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REVIEW PAPER

Titanium Dioxide Nanoparticles as Oxidative Stress Inducers: A Review on Noxious Corollary

Moksh Tandon, Akshita Gupta, and Anupreet Kaur*

[1] *Dept. of Biotechnology, University Institute of Engineering and Technology, Panjab University, Chandigarh, 160014, India*

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ABSTRACT

Unquestionably, the employment of nanotechnology in every enterprise depicts a future for sustainable development due to the cheap and clean availability of nanomaterials. Evidently, the researchers have largely centered on the blessings of nanomaterials in cosmetics and food industries failing to center the negative effects it could impose on human fitness and the environment. Titanium dioxide (TiO_2) is one such nanoparticle that despite its elite properties, is responsible for the generation of oxidative stress. This review compiles some significant research carried out for the assessment of accelerated oxidative stress markers and the presence of Titania traces in human samples and sea organisms; manifesting the way they are damaging the living mechanisms. The release of the nanoparticles into the environment somehow advances towards land and water contaminating the soil, rivers, and oceans and having a derogatory effect on the natural running phenomena of soil organisms, sea algae, and mussels. This review presents the latest findings and indicates making some strategies to reduce the use of nanomaterials to a significant but limited amount making sure that it is not responsible for any impairment to the humans and its surroundings.

Keywords: *Environmental contamination; Reactive Oxygen Species (ROS); Sunscreen; TiO_2 nanoparticle; Toxicity.*

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INTRODUCTION

DNA damage by reactive oxygen species (ROS), lung inflammation, genotoxicity, apoptosis, and inflammation are among the significant concerns that need to be taken into consideration if people are exposed to Titanium dioxide (TiO_2). TiO_2 nanoparticles could be synthesized in various sizes, going from 1-1000nm which is utilized for the most part in sunscreens, toothpaste, paints and coatings, agribusiness, medication and drug delivery in light of its low cost, chemical stability, antimicrobial properties and so forth[1], [2]. But it's also extremely important to establish that these titania incorporated materials are good for usage[3], [4]. Anatase (yellow to blue), rutile(deep red), and brookite(brown to black) are three predominantly occurring crystalline forms of TiO_2 nanoparticles[5]–[7]. Studies indicate that anatase is the most efficient and used form among these three[5]. Some studies say anatase is also more cytotoxic when compared to the rutile form[8]. However, rutile is viewed as the most favored one to be utilized in sunscreens due to its high absorbance index[9]. Brookite is quite difficult to synthesize and is known to have a high photocatalytic activity[10]. Among the countless beneficial properties of TiO_2, the most important one is its photocatalytic activity and high refractive index. It is speculated that there are three routes through which titanium dioxide enters the human body; 1) dermal entrance by the usage of sunscreens and topical treatments 2) oral

* Corresponding Author Email: *anupreet410@gmail.com*

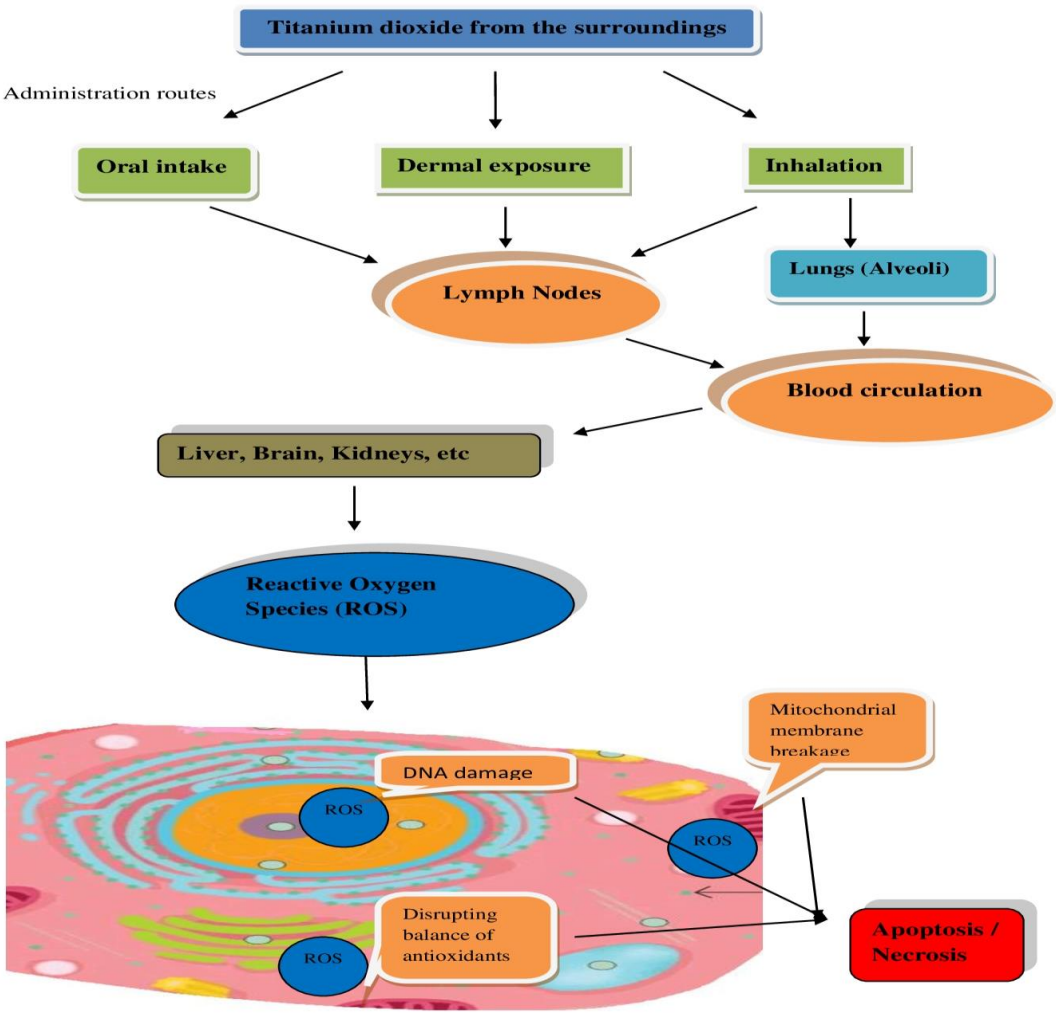

Fig. 1. Mechanism of toxicity induction and cell death by TiO_2 nanoparticles absorption

admission which is through consuming food and 3) inhalation at working environments[11]. Once inside the body, it is phagocytized into cells, where it might bind to the mitochondria, destructing its membranes and electron transport chains or it might attach to the cell nucleus causing DNA damage and altering the gene expression, eventually stimulating loss of cell functionality(Fig.1)([11]–[14]. Titanium dioxide (TiO_2) regardless of its various points of interest for which it is being utilized in many industries like food, paper, and cosmetics is the main concern for causing toxicity in humans, sea creatures, and the environment. In India, more than half of the population uses sunscreen on a daily basis[15]. The affectability towards titanium is high to the point that even the individuals working in a basic printing shop are at a serious danger of TiO_2 being invested in their bodies. Titanium dioxide in 2010 has also been classified as a group 2B carcinogen by the International agency for research on cancer[16].

Titanium dioxide is not only detrimental to humans but also has severe effects on marine life. TiO_2 released into the environment is eventually getting blended with the ocean water which makes the water unfit for the organisms to live[17]. The major release sources of this chemical into the environment are via sunscreens, health care products, effluents from industries, volatile particles from factories, anti-fouling components of paints, and from many other routes. Consequently, the sea waters are getting contaminated and therefore we are witnessing worse effects on humans and marine creatures. The concentration of TiO_2 nanoparticles released from

paints and coatings in the water when compared with other nanoparticles was calculated to be a number as high as 3.5×10^8 particles/L[18].

Titanium dioxide is reported to be one of the leading causes of the generation of reactive oxygen species (ROS) such as hydroxyl radicals($OH^{\cdot}$), hydrogen peroxide (H_2O_2), superoxide anions(O_2^-) that causes oxidative stress[18]–[20]. Oxidative stress occurs when there is an imbalance of free radicals and antioxidants in the body. Uncontrolled oxidative stress can trigger the aging process and may accelerate tissue and DNA damage. Health conditions linked to oxidative stress are cancer, Alzheimer's disease, diabetes, chronic fatigue syndrome, cardiovascular diseases, etc[21], [22]. Blend of TiO_2 with sunscreen lotions shows properties to reflect, scatter, and absorb UV radiations, resulting in photocatalysis and generation of ROS. Instead of preventing the oxidative stress generation by UV radiation, Titanium dioxide nanoparticles contribute to its formation. Researchers state the fact that TiO_2 is a ROS generator and therefore it encourages many mutations in humans. Many studies have been conducted that measures the markers of oxidative stress in humans as well as in marine animals, for example, lipid oxidation gives rise to unstable markers of oxidative stress in the cell-like 4-hydroxy-trans-hexenal(HHE) and 4-hydroxy-trans-nonenal(HNE); that have the affinity for proteins and DNA and are considered cytotoxic[16]. Modulation and doping of nanoparticles are expansively being used these days since they improve their properties. Transition metals like Zn, Cu, Ag, Fe, Al, Ni, and several others are considered as dopants for nanoparticle-based semiconductors. Doping of TiO_2 nanoparticles with Cu is recognized to improve its photocatalytic activity and hence is reported to be used for nanomedicine, agriculture, defense industry, and remediation of water [23], [24]; Although, these doped nanoparticles apparently have some side effects as well. Cu doped TiO_2 nanoparticles reportedly lead to increased toxicity and ROS generation[5].

This review summarizes the toxic effects of titanium dioxide (TiO_2) nanoparticles through examinations conducted on human cell lines, human exposure to TiO_2 in everyday life, and on marine organisms like sea mussels and marine algae. It additionally centers around the inception of reactive oxygen species (ROS) and reasoning that titanium dioxide should be answerable for causing oxidative stress instead of turning it away.

OXIDATIVE STRESS AND REACTIVE OXYGEN SPECIES

The disparity between the free radicals and antioxidants in the body promotes oxidative stress. This irregular number allows the free radicals to act together with other molecules and persuade chemical reactions well-known as oxidation reactions which could be beneficial or harmful. There could be such a significant number of reasons for increased oxidative stress in the body such as exercises, inflammation, or some environmental conditions. Environmental conditions like cigarette smoke, pollution, radiation are also recognized to cause many diseases leading to the generation of free radicals[25].

In the recent scenario, titanium dioxide-based nanomaterials have picked up swiftness in about every business starting from cosmetics to the textile and many more. With the increased application of titanium dioxide in sunscreens, lotions, toothpaste, and in many medicines, individuals have become more prone to their toxic effects in which the major concern is the generation of damaging oxidants. These oxidants include hydrogen peroxide, superoxide anions, and hydroxyl radicals, collectively known as reactive oxygen species (ROS)[26]. All the organisms have their own antioxidant mechanisms such as low molecular weight antioxidant molecules like glutathione (GSH), melatonin, some enzymes like superoxide dismutase (SOD), catalase (CAT) and many more. GSH works by destroying H_2O_2 and SOD destroying superoxide radicals. Oxidative damage to the essential biomolecules results in alterations in some biological functions such as signal transduction and gene expression for mitogenesis and mutagenesis[7], [27]. As the size and surface area of TiO_2 nanoparticles show greater affinity to produce reactive oxygen species, it could be anticipated from the studies that oxidative stress pathway has a role in injuries induced by nano TiO_2[28].

Mechanism of reactive oxygen species generation

The mechanism of ROS generation differs from nanoparticle to nanoparticle. Titanium dioxide, Zinc oxide, and silver nanoparticles tend to deposit on the cellular surface of organelles or inside the sub-cellular organelles eventually exerting a cascade of oxidative stress giving rise to the reactive oxygen species.[29] Most of the metal-based nanoparticles give rise to ROS via Fenton based reactions[30]. A Fenton reaction takes place when

a transition metal ion reacts with H_2O_2 to form an $OH^{\cdot}$ radical and an oxidized metal ion. These hydroxyl ions are considered to be extremely reactive with biological entities inside a cell. ROS include superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). There are certain factors involved in the generation of reactive oxygen species by metal oxide nanoparticles such as 1)active redox cycling on the surface of NP due to transition metal-based NPs, 2) pro-oxidant functional groups on the reactive surface of NPs and 3) particle-cell interactions[29], [31].

Transition metals are fit for surface adjustment in order to be more stable and have a better binding capacity. A relatively inert metal or metal oxide could become more reactive when prepared in nano dimensions. A high surface area to volume ratio makes these metal nanoparticles more inclined to ecological stressors like free radical generation[32]. Metals could produce free radicals by the means of Fenton-type reactions that react with cellular macromolecules and induce oxidative stress. It has been reported that pro-oxidant metals like Cu and Fe react with the lipids and proteins present in the biomembranes giving rise to the DNA damaging end products like malondialdehyde (MDA) that act as inflammatory mediators and risk factors for carcinogenesis. Exposure to these pro-oxidant metals like Ti, Cu, Fe, Si induces lipid peroxidation, skin, bladder, liver, and lung cancers[29], [33]. The immune cells of the lungs such as alveolar macrophages and neutrophils play the role of ROS inducers. Therefore the nanoparticles could activate the cellular redox system in the lungs. NPs react with cells and induce their pro-oxidant effects via intracellular ROS generation. In addition, this absorption of certain chemical entities on the surface of nanoparticles gives rise to the inflammation-based ROS generation [29],[34].

Size-dependent toxicity of TiO_2 nanoparticles

Continuing with the same line of reasoning, the metal oxides tend to improve their stability, binding, and reactivity when being converted into nanostructures[29]. According to the study conducted by Xiong et al., 2012, a converse connection between the phototoxicity and the size of nanoparticles was observed. They concluded the fact that phototoxicity of nanoparticles is because of their high surface area to volume ratio making more TiO_2 molecules to be surface exposed[35]. It has additionally been accounted that smaller particles tend to generate more hydroxyl radicals. These hydroxyl radicals are exceptionally reactive and have the potential to damage any bimolecular entity. As smaller size provides more surface area to biomolecules in order to attach to the nanomaterial surface, it could be proved that the formed hydroxyl radical can decimate more biomolecules that come in contact with the nanomaterial.

This study also concluded that the size-dependent toxicity of TiO_2 nanoparticles could be because of two main reasons that are, size-dependent ROS generation and size-dependent biomolecules absorption[35], [36]. When TiO_2 nanoparticles are excited using a UV light, the electrons (e^-) in the valance band leave for the conduction band making a hole (h^+) in the valance band. The holes (h^+) in the valance band then interact with the water (H_2O) and give rise to hydroxyl radicals ($OH^{\cdot}$) which because of its high reactivity could damage any bio-molecular entity. Additionally, the electrons (e^-) in the conduction band could interact with an oxygen atom and reduce it into superoxide ions (O_2^-) which will eventually form hydrogen peroxide in the cells after reacting with the water present in the environment. The mechanism is depicted in Fig 2.

ROUTES OF ADMINISTRATION OF TIO_2 NANOPARTICLES

Dermal exposure of titanium dioxide nanoparticles

Dermal exposure to nano TiO_2 containing sunscreens and other topical lotions is raising many eyebrows. Sunscreens are used by roughly one-third of the world population in order to avoid harmful UVA(320-400nm) and UVB(290-320nm) radiations emitted by the sun[37], [38]. The TiO_2 employed in sunscreens acts as an inorganic sun blocker in order to absorb UVB radiations[1], [7]. Mostly the rutile form of TiO_2 is preferred to be used in sunscreens because it is less photo reactive[39]. The size of the commercial TiO_2 used in lotions is around 30-40nm. As the ultrafine nanoparticles seemingly do not go through human skin beyond the stratum corneum, the outermost layer of the skin, there are traces of evidence that TiO_2 exists in blood plasma and urine samples taken from volunteers after a certain time of sunscreen application[16]. If these nanoparticles travel ahead of the stratum corneum, the oxidative stress-induced causes adverse cellular effects and potentially cancer[16]. In accordance with a study, six volunteers

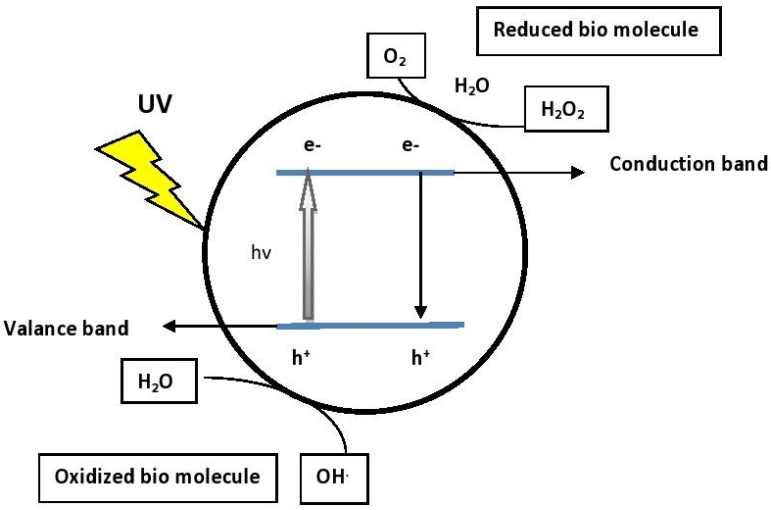

Fig. 2. Mechanism of ROS generation by TiO_2 nanoparticles

were studied for their blood plasma, urine, and exhaled breath condensate (EBC) for 11 days. The volunteers were selected on the basis of their exposure in three forms viz. 1) nano TiO_2 sunscreen 2) exposure to UV radiation 3) TiO_2 sunscreen+ exposure to UV radiation. The samples were acquired at different time intervals for 11 days.

The data gave significant insight into the TiO_2 concentration that was detected in 6 volunteers after the application of sunscreens and exposure to UV[16]. The first test in which only the sunscreen was applied to the volunteers testified significant traces of titanium in the subjects without elevating the markers of oxidative stress. Therefore, indicating that the sunscreens containing TiO_2 nanoparticles do not elevate biomarkers of oxidative stress but get absorbed into the skin. The second test in which volunteers were exposed to UV radiations only, showed an increase in the level of biomarkers associated with oxidative stress, which means UV radiations are associated with the generation of ROS. The application of sunscreen in the third test along with UV exposure did not prove to have a positive effect on those elevated markers as their levels remained prominent. Thinner skin in women compared to men was also considered as a major factor among others like hormone metabolism, hair growth, fat accumulation, etc. for the augmented penetration of TiO_2[40], [41]. Thus it was speculated that TiO_2 gets absorbed in the skin and might lead to ROS generation on exposure to UV.

Although many studies have suggested that most of the microfine and ultrafine TiO_2 nanoparticles in sunscreen formulations do not cross the outermost layer of skin (stratum corneum) as an insignificant amount of Titania was detected in lymph nodes and liver, stating that dermal exposure through application of sunscreens and other TiO_2 containing lotions are quite safe to be applied on daily basis[1], [11]. But there are some studies and reviews that suggest the opposite. A brief account of such studies is presented in Table 1. It is also factual that when sunscreens are applied to the cracked or injured part of the skin some amount of Titania enters the body and may interact with the bloodstream or build up in any of the body parts ultimately causing oxidative stress. Thus a prolonged exposure to lotions containing titanium nanoparticles could be absorbed into the skin and if not washed properly it gives rise to health problems.

Inhalation of titanium dioxide nanoparticles

Nanoparticles may get suspended in the air throughout manufacturing, distribution, and utilization. These hovering nanoparticles in the atmosphere could be inhaled, leading to harmful impacts on the primary target that is the respiratory tract[11]. Many studies have reported inflammation in the lungs of humans and mice due to the inhalation of TiO_2 nanoparticles present in the air[42]. Inhalation is considered one of the major routes of TiO_2 exposure especially at work places[43]. Choi et al.,(2010) reported that upon inhalation these nanoparticles can travel to different organs via the bloodstream. The laser light utilized

Table1. Studies suggestive of the penetration of TiO_2 nanoparticles from sunscreens into the skin

Model type	Dosage	Penetration evidence	References
1. Human volunteers			
• Male	2 mg/cm^2 skin, 2 applications/day for 3 Months	Traces of TiO_2 found in Urine, blood plasma and Exhaled breath condensate (EBC)	(Effros *et al.*, 2003; Lischkova *et al.*, 2019; Pelclova *et al.*, 2019)
• Females	2 mg/cm^2 skin, 2 applications/day for 3 Months	Comparatively more amount of TiO_2 was found in urine, blood plasma and EBC of females	
2. Middle aged males	2 mg/cm^2, 6 applications/day for 7 days	TiO_2 nanoparticles penetrated the skin beyond stratum corneum into viable cells of epidermis	(Mavon *et al.*, 2006; Schilling *et al.*, 2010; Næss *et al.*, 2016)
3. Mice and Pig	5% Anatase(4 and10nm)and rutile (25,60 and90nm), for 30 days	TiO_2 was detected in Stratum granulosum, prickle cell layer, and basal cell layer, but not in the dermis. Only 4nm nanoparticles reached dermis	(Gamer, Leibold and Van Ravenzwaay, 2006; Wu *et al.*, 2009; Robertson, Sanchez and Roberts, 2010)

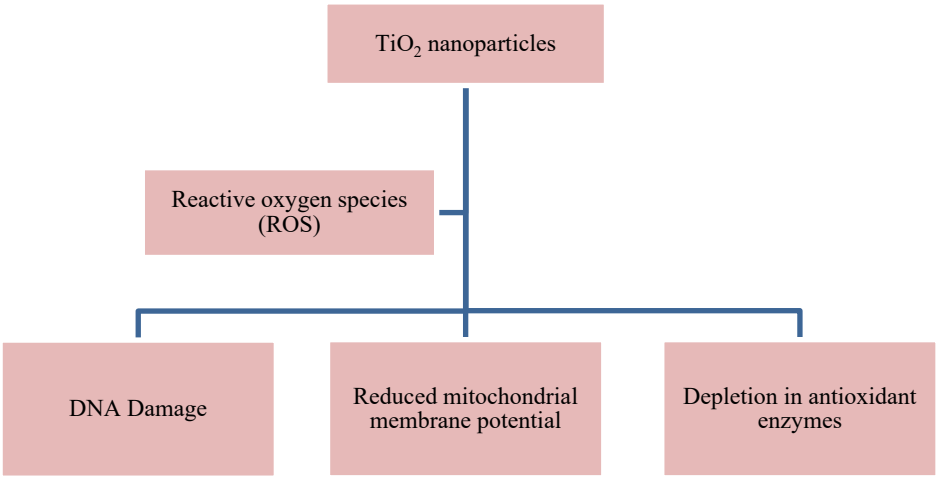

Fig. 3. Damage caused by reactive oxygen species

in a printing press is an eminent source of radiating expansive sums of nanoparticles into the surroundings and the people working there are very much vulnerable to getting serious health issues. Fig. 3 depicts the damage caused by TiO_2 nanoparticles by the induction of ROS.

Moreover, this exposure to TiO_2 is identified to stimulate the formation of reactive oxygen species. Production of hydroxyl radicals surfaced as the lungs of a rat were exposed to titanium dioxide nanoparticles[17]. Polyunsaturated fatty acids that are located in the plasma membrane are oxidized by these ROS which results in the breakdown product formation, malondialdehyde (MDA) of lipid peroxidation[11]. The high reactivity index of nano titanium dioxide is the leading cause of ROS mediated cytotoxicity and genotoxicity. In the human lungs, it has been reported that the inhaled TiO_2 could rise free radicals and cause genotoxicity mainly by DNA adduct formation. Reports have also suggested that, in the human bronchial epithelial cells, induction of ROS has decreased the level of intracellular GSH [45], [46].

The liver is the main distributor of organic and inorganic compounds that enter the circulation through various routes. It has been indicated that most of the TiO_2 that enters the body is accumulated in the liver, kidney, and spleen causing oxidative stress, and lipid peroxidation by decreasing SOD and GSH activity[47]. There are scarce studies reporting the effect of TiO_2 on human kidneys, liver, or lungs but one such study conducted on zebra

fish, concluded that acute exposure caused the $OH^{\cdot}$ radicals to boost significantly[11]. These studies confirmed that Titania exposure gives rise to a free radical generation that may lead to depleted GSH, reduced mitochondrial membrane potential in rodents, and impair nephric functions of kidneys.

The effects of pure TiO_2 in comparison with Cu doped TiO_2 were studied[5]. Copper doping is identified to improve the photocatalytic efficiency of Titania which eventually could be applied to many fields like environmental remediation, medicine, and many more. Although the contact of Cu and TiO_2 mixture with humans is rare but is recognized to cause lung disorders. The scientists examined the exposure of human lung epithelial (A549) cell line with pure and Cu doped TiO_2 through different assays and characterization techniques like MTT assay, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and others. The accomplished results indicated that A549 cells when treated with Cu doped TiO_2 had a higher cytotoxic response than the treatment with pure TiO_2. The cell viability was observed to drop from 86% to 42% due to pure TiO_2 and 77% to 33% owing to Cu doped TiO_2 at concentrations ranging from 25-200μg/ml. In order to evaluate whether or not the cytotoxic response is mediated by ROS, A549 cells were also treated with pure and Cu doped TiO_2 in the absence and presence of ROS scavenger N-acetyl cysteine (NAC). NAC was not only capable of scavenging ROS generated by pure and Cu doped TiO_2 but also was able to control the cell viability drop. Hence, concluding that the cytotoxic response leads to ROS generation.

Doping of TiO_2 nanoparticles isn't constrained to just Cu but numerous different metals are also utilized to improve the properties of Titanium dioxide. As indicated by the investigation completed by Balbi et al., 2016 TiO_2 nanoparticles were doped with $Fe^{3+,}$ and tests were carried out on humans as well as on marine models. The doped TiO_2 samples were synthesized by sol-gel method utilizing titanium isopropoxide as a precursor for titania, 2-propanol as a solvent, and iron (III) as dopants[48]. The experiment was carried out on human umbilical vein endothelial cells (HECV) and mussels (*Mytilus galloprovincialis*) hemocytes. In both of these models, the toxicity of Fe^{3+} doped TiO_2 was recorded as an increase in ROS/RNS formation. In both HECV cell lines and mussel hemocytes, the vitality of cells was undermined, and photocatalytic activity was also declined. Furthermore, it was inferred that because of the small size of these doped nanoparticles they could undoubtedly reach the blood circulation regardless of the route of administration and aggregate near endothelial tissues causing inflammation and oxidative stress[49].

Amongst others, zinc (Zn) and aluminum (Al) are likewise considered as potential dopants for TiO_2 nanoparticles following the mechanism of sol-gel synthesis. Analysts considered the impact of Zn and Al doping in MCF-7 cells of the human breast cancer cell line and the outcomes obviously expressed that doping caused cytotoxicity and oxidative response in the cell line[50], [51]. These dopants reportedly actuated cell viability reduction, membrane damage, and cell cycle arrest, and glutathione depletion. An increase in Zn doping increased the cytotoxic response and also down-regulated the superoxide dismutase gene. On the other hand, Al doping up-regulated apoptotic gene proposing apoptosis of MCF-7 cells via the mitochondrial damage pathway. Thus in addition to the enhancement of photocatalytic activity and bioremediation potential of TiO_2 nanoparticles via doping, these dopants are largely responsible in the generation of cytotoxicity in humans as well as in the marine creatures.

Oral intake of titanium dioxide nanoparticles

With the changing lifestyles and eating habits of people, it has become obligatory to reassess and set some ground rules for the consumption of TiO_2 by humans which is initially considered superfluous by the World Health Organization(WHO) expert group on food additives[2]. Titania is found in food both in bulk form and in nano form[60]. TiO_2 is used in consumer products because of its high refractive index and higher opacity. The worldwide production of nano TiO_2 was reported to be 10% in 2009 and chewing gums, candies, and jellies were found to contain the majority of TiO_2. It has been estimated that approximately 50% of TiO_2 will be manufactured in nano form by 2023[42]. Owing to titanium dioxide's brilliant photocatalytic activity it is chiefly used as a white pigment in most of the products. In the wake of the need for setting a minimal consumption need of these nanoparticles, studies have evidenced that TiO_2 is not suitable when consumed orally. A study was conducted in 2012 in which researchers characterized commercially available food-grade TiO_2 (E171)[60]. Morphology and size of this food additive were examined by TEM. The size came out to be in the range

Table 2. Toxic effects imposed by exposure of TiO_2 nanoparticles

Mode Model	Type of nanoparticle	Route of administration	Effects	References
Human	Anatase, brookite, rutile or mixture	Oral,dermal, inhalation	Genotoxicity; cardiovascular system malformation; oxidative stress; increase in diastolic blood pressure; disturbed Blood Brain Barrier; Liver fibrosis	(Ghosh, Chakraborty and Mukherjee, 2013; Hong and Zhang, 2016; Baranowska-wójcik *et al.*, 2019)
Rats	Anatase TiO2, mixture of anatase and rutile	Intravenous, oral	Oxidative stress, liver and heart damage; cardiac muscle damage; decreased neurogenesis; increased number of apoptotic cells in hippocampus	(Zhu *et al.*, 2008; Schneider and Lim, 2018; Baranowska-wójcik *et al.*, 2019)
Mice	Anatase TiO_2;Food grade TiO_2	Oral; intravenous	Colon cancer; spleen damage; chronic gastritis; oxidative stress; inflammation in lungs; necrosis of liver cells	(Wang *et al.*, 2007; Baranowska-wójcik *et al.*, 2019)
Mediter-ranean mussels	Mostly anatase	Dermal, oral	Hormonal imbalance; lysosomal alteration in digestive glands; changes in immune parameters; loss of the ability to be ecosystem engineers; oxidative stress	(Sureda *et al.*, 2018; Hou *et al.*, 2019)
Marine algae	Mostly anatase, Rutile	Dermal	Growth inhibition; lipid peroxidation; cytotoxicity; genotoxicity ; decreased membrane integrity	(Xia *et al.*, 2015; Sureda *et al.*, 2018)
Fish	Anatase, rutile, brookite or mixture	Dermal, oral, Through gills	Genotoxicity; cytotoxicity; decrease in cell viability; loss of balance; hyperactivity; oedema; thickening of primary gills lamella	(Reeves *et al.*, 2008; Lee *et al.*, 2012; Pirsaheb *et al.*, 2019)
Earth-worms	Anatase, rutile, brookite or mixture	Oral, dermal	Decrease in enzyme activity; Mitochondria showed loss of cristae; abnormal structural change; DNA damage; depletion in protein thiols; Oxidative stress	(Hu *et al.*, 2010; Dec and Virginia, 2014)

of 40-200nm which was taken as proof that these food additives are of nano range. Results concluded that the different TiO_2 concentrations brought about an acute cellular toxicity in the viable cells in a dose-dependent not time-dependent manner at lower doses. The cellular viability was reduced by 10%-20% at a low dose but at a higher dose the cellular viability was restored to normal. Treatment with TiO_2 elevated the reactive oxygen species in dose and time-dependent manner. The intracellular level of ROS in the cells was evidently and radically altered relative to control cells. Cellular uptake as another parameter was measured by flow cytometry. It indicated that a higher concentration of TiO_2 caused higher side scatter which means cell density of nanoparticles increased and forward scatter was decreased, indicating a smaller size nanoparticle.

Another similar study conducted by[19], on the effects of TiO_2 exposure on the human hepatic cell line, WRL-68 proved that the exposure of TiO_2 is bound to cause oxidative stress in the cell line and hence reactive oxygen species generation is induced. Table 2 contains some of these studies conducted on various models and how they are affected by TiO_2 exposure.

Estimation of TiO_2 uptake by the cell line was done by flow cytometry and the measurement of reactive oxygen species was done through assays like MTT and neutral red uptake (NRU). It has been reported that the nano TiO_2 could be incorporated into cell membranes and may get endocytosed from the extracellular fluid that eventually causes damage and the destruction of organelles[61]. Results demonstrated that as the nanoparticles intake by the cells increased, the oxidative stress also increased proportionally. ROS has a major role in the toxicity of nanoparticles, there was also an increase in ROS generation in WRL-68 cell line when it was

treated with TiO_2 nanoparticles. In both cases as the concentration of TiO_2 increased, cellular uptake and ROS were augmented. In contrast to the previous study, in this experiment proliferation of the cells was observed in order to increase with an elevation in the concentration of TiO_2. This showed that cell viability was increased and apoptosis was reduced. Apoptosis is usually observed when the internal environment of the cell is compromised whereas in this case, TiO_2 seems to have no negative effect on viability.

OXIDATIVE STRESS GENERATION IN SEA ORGANISMS.

In an article which was titled "The Maturing Nanotechnology Market: Products and Applications published by BCC research, it was stated that by the year 2021 the nanotechnology market should reach 90.5 billion dollars at an annual growth rate of 18.2%. These nanoparticles are inevitably discharged into the environment and eventually get fused into the oceans. With the escalating exploitation of nanoparticles in science and technology, it has become vital to comprehend their eco-toxicological impacts on marine life. These nanoparticles are making their way to the oceans and rivers mainly through sunscreens and food items that consequently hinder with the marine life[22], [71], [72]. The three main sources from which these nanoparticles are released into the ecosystem are sewage, personal care products, and anti-fouling agents in paints and coatings[73]–[75]. Since the sea is considered the final recipient of all the pollution from land, scientists have carried out numerous studies to prove the presence of titanium dioxide nanoparticles in water [66][76][77].

The use of sunscreens in order to protect against UVA and UVB radiations on the beach is considered to be one of the leading causes of contamination by titanium dioxide at sea. A particular study contended that the injection of TiO_2 into the sea produces ROS in marine species such as Mediterranean mussels (*Mytillusgalloprovincialis*), with proof of titanium traces found in their gills[78]–[80]. Furthermore, it was reported that sunscreens contain many other organic compounds with hazardous effects to the environment such as benzophenones, cinnamates which play the role of UV filters. However, these organic filters have been reported to have an unconstructive impact on the hormonal balance of animals and no known effect on human models [81], [82]. Various studies were also performed to assess the levels of oxidative stress biomarkers in mussels. The results concluded that as the mussels are treated with increasing concentration of sunscreens, Titania accumulation in the gills become more notable as compared to the control groups. The biomarkers of oxidative stress like SOD, catalase, and others show an increase when treated with 0.2g and 2.0g of sunscreen[20]. Thus, the damage caused by free radicals to mussels makes them eventually lose their ability to be ecosystem engineers, modifying aquatic habitat so that making them suitable for themselves and other organisms to live in.

The growth inhibition, oxidative stress, and accumulation of TiO_2 in marine algae and cytotoxicity and genotoxicity in goldfish skin cells have been illustrated in many other studies[66]. It is concluded that the growth of marine algae was inhibited when the TiO_2 nanoparticles of 21nm and 60nm were internalized[18]. Researchers suggested that the 21nm nanoparticles were more harmful than 60nm particles. It was found that the growth was promoted at lower concentrations of TiO_2 and inhibited at higher concentrations corresponding to hormesis[19].

Another study was performed on goldfish skin cells that detected oxidative damage and genotoxicity due to the interaction and accumulation of TiO_2 nanoparticles. Co-exposure of goldfish cells to TiO_2 nanoparticles and UVA radiations was studied that showed a decrease in cell viability[66]. At the highest concentration of nanoparticles, the viability was observed to drop by two folds. Similarly, when the cells are treated with different doses of TiO_2, they indicate a considerable boost up in oxidative damage, and when these damaged cells were incubated under UVA, further express an increase in the oxidative damage by ROS generation[83].

TOXICITY STUDIES OF NANO TiO_2

In the year 2011, the International Agency for Research on Cancer classified TiO_2 as group 2B carcinogen[16]. The research was focused on the fact that when tested on mice, TiO_2 induces cancer of respiratory tract. Subsequently, it was apprehended that TiO_2 may also cause tumors in humans since the mechanism that causes lung cancer in mice seems identical in humans[1]. A few studies have reported the likely risk of NPs on human health, based on inflammatory reactions caused by ferric oxide NPs in rats and toxic effects of silica NPs on fibroblast and tumor cells [64], [84]. As discussed in as previously discussed many authors have identified the toxicity of nanoparticles in various spe-

Table 3. Effects of nanoparticle exposure on an earthworm

Set up	Nanoparticle content	Enzyme activity (SOD,CAT and MDA)	DNA damage	Mitochondrial damage
Control	No NP traces	Normal activity	No damage	No damage
Experimental	NP traces increased with dosage	SOD – NO significant change CAT- Activity decreased at 5g kg^{-1} soil MDA- Activity decreased at 5g kg^{-1} soil	Significant damage observed at dosage above 1g kg^{-1} soil	Mitochondria showed loss of cristae, abnormal structural change

cies varies depending upon the size and form of nanoparticles with which they have come in contact. Especially toxicity increases as particle size reduce [85]. Light has also been shown to play a critical role in the toxicity of TiO_2 nanoparticles. Experimentally it has been established that when certain microorganisms were treated with TiO_2 nanoparticles under light and dark conditions, the various effects of nanoparticles were observed. *E.coli*, for example, showed a higher susceptibility to growth inhibition in light conditions when treated with median lethal concentration (LC_{50}) of nanoparticles than in the dark[86], [87].

Similarly, the antibacterial analysis demonstrated TiO_2 nanoparticle's antimicrobial activity against *B. subtilis* and *E. coli* increased 2.5 and 1.8 times in the presence of light, respectively [65]. Thus, the toxic impact of nanoparticles on the microorganisms could also induce an environmental imbalance to the natural microbiota. Table 3 compiles the toxic effects caused by nano TiO_2 on various experimental models.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are viewed as vital factors in the apoptosis of cells. Excessive ROS development has recorded to initiate membrane permeability, damaging the respiratory chain in order to start the apoptosis process[19]. The oxidative stress that generates due to the inflammatory response in the lungs, surrounding the macrophages and neutrophils is generally answerable for the damaging impacts on multi-cellular organisms[31]. At a specific time, production of ROS outperforms the antioxidant count in the cells and oxidizes the biomolecules inducing many modifications in the DNA. The reaction of ROS and RNS with DNA brings about fragmentation of DNA with loss of bases and strand breaks [70]. DNA damage has been associated with the killing of both bacterial and mammalian cells by oxygen radicals. Accumulation of ROS/RNS by TiO_2 nanoparticles has also contributed to kidney damage as well. According to the experiments ran on mice, it was observed that after the accumulation of these reactive species, nuclear factor (NF)-κB was activated which in turn promoted the expression of tumor necrosis factor (TNFα). This suggested that injury to the kidneys is associated with nanoparticle-induced ROS/RNS[88]. Moreover, the toxicity of TiO_2 was studied with the treatment of mice with these nanoparticles, which indicates that deposition of nano TiO_2 in the spleen resulted in the over-production of ROS which further increased splenic inflammation and necrosis in time-dependent manner[89]. The deposition of nanoparticles in the heart of the mice subjected to inflammation, cardiac biochemical dysfunction, cell necrosis, and ROS production disturbed the antioxidant population in the heart[90].

Cytotoxicity and genotoxicity caused by TiO_2 could be related to its photocatalytic effect. TiO_2's photocatalytic activity is considered to be of great importance in applications such as environmental remediation and wastewater treatment but it also contributes to the production of free radicals in the environment. These free radicals, as discussed in above sections are the major reason for causing toxicity in cells. It has been reported that the anatase form of TiO_2 shows a superior photocatalytic activity than the rutile form that makes it more liable to cause toxicity[91]. It is speculated that nanoparticles get accumulated in the human body, though after limited but continuous exposure for a longer period of time. Scientists nowadays have shown a surge in the Titanium dioxide nanoparticles due to its proficient photocatalytic activity. On being exposed to UV, the array of ROS creat-

ed by TiO_2 can prompt apoptosis which could be helpful in the treatment of tumor cells. Owing to this ability, TiO_2 has been perceived as a potential candidate for photodynamic therapy (PDT), which is the treatment of a wide range of tumor conditions and antimicrobial activity[92]. TiO_2 could be effectively utilized for PDT, as well as their composites. Despite this useful property of TiO_2, its toxicity poses a hindrance to its wide-scale utilization for PDT. Thus to evaluate the phototoxicity of TiO_2 a study was carried out on HaCaT human skin keratinocytes using anatase and the rutile form of TiO_2 nanoparticles[93]. The outcome indicated that TiO_2 NPs are unsafe to human skin due to the production of ROS which has a detrimental effect on the skin's keratinocyte cells. Additionally, it was recorded in this study that the rutile form of the NP was less toxic than the anatase form. Another investigation was led on human monocyte leukemia (THP-1) which indicated that cell viability due to TiO_2 exposure in PDT was significantly reduced[94]. Still, in order to defeat this disadvantage a mix of TiO_2 with other photosensitizers (PS) is viewed as effectual in reducing the cytotoxicity of both TiO_2 and photosensitizer like porphyrin[92]. The combination of TiO_2 with other photosensitizers is achieved through encapsulation, adsorption, and covalent linkage which enhances its ability to be excited by the visible light and not just limited to UV exposure[92].

Numerous metal oxides in nano form (3-200nm) are used as vehicles in photodynamic therapy. The nanoparticles used include dendrimers, liposomes, viral nanoparticles, nanotubes, magnetic nanoparticles, and carbon material. In contrast to TiO_2, zinc oxide (ZnO) nanoparticles are likewise viewed as efficient in PDT as a photosensitizer due to their good physicochemical properties as drug delivery agents[95]. The significant advantage of using ZnO in PDT is its property of generating visible light upon irradiation by X-ray. As most of the PS absorbs light at a low wavelength, ZnO is considered to improve PDT as it may be used as a source to irradiate on deep routed tumors[96]; however, as far as the safety and cytotoxicity are concerned, it has been accounted that in ZnO-PS frameworks, the solubility of ZnO in extracellular matrix prompts the increase of Zn^+ in an intracellular matrix which therefore builds the cytotoxicity[95]. Fullerene, the C_{60} form of carbon has also gained interest as a potential PS for PDT and other medical applications. Functionalized fullerenes with carboxylic acid groups have discovered new applications in PDT, owing to their electron and energy transferability. Similarly, graphene or graphene oxide and many more have found their potential applications as photosensitizers[97],[98],[95].

Nanotechnology has made its way to almost every industrial sector and in the food sector, candies are considered to be containing the highest quantity of nanoTiO_2. On the other hand cement and concretes contain about only 2% Titania. Sunscreens that are responsible for the release of Titania into water bodies contain more than 25% of TiO_2, paints and coatings also contain a higher concentration of Titania[99]. A diagrammatic representation of Titania's percentage estimate in various products is shown in Fig.4[88]. Titania nanoparticles could cause morphological changes in cells on steady exposure and the most vital organ in the human body, the brain is also affected[20]. Nanoparticles are widely acknowledged to go into deeper matrices of the human body in order to fulfill its application in drug delivery. Since these particles can cross any barrier, the most austere and sturdy, the blood-brain barrier is also affected[100]. On subjection, nanoparticles can make their way to the central nervous system, damaging brain cells and, showing some cytotoxic effects with inhibition to growth[101]. Nanoparticles could also be seen accumulated in the cerebral cortex, cerebellum, and hippocampus region. Wang et al.,(2008) reported that TiO_2 nanoparticles are first absorbed by the olfactory bulb then get transferred to the hippocampus and CNS with extended time and cause changes in the nerves and cellular morphology. With this accumulation, memory cells and learning ability are also impaired.[26]

Due to their worldwide use, nanoparticles are receiving much attention. One of the main areas of contamination and toxicity by nanoparticles is soil. Soil is the home to millions of creatures and plants that will ultimately take up the nanoparticles present in it. NPs may hinder their growth mechanisms and enter the food chain, thus reaching the topmost predators. It is evident that TiO_2 is responsible for the depletion of plant growth-promoting bacteria(PGPB) from the soil thus hindering the plants proper growth in the TiO_2 contaminated soil[103]–[105]. Scientists recognized that the living mechanisms of soil organisms are affected by the presence of TiO_2 in their environment. 12 earthworms were put in wide-mouth bottles in artificial soils, provided with sufficient light and food sources. One bottle

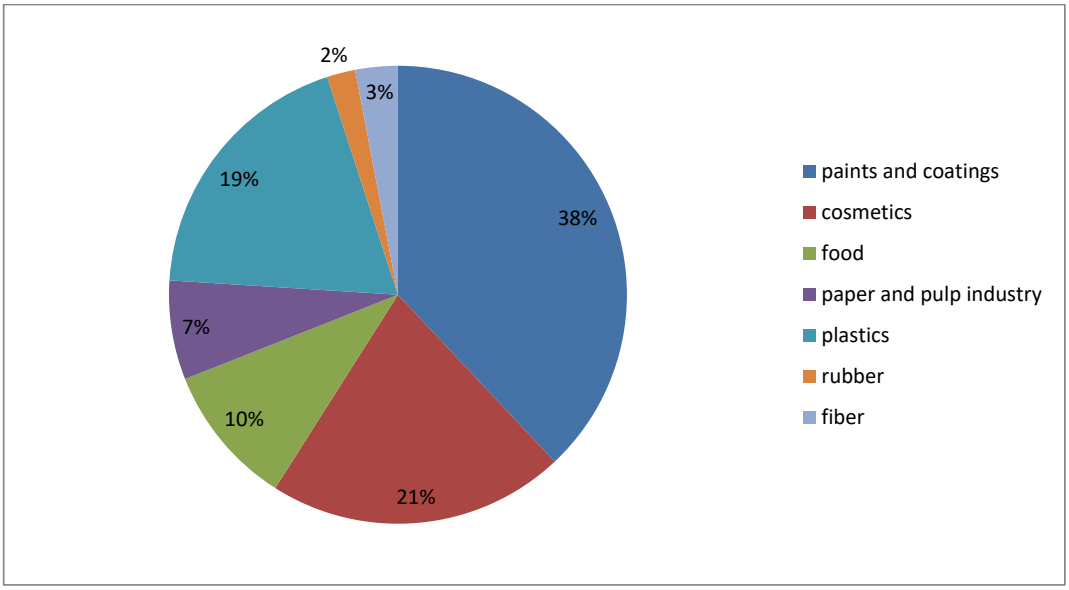

Fig. 4. Employment of TiO_2 nanoparticles in various sectors

was kept as control and in the other bottles, dried powder of TiO_2 and ZnO nanoparticles were added in different concentrations(0.1,0.5,1.0 and5.0 g Kg^{-1}). When all of the tests were conducted, the earthworms were put in Petri plates on moist filter paper for 24h and were permitted to remove their gut contents. The recorded results after the 7-day exposure are depicted in Table 3[69]:

This experiment performed on the earthworms reasoned that there were no huge changes in the mechanisms of the control group. While in the experimental group, increment in traces of nanoparticles was found in earthworms with an expansion in the dose. A noteworthy decrease in CAT and MDA activity was recorded at the nanoparticle concentration of 5g/Kg. Mitochondria also exhibited an abnormal structural change and a significant amount of DNA damage was observed above the dosage concentration of 1g/Kg.

Direct contact with nanoparticles could cause more serious damage to the human body depending upon the route of administration. Oral toxicity issues are derived from oral administration such as from food consumption and dermal toxicity problems due to sunscreen application and other lotions containing titanium dioxide nanoparticles. Lastly, the most important concern of nanoparticles dust inhalation is from the exposure in workplaces. The risk assessment of TiO_2 nanoparticle exposure was done studying its effects on fetal development. Researchers reported that on the exposure of TiO_2 (100mg/Kg) to pregnant rats, apoptotic cell count increased significantly in the hippocampus followed by a decrease in neurogenesis. Also, premature ovarian failure was noted by the ingestion of the nanoparticles. These trials in mouse models indicate that TiO_2 has an adverse impact on pregnancy, but no such risk in humans has been reported yet[21].

CONCLUSIONS

Nanotechnology has blasted into one of the fundamental territories today and is utilized to change current particle attributes. With the expanding applications of nanomaterials in nourishment and cosmetic sectors, monitoring the elimination of nanoparticles into the environment has become the utmost significance. The abolition of these nanoparticles is quite harmful to the environment as it distorts the ecosystem and hinders many natural procedures. The essential explanation of limiting the utilization of or decrease in the degrees of nanoparticles in purchaser items is to abridge their contact with people to forestall any medical issues. It is apparent now that TiO_2 nanoparticles are equipped for causing oxidative stress in humans as well as in marine creatures, regardless of their several supportive qualities. Oxidative stress incited by TiO_2 causes serious issues of wellbeing after some time, for example, malignancies, changes in cell morphology, DNA damage, necrosis of cells, and harmful impact on the brain, cardiovascular

problems, and so forth. It is likewise perceived that genotoxicity and cytotoxicity are brought about by photocatalytic impact which is viewed as one of the most yielding properties of titanium dioxide nanoparticles. It is important to comprehend that the rising manufacture of TiO_2 nanoparticles based beauty care products is likewise making a danger of respiratory and stomach related problems in people owing to their use in nano dimensions. Despite the fact that the utilization of nanoparticles has made a lot of things simpler for the customers yet their downsides should equally be considered before consolidating these into any item.

Therefore, it is recommended that the product design should comprise of minimum amounts of nanoparticles with a mandatory direction of use for the consumers, setting a limit to daily exposure and intake of nanoparticle-based products. Additionally, a substitute of these nanoparticles can be joined into utilization so the ideal motivation behind the nanoparticle is satisfied without their being destructive to the people and the environment.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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