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 $5 \text{ mg L}^{-1} nC_{60}$ 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.

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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 nano-

toxicity 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 (correspond-

ing to a volume of 0.132 $\mu L cm^{-1}$ 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, Malvern, 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 $\mu g L^{-1}$ each of α -HCH, γ -HCH, δ -HCH, and 1 $\mu g L^{-1}$ 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 $\mu g L^{-1}$ 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

Table 1

Overview of partitioning coefficients ($\log K_{PDMS}$), uptake rate constants (k_1), elimination rate constants (k_2), equilibrium time (t_{eq}), equilibrium concentration in fiber ($C_{fiber,eq}$) for organochlorine compounds in the systems consisted of water or aqueous suspensions of fullerene (nC_{60}) solutions with the negligible-depletion solid-phase microextraction (nd-SPME) fibers.

Compounds	Aqueous solubility, 25 °C (mg L ⁻¹)	$\log K_{OW}$	$\log K_{PDMS}$	Ref	k_1 ($\times 10^4$ d ⁻¹) ^b		k_2		$t_{eq,90\%}$ (d)		$C_{PDMS,eq}$ (mean $\pm$ SD, mg L ⁻¹)		$K_{lipid,PDMS}^i$
					Water	nC_{60}	Water (d ⁻¹)	Water, 95% confidence intervals	Water	nC_{60}	Water	nC_{60}	
α -HCH	1.629 ^a		2.65 (0.02)	2.66 ^c	-	-	-	-	<1	<1	22.1 $\pm$ 0.9	22.5 $\pm$ 0.5	37.5
γ -HCH	7.562 ^a		2.82 (0.01)	2.59 ^c	-	-	-	-	<1	<1	33.4 $\pm$ 0.7	34.1 $\pm$ 0.7	37.5
δ -HCH	-		2.17 (0.01)	2.03 ^c	-	-	-	-	<1	<1	7.40 $\pm$ 0.23	7.42 $\pm$ 0.09	37.5
HCB	0.00484 ^b		4.68 (0.02)	4.75 ^g	8.56 (2.32)	5.61 (0.41)	1.78 (0.48)	0.45–3.11	1.29 (0.35)	1.97 (0.12)	48.0 $\pm$ 1.7	33.6 $\pm$ 0.4	15.3
p,p'-DDD	0.0199 ^b		6.22 ^e	5.45 ^g	42.5 (12.7)	39.0 (3.3)	0.91 (0.26)	0.17–1.65	2.53 (0.73)	2.76 (0.17)	467 $\pm$ 31	219 $\pm$ 3	63.9
PCB-77	0.00272 ^c		6.63 ^f	5.31 ^g	14.0 (2.0)	19.7 (2.8)	0.39 (0.05)	0.25–0.54	5.83 (0.79)	4.14 (0.51)	353 $\pm$ 17	72.7 $\pm$ 3.2	18.5
p,p'-DDT	0.00496 ^b		6.91 ^d	6.00 ^g	11.2 (4.9)	6.42 (2.52)	0.31 (0.12)	0–0.66	7.43 (2.99)	12.9 (4.26)	361 $\pm$ 59	10.9 $\pm$ 2.2	45.2
p,p'-DDE	0.0140 ^b		6.96 ^{d,e}	6.05 ^g	41.1 (6.3)	31.3 (2.5)	0.33 (0.06)	0.16–0.50	7.02 (1.33)	9.22 (0.37)	1253 $\pm$ 93	178 $\pm$ 2	24.8

The standard error (SE) derived from the fit to Eq. (S1) of the corrected uptake curves are indicated in parenthesis. SD denotes standard deviation.

^a Xiao et al. (2004).

^b Shen and Wania (2005).

^c Huang and Hong (2002).

^d Sabljic et al. (1995).

^e Paschke and Popp (2003).

^f Sabljic et al. (1993).

^g Obtained or calculated from the equation ($\log K_{PDMS,water} = 1.00 \log K_{OW} - 0.91$; $\log K_{OW} = 4.5 - 7.5$, $n = 17$, $r^2 = 0.99$, $s = 0.10$) described by Mayer et al. (2000a).

^h k_1 values calculated as $K_{PDMS} \times k_2$.

ⁱ $K_{lipid,PDMS}$ assigned based on Jahnke et al. (2008).

the current experiment. The test fish were raised in dechlorinated tap water (using active carbon column) in a flow-through system at a constant temperature of 25 ± 2 °C and a photo-period of 16:8 h (light:dark). Before the exposure, the fish were not fed for 3 d.

The bioaccumulation experiment was conducted in a static aquatic system with a test solution of 4 L, and the test solutions were renewed each day. The test solutions were prepared by spiking into dechlorinated tap water (using active carbon column) and 5.0 mg L⁻¹ nC_{60} , respectively (obtained by diluting the nC_{60} stock solution with dechlorinated tap water) with 2 μ g L⁻¹ each of α -HCH, γ -HCH, δ -HCH, and 1 μ g L⁻¹ each of HCB, p,p'-DDE, p,p'-DDD, p,p'-DDT, and PCB77. Dechlorinated tap water was used as the control medium. The solutions were aged for 7 d to equilibrate the OCCs and nC_{60} by shaking at intervals prior to exposure. Nine medaka were then introduced into each test solution. The exposure lasted for 12 d, during which the medaka received no food. Three medaka in each treatment were sampled after exposure for 0, 1, 5 and 12 d, respectively.

The medaka (excluding the head and internal organs) were freeze-dried for 3 d at -40 °C, and then each one was ground to powder to produce a sample for ultrasonic extraction. The extraction procedure and qualification of the OCCs are modified from that described in our previous work (Wang et al., 2007; Yang et al., 2007), and the details are shown in Supplementary material. The recoveries for the target compounds were between 78.2% and 92.6%. Detailed QA/QC procedures can be found in our previous paper (Yang et al., 2004). The lipid-normalized OCC concentration in medaka (C_{fish} , mg kg⁻¹ lipid) was calculated on the basis of the measured lipid content in medaka ($(25.24 \pm 0.96)\%$ dry weight). Details for the determination of the lipid content of medaka were described in Supplementary material.

2.5. Data analysis

All experimental data of the uptake profiles to nd-SPME fiber were fitted to a first-order one-compartment uptake model modified from reference (Sijm and Vanderlinde, 1995; Leslie et al., 2002) using Graphpad Prism (V 4.1, GraphPad Software, San Diego, CA, USA), the details were described in data analysis section (Supplementary material). Mean values were calculated from the three replicates and the standard deviations were presented. A one-way analysis of variance (ANOVA) test was performed to assess the significance of differences, and differences were considered statistical significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of nC_{60} on the free fraction of OCCs

The free fraction of OCCs was measured by nd-SPME in equilibrium mode. For the less hydrophobic HCHs and HCB ($\log K_{OW} < 6$), a small decline of the free fraction was observed with increasing nC_{60} concentration (Fig. 1a); ANOVA test showed that these small differences in free fraction were only statistically significant for δ -HCH and HCB ($p < 0.05$), but not for α -HCH and γ -HCH ($p > 0.05$). For the highly hydrophobic DDTs and PCB77 ($\log K_{OW} > 6$), however, significant reduction of the free fractions was observed with increasing nC_{60} concentration ($p < 0.05$) (Fig. 1b). It was noteworthy that a minimum of the free fractions was nearly reached at 10 or 25 mg L⁻¹ of nC_{60} , which were also observed in our previous study (Hu et al., 2008). The binding of OCCs to nC_{60} strengthened with increasing hydrophobicity of the OCCs, the highest reduction of C_{free} was thus observed for the most hydrophobic OCCs (Fig. S2). The free fraction in the suspension of

Fig. 1. Effect of the concentration of aqueous suspensions of fullerene (nC_{60}) on the free fraction of organochlorine compounds. Solutions with various nC_{60} concentrations spiked with 50 $\mu\text{g L}^{-1}$ each of α -HCH, γ -HCH, δ -HCH, and 1 $\mu\text{g L}^{-1}$ each of HCB, p,p'-DDE, p,p'-DDD, p,p'-DDT, PCB77 and aged 7 d, then conducted the equilibrium nd-SPME for 14 d. (a) HCHs and HCB; (b) DDTs and PCB77.

5 mg L^{-1} nC_{60} was reduced by between 11.5% and 88.4% relative to pure water. Such a decrease in the free fraction leads to a reduced diffusive uptake of non-bound OCCs into aquatic organisms as well as to a reduced equilibrium partitioning concentration in the fish.

According to the method described in measurement of sorption coefficients to nC_{60} in [Supplementary material](#), the sorption coefficients of OCCs between nC_{60} and water (K_{C60}) were determined via the measurement of C_{free} . As can be seen from [Fig. S3 \(Supplementary material\)](#), the $\log K_{C60}$ values were well linearly correlated with the corresponding hydrophobicity ($\log K_{OW}$), with correlation coefficient (R^2) of 0.958 ($p < 0.0001$). This result indicates the significant role of non-polar forces in OCCs- nC_{60} interactions, which was also observed in our previous work for PAHs (Hu et al., 2008).

3.2. Uptake of OCCs to nd-SPME fibers

The uptake of the OCCs studied in the absence and presence of nC_{60} , respectively, were fitted to [Eq. \(S1\) \(Supplementary material\)](#), with parameters obtained or further calculated from the fittings shown in [Table 1](#). For both the water and nC_{60} suspensions, the elimination rate constant k_2 decreased with the increase of $\log K_{OW}$, which was similar to previous studies for both polar (Leslie et al., 2002) and non-polar organic compounds (Mayer et al., 2007) using SPME or SPME-related techniques. The decrease of k_2 with the increase of $\log K_{OW}$ of OCCs suggests that the uptake was limited by diffusion through the unstirred aqueous boundary layer adjacent to the fiber (Leslie et al., 2002). The uptake rate constant k_1 listed in [Table 1](#) showed no significant trends with increasing $\log K_{OW}$, again suggesting that the aqueous boundary layer limits the uptake (Leslie et al., 2002).

[Table 1](#) also showed that the presence of 5 mg L^{-1} nC_{60} slightly reduced the k_2 values for all the OCCs except for PCB77, with the

$k_{2,nC60}/k_{2,\text{water}}$ ratios (with SE values in the corresponding parentheses) generally ranged from 0.57 (0.42) to 0.92 (0.30) but 1.41 (0.36) for PCB77. Although this reduction was not statistically significant for any of the studied compound by comparing the listed 95% confidence intervals ($p > 0.05$), it was noteworthy that the slight reduction should not be ignored. This result agreed with our previous study, where nC_{60} led to a decrease in the SPME sampling kinetics for chrysene (Hu et al., 2008). However, it was very different from previous work (Oomen et al., 2000; Mayer et al., 2007) with other natural and artificial aqueous matrixes, such as humic acid solutions and proteins, which were found to enhance the uptake kinetics of organic compounds to the solid sampling phase. This difference can likely be explained by differences in the dissociation or desorption kinetics of the bound analytes, which need to be sufficiently fast to allow the bound form to contribute to the mass transfer of the analyte into the SPME fiber coating (Oomen et al., 2000; Benhabib et al., 2009). The unique micropore structure of nC_{60} slowed the desorption of OCCs from nC_{60} (Cheng et al., 2004). Moreover, fouling of nC_{60} on the fiber surface might hamper mass transfer of the chemicals into the fiber. The independent k_2 values for the PCB77 studied may be attributed to its structure, which remains to be investigated further.

Minor reduction of the k_2 values for most of the OCCs by nC_{60} retarded slightly the equilibration, i.e. the equilibration time t_{eq} (with SE values in the corresponding parentheses) for p,p'-DDT was prolonged to 12.9 (4.26) d in nC_{60} compared to 7.43 (2.99) d in water; whereas increase of the k_2 values by nC_{60} shorten the equilibration, i.e. 4.14 (0.51) d with nC_{60} and 5.83 (0.79) d without. Although there are no statistically significant differences between the k_2 values or the equilibration times for all the OCCs in solutions with and without nC_{60} ($p > 0.05$), the slight kinetic retardation or enhancement should be not neglected as we discussed before. However, 14 d was sufficient for all the OCCs to reach their extraction equilibrium.

3.3. Uptake of OCCs to medaka

The uptake profiles of OCCs to medaka are shown in [Fig. 2](#). The presence of nC_{60} affected the dynamic bioaccumulation of OCCs to medaka. For the less hydrophobic HCHs and HCB ($\log K_{OW} < 6$), an overall trend to a minor enhancement of bioaccumulation was observed in the presence of 5 mg L^{-1} nC_{60} compared to its absence ([Fig. 2a](#)). ANOVA tests indicated that in most cases there was no statistically significant difference for the mean C_{fish} of each analyte between the solutions with and without nC_{60} ($p > 0.05$, $n = 3$ for each group), except for the case of δ -HCH with an exposure time of 5 d and for HCB with an exposure time of 12 d. For the highly hydrophobic DDTs and PCB77 ($\log K_{OW} > 6$), however, an overall trend showing a significant reduction of the bioaccumulation was observed in the solutions containing nC_{60} relative to those without it at various exposure times (1, 5 and 12 d) ($p < 0.05$, $n = 3$ for each group) ([Fig. 2b](#)). It was noteworthy that, in spite of the relatively high exposure concentration of nC_{60} (5 mg L^{-1}) compared with that usually detected in the real aquatic environment (ng L^{-1} – $\mu\text{g L}^{-1}$) (Koelmans et al., 2009), the impact on OCCs uptake in fish was not very dramatic (maximally a factor of 2, even for the most hydrophobic compounds). Thus, environmentally relevant ng L^{-1} or $\mu\text{g L}^{-1}$ nC_{60} concentrations will have hardly or no effect on exposure to HOCs.

The overall impact of nC_{60} on the dynamic bioaccumulation of OCCs in medaka comes from a combination of the following two effects. On the one hand, the presence of nC_{60} can reduce the C_{free} of a chemical and thus the diffusive uptake of its non-bound form. As shown in [Fig. S2](#), the free fraction (ratio of $C_{\text{free},nC60}$ to $C_{\text{free},\text{water}}$) decreased slightly for OCCs with $\log K_{OW} < 6$ ($C_{\text{free},nC60}/C_{\text{free},\text{water}} = 0.75$ – 0.88), while decreasing dramatically for OCCs with

Fig. 2. Effect of aqueous suspensions of fullerene (nC_{60}) on the bioaccumulation of organochlorine compounds to medaka ($n = 3$). An asterisk indicates exposures that are significantly elevated or depressed relative to the corresponding " C_{60} 0 mg L⁻¹" exposure ($p < 0.05$). (a) The less hydrophobic HCHs and HCB ($\log K_{OW} < 6$); (b) the highly hydrophobic DDTs and PCB77 ($\log K_{OW} > 6$). ■ = blank; □ = C_{60} 0 mg L⁻¹; ▨ = C_{60} 5 mg L⁻¹.

$\log K_{OW} > 6$ ($C_{free+C60}/C_{free+water} = 0.11\text{--}0.37$). On the other hand, OCCs may be taken up together with the particles as it was re-

ported that fullerenes can penetrate both cell membranes (Foley et al., 2002) and living tissues (Rouse et al., 2007; Xia et al.,

2009) or the uptake kinetics of OCCs might be accelerated by nC_{60} ; any of the above two processes can enhance the bioaccumulation of the OCCs. Bioaccumulation studies with phenanthrene in *Daphnia magna* indicated that nC_{60} enhanced the steady-state bioaccumulation by 1.7 times, also suggesting the nC_{60} associated phenanthrene was bioavailable (Baun et al., 2008). The diffusivity of nC_{60} that are 2–3 orders of magnitude larger than the tested chemicals will have diffusion coefficients that are more than one order of magnitude lower than the free chemicals. With a maximum bound fraction under 88% in 5 mg L^{-1} of nC_{60} (Fig. 1), the contribution of the bound fraction to the kinetics purely based on diffusion will not exceed a factor 2. Whether nC_{60} enhance or reduce the transport of compounds to the hydrophobic phase depends mostly on the behavior of particles. If nC_{60} does not enter the hydrophobic phase, the extraction/accumulation processes will not become slower because the rates scale with the concentration of the extracted species, i.e. the free form of the compound. If a fraction of very small nC_{60} does enter the hydrophobic phase, then their lower diffusion coefficient will tend to slow down the equilibration. As a result, the apparent kinetic rate constants can be either enhanced by the non-entering nC_{60} facilitated transport or reduced by the slow diffusion of nC_{60} with adsorbed target compounds (Mayer et al., 2007). For the DDTs and PCB77, the decrease of C_{free} plays the major role and thus results in reduced bioaccumulation. For HCHs and HCB, the possible enhancement of the dynamic uptake kinetics might play a major role, so that the bioaccumulation was slightly enhanced; however, further work needs to be done in this aspect.

3.4. Correlation between C_{fish} and C_{fiber} of OCCs

The relationship between the concentrations of eight individual OCCs in medaka (C_{fish}) and that in SPME fiber (C_{fiber}) was studied at 0 and 5 mg L^{-1} nC_{60} level, respectively. The correlation of C_{fish} at 12-d bioaccumulation with C_{fiber} at 12 d accumulation was not significant ($p > 0.05$) due to the accumulation being far from equilibrium for the fish but near equilibrium for the fiber. Therefore, the kinetic SPME accumulation data at 1 and 3 d were adopted for the correlation study (Fig. 3a–b). It was observed that the correlation of C_{fish} at 12-d bioaccumulation with C_{fiber} at 1 d and 3 d was pronounced with $p = 0.007$ – 0.013 and $R^2 = 0.666$ – 0.723 in the nC_{60} absent solutions, whereas this correlation became much worse ($R^2 = 0.423$ – 0.440) and was not significant at 95% confidence level ($p = 0.073$ – 0.081) in the presence of 5 mg L^{-1} nC_{60} . The pronounced correlation of C_{fish} with C_{fiber} in the nC_{60} absent solutions demonstrated the validity of nd-SPME for evaluating the bioavailability of OCCs. The lack of correlation between C_{fish} and C_{fiber} of OCCs in nC_{60} also suggests that the uptake mechanism of OCCs to medaka is different from that to nd-SPME fiber in nC_{60} suspensions, i.e. rather than only the freely dissolved OCCs is available for the SPME fiber, the nC_{60} -bound OCCs might be available for medaka; this would in turn limit the ability of nd-SPME to mimic the dynamic uptake of a chemical to biota in nC_{60} suspensions.

3.5. Prediction of equilibrium bioaccumulation of OCCs in nC_{60} with nd-SPME

Using the k_2 value for marine fish (0.002 d^{-1}) reported in the literature (Wang and Wang, 2005), the equilibration time for the accumulation of highly hydrophobic DDT in fish was estimated by $t_{\text{eq}} = \ln(0.10) \times (-k_2) = 1151 \text{ d}$. Even though nC_{60} could enhance the uptake kinetics of OCCs to medaka and thus reduce t_{eq} , the equilibration time would still be very long. Moreover, large amounts of OCC-spiked nC_{60} solutions that were very tedious to prepare had to be used in the medaka exposure experiment. These lead to severe obstacles in estimating the equilibrium concentra-

Fig. 3. The concentration of eight individual organochlorine compounds in medaka after bioaccumulation for 12 d (C_{fish}) versus that in the SPME fibers after extraction for 1 and 3 d (C_{fiber}) in solutions containing 0 and 5 mg L^{-1} nC_{60} , respectively. (a) 0 mg L^{-1} nC_{60} ; (b) 5 mg L^{-1} nC_{60} .

tion in medaka via exposure experiments. Although passiving dosing might solve these problems in fish exposure (Ter Laak et al., 2009), in this present study equilibrium partitioning concentrations of OCCs in medaka ($C_{\text{fish,eq}}$) were predicted by $C_{\text{fish,eq}} = K_{\text{lipid,PDMS}} \times C_{\text{fiber,eq}}$ (Eq. (S5) in Supplementary material), where $C_{\text{fiber,eq}}$ is the measured OCC equilibrium concentrations in the nd-SPME PDMS fiber and $K_{\text{lipid,PDMS}}$ is the partition ratio of OCCs between the medaka lipid and the PDMS fiber (Table 1). The 12 d bioaccumulation in medaka ($\log C_{\text{fish,12d}}$ (measured)) was plotted against the corresponding predicted equilibrium concentration ($\log C_{\text{fish,eq}}$ (predicted)) for the OCCs in solutions with and without nC_{60} (Fig. 4). As expected, the data points for the most hydrophobic OCCs were well below the perfect fit line (1:1 line) due to the non-equilibrium bioaccumulation to the fish, whereas the HCH isomers were close to the perfect fit line. Moreover, the difference between solid and open symbols for each compound indicated the effect of the nC_{60} , i.e. nC_{60} only slightly increased the bioaccumulation of OCCs with $\log K_{\text{OW}} < 6$ (e.g. HCHs and HCB), but remarkably reduced the bioaccumulation of OCCs with $\log K_{\text{OW}} > 6$.

Fig. 4 indicates that for all the studied OCCs the measured C_{fish} at non-equilibrium was lower than the nd-SPME predicted $C_{\text{fish,eq}}$ in both water and nC_{60} , except for δ -HCH with slightly higher C_{fish} than the predicted $C_{\text{fish,eq}}$. This result implies that nd-SPME may be applicable for predicting the equilibrium bioaccumulations in

Fig. 4. Measured concentrations of organochlorine compounds in medaka after 12 d exposure (C_{fish} (measured)) are plotted against predicted equilibrium concentrations in this fish ($C_{\text{fish,eq}}$ (predicted)) as estimated with equilibrium SPME. Open symbols refer to bioaccumulation from water and solid symbols refer to bioaccumulation in solutions containing $5 \text{ mg L}^{-1} \text{ nC}_{60}$. Distances between open and closed symbols indicate the effect of the nC_{60} , and vertical distances to the perfect fit line indicate how far the measured concentrations are from the predicted equilibrium level.

nanoparticle suspensions. However, this needs to be confirmed by additional long-term exposure tests. Theoretically, the applicability of nd-SPME for predicting the equilibrium bioaccumulations in nanoparticle suspensions depends on the possibility of bioaccumulation of nanoparticles to biota. In cases where the nanoparticles were accumulated by an organism, the chemicals bound to them can also be accumulated; thus, nd-SPME is invalid as the nanoparticles are not accumulated and the freely dissolved chemicals only are accumulated in SPME fibers (Benhabib et al., 2009).

4. Conclusions

The results of this study suggest that the release of nC_{60} at mg L^{-1} level into the environmental aqueous system could alter the exposure of aqueous organisms to OCCs. The presence of nC_{60} reduced the C_{free} of OCCs as well as the uptake rate constants for SPME, which was limited to the diffusive uptake of non-bound OCCs. The overall effect of nC_{60} on the bioaccumulation of OCCs in aquatic organisms will depend on whether the reduction of the diffusive uptake of non-bound OCCs or the additional uptake of the bound OCCs was predominant. The present study suggests that nC_{60} can both reduce and enhance the bioaccumulation of OCCs, and future research should be directed at identifying and studying exposure scenarios in which nanoparticles are likely to enhance the uptake of OCCs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2010.05.042.

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