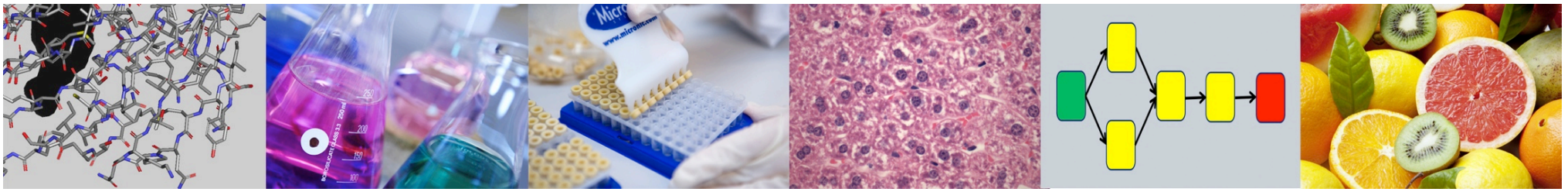
EuroMix handbook for mixture risk assessment

Johanna Zilliacus, Karolinska Institutet, Sweden
EuroMix training material
May 2019



Outline

- EuroMix project
- Mixture risk assessment
- EuroMix handbook
  - EuroMix toolbox
  - Problem formulation
  - Hazard assessment
  - Exposure assessment
  - Risk characterisation
  - Tiering approaches
  - Uncertainty analysis
- Conclusions

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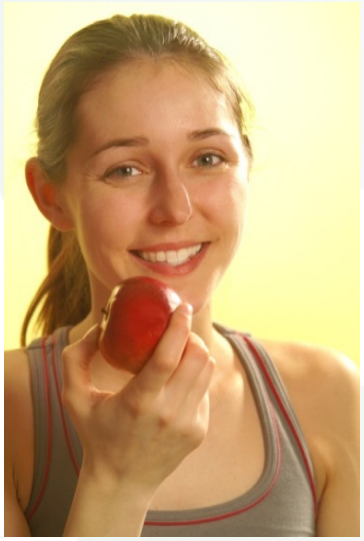
Exposure to many different substances – mixtures



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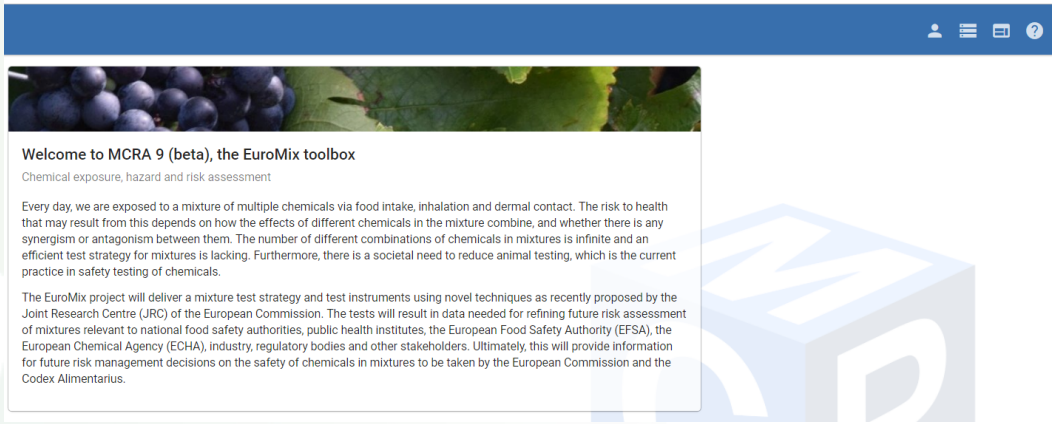
EuroMix methodology and tools

EuroMix handbook

- Methodology and tools for mixture risk assessment
- Examples

EuroMix toolbox

- Web based toolbox for mixture risk assessment
- Data repository



EuroMix handbook and the EuroMix toolbox provide practical support to apply the recent OECD and EFSA guidance documents in mixture risk assessment

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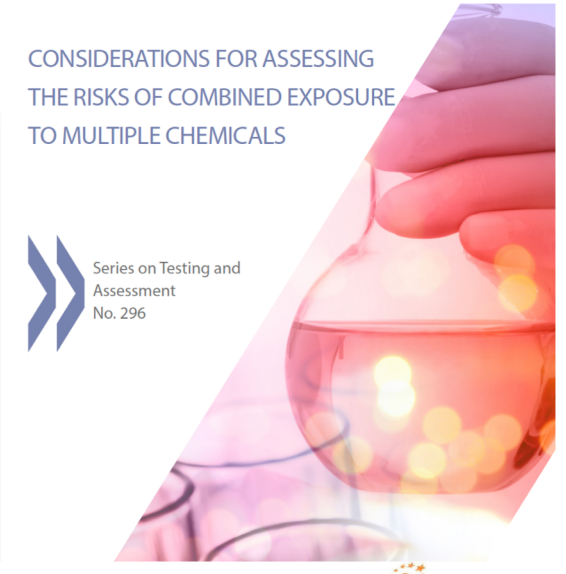
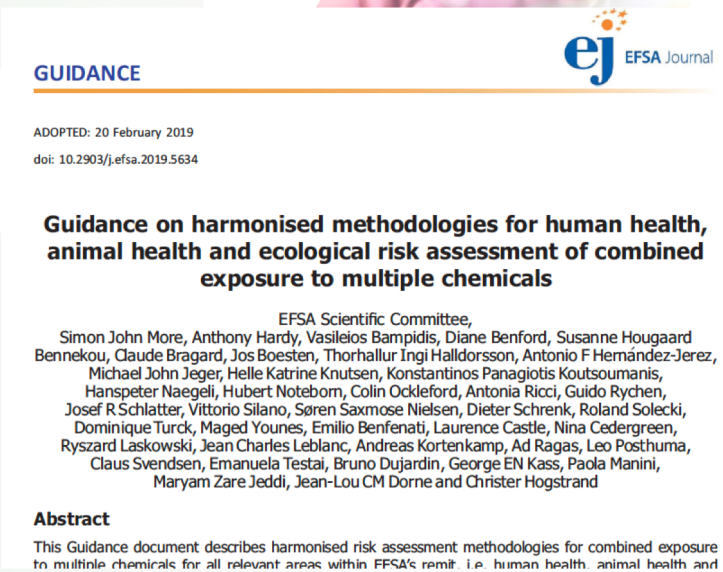
Recent international guidance on mixture risk assessment

OECD: Considerations for Assessing the Risks of Combined Exposure to Multiple Chemicals, 2018

EFSA: Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals, 2019

EuroMix handbook is in line with the recent documents





GUIDANCE

EFSA Journal

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Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
Simon John More, Anthony Hardy, Vasileios Bampidis, Diane Benford, Susanne Hougaard Bennekou, Claude Bragard, Jos Boesten, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Michael John Jeger, Helle Katrine Knutsen, Konstantinos Panagiotis Koutsoumanis, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Josef R Schlatter, Vittorio Silano, Søren Saxmose Nielsen, Dieter Schrenk, Roland Solecki, Dominique Turck, Maged Younes, Emilio Benfenati, Laurence Castle, Nina Cedergreen, Ryszard Laskowski, Jean Charles Leblanc, Andreas Kortenkamp, Ad Ragas, Leo Posthuma, Claus Svendsen, Emanuela Testai, Bruno Dujardin, George EN Kass, Paola Manini, Maryam Zare Jeddi, Jean-Lou CM Dorne and Christer Hogstrand

Abstract

This Guidance document describes harmonised risk assessment methodologies for combined exposure to multiple chemicals for all relevant areas within EFSA's remit, i.e. human health, animal health and

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Risk assessment of mixtures of substances

Whole mixture approach

- Toxicity and exposure data on whole mixture
- Many possible mixtures
- Mixtures change composition

Component-based approach

- Toxicity and exposure data on individual substances in the mixture
- Predict toxicity of mixture

- EuroMix is using the component-based approach

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Combine toxicities of substances in a mixture

- Dose addition
  - Substances with similar toxicity
  - Substances in mixtures treated as dilutions of each other scaled for the potencies
  - Default, conservative model
- Response addition
  - Toxicologically independent substances
  - Joint probability of independent events
- Interactions
  - Synergistic: 1 + 1 > 2
  - Antagonistic: 1 + 1 < 2
  - Rare, handled case by case
- Dose addition is the default model in the EuroMix tools
- EuroMix mixture experiments to identify possible interactions

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Dose addition using relative potency factors (RPFs)

The toxic potency of each substance is combined using 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 index substance equivalents

EuroMix toolbox is based on the RPF methodology

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Dose addition using relative potency factors (RPFs)

- Toxicity of index substance (Point of departure (POD), NOAEL/BMD): POD_{index}
- Toxicity of each substance in mixture (Point of departure, NOAEL/BMD): $POD_{1,}$ $POD_{2,}$ $POD_{3,}$ POD_{4} …
- Calculate Relative potency factor (RPF) for each substance: $RPF_1 = POD_{index} / POD_1$

- Exposure to mixture is scaled based on RPFs
- $Exp_{mix} = Exp_1 \times RPF_1 + Exp_2 \times RPF_2 + \ldots$

- Margin of Exposure (MOE) is calculated:
- $MOE = POD_{index}/Exp_{mix}$
- MOE > assessment factors, combined risk of mixture is usually considered acceptable

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Grouping of substances

Group of substances included in the mixture risk assessment

Grouping based on e.g.:

- Common exposure source
- Structural similarities
- Toxicological considerations

EuroMix developed methods for grouping based on toxicological considerations

EuroMix toolbox can also be used for other types of grouping

Grouping can be done at different levels

- Organ
- Specific effect (adverse outcome)
- Specific mode of action/adverse outcome pathway

Grouping at level of specific effect will probably be most common

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EuroMix methodology and tools

- Component-based approach
- Grouping based on toxicological considerations
- Dose addition as default model
- Relative potency factors (RPF) approach
- Probabilistic exposure assessment
- Mainly dietary exposure

Very flexible

- Also applicable for other grouping principles (structure, exposure…)
- Any type of substance
- RPFs based on ADI/TDI, NOAEL/BMD for critical or specific effect or same potency for all substances
- Methodology to handle data poor substances

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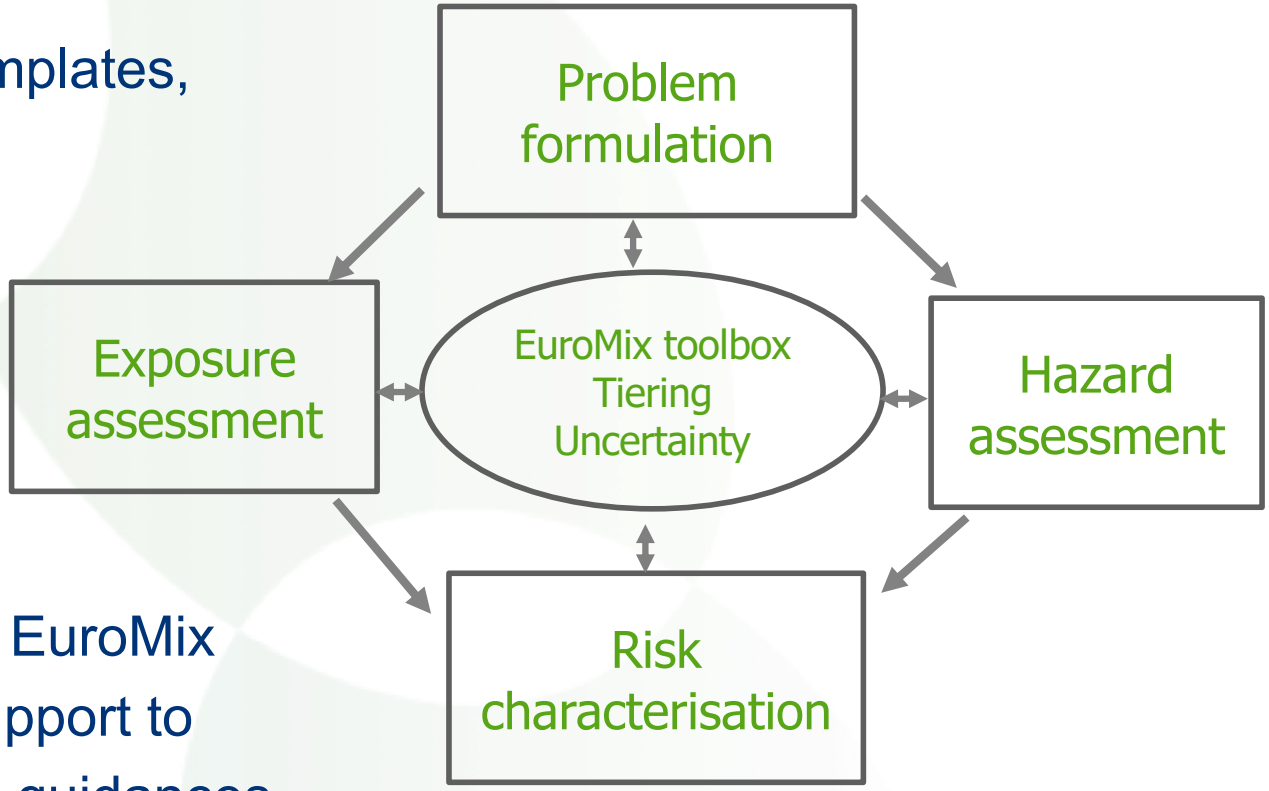
EuroMix handbook for mixture risk assessment

Practical handbook with templates, instructions and examples:

- Problem formulation
- Hazard assessment
- Exposure assessment
- Risk characterisation



EuroMix handbook and the EuroMix toolbox provide practical support to apply the OECD and EFSA guidances

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EuroMix handbook – sections (1)

- Abbreviations
- Introduction
- EuroMix toolbox
- Problem formulation
- Hazard assessment
    - Identification and assessment of AOP networks
    - Collection and assessment of toxicity data from literature
    - Tiered testing strategies
    - Grouping of substances based on toxicological considerations
    - Relative potency factors
    - Mixture experiments

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EuroMix handbook – sections (2)

- Exposure assessment
  - Probabilistic exposure assessment
  - Absence of measured concentration data
  - Non-dietary exposure assessment
- Risk characterisation
  - Dose addition
  - Margin of exposure
  - Selection of main mixtures based on exposure and hazard data
- Tiering approaches
- Uncertainty analysis
- References
- Glossary

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EuroMix handbook – sections (3)

Annexes

- Detailed methodology and templates
- Examples
- Training material for EuroMix toolbox

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EuroMix handbook – sections (1)

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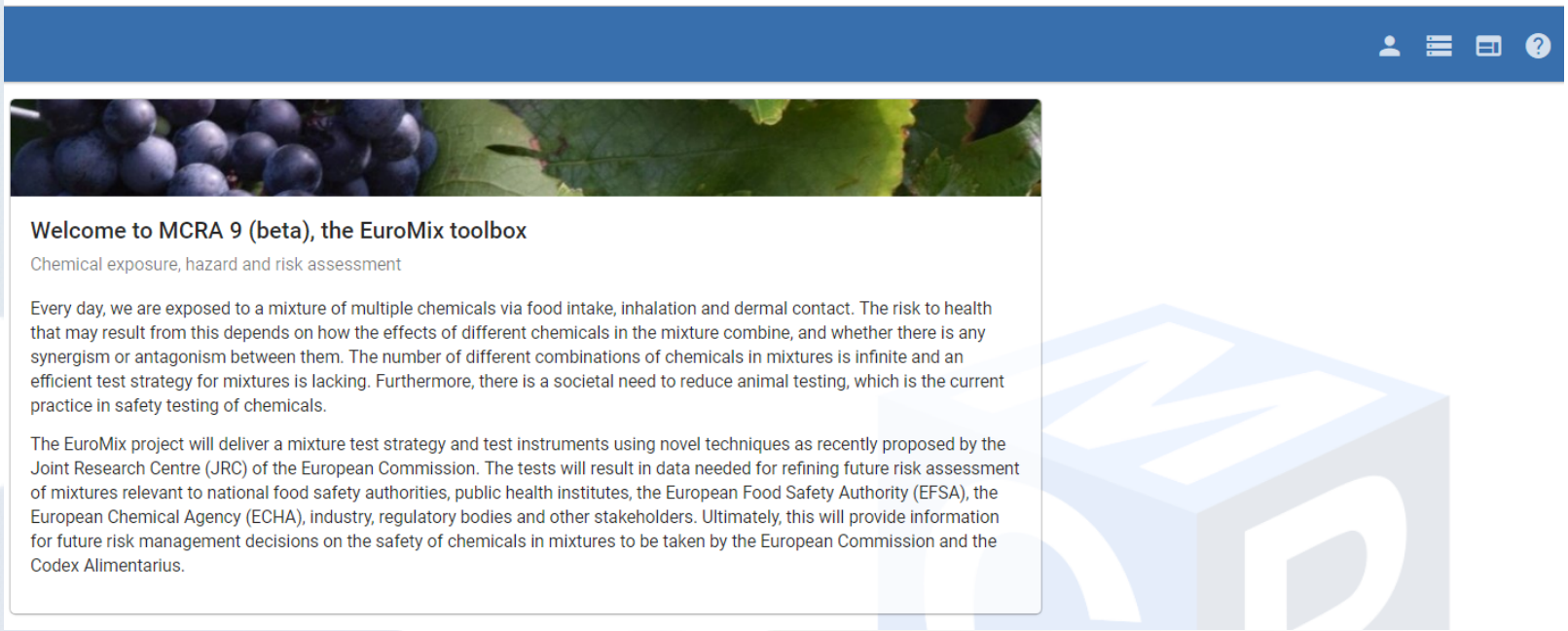
EuroMix toolbox

Web-based toolbox for mixture risk assessment

Based on previous MCRA tool

Upload toxicity and exposure data

Calculate exposure levels, relative potency factors, risk levels



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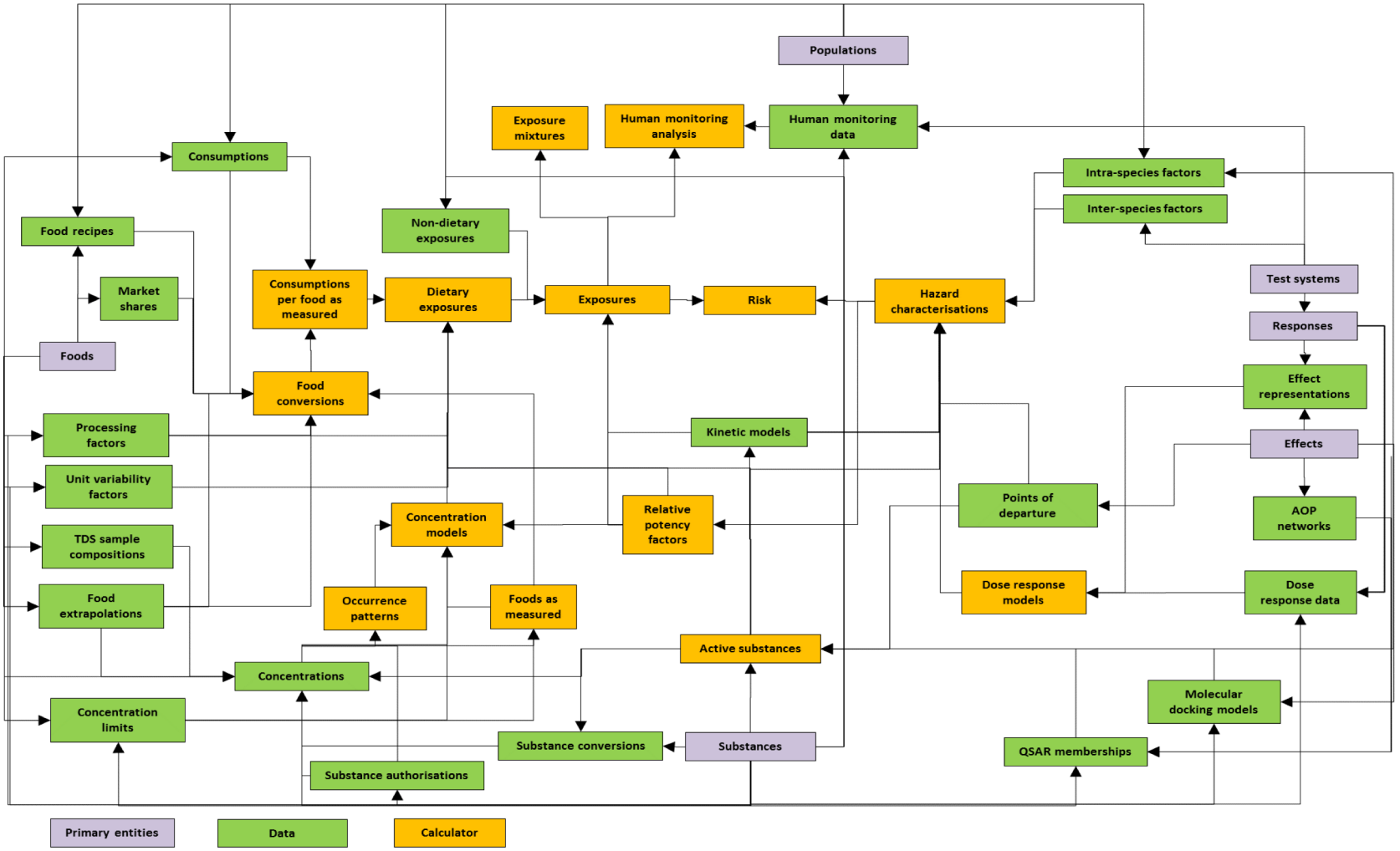
EuroMix toolbox-modules



EuroMix toolbox-modules

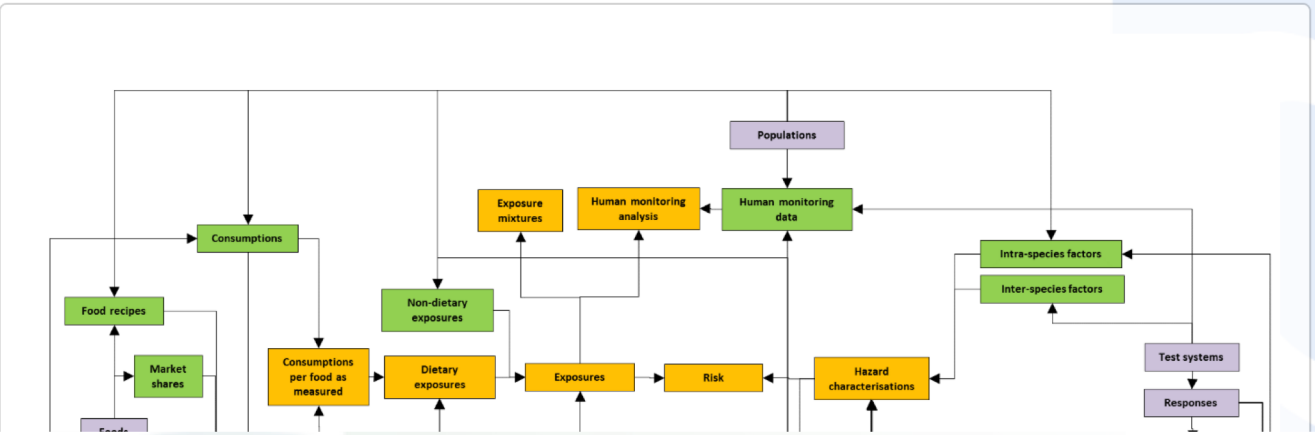
MCRA 9 - EuroMix toolbox
Exposure, Hazard & Risk Assessment

Help / Modules overview

- Primary entities
- Consumption
- Occurrence
- Exposure
- Hazard
- Risk
- In-silico
- Kinetic

Modular design

The models and data of the MCRA 9 toolbox are bundled in the modular design of which the diagram is shown below. It shows how the data and models are related to each other. Each module (i.e., each block in the diagram) represents a certain type of data that can be provided as a data source or it may be computed from other data. In the diagram, the purple boxes represent primary entity modules (or scoping modules), the green boxes represent data modules, and the orange boxes represent modules for which the data can be calculated from other data. In the toolbox, each module corresponds with an action that can be configured and for which output may be produced.



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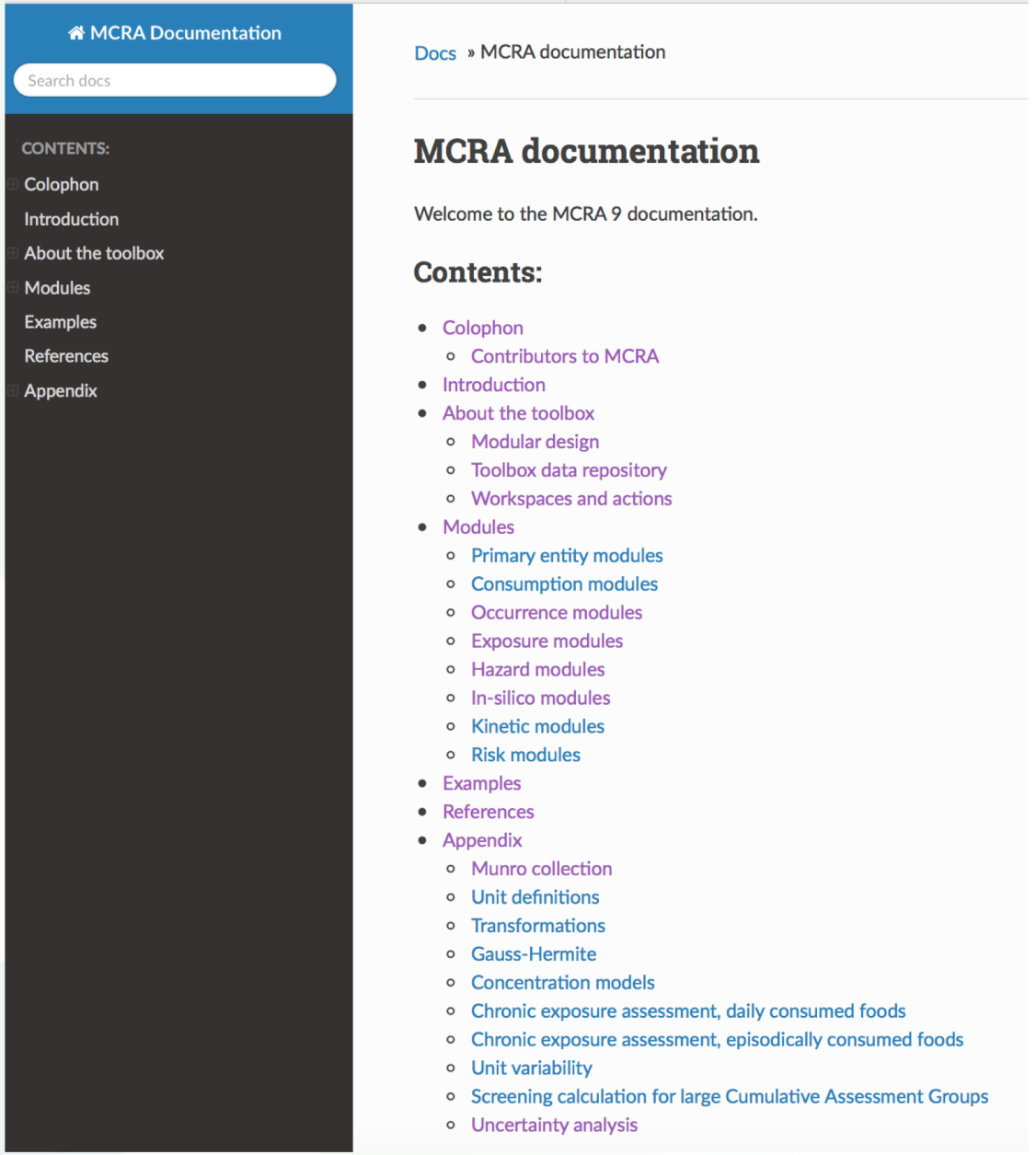
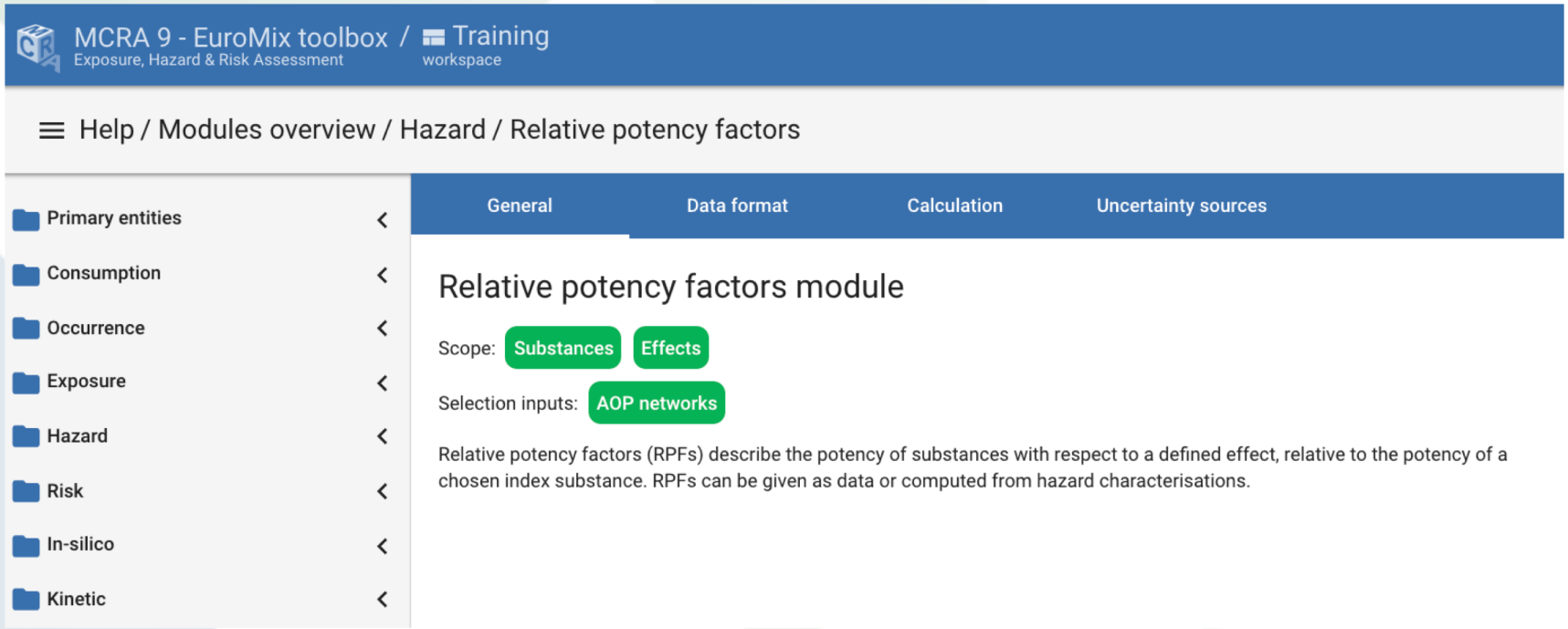
EuroMix toolbox user manual

Web-based user manual

- Descriptions of each module
- Settings
- Data formats



Examples: Relative potency factors



Calculate relative potency factors (RPF) based on

- NOAELs/BMDs from literature
- In vitro or in vivo dose response modelling
- TTC values

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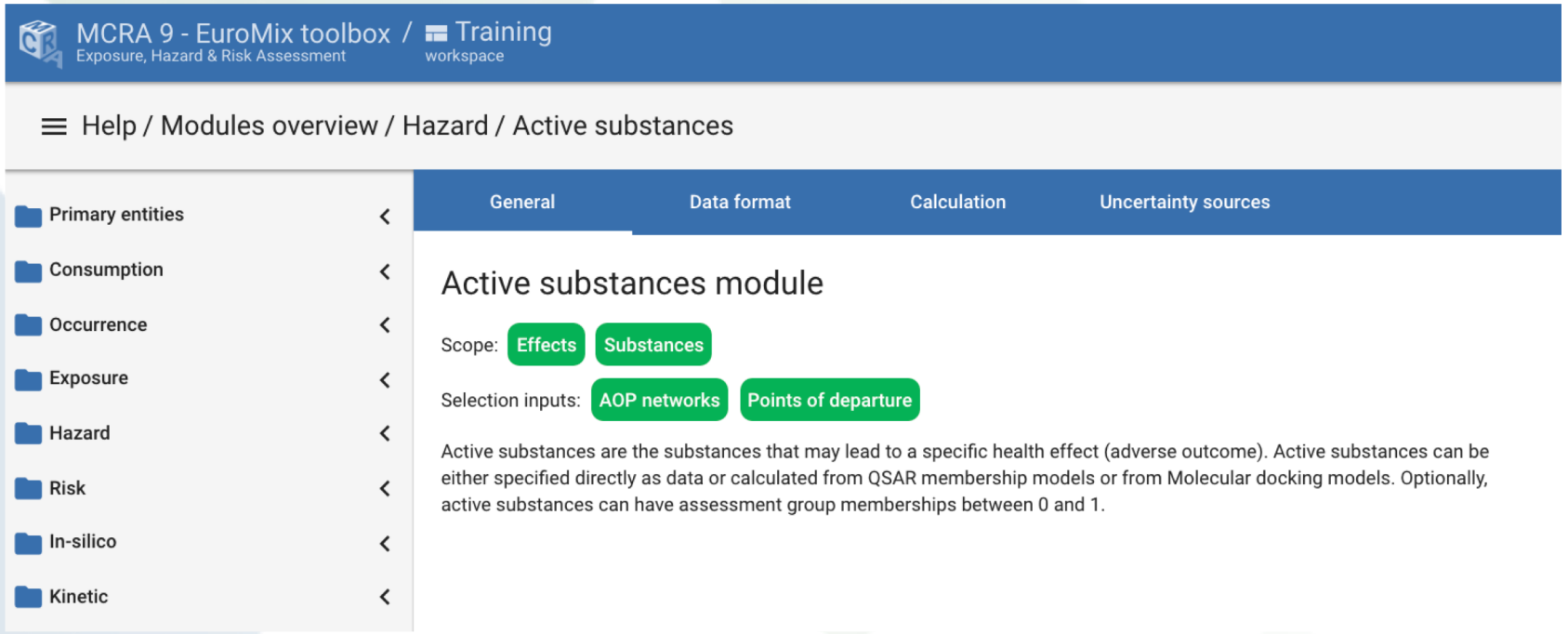
Example: Active substances/ Assessment groups



Specify substances included in an assessment group

- List of substances
- Calculate based on in silico models

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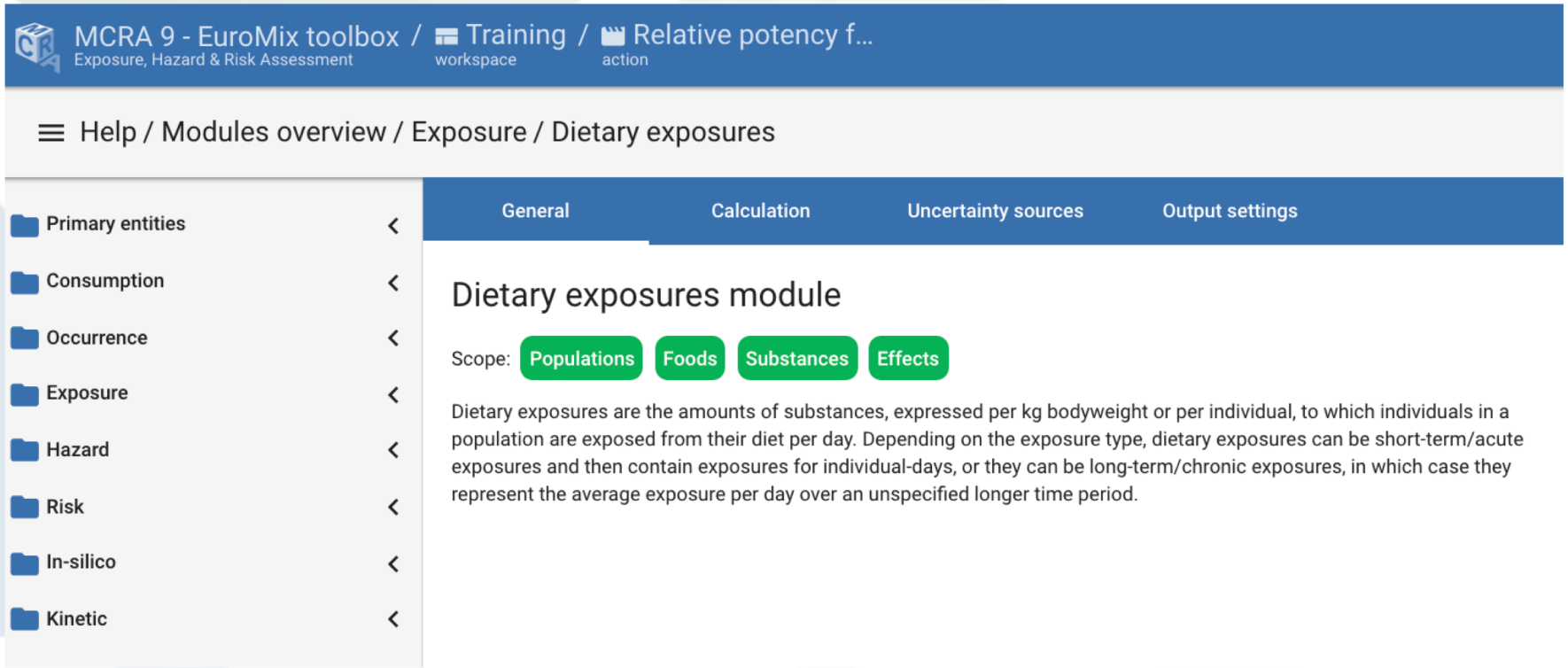
Example: Combined dietary exposure



Calculate combined dietary exposure and margin of exposure (MOE) using

- Consumption data
- Concentration data
- Relative potency factors (RPF)

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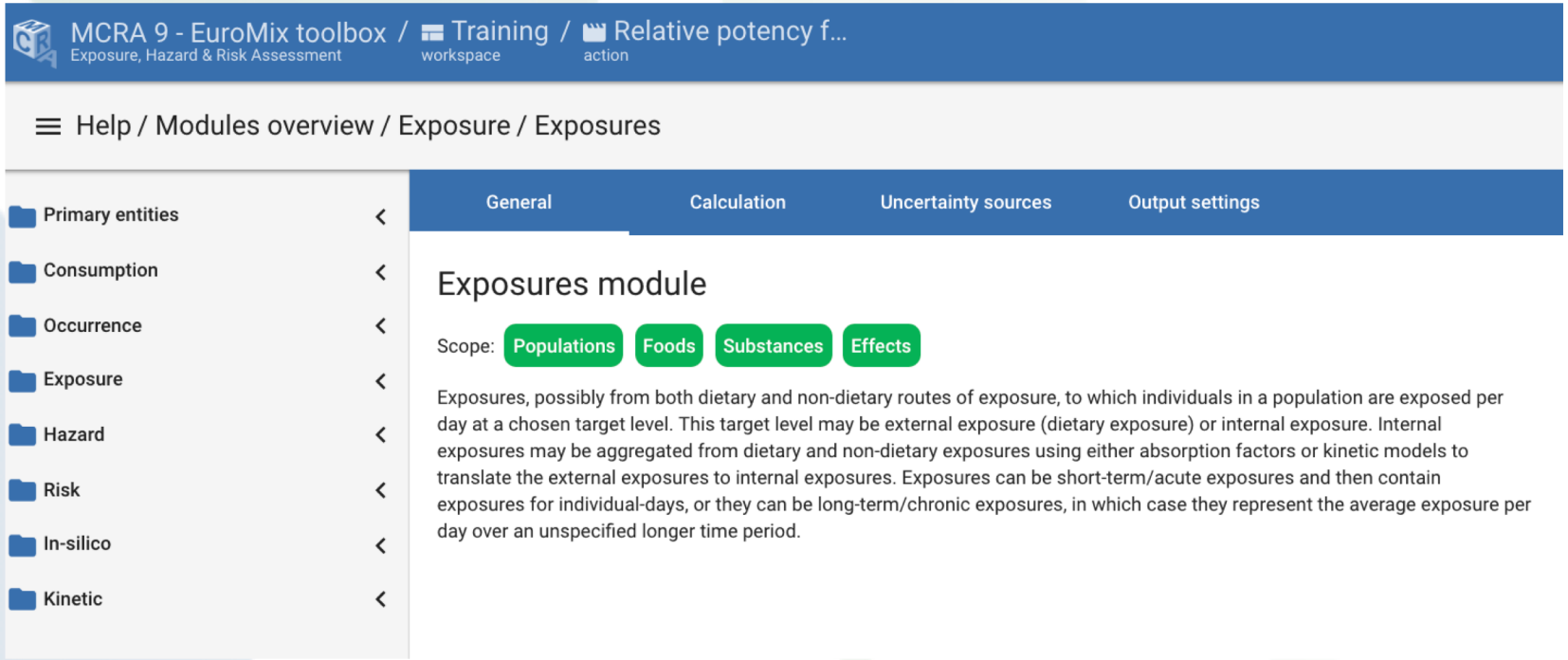
Example: Aggregate exposure



Calculate aggregate exposure and margin of exposure (MOE) using

- Dietary exposure
- Non-dietary exposure
- Relative potency factors (RPF)

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- **Problem formulation**
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  - Relative potency factors
  - Mixture experiments

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Problem formulation for mixture risk assessment

- Risk assessment question
- Description of the mixture
- Conceptual model
- Methodological approach
- Analysis plan

- EuroMix developed a template for the problem formulation and analysis plan

Problem formulation element	Description	Recorded information
Risk assessment question	Aim of the assessment and/or specific questions to be addressed.	
Description of mixture	Evidence for common toxicological effect of the mixture components	
	Evidence for co-exposure	
Conceptual model	Regulatory framework or specific regulatory remit	
	Substances	
	Exposure source(s) (e.g. food (specify if possible), drinking water, cosmetics, consumer products (specify), air, soil)	
	Exposure pathway(s) (e.g. oral, dermal, inhalation - specify as needed)	
	Population group (e.g. general population, workers, school children, pregnant women)	
	Population age (infant, toddler, child, teen, adult, elderly)	
	Toxicological effect	
	Level of grouping (common target organ, common adverse outcome (specific effect on the organ level) or common specific mode of action/AOP)	
Methodology	Data availability for toxicity described in general terms	
	Data availability for exposure described in general terms	
	Identification and assessment of AOP networks	
	Grouping of substances based on toxicological effect	
	Collection of toxicity data	
	Description of approach for dealing	

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Problem formulation-example

Problem formulation element	Description	Recorded information
Risk assessment question	Specific questions to be addressed	What is the risk for the adult population in the Netherlands to develop liver steatosis due to combined dietary exposure to pesticide residues in food?
Description of mixture	Evidence for common toxicological effect of the mixture components	Liver steatosis is identified as an effect in *in vivo* studies of several pesticides (RIVM, ICPS, ANSES, 2013, 2016)
	Evidence for co-exposure	Monitoring studies show that European food contains low levels of pesticide residues (EFSA 2018d)
Conceptual model	Regulatory framework or remit	Not applicable
	Substances categories	Pesticide residues
	Exposure source(s) (e.g. food, drinking water, cosmetics, consumer products, air, soil)	Food and drinking water
	Exposure route(s) (e.g. oral, dermal, inhalation - specify as needed)	Oral
	Population group (e.g. general population, workers, school children, pregnant women, country)	General population in the Netherlands
	Population age (infant, toddler, child, teen, adult, elderly)	Adults
	Toxicological effect	Liver steatosis
	Level of grouping (common target organ, common effect/adverse outcome or common specific mode of action/AOP)	Common effect/adverse outcome

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Problem formulation-example

Methodology	Data availability for toxicity described in general terms, including the type of data	Regulatory in vivo toxicity studies reported in EFSA draft assessment reports, other assessment reports from international bodies. In vivo studies in scientific papers. Study characteristics, NOAEL, LOAEL, but in most cases not dose-response data. Results from QSAR modelling and in vitro studies.
	Data availability for exposure described in general terms, including the type of data	Consumption data from national dietary surveys in Netherlands. Concentration data from European monitoring studies.
	Use of AOP networks to support grouping of substances into assessment groups and/or identification of measurable effect/key events	AOP networks for liver steatosis used to support grouping and identify measurable key events
	Collection of toxicity data from literature	Literature search to identify toxicity data from reports and scientific papers, selection of studies on included substances and effect included to the AOP network, assessing reliability of scientific papers using SciRAP tool.
	Generation of toxicity data	In case relevant and reliable toxicity data is not identified from literature, in silico data and in vitro data for selected KEs in the AOP network is generated.
	Grouping of substances based on toxicological considerations	Grouping at the level of common effect, liver steatosis, using in silico, in vitro and in vivo data using two experts. Grouping reported as included/not included.

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- Abbreviations
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  - Collection and assessment of toxicity data from literature
  - Tiered testing strategies
  - Grouping of substances based on toxicological considerations
  - Relative potency factors
  - Mixture experiments

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Toxicity data for mixture risk assessment

Toxicity data needed for

- Grouping into assessment groups
- Relative potency factors

- **In vivo data**
  - Not always available or feasible to produce for all substances
- **In vitro data**
  - Inform grouping
  - Relative potency factors using in vitro to in vivo extrapolation (IVIVE)
  - Tiered testing strategies and set priorities for in vivo testing
- **In silico data**
  - Inform grouping
  - Tiered testing strategies and set priorities for in vitro testing
- **TTC values**
  - Lack of in vitro or in vivo data

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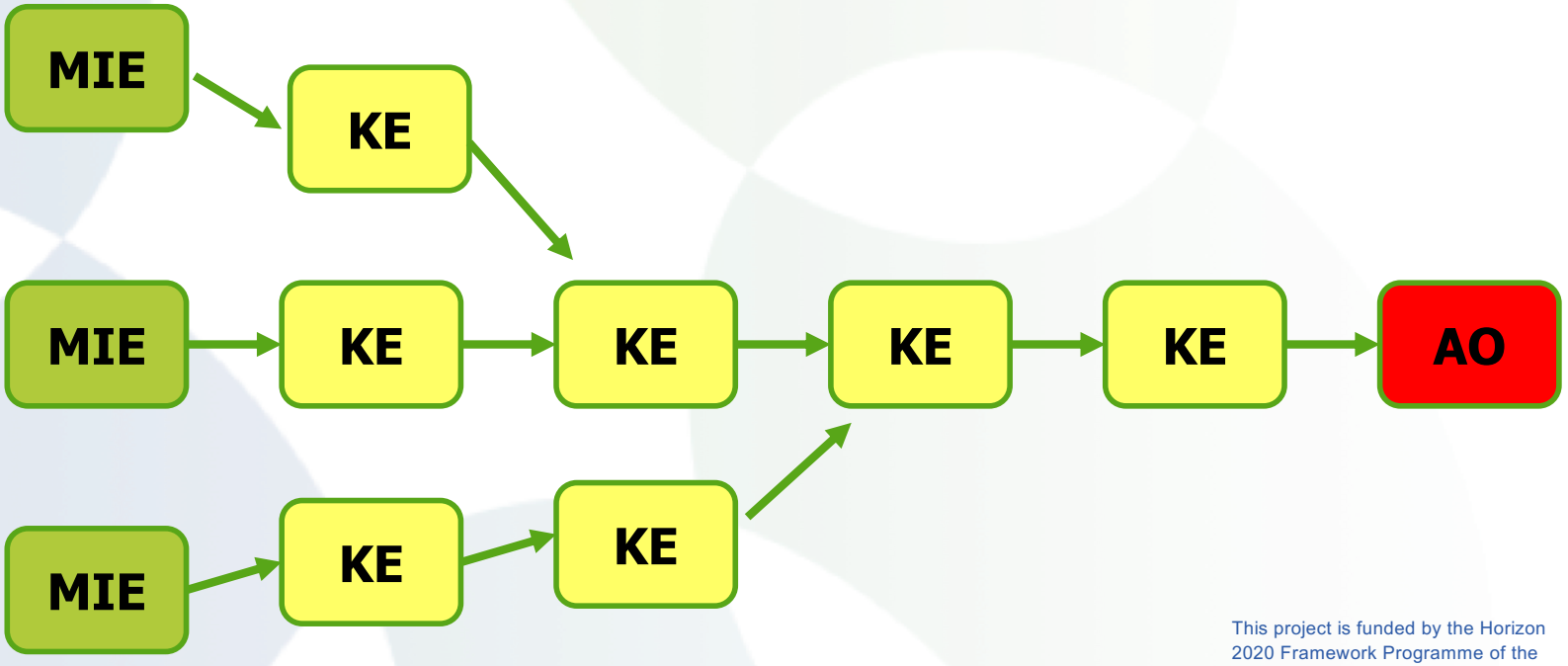
Adverse outcome pathway (AOP) networks

- Adverse outcome pathway (AOP) networks can be used as basis for grouping of substances and to identify suitable assays for testing toxicity in vitro
- However, mixture risk assessment in the EuroMix toolbox does not require any information from AOPs, only one effect has to be specified for the assessment.



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Identification and assessment of AOP networks

Methodology based on OECD AOP methodology and handbook (2018)

- Identify any existing AOPs
- Develop new AOP starting from Adverse outcome (AO)
- Identify key events (KE) and KE relationships
- Focus on easily measured KEs
- Complete AOP not necessary
- Assess the postulated AOP
- Describe the AOP in the tables for use in EuroMix toolbox

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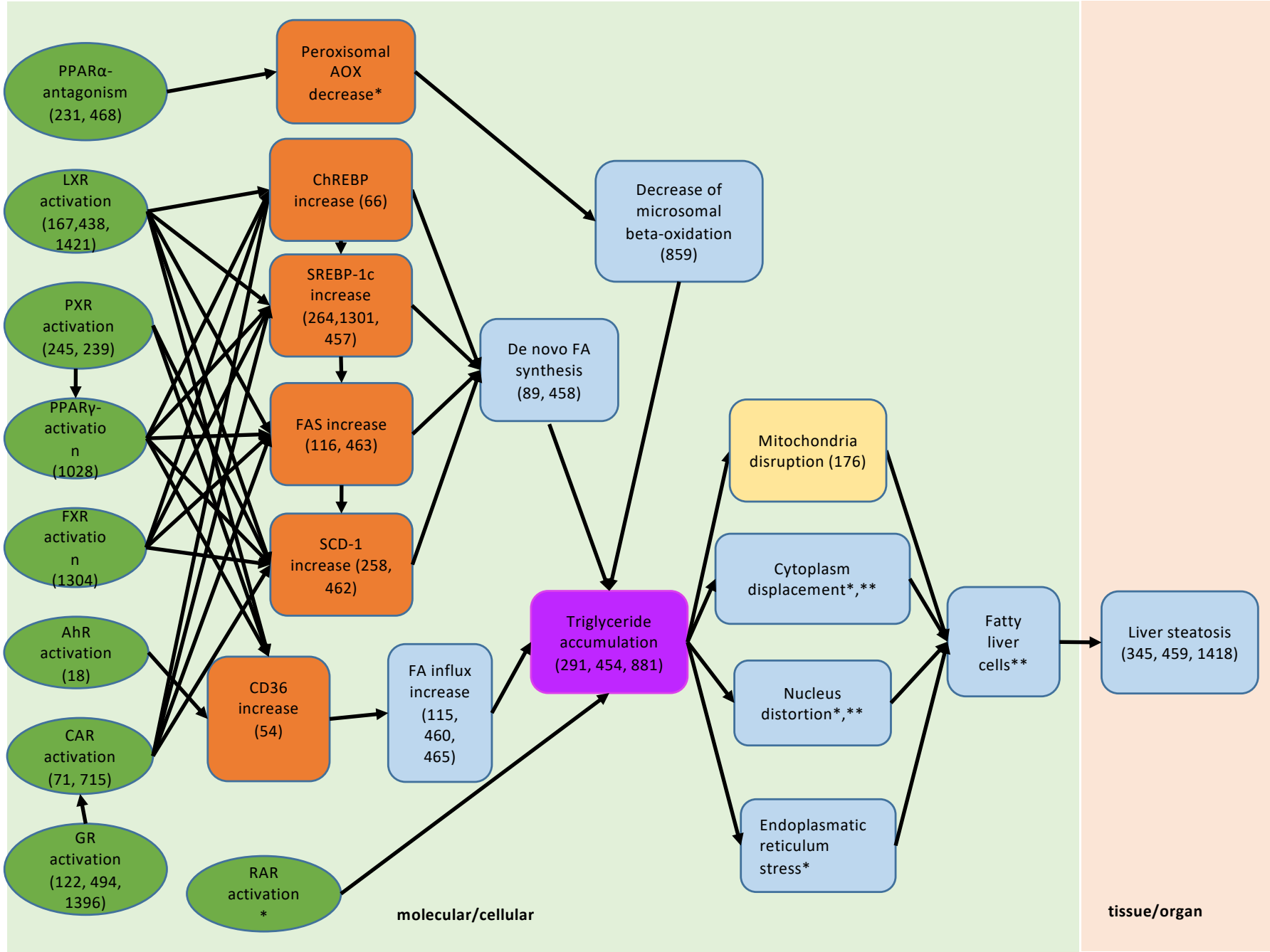
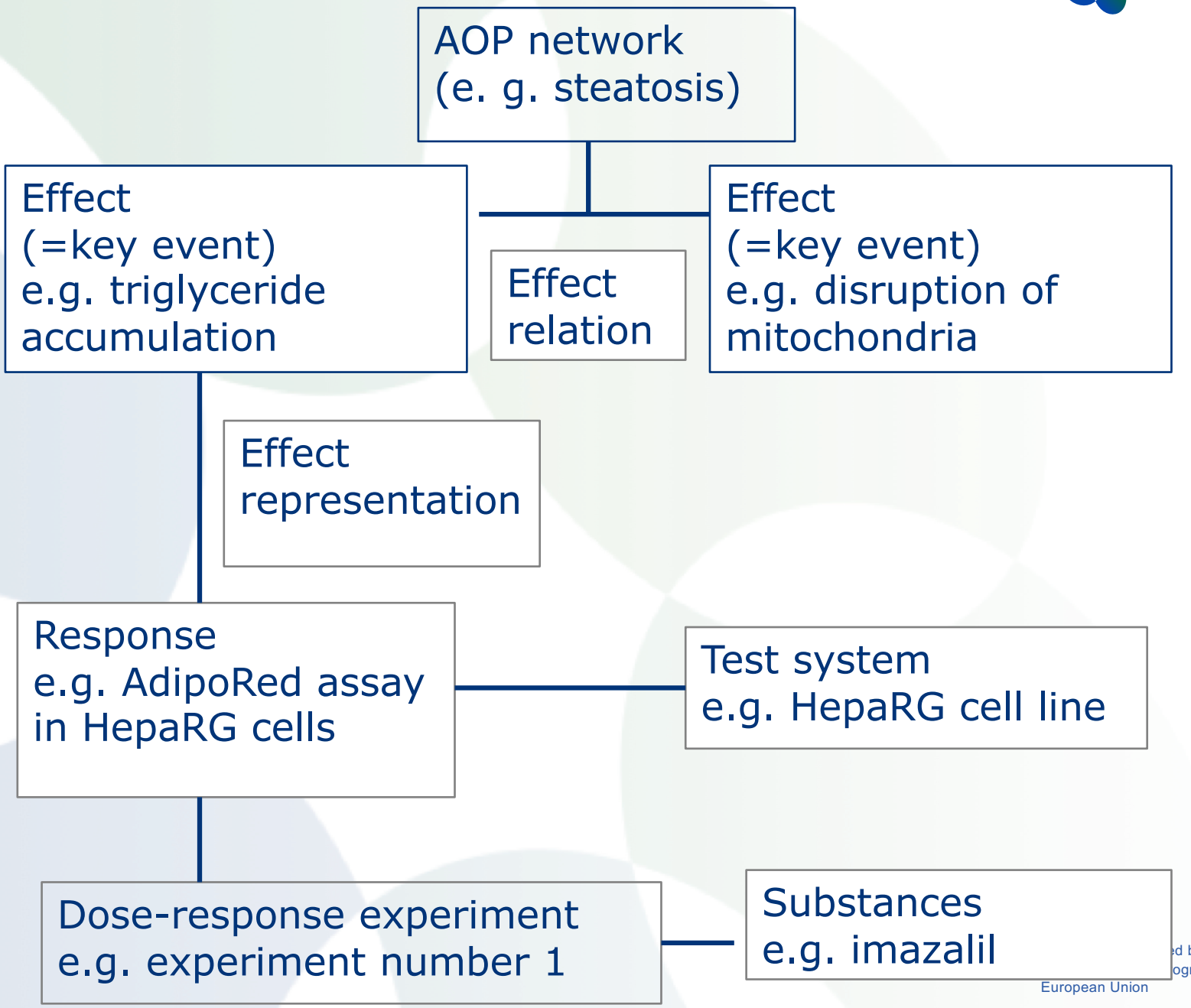
AOP network for liver steatosis



Modules for hazard data



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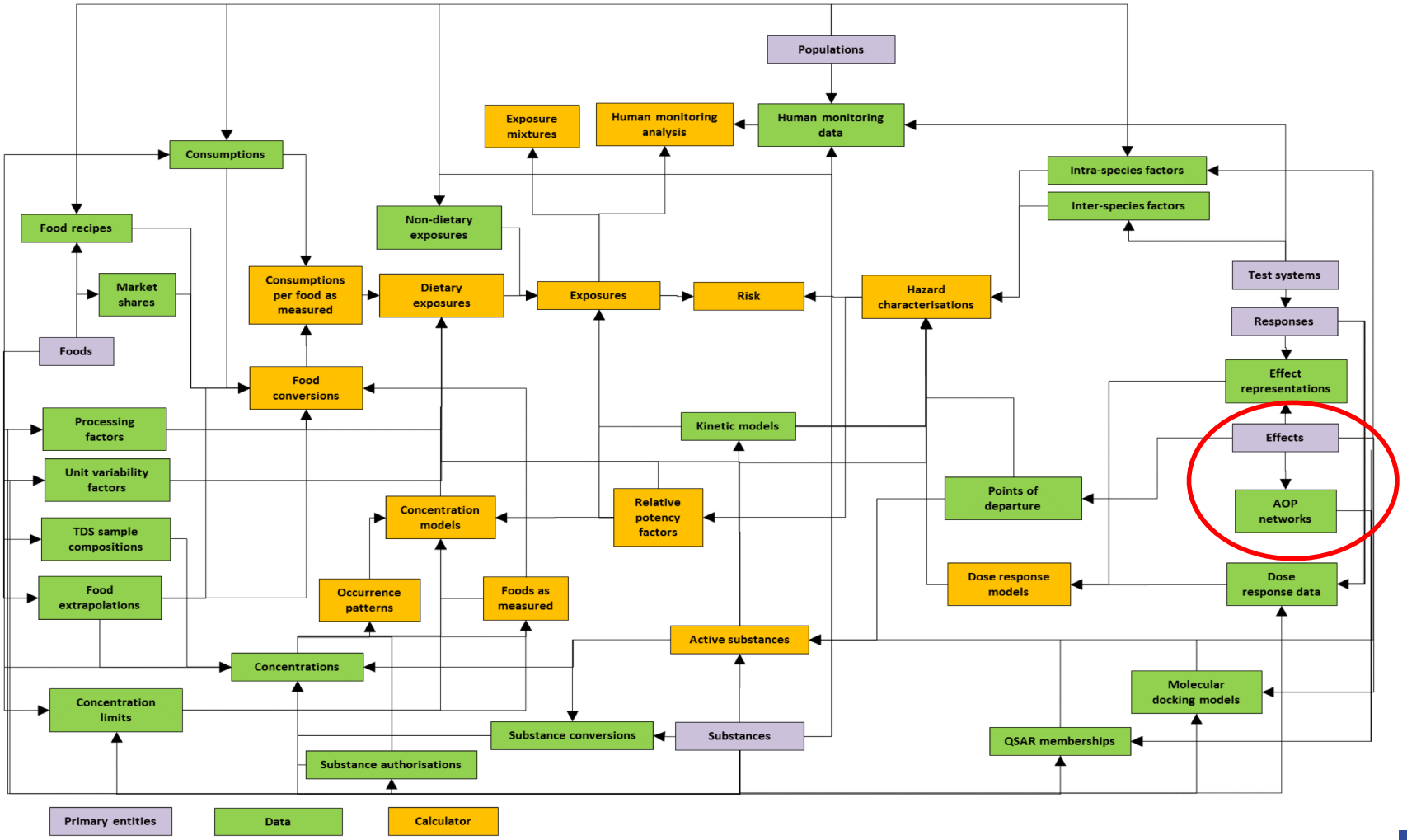
AOP network in EuroMix toolbox

Coding of Effects (key events) and Effect relations (key event relationships) according to AOP wiki

	A	E	F	G	H	I	J	K
1	idEffect	BiologicalOrganisation	KeyEventProcess	KeyEventObject	KeyEventAction	KeyEventCell	KeyEventOrgan	AOPwiki
2	PPARalpha-antagonism-liver	Molecular	peroxisome proliferator activated re	peroxisome proliferator-activated	decreased	hepatocyte	liver	231,468
3	LXR-act-liver	Molecular	signaling	oxysterols receptor LXR-alpha ANI	increased	hepatocyte	liver	167,483,1421
4	PXR-act-liver	Molecular	signaling	nuclear receptor subfamily 1 grou	increased	hepatocyte	liver	245,239
5	PPARgamma-act-liver	Molecular	peroxisome proliferator activated re	peroxisome proliferator-activated	increased	hepatocyte	liver	1028
6	FXR-act-liver	Molecular	signaling		increased	hepatocyte	liver	1304
7	AhR-act-liver	Molecular	aryl hydrocarbon receptor activity	aryl hydrocarbon receptor	increased	hepatocyte	liver	18
8	CAR-act-liver	Molecular	signaling	nuclear receptor subfamily 1 grou	increased	hepatocyte	liver	71,715
9	GR-act-liver	Molecular	glucocorticoid receptor activity	glucocorticoid receptor	increased	hepatocyte	liver	
10	RAR-act-liver	Molecular	signaling		increased	hepatocyte	liver	
11	AOX-decr-liver	Molecular	fatty acid beta-oxidation		decreased	hepatocyte	liver	
12	ChREBP-incr-liver	Molecular	signaling	carbohydrate-responsive element	increased	hepatocyte	liver	
13	SREBP-1c-incr-liver	Molecular	SREBP signaling pathway	sterol regulatory element-binding	increased	hepatocyte	liver	
14	FAS-incr-liver	Molecular	fatty acid synthase activity	fatty acid synthase	increased	hepatocyte	liver	
15	SCD1-incr-liver	Molecular	gene expression	acyl-CoA desaturase	increased	hepatocyte	liver	
16	CD36-incr-liver	Molecular	gene expression	platelet glycoprotein 4	increased	hepatocyte	liver	
17	microsomalbetaox-decr-liver	Molecular				hepatocyte	liver	
18	denovoFA-incr-liver	Cellular	fatty acid biosynthetic process	fatty acid	increased	hepatocyte	liver	
19	FAinflux-incr-liver	Cellular	positive regulation of fatty acid trans	fatty acid	increased	hepatocyte	liver	
20	triglyceride-accum-liver	Cellular	triglyceride biosynthetic	triglyceride	increased	hepatocyte	liver	
21	cytoplasm-displ-liver	Cellular				hepatocyte	liver	
22	nucleus-distort-liver	Cellular				hepatocyte	liver	
23	mitochondrial-disrupt-liver	Cellular	mitochondrion disassembly	mitochondrion	functional change	hepatocyte	liver	
24	ER-stress-liver	Cellular				hepatocyte	liver	
25	FattyCells-liver	Tissue					liver	
26	Steatosis-liver	Organ		fatty liver	occurence	*N/A*	liver	

	A	B	C	D
1	idAOPN	idUpstreamKeyEvent	idDownstreamKeyEvent	AOPwikiKER
2	AOPN-steatosis	PPARalpha-antagonism-liver	AOX-decr-liver	
3	AOPN-steatosis	LXR-act-liver	ChREBP-incr-liver	174
4	AOPN-steatosis	LXR-act-liver	SREBP-1c-incr-liver	177,479
5	AOPN-steatosis	LXR-act-liver	FAS-incr-liver	175
6	AOPN-steatosis	LXR-act-liver	SCD1-incr-liver	176
7	AOPN-steatosis	LXR-act-liver	CD36-incr-liver	173
8	AOPN-steatosis	PXR-act-liver	SCD1-incr-liver	526
9	AOPN-steatosis	PXR-act-liver	CD36-incr-liver	529
10	AOPN-steatosis	PXR-act-liver	PPARgamma-act-liver	
11	AOPN-steatosis	PPARgamma-act-liver	ChREBP-incr-liver	
12	AOPN-steatosis	PPARgamma-act-liver	SREBP-1c-incr-liver	
13	AOPN-steatosis	PPARgamma-act-liver	FAS-incr-liver	
14	AOPN-steatosis	PPARgamma-act-liver	SCD1-incr-liver	
15	AOPN-steatosis	PPARgamma-act-liver	CD36-incr-liver	
16	AOPN-steatosis	FXR-act-liver	ChREBP-incr-liver	
17	AOPN-steatosis	FXR-act-liver	SREBP-1c-incr-liver	
18	AOPN-steatosis	FXR-act-liver	FAS-incr-liver	
19	AOPN-steatosis	FXR-act-liver	SCD1-incr-liver	
20	AOPN-steatosis	AhR-act-liver	CD36-incr-liver	495
21	AOPN-steatosis	CAR-act-liver	ChREBP-incr-liver	
22	AOPN-steatosis	CAR-act-liver	SREBP-1c-incr-liver	
23	AOPN-steatosis	CAR-act-liver	FAS-incr-liver	
24	AOPN-steatosis	CAR-act-liver	SCD1-incr-liver	
25	AOPN-steatosis	GR-act-liver	CAR-act-liver	
26	AOPN-steatosis	RAR-act-liver	triglyceride-accum-liver	
27	AOPN-steatosis	AOX-decr-liver	microsomalbetaox-decr-liver	
28	AOPN-steatosis	ChREBP-incr-liver	denovoFA-incr-liver	77,483
29	AOPN-steatosis	ChREBP-incr-liver	SREBP-1c-incr-liver	
30	AOPN-steatosis	SREBP-1c-incr-liver	denovoFA-incr-liver	294
31	AOPN-steatosis	SREBP-1c-incr-liver	FAS-incr-liver	
32	AOPN-steatosis	FAS-incr-liver	denovoFA-incr-liver	133,492,1422
33	AOPN-steatosis	FAS-incr-liver	SCD1-incr-liver	
34	AOPN-steatosis	SCD1-incr-liver	denovoFA-incr-liver	284,1404
35	AOPN-steatosis	CD36-incr-liver	FAinflux-incr-liver	66,501
36	AOPN-steatosis	microsomalbetaox-decr-liver	triglyceride-accum-liver	
37	AOPN-steatosis	denovoFA-incr-liver	triglyceride-accum-liver	110,484
38	AOPN-steatosis	FAinflux-incr-liver	triglyceride-accum-liver	132,504,1658

EuroMix toolbox-modules



Collection and assessment of toxicity data from literature

Toxicity data for grouping of substances and for calculation of RPFs

Methodology based on systematic review and weight of evidence methodology

- Purpose of data collection
- Search for studies from reports, scientific publications
- Select the studies that contain relevant data
- Collect data from the studies
- Assess the data for reliability and relevance

Template for data collection using EuroMix toolbox data format

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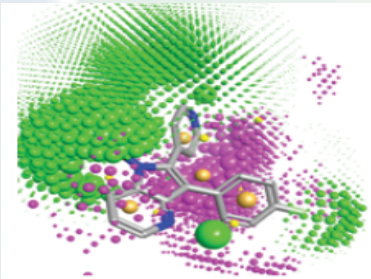
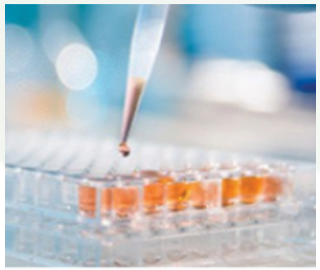
Tiered testing strategy based on AOP networks

In silico



In vitro



In vivo

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Tiered testing strategy based on AOP networks

Methodology

- Identify KEs that can provide info for grouping or RPFs in the AOP network
- Identify in silico, in vitro and in vivo assays for the KEs
- Assess the
  - relevance of the assays
  - reliability of the assays
  - availability and feasibility in terms of costs and resources
  - information provided for grouping, RPFs, prioritisation for further testing
- Select assays to be included based on the assessments
- Describe the assays (test systems and responses) in the tables for use in EuroMix toolbox

- Template for description of tiered testing strategy

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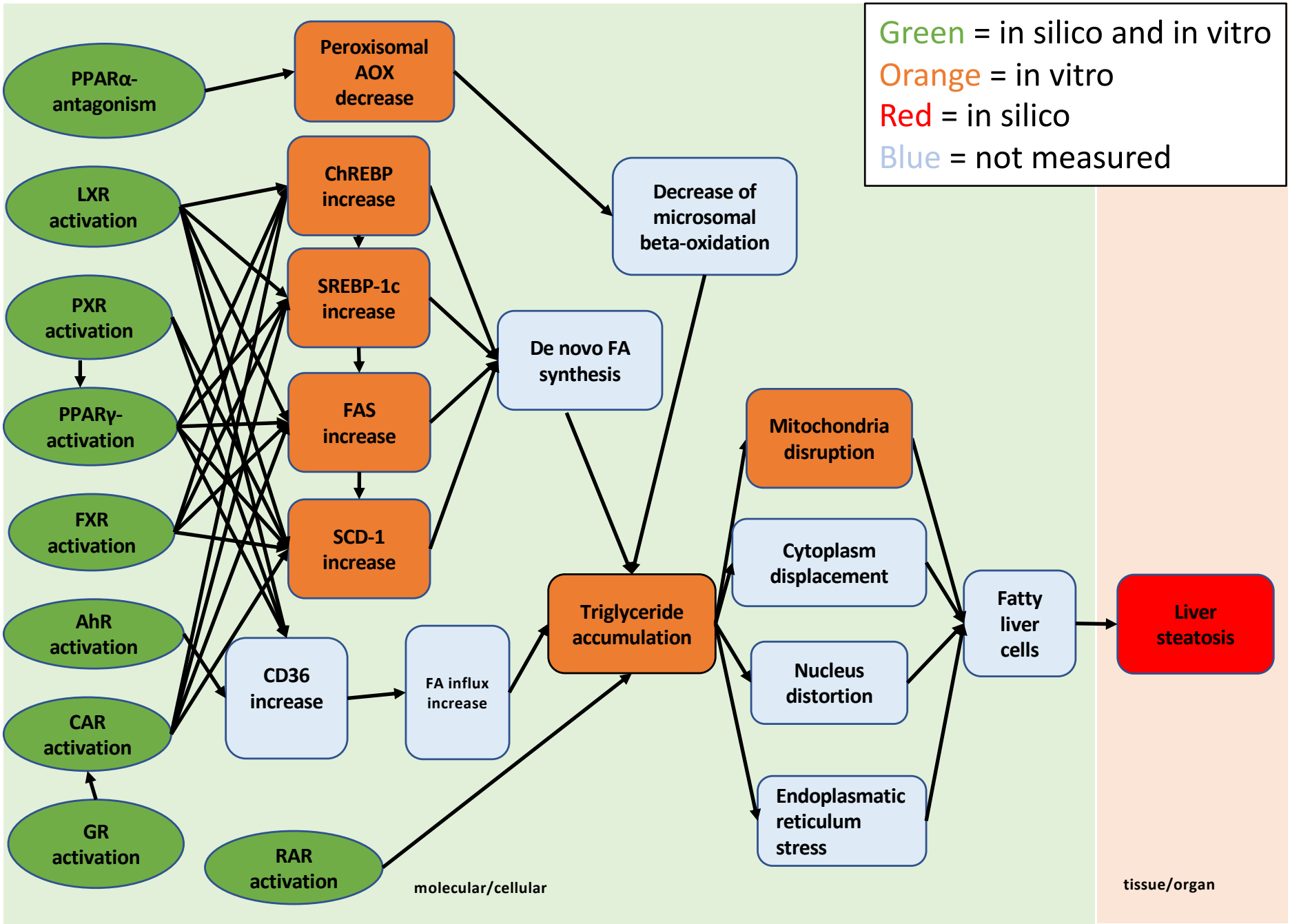
AOP based testing strategy for liver steatosis



Template for tiered testing strategy

KE number in AOP network	KE name	*In silico* model/*in vitro* assay for measuring the KE	Relevance of the *in silico* model/*in vitro* assay	Reliability of the *in silico* model/*in vitro* assay	Availability and feasibility of *in silico* model/*in vitro* assay	Information provided by the *in silico* model/*in vitro* assay for the mixture risk assessment (e.g. for grouping, RPFs and/or prioritisation for further testing)
MIE1						
MIE2						
KE1						
KE2						
KE3						
KE4						
KE5						
KE6						
KE7						
AO						

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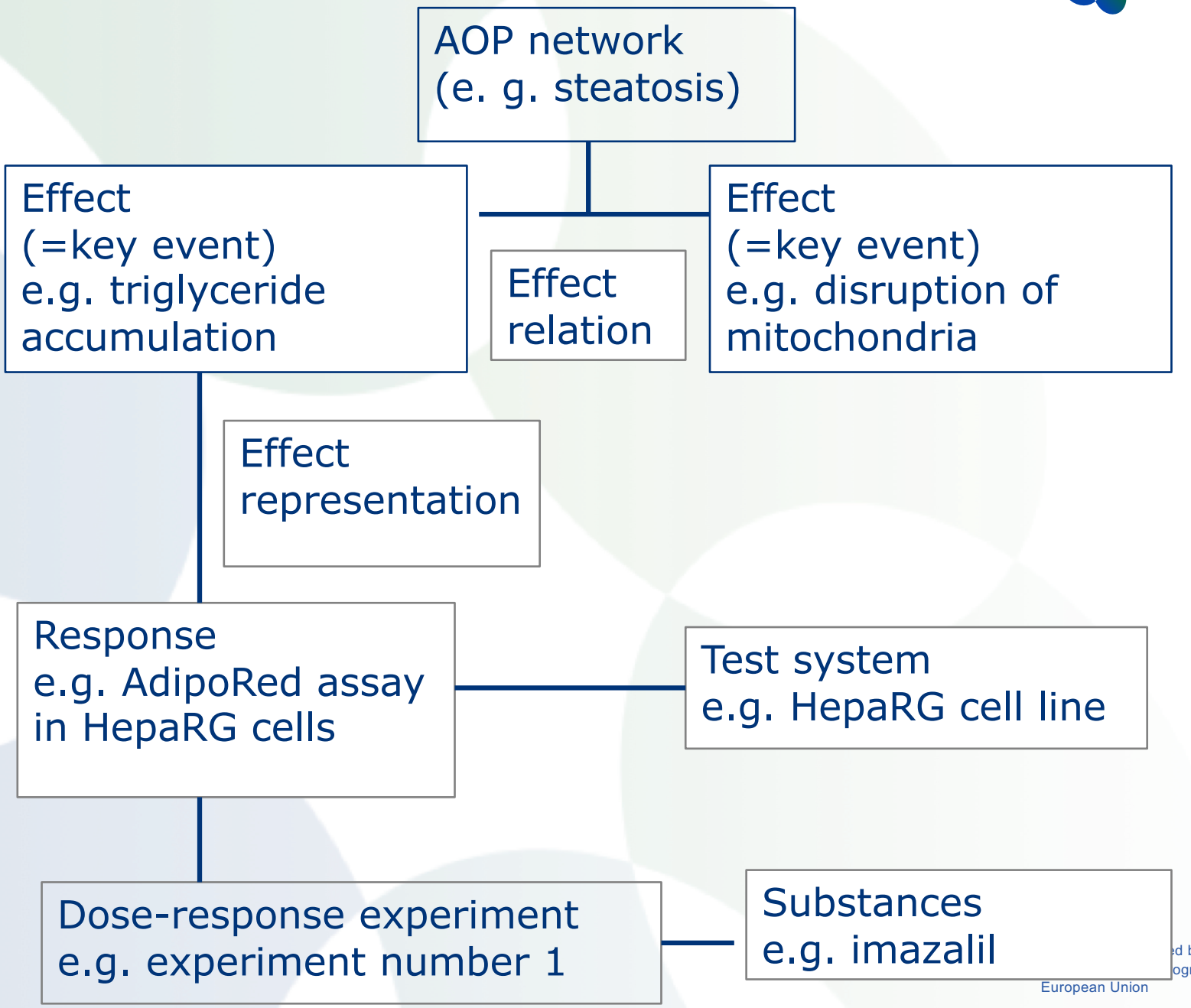
Modules for hazard data



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Assays (test systems and responses in EuroMix toolbox

Coding of test systems, responses and effect representations (connecting response and effect)

	A	E	F	G	H	I	J
1	idTestSystem	TestSystemType	Organ	GuidelineMethod	Species	Strain	RouteExposure
2	HepG2	CellLine	liver		human		
3	HepaRG	CellLine	liver		human		
4	HEK293T	CellLine	kidney		human		
5	Rat28Day	InVivo		OECD TG 407	rat	SD	Oral

	A	E	F	G	H
1	idResponse	idTestSystem	GuidelineMethod	TimePoint	ResponseType
9	HepG2-RGA-AhR	HepG2		24 h	ContinuousMultiplicative
10	HepG2-RGA-CAR	HepG2		24 h	ContinuousMultiplicative
11	HepG2-RGA-FXR	HepG2		24 h	ContinuousMultiplicative
12	HepG2-RGA-GR	HepG2		24 h	ContinuousMultiplicative
13	HepG2-RGA-LXR	HepG2		24 h	ContinuousMultiplicative
14	HepG2-RGA-PPARalpha	HepG2		24 h	ContinuousMultiplicative
15	HepG2-RGA-PPARgamma	HepG2		24 h	ContinuousMultiplicative
16	HepG2-RGA-PXR	HepG2		24 h	ContinuousMultiplicative
17	HepG2-RGA-RARalpha	HepG2		24 h	ContinuousMultiplicative
196	HepaRG-HCS-triglyceride-24h	HepaRG		24h	ContinuousMultiplicative
197	HepaRG-HCS-triglyceride-72h	HepaRG		72h	ContinuousMultiplicative
198	HepaRG-GC-triglyceride-C44	HepaRG		72h	ContinuousMultiplicative
199	HepaRG-GC-triglyceride-C46	HepaRG		72h	ContinuousMultiplicative
200	HepaRG-GC-triglyceride-C48	HepaRG		72h	ContinuousMultiplicative
201	HepaRG-GC-triglyceride-C50	HepaRG		72h	
202	HepaRG-GC-triglyceride-C52	HepaRG		72h	
203	HepaRG-GC-triglyceride-C54	HepaRG		72h	
204	HepaRG-GC-triglyceride-C56	HepaRG		72h	
205	HepaRG-AdipoRed-72h	HepaRG		72h	
206	HepaRG-AdipoRed-24h	HepaRG		24h	
207	Rat28day-FattyCells	Rat28day		28d	

	A	B	C	D
1	idEffect	idResponse	BenchMarkResponse	BenchMarkResponseType
3	PPARalpha-antagonism-liver	HepG2-RGA-PPARalpha	0,8	Factor
7	LXR-act-liver	HepG2-RGA-LXR	0,8	Factor
9	PXR-act-liver	HepG2-RGA-PXR	0,8	Factor
35	triglyceride-accum-liver	HepaRG-HCS-triglyceride-24h	0,8	Factor
36	triglyceride-accum-liver	HepaRG-HCS-triglyceride-72h	0,8	Factor
39	triglyceride-accum-liver	HepaRG-GC-triglyceride-C48	0,8	Factor
44	triglyceride-accum-liver	HepaRG-AdipoRed-72h	0,8	Factor
45	triglyceride-accum-liver	HepaRG-AdipoRed-24h	0,8	Factor
46	triglyceride-accum-liver	Rat28Day-GC-triglyceride-C44		

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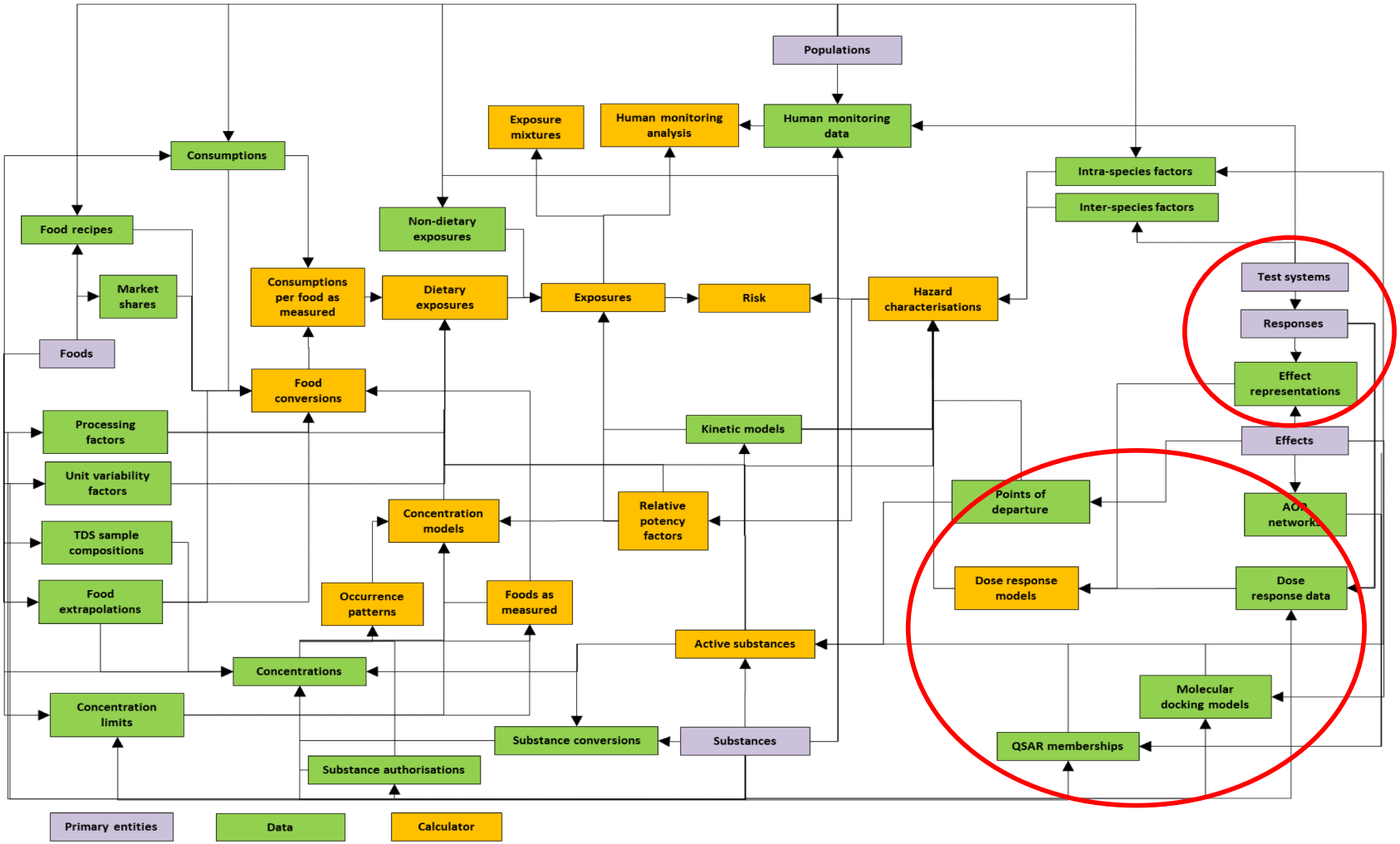
EuroMix toolbox-modules



Grouping of substances

Group of substances included in the mixture risk assessment

Grouping based on e.g.:

- Common exposure source
- Structural similarities
- Toxicological considerations

EuroMix developed methods for grouping based on toxicological considerations

EuroMix toolbox can also be used for other types of grouping

Grouping can be done at different levels

- Target organ
- Specific effect (adverse outcome)
- Specific mode of action/AOP

Grouping at level of specific effect will probably be most common

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Grouping of substances based on toxicological considerations

Methodology

- Level of grouping (target organ, common effect/AO, common specific mode of action /AOP)
- AOP network
- Substance category
- Collect toxicity data (in silico, in vitro, in vivo, human)
- Organise data in lines of evidence
- Assess data for relevance and reliability
- Decide on group membership using weight of evidence approach
- Report group membership in table for use in EuroMix toolbox (either 0 (not included) or 1 (included) or a value between 0-1 indicating the probability for belonging to the assessment group)

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Template for organising data for grouping

Substance	Key event in the AOP network (organised according to MIE, intermediate KEs, AO)	Study type (organised according to *in silico*, *in vitro*, *in vivo* data, human study)	Assay (specific assay used)	Main study result (e.g. positive, negative, BMDL, NOAEL)	Reliability (low, medium, high)	Relevance (low, medium, high)
	MIE	*In silico*				
		In vitro				
		In vivo				
		Human				
	Each intermediate KE	*In silico*				
		In vitro				
		In vivo				
		Human				
	AO	*In silico*				
		In vitro				
		In vivo				
		Human				

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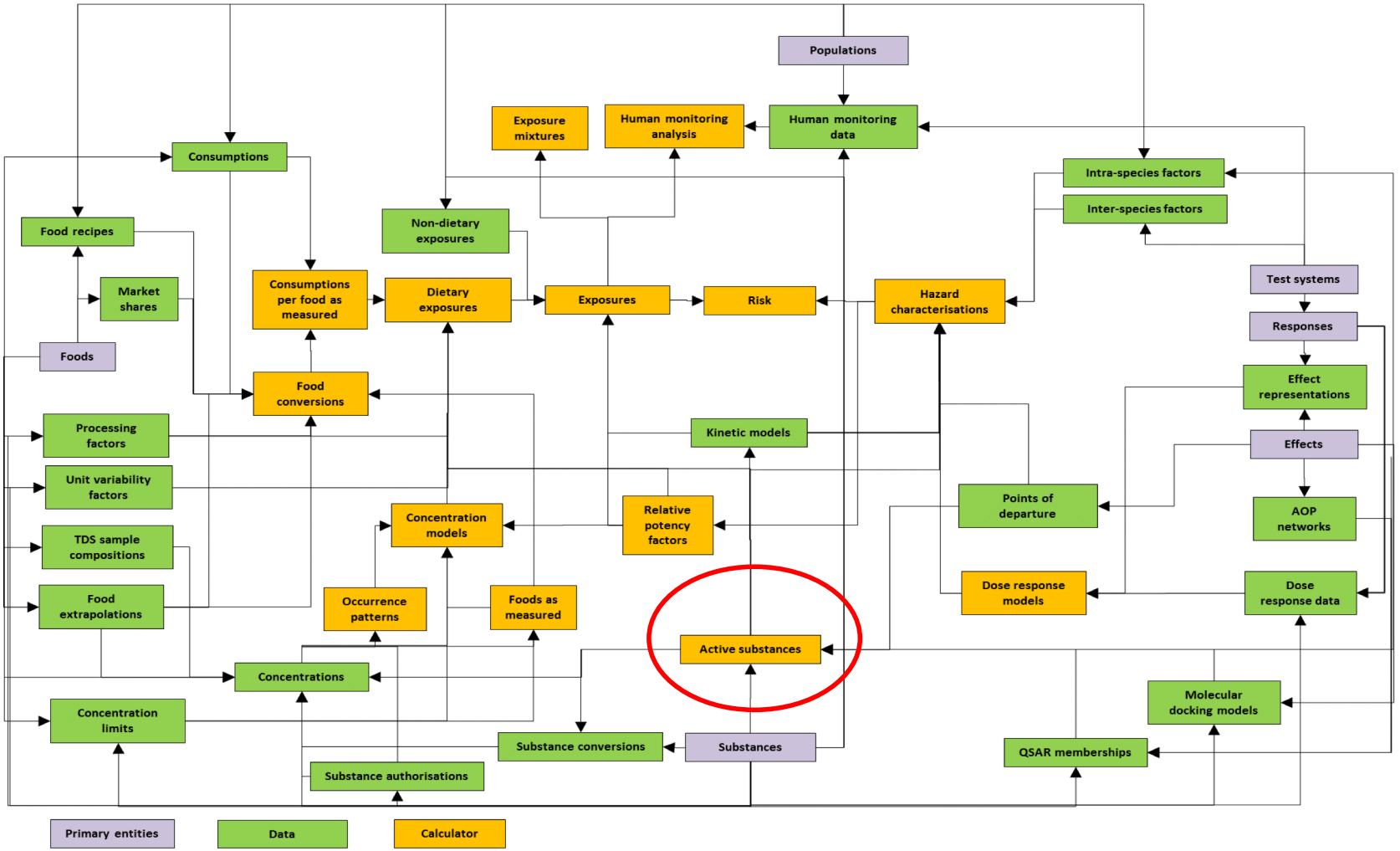
EuroMix toolbox-modules



Dose addition using relative potency factors (RPFs)

- Toxicity of index substance (Point of departure (POD), NOAEL/BMD): POD_{index}
- Toxicity of each substance in mixture (Point of departure, NOAEL/BMD): $POD_{1,}$ $POD_{2,}$ $POD_{3,}$ POD_{4} …
- Calculate Relative potency factor (RPF) for each chemical:
- $RPF_1 = POD_{index} / POD_1$

- Exposure to mixture is scaled based on RPFs
- $Exp_{mix} = Exp_1 \times RPF_1 + Exp_2 \times RPF_2 + \ldots$

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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 index substance/ PoD substance

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

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Sources for Relative potency factors

- RPFs based on
  - ADI/TDI
  - NOAEL/BMD for critical effect
  - NOAEL/BMD for specific effect
  - same RPF for all substances

- Data sources for RPFs
  - RPF data from literature
  - NOAELs from literature
  - Experimental dose response data
  - TTC values when no in vitro or in vivo data is available

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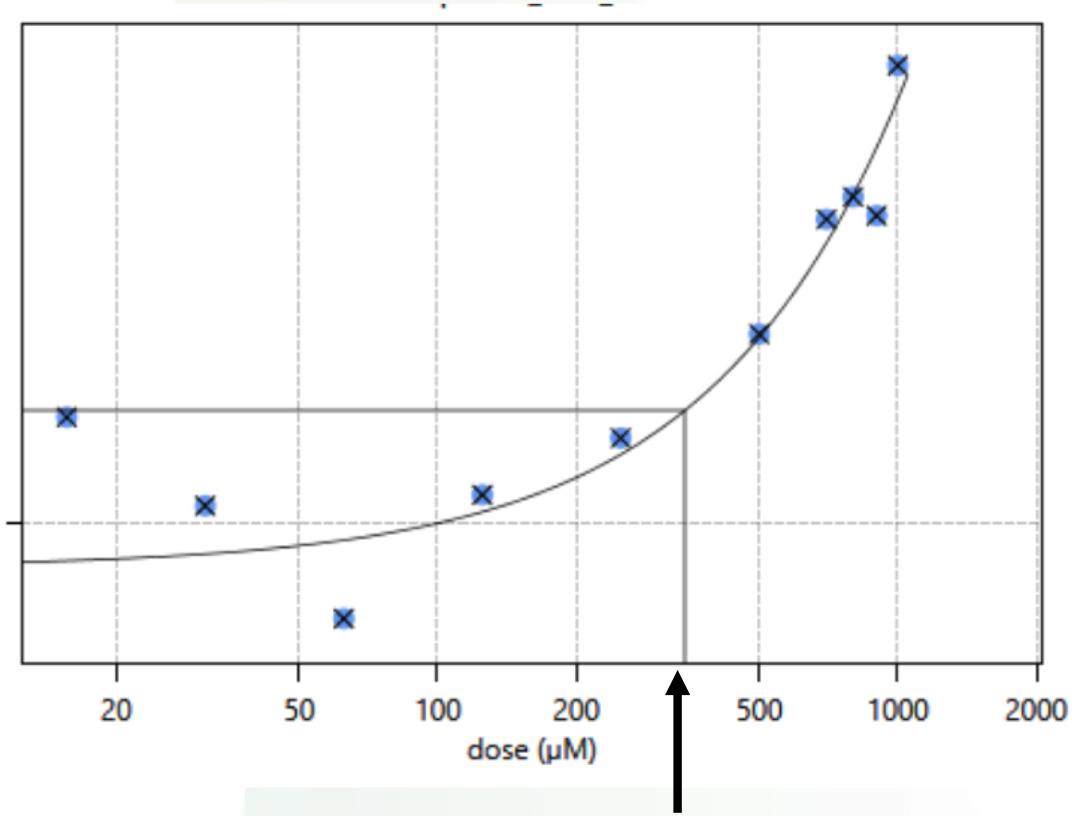
Benchmark dose response modelling

Benchmark dose software Proast integrated into EuroMix toolbox

Benchmark response (BMR)



Benchmark dose (BMD)

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Benchmark dose response modelling

Two scenarios for RPFs using Benchmark dose method:

- Purpose to derive BMDs to calculate RPFs for a group of substances, but the BMDs will not be used as a POD for the risk assessment

  BMR selected anywhere on dose response curve

- Purpose to derive BMDs to be used as PODs in a risk assessment, e.g. for index substance

  BMR should be chosen to reflect a no effect level, according to the EFSA guidance

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Index substance

Criteria for selection of index substance

- confidence that the substance is representative for the specific assessment group
- confidence that the substance causes the effect that is the basis for the risk assessment
- the POD is derived from an *in vivo* study for the effect in focus for the mixture risk assessment
- quality and quantity of toxicity data, resulting in a high confidence in the POD

The index substance does not have to be the most toxic substance (i.e. lowest POD) in the assessment group

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Selection of point of departure (POD)

Several PODs available from several studies measuring same or different responses

- Comparability within the assessment group
  - Same response for all substances
- Responses from different KEs in the AOP network
  - Relevance of response, downstream in AOP
- Several PODs for same response
  - Most reliable, conservative, overall POD
- Selection of POD in the EuroMix toolbox
  - Lowest or mean

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In vitro to in vivo extrapolation (IVIVE)

- RPFs calculated using in vitro data, at the level of the in vitro system, the cell
- Dietary exposure data is expressed as external exposure, intake
- In vitro to in vivo extrapolation (IVIVE) is needed to use the in vitro RPFs in the dietary exposure assessment
- Two options to extrapolate are implemented in the EuroMix toolbox:
  - Inverse dosimetry using absorption factors
  - Inverse dosimetry using PBK models
- In both options is the in vitro BMD for each substance multiplied by a factor to extrapolate to the external BMD

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Imputation of missing point of departure data

POD based on Munro collection of TTC values

- Database of NOAEL values compiled by Munro et al 1996 can be used
- NOAELs can be divided into Cramer classes
- 5th percentile of the NOAELs in each class can be calculated and used as a conservative estimate of NOAEL
- Mean value or uncertainty sampling of all values also possible

POD based on existing PODs in the assessment group

- 5th percentile, mean or uncertainty sampling of all values

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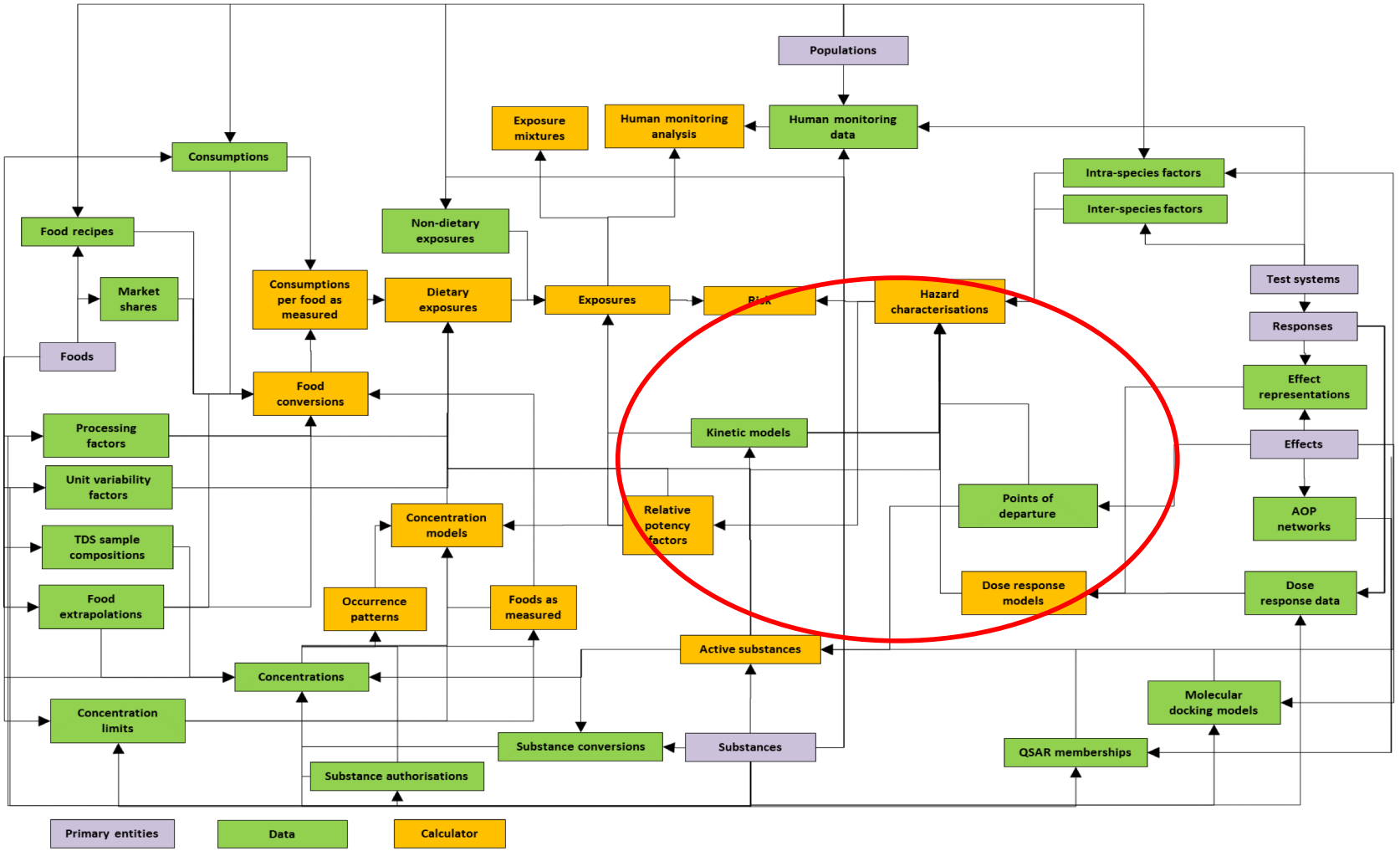
EuroMix toolbox-modules

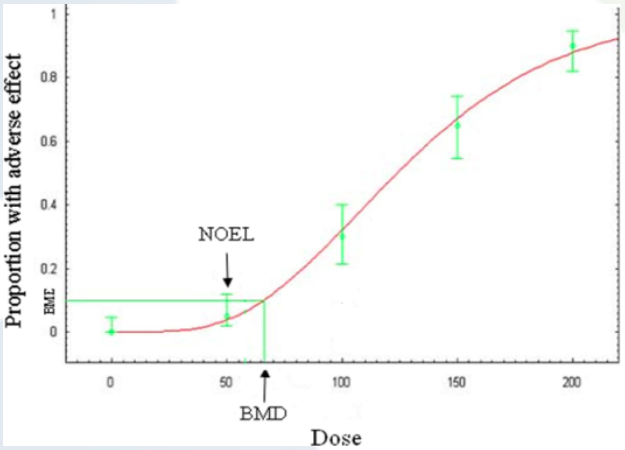


Mixture testing

test substances

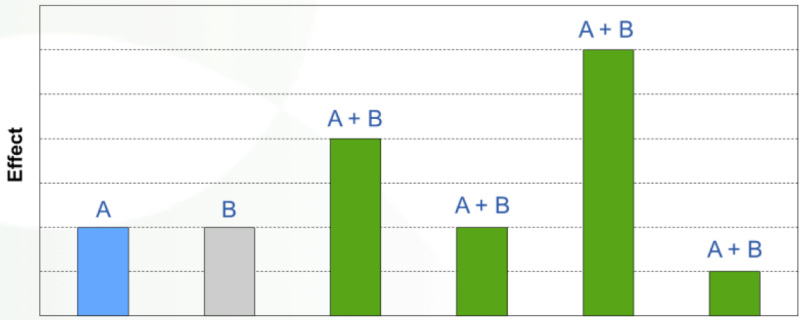
single substances

- dose response curves
- Relative Potency Factors (RPF)



binary mixtures

- equipotent mixtures
- dose additivity?
- interactions?



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Mixture testing

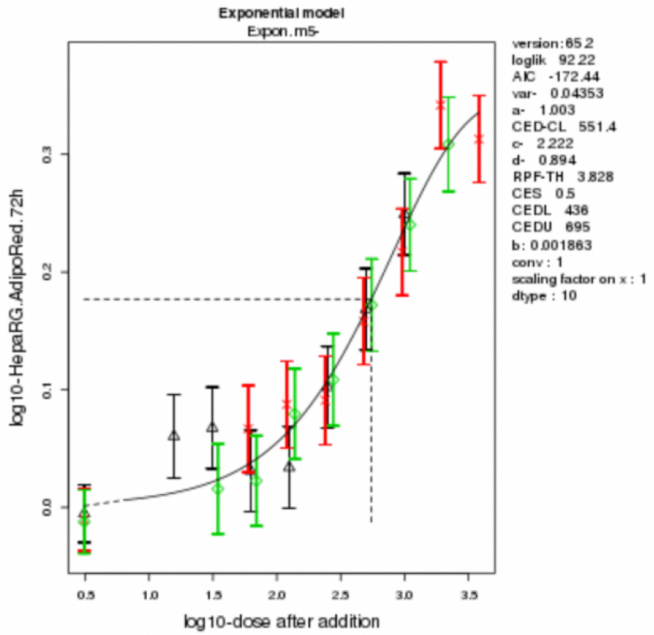
EuroMix

Methodology

- Equal potency of substances
- RPFs of individual substances needed
- Several doses of individual substances and binary mixture
- Results analysed using benchmark dose method

Substance 1 RPF=1	Substance 2 RPF=5
Dose in e.g. µM	
0	
1	
2	
4	
8	
16	
32	
	0
	1/5=0.2
	2/5=0.4
	4/5=0.8
	8/5=1.6
	16/5=3.2
	32/5=6.4
0	0
1/2=0.5	1/5/2=0.1
2/2=1	2/5/2=0.2
4/2=2	4/5/2=0.4
8/2=4	8/5/2=0.8
16/2=8	16/5/2=1.6
32/2=16	32/5/2=3.2

Black triangles and red crosses: single substances Green diamonds: mixture



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EuroMix handbook – sections (2)

- **Exposure assessment**
  - Probabilistic exposure assessment
  - Absence of measured concentration data
  - Non-dietary exposure assessment
- Risk characterisation
  - Dose addition
  - Margin of exposure
  - Selection of main mixtures based on exposure and hazard data
- Tiering approaches
- Uncertainty analysis
- References
- Glossary

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Probabilistic dietary exposure assessment

Probabilistic exposure assessment in EuroMix toolbox is based on previous MCRA tool and in line with EFSA guidance

Distribution of exposure and quantification of uncertainty

Acute - effect caused by a short time or single exposure

Chronic - effect caused by a longer time exposure

Food consumption data from consumption surveys

Concentration data from measurement of levels of substances in food

Conversion of food-as-eaten to foods-as-measured

Processing factors

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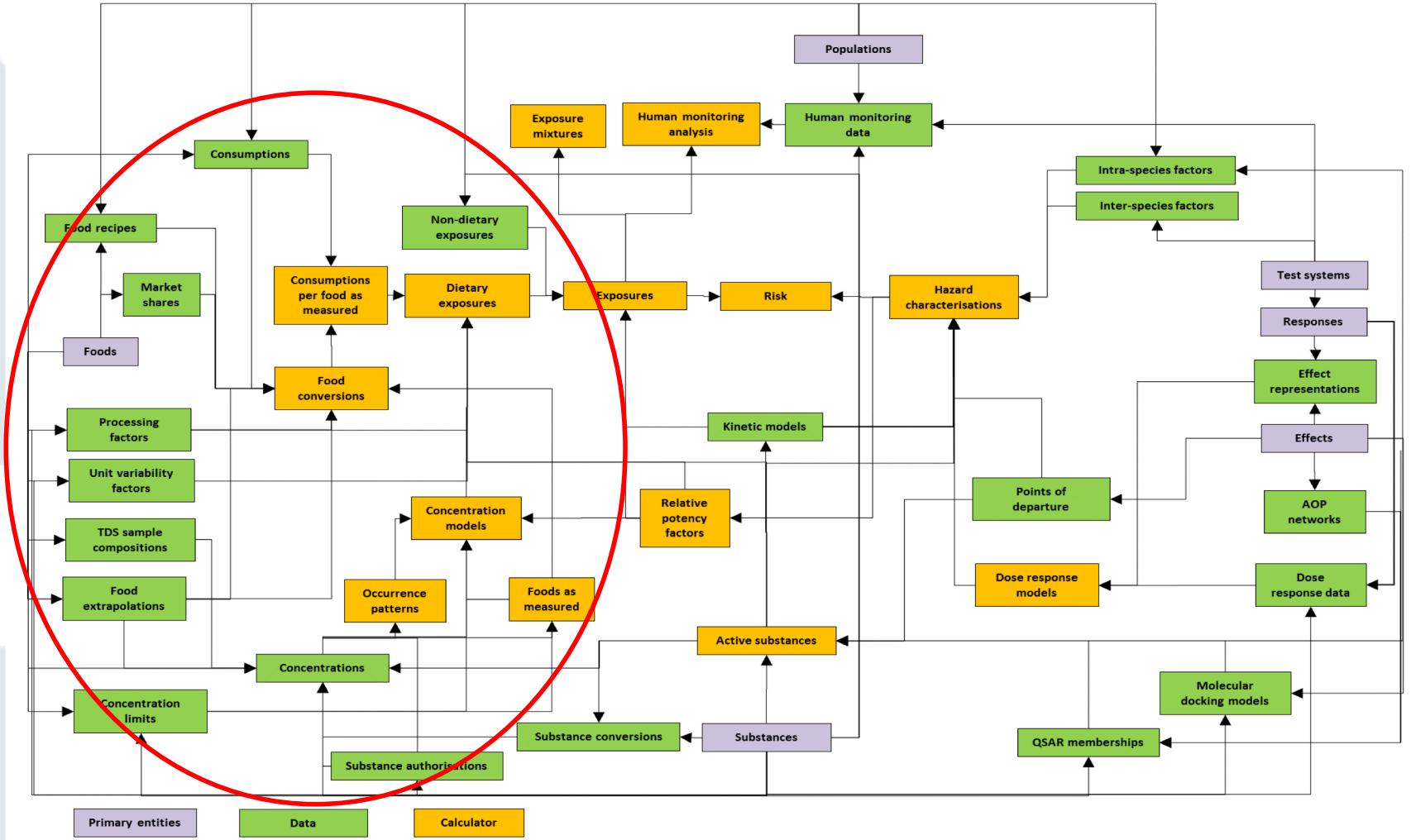
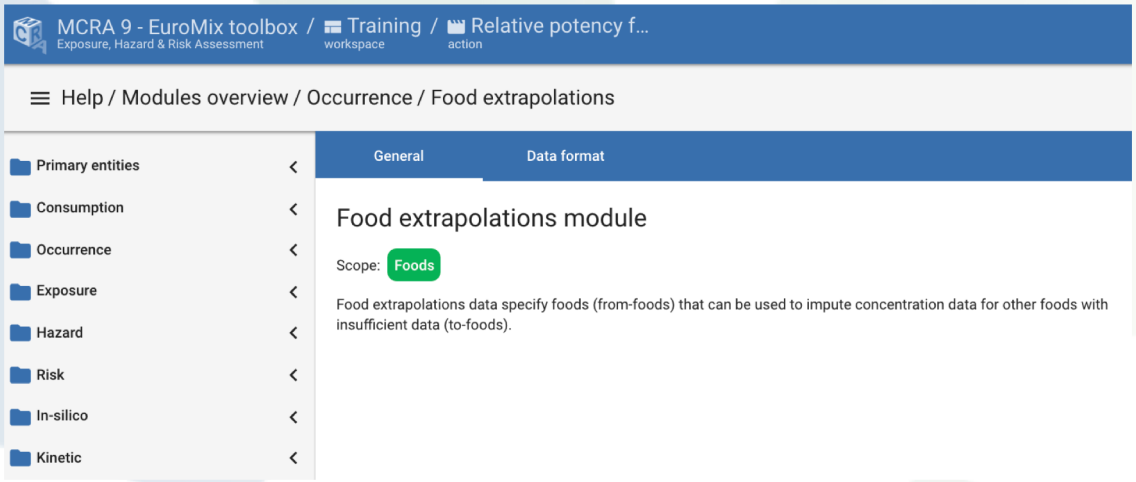
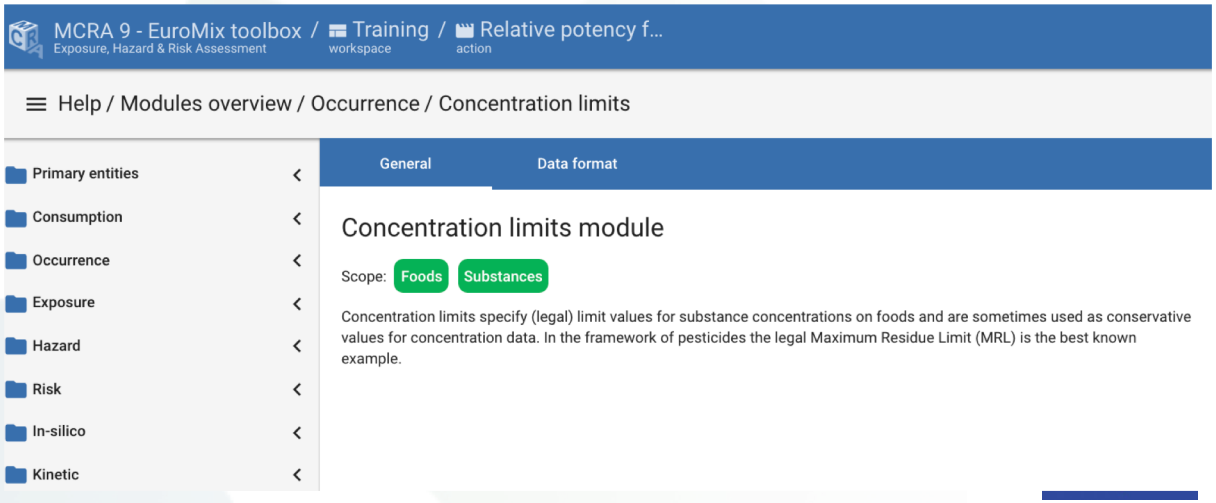
EuroMix toolbox-modules



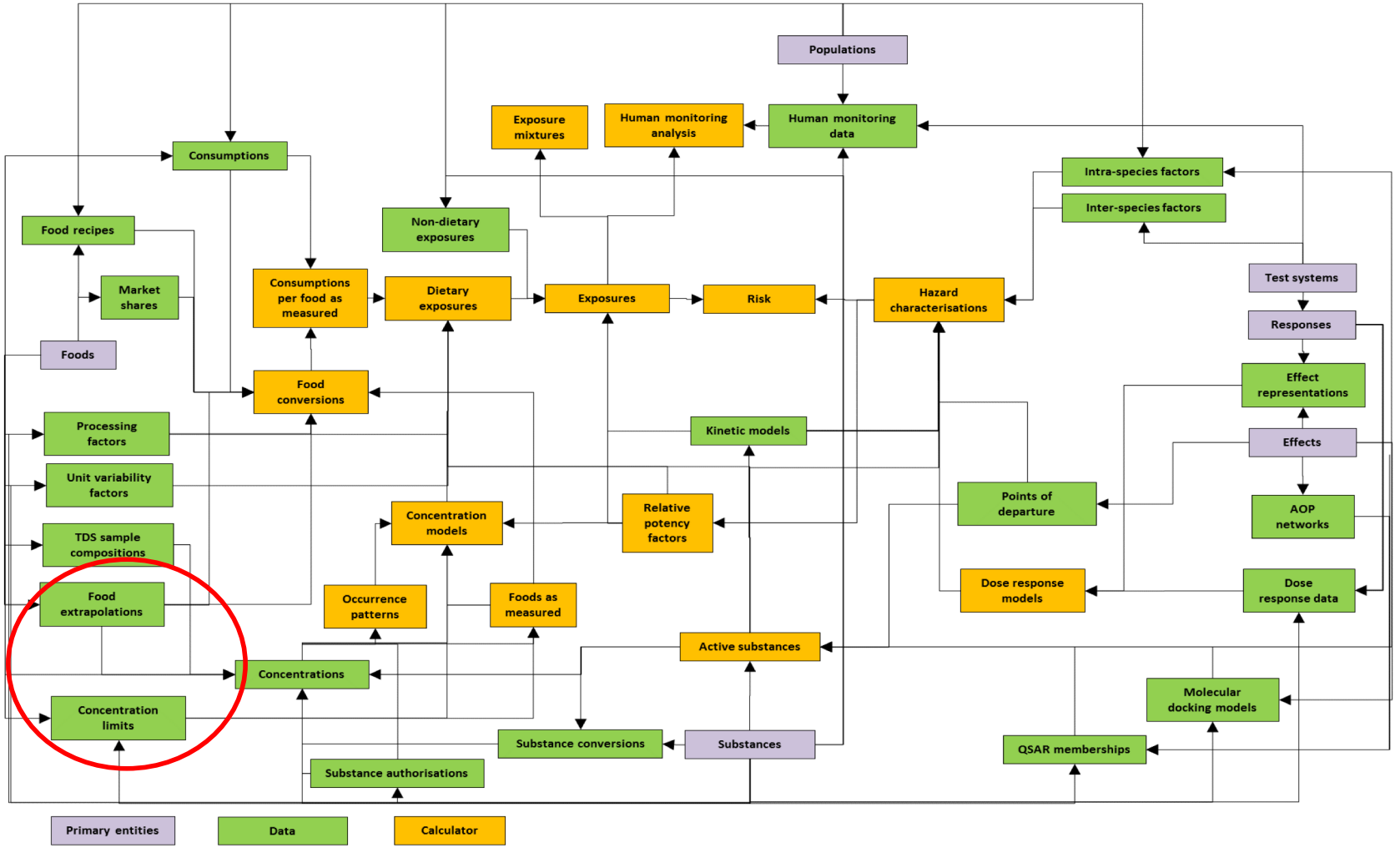
Absence of measured concentration data

- Extrapolation from other foods
- Use of legal limits in food





EuroMix toolbox-modules



Non-dietary exposure assessment

- Model non-dietary exposures in external programmes
- Import non-dietary exposure into EuroMix toolbox
- Combine with dietary exposure

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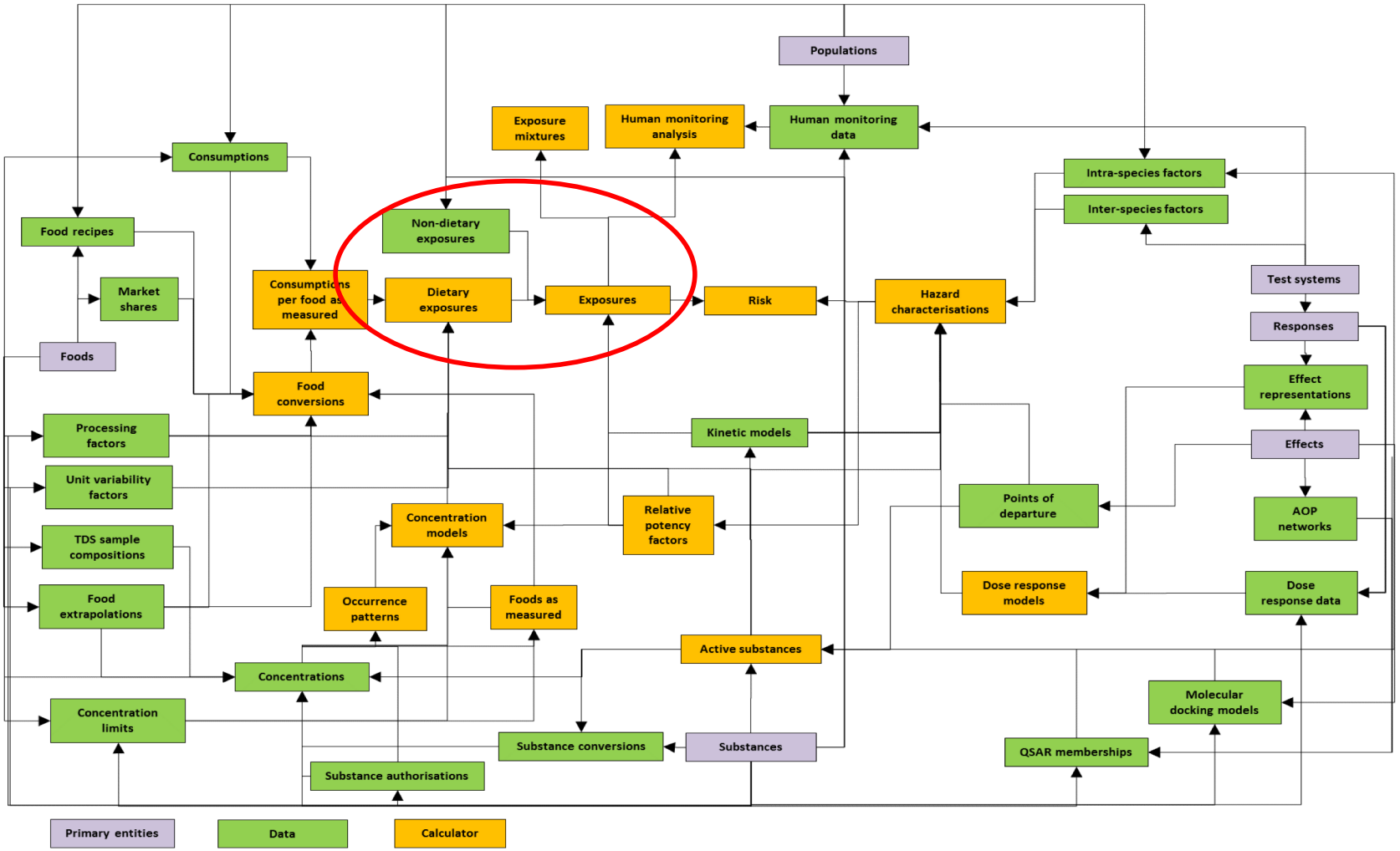
EuroMix toolbox-modules



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EuroMix handbook – sections (2)

- Exposure assessment
  - Probabilistic exposure assessment
  - Absence of measured concentration data
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- **Risk characterisation**
  - Dose addition
  - Margin of exposure
  - Selection of main mixtures based on exposure and hazard data
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- Glossary

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Risk characterisation

Dose addition default model

Margin of exposure (MOE)

$MOE = POD_{index}/Exp_{mix}$

MOE > assessment factors, combined risk of mixture is usually considered acceptable

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Margin of exposure-example

Action settings | Sub-action results | Dietary exposures

- Exposures by food
- Exposures by substance
- Exposures by food and substance
- Observed individual means
  - Graph total
  - Graph upper tail
  - Percentiles

Reference: Flusilazole, PoD = 530 µg/kg bw/day

Mean exposure: 0,292 (µg/kg bw/day)

Percentage	Exposure (µg/kg bw/day)	Percentage of PoD (%)	Margin of exposure
50.00	0.1249	0.02	4242
90.00	0.7777	0.15	681.5
95.00	1.087	0.21	487.5
99.00	1.914	0.36	277
99.90	3.058	0.58	173.3
99.99	4.423	0.83	119.8

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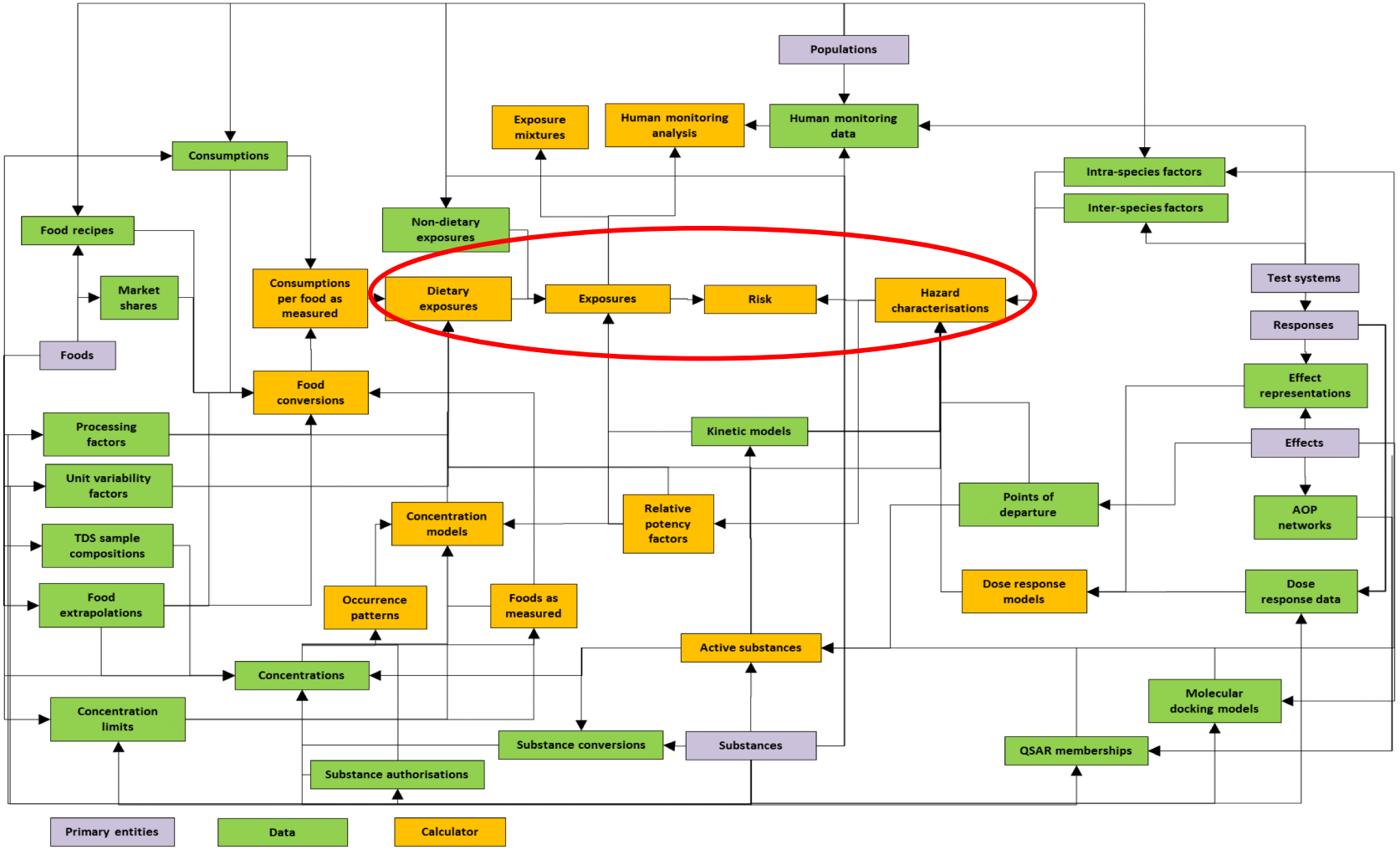
EuroMix toolbox-modules



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Selection of main mixtures

- Selection of most common mixtures based on food consumption patterns, concentration data and RPFs
- Statistical method Sparse Nonnegative Matrix Underapproximation (SNMU)

- Can be used for prioritisation for refinement and mixture testing

Table 2
Characteristics of the exposure estimates (mean, median, P5 and P95 in µg/kg bw/day), SNMU weights and contributions to the total exposure for the main mixture following the four scenarios in each country.

	Name compound	RPF	Belgium (BE)						Czech Republic (CZ)						Cyprus (CY)					
			SNMU weight	Contrib.	Mean	Median	P5	P95	SNMU weight	Contrib.	Mean	Median	P5	P95	SNMU weight	Contrib.	Mean	Median	P5	P95
			1356 individuals. Variance: 75.6%						1666 individuals. Variance: 63.7%.											
Scenario 1 (Adults, chronic, merged)	Imazalil	0.13	85%	44%	0.98	0.22	0	3.80	65%	31%	0.41	0.09	0.002	1.1						
	Dithiocarbamates	0.53	13%	39%	0.22	0.17	0.02	0.53	25%	39%	0.13	0.09	0.016	0.35						
	Carbendazim and benomyl	0.2	1%	2%	0.03	0.02	0.002	0.10	2%	4%	0.03	0.02	0.003	0.08						
	Cypermethrin	0.28	1%	4%	0.04	0.03	0.01	0.09	3%	8%	0.05	0.04	0.013	0.12						
	Triadimefon and triadimenol	0.59							2%	4%	0.01	0.002	0	0.06						
	Thiacloprid	0.44							2%	4%	0.01	0.005	0.001	0.06						
	Deltamethrin	0.53							1%	4%	0.01	0.008	0.002	0.04						
			1356 individuals. Variance: 95.9%						756 individuals. Variance: 99.3%.											

Crépet et al 2019. Int J Hyg Environ Health. 222:291-306

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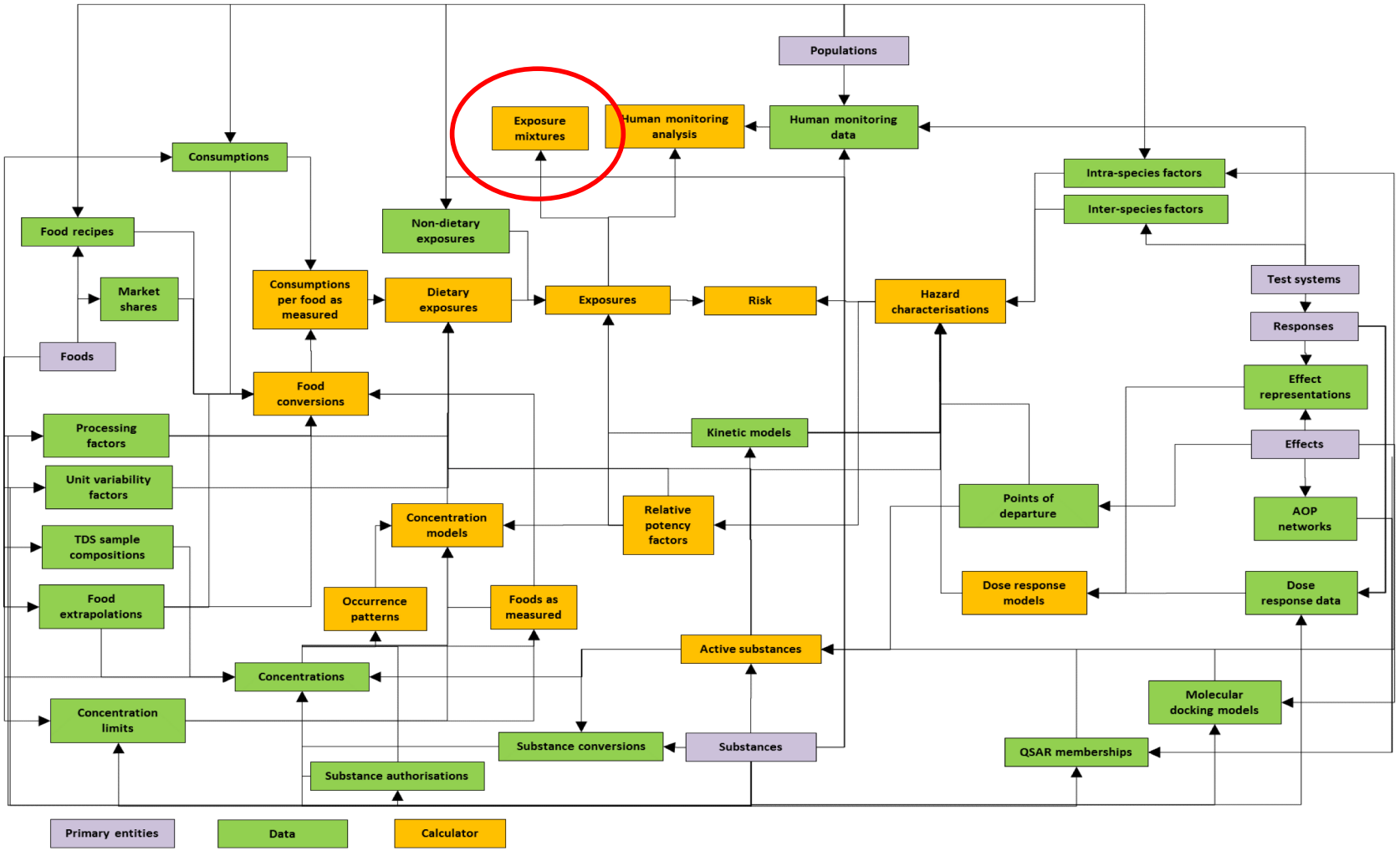
EuroMix toolbox-modules



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Tiering approches

Conservative approach at low tier

If MOE is sufficiently protective, refinement and higher tiers are not needed

Different tiers are available in the EuroMix methodology and toolbox

Step in assessment	Possibilities for tiering
Hazard assessment	
Grouping into assessment groups	At lower tier all substances that have a common target organ can be grouped forming a large assessment group and at higher tiers substances can be grouped based on a common effects/adverse outcome (see section 5.4)
Potency of substances	At lower tiers can the lowest POD for the substances in the group be used for all substances and at higher tiers can substance-specific PODs used (see section 5.5)
Effect used for RPFs	At lower tiers can the critical effect (lowest POD for substance for any effect) be used and at higher tiers can the specific effect that is the basis for grouping be used (see section 5.5)
Missing toxicity data	At lower tiers can Munro NOAELs be used and at higher tiers can new toxicity data generated (see section 5.5.6)
Exposure assessment	
Consumption data	At lower tiers can physiological limits of consumption be used and at higher tiers individual data from consumption surveys (see section 6.1)
Concentration data	At lower tiers can maximum permitted levels be used and at higher tiers data from representative monitoring studies (see section 6.1)
Missing concentration data	At lower tiers can maximum permitted levels be used and at higher tiers can new concentration data generated (see section 6.2)

Uncertainty analysis

Uncertainties related to the different steps the mixture risk assessment

- Identify and describe uncertainties
- Quantify uncertainties if possible

Template for uncertainty analysis

EuroMix toolbox provides many possibilities for uncertainty analysis

Aspect	Identified uncertainties	Analysis of uncertainty (qualitative or quantitative)
Grouping of substances into assessment groups		
Choice of toxicity data to derive POD		
Calculation of RPFs		
Extrapolation between *in vitro* and *in vivo* studies		
Lack of toxicity data		
Consumption data		
Concentration data		
Non-detects, concentration measurements below the limit of detection		
Lack of concentration data		
Conversion of food-as-eaten to food-as-measured and processing factors		
Other (non-dietary) routes of exposure		
Use of the dose addition model		
Possible interactions (synergism or antagonism)		

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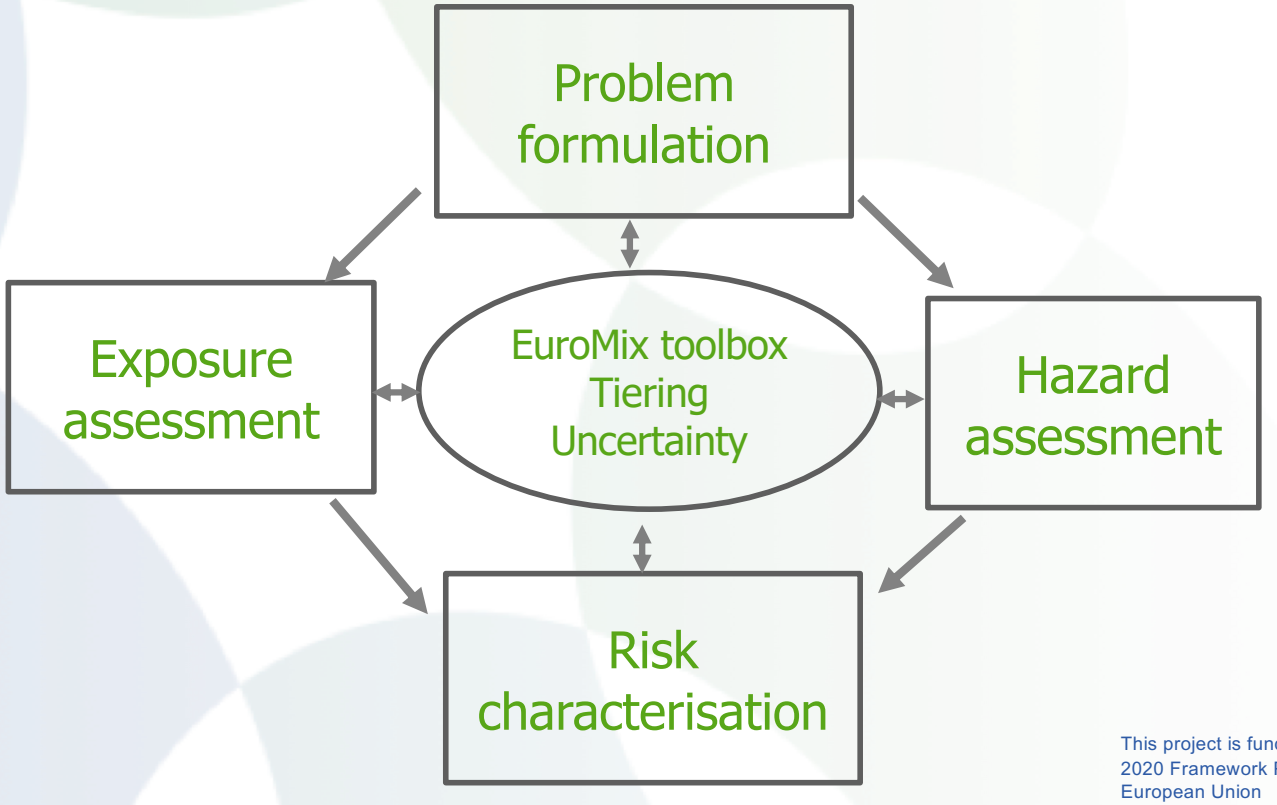
Conclusions

- EuroMix handbook and EuroMix toolbox provides practical support to perform mixture risk assessment and testing of substances in line with recent OECD and EFSA guidance



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EuroMix participants

22 beneficiaries from 16 countries linked to international organisations including WHO, FAO and EFSA.
EuroMix is coordinated by RIVM.