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ANALYSIS OF FULLERENE-C_{60} AND KINETIC MEASUREMENTS FOR ITS ACCUMULATION AND DEPURATION IN *DAPHNIA MAGNA*

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Abstract—A simple method for analyzing masses of water suspended fullerenes (nC_{60}) in *Daphnia magna* by extracting to toluene and measuring by ultraviolet-vis spectrophotometry was developed. This method was used to assess bioaccumulation and depuration rates by daphnia after nC_{60} exposure in artificial freshwater. Accumulation was rapid during the first few hours, and based on accumulation modeling, 90% of the steady-state concentration was reached in 21 h. After exposure for 24 h to a 2 mg/L fullerene solution, the daphnia accumulated 4.5 ± 0.7 g/kg wet weight, or 0.45% of the organism wet mass. Daphnids exposed to 2 mg/L fullerenes for 24 h eliminated 46 and 74% of the accumulated fullerenes after depuration in clean water for 24 and 48 h, respectively. Transmission electron microscopy revealed that the majority of the fullerenes present in the gut of daphnids were large agglomerates. The significant fullerene uptake and relatively slow depuration suggest that *D. magna* may play a role as a carrier of fullerene from one trophic level to another. Additionally, *D. magna* may impact the fate of suspended fullerene particles in aquatic ecosystems by their ability to pack fullerene agglomerates into larger particles than were found in the exposure water, and then excrete agglomerates that are not stable in water, causing them to settle out of solution. This process decreases fullerene exposure to other aquatic organisms in the water column but may increase exposure to benthic organisms in the sediment. Environ. Toxicol. Chem. 2010;29:1072–1078. © 2010 SETAC

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INTRODUCTION

A fullerene (C_{60}) is an allotrope of carbon, and also one kind of carbon nanoparticle. Nano-size usually indicates that a particle has one characteristic dimension between 1 and 100 nm, a property that provides fullerenes with unique properties different from those exhibited by larger carbon particles. This trait has led to a wide range of current and potential applications for fullerenes in industry, consumer goods, and medicine [1,2]. As a result of their ability to penetrate cells, for example, fullerenes can be used as anti-cancer agents to kill tumor cells, and they can be used to carry a drug molecule to a special location or receptor in the cell [2]. These same unique and useful properties in one application may have completely different and potentially harmful effects to organisms if fullerenes are released to the environment by means of waste disposal, or accidentally during or after use.

Despite the broad range of research conducted on the application potentials of fullerenes and other nanoparticles, their toxicological and environmental effects are still not well known, especially in aquatic environments [3]. Chemical safety legislation established by the European Union (REACH) presently manages fullerenes with the same guidelines as for bulk carbon. However, unlike bulk carbon, there is substantial evidence that water-suspended fullerenes may have harmful effects on human cells [4,5], bacteria [6,7], and also to whole animals [8,9]. Toxic impacts reported include necrotic cell death caused by fullerenes' capacity to generate reactive oxygen species [10–13], exhibiting antibacterial activity, and altering bacterial membrane lipid compositions of both Gram-positive and -negative bacteria [6,7]. Also, DNA damage to human lymphocytes [5] and pulmonary toxicity in rats [8] has been observed. Similar effects on aquatic species have been detected, such as pathological changes in fish embryos [14,15], behavioral changes in *Daphnia magna* [16] and reduced offspring production in *D. magna* [17].

An important property of C_{60} in aquatic ecotoxicology is its dualistic character in water solubility: as a molecule it is very poorly soluble in water and its octanol-water partition coefficient log K_{OW} has been measured to be 6.67 [18], but it can also be suspended in water as colloidal nano-size agglomerates relatively easily. Fullerenes can form these negatively charged agglomerates of several C_{60} molecules surrounded by water molecules, namely nC_{60}, by means of natural processes like water flow and mixing, as well as by vigorous stirring in the laboratory [19–21]. Aggregation allows fullerenes to remain suspended for weeks or months [22], and this mobility likely makes them more available for aquatic organisms like *D. magna*.

Daphnia magna is an organism widely used as an indicator in aquatic environmental risk assessment. Because daphnia filter large volumes of water and water-suspended particles, it is a significant target of water suspended xenobiotics. *Daphnia magna* also plays an important role in freshwater food chains ([23–25]; http//www.ncbi.nlm.nih.gov/entrez/query. fcgi?db=Books). These features make *D. magna* a particularly useful test animal for assessing the accumulation of nanomaterials, because uptake of nanoparticles such as fullerenes by *D. magna* could result in transfer throughout food chains.

Previous research indicates that nC_{60} can be taken into the guts of daphnia and maximum uptake was approximately 2.3 μg

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per mg of wet tissue after 48 h of exposure to a 30 mg/L solution [17]. However, to our knowledge there is no assessment of fullerenes' accumulation and depuration kinetics for *D. magna*. This may stem in part from the lack of a simple and rapid method for quantifying fullerenes in biological samples.

The main focus for the present study was to assess to what extent *D. magna* accumulates fullerenes. To achieve this objective, a simple and rapid method for quantifying fullerenes in daphnia samples was developed and used to measure fullerene accumulation and depuration rates in *D. magna*. This information will help guide a scientifically sound risk assessment of fullerenes released into aquatic ecosystems.

MATERIALS AND METHODS

Chemicals and experimental waters

Crystalline C_{60}-fullerene (98 %) was purchased from Sigma-Aldrich, USA. Artificial freshwater was made by adding analytical grade salts ($CaCl_2$ x $2H_2O$ 58.8 mg/L, $MgSO_4$ x 2 H_2O 24.7 mg/L, $NaHCO_3$ 13.0 mg/L, and KCl 1.2 mg/L; hardness $[Ca^{+2}]+[Mg^{+2}] = 0.5$ mM) to Millipore-water and adjusting the pH to 6.8. This artificial freshwater corresponded to Finnish lakes with its pH and hardness. This low hardness also helped minimize C_{60} precipitation. Sodium chloride (NaCl; WVR International) and toluene (Baker) used in the extraction of fullerene were analytical grade.

Preparation and analysis of the nC_{60} suspension

Stock suspensions of nC_{60} were made by mixing 250 mg of crystalline fullerene in 500 ml of artificial freshwater at 1000 rpm for four weeks by magnetic stirring at $20 \pm 2°C$. The fullerene suspension was then filtered with glass fibers (1-μm pore size) to remove the largest fullerene agglomerates. The concentration of fullerenes in suspension was analyzed by extracting fullerenes to toluene and recording spectra from 280 to 600 nm using a Shimadzu 1601 PC spectrophotometer and recording a calibration curve at 335 nm [19]. The fullerene concentration in the stock suspension was 200 mg/L. The efficiency of this extraction method was checked using a total organic carbon analyzer (Shimadzu TOC-5000A with ASI-5000A auto sampler) and was found to be greater than 97%.

To characterize the fullerenes from the stock solution using transmission electron microscopy (TEM), the stock suspension was diluted 1:40 with artificial freshwater to reduce particle aggregation during drying. This dilution resulted in a fullerene concentration of 5 mg/L, which was close to that used for the accumulation experiments. Eight microliters of the diluted nC_{60} suspension was dropped on Formvar polyvinyl resin-coated 150-mesh copper grids, and the samples were air dried before analysis with a Zeiss 900 transmission electron microscope operating at 80 kV incident beam energy with magnification ranging from ×3,000 to 140,000. Average particle size was measured by dynamic light scattering (DLS) Zetasizer Nano ZS (Malvern Instruments).

Test organisms

Daphnia magna were obtained from a culture maintained at the University of Joensuu. Organisms were grown in artificial freshwater (Ca+Mg hardness 2.5 mM, pH 6.5 to 7.1) with a photoperiod of 16:8 h light:dark at $20 \pm 2°C$. The population was fed three times a week with a green algae culture of *Scenedesmus sp* (dominant species), *Monoraphidium contortum* and *Selenastrum capricornutum*. Organisms used in tests were 5 to 7 d old at the beginning of experiments.

Transmission electron microscope observations of D. magna

The shape and size of fullerene agglomerates and their distribution in the guts of daphnids were assessed using TEM. *Daphnia magna* samples were prepared by fixing whole organisms with a 1:1 ratio of 4% glutaraldehyde and 0.2% Na-cacodylate buffer (pH 7.5) overnight in a refrigerator and then added to a Na-cacodylate buffer for at least 15 min. For post-fixation, daphnia were placed in a solution composed of a 1:1 ratio of 2% OsO_4 and 0.2% 0.1 M Na-cacodylate buffer (pH 7.5). Daphnia were then added to fresh Na-cacodylate buffer for 15 min. Dehydration steps were made in an upward acetone series. They were consecutively placed in solutions of 30, 60, or 90% acetone for 10 min each, and then in 100% acetone for 10 min three times. The daphnia were then added for 30 min each to 1:1 and then 1:3 solutions of acetone and Epon™ epoxy embedding medium (Fluka). Infiltration to 100% Epon™ was made overnight, and the samples were then cast. After casting, 80-nm-thick slices were cut vertically in the area of the intestine and placed on Formvar™ coated copper grids.

Quantification method of nC_{60} accumulated by Daphnia magna

For quantitative analyses of fullerenes in daphnia, 20 organisms were exposed to 240 ml of a 2 mg/L nC_{60} suspension for 24 h. Four replicates were tested, and organisms in artificial freshwater only were included as a control. After exposure, the organisms were removed from fullerene-spiked and control waters, placed on filter paper, and rinsed with clean Millipore™ water to remove fullerenes that may have been stuck on the carapace and antennae [23]. The daphnia were then carefully dried with blotting paper. Between 1 and 2 mg of organism mass wet weight per sample was weighed carefully by microbalance (Sartorius 4503 Micro) and placed in 10-ml glass tubes. Then NaCl solution (1.5 ml, 2%) was added as the homogenizing media. Samples were homogenized with a probe tip sonicator (Vibra Cell, Sonics & Materials) for 2 min. After homogenization, samples were extracted to 1.5 ml of toluene by vortex shaking for 15 s and then by bath sonication for 5 min. Spectra of toluene were recorded by ultraviolet-vis spectrophotometry. Fullerene peaks were identified at 335 and 407 nm. The extraction of control organisms did not yield a peak at 335 or 407 nm or at any other section of the spectrum.

Accumulation and depuration experiments

Daphnia were transferred to clean artificial freshwater for at least 1 h before the beginning of accumulation or depuration experiments to allow them to empty their guts and acclimate to the test water. Then, 20 test organisms per beaker were transferred to four replicate glass beakers containing 240 ml of artificial freshwater, and aliquots from the fullerene stock suspension were added to each exposure beaker to yield 2 and 0.5 mg/L concentrations. Preliminary standard acute toxicity tests (U.S. Organisation for Economic Co-operation and Development acute immobilization test, 24 h, concentrations 0, 0.2, 2, 7, 15, 30, and 50 mg/L, four replicates) revealed 20% effective concentration and no-effect concentration values of 30 and 2 mg/L, respectively, and thus concentrations of 2 and 0.5 mg/L were chosen for accumulation experiments. Daphnia were also added to artificial freshwater without nC_{60}. The test beakers were kept in the dark between sampling times to minimize the effect of light. To assess bioaccumulation rates, samples of 1 to 2 mg wet weight organism mass were collected after 15 min, 30 min, 1 h, 4 h, and 24 h of exposure, and fullerene

concentrations determined as described in the fullerene quantification section. Control organisms were included at each time point. Preliminary experiments with daphnia exposed to 2 mg/L fullerenes did not reveal a significant difference (*t* test, $\alpha > 0.05$) in the concentrations of accumulated fullerenes after 24 h or 48 h of exposure, so 24 h was selected as the endpoint for the bioaccumulation tests. Accumulation of fullerenes by daphnia was also assessed microscopically using a light microscope (Leitz Wetzlar Dialux 20) and a digital camera.

The potential for fullerene precipitation at concentrations of 2 and 0.5 mg/L without daphnia was checked by measuring the absorbance at 335 nm using ultraviolet-vis spectroscopy for quadruplicate samples taken across a 24-h period. The aqueous fullerene concentration with daphnia was measured at the start of each exposure and at every sampling time using ultraviolet-vis spectrophotometry.

Depuration experiments were conducted after daphnia had been exposed to a 2 mg/L fullerene solution in artificial freshwater for 24 h. Depuration experiments were repeated four times. After fullerene exposure, the organisms were rinsed in a beaker of clean artificial freshwater to remove the fullerenes from their carapace and antennae, and then the daphnia were transferred into 240 ml of clean artificial freshwater for depuration. Depuration sampling times were 0, 1, 4, 24, and 48 h. Fullerene concentrations were measured in quadruplicate replicates for each data point using the quantification method described above.

Calculations and modeling

Calculations and data handling were made by Microsoft Excel® and SPSS16 for Windows.

Accumulation data as individual data points was fit to a first-order one-compartment kinetic model [26] that accounts for the decreasing water concentration [27]:

$$C_a(t) = \left[\frac{\left(ku \times C_w^{t=0}\right)\left(e^{-\lambda t} - e^{-k_e t}\right)}{k_e - \lambda}\right] \quad (1)$$

where $C_a(t)$ is the concentration of fullerene in the organism (mg/kg wet weight); k_u is the conditional uptake rate coefficient L/(kg · h; k_e is the conditional elimination rate constant (1/h); $C_w^{t=0}$ is the concentration of fullerene in water (mg/L) at the time zero; *t* is the time (h); and the lambda value (λ, 1/h) is the linear regression slope of the natural logarithm of fullerene concentration in the aqueous phase during exposure.

Depuration data was fit using a first-order decay model:

$$C(t) = C_0 e^{-k_e t} \quad (2)$$

where C_0 is the concentration of fullerene (mg/kg) in the beginning of depuration and $C(t)$ concentration at the time *t*; and k_e is the elimination rate coefficient (l/h).

RESULTS

Characterization of nC_{60}

Representative TEM images of nC_{60} showed spherical shape fullerene agglomerates which had sizes between 30 and 600 nm (average 200 ± 120, $n = 320$, Fig. 1). Average particle size measured by DLS was 235 ± 1 nm ($n = 5$). Transmission electron microscopy images of nC_{60} exposed *D. magna* showed that fullerene nanoparticles were tightly packed in the gut lumen. Most of the fullerenes observed in the organisms' guts also were larger (average 1100 ± 500 nm, $n = 250$) and more angular than those suspended in water (Fig. 2). Some nC_{60} was located between the microvilli too. Interestingly, particles sized approximately 10 nm were observed on the surface of microvilli of exposed daphnia. These particles were not found in the control daphnia and are assumed to represent small fullerene particles.

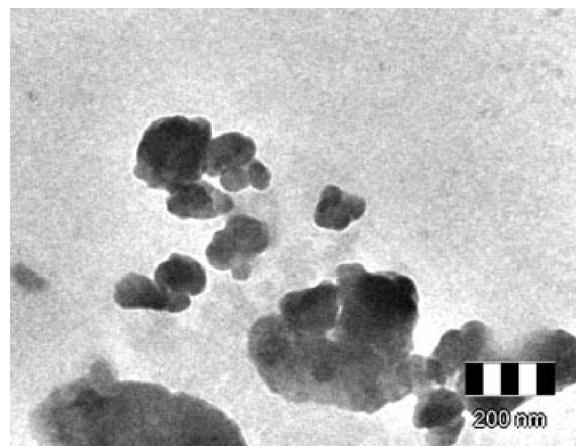

Fig. 1. Transmission electron micrograph of nC_{60} suspension (magnification ×50,000).

Method development for fullerene quantification in D. magna

The method for quantifying accumulated fullerene in *D. magna* was workable and simple. The recovery of the extraction procedure was verified by adding 1 to 20 μg of C_{60} into test tubes containing known masses of daphnia ranging from 1 to 3 mg and then following the extraction and spectrophotometry procedure described above. The recovery ranged from 85 to 98%, thus indicating a high recovery. This high recovery was repeatable (data not shown).

Accumulation and depuration experiments

Accumulation occurred rapidly during the first 2 h as shown in Figure 3, and it reached 90% of the calculated steady-state in 21 h. A composite average of five accumulation data points taken after 24 h of exposure to a 2 mg/L fullerene solution yielded a value of 4500 ± 0.7 mg/kg wet weight. The conditional accumulation rate coefficient k_u (±standard deviation) was 1660 ± 280 L/(kg · h) for exposure concentration 0.5 mg/L, and 400 ± 70 L/(kg · h) for 2 mg/L, respectively.

Bioconcentration factors (BCFs) were calculated as the concentration of fullerenes in the organisms divided by that in the aqueous solution at the time of sampling. Bioconcentration factors after 24 h in 2 mg/L was 2000 on a wet mass basis, and for an exposure concentration of 0.5 mg/L, it was 7600.

Depuration kinetics are shown in Figure 4. After 24 h of depuration, $54 \pm 10\%$ ($n = 4$) of the fullerenes remained in the daphnia. Although there was more variability, a significant concentration of fullerene particles ($26 \pm 14\%$) was still measured after 48 h.

The modeled elimination rate constant k_e was 0.11 ± 0.02 l/h for 2 mg/L exposure and 0.09 ± 0.02 1/h for 0.5 mg/L. The depuration rate coefficient k_d determined by the first-order decay model was 0.023 ± 0.007 1/h, which is smaller than the values determined by the modeling of the accumulation data. This difference likely stems from the facilitated excretion of fullerenes with the ingestion of new fullerene particles during the accumulation experiments.

To assess the fullerene settling rate in the absence of daphnia, suspended fullerene concentrations were tested across

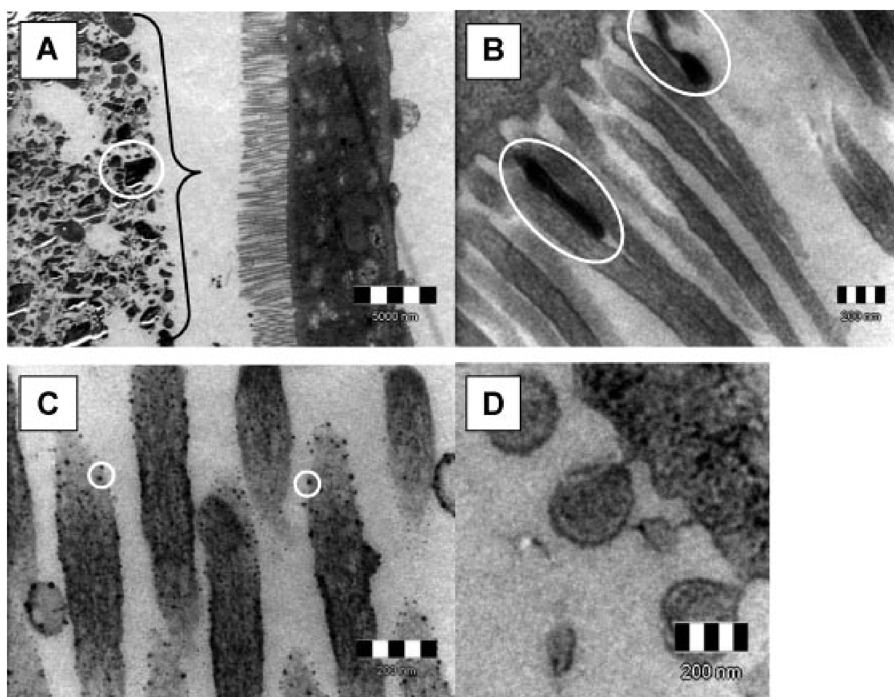

Fig. 2. (**A**) Transmission electron micrograph image of the gut of exposed daphnia (×3000). C_{60} agglomerate mass on the left of the bracket with an individual agglomerate circled, and microvilli and the wall of the gut on the right. (**B**) Some C_{60} agglomerates (circled) are positioned between microvilli (×50,000). (**C**) Apparent fullerene agglomerates (circled) on the surface of and inside the microvilli (×85,000). (**D**) The microvilli of the control *Daphnia* (×140,000).

a 24-h interval and 96 ± 4% of the initial concentration remained suspended after 24 h. However, the aqueous fullerene concentration decreased by 31 ± 9% during a 24-h period when daphnia were present, and the lambda values from modeling the accumulation experiments were −0.056 for 0.5 mg/L and −0.043 for 2 mg/L, thus indicating that daphnia accelerate fullerene settling.

DISCUSSION

According to TEM observations, nC_{60} agglomerates in the gut of organisms were larger and more angular than in the water suspension. This alteration may be a result of mechanical packing when daphnia accumulate nC_{60} very rapidly. It is known that solution chemistry such as pH, ionic strength and fullerene concentration affect the aggregation forms of fullerenes [17]. It is possible that the gut conditions may have had a similar effect and that some agglomerates may reform in the gut lumen as a result of the gut conditions. It is also possible that larger particles were preferentially retained in the guts of the organisms. *Daphnia magna* can be grouped as a fine mesh filter-feeders meaning its filtering apparatus has a mesh size 0.24 to 0.64 μm [23] and the ability to preferentially catch and ingest particles within this range, which fits the size range of the larger nC_{60} agglomerates in the exposure water [23]. Although the highest particle intake activity by *D. magna* has been shown to occur at particle sizes of approximately 0.5 μm, it has also

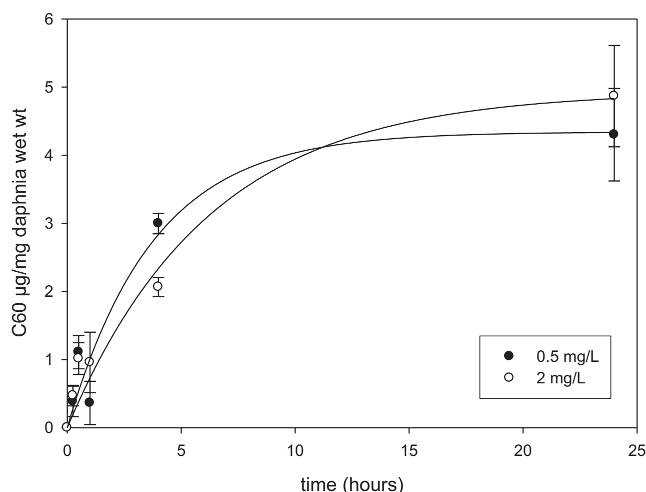

Fig. 3. Accumulation kinetics of fullerenes in *Daphnia magna*. Each data point is the average of quadruplicate samples and the error bars are the standard deviations. The lines are a first-order exponential fit to the data.

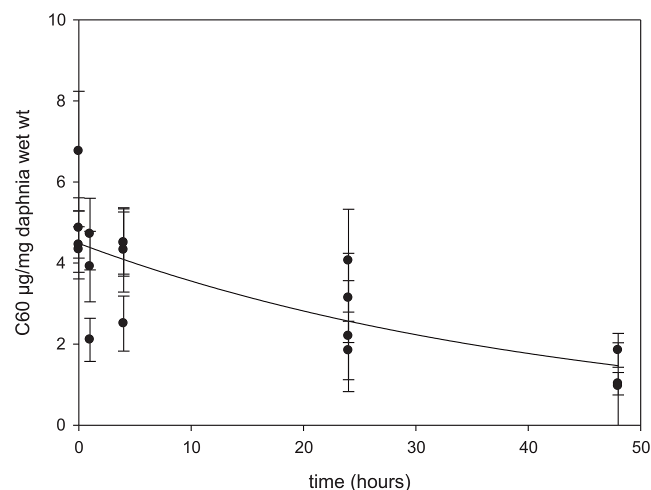

Fig. 4. Depuration kinetics of fullerenes in *Daphnia magna* after exposure to 2 mg/L fullerenes for 24 h as a combination of four depuration experiments. Each data point is the average of quadruplicate samples and the error bars are the standard deviations. The line is the first-order decay fit to the data.

been shown that *D. magna* can intake and use smaller size bacteria as a food source and for nutrients [24].

Some nC_{60} agglomerates were observed between the microvilli. One potential toxicological effect of this is that the fullerenes packed between the villi may disturb the function of the digestion channel of daphnia. Additionally, daphnia may have to expend additional energy to excrete packed fullerenes. For long-term exposure to fullerenes [16,17], alterations to energy use might affect the daphnia's filtration rate and thus also ingestion and nutrient supply, which are important sub-chronic or chronic toxic effects. Fullerene particles approximately 10 nm were probably seen in the surfaces of microvilli, although the majority of fullerenes appeared as much larger agglomerates. Daphnia normally consume bacteria and algae, the size of which should be significantly larger than 10 nm, but daphnia may decompose algae or bacteria for absorption across the membranes of the microvilli because they have absorption mechanism for molecules only, not for particles [25]. This decomposing mechanism may explain the apparent presence of small fullerene agglomerates on the surfaces of microvilli. Heinlaan et al. [28] showed that metal oxide nanoparticles do not have to enter the cells to cause toxicity. The same could conform to carbon nanoparticles as well.

Accumulation of nC_{60} occurred very rapidly initially, and the yields of 4 to 5 g/kg wet weight after 24 h indicate that *D. magna* accumulate fullerenes effectively from exposure water. These quantitative results agree with those determined qualitatively through light microscopy. After less than 30 min of exposure, fullerenes were seen in the upper portion of the gut while some green algae still fills the lower portion of the gut (Fig. 5). After 1 h, the guts appeared to be filled with fullerene particles. This rapid accumulation of substantial concentrations of fullerenes indicates that uptake should be taken into consideration when assessing the potential environmental effects of fullerenes, especially because *D. magna* has an important role in food chains. In addition to the potential toxic effects of fullerenes themselves, their ability to adsorb other contaminants must be taken to account when assessing fullerenes' risks to aquatic organisms [29–32]. For example, polyaromatic hydrocarbons, which are found in notable concentrations in the environment, may accumulate with fullerenes to organisms if they are concurrently present. Baun et al. reported that phenanthrene sorbed to fullerene agglomerates can accumulate in the daphnia and the toxicity of phenanthrene increased by 60% compared with toxicity without fullerenes [29].

Earlier it has been shown that fullerene intake by *D. magna* was approximately 2.3 μg per mg of wet tissue after 48 h of exposure at a fullerene concentration of 30 mg/L [15]. These results are 51% of those observed here for daphnia exposed to a 2-mg/L fullerene suspension for 24 h. This difference may stem from the different ages of the daphnia used, from feeding of the daphnia during the uptake experiments by Oberdörster et al. [17], and from different extraction methods. If the fullerene accumulation results determined here are adjusted to a dry weight basis by assuming that the daphnia dry weight is roughly 8% of the daphnia wet weight as indicated by preliminary results not shown, the fullerene concentration in the daphnia is approximately 56 ± 9 μg per mg of dry tissue after 24 h of exposure to a 0.5 mg/L solution. This result is nearly identical to the 63 ± 15 μg of accumulated multi-walled carbon nanotubes (MWNTs) per mg of dry daphnia tissue after 48 h of exposure to a solution with an initial nanotube concentration of 0.4 mg/L determined by Petersen et al. [33]. These MWNTs had diameters ranging mainly from 30 to 70 nm and an average length of 407 nm, which suggests that carbon nanoparticles of this size will have similar high accumulation by *D. magna*. The lack of fullerene uptake into daphnia tissues is similar to what was observed with carbon nanotubes and other invertebrates [33–36].

From the light and electron microscopy results, the vast majority of the fullerenes observed appeared to be in the guts of the organisms and not absorbed into its tissues. Calculated BCF was 2000 for an exposure concentration of 2 mg/L, and 7600 for 0.5 mg/L, respectively. However, this term does not have the same significance with fullerenes as it does with typical organic chemicals because fullerenes do not appear to be readily absorbed into the organism tissues. Additionally, the mass of fullerenes in the organisms did not change based upon the aqueous concentration, and therefore the volume of the gut may be the factor that limits fullerene accumulation by *D. magna* at these concentrations. In this respect, it is unclear whether terms such as bioconcentration factors are relevant for nanoparticle risk assessment. The relevance of the gut volume to the accumulation kinetics is further confirmed by the similar elimination rate coefficients for the two test concentrations and by the fact that the uptake rate coefficients changed proportionally to the aqueous phase concentration.

Daphnia were able to depurate fullerenes after they were transferred to clean artificial freshwater, but the depuration was not complete after 48 h. The organisms were not fed during depuration experiments. It is likely that feeding would have accelerated the rate of depuration. Previously, the consumption of algae helped daphnia excrete ingested MWNTs and sediment particles [33,37], and a similar effect would likely occur with

Fig. 5. Light microscopy of *Daphnia magna* after (**A**) 30 min exposure (an arrow shows the zone between dark fullerene packed gut and the remainder of the gut believed to only contain algae) and (**B**) 1-h exposure.

fullerenes. In natural conditions both fullerenes and algae may be present, and so the interactions between them should be investigated. Unlike MWNTs and ingested sediment particles, the fullerenes were largely excreted by the daphnia in the absence of food. The cause of this discrepancy between the elimination behaviors for the carbon nanotubes and fullerenes is unclear.

Additionally, daphnia enhanced fullerene settling out of solution and thus would be expected to impact the fate of fullerenes in aquatic ecosystems. This settling may be due to daphnia's ability to pack fullerene agglomerates into larger particles than found in the exposure water, and then excrete agglomerates that are not stable in water.

CONCLUSIONS

This study presents a simple, rapid, and repeatable method with high recovery for quantifying fullerenes in *D. magna*. The present study also provides accumulation and depuration kinetics in *D. magna* when exposed to nonacutely toxic concentrations of nC_{60} in artificial freshwater. According to the findings, fullerene nanoparticles can be rapidly taken into *D. magna* in substantially higher concentrations than in the exposure water. Accordingly, *D. magna* may play a role as a carrier of fullerenes from one trophic level to another. Additionally, *D. magna* may substantially impact the fate of suspended fullerene particles in aquatic ecosystems by causing them to settle out of solution, and thereby decrease fullerene exposure to other aquatic organisms in the water column, but increase exposure to benthic organisms in the sediment. For fullerene risk assessment, it should be considered whether BCFs are relevant terms, because fullerenes do not appear to be readily absorbed into the organism tissues, and the accumulated mass of fullerenes appear to be limited by the volume of the gut rather than the aqueous concentration.

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