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Bioavailability of organochlorine compounds in aqueous suspensions of fullerene: Evaluated with medaka (*Oryzias latipes*) and negligible depletion solid-phase microextraction

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ABSTRACT

The wide application of engineered nanomaterials, such as fullerene (C_{60}), will inevitably lead to their release into the aqueous environment, which may alter the bioavailability of organic compounds to aquatic organisms. Negligible depletion solid-phase microextraction (nd-SPME) together with medaka (*Oryzias latipes*) bioaccumulation were used to study the effects of aqueous suspensions of fullerene (nC_{60}) on the bioavailability of eight organochlorine compounds (OCCs) (log K_{OW} 3.76–6.96). Freely dissolved concentrations of OCCs decreased by 11.5–88.4% at addition of 5 mg L^{-1} nC_{60} as indicated by reduced equilibrium concentrations in the SPME fiber coating, the highest reduction being observed for the most hydrophobic OCCs. Medaka bioaccumulation study demonstrated that at the kinetic uptake regime, nC_{60} significantly decreased the bioaccumulation of the high hydrophobic OCCs (log K_{OW} > 6), but slightly enhanced the bioaccumulation of the less hydrophobic OCCs (log K_{OW} < 6). The OCC concentrations in medaka (C_{fish}) at the kinetic uptake regime linearly correlated with that in nd-SPME fiber (C_{fiber}) without nC_{60} (p = 0.007–0.013, R^2 = 0.666–0.723), but this correlation deteriorated with the presence of nC_{60} (p = 0.073–0.081, R^2 = 0.423–0.440). These results suggest that in nC_{60} the uptake mechanism of OCCs to medaka is different from that to nd-SPME fiber. While only the freely dissolved OCCs are available to nd-SPME fiber, both the freely dissolved and the nC_{60} associated OCCs contributed to the accumulation of OCCs to medaka.

1. Introduction

Since its first discovery in 1985 (Kroto et al., 1985), fullerene has attracted great interest in a range of fields from material chemistry to biological applications (Bosi et al., 2003) because of its unique structure and physical chemical features. In view of its large production and wide applications, fullerene will inevitably be released into the environment and thus raise environmental and health concerns due to its probable toxicity (Colvin, 2003). Fullerene could enter living organisms via several routes, especially the respiratory and digestive systems and the skin (Oberdorster et al., 2005). Various studies have demonstrated that carbon nanomaterials (CNMs), including fullerene, can penetrate both cell membranes (Foley et al., 2002) and living tissues (Rouse et al., 2007; Xia et al., 2009), and cause both in vitro (Wang et al., 2009, 2010) and in vivo (Oberdorster et al., 2006; Tao et al., 2009) toxicity. New insights in nanotoxicity suggest that the toxicity of CNMs is due not only to their own intrinsic toxicity, but also to the effect of these CNMs on the fate, transport and exposure of toxic substances (Colvin, 2003; Yang et al., 2006; Xia et al., 2010). Previous studies (Cheng et al., 2004; Yang et al., 2006) demonstrated that common organic pollutants can be strongly sorbed by CNMs, suggesting that their fate, transport and exposure may be significantly modified by the presence of CNMs. Recent modeling by Hofmann and von der Kammer (2009) indicates that CNMs may or may not have an effect on the transport and distribution of hydrophobic organic compounds (HOC). Moreover, despite its insoluble nature, fullerene can form stable aqueous suspensions containing high concentrations of C_{60} aggregates (nC_{60}) by some environmentally relevant processes, including the extended mixing of fullerene with water (Cheng et al., 2004; Lyon et al., 2006) and interaction with natural organic matter (NOM) (Espinasse et al., 2007; Terashima and Nagao, 2007). These aqueous suspensions of nC_{60} are negatively charged and have a high mobility in porous media (Espinasse et al., 2007), suggesting potential migration in surface and groundwater systems, allowing them to reach the receptor

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organisms. Accordingly, the mobility and bioavailability of the nC_{60} associated pollutants may be enhanced.

To the best of our knowledge, only a few studies (Baun et al., 2008; Ferguson et al., 2008; Petersen et al., 2009; Xia et al., 2010) have dealt with the effects of CNMs on the bioavailability of organic pollutants, and the mechanisms remain ambiguous. Ferguson et al. (2008) demonstrated that sediment amendment with single-walled carbon nanotubes significantly impacted the bioavailability of adsorbed HOCs to deposit-feeding organisms, such as reducing the bioaccumulation of HOCs in *polychaete*. Petersen et al. (2009) indicated that carbon nanotubes in soil either decreased or had no effect on HOC bioaccumulation by earthworms depending on the concentration of CNMs. Both the above research on carbon nanotubes in sediment (Ferguson et al., 2008) and soil (Petersen et al., 2009) suggested that they act similarly to black carbons (Cornelissen et al., 2005). Modeling research by Koelmans et al. (2009) indicates that CNMs would not be expected to impact HOC availability in soil or sediment ecosystems except at extremely high nanomaterial concentrations. However, as for the case of CNMs in the aquatic ecosystem (Baun et al., 2008), the effects seem to be very complicated. Baun et al. (2008) studied the effects of nC_{60} on contaminant toxicity in aqueous systems with algae and *Daphnia magna*. It was found nC_{60} increased the toxicity of phenanthrene, reduced the toxicity of pentachlorophenol, and had no impact on the toxicity of atrazine and methyl parathion.

The diffusive uptake of a compound is more usually related to its freely dissolved concentration (C_{free}) than to its total concentration (Escher and Hermens, 2004). The effects of CNMs on the uptake of a compound might thus be better explained via the C_{free} that can be measured with negligible depletion solid-phase microextraction (nd-SPME) (Mayer et al., 2000a,b; Hu et al., 2006; Yang et al., 2008). The C_{free} of polycyclic aromatic hydrocarbons (PAHs) in nC_{60} suspensions was measured in our previous study (Hu et al., 2008). The sorption of PAHs to nC_{60} resulted in a remarkable decline of their C_{free}, suggesting a reduced diffusive uptake of non-bound HOCs even at relatively low concentrations of nC_{60}. However, an increasing number of studies have shown that C_{60} fullerene and fullerene derivatives can traverse cell membranes (Foley et al., 2002; Rouse et al., 2007) and accumulate in living organisms (Oberdorster et al., 2006; Tao et al., 2009; Xia et al., 2009), which suggested that HOCs may also be transported when bound to the nC_{60}. Additionally, just as the artificial and natural organic matter, nC_{60} may also enhance the diffusive mass transfer of the HOCs through the aqueous boundary layer at the organisms (Mayer et al., 2007; ter Laak et al., 2009), which is often considered the rate-limiting step for diffusive uptake (Sijm and Vanderlinde, 1995). Thus, it is of importance to study the uptake kinetics and accumulation of HOCs in nd-SPME fiber to understand the impacts of nC_{60} on the bioaccumulation of HOCs in fish.

In this present study, the influence of nC_{60} on the bioavailability of HOCs was evaluated in bioconcentration tests with the model fish medaka that were exposed to a range of organochlorine compounds (OCCs, log K_{OW} = 3.76–6.96). Equilibrium sampling by nd-SPME was applied to measure C_{free} as well as the effect of nC_{60} on C_{free}. Finally, correlations between the concentrations of eight individual OCCs in Medaka (C_{fish}) and that in SPME fiber (C_{fiber}) was investigated to test the feasibility of nd-SPME as a biomimetic method for fish bioaccumulation in nC_{60} suspensions.

2. Materials and methods

2.1. Materials and chemicals

Poly(dimethylsiloxane) (PDMS) coated glass fibers with a core diameter of 110 μm and a coating thickness of 30 μm (corresponding to a volume of 0.132 μL cm^{-1} PDMS), obtained from Poly Micro Industries (Phoenix, AZ, USA), were used as disposable nd-SPME fibers. The fibers were cut into 1 cm pieces, and then washed twice in analytical grade acetone prior to use.

The C_{60} fullerene with a purity >99.5% was obtained from Aldrich (Steinheim, Germany). The following standards of OCCs with a purity >99.5% were purchased from Labor Ehrenstorfer (Augsburg, Germany): α-hexachlorocyclohexane (α-HCH), γ-HCH, δ-HCH, hexachlorobenzene (HCB), 1,1-dichloro-2,2-bis-4-chlorophenyl-ethene (p,p′-DDE), 1,1-dichloro-2,2-bis-4-chlorophenyl-ethylene (p,p′-DDD), 1,1,1-trichloro-2,2-bis-4-chlorophenyl-ethane (p,p′-DDT), 3,3′,4,4′-Tetrachlorobiphenyl (PCB77). Individual stock solutions of the standards were prepared in liquid chromatography-grade methanol (J.T. Baker, Phillipsburg, NJ, USA) and working solutions were prepared daily by appropriate dilution of the stock solutions with water. The internal standard decachlorobiphenyl (PCB209) was purchased from Accustandard, Inc. (New Haven, CT, USA), and the stock solution was prepared in *n*-hexane. All solvents, such as toluene, *n*-hexane and dichloromethane, were of pesticide residual grade (J.T. Baker, Phillipsburg, NJ, USA). All the water used was ultra-purified to >18 Ω (EASY-pure LF, Barnstead International, Dubuque, IA, USA).

2.2. nC_{60} preparation and characterization

nC_{60} stock suspensions were prepared by the solvent exchange method and the concentration was determined by UV–visible spectrophotometric method described in our previous work (Hu et al., 2008). The details were also shown in Supplementary material. The particle size distribution of nC_{60} suspensions was measured by laser particle size analysis (Mastersizer 2000, Malver, Worcestershire, UK) and corroborated by transmission electron microscopy (H-7500, Hitachi, Tokyo, Japan). The $d_{(0.5)}$ value was 194 ± 2.1 nm (n = 3), and the typical Transmission Electron Microscope image is shown in Fig. S1 (Supplementary material).

2.3. Evaluation with nd-SPME

The nd-SPME procedure was modified from our previous study (Hu et al., 2008). After extraction during the prescribed time, the extracted OCCs on nd-SPME fiber were desorbed with *n*-hexane and analyzed with a gas chromatograph–electron capture detector (GC–ECD) system (see Supplementary material for details). Sample solutions spiked with 50 μg L^{-1} each of α-HCH, γ-HCH, δ-HCH, and 1 μg L^{-1} each of HCB, p,p′-DDE, p,p′-DDD, p,p′-DDT, PCB77 were used throughout unless otherwise specified. The test concentrations for all these compounds were within their water solubility (Table 1). Mixtures of test solutions were used throughout as the concentration of each compound was at low μg L^{-1} level and the competitive sorption between analytes in nC_{60} was negligible.

To investigate the effect of nC_{60} on the uptake kinetics of OCCs into PDMS fibers, uptake profiles were created in pure water and in 5.0 mg L^{-1} nC_{60}, respectively. The solutions were pre-aged for 7 d to ensure sorption equilibrium between OCCs and nC_{60} (data not shown) and then sampled for 1, 2, 3, 5, 7 and 10 d with the nd-SPME procedure.

To study the influence of nC_{60} concentration on the free fractions of OCCs, solutions with 0, 1, 2.5, 5, 10 and 25 mg L^{-1} of nC_{60} were spiked with OCCs and shaken for 7 d to ensure equilibrium between OCCs and nC_{60}, after which the C_{free} of OCCs were determined with nd-SPME.

2.4. Evaluation with medaka

A batch of fully mature medaka with a mean body weight of 550 ± 100 mg and a mean body length of 25 ± 3 mm was used for