Titanium Dioxide (TiO_2)

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Citation for most recent IARC review

IARC Monograph 93 (in press)

Current evaluation

Conclusion from the previous review:
Titanium dioxide is *possible carcinogenic to humans (Group 2B)* based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies.

Exposure and biomonitoring

Occupational exposure

Titanium dioxide is produced from iron titanate or titanium slag by digesting with sulfuric acid or from ores with a high titanium content by heating with coke and chlorine to form titanium tetrachloride, then oxidizing to titanium chloride. Production workers are exposed to sulfuric acid mist or hydrochloric acid and titanium dioxide dust. The occupational

epidemiology studies do not differentiate production of fine titanium dioxide from that of ultrafine (or nano) titanium dioxide. A relative risk assessment ranked manufacture of nano-titanium dioxide at 62 (range 0-100), similar to the assigned rank of automotive lead battery manufacture (Robichaud, et al. 2005)

Four U.S. companies manufacture 1.3 million metric tons/year of bulk titanium dioxide, 25% of global production, with the chloride process at eight sites. The percentage of titanium dioxide manufactured as nanoparticles has been estimated as 2.5% in 2009 and about 10% by 2015 (Robichaud et al., 2009).

Liao and colleagues transformed mass exposure data from two recent epidemiology studies to surface area measurements for nano-titanium dioxide, calculating that 1 g titanium dioxide had 50 m^2 surface. Calculated concentrations were 0.168 m^2 for packers, the highest exposed job, in U.S. production plants and 0.387 m^2 for surface treaters in European plants (Liao et al., 2008).

Workers in industries using titanium dioxide are also exposed. Levels of titanium dioxide exposure in user industries have not been reported. U.S. user industries include paints and pigment (57%); plastics (26%); paper (13%); cosmetics, catalysts, ceramics, printing inks, roofing granules, glass, and welding fluxes (Robichaud et al., 2009).

Environmental exposures

There is conflicting evidence as to whether nanoparticles of titanium dioxide can pass through the skin (Kiss et al., 2008; Wu et al., 2009). If they can, the presence of titanium dioxide in a large variety of cosmetic powders and creams may be a cause of concern.

Cancer in humans

(*inadequate*)

The previous IARC review Monograph (93, in press) evaluated three retrospective cohort studies of titanium dioxide production workers and one case-control study published through 2006 A 2008 re-evaluation of two previously conducted case-control studies found no association with lung cancer (Ramanakumar et al., 2008).

No studies have been conducted of workers using titanium dioxide as a pigment in the manufacture of cosmetics, paints, varnishes, lacquers, paper, plastics, ceramics, rubber, or printing ink.

Cancer in experimental animals

(*sufficient*, Monograph 93, 2006)

Elevated lung cancer was observed in two chronic inhalation studies in rats exposed to fine (Lee et al., 1985) or ultrafine (Heinrich et al., 1995) TiO_2.

Lee et al. ***(1985)***

Rats (female CD Sprague-Dawley-derived) were exposed by whole body inhalation to fine, rutile TiO_2 (aerodynamic mass median diameter of 1.5-1.7 μm) for 6 hr/day, 5 days/week, for

up to two years, to 0, 10, 50, or 250 mg/m^3 (84% respirable; <13 μm MMAD); 80 rats were exposed for two years, and all surviving rats were killed at the end of exposure. No increase in lung tumors was observed at 10 or 50 mg/m^3. At 250 mg/m^3, bronchioalveolar adenomas were observed in 12/77 male rats and 13/74 female rats. In addition, squamous cell carcinomas were reported in 1 male and 13 females at 250 mg/m^3. These squamous cell carcinomas were later reclassified as proliferative keratin cysts (Carlton 1994), or as a range of responses from pulmonary keratinizing cysts through pulmonary keratinizing eptheliomas to frank pulmonary squamous carcinomas (Boorman et al., 1996). A recent reanalysis of the 16 tumors originally classified as cystic keratinizing squamous cell carcinomas in Lee et al. (1985) had a similar interpretation: two were re-classified as squamous metaplasia, one as a poorly keratinizing squamous cell carcinoma, and 13 as nonneoplastic pulmonary keratin cysts (Warheit and Frame 2006).

Heinrich et al. (1995)

Female Wistar rats were exposed to ultrafine TiO_2 (80% anatase/20% rutile; 15-40 nm primary particle size; 0.8 μm MMAD; 48 ($\pm$ 2.0) m^2/g specific surface area) at an average concentration of 10 mg/m^3, 18 h/d, 5d/wk, for up to 24 months (actual concentrations were 7.2 mg/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months, and 9.4 mg/m^3 for 16 months). After the 2-year exposure, the rats were kept in clean air for an additional 6 months. After 24 months of exposure, four of the nine rats examined had developed tumors (including a total of 2 squamous cell carcinomas, 1 adenocarcinoma, and 2 benign squamous cell tumors). At 30 months (6 months after the end of exposure), a statistically significant increase in adenocarcinomas was observed (13 adenocarcinomas, in addition to 3 squamous cell carcinomas and 4 adenomas, in 100 rats). In addition, 20 rats had benign keratinizing cystic squamous-cell tumors. Only 1 adenocarcinoma, and no other lung tumors, was observed in 217 nonexposed control rats.

NMRI mice were also exposed to ultrafine TiO_2 in Heinrich et al. (1995). The lifespan of NMRI mice was significantly decreased by inhaling approximately 10 mg/m^3 ultrafine TiO_2, 18 hr/day for 13.5 months (Heinrich et al., 1995). This exposure did not produce an elevated tumor response in the NMRI mice, but the 30% lung tumor prevalence in controls may have decreased the sensitivity for detecting carcinogenic effects in this assay.

Recent studies: No subsequent carcinogenicity studies of TiO_2 in animals were found in the literature since those evaluated in Monograph 93 (in press).

Mechanisms of carcinogenicity

Titanium dioxide is poorly soluble low toxicity (PSLT) particles, which can elicit overloading of lung clearance, chronic inflammation, and lung tumors in rats following prolonged exposure at sufficiently high concentrations of particles (Monograph 93 (in press); Baan 2007). Overloading of lung clearance occurs at much lower mass concentrations of ultrafine TiO_2 (10 mg/m^3) than fine TiO_2 (50 or 250 mg/m^3) (Bermudez et al., 2002, 2004). Lung tumors also develop a lower mass concentration of ultrafine TiO_2 (~10 mg/m^3) (Heinrich et al., 1995) compared to fine TiO_2 (250 mg/m^3) (Lee et al., 1985) following chronic inhalation in rats. Particle surface area dose was found to be most predictive of the pulmonary

inflammation and tumor responses in rats when the dose-response relationships are compared for various types and sizes of PSLT including TiO_2 (Driscoll 1995; Dankovic et al., 2007).

Most evidence suggests that TiO_2 and other PSLT-elicited lung tumors develop via a secondary genotoxic mechanism involving chronic inflammation, cell proliferation, and oxidative stress (Schins and Knaapen 2007). Overloading of lung clearance is accompanied by pulmonary inflammation, production of reactive oxygen and nitrogen species, depletion of antioxidants and/or impairment of other defense mechanisms, cell injury, cell proliferation, fibrosis, and as observed in rats, induction of mutations and eventually cancer (Monograph 93 (in press); Baan 2007).

Rats were more sensitive to the adverse effects of inhaling either fine or ultrafine TiO_2 than either mice or hamsters (Bermudez et al., 2002, 2004). Both mice and rats developed overloading of lung clearance (at 50 mg/m^3 of fine TiO_2 and 10 mg/m^3 of ultrafine TiO_2), although rats developed more persistent neutrophilic inflammation, cell proliferation, and fibrotic responses than did mice at 52 weeks after a 13-week inhalation exposure to 250 mg/m^3 fine TiO_2 (Bermudez et al., 2002). Rats were also more sensitive than mice or hamsters to adverse lung effects of inhaled carbon black (Elder et al., 2005). In each of these studies, hamsters had rapid lung clearance and thus low retained dose and response.

Although studies in humans have not shown a direct link between inhaled PSLT and lung cancer, many of the steps in the mechanism observed in rats have also been observed in humans who work in dusty jobs, including increased particle lung retention and pulmonary inflammation in workers exposed to coal dust or crystalline silica (Castranova 2000; Kuempel et al., 2001; Lapp and Castranova 1993); and elevated lung cancer has been observed in some studies of workers exposed to carbon black (Sorahan and Harrington 2007), crystalline silica (Rice et al., 2001; Attfield and Costello 2004), and diesel exhaust particles (Stayner et al., 1998).

An alternative genotoxic mechanism for nanoscale particles may involve direct interaction with DNA (Schins and Knaapen 2007). Nano-TiO_2 particles have been observed inside lung epithelial cells and cell organelles, including the nucleus, of rats 24-hours after a 1-hr inhalation exposure to 0.1 mg/m^3 nanoscale TiO_2 (4 nm primary particle diameter; 22 nm count median diameter; 330 m^2/g specific surface area) (Geiser et al., 2005). Nano-TiO_2 particles were ineffectively cleared by alveolar macrophages and were also observed in all major lung tissue compartments and within capillaries (Geiser et al., 2008).

Recent studies

Several *in vitro* studies have shown that TiO_2 produced reactive oxygen species (ROS) and induced oxidative DNA damage (Gurr et al., 2005; Türkez and Geyikoğlu 2007; Wang et al., 2007). Sayes et al. (2006) reported that nano-anatase produced more ROS and was more cytotoxic than nano-rutile, but only after UV irradiation. Fenoglio et al. (2009) observed that oxygen and carbon-centered free radical generation was associated with the surface area of micro- or nano-sized anatase TiO_2, and that while superoxide production was related to exposure to sunlight, other free radical species were generated in the dark. TiO_2 nanoparticles did not induce DNA breakage (measured by Comet assay) in human lung fibroblast or

bronchial epithelial cell cultures, but did induce a high level of oxidative DNA adduct formation (8-hydroxyl-2-deoxyguanosine or 8-OHdG) (Bhattacharya et al., 2009).

Inflammation in bronchoalveolar lavage (BAL) fluid and in whole blood was examined 24 hours after a single intratracheal instillation (IT) dose of TiO_2 rutile nanorods (1 or 5 mg/kg) in Wistar rats (Nemmar et al., 2008). At both doses, the neutrophilic inflammation in BAL fluid was significantly elevated compared to vehicle controls. The number of monocytes and granulocytes in blood was dose-dependently elevated, while the platelets were significantly reduced at the higher dose, indicating platelet aggregation.

Nanoscale TiO_2 elicited a significantly greater increase in chemokines (associated with pulmonary emphysema and alveolar epithelial cell apoptosis) than did the microscale TiO_2 one week after a single IT dose in a study of adult male ICR mice. Animals were treated by IT administration of a single dose of 0.1 or 0.5 mg per mouse of either nanoscale TiO_2 (rutile, 21 nm average particle size; specific surface area of 50 m^2/g) or microscale TiO_2 (180-250 nm diameter; specific surface area 6.5 m^2/g) (Chen et al., 2006).

Three recent studies compared the pulmonary responses to various types of nanoscale or microscale TiO_2. A similar experimental design was used in each study, including IT dosing of male Crl:CD(SC):IGS BR rats, to a particle dose of either 1 or 5 mg/kg. BAL was performed at 24 hours, 1 week, 1 month, and 3 months after instillation (Warheit et al., 2006a,b; 2007):

In Warheit et al. (2006a), rats were administered IT doses of either 1 or 5 mg/kg of "R-100" or "Pigment A" (two types of hydrophilic TiO_2), carbonyl iron, or Min-U-Sil quartz. Primary average particle sizes were 300 nm, 290 nm, ~1.2 µm, or ~1.5 µm, respectively (Warheit et al., 2006a). Significantly elevated polymorphonuclear leukocytes (PMNs) in BAL fluid were observed for the two types of TiO_2 or carbonyl iron at 24 hours post-exposure, but not at the later time points.

Warheit et al. (2006b) compared nanoscale TiO_2 rods (anatase, 92-233 nm length, 20-35 nm width; 26.5 m^2/g specific surface area), nanoscale TiO_2 dots (anatase, 5.8-6.1 nm spheres; 169 m^2/g specific surface area), and microscale rutile TiO_2 (300 nm primary particle diameter; 6 m^2/g specific surface area). A statistically significant increase in the percentage of PMNs in BAL fluid was seen at the 5 mg/kg dose for all three TiO_2 materials tested (which was higher in the rats administered the nanoscale TiO_2) but returned to control levels at the 1-week time point. There were no statistically significant lung responses (inflammation or histopathology) to either the nanoscale or microscale TiO_2 at either dose (1 or 5 mg/kg) compared to controls at the 1-week to 3-month time points. Because of the low response in rats to either nanoscale or microscale TiO_2 at the doses used in this study, there were insufficient data to compare the dose-response relationships of TiO_2 by particle size.

In Warheit et al. (2007), the lung inflammation, cytotoxic, cell proliferation, and histopathological responses of two types of ultrafine rutile TiO_2, fine rutile TiO_2, and ultrafine 80/20% anatase/rutile TiO_2, and quartz particles were compared. Although the specific surface area of these particles varied from 5.8 to 53 m^2/g, the median particle sizes in the

phosphate buffered saline (PBS) instillation vehicle were similar (2.1-2.7 μm), suggesting that particle agglomeration had occurred reducing the effective surface area. The pulmonary responses (percent PMNs or percent proliferating tracheobronchial epithelial cells) in rats exposed to either type of ultrafine rutile TiO_2 or to fine rutile TiO_2 did not differ significantly from controls at either dose or any time point. The rats exposed to anatase/rutile TiO_2 had significantly greater responses (percent PMNs and percent of proliferating tracheobronchial epithelial cells) at the 5 mg/kg dose than the PBS controls 24-hr and 1-wk after IT, but not at 1 or 3 months. The two ultrafine rutile TiO_2 preparations had been passivated with amorphous silica and alumina coatings to reduce their chemical and photo-reactivity to a low level similar to that of the fine rutile TiO_2, while the ultrafine anatase/rutile TiO_2 was not passivated and was more chemically reactive based on a Vitamin C assay measuring oxidation potential.

Grassian et al. (2007) investigated lung responses in male C57Bl/6 mice exposed to nano-TiO_2 (2-5 nm diameter; 210 m^2/g specific surface area) by whole-body inhalation for either 4 hr (acute) or 4 hr/d for 10 d (subacute). The airborne exposure concentrations were 0.77 or 7.22 mg/m^3 (acute) or 8.88 mg/m^3 (subacute). The TiO_2 primary particle size was 2-5 nm, and the specific surface area was 210 m^2/g. No adverse effects were observed after the 4 hour exposure. Mice in the subacute study were necropsied at the end of the exposure period and at 1, 2, and 3 weeks post-exposure. A "significant but modest" inflammatory response was observed in the mice at 0, 1, or 2 weeks after the subacute exposures, with recovery at the 3rd week post-exposure.

Grassian et al. (2007a) compared the pulmonary toxicity of two sizes of TiO_2 nanoparticles (~5 nm and ~21 nm primary particle diameter, and BET surface area of 219 and 41 m^2/g, respectively) by inhalation and intra-nasal instillation in mice. The crystal structure of these two particles also varied (5 nm was anatase and 21 nm was anatase/rutile, and TEM images showed that the 5 nm particles formed closely compacted agglomerates whereas the 21 nm particles were more loosely agglomerated. The aerosol sizes were 120-123 GM (GSD 1.56) and 139-153 (GSD 1.4) nm. Instillation doses for the 5 nm TiO_2 particles were 5, 20, and 30 μg/mouse, and for the 21 nm TiO_2 particles were 25, 100, and 150 μg/mouse. Inhalation exposures to each particle size were approximately 0.8 and 7 mg/m^3 for 4 hours. Lung responses were examined by BAL and histopathology at 24 hours post-exposure (also at 4 hr for inhalation). By instillation, the pulmonary neutrophilic inflammation was somewhat greater on a mass basis for the 5 nm particles, but was less by BET-surface area. By inhalation, inflammation was similar at 24 hours post-exposure for equivalent mass concentrations of the 5 and 21 nm particles. These findings reinforce previous studies showing that the physical and chemical properties of particles influence toxicity, and that the contribution of a given factor cannot be determined unless the other factors are controlled. In this study, the agglomeration state varied between the two particle samples such that the measured particle sizes and surface areas may not have been a good measure of those to which the lung cells were exposed.

Sager et al. (2008) and Sager and Castranova (2009) investigated the role of particle surface area on pulmonary inflammation in male Fischer 344 rats treated with either fine or ultrafine TiO_2 by intratracheal instillation. The mass doses of ultrafine TiO_2 (primary particle size 21

nm; specific surface area 48 m^2/g; 0.26, 0.52, and 1.04 mg/rat) and fine TiO_2 (primary particle size 1,000 nm; specific surface area 2.3 m^2/g; 5.35, 10.7, and 21.4 mg/rat) corresponded to an equivalent surface area dose (0.031, 0.062, 0.12 cm^2 particles / cm^2 alveolar epithelial cell surface of the lungs). At each post-exposure time point (1, 7, or 42 days) and mass dose, the ultrafine TiO_2 was least 41 times more potent than fine TiO_2 in eliciting pulmonary inflammation (as measured by neutrophil cell count in BAL fluid, relative to control (saline only) rats). When dose was expressed as particle surface area (measured by BET gas absorption), the ultrafine TiO_2 as less than two times more potent than fine TiO_2, and this difference was not statistically significant. This study also showed that ultrafine TiO_2 translocated from the lungs to the lung-associated lymph nodes to a greater extent than fine TiO_2.

Overall, these recent studies are consistent with roles for both particle surface area and particle surface reactivity in the pulmonary responses to TiO_2 and other inhaled particles. Particle surface area and reactivity have been shown to influence the pulmonary inflammation response to various types of inhaled particles including PSLT and crystalline silica (Duffin et al., 2007; Dankovic et al., 2007).

In study in mice of TiO_2 (by oral gavage), Wang et al. (2007) observed that nanoscale and fine TiO_2 translocated to the liver, kidney spleen, and lungs, and that liver damage occurred in mice administered nanoscale TiO_2 (at the high dose of 5 g/kg). This study suggests the need to investigate possible adverse effects or carcinogenicity in other organs and by other routes of exposure, especially to nanoscale TiO_2.

Biomarkers of exposure

No biomarkers of exposure were identified.

Biomarkers of effect

A number of recent studies have identified markers of inflammation, including granulocyte macrophage colony stimulating factor (GM-CSF) mRNA expression and secretion in a human bronchial epithelial cell line (16HBE14o-) (Hussain et al., 2009) and interleukin (IL)-8 production in a human alveolar epithelial type II cell line (A549), which is a pro-inflammatory cytokine also produced *in vivo* (Duffin et al., 2007). MicroRNA signatures may provide a marker linking inflammation, immune response, and cancer (Hussain and Harris 2007), although this has not been examined specifically in conjunction with particle-elicited inflammation. Markers of oxidative DNA damage (e.g., 8-OHdG) may provide an indication of the particle-elicited oxidative stress. However, none of these markers is specific to TiO_2, nor was it determined how feasible these biomarkers would be for testing in exposed populations.

Research needs and recommendations

Possible cohort for future epidemiologic studies

Epidemiological studies with well-characterized exposures and adequate follow-up are needed, especially for workers producing or using nanoscale TiO_2. Exposure data should include information on particle size, crystal structure, and surface properties. A possible cohort for

epidemiologic studies would include workers in industries using TiO_2, particularly the ultrafine (nanoscale) TiO_2 now used extensively in the cosmetics industry. Workers handling or mixing TiO_2 powders with other ingredients would probably be at the greatest exposure. NIOSH is currently conducting exposure studies of TiO_2 users and identifying possible cohorts.

Toxicology studies:

Experimental studies are needed to elucidate the biological mechanisms between particle-induced inflammation and lung cancer. A study examining the relationship between TiO_2 exposure in workers and validated markers of oxidative stress, with quantitative comparison in rodent studies, could provide data on interpretation of the animal studies for predicting lung cancer risk in humans.

Studies are needed that provide mechanistic linkages between the biological responses observed in short-term or subchronic studies and the adverse health effects observed with chronic exposure. The same species, strain, and gender should be used. For example, different mouse strains were used in Heinrich et al. (1995) and in Bermudez et al. (2004), making it difficult to determine whether the subchronic inflammation results are relevant to the chronic lung responses observed in another mouse strain.

The observation of inhaled discrete nanoscale TiO_2 particles inside rat alveolar epithelial cell organelles including the nucleus (Geiser et al., 2005) suggests that possible direct genotoxic mechanisms for lung cancer should be examined.

Given the increasing applications of nano-TiO_2 in consumer products (e.g., food or food packaging and skin care products), there is a need to develop better techniques to detect TiO_2 in tissues and to examine possible carcinogenicity of nano-TiO_2 by other routes of exposure (oral, dermal). A chronic feeding study of nanoscale TiO_2 may be appropriate.

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