CARBON SOLUTIONS, INC

January 21, 2010

Jeffrey Wong, Ph.D.
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Re: Chemical Information Call-In: Carbon Nanotubes

Dear Dr. Wong,

Enclosed is the response of Carbon Solutions Inc to the questions included in the chemical information call-in notice of the California Department of Toxic Substances Control dated January 22, 2009.

We hope that the submitted information will help the effort led by CalEPA in developing sound science and safety in chemical practices.

Please, feel free to contact me if you need further information.

Sincerely,

Elena Bekyarova

CEO
Carbon Solutions Inc

1) What is the value chain for your company? For example, in what products are your carbon nanotubes used by others? In what quantities? Who are your major customers?

Carbon Solutions Inc. (CSI) was established in 1998 when the carbon nanotube field was in its infancy and milligram quantities of single-walled carbon nanotubes (SWNTs) had just started to become available. The driving force behind this effort was the belief that CSI scientists could provide specifically tailored, high quality materials to the scientific community and that this would facilitate a fundamental understanding of the outstanding properties of carbon nanotubes and allow their translation into functional materials, structures and devices. The startup funding for Carbon Solutions was provided by NSF Phase 1 and 2 SBIR awards which allowed the company to make available functionalized SWNTs for use in applications which required solvent solubility or enhanced chemical and physical properties.

This remains true today and the major CSI customers are still universities, national labs, research institutions, specialty chemical retail companies and R&D groups of small and large companies. Despite the claims that carbon nanotubes may revolutionize existing industrial processes and products, their translation into the worldwide commercial market is limited. To the best of our knowledge CSI materials have not been incorporated into commercial products. In 2009, about 70% of the customers were researchers from universities, national labs and research institutions. By quantity, about 72% of the total amount of the CSI SWNT products was purchased by these customers. The functionalized SWNT materials are purchased at the milligram scale and typical orders of purified SWNTs are in the range of 0.5-1 g.

From this data and based on discussions with our customers, we believe that the materials sold by Carbon Solutions are currently used for fundamental research and R&D purposes only.

2) What sampling, detection and measurement methods are you using to monitor (detect and measure) the presence of your chemical in the workplace and the environment? Provide a full description of all required sampling, detection, measurement and verification methodologies. Provide full QA/QC protocol.

Quantitative analysis of carbon nanotubes remains a challenging task. The complexity of the problem is best characterized by the fact that the scientific community has not yet agreed on a

quantitative technique, which provides unambiguous quantitative measurements of bulk SWNT samples. Various analytical techniques, such as Scanning and Transmission Electron Microscopy (SEM and TEM), Thermo-Gravimetric Analysis (TGA), Raman and near-infrared (NIR) spectroscopy are used for analysis of SWNT materials and each of these techniques has advantages and limitations.[1]

Monitoring SWNTs in Solid and Liquid Phases: We use the NIR spectroscopic technique to monitor the presence of electric arc produced SWNTs in solid (bulk powder materials) and solution phase (dispersions). This approach is based on the distinct spectral features, which originate from the SWNT interband transitions and are absent in other forms of carbon materials as shown in Figure 1.

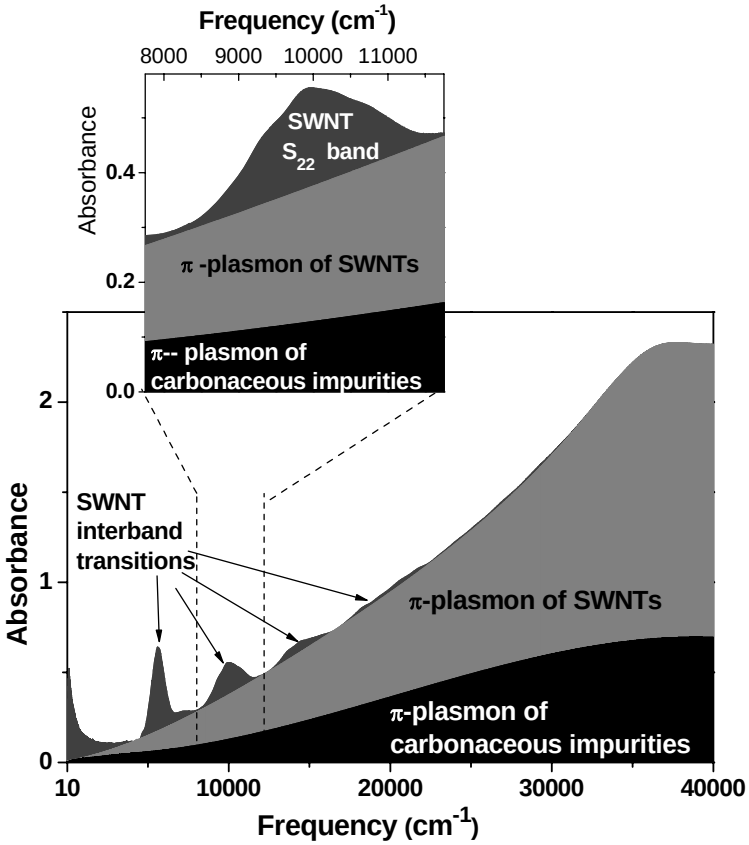

Figure 1. Typical wide range spectrum of SWNT sample showing distinct interband transition features. The presence of nanotubes and their concentration in any bulk liquid or solid (powder) samples can be evaluated by calculating area of S_{22} interband transition peak normalizing to the area of total absorbance in the same spectral range.

The NIR technique has been shown to provide a rapid, convenient, and unambiguous measure for the presence of SWNTs. Figure 2 illustrates the NIR spectra of three samples – a purified SWNT material (sample A), waste material with 10% content of SWNTs (sample B) and waste material (sample C), which does not contain SWNTs. For example, spectrum of sample A shows a strong S_{22} peak which corresponds to a SWNT content in the sample of 89%, sample B shows a very weak S_{22} peak corresponding to a SWNT concentration of only 10%, and sample C shows no visible S_{22} feature indicating the absence of SWNTs. Thus, using this spectroscopic technique and sample preparation methods described in Appendix A we can determine SWNT content in any bulk liquid or solid material.

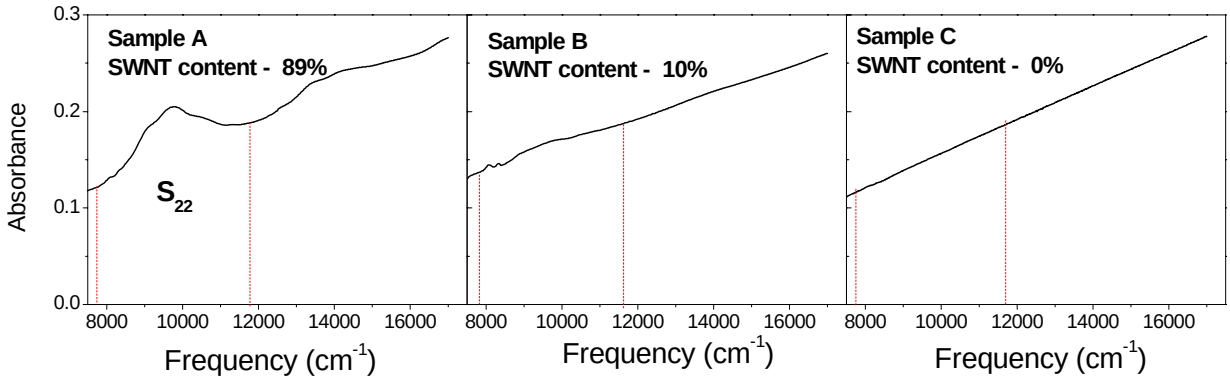

Figure 2. Near-IR (NIR) spectra of samples with relative SWNT carbonaceous purities of 89%, 10% and 0%, as estimated by integrating the area of the second interband transition (S_{22}) of SWNTs.[2]

The sample preparation and measurement procedures are developed following the procedures described in detail in the NIST Recommended Practice Guide "Measurement Issues in Single Wall Carbon Nanotubes" and are included in APPENDIX A.

In addition, Solution Phase Raman can be utilized with similar success for monitoring the presence of SWNTs.[3]

Monitoring Airborne SWNTs: Carbon Solutions invited the Nanotechnology Field Team of NIOSH to conduct an on-site assessment of the potential sources of airborne carbon nanotubes in our laboratory facilities and to evaluate our risk management program. In November 2009 the team performed sampling and data collection of the processes at various stages of SWNT manufacture in order to evaluate airborne release of carbon nanotubes at potential emission

sources. The measurements were conducted over a period of 3 days and covered all SWNT standard processes and CSI handling procedures.

A number of direct-reading condensation particle counters for various particle sizes were positioned as close as possible to the potential point of emission for a given process as well as in other selected locations in the lab. Air-filtered samples were also collected for particle size and morphology determination by transmission electron microscopy. Ambient/background particle number concentration measurements were collected inside each laboratory before each process.[4] The collected data are being processed and analyzed by NIOSH and will become available to us within several months. Below we summarize the preliminary information from NIOSH which was provided at the end of the study:

1) Harvesting of synthesized SWNTs from the reactor chamber inside the synthesis lab. This procedure generates observable levels of airborne emission in the synthetic room during the SWNT collection procedure (quantitative data regarding particle size distribution and concentrations will be available later). Preliminary data did not show particle counts above the background level in adjacent laboratory rooms or within the personal protective equipment. After the conclusion of the harvesting process the particle count reduces with time and measurements next morning were at the background level.

Summary of current relevant safety measures:

a) Synthetic room operating at a negative pressure differential
b) Full face-piece respirators – HEPA filtered
c) Full-cover protective gear
d) Gloves - double gloves
e) Shoe covers
f) HEPA filtered powder hood
g) Sticky mats in front of the reactor chamber and entrance door.
h) HEPA filtered vacuum cleaners
i) Change room separating the synthetic lab and general lab area

2) Chemical processing of SWNT materials

This procedure generates relatively small amount of airborne SWNTs, mainly during loading of the SWNT soot in the reaction vessel and during addition of reagent (e.g. acid, water, organic solvents).

Summary of relevant safety measures for this process:

a) Loading of SWNT soot in a HEPA filtered powder hood
b) Respirator with P100 filters
c) Gloves and safety glasses

3) What is your knowledge about the current and projected presence of your chemical in the environment that results from manufacturing, distribution, use, and end-of-life disposal?

As detailed in reference to question 5, Carbon Solutions Inc (CSI) has implemented a suite of engineering and administrative controls, based on available technology to control the entry of nanomaterials into the environment. To evaluate the effectiveness of these measures, NIOSH was invited to conduct an on-site study. Preliminary data from the NIOSH evaluation of CSI operations indicate that the current CSI risk management program is efficient in capturing airborne SWNTs. Based on the detailed analysis report, the controls will be adjusted and optimized. The rational risk management program developed at CSI, minimizes the entry of SWNTs into the environment.

Handling and treatment methods for carbon nanotubes in the liquid phase and end-of-life (waste) is discussed with reference to question 6. Products are distributed in capped, thick-glass containers, sealed appropriately with adhesive polymer based materials, to prevent the release and spread of the packaged materials. Packaging is carried out under the same precautionary conditions as used during manufacturing and processing. Our customers are researchers, with training in nanotechnology and related fields, and thus we believe that with appropriate nanotechnology safety programs in place, the release into the environment, which results from distribution of our SWNT materials, is negligible. The amounts of ordered materials are also very small – milligram and gram scale.

Potential Sources of SWNT release:

- Production - SWNTs are synthesized in a closed chamber and there is no release of material during the production.
- Harvesting of SWNT soot from the synthetic chamber – opening the chamber poses the potential for release of airborne material. Currently, to prevent release of the SWNTs in the environment, the synthetic chamber is placed in a room specially constructed to operate at a negative pressure differential to the remainder of the facility. Preliminary particle count measurements in adjacent laboratory rooms do not show particle counts above the background level, indicating that this measure is efficient in preventing release of SWNTs outside of the synthetic room. The room is designed with a local ventilation system. A HEPA filtered powder hood is installed inside the room and the SWNT soot is transferred into sealed containers in the hood. We are developing an enclosure of the synthetic chamber, which would minimize and ultimately eliminate the risk of SWNT release.
- Processing of SWNT materials – this include dispersion in solvents for chemical processing and analysis. The dry SWNT material is loaded into the containers for dispersion in the powder hood and the sonication is performed in sealed containers using a bath sonicator placed in a chemical fume hood.
- Storage of SWNT materials – the materials are stored in capped containers, sealed appropriately with adhesive polymer based materials, to prevent its release and spread.
- Spills – there are in place procedures for handling of spills (Appendix C)
- Waste – materials containing SWNTs are collected as hazardous waste, as addressed in reference to question 6.

In summary, there are existing engineered control techniques to minimize airborne SWNT material, which are effective in capturing nanoparticles. Current knowledge indicates that a well designed ventilation system with high-efficiency particulate (HEPA) filters should effectively remove nanomaterials.[5, 6] We take all reasonable measures to minimize release of SWNTs into the environment and to capture SWNTs released during handling: HEPA filters, sticky mats, sealed containers, manipulation (handling) of large quantities of carbon nanotubes in a restricted area specially constructed to operate at a negative pressure differential to the remainder of the facility, disposal of materials containing SWNTs as hazardous waste. Currently all procedures, which are expected to have a high potential for the release of airborne SWNTs, are performed in a powder hood equipped with HEPA filter, which is installed in the room under negative pressure differential to the remainder of the facility. We have further evaluated these

measures with the help of NIOSH. We continue to improve existing procedures and to introduce new engineered controls as a part of our risk management program, including source enclosure. Until complete understanding of the health risks of carbon nanotubes becomes available, Carbon Solutions will utilize the existing techniques to capture released SWNT materials and assure protection of the workers and the environment.

4) What is your knowledge about the safety of your chemical in terms of occupational safety, public health and the environment?

There is an increased scientific interest in the toxicological properties, environmental fate and safe handling of carbon nanotubes. Several centers have been established in the United States, which are focused on these issues and we follow their active research activities and scientific publications, which provide state of the art knowledge of the field:
Center for Biological and Environmental Nanotechnology at Rice University,[7] Center for Environmental Implications of Nanotechnology at UCLA, [8] Center for Environmental Implications of Nanotechnology at Duke University.[9]

NIOSH established a Nanotechnology Field Research Team[10] to expand its knowledge and understanding of the potential health and safety risks that are faced by workers who come into contact with engineered nanoparticles in the nanotechnology industry. Some information on the potential health concerns is provided by NIOSH in the “Approaches to safe nanotechnology”, Chapter 5.[5]

The knowledge about the safety of the SWNTs continues to evolve. The toxicity studies of single-walled carbon nanotubes have given contradictory results for a number of reasons: varying nature and degree of metal contaminations in the carbon nanotube samples, different agglomeration state of the nanotubes, interaction of the SWNTs with the medium and various dyes commonly used to assess cytotoxicity, physical interference in the spectrophotometric measurements due to light absorbance and scattering. Recent reviews published by NIOSH[11] and an international research team conducting a study on medical applications of SWNTs[12] give a comprehensive analysis of the toxicological studies of SWNTs and highlights critical issues concerning the conduct and evaluation of the studies.

It is worth noting that Carbon Solutions Inc has expertise in the preparation of functionalized SWNT materials. Studies suggest that chemical modification affects the biocompatibility of SWNTs and it is known that water soluble SWNTs have relatively low toxicity.[13, 14]

There is active research on the use of carbon nanotubes in biomedicine and there are a number of scientific publications, which highlight the potential of these materials for the development of novel nanotube-based therapeutic and diagnostic tools. For example, carbon nanotubes show promise in the battle against cancer[15-21] and some of the studies utilize CSI functionalized SWNT products.[22]

Functionalized SWNT materials, similar in chemical nature to our materials, have shown promise as scaffolds for neuron growth and interfacing,[12, 23-29] bone growth[30, 31] and advanced imaging techniques.[32-34]

As described in reference to question 2 and 5, CSI has developed engineering and administrative controls to assure a safe workspace and environment. Until complete understanding of the risk factors associated with carbon nanotubes develops, CSI will continue to use precautionary measures in working with these materials and to utilize the latest developments in the safe handling of carbon nanotubes. The key to maintaining safety is a trained and aware workforce.

5) **What methods are you using to protect workers in the research, development and manufacturing environment?**

At CSI, minimizing the potential risk for exposure of the workers and preventing the release of carbon nanotubes into the environment is the topmost priority. Within the limits of the information available about the health risks associated with exposure to single-walled carbon nanotubes and the fact that methods for quantitative assessment of carbon nanotubes in the environment are still under development, the company has implemented a risk management program to minimize worker exposure to SWNTs, particularly in airborne form. The risk management program has three components:

1. Worker's Training: The scope and content of the training is described in APPENDIX C. Safety issues are discussed in a meeting with the employees weekly**;** the risk management

program is dynamic and implements the latest knowledge about nanomaterials and their safe handling.

2. Engineered Controls:

a) The laboratory space is designed for work with nanomaterials and equipped with local ventilation system, chemical fume hoods, HEPA filters and sticky mats.

b) The laboratory space is divided into separate rooms and the processes are categorized in three groups according to the risk of carbon nanotube release – low, intermediate and high risk.

c) The processes with high risk of carbon nanotube release are separated in a room kept under negative pressure, equipped with powder hood and HEPA filters.

3. Personal Protective Equipments: The selection and use of appropriate PPE is described in APPENDICES B and C.

6) When released, does your material constitute a hazardous waste under California Health &Safety Code provisions? Are discarded off-spec materials a hazardous waste? Once discarded are the carbon nanotubes you produce a hazardous waste? What are your waste handling practices for carbon nanotubes?

The waste from carbon nanotube manufacturing and processing are treated as a hazardous waste.

Solid waste, which results from processing of carbon nanotubes, is collected in sealed containers. The containers are labeled, together with a list of all chemical constituents as hazardous waste.

Liquid suspensions containing nanomaterials are collected in sealed containers. The suspensions are then filtered to collect the carbon nanotubes, which are collected as solid waste. Water based acidic suspensions are first neutralized before filtering. The neutralization facilitates the precipitation of carbon nanotubes from the dispersions and filtration is then used to isolate the solid waste.

It should be noted that carbon nanotubes consist of carbon, which is relatively easy to oxidize with strong oxidants, including air at elevated temperatures. Thus, methods for destruction of this nanomaterial are relatively easy to develop – examples include exhaustive reflux in nitric acid,[35] sulfuric acid or a mixture of the two; use of potassium permanganate; prolonged heating

in air at temperatures above 300°C to burn the carbon nanotubes. We are developing a procedure for decomposition of waste SWNTs by treatment with strong oxidizing acids; this will ultimately reduce the quantities of waste SWNT material requiring disposal.

Paper, wipes, PPE and other items with carbon nanotube contamination are collected in air-tight plastic bag stored in the laboratory hood. The full bags are transferred into closed containers and labeled with a proper waste label.

References:

1. Itkis, M. E.; Perea, D.; Jung, R.; Niyogi, S.; Haddon, R. C., Comparison of Analytical Techniques for Purity Evaluation of Single-Walled Carbon Nanotubes. *J. Am. Chem. Soc.* **2005,** 127, 3439-3448.
2. Itkis, M. E.; Perea, D.; Niyogi, S.; Rickard, S.; Hamon, M.; Hu, H.; Zhao, B.; Haddon, R. C., Purity Evaluation of As-Prepared Single-Walled Carbon Nanotube Soot by Use of Solution Phase Near-IR Spectroscopy. *Nano Lett.* **2003,** 3, 309-314.
3. Nishide, D.; Miyata, Y.; Yanagi, K.; Tanaka, T.; Kataura, H., PERIPUTOS: Purity evaluated by Raman intensity of pristine and ultracentrifuged topping of single-wall carbon nanotubes. *Physica Status Solidi B-Basic Solid State Physics* **2009,** 246, (11-12), 2728-2731.
4. Johnson, D. R.; Methner, M. M.; Kennedy, A. J.; Steevens, J. A., Potential for Occupational Exposure to Engineered Carbon-Based Nanomaterials in Environmental Laboratory Studies. *Environ. Health Prospect* **2010,** 118, 49-54.
5. Approaches to Safe Nanotechnology. http://www.cdc.gov/niosh/topics/nanotech.
6. DOE, Nanoscale Science Research Centers Approach to Nanomaterial ES&H. In Vol. http://www.er.doe.gov/bes/DOE_NSRC_Approach_to_Nanomaterial_ESH.pdf.
7. Center for Biological and Environmental Nanotechnology, Rice University. http://cben.rice.edu/.
8. Center for Environmental Implications of Nanotechnology, UCLA. http://cein.cnsi.ucla.edu/.
9. Center for Environmental Implications of Nanotechnology, Duke University. http://www.ceint.duke.edu/.
10. Nanotechnology Field Research Team. http://www.cdc.gov/niosh/docs/2008-120.

11. Shvedova, A. A.; Kisin, E. R.; Porter, D.; Schulte, P.; Kagan, V. E.; Fadeel, B.; Castranova, V., Mechanisms of Pulmonary Toxicity and Medical Applications of Carbon Nanotubes: Two Faces of Janus? *Pharmac. Therap.* **2009,** 121, (2), 192-204.
12. Kotov, N. A.; Winter, J. O.; Clements, I. P.; Jan, E.; Timko, B. P.; Campidelli, S.; Pathak, S.; Mazzatenta, A.; Lieber, C. M.; Prato, M.; Bellamkonda, R.; Silva, G. A.; Kam, N. W. S.; Patolsky, F.; Ballerini, L., Nanomaterials for Neural Interfaces. *Adv. Mater.* **2009,** 21, 3970-4004.
13. Sayes, C. M.; Liang, F.; Hudson, J. L.; Mendez, J.; Guo, W. H.; Beach, J. M.; Moore, V. C.; Doyle, C. D.; West, J. L.; Billups, W. E.; Ausman, K. D.; Colvin, V. L., Functionalization Density Dependence of Single-Walled Carbon Nanotubes Cytotoxicity *in vitro*. *Toxicol. Lett.* **2006,** 161, (2), 135-142.
14. Dumortier, H.; Lacotte, S.; Pastorin, G.; Marega, R.; Wu, W.; Bonifazi, D.; Briand, J. P.; Prato, M.; Muller, S.; Bianco, A., Functionalized Carbon Nanotubes are Non-Cytotoxic and Preserve the Functionality of Primary Immune Cells. *Nano Lett.* **2006,** 6, (7), 1522-1528.
15. Shi, X. Y.; Wang, S. H.; Shen, M. W.; Antwerp, M. E.; Chen, X. S.; Li, C.; Petersen, E. J.; Huang, Q. G.; Weber, W. J.; Baker, J. R., Multifunctional Dendrimer-Modified Multiwalled Carbon Nanotubes: Synthesis, Characterization, and In Vitro Cancer Cell Targeting and Imaging. *Biomacromol.* **2009,** 10, (7), 1744-1750.
16. Biris, A. S.; Galanzha, E. I.; Li, Z. R.; Mahmood, M.; Xu, Y.; Zharov, V. P., In vivo Raman Flow Cytometry for Real-time Detection of Carbon Nanotube Kinetics in Lymph, Blood, and Tissues. *J. Biomed. Opt.* **2009,** 14, (2), 021006.
17. Biris, A. S.; Boldor, D.; Palmer, J.; Monroe, W. T.; Mahmood, M.; Dervishi, E.; Xu, Y.; Li, Z. R.; Galanzha, E. I.; Zharov, V. P., Nanophotothermolysis of Multiple Scattered Cancer Cells with Carbon Nanotubes Guided by Time-resolved Infrared Thermal Imaging. *J. Biomed. Opt.* **2009,** 14, (2), 021007.
18. Liu, H.; Ryu, S.; Chen, Z.; Steigerwald, M. L.; Nuckolls, C.; Brus, L. E., Photochemical Reactivity of Graphene. *J. Am. Chem. Soc.* **2009,** 131, 17099-17101.
19. Liu, Z.; Fan, A. C.; Rakhra, K.; Sherlock, S.; Goodwin, A.; Chen, X. Y.; Yang, Q. W.; Felsher, D. W.; Dai, H. J., Supramolecular Stacking of Doxorubicin on Carbon Nanotubes for In Vivo Cancer Therapy. *Angewandte Chemie-International Edition* **2009,** 48, (41), 7668-7672.
20. Liu, Z.; Chen, K.; Davis, C.; Sherlock, S.; Cao, Q. Z.; Chen, X. Y.; Dai, H. J., Drug Delivery with Carbon Nanotubes for *in vivo* Cancer Treatment. *Cancer Res.* **2008,** 68, (16), 6652-6660.
21. National Cancer Institute, Alliance for Nanotechnology in Cancer. http://nano.cancer.gov/action/news/2009/june/nanotech_news_2009-06-26g.asp.

22. Marches, R.; Chakravarty, P.; Musselman, I. H.; Bajaj, P.; Azad, R. N.; Pantano, P.; Draper, R.; Vitetta, E. S., Specific Thermal Ablation of Tumor Cells Using Single-Walled Carbon Nanotubes Targeted by Covalently-Coupled Monoclonal Antibodies. *Int. J. Cancer* **2009,** 125, 2970-2977.

23. Mattson, M. P.; Haddon, R. C.; Rao, A. M., Molecular Functionalization of Carbon Nanotubes and Use as Substrates for Neuronal Growth. *J. Mol. Neurosci.* **2000,** 14, 175-182.

24. Hu, H.; Ni, Y.; Montana, V.; Haddon, R. C.; Parpura, V., Chemically-Functionalized Carbon Nanotubes as Substrates for Neuronal Growth. *Nano Lett.* **2004,** 4, 507-511.

25. Ni, Y.; Hu, H.; Malarkey, E. B.; Zhao, B.; Montana, V.; Haddon, R. C.; Parpura, V., Chemically Functionalized Water Soluble Single-Walled Carbon Nanotubes Modulate Neurite Outgrowth. *J. Nanosci. Nanotechnol.* **2005,** 5, 1707-1712.

26. Hu, H.; Ni, Y.; Mandal, S. K.; Montana, V.; Zhao, B.; Haddon, R. C.; Parpura, V., Polyethyeneimine Functionalized Single-Walled Carbon Nanotubes as a Substrate for Neuronal Growth. *J. Phys. Chem. B* **2005,** 109, 4285-4289.

27. Malarkey, E. B.; Reyes, R. C.; Zhao, B.; Haddon, R. C.; Parpura, V., Water Soluble Single-Walled Carbon Nanotubes Inhibit Stimulated Endocytosis in Neurons. *Nano Lett.* **2008,** 8, 3538-3542.

28. Malarkey, E. B.; Fisher, K. A.; Bekyarova, E.; Liu, W.; Haddon, R. C.; Parpura, V., Conductive Single-Walled Carbon Nanotube Substrates Modulate Neuronal Growth. *Nano Lett.* **2009,** 9, 264-268.

29. Jan, E.; Hendricks, J. L.; Husaini, V.; Richardson-Burns, S. M.; Sereno, A.; Martin, D. C.; Kotov, N. A., Layered Carbon Nanotube-Polyelectrolyte Electrodes Outperform Traditional Neural Interface Materials. *Nano Lett.* **2009,** 9, (12), 4012-4018.

30. Zhao, B.; Hu, H.; Mandal, S. K.; Haddon, R. C., A Bone Mimic Based on the Self-Assembly of Hydroxyapatite on Chemically Functionalized Single-Walled Carbon Nanotubes. *Chem. Mater.* **2005,** 17, 3235-3241.

31. Zanello, L. P.; Zhao, B.; Hu, H.; Haddon, R. C., Bone Cell Proliferation on Carbon Nanotubes. *Nano Lett.* **2006,** 6, (3), 562-567.

32. Welsher, K.; Liu, Z.; Daranciang, D.; Dai, H., Selective Probing and Imaging of Cells with Single Walled Carbon Nanotubes as Near-Infrared Fluorescent Molecules. *Nano Lett.* **2008,** 8, (2), 586-590.

33. Liu, Z. A.; Li, X. L.; Tabakman, S. M.; Jiang, K. L.; Fan, S. S.; Dai, H. J., Multiplexed Multicolor Raman Imaging of Live Cells with Isotopically Modified Single Walled Carbon Nanotubes. *J. Am. Chem. Soc.* **2008,** 130, (41), 13540-13541.
34. Welsher, K.; Liu, Z.; Sherlock, S. P.; Robinson, J. T.; Chen, Z.; Daranciang, D.; Dai, H. J., A route to Brightly Fluorescent Carbon Nanotubes for Near-Infrared Imaging in Mice. *Nat. Nanotechnol.* **2009,** 4, (11), 773-780.
35. Hu, H.; Zhao, B.; Itkis, M. E.; Haddon, R. C., Nitric Acid Purification of Single-Walled Carbon Nanotubes. *J. Phys. Chem. B* **2003,** 107, 13838-13842.

APPENDIX A

Sample Preparation for NIR Measurements for Monitoring SWNTs in Liquid Phase:

1. For measurement of SWNT in solution phase collect all liquids, which result from processing SWNT materials (washing, filtration) in a container. Work in a chemical fume hood.
2. Homogenize by mechanical mixing.
3. Take a representative sample (10 mL) by a pipette and place in a vial.
4. Sonicate the sealed vial with the liquid for 10-15 min in a bath sonicator.
5. Visually observe the dispersion. If the dispersion is stable and visually non-scattering, proceed to step 6. If the dispersion is poor, continue to sonicate until all solid material is well dispersed. If the dispersion is very concentrated (appears dark and almost non-transparent) dilute with solvent and continue to sonicate.
6. Make appropriate dilutions to obtain a pale transparent dispersion.
7. Take spectra in the optical range of 7500 cm^{-1} to 12000 cm^{-1}. Note: collect background using the same solvent as in the analyzed dispersion (typically water)
8. Use the procedure for relative carbonaceous purity of SWNTs, described in the NIST Recommended Practice Guide "Measurement Issues in Single Wall Carbon Nanotubes" – pages 20 - 22, to calculate the purity of the sample. This represents the concentration of SWNTs in the carbon material, which is dispersed in the solvent. If the dispersion contains SWNTs, take a note of the nanotube content.
9. Combine all fractions used for analysis. Filter and collect as a solid waste. Label the container and include "Contains nanomaterials" in the content note.

Sample Preparation for NIR Measurements for Monitoring SWNTs in Solid Phase (powders):

1. For measurement of SWNT in a solid waste, homogenize the sample by mechanical mixing. Work in a chemical fume hood. Use P100-type respirator.
2. Take a representative sample (~50 mg) and disperse in 100 mL dimethylformamide (DMF) by ulrtasonication in a sealed vial using a bath sonicator for 30 min.
3. Add a few drops of the concentrated dispersion, collected by pipette from different regions of the sample, and dilute to 10 mL with fresh DMF.

4. Sonicate for 10 min.
5. Use additional 10 mL-scale dilution-ultrasonication cycles to reduce the concentration of the dispersion to ≈ 0.01 mg/mL.
6. Take spectra in the optical range of 7500 cm^{-1} to 12000 cm^{-1}.
7. Use the procedure for relative carbonaceous purity of SWNTs, described in the NIST Recommended Practice Guide "Measurement Issues in Single Wall Carbon Nanotubes" – pages 21 and 22, to calculate the purity of the sample. This represents the concentration of SWNTs in the carbon material, which is dispersed in the solvent. If the dispersion contains SWNTs, take a note of the nanotube content.
8. Collect the solid waste in a waste container and label; include "Contains nanomaterials" in the content note.

APPENDIX B

Safety Procedures for Harvesting SWNTs from the Synthetic Chamber

1. **Before entering the synthetic room:**

   - Wear clean “Tyvec” coverall
   - Wear shoe covers
   - Wear gloves, which cover hands and wrists completely through overlapping sleeve of the coverall; use of a second pair of gloves is recommended
   - Use only HEPA or ULPA particulate cartridges (PAPRFC1 or similar)
   - Check if the cartridges are clean and ready for use before each use
   - Make sure the respirator motor is working properly, perform a check with the test tube
   - Use PAPR (Powered Air Purifying Respirator - “Bullard” PA30 or similar) or supplied air respirator
   - Make sure the exhaust switch is always on

2. **Harvesting SWNT soot**:

   - Turn on the respirator’s motor
   - Make sure that the synthetic chamber is turned off
   - Assemble all accessories used for harvesting of SWNTs - trays, spatula, jars, in the powder hood.
   - Open the chamber
   - Collect the nanotubes into a tray
   - Transfer the tray into the powder hood
   - Transfer the collected material into jars and take the weight
   - Label the jar with batch number, amount of collected material, and date
   - Clean the chamber, work bench, powder hood, the utilized equipment and the room with HEPA-filtered vacuum cleaner and wet-wipe the surfaces.
   - Clean coverall with the vacuum cleaner.

3. **After harvesting SWNT soot**

- Go to the change-room and take off coverall, shoes cover, the first pair of gloves and put in a plastic bag for disposal; close the bag before exiting the room. Wet-wipe the motor, breathing tube and respirator hood if necessary and store in a plastic bag.
- Next morning wet-wipe the floor and surfaces (bench, chamber) once again. Use half-face respirator with a P100 cartridge.

CAUTIONARY NOTES:

- Do not use the respirator with low flow. If the “low air flow” alarm goes off check the connection of the breathing tube to the hood. If the alarm sound continues stop working, quickly clean yourself with the HEPA vacuum cleaner and leave the room.
- Do not exit the synthetic room in contaminated clothes.
- Do not remove any parts of the personal protective clothing before cleaning yourself with the HEPA vacuum cleaner
- Do not enter the synthetic room without a respirator and a lab coat
- Do not enter in the synthetic room at least one hour after harvesting SWNT soot

Purification Processes

Weigh the SWNT soot in the synthetic room.

Before entering the synthetic room:

- Wear clean “Tyvec” coverall
- Wear shoe covers
- Wear gloves, which cover hands and wrists completely through overlapping sleeve of the coverall; use of a second pair of gloves is recommended
- Use only HEPA or ULPA particulate cartridges (PAPRFC1 or similar)
- Check if the cartridges are clean and ready for use before each use

- Make sure the respirator motor is working properly, perform a check with the test tube
- Use PAPR (Powered Air Purifying Respirator - “Bullard” PA30 or similar) or supplied air respirator

In the synthetic room:

- Take the bottle with AP-SWNT soot and place in the HEPA-filtered powder hood
- Assemble all necessary equipment – glassware, spatula, balance
- Weigh the material in a round bottom flask
- Seal the flask with septa or parafilm and set aside in the powder hood
- Close the bottle with the remaining AP-SWNT soot
- Clean all used equipment and contaminated surfaces with HEPA-filtered vacuum cleaner
- Wet-wipe the flask
- If necessary clean coverall with the vacuum cleaner.
- Take off first set of gloves, coverall and respirator in the change room

In the wet-chemical lab

- Transfer the round bottom flask with the SWNT material from the synthetic to the wet lab. Make sure the flask is sealed. Use respirator – type P100. Use safety gloves
- Place the flask inside the chemical fume hood; secure
- Open the flask and slowly add all chemicals; use apron
- At the end of the process dispose waste in appropriate waste containers.

APPENDIX C

GUIDELINES FOR WORKING WITH SWNTs

1. Storage, labeling and handling

1.1 Storage:

- Carbon nanotubes or nanomaterials are stored in sealed labeled containers.
- Equipments and tools used to handle dry carbon nanotube power are stored in a powder hood equipped with HEPA filter.
- Outer surface of the container and storage area are cleaned with wet-wipes after use to avoid carbon nanotube contamination.
- Do not dry clean or use compressed air to clean carbon nanotubes in storage area.

1.2 Labeling:

- The containers should be labeled clearly such as “as-prepared carbon nanotubes” or “purified carbon nanotubes”.
- The label also indicates any other information pertaining to safe handling.
- If the carbon nanotube or the nanoparticles are dispersed in a solvent, indicate the solvent and concentration.

1.3 Handling:

- All manipulations of powder carbon nanotubes are done in a HEPA filtered power-hood. Wear appropriate respiratory protection and personal protective equipment (PPE).
- Carbon nanotubes dispersed in a liquid or solid phase are also handled with appropriate personnel protection equipment in a fume hood.
- Work area is cleaned using a wet-wiping method and/or HEPA-filtered vacuum cleaner. Dry sweeping is prohibited to clean work areas.
- The carbon nanotubes are transported in a sealed container.

2. Personal Protection

2.1 General requirements: Appropriate personal-protective equipment is used always in the work area to reduce the risk for exposure to nanomaterials. Each laboratory procedure involving carbon nanomaterial has been reviewed and evaluated to determine the use of appropriate personal protective equipment.

2.2 Clothing:

- Appropriate protective clothing with low permeability, safety glasses and closed-toed shoes.
- Use disposable laboratory coats and dispose them after use.
- Non-disposable laboratory coats are recommended for low-risk exposure procedure.

2.3 Gloves:

- Appropriate gloves are chosen after considering the resistance of the glove to the chemical attack by both the nanomaterial and, if suspended in liquids, the liquid. A table with the type of gloves and their chemical resistance is located in the wet chemical lab.
- Nitrile gloves are used when handling carbon nanotubes in liquids and use of double gloves is recommended to reduce skin exposure.
- Gloves are changed frequently during the experiments to avoid cross contamination.
- The contaminated gloves are kept in a closed air-tight plastic bag in the fume hood until disposed as hazardous waste.
- Wash hands before leaving the work area.

2.4 Respiratory Protection:

- Appropriate respirator and cartridge combination is worn when handling carbon nanotube powder.
- The respirator should be at a minimum half-mask, P-100 or N-100 cartridge-type respirator.
- Respirators of class N-95 can be worn during processes with low risk of airborne carbon nanotubes (the SWNTs are well dispersed in solution or polymer; processes in which SWNTs are in sealed containers).

- Do not use “dust masks”

3. Nanomaterial-Bearing Waste

- Nanomaterial-bearing waste is considered any waste consisting of pure nanomaterials (e.g. carbon nanotubes), items contaminated with nanomaterials (e.g. wipes/gloves/ disposable coats), liquid or solid matrixes containing nanomaterials.
- Nanomaterial-bearing waste is prohibited to be disposed off directly into the sanitary sewer system (i.e. down a sink drain) or disposed in the regular trash.
- Nanomaterial-bearing waste is collected in a sealed container or air-tight plastic bags that are compatible with the contents, in good condition, and that afford adequate containment to prevent escape of the Nanomaterials.
- Nanomaterial-bearing waste is labeled as “Hazardous Waste” and identified as waste containing nanomaterials with additional information for example, “gloves and wipes contaminated with carbon nanotubes”. When the bag/container is full it is sealed in a container for disposal.

4. Spills

- Use HEPA-filtered vacuum cleaners to clean up spills. Use wet wiping to clean surfaces.
- Do not use dry sweep or compressed air.
- The spill materials are collected and disposed as nanomaterial-bearing hazardous waste.

5. Training of technical personnel

Workers receive training prior to working with carbon nanotubes. This includes reading of the laboratory specific chemical safety procedures and specifically safe handling of carbon nanotubes. During the training the workers become familiar with:

- Engineered controls employed
- Use of PPE
- Techniques for safe handling of carbon nanotubes (**Appendix B**)

- Cleaning of potentially contaminated surfaces and steps to be taken in the event of an exposure incident or spill
- Handling of potentially contaminated laboratory garments and protective clothing
- Specific nanomaterial-related health and safety risks