The importance of a comprehensive toxicity assessment of nanopesticides in the environment:

comparing toxic effects of tebuconazole (TBZ) in different formulations on the nematode *C. elegans*

Mahleh EghbaliNejad[1], Sebastian Höss[2], Jakub Hofman[1]

INTRODUCTION

❖**Indiscriminate application of pesticides** has led to ecological and health adverse effects, demanding new pesticides[1].

❖**Nanopesticides (NPs), potentially a promising solution for concerns** in the agriculture sector (benefits e.g., improved pesticide solubility& pest` penetration, decrease loss of active ingredients (a.i.) due to their small size, high surface area)[1].

❖Potential **safety risks of NPs to non-target organisms are emerging concern** for both a.i. and the nanocarriers (NCs)[2].

❖ **Poly(ε-caprolactone) (PCL)** is a biodegradable and biocompatible polymer, **Nanostructure lipid carrier (NLC),** 2nd generation of lipid carrier used mostly in medical and agriculture area.

❖**Nematode** *C. elegans* is used as a test organism due to simplicity, accuracy, repeatability, and low cost of the experiments.

PURPOSE

❖ **Toxicity assessment of different formulations of TBZ** on *C. elegans* reproduction in aquatic matrix.

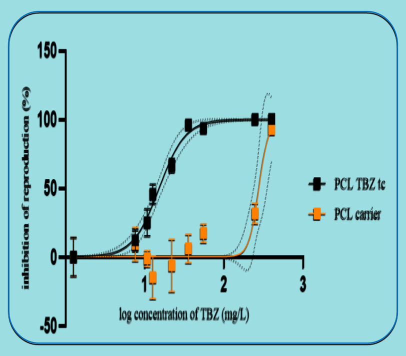
HYPOTHESIS

❖ **Different formulations of TBZ** may show varying toxic effect on *C. elegans* reproduction?

❖ **Mode of action** of NPs may be different compared to their associated NCs without pesticide?

❖ **A comprehensive toxicity assessment** is necessary to evaluate the potential risks of these formulations in the environment!

METHOD& MATERIAL

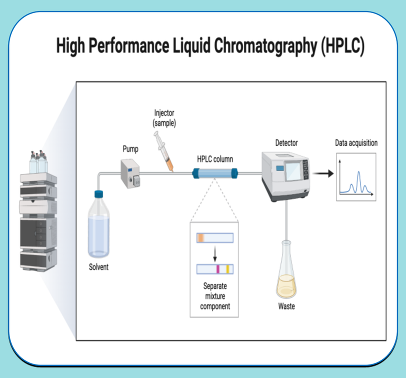


Ecotoxicity test (fig 1)

❖ Tested compounds: Pure TBZ(10 concentrations), NLC NF& NLC NC(8concentrations), PCL NF&PCL NC(8concentrations) Folicur TBZ (TBZ Commercial,10 concentrations),, (table1, Fig 2))

❖ Based on ISO 10872 t=96, endpoint= *C.elegans* reproduction (here reporting as %inhibition of reproduction (%IR)

❖ E.coli suspension 1000FAU



Chemistry

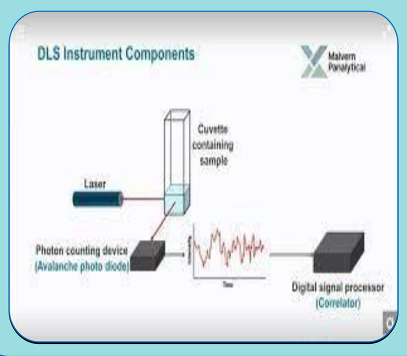
↓ Preparation of test compounds

↓ Incubation for 48h (t=20˚C,gentle shaking, to get max release TBZ)

↓ adding E.coli suspension (1000FAU), incubation for 2h

↓ Removing aliquot for t=50h verification of TC and free TBZ

↓ Incubating for 146h , aliquot for TC and free TBZ at t=146h



Physics characterization

❖ After preparation of chemicals, particles` size, PdI, particle concentration (PC) were measured by MADLS (dynamic light scattering).

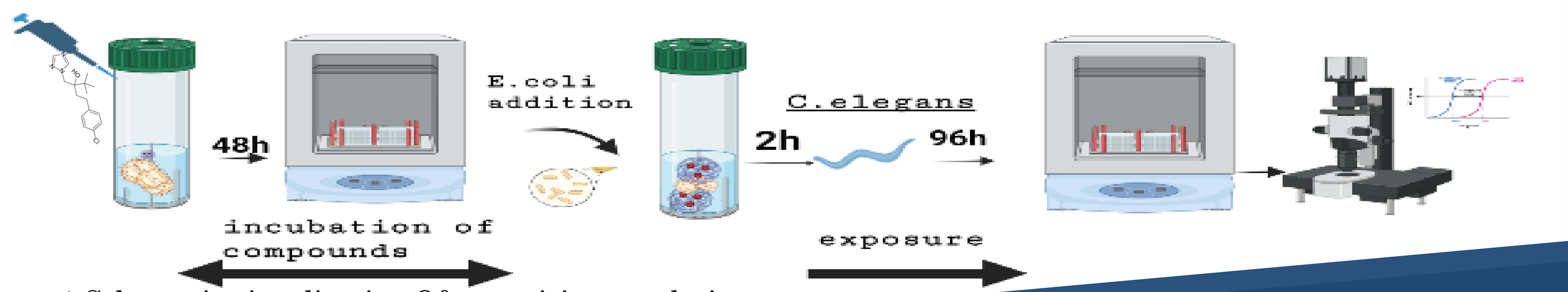


Figure 1 Schematic visualization Of ecotoxicity test design

Compounds	TC*µg/ml	ZA** (nm)	PdI***	ZP ^	PC ^^(particles/ml)	EE ^^^
NLC NF	571.16 ± 35	329±28	0.298±0.07	-28	4.57E+09	91% ± 1
NLC NC	/// //	318±3	0.2±0.029	-27.4	2.37E+09	////
PCL NF	1660 ± 120	242.5±2.5	0.176±0.005	-28.56	2.09E+09	98%
PCL NC	/// //	261±3	0.203±0.04	-27.6±1	1.41E+09	///

Table1 NFs and NCs characterizations (*TC=total concentration of TBZ, ZA** zeta average, PdI***=Polydispersity index, ZP^=Zeta potential, PC^^=Particles concentration, EE^^^= Encapsulation of efficiency

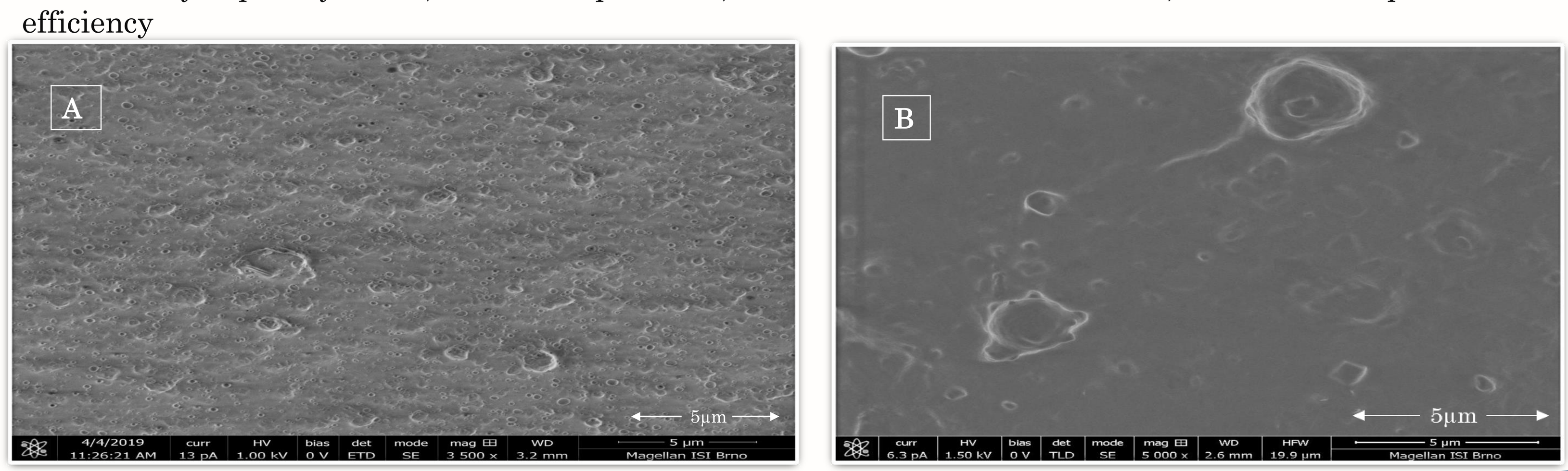


Figure 2, SEM images of A)NLC-TBZ, B) PCL-TBZ

RESULTS

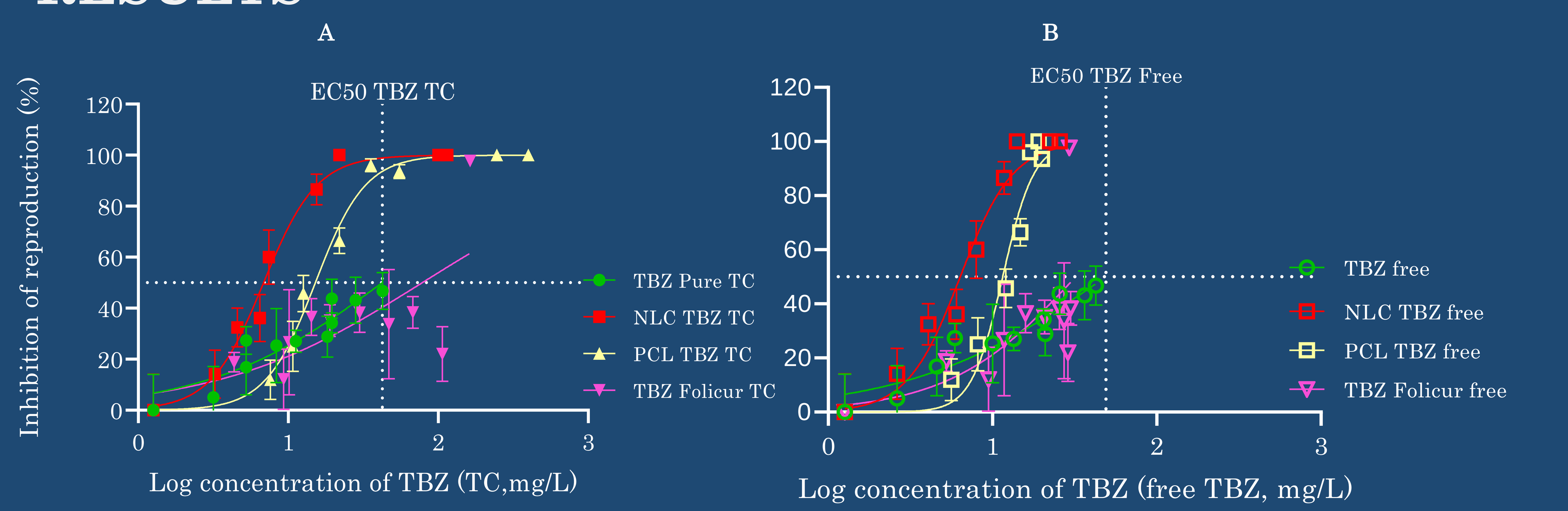


Figure 3 (A) dose-response curve of TC for tested compounds, EC50 of TBZ Pure=42.3, NLC-TBZ= 6.8, PCL-TBZ=15.2, TBZ Folicur=78.2 mg/l. (B) Dose-response curve of free TBZ for tested compounds: EC50 of TBZ Pure=49.12, NLC-TBZ=6.4, PCl-TBZ=11.5, TBZ Folicur= 32.2 mg/l.

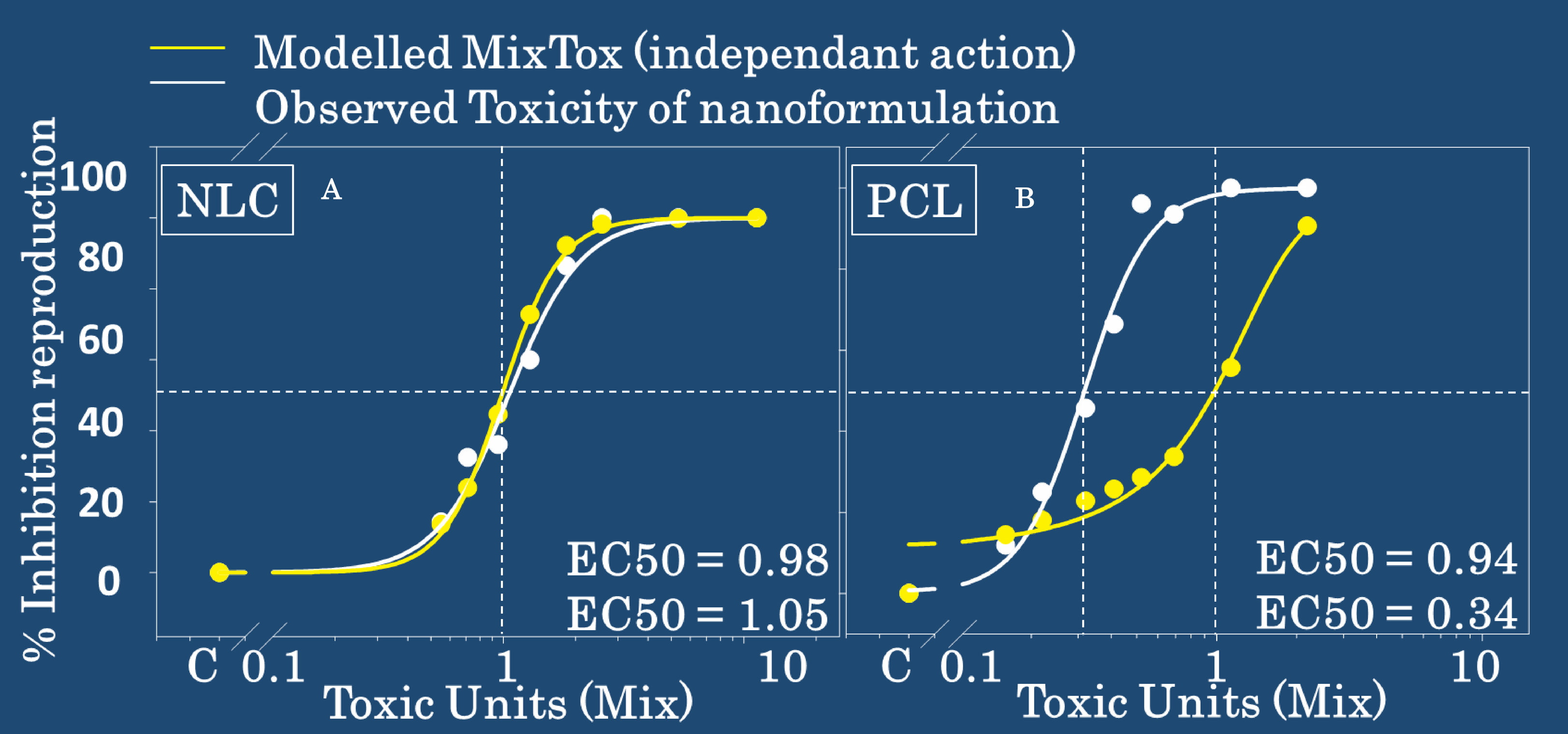


Figure 4 (A) Mixture toxicity model NLC, EC50≥ 1= combination effect (B) Mixture toxicity model PCL, EC50<1= synergism!)

CONCLUSIONS & limitation

❖ Toxicity assessment of different formulations of TBZ showed varying effects, NFs showed higher toxicity on C. elegans (IR%).

❖ Both NFs (NLC, PCL) showed a joint toxicity of free TBZ and the associated NCs(mixture toxicity). For NLC-NF, TBZ toxicity was masked by a strong NC` effect. For PCL NF, a synergistic mixture toxicity could be observed (observed > modeled toxicity)[3].

❖ NFs improved **TBZ properties** (higher solubility..).

❖ There is insufficient suitable analytical metrology to monitor the changes of physical characterizations of NFs.

SUGGESTION

❖ **Regulation** and **scientific** evaluation of nanopesticides is very **urgent.**

❖ **Artificial intelligence** can have deeper contribution to properties `prediction and nanosafety.

REFERENCES

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Affiliation

[1]Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University

[2]Ecossa, Starnberg, Germany

*Presenting author: Mahleh.eghbalinejad@recetox.muni.cz

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ecossa