The uptake of ZnO and CuO nanoparticles in the water-flea *Daphnia magna* under acute exposure scenarios

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**A B S T R A C T**

In this study the uptake of ZnO and CuO nanoparticles by *Daphnia magna* was tested. Daphnids were exposed during 48 h to acute concentrations of the nanoparticles and corresponding metal salts. The *Daphnia* zinc and copper concentration was measured and the nanoparticles were localized using electron microscopy. The aggregation and dissolution in the medium was characterized. A fast dissolution of ZnO in the medium was observed, while most CuO formed large aggregates and only a small fraction dissolved. The *Daphnia* zinc concentration was comparable for the nanoparticles and salts. Contrarily, a much higher *Daphnia* copper concentration was observed in the CuO exposure, compared to the copper salt. CuO nanoparticles adsorbed onto the carapace and occurred in the gut but did not internalize in the tissues. The combined dissolution and uptake results indicate that the toxicity of both nanoparticle types was caused by metal ions dissolved from the particles in the medium.

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1. Introduction

The last decade the production and application of metal oxide nanoparticles is strongly increasing. ZnO nanoparticles are being widely used in sunscreens, cosmetics, paints, plastics (Ma et al., 2013), while CuO nanoparticles are being used in gas sensors (Chowdhuri et al., 2004), batteries (Zhang et al., 2005), plastics and metallic coatings (Hernández Battez et al., 2010). This wide interest in nanoparticles occurs as a result of their very small size (1–100 nm in at least one dimension) and associated specific physical and chemical properties. The large scale application of nanomaterials also makes them prone to end up in the aquatic environment, where their specific properties may cause adverse effects to aquatic species.

ZnO and CuO nanoparticles are toxic to *Daphnia magna* under acute exposure scenarios (Jo et al., 2012; Wiencz et al., 2009; Zhao et al., 2012; Zhu et al., 2009). Several authors have suggested that the acute toxicity of these ZnO (Franklin et al., 2007; Heinlaan et al., 2008) and CuO (Aruoja et al., 2009; Heinlaan et al., 2008) nanoparticles to different aquatic species is caused by the release of free metal ions. It is known that metal oxide nanoparticles are highly dynamic when entering the aquatic environment and do not just remain as single particles. Zinc oxide nanoparticles have been shown to dissolve (e.g. Kasemets et al. (2009)) but at the same time aggregate (e.g. Keller et al. (2010)). These dissolution (Kasemets et al., 2009; Mortimer et al., 2010) and aggregation (Jo et al., 2012; Zhao et al., 2011) patterns have also been shown for copper oxide nanoparticles. The dissolution of nanoparticles depends on different factors including the exposure concentration (Li and Wang, 2013), the size of the nanoparticles (David et al., 2012) and water chemistry (Li and Wang, 2013). The formation of aggregates depends mostly on the surface charge of the particles. Highly negative or positive charged nanoparticles will repel each other, resulting in a higher stability. When the surface charge is low, nanoparticles will aggregate (Bagwe et al., 2006).

As a result of the nanoparticle dynamics, it is possible that not only ZnO and CuO nanoparticles but also their derivates are taken in by daphnids. Metal ions dissolved from the nanoparticles may be taken in by ion channels or ion pumps located in the membranes of gill epithelial cells (Bianchini and Wood, 2008; Simkiss and Taylor, 1989). On the other hand there is also a possibility that nanoparticles or their aggregates are ingested by *D. magna*, as shown for CuO (Heinlaan et al., 2011) and ZnO nanoparticles (Li and Wang, 2013). After ingestion, the nanoparticles may occur as dispersed nanoparticles or aggregates in the gut (Heinlaan et al., 2011) or dissolve in the gut or in the cells after uptake by endocytosis due to...
lower pH values (Studer et al., 2010). Li and Wang (2013) indicate that ZnO nanoparticles are ingested by *Daphnia* and subsequently largely dissolve in the gut. However, in this study (Li and Wang, 2013), the uptake of ZnO nanoparticles in *D. magna* was only studied during a very short exposure period (up to 8 h of exposure). CuO nanoparticles were also shown to be ingested by *Daphnia* (Heinlaan et al., 2011). Since in this study, less CuO nanoparticles were observed in the gut after 48 h of exposure than after 24 h of exposure, there is a possibility that some CuO nanoparticles dissolved as well.

Despite the high diversity of commercially available nanoparticles, only a limited number of studies have investigated the uptake of ZnO and CuO nanoparticles in *Daphnia magna*. Without knowledge on the nanoparticle dynamics in the medium and the uptake by *D. magna*, it remains unclear whether the observed acute toxicity is due to the formed toxic ions. Therefore, we studied the nanoparticle dynamics (aggregation and dissolution state) in the *Daphnia* test medium during the exposure and measured the total zinc and copper concentration of *Daphnia* when exposed to high acute concentrations of the ZnO and CuO nanoparticles. In addition, to distinguish between the metal ions and the nanoparticles that are taken in by *D. magna*, metal concentrations were also measured when exposed to corresponding metal salts.

### 2. Materials and methods

#### 2.1. Tested nanoparticles and metal salts

In this study, the ZnO nanodispersion (NanoTek 40 weight % in water colloidal dispersion, Alfa Aesar Germany, 80 nm), ZnO nanopowder (NanoSun, Micromisers PTY Australia, 30 nm) and ZnCl₂ (Sigma–Aldrich Belgium, >98%) were used to determine the acute uptake by *Daphnia*. Similar experiments were performed with the CuO nanopowder (Sigma–Aldrich Belgium, <50 nm) and CuCl₂·2H₂O (ICN BioMedicals Belgium). The nanoparticle size and shape were characterized using Transmission Electron Microscopy (TEM; FEI Philips CM30 equipped with a Gatan imaging filter).

#### 2.2. Test species

The freshwater crustacean *Daphnia magna* was used as a test species. *D. magna* were reared in biofilter-treated tap water (pH 8.4–8.5, conductivity 153 μs/cm) under a constant light–dark cycle (14 h light/10 h dark) at 20 °C. Three times a week, the water was refreshed and *D. magna* were fed with 4 × 10⁵ algae cells/ml (Pseudokirchneriella subcapitata and Chlamydomonas reinhardtii in a 3:1 ratio).

#### 2.3. Acute uptake of metal oxide nanoparticles and metal salts by *Daphnia magna*

Neonates of *D. magna* (>24 h) were exposed in OECD recommended IS test medium (CaCl₂·2H₂O: 0.294 g/l, MgSO₄·7H₂O: 0.123 g/l, NaHCO₃: 0.065 g/l, KCl: 0.006 g/l) to nominal acute immobilization EC₅₀ concentrations (previously tested) of ZnO nanodispersion (1.12 mg Zn/l ZnO nanoparticle, 2.25 mg Zn/l ZnO nanosun, 2.30 mg Zn/l ZnCl₂ during the uptake experiments, unfiltered (containing all zinc present in the water column) and filtered (450 nm Acrodisc PP syringe filter, Pall life sciences; containing all zinc from small aggregates <450 nm, individual nanoparticles and dissolved fraction present in the water column)) water samples were taken in triplicate from the medium. These samples were taken directly after addition of the daphnids to the different solutions (which was 1–2 h after having made the stock solutions), to which we will refer to as 0 h and 24 h and 48 h later, to which we will refer to as 24 h and 48 h. The zinc exposure concentration of these samples was determined by ICP-OES (Thermo Scientific 6000 series) after acidification of the samples to 1% HNO₃. To study the dynamics of ZnO nanoparticles and ZnCl₂, different medium samples were taken from vials exposed under the same conditions as in the actual uptake experiment. Samples were taken in triplicate after 0.6, 48, 54, 96, 240, 288 h of exposure. Different filtration methods were applied to the samples to characterize the nanoparticle dynamics by distinguishing between the total nanoparticle and dissolved zinc concentration i.e. the aggregated fraction (retained on a 100 nm or 450 nm filter), the nanoparticle fraction (retained on a 1 kDa filter but passing through a 100 nm filter) and the dissolved fraction (passing through a 1 kDa). The 100 nm (Paradisc PTFE, Whatman) and 450 nm (Acrodisc PP, Pall life sciences) filtrations were performed with syringe filters. The 1 kDa ultrafiltrations were performed with Microsep centrifuge filters (Pall Life Sciences), using a 1 h centrifugation at 5000 g (Beckman Avanti J25). All samples were acidified to 1% HNO₃ and the zinc concentration was analyzed by ICP-MS (Thermo Scientific Element 2 XR; Agilent technologies 7700 series) or ICP-OES (Thermo scientific 6000 series).

Since the dissolution of CuO nanoparticles is known to be lower than the dissolution of ZnO nanoparticles, the dynamics of these nanoparticles were instead measured in the acute uptake experiment (but with less sampling points than in the zinc dynamics experiment). Medium samples (1 kDa, 100 nm, 450 nm filtered and unfiltered) were taken in triplicate from the CuO nanoparticle (nominal concentration of 14.38 mg Cu/l) and CuCl₂·2H₂O (nominal concentration of 0.04 mg Cu/l) exposure after 0, 24 and 48 h of exposure and were analyzed similarly as in the zinc dynamics experiment.

All samples (from the acute uptake and dynamics experiment) for all exposures (zinc and copper) were taken from the water column. As a result, nanoparticle aggregates precipitated to the bottom of the vial were not included.

The pH (7.7–8.2), oxygen concentration (~80%) and temperature (19–21 °C) were measured several times during the exposure (Hach HQ30d-fluo) and showed little variation.

#### 2.5. Data analysis and statistics

GraphPad Prism (version 6) was used for all data visualizations and statistics. The differences in total zinc or copper concentration (after 0, 24, 48 h of exposure) of the unexposed (blanks) and exposed daphnids was compared using two-way ANOVA tests. In these tests, the effects of the exposure (differences between exposed daphnids and unexposed daphnids), the exposure time (differences between 0, 24, 48 h of exposure) and the interaction between exposure and time were tested. Differences in zinc concentration in the unfiltered and filtered (450 nm) medium samples during the *Daphnia* uptake experiments were tested using unpaired t-tests at each time point (0, 24 and 48 h of exposure). One-way ANOVA with Tukey’s post tests were used to test for significant differences between the zinc or copper concentrations in the unfiltered, 450 nm, 100 nm and 1 kDa filtered samples at each time point in the uptake experiment (for copper) and the dynamics experiment (for zinc).

### 3. Results

#### 3.1. Nanoparticle characterization

The electron microscopic analysis of the nanoparticles is indicated in Fig. 1. The ZnO nanopowder (Fig. 1a) consists of uncoated round particles with an average size (with standard deviation) of...
The ZnO nanodispersion (Fig. 1b) consists of coated (unknown coating type) particles with a larger diversity in size (with an average size of 39.2 ± 22.3 nm) and shape (both round, rectangular and elongated). The CuO nanoparticles (Fig. 1c) are round uncoated particles with an average size of 21.3 ± 10.2 nm.

3.2. Exposure conditions and nanoparticle dynamics

The measured zinc concentrations (for the nanopowder and zinc salt) in the uptake experiment corresponded well with the measured zinc concentrations in the dynamics experiment (Fig. 2). The zinc salt (Fig. 2c) completely dissolved in the exposure solution of the uptake experiment (unpaired t-test indicating no significant differences between the filtered and unfiltered samples after 0 h (p = 0.9053), 24 h (p = 0.9309), 48 h (p = 0.9399) of exposure). Also in the dynamics experiment, the zinc salt remained completely dissolved throughout the exposure. Although significant differences are indicated between the measured concentrations after filtration over the different filters, these differences in zinc concentration were very small.

Directly after exposing the daphnids to the ZnO nanodispersion (0 h, uptake experiment, Fig. 2b), some nanoparticle aggregates were formed in the medium (t-tests p = 0.0001 indicating significant differences between the filtered and unfiltered samples). After 24 h of exposure, the differences in zinc concentration between the filtered and unfiltered samples decreased (t-test p = 0.0028) and after 48 h of exposure these differences were no longer significant (t-test p = 0.3969), suggesting that these nanoparticle aggregates had completely dissolved.

For the ZnO nanopowder (Fig. 2a), a similar initial aggregation of some of the particles was observed directly after exposure (t-test p = 0.0117 indicating significant differences between the filtered and unfiltered samples), in the uptake experiment. A fast dissolution of these particles occurred, with no significant differences
between the zinc concentrations in the unfiltered and filtered samples after 24 h ($p = 0.2013$) and 48 h ($p = 0.2667$) of exposure. The dynamics experiment confirmed these kinetics for the ZnO nanopowder. Directly after exposure (one-way ANOVA $p < 0.0001$ indicating significant differences in zinc concentration between the different treatments) and after 6 h of exposure (one-way ANOVA $p = 0.0174$), nanoparticle aggregates larger than 450 nm were present in the medium (Tukey's post test showed significant differences in zinc concentration between the unfiltered and different filtered samples but not between the different filtered samples). However, a large fraction of the ZnO nanoparticles was already dissolved (on average 59.8% with min: 58.5 - max: 60.9%) directly after exposing the daphnids to the nanoparticles (within 1-2 h after having made the stock solution). After 48 h of exposure, the nanoparticles had completely dissolved and the concentration of the different fractions remained constant during the experiment (288 h).

The increase in zinc concentration (in the uptake and dynamics experiment) in the different filtered and unfiltered samples from 0 to 48 h of exposure to the nanopowder and nanodispersion also indicates this aggregation and subsequent dissolution. During the initial exposure, the medium may not be sampled in a representative way due to local aggregation, while after 48 h of exposure all particles had dissolved and the medium is more uniform. During the exposure, the ZnO nanoparticles did not visually precipitate to the bottom of the exposure vessel. In the zinc exposure, average values of $8.20 \pm 0.22$ mg/l (93.7 $\pm$ 1.8%), $7.89 \pm 0.12$ and $20.6 \pm 0.5$ °C were measured for oxygen, pH and temperature.

The measured copper concentrations (from the acute uptake experiment) in the different filtered and unfiltered samples are indicated after 0, 24, 48 h of exposure to the CuO nanopowder (Fig. 3a) and CuCl2.2H2O (Fig. 3b).

The metal salt was completely dissolved in the exposure solution. Significant differences in copper concentration between the different filtered and unfiltered samples can still be seen but concentration differences are very low.

In the CuO exposure, most of the nanoparticles were aggregated to sizes larger than 450 nm. One-way ANOVA tests showed significant differences in copper concentration between the different filtered and unfiltered samples after 0 ($p < 0.0001$), 24 ($p = 0.0068$), 48 h ($p < 0.0001$) of exposure, with Tukey's post tests indicating significant differences between the copper concentration in the unfiltered and different filtered samples but not between these different filtered samples. After 24 and 48 h of exposure, some CuO nanoparticle aggregates visually precipitated to the bottom of the exposure vessels and were as such not included in the water samples. Only a very small fraction of the CuO nanoparticles was dissolved directly after exposure (on average 0.076% with min: 0.072 – max: 0.079%). This dissolved fraction stayed constant during the exposure (on average 0.074% with min: 0.064 – max: 0.080% after 48 h of exposure). Although exposed to much higher copper concentrations, the measured dissolved concentration (passing through 1 kDa filter) of the CuO nanoparticle concentration ($0.010 \pm 0.002$ mg Cu/l) corresponded well to the dissolved Cu salt concentration ($0.014 \pm 0.003$ mg Cu/l). In the copper exposure the average pH was $7.85 \pm 0.04$, while the temperature was $21.0 \pm 0.4 \degree C$.

3.3. Acute uptake of metal oxide nanoparticles and metal salts by Daphnia magna

An increase in the total zinc body concentration (i.e. metal adsorbed on the carapace and ingested metal and internalized metal) of the daphnids can be observed during the exposure to the ZnO nanopowder (Fig. 4a), ZnO nanodispersion (Fig. 4b) and ZnCl2 (Fig. 4c). After 48 h of exposure to $1.78 \pm 0.02$ mg Zn/l ZnO nanopowder, the zinc concentration reached a maximum average value of $4.23 \pm 2.51$ µg Zn/mg dry weight. When exposed to $1.70 \pm 0.05$ mg Zn/l of the ZnO nanodispersion, the body concentration average maximum value was $3.87 \pm 1.31$ µg Zn/mg dry weight, similar to the nanopowder. Maximum zinc concentrations of $2.62 \pm 1.39$ µg Zn/mg dry weight were measured when exposed to $1.88 \pm 0.09$ mg Zn/l of the zinc salt.

Using electron microscopy, no ZnO nanoparticles were visible in any of the Daphnia tissues or attached to their carapace after 24 h of exposure to the ZnO nanopowder and nanodispersion.

The total copper body concentration (i.e. metal adsorbed on the carapace and ingested metal and internalized metal) of the daphnids exposed to $13.35 \pm 0.10$ mg Cu/l CuO nanopowder reached high maximum values of $62.09 \pm 11.26$ µg Cu/mg dry weight after 24 h of exposure and afterwards dropped to $6.73 \pm 0.92$ µg Cu/mg dry weight after 48 h of exposure (Fig. 5a). An increase in total copper was observed in the daphnids exposed to $0.031 \pm 0.001$ mg Cu/l copper salt during 48 h (Fig. 5b). After 48 h of exposure to the metal salt, an average concentration of $0.35 \pm 0.03$ µg Cu/mg dry weight was reached.

After 24 h of exposure, CuO nanoparticles were clearly visible using electron microscopy. The CuO nanoparticles adsorbed onto the outside carapace of D. magna (Fig. 6a) but were not able to penetrate it. After the first sampling (24 h), most daphnids were visually covered with CuO nanoparticle aggregates. After the second sampling (48 h) this was no longer the case. The CuO

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**Fig. 3.** Measured copper concentration (with standard deviations of three replicates) in the unfiltered and different filtered (450 nm, 100 nm, 1 kDa) medium samples when exposed to the CuO nanopowder (a) and CuCl2.2H2O (b). At each time point, one-way ANOVA tests indicate differences in copper concentration between the unfiltered, 450 nm, 100 nm and 1 kDa filtered samples. The blanks (not indicated on graph) in the unfiltered samples, corresponding the nanoparticle and salt exposure, were below detection limit (<0.002 mg Cu/l).
nanoparticles were also visible in the gut (Fig. 6b). The EDX results (Fig. 6c) confirmed that it were indeed CuO particles that were present in the gut. Internalization of these nanoparticles in the tissues or cells was not observed.

4. Discussion

4.1. ZnO nanoparticle dynamics and uptake by D. magna

Directly after addition of the daphnids to the ZnO nanoparticle exposure solutions (Fig. 2a; measured total exposure of \(1.89 \pm 0.02\) mg Zn/l), a large part of the nanoparticles was already dissolved (on average 59.8\% with min: 58.5 – max: 60.9\%). However, during this initial exposure, the ZnO nanoparticles had also formed aggregates with sizes larger than 450 nm (Fig. 2a and b), similar to the results found when exposed to lower concentrations (\(\leq 0.131\) mg Zn/l) as shown by Adam et al. (2014). This initial aggregation may be largely due to medium characteristics such as the pH (approaching the point of zero charge situated at pH 8 according to Tso et al. (2010)) and to the ionic strength (0.0119 M as calculated with Visual Minteq, at which aggregation occurs according to Zhou and Keller (2010)). As a result, from the onset the daphnids were exposed to a combination of dissolved zinc and nanoparticle aggregates. During the exposure, the initially formed nanoparticle aggregates started to dissolve. Within 48 h of exposure, the aggregates had completely dissolved. At this time the measured dissolved concentration from the nanopowder (1.84 \(\pm\) 0.08 mg Zn/l) was similar to the dissolved zinc salt (1.82 \(\pm\) 0.05 mg Zn/l). When exposed to lower concentrations (\(\leq 0.131\) mg Zn/l) of the ZnO nanoparticles under the same conditions, similar total dissolution values were found (Adam et al., 2014). Li and Wang (2013) showed different dissolution values. As such, when exposed to concentrations (1.61 mg Zn/l) similar to our exposure (1.70–1.88 mg Zn/l), only 20\% of the nanoparticles
dissolved (measured after filtration over a 3 kDa filter) in simplified M7 medium at pH 8.2, with equilibrium observed within 2 h.

Despite the appearance that the ZnO nanodispersion (nominal EC\textsubscript{50}: 1.12 mg Zn/l) was more toxic than the ZnO nanopowder (nominal EC\textsubscript{50}: 2.25 mg Zn/l) and the zinc salt (nominal EC\textsubscript{50}: 2.30 mg Zn/l), based on the nominal exposure concentrations used in our study, the toxicity of these chemicals was very similar based on the measured zinc concentrations (not indicated in this study). Also in this study, very similar exposure concentrations were measured (Fig. 2) for the nanodispersion (1.70 ± 0.05 mg Zn/l), nanopowder (1.78 ± 0.02 mg Zn/l) and zinc salt (1.88 ± 0.09 mg Zn/l) after 48 h of exposure (when all aggregates had dissolved) in the uptake test.

When exposed to comparable measured concentrations of the ZnO nanopowder (Fig. 4a), ZnO nanodispersion (Fig. 4b) and ZnCl\textsubscript{2} (Fig. 4c), a similar (overlapping confidence intervals) total zinc concentration in D. magna was obtained, indicating a similar ingestion and/or adsorption to the carapace and/or internalization in the tissues or cells. According to Li and Wang (2013), the attachment of ZnO nanoparticles to the carapace is negligible, while only 10–16% of the total zinc from the metal salt attaches to the outside of the daphnids (Muyssen and Janssen, 2002). As a result, it is expected that most of the measured zinc is internal zinc present in the form of dissolved (nanoparticle and salt exposure) or aggregated ZnO nanoparticles (nanoparticle exposure). Li and Wang (2013) have shown that ZnO nanoparticles are ingested by daphnids when exposed during 40 min to 1.58 mg Zn/l. Within this 40 min exposure, an initial increase in zinc concentration (of up to almost 6 \(\mu\)g Zn/mg dry weight) was seen during the first 20 min of exposure, followed by an elimination of zinc from the daphnids (to about 1.5 \(\mu\)g Zn/mg dry weight). However, when exposed during a longer period (as in our study) of up to 24 and 48 h, the total zinc concentration kept increasing. It has been shown that zinc becomes toxic at exposure concentrations equal to or higher than 0.8 mg Zn/l corresponding to a zinc body content of 0.468 ± 0.080 mg Zn/mg dry weight in D. magna (in this study daphnids were acclimated during 2–10 generations to the zinc concentrations; Muyssen and Janssen (2002)). In our study, the daphnids were exposed to more than twice this concentration (1.70–1.88 mg Zn/l), at which the zinc body content reached more than double this concentration (up to 4.22 ± 2.51 \(\mu\)g Zn/mg dry weight). As a result, the daphnids could no longer regulate these high zinc concentrations (by reduced uptake or elimination) and died. In this exposure, 50% of the daphnids died (daphnids were exposed to EC\textsubscript{50} concentrations) due to the increasing uptake of zinc. The similar dissolution (after 48 h of exposure), toxicity and uptake of zinc by Daphnia when exposed to the ZnO nanoparticles and the zinc salt indicates that the caused nanoparticle toxicity (EC\textsubscript{50} exposure concentrations) at the acute level is due to the toxic ions.

Fig. 6. STEM analysis of sections through the carapace (a; from left to right: carapace, nanoparticles) and the gut (b; from left to right: epithelium, microvilli, lumen with nanoparticles) and EDX (c, through particle in gut) of D. magna after 24 h of exposure to the CuO nanopowder (measured exposure of 13.4 ± 0.1 mg Cu/l; black particles on image).
4.2. CuO nanoparticle dynamics and uptake by D. magna

Directly after exposure (0 h), only a very small fraction of the nanoparticles (Fig. 3a; measured total exposure of 13.35 ± 0.10 mg Cu/l) was dissolved (0.076% with min: 0.072 – max: 0.079%). After 48 h of exposure to the nanoparticles, the concentration of this dissolved fraction remained constant. Heinlaan et al. (2011) also showed low dissolution of CuO nanoparticles: between 0.005 and 0.02 mg Cu/l, indicating about 0.16–0.63% dissolution of the total concentration when exposed to 3.2 mg Cu/l under similar exposure conditions. At an exposure concentration of 0.1 mg/l about 2.7–10.6% (measured at pH 8.6 and 6.8) of the CuO nanoparticles was shown to dissolve (Fan et al., 2012). Throughout our exposure, the CuO nanoparticles were heavily aggregated, with aggregate sizes larger than 450 nm. Heinlaan et al. (2011) and Jo et al. (2012) also reported aggregation of CuO nanoparticles. The high aggregation of CuO nanoparticles can be partly explained by the ionic strength (of 0.0119 M), which increases the aggregation at higher concentrations (Sousa and Teixeira, 2013). The main factor influencing the aggregation is the pH. The aggregation is highest when the pH approaches the point of zero charge. Different PZC values have been suggested for CuO nanoparticles. Li and Chang (2004) and Sousa and Teixeira (2013) indicate that the PZC is situated at a pH of 9.9 (needle-shaped particles with one dimension <50 nm) and 10 (round particles <50 nm), while Guedes et al. (2009) indicates that it is situated at pH 7.9 (measured size of 1.9 μm), close to the pH in our exposure (7.85 ± 0.04).

The measured total copper concentration of the daphnids exposed to the CuO nanoparticles (Fig. 5a) was much higher than for the ones exposed to the copper salt (Fig. 5b). After 24 h of exposure, the total measured copper concentration of the daphnids exposed to the CuO nanoparticles reached higher values (62.09 ± 11.26 μg Cu/mg dry weight) than after 48 h (6.73 ± 0.92 μg Cu/mg dry weight) of exposure. This higher concentration after 24 h of exposure may be explained by nanoparticles attached externally to the carapace of the daphnids, as shown by the electron microscopic study (Fig. 6a). After 24 h, most of the sampled daphnids were visually covered in aggregated copper oxide nanoparticles. However, after 48 h of exposure no nanoparticle aggregates were visually attached to the daphnids. This decrease of the externally adsorbed nanoparticles from 24 to 48 h can be explained by the Daphnia molting after 24 h. The external attachment of nanoparticles to D. magna and subsequent release after molting was also observed for TiO2 nanoparticles (Dabrunz et al., 2011). After 24 and 48 h of exposure, a large fraction of the nanoparticles was also ingested by D. magna and could be localized in the gut (Fig. 6b). However, our results showed no internalization of the CuO nanoparticles in any of the D. magna cells or tissues. The accumulation of nanoparticles in the intestine was confirmed by Fan et al. (2012). Heinlaan et al. (2011) indicated that already 10 min after exposure to 4 mg CuO/l, nanoparticles were ingested by D. magna and could be located in the midgut but did not internalize in the midgut epithelial cells after 48 h of exposure. In our study, the daphnids were exposed to similar acute effect concentrations of the nanoparticles and the metal salt (immobilization EC50). Even though the total D. magna copper concentration (i.e. ingested and/or adsorbed to carapace and/or internalized in tissues or cells) from the nanoparticles was higher than the uptake of copper from the metal salt, the small dissolved fraction of the nanoparticles (0.010 ± 0.002 mg Cu/l) measured in the medium (passing through a 1 kDa filter) (Sousa and Teixeira, 2013) indicated very well with the dissolved fraction of the metal salt (0.014 ± 0.003 mg Cu/l). This indicates that when expressed on a dissolved scale, similar effect concentrations (EC50) are observed for the CuO nanoparticles and Cu salts. These similar effect concentrations indicate that the ingested nanoparticles did not dissolve in the gut of the daphnids and the externally adsorbed particles did not cause additional toxic effects. The combined toxicity (EC50 exposure concentrations) and dissolution results indicate that even tough nanoparticle aggregates are ingested by the daphnids and adsorbed on their carapace, the observed toxicity under these conditions is caused by the toxic ions formed during dissolution of the nanoparticles in the exposure medium, comparable with the copper salt.

5. Conclusions

In this study, the dynamics of ZnO and CuO nanoparticles and their uptake by D. magna were characterized and compared with corresponding metal salts. The ZnO nanoparticles showed complete dissolution within 48 h of acute exposure. In addition, a very similar increase in Daphnia zinc concentration was observed in the nanoparticles and metal salt exposure. In the CuO exposure, a high nanoparticle aggregation in the exposure medium and a high total Daphnia copper concentration was observed. However, the dissolved nanoparticle fraction corresponded very well to the dissolved copper salt fraction. As a result, when expressed on a dissolved scale, similar effect concentrations (EC50) are observed for the CuO nanoparticles and Cu salts. In conclusion, the combined dissolution, uptake and toxicity (EC50 exposure concentration) results of the ZnO and CuO nanoparticles indicate that the acute toxicity of both nanoparticle types to D. magna is caused by the metal ions formed during dissolution of the particles in the exposure medium. Future work should focus on long-term uptake and effects of these nanoparticles, since these data are still largely lacking.

Conflict of interest

The authors report no conflicts of interest.

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