



# SPRINT

SUSTAINABLE PLANT PROTECTION TRANSITION

## WP4.1 ECOTOXICOLOGICAL ASSESSMENT



universidade  
de aveiro

Nelson Abrantes, University of Aveiro

**MUNI**

Paula Tourinho, Masaryk University



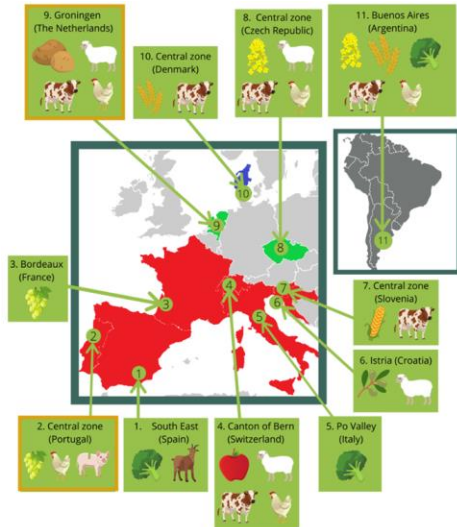
Funded by  
the European Union

# Main objective:

Assess the effects of  
**plant protection  
product (PPP)  
mixtures**  
on aquatic and  
terrestrial ecosystems



# Ecotoxicity testing strategy



**11** pesticide mixtures  
(in 11 case study sites)

combination  
of **5**  
pesticides



ELSEVIER

Contents lists available at ScienceDirect

Science of the Total Environment

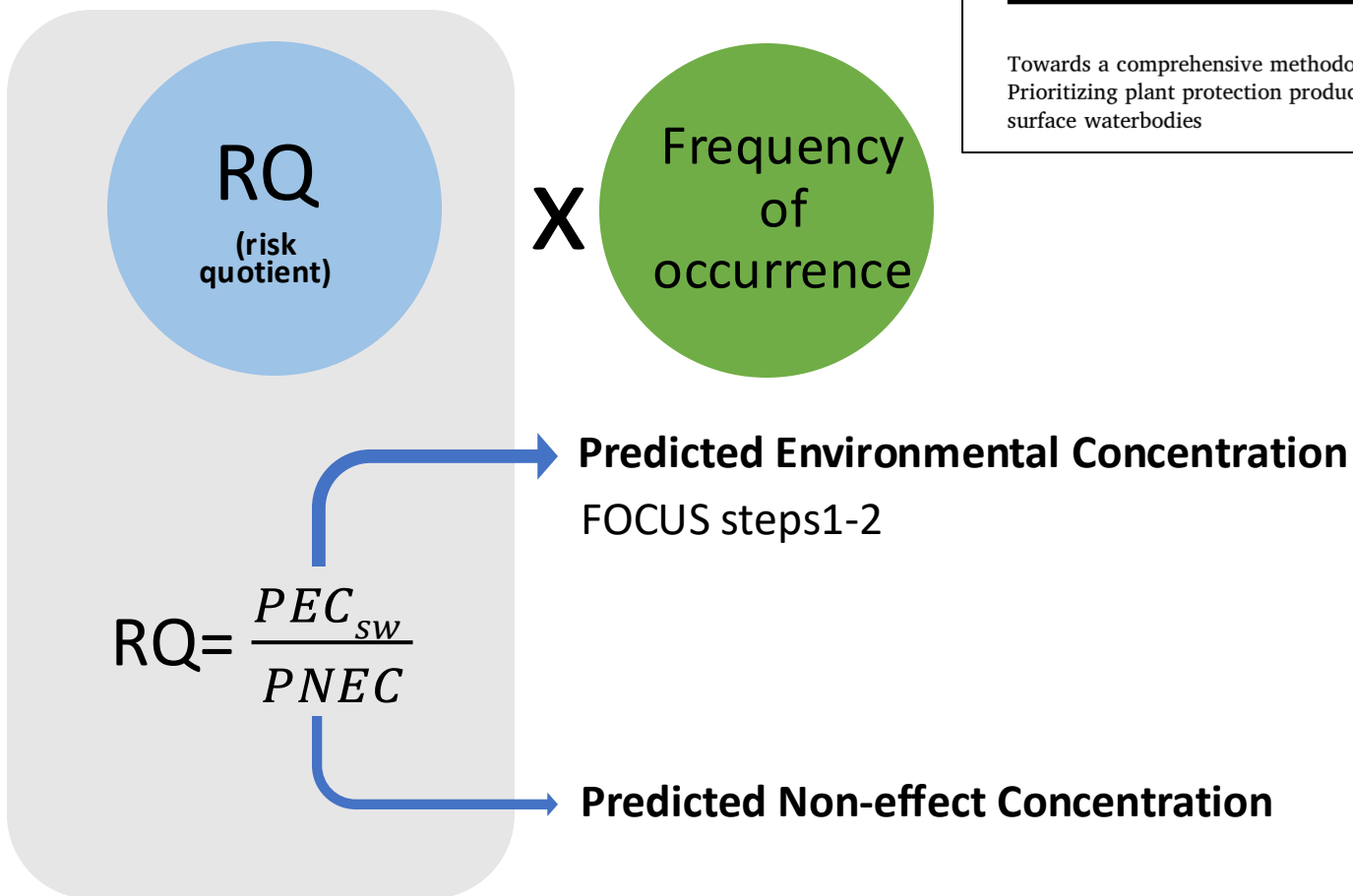
journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)



Towards a comprehensive methodology for ecotoxicological assessment:  
Prioritizing plant protection products for mixture testing in edge-of-field  
surface waterbodies

Selection of  
the TOP 5  
PPPs

For each pesticide:





Selection of  
the TOP 5  
PPPs

For each pesticide:

Soil invertebrates and  
microbes

RQ  
(risk  
quotient)

$$RQ = \frac{PEC_{soil}}{PNEC}$$

Bees and beneficial  
insects

RQ  
(risk  
quotient)

$$RQ = \frac{Applic.rate}{LD50/LR50}$$

X

Frequency  
of  
occurrence

Prioritization of currently used pesticides in soils of main European cropping systems and an Argentinian cropping system for assessment of mixture toxicity and risk on terrestrial biota

Olukayode Jegede <sup>a</sup>✉, Paula S. Tourinho <sup>b</sup>✉, Violette Geissen <sup>a</sup>, Jakub Hofman <sup>b</sup>

# MIXTURE COMPOSITION

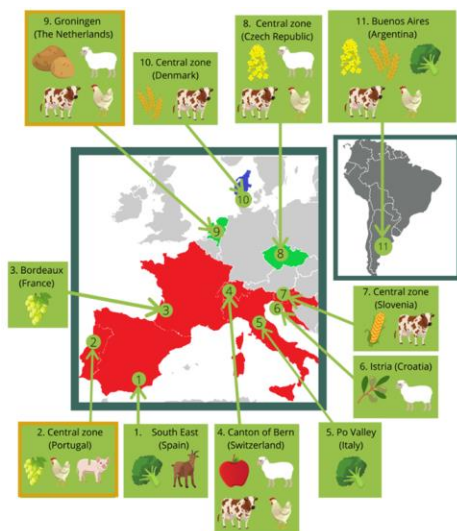


- Herbicides
- Fungicides
- Insecticides
- Metabolites

CSS	AQUATIC SPECIES
ES	Chlorantraniliprole
	Fludioxonil
	Azoxystrobin
	Fluopicolide
	Acetamiprid
PT	Terbuthylazine
	Dimethomorph
	Ethofumesate
	Tebuconazole
	Glyphosate
FR	Chlorantraniliprole
	Zoxamide
	Fluopicolide
	Trifloxystrobin
	Fluopyram
CH	Terbuthylazine
	Ethofumesate
	Tebuconazole
	Iprovalicarb
	Glyphosate
IT	Terbuthylazine
	Azoxystrobin
	Glyphosate
	Spinetoram
	Acetamiprid
HR	Terbuthylazine
	Glyphosate
	Trifloxystrobin metabolite CGA 321113
SL	AMPA
	Terbuthylazine
	Azoxystrobin
	Fludioxonil
	Mecoprop (P)
CZ	Glyphosate
	Terbuthylazine
	Fluopicolide
	Fludioxonil
	Fenhexamid
NL	Tebuconazole
	Terbuthylazine
	Azoxystrobin
	Tebuconazole
	Dimethenamid (P)
DK	Metobromuron
	Glyphosate
	Fluopyram
	Metalaxyl Metabolite CGA
	Cyproconazole
AR	Metribuzin
	2,4-D
	HCPA
	Glyphosate

CSS	Soil invertebrates	Plants	Microbes	Bees and insects
SP	Oxyfluorfen	AMPA	Metrafenone	lambda Cyhalothrin
	Chlorantraniliprole	Glyphosate	Propamocarb (hydrochloride)	Acetamiprid
	Difenoconazole	Metazachlor	Pendimethalin	Chlorantraniliprole
	Boscalid	Propyzamide	Boscalid	Glyphosate
	Propyzamide	Difenoconazole	Difenoconazole	Propamocarb (hydrochloride)
PT	Chlorantraniliprole	Glyphosate	Metrafenone	Spirotetramat
	Boscalid	AMPA	Boscalid	Glyphosate
	Difenoconazole	Iprovalicarb	Penconazole	Spiroxamine
	AMPA	Penconazole	Fludioxonil	Tebuconazole
	Azoxystrobin	Metolachlor oxanilic	Tebuconazole	Cyprodinil
FR	Chlorantraniliprole	Glyphosate	Metrafenone	lambda Cyhalothrin
	Boscalid	AMPA	Boscalid	Trifloxystrobin
	Difenoconazole	Fenbuconazole	Dimethomorph	Glyphosate
	AMPA	Metrafenone	Glyphosate	Spiroxamine
	Cyflufenamid	Difenoconazole	AMPA	Metrafenone
SW	Difenoconazole	AMPA	Methoxyfenozide	Emamectin
	Methoxyfenozide	Difenoconazole	Tebuconazole	Pirimicarb
	Myclobutanil	Myclobutanil	AMPA	Myclobutanil
	AMPA	Emamectin	Difenoconazole	Tebuconazole
	Pirimicarb	Tebuconazole		Difenoconazole
IT	Chlorantraniliprole	Propyzamide	Metrafenone	lambda Cyhalothrin
	Oxyfluorfen	Pendimethalin	Boscalid	Pendimethalin
	Boscalid	lambda Cyhalothrin	Pendimethalin	Chlorantraniliprole
	Propyzamide	Oxyfluorfen	Propamocarb (hydrochloride)	Tebuconazole
	Difenoconazole	Difenoconazole	Tebuconazole	Boscalid
CR	Boscalid	Glyphosate	Boscalid	Phosmet
	AMPA	AMPA	Tebuconazole	Acetamiprid
	Dimethomorph	Deltamethrin	AMPA	Trifloxystrobin
	Tebuconazole	Trifloxystrobin	Dimethomorph	Glyphosate
	Glyphosate	Tebuconazole	Glyphosate	Tebuconazole
SL	Bixafen	Metolachlor (S)	Terbuthylazine	CAPTAN
	AMPA	Terbuthylazine	Tebuconazole	Terbuthylazine
	Tebuconazole	Thiencarbazone-methyl	Thiencarbazone-methyl	Bixafen
	Terbuthylazine	AMPA	AMPA	Tebuconazole
	Metolachlor (S)	Terbuthylazine desethyl		Thiencarbazone-methyl
CZ	Boscalid	AMPA	Metrafenone	lambda Cyhalothrin
	Prochloraz	Diflufenican	Boscalid	tau-Fluvalinate
	AMPA	Mecoprop P	Flupyradifurone	Acetamiprid
	Dimoxystrobin	Dimethenamid (P)	Dimoxystrobin	Flupyradifurone
	Azoxystrobin	Deltamethrin	Tebuconazole	Spiroxamine
NT	Bixafen	Metribuzin	Boscalid	lambda Cyhalothrin
	Boscalid	Metobromuron	Metobromuron	Prochloraz BTS 44595
	Azoxystrobin	AMPA	Fluoxastrobin	Esfenvalerate
	AMPA	Diflufenican	AMPA	Prosulfocarb
	Pirimicarb	Prosulfocarb	Prochloraz BTS 44595	Metobromuron
DE	Boscalid	AMPA	Boscalid	Esfenvalerate
	AMPA	Diflufenican	Pendimethalin	Glyphosate
	Diflufenican	Fluopyram	AMPA	Fluopyram
	Fluopyram	Pendimethalin		Pendimethalin
	Pendimethalin	Boscalid		Boscalid
AR	AMPA	Glyphosate	Methoxyfenozide	folpet
	methoxyfenozide	AMPA	AMPA	Glyphosate
	Azoxystrobin	Diflufenican	Glyphosate	Fluxapyroxad
	Fluxapyroxad	2,4-D (free)	Folpet	Bixafen
	Bixafen	lambda-Cyhalothrin		lambda Cyhalothrin

# Ecotoxicity testing strategy



- Control (no treatment)
- MEC (measured environmental concentration)
- PEC (predicted environmental concentration)
- 3x PEC
- 5x PEC

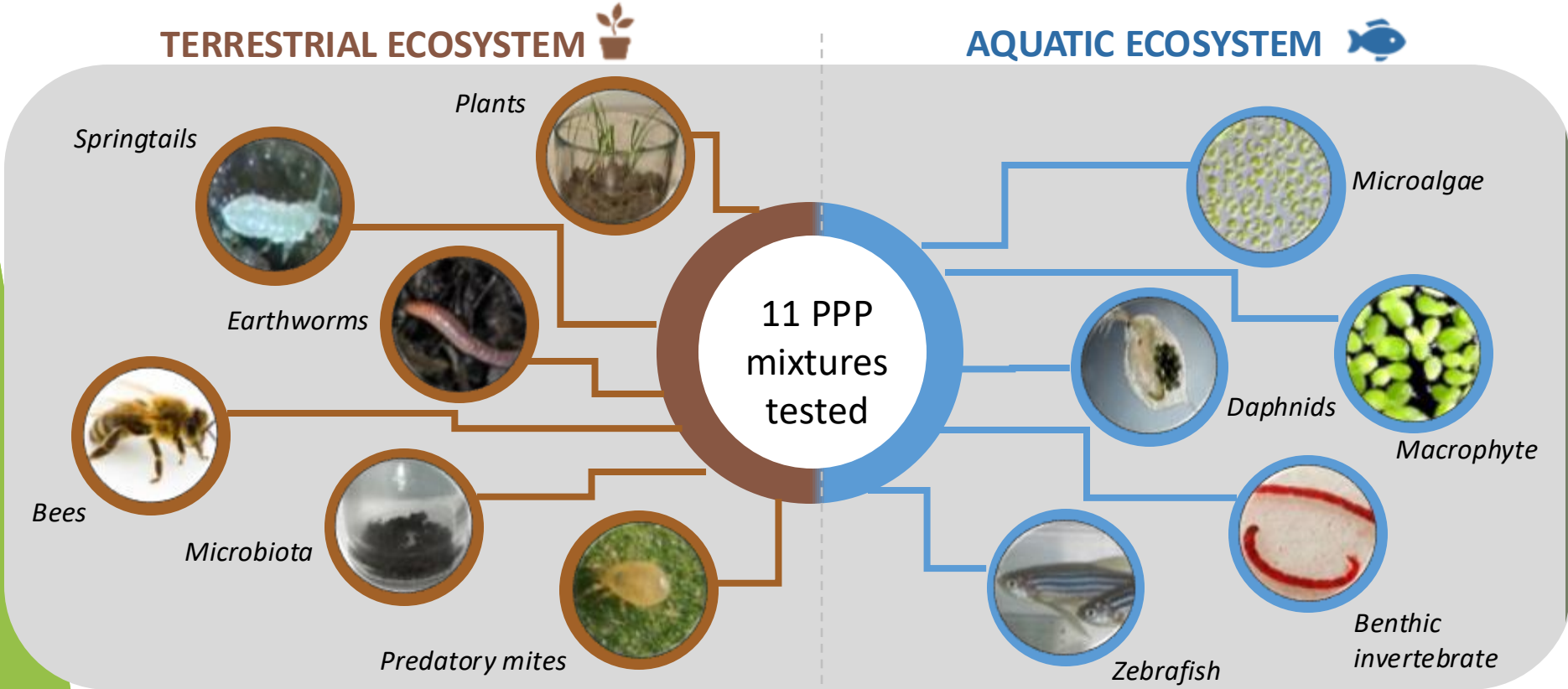
**11** pesticide mixtures  
(in 11 case study sites)

combination  
of **5**  
pesticides

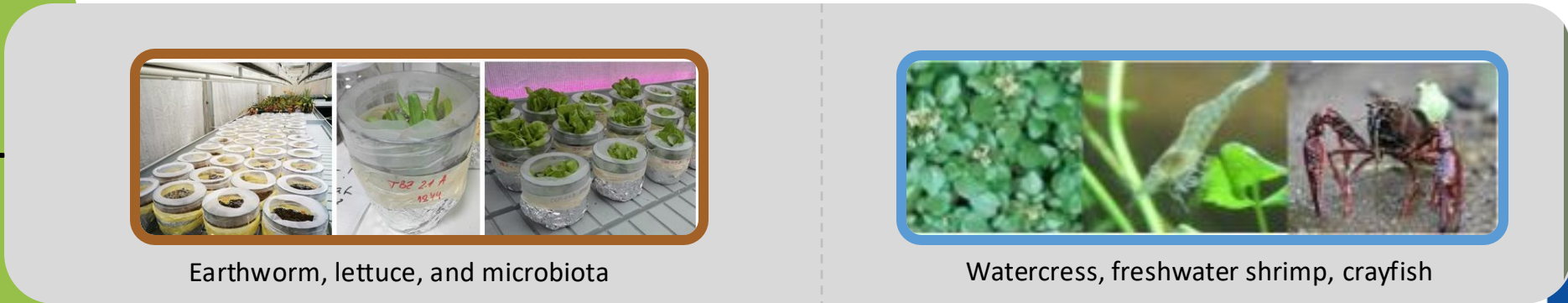
**5**  
concentrations

# Overview of the Ecotoxicological Assessment

Monospecies assays:



Multispecies:



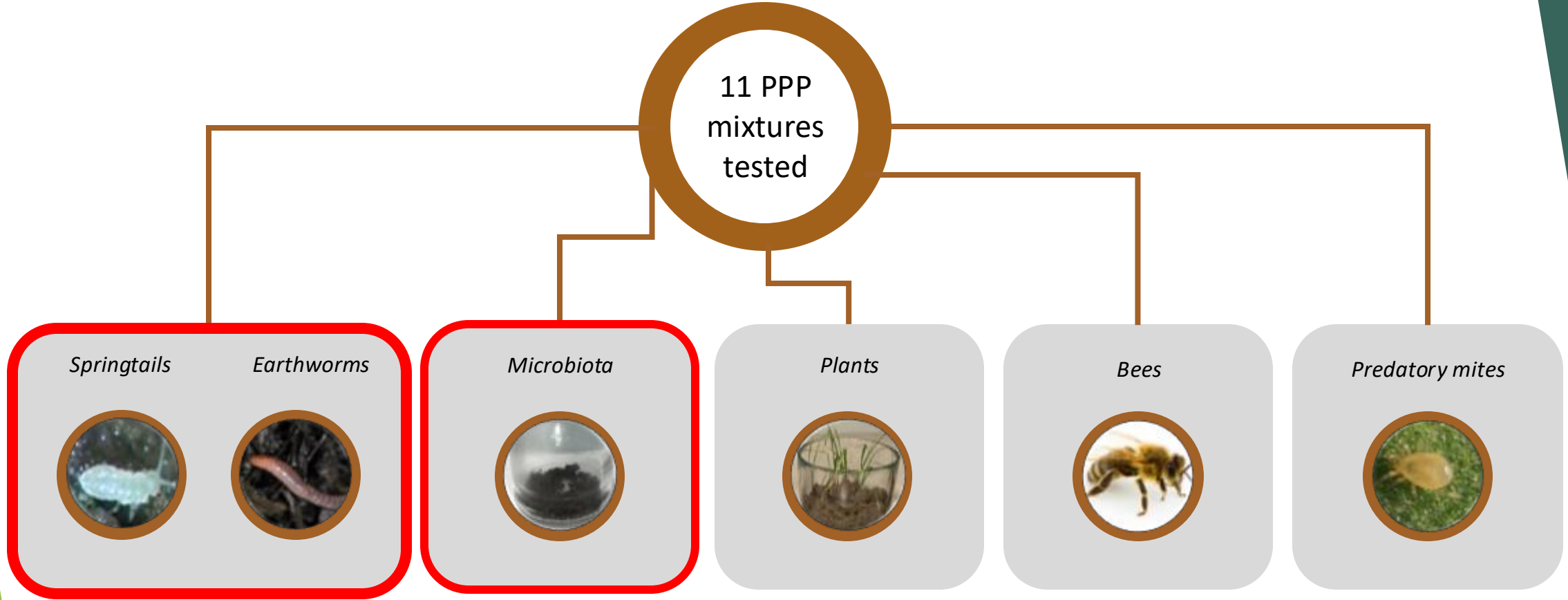
**New ecotoxicological insights on 11 realistic mixtures of PPP** and their impact on soil and aquatic species.

New ecotoxicological data on the **effects of PPP mixtures on native** soil and aquatic species.

News health indicators.

New information on the potential for PPP bioaccumulation and trophic transfer.





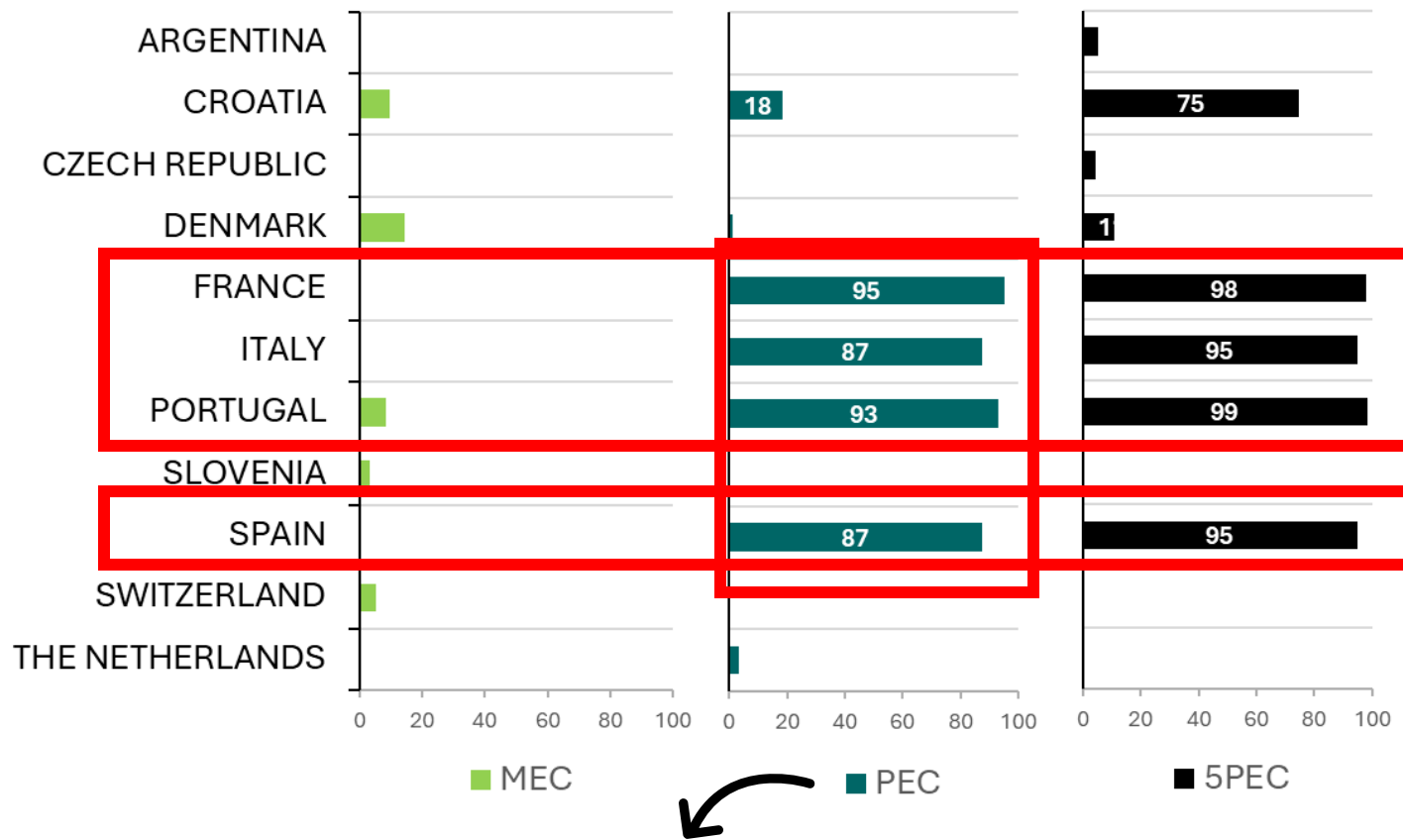
# Springtails

*Folsomia candida*



Reproduction  
(28 d)

### % of effect in each concentration



# Earthworms

*Eisenia fetida*



Reproduction  
(56 d)

### % of effect in each concentration



Concentrations  $\leq$  'Safe levels'

High toxicity

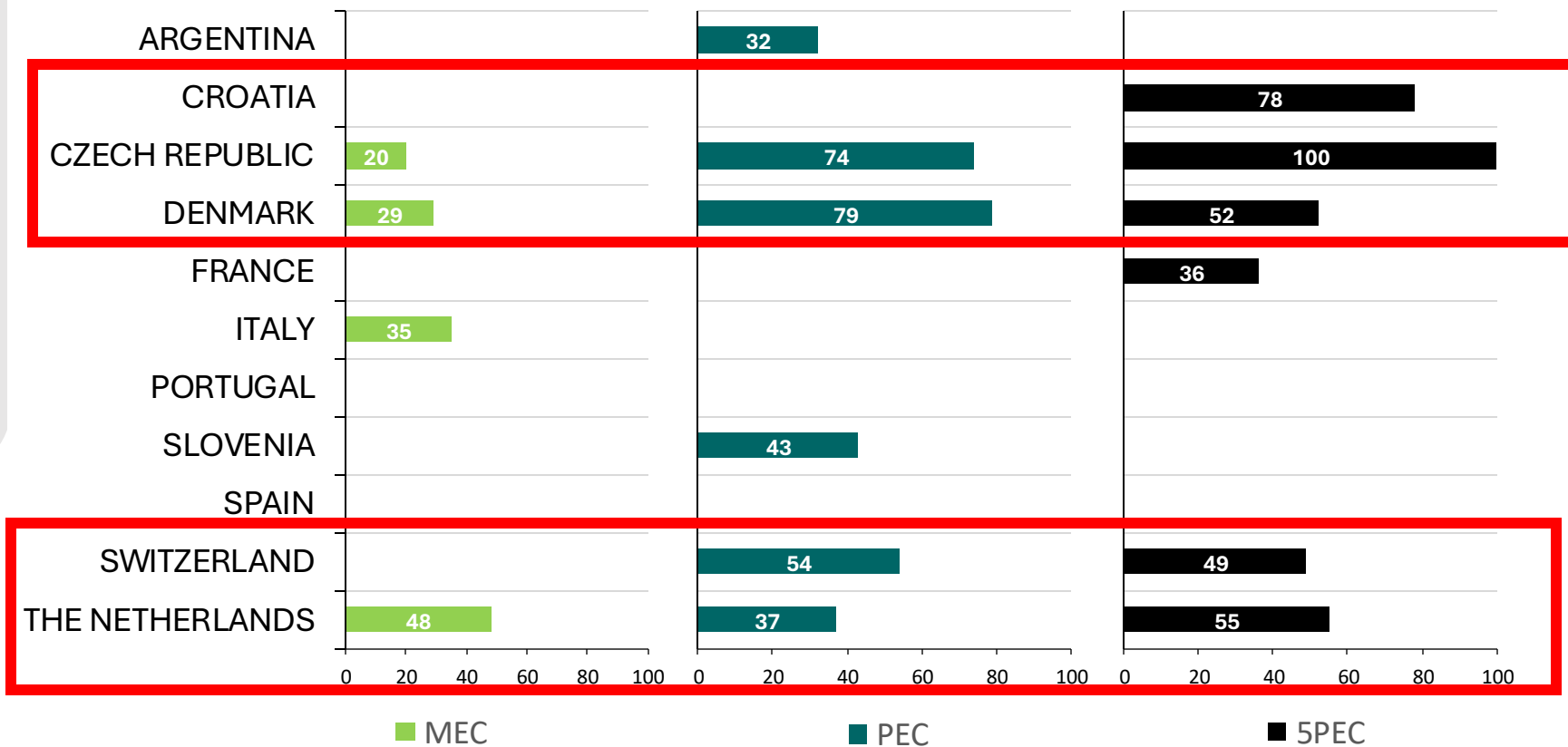
# Earthworms

*Lumbricus rubellus*



Reproduction  
(56 d)

## % of effect in each concentration



No data on 'Safe levels'

# Significant effects:



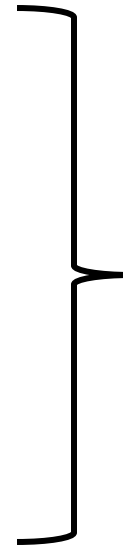
*F. Candida* → ES, FR, HR, IT, PT



*E. fetida* → AR, CZ, HR, PT, SI



*L. rubellus* → CH, CZ, DK, NL



11 mixtures

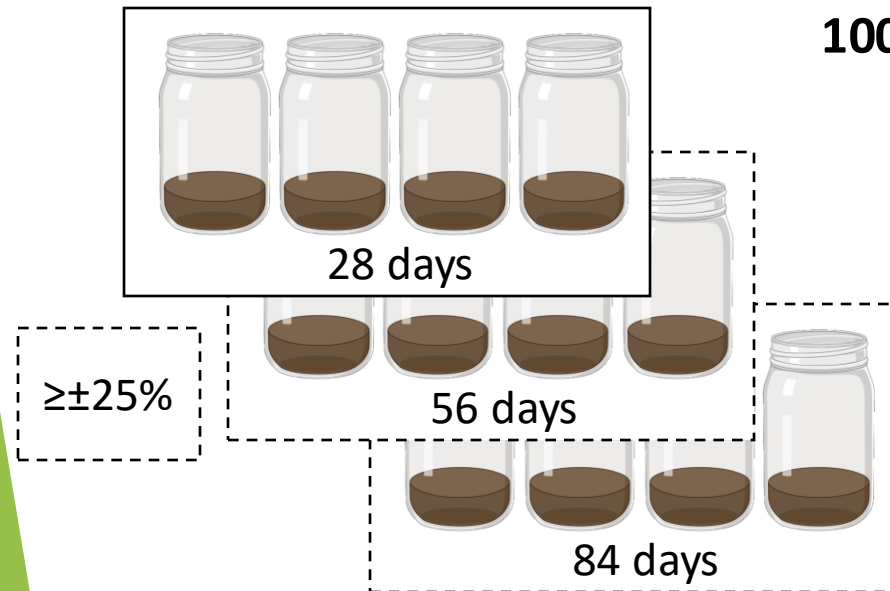
**Different mixtures**

# Soil microbes

## OECD guidelines 216 and 217

- Carbon transformation
- Nitrogen transformation

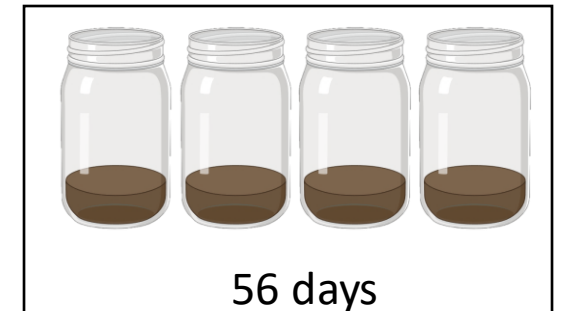
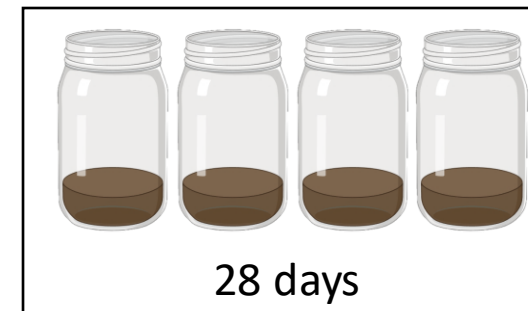
Threshold  
< 25% up to  
100 days



## Novel soil indicator

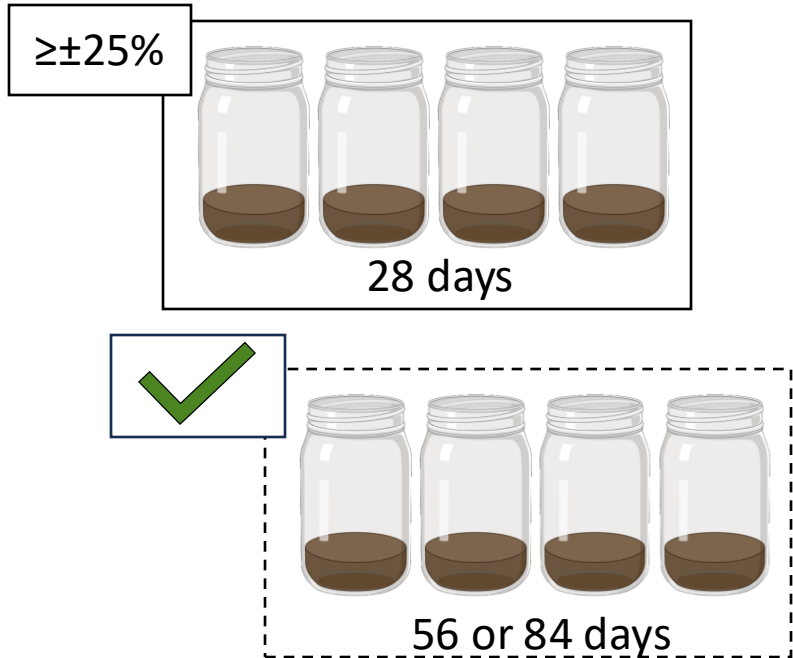
### Microbial function and structure

- Enzyme activities
- Fungal ITS fragment
- Functional genes
- DNA extraction
- 16S rRNA genes
- 16S archaea



## OECD tests

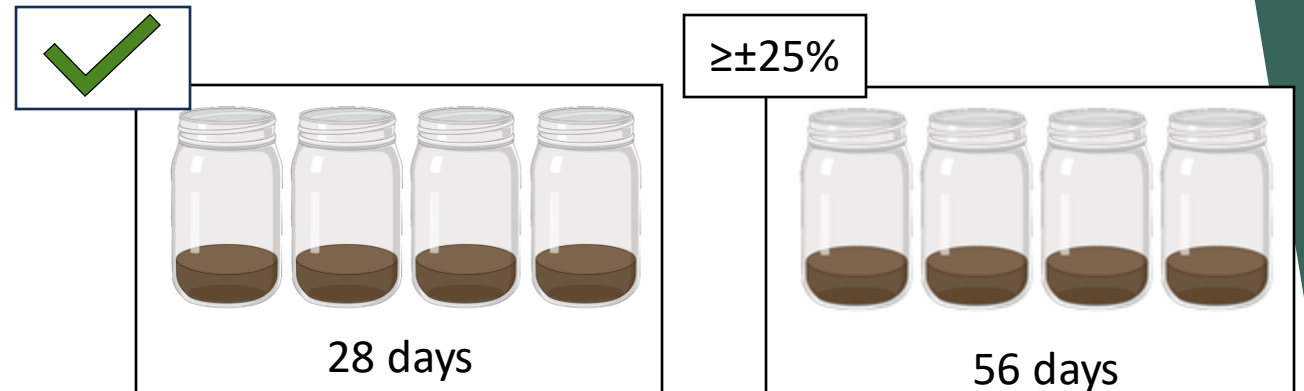
5 mixtures



 Risk assessment

## Novel soil indicator

- All mixtures → endpoints
- Effects observed only at 56 days



Risk assessment

# Conclusions

- Effects observed at lowest concentrations tested



Bees



Terrestrial plants



Predatory mites



Toxicity was higher than expected → synergistic effects

## New SPRINT indicators (non-standard tests)

Sensitivity → native species vs standard species



Prolongation of exposure period → “late” effects on microorganisms

≥±25%

