



<https://openaccess.leidenuniv.nl>

### **License: Article 25fa pilot End User Agreement**

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with explicit consent by the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' pilot project. In this pilot research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and/or copyrights owner(s) of this work. Any use of the publication other than authorised under this licence or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please contact the Library through email: [OpenAccess@library.leidenuniv.nl](mailto:OpenAccess@library.leidenuniv.nl)

### **Article details**

Xiao Y., Peijnenburg W.J.G.M., Chen G. & Vijver M.G. (2018), Impact of water chemistry on the particle-specific toxicity of copper nanoparticles to *Daphnia magna*, *Science of the Total Environment* 610-611: 1329-1335.

Doi: 10.1016/j.scitotenv.2017.08.188



# Impact of water chemistry on the particle-specific toxicity of copper nanoparticles to *Daphnia magna*

Yinlong Xiao<sup>a,b,\*</sup>, Willie J.G.M. Peijnenburg<sup>a,c</sup>, Guangchao Chen<sup>a</sup>, Martina G. Vijver<sup>a</sup>

<sup>a</sup> Institute of Environmental Sciences (CML), Leiden University, P. O. Box 9518, 2300 RA Leiden, The Netherlands

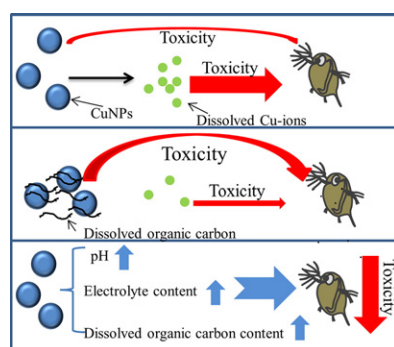
<sup>b</sup> College of Environmental Sciences, Sichuan Agricultural University, Wenjiang 611130, PR China

<sup>c</sup> National Institute of Public Health and the Environment, Center for the Safety of Substances and Products, P. O. Box 1, 3720 BA Bilthoven, The Netherlands

## HIGHLIGHTS

- Connection between the dynamic fate characterization of CuNPs and their toxicity was drawn.
- Toxicity of CuNP suspension varies in dynamic and static exposure treatments, when organic matter (OM) was added.
- Toxicity of CuNP suspensions results from the combined effect of the particles and their released ions.
- The particle-specific toxicity of CuNPs decreased with increasing pH and contents of divalent cations and OM.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 12 July 2017

Received in revised form 17 August 2017

Accepted 17 August 2017

Available online 30 August 2017

Editor: D. Barcelo

### Keywords:

Copper nanoparticles

Water chemistry

Fate

Toxicity

*Daphnia magna*

## ABSTRACT

Toxicity of metallic nanoparticle suspensions ( $NP_{(total)}$ ) is generally assumed to result from the combined effect of the particles present in suspensions ( $NP_{(particle)}$ ) and their released ions ( $NP_{(ion)}$ ). Evaluation and consideration of how water chemistry affects the particle-specific toxicity of  $NP_{(total)}$  are critical for environmental risk assessment of nanoparticles. In this study, it was found that the toxicity of Cu  $NP_{(particle)}$  to *Daphnia magna*, in line with the trends in toxicity for Cu  $NP_{(ion)}$ , decreased with increasing pH and with increasing concentrations of divalent cations and dissolved organic carbon (DOC). Without the addition of DOC, the toxicity of Cu  $NP_{(total)}$  to *D. magna* at the LC50 was driven mainly by Cu  $NP_{(ion)}$  (accounting for  $\geq 53\%$  of the observed toxicity). However, toxicity of Cu  $NP_{(total)}$  in the presence of DOC at a concentration ranging from 5 to 50 mg C/L largely resulted from the  $NP_{(particle)}$  (57%–85%), which could be attributable to the large reduction of the concentration of Cu  $NP_{(ion)}$  and the enhancement of the stability of Cu  $NP_{(particle)}$  when DOC was added. Our results indicate that water chemistry needs to be explicitly taken into consideration when evaluating the role of  $NP_{(particle)}$  and  $NP_{(ion)}$  in the observed toxicity of  $NP_{(total)}$ .

© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

The fast development of nanotechnology over the past decade has boosted the manufacture and application of engineered nanomaterials in industrial and consumer products. For example, Cu nanoparticles (CuNPs) currently are widely utilized in antimicrobials,

\* Corresponding author at: College of Environmental Sciences, Sichuan Agricultural University, Wenjiang 611130, PR China.

E-mail address: [xiao@cml.leidenuniv.nl](mailto:xiao@cml.leidenuniv.nl) (Y. Xiao).

semiconductors, catalysis and skin products. The rapid increase in their manufacture, use and disposal inevitably results in an increasing likelihood for CuNPs to be released into aquatic environments (Vale et al., 2016). Many studies have found that CuNPs are highly toxic to a wide range of organisms, such as algae (Adam et al., 2015a; Zhao et al., 2016), mussels (Hu et al., 2014), crustaceans (Song et al., 2015a; Xiao et al., 2016), and fishes (Hua et al., 2014; Song et al., 2015b). Hence, concerns regarding the environmental safety of CuNPs deserve to be emphasized.

Although a growing number of studies involved in nanotoxicology have been conducted over the past decade, issues regarding the mechanisms of toxicity of NPs are still under debate, especially the topic whether particles themselves or their released ions are the main drivers for the toxicity of suspensions of slowly dissolving metallic NPs. Some recent studies found that the toxicity of metallic NPs was mainly due to their released ions (referred to as  $NP_{(ion)}$  hereafter) (Jo et al., 2012; Adam et al., 2015b), while others revealed that the cause underlying the NPs toxicity was largely attributable to the NPs themselves (referred to as  $NP_{(particle)}$  hereafter) (Hua et al., 2014; Santo et al., 2014; Wang et al., 2016). These inconsistent conclusions may result from ignoring the effects of the physicochemical properties of test medium on the fate and toxicity of  $NP_{(particle)}$  and  $NP_{(ion)}$ . In fact, once emitted to aquatic environments, metallic NPs are commonly subject to undergo a series of environmental processes, such as dissolution and aggregation followed by sedimentation. As a consequence of these processes, a metallic NP suspension is generally a mixture of  $NP_{(particle)}$  and  $NP_{(ion)}$ . Factors capable of influencing these environmental processes have the potential to affect the fate and toxicity of  $NP_{(particle)}$  and  $NP_{(ion)}$  in water systems, which may further result in the change of the contribution of  $NP_{(particle)}$  and  $NP_{(ion)}$  to the toxicity of NP suspensions. Currently, it is widely known that water chemistry parameters, such as pH (Mohd Omar et al., 2014), electrolytes (especially divalent cations) and natural organic matter (NOM) (Mukherjee and Weaver, 2010; Grillo et al., 2015), can impact the environmental behavior and fate of NPs and the toxicity of NP suspensions to biota. However, how the water chemistry affects the particle-specific toxicity and the relative contribution of  $NP_{(particle)}$  and  $NP_{(ion)}$  to the observed toxicity of NP suspensions remains an elusive question (Minetto et al., 2016).

In this study, the behavior, fate and toxicity of CuNPs and copper ions to *Daphnia magna* across a range of water chemistry parameters were assessed. Furthermore, the relative contribution of  $NP_{(particle)}$  and  $NP_{(ion)}$  to the toxicity of CuNP suspensions upon varying water chemistry was determined.

## 2. Materials and methods

### 2.1. Testing materials and organisms

CuNPs (nominal size, 25 nm; specific surface area, 30–50 m<sup>2</sup>/g; purity, 99.9%; shape, spherical) were obtained from IoLiTec (Heilbronn, Germany). Aldrich humic acid (sodium salt) (HA) was used as a standardized natural dissolved organic carbon (DOC). A stock solution was prepared by dissolving HA in 0.002 N NaOH in deionized water. The HA solution was then stirred overnight and filtered through a 0.2 µm cellulose acetate membrane and subsequently stored at 4 °C prior to experiments. The total organic carbon (TOC) content of the prepared stock solution was measured by a TOC analyzer (TOC-VCPH, Shimadzu Corporation). *Daphnia magna* was selected as the model organism for toxicity testing. The test organisms were fed with freshly cultured *Pseudokirchneriella subcapitata* every three days and maintained inside a controlled-temperature chamber under a 16:8 light-dark cycle (20 ± 1 °C). At intervals of about 4 months, the sensitivity of the daphnid culture was checked with the reference toxicant K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> to ensure the sensitivity of the daphnid culture remained within the limits as set by the OECD guideline (24 h 50% effective concentration = 0.6–2.1 mg/L K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) (OECD, 2004).

### 2.2. Preparation of suspensions of CuNPs

ISO standard testing medium (STM), recommended by OECD, was used to prepare CuNP suspensions. The STM (pH 7.8 ± 0.2) contained 2 mM of CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.5 mM of MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.77 mM of NaHCO<sub>3</sub>, and 0.08 mM of KCl. Specifically, stock suspension of CuNPs (250 mg/L) was prepared in MilliQ water after 30 min of bath-sonication to disperse the particles, prior to each experiment. The prepared stock suspension of CuNPs was then immediately diluted to the STM. In order to understand the influence of water chemistry on the fate and toxicity of CuNPs, CuNP suspensions in which the water chemistry was modified, were prepared immediately by a series of dilution of the prepared stock suspension of CuNPs. The modification of water chemistry of the exposure media was achieved by altering the most critical environmental factors assumed to affect NP toxicity, which is pH, and divalent cation and DOC concentrations. The overview of the testing scheme with the details of the different trials is presented in Table 1. For the effects of pH, besides at pH 7.8, suspensions of CuNPs at pH 6 and 9 (adjusted by addition of 0.1 M NaOH or 0.1 M HCl) were also prepared; for the divalent cation treatments, suspensions of CuNPs with 0, 2.5 and 5 mM of cations were prepared by adding CaCl<sub>2</sub>·2H<sub>2</sub>O and MgSO<sub>4</sub>·7H<sub>2</sub>O in a fixed molar ratio of 4:1; for assessing the effects of DOC on toxicity, CuNP suspensions with 0, 5, 25 and 50 mg C/L (carbon per liter) were prepared by diluting the stock HA solutions. The ranges of the water chemistry parameters were selected to accommodate the optimal conditions for growth of *D. magna* and they encompass the range commonly observed in natural environments (Vijver et al., 2008; Ottoufelling et al., 2011; Hammes et al., 2013). Moreover, most previous studies regarding the fate and toxicity of NPs were performed under static condition (i.e., stored without disturbance along the exposure duration). However, by definition, the 'real' environment is dynamic (Godinez and Darnault, 2011; Lv et al., 2016), and accordingly, fate and toxicity of NPs under dynamic exposure condition deserve to be studied. To compare the fate and toxicity of CuNPs to *D. magna* under static and dynamic conditions, one set of the prepared CuNP suspensions was maintained statically under a 16:8-h light-dark cycle (20 ± 1 °C) during 48 h of incubation and the other set of CuNP suspensions was stored on a laboratory shaker with a vibration speed of 140 rpm under identical conditions (i.e., 16:8-h light-dark cycle and 20 ± 1 °C). It was verified (visual observation) that the vibration speed applied (140 rpm) had no adverse effects on the well-being of *D. magna* throughout the 48 h of exposure.

**Table 1**

Overview of the experimental setup for testing the fate and toxicity of CuNPs across a range of water chemistry.

Trial no.	Condition	pH	Cation conc. (mM)	DOC conc. (mg/L)
1	Static	6	2.5	0
2	Static	7.8	2.5	0
3	Static	9	2.5	0
4	Static	7.8	0	0
5	Static	7.8	5	0
6	Static	7.8	2.5	5
7	Static	7.8	2.5	25
8	Static	7.8	2.5	50
9	Dynamic	6	2.5	0
10	Dynamic	7.8	2.5	0
11	Dynamic	9	2.5	0
12	Dynamic	7.8	0	0
13	Dynamic	7.8	5	0
14	Dynamic	7.8	2.5	5
15	Dynamic	7.8	2.5	25
16	Dynamic	7.8	2.5	50

Conc. = concentration.

### 2.3. Physicochemical characterization of CuNPs

The morphology and primary size of the CuNPs in the STM were characterized using transmission electron microscopy (TEM, JEOL 1010, JEOL Ltd., Japan). The primary particle size of CuNPs was analyzed using Nano Measurer 1.2 (Fudan University, China). The hydrodynamic diameters of CuNPs upon various exposure scenarios prepared above were measured in triplicate immediately after preparation (which was around 1 h for the preparation of CuNP suspensions, to which we referred to as 1 h hereafter) and after 24 and 48 h of preparation by dynamic light scattering (DLS) on a Zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., UK), at a scattering angle of 90° and a temperature of 20 °C. The zeta potential of each copper suspension at the same time point was measured by ZetaPALS software based on the Smoluchowski equation.

The changes of the total Cu concentration and dissolution profile in the exposure suspensions upon modification of pH, cation and DOC concentrations within 48 h were monitored separately. This was done at an actual CuNP concentration of about 800 µg/L, which is in the range (10–920 µg/L) of the predicted CuNP concentration in aquatic environments (Chio et al., 2012). The prepared CuNP suspensions across a range of water chemistry, as presented in Table 1, were kept for increasing time periods (1, 12, 24, 36 and 48 h). At each sampling time point, 2 independent CuNP suspensions with the same water chemistry as duplicates were used to measure the concentration of each Cu fraction. For each suspension, a 5 mL sample was collected carefully from the position around 2 cm below the surface of each suspension and then digested by 65% nitric acid at room temperature for at least 1 d before being analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). In this way, the total Cu concentration in the water column (i.e., the sum of the dissolved Cu and particulate Cu) could be measured. After sampling for the total Cu concentration measurement, a 10 mL of each suspension was pipetted from the water column and subsequently centrifuged at 30,392g for 30 min at 4 °C (Sorvall RC5B plus centrifuge, Fiberlite F21-8 × 50 y rotor). The supernatants were then filtered through a syringe filter with 0.02 µm pore diameter (Anotop 25, Whatman). The filtrates were digested by nitrate acid and ICP-OES was used to determine the dissolved Cu concentration.

### 2.4. Acute toxicity testing

All acute toxicity tests in this study were carried out according to OECD Guideline 202. Five neonates (<24 h) were exposed for 48 h to each suspension of CuNPs (referred to as CuNP<sub>(total)</sub> hereafter) prepared according to Table 1. During the 48 h acute toxicity test, daphnids were not fed. In order to obtain the dose-response curves of CuNP<sub>(total)</sub> to daphnids, a series of exposure concentrations for CuNP<sub>(total)</sub> with the same water composition was employed to expose the daphnids. Each concentration tested, consisted of 4 replicates. To calculate the toxic effects of the dissolved ions released from CuNPs (referred to as CuNP<sub>(ion)</sub> hereafter), the dose-response curves of Cu(NO<sub>3</sub>)<sub>2</sub> solutions to daphnia neonates for 48 h across a range of water chemistry were also determined.

### 2.5. Data analysis

The specific modes of action of NP<sub>(ion)</sub> and NP<sub>(particle)</sub> remain unclear. Nevertheless, some recently published papers found that the mode of action of NP<sub>(particle)</sub> differed from that of NP<sub>(ion)</sub> (Poynton et al., 2011; Poynton et al., 2012; Rainville et al., 2014). Hence, it was assumed that the modes of action of CuNP<sub>(ion)</sub> and CuNP<sub>(particle)</sub> would be dissimilar. In this circumstance, the toxic effects of CuNP<sub>(particle)</sub> can be deduced by using the response addition model (Backhaus et al., 2000):

$$E_{(total)} = 1 - [(1 - E_{(ion)}) (1 - E_{(particle)})] \quad (1)$$

where  $E_{(total)}$ ,  $E_{(ion)}$  and  $E_{(particle)}$  represent the toxic effects caused by the nanoparticle suspensions, and the ions and the NPs present in the suspensions (scaled from 0 to 1), respectively. In the present study,  $E_{(total)}$  was measured experimentally. The time weighted average (TWA) ion concentration at each exposure concentration of CuNPs, calculated from Eq. (2), was used to analyze the toxicity caused by copper ions (i.e.,  $E_{(ion)}$ ) in the suspensions of CuNPs, according to the concentration-response curves of Cu(NO<sub>3</sub>)<sub>2</sub> towards *D. magna*. This makes  $E_{(particle)}$  as the only unknown, allowing for direct calculation of the effects caused by a specific concentration of NP<sub>(particle)</sub>.

$$C_T = \frac{C_1 T_1 + C_2 T_2 + C_3 T_3 + \dots C_n T_n}{T_1 + T_2 + T_3 + \dots T_n} \quad (2)$$

where  $C_T$  is the TWA concentration and  $C_i$  is the analyte concentration observed for time  $T_i$ , and so on, until time  $T_n$ .

The median lethal concentration (LC50) and the related 95% confidence intervals (CI) were calculated using the log (inhibitor) versus normalized response-variable slope function in Graphpad Prism 5.

## 3. Results

### 3.1. Physicochemical characterization of CuNPs

The image captured by the transmission electron microscopy demonstrated that the pristine shape of the CuNPs was spherical and CuNPs aggregated rapidly after submersion into the exposure medium (Fig. S1). Size analysis was not performed, as no individual well-defined NPs could be determined by TEM. The hydrodynamic diameters and zeta-potentials of CuNP suspensions across a range of water chemistry were presented in Table 2. At a cation concentration of 2.5 mM, in both the static and dynamic exposure treatments the NPs aggregated to micro-size aggregates after 48 h of incubation in the testing media with pH ranging from 6 to 9 and without the addition of DOC (trials 1–3 and 9–11). The hydrodynamic diameter of CuNPs remained around 518 nm after 48 h of incubation in the static treatment without the addition of cations (trial 4). However, the addition of divalent cations enhanced the extent of aggregation of the NPs (trials 2, 4 and 5). The zeta-potential of the NP suspension without the addition of divalent cations in the static treatment was around −30 mV within 48 h of incubation, while it decreased to around −10 mV at 5 mM of cations. In the static treatments, the aggregate size of CuNPs after 48 h of incubation was around 500 nm with the addition of DOC at a concentration ranging

**Table 2**

Hydrodynamic diameter and zeta-potential of CuNPs during 48 h of incubation in systems with various water chemistry.

Trial no.	Hydrodynamic diameter (nm) <sup>a</sup>			Zeta-potential (mV) <sup>a</sup>		
	1 h	24 h	48 h	1 h	24 h	48 h
1	754 ± 217	903 ± 194	1383 ± 360	−13 ± 2	−14 ± 2	−9 ± 1
2	637 ± 105	1008 ± 116	1650 ± 335	−17 ± 1	−12 ± 3	−10 ± 1
3	745 ± 93	1307 ± 172	2436 ± 490	−9 ± 2	−6 ± 3	−5 ± 4
4	465 ± 84	641 ± 173	518 ± 80	−34 ± 4	−27 ± 1	−26 ± 1
5	715 ± 134	1474 ± 144	1865 ± 132	−10 ± 2	−10 ± 2	−8 ± 1
6	369 ± 41	486 ± 21	512 ± 23	−18 ± 1	−16 ± 1	−16 ± 2
7	373 ± 60	457 ± 23	468 ± 16	−19 ± 1	−18 ± 1	−17 ± 1
8	359 ± 19	445 ± 27	495 ± 17	−19 ± 1	−16 ± 3	−15 ± 1
9	ND	1078 ± 219	1617 ± 293	ND	−9 ± 3	−7 ± 3
10	ND	1029 ± 239	1761 ± 985	ND	−8 ± 4	−6 ± 3
11	ND	2050 ± 319	1203 ± 562	ND	−7 ± 1	−3 ± 2
12	ND	414 ± 82	891 ± 390	ND	−23 ± 4	−19 ± 1
13	ND	879 ± 169	1237 ± 219	ND	−5 ± 3	−4 ± 3
14	ND	282 ± 10	221 ± 23	ND	−12 ± 2	−12 ± 1
15	ND	142 ± 25	118 ± 14	ND	−12 ± 1	−11 ± 1
16	ND	127 ± 10	127 ± 9	ND	−11 ± 1	−11 ± 2

ND means not determined.

<sup>a</sup> Hydrodynamic diameter and zeta-potential are expressed as the mean ± standard deviation (n = 3).



from 5 to 50 mg C/L (trials 6–8). In the dynamic treatments, the aggregate sizes of CuNPs were around 200 nm at 5 mg C/L and 100 nm at 25 and 50 mg C/L within 48 h of incubation (trials 14–16).

After 48 h of incubation, 91%, 76% and 60% of the total added CuNPs still remained in the water column in the static exposure treatments at pH 6, 7.8 and 9, respectively (Fig. 1A). In the static treatments, 78%, 64% and 54% of the total added CuNPs were dissolved after 48 h of incubation at pH 6, 7.8 and 9, respectively (Fig. 1B). The profiles of the total amount of Cu and CuNP<sub>(ion)</sub> remaining in the water column during 48 h of incubation in the static and dynamic exposure settings across the range of pH from 6 to 9 were similar (Fig. 1A–B). At the cation concentrations of 0, 2.5 and 5 mM, the total amount of Cu remaining in the water column after 48 h was 88%, 76% and 71% in the static treatments and 94%, 83% and 52% in the dynamic treatments, respectively (Fig. 1C). Around 65% of the total added Cu was dissolved at the cation concentrations ranging from 0 to 5 mM in both the static and the dynamic treatments, except at the concentration of 5 mM in the dynamic treatment, as 48% of the CuNPs was dissolved after 48 h of incubation (Fig. 1D). Around 63%, 73% and 76% of the total added CuNPs remained in the water column after 48 h of incubation in the static treatments at 5, 25 and 50 mg C/L, respectively (Fig. 1E). In the dynamic treatments, approximately 85% of the initially added CuNPs remained in the water column after 48 h of incubation across the DOC concentration range from 0 to 50 mg/L. The addition of DOC significantly reduced the amount of

CuNP<sub>(ion)</sub> in the water column. In both the static and dynamic treatments, the amount of CuNP<sub>(ion)</sub> decreased to around 20% after 48 h of incubation in all the cases with the addition of DOC from 5 to 50 mg/L (Fig. 1F).

### 3.2. Acute toxicity of CuNPs to *Daphnia magna*

The LC50 of CuNP<sub>(total)</sub> across a range of water chemistry is provided in Table 3. In the static treatments, the LC50 values of CuNP<sub>(total)</sub> were 0.024, 0.050 and 0.094 mg/L at pH 6, 7.8 and 9, respectively. The LC50 values of CuNP<sub>(total)</sub> in the dynamic treatments were similar to those in the static treatments at the same pH, which were 0.030, 0.049 and 0.084 mg/L at pH 6, 7.8 and 9, respectively. In the static treatments, the LC50 of CuNP<sub>(total)</sub> increased from 0.026 mg/L without the addition of cations to 0.076 mg/L at 5 mM of cations. The LC50 of CuNP<sub>(total)</sub> upon the dynamic exposure trial was similar to that upon the static trial at the same cation concentration, except at the cation concentration of 5 mM, at which the LC50 of CuNP<sub>(total)</sub> was 0.152 mg/L in the dynamic treatment, about a factor of 2 higher than the LC50 obtained in the static treatment. The LC50 of CuNP<sub>(total)</sub> significantly increased upon the addition of DOC. In the static treatments, the LC50 of CuNP<sub>(total)</sub> increased from 0.050 mg/L without addition of DOC to 0.515, 2.166 and 3.591 mg/L at 5, 25 and 50 mg C/L, respectively; in the dynamic

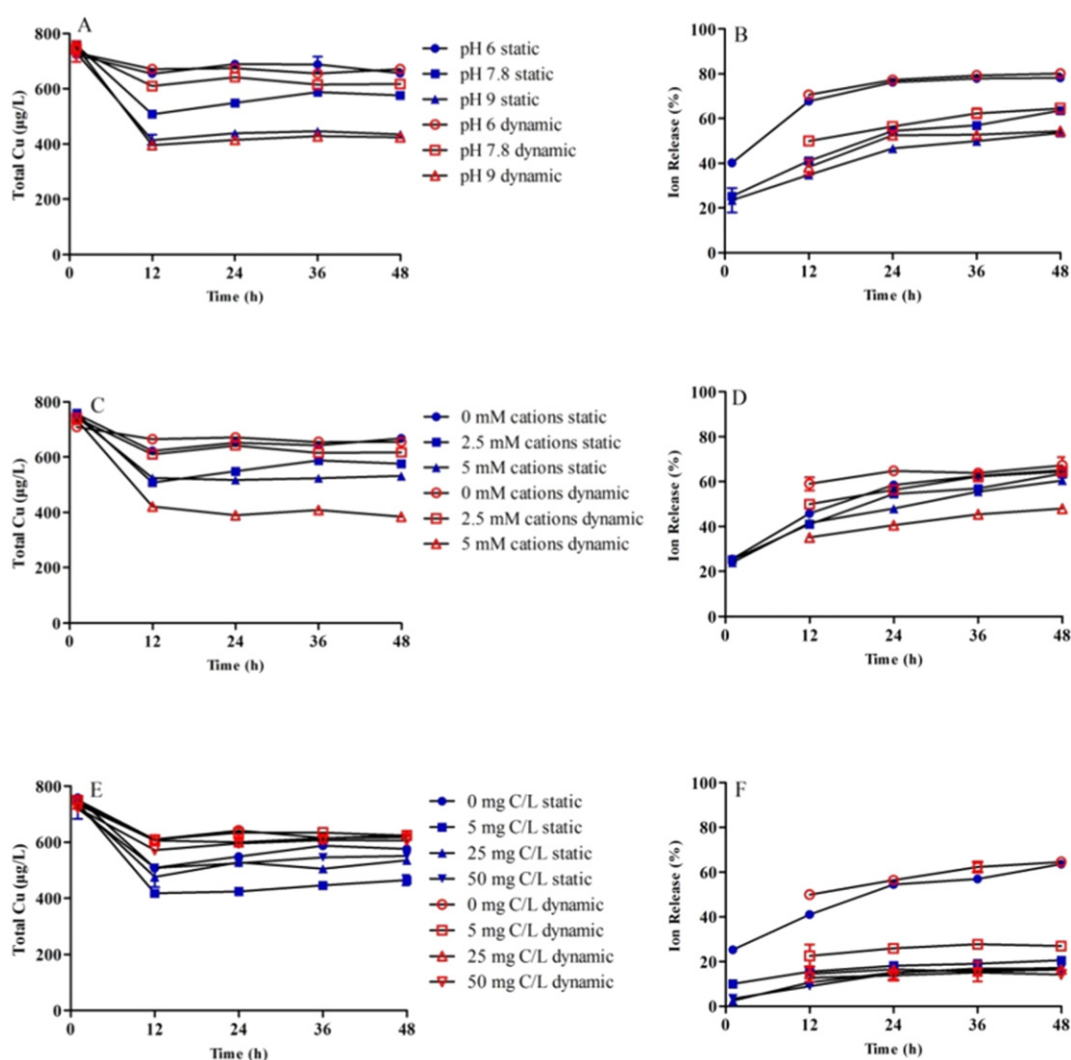


Fig. 1. Time profiles of the total amount of Cu and dissolved Cu in suspensions of CuNPs within 48 h of incubation in the static and dynamic exposure treatment as a function of pH (A–B), of concentrations of divalent cations (C–D) and of DOC (E–F). All data are presented as the mean  $\pm$  standard deviation ( $n = 2$ ).

**Table 3**

The median lethal concentration (LC50) of CuNP<sub>(total)</sub> and CuNP<sub>(particle)</sub> after 48 h of exposure to *D. magna* upon various exposure conditions and the relative contribution of CuNP<sub>(particle)</sub> and CuNP<sub>(ion)</sub> to the toxicity of CuNP<sub>(total)</sub> at the LC50.

Trial no.	Condition	pH	Cation conc. (mM)	DOC conc. (mg/L)	LC50 (95% CI, mg/L)		Relative contribution at LC50 (%)	
					CuNP <sub>(total)</sub>	CuNP <sub>(particle)</sub> <sup>a</sup>	CuNP <sub>(ion)</sub>	CuNP <sub>(particle)</sub>
1	Static	6	2.5	0	0.024 (0.022–0.026)	0.011 (0.011–0.012)	100	0
2	Static	7.8	2.5	0	0.050 (0.048–0.053)	0.040 (0.031–0.052)	53	47
3	Static	9	2.5	0	0.094 (0.084–0.106)	0.089 (0.061–0.130)	68	32
4	Static	7.8	0	0	0.026 (0.022–0.031)	0.021 (0.018–0.025)	72	28
5	Static	7.8	5	0	0.076 (0.069–0.082)	0.058 (0.044–0.076)	60	40
6	Static	7.8	2.5	5	0.515 (0.414–0.640)	1.913 (0.309–11.850)	43	57
7	Static	7.8	2.5	25	2.166 (2.009–2.335)	2.142 (1.916–2.393)	38	62
8	Static	7.8	2.5	50	3.591 (3.273–3.939)	3.939 (3.324–4.669)	33	67
9	Dynamic	6	2.5	0	0.030 (0.025–0.036)	0.018 (0.018–0.018)	100	0
10	Dynamic	7.8	2.5	0	0.049 (0.046–0.053)	0.038 (0.031–0.045)	70	30
11	Dynamic	9	2.5	0	0.084 (0.071–0.098)	0.081 (0.063–0.104)	64	36
12	Dynamic	7.8	0	0	0.022 (0.019–0.025)	–0.015 <sup>b</sup>	62	38
13	Dynamic	7.8	5	0	0.152 (0.132–0.176)	–0.171 <sup>b</sup>	100	0
14	Dynamic	7.8	2.5	5	0.318 (0.266–0.380)	0.311 (0.219–0.441)	33	67
15	Dynamic	7.8	2.5	25	1.634 (1.470–1.817)	1.568 (1.404–1.750)	28	72
16	Dynamic	7.8	2.5	50	2.153 (1.923–2.411)	1.930 (1.717–2.169)	15	85

CI: confidence intervals. Conc. = concentration.

<sup>a</sup> CuNP<sub>(particle)</sub> was estimated from Eq. (1).

<sup>b</sup> Means the data is not accurate. Statistics for comparison of LC50 of CuNP<sub>(total)</sub> and CuNP<sub>(particle)</sub> among dynamic and static treatment groups are given in Supplementary information (Tables S2–S7).

treatments, the LC50 values of CuNP<sub>(total)</sub> were 0.318, 1.634, and 2.153 mg/L at 5, 25, and 50 mg C/L, respectively.

In the absence of DOC, the 48-h LC50 of Cu(NO<sub>3</sub>)<sub>2</sub> increased with increasing pH, which was 0.016, 0.028 and 0.048 mg/L at pH 6, 7.8 and 9, respectively (Table S1). At pH 7.8 and in the absence of DOC, the LC50 values of Cu(NO<sub>3</sub>)<sub>2</sub> were 0.015, 0.028 and 0.043 mg/L with the addition of 0, 2.5 and 5 mM of the divalent cations, respectively. Toxicity of Cu(NO<sub>3</sub>)<sub>2</sub> to *D. magna* was greatly mitigated by the addition of DOC, with the LC50 increasing from 0.028 mg/L without the addition of DOC to 0.133, 0.577 and 0.970 mg/L with the addition of 5, 25 and 50 mg C/L, respectively (Table S1). The dose-response curves of Cu(NO<sub>3</sub>)<sub>2</sub> across the ranges of pH, divalent cation and DOC concentrations used in this study for CuNPs are presented in the supplementary information (Fig. S2).

In the static treatments, the LC50 of CuNP<sub>(particle)</sub> increased from 0.011 mg/L at pH 6 to 0.040 mg/L at pH 7.8 and 0.089 mg/L at pH 9; the LC50 of CuNP<sub>(particle)</sub> increased from 0.021 mg/L without the addition of divalent cations to 0.058 mg/L upon the addition of 5 mM of cations; the LC50 of CuNP<sub>(particle)</sub> increased from 0.040 mg/L in the absence of DOC to 3.939 mg/L upon the addition of 50 mg C/L (Table 3). Similar to the LC50 in the static exposure treatments, the LC50 of CuNP<sub>(particle)</sub> in the dynamic exposure treatments also showed increasing trends with increasing pH and with increasing concentrations of cations and DOC (Table 3), indicating that the toxicity of CuNP<sub>(particle)</sub> decreased with increasing pH and with increasing concentrations of cations and DOC in both the static and dynamic exposure treatments. The dose-response curves with the endpoint mortality of *D. magna* calculating based on the response addition model, are provided in the supplementary information (Figs. S3–S5).

### 3.3. Relative contribution of CuNP<sub>(particle)</sub> and CuNP<sub>(ion)</sub> to toxicity

The relative contribution of CuNP<sub>(particle)</sub> and CuNP<sub>(ion)</sub> to the toxicity of CuNP<sub>(total)</sub> to *D. magna* at the LC50 levels is given in Table 3. According to the calculation results based on the response addition model, the toxicity of CuNP<sub>(total)</sub> to *D. magna* at the LC50 level in the absence of DOC was mainly caused by CuNP<sub>(ion)</sub>. In both the static and dynamic treatments, >53% of the toxicity of CuNP<sub>(total)</sub> could be explained by CuNP<sub>(ion)</sub> at pH ranging from 6 to 9. At pH 7.8 and in the static exposure treatments, 72%, 53% and 60% of the observed toxicity could be attributed to CuNP<sub>(ion)</sub> upon the addition of 0, 2.5 and 5 mM of cations,

respectively. Similarly, in the dynamic treatments toxicity of CuNP<sub>(total)</sub> was predominantly contributed by CuNP<sub>(ion)</sub> (≥62%) at the divalent cation concentrations from 0 to 5 mM. However, upon the addition of DOC at concentrations from 5 to 50 mg/L, the relative contribution of CuNP<sub>(particle)</sub> to the overall toxicity was higher than that of CuNP<sub>(ion)</sub>. In the static exposure treatments, the relative contribution of CuNP<sub>(ion)</sub> to the overall toxicity decreased from 53% without the addition of DOC to 43%, 38% and 33% upon the addition of 5, 25 and 50 mg C/L, respectively; in the dynamic exposure treatments, the relative contribution of CuNP<sub>(ion)</sub> to the overall toxicity shifted from 70% without the addition of DOC to 33%, 28% and 15% with the addition of 5, 25 and 50 mg C/L, respectively.

## 4. Discussion

### 4.1. Behavior and fate of CuNPs upon modification of water chemistry

In this study, CuNPs aggregated to a higher extent in the exposure matrices with a higher concentration of divalent cations (Table 2). The enhanced aggregation was due to the compression of the double-layer of NPs imposed by the cations, as the absolute value of the zeta-potential of CuNP suspension decreased with the addition of the cations (Table 2). In natural waters, DOC is ubiquitous and has been identified in many studies as a key factor in determining the fate of metallic NPs in environments (Conway et al., 2015; Zou et al., 2015; Lawrence et al., 2016; Joo and Zhao, 2017). Consistent with the findings of other studies (Adeleye et al., 2014; Conway et al., 2015), we also found that the addition of DOC inhibited the further aggregation of CuNPs. Furthermore, the inhibiting effect of DOC on the aggregation of the CuNPs was stronger in the dynamic exposure treatments than in the static exposure treatments, as reflected by the smaller average sizes of CuNPs in the dynamic exposure treatments (Table 2). This is probably due to the increased shear forces upon dynamic flow, which consequently results in the disaggregation of NPs (Metreveli et al., 2015; Lv et al., 2016). In agreement with other studies (Adeleye et al., 2014; Odzak et al., 2014), the percent dissolution of the CuNPs was enhanced with increasing pH. The addition of DOC significantly reduced the concentration of CuNP<sub>(ion)</sub> in both the static and dynamic exposure treatments. The reduction of the concentration of NP<sub>(ion)</sub> upon addition of DOC in the water column was also reported by some other studies (Conway et al., 2015; Zhou et al., 2016). The possible mechanisms underlying

the reduction effects of DOC include complexation and surface adsorption to block the oxidation sites of NPs by DOC (Dubas and Pimpan, 2008; Liu and Hurt, 2010), steric exclusion of water from the surface of the particles by DOC coating, and/or reduction of the availability of  $H^+$  which may bind to DOC molecules (Yoon et al., 2005; Adeleye et al., 2014).

#### 4.2. Toxicity of CuNPs upon modification of water chemistry

In this study, the toxicity of CuNPs to *D. magna* was strongly dependent on the water chemistry of the exposure medium. Both  $CuNP_{(ion)}$  and  $CuNP_{(particle)}$  were more toxic at lower pH. The higher toxicity of  $CuNP_{(ion)}$  at lower pH is due to the increasing percentage of free  $Cu^{2+}$  species (Odzak et al., 2014; Xiao et al., 2016), which is generally considered to be the most toxic species among all dissolved Cu species (de Schampelaere and Janssen, 2002). The increasing toxicity of  $CuNP_{(particle)}$  under reduced pH may be explained by the reduced resistance of *D. magna* to  $CuNP_{(particle)}$ , as it has been found that acid stress could influence the membrane permeability of *D. magna* (Locke, 1991; Glover and Wood, 2005). The toxicity of  $CuNP_{(total)}$  increased with a reduction in the divalent cation concentration. This finding is the net effect of the reduction in the toxicity of both  $CuNP_{(ion)}$  and  $CuNP_{(particle)}$  upon the increasing concentrations of the cations. The reduced toxicity of  $CuNP_{(particle)}$  with the addition of cations may result from the enhanced aggregation imparted by the cations as mentioned above, which could decrease the effective surface area of  $CuNP_{(particle)}$  to *D. magna* and consequently reduced the toxicity of  $CuNP_{(particle)}$ . According to the biotic ligand model (BLM) (Di Toro et al., 2001), the enhanced competition between  $Ca^{2+}$  and  $Mg^{2+}$  and the  $CuNP_{(ion)}$  for binding sites on the biotic ligands of daphnids upon the increasing cation concentrations probably resulted in the mitigation of the toxicity of  $CuNP_{(ion)}$ . In the presence of DOC, consistent with many other studies (Blinova et al., 2010; Gunsolus et al., 2015), the toxicity of  $CuNP_{(total)}$  was highly mitigated. In the static exposure treatments, the toxicity of  $CuNP_{(total)}$  decreased around 10, 43, and 72 times with the addition of 5, 25 and 50 mg C/L, respectively, compared to the situation in which no DOC was added. The mitigation effects of DOC on the observed toxicity were derived from the finding that both the toxicity of  $CuNP_{(ion)}$  and  $CuNP_{(particle)}$  to *D. magna* was decreased with the addition of DOC. The decrease in toxicity for  $CuNP_{(ion)}$  and  $CuNP_{(particle)}$  with the addition of DOC may be due to the complexation of  $CuNP_{(ion)}$  with DOC and the passivation of the particle surface by DOC adsorption (Fabrega et al., 2009). In the dynamic exposure treatments, the mitigating effects of DOC on the toxicity of  $CuNP_{(total)}$  were weakened, compared to those observed in the static exposure treatments. The toxicity of  $CuNP_{(total)}$  in the dynamic exposure treatments was around 38%, 25% and 40% higher than the toxicity of  $CuNP_{(total)}$  in the static treatments upon the addition of 5, 25 and 50 mg C/L, respectively. The dissolution profiles upon the addition of DOC in the static and dynamic exposure treatments were similar within 48 h of incubation (Fig. 1F), whereas the aggregation extents of CuNPs were smaller within the 48 h of incubation in the dynamic treatments than in the static treatments when DOC was added (Table 2). Hence, the higher toxicity of  $CuNP_{(total)}$  as found in the dynamic exposure treatments, compared with the toxicity observed in the static exposure treatments when DOC was added, probably resulted from the reduction in the hydrodynamic diameters of particles.

#### 4.3. Relative contribution of $CuNP_{(particle)}$ and $CuNP_{(ion)}$ to toxicity

Evaluation of the relative contribution of  $NP_{(particle)}$  and  $NP_{(ion)}$  to the suspension toxicity upon varying water chemistry is critical for environmental risk assessment. This would allow us to make process-based predictions of fate and ecological responses. Our results clearly evidenced that even for the same type of CuNPs, the relative contribution of  $CuNP_{(particle)}$  and  $CuNP_{(ion)}$  to the observed toxicity was greatly altered by the physicochemical characteristics of the exposure medium.

In the absence of DOC, the toxicity of  $CuNP_{(total)}$  to *D. magna* at the LC50 level was mainly caused by  $CuNP_{(ion)}$  ( $\geq 53\%$  of the observed toxicity). Similarly, the toxicity of CuO NPs (with a nominal size  $< 50$  nm) to *D. magna* in exposure media without DOC and at pH 7.6 was found by Jo et al. (2012) to be largely attributable to the  $NP_{(ion)}$ . Also, the  $NP_{(ion)}$ , revealed by Heinlaan et al. (2008), was the main driver for the toxicity of CuO NPs (with a nominal size around 30 nm) to *D. magna* in exposure medium without addition of DOC and at pH within the range 7.3–7.8. In the cases that ions are dominating the toxicity, BLMs can give large insights in the NP suspensions-induced response assessment. On the other hand, in the presence of DOC at concentrations ranging from 5 to 50 mg/L, the toxicity of  $CuNP_{(total)}$  was largely explained by the contribution of  $CuNP_{(particle)}$  (Table 3). The alteration of the roles of  $CuNP_{(particle)}$  and  $CuNP_{(ion)}$  in the toxicity of CuNP suspension by DOC could result from the large reduction in dissolution of the particles on top of the observed enhancement of the stability of  $CuNP_{(particle)}$  in the water column. The contribution of particles to the toxicity of CuNP suspension could result from the particle-mediated toxicity. Determining the precise mechanisms underlying the toxicity of  $NP_{(particle)}$  was beyond the scope of this research, while previous studies have indicated that the toxic effects of  $NP_{(particle)}$  may be associated with the induction of oxidative stress (Ivask et al., 2014), inflammation (Piret et al., 2012), membrane deterioration and/or intracellular dissolution of  $CuNP_{(particle)}$  (Minocha and Mumper, 2012). It is worth to note that the relative contribution of  $CuNP_{(particle)}$  to toxicity with the addition of DOC at concentrations from 5 to 50 mg/L in the dynamic exposure treatments was 10–18% higher than that in the static exposure treatments. This may be derived from the additional stabilization effects of DOC on CuNPs in the dynamic treatments. These observations imply that the particle dynamics in aqueous environment are of importance as well. Our results highlight the importance of water chemistry on the roles of  $NP_{(particle)}$  and  $NP_{(ion)}$  in the observed toxicity.

## 5. Conclusions

This study demonstrates that the particle-specific toxicity of CuNPs strongly depends on water chemistry of the exposure medium. In the absence of DOC, the toxicity of  $CuNP_{(ion)}$  and  $CuNP_{(particle)}$  was decreased upon increasing pH and increasing concentrations of divalent cations. Toxicity of  $CuNP_{(total)}$  was mainly driven by  $CuNP_{(ion)}$  when no DOC was added. In addition, toxicity of CuNP suspensions with the addition of DOC at concentrations from 5 to 50 mg C/L under the dynamic exposure modality was approximately 25–40% higher than that under the static exposure modality. The toxicity of  $CuNP_{(ion)}$  and  $CuNP_{(particle)}$  with the addition of DOC was largely mitigated. As a result of the large reduction in the concentration of  $CuNP_{(ion)}$  and the enhancement of the stability of  $CuNP_{(particle)}$  when DOC was added, the toxicity of  $CuNP_{(total)}$  was mainly attributable to the  $CuNP_{(particle)}$  in case of the addition of DOC, especially under the dynamic exposure modality. Our results highlight the need of dynamic fate characterization of metallic NPs in aquatic environments along the exposure duration in order to interpret their ecotoxicity.

## Acknowledgements

Both Yinlong Xiao and Guangchao Chen are financially supported by the China Scholarship Council (CSC) (Grant No. 201306910009). M. G. Vijver was funded by VIDI 864.13.010. The research described in this work was supported by the European Union Seventh Framework Programme under EC-GA No. 604602 'FUTURENANONEEDS'.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.08.188>.



## References

- Adam, N., Schmitt, C., De Bruyn, L., Knapen, D., Blust, R., 2015a. Aquatic acute species sensitivity distributions of ZnO and CuO nanoparticles. *Sci. Total Environ.* 526, 233–242.
- Adam, N., Vakurov, A., Knapen, D., Blust, R., 2015b. The chronic toxicity of CuO nanoparticles and copper salt to *Daphnia magna*. *J. Hazard. Mater.* 283, 416–422.
- Adeleye, A.S., Conway, J.R., Perez, T., Rutten, P., Keller, A.A., 2014. Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. *Environ. Sci. Technol.* 48, 12561–12568.
- Backhaus, T., Scholze, M., Grimme, L., 2000. The single substance and mixture toxicity of quinolones to the bioluminescent bacterium *Vibrio fischeri*. *Aquat. Toxicol.* 49, 49–61.
- Blinova, I., Ivask, A., Heinlaan, M., Mortimer, M., Kahru, A., 2010. Ecotoxicity of nanoparticles of CuO and ZnO in natural water. *Environ. Pollut.* 158, 41–47.
- Chio, C.P., Chen, W.Y., Chou, W.C., Hsieh, N.H., Ling, M.P., Liao, C.M., 2012. Assessing the potential risks to zebrafish posed by environmentally relevant copper and silver nanoparticles. *Sci. Total Environ.* 420, 111–118.
- Conway, J.R., Adeleye, A.S., Gardea-Torresdey, J., Keller, A.A., 2015. Aggregation, dissolution, and transformation of copper nanoparticles in natural waters. *Environ. Sci. Technol.* 49, 2749–2756.
- de Schampelaere, K.A., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. *Environ. Sci. Technol.* 36, 48–54.
- Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ. Toxicol. Chem.* 20, 2383–2396.
- Dubas, S.T., Pimpan, V., 2008. Humic acid assisted synthesis of silver nanoparticles and its application to herbicide detection. *Mater. Lett.* 62, 2661–2663.
- Fabrega, J., Fawcett, S.R., Renshaw, J.C., Lead, J.R., 2009. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ. Sci. Technol.* 43, 7285–7290.
- Glover, C.N., Wood, C.M., 2005. Physiological characterisation of a pH- and calcium-dependent sodium uptake mechanism in the freshwater crustacean, *Daphnia magna*. *J. Exp. Biol.* 208, 951–959.
- Godinez, I.G., Darnault, C.J., 2011. Aggregation and transport of nano-TiO<sub>2</sub> in saturated porous media: effects of pH, surfactants and flow velocity. *Water Res.* 45, 839–851.
- Grillo, R., Rosa, A.H., Fraceto, L.F., 2015. Engineered nanoparticles and organic matter: a review of the state-of-the-art. *Chemosphere* 119, 608–619.
- Gunsolus, I.L., Mousavi, M.P., Hussein, K., Bühlmann, P., Haynes, C.L., 2015. Effects of humic and fulvic acids on silver nanoparticle stability, dissolution, and toxicity. *Environ. Sci. Technol.* 49, 8078–8086.
- Hammes, J., Gallego-Urrea, J.A., Hasselöv, M., 2013. Geographically distributed classification of surface water chemical parameters influencing fate and behavior of nanoparticles and colloid facilitated contaminant transport. *Water Res.* 47, 5350–5361.
- Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.C., Kahru, A., 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO<sub>2</sub> to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere* 71, 1308–1316.
- Hu, W., Culloty, S., Darmody, G., Lynch, S., Davenport, J., Ramirez-Garcia, S., Dawson, K.A., Lynch, I., Blasco, J., Sheehan, D., 2014. Toxicity of copper oxide nanoparticles in the blue mussel, *Mytilus edulis*: a redox proteomic investigation. *Chemosphere* 108, 289–299.
- Hua, J., Vijver, M.G., Richardson, M.K., Ahmad, F., Peijnenburg, W.J.G.M., 2014. Particle-specific toxicity effects of differently shaped ZnO nanoparticles to zebrafish embryos (*Danio rerio*). *Environ. Toxicol. Chem.* 33, 2859–2868.
- Ivask, A., Juganson, K., Bondarenko, O., Mortimer, M., Aruoja, V., Kasemets, K., Blinova, I., Heinlaan, M., Slaveykova, V., Kahru, A., 2014. Mechanisms of toxic action of Ag, ZnO and CuO nanoparticles to selected ecotoxicological test organisms and mammalian cells in vitro: A comparative review. *Nanotoxicology* 8, 57–71.
- Jo, H.J., Choi, J.W., Lee, S.H., Hong, S.W., 2012. Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: the importance of their dissolved fraction varying with preparation methods. *J. Hazard. Mater.* 15, 301–308.
- Joo, S.H., Zhao, D., 2017. Environmental dynamics of metal oxide nanoparticles in heterogeneous systems: a review. *J. Hazard. Mater.* 332, 29–47.
- Lawrence, J.R., Swerhone, G.D.W., Dynes, J.J., Hitchcock, A.P., Korber, D.R., 2016. Complex organic corona formation on carbon nanotubes reduces microbial toxicity by suppressing reactive oxygen species production. *Environ. Sci. Nano* 3, 181–189.
- Liu, J., Hurt, R.H., 2010. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ. Sci. Technol.* 44, 2169–2175.
- Locke, A., 1991. Zooplankton responses to acidification: a review of laboratory bioassays. *Water Air Soil Pollut.* 60, 135–148.
- Lv, X., Tao, J., Chen, B., Zhu, X., 2016. Roles of temperature and flow velocity on the mobility of nano-sized titanium dioxide in natural waters. *Sci. Total Environ.* 565, 849–856.
- Metreveli, G., Philippe, A., Schaumann, G.E., 2015. Disaggregation of silver nanoparticle homoaggregates in a river water matrix. *Sci. Total Environ.* 535, 35–44.
- Minetto, D., Volpi Ghirardini, A., Libralato, G., 2016. Saltwater ecotoxicology of Ag, Au, CuO, TiO<sub>2</sub>, ZnO and C60 engineered nanoparticles: an overview. *Environ. Int.* 92–93, 189–201.
- Minocha, S., Mumper, R.J., 2012. Effect of carbon coating on the physico-chemical properties and toxicity of copper and nickel nanoparticles. *Small* 8, 3289–3299.
- Mohd Omar, F., Abdul Aziz, H., Stoll, S., 2014. Aggregation and disaggregation of ZnO nanoparticles: influence of pH and adsorption of Suwannee River humic acid. *Sci. Total Environ.* 468–469, 195–201.
- Mukherjee, B., Weaver, J.W., 2010. Aggregation and charge behavior of metallic and non-metallic nanoparticles in the presence of competing similarly-charged inorganic ions. *Environ. Sci. Technol.* 44, 3332–3338.
- Odzak, N., Kistler, D., Behra, R., Sigg, L., 2014. Dissolution of metal and metal oxide nanoparticles in aqueous media. *Environ. Pollut.* 191, 132–138.
- OECD, 2004. OECD Guideline 202. OECD Guidelines for Testing of Chemicals. *Daphnia* sp., Acute Immobilization Test (Paris).
- Ottofuelling, S., Von der Kammer, F., Hofmann, T., 2011. Commercial titanium dioxide nanoparticles in both natural and synthetic water: comprehensive multidimensional testing and prediction of aggregation behavior. *Environ. Sci. Technol.* 45, 10045–10052.
- Piret, J.P., Jacques, D., Audinot, J.N., Mejia, J., Boilan, E., Noel, F., Fransolet, M., Demazy, C., Lucas, S., Saout, C., Toussaint, O., 2012. Copper(II) oxide nanoparticles penetrate into HepG2 cells, exert cytotoxicity via oxidative stress and induce pro-inflammatory response. *Nano* 4, 7168–7184.
- Poynton, H.C., Lazorchak, J.M., Impellitteri, C.A., Smith, M.E., Rogers, K., Patra, M., Hammer, K.A., Allen, H.J., Vulpe, C.D., 2011. Differential gene expression in *Daphnia magna* suggests distinct modes of action and bioavailability for ZnO nanoparticles and Zn ions. *Environ. Sci. Technol.* 45, 762–768.
- Poynton, H.C., Lazorchak, J.M., Impellitteri, C.A., Blalock, B.J., Rogers, K., Allen, H.J., Loguinov, A., Heckman, J.L., Govindaswamy, S., 2012. Toxicogenomic responses of nanotoxicity in *Daphnia magna* exposed to silver nitrate and coated silver nanoparticles. *Environ. Sci. Technol.* 46, 6288–6296.
- Rainville, L.C., Carolan, D., Varela, A.C., Doyle, H., Sheehan, D., 2014. Proteomic evaluation of citrate-coated silver nanoparticles toxicity in *Daphnia magna*. *Analyst* 139, 1678–1686.
- Santo, N., Fascio, U., Torres, F., Guazzoni, N., Tremolada, P., Bettinetti, R., Mantecchia, P., Bacchetta, R., 2014. Toxic effects and ultrastructural damages to *Daphnia magna* of two differently sized ZnO nanoparticles: does size matter? *Water Res.* 53, 339–350.
- Song, L., Vijver, M.G., de Snoo, G.R., Peijnenburg, W.J.G.M., 2015a. Assessing toxicity of copper nanoparticles across five cladoceran species. *Environ. Toxicol. Chem.* 34, 1863–1869.
- Song, L., Vijver, M.G., Peijnenburg, W.J.G.M., Galloway, T.S., Tyler, C.R., 2015b. A comparative analysis on the in vivo toxicity of copper nanoparticles in three species of freshwater fish. *Chemosphere* 139, 181–189.
- Vale, G., Mehennaoui, K., Cambier, S., Libralato, G., Jomini, S., Domingos, R.F., 2016. Manufactured nanoparticles in the aquatic environment—biochemical responses on freshwater organisms: a critical overview. *Aquat. Toxicol.* 170, 162–174.
- Vijver, M.G., de Koning, A., Peijnenburg, W.J.G.M., 2008. Uncertainty of water type-specific hazardous copper concentrations derived with biotic ligand models. *Environ. Toxicol. Chem.* 27, 2311–2319.
- Wang, D., Lin, Z., Wang, T., Yao, Z., Qin, M., Zheng, S., Lu, W., 2016. Where does the toxicity of metal oxide nanoparticles come from: the nanoparticles, the ions, or a combination of both? *J. Hazard. Mater.* 5, 328–334.
- Xiao, Y., Peijnenburg, W.J.G.M., Chen, G., Vijver, M.G., 2016. Toxicity of copper nanoparticles to *Daphnia magna* under different exposure conditions. *Sci. Total Environ.* 563–564, 81–88.
- Yoon, T.H., Johnson, S.B., Brown, G.E., 2005. Adsorption of organic matter at mineral/water interfaces. IV. Adsorption of humic substances at boehmite/water interfaces and impact on boehmite dissolution. *Langmuir* 21, 5002–5012.
- Zhao, J., Cao, X., Liu, X., Wang, Z., Zhang, C., White, J.C., Xing, B., 2016. Interactions of CuO nanoparticles with the algae *Chlorella pyrenoidosa*: adhesion, uptake, and toxicity. *Nanotoxicology* 10, 1297–1305.
- Zhou, W., Liu, Y.L., Stallworth, A.M., Ye, C., Lenhart, J.J., 2016. Effects of pH, electrolyte, humic acid, and light exposure on the long-term fate of silver nanoparticles. *Environ. Sci. Technol.* 50, 12214–12224.
- Zou, X., Shi, J., Zhang, H., 2015. Morphological evolution and reconstruction of silver nanoparticles in aquatic environments: the roles of natural organic matter and light irradiation. *J. Hazard. Mater.* 292, 61–69.