Nanotoxicology References

A bibliography of references and abstracts of risk-related research studies on nanomaterials compiled by Environmental Defense

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Nanotoxicology Abstracts

J Am Chem Soc **123**, 10460-10467, 2001
Stable Colloidal Dispersions of Fullerenes in Polar Organic Solvents
Rossitza G. Alargova,* Shigeru Deguchi, and Kaoru Tsujii

Colloidal dispersions of C_{60} and C_{70} were prepared by simply mixing a fullerene solution in a good solvent with a poor polar organic solvent for fullerenes. The process was very easy and fast and the formation of particles with average diameter in the colloidal range was detected immediately after the components were mixed. The formation and the properties of the fullerene particles were studied mainly with dynamic light scattering and high-resolution transmission electron microscopy. The most interesting findings are the long-term colloid stability of the samples in the absence of any stabilizers, the relatively narrow size distribution, and the different average sizes of the particles formed by C_{60}, C_{70}, and their mixtures. The influence of various factors such as fullerene concentration, mixing procedure, solvent properties, and C_{60}/C_{70} ratio was investigated. It is shown that the smaller particles are formed when the total fullerene concentration in the good solvent is decreased and that the fullerene particles have crystalline structure. The measured negative values for the electrophoretic mobility of the particles suggest that fullerene dispersions in polar organic solvents are stabilized by repulsive electrostatic interactions.

Chem Phys Lett **364**, 8-17, 2002
Comparative analysis of two aqueous-colloidal solutions of C_{60} fullerene with help of FTIR reflectance and UV-Vis spectroscopy
G. V. Andrievsky, V. K. Klochkov[a], A. B. Bordyuh and G. I. Dovbeshko

Two types of fullerene-water colloidal systems: molecular-colloidal C_{60} solution in water (C_{60}FWS) and typical monodisperse C_{60} hydrosol are compared in this work. It was confirmed that C_{60}FWS consists of isolated C_{60} molecules in hydrated state, $C_{60}@\{H_2O\}_n$, and of their small spherical C_{60} clusters of different sizes. It was shown that C_{60}FWS simultaneously has the properties of both true solutions and colloidal systems. The origin of supramolecular complexes $C_{60}@\{H_2O\}_n$ stabilization is explained both by the weak donor-acceptor interactions of unpaired electrons of H_2O oxygen atoms with fullerene molecule and by formation of ordered, H-bounded and sphere-like hydrated shells around the fullerene. Spectra of surface enhanced infrared absorption (SEIRA) of C_{60} on gold substrate were recorded in FTIR reflectance mode. In the 400-900 cm^{-1} region some additional vibration bands of C_{60}, which are forbidden in IR spectra, have been registered. Earlier the similar bands had been observed in inelastic neutron scattering.

Appl Catalysis B-Environmental **39**, 161-169, 2002
Photodestruction of dichloroacetic acid catalyzed by nano-sized TiO_2 particles
D. W. Bahnemann, S. N. Kholuiskaya, R. Dillert, A. I. Kulak and A. I. Kokorin

The photocatalytic activity of TiO_2 nanoparticles of different size has been studied by following the photodegradation of dichloroacetic acid (DCA), and was tested by measuring the initial rate of the H^+ and Cl^- ion formation. The catalyst efficiency was found to be in correlation with the specific surface area of the semiconductor particles. The efficiency of the nanocrystalline TiO_2 photocatalyst could be increased by doping the particles with copper ions. The structure and the partitioning of different surface copper complexes has been studied using ESR technique. The influence of the pH value of the solutions, as well as of the adsorbed copper ion content, on the efficiency of the DCA photodegradation is discussed in the paper.

Bioconjugate Chem., **15** (1), 79 -86, 2004
Noninvasive Imaging of Quantum Dots in Mice
Byron Ballou, B. Christoffer Lagerholm, Lauren A. Ernst, Marcel P. Bruchez, and Alan S. Waggoner

Quantum dots having four different surface coatings were tested for use in in vivo imaging. Localization was successfully monitored by fluorescence imaging of living animals, by necropsy, by frozen tissue sections for optical microscopy, and by electron microscopy, on scales ranging from centimeters to nanometers, using only quantum dots for detection. Circulating half-lives were found to be less than 12 min for amphiphilic poly(acrylic acid), short-chain (750 Da) methoxy-PEG or long-chain (3400 Da) carboxy-PEG quantum dots, but approximately 70 min for long-chain (5000 Da) methoxy-PEG quantum dots. Surface coatings also determined the in vivo localization of the quantum dots. Long-term experiments demonstrated that these quantum dots remain fluorescent after at least four months in vivo.

Environ Health Perspect, **109 Suppl 4**, 613-618, 2001
Agglomerates of ultrafine particles of elemental carbon and TiO2 induce generation of lipid mediators in alveolar macrophages.
Beck-Speier I, Dayal N, Karg E, Maier KL, Roth C, Ziesenis A, Heyder J.

Agglomerates of ultrafine particles (AUFPs) may cause adverse health effects because of their large surface area. To evaluate physiologic responses of immune cells, we studied whether agglomerates of 77-nm elemental carbon [(EC); specific surface area 750 m2/g] and 21 nm titanium dioxide (TiO(2) particles (specific surface area 50 m(2)/g) affect the release of lipid mediators by alveolar macrophages (AMs). After 60-min incubation with 1 microg/mL AUFP-EC (corresponding to 7.5 cm(2) particle surface area), canine AMs (1 x 10(6) cells/mL) released arachidonic acid (AA) and the cyclooxygenase (COX) products prostaglandin E(2) (PGE(2), thromboxane B(2), and 12-hydroxyheptadecatrienoic acid but not 5-lipoxygenase (5-LO) products. AUFP-TiO(2) with a 10-fold higher mass (10 microg/mL) than AUFP-EC, but a similar particle surface area (5 cm(2) also induced AMs to release AA and COX products. Agglomerates of 250 nm TiO(2) particles (specific surface area 6.5 m(2)/g) at 100 microg/mL

mass concentration (particle surface area 6.5 cm(2) showed the same response. Interestingly, 75 cm(2)/mL surface area of AUFP-EC and 16 cm(2)/mL surface area of AUFP-TiO(2) additionally induced the release of the 5-LO products leukotriene B(4) and 5-hydroxyeicosatetraenoic acid. Respiratory burst activity of stimulated canine neutrophils was partially suppressed by supernatants of AMs treated with various mass concentrations of the three types of particles. Inhibition of neutrophil activity was abolished by supernatants of AMs treated with COX inhibitors prior to AUFP-incubation. This indicates that anti-inflammatory properties of PGE(2) dominate the overall response of lipid mediators released by AUFP-affected AMs. In conclusion, our data indicate that surface area rather than mass concentration determines the effect of AUFPs, and that activation of phospholipase A(subscript)2(/subscript) and COX pathway occurs at a lower particle surface area than that of 5-LO-pathway. We hypothesize a protective role of PGE(2) in downregulating potential inflammatory reactions induced by ultrafine particles.

Toxicological Sciences, **77**, 347-357, 2004

Pulmonary Responses of Mice, Rats, and Hamsters to Subchronic Inhalation of Ultrafine Titanium Dioxide Particles

Edilberto Bermudez, James B. Mangum, Brian A. Wong, Bahman Asgharian, Paul M. Hext, David B. Warheit and Jeffrey I. Everitt

A multispecies, subchronic, inhalation study comparing pulmonary responses to ultrafine titanium dioxide (uf-TiO_2) was performed. Female rats, mice, and hamsters were exposed to aerosol concentrations of 0.5, 2.0, or 10 mg/m^3 uf-TiO_2 particles for 6 h/day, 5 days/week, for 13 weeks. Following the exposure period, animals were held for recovery periods of 4, 13, 26, or 52 weeks (49 weeks for the uf-TiO_2-exposed hamsters) and, at each time point, uf-TiO_2 burdens in the lung and lymph nodes and selected lung responses were examined. The responses studied were chosen to assess a variety of pulmonary parameters, including inflammation, cytotoxicity, lung cell proliferation, and histopathological alterations. Retained lung burdens increased in a dose-dependent manner in all three species and were at a maximum at the end of exposures. Mice and rats had similar retained lung burdens at the end of the exposures when expressed as mg uf-TiO_2/mg dry lung, whereas hamsters had retained lung burdens that were significantly lower. Lung burdens in all three species decreased with time after exposure, and, at the end of the recovery period, the percentage of the lung particle burden remaining in the 10 mg/m^3 group was 57, 45, and 3% for rat, mouse, and hamster, respectively. The retardation of particle clearance from the lungs in mice and rats of the 10 mg/m^3 group indicated that pulmonary particle overload had been achieved in these animals. Pulmonary inflammation in rats and mice exposed to 10 mg/m^3 was evidenced by increased numbers of macrophages and neutrophils and increased concentrations of soluble markers in bronchoalveolar lavage fluid (BALF). The initial neutrophil response in rats was greater than in mice, whereas the relative increase of macrophages was less than in mice. The neutrophilic response of rats, but not mice, declined in a time-dependent manner correlating with declining lung burdens; however, the fraction of recovered neutrophils at 52 weeks postexposure was equivalent in the two species. Consistent increases in soluble indicators of toxicity in the BALF (LDH and protein) occurred principally in rats and mice exposed to 10 mg/m^3 and diminished with time postexposure. There were no significant changes

in cellular response or with markers indicating toxicity in hamsters, reflecting the capacity of these animals to rapidly clear particles from the lung. Progressive epithelial and fibroproliferative changes were observed in rats of the 10 mg/m^3 group. These lesions consisted of foci of alveolar epithelial proliferation of metaplastic epithelial cells (so-called alveolar bronchiolization) circumscribing aggregated foci of heavily particle-laden macrophages. The observed epithelial proliferative changes were also manifested in rats as an increase in alveolar epithelial cell labeling in cell proliferation studies. Associated with these foci of epithelial proliferation were interstitial particle accumulation and alveolar septal fibrosis. These lesions became more pronounced with increasing time postexposure. Epithelial, metaplastic, and fibroproliferative changes were not noted in either mice or hamsters. In summary, there were significant species differences in the pulmonary responses to inhaled uf-TiO_2 particles. Under conditions where the lung uf-TiO_2 burdens were equivalent, rats developed a more severe inflammatory response than mice and, subsequently, developed progressive epithelial and fibroproliferative changes. Clearance of particles from the lung was markedly impaired in mice and rats exposed to 10 mg/m^3 uf-TiO_2, whereas clearance in hamsters did not appear to be affected at any of the administered doses. These data are consistent with the results of a companion study using inhaled pigmentary (fine mode) TiO_2 (Bermudez *et al.*, 2002) and demonstrate that the pulmonary responses of rats exposed to ultrafine particulate concentrations likely to induce pulmonary overload are different from similarly exposed mice and hamsters. These differences can be explained both by pulmonary response and by particle dosimetry differences among these rodent species.

Inhalation Toxicology, **14**, 311-324, 2002
Particle Toxicology: From Coal Mining to Nanotechnology
Paul J. A. Borm

Particle research has been historically closely connected to industrial activities or materials, such as coal, asbestos, man-made mineral fibers, and more recently ambient particulate matter (PM). It is the purpose of this review to combine insights and developments in particle toxicology with the historical context of exposure and organizations sponsoring such research in Europe. In supporting research on particle-induced respiratory effects and mechanisms, research programs of the European Community on Steel and Coal (ECSC) have played a tremendous role. Current particle research in Europe is dominated by PM, and funded by the World Health Organization (WHO), European Union Framework programs, and the Health Effects Institute (HEI). Differences between historical and current research in particle toxicology include the exposure concentrations, particle size, target populations, endpoints, and length of exposure. Inhaled particle effects are no longer confined to the lung, since particles are suggested to translocate to the blood while lung inflammation invokes systemic responses. Finally, the particle size and concentrations have both been reduced about 100-fold from 2-5 mg/m^3 to 20-50 mg/m^3 and from 1-2 μm to 20-100 nm (ultrafine) as domestic fuel burning has decreased and vehicle sources have increased and attention has moved from coal mining industry to general environment. There is, however, a further occupational link to nanotechnology, which continuously produces new materials in the ultrafine range. Although inhalation exposure is considered to be minimal in this technology, some particles are produced to be used for carrier purpose in medical applications. Based on our current knowledge of particle toxicology, it is highly desirable that

toxicology and technology are linked in this extremely rapid developing area, to learn more about potential risks and also to develop knowledge on the role of surface and size in particle toxicity.

Inhalation Toxicology, **12 Suppl 3**, 7-14, 2000
FROM COAL MINE DUST TO QUARTZ: Mechanisms of Pulmonary Pathogenicity
Vincent Castranova

Exposure to coal mine dust or crystalline silica can result in the initiation and progression of interstitial lung disease. Pathogenesis is the consequence of damage to lung cells and resulting lung scarring associated with activation of fibrotic processes. This review presents the radiologic and histologic characteristics of simple and complicated coal workers' pneumoconiosis (CWP) as well as pathological indices of acute and chronic silicosis. This presentation also reviews the results of in vitro, animal, and human investigations that elucidate mechanisms involved in the development of these pneumoconioses. Results support the involvement of four basic mechanisms in the etiology of CWP and silicosis: 1. Direct cytotoxicity of coal dust or silica, resulting in lung cell damage, release of lipases and proteases, and eventual lung scarring. 2. Activation of oxidant production by pulmonary phagocytes, such as alveolar macrophages. When oxidant production exceeds antioxidant defenses, lipid peroxidation and protein nitrosation occur, resulting in tissue injury and consequent scarring. 3. Activation of mediator release from alveolar macrophages and alveolar epithelial cells. Chemokines recruit polymorphonuclear leukocytes and macrophages from the pulmonary capillaries into the air spaces. Once within the air spaces, these leukocytes are activated by proinflammatory cytokines to produce reactive species, which increase oxidant injury and lung scarring. 4. Secretion of growth factors from alveolar macrophages and alveolar epithelial cells. Release of such mediators stimulates fibroblast proliferation and induces fibrosis. In conclusion, results of in vitro and animal studies have provided the basis for proposing mechanisms that may lead to the initiation and progression of CWP and silicosis. Data obtained from exposed workers has lent support to these proposals. The mechanistic understanding obtained for the development of CWP and silicosis should be useful in elucidating the possible pathogenicity of other inhaled particles.

Am J Physiol Lung Cell Mol Physiol, **274**, L81-L86, 1998
Comparison of the uptake of fine and ultrafine TiO_2 in a tracheal explant system
A. Churg, B. Stevens, and J. L. Wright

To examine the relationship between particle uptake by pulmonary epithelial cells and particle size, we exposed rat tracheal explants to fine particles (FPs; 0.12 μm) or ultrafine particles (UFPs; 0.021 μm) of titanium dioxide for 3 or 7 days. By electron microscopy, particles were found in the epithelium at both time points, but in the subepithelial tissues, they were found only at 7 days. The volume proportion of both FPs and UFPs in the epithelium increased from 3 to 7 days; it was greater for UFPs at 3 days but was greater for FPs at 7 days. The volume proportion of particles in the subepithelium at 7 days was equal for both dusts, but the ratio of epithelial to subepithelial volume proportion was ~2:1 for FPs and 1:1 for UFPs. Mean volume of individual particle aggregates was similar for both dusts at 3 days but was markedly smaller for FPs at 7 days. These observations suggest that the behavior of particles of different size is

complex: UFPs persist in the tissues as relatively large aggregates, whereas the size of FP aggregates becomes smaller over time. UFPs appear to enter the epithelium faster, and once in the epithelium, a greater proportion of them is translocated to the subepithelial space compared with FPs. However, if it is assumed that the volume proportion is representative of particle number, the number of particles reaching the interstitial space is directly proportional to the number applied; i.e., overall, there is no preferential transport from lumen to interstitium by size.

Nature Biotechnology, **21**, 1166–1170, 2003
The potential environmental impact of engineered nanomaterials
Vicki L Colvin

With the increased presence of nanomaterials in commercial products, a growing public debate is emerging on whether the environmental and social costs of nanotechnology outweigh its many benefits. To date, few studies have investigated the toxicological and environmental effects of direct and indirect exposure to nanomaterials and no clear guidelines exist to quantify these effects.

Toxicology Letters, **155**, 73-85, 2005
Effect of single wall carbon nanotubes on human HEK293 cells
Daxiang Cui, Furong Tian, Cengiz S. Ozkan, Mao Wang and Huajian Gao

The influence of single-walled carbon nanotubes (SWCNTs) on human HEK293 cells is investigated with the aim of exploring SWCNTs biocompatibility. Results showed that SWCNTs can inhibit HEK293 cell proliferation, decrease cell adhesive ability in a dose- and time-dependent manner. HEK293 cells exhibit active responses to SWCNTs such as secretion of some 20-30 kd proteins to wrap SWCNTs, aggregation of cells attached by SWCNTs and formation of nodular structures. Cell cycle analysis showed that 25 μg/ml SWCNTs in medium induced G_1 arrest and cell apoptosis in HEK293 cells. Biochip analysis showed that SWCNTs can induce up-regulation expression of cell cycle-associated genes such as *p16*, *bax*, *p57*, *hrk*, *cdc42* and *cdc37*, down-regulation expression of cell cycle genes such as *cdk2*, *cdk4*, *cdk6* and *cyclin D3*, and down-regulation expression of signal transduction-associated genes such as *mad2*, *jak1*, *ttk*, *pcdha9* and *erk*. Western blot analysis showed that SWCNTs can induce down-regulation expression of adhesion-associated proteins such as laminin, fibronectin, cadherin, FAK and collagen IV. These results suggest that down-regulation of G_1-assoicated *cdks* and *cyclins* and upregulation of apoptosis-associated genes may contribute to SWCNTs induced G_1 phase arrest and cell apoptosis. In conclusion, SWCNTs can inhibit HEK293 cells growth by inducing cell apoptosis and decreasing cellular adhesion ability.

Chem. Commun., **8**, 663 – 669, 1999
Medicinal chemistry with fullerenes and fullerene derivatives
Tatiana Da Ros and Maurizio Prato

The study of the biological applications of fullerenes has attracted increasing attention despite the low solubility of the carbon spheres in physiological media. The organic functionalisation of fullerenes has helped solubilisation by covalent attachment of hydrophilic appendages. Therefore,

recently synthesised fullerene derivatives reach satisfactory concentrations in water. However, the tendency of the fullerenes to form clusters is enhanced in polar media, where better solubilisation can be achieved by means of multiple functionalisation or using micellar systems. Once homogeneously dissolved, the fullerenes and fullerene derivatives exhibit an interesting range of biological activities, especially promising in the field of photodynamic therapy, HIV, neuroprotection and apoptosis.

Croatica Chemica Acta, **74**, 743-755, 2001
Biological Applications of Fullerene Derivatives: A Brief Overview
Tatiana Da Ros, Giampiero Spalluto, and Maurizio Prato

Starting soon after the production of fullerenes in 1990, many efforts have been devoted to the application of C60 and its derivatives. In fact, •60•fullerene possesses a variety of interesting biological properties, such as HIV-P inhibition, DNA photocleavage, neuroprotection, apoptosis, *etc*. Unfortunately, the low solubility in biological fluids limits the use of these compounds as new pharmacophores for structure-activity relationship studies in medicinal chemistry. This article briefly summarizes recent studies on the functionalization of C60 aimed at increasing water solubility as well as the preliminary studies performed on biological targets. In particular, the HIV-P inhibition, DNA photocleavage and antibacterial activity are discussed.

Langmuir, **17**, 6013 -6017, 2001
Stable Dispersions of Fullerenes, C_{60} and C_{70}, in Water. Preparation and Characterization
Shigeru Deguchi, Rossitza G. Alargova, and Kaoru Tsujii

Stable aqueous dispersions of fullerenes, C_{60} and C_{70}, were prepared by simply injecting into water a saturated solution of fullerene in tetrahydofuran (THF), followed by THF removal by purging gaseous nitrogen. To our knowledge, this is the first report of the stable dispersion of C_{70} in water. Fullerenes are dispersed as monodisperse clusters in water, 60 nm in diameter. High resolution transmission electron microscopy revealed the polycrystalline nature of the cluster. The preparation of the dispersion is very easy to perform, and the dispersions thus obtained are of excellent colloidal stability even though no stabilizing agent is used. It was found that the surface of the cluster is negatively charged and the electrostatic repulsion between the negatively charged cluster surfaces is important for the stability of the dispersions.

Nano Letters, **4** (1), 11 -18, 2004
Probing the Cytotoxicity of Semiconductor Quantum Dots
Austin M. Derfus, Warren C. W. Chan, and Sangeeta N. Bhatia

With their bright, photostable fluorescence, semiconductor quantum dots (QDs) show promise as alternatives to organic dyes for biological labeling. Questions about their potential cytotoxicity, however, remain unanswered. While cytotoxicity of bulk cadmium selenide (CdSe) is well documented, a number of groups have suggested that CdSe QDs are cytocompatible, at least with some immortalized cell lines. Using primary hepatocytes as a liver model, we found that

CdSe-core QDs were indeed acutely toxic under certain conditions. Specifically, we found that the cytotoxicity of QDs was modulated by processing parameters during synthesis, exposure to ultraviolet light, and surface coatings. Our data further suggest that cytotoxicity correlates with the liberation of free Cd^{2+} ions due to deterioration of the CdSe lattice. When appropriately coated, CdSe-core QDs can be rendered nontoxic and used to track cell migration and reorganization in vitro. Our results provide information for design criteria for the use of QDs in vitro and especially in vivo, where deterioration over time may occur.

Biochemical and Biophysical Research Communications, **294**, 116-119, 2002
Cellular localisation of a water-soluble fullerene derivative
Sarah Foley, Colin Crowley, Monique Smaihi, Claude Bonfils, Bernard F. Erlanger, Patrick Seta and Christian Larroque

Fullerenes are a new class of compounds with potential uses in biology and medicine and many insights were made in the knowledge of their interaction with various biological systems. However, their interaction with organised living systems as well as the site of their potential action remains unclear. In this work, we have demonstrated that a fullerene derivative could cross the external cellular membrane and it localises preferentially to the mitochondria. We propose that our finding supports the potential use of fullerenes as drug delivery agents as their structure mimics that of clathrin known to mediate endocytosis.

Chem. Commun., **1**, 121 – 123, 2005
Semiconductor quantum dots and free radical induced DNA nicking
Mark Green and Emily Howman
There is a growing interest in the use of semiconductor quantum dots as fluorescent markers in biological applications. However, there are concerns regarding the potential environmental impact and toxic nature of these nanomaterials. In this study, we have investigated the interaction of water-soluble semiconductor quantum dots with supercoiled DNA.

Biomaterials, **24**, 4529-4537, 2003
Surface-engineered nanoparticles for multiple ligand coupling
Ruxandra Gref, Patrick Couvreur, Gillian Barratt and Evgueni Mysiakine

The design of surface-engineered nanoparticles for targeting to specific sites is a major challenge. To our knowledge, no study in the literature deals with ligand functionalization of biodegradable nanoparticles through biotin–avidin interactions. With the aim of conceiving small-sized nanoparticles which can be easily functionalized with a variety of ligands or mixtures thereof, biotinylated and PEGylated biotin-poly(ethylene glycol)-poly(ε-caprolactone) (B-PEG-PCL) copolymers were synthesized and used to prepare nanoparticles of around 100 nm. Avidin, followed by biotinylated wheat germ agglutinin as a model lectin, were coupled to their surface by taking advantage of the strong biotin–avidin complex formation. The cytotoxicity of the nanospheres towards Caco-2 cells in culture was negligible (more than 82% cell survival for nanoparticle concentrations up to 300 μg/well). The amount of radiolabeled poly(lactic acid) (PLA) or PEG-PLA nanoparticles associated with Caco-2 cells was only 0.7% and 1.5% of the

amount added, respectively. This value was increased to 8.5% when a sufficient amount of lectin was bound to the PEG-PLA copolymer. After further studies, the biotin-PEG-coated nanoparticles could be helpful tools for studying the interaction between cells and functionalized nanoparticles with various surface characteristics (PEG layer density and thickness, ligand type and density).

Journal of Nanobiotechnology, **2**, 12, 2004
Nanoparticles – known and unknown health risks
Peter HM Hoet, Irene Brüske-Hohlfeld and Oleg V Salata

Manmade nanoparticles range from the well-established multi-ton production of carbon black and fumed silica for applications in plastic fillers and car tyres to microgram quantities of fluorescent quantum dots used as markers in biological imaging. As nano-sciences are experiencing massive investment worldwide, there will be a further rise in consumer products relying on nanotechnology. While benefits of nanotechnology are widely publicised, the discussion of the potential effects of their widespread use in the consumer and industrial products are just beginning to emerge. This review provides comprehensive analysis of data available on health effects of nanomaterials.

Nano Letters, **4** (11), 2163 -2169, 2004
Physicochemical Properties and Cellular Toxicity of Nanocrystal Quantum Dots Depend on Their Surface Modification
Akiyoshi Hoshino, Kouki Fujioka, Taisuke Oku, Masakazu Suga, Yu F. Sasaki Toshihiro Ohta, Masato Yasuhara, Kazuo Suzuki, and Kenji Yamamoto

Nanocrystal quantum dots (QDs) have been applied to molecular biology because of their greater and longer fluorescence. Here we report the potential cytotoxicity of our characterized QDs modified with various molecules. Surface modification of QDs changed their physicochemical properties. In addition, the cytotoxicity of QDs was dependent on their surface molecules. These results suggested that the properties of QDs are not related to those of QD-core materials but to molecules covering the surface of QDs.

Chem Biol Interact, **114**, 145-159, 1998
Oxidative damage induced by the fullerene C_{60} on photosensitization in rat liver microsomes
Jayashree P. Kamat, Thomas P. A. Devasagayam, K. I. Priyadarsini, Hari Mohan and Jai P. Mittal

We have examined the ability of a commonly used fullerene, C_{60}, to induce oxidative damage on photosensitization using rat liver microsomes as model membranes. When C_{60} was incorporated into rat liver microsomes in the form of its cyclodextrin complex and exposed to UV or visible light, it induced significant oxidative damage in terms of (1) lipid peroxidation as assayed by thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides and conjugated dienes, and (2) damage to proteins as assessed by protein carbonyls and loss of the membrane-bound enzymes. The oxidative damage induced was both time- and concentration-dependent. C_{60} plus

light-induced lipid peroxidation was significantly inhibited by the quenchers of singlet oxygen ($^1O_2$), β-carotene and sodium azide, and deuteration of the buffer-enhanced peroxidation. These observations indicate that C_{60} is an efficient inducer of peroxidation and is predominantly due to $^1O_2$. Biological antioxidants such as glutathione, ascorbic acid and α-tocopherol significantly differ in their ability to inhibit peroxidation induced by C_{60}. Our studies, hence, indicate that C_{60}, on photosensitization, can induce significant lipid peroxidation and other forms of oxidative damage in biological membranes and that this phenomenon can be greatly modulated by endogenous antioxidants and scavengers of reactive oxygen species.

Environ. Sci. Technol., **34** (7), 1132 -1142, 2000
Size and Composition Distribution of Fine Particulate Matter Emitted from Motor Vehicles
Michael J. Kleeman, James J. Schauer, and Glen R. Cass

A dilution source sampling system is augmented to measure the size-distributed chemical composition of fine particle emissions from motor vehicles. Measurements are made using an optical particle counter (OPC), a differential mobility analyzer (DMA)/condensation nucleus counter (CNC) combination, and a pair of microorifice uniform deposit impactors (MOUDIs). The sources tested with this system include catalyst-equipped gasoline-powered light-duty vehicles, noncatalyst gasoline-powered light-duty vehicles, and medium-duty diesel trucks. Chemical composition analysis demonstrates that particles emitted from the gasoline-powered vehicles tested are largely composed of organic compounds while particles emitted from diesel engines contain roughly equal amounts of organic compounds and elemental carbon. The particle mass distributions from all mobile sources tested have a single mode that peaks at approximately 0.1-0.2 μm particle diameter. Of the two diesel vehicles tested, the vehicle with the lowest fine particle emissions rate released the largest number of ultrafine particles, a finding similar to that of Bagley et al. (*Characterization of fuel and aftertreatment device effects on diesel emissions*; Technical Report 76; Health Effects Institute: Cambridge, MA, 1996). Particle size distribution measurements taken throughout the FTP urban driving cycle used to test all of the vehicles described in this paper reveal that particulate mass emission rates and particulate size distributions from the vehicles tested here are similar during the cold start and hot start segments of the driving cycle.

Chemosphere, **53**, 71-77, 2003
Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light
Klaus P. Kühn, Iris F. Chaberny, Karl Massholder, Manfred Stickler, Volker W. Benz, Hans-Günther Sonntag and Lothar Erdinger

Particularly in microbiological laboratories and areas in intensive medical use, regular and thorough disinfection of surfaces is required in order to reduce the numbers of bacteria and to prevent bacterial transmission. The conventional methods of disinfection with wiping are not effective in the longer term, cannot be standardized, are time- and staff-intensive and use aggressive chemicals. Disinfection with hard ultraviolet C (UVC) light is usually not satisfactory, as the depth of penetration is inadequate and there are occupational medicine risks. Photocatalytic oxidation on surfaces coated with titanium dioxide (TiO_2) might offer a possible

alternative. In the presence of water and oxygen, highly reactive OH-radicals are generated by TiO_2 and mild ultraviolet A (UVA). These radicals are able to destroy bacteria, and may therefore be effective in reducing bacterial contamination. Direct irradiation with UVC however can produce areas of shadow in which bacteria are not inactivated. Using targeted light guidance and a light-guiding sheet (out of a UVA-transmittant, Plexiglas®, for example), as in the method described in the present study, bacterial inactivation over the entire area is possible. The effectiveness of the method was demonstrated using bacteria relevant to hygiene such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecium*. For these bacteria, a reduction efficiency (RE) more than 6 $\log_{10}$ steps in 60 min was observed. Using *Candida albicans*, a RE of 2 $\log_{10}$ steps in 60 min was seen. Light and scanning electron microscopic examinations suggest that the germ destruction achieved takes place through direct damage to cell walls caused by OH-radicals.

Skin Pharmacol Appl Skin Physiol, **12**, 247-256, 1999
Penetration of Titanium Dioxide Microparticles in a Sunscreen Formulation into the Horny Layer and the Follicular Orifice
Juergen Lademann, Hans-Juergen Weigmann, Christiane Rickmeyer, Hans Barthelmes, Hans Schaefer, Gerhard Mueller, Wolfram Sterry

Coated titanium dioxide (TiO_2) microparticles are commonly used as UV filter substances in commercial sunscreen products. The penetration of these microparticles into the horny layer and the orifice of the hair follicle was investigated. The distribution of the microparticles in the horny layer was analyzed using the method of tape stripping in combination with spectroscopic measurements. Deeper layers of the stratum corneum were devoid of TiO_2 even after repetitive application of sunscreen preparation when analyzing interfollicular areas. Only in the areas of the pilosebaceous orifices could microparticles be identified. The penetration of TiO_2 was investigated in histological skin sections. A biopsy was taken from a skin area from which the horny layer had been removed by tape stripping. In isolated areas, a penetration of coated TiO_2 into the open part of the follicle was observed. The amount of TiO_2 found in a given follicle was less than 1% of the applied total amount of sunscreens. A penetration of microparticles into viable skin tissue could not be detected

Toxicological Sciences **77**, 126-134 (2004)
Pulmonary Toxicity of Single-Wall Carbon Nanotubes in Mice 7 and 90 Days After Intratracheal Instillation

Chiu-Wing Lam, John T. James, Richard McCluskey and Robert L. Hunter

Nanomaterials are part of an industrial revolution to develop lightweight but strong materials for a variety of purposes. Single-wall carbon nanotubes are an important member of this class of materials. They structurally resemble rolled-up graphite sheets, usually with one end capped; individually they are about 1 nm in diameter and several microns long, but they often pack tightly together to form rods or ropes of microscopic sizes. Carbon nanotubes possess unique electrical, mechanical, and thermal properties and have many potential applications in the electronics, computer, and aerospace industries. Unprocessed nanotubes are very light and could become

airborne and potentially reach the lungs. Because the toxicity of nanotubes in the lung is not known, their pulmonary toxicity was investigated. The three products studied were made by different methods and contained different types and amounts of residual catalytic metals. Mice were intratracheally instilled with 0, 0.1, or 0.5 mg of carbon nanotubes, a carbon black negative control, or a quartz positive control and euthanized 7 d or 90 d after the single treatment for histopathological study of the lungs. All nanotube products induced dose-dependent epithelioid granulomas and, in some cases, interstitial inflammation in the animals of the 7-d groups. These lesions persisted and were more pronounced in the 90-d groups; the lungs of some animals also revealed peribronchial inflammation and necrosis that had extended into the alveolar septa. The lungs of mice treated with carbon black were normal, whereas those treated with high-dose quartz revealed mild to moderate inflammation. These results show that, for the test conditions described here and on an equal-weight basis, if carbon nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz, which is considered a serious occupational health hazard in chronic inhalation exposures.

Environ. Sci. Technol., **38**, 5164 -5169, 2004
Laboratory Assessment of the Mobility of Nanomaterials in Porous Media
Hélène F. Lecoanet, Jean-Yves Bottero, and Mark R. Wiesner

The production of significant quantities of engineered nanomaterials will inevitably result in the introduction of these materials to the environment. Mobility in a well-defined porous medium was evaluated for eight particulate products of nanochemistry to assess their potential for migration in porous media such as groundwater aquifers and water treatment plant filters. Contrary to the assertion that nanomaterials present monolithic environmental risks, here we show that these nanomaterials exhibit widely differing transport behaviors. Fullerene-based nanomaterials that had been functionalized to facilitate dispersal in water displayed the highest mobilities, with a calculated potential to migrate approximately 10 m in unfractured sand aquifers. Colloidal aggregates of C_{60}, which have been the focus of recent toxicity studies, were among the least mobile of the nanomaterials evaluated.

Environ. Sci. Technol., **38** (16), 4377 -4382, 2004
Velocity Effects on Fullerene and Oxide Nanoparticle Deposition in Porous Media
Hélène F. Lecoanet and Mark R. Wiesner

Products of nanochemistry have been proposed in a number of applications ranging from soil stabilization and cosmetics to groundwater remediation. A fundamental understanding of the transport properties of these materials is essential to assess their efficacy and environmental impact in such applications. In this work, we consider the effect of flow on nanoparticle transport and deposition in porous media. The transport of three aqueous suspensions of fullerenes in a well-characterized porous medium is compared with that of two oxide nanomaterials at two flow rates. Despite significant differences in surface chemistry and size, the fullerenes exhibited an unexpected and similar breakthrough behavior at the higher flow rate. A striking characteristic of the fullerene breakthrough curves obtained at the higher Darcy velocity was an initial enhancement in nanoparticle deposition shortly after the passage of the first pore volume of

suspension, followed by an increase in passage. This velocity-sensitive "affinity transition" in the initial deposition of nanoparticles in the porous medium was observed for fullerene-based materials only at the higher velocity and was in no case observed for silica or titania nanoparticles. The removal of fullerene-based nanoparticles was observed to converge to a level that was independent of flow velocity, suggesting that under these conditions time scales for attachment or reorganization on the surface are greater than the time scale for transport to collector surfaces.

World J Gastroenterol, **9**, 1968-1971, 2003
Effects of hydroxyapatite nanoparticles on proliferation and apoptosis of human hepatoma BEL-7402 cells
Liu ZS, Tang SL, Ai ZL.

AIM: To study the effect of hydroxyapatite (HAP) nanoparticles on human hepatoma cell line BEL-7402 in vitro. METHODS: The human hepatoma cell line BEL-7402 was cultured and treated with HAP nanoparticles at various concentrations. Growth suppression was detected with MTT colorimetric assay, cell apoptotic alterations were evaluated by cytochemical staining (Hoechst 33258), transmission electron microscopy (TEM), and flow cytometry (FCM). RESULTS: HAP nanoparticles inhibited the growth of hepatoma cells in a dose-dependent manner, with IC50 values of 29.30 mg/L. Treated with 50-200 mg/L HAP nanoparticles for 48 h, BEL-7402 cells apoptosis with nuclear chromatin condensation and fragmentation as well as cell shrinkage and the formation of apoptotic bodies were observed under cytochemical staining and transmission electron microscopy. FCM analysis showed hypodiploid peaks on histogram, the apoptotic rates at the concentrations of 50, 75, 100, 150 and 200 mg/L of HAP nanoparticles were 20.35+/-2.23 %, 25.35+/-1.92 %, 29.34+/-4.61 %, 44.92+/-3.78 % and 53.64+/-3.49 %, respectively, which were all significantly higher than that of control group 2.23+/-0.14 %. There was a significant correlation between HAP nanoparticle concentration and apoptotic rate (r=0.994, P<0.01). CONCLUSION: HAP nanoparticles not only inhibit proliferation but also induce apoptosis of human hepatoma cell line BEL-7402 in vitro.

Applied and Environmental Microbiology, **65**, 4094-4098, 1999
Bactericidal Activity of Photocatalytic TiO_2 Reaction: toward an Understanding of Its Killing Mechanism
Pin-Ching Maness, Sharon Smolinski, Daniel M. Blake, Zheng Huang, Edward J. Wolfrum, and William A. Jacoby

When titanium dioxide (TiO_2) is irradiated with near-UV light, this semiconductor exhibits strong bactericidal activity. In this paper, we present the first evidence that the lipid peroxidation reaction is the underlying mechanism of death of *Escherichia coli* K-12 cells that are irradiated in the presence of the TiO_2 photocatalyst. Using production of malondialdehyde (MDA) as an index to assess cell membrane damage by lipid peroxidation, we observed that there was an exponential increase in the production of MDA, whose concentration reached 1.1 to 2.4 nmol $\cdot$ mg (dry weight) of cells $^{-1}$ after 30 min of illumination, and that the kinetics of this process paralleled cell death. Under these conditions, concomitant losses of 77 to 93% of the cell

respiratory activity were also detected, as measured by both oxygen uptake and reduction of 2,3,5-triphenyltetrazolium chloride from succinate as the electron donor. The occurrence of lipid peroxidation and the simultaneous losses of both membrane-dependent respiratory activity and cell viability depended strictly on the presence of both light and TiO_2. We concluded that TiO_2 photocatalysis promoted peroxidation of the polyunsaturated phospholipid component of the lipid membrane initially and induced major disorder in the *E. coli* cell membrane. Subsequently, essential functions that rely on intact cell membrane architecture, such as respiratory activity, were lost, and cell death was inevitable.

J Toxicol Environ Health A., **67(1)**, 87-107, 2004
Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material.
Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V.

Carbon nanotubes represent a relatively recently discovered allotrope of carbon that exhibits unique properties. While commercial interest in the material is leading to the development of mass production and handling facilities, little is known of the risk associated with exposure. In a two-part study, preliminary investigations have been carried out into the potential exposure routes and toxicity of single-walled carbon nanotube material (SWCNT)--a specific form of the allotrope. The material is characterized by bundles of fibrous carbon molecules that may be a few nanometers in diameter, but micrometers in length. The two production processes investigated use-transition metal catalysts, leading to the inclusion of nanometer-scale metallic particles within unrefined SWCNT material. A laboratory-based study was undertaken to evaluate the physical nature of the aerosol formed from SWCNT during mechanical agitation. This was complemented by a field study in which airborne and dermal exposure to SWCNT was investigated while handling unrefined material. Although laboratory studies indicated that with sufficient agitation, unrefined SWCNT material can release fine particles into the air, concentrations generated while handling material in the field were very low. Estimates of the airborne concentration of nanotube material generated during handling suggest that concentrations were lower than 53 microg/m(3) in all cases. Glove deposits of SWCNT during handling were estimated at between 0.2 mg and 6 mg per hand.

Chem. Soc., Faraday Trans., **24**, 4343 – 4346, 1997
Colloidal dispersions of fullerene C_{60} in water: some properties and regularities of coagulation by electrolytes
Nikolay O. Mchedlov-Petrossyan , Vladimir K. Klochkov and Grigoriy V. Andrievsky

The ultramicroheterogeneous dispersion of buckminsterfullerene, C_{60}, in water was found to be a typical hydrophobic colloidal system. The studied sol was polydisperse (*d* from several nanometers to *ca*. 200 nm), with negatively charged surfaces of the particles. The coagulation by inorganic electrolytes occurs in accordance with the Schulze-Hardy rule; the reciprocal coagulation points for counter-ion charges *z*=1, 2 and 3 are related as =1:20:1500. The results are discussed in terms of the Derjaguin-Landau-Verwey-Overbeek theory. In the case of organic electrolytes, including colloidal cationic surfactants, their coagulation points decrease with

increasing hydrophobicity and surface activity of the cations. An excess of cetyltrimethylammonium bromide stabilises the fullerene dispersion. The coagulating action of the tetrabutylammonium ion depends on the nature of the co-ion. Polyvinyl pyrrolidone and sodium dodecyl sulfate protect the C_{60} sol from coagulation.

Biochemical and Biophysical Research Communications, **277**, 711-717, 2000
C60 Carboxyfullerene Exerts a Protective Activity against Oxidative Stress-Induced Apoptosis in Human Peripheral Blood Mononuclear Cells
Daniela Monti, Laura Moretti, Stefano Salvioli, Elisabetta Straface, Walter Malorni, Roberto Pellicciari, Gennaro Schettini, Michela Bisaglia, Carlo Pincelli, Cristiana Fumelli, Massimiliano Bonafè and Claudio Franceschi

C60 carboxyfullerene is a novel buckminsterfullerene-derived compound that behaves as a free-radical scavenger. In the present report, we investigated whether this drug exerts a protective activity against oxidative stress-induced apoptosis. Human peripheral blood mononuclear cells (PBMCs) were challenged by 2-deoxy-Ì-ribose (dRib) or TNF-á plus cycloheximide as agents that trigger apoptosis by interfering with the redox status of cell and mitochondrial membrane potential. We found that carboxyfullerene was able to protect quiescent PBMCs from apoptosis caused either by 2-deoxy-Ì-ribose or TNF-á plus cycloheximide by a mechanism partially involving the mitochondrial membrane potential integrity, known to be associated with early stages of apoptosis. These results represent the first indication for a target activity of buckminsterfullerenes on cells of the immune system and their mitochondria.

Environ. Sci. Technol., **38** (5), 1600 -1604, 2004
Photocatalytic Degradation of Organic Compounds over Combustion-Synthesized Nano-TiO_2
K. Nagaveni, G. Sivalingam, M. S. Hegde, and Giridhar Madras

The photocatalytic degradation of various organics such as phenol, *p*-nitrophenol, and salicylic acid was carried out with combustion-synthesized nano-TiO_2 under UV and solar exposure. Under identical conditions of UV exposure, the initial degradation rate of phenol with combustion-synthesized TiO_2 is 2 times higher than the initial degradation rate of phenol with commercial Degussa P-25 TiO_2. The intermediates such as catechol (CC) and hydroquinone (HQ) were not detected during the degradation of phenol with combustion-synthesized TiO_2, while both the intermediates were detected when phenol was degraded over Degussa P-25. This indicates that the rates of secondary photolysis of CC and HQ occur extremely faster than the rates at which they are formed from phenol and further implies that the primary hydroxylation step is rate limiting for the combustion-synthesized TiO_2 aided photodegradation of phenol. The degradation rates of salicylic acid and *p*-nitrophenol were also investigated, and the rates were higher for combustion-synthesized titania compared to Degussa P-25 TiO_2. Superior activity of combustion-synthesized TiO_2 toward photodegradation of organic compounds can be attributed to crystallinity, higher surface area, more surface hydroxyl groups, and optical absorption at higher wavelength.

Mutation Research, **394**, 125-132, 1997
The photogenotoxicity of titanium dioxide particles
Yuzuki Nakagawa, Shinobu Wakuri, Kyoko Sakamoto and Noriho Tanaka

We employed a series of in vitro genotoxicity assays —a single cell gel (SCG) assay with mouse lymphoma L5178Y cells, a microbial mutation assay with *Salmonella typhimurium*, a mammalian cell mutation assay with L5178Y cells, and a chromosomal aberration assay with Chinese hamster CHL/IU cells— to evaluate the photogenotoxicity of titanium dioxide (TiO_2) particles. Without UV/visible light irradiation, TiO_2 particles exhibited no or weak genotoxicity. With irradiation, however, TiO_2 particles exhibited significant genotoxicity in the SCG and chromosomal aberration assays. Therefore, we concluded that TiO_2 particles are photogenotoxic.

Environmental Health Perspectives, **112**, 1058-1062
Manufactured Nanomaterials (Fullerenes, C60) Induce Oxidative Stress in the Brain of Juvenile Largemouth Bass
Eva Oberdörster

Although nanotechnology has vast potential in uses such as fuel cells, microreactors, drug delivery devices, and personal care products, it is prudent to determine possible toxicity of nanotechnology-derived products before widespread use. It is likely that nanomaterials can affect wildlife if they are accidentally released into the environment. The fullerenes are one type of manufactured nanoparticle that is being produced by tons each year, and initially uncoated fullerenes can be modified with biocompatible coatings. Fullerenes are lipophilic and localize into lipid-rich regions such as cell membranes *in vitro*, and they are redox active. Other nano-sized particles and soluble metals have been shown to selectively translocate into the brain via the olfactory bulb in mammals and fish. Fullerenes (C_{60}) can form aqueous suspended colloids (nC_{60}); the question arises of whether a redox-active, lipophilic molecule could cause oxidative damage in an aquatic species. The goal of this study was to investigate oxyradical-induced lipid and protein damage, as well as impacts on total glutathione (GSH) levels, in largemouth bass exposed to nC_{60}. Significant lipid peroxidation was found in brains of largemouth bass after 48 hr of exposure to 0.5 ppm uncoated nC_{60}. GSH was also marginally depleted in gills of fish, and nC_{60} increased water clarity, possibly due to bactericidal activity. This is the first study showing that uncoated fullerenes can cause oxidative damage and depletion of GSH *in vivo* in an aquatic species. Further research needs to be done to evaluate the potential toxicity of manufactured nanomaterials, especially with respect to translocation into the brain.

International Archives of Occupational and Environmental Health, **74**, 1-8, 2001
Pulmonary effects of inhaled ultrafine particles
Günter Oberdörster

Abstract Introduction and Objectives: Recent epidemiological studies have shown an association between increased particulate urban air pollution and adverse health effects on susceptible parts of the population, in particular the elderly with pre-existing respiratory and cardiovascular diseases. Urban particles consist of three modes: ultrafine particles, accumulation mode particles

(which together form the fine particle mode) and coarse mode particles. Ultrafine particles (those of <0.1 wm diameter) contribute very little to the overall mass, but are very high in number, which in episodic events can reach several hundred thousand/cm^3 in the urban air. The hypothesis that ultrafine particles are causally involved in adverse responses seen in sensitive humans is based on several studies summarized in this brief review. Methods and Results: Studies on rodents demonstrate that ultrafine particles administered to the lung cause a greater inflammatory response than do larger particles, per given mass. Surface properties (surface chemistry) appear to play an important role in ultrafine particle toxicity. Contributing to the effects of ultrafine particles is their very high size-specific deposition when inhaled as singlet ultrafine particles rather than as aggregated particles. It appears also that ultrafine particles, after deposition in the lung, largely escape alveolar macrophage surveillance and gain access to the pulmonary interstitium. Inhaled low doses of carbonaceous ultrafine particles can cause mild pulmonary inflammation in rodents after exposure for 6 h. Old age and a compromised/sensitized respiratory tract in rodents can increase their susceptibility to the inflammatory effects of ultrafine particles significantly, and it appears that the aged organism is at a higher risk of oxidative stress induced lung injury from these particles, compared with the young organism. Results also show that ultrafine particle effects can be significantly enhanced by a gaseous co-pollutant such as ozone. Conclusions: The studies performed so far support the ultrafine particle hypothesis. Additional studies are necessary to evaluate mechanistic pathways of responses.

Inhal. Toxicol., **16**, 437-445, 2004

Translocation of Inhaled Ultrafine Particles to the Brain

G. Oberdörster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox

Ultrafine particles (UFP, particles < 100 nm) are ubiquitous in ambient urban and indoor air from multiple sources and may contribute to adverse respiratory and cardiovascular effects of particulate matter (PM).Depending on their particle size, inhaled UFP are efficiently deposited in nasal, tracheobronchial, and alveolar regions due to diffusion. Our previous rat studies have shown that UFP can translocate to interstitial sites in the respiratory tract as well as to extrapulmonary organs such as liver within 4 to 24 h postexposure. There were also indications that the olfactory bulb of the brain was targeted. Our objective in this follow-up study, therefore, was to determine whether translocation of inhaled ultrafine solid particles to regions of the brain takes place, hypothesizing that UFP depositing on the olfactory mucosa of the nasal region will translocate along the olfactory nerve into the olfactory bulb. This should result in significant increases in that region on the days following the exposure as opposed to other areas of the central nervous system (CNS). We generated ultrafine elemental13C particles (CMD=36 nm; GSD=1.66) from [13C] graphite rods by electric spark discharge in an argon atmosphere at a concentration of 160µg/m3. Rats were exposed for 6 h, and lungs, cerebrum, cerebellum and olfactory bulbs were removed 1, 3, 5, and 7 days after exposure.13C concentrations were determined by isotope ratio mass spectroscopy and compared to background13C levels of sham-exposed controls (day 0). The background corrected pulmonary13C added as ultrafine13C particles on day 1 postexposure was 1.34µg/lung. Lung13C concentration decreased from 1.39µg/g (day 1) to 0.59µg/g by 7 days postexposure. There was a significant and persistent

increase in added13C in the olfactory bulb of 0.35μg/g on day 1, which increased to 0.43μg/g by day 7. Day 113C concentrations of cerebrum and cerebellum were also significantly increased but the increase was inconsistent, significant only on one additional day of the postexposure period, possibly reflecting translocation across the blood–brain barrier in certain brain regions. The increases in olfactory bulbs are consistent with earlier studies in nonhuman primates and rodents that demonstrated that intranasally instilled solid UFP translocate along axons of the olfactory nerve into the CNS. We conclude from our study that the CNS can be targeted by airborne solid ultrafine particles and that the most likely mechanism is from deposits on the olfactory mucosa of the nasopharyngeal region of the respiratory tract and subsequent translocation via the olfactory nerve. Depending on particle size, > 50%of inhaled UFP can be depositing in the nasopharyngeal region during nasal breathing. Preliminary estimates from the present results show that•20%of the UFP deposited on the olfactory mucosa of the rat can be translocated to the olfactory bulb. Such neuronal translocation constitutes an additional not generally recognized clearance pathway for inhaled solid UFP, whose significance for humans, however, still needs to be established. It could provide a portal of entry into the CNS for solid UFP, circumventing the tight blood–brain barrier. Whether this translocation of inhaled UFP can cause CNS effects needs to be determined in future studies.

J Mater Sci Mater Med, **15**, 321-325, 2004
Effects of nano-scaled particles on endothelial cell function in vitro: studies on viability, proliferation and inflammation.
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Recent studies give support for a connection between the presence of inorganic particles (of microm and nm size) in different organs and tissues and the development of inflammatory foci, called granulomas. As the potential source of particles (e.g. porcelain dental bridges) and the location of particle detection were topographically far apart, a distribution via the blood stream appears highly probable. Thus, endothelial cells, which line the inner surface of blood vessels, would come into direct contact with these particles, making particle-endothelial interactions potentially pathogenically relevant. The objective of this study was to evaluate the effects that five different nano-scaled particles (PVC, TiO2, SiO2, Co, Ni) have on endothelial cell function and viability. Therefore, human endothelial cells were exposed to different amounts of the above-mentioned particles. Although most particle types are shown to be internalised (except Ni-particles), only Co-particles possessed cytotoxic effects. Furthermore, an impairment of the proliferative activity and a pro-inflammatory stimulation of endothelial cells were induced by exposure to Co- and, to a lesser extent, by SiO2-particles. If a pro-inflammatory stimulation of endothelial cells occurs in vivo, a chronic inflammation could be a possible consequence.

Environmental Health Perspectives, **110**, 797-800, 2002
Evidence That Ultrafine Titanium Dioxide Induces Micronuclei and Apoptosis in Syrian Hamster Embryo Fibroblasts
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Inhaled ultrafine titanium dioxide (UF-TiO_2) particles cause pronounced pulmonary inflammation, in contrast to fine TiO_2. Previous studies provide evidence for the production of reactive oxygen species by alveolar macrophages, after overloading with UF-TiO_2 particles and cytotoxicity of UF-TiO_2 in rat lung alveolar macrophages. UF-TiO_2 also causes pulmonary fibrosis and lung tumors in rats. UF-TiO_2 particles are photogenotoxic, but in general, information on the genotoxicity of UF-TiO_2 is still limited. We studied the potential of UF-TiO_2 (particle size $\leq$20 nm) and fine TiO_2 (particle size > 200 nm) to induce chromosomal changes, which can be monitored by the formation of micronuclei (MN) in Syrian hamster embryo (SHE) cells. We also analyzed UF-TiO_2-treated cells for apoptosis induction. The MN assay revealed a significant increase in MN induction ($p \leq 0.05$) in SHE cells after treatment with UF-TiO_2 (1.0 $\mu g/cm^2$) for 12 hr (mean, 24.5 MN/1,000 cells), 24 hr (mean, 31.13 MN/1,000 cells), 48 hr (mean, 30.8 MN/1,000 cells), 66 hr (mean, 31.2 MN/1,000 cells), and 72 hr (mean, 31.3 MN/1,000 cells). Bisbenzimide staining of the fixed cells revealed typical apoptotic structures (apoptotic bodies), and the apoptosis-specific "DNA ladder pattern" resulting from internucleosomal cleavage was identified by gel electrophoresis. Furthermore, transmission electron microscopy of the exposed cells revealed the typical chromatin compaction of apoptosis.
Key words: apoptosis, genotoxicity, kinetochores, micronuclei, ultrafine titanium dioxide.

Antimicrobial Agents and Chemotherapy, **40**, 2262-2265, 1996
Pharmacokinetics of a water-soluble fullerene in rats
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Fullerenes are the recently discovered third allotropic form of carbon. The biological activities of these compounds are being studied for various purposes. The bis(monosuccinimide) derivative of p p'-bis(2- amino-ethyl)-diphenyl-C60 (MSAD-C60) is a water-soluble fullerene derivative. MSAD-C60 has been shown to have antiviral activity against human immunodeficiency virus types 1 and 2 in vitro and to have virucidal and anti-human immunodeficiency virus protease activities. Moreover, MSAD-C60 has been shown to be well tolerated in mice after intraperitoneal administration. The purpose of the present study was to develop a high-performance liquid chromatographic analytical methodology for MSAD-C60 and to characterize the preclinical pharmacokinetics of the compound in rats. Following intravenous administration of the fullerene derivative at a dose of 15 mg/kg of body weight, the concentrations of MSAD-C60 in plasma declined either bi- or triexponentially. The mean terminal-phase half-life of MSAD-C60 was 6.8 +/- 1.1 h (mean +/- standard deviation). Binding studies indicated that the compound is greater than 99% bound to plasma proteins. The average total clearance of the compound was 0.19 +/- 0.06 liter/h/kg. Urine samples obtained 24 h after intravenous administration did not contain detectable levels of the compound, indicating the absence of a significant renal clearance mechanism. The steady-state volume of distribution of MSAD-C60 averaged 2.1 +/- 0.8 liters/kg, indicating that the compound distributes into tissues. At a dose of 15 mg/kg, MSAD-C60 appeared to be well tolerated. However, a dose of 25 mg/kg resulted in shortness of breath and violent movement of the rats, followed by death within 5 min of dosing. Further controlled toxicity studies are needed to fully evaluate the toxicity of the compound.

Journal of Photochemistry and Photobiology B: Biology, **67**, 157-162, 2002
Cytotoxicity and photocytotoxicity of a dendritic C_{60} mono-adduct and a malonic acid C_{60} tris-adduct on Jurkat cells
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The cytotoxic and photocytotoxic effects of two water-soluble fullerene derivatives, a dendritic C_{60} mono-adduct and the malonic acid C_{60} tris-adduct were tested on Jurkat cells. Cell growth and vitality were determined by a cell counting and staining technique. After 2 weeks cultivation in the presence of the fullerene derivatives, it was found that only the dendritic mono-adduct inhibits cell growth (within 2 weeks the cell number decreased to 19%), whereas the tris-malonic acid adduct has little effect. The growth inhibition is reversible; cultivating the same cells further in the absence of fullerene, the cell number increased to 106.4%. Other experiments showed that these fullerene derivatives become toxic when irradiated with UVA or UVB light. The cell death is mainly caused by membrane damage and it is UV dose-dependent. Tris-malonic acid fullerene was found to be more phototoxic than the dendritic derivative. This result is in contrast to the singlet oxygen quantum yields determined for the two compounds. We propose that the two fullerene derivatives may interact with the cell membrane in different ways thus causing the observed effects. Further experiments will be done to determine the location and concentration of the two compounds in and on the cells.

Applied Catalysis B: Environmental, **44**, 263-284, 2003
Photocatalytical inactivation of *E. coli*: effect of (continuous–intermittent) light intensity and of (suspended–fixed) TiO_2 concentration
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A detrimental effect on the survival of bacteria *Escherichia coli* K12 was observed after photocatalytic exposure. The reactions have been carried out in a batch photoreactor using mainly titanium dioxide (TiO_2) P-25 Degussa as a catalyst. Illumination was produced by a Hanau Suntest lamp. Some parameters, such as light intensity, extend of continuous irradiation, catalyst concentration and temperature have a positive effect on disinfection. Intermittent illumination results in an increase in the time required for *E. coli* inactivation. No bacterial growth was observed after illumination of a contaminated TiO_2 suspension. In contrast, without catalyst, illuminated bacteria recovered its initial concentration after 3 h in the dark. Bacterial inactivation in the absence of catalyst was more affected than that with catalyst when increasing light intensity from 400 to 1000 W/m^2. TiO_2 concentrations higher than 1 g/l do not significantly increase the initial inactivation rate for both intensities. However, at 1000 W/m^2 a modification of TiO_2 concentration ranging between 0.25 and 1.5 g/l did not affect the total inactivation time, as with 400 W/m^2.
Water turbidity negatively affects the photocatalytic inactivation of bacteria. TiO_2 immobilized on Nafion® membranes inactivates *E. coli* with efficiencies close to those observed for bacterial suspension containing the same concentration of suspended TiO_2. For fixed TiO_2 on glass, the dose ($W\ min/m^2$) necessary for the total inactivation decreases by increasing the fixed TiO_2 amount. Fixed TiO_2 P-25 was more active to photocatalytic inactivation when compared with

immobilized rutile and anatase. However fixed rutile enhances *E. coli* inactivation as anatase. The effect of temperature and turbidity were made using wastewater sample.

Nano Letters, **4**, 1881 -1887, 2004
The Differential Cytotoxicity of Water-Soluble Fullerenes
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We show that the cytotoxicity of water-soluble fullerene species is a sensitive function of surface derivatization; in two different human cell lines, the lethal dose of fullerene changed over 7 orders of magnitude with relatively minor alterations in fullerene structure. In particular, an aggregated form of C_{60}, the least derivatized of the four materials, was substantially more toxic than highly soluble derivatives such as C_3, $Na^{+}_{2-3}[C_{60}O_{7-9}(OH)_{12-15}]^{(2-3)-}$, and $C_{60}(OH)_{24}$. Oxidative damage to the cell membranes was observed in all cases where fullerene exposure led to cell death. We show that under ambient conditions in water fullerenes can generate superoxide anions and postulate that these oxygen radicals are responsible for membrane damage and subsequent cell death. This work demonstrates both a strategy for enhancing the toxicity of fullerenes for certain applications such as cancer therapeutics or bactericides, as well as a remediation for the possible unwarranted biological effects of pristine fullerenes.

Advanced Drug Delivery Reviews, **54**, S157-S163, 2002
Distribution of sunscreens on skin
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The effectiveness of sunscreens was originally achieved by incorporation of soluble organic UV absorbers such as cinnamates and others into cosmetic formulations. Determinations of the sun protection factor (SPF) of emulsions containing different organic UV absorbers clearly indicate that the efficacy depends on the absorption characteristics of each single UV filter substance. Nowadays, micronised pigments such as titanium dioxide or zinc oxide have also been found to be protective against harmful UV rays. Our investigations using optical and electron microscopy proved that neither surface characteristics, particle size nor shape of the micronised pigments result in any dermal absorption of this substance. Micronised titanium dioxide is solely deposited on the outermost surface of the stratum corneum and cannot be detected in deeper stratum corneum layers, the human epidermis and dermis.

Journal of Toxicology and Environmental Health Part A, **66**, 1909 – 1926, 2003
Exposure to Carbon Nanotube Material: Assessment of Nanotube Cytotoxicity using Human Keratinocyte Cells

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Carbon nanotubes are new members of carbon allotropes similar to fullerenes and graphite. Because of their unique electrical, mechanical, and thermal properties, carbon nanotubes are important for novel applications in the electronics, aerospace, and computer industries. Exposure to graphite and carbon materials has been associated with increased incidence of skin diseases, such as carbon fiber dermatitis, hyperkeratosis, and naevi. We investigated adverse effects of single-wall carbon nanotubes (SWCNT) using a cell culture of immortalized human epidermal keratinocytes (HaCaT). After 18 h of exposure of HaCaT to SWCNT, oxidative stress and cellular toxicity were indicated by formation of free radicals, accumulation of peroxidative products, antioxidant depletion, and loss of cell viability. Exposure to SWCNT also resulted in ultrastructural and morphological changes in cultured skin cells. These data indicate that dermal exposure to unrefined SWCNT may lead to dermal toxicity due to accelerated oxidative stress in the skin of exposed workers.

Nature Medicine, **10**, 993 – 998, 2004
Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy
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Metastasis is an impediment to the development of effective cancer therapies. Our understanding of metastasis is limited by our inability to follow this process in vivo. Fluorescence microscopy offers the potential to follow cells at high resolution in living animals. Semiconductor nanocrystals, quantum dots (QDs), offer considerable advantages over organic fluorophores for this purpose. We used QDs and emission spectrum scanning multiphoton microscopy to develop a means to study extravasation in vivo. Although QD labeling shows no deleterious effects on cultured cells, concern over their potential toxicity in vivo has caused resistance toward their application to such studies. To test if effects of QD labeling emerge in vivo, tumor cells labeled with QDs were intravenously injected into mice and followed as they extravasated into lung tissue. The behavior of QD-labeled tumor cells in vivo was indistinguishable from that of unlabeled cells. QDs and spectral imaging allowed the simultaneous identification of five different populations of cells using multiphoton laser excitation. Besides establishing the safety of QDs for in vivo studies, our approach permits the study of multicellular interactions in vivo.

J. Med. Chem., **42** (22), 4614 -4620, 1999
C_{60} and Water-Soluble Fullerene Derivatives as Antioxidants Against Radical-Initiated Lipid Peroxidation
I Chen Wang, Lin Ai Tai, Don Dar Lee, P. P. Kanakamma, Clifton K.-F. Shen, Tien-Yau Luh, Chien Hong Cheng, and Kuo Chu Hwang

C_{60}, vitamin E, and three C_{60} derivatives (polar **1** and water-soluble C_3/D_3C_{60}s) were examined for their antioxidant effects on prevention of lipid peroxidation induced by superoxide and hydroxyl radicals. The protection effect on lipid peroxidation was found to be in the sequence: $C_{60} \geq$ vitamin E > **1** > none, for liposoluble antioxidants, and $C_3C_{60} \gg D_3C_{60}$ > none, for water-soluble ones. Fluorescence quenching of $PyCH_2COOH$ (Py = pyrene) by both C_3- and D_3C_{60}s shows that the Stern-Volmer constant, K_{SV}, is about the same for both quenchers in aqueous

solution. Upon addition of liposomes, the fluorescence quenching becomes more efficient: 5-fold higher in K_{SV} for C_3C_{60} than for D_3C_{60}. When $Py(CH_2)_nCOOH$ (n = 1, 3, 5, 9, or 15) was incorporated in lipid membranes, the K_{SV}s all were small and nearly equal for D_3C_{60} but were quite large and different for C_3C_{60} with the sequence: n = 1 < 3 < 5 < 9 < 15. The better protection effect of C_3C_{60} on lipid peroxidation than that of D_3C_{60} is attributed to its stronger interaction with membranes. Overall, the antioxidation abilities of the compounds examined were rationalized in terms of the number of reactive sites, the location of antioxidant in lipid membranes, and the strength of interactions between antioxidants and membranes.

Toxicological Sciences, **77**, 117-125, 2004

Comparative Pulmonary Toxicity Assessment of Single-wall Carbon Nanotubes in Rats

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The aim of this study was to evaluate the acute lung toxicity of intratracheally instilled single-wall carbon nanotubes (SWCNT) in rats. The lungs of rats were instilled either with 1 or 5 mg/kg of the following control or particle types: (1) SWCNT, (2) quartz particles (positive control), (3) carbonyl iron particles (negative control), (4) phosphate-buffered saline (PBS) + 1% Tween 80, or (5) graphite particles (lung tissue studies only). Following exposures, the lungs of PBS and particle-exposed rats were assessed using bronchoalveolar lavage (BAL) fluid biomarkers and cell proliferation methods, and by histopathological evaluation of lung tissue at 24 h, 1 week, 1 month, and 3 months postinstillation. Exposures to high-dose (5 mg/kg) SWCNT produced mortality in ~15% of the SWCNT-instilled rats within 24 h postinstillation. This mortality resulted from mechanical blockage of the upper airways by the instillate and was not due to inherent pulmonary toxicity of the instilled SWCNT particulate. Exposures to quartz particles produced significant increases versus controls in pulmonary inflammation, cytotoxicity, and lung cell parenchymal cell proliferation indices. Exposures to SWCNT produced transient inflammatory and cell injury effects. Results from the lung histopathology component of the study indicated that pulmonary exposures to quartz particles (5 mg/kg) produced dose-dependent inflammatory responses, concomitant with foamy alveolar macrophage accumulation and lung tissue thickening at the sites of normal particle deposition. Pulmonary exposures to carbonyl iron or graphite particles produced no significant adverse effects. Pulmonary exposures to SWCNT in rats produced a non-dose-dependent series of multifocal granulomas, which were evidence of a foreign tissue body reaction and were nonuniform in distribution and not progressive beyond 1 month postexposure (pe). The observation of SWCNT-induced multifocal granulomas is inconsistent with the following: (1) lack of lung toxicity by assessing lavage parameters, (2) lack of lung toxicity by measuring cell proliferation parameters, (3) an apparent lack of a dose response relationship, (4) nonuniform distribution of lesions, (5) the paradigm of dust-related lung toxicity effects, (6) possible regression of effects over time. In addition, the results of two recent exposure assessment studies indicate very low aerosol SWCNT exposures at the workplace. Thus, the physiological relevance of these findings should ultimately be determined by conducting an inhalation toxicity study.

Toxicology in Vitro, **16**, 41-46, 2002
Photo-induced cytotoxicity of malonic acid [C_{60}]fullerene derivatives and its mechanism
X. L. Yang, C. H. Fan and H. S. Zhu

The biological activities of fullerenes have attracted extensive attention in recent years. The aim of this paper is to study the relation of the photo-induced cytotoxicity of fullerene derivatives to their chemical structures as well as the possible cellular mechanism involved in the photocytotoxicity. Three C_{60} derivatives with two to four malonic acid groups (DMA C_{60}, TMA C_{60} and QMA C_{60}) were prepared and the cytotoxicity of these compounds against HeLa cells was determined by MTT. Cell cycle was measured by flow cytometry. The results showed that the cytotoxicity of the malonic acid C_{60} derivatives was irradiation- and dose-dependent. The sequence of their photo-induced growth inhibition was DMA C_{60}>TMA C_{60}>QMA C_{60}. Hydroxyl radical quencher mannitol (10 m**M**) was not able to prevent cells from the damage induced by irradiated DMA C_{60}. DMA C_{60}, together with irradiation, was found to have an ability of inducing a decrease in the number of G_1 cells from 63 to 42% and a rise in that of G_2+M cells from 6 to 26%. These data indicated that the number of malonic acid molecules added to C_{60} played an important role in the phototoxicity, and the blockage of cell cycle might be a mechanism of this activity

The Journal of Chemical Physics, **121**, 12600-12605, 2004
Single electron emission from the closed-tips of single-walled carbon nanotubes
Gang Zhou, Wenhui Duan, and Binglin Gu

The single electron emission behaviors and characteristics from the well-defined quantized energy levels, corresponding to localized electronic states at the dome-structure tips, in single-walled carbon nanotubes (SWNTs) are investigated and illuminated by use of the energy level emission model in combination with the first-principles calculations on the electronic structures. Under the external electric field, the confined electrons are emitted simultaneously from each quantized energy level by virtue of the resonant tunneling effects. With increasing applied voltage, the emission current increases monotonically and exponentially up to the first peak value, and then steps into the increasing and decreasing "sawtoothlike" variations in sequence. The negative differential resistance or conductivity and the maximum current for SWNTs are simulated. The influences of localized electronic states and curvatures of the different closed tips on the single electron emission behaviors of SWNTs are evaluated and discussed. Also a few issues and applications relevant to electron emission of carbon nanotubes are addressed.

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