European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2011; 15: 481-508

Toxicological effects of titanium dioxide nanoparticles: a review of *in vitro* mammalian studies

I. IAVICOLI, V. LESO, L. FONTANA, A. BERGAMASCHI

Institute of Occupational Medicine, School of Medicine, Catholic University of the Sacred Heart, Rome (Italy)

Abstract. – *Background and Objective:* **Recent rapid advances in nanotechnology raise concerns about development, production route, and diffusion in industrial and consumer products of titanium dioxide nanoparticles (TiO_2-NPs). In fact, compared to recent increase in applications of this nanomaterial, the health effects of human exposure have not been systematically investigated. The aim of this review was to provide a comprehensive overview on the current knowledge regarding the effects of TiO_2-NPs on mammalian cells.**

Evidence and Information Sources: **This review is based on an analysis of the current literature on this topic.**

State of the Art: **Fine TiO_2 particles have been considered as safe and to pose little risk to humans, suggesting that exposure to this material is relatively harmless. However, available data in the literature showed that TiO_2-NPs can cause several adverse effects on mammalian cells such as increase of reactive oxygen species (ROS) production and cytokines levels, reduction of cell viability and proliferation, induction of apoptosis and genotoxicity.**

Perspectives and Conclusions: **Additional research is needed to obtain up-to-date knowledge on health effects of TiO_2-NPs and to avoid any potential risk correlated to their exposure. Consequently, future studies need to: (1) use an homogeneous and rigorous exposure classification to clarify how the physicochemical properties of TiO_2-NPs correlate with their toxicological effects; (2) assess the potential adverse effects of low level exposures to TiO_2-NPs, as most of the information currently available originates from studies in which exposure levels were excessively and unrealistically high; (3) identify the possible roles of TiO_2-NPs in genotoxicity and carcinogenicity (4) carry out epidemiologic studies of exposed workers to provide an assessment of possible risks correlated to the occupational exposure to TiO_2-NPs.**

Key Words:

Titanium dioxide, Nanoparticles, *In vitro* studies, Effects, Genotoxicity.

Introduction

Nanotechnology is a broad interdisciplinary branch of science, grouping physical, chemical, biological, and engineering expertise, that has the ability to manipulate matter at the level of single atoms and small groups of atoms to produce new structures, materials and devices with unique physical and chemical properties[1]. Research in nanoscale technologies is growing rapidly worldwide. The U.S. National Science Foundation[2] estimates that, by 2015, nanotechnology will have a $1 trillion impact on the global economy and will employ 2 million workers. The industrial applications of nanomaterials are very wide including those that may lead to more efficient water purification, stronger and lighter building materials, increased computing power and speed, improved generation and conservation of energy, and new tools for the diagnosis and treatment of diseases[3]. To achieve these multiple objectives nanotechnology, in the manufacturing of different consumer and industrial products, uses several types of nanoparticles (NPs) such as carbon nanotubes, silica and metal NPs.

Among the manufactured NPs, titanium dioxide NPs (TiO_2-NPs) are the earliest industrially produced nanomaterials[4] and, according to the U.S. National Nanotechnology Initiative, they are one of the most highly manufactured in the world[5]. In fact, they are widely used in paints, printing ink, rubber, paper, cosmetics, pharmaceuticals, sunscreens, car materials, cleaning air products, bio-medical ceramic and implanted biomaterials, sterilization, industrial photocatalytic processes and decomposing organic matters in wastewater[6-8].

Titanium dioxide is a natural, highly insoluble, thermally stable and non-flammable, non-silicate mineral oxide found primarily in the form of the minerals rutile, anatase, brookite and as the iron-

Corresponding Author: Ivo Iavicoli MD, PhD; e-mail: iavicoli.ivo@rm.unicatt.it.

containing mineral ilmenite[9-13]. The major natural source of TiO_2 is ilmenite, whereas rutile and anatase polymorphs are mainly manufactured commercially[13]. Though both rutile and anatase belong to the tetragonal crystal system, rutile has a denser arrangement of atoms. The luster and hardness of anatase and rutile are also similar, but their cleavages differ. Common impurities in rutile include iron, tantalum, niobium, chromium, vanadium and tin, whereas those in anatase include iron, tin, vanadium and niobium[14]. Due to its excellent physicochemical properties of good fatigue strength, resistance to corrosion, machineability, biocompatibility, whitening and photocatalysis, as well as its excellent optical performance and electrical properties, TiO_2 has a wide range of applications[15,16]. In fact, it is used mainly in paints, varnishes, lacquer, paper, plastics, ceramics, printing ink, welding rod coatings, floor coverings, catalysts, coated fabrics and textiles, cosmetics, food colorants, glassware, pharmaceuticals, roofing granules, rubber tire manufacturing and in the production of electronic components and dental impressions[14,17-19].

The potential adverse health effects of TiO_2 have been investigated in different experimental investigations[20-23] and in some epidemiological studies[24-27]. On the basis of results and data collected by researchers TiO_2 was considered to exhibit relatively low toxicity[28] and inhaled fine particles of this material are generally regarded, at least under nonoverload conditions[29,30], biologically inactive and physiologically inert[21,29,31,32]. The International Agency for Research on Cancer (IARC) has recently reclassified TiO_2[33] (IARC, 2006) as "possibly carcinogenic to humans" (group 2B) because high concentrations of pigment-grade (< 2.5 μm) and ultrafine (< 100 μm) TiO_2 dust can cause respiratory tract cancer in exposed rats[34,35]. However, the epidemiological studies of workers exposed to pigment-grade TiO_2 have not be able to detect an association between occupational exposure and an increased risk for lung cancer[36,37].

With the advent of nanotechnology, some of the assumptions concerning the safety of TiO_2 would be challenged. In fact, the chemical, optical, magnetic, biological and structural properties of NPs may differ considerably from those of larger particles composed of the same materials[38,39]. Since available data in the literature show that the physico-chemical characteristics of NPs are closely associated with their biological effects[40-45], and considering that they are deeply unlike in TiO_2-NPs, respect to their bulk counterpart, it is reasonable to assume that TiO_2-NPs have also a quite different toxicological profile. In the literature there are several *in vitro* and *in vivo* studies that have investigated the toxicological effects of TiO_2-NPs, even if the former studies are much more numerous and extremely useful means to investigate underlying mechanistic processes and to decide on the dose levels for the latter studies. Nevertheless, there is a lack of an overall evaluation on these effects to define an adequate risk assessment, in particular to protect exposed workers and general population subjects. Therefore, in this review we will evaluate current knowledge regarding the *in vitro* toxic effects of TiO_2-NPs to identify research areas where further studies should be carried out in order to reach a fuller understanding of TiO_2-NPs toxicity.

In vitro studies

Lung Cells

Studies conducted on lung cells (Table I) demonstrate that several parameters seem to be correlated with the toxicity of the TiO_2-NPs, principally the form of the TiO_2, but also the size, shape, surface chemistry and surface area of NPs.

Concerning the form of TiO_2, different studies have reported several toxic effects due to anatase TiO_2-NPs relative to other forms. In fact, anatase TiO_2-NPs have been found to produce greater responses, particularly a reduction of cell viability[46-51], an increase of inflammatory indices (e.g., lactate dehydrogenase (LDH) and interleukin (IL)-8)[46] and an increase in radical oxygen species (ROS) generation[46,49,51-54], that in some studies was shown to be dose-dependent[49,51]. Finally, anatase NPs are also able to induce cell death by an intrinsic apoptosis pathway[51]. This higher toxicity of anatase TiO_2-NPs may be correlated to their photocatalytic activity, as suggested by Sayes et al[46].

Furthermore, though the aforementioned studies were performed with all due diligence, it is important to note that is difficult to extrapolate the toxic effects observed *in vitro* studies with monocellular cultures to the real *in vivo* situation of the lung, and consequently, these findings should be considered with caution. Indeed, this point of view is confirmed by the fact that in a triple culture model that simulates the real cellular conformation of the lung, exposed to anatase TiO_2-NPs, no statistically significant increases in

Table I. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on lung cells.

Lung cells					
Reference	**Crystal phase composition**	**Particle size (Nm)**	**Doses of exposure**	**Cell lines**	**Results**
Stearns et al, 2001 – (67)	TiO_2	50	0.25, 5, 40 µg/ml	A549 human lung epithelial cells	• Phagocytized TiO_2 in vacuoles (early) or lamellar bodies; • TiO_2 externally associated with plasma membrane.
Gurr et al, 2005 – (52)	Anatase TiO_2	10; 20 200 > 200	10 µg/ml	Human bronchial epithelial cells (BEAS-2B)	• Induction of oxidative stress; • Inhibition of cell growth.
	Rutile TiO_2	200			
Sayes et al, 2006 – (46)	100% anatase TiO_2 60% anatase - 40% rutile mixture TiO_2 100% rutile TiO_2	10.1 ± 1.0 5.2 ± 0.34 3.2 ± 0.34	3 µg/ml-3 mg/ml for 48 hr	A549	• High LDH release; • Decreased mithocondrial activity; • Enhanced IL-8 production.
Chen et al, 2006 – (61)	≥ 99.5% pure rutile TiO_2	21	0.1, 0.2, 0.5 µg/ml	Human monocytes THP-1 cells; A549	• Increased PIGF mRNA and protein levels and increased cxcl-5 mRNA levels; • Increased MCP-1(CCL2) protein level (THP-1 cells); No or only a slight effect on the above-mentioned parameters
	TiO_2	180-250	0.2 µg/ml		
Xia et al, 2006 – (57)	P25 Degussa TiO_2: 80% anatase - 20% rutile	20-30	10 µg/ml	Murine alveolar macrophage cells (RAW 264.7)	• TiO_2 failed to produce ROS, had no effect on GSH depletion and on Heme Oxygenase-1 expression; • TiO_2 taken up into lose-fitting phagosomes.
Park et al, 2007 – (68)	TiO_2	30; 1 µm	5- 200 µg/ml	A549	• Increase of granularity of cells (micro TiO_2 > nano TiO_2). • Micro and nano TiO_2 located in cytosol as aggregates. • 14-17% of apoptotic cells (micro and nano TiO_2); • ~ 7-8% DNA fragmentation (micro and nano TiO_2).
Soto et al, 2007) – (47)	Anatase TiO_2	5-40	5 µg/ml	RAW 264.7; Human alveolar macrophage cells (THB-1); A549.	• Anatase EC50 (cell death) was ~10, ~5, ~2 µg/ml for RAW 264.7, THB-1 and A549, respectively.
	Rutile TiO_2	2-60			
Singh et al, 2007 – (58)	Pure anatase TiO_2 P25 Degussa TiO_2: 80% anatase - 20% rutile	40-300 20-80	3, 16, 80, 400 µg/cm^2	A549	• TiO_2 incorporated in vacuoles and lamellar bodies; • Increased ROS production (ultrafine TiO_2); • Increased IL-8 levels (ultrafine TiO_2).
Monteiller et al, 2007 – (62)	TiO_2; TiO_2-NPs	N.A.	15- 250 µg/ml	A549	• Increase of IL-8 mRNA and protein levels; • GSH depletion with both TiO_2.
Bhattacharya et al, 2008 – (53)	Anatase TiO_2 Vanadium pentoxide (V_2O_5) treated anatase TiO_2	30-50 30-50	1-100 µg/cm^2	Chinese hamster lung fibroblasts (V79)	• Reduction of cell viability; • Induction of ROS.

Continued

Table I. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on lung cells.

Lung cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Bhattacharya et al, 2008 – (53)	Anatase TiO_2 Vanadium pentoxide (V_2O_5) treated anatase TiO_2	30-50 30-50	1-100 μg/cm^2	Chinese hamster lung fibroblasts (V79)	• Reduction of cell viability; • Induction of ROS.
Bhattacharya et al, 2009 – (49)	Anatase TiO_2	< 100	2-50 μg/cm^2	BEAS-2B; Human diploid bronchial fibroblasts (IMR 90)	• TiO_2-NPs were clustered in cytoplasm vacuole like structures or lysosomes; • Cytotoxic effects observed only in IMR90; • Dose-dependent intracellular ROS production.
Park et al, 2008 – (59)	P25 Degussa TiO_2	21	5-40 μg/ml	BEAS-2B	• Dose and time dependent reduction of cell viability; • Dose and time dependent increase of ROS production; • Decreased intracellular reduced GSH; • Induction of oxidative stress related genes; • Increased levels of IL-1, IL-6, IL-8, TNF-α and CXCL2; • Induction of apoptosis.
Simon-Deckers et al, 2008 – (60)	P25 Degussa TiO_2, 75% anatase CEA TiO_2, 95% anatase Sigma TiO_2, 100% anatase Sigma rutile TiO_2, 100% rutile	25 ± 7 12 ± 3 142 ± 36 9 ± 3	0.25-100 μg/ml	A549	• Cell membrane damage; • Reduction of cell viability; • TiO_2-NPs located in the cytoplasm, isolated or in vacuoles.
Rothen-Rutishauser et al, 2007 – (55)	99.9% anatase TiO_2	0.02-0.03 μm	5 μg/ml	A549 Human monocyte derivated macrophages (MDM); Human monocyte derivated dendritic cells (MDDC)	• Membrane-bound aggregates; • Single particles and small aggregates free in cytoplasm in all cell types; • No increase in TNF-α release.
Rothen-Rutishauser et al, 2008 – (54)	99.9% anatase TiO_2	0.02-0.03 μm	5 μg/ml	A549; MDM; MDDC.	• Single particles unsurrounded by membranes and in membrane-bound agglomerates; • Increased ROS production; • No increase in TNF-α release.
Muller et al, 2010 – (56)	Anatase TiO_2	20-30	2.5 μg/ml	A549; MDM; MDDC.	• Internalization of TiO_2-NPs in all cell lines and culture types; • Induction of ROS (except in MDDCs); • In the triple co-culture the TAC, IL-8 and TNF-α values were lower than expected values.

Table I. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on lung cells.

Lung cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Hussain et al, 2009 – (63)	99.9% anatase TiO_2; 65% anatase - 35% rutile TiO_2	15 25-75	5-160 μg/cm^2	Human bronchial epithelial cells (16HBE14o-)	• Aggregates inside endosomes or free in the cytoplasm; • Increased ROS production in HE positive cells; • 15 nm TiO_2 particles increased GM CSF mRNA and GM-CSF release.
Val et al, 2009 – (50)	99.9% anatase TiO_2	15	0-160 μg/cm^2	16HBE14o-	• Dose dependent citotoxic effects; • Dose dependent increase of GM-CSF, IL-6, TNF-α mRNA; • Increased intracellular cytokine contents; • Absorption of GM-CSF and TNF-α by TiO_2-NPs.
Falck et al, 2009 – (48)	99.5% pure rutile TiO_2 coated with SiO_2 (< 5%) 99.9% rutile TiO_2 99.7% anatase TiO_2	10 × 40 < 5 μm < 25	1-160 μg/cm^2	BEAS 2B	• Fine rutile showed the highest cytoxicity, followed by anatase, while nanosized rutile was the least cytotoxic
Hamilton et al, 2009 – (64)	Anatase TiO_2 nanospheres Anatase TiO_2 long nanobelts Anatase TiO_2 short nanobelts	60-200 width: 60-300; length 15-30 width: 60-300; length 0.8-4 μm	0-250 μg/ml	C57BL/6 primary murine alveolar macrophages (AM); MARCO null mice AM; C11-BODIPY (581-591) loaded AM	• TiO_2 nanospheres and short nanobelt were taken up in lysosomes, long nanobelt were "free-floating" in cytoplasm; • MARCO receptor was involved in TiO_2 nanospheres uptake. • Long nanobelt were cytotoxic and increased Cathepsin B levels; • Increased ROS levels and lipid peroxidation; • Increased IL-1β and IL-18 levels.
Magrez et al, 2009 – (65)	Na (x) $TiO_{2+\delta}$ or Hy $TiO_{2+\delta}$	12 nm nanotubes 75 nm nanowires	0.02-2 μg/ml cells (H596)	Human lung tumor	• Nanofilaments impaired cell proliferation; • $HyTiO_{2+\delta}$ were more toxic than $Na(x)TiO_{2+\delta}$ forms; • Nanofilaments were inside cells in needle-like structures and cell morphology was altered.
Shi et al, 2010 – (51)	99.7% anatase TiO_2	< 25	0-100 μg/ml	BEAS-2B	• Dose dependent reduction of cell viability; • Induction of apoptosis; • Dose dependent increase of ROS.
Rossi et al, 2010 – (66)	Rutile TiO_2 Rutile/anatase TiO_2 Anatase TiO_2 Silica coated rutile TiO_2	< 5 μm 30-40 25 ≈10 × 40 nm, needle-like	0-500 μg/ml	RAW 264.7; MDM; Human pulmonary fibroblasts (MRC-9)	• Induction of TNF-α protein (RAW 264.7) and TNF-α mRNA and protein (MDM) by silica coated NPs; • Induction of CXCL-1 and CXCL-8 mRNA expression (MDM) by silica coated NPs; • Stimulation of MRC-9 fibroblasts with silica coated TiO_2 activated macrophage supernatants elicited CXCL-1 and CXCL-8 mRNA.

CCL2, chemokine (C-C motif) ligand 2; CXCL-1, chemokine (C-X-C motif) ligand 1; CXCL2, chemokine (C-X-C motif) ligand 2; cxcl-5, chemokine (C-X-C motif) ligand 5; CXCL-8, chemokine (C-X-C motif) ligand 8; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSH, reduced glutathione; HE, hydroethidine; IL-1, interleukin- 1; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; IL-18, interleukin-18; LDH, lactate dehydrogenase; MCP-1, monocytes chemoattractant protein-1; N.A., not applicable; PIGF, placenta growth factor; ROS, reactive oxygen species; TAC, total antioxidant capacity; TiO_2-NPs, titanium dioxide nanoparticles; TNF-α, tumor necrosis factor-α.

ROS production and inflammatory parameters (i.e., IL-8 and TNF-α) were observed[54-56]. Surprisingly, however, these parameters were actually lower than the expected values experimentally calculated from the monocultures used by the Authors.

Other investigations have reported conflicting data on the effects of another form of TiO_2-NPs: P25 Degussa. In fact, it was observed that P25 Degussa NPs are unable to induce ROS production[57], and subsequently, other research groups have shown that these NPs induce ROS generation[58,59], IL-8 release[58], decrease of glutathione (GSH) levels[59] and a time- and dose-dependent reduction of cell viability[59]. Interestingly, increased ROS generation appeared to stimulate cytotoxicity by an apoptotic process, even if only in BEAS-2B cells[59].

Concerning the rutile form, several studies[46-48,60] reported the minimal toxicity of this type of NPs even if in a single study[61] toxic effects were observed. In particular it was shown a significant induction of placenta growth factor expression in the monocytes and A549 cells at the mRNA, protein and secreted protein levels.

Another study[60] analyzed the effects of exposure to NPs composed of different forms of TiO_2 in A549 cells and found that the size of the NPs has an important role in determining toxic effects. In fact, the Authors observed that the smallest anatase and P25 TiO_2-NPs exerted the greatest cytotoxic effects.

Conversely, other Authors have highlighted the importance of the surface area of TiO_2-NPs as being correlated to a stronger pro-inflammatory effect and oxidative stress[62,63].

Concerning the shape, as parameter of TiO_2-NP toxicity recenty it has been observed that nanobelt- or nanofilament-shaped TiO_2-NPs, as opposed to other shapes of the same nanomaterial, induce cytotoxicity[64,65], likely via apoptosis[64].

The toxicity of TiO_2-NPs seems to be also correlated to the surface chemistry of nanomaterials[65], which affects inflammatory responses[66]. In fact, different from other anatase and rutile NPs, only exposure of murine and human macrophages to silica-coated NPs resulted in significant induction of TNF-α and neutrophil-attracting chemokines.

Finally in several *in vitro* researches it was evaluated also the internalization of NPs in lung cells, another important aspect of TiO_2-NP toxicity, which was shown to be dose-[67,68] and time-[67] dependent. However, internalization is neither influenced by the form of the TiO_2-NPs[58,60,68] nor by their shape[64,65]. Particles were detected in the cytosol of cells, particularly in the peri-region of the nucleus, and trapped in vacuoles, lamellar bodies and/or lysosomes[49,58,59] even though free nanoparticles[60] and needle-like nanofilament structures[65] were observed at early treatment times. This phenomenon has been observed in all types of lung cells examined to date and in the triple cell co-culture model[55,56]. The internalization was also correlated with the oxidative stress and inflammatory responses[63].

Nervous Cells

Different *in vitro* researches have evaluated the toxic effects of TiO_2-NPs and their internalization in nervous cells (Table II). The findings suggest that an important parameter determining NP toxicity is the form of TiO_2, in particular, P25 Degussa[69-72] and anatase NPs[73]. Furthermore, a recent study[74] correlated the toxicity of these NPs with their surface chemistry investigating the effects of rutile TiO_2-NPs coated with SiO_2 on the immortalized murine neural stem cell line. After 24 h of incubation with these NPs, a dose-dependent decline in proliferation of cells and a differentiation tendency towards neurons from exposed neural stem cells were observed. Indeed, the Authors correlated these findings with the ability of TiO_2-NPs to induce neuronal differentiation. To gain insight into the possible molecular mechanism(s) of this neuronal differentiation, Liu et al[74] mapped target proteins in lysates of stem cells treated and untreated with TiO_2-NPs, and nine protein levels significantly changed. Subsequent mass spectrometric assay demonstrated that these altered proteins were signaling molecules, molecular chaperones, cytoskeletal components, and nucleoproteins. Furthermore, the Authors conducted also a gene expression analysis. They concluded by suggesting that the regulation of protein kinase C epsilon gene expression is indirectly correlated with the neuronal differentiation of this stem cells exposed to TiO_2-NPs.

The aforementioned researches also report the internalization of TiO_2-NPs by different types of neural cells, in particular, microglia cells[69,70], dopaminergic neurons[70] and neural stem cells[74]. Particles were detected in the cytosol of cells as aggregates in membrane-bound vacuoles or in endosomal vesicles[69,70,74].

Table II. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on nervous cells.

Nervous cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Long et al, 2006 – (69)	Degussa P25 TiO_2: 70% anatase - 30% rutile	~ 30	2.5-120 mg/kg	Immortalized mous e brain microglia (BV2)	• Rapid and sustained release of H_2O_2 and O_2^-; • Small clusters phagocytised and internalized into the cells cytoplasm; • Disrupted and swollen mithocondrial lying.
Long et al, 2007 – (70)	Degussa P25 TiO_2: 70% anatase - 30% rutile	300-350; 800-1900 (aggregate size)	2.5-120 mg/kg	BV2; Rat N27 mesencephalic neurons; Primary cultures of embryonic rat striatum	• Rapid release of H_2O_2 and of O_2^- (BV2 cells); • Internalization in BV2 and N27 cytoplasm; • Increased activity of caspase 3/7 in BV2 and N27 cells; • Induction of apoptosis in embryonic striatum cells • Up-regulation of genes involved with apoptosis, death receptor families, calcium signaling, inflammation, cell cycling and oxidative stress; • Down-regulation of genes associated with adaptive change, energy production and hypoxia pathways, Nrf2-mediated oxidative stress. • Increased ATP in N27.
Lai et al, 2008 – (73)	> 99% pure beta-rutile TiO_2 99.7% pur e anatase TiO_2	1-1.3 μm < 25	0.1-100 μg/ml	Human astrocytoma U87 cells; Human fibroblasts (HFF1)	• Micro and nano TiO_2 particles caused a reduction of cell viability in U87 and HFF1 cells
Liu et al, 2010 – (72)	P25 TiO_2 (largely anatase)	21	1-100 μg/ml	PC12 rat pheochromocytoma cells	• Reduction of cell viability; • Increase of percentage of apoptotic cells; • Dose-dependent increase of ROS levels.
Liu et al, 2010 – (74)	Rutile TiO_2 coated by SiO_2	80-100	0-250 μg/ml	Mouse neural stem cells (NSCs) line C17.2	• Internalisation into cytoplasm (endosome vesicles); • Dose-dependent inhibition of cellular proliferation; • Up-regulated proteins: tumor reaction antigen gp96; pyrophosphatase (inorganic)1; Rho GDP dissociation inhibitor (GDI) alpha; • Down-regulated proteins: T-complex protein 1, pyrophosphoryle 1- like 1, peroxiredoxin-6 nuclear mitotic apparatus protein isoform CRA_b, tropomyosin 4,vimentin.
Shin et al, 2010 – (71)	100% rutile TiO_2 TiO_2 (80% anatase - 20% rutile)	1 μm (fine) 21 nm (ultrafine)	25-200 μg/ml	BV2 stimulated or not with LPS	• Ultrafine TiO_2 increased the LPS induced TNF- α; • Ultrafine TiO_2 augmented NF-kB activity in LPS stimulated cells.

H_2O_2, hydrogen peroxide; LPS, Lypopolysaccaride; NF-κB, nucleic factor-KB; Nrf2, Nuclear Factor-E2-related factor 2; O_2^-, superoxide; ROS, reactive oxygen species; TiO_2-NPs, titanium dioxide nanoparticles; TNF-α, tumor necrosis factor-α.

Dermal and Mucosal Cells

The form of TiO_2 is also an important parameter of TiO_2-NP toxicity in dermal cells (Table III). In various studies[46,75-78], it has been reported that anatase TiO_2 is correlated with toxicity in human and murine dermal cells, though a recent study[79] failed to report these effects on primary human epithelial cells. The photocatalytic activity of the anatase form may explain this greater toxicity.

Some of the aforementioned studies have also reported NP internalization by dermal cells. In particular, it was reported that anatase TiO_2-NPs are internalized[75,78] in the cytoplasm and, surprisingly, as aggregates in the nucleus of a small percentage of cells[79]. Kiss et al[75] also reported that NP internalization is correlated to the type of cells.

Pan et al[77] showed that NP internalization in human dermal fibroblasts is independent of the NP forms; only anatase NPs induced vesiculated nuclei. Furthermore, these Authors underscored the importance of the NP surface chemistry, which, if changed, altered adhesion to and penetration of the cell membrane.

Unfortunately, the scientific value of these internalization investigations is limited because other *in vitro* studies have demonstrated that TiO_2-NPs do not penetrate intact human or pig skin. In fact, they were found only in the superficial layer of the stratum corneum but not in the epidermis and dermis. This phenomenon was observed with NPs of various forms, sizes, surface chemistries, and shapes, as well as with different applied formulations[80-84]. Finally, the lack of TiO_2-NP penetration in the skin was also observed in hair-removed skin, a model of damaged skin[84].

Cardiovascular Cells

Currently, there is only one *in vitro* study that investigated the effects of TiO_2-NPs on cardiac cells (Table IV). To this aim, Helfenstein et al[85] exposed patterned growth strands of cultured neonatal rat ventricular cardiomyocytes to anatase TiO_2-NPs. After 24 h it was observed a dose-dependent increase in impulse conduction velocity, maximal action potential upstroke velocity and ROS production. Moreover, laser scanning microscopy revealed that the myofibrillar structure in cells treated with a 0.25 μg/ml TiO_2 preparation was less organized than in unexposed cells. After a 24-h incubation with 2.5 μg/ml TiO_2-NPs, TEM analysis demonstrated that Z lines were thicker and not as regular as in control cells. On the basis of these results, the Authors concluded that TiO_2-NPs directly cause cardiac cell damage, affecting the function of the cells. However, further researches are needed to verify if these effects are also caused by TiO_2-NPs with different characteristics and using other cardiac cell lines.

Few studies have investigated the effects of TiO_2-NPs on vascular cells (Table IV). Some studies[86-88] examined the effects of NPs on human and murine endothelial cells but did not find significant effects, though the experimental models are limited. In particular, the Authors did not indicate the form of TiO_2 used, which, as we have previously seen, is an important parameter correlated with the toxicity of NPs. The lack of significant effects following TiO_2-NP exposure was also observed by Courtois et al[89,90] using rat arteries that simulate the *in vivo* situation.

In conclusion, further research is needed, taking in consideration the characterization of the NPs and using experimental models that simulate the *in vivo* situation, to clarify the effects of TiO_2-NPs on vascular cells.

Hepatic and Intestinal cells

Few Authors have investigated the effects of TiO_2-NPs on hepatic and intestinal cells, and there are not *in vitro* investigations on gastric cells (Table V). Indeed, there are only four studies in the literature[91-94] on the toxicity of TiO_2-NPs on hepatic cells, and it seems that these NPs do not exert direct effects, with the exception of increase production of malonaldehyde (MDA) and ROS. On the contrary, after TiO_2-NP exposure, significant alterations were observed with contemporary exposure of cells to visible light or to dichlorodiphenyltrichloroethane (p,p'-DDT).

To date, there are only two articles that investigated the effects of TiO_2-NPs on intestinal cells. In the first[95], human colon carcinoma cells were exposed to TiO_2-NPs with or without UVA irradiation. The Authors observed that TiO_2-NPs alone were not cytotoxic. However, when the cells were exposed to UVA irradiation and TiO_2-NPs, they died at a much faster rate that was correlated to the NP concentration and duration of irradiation. The second study by Koeneman et al[96] investigated the pathways by which a mixture of anatase and rutile TiO_2-NPs crossed a model of the intestinal epithelium layer composed of human Caco-2 cells. The Authors reported that the NPs were able to cross the intestinal epithelium layer by transcytosis without damaging its in-

Table III. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on dermal and mucosal cells.

Dermal and mucosal cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Gamer et al, 2006 – (80)	T-Lite SF-S emulsion with 10% needle-like TiO_2 particles coated with silica (2-5wt %) and methicone (4.5-6.5%) T-Lite SF emulsion with 10% needle-likeTiO_2 particles coated with methicone (3.5-5.5%)	30-60 × 10	4 mg/cm^2	Full thickness intact porcine skin	• The mean total Ti recoveries were 98-100% and 86-93% in the T-Lite SF-S and T-Lite S, respectively; • All Ti applied was removed by washing; • The Ti amount found in tape strips and skin preparations was in the order of analytical determination limit; • No Ti in the receptor fluid.
Sayes et al, 2006 – (46)	100% anatase TiO_2 60% anatase - 40% rutile mixture TiO_2 100% rutile TiO_2	10.1 ± 1.0 5.2 ± 0.34 3.2 ± 0.34	3 μg/ml-3 mg/ml	Human dermal fibroblast (HDF).	• Dose and time-dependent decrease of cell viability; • Anatase increased LDH release; • Anatase decreased mithocondrial activity; • Anatase induced greater levels of ROS compared to rutile.
Mavon et al, 2007 – (81)	UV emulsion with 3% T805 Degussa TiO_2 particles hydrophobically coated with trimethyloctylsilane	20	2 mg/cm^2	Abdominal human skin explants	• 50.7 μg/cm^2 TiO_2 (> 94.2%) was recovered in the stratum corneum, 5.6% in epidermis, < 0.1% in dermal compartment (88.8% recovery); • No TiO_2 in the receptor fluid; • TiO_2 was found in the first 10 tape strips.
Vileno et al, 2007 – (134)	Anatase TiO_2	5	4 μg/ml	Normal human skin fibroblasts CCL-110 (ATCC)	• UVC alone or with TiO_2 and UVA in the presence of TiO_2 decreased cells stiffness; • β-carotene prevented photo-oxidative stiffness alterations.
Kiss et al, 2008 – (75)	Anatase TiO_2	9	0.15-15 μg/ cm^2	Human immortalized HaCaT keratinocyte cell line; HDF; Human immortalized sebaceous gland cell line (SZ95); Primary human melanocytes.	• TiO_2 was detected in the perinuclear area of HDFs and melanocyte cytoplasm; • Slow and reversible elevation in [Ca^{2+}] in fibroblasts and melanocytes. • Dose and time dependent decrease of cellular growth; • Differentiation markers: TiO_2 decreased involucrin, desmoglein-1 and P-Cadherin levels on keratinocytes
Jin et al, 2008 – (78)	100% anatase TiO_2	5	3-600 μg/ml	Mouse subcutaneous fibroblasts (L929)	• Round and shrank cells as TiO_2 dose increased, condensated fragmented chromatin and necrosed cells; • Increased lysosomes, and damaged organelles; • Dose-dependent reduction of cell viability; • Increased ROS and LDH levels and decreased GSH and SOD levels.

Continued

Table III. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on dermal and mucosal cells.

Dermal and mucosal cells					
Reference	**Crystal phase composition**	**Particle size (Nm)**	**Doses of exposure**	**Cell lines**	**Results**
Pan et al, 2009 – (77)	Rutile TiO_2 Anatase TiO_2	15.0 ± 3.5 200 ± 13	0.4, 0.8 mg/ml 0.1-0.5 mg/ml	HDF	• Decrease in the cells area (rutile), with actin fibers thinner and less extended (rutile and anatase TiO_2). • Both particles types were internalised in cytoplasm; • Reduction of cell viability; • Weak traction forces, ~ 59% reduction in migration and less than 80% control decrease in contract matrix collagen.
Wu et al, 2009 – (82)	99.5% pure anatase hydrophobic TiO_2 99.5% pure rutile hydrophilic TiO_2	5 ± 1; 10 ± 1 25 ± 5; 60 ± 10; 90 ± 10.	5% (w/w) solution applied on 2 cm × 2 cm area	Isolated ear porcin skin	• No Ti was detected in receptor fluid; • Only a trace amount of Ti was detected in the first 5 tape strips (uppermost layers of the stratum corneum); • TiO_2 was not detected in epidermis and dermis.
Van der Merwe et al, 2009 – (83)	Nanocrystalline TiO_2	< 1	50 mg/cm^2	Dermatomed human skin	• Skin absorption was not detectable as assessed by a Bronaugh-type flow-trough diffusion cell system.
Fujita et al, 2009 – (76)	Bulk 100% anatase TiO_2 (ST-01) Bulk 100% anatase TiO_2 (ST-21) Bulk 100% anatase TiO_2 (ST-41)	7 20 200	47.0, 58.8, 60.2 μg/ml	Human keratinocyte HaCaT cells	• Reduction of cell viability; • Over-representation of genes involved in inflammation and cell adhesion. • 7 nm TiO_2 altered expression levels of MMP-9 and MMP-10, fibronectin FN-1, integrin ITGB-6, and mucin MUC-4.
Zhao et al, 2009 – (135)	P25 Degussa TiO_2 (80% anatase - 20% rutile) Rutile TiO_2	21 < 5 μm	0.1-100 μg/cm^2	Mouse epidermal JB6 cells	• Dose-dependent cytotoxic effects (higher for TiO_2-NPs); • Induction of apoptosis (TiO_2-NPs); • Activation of caspase 8, Bid, BAX and caspase 3, PARP cleavage, citochrome c and decrease of Bcl – 2.
Senzui et al, 2010 – (84)	Uncoated rutile TiO_2 Alumina/silica/ silicon coated rutile TiO_2 Mixture of alumina coated and silicon coated rutile TiO_2	35 and 250 35 10 × 100	2 μl of 10% TiO_2 suspension applied on 1 cm^2	Intact, stripped and hair removed skin of Yucatan micropigs	• No penetration in intact or stripped skin; • Ti concentration was higher in hair-removed skin treated with coated 35 nm TiO_2; • Ti was observed into vacant hair follicles but not into dermis or viable epidermis.
Hackenberg et al, 2010 – (79)	Anatase TiO_2	25	10-100 μg/ml	Epithelial cells of human nasal mucosa	• Uptake of TiO_2 (11% cells) and nucleus invasion (4% cells). • No reduction of cell viability.

GSH, reduced glutathione; LDH, lactate dehydrogenase; MMP-9, matrix metalloproteinase-9; MMP-10, matrix metalloproteinase-10; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; SOD, superoxide dismutase; Ti, titanium; TiO_2-NPs, titanium dioxide nanoparticles; UVA, ultraviolet A; UVC, ultraviolet C.

Table IV. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on cardiovascular cells.

Cardiovascular cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Helfenstein et al, 2008 – (85)	Anatase TiO_2	20-30	0.0025-2.5 µg/ml	Neonatal rat ventricular cardiomyocytes (NRVM)	• Increased impulse conduction velocity and maximal action potential upstroke; • Dose-dependent increase of ROS levels; • Myofibrillar structure resulted less organized, with Z lines thicker and not regular compared to control cells.
Peters et al, 2004 – (86)	TiO_2	70 (range 20-160)	0.5-50 µg/ml	Human dermal microvascular endothelial cells (HDMEC)	• TiO_2 was internalised into autophagic vacuoles; • No reduction of cell viability and no effect on cell proliferation; • Increased IL-8 release.
Courtois et al, 2008 – (89)	TiO_2	15 0.14 µm	200 µg/ml	Segments of rat intrapulmonary arteries and/or bovine endothelial cells	• Both TiO_2 particles did not impair Ach-induced relaxation.
Courtois et al, 2010 – (90)	Degussa P25 TiO_2	15	0-200 µg/ml	Intralobar pulmonary rat arteries	• No alterations of vascular tone; • Contractile response to KCl or PGF2α and relaxant response to Ach were not modified.
Yu et al, 2010 – (87)	TiO_2	28	0-10 µg/ml	Pulmonary C57BL/6J mice vascular endothelial cells (MPMVEC)	• No reduction of cell viability; • No increase of DCF fluorescence and p38 phosphorylation; • No time response increase of PAI-1 expression.
Peng et al, 2010 – (88)	TiO_2 nanotubes	30 100	N.A.	Human aortic endothelial cells (HAECs)	• Enhanced endothelial cell proliferation and motility; • Decreased inflammatory and coagulation molecules.

Ach; acetylcholine; DCF, dichlorofluorescein; IL-8, interleukin-8; KCl, potassium chloride; N.A., not applicable; PAI-1, plasminogen activator inhibitor-1, PGF2α, prostaglandin 2Fα; ROS, reactive oxygen species; TiO_2-NPs, titanium dioxide nanoparticles.

Table V. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on liver and intestinal cells.

Liver and intestinal cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Linnainmaa et al, 1997 – (91)	P25 Degussa uncoated anatase TiO_2 UV - TITAN M160 rutile TiO_2 coated\ with alumina and\ stearic acid	20	0-200 μg/cm^2	Rat liver epithelial cells (RLE)	• Neither of the ultrafine TiO_2 was toxic to cells with or without UV irradiation.
Zhang and Sun, 2004 – (95)	TiO_2	21.2	200-1000 μg/ml	Human colon carcinoma cell lines (Ls-174-t)	• Reduction of cell viability after UVA irradiation. TiO_2 alone resulted non cytotoxic; • Condensed cellular shape ad fragmented nuclei were observed.
Hussain et al, 2005 – (92)	TiO_2	40	0-250 μg/ml	Immortalized rat liver cells (BRL 3A)	• No cytotoxic effects was observed
Wang et al, 2007 – (93)	Ce element (IV) doped anatase TiO_2 (CDT)	21	10 μg/cm^3	Hepatoma cell line (Bel 7402)	• Induction of apoptosis with visible light illumination for 10 min.
Koeneman et al, 2010 – (96)	Mixture of anatase and rutile TiO_2	< 40	1-1000 μg/ml	Human brush border expressing intestinal cell line, Caco-2	• TEER dropped 6 days after chronic exposure to 1000 μg/ml TiO_2; • γ catenin could be seen in epithelial sheet at 1-100 μg/ml, but not at 1000 μg/ml TiO_2 after 10 day treatment; • Weak reduction of cell viability; • Changes in microvillar organization and increased intracellular free calcium.
Shi et al, 2010 – (94)	P25 Degussa TiO_2 (80% anatase - 20% rutile)	25	0-1 μg/ml TiO_2 combined with 0-0.1 μmol/l p,p^I-DDT	Human fetus hepatic cell line L-02.	• No dose and time response differences were observed between any treatment groups for cell viability and apoptosis; • Every concentration of TiO_2 alone increased MDA levels, while only 1 μg/ml TiO_2 alone increased ROS levels; • Mixed TiO_2 and p,p^I - DDT had higher ROS and MDA compared to TiO_2 and p,p^I - DDT alone.

MDA, malonaldehyde; p,p^I-DDT, dichlorodiphenyltrichloroethane; ROS, reactive oxygen species; TEER, transepithelial electrical resistance; TiO_2-NPs, titanium dioxide nanoparticles; UV, ultraviolet; UVA, ultraviolet A.

tegrity and only causing subtle effects. The transcytosis of the epithelial cells, even at low levels, demonstrates the ability of NPs to be internalized by cells and supports the role of the gastrointestinal tract as a possible route of TiO_2-NP entry into organisms. The alterations reported in individual cells, although non-lethal, could impair the intestinal mucosal homeostasis, as well as nutrient absorption, with potentially health-impacting consequences, particularly in cases of chronic exposure. Moreover, these results suggest the need to discover markers of early tissue damages to detect toxic effects of TiO_2-NPs.

Finally, further researches are needed to verify the effects of TiO_2-NPs on hepatic and gastrointestinal cells, comparing to their characterization and NP internalization in these cells. Indeed, the limited number of the studies did not allow us to correlate any NP parameters (e.g., the form, size, surface area, etc. of the TiO_2-NPs) with adverse effects.

Hematopoietic Cells

Data regarding the effects of TiO_2-NPs on hematopoietic cells are heterogeneous due to the extremely different characteristics of these cells, particularly regarding their functional properties and the various endpoints evaluated by the published studies (Table VI).

White Blood Cells

Several Authors have investigated the cytotoxic effects of TiO_2-NPs on white blood cells. In particular, the cytotoxic effects of P25 Degussa TiO_2-NPs were studied on human peripheral blood lymphocytes by Kang et al[19] that reported a dose- and time-dependent reduction in cell viability, a dose-dependent increase of ROS in TiO_2 treated cells which was inhibited by the addition of N-acetylcysteine. In a subsequent study[97], the same researchers found that P25 Degussa TiO_2-NPs caused apoptosis of human peripheral blood lymphocytes. Data regarding mixtures of anatase and rutile are conflicting. Both necrotic and apoptotic cells were detected when human lymphoma cells were treated with a suspension of mixed anatase and rutile NPs[98]. Conversely, Wan et al[99] failed to observe cytotoxic effects of similar TiO_2NPs on the same cell line.

The cytotoxic effects of TiO_2-NPs on human lymphoblastoid cells were observed by Wang et al[100].

In this investigation cell viability, decreased in a dose-dependent manner, but only the highest NP dose induced a time-dependent viability reduction. TiO_2-NPs inhibited population growth according to a dose- and time-dependent relationship. Apoptosis was observed too. Unfortunately, details on the form of TiO_2 used are missing.

Similar effects were also observed in macrophage cell lines, particularly a decrease in cell viability and an increase in apoptosis[101]. However, no description of the TiO_2 form used is available. These findings also provide information on the functional impairment of white blood cells induced by TiO_2-NPs. Indeed, impairment of the phagocytic ability, phagosomal transport and cytoskeletal stiffness has been reported in murine macrophages. Previously, similar results were reported by Renwick et al[102], though in this study, phagocytosis was more strongly inhibited by ultrafine TiO_2 than by fine TiO_2. Unfortunately, the form of the TiO_2 used is not described.

The pro-inflammatory action of TiO_2-NPs has also been described[16,103,104]. In particular, Schanen et al[103] investigated the immunogenicity of anatase TiO_2-NPs, rutile TiO_2-NPs and TiO_2 nanotubes on a human immunologic system (MIMIC) composed of blood vein endothelial cells and monocyte-derived dendritic cells (DCs). In particular it was observed an increase of pro-inflammatory cytokines, such as IL-1 α, IL-1β, IL-6, IL-8, INF-γ and TNF-α, and the maturation of DCs and the expression of co-stimulatory molecules on their surfaces. Finally, TiO_2-NP-pulsed DCs were able to activate naive CD4(+) T cells and promote their proliferation.

In a recent study[104], human neutrophils exposed to anatase TiO_2-NPs manifested morphological changes, such as irregular shape, in a dose-dependent manner. The exposure to NPs caused also a rapid tyrosine phosphorylation of multiple proteins, including P38 and the Erk-1/2 protein kinases.

Conversely, Morishige et al[16] investigated the role of TiO_2 form and NP size/shape in the increased production of IL-1β detected in THP-1 cells treated with TiO_2 particles with or without LPS stimulation. Considering the TiO_2 form, rutile and spicular particles caused greater IL-1β production than anatase NPs at all concentrations. Considering particle size, the smallest (10-nm) anatase particles and the largest (<5-μm) rutile particles induced higher IL-1β levels than other particles of the same TiO_2 form, respectively. Moreover, the spicular rutile TiO_2 particles caused greater IL-1 β production than

Table VI. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on hematopoietic cells.

Hematopoietic cells					
Reference	**Crystal phase composition**	**Particle size (Nm)**	**Doses of exposure**	**Cell lines**	**Results**
Renwick., et al, 2001 – (102)	TiO_2	29 250	0.0975-0.78 $\mu g/mm^2$	Mouse (BALB/C) tumor monocytic-macrophages cell line (J774.2)	• Both TiO_2 types reduced and increased percentage of J774.2 cells capable and unable of phagocytosing indicator beads, respectively.
Möller et al, 2002 – (101)	TiO_2	20 220	10-320 μg/ml	Beagle dog alveolar macrophages; (BD-AM) BALB/c/NIH mouse macrophages (J744A.1)	• Increased retard relaxation in both cell types; • Increased cytoskeleton stiffness in both cell types; • Reduction of cell proliferation in J744A.1; • Impaired phagocytic capacity in J744A.1 cells; • Reduction of cell viability and enhanced apoptosis by 20 nm TiO_2 particles compared to 220 nm.
Wang et al, 2007 – (100)	99% pure TiO_2	N.A.	0-130 μg/ml	Human B-cell lymphoblastoid cell line (WIL2-NS)	• Dose and time dependent decrease of cell viability; • Dose dependent increase of apoptotic cells.
Kang et al, 2008 – (19)	P25 Degussa TiO_2 (70-85% anatase, 30-15% rutile)	25	0-100 μg/ml	Human peripheral blood lymphocytes	• Dose and time dependent decrease of cell viability; • Dose dependent increase of ROS levels.
Kang et al, 2009 – (97)	P25 Degussa TiO_2 (70-85% anatase, 30-15% rutile)	25	20-100 μg/ml	Human peripheral blood lymphocytes	• Increased sub-G1 cells percentage; • Activation of caspase 9, 3, 8 - Bid and PARP cleavage; • Mithocondrial depolarisation.
Vamanu et al, 2008 – (98)	Anatase and rutile 99% pure TiO_2	< 1 00	0.005-4 mg/ml	Human histiocytic lymphoma cells (U937)	• Induction of apoptosis; • TEM images revealed aspects of apoptosis and necrosis.
Wan et al, 2008 – (99)	90% anatase and 10% rutile TiO_2	20	0-20 μg/ml	U937	• No effect observed on cell viability; • No increase of ROS levels; • No significant expression changes neither in mRNA, nor in pro- MMP2 and MMP9 activities.
Schanen et al, 2009 – (103)	Anatase TiO_2 Rutile TiO_2 TiO_2 nanotubes	7-10 15-20 diameter: 10-15; length: 70-150	0.05-100 μM	Human immunological construct (MIMIC): endothelial (HUVEC) and monocytes-derived dendritic cells (PBMC)	• Increased IL-1 α, IL-1β, IL-6, IL-8 , INF-γ, and TNF-α levels; • Increased of maturation (CD83, CCR7) and costimulatory (CD86) molecules on dendritic cells.

Continued

Table VI. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on hematopoietic cells.

Hematopoietic cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Morishige et al, 2010 – (16)	Anatase spherical TiO_2 Rutile spherical TiO_2 Rutile spicular TiO_2	< 50 µm; < 25 nm; 10 nm < 5 µm; 30-40 nm 10 × 40	20-500 µg/ml in absence or presence of LPS	Human acute monocytic leukaemia cell line (THP-1)	• Rutile induced higher IL-1β, without LPS, while anatase and spicular rutile induced higher IL-1β, with LPS; • Considering the form, rutile induced higher IL-1β than anatase; • Considering the size, 10 nm anatase and < 5 µm rutile induced higher IL-1β than the other anatase and rutile particles, respectively;
Gonçalves et al, 2010 – (104)	Anatase TiO_2	4-6	0-100 µg/ml	Human neutrophils	• Dose-dependent induction of irregular shape; • Induction of tyrosine phosphorylation; • Activation of P38 and Erk-1/2 MAPK; • Dose-dependent decrease of apoptosis; • Increase of IL-8 levels.
Rothen-Rutishauser et al, 2006 – (105)	99.9% anatase TiO_2	0.02-0.03 µm.	5 µg/ml	Human red blood cells	• TiO_2 particles and small aggregates were found inside cells; • Aggregates larger than 0.2 µm were attached to the membrane, not within cells.
Li et al, 2008 – (106)	99.8% anatase TiO_2	20 200	0-800 µg/ml	Rabbit erythrocytes	• Increased erythrocyte sedimentation rate (nano TiO_2); • Induction of erythrocytes agglutination (nano TiO_2); • Dose-dependent increase of hemolysis; • Nano TiO_2 aggregates were mainly attached along the membrane; • Dose-dependent increase of MDA (nano TiO_2).
Aisaka et al, 2009 – (107)	Anatase TiO_2 Rutile TiO_2 Amorphous TiO_2	< 25 < 5000 < 25 < 50	0-20 mg/ml	Human erythrocytes	• The TiO_2 estimated dose (µg/ml) causing 50% hemolysis was 444, 564, 4960, 32414 for anatase < 5000 nm, anatase < 25 nm, rutile and amourphous, respectively; • Micro-anatase was 73, 11 and 1.3 times more potent than amorphous, rutile, and nano-anatase, respectively.
Bihari et al, 2009 – (109)	99.5% rutile TiO_2	~10 × 40	0.1-0.2 mg/ml for 10 min	Human platelets	• CD62P (P selectin) was not changed by TiO_2; • No effect was observed on platelet granulocyte complexes.
Nemmar et al, 2008 – (108)	Rutile TiO_2 nanorods	4-6	0.4-10 µg/ml	Rat whole blood	• Dose-dependent increase of platelet aggregation.

INF-γ, interferon-γ; IL-1 α, interleukin- 1α; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; LPS, Lypopolysaccaride; MAPK, mitogen-activated protein kinase; MDA, malonaldehyde; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; N.A., not applicable; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; TEM, transmission electron microscopy; TiO_2-NPs, titanium dioxide nanoparticles; TNF-α, tumor necrosis factor-α.

spherical rutile particles, which only differed from the spicules in shape. In addition, TiO_2 induced IL-1β production by caspase-1, ROS and cathepsin B.

In conclusion, these investigations suggest that the form, size and shape of NPs influence the effects of TiO_2-NPs, in particular, on the pro-inflammatory action of white blood cells. However, further studies are needed to clarify the role of these characteristics in the TiO_2-NP toxicity on white blood cells.

Red Blood Cells

There are few papers in the literature that investigated the response of red blood cells to TiO_2-NP exposure. Rothen-Rutishauser et al[105] incubated human red blood cells with anatase TiO_2-NPs for 4 to 24 h. TiO_2-NPs or < 0.2-μm small aggregates were detected inside cells, while larger aggregates were observed only outside the cell, though attached to the membrane. These results are very important considering of the fact that erythrocytes have no specific phagocytic properties.

Furthermore, on the basis of their experiments, Li et al[106] suggested that the membrane attachment of similar NPs could change the native surface properties of the red blood cells, leading to hemoagglutination, while TiO_2-NP trans-membrane insertion breaks the erythrocytes causing hemolysis[106]. Hemolysis was also observed in human erythrocytes in a subsequent study[107]. The Authors exposed these erythrocytes to anatase TiO_2-NPs, anatase TiO_2 microparticles, rutile TiO_2 microparticles and amorphous TiO_2-NPs for 1 h. Hemolysis was then assessed by the percentage of hemoglobin released from the cells. Anatase TiO_2 microparticles resulted in approximately 73, 11, 1.3 times more hemolysis than amorphous TiO_2, rutile TiO_2 and nano-sized anatase TiO_2 particles, respectively. These findings suggest that hemolysis may also be correlated with the form of TiO_2.

Platelets

Data regarding platelet aggregation are conflicting. Nemmar et al[108] demonstrated dose-dependent platelet aggregation caused by rutile TiO_2 nanorods. However, Bihari et al[109] showed that incubation of human whole blood with rutile TiO_2-NPs for 10 min did not affect platelet P-selectin expression, nor the formation of platelet-granulocyte complexes.

Thus, further researches are needed to verify if these contrasting results are correlated to the different shape or size of these TiO_2 nanomaterials or if NPs with other characteristics cause similar or different effects.

Reproductive Cells

There are only three studies that investigated the effects of TiO_2-NPs on reproductive cells (Table VII). In the first study, Komatsu et al[110] studied the direct effect of TiO_2-NPs on the mouse testis Leydig cell line. However, the form of TiO_2 used was not detailed. The findings showed that NPs were internalized by the cells and were found as agglomerates in the cytoplasm but not inside the nucleus. Furthermore it was also detected a dose-dependent reduction in cell viability. A concentration of 100 μg/ml TiO_2-NPs for 24 h reduced proliferation, which, however, was restored after this time point. Additionally, no oxidative stress was determined by TiO_2-NP exposure.

The other two studies were conducted on a cell line of the female reproductive system, supporting a potential role of ROS production in inducing reproductive cellular alterations[111,112].

In conclusion, additional studies are needed to verify these findings and the presence of other effects, particularly those implicated in mutagenesis.

Renal Cells

In the literature, there is only one study[113] that investigated the *in vitro* effects of TiO_2-NPs on renal cells (Table VII). This report indicates that TiO_2-NPs are cytotoxic for tubular but not mesangial cells. Further, the size of NPs seems to be implicated in the onset of this damage. Finally other *in vitro* studies are needed to verify these results and the observed internalization of TiO_2-NPs.

Bone Cells

There are two papers in the literature concerning the effects of TiO_2-NPs on bone cells[114,115] (Table VII). The Authors demonstrated that in MG-63 cells cultured on anatase-coated disks, several genes were significantly up- or down-regulated. These genes are implicated in signal transduction, immunity, cell cycle regulation, lysosome composition and vesicular transport, cell adhesion, proliferation, apoptosis, and as cytoskeletal and extracellular matrix components. Unfortunately the release of TiO_2-NPs into the cell culture medium was not quantified. Thus, altered gene regulation cannot be correlated to specific doses of NPs released.

Table VII. *In vitro* studies that investigated the adverse effects of TiO_2-NPs on other mammalian cells (reproductive, renal, bone and muscular cells).

Other mammalian cells					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Komatsu et al, 2008 – (110)	TiO_2	2-70	0 -1000 µg/ml	Mouse testis Leydig cell line TM3	• Dispersed TiO_2 agglomerates in the cytoplasm but not in nucleus; • Dose-dependent reduction of cell viability; • Reduction of cell proliferation.
Uchino et al, 2002 – (111)	F-6 TiO_2, 91% anatase, 9% rutile F-4 TiO_2, 75% anatase, 25% rutile Anatase form TiO_2, 81% anatase, 19% rutile F-1 TiO_2, 78% anatase, 22% rutile St-2 TiO_2, 99% anatase, 1% rutile Amorphous TiO_2 St-3 TiO_2, > 1% anatase, < 99% rutile F1-R TiO2, 3% anatase, 97% rutile	15 30 90 17 37 93	0-100 µg/ml	Chinese hamsters ovary cells (CHO)	• Dose-dependent reduction of cell viability (anatase form) after UVA irradiation; • High concentration of anatase form TiO_2 (100 µg/ml) induced cytotoxicity without UVA irradiation; • Rutile did not affect viability.
Dodd et al, 2009 – (112)	Anatase TiO_2	5	10-500 µg/ml	Chinese hamsters ovary-K1 cells (CHO-K1)	• Production of OH- and CO_2- radicals after UVA irradiation.
L'Azou et al, 2008 – (113)	98% anatase/ rutile TiO_2 65% anatase/ rutile TiO_2	15 25-75	0.625-160 µg/cm^2	Human mesangial cell line (IP15); Normal pig kidney proximal epithelial tubular cell line (LLC-PK1)	• Induction of cytotoxic effects; • Vesicles of F-actin • Abnormal cell size and surface detachment were observed in both cell lines; • 15 nm TiO_2 were incorporated into cytoplasmic vesicles.
Sollazzo et al, 2008 – (114)	Anatase TiO_2.	N.A.	Cells cultured on anatase TiO_2 coated disks	Osteoblast cell line (MG63)	• Up- or down-regulation of genes involved in signal transduction, immunity, cell cycle regulation, lysosomes composition and vesicular transport, cell adhesion, proliferation and apoptosis.
Di Virgilio et al, 2010 – (115)	100% anatase TiO_2	15	0-300 µg/ml	Rat osteosarcoma -derived cell line (UMR 106)	• Induction of cytotoxic effects; • Vesicles with phagocyted materials were observed in treated cells.
Berntsen et al, 2010 – (116)	Degussa TiO_2	25	0-200 µg/ml	Human airway smooth muscle cells (HASM)	• Increase of cell viability; • Altered cell stiffness, histamine contraction and isoprotrenol relaxation.
Peng et al, 2010 – (88)	TiO_2 nanotubes	30	N.A.	Vascular smooth muscle cells (VSMCs)	• Reduction of cell proliferation; • Reduction of inflammatory and coagulation molecules.

CO_2^-, carboxyl radical; N.A., not applicable; OH-, hydroxyl radical; TiO_2-NPs, titanium dioxide nanoparticles; UVA, ultraviolet A.

Further, Di Virgilio et al[115] studied the cytotoxic effects of 5 to 300 μg/ml anatase TiO_2-NPs applied for 24 h to rat osteosarcoma-derived cells. A significant increase in adsorbance was determined at ≥150 μg/ml and ≥25 μg/ml NPs, in both neutral red uptake and MTT assays, respectively. The trypan blue method revealed that cell proliferation also decreased with exposure to NP concentrations of up to 100 μg/ml for 24 to 96 h. TEM was also used to observe intracellular vesicles with phagocytosed material in cells treated with 50 or 100 μg/ml TiO_2-NPs for 24 h.

The findings of these studies, though limited, suggest toxic effects of TiO_2-NPs on bone cells, which should be further tested by *in vivo* studies. However, to our knowledge, such studies are lacking.

Muscular Cells

There are two recent articles that evaluated the effects of TiO_2-NPs on smooth muscle cells (Table VII). In the first study[116], human airway smooth muscle cells were exposed to Degussa TiO_2-NPs. The researchers measured cell viability and cell stiffness and agonist-induced contractility, respectively. TiO_2 dose-dependently increased the nominal viability by up to 20% at 200 μg/ml. Further, doses of up to 50 μg/ml TiO_2 altered cell stiffness, the contraction induced by histamine and relaxation induced by isoproterenol by no more than 50%; these alterations exhibited no clear dose dependence. In the other study[88], gene expression analysis of vascular smooth muscle cells exposed to TiO_2 nanotubes for 24 h revealed a reduction in proliferation and in the expression of molecules involved in inflammation and coagulation.

The findings of these studies suggest that TiO_2-NPs affect smooth muscle cells independent of the form of TiO_2 and the shape of the NPs. Regardless, further research is required to clarify and evaluate other effects.

Genotoxicity

Data regarding the genotoxicity induced by TiO_2-NPs are conflicting and heterogeneous (Table VIII). Different studies have principally reported micronuclei (MN) formation[11,19,48,52,53,93,94,100,117] or the presence of multinucleated cells[100,118]. Some of these investigations reported also other genotoxic effects, such as inter-nucleosomal fragmentation[11] and DNA damage[19,48,52,94]. In particular, Wang et al[100] also observed an increased frequency of mutations and an increase in DNA tail length. An increased mutation frequency was also recently reported by Xu et al[119]. The other effect (i.e., an increase in DNA tail length) reported by Wang et al[100] was also previously shown by Nakagawa et al[120] who observed an increase in the frequency of aberrant chromosomes.

Finally, recentely, Bhattacharya et al[49] reported an increase in DNA adduct formation and the absence of DNA damage.

Similar to the results above, other researches did not confirm the genotoxic effects (e.g., DNA damage[79], chromosome aberration[121] and MN generation with or without UV irradiation[91]) of TiO_2-NPs.

All of these conflicting data do not reveal a clear correlation between genotoxicity and the characteristics of NPs, likely due to the different cell lines and TiO_2-NPs used, the various endpoints investigated, and the assays utilized. In any event, the studies suggest that care should be taken when considering parameters such as UV irradiation[120], co-exposure with other substances[94] and NP surface chemistry (which may play a role or have synergistic effects in inducing genotoxic alterations[53]) for a correct interpretation of these results. Ultimately, further investigations are needed to clarify the genotoxic role of TiO_2-NPs.

Discussion

The recent rapid growth of nanotechnology and the specific attractive properties of nanomaterials have led to the widespread application of TiO_2-NPs in the field of life sciences, as well as in the biotechnology, pharmaceutical, cosmetics and textile industries. Several concerns regarding the potentially greater biological activity of TiO_2 in the nanoparticulate form, related to the smaller size and corresponding larger surface area[122], and the increasing worldwide TiO_2-NP distribution, with the consequent augmented likelihood of human exposure, have emerged. Moreover, the yet-limited knowledge of TiO_2-NP toxicological properties do not allow one to extrapolate toxicological conclusions to TiO_2-NPs.

Based on the studies that we reviewed, though they differ in the cell lines, the intensity and duration of exposure, endpoint parameters, and measurement techniques, interesting and critical points emerge that we argue will be the object of future research.

Table VIII. *In vitro* studies that investigated the genotoxic effects of TiO_2-NPs on mammalian cells.

Genotoxicity					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Nakagawa et al, 1997 – (120)	P25 anatase TiO_2 WA anatase TiO_2 WR rutile TiO_2 TP-3 rutile TiO_2	0.021 µm 0.255 µm 0.255 µm 0.42 µm	0-3200 µg/ml	Mouse lymphoma cell line (L5178Y); Chinese hamster cell line CHL/IU	• Dose-dependent increase in mean tail length (p-25, WA and TP-3); • P25 TiO_2 showed a dose-dependent increase in chromosome aberration frequency with irradiation.
Linnainmaa et al, 1997 – (91)	P25 Degussa uncoated anatase TiO_2 UV - TITAN M160 rutile TiO_2 coated with alumina and stearic acid Pigmentary uncoated anatase TiO_2 with USP purity	20 170	0-200 µg/cm^2	RLE	• Neither of the ultrafine TiO_2 was toxic to cells with or without UV irradiation; • None TiO_2 samples increased MN with or without UV irradiation.
Rahman et al, 2002 – (11)	TiO_2	≤ 20 > 200	0.5-10 µg/cm^2	Syrian hamster embryo cells (SHE)	• Ultrafine TiO_2 increased MN; • Induction of cytotoxic effects (ultrafine TiO_2); • Compaction and marginalization of chromatin at the nuclear periphery; • Internucleosomal fragmentation of the DNA.
Gurr et al, 2005 – (52)	Anatase TiO_2 Rutile TiO_2	10 and 20 > 200 200 200	10 µg/ml	BEAS-2B	• Induction of oxidative DNA damage by 10, 20 nm anatase and 200 nm rutile; • 10 and 200 nm sized anatase increased MN.
Wang et al, 2007 – (93)	Ce element (IV) doped anatase TiO_2 (CDT)	21	10 µg/cm^3	Hepatoma cell line (Bel 7402)	• Detection of MN after 4 hr exposure with visible light illumination.
Wang et al, 2007 – (100)	99% pure TiO_2	N.A.	0-130 µg/ml	WIL2-NS	• Increased micronucleated binucleated cells; • Decrease of cytokinesis block proliferation index; • 5-fold increase in olive tail moment (Comet assay); • 2.5-fold increase in mutation frequency (HPRT).
Warheit et al, 2007 – (121)	79% rutile-21% anatase TiO_2	140 ± 44	25-100; 62.5-250; 750-2500 µg/ml	Chinese hamster ovary cells	• The percentage of cells with aberration in the treated groups was not increased above that of controls at any concentration.
Bhattacharya et al, 2008 – (53)	Anatase TiO_2 Vanadium pentoxide (V_2O_5) treated anatase TiO_2	30-50	1-100 µg/cm^2	Chinese hamster lung fibroblasts (V79)	• Genotoxic effects were induced by V_2O_5 treated anatase, not by untreated anatase; • Threefold increase of MN (V_2O_5 anatase).

Continued

Table VIII. *In vitro* studies that investigated the genotoxic effects of TiO_2-NPs on mammalian cells.

Genotoxicity					
Reference	Crystal phase composition	Particle size (Nm)	Doses of exposure	Cell lines	Results
Kang et al, 2008 – (19)	P25 Degussa TiO_2 (70-85% anatase, 30-15% rutile)	25; ~30 nm in XRD	0-100 µg/ml	Human peripheral blood lymphocytes	• Dose-dependent increase of MN; • Dose and time dependent increased DNA breakage; • Accumulation of p53 and activation of DNA damage checkpoint kinases.
Falck et al, 2009 – (48)	99.5% pure rutile TiO_2 coated with SiO_2 (< 5%) 99.9% rutile TiO_2 99.7% anatase TiO_2	10 × 40 < 5 µm < 25	1-100 µg/cm^2	BEAS 2B	• Induction of DNA damage (fine rutile and anatase); • Increased frequency of micronucleated BEAS 2B cells (anatase).
Bhattacharya et al, 2009 – (49)	Anatase TiO_2	< 100	2-50 µg/cm^2	BEAS-2B; IMR 90	• No induction of DNA-breakage; • Induction of 8-OHdG.
Xu et al, 2009 – (119)	99.7% anatase TiO_2 99.9% anatase TiO_2 ≥ 99% anatase TiO_2	5 40 325 mesh in diameter	0.1-100 µg/ml for 24 hr; 0.1-30 µg/ml for 3 days	Gpt delta transgenic mouse primary embryo fibroblasts (MEF)	• Dose-dependent reduction of cell viability (40 nm TiO_2); • Increase of mutation frequencies at red/gam gene loci; • Induction of oxidative stress.
Huang et al, 2009 – (117)	TiO_2	15	0-50 µg/ml for 24-72 hr; 10 µg/ml every 3 days up to 12 wks	NIH 3T3; Human HFW fibroblasts	• Increase of ROS production; • Dose and time-dependent activation of ERK1/2 cascade; • Increase of BNMN cells; • Increase of MN in long term exposure; • G2/M delay, affection of progression at anaphase and telophase, higher subG1 phase population at long term exposure.
Onuma et al, 2009 – (118)	Hydrophilic rutile TiO_2 (84-92%) treated with $ZrO2Al(OH)_3$ Hydrophobic rutile TiO_2 (77-86%) treated with $ZrO2Al(OH)_3$ and steric acid	40-70 minor axis; 200-300 major axis	312 µg/ml	QR2 fibrosarcoma cells	• Increase of ROS levels; • Higher citotoxicity in hydrophobic than hydrophilic TiO_2; • Tumorigenic conversion was observed in both TiO_2 types co-cultures; • Hydrophobic TiO_2 led to interstices around nuclear membrane and multinucleated cells.
Shi et al, 2010 – (94)	P25 Degussa TiO_2 (80% anatase-20% rutile)	25	0-1 µg/ml TiO_2	Human fetus hepatic cell line L-02	• Synergistic action of TiO_2 and p,p^I – DDT as regards DNA breaks and MN; • Increase of 8-OHdG formation.
Hackenberg et al, 2010 – (79)	Anatase TiO_2	< 25	10-100 µg/ml	Epithelial cells of human nasal mucosa	• No DNA damage was observed by comet assay.

8-OHdG, 8-hydroxyl-2-deoxyguanosine; BNMN, binucleated micronucleated; ERK 1/2, extracellular-receptor kinase 1/2; HPRT, hypoxanthine-guanine phosphoribosyltransferase, MN, micronulei; p,p^I-DDT, dichlorodiphenyltrichloroethane; ROS, reactive oxygen species; TiO_2-NPs, titanium dioxide nanoparticles; UV, ultraviolet.

Firstly, it should be noted that most of the effects observed in *in vitro* studies were obtained using excessively and unrealistically high doses of NPs[15,123]. Thus, the direct extrapolation of these results to humans under realistic, lower exposure scenarios must be questioned[124]. However, though the results of such studies have to be interpreted with caution and the relevance of these data is dubious, they could lead to focused future works on the detection of early signs of more severe damages induced by higher dose exposures.

From our review, it is evident that additional research is needed to more thoroughly investigate the toxic effects derived from NP exposure, particularly in relation to the particle characterization, which, in our opinion, is the first step to reach a comprehensive and appropriate identification of the TiO_2-NP hazards. Various physicochemical features (e.g., size, shape, composition, charge, crystallinity, solubility, added functional groups and impurities) can be combined in any particular type of NP, leading to different toxic potentials[125-127]. Unfortunately, the lack of and the heterogeneity in the TiO_2-NP parameters examined in different studies leads to a non-homogeneous classification of the exposures and to a biased assessment of the exposure-response relationships[125]. To overcome this potential bias, a homogeneous exposure classification should be used in future studies to thoroughly clarify how the physicochemical properties of TiO_2 nanocrystals correlate with their toxicological effects. Useful parameters for systematic categorization could include the TiO_2 form, number concentration, surface area, mass concentration, weighted size distribution, state of agglomeration, surface reactivity (e.g., the ability to produce radicals and zeta potential), chemical composition and morphology[38].

Moreover, an argument that merits greater focus in future research (due to the limited number of studies in that regard) is the possible roles of TiO_2-NPs in genotoxicity and carcinogenicity. The IARC recently classified TiO_2 as possibly carcinogenic to humans (Group 2B)[33]. TiO_2-NPs had a carcinogenic effect on lungs in rats[128], and several studies have reported positive *in vitro* and *in vivo* genotoxicity of this nanomaterial[11,19,48,52,53,93,94,100,117-119,129], though these effects were not detected in other studies[79,91,121,130]. Differences in findings between studies may be due to how TiO_2-NPs differ in terms of production, particle size, degree of aggregation, preparation method, incubation or exposure conditions, dose and susceptibility between cell types[129,131,132], implying that more studies are required to determine the conditions in which TiO_2-NP genotoxicity occurs. However, for these engineered nanomaterials, there is simply no adequate epidemiological information to conclude whether or not there is an association between exposure and lung cancer risk to date[133]. In particular, it will be interesting to elucidate if TiO_2-NPs act by inducing modifications directly on DNA or regulating gene expression, namely through mutagenic or epigenetic mechanisms, respectively. Moreover, because carcinogenesis is a multistep process, the role of TiO_2-NP action in inducing the initiation event or in the subsequent processes of promotion or progression should be clarified. Tests evaluating mutagenicity should be widely performed in both *in vitro* and *in vivo* studies to confirm the alterations induced by TiO_2-NPs and in relation to the particle characteristics to understand the mechanisms of action and to establish threshold values.

The use of nanotechnological products will likely increase sharply over the next decade. Because of this increase, NP-related research should carefully balance the improvement of nanotechnology and human health through the optimal properties of nanomaterials with the need to identify potential hazards derived from unintentional or intentional exposures[122]. In fact, the same NP properties that may be useful for the development of nanomedicine and specific industrial processes (e.g., antioxidant activity, carrier capacity for therapeutics and catalytic capacity for chemical reactions) could be harmful when NPs interact with cells by inducing toxicity, oxidative stress or cellular dysfunction[122]. A database composed of the results of toxicological tests could provide a comprehensive set of information useful for TiO_2-NP material safety data sheets, as well as a basis for potential NP risk assessments and risk management[122]. Currently, the public awareness of nanotechnology in everyday life is limited. However, some pressing concerns are related to the risks posed by occupational exposure, particularly during manufacturing or processing for industrial applications. Nevertheless, evidence is not extensive or definitive, and there are no published studies on the risks of occupational exposure to engineered NPs[125]. Moreover, studies on long-term exposure and chronic effects have not been carried out to date because such exposure is relatively recent and generally occurs in controlled situa-

tions. Therefore, given the increasing use of TiO_2-NPs, epidemiologic investigations of exposed workers will be needed in the near future to provide an assessment of possible risks. Epidemiologic investigation will form an important link in understanding health outcomes associated with exposure to potentially hazardous materials. Such studies will form the basis for quantitative risk estimations to establish levels that protect human health. Currently, only the U.S. National Institute for Occupational Safety and Health[14] has recommended that exposure limits for nano-TiO_2 should be 0.1 mg/m^3 as a Time Weighted Average (TWA) concentration for up to 10 h/day during a 40-h work week. In this occupational context, a better knowledge of the characteristics of different types of TiO_2-NPs could be useful both for improving the performance of nanomaterials, extending their specific applications in technological products and for detailing properties (such as photocatalytic ability) that could enhance cell alterations under particular exposure conditions (e.g., in presence or absence of UV-light irradiation). Moreover, a deeper understanding of TiO_2-NP biokinetics, target organs and potential health effects would be helpful in determining biological monitoring methods and establishing appropriate individual defense systems for workers exposed to TiO_2-NPs.

Our review highlights the lack of homogeneity among NP studies, which prevents a comprehensive interpretation of results and underscores the need to validate standard methods of measurement and methodological strategies that should, we argue, be the object of national or international consensus standards[38,125]. Moreover, the lack of sufficiently standardized toxicological data on engineered TiO_2-NPs and conflicting results regarding their potential hazard impede an adequate risk assessment. Future studies using validated methods and strategies are needed in order to acquire a deeper understanding of the risks related to TiO_2-NP exposure. This step could be extremely important to achieve adequate risk communication and management. To date, because certain data regarding TiO_2-NPs are limited, these phases should be conducted with great caution, giving correct TiO_2-NP risk information to workers, adopting valuable measures of environmental and biological monitoring to obtain a correct evaluation of the exposure and providing measures of collective and individual protection (particularly of the respiratory airways) at present considered a primary route of exposure.

References

1) NIOSH (National Institute for Occupational Safety and Health). Approaches to Safe Nanotechnology: managing the health and safety concerns associated with engineered nanomaterials. Centers for Disease Control and Prevention, Atlanta, GA; 2009.

2) NSF (National Science Foundation). Societal implications of nanoscience and nanotechnology. Nanoscale Science, Engineering, and Technology (NSET) Workshop Report, Arlington, VA; 2001.

3) Card JW, Zeldin DC, Bonner JC, Nestmann ER. Pulmonary applications and toxicity of engineered nanoparticles. Am J Physiol. Lung Cell Mol Physiol 2008; 295: L400-L411.

4) Baan R, Straif K, Groose Y, Secretan B, El Ghissassi F, Cogliano V. WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of carbon black, titanium dioxide, and talc. Lancet Oncol 2006; 4: 295-296.

5) Liang G, Pu Y, Yin L, Liu R, Ye B, Su Y, Li Y. Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress. J Toxicol Environ Health A 2009; 72: 740-745.

6) Gelis C, Girard S, Mavon A, Delverdier M, Pailous N, Vicendo P. Assessment of the skin photoprotective capacities of an organo-mineral broad-spectrum sunblock on two ex vivo skin models. Photodermatol Photoimmunol Photomed 2003; 19: 242-253.

7) Sun D, Meng TT, Loong H, Hwa TJ. Removal of natural organic matter from water using a nanostructured photocatalyst coupled with filtrating membrane. Water Sci Technol 2004; 49: 103-110.

8) Wang J, Chen C, Liu Y, Jiao F, Li W, Lao F, Li Y, Li B, Ge C, Zhou G, Gao Y, Zhao Y, Chai Z. Potential neurological lesion after nasal instillation of TiO(2) nanoparticles in the anatase and rutile crystal phases. Toxicol Lett 2008; 183: 72-80.

9) WHO (World Health Organization). Titanium dioxide. EHC 24. Geneva: World Health Organization; 1982.

10) Jin T, Berlin M. Titanium. in: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, eds. Handbook of the Toxicology of Metals, 3rd edition. Elsevier; 2007.

11) Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss DG, Schiffmann D. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ Health Perspect 2002; 110: 797-800.

12) Hedenborg M. Titanium dioxide induced chemiluminescence of human polymorphonuclear leukocytes. Int Arch Occup Environ Health 1988; 61: 1-6.

13) Duan Y, Liu J, Ma L, Li N, Liu H, Wang J, Zheng L, Liu C, Wang X, Zhao X, Yan J, Wang S, Wang H, Zhang X, Hong F. Toxicological characteristics of nanoparticulate anatase titanium dioxide in mice. Biomaterials 2010; 31: 894-899.

14) NIOSH (National Institute for Occupational Safety and Health). NIOSH current intelligence bulletin: evaluation of health hazard and recommendations for occupational exposure to titanium dioxide Centers for Disease Control and Prevention, Atlanta, GA; 2005.

15) Chen J, Dong X, Zhao J, Tang G. *In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection. J Appl Toxicol 2009; 29: 330-337.

16) Morishige T, Yoshioka Y, Tanabe A, Yao X, Tsunoda S, Tsutsumi Y, Mukai Y, Okada N, Nakagawa S. Titanium dioxide induces different levels of IL-1 beta production dependent on its particle characteristics through caspase-1 activation mediated by reactive oxygen species and cathepsin B. Biochem Biophys Res Commun 2010; 392: 160-165.

17) ACGIH (American Conference of Governmental Industrial Hygienists). Titanium dioxide. In: Documentation of the threshold limit values for chemical substances. 7th ed. Cincinnati, OH; 2001.

18) Liu H, Ma L, Zhao J, Liu J, Yan J, Ruan J, Hong F. Biochemical toxicity of nano-anatase TiO_2 particles in mice. Biol Trace Elem Res 2009; 129: 170-180.

19) Kang SJ, Kim BM, Lee YJ, Chung HW. Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. Environ Mol Mutagen 2008; 49: 399-405.

20) Christie H, Mackay RJ, Fisher AM. Pulmonary effects of inhalation of titanium dioxide by rats. Am Ind Hyg Assoc J 1963; 24: 42-46.

21) Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. Toxicol Appl Pharmacol 1985; 79: 179-192.

22) Lee KP, Henry NW 3rd, Trochimowicz HJ, Reinhardt CF. Pulmonary response to impaired lung clearance in rats following excessive TiO_2 dust deposition. Environ Res 1986; 41: 144-167.

23) Bernard BK, Osheroff MR, Hofmann A, Mennear JH. Toxicology and carcinogenesis studies of dietary titanium dioxide-coated mica in male and female Fischer 344 rats. J Toxicol Environ Health 1990; 29: 417-429.

24) Daum S, Anderson HA, Lilis R, Lorimer WV, Fischbein SA, Miller A, Selikoff IJ. Pulmonary changes among titanium workers. Proc R Soc Med 1977; 70: 31-32.

25) Ophus EM, Rode L, Gylseth B, Nicholson DG, Saeed K. Analysis of titanium pigments in human lung tissue. Scand J Work Environ Health 1979; 5: 290-296.

26) Rode LE, Ophus EM, Gylseth B. Massive pulmonary deposition of rutile after titanium dioxide exposure: Light-microscopical and physico-analytical methods in pigment identification. Acta Pathol Microbiol Scand A 1981; 89: 455-461.

27) Chen JL, Fayerweather WE. Epidemiologic study of workers exposed to titanium dioxide. J Occup Med 1988; 30: 937-942.

28) ILSI Risk Science Institute Workshop Partecipants. The relevance of the rat lung response to particle overload for human risk assessment: A workshop consensus report. Inhal Toxicol 2000; 12: 1-17.

29) Baggs RB, Ferin J, Oberdorster G. Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. Vet Pathol 1997; 34: 592-597.

30) Warheit DB, Hansen JF, Yuen IS, Kelly DP, Snajdr SI, Hartsky MA. Inhalation of high concentrations of low toxicity dusts in rats results in impaired pulmonary clearance mechanisms and persistent inflammation. Toxicol Appl Pharmacol 1997; 145: 10-22.

31) Ferin J, Oberdörster G. Biological effects and toxicity assessment of titanium dioxides: anatase and rutile. Am Ind Hyg Assoc J 1985; 46: 69-72.

32) Lindenschmidt RC, Driscoll KE, Perkins MA, Higgins JM, Maurer JK, Belfiore KA. The comparison of a fibrogenic and two nonfibrogenic dusts by bronchoalveolar lavage. Toxicol Appl Pharmacol 1990; 102: 268-281.

33) IARC (International Agency for research on Cancer). Titanium dioxide Group 2B IARC Monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer: vols.9. Lyon: World Health Organization; 2006.

34) Borm PJ, Schins RP, Albrecht C. Inhaled particles and lung cancer, part B: paradigms and risk assessment. Int. J. Cancer. 2004; 110: 3-14.

35) Dankovic D, Kuempel E, Wheeler M. An approach to risk assessment for TiO_2. Inhal Toxicol 2007; 19(Suppl 1): 205-212.

36) Boffetta P, Soutar A, Cherrie JW, Granath F, Andersen A, Anttila A, Blettner M, Gaborieau V, Klug SJ, Langard S, Luce D, Merletti F, Miller B, Mirabelli D, Pukkala E, Adami HO, Weiderpass E. Mortality among workers employed in the titanium dioxide production industry in Europe. Cancer Causes Control 2004; 15: 697-706.

37) Fryzek JP, Chadda B, Marano D, White K, Schweitzer S, McLaughlin JK, Blot WJ. A cohort

mortality study among titanium dioxide manufacturing workers in the United States. J Occup Environ Med 2003; 45: 400-409.

38) Borm, PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdorster E. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol 2006; 14: 3-11.

39) Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. Science 2006; 311: 622-627.

40) Donaldson K, Stone V, Tran CL, Kreyling W, Borm PJA. Nanotoxicology: A new frontier in particle toxicology relevant to both the workplace and general environment and to consumer safety. Occup Environ Med 2004; 61: 727-728.

41) Warheit DB, Webb TR, Reed KL. Pulmonary toxicity studies with TiO_2 particles containing various commercial coatings. Toxicologist 2003; 72: 298A.

42) Warheit, DB, Webb TR, Sayes CM, Colvin VL, Reed KL. Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. Toxicol Sci 2006; 91: 227-236.

43) Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM. Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: differential responses related to surface properties. Toxicology 2007; 230: 90-104.

44) Carnevali C, Morazzoni F, Scotti R, Bellobono IR, Giusti M, Sommaria M, D'Arienzo M, Testino A, Musinu A, Cannas C. Nanocrystalline TiO_2 with enhanced photoinduced charge separation as catalyst for the phenol degradation. Int J Photoenergy 2006 (Article ID 90809): 1-6.

45) Tsuji JS, Maynard AD, Howard PC, James JT, Lam CW, Warheit DB, Santamaria AB. Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles. Toxicol Sci 2006; 89: 42-50.

46) Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB, Colvin VL. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci 2006; 92: 174-185.

47) Soto K, Garza KM, Murr LE. Cytotoxic effects of aggregated nanomaterials. Acta Biomater 2007; 3: 351-358.

48) Falck GC, Lindberg HK, Suhonen S, Vippola M, Vanhala E, Catalán J, Savolainen K, Norppa H. Genotoxic effects of nano-sized and fine TiO_2. Hum Exp Toxicol 2009; 28: 339-352.

49) Bhattacharya K, Davoren M, Boertz J, Schins RP, Hoffmann E, Dopp E. Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells. Part Fibre Toxicol 2009; 21: 6-17.

50) Val S, Hussain S, Boland S, Hamel R, Baeza-Squiban A, Marano F. Carbon black and titanium dioxide nanoparticles induce pro-inflammatory responses in bronchial epithelial cells: need for multiparametric evaluation due to adsorption artifacts. Inhal Toxicol 2009; 21(Suppl 1): 115-122.

51) Shi Y, Wang F, He J, Yadav S, Wang H. Titanium dioxide nanoparticles cause apoptosis in BEAS-2B cells through the caspase 8/t-Bid-independent mitochondrial pathway. Toxicol Lett 2010; 196: 21-27.

52) Gurr JR, Wang AS, Chen CH, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 2005; 213: 66-73.

53) Bhattacharya K, Cramer H, Albrecht C, Schins R, Rahman Q, Zimmermann U, Dopp E. Vanadium pentoxide-coated ultrafine titanium dioxide particles induce cellular damage and micronucleus formation in V79 cells. J Toxicol Environ Health A 2008; 71: 976-980.

54) Rothen-Rutishauser B, Mueller L, Blank F, Brandenberger C, Muehlfeld C, Gehr P. A newly developed *in vitro* model of the human epithelial airway barrier to study the toxic potential of nanoparticles. ALTEX 2008; 25: 191-196.

55) Rothen-Rutishauser B, Mühlfeld C, Blank F, Musso C, Gehr P. Translocation of particles and inflammatory responses after exposure to fine particles and nanoparticles in an epithelial airway model. Part Fibre Toxicol 2007; 25: 4-9.

56) Müller L, Riediker M, Wick P, Mohr M, Gehr P. Rothen-Rutishauser B. Oxidative stress and inflammation response after nanoparticle exposure: differences between human lung cell monocultures and an advanced three-dimensional model of the human epithelial airways. J R Soc Interface 2010; 7(Suppl 1): S27-S40.

57) Xia T, Kovochich M, Brant J, Hotze M, Sempf J, Oberley T, Sioutas C, Yeh JI, Wiesner MR, Nel AE. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett 2006; 6: 1794-1807.

58) Singh S, Shi T, Duffin R, Albrecht C, Van Berlo D, Höhr D, Fubini B, Martra G, Fenoglio I, Borm PJ, Schins RP. Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO_2: role of the specific surface area and of surface methylation of the particles. Toxicol Appl Pharmacol 2007; 222: 141-151.

59) Park EJ, Yi J, Chung KH, Ryu DY, Choi J, Park K. Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. Toxicol Lett. 2008; 180: 222-229.

60) Simon-Deckers A, Gouget B, Mayne-L'hermite M, Herlin-Boime N, Reynaud C, Carrière M. *In vitro* investigation of oxide nanoparticle and carbon nanotube toxicity and intracellular accumulation in A549 human pneumocytes. Toxicology 2008; 253: 137-146.

61) Chen HW, Su SF, Chien CT, Lin WH, Yu SL, Chou CC, Chen JJ, Yang PC. Titanium dioxide nanoparticles induce emphysema-like lung injury in mice. FASEB J 2006; 20: 2393-2395.

62) Monteiller C, Tran L, MacNee W, Faux S, Jones A, Miller B, Donaldson K. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells *in vitro*: the role of surface area. Occup Environ Med 2007; 64: 609-615.

63) Hussain S, Boland S, Baeza-Squiban A, Hamel R, Thomassen LC, Martens JA, Billon-Galland MA, Fleury-Feith J, Moisan F, Patron JC, Marano F. Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: role of particle surface area and internalized amount. Toxicology 2009; 260: 142-149.

64) Hamilton RF Jr, Wu N, Porter D, Buford M, Wolfarth M, Holian A. Particle length-dependent titanium dioxide nanomaterials' toxicity and bioactivity. Part Fibre Toxicol 2009; 31: 6-35.

65) Magrez A, Horváth L, Smajda R, Salicio V, Pasquier N, Forró L, Schwaller B. Cellular toxicity of TiO_2-based nanofilaments. ACS Nano 2009; 3: 2274-2280.

66) Rossi EM, Pylkkänen L, Koivisto AJ, Vippola M, Jensen KA, Miettinen M, Sirola K, Nykäsenoja H, Karisola P, Stjernvall T, Vanhala E, Kiilunen M, Pasanen P, Mäkinen M, Hämeri K, Joutsensaari J, Tuomi T, Jokiniemi J, Wolff H, Savolainen K, Matikainen S, Alenius H. Airway exposure to silica-coated TiO_2 nanoparticles induces pulmonary neutrophilia in mice. Toxicol Sci 2010; 113: 422-433.

67) Stearns RC, Paulauskis JD, Godleski JJ. Endocytosis of ultrafine particles by A549 cells. Am J Respir Cell Mol Biol 2001; 24: 108-115.

68) Park S, Lee YK, Jung M, Kim KH, Chung N, Ahn EK, Lim Y, Lee KH. Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells. Inhal Toxicol 2007; 19(Suppl 1): 59-65.

69) Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol 2006; 40: 4346-4352.

70) Long TC, Tajuba J, Sama P, Saleh N, Swartz C, Parker J, Hester S, Lowry GV, Veronesi B. Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons *in vitro*. Environ Health Perspect 2007; 115: 1631-1637.

71) Shin JA, Lee EJ, Seo SM, Kim HS, Kang JL, Park EM. Nano-sized titanium dioxide enhanced inflammatory responses in the septic brain of mouse. Neuroscience 2010; 165: 445-454.

72) Liu S, Xu L, Zhang T, Ren G, Yang Z. Oxidative stress and apoptosis induced by nano-sized titanium dioxide in PC12 cells. Toxicology 2010; 267: 172-177.

73) Lai JC, Lai MB, Jandhyam S, Dukhande VV, Bhushan A, Daniels CK, Leung SW. Exposure to titanium dioxide and other metallic oxide nanoparticles induces cytotoxicity on human neural cells and fibroblasts. Int J Nanomedicine 2008; 3: 533-545.

74) Liu X, Ren X, Deng X, Huo Y, Xie J, Huang H, Jiao Z, Wu M, Liu Y, Wen T. A protein interaction network for the analysis of the neuronal differentiation of neural stem cells in response to titanium dioxide nanoparticles. Biomaterials 2010; 31: 3063-3070.

75) Kiss B, Bíró T, Czifra G, Tóth BI, Kertész Z, Szikszai Z, Kiss AZ, Juhász I, Zouboulis CC, Hunyadi J. Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. Exp Dermatol 2008; 17: 659-667.

76) Fujita K, Horie M, Kato H, Endoh S, Suzuki M, Nakamura A, Miyauchi A, Yamamoto K, Kinugasa S, Nishio K, Yoshida Y, Iwahashi H, Nakanishi J. Effects of ultrafine TiO_2 particles on gene expression profile in human keratinocytes without illumination: involvement of extracellular matrix and cell adhesion. Toxicol Lett 2009; 191: 109-117.

77) Pan Z, Lee W, Slutsky L, Clark RA, Pernodet N, Rafailovich MH. Adverse effects of titanium dioxide nanoparticles on human dermal fibroblasts and how to protect cells. Small 2009; 5: 511-520.

78) Jin CY, Zhu BS, Wang XF, Lu QH. Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. Chem Res Toxicol 2008; 21: 1871-1877.

79) Hackenberg S, Friehs G, Froelich K, Ginzkey C, Koehler C, Scherzed A, Burghartz M, Hagen R, Kleinsasser N. Intracellular distribution, geno- and cytotoxic effects of nanosized titanium dioxide particles in the anatase crystal phase on human nasal mucosa cells. Toxicol Lett 2010; 195: 9-14.

80) Gamer AO, Leibold E, Van Ravenzwaay B. The *in vitro* absorption of microfine zinc oxide and titanium dioxide through porcine skin. Toxicol *in vitro* 2006; 20: 301-307.

81) Mavon A, Miquel C, Lejeune O, Payre B, Moretto P. *In vitro* percutaneous absorption and *in vivo* stratum corneum distribution of an organic and a mineral sunscreen. Skin Pharmacol Physiol 2007; 20: 10-20.

82) Wu J, Liu W, Xue C, Zhou S, Lan F, Bi L, Xu H, Yang X, Zeng FD. Toxicity and penetration of TiO_2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. Toxicol Lett 2009; 191: 1-8.

83) Van der Merwe D, Tawde S, Pickrell JA, Erickson LE. Nanocrystalline titanium dioxide and magnesium oxide *in vitro* dermal absorption in human skin. Cutan Ocul Toxicol 2009; 28: 78-82.

84) Senzui M, Tamura T, Miura K, Ikarashi Y, Watanabe Y, Fujii M. Study on penetration of titanium dioxide (TiO(2)) nanoparticles into intact and damaged skin *in vitro*. J Toxicol Sci 2010; 35: 107-113.

85) Helfenstein M, Miragoli M, Rohr S, Müller L, Wick P, Mohr M, Gehr P, Rothen-Rutishauser B. Effects of combustion-derived ultrafine particles and manufactured nanoparticles on heart cells *in vitro*. Toxicology 2008; 253: 70-78.

86) Peters K, Unger RE, Kirkpatrick CJ, Gatti AM, Monari E. Effects of nano-scaled particles on endothelial cell function *in vitro*: studies on viability, proliferation and inflammation. J Mater Sci Mater Med 2004; 15: 321-325.

87) Yu M, Mo Y, Wan R, Chien S, Zhang X, Zhang Q. Regulation of plasminogen activator inhibitor-1 expression in endothelial cells with exposure to metal nanoparticles. Toxicol Lett 2010; 195: 82-89.

88) Peng L, Barczak AJ, Barbeau RA, Xiao Y, LaTempa TJ, Grimes CA, Desai TA. Whole genome expression analysis reveals differential effects of TiO_2 nanotubes on vascular cells. Nano Lett 2010; 10: 143-148.

89) Courtois A, Andujar P, Ladeiro Y, Baudrimont I, Delannoy E, Leblais V, Begueret H, Galland MA, Brochard P, Marano F, Marthan R, Muller B. Impairment of NO-dependent relaxation in intralobar pulmonary arteries: comparison of urban particulate matter and manufactured nanoparticles. Environ Health Perspect 2008; 116: 1294-1299.

90) Courtois A, Andujar P, Ladeiro Y, Ducret T, Rogerieux F, Lacroix G, Baudrimont I, Guibert C, Roux E, Canal-Raffin M, Brochard P, Marano F, Marthan R, Muller B. Effect of engineered nanoparticles on vasomotor responses in rat intrapulmonary artery. Toxicol Appl Pharmacol 2010; 245: 203-210.

91) Linnainmaa K, Kivipensas P, Vainio H. Toxicity and cytogenetic studies of ultrafine titanium dioxide in cultured rat liver epithelial cells. Toxicol *in vitro* 1997; 11: 329-335.

92) Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol *in vitro* 2005; 19: 975-983.

93) Wang L, Mao J, Zhang GH, Tu MJ. Nano-cerium-element-doped titanium dioxide induces apoptosis of Bel 7402 human hepatoma cells in the presence of visible light. World J Gastroenterol 2007; 13: 4011-4014.

94) Shi Y, Zhang JH, Jiang M, Zhu LH, Tan HQ, Lu B. Synergistic genotoxicity caused by low concentration of titanium dioxide nanoparticles and p,p'-DDT in human hepatocytes. Environ Mol Mutagen 2010; 51: 192-204.

95) Zhang AP, Sun YP. Photocatalytic killing effect of TiO_2 nanoparticles on Ls-174-t human colon carcinoma cells. World J Gastroenterol 2004; 10: 3191-3193.

96) Koeneman BA, Zhang Y, Westerhoff P, Chen Y, Crittenden JC, Capco DG. Toxicity and cellular responses of intestinal cells exposed to titanium dioxide. Cell Biol Toxicol 2010; 26: 225-238.

97) Kang SJ, Kim BM, Lee YJ, Hong SH, Chung HW. Titanium dioxide nanoparticles induce apoptosis through the JNK/p38-caspase-8-Bid pathway in phytohemagglutinin-stimulated human lymphocytes. Biochem Biophys Res Commun 2009; 386: 682-687.

98) Vamanu CI, Cimpan MR, Høl PJ, Sørnes S, Lie SA, Gjerdet NR. Induction of cell death by TiO_2 nanoparticles: studies on a human monoblastoid cell line. Toxicol *in vitro* 2008; 22: 1689-1696.

99) Wan R, Mo Y, Zhang X, Chien S, Tollerud DJ, Zhang Q. Matrix metalloproteinase-2 and -9 are induced differently by metal nanoparticles in human monocytes: The role of oxidative stress and protein tyrosine kinase activation. Toxicol Appl Pharmacol 2008; 233: 276-285.

100) Wang J, Sanderson BJ, Wang H. Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells. Mutat Res 2007; 628: 99-106.

101) Möller W, Hofer T, Ziesenis A, Karg E, Heyder J. Ultrafine particles cause cytoskeletal dysfunctions in macrophages. Toxicol Appl Pharmacol 2002; 182: 197-207.

102) Renwick LC, Donaldson K, Clouter A. Impairment of alveolar macrophage phagocytosis by ultrafine particles. Toxicol Appl Pharmacol 2001; 172: 119-127.

103) Schanen BC, Karakoti AS, Seal S, Drake DR 3rd, Warren WL, Self WT. Exposure to titanium dioxide nanomaterials provokes inflammation of an *in vitro* human immune construct. ACS Nano 2009; 3: 2523-2532.

104) Gonçalves DM, Chiasson S, Girard D. Activation of human neutrophils by titanium dioxide (TiO_2) nanoparticles. Toxicol *in vitro* 2010; 24: 1002-1008.

105) Rothen-Rutishauser BM, Schürch S, Haenni B, Kapp N, Gehr P. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ Sci Technol 2006; 40: 4353-4359.

106) Li SQ, Zhu RR, Zhu H, Xue M, Sun XY, Yao, SD, Wang, SL. Nanotoxicity of TiO(2) nanoparticles to erythrocyte *in vitro*. Food Chem Toxicol 2008; 46: 3626-3631.

107) Aisaka Y, Kawaguchi R, Watanabe S, Ikeda M, Igisu H. Hemolysis caused by titanium dioxide particles. Inhal Toxicol 2008; 20: 891-893.

108) Nemmar A, Melghit K, Ali BH. The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO_2 nanorods in rats. Exp Biol Med (Maywood) 2008; 233: 610-619.

109) Bihari P, Holzer M, Praetner M, Fent J, Lerchenberger M, Reichel CA, Rehberg M, Lakatos S, Krombach F. Single-walled carbon nanotubes activate platelets and accelerate thrombus formation in the microcirculation. Toxicology 2010; 269: 148-154.

110) Komatsu T, Tabata M, Kubo-Irie M, Shimizu T, Suzuki K, Nihei Y, Takeda K. The effects of nanoparticles on mouse testis Leydig cells *in vitro*. Toxicol *in vitro* 2008; 22: 1825-1831.

111) Uchino T, Tokunaga H, Ando M, Utsumi H. Quantitative determination of OH radical generation and its cytotoxicity induced by TiO(2)-UVA treatment. Toxicol *in vitro* 2002; 16: 629-635.

112) Dodd NJ, Jha AN. Titanium dioxide induced cell damage: a proposed role of the carboxyl radical. Mutat Res 2009; 660: 79-82.

113) L'azou B, Jorly J, On D, Sellier E, Moisan F, Fleury-Feith J, Cambar J, Brochard P, Ohayon-Courtès C. *In vitro* effects of nanoparticles on renal cells. Part Fibre Toxicol 2008; 19: 5-22.

114) Sollazzo V, Calmieri A, Pezzetti F, Scarano A, Martinelli M, Scapoli L, Massari L, Brunelli G, Caramelli E, Carinci F. Genetic effect of anatase on osteoblast-like cells. J Biomed Mater Res B Appl Biomater 2008; 85: 29-36.

115) Di Virgilio AL, Reigosa M, De Mele MF. Response of UMR 106 cells exposed to titanium oxide and aluminum oxide nanoparticles. J Biomed Mater Res A 2010; 92: 80-86.

116) Berntsen P, Park CY, Rothen-Rutishauser B, Tsuda A, Sager TM, Mollina RM, Donaghey TC, Alencar AM, Kasahara DI, Ericsson T, Millet EJ, Swenson J, Tschumperlin DJ, Butler JP, Brain JD, Fredberg JJ, Gehr P, Zhou EH. Biomechanical effects of environmental and engineered particles on human airway smooth muscle cells. J R Soc Interface 2010; 7(Suppl 3): S331-340.

117) Huang S, Chueh PJ, Lin YW, Shih TS, Chuang SM. Disturbed mitotic progression and genome segregation are involved in cell transformation mediated by nano-TiO_2 long-term exposure. Toxicol Appl Pharmacol 2009; 241: 182-194.

118) Onuma K, Sato Y, Ogawara S, Shirasawa N, Kobayashi M, Yoshitake J, Yoshimura T, Iigo M, Fujii J, Okada F. Nano-scaled particles of titanium dioxide convert benign mouse fibrosarcoma cells into aggressive tumor cells. Am J Pathol 2009; 175: 2171-2183.

119) Xu A, Chai Y, Nohmi T, Hei TK. Genotoxic responses to titanium dioxide nanoparticles and fullerene in gpt delta transgenic MEF cells. Part Fibre Toxicol 2009; 6: 3.

120) Nakagawa Y, Wakuri S, Sakamoto K, Tanaka N. The photogenotoxicity of titanium dioxide particles. Mutat Res 1997; 394: 125-132.

121) Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM. Development of a base set of toxicity tests using ultrafine TiO- particles as a component of nanoparticle risk management. Toxicol Lett 2007; 171: 99-110.

122) Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005; 113: 823-839.

123) Guo LL, Liu XH, Qin DX, Gao L, Zhang HM, Liu JY, Cui YG. Effects of nanosized titanium dioxide on the reproductive system of male mice. Zhonghua Nan Ke Xue 2009; 15: 517-522.

124) Rushton EK, Jiang J, Leonard SS, Eberly S, Castranova V, Biswas P, Elder A, Han X, Gelein R, Finkelstein J, Oberdörster G. Concept of assessing nanoparticle hazards considering nanoparticle dosemetric and chemical/biological response metrics. J Toxicol Environ Health A 2010; 73: 445-461.

125) Schulte PA, Schubauer-Berigan MK, Mayweather C, Geraci CL, Zumwalde R, McKernan JL. Issues in the development of epidemiologic studies of workers exposed to engineered nanoparticles. J Occup Environ Med 2009; 51: 323-335.

126) Höhr D, Steinfartz Y, Schins RP, Knaapen AM, Martra G, Fubini B, Borm PJ. The surface area rather than the surface coating determines the acute inflammatory response after instillation of fine and ultrafine TiO_2 in the rat. Int J Hyg Environ Health 2002; 205: 239-244.

127) Sayes CM, Reed KL, Warheit DB. Assessing toxicity of fine and nanoparticles: comparing *in vitro* measurements to in vivo pulmonary toxicity profiles. Toxicol Sci 2007; 97: 163-180.

128) Heinreich U, Fuhst R, Rittinghause S, Creutzenberg O, Bellmann B, Koch W, Levsen K. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black and titanium dioxide. Inhal Toxicol 1995; 7: 533-556.

129) Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. Titanium dioxide nanoparticles induce DNA damage and genetic instability *in vivo* in mice. Cancer Res 2009; 69: 8784-8789.

130) Rehn B, Seiler F, Rehn S, Bruch J, Maier M. Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. Toxicol Appl Pharmacol 2003; 189: 84-95.

131) Tao F, Kobzik L. Lung macrophage-epithelial cell interactions amplify particle-mediated cytokine release. Am J Respir Cell Mol Biol 2002; 26: 499-505.

132) Thevenot P, Cho J, Wavhal D, Timmons RB, Tang L. Surface chemistry influences cancer killing effect of TiO_2 nanoparticles. Nanomed Nanotechnol Biol Med 2008; 4: 226-236.

133) Roller M. Carcinogenicity of inhaled nanoparticles. Inhal Toxicol 2009; 21(Suppl 1): 144-157.

134) Vileno B, Lekka M, Sienkiewicz A, Jeney S, Stoessel G, Lekki J, Forró L, Stachura Z. Stiffness alterations of single cells induced by UV in the presence of nanoTiO_2. Environ Sci Technol 2007; 41: 5149-5153.

135) Zhao J, Bowman L, Zhang X, Vallyathan V, Young SH, Castranova V, Ding M. Titanium dioxide (TiO_2) nanoparticles induce JB6 cell apoptosis through activation of the caspase-8/Bid and mitochondrial pathways. J Toxicol Environ Health A 2009; 72: 1141-1149.