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Embryonic toxicity changes of organic nanomaterials in the presence of natural organic matter

Ki-Tae Kim^{a,b}, Min-Hee Jang^c, Jun-Yeol Kim^a, Baoshan Xing^d, Robert L. Tanguay^b, Byeong-Gweon Lee^e, and Sang Don Kim^{a,*}

^aDepartment of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261 Cheom-dan Gwagi-ro, Buk-gu, Gwangju 500-712, Republic of Korea

^bDepartment of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331, USA

^cEnvironmental Toxicology Team, Korea Institute of Toxicology (KIT), Yuseong, Daejeon, 305-343, Republic of Korea

^dDepartment of Plant, Soil and Insect Science, University of Massachusetts, Amherst, MA 01003, USA

^eDepartment of Oceanography, Chonnam National University, 300 Yongbong-Dong, Gwangju 500-775, Republic of Korea

Abstract

When elucidating the potential fate and bioavailability of nanomaterials (NMs) in an aquatic system, it is important to consider the interactions between NMs and natural organic matter (NOM). The present study compared the toxicities of carbon-based NMs, with disparate physicochemical properties, on Japanese medaka (*Oryzias latipes*) embryos after the addition of NOM. The measured embryonic toxicity parameters were mortality, malformation and hatching delay. Various physicochemical properties of water suspended fullerenes (nC₆₀) and multi-walled carbon nanotubes (MWNTs) were modulated by organic exchange (Tol/nC₆₀), stirring over time (Aqu/nC₆₀) and acid treatment (f-MWNTs) followed by characterization. Tol/nC₆₀ produced relatively more hydrophobic surfaces and exhibited smaller closed spherical agglomerates than Aqu/nC₆₀. Acid-treated f-MWNTs displayed functionalized hydrophilic surfaces compared to raw MWNTs (r-MWNTs). The resultant embryonic toxicities, in the absence of NOM, were ranked in the order: f-MWNTs > Tol/nC₆₀ > Aqu/nC₆₀. As the NOM concentrations were increased, no changes in embryonic toxicities were observed on exposure of Aqu/nC₆₀ or r-MWNTs; whereas, the toxicities were reduced on exposure to Tol/nC₆₀ and f-MWNTs, due to a disappearance of hydrophobic primary spherical aggregates and partial coating, respectively. These data suggest that in the presence of NOM, the morphological differences of NMs, as well as their physicochemical properties, play a significant role in their reactions and subsequent medaka embryonic nanotoxicity.

Corresponding Author: Sang Don Kim, Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology, 261 Cheom-dan Gwagi-ro, Buk-gu, Gwangju 500-712, Korea, Tel: +82-62-715-2445, Fax: +82-62-715-2434, sdkim@gist.ac.kr.

Keywords

Fullerene (nC₆₀); Multiwalled carbon nanotube; Nanotoxicity; Japanese medaka embryos; Natural organic matter

1. Introduction

Due to increasing concerns that engineered nanomaterials (NMs) are adversely affecting organisms, many studies have focused on evaluating their health and environmental risks (Lam et al., 2007; Maynard, 2006; Klaine et al., 2008). While attention has been given to the toxicological effects of pristine materials in solution, we recognize that NMs released into aquatic ecosystems interact with environmental components, such as dissolved or particulate organic matter and various contaminants, and that this may alter their toxicity. In particular, natural organic matter (NOM), a complex and heterogeneous mixture of organic molecules of different molecular weights, has received great attention due to its ubiquitous presence in aquatic systems; its concentration can reach 100 mg/L in freshwater (Paul et al., 2006). It is important to consider the interactions between NMs and NOM when elucidating the potential fate and toxicity of NMs in an aquatic system.

The colloidal behavior of NMs is influenced by NOM, that is, enhanced colloidal stability and mobility of NMs. The aggregation or agglomeration of NMs originating from their intrinsically strong hydrophobicity is overcome by an NOM coating, resulting in stable NMs suspensions. NOM coating increased steric repulsion whose main mechanism is π - π interactions for organic NMs (Hyung et al., 2007; Lin and Xing 2008; Chappell et al., 2009). Secondly, the physicochemical properties of NMs (e.g. size, morphology and surface chemistry) are changed by interactions with NOM (Xie et al., 2008; Pan and Xing, 2008). These changes in the physicochemical properties alter the adsorption capacity of NMs to contaminants (Pan and Xing, 2008). An NOM coating producing a negatively charged surface on the NMs due to polar functionalities make the hydrophobic sites of NMs less favorable to hydrophobic organic chemicals. Multiwalled carbon nanotubes (MWNTs) when dispersed by NOM inhibited the capacity of NOM to complex with inorganic heavy metal (i.e., copper), which liberated more free copper (Kim et al., 2009).

The toxicity of NMs such as MWNTs and fullerene C₇₀ is enhanced by NOM coatings as they became more bioavailable to aquatic organisms and plants due to the formation of stable suspensions when associated with NOM (Lin et al., 2009), as explained above. However, toxicity change pattern seems to be NM-type and test-species dependent. While the toxicity of water suspended fullerene (nC₆₀) coated with NOM to *Escherichia coli* decreased due to indirect contact (Li et al., 2008), the inactivation of *E. coli* was not affected by single-walled carbon nanotubes (SWNTs) associated with NOM (Kang et al., 2009). Nanocopper toxicity was found to decrease with increasing dissolved organic carbon (DOC) concentrations in a *Ceriodaphnia dubia* bioassay; whereas, nanocopper toxicity increased in a MetPLATE bioassay, a bacterial-enzyme toxicity test based on the inhibition of β -galactosidase (Gao et al., 2009). Unfortunately, little studies provide clear reasons on these variations. Further, little systematic information is available on the ecological responses of aquatic species exposed to carbon-based NMs associated with NOM.

Japanese medaka (*Oryzias latipes*) embryos were used to investigate the toxicity of NMs in the absence or presence of NOM. Medaka were chosen for facile husbandry and fecundity in addition to known chemical sensitivity. Recently, we investigated the toxicities of nC₆₀ having different physicochemical properties by using medaka embryos (Kim et al., 2010). The embryonic fish model is of special interest, as it is well-established as a sensitive alternative tool for providing systematic toxicity information and basic biological development processes (Embry et al., 2009).

In the present study, the physicochemical properties of selected carbon-based NMs (i.e., nC₆₀ and MWNTs) were modified and compared with the embryonic toxicity as a function of increasing NOM concentrations. We hypothesized that “the interactions between NMs and NOM were dependent on the different physicochemical properties of NMs”, resulting in different patterns of reduction or enhancement of their toxicity. All NMs were carefully characterized to observe the relationship between the toxicity results and changes in the physicochemical properties caused by NOM association before and during toxicity assessments.

2. Materials and methods

2.1. nC₆₀ preparation

Fullerene (C₆₀, 99.5% purity) was purchased from Aldrich (Milwaukee, WI, USA) and toluene from Fisher Scientific (Pittsburgh, PA, USA), the materials were used as received. Tol/nC₆₀ and Aqu/nC₆₀ were prepared by the organic exchange and stirring over time methods, respectively. Previous studies have shown different physicochemical properties of nC₆₀ produced by solvent exchange (Tol/nC₆₀) and stirring overtime (Aqu/nC₆₀) methods (Xie et al., 2008; Andrievsky et al., 2002; Brant et al., 2005; Duncan et al., 2008). To obtain Tol/nC₆₀, 20 mg of C₆₀ in 10 mL of toluene was prepared, to which 100 mL Milli-Q water was added. Toluene was subsequently removed by probe sonication (CPX 500, Cole Parmer Instrument Company, USA) at 80–100 W for 3 h (25 min cycle, 5 min interruption). Residual toluene was measured using a purge & trap couple to a GC/MS (Shimadzu QP–5050, Japan). Fluorobenzene was used as an Internal Standard in the chemical analysis. Aqu/nC₆₀ was prepared by the stirring over time method, which is accepted as a more environmentally relevant method due to the lack of a co-solvent. 80 mg of C₆₀ was added to 100 mL of Milli-Q water and then vigorously stirred at 500 rpm for 4 weeks. Tol/nC₆₀ and Aqu/nC₆₀ were then filtered using GF/A filters, with a pore size of 1.6 µm (Whatman, Springfield, UK). An oxidation-extraction protocol, using UV–Vis spectrophotometry (UV–1601, Shimadzu, Kyoto, Japan), was employed to determine the concentration of the nC₆₀ stock suspension (Fortner et al., 2005; Xie et al., 2008; Kim et al., 2010). After prepared samples of Tol/nC₆₀ and Aqu/nC₆₀ were destabilized using potassium perchlorate (KClO₄), they were extracted into toluene. The concentrations of each nC₆₀ samples were then determined by UV spectra at 334 nm. The calibration curve was established by extracting known amount of C₆₀ in toluene.

2.2. f- MWNTs preparation

Raw MWNTs (r-MWNTs) (95% purity), which were synthesized by chemical vapor deposition (CVD), were purchased from Hanhwa Nanotech (Seoul, Korea) and used as received. The procedures for acid-treatment to obtain functionalized MWNTs (f-MWNTs) were established on a trial and error basis by modifying previous studies (Ma et al., 2006; Wang et al., 2009). Briefly, 80 mg of r-MWNTs was transferred into aqua regia, i.e. a 3:1 (v/v) ratio of nitric acid and sulfuric acid, and then refluxed after covering with parafilm with magnetic stirring for 12 h at 80–100°C in the hood. After oxidation, the MWNTs aqua regia was sonicated for 3 h to help disperse the f-MWNTs (Bransonic 8510, Bransonic Ultrasonic Corporation, CT, USA). After decanting the liquid portion, the remaining solids were rinsed with Milli-Q water until the pH of the wash water reached approximately 6.5. The solids were then dried overnight at 80°C, with the samples stored in desiccators prior to use. Approximately 20 mg of f-MWNTs in a 50 mL vial were sonicated for 20 min to prepare a f-MWNTs stock suspension for use in the bioassay followed by dilution to desired concentrations.

2.3. NMs-NOM preparation

Suwannee River NOM (SR-NOM) was purchased from the International Humic Substances Society (IHSS) (St. Paul, MN, USA) and used as a representative NOM. NOM dissolved in Milli-Q water was filtered using a 0.22- μ m cellulose membrane filter (Millipore, Bellerica, MA, USA). The total organic carbon (TOC) composition was measured using a TOC analyzer (Sievers, Boulder, CO, USA). The concentrations of NOM were based on the TOC concentrations, with a high TOC concentration NOM solution diluted with Milli-Q water to the desired TOC concentrations. r-MWNTs can be easily precipitated without NOM, meaning they are not bioavailable. An evaluation of the r-MWNTs toxicity was excluded in the absence of NOM because they cannot be dispersed in Milli-Q water.

A NMs suspension-NOM mixture was stirred at a 3 speed for two days to attain equilibrium for the desired TOC concentrations for each of Tol/nC₆₀, Aqu/nC₆₀ and f-MWNTs. In the case of the r-MWNTs-NOM mixture, probe sonication procedures, set-up in our previous study, were followed to obtain consistent concentrations (Kim et al., 2009). The concentration of r-MWNTs was varied by stirring (Hyung et al., 2007). Briefly, a nominal weight of r-MWNTs (30–35 mg) was transferred to 25 mL of the desired concentration of TOC in a 100 mL glass centrifuge tube (Kimble Kontes, Vineland, NJ, USA) and dispersed by probe sonication (Cole Parmer, Vernon Hills, IL, USA) at 30 W for 5–7 min (5 s cycle, 2 s interruption). Four rounds of sonication were conducted after the addition of the 25 mL of NOM. The r-MWNTs-NOM mixture was then placed at room temperature for 24 h to settle any undispersed MWNTs. The actual concentration of the dispersed r-MWNTs was calculated by subtracting the settled r-MWNTs from the initial weight added. The concentration was determined to be approximately 20 mg/L for each desired TOC concentration. Prior to use, the r-MWNTs-NOM were kept at room temperature. All r-MWNTs-NOM mixture concentrations refer to the mass of r-MWNTs dispersed in NOM.

2.4. Characterization of NMs

The particle size, morphology and zeta potential (ζ) of the NMs were characterized in the absence or presence of NOM using dynamic light scattering (DLS, ELS 8000, Otsuka Electronics, Osaka, Japan) and transmission electron microscopy (TEM, JEM-2100, Jeol, Tokyo, Japan). The chemical properties of the NMs suspensions were characterized for weight of metal impurities using thermo-gravimetric analysis (TGA) and for the ratio of the G-band ($\sim 1580\text{ cm}^{-1}$) to the D-band ($\sim 1350\text{ cm}^{-1}$) using a SPEX-1403 laser Raman spectrometer (SPEX Corporation, USA). The elemental composition of the NMs was determined using X-ray photoelectron spectroscopy (XPS, Multilab 2000, Thermo Electron Corporation, USA), and the surface modification measured using Fourier transform infrared spectroscopy (FTIR, Jasco FTIR-460 Plus, Tokyo, Japan), as previously described (Kang et al., 2009; Kim et al., 2009). TGA and XPS measurements were carried out on the r-MWNTs and f-MWNTs only, with FTIR conducted in the liquid phase for Tol/nC₆₀ and Aqu/nC₆₀, as a large solid sample volume is required for nC₆₀ samples. All characterizations were performed at pH 6.8–7.0 or very similar, using Hank's buffer in the bioassay.

2.5. Embryonic toxicity test

Adult Japanese medaka (*Oryzias latipes*) was obtained from Korea Institute of Toxicology (KIT) at the Korea Research Institute of Chemical Technology (KRICT), and cultured at 25 °C in a 16 h light/8 h dark cycle in the laboratory. Reconstituted media including 1200 mg of CaSO₄·2H₂O, 1200 mg of MgSO₄, 1920 mg of NaHCO₃, and 80 mg of KCl in 20 L deionized water was used. Freshly hatched brine shrimp (*Artemia salina*) (Golden West Artemia Inc., Morgan, Utah, USA) was fed twice daily. The embryos were reared in embryo rearing media (ERM) including 1986 mg of NaCl, 60 mg of KCl, 106 mg of CaCl₂·2H₂O, 144 mg of MgSO₄, and 2 mg of methylene blue in 2 L deionized water.

The embryos were partially dechorionated to increase their bioavailability despite the toxicity can be overestimated due to the presence of chorion acting as natural protection (Cheng et al., 2007). The dechorionated embryos using pronase (Villalobos et al., 2000) were transferred into 48-well plates and exposed to 1 mL of the test suspensions, containing Hank's buffer and NOM-free NMs or NMs-NOM mixture. The exposure was conducted on early gastrula stage (Iwamatsu, 2004). The suspensions were renewed daily for 4 days to maintain consistent exposure concentrations. After 4 days, the embryos were transferred into Hank's buffer containing no test suspension. The mortality, morphological malformations and hatching delay were monitored.

For comparison of significant differences of embryonic toxicity in the absence and presence of NOM, statistical analysis were performed using Statview based on a one-way analysis of variance (ANOVA) post hoc test ($p < 0.05$).

3. Results and Discussion

3.1. Characteristics of NMs

NMs exhibiting different physicochemical properties were prepared, as shown in Table 1 and Figures. Tol/nC₆₀ formed closely packed aggregates of small spherical particles;

whereas, Aqu/nC₆₀ formed amorphous mesoscale aggregates. The average size of the Tol/nC₆₀ particles was 226 ± 2.4 nm, and that of the Aqu/nC₆₀ was 245 ± 5.8 nm. However, single particles less than 100 nm size were observed in the DLS size distribution and TEM images of Tol/nC₆₀. The FTIR spectra (Figure S1) indicated that Aqu/nC₆₀ had a hydrophilic surface formed by the adsorption of hydroxyl ions or due to surface-mediated hydrolysis when stirred in water. Two peaks were measured at 1700 and 3400 cm⁻¹, indicating the presence of oxygen moieties, such as OH⁻ and C=O (Pavia et al., 2001; Kim et al., 2009). No chemical modification was observed in the powdered C₆₀, indicating that the detected surface functional groups had not originated from the pristine powdered C₆₀. The UV spectra of Tol/nC₆₀ and Aqu/nC₆₀ were typically those of nC₆₀ (Figure S1); the peaks at 330 and 380 nm represented solvated C₆₀, and the broad band between 400 and 600 nm indicated the aggregated form of C₆₀ (i.e., nC₆₀). f-MWNTs were preferentially functionalized by acid-treatment. The oxygen composition, as indicated from the XPS, was increased from 2.3 to 10.8%, as shown in Table 1 and Figure 1. An oxidized surface modification was confirmed from the FTIR spectra in Figure 1. The peaks at around 1700 and 3700 cm⁻¹, not detected in the pristine r-MWNTs, were observed, indicating the generation of oxygen moieties on the f-MWNTs surface. Several functional groups, such as hydroxyl, carboxyl and carbonyl, have been reported to be introduced onto the surface of r-MWNTs by acid-treatment (Li et al., 2002). Metal impurities, such as cobalt (Co) and iron (Fe), contained within the MWNTs depending on manufacturing process (Lam et al., 2007), were removed by acid-treatment. The percentage of metal residues was significantly reduced according to TGA in Table 1 and Figure 1. In the TEM images, the f-MWNTs were open-ended and formed as a single tube, but coiled forms were still observed in Figure 2. However, the r-MWNTs were mainly observed as coiled forms, which were complicatedly tangled in the absence of NOM. The diameter of the r-MWNTs was determined to be 10–25 nm, which was larger than specified in the manufacturer's information (10–15 nm). The ratio of the G-band to the D-band in the Raman spectra was slightly increased in Figure 1, meaning the structural defects in the f-MWNTs.

3.2. Embryonic toxicity without NOM

Co-exposure to NMs and toluene could result in enhanced toxicity, thus residual toluene was quantified. The concentration was determined to be 0.347 µg/L in 5 mg/L of Tol/nC₆₀. This would be too low to induce any toxicity toward medaka embryos in the concentration range. The median lethal concentration (LC₅₀) of toluene toward medaka embryos was reported to be at the mg/L level (Teuschler et al., 2005). The concentration dependent responses of NMs in the absence of NOM are shown in Figure 3. The embryonic toxicities were in the order: f-MWNTs > Tol/nC₆₀ > Aqu/nC₆₀. Embryo malformations, such as heart abnormalities, absence of a swim bladder, caudal fin malformation, and pericardial and peritoneal edema, were significantly observed on exposure of f-MWNTs; a hatching delay was observed in Tol/nC₆₀. Forty percent mortality was observed at the highest f-MWNTs concentration (2000 µg/L), compared to the 30 and 25% mortalities for Tol/nC₆₀ and Aqu/nC₆₀, respectively. Usenko et al. (2008) observed about 50% mortality of dechorinated zebrafish (*Danio rerio*) embryos at 200 µg/L of fullerenes C₆₀ and C₇₀ suspended in DMSO. This result can be explained by the variation in characteristics of dechorinated embryos between the test fish species and different type of fullerenes by different preparation methods. The

toxicity of Tol/nC₆₀ was similar to that of Aqu/nC₆₀, being only slightly more toxic, probably due to its smaller size and hydrophobicity, facilitating its penetration into or contact with the embryos. It has been reported that small nC₆₀ particles induce higher antibacterial activity (Lyon et al., 2006), and that hydrophobic pristine C₆₀ translocated in a lipid bilayer model more easily than its hydrophilic derivatives (Qiao et al., 2007). Functionalization may also play a significant role in toxicity as f-MWNTs were more toxic than Tol/nC₆₀ despite their hydrophilic surface. The Morphological difference might be one of explanations. Jia et al. (2005) proposed differences in the geometric structures to describe the higher cytotoxicity of nanotubes compared to C₆₀. The reactivity induced by oxygen moieties on the f-MWNTs surface, which are also generated by acid oxidation, could be another possibility. Functional groups on oxygen moieties, such as carboxyl and carbonyl, might induce membrane damage, such as membrane potential reduction or oxidative stress, when brought into contact. f-MWNTs were observed clinging to the chorion surface of embryos and to the surface of hatched juveniles during the exposure. Kang et al. (2009) found that functionalized MWNTs induced the highest bacterial toxicity among various physicochemical modified MWNTs, due to the elevated dispersion enhancing the contact with bacterial cell.

3.3. Embryonic toxicity changes with NOM

Changes in the embryonic toxicity of NMs of 2000 µg/L were investigated at an NOM concentration from 0 to 10 mg TOC/L. No significance of embryonic toxicity in NOM alone to the control was observed up to 10 mg TOC/L. As a result, changes in the embryonic toxicity varied according to the NMs. The mortality on exposure to Tol/nC₆₀ and f-MWNTs decreased; whereas, no change in the mortality was observed with Aqu/nC₆₀ and r-MWNTs (Figure 4). While the mortality on exposure to Tol/nC₆₀ proportionally decreased with increasing NOM concentration, the mortality on exposure to f-MWNTs was reduced to below 10% upon the addition of NOM. One mg TOC/L of NOM was enough to mitigate the mortality induced by f-MWNTs, indicating the overestimation of the toxicity of Tol/nC₆₀ and f-MWNTs without considering NOM in aquatic systems. In the hatching delay, no significant changes were observed in Tol/nC₆₀ and r-MWNTs whereas an increase of hatching delay was seen in Aqu/nC₆₀ and f-MWNTs with increasing NOM concentrations (Figure 4c).

In the case of Tol/nC₆₀, the attenuation of mortality was probably due to the disappearance of the hydrophobicity by NOM association. NOM adsorption induced the impacts of hydration or hydrolysis due to the presence of the functional groups on NOM. Indirect contact due to steric NOM adsorption would be a major reason for the reduced embryonic toxicity of Tol/nC₆₀. As illustrated in the TEM images in Figure 2, the closed aggregates of spherical small particles disappeared. This is not the chemical transformation of nC₆₀ caused by NOM adsorption (Xie et al., 2008) but simple the morphological changes. In comparison, in the case of Aqu/nC₆₀, amorphous mesoscale aggregates were also observed in the absence and presence of NOM. No morphological differences caused by NOM adsorption observed in Tol/nC₆₀ seem to occur in Aqu/nC₆₀, resulting in no changes in the embryonic toxicity by the addition of NOM. The hydrophobic π - π interaction was reported to be a primary mechanism for organic NMs, where electrostatic attraction and ligand exchange played

important roles in the interaction between inorganic NMs and NOM (Hyung et al., 2007; Lin and Xing, 2008; Yang et al., 2009).

In the case of r-MWNTs, NOM adsorption onto r-MWNTs did not efficiently protect the embryos from contact with r-MWNTs due to the partial coating. Comparing to nC₆₀, the low sorption between r-MWNTs and NOM were limited, due to the long length of MWNTs. It is likely that NOM can be strongly adsorbed onto round-shape than rod-type of NMs. The arrows in Figure 2(f) indicate parts of the r-MWNTs not coated with NOM. In contrast, f-MWNTs were completely coated with NOM (Figure 2). It was noted that the r-MWNTs were partially coated, despite probe sonication; however, the f-MWNTs were completely coated by stirring for 2 d with no further artificial treatment required. Complete NOM coating was possible for the f-MWNTs due to the formation of single tubes and the presence of functional groups on the f-MWNTs increasing the electrostatic interactions with NOM. Therefore, it was concluded that the physicochemical and morphological differences of NMs resulted in different reactivities of NOM to NMs, leading to different patterns of toxicity changes for NMs in the presence of NOM. Further TEM images, both with and without Hank's media, in the absence and presence of NOM, are provided in Figure S2 in the Supplementary materials.

4. Conclusions

There have been many reports in the literature that nanotoxicity is controlled by different physicochemical properties of NMs when generated via various preparation methods and with physicochemical modifications. This study has demonstrated that the different physicochemical properties of NMs affect their interactions with NOM, resulting in alterations of toxicological responses. No toxicity reduction was observed in Aqu/nC₆₀ and r-MWNTs, while the toxicities of Tol/nC₆₀ and f-MWNTs were reduced by NOM association due to the disappearance of hydrophobicity in Tol/nC₆₀ and complete coating of NOM in f-MWNTs, respectively. The adsorption of NOM onto NMs is known to depend on the NOM source and water quality parameters, such as pH and ionic strength; therefore, these should also be investigated in future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:

Figure 1. Physicochemical properties of r-MWNTs and f-MWNTs. FTIR spectra (a), TGA analysis (b), XPS analysis (c), and Raman spectroscopy (d).

Figure 2. TEM images of Tol/nC₆₀ (a, b), Aqu/nC₆₀ (c, d), r-MWNTs (e, f) and f-MWNTs (g, h). Left-hand images (a, c, e, g) were observed in the absence of NOM. Arrows indicate r-MWNTs not coated with NOM.

Figure 3. Concentration-dependent influence of NMs on medaka embryos in the absence of NOM; (a) Mortality, (b) Malformation and (c) Hatching delay. Mortality was counted within 96 h of exposure. The percentage of abnormalities and delayed development represents the number of affected embryos per living embryos for 14 d. Error bars represent the standard errors. All tests were conducted in triplicate, with 12 embryos used for each treatment. Asterisks represent the significant differences relative to the control based on one-way ANOVA ($p < 0.05$). ● = Tol/nC₆₀; ▽ = Aqu/nC₆₀; ■ = r-MWNTs; ◇ = f-MWNTs.

Figure 4. Changes in the embryonic toxicity of NMs in the presence of NOM. (a) Mortality, (b) Malformation and (c) Hatching delay. Error bars represent the standard errors. All tests were conducted in triplicate, with 12 embryos exposed to each treatment. Asterisks represent the significant differences relative to the results in the absence of NOM based on one-way ANOVA ($p < 0.05$). □ = f-MWNTs; ▨ = Tol/nC₆₀; ▩ = Aqu/nC₆₀.

Table 1

Physicochemical properties of selected carbon-based nanomaterials

mg TOC/L	Diameter (±SD) (nm) ^a				Zeta potential (ζ)				RM (%) ^b	G/D ^c	Oxygen composition (%) ^d
	0	1	5	10	0	1	5	10			
Tol/nC ₆₀	226±2.4	208±3.4*	228±4.2	210±1.8*	-28.5±0.4	-18.8±4.2*	-23.7±3.6*	-17.0±2.7*	r-MWNTs	1.4	2.3
Aqu/nC ₆₀	245±5.8	269±3.6*	270±6.7*	260±7.2	-35.1±0.9	-14.7±2.1*	-13.3±1.6*	-35.4±6.7	f-MWNTs	0.7	10.8

^a average diameter from dynamic light scattering (DLS).

^b the mass (%) of metal impurities after thermo gravimetric analysis (TGA).

^c the ratio of G-band (~1580 cm⁻¹) to D-band (~1350 cm⁻¹) peak heights at λ_{laser}=532 nm in Raman spectroscopy.

^d oxygen content (%) from X-ray photoelectron spectroscopy (XPS) analysis.

The diameter of r-MWNTs was determined to be 10–25 nm in TEM images. The size and length of r-MWNTs were reported to be 10–15 nm and 10–20 μm from manufacturer. Asterisks represent the significant difference to the control (i.e., no NOM) determined by one-way ANOVA (*p*<0.05).