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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · APRIL 2013

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Risks of Single-Walled Carbon Nanotubes Acting as Contaminants-Carriers: Potential Release of Phenanthrene in Japanese Medaka (*Oryzias latipes*)

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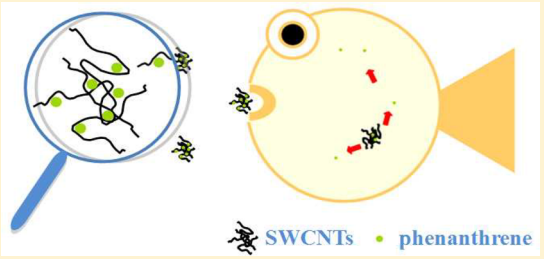
Supporting Information

ABSTRACT: The performance of carbon nanotubes (CNTs) acting as contaminants-carriers in vivo is critical for understanding the environmental risks of CNTs. In this study, the whole-body accumulation and tissue distribution of phenanthrene in Japanese medaka was examined in the presence of single-walled carbon nanotubes (SWCNTs) and the potential release of phenanthrene was investigated from two types of SWCNTs suspensions that differed in surface charge and stability. The results showed that the coexistence of SWCNTs facilitated the accumulation of phenanthrene in the digestive track of fish and therefore enhanced the whole-body phenanthrene concentration by 2.1 fold after exposure for 72 h. Meanwhile, 6.4−48 and 20−34 times higher phenanthrene concentrations were measured in the liver and brain of fish exposure to the two mixtures, respectively, when comparing with the phenanthrene alone treatment with equal concentration of soluble phenanthrene. The extra phenanthrene was from the SWCNTs-associated phenanthrene that accumulated in the digestive track indicating the release of phenanthrene from SWCNTs did occur in fish. Moreover, the neutrally charged SWCNTs showed different agglomeration behaviors from the negatively charged SWCNTs, which could affect the accumulation of SWCNTs in the digestive track of fish and subsequently influence the retention of phenanthrene associated with the carbon nanotubes.



INTRODUCTION

With the rise of nanotechnology, the unique physicochemical properties of carbon nanotubes (CNTs) have expanded their application in industrial and consumer products.[1] The surge in production of CNTs will increase the accessibility of nanoparticles (NPs) to the environment. The safety issues of CNTs have aroused great attention among researchers from human-health-related studies[2,3] to concerns with environmental organisms.[4−6] One of the environmental risks of CNTs is their potential ingestion by the ecological receptors,[7,8] and the surface properties might have impacts on the biological uptake and depuration of NPs.[9−11]

Regardless of the environmental behaviors of CNTs themselves, CNTs with large surface area would affect the transport, fate, and bioavailability of organic contaminants in the environment,[12−14] such as polycyclic aromatic hydrocarbons (PAHs).[15,16] Previous studies have shown reversible adsorption of PAHs on CNTs,[17] and the presence of biomolecules in the simulated digestive fluids could enhance the desorption of phenanthrene on CNTs[18] implying the CNTs-associated pollutants could be bioavailable. However, little is known about the release of organic contaminants from CNTs in vivo. Unlike CNTs, the desorption hysteresis of PAHs[17] and 17α-ethinylestradiol[19] was observed for fullerene due to the rearrangement of fullerene aggregates and the penetration of sorbate into the closed interstitial spaces.[17] Recently, Park et al. reported that the association with fullerene could reduce the bioavailability of 17α-ethinylestradiol in zebrafish.[20,21] These studies indicated that NPs might have impacts on the bioavailability of NPs-associated contaminants and the extent to which would partly depend on the adsorption−desorption. Therefore, bridging adsorption of organic contaminants on CNTs and the bioavailability of CNTs-associated organic contaminants are critical for understanding the environmental risks of CNTs.

In the aquatic environment, ubiquitous PAHs could be taken up by fish and the distribution of PAHs was different among tissues.[22,23] CNTs are supposed to interact with PAHs and then be ingested by fish. It is possible that CNTs-associated PAHs are primarily present in certain tissues and then will be distributed to other tissues if PAHs are released from CNTs with their digestion by fish. In the present study, two types of SWCNTs suspensions with different surface charge and stability were prepared; phenanthrene and Japanese medaka (*Oryzias latipes*) were selected as a model organic contaminant and a test organism, respectively. The adsorption−desorption of phenanthrene on SWCNTs was examined. Moreover, the

Received: November 2, 2012
Revised: April 1, 2013
Accepted: April 11, 2013
Published: April 11, 2013

dx.doi.org/10.1021/es304479w | Environ. Sci. Technol. 2013, 47, 4704−4710

Table 1. Representative Characterization Data of Solid SWCNTs and SWCNTs Suspensions

	surface area and pore structure			surface charge			surface acidic group content (mmol/g)			
sample	surface area[a] (m^2/g)	mesopore diameter[b] (nm)	mesopore volume[b] (cm^3/g)	pH	concentration (mg/L)	ζ[c] (mV)	hydroxyl groups	carboxyl groups	weak acid	total acidic groups
R−S	499	8.0	1.2	7.08	10	−0.40 ± 0.10				
P−S	413	5.9	0.71	7.06	10	−1.9 ± 0.60	ND[d]	0.022	0.023	0.045
SP−S				7.03	8	−51 ± 2.9	ND	0.022	0.028	0.050

[a]Surface area values were calculated by multipoint Brunauer−Emmett−Teller (BET) method. [b]Average mesopore diameter and volume were calculated from desorption isotherms by the Barrett−Joyner−Halenda (BJH) method. [c]ζ is zeta potential. [d]ND, not detectable.

effects of SWCNTs on the whole-body accumulation and the tissue distributions of phenanthrene were assessed, and the release of phenanthrene from SWCNTs in fish was tested and verified.

MATERIALS AND METHODS

Synthesis and Characterization of SWCNTs. SWCNTs synthesized by the CVD method were purchased from Shenzhen Nanotech Port Co. Ltd. of China. The raw SWCNTs (R−S) were purified by using a modified method described by Liu et al.[24] Briefly, a raw sample of nanotubes (5 g) was refluxed in 600 mL of 2.6 M nitric acid for 11 h, filtered, and washed with deionized water. Standard suspensions (1 mg/mL) of SWCNTs were prepared by bath sonicating 1 mg of P−S with 1 mL of 25 mg/mL PEG solution for 30 min. Bath and probe sonication was performed to prepare two types stock suspensions of purified SWCNTs (15 mg/L) respectively. For preparation the P−S suspensions, nanotubes were bath sonicated for 30 min with standard medium (290 mg/L $CaCl_2$, 120 mg/L $MgSO_4{\cdot}7H_2O$, 65 mg/L $NaHCO_3$ and 6 mg/L KCl). For preparation better dispersions of P−S, nanotubes were probe-sonicated for 3 h (ensuring all precipitates became suspended) with the standard medium by using an ultrasonic processor (Shangchao FC-450 V, China) operated at 400 W. Probe sonication was conducted by using a 20 s on/ 60 s off pulse sequence with a 20 mm diameter probe tip. The prolonged and intensive probe sonication made P−S present in smaller suspended SWCNTs, which were denoted as SP−S.

The solid SWCNTs samples were observed directly using a scanning electron microscope (SEM) (Leo 1530, Germany). SWCNTs were dispersed and then were dropped on the copper grids and dried for transmission electron microscope (TEM) observation (Hitachi 7500, Japan). Raman spectra of SWCNTs were obtained using a Renishaw RM 1000 microscopic confocal Raman spectroscope equipped with a laser excitation of 633 nm (He−Ne laser). Thermal gravimetric analysis (TGA) (Netzsch STA 409 C131F, Germany) was conducted under a flow of air and heated to 1000 °C at a rate of 10 °C/min. Surface acidic groups of SWCNTs were measured by Boehm titration method[25] using a 716 DMS automatic potential titrator (Metrohm, Switzerland). Zeta potential of SWCNTs suspensions was determined using a Malvern Zetasizer 2000 instrument. Nitrogen adsorption−desorption isotherms of SWCNTs (Figure S5 of the Supporting Information) were measured using a Micromeritics ASAP 2000 instrument. Surface area was calculated by multipoint Brunauer−Emmett−Teller (BET) method, and the average mesopore diameter and volume were calculated from the desorption branches of the isotherms by the Barrett−Joyner−Halenda (BJH) method.

Chemicals. Phenanthrene (99.8% purity, Sigma−Aldrich) was dissolved in DMSO to prepare a stock solution (1 g/L). All chemicals needed to prepare a SDS-PAGE gel were purchased from Amresco Inc.

Test Organism. Japanese medaka (*Oryzias latipes*) was selected from a brood stock that originated from the Laboratory of Freshwater Fish at the Bioscience Center of Nagoya University of Japan. Fish were cultured in charcoal-dechlorinated tap water (pH 7.2−7.6) at 25 ± 1 °C (16:8 h light/dark photoperiod) and were fed with brine shrimp once a day. Four month old fish with an average length of 38 ± 2 mm weighing 600 ± 100 mg (n = 200) were used. Prior to the exposure experiment, selected fish were cultured in the standard medium overnight without feeding.

Exposure Assay. Prior to the start of exposure, adsorption and desorption of phenanthrene on the two types of SWCNTs suspensions were investigated at 25 °C by batch experiments (procedures are described in the Supporting Information). Also, preadsorption of phenanthrene on SWCNTs suspensions was conducted. Briefly, following pH adjustment to 7 by 0.1 M NaOH, 15 mg/L SWCNTs stock suspensions and 60 μg/L phenanthrene solution were mixed in aluminum foil wrapped glass bottles. Then, the bottles were sealed with aluminum foil-lined screw caps and shaken at 150 rpm (25 °C) for 5 days to reach adsorption equilibrium. After centrifugation (3000 g for 5 min), the concentrations of soluble phenanthrene were measured (procedures are described in the Supporting Information). The bottles without addition of carbon nanotubes were used for phenanthrene loss assessment, which showed that the loss was less than 3% of the initial concentration. Therefore, phenanthrene removed from the aqueous phase was adsorbed by SWCNTs.

When the adsorption equilibrium was reached, 200 mL of a mixture of SWCNTs and phenanthrene was added in an amber bottle (250 mL), one fish was transferred into it, and then the bottle was covered with a glass stopper and stored at 25 ± 1 °C (16:8 h light/dark photoperiod). Fish were exposed to solutions proximately containing 3 and 60 μg/L phenanthrene (prepared with the standard medium) as controls. In total, there were four treatments: [1] Three μg/L phenanthrene alone, Phe(3); [2] 60 μg/L phenanthrene alone, Phe(60); [3] Phe(60) + P−S; [4] Phe(60) + SP−S. The concentrations of soluble phenanthrene in each treatment are listed in Table 2. Ten fish were exposed for each treatment at each exposure time. The mixtures or the phenanthrene solutions were not

Table 2. Concentrations of Soluble Phenanthrene in Each Treatment

treatment	Phe(60)	Phe(60) + P−S	Phe(60) + SP−S	Phe(3)
soluble phenanthrene concentration (μg/L)	59.5 ± 1.8	2.9 ± 0.3	2.9 ± 0.5	2.8 ± 0.2

dx.doi.org/10.1021/es304479w | Environ. Sci. Technol. 2013, 47, 4704−4710

renewed during the whole experiment. The SWCNTs settling experiments were conducted (procedures are described in the Supporting Information) to evaluate the stabilities of the two mixtures during the three days. Moreover, the bottles merely containing phenanthrene solution without fish were used for phenanthrene loss assessment, which showed that the loss was less than 8% of the initial concentrations at 72 h.

At each time point (1, 4, 12, 24, and 72 h), 10 bottles in each treatment were removed, all fish were sacrificed in an ice−water bath, and then placed on filter papers to get rid of the surface water on their body and weighted. Five fish were stored at −80 °C for measurement of the whole-body phenanthrene levels. The remaining five fish were dissected; the main tissues (liver, digestive track, gill, and brain) were weighted and then stored at −80 °C for measurement of the tissue contents of phenanthrene. The presence of a black substance was checked via an optical microscope, and SWCNTs were mainly found in the digestive track of fish, which were confirmed by the Raman spectroscope afterward. To minimize the fluorescence interference originated from the tissue, the black substance in the digestive track was squeezed out and dried. The Raman spectra were excited with a 514 nm laser line.

Extraction of Phenanthrene and SWCNTs. The sampled whole body and tissues of fish were homogenized with 400 μL of 0.1 M PBS buffer (pH 7.4) in ice−water bath and then were mixed with 200 μL of 1 mM $CaCl_2$, 200 μL of 1 mM $MgCl_2$, and 100 μL of 10% SDS to break down the tissues[26] and to ensure cell lysis.[27] Then, 1 mL of acetonitrile (HPLC grade) was added into the homogenates. After bath sonication for 45 min at 20 °C, the samples were stored at 4 °C for 24 h. Following phenanthrene extraction, the samples were centrifuged for 10 min at 10 000 *g* and the supernatants were used for HPLC analysis of the concentration of phenanthrene. The pellets of the digestive track were kept for quantification of SWCNTs. The content of phenanthrene was calculated by the wet weight of tissue dividing the mass of phenanthrene in the tissue. Recovery checks were performed to validate the procedure for phenanthrene extraction. The sampled tissues were spiked with 100 μL of phenanthrene stock solution and then were treated in the same manner. The calculated extraction recovery was 63−70% for phenanthrene.

Quantification of SWCNTs in the Digestive Track of Fish. The mass of SWCNTs in the digestive track of fish was quantified using a modified method described by Wang et al.[27] A 30% polyacrylamide gel stock solution was prepared by mixing acrylamide and bisacrylamide in a ratio of 29:1. Each gel had two layers: an upper larger pore stacking gel (6% polyacrylamide, pH 6.8) and a lower smaller pore resolving gel (10% polyacrylamide, pH 8.8). Following extraction of phenanthrene, the pellets of the digestive track were homogenized with 100 μL of 5% sulfosalicylic acid and diluted 2 fold with 20% glycerol, and then 10 μL of samples were loaded into wells of the gel. After electrophoresis at 100 V for 30 min, the optical images of the gel were acquired in Epi White mode using a Gel Doc XR imaging system (Bio-Rad, U.S.A). The pixel intensities of the digitized gels were quantified by using the Quantity One 1-D software. The intensities of all samples were background-subtracted from the intensities of control gels that did not contain SWCNTs. The mass of SWCNTs in samples was quantified by SWCNTs calibration curves (Figure S8 of the Supporting Information) prepared with the SWCNTs standard suspensions. The content of SWCNTs was calculated by the wet weight of tissue dividing the mass of SWCNTs in the tissue. For preparation the SWCNTs calibration curves, the SWCNTs standard suspensions were diluted 20 fold with 20% glycerol and were subsequently loaded into the wells of the gel with various volumes. The SWCNTs-containing samples should be diluted to meet the limit of detection and should be bath sonicated before being loaded into each well of the gel.

Statistical Analysis. All data are expressed as means and standard deviations. One-way ANOVA with Tukey's multiple comparison test was used to determine the statistical differences in phenanthrene content among treatments. Statistical difference was set at $p < 0.05$.

RESULTS AND DISCUSSION

Structure and Surface Properties of SWCNTs. SEM micrographs showed the granulate impurities in the R−S aggregates were removed through purification treatment (parts a and b of Figure S1 of the Supporting Information), and the content of catalytic metal impurities in P−S was reduced to 6% according to thermal gravimetric analysis (parts a and b of Figure S2 of the Supporting Information). The prolonged and intensive sonication procedure was effective to elevate the stability of SWCNTs in aqueous phase without damaging the structure of nanotubes because the intensity of the D-band (∼1327 cm^{-1}) in Raman spectra of SP−S representing SWCNTs defects was not significantly changed (part c of Figure S3 of the Supporting Information). The large aggregates of P−S were prone to precipitate ($\zeta = -1.9 \pm 0.60$ mv) and SP−S with more negative zeta potential (−51 ± 2.9 mv) was more stable in water (Table 1), which was confirmed by the adsorption spectra of SWCNTs suspensions measured within 72 h (Figure S4 of the Supporting Information). The absorbance at 800 nm of the suspensions of P−S and SP−S was decreased by 61% and 9% at 72 h, respectively (Figure S4 of the Supporting Information). Therefore, the biological uptake of the two SWCNTs might be different.

Adsorption and Desorpiton of Phenanthrene on SWCNTs. The adsorption process of PAHs on CNTs could be well interpreted by the Polanyi theory.[13] Similarly, a better fitting of adsorption isotherms (Figure S7 of the Supporting Information) by the Polanyi−Manes model (PMM) than Freundlich and Langmuir models was indicated by the lower mean weighted square error (MWSE) and the higher relative coefficients (r^2) (Table S2 of the Supporting Information). The high adsorption capacity for phenanthrene (200−251 mg/g) (Table S2 of the Supporting Information) indicates SWCNTs may have impacts on the transport and fate of phenanthrene once released to the environment. Dispersion of large aggregates to smaller aggregates is expected to increase the surface area of CNTs and consequently promotes the adsorption of organic contaminants.[28] This could be applicable for interpreting the slightly higher phenanthrene adsorption capacity of SP−S than that of P−S (Figure 1). The isotherms of P−S and SP−S did not show significant adsorption−desorption hysteresis (Figure 1), which were in agreement with the results reported by Yang and Xing.[17] The intra-aggregates spaces and intertube spaces in CNTs may become pathways and sorption sites for PAHs;[17] however, these spaces were not closed[17] and therefore reversible adsorption of phenanthrene on SWCNTs occurred (Figure 1). Desorption of phenanthrene indicates the potential release of phenantherene from SWCNTs when taken up by fish.

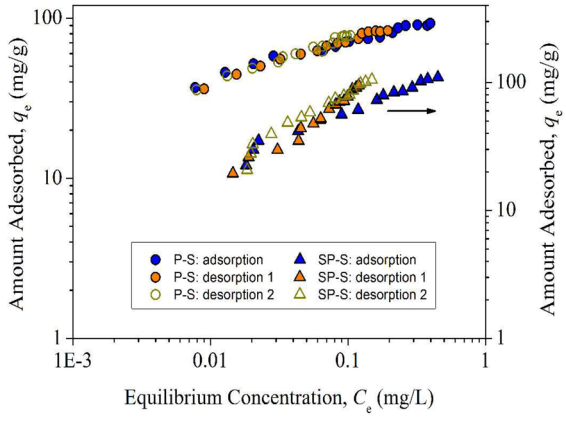


Figure 1. Adsorption−desorption isotherms of phenanthrene by SWCNTs. Data points are mean values that were calculated from triplicate samples.

Whole-Body Phenanthrene Levels in Fish with/without SWCNTs. The measured whole-body levels of phenanthrene in Japanese medaka are shown in Figure 2. For fish that

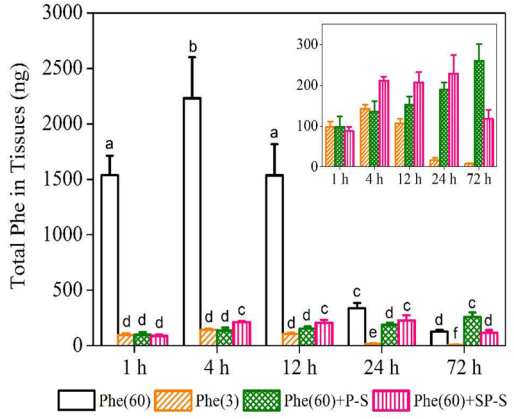


Figure 2. Whole body levels of phenanthrene in Japanese medaka with or without addition of SWCNTs. Mean and standard deviation values were calculated from five replicate samples. Bars with different letters above them are significantly different (one-way ANOVA with Tukey's multiple comparison test, $p < 0.05$). Inset, enlarged view of the data presented in the larger figure.

were exposed to Phe(60), the whole-body phenanthrene level (2000 ± 300 ng wet mass) reached a steady-state concentration at 4 h and then gradually decreased as a function of time. Valdez Domingos et al. have shown that phenanthrene could be eliminated rapidly by *Fundulus heteroclitus* and the elimination half-lives was 2.3 ± 0.3 d.[23] The presence of SWCNTs significantly reduced the whole-body phenanthrene levels in fish at the beginning 12 h, for Phe(60) + P−S and Phe(60) + SP−S, the whole-body phenanthrene levels were decreased by 90% and 86%, respectively ($p < 0.05$). This might be due to the concentration-dependent bioaccumulation of phenanthrene,[29,30] and the soluble phenanthrene concentrations in the mixtures were significantly decreased by 96% through preadsorption (Table 2). However, after exposure to Phe(60) + P−S for three days, the whole-body phenanthrene level in fish increased to 200 ± 40 ng wet mass, which was significantly ($p < 0.05$) higher than those of fish exposure to other treatments (Figure 2).

These phenomena were somewhat unsurprising as several studies have noted that carbon nanoparticles could decrease the concentrations of organic contaminants in organisms that lived in soils or sediments as other carbon sorbents (e.g., activated carbon).[31−34] Petersen et al. observed that the bioaccumulation factor values of pyrene in *Eisenia fetida* were decreased by 25−50% upon 3 mg/g CNTs amendment.[35] Similarly, Feguson et al. reported that the presence of 5 mg/g SWCNTs could significantly reduce the bioaccumulation of PAHs in *Streblospio benedicti*.[36] In addition, Shen et al. have shown that the decrease rate of biota-sediment accumulation factor of PAHs changed after increasing the CNTs concentration to 1.5%.[37] However, different findings might be observed during exposure in the water column because NPs could facilitate the bioaccumulation of environmental pollutants by acting as contaminants-carriers.[38−40] Baun et al. confirmed that the steady-state concentration of phenanthrene in *Daphnia magna* was increased by 1.7 fold upon exposure to 3 mg/L fullerene suspensions.[41] Thus, questions are raised on the potential release of contaminants if the NPs-associated contaminants are taken up by organisms, especially under the influence of biomolecules (e.g., pepsin and bile salt) presented in digestive fluids.[18]

To investigate the release of phenanthrene from SWCNTs in vivo, fish were exposed to the phenanthrene alone with similar concentrations of soluble phenanthrene to the mixture of phenanthrene and SWCNTs. After preadsorption, the soluble phenanthrene either in Phe(60) + P−S or in Phe(60) + SP−S was 2.9 ± 0.5 μg/L; therefore, Phe(3) with 2.8 ± 0.2 μg/L soluble phenanthrene was performed as a control for the two mixtures (Table 2). The expected results were: (1) the whole-body phenanthrene level in fish that was exposed to the mixture should be not less than that of fish treated by phenanthrene alone because both of them had equal mass of soluble phenanthrene; and (2) the contents of phenanthrene in specific tissues of fish might be different between the mixture and phenanthrene alone if extra mass of phenanthrene that was associated with SWCNTs (38−59 μg) released from nanotubes (the SWCNTs themselves did not release detectable concentrations of phenanthrene). After exposure for 24 h, the whole-body phenanthrene levels in fish that were exposed to Phe(60) + P−S and Phe(60) + SP−S were 9.4 and 18 times higher than that of fish treated by Phe(3), respectively. The results were consistent with our prediction and the increases in the whole-body phenanthrene levels should come from the SWCNTs-associated phenanthrene. The potential release of phenanthrene would be further analyzed through the tissue distribution of phenanthrene in fish.

Tissue Distribution of Phenanthrene in Fish with/without SWCNTs. After exposure for 1 h, phenanthrene could be measured in selected tissues of all fish (Figure 3). For fish that were exposed to phenanthrene alone, the contents of phenanthrene in all tissues reached steady-state concentrations at 4 h; the liver and digestive track phenanthrene levels ranged from 0.2 to 21 ng/mg wet and 0.6−12 ng/mg wet, respectively; and the gill and brain phenanthrene levels ranged from 0.3 to 12 ng/mg wet and 0.4−14 ng/mg wet, respectively (Figure 3). After that, the phenanthrene contents in all tissues decreased as a result of metabolism, 90−97% reduction was found in all tissues of fish treated by Phe(60) at 72 h (Figure 3). The main uptake routes for organic contaminants' access to fish are sorption on gills and ingestion into digestive track through the mouth. Parent contaminants adsorbed by the gill will be

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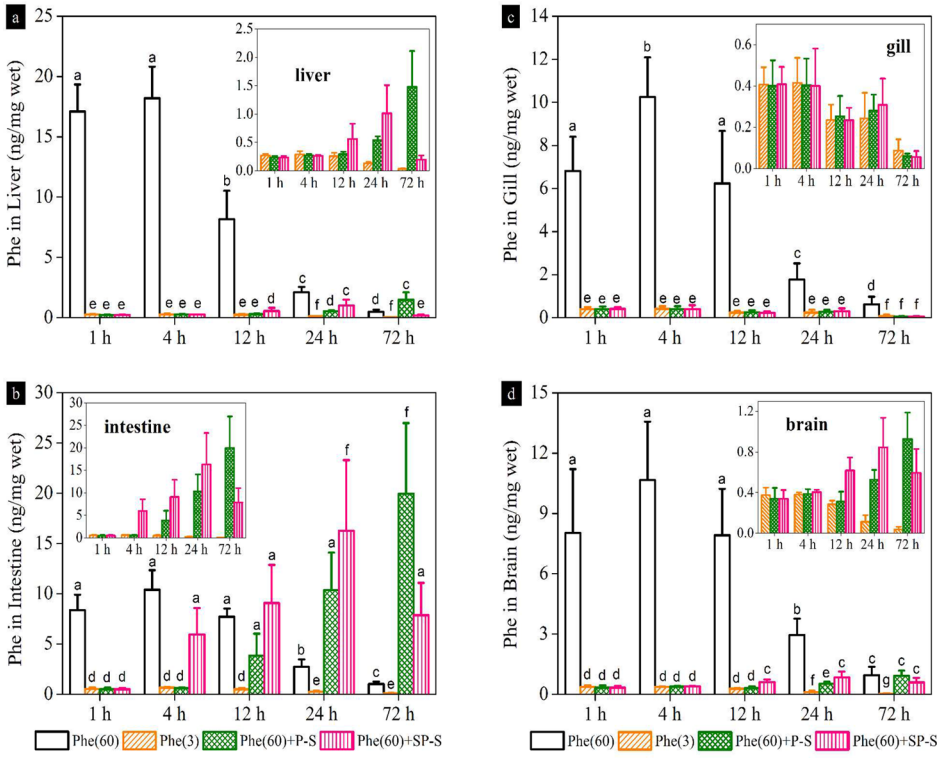

Figure 3. Tissue distribution of phenanthrene in Japanese medaka with or without addition of SWCNTs. Mean and standard deviation values were calculated from five replicate samples. Bars with different letters above them are significantly different (one-way ANOVA with Tukey's multiple comparison test, $p < 0.05$). Inset, enlarged view of the data presented in the larger figure.

transported through the whole body with the arterial blood flow and then will be sent back to the heart by the venous blood flow, passing through and being metabolized in the urinary and hepatobiliar (liver−bile−intestine) system; through oral intake, parent contaminants get into the digestive track and then will be metabolized in the same way.[23,42] The soluble phenanthrene could be directly taken up, metabolized, and eliminated by fish, whereas for the SWCNTs-associated phenanthrene the accumulation of which would largely depend on the ingestion and elimination of SWCNTs. In comparison with Phe(60), the coexistence of SWCNTs enhanced the accumulation of phenanthrene in the digestive track, and the digestive track phenanthrene concentrations were increased by 3.3−6.7 fold upon the two mixtures exposure for 24 h ($p < 0.05$) (part b of Figure 3). Meanwhile, phenanthrene levels in the digestive track of fish that were exposed to Phe(60) + P−S and Phe(60) + SP−S were 40 and 67 times higher than that of fish treated by Phe(3), respectively ($p < 0.05$) (part b of Figure 3). Therefore, it is likely that phenanthrene would be released from SWCNTs with the retention and digestion of carbon nanotubes by fish.

With equal concentrations of soluble phenanthrene, the presence of the SWCNTs-associated phenanthrene did not significantly affect the contents of phenanthrene in selected tissues (except digestive track) in the beginning 4 h exposure (Figure 3). However, after exposure to Phe(60) + P−S and Phe(60) + SP−S for 24 h, the liver and brain phenanthrene concentrations were significantly ($p < 0.05$) increased by 4.0−10 and 3.5−12 fold when comparing to Phe(3), respectively (parts a and d of Figure 3). The results indicated the release of phenanthrene from SWCNTs did occur in Japanese medaka. Another question came up − where did the extra amounts of phenanthrene come from? As shown in part c of Figure 3, there was no significant difference among the contents of phenanthrene in the gills of fish that were exposed to the three treatments with similar soluble phenanthrene concentration. Therefore, the extra phenanthrene in the liver and brain should come from the SWCNTs-associated phenanthrene that accumulated in the digestive track of fish. Unlike Phe(60) + P−S, with the reduction of the digestive track phenanthrene concentration, the contents of phenanthrene in the liver and brain decreased after exposure to Phe(60) + SP−S for 72 h (Figure 3). Thus, the accumulation of phenanthrene released to the liver and brain was relevant to the retention of the SWCNTs-associated phenanthrene in the digestive track. Moreover, the release potential of phenanthrene from P−S and SP−S in fish was in accordance with the adsorption−desorption isotherms in which reversible adsorption of phenanthrene was observed for both of them (Figure 1).

Ingestion of SWCNTs by Fish and Its Effect on the Accumulation of Phenanthrene. Checked by the optical microscope and Raman spectroscope, SWCNTs were mainly found in the digestive tracks of fish treated by the mixture of phenanthrene and SWCNTs (Figure S9 of the Supporting Information). The results obtained by the SDS-PAGE method showed that SWCNTs could be detected in the digestive track of fish upon Phe(60) + SP−S exposure for 4 h and the contents of SWCNTs in the digestive track reached steady-state concentration at 24 h (Figure 4). While in Phe(60) + P−S, SWCNTs could be measured in the digestive track of fish 12 h later, and then the content of SWCNTs continuously increased to 20 ± 7.0 μg/mg wet at 72 h, which were 1.9 times higher than that of fish treated by Phe(60) + SP−S (Figure 4).

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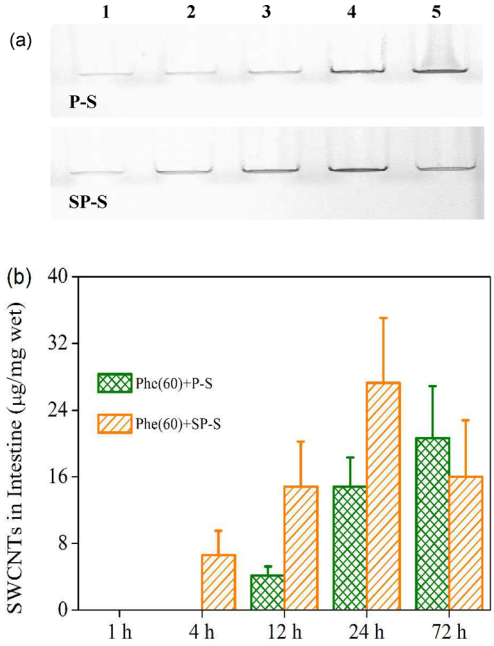

Figure 4. Accumulation of SWCNTs in the digestive track of Japanese medaka. (a) Digital images of SWCNTs-containing tissue samples on the top of the stacking gel. Lane 1−5 represents fish exposure for 1, 4, 12, 24, and 72 h, respectively. (b) The contents of SWCNTs in the digestive track of fish as a function of time. Mean and standard deviation values were calculated from five replicate samples.

The accumulation of P−S in the digestive track was different from that of SP−S, which may be governed by the agglomeration behaviors of SWCNTs. The 72 h settling experiments confirmed that the SP−S was more stable than the P−S in water, that is, the P−S tended to aggregate and precipitate (Figure S5 of the Supporting Information). Fish are filter feeders and therefore the well-dispersed SP−S could be easily taken up and be detected in the digestive track of Japanese medaka after exposure for 4 h. Besides, the surface charge may have contributed to the decrease of SP−S contents in the digestive track of fish. Petersen et al. found that the ingestion and elimination of CNTs were not apparently different among *Daphnia magna* or earthworms that were exposed to CNTs with different surface charge.[9,10] However, Zhu et al. observed that the negatively charged gold nanoparticles were taken up and digested by Japanese medaka more easily than the neutrally charged ones because the negatively charged Au NPs were expected to have electrostatic repulsion with mucus layers that cover the surfaces of the intestine, and the negligible repulsive interaction with the neutral Au NPs could be mediated by cations presented in the mucus layers.[11] In this study, the interaction between the SWCNTs differed in surface charge and the chemicals (e.g., glycoproteins) present in the digestive track may be different, and the negatively charged SP−S were more easily eliminated by Japanese madaka than the neutrally charged P−S.

As mentioned above, the agglomeration behaviors and surface charge may have impacts on the accumulation of SWCNTs and subsequently affect the retention of the SWCNTs-associated phenanthrene in the digestive track of fish. Thus, phenanthrene associated with the easily aggregated and neutrally charged P−S would exist longer than the well dispersed and negatively charged SP−S associated phenanthrene in the digestive track of fish. As a result, the presence of P−S significantly ($p < 0.05$) increased the whole-body phenanthrene levels in fish at 72 h (Figure 2). Moreover, phenanthrene adsorbed on P−S would have a greater chance of being released from carbon nanotubes with the digestion of SWCNTs by fish resulting in an increase of phenanthrene in the liver and brain (parts a and d of Figure 3).

In conclusion, the presence of single-walled carbon nanotubes could facilitate the bioaccumulation of phenanthrene in Japanese medaka, and the increase of whole-body phenanthrene levels could be due to the retention of SWCNTs-associated phenanthrene in the digestive track of fish. With the digestion of carbon nanotubes, phenanthrene could be released from SWCNTs resulting in the increase of phenanthrene concentrations in the liver and brain of fish. The release of phenanthrene from SWCNTs observed in vivo indicates the potential environmental risks of carbon nanotubes acting as contaminants-carriers, and the subsequently biological effects should be assessed. Moreover, the role of agglomeration in the biological uptake and depuration of carbon nanotubes should be further studied because the aggregated SWCNTs may have more adverse effects than the well-dispersed carbon nanotubes.

■ ASSOCIATED CONTENT

Supporting Information

Additional details of experimental methods and results as well as other supporting tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Basic Research Program of China (Grant No.: 2011CB933704) and the National Natural Science Foundation of China (Grant Nos.: 51025830, 21007081, and 50921064). We thank Dr. Jinmiao Zha, Yutao Cheng, and Simin Huang for experimental assistance and all anonymous reviewers for their constructive comments and suggestions.

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