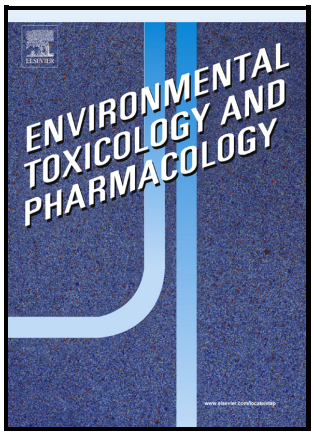
Nanosilica size-dependent toxicity in *Ceriodaphnia reticulata* (Cladocera)



Victoria Soledad Andrade, Analía Ale, Sofia Municoy, Carla Bacchetta, Martín Federico Desimone, María Florencia Gutierrez, Jimena Cazenave

PII: S1382-6689(23)00180-1

DOI: https://doi.org/10.1016/j.etap.2023.104238

Reference: ENVTOX104238

To appear in: *Environmental Toxicology and Pharmacology*

Received date: 7 June 2023
Revised date: 11 July 2023
Accepted date: 28 July 2023

Please cite this article as: Victoria Soledad Andrade, Analía Ale, Sofia Municoy, Carla Bacchetta, Martín Federico Desimone, María Florencia Gutierrez and Jimena Cazenave, Nanosilica size-dependent toxicity in *Ceriodaphnia reticulata* (Cladocera), *Environmental Toxicology and Pharmacology,* (2023) doi:https://doi.org/10.1016/j.etap.2023.104238

Nanosilica size-dependent toxicity in *Ceriodaphnia reticulata* (Cladocera)

Andrade, Victoria Soledad[a]*; Ale, Analía[a]; Municoy, Sofia[b]; Bacchetta, Carla[a]; Desimone, Martín Federico[b]; Gutierrez, María Florencia[ac]; Cazenave, Jimena[ad]

[a] Instituto Nacional de Limnología (CONICET-UNL), Santa Fe, Argentina.

[b] Universidad de Buenos Aires, Instituto de Química y Metabolismo del Fármaco (IQUIMEFA, CONICET-UBA), Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental, Buenos Aires, Argentina.

[c] Escuela Superior de Sanidad “Dr. Ramon Carrillo” (FBCB-UNL), Santa Fe, Argentina.

[d] Departamento de Ciencias Naturales, Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral (FHUC-UNL), Santa Fe, Argentina.

*vandrade@inali.unl.edu.ar Instituto Nacional de Limnología (CONICET-UNL), Ciudad Universitaria, 3000. Santa Fe, Argentina. +54 0342 4511645. https://orcid.org/0000-0002-1860-9792

Abstract

Silica nanoparticles (SiNP) are the most produced nanomaterials due to their variety of applications. When released to environments, surface water bodies are their main final sink. SiNP toxicity is still inconclusive and may vary according to particle properties such as their size. We analyzed the size-related effects of SiNP (22 and 244 nm) on mortality, life history traits, and oxidative stress in the cladoceran *Ceriodaphnia reticulata*. The smaller SiNP ($LC50_{72h}$: 105.5 µg/ml) were more lethal than the larger ones ($LC50_{72h}$ >500 µg/ml). The 22 nm-sized SiNP decreased the number of molts and neonates, increased

superoxide dismutase and inhibited glutathione S-transferase activities, while larger SiNP did not exert substantial effects on the organisms at the tested concentrations. In conclusion, SiNP toxicity depended on their size, and this information should be considered for regulatory purposes and to the development of safe-by-design nanoproducts to ultimately guarantee the environment protection.

Keywords: Silica nanoparticles; Nanotoxicity; Microcrustacean; Mortality; Life history traits; Oxidative stress

1. Introduction

Nanotechnology industry is continuously increasing and diversifying due to nanoparticles (NP) unique characteristics (Salem et al., 2022). Silica nanoparticles (SiNP) are the most widely produced ones with an estimated global consumption of more than four million tons per year (IHS, 2021). Due to their particular properties like variable size, hydrophobicity, large pore volume, and economic synthesis, they have a wide variety of applications such as drug delivery, biomedicine, food, biotechnology, pesticides, personal care products, environmental remediation, wastewater purification, semiconductors, and ceramics (Akhter et al., 2022). Although SiNP are usually considered biocompatible, they have been proved to exert oxidative stress, inflammation, autophagy, fibrosis, deregulation of cellular energetics, and apoptosis in human models (Huang et al., 2022; Liu and Sayes, 2022).

The SiNP are released to the environment throughout their entire life cycle, and surface water are their main final sink. Accordingly, their predicted environmental concentrations range is approximately between 0.12 and 2.6 µg/L (Wang et al., 2016; Wang and Nowack, 2018), although empirical data on environmental concentrations are currently lacking. Despite that little is known about their potential toxic effects on non-target organisms, some reports showed that SiNP exerted deleterious effects on freshwater biota such as algae, crustaceans, mussels, and fish (Ale et al., 2021; Book and

Backhaus, 2022). Cladocerans have been widely used as model non-target organisms as they are highly sensitive to contaminants, and are ubiquitous in freshwater systems where they constitute intermediate links in trophic networks contributing to nutrients and energy cycling (Ferdous and Muktadir, 2009; Mano and Tanaka, 2016). SiNP ecotoxicological studies in Cladocera are controversial since, while some studies affirm that they are safe (Book et al., 2019; Karimi et al., 2019; Lapin et al., 2018; Rivero Arze et al., 2021), others reported lethal and sublethal effects such as bioaccumulation, genotoxicity, behavior, growth, and reproduction (Kim et al., 2021; Maia et al., 2023; Puerari et al., 2021; Shariati et al., 2020; Vicentini et al., 2017). Since the available reports were mainly focused on *Daphnia magna*, such information should be complemented with sublethal effects on more sensitive and representative species of holotropical regions. Despite oxidative stress has been pointed as the main SiNP mechanism of action (Ale et al., 2021; Book and Backhaus, 2022), to the best of our knowledge, there are no reports of this mechanism in crustaceans. The analysis of a battery of biomarkers gives an early response to contaminants which, complemented with mortality and life history traits, constitutes an integrated response.

As nanotoxicity widely depends on intrinsic (defined when being synthesized) and extrinsic properties (environmental conditions), SiNP toxicity have shown to vary according to their external charge, shape, size, and pore size (Book et al., 2019; Huang et al., 2022; Zhu et al., 2019). Particularly, their size has been pointed as one of the most determining characteristics, being the smaller SiNP, the more toxic due to their higher surface-volume ratio, which usually implies greater reactivity, and therefore, greater toxicity. Moreover, it has been reported that smaller NP are more likely to penetrate biological membranes and then exert cytotoxicity (Clément et al., 2013; Liu and Sayes, 2022). However, this hypothesis needs to be studied in greater depth because some controversial results have been achieved. Such information is a very important input for the safe-by-design approach in nanotechnology industry. Having stated that, the aim of

this study was to analyze the size-related effects of SiNP on mortality, life history traits, and oxidative stress in *Ceriodaphnia reticulata* (Jurine).

2. Materials and Methods

2.1 Silica nanoparticles

Small SiNP (22 nm-sized) were purchased from LUDOX® (TM-50 colloidal silica). The bigger ones, (244 nm-sized) were synthetized through Stöber method (Stöber et al., 1968; Thomassen et al., 2010). Briefly, tetraethylorthosilicate was added dropwise to a stirred solution of ultrapure water, absolute ethanol, and ammonium hydroxide. The concentration of reagents used determines the size of the particles. The solution was stirred overnight at room temperature. The obtained suspension of 244 nm-SiNP and the commercial 22 nm-SiNP were dialyzed using cellulose membranes (MEMBRA-CEL MD44 14x100) against distilled water until a neutral pH was reached. The particles were washed twice with absolute ethanol, once with deionized water, and then centrifuged (10,000 *g*). Finally, the SiNP were suspended in distilled water. SiNP were characterized through transmission electron microscopy (TEM), Scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR), obtained over the range of 4000–500 cm^{-1} using an FTIR-Raman Nicolet iS 50 (Thermo Scientific). The zeta potential of SiNP was estimated through a Zetasizer Nano-Zs (Malvern Instruments, UK) using polycarbonate capillary cells (DTS1070, Folded Capillary cell).

Stock solutions were prepared in ultrapure water before each bioassay and stored in darkness to prevent any prior transformation (e.g., agglomeration or aggregation). SiNP were sonicated (5 min, 40kHz) and vortexed to properly resuspend them before aliquoting to prepare stock solutions. Dissolved oxygen (DO, mg/L), conductivity (µs/cm), and pH were measured (Hanna multiparameter portable meter) at the beginning and the end of the bioassays.

2.2 Test organisms

Ceriodaphnia reticulata individuals were collected from a lake of the Paraná River basin and cultured under laboratory conditions for two months in order to obtain ten consecutive generations. The neonates for the toxicological experiments were developed from a parthenogenetic female isolated from the initial stock culture. The culture conditions were 12/12 day/night photoperiod, 21±1 °C, in dechlorinated and aerated tap water (pH: 7.1, conductivity: 700 µs/cm, total hardness: 180 mg/L $CaCO_3$, alkalinity 120 mg/L $CaCO_3$, 39 mg/L Ca^{++}, 20 mg/L Mg^{++}, 146 mg/L HCO_3^-). Culture water was changed three times a week and the organisms were fed with *Tetradesmus obliquus* (Turpin) MJ Wynne (before *Scenedesmus obliquus*) every other day.

The strain of *T. obliquus* was also isolated from a lake of the Paraná River basin. The axenic culture was grown in Detmer modified medium for green algae (Watanabe, 1960) (KH_2PO_4: 50, KCl: 50, $MgSO_4$-$7H_2O$: 360, Ca $(NO_3)_2$-$4H_2O$: 360, Cl_3Fe^+: 5, H_3BO_3, 2.86, $C_4H_6O_6$: 5, $MnCl_2$-$4H_2O$: 1.81, Cl_2Cu: 0.05, $ZnSO_4$-$7H_2O$: 0.23 mg/L), at 25 °C with warm-white LED light (2600 lx) and constant aeration. At the exponential growth phase, the culture was cropped and algae were resuspended in sterile distilled water and stocked in darkness at -4 °C.

2.3 Acute toxicity

To obtain the lethal concentration 50 (LC50) of each SiNP size (22 and 244 nm), acute toxicity tests were performed according to APHA, (2017) with some modifications. *C. reticulata* neonates (< 24 h) were exposed to five concentrations of SiNP (10, 50, 100, 150, and 500 µg/ml) plus a control (0 µg/ml). Each treatment was 4 times replicated with five organisms each in 50 mL beakers. Culture conditions were the same as described before. The beakers were kept in an orbital shaker during experiments to simulate slow natural water movements (50 rpm) and prevent NP precipitation (Karimi et al., 2019). Mortality, determined as immobility after a stimulus, was recorded at 24, 48, and 72 h.

2.4 Life history traits and oxidative stress

Adults of *C. reticulata* (6 days old) were exposed during 72 h to two sublethal concentrations of both SiNP sizes. These concentrations were selected according to the LC50 values (50 and 100 µg/ml) plus a control. Five replicates of 250 ml with 30 organisms each were maintained under the same laboratory conditions as described before. Adult molts and neonates were daily removed. Molt number and neonate number (offspring) and size were recorded as life history response variables. At the end of the experiment, the adults were collected in pools of 30 organisms (1 per replicate) and stored at -80 °C.

For enzyme extraction, each pool of organisms was homogenized in 0.1 M sodium phosphate buffer, pH 6.5 containing 20% (v/v) glycerol, 1.4 mM dithioerythritol, and 1 mM EDTA. The homogenates were centrifuged at 4 °C at 20,000 *g* for 30 min, and the supernatant was stored at −80 °C to then assess all oxidative stress determinations (Bacchetta et al., 2014). Superoxide dismutase (SOD) activity was estimated by its ability to inhibit the epinephrine autoxidation (Misra and Fridovich, 1972); catalase activity (CAT) was determined following Beutler, (1982) adapted for microplate spectrophotometer; and glutathione S-transferase activity (GST) was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to Habig et al., (1974) with modifications for microplate assays. Lipid peroxidation (LPO) was estimated through the formation of thiobarbituric reactive substances (TBARS) according to Yagi, (1976). Briefly, a solution of trichloroacetic acid (TCA) 20% and butylhydroxytoluene (BHT) 4% was added to the samples, and they were centrifuged at 3200 *g* for 5 min. Then, thiobarbituric acid (TBA) 0.7% was added and the samples were placed in a heat bath at 100 °C for 1 h before measuring. All measurements were performed in triplicate and expressed in terms of the sample protein content (Bradford, 1976).

2.5 Statistical analysis

Prior to analysis homogeneity of variances and normality were checked when appropriate through Levene and Kolmogorov-Smirnov tests, respectively.

The means of physicochemical variables (oxygen, pH, and conductivity) were compared between treatments and through time with repeated measured analysis of variance (RM-ANOVA, Tukey post-test), with R studio package “rstatix” (Kassambara, 2020). The LC50 for each SiNP (22 and 244 nm) was estimated based on the mortality data at 24, 48, and 72 h when possible through Probit analyses (Finney, 1971) performed with the “drc” R studio package (Ritz et al., 2015). The means of neonate and molt number of *C. reticulata* exposed to each SiNP (22 and 244 nm) at each observation time (24, 48, and 72 h) were compared to control through ANOVA (Dunnett post-test) after checking model assumptions. The control means of neonates and molts were compared through time (24, 48, and 72 h) with RM-ANOVA (Tukey post-test). The enzymatic activities and LPO means of *C. reticulata* exposed to each SiNP (22 and 244 nm) were compared to control through ANOVA (Dunnett post-test).

3. Results

3.1 Silica nanoparticles

The SiNP characterization is shown in Figure 1. TEM (Figs. 1. a. and b.) and SEM (Figs. 1. c. and d.) images show spherical particles with a mean diameter of 22 ± 3 nm for the smaller SiNP (Fig. 1. e.) and 244 ± 10 nm in case of the bigger SiNP (Fig. 1. f.). The Z potential analysis of both the 22 and 244 nm SiNP showed a highly negative charge of -33.0 ± 0.7 mV and -29.6 ± 1.6 mV, respectively, at neutral pH. For further characterization, a FTIR analysis of 22 nm-SiNP and 244 nm-SiNP was performed (Fig. 1. g.). The spectra of both colloidal SiNP revealed the asymmetric vibration band of Si–O and Si –OH at 1071 and 970 cm^{-1}, respectively, and the symmetric vibration peak of Si –O at 785 cm^{-1} (Baudou et al., 2020).

The physicochemical variables of exposure media did not vary significantly between treatments or through time (RM-ANOVA $p > 0.05$) (DO: 8.9 ± 0.03 mg/L; conductivity: 696.4 ± 5.1 µs/cm; pH: 7.1 ± 0.4).

3.2 Acute toxicity

The smaller SiNP (22 nm) were more toxic than the biggest ones (244 nm) (Fig. 2). The $LC50_{72h}$ of SiNP-22nm was 105.2 µg/ml, while that of SiNP-244nm was >500 µg/ml (Table 1). However, it is worth noting that a decrease in the mortality percentage of the smaller SiNP was observed at 500 µg/ml which may be related to NP agglomeration at high concentrations.

3.3 Life history traits and oxidative stress

The molt number of *C. reticulata* adults exposed to the smaller SiNP (22 nm) significantly decreased when compared to control after 48 h of exposure both at 50 and 100 µg/ml (ANOVA, $p = 0.01$). The number of molts significantly decreased after 48 h in organisms exposed to 100 µg/ml of the bigger SiNP (244 nm) (ANOVA, $p = 0.002$) (Fig 3. a.). The neonate number of *C. reticulata* exposed to the smaller SiNP for 48 h significantly decreased compared to control in both tested concentrations (ANOVA, $p = 0.012$) (Fig. 3. b.). The neonate body size was not significantly affected by the SiNP (Fig. 3. c.). Both molt and neonate number decreased significantly in time in controls (RM-ANOVA, $p < 0.001$ and 0.001 respectively) (Figs. 3. a. b.).

The SOD activity significantly increased in *C. reticulata* organisms exposed to 100 µg/ml of the smaller SiNP (22 nm) for 72 h compared to control (ANOVA, $p = 0.025$) (Fig. 4 a.). The GST activity was inhibited significantly by the smaller SiNP at 100 µg/ml (ANOVA, $p = 0.016$) (Fig. 4. c.). However, no differences were found in the case of LPO levels. The bigger SiNP (244 nm) did not significantly affect neither the antioxidant enzymes nor exert oxidative damage in lipids in *C. reticulata* under the tested concentrations (Fig. 4).

4. Discussion

4.1 Silica nanoparticles

Two different sizes of SiNP were studied. TEM and SEM images and size distribution analysis (Fig. 1) clearly exhibit spherical nanoparticles with a main diameter of 22 and 244 nm, respectively. The obtained Z potential values indicate that the negatively charged particles repel each other, resulting in highly stable SiNP suspensions. The greater absolute value of negative Z potential obtained for smaller particles compared to the large particles can be attributed to the fact that smaller particles are more influenced by the Brownian motion tending to collide with other particles and producing an increment of the surface charge (Jiang et al., 2009; Nakatuka et al., 2015).

4.2 Acute toxicity

The smaller SiNP (22 nm) exerted higher lethality in *Ceriodaphnia reticulata* than the bigger ones (244 nm). This agrees with several studies such as Clément et al., (2013), Kim and Choi, (2008), and Lee et al., (2009), who reported that *Daphnia magna* mortality was SiNP-size dependent, being the smaller particles the more toxic. Size is considered one of the most determining properties of NP toxicity. It is usually attributed to the higher surface-volume ratio of smaller NP, which increases the particles surface energy and thereby their reactivity (Jiang et al., 2009). Moreover, smaller NP can easily penetrate biological membranes and exert cytotoxicity (Liu and Sayes, 2022; Santo et al., 2014). This can also be related to the fact that a smaller NP size implies a higher number of NP in the same mass, as discussed by Hull et al., (2012) and Kennedy et al., (2015).

4.3 Life history traits and oxidative stress

When *C. reticulata* was exposed to the 22 nm-sized SiNP for 48 h, a significative decrease in molt and neonate numbers was observed. Accordingly, several studies reported that SiNP may cause impairments in growth and reproduction in *D. magna* (Kim et al., 2021; Puerari et al., 2021; Vicentini et al., 2017). The authors argued that it could be due to swimming and feeding behavior alterations and SiNP accumulation (Kim et al., 2021; Maia et al., 2023; Yang et al., 2014) which can affect the energy availability to

cope with the exposure stress. On the other hand, the larger SiNP only significantly affected the molt number at the higher tested concentration (100 µg/ml), showing to be less toxic than the smaller ones which is in congruence with the observed in the mortality assay.

The smaller SiNP induced an antioxidant enzymatic response in *C. reticulata*, while the biggest SiNP did not affect these endpoints at the tested concentrations. This agrees with the observed for mortality and life history traits, showing that oxidative stress could have been the main mechanism of toxicity. SiNP effects in oxidative stress were not previously reported in crustaceans but in other organisms such as fish and humans (Ale et al., 2021; Liu and Sayes, 2022). In the present study, the SiNP-22nm exerted an increase in the SOD activity of *C. reticulata*. In concordance, Kim et al., (2010) reported that titanium oxide NP enhanced SOD activity in *D. magna*, and this effect was highly dependent on the particle size. Also, the activation of SOD activity was reported for *Artemia salina* exposed to iron oxide (Zhu et al., 2017) and magnetic silver NP (Demarchi et al., 2020), and for the marine copepod *Centropages ponticus* exposed to nickel oxide NP (Djebbi et al., 2021). Nevertheless, other studies founded that SOD activity was inhibited in *A. salina* exposed to silver nanowires (An et al., 2019) and in freshwater shrimp *Macrobrachium rosenbergii* exposed to titanium dioxide NP (Guo et al., 2023). Mansano et al., (2018) reported that copper oxide NP induced reactive oxygen species (ROS) generation in the neotropical cladoceran *C. silvestrii*. SOD constitutes the first barrier of the antioxidant defense system as catalyze the degradation of ROS to prevent oxidative damage (Marklund and Marklund, 1974). As the main toxicity mechanisms for SiNP has reported to be oxidative stress, partly through the generation of ROS (Ale et al., 2021), the SOD activity activation evidenced in the present study may have prevented *C. reticulata* from oxidative damage, as oxidative damage in lipids were not observed.

The GST is a group of enzymes that act in the detoxification system, as catalyze the conjugation of reduced glutathione with xenobiotics, therefore GST levels are usually related with organisms susceptibility to contaminants (Habig et al., 1974). The smaller SiNP inhibited the GST activity of *C. reticulata*. This effect was also reported for *D. magna* exposed to copper and zinc oxide NP (Mwaanga et al., 2014) and for *A. salina* exposed to stannic oxide NP (Gambardella et al., 2014). However, an increase in GST activity was reported for *D. magna* exposed to titanium oxide NP (Kim et al., 2010) and for the marine copepods *C. ponticus* and *Tigriopus japonicus* exposed to nickel oxide NP (Djebbi et al., 2021) and sunscreens with zinc oxide NP (Wong et al., 2020), respectively. Although SiNP induced an antioxidant response in *C. reticulata*, this did not trigger oxidative damage in cell membranes, since no effects were observed in LPO levels. However, this response may imply changes in energy allocation as observed in the reduction of growth and reproduction.

5. Conclusion

The SiNP induced mortality, oxidative stress, and affected life history traits of *C. reticulata*. As SiNP ecotoxicity depended on their size, being the smaller ones the most toxic, this study highlights the relevance of assessing the effects of different nanoparticles intrinsic properties in sensitive non-target organisms such as cladocerans. The importance of this study lies in the fact that it is the first one evaluating oxidative stress generated by SiNP in crustaceans, therefore further investigations are highly encouraged. This piece of information is crucial for regulatory purposes and contributes to the develop of safe-by-design nanoproducts to ultimately guaranteed the environment protection.

Acknowledgments: We thank Celeste Mora (INALI, CONICET-UNL) for technical assistance in antioxidant enzymes measurements.

Funding: This work was supported by the Agencia Nacional de Promoción Científica y Técnica [grant number PICT 2018-01271, PI: Jimena Cazenave; and grant number PICT-2020-01206, PI: Analía Ale] and the Universidad de Buenos Aires [UBACYT 20020150100056BA and PIDAE 2022, PI: Martín Desimone].

Availability of data: Data associated to this document are available from the corresponding author.

References

Akhter, F., Atta, A., Mahmood, R., Abbasi, N., Ahmed, S., Mukhtiar, W., Mallah, A., Anees-ur-Rehman, H., Chandio, Z.A., 2022. A Comprehensive Review of Synthesis , Applications and Future Prospects for Silica Nanoparticles (SNPs). Silicon 14, 8295–8310. https://doi.org/10.1007/s12633-021-01611-5

Ale, A., Gutierrez, M.F., Rossi, A.S., Bacchetta, C., Desimone, M.F., Cazenave, J., 2021. Ecotoxicity of silica nanoparticles in aquatic organisms : An updated review. Environ. Toxicol. Pharmacol. 87, 103689. https://doi.org/10.1016/j.etap.2021.103689

An, H.J., Sarkheil, M., Park, H.S., Yu, I.J., Johari, S.A., 2019. Comparative toxicity of silver nanoparticles (AgNPs) and silver nanowires (AgNWs) on saltwater microcrustacean , Artemia salina. Comp. Biochem. Physiol. , Part C 218, 62–69. https://doi.org/10.1016/j.cbpc.2019.01.002

APHA, 2017. Standard Methods for the Examination of Water and Wastewater, 23rd ed. American Public Health Association, American Water Works Federation. Water Environment Association, Washington, D.C. https://doi.org/10.1016/B978-0-12-382165-2.00237-3

Bacchetta, C., Rossi, A., Ale, A., Campana, M., Parma, M.J., Cazenave, J., 2014.

Combined toxicological effects of pesticides: A fish multi-biomarker approach. Ecol. Indic. 36, 532–538. https://doi.org/10.1016/j.ecolind.2013.09.016

Baudou, F.G., Fusco, L., Giorgi, E., Diaz, E., Municoy, S., Desimone, M.F., Leiva, L., De Marzi, M.C., 2020. Physicochemical and biological characterization of nanovenoms, a new tool formed by silica nanoparticles and Crotalus durissus terrificus venom. Colloids Surfaces B Biointerfaces 193, 111128. https://doi.org/10.1016/j.colsurfb.2020.111128

Beutler, E., 1982. Catalase In Red Cell Metabolism, a Manual of Biochemical Methods. Grune and Stratton. Inc, New York.

Book, F., Backhaus, T., 2022. Aquatic ecotoxicity of manufactured silica nanoparticles : A systematic review and meta-analysis. Sci. Total Environ. 806, 150893. https://doi.org/10.1016/j.scitotenv.2021.150893

Book, F., Ekvall, M.T., Persson, M., Lönnerud, S., Lammel, T., Sturve, J., Backhaus, T., 2019. Ecotoxicity screening of seven different types of commercial silica nanoparticles using cellular and organismic assays: Importance of surface and size. NanoImpact 13, 100–111. https://doi.org/10.1016/j.impact.2019.01.001

Bradford, M., 1976. A rapid and sensitive method for the quanti- fication of microgram quantities of proteins utilizing the princi- ple of protein-dye binding. Anal. Biochem. 72, 248–254.

Clément, L., Zenerino, A., Hurel, C., Amigoni, S., Taffin de Givenchy, E., Guittard, F., Marmier, N., 2013. Toxicity assessment of silica nanoparticles, functionalised silica nanoparticles, and HASE-grafted silica nanoparticles. Sci. Total Environ. 450–451, 120–128. https://doi.org/10.1016/j.scitotenv.2013.01.042

Demarchi, C.A., da Silva, L.M., Niedźwiecka, A., Ślawska-Waniewska, A., Lewińska, S., Dal Magro, J., Fossá Calisto, J.F., Martello, R., Rodrigues, C.A., 2020.

Nanoecotoxicology study of the response of magnetic O-Carboxymethylchitosan loaded silver nanoparticles on Artemia salina. Environ. Toxicol. Pharmacol. 74. https://doi.org/10.1016/j.etap.2019.103298

Djebbi, E., Bonnet, D., Pringault, O., Tlili, K., Yahia, M.N.D., 2021. Effects of nickel oxide nanoparticles on survival, reproduction, and oxidative stress biomarkers in the marine calanoid copepod Centropages ponticus under short-term exposure. Environ. Sci. Pollut. Res. 28, 21978–21990. https://doi.org/10.1007/s11356-020-11781-1

Ferdous, Z., Muktadir, A., 2009. A Review : Potentiality of Zooplankton as Bioindicator. Am. J. Appl. Sci. 6, 1815–1819.

Finney, D., 1971. Probit analysis. Cambridge University Press, London, U.K.

Gambardella, C., Mesarič, T., Milivojević, T., Sepčić, K., Gallus, L., Carbone, S., Ferrando, S., Faimali, M., 2014. Effects of selected metal oxide nanoparticles on Artemia salina larvae: Evaluation of mortality and behavioural and biochemical responses. Environ. Monit. Assess. 186, 4249–4259. https://doi.org/10.1007/s10661-014-3695-8

Guo, Z., Zuo, J., Feng, J., Li, J., Zhang, S., Ma, K., 2023. Impact of Titanium Dioxide-Graphene Oxide (TiO2-GO) Composite Nanoparticle on the Juveniles of the Giant River Prawn, Macrobrachium rosenbergii: Physio-Biochemistry and Transcriptional Response. Mar. Biotechnol. 25, 45–56. https://doi.org/10.1007/s10126-022-10180-6

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S transferases. The first enzymatic step in mercapturic acid formation. J. Biol. chemestry 249, 7130–7139. https://doi.org/10.1016/s0021-9258(19)42083-8

Huang, Y., Li, P., Zhao, R., Zhao, L., Liu, J., Peng, S., Fu, X., Wang, X., Luo, R., Wang,

R., Zhang, Z., 2022. Silica nanoparticles: Biomedical applications and toxicity. Biomed. Pharmacother. 151, 113053. https://doi.org/10.1016/j.biopha.2022.113053

Hull, M., Kennedy, A.J., Detzel, C., Vikesland, P., Chappell, M.A., 2012. Moving beyond Mass: The Unmet Need to Consider Dose Metrics in Environmental Nanotoxicology Studies. Environ. Sci. Technol. 46, 10881–10882. https://doi.org/dx.doi.org/10.1021/es3035285

IHS, 2021. Silicates and Silicas, in: Chemical Economics Handbook. Information Handling Services.

Jiang, J., Oberdörster, G., Biswas, P., 2009. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. J. Nanoparticle Res. 11, 77–89. https://doi.org/10.1007/s11051-008-9446-4

Karimi, S., Troeung, M., Wang, R., Draper, R., Pantano, P., Crawford, S., Aravamudhan, S., 2019. Acute and chronic toxicity to Daphnia magna of colloidal silica nanoparticles in a chemical mechanical planarization slurry after polishing a gallium arsenide wafer. NanoImpact 13, 56–65. https://doi.org/10.1016/j.impact.2018.12.004

Kassambara, A., 2020. rstatix: Pipe-friendly framework for basic statistical tests. R Packag. version 0.6.0.

Kennedy, A.J., Hull, M.S., Diamond, S., Chappell, M., Bednar, A.J., Laird, J.G., Melby, N.L., Steevens, J.A., 2015. Gaining a Critical Mass: A Dose Metric Conversion Case Study Using Silver Nanoparticles. Environ. Sci. Technol. 49, 12490–12499. https://doi.org/10.1021/acs.est.5b03291

Kim, K.T., Klaine, S.J., Cho, J., Kim, S.H., Kim, S.D., 2010. Oxidative stress responses of Daphnia magna exposed to TiO2 nanoparticles according to size fraction. Sci.

Total Environ. 408, 2268–2272. https://doi.org/10.1016/j.scitotenv.2010.01.041

Kim, S.-M., Choi, J., 2008. Genotoxicity of CeO_2, SiO_2 and TiO_2 Nanoparticles in the Freshwater Crustacean Daphnia Magna. Environ. Health Toxicol. 23, 79–85.

Kim, Y., Samadi, A., Gwag, E.H., Park, Jayoung, Kwak, M., Park, Jihoon, Lee, T.G., Kim, Y.J., 2021. Physiological and behavioral effects of sio2 nanoparticle ingestion on daphnia magna. Micromachines 12. https://doi.org/10.3390/mi12091105

Lapin, A.A., Kalayda, M.L., Potapov, V. V, Zelenkov, V.N., Voropaeva, N.L., 2018. The influence of hydrothermal nanosilica powder aquaspersions on the vital capacity of Daphnia magna Straus crustaceans. Int. J. Nanotechnol. 15, 422–432. https://doi.org/https://doi.org/10.1504/IJNT.2018.094799

Lee, S.W., Kim, S.M., Choi, J., 2009. Genotoxicity and ecotoxicity assays using the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius to screen the ecological risks of nanoparticle exposure. Environ. Toxicol. Pharmacol. 28, 86–91. https://doi.org/10.1016/j.etap.2009.03.001

Liu, J.Y., Sayes, C.M., 2022. A toxicological profile of silica nanoparticles. Toxicol. Res. (Camb). 11, 565–582. https://doi.org/https://doi.org/10.1093/toxres/tfac038

Maia, M.T., Delite, F., da Silva, G., Ellis, L.J.A., Papadiamantis, A.G., de Paula, A., Lynch, I., Martinez, D.S.T., 2023. Combined Toxicity of Fluorescent Silica Nanoparticles with Cadmium in Ceriodaphnia Dubia: Interactive Effects of Natural Organic Matter and Green Algae Feeding. Available SSRN 38. https://doi.org/http://dx.doi.org/10.2139/ssrn.4415194

Mano, H., Tanaka, Y., 2016. Mechanisms of compensatory dynamics in zooplankton and maintenance of food chain efficiency under toxicant stress. Ecotoxicology 25, 399–411. https://doi.org/10.1007/s10646-015-1598-2

Mansano, A.S., Souza, J.P., Cancino-Bernardi, J., Venturini, F.P., Marangoni, V.S., Zucolotto, V., 2018. Toxicity of copper oxide nanoparticles to Neotropical species Ceriodaphnia silvestrii and Hyphessobrycon eques. Environ. Pollut. 243, 723–733. https://doi.org/10.1016/j.envpol.2018.09.020

Marklund, S., Marklund, G., 1974. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. Eur. J. Biochem. 47, 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175. https://doi.org/10.1016/s0021-9258(19)45228-9

Mwaanga, P., Carraway, E.R., van den Hurk, P., 2014. The induction of biochemical changes in Daphnia magna by CuO and ZnO nanoparticles. Aquat. Toxicol. 150, 201–209. https://doi.org/10.1016/j.aquatox.2014.03.011

Nakatuka, Y., Yoshida, H., Fukui, K., Matuzawa, M., 2015. The effect of particle size distribution on effective zeta-potential by use of the sedimentation method. Adv. Powder Technol. 26, 650–656. https://doi.org/10.1016/j.apt.2015.01.017

Puerari, R.C., Ferrari, E., Oscar, B.V., Simioni, C., Ouriques, L.C., Vicentini, D.S., Matias, W.G., 2021. Acute and chronic toxicity of amine-functionalized SiO2 nanostructures toward Daphnia magna. Ecotoxicol. Environ. Saf. 212, 111979. https://doi.org/10.1016/j.ecoenv.2021.111979

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10, 1–13. https://doi.org/10.1371/journal.pone.0146021

Rivero Arze, A., Mouneyrac, C., Chatel, A., Manier, N., 2021. Comparison of uptake and elimination kinetics of metallic oxide nanomaterials on the freshwater microcrustacean Daphnia magna. Nanotoxicology 15, 1168–1179.

https://doi.org/10.1080/17435390.2021.1994668

Salem, S.S., Hammad, E.N., Mohamed, A.A., El-Dougdoug, W., 2022. A Comprehensive Review of Nanomaterials: Types, Synthesis, Characterization, and Applications. Biointerface Res. Appl. Chem. 13. https://doi.org/10.33263/BRIAC131.041

Santo, N., Fascio, U., Torres, F., Guazzoni, N., Tremolada, P., Bettinetti, R., Mantecca, P., Bacchetta, R., 2014. Toxic effects and ultrastructural damages to Daphnia magna of two differently sized ZnO nanoparticles: Does size matter? Water Res. 53, 339–350. https://doi.org/10.1016/j.watres.2014.01.036

Shariati, F., Poordeljoo, T., Zanjanchi, P., 2020. The Acute Toxicity of SiO2 and Fe3O4 Nano-particles on Daphnia magna. Silicon 2941–2946. https://doi.org/https://doi.org/10.1007/s12633-020-00393-6

Stöber, W., Fink, A., Bohn, E., 1968. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. J. Colloid Interface Sci. 26, 62–69. https://doi.org/https://doi.org/10.1016/0021-9797(68)90272-5

Thomassen, L.C.J., Aerts, A., Rabolli, V., Lison, D., Gonzalez, L., Kirsch-Volders, M., Napierska, D., Hoet, P.H., Kirschhock, C.E.A., Martens, J.A., 2010. Synthesis and characterization of stable monodisperse silica nanoparticle sols for in vitro cytotoxicity testing. Langmuir 26, 328–335. https://doi.org/10.1021/la902050k

Vicentini, D.S., Puerari, R.C., Oliveira, K.G., Arl, M., Melegari, S.P., Matias, W.G., 2017. Toxicological impact of morphology and surface functionalization of amorphous SiO2 nanomaterials. NanoImpact 5, 6–12. https://doi.org/10.1016/j.impact.2016.11.003

Wang, Y., Kalinina, A., Sun, T., Nowack, B., 2016. Probabilistic modeling of the fl ows and environmental risks of nano-silica. Sci. Total Environ. 545–546, 67–76.

https://doi.org/10.1016/j.scitotenv.2015.12.100

Wang, Y., Nowack, B., 2018. Environmental Risk Assessment of Engineered Nano-SiO2, Nano Iron Oxides, Nano-CeO2, Nano-Al2O3, and Quantum Dots. Environ. Toxicol. Chem. 37, 1387–1395. https://doi.org/10.1002/etc.4080

Watanabe, A., 1960. List of algal strains in collection at the institute of applied microbiology, university of tokyo. J. Gen. Appl. Microbiol. 6, 283–292. https://doi.org/10.2323/jgam.6.283

Wong, S.W.Y., Zhou, G., Leung, P.T.Y., Han, J., Lee, J., Kwok, K.W.H., Leung, K.M.Y., 2020. Sunscreens containing zinc oxide nanoparticles can trigger oxidative stress and toxicity to the marine copepod Tigriopus japonicus. Mar. Pollut. Bull. 154, 111078. https://doi.org/10.1016/j.marpolbul.2020.111078

Yagi, K., 1976. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med. 15, 212–216.

Yang, S., Ye, R., Han, B., Wei, C., Yang, X., 2014. Ecotoxicological effect of nano-silicon dioxide particles on Daphnia Magna. Integr. Ferroelectr. 154, 64–72. https://doi.org/10.1080/10584587.2014.904143

Zhu, B., He, W., Hu, S., Kong, R., Yang, L., 2019. The fate and oxidative stress of different sized SiO2 nanoparticles in zebra fish (Danio rerio) larvae. Chemosphere 225, 705–712. https://doi.org/10.1016/j.chemosphere.2019.03.091

Zhu, S., Xue, M.Y., Luo, F., Chen, W.C., Zhu, B., Wang, G.X., 2017. Developmental toxicity of Fe3O4 nanoparticles on cysts and three larval stages of Artemia salina. Environ. Pollut. 230, 683–691. https://doi.org/10.1016/j.envpol.2017.06.065

Figure captions

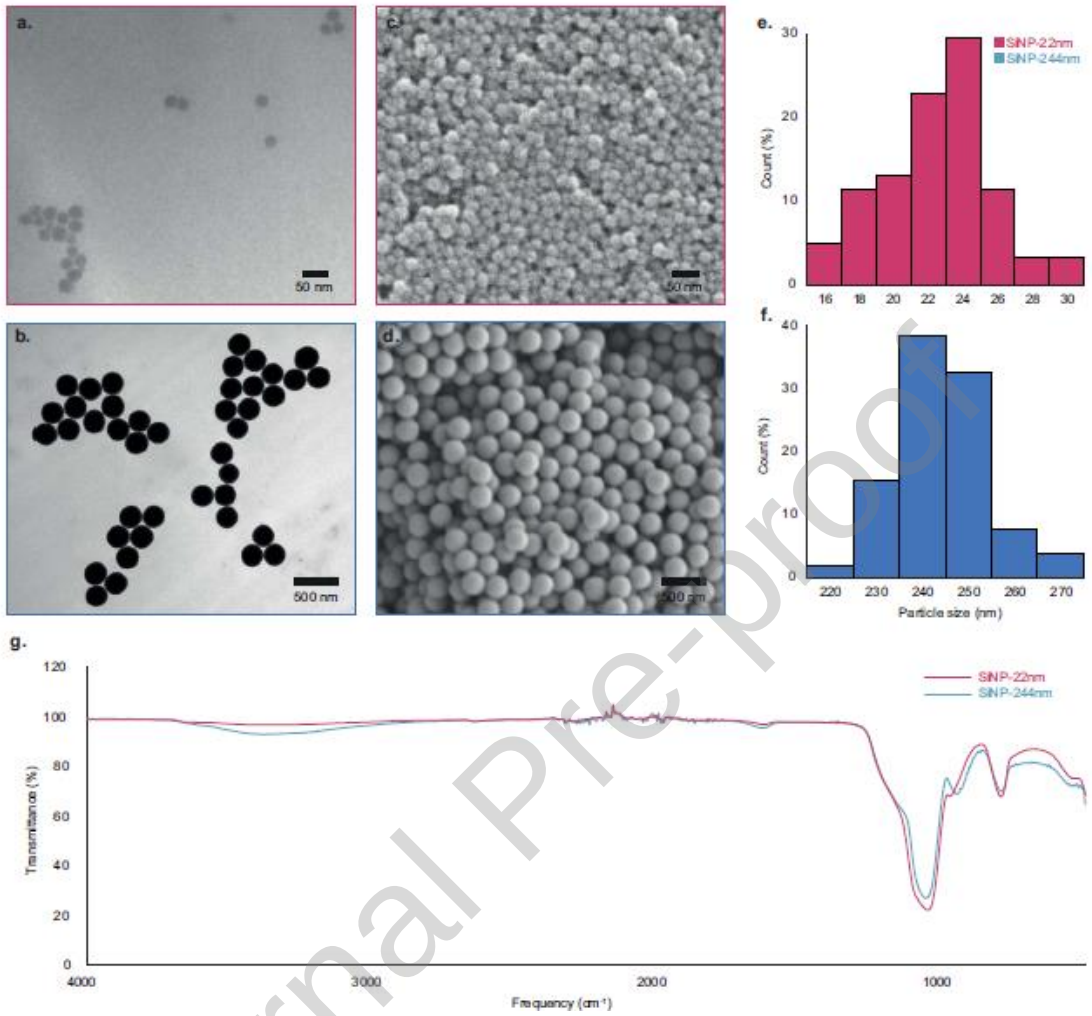


Fig. 1 Transmission electron microscopy (TEM) of **a.** 22 and **b.** 244 nm SiNP. Scanning electron microscopy (SEM) of **c.** 22 and **d.** 244 nm SiNP. Relative frequency histogram of **e.** 22 and **f.** 244 nm SiNP. **g.** Fourier transform infrared spectroscopy (FT-IR) of SiNP of 22 and 244 nm

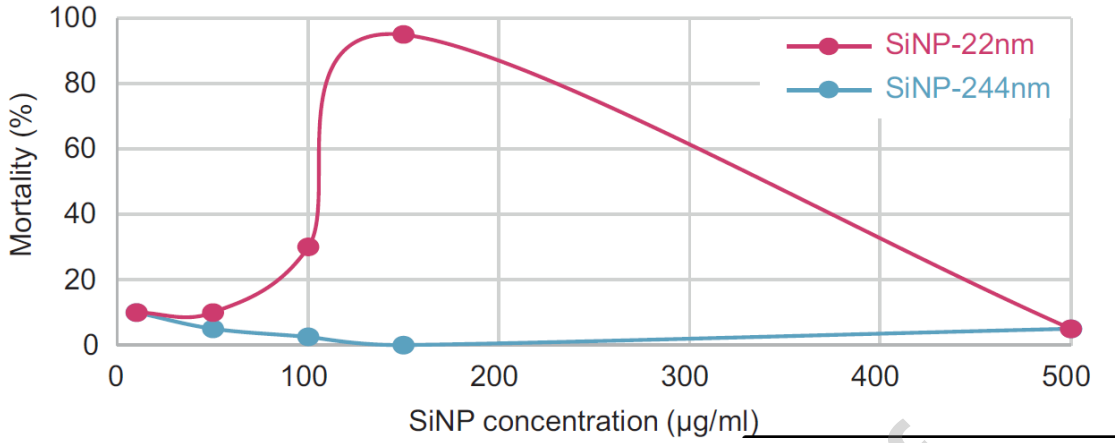


Fig. 2 Dose response curves (48 h) from *C. reticulata* acute toxicity test for SiNP of 22 and 244 nm

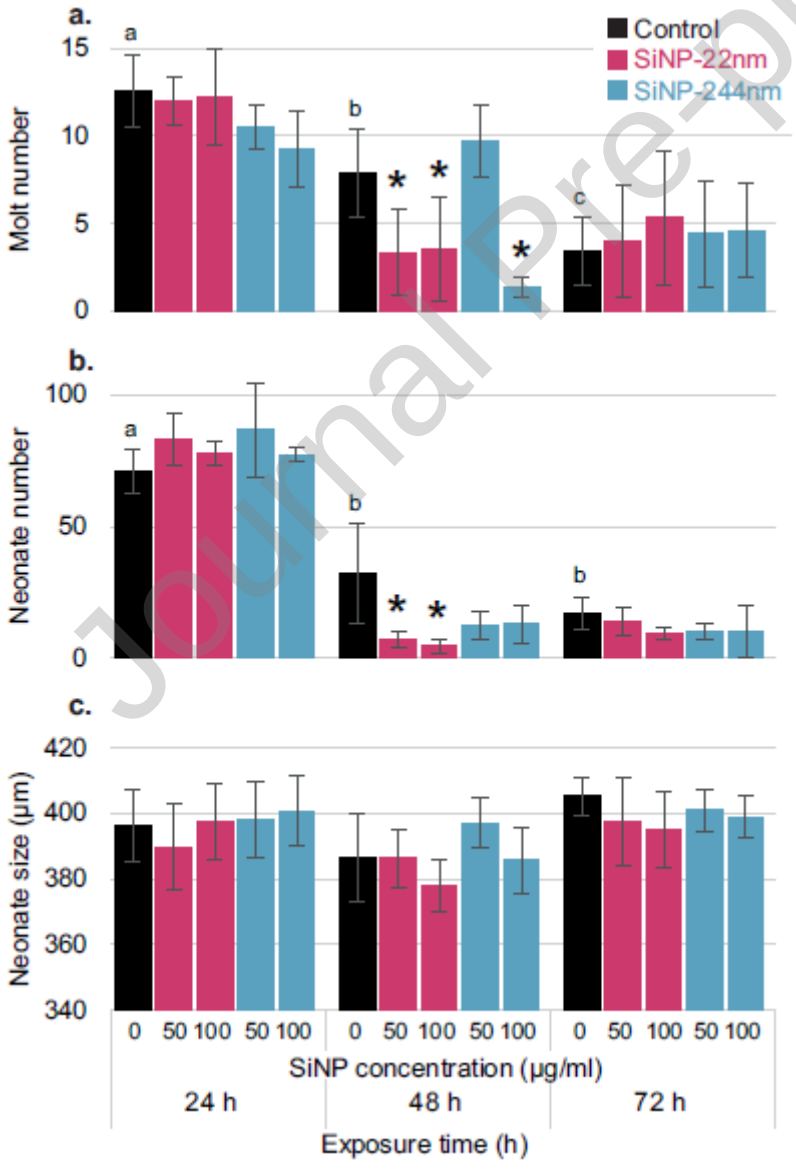


Fig. 3 Mean values and standard deviation of *C. reticulata* **a.** molt number, **b.** neonate number, and **c.** neonate size at 24, 48, and 72 h of exposure to SiNP of 22 and 244 nm. * indicate significant differences from control. Letters indicate differences between controls over time

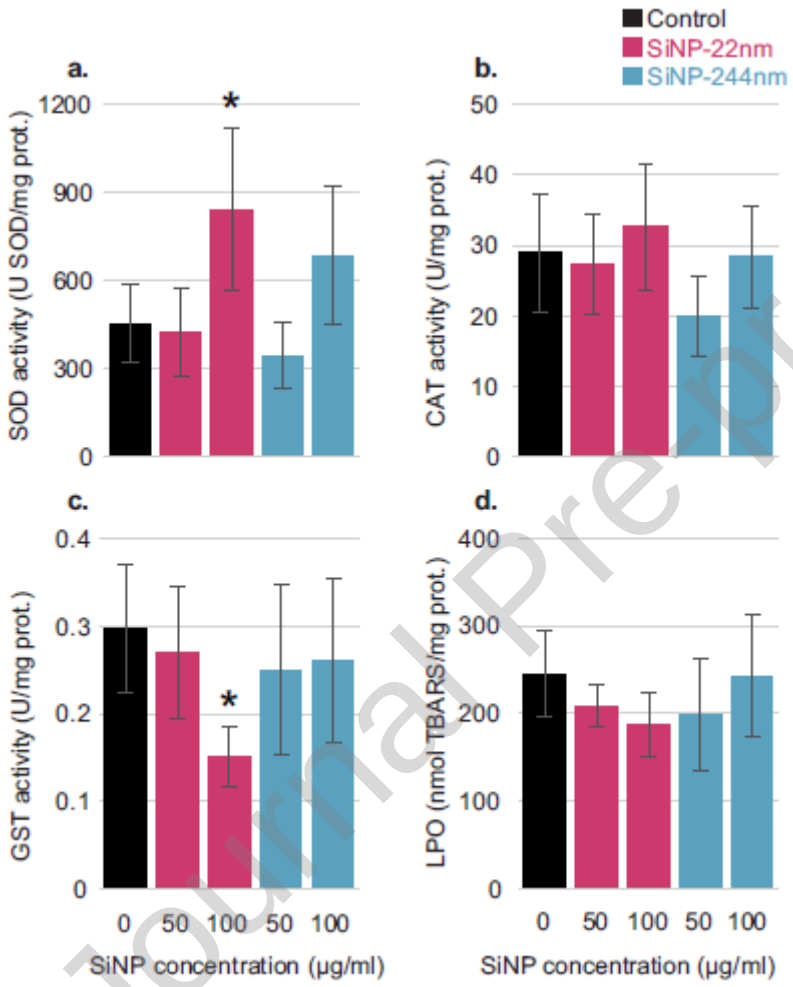


Fig 4. Mean values and standard deviation of antioxidant enzyme activities **a.** Superoxide dismutase (SOD) (U SOD/mg prot.), **b.** Catalase (CAT), and **c.** Glutathione-s-transferase (GST) (U/mg prot.), and **d.** Lipid peroxidation levels (LPO) (nmol TBARS/mg prot.) in *C. reticulata* exposed to SiNP of 22 and 244 nm for 72 h. * indicate significant differences from control

Table 1 Lethal concentrations 50 (LC50) and their confidence intervals (CI) for *C. reticulata* exposed to SiNP (22 and 244 nm) during 24, 48, and 72 h

	LC50 (µg/ml)		CI (95%)
SINP-22 nm	24 h	**122.5**	110.3 – 134.6
	48 h	**109.6**	94.8 – 124.4
	72 h	**105.2**	94 – 116.5
SiNP-244 nm		**> 500**	-

Author contributions:

Victoria S. Andrade: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. Analía Ale: Investigation, Funding acquisition, Methodology, Writing - review & editing. Sofía Municoy: Formal analysis, Investigation, Writing - review & editing. Carla Bacchetta: Methodology, Writing - review & editing. Martín F. Desimone: Funding acquisition, Methodology, Resources, Writing - review & editing. María F. Gutierrez: Conceptualization, Methodology, Project administration, Resources, Writing - review & editing. Jimena Cazenave: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Silica nanoparticles (SiNP) toxicity depends on intrinsic properties such as size
- 22 nm SiNP are more lethal than 244 nm SiNP
- 22 nm SiNP impair growth and reproduction of *Ceriodaphnia reticulata*
- 22 nm SiNP cause oxidative stress in *C. reticulata*