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Nanoparticles And Reproductive Toxicity: An Overview

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ABSTRACT

Nanotechnology is a branch of science which deals with different technological systems, construction of new materials and devices with a wide range of potential applications at the atomic and molecular level. Fascinating physicochemical properties of nanoparticles offer great opportunities of its applications in chemical, biological, and industrial world. But manufactured or engineered nanomaterials, nanoparticles and nanodevices by different methods released into the environment intentionally and unintentionally without any safety test. Thus toxicity of these nanoparticles has become the subject of concern because they can move freely as compared to the large-sized particles; therefore, they can be more toxic than bulky materials. The consequently increasing exposure of these NPs to human has raised concerns regarding health and safety. This review article delineates the toxic effects of different types of nanoparticles on the human health specifically reproductive health through different sources like water, air, contact with skin.

Keywords: Reproductive toxicity, Titanium dioxide nanoparticles, Silver nanoparticles, Carbon Black nanoparticles, Gold nanoparticles

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INTRODUCTION

The term “nanoparticle” is a mixture of the words “nanos” [Greek: the dwarf] and “particulum” [Latin: particle]. In the scientific context, “nano” primarily refers to a specific order of magnitude, namely 10^{-9}. ISO describes the term nanoparticle as a material with one, two or three external dimensions in the nanoscale, with nanoscale being the size range from approximately 1 nm to 100 nm. Nanoparticles [NPs] are not solely a product of modern technology, but are also created by natural processes such as volcanic ash, ocean spray and mineral composites or forest fires. Naturally occurring nanoparticles also include ultrafine sand grains of mineral origin [e.g. oxides, carbonates].

This leads to the development of nanotechnology which produces new structures, materials, devices and nanoparticles involving manipulation of matter on a near-atomic scale. Thus producing NPs with unique physicochemical properties such as small size, large surface area to mass ratio, shape, crystallinity, surface charge, reactive surface groups, dissolution rate, state of agglomeration, or dispersal that confer them properties substantially different from those of the bulk particles of the same composition [1-3]. These properties offer immense opportunities for the development of new NP industrial applications increasing their worldwide distribution and enhancing the likelihood of environmental and human exposure [4].

Numbers of researchers from different parts of world are engaged in developing deep into the field of nanotechnology. Nanoscience and nanotechnology promise for creating new materials with enhanced properties and potential applications [5, 6].

Applications of Nanoparticles

Figure 1 and table 1 represents application of nanoparticles in different areas. Numbers of nanoparticles whether organic or inorganic, are applied in different sectors for various purposes. The engineered nanomaterials production in 2004 was estimated as 2000 tonnes and increasing to 58,000 tonnes by 2011-2020 [7]. The worldwide market of different industries of biotechnology, drug development and drug delivery containing nanoparticles was valued at $17.5 billion in 2011 and should reach nearly $21.6 billion in 2012. The expected reach of total market value in 2017 is $53.5 billion after increasing at a five-year compound annual growth rate [CAGR] of 19.9%, whereas the projected increase in drug delivery systems from $11.3 billion in 2012 to $30.9 billion in 2017, a CAGR of 22.2%. Drug development and formulation should total nearly $9.4 billion in 2012 and nearly $20.5 billion in 2017, a CAGR of 16.9%. Figure 2 shows the total products containing Nanoparticles and further its use in health and fitness sector. Similarly table 2 represents the global market of nanotechnology.

Toxicity of Nanoparticles

Nanotoxicology is a branch of bionanoscience which deals with the study and application of toxicity of nanomaterials [13]. These studies help to understand and determine

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the extent to which these nanoparticles may pose a threat to the environment and to human beings [14].

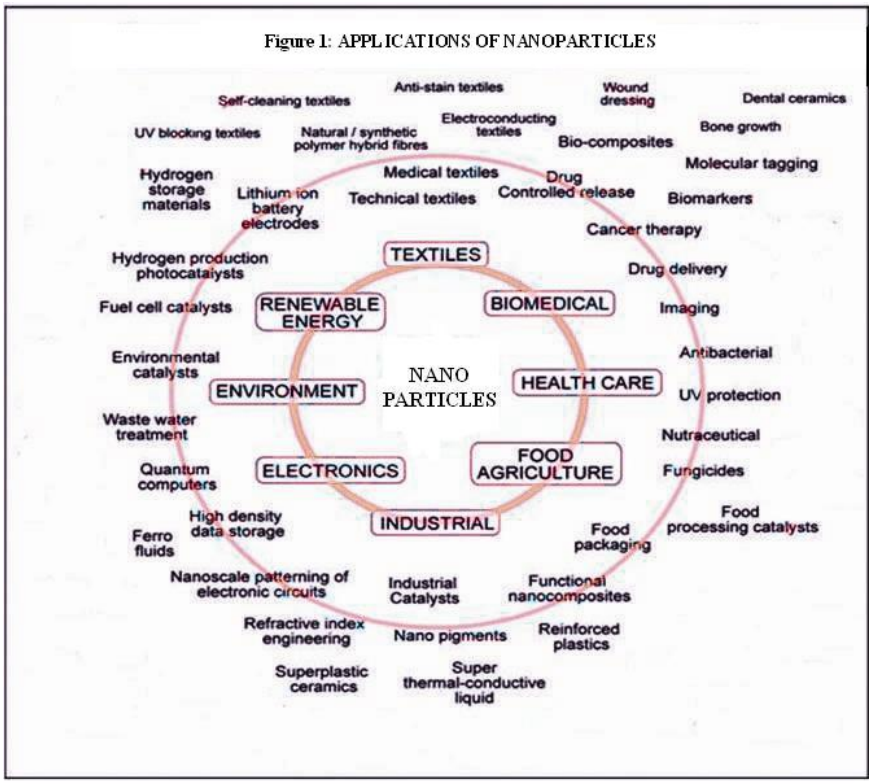

Figure 1: Applications of Nanoparticles [8]

Route of Exposure

Nanomaterials can enter the human body by various routes, such as breathing, eating, and touching the skin [figure 3].

Skin is the largest primary defense system in our body and directly comes into contact with many toxic agents. Skin exposed to nanomaterials/ nanoparticles by application of topical creams and other drug treatments [16-18].

The inhalation serves as a major portal for ambient particulate materials. The deposition of inhaled ultrafine particles [aerodynamic-diameter <100 nm] mainly takes place in the alveolar region [16, 17]. After absorption across the lung epithelium, nanomaterials can enter the blood and lymph to reach cells in the bone marrow, lymph nodes, spleen and heart [17, 19] and results in coagulation and cardiac rhythm [20, 21].

Nanoparticles can be ingested directly in food, water, cosmetics, drugs and drug delivery devices or can enter through nasal region [17, 18]. Nanocopper was also reported to cause

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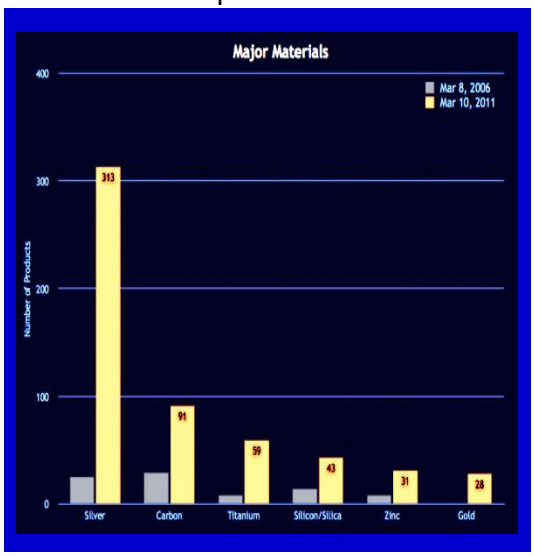
pathological damage to liver, kidney and spleen. Occurrence of systemic argyria after ingestion of colloidal nanosilver proves its translocation from the intestinal tract [22].



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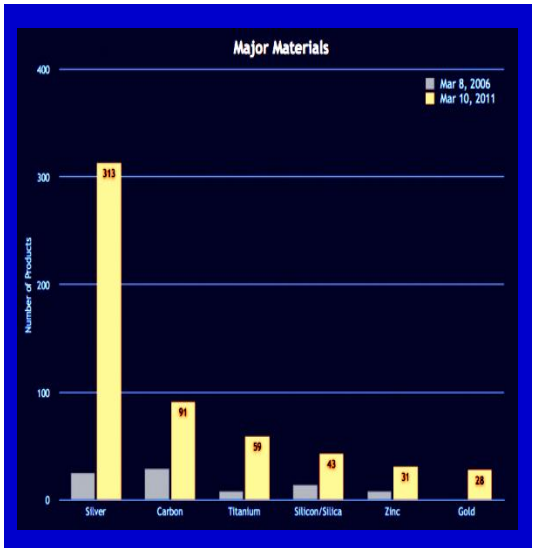

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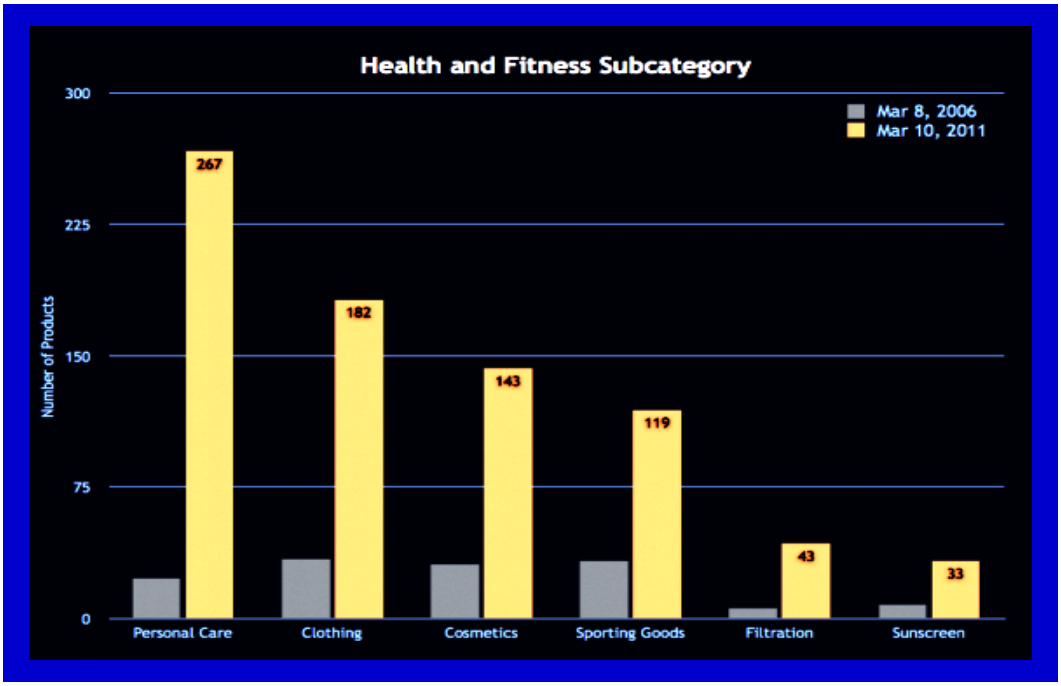

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Figure 2:[a] Total product listed from 2005 onwards
[b] Major material that are used as nanoaparticle in product formation.
[c] This diagram is showing utilization of nanoparticle containing product in Health and Fitness
Source: Online nanotechnology consumer products inventory report based on consumer products, March 10, 2011

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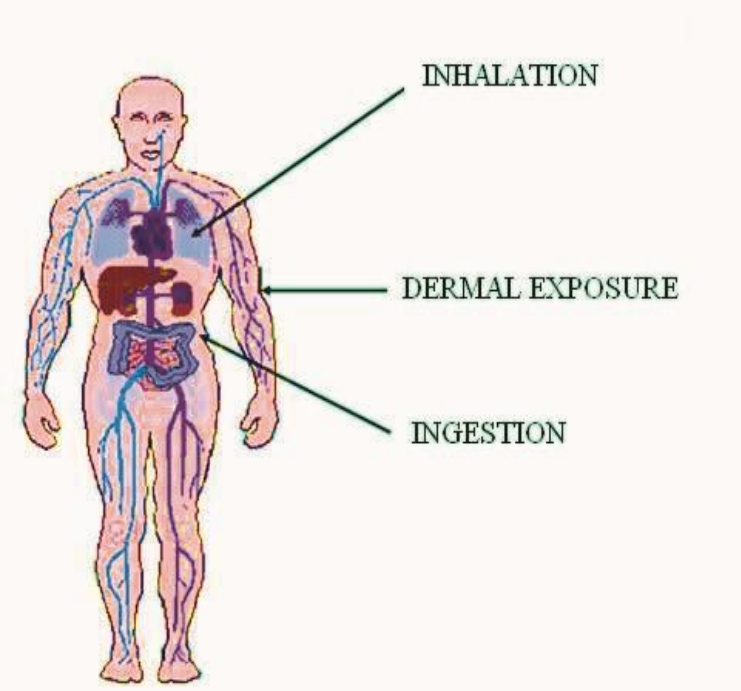

Figure 3: **Route of exposure of Nanoparticles [15]**

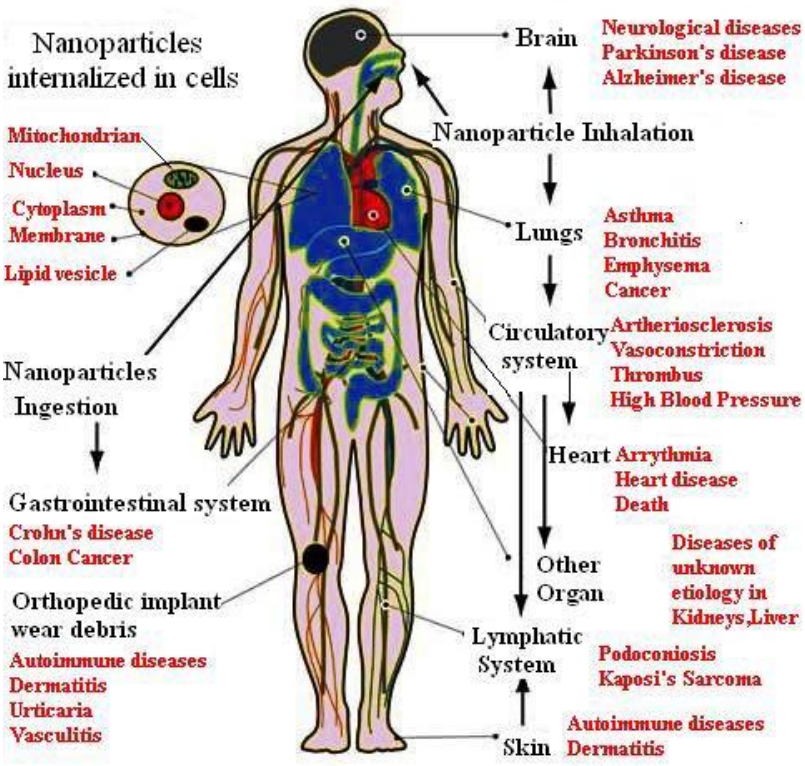

Figure 4: Schematics of human body with pathways of exposure to nanoparticles, affected organs and associated diseases from epidemiological, *in vivo* and *in vitro* studies. [13]

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Table1. Selected Applications of Metal, Metal oxide and Inorganic Nanoparticles

Nanoparticle	Abbreviation	Applications
Aluminum	Al	Fuel additive/propellant, explosive, wear resistant, coating additive.
Carbon nanotube	CNT	Paper batteries, high-tensile carbon cables for space elevator. Electrostatic and thermal dissipation, electromagnetic shielding.
Cerium [oxide]	CeO_2	Polishing and computer chip manufacturing, fuel additive to decrease emissions.
Copper	Cu	Antimicrobial [i.e., antiviral, antibacterial, antifouling, antifungal], antibiotic treatment alternatives, nanocomposite coating, catalyst, lubricants, inks, filler materials for enhanced conductivity and wear resistance.
Fullerene		Drug delivery, used in the design of safety goggles in intense light situations.
Gold	Au	Cellular imaging, photodynamic therapy.
Iron [oxide]	Fe,Fe_3O_4, Fe_2O_3	Magnetic imaging, environmental remediation.
Manganese [oxide]	Mn	Catalyst,batteries
Multi Walled Carbon NanoTube	MWCNT	Cytotoxicity: alveolar macrophages at high dose
Nickel [oxide]	Ni	Conduction, magnetic properties, catalyst, battery manufacturing, printing inks.
Silica	SiO_2	Fabrication of electric and thermal insulators, catalyst supports, drug carriers, gene delivery, adsorbents, molecular sieves, and filler materials.
Silver	Ag	Antimicrobial, photography, batteries, electrical
Single Walled Carbon Nano Tube	SWCNT	Cytotoxicity: alveolar macrophages at low dose; transient inflammatory and cell injury
Titanium dioxide	TiO_2	Photocatalyst, antibacterial coating, sterilization, paint, cosmetics, sunscreens.
Zinc [oxide]	Zn, ZnO	Skin protectant, sunscreen Carbon nanotube.

So far the knowledge regarding the health and safety aspects of NPs is still in its initial phase and greater efforts is needed to understand interaction of these NPs with the human body [23, 24]. The lack of studies investigating human toxicity from exposure to nanoparticles and their entry portals and interactions with the body make risk assessment for these materials challenging. In this regard, toxicity caused by these nanoparticles is gaining attention of toxicologists and regulatory scientists [25] specifically in relation to the unique properties of NPs that may render them potentially more dangerous than their fine-sized counterpart and may pose unexpected adverse health effects to exposed people [26, 3]. The safety issues and areas of agreement and disagreement regarding risk, exposure and hazards of nanoparticles is matter of concern [27].

Table 2 : Nanotechnology Global market

Country /region	Year	Market	Amount	Source
Worldwide	2003	Estimated turnover with nano products	Double-digit billion us dollar	[9]
Worldwide	2003	Public research funding	3 billion us dollar [estimation]	[9]
Worldwide	2005	Total investment in nanotechnology	5-7 billion us dollar [estimation]	[10]
Worldwide	2008	Global market for nano products	700 billion us dollar	[11]
Worldwide	2008	Global market for nano products	700 billion euro [estimation]	[12]
Worldwide	2010	Estimated turnover with nano products	Triple-digit billion us dollar	[9]
Worldwide	2010	Global market for nano products	148 billion us dollar [estimation]	[12]
Worldwide	2011	World nanomaterials demand	4.1 billion us dollar	[10]
Worldwide	2015	Estimated turnover with nano products	Four-digit billion us dollar	[9]

These nanoparticles easily gain access through the body's membranes and within cells, enters into cell mitochondria [28] and the cell nucleus [29], where they can induce major structural damage to mitochondria [30, 31], cause DNA mutation [32], and even result in cell death [33]. Unfortunately this also means that they may accumulate or override our normal control systems that manage our complex biochemistry, with unidentified health effects.

Various nanomaterials are found to be harmful to humans, animals, and environmental systems [25] then also these nanomaterials are being manufactured commercially and released into the environment without any health and safety testing or environmental impact assessment. Biological effects caused by nanoparticle deposition are related to their physical and chemical parameters. Different health impacts are associated with nanoparticles when compared to fine-sized [bulk] particle-types of similar chemical composition.

Numbers of disease associated with nanoparticle exposure are as follows in figure 4:

Despite of this, large number of products containing nanoparticles or nanomaterials are commercially available, including sunscreens, cosmetics, foods, fertilizers, clothing, industrial catalysts, fuel cells, sports equipment, computer and television screens, and medical equipment [34, 35,7].

The existing body of toxicological literature [36] suggests clearly that nanoparticles may have a greater risk of toxicity than larger particles. This body of evidence has been sufficient for the world's oldest scientific organization to warn that we should not release consumer products containing nanomaterials until we have vastly improved requirements for safety testing [37].

Factor Responsible For Toxicity Of Nanoparticles

Size

Size, is a key factor in determining the potential toxicity of a particle. As the size reduces to the nanoscale there is large increase in surface to volume ratio, so more molecules of the chemical are present on the surface, thus enhancing the intrinsic toxicity. These nanoparticles being small in size are toxic to human once in the blood stream, spleen, bone marrow and nervous system can be transported around the body. They are taken up by organs, tissue, cell cultures and results in oxidative stress, inflammatory cytokine production and cell death. Within the cell, nanoparticles cause mutation in DNA of cell nucleus and induce major structural damage to mitochondria, even resulting in cell death [38]. Size is therefore a key factor in determining the potential toxicity of a particle. The expression of a dose response relationship on the basis of particle size resulted in a similar dose response relationship between low solubility-low toxicity, particles of different sizes.

Chemical Composition

The toxicity of nanoparticles depends upon chemical composition and the intrinsic toxicological properties of the chemical. The effect of carbon black has been shown to be more severe than that of titanium dioxide, while for both compounds the nanoparticles induced lung inflammation and epithelial damage in rats at greater extent than their larger counterparts. Metallic iron was able to potentiate the effect of carbon black nanoparticles, resulting in enhanced reactivity, including oxidative stress.

Reactive Oxygen Species [ROS]

The greater chemical reactivity of nanoparticles results in increased production of reactive oxygen species [ROS], including free radicals [39]. Different type's nanomaterials like carbon fullerenes, carbon nanotubes, and nanoparticle metal oxides are found to be responsible for reactive oxygen species production [40]. ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity. These reactive oxygen species result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA [41].

Shape

Shape is also likely to be an important factor although there is little definitive evidence. Importance of shape in causing toxicity can be understood using fibres as significant example, especially in relation to inhalation, where the physical parameters of thinness and length appear to determine respirability and inflammatory potential.

Surface

For the same mass of any particular material, the combined surface area of a particle is inversely proportional to particle size. If the toxic properties of particles are determined by interactions occurring at the interface between particles and biological systems, then toxic response should correlate with the total surface area of particles. Indeed, it was observed in animal studies that the inflammatory response to inhaled TiO_2 particulates of different sizes, including those at the nanoscale size range, varied as a function of surface area [42].

Solubility

Solubility of nanoparticle also affects toxicity. Surface area and structural morphology are the factors determining solubility of nanoparticle toxicity. Low solubility low toxicity was observed in case of SiO_2, polystyrene. Low solubility high reactivity was observed in Quartz, CdSe, quantum dots. Ag, ZnO has high solubility.

Only few of *in vivo* and *in vitro* studies on reproductive toxicity of manufactured and natural nanomaterials have been done to assess the toxic effects of nanoparticles on humans and environment [43, 44, 45].

Reproductive Toxicity

Titanium dioxide Nanoparticles

Titanium dioxide [TiO_2] is fine, white, crystalline, odorless, low-solubility powder. It is a natural, thermally stable and nonflammable, nonsilicate mineral oxide found primarily in the form of the minerals rutile, anatase, brookite, and as the iron-containing mineral ilmenite [46, 47, 48, 49]. TiO_2 is a versatile compound that has broadly been used in nanoparticulate form [49]. Nanosized TiO_2 particles are among those most widely manufactured on a global scale [50]. TiO_2 NPs are widely used in paints, printing ink, rubber, paper, cosmetics, sunscreens, car materials, cleaning air products, industrial photocatalytic processes and decomposing organic matters in wastewater due to their unique physical, chemical and biological properties [including the inherent advantages of physical stability, anticorrosion and nanoscale-enhanced photocatalysis] [51].

The manufacturing and demand of Titanium Dioxide [TiO_2] nanoparticles is a significant and strong in current scenario. 50,400 tons of nanoparticle TiO_2 was produced in 2010, representing 0.7% of the overall TiO_2 market. The production is expected to increase to 201,500 tons by 2015 [52].

Adverse effects on spermatogenesis and histopathology was observed in *in vivo* studies with increased allergic susceptibility in offspring of mouse dams intranasally insufflated with respirable-size titanium dioxide [TiO_2] and also changes in gene expression in the brain of mouse offspring after maternal subcutaneous injection of TiO_2 nanoparticles [53].

Intraperitoneal injections of TiO_2 NPs cause reductions in sperm density and motility and an increase in sperm abnormality and germ cell apoptosis in mice. [54].

Aggregates of TiO_2 NPs in Leydig cells, Sertoli cells, and spermatids in the testes were observed when mice were prenatally exposed to anatase TiO_2 NPs via subcutaneous injections [45]. Seminiferous tubules become disorganized and disrupted, tubule lumens with few mature sperm and decreases in daily sperm production, epididymal sperm motility, and numbers of Sertoli cells were also observed [44, 53].

The potential of TiO_2 NPs impair the function of male mouse reproductive system. This study evaluated the direct effect of NPs on testis-constituent cells, and examined the effect of TiO_2 on mouse Leydig TM3 cells, the testosterone-producing cells of the testis [55].

Such limited knowledge regarding the reproductive toxicological effects of TiO_2 NPs and the relevance of this topic makes future investigations a matter of urgency.

Silver Nanoparticles

Silver nanoparticles [Ag NPs] are most commonly used nanoparticles to many household products such as bedding, washers, water purification systems, tooth paste, shampoo, fabrics, deodorants, filters, paints, kitchen utensils, toys, and humidifiers to impart antimicrobial properties[56, 57, 58, 59, 60, 61]. It is also used in glues, inks, pastes, polymers, coatings, etc., to make them thinner conducting pastes and coatings.

Ag nanoparticles are also able to assess human reproductive system through a variety of commercial products such as contraceptive devices and feminine hygiene products. Studies have shown that Ag nanoparticles cause toxicity to germ line stem cells through reduction in mitochondrial function and induction of membrane leakage and apoptosis [62].

Work on dispersed silver nanoparticles obtained via biochemical synthesis has shown a lethal effect on mammalian organisms when injected *in vivo* in mice [63]. Colloidal silver is widely used in anti-microbial formulations and dressings in drug delivery system [64].Toxicity of colloidal silver nanoparticles and a suspension of silver nanoparticles were compared in Daphnia magna [65]. Silver nanoparticles [40 and 80 nm] cause impairment of mitochondrial function [66].

Adverse impacts of silver nanoparticles [70 nm] on liver, spleen and kidney were observed in a high dose-treated group [1 and 2 mg/kg] in rat's living tissues [67]. Three different characteristic sizes [10, 50, and 100 nm] AgNPs shows size-dependent cellular toxicity against several cell lines including MC3T3-E1 and PC12 were studied [68]. Similarly repeated-dose toxicity was observed with small-sized AgNPs [22 nm, 42 nm, and 71 nm] [69].

Birth defects related to the male reproductive system affecting sperm cells were observed when silver nanoparticles were given during development. These silver particles can

cross the mom's placenta and directly affect the baby [70]. In another study of reproductive toxicity human testicular carcinoma cell line and testicular cells from two strains of mice, one of the two is modified at genetic level to serve as a model for human male reproductive toxicity. Silver nanoparticles inhibited normal cell function and caused more cell death when cells were exposed to two different sizes of silver nanoparticles [20 nm and 200nm] over different concentrations and time periods. A concentration-dependent increase in DNA damage in the human cells was observed in 200 nm size silver particles [71].

Carbon Based Nanoparticles

New form of carbon molecule, carbon nanotubes was discovered barely a decade ago. These are hollow cylinders having hexagonal network of carbon atoms of as small as 0.7 nm diameters and reach several millimeters in length [33]. Each end can be opened or closed by a fullerene half-molecule. Nanotubes can be single layer or multi layers of coaxial cylinders of increasing diameters in a common axis [Iijima, 1991]. Multilayer carbon nanotubes can reach diameters of 20 nm [72]. They are chemically and thermally very stable [73].

Depending on the specific process by which it is manufactured, carbon black also known as acetylene black, channel black, furnace black, lamp black or thermal black. It is amorphous form of carbon with high surface-area-to-volume ratio. Carbon black is used as a pigment and reinforcement in rubber and plastic products. As a pigment and in automobile tires is the most common use [70%] of carbon black. It also helps conduct heat away from the tread and belt area of the tire there by reducing thermal damage and increasing tire life. Carbon black particles are also employed in some radar absorbent materials and in photocopier and laser printer toner. Total production was around 8,100,000 metric tons [8,900,000 short tons] in 2006 [74]. Carbon black printex 90 is a well-known ingredient in rubber, plastics, inks, and paints with an annual production about 10 million tonnes [75, 76]. It is estimated that the global annual production of nanotubes and fiber was 65 tons equal to €144 million worth and it is expected to surpass €3 billion by 2010 representing an annual growth rate of well over 60% [77]. Even though the information about the production of carbon-based nanomaterials is scarce, the annual production volumes of for instance quantum dots, nano-metals, and materials with nanostructured surfaces are completely unknown.

The adverse effects of carbon nanoparticles on the male reproductive system were investigated using three different sizes [14, 56 and 95 nm] of carbon black nanoparticles. The serum testosterone levels were elevated significantly in the 14- and 56-nm carbon nanoparticles-exposed groups [78]. Repeated intravenous injections of water-soluble multi-walled carbon nanotubes into male mice can cause reversible testis damage without affecting fertility. Nanotubes accumulated in the testes, generated oxidative stress, and decreased the thickness of the seminiferous epithelium in the testis [79].

14-nm carbon nanoparticles was administered intratracheally to mice showed partial vacuolation of seminiferous tubules and reduction in cellular adhesion of seminiferous epithelia [80]. The highly toxic nature and reactive surface chemistry of the carbon black nanoparticles

has been found to affect lungs by inducing type II cell line to release chemotaxins for alveolar macrophages [81]. The toxicity of agglomerated carbon black was assessed in sprague-dawley rats [82]. Pulmonary toxicity of inhaled carbon black in rats was observed [83]. *In vivo* and *in vitro* studies on the reproductive and developmental toxicity of manufactured nanomaterials including metallic and metal oxide-based particles shows adverse effects on spermatogenesis in mouse offspring after maternal intratracheal instillation of carbon black nanoparticles [53].

Gold Nanoparticles

Gold nanoparticles [GNPs] has become very popular due to their unique electronic, optical, thermal, chemical, biological properties and their potential catalytic applications in various fields such as biology, medicine, physics, chemistry, material science and other interdisciplinary fields [64*].* Gold nanoparticles have bioapplications in four areas labelling, delivery, heating, and sensing. *In vitro* toxicity of gold nanoparticle on the spermatozoa has shown adverse effect on motility of sperm and also causes fragmentation of sperm [84]. Further reports are there showing toxicity of gold nanoparticle [85]. Another research shows elevated plasma testosterone levels in male mice without affecting fertility after administration of Gold nanoparticles [86].

CONCLUSION

Nanotechnology is producing new products and materials with brand-new properties. The current knowledge of the toxic effects of nanoparticles is relatively limited. The available data on nanoparticles shows that they can be distributed in the body and accumulate in several organs by escaping through the different protective barriers. The potential risk due to release of nanoparticles from manufacturing and /or processing units on safety and reproductive health of local human populations, and on environment still in its formative stage. Significant accumulations have been shown in the lungs, brain, liver, spleen and bones. It is unclear at the present time the extent to which these nanoparticles affects reproductive organ and fertility from knowledge of their physicochemical properties. These studies indicate that although NPs have far-reaching applications, they also have the potential to cause adverse effects at the cellular, subcellular, and protein levels. Strict preventive measures are needed to avoid any risk of disease in researchers and students who are in contact of these nanoparticles during its synthesis and also workers who manufacture, transform or use nanoparticles.

REFERENCES

[1] Handy RD, Shaw BJ. Health Risk and Society 2007; 9(2): 125–144.
[2] Ling MP, Chio CP, Chou WC. Environmental Science and Pollution Research, 2011;18(6): 877–889.
[3] Li N, Nel AE. Journal of Occupational and Environmental Medicine. 2011; 53 (6): S74–S79.
[4] Iavicoli I, Leso V, Bergamaschi A. Journal of Nanomaterials 2012; 36:1-36
[5] Zeng, H, Sun S. Advanced Functional Materials, 2008; 18(3): 391–400.

[6] Liu Z, Kiessling F, Gatjens J. Journal of Nanomaterials, 2010; 1–15.
[7] Nowack, Bucheli TD. Environmental Pollution 2007; 150(1): 5–22.
[8] Tsuzuki T. Int. J. of Nanotechnology, 2009; 6(5/6): 567 – 578.
[9] Hett A, Nanotechnology - Small matter, many unknown, 2004, accessed on 13 November2008. http://www.swissre.com/pws/research%20publications/risk%20and%20expertise/risk%20perception/nanotechnology_small_matter_many_unknowns_pdf_page.html
[10] Hannah W, Thompson PB. Journal of Environmental Monitoring 2008; 10: 291-300.
[11] Hassan E, Sheehan J. Scaling-up nanotechnology, 2008 http://www.oecdobserver.org/news/fullstory.php/aid/1005/Scalingup_nanotechnology.html
[12] Luther W, Malanowski N, Innovations- und Technikanalyse: Nanotechnologie alswirtschaftlicher Wachstumsmarkt, 2004; http://www.bmbf.de/pub/nanotech_als_wachstumsmarkt.pd
[13] Buzea C, Pacheco I, Robbie K. Biointerphases 2007; MR17-MR71
[14] Mahmoudi M, Hofmann H, Rothen-Rutishauser B, Petri-Fink A. Chemical Reviews, 2012; 112 (4): 2323–2338
[15] Colvin VL. Nature Biotechnology 2003; 2(1): 1166-1170.
[16] Curtis J, Greenberg M, Kester J, Phillips S, Krieger G. Toxicol. Sci. 2006; 25, 245–260.
[17] Hagens WI, Oomen AG, de Jong WH, Cassee FR, Sips AJ. Regul. Toxicol. Pharmacol. 2007; 49, 217–219.
[18] Oberdorster G, Oberdorster E, Oberdorster J. Health Perspect 2005a; 113, 823–839.
[19] Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H. Part. Fibre Toxicol. 2005b; 2, 8–43.
[20] Nurkiewicz TR, Porter DW, Barger M, Millecchia L, Rao KM, Marvar PJ, Hubbs AF, Castranova V, Boegehold MA. Health Perspect. 2006; 114: 412–419.
[21] Yeates DB, Mauderly JL. Environ.Health Perspect. 2001; 4: 479–481.
[22] Chung IS, Lee MY, Shin DH, Jung HR. Int. J. Dermatol. 2010; 49: 1175–1177.
[23] Clift MJD, Gehr P, Rothen-Rutishauser B, Archives of Toxicol, 2011; 85 (7): 723–731.
[24] Yokel RA, MacPhail RC. J Occup Med Toxicol. 2011; 6 (1) article 7.
[25] Oberd¨orster G, Oberd¨orster E, Oberd¨orster J. Environmental Health Perspectives 2005; 113(7): 823–839.
[26] Nel, AE, Xia T, Madler L, Li N. Science 2006; 311(5761): 622–627.
[27] Stern ST, McNeil SE. Toxicol. Sci. 2008;101(1): 4–21
[28] Geiser M, Rothen-Rutishauser B, Kapp N. Environmental Health Perspectives 2005; 113(11):1555–1560.
[29] Savi´c R, Luo L, Eisenberg A, Maysinger D. Science 2003; 300(5619): 615–618.
[30] Hoshino K, Fujioka T, Oku. Microbiology and Immunology 2004; 48(12): 985–994.
[31] Salnikov V, Luky´anenko Y O, Frederick CA, Lederer WJ, Luky´anenko V. Biophysical Journal 2007; 92(3): 1058–1071.
[32] Donaldson K, Stone V. Annali dell'Istituto Superiore di Sanita 2003; 39(3): 405–410.
[33] Hett A, "Nanotechnology: small matter, many unknowns," Swiss Re publications 2004.
[34] Arvidson. Toxicol. 1994; 88 (1–3): 1–14.

[35] Cross S E, Innes B, Roberts MS, Tsuzuki T, Robertson TA, McCormick P. Skin Pharmacology and Physiology 2007; 20(3): 148–154.
[36] Hoet P H, Br¨uske-Hohlfeld I, OV Salata. Journal of Nanobiotechnology 2004; 2 article 12.
[37] Duffin, R, Mills NL, Donaldson K. Yonsei Medical Journal 2007; 48(4): 561–572.
[38] Shinde SK, Grampurohit ND, Gaikwad DD, Jadhav SL, Gadhave MV Shelke PK. Asian Pacific Journal of Tropical Disease 2012; 2(4): 331–334
[39] Brown JS, Zeman KL, Bennett WD. American Journal of Respiratory and Critical Care Medicine 2002; 166 (9): 1240–1247.
[40] Marquis J, Love SA, Braun KL, Haynes K L. Analyst 2009; 134(3): 425–439.
[41] Kulmala M, Vehkam¨aki H, Pet¨aj¨a T. Journal of Aerosol Science 2004; 35(2): 143–176.
[42] Oberdorster G, Philos. Trans. R. Soc. Lond. A. 2000; 2719-2740.
[43] Shimizu M, Tainaka H, Oba T, Mizuo K, Umezawa M, Takeda K. Particle and Fibre Toxicology 2009; 6 article 20.
[44] Takeda K, Suzuki K I, Ishihara A. Journal of Health Science 2009; 55(1): 95–102.
[45] Takahashi Y, Shinkai Y, Mizuo K, Oshio S, Takeda K. Journal of Toxicological Sciences 2010; 35(5): 749–756.
[46] Jin T, Berlin M, “Titanium,” in Handbook of the Toxicology of Metals, G. F. Nordberg, B. A. Fowler, M. Nordberg, and L. T. Friberg, Eds. 2007; Elsevier, 3rd edition.
[47] Rahman Q, Lohani M, Dopp E. Environmental Health Perspectives 2002; 110(8): 797–800.
[48] Hedenborg M. International Archives of Occupational and Environmental Health 1998; 61(1-2): 1–6.
[49] Duan Y, Liu J, Ma L. Biomaterials 2010; 31(5): 894–899.
[50] Liang G, Pu Y, Yin L. Journal of Toxicology and Environmental Health Part A, 2009; 72(11): 740–745.
[51] Wang J, Chen C, Liu Y. Toxicol. Letters 2008; 183(1–3): 72–80.
[52] www.aarkstore.com/report/the world market for nanoparticle titanium dioxide: Production, Revenues, Markets and Producers.
[53] Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Repro. Toxicol. 2010; 30(3): 343–352.
[54] Guo LL, Liu XH, Qin DX, et al. Zhonghua Nan Ke Xue 2009; 15(6): 517–522.
[55] Komatsu T, Tabata M, Kubo-Irie M, Shimizu T, Suzuki KI, Nihei Y, Takeda K. Toxicol. In Vitro 2008; 22:1825–1831.
[56] Samuel U, Guggenbichler J. Int J Antimicrob Agents 2004, 23(1): 75–78.
[57] Cioffi N, Ditaranto N, Torsi L, Picca R, De Giglio E, Sabbatini L, Novello L, Tantillo G, Bleve-Zacheo T, Zambonin P. Anal Bioanal Chem. 2005; 382: 1912–1918.
[58] Cioffi N, Torsi L, Ditaranto N, Tantillo G, Ghibelli L, Sabbatini L, Bleve-Zacheo T, D'Alessio M, Zambonin PG, Traversa E. Chem Mater 2005, 17: 5255–5262. J, 2007; 4: 186–191.
[59] Vigneshwaran N, Kathe A, Varadarajan P, Nachane R, Balasubramanya R. J Nanosci Nanotechnol 2007; 7: 1893–1897.
[60] Percival S, Bowler PG, Dolman J. Int Wound J 2007; 4: 186–191.
[61] Jain J, Arora S, Rajwade J, Khandelwal S, Paknikar K. Mol Pharm. 2009; 6: 1388–1401.

[62] Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC. Toxicological Sciences 2005; 88(2): 412-419
[63] Ordzhonikidze CG, Ramaiyya LK, Egorova EM, Rubanovich1 AV. Acta Naturae 2009; 1(3):99-101.
[64] Salata OV. J Nanobiotech. 2004; 2(1):3.
[65] Asghari S, Johari SA, Lee JH, Kim YS, Jeon YB, Choi HJ, Moon MC, Yu IJ. J. Nanobiotech. 2012;10:14
[66] Teodoro JS, Simoes AM, Duarte FV, Rolo AP, Murdoch RC, Hussain SM, Palmeira CM. Toxicol. In Vitro 2011; 25(3): 664–670.
[67] Sardari RRR, Zarchi SR, Talebi A, Nasri S, Imani S, Khoradmehr A, Sheshde SAR. African J. Microbio. Res. 2011; 6(27): 5587-5593.
[68] Kim TH, Kim M, Park HS, Shin US, Gong MS, Kim HW. J. Biomed. Mater. Res A. 2012; 100(4):1033-43.
[69] Park EJ, Bae E, Yi J, Kim Y, Choi K, Lee SH, Yoond J, Leed BC, Park K. Toxicol. Pharmacol. 2010; 30(2):162–168.
[70] Nyland JF. Environmental News Science 2010.
[71] Science Daily, Are Silver Nanoparticles Harmful 2012.
[72] Aitken RJ, Creely KS, Tran CL, Nanoparticles: an occupational hygiene review. Sudbury, Suffolk, G.-B. HSE, 2004: 100.
[73] Hameed Hyder MA, Nanotechnology and environment, potential applications and environmental implications of nanotechnology. Master's Thesis, Techniche Universitat Hamburg-Harburg, Allemagne, 2003.
[74] International Carbon Black Association, What is Carbon Black 2009: 04-14
[75] Ostiguy C, Soucy B, Lapointe G, Woods C, Menard L, Trottier M, IRSST Health effects of nanoparticles, 2007, second edition. Report R-589 67-74.
[76] Tiede K, Hassellov M, Breitbarth E, Chaudhry Q, Boxall ABA. J. Chromatography A 2009; 1216: 503-509
[77] Cientifica Nanotube Production Survey. http://www. cientifica. eu/index. php?option=com_content&task=view&id=37&Itemid=74 2005.
[78] Yoshida S, Hiyoshi K, Ichinose T, Takano H, Oshio S, Sugawara I, Takeda K, Shibamoto T. Int J Androl. 2009; 32(4):337-42
[79] Yuhong B, Zhang Y, Zhang J, Qingxin M, Zhang W, Elizabeth RB, Scott ES, Bing Y. Nat Nanotechnol. 2010; 5(9): 683–689
[80] Yoshida S, Hiyoshi K, Oshio S, Takano H, Takeda K, Ichinose T. Fertil Steril. 2010; 93(5):1695-9
[81] Barlow PG, Baker AC, Donaldson K, MacCallum J, Stone V. Part. Fibre Toxicol. 2005; 2:11
[82] Lim CH, Kang M, Han JH, Yang JS. Environ Health Toxicol. 2012; 27
[83] Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, Chang IY, Cheng YS, Gillett NA, Griffith WC. Res. Rep. Health Eff. Inst. 1998; (68 Pt 1):1-75;
[84] Wiwanitkit V, Sereemaspun A, Rojanathanes R Fertil Steril. 2009; 91(1):e7-8.
[85] Taylor U, Barchanski A, Garrels W, Klein S, Kues W, Barcikowski S, Rath D. Adv Exp Med Biol. 2012; 733:125-33.
[86] Li WQ, Wang F, Liu ZM, Wang YC, Wang J, Sun F, (2012): Gold Nanoparticles Elevate Plasma Testosterone Levels in Male Mice without Affecting Fertility. Small 2012.