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Potential Harmful Effects of Bulk Titanium Dioxide in Risk and Hazard Assessment

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In 2010, approximately 400,000 tons of titanium dioxide (TiO_2) was produced, used and imported in Korea. TiO_2 is mainly used as a pigment in paints and paint additives and as a white colorant in foods, cosmetics, drugs, sunscreen, textiles, and toothpaste. To control risks and manage the health and safety concerns of TiO_2, the potential risk assessment of TiO_2 in humans was investigated in this article. All available reliable studies that investigated TiO_2 toxicity were collected by searching the literature for relevant papers. For filling the data gap, the reproductive and developmental toxicity of TiO_2 was evaluated using rats. The particle size of TiO_2 assessed in this report was >100 nm (except nano-sized TiO_2). In repeated inhalation toxicity test, the lung lymph node burdens of TiO_2 increased in a concentration-dependent manner and inflammation was noted in bronchoalveolar lavage cells. The tumors observed in rats are thought to be secondary to particle overload in inhalation carcinogenicity studies. There was no indication of any reproductive toxicity in parent animals or developmental toxicity in pups. This study indicated that TiO_2 possesses properties that have potential harmful impact on human health due to repeated dose toxicity and carcinogenicity via inhalation.

Key words: Carcinogenicity, Repeated dose toxicity, Risk assessment, Titanium dioxide

1. Introduction

Titanium dioxide(TiO_2) is used for various purposes including a white pigment, inks, adhesives, synthetic resins, plastic, rubber products and paper products.[1] TiO_2 is mainly used as a pigment in paints and paint additives in Korea. It is also used as a white colorant in foods, cosmetics and drugs, sunscreen, textiles and toothpaste.[2] From 1916 to 2011, 165,050,000 tons of TiO_2 have been produced worldwide.[3] In Korea, the production, use and import volumes of bulk TiO_2 were 63,239, 227,446 and 126,748 tons in 2010, respectively. While the vast amount of TiO_2 materials has been consumed for several decades, there is no sufficient information about TiO_2 to estimate the risk for general population. Therefore, TiO_2 properties and their toxic effects as well as use patterns need to be determined in order to predict their potential risk and influences to humans.

TiO_2 occurs naturally in a crystalline form: rutile, anatase and brookite. Common crystalline forms include rutile, anatase or their mixture.[4] The product is primarily the anatase form. Rutile is chlorinated and the titanium tetrachloride is converted to the rutile form of TiO_2 by vapour-phase oxidation.[5] In this study, the types of TiO_2 were not considered. This article did not also cover the nanoparticle form of TiO_2 as the specific properties of materials above 100 nm.

High concentrations of TiO_2 in food can be exposed to workers when used as a whitener in the manufacture of dairy products such as cheese.[6] In addition, concerns are raised over possible human

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effects of TiO_2 when used as the additive for bread flours and replacing the normally used flour-bleaching agents[7], and the clouding agent for incorporation in dry beverage mixes[8], and in tobacco wrapping.[9] In the occupational environment, exposure mainly occurs during the processing of titanium-containing minerals and TiO_2.[10] The majority of workers are occupationally exposed to TiO_2 dust by inhalation system. For understanding human effects of TiO_2, its concentration and distribution of TiO_2 particles in ambient and occupational environments are important factors and the results of TiO_2 in short- and long-term mammalian studies are essential elements.

Although several data are available about toxic effect and exposure of TiO_2, these studies were almost explored the risks of occupational exposure to TiO_2. Therefore it needs to consider for exposure assessment for consumer exposure to TiO_2 as well as workers.

The aim of this study was to summarize the health effects of TiO_2 associated with oral, skin/eye, respiratory routes of exposure, and to evaluate the risk of TiO_2. To assess the hazards posed by TiO_2, we conducted its fertility and developmental toxicity test of TiO_2 because any experimental studies demonstrating reproductive impacts could not be found.

2. Materials and Methods

2.1. Methodology of data collection

To collect specific and scientific data and information about toxicity and exposure of TiO_2, the study prior to December 2012 was carried out on 5 different topics using search engine PubMed and Google site.

To choose the reliable articles and reports, published and peer-reviewed studies that investigated mammalian toxicity by exposure route and human exposure of TiO_2 were just selected.

1) Oral toxicity data of TiO_2
2) Skin and eye toxicity data of TiO_2
3) Inhalation toxicity data of TiO_2
4) Genotoxicity and carcinogenicity data of TiO_2
5) Use and human exposure data of TiO_2

2.2. Reproductive and developmental toxicity test

This study was performed to evaluate the reproductive/developmental toxicity of TiO_2 by gavage to male and female rats following OECD TG 421: Reproduction/Developmental Toxicity Screening Test.[11] TiO_2 was purchased from Sigma-Aldrich (CAS No.13463-67-7, Product No. 14021). Stock suspensions of TiO_2 were prepared immediately before each experiment. Dose levels were determined based on the information from the result of NOAEL>1,000 mg/kg bw/day from repeated dose studies.[12,13] Sprague-Dawley rats were treated by gavage at doses of 0 (amount of vehicle: 1% methylcellulose solution, 10 mL/kg) or 1,000 mg/kg bw/day (limit test). Males in the main group (10 rats per group) were administered for a minimum total of 4 weeks (for 2 weeks prior to mating, mating period and 2 weeks post mating period), and females in the main group (10 rats per group) were administered for two weeks prior to mating, throughout gestation and until day 4 after delivery. The results of this study included measurements (weighing, food/water consumption) and daily and detailed observations, preferably each day at the same time, as well as gross necropsy and histopathology. In gestation and lactation periods, abortion, premature delivery, dystocia or prolonged parturition, gestation lengths, litter size, delivery index, stillbirths, sex and body weights of pups, runts and viability of pups were observed. The findings of this toxicity study were examined in terms of the observed effects, necropsy and microscopic findings. One-way analysis of variance (ANOVA) was used to estimate overall significance. Data were presented as mean±standard deviation. A probability level less than 5% ($p<0.05$) was considered significant.

3. Results and Discussion

3.1. Oral exposure and toxic effects

Three studies of acute oral toxicity were performed according to OECD TG 420, TG 425 and TG 401.[13,14] Significant increase of titanium concentration in spleen and brain was observed in exposure mice to 5,000 mg/kg. Based on these results, the acute oral LD_{50} value was higher than 2,000 mg/kg bw.

Two studies of repeated oral toxicity were performed according to OECD TG 407.[12] At 250, 500 or 1,000 mg/kg bw/day, treatment related effects were observed such as compound-colored feces, some hematological and clinical chemistry parameters, and liver and thymus weight changes. But these effects did not consider toxic phenomenon by TiO_2. The other study was conducted at 24,000 mg/kg bw/day for 28 days, and no substance related effects were observed.[13] Therefore the oral repeated no-observed-adverse-effect level (NOAEL) for TiO_2 was over 24,000 mg/kg/day, based on the lack of any adverse effects at this dose.

3.2. Skin and eye exposure and toxic effects

ECHA performed acute dermal and irritation/corrosion tests [OECD TG 404 and 405] of TiO_2.[13] In rabbit skin treated with TiO_2, the Draize scores were "0" at all examination points. In all rabbit eyes treated with TiO_2, the conjunctival redness (score of 1 or 2) was observed at the 1- and 24-hour examinations. However, fluorescein stain examinations were negative for corneal injury. Based on these results, TiO_2 is not irritating to the skin and eye.

In the buehler test with TiO_2 in accordance with OECD TG 406, sensitization reactions were not observed for both 24 and 48 hours after the challenge application.[13] Therefore TiO_2 is not a dermal sensitizer.

3.3. Inhalation exposure and toxic effects

In an inhalation toxicokinetic study, about 16% of TiO_2 was retained with a clearance half-life of 51-53 days.[15]

In nose-only exposure for 4 hours with TiO_2 (particle size <3.5 μm was 56%, Mass Median Aerodynamic Diameter (MMAD) 3.2 μm) and TiO_2 (particle size <3.5 μm was 20%, MMAD 7.0 μm) according to OECD TG 403, SD had gross pathology that revealed pale and mottled lungs by test materials.[13] These findings were the phenomena by concentrated TiO_2 in lungs, but were not considered clinical signs.

Fedulov et al. investigated whether inflammatory responses to air pollution particles or TiO_2 particles were enhanced during pregnancy and whether exposure to particles could cause increased neonatal susceptibility to asthma.[16] Pregnant mice were shown to have a more significant level of respiratory sensitization than non-pregnant mice after TiO_2 exposure. Neonates of mothers exposed to TiO_2 showed increased allergic susceptibility, while offspring of mothers exposed to TiO_2 showed increased airway hyper-responsiveness and allergic inflammation. Therefore TiO_2 may have the potential to be a respiratory sensitizer of offspring.

For analyzing repeated dose toxicity of TiO_2, SD rats were exposed to TiO_2 by inhalation (whole body) at nominal concentrations of 0, 10, 50 and 250 mg/m^3 for 6 hours/day, 5 days/week for 24 months.[17] Irregular respiration, abnormal lung noise and stained and/or wet perineum were observed in TiO_2 exposed rats. Mean lung weights were 1.52 to 2.59 fold and 1.53- to 3.38-fold greater for male and female rats, respectively. Mean thymus weights were as high as 1.37-fold greater than controls. Furthermore TiO_2 deposits were observed on skin and the mucosa of the nasal cavity, trachea, bronchus and gastrointestinal tract of rats. The pleural surfaces of the lungs contained scattered white foci, and subpleural cholesterol granulomas that appeared on the lungs were observed in the 50 and 250 mg/m^3 treatment group. The tracheabronchial lymph nodes were markedly swollen and appeared as chalky masses in all exposure groups.

After 2 years of exposure, TiO_2 retention in dried lung was 3.1% at the low dose, 16.9% at the middle dose and 28% at the high dose with a foamy macrophage, Type II pneumocyte hyperplasia, alveolar proteinosis, alveolar bronchiolarisation, and cholesterol granulomas. Based on these findings, the TiO_2 concentration of 10 mg/m^3 is considered as LOAEC for repeated respiratory exposure. In another repeated dose study of TiO_2, lung and lung-associated lymph node of Fischer 344 rats exposed to 0, 10, 50 and 250 mg/m^3 had increased burdens of TiO_2 in a concentration-dependent manner.[18] Pulmonary overload was achieved in rats at 50 and 250 mg/m^3 and inflammatory response remained elevated in 250 mg/m^3-exposure recovery group. Additionally TiO_2-related findings including progressive epithelial and fibro-proliferative changes in pulmonary lesions were observed in 250 mg/m^3.

In summary, the several inhalation studies showed that airborne concentration of 10 mg/m^3 TiO_2 was estimated as the LOAEC based on lung responses as a lymphoid hyperplasia of the lung-associated lymph nodes.

3.4. Genotoxicity and carcinogenicity

Investigations on genotoxicity of TiO_2 applying a bioassay battery were reviewed. In an Ames test [OECD TG 471] with strains of *Salmonella typhimurium*, and *Escherichia coli*, TiO_2 did not induce gene mutations with and without metabolic activation.[13] TiO_2 did not also induce chromosomal aberrations [OECD TG 473] in Chinese Hamster Ovary (CHO) cells with and without metabolic activation[13], but induced micronucluses *in vivo*.[19] And TiO_2 also induced micronucluses in CHO-K1 cells and human lymphocytes *in vitro* study.[20,21] In the hprt gene mutation assay, the hprt mutation frequency was significantly increased in alveolar type II cells of rats after *in vivo* exposure of TiO_2.[22] In summary, some studies showed that TiO_2 is not a genotoxic material, but positive results observed in other studies were thought to be a consequence of oxidative stress mediated DNA damage and revealed potential genotoxic impacts of TiO_2 (Table 1).

There was no evidence of carcinogenicity at any dose levels in oral studies. However, from inhalation studies, the carcinogenic evidences, bronchioloalveolar adenomas, squamous metaplasias, pulmonary keratin cysts and squamous carcinoma cell were observed. In Warheit et al. study, bronchioloalveolar adenomas, squamous metaplasias, pulmonary keratin cysts and squamous cell carcinomas were observed in the TiO_2 treatment group.[17] The tumor findings are considered to result from prolonged inflammation and fibrogenesis due to particle overload of the clearance mechanism of the lung.[18] In addition, TiO_2 is classified by the International Agency for Research on Cancer (IARC) (2010) as group 2B (Possibly carcinogenic to humans).[24]

3.5. Use and human exposure

TiO_2 is the most important titanium compound that is extensively used. It is estimated that over half of all non-permanent white or light-colored sur-

Table 1. Summary of genotoxicity results

Type of genotoxicity	Type of study	Concentration range	Result
Bacterial reverse mutation assay (Ames test)	*Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2uvrA	100-5,000 μg/plate (±S9)	negative[13]
Chromosomal aberration test	CHO cells	125-2,500 μg/mL (±S9)	negative[13]
In vivo micronucleus test	mouse bone marrow cells	0-1,500 mg/kg bw	positive [19]
In vitro micronucleus test	CHO-K1 cells	1-20 μM	positive[20]
	human peripheral blood lymphocytes	1-10 μM	positive[21]
Sister chromatid exchange assay	CHO-K1 cells	1-5 μM	positive[20]
	human peripheral blood lymphocytes	1-10 μM	positive[21]
hprt gene mutation assay	alveolar type II cells in rats	10 and 100 mg/kg bw	positive[22]

face coatings include a TiO_2 levels of 0.1-0.3 kg/L. The application of TiO_2 is growing in the plastics industry, because of its resistance to degradation by ultraviolet light, high refractive index, whiteness, and chemical inertness.[25] In addition, TiO_2 is used for various purposes, including ceramic capacitors, electromechanical transducers, welding-rod coatings, and in the production of glass fibers, floor coverings, mainly of the synthetic resin types, rubber tires, porcelain enamels, inks, wall coverings, artificial leather, oilcloth, upholstery materials, and other coated fabrics.

In several reviews of TiO_2, they concluded that it was acceptable for general food and cosmetics additives. Also its use would not pose any safety concerns[26] as TiO_2 is not absorbed via the gastrointestinal tract or through the skin. However, inhalation exposure to high concentrations of TiO_2 has shown to result in pulmonary carcinogenic effects in rats. By occupational exposure of TiO_2, 35 among 120 workers had fluctuations in hematological parameters and 3 workers had symptoms of early stages of pneumofibrosis.[27]

The industrial use process of TiO_2 as a pigment in a commercial formulation is as follows: TiO_2 powder is blended with other raw materials mixed until well-dispersed. After dilution with suitable solvents the formulation containing TiO_2 is packed into containers. In industry facilities of Korea, TiO_2 is handled in closed systems and is used commercially as a powder. According to monitoring data, TiO_2 has been not detected in the workplace from 2009 to 2011.[1] But the management of workers is an essential factor with personal protective equipment such as a mask with dustproof filter in the workplace.

3.6. Reproductive/developmental toxic effects of TiO_2

While there were no toxic effects by orally dosing TiO_2, we pointed out the remaining knowledge gaps concerning the reproductive/developmental toxicity. No treatment-related changes were observed in clinical signs, body weight, food consumptions, necropsy findings and organ weights of testis and epididymis (Table 2). The tubular cell vacuolation of seminiferous tubule and inflammatory cell foci of male prostate were observed (Table 3). But, these symptoms were not considered test substance-related, since these were also found in the vehicle control group. No treatment-related effect was seen in the TiO_2 groups on the following parameters examined: the mating and gestation length, the number of corpora lutea, implantations and delivery index, body weight of pups found in both sexes on Day 0 and 4 of lactation, and pre- and post-implantation losses (Table 4 and 5). From observation of live pups at birth, there were no externally malformed pups in any groups. At necropsy of pups, no gross finding was observed in any groups (data not shown). Based on the results, NOAEL of TiO_2 is considered to be over 1,000 mg/kg bw/day in both sexes for general toxicity, reproductive capability and F1 neonates.

3.7. Human health risk assessment of TiO_2

Based on findings from various studies on mammals, oral and skin exposure of TiO_2 is not bioavailable to decompose in organ, and any oral acute

Table 2. The organ weights of male rats exposed TiO_2

Dose (mg/kg/day)	0	1,000
Testes (g)	3.33±0.34	3.03±0.36
(% of body weight)	0.77±0.08	0.69±0.08
Epididymides (g)	1.21±0.10	1.14±0.12
(% of body weight)	0.28±0.02	0.26±0.03

Table 3. Summary of microscopic observations in male reproductive systems

Dose (mg/kg/day)	0	1,000
Testes		
Tubular cell vacuolation (minimal)	2/10	2/10
Tubular atrophy (minimal)	0/10	1/10
Epididymides		
Inflammatory cell foci (minimal)	3/10	0/10
Cellular debris (minimal)	0/10	1/10
Oligospermia (minimal)	0/10	1/10
Prostate		
Inflammatory cell foci (minimal)	4/10	4/10

Table 4. Mating and gestation results in rats exposed TiO_2

Dose (mg/kg/day)	No. of females placed with male	No. of females mated	No. of females pregnant	Mating index (%)	Female fertility index (%)	Mating period (days)	Gestation period (days)
0	10	10	10	100	100	4.7±4.8	21.7±0.4
1,000	10	10	10	100	100	3.7±3.1	21.9±0.5

Mating index (%): (No. of females mated / No. of females placed with males) × 100
Male fertility index (%): (No. of males impregnating a female / No. of males placed with females) × 100
Female fertility index (%): (No. of females pregnant / No. of females placed with males) × 100

Table 5. Delivery and pups observations in rats exposed TiO_2

Dose (mg/kg/day)	No. of corpus lutea	No. of implantations	Implantation loss rate (%)	Live birth index (%)	No. of pups born (day 0)	No. of live pups (day 4)	Viability index day 4 (%)	Gestation index (%)
0	15.6±1.9	14.8±1.5	2.7	97.3	13.0±2.6	12.9±2.6	99.2	100
1,000	17.7±3.4	13.4±3.3	0.7	99.3	11.9±4.8	11.9±4.8	100	100

Implantation loss rate (%): ((No. of implantations – No. live neonates) / No. of implantations) × 100
Live birth index (%): (No. of live pups in postnatal day 0 / No. of implantations) × 100
Viability index day 4 (%): (No. of pups surviving on postnatal day 4 / No. of pups born alive in postnatal day 0) × 100
Gestation index (%): (No. of females with live pups / No. of pregnant females) × 100

toxic effect is not observed. No signs of irritation and sensitization toxicity were reported in animals treated TiO_2. There are no effect related TiO_2 exposure in reproductive toxicity in parent animals and developmental toxicity in pups.

However inhalational exposure of TiO_2 caused titanium concentrations to be concentrated in lung and lymph node.[13] TiO_2 has clear potential for absorption by mammals after breathing, as well as for storage in other organs where it can cause tissue damage and alter biochemical parameters. These properties, however, are most likely dependent on the concentration and exposure route of TiO_2. In acute exposure, TiO_2 burdens brought about bottled and pale lung and play as a potential respiratory sensitizer in pregnant mother and offspring by inhaled treatment.[13,14] Based on these literatures, the TiO_2 exposure is considered to affect young and pregnant people, and furthermore can have severe effects on old people not having perfect immune system. Although several experts said TiO_2 is a safe additive for consumers, the weak and the vulnerable need to be protected from nose route exposure.

The Occupational Safety and Health Administration (OSHA) suggested the permissible exposure limit for TiO_2 as 15 mg/m^3 and the National Institute for Occupational Safety and Health (NIOSH) recommended that TiO_2 be classified as a potential occupational carcinogen and that exposures be controlled as low as feasible in USA. These recommendations were based on the observation of lung tumors in a chronic inhalation study in rats at 250 mg/m^3 of TiO_2[27] Later, several inhalation studies showed a statistically significant increase in lung cancer in rats exposed to 10 mg/m^3 of TiO_2.[19] Before 2 years-exposure, pulmonary inflammation responses appeared and then that effect aggravated lung cancer in rats. Some epidemiologic studies have not found a relationship between exposure to total or respirable TiO_2 and lung cancer[29-31], but IARC reviewed relevant literatures on TiO_2 and concluded that there was sufficient evidence of carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans (Group 2B), "possibly carcinogenic to humans".[24] Therefore, on the basis of many studies and the pattern of pulmonary inflammatory responses, TiO_2 has been determined a potential occupational carcinogen.

In Korean's industrial places, TiO_2 is under con-

trol in accordance with international occupational exposure limit as TWA 10 mg/m^3. Fortunately the occupational external exposure is managed by filter facilities and TiO_2 has been not detected in the workplace from 2009 to 2011.[1)] Therefore occupational exposure is considered to be negligible in tightly sealed and well-constructed facilities.

However, the concern of occupational respiratory exposure remains in many small factories. Several factories have no capacity to equip with adequate facilities for protecting process of workers. A pneumoconiosis case by TiO_2 accompanied by lung cancer is reported for humans engaged in packing TiO_2 for about thirteen years.[32)] In that case, workers should understand the potentially recognized risk to their health, with training for proper work procedures and personal protective equipment of filtering dust, fume and mist of TiO_2.

The color additive TiO_2 for food may be safely used and US FDA suggested that the quantity of dose not exceed 1 % by weight of the food.[26)] But, increasing use of spray products containing TiO_2 could be harmful to infant and young children. No consumer inhalation exposure limit is yet existed. When the spray type goods are used, the parents should protect their children from exposure to TiO_2. Further work should focus on consumer respiratory exposure and effects of these nanoparticles, since these data are still largely lacking.

4. Conclusion

As sufficient data are available to assess the risks of inhalational exposure to TiO_2, there are concerns to consumer pulmonary exposure. And the workers in facilities producing or using TiO_2 needs to protect its particle respiratory exposure. TiO_2 can be a respiratory sensitizer and pulmonary effecter or carcinogen in high exposure-response relationship. Therefore the need for properly using personal protective equipment, and workplaces have to be managed under control in accordance with the occupational safety and health acts.

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