Review Article

Current Studies into the Genotoxic Effects of Nanomaterials

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Nanotechnology has created opportunities for engineers to manufacture superior and more efficient devices and products. Nanomaterials (NMs) are now widely used in consumer products as well as for research applications. However, while the lists of known toxic effects of nanomaterials and nanoparticles (NPs) continue to grow, there is still a vast gap in our knowledge about the genotoxicity of NMs. In this paper, we highlight some NMs of interest and discuss the current *in vivo* and *in vitro* studies into genotoxic effects of NMs.

1. Introduction

Materials in the nanoscale are used in many commercial products and industrial practices in the new millennium. They are now increasingly found in plastic wares, clothing, cosmetics, electrical appliances, and even food products. Their applications also extend into the biomedical field and healthcare, particularly in medical imaging and diagnosis, pharmaceuticals, drug delivery, and therapy [1]. The demand for nanomaterials (NMs) in the market in the areas defined above is escalating and estimated to reach sales of up to US$1 trillion by 2015 [2]. The recent burgeoning research interest and development of NMs, nanotechnology, and nanomedicine have led to a vast potential for novel ways of rapid disease diagnosis, treatment, and enhancement of the quality of life. NMs consist of one or more components present in various forms that possess at least one-dimensional structure of diameters in the range of 1 to 100 nm [3]. Engineered NMs, including nanoparticles (NPs) and nanofibres, are generally categorized into four classes, which include carbon-based materials, metal-based materials (quantum dots, nanosilver, and nanogold), dendrimers (nanosized polymers), and composites. Their characteristic features are durability, high conductivity, and reactivity [4].

Many researchers have commented that in actuality, there is still much more to be understood about nanomaterials, especially with regard to the health risks and hazards. The Royal Society and Royal Academy of Engineering first raised this concern in 2004 [5]. This has paved the way for a rapid increase in investigational studies in the toxicity of nanobased materials, in particular, genotoxicity studies of NMs and nanoparticles (NP). A quick search through the Pubmed literature database shows that the bulk of the research articles on NM genotoxicity were published within the past 3 years. As the development of nanotechnological applications continue to grow, it is anticipated that there will be an even greater demand for safety and health and risk assessments studies in the coming years. There have been excellent reviews regarding the methodologies for studying NM-induced toxicity [6–8].

In this paper, we would like to briefly discuss the methodologies currently available for genotoxic studies and present a survey of the *in vitro* and *in vivo* genotoxicological studies of NMs conducted in recent years.

2. Methodologies in Genotoxicity Studies

The study of NM toxicology has its roots in ultrafine particle study, mostly starting out as particulate matter (PM10) and

carbon black. The first wave of nanotoxicological studies were assessments of NM cytotoxicity which had been comprehensively outlined by Lewinski et al. [9]. Currently, there is an increasing focus on specific nanotoxic effects, and thus the advent of a subfield called "nanogenotoxicology" [10] which generally refers to the study of toxic effects of NMs on genomic stability and integrity. Common *in vitro* tests for measuring insults to DNA would centre on single-strand and double-strand breaks, mutations, deletions, chromosomal aberrations, impairment in DNA repair and cell-cycle while tumorigenesis and carcinogenicity are the main focus in *in vivo* studies. There are as many different kinds of NMs as there are elements and compounds. NMs, depending on the size, shape, elemental materials, and the surface functional groups were observed to have a range of detrimental effects on cells. Compounding the difficulties in toxicological studies, Stone et al. [6] and Landsiedel et al. [7] reiterated that based on existing knowledge, specific NMs probably induce definitive genotoxic effects. Nevertheless, some of the more common tests used in current genotoxic studies are described below.

2.1. In Vitro Techniques and Approaches

2.1.1. Ames Test (Bacterial Reversion Mutation Test). This test is used to assess the mutagenicity of a chemical compound [11]. Various strains of the histidine dependent bacterium, *Salmonella typhimurium*, contain mutations in the genes that impair synthesis of histidine required for cell growth. Test substances or compounds are added to different areas on the agar plate, and the bacterium is then plated onto the minimal histidine media. The test compound is deemed to have mutagenic potential if it is able to cause mutations that allow the bacterium to revert back its histidine synthesis ability. The downside of this test is that it is difficult to translate prokaryotic data for eukaryotic genotoxicity testing, and the test is known to generate false positive results [12]. Specific to NM toxicity testing, there are doubts if the Ames test is accurately representative of genotoxicity. Some NMs are not able to cross the bacterial wall, and some kill the test organism as they are bactericidal [7]. Therefore, data should be followed up with other tests after the initial screening.

2.1.2. Comet Assay (Single-Cell Gel Electrophoresis Assay). This is a simple, inexpensive, and sensitive technique to test for DNA damage. It was first described in 1988 by Singh et al. [13] and has since become the standard test for DNA damage. Cell samples from *in vitro* or *in vivo* experiments are first suspended in low melting point agarose and cast onto microscope slides. The cells are lysed so that only the DNA remains, which is then made to undergo electrophoresis in order to separate the DNA strands based on molecular weight. The DNA strains are subsequently stained with, for example, SYBR green dye and viewed under a fluorescence microscope. Under specific conditions, this test is able to distinguish single-and double-strand breaks in DNA. It is a quick way to assess DNA lesions and extent of genotoxicity in individual eukaryotic cells. However, due to its sensitivity, samples should be handled appropriately to ensure reproducibility of the results.

2.1.3. Micronucleus Test (MN)/Cytokinesis Block Micronucleus Test (CBMN). This assay is based on scoring the number of micronuclei (MNi) in treated cells [14]. MNi are formed during anaphase from chromosomal fragments or whole chromosomes that are left behind when the nucleus divides. Over time, the assay has evolved to include a pretreatment with cytochalasin-B (Cyt-B), a cytokinesis blocking agent that inhibits cell-division, thereby giving the cells a binucleated appearance. This enables more accurate scoring and the ability to sieve out the dividing cells (where MNi would be found) from the nondividing ones, thereby reducing the incidence of false positives. The CBMN method is now routinely used for measuring chromosome breakage, impairment in DNA repair, chromosome loss, nondisjunction, necrosis, apoptosis and cytostasis.

2.1.4. Hydroxy-Deoxyguanosine (8-OHdG) Analysis. Oxidative stress is considered one of the foremost reasons for DNA damage. Reactive oxygen species (ROS) generated in metabolizing cells could attack DNA base guanine forming the 8-OHdG lesions, which is known to have mutagenic potential and hence used routinely as a biomarker for carcinogenesis [15]. There are a few methods to measure the extent of 8-OHdG lesions and the most established is HPLC (high-performance lipid chromatography), which is often coupled with mass spectrometry, also known as the HPLC-MS/MS. Other methods include performing antibody probes for DNA repair proteins or posttreatment with the enzyme formamidopyrimidine DNA N-glycosylase before quantitative analysis with the comet assay to determine DNA strand breaks [16].

2.2. In Vivo Approaches. There is a need for validation of animal models for studies in NM toxicity. The difficulties lie in devising the correct approach in interpreting the studies and deciding on the parameters that should be considered in examining NM toxicity in *in vivo* systems. Many investigators have administered NMs through inhalation exposure or orally, ingestion by feed or water supply, and direct instillation or injection into the body. Usually, the subsequent bioavailablity and translocation of the NMs are evaluated, including the organ of entry as well as in other organs where accumulation is more significant. The tests used for assessment of genotoxicity are similar to those used in the *in vitro* studies.

3. Nanomaterials and their Genotoxic Status

A summary of some of the current genotoxic studies in nanomaterials are shown in Tables 1 and 2, which display the in *vivo* and *in vitro* studies, respectively.

3.1. Carbon Fullerenes. Carbon fullerenes, which are ultrafine particulate matter, are one of the most ubiquitous NMs found [46]. They are generally present in polluted air as

TABLE 1: Selected *in vivo* genotoxicity studies on NMs.

Type of NP	Size and form	Experimental design/genotoxic tests	Summary of findings	References
C60 fullerenes	spheres	Bone marrow micronucleus test on ICR mice	No *in vivo* clastogenic ability of C_{60} up to 88 mg/kg	Shinohara et al.; 2009 [17]
C60 Single-walled carbon nanotubes (SWCNT)	spheres	Oral administration at doses of 0.064 and 0.64 mg/kg of body weight. 8-OHdG analysis	Both NPs were associated with increase in 8-OHdG in liver and lungs. No impairment of DNA repair system	Folkmann et al.; 2009 [18]
SWCNT Multi-walled carbon nanotubes (MWCNT)	nanotubes	Oral administration and urinary samples collected for Ames test	No urinary mutagenicity	Szendi and Varga 2008 [19]
Carbon black (CB) C60 SWCNT AuNP Cd quantum dots (QDs)	nanospheres	Apo E knockout mice Timepoints at 3 and 24 hours; NP administered by instillation	Increase in cytokines gene expression. ApoE $-/-$ mice are sensitive to particle induced inflammation. DNA damage in order of. QD>CB>SWCNT> C_{60}, Au	Jacobsen et al.; 2009 [20]
TiO$_2$	anatase/rutile 21 nm	TiO$_2$ ingested through drinking water at concentrations of 60, 120, 300, 600 μg/mL. Comet assay MN test gamma-H2AX immunostaining 8-OHdG analysis	Increase in 8-OHdG and gamma-H2AX foci. indicative of DNA double-strand breaks. MN. shows increase in DNA deletions.	Trouiller et al.; 2009 [21]
Ag	60 nm	Oral administration in Sprague-Dawley rats over a period of 28 days; doses at 30, 300 and 1000 mg/kg.	No significant genotoxicity in bone marrow. (micronucleated erythrocytes)	Kim et al.; 2008 [22]
Silica	amorphous 37 and 83 nm	Inhalation study where mice were exposed to 3.7×10^7 and 1.8×10^8 particles/cm^3	No significant pulmonary, inflammatory, genotoxic or adverse lung histopathological effects	Sayes et al.; 2010 [23]

they are often released in soot resulting from the process of fuel combustion. Engineered carbon fullerenes are stable, soccer ball-like carbon atoms with hexagonal and pentagonal shapes. The most notable fullerene would be C60, a highly reactive biomolecules that has the ability to cross blood brain barrier (BBB) [47]. C60 fullerene is highly used in industry as catalysts, reactive oxygen species scavengers [48] and tools in drug delivery systems [49].

Since the early 1990s, there have been concerns about the potential dermal and inhalation effects of fullerenes due to their strong oxidizing and phototoxic properties [50]. *In vitro* experiments have shown C60 to be generally noncytotoxic with no mutagenic response [17, 24] in Chinese hamster ovary (CHO-K1) cells and mouse lung epithelial cells [28] using the Ames test and CBMN tests, respectively. Another report has found that C60 treatment also increases formamidopyrimidine [fapy]-DNA glycosylase (FPG) sensitive sites, accounting for short-term DNA strand damage. Xu et al. observed that C60 induced an increase in mutation yield in primary mouse embryo fibroblast cells and dose-dependent formation of free radical ONOO$^-$ [25] using dihydrorohodamine radical probes. However, in the *in vivo* setting, C60 treatment was found to be associated with increased DNA damage 8-hydroxydeoxyguanosine (8-OHdG) in mouse lung and liver [18]. Not surprisingly, inflammatory cytokines such as the interleukins and MIP and MCP genes were found to be upregulated although C60 extent of damage was lower as compared to other NMs.

3.2. Carbon Nanotubes. Carbon nanotubes are the byproducts of combustion, which are commonly present in air pollution and soot. Engineered carbon nanotubes can also

Table 2: Selected *in vitro* genotoxicity studies on NMs.

Type of NP	Size and form	Experimental design/genotoxic tests	Summary of findings	References
Carbons				
C_{60}	0.92 m2/g surface area	Ames test	No mutagenic response, and no incidence of chromosomal aberration	Shinohara et al.; 2009 [17]
C_{60}	polyhydroxylated	CHO-K1 cells chromosome aberration assay CBMN test	No genotoxicity at all doses (11–221 μM)	Mrdanović et al., 2009 [24]
C_{60}	nanospheres	Mouse primary embryo fibroblasts Dihydrorhodamine 123 radical probe	Increased mutation yield and induces kilo-based pair deletion mutations in transgenic mouse cells. Dose-dependent formation of $ONOO^-$	Xu et al.; 2009 [25]
SWCNT -MWSCNT	nanotubes	Human lymphocytes in culture CBMN test Sister Chromatid Exchange (SCE) assay	No genotoxicity effects but SWCNT induces mitotic inhibition	Szendi and Varga; 2008 [19]
MWSCNT	agglomerates	V79 cells treated for 18 h and 30 h at 2.5, 5 and 10 μg/mL. Chromosome aberration test Ames test	No mutagenic or clastogenic effects	Wirnitzer et al., 2009 [26]
MWSCNT	nanotubes	Ames test on *Salmonella typhimurium* TA 98 and TA 100 strains, and on *Escherichia coli* WP2uvrA strain, in presence and in absence of the metabolic activation system S9	No mutagenic effects	Di Sotto et al.; 2009, [27]
C_{60} SWCNT Carbon black (CB)	0.7 nm (C60) 0.9–1.7 nm (SWCNT) 14 nm (CB)	FE1-muta trademark mouse lung epithelial cell line comet assay FE1-MML mutagenicity analysis c11 mutation analysis	No cell death. Slower proliferation and cell-cycle arrest at G_1 with SWCNT. Mutant frequency unaffected by 576 h exposure	Jacobsen et al., 2008 [28]
Metals				
Alumina (Al_2O_3) Cobalt Chromium alloy (CoCr)	bare	Human primary fibroblasts over 5 days CBMN assay gamma-H2AX immuno staining cytogenetic analysis (FISH)	At 24 h, Al_2O_3 increase micronucleus binucleated cells, chromosomal loss, gain, and polyploidy. At 24 h, CoCr induce dose-dependent increase in micronucleus binucleated cells, chromosomal loss, gain, deletions, and polyploidy.	Tsaousi et al.; 2010, [29]
Co	20 nm 500 nm	Balb/3T3 cells at 1–100 μM dose concentrations. CBMN test Comet assay	Significant results for CBMN and comet assay but no dose-dependency. Increase of type III foci	Ponti et al.; 2009 [30]
Co	100–500 nm	Peripheral blood leulocytes at 24, 48 h timepoints in 10^{-5}M and 10^{-4}M dose concentrations CBMN test Comet assay	Induces DNA damage Genotoxic effects modulated by donor characteristics and/or Co2+ release.	Colognato et al.; 2008 [31]

TABLE 2: Continued.

Type of NP	Size and form	Experimental design/genotoxic tests	Summary of findings	References
Al_2O_3 TiO_2	nanoparticles	CHO-K1 cells Micronucleus (MN) test Sister chromatid exchange (SCE)	MN frequencies increase at 0.5 and 1 μg/mL TiO2 and 0.5–10 μg/mL AL2O3. SCE higher at 1–5 μg/mL TiO2 treatment, and at 1–25 μg/mL Al_2O_3	Di Virgillio et al.; 2010 [32]
TiO_2	rutile/anatase fine rutile	Human bronchial epithelial cells (BEAS 2B) with 1–100 μg/cm^2 at 24, 48, and 72 h. Comet assay MN test	Both induce DNA damage at all treatment times. Only nanosize rutile increase frequency of MN cells at 10, 60 μg/cm^2, 72 h.	Falck et al.; 2009 [33]
TiO_2	with p,p′-DDT	Human embryo L-02 hepatocyte 0.01, 0.1, 1 μg/mL treatment concentrations Flow cytometry with DCFH-DA probe 8OHdG analysis Comet assay MN test	TiO$_2$ enhances photocatalysis. Increases oxidative stress, DNA adducts, DNA strand breaks, and chromosome damage	Shi et al.; 2010 [34]
TiO_2	2–30 nm (mean at 15 nm)	NIH3T3 human fibroblasts HFW cells Short-term treatment at 24, 48 and 72 h. Long-term treatment, cell passage every 3 days with NP media. Flow cytometry with H2DCFDA probes Cell-cycle analysis Cell-division analysis Confocal microscopy	Short-term increased cell survival and growth. Long-term G$_2$/M delay and slower cell-division with aberrant multipolar spreads. Overall disturbance in cell-cycle progression, duplicate genome segregation, and chromosomal instability	Huang et al.; 2009 [35]
TiO_2 Fe_2O_3	anatase <100 nm <100 nm	Human lung fibroblasts IMR-90 and BEAS-2B cells Electron paramagnetic resonance (EPR) 8-OHdG analysis	TiO$_2$ treatment showed no DNA breakage, DNA adduct nor free radical generation. Fe$_2$O$_3$ had significant DNA damage after 24 h in IMR-90 cells	Bhattacharya et al.; 2009 [36]
TiO_2	nanoparticles rutile anatase	Mouse primary embryo fibroblasts Dihydrorhodamine 123 radical probe	Increased mutation yield and induces kilo-based pair deletion mutations in transgenic mouse cells. Dose-dependent formation of ONOO$^-$	Xu et al.; 2009 [25]
TiO_2	100 nm	Human lymphoblastoid cells. Treatment with 26, 65, 130 μg/mL at 6, 24, 48 h. CBMN test Comet assay Hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutation assay	130 μg/mL treatment increases MNBC frequency 2-3 folds and 2.5 fold in mutation frequency. 65 μg/mL treatment induce 5 fold increase in comet tail moments	Wang et al.; 2007 [37]

Table 2: Continued.

Type of NP	Size and form	Experimental design/genotoxic tests	Summary of findings	References
ZnO	nanospheres	Human epidermal cell line (A431) Treatment at 0.8, 0.008g/mL Comet assay	Significant DNA damage in comet assay. Induces oxidative stress	Sharma et al.; 2009 [38]
Ag	30 nm, nanospheres	Medaka fish cell lines Treatment at 0.05, 0.1, 0.3 μg/cm^2	Chromosomal aberration and aneuploidy	Wise et al.; 2010 [39]
Ag	6–20 nmstarch coated	IMR-90 and human glioblastoma cells U251 Comet assay CBMN Annexin V propidium iodide staining	DNA aberrations more prominent in cancer cells with more chromosomal aberrations.	Asharani et al.; 2009 [40]
Ag	25 nm polysaccharide surface functionalized and uncoated nanospheres	Mouse embryonic stem cells and embryonic fibroblasts Immuno blot Immunoflorescence	Upregulation of p53, Rad 51 and phosphorylated H2AX protein expression. Coated AgNP show more severe damage than uncoated AgNP	Ahamed et al.; 2008 [41]
Au	20 nm Serum coated	Human fetal lung fibroblasts cells (MRC-5) treated with nAu at 0, 0.5 and 1 nm concentrations. 8-OHdG analysis	Significant DNA damage in 1 nm treatment compared to control.	Li et al.; 2008 [42]
Platinum (Pt NP)	5–8 nm capped with poly-vinyl alcohol	Human cell line	p53 activation, p21 downregulation. Increase of DNA damage, arrest at cell-cycle S phase and apoptosis	Asharani et al.; 2010 [43]
Other Nanomaterials				
Nanoceria (CeO$_2$)	nanoparticles	Human lens epithelial cells at 5, 10 μg/mL concentrations SCE Comet assay (alkaline)	No DNA damage nor SCE	Pierscionek et al.; 2010 [44]
Polymer NP	lyophilized PELGE and PLGAnp	CHO cells MN test SCE	No significant difference in MN assay and no cell-cycle delay. SCE found to be higher in 5 kinds of PELGE-NP than in negative controls	He et al.; 2009 [45]

come in a variety of shapes and conformations, with the most common being the single-walled carbon nanotubes (SWCNTs) and the multiwalled carbon nanotubes (MWCNTs). They are also found in a wide range of applications in the industry as composites, polymers, as well as in the biomedical and pharmaceutical fields. Great physical strength, flexibility, electrical conductivity, insolubility and nonbiodegradability are among the valued properties of carbon nanotubes [51]. On the other hand, it has been postulated that these nanotubes could possess health hazards upon inhalation as their durability, biopersistency, and long and thin shape resembling asbestos fibers [52]. In addition, trace contaminations with iron and nickel have been reported to be the major cause of toxicity in carbon nanotubes [53].

There is a scarcity of information regarding SWCNTs and genotoxicity. SWCNTs have been reported to induce slower proliferation rate and cell-cycle arrest at G1 phase in mice lung epithelial cells [28] and mitotic inhibition in human lymphocyte cultures [19]. In *in vivo* experiments, oral administration of SWCNTs in mice is found to be associated with increase in 8-OHdG levels in liver and lung [18]. SWCNTs, compared to carbon black, only causes moderate inflammation in ApoE knockout mice [20]. However,

agglomerates of MWCNTs were found to possess neither clastogenic nor mutagenic effects [19, 26, 27] when put under the Ames test and chromosome aberration test.

3.3. Titanium Dioxide and Zinc Oxide Nanoparticles (TiO$_2$ and ZnO NPs).

TiO$_2$ and ZnO NPs, which have the properties of high refractive index and brightness, are regularly used as whitening pigments or reflective optical coats [54]. These specific properties lead to the application in commercial products such as paint and whitening agents in food products [55]. Nanoparticulate suspensions of ZnO and TiO$_2$ also appear transparent in air and liquid under visible light. As such, ultrafine TiO$_2$ is also extensively used in cosmetics, skin care, and sunscreen products, as their application does not leave unsightly white residue on skin unlike bulk TiO$_2$ [56]. ZnO is quite well known to be cytotoxic to cells in culture [57], while the toxicity of TiO$_2$NP is rapidly gaining attention due to the increased use and applications in many accessible medical and cosmetic products. TiO$_2$NP comes in two common shapes, namely, the rutile and anatase forms. Although both are found to be genotoxic, one study showed that the anatase form induced greater DNA damage in human bronchial epithelium [33]. TiO$_2$NP could also increase cell sensitivity to phototoxicity [34], as well as induce more DNA adducts, strand breaks, base-pair mutations and chromosomal damage [21, 25, 37]. Interestingly, Huang et al. reported that while long-term exposure to TiO$_2$NP slowed down cell-division and induced aberrant multipolar spreads, chromatin alignment, and segregation, short-term exposure increased cell survival and growth and number of multinucleated cells [35]. Another group of investigators did not observe DNA breakage under TiO$_2$NP treatment but found positive DNA adduct formation and free radical generation [36].

Although ZnONPs are probably the less studied of the two, there is also evidence to suggest that they may also induce significant DNA damage through oxidative stress, albeit with less obvious effects than in TiO$_2$NPs [38].

3.4. Aluminium Oxide Nanoparticles (Al$_2$O$_3$NPs).

Al$_2$O$_3$NP, or alumina NP as it is commonly known, belongs to a class of materials known as nanoceramics. It is widely used in industrial and medical product such as orthopaedic parts and composite repellants. However, the toxic and genotoxic effects of Al$_2$O$_3$NP are not well known, and there are very few research studies on the toxicity of this material. Thus far, Al$_2$O$_3$NPs were found to significantly increase micronucleus frequencies, chromosomal loss, and gain mutations as well as polyploidy but no sister chromatid exchanges were found to take place [29, 32].

3.5. Cobalt and Cobalt-Chromium Nanoparticles (CoNPs and CoCrNPs).

Cobalt and its alloy are commonly used in hip joint replacements and other orthopedic joint replacements. Unfortunately, the friction produced in movement of the replacement joints generate NPs of the metal which could reach out and affect the surrounding tissue and even lymphocytes, thereby lead to some concerns regarding the genotoxicity observed from clinical studies [58]. Hence, much interest was generated to study the effects of these wear particles and a significant amount of research into Co and CoCr NPs are centered around these issues. The results, although not surprising, are generally aligned to positive indications of genotoxicity. Analysis of peripheral blood leukocytes of patients with cobalt alloy joint replacements showed positive DNA damage in comet assays [31]. However, it was also suggested that these results could possibly be modulated by donor characteristics and may be due to Co^{2+} release instead of CoNPs per se. Recent studies show that by 24 h, CoCrNPs induced a dose-dependant increase in micronucleus containing cells as well as chromosomal loss, gains, deletions, and polyploidy [29]. In a separate study with CoNPs on Balb/3T3 cells, there were significant results in micronuclei and comet assay for NP induced DNA damage but the results were not dose-dependent [30].

3.6. Quantum Dots.

Quantum dots are crystalline semiconducting NPs. They are comprised of a metalloid crystalline core and a "cap" or "shell" that shields the core or renders the dots biologically compatible [4]. The metalloid crystalline core is normally made up of heavy metals like cadmium and lead or sometimes from other semiconductor, noble, and transition metals. These are also quantum dots that are coated with materials such as polyethylene glycol, zinc sulphide, or polyacrate [59] Quantum dots are used in composites, paints, inks, solar cells, and optoelectronics [4]. Due to their bright fluorescence, narrow emission, broad UV excitation, and photostability, they have been used as alternative fluorescent dyes for labeling cell structure *in vitro* and for fluorescence imaging *in vivo* [60].

They are considered one of the most toxic of substances and there are many studies showing the acute cytotoxicity of quantum dots [61]. The cadmium and lead metals themselves are considered potent human carcinogens. Cadmium induces DNA damage and mutation through ROS production and inhibition of DNA repair and methylation [62]. It also incites disruption of E-cadherin cell-to-cell adhesion which could lead to tumor formation. Lead and its compounds are listed under group 2B of possible human carcinogens in IARC reports [63], as they are found to induce lipid peroxidation and inhibit enzymes and antioxidants thereby putting the cell under an environment of oxidative stress [64]. However, few have ventured into exploring the genotoxicity of such QDs. One experiment with Apo E knockout mice showed that such mice were more sensitized to QD-induced inflammation, upregulating gene expressions of cytokines, IL-6, Mip 2 and Mcp signaling molecules [20].

3.7. Silver and Gold Nanoparticles (AgNPs and AuNPs).

AgNPs and AuNPs are the most marketable NPs and widely used in consumer products. AgNPs are particulary known for their antimicrobial qualities, while AuNPs are used in bioimaging and diagnosis applications. They are also easily synthesized from their salt compounds and are convenient

to handle, which also makes them another popular choice of NMs to work with. What is of concern is that several studies have found AgNPs to be toxic in aquatic animals [65] and AuNPs to possess some degree of toxicity *in vitro* [66]. Many researchers have focused on AgNPs because of the acute toxicity shown *in vitro* experiments. AgNPs were found to induce DNA damage in human glioblastoma cells as well as chromosomal aberrations in human fibroblast cells [40]. Other genotoxic reactions include upregulation of p53 and DNA repair protein Rad51 observed in mouse embryonic stem cells and fibroblasts [41]. In the same study, AgNP when functionalized with polysaccharide on its surface was more DNA damaging than uncoated AgNPs. In long-term rodent studies, oral administration of high-dose AgNPs for 28 days resulted in liver damage but no significant genotoxicity in erythrocytes and bone marrow [22]. A number of studies have also shown that AgNP treatment induced DNA damaging effects on aquatic and plant cells with impairment of cell-division [39, 65]. Although less dramatic than AgNPs, AuNPs are also able to induce DNA damage in the form of single-strand lesions in human lung fibroblasts [42].

3.8. Other Nanoparticles. There are a few research groups working with new types of NPs. The rare earth metal cerium oxide NPs (nanoceria) is one example. Researchers have found nanaoceria to be a radical scavenger with antiinflammatory effects [67] which causes no DNA damage [44]. They are currently being developed for application in human lens epithelium. Although this is a promising NM for future applications, it has also been reported that nanoceria exerts differential growth in soybean seedlings [68]. Silica NPs, or often known as mesoporous silica, are also popular materials for development of drug delivery and cell-imaging systems [69, 70]. There are few genotoxicity studies on silica NPs but a notable one by Sayes et al. [23] has shown that there are no significant inflammatory or genotoxic effects in mouse lungs on short-term exposure. Metal NPs such as platinum NPs (PtNPs) and iron oxide Fe_2O_3NPs are also popular alternatives. There is one report on PtNP toxicity which showed an increase in DNA damage concurrent with p53, p21 downregulation, and cell-cycle arrest at the S-phase [43]. Fe and Fe_2O_3NPs are also known to be toxic and can cause significant DNA damage [36].

Other particles of note are the nanopolymers. Although there is a wide variety of such nanopolymers, they are generally known as a family of compounds that consists of chain units, which could be fashioned into nano-sized particles. These are also largely being developed for use in drug delivery [71]. Current genotoxicity studies suggest that some of these nanopolymers show antiinflammatory properties and also non or limited DNA damage [45, 72]. However, a recent report has implicated long-term nanopolymer exposure to pulmonary fibrosis and granuloma formation, resulting in two fatal deaths [73]. This case cannot be taken in isolation and others have raised the concern that the workplace condition as well as health or other pre-dispositions of the workers involved should be considered [74].

4. NMs and Carcinogenesis

While it has been shown in many *in vitro* experiments that NMs are able to induce DNA damage and some form of mutagenesis, there is still a lack of evidence for tumorigenicity of NPs. Of note, *in vivo* studies involving MWCNT has demonstrated formation of mesotheliomas in rodents in works by Takagi and colleagues [75] and Sakamoto et al. [76]. Wide spread deposition of MWCNTs were observed in the peritoneal cavity where the nanotubes were injected. In the study by Sakamoto et al., they have even found mesotheliomas in the peritoneal cavity away from the original site of injection, suggesting that MWCNTs may easily translocate and also exert effects away from organ of exposure. Both studies emphasized on the persistency, size and shape on the carcinogenic potential of MWCNTs. While such studies may provide some insight into the outcome of NM toxicity, one must take into account the differences in how the nanotubes were prepared as well as the experimental design. Muller et al. conducted similar tests on MWCNTs but reported no carcinogenicity after a 2 year period of exposure [77]. They speculate that tumor formation could be dependent on size and length of the nanotubes administered and the p53 knockout mice used in the Takagi study produced a more sensitive carcinogenic reaction. However, NMs can induce oxidative stress and trigger inflammatory responses, which could form the starting point for carcinogenesis to occur. NMs that are highly reactive are also more likely to absorb endogenous substances, react with proteins and enzymes, trigger cytokine release. This would mediate inflammatory responses and potentially initiate a series of toxic responses far from the initial site of deposition [78, 79]. C60 fullerene, for example, was reported to cause photo-induced DNA damage by interacting with biological reducing agents such as NADH to cleave supercoiled DNA [80]. Similarly, exposure to carbon nanotubes in atmospheric air pollution has been associated with adverse cardiovascular effects by causing aortic DNA damage, platelet aggregation and enhances vascular thrombosis through inflammatory events [81].

Biopersistence of NMs pose a certain degree of adverse health effect. For instance, when the clearance rate is slower than the accumulative rate, the NMs will remain in the lungs; and those containing mutagenic substances will increase the risk of developing cancer. To address this concern, Sera et al. conducted a mutagenicity test using 3 different strains of *Salmonella* and found C60 Fullerene to exert mutagenic activity due to the oxidized phospholipids in rat liver microsms [82].

There are also certain shortcomings in the current research field. The short-term nature of toxicology tests in the treatment period for NMs generally lasts only up to three days, which implies that testing is limited to acute toxicity. *In vitro* and *in vivo* genotoxicity testing will have to be conducted for longer periods to observe if there are long-term effects of NMs such as tumour formation and carcinogenesis. Treatment intervals will have to go beyond days to weeks or even months in animal studies. It will also be useful to look at the clearance of NMs from the body and

to study if there is a preference for accumulation in certain organs and any effect from biopersistence of such NMs.

On the public front, safety measures have been implemented to safeguard the public health. The International Agency for Research on Cancer (IARC) recently classified TiO_2 as a potential Group 2B human carcinogen. This decision was made on the experimental animal carcinogenicity data [83]. There had been four previous epidemiological studies conducted among the male production workers at TiO_2 industry from Western Europe and North America. After comparing the risk for lung and kidney cancer with the general population, they concluded that these data were not supportive enough to conclude the association between occupational exposure of TiO_2 and cancer risk. Hence, data collected were inadequate in classifying TiO_2 as potential carcinogen. However, there was sufficient animal carcinogenicity data that provided evidence of TiO_2-induced carcinogenicity. Several TiO_2 exposure routes were chosen for experimental animal studies. These include oral, inhalation, intratracheal, subcutaneous injection, and intraperitoneal administration. Researchers observed an increase in tumor incidence in these experimental animals upon TiO_2 exposure. After considering other relevant data such as clearance kinetics of TiO_2 and micronucleus formation, a conclusion that TiO_2 possess possible carcinogenicity to human was made.

5. Conclusion

The field of nanotoxicology, besides investigations on the adverse effects of NMs, also include continuous monitoring and risk assessment of NMs. Despite the many nanotoxicological studies that are ongoing, there are questions that need to be answered and addressed. There is difficulty in interpreting data in view of variable parameters utilized in the study, for example, the sizes of the NMs and its composing materials. The most critical research gap is the lack of studies on real-time NM exposure. Moreover, there is a need for long-term nanomaterials exposure assessment for studies on tumourigenesis. At the industry level, close monitoring and followup on the levels of emissions from NM production industries are essential in protecting public health and our environment. However, there still exists a lack of appropriate epidemiological studies and equipment for accurate collection of data in assessing the real risk of NM exposure in the workplace. Despite the promising applications of NMs, there are still doubts regarding their safety. There is some certainty that NMs do pose a certain degree of health risk that would require further investigation. A proper guideline on NM usage is imperative to ensure the safety of NMs for consumer usage and environment.
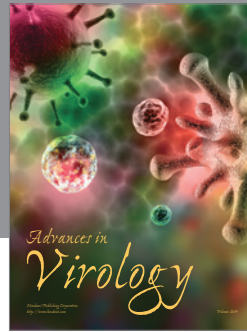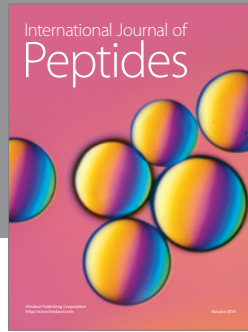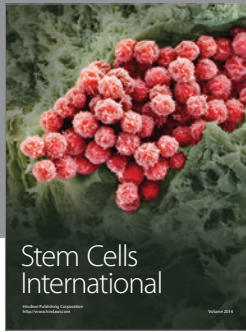
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