

STUDIES ON THE TOXICOLOGICAL EFFECTS OF ENGINEERED NANOPARTICLES IN ENVIRONMENT – A REVIEW

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ABSTRACT

This paper considers the standard ecotoxicity tests done so far for assessing the hazards of engineered nanoparticles and potential negative effects that engineered nanoparticles may have on environment. Research and development in nanotechnologies increase strongly and attract substantial funding, but environmental consequences of resulting materials and applications are poorly known. We prepared nanoparticles by continuous sonication and by ultra filtration. *Artemia salina* was differentiated to five life stages based on certain easily distinguished features and were exposed to different concentrations of Titanium Dioxide and Fullerene using 48 hours acute toxicity testing. Images of the particle solutions were recorded using TEM and the median lethal concentration was determined in each life stage. Exposure to sonicated nanoparticles shows varied mortalities in different stages of *Artemia salina* where as filtered solutions showed increasing mortality with increase in concentration. We conclude that uncontrolled and unobserved release of these nanoparticles either as byproducts or medical wastes, could have a large negative consequence on the aquatic and terrestrial organisms, particularly on the humans.

Keywords: Engineered nanoparticles, toxicity testing, *Artemia salina*, Negative effects.

I. INTRODUCTION

Nanotechnology is the development and manufacture of materials in the nanometer size range (at least one dimension less than 100 nm) and their application. Nanoparticles deserve special attention, because compounds in this miniature size range have chemical properties that differ from those of their larger counterparts. As the size of the particle decreases, the number of atoms exposed on the surface and the amount of energy available for release increase (Banfield and Navrotsky, 2001). Due to their small size, a relatively large proportion of the atoms and molecules making up the particles are exposed at the particle surface compared to larger particles. This structural difference coupled with the relatively large surface area per unit mass of nanoparticles allow such materials to exhibit properties that differ from bulk chemicals, making them useful in a wide variety of applications including electronics, paints, cosmetics, medicines, foods, textiles and environmental remediation. Today, nano-scale zinc oxides are used in sunscreen lotions and scratch-resistant glass while carbon nanotubes are incorporated into tennis rackets, and nano-engineered chemical treatments performed on fabrics to render them stain-resistant. The National Science Foundation has estimated that nanotechnology applications may be

valued at more than 1 trillion dollars in the global economy by 2015 (Bergeson and Auerbach, 2004). Apart from consumer products, nanomaterials are being tested for medical and environmental uses.

A. Health concerns of nanoparticle toxicity

Research has shown that many types of nanomaterials can be toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production, DNA mutation and even cell death (Oberdoster *et al.*, 2005a). Materials are more readily taken up by the human body than larger sized particles and are able to cross biological membranes that larger sized particles normally cannot (Holsapple *et al.*, 2005). Once in the blood stream, nanomaterials are transported around the body and can be absorbed into vital organs including the heart, kidney, liver and spleen (Oberdoster *et al.*, 2005b).

Unlike larger particles and materials, nanomaterials may be transported within cells and be taken up by cell mitochondria (Li *et al.*, 2003) and the cell nucleus (Gieser *et al.*, 2005), where they can cause major structural damage. The small size, greater surface area and greater chemical reactivity of nanomaterials results in increased production of reactive oxygen species, including free radicals (Nel *et*

al., 2006). Production of reactive oxygen species has been found in a diverse range of nanomaterials including nanoparticle metal oxides (Dunford *et al.*, 1997; Warmer *et al.*, 1997; Savic *et al.*, 2003; Hussian *et al.*, 2005; Long *et al.*, 2006 ; Zhang and Sun, 2003) commonly used in sunscreens and cosmetics and carbon fullerenes (Yamakoshi, *et al.*, 2004) that are used in some face creams and moisturizers. Reactive oxygen species and free radical production is one of the primary mechanisms of nanotoxicity; it may result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA (Nel *et al.*, 2006).

There is evidence that nanomaterials can enter the human body through several mediums. The human skin, intestinal tract, and lungs are always in direct contact with the environment (Hoet *et al.*, 2004). Some toxicological studies have been conducted in relation to the inhalation of NP made from low toxicity materials such as carbon black (Li *et al.*, 1999), TiO₂ (Ferin *et al.*, 1992) and polystyrene (Brown *et al.*, 2001). Such studies demonstrate that the toxicity of these materials is related to their ability to induce oxidative stress and inflammation in the lung leading to impacts on lung and cardiovascular health. The cells of the body contain a number of antioxidant defense molecules (e.g. glutathione and vitamin E) that protect cells against reactive oxygen species (ROS) and free radicals that due to their electrophilic properties damage proteins, lipids and DNA. Such ROS and free radicals include superoxide anion radicals (O₂⁻) and hydroxyl radicals (OH[•]) and are generated at the surface of NP (Stone *et al.*, 1998, Wilson *et al.*, 2002). Depletion of antioxidant defense molecules by these ROS and production of free radicals lead to oxidative stress and damage to cells. Nanoparticles causing oxidative stress may also lead to the production of reactive nitrogen, sulphur and other species (i.e. RNS, RSS and others) stressing the body in a similar manner to the effect of ROS. In the case of RSS (Giles and Jacob, 2002), reactive sulphur substances such as thiol radicals and disulphides can oxidize and ultimately inhibit thiol proteins and enzymes.

Oxidative stress can also cause cell damage and activate inflammation (Donaldson *et al.*, 2000) . This involves activation of various white blood cells within the immune system. Such cells include macrophages that migrate to the site of particle deposition and then

engulf the particles by phagocytosis. The macrophages then remove the particles from the lung surface by either moving out of the airways along with mucus, or by migrating into the body's lymph nodes. Successful particle clearance allows the inflammation to subside and any tissue damage to be repaired. Unsuccessful particle clearance leads to persistent inflammation and oxidative stress that causes cellular damage leading to a variety of disease effects (Donaldson *et al.*, 2003). Acute effects associated with particulate air pollution result in increased hospitalization and deaths from both respiratory and cardiovascular diseases. Such effects have been attributed to airborne nanoparticles (Peters *et al.*, 1997). Acute effects tend to affect people who are susceptible due to pre-existing inflammatory diseases. Chronic effects associated with other respirable particles, such as environmental particulate air pollution, crystalline silica (alpha-quartz) and asbestos fibres, include fibrosis lung tissue making breathing difficult) and cancer.

The long term health effects of respirable nanoparticles as yet have not been elucidated. Epidemiological evidence from industrial processes, such as the manufacture of carbon black, where workers may potentially be exposed to nanoparticles, is not clear-cut. Studies which investigate the ability of nanoparticles made from low toxicity materials to generate oxidative stress and inflammation suggest that potency is dependent upon their surface area (Brown *et al.*, 2001, Duffin *et al.*, 2002). Furthermore, studies that compare low toxicity materials and particles made from more noxious substances, such as nickel or alphaquartz, demonstrate that biological reactivity is a function of both surface reactivity and surface area (Duffin *et al.*, 2002). For non-spherical particles such as fibres, it is well recognized that dimensions and durability are important factors in determining their ability to induce fibrosis and cancer (Warheit *et al.*, 1995, Donaldson *et al.*, 1989). It is conceivable that these properties will also be relevant to nanoparticles such as nanotubes, nanowhiskers and nanofibres. For new, more novel forms of engineered nanoparticles it is likely that a combination of the factors described above will be important in determining their ability to induce adverse effects in human and wider environmental systems.

Experiments on animals indicate that nanoparticles do affect the cell structure and in some

cases even reflect the inflammation in organs leading to carcinogenic developments (Driscoll *et al.*, 1997). Another consideration is the development of nanotech-associated health complications in workers engaged directly in the production of these particles (Dowling, 2004; Lyman, 2003; Wolfgang, 2004). The direct and sustained exposure to these particles at various stages makes them the most vulnerable category. Without stringent safety measures and containment procedures, nanoparticles released through exhaust, leaks, cleaning and maintenance of the facility would saturate the working area, increasing the level of exposure to the worker (Lyman, 2003).

B. Environmental concerns of nanoparticles toxicity

In addition to the health hazards that nanoparticles may pose when directly inhaled, ingested, or applied to the human body, general environmental risks may also exist when such particles enter the soil, the water, or the air. Many of the current and intended uses of nanoparticles are in relation with environment including remediation (removal of pollutants from contaminated water or soil where large quantities are used in e.g. permeable reactive barrier), water treatment filters and control of algal growth in water systems (Biswas and Wu, 2005). The rapid growth of nanotechnologies will also lead to increased accidental and purposeful release of nanoparticles into the environment. It is, therefore, important that overall impacts and risks of newly engineered nanoparticles released into the environment are addressed (Colvin, 2003, Nature, 2003, Oberdorster *et al.* 2005). A study by Oberdorster *et al.* (2004) on the effects of buckyballs on bass proved that other species are also highly vulnerable to the effects of nanomaterials. In this same study, it was also observed that nanomaterials also affected microbes; the result was a 100% mortality rate. Although there are no other significant studies to substantiate this further, it is logical that nanomaterials could affect the ecosystem.

When nanoparticles are released into the environment they may be released into air, water or into soil allowing access to a variety of different species ranging from single celled organisms such as bacteria and algae through to more complex vertebrates such as fish and aquatic mammals (Oberdorster, 2004, Oberdorster *et al.*, 2006). Impacts on any of the organisms within the ecosystem may have consequences on the remainder of the ecosystem by

affecting food chains and interactions between different species. It is, therefore, important to assess the fate and distribution of nanoparticles released into the environment in order to ascertain which environments and species are most at risk of significant exposure. Fate and distribution are likely to be different depending on the chemical and physical characteristics of the particles in relation to hydrophobicity and particle surface charge. As yet, we have been unable to identify any studies that have demonstrated the ability to track nanoparticles in the environment or to assess their fate. In addition to determining the fate and distribution of nanoparticles in the environment, it is also essential to assess their potential toxicity to a wide range of species (Lovern *et al.*, 2006).

C. Nanotoxicity studies in terrestrial organisms

To date in relation to ecotoxicology, the effects of nanoparticles on terrestrial microorganisms has been the area most widely studied. For example, silver nanoparticles can be used in wound dressings, although it is not clear whether these antimicrobial effects are simply due to the silver rather than the particulate form. However, Sondi and Salopek-Sondi (2004) provided evidence of silver nanoparticles accumulation in the membrane of the bacterium *Escherichia coli* causing cell wall pits leading to cell permeability changes and ultimate death. Lee, 2005 recently demonstrated that UV irradiation of TiO₂-coated multi-walled nanotubes (MWNTs) was more useful at killing bacterial endospores than either UV light alone or UV irradiated TiO₂ particles. However, the composite of nanotubes and TiO₂ appeared to cause endospore aggregation so that some of the spores were protected and survived, a phenomenon not observed with the other treatments. Magnetic nanoparticles have also been developed as a tool to remove bacteria from aquatic environments (Watson *et al.*, 2000). The large surface area of nanoparticles makes them ideal for use as absorbents and so a number of products are being developed for the destruction or removal from the environment of bacteria, including biological warfare agents (Koper & Klabunde, 2000).

In addition to effects on bacteria, TiO₂-coated hollow glass beads have been shown to inhibit the photosynthetic activity of cyanobacteria, and diatoms, suggesting potential useful applications in preventing excessive algal growth (Kim and Lee, 2005). Yang and

Watts (2005) investigated the phytotoxicity of 13nm aluminium nanoparticles (Al-NPs) on root growth by the seeds of five different plant species. The Al-NPs inhibited root growth at high concentrations (2mg ml⁻¹), while larger Al particles of 200-300nm had no effect. The inhibiting effects on root growth were decreased by the addition of dimethyl sulphoxide (DMSO), a molecule that scavenges free radicals such as hydroxyl radicals, suggesting that oxidative stress may play a role in the effects of the NP on root elongation. The toxicity of copper NPs (23.5 nm) was assessed in vivo based on LD₅₀, morphological changes, pathological examinations and blood biochemical indices of experimental mice (Chen *et al.*, 2006).

In addition, acute tests on other target organisms have not as yet been published, and so there is still a lack of knowledge in this area. It is clear that different nanomaterials and test procedures are factors in the results obtained, but these need to be further explored.

D. Nanotoxicity studies in aquatic organisms

Aquatic environments may also be threatened by pollution from nanomaterials. Oberdörster (2004) exposed juvenile largemouth bass to C₆₀ fullerenes and investigated the resulting induced oxidative stress. Her research indicated a trend toward a decrease of glutathione (GSH) in the gills and an increase of lipid peroxidation in the liver. Gills are important in extracting oxygen from ambient water and are priority organs in xenobiotic exposure. Redox-active particles encountered by the gills should therefore induce antioxidant enzyme production and consume GSH. Meanwhile, the brain has a blood-brain barrier that prohibits exposure of the brain to xenobiotics. Lipid peroxidation in the brain would be an indicator that nanomaterials have reached this organ, but unfortunately there is no evidence indicating that nanomaterials reach the brain of fish (Oberdörster *et al.* 2004). Yamago *et al.* (1995) studied the in vivo biological behavior of a 14C-labeled water-miscible C₆₀ fullerene in rats. Fullerenes administered orally were easily eliminated in the feces, but those injected intravenously were retained in the body after 1 week. The intravenously injected fullerenes were distributed mainly in the liver (91.7% of dose), and simultaneously some were able to penetrate slightly the blood-brain barrier. Nanoparticles were also detected in the brain, testis, liver, and blood of *Oriziyas latipes* (Kashiwada, 2006). Concentrations of nanoparticles in the blood of male and female medaka

were 16.5 and 10.5 ng/mg blood protein, respectively. Liver cells of rainbow trout *Oncorhynchus mykiss* exposed to TiO₂ NPs showed minor fatty change and lipidosis, and some hepatocytes showed condensed nuclear bodies (Federici *et al.*, 2007). Lovern and Klaper (2006) treated *Daphnia magna* with C₆₀ or TiO₂. The particles were prepared by either sonication in medium for 30 minutes to break up the aggregates, or by solubilisation in the organic solvent tetrahydrofuran (THF). The sample prepared in THF was filtered and evaporated in order to attempt to remove the solvent from the particles (see below) prior to addition to the invertebrates. The TiO₂ and the C₆₀ particles were more potent at killing the organisms when prepared in THF than when prepared by sonication, and the C₆₀ was more potent than the TiO₂. Lovern and co-workers have also observed that nanoparticles induced irregular swimming behaviour of *Daphnia magna*.

E. Fullerene and titanium dioxide toxicity

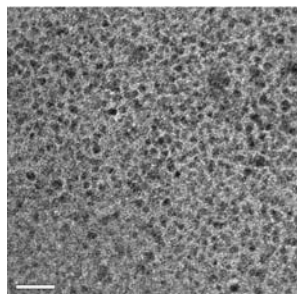
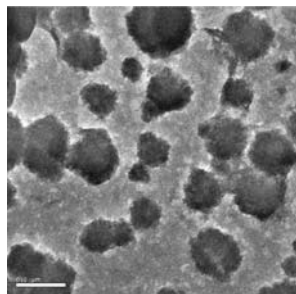
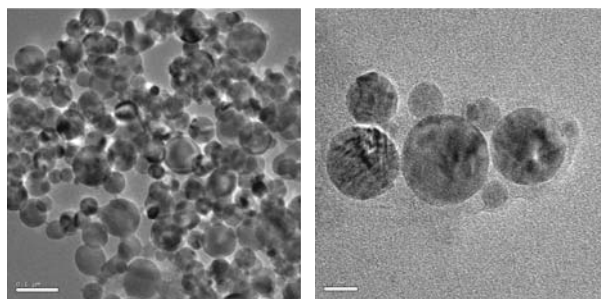
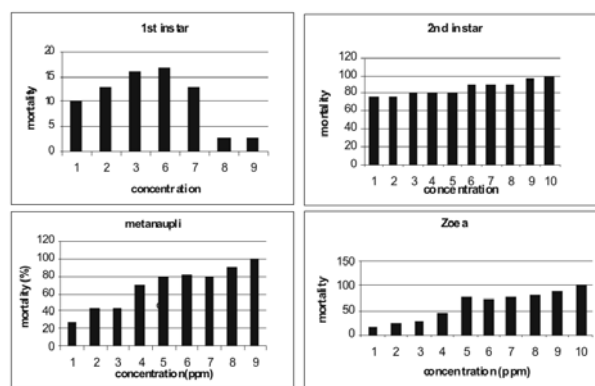
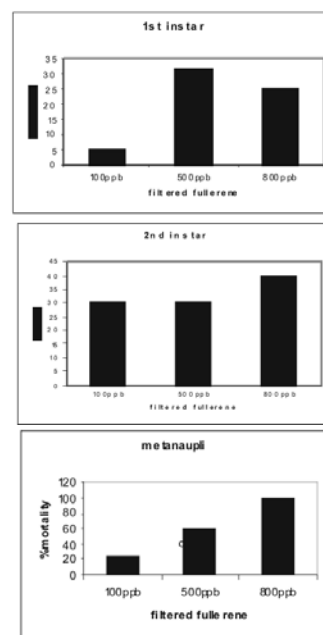
Exposure to titanium dioxide nanoparticle, used in large numbers of sunscreens, cosmetics and personal care products, has been shown to cause far greater cell damage than larger particles of titanium dioxide. It has been demonstrated that 500nm titanium dioxide particles have only a small ability to cause DNA strand breakage, whereas exposure to 20nm particles of titanium dioxide causes complete destruction of supercoiled DNA, even at low doses and in the absence of exposure to UV (Donaldson, 1995). Also in the absence of UV light, in vitro exposure to nanoparticle titanium dioxide resulted in the production of reactive oxygen species in human brain cells although it is not yet known whether these reactive oxygen species damage brain neurons. Pilot data indicate that nanoparticle titanium dioxide results in cell death in cultured neurons at concentrations 20ppm after 24 hours exposure (Long *et al.*, 2006).

The toxicity of fullerenes, currently being used in some face creams and moisturizers, remains poorly understood. However some early experiments have demonstrated the potential for some forms of fullerenes to be toxic. Carbon fullerenes (buckyballs) have been found to cause brain damage in fish (Oberdörster, 2004), kill water fleas and have bactericidal properties (Fortner, 2005). Even low levels of exposure to water soluble fullerenes have been shown to be toxic to human liver cells carcinoma cells and dermal fibroblasts in vitro (Sayes, 2004). Fullerene-based amino acid

nanoparticles have been found to decrease the viability of human epidermal keratinocytes and initiate a pro-inflammatory response (Rouse *et al.*, 2006). Toxicity appears to be a function of both surface structure (Says, 2004) and also the extent of aggregation, where different solvents or emulsion bases are key variables in the formation of aggregates (Rouse *et al.*, 2006).

Fullerene has the capacity for 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 (Oberdoster, 2004).

Centre for ocean Research of Sathyabama University experimented with two nanoparticles, $nTiO_2$ and nC_{60} and assessed their toxicological effects on marine invertebrates, *Artemia salina* (Figure 9a-b) and *Crassostrea madrasensis*. The nanoparticles were prepared using two reliable methods namely, sonication and filtration, both of which resulted in a reduction in size of the regular sized particles. Sonicated fullerenes were observed to be much smaller in size than $nTiO_2$ obtained by the same method (Figures 1-4). Also filtered nanoparticles are found to be smaller than their sonicated counterparts. The results of the present study are important because it is the first study to assess the impact of nanoparticles on the different life stages of *Artemia salina* and *Crassostrea madrasensis*. Amongst the two nanoparticles, the highest toxicity was observed in the second larval stage of *Artemia* exposed to nC_{60} , data obtained from Probit analysis (Figure 5 & 6). Also filtered TiO_2 was found to cause higher mortality than sonicated TiO_2 (Figures 7 & 8). Thus the size, method of preparation and the nature of particles was seen to account for toxicological effects.

Fig. 1. Sonicated nC_{60} Fig. 2. Filtered nC_{60} Fig. 3. Sonicated $nTiO_2$ Fig. 4. Filtered $nTiO_2$ Fig. 5. Mortality of *A. salina* life stages on exposure to sonicated C_{60} Fig. 6. Mortality of *A. salina* life stages on exposure to filtered C_{60}

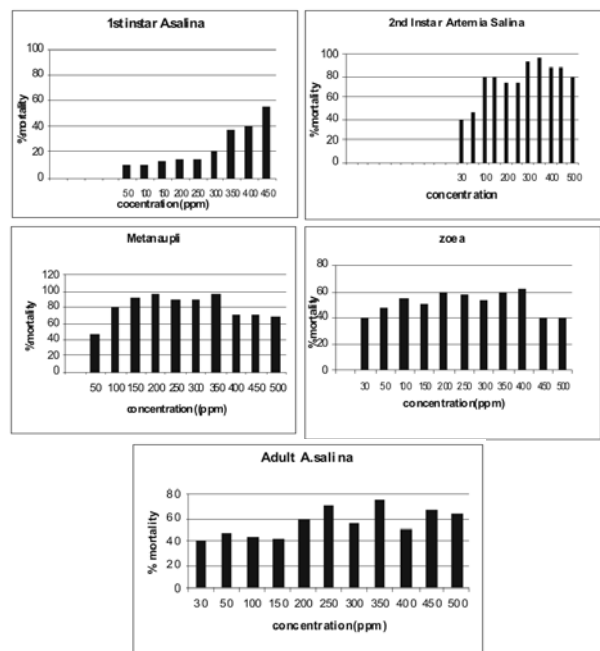


Fig. 7. Mortality of *A.salina* life stages on exposure to sonicated TiO_2

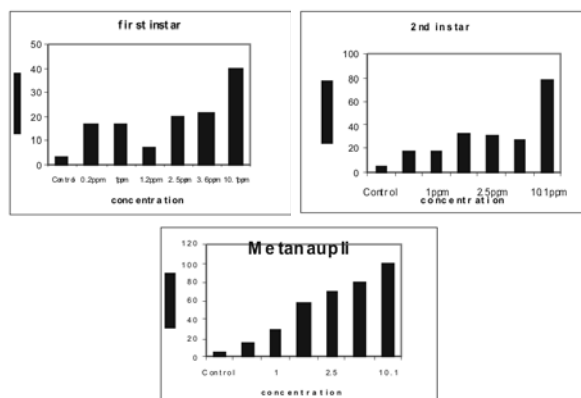


Fig. 8. Mortality of *A.salina* life stages on exposure to filtered TiO_2

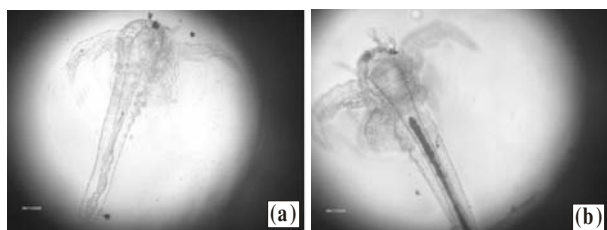


Fig 9a-b. Metanauplii stage of *A.salina* before and after exposure, intestine filled with particles

II. CONCLUSION

The result of the present study gains significance from the fact that it is one of the first to examine the impact of nanoparticles on various life stages of a marine invertebrate. This work has shown that standard toxicity testing can be used to examine nanoparticle toxicity in lower level of organisms. *Artemia salina* is one of the most valuable test organisms available for marine ecotoxicity testing as it is widely used as a nutritious live food source to the larvae of a variety of marine organism, which makes them the most convenient, least labor-intensive live food available for aquaculture and will be impacted greatly by release of nanoparticles into aquatic systems because of their high levels of interaction with the environment through non selective filter feeding. Understanding the potential impacts of these particles can help in identifying the most appropriate nanotechnology that will preserve the marine aquatic environment while also advancing medical and environmental technology. This result also attributes to our limited knowledge regarding the overall implications of these two nanoparticles on marine ecosystems. As the nanoparticles have proved to be detrimental in this acute toxicity test, its effect in the long run is to be carefully analysed. Considering that the smaller nanoparticles turned out to be toxic in the present test, it cannot be excluded that the overall ecotoxicological potential will increase in future.

Hence it can be concluded that, the uncontrolled and unobserved release of these nanoparticles either as byproducts or medical wastes, could have a large negative consequence on the aquatic and terrestrial organisms, particularly on the humans. Hence, studies on the fate and effects of nanomaterials in the environment and in the living organisms are needed to more clearly define the benefits and potential risks of this promising technology.

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