MINI-REVIEW

Toxicology of Engineered Nanomaterials - A review of Carcinogenic Potential

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Abstract

Nanotechnology has considerable socioeconomic potential. Benefits afforded by engineered nanoparticles (NP: defined as being less than 100 nm in diameter) are expected to be significant in fields such as plastics, energy, electronics, aerospace and medicine. However, NPs are being introduced into the market without adequate assessment of their potential toxicities. It is urgently important to conduct risk assessment of commercial NPs and establish a framework enabling risk management which is not subordinate to their commercial production. An overview of currently available carcinogenicity risk evaluation results of NP materials raises serious questions as to their safety. NP sized titanium dioxide ($nTiO_2$) and carbon black (nCB) are carcinogenic to the lung of female rats, and the tumors preferentially include squamous cell morphology. Carbon nanotubes (CNT) induce mesotheliomas when applied intraperitoneally in rats and mice. Data for Fullerenes are insufficient to evaluate carcinogenic risk. Sub-chronic toxicity data indicate that, in general, NPs form aggregates and agglomerates and cause foreign body reactions at their applied sites with inflammatory cell, including macrophage, infiltration. These findings are similar to the biological effects of asbestos, a potent carcinogen, and indicate that careful assessment of NPs is indispensable.

Key words: Nanoparticles - toxicology - carcinogenicity - titanium dioxide - carbon black

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Principles of Safety

The safety of our living environment can be secured by the balanced function of three elements: risk assessment, risk management and risk communication (Figure 1). The first of these elements, risk assessment, must be addressed first, since without reliable risk assessment, risk communication and risk management can not function. Importantly, for reliable risk assessment long-term animal studies are indispensable.

These principles, of course, hold true for engineered nanoparticles. Unfortunately, the risk assessment data for engineered nanoparticles are rather fragmentary. However, the available findings do present a disturbing picture of potential carcinogens entering the market place. Engineered nanoparticles included in this review include nano-size titanium dioxide ($nTiO_2$), carbon black (nCB), single-walled carbon nanotubes (SWCNT), multiple-walled carbon nanotubes (MWCNT) and fullerenes (C60).

Metals and Metal-derived Nanoparticles: Titanium dioxide ($nTiO_2$)

In an inhalation study, female rats were exposed to air containing $nTiO_2$ (28nm in diameter) at a concentration of 7.5 mg/m^3 for 4 months, then at a concentration of 15 mg/m^3 for 4 months, and finally at a concentration of 10 mg/m^3 for 16 months, then killed at month 30. The incidence of lung tumors (19%), benign and malignant squamous and alveolar cell tumors combined, was significantly increased compared to the clean air control group (0.5%) (Heinrich et al., 1995).

Figure 1. Principles of Safety of the Living Environment. Safety of the living environment can be secured by the balanced function of three elements: risk assessment, risk management and risk communication

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In another study, female rats were administered $nTiO_2$ by intratracheal instillation. In this study, several dosing strategies were used. Hydrophilic nano-sized $nTiO_2$ (21-25 nm in diameter) was applied in 5 doses of 3 mg each, 5 doses of 6 mg each, or 10 doses of 6 mg each. Hydrophobic nano-sized $nTiO_2$ (21 nm in diameter) was applied in 15 doses of 0.5 mg each or 30 doses of 0.5 mg each. Hydrophilic anatase $nTiO_2$ (200 nm in diameter) was applied in 10 doses of 6 mg each or 20 doses of 6 mg each. The $nTiO_2$, hydrophilic and hydrophobic $nTiO_2$ and hydrophilic anatase $nTiO_2$, was suspended in PBS with 0.5% Tween 80. The instillation was done once weekly. The animals were observed for up to 30 weeks. The incidences of lung tumors (52-69.6%), adenomas/carcinomas and squamous cell epitheliomas/carcinomas combined, in rats receiving hydrophilic $nTiO_2$ were significantly increased over untreated controls (0%). Anatase $nTiO_2$ also induced significant incidences of lung tumors (29.5-63.6%), and these tumors were similar to those induced by hydrophilic $nTiO_2$. The incidences of benign and malignant lung tumors in the hydrophobic $nTiO_2$ groups (6.7%) was not significant (Pott and Roller, 2005).

In another inhalation study, female rats were treated with TiO_2 (size, not indicated) at a concentration of 11.3mg/m^3 for 24 months followed by observation for 6 months. Incidences of cystic keratinizing epitheliomas (11.7%) and squamous cell carcinomas (4.8%) were significantly greater than the control group (0.5%) (Rittinghausen et al., 1997). $nTiO_2$ is not carcinogenic to the skin because it does not penetrate the dermal tissue (Newman et al., 2009). Based on the studies outlined here, $nTiO_2$ is evaluated by WHO/IARC as a Group 2B compound (possibly carcinogenic to humans) (Baan, 2007).

Carbon-derived Nanoparticles

1. Carbon black (nCB)

Because of its long history of production and consumption, the highest number of reports concerning the carcinogenicity of carbon-derived nanomaterials are about nCB.

a) Rat studies. In an inhalation study, female rats were exposed to nCB (Printex90, 14 nm in diameter) at a concentration of 7.5 mg/m^3 for 4 months and then at a concentration of 12 mg/m^3 for 20 months followed by clean air for 6 months. The incidence of lung tumors (39%), benign and malignant squamous cell tumors and bronchio-alveolar cell tumors combined, was significantly increased as compared to the clean air group (0.5%) (Heinrich et al., 1995).

In another study, nCB (Printex90, 14 nm in diameter and Lamp Black101, 98nm in diameter) was administered to female Wistar rats by intratracheal instillation. Printex90 was washed in boiling toluene and suspended in saline containing 0.25% Tween 80, then administered once per week for 3 weeks at a dose of 0.66 mg per rat for 3 weeks then once per week for 13 weeks at a dose of 1.0 mg per rat. Animals were observed for up to 800 days from the beginning of the study. The incidence of cystic keratinizing epitheliomas and bronchio-alveolar cell tumors combined in Printex90 treated rats (21%) was similar to that observed in rats treated with benzo[a]pyrene and diesel emission particles and significantly elevated compared to the vehicle control group (0%). Lamp Black 101 treated rats also showed a significant increase in lung tumors compared to the control group (Dasenbrock et al., 1996).

In a second inhalation study, female Wistar rats were exposed to nCB (Printex90, 14 nm in diameter) at a concentration of 11.3 mg/m^3 for four months and then at a concentration of 12.2 mg/m^3 for the following 20 months. The incidence of cystic keratinizing tumors (20%), epitheliomas and carcinomas combined, was significantly increased compared to the clean air control group (0%) (Rittinghausen et al., 1997).

In the same series of experiments as the one outlined above, Printex90 was administered to female Wistar rats by intratracheal instillation. Printex90 was suspended in saline with 0.25% Tween 80 and administered 16-17 times (total dose 15 mg per animal). Animals were observed for up to 24 months. In rats receiving Printex90, the incidence of cystic keratinizing epitheliomas (19%) was significantly increased compared to saline treated animals (0%). Rats were also treated with purified Lamp Black: the treatment regimen was the same as for Printex90. Tumor incidence in these rats (6.3%) was not significantly elevated (Rittinghausen et al., 1997).

In a fifth study, nCB (Printex90, 14 nm in diameter and Lamp Black 101, 98nm in diameter; Degussa) was administered to female Wistar rats by intratracheal instillation. Printex90 was washed in boiling toluene and suspended in saline containing 0.25% Tween 80. The Printex90 was applied once per week for 3 weeks at a dose of 0.66 mg per rat for 3 weeks then once per week for 13 weeks at a dose of 1.0 mg per rat. Animals were observed for up to 800 days from the beginning of the study. The incidence of cystic keratinizing epitheliomas and bronchio-alveolar cell tumors combined in Printex90 treated rats (21%) was similar to that observed in rats treated with benzo[a]pyrene and diesel emission particles and significantly elevated compared to the vehicle control group (0%). Lamp Black101 treated rats also showed a significant increase in lung tumors compared to the control group (Dasenbrock et al., 1996).

In a sixth study, nCB (Printex90 or purified Lamp Black 101) was administered to female SPF Wistar rats intratracheal instillation. Animals were observed for up to 30 months. Several dosing strategies were used: animals were treated from 5 to 20 times with 1.5 to 6 mg nCB. Total lung tumor incidence for each treatment regimen (Printex90, 56% to over 80%; Lamp Black, 44% to 70%) was significantly elevated compared to the control group (2%) (Pott and Roller, 2005).

Based on the studies outlined here, nCB is evaluated by WHO/IARC as a Group 2B compound (possibly carcinogenic to humans) (Baan, 2007).

b) Mouse and Hamster Studies. No data are available as to carcinogenicity and related studies in mice and hamsters.

c) Summary. nCB is carcinogenic to the female rat

inducing cystic keratinizing epitheliomas and bronchio-alveolar cell lung tumors. Mechanisms to explain why cystic squamous cell tumors were induced exclusively in female rats have not yet been elucidated. No data for carcinogenicity of nCB in mice and hamsters are available.

2. Carbon nanotubes

There are two types of carbon nanotubes: SWCNT are tube structures with a diameter of close to 1 nanometer and composed of a one-atom-thick layer of graphite (Figure 2, left), and MWCNT are tube structures with a diameter of close to 1 nanometer and composed of two or more layers of graphite atoms (Figure 2, right).

A) SWCNT; a) Rat studies. In a short to medium-term (6 months or less) study, SWCNT (diameter 1.4 nm, length more than 1 mm agglomerate at use) was administered to male Crl:CD(SD)IGS BR rats intratracheal instillation. The SWCNT particles were suspended in PBS with 1% Tween 80 and administered one time at 1 or 5 mg/kg. Animals were observed up to 12 weeks. Multifocal small granulation tissue with deposition of aggregates of SWCNT were observed in the lung. Inflammatory cell counts of bronchoalveolar lavage (BAL) did not clearly correlate with the degree of the inflammatory reaction. Similar inflammatory changes were also observed in rats which received quarts particles (crystalline, diameter 1-3 mm). No tumors were found in any of the groups (Warheit et al., 2004).

b) Mouse studies. In a short to medium-term (6 months or less) study, SWCNT (diameter 0.8-2.2 nm, length not indicated) and cup-stacked CNT (mean diameter 80 nm, length not more than 100 mm) were administered to female BALB/c mice by subcutaneous injection. The SWCNT and CNT particles were suspended in saline and animals were injected at 2 mg per animal then observed for up to 12 weeks. The iron content of these materials was 3.5-5.0% (weight). Although inflammatory lesions in the injected site were observed, no tumors were found (Koyama et al., 2006).

In another short to medium-term (6 months or less) study, SWCNT (iron content, 26.9% weight) was administered to male B6C3F1 mice by intratracheal instillation. Unpurified SWCNT and purified (polycyclic aromatic hydrocarbon-free) SWCNT suspended in heat-inactivated mouse serum at a concentration of 2 mg/ml was administered one time at 0.1 or 0.5 ml/mouse. The animals were then observed for 7 or 90 days. Granulation tissue formation with epithelioid cell reaction was observed in the bronchioles, respiratory ducts and alveoli. The inflammatory reaction in animals given SWCNT was more prominent than animals treated with carbon black. Neoplastic lesion development was not observed (Lam et al., 2004).

B) MWCNT: a) Rat studies. In a short to medium-term (6 months or less) study, MWCNT (average 15 carbon layers, approximate inner diameter 5 nm, outer diameter 9-10 nm) was administered to female SD rats by intratracheal instillation. The MWCNT particles were suspended in saline with 1% Tween 80 and administered one time at doses of 0.5, 2.0 and 5 mg/rat in 0.5 ml saline. The rats were killed on day 60. Small granulation tissue with deposition of MWCNT was observed in the bronchi, bronchio-alveolar space and some alveoli. No tumors were found (Muller et al., 2005).

In another study, MWCNT (MUTSUI MWCNT-7, 3500 ppm iron content; diameter 70-100 nm; approximate length 1-4 μm) was administered to male F344/DuCrlj rats by a single intrascrotal injection. The MWCNT particles were suspended in 0.5% methyl cellulose with 1.0% Tween 80 and administered at a dose of 20 mg/mouse. The animals were then observed until week 104. Another group of mice were treated with crocidolite (UICC grade asbestos). The incidence of disseminated mesothelioma in the peritoneal cavity was 86% in MWCNT and 0% in crocidolite groups (Sakamoto et al., 2009).

In a third study, MWCNT (11.3 nm in mean diameter, approximate length 0.7 μm) was administered to three groups of male Wistar rats by a single intraperitoneal injection. MWCNT with structural defects was suspended in PBS and administered at a dose of 2 or 20 mg/rat and MWCNT without structural defects was suspended in PBS and administered at a dose of 20 mg/rat. The animals were then observed for up to 104 weeks. Another group of rats was treated with 2 mg crocidolite (UICC grade asbestos) per rat. The incidence of mesothelioma in the group administered crocidolite was 34.6%, but the incidence of mesothelioma in the MWCNT groups (up to 6%) was not statistically higher than the incidence (3.8%) in the vehicle control group (Muller et al., 2009).

b) Mouse studies. In a short to medium-term (6 months or less) study, MWNT (diameter 20-150 nm, length 10-20 mm) was administered to female BALB/c mice by a single subcutaneous injection. The nano-particles were suspended in saline administered at 2 mg per mouse. The animals were then observed for up to 12 weeks. The iron content of these materials was 3.5-5.0% (weight). Although inflammatory lesions at the injected site were observed, no tumors were found (Koyama et al., 2006).

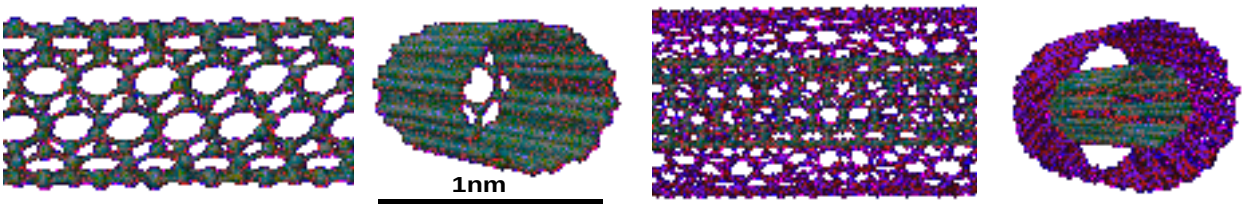

Figure 2. Structure of a Single-walled Carbon Nanotube (SWCNT) and a Multiple-walled Carbon Nanotube (MWCNT). (Courtesy of Dr. M. Ata; National Institute of Advanced Industrial Science and Technology, Japan)

In another short to medium-term (6 months or less) study, two types of MWCNT, pure MWCNT and N-doped (nitrogen attached on the surface) MWCNT (approximately 30-50 nm in diameter and 100-300 mm in length) were administered intranasally, intratracheally, orally or intraperitoneally to male CD1 mice. The MWCNT particles were suspended in phosphate buffered saline (PBS) and administered one time at doses of 1, 2.5 or 5 mg/mouse. The animals were killed after 30 days. Intratracheal administration of MWCNT resulted in inflammatory lesions surrounding aggregates of MWCNT and hyperplastic change of the bronchial epithelium in a dose dependent manner. These changes were less intense in N-doped MWCNT. No obvious clinical symptoms were noted in mice treated though other routes (Carrero-Sanchez et al., 2006).

In a third study, MWCNT (MUTSUI MWCNT-7, 3500 ppm iron content; diameter 100 nm; approximate length 1-5 μm) was administered to male p53 (+/-) mice with a C57BL/6 background by intraperitoneal injection. The MWCNT was suspended in 0.5% methyl cellulose with 1.0% Tween 80 and administered one time at a dose of 3 mg/mouse. The animals were then observed for 25 weeks. Other groups of mice were treated with C60 and crocidolite (UICC grade asbestos) at a dose of 3 mg/mouse. The incidence of mesothelioma in the peritoneal cavity was 14/16 (87.5%) in the MWCNT group and 0% in the C60 group and 14/18 (77.8%) in the crocidolite group (Takagi et al., 2008).

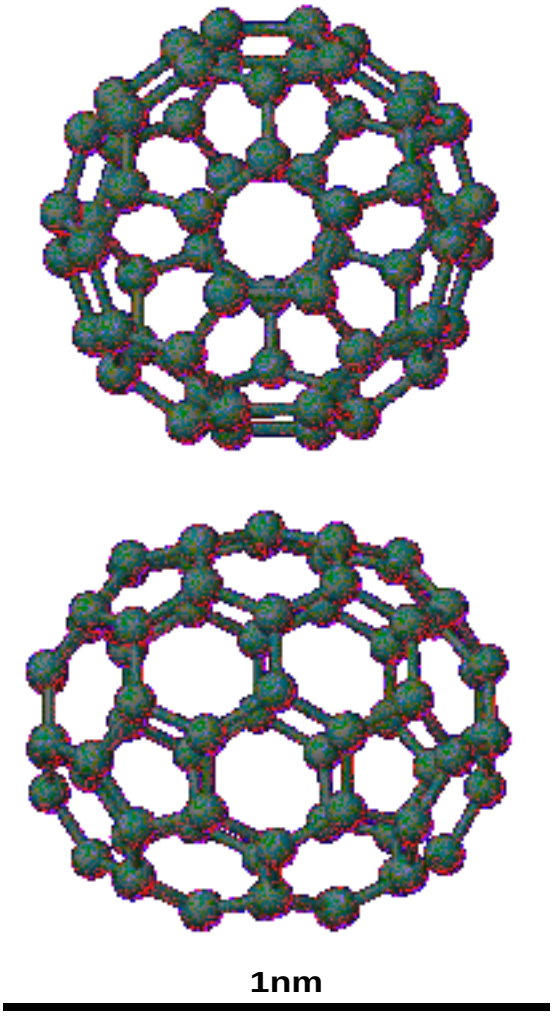

Figure 3. Structure of Fullerene 60 (C60) and Fullerene 70 (C70). (Courtesy of Dr. M. Ata; National Institute of Advanced Industrial Science and Technology, Japan)

c) Summary. Apparently contradictory results are reported in the rat studies. In one study, intrascrotal injection of MWCNT (approximate length 1-4 μm) resulted in mesothelioma in the peritoneal cavity, but intrascrotal injection of crocidolite did not induce tumor formation; in another study, mesothelioma was induced by intraperitoneal injection of crocidolite but intraperitoneal injection of MWCNT (approximate length 0.7 μm) did not induce tumor formation. Obviously, further studies to the confirm carcinogenic potential of MWCNT, especially MWCNT of different lengths, in rats is required. SWCNT and MWCNT induced a small amount of granulation tissue formation in the bronchioles and bronchio-alveolar area in mice. In a critical study, MWCNT was found to induce mesothelioma in p53-/+ mice. Data pertaining to the carcinogenicity of SWCNT were negative in both rats and mice.

3) Fullerenes (C60/70)

Fullerene was named after Richard Buckminster Fuller, an architect who popularized the geodesic dome which resembles a spherical fullerene in appearance. C60 is composed of 60 carbons arranged at the corners of each hexagon and a bond along each edge resembling a soccer ball (Fig. 3). C70 is composed of 70 carbons (Fig. 3) and is similar in structure to C60. C60 has photocatalytic activity releasing reactive oxygen species in the presence of light.

a) Rat studies. No data is available as to carcinogenicity and related studies in rats.

b) Mouse studies. Fullerene was tested for skin tumor promotion. Female CD-1 mice were initially painted with 7,12-dimethylbenz[a]anthracene (DMBA): 20 nmol DMBA dissolved in 200 mL acetone was applied one time to shaved dorsal skin. 1 week later, a C60/C70 mixture (6:1) dissolved in benzene was applied twice a week for 25 weeks. No tumors were found in the skin. Another group of mice was treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) (5mg in 200 mL acetone), a known skin tumor promoter. The incidence of tumor development in the TPA treated mice (100%) was significantly greater than in the acetone treated controls (0%). In a co-occurrent experiment ornithine decarboxylase (ODC) activity and DNA synthesis, measured by 3H-thymidine incorporation, were also increased by TPA treatment but not by C60/C70 (Nelson et al., 1993).

c) In vitro studies related to carcinogenesis. Fullerene C60 was treated with polyvinylpyrrolidone (PVP) and then dissolved in water. In the presence of rat liver microsomes, treated C60 was mutagenic for Salmonella strains TA102, TA104 and YG3003, but only when exposed to visible light and not in the absence of light. Mutagenicity was

reduced in the presence of antioxidants. The results suggest that singlet molecular oxygen radicals were generated by irradiating C60 with visible light. Further experiments indicated that the mutagenicity was due to oxidized phospholipids in the rat liver microsomes, in particular those present in the HPLC isolated linoleate fraction (Sera et al., 1996). Similarly, C60 induced peroxidation of lipids which caused oxidative liver cell injury in the presence of microsomes from hepatocytes (Kamat et al., 1998).

d) Summary. *In vitro*, C60 caused DNA damage when irradiated with visible light. In one *in vivo* study using mice, C60/70 did not show skin tumor promotion activity in a 26-week initiation-promotion protocol. No long-term carcinogenicity studies were reported.

Overall Evaluation and Proposal for the Future

During the development and marketing of nanomaterials, risk assessment of these new products has been perfunctory at best. While nanomaterials have undeniable benefits, their use also has undeniable potential risk. This risk must be addressed in an unbiased and thorough manner. Only after the toxicity of the various nanomaterials is understood can their true benefits be realized.

In rodent studies, $nTiO_2$ whether administered by inhalation or intratracheal instillation was shown to induce lung tumors with characteristic squamous cell morphology in female rats. These nanomaterials did not induce lung tumors in male rats. Our own studies have also shown that instillation of $nTiO_2$ into the lungs of female rats showed tumor promoting activity and resulted in elevated ROS-mediated damage and production of inflammatory cytokines. It is reasonable to assume that other metal-derived nanoparticles, such as aluminium and copper nanoparticles, and metal containing nanoparticles, for example nCB-metal mixtures and SWCNT and MWCNT preparations, are also capable of producing these effects. Nanoparticles such as $nTiO_2$, nCB, SWCNT and MWCNT when intratracheally administered, were detected by light microscope as aggregates or agglomerates and these forms

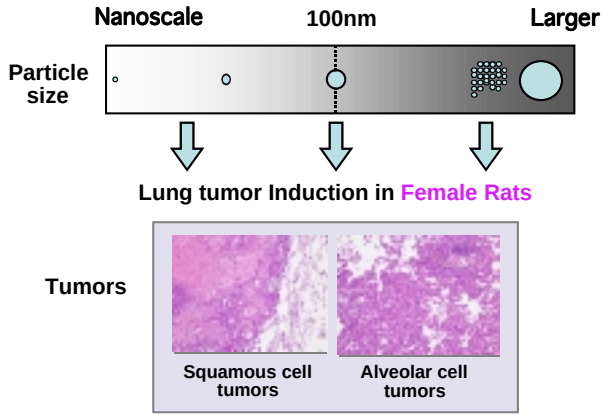

Figure 4. Schematic Presentation of Carcinogenic Effects of TiO_2, Carbon Black. Carcinogenic effects were elicited by both nano-scale and larger sized particles

are reported to induce foreign body granulation tissue with various degree of inflammatory reaction. Although the relevance of foreign body-induced chronic inflammation to carcinogenesis is not clearly established, it is possible that reactive oxygen species (ROS) produced by macrophages attempting to destroy the foreign material in the inflammation site may cause DNA damage associated with carcinogenesis. Another possible contributing factor is metal, for example from metal-derived nanoparticles such as TiO_2 or from metal contaminants: these metals could also be involved in ROS production. Thus, it is possible that the observed carcinogenic effect is not specific to nanoparticles but rather associated with their ability to induce persistent foreign body-induced chronic inflammation and/or introduce metals into susceptible sites. For example, TiO_2 and carbon blacks larger than 100nm in diameter are known to induce lung tumors including similar squamous cell morphology (Nikula, 2000); (Pott and Roller, 2005) and both of these materials (larger than nano size) are classified as into group 2B (possibly carcinogenic to humans) by WHO/IARC.

Mechanisms for mesothelioma induction by MWCNT in mice and rats have not been elucidated yet. A possible contributing factor is metal: Transition metals, such as iron, are commonly used as a catalytic center in the formation of CNTs, and contaminating metal in SWCNT and MWCNT particles could catalyze the formation of ROS by the Fenton reaction (Liu and Okada, 1994). One example of this type of toxicity is that human keratinocytes exposed to SWCNT were killed by ROS in the media (Shvedova et al., 2003). Another possible contributing factor is the length of the MWCNT (Pott and Roller, 2005; Muller et al., 2009; Sakamoto et al., 2009).

As noted at the beginning of this review, for reliable risk assessment long-term animal studies are indispensable. This is particularly true for risk assessment of potential carcinogens. The standard for the evaluation of the carcinogenic potential of a test chemical is testing in two rodent species, generally rats and mice, of each sex, at 3 doses (0, low and high) of the test chemical for up to two years. In the studies conducted to date concerning the carcinogenic risk presented by nanoparticles, there is a noticeable lack of long term testing: No long-term tests of any type have been reported for either SWCNT or fullerenes. Importantly, the primary goal of risk assessment is not to simply ban a product from the market place, but rather to determine product safety and establish guidelines lines for its production and use and promote consumer confidence. Given the known ability of many nanomaterials to induce mechanisms which are active in humans that are risk factors for carcinogenesis, for example ROS and inflammatory cytokine production, the continued introduction of these materials into the market is alarming. Establishing the safety of these materials is urgently needed.

In this short review, available in vivo data concerning the carcinogenic effects of $nTiO_2$, nCB, SWCNT and MWCNT, and Fullerenes is outlined. Of these, $nTiO_2$ and nCB are classified as possibly carcinogenic to humans. Testing of the carcinogenic activity of MWCNT produced

mixed results. SWCNT and fullerenes have no carcinogenic activity in the studies conducted to date, however, toxicity testing of these materials has been quite limited and both of these materials have the potential to produce ROS. The observations noted here may apply to possible carcinogenic risk of other nanoparticles because of shared mechanisms of induction of inflammatory lesions and ROS generation. Our conclusions are that nanoparticles are clearly potentially toxic/carcinogenic to humans and their toxicity must be assessed, and their production and use managed appropriately.

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References

Baan RA (2007). Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. *Inhal Toxicol*, **19 Suppl 1**, 213-28.

Carrero-Sanchez JC, Elias AL, Mancilla R, et al (2006). Biocompatibility and toxicological studies of carbon nanotubes doped with nitrogen. *Nano Lett*, **6**, 1609-16.

Dasenbrock C, Peters L, Creutzenberg O, Heinrich U (1996). The carcinogenic potency of carbon particles with and without PAH after repeated intratracheal administration in the rat. Toxicol Lett, 88, 15-21.

Emerich DF, Thanos CG (2003). Nanotechnology and medicine. *Expert Opin Biol Ther*, **3**, 655-63.

Heinrich U, Fuhst R, Rittinghausen S, et al (1995). Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. *Inhal Toxicol*, **7**, 533-56.

Kamat JP, Devasagayam TP, Priyadarsini KI, Mohan H, Mittal JP (1998). Oxidative damage induced by the fullerene C60 on photosensitization in rat liver microsomes. *Chem Biol Interact*, **114**, 145-59.

Koyama S, Endo M, Kim YA, et al (2006). Role of systemic T-cells and histopathological aspects after subcutaneous implantation of various carbon nanotubes in mice. *Carbon*, **44**, 1079-92.

Lam CW, James JT, McCluskey R, Hunter RL (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol Sci*, **77**, 126-34.

Liu M, Okada S (1994). Induction of free radicals and tumors in the kidneys of Wistar rats by ferric ethylenediamine-N,N'-diacetate. *Carcinogenesis*, **15**, 2817-21.

Muller J, Delos M, Panin N, et al (2009). Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. *Toxicol Sci*, **110**, 442-8.

Muller J, Huaux F, Moreau N, et al (2005). Respiratory toxicity of multi-wall carbon nanotubes. *Toxicol Appl Pharmacol*, **207**, 221-31.

Nelson MA, Domann FE, Bowden GT, et al (1993). Effects of acute and subchronic exposure of topically applied fullerene extracts on the mouse skin. *Toxicol Ind Health*, **9**, 623-30.

Newman MD, Stotland M and Ellis JI (2009). The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens. *J Am Acad Dermatol*, **61**, 685-92.

Nikula KJ (2000). Rat lung tumors induced by exposure to selected poorly soluble nonfibrous particles. *Inhal Toxicol*, **12**, 97-119.

Pott F, Roller M (2005). Carcinogenicity study with nineteen granular dusts in rats. *Eur J Oncol*, **10**, 249.

Rittinghausen S, Mohr U, Dungworth DL (1997). Pulmonary cystic keratinizing squamous cell lesions of rats after inhalation/instillation of different particles. *Exp Toxicol Pathol*, **49**, 433-46.

Roco MC (2003). Nanotechnology: convergence with modern biology and medicine. *Curr Opin Biotechnol*, **14**, 337-46.

Sakamoto Y, Nakae D, Fukumori N, et al (2009). Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. *J Toxicol Sci*, **34**, 65-76.

Sera N, Tokiwa H, Miyata N (1996). Mutagenicity of the fullerene C60-generated singlet oxygen dependent formation of lipid peroxides. *Carcinogenesis*, **17**, 2163-9.

Shvedova AA, Castranova V, Kisin ER, et al (2003). Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J Toxicol Environ Health A*, **66**, 1909-26.

Takagi A, Hirose A, Nishimura T, et al (2008). Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. *J Toxicol Sci*, **33**, 105-16.

Warheit DB, Laurence BR, Reed KL, et al (2004). Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol Sci*, **77**, 117-25.