Study of environmental risks incurred by leakage of lithium cells to the food chain in a freshwater ecosystem
Dai Zhao-xia, Yin Ying, Guo Hong-yan and Wang Shi-he

ABSTRACT

Water flea (Daphnia magna) and fish (Carassius auratus) at trophic level were used for comprehensive evaluation of environmental risks incurred by manufactured nanomaterial (nNi(OH)₂) as leaked from lithium cells to the food chain in freshwater ecosystem. The 48, 72 and 96 h acute toxicities of water suspensions of nNi(OH)₂ to the flea and the fish were tested, using the immobilization and the mortality as toxicological endpoints. The results showed that the water flea was more highly sensitive to nNi(OH)₂ than the fish. Then, the fish were exposed to 1.0 mg/L nNi(OH)₂ for 6, 12, 24, 36, 48, 60, 72 and 96 h, and the relationship between the concentrations in the water and the fish were described by a bioconcentration factor (BCF). After calculation, lgBCF is 1.61. Reactive oxygen species (ROS) generation was studied after fish were exposed to 1.0 mg/L water suspensions of nNi(OH)₂ for 24 h. As proved by electron paramagnetic resonance, nNi(OH)₂ may induce the generation of hydroxyl radical in the fish, and nNi(OH)₂ as concentrated in the fish may incur redox reaction and produce redox metabolic intermediates. As one of the important toxic mechanisms of nNi(OH)₂ to the fish, the oxidative stress mechanism requires further study.

Key words | Carassius auratus, Daphnia magna, nNi(OH)₂

INTRODUCTION

Owing to its unique physical and chemical properties, nanomaterial has wide application, which may bring forward enormous benefits to human beings. As a classical example, the lithium cell manufactured with nNi(OH)₂ has been widely applied to mobile phones, laptops, portable cameras and electrically propelled vehicles in recent years (Kelly et al. 2001; Thomas & Sayre 2005). However, improper utilization of this nanomaterial by human beings may result in irrecoverable losses. Nanomaterial may also become a nanopollutant in various ways (wastewater treatment, atmospheric sedimentation, landfill wastes, surface runoff, etc.) Furthermore, nanomaterials containing nNi(OH)₂ can be released to the environment intentionally or accidentally over time during their use and life cycle, and their residues therefore can lead to direct and indirect exposure to the environment and human beings (Colvin 2003). In fact, it has already been aware of the toxicity of nanomaterials and particles has already been shown (Dreher 2004; Wang & Hu 2005; Moore 2006). For example, Adams reported ecological toxicity of suspensions of three photosensitive nanomaterials (TiO₂, SiO₂ and ZnO) to Bacillus subtilis and Escherichia coli. As discovered, SiO₂, TiO₂ and ZnO water suspensions had different toxicities, among which ZnO suspension had the highest toxicity (Adams et al. 2006). Currently, literatures focusing on acute toxicity of nNi(OH)₂ to aquatic organisms are few. Study of acute toxicity of nNi(OH)₂ to aquatic organisms can provide insufficient data on toxicity of compounds to aquatic organisms. With regard to toxicological effect as produced by pollutants inside organisms, accumulation inside the organisms is more important than that in the external water body or inside the sediments. As pollutants result in toxicological effect only inside organisms in most cases, absorption rate and concentration level of such substances inside organisms are most important.

Nanomaterials are easily concentrated in aquatic organisms. Many nanomaterials, especially the artificial ones, have higher durability and can be easily absorbed by large particles. For instance, numerous nanoparticles (NPs) may come into the food chain once absorbed by primary consumers such as the water flea (Borm et al. 2006). Water flea and fish constitute important parts of the freshwater food chain. The flea is at a low tropic level of the food chain, and chronic toxicity...
has been indicated by data on evaluation of its instantaneous toxicity response (Giesy et al. 1988). Fish is at a high trophic level of the food chain. With regard to toxicity of NPs to aquatic organisms, most studies focus on spectacular fish without edible value (zebra fish, Japanese medaka and swordtail). Therefore, study of the toxicological impact of nanomaterials on Carassius auratus has very important significance. On account of such a situation, this study aims to evaluate risk incurred by nNi(OH)\(_2\) as leaked from lithium cells to the food chain in freshwater ecosystems with the help of two organisms at trophic level (Daphnia magna and Carassius auratus). Some reports show that the concentration of nanomaterial was low, about 1–100 ng/L, in the water environment, but in a study of stable water suspensions, concentrations were at least 1 and 35 mg/L (Li et al. 2011). This study used 1.0 mg/L \(n\)Ni(OH)\(_2\) water suspensions as the laboratory experimental condition.

**MATERIALS AND METHODS**

**Chemicals**

\(\alpha\)-Phenyl-N-tert-butynitrone (PBN, purity >98\%) and dimethylsulfoxide (DMSO, purity >99.9\%) were purchased from Sigma-Aldrich Company (USA); other reagents, of analytical grades, were obtained domestically.

**Preparation of nanoparticle suspensions**

Powders of \(n\)Ni(OH)\(_2\) (280 nm, 40 mm advertised particle size) were obtained from the School of Chemistry & Chemical Engineering, Nanjing University. The powders were added to Milli-Q water in accordance with the Guideline 202 of Organisation for Economic Cooperation and Development (OECD 2004). The suspensions were shaken vigorously at room temperature to obtain a final concentration of 100 mg/L.

**Fish collection and treatment**

Fish were purchased from a local aquatic breeding base, with average body length and weight about 9.78 cm and 30.8 g, respectively. Before exposure, the fish were acclimated to the water, which had been dechlorinated by active carbon for 10 days, with the total mortality of the fish near zero. Air flow was constant and artificial dry foods were provided once a day. The fish were exposed to each toxicant concentration in the series 0.0 (control), 0.6, 1.0, 1.5, 2.0 and 3.0 mg/L \(n\)Ni(OH)\(_2\). Mortality was observed at time intervals of 48, 72 and 96 h. During the experiment, water hydrogen ion concentration (pH value) was 7.37 ± 0.5 and water temperature was 22 ± 1°C.

In order to obtain the relationship between the concentrations of \(n\)Ni(OH)\(_2\) in water and fish, the fish were randomly selected and put into a glass aquarium with 45 L test solution (1.0 mg/L) for static exposure without renewal of the test solutions, designated as group 1. In this group, two fish samples and two water samples for parallel samples were taken from the aquarium at 0, 6, 12, 24, 36, 48, 60, 72, and 96 h. As soon as fish were taken out from the water, their weights and lengths were measured. Then their livers were subdivided into portions for chemical and biochemical analyses after being rinsed in physiological salt water. The livers of every parallel sample were combined and immediately stored at −20°C until analysis. On the other hand, water samples were taken from another aquarium as group 2, containing the same test solution but without fish with the same time interval, and analyzed.

**Water flea culture and acute toxicity assay**

*Daphnia magna* were cultured in ASM-1 medium (Gorham et al. 1964) at 21 ± 1°C with continuous 2,500 lux light (Sylvania Gro Lux, 15 W) and aeration (0.2 μm filtered air). *Daphnia magna* (from Nanjing Institute of Geography & Limnology, Chinese Academy of Sciences) was cultured at 21 ± 1°C, with a 16:8 light/dark photoperiod in uncontaminated natural water (total hardness of 200 mg CaCO\(_3\)/L and a conductivity of 600 mS/cm, pH 7.8–8.2).

Static toxicity assays were used to determine the acute toxicity of \(n\)Ni(OH)\(_2\) to the invertebrates. Test containers used for toxicity assays with *Daphnia magna* consisted of six 4-L plexi-glass aquaria that contained 20 invertebrates. Neonates from the third to sixth brood not older than 24 h were used in all tests. Twenty invertebrates were exposed to each toxicant concentration in the series 0.0 (control), 0.6, 1.0, 1.5, 2.0 and 3.0 mg/L \(n\)Ni(OH)\(_2\). Each concentration was replicated. Immobility was observed at time intervals of 48, 72 and 96 h. The values of pH and dissolved oxygen were monitored during the whole process of the experiment. The test solution was not replaced during the assay. However, it was ensured that there were enough treatments to get a good description of the whole dose–response curve. Experiments were run at light:dark regime of 16 h:8 h and 23 ± 2°C for 48, 72 and 96 h. No foods were added during these periods. The aim of the test was to determine the median effective concentration, EC\(_{50}\), which is the
concentration at which 50% of the exposed organisms are affected by measured effects (Newman 1995).

**Analysis of nNi(OH)₂**

HNO₃ and H₂O₂ (30%) were used to decompose the fish, and the analysis of nNi(OH)₂ was done by the zero adjustment method with air-acetylene using an atomic absorption spectrometer at the wavelength of 232 nm to measure the absorbency. The recovery of analysis in the whole fish sample was 85.9 ± 2.49% (n = 6). And in the water sample it was 98.2 ± 0.51% (n = 6).

**PBN adduct extraction and electron paramagnetic resonance analysis**

The fish that were taken out after 24 h of exposure were dissected. For each fish, the liver was removed and rinsed in ice-cold physiological salt water. The whole operation was conducted in an incubation system with a continuous purging of N₂. Then, the fish livers were immediately weighed, and a fraction (0.1 g) was picked out and homogenized quickly in 1.0 mL 50 mM PBN (dissolved in DMSO) using a Teflon pestle in a Potter homogenizer. Then 0.1 mL supernatants were transferred to a capillary tube with a diameter of 0.9 mm, and frozen by liquid nitrogen for electron paramagnetic resonance (EPR) analysis. The EPR spectra were recorded with a Bruker EMX 10/12 X-band spectrometer at room temperature. The operation conditions were: center field, 3,470 G; scan range, 200 G; modulation frequency, 100 kHz; modulation amplitude, 0.5 G; receiver gain, 5 × 104 scans; five times; microwave power, 20 mW.

**Data analysis**

At 48, 72 and 96 h, EC₅₀ and associated 95% confidence intervals, were calculated using the EPA Computer Probit Analysis program (Version 1.5). Statistical analyses were carried out using standard ANOVA techniques followed by Tukey’s honestly significant difference test (SPSS Ver. 14.0). Differences were statistically significant when p < 0.05.

**RESULTS AND DISCUSSION**

**Study of acute toxicological effect**

In the natural ecosystem, flea and fish constitute an important aquatic food chain. nNi(OH)₂ as leaked from lithium cells into water is inevitably harmful to such aquatic organisms. For flea and fish, acute toxicity of nNi(OH)₂ is listed in Table 1. From this table, it can be seen that the flea has a higher sensitivity to nNi(OH)₂ than the fish. Therefore, it is applicable to use Daphnia magna for ecological monitoring of wastewater discharge in order to control production waste drainage of this compound and protect the aquatic ecosystem.

It also can be seen that toxicity of compounds to flea and fish will become more and more obvious by the increasing of the exposure time (Table 1). On the one hand, it indicates that toxicity of nNi(OH)₂ to organisms is probably determined by its accumulation in the organism body. On the other hand, nNi(OH)₂ may generate intermediates of higher activity inside organisms through metabolism. Nevertheless, this needs to be proved by further study.

nNi(OH)₂ at concentrations of 0.6 mg/L produced a slight immobilization (6.67% of the control) and 0% mortality in Daphnia magna. This was not significantly different from the control (p < 0.05). A concentration of 3.0 mg/L, however, caused 100% immobilization and mortality. The 48 h EC₅₀ of immobilization for nNi(OH)₂ was calculated as 2.2616 mg/L.

Few studies have focused on environmental effects related to the disposal or accidental spillage of nNi(OH)₂. The results of this study demonstrate that nNi(OH)₂ had different effects on the immobilization and mortality of Daphnia magna, although a similar dose–response pattern was observed. Daphnia magna was more susceptible to nNi(OH)₂ than nTiO₂; for example, Daphnia magna resulted in 73.33% immobilization at 1.0 mg/L, but nTiO₂ caused only 9% immobilization at the same concentration. The 48 h EC₅₀ of immobilization for nTiO₂ and nZnO water suspensions were 35.306 and 0.622 mg/L, respectively (Zhu et al. 2009). The nZnO suspension appears to be the

**Table 1** | Acute toxicity of nNi(OH)₂ to Daphnia magna and Carassius auratus

<table>
<thead>
<tr>
<th>Toxication time (h)</th>
<th>Daphnia magna EC₅₀ (mg/L)</th>
<th>Confidence interval 95%</th>
<th>Carassius auratus EC₅₀ (mg/L)</th>
<th>Confidence interval 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>1.4634</td>
<td>1.0758–1.7061</td>
<td>7.8164</td>
<td>5.2016–11.7456</td>
</tr>
<tr>
<td>96</td>
<td>0.4540</td>
<td>0.3266–0.6310</td>
<td>5.8374</td>
<td>4.2665–7.9867</td>
</tr>
</tbody>
</table>
most toxic to *Daphnia magna*, while *n*TiO₂ was the least toxic NP tested. The effects of such NPs on the immobilization of *Daphnia magna* were in the order *n*ZnO > *n*Ni(OH)₂ > *n*TiO₂.

Presently, there is no uniform standard for evaluation of biological toxicity of chemicals in China. This thesis aims to define the level of hazards presented by pollutants to aquatic organisms based on the hazard grading standard in the latest criterion for evaluation of hazards of chemical as issued by the Ministry of Environmental Protection (1989) and the toxicity grading standard of the Institute of Hydrobiology, Chinese Academy of Sciences (Ministry of Environmental Protection 1990). According to the experiment on acute toxicity of *n*Ni(OH)₂ to flea and fish, such toxicity belongs to the medium category, which may bring forth certain hazards. Therefore, it is necessary to enhance its monitoring and evaluation.

**Dynamic variation of *n*Ni(OH)₂ in water and fish**

The concentrations of *n*Ni(OH)₂ in water for group 1 and 2 at the different exposure durations are indicated in Figure 1. The results show that the concentrations of *n*Ni(OH)₂ in water for the group 1 decreased rapidly in the first 24 h, and then maintained equilibrium. In contrast, the concentrations of *n*Ni(OH)₂ in water for the group 2 decreased slightly. In this study, the removals of *n*Ni(OH)₂ in water over a 96 h period for group 1 and 2 were approximately equal to 92 and 18%, respectively. Figure 2 illustrates the concentrations of *n*Ni(OH)₂ in fish at different exposure times. The concentrations of *n*Ni(OH)₂ in fish rapidly increased shortly after the start of the exposure and reached a maximum level approximately at 24 h. It decreased after 24 h and maintained stabilization after 48 h.

![Figure 1](https://iwaponline.com/wst/article-pdf/67/7/1599/440833/1599.pdf)  
*Figure 1* | The concentration of *n*Ni(OH)₂ in water for groups during 96 h of exposure.

The relationship between the concentrations in water and fish can be described by BCF: BCF = Cᵢ/Cₑ, where BCF is the bioconcentration factor, Cᵢ (mg/g wet wt) is the equilibrium concentrations of *n*Ni(OH)₂ in fish, and Cₑ (mg/mL) is the equilibrium concentrations of *n*Ni(OH)₂ in water. After calculation, the lgBCF value is 1.61.

The removal of 18% *n*Ni(OH)₂ in water for the group 2 over time may be attributed to photodecomposition and evaporation. The additional losses (74%) may be due to the factors such as uptake, or degradation by fish. To differentiate the possibilities of *n*Ni(OH)₂ being (a) absorbed and stored in fish, and (b) degraded by fish, residual concentrations were measured in fish. The concentrations of *n*Ni(OH)₂ in fish showed that the amount of *n*Ni(OH)₂ accumulation by fish accounted for 16.4% of the total loss of *n*Ni(OH)₂ in water. Thus, it can be concluded that up to 57.6% of the *n*Ni(OH)₂ decrease in water was due to metabolism by fish. The result is consistent with the low lgBCF (=1.61). Hence, we can conclude that *n*Ni(OH)₂ bioaccumulation in fish is significantly affected by the fast metabolic clearance. These results are consistent with previous studies; for example, some organic chemicals such as organochlorine pesticides and polychlorinated biphenyls can be greatly accumulated by organisms (Zhou *et al.* 1999), and other organic chemicals like polycyclic aromatic hydrocarbons can not only be bioaccumulated but also be metabolized (Baussant *et al.* 2001).

**Production of reactive oxygen species by induction of *n*Ni(OH)₂ for 24 h**

Twenty-four hours after the exposure, reactive oxygen species (ROS) production marked by EPR spectra signals of samples...
were detected (Figure 3). This indicated that the radicals generated in vivo in fish liver were trapped by PBN and formed the PBN-radical adduct, which was detected by EPR spectrometry. As compared to the controls, \( n\text{Ni(OH)}_2 \) significantly induced ·OH production marked by the intensity of the prominent spectra (Figure 3). The production of ·OH was induced by 194.4% after exposure to 1.0 mg/L \( n\text{Ni(OH)}_2 \) for 24 h \( (p < 0.01) \).

The control group showed a rather weak ·OH signal of EPR spectra. This could be explained by ·OH formation during normal cellular functions. The environmental pollutants may accelerate the rate of ROS generation.

According to a study of the impact of \( n\text{ZnO} \) aqueous suspension on survival rate of zebra fish, toxicological action of \( n\text{ZnO} \) aqueous suspension on embryo of zebra fish is not simply caused by the zinc ion (Zhuet al. 2007). There might be contact-dependent toxicological action. In other words, \( n\text{ZnO} \) in the \( n\text{ZnO} \) aqueous suspension can reach the surface of the egg membrane of the fertilized ovum of zebra fish or the epidermis of larval fish in certain ways (such as absorption or sediment) to the extent of incurring damages to cell membrane and even hazards to the zebra fish. When \( n\text{ZnO} \) aqueous suspension is exposed, death of larval fish and the embryo of zebra fish is associated with barriers to the blood stream. This indicates that a profound study of the physiological and biochemical reactions inside organisms under the stress of NPs is of significance, to further reveal the biological toxicological molecule of artificial NPs and the cell action mechanism. According to existing literature, \( n\text{ZnO} \) mainly has three biological toxicological mechanisms. (1) NPs may induce cells to produce the activated oxygen free radical. The activated oxygen free radical has high oxidation, which may damage cell fat, protein and DNA macro-molecule substance. It may also produce lipid superoxide and protein hydroxyl, cause damage to DNA, and eventually result in apoptosis (Wesselkamper et al. 2005). (2) \( n\text{ZnO} \) particles in the suspension may release \( \text{Zn}^{2+} \) to the extent of incurring toxicity (Wesselkamper et al. 2005). (3) NPs may also cause dysfunction to the cell membrane, and disturb normal life activity of the cell (Baveye & Laba 2008). The hydroxy radical belongs to free radicals in the organism body, which have the most serious hazard to the cell and tissue. It may attack protein, unsaturated fatty acid and DNA macro-molecule to the extent of inducing over-oxidation to protein and lipid as well as oxidizing damages to DNA. According to the mode of increase in free radical accompanied by extension of exposure time in this experiment, \( n\text{Ni(OH)}_2 \) may result in oxidizing damages to the liver of Carassius auratus.

**CONCLUSIONS**

The present results demonstrate the hepatic antioxidant homeostasis of fish exposed to \( n\text{Ni(OH)}_2 \), which provides an effective tool for environmental biomonitoring and some useful data for guaranteeing environmental safety. In summary, \( n\text{Ni(OH)}_2 \) can be accumulated and metabolized in fish. The bioaccumulation of \( n\text{Ni(OH)}_2 \) in fish is low with \( \text{lgBCF} \) equal to 1.61. \( n\text{Ni(OH)}_2 \) bioaccumulation in fish is significantly affected by a fast metabolic clearance in fish. The possible poisoning mechanism of fish can be explained as an oxidative stress mechanism: while \( n\text{Ni(OH)}_2 \) transforms in the fish liver, oxidation and reduction recycle are formed and much reactive oxygen is generated, causing fish liver to suffer from oxidative stress. Furthermore, it was proved that fish were able to metabolize \( n\text{Ni(OH)}_2 \) and redox-active metabolites were produced. These results fill a gap in the literature on the fate of \( n\text{Ni(OH)}_2 \) and the antioxidant responses in fish exposed to \( n\text{Ni(OH)}_2 \). However, many factors have been ignored in the experiment; the fish living environment of the study is very different from the actual one with various conditions. Therefore, as one of the toxication mechanisms of \( n\text{Ni(OH)}_2 \) to Carassius auratus, the oxidation stress mechanism requires further study under the different antioxidant parameters such as the activities of catalase, the superoxide dismutase, the glutathione S-transferase, and the contents of reduced glutathione. Then their protective functions and the detailed relations among these correlated parameters will be built.

**ACKNOWLEDGEMENTS**

This research was supported by State Key Laboratory of Pollution Control and Resources Reuse of China
(No. PCRRF06001) and also by Practice and Innovation Training Program for Undergraduates in Universities as funded by Jiangsu provincial department of education (No. 2010768).

REFERENCES


Moore, M. N. 2006 Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ. Int. 32 (8), 967–976.


First received 17 June 2012; accepted in revised form 26 November 2012