effects (5)

1. Effects data source: The green tick means that the data connection is ok.
2. Effects selection: Effects: 2 in scope means that your file contains 2 Effect.
3. Optional: You can click on the pencil to see the code and name of the Effects
4. Effect settings: Click to select the Focal effect steatosis-liver
5. Click on Save changes
6. Go back to action settings in the navigation

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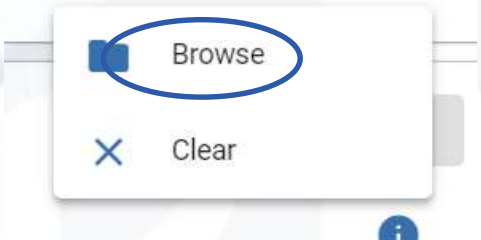
Specify Relative potency factors data (1)

1. A ndicates that you can insert data
2. Click on pencil
3. After you clicked on pencil a window pop-up, select Browse and you will be connected to your data folders



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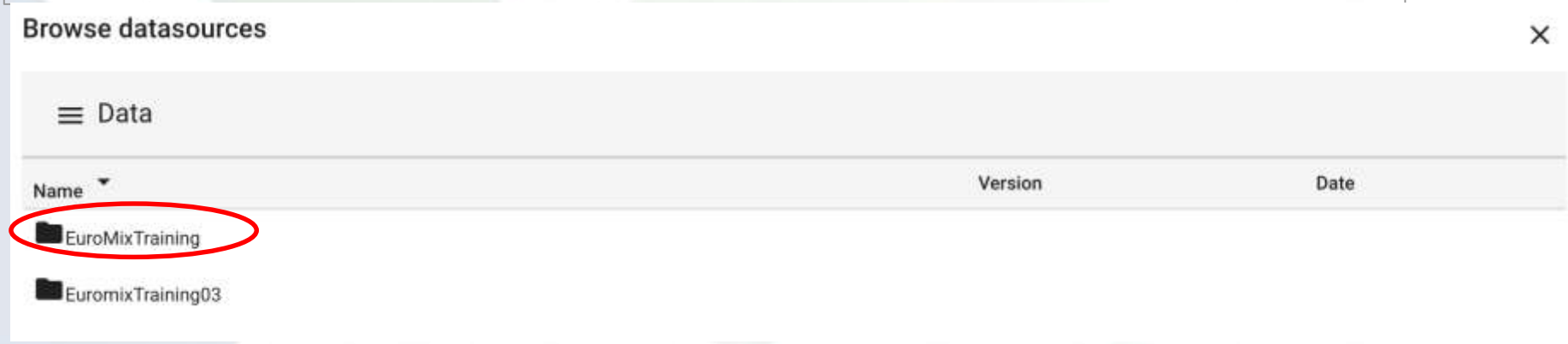
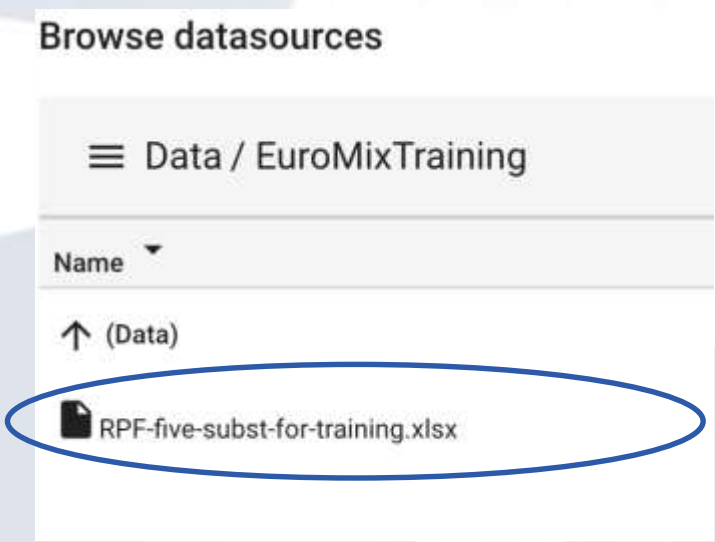
Specify Relative potency factors data (2)

1. Click on EuroMixTraining
2. A second window will open. Click on RPF-five-subst-for-training.xlsx. The file contains Relative potency factor data.





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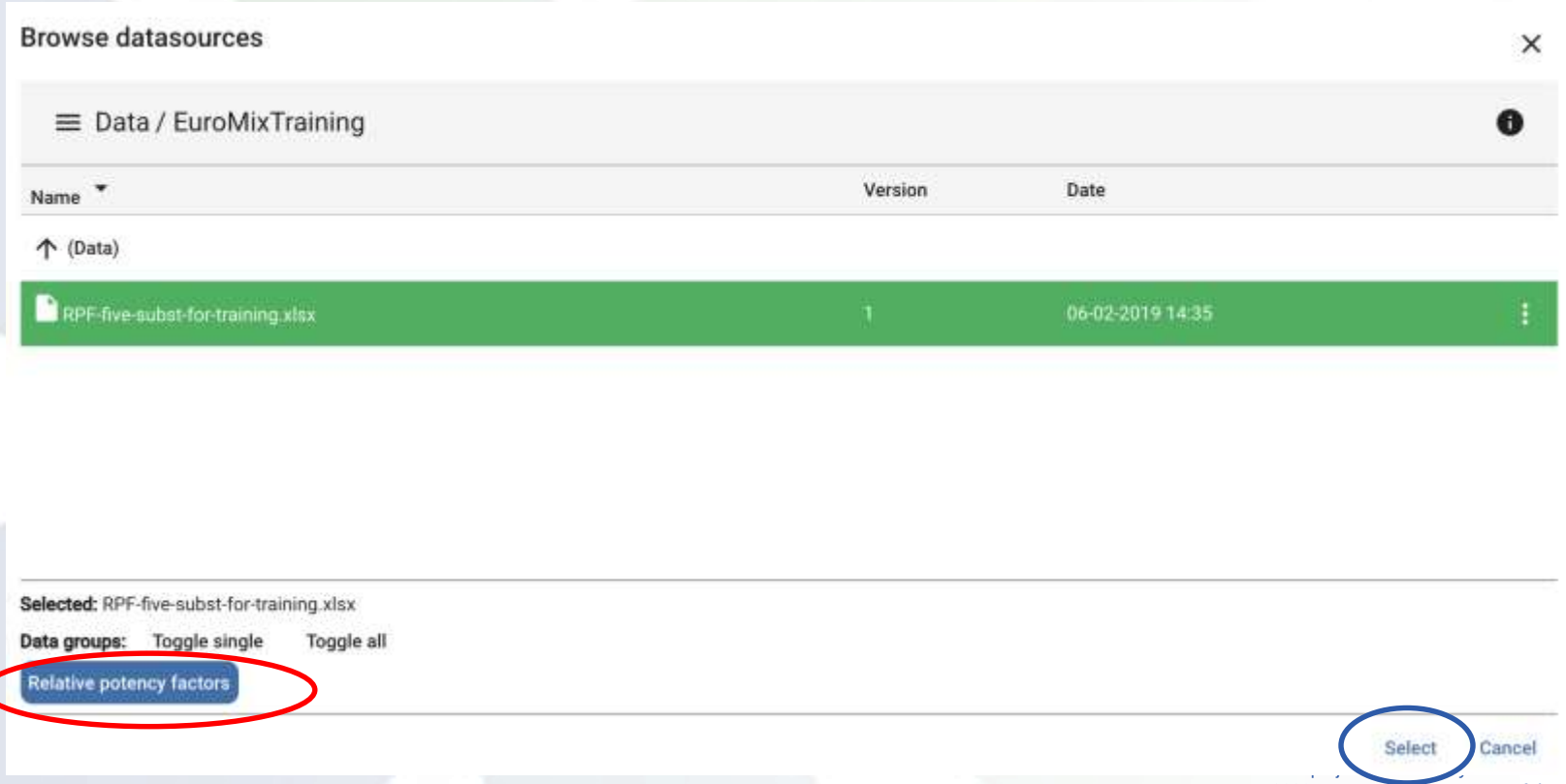
Specify Relative potency factors data (3)

1. The file RPF-five-subst-for-training.xlsx contains Relative potency factor data
2. Check that Relative potency factors is selected and blue.
3. Click on Select.



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Specify Relative potency factors data (4)

1. Relative potency factors data source: The green tick means that the data connection is ok
2. Now all settings are done, Click on Summary in the navigation

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Summary overview

1. In the summary overview you can see the settings.
2. If all settings are ok, click on run to start the run.

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Run the calculation of relative potency factors

1. After you start running the calculation, you see a screen with waiting for activation. This takes 10-20 seconds.
2. Then waiting for activation changes into running. This might take 3-4 minutes. If it takes longer than 10 minutes abort the job.
3. After 3-4 minutes you see Run to completion.
4. To see the result, click on Relative potency factors training.

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View the output (1)

1. Action settings shows the settings for the run.
2. Sub-action results shows intermediary results of the run.
3. Relative potency factors shows the final result of the run.
4. Click on Relative potency factors to view the results.

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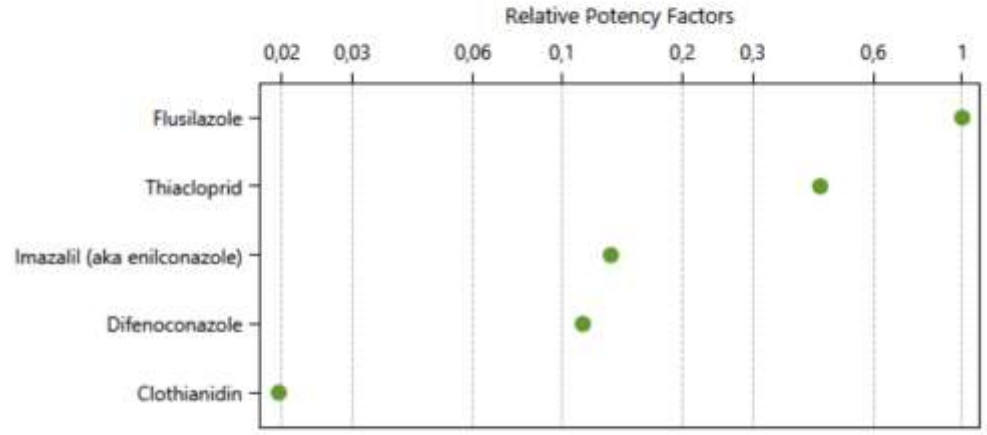
View the output (2)

1. Results are shown in a graph and a table
2. The relative potency factors are used for the dose addition approach

Results / Relative potency factors-exercise 1

Action settings | Sub-action results | Relative potency factors

Relative potency factors



Substance name	Substance code	RPF (nominal) scaled to reference
Clothianidin	RF-0101-001-PPP	0.0196
Difenoconazole	RF-0133-001-PPP	0.113
Flusilazole	RF-0218-001-PPP	1
Imazalil (aka enilconazole)	RF-0246-001-PPP	0.133
Thiacloprid	RF-0417-001-PPP	0.442

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