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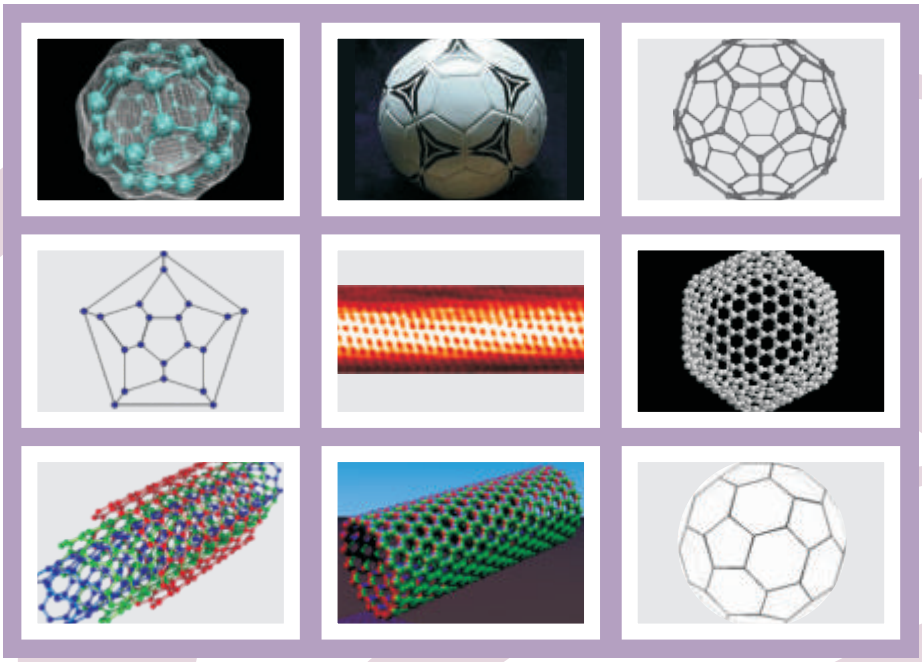

Indian Institute of Toxicology Research
Lucknow, India

IN THIS ESSUE

EDITORIAL

Nanotoxicology has brought in a revolution in the 21st century. Nanomaterials possess unique properties as they have very small size but large surface area when compared with their bulk counterparts. While these materials are finding applications in large number of products it is important to assess risk to environment and human beings due to their excessive use. Nanoparticles can be divided into three categories 1.) combustion-derived nanoparticles (like diesel soot), 2.) manufactured nanoparticles like carbon nanotubes 3.) naturally occurring nanoparticles from volcanic eruptions etc. The most studied nanoparticles are titanium dioxide, alumina, zinc oxide, carbon black, and carbon nanotubes, and "nano-C60". Since, nanoparticles have much target organ surface area to unit mass ratio it may lead to inflammatory effects in animal tissues in contact like lung. Sometimes nanoparticles translocate from their site of contact to distant sites for example brain. Thus, a new approach has been adopted to study toxic effects of nanoparticles since, instead of being confined to the target organ, their effects on brain, blood, liver, skin and gut also needs to be assessed. One can say that nanotoxicology has revolutionised particle toxicology and rejuvenated it. The extremely small size of nanomaterials also means that they are more capable of gaining entry into the human body as compared to particles of larger size. Behaviour of nanoparticles inside the body is still a major question that needs to be resolved as it is a function of their size, shape and surface reactivity with the surrounding tissues. One can assume that a large number of particles could overload the body's phagocytes (cells responsible to fight foreign matter), and thus trigger the stress reaction that leads to inflammation and weaken the body's defence against any other pathogen. There is also a question regarding, what happens if non-degradable or slowly degradable nanoparticles accumulate in body organs, and interact or interfere with biological processes inside the body. The large surface area of nanoparticles enables their quicker adsorption on exposure to tissues/fluids and at the same time has the potential to affect the regulatory mechanisms of enzymes and other proteins. They can cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot. Since mechanism to regulate nanotech-based products are still evolving, there are many products that could possibly pose health risk to humans due to long-term use. As the use of nanomaterials increases worldwide, concerns for worker and user safety along with adverse effects of environmental load are increasing. Now it is clear that size is a key factor in determining the potential toxicity of a particle. However there are other important factors viz: chemical composition, shape, surface structure, surface charge, aggregation and solubility and the presence or absence of functional groups. Thus, it is difficult to generalise about health risks associated with exposure to nanomaterials – and each new nanomaterial needs to be assessed individually with all the properties taken into consideration.

Editors
Dr. Poonam Kakkar
Dr. Anvita Shaw

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Distributed Information Centre on Toxic Chemicals
Environmental Information System (ENVIS)
at Indian Institute of Toxicology Research
Lucknow, India

ODDS AND ENDS

Nanotoxicology: an interdisciplinary challenge.

The increasing consumption of products containing nanomaterials that can be currently observed and forecasts of new developments and applications fan the fear of individuals and organizations regarding new risks to health. Considering experiences gained from previous technology developments, such fears are not completely unfounded. But are they really justified? And is it justified, moreover, to speak of "nanotoxicology" as a new discipline? Authors in this review seek to cast light on the phenomena that may occur as nanoobjects interact with cells, tissues, and organisms. Furthermore, authors demonstrate that the many data made available on the biological effects of nanomaterials do not always come from studies that can be considered reliable. They have pointed out the aspect of reliability with specific examples from the literature and do not address specific (nano)materials. In particular, inadequate methods have been described together with recommendations how to avoid this in the future, thereby contributing to a sustainable improvement of the available data.

Angew Chem Int Ed Engl. 2011 Feb 7;50(6):1260-78. doi: 10.1002/anie.201001037.

Alterations in welding process voltage affect the generation of ultrafine particles, fume composition, and pulmonary toxicity.

The goal of this study was to determine if increasing welding voltage changes the physico-chemical properties of the fume and influences lung responses. Rats inhaled 40 mg/m(3) (3 h/day × 3 days) of stainless steel (SS) welding fume generated at a standard voltage setting of 25 V (regular SS) or at a higher voltage (high voltage SS) of 30 V. Particle morphology, size and composition were characterized. Bronchoalveolar lavage was performed at different times after exposures to assess lung injury. Fumes collected from either of the welding conditions appeared as chain-like agglomerates of nanometer-sized primary particles. High voltage SS welding produced a greater number of ultrafine-sized particles. Fume generated by high voltage SS welding was higher in manganese. Pulmonary toxicity was more substantial and persisted longer after exposure to the regular SS fume. In summary, a modest raise in welding voltage affected fume size and elemental composition and altered the temporal lung toxicity profile.

Nanotoxicology. 2011 Feb 1. (doi:10.3109/17435390.2010.550695)

State of the art of nanocrystals - Special features, production, nanotoxicology aspects and intracellular delivery.

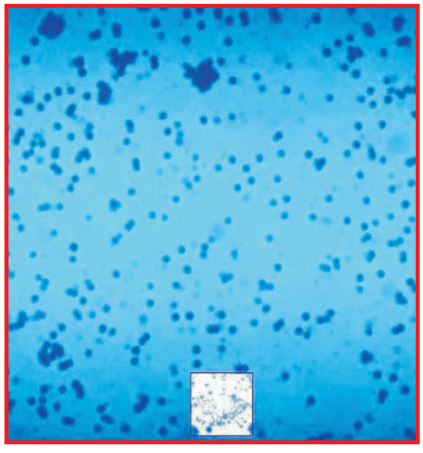
Drug nanocrystals are the latest, broadly introduced nanoparticulate carrier to the pharmaceutical market from the year 2000 onwards. The special features of nanocrystals for the delivery of poorly soluble drugs are briefly reviewed (saturation solubility, dissolution velocity, adhesiveness). The industrially relevant bottom up (precipitation) and top down production technologies (pearl milling, high pressure homogenization, combination technologies) are presented. As nanotoxicological aspects, the effect of size, degradability versus biopersistency and intracellular uptake are discussed, classifying the nanocrystals in the low/non-risk group. Intracellular uptake plays a minor or no role for dermal and oral nanocrystals, but it plays a key role for intravenously injected nanocrystals (e.g. nevirapine, paclitaxel, itraconazole). Uptake by the macrophages of the mononuclear phagocytic system (MPS, liver spleen) can modify/optimize blood profiles via prolonged release from the MPS (itraconazole), but also target toxicity by too high organ concentrations and thus cause nanotoxicity. The balance in the competitive intracellular uptake by MPS and the target cells (e.g. blood-brain barrier) decides about therapeutic efficiency. The concept of "differential protein adsorption" to modulate this balance is shown for its applicability to nanocrystals for intracellular delivery to the cells of the blood-brain barrier (atovaquone).

European Journal of Pharmaceutics and Biopharmaceutics, Volume 78, Issue 1, May 2011, Pages 1-9

Measurement of airborne nanoparticle surface area using a filter-based gas adsorption method for inhalation toxicology experiments.

Measurement of the surface area of airborne nanoparticles as administered to an experimental subject is critical for characterizing exposures during inhalation experiments. A filter-based surface

area measurement methodology is described herein that allows for such determinations. Krypton gas adsorption was used to determine total particle surface area. Track-etched polycarbonate 0.4 µm pore filters were chosen as the collection substrate for metal oxide particles due to their highly reproducible surface areas and low background weights. The subject nanomaterials included two different batches of ultrafine TiO(2), TiO(2) nanorods, and SiO(2). The instrument detection limit for surface area was 200 cm(2) (0.02 m(2)). Ninety percent confidence interval estimates of method accuracy were 17.7-23.5% with a point estimate of 20.8%. The filter-based surface area measurement strategy is demonstrated to be a viable sampling and analysis methodology that provides much needed physical characterization information of particles as administered in an animal inhalation chamber.



SiO_2 nano particle

Nanotoxicology. 2011 Jan 24. (doi:10.3109/17435390.2010.546951)

Statistical analysis of nanoparticle dosing in a dynamic cellular system.

The delivery of nanoparticles into cells is important in therapeutic applications and in nanotoxicology. Nanoparticles are generally targeted to receptors on the surfaces of cells and internalized into endosomes by endocytosis, but the kinetics of the process and the way in which cell division redistributes the particles remain unclear. Here we show that the chance of success or failure of nanoparticle uptake and inheritance is random. Statistical analysis of nanoparticle-loaded endosomes indicates that particle capture is described by an over-dispersed Poisson probability distribution that is consistent with heterogeneous adsorption and internalization. Partitioning of nanoparticles in cell division is random and asymmetric, following a binomial distribution with mean probability of 0.52-0.72. These results show that cellular targeting of nanoparticles is inherently imprecise due to the randomness of nature at the molecular scale, and the statistical framework offers a way to predict nanoparticle dosage for therapy and for the study of nanotoxins.

Nature Nanotechnology, 6, p 170–174, 2011 .

Darkfield-Confocal Microscopy detection of nanoscale particle internalization by human lung cells.

Concerns over the health effects of nanomaterials in the environment have created a need for microscopy methods capable of examining the biological interactions of nanoparticles (NP). Unfortunately, NP are beyond the diffraction limit of resolution for conventional light microscopy (~200 nm). Fluorescence and electron microscopy techniques commonly used to examine NP interactions with biological substrates have drawbacks that limit their usefulness in toxicological investigation of NP. EM is labor intensive and slow, while fluorescence carries the risk of photobleaching the sample and has size resolution limits. In addition, many relevant particles lack intrinsic fluorescence and therefore cannot be detected in this manner. To surmount these limitations, we evaluated the potential of a novel combination of darkfield and confocal laser scanning microscopy (DF-CLSM) for the efficient 3D detection of NP in human lung cells. The DF-CLSM approach utilizes the contrast enhancements of darkfield microscopy to detect objects below the diffraction limit of 200 nm based on their light scattering properties and interfaces it with the power of confocal microscopy to resolve objects in the z-plane. Validation of the DF-CLSM method using fluorescent polystyrene beads demonstrated spatial colocalization of particle fluorescence (Confocal) and scattered transmitted light (Darkfield) along the X, Y, and Z axes. DF-CLSM imaging was able to detect and provide reasonable spatial locations of 27 nm $T_iO(2)$ particles in relation to the stained nuclei of exposed BEAS 2B cells. Statistical analysis of particle proximity to cellular nuclei determined a significant difference between 5 min and 2 hr particle exposures suggesting a time-dependant internalization process. DF-CLSM microscopy is an alternative to current conventional light and electron microscopy methods that does not rely on particle fluorescence or contrast in electron density. DF-CLSM is especially well suited to the task of establishing the spatial localization of nanoparticles within cells, a critical topic in nanotoxicology. This technique has advantages to 2D darkfield microscopy as it visualizes nanoparticles in 3D using confocal microscopy. Use of this technique

should aid toxicological studies related to observation of NP interactions with biological endpoints at cellular and subcellular levels.

Part Fibre Toxicol. 2011 Jan 19;8(1):2.

Exposure to nanoparticles and hormesis.

Nanoparticles are particles with lengths that range from 1 to 100 nm. They are increasingly being manufactured and used for commercial purpose because of their novel and unique physicochemical properties. Although nanotechnology -based products are generally thought to be at a pre-competitive stage, an increasing number of products and materials are becoming commercially available. Human exposure to nanoparticles is therefore inevitable as they become more widely used and, as a result, nanotoxicology research is now gaining attention. However, there are many uncertainties as to whether the unique properties of nanoparticles also pose occupational health risks. These uncertainties arise because of gaps in knowledge about the factors that are essential for predicting health risks such as routes of exposure, distribution, accumulation, excretion and dose-response relationship of the nanoparticles. In particular, uncertainty remains with regard to the nature of the dose-response curve at low level exposures below the toxic threshold. In fact, in the literature, some studies that investigated the biological effects of nanoparticles, observed a hormetic dose-response. However, currently available data regarding this topic are extremely limited and fragmentary. It therefore seems clear that future studies need to focus on this issue by studying the potential adverse health effects caused by low-level exposures to nanoparticles.

Dose Response. 2010 Aug 12;8(4):501-17.

The New Toxicology of Sophisticated Materials: Nanotoxicology and Beyond.

It has long been recognized that the physical form of materials can mediate their toxicity - the health impacts of asbestiform materials, industrial aerosols and ambient Particulate Matter are prime examples. Yet over the past twenty years toxicology research has suggested complex and previously unrecognized associations between material physicochemistry at the nanoscale and biological interactions. With the rapid rise of the field of nanotechnology and the design and production of increasingly complex nanoscale materials, it has become ever-more important to understand how the physical form and chemical composition of these materials interact synergistically to determine toxicity. As a result, a new field of research has emerged - nanotoxicology. Research within this field is highlighting the importance of material physicochemical properties in how dose is understood, how materials are characterized in a manner that enables quantitative data interpretation and comparison, and how materials move within, interact with and are transformed by biological systems. Yet many of the substances that are the focus of current nanotoxicology studies are relatively simple materials that are at the vanguard of a new era of complex materials. Over the next fifty years, there will be a need to understand the toxicology of increasingly sophisticated materials that exhibit novel, dynamic and multifaceted functionality. If the toxicology community is to meet the challenge of ensuring the safe use of this new generation of substances, it will need to move beyond "nano" toxicology and towards a new toxicology of sophisticated materials. Here, we present a brief overview of the current state of the science on the toxicology of nanoscale materials, and focus on three emerging toxicology-based challenges presented by sophisticated materials that will become increasingly important over the next fifty years: identifying relevant materials for study, physicochemical characterization and bio-interactions.

Toxicological Sciences Volume120, Issuesuppl 1 Pp. S109-S129.

Low-toxic and safe nanomaterials by surface-chemical design, carbon nanotubes, fullerenes, metallofullerenes, and graphenes.

The toxicity grade for a bulk material can be approximately determined by three factors (chemical composition, dose, and exposure route). However, for a nanomaterial it depends on more than ten factors. Interestingly, some nano-factors (like huge surface adsorbability, small size, etc.) that endow nanomaterials with new biomedical functions are also potential causes leading to toxicity or damage to the living organism. Is it possible to create safe nanomaterials if such a number of complicated factors need to be regulated? We herein try to find answers to this important question. We first discuss chemical processes that are

applicable for nanosurface modifications, in order to improve biocompatibility, regulate ADME, and reduce the toxicity of carbon nanomaterials (carbon nanotubes, fullerenes, metallofullerenes, and graphenes). Then the biological/toxicological effects of surface-modified and unmodified carbon nanomaterials are comparatively discussed from two aspects: the lowered toxic responses or the enhanced biomedical functions. We summarize the eight biggest challenges in creating low-toxicity and safer nanomaterials and some significant topics of future research needs: to find out safer nanofactors; to establish controllable surface modifications and simpler chemistries for low-toxic nanomaterials; to explore the nanotoxicity mechanisms; to justify the validity of current toxicological theories in nanotoxicology; to create standardized nanomaterials for toxicity tests; to build theoretical models for cellular and molecular interactions of nanoparticles; and to establish systematical knowledge frameworks for nanotoxicology.

Nanoscale, 2011, 3, 362-382

Cationic nanoparticles induce caspase 3-, 7- and 9-mediated cytotoxicity in a human astrocytoma cell line.

On a daily basis we are exposed to cationic nanoparticulates in many different ways. They are known to distribute to many organs of the body, and while some evidence suggests that these nanoparticles are toxic to cells, the mechanism of their toxicity is not clear. Here we apply a combination of biochemical and imaging techniques to study the mechanism by which amine-modified polystyrene nanoparticles induce cell death in a human brain astrocytoma cell line. Flow cytometry analysis of cells exposed to cationic nanoparticles revealed an increase in cell membrane permeability of the dyes YoPro-1 and propidium iodide, indicating onset of an apoptotic followed by a secondary necrotic response. Activation of caspases 3/7 and 9 and cleavage of poly(ADP-ribose) polymerase (PARP)-1 was also detected, providing clear molecular evidence of the apoptotic pathway induced by the nanoparticles. Transmission electron microscopy also revealed that these nanoparticles induce morphological changes in lysosomes and mitochondria, consistent with our observation of a rapid increase in the formation of reactive oxygen species in these cells. Together these results suggest that amine-modified polystyrene nanoparticles can mediate cell death through an apoptotic mechanism mediated by damage to the mitochondria.

Nanotoxicology. 2010 Dec 15. (doi/pdf/10.3109/17435390.2010.539713)

Stable nanoparticle aggregates/ agglomerates of different sizes and the effect of their size on hemolytic cytotoxicity.

To study the toxicity of nanoparticles under relevant conditions, it is critical to disperse nanoparticles reproducibly in different agglomeration states in aqueous solutions compatible with cell-based assays. Here, we disperse gold, silver, cerium oxide, and positively-charged polystyrene nanoparticles in cell culture media, using the timing between mixing steps to control agglomerate size in otherwise identical media. These protein-stabilized dispersions are generally stable for at least two days, with mean agglomerate sizes of 23 nm silver nanoparticles ranging from 43-1400 nm and average relative standard deviations of less than 10%. Mixing rate, timing between mixing steps and nanoparticle concentration are shown to be critical for achieving reproducible dispersions. We characterize the size distributions of agglomerated nanoparticles by further developing dynamic light scattering theory and diffusion limited colloidal aggregation theory. These theories frequently affect the estimated size by a factor of two or more. Finally, we demonstrate the importance of controlling agglomeration by showing that large agglomerates of silver nanoparticles cause significantly less hemolytic toxicity than small agglomerates.

Nanotoxicology. 2010 Dec 13 (doi:10.3109/17435390.2010.536615)

Nanotechnology and nanomaterials: toxicology, risk assessment, and regulations.

Nanomaterials have very unique chemical and physical properties that suggest potential health hazards, but limited health and safety information exists for engineered nanomaterials. This review identifies a need for expanding efforts for addressing health and safety concerns in nanotechnology development and in nanotoxicology of engineered nanomaterials. The efforts include

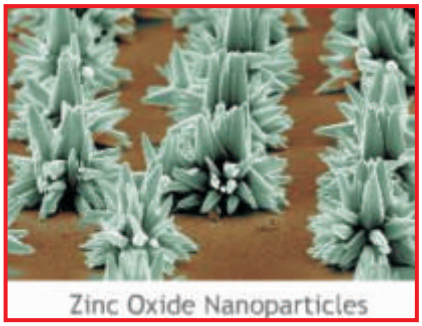
research to generate data for safety evaluation, toxicologic evaluation of potential human health effects, risk assessment to support risk-management decision-making, and regulations development to protect human health and the environment. The federal government's current understanding is that existing statutory authorities are adequate to address oversight of nanotechnology and its applications. On the other hand, the present review identifies weaknesses in the current research efforts and inadequacies in existing regulations. A collaborative effort involving multidisciplinary groups is a key element to address the related needs and issues. While federal agencies with regulatory responsibilities are looked upon to develop and implement sound policies and regulations to protect public health and the environment, state agencies may be required to initiate policies which rapidly incorporate new innovations and address public concerns. To address current and futures need related to nanotechnology, the responsible state agencies need to fill the information gaps and address the health and environmental issues. In California, activities have been initiated, but legislative authority and resources are required to provide risk assessment and health protection in an efficient and timely manner.

J Nanosci Nanotechnol. 2010 Dec;10(12):8646-57.

Cytotoxicity of zinc oxide nanoparticles: importance of microenvironment.

While ZnO particles are widely used in many fields, including personal care products, the high toxicity of ZnO nanoparticles has been reported and aroused great health concerns. In this study, the cytotoxicity of ZnO nanoparticles was evaluated and, in particular, the role of micro-environment in their toxicity was investigated. Our results show that ZnO nanoparticles are highly toxic to NIH/3T(3) cells, inducing viability loss, membrane leakage and morphology changes. The microenviroment, here the CO(2) atmosphere under cell culture condition, promoted the solubilization of ZnO nanoparticles. Then the released Zn from ZnO nanoparticles induces the cytotoxicity. The importance of microenvironment on the ZnO nanotoxicity is presented and the implications to future Nanotoxicology studies are discussed.

J Nanosci Nanotechnol. 2010 Dec;10(12):8638-45.


Zinc Oxide Nanoparticles

Using activated carbon nanoparticles to decrease the genotoxicity and teratogenicity of anticancer therapeutic agents.

One of the key obstacles against the success in cancer chemotherapy is the toxic and side effects of the chemotherapeutic agents. The avoidance of these toxic and side effects will greatly improve the therapeutic effects of anticancer drugs while decrease the pains of the patients. Here we show that activated carbon nanoparticles (ACNP), one of the mesoporous nanoparticles, can decrease the genotoxicity and teratogenicity of mitomycin C (MMC). To study the effects of ACNP on genotoxicity and teratogenicity of MMC, methods of PCE micronucleus test, Chinese hamster lung cell chromosome aberration experiment and rat teratogenicity were employed to observe the differences in genotoxicity and teratogenicity between ACNP-adsorbed MMC (ACNP-MMC) and free MMC. Results demonstrated that free MMC 0.16-5.0 microg/kg significantly increased the positive rate of PCE micronucleus test, the chromosome aberration rate and rat teratogenecity, but ACNP-MMC did not increased these heredity and reproduction toxicological indexes in a dose range of 0.625-10.0 microg/kg. From these results, it can be concluded that ACNP-MMC have significant effects to decrease the genotoxicity and teratogenicity effects of MMC. These results will have a considerable impact on the strategy of anticancer chemotherapy.

J Nanosci Nanotechnol. 2010 Dec;10(12):8603-9.

STEM mode in the SEM: A practical tool for nanotoxicology.

The addition of a transmitted electron detector to a scanning electron microscope (SEM) allows the recording of bright and dark field scanning transmission electron microscope (STEM) images and the corresponding in-lens secondary electron images from the same region of a thin sample. These combined imaging techniques have been applied here to the analysis of ultrathin sections of cells exposed *in*

vitro to nanomaterials for toxicology investigation. Electron microscopy in general permits the exact nature of the interaction of nanomaterials and cells to be elucidated, and in addition the use of STEM mode in the SEM enables the easy identification and exclusion of artefacts produced by ultramicrotome sectioning. The imaging and analysis obtained by using the STEM mode in the SEM configuration from three different nanomaterial systems of importance (iron oxide nanoparticles, single-walled carbon nanotubes and cadmium selenide quantum dots) indicate that it is a simple, practical and cost-effective tool for nanotoxicological research.

Nanotoxicology. 2010 Nov 19. (doi:10.3109/17435390.2010.535622)

Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment - issues and recommendations.

Studies designed to investigate the environmental or biological interactions of nanoscale materials frequently rely on the use of ultrasound (sonication) to prepare test suspensions. However, the inconsistent application of ultrasonic treatment across laboratories, and the lack of process standardization can lead to significant variability in suspension characteristics. At present, there is widespread recognition that sonication must be applied judiciously and reported in a consistent manner that is quantifiable and reproducible; current reporting practices generally lack these attributes. The objectives of the present work were to: (i) Survey potential sonication effects that can alter the physicochemical or biological properties of dispersed nanomaterials (within the context of toxicity testing) and discuss methods to mitigate these effects, (ii) propose a method for standardizing the measurement of sonication power, and (iii) offer a set of reporting guidelines to facilitate the reproducibility of studies involving engineered nanoparticle suspensions obtained via sonication.

Nanotoxicology. 2010 Dec 2. (doi:10.3109/17435390.2010.528846)

DNA damage and alterations in expression of DNA damage responsive genes induced by TiO(2) nanoparticles in human hepatoma HepG2 cells.

We investigated the genotoxic responses to two types of TiO(2) nanoparticles (<25 nm anatase: TiO(2)-An, and <100 nm rutile: TiO(2)-Ru) in human hepatoma HepG2 cells. Under the applied exposure conditions the particles were agglomerated or aggregated with the size of agglomerates and aggregates in the micrometer range, and were not cytotoxic. TiO(2)-An, but not TiO(2)-Ru, caused a persistent increase in DNA strand breaks (comet assay) and oxidized purines (Fpg-comet). TiO(2)-An was a stronger inducer of intracellular reactive oxygen species (ROS) than TiO(2)-Ru. Both types of TiO(2) nanoparticles transiently upregulated mRNA expression of p53 and its downstream regulated DNA damage responsive genes (mdm2, gadd45α, p21), providing additional evidence that TiO(2) nanoparticles are genotoxic. The observed differences in responses of HepG2 cells to exposure to anatase and rutile TiO(2) nanoparticles support the evidence that the toxic potential of TiO(2) nanoparticles varies not only with particle size but also with crystalline structure.

Nanotoxicology. 2010 Nov 10. (doi/abs/10.3109/17435390.2010.507316)

Genotoxic analysis of silver nanoparticles in Drosophila.

Health risk assessment of nanomaterials is an emergent field, genotoxicity being an important endpoint to be tested. Since *in vivo* studies offer many advantages, such as the study of the bioavailability of nanomaterials to sensitive target cells, we propose Drosophila as a useful model for the study of the toxic and genotoxic risks associated with nanoparticle exposure. In this work we have carried out a genotoxic evaluation of silver nanoparticles in Drosophila by using the wing somatic mutation and recombination test. This test is based on the principle that loss of heterozygosis and the corresponding expression of the suitable recessive markers, multiple wing hairs and flare-3, can lead to the formation of mutant clones in larval cells, which are expressed as mutant spots on the wings of adult flies. Silver nanoparticles were supplied to third instar larvae at concentrations ranging from 0.1-10 mM. The results showed that small but significant increases in the frequency of total spots were observed, thus indicating that silver nanoparticles were able to induce genotoxic activity in the wing spot assay of *D. melanogaster*, mainly via the induction of somatic recombination. These positive results obtained with silver nanoparticles contrast with the negative findings obtained when silver nitrate was tested.

Nanotoxicology. 2010 Nov 1. (doi/pdf/10.3109/17435390.2010.529176)

Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods.

Silver nanoparticles (Ag-nps) are used as a natural biocide to prevent undesired bacterial growth in clothing and cosmetics. The objective of this study was to assess the antibacterial efficacy of Ag-nps of different sizes, surface conditions, and synthesis methods against *Escherichia coli*, Ag-resistant *E. coli, Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and Salmonella sp. Ag-nps samples were synthesized by: Base reduction with unmodified surfaces and used as synthesized ('unwashed'; 20, 50 and 80 nm) or after 20 phosphate buffer washes ('washed'; 20, 50 and 80 nm), or synthesized by laser ablation with carbon-stabilized surfaces ('carbon-coated'; 25 and 35 nm). Unwashed Ag-nps were toxic to all bacterial strains at concentrations between 3.0-8.0 µg/ml. The washed Ag-nps and carbon-coated Ag-nps were toxic to all bacterial strains except Ag-resistant *E. coli* at concentrations between 64.0-1024.0 µg/ml. Ag-resistant *E. coli* died only when treated with unwashed Ag-nps or its supernatant, both of which contained formaldehyde.

Nanotoxicology. 2010 Nov 1. (doi:10.3109/17435390.2010.525669)

Possible genotoxic mechanisms of nanoparticles: criteria for improved test strategies.

We review the mechanisms and pathways whereby nanoparticles might cause genotoxicity. Primary and secondary mechanisms are discussed in relation to the general particle toxicology paradigm. We also discuss how we might improve genotoxicity assays for nanoparticles. In this context we describe the role of the dispersion and the protein corona, the most relevant metric, choice of controls and new endpoints for genotoxicity along with the need for a structure activity model of NP genotoxicity.

Nanotoxicology. 2010 Dec;4:414-20.

Rationale of genotoxicity testing of nanomaterials: regulatory requirements and appropriateness of available OECD test guidelines.

The development of an environmental health and safety risk management system for nanoscale particle-types requires a base set of hazard data. Accurate determination of health and environmental risks of nanomaterials is a function of the integration of hazard and exposure datasets. Recently, a nanoparticle risk assessment strategy was promulgated and the components are described in a document entitled "Nanorisk framework" (www.nanoriskframework.com). A major component of the hazard evaluation includes a proposed minimum base set of toxicity studies. Included in the suggested studies were substantial particle characterization, a variety of acute hazard and environmental tests, concomitant with screening-type genotoxicity studies. The implementation of well-accepted genotoxicity assays for testing nanomaterials remains a controversial issue. This is because many of these genotoxicity tests were designed for screening general macroparticle chemicals and might not be suitable for the screening of nanomaterials (even of the same compositional material). Furthermore, no nanoparticle-type positive controls have been established or universally accepted for these tests. Although it is the comparative results of the test material vs the negative or vehicle control that forms the basis for interpreting the results and potency of test materials in genetic toxicology assays, the lack of a nanoparticle-type positive control questions the suitability of the tests to identify nanomaterials with genotoxic properties. It is also not possible to establish historical positive control ranges that would confirm the sensitivity of the tests. Although several genetic toxicology tests have been validated for chemicals according to the Organisation for Economic Co-operation and Development (OECD) test guidelines, the relevance of these assays for nanoparticulate materials remains to be determined. In an attempt to remedy this issue, the OECD has established current projects designed to evaluate the relevance and reproducibility of safety hazard tests for representative nanomaterials, including genotoxicity assays (i.e., Steering Group 3 – Safety Testing of Representative Nanomaterials). In this article, we discuss our past approaches and experience in conducting genotoxicity assays (1) for a newly developed ultrafine $T_iO(2)$ particle-

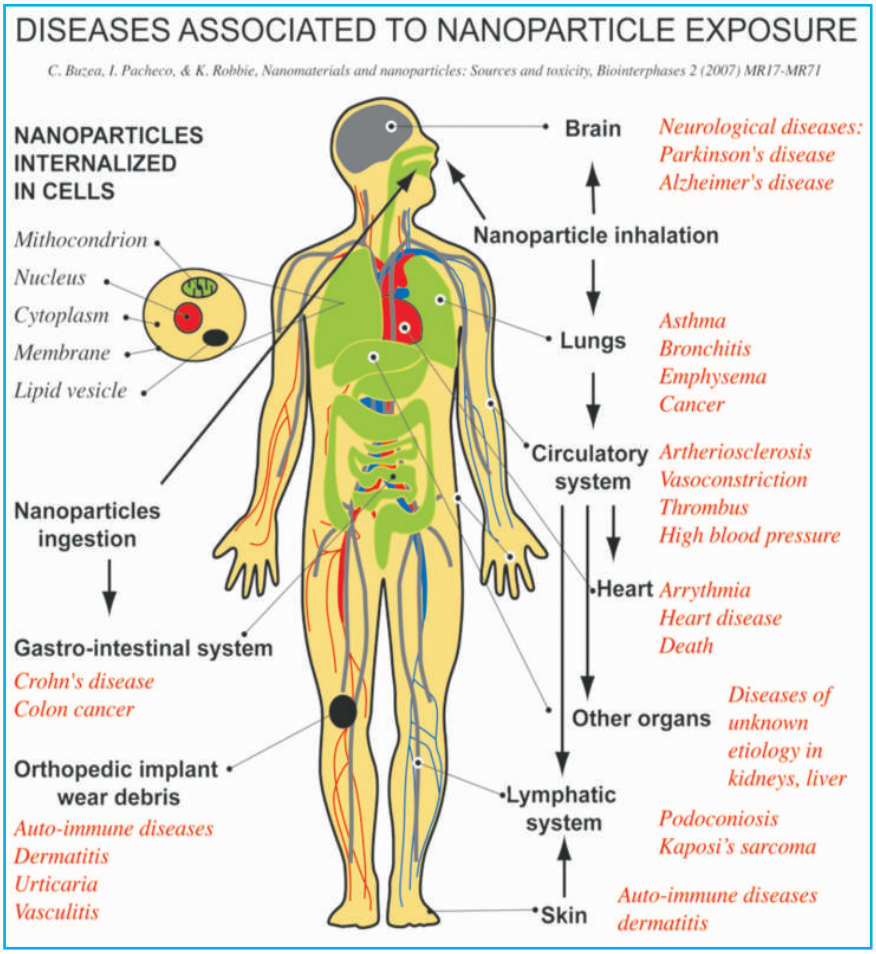
type; and (2) in a recent inhalation study, evaluating micronucleus formation in rat erythrocytes following exposures to engineered amorphous nanosilica particles. It seems clear that the development of standardized approaches will be necessary in order to determine whether exposures to specific nanoparticle-types are associated with genotoxic events. The appropriateness of available genotoxicity test systems for nanomaterials requires confirmation and standardization. Accordingly, it seems reasonable to conclude that any specific regulatory testing requirements for nanoparticles would be premature at this time.

Nanotoxicology. 2010 Dec;4:409-13.

Gene toxicity studies on titanium dioxide and zinc oxide nanomaterials used for UV-protection in cosmetic formulations.

Titanium dioxide and zinc oxide nanomaterials, used as UV protecting agents in sunscreens, were investigated for their potential genotoxicity in *in vitro* and *in vivo* test systems. Since standard OECD test methods are designed for soluble materials and genotoxicity testing for nanomaterials is still under revision, a battery of standard tests was used, covering different endpoints. Additionally, a procedure to disperse the nanomaterials in the test media and careful characterization of the dispersed test item was added to the testing methods. No genotoxicity was observed *in vitro* (Ames' Salmonella gene mutation test and V79 micronucleus chromosome mutation test) or *in vivo* (mouse bone marrow micronucleus test and Comet DNA damage assay in lung cells from rats exposed by inhalation). These results add to the still limited data base on genotoxicity test results with nanomaterials and provide congruent results of a battery of standard OECD test methods applied to nanomaterials.

Nanotoxicology. 2010 Dec;4:364-81.



Source:http://www.ask.com/wiki/Nanotoxicology

Nanotoxicology: no small matter.

Engineered nanomaterials i.e. materials deliberately manufactured on a nanoscale offer exciting new opportunities in technology and medicine. However, the increasing use of nanomaterials in society also raises concerns as to their possible adverse effects on human health and the environment. This review considers the potential application of high-throughput screening approaches to assess hazards of engineered nanomaterials. The disciplinary identity of toxicology is also discussed as attention shifts towards nanoscale objects.

Nanoscale. 2010 Dec 1;2(12):2514-20.

Presence and risks of nanosilica in food products.

This study uniquely describes all steps of the risk assessment process for the use of one specific

nanomaterial (nanosilica) in food products. The aim was to identify gaps in essential knowledge and the difficulties and uncertainties associated with each of these steps. Several food products with added silica (E551) were analyzed for the presence, particle size and concentration of nanosilica particles, using experimental analytical data, and the intake of nanosilica via food was estimated. As no information is available on the absorption of nanosilica from the gastrointestinal tract, two scenarios for risk assessment were considered. The first scenario assumes that the silica is absorbed as dissolved silica, while the second scenario assumes that nanosilica particles themselves are absorbed from the gastrointestinal tract. For the first scenario no adverse effects are expected to occur. For the second scenario there are too many uncertainties to allow proper risk assessment. Therefore, it is recommended to prioritize research on how nanosilica is absorbed from the gastrointestinal tract.

Nanotoxicology. 2010 Sep 24. (doi:10.3109/17435390.2010.519836)

Physico-chemical features of engineered nanoparticles relevant to their toxicity.

Nanotoxicology studies require investigations of several physico-chemical aspects of the particle/body fluid interaction, here described by reviewing recent literature in the light of new experimental data. Current characterization mostly covers morphology and metric-related characteristics (form, chemical composition, specific surface area, primary particle size and size distribution), and is mandatory in any experimental study. To unveil toxicity mechanisms, several other physico-chemical properties relevant to (geno) toxicity need to be assessed, typically the release or quenching of radical/ROS (Reactive Oxygen Species), the presence of active metal ions, evidence of structural defects. Major tasks for physical chemists working on nanoparticles-induced genotoxicity are described with some examples: (i), Tailored preparation of the same material in different sizes; (ii) particle modification changing a single property at a time; and (iii) identification of appropriate reference materials. Phenomena occurring during the contact between nanoparticles and cellular media or biological fluids (dispersion, agglomeration/aggregation, protein adsorption) are discussed in relation to the surface properties of the nanoparticles considered.

Nanotoxicology. 2010 Dec;4:347-63.

Uptake and cytotoxic effects of multi-walled carbon nanotubes in human bronchial epithelial cells.

Carbon nanotubes (CNT) are cytotoxic to several cell types. However, the mechanism of CNT toxicity has not been fully studied, and dosimetric analyses of CNT in the cell culture system are lacking. Here, we describe a novel, high throughput method to measure cellular uptake of CNT using turbimetry. BEAS-2B, a human bronchial epithelial cell line, was used to investigate cellular uptake, cytotoxicity, and inflammatory effects of multi-walled CNT (MWCNT). The cytotoxicity of MWCNT was higher than that of crocidolite asbestos in BEAS-2B cells. The IC(50) of MWCNT was 12 µg/ml, whereas that of asbestos (crocidolite) was 678 µg/ml. Over the course of 5 to 8 h, BEAS-2B cells took up 17-18% of the MWCNT when they were added to the culture medium at a concentration of 10 µg/ml. BEAS-2B cells were exposed to 2, 5, or 10 µg/ml of MWCNT, and total RNA was extracted for cytokine cDNA primer array assays. The culture supernatant was collected for cytokine antibody array assays. Cytokines IL-6 and IL-8 increased in a dose dependent manner at both the mRNA and protein levels. Migration inhibitory factor (MIF) also increased in the culture supernatant in response to MWCNT. A phosphokinase array study using lysates from BEAS-2B cells exposed to MWCNT indicated that phosphorylation of p38, ERK1, and HSP27 increased significantly in response to MWCNT. Results from a reporter gene assays using the NF-κB or AP-1 promoter linked to the luciferase gene in transiently transfected CHO-KI cells revealed that NF-κB was activated following MWCNT exposure, while AP-1 was not changed. Collectively, MWCNT activated NF-κB, enhanced phosphorylation of MAP kinase pathway components, and increased production of proinflammatory cytokines in human bronchial epithelial cells.

Toxicol Appl Pharmacol. 2010 Nov 15;249(1):8-15.

Impact of silver nanoparticles on human cells: effect of particle size.

This work investigated the cytotoxicities of three silver nanoparticles (SNPs) SNP-5, SNP-20 and SNP-50 with different sizes (approximately 5 nm, approximately 20 nm and approximately 50 nm) using four human cell models (A549, SGC-7901, HepG2 and MCF-7). Endpoints included cell morphology, cell viability, cellular membrane integrity, oxidative stress and cell cycle progression. Observable deleterious effects on the cell morphologies and membrane integrity were induced by SNP-5 and SNP-20. SNPs elevated the ROS levels in cells and arrested the cells at S phase. Apoptosis occurred for 4-9% of the exposed cells. All these cellular responses as well as EC50 values were found to be size-dependent for the tested SNPs. Ultrastructural observations confirmed the presence of SNPs inside cells. Elemental analysis of silver in cells by ICP-MS showed that smaller nanoparticles enter cells more easily than larger ones, which may be the cause of higher toxic effects. The findings may assist in the design of SNP applications and provide insights into their toxicity.

Nanotoxicology. 2010 Sep;4(3):319-30.

Attenuating Risks Through Regulation: Issues for Nanotechnology in India.

The problems in developing risk-based regulation are particularly compounded for a developing country like India, owing to a lack of resources, expertise and regulatory mandate. Nevertheless, it is of utmost importance considering that in the event of some of the risks materializing, developing countries would be ill-equipped to handle and mitigate them. This paper attempts to examine the existing regulatory framework, taking the particular case of the chemicals legislation, and examine whether it could be extended to the regulation of nanomaterials and their risks. Our study indicates that the Manufacture, Storage and Import of Hazardous Chemical Rules, 1989 could be applied to nanomaterials but would require substantial amendments. The legislation is very laudable in that it provides for very detailed and minute information reporting and disclosures. This is believed to be very crucial for 'information regulation' of nanotechnology as an intermediate step between unfettered self-regulation and traditional 'command and control' regulation and which could help address the present information deficit and help gain regulatory experience.

Journal of Biomedical Nano-

DID YOU KNOW ?

A nanometer is one billionth of a meter – about one ten-thousandth the diameter of a human hair. Nanotechnology encompasses nanoscale science, engineering and technology, and involves imaging, measuring, modeling, and manipulating matter at this length scale. Nanoscale materials may have organizations and properties different than the same chemical substances displayed at a larger scale.

(http://www.epa.gov/opptintr/nano/nano-facts.htm)

CURRENT CONCERNS

There is a feeling that with the increase in mass production of engineered nanoparticles like carbon nanotubes, there are chances that these particles will be present in abundant amount in the environment and will interact with water, soil and air, and finally enter the food chain. So study of the behaviour and impacts of nanomaterials in the biotic and abiotic environment as well as human health is a challenging task for the scientists/toxicologists.

REGULATORY TRENDS

There are no provisions in Community legislation dealing specifically with nanomaterials. However, without excluding the possibility that a need would be identified for specific labelling requirements, nanomaterials have to comply with the existing provisions of Community law addressing the labelling of products, warnings to consumers and users based on the properties of products, instructions for use, or any other information requirements. Also relevant are the provisions in REACH with obligations of data dissemination about environment, safety and health risks via Safety Data Sheets up and down the supply chain, to industrial users and via the Internet to the public at large. Chemical safety reports will be produced for substances placed on the market in quantities at or above the fixed quantity and a data base with the purpose to make publicly available non confidential data about chemical substances will be kept by the European Chemicals Agency. Attention is also drawn to provisions in Community law creating a right of access to information in relation to programmes mainly implementing legislation on environmental protection. The obligation to provide information in relation to the use of nanomaterials and nanotechnologies should be distinguished from manufacturers' claims regarding the presence of particular characteristics associated with the use of nanomaterials and nano technologies.

Commission of the European Communities, Regulatory Aspects of Nanomaterials, Brussels, 17.6.2008, Com(2008) 366 Final [Sec(2008) 2036]

ON THE LIGHTER SIDE

- A man was driving down a country road, when he spotted a farmer standing in the middle of a huge field of grass. He pulled the car over to the side of the road and noticed that the farmer is just standing there, doing nothing, looking at nothing. The man got out of the car, walked all the way out to the farmer and asked him, "Ah excuse me mister, but what are you doing?"

The farmer replied, "I'm trying to win a Nobel Prize."

"How?" asked the man, puzzled.

"Well I heard they give the Nobel Prize to people who are out standing in their field."

- **Albert einstein quotes**

“Two things are infinite: the universe and human stupidity; and I'm not sure about the universe”

"You do not really understand something unless you can explain it to your grandmother." -

"Gravitation can not be held resposible for people falling in love"

“The wireless telegraph is not difficult to understand. The ordinary telegraph is like a very long cat. You pull the tail in New York, and it meows in Los Angeles. The wireless is the same, only without the cat”

ON THE WEB

- http://www.cdc.gov/niosh/topics/nanotech/ describes Workplace Safety & Health Topics
- http://www.hse.gov.uk/research/rrhtm/rr274.htm Health and Safety Executive (HSE) report that provides information on routes, sources, and levels of nanoparticle exposure, control measures, trends, etc.
- http://www.biotecharticles.com/Nanotechnology-Article/Nanotoxicology-Study-of-Toxicity-of-Nanomaterials-655.html Introduction to nanotoxicology

CONFERENCES

- **5th International Conference** on Nanotechnology, Occupational and Environmental Health

  09/08/2011, Boston, MA, USA
  www.uml.edu/nano/nanoehs/Conferences/Conferences.html

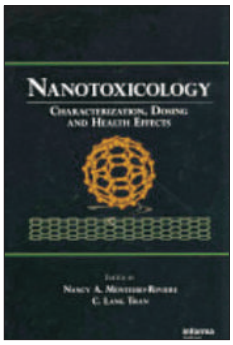
- **6th International Conference** on the Environmental Effects of Nanoparticles and Nanomaterials

  19/09/2011, London, UK
  www.sebiology.org/management/meetings/NANO/index.htm

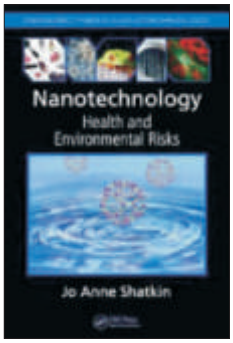
- **Senn 2012:** Safety of engineered nanomaterials and nanotechnologies

  28/10/2012, Helsinki, Finland
  www.ttl.fi/senn2012

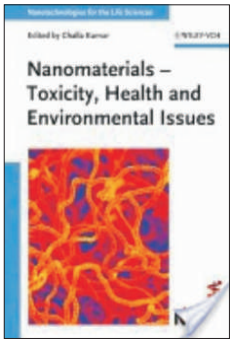
BOOK STOP



Title : Nanotoxicology: characterization, dosing and health effects
Authors Nancy A. Monteiro-Riviere, C. L. Tran
Editors Nancy A. Monteiro-Riviere, C. L. Tran
Edition illustrated
Publisher Informa Healthcare, 2007
ISBN 1420045148, 9781420045147
Length 434 pages



Title : Nanotechnology: health and environmental risks perspectives in nanotechnology
Author Jo Anne Shatkin
Edition illustrated
Publisher CRC Press/Taylor & Francis Group, 2008
ISBN 1420053639, 9781420053630
Length 167 pages



Title : Nanomaterials: toxicity, health and environmental issues, Volume 5
Nanotechnologies for the life sciences, ISBN 352731301X, 9783527313013
Author Challa S. S. R. Kumar
Editor Challa S. S. R. Kumar
Edition illustrated
Publisher Wiley-VCH, 2006
ISBN 3527313850, 9783527313853
Length 333 pages

MINI PROFILE OF TITANIUM DIOXIDE

NAME OF CHEMICAL: TITANIUM DIOXIDE

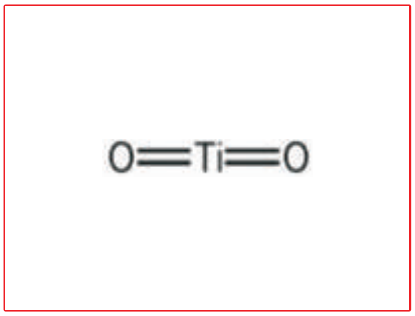
SYNONYMS: anatase-; atlas-white-titanium-dioxide-; austiox-r-cr-3-; bayeritian-; bayertitan-; bayertitan-a-; bayertitan-r-u-f-; baytitan-; blend-white-9202-; brookite-; calcotone-white-t-; ci-pigment-white-6-; fil-cream-; hombitan-; kronos-titanium-dioxide-; levanox-white-rkb-; orgasol-1002d-white-10-extra-cos-; rayox-; ro-2-; runa-arh-200-; runa-rh20-; rutile-; rutiox-cr-; tiofine-; tiona-td-; tioxide-; tioxide-ad-m-; tioxide-r-cr-; tioxide-rhd-; tioxide-rsm-; tioxide-r-xl-; tipaque-; tipaque-r-820-; ti-pure-; ti-pure-r-900-; titafrance-; titandioxid-(sweden); titania-; titanic-anhydride-; titanic-oxide-; titanium-oxide-; titanium-oxide- (tio2); titanium-peroxide- (tio2); titanox-; titanox-2010-; titanox-ranc-; trioxide- (s); tronox-; unitane-;

RTECS NUMBER (NIOSH): XR2275000

CAS RN: 13463-67-7

MOLECULAR WEIGHT: 79.9

MOLECULAR FORMULA: TiO_2



PROPERTIES: White, tetragonal crystals; odorless; tasteless; m.p.1843°C; b.p.2500-3000 °C; density: 3.9-4.3 g/cm³; soluble in hot concentrated sulfuric acid; in hydrofluoric acid, insoluble in hydrochloric acid, nitric acid or diluted sulfuric acid, insoluble in org solvents, soluble in alkali, insoluble in water.

USES: Airfloated ilmenite is used for titanium pigment manufacture. Rutile sand is suitable for welding-rod-coating materials, as ceramic colorant, as source of titanium metal. As color in the food industry. Anatase titanium dioxide is used for welding-rod-coatings, acid resistant vitreous enamels, in specification paints, exterior white house paints, acetate rayon, white interior air-dry and baked enamels and lacquers, inks and plastics, for paper filling and coating, in water paints, tanners leather finishes, shoe whiteners, and ceramics(6).

TOXICITY DATA:

orl-rat TDLo: 60 gm/kg------- Hypermotility, diarrhea; Other changes(Gastrointestinal)
ihl-rat TCLo: 10 mg/m3/13W-I (RTECS 2008 AN: XR2275000)

STORAGE and DISPOSAL: Keep container tightly closed. Keep container in a cool, well-ventilated area. Waste must be disposed off in accordance with federal, state and local environmental control regulations.

Route	Symptoms	First aid	Target organ
Inh. & Ing.	Irritation to the respiratory tract, gastrointestinal (digestive) tract irritation with nausea, vomiting and diarrhea	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious Person. Get medical attention.	lungs, upper respiratory tract
Cont.	Irritation and redness.	Rinse and then wash skin with water and soap. First rinse with plenty of water for several minutes (remove contact lenses if possible), then take to a doctor.	Skin & Eyes