

Ecotoxicological indicators : How to assess and link pesticides' exposure and impacts in agricultural landscapes?



Colette BERTRAND

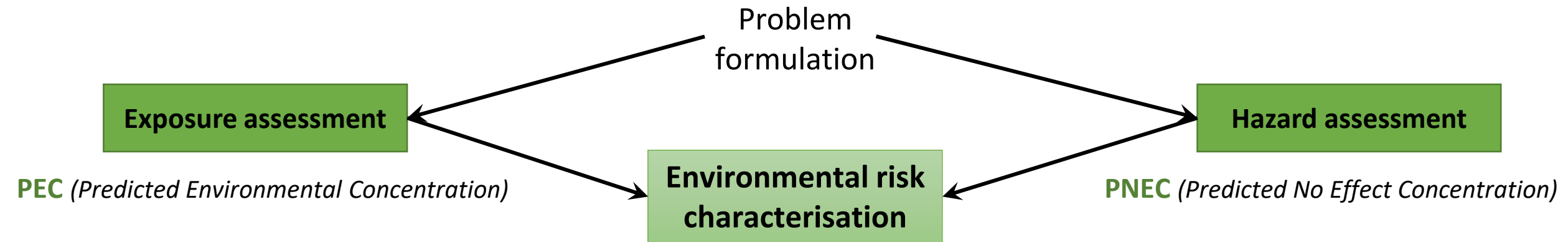
Pôle Ecotoxicologie - UMR EcoSys
INRAE

Clémentine FRITSCH

UMR Chrono-environnement
CNRS

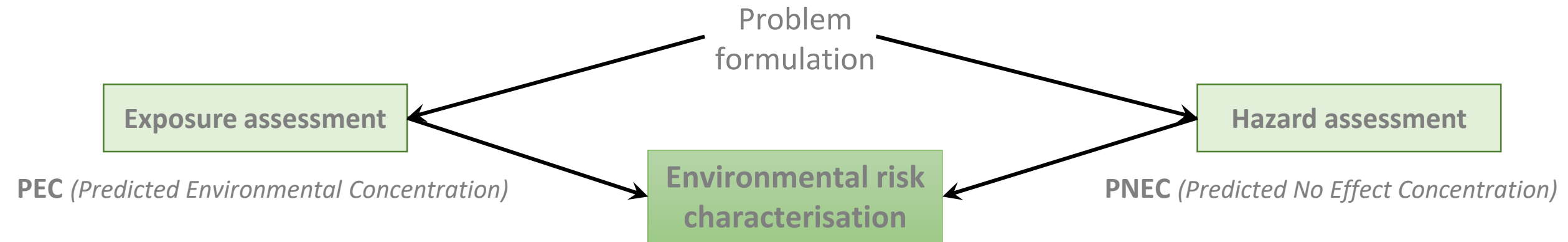
Post-registration monitoring of the environmental impact of pesticides ?

The purpose of registration is to ensure that pesticides, when used according to directions for use, will be effective for their intended purpose, while **not posing unacceptable risks** to users, consumers of treated food, and **wildlife or other non-target organisms**.



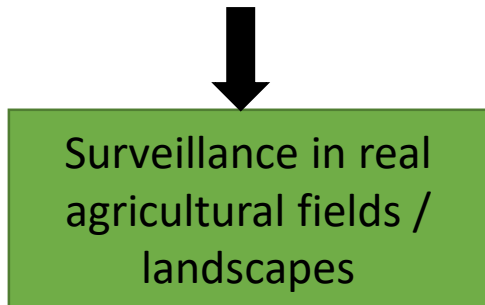
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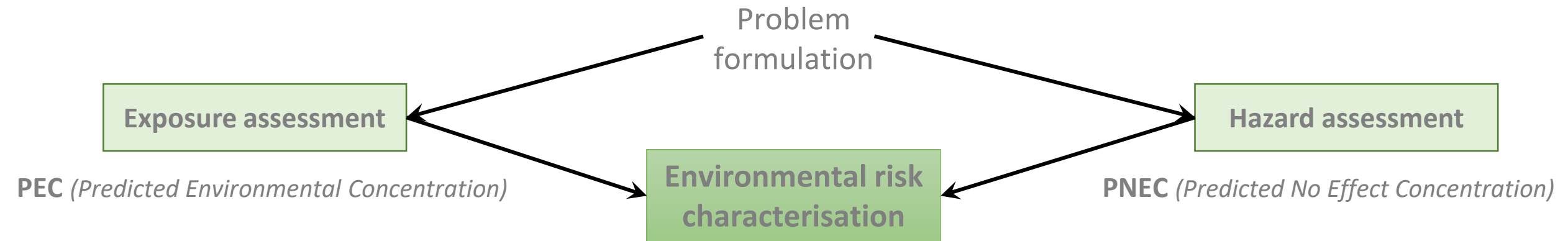
Post-registration activities provide a means of measuring the **validity of predictions based on registration data regarding **environmental effects****

If results of field surveillance raise doubts → further studies may be required or appropriate regulatory sanctions imposed



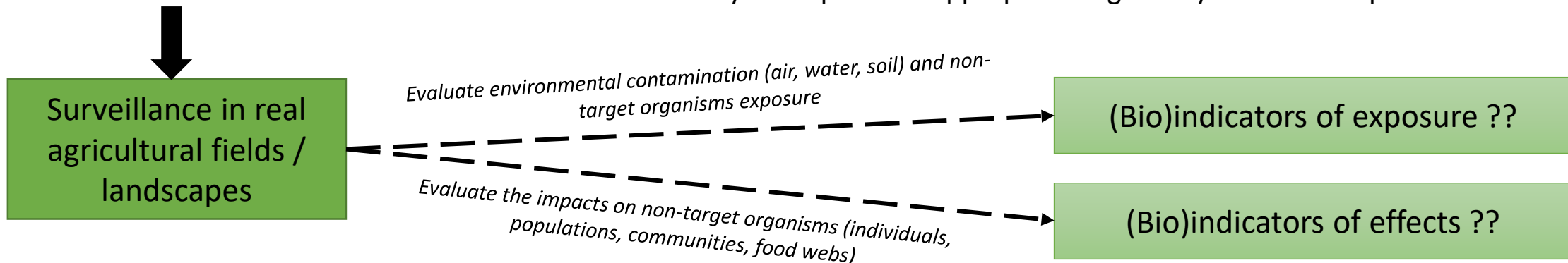
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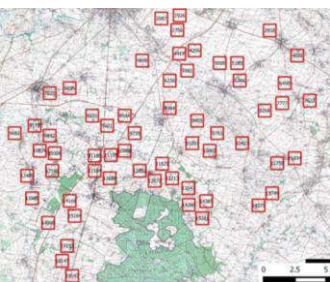
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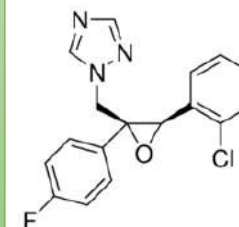
Measuring concentrations in water, air and soils

The actual risk that multiple plant protection products residues might pose to non-target species is difficult to assess due to the lack of clear evidence of their actual concentrations

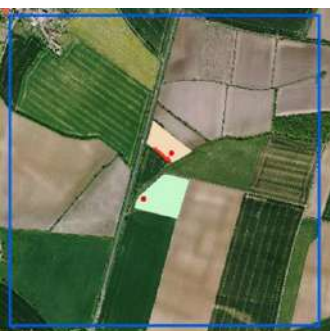
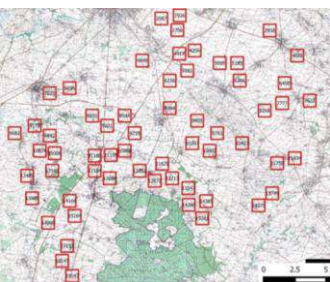
Measuring concentrations in water, air and soils



Epoxiconazole



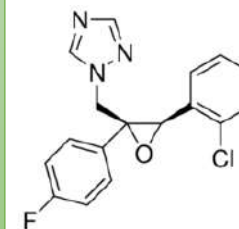
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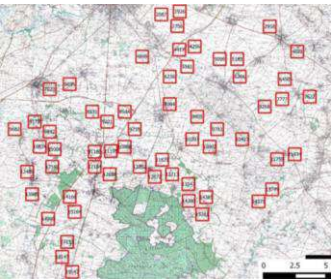
Epoxiconazole detected in 81% of the samples (145 / 180)

Concentration mean : 28 ng/g

Concentration max : 283 ng/g



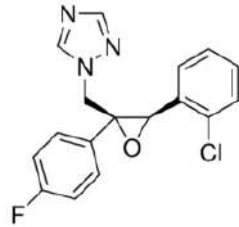
Measuring concentrations in water, air and soils



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Class	Pesticide	Recommended dose (ng/g)	PEC _{soil} initial (ng/g)	LC50 acute earthworm (ng/g)	NOEC reproduction earthworm (ng/g)
Fungicide	Epoxiconazole	153	128	> 62500	84

**MEC > PEC
for 8 soil samples**

7 cereal fields ; 1 hedgerow

**TER earthworms (NOEC / MEC)
≤5 for 52 soil samples**

*38 cereal fields (1 OF) ; 6 hedgerows ;
8 grasslands*

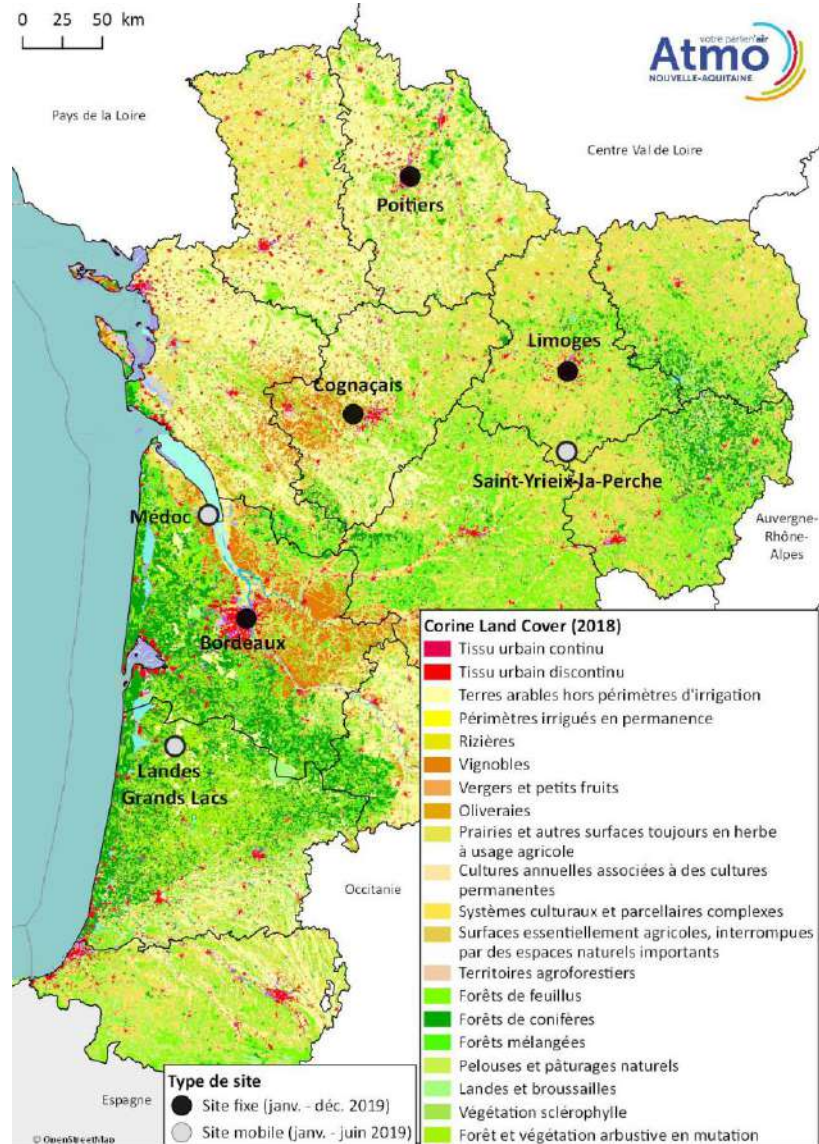
Measuring concentrations in water, air and soils

REAL ASSESSMENT OF THE ENVIRONMENTAL CONTAMINATION ?

→ Challenging due to the large number of potential contaminants

Measuring concentrations in water, air and soils

Fédération des associations
de surveillance de la
qualité de l'air



39 Herbicides
31 Fongicides
34 Insecticides

*AFNOR XPX 43-059 standard
gas chromatography or liquid chromatography + tandem mass
spectrometry*



Measuring concentrations in water, air and soils

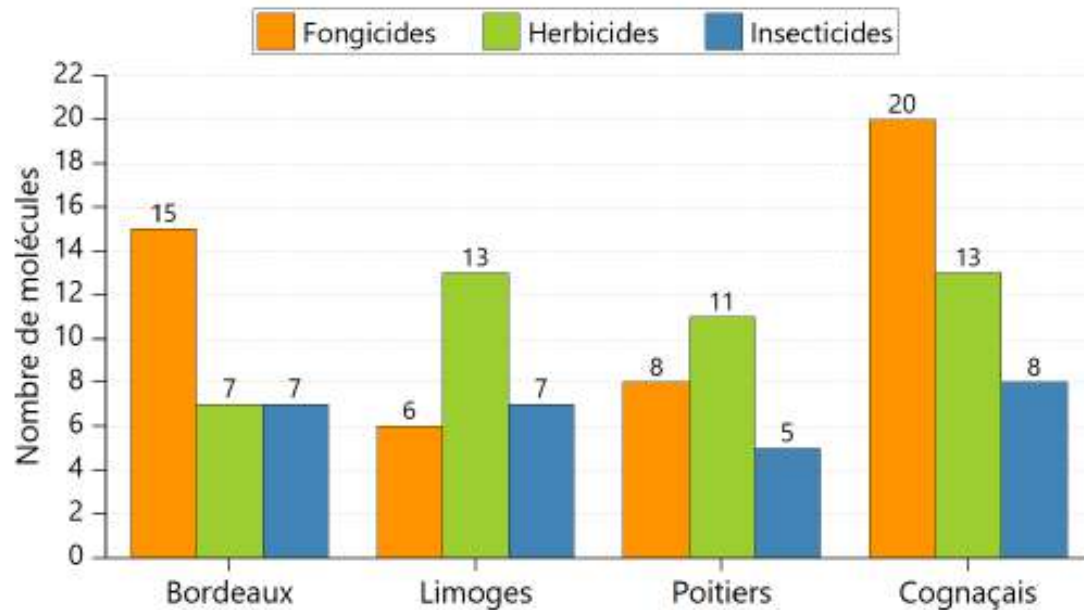


Figure 10 : Nombre de molécules détectées en 2019

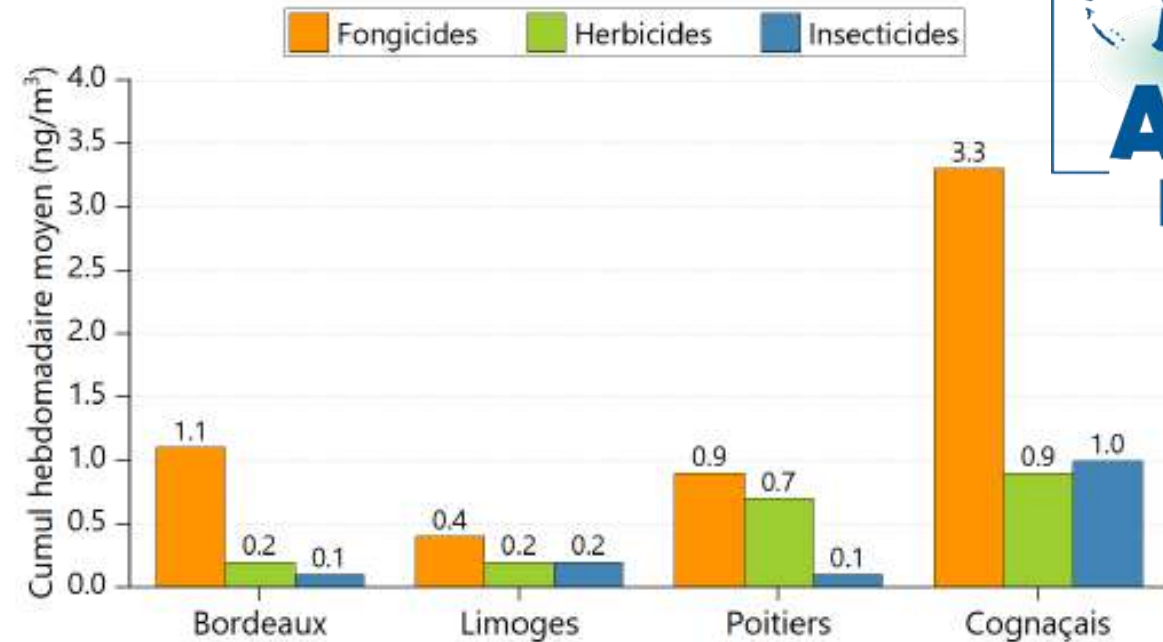


Figure 12 : Cumuls hebdomadaires moyens des concentrations en 2019

REAL ASSESSMENT OF THE ENVIRONMENTAL CONTAMINATION ?

- Challenging due to the large number of potential contaminants
- Most scientific studies and routine monitoring programs only include a “limited” number of pesticides
- Metabolites??
- But promising new analytical methods → Large pesticide multiresidue screening methods

Measuring concentrations in water, air and soils

→ Pesticide multiresidues in the environment (water + air + soil)... what to do with this data??

→ Lack of reference of the mixtures (and concentrations) of pesticides that can be found in different agropedoclimatic contexts

→ How to compare environments where the mixtures found are not the same?

→ Difficulty in estimating the risk of mixtures to biodiversity

the combined exposure to multiple chemicals can trigger stronger (or occasionally weaker) (eco)toxicological effects than exposure to individual chemicals alone

$$RQ_i = MEC / PNEC$$



$$RQ_{\text{mix}} = \sum RQ_i$$

no risk ($RQ < 0.01$), lower risk ($0.01 \leq RQ < 0.1$), moderate risk ($0.1 \leq RQ < 1$) and higher risk ($RQ \geq 1$)

Need to be able to test the effects of mixtures under real exposure conditions

Measuring concentrations in biological matrices

→ **REAL ASSESSMENT OF BIODIVERSITY EXPOSURE ?**

Take into account exposure by ingestion

Measuring concentrations in biological matrices

→ REAL ASSESSMENT OF BIODIVERSITY EXPOSURE ?

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Pollen ; Nectar ; Honey

Measuring concentrations in biological matrices

→ REAL ASSESSMENT OF BIODIVERSITY EXPOSURE ?

Take into account exposure by ingestion



Pollen ; Nectar ; Honey

Same locks as mentioned above

Lack of reference of the mixtures, difficult to compare environments with different mixtures, difficulty in estimating the risk of mixtures to biodiversity

+

**How to integrate different exposure routes
(contact / ingestion / inhalation) ?**

whose contribution is different according to the organisms considered

Measuring concentrations in biological matrices

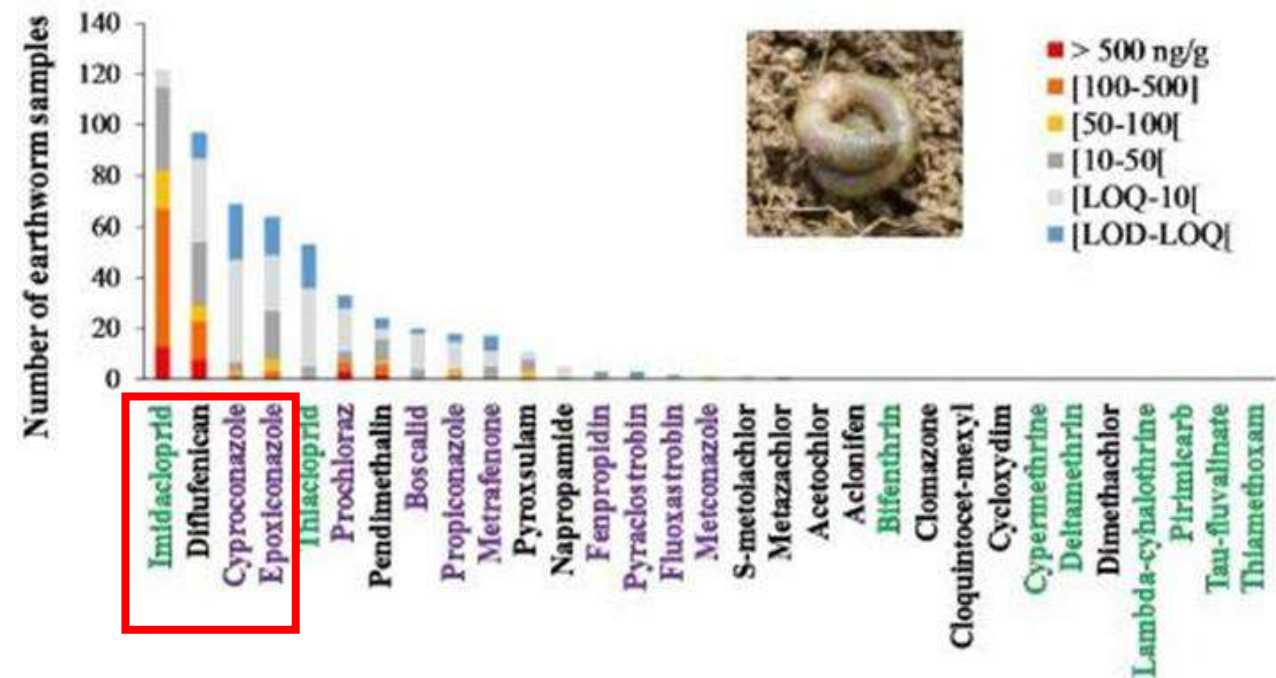
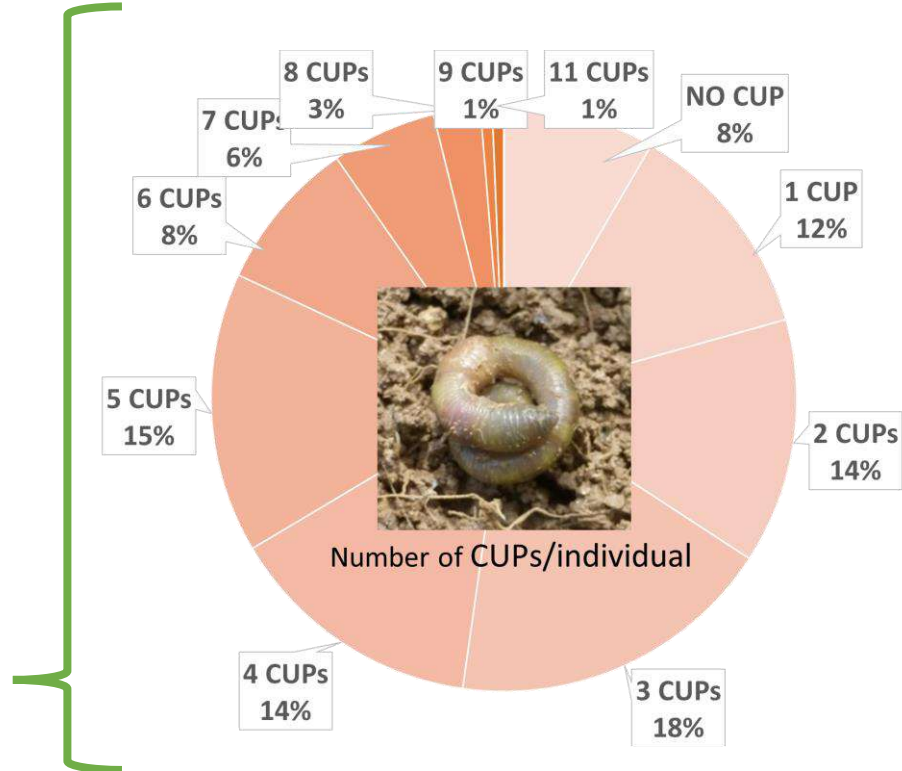
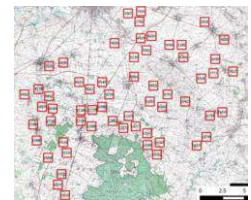
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→ Biomonitoring data can be used to quantitatively estimate internal dose or absorbed dose from all exposure routes and can be useful to provide information on co-exposure.

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→ REAL ASSESSMENT OF BIODIVERSITY EXPOSURE ?

→ Biomonitoring data can be used to quantitatively estimate internal dose or absorbed dose from all exposure routes and can be useful to provide information on co-exposure.

→ ... what to do with this data??

→ The estimation of actual exposure from biomonitoring data requires an understanding of the compound's toxicokinetics information

Need to be able to interpret this data => Link it with toxicological information

→ comparer les concentrations internes mesurées avec des valeurs de référence?

Besoin de disposer de « Toxicity reference values (TRVs) »

The 2 extremes of (regulatory) studies on effects

Laboratory Tests



Genes
Cells
Organs
Individuals

Assessment of EFFECTS of pesticides

Individuals
Populations
Communities
Functions

In situ Biomonitoring



The 2 extremes of (regulatory) studies on effects

In vivo assays

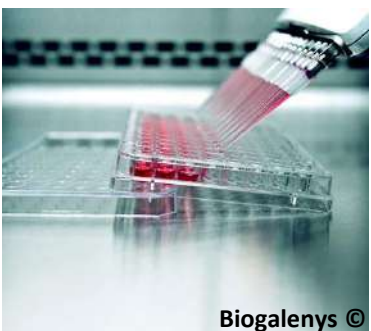


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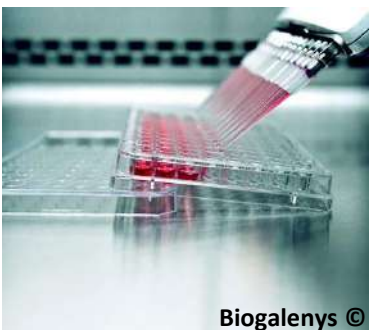
In vivo assays



Laboratory Tests



In vitro assays



- Toxicology, Toxicokinetics / toxicodynamics, Mechanisms of effects
 - **Biomarkers**
 - *genotoxicity, disorders in physiology or metabolism, endocrine disruption, teratotoxicity, pathogenicity, embryotoxicity, carcinogenicity, etc*
- **Indicators:**
 - **Toxicological thresholds**
 - *LC50/LD50, NOAEC/NOAEL, LOAEC/LOAEL*

The 2 extremes of (regulatory) studies on effects

In vivo assays



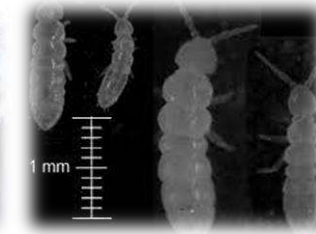
Examples:
Standardized normalized
(ISO, OECD) tests



Eisenia fetida
Source : worm-farm.co.za



Honeybees
Source : OECD

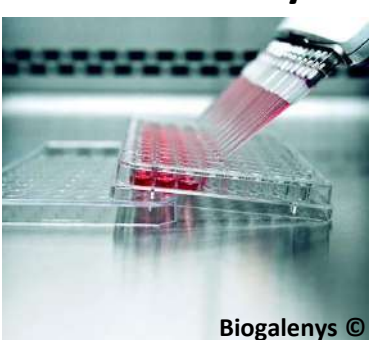


Springtails
Source : OECD

Laboratory Tests



In vitro assays



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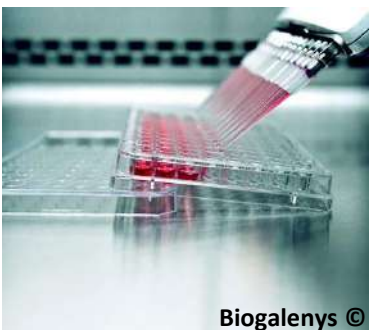
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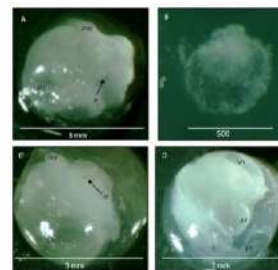


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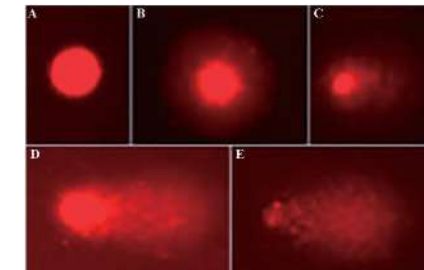
Examples:
Standardized alternative
bioassays

- Toxicology, Toxicokinetics / toxicodynamics, Mechanisms of effects
 - **Biomarkers**
 - *genotoxicity, disorders in physiology or metabolism, endocrine disruption, teratotoxicity, pathogenicity, embryotoxicity, carcinogenicity, etc*
- **Indicators:**
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*Embryotoxicity
Terrestrial
snails*

Source : Druart C.



*Comet Assay
insects*

Source : Bioone.org

The 2 extremes of (regulatory) studies on effects

Passive Biomonitoring

Individuals
Populations
Communities
Functions

Poisoning



In situ Biomonitoring



Biodiversity



The 2 extremes of (regulatory) studies on effects

■ Indicators:

• Mortality / Morbidity

➤ *Pathogenic status, health status, signs of intoxication (necropsy, residues of PPPs in tissues/GI tract), etc*

■ Toxicovigilance, Epidemiosurveillance, Phytopharmacovigilance

Passive Biomonitoring

Poisoning



***In situ* Biomonitoring**



Biodiversity



The 2 extremes of (regulatory) studies on effects

Indicators:

Mortality / Morbidity

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Example: SAGIR Network (OFB)



Poisoning



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Passive Biomonitoring

***In situ* Biomonitoring**



Biodiversity



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Passive Biomonitoring

- Biodiversity, biological conservation, ecosystem functioning/services

■ Indicators:

• Biodiversity and ecosystem functioning

- Occurrence, richness, abundance of 1 or several taxa, soil functioning, potential for biological pest control

• Population dynamics

- Temporal dynamics of some taxa population, reproduction outcomes, etc

Poisoning



In situ Biomonitoring



Biodiversity



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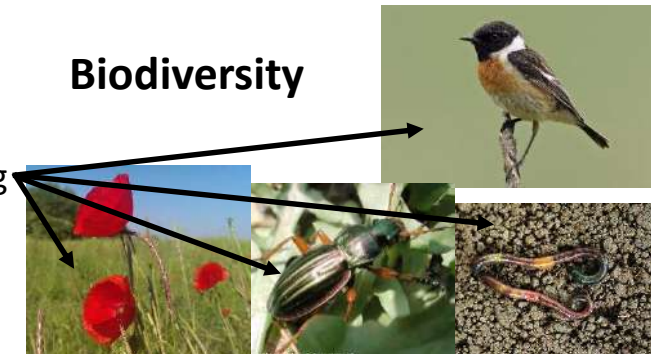
Example:

Survey of unintentional effects
(suivi ENI - ECOPHYTO plan)

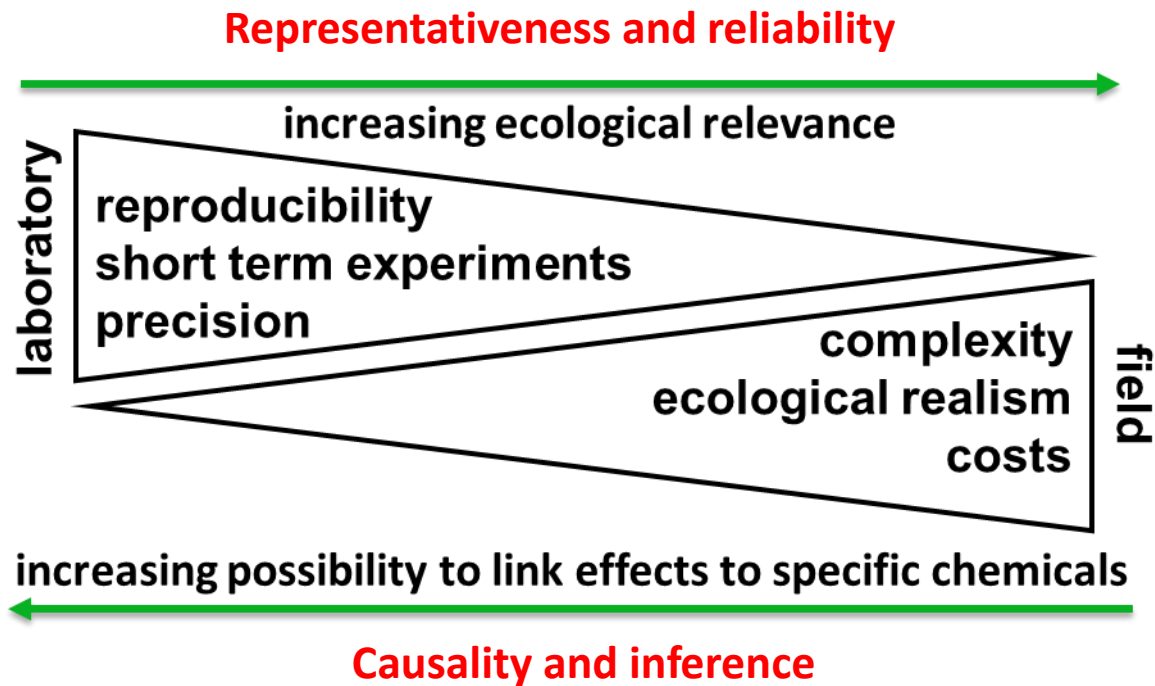


- Field monitoring of 4 taxa
- 500 plots at national scale

Biodiversity



The pros and cons of each approach



The pros and cons of each approach

Short duration

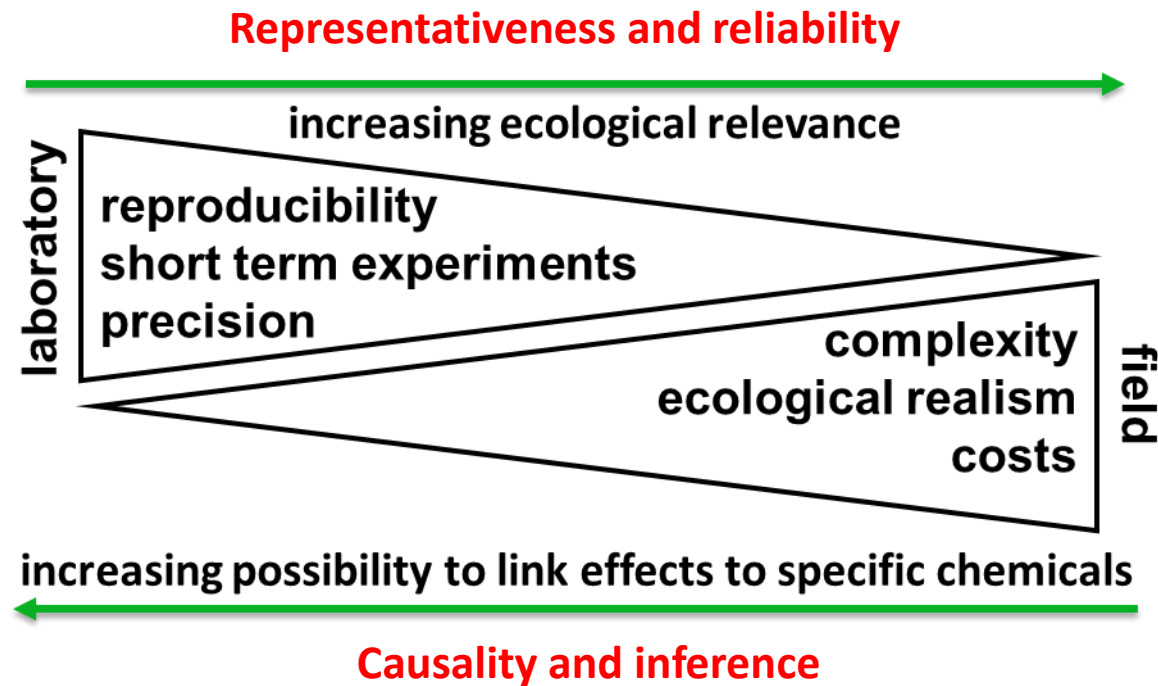
Single exposure

Acute/Repro toxicity

Full life cycle

Chronic / repeated exposure

Low to acute dose



The pros and cons of each approach

Short duration

Single exposure

Acute/Repro toxicity

Mono-substance

Mono-source

Direct toxic effects

Experimental species
Cell lines

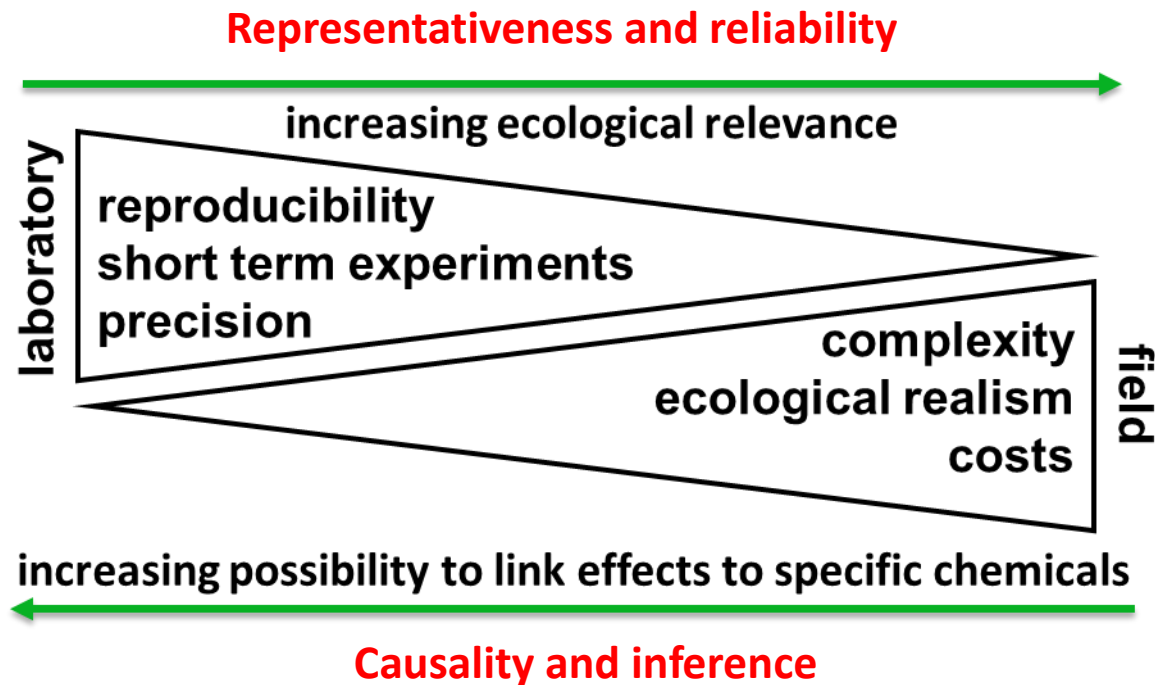
Full life cycle

Chronic / repeated exposure

Low to acute dose

Multiple exposure (mixtures)

Multimedia exposure

Direct & indirect effects (e.g.
trophic cascade)Free-ranging species
(sensitivity/vulnerability, traits,
conservation issues)

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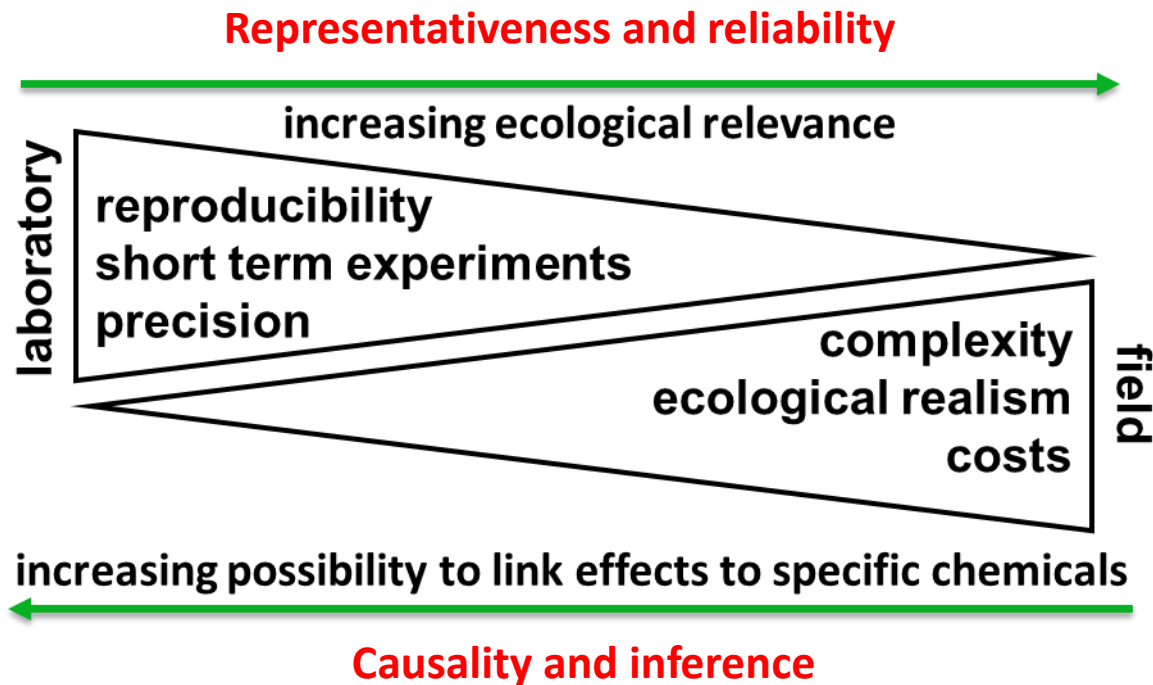
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Acute/Repro toxicity

Mono-substance

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Direct toxic effects

Experimental species
Cell linesStandardized experimental
spaceOptimal & controlled
environmental conditions

Full life cycle

Chronic / repeated exposure

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Multiple exposure (mixtures)

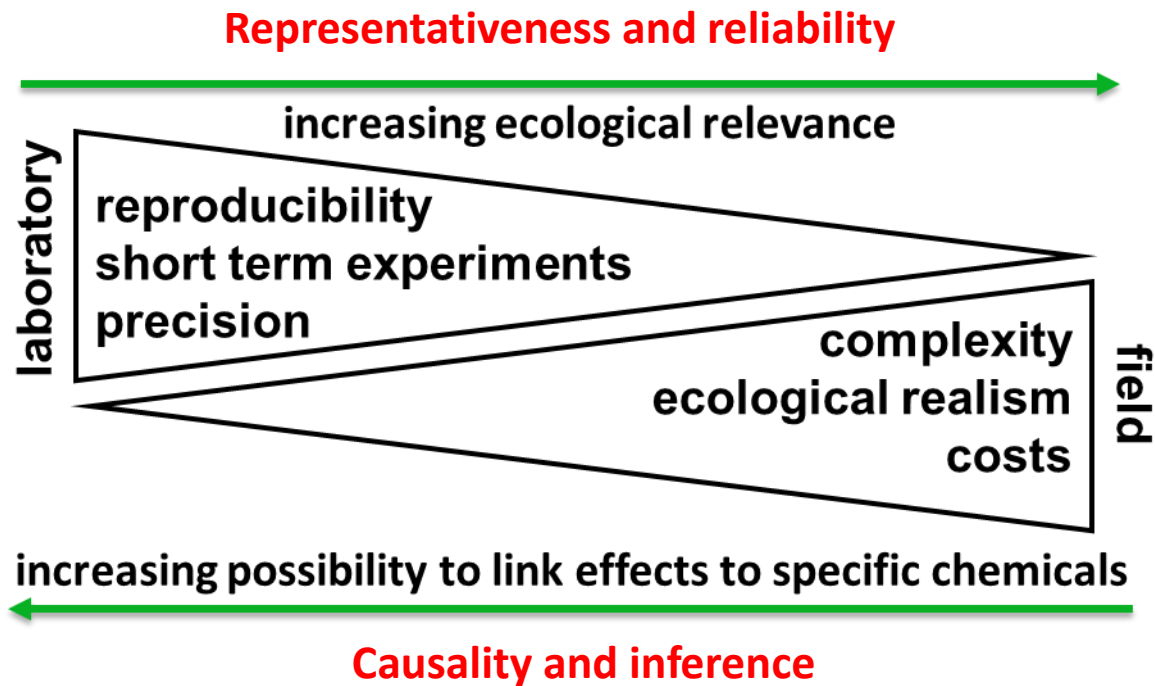
Multimedia exposure

Direct & indirect effects (e.g.
trophic cascade)Free-ranging species
(sensitivity/vulnerability, traits,
conservation issues)Farming practices (e.g. fertilizers,
chemicals, tillage)Multi-stress (land use, climate,
food resources, predation,
competition, pathogens
anthropogenic disturbances (light,
noise, chemical pollution)

The pros and cons of each approach

MISMATCH WITH
REALITY OF USE

ACTUAL EXPOSURE
AND IMPACTS



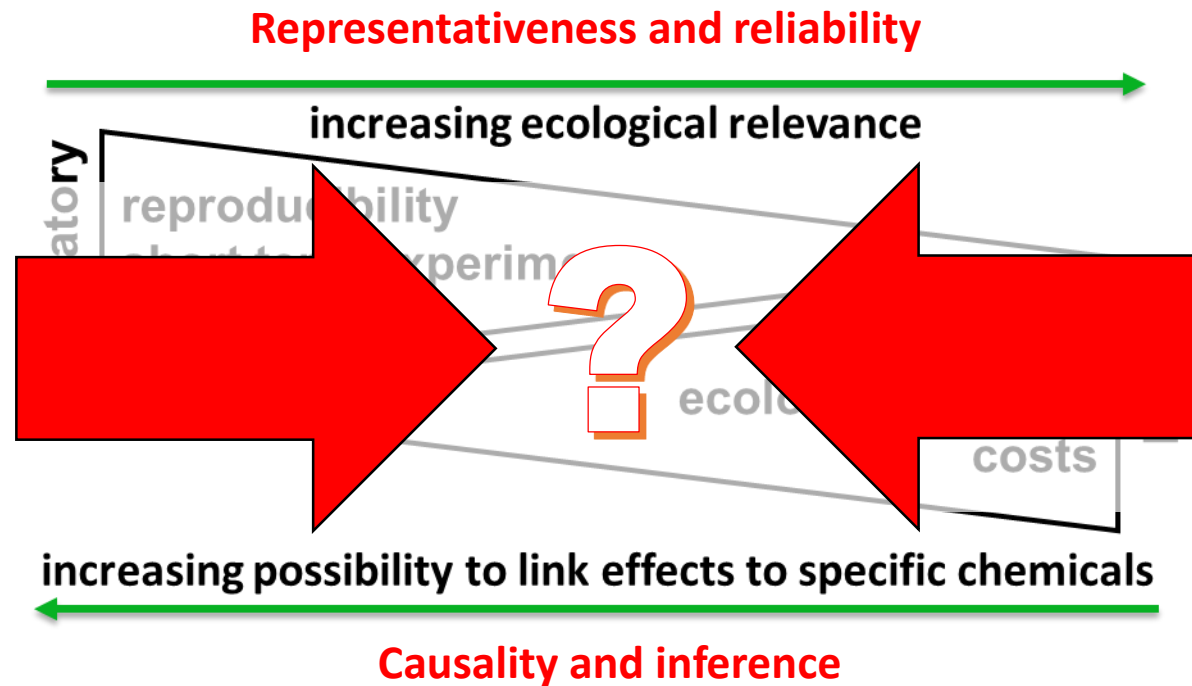
SIGNIFICANCE OF
DOSE-RESPONSE

CONFOUNDING FACTORS
WORSENING FACTORS

Bridge the gap: Scientific issues and Needs to support survey, management and regulation

MISMATCH WITH
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ACTUAL EXPOSURE
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SIGNIFICANCE OF
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Lab Biomarkers in field studies/field species in the lab

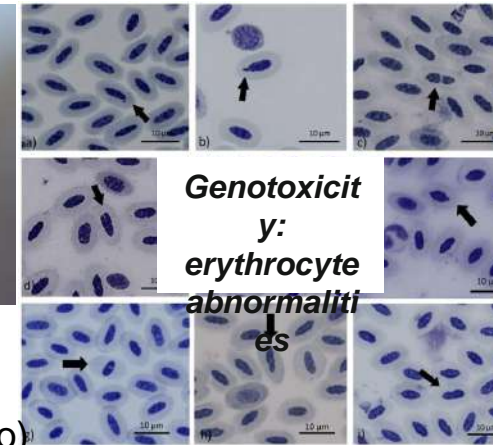
Examples:

Genotoxicity, body condition, plasma
biochemistry, oxidative stress



American kestrel
Falco sparverius

Agricultural area (Mexico)



*Genotox varied with
season, size/health,
and coverage of native vegetation
around capture site.
Genotox observed coincide with the
time when agrochemicals are
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Lab Biomarkers in field studies/field species in the lab

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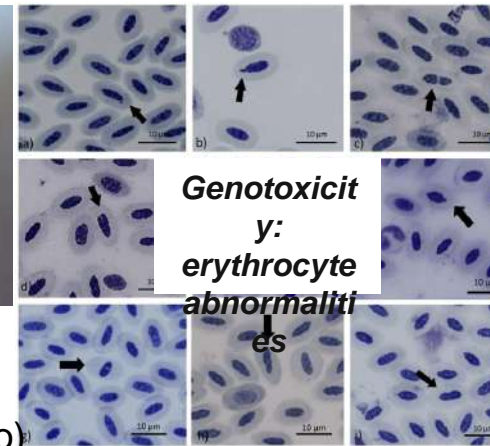
Small
mustelids

Denmark



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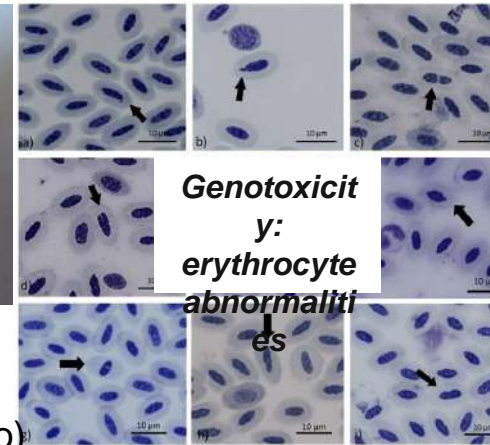
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Red-legged
partridges *Alectoris
rufa*



*Changes in plasma
biochemistry*

*Changes in markers of
oxidative stress*

*Various effects
according to timing*

Imidacloprid-
treated seeds



Repeated exposure

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Genotoxicity, body condition, plasma
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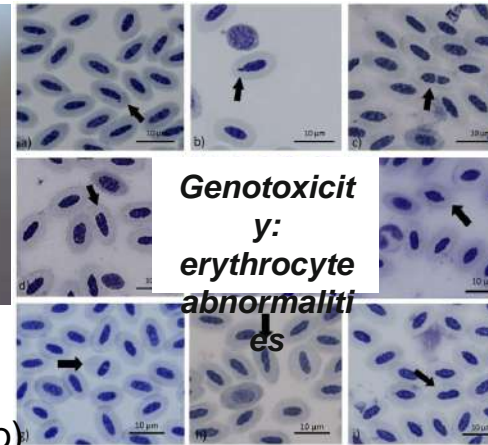
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Repeated exposure

These markers are known to vary with various toxicants/stressors:

- Various pesticides, other xenobiotics (e.g. organic pollutants, metals)
- Radiations
- Pathogens/parasites
- Various stressors (e.g. food depletion, reproduction investment, climatic constraints)

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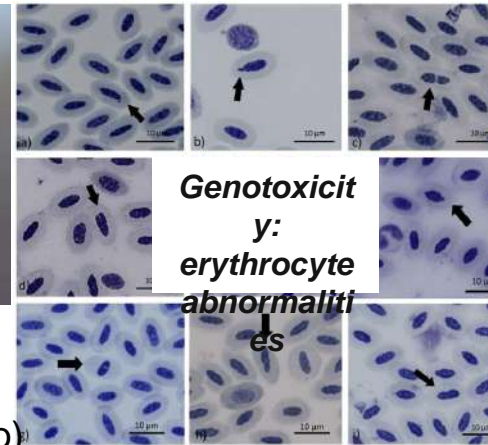
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- Various stressors (e.g. food depletion, reproduction investment, climatic constraints)

→ Reflect actual health status of animals

→ Lack of specificity: difficult to interpret variations (pesticides?)

→ Difficulty to link to effects at population / community level

Lab reaching field, field reaching lab?



Apply management tools
Support decision-making

→ **Challenges**

- Gain representativeness in controlled experiments
- Gain inference on specific pesticide role from field surveys

Lab reaching field, field reaching lab?

Active biomonitoring

Examples:
Semi-field experiments
(Experimental stations)

"La Cage"

INRAE



Lab reaching field, field reaching lab?

Active biomonitoring

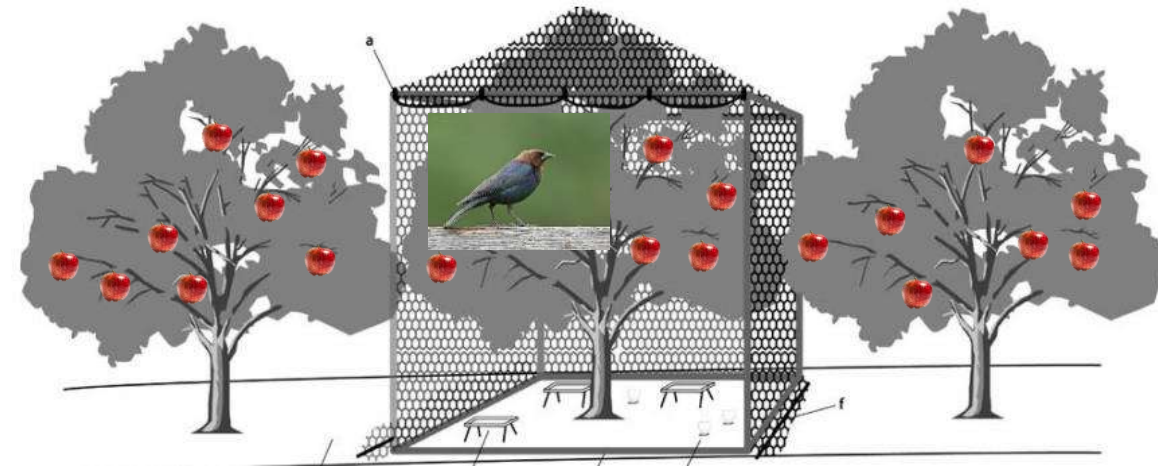
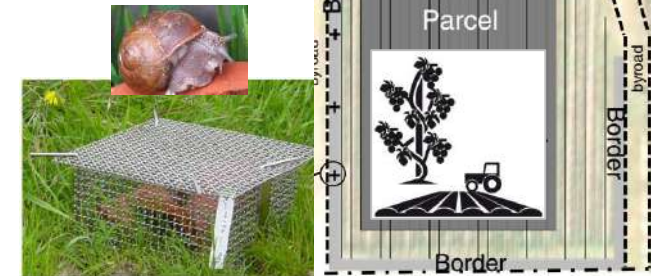
Examples:
Semi-field experiments
(Experimental stations)

"La Cage"

INRAE



Examples:
Snail caging, bird enclosure



Lab reaching field, field reaching lab?

Active biomonitoring

Indicators:

- Exposure
- Individual markers (*e.g. genotox, physiology, etc*)
- Population parameters (*e.g. survival, reproduction, abundance, etc*)
- Interactions (*e.g. trophic interactions*)

Examples:
Semi-field experiments
(Experimental stations)

"La Cage"

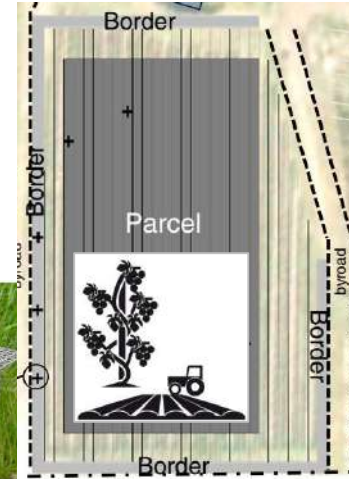
INRAE



→ Semi-field experiments:
bias such as edge effects,
size-effects, density-effects

Examples:
Snail caging, bird enclosure

→ Active biomonitoring:
not fully ecologically representative
(*e.g. life-cycle and carry-over effects, multi-stress*)



→ Better ecological relevance than in lab

(real doses and practices, multiple sources, multiple exposure, actual environmental conditions)

→ Better link to specific pesticide causality than passive biomonitoring

(enclosure: movements, predation, competition, etc. are controlled; climate, practices, land use, etc. known and can be taken into account)

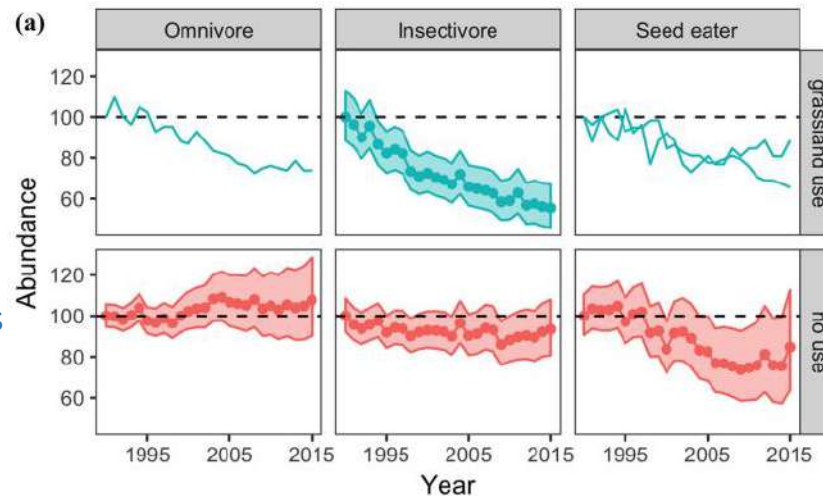
Lab reaching field, field reaching lab?

Long-term large-scale surveys Epidemiological approaches

- **Indicators:**
 - Trends populations/ communities
(dynamics, biological traits, etc)
 - Trends use of pesticides
(specific chemical families, specific uses, etc)
 - Links with other concomitant spatial/temporal changes
(climate, land use, etc)
 - Links with other taxa
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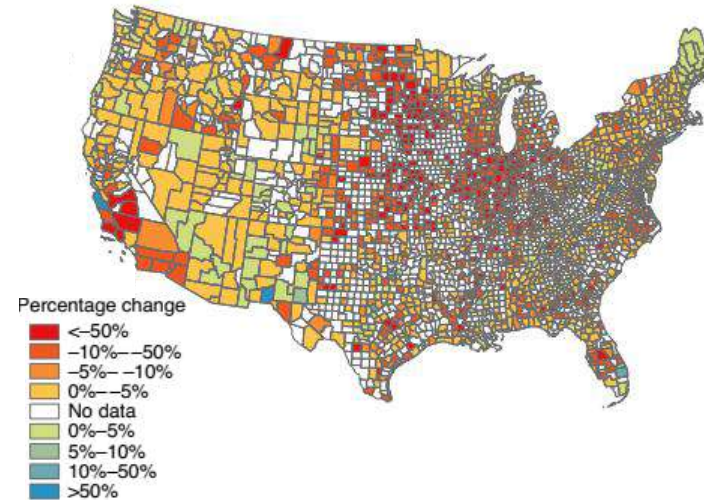
Examples: National Bird Census

EU: Bird population changes according to grassland use and diet



USA: Bird population changes according to neonicotinoid use

Insectivorous bird population change due to neonicotinoid use from 2008 to 2014



Denmark: declines of both insects and birds



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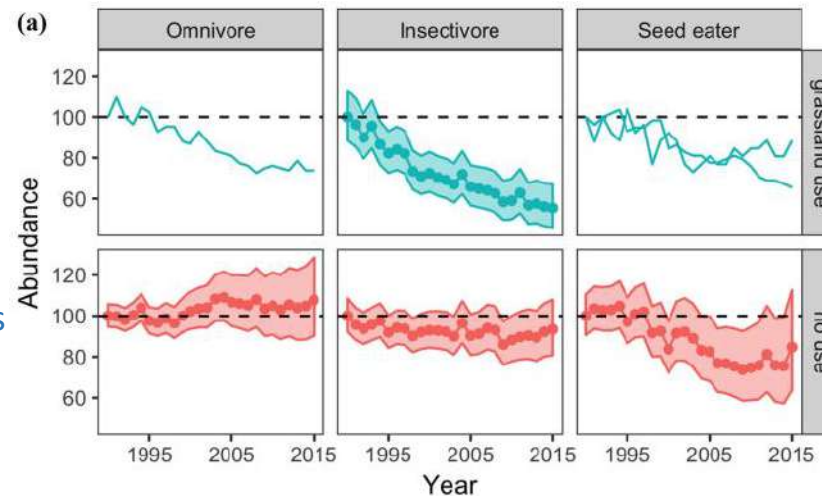
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- ➔ Real trends under multi-stress context & actual dynamics at relevant ecological scales
- ➔ Independence from local/site-specific situations and temporary events
- ➔ Statistics and meta-analyses help in disentangling/ranking the role of various factors

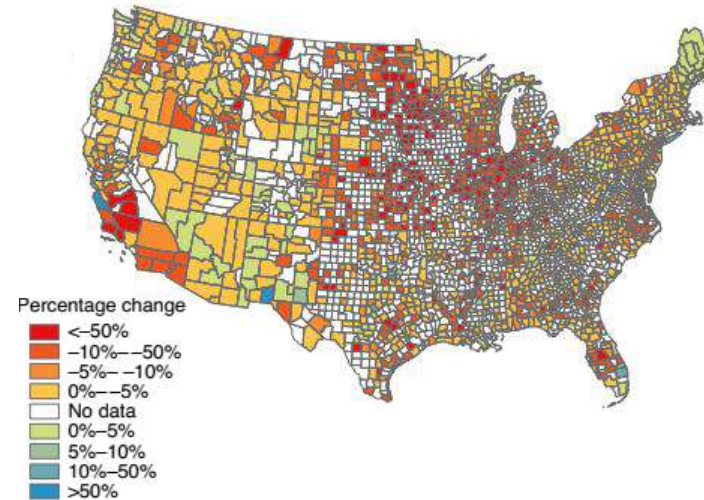
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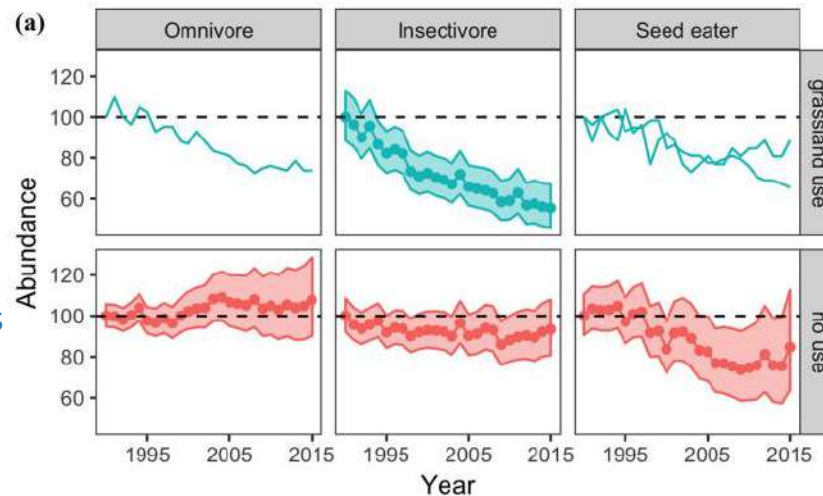
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- ➔ Still correlative, need for additional information (e.g. Hill's criteria)
- ➔ Long-term trends: need for time and data accumulation before decision-making
- ➔ Inherent issues of long-term large-scale comparisons and surveys (cf adaptive monitoring, costs)

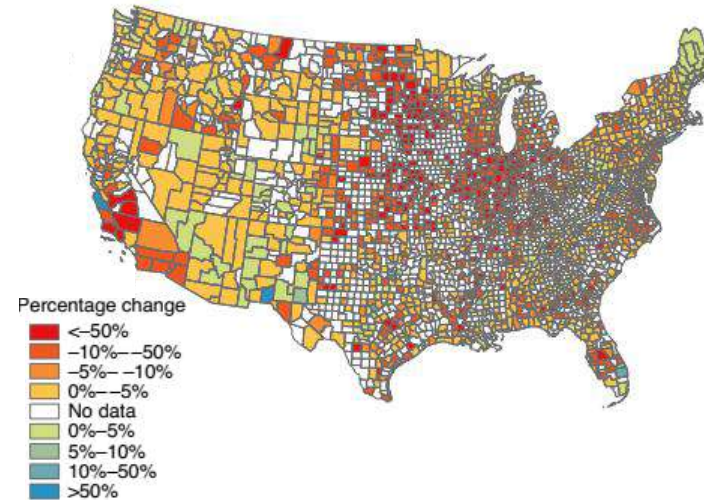
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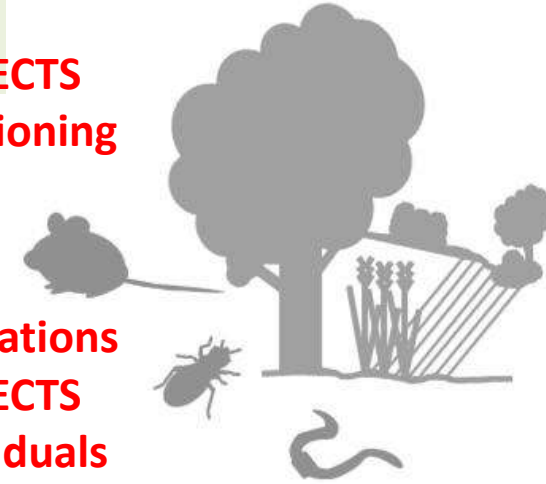
Future directions

- Develop frameworks of complementary assessment of indicators in the field
 - exposure/accumulation, individual health markers and population potential outcomes at once
 - link with functioning and apply on food webs
 - improve knowledge about mechanisms and understanding of impacts to allow prediction and support to management
- Create baselines and benchmarks for PPPs in environment (water, air, soil) and in organisms
 - detection molecules/practices/ecosystem at risk, survey temporal and spatial trends (regulation efficacy, hot-spot to be managed, etc.)

EFFECTS
Functioning

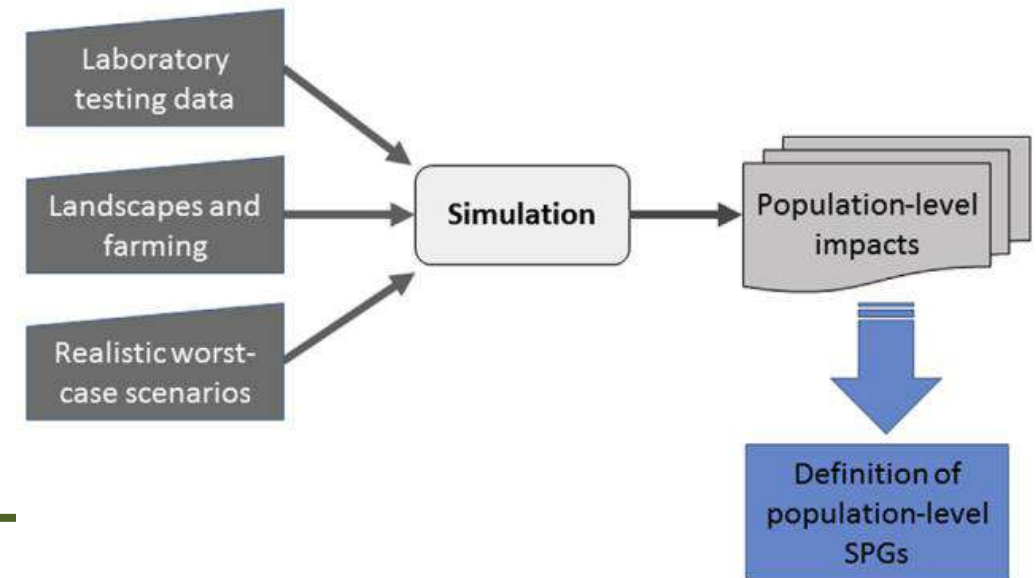
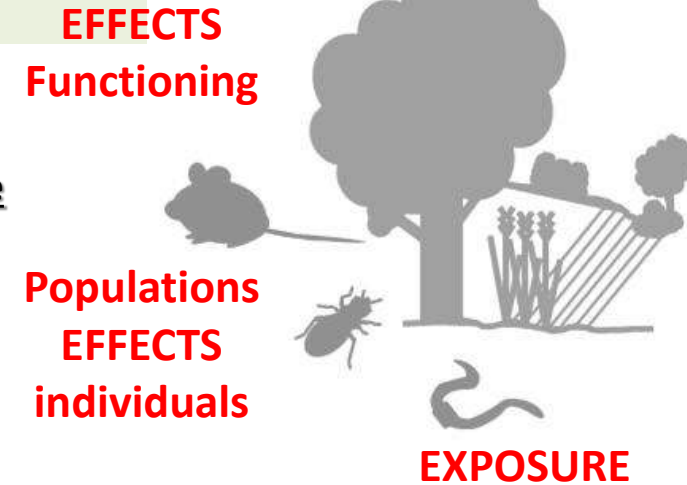
Populations
EFFECTS
individuals

EXPOSURE



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- Use of modelling as a complement
 - comprehensive and predictive approach of mechanisms (TK/TD, AOP), test of scenarios for spatial planning, exploration of long-term expected trends
 - support for decision-making



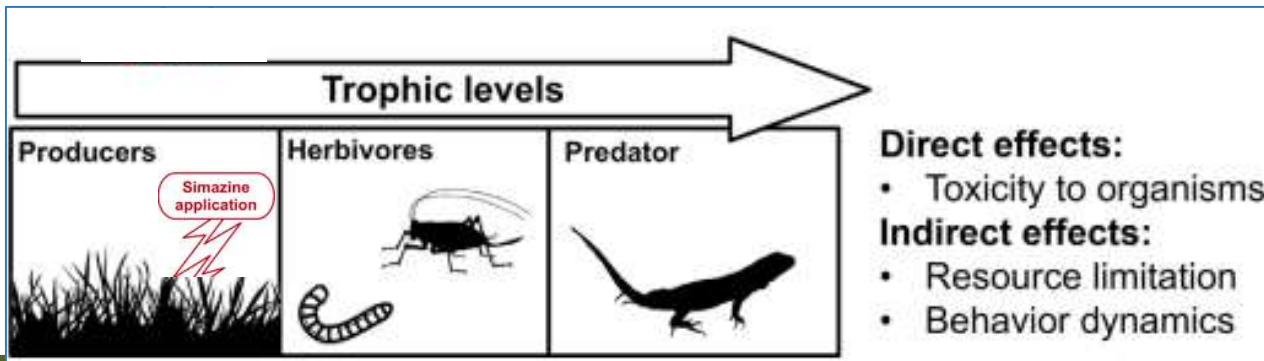
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exposure/accumulation, individual health markers and population potential outcomes at once
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- Enhance PNEC relevance and effect assessment to match ecological realism and adapt to 3R principles
 - re-design laboratory tests to improve their representativeness: natural soils, sequence of molecules and timing of exposure
 - re-think laboratory assay to link exposure to responses: assessment of bioaccumulation during tests of effects
 - improve ecological relevance direct & indirect effects: tri-trophic and multi-species tests

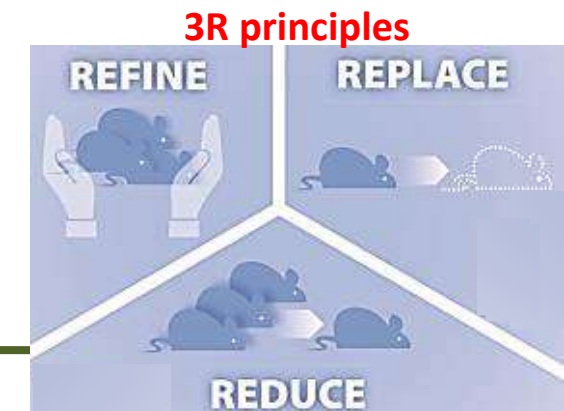
EFFECTS
Functioning

Populations
EFFECTS
individuals

EXPOSURE



Use of animal models in scientific research



THANK YOU



Colette BERTRAND

Pôle Ecotoxicologie – UMR EcoSys
INRAE

Clémentine FRITSCH

UMR Chrono-environnement
CNRS