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Review:

Significance of physicochemical and uptake kinetics in controlling the toxicity of metallic nanomaterials to aquatic organisms*

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Abstract: With the extensive applications of metallic-based nanomaterials (MNs), concerns are growing of their potential impact on aquatic organisms. Unlike traditional metal pollutants, MNs have different surface properties and compositions, which may modify their impact on aquatic environments as well as their bioavailability to aquatic organisms. Kinetic processes of MNs, such as dissolution, stabilization, aggregation, and sedimentation, are important in determining their bioavailability and subsequent toxicity to aquatic organisms. Among all of the physicochemical kinetics, the dissolution of MNs attracts the most attention, due to their potential toxicity generated by dissolved ions. This review summarizes the dissolution behavior of three common MNs, i.e., ZnO nanoparticles (ZnO-NPs), Ag nanoparticles (Ag-NPs), and TiO_2 nanoparticles (TiO_2-NPs), in toxicological studies. A kinetic model was developed to evaluate the contribution of dissolved ion on the total MN accumulation. Finally, toxicological data of the MNs to algae, zooplankton, and fish are summarized and interpreted based on their kinetics. Different dissolution rates were observed for ZnO-NPs, Ag-NPs, and TiO_2-NPs, and their solubility also varied during different toxicological studies, leading to a variable but increasing waterborne ion concentration during exposure. The bioavailability of these MNs and corresponding ions also varied for different aquatic organisms (e.g., algae, zooplankton, and fish). Specifically, the MNs appeared to be more bioavailable to daphnids, rendering a minor contribution of ion during short-term exposure. Generally, dissolved ion contributed partially to toxicity of ZnO-NPs and Ag-NPs, while the toxicity of TiO_2-NPs was mainly due to the generated reactive oxygen species (ROS). Additionally, the role of dissolved ion in both MN bioaccumulation and toxicity intensified during chronic exposure as a result of dissolution, thus it is critical to monitor the dissolution of MNs in toxicological studies. This review emphasizes the importance of integrating physicochemical kinetics and uptake kinetics in evaluating the bioavailability and toxicity of both MNs and dissolved ions.

Key words: Metallic-based nanomaterials (MNs), Dissolution, Kinetics, Aquatic organisms, Toxicity
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1 Introduction

The application of metallic-based nanomaterials (MNs) now exists in every corner of modern life, e.g., cosmetics, textiles, and paints. For some MNs, e.g., TiO_2 nanoparticles (TiO_2-NPs) and ZnO nanoparticles (ZnO-NPs), the annual worldwide production exceeds hundreds of tons (Piccinno *et al.*, 2012). The extensive use of these MNs will eventually result in an increased MN input into the aquatic environment. Due to the complexity of the environmental matrix in affecting the analytical processes (Farré *et al.*, 2009), reports of MN concentrations in the aquatic environment are still limited, but the concerns toward the potential impact of these MNs on aquatic organisms are growing (Griffitt *et al.*, 2008). Various toxicological studies have been conducted to screen the toxicity of MNs on aquatic organisms (e.g., algae, zooplankton, and fish) (Baun *et al.*, 2008; Farré *et al.*, 2009; Scown *et al.*, 2010). However, unlike the traditional trace metal pollutants, the MNs are very

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dynamic in an aquatic environment. Kinetic processes, such as aggregation, dissolution, and sedimentation, take place immediately after these MNs are released into the aquatic environment (Fig. 1) (Klaine *et al.*, 2008). Thus, greater attention should be paid to the physicochemical properties of these MNs in toxicological studies, because they can significantly affect the kinetic processes mentioned above (Auffan *et al.*, 2010; Podila and Brown, 2013).

In connection with MNs toxicity, one of the most common debates is the role of dissolved ion in contributing to MNs' toxicity (Lubick, 2008; Shaw and Handy, 2011; Misra *et al.*, 2012). To separate the impact of ions in MN toxicity testing, most studies also run a parallel ion toxicity test for comparison of the results. However, MNs as dynamic entities are not stable and the ion concentration keeps increasing within the dissolution period, which differs from traditional static exposures with a relatively constant exposure concentration. Therefore, a kinetic interpretation of both the accumulation and resulting toxicity is needed in order to define the contribution of ions. For this purpose, the present study reviews the dissolution of three types of MNs, i.e., ZnO-NPs, Ag nanoparticles (Ag-NPs), and TiO_2-NPs, in toxicological studies. These three types of MNs are mainly chosen because of their different dissolution behavior. The subsequent uptake of these MNs and the corresponding ions within algae, zooplankton, and fish are reviewed. We then develop a kinetic uptake model in *Daphnia magna* by integrating the dissolution kinetics and uptake kinetics of MNs to quantify the contribution of ions during short-time exposure. Finally, the MNs toxicity data (mostly acute data) are gathered and interpreted based on the kinetic process.

2 Dissolution of MNs in the aquatic environment

Once released into the aquatic environment, the dissolution of some MNs will immediately occur, raising a question whether the toxicity of MNs is due to the ions or MNs. This has been a central question in nanotoxicology. Before such a question can be realistically answered, the dynamic dissolution of these MNs in the studied medium needs to be understood. Several environmental factors (e.g., dissolved natural organic matter, ionic strength, type of electrode, and pH) as well as the intrinsic properties of the MNs (e.g., size, aggregation state, coating, and concentration) affect this process (Klaine *et al.*, 2008). To describe the dissolution of MNs, models evaluating MNs dissolution are briefly discussed and the solubility of the MNs mentioned above in nanotoxicological studies are elaborated.

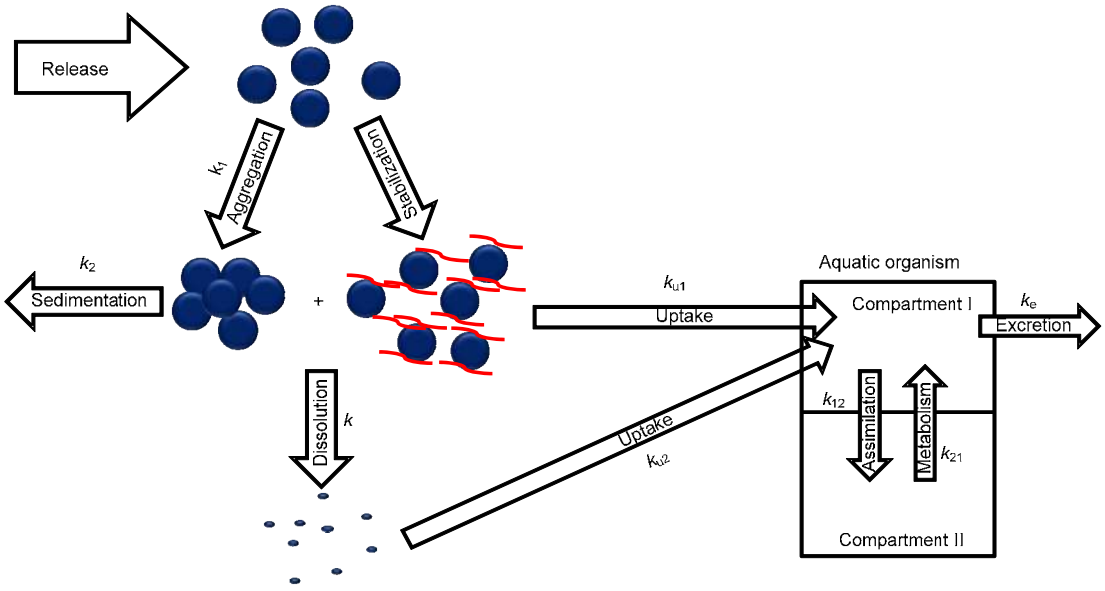

Fig. 1 Kinetic scheme of MNs in aquatic environments

k, k_1, and k_2 are the rate constants for dissolution, aggregation, and sedimentation of MNs, respectively. k_{u1} and k_{u2} are the uptake rate constants for MNs and dissolved ions, respectively. k_{12} and k_{21} are the rate constants for metals being transferred between compartments I and II, respectively. k_e is the efflux rate constant of silver

2.1 Kinetic models for dissolution of MNs

In simply considering the particle surface as a major factor affecting particle dissolution, the traditional Noyes-Whitney equation is frequently applied (Dokoumetzidis and Macheras, 2006). A common mathematic expression is given as

$$\frac{\mathrm{d}m}{\mathrm{d}t}=\frac{DA}{h}(C_\mathrm{s}-C), \tag{1}$$

where dm/dt is the dissolution rate, D is the diffusion coefficient, A is the surface area, h is the thickness of diffusion layers, C_s and C are the saturated and bulk concentrations, respectively. Thus, particles with a smaller size (relatively high surface area) are considered to have a faster dissolution rate. h has been experimentally found to decrease with particle size leading to faster transport of solvated molecules to bulk solution and hence a more rapid dissolution (Tinke *et al.*, 2005). The Noyes-Whitney equation has been applied in predicting the initial drug release rate in MNs by comparing this with the bulk materials (Heng *et al.*, 2008). However, the direct calculation of the dissolution rate using the Noyes-Whitney equation is difficult due to the lack of a reliable theoretical approach for their prediction. D and h are often determined experimentally (MacInnis and Brantley, 1992; Wang and Flanagan, 2002). Alternatively, the Ostward-Freundlich equation derived from thermodynamics provides a solution for quantifying the solubility of particles (Hunter, 2001), which can be given as

$$\frac{S}{S_\mathrm{o}}=\exp\left(\frac{2\gamma V}{RTr}\right), \tag{2}$$

where S is the solubility of fine particles with an inscribed radius r, S_o is the solubility of the bulk material, V is the molar volume (m^3/mol), γ is the surface energy (J/m^2), R is the gas constant, and T is the temperature (K). A prediction of the particle dissolution rate needs a precise estimation of γ, but it differs with the current particle surface morphology and type (Mihranyan and Strømme, 2007). David *et al*. (2012) estimated the γ of ZnO-NPs by analyzing the equilibrium free Zn^{2+} concentration in a medium with different sized ZnO-NPs. They were able to predict the dissolution rate of large ZnO-NPs aggregates. However, complications arise in the applications of the Ostwald-Freundlich equations for very small particles (<several nanometers in diameter), since the surface stress (the reversible work per unit area required to elastically stretch a surface) will affect the surface energy (Hofmeister *et al.*, 1997). The accompanying aggregation process, especially at the beginning of dissolution, leads to an increasing diameter and modifies the surface morphology, which is considered as the major reason for poor prediction of the ZnO-NPs (4 nm) dissolution (Bian *et al.*, 2011).

An empirical model on Ag-NPs (50 nm) dissolution was proposed by Liu and Hurt (2010) by considering the impacts of pH, temperature, dissolved oxygen, and natural organic matters. This empirical model was less applicable for Ag-NPs with different sizes, since their activation energy for the dissolution process was different (Zhang *et al.*, 2011). Most of the previous dissolution studies only determined a single or several alternate factors affecting MNs' dissolution, and a comprehensive mathematical prediction of the dissolution process in complex environmental matrix is still lacking. Since MNs with different chemical components may dissolute differently, representative MNs (e.g., ZnO-NPs, Ag-NPs, and TiO_2-NPs) are chosen and the favorable environmental conditions for their dissolution, as well as the dissolution behavior of MNs in toxicological studies, are discussed in the following sections.

2.2 ZnO-NPs

The dissolution process of ZnO-NPs in water firstly involves the hydration of the surface ZnO to $Zn(OH)_2$ (Han *et al.*, 2010) and can be simply written as (David *et al.*, 2012)

$$\mathrm{ZnO(s)+H_2O(l)\leftrightarrow Zn^{2+}(aq)+2OH^-(aq)}.$$

Neutralization of the generated OH^- can facilitate the dissolution process. Thus, a low pH was favorable for ZnO-NPs dissolution, as it was discovered that the maximum Zn^{2+} concentration increased by almost 100 times when the medium pH decreased from 9 to 7 (Miao *et al.*, 2010). Along with pH, the increasing ionic strength also facilitated the dissolution by providing binding ligands (e.g., Cl^- and SO_4^{2-}) to dissolved Zn^{2+}. Temperature can also affect the

dissolution of ZnO-NPs; more dissolution of ZnO-NPs in the Roswell Park Memorial Institute medium was observed at 20 °C than at 37 °C (Reed *et al.*, 2012). The impact of natural organic matter depends on whether they are acting as chelating agents or blocking agents (adsorbed on the particle surface) (Miao *et al.*, 2010; Mudunkotuwa *et al.*, 2012). Remarkably, re-precipitation of dissolved Zn^{2+} occurred when they reacted with carbonate (CO_3^{2-}) or phosphate (PO_4^{3-}) forming precipitates (e.g., $ZnCO_3$ and $Zn_3(PO_4)_2$) (Pan *et al.*, 2010; Reed *et al.*, 2012).

The time required for ZnO-NPs to achieve saturable Zn^{2+} concentration varied in different toxicological studies. David *et al*. (2012) demonstrated that the dissolution of ZnO-NPs (e.g., 20 and 71 nm) reached equilibrium at about 100 min and fast dissolution was observed within the first 20 min in a buffered medium (pH 7.7–8.4, ionic strength 0.1 mol/L). Fast dissolution was also observed within the first 1 h in another dissolution kinetic study of 4 nm ZnO-NPs and equilibrium was reached at about 10 h (Bian *et al.*, 2011), whereas a longer time was needed (e.g., 50–70 h) in some toxicological studies (Franklin *et al.*, 2007; Wong *et al.*, 2010; Peng *et al.*, 2011). For most ZnO-NPs toxicological studies, dissolution kinetics was not quantified and only a few studies measured the final dissolved Zn concentration in the medium (Table 1). The percentage of dissolved Zn also varied for different ZnO-NPs and testing systems, ranging from 0.19% to 20%. Therefore, it is difficult to draw a consensus of the percentage of dissolved Zn in these toxicological studies. A typical dissolution curve of ZnO-NPs is simulated in Fig. 2a (p.578), assuming 10% dissolution and a 1 h^{-1} dissolution rate (for details, see Section 3.3). Consistent with other kinetic studies, the dissolution process reached temporal equilibrium within several hours, which was shorter than the traditional toxicological study duration (e.g., 48–96 h). More evidence suggested that the dissolution continued slowly during prolonged exposure (e.g., chronic 21 d exposure), during which all ZnO-NPs were considered to dissolute totally (Lopes *et al.*, 2014).

2.3 Ag-NPs

A redox reaction was involved in the dissolution process of Ag-NPs with the oxidation of zero-valent Ag to Ag^+ on the particle surface (Marambio-Jones and Hoek, 2010), which is given as

$$\mathrm{Ag(s)}+\frac{1}{2}\mathrm{O_2(aq)}+2\mathrm{H^+(aq)}\leftrightarrow 2\mathrm{Ag^+(aq)}+\mathrm{H_2O(l)}.$$

High H^+ and O_2 concentrations were favorable for the dissolution reaction, which was confirmed in a dissolution kinetic study conducted by Liu and Hurt (2010). Also, Ag-NPs with a smaller size provided more surface area for this reaction, and the smaller citrate coated Ag-NPs had the fastest Ag^+ release rate (Zhang *et al.*, 2011; Ma *et al.*, 2012). However, the dissolution of Ag-NPs was inhibited when they were stabilized by some surfactants (e.g., Tween 80 and sodium dodecyl sulfate) even when they were not aggregated, presumably due to the build-up of a barrier between the particle and oxidants (Li *et al.*, 2012). In a natural environment, the presence of natural organic matter could well stabilize Ag-NPs from aggregation (Delay *et al.*, 2011), whereas their impact on Ag-NPs dissolution depended on the mechanism of dissolved organic matter sorption as well as the solution composition (Brantley, 2008). Although increasing salinity can provide more binding ligands (e.g., Cl^-) for dissolved Ag, Ag-NPs preceded at a fast aggregation in high ionic environments and a declining dissolution rate was observed (Liu and Hurt, 2010). However, as a thermodynamic unstable form, Ag-NPs were generally considered to go through continuous dissolution in aquatic environments containing dissolved oxygen (Liu and Hurt, 2010; Fabrega *et al.*, 2011).

Compared with ZnO-NPs, Ag-NPs were relatively stable during toxicological exposure. Although the proportion of dissolved Ag also varied remarkably in different toxicological studies (Table 1), most of this dissolved Ag came from the initial rapid dissolution or from the stock. Sotiriou *et al.* (2012) found that the rapid dissolution of Ag occurred within 5 min, which was far shorter than the regular preparation time in toxicological studies. The subsequent slow dissolution was negligible for most of the short-term toxicity tests (e.g., 24–96 h) (Griffitt *et al.*, 2009; Laban *et al.*, 2010; Zhao and Wang, 2011; Yang *et al.*, 2012). However, the dissolved Ag built up gradually with the extension of the duration time and 100% dissolution was achieved in air-saturated deionized water after 130 d (Liu and Hurt, 2010). As another important source of dissolved Ag, the dissolution of stock Ag-NPs solutions also preceded progressively at various storage temperatures, ranging from 10% to 70% after 100 d for citrate coated Ag-NPs (Kittler

Table 1 Solubility of ZnO-NPs and Ag-NPs in toxicological studies

NPs type	Particle characterization			Test medium	Separation method	Dissolution test duration (h)	Total conc. (mg/L)	Ion conc. (mg/L)	Solubility (%)	Reference
	TEM Size (nm)	DLS size (nm)	Zeta-potential (mV)							
ZnO-NPs	30	73–480		FW (pH 7.6)	Dialyze	72	100	16	16.0	Franklin *et al.*, 2007
	26.2	2300		SW (pH 8.0, 30 psu)	Filtration (0.1 μm)	96	61.2	3.7	5.8	Wong *et al.*, 2010
	20	1030	−39.4	FW	Centrifugation (5000 r/min)	144	816.5	1.54	0.2	Ji *et al.*, 2011
	6.3	313–2149		SW	Centrifugation (8300*g*) and filtration (0.05 μm)	96	31	1.59	5.1	Peng *et al.*, 2011
	15.7	363–1588		SW	Centrifugation (8300*g*) and filtration (0.05 μm)	96	31	1.41	4.6	Peng *et al.*, 2011
	30	142–517		FW (pH 7.6)	Centrifugation ($1\times10^4 g$)	24	81.6	6	7.5	Bai *et al.*, 2010
	30	1061–4533	−3	FW (pH 8.3)	Centrifugation (19 064*g*) and filtration (0.2 μm)	48	100	0.36–0.50	0.4–0.5	Lopes *et al.*, 2014
	80–100	1353–3560	−3	FW (pH 8.3)	Centrifugation (19 064*g*) and filtration (0.2 μm)	48	100	0.34–0.43	0.3–0.4	Lopes *et al.*, 2014
	>200	1565−3365	−15	FW (pH 8.3)	Centrifugation (19 064*g*) and filtration (0.2 μm)	48	100	0.3–0.8	0.3–0.8	Lopes *et al.*, 2014
	27.2			FW	Ultra-filtration (10 kDa)	24	2 or 9	0.4	4.4–20	Poynton *et al.*, 2011
	<200			FW	Centrifugation ($3\times10^5 g$)	48	81.6	1.2	1.5	Wiench *et al.*, 2009
	20	2196		FW (pH 8.0)	Filtration (0.05 μm)	96	8.16	1.01	10.1	Zhu *et al.*, 2009b
Ag-NPs	25	44	−36.6	FW (pH 7.8)	Diffusive gradient in thin films technique	192	5.6	0.048	0.9	Navarro *et al.*, 2008a
	25	44		FW (pH 7.8)	Ultra-filtration (3 kDa)	3	530	3.71–4.24	0.7–0.8	Navarro *et al.*, 2008a
	25	44	−36.6	FW (pH 7.8)	Electrode	192	NA	NA	0.7–1.2	Navarro *et al.*, 2008a
	60–70			SW (pH 8.2)	Ultra-filtration (1 kDa)	168	495 100	2360	0.5	Miao *et al.*, 2009
	60	307	−28.5	FW (pH 7)	Filtration (0.45 μm)	24	10–10 000	2.2–0.55	0.0055–22	Oukarroum *et al.*, 2012
	60	449	−15.0	SW (pH 6)	Filtration (0.45 μm)	24	10–10 000	1.3–7.6	0.8–13	Oukarroum *et al.*, 2012
	10–50	77−310		FW	Centrifugation ($1\times10^5 g$)	48	NA	NA	2−8	Kennedy *et al.*, 2010
	21–60			FW	Centrifugation (15 000 r/min) and filtration (20 nm)	96	62.5–20 000	23–94	0.5–3.7	Laban *et al.*, 2010
	26.6		−27	FW	Filtration (0.2 μm)	48	51	4.2–0.9	1.8–8.2	Griffitt *et al.*, 2009
	26.6 (SEM)		−27	FW (pH 8.2)	Centrifugation ($1\times10^6 g$)	48	NA	NA	0.07	Griffitt *et al.*, 2008
	20	40–50	−19.6	FW	Ultra-filtration (3 kDa)	48	NA	NA	0.5–1.0	Zhao and Wang, 2011
	<100	124	−22.5	FW	Ultra-filtration (3 kDa)	48	10–50	0.37–1.0	2.4–4.0	Zhao and Wang, 2012a
	10–20	80	−19.5	FW	Ultra-filtration (3 kDa)	48	1–8	0.27–0.98	12.3–27	Zhao and Wang, 2012a
	10–20	65	−22.6	FW	Ultra-filtration (3 kDa)	24	1000	380	38.0	Zhao and Wang, 2012a
	5	90		FW	Filtration (0.025 μm)	72	NA	NA	25.7	Yang *et al.*, 2012
	38	70		FW	Filtration (0.025 μm)	72	NA	NA	2.0	Yang *et al.*, 2012
	22	70		FW	Filtration (0.025 μm)	72	NA	NA	2.0	Yang *et al.*, 2012
	8	110		FW	Filtration (0.025 μm)	72	NA	NA	1.3	Yang *et al.*, 2012
	7	50–400		FW	Filtration (0.025 μm)	72	NA	NA	0.1	Yang *et al.*, 2012

TEM: transmission electron microscopy; SEM: scanning electron microscopy; DLS: dynamic light scattering; FW: freshwater; SW: seawater; NA: not available

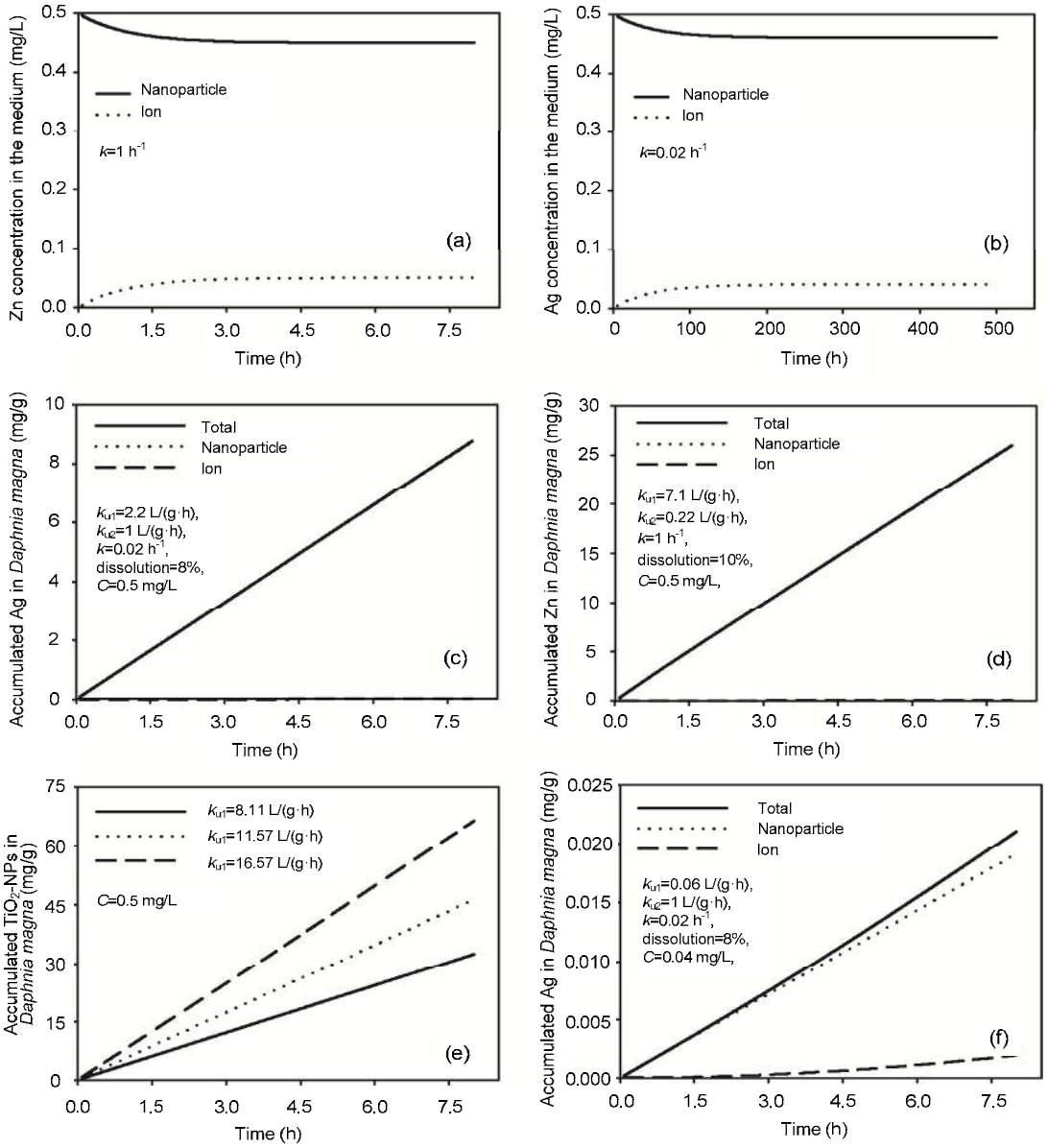

Fig. 2 Modeling results of MNs dissolution and uptake of both MNs and ions by *Daphnia magna*

(a) Dissolution kinetic of ZnO-NPs (0.5 mg/L); (b) Dissolution kinetic of Ag-NPs (0.5 mg/L); (c) Uptake of Ag-NPs (0.5 mg/L); (d) Uptake of ZnO-NPs (0.5 mg/L); (e) Uptake of different types of TiO_2-NPs (0.5 mg/L); (f) Uptake of Ag-NPs (0.04 mg/L)

et al., 2010). Therefore, it is important to perform some clean-up procedures (e.g., dialyze, centrifugation, and resuspension) for aged Ag-NP stocks before conducting nanotoxicological studies. For most of the reviewed toxicological studies, although there were some extreme cases (e.g., 12.3%–38%), the dissolved Ag accounted for less than 8% of total Ag mass and the dissolution was rather slow during short-term exposures (Yang *et al.*, 2012; Zhao and Wang, 2011), which can also be inferred from the simulation (Fig. 2b).

2.4 TiO_2-NPs

TiO_2-NPs were rather inert compared to the two above-mentioned MNs, whereas slow hydrolysis still exists especially under some extreme conditions (e.g., low or high pH, high temperature). This can be simply expressed as (Schmidt and Vogelsberger, 2006)

$$TiO_2(s)+(4-n)H^+ +(n-2)H_2O \leftrightarrow Ti(OH)_n^{4-n}.$$

Similar to Ag-NPs, high H^+ was favorable for the dissolution of TiO_2-NPs, while the pH needed to be extremely low to obtain an oblivious dissolution. The bulk TiO_2 (rutile) was found less soluble at near neutral pH (e.g., 4–8), whereas the solubility increased remarkably when pH decreased to 2 (Knauss *et al.*, 2001). For TiO_2-NPs, slight dissolution was observed when the pH went down to 3 and further increased by >100 fold as the pH decreased to 1.5, whereas the overall proportion of soluble Ti was less than 0.0038%, even after 125 d (Schmidt and Vogelsberger, 2006). Therefore, the dissolution of TiO_2-NPs was supposed to be rather slow in natural aquatic environment and was often ignored in short-term toxicological studies (Heinlaan *et al.*, 2008; Zhu *et al.*, 2009a; Ji *et al.*, 2011).

3 Uptake of NMs by aquatic organisms

3.1 Kinetic models on metal and MNs uptake

Several models have been developed in describing the dissolve uptake of metals. The common linear relationship between uptake and dissolved metal concentration can be written as

$$I_w = k_u C_w. \tag{3}$$

This model assumes that the uptake rate (I_w) of metals is proportional to the metal concentration (C_w) in water, k_u is the uptake rate constant, and can be obtained by linear regression of different I_w under different C_w during short-term exposure (Wang *et al.*, 1996; Wang and Fisher, 1997). However, in some organisms, the uptake of metals was non-linear as a function of water metal concentration, exhibited by the Freundlich or Michaelis-Mention equation (Wang, 2011). In this review, only the linear model is elucidated.

While different from the pure uptake of metals, the uptake of MNs involves the uptake of several species (e.g., MNs, dissolved ion, and MNs binding ion). Therefore, by assuming that the MNs are homogenously disseminated in the medium and the uptake of the particle and ion is independent, the apparent uptake rate (I'_w) of MNs can be expressed as

$$I'_w = k_{u1}(C_{NP}+C_{BI})+k_{u2}C_w, \tag{4}$$

where C_{NP} is the concentration of the MNs, C_{BI} is the concentration of MNs binding with ions, and C_w is the concentration of dissolved ions. Similarly, for some "inert" MNs (dissolution was negligible), k_{u1} can be obtained through linear regression of different I'_w under different C_{NP} during short-term exposure. However, for MNs with significant dissolution, uptake of ions can be deducted either by adding metal chelates (reducing bioavailability of ions) (Zhao and Wang, 2010; 2012b) or deducting the accumulated ions at each time-point by applying the ion uptake kinetic model (Wang and Wang, 2014). k_{u1} can subsequently be calculated similarly as k_{u2} using the obtained "pure" uptake of MNs. Additionally, with the proceeding dissolution, C_w keeps increasing and then leads to an elevated accumulation of ionic metals during exposure. Since the traditional separation methods (e.g., filtration and centrifugation) were unable to distinguish the adsorbed ion from the MNs, the adsorbed ion can be regarded as a part of the MNs. Also, the excretion of accumulated metals can be ignored during short-term exposure, thus the accumulation was equal to the uptake. The total accumulated metals at the time-point t can be simplified as

$$C=\int_0^t (k_{u1}C_{NP}+k_{u2}C_w)\mathrm{d}t. \tag{5}$$

As mentioned above, continuous dissolution may proceed during short-term toxicity testing for MNs like ZnO-NPs and Ag-NPs, rendering an increasing ionic metal concentration and decreasing the MNs concentration in the medium. Additionally, considering the variability of bioavailability of different metals in different organisms, the contribution of accumulated ionic metals may also vary. Therefore, the uptake of the above-mentioned three types of MNs in three types of aquatic organisms (i.e., algae, zooplankton, and fish) was evaluated. Finally, the uptake kinetic model was developed for *Daphnia magna*.

3.2 Algae

For the uptake of MNs into algae, the MNs need to cross two barriers (i.e., cell wall and plasma membrane). Cell walls in algae usually consist of cellulose and semi-permeable, allowing the passage of small molecules. The diameters of these passage pores were in the range of 5–20 nm (Zemke-White *et al.*, 2000) and might change during reproduction, with the newly synthesized cell wall being more permeable (Wessels, 1993; Ovečka *et al.*, 2005). Therefore, smaller MNs (<20 nm) can easily pass the cell walls and there are still some possibilities for the uptake of bigger MNs during reproduction. Once across the cell walls, endocytosis was considered as a major uptake route for incorporating MNs into the cells (Rejman *et al.*, 2004; Clift *et al.*, 2008; Lu *et al.*, 2009; He *et al.*, 2010). A size limitation exists for different endocytosis processes (e.g., phagocytosis and pinocytosis) (Conner and Schmid, 2003; Hirota and Terada, 2012). Beside size, shape, surface charge, surface functional groups, and hydrophilicity of the MNs as well as experimental conditions also affected the uptake process for cells (Kettler *et al.*, 2014). However, a quantitative uptake study of MNs by algae is still lacking, especially for the above-mentioned three types of MNs. As for the uptake of ionic metals (e.g., zinc and silver), Miao and Wang (2004) found that the uptake of zinc (concentration range: 1.2–200 nmol/L) by coastal diatom (*Thalassiosira pseudonana*) exhibited a Michaelis-Mention relationship, with the maximum uptake rate of 26.3 μg/(g·h). A similar relationship was also found in the uptake of zinc by green algae *Chlorella vulgaris* and *Chlorella salina* (Ting *et al.*, 1989; Garnham *et al.*, 1992).

As for ionic silver, a continuous uptake was observed (within 60 min) in two green algae *Pseudokirchneriella subcapitata* and *Chlorella pyrenoidosa*, with the average internalization rates of 1.009×10^{-6} μg/(cell·min) and 7.37×10^{-7} μg/(cell·min), respectively (Lee *et al.*, 2004). A non-linear uptake kinetic model in *Chlamydomonas reinhardtii* was developed by Piccapietra *et al.* (2012), with uptake rate constants for wild and mutant (no cell wall) of 2.5×10^{-5}–6.9×10^{-5} ml/(cell·min) and 1.94×10^{-4}–3.66×10^{-4} ml/(cell·min), respectively, whereas the uptake of Ag-NPs cannot be simulated either by linear or nonlinear models. Studies on the uptake kinetics of ZnO-NPs or TiO_2-NPs in algae were scarce. For reference, the uptake kinetics of CdTe quantum dots (Cd concentration: 1.3–4.7 mg/L) in freshwater alga *Ochromonas danica* followed the first-order kinetics with the uptake rate constant of 2.15×10^{-9}–4.92×10^{-9} ml/(cell·min) (Wang *et al.*, 2013). However, due to the lack of uptake kinetic studies, a systematic comparison between ionic metals and corresponding MNs could not be carried out.

3.3 Zooplankton

Uptake of MNs by zooplanktons was considered closely related to their feeding strategies, especially for suspension filter-feeders (e.g., daphnids and copepods). MNs may appear similar as food particles when captured by the animals (Hou *et al.*, 2013). High bioconcentration factors (BCFs) of TiO_2-NPs ($10^{4.75}$–$10^{5.05}$ L/kg) and Ag-NPs ($10^{3.16}$–$10^{4.66}$ L/kg) have been found in *Daphnia magna*, with a visually choked gut (Zhu *et al.*, 2010; Zhao and Wang, 2010; 2012a). The capture efficiency increased with increasing particle size (Ward and Kach, 2009; Zhao and Wang, 2012b). However, as mentioned above, dissolution of MNs may proceed continuously during accumulation, and the uptake of dissolved ions may lead to an overestimated BCF of MNs. To quantify the contribution of dissolved ions during the uptake process, three types of MNs (i.e., ZnO-NPs, Ag-NPs, and TiO_2-NPs) with dissolution kinetic as well as biokinetic uptake parameters in *Daphnia magna* were gathered from previous studies. We then model their contribution using Modelmaker 4.0 (Cherwell Scientific Ltd., UK). Kinetic parameters (e.g., solubility, dissolution rate constant, and uptake rate constant) of both MNs and corresponding ions are listed in Table 2. For the modeling, the first-order dissolution kinetics (Noyes-Whitney equation) has been applied in describing the dissolution kinetics of ZnO-NPs and Ag-NPs (Figs. 2a and 2b), which was also proposed in several dissolution kinetic studies (Zhang *et al.*, 2011; David *et al.*, 2012; Piccapietra *et al.*, 2012), whereas the dissolution of TiO_2-NPs was considered negligible. The short-term (0.5–8 h) uptake of these MNs was found to be proportional to the ambient particle concentration, also exhibiting the first-order kinetics (Zhao and Wang, 2010; 2012a; Li and Wang, 2013). Therefore, a synthetic uptake

Table 2 Summary of dissolution (k) and uptake rate constants (k_u) of ZnO-NPs, Ag-NPs, and TiO_2-NPs and corresponding ions

Agent	Solubility (%)	k (h)	k_u in *Daphnia magna* (L/(kg·h))	Reference
Zn ion			0.094–0.359 (at 2 μg/L)*	Tan and Wang, 2008
ZnO-NPs	0.19–20	0.60–0.14 (20–71 nm)	7.02–7.2 (46–56 nm, at 0.5–2 mg/L)	David *et al.*, 2012; Li and Wang, 2013
Ag ion			0.26 (at 8–880 ng/L); 0.75–1.34 (at 0.7 μg/L)	Lam and Wang, 2006; Zhao and Wang, 2012b
Ag-NPs	0.005–38	0.01–0.55 (4.8–80 nm)	0.25–0.86 (20–100 nm, at 1–4 μg/L); 0.06 (40–50 nm, at 2–40 μg/L); 2.2 (40–50 nm, at 40–500 μg/L)	Liu and Hurt, 2010; Zhang *et al.*, 2011; Zhao and Wang, 2010; 2012b
TiO_2-NPs	0	0	5.75–23.6 (30–200 nm, at 0.1–10 mg/L)	(Unpublished data)

* The values in bracket indicate the particle size range and exposure concentration range

kinetic simulation was developed. Considering the variation of solubility, the dissolution, and the uptake rate constant, a generalized value was applied (Figs. 2c–2f). Dissolution (except TiO_2-NPs) and uptake of MNs (0.5 mg/L) are modeled. Specifically, uptake of Ag-NPs at low concentration (40 μg/L) and three types of TiO_2-NPs (50 nm) with different surface properties are also modeled for comparison.

Generally, the contribution of ionic metal to the total accumulated metal in *Daphnia magna* was not significant (Figs. 2c, 2d, and 2f). Although the uptake rate constant of silver ions was 16.6 folds of the Ag-NPs at a low concentration of Ag-NPs (Zhao and Wang, 2010), the contribution of ionic silver only accounted for 9.1% of the total accumulated silver (Fig. 2f). For ZnO-NPs with a fast dissolution (Fig. 2a), the contribution of zinc ions was also minor. For uptake at a high concentration (0.5 mg/L) of MNs, the uptake rate constants varied for different particles, but were generally in the same order and higher than the corresponding ions. Additionally, even for the same sized TiO_2-NPs (Fig. 2e), surface properties can significantly affect the uptake rate constants.

In this model, only moderate dissolution (e.g., 8% for Ag-NPs and 10% for ZnO-NPs) of MNs was considered. However, in some extreme cases, 27% of Ag-NPs was dissolved (Zhao and Wang, 2011), and 25.8% of accumulated silver was estimated to be due to the uptake of ionic Ag. Therefore, it is important to monitor the dissolution behavior of MNs during toxicity studies, especially at low exposure concentrations. Moreover, the impact of surface properties on uptake cannot be ignored when considering the toxicity of same chemical component MNs.

3.4 Fish

Gill and digestion tract were considered as the major uptake sites of waterborne MNs in fish (Handy *et al.*, 2008b). Similar to the uptake of ions, MNs firstly need to diffuse through the unstirred layer (USL) formed over the epithelial cells of the gill or gut (Handy and Eddy, 2004; Handy *et al.*, 2008b). Movement of MNs from the bulk water into the USL is controlled by the perikinetic forces (shear forces) at this interface (Handy *et al.*, 2008a). Several factors, such as the speed of the bulk water flow, particle size and shape, and the viscosity of the USL, can affect this process (Handy *et al.*, 2008a). After crossing the USL, MNs can bind with viscous mucus and may be more readily transported for positively charged MNs than for other substances (Smith *et al.*, 2007; Cho *et al.*, 2009). The subsequent uptake of MNs was through the endocytosis of the gill or gut epithelial cells. Detailed uptake mechanisms and subsequent distribution were reviewed by Handy *et al.* (2008b).

To quantitatively evaluate the uptake of MNs in fish, Wang and Wang (2014) investigated the uptake of Ag-NPs and $AgNO_3$ by Japanese medaka at different salinities (e.g., 1, 5, 15, and 30 psu). The uptake rate constants (k_u) of $AgNO_3$ (0.13–0.93 L/(kg·h), 47.6 μg/L) were generally higher than those of Ag-NPs (<0.27 L/(kg·h), 87 μg/L) across all the study salinities. Limited bioavailability of commonly used citrate coated Ag-NPs was observed at high salinities (30 psu), presumably due to the fast aggregation. Similar results were also found in another study (unpublished data), in which the uptake of $AgNO_3$ (2.1 L/(kg·h), 0.5–16 μg/L) by zebrafish was significantly higher than that of tannic acid coated Ag-NPs

(0.3 L/(kg·h), 100–500 μg/L) and citrate coated Ag-NPs (0.125 L/(kg·h), 100–500 μg/L). The uptake rate constants of $AgNO_3$ quantified in some other freshwater fish (e.g., rainbow trout, fathead minnows, Gulf toadfish, European eel, and midshipman) ranged from 0.979 to 32.38 L/(kg·h) (Bury *et al.*, 1999; Webb and Wood, 2000; Wood *et al.*, 2002; 2004). It appears that the bioavailability of ionic silver was higher than that of Ag-NPs in freshwater fish, but more kinetic studies are needed to verify this assumption.

For Zn, the k_u of zinc ions in freshwater rainbow trout (*Oncorhynchus mykiss*) was estimated to be 0.1–0.33 L/(kg·h) (Alsop and Wood, 1999; Barron and Albeke, 2000). In marine fish black sea bream (*Acanthopagrus schlegeli*) and mangrove snapper (*Lutjanus argentimaculatus*), the k_u was 0.229 and 0.417 L/(kg·h), respectively (Xu and Wang, 2002; Zhang and Wang, 2007). These values were nearly three orders of magnitude lower than that of daphnids. However, no data is available for the k_u of ZnO-NPs, neither is the TiO_2-NPs, thus there is a great need to quantify the uptake of these MNs in fish.

4 Toxicity of MNs in aquatic organisms

Generally, the toxicity of MNs in aquatic organisms was considered to result from released ions, reactive oxygen species (ROS), or MNs themselves. For specific MNs, the contribution of these components may also vary (Navarro *et al.*, 2008a; Matranga and Corsi, 2012). For MNs, such as ZnO-NPs and Ag-NPs, the toxicity generated from the dissolved ions is usually a major concern (Ma *et al.*, 2013; Reidy *et al.*, 2013), while the toxicity of TiO_2-NPs was mainly associated with their oxidative stress (Sharma, 2009). The explicit biochemical toxic mechanisms could be found in several reviews (Navarro *et al.*, 2008a; Sharma, 2009; Matranga and Corsi, 2012; Reidy *et al.*, 2013). We will focus on quantitative toxicity data (mostly acute toxicity) of these MNs as well as their corresponding ions. A subsequent interpretation of the observed toxicity is then given from the perspective of kinetic processes, including the physicochemical kinetics of MNs and toxicokinetics of aquatic organisms.

4.1 Quantitative evaluation of toxicity of MNs: ion or particle?

The available literature on the toxicity data of ZnO-NPs, Ag-NPs, and TiO_2-NPs and corresponding ions on three types of aquatic organisms (i.e., algae, zooplankton, and fish) are listed in Tables 3–5. In general, a great variability of MNs toxicity was observed in different aquatic organisms for different MNs, and the contribution of ions in the toxicity of MNs also varied.

4.1.1 Algae

For algae, the estimated 72-h or 96-h IC50/EC50 (IC50 is the 50% inhibitory concentration; EC50 is the 50% effective concentration; usually using growth inhibition as the endpoint) of ZnO-NPs ranged from 0.042 to 4.56 mg/L, and the corresponding value for ions was 0.042–3.48 mg/L (Table 3). Five of the literature reviewed investigated the 72-h or 96-h IC50/EC50 of bulk ZnO, with the values ranging from 0.037 to 3.57 mg/L. Almost 50% of the literature reviewed attributed the ZnO-NPs toxicity mainly to the dissolved zinc ions, whereas the other studies considered the impacts due to specific MNs. Considering the sensitivity of different species, freshwater green alga *Pseudokirchneriella subcapitata* was the most susceptive (72-h EC50: <0.05 mg/L) to both ZnO-NPs and zinc ions, while for marine algae, such as *Chaetoceros gracilis*, *Phaeodactylum tricornutum*, or *Chlorella* sp., several orders of higher magnitudes of concentration of Zn were needed to cause the 50% inhibition of growth (Franklin *et al.*, 2007; Aruoja *et al.*, 2009; Ji *et al.*, 2011; Peng *et al.*, 2011; Lee and An, 2013). Compared to ZnO-NPs, similar high toxicity of Ag-NPs was found in *Pseudokirchneriella subcapitata*, with the estimated 96-h EC50 to be 9.9–190 μg/L (Griffitt *et al.*, 2008; Kennedy *et al.*, 2010). However, higher concentration of Ag-NPs was needed, especially for marine species (up to 1 mg/L) to generate a toxic effect (Miao *et al.*, 2009; Oukarroum *et al.*, 2012). Partial contribution of silver ions to Ag-NPs toxicity to *Chlamydomonas reinhardtii* was found in the inhibition of photosynthesis (Navarro *et al.*, 2008b), whereas the role of silver ion has not been manifested in the other literature reviewed. For TiO_2-NPs, the estimated 72-h EC50 was at the mg/L

Table 3 Toxicity of ZnO-NPs, Ag-NPs, and TiO_2-NPs on algae

Algae	TEM size (nm)	Conc. (mg/L)	Duration (h)	Nanoparticle toxicity	Ion toxicity	Bulk material toxicity	Contribution of ion to nanotoxicity	Reference
ZnO-NPs: freshwater								
Pseudokirchneriella subcapitata	30	0.025–0.600	72	IC50: 49 μg/L	IC50: 69 μg/L	IC50: 63 μg/L	Mainly	Franklin *et al*., 2007
	50–70	0–0.5	72	EC50: 42 μg/L; NOEC*: 17 μg/L	EC50: 42 μg/L; NOEC: 5 μg/L	EC50: 37 μg/L; NOEC: 20 μg/L	Mainly	Aruoja *et al*., 2009
	<100	0–0.3	72	EC50: <0.05 mg/L; NOEC: <0.05 mg/L				Lee and An, 2013
Chlorella sp.	20	0–1000	144	IC50: 803 mg /L; NOEC: 5 mg/L	EC30: 2 mg/L; NOEC: 1 mg/L	IC50: 803 mg/L; NOEC: 50 mg/L	Partly	Ji *et al*., 2011
ZnO-NPs: marine								
Thalassiosia pseudonana	26.2		96	IC50: 4.56 mg/L	IC50: 3.48 mg/L	IC50: 6.65 mg/L	Mainly	Wong *et al*., 2010
	20		48	EC50: 25.5 μg/L	EC50: 76.5 μg/L		Mainly	Miao *et al*., 2010
	6.3, 15.7	0–62	72	IC90: 8 mg/L			Partly	Peng *et al*., 2011
Skeletonema costatum	26.2		96	IC50: 1.90 mg/L	IC50: 1.23 mg/L	IC50: 2.38 mg/L	Mainly	Wong *et al*., 2010
Chaetoceros gracilis	6.3, 15.7	0–62	72	IC94: 8 mg/L			Partly	Peng *et al*., 2011
Phaeodactylum tricornutum	6.3, 15.7	0–62	72	IC55: 8 mg/L			Partly	Peng *et al*., 2011
Dunaliella tertiolecta	900 (DLS)	0.1–10	96	EC50: 1.94 mg/L; NOEC: 0.08 mg/L	EC50: 0.65 mg/L; NOEC: 0.01 mg/L	EC50: 3.57 mg/L; NOEC: 0.80 mg/L	Partly	Manzo *et al*., 2013
Ag-NPs: freshwater								
Chlamydomonas reinhardtii	25		2–5	2-h EC50: 112 μg/L	2-h EC50: 19.6 μg/L		Partly	Navarro *et al*., 2008a
Chlorella vulgaris	60		24	EC33: 1 mg/L				Oukarroum *et al*., 2012
Pseudokirchneriella subcapitata	29–42		96	EC50: 9.9 μg/L				Kennedy *et al*., 2010
	26.8		96	IC50: 190 μg/L				Griffitt *et al*., 2008
Ag-NPs: marine								
Thalassiosira weissflogii	60–70		48	No significant toxic effects at 2.36 mg/L				Miao *et al*., 2009
Dunaliella tertiolect	60		24	EC44: 1 mg/L				Oukarroum *et al*., 2012
TiO_2-NPs: freshwater								
Chlorella sp.	5–10 Anatase	0–1000	144	EC30: 30 mg/L; IC70.5: 1000 mg/L				Ji *et al*., 2011
	50 Rutile	1000	144	No significant toxicity effects at 1000 mg/L		No significant toxicity effects at 1000 mg/L		Ji *et al*., 2011
Pseudokirchneriella subcapitata	25–70	0–100	72	EC50: 58.3 mg/L; NOEC: 0.984 mg/L		EC50: 35.9 mg/L; NOEC: 10.1 mg/L		Aruoja *et al*., 2009
	<100		72	IC25: >100 mg/L				Blaise *et al*., 2008
	<10	0–250	72	EC50: 241 mg/L				Hartmann *et al*., 2010
	140 (DLS)	0–100	72	EC50: 87 mg/L				Warheit *et al*., 2007
	21	0–10	72	EC50: 2.53 mg/L; NOEC: <0.05 mg/L				Lee and An, 2013
Chlamydomonas reinhardtii		0–100	192	EC50: >100mg/L		EC50: >100 mg/L		Gunawan *et al*., 2013
	21	0–100	120	IC20:100 mg/L				Wang *et al*., 2008
Desmodesmus subspicatus	25	0–50	72	EC50:44 mg/L				Hund-Rinke and Simon, 2006
TiO_2-NPs: marine								
Phaeodactylum tricornutum	15, 25, 32	0–100	72	EC50: 10.9–14.3 mg/L		EC50: 24–36 mg/L		Clément *et al*., 2013

* NOEC: no observed effect concentration

levels (2.53–58.3 mg/L) and in some cases the EC50 was outside of the studied concentration range (e.g., 0–100 mg/L) (Wang *et al.*, 2008; Gunawan *et al.*, 2013). Since the dissolution of TiO_2-NPs was less likely to proceed in studied mediums (Schmidt and Vogelsberger, 2006), the contribution of ions to the TiO_2-NPs toxicity was negligible. Some studies indicated that the toxicity was more likely due to the generated ROS (Sharma, 2009).

4.1.2 Zooplankton

Compared to algae, a relatively narrow range of 48- or 96-h LC50 (50% lethal concentration) of ZnO-NPs was estimated in several zooplanktons (e.g., *Tigriopus japonicas*, *Elasmopus rapax*, and *Daphnia magna*), ranging from 0.85 to 3.2 mg/L (Table 4). The 48- or 96-h LC50 of zinc ions was slightly lower (0.76–1.14 mg/L) and the 48- or 96-h LC50 of bulk ZnO was 0.37–1 mg/L. More than 50% of the literature reviewed attributed the toxicity of ZnO-NPs to zinc ions, whereas only a few indicated a particle specific toxicity (e.g., differentially gene expression in *Daphnia magna*) (Poynton *et al.*, 2011). The 48-h LC50 of Ag-NPs *Daphnia magna* was 0.0018–0.236 mg/L, which was two orders of magnitude lower than that of ZnO-NPs, and the corresponding 48-h LC50 for silver ions was 0.8–12.9 μg/L. Of all the eight literature reviewed, half of the studies attributed the Ag-NPs toxicity to dissolved silver ions and no mortality was observed even at 500 μg/L Ag-NPs by chelating the dissolved Ag^+ with cysteine (Zhao and Wang, 2012a). As for TiO_2-NPs, a high concentration of TiO_2-NPs (48-h or 72-h LC50: >1.3 mg/L) was needed to generate 50% mortality, and in some extreme cases no toxicity effect was observed on the *Daphnia magna*, even at 20 g/L TiO_2-NPs (Heinlaan *et al.*, 2008). Additionally, great variations of 48-h LC50 were found between different types of TiO_2-NPs, with the smaller size and anatase type more toxic (48-h LC50: 7.75–35.3 mg/L) (Zhu *et al.*, 2009a; Das *et al.*, 2013; Clément *et al.*, 2013).

4.1.3 Fish

Comparatively, fewer acute toxicity studies of these three MNs have been conducted on fish (Table 5, p.586). Toxic effects, such as mortality, retarded hatching, reduced larval body length, and tail malformation, had been observed in zebrafish embryos after exposure to 50 and 100 mg/L ZnO-NPs for 96 h, while zinc ion (2.152 mg/L) could also cause a retarded hatching as noted in the same study (Zhu *et al.*, 2009b; Bai *et al.*, 2010). The 96-h LC50 of ZnO-NPs and bulk ZnO to larval zebrafish was estimated to be 1.79 mg/L and 1.55 mg/L, respectively (Ward and Kramer, 2002; Zhu *et al*., 2008). However, for adult Japanese medaka (*Oryzias melastigma*), no significant change on superoxide dismutase (SOD) and metallothionein (MT) gene expression was detected after exposure to ZnO-NPs (4 or 40 mg/L) for 96 h, or conversely with the zinc ions (Wong *et al.*, 2010). Zinc ions were considered to be partially responsible for the observed ZnO-NPs toxicity based on the limited literature reviewed, but more studies are needed to draw a generalized conclusion.

For Ag-NPs, significant accumulation of Ag-NPs in gill and subsequent alteration in gene expression were observed in adult zebrafish after exposure to 50 μg/L Ag-NPs for 48 h (Griffitt *et al.*, 2009). The 48-h LC50 of Ag-NPs and silver ions for adult zebrafish were estimated to be 7.07 and 0.022 mg/L, respectively (Griffitt *et al.*, 2008). Minor contribution of silver ions was found in the toxicity study of Ag-NPs (5–500 μg/L) to zebrafish embryos (Asharani *et al.*, 2008), but again more studies are needed to draw a generalized conclusion. Size-dependent toxicity was observed for TiO_2-NPs to zebrafish embryos, with the 120-h LC50 of TiO_2-NPs (5.7 and 12.4 nm) to be 23.4 and 610 mg/L, respectively, but the value was over 1000 mg/L for 15 nm sized TiO_2-NPs (Kim *et al.*, 2014). Another study showed no impact of TiO_2-NPs (size<20 nm) and bulk TiO_2 on the hatching of zebrafish embryos, even when the concentration reached as high as 500 mg/L (Zhu *et al*., 2008). Similar low toxicity of TiO_2-NPs was also observed in *Oncorhynchus mykiss*, *Piaractus mesopotamicus*, and *Carassius auratus* with no or minor impact at an exposure concentration of 100 mg/L (Warheit *et al.*, 2007; Ates *et al.*, 2013; Clemente *et al.*, 2013). However, direct intraperitoneal injection of 1 ml (1 mg/L) significantly increased the antioxidant genes (e.g., CAT, GST, and SOD) expression within 6 h (Varela-Valencia *et al.*, 2014), indicating the low bioavailability of waterborne TiO_2-NPs as a possible reason for observed low toxicity.

Table 4 Toxicity of ZnO-NPs, Ag-NPs, and TiO_2-NPs on zooplankton

Zooplankton	TEM size (nm)	Duration (h)	Nanoparticle toxicity	Ion toxicity	Bulk material toxicity	Contribution of ion to nanotoxicity	Reference
ZnO-NPs: freshwater							
Thamnocephalus platyurus	50–70	24	LC50: 0.18 mg/L; NOEC: 0.03mg/L			Mainly	Heinlaan *et al*., 2008
Daphnia magna	30	24, 48, 504	48-h LC 50: 1.02 mg/L; 24-h EC50: 1.41 mg/L (feeding inhibition); 21-d EC50: 0.26 mg/L (reproduction)	48-h LC50: 0.76 mg/L; 24-h EC50: 0.8 mg/L (feeding inhibition); 21-d EC50: 0.25–0.4 mg/L (reproduction)	48-h LC50: 0.89 mg/L; 24-h EC50: 1.89 mg/L (feeding inhibition); 21-d EC50: 0.3 mg/L (reproduction)	Partly	Lopes *et al*., 2014
	80–100	24, 48, 504	48-h LC50: 1.10 mg/L; 24-h EC50: 2.0 mg/L (feeding inhibition); 21-d EC50: 0.36 mg/L (reproduction)			Mainly	Lopes *et al*., 2014
	27.2	24	Alteration of gene expression in cyto-skeletal transport, cellular respiration, and reproduction			Partly	Poynton *et al*., 2011
	50–70	48	LC50: 3.2 mg/L; NOEC: 0.5 mg/L			Mainly	Heinlaan *et al*., 2008
		48	EC50: 1 mg/L		EC10: 0.60mg/L; EC50: 1 mg/L		Wiench *et al*., 2009
	20	48	EC50: 0.622 mg/L; LC50: 1.511 mg/L				Zhu *et al*., 2009a
ZnO-NPs: marine							
Tigriopus japonicus	26.2	96	LC50: 0.85 mg/L	LC50: 1.14 mg/L	LC50: 0.43 mg/L	Mainly	Wong *et al*., 2010
Elasmopus rapax	26.2	96	LC50: 1.19 mg/L	LC50: 0.80 mg/L	LC50: 0.37 mg/L	Mainly	Wong *et al*., 2010
Ag-NPs: freshwater							
Daphnia pulex	26.7	48	LC50: 40 μg/L	LC50: 8 μg/L		Partly	Griffitt *et al*., 2008
Daphnia magna	29–41	48	LC50: 1.8–97 μg/L	LC50: 0.7 μg/L			Kennedy *et al*., 2010
	5–25	48	LC50: 2–4 μg/L	LC50: 2.3 μg/L		Mainly	Asghari *et al*., 2012
	20	48, 504	No mortality at 500 μg/L Ag-NPs after Ag^+ was complexed by cysteine (48 h)	48-h LC50: 2.51 μg/L 21-d LC50: 1.6 μg/L		Mainly	Zhao and Wang, 2011
	10–100	48	LC50: Ag-NPs-L: 28.7 μg/L; Ag-NPs-P: 2.0 μg/L; Ag-NPs-S*: 1.1 μg/L	LC50: 0.88 μg/L		Mainly	Zhao and Wang, 2012a
	12.5	48	LC50: 40.2–65.7 μg/L	LC50: 2.2–12.9 μg/L			Blinova *et al*., 2013
	8.4	48	LC50: 54–236.3 μg/L				Blinova *et al*., 2013
	1–10	48	LC50: 2.75 μg/L				Das *et al*., 2013
TiO_2-NPs: freshwater							
Daphnia magna	1–10	48	LC50: 7.75 mg/L				Das *et al*., 2013
	25–70	48	LC50: 20000 mg/L				Heinlaan *et al*., 2008
		48, 504	48-h EC50: >100 mg/L; 21-d NOEC: 3 mg/L; 21-d EC50: 26.6 mg/L				Wiench *et al*., 2009
	<20	48	EC50: 35.3 mg/L; LC50: 143.387 mg/L				Zhu *et al*., 2009a
	140 (DLS)	48	EC50: >100 mg/L				Warheit *et al*., 2007
	15, 32, 25	72	EC50: 1.30–3.44 mg/L		EC50: 94.71–250 mg/L		Clément *et al*., 2013
Thamnocephalus platyurus	25–70	24	LC50: >20 g/L; NOEC: >20 g/L				Heinlaan *et al*., 2008
Brachionus plicatilis	15, 32, 25	48	EC50: 5.37–267.3 mg/L		EC50: 5.37–107 mg/L		Clément *et al*., 2013

* L, P, and S represent lactate, polyvinylpyrrolidone, and sodium dodecylbenzene sulfonate, respectively

Table 5 Toxicity of ZnO-NPs, Ag-NPs, and TiO_2-NPs on freshwater fish

Fish	TEM size (nm)	Duration (h)	Nanoparticle toxicity	Ion toxicity	Bulk material toxicity	Contribution of ion to nanotoxicity	Reference
ZnO-NPs							
Oryzias melastigma	26.2	96	No significant change on SOD and MT RNA expression		Significant up-regulation of SOD and MT RNA expression	Mainly	Wong *et al*., 2010
Danio rerio (embryo)	30	96	Mortality of embryos: 50 and 100 mg/L; Retarded hatching: 1–25 mg/L; Reduced larval growth and tail malformation: 10–100 mg/L	Hatching time increased significantly (2.15 mg/L)		Partly	Bai *et al.*, 2010
Danio rerio (larvae)	20	96	96-h LC50: 1.79 mg/L; 84-h EC50: 2.06 mg/L (hatching)		96-h LC50: 1.55 mg/L; 84-h EC50: 2.06 mg/L (hatching)		Zhu *et al*., 2008
	20	96	Reduced hatching and induction of pericardial edema at 72 h (50 and 100 mg/L)			Partly	Zhu *et al*., 2009b
Ag-NPs							
Danio rerio	26.6	48	LC50: 7.07 mg/L (adult); LC50: 7.20 mg/L (juvenile)	LC50: 22 μg/L (adult); LC50: >10 mg/L (juvenile)		Minor	Griffitt *et al*., 2008
	26.6	48	Different gene expressions			Partly	Griffitt *et al*., 2009
Danio rerio (embryo)	5–20	72	Abnormal body axes, twisted notochord, slow blood flow, pericardial edema and cardiac arrhythmia (5–500 μg/L)	No significant defect in developing embryos (0.26–2.14 μg/L)		Minor	Asharani *et al*., 2008
TiO_2-NPs							
Danio rerio (embryo)	<20	96	No impact on hatching (500 mg/L)		No impact on hatching (500 mg/L)		Zhu *et al*., 2008
	5.7, 12.4, 15.0	120	LC50: 23.4 mg/L (5.7 nm); LC50: 610 mg/L (12.4 nm); LC50: >1000 mg/L (15 nm)				Kim *et al*., 2014
Danio rerio	20.5	48	Different gene expressions				Griffitt *et al.*, 2009
Oncorhynchus mykiss	140 (DLS)	96	EC50: >100 mg/L				Warheit *et al*., 2007
Oreochromis niloticus	7, 14, 21	24	Alteration of CAT, GST, and SOD gene expression (1.0 mg/L)				Varela-Valencia *et al.*, 2014
Carassius auratus	432 (DLS)	120	Inhibition the growth by 19.7% at 100 mg/L				Ates *et al.*, 2013
Piaractus mesopotamicus	543.9 (DLS)	96	No mortality at 100 mg/L				Clemente *et al.*, 2013

CAT: catalase; GST: glutathione S-transferase; DLS hydrodynamic size is used when a TEM size is not available

4.2 Interpreting the toxicity of MNs and ions: importance of kinetic process for algae, daphnia, and fish

To disengage the impact of ions in the MNs toxicity test, several approaches were applied. The most commonly used approach was to measure the dissolution of MNs during toxicity testing and compare those results with the parallel ion toxicity testing (Franklin *et al*., 2007; Asharani *et al*., 2008; Griffitt *et al*., 2008; Zhu *et al*., 2008; Aruoja *et al*., 2009; Bai *et al*., 2010; Wong *et al*., 2010; Ji *et al*., 2011; Lopes *et al*., 2014). Other approaches, such as adding chelates

(e.g., cysteine) to reduce the ion bioavailability or studying the toxicity of the filtrates, were also adopted (Zhao and Wang, 2012a; 2012b; Li and Wang, 2013). However, all of these approaches considered that the dissolved ions released from the MNs were constant during their exposure, which is often not necessarily the real case. We attempted to integrate the dissolution and uptake kinetics of MNs to define the role of ions during short-term exposure. Due to the lack of kinetic studies of the MNs uptake in algae, it was not possible to establish the integrated model for algae. Remarkably, the well-developed model in *Daphnia magna* indicated the minor uptake of ions during short-time exposure was mainly because of the high bioavailability of MNs.

Considering the toxicity of TiO_2-NPs to *Daphnia*, although a high bioaccumulation of TiO_2-NPs can be commonly observed (Hou *et al.*, 2013), their acute toxicity was comparatively low. For MNs (e.g., ZnO-NPs and Ag-NPs) with dissolution, although the contribution of dissolved ions to the total accumulated mass was negligible, their toxicity was extraordinary high, especially for silver ions. Therefore, when considering the toxicity of MNs, the dissolution of MNs as well as specific metal toxicity to certain organisms should not be ignored. Additionally, attention should be paid to the environmentally relevant low concentration of MNs exposure, at which the bioavailability may decrease and become even lower than the dissolved ions (Zhao and Wang, 2012b), leading to an elevated accumulation of ions instead of MNs. For fish, the sites of action of these MNs need to be taken into account when considering their toxicity. The gill and digestion tracts are generally considered to be the primary interaction sites (Handy *et al.*, 2008b). Although the gill was generally considered more sensitive than the gut (Wang, 2013), the permeability of gill cells to MNs appeared to be lower than ions, especially to the aggregated MNs (Handy *et al.*, 2008b). The low bioavailability of Ag-NPs to zebrafish may partly explain the relatively low toxicity of Ag-NPs compared to silver ions (Griffitt *et al.*, 2008). However, there is still a great need for a kinetic uptake study of different MNs to different aquatic organisms. Future studies of integrating toxicodynamics with toxicokinetics may better quantify the observed toxicity (Tan and Wang, 2012).

5 Conclusions

As a dynamic entity, several kinetic processes (e.g., dissolution, aggregation, stabilization, and sedimentation) were involved in the transformation of MNs in the aquatic environment. Different uptake kinetics were involved for the uptake of MNs and dissolved ions. Therefore, unlike the traditional metal exposure, a kinetic point of view should be addressed in MN toxicological studies. Different dissolution rates were observed in ZnO-NPs, Ag-NPs, and TiO_2-NPs, and their solubilities were also variable in different toxicological studies, leading to a variable and increasing waterborne ion concentration during exposure. On the other hand, varied bioavailability of these MNs and corresponding ions were also found in different aquatic organisms (e.g., algae, zooplankton, and fish). Specifically, the MNs appeared to be more bioavailable to daphnids, rendering a minor contribution of ions during short-term exposure. In addition, partial toxicity of ZnO-NPs and Ag-NPs was attributed to the dissolved ions, while the toxicity of TiO_2-NPs was mainly due to the generated ROS. Overall, this review emphasizes the importance of integrating physicochemical kinetics and uptake kinetics in evaluating the bioavailability and toxicity of both MNs and dissolved ions. Despite the need for more uptake kinetic studies, the overall understanding of the physiochemical kinetics of MNs (e.g., aggregation, sedimentation, and dissolution) in aquatic environment is also needed to have a systematic evaluation of the bioavailability of MNs to aquatic organisms. In future toxicological studies, more chronic exposures at the environmentally relevant low concentrations are desirable. Transformation of MNs within biological tissues should also be investigated to better understand their toxic mechanisms.

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中文概要：

本文题目：从金属纳米颗粒的理化性质以及生物吸收动力学角度探究金属纳米颗粒对水生生物的毒性

Significance of physicochemical and uptake kinetics in controlling the toxicity of metallic nanomaterials to aquatic organisms

研究目的：研究金属纳米颗粒在进入水体后的一系列动力学过程对金属纳米颗粒的生物可利用性和毒性可能产生的影响。

研究方法：针对在毒性测试中金属纳米颗粒的解析现象，选取三种常见的金属纳米颗粒（纳米氧化锌、纳米银和纳米二氧化钛），总结了它们在毒性测试中的解析动力学、溶解性以及毒性。同时，综合水生生物对金属纳米颗粒以及离子的吸收动力学，利用动态模型进行模拟，阐述解离的离子在生物对金属纳米颗粒吸收中的贡献。

重要结论：在评价金属纳米颗粒和解析离子对水生生物的生物利用度和毒性的测试过程中，需要综合考虑金属纳米颗粒的理化性质以及生物吸收动力学过程。

关键词组：金属纳米颗粒；解析；动力学；水生生物；毒性