

Comparative Pulmonary Toxicity Assessments of C₆₀ Water Suspensions in Rats: Few Differences in Fullerene Toxicity in Vivo in Contrast to in Vitro Profiles

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ABSTRACT

It has previously been reported that the in vitro cytotoxic effects of water-soluble fullerene species are a sensitive function of their surface derivatization status. In a recent study, it was reported that doses of an aggregated form of underivatized C₆₀, termed nano-C₆₀, were 3–4 orders of magnitude more toxic to human dermal fibroblasts, lung epithelial cells, and normal human astrocytes when compared to identical exposures of these cell types to a fully derivatized, highly water-soluble derivative, C₆₀(OH)₂₄. Accordingly, the aim of this study was to test and validate these in vitro findings by comparing the in vivo pulmonary toxicity effects in rats of intratracheally instilled nano-C₆₀ and C₆₀(OH)₂₄. In two combined studies, groups of rats were instilled with doses of either 0.2, 0.4, 1.5, or 3.0 mg/kg of nano-C₆₀, C₆₀(OH)₂₄, or α -quartz particle types using Milli-Q water as the vehicle. Subsequently, the lungs of vehicle and particle-exposed rats were assessed using bronchoalveolar lavage (BAL) fluid biomarkers, oxidant and glutathione endpoints, airway and lung parenchymal cell proliferation methods, and histopathological evaluation of lung tissue at 1 day, 1 week, 1 month, and 3 months postinstillation exposure. Exposures to both nano-C₆₀ or water-soluble C₆₀(OH)₂₄ produced only transient inflammatory and cell injury effects at 1 day postexposure (pe) and were not different from water instilled controls at any other pe time periods. An increase in lipid peroxidation endpoints vs controls was measured in BAL fluids of rats exposed to 1.5 and 3 mg/kg of nano-C₆₀ at 1 day and 3 month pe time points. In addition, no adverse lung tissue effects were measured at 3 months postinstillation exposures to the highest dose of the two types of fullerenes. In contrast, pulmonary exposures to quartz particles in rats produced dose-dependent lung inflammatory responses characterized by neutrophils and foamy lipid-containing alveolar macrophage accumulation as well as evidence of early lung tissue thickening consistent with the development of pulmonary fibrosis. The results demonstrated little or no difference in lung toxicity effects between the two fullerene samples when compared to controls, and these data are not consistent with the previously reported in vitro effects. The findings exemplify both the difficulty in interpreting and extrapolating in vitro toxicity measurements to in vivo effects and highlight the complexities associated with probing the relevant toxicological responses of fullerene nanoparticle systems.

Fullerene water suspensions are becoming increasingly popular materials for use in the biomedical, electronic, and semiconductor industries.^{1–3} Their toxicity, in in vivo systems, is an important property for defining and limiting these applications.^{4–7} Fullerene water suspensions can be used as a model system to examine the in vivo pulmonary health effects of nanomaterials.⁸

This paper examines the differential toxicity of two different fullerene, specifically C₆₀, water suspensions (Figure

1). The first, termed nano-C₆₀, is an aggregate of pristine C₆₀ cages, with minimal amount of substitutions on the aggregate's surface. The second is a derivatized C₆₀ cage, termed C₆₀(OH)₂₄, where 24 OH groups are substituted on the surface of each individual C₆₀ cage. Previously, it was hypothesized that the nano-C₆₀ aggregate could cause oxidative damage to cellular membranes, thus contributing to its toxicity in vitro. As the fullerene cage becomes fully derivatized (i.e., 24 substitutions on each cage), the oxidative potential decreases due to the breakage of the π orbital structure. This rationale was supported by reported evidence that C₆₀ in the presence of oxygen or water could result in radical generation.^{9–11} One particular study found evidence

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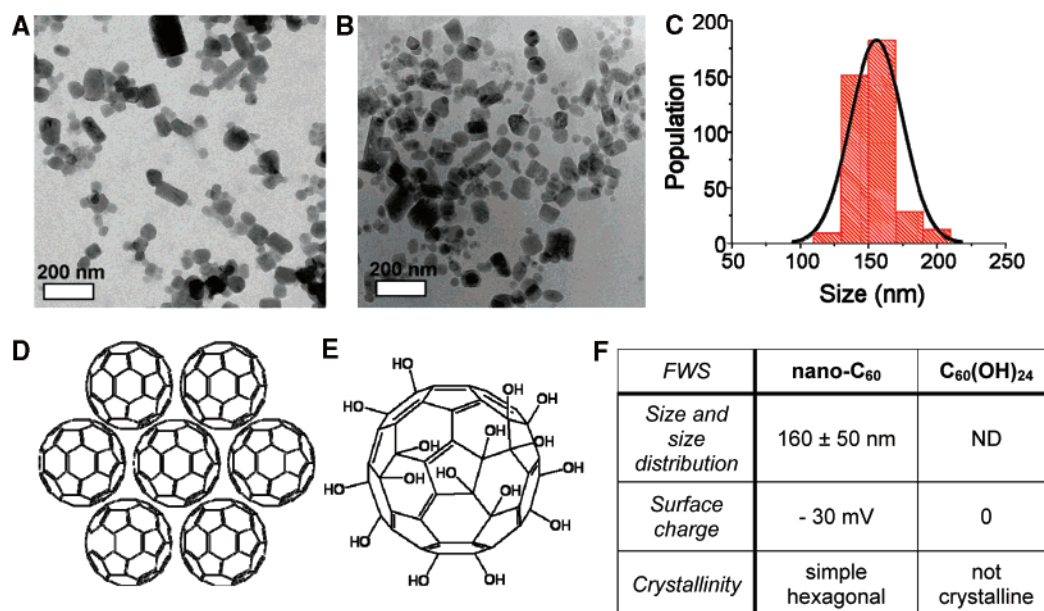


Figure 1. Characterization of the C₆₀ Water Suspensions. The characterization of the FWS used in the intratracheal instillation studies described here. (A and B) Transmission electron micrographs of the nano-C₆₀ suspension. (C) Size histogram of the nano-C₆₀ suspension population. Chemical structural drawings of (D) nano-C₆₀ and (E) C₆₀(OH)₂₄ water suspensions. (F) Table reporting size and size distribution, surface charge, and crystallinity data of the nano-C₆₀ and C₆₀(OH)₂₄ water suspensions. ND stands for “could not be determined”.

for free radicals that were generated in the presence of atmospheric oxygen and C₆₀. The radical was hypothesized to be the superoxide anion (O₂*⁻), a radical species primarily responsible for peroxidation of the lipid bilayer.¹²

Because of the potential for fullerenes becoming increasingly available and because fullerenes exhibit complex chemistries in the presence of oxygen and water, we set out to define the *in vivo* pulmonary toxicology of nano-C₆₀ and C₆₀(OH)₂₄ fullerene water suspensions. Traditionally, pulmonary toxicity studies in rats have demonstrated that low solubility nanoparticles produce more potent inflammatory responses than larger particles of similar chemical composition at the same mass concentrations or doses.^{13,14} However, because fullerenes exhibit different chemical and physical properties than their bulk counterpart, carbon black, it is difficult to draw that conclusion.

In 2004, Oberdorster reported that these C₆₀ aggregates, prepared using a solvent extraction methodology, were found to elevate lipid oxidation levels in the brains of fish.¹⁵ Later, Sayes and others reported increased lipid peroxidation and total glutathione in human cells in culture.^{16–20} In contrast, a number of studies have shown that sparingly derivatized C₆₀ water suspensions do not decrease cellular viability. These C₆₀ systems, however, were produced using alternate methodologies. The *in vivo* validation of C₆₀ water suspensions prepared in the solvent extraction method is important in determining the validity of some nanoparticle toxicity studies. Regulatory decisions cannot be made based on a set of studies, rather a culmination of studies conducted in collaboration with researchers from many scientific disciplines. We set out to test the hypothesis that the aggregated form of C₆₀ cages in an aqueous solution could cause lipid or membrane oxidation in the bronchoalveolar lavage (BAL) fluid of rats. Fullerene water suspensions, in reference to

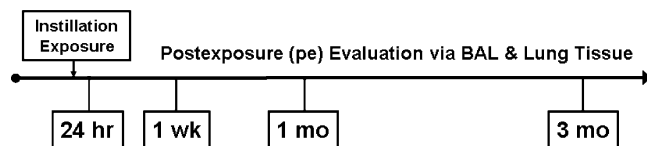
this study, are colloiddally stabilized aqueous dispersions of fullerene aggregates, including any derivatization or other modification of surface fullerenes that facilitate stable suspension.

Sayes et al.¹⁶ reported that doses of an aggregated form of underivatized C₆₀, termed nano-C₆₀, were 3–4 orders of magnitude more toxic to human dermal fibroblasts, lung epithelial cells, and normal human astrocytes when compared to identical exposures of these cell types to a fully derivatized, highly water-soluble derivative, C₆₀(OH)₂₄. Similarly, Isakovic and co-workers confirmed the greater toxicity of nano-C₆₀ in a variety of *in vitro* cell types.¹⁹ Because the different types of fullerene species exhibit variable physicochemical properties, it is conceivable that the toxicological properties could vary, as well. To test this hypothesis, a pulmonary bioassay with the two fullerene species was conducted in rats utilizing intratracheal instillation as the route of *in vivo* particle exposure (Table 1). By comparing the potency of inflammation and cytotoxic lung effects of these two C₆₀ water suspensions at equivalent mass doses to a standard active reference α -quartz particle suspension, Min-U-Sil 5 (positive control), we were able to deduce that, relative to controls, for *both fullerene species* there were (1) no alterations in lung inflammation endpoints, (2) no evidence of adverse biochemical endpoints, and (3) no differences in lung tissue sections of exposed rats. Thus, unlike results from earlier *in vitro* studies, no significant differences in toxicity were measured between the two fullerene water suspensions. Minor differences among the fullerenes included evidence for carbon-centered radicals in the nano-C₆₀ water suspension, but not in the C₆₀(OH)₂₄ system, and an increase in lipid peroxidation levels after exposure to the nano-C₆₀ water suspension at the 1 day and 3 month postexposure time points.

Table 1. The Experimental Design of the Study^a

Exposure Groups

- Milli-Q ultrapure water (vehicle control)
- Min-U-Sil 5 (α -quartz) particles (positive control)
- FWS (0.2, 0.4, 1.5 and 3.0 mg/kg)
 - Nano- C_{60} water suspension
 - $C_{60}(OH)_{24}$ water suspension



^a Groups of rats were intratracheally instilled with Milli-Q ultrapure water (vehicle control), Min-U-Sil 5 (α -quartz, positive control), nano- C_{60} water suspension, or $C_{60}(OH)_{24}$ water suspension. After instillation, BAL fluid from the lungs of the exposed animals was analyzed for cytotoxicity and inflammation endpoints. Also, the lung tissue was analyzed for injury. Analysis was performed at four different times (1 h, 1 week, 1 month, and 3 months) postinstillation.

General Experimental Design. The major objective of this study was to assess the pulmonary toxicity of intratracheally instilled, underivatized C_{60} , termed nano- C_{60} when compared to a fully derivatized, highly water-soluble derivative, $C_{60}(OH)_{24}$. The general approach to this study was to conduct substantial particle characterization assessments including surface activity studies (electron spin resonance and hemolytic potential) of the C_{60} water suspensions, concomitant with in vivo pulmonary bioassay studies, and evidence for lipid peroxidation and glutathione production in cells recovered from the lungs of fullerene water suspensions (FWS) and quartz-exposed rats.

The fundamental features of this pulmonary bioassay are dose–response evaluations and time-course assessments to determine the sustainability of any observed effect. In addition, quartz particles served as a positive control for this experiment (instillation volumes averaged 800 μ L per animal), and Milli-Q water served as a negative control (instillation volumes were 500 μ L per animal). Thus, the major endpoints of this study were the following: (1) time course and dose/response intensity of pulmonary inflammation and cytotoxicity; (2) airway and lung parenchymal cell proliferation; and (3) histopathological evaluation of lung tissue.

FWS preparation and characterization, including suspension concentration, electron spin resonance (ESR) of C_{60} water suspensions, and hemolytic potential of C_{60} water suspensions are described in the Supporting Information. Pulmonary toxicity of particles, including pulmonary lavage, lactate dehydrogenase release, total protein, and statistical analyses are also included in the supplemental information. Last, biochemical analyses of BAL fluid, including the lipid peroxidation and total glutathione biochemical techniques are described in the Supporting Information.

Particle Characterization Results. Particle size and size distribution, morphology, crystallinity, purity, and surface charge were analyzed. The average particle size in the nano- C_{60} water suspension was 160 ± 50 nm. These particles are

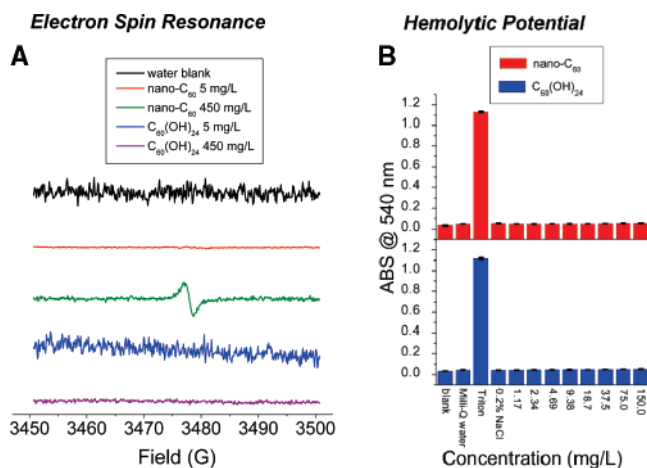


Figure 2. The ex vivo determination of C_{60} water suspension biological reactivity. (A) The ESR of the C_{60} water suspensions at 100 K. The spectrum of nano- C_{60} suspension at 450 mg/L shows presence of carbon-centered radical species, while neither of the $C_{60}(OH)_{24}$ suspensions spectra shows any radical species. However, (B) the hemolytic potential of the two C_{60} water suspensions indicated that neither suspension had any effect on erythrocyte hemolysis.

crystalline (simple hexagonal) and have an average surface charge of -30 mV. The $C_{60}(OH)_{24}$ fullerene molecules were soluble in the aqueous phase. No sizing data, nor crystallinity, could be obtained. The surface charge of these particles measured a net 0 mV.

Ex Vivo Determination of Radical Species Results. ESR and Hemolysis. The presence of radical species was measured using two independent techniques: ESR spectroscopy and hemolytic potential (Figure 2). ESR spectra were acquired for nano- C_{60} and $C_{60}(OH)_{24}$ fullerene water suspensions at two concentrations (80 and 450 mg/L), as well as a Milli-Q water blank. No transition was observed in the water blank, 80 mg/L nano- C_{60} , 80 mg/L $C_{60}(OH)_{24}$, or 450 mg/L $C_{60}(OH)_{24}$. However, a singlet ($g_e = 2.0025$, $\Delta H = 1.7$ G) resonance was observed in the suspension of 450 mg/L nano- C_{60} , very likely attributable to a carbon-centered free radical.

The hemolytic potential assay demonstrates the differential surface activity particles when incubated with red blood cells. The experiment was performed on both C_{60} water suspensions. Neither suspension had any effect on erythrocyte hemolytic effects.

Bronchoalveolar Lavage Fluid Results. Biochemical and Cytotoxicity Endpoints. No increases in BAL fluid lactate dehydrogenase values versus vehicle controls were measured in the lungs of rats exposed to either FWS (3 mg/kg) at any postexposure (pe) time point (i.e., 1 day, 1 week, or 1 or 3 months). In contrast, exposures to high dose α -quartz particles (3 mg/kg) produced a persistent increase in BAL fluid LDH values versus controls throughout the 3 month pe period (Figure 3). No increases in BAL fluid micrototal-protein values vs controls were measured in the lungs of rats exposed to either FWS type (3 mg/kg) at any pe time period. In contrast, exposures to high dose α -quartz particles (3 mg/kg) produced sustained increases vs controls in BAL fluid microprotein values at 1 day, 1 week, and 1 and 3

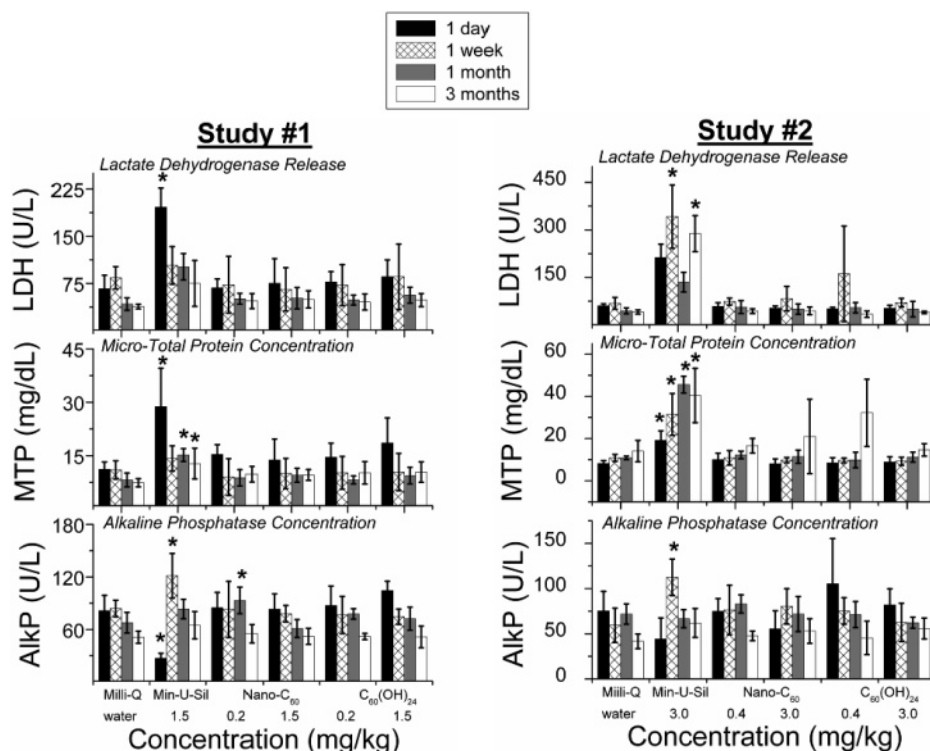


Figure 3. BAL fluid analyses for lactate dehydrogenase release, micro-total-protein concentrations, and alkaline phosphatase concentrations. The cytotoxicity endpoints from the FWS-instilled rats. Groups of animals were exposed to either a low dose (0.2 mg/kg) or high dose (1.5 mg/kg) for study 1 or a low dose (0.4 mg/kg) or high dose (3.0 mg/kg) for study 2 of either water suspension. Any significant effects would be expected to be measured in study 2. Lactate dehydrogenase (LDH) is shown on top, micro-total-protein (MTP) is shown in the middle, and alkaline phosphatase (ALKP) values are shown below. * indicates statistical significance ($p < 0.05$).

months pe (Figure 3). No significant increases in BAL fluid alkaline phosphatase values relative to vehicle controls were measured among any of the groups at any time period pe (Figure 3).

Bronchoalveolar Lavage Fluid Results. Pulmonary Inflammation. Intratracheal instillation exposures to nano- C_{60} or $C_{60}(OH)_{24}$ fullerene water suspensions produced a transient, short-lived, pulmonary inflammatory response, as evidenced by an increase in the percentages/numbers of BAL-recovered neutrophils, measured at 1 day pe but was not sustained at other time points. In contrast, intratracheal instillation exposures to high dose α -quartz particles (3 mg/kg) produced persistent pulmonary inflammatory responses, as measured through 3 months pe (Figure 4). Further, the numbers of cells recovered by the BAL from the lungs of rats exposed to high dose α -quartz particles were substantially higher than any of the other groups for all pe time periods (Figure 4).

To summarize the results from BAL fluid biomarker studies, pulmonary exposures to α -quartz particles produced a sustained, dose-dependent, lung inflammatory response, concomitant with cytotoxic effects, measured from 1 day through 3 months postexposure. Exposures to the two different FWS types (3 mg/kg) produced small, transient pulmonary inflammatory responses, but these effects were not sustained. No significant increases compared to vehicle controls were measured for any of the BAL fluid cytotoxicity endpoints in any of the FWS-exposed groups.

Lung Tissue Study Results. Lung Weights. Lung weights in high dose α -quartz-exposed (3 mg/kg) rats were slightly increased when compared to vehicle control animals at 1 week and 1 month pe and substantially increased at 3 months pe (data not shown).

Lung Tissue Study Results. Cell Proliferation. Tracheo-bronchial and lung parenchymal cell proliferation rates (% immunostained cells taking up BrdU) were measured in high dose FWS or α -quartz-exposed (3 mg/kg) rats and corresponding controls at 1 day, 1 week, 1 month, and 3 months pe. Transient increases in cell labeling indices were measured in high dose α -quartz particles (3 mg/kg) animals at 1 day pe, but these effects were not sustained (data not shown). Significant increases in lung parenchymal cell proliferation indices were measured in the lungs of α -quartz exposed rats at 1 and 3 months pe (Figure 1 in Supporting Information).

To summarize the results of airway and alveolar cell proliferation studies, when compared to the FWS or Milli-Q water controls, pulmonary exposures to high dose α -quartz particles (3 mg/kg) produced higher lung parenchymal cell proliferation rates at 1 and 3 months pe, suggesting a greater likelihood to result in adverse pulmonary effects over time with continued exposures.

Oxidative Stress Results. Lipid Peroxidation and Glutathione Production. Statistically significant increases in lipid peroxidation values were measured in the lungs of rats exposed to 1.5 and 3 mg/kg of nano- C_{60} suspensions at 1 day and 3 months pe (Figure 5). No increases in lipid

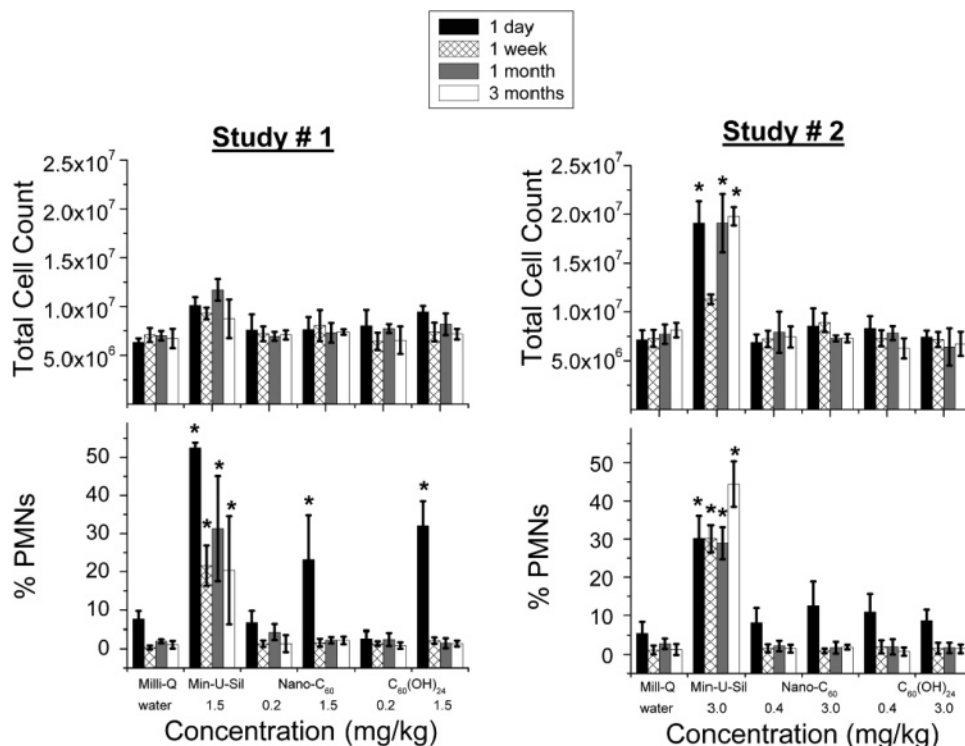


Figure 4. BAL fluid analyses for total cell numbers and % PMNs. The cytotoxicity endpoints from the FWS-instilled rats. Groups of animals were exposed to either either a low dose (0.2 mg/kg) or high dose (1.5 mg/kg) for study 1 or a low dose (0.4 mg/kg) or high dose (3.0 mg/kg) for study 2 of either water suspension. Percent polymorphonuclear leukocyte (% PMN) is shown on top and total cell number is shown below. * indicates statistical significance ($p < 0.05$).

peroxidation were measured in the lungs of rats exposed to α -quartz particles or derivatized C_{60} species at any post-exposure time periods.

No significant changes in total glutathione were measured in the lungs of rats exposed to either of the FWS-types (3 mg/kg) or to α -quartz particles (3 mg/kg) at any pe time period.

Histopathological Evaluation Results. Histopathological analyses of lung tissues revealed that pulmonary exposures to nano- C_{60} or $C_{60}(OH)_{24}$ fullerene water suspensions produced no significant adverse effects when compared to water instilled control animals, as evidenced by the normal lung architecture observed in the exposed animals at postinstillation exposure time periods ranging from 1 day to 3 months (Figure 6).

In contrast, histopathological analyses of lung tissues revealed that pulmonary exposures to α -quartz particles in rats produced dose-dependent lung inflammatory responses characterized by neutrophils and foamy (lipid-containing) alveolar macrophage accumulation. In addition, lung tissue thickening as a prelude to the development of fibrosis was evident and progressive (Figure 6).

The objective of this pulmonary bioassay study was to assess the acute lung toxicity of intratracheally instilled, nano- C_{60} or $C_{60}(OH)_{24}$ fullerene water suspensions in rats. Using a pulmonary bioassay methodology, the lung toxicity of instilled, different FWS types were compared with a positive control particle type, α -quartz. Exposures to either nano- C_{60} or $C_{60}(OH)_{24}$ suspensions produced no significant

adverse pulmonary effects. Results from the BAL fluid and cell proliferation evaluations demonstrated that pulmonary exposures to α -quartz particles, particularly at the higher dose, produced significant adverse effects as compared to the controls in pulmonary inflammation, cytotoxicity, and lung parenchymal cell proliferation indices. In contrast, both fullerene water suspensions produced only transient and reversible inflammation, due primarily to the method of bolus exposure (intratracheal instillation) rather than to the effects of the deposited fullerenes in the lung. Similarly, exposures to the vehicle (Milli-Q water) resulted only in short-term and reversible lung inflammation, also likely related to the effects of the instillation procedure. Histopathological evaluation demonstrated that α -quartz particle exposures produced pulmonary inflammation, foamy macrophage accumulation, and tissue thickening (i.e., fibrosis). Exposures to either of the FWS types produced no adverse tissue reactions in the lungs.

Some reports have correlated the concentration of peroxy radicals on the cell's lipid bilayer (i.e., lipid peroxidation or membrane oxidation) to changes in glutathione production or total glutathione concentrations.^{15,17,21} In the present study, although we measured significant increases in lipid peroxidation endpoints in the BAL-recovered cells exposed to the nano- C_{60} water suspension at the 24 h and 3 month pe time points (for 0.4 and 3.0 mg/kg exposures), we did not measure changes in total glutathione concentrations at the corresponding time points. We did, however, measure increased GSH levels at the 1 week time point (0.4 and 3.0 mg/kg nano- C_{60}

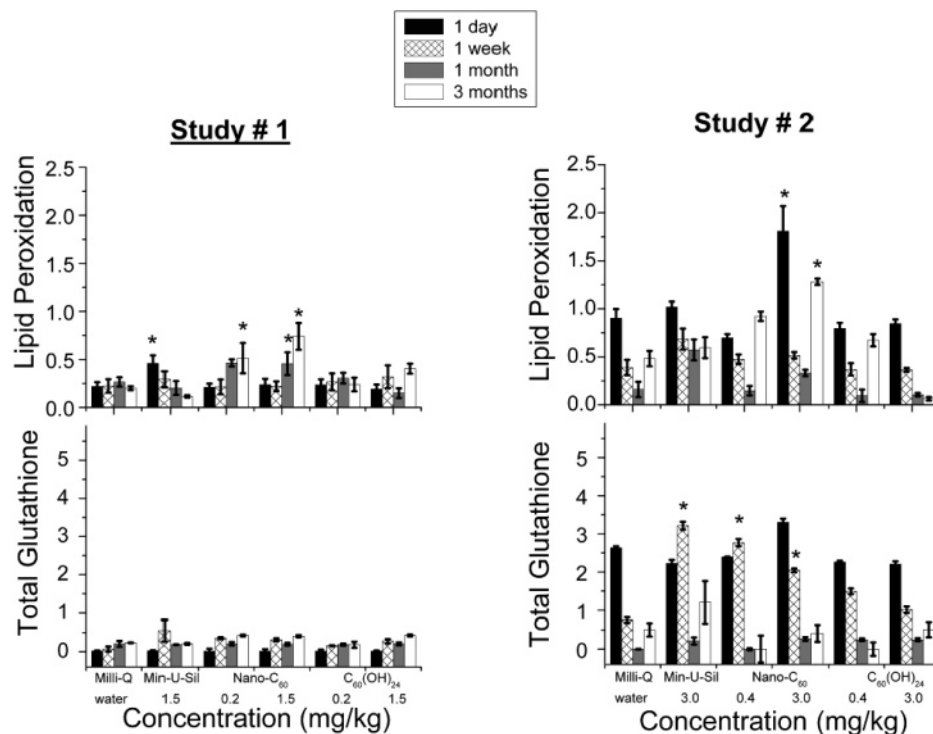


Figure 5. BAL fluid analyses for lipid peroxidation and total glutathione production of cells. The lipid peroxidation and total glutathione concentrations from the FWS-instilled rats. Groups of animals were exposed to either a low dose (0.2 mg/kg) or high dose (1.5 mg/kg) for study 1 or a low dose (0.4 mg/kg) or high dose (3.0 mg/kg) for study 2 of either water suspension. Lipid peroxidation, as measured by MDTA concentrations, is shown on top and total glutathione, as measured by GSH concentrations on bottom. * indicates statistical significance ($p < 0.05$).

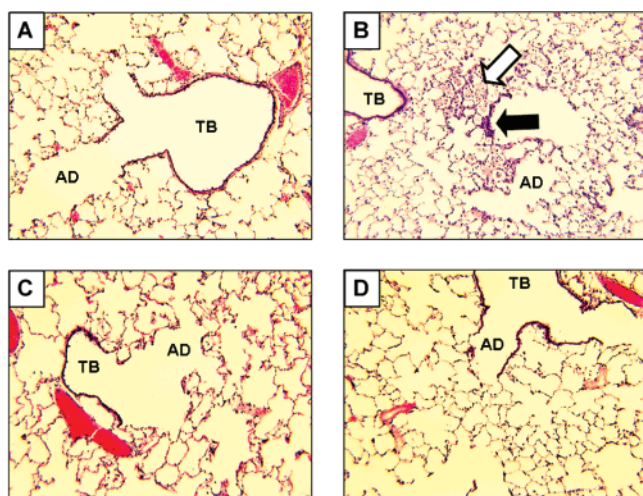


Figure 6. Lung tissue analysis. Light micrographs of lung tissue of rats exposed to (A) Milli-Q water vehicle control, (B) Min-U-Sil positive control, (C) nano- C_{60} water suspensions, and (D) $C_{60}(OH)_{24}$ water suspensions (all at 3 mg/kg) at 3 months postinstillation exposure. The micrographs illustrate the terminal bronchiole (TB) and corresponding alveolar ducts (AD), and demonstrate normal lung architecture in graphs A, C, and D, indicating that exposures to FWS produced no adverse pulmonary effects. Micrograph B depicts the prominence of foamy alveolar macrophage accumulation (white arrow) and tissue thickening (black arrow) in the distal lung. (magnification = 40 \times)

exposed rats). It is important to note, however, that the increases in lipid peroxidation endpoints did not correlate

with any of the other endpoints including cytotoxicity, inflammation, cell proliferation, or lung histopathological findings.

Numerous studies in rats and corresponding reviews on the topic have indicated that exposures to nanoscale or ultrafine particles (generally defined as particles in the size range <100 nm) produce greater inflammatory and cytotoxic effects when compared to exposures to larger-sized particles at equivalent mass concentrations.^{13,14} Surface area metrics have been implicated in playing an important role in the development of nanomaterial toxicity. These authors have suggested that two main mechanisms may be operative in the development of nanotoxicological effects which differentiate these materials from their bulk counterparts. These effects are postulated to be (1) the greater potential for deposited nanomaterials to escape macrophage surveillance and, as a consequence, translocate from air space to interstitium or vascular sites and thus cause either interstitial inflammation/fibrosis or translocate to other organs and/or (2) the direct effect of nanomaterials on cells to cause injury and oxidative stress.^{13,14} Moreover, in other studies, ultrafine carbon black, nickel, and TiO_2 particles have produced more potent lung inflammation and cytotoxicity than bulk materials of similar composition in the lungs of rats.^{22–26} However, it should be noted that other investigators have demonstrated that surface reactivity of particle types is likely to be a more important factor than particle size/surface area in producing pulmonary inflammation and cytotoxicity following particle exposures.^{27,28}

In assessing the accuracy and reproducibility of in vitro toxicity assays with fine or nanoscale particle-types, previous studies have reported little correlation between the relative toxicity of particles when comparing lung toxicity rankings following in vivo instillation compared to in vitro cell culture exposures. Recently we evaluated the capacity of in vitro screening studies to predict in vivo lung toxicity effects of several fine or nanoscale particles in rats. In the in vivo component of the study, rats were exposed by intratracheal instillation to the following particle types: (1) carbonyl iron; (2) crystalline silica (Min-U-Sil 5, α -quartz); (3) precipitated amorphous silica; (4) nanosized zinc oxide; (5) fine-sized zinc oxide. After exposures, the lungs of PBS and particle-instilled rats were lavaged and inflammation and cytotoxicity biomarkers were measured at 24 h, 1 week, 1 month, and 3 months postexposure. For the in vitro component of the study, three different cell culture conditions were utilized. Cultures of (1) rat lung epithelial cells, (2) primary alveolar macrophages (collected via bronchoalveolar lavage (BAL) from unexposed rats), and (3) lung epithelial cell/alveolar macrophage cocultures were incubated with the particle types listed above at several concentrations. The culture fluids were evaluated for cytotoxicity endpoints (LDH, MTT) and inflammatory cytokines (MIP-2, TNF- α , and Interleukin-6) at time points ranging from 1 to 48 h. The comparative results demonstrated little correlation between in vitro and in vivo toxicity measurements, and it was concluded that in vitro cellular systems will need to be further developed, standardized, and validated (relative to in vivo effects) in order to provide useful screening data on the relative toxicity of inhaled particles.²⁹

The hazard database regarding fullerene toxicity is rather limited. Dhawan and co-workers reported that stable colloidal dispersions of C₆₀ fullerenes in water are genotoxic.³⁰ Rouse et al. investigated fullerene-based amino acid nanoparticle interactions with human epidermal keratinocytes and concluded that higher doses could produce cytotoxicity and induce inflammatory cytokines.³¹ Sayes et al. and Isakovic et al. compared the cytotoxicity mechanisms of pristine versus hydroxylated fullerenes in human cell lines and mouse, rat, and human cell lines, respectively.^{16,18} The results of both studies indicated substantial differences in the potency of pristine nano-C₆₀ to produce toxicity relative to the hydroxylated fullerene test samples. It is important to note that all of the studies described above were conducted under in vitro cell culture conditions.

In a previously reported study by Sayes et al., it was concluded that exposures to nano-C₆₀ water suspensions induced significant cytotoxic effects in three different human cell lines.^{16,17} Moreover, the findings indicated a differential cytotoxic response to the FWS-types based upon derivatization or hydroxylation: as the concentration of surface modifications on the fullerene cage structure increased, the LC₅₀ (fullerene concentration where 50% cellular viability is measured) decreased, thus decreasing the toxic nature of the fullerene species. This differential has been used as an example for a basis of innovation, i.e., others have proposed that nanomaterials can be engineered for safety based upon

the results of in vitro toxicity assays. However, the development of this concept may be premature. In the study presented herein, a sustained cytotoxic response was measured following pulmonary exposures to crystalline silica, the positive control particle-type; however, no differences in cytotoxicity, inflammation parameters, cell proliferation or lung histopathological endpoints were measured when comparing the two fullerene species, i.e., the nano-C₆₀ and fully hydroxylated C₆₀ to vehicle control parameters. The conclusions of this study are not unique, as recent comparative studies have demonstrated a lack of correlation when comparing the results of in vitro and in vivo toxicity assessments following exposures to fine and/or nano-metal-oxide particle types.²⁹

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Supporting Information Available: Descriptions of particle preparation and characterization, pulmonary toxicity of particles, and biochemical techniques and a figure comparing lung weights. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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