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Letter to the Editor

RETURNING ONCE AGAIN TO THE QUESTION ABOUT "TOXICITY" OF PRISTINE C_{60} FULLERENES

Comments on "Ecotoxicology of carbon-based engineered nanoparticles: Effects of fullerene (C_{60}) on aquatic organisms. Carbon 44 (2006) 1112-20

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Abstract.

In this letter we discuss the facts which were the reason for erroneous conclusions about high toxicity of water nanodispersions of pristine fullerenes. Analysis of these facts testifies that authors who are finding out special toxic properties of C_{60} fullerenes, actually investigated the biological activity of both chemically modified (oxidized) C_{60} and crystal-solvated forms of pristine C_{60} which contained toxic molecules of organic solvent (THF) and/or products catalytic degradations of the last. As a whole, pristine fullerenes do not constitute any environmental danger and it is recommended to handle pure fullerenes in the same way as with usual carbon black.

Keywords: Fullerene nanoparticles; Bioactivity; Toxicity; Ecotoxicity.

In recent years there have been many attempts to prove that C_{60} fullerenes are toxic and dangerous for environment. Those experiments were conducted on water nanodispersions (nano-C_{60} or nC_{60}) [1-7], but the physical and chemical properties were not studied comprehensively. The toxic effects of nano-C_{60} were wrongly attributed to the pure C_{60}, and the results of such of investigations, which in 2004-2005 have

intimidated the world with high toxicity of fullerenes, continue to mislead still both the scientific world and the public.

However, in general, it is reasonable to note that, prior to discussion of the theoretical questions about very far consequences of the pure fullerenes influence on an environment, it is necessary to solve first of all the problem on their acute and chronic toxicity. For this purpose, as it is known, quite specific tests and models are used (mainly with experimental animals). In these cases the complete (objective) knowledge about physical-chemical properties of tested object is the indispensable condition.

Granting this, we have already published a critique on this topic in a recent review [9] showing that pure C_{60} does not represent any significant danger for living organisms. We also gave a number of arguments stating that the supramolecular system of "pristine fullerene - water" has a wide spectrum of positive and unique biological activity [10-11,].

Recent new publications, including [12] studying toxicity of fullerenes with procedures for preparation of C_{60} water dispersion (nano-C_{60}) proposed by Deguchi and Colvin group [13, 2-5]. The conclusions drawn in these publications about essential toxicity of pristine C_{60} are wrong that could be shown by detailed analysis of all available in publications data. For example, Isacovich and co-authors [12], first time for last years and in contrast to publications by Colvin group [4,5], showed in their paper the representative IR and UV spectra of nano-C_{60}. Those spectra unambiguously prove that colloidal particles of nano-C_{60} by no means are pure C_{60}! It is absolutely obvious that such nano-C_{60} dispersions consist of various aggregates of solvated fullerenes. It is important that such aggregates, even after drying, retain noticeable amount of toxic tetrahydrofuran (THF) and/or products of its degradation. Knowing these facts it is hard to understand why authors of articles [1-6, 11] persistently attribute cytotoxic effects to action of pure C_{60}.

After our critique in 2004-2005, Oberdörster group repeated their experiments using this time another method for nC_{60} preparation, essentially without toxic organic solvent THF [7,8]. In order to make new C_{60} water dispersion, “water-soluble (nC_{60})”, Oberdörster and co-authors used continuous stirring of crystalline

C_{60} powder with water for at least 2 months under solar light and air oxygen[1]. As a result they obtained colloidal dispersion containing 35 ppm (35 mg/L) of C_{60} nanoaggregates (10-200 nm), surface of which is covered by partially hydroxylated fullerenes, although authors in their article do not show any strict physical or chemical proofs for it.

In this connection it is worthy to remind that spontaneous (cluster) solubility of C_{60} crystals, even at ultrasonic agitation, does not exceed 10^{-8} mg/L. Additionally, if fullerene molecules are exposed simultaneously to water, solar light and oxygen from air then this leads to the formation of different oxidized forms of C_{60} molecule [14]. These oxidized C_{60} may contain oxo-, hydroxyl- and easy hydrolyzable epoxy-groups which facilitates the further oxidation and destruction of carbon shell of C_{60} by sufficiently strong oxidizers (e.g., bleach or magnesium persulfate [7], but excluding magnesium perchlorate which indeed was simple coagulating agent [15-17] for nano-C_{60} dispersions [5], but it was not an oxidizer).

Thus, it is obvious, that colloidal nanoparticles of “water-soluble (nC_{60})” synthesized by Oberdörster and co-authors must contain on their surface not only hydroxyl-, but also another oxygen-containing groups. Similar chemical groups are more polar than C_{60} molecule and its presence at C_{60} surface is the major reason for the stability of such heterogeneous nanocolloids of C_{60}. Additionally, this is the only reason why authors [7] could reach so high concentration of “water-soluble (nC_{60})”, 35 mg/L, which exceeds the spontaneous (cluster) solubility of "high" hydrophobic C_{60} crystals by a factor of more than million (nevertheless, about hydrophilicity and high affinity C_{60} micro-crystals to water see ref.[18]!).

Therefor, any interaction with living cells, with living organisms, as well as any toxic effects of “water-soluble (nC_{60})” must be caused by the presence of polar oxygen-containing chemical groups on the surface of fullerene particles, but not by pure C_{60}. In consequence of this the title of the paper by Oberdörster and co-authors should be modified to indicate that study of toxic effects was conducted on ”water-soluble (nC_{60})” with modified (oxidized) surface, but not just “fullerene (C_{60})”.

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1 - Oberdörster E. Informal information on the toxicity of engineered nanomaterials; 2005. Available from: http://www.nanotox.info.

Next, Oberdörster in her article [7,8] admits in fact that ”water-soluble (nC_{60})” are much less toxic than former nC_{60} or nano-C_{60} synthesized by Colvin methods (THF+C_{60}+H_2O) [2,5].

Indeed, tests with *Daphnia magna* shown in her paper had not revealed any noticeable toxic effects in 2 to 4 days at concentration of ”water-soluble (nC_{60})” up to 35 ppm (35 mg/L). In complete contrast to these results, earlier Oberdörster observed obvious mortality of *Daphnia magna* at LC_{50} ~0.5 ppm (0.5 mg/L) over the same period of time when nC_{60} or nano-C_{60} was used instead [1].

In addition to this we would like to remind that toxic concentration of nano-C_{60} for human dermal fibroblasts (HDF) was found to be very low - 0.02 ppm (0.02 mg/L). It was shown in previous research by Colvin group [3].

From the facts described above we conclude that toxicological characteristics of “water-soluble (nC_{60})” strongly, by 1 to 2 orders of magnitude, differ from former nC_{60} or nano-C_{60}, and that this difference is naturally connected with different physical-chemical composition of colloidal particles in such dispersions [17].

Now, accounting all above mentioned, one can ask a fair question: if nano-C_{60}, nC_{60} and “water-soluble (nC_{60})” expose so different toxicity, then is it correct to attribute their toxic effect to pure C_{60}? Or real reasons of their toxic effect are quite another?

In connection with these questions, we point that in well known paper by Colvin group, in the experiments with HDF, the toxic concentration of oxygen-containing derivatives of C_{60} was equal to 40 ppm (40 mg/L) for $Na^{+}_{2-3}[C_{60}O_{7-9}(OH)_{12-15}]^{(2-3)}$, 10 ppm (10 mg/L) for $C_{60}\{C(COOH)_2\}_3$ [3] and 10 ppm if $C60(H_2O)_{24}$ was tested with human vascular endothelial cells [19]. These values are very close to those found from the experiments with “water-soluble (nC_{60})” and are much less (by approximately 2 orders of magnitude) than same values for nano-C_{60}.

Thus, after careful analysis of all above mentioned facts we have to come to a single possible conclusion that all previously discovered toxic effects of nano-C_{60}, nC_{60} and “water-soluble (nC_{60})” are caused not by pure

fullerenes but rather by their solvates (C_{60} clusters impregnated by THF) [9], and C_{60} nanoparticles with chemically modified surface.

Neither Oberdörster nor Colvin, nor Isacovic can tell anything about toxicity of pure C_{60}, because none of them had such object in their biological experiments. As a result, all their data for fullerene toxicity completely contradict many known facts about non-toxicity of C_{60}, (see also the recent works [20-22]) and moreover, they contradict facts about amazing biological activity of hydrated C_{60} (HyFn) used in a form of its aqueous molecular-colloidal solutions – FWS [9-11, **1**].

Going back to the question of ecological danger of pristine C_{60}, we would like to point that in experiments by Oberdörster and co-authors some negative biological effects observed for water-living species appeared at concentration of "water-soluble (nC_{60})" 2.5 ppm (2.5 mg/L) and higher. Hereto it should be noted that aquatic organisms under test lived long time in the quite turbid (troubled) medium containing the nC60 at concentrations of 2-35 ppm and consisting of nanoparticles with size up to 200 nm. In this connection there are fair questions, whether a some negative effects of nC60 nanoparticles (that are really hydrophilic! [18]) can to be in fact stipulated by the coarse clogging with nanoparticles of vitally important openings and channels for food and water consumption at Daphnia Magna? [7, 8, 23] What is known about similar effects, including bioaccumulation, for another nano-materials (e.g., for carbon black, standard urban particulate matter (SRM1648)?

Nevertheless, let us solve an imaginable task, where we use some natural water reservoir like a small lake. Let size of such lake be 1000 m x 500 m x 4 m. Then volume of the water in this lake is $2*10^9$ L. After multiplying this volume by concentration of "water-soluble (nC_{60})" 2.5 mg/L we get 5 tons! In other words, in order to reproduce Oberdörster's experiment in nature one has pour 5 tons of pristine C_{60} in this hypothetical lake, then to mix intensively (e.g. using tornado like it was described in footnote **2**) during 2 months at good solar radiation to see how do water-living organisms feel. It is clear that such task cannot be performed under natural conditions, especially if we have to work with such natural reservoirs like rivers, seas and oceans. From other side, if someone will try to make the environmental harm using toxic property of

Colvin's nano-C_{60}, then it is needed to pour in such hypothetical lake about 2800 ton (35 rail tank car) of the saturated solution of C_{60} (ca 9 mg/L [5,13]) in THF. Such attempts are equal to a big diversion. Only crazy man would do that. But such an idea is absolutely not realistic and not possible to perform in practice: it would be much easier to break down the tanker with oil.

In conclusion of this discussion on the toxicity of fullerenes, we would like to stress that we are absolutely sure that pure fullerenes, even if they happen to appear in our natural water reservoirs, do not constitute some significant danger for warm-blooded animals and mammalians (see also [20-22]), even if these animals will eat the Daphnia ([7,8]) "charged" by fullerenes nanoparticles.

Therefor it is recommended to handle pure fullerenes in the same way as with usual carbon black (U.S. Dept. of Labor, OSHA's Hazard Communication Standard, 29 CFR 1910.1000 and 29 CFR 1910.1200).

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