ISSN 1995-0780, Nanotechnologies in Russia, 2013, Vol. 8, Nos. 7–8, pp. 565–572. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © D.G. Deryabin, E.S. Aleshina, A.S. Vasilchenko, L.V. Efremova, O.S. Klokova, 2013, published in Rossiiskie Nanotekhnologii, 2013, Vol. 8, Nos. 7–8.

# Toxicity of Carbon-Based Nanomaterials against *Escherichia coli* Depends on Dispersion Efficacy of Their Water Suspensions

**D. G. Deryabin, E. S. Aleshina, A. S. Vasilchenko, L. V. Efremova, and O. S. Klokova**

*Orenburg State University, pr. Pobedy 13, Orenburg, 460018 Russia*

*e-mail: dgderyabin@yandex.ru*

Received July 25, 2012; accepted for publication April 11, 2013

**Abstract**—The relationships between surface wettability, dispersion efficacy in water suspensions and biotoxicity of nine carbon-based nanomaterials (CBN) samples represented by nanotubes, nanofibres and fullerenes are established. It is shown that presence of polar groups on the surface of similar in structure CBNs increases their hydrophilicity, reduces the particles size in water suspensions, and manifests itself by growth of the toxicity level in the luminescent screening assay based on *Escherichia coli* with the cloned *luxCDABE*-genes *Photobacterium leiognathi*. The artificial increase in individual CBN dispersion efficacy by primary suspension in the aprotic solvent dimethyl sulfoxide with the following transfer into an aqueous environment, also has led to growth of the registered biological activity. At the same time, the dispersion efficacy of morphologically diverse CBN is not the main cause of distinctions in their biotoxicity..

**DOI:** 10.1134/S1995078013040022

## INTRODUCTION

The urgency of the investigation of the antibacterial activity of carbon-based nanomaterials (CBN) is dictated by two major problems. First, microorganisms are constant components of natural ecosystems and trophic chains and, hence, are important tools for assessing risks related to the release of CBN into the environment [1]. Second, the suggested development of a new generation based on the CBN nanocompounds [2] and nanocoatings [3] with antibacterial properties dictates the necessity of investigating and controlling formed by the activity. Interest in both cases is establishing the dependence of antibacterial properties on the physico-chemical characteristics of the CBN, which determine the nature of their interaction with living systems to a great extent [4]. Of particular the features of nanocarbon particle organization, it is of interest to investigate their interaction with the aqueous environment [5] as well as the properties of the formed water suspensions [6], which are of great importance for nanocarbon biological activity according to the accumulated data. At the same time, there are very few observations of the role of the abovementioned characteristics in a manifestation of the CBN antibacterial properties [7], which requires a continuation and expansion of the research in the indicated directions.

A wide variety of Gram-negative and Gram-positive bacteria with different cell sizes and shapes are used currently as test objects for investigating nanocarbon biological activity [8, 9], which makes it possible to estimate the peculiarities of the effects of the CBN on certain types of microorganisms with different character of the organization of surface structures. At the same time, the studies using model laboratory strains, among which *Escherichia coli* is used very often [10, 11], usually are preceded experiments with natural and clinical isolates. Three main points testify in favor of this: (i) the availability of *E. coli* in many natural ecosystems is common and considered a sanitary indicator; (ii) *E. coli* plays a role in the infection pathology of the in humans and animals and, also, it is very similar to other pathogenic species of the *Enterobacteriaceae* family; and (iii) detailed molecular-genetic characteristics of the *E. coli* are available, which facilitates the development of genetic constructs with specific bioindicator properties based on the parent *E. coli*. In particular, the current national standard on microbiological and molecular-genetic testing of nanomaterials [12] recommends employing the recombinant luminescent *E. coli* stain for these purposes with the use of a quantitative assessment of the luminescence inhibition as an indicator of their biotoxicity.

The goal of this study was to investigate the role of the dispersion of the CBN water suspensions, which is determined by their own physicochemical features or reached by the use of special methods of suspension preparation in the development of the toxic effect towards the sensor stain of the luminescent *Escherichia coli*.

**Table 1.** General characteristics of the investigated CBN

| Designation | Characteristics | Manufacturer |
|---|---|---|
| SWNT-1 | SWNTs 1.2–1.5 nm in diameter and 1–5 μm in length containing 2–5% COOH-groups on the surface | A |
| SWNT-2 | Same nanotubes shortened to 0.2–0.5 μm with 5–10% COOH-group content | A |
| SWNT-3 | Same nanotubes with COOH-groups annealed under vacuum | A |
| MWNT | MWNTs 7–48 nm in diameter and 0.5–5.8 μm in length | B |
| NF | Carbon NFs 30–60 nm in diameter and 1–2 μm in length | C |
| fNF | Same NFs functionalized by $HNO_3$ and $H_2SO_4$ treatment | D |
| $C_{60}$ | $C_{60}$-fulleren, diameter 0.71 nm | B |
| $C_{60}(OH)_{\sim 24}$ | Fullerenol, diameter 1.6 nm | B |
| $C_{70}$ | $C_{70}$-fulleren, 0.7 × 0.9 nm | B |

Note: (A) OOO Karbonlait, Moscow region, Dolgoprudnyi, Russia; (B) Institute of Problems of Chemical Physics, Russian Academy of Sciences, Moscow region, Chernogolovka, Russia; (C) OOO NTTs GraNaT, Moscow region, Elektrostal', Russia; (D) D. Mendeleev University of Chemical Technology of Russia, Moscow, Russia

## MATERIALS AND METHODS

Nine commercially available and laboratory preparations of CBN with at least 95% purity and the general characteristics presented in Table 1 were used in the investigations. Their list included three samples of single-walled carbon nanotubes (SWNTs) with various lengths and degrees of surface functionalization by COOH groups; multiwalled carbon nanotubes (MWNTs); nanofibers (NFs) and a variant of NFs that underwent the acidic functionalization procedure (fNFs) [13]; and $C_{60}$-, $C_{70}$-fullerens, and fullerenol ($C_{60}(OH)_{\sim 24}$). Amorphous carbon (AC) was used as a comparison object.

The work of adhesion ($W_a$) that was determined from the results of experimental measurements of equilibrium contact angles wetting [14] was used as an integral parameter characterizing the interaction of the CBN with a polar solvent (water). For this purpose, 2 μl of distilled water was applied onto the preformed CBN surfaces at 20 ± 1°C and a photo was taken after the stable contact between the drop and the tested surface was established, from which values of the contact angles (θ) were determined on the left and on right interfaces of the liquid and solid phases. The work of adhesion was calculated according to the equation $W_a = \sigma(1 + \cos\theta)$, where σ is the coefficient of the water-surface tension at 20°C, taken for 72.86 × 0.001 N/m; θ is the averaged value of the static contact angle.

The degree of CBN dispersion was investigated with sedimentation analysis on centrifugation with the values of 100, 1000, and 10000 g (MiniSpin, Eppendorf, Germany) with the subsequent testing of the supernatants on a spectrofluorimeter Flyuorat-02 Panorama (NPF Lyumeks, Russia) and expression of the share of the settled particles as a percent of the initial optical density of the suspension. Based on these data, the radius of nanocarbon particles ($r$) settled at a certain value of the acceleration of gravity ($g$) was calculated considering the values of the relative density of water (ρ) and each of the investigated CNMs ($\rho_0$) and using the following equation: $r = \sqrt{\frac{9H\eta}{2gt(\rho - \rho_0)}}$, where $H$ is the height of liquid, η is the solvent viscosity, and $t$ is sedimentation time.

The investigated CBN suspensions and their mixtures with *E. coli* (20 μl) were applied on a fresh mica cleavage, dried at 93% relative humidity and 20 ± 1°C. The obtained objects were investigated by atomic force microscope in a contact mode using an SMM-2000 multimicroscope (Proton-MIET, Russia). MSCT-AUNM cantilevers (Veeco, United States) with a spring constant 0.01 N/m and tip curvature radius of 10 nm were used for scanning. Qantitative morphometric analysis of the images was conducted using the standard microscope software as described previously [15]. From 30 to 50 randomly selected objects were analyzed in the scanning series for the purpose of a statistical evaluation, which made it possible to measure the required size characteristics.

The CBN toxic properties were investigated by a modified bioluminescent method, which was justified earlier [16]. The features of the method were (1) a special procedure for nanocarbon suspension pretreatment, (2) an extended contact time of the sensor luminescent microorganism with CBN completely revealing their biological activity, and (3) the use of a special algorithm to estimate the bacterial luminescence inhibition that made it possible to eliminate the effect of nanocarbon optical properties on the results of the research.

To prepare the CBN suspension, aliquots of 4 mg (in the case of nanotubes, NFs, and AC) or 4 mM (in the case of fullerens) were placed into a glass flask where 1 mL of distilled water or dimethyl sulfoxide (DMSO) was added and the flask was vigorously mixed by pipetting and treated with ultrasound in an ultrasonic bath (PKF Sapfir, Russia) operating at 35 kHz and specific sound power 30 W $dm^{-3}$ for 30 min.

A recombinant *Escherichia coli* K12 TG1 strain with cloned *luxCDABE*-genes of the natural marine luminescence microorganism *Photobacterium leiognathi* produced by ZAO NVO Immunotekh (Russia) commercially available as Ecolum and recommended

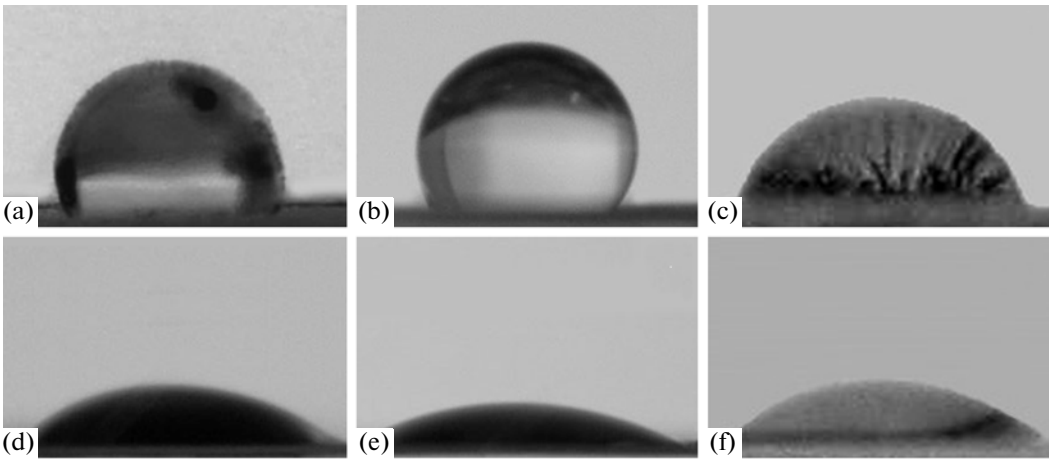


**Fig. 1.** Examples of typical contact angles of the CNM surface: SWNT-3 (a), NF (b), C60 (c), SWNT-2 (d), fNF (e), and $C60(OH)_{\sim 24}$ (f).

by the current national standard [12] for assessing the biological activity of nanoparticles and nanomaterials was used as a test object.

Series of twofold dilutions of 100 μl of the CBN up to 1 : 1024 were prepared for biotesting in nontransparent microplates (Thermo, United States) using distilled water or 2.5% DMSO solution, depending on the initial nature of the suspension. Controls without the investigated CBN were prepared respectively. Next, 100 μl of bacterial biosensors was placed into each well and the plate was placed immediately into a measuring chamber of a LM-01T luminometer (Immunotech, Czech Republic); the luminescence intensity was measured every 5 min for 180 min. The plates were shaken between measurements on a ST-3 shaker (Elmi, Latvia) with a 1.5 mm rotation radius and a rotation rate of 100 rpm to prevent the sedimentation of the suspensions.

The mathematical algorithm $\frac{Ik_{0\,\min} \cdot Io_{n\,\min}}{Ik_{n\,\min} \cdot Io_{0\,\min}}$, where *Ik* and *Io* are the luminescence intensity of the control and test samples at 0 and *n* minutes, was used for calculating the bioluminescence index characterizing the effect of the investigated samples on the luminescence of the sensor microorganism. The $EC_{50}$ values were calculated based on these data, which corresponded to the CBN concentration, causing the 50% inhibition of the luminescence of the sensor organism in comparison with the control.

The results were statistically processed by the method of variance using the software package Statistica V8 (StatSoft Inc., United States).

## RESULTS

The investigation into wetting the CBN with water was complicated by the hysteresis phenomenon, which is caused mainly by the pronounced microrelief of the formed surfaces. In connection with this, a series of 3–4 measurements was conducted for each investigated object and the average values of contact angles (θ) and the derived values of the work of adhesion ($W_a$) were calculated on their basis. The results indicated regular changes in the degree of wetting of the investigated CBN depending on the level of their structuredness and availability of carboxyl (–COOH), hydroxyl (–OH), and other polar groups on their surface (Fig. 1). In particular, a number of highly structured nanocarbon compounds form nonwettable hydrophobic surfaces, which is most pronounced for the SWNT-3 sample ($\theta = 101.6 \pm 1.2°$; $W_a = 58.3 \pm 9.7$ N/m) and NF ($\theta = 133.8 \pm 6.9°$; $W_a = 22.6 \pm 0.7$ N/m), which significantly differ in these parameters from the sample of AC that was used ($\theta = 33.8 \pm 0.9°$; $W_a = 133.3 \pm 1.9$ N/m). On the other hand, the saturation of the CBN surface with polar groups of different compositions significantly increased their hydrophilicity, especially for the fNF ($\theta = 24.7 \pm 1.3°$; $W_a = 139.2 \pm 2.3$ N/m) and fullerenol ($\theta = 29.1 \pm 0.5°$; $W_a = 134.0 \pm 0.6$ N/m), the wettability of which with water was even higher than for the AC preparation.

Most of the CBN water suspensions were classified as polydisperse according to the data of sedimentation analysis; that is they were characterized with a wide range of the probable particle size distribution. A number of highly structured nanocarbon compounds formed predominantly coarse systems, which pelleted already at 100 g. This was mostly related to the SWNT-3, MWNT, and NF samples, with the fraction of large particles being 94.9 ± 4.5%, 90.9 ± 4.4%, and 71.6 ± 3.4%, respectively. Our calculations made it possible to estimate the average size of the mentioned CBN as being above 1000 nm and suggesting the existence of nanotube and NF aggregates in the suspensions. With this in mind, the saturation of the CBN surface with polar groups together with the shortening

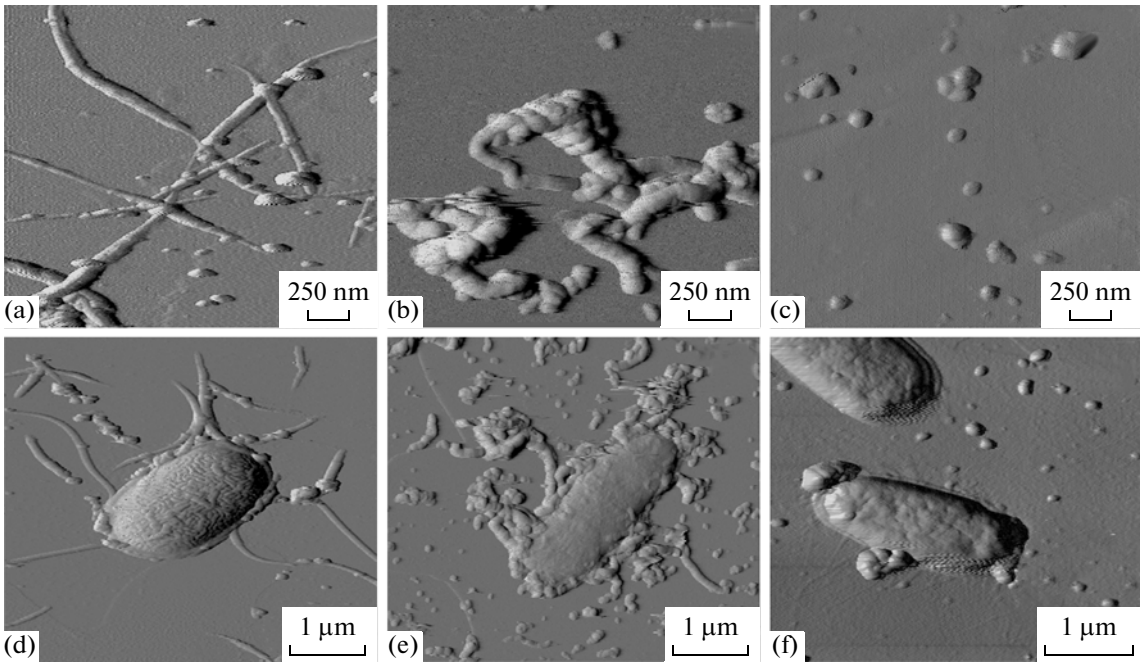


**Fig. 2.** Atomic force microscopy images of the SWNT-2 (a), fNF (b) and C60(OH)$_{\sim 24}$ (c) particles and their spatial contacts with *E. coli* K12 TG1 cells (d, e, f).

of their length resulted in an increase in the dispersion degree of the SWNT-2 suspension and, above all, of the fNF (the average particle size in the suspension was 941.9 ± 299.3 and 160.9 ± 38.1 nm, respectively); the latter formed a sedimentation-resistant colloid system. The investigated C60- and C70-fulleren suspensions were in turn represented by a particle size with an average diameter of 275.0 ± 111.7 and 524.4 ± 194.6 nm, which could be explained by the significantly smaller size of the individual nanocarbon compound forming particles when compared with nanotubes and NFs. Nevertheless, the size of the particles of the n($C_{60}$) and n($C_{70}$) composition was more than eight orders of magnitude larger than single molecules, which again was most probably the result of their pronounced aggregation in an aqueous environment. The saturation of the fullerenol surface with hydroxyl groups on the other hand resulted in an almost twofold decrease in the particle size (187.2 ± 83.0 nm) in comparison with the C60-fulleren.

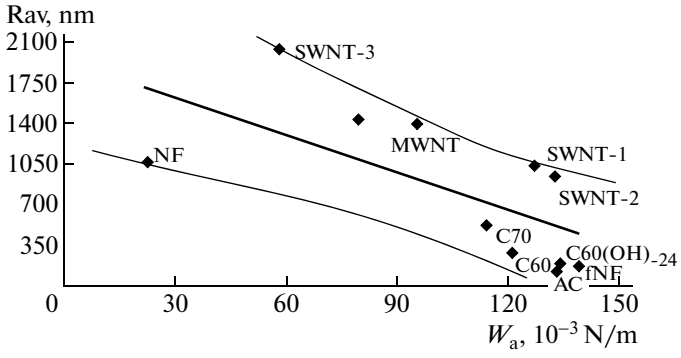


**Fig. 3.** Dependence of the average size of CBN particles (Rav) in suspension (ordinate axis) for hydrophility surface characterized by the $W_a$ value (abscissa axis).

Additional information on the sizes and shapes of the CBN particles deposited from the suspensions was obtained with the use of atomic force microscopy (Fig. 2). In particular, the SWNT-2 sample was visualized as individual formations of the 7.3 ± 4.4 (2.7–20) nm width/height and 1.0 ± 0.5 (0.2–1.6) μm length (Fig. 2a), which was in good agreement with the sedimentation-analysis data presented above. The fNF particles on the other hand were visualized with atomic force microscopy as slightly larger helical coiled structures with 101.6 ± 23.3 (52.0–114.0) nm width/height and 1.4 ± 0.6 (0.3–2.0) μm length (Fig. 2b). Lastly, the fullerenol particles were spherical formations with a 170.2 ± 29.0 (50.5–230.0) nm diameter corresponding to the aggregates of more than 1 million C$_{60}$(OH)$_{\sim 24}$ molecules according to the conducted calculations (Fig. 2c).

The results raised a question about the dependence of the degree of dispersion of the CBN water suspensions on the degree of their wettability with polar solvents (water). The results of correlation analysis conducted in the process indicated the existence of the pronounced inverse dependence ($r = -0.660$; $P < 0.05$) of the average calculated nanocarbon particle radius and the experimentally registered $W_a$ values (Fig. 3). Hence, the following relationship can be demon-

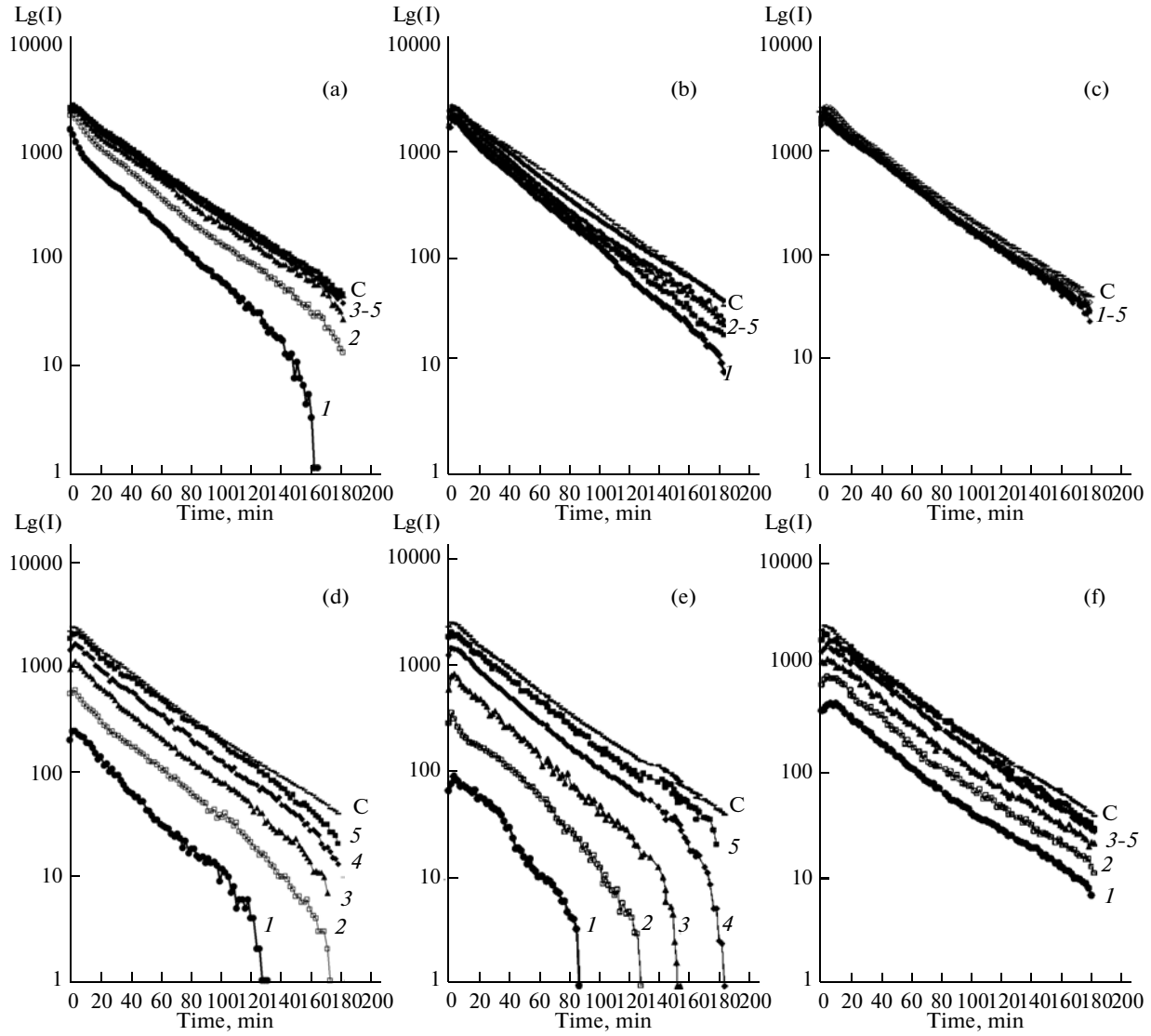


**Fig. 4.** Examples of the luminescence dynamics of the *E. coli* K12 TG1 with cloned c *luxCDABE*-genes of *P. leiognathi* on contact with CBN water suspensions. Designations: (a) SWNT-3, (b) NF, (c) C60, (d) SWNT-2, (e) fNF, and (f) $C60(OH)_{\sim 24}$, used at concentrations 100 (*1*), 50 (*2*), 25 (*3*), 12.5 (*4*), and 6.25 (*5*) μg/mL for SWNTs and NF or μM for fullerens; (C) control. X-axis: time of contact, min; Y-axis: registered luminescence intensity (I).

strated for the entire set of investigated CBN: the better the wettability of the individual nanocarbon compound was, the higher the degree of dispersion of its water suspension was observed. At the same time it must be mentioned that carbon nanotubes with similar wettability levels formed less disperse suspensions, which can be explained by the geometry of the individual forming nanoparticles (Table 1), as well as by the differences in the absolute values of van der Waals interactions depending on these parameters [17].

The final result of the different degree of CBN wettability and the size of carbon nanoparticles defined by it was the differences in their biological activity (toxicity) evaluated in the tests of the bioluminescence inhibition of the sensor *E. coli* K12 TG1 strain with the cloned *luxCDABE*-genes of the naturally bioluminescent marine *P. leiognathi*.

An analysis of the raw data (Fig. 4) indicated that the increase in the dispersion degree of the CBN suspensions significantly decreased their light transmission ability and, hence, prevented the transfer of the signal from the biosensor to the photodetector. This resulted in a decrease in the luminescence intensity developing in the first seconds of the reaction-system existence, which linearly depended on the values of light absorption of the tested suspensions but was unrelated to their biological activity [16]. On the other hand, the continuing contact of the sensor microorganism with the CBN resulted in true bioluminescence inhibition developing in time (toxic effect),

**Table 2.** Values of the toxicity parameter $EC_{50}$ (μg/ml for CNT, NF, and AC or μM for fullerens) determined for the CBN suspensions formed in water or DMSO in testing of their effect on the bioluminescence level of the *E. coli* K12 TG1 sensor strain with cloned *luxCDABE*-genes of *P. leognathi*

| Investigated CBN | Solvent used for primary suspension preparation | |
|---|---|---|
| | Water | DMSO |
| SWNT-1 | 62.0 ± 3.6 | 18.0 ± 0.7 |
| SWNT-2 | 54.0 ± 2.8 | 17.0 ± 0.9 |
| SWNT-3 | 78.3 ± 3.9 | 46.0 ± 2.9 |
| MWNT | >100 | 28.0 ± 0.1 |
| NF | >100 | 20.0 ± 0.9 |
| fNF | 42.0 ± 1.3 | 9.0 ± 0.1 |
| $C_{60}$ | >100 | 84.0 ± 4.3 |
| $C_{60}(OH)_{\sim 24}$ | >100 | >100 |
| $C_{70}$ | >100 | 50.0 ± 2.5 |
| AC | 40.0 ± 2.0 | 8.0 ± 0.1 |

which was estimated with the respective mathematical algorithm and presented as $EC_{50}$ (Table 2).

The fact that the registered toxicity values of the group of CBN similar in structure (SWNTs, NFs, and fullerens) were found to be inversely proportional to the size of the carbon nanoparticle in the tested system was an important conclusion of this part of the study.

In particular, the statement presented above can be illustrated by the results of the biological activity investigation of the SWNT (Figs. 4a, 4d) in the tested set of the CBN exhibiting a toxic effect. The process of the COOH-group annealing, causing an increase in hydrophobicity and, thus, an increase in particle size in the suspension, resulted in a certain reduction in the toxicity of the SWNT-3 object ($EC_{50}$ = 78.3 ± 3.9 μg/mL) in comparison with the initial SWNT-1 sample ($EC_{50}$ = 62.0 ± 3.6 μg/mL), while the saturation of the nanotube surface with polar groups together with their shortening resulted in opposite results on the testing of the SWNT-2 sample ($EC_{50}$ = 54.0 ± 2.8 μg/mL).

Similar dependencies were demonstrated in the group of NFs (Figs. 4b, 4e), where the saturation of the surface with polar groups with the simultaneous cleavage of the initial NF into shorter fragments resulted in a pronounced increase in the registered biological activity of the fNF object. The results allowed us to classify the initial sample of hydrophobic and poorly dispersed NF as nontoxic; that is it did not exhibit significant biological activity in the bioindicator system. On the other hand, the fNF that formed a highly dispersed colloid system caused the development of a toxic effect ($EC_{50}$ = 42.0 ± 2.0 μg/mL), which makes them one of the most biologically active compounds in the investigated set of CBN (Table 2).

The investigated MWNTs, C60- (Fig. 4c) and C70-fulleren water suspensions, in turn did not inhibit the sensor organism, revealing themselves as nontoxic. The investigated fullerenol object caused only an optical decrease in the luminescence intensity of the bioindicator and did not lead to the development of the detected biotoxicity, despite the high dispersion degree of water suspension (Fig. 4f).

The detection of toxic properties of the AC object ($EC_{50}$ = 40.0 ± 2.0 μg/mL) that exceeded the ones of all other investigated CBN is of interest considering the aforesaid. This made it possible to suggest not growth but a decrease in carbon toxic properties upon the formation of nanostructures.

The differences in the specific surface of the tested nanocarbon compounds, which is progressively increasing with a decrease in the particle size in the suspension and, thus, increasing the probability of the spatial contact of the CBN with the surface of the target cells (the initial and most important step in the realization of their biological activity [18]), is probably the reason for the indicated relationships.

An example of such an interaction of the most dispersed SWNT-2 and fNF (Figs. 2d, 2e) was visualized with atomic force microscopy as a multiple in quantity and variable in location contact of the carbon nanoparticles to the entire bacterial cell surface. On the other hand, a similar interaction of fullerene particles with bacterial cells (Fig. 2f) was observed as a single-point contact, which corresponded to the results of the bioluminescence analysis presented above.

More evidence of the significance of the CBN degree of dispersion for determining their biotoxicity was obtained in a series of experiments employing the primary dispersion of the tested compounds in the aprotic solvent dimethyl sulfoxide, which has permittivity lower than water ($\varepsilon_{DMSO}$ = 45 versus $\varepsilon_{water}$ = 81). According results of sedimentation analysis, this resulted in a significant decrease in the average radius of the CBN particles, which was most pronounced for poorly wettable highly structured carbon nanocompounds. A determination of the permissible level of the disperser content that does not affect the viability and bioluminescence level of the sensor strain was an additional condition of the study; it was set at a level of 1.25% of the final volume of the reaction mixture (5.7-fold lower than the DMSO $EC_{50}$ values for the *E. coli* K12 TG1). Furthermore, it was shown in a separate series of experiments involving the simultaneous or sequential introduction of the CBN and DMSO that the introduction of the latter changed the level of the nanocarbon biological activity via an increase in degree of dispersion of its suspensions, but not via the increased sensitivity of the sensor microorganism.

One conclusion of this part of the study was that the increase in the degree of dispersion of the individual

CBN achieved through the use of DMSO resulted in the growth of their biotoxicity for 9 out of 10 of the investigated carbon compounds, which was characterized by a 1.7 to 5.0-fold decrease of the nominal $EC_{50}$ values for the SWNT, fNF, and AC object (Table 1). The consistence of this result was confirmed by correlation data analysis, indicating that the toxicological parameters $EC_{50}$ in the experiment series with and without DMSO were interdependent ($r = 0.653$, $P < 0.05$).

It can be stated based on the entire wealth of the obtained results that the degree of dispersion of the nanocarbon suspension could be of importance in the manifestation of their biological activity for the entire set of investigated CBN presented by morphologically different compounds. However, the calculated dependence of the CBN biotoxicity level ($EC_{50}$) on their size characteristics (average particle radiuses in the suspensions, *r*) is in general agreement with the trends described above, demonstrating a low value of the correlation coefficient ($r = 0.272$; $P > 0.05$). Hence, if the hydrophobic–hydrophilic properties of the structurally uniform CBN and the degree of dispersion in suspension determined by them defined the differences in the levels of their biological activity, the significance of these factors was not as obvious in the CBN that are variable in shape and structure. The most likely reasons for that were the different mechanisms of the damaging effect exhibited by structurally different CBN with similar values of the specific surface to target the bacterial cells [19], which resulted in the development of a quantitatively different toxic effect.

## DISCUSSION

The formation of carbon nanoparticles with different structures results in significant changes in the physicochemical characteristics of the synthesized compounds. In particular, one of the examples is the extremely low wettability of the CBN (nanotubes in particular), which is determined according to molecular modeling by the energy of the bond cleavage between the water molecules during surface hydration [20] and which are characterized as hydrophobic and superhydrophobic compounds according to the experimental results of the contact angle studies [21]. In this context, the results of this study confirm a lack of wettability or limited wettability of most of the investigated CBN, which causes the formation of sufficiently large aggregates of nanocarbon particles in water suspensions. On the other hand, the data indicate that the saturation of the CBN surface with polar groups significantly increases the degree of wettability, which results in an increase in the dispersion degree of their water suspensions. The abovementioned is in good agreement with the known data that the covalent binding of polar sulfo-groups to the surface of initially nonwettable nanotubes [22] or the increase in the hydroxylation degree of the $C_{60}$-fulleren [23] results in a significant increase in solubility and a decrease in the particle size in the suspension; it also offers the possibility for the formation of stable water dispersions of the CBN.

A difference in the biological activity (toxicity) of the CBN, including toxicity to the representatives of the microorganism community, is an important consequence of such behavior in water suspensions. Thus, the data obtained in the experiments on the luminescence inhibition of the *E. coli* with cloned *luxCDABE*-genes of *P.leiognathi* made it possible to state the absence of toxicity of poorly wettable SWNTs, MWNTs, NFs, and C60- and C70-fullerens; hence, the ecological risks of their release into aqueous ecosystems seem slightly overestimated [24]. On the other hand, an increase in wettability and the degree of dispersion of the CBN water suspensions determined by it resulted in an increase in its toxic properties, which was earlier shown for nanotubes [4] and fullerens [23]; in the used biotest system it was most pronounced in the pairs of compounds with similar morphological organization: SWNT-3 SWNT-2 and NF fNF.

Moreover, an increase in the degree of dispersion of individual CBN by creating an adsorption layer on their surface from the DMSO molecules followed by a transfer of the formed suspension in the aqueous environment also resulted in an increase in the registered biological activity. The goal of the use of the dispersant in this work was to fully evaluate the CBN toxicity under laboratory conditions [16], while the same effects could be observed in natural ecosystems in the presence of organic substances found in nature [25].

Hence, the results testify in favor of the reality of ecological risks of the release of highly wettable nanoparticles in the environment, as well as an increase in the degree of dispersion resulting from the effect of surface-active chemical factors. The same data indicate the necessity of covalent or noncovalent modifications of the nanocarbon surface, providing a high extent of hydrophilization for diagnostic or therapeutic applications of the CBN in living systems.

According to the results of this study, the increase in the specific surface could be the main reason determining the dependence of the CBN biological activity on the degree of dispersion of the water suspensions [18], which provide multiple-point contacts of nanocarbon particles and target bacterial cells. The specific surface of the coarse suspensions, on the other hand, is significantly lower, which, considering the size of the objects, makes it possible to suggest not the effect of the CBN on bacterial target cells but the adsorption of bacterial cells on large aggregates of nanocarbon.

All the data suggesting a link between the degree of dispersion indicators and biotoxicity in the group of compounds with similar morphology indicate that, in the set of CBN with varying structures and geometries, the degree of dispersion that is reached defines no more than 10% of variability in the registered biological activity. Taking in consideration the abovemen-

tioned, the formation of the spatial contact of the CBN with the target bacterial cells can be regarded as an important initial (but not the sole) mechanism of biotoxicity, which, according to the accumulated data, is determined by the following disruption of the cytoplasmic membrane integrity [10], the development of oxidative stress [23], and protein and DNA damage [19], as well as other mechanisms that are of interest for future investigations into nanocarbon biological activity.

## ACKNOWLEDGMENTS

This work was supported by the Federal Target Program "Scientific and Scientific-Pedagogical Personnel of Innovative Russia" for 2009–2013 (project no. 8597).

## REFERENCES

1. G. G. Onishchenko, "The way to secure sanitary-hygienic well-being for population when nanomaterial and nanotechnologies are used widely," Gigiena Sanit., No. 2, 4–7 (2010).
2. A. J. Huh and Y. J. Kwon, "Nanoantibiotics": A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era," J. Control. Release **156** (2), 128–145 (2011).
3. A. Tiraferri, C. D. Vecitis, and M. Elimelech, "Covalent binding of single-walled carbon nanotubes to polyamide membranes for antimicrobial surface properties," ACS Appl. Mater. Interfaces **3** (8), 2869–2877 (2011).
4. S. Kang, M. S. Mauter, and M. Elimelech, "Physicochemical determinants of multiwalled carbon nanotube bacterial cytotoxicity," Environ. Sci. Technol. **42** (19), 7528–7534 (2008).
5. A. Mathur, S. Wadhwa, J. A. Byrne, S. S. Roy, and J. A. McLaughlin, "Experimental demonstration of hydrophobicity variation in carbon nanotubes by surface modifications," e-J. Surf. Sci. Nanotech. **7**, 334–336 (2009).
6. D. Y. Lyon, L. K. Adams, J. C. Falkner, and P. J. Alvarez, "Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size," Environ. Sci. Technol. **40** (14), 4360–4366 (2006).
7. M. S. Mauter and M. Elimelech, "Environmental applications of carbon-based nanomaterials," Environ. Sci. Technol. **42** (16), 5843–5859 (2008).
8. L. R. Arias and L. Yang, "Inactivation of bacterial pathogens by carbon nanotubes in suspensions," Langmuir **25** (5), 3003–3012 (2009).
9. L. Dong, A. Henderson, and C. Field, "Antimicrobial activity of single-walled carbon nanotubes suspended in different surfactants," J. Nanotechnol. (2012). doi:10.1155/2012/928924
10. S. Kang, M. Herzberg, D. F. Rodrigues, and M. Elimelech, "Antibacterial effects of carbon nanotubes: size does matter!," Langmuir **24** (13), 6409–6413 (2008).
11. S. Liu, M. Hu, T. H. Zeng, R. Wu, R. Jiang, J. Wei, L. Wang, J. Kong, and Y. Chen, "Lateral dimension-dependent antibacterial activity of graphene oxide sheets," Langmuir **28** (33), 12364–12372 (2012).
12. *Metodological* (Practical Recomendations) *no. 1.2.2634-10: Microbiological and Molecular-Genetic Way for Estimating How Nanomaterials Effect to Microbiocoenosis Specimen* (Russian Federation Center of Hygiene and Epidemiology, Moscow, 2010).
13. E. G. Rakov, N. T. Hung, and N. M. Tuong, "Acid functionalization of carbon nanofibers," Inorg. Mater. **46** (10), 1077–1083 (2010).
14. A. M. Emelyanenko, "Wettability of interphase boundaris as an indicator of their properties and states," Fizikokhim. Poverkhn. Zashchita Mater. **44** (5), 453–463 (2008).
15. D. G. Deryabin, A. S. Vasil'chenko, E. S. Aleshina, A. S. Tlyagulova, and A. N. Nikiyan, "An investigation into the interaction between carbon-based nanomaterials and Escherichia coli cells using atomic force microscopy," Nanotechnol. Russ. **5** (11–12), 857 (2010).
16. D. G. Deryabin, E. S. Aleshina, and L. V. Efremova, "Application of the inhibition of bacterial bioluminescence test for assessment of toxicity of carbon-based nanomaterials," Microbiol. **81** (4), 532–538 (2012).
17. G. A. Rance, D. H. Marsh, S. J. Bourne, T. G. Reade, and A. N. Khlobystov, "Van der Waals interactions between nanotubes and nanoparticles for controlled assembly of composite nanostructures," ACS Nano **4** (8), 4920–4928 (2010).
18. A. S. Karakoti, L. L. Hench, and S. Seal, "The potential toxicity of nanomaterials – the role of surfaces," JOM **58** (7), 77–82 (2006).
19. M. J. Riding, F. L. Martin, J. Trevisan, V. Llabjani, I. I. Patel, K. C. Jones, and K. T. Semple, "Concentration-dependent effects of carbon nanoparticles in gram-negative bacteria determined by infrared spectroscopy with multivariate analysis," Environ. Pollut. **163**, 226–234 (2012).
20. J. H. Walther, R. Jaffe, T. Halicioglu, and P. Koumoutsakos, "Carbon nanotubes in water: structural characteristics and energetics," J. Phys. Chem **105** (41), 9980–9987 (2001).
21. H. Liu, J. Zhaia, and L. Jiang, "Wetting and anti-wetting on aligned carbon nanotube films," Soft Matter **2**, 811–821 (2006).
22. O. A. Pyshkina, T. V. Panova, Zh. A. Boeva, A. A. Lezov, G. E. Polunina, A. V. Lezov, and V. G. Sergeev, "Behavior of Multiwalled Carbon Nanotubes Functionalized with Sulfo Groups in Aqueous Salt Solutions," Nanotechnologies in Russia, No. 11–12, 629–634 (2012).
23. S. -R. Chae, A. R. Badireddy, J. F. Budarz, S. Lin, Y. Xiao, M. Therezien, and M. R. Wiesner, "Heterogeneities in fullerene nanoparticle aggregates affecting reactivity, bioactivity, and transport," ACS Nano **4** (9), 5011–5018 (2010).
24. S. J. Klaine, P. J. Alvarez, G. E. Batley, T. F. Fernandes, R. D. Handy, D. Y. Lyon, S. Mahendra, M. J. McLaughlin, and J. R. Lead, "Nanomaterials in the environment: behavior, fate, bioavailability, and effects," Environ Toxicol Chem. **27** (9), 1825–1851 (2008).
25. H. Hyung, J. D. Fortner, J. B. Hughes, and J. H. Kim, "Natural organic matter stabilizes carbon nanotubes in the aqueous phase," Environ. Sci. Technol. **41** (1), 179–184 (2007).

*Translated by L. Brovko*