



Nanopesticides ecotoxicity towards traditional ones: A case of study with *Daphnia magna* and λ -cyhalothrin

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HIGHLIGHTS

- Novel nanopesticides (NP) ecotoxicity differ from traditional formulations (TP).
- The NP had a delayed but greater lethality in *Daphnia magna* than the TP.
- Temperature rise increased pesticides lethality, neurotoxicity, and energy depletion.
- This work contributes to eco-safer NP development in a warming world (WW) context.
- NP development and WW mitigation and adaptation strategies must be aligned.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Alvine C.Mehinto

Keywords:

Pyrethroid
Encapsulated pesticide
Climate change
Biomarker
Crustacea

ABSTRACT

Nanotechnology has contributed to agriculture industry with novel products to improve the targeted delivery of active ingredients (a.i.), enable gradual release, avoid premature degradation, and increase efficacy. The properties of nanopesticides make their drift and environmental behavior more unpredictable than traditional formulations. Our aim was to compare the ecotoxicity of two insecticides with λ -cyhalothrin as a.i.: a nano-based one and a traditional emulsified, considering the incidence of temperature according to climate change projections (20 and 24 °C). We evaluated their effects on *Daphnia magna* survival, body stores, and butyrylcholinesterase (BChE) activity. Although after 24 h the traditional formulation had greater lethality than the nanopesticide, after 48 h this pattern was reversed. At 24 °C the lethality of both pesticides increased. BChE

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<https://doi.org/10.1016/j.chemosphere.2025.144371>

Received 28 December 2024; Received in revised form 17 March 2025; Accepted 28 March 2025

Available online 3 April 2025

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activity was inhibited at 24 °C by both pesticides. In general, the increase in temperature negatively affected protein and glycogen content. The traditional formulation reduced glycogen content at 24 °C. A decrease in lipid content and the whole energy budget was observed in organisms exposed to the traditional formulation at both temperatures. Although the nano-based pesticide developed a delayed toxicity, it was more lethal than the traditional one in the long term. The temperature rise worsened the pesticides effects in terms of decreased survival, BChE activity inhibition, and energy reserves depletion. The development and regulation of new eco-safer nanopesticides needs to be complemented by their ecotoxicological assessment. It is imperative to analyze their impact in the context of climate change in order to develop mitigation and adaptation strategies.

1. Introduction

Nanotechnology industry has been rapidly developed due to the desired properties of nanoscale products. In the last decade, it has contributed to agriculture industry with novel products for pest control and fertilization (Ding et al., 2023). In this context, nanopesticides emerged in different ways such as nanoparticles with biocides properties *per se* (e.g., copper and alumina nanoparticles), nanoemulsions, or nanoencapsulations of traditional pesticides. The development of nanoencapsulations aims to improve the targeted delivery of the active ingredient (a.i.) to the site of action, enables a gradual release, and protects it from premature degradation, contributing to increase efficacy and reduce the amount of applied products (Ding et al., 2023; Hennig et al., 2023). The unique properties of nanopesticides, due to their nanometric scale, make their drift and environmental behavior more unpredictable than traditional formulations. Indeed, nanoencapsulation can enable higher solubility of lipophilic compounds, promoting their bioavailability in the water column compartment (Ding et al., 2023; Son et al., 2015).

λ -Cyhalothrin is a type II pyrethroid insecticide broadly applied and detected in surface water at concentrations ranging from 0.01 to 0.14 $\mu\text{g/L}$ (He et al., 2008; Mac Loughlin et al., 2022; Papadakis et al., 2015; Wang et al., 2023). This insecticide is considerably toxic for aquatic invertebrates (He et al., 2008; Ranatunga et al., 2023). Type II pyrethroids act primarily on sodium, chloride, and calcium channels by preventing them from closing normally, which results in continuous nerve impulse. They have been reported to affect cholinesterases (ChE) activity in cladocerans and fishes (Badiou and Belzunces, 2008; Carr and Chambers, 2005; Ren et al., 2017; Santana et al., 2021; Toumi et al., 2015). Pyrethroids may also affect metabolic reserves due to the energy invested in detoxification (Finotello et al., 2017; Fouzai et al., 2023; Martínez-Jerónimo et al., 2013; Rodríguez-Estrada et al., 2016). Both traditional and nano-based formulations containing λ -cyhalothrin as a.i. are available in the market, despite that, little is known about their comparative toxicity and fate in freshwater environments (Ale et al., 2023). Son et al. (2015) reported that the lethality of encapsulated λ -cyhalothrin on *Daphnia magna* cladoceran was higher for the smaller fraction of capsules and under optimal pH and ionic strength conditions. Likewise, Huang et al. (2022) showed that the smaller nanocapsules loaded with λ -cyhalothrin were more lethal and caused higher malformations in *Danio rerio* fish when compared to the micro-sized capsules. In addition, Meredith et al. (2016) observed that encapsulated λ -cyhalothrin was more lethal to *D. rerio* than the a.i. alone.

Climate change due to human greenhouse gas emissions is one of the main imminent threats for freshwater systems (Johnson et al., 2024; van Vliet et al., 2023). The IPCC (2023) projects an increase of the pre-industrial mean atmospheric temperature by up to 4 °C by 2100. Temperature rise is known to affect freshwater organisms, mainly ectotherms, increasing their metabolism and energy consumption (Garreta-Lara et al., 2018; Hudson, 2021). This factor interacts synergistically with several contaminants including pesticides (e.g., García-Astillero et al., 2024; Huang et al., 2023; López-Valcárcel et al., 2023). Moreover, climate change could rise pesticide pollution in surface water through different mechanisms. Weather events including storms are expected to increase in frequency and intensity contributing

to pesticide drift by runoff (Andrade et al., 2021; Bolan et al., 2024; Otieno et al., 2013). Also, climate change might alter the spatial and temporal distribution of pests and cultivated areas, therefore an increase in pesticide use is expected (Delcour et al., 2015; Kattwinkel et al., 2011; Noyes et al., 2009).

In this context, it results imperative to compare the toxicity of both traditional and novel nanopesticides under a warming world scenario, to ultimately contribute to the regulation and development of eco-safer emerging nanoproducts, and to trigger mitigation and adaptation strategies. The aim of the present study was to compare the ecotoxicity of two insecticides with λ -cyhalothrin as a.i.: a nano-based one and a traditional emulsified, considering the incidence of temperature increase. We evaluated their effects on *Daphnia magna* Straus, 1820 survival, body stores, and butyrylcholinesterase (BChE) activity.

We hypothesize higher toxicity of the traditional pesticide in the short term, and a delayed toxicity of the nano-based one due to gradual release of the a.i. from capsules. We expect high lethality of both pesticides in *D. magna*, an inhibition of BChE activity, and a decrease in body stores. All these responses are expected to be worsened by the temperature increase.

2. Materials and methods

2.1. Materials and reagents

Two commercial formulations containing λ -cyhalothrin [(RS)-alpha-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethyl-cyclopropane carboxylate] as active ingredient were purchased: a traditional water-soluble emulsified: ZERO®, 5 %, GLEBA, solvent and emulsifiers not described; and a nano-based formulation with encapsulated a.i.: KARATE ZEON®, 25 %, SYNGENTA AGRO, solvent and capsules: hydrocarbons, C10–C13, aromatics (10–20 %), lignosulfonic acid, sodium salt, sulfomethylated (1–5 %), naphthalene (0.1–0.25 %), ammonium (0.1–0.25 %), 1,2-benzisothiazol-3(2H)-one (0.05–0.1 %).

2.2. Test organisms

Daphnia magna was cultured following APHA (2017) with some modifications. The organisms were grown in dechlorinated and aerated tap water (pH: 7, conductivity: 196 $\mu\text{S/cm}$, dissolved oxygen: 8.6 mg/L, total hardness: 80 mg/L CaCO_3 , alkalinity 140 mg/L CaCO_3 , 26 mg/L Ca^{++} , 3.4 mg/L Mg^{++} , and 171 mg/L HCO_3^-), with 16:8 day:night photoperiod. Water media was completely changed once a week, and organisms were feed three times a week with a trout chow-yeast-alfalfa suspension (APHA, 2017 method) and *Tetrademus obliquus* algae. *T. obliquus* was grown in sterile modified Detmer medium for green algae (Watanabe, 1960) (KCl: 50, KH_2PO_4 : 50, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$: 360, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 360, Cl_3Fe^+ :5, $\text{C}_4\text{H}_6\text{O}_6$:5, H_3BO_3 : 2.86, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$: 1.81, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.23, Cl_2Cu : 0.05 mg/L), at 25 °C, with constant aeration and warm-white LED light (50 $\mu\text{mol/m}^2\text{s}$). Algae were cropped in the exponential growth phase, resuspended in sterile distilled water, and stocked in the dark at −4 °C. Two parallel stock cultures of *D. magna* were established at 20 and 24 °C, considering the IPCC (2023) projections of temperature increase by 2100: limit warming to 4 °C at a high

greenhouse gas emission scenario (SSP3-7.0: Doubling of CO₂-equivalent emissions by 2100).

2.3. Pesticide characterization and degradation

To investigate the morphology of the nano-based formulation (KARATE ZEON®, 25 %, SYNGENTA AGRO), transmission electron microscopy (TEM) was employed using a Zeiss EM109T electron microscope. Briefly, a droplet of the sample was deposited onto carbon-coated copper grids and allowed to air-dry for a few minutes. To further characterize the formulation, Dynamic Light Scattering (DLS) analysis was performed.

The degradation of λ -cyhalothrin of both formulations (nano-based and traditional) was assessed at the bioassay temperatures: 20 and 24 °C during 96 h (i.e. biomarkers exposure time). Also, the release of the a.i. from the capsules of the nanopesticide was analyzed under the same conditions by filtrating the sample through 0.22 μ m pore-size nylon membrane (Gamafil, Argentina). λ -Cyhalothrin concentrations of both filtered and unfiltered samples were assessed as follows: 40 mL of sample was subjected to a liquid-liquid extraction using 2 mL of hexane (pre-saturated with ultrapure water) and 0.4 g of NaCl. The λ -cyhalothrin concentrations were determined by gas chromatography with electron capture detector (GC-ECD). The methodology was validated in accordance with [SANTE/12830/2020 Rev. 2 \(2023\)](#). The validation process included a detailed evaluation of recovery, selectivity, limits of detection (LOD), limits of quantification (LOQ), linearity, precision, and accuracy. Pesticide recovery was assessed using three replicates at two concentration levels (10 and 50 μ g/L). Average recoveries ranged between 70 and 120 %, with a relative standard deviation (RSD) below 20 %. The LOD and LOQ were determined at 0.3 and 1.0 μ g/L, respectively, based on the signal-to-noise ratio of five blank injection replicates, calculated as 3 times S/N for the LOD and 10 times S/N for the LOQ. The LOQ was subsequently verified through a recovery assay (see Supplementary material).

2.4. Lethality

Acute toxicity tests were performed to obtain the lethal concentration 50 (LC50) of each pesticide: nano-based and traditional, at two temperatures: 20 and 24 °C with 16:8 day:night photoperiod. [APHA \(2017\)](#) protocol was followed with some modifications. *D. magna* neonates (<24 h) were exposed to different concentrations (0–16 μ g/L) of each formulation with a dilution factor of 2 ([Table 1](#)). Two intermediate dilutions were performed for each formulation in distilled water to minimize error. λ -Cyhalothrin concentration in the second intermediate

Table 1

λ -Cyhalothrin exposure concentrations for traditional and nano-based formulations at two temperatures to assess lethality and biomarkers (butyrylcholinesterase and body stores) in *Daphnia magna*.

λ -cyhalothrin concentration (μ g/L)			
Traditional ZERO®		Encapsulated KARATE ZEON®	
20 °C	24 °C	20 °C	24 °C
Mortality			
0	0	0	0
0.063	0.063	0.063	0.063
0.125	0.125	0.125	0.125
0.25	0.25	0.25	0.25
0.5	0.5	0.5	0.5
1	1	1	1
2	2	2	2
4		4	
		8	
		16	
Biomarkers			
0	0	0	0
0.01	0.01	0.01	0.01

dilution (400 μ g/L) before the final dilution in dechlorinated and aerated tap water for each tested concentration was confirmed as described before. Five replicates were performed in 50 mL beakers with five organisms each. Lethality, determined as immobility after stimulus, was recorded after 24 and 48 h without media renewal (static).

2.5. Biomarkers

D. magna neonates (<24 h) were exposed to 0.01 μ g/L (10 % of the 48 h LC50s) of each pesticide: nano-based and traditional plus a control, at both temperatures: 20 and 24 °C with 16:8 day:night photoperiod. This concentration is below the detected in freshwater environments ([Mac Loughlin et al., 2022](#); [Wang et al., 2023](#)). Two intermediate dilutions were performed for each formulation in distilled water to minimize error. λ -Cyhalothrin concentration in the second intermediate dilution (400 μ g/L) before the final dilution in dechlorinated and aerated tap water for the tested concentration was confirmed as described before. Five replicates were performed in 300 mL beakers with 15 organisms each. Organisms were fed every two days with 100 μ L of algae suspension (*T. obliquus*, 106 cells/ml) and trout chow-yeast-alfalfa complement ([APHA, 2017](#)). The experiment was static (without media renewal) and lasted 96 h in order to avoid reproduction as it can interfere on the analyzed endpoints.

2.5.1. Butyrylcholinesterase activity

Butyrylcholinesterase (BChE) activity was assessed as *D. magna* ChE showed a preference for butyrylcholine over acetylcholine as substrate ([Li and Tan, 2011](#); [Yang et al., 2013](#)). Briefly, 15 organisms from each experimental unit were homogenized in 200 mL of ice-cold 1.15 % KCl in Na, K phosphate buffer (0.01 M, pH 7.4, containing 0.02 M EDTA). The homogenates were centrifuged at 10,000 \times g (for 15 min at 4 °C) and the supernatant was used for the measurement of BChE activity using a commercial kit (Wiener Lab®) adapted to a microplate reader. All measurements were performed in triplicate and expressed per milligram of protein ([Bradford, 1976](#)).

2.5.2. Body stores

Macromolecules related to the energetic reserves of *D. magna* were assessed. A pool of 15 organisms was employed to quantify the glucose-glycogen content. After alkaline disruption, glycogen was precipitated by ethanol and then hydrolyzed and dehydrated using anthrone reagent. The generated colored complexes were measured spectrophotometrically at 620 nm ([Seifter et al., 1950](#)). For lipids and protein analysis a pool of 30 organisms per experimental unit was employed. Total lipids were extracted with a chloroform: methanol mixture (2:1) and washed afterwards with a NaCl solution ([Folch et al., 1957](#)). After centrifugation, the organic-lipid layer was separated and evaporated under a stream of N₂. The dried lipid extract was weighed to obtain the grams of total lipids of the sample ([Bligh and Dyer, 1959](#)). Protein determination was carried out following [Lowry et al. \(1951\)](#): the proteins were first treated with an alkaline copper solution and then the Folin Ciocalteu phenol reagent was added. The proteins cause the reduction of phosphomolybdic and phosphotungstic acids, forming a complex with a characteristic blue color, which absorbance was measured at 660 nm. Energetic reserves were expressed as micrograms per individual. The whole energy budget was calculated with the corresponding energy equivalents: 17 kJ/g glycogen +39.5 kJ/g lipids +24 kJ/g proteins, and expressed as joules per individual ([Smolders et al., 2003](#)).

2.6. Analysis of data

The LC50 for each pesticide (nano and traditional formulations) and temperature (20 and 24 °C) were estimated based on the lethality data recorded after 24 and 48 h of exposure through Probit analyses ([Finney, 1971](#)) performed with the “drc” Rstudio package ([Ritz et al., 2015](#)). The means of BChE activity, body stores (protein, glycogen, and lipids), and

whole energy budget of *D. magna*, were compared among treatments (control, nanopesticide, and traditional formulation) and temperatures (20 and 24 °C) through two-way analysis of variance (ANOVA, Tukey post-test). When the assumptions of normality and homoscedasticity were not met, Kruskal-Wallis test was performed. Comparison of means were performed with the “stat” Rstudio package (Bolar, 2019).

3. Results

3.1. Pesticide characterization and degradation

Transmission electron microscopy (TEM) analysis was employed to visualize the size and morphology of the nano-based formulation. TEM images (Fig. 1 a.-c.) revealed spherical nanoparticles with some degree of agglomeration. DLS analysis (Fig. 1 d.) further confirmed the particle size distribution, indicating two distinct populations: a dominant population with a hydrodynamic diameter of 1752 ± 421 nm and a smaller population at 467 ± 97 nm. Although the average hydrodynamic sizes were in the micrometer range, smaller particles in the nanometer range were observed. This is consistent with the TEM images, which show that the morphology of the capsules is spherical and that they are mainly in the micrometer size range (Fig. 1 a.-c.). The TEM images also reveal a less dense central area, consistent with the presence of a cavity inside the capsule, where the active substance is loaded. DLS characterization confirmed the presence of both micro- and nano-capsules. This agrees with the literature data, where polydisperse suspensions with elements in the nanometer size range have been described.

At the initial time, the a.i. was not detected outside the capsules in the exposure medium. After 96 h, a greater decrease of the a.i. was observed at 24 °C compared to 20 °C (47 % nano and 29 % traditional).

The percentage of a.i. released from the capsules was 63 % at 20 °C and 40 % at 24 °C. However, these values may be influenced by the simultaneous degradation of the a.i. (67 % of degradation of filtrated a.i., i.e. λ -cyhalothrin outside the capsules, at 24 °C).

3.2. Lethality

After 24 h, the traditional formulation had greater lethality (LC50: 2.8 $\mu\text{g/L}$) than the nanopesticide (LC50: 13.4 $\mu\text{g/L}$) at 20 °C (Fig. 2). However, at 48 h an inverse pattern was observed as the nanopesticide showed to be more lethal (LC50_{20°C}: 0.07 and 0.18 $\mu\text{g/L}$ for nano and traditional, respectively). At 24 °C the 24 h lethality of both pesticides increased (LC50: 1.65 y 1.24 $\mu\text{g/L}$ for nano and traditional, respectively). After 48 h, the 24 °C lethality of the traditional formulation was higher compared to 20 °C (LC50_{24°C}: 0.08 $\mu\text{g/L}$), while the 48-h lethality of the nanopesticide was not influenced by temperature (LC50_{24°C}: 0.07 $\mu\text{g/L}$) (Fig. 2).

3.3. Biomarkers

3.3.1. BChE activity

BChE activity was inhibited at 24 °C by both pesticides ($p = 0.05$). The nanopesticide induced a 65 % inhibition and the traditional formulation, 61 %. An interaction between treatments and temperature was observed ($p = 0.038$) (Fig. 3).

3.3.2. Body stores

The increase in temperature (24 °C) negatively affected protein and glycogen content ($p < 0.001$) (Fig. 4 a., b.). An interaction between treatments and temperature was observed for glycogen ($p = 0.001$). The

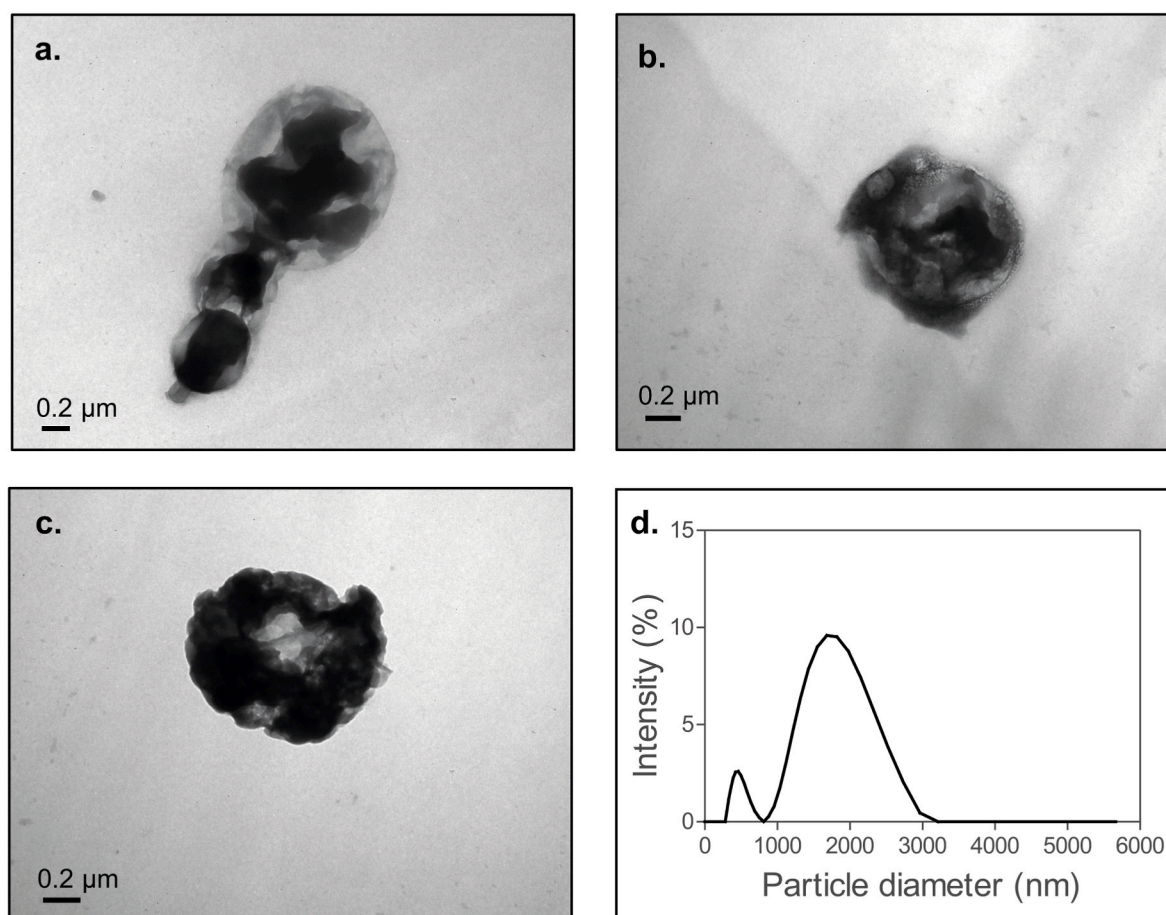


Fig. 1. Nano-based formulation characterization. Transmission electron microscopy (TEM) images (a., b., c.). Dynamic Light Scattering (DLS) analysis (d.).

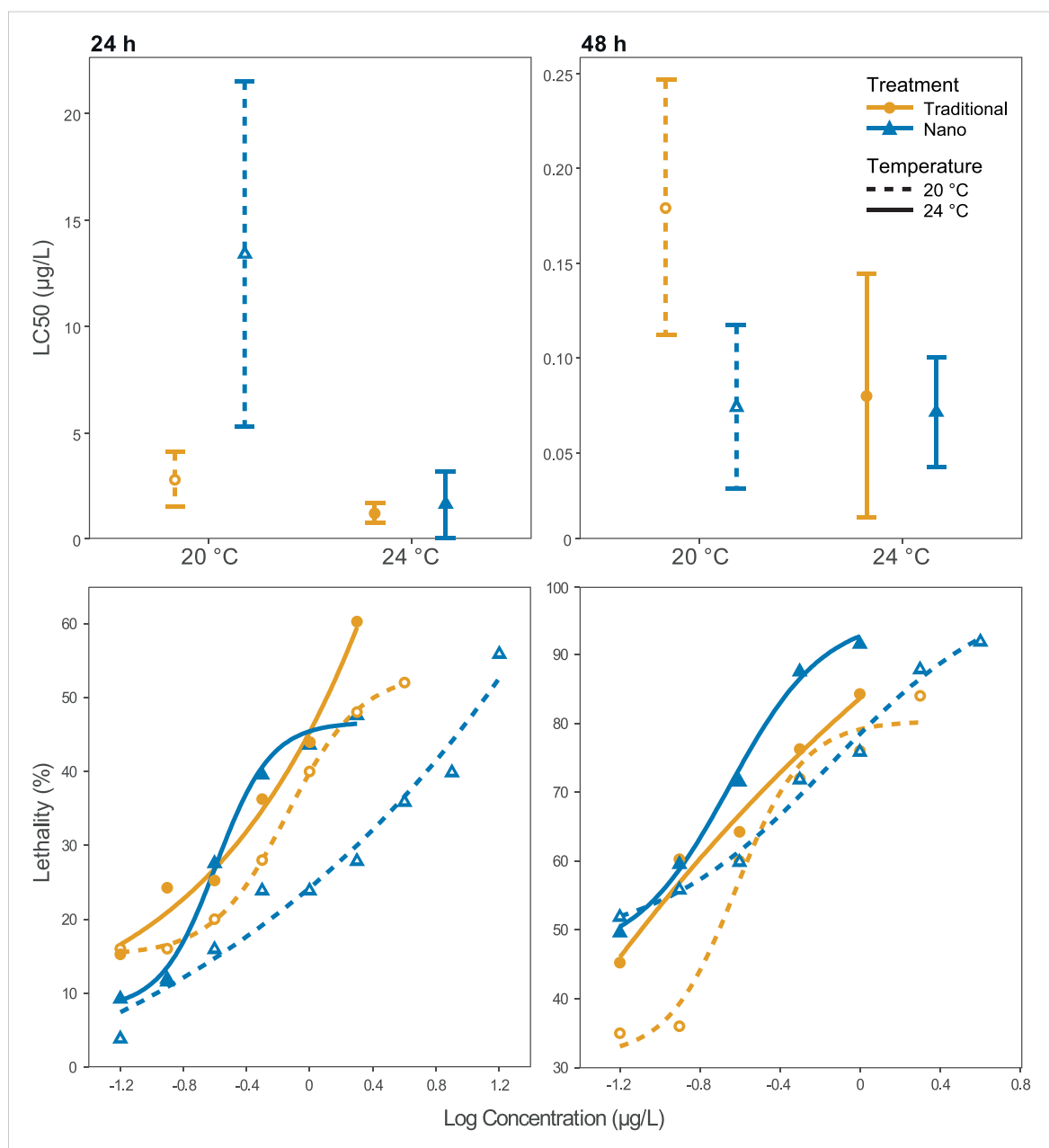


Fig. 2. Lethal concentration 50 (LC50, µg/L), 95 % confidence intervals, and dose-response curves of traditional and nano-based pesticides at different temperatures and exposure times on *Daphnia magna*.

traditional formulation reduced glycogen compared to control at 24 °C ($p = 0.037$) (Fig. 4 a.). A decrease in lipid content and the whole energy budget was observed in organisms exposed to the traditional formulation at both temperatures compared to control and the nano-based formulation ($p = 0.01$) (Fig. 4 c and d.). The whole energy budget was mainly composed of lipids, followed by proteins (Fig. 4 d.). The nano-pesticide did not affect the energetic reserves of *D. magna* ($p > 0.05$).

4. Discussion

4.1. Pesticide characterization and degradation

We found that TEM images correlate with the DLS results since we were able to appreciate the populations found in the DLS study. The TEM and DLS analysis revealed a polydisperse system with a predominant population of micro-sized capsules, as evidenced by the average

hydrodynamic diameter of 1752 nm. However, the presence of a smaller population with a hydrodynamic diameter of 467 nm, along with the observation of nanoparticles in the TEM images, suggests the co-existence of nano-sized capsules in the formulation. This finding is consistent with previous reports, further supporting the complex nature of the system (Gomes et al., 2023; Paz-Trejo et al., 2022). The observed spherical morphology and the presence of a central cavity in the capsules in the TEM images are indicative of their potential as drug delivery carriers.

Pesticide degradation is known to depend on temperature, as it can enhance microbial degradation (Kaur et al., 2023). Particularly, the degradation of λ -cyhalothrin in soil is improved by temperature (Tariq et al., 2006).

We found that at the initial time there were no a.i. outside the capsules. In this sense, Huang et al. (2022) reported that the total release of λ -cyhalothrin from nanocapsules occurred after 24 h. The difference in

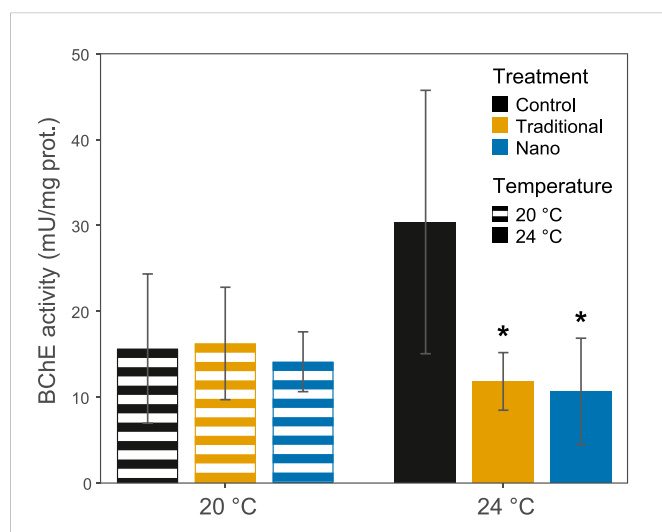


Fig. 3. Butyrylcholinesterase activity (BChE, mU/mg prot., mean values and standard deviation) of *Daphnia magna* exposed to traditional and nano-based pesticides at different temperatures. *indicates significant differences.

the release of a.i. from the capsules at different temperatures (63 % at 20 °C and 40 % at 24 °C) could not be interpreted without considering the higher degradation of the a.i. released (i.e. λ -cyhalothrin outside the capsules) at 24 °C compared to 20 °C.

4.2. Lethality

The lower lethality of the nanopesticide compared to the traditional formulation at 24 h (20 °C) may be due to the gradual release of the a.i. from the capsules, resulting in a delay in the organisms exposure. This is consistent with the observed release of a.i. from the capsules, as no a.i. was detected outside of the capsules at the initial time. However, this pattern was reversed after 48 h, and although the nanopesticide had a delayed effect, it was more lethal for *D. magna* after 48 h, showing that their comparative toxicity was timing dependent. This agrees with the observed by Huang et al. (2022), who reported that the total release of the λ -cyhalothrin from capsules occurred after 24 h. There are very few studies comparing the toxicity of encapsulated and traditional formulations of pesticides or the isolated a.i. Only one study compared the toxicity of an encapsulated λ -cyhalothrin formulation with the a.i., and reported that the first was more toxic than the second in *Danio rerio* fish (Meredith et al., 2016). However, other encapsulated pyrethroids such as bifenthrin and cypermethrin were less toxic for fish species than their traditional counterparts (Amjad et al., 2018; Blewett et al., 2019). Likewise, a nanoencapsulation of the organophosphate insecticide dimethoate was less toxic for *D. rerio* than a traditional formulation and the a.i. alone (Vallim et al., 2022). There are also controversial reports on comparative toxicity for herbicides. Atrazine-loaded nanocapsules were less toxic than the isolated a.i. in *Prochilodus lineatus* fish (de Andrade et al., 2019) and *Pseudokirchneriella subcapitata* algae (Clemente et al., 2014); however, the opposite was reported for *Daphnia similis* (Clemente et al., 2014) and *Chironomus sancticarloi* insect larvae (de Albuquerque et al., 2021). It is also worth mentioning that in the present study both pesticides were lethal to *D. magna* at environmentally relevant concentrations (He et al., 2008; Mac Loughlin et al., 2022; Papadakis et al., 2015; Wang et al., 2023), which poses a risk to this species and other more sensitive.

On the other hand, the higher temperature (24 °C) increased the lethality of both formulations at 24 h (56 % for the traditional and 88 % for the nano-based) and the traditional formulation at 48 h (55 %). There are no reports in the literature analyzing the effects of nanopesticides under temperature increase. Temperature was shown to

worsen pesticides (and other contaminants) toxicity mainly due to increased metabolism, leading to higher energy consumption and pesticides uptake rate (Huang et al., 2023; Khan and Khan, 2008). Synergistic interactions at the population level were reported for temperature and chlorpyrifos (García-Astillerio et al., 2024) or glyphosate (López-Valcárcel et al., 2023) in *D. magna*. Also, Huang et al. (2023) found that a temperature increase worsened the uptake and biotransformation rates of imidacloprid and flupyradifuron, also enhancing lethality and feeding inhibition behavior in the amphipod *Gammarus pulex*.

Conversely, the 48-h lethality of the nanopesticide did not vary according to the temperature. One possible explanation might be that the extra stress imposed by the temperature increase was countered by the high degradation (67 %) of the filtrated λ -cyhalothrin (i.e., a.i. outside the capsules) observed at 24 °C. However, more research on this topic is needed.

4.3. Biomarkers

4.3.1. BChE activity

BChE activity was inhibited by both formulations at the higher temperature (24 °C) after the chronic exposure (0.01 μ g/L), whereas both factors significantly interacted. Similarly, Laetz et al. (2014) reported that a temperature increase exacerbated the inhibition of ChE activity by ethoprop and malathion in *Oncorhynchus kisutch* fish. Likewise, Fuller et al. (2021) found that the inhibition of ChE activity in brain of *Menidia beryllina* fish by chlorpyrifos was aggravated by temperature increase.

The observed inhibition of BChE activity was similar for both formulations (traditional and nano). Although it is not their main mechanism of action, it has been reported that pyrethroids can inhibit ChE in different organisms such as fish (Cazenave et al., 2024; Santana et al., 2021; Velišek et al., 2007), shrimp (Tu et al., 2012), rat (Abdel-Daim and El-Ghoneimy, 2015), and human (Holyńska-Iwan and Szweczyk-Golec, 2020). Toumi et al. (2015) and Ren et al. (2017) reported that deltamethrin, a type II pyrethroid, inhibit acetylcholinesterase in *D. magna*. Type II pyrethroids cause membrane depolarization of the nerve terminal resulting in severe disruption of synaptic transmission (He et al., 2008). Pyrethroids may also exert indirect effects that ultimately promote the accumulation of neurotransmitters: I) acting on nicotinic acetylcholine receptors (Gupta and Crissman, 2013); II) causing up regulation of acetyltransferase (Toumi et al., 2014); III) inducing oxidative stress and damaging membrane proteins, including ChE (Tu et al., 2012); IV) altering gene expression, potentially affecting ChE biosynthesis; V) altering the anchoring of ChE to the synaptic membrane (Badiou and Belzunces, 2008); and VI) interacting with the active site of ChE due to their lipophilic nature (Colovic et al., 2013; Holyńska-Iwan and Szweczyk-Golec, 2020).

4.3.2. Body stores

Protein and glycogen content were negatively affected by the increase in temperature. Thermal stress accelerate metabolism and induce oxidative stress and hypoxia on *D. magna* (Khan and Khan, 2008; Samanta et al., 2020; Sanpradit and Peerakietkhajorn, 2023). These impairments imply the disruption of the energy homeostasis, resulting in trade-offs as energy is allocated to deal with stress over other fitness functions (Müller et al., 2018; Samanta et al., 2020). Protein metabolism is essential for growth, therefore the consumption of this energy reserve to face stress may impair organisms development (Sanpradit and Peerakietkhajorn, 2023).

Glycogen content was negatively affected by the traditional formulation at 24 °C. It was the most affected energy reserve with an average reduction of 40 %. Glycogen constitutes the first energy source to cope with stress as it is rapidly available (Smolders et al., 2003). Pyrethroids were shown to affect this energy source in freshwater invertebrates. For instance, carbohydrates depletion was reported for *D. magna* exposed to

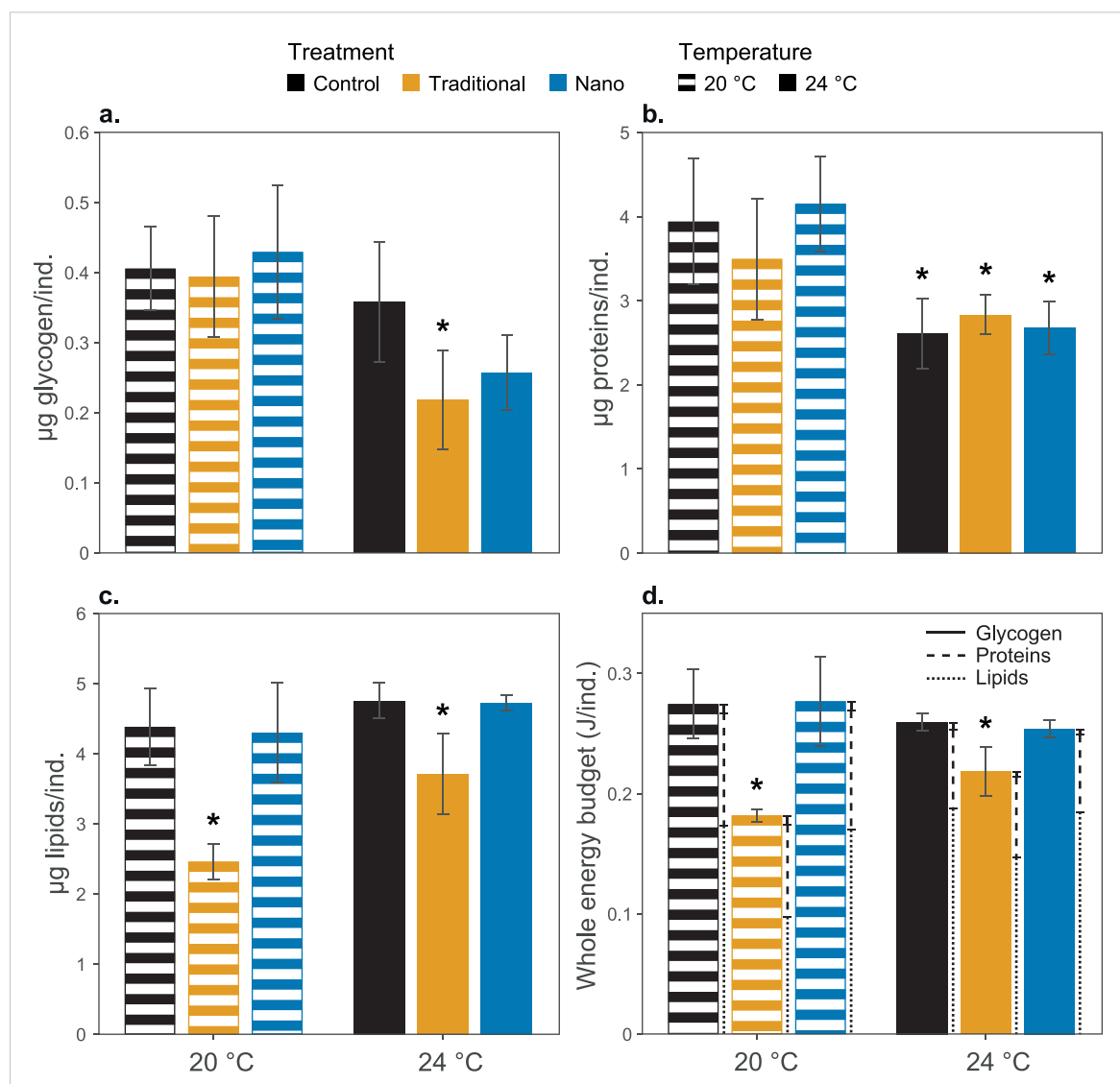


Fig. 4. a. Glycogen, b. protein, and c. lipid content (µg/ind.) of *Daphnia magna* exposed to traditional and nano-based pesticides at different temperatures. d. Whole energy budget of *D. magna* (columns) and proportional contribution of each body store to this budget (lines). * indicates significant differences.

deltamethrin (Rodrigues et al., 2023) and cypermethrin (Christensen et al., 2005), and for *Gammarus pulex* amphipod exposed to λ -cyhalothrin (Heckmann et al., 2005).

Lipid content was negatively affected by the traditional formulation (average decrease: 32 %) regardless of temperature. The same pattern was observed for the whole energy budget, as lipids were the main energy reserve in terms of J/ind. of *D. magna*. Similarly, cypermethrin exposure induced lipid (and total energy content) depletion in *Danio rerio* fish (Rodríguez-Estrada et al., 2016). Organisms seemed to use part of their lipid budget to cope with the stress by detoxifying, repairing damaged cells, and ultimately maintaining homeostasis (Sanpradit and Peerakietkhajorn, 2023). In addition, cypermethrin and λ -cyhalothrin were reported to impair the feeding efficiency and swimming ability of *D. magna*, thereby limiting food intake and subsequently available energy through behavioral neurotoxic effects (Bownik et al., 2019; Christensen et al., 2005). As lipids are essential for the reproduction of organisms, the impact on these reserves may have implications at the population level (Rodrigues et al., 2023; Sancho et al., 2009).

The nanopesticide did not affect the energetic reserves of *D. magna* at the tested concentration (0.01 µg/L) and exposure time (96 h). This might be related to the delayed toxicity observed in the lethality

bioassay. Although both formulations showed a similar response in terms of BChE activity, the body stores of organisms tend to be affected at longer times of exposure. Therefore, a delay in exposure for the nanopesticide may have reduced its effect on energy reserves. However, such observations need to be further studied including multiple exposure times and concentrations of pesticides.

5. Conclusion

The ecotoxicological assessment of nanopesticides is incipient, the present work constitutes a contribution to their comparative analysis with traditional formulations also considering global warming projections. Although the nano-based formulation had delayed toxicity, it was more lethal than the traditional one in the long term. The temperature rise according to climate change projections worsened the formulations effects in terms of decreased survival, inhibition of BChE activity, and energy reserves depletion. The development and regulation of new eco-safer nanopesticides needs to be complemented and fed back by their ecotoxicological assessment. Moreover, it is imperative to analyze their impact in the context of climate change in order to develop mitigation and adaptation strategies.

CRediT authorship contribution statement

Victoria Soledad Andrade: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Analía Ale:** Writing – review & editing, Methodology, Investigation. **Andrea Silvana Rossi:** Writing – review & editing, Investigation, Formal analysis. **Jimena Cazenave:** Writing – review & editing, Investigation, Formal analysis. **Pablo Edmundo Antezana:** Writing – review & editing, Formal analysis. **Florencia Valentina Magni:** Writing – review & editing, Formal analysis. **María Rosa Repetti:** Writing – review & editing, Formal analysis. **Martín Federico Desimone:** Writing – review & editing, Resources, Methodology, Funding acquisition. **María Florencia Gutierrez:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Funding

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET [PIP 11220220100255CO, and PIP 0826] and Universidad de Buenos Aires, UBACYT [20020150100056BA and PIDAE2022].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thanks Patricio Francisco Alvarenga (INALI-CONICET-UNL) for his assistance in organisms culture maintenance, and Celeste Mora (INALI-CONICET-UNL) for her assistance in biomarkers determinations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2025.144371>.

Data availability

Data will be made available on request.

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