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## **An approach to choose the most relevant time of exposure for biomarker measurement in earthworms exposed to pesticides**

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# AN APPROACH TO CHOOSE THE MOST RELEVANT TIME OF EXPOSURE FOR BIOMARKER MEASUREMENT IN EATHWORMS EXPOSED TO PESTICIDES

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## Context

Biomarkers = good tools for environment quality assessment

No clear trend/ contradictory responses for biomarkers in soil organisms exposed to pesticides

Due to :

- The **high heterogeneity in laboratory protocols** (species, concentrations, contaminants nature, exposure time, analysis methods) ?
- a **lack of relevance in the choice of the exposure time** ?



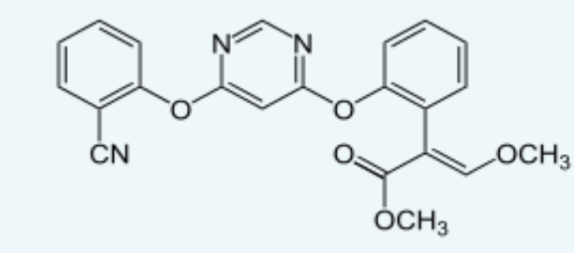
Soil from an uncontaminated fallow (INRA Versailles, France)  
5-20 cm depth  
5 mm sieved



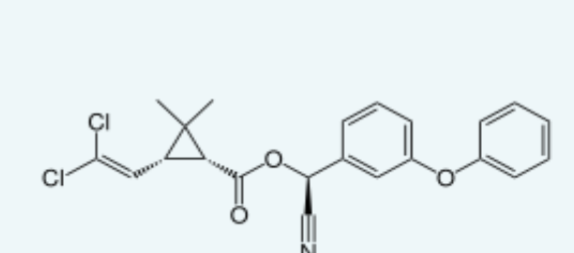
*Aporrectodea icterica*

Earthworms = ecosystem engineers  
• **Recycling organic material**  
• **Increasing nutrient availability**  
• **Improving soil structure**  
• **Increasing pastoral productivity**

- Relevant organisms for soils quality assesment



AMISTAR® (Azoxystrobin)  
fungicide of the strobilurin family  
250 G/L ; 1L/ha and 100 L/ha



CYTHRINE MAX® (Cypermethrin)  
insecticide of the pyrethrinoids family  
500 G/L ; 0,25 L/ha and 25 L/ha



- Commonly applied in French cereal crops
- Harmful for the environment and human health

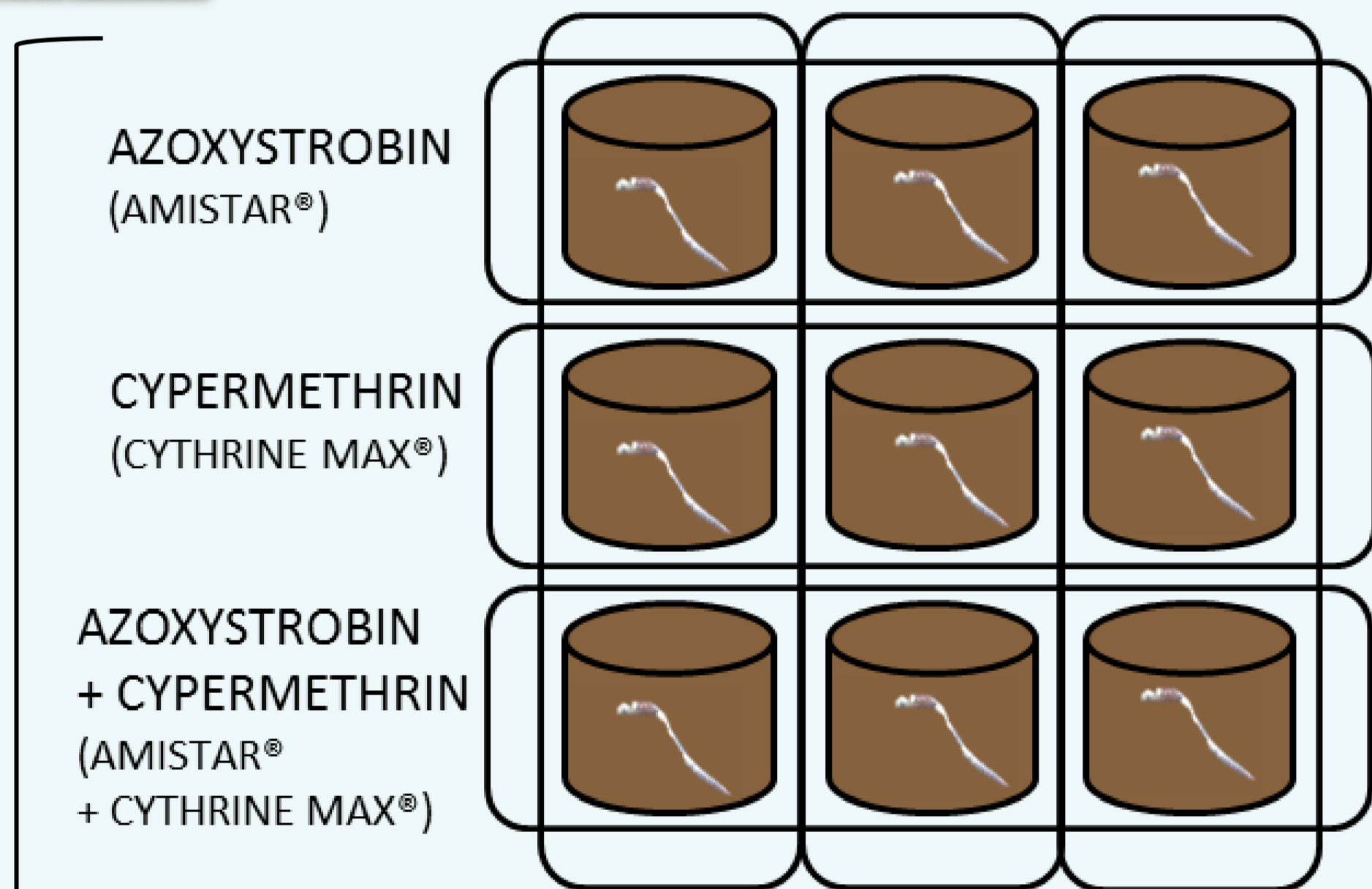
Can we determine the kinetic responses of biomarkers involved in earthworm physiology (defense against oxidative stress, detoxification mechanisms, energy reserves) with time ?

## Experimental design



3 doses

DOSE 0 without pesticide  
DOSE 1 agronomic rate  
DOSE 10 10 times the agronomic rate



7 times of exposure : 0, 2, 4, 7, 10, 14, 21 days

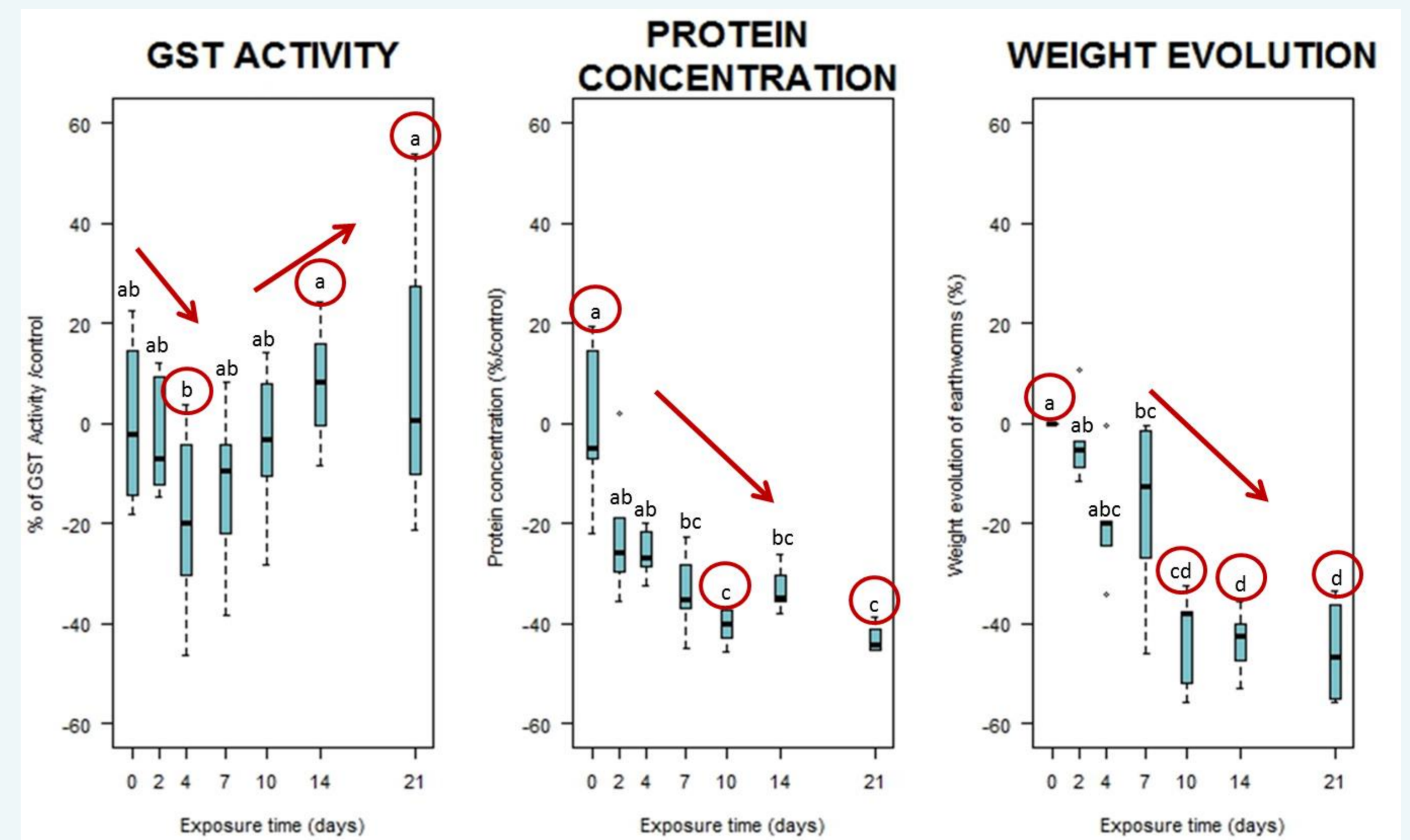
➔ 10 earthworms per conditions  
➔ 430 earthworms

Measurements of biochemical biomarkers involved in :

- **Oxidative stress** (Catalase, Superoxide dismutase, Lipid peroxidation)
- **Detoxification mechanisms** (Glutathione-S-Transferase, Glutathione Peroxidase)
- **Energy reserves** (Lipids, Glycogen, Proteins)
- **Others** (Acetylcholinesterase, weight evolution)

## Preliminary results

Earthworms exposed to:  
Cypermethrin – 10 times the recommended dose



➔ **Significant differences between the time of exposure** were found for Glutathione-S-Transferase activity, Catalase activity, Protein concentration, Weight evolution

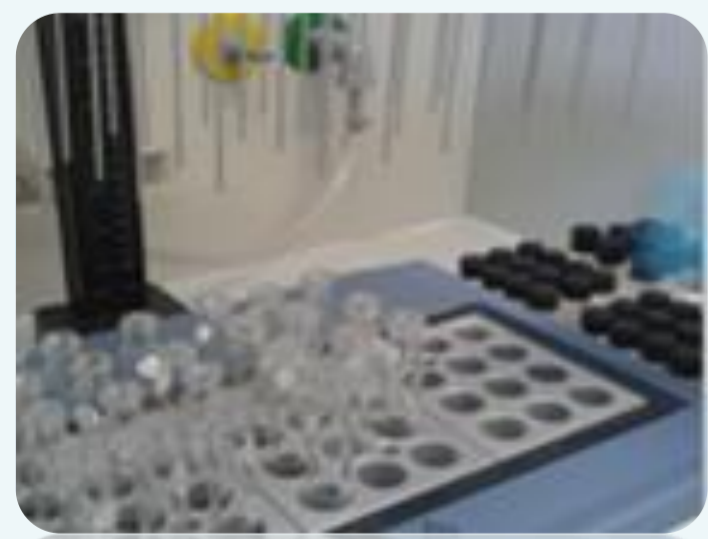
➔ **No significant effects of the dose**

## Take Home Message

Time of exposure effect > Dose effect

**Important to take into account the time of exposure in ecotoxicology studies rather than focusing on the dose only**

Only half of the samples were analyzed and other biochemical biomarkers are being analysed (Lipid, Glycogen, Lipid Peroxidation). However preliminary results seems to be promising to help us to determine the kinetic responses of biomarkers involved in earthworm physiology



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