TOXICITY STUDIES OF FULLERENES

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Abstract
Nanomaterials, due to their unique physical, chemical, and magnetic properties have great potential for industrial development. As the nanotechnology industry promises to accelerate over the coming years, nanoparticles may pose serious health and environment threats. It is estimated that the amount of manufactured nanoparticles will get doubled by 2020. Nanoparticles can come loose during their use and subsequently get into drain water, unavoidably dispersing into the environment. The accumulation of nanoparticles and their associated nanotoxicity would be expected to have a major impact on health and environment. Human contact with nanomaterials can take various forms: inhalation, ingestion, and absorption through the skin. These have the potential to cause damage to the human body in different ways. Recent studies have shown that nanoparticles can cross the blood-brain barrier and exhibit effects on the central nervous system. In general, smaller particles are more bioactive and toxic. Their ability to interact with other living systems increases because they can easily cross the skin, lung, and in some cases the blood/brain barriers. Once inside the body, there may be further biochemical reactions like the creation of free radicals that damage cells. While the body has built-in defenses for natural particles it encounters, the danger of nanotechnology is that it is introducing entirely new type of particles. When administered dermally, nanoparticles have been known to localize to regional lymph nodes. There is a pressing need to develop rapid testing assays to assess the potential toxicity of engineered nanomaterials. Present report is an attempt for understanding of the effect of Fullerenes (C_{60})on health and environment. There is no doubt that they have interesting and useful properties. Strong economic drives and competition in the marketplace may be taking priority over precise scientific caution when it comes to public health and possible dangers of nanotechnology. The potential for these nanoparticles to enter the body and possible toxicity has been tried to explore.

Keywords: Fullerenes, Nanomaterials, Nanotoxicity.

1.1. Introduction to Nanotechnology and Nanoscale Materials

Nanotechnology is an emerging technology which is capable of revolutionizing our approaches to many problems. It is defined as the ability to visualize, analyze, and manipulate materials on molecular scales of approximately 0.1 to 100 nm. It includes a wide range of applications across a wide range of technology platforms, including scanning, tunneling, and atomic force microscopy, next generation semiconductors, nanoparticles, nano-bio molecules, thin film technologies, nano-sensors and other nano-devices, quantum computing, and many, many more.

The value of nanomaterials in many technology areas is very high because of their versatile properties. Nanomaterials are defined as having at least one dimension in the 1-100 nm range. The possibilities of nanomaterials appear endless with current research and development in the areas of electronics and optics, drug delivery, cosmetics, and clothing, as well as groundwater remediation and energy sources. Some nanomaterials have been used for years, such as titanium dioxide which has been used in sunscreens and aluminum oxide which is used in deodorants. Dendrimers are being developed for uses in solar energy antennae and drug delivery mechanisms. Other nanomaterials are being added to clothing for permanent waterproofing and superior stain resistance. These are a few of the many examples of how nanomaterials are being developed and affect our every day life.

1.2. Impact of Nanomaterials on Human Health and Environment

Human beings are in constant contact with the environment. Skin is generally an effective barrier to foreign substances but the lungs and gastro-intestinal tract are more susceptible. These ways are the most likely points of entry for natural or anthropogenic nanoparticles. Other possible routes of exposure, primarily limited to engineered materials are injections and implants. Nanotechnology, no doubt has direct beneficial applications for medicine and the environment, but like all technologies it may have unintentional effects. The progress in nanotechnology based products has raised concerns about the impacts of unintentional nanoparticle exposure on human health and ecological communities. While taking advantage of this new technology for health, environmental, and sustainability benefits, science needs to examine the environmental and health implications as nanoparticles present possible dangers, both medically and environmentally. At present, we are at the best possible time to begin to study the impact of nanomaterials on human health.

In recent years, nanotoxicity has received growing attention from environmental and health agencies all over the world as nanoparticles have become one of the emerging pollutants due to their frequent detection in the environment [1-5]. Nanoparticles because of their small size are able to pass through cell membranes in organisms, move through the body and brain and cause biochemical damage. Their interactions with biological systems are relatively less explored. Despite the relatively fast growing numbers of studies on ecological/environmental risk, the number of publications related to studies of nanotoxicity remains small. It becomes important to understand and analyze different aspects of nanoparticle exposures to humans, the associated health risks. Data on nanoparticles, such as increasing production volumes and commercialization, capabilities to cross biological barriers, and increased biological activities of nanoparticles when compared to bulk counterparts, have worried some researchers, policy-makers, and investors about their potential impacts on human health and environment. However, until recently the potential negative effects of nanomaterials on human health and the environment were rather uncertain. Number of laboratory studies have indicated that exposure to some nanoparticles can lead to adverse effects in the lungs and the brain of test animals [6, 7]. Inhalation of nanoparticles is problematic because the particles are often small enough that alveolar macrophages cannot detect or scavenge the particles for elimination. By evading alveolar macrophages, nanoparticles can enter the lymphatic and circulatory systems to be distributed throughout the body within 24hours [6]. This has been studied with a number of particles. Inhaled particles can also be transported to the brain via the olfactory or trigeminal nerves. This process was first noted in studies from the 1940s, and the current hypothesis is that these nano-sized particles move in a similar mechanism as viruses.

In general, the toxicological data of nanoparticles is insufficient due to the small number of studies, the short exposure period, the different composition of the nanoparticles tested (diameter, length and agglomeration), and the often-unusual exposure route in the work environment, among other factors. Additional studies are essential to assess the risk associated with inhalation exposure. However, In order to assess the impact of nanomaterials on human health and environment, insight is required into current and future trends with respect to the development and commercialization of nanotechnology. Much of the nanotechnology attention has been on the carbon nanomaterials, particularly concerning the toxicity of carbon nanomaterials. In this report, the current state of knowledge of Fullerenes toxicity are discussed and the most significant findings are highlighted. The objective of the study is to identify existing issues within the Fullerenes C_{60} related health issues and propose possible suggestions.

Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms**.** C_{60} is a hollow sphere, resembling a soccer ball, composed of interconnected carbon pentagons and hexagons Fullerenes can be subjected to extreme pressures and recover their original shape when the pressure is released. These molecules are not modified and do not combine with each other.

However, when fullerenes are manufactured, certain carbon atoms can be replaced with other atoms and form bondable molecules, thus producing a hard but elastic material. The surface chemical composition can be tailored and different organic chains can be added, or they can be included into carbon nanotubes. Since fullerenes are empty structures with dimensions comparable to several biologically active molecules, they can be packed with different substances and find medical applications. Findings of this study are expected to highlight research needs in the area related to toxicity of Fullerenes.

2. Fullerenes (C_{60})

Fullerenes represent a group of nanoparticles discovered in 1985 with widely different properties. Fullerenes (also known as "buckyballs") are a class of cage-like carbon compounds composed of fused, pentagonal and/or hexagonal sp^2 carbon rings like the patches on a soccer ball. The basic fullerene is made of 60 carbon atoms (C_{60}) and has a diameter of approximately 1 nm. Engineered carbon fullerenes are stable, soccer ball-like carbon atoms with hexagonal and pentagonal shapes. The fullerenes are one type of manufactured nanoparticle that is being produced by tons each year. Carbon fullerenes, which are ultrafine particulate matter, are one of the most ubiquitous nanomaterials found. They are generally present in polluted air as they are often released in soot resulting from the process of fuel combustion. Fullerenes are lipophilic and localize into lipid-rich regions such as cell membranes in vitro, and they are redox active.

Current literature on the effects of fullerene exposure appears contradictory due to some reasons. First, the term fullerene is used to refer to C_{60}, C_{70}, and their derivatives, as well as other clusters with high numbers of carbon atoms. Consequently, summary statements that report on the effects of "fullerenes" become a source of confusion. It is important to take into consideration the functionalization of the material when making toxicity statements as well as the type of toxicological assay, age of the organism and route of exposure. Second, the purity of the fullerene material affects its toxic potential. It has been noted that purified C_{60} did not cause human platelet aggregation *in vitro* or rat vascular thrombosis *in vivo.* However, purified samples are rare and costly, and will not likely be utilized for mass production except for specific purposes. As such, the toxic potential of commercially available fullerenes is important since some variations are already being manufactured by the ton. Third, the method of solubilizing the C_{60} or its derivatives plays a large role in the toxicity of the fullerene. In cell culture studies of C_{60} and $C_{60}(OH)_{24}$, it has been reported that underivatized fullerenes were at least three orders of magnitude more toxic than their hydroxylated counterpart.

The most notable fullerene would be C_{60}, a highly reactive biomolecules that has the ability to cross blood brain barrier [8]. C_{60} fullerene is highly used in industry as catalysts, reactive oxygen species scavengers [9] and tools in drug delivery systems [10]. Since the early 1990s, there have been concerns about the potential dermal and inhalation effects of fullerenes due to their strong oxidizing and phototoxic properties [11]. Some studies suggest that when exposed to light, fullerenes can have cytotoxic effects, effect embryo development, cleave DNA, distribute rapidly to many tissues where they retain for long time. Cytotoxic effects of fullerenes may be due to lipid peroxidation of cell membranes and resulting leakiness of membranes. It has been found that C_{60} treatment also increases formamidopyrimidine [fapy]-DNA glycosylase (FPG) sensitive sites, accounting for short-term DNA strand damage. Xu et al. observed that C_{60} induced an increase in mutation yield in primary mouse embryo fibroblast cells and dose-dependent formation of free radical $ONOO^-$ using dihydrorohodamine radical probes [12]. However, in the *in vivo* setting, C_{60} treatment was found to be associated with increased DNA damage 8-hydroxydeoxyguanosine (8-OHdG) in mouse lung and liver [13].

Water-soluble fullerene (nC_{60}) has been shown to induce lipid peroxidation in brain of juvenile largemouth bass [14]. Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in brain of juvenile largemouth bass. Gharbi *et al.* (2005) [15] observed no acute or subacute effects in rats exposed to C_{60} in a dose of 2g/kg body weight (bw) 14 and 21 days post exposure, respectively. Sayes *et al.* [16], however, did observe an increase in the percentages/numbers of Broncho alveolar lavage recovered neutrophils (i.e. white blood cells) after intratracheally instillation of C_{60} and hydroxylated C_{60} i.e. $C_{60}(OH)_{24}$ just 1 day post-exposure. Sayes *et al.* also observed a significant increase in lipid peroxidation values and an increase in level of glutathione (GSH), after 1 week.

Lai *et al.* [17] also observed a significant increase in lipid peroxidation products after intravenous administration of 1 mg $C_{60}(OH)_{18}$ per kg into male mongrel dogs previously induced with infusion/reperfusion injury. Tsuchiya *et al.* [18] observed shrunken membrane and narrow blood vessels on the yolk sac on 11 day pregnant mice and embryo death 18 hours after intraperitoneal injection of between 25-137 mg C_{60} per kg. Adverse effects of functionalized C_{60} have been observed as well for instance by Yamago *et al.* [19]. Chen *et al.* (1998) observed a LD50 of 600 mg $C_{60}((CH_2)_4SO_2Na)_{4\text{-}6}$/kg bw in female rats after intraperitoneal administration of between 0 and 2,500 mg/kg bw for 2 weeks[20]. Whereas Yamago *et al.* observed symptoms of discomfort and weight loss in female mice after a single intraperitoneal injection of between 200-500 mg/ per kg.

The cytotoxic effects of C_{60} have been studied extensively *in vitro* using a number of different cell strains, different test procedures including a range of different solvents to get C_{60} into suspension. Studies on the cytotoxicity of C_{60} towards cancer cell are ambiguities and while some have reported observing no signs of cytotoxicity of any kind after exposure to fairly high concentrations of C_{60}, suspended in toluene, methanol and by sonication [21] others do observe cytotoxicity of C_{60} suspended in tetrahydrofuran (THF) [22]. However, these studies are hard to interpret since they often use different transformed and/or damaged cell strains and different ways of suspending C_{60} some even consider the cytotoxicity of C_{60} under the influence of light. Surface chemistry has been found to have an important influence on the toxicity of C_{60}. Dose-dependent cytotoxicity of hydroxylated C_{60} and functionalized C_{60} has been observed for instance by Yamawaki and Iwai [23] who observed a dose-dependent decrease in cell density and lactate dehydrogenase (LDH) release in human umbilical vein endothelial cells cavity after exposure to $C_{60}(OH)_{24}$.

Sayes *et al.* [24] found that the toxicity of C_{60} towards human skin fibroblasts and liver carcinoma cells varied by seven orders of magnitude depending on the number of functional groups. Aggregates of C_{60} were found to be substantially more toxic than highly soluble derivatives $C_{60}(OH)_{24}$. Rouse *et al.* [25] observed a dose-dependent decrease in the viability of human epidermeal keratinocytes after exposure to C_{60} phenylalanine while exposing HeLa cells to di-, tri-, quadrimalonic acid C_{60} was observed by Yang *et al.* [26] to cause irradiation and dose-dependent cytotoxicity. A number of studies also report finding no cytotoxic effects on macrophages, human keratinocytes, human skin and human fibroblasts cells after exposure to $C_{60}((CH_2)_4SO_3Na)_{4\text{-}6}$, $C_{60}[C(COOH)_2]$, Polyhydroxy C_{60}, and *N* ethyl- polyamino C_{60} [27].

A number of studies have reported hydrolated C_{60} being able to reduced cell and neuronal death induced by: Doxorubicin (Bogdanovic *et al.* 2004), sodium nitroprusside and H_2O_2, amino-3-hydroxy-5-methyl-4-isxazole propionic acid or kainite, iron, serum, UVB-irradiation. Others have reported that glutathione, ascorbic acid and tocopherol were capable of inhibiting membrane damage induced by hydroxylated C_{60} [28]. A number studies on the environmental effects of C_{60} have been done as well. Oberdorster [6] observed significant increase in lipid peroxidation of the brain of juvenile largemouth bass after exposure to uncoated fullerenes (99.5%) in concentrations of 0.5 and 1 ppm after exposure for 48 h. In a long-term study Oberdorster *et al.* [29] investigated the effect of hydroxylated C_{60} on the reproduction and survival rate of *Daphnia magna* and observed an

increased cumulative mortality and significant delay in molting and reduced offspring after exposure to 1-5 ppm for 21 days. The hypothesis that C_{60} could act as a vector for the transport of other toxic chemicals has also been investigated.

The effect on various kinds of bacterial strains has also been tested. Chiron *et al.* [30] tested micronized C_{60} and observed no effect on microbial growth of 22 collection strains including 6 strains of *S. typhimurium*, 5 strains of *E. coli*, and 2 strains of *P. aeruginosa*, *S. aureus* and *L. monocytogenes* (43.2 *μ*g/mL). Babynin *et al.* [31] found that the occurrence of mutations in *S. typhimurium* strain BA13 depends on the type of molecular group with which fullerene interacts whereas Fortner *et al.* [32] observed that bacteria growth was media dependent. No growth was observed for either *E. coli* DH5 or *B. subtilis* CB315 exposed to > 0.4 mg/L C_{60} at pH= 7 under anaerobic or aerobic conditions using a Minimal Davis media. Tests conducted by environmental toxicologist Eva Oberdorster found extensive brain damage to fish exposed to fullerenes for a period of just 48 hours at a relatively moderate dose of 0.5 parts per million. The fish also exhibited changed gene markers in their livers, indicating their entire physiology was affected. In a concurrent test, the fullerenes killed water fleas, an important link in the marine food chain. Oberdorster could not say whether fullerenes would also cause brain damage in humans but cautioned that more studies are necessary and that the accumulation of fullerenes over time could be a concern, particularly if they were allowed to enter the food chain.

For oral administration, 98 percent of fullerenes are eliminated within 48 hours via feces and urine. Oberdoster explained that the 2 percent that is not excreted is found throughout the rest of the body. Intravenous dosing is rapidly transported to the liver (73–92 percent), the spleen (up to 2 percent), lung (up to 5 percent), kidney (up to 3 percent), heart (approximately 1 percent), and the brain (approximately 0.84 percent) within 3 hours. After 1 week, 90 percent of intravenously administered fullerenes are still in the body, noted Oberdorster. Some results indicate that fullerenes travel freely through soil and could be absorbed by earthworms.

Table1. Selected Cytotoxic studies of Fullerenes

Fullerene	Effects observed	References
C_{60} water suspension	Antibacterial; cytotoxic to human cell lines; taken up by human keratinocytes; stabilizes proteins	Lyon, D. Y.; et al. [33] Sayes, C. M.; et al.[34]
C_{60} encapsulated in poly(vinylpyrrolidone), cyclodextrins, or poly(ethylene glycol)	Damages eukaryotic cell lines;antibacterial	Kai, Y.; et al.[35] Kamat, J. P.; et al.[36]
Hydroxylated fullerene	Oxidative eukaryotic cell damage	Kamat, J. P.; et al.[37]
Carboxyfullerene (malonic acid derivatives)	Bactericidal for Gram-positive bacteria; cytotoxic To human cell lines	Tsao, N.; et al. [38] Tsao, N.; et al.[39]
Fullerene derivatives with pyrrolidine groups	Antibacterial; inhibits cancer cell proliferation; cleave plasmid DNA	Mashino, T.; et al.[40]
Other alkane derivatives of C_{60}	Antimutagenic; cytotoxic; induces DNA damage in plasmids; inhibits protein folding; antibacterial; accumulates in rats' livers	Babynin, E. V.; et al.[41] Tokuyama, H.; Yamago, S.; Nakamura.[42] .

3.1. Discussion

The scientific literature on the environmental, health and safety of Fullerenes has been reviewed. It is clear from studies that air exposure and their dose is important for cytotoxic effects. The studies examining the toxicity of Fullerenes on human systems are still emerging and the subject of much debate. It is obvious from the studies that Fullerenes can accumulate in the body, depending on the dosing route. It is unknown as to what extent, Fullerenes or other nanomaterials have found use in marketed products. Manufacturers claim that their products contain registered (™) descriptions of materials but no indication is given of their physico-chemical identity.

It has been seen that maximal doses of fullerenes caused cytotoxic injury and/or death and inhibited cell growth. Fullerenes interact with biological systems by, enzyme inhibition, causing phototoxic reactions, being scavengers of reactive oxygen species and free radicals, in addition to being able to initiate free radical reactions. Absorption, distribution and excretion strongly depend on the properties of the side chains. The pristine C_{60} has a very long biological half-life, whereas the most water-soluble derivatives are eliminated from the exposed animals within weeks. A long biological half-life raises concern about bioaccumulation and long-term effects. In general, the acute oral, dermal and airway toxicity is low. Few experimental studies of repeated dose toxicity, reproductive toxicity and carcinogenic effect are accessible. The data suggest that direct DNA damaging effects are low, but formation of reactive oxygen species may cause inflammation and genetic damage. Few studies have been conducted on the metabolism of fullerenes, and those few used functionalized C_{60}. Many organic compounds are metabolized in the liver; however the fullerenes and their derivatives have remained unchanged in the liver. Environmental conditions, such as pH and ionic strength, also play a role in the characterization of C_{60} agglomerations.

Much of data on the effects of fullerene exposure has been gained using *in vitro* methods. These data may be of limited use in predicting *in vivo* responses especially since those results may be dependent on the cell culture system chosen for the experiment.

3.2. Conclusion

The applications of nanomaterials are increasing and it is likely that exposure to nanoparticles will become more common with the advancement of nanotechnology. The overall potential risks are likely to increase if no control actions are taken. Among the main factors, life-cycle of nanoparticles is clearly of importance. There are different human exposure scenarios during the life cycle of nanoparticles, including those during production, processing and distribution, use and application, storage, and waste disposal and recycling. Humans may also be exposed indirectly through contamination of the food chain by manufactured nanoparticles. Environmental species are also exposed via the different contaminated media, the air, the water and the soil/sediment system. The route of exposure to nanoparticles is likely to have a bearing on the uptake by biota in the environment and on the resulting toxicity. Several tests on Fullerenes have already been performed which have lead scientists to have some presumptions about their toxicity. But, extrapolation of animal data to humans is not straightforward. Some in vitro studies suggest the Fullerenes cytotoxicity, namely in the case of prolonged exposure. More work has to be done to understand in vivo transport, the mechanism of toxicity, and the process of elimination and accumulation in vivo. Such investigations will not only promote and support the protection of human health and the environment but, will also aid industry and regulatory bodies in maximizing the favorable use of these materials, by minimizing risks of this technology. Further investigations into the interaction of C60 *in vivo* are necessary. It is important to acknowledge that not each material will impact the environment that they are released into; though, the potential should be investigated to ensure human and environmental health are not at risk. So, it is wise to approach the progress of nanotechnology not only with excitement, but with caution.

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