March 14, 2007

Prof. Paul Pantano
Department of Chemistry
University of Texas at Dallas
Richardson TX 75083-0688

Ms. No.: JA070971F-34-52
Title: Single-Walled Carbon Nanotube Interactions with HeLa Cells

Dear Prof. Pantano:

The reviews of your manuscript follow. As you will see, in addition to raising a number of technical issues, both reviewers indicate that this manuscript is not appropriate for JACS. The manuscript focuses on analytical methodologies for assessing nanotube uptake in cells and the distribution of the nanotubes within cells. This is nicely performed research but does not appear to provide new chemical insights that are anticipated in a general chemistry journal. In addition, reviewer II correctly notes that this manuscript and ref 19 report very similar studies using different materials. Thus, based on these reviews and my own reading of your manuscript, I cannot accept it for publication in JACS. Perhaps after the reviewers' comments have been addressed, submission to a more specialized journal would be appropriate.

Thank you for considering JACS for this work.

Sincerely,

Henry S. White
Associate Editor

Enclosures: Referee Reports I and II
Rating

Is the Manuscript likely to be of interest to the broad readership? Yes
Are the conclusions adequately supported by the data presented? Yes
Are the literature references appropriate and correct? Yes
Does the nomenclature used conform with accepted practice? Yes
Are hazardous procedures clearly defined as such? N/A

Recommendation: Do Not Publish.

Additional Comments: The paper is well written and demonstrates all possible experimental characterization of SWNTs and also their uptake results with HeLa cells.

The major concern is that authors submitted another paper on the exactly the same subject and the only difference is the type of CNTs used. In reference 19, authors claim that they have done the same study for the HiPCo SWNTs and they reported that. In this paper in JACS the only major difference is the type of SWNT which are purchased from CoMoCAT this time.

Reviewer is in major doubt about the scientific contribution of very similar two papers and recommends pulling one of the submissions back.
Rating

Is the Manuscript likely to be of interest to the broad readership? No
Are the conclusions adequately supported by the data presented? Yes
Are the literature references appropriate and correct? Yes
Does the nomenclature used conform with accepted practice? Yes
Are hazardous procedures clearly defined as such? N/A

Recommendation: Publish elsewhere.

Additional Comments:

See attached.
In this paper Yehia et al. studied the interactions of CoMoCAT single walled carbon nanotubes with HeLa cells. They used a series of different analytical techniques to characterize the material suspended/solubilized in the cell culture media and eventually its cellular internalization and impact on the cell viability. Overall the work is impressive and carefully conducted.

However, I believe that the results described are not appropriate for JACS. The authors should submit this manuscript to a journal focused on toxicity and health studies of new materials.

In a revised version of the paper, the authors should tone down their claim about standardization of their method of characterization of the nanotube cytotoxicity. They used only a single specific type of tubes and I believe this is not sufficient to establish the series of techniques used in this paper as general standards.

The characteristics of the starting carbon nanotubes should be given in order to immediately compare them with the tubes isolated in the cell culture media. The authors should explain how they calculated the concentration of the nanotubes after the different centrifugation cycles. They wrote that the starting suspension is 1 mg/ml and only at page 21 they report, for the first time, that the solutions of the nanotubes they are using contain 10 micrograms per milliliter, without giving any detail in the text on the calculation of this value.

It is very difficult to understand if the structures shown in Figure 4A are complexed carbon nanotubes. Did the authors try to use a different electron microscopy technique for comparison?

The description of the TGA analysis is not clear. Since there is any clear difference in the curve of DMEM alone and DM-SWNT, the derivative weight-percent plot seems very important, but the authors just cited this calculation as data not shown. I believe that this should be shown. This would support the presence of the nanotubes that seems not to influence the TGA curve of DMEM. The amount of nanotubes present in the complex is given, but the authors did not report the relative total amount of the components contained in the medium. How these components affect the TGA analysis? The curve of DM-SWNT in the Figure 5 does not support the presence of the tubes into the sample.

The authors should explain how the cells incubated at 4°C behave after 60 h. Are they still alive? Certainly all functions are blocked and the membrane should be completely rigid. After such long treatment at low temperature it is difficult to believe that the cells have any active mechanism operating. It would be more accurate if the analysis at low temperature is conducted by analyzing the time dependence. What happens if the cells are incubated at 4°C for shorter times (0.5 h, 1 h, 2h, 4h, etc.) and subsequently added with the nanotubes solution and let to interact with them for different times?

The Raman curves reported in the Figure 8 are not reliable. They have no statistical significance. The internalization might certainly be time dependent, but the intensity of the nanotube detection inside the cell depends on the exact point on the cell toward which the laser is directed. The authors should look at different cytoplasmatic areas and subsequently make an average of the intensities of their signal at the different time points. A single measure is not sufficient and not representative.

The authors should be more careful in the description of the dimensions of the clusters inside the vesicles. On which basis they wrote that the nanotubes are in the range of 5 to 20 nm in diameter and between 50 and 300 in length. Inside the vesicles shown on Figure 10, the tubes look like black globular aggregates.

The mass analysis technique is not a spectroscopy. Mass spectroscopy should be changed to mass spectrometry. The letters on Figure 13 and S5 are missing.