

# 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





#### **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



### **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



### Example



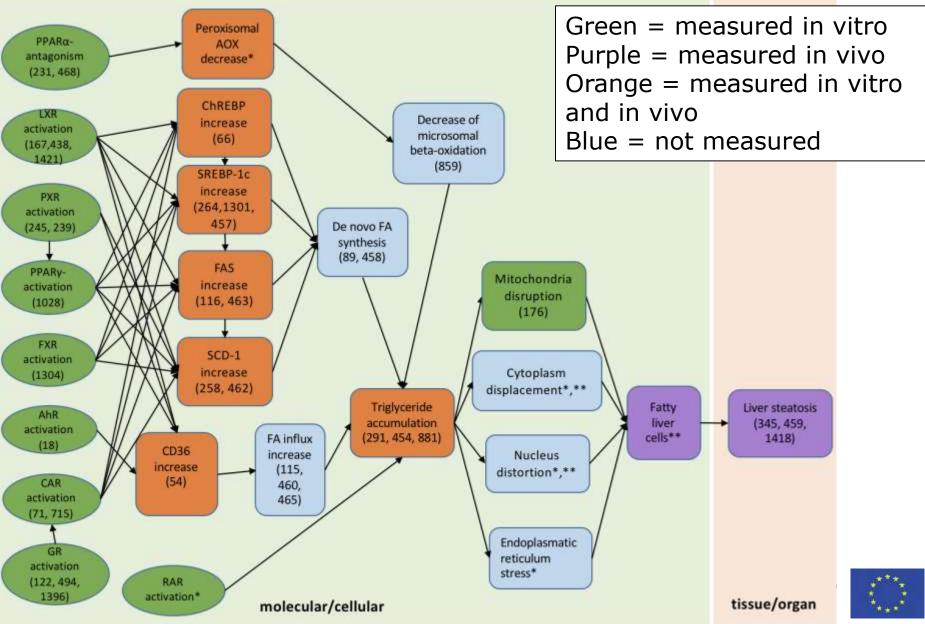
- The example is based on five\* substances that have been grouped in an assessment group for liver steatosis
- Substances: Clothianidin, Flusilazole, Difenoconazole, Thiacloprid, Imazalil
- Reference substance: Flusilazole
- Exercise 1: RPFs based on in vivo NOAELs from literature
- Exercise 2: NOAELs 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



#### **Steatosis AOP network**





#### **Modules for hazard data**



Relative potency factors

Point of departure

Dose response models

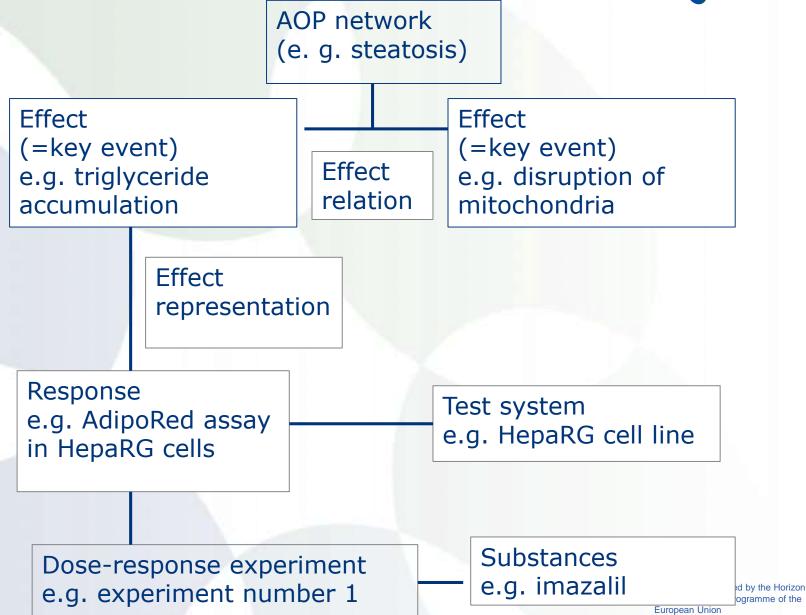
Dose-response data

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#### **Modules for hazard data**





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

1	A		C	0		1.23
1	kdEffect.	CodeSystem	Name	Description	<b>BiologicalOrganisation</b>	KeyEventProcess
2	P#ARalpha-antagonium-liver	EuroMix	PPA/lalpha-antagonism-liver	Antagorism of Peroxisome proliferator-activated receptor alpha (#1/	Molecular	perceisome proliferator act
3	LXR-act-liver	EuroMix	UXR-act liver	Activation of Liver X receptor (LXR) signaling in liver	Molecular	signating
-4	PXR-act-liver	EuroMix	PXR-act-liver	Activation of Pregnane X receptor (PXR) signaling in liver	Molecular	signaling
5	PPA/tgamma-act-liver	EuroMix	PPA/Igamma-act-liver	Activation of Peroxisome proliferator-activated receptor gamma (PP)	Molecular	peroxisome proliferator acti
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	anyl hydrocarbon receptor as
.8	CAR-act-liver	EuroMix	CAR-act-liver	Activation of Constitutive andrestane receptor (CAR) signaling in live	Molecular	signaling
9	GR-pct-liver	EuroMix	GR-act-liver	Activation of Glucocorticoid receptor (GR) signaling in liver	Molecular	glucocorticoid receptor activ
10	RAR-act-liver	EuroMix	RAR-act-liver	Activation of Retinoic acid receptor (RAII) signaling in liver	Molecular	signaling
11	HSD17810 decr-liver	EuroMix	HSD17B10-decr-liver	Decreased HSD17910 enzyme level or activity in liver	Molecular	gone expression
12	ADX inhib-liver	DuroMix	A00-inhib-liver	Decreased fatty acyl-CoA oxidase (AOX) level or activity in liver	Molecular	
13	ChitEBP-incr-liver	EuroMix	ChREBP-incr-liver	increased carbohydrate response element binding protain (ChREBP)	Molecular	signaling
14	SREBP-3c-Incr-liver	EuroMix	SREBP-1c-inkr 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 synthese (FAS) level or activity in liver	Molecular	fatty acid synthese activity
16	SCD1-intr-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-Intr-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-deor-liver	Decreased mitochondrial fatty acid beta-oxidation enzyme level or an	Molecular	fatty acid beta-oxidation
20	denovaFA-incr-liver	EuroMix	denovaFA-incr-liver	Increased de novo fatty acid synthesis liver (enzyme level or activity)	Cellular	fatty acid biosynthetic proci
21	FAinflux-incr-liver	EuroMix	FAinflux-incr-liver	Increased fatty acid influx to liver	Cellular	positive regulation of fatty



#### **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

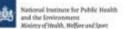
	A	В	С	D
1	idEffect	active Substance	idSubstance	RPF
2	Steatosis-liver	Clothianidin	RF-0101-001-PPP	0,01962963
3	Steatosis-liver	Difenoconazole	RF-0133-001-PPP	0,112765957
4	Steatosis-liver	Flusilazole	RF-0218-001-PPP	1
5	Steatosis-liver	Imazalil (aka enilconazole)	RF-0246-001-PPP	0,1325
6	Steatosis-liver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



### 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







#### MCRA 9.0 Beta

MCRA stands for Monte Carlo Risk Assessment. MCRA is a web-based system for probabilistic exposure and risk assessment of chemicals in the diet.

The MCRA system brings together statistical models, shared data and data uploaded by the user. MCRA also provides Cumulative Exposure Assessment for chemicals grouped in a Cumulative Assessment Group for

which a single health effect is considered relevant.

Optionally exposure from other routes can be added in an Aggregate Exposure Assessment.

MCRA was developed in EU project ACROPOLIS and is further developed in actions for EFSA and in EU project EuroMix

#### Publications and reports using MCRA

Username	
Password	
Go to registration	LOGI

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Contact: Prof. J.D. van Klaveren National Institute for Public Health and the Environment (RIVM MCRA is developed by Wageningen University & Research, Biometris for RIVM (2007 - 2019)

### Login EuroMix toolbox



Log in

Register

<u>...</u>

Log in: click on
 Then log in

# 8

#### MCRA 9 - EuroMix toolbox



#### Welcome to MCRA 9 (beta), the EuroMix toolbox

Chemical exposure, hazard and risk assessment

Every day, we are exposed to a mixture of multiple chemicals via food intake, inhalation and dermal contact. The risk to health that may result from this depends on how the effects of different chemicals in the mixture combine, and whether there is any synergism or antagonism between them. The number of different combinations of chemicals in mixtures is infinite and an efficient test strategy for mixtures is lacking. Furthermore, there is a societal need to reduce animal testing, which is the current practice in safety testing of chemicals.

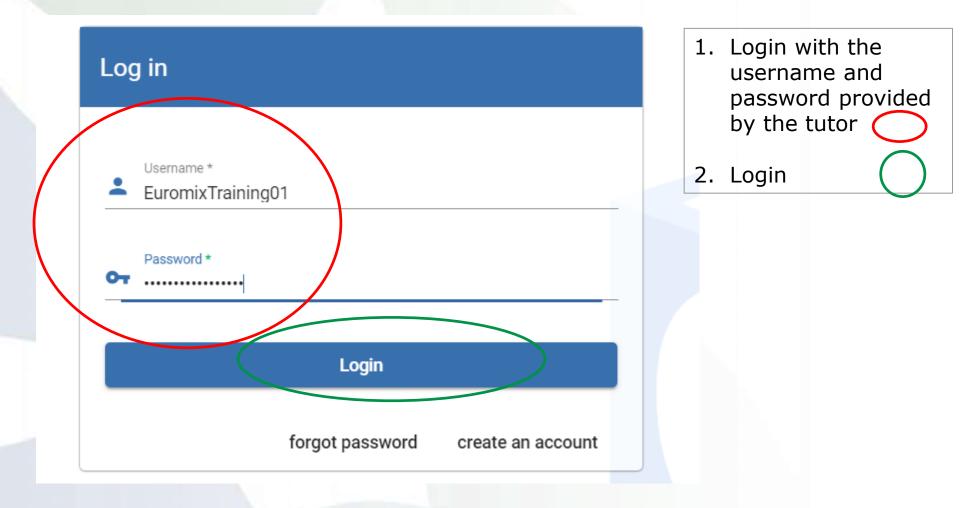
The EuroMix project will deliver a mixture test strategy and test instruments using novel techniques as recently proposed by the Joint Research Centre (JRC) of the European Commission. The tests will result in data needed for refining future risk assessment of mixtures relevant to national food safety authorities, public health institutes, the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), industry, regulatory bodies and other stakeholders. Ultimately, this will provide information for future risk management decisions on the safety of chemicals in mixtures to be taken by the European Commission and the Codex Alimentarius.

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#### **Insert username password**





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#### **Data folders**



#### 1. Click on Data



#### Welcome to MCRA 9 (beta), the EuroMix toolbox

Chemical exposure, hazard and risk assessment

Typical **action types** which this system can perform are: dietary exposure assessment, hazard dose assessment and risk assessment. Actions are structured in a network of modular calculators [see Overview].

You have to organize your work in one or more **workspaces**. The work consists of specifying **tasks** of a specific action type. Tasks may need subtasks of other action types.

After running a task, **outputs** are available. Outputs are in the form of screen reports and print reports, and may also include data that may be useful as input in other tasks.

All tasks need input data, and some tasks produce output data. Data, but also saved tasks and outputs, are organised in a data repository, which includes shared items from other users and user groups.

Start by clicking Workspaces or Data, or use wizard options

WORKSPACES



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#### **Data folders**



#### 1. Click on data

 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

MCRA 9 - EuroMix toolbox Ó Exposure, Hazard & Risk Assessment ■ Data / EuromixTraining01 Version Name (Data) MCRA 9 - EuroMix toolbox Exposure, Hazard & Risk Assessment ≡ Data Name EuroMix Training EuromixTraining01

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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 (

MCRA 9 - EuroMix toolbox Esposure, Hazard & Risk Assessment		💄 Euromix Training03		9
■ Data / EuroMixTraining			0	:
Name	Version	Date -		
个 (Data)				
HepaRG-AdipoRed-five-subst-for training.xlsx	1	26-01-2019 16:20		;
Substances-five-for training.xlsx	1	26-01-2019 15:24		:
TestSystems-Responses-EffectRepresentations-for training.xlsx	1	26-01-2019 12:47		:
AOPN-Effects-EffectRelations-for training.xlsx	1	26-01-2019 12:47		:

## Actions needs to be done in workspaces EuroMix

#### 1. Click on workspaces and create a new workspace



#### Welcome to MCRA 9 (beta), the EuroMix toolbox

Chemical exposure, hazard and risk assessment

Typical **action types** which this system can perform are: dietary exposure assessment, hazard dose assessment and risk assessment. Actions are structured in a network of modular calculators [see Overview].

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Start by clicking Workspaces or Data, or use wizard options

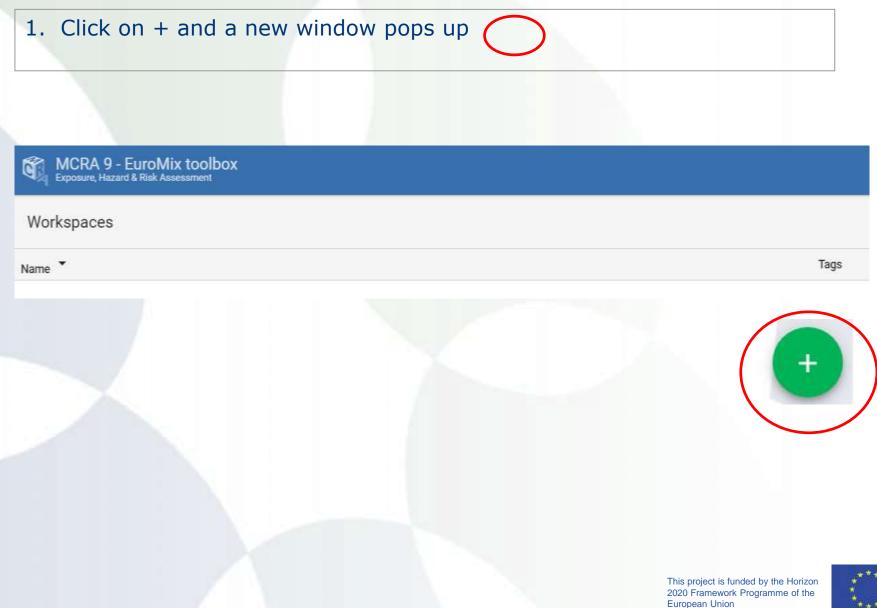


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#### **Create a new workspace (1)**



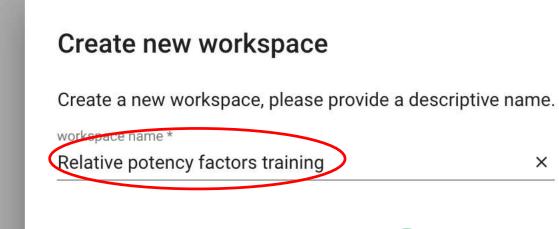


### **Create a new workspace (2)**





2. Click on ok





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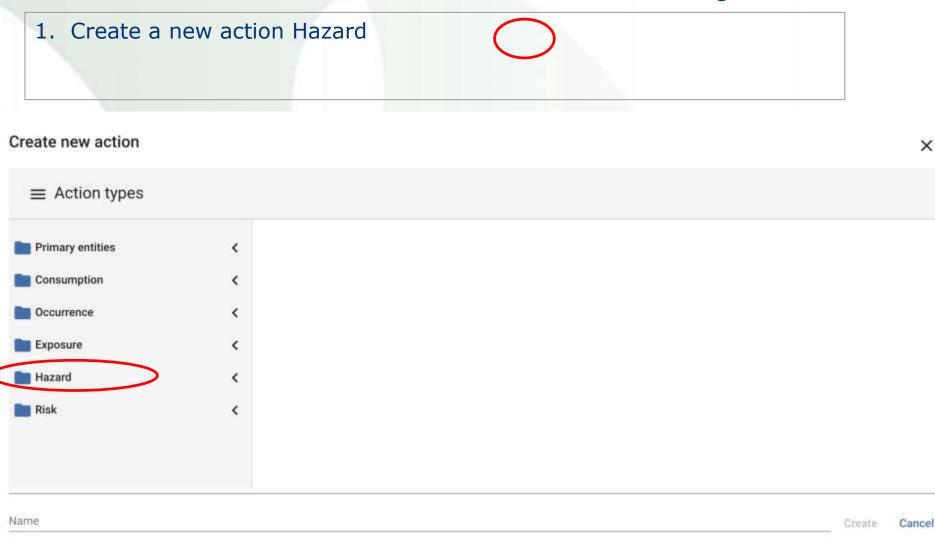


### **Create an action in the workspace (1)**



Click on + and	a new window	pops up	
MCRA 9 - Eur	oMix toolbox /	Relative potency f rorkspace	
Actions	Data	II. Results	Properties
/orkspace actio	ns		
iis workspace does no	t contain actions.		
			This project is funded by the 2020 Framework Programm

### **Create an action in the workspace (2)**



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×

EuroMix

### **Create an action in the workspace (3)**

Name



eate new action			
$\equiv$ Action types			
Primary entities	<	A Hazard characterisations	
Consumption	<	Inter-species conversions	
Occurrence	<	Intra species factors	
Exposure	<	Kinetic models	
Hazard	<	Molecular docking models	
Risk	<	Points of departure	

Create Cancel

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### **Create an action in the workspace (4)**



Create new action				3
Action type: Relation Primary entities	ative potency factors	Calculation	Uncertainty sources	
Consumption	< Relative n	otency factors m	odule	
Occurrence	<		loudie	
Exposure	Scope: Substan			
Hazard	<		factore (DDEe) can be niven as data r	or computed from target hazard doses. If
<b>Risk</b>	possible, comput	ation from target hazard dos the Assessment group mem	ses will be used (overruling RPF data)	<ul> <li>The index substance for the RPFs is chosen in be specified by providing or calculating</li> </ul>
ame				
Relative potency factors-exercise	e 1			Create Cance

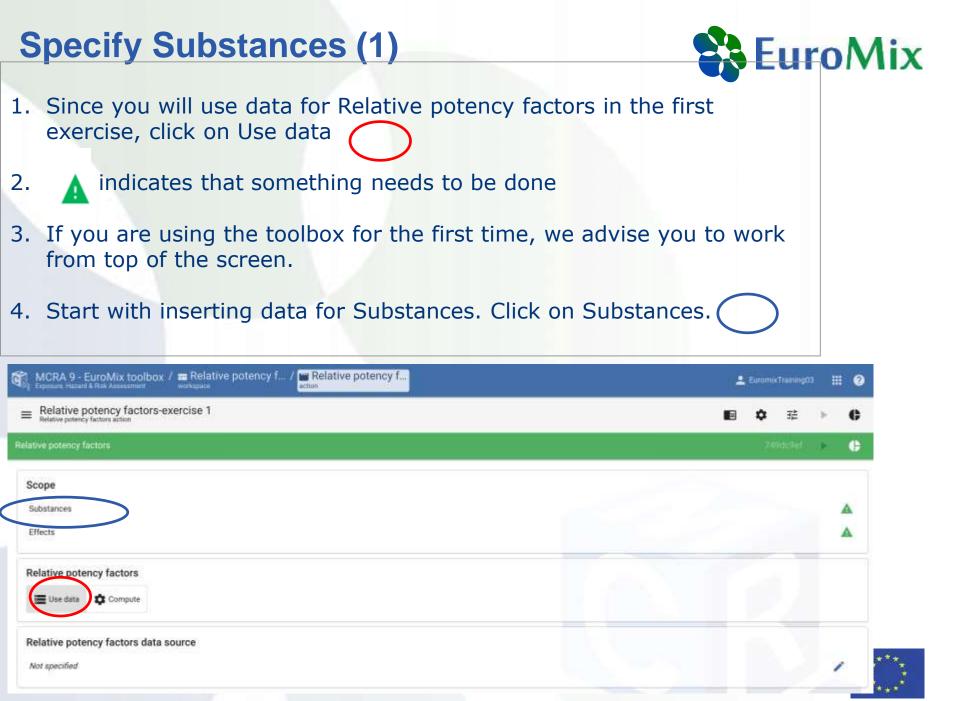
#### **Action settings**



	tered the summary overview.			
2. 00 10 0	action settings			
MCRA 9 - EuroMix	toolbox / # Relative potency f / # Relative potency f		🛓 EuromixTraining0	∞ Ⅲ @
Relative potency f Relative potency factors action	factors-exercise 1		■ 💠 🛱	> 0
Summary				1
General				1
Name Description Tags	Relative potency factors-exercise 1 no description no tags			
Scope		1100		
Effects Substances	No data source selected No data source selected			
Data				
Relative potency factors	No data source selected			

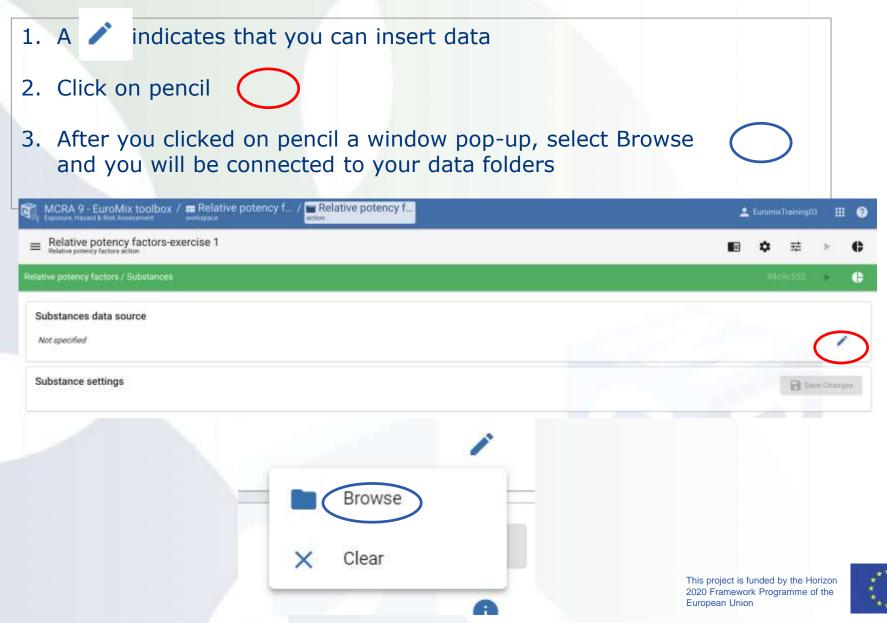
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### **Specify Substances (2)**





#### **Specify Substances (3) Euro**Mix 1. Click on EuroMixTraining 2. A second window will open. Click on Substances-five-for-training.xlsx. The file contains information on Substances. Browse datasources X ≡ Data Name \* Version Date EuroMixTraining EuromixTraining03 Browse datasources × E Data / EuroMixTraining 6 Name Version Date ^ ↑ (Data) Substances-five-for training.xlsx 26-01-2019 15:24 This project is funded by the Horizon

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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.

Browse datasources			×
■ Data / EuroMixTraining			0
Name	Version	Date 🕇	

#### ↑ (Data)

Substances

Substances five for training xlsx	Ť.	26-01-2019 15:24	ŧ
Secondary input data exposure.mdb	1	21-01-2019 14:36	I
Concentrations-klein.mdb	1	21-01-2019 13:59	:

#### Selected: Substances-five-for training.xlsx

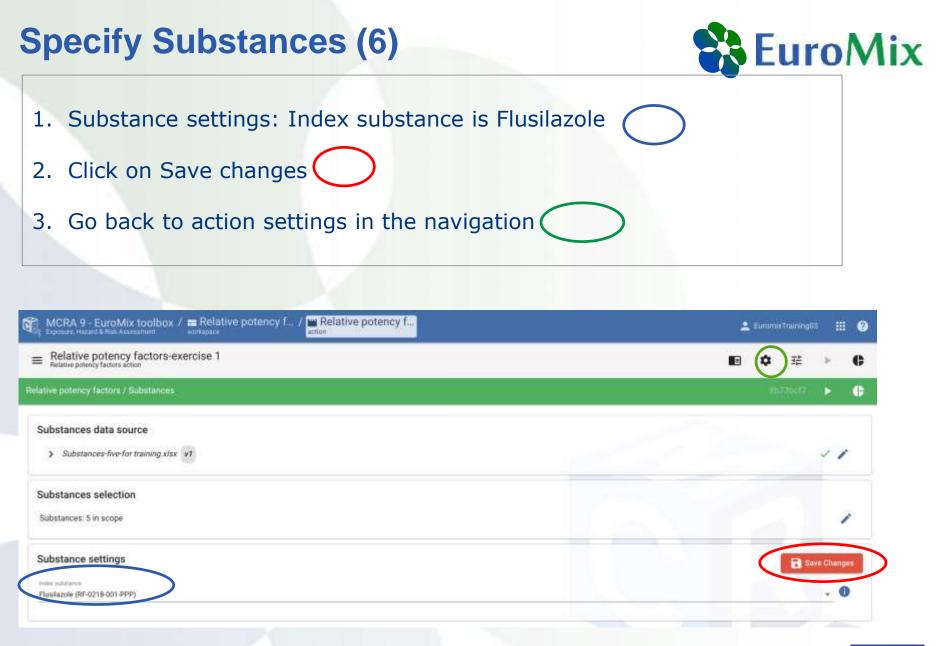
Toggle single Data groups:

Toggle all





#### **Specify Substances (5) EuroMix** 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 MCRA 9 - EuroMix toolbox / Relative potency f... / Relative potency f... EurominTraining03 11 (2) Relative potency factors-exercise 1 = Relative potency factors action 54371290A Substances data source > Substances-five-for training xlsx v1 Substances selection Substances: 5 in scope Substance settings Save Changes bo substance . 0 -001-PPP) Clothianidin (RF-010) 2020 Framework Programme or the European Union



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### **Specify Effects (1)**



- 2. Now the specific Effect must be selected.
- 3. Click on Effects.

MCRA 9 - EuroMix toolbox / Relative potency f / Relative potency f / come	*	Euromix	(Training0)	1	II (?
Relative potency factors-exercise 1 Instative potency factors action		٥	荜	Þ	¢
slative potency factors		Dog	dac97	×	¢
Scope					$\sim$
Substances (5 in scope) Effects					
Relative potency factors		-			
🗮 Use data 🌼 Compute					
Relative potency factors data source				1	
Not specified				10	1

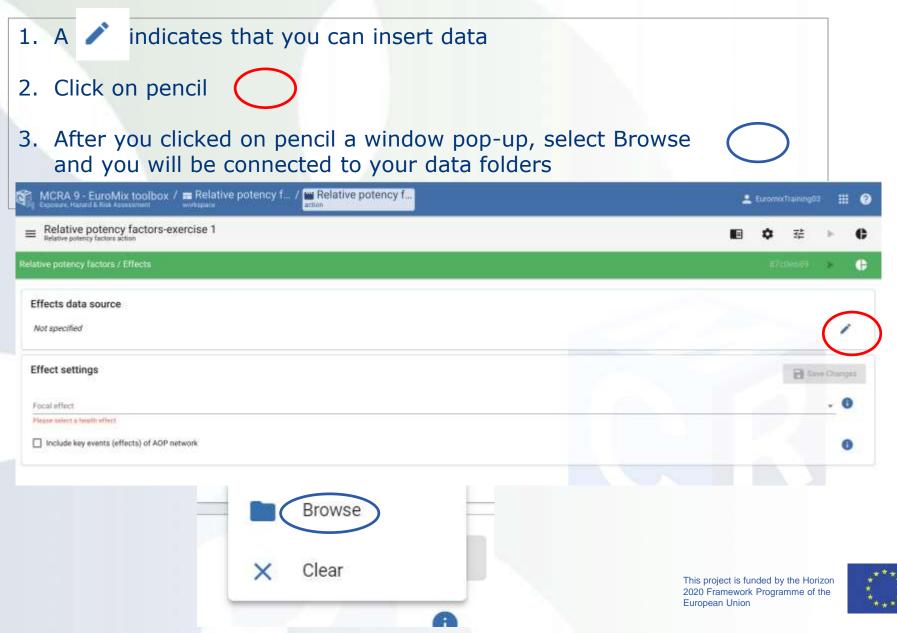
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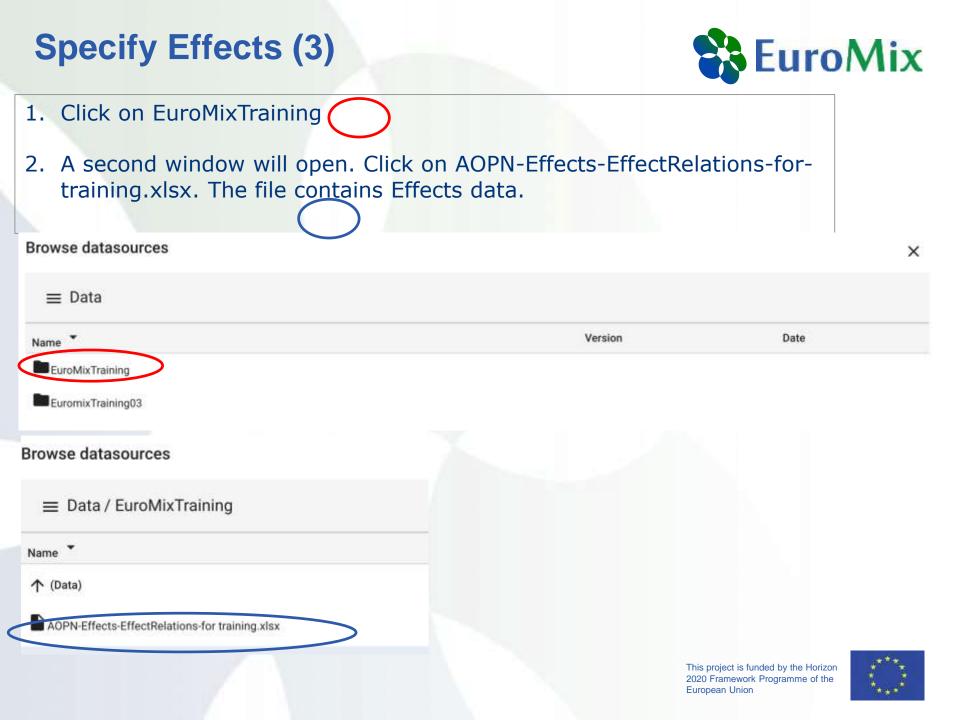


**Euro**Mix

### **Specify Effects (2)**







### **Specify Effects (4)**



1. The file AOPN-Effects-EffectRelations-for- data.	-training	.xlsx contains	Effects
2. Check that Effects is selected and blue.	$\bigcirc$		
3. Click on Select.			
Browse datasources			× _
■ Data / EuroMixTraining			0
Name 🔭	Version	Date	
↑ (Data)			
AOPN-Effects-EffectRelations-for training.xlsx	1	26-01-2019 12:47	:
Secondary input data exposure.mdb	1	21-01-2019 14:36	:
Selected: AOPN-Effects-EffectRelations-for training.xlsx			
Data groups: Toggle single Toggle all			
Effects AOP networks			

Select Cancel

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### **Specify Effects (5)**



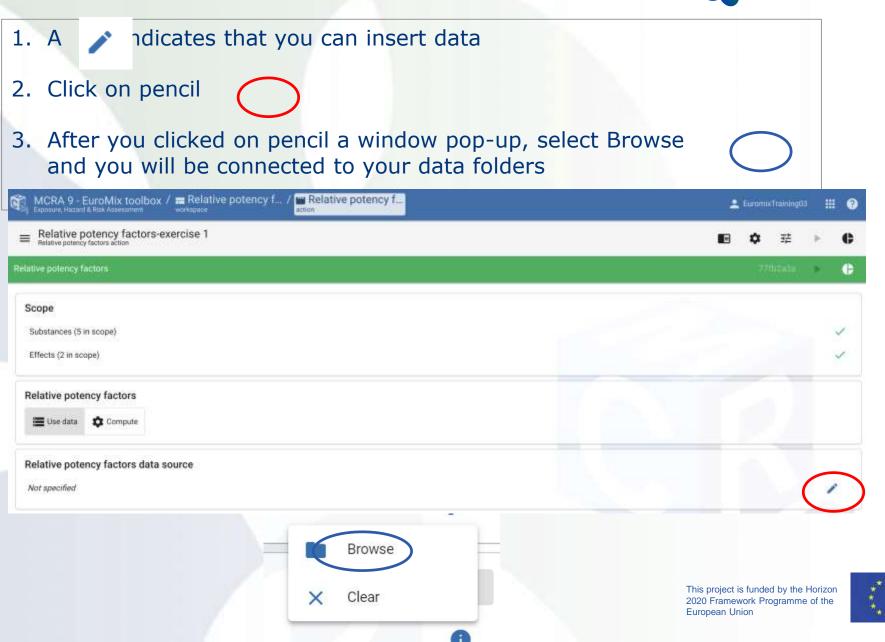
- Effect. 3. Optional: You can click on the pencil to see the code and name of the Effects
- 4. Effect settings: Click to select the Focal effect steatosis-liver
- 5. Click on Save changes
- 6. Go back to action settings in the navigation 🤇

C MCRA 9 - EuroMix toolbox / Relative potency f / Relative potency f / Relative potency f	💄 Euromis Tearing 33 💠 🥝
Relative potency factors-exercise 1 Relative potency factors action	<b>€</b> \$ # > \$
lelative potency factors / Effects	Bredeseo 👘 👘 🕒
Effects data source AOPN-Effects-EffectRelations-for training.xlsx v1	
Effects: 2 in scope	
Effect settings	Save Changes
Preventation of the second sec	0
	2020 Framework Programme of the European Union



EuroMix

## Specify Relative potency factors data (1) EuroMix



#### Specify Relative potency factors data (2) EuroMix 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. Browse datasources × ≡ Data Version Date Name EuroMixTraining EuromixTraining03 Browse datasources Data / EuroMixTraining Name ↑ (Data) RPF-five-subst-for-training.xlsx This project is funded by the Horizon

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## Specify Relative potency factors data (3) EuroMix

- 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.

Browse datasources			×
■ Data / EuroMixTraining			0
Name *	Version	Date	
↑ (Data)			
RPF-five-subst-for-training xlsx		06-02-2019 14:35	
elected: RPF-five-subst-for-training.xlsx			
Data groups: Toggle single Toggle all			
Relative potency factors			$\frown$
			Select Cancel
		2020 Fra Europear	mework Programme of the

#### Specify Relative potency factors data (4) SE EuroMix 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 MGRA 9 - EuroMix toolbox / 🚍 Relative potency f... / 🞬 Relative potency f... 🚊 Euromix Training03 # odition Relative potency factors-exercise 1 = Relative potency factors act C E Scope Substances (5 in scope) 1 Effects (2 in scope) 1 Relative potency factors 🗮 Use data 🌼 Compute Relative potency factors data source > RPF-five-subst-for-training xlsx v1

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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.

	olbox / m Relative potency f / W Relative potency f	17.7	
Relative potency factors action	tors-exercise 1	<b>■ ¢</b> ₽	: ( <b>)</b> ()
nmary			
General			1
Name	Relative potency factors-exercise 1		
Description	na description		
Tags	no tags		
Scope			
Effects	AOPN-Effects-EffectRelations-for training.xlsx v1		~
Substances	Substances-five-for training xIsx v1		~
Data			
Relative potency factors	RPF-five-subst-for-training xisx v1		4

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# Run the calculation of relative potency factors



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- 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.

Output	Claius	Message	Date Running	time		
Relative potency factors training	Westing for activation	Job scheduled for execution (waiting for available resources)	21		8	:
		- T .		1		
lesults						2
Output	Status	Message	Date Running time			
Relative potency factors training	Running	Computing dose response models	e (†		0	:
tesults						
Output	Status	Message	Date	Running time		
Relative potency factors training	Ran to completion		27-01-2019 10:39	00:00:03		

### View the output (1)



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

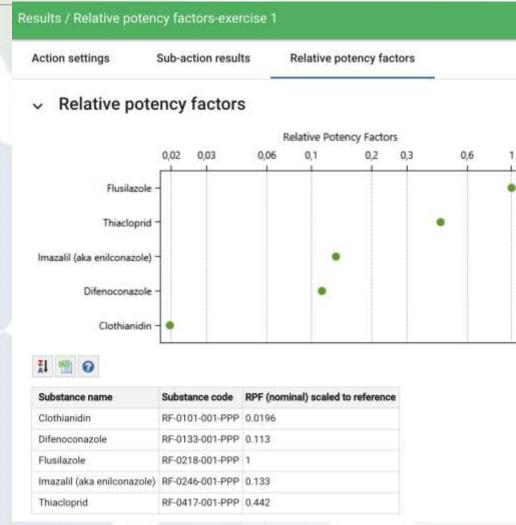
MCRA 9 - EuroMix toolbox / Relative potency f / Relative potency f / Relative potency f	8	, Euromix	(TrainingO)		. 9
Relative potency factors-exercise 1 Relative potency factors action		۵	荘	•	¢
Results / Relative potency factors-exercise 1       Action settings     Sub-action results       Relative potency factors					
<ul> <li>Action settings</li> <li>Data sources</li> <li>Relative potency factors</li> <li>Run settings</li> <li>Uncertainty settings</li> <li>Output settings</li> </ul>					99999



### 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



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