THE IMPACT OF CHLORPYRIFOS AND ITS FORMULATIONS ON THE ACETYLCHOLINESTERASE ACTIVITY IN NON-TARGET SOIL ORGANISMS

MUNI | RECETOX

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Introduction

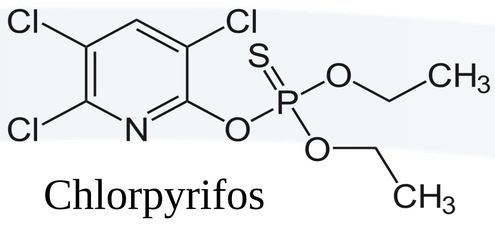
The Ecological Risk Assessment of pesticides requires data regarding their effects to terrestrial non-target species. Commercial pesticides formulations, however, contain a significant proportion (> 90%) of so-called inert ingredients, which may greatly enhance or lessen the toxicity of a formulation. Chlorpyrifos is a broad-spectrum organophosphate insecticide that is used globally on wide range of crops to control pest. This substance disrupt the nervous system via the inhibition of the cholinesterase activity which catalyzes the hydrolysis of the neurotransmitter acetylcholine. Overuse of pesticides have adverse impact on beneficial invertebrates, such as snails and earthworms. To evaluate the sublethal effect of pesticides in organisms, activity of acetylcholinesterase (AChE) is frequently used as a specific biomarker of exposure to the organophosphate pesticides.

Objectives

- **The determination of physiological levels of AChE in snails *Helix aspersa* and earthworms *Eisenia andrei*** in different tissues (head and haemolymph in snails; head and body in earthworms; Table 1).
- **The impact of the pure chlorpyrifos** (a.i.) **and its commercial formulations** (Dursban® 480 EC, Pyrisimex® 480 EC, Pyrifos® 480 EC, Nurelle D®).
- Evaluation of **the difference in sensitivity** of tested organisms (*in vitro* exposures to above mentioned pesticides).

Sample	AChE activity (nmol/min/mg protein)
***H. aspersa* (head)**	20.51 ± 1.52
***H. aspersa* (haemolymph)**	79.15 ± 8.74
***E. andrei* (head)**	63.69 ± 5.35
***E. andrei* (body)**	67.71 ± 7.14

Table 1: The physiological levels of AChE measured in different tissues of the snail *H. aspersa* and the earthworm *E. andrei*.



Chlorpyrifos

Materials & Methods

- **Bradford assay** for protein analysis. Colorimetric protein assay based on an absorbance shift of the dye Coomassie Brilliant Blue G-250.
- **Ellman's method** for the cholinesterase activity measurement. Colorimetric method based on the thiol-groups determination.
- All methods were performed in 96-well plates.

Results

- **The highest physiological activity** in gastropod was measured **in haemolymph**. AChE activity in earthworms had very similar values in the head and the body (Table 1).
- *In vitro* study showed AChE **inhibition in a concentration dependent manner** (Figure 1)**.**
- **The pure a.i.** caused **no effect** in **snail haemolymph** compare to other tissues (Figure 1A). The values on the graph show some inhibition/stimulation, but this is due to variability in physiological values (Table 1).
- **The most sensitive responses** to exposures to **commercial formulations** were found in ***H. aspersa* haemolymph** and ***E. andrei* whole body** homogenate (Figure 1B; Table 2).
- **The inhibition** (based on the IC50s comparison) **increased** in the following order **a.i. < Dursban® < Pyrifos® < Pyrisimex® < NurelleD® (earthworm head tissue)** and **a.i.< Dursban® < NurelleD® < Pyrisimex® < Pyrifos® (snail haemolymph**; Table 2**)**.

Conclusion

- The **commercial pesticides** caused significantly **higher AChE inhibition** compare to the technical a.i. in both model organisms (Figure 1A and 1B; Table 2).
- The **toxicity testing of both a.i. and commercial formulation** provide **more realistic reports** on the overall ecotoxicological impact of pesticides on sensitive non-target organisms.
- **Snails and earthworms** are **good biomarkers** of exposure due to their **sensitive response** to the presence of the pesticide.

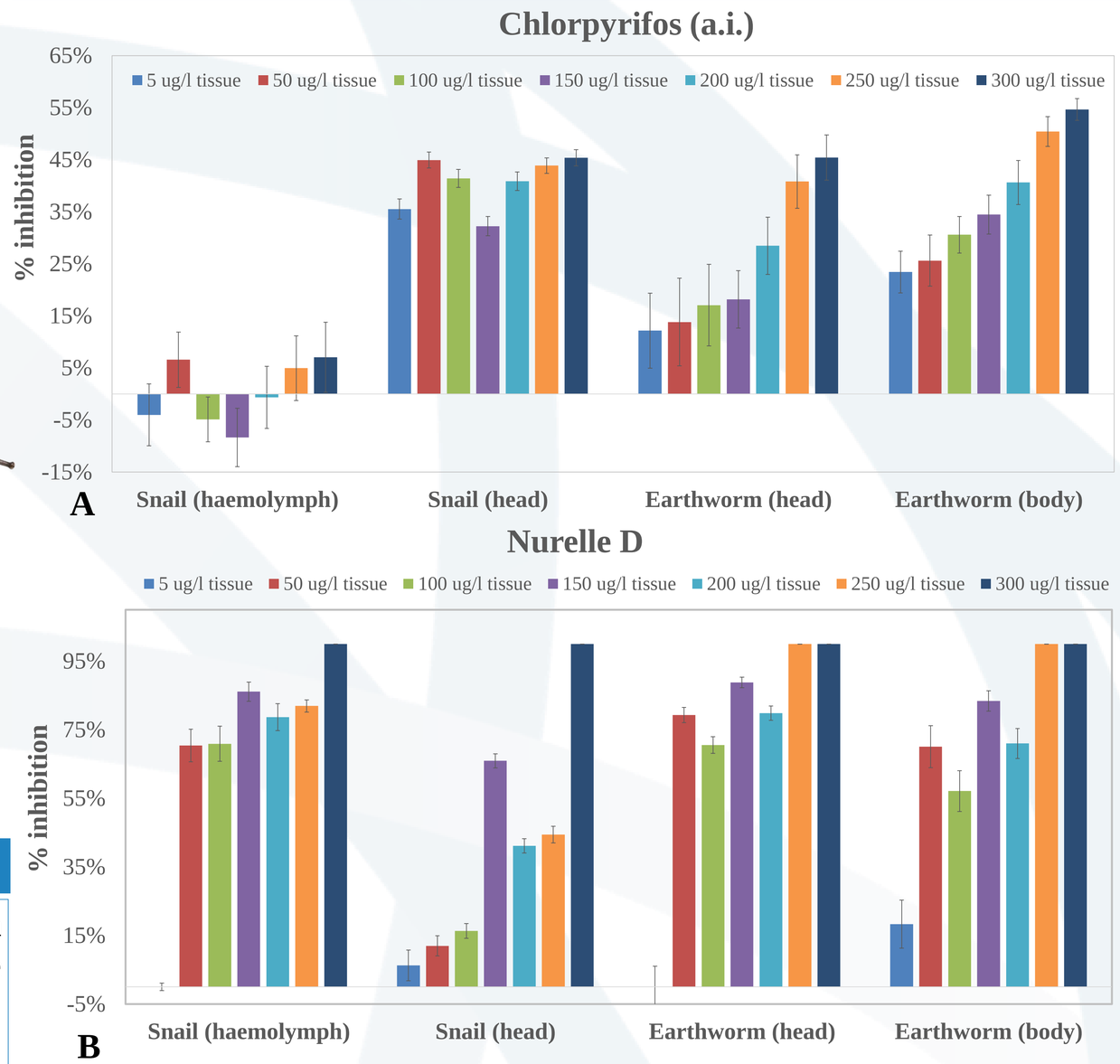


Figure 1: The inhibition of acetylcholinesterase caused by chlorpyrifos a.i. (A) and Nurelle D (B) at different tested concentrations. In order to compare pure a.i. with commercial formulation, the pesticides Nurelle D® was selected.

% of inhibition	*H. Aspersa* (haemolymph)	*H. Aspersa* (head)	*E. Andrei* (head)	*E. Andrei* (body)
Chlorpyrifos (pure a.i.)	7%	45%	14%	26%
NurelleD®	70%	12%	79%	70%
Dursban®	38%	48%	38%	92%
Pyrifos®	79%	1%	68%	61%
Pyrisimex®	77%	39%	76%	70%

Table 2: The difference in inhibition between pure a.i. (chlorpyrifos) and commercial formulations. The data shows the inhibition at one concentration level (50 µg/l tissue).

Acknowledgement

This research was supported by the RECETOX Research Infrastructure (LM2018121), CETOCOEN EXCELLENCE Teaming 2 project supported by Horizon2020 (857560) and the Czech ministry of Education, Youth and Sports (02.1.01/0.0/0.0/18_046/0015975).