Acute Toxicity of Colloidal Silicon Dioxide Nanoparticles on Amphibian Larvae: Emerging Environmental Concern

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RESEARCH PAPER

Acute Toxicity of Colloidal Silicon Dioxide Nanoparticles on Amphibian Larvae: Emerging Environmental Concern

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Abstract

Emerging contaminants derive from pharmaceuticals, pesticides, disinfection by-products, home and care products, and wood preservation and industrial chemicals that contain specific drugs, metals, metal oxides and metalloids as nanoparticles (NPs) in their formulations. Although the use of silicon dioxide (SiO_2) NPs in commercial products increases, its impacts on the environment and on animal and human health are largely unknown. Thus, the aim of this study was to evaluate the ecotoxicity of colloidal SiO_2-NPs in *Rhinella arenarum* larvae exposed to 0.001, 0.01, 0.1, and 1 mg/L colloidal SiO_2-NPs for 48 h. Biotoxicological endpoints (median lethal concentration-LC_{50}; 95% confidence limits), the no-observed-effect concentration (NOEC), the lowest-observed-effect concentration (LOEC), Toxic Units (TU), oxidative stress enzyme activity (glutathione S-transferase-GST), and genotoxicity (frequency of micronuclei, and other erythrocyte nuclear abnormalities-ENAs) were measured in exposed larvae. Scanning electron microscopy equipped with an energy dispersive X-ray system allowed detecting that SiO_2-NPs aggregate on the dorsal skin of SiO_2-treated larvae. The 48 h LC_{50} of colloidal SiO_2-NPs was 0.0251 mg/L (0.0163- 0.0338 mg/L). The NOEC and LOEC values after 48 h were 0.001 mg/L and 0.01 mg/L, respectively. According to the hazard classification system for wastewaters discharged into the aquatic environment, the colloidal SiO_2-NPs evaluated are Class V, i.e., of very high acute toxicity (TU = 3984.06). At 48 h of exposure to NOEC, GST activity and ENAs frequency were significantly increased (118.75 and 58%, respectively) with respect to controls. The results of the present study indicate that, at low concentration, colloidal SiO_2-NPs exerted high toxicity on *R. arenarum* tadpoles.

Keywords *Rhinella arenarum* · Nanotoxicity · Emerging contaminants · Biomarkers · Personal care products

Introduction

There is increasing widespread concern about the potential impacts of emerging contaminants (ECs) on the environment as well as on wildlife and human health (Kendall et al. 2016). ECs are defined as synthetic or naturally occurring chemicals that have appeared in freshwater ecosystems and potable water during the last decades (Sgroi et al. 2017). Pharmaceuticals, personal care products and endocrine disrupting compounds are among the prime examples of ECs. ECs enter water systems from different sources, such as human excretion (sewage and hospital effluents), clandestine or untreated disposal of animal manure from feedlots, leaching and runoff from agricultural fields that use organic fertilizers, or from industries (Archer et al. 2017). However, monitoring data sets (previously limited by analytical capabilities) and accurate risk assessment and environmental legislation are currently lacking (Lindsey et al. 2001; Klavarioti et al. 2009; Al-Odaini et al. 2013).

Some ECs recently detected in waters are nanoparticles (NPs) (Sauvé and Desrosiers 2014), named as engineered nanomaterials. Nanotechnology has created new type of materials, which have revolutionized the world with a large

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range of applications (Bhushan 2004). Nanomaterials are diverse types of small-scale materials that have structural elements smaller than 100 nm (nano-sized particles or NPs), in at least one dimension (EPA 2015; Calderón-Jiménez et al. 2017) and have facilitated the development of new cosmetics, pharmaceuticals, personal care products, sunscreen, powdered food, insecticides, and biocidal products for human ectoparasites (e.g., Jones et al. 2008; Gandhi et al. 2016). The use of NPs in cosmetic or personal care products poses significant challenges, because NPs frequently occur at low concentrations and are often incompatible with the analytical instruments that would be required for their identification, quantification and characterization (Contado 2015).

The number of consumer products that have incorporated NPs into their formulations has grown from a total of 54 products identified in 2005 to over 1800 nanomaterial- and NP-containing consumer products in 2014 in 32 countries (Vance et al. 2015). One of the main concerns regarding long-term environmental and human exposures to NPs is the limited information (Sajid et al. 2015). As a consequence, there is a need to evaluate their toxicity (Ray et al. 2009; Ostroumov and Kotelevtsev 2011). In general, the chemical dynamics of NPs is different from that of non-particulate contaminants, so new paradigms will be needed for the NPs present in the water, soil, sediment and biota (Mahdi et al. 2017). Silicon dioxide-based NPs (known as silica) (SiO_2-NPs) are one of the main NPs used for personal care products for children and as bio-pesticides for veterinary treatments, human health, and as pesticides in agriculture (Barik et al. 2008, 2012). The products that contain SiO_2-NPs in complex matrices such as water and soils (Sferratore et al. 2006) require risk evaluation and characterization in non-target organisms (Sauvé and Desrosiers 2014).

Different investigations have demonstrated that SiO_2-NPs in zebrafish (*Danio rerio*) (embryos and larvae) cause embryonic developmental toxicity by oxidative damage, which results in persistent effects on larval behavior (Duan et al. 2013, Ye et al. 2013). Moreover, Ambrosone et al. (2014) reported that SiO_2-NPs treatment of *Hydra vulgaris* (Cnidaria) leads to the modification of homeostasis and modulation of gene expression. In mice, it has been demonstrated that SiO_2-NPs with a diameter of 100 nm induce liver injury (Nishimoria et al. 2009; Hasezaki et al. 2011). Furthermore, insecticides containing NPs such as titanium dioxide NPs (TiO_2-NPs) in pediculicidal formulations (Gandhi et al. 2016) act mechanically by obstructing the respiratory openings of the cuticle surfaces of the insects and produce abrasions or block the spiracle, thus leading to biological and behavioral changes, including the reduction of movements, feeding, and death. Likewise, Shrivastava et al. (2007) and Wijnhoven et al. (2009) have suggested that Ag-NPs could accumulate in fish skin and affect cellular modulation and therefore inhibit bacterial growth.

In the present study, larvae of the common South American toad *Rhinella arenarum* were selected as test organisms. This species has an extended geographical distribution and is frequently present in natural and artificial aquatic ecosystems (e.g., forests, wetlands, agricultural lands, and urban regions) (Bionda et al. 2015). This species is suitable and useful as a laboratory experimental model for monitoring aquatic ecosystems and its sensitivity to some xenobiotics has been proven in many biomarker studies (e.g., Venturino et al. 2003; Lajmanovich et al. 2014). It is important to highlight the ecological role of amphibian tadpoles as a link between terrestrial and aquatic habitats (Altig et al. 2007), in relation to the potential for NPs to be taken up by organisms and be transferred in food webs (Bundschuh et al. 2016).

Some researchers have evaluated the undesired biological effects of ECs (e.g., McConnell and Sparling 2010; Melvin 2016; Peltzer et al. 2017), including some NPs, on amphibian tadpoles (e.g., Hinther et al. 2010; Salvaterra et al. 2013; Nations et al. 2015; Thompson et al. 2017), but little is known about the ecotoxicity of SiO_2-NPs on anuran larvae. For these reasons, the aim of this study was to investigate the acute and sublethal effects of colloidal SiO_2-NPs using larvae of the common toad *R. arenarum* as a biological model. The development of such information may allow the assessment and characterization of potential ecological risks following future massive use of NPs as ECs.

Materials and Methods

Test Organisms

Premetamorphic larvae at Gosner stages (GS) 26–30 (Gosner 1960) of *R. arenarum* (n = 150), with average size (snout-tail tip) 16 ± 0.25 mm and weight 0.045 ± 0.005 g, were collected from the temporary pond called "Lago Parque del Sur" (31°39'53.90″S—60°42′51.20″W, Santa Fe Province, Argentina) in November 2016. In this site, pesticides are never applied because pesticide application is restricted by law because they might have a toxic effect on human and wildlife health (Martinuzzi et al. 2016). Larvae were acclimated in laboratory conditions for 48 h at a 12-h light/dark cycle with dechlorinated tap water (DTW), pH 7.2 ± 0.05, conductivity of 165 ± 12.5 μmhos cm^{-1}, dissolved oxygen concentration of 6.5 ± 1.5 mg L^{-1}, and hardness of 48.5 mg L^{-1} of $CaCO_3$ at 23 ± 3 °C, and fed on boiled lettuce (*Lactuca sativa*) at the beginning of the experiment.

The bioassays using larvae were approved by the bioethical committee of the Facultad de Bioquímica y Ciencias Biológicas, Universidad del Litoral, Santa Fe, Argentina (Res. CD No.: 388/06), and the experimental protocol was according to the norms of ASIH-American Society of Ichthyologists and Herpetologists (2004) criteria.

NPs and Experimental Design

For short-term (48-h) static toxicity tests, a personal care formulation with a colloidal suspension of SiO_2-NPs used to control ectoparasite insects was purchased in a local pharmacy. Sedimentation and re-dispersion were used to obtain only NPs and remove agents and surfactants from the NPs (Anon. 2008). The method consisted in centrifugation at 10,000 rpm for 20 min; and then, to obtain the final concentration of NPs, the pellet was weighed by electronic balance (Ohaus®, ± 0.0001 g) and dissolved in deionized water (Anon 2008; Gandhi et al. 2016). Stock solutions containing colloidal SiO_2-NPs were prepared for the toxicity tests. Test concentrations were prepared by diluting the stock solution in DTW. These SiO_2-NPs were previously characterized using a Scanning Electron Microscope (SEM) (FEI-Quanta™200) equipped with an Energy Dispersive X-ray (EDX) system. The same procedure was followed with dehydrated treated larvae. Particles on the images obtained were measured using ImageJ software (available free over the Internet at: http://rsb.info.nih.gov/ij/index.html); the average primary particle diameter was calculated from 20 to 30 particles.

Range-finding toxicity tests consisted in exposing larvae to colloidal SiO_2-NPs to estimate the median lethal concentration (LC_{50}), the no-observed-effect concentration (NOEC), and the lowest-observed-effect concentration (LOEC). Ten tadpoles/container were exposed to 0.001, 0.01, 0.1, and 1 mg/L colloidal SiO_2-NPs and a control (only DTW) in glass aquaria (13 cm in diameter and 14 cm in height) with 1 L DTW. Both the control and the test solutions were made in triplicate. Treatments were randomly assigned to the experimental containers, as was the order in which the glass containers were sampled. Because of the lack of data on the environmental concentration of NPs deposited on water bodies and uncertainties associated with the fate of these xenobiotics for biomarker assessment, a subsample of tadpoles per control and NOEC-48 h exposures were euthanized in accordance with the ASIH (2004) guidelines and with approval of the animal ethics committee of the Facultad de Bioquímica y Ciencias Biológicas, Universidad del Litoral, Santa Fe, Argentina. The residual water of the experiments was disposed by the Waste Management Program of the same institution.

Antioxidant Enzymes

Each larva was homogenized (on ice) in 0.1% t-octylphenoxypolyethoxy-ethanol (triton X-100) in 25 mM tris (hydroxyl methyl) aminomethane hydrochloride (pH 8.0), using a polytron. Suspensions were centrifuged at 10,000 rpm for 15 min at 4 ± 1 °C and the supernatant (crude extract) was extracted. The Biuret method was used to determine protein concentration in the supernatants (Kingsley 1942). When sample volume was enough, enzyme kinetics assays were carried out in duplicate. Glutathione S-transferase (GST) activity was determined spectrophotometrically using the method described by Habig et al. (1974) and adapted by Habdous et al. (2002) for mammal serum GST activity. The enzyme assay was performed at 340 nm in 100 mM Na–phosphate buffer (pH 6.5) (F.V. = 920 µL), 20 µL of 0.2 mM 1-chloro-2, 4-dinitrobenzene, 50 µL of 5 mM reduced glutathione, and the sample. Enzyme kinetics assays were performed at 25 °C and whole GST activity was expressed as nmol min^{-1} mg^{-1} protein using a molar extinction coefficient of $9.6 \times 10^3 M^{-1}$ cm^{-1}.

Genotoxicity

One smear per larva was prepared on clean slides with blood samples obtained by cardiac puncture, then fixed and stained using the May-Grünwald-Giemsa method (Lajmanovich et al. 2005). It is important to consider that red blood cells in amphibians are nucleated and undergo cell division in the circulation, particularly during the developmental stages. In mature erythrocytes, the frequencies of micronuclei (MN) and other erythrocyte nuclear abnormalities (ENAs) such as binucleated erythrocytes (BER), erythroplastids (EP), kidney-shaped nuclei (KN), lobed nuclei (LN), multi-micronucleated erythrocytes (MMER), and notched nuclei (NN), were recorded according to the procedures of Guilherme et al. (2008). The ENAs value was the sum of BER + EP + KN + LN + MMER + NN (Lajmanovich et al. 2014). Coded and randomized slides were examined blind by a single operator.

Data Analyses

The lethal concentration (LC_{50}) values and their respective 95% confidence limits were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977). Mortality data were statistically evaluated using the Dunnett's test for post hoc comparison of means to determine NOEC and LOEC (U.S.EPA 1989). Taking into account that NPs emitted by wastewater are considered to be widely present in the natural environment (Li et al. 2016), the toxicity

value (LC_{50}) was transformed into Toxic Units (TU) according to the following equation: TU = 100/LC_{50} and classified under the hazard classification system for wastewaters discharged into the aquatic environment (Table 1; Persoone et al. 2003). The data of GST activity were expressed as means ± standard error (SEM). The Mann–Whitney *U* test was used to compare enzymatic activities between control and SiO_2-NPs-treated larvae. Data of MN and other ENAs were analyzed using the binomial proportion test (Margolin et al. 1983). These statistical methods were performed using BioEstat software 5.0 –(Ayres et al. 2008). A value of $p < 0.05$ was considered significant.

Results

SEM analyses of the colloidal SiO_2-NPs clearly showed spherical shapes, mostly aggregated, of an average particle size of 102 ± 12 nm (Fig. 1a). EDX spectroscopy confirmed the purity of colloidal SiO_2-NPs with high Si contents (Fig. 1b).

Table 1 Criteria of classification for hazardous substances for wastewaters discharged (Persoone et al. 2003)

TU	Class	Toxicity
< 0.4	Class I	No acute toxicity
< 0.4 < TU < 1	Class II	Slight acute toxicity
1 < TU < 10	Class III	Acute toxicity
10 < TU < 100	Class IV	High acute toxicity
TU > 100	Class V	Very high acute toxicity

Acute Toxicity Tests

No mortality was observed in the controls. The 48 h colloidal SiO_2-NPs acute LC_{50} value (95% CL) calculated based on the Trimmed Spearman-Karber was 0.0251 mg/L (0.0163% - 0.0338 mg/L). The LC_{50} values were stabilized at 24 h of exposure. The NOEC value was 0.001 mg/L, whereas the LOEC value was 0.01 mg/L. The highest concentration of colloidal SiO_2-NPs (1 mg/L) killed all exposed larvae. The value for TU was 3984.06, which, according to the hazard classification system for wastewaters discharged into the aquatic environment, is considered Class V, i.e., of very high acute toxicity (TU > 100; Table 1).

Morphological observations of untreated dehydrated larvae exhibited normal external surface (Fig. 2a). The skin of the larvae treated with 0.01 mg/L colloidal SiO_2-NPs (LOEC value) showed several NPs absorbed or adhered, although no intercellular epidermal edemas or focal dermal inflammations were observed (Fig. 2b, c). EDX spectroscopy exhibited the chemical components of these NPs and confirmed the high percentage of Si on the tadpoles skin, which was similar to that of the colloidal SiO_2-NPs sample (Fig. 2d).

GST Activity

The mean value of GST activity in controls was 105.88 ± 12.93 nmol min^{-1} mg^{-1} total protein at 48 h. GST activity was highly significantly increased (118.75%) by colloidal SiO_2-NPs at NOEC exposure (U = 81.00; $p < 0.01$) (Fig. 3).

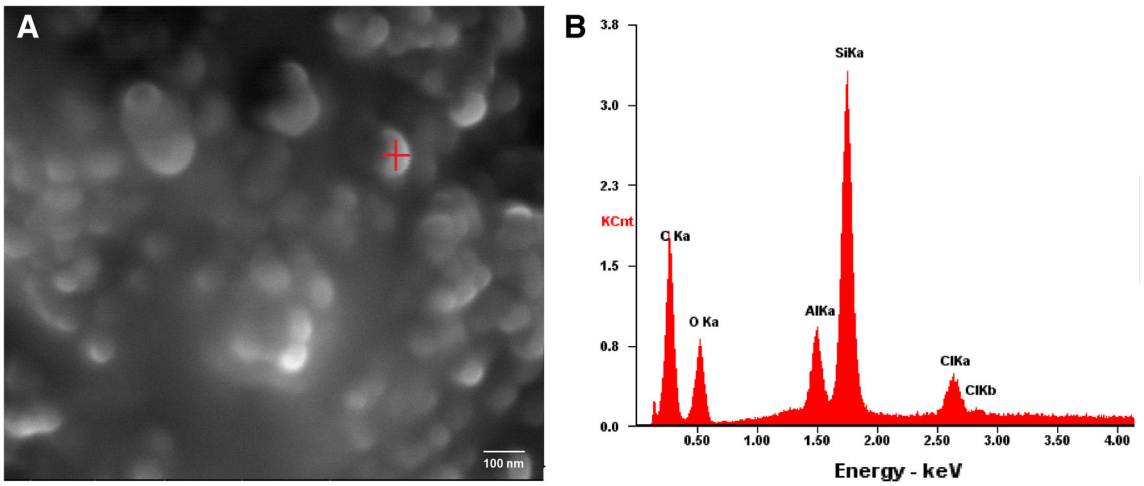

Fig. 1 **a** Scanning Electron Microscopy (SEM) image of 99% of colloidal silicon dioxide nanoparticles (SiO_2-NPs). **b** SEM–EDX spectra of SiO_2-NPs. The vertical axis corresponds to the intensity (counts per second). The highest peak is due to the silice (Si) contents on the sample

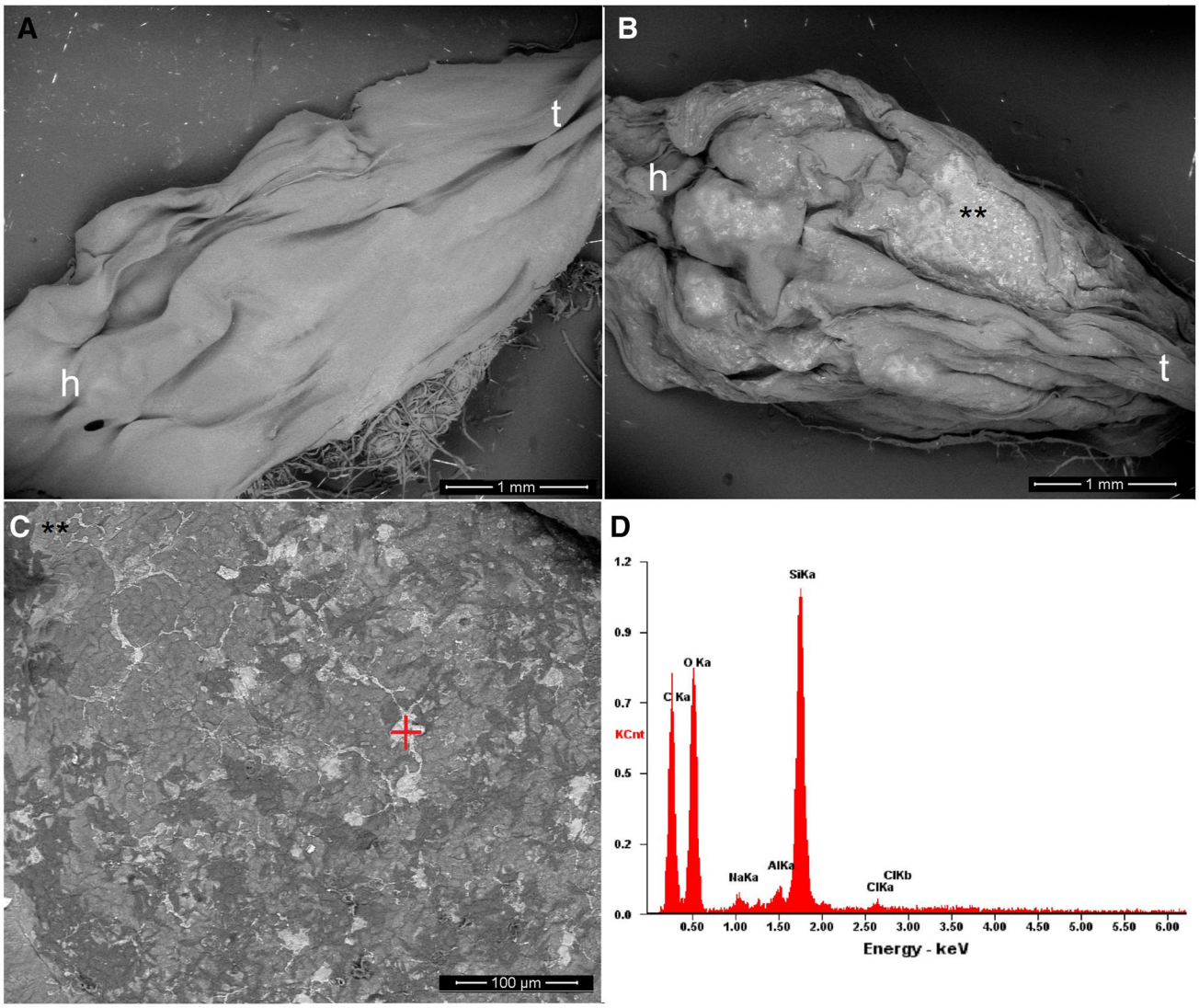

Fig. 2 Scanning electron microscopy (SEM) images of *R. arenarum* tadpoles (see reference: *h* head, *t* tail) exposed to colloidal silicon dioxide nanoparticles (SiO_2-NPs). **a** Control with dechlorinated tap water (DTW). **b** Tadpole dead at the LOEC value (0.01 mg/L) (**) see detail of SiO_2-NPs aggregates adhered to the dorsal skin of *R. arenarum* larvae. **(C)** Magnification of area exposed (**) in Fig. 2b. **d** SEM–EDX spectra for SiO_2-NPs aggregates indicated by the red mark in Fig. 2c. The vertical axis corresponds to the intensity (counts per second). The highest peak is due to the silice (Si) contents on the skin sample

Effect of SiO_2-NPs on ENAs

Normal mature erythrocytes of *R. arenarum* larvae are oblong/oval-shaped with a central nucleus visibly structured and a well-defined boundary, which enabled the recognition of fragments in their cytoplasm (Fig. 4a). The MN quantified were spherical nuclear fragments separated from the nucleus (Fig. 4b). Binucleated, erythroplastid, kidney-shaped, multi-micro nucleated, and notched nuclei were also observed in larvae treated with colloidal SiO_2-NPs at the NOEC value (0.001 mg/L) (Fig. 4c–h).

After 48-h exposure, at the NOEC value of SiO_2-NPs (0.001 mg/L), blood of *R. arenarum* larvae showed a significant increase (58%) in the frequency of ENAs respect to controls ($z = -2.25$; $p < 0.01$) (Fig. 5).

Discussion

NPs are being increasingly used in imaging, diagnosis, care products, cosmetics, and drug delivery, but their toxicity in aquatic organisms has only recently begun to be investigated. In the present study, very low concentrations (< 1 mg/L) of colloidal SiO_2-NPs induced high lethal toxicities in *R. arenarum* larvae with an LC_{50} 48 h value of 0.0251 mg/L. The TU calculated estimate the risk associated as a result of the discharge of effluents containing

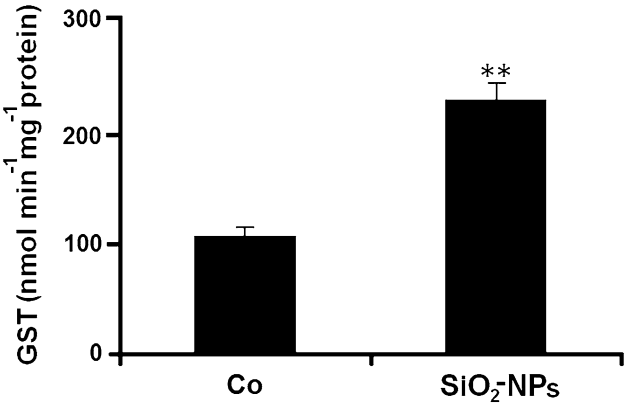

Fig. 3 Glutathione S-transferase (GST) activity in SiO_2-NPs-treated *R. arenarum* larvae at 48 h of exposure. (Co) control. SiO_2-NPs: 0.001 mg/L. Bars represent the mean ± SEM, $n = 10$. Significant difference was $^{**}p < 0.01$ with respect to the control (Mann–Whitney *U* test). $n = 10$

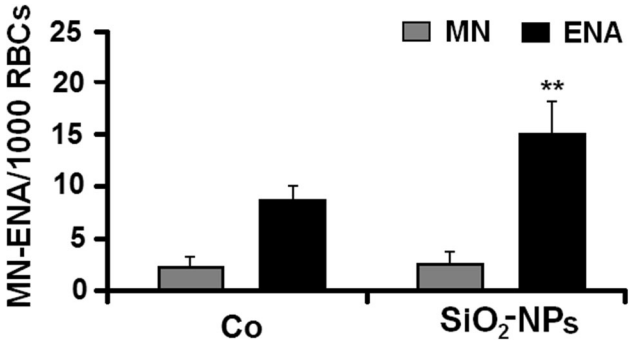

Fig. 5 Induction of micronuclei (MN) and erythrocyte nuclear abnormalities (ENAs) (per 1000 red blood cells; RBCs) in *R. arenarum* larvae at 48 h of exposure to colloidal silicon dioxide nanoparticles (SiO_2-NPs). (Co) control. SiO_2-NPs: 0.001 mg/L. Bars represent the mean ± SEM, $n = 10$. Significant difference was $^{**}p < 0.01$ with respect to the Co (binomial proportion's test). $n = 10$

these test compounds. As mentioned above, according to the hazard classification system applied for wastewaters discharged into the aquatic environment (Persoone et al. 2003), colloidal SiO_2-NPs belong to Class V, which indicates a very high acute toxicity.

It is generally recognized that SiO_2-NPs are not toxic (e.g., Ryu et al. 2014; Caltagirone et al. 2015; Diab et al. 2017). However, the increase in the use of NPs in many industry fields has prompted the careful investigation of their toxicity in non-target organisms. Here, we determined different biological effects elicited by SiO_2-NPs on *R. arenarum* larvae. Apparently, as shown in Fig. 2, these NPs were absorbed or adhered onto the tadpole's skin, possibly by the affinity to lipids and caused the death simply by physical means, similarly to that described for larvicidal and pediculicidal effects by TiO_2-NPs (Gandhi et al. 2016). It should be noted that the mode of action of insecticidal compounds for ectoparasite control that contain SiO_2-NPs in their formulations is through dehydration of the insect cuticle by physical sorption of lipids, and they are also expected to cause damage in the plasma cell membrane, resulting in cell lysis and death of the organism solely by physical means-asphyxiation mechanism (Tiwari and Behari, 2009). Recent studies on the in vitro toxicity of SiO_2-NPs have shown that their toxicity is mediated by adsorption of NPs to extracellular components as serum proteins (Napierska et al. 2010; Zhang et al. 2012). Also, Ambrosone et al. (2014) suggested that, in the case of not-target organisms (e.g., *H. vulgaris*) in contact with amorphous SiO_2-NPs, this interaction first occurs with the external cuticle and induces a progressive morpho-physiological alteration (i.e., changes in hydrostatic pressure) and normal behavior (feeding behavior).

Several studies have reported oxidative stress and pathological changes in aquatic species, specifically in fishes after exposure to TiO_2-NPs (e.g., Federici et al. 2007). An increased activity of GST can reveal disorders that could be indicative of redox alterations related to a possible oxidative stress situation (Oruç et al. 2004). After 48 h, *R. arenarum* larvae treated with colloidal SiO_2-NPs at the NOEC value (0.001 mg/L) showed an increase in GST activity in relation to the controls. Similarly, an increase in GST activity induced by SiO_2NPs has been reported in various cell lines after 48 of exposure (Munteanu et al. 2010). However, the antioxidant adaptation system to SiO_2-NPs is insufficient to prevent the formation of reactive oxygen species (ROS) and thus biomolecules

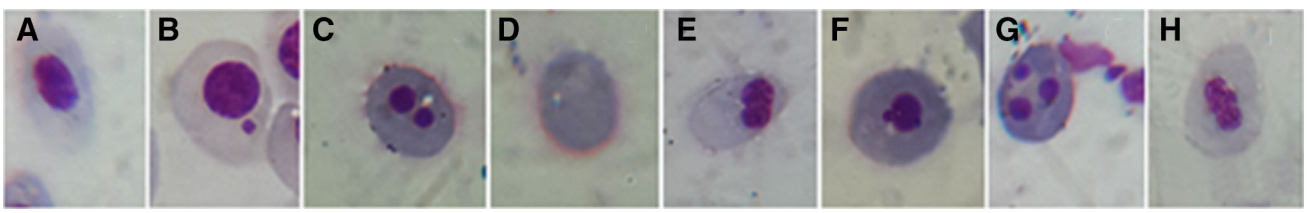

Fig. 4 Detail of red blood cells observed in SiO_2-NPs-treated *R. arenarum* larvae. **a** normal mature erythrocyte (NE); **b** micronuclei (MN); **c** binucleated erythrocyte (BER); **d** erythroplastid (EP); **e** kidney-shaped nuclei (KN); **g** multi-micronucleated erythrocyte (MEER); **h** notched nuclei (NN). May Grünwald-Giemsa 100X

are damaged (Petrache Voicu et al. 2015). SiO_2-NPs induce ROS production in *R. arenarum* larvae and, consequently, a response of antioxidative defences (GST). Similarly, GST activity could be induced to neutralize SiO_2-NPs toxicity and could thus be a suitable biomarker for the evaluation of colloidal SiO_2-NPs at very low exposure in potentially contaminated aquatic ecosystems.

Some NPs (e.g., TiO_2) may generate ROS, which also can lead to DNA damage (Jaeger et al. 2012). For example, Bacchetta et al. (2017) reported genotoxicity and oxidative stress in fish after short-term exposure to silver NPs. MN and other ENAs such as kidney shaped, lobed and segmented nuclear abnormalities, binucleated erythrocytes, erytrhoplastids, kidney-shaped nuclei, lobed nuclei, and others have been used by many authors as suitable indicators for the assessment of genotoxicity of xenobiotics on fishes and amphibians (Ayllon and García-Vazquez 2000; Gravato and Santos 2002; Pacheco and Santos 1997; Lajmanovich et al. 2014), and for natural environment biomonitoring programs (Phan et al. 2007; Attademo et al. 2011). Different frequencies of MN and other ENAs may be caused by specific genotoxic events, which are subjected to different mechanisms of mutagenicity (Bolognesi et al. 2006). The synchronized expressions of ENAs and MN in red blood cells are considered as indicators of cytotoxicity and genetic toxicology, respectively (Grisolia et al. 2009). In the present study, total ENAs of *R. arenarum* tadpoles exposed to colloidal SiO_2-NPs increased significantly. Therefore, this study is the first report investigating the cytotoxic effects of these types of substances by ENAs in amphibians. There is limited evidence concerning whether or not SiO_2-NPs are genotoxic and opposed results have been reported (e.g., Wang et al. 2007; Kwon et al. 2014). However, the latest researches confirmed the genotoxic potential of these NPs (e.g., Demir and Castranova, 2016; Åkerlund et al. 2017; Scherzad et al. 2017; de Souza et al. 2018). In this sense, several researches have also pointed out that MN assays are more sensitive and frequently used to confirm the genotoxicity of NPs (Kisin et al. 2007; Landsiedel et al. 2009). The molecular mechanisms (genotoxicity, cytotoxicity) of SiO_2-NPs are not quite clear and further investigations are needed.

Conclusions

Many reports about the toxicity of SiO_2-NPs either mention the effects of pure ingredients or are carried out in vitro or in cell cultures. However, the present results showed the biotoxicity effects of SiO2-NPs contained in a biocidal commercial product on a non-target aquatic vertebrate. The LC_{50} here recorded for SiO_2-NPs-treated *R. arenarum* larvae indicates that high toxicities were induced by very low concentrations of this xenobiotic. The present results indicate that these NPs have high acute biotoxicity and are thus potentially deleterious to aquatic ecosystems. Also, colloidal SiO_2-NPs at NOEC value can induce signs of cytotoxicity. However, further studies are necessary to elucidate the role of the possible synergy of the surfactants, additives, and dispersing agents with SiO_2-NPs within a commercial product that can produce high lethal toxicity, oxidative stress, and genotoxicity in amphibian larvae.

A combination of ecotoxicological studies on non-target organisms and a multi-scale monitoring program, fate and risk assessment tools with respect to NPs as emerging contaminants is required as a base for sustainable water resource management and overall protection of ecological communities in the aquatic environment.

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