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Accumulation and elimination of iron oxide nanomaterials in zebrafish (*Danio rerio*) upon chronic aqueous exposure

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ABSTRACT

A 52-day continuous semi-static waterborne exposure (test media renewed daily) regimen was employed to investigate the accumulation and elimination profiles of two iron oxide nanomaterials (nano-Fe_2O_3 and nano-Fe_3O_4) in zebrafish (*Danio rerio*). Adult zebrafish were exposed to nanomaterial suspensions with initial concentrations of 4.0 and 10.0 mg/L for 28 days and then were moved to clean water for 24 days to perform the elimination experiment. Fe content was measured in fish body and feces to provide data on accumulation and elimination of the two iron oxide nanomaterials in zebrafish. The experiment revealed that: (1) high accumulation of nano-Fe_2O_3 and nano-Fe_3O_4 were found in zebrafish, with maximum Fe contents, respectively, of 1.32 and 1.25 mg/g for 4.0 mg/L treatment groups and 1.15 and 0.90 mg/g for 10.0 mg/L treatment groups; (2) accumulated nanoparticles in zebrafish can be eliminated efficiently (the decrease of body burden of Fe conforms to a first-order decay equation) when fish were moved to nanoparticle-free water, and the elimination rates ranged from 86% to 100% by 24 days post-exposure; and (3) according to analysis of Fe content in fish excrement in the elimination phase, iron oxide nanomaterials may be adsorbed via the gastrointestinal tract, and stored for more than 12 days.

Introduction

Nanotechnology and the production of nanoparticles (NPs) are exponentially growing, but research into the toxicological impact of nanoparticles on human health and the environment is still in its infancy (Elsaesser and Howard, 2012). Iron oxide nanomaterials are widely used in remediation and biomedical applications such as drug delivery (Gupta and Gupta, 2005), cell labeling (Chen et al., 2011), and magnetic resonance imaging (Puppi et al., 2011; Rümenapp et al., 2012). The research on health risks and ecological impacts of iron oxide nanomaterials is very limited because iron oxide nanomaterials are generally regarded as non- or low-toxic materials (Karlsson et al., 2008, 2009; Soenen and De Cuyper, 2010). However, recent studies have revealed that iron oxide nanomaterials pose a potential health risk. Superparamagnetic iron oxide nanoparticles show cytotoxicity (Mahmoudi et al., 2010). Zhu et al. (2009a, 2011) also found that ferric oxide nanoparticles had potential lung and systemic cumulative toxicity in rats, and intravascular iron oxide nanoparticles may induce human endothelial inflammation and dysfunction. Moreover, iron oxide nanomaterials could serve as significant carriers of toxic chemicals (Guan et al., 2008; Tang et al., 2009) and increase exposures to adsorbed pollutants.

In the last decade, a wealth of acute toxicity studies focused on the short-term effects of engineering nanomaterials (ENMs). Works that have been done on chronic endpoints, such as biological and ecological accumulation, only began to increase in the last 3–4 years (Hou et al., 2013). As a result of the extensive

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application of metal oxide nanomaterials, the aquatic environment as a sink of most contaminants is particularly at risk of exposure to ENMs. Recently, studies on the accumulation of metal oxide nanomaterials in aquatic organisms have been carried out. TiO_2 was a relatively common research nanomaterial for aquatic organisms including daphnia, carp and zebrafish (Zhang et al., 2007; Sun et al., 2009; Zhu et al., 2010a, 2010b). Meanwhile, the exposure time is crucial for the toxic effects of metal oxide nanomaterials on aquatic organisms. Compared with 24 hr exposure, the accumulated content and mortality of brine shrimp (*Artemia salina*) exposed to nano-TiO_2 and nano-ZnO increased remarkably at 96 hr (Ates et al., 2013a,2013b). In addition, the cubic and octahedral Cu_2O micro/nanocrystals both showed Cu accumulation toward *Daphnia magna* after a 3-day exposure (Fan et al., 2012). Montes et al. (2012) demonstrated the accumulation of Ce and Zn in soft tissues of a marine suspension-feeder, *Mytilus galloprovincialis*, exposed to nano-CeO_2 and nano-ZnO for 96 hr. Similarly, Gomes et al. (2012) found the accumulation and toxicity of nano-CuO in digestive gland of *M. galloprovincialis* exposed to nano-CuO for 15 days. Given that the exposure of aquatic organisms to NPs is probably long-term, the uptake and accumulation of NPs by aquatic organisms could impact the overall effects of nanomaterials and background toxins. Therefore, the chronic exposure of aquatic organisms to metal oxide nanomaterials as well as their clearance processes are worthy of examination.

As a common model organism, zebrafish (*Danio rerio*) is widely used in ecological toxicity research for ENMs. The objective of this study was to investigate the accumulation and elimination behavior of iron oxide nanomaterials (nano-Fe_2O_3 and nano-Fe_3O_4) in zebrafish under chronic exposure according to the total Fe content in fish body. Meanwhile, the elimination routes of NPs were examined through the total Fe content in their excrement. Little data have been found on NPs in the excrement of aquatic organisms. Therefore, this aspect is important for understanding the process of accumulation and elimination of iron oxide nanomaterials in aquatic vertebrates under long-term exposure.

1. Materials and methods

1.1. Materials

Experimental subjects were commercially available adult blue zebrafish (mixed gender) with 29.7 ± 1.8 mm body length and 0.29 ± 0.09 g mass. Prior to nanomaterial exposure, zebrafish were acclimated to the experimental condition. They were maintained for more than two weeks in a semiautomatic circulating water system with tap water, and fed by dry food from the market once per day. The total mortality of zebrafish was less than 3% during cultivation. The powders of nano-Fe_2O_3 and nano-Fe_3O_4 were uncoated at >99% purity. The size distributions were measured by nanoparticle size and zeta potential analyzer (Zetasizer Nano ZS90, Malvern, UK).

1.2. Experimental design

Semi-static experiments (designed according to OECD Guideline 305, 2011) including a 28-day uptake period and a 24-day elimination period were performed to measure the profile of accumulation and elimination in zebrafish exposed to nano-Fe_2O_3 and nano-Fe_3O_4. The results of preliminary experiments showed that visible precipitation occurred within a few hours for suspensions >10.0 mg/L and that operating errors in preparation were bigger for suspensions <4.0 mg/L, so the concentrations of the experimental suspensions were set to 4.0 and 10.0 mg/L. Four NP treatment groups were expressed as Fe_2O_3-4.0, Fe_2O_3-10.0, Fe_3O_4-4.0, and Fe_3O_4-10.0, respectively. The zebrafish (initially 55 individuals per exposure) were exposed to NP suspensions for 28 days, and then were removed to clean water (tap water pre-aerated for 24 hr) until day 52. Samples were taking with increasing intervals to optimally capture the accumulation and excretion profiles: days 0, 2, 5, 9, 14, 19, 24, 28, 30, 34, 40, 46, and 52. At each time point, four zebrafish were randomly sampled, pooled, and the total Fe amounts measured. On days 30, 34, 40, 46, and 52, fish excrement was also sampled and total Fe content measured. In addition, a control group (NP-free tap water) was set up, and four fish were sampled on days 0, 28 and 52 to determine the background values of total Fe content in the fish body and excrement. The exposure solution was refreshed every day with a new suspension of NPs to maintain the exposure at relatively consistent level at accumulation phase, and clean water renewal was also performed daily during the elimination phase. During the whole test period, in order to simulate real environmental conditions where food was available, zebrafish were allowed to be fed daily with dry food (Fe concentration of about 2 mg/g) after test medium changes, and the supplied diet was based on the amount of zebrafish contained in test tank (~2 mg dry food per fish). Sampling and feeding were conducted just before and just after test medium renewal, respectively. Furthermore, the mortality and behavior immobilization were also recorded. The experiments were performed at room temperature (23 ± 2°C) with a natural light–dark cycle.

1.3. Preparation and measurement of NP suspensions

Nano-Fe_2O_3 and nano-Fe_3O_4 powders were dispersed in glass beakers containing 5 L of tap water (pre-aerated for 24 hr, pH 7.7 and dissolved oxygen at 8.54 ± 0.18 mg/L) to a concentration of 4.0 and 10.0 mg/L. No dispersing agent was added to the suspension of nanomaterials in order to avoid any confounding toxicity. The test medium was sonicated (250 W, 40 kHz, 24°C) in a water bath using ultrasonic cleaners (KQ-250DE, Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, Jiangsu Province, China) for 10 min and then hand-stirred for 1 min by three times. The suspensions were generated immediately prior to use in each experiment.

The 25 mL of NP suspension was sampled from the middle part of the test tank into 50 mL glass beakers and evaporated. Dried iron oxide NPs adsorbed onto beakers were decomposed into Fe ions by heating with HNO_3–H_2O_2 (6:1, V/V) until the pellets were completely dissolved into a colorless solution. After cooling, the solutions were moved to 25 mL volumetric flasks. Released total Fe content was determined using an inductively coupled plasma mass spectrometer (ICP-MS, ELAN DRC-e, PerkinElmer SCIEX, Waltham, Massachusetts, USA). The tank NP concentration was indicated by measured total Fe content.

1.4. Quantification of NPs in fish tissue and excrement

At each sampling time point during the experiment, four zebrafish from each exposure were rinsed with cool physiological