Predicted phototoxicities of carbon nano-material by quantum mechanical calculations

Don Betowski*

National Exposure Research Laboratory, Exposure Methods and Measurement Division, U.S. Environmental Protection Agency, 944 E. Harmon Avenue, P.O. Box 93478, Las Vegas, NV 89193-3478, USA

*Corresponding author. Tel: +1-702-798-2116

E-mail address: betowski.don@epa.gov

Abstract

The purpose of this research is to develop a predictive model for the phototoxicity potential of carbon nanomaterials (fullerenols and single-walled carbon nanotubes). This model is based on the quantum mechanical (ab initio) calculations on these carbon-based materials and comparison of the triple excited states of these materials to published work relating phototoxicity of polynuclear aromatic hydrocarbons (PAH) to their predictive triple excited state energy. A successful outcome will add another tool to the arsenal of predictive methods for the U.S. EPA Program Offices as they assess the toxicity of compounds in use or coming into commerce.

The basis of this research is obtaining the best quantum mechanical structure of the carbon nanomaterial and is fundamental in determining other properties. Therefore, these properties, like possible phototoxicity, will have influence in directing the future uses of these materials.

This project relies heavily on the interaction of the predictive results (physical chemistry) and the experimental results obtained by U.S. EPA biologists and toxicologists. The results of the experiments (toxicity testing) will help refine the predictive model, while the predictions will alert scientists to red flag compounds. It is hoped that a guidance document for the U.S. EPA will be forthcoming to help determine the toxicity of compounds. The results of this research provide a screening tool that would rely on further testing for those compounds found by these predictions to be a phototoxic to health and the environment.

Keywords: phototoxicity, fullerenols, single-walled carbon nanotubes, ab initio calculations.

1. Introduction

Nanotechnology has been presented as a two-edged sword. The advances that can be, and have been, made in materials, electronics, and medicine are remarkable. Yet, at the same time the situation of opening Pandora's box is also real, in the sense that unknown dangers to the environment and health can result. Nanotechnology has already generated novel types of matter such as fullerenes and carbon nanotubes. The problem is the final disposition of these and other nanomaterials when they enter the environment and what are their effects? Some researchers have been worried about the ethical gap that this new technology has created (Mnyusiwalla et al., 2003)

Maynard et al. (2006) called for methods to evaluate the toxicity of engineered nanomaterials in the next five to fifteen years, validate screening tests, develop viable alternatives to in vivo tests, and determine the toxicity of fibre-shaped nanoparticles. Among the suggestions put forward in this paper are providing alternatives to in vivo testing for simulating and predicting nanomaterial behavior in living organisms on ethical and economic bases.

Among the most widely used carbon nanomaterials are fullerenes (and their derivatives) and carbon nanotubes, both single- and double-walled versions. Buckminsterfullerene, C_{60}, which came out of the laser-vaporization supersonic cluster beam technique study on clusters of carbon (R.E. Smalley, 1992), was recognized as a separate form of carbon, its structure being similar to a geodesic dome. The discovery of fullerene stimulated much research on C_{60} and many derivatives resulted. One of the series of compounds derived from fullerene is the hydroxylated form called fullerenol or fullerol, $C_{60}(OH)_n$. Functional fullerenes, such as the

fullerenols, potentially have more applications than basic fullerenes due to their water solubility (Gao et al., 2011). Since the fullerenols are water soluble, they may be capable of bypassing the brain and ocular barriers and be useful in medical uses as drug carriers (Calvo, et al., 1996); Roberts et al., 2008.)

However, also because of this water solubility, the potential for water and soil environment contamination by these fullerene derivatives is greater (Gao et al., 2009 ; Gao et al., 2011). Zakharenko et al. (1997) determined that water-soluble fullerenes are not genotoxic, but water-soluble fullerenes being retained in the body could raise concerns about chronic toxic effects (Yamago et al., 1995). Roberts et al. (2008) have found that fullerenols are both cytotoxic and phototoxic to human lens epithelial cells in the presence of either UVA or visible light. These finding will be examined by extending work on the theoretical phototoxicity of PAHs to the study of fullerenols (Betowski et al., 2002).

Included in this present work are some basic single-walled carbon nanotubes (SWCNT). SWCNTs are carbon cylindrical tubes composed of benzene rings. There are many different forms of the SWCNTs, which can represented by the indices n, m. If n=m, they are classified as armchair nanotubes, if m=0, they are called zigzag nanotubes, and the rest are called chiral. In addition to the dimensions n, m, nanotubes are also characterized by the number of units contributing to their length from 1 to p, where p can be a very large number. Nanotubes have been used in various fields from electronics to medicine.

Several authors studied the connection between toxicity and the excited states of the subject molecule (Newsted and Giesy, 1987; Mekenyan et al., 1994). The triplet excited state energy of the molecule was found to play an important role in predicting median lethal time (LT50) for polynuclear aromatic hydrocarbons (PAHs). The number of photons hitting the earth

plays a role in phototoxicity, because various wavelength regions in natural sunlight have different number of photons. The higher energy photons are absorbed by the atmosphere before they reach the surface. If all wavelengths had similar number of photons, the phototoxicity curve would be linear increasing with the energy of the photons (or decreasing with the increasing wavelength of the photons). Because of this uneven distribution of photons, the phototoxicity curve shows a parabolic relationship. This study will use the curve from Betowski et al. (2002), which is shown on the Figure 1, and this curve will be used as a model for nanomaterials based on the toxicities and calculated triplet states for PAHs.

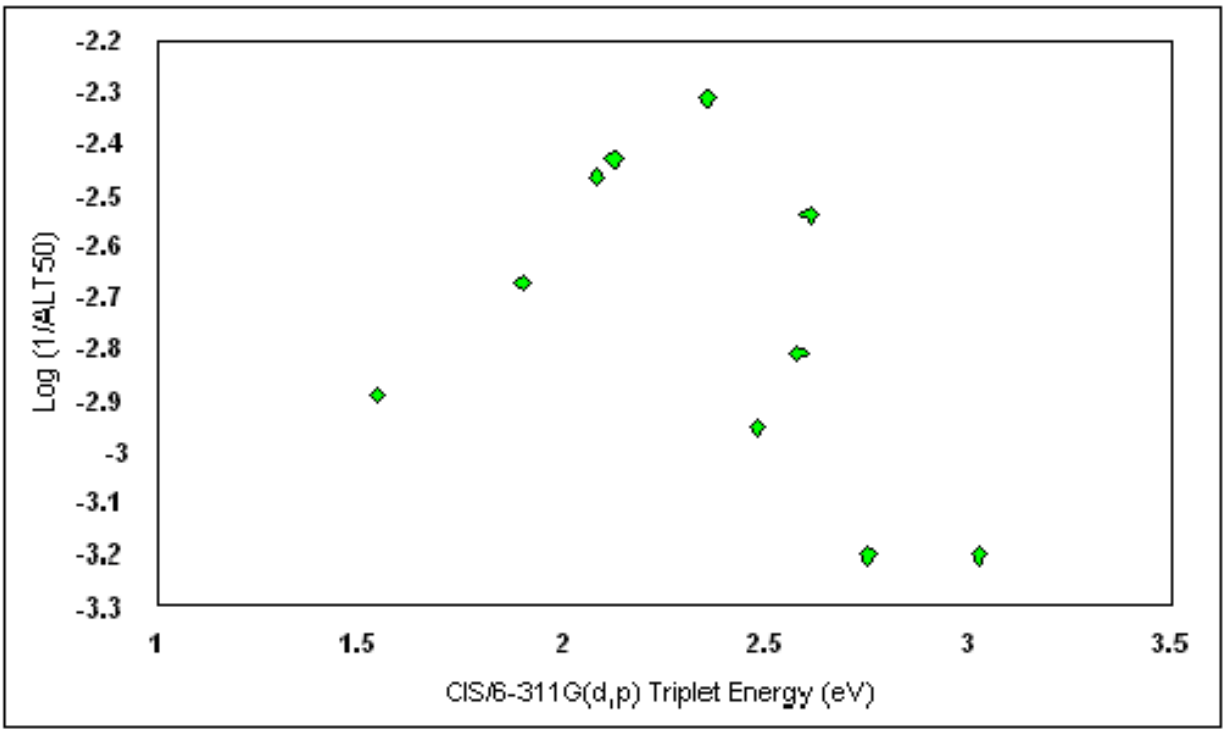

Figure 1. Phototoxicity of PAHs as a function of their triplet excited states; phototoxic range approximately from 1.1 to 2.5 eV (Betowski et al., 2002)

2. Methods

The toxicities of the PAHs used for the model are those reported by Newsted and Giesy (1987). Calculations on the fullerenols and carbon nanotubes were performed using the Gaussian 09 programs (M. J. Frisch et al., 2009). Complete geometry optimizations for the neutral forms of all molecules were first carried out using ab initio calculations at several levels of theory. Because of the size of these molecules, the following levels of theory were undertaken: am1 (semi-empirical), HF/3-21G, HF-6-31G* and/or HF-6-311G**. The calculations at HF/3-21G were followed by frequency calculations in order to verify that the stationary points obtained were true minima. In some instances, calculations at B3LYP/6-311G(d,p) were used and compared with the HF/6-311G** calculations for the geometry optimizations. Triplet excited state calculations were performed using the configuration interaction singles (CIS) approach with the 6-311G(d,p) basis set. The structures of the fullerenols were designed to reflect the highest symmetry. In some cases, two different point groups were chosen as the starting geometry. In one case ($C_{60}(OH)_{26}$), two structures with C_1 symmetry were tried. In those cases, we chose the geometry that gave the lower energy in the HF/6-311G(d,p) calculation as representative of that structure, $C_{60}(OH)_n$, and that structure was used to determine the triplet energy.

The starting geometries for the single-walled carbon nanotubes (SWCNT) were obtained from the Nanotube Website (http://www.nanotube.msu.edu/tubeASP/) with a

web-accessible carbon nanotube generation applet by Roberto Veiga. These geometries were then used as the starting point for the ab initio geometry optimizations.

3. Results and discussion

$C_{60}(OH)_{26}$ is one of the fullerenols under study and its structure is displayed in Figure 2. It has C_1 symmetry. However, as indicated above, there is an additional structure that has the same formula and the same symmetry, C_1, which is shown in Figure 3. Since the fullerenols, $C_{60}(OH)_{22\text{-}26}$ were the compounds studied by Roberts et al. (2008), the fullerenols with 22-26 hydroxyl groups were important to compare the calculated with the experimental phototoxicity. For example, the triplet energy calculated for $C_{60}(OH)_{26}$ (Table 1), shown in Figure 2, is 1.72 eV. Using the plot in Figure 1, which is based on PAH toxicity, as a model for carbon-based nanomaterial toxicity, the triplet energy of 1.72 eV corresponds to a log (1/ALT50) (toxicity) of approximately -2.8. Any log(1/ALT50) value of greater than -3.1 is phototoxic, and the toxicity increases in this curve, in the case of PAHs, to -2.3, which is extremely toxic. The other structure, shown in Figure 3, is 0.23 eV, which is not in the phototoxic region. Table 1 indicates that the Figure 2 species has a lower total energy, when optimized with the HF/6-311G(d,p) calculations. Since the optimum structure derived from calculations generates the lowest energy, the preferred structure is shown in Figure 2. Thus, 1.72 eV is expected to be the correct triplet energy for $C_{60}(OH)_{26}$. $C_{60}(OH)_{22}$ with a triplet energy of 1.33 eV is expected to be slightly phototoxic, while $C_{60}(OH)_{24}$ is barely phototoxic. Most of the other fullerenols, $C_{60}(OH)_{4n}$, where n =1 to 8, fall in the phototoxic range.

As can be seen, the triplet energies for the fullereneols, optimized with the B3LYP procedure, are lower in Table 2 than in Table 1, although most remain in the phototoxic region except for $C_{60}(OH)_{24}$. Roberts et al. found that fullerenols ($C_{60}(OH)_{22-26}$) are phototoxic to human lens epithelial cells (Roberts et al., 2008), which is in agreement to the calculations in this study.

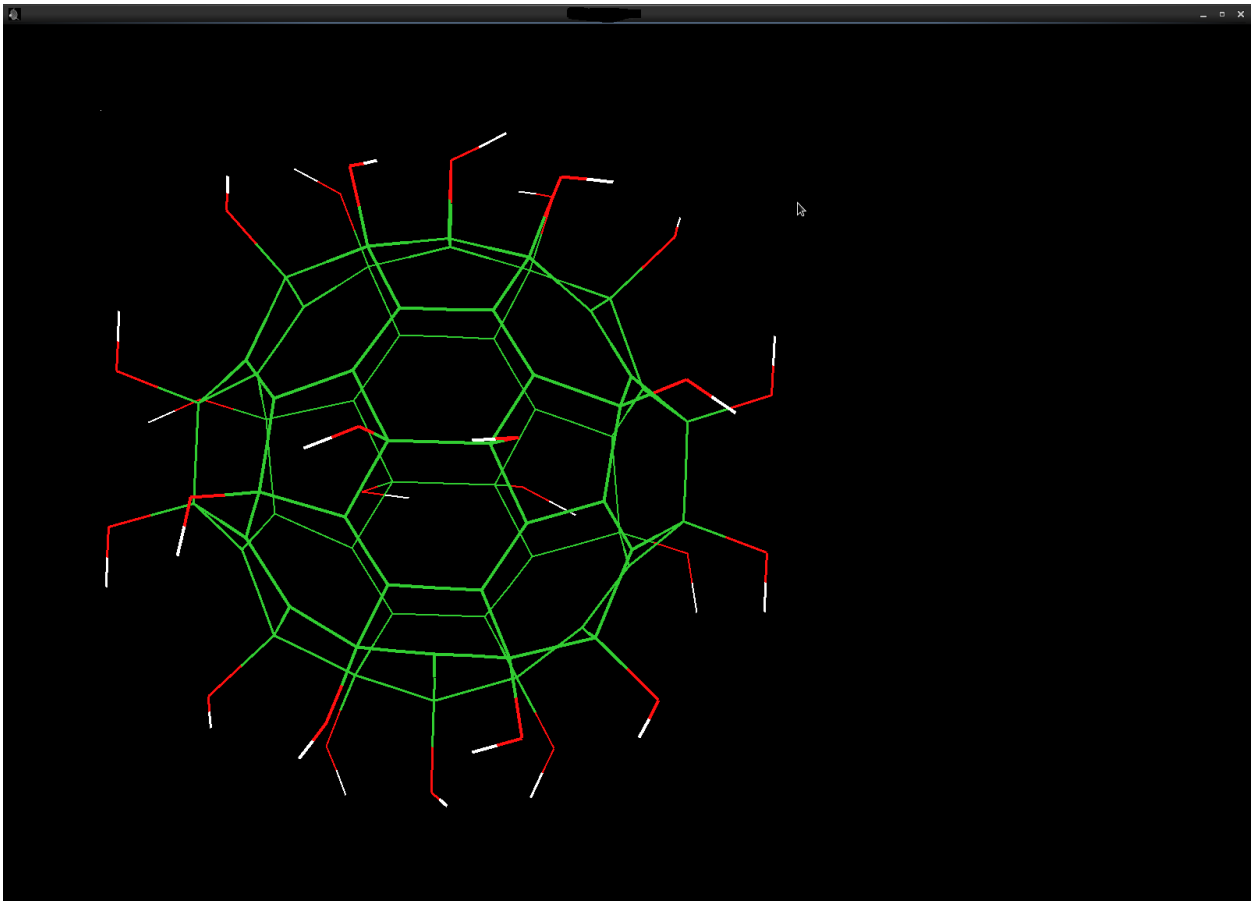

Figure 2. Structure (1) of $C_{60}(OH)_{26}$

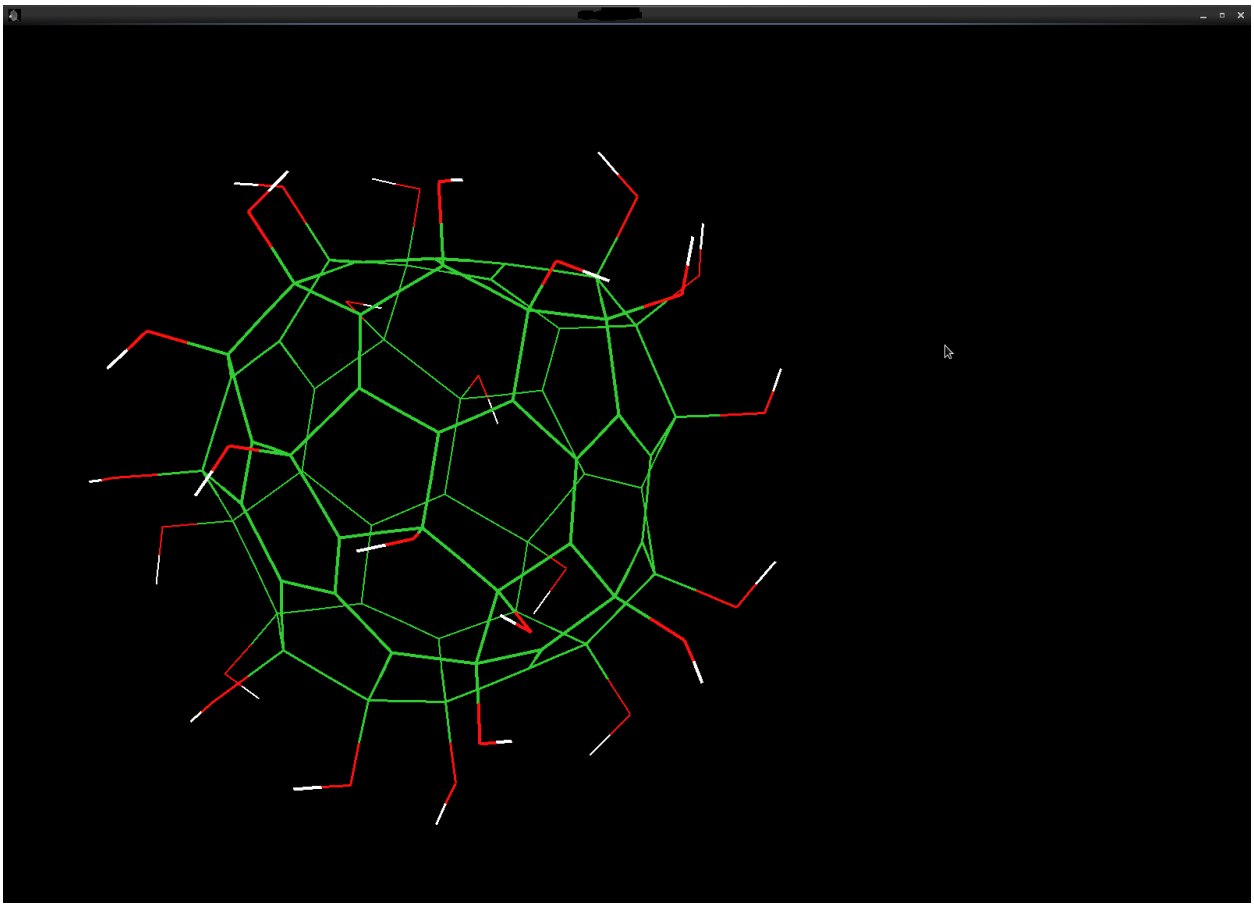

Figure 3. Structure (2) of $C_{60}(OH)_{26}$

Table 1. Triple state energies for a series of fullerenols, $C_{60}(OH)_n$. Geometries were calculated at HF/6-311G(d,p).

Fullerenols	Triplet Energy (eV) RCIS/6-311G(d,p) Symmetry [Total Energy]	Triplet Energy (eV) RCIS/6-311G(d,p) Symmetry [Total Energy]
$C_{60}(OH)_4$	1.88 D_{2h} [-2574.00353798]	
$C_{60}(OH)_8$	1.83 D_{2h} [-2875.82111174]	
$C_{60}(OH)_{12}$	1.69 C_{2h} [-3177.641173]	1.69 C_I[-3177.641170]
$C_{60}(OH)_{16}$	1.70 C_I[-3479.53239]	1.77 D_2[-3479.50094]
$C_{60}(OH)_{20}$	1.69 D_2 [-3781.31157682]	
$C_{60}(OH)_{22}$	1.33 C_2[-3932.24229]	1.07 C_1[-3932.1726408]
$C_{60}(OH)_{24}$	1.03 C_2[-4082.99553]	1.03 C_1[-4083.00069215]
$C_{60}(OH)_{26}$	1.72 C_1[4234.01728886] (Figure 2)	0.232 C_1[4234.00940676] (Figure 3)
$C_{60}(OH)_{28}$	1.61 C_2[-4385.05143890]	
$C_{60}(OH)_{32}$	1.44 C_2[-4686.54330161]	
C_{60}	1.97 I_n [-2272.18418517]	

Table 2. Triple state energies for a series of fullerenols, $C_{60}(OH)_n$. Geometries were calculated at B3LYP/6-311G(d,p).

Fullerenols	Triplet Energy (eV) RCIS/6-311G(d,p) Symmetry [total energy]	Triplet Energy (eV) RCIS/6-311G(d,p) Symmetry [total energy]
$C_{60}(OH)_4$	1.70 D_{2h} [-2589.92330215]	
$C_{60}(OH)_8$	1.65 D_{2h} [-2893.25325861]	
$C_{60}(OH)_{12}$	1.56 C_{2h}[-3196.582839]	1.56 C_I[-3196.58296584]
$C_{60}(OH)_{16}$	1.49 C_I[-3499.99994543]	1.49 D_2[-3499.95374488]
$C_{60}(OH)_{20}$	1.24 D_2 [-3803.27229706]	
$C_{60}(OH)_{24}$	0.321 C_2[-4106.59071049]	0.317 C_1 [-4106.59357743]
$C_{60}(OH)_{26}$	1.38 C_1[-4258.26524553]	
C_{60}	1.80 $_n$ [-2286.59087138]	

Similar calculations were made on single-walled carbon nanotubes. An assortment of the three different forms of the SWCNT was examined: {n, m}, where m=0, m=n, and m≠n≠0.

For the most part nanotubes with one or two units were studied, but for {3,3}, one to six units were examined. SWCNTs are likely to have dimensions such that their length (number of units) is much greater that their diameters. It is instructive to see the trend of increasing length to constant diameter, as far as the triplet energies calculated for each increasing unit. The SWCNTs studied were generally below the phototoxic limit with the exception of {3,3}, as shown in Table 3. However, as the length increases by adding units, the triplet energy decreases (except for one unit, which shows a negative triplet energy), and by six units {3,3} is barely the phototoxic. As with the fullerenols, the geometries calculated with the B3LYP/6-311G(d,p) method give lower triplet energies than the geometries based on the HF/6-311G(d,p) calculations for the examples in the Table 3.

Table 3. Single-walled carbon nanotubes

Geometry optimization @ HF/6-311G(d,p)

SWCNT	Triplet Energy (eV) RCIS/6-311G(d,p)
{5,5} 2 units	1.38
{9,0} 2 units	0.44
{10,10} 2 units	1.41
{6,0} 2 units	1.09
{8,2} 1 unit	0.533
{4,2} 1 unit	0.47
{9,9} 2 units	1.41
{9,9} 3 units	1.18
{3,1} 1 unit	-0.578
{3,3} 2 units	2.28
{3,3} 3 units	2.22
{3,3} 4 units	1.49
{3,3} 5 units	1.23
{3,3} 6 units	1.02

Geometry optimization @ B3LYP/6-311G(d,p)

SWCNT	Triplet Energy (eV) RCIS/6-311G(d,p)
{5,5} 2 units	1.08
{10,10} 2 units	1.12

4. Summary and conclusions

Following a model used to predict phototoxicity on PAHs, similar predictions for fullerenols and SWCNTs have been made. Fullerenols and SWCNTs are classified as nanomaterials, and there is much interest in their environmental effects, both on human health and the ecology. Roberts et al.(2008) found that the C_{22} to C_{26} fullerenols are phototoxic to human lens epithelial cells. Our predictions indicate that the C_{22} and C_{26} fullerenols are probably phototoxic and the C_{24} fullerenol is at the edge of the phototoxic region, when it is optimized with the HF/6-311G(d,p) calculation, and out of the phototoxic region, when the B3LYP/6-311G(d,p) calculation is used. From Figure 1 the phototoxic region extends approximately 1.1 eV to 2.5 eV. Taking the liberty of extrapolating the phototoxic region from 1.5 eV to 1.1 eV, the assumption is the phototoxic curve is linear in that region. In fact, doing a linear regression of the five points on the left side of the curve gives an r^2 of 0.979 (Betowski et al., 2002). In this region the phototoxicity appears to increase with increasing excited state energy. The calculations for the other fullerenols, $C_{60}(OH)_{4n}$, where n=1 to 8, show them to be in the phototoxic region, except the aforementioned C_{24} fullerenol. It is advisable to heed some caution when looking at these numbers for the fullerenols, as structure appears to make a great difference in determining the triplet excited states. For some of the fullerenols, (e.g., $C_{60}(OH)_{12}$ and $C_{60}(OH)_{16}$, shown in Table 1) the starting symmetries do not make a difference, but as an extreme example $C_{60}(OH)_{26}$ shows great differences in the triplet excited states of different orientations of the hydroxyl groups even though both examples are C_1 symmetry. In this work, effort was made to generate the most symmetric

structures. Those fullerenols that optimized without any imaginary frequencies, i.e., bein at a local minimum structure, were chosen for calculation of triplet states.

The SWCNTs show little phototoxicity as determined by the triplet excited states. There are some in the phototoxic region, e.g., {10,10} with two units at 1.4136 eV, but as in the case of {3,3} and even {9,9} the triplet excited state trend is to decrease as the length increases. Since these nanotubes are expected to have great length, it is expected that they will be out of the phototoxic region as their length increases.

ACKNOWLEGMENTS

Special acknowledgement for the computer time at the Environmental Modeling and Visualization Laboratory provided for this project. The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to Agency's administrative review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

Betowski, L. D., Enlow, M., Riddick, L, 2002. Computers & Chemistry, 26(4),371-377.

Calvo, P., Sanchez, A., Martinez, J., Lopez, M.I., Calonge, M., Pastor, J.C., Alonso, M.J. 1996. Pharm. Res. 13, 311–315

M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria,
M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci,
G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian,
A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada,
M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima,
Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr.,
J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers,
K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand,
K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi,
M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross,
V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann,
O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski,
R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth,
P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels,
O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski,
and D. J. Fox, 2009. Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford CT.

Jie Gao, Sejin Youn, Anna Hovsepyan, Verónica L. Llaneza, Yu Wang, Gabriel Bitton and Jean-Claude J. Bonzongo. 2009. Environ Sci & Technol 43: 3322–3328.

Jie Gao, Yihai Wang, Kevin M. Folta, Vijay Krishna, Wei Bai, Paul Indeglia, Angelina Georgieva, Hideya Nakamura, Ben Koopman, Brij Moudgil. 2011. PLoS ONE 6(5): e19976. doi:10.1371/journal.pone.0019976.

Andrew D. Maynard, Robert J. Aitken, Tilman Butz, Vicki Colvin, Ken Donaldson, Günter Oberdörster, Martin A. Philbert, John Ryan, Anthony Seaton, Vicki Stone, Sally S. Tinkle, Lang Tran, Nigel J. Walker & David B. Warheit. 2006. *Nature* **444**, 267-269.
Anisa Mnyusiwalla, Abdallah S Daar, and Peter A Singer Nanotechnology 14 (2003) R9–R13.

Mekenyan, O.G., Ankley, G.T., Veith, G.D., Call, D.J., 1994. Chemosphere 28, 567.

Nanotube Website: http://www.nanotube.msu.edu/tubeASP/

Newsted, J.L., Giesy, J.P., 1987. Environ. Toxicol. Chem. 6, 445.

Joan E. Roberts, Albert R. Wielgus, William K. Boyes, Usha Andley, Colin F. Chignell. 2008. Toxicology and Applied Pharmacology 228, 49–58

R.E. Smalley, 1992. Acc. Chem. Res, 25, 98-105.

Yamago, S., Tokuyama, H., Nakamura, E., Kikuchi, K., Kananishi, S., Sueki, K., Nakahara, H., Enomoto, S., Ambe, F., 1995. Chem. Biol. 2, 385–389.

Zakharenko, L.P., Zakharov, I.K., Vasiunina, E.A., Karamysheva, T.V., Danilenko, A.M., Nikiforov, A.A. 1997. Genetika 33, 405–409.