Trophic transfer and biomagnification of fullerenol nanoparticles in an aquatic food chain†

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Understanding the trophic transfer and biomagnification potential of nanomaterials in aquatic food chains is crucial for assessing the environmental risks of such materials. To analyse the fate of fullerenols in an aquatic food chain, we quantitatively investigated the bioaccumulation, tissue-specific distribution, depuration, trophic transfer and biomagnification potential of 13C-labelled fullerenols in a three-level aquatic food chain. The fullerenol nanoparticles accumulated in Scenedesmus obliquus through water exposure, and the latter were ingested by Daphnia magna before being transferred to Danio rerio. The tissues of D. rerio were ranked from highest to lowest fullerenol concentration as follows: intestine > liver > muscle > gills > brain. The biomagnification factor (BMF) value of fullerenols from S. obliquus to D. magna was 3.20, while the calculated fitted BMF from D. magna to D. rerio was less than 1 (BMFf = 0.54).

Thus, fullerenols were significantly biomagnified from the first to the second trophic level but not from the second to the third trophic level. Although fullerenols eventually enter fish tissues via the food chain, a lack of biomagnification greatly reduce the potential risk to high-trophic-level aquatic organisms. These results will be useful for environmental and ecological risk assessments of carbon nanomaterials.

Environmental significance

The unique properties of manufactured nanomaterials that have wide applications in numerous fields also result in concerns surrounding their environmental impact and ecological risks. Ecotoxicological studies have suggested that the biomagnification of any pollutant is the most dangerous factor in terms of human health and the environment. To address a gap in knowledge in carbon nanomaterials in three-level aquatic food chains and assess their environmental risk, we quantitatively and systematically study the trophic transfer and biomagnification potential of fullerenols, one of the mostly used carbon nanomaterials, in a three-level aquatic food chain. This work is significant for not only aquatic environmental and ecological risk assessments but also future research on the environmental behaviour and fate of carbon nanomaterials.

Introduction

With the increasing use of nanomaterials in commercial applications,1 nanomaterials inevitably enter the aquatic environment, and can negatively affect aquatic organisms and ultimately human health via trophic transfer through the food chain. Toxicity, bioaccumulation and food chain transfer are crucial aspects of the environment risk assessment of nanomaterials. There are reports that specific nanomaterials, such as fullerences, carbon nanotubes, ZnO, TiO2, CeO2, and quantum dots (QDs), are toxic to some aquatic organisms such as fish, Daphnia, and algae.2–7 The bioaccumulation of nanomaterials in invertebrates and fish species was also found.8 For example, fullerences, the first carbon nanomaterial discovered, were shown to accumulate in Daphnia magna after water phase exposure.7,9,10 The trophic transfer of nanomaterials has been detected in high trophic levels of aquatic food chains, such as the transfer of QDs from algae to Ceridaphnia dubia11 and the transfer of QDs or TiO2 from Daphnia magna to Danio rerio.12,13 Moreover, trophic transfer studies on fullerences have shown that their concentrations in D. magna were five times greater after dietary exposure than those after water exposure.14,15 This indicates that the environmental risk posed by nanomaterials to organisms is higher from trophic transfer than from water exposure. The bioconcentration factor (BCF)
and the biomagnification factor (BMF) are crucial considerations in the assessment of the environmental risks posed by nanomaterials in aqueous media or food chains. To date, only the biomagnification of TiO$_2$ nanoparticles has been observed in an aquatic food chain from *Scenedesmus obliquus* to *D. magna*. However, the biomagnification of nanoparticles has not been rigorously examined in aquatic food chains. Thus, the trophic transfer and biomagnification potential of a given nanomaterial must be understood to evaluate the environmental risk in aquatic systems.

Currently, most studies on the trophic transfer of nanomaterials in aquatic environments have focused on two-level food chains. However, a productive and stable aquatic ecosystem contains a food web consisting of phytoplankton (primary producers), invertebrates (secondary consumers) and vertebrates (top consumers), all of which are extremely important for maintaining aquatic ecological equilibrium. Compared with research on two-level food chains, research on three-level food chains, especially those including vertebrates, provides a more thorough understanding on the multistage trophic transfer and biomagnification of nanoparticles for assessing the ecological risks posed by nanoparticles. To the best of our knowledge, very few studies have analysed the trophic transfer of nanomaterials in three-level aquatic food chains. Such analysis will help us to understand the environmental and ecological risks of nanomaterials. Moreover, through complete food chain transmission, nanomaterials can eventually be distributed/accumulated in high-trophic-level organisms such as fish. Understanding the biodistribution of nanomaterials in organs and tissues is essential to elucidate the mechanisms of nanomaterial effects and potential toxicity, as well as to assess the ecological risks of biological absorption of nanomaterials at different trophic levels in the ecosystem.

Fullerene nanomaterials, a class of water-soluble polyhydroxylated carbon nanomaterials derived from fullerenes, are increasingly used in medicine and other industries due to their extensive biological activities. Stable isotope ($^{13}$C) labelling of the carbon skeleton is a well-established approach to quantify carbon nanomaterials in biological samples by analysing the $^{13}$C/$^{12}$C ratio by isotope ratio mass spectrometry (IRMS). This technique has the advantages of overcoming the high background levels of carbon in the environment or in organisms and avoiding the drawbacks of radioactive labelling, such as detachment, radioactive waste generation, and the requirement for specific experimental approval. Moreover, $^{13}$C-labelling of the carbon skeleton does not damage the stability or intrinsic structure of carbon nanomaterials enabling them to be traced in the ecosystem.

In this study, we selected *S. obliquus* (phytoplankton), *D. magna* (Cladocera), and *D. rerio* (fish) as model organisms to systematically investigate the trophic transfer, biomagnification, and biodistribution/depuration of $^{13}$C-skeleton-labelled fullerene nanoparticles in a three-level food chain. Through IRMS, we precisely determined the concentrations of fullerene nanoparticles in these organisms for different sampling times. We then systematically analysed and calculated the BMFs in the three-level food chain. The transfer and biomagnification in higher organisms were verified from the perspective of tissue distribution and transfer. Our data clarify the fate of fullerene nanomaterials in this ecosystem, advancing our understanding of the ecological risks of carbon nanomaterials.

**Materials and methods**

**Aqueous phase exposure of *S. obliquus* to $^{13}$C-labelled fullerensols**

*S. obliquus* was cultured in 2 L conical flasks in BG11 medium for 13 d until the exponential growth phase was reached, and then used in the aqueous exposure experiment. *S. obliquus* (1 x $10^6$ cells per ml) was exposed to the exposure solution with 1 mg L$^{-1}$ fullerol for 36 h under continuous gentle aeration. During exposure, the conical flask was shaken once every 12 h. Three replicates were prepared for the exposure and control groups. At intervals of 2, 4, 6, 8, 10, 12, 24, and 36 h, 20 mL algal suspension was collected for the quantification of fullerols in algae. To separate algal cells from the exposure solution, each sample was centrifuged in 50 mL centrifuge tubes, followed by three successive clean-up steps consisting of washing with phosphate buffered saline and centrifugation (5000 rpm, 10 min, 4 °C). A total of 93.3% of the fullerols was removed from the algal suspension (Fig. S1†). Moreover, the ratio of the fullerol concentration in algae after cleaning to that before cleaning gradually decreased with the increase in the number of centrifugation and clean-up steps (Table S4†). These results suggested that it was feasible to separate algae from the suspension solution by centrifugal cleaning. The pellets of algal cells were stored at −20 °C. For isotope analysis, algal samples were freeze-dried in a freeze dryer (Boyikang, Beijing, China) and weighed (Mettler Toledo microbalance, XP6, Switzerland, precision 1% mg) into tin capsules to determine the $^{13}$C-labelled fullerol concentrations. According to the steady-state uptake time confirmed above, *S. obliquus* was exposed to $^{13}$C-labelled fullerols for 24 h. This was followed by three resuspension and centrifugation steps to prepare the algae for *D. magna*. Both exposed and unexposed algae were stored at 4 °C until use in the dietary exposure experiment of *D. magna*.

**Exposure of *D. magna* to $^{13}$C-labelled fullerols by feeding with *S. obliquus***

The aim of the dietary exposure experiment was to confirm the time to reach steady state (i.e., the correct exposure time) and the content of $^{13}$C-labelled fullerols in the diet (i.e., initial nominal dietary fullerol content) for subsequent experiments. After 1 d without feeding, seven-day-old *D. magna* was transferred to clean artificial freshwater (KCl, 1.2 mg L$^{-1}$; CaCl$_2$·2H$_2$O, 58.6 mg L$^{-1}$; NaHCO$_3$, 13.0 mg L$^{-1}$; MgSO$_4$·2H$_2$O, 24.5 mg L$^{-1}$) for 2 h to allow for gut purging and acclimatization. These experiments consisted of three
replicates and a control group. Each replicate consisted of a glass beaker containing 150 organisms fed with 13C-labeled fullerenol-containing S. obliquus at a concentration of 1.0 \times 10^9 cells per L. This amount ensured that sufficient food was available. In the control beakers, D. magna were fed with unexposed S. obliquus at the same concentration. Five D. magna from each glass beaker were sampled after 2, 4, 6, 8, 10, 12, 24, 36, and 48 h of exposure. The sampled D. magna were rinsed five times with ultrapure water to remove any S. obliquus attached to their carapaces. We then determined the fullerenols in the trophic transfer from exposure and control groups were evenly allocated to eight glass experimental tanks, respectively. Each specimen in each group was set up in triplicate. D. rerio specimens in each beaker were fed with either exposed or unexposed D. magna for an uptake period of 28 d and then fed with unexposed D. magna for a depuration period of 15 d based on preliminary experiments and the related literature. The average feed weight for each fish per day was 0.01 g wet weight of D. magna (approximately 17 D. magna per D. rerio). There was no residual food observed throughout the experiment. Eight D. rerio individuals were selected as samples from each group 24 h after feeding on days 2, 7, 14, 21, 28, 31, 33, 37, and 43 of exposure, respectively. Of the eight D. rerio specimens selected, five were used for tissue sample collection, and three for whole D. rerio analysis. Several tissue samples, including the liver, intestine, gills, brain, and muscle, were dissected from D. rerio. All samples were freeze-dried and weighed into tin capsules for carbon isotope analysis to determine 13C-labelled fullerenol concentrations. At the same time, the concentration of fullerenols in the freshwater after exposure was detected, which was below the detection limit. Thus the steady-state concentration (6.93 ± 0.56 mg per g dw) of fullerenols in D. magna was the nominal concentration of fullerenols in the trophic transfer from D. magna to D. rerio.

Results and discussion

Characterization of 13C-labelled fullerenol nanoparticles

For aquatic exposure, 13C-labelled fullerenols (13C-fullerenol) were added to artificial freshwater at a final concentration of 1.0 mg L⁻¹. The fullerenol nanoparticle solution was homogeneous and stable. The size distribution and zeta potential of these 13C-fullerenol nanoparticles were determined by dynamic light scattering (DLS) to be 174.6 ± 22.0 nm (n = 3) and –58.5 ± 2.3 mV (n = 3), respectively (Fig. 1A and B). Analysis by transmission electron microscopy (TEM; Fig. 1C) showed that fullerenol nanoparticles in artificial freshwater agglomerated into spherical structures, with diameters ranging from approximately 30–100 nm. Additional details of the precise structure of 13C-fullerenol nanoparticles are available in our previous reports. Briefly, fullerenol nanoparticles were added to 13C-labelled fullerenol concentration in D. magna by IRSM (Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany). At the same time, the concentration of fullerenols was the same as those used for the acclimated culture conditions. D. rerio from exposure and control groups were evenly allocated to eight glass experimental tanks, respectively. Each group was set up in triplicate. D. rerio specimens in each beaker were fed with either exposed or unexposed D. magna for an uptake period of 28 d and then fed with unexposed D. magna for a depuration period of 15 d based on preliminary experiments and the related literature. The average feed weight for each fish per day was 0.01 g wet weight of D. magna (approximately 17 D. magna per D. rerio). There was no residual food observed throughout the experiment. Eight D. rerio individuals were selected as samples from each group 24 h after feeding on days 2, 7, 14, 21, 28, 31, 33, 37, and 43 of exposure, respectively. Of the eight D. rerio specimens selected, five were used for tissue sample collection, and three for whole D. rerio analysis. Several tissue samples, including the liver, intestine, gills, brain, and muscle, were dissected from D. rerio. All samples were freeze-dried and weighed into tin capsules for carbon isotope analysis to determine 13C-labelled fullerenol concentrations. At the same time, the concentration of fullerenols in the freshwater after exposure was detected, which was below the detection limit. Thus the steady-state concentration (6.93 ± 0.56 mg per g dw) of fullerenols in D. magna was the nominal concentration of fullerenols in the trophic transfer from D. magna to D. rerio.

Bioconcentration and bioaccumulation of 13C-labelled fullerenol nanoparticles in S. obliquus and D. magna

S. obliquus is a primary producer in the food chain and plays an important role in the equilibrium of aquatic ecosystems. Carbon nanoparticles can be taken up by S. obliquus after entering an aquatic ecosystem, leading to photosynthetic toxicity. Moreover, nanoparticles accumulated in prey can also be transferred and potentially cause secondary toxic effects on predators. D. magna connects the microbial loop with the multicellular food web, and can accumulate large amounts of nanoparticles in the body, which may be due to its unique uptake system of filter nanoparticles. Thus, nanoparticles accumulated in D. magna may be transferred to higher consumers through dietary exposure. Because D. magna is an essential component of a major potential nanoparticle exposure pathway for aquatic organisms at high trophic levels, it is often selected as a model species in standard toxicological studies and ecological risk assessments of nanoparticles. Consequently, we selected S. obliquus and D. magna as the primary and secondary organisms in the three-level trophic food chain.

We first exposed S. obliquus to fullerenols in water for 36 h, during which time the fullerenol concentration was monitored by IRMS. The concentration of fullerenols in S. obliquus increased rapidly (Fig. 2A) during the first 12 h but then remained nearly constant until the end of the incubation period. The maximum body-burden of fullerenols in S. obliquus was calculated to be 2.17 ± 0.02 mg per g dry weight (using ESI† eqn (1)) and did not change significantly between 12 and 36 h (p > 0.05). The BCF was 2170 L per kg dry weight under this experimental condition according to eqn (4) (ESI†). After incubation, the pre-exposed S. obliquus was analysed by TEM and collected as food for D. magna. As shown in Fig. 3, compared with the status of algal cells in the control group, dark dots with diameters between 18 and 70 nm were present in the algal cells of the exposure group. Moreover, in order to eliminate the interference of dye, no
dye was used in preparing the ultrathin TEM section of the samples. In this premise, the morphology of these black dots in *S. obliquus* under TEM was the same as that of fullerenol nanoparticles, demonstrating that *S. obliquus* had absorbed fullerenol nanoparticles.

In the dietary exposure experiment, the collected *S. obliquus* was fed to *D. magna*. No mortality was observed in any *D. magna* group over the course of the experiment. The breeding period lasted 48 h, during which time the concentration of $^{13}$C-fullerenols in *D. magna* was measured by IRMS for different sampling times. As shown in Fig. 2B, the fullerenol concentration in *D. magna* increased rapidly during the first 12 h and then increased slowly as it moved towards steady state from 12 h to 24 h. Steady state was reached at approximately 24 h, after which time there was no significant variation in the cumulative fullerenol concentration ($p > 0.05$) for the three sample times (i.e., 24, 36, and 48 h) as determined by one-way analysis of variance (ANOVA). After breeding, exposed *D. magna* was collected for subsequent experiments. The body burden of $^{13}$C-fullerenols in *D. magna* was calculated to be $6.93 \pm 0.56$ mg per g dry weight.

**Bioaccumulation and depuration of $^{13}$C-labelled fullerenol nanoparticles in *D. rerio* through dietary exposure**

As high-trophic-level organisms in aquatic ecosystems, fish play an important role in the association between the invertebrate food web and the vertebrate food web. Although some studies have reported on the accumulation of nanoparticles in fish, few studies have documented the transfer of nanoparticles from primary producers to higher consumers. Hydrophilic fullerenols are very soluble (water
solubility $> 100$ g L$^{-1}$) and stable in solution for at least 9 months.\textsuperscript{40} To investigate the potential trophic transfer of fullerenols to high-trophic-level organisms, we selected \textit{D. rerio} as the third-level organism.

Our experiment consisted of a 28 d uptake period and a 15 d depuration period. No mortality was observed in any \textit{D. rerio} group throughout the entire experiment. During the uptake period, \textit{D. rerio} were fed with \textit{D. magna} containing $6.93 \pm 0.56$ mg per g dry weight fullerenols. After the uptake period, the exposed \textit{D. rerio} were transferred to freshwater for depuration. As shown in Fig. 4, the body burden of fullerenols continued to increase during the 28 d uptake period, reaching $3.70 \pm 0.27$ mg per g dry weight fullerenols. During the depuration period, the body burden decreased gradually to reach $0.56 \pm 0.02$ mg per g dry weight at the end of day 15. Similar uptake and depuration patterns have been reported for $^{14}$C-labelled graphene taken up by \textit{D. rerio} through dietary exposure,\textsuperscript{41} amphiphilic polymer coated CdSe/ZnS QDs taken up by \textit{D. rerio} through a two-level food chain,\textsuperscript{13} and carboxylated CdSe/ZnS QDs taken up by \textit{Brachionus calyciflorus} through a three-level food chain.\textsuperscript{18}

Because the bioaccumulation and depuration kinetics of fullerenols were comparable to those of traditional contaminants and engineered nanomaterials such as QDs,\textsuperscript{18,42,43} we selected a first-order kinetic model to simulate uptake and depuration (dashed curve in Fig. 4). The determination coefficient ($R^2$) of 0.80 indicated that the model was appropriate for this experiment. According to the curve-fitting kinetics, the fitted plateau concentration for a whole fish was 3.73 mg per g dry weight, and the fitted concentration on day 28 was 3.16 mg per g dry weight (Table S1†). The uptake rate constant of fullerenol ($k_u$) was calculated

![Fig. 3 TEM images of \textit{S. obliquus}: (A–C) exposure group; (D) control group. The red circles mark the fullerenol nanoparticles in \textit{S. obliquus}.](image)

![Fig. 4 Uptake and depuration of $^{13}$C-fullerenol nanoparticles in \textit{D. rerio} after dietary exposure. The data are presented as the mean ± SD ($n = 3$).](image)
to be 0.036 d⁻¹, which was lower than the depuration rate constant (k_e) of fullerenol (0.067 d⁻¹; Table S1†). Thus, the depuration rate was greater than the uptake rate. According to our calculation, the time required to purify half of the accumulated concentration on the 28th day (3.16 mg per g dry weight) is 10.35 days; the time required to purify 90% of the accumulated concentration is about 35 days.

Currently, only a few studies have analysed the uptake kinetics of carbon nanomaterials by fish, making it difficult to compare and analyse the results obtained in our present study. For example, compared with that of multi-walled carbon nanotubes,44 the fullerenol in this study showed different bioaccumulation behaviours. Our results show that fullerenols continued to accumulate in D. rerio throughout the uptake period and had not reached steady state after 28 d of exposure. In contrast, multi-walled carbon nanotubes rapidly associated with D. rerio over time and reached steady state within 24 h. The k_u and k_e of multi-walled carbon nanotubes in D. rerio during water exposure were calculated to be 9.64 L kg⁻¹ h⁻¹ and 0.14 h⁻¹, respectively,44 which are much higher than the rates calculated for fullerenols in our study. These differences may be due to differences in the shape, biocompatibility, and/or exposure pathway of the nanoparticles.

**Tissue-specific accumulation and depuration of ¹³C-labelled fullerenol nanoparticles in D. rerio through dietary exposure**

Nanoparticles will inevitably affect various tissues in organisms, following their organismal uptake. To clarify the fate, biological effects and further confirm the capacity for trophic transfer of fullerenols in high-trophic-level organisms, we analysed the bioaccumulation and depuration of fullerenol nanoparticles in different tissues of D. rerio. Fullerenols were detected in the liver, intestine, gills, muscle, and brain of D. rerio after they were fed with D. magna containing 6.93 ± 0.56 mg per g dry weight fullerenols. The accumulation and depuration of fullerenols in different tissues of D. rerio are shown in Fig. 5. After the 28 d uptake and 15 d depuration periods, fullerenol nanoparticles were mainly distributed in the intestine and liver. During the 28 d uptake period, the fullerenol concentrations in the tissues increased over time with maximum accumulation on day 28 or day 21 (Fig. 5). Over the 28 d of exposure, the concentrations of fullerenols in the liver, intestine, and muscle tended to increase. Although the concentrations of fullerenols in the gills and brain tended to increase from day 0 to day 21 of exposure, the concentration slightly decreased in the gills and remained almost unchanged in the brain from day 21 to day 28 of exposure. The uptake of fullerenols showed a general time-dependent pattern in all tissues. At the end of the uptake period, fullerenols were distributed differently among these tissues. The highest fullerenol concentration was in the intestine (8.48 ± 0.57 mg per g dry weight), where the level was almost 10 times that in the brain (0.86 ± 0.09 mg per g dry weight). The D. rerio tissues could be ranked, from highest to lowest fullerenol concentration, as follows: intestine > liver > muscle > gills > brain. These results indicated that the liver and intestine were the major target organs of fullerenols.

In dietary exposure, the gastrointestinal tract was the initial target tissue, which was consistent with the large amount of fullerenols accumulated in the intestine. Therefore, fullerenols likely passed through intestinal membranes and enter the circulatory system where they are subsequently distributed to other tissues. A study on medaka (Oryzias latipes) found that nanoparticles could pass through intestinal membranes and enter the blood circulatory system, from which they were further distributed to other parts of the body.45 In the present study, the liver had the second highest fullerenol concentration after that in the intestine. This was largely due to the detoxifying function of the liver, which was the first organ exposed to the material that had entered circulation. The fullerenols that accumulated in the liver likely passed through the liver–bile–intestine metabolic system. The distribution of ¹³C-labelled fullerenols in D. rerio was similar to that of ¹⁴C-labelled fullerenes in rats,46 in that nanoparticles were distributed throughout the bodies of both species via blood flow. Some fullerenols penetrated the blood–brain barrier to reach the brain, although the fullerenol concentration in the brain was low. Examination of the distribution of nanoparticles in O. latipes showed that a particle

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**Fig. 5** Uptake and depuration of fullerenol nanoparticles in the tissues of D. rerio after dietary exposure. The data are presented as the mean ± SD (n = 5).
with a size of 39.4 nm can penetrate the blood–brain barrier after 7 d of exposure. In addition, a previous exposure study indicated that fullerene nanoparticles could reach the brain of largemouth bass through olfactory neurons, which was also a possibility pathway for carbon nanoparticles to enter the brain of fish. During the depuration period, the fullerenol concentrations in different tissues of D. rerio decreased gradually over time. After the 15 d depuration period, some residual fullerenols remained in tissues.

Analysing the tissue distribution of nanoparticles can help identify which tissues and organs are at greater risk of exposure and predict negative downstream effects. Redox-active, small nanoparticles can penetrate organs by causing inflammation, and then passing into the inflamed tissue to possibly cause damage to the liver, spleen, and other organs. Most studies on the distribution of nanomaterials in tissues have focused on water exposure. For instance, a study on Cyprinus carpio (carp) exposed to silver particles in water showed that more silver accumulated in the gills, gastrointestinal tract, and liver than in the brain, muscle, and blood. Similarly, the tissues of carp were ranked, from highest to lowest CuO NP concentration, as follows: liver > gills > muscle > intestine. The results of those investigations indicate that the tissue distribution of nanoparticles is closely related to the exposure pathway and the type of nanomaterial. However, in addition to the tissue distribution, the tissue-targeted absorption and potential tissue-specific toxicity of nanomaterials are important factors. The accumulation of nanoparticles in critical tissues such as the digestive system tissues, brain, and liver not only poses risks to the organism itself but also to higher organisms through the food chain transfer. Thus, the results of our study provide a theoretical basis for assessing the ecological risks of carbon nanoparticles.

Analysis of tissue dynamics simulation curves may explain the process of the absorption and metabolism of nanoparticles in tissues and identify possible target tissues. By plotting the amounts taken up by tissues and depurated from tissues at different time points, the kinetic parameters of absorption and metabolism in different tissues may be obtained by simulation analyses. As shown in Fig. 6 and Table S2, the uptake and depuration of fullerenol nanoparticles in tissues followed first-order kinetics. After 15 d of depuration, some fullerenols remained in tissues, and curves fit to plateaus of 6.70, 6.63, 5.20, 3.31 and 1.50 mg per g dry weight in the intestine, liver, muscle, gills, and brain, respectively. The $k_u$ and $k_e$ were calculated for the different tissues. The intestine, liver, gills, and muscle, and gill had higher uptake rates ($k_u$ values of 0.088, 0.066, 0.054, and 0.048 d$^{-1}$, respectively) but faster elimination rates ($k_e$ values of 0.091, 0.069, 0.113 and 0.064 d$^{-1}$, respectively) than other tissues. Thus, fullerenol had short half-lives in the intestine, liver, gills, and muscle (7.62, 10.05, 6.13, and 10.83 d, respectively). The depuration rates of fullerenols were faster than the uptake rates, indicating that fullerenols would not be retained in tissues, which once again confirmed that there was no risk of biomagnification in D. rerio. The lowest fullerenol concentration was in the brain (ANOVA post hoc Tukey test, $p < 0.05$), probably because of the very slow uptake rate (0.008 d$^{-1}$). The brain retained fullerenols for the longest time (62.23 d$^{-1}$). The observation that fullerenols were retained for long periods in some tissues, especially the brain, may raise concerns about chronic toxicity.

In the present study, we analysed the distribution and fitted first-order kinetics for fullerenol in each tissue, which allowed us to calculate tissue-specific uptake and depuration rates of the nanoparticles by taking advantage of $^{13}$C labelling. A previous toxicokinetics study in carp exposed to aquatic silver nanoparticles found that the muscle and brain had the highest rates of total silver uptake ($k_u$ values of 0.015 and 0.006 h$^{-1}$, respectively) and the fastest elimination rates ($k_e$ values of 0.082 and 0.021 h$^{-1}$, respectively), resulting in short half-lives and short retention times in those tissues. However, that study’s findings that the brain and muscle were the main sites of particle accumulation are inconsistent with our results. This discrepancy may be due to differences in the nanoparticle administration methods, nanomaterial characteristics, and/or model organisms. It is well known that nanomaterials are unique pollutants, and their uptake rates and adverse effects depend on the routes of exposure and internalization into cells and organisms. Based on our experimental data and the kinetic analyses above, we concluded that fullerenols could rapidly distribute into many tissues and were not completely eliminated through depuration. Thus, the trophic transfer of fullerenols through the food chain posed some risks to organisms at high trophic levels.

Transfer and biomagnification of $^{13}$C-labelled fullerenol nanoparticles through the S. obliquus-D. magna-D. rerio food chain

The transfer of nanomaterials in the ecosystem from low- to high-trophic-level organisms can pose risks to ecosystem services such as nutrient recycling, biogeochemical cycling, energy transformation, and carrying capacity. Ecotoxicological studies have suggested that the
biomagnification of any pollutant is the most dangerous factor in terms of human health and the environment.\textsuperscript{46} Thus, it is a challenging and critical necessity of scientific analysis to establish whether specific nanomaterials have the potential for biomagnification through different trophic levels. We calculated the transfer and biomagnification potential of fullerenol nanoparticles along the \textit{S. obliquus}–\textit{D. magna}–\textit{D. rerio} aquatic food chain (Fig. 7). \textit{S. obliquus} was exposed to fullerenols in water and then fed to \textit{D. magna}, and finally the fullerenol-containing \textit{D. magna} was fed to \textit{D. rerio}.

Based on our results on the bioaccumulation of fullerenol nanoparticles in three different organisms and the formulae described in our previous work,\textsuperscript{24} we examined the fullerenol concentrations \textit{in vivo} in \textit{S. obliquus}, \textit{D. magna} and \textit{D. rerio} in the potential steady-state equilibrium exposure period, and calculated the BMFs for two- or three-level food chains (Table 1). Our results showed that fullerenols were transferred across the three trophic levels from \textit{S. obliquus} to \textit{D. magna} to \textit{D. rerio}. Fullerenol nanoparticles accumulated in \textit{S. obliquus} through water exposure. At the bottom two levels (\textit{S. obliquus}–\textit{D. magna}) of the three-level food chain, the fullerenol concentration reached steady state in \textit{D. magna} after only 24 h of feeding, and the BMF value of fullerenol nanoparticles was 3.20. In the top two levels of the food chain (\textit{D. magna}–\textit{D. rerio}), more than 28 days of feeding were required for the fullerenol concentration to reach steady state in \textit{D. rerio}, and the value of the fitted plateau (BMF\(_f\)) was calculated to be 0.54. According to our calculations, it would take approximately 60 d to reach steady state in \textit{D. rerio} (to reach 99\% of the plateau concentration). The magnification of pollutants in a food web occurs when the BMF \(> 1\).\textsuperscript{49} Thus, the high BMF (3.20) of fullerenol nanoparticles meant that biomagnification was likely to occur in the \textit{S. obliquus}–\textit{D. magna} food chain.

The transfer and magnification of nanomaterials in aquatic ecosystems is very complex. Biomagnification of TiO\(_2\) nanoparticles was previously found in the same two-level food chain (\textit{S. obliquus}–\textit{D. magna}) with BMFs of 7.8–2.2.\textsuperscript{17} Gold nanoparticles and CdSe QDs have been shown to be biomagnified across a two-level food chain.\textsuperscript{20,51} However, BMF values of \(< 1\) were calculated for other nanoparticles in two-level aquatic food chains, such as graphene transfer from \textit{Tetrahymena thermophila} to \textit{Daphnia magna}.,\textsuperscript{41} carboxylated QDs transfer from \textit{Pseudokirchneriella subcapitata} to \textit{Ceriodaphnia dubia},\textsuperscript{11} and aluminium oxide nanoparticles transfer from \textit{Chlorella ellipsoidea} to \textit{Ceriodaphnia dubia}.\textsuperscript{34}

Thus, biomagnification of nanomaterials does not occur in all two-level food chains. In a two-level food chain, the biomagnification of these nanomaterials may or may not occur, depending on the size, specific surface area, type, and physicochemical properties of the nanomaterials, the organism at each trophic level, and the exposure conditions.

At the top two levels of the three-level food chain model in our study, the BMF\(_f\) (0.54) \(< 1\) indicated that biomagnification did not occur from \textit{D. magna} to \textit{D. rerio}. In addition, the depuration rate was faster than the uptake rate, indirectly implying that the biomagnification of fullerenols by zebrafish through the food chain would not be expected. Other studies on the \textit{D. magna}–\textit{D. rerio} two-level food chain calculated low BMFs for other nanoparticles, e.g., 0.0002–0.009 for graphene,\textsuperscript{41} 0.04–0.19 for aluminium oxide nanoparticles,\textsuperscript{34} 0.009–0.024 for TiO\(_2\),\textsuperscript{12} and 0.004–0.04 for Cd QDs.\textsuperscript{13} These previous findings suggest that the biomagnification of carbon nanomaterials and metal/metal oxide nanomaterials is unlikely in this two-level food chain. Although low BMFs were calculated for metal/metal oxide nanomaterials and carbon nanomaterials with poor dispersibility, the BMF values differed by an order of magnitude. The BMF value of fullerenols for the three-level transfer was several orders of magnitude larger than those of other nanoparticles for a two-level transfer. This may be related to the nature of the nanomaterial itself and the number of the trophic level. The polyhydroxylated surface of fullerenols makes them more water soluble, biocompatible, and dispersive than unmodified carbon nanomaterials. The transfer of nanomaterials through more trophic levels may mean that they pose greater risks to higher organisms. Further studies are required to evaluate the relative potential risks of fullerenol nanoparticles in aquatic environments.

Based on the calculated BMFs, we speculated that biomagnification of fullerenols occurred in the first two

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**Fig. 7** Schematic diagram of the trophic transfer and biomagnification of fullerenol nanoparticles in an aquatic food chain.
trophic levels, but not at the higher trophic level. From the perspective of a three-level food chain, our results confirmed the trophic transfer of fullerenol nanoparticles up to three trophic levels, with no biomagnification at the advanced organism level (fish), with a BMF of 0.54. This result was consistent with the fate of QDs in a three-level aquatic food chain (Astasia longa–Moina macrocopa–D. rerio). This suggests that fullerenols, similar to QDs, pose greater ecological risk at lower trophic levels than at higher trophic levels in aquatic environments.

To date, biomagnification of nanoparticles has not been observed in three-level aquatic food chains, but it has been observed in three-level terrestrial food chains (Phaseolus vulgaris–Epilachna varivestis–Podisus maculiventris). Moreover, unlike the environmental behaviours of nanoparticles in the three-level aquatic food chain, biomagnification occurred only at the higher trophic levels (E. varivestis larvae–P. maculiventris) and not at the first two levels (P. vulgaris–E. varivestis larvae). Clearly, there are differences in the transfer and biomagnification of nanoparticles between aquatic and terrestrial food chains. Earlier studies have shown that the types of organisms in different food chains affect the biomagnification and toxic effects of metal contaminants in aquatic ecosystems. Similarly, the biomagnification of nanoparticles can depend on environmental factors, exposure conditions (time and concentration), the ecosystem structure in food chains, and the properties of nanomaterials. Further studies are required to clarify the effects of these factors on nanoparticle biomagnification.

### Conclusion

We used $^{13}$C stable isotope labelling to track and quantify fullerenols in an aquatic ecosystem. This approach allowed us to systematically analyse their transfer and biomagnification potential in a three-level aquatic food chain. Our results provided the first direct evidence that fullerenol nanoparticles could be transferred within three trophic levels (S. obliquus–D. magna–D. rerio). The biomagnification of fullerenol nanoparticles in the three-level food chain occurred between the bottom two levels (S. obliquus–D. magna) but not between the top two levels (D. magna–D. rerio). The depuration rate of fullerenols in D. rerio was faster than the uptake rate, providing further evidence that fullerenols were not biomagnified through this three-level food chain. Fullerenol nanoparticles were distributed in almost all the tissues of D. rerio, with large amounts in the intestine and liver and small amounts in the brain. Uptake and depuration were fastest in the intestine and the gills, respectively. The depuration rates of fullerenol nanoparticles were significantly faster than the uptake rates during the ingestion and elimination exposure times. The tissue distribution and depuration results confirmed that most fullerenol nanoparticles would not be retained in tissues, providing further evidence that there was no risk of biomagnification in D. rerio. In brief, fullerenol nanoparticles may not pose significant risks to advanced organisms (such as fish), although they have the potential for bioaccumulation and biomagnification in primary and secondary organisms in aquatic food chains. This work is significant for not only aquatic environmental and ecological risk assessments but also future research on the environmental behaviour and fate of carbon nanomaterials. Further studies are required to evaluate the potential biomagnification and toxicological effects of long-term and/or high-dose exposure to nanomaterials in complex aquatic ecosystems. In addition, the consequences of the transformation and/or metabolism of fullerenol nanomaterials should be determined.
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Ethical statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Chinese Academy of Sciences University and approved by the Animal Ethics Committee of the Institute of Urban Environment, Chinese Academy of Sciences.

Conflicts of interest

The authors of this study declare no potential conflict of interest.

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References


33 X. Tao, Y. Yu, J. D. Fortner, Y. He, Y. Chen and J. B. Hughes, Effects of aqueous stable fullerene nanocrystal (nC60) on Scenedesmus obliquus: evaluation of the sub-lethal photosynthetic responses and inhibition mechanism, Chemosphere, 2015, 122, 162–167.

34 S. Pakrashi, S. Dalai, N. Chandrasekaran and A. Mukherjee, Trophic transfer potential of aluminium oxide nanoparticles using representative primary producer (Chlorella ellipsoides) and a primary consumer (Ceriophyllum dubia), Aquat. Toxicol., 2014, 142, 74–81.


48 G. S. Gupta, R. Shanker, A. Dhawan and A. Kumar, Impact of nanomaterials on the aquatic food chain, in Nanoscience in


