Acute and chronic toxicity of amine-functionalized SiO$_2$ nanostructures toward *Daphnia magna*

Rodrigo Costa Puerari [a], Emeline Ferrari [b], Bianca Vicente Oscar [a], Carmen Simioni [c], Luciane Cristina Ouriques [c], Denice Schulz Vicentini [a], William Gerson Matias [a],[*],[1]

[a] *Department of Sanitary and Environmental Engineering, Federal University of Santa Catarina, Florianópolis, Brazil*
[b] *Department of Basic and Applied Sciences, University of Lorraine, Metz, France*
[c] *Department of Cell Biology, Embryology and Genetics Federal University of Santa Catarina, Florianópolis, Brazil*

ARTICLE INFO

ABSTRACT

Silicon oxide (SiO$_2$) nanostructures (SiO$_2$NS) are increasingly being incorporated into an array of products, notably in the food, pharmaceutical, medical industries and in water treatment systems. Amorphous SiO$_2$NS have low toxicity, however, due to their great versatility, superficial modifications can be made and these altered structures require toxicological investigation. In this study, SiO$_2$NS were synthetized and amine-functionalized with the molecules (3-aminopropyl)triethoxysilane (APTMS) and 3-[2-(2-aminoethylamino)ethylamino]propyl-trimethoxysilane (AEAEAPTMS), named SiO$_2$NS@1 and SiO$_2$NS@3, respectively. The bare SiO$_2$NS, SiO$_2$NS@1 and SiO$_2$NS@3 samples were characterized and the influence of the culture medium used in the toxicological assays was also evaluated. The effect of amine functionalization of SiO$_2$NS was investigated through acute and chronic toxicity assays with *Daphnia magna*. Modifications to ultrastructures of the intestine and eggs of these organisms were observed in TEM and SEM analysis. The toxicity was influenced by the surface modifications and a possible Trojan horse effect was highlighted, particularly in the case of chronic exposure. Exposure to all NSs promoted alterations in the microvilli and mitochondria of the *D. magna* intestine and some damage to egg cells was also observed. The results demonstrate the importance of carrying out a full characterization of these materials, since surface modifications can enhance their toxic potential.

1. Introduction

Nanotechnology products can be grouped into five major categories (metal, carbonaceous, silicon, not advertised and other) according to the Nanotechnology Consumer Product Inventory (CPI), created by the Woodrow Wilson International Center for Scholars and the Project on Emerging Nanotechnology (Vance et al., 2015). It has been noted that silver, titanium oxide and silicon dioxide nanoparticles (NPs) correspond to approximately 25% of all nanomaterials that are incorporated into products reaching the market (Inshakova and Inshakov, 2017). Silicon-based NPs are increasingly being incorporated in commonly used products. In 2015, the global consumption of SiO$_2$ was 198 kilotons and the projection for 2022 is estimated at 786 kilotons (Inshakova and Inshakov, 2017). This increase can be explained by the versatility of SiO$_2$ nanostructures (SiO$_2$NS). These nanostructures (NSs) can be synthetized in different shapes, such as nanoparticles (NPs), nanorods (NRs) and nanotubes (NTs), and their characteristics include low cost, ease of synthesis and nanoscale control (Maser et al., 2015; Napierska et al., 2010). SiO$_2$NS are widely applied in food products and other applications include cosmetics, medicinal products, biotechnology and agriculture, drugs, paints, perfumes and building materials (Maser et al., 2015; Napierska et al., 2010; Ude et al., 2019; Van der Zande et al., 2014). More recently, SiO$_2$NS became an interesting nanomaterial for application in water treatment systems. The large surface area of the porous morphology of SiO$_2$NS facilitates contact with possible contaminants in effluents and thus these NSs have been applied in filtration membranes (Elma et al., 2012; Lv et al., 2016; Puerari et al., 2020). However, there is growing concern regarding the wide use of SiO$_2$NS in various products, since they could be released into the environment (Reijnders, 2009).

* Corresponding author.
E-mail address: william.g.matias@ufsc.br (W.G. Matias).
[1] Laboratório de Toxicologia Ambiental, LABTOX – Depto. de Engenharia Sanitária e Ambiental, Universidade Federal de Santa Catarina – Campus Universitário Trindade – CEP: 88040–970 – Florianópolis – SC – Brazil – Fax: +55–48-3721–9823.

Several reports confirm that amorphous SiO$_2$ has low toxicity, but the World Health Organization (WHO) is developing protection guides for industrial workers exposed to nanomaterials, including amorphous SiO$_2$NS. Although a report produced by Lee et al. (2017) indicated that exposure to SiO$_2$NS poses no danger in terms of acute toxicity, skin or eye damage, respiratory or skin sensitization, germ cell mutagenicity and reproductive toxicity, there was insufficient data for classification regarding carcinogenicity and specific target organ toxicity after single exposure. However, potential toxic effects of amorphous SiO$_2$NS cannot be rule out. In fact, in the above cited report, considering the "specific target organ toxicity after repetitive exposure" parameter, SiO$_2$NS were classified as Category 2, that is, "substances that, based on evidence from animal experimentation studies, may have the potential to be harmful to human health after repeated exposure" (Lee et al., 2017).

The size and shape of NSs play a crucial factor in toxicity. In a previous study by our group, tubular (SiO$_2$NT) and spherical (SiO$_2$NH) shaped SiO$_2$NS were synthetized and their toxicity towards *Daphnia magna* and Vero cells was evaluated (Vicentini et al., 2017). The surface area of SiO$_2$NH was higher than that of SiO$_2$NT and this may have contributed to the higher toxicity of spherical compared with tubular particles observed in acute and chronic assays with *D. magna* and cytotoxicity tests with Vero cells (Vicentini et al., 2017). However, another factor to be considered when evaluating the toxicity of SiO$_2$NS is their surface modification. Functionalization is a technique used to anchor molecules on the surface of materials through covalent or electrostatic bonds and this provides them with different proprieties based on the type of molecule used. Depending on the desired application, the functionalized material may have, for example, a modified surface charge or increased ion absorption capacity (Bhattacharjee et al., 2013; Ko et al., 2013a). These types of significant modifications need to be investigated as they may alter the toxicity of the materials.

Few authors have addressed the toxicity of functionalized SiO$_2$NS. A previous study showed that amine-functionalized SiO$_2$NS had greater toxic potential than bare SiO$_2$NS towards Vero cells (Puerari et al., 2019). In acute toxicity assays with *D. magna*, the amine functionalization of SiO$_2$NP with the molecule (3-aminopropyl)triethoxysilane (APTMS) led to a lower toxicity when compared to the bare SiO$_2$NP (Clément et al., 2013). However, the amine functionalization of SiO$_2$NT and SiO$_2$NH with the molecule 3-[2-(2-aminoethylamino)ethylamino] propyltrimethoxysilane (AEAEAPTMS) promoted a higher toxicity of the SiO$_2$NS toward *D. magna* (Vicentini et al., 2017) in both acute and chronic assays. This highlights the fact that the toxicity of functionalized materials is also dependent on the type of molecule is used for the surface modification (Montes-Fonseca et al., 2015).

In this context, the aim of this research was to ascertain the difference between uncoated and amine-functionalized SiO$_2$NS in terms of toxicity. Thus, SiO$_2$NS were synthetized and then amine-functionalized with either APTMS (primary amine) (SiO$_2$NS@1) or with AEAEAPTMS (tri-amine) (SiO$_2$NS@3) to evaluate their toxicity towards the freshwater microcrustacean *D. magna*. These materials were applied on nanofiltration membranes in a previous study (Puerari et al., 2020). Bare SiO$_2$NS, SiO$_2$NS@1 and SiO$_2$NS@3 were characterized to verify their shape, functionalization and behavior in different culture media. The toxicity was evaluated through acute and chronic assays. Ultrastructural alterations in the exposed organisms were investigated through transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Synthesis and amine functionalization of SiO$_2$NS

The procedures used for the synthesis of SiO$_2$NS and their amine functionalization were based on the methodology proposed by Vicentini et al. (2017). The molecule APTMS was purchased from Sigma-Aldrich® (St. Louis, USA) and AEAEAPTMS from Acros Organics® (New Jersey,

USA). The estimated molecular lengths are approximately 0.9 nm and 1.7 nm (Ko et al., 2013a; Yokoi et al., 2004), respectively.

2.2. Characterizations

The morphology, distribution, average diameter and agglomeration potential of the bare SiO$_2$NS, SiO$_2$NS@1 and SiO$_2$NS@3 were evaluated by TEM. The samples were diluted in ultrapure water (UPW) (resistivity 18.2 MΩ-cm) at a concentration of 1000 mg L^{-1} and then sonicated at 165 W for 1.5 min in an ultrasonic sonicator (Q500 Sonicator 500 W, QSonica, USA). In the next step, 50 μL of each sample was dripped onto carbon grids. Analysis was conducted using a TEM JEM 1011 microscope (JOEL Ltd., Tokyo, Japan) operated at 100 kV. Under the same conditions, all of the NSs were also diluted in ISO (used in the acute toxicity tests) and M4 (used in the chronic toxicity tests) culture medium in order to investigate the influence of the medium on the material. The diameters of all SiO$_2$NS were expressed as mean size ± standard deviation and were obtained after analysis using ImageJ particle analysis software (version 1.52a).

Surface charge and stability were investigated by measuring the zeta potential (ZP) of the samples through phase analysis light scattering (PALS). Effective diameter (ED) was measured by dynamic light scattering (DLS) and the polydispersity index (PDI) was also obtained. All of these analysis procedures were conducted on a Nanobrook 90Plus PALS analyzer (Brookhaven®, New York, USA). For the procedures, NSs in powder were diluted in UPW, ISO and M4 at a concentration 250 mg L^{-1} and then sonicated at 165 W for 1.5 min

The samples were also characterized in terms of surface area by the Brunauer−Emmett−Teller (BET) method and the structure was examined by X-ray diffraction (XRD). To confirm the functionalization, samples were analyzed by Fourier transform infrared (FTIR) spectroscopy. All of these analysis procedures have been reported in a previous publication (Puerari et al., 2019).

2.3. Toxicological evaluation

2.3.1. Daphnia magna culture

The freshwater microcrustacean (*Daphnia magna*) was cultivated following the guidelines published in ISO 6341 (ISO, 2012) and NBR 12713 (ABNT, 2009, 2017) and during the tests the pH, hardness and dissolved oxygen were maintained within the limits stipulated: 7.6–8.0; 175–225 mg CaCO$_3$ L^{-1}; > 1.0 mg L^{-1}, respectively. The organisms (exclusively females) were kept in 2 L beakers containing reconstituted water (M4 medium) with a density of one adult organism per 50 mL of medium. The temperature was controlled at 20 ± 2 ℃ and the photoperiod was 16 h of light/8 h of dark. The medium was changed and the organisms were fed with the green alga *Scenedesmus subspicatus* (approximately 10^6 cells mL^{-1} per organism) three times a week.

Details on the composition and concentrations of the salts used to obtain M4 and ISO media can be found in Supplementary Material 1.

2.3.2. Acute toxicity assays

Acute toxicity assays were conducted based on ISO 6341 (ISO, 2012) and NBR 12713 (ABNT, 2009, 2017). Neonates (2–26 h) were placed in beakers and exposed to the samples and to a negative control for 48 h. The endpoint evaluated was immobility. The SiO$_2$NS, SiO$_2$NS@1 and SiO$_2$NS@3 were diluted in ISO medium, to obtain stock solutions at a concentration of 10 g L^{-1}, and sonicated at 165 W for 1.5 min. Neonates (10 per beaker) were exposed to a range of concentrations varying from 0.1 g L^{-1} to 10 g L^{-1}. These concentrations were based on a previous study by our group (Vicentini et al., 2017) where organisms were exposed to different materials, in order to allow a comparison of the results. Control tests were conducted with only ISO medium. After 48 h of exposure, immobile organisms were counted for each concentration. The data obtained were statistically analyzed using the Trimmed Spearman−Karber method and expressed as mean EC$_{50,48 h}$ ± standard

deviation. All treatments were carried out in triplicate.

The sensitivity of the culture was measured weekly by exposing neonates to potassium dichromate ($K_2Cr_2O_7$) diluted in ISO medium at concentrations of 0.7, 0.9, 1.1 and 1.3 mg L^{-1}. Organisms were exposed for 24 h and $EC_{50,24 h}$ values between 0.6 and 1.7 mg L^{-1} indicate the validation of the assays (Gonçalves et al., 2018).

2.3.3. Chronic toxicity assays

The chronic toxicity assays with *D. magna* were conducted following the guidelines published in ISO 10706 (ISO, 2000) and NBR 13373 (ABNT, 2009, 2017) with adaptations (Vicentini et al., 2019) and the organisms were exposed for 21 days to sublethal concentrations. All SiO₂NS samples were diluted in M4 medium at a concentration of 500 mg L^{-1}. Stock solutions were then sonicated at 165 W for 1.5 min. Bare SiO₂NS was evaluated at concentrations of 6.25, 12.50, 25.00, 50.00, 100.00, 250.00 and 500.00 mg L^{-1} while for both SiO₂NS@1 and SiO₂NS@3 the concentrations used were 0.78, 1.56, 3.12, 6.25, 12.50, 25.00 and 50.00 mg L^{-1}. Control tests were performed with only M4 medium. The tests were conducted with 10 beakers per concentration with one neonate per beaker. Test solutions (samples and control) were changed 3 times a week and the organisms were fed with the green alga *S. subspicatus*. The beakers were maintained under the same conditions of temperature and luminosity as the culture. At the end of exposure, the parameters analyzed were longevity (% of survivors), reproduction (neonates per brood), growth (length in mm) and time (days) until first brood of each organism. The results were treated statistically using one-way ANOVA analysis of variance (EPA, 2002) followed by Dunnett's post-hoc test using Dunnett Program (version 1.5). The results were expressed as "observed effect concentration" (OEC) and "no observed effect concentration" (NOEC). The results are presented as mean ± standard deviation and differences between control group and treatments were considered statistically significant for $p < 0.05$.

2.3.4. Morphological analysis of exposed organisms

Preparation of the samples for TEM and SEM analysis was based on the method described by Puerari et al. (2016) with modifications. The number of *D. magna* survivors was recorded after 21 days of exposure to the control (M4) and to all NSs tested. For bare SiO₂NS, the organisms analyzed were obtained from the test conducted at 50 mg L^{-1} while for SiO₂NS@1 and SiO₂NS@3 they were taken from the test conducted at 3.12 mg L^{-1}. These concentrations were selected based on the results of chronic toxicity tests.

Prior to the TEM analysis, the exposed organisms were fixed with 2.5% glutaraldehyde solution and 0.1 M sodium cacodylate buffer (pH 7.2) for 24 h. The intestine and eggs of exposed organisms were then analyzed. Organisms were sectioned using a scalpel and the intestine and eggs were separated. Post-fixation of intestines and eggs was performed with 1.0% osmium tetroxide and 0.1 M sodium cacodylate buffer. The next step consisted of successive washes with 0.1 M sodium cacodylate buffer and dehydration with increasing concentrations of acetone. After this stage, infiltration was carried out with Spurr resin (Spurr, 1969) in increasing concentrations up to 100%. Ultrathin sections (60–70 nm) were prepared using a diamond knife and the samples were then stained with aqueous uranyl acetate followed by 5% lead citrate. Analysis was performed with a TEM JEM 1011 microscope (JOEL Ltd., Tokyo, Japan) operated at 80 kV.

In the case of the SEM, the whole body of each *D. magna* was fixed and the only difference in the procedure was the degree of dehydration that occurred with increasing concentrations of ethanol. After complete dehydration, samples were dried to the critical point in an EM-DPC-030 dryer (Leica, Heidelberg, Germany), deposited on metallic supports (stubs) and coated with Au. Samples were then analyzed using an SEM JSM 6390 LV microscope (JEOL Ltd., Tokyo, Japan) at 8 kV. Energy dispersive spectroscopy (EDS) was also performed to identify the chemical elements in the samples.

3. Results and discussion

Micrographs obtained in the TEM analysis are shown in Fig. 1. The following samples were analyzed: bare SiO₂NS diluted in UPW (Fig. 1A and B), in ISO medium (Fig. 1C and D) and in M4 (Fig. 1E and F); SiO₂NS@1 diluted in UPW (Fig. 1G and H), in ISO medium (Fig. 1I and J) and in M4 (Fig. 1K and L); and SiO₂NS@3 diluted in UPW (Fig. 1M and N), in ISO medium (Fig. 1O and P) and in M4 (Fig. 1Q and R). All of the NSs were spherical and porous, with average diameters of < 100 nm. The size distribution histograms for each type of NS in UPW are provided in Supplementary Material 2. Particles connected to each other by SiO₂ wires were observed in all micrographs.

In the case of UPW, a significant difference in the size of the NSs was observed. The average diameters for SiO₂NS, SiO₂NS@1 and SiO₂NS@3 were 48.2 ± 13.1 nm, 80.2 ± 18.1 nm and 84.7 ± 19.5 nm, respectively, and the micrographs show an increase in the thickness of the SiO₂ wires that connect the particles when the NSs were amine-functionalized (Puerari et al., 2019). The increase in the size of SiO2NS can be explained by functionalization. The size of the APTMS and AEAEAPTMS molecules is 0.9 nm and 1.7 nm, respectively (Ko et al., 2013a). One of the possibilities of linking functionalizing molecules with nanostructures is the formation of multilayers due horizontal and vertical polymerization with neighboring silanes (Ko et al., 2013b). In addition, when using AEAEAPTMS, reactions can also occur between NH and other molecules, increasing the complexity of the network formed on the surface of SiO₂NS. Based on studies by Ko et al. (2013a), (2013b), a scheme was assembled showing the functionalizing molecules APTMS and AEAEAPTMS. A possible attachment between the molecules and the surface of SiO₂NS was also presented. This scheme can be seen in Supplementary Material 3.

When the NSs were diluted in ISO and M4 media, there was a tendency toward agglomeration. This is probably due the presence of salts in their composition that enhance the ionic strength of the medium. This effect has been reported in literature for ZnO nanorods (ZnONR) dispersed in the same culture media (Gonçalves et al., 2018).

The pH, ZP, ED and PDI values for the NSs diluted in UPW, ISO and M4 media and the results obtained in the acute toxicity assays are shown in Table 1. The pH and ZP values were higher when the NSs were amine-functionalized, as observed in a previous study and attributed to the presence of amine groups at the surface of the materials (Puerari et al., 2019; Vicentini et al., 2017).

In all scenarios evaluated, the ED of the particles increased when the NSs were dispersed in ISO and M4 media in comparison to UPW and the PDI results indicated that the particle diameters were monodisperse, with a narrow range of ED values. The ED results are consistent with the agglomeration shown in Fig. 1, since in this analysis significant differences were observed in the diameters of all materials when comparing the dilutions in the two culture media with those in UPW.

In Table 1, a reduction in stability is also observed when the ZP values of SiO₂NS@1 and SiO₂NS@3 diluted in ISO and M4 are compared to the corresponding samples diluted in UPW. This is associated with the composition of the media (Gonçalves et al., 2018; Rossetto et al., 2014), reinforcing the hypothesis that the ionic strength of culture medium influences the intrinsic characteristics of nanomaterials and the consequences of this must be taken into account in nanotoxicology. In this study, the culture media ISO and M4 increased the ED of all NSs, consequently decreased the stability and promoted agglomeration. Although not performed in the present study, it is recommended for future research the characterization of all SiO₂NS in the exposure periods used in toxicological tests.

Amine functionalization was confirmed by the FTIR spectroscopy results. As previously reported, all materials were amorphous and the amine functionalization promoted a decrease in the surface area of SiO₂NS@1 (283.1 m² g⁻¹) and SiO₂NS@3 (286.8 m² g⁻¹) when compared to the bare SiO₂NS (444.0 m² g⁻¹) (Puerari et al., 2019). The same behavior was observed in other studies that functionalized SiO₂
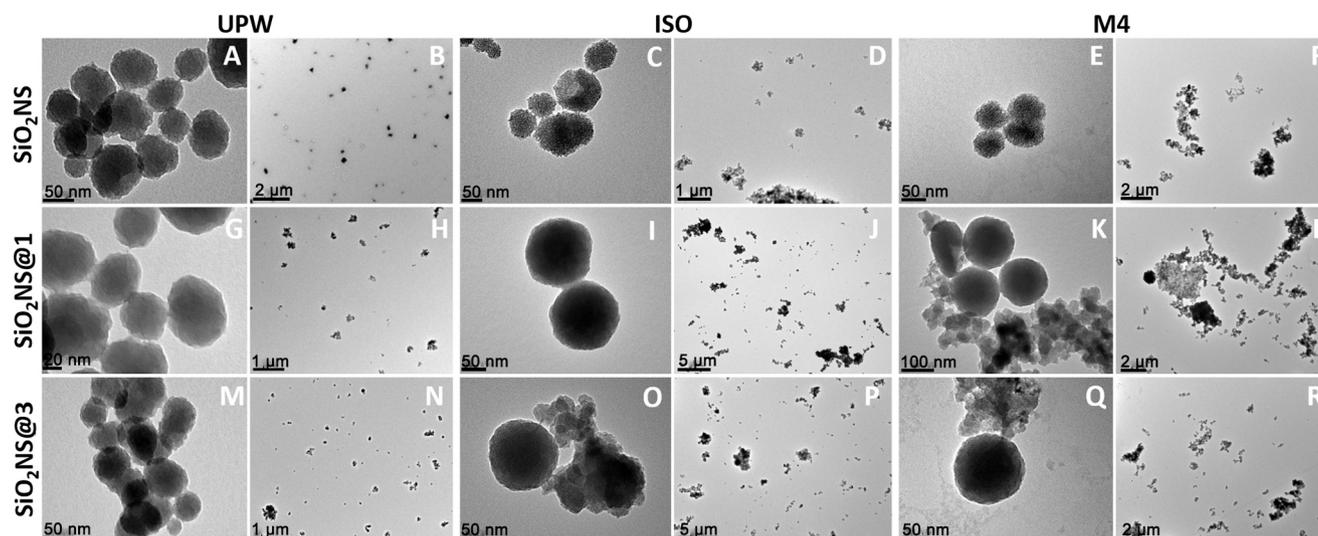
Fig. 1. TEM micrographs: SiO₂NS in UPW (A) and (B), ISO (C) and (D) and M4 (E) and (F); SiO₂NS@1 in UPW (G) and (H), ISO (I) and (J) and M4 (K) and (L); SiO₂NS@3 in UPW (M) and (N), ISO (O) and (P) and M4 (Q) and (R).

Table 1
Values obtained for pH, ZP, ED and PDI for each type of NS in UPW and in the culture media and results of acute toxicity assays expressed in $EC_{50,48\,h}$.

		SiO₂NS	SiO₂NS@1	SiO₂NS@3
UPW	pH	5.35[a]	6.20[a]	6.41[a]
	ZP (mV)	-22.87 ± 0.57[a]	39.24 ± 0.28[a]	44.09 ± 0.51[a]
	ED (nm)	794.51 ± 33.19[a]	867.99 ± 20.39[a]	855.32 ± 28.69[a]
	PDI	0.29 ± 0.01	0.27 ± 0.04	0.29 ± 0.02
ISO	pH	7.30	7.93	8.19
	ZP (mV)	-22.27 ± 0.26	30.52 ± 0.33	32.90 ± 0.51
	ED (nm)	1193.34 ± 22.80	2084.90 ± 55.80	2049.12 ± 90.61
	PDI	0.28 ± 0.03	0.27 ± 0.02	0.30 ± 0.01
M4	pH	7.36	7.83	8.21
	ZP (mV)	-22.15 ± 0.78	31.42 ± 0.16	29.73 ± 0.87
	ED (nm)	1386.49 ± 10.44	2248.83 ± 40.27	2181.59 ± 82.28
	PDI	0.30 ± 0.00	0.30 ± 0.01	0.30 ± 0.00
$EC_{50,48\,h}$ (g L⁻¹)		2.20 ± 0.48	7.88 ± 0.70	0.22 ± 0.03

^a Puerari et al. (2019).

nanostructures with APTMS and AEAEAPTMS molecules (Ko et al., 2013a; Vicentini et al., 2017).

After the extensive characterization of the samples, the toxicological evaluation was conducted. The overall sensitivity of the *D. magna* culture tested in this research was $EC_{50,24\,h} = 0.90 \pm 0.16$ mg L⁻¹ and this validates the results obtained in the acute toxicity assays. Based on the $EC_{50,48\,h}$ (Table 1) and according to the European directive EU-Directive 93/67/EEC and a study by Bondarenko et al. (2013), for aquatic organisms bare SiO₂NS, SiO₂NS@1 and SiO₂NS@3 are not classified as toxic substances, since the $EC_{50,48\,h}$ value found was greater than 0.1 g L⁻¹ for the three materials investigated. Shariati et al. (2020) used a commercial nano silica powder with 20–30 nm diameter to evaluate the acute toxicity toward *D. magna* and they found a lethal concentration (LC_{50}) after 48 h of 48.86 mg L⁻¹. Yang et al. (2014) found a EC_{50} of 148.871 mg L⁻¹ and a LC_{50} of 660.943 mg L⁻¹ when *D. magna* were exposed to commercial SiO₂NP (10–20 nm). The difference in the toxicity of these studies to the present herein could be explained due the SiO₂NS synthesis. It is worth mentioning that SiO₂NS micrographs (Fig. 1) showed particles connected by wires of SiO₂. This may have promoted a net of SiO₂NS and the uptake of particles by *D. magna* could be limited due this factor, which can explain the very low toxicity.

The lowest acute toxicity was observed when organisms were exposed to SiO₂NS@1. This finding is consistent with results reported by Clément et al. (2013), where the amine functionalization of SiO₂NP with

the APTMS molecule promoted a decrease in toxicity in acute assays with *D. magna*. This was attributed to an increase in the NM diameter from 14 nm for SiO₂NP to 22 nm for amine-functionalized SiO₂NP (Clément et al., 2013). However, in the study reported herein, SiO₂NS@3 was more toxic than bare SiO₂NS, even though it has a smaller surface area and a larger diameter. A similar effect was found in the functionalization of SiO₂NT with the AEAEAPTMS molecule, that is, despite the increase in size and decrease in surface area, the amine-functionalized SiO₂NT showed greater toxicity than bare SiO₂NT (Vicentini et al., 2017). Thus, although size and area are important factors, these should not be considered the only parameters for assessing the toxicity of materials, since other factors such as morphology, type of coating and surface energy can contribute to toxic effects (Liu et al., 2016; Vicentini et al., 2017). In general, positively-charged particles are more toxic than negatively-charged particles, as they interact more easily with negatively-charged cell membranes. However, surface hydrophobicity is also important for nanotoxicology (Fröhlich, 2012), as hydrophobic substances adhere easily to negatively-charged biological materials (Yang et al., 2014). Also, Hurel et al. (2018) investigated the functionalization of TiO₂, CeO₂ and SiO₂ NPs and concluded that surface functionalization with amine groups reduced the NP hydrophobicity. In acute toxicity assays with *D. magna*, the cited authors found that hydrophilic NPs were less toxic than hydrophobic NPs (Hurel et al., 2018). Thus, one hypothesis for the greater toxicity of SiO₂NS@3 is the possible cyclization of the AEAEAPTMS molecule, as reported by Puerari et al. (2020). Cyclization of the molecule could lead to a decrease in the hydrophilicity and this can result in greater interaction with the cells of the organism, since particles that are more hydrophobic than cell membranes tend to undergo phagocytosis more easily than those that are more hydrophilic (Chen et al., 1997). When inserted into nanofiltration membranes, SiO₂NS@3 are expected to confer more hydrophilicity since they have more amine groups in their structure than SiO₂NS@1. However, contact angle and ultrapure water flow assays showed that SiO₂NS@1 conferred more hydrophilicity to the membranes than SiO₂NS@3 (Puerari et al., 2020). Despite not being a direct measure of the NS, these results reinforce the hypothesis of AEAEAPTMS cyclization and consequent lower hydrophilicity of SiO₂NS@3 in relation to SiO₂NS@1.

The results obtained after 21 days of *D. magna* exposure to SiO₂NS, SiO₂NS@1 and SiO₂NS@3 are shown in Table 2. In contrast to the acute assays, both amine-functionalized materials showed a greater toxic effect than the bare SiO₂NS in the chronic exposure tests. The greatest effects on longevity, reproduction and growth were observed on

Table 2

Results obtained in chronic toxicity assays where *D. magna* was exposed to SiO₂NS, SiO₂NS@1 and SiO₂NS@3.

	Concentration (mg L^{-1})	L (%)	R (neonates/brood)	G (mm)	D (days)
SiO₂NS	Control	100	10.50 ± 2.16	3.94 ± 0.12	9.60 ± 0.47
	6.25	100	9.70 ± 1.10	3.81 ± 0.19	9.40 ± 0.46
	12.5	100	9.60 ± 0.62	3.79 ± 0.24	9.70 ± 1.12
	25	100	9.13 ± 1.09	3.68 ± 0.28	10.67 ± 1.12
	50	100	8.70 ± 1.04*	3.49 ± 0.48*	11.38 ± 3.16*
	100	60*	6.50 ± 0.71*	2.70 ± 0.40*	18.50 ± 0.71*
	OEC (mg L^{-1})	100	50	50	25
	NOEC (mg L^{-1})	50	25	25	12.5
SiO₂NS@1	Control	100	10.50 ± 2.16	3.94 ± 0.12	9.60 ± 0.47
	0.78	100	9.50 ± 1.22	3.81 ± 0.12	9.88 ± 1.32
	1.56	100	9.00 ± 1.29	3.58 ± 0.14	9.90 ± 1.32
	3.12	70*	7.55 ± 1.34*	3.40 ± 0.21*	9.70 ± 1.28
	6.25	70*	7.56 ± 0.61*	3.10 ± 0.12*	10.20 ± 1.78
	12.5	70*	6.79 ± 1.56*	3.00 ± 0.08*	12.75 ± 1.49*
	OEC (mg L^{-1})	3.12	3.12	3.12	12.5
	NOEC (mg L^{-1})	1.56	1.56	1.56	6.25
SiO₂NS@3	Control	100	10.50 ± 2.16	3.94 ± 0.12	9.60 ± 0.47
	0.78	100	10.50 ± 0.96	3.77 ± 0.21	9.80 ± 0.79
	1.56	100	9.30 ± 0.87	3.70 ± 0.18	10.20 ± 1.65
	3.12	100	8.83 ± 0.97*	3.69 ± 0.22	11.44 ± 1.88*
	6.25	100	6.57 ± 0.89*	3.07 ± 0.72*	12.14 ± 1.77*
	12.5	60*	0*	2.10 ± 0.42*	–
	OEC (mg L^{-1})	12.5	3.12	6.25	3.12
	NOEC (mg L^{-1})	6.25	1.56	3.12	1.56

L − longevity (survivors); R − reproduction; G − growth; D − days until first brood;
* Significant difference in relation to the control (p < 0.05).

exposure to SiO₂NS@1 while exposure to SiO₂NS@3 had the greatest effect on the number of days until first brood.

Since this was a chronic exposure test, the increase in SiO₂NS@1 toxicity could be related to the Trojan horse effect. In the acute assays, the presence of 1 amine group in the molecule attached to SiO₂NS@1 may not be sufficient to promote this effect on *D. magna* and the lower acute toxicity in comparison to bare SiO₂NS is actually due to the increase in size and decrease in surface area. However, repeated exposure during the organism life cycle promotes a longer contact time between SiO₂NS@1 and *D. magna*, effectively resulting in the Trojan horse effect. As stated by Xiong et al. (2009), the presence of hydrophilic functional groups, such as amine, is considered a requirement to allow conjugation with biological molecules. Another factor to be considered is intake through feeding, since the algae could be contaminated with SiO₂NS@1, leading to bioamplification in the microcrustaceans. Previous reports indicate that SiO₂NP adhere to the cells of green microalgae (Manzo et al., 2015; Shariati and Shirazi, 2019; Van Hoecke et al., 2008). In a study by Manzo et al. (2015), the cells of the microalga *Dunaliella tertiolecta* were completely covered with NPs after exposure to 125 mg L^{-1} of SiO₂NP for 96 h, without loss of integrity or shape. Thus, in the study reported herein, since feeding and contamination were carried out three times a week, the algae used could be coated with SiO₂NS, which would be ingested by *D. magna*. In addition, the presence of 3 amine groups in the molecule used in SiO₂NS@3 may favor the appearance of acute responses due to the Trojan horse effect and the behavior in the chronic test was similar to that observed for SiO₂NS@1. Evidence that APTMS and AEAEAPTMS molecules promoted a Trojan horse effect when used in the amine-functionalization of SiO₂NS was observed in a previous study using Vero cells (Puerari et al., 2020). This topic, however, needs further investigation.

The reproduction of the organisms was one of the most affected parameters and this is evidenced by the results for the number of neonates per brood (Table 2 − R) and the days until first brood (Table 2 − D). No toxic effects on reproduction are reported in the literature for organisms exposed to amorphous forms of silica, including *D. magna* (Fruijtier-Pölloth, 2012; Lee et al., 2009). However, the biological activity and cytotoxicity may be related to the surface characteristics of the particle interacting with the medium (Fruijtier-Pölloth, 2012). Since functionalization alters the surface characteristics of the material, the

observed effect on the reproduction of *D. magna* can be attributed to the presence of amine-functionalizing molecules. It should be noted that exposure to 12.5 mg L^{-1} of SiO₂NS@1 inhibited the reproduction of the organisms over a period of 21 days in the chronic toxicity assays. As SiO₂NS@1 and SiO₂NS@3 have a greater tendency to agglomerate in M4 medium (Table 1) and probably have a greater affinity with the organisms (Table 3), the ingestion of particles agglomerated over time may have reduced the intake of algae, affecting the energy reserves of daphnia and their reproduction (Glazier and Calow, 1992).

Based on the results of the chronic toxicity assays, in which the *D. magna* reproduction was affected, the organisms selected for the microscopic investigation were those exposed to concentrations of 50 mg L^{-1} of bare SiO₂NS and 3.12 mg L^{-1} of SiO₂NS@1 and SiO₂NS@3, corresponding to the respective OEC for each material considering the reproduction parameter. Fig. 2 shows the SEM micrographs obtained for the antennae (Fig. 2-A, B, C and D) and ciliary filaments in the region of the gills (Fig. 2E−H) of *D. magna* exposed to M4 medium, SiO₂NS@1, SiO₂NS@1 and SiO₂NS@3.

The micrographs of *D. magna* samples exposed to M4 medium showed the presence of filaments on the antennae (Fig. 2A) and on the ciliary filaments in gill region (Fig. 2E). However, when *D. magna* samples were exposed to the NSs, fusion of the antenna filaments (Fig. 2B) and a decrease in the number of these filaments (Fig. 2C and D) were observed. A lack of organization was also observed in the case of ciliary filaments (Fig. 2F−H) when compared to the control. Similar effects resulting from the *D. magna* exposure to other nanomaterials are reported in the literature (Puerari et al., 2016). Although the gill region is not the main location where gas exchange occurs, changes in this

Table 3

Mass percentage of elements in the *D. magna* antennae according to the EDS analysis.

	Carbon (C) (%)	Oxygen (O) (%)	Calcium (Ca) (%)	Silicon (Si) (%)
Control	66.54 ± 0.66	29.51 ± 0.58	3.95 ± 0.18	ND[a]
SiO₂NS	63.07 ± 3.25	33.06 ± 2.64	3.38 ± 0.43	0.49 ± 0.25
SiO₂NS@ 1	65.70 ± 2.78	30.35 ± 2.20	2.98 ± 0.33	0.97 ± 0.28
SiO₂NS@ 3	59.33 ± 3.05	36.33 ± 2.41	3.47 ± 0.43	0.88 ± 0.26

[a] ND = not detected.
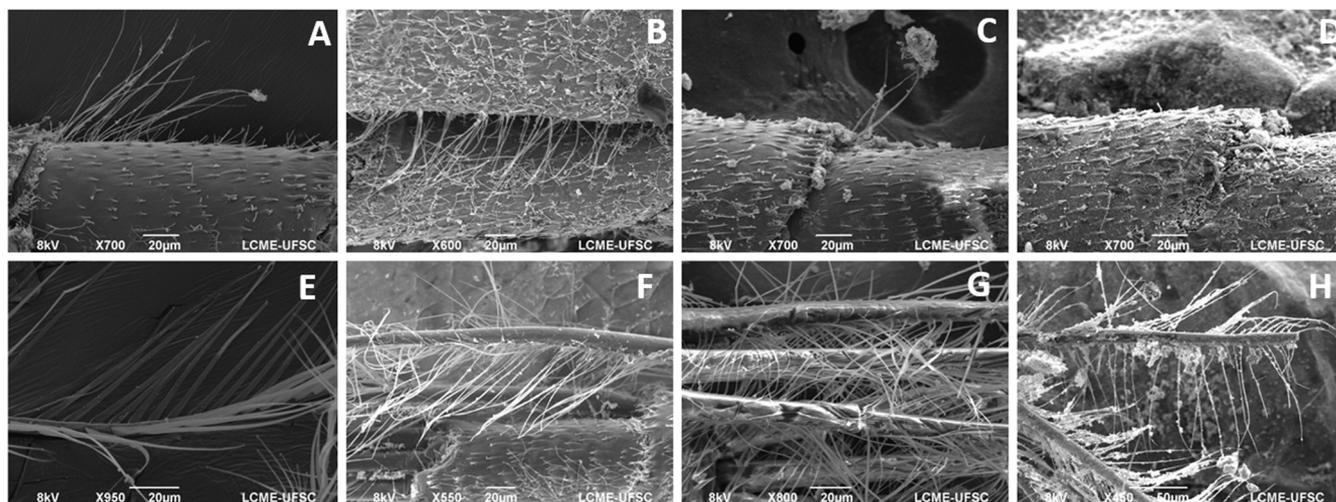
Fig. 2. SEM micrographs of *D. magna* exposed to: (A) and (E) M4 medium (control); (B) and (F) SiO$_2$NS; (C) and (G) SiO$_2$NS@1; (D) and (H) SiO$_2$NS@3. The letters A, B, C and D correspond to the antennae region of the organisms. The letters E, F, G and H correspond to the gill region. The micrographs were obtained after chronic exposure.

region can compromise the respiratory capacity of the organisms (Yang et al., 2014). Also, changes in the *D. magna* antennae hinder the swimming capacity, which makes feeding difficult since these are filter-feeders. In a previously reported comparative study on SiO$_2$NP (10–20 nm of diameter) and the bulk form of this material (5–10 µm of diameter), the nanometric form was present in the digestive tract, characterizing ingestion, and in the gills, characterizing absorption, while the bulk form was only ingested by *D. magna* (Yang et al., 2014). Therefore, the presence of effects on the ciliary filaments observed from the micrographs could be attributed to absorption of the NSs.
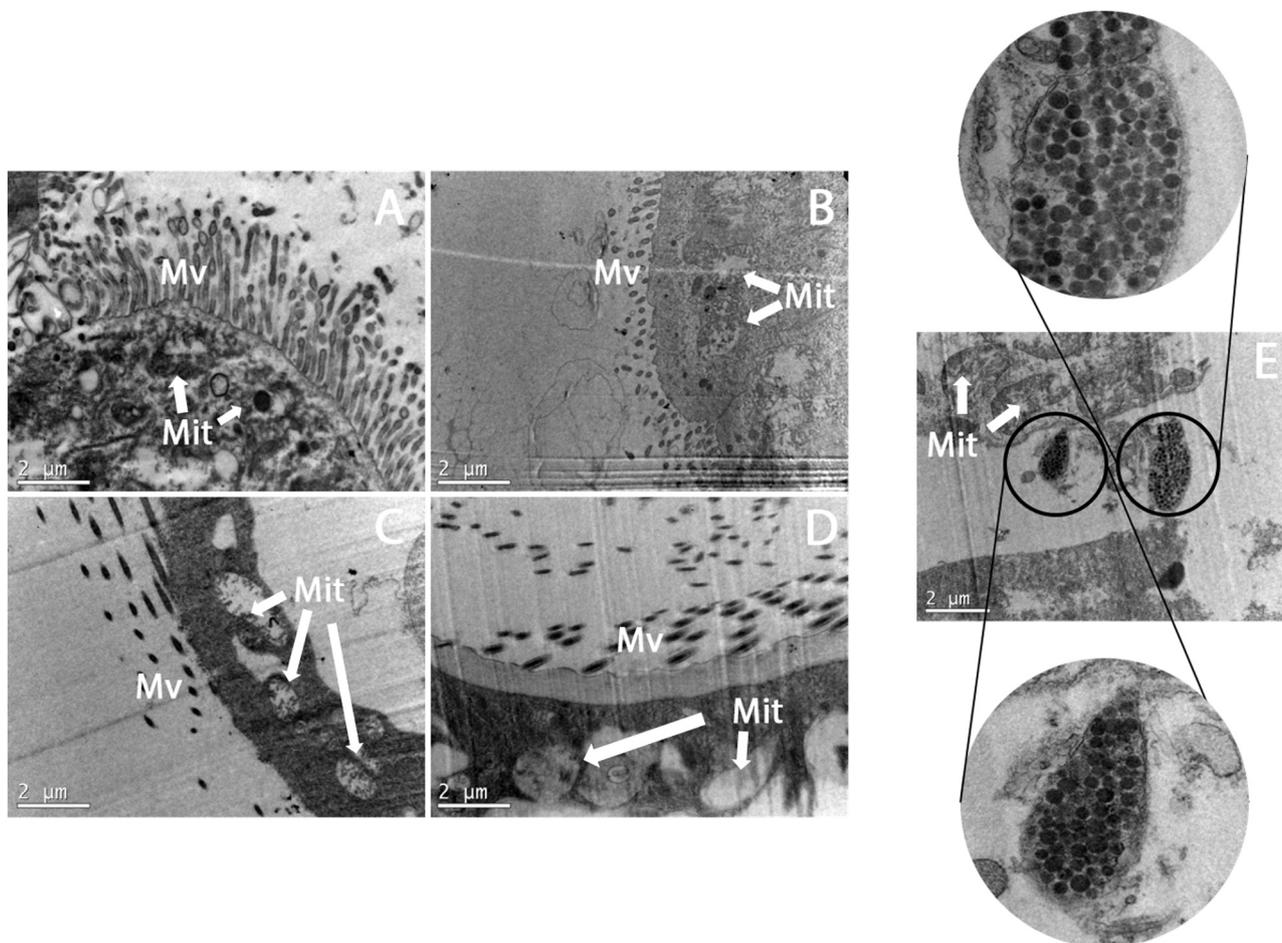
Fig. 3. TEM micrographs of *D. magna* exposed to: (A) M4 medium; (B) SiO$_2$NS; (C) SiO$_2$NS@1 and (D) SiO$_2$NS@3. The details in (E) are of *D. magna* exposed to SiO$_2$NS@1. Mv = microvilli; Mit = mitochondria. The micrographs are from the intestine region of the organisms after chronic exposure.

The stubs used in the SEM technique were also subjected to EDS analysis. The results are shown in Table 3 and the data were collected from the *D. magna* antennae. No silicon (Si) was detected in the control samples, in contrast to the organisms exposed to the NSs.

Although exposure to SiO$_2$NS was carried out at a concentration greater than that used in the case of SiO$_2$NS@1 and SiO$_2$NS@3 (50 mg L^{-1} > 3.12 mg L^{-1}), the highest amount of Si was found in the antennae of organisms exposed to SiO$_2$NS@1, followed by SiO$_2$NS@3. Taking into account that nanometric SiO$_2$ can be ingested and absorbed by *D. magna* (Yang et al., 2014), the greater presence of Si in the case of SiO$_2$NS@1 and SiO$_2$NS@3 is attributed to the fact that these nano-structures are coated with amine-functionalizing molecules, enhancing their affinity with the organisms. Therefore, the damage observed in the antennae may be due to an affinity between amine and the exoskeleton of *D. magna*. Another possible factor is contact between SiO$_2$NS@1 and SiO$_2$NS@3 agglomerates and this region during swimming.

The TEM micrographs of the intestine of *D. magna* exposed to the control and to the NSs are shown in Fig. 3. In the control sample (Fig. 3A), the presence of well-formed mitochondria (Mit) and the presence of microvilli (Mv) in the intestine wall can be noted. With exposure to all of the NSs, the organisms showed mitochondria with swelling and the loss of mitochondrial crystals (crystolysis). In addition, a decrease in the microvilli structure can be observed in Fig. 3B–D.

The observed damage to the microvilli may have occurred due to the accumulation of NSs in the intestine of exposed individuals, since these insoluble materials may aggregate, preventing their elimination due to the increased diameter (Yang et al., 2014). A loss of microvilli in the intestine of *D. magna* after exposure to NPs has been reported in the literature (Puerari et al., 2016). Microvilli are small processes that project from the cell membrane surface and act in the absorption of nutrients. Thus, changes in these ultrastructures may lead to a deficiency in the amount of nutrients absorbed by the body, interfering in the metabolism (Yang et al., 2010), which would also explain the observed effects on the growth parameter in chronic assays. Also, adverse effects on the mitochondria of intestinal epithelial cells can impair some biochemical and metabolic processes, such as the production of ATP and NADPH. This organelle is also involved in the production of reactive oxygen (ROS) and nitrogen (RNS) species, being the main site of the formation/accumulation of intracellular reactive species (Kowaltowski and Vercesi, 1999). Effects related to damage of the intestine following exposure to SiO$_2$ have been reported in literature. Ude et al. (2019) evaluated the in vitro toxicity of SiO$_2$NM toward undifferentiated Caco-2 cells, differentiated Caco-2 cells, Caco-2/HT29-MTX and Caco-2/Raji B co-cultures, these cell lines being chosen since they resemble different aspects of the intestine in vivo. Despite the low cytotoxicity, production of IL-8 was observed, a marker used in studies on intestinal bioreactivity in in vitro models. The same effect was observed after the exposure of undifferentiated Caco-2 cells to SiO$_2$NM with a particle size of 15 nm (Tarantini et al., 2015). The production of IL-8 can be a response to inflammatory activity (Ude et al., 2019). In addition, Yang et al. (2014) reported probable pathological changes in the intestinal cells of *D. magna*, since high concentrations of SiO$_2$ NPs caused rupture of the gut. Thus, the chronic test results reported herein can be attributed to damage in the intestinal region of *D. magna*, since ROS production, metabolic changes and a decrease in nutrient absorption could result from adverse effects on the microvilli and mitochondria.

In addition, in TEM micrographs of the organisms exposed to SiO$_2$NS@1, the presence of vacuoles containing this NS was observed in the *D. magna* cells close to deformed mitochondria (Fig. 3-E). This reinforces the hypothesis that the Trojan horse effect is promoted by the amine functionalization of the materials. However, this is not a conclusive result, since the same behavior was not observed in organisms exposed to SiO$_2$NS@3. Adverse effects on mitochondria after internalizing SiO$_2$NS@ 1 could lead to the production of ROS and lipoperoxidation and these two processes have been previously observed

after the exposure of different cell cultures to amorphous SiO$_2$NS (Lin et al., 2006; Napierska et al., 2010; Puerari et al., 2019).

Since reproduction was the parameter most affected in the chronic assays, the possibility of an intracellular effect on the eggs of *D. magna* exposed to all SiO$_2$NS was investigated. The TEM micrographs obtained are shown in Fig. 4.

In contrast to the control (Fig. 4A), swelling and crystolysis in the mitochondria were observed (Fig. 4B and C), as well as swelling in the membranes of the cell nucleus (Fig. 4C and D) for organisms exposed to the amine-functionalized NSs. In chronic toxicity assays, Gonçalves et al. (2018) exposed *D. magna* to ZnONR without and with amine functionalization with the AEAEAPTMS molecule. The authors collected the first and twelfth broods of neonates from each concentration investigated and these neonates were exposed only to M4 medium, with feeding under the same conditions as the culture. As a result, even without the addition of ZnONR during the life cycle, chronic effects were observed in the organisms due to maternal exposure (Gonçalves et al., 2018). Thus, the deformities in *D. magna* egg cells observed in the present study may lead to adverse effects in future generations of this organism, even if they have no direct contact with the NSs. This could disrupt the population dynamics, which is of concern given that *D. magna* is an important species in aquatic food chains (Gonçalves et al., 2018).

All of the results reported herein demonstrate that, when amine-functionalized, even nanomaterials of low toxicity, such as amorphous SiO$_2$NS, can have significant toxic effects on organisms subjected to chronic exposure. The possibility of NS functionalization thus needs to be evaluated in conjunction with toxicological studies to achieve the proper characterization of the materials, since surface modifications to NSs can alter their toxic potential.

4. Conclusions

The amine functionalization of the SiO$_2$NS synthetized in this study promoted significant alterations to the particle stability and size. The dispersion of NSs in the culture media M4 and ISO promoted greater agglomeration of particles in relation to UPW, as well as promoting an increase in their ED. The acute toxicity assays with *D. magna* indicated toxicity in the following order (from most to least toxic): SiO$_2$NS@3 > bare SiO$_2$NS > SiO$_2$NS@1. However, none of the NSs would be classified as toxic due to the high EC$_{50.48\ h}$ values obtained. In the chronic toxicity assays, the bare SiO$_2$NS was the least toxic of the three NSs and the reproduction of *D. magna* was one of the parameters most affected by exposure to all NS samples. In addition, alterations were observed in the antennae and gills of *D. magna* exposed to NSs and a higher concentration of Si was observed in organisms exposed to SiO$_2$NS@1 and SiO$_2$NS@3, which is consistent the hypothesis of a Trojan horse effect being promoted by amine functionalization of the molecules. In the intestine of exposed *D. magna*, ultrastructural changes were found in the mitochondria and microvilli. Ultrastructural deformations were also observed in the mitochondria and in the cell nucleus membrane of the eggs of *D. magna* exposed to NSs. The results highlight the need for the toxicological assessment of materials subjected to surface modifications.

CRediT authorship contribution statement

Rodrigo Costa Puerari: Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Emeline Ferrari**: Methodology, Validation, Investigation, Data curation. **Bianca Vicente Oscar**: Formal analysis, Investigation, Data curation. **Carmen Simioni**: Methodology, Resources. **Luciane Cristina Ouriques**: Methodology, Resources. **Denice Schulz Vicentini**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Supervision, Funding acquisition. **William Gerson Matias**: Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Project
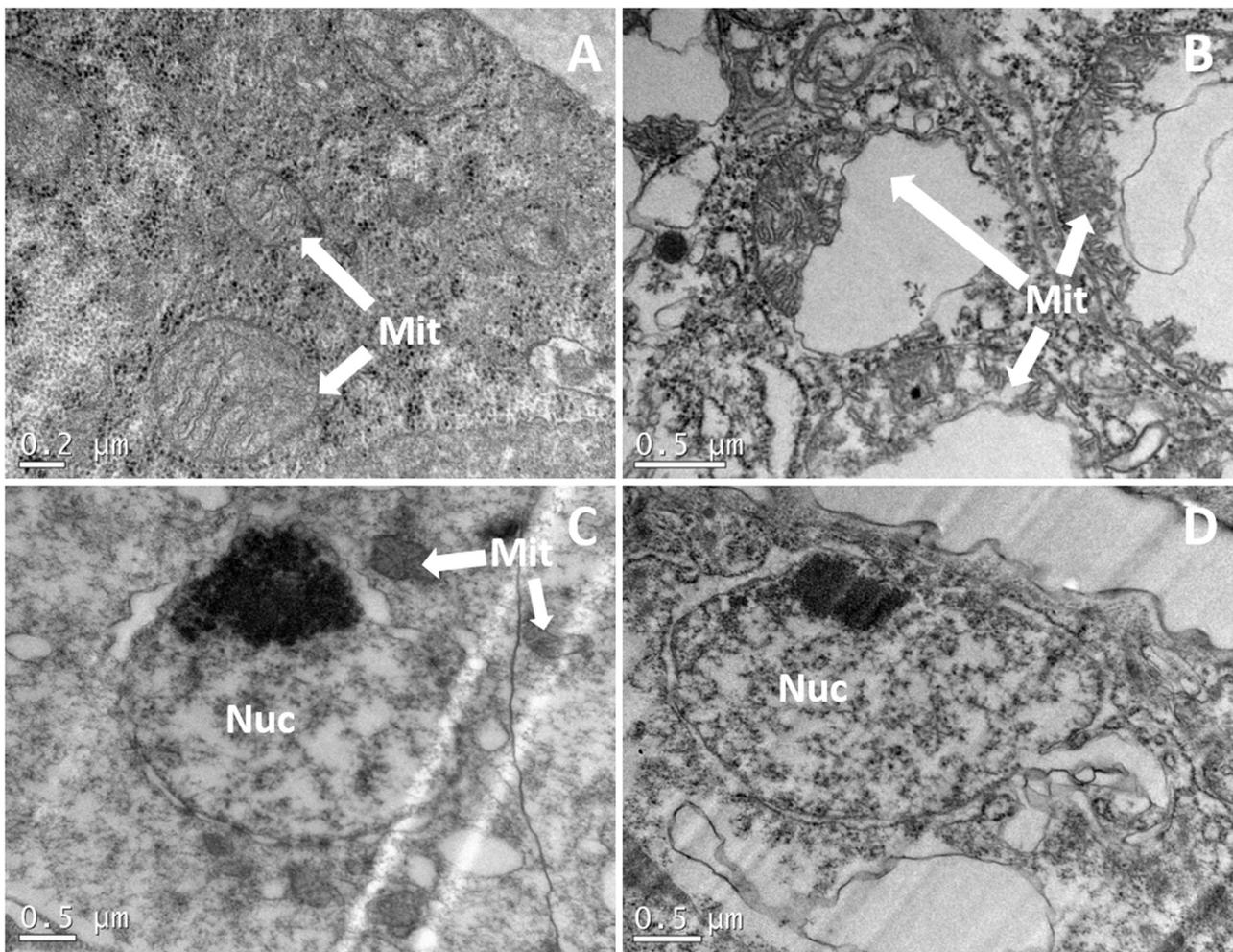
Fig. 4. TEM micrographs of eggs of *D. magna* exposed to: (A) M4 medium; (B) SiO_2NS; (C) $SiO_2NS@1$; (D) $SiO_2NS@3$. Mit = mitochondria; Nuc = nucleus.

administration, Funding acquisition.

Declaration of 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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.111979.

References

ABNT, 2017. ABNT NBR 13373:2017 aquatic ecotoxicology – chronic toxicity – test method with Ceriodaphnia spp (Crustacea, Cladocera).

ABNT, 2009. ABNT NBR 12713:2009 aquatic ecotoxicology – acute toxicity – test with Daphnia spp (Cladocera, Crustacea).

Bhattacharjee, S., Rietjens, I.M.C.M., Singh, M.P., Atkins, T.M., Purkait, T.K., Xu, Z., Regli, S., Shukaliak, A., Clark, R.J., Mitchell, B.S., Alink, G.M., Marcelis, A.T.M., Fink, M.J., Veinot, J.G.C., Kauzlarich, S.M., Zuilhof, H., 2013. Cytotoxicity of surface-functionalized silicon and germanium nanoparticles: the dominant role of surface charges. Nanoscale 5, 4870. https://doi.org/10.1039/c3nr34266b.

Bondarenko, O., Juganson, K., Ivask, A., Kasemets, K., Mortimer, M., Kahru, A., 2013. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Arch. Toxicol. 87, 1181–1200. https://doi.org/10.1007/s00204-013-1079-4.

Chen, H., Langer, R., Edwards, D.A., 1997. A film tension theory of phagocytosis. J. Colloid Interface Sci. 190, 118–133. https://doi.org/10.1006/jcis.1997.4865.

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.

Elma, M., Yacou, C., Wang, D.K., Smart, S., Diniz da Costa, J.C., 2012. Microporous silica based membranes for desalination. Water 4, 629–649. https://doi.org/10.3390/w4030629.

EPA, 2002. EPA-821/R02–013 short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. Washington, DC.

Fröhlich, E., 2012. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. Int. J. Nanomed. 7, 5577–5591. https://doi.org/10.2147/IJN.S36111.

Fruijtier-Pölloth, C., 2012. The toxicological mode of action and the safety of synthetic amorphous silica – a nanostructured material. Toxicology 294, 61–79. https://doi.org/10.1016/j.tox.2012.02.001.

Glazier, D.S., Calow, P., 1992. Energy allocation rules in Daphnia magna: clonal and age differences in the effects of food limitation. Oecologia 90, 540–549. https://doi.org/10.1007/BF01875448.

Gonçalves, R.A., de Oliveira Franco Rossetto, A.L., Nogueira, D.J., Vicentini, D.S., Matias, W.G., 2018. Comparative assessment of toxicity of ZnO and amine-functionalized ZnO nanorods toward Daphnia magna in acute and chronic multigenerational tests. Aquat. Toxicol. 197, 32–40. https://doi.org/10.1016/j.aquatox.2018.02.002.

Van Hoecke, K., De Schamphelaere, K.A.C., Van Der Meeren, P., Lucas, S., Janssen, C.R., 2008. Ecotoxicity of silica nanoparticles to the green alga Pseudokirchneriella

subcapitata: Importance of surface area. Environ. Toxicol. Chem. 27, 1948–1957. https://doi.org/10.1897/07-634.1.

Hurel, C., Bignon, C., Said-Mohamed, C., Amigoni, S., Devers, T., Guittard, F., 2018. Functionalized and grafted TiO2, CeO2, and SiO2 nanoparticles—ecotoxicity on Daphnia magna and relevance of ecofriendly polymeric networks. Environ. Sci. Pollut. Res. 25, 21216–21223. https://doi.org/10.1007/s11356-018-2251-4.

Inshakova, E., Inshakov, O., 2017. World market for nanomaterials: structure and trends. MATEC Web Conf. 129, 1–5. https://doi.org/10.1051/matecconf/201712902013.

ISO, 2012. ISO 6341:2012 − water quality – determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) − acute toxicity test. Switzerland.

ISO, 2000. ISO 10706:2000 − water quality − determination of long term toxicity of substances to Daphnia magna Straus (Cladocera, Crustacea), ISO/TC 147/SC5 − water quality/Biological methods. Switzerland.

Kowaltowski, A.J., Vercesi, A.E., 1999. Mitochondrial damage induced by conditions of oxidative stress. Free Radic. Biol. Med. 26, 463–471. https://doi.org/10.1016/S0891-5849(98)00216-0.

Ko, Y.G., Lee, H.J., Oh, H.C., Choi, U.S., 2013a. Cu2+ sequestration by amine-functionalized silica nanotubes. J. Hazard. Mater. 260, 489–497. https://doi.org/10.1016/j.jhazmat.2013.06.005.

Ko, Y.G., Lee, H.J., Oh, H.C., Choi, U.S., 2013b. Amines immobilized double-walled silica nanotubes for CO2 capture. J. Hazard. Mater. 250–251, 53–60. https://doi.org/10.1016/j.jhazmat.2013.01.035.

Lee, N., Hong Lim, C., Kim, T., Kyung Son, E., Sob Chung, G., Jun Rho, C., Rook Lee, S., Je Yu, I., 2017. Which hazard category should specific nanomaterials or groups of nanomaterials be assigned to and how? World Heal. Organ. 62.

Lee, S., Kim, S., 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.

Lin, W., Huang, Y.-W., Zhou, X.-D., Ma, Y., 2006. In vitro toxicity of silica nanoparticles in human lung cancer cells. Toxicol. Appl. Pharmacol. 217, 252–259. https://doi.org/10.1016/j.taap.2006.10.004.

Liu, L., Sun, M., Zhang, H., Yu, Q., Li, M., Qi, Y., Zhang, C., Gao, G., Yuan, Y., Zhai, H., Chen, W., Alvarez, P.J.J., 2016. Facet energy and reactivity versus cytotoxicity: the surprising behavior of CdS nanorods. Nano Lett. 16, 688–694. https://doi.org/10.1021/acs.nanolett.5b04487.

Lv, Y., Du, Y., Qiu, W.-Z., Xu, Z.-K., 2016. Nanocomposite membranes via the co-deposition of polydopamine/polyethyleneimine with silica nanoparticles for enhanced mechanical strength and high water permeability. ACS Appl. Mater. Interfaces, 6b13761. https://doi.org/10.1021/acsami.6b13761.

Manzo, S., Buono, S., Rametta, G., Miglietta, M., Schiavo, S., Di Francia, G., 2015. The diverse toxic effect of SiO2 and TiO2 nanoparticles toward the marine microalgae Dunaliella tertiolecta. Environ. Sci. Pollut. Res. 22, 15941–15951. https://doi.org/10.1007/s11356-015-4790-2.

Maser, E., Schulz, M., Sauer, U.G., Wiemann, M., Ma-Hock, L., Wohlleben, W., Hartwig, A., Landsiedel, R., 2015. In vitro and in vivo genotoxicity investigations of differently sized amorphous SiO2 nanomaterials. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 794, 57–74. https://doi.org/10.1016/j.mrgentox.2015.10.005.

Montes-Fonseca, S.L., Sanchez-Ramirez, B., Luna-Velasco, A., Arzate-Quintana, C., Silva-Cazares, M.B., Gonzalez Horta, C., Orrantia-Borunda, E., 2015. Cytotoxicity of protein-carbon nanotubes on J774 macrophages is a functionalization grade-dependent effect. BioMed. Res. Int. 2015, 1–8. https://doi.org/10.1155/2015/796456.

Napierska, D., Thomassen, L.C.J., Lison, D., Martens, J. a, Hoet, P.H., 2010. The nanosilica hazard: another variable entity. Part. Fibre Toxicol. 7, 39. https://doi.org/10.1186/1743-8977-7-39.

Puerari, R.C., da Costa, C.H., Vicentini, D.S., Fuzinatto, C.F., Melegari, S.P., Schmidt, T. C., Bouzon, Z.L., Matias, W.G., 2016. Synthesis, characterization and toxicological evaluation of Cr2O3 nanoparticles using Daphnia magna and Aliivibrio fischeri. Ecotoxicol. Environ. Saf. 128. https://doi.org/10.1016/j.ecoenv.2016.02.011.

Puerari, R.C., Ferrari, E., de Cezar, M.G., Gonçalves, R.A., Simioni, C., Ouriques, L.C., Vicentini, D.S., Matias, W.G., 2019. Investigation of toxicological effects of

amorphous silica nanostructures with amine-functionalized surfaces on Vero cells. Chemosphere 214, 679–687. https://doi.org/10.1016/j.chemosphere.2018.09.165.

Puerari, R.C., Gonçalves, R.A., Justino, N.M., Vicentini, D.S., Matias, W.G., 2020. The influence of amine-functionalized SiO2 nanostructures upon nanofiltration membranes. Environ. Nanotechnol. Monit. Manag. 13, 100287 https://doi.org/10.1016/j.enmm.2020.100287.

Reijnders, L., 2009. The release of TiO2 and SiO2 nanoparticles from nanocomposites. Polym. Degrad. Stab. 94, 873–876. https://doi.org/10.1016/j.polymdegradstab.2009.02.005.

Rossetto, A.L.D.O.F., Melegari, S.P., Ouriques, L.C., Matias, W.G., 2014. Comparative evaluation of acute and chronic toxicities of CuO nanoparticles and bulk using Daphnia magna and Vibrio fischeri. Sci. Total Environ. 490, 807–814. https://doi.org/10.1016/j.scitotenv.2014.05.056.

Shariati, F., Poordeljoo, T., Zanjanchi, P., 2020. The acute toxicity of SiO2 and Fe3O4 nano-particles on Daphnia magna. Silicon 12, 2941–2946. https://doi.org/10.1007/s12633-020-00393-6.

Shariati, F., Shirazi, M.A., 2019. Effect of SiO2 nanoparticles on chlorophyll, carotenoid and growth of green micro-algae Dunaliella salina. Nanomed. Res. J. 4, 164–175. https://doi.org/10.22034/NMRJ.2019.03.005.

Spurr, A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31–43. https://doi.org/10.1016/S0022-5320(69)90033-1.

Tarantini, A., Lanceleur, R., Mourot, A., Lavault, M.T., Casterou, G., Jarry, G., Hogeveen, K., Fessard, V., 2015. Toxicity, genotoxicity and proinflammatory effects of amorphous nanosilica in the human intestinal Caco-2 cell line. Toxicol. Vitr. 29, 398–407. https://doi.org/10.1016/j.tiv.2014.10.023.

Ude, V.C., Brown, D.M., Maciaszek, K., Stone, V., Johnston, H.J., 2019. Comparing the sensitivity of different intestinal Caco-2 in vitro monocultures and co-cultures to amorphous silicon dioxide nanomaterials and the clay montmorillonite. NanoImpact 15, 100165. https://doi.org/10.1016/j.impact.2019.100165.

Vance, M.E., Kuiken, T., Vejerano, E.P., McGinnis, S.P., Hochella, M.F., Hull, D.R., 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J. Nanotechnol. 6, 1769–1780. https://doi.org/10.3762/bjnano.6.181.

Vicentini, D.S., Nogueira, D.J., Melegari, S.P., Arl, M., Köerich, J.S., Cruz, L., Justino, N. M., Oscar, B.V., Puerari, R.C., da Silva, M.L.N., Simioni, C., Ouriques, L.C., Matias, M.S., de Castilhos Junior, A.B., Matias, W.G., 2019. Toxicological evaluation and quantification of ingested metal-core nanoplastic by Daphnia magna through fluorescence and inductively coupled plasma-mass spectrometric methods. Environ. Toxicol. Chem. 38, 2101–2110. https://doi.org/10.1002/etc.4528.

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.

Xiong, L.Q., Chen, Z.G., Yu, M.X., Li, F.Y., Liu, C., Huang, C.H., 2009. Synthesis, characterization, and in vivo targeted imaging of amine-functionalized rare-earth up-converting nanophosphors. Biomaterials 30, 5592–5600. https://doi.org/10.1016/j.biomaterials.2009.06.015.

Yang, X.Y., Edelmann, R.E., Oris, J.T., 2010. Suspended C60 nanoparticles protect against short-term UV and fluoranthene photo-induced toxicity, but cause long-term cellular damage in Daphnia magna. Aquat. Toxicol. 100, 202–210. https://doi.org/10.1016/j.aquatox.2009.08.011.

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.

Yokoi, T., Yoshitake, H., Tatsumi, T., 2004. Synthesis of amino-functionalized MCM-41 via direct co-condensation and post-synthesis grafting methods using mono-{,} di- and tri-amino-organoalkoxysilanes. J. Mater. Chem. 14, 951–957. https://doi.org/10.1039/B310576H.

Van der Zande, M., Vandebriel, R.J., Groot, M.J., Kramer, E., Herrera Rivera, Z.E., Rasmussen, K., Ossenkoppele, J.S., Tromp, P., Gremmer, E.R., Peters, R.J.B., Hendriksen, P.J., Marvin, H.J.P., Hoogenboom, R.L.A.P., Peijnenburg, A.A.C.M., Bouwmeester, H., 2014. Sub-chronic toxicity study in rats orally exposed to nanostructured silica. Part. Fibre Toxicol. 11, 1–19. https://doi.org/10.1186/1743-8977-11-8.