

## Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration

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### Abstract

In order to evaluate the toxicity of TiO<sub>2</sub> particles, the acute toxicity of nano-sized TiO<sub>2</sub> particles (25 and 80 nm) on adult mice was investigated compared with fine TiO<sub>2</sub> particles (155 nm). Due to the low toxicity, a fixed large dose of 5 g/kg body weight of TiO<sub>2</sub> suspensions was administrated by a single oral gavage according to the OECD procedure. In 2 weeks, TiO<sub>2</sub> particles showed no obvious acute toxicity. However, the female mice showed high coefficients of liver in the nano-sized (25 and 80 nm) groups. The changes of serum biochemical parameters (ALT/AST, LDH) and pathology (hydropic degeneration around the central vein and the spotty necrosis of hepatocytes) of liver indicated that the hepatic injury was induced after exposure to mass different-sized TiO<sub>2</sub> particles. In addition, the nephrotoxicity like increased BUN level and pathology change of kidneys was also observed in the experimental groups. The significant change of serum LDH and alpha-HBDH in 25 and 80 nm groups showed the myocardial damage compared with the control group. However, there are no abnormal pathology changes in the heart, lung, testicle (ovary), and spleen tissues. Biodistribution experiment showed that TiO<sub>2</sub> mainly retained in the liver, spleen, kidneys, and lung tissues, which indicated that TiO<sub>2</sub> particles could be transported to other tissues and organs after uptake by gastrointestinal tract.

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**Keywords:** Acute toxicity; Fixed dose procedure; Titanium dioxide; Nanoparticles; Mice

### 1. Introduction

Titanium dioxide (TiO<sub>2</sub>), a noncombustible and odorless white powder, naturally exists in anatase, rutile and brookite. It is frequently used as a white pigment for a wide range of paints, paper, plastics, ceramics, and the like. TiO<sub>2</sub> becomes transparent at the nanoscale (particle size <100 nm), is able to absorb and reflect UV

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light, and has been used in sunscreens. The US Food and Drug Administration (FDA) established a regulation for TiO<sub>2</sub> as the color additive for food (FDA, 2002). Nowadays, nano-sized TiO<sub>2</sub> is produced abundantly and used widely because of its high stability, anticorrosion and photocatalysis.

More and more nanoparticles are entering into the environment with the increasing development of nanotechnology. The small size and large surface area endow them with an active group or intrinsic toxicity. The impacts of nanoparticles on human and the environment have been put forward recently by some scientists and organizations (RS & RAE, 2004; Service, 2003; Warheit, 2004). The acute toxic effects of nano-copper particles and nano-zinc powder in healthy adult mice have been accomplished in our laboratory (Chen et al., 2006; Wang et al., 2006). We found that the mice showed color change in both spleen and kidney as well as atrophy of spleen after ingesting nano-copper particles (Chen et al., 2006) and the mice treated by nano-zinc suspensions showed the symptoms of lethargy, nausea, vomiting and diarrhea at the beginning days, but the mice treated by micro-zinc were not (Wang et al., 2006). Nano-sized TiO<sub>2</sub>, for example, can produce free radicals (i.e., reactive species of molecules) and exert a strong oxidizing ability. Dunford (Dunford et al., 1995) reported that sunlight-illuminated TiO<sub>2</sub> catalyses DNA damage both *in vitro* and in human cells. Others have proposed that in some conditions, nanoscale TiO<sub>2</sub> could be used to fight cancer or even anthrax (Cai et al., 1992; Xiong et al., 2003).

Concerning TiO<sub>2</sub> toxicity, the analysis of bronchoalveolar lavage (BAL) fluid and histopathological changes for lung responses had been reported using bronchial instillation and inhalation methods in mice, rats, and hamsters (Bermudez et al., 2002, 2004; Driscoll et al., 1990a,b; Henderson et al., 1995; Warheit et al., 2005). Increased numbers of neutrophils and phagocytes in BAL fluid and the deposition of particles in lung were observed after intratracheal instillation of TiO<sub>2</sub> in rats and hamsters. Acute pulmonary toxicity of ultrafine particles (Teflon, carbon black, TiO<sub>2</sub>, Pt, iron oxide, etc.) revealed rapid translocation of ultrafine Teflon particles across the epithelium after their deposition (Oberdörster et al., 2000). Pulmonary inflammatory response was found only for 20 nm TiO<sub>2</sub>, but not for 250 nm TiO<sub>2</sub> particles.

The use of nano-sized TiO<sub>2</sub> is still prevalent, though it has the pulmonary toxicity after intratracheal inhalation/instillation into the organism. People seek to harness this photo-reactive property, including solar cell research, water cleanup techniques, and even self-cleaning windows that can automatically remove dirt

under natural UV light. ETC Group in Canada stressed that, although a moratorium is the only responsible avenue opened at this time, it need not be long-lasting (ETC, 2003).

Uptake of engineered nanoparticles into human body has several different routes. A potential exposure route for general population is the oral ingestion because TiO<sub>2</sub> is used as a food additive in toothpaste, capsule, cachou, and so on. The quantity of titanium dioxide does not exceed 1% by weight of the food according to the Federal Regulations of US Government. Until now, most studies on the toxicity of TiO<sub>2</sub> particles in mammals were focused on the pulmonary impact of inhaled TiO<sub>2</sub> nanoparticles or dermal exposure, but no available work has been undertaken on the impacts of oral exposure of TiO<sub>2</sub> and neither on its quantitative distribution *in vivo*.

Thus, in present paper, the purposes of testing for acute oral TiO<sub>2</sub> toxicity are to obtain information on the biological response of a chemical and to gain insight into the targets of its action. The fixed-dose procedure is a more humane method to replace the LD50 in acute toxicity testing, which was first proposed by the British Toxicology Society (BTS, 1984). After an international validation, the procedure was incorporated into the Organization for Economic Co-operation and Development (OECD) Guideline 420 in 1992 (OECD, 1992). It was concluded that the generated data could be used both for risk assessment and for ranking chemicals for classification. In this study, the acute oral toxicity of different sized TiO<sub>2</sub> was evaluated according to the fixed dose procedures (OECD, 1992). Furthermore, the changes of coefficients of tissues to body weight, histopathology, biochemical parameters of blood, and distribution of titanium in tissues were investigated after administration via gastrointestinal tract in mice.

## 2. Materials and methods

### 2.1. Chemicals and preparation

Nano-sized TiO<sub>2</sub> (Hangzhou Dayang Nanotechnology Co. Ltd., 80 and 25 nm) and fine TiO<sub>2</sub> (Zhonglian Chemical Medicine Co.) particles were used in this experiment. The purity was analyzed by X-ray fluorescence technique.

A 0.5% hydroxypropylmethylcellulose K4M (HPMC, K4M) was used as a suspending agent. A 3 g of each TiO<sub>2</sub> powder was dispersed onto the surface of 0.5%, w/v HPMC solution (12 ml), and then the suspending solutions containing TiO<sub>2</sub> particles were treated by ultrasonic for 15–20 min and mechanically vibrated for 2 or 3 min. The sizes of particles were tested using transmission electron microscopy (TEM). The sizes observed by TEM are in coincidence with the nominal sizes (Wang et al., *in press*). The size of fine TiO<sub>2</sub> is 155 ± 33 nm.

## 2.2. Animals and treatment

CD-1 (ICR) mice of 40 female and 40 male ( $19 \pm 2$  g) were purchased from Beijing Vitalriver Experimental Animal Technology Co. Ltd. Animals were housed in stainless steel cages by sex in a ventilated animal room. Room temperature was maintained at  $20 \pm 2$  °C, relative humidity at  $60 \pm 10\%$ , and a 12 h light/dark cycle. Distilled water and sterilized food for mice were available *ad libitum*. They were acclimated to this environment for 5 days prior to dosing. All procedures used in this experiment were compliant with the local ethics committee.

Animals were randomly divided into four groups: control group and three experimental groups (25, 80 nm, and fine groups). Before treatment, animals were fasted over night. After vigorous stirring, TiO<sub>2</sub> suspension (single dose of 5 g/kg body weight) was given to mice by a syringe via gastrointestinal tract in a minute. Control mice were given 0.5% HPMC. Food and water were provided 2 h later.

The symptom and mortality were observed and recorded carefully during the first 24 h. Two female mice treated with fine particles, two female mice treated with 25 nm particles and one female mouse treated with 80 nm particles were spiritless, inactivity and anorectic. Unfortunately, these mice died within 2 days. At the third day, one male mouse in the fine group and one female mouse in the 25 nm group were dead. After death, the mice were sacrificed immediately, a large amount of TiO<sub>2</sub> was found in the front arm and much blood was clogged in thorax. We think the death was not induced by the TiO<sub>2</sub> toxicity, but the ruptured oesophagus during oral administration by mistake. However, no abnormal behavior and symptom were observed in the survivors. Two weeks later, the remaining animals were sacrificed after being anaesthetized by ether. Blood samples were collected from the eye vein by removing the eyeball quickly. Serum was harvested by centrifuging blood at 2500 rpm for 10 min and red cells were kept for analyzing titanium content. The tissues and organs, such as heart, liver, spleen, kidneys, lung, brain, and testicle (ovary), were excised and weighed accurately. We did not observe the ruptured oesophagus or other injury for the remaining mice during the autopsy. A part of tissues and organs were stripped and immediately fixed in a 10% formalin solution for further histopathological diagnosis. The remaining samples were stored at  $-65$  °C for other analysis.

## 2.3. Coefficients of liver, kidneys and spleen

After weighing the body and tissues, the coefficients of liver, kidneys, and spleen to body weight were calculated as the ratio of tissues (wet weight, mg) to body weight (g).

## 2.4. Blood biomarker assay

In the present study, liver function was evaluated with serum levels of total bilirubin levels (TBIL), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Nephrotoxicity was determined by

uric acid (UA), blood urea nitrogen (BUN) and creatinine (Cr). The enzymes of creatine kinase (CK), lactate dehydrogenase (LDH) and alpha-hydroxybutyrate dehydrogenase (HBDH) were assayed for evaluating cardiac damage using a Biochemical Autoanalyzer (Type 7170, Hitachi, Japan).

## 2.5. Histopathological examination

For pathological studies, all histopathological tests were performed using standard laboratory procedures. The tissues were embedded in paraffin blocks, then sliced into 5 μm in thickness and placed onto glass slides. After hematoxylin–eosin (HE) staining, the slides were observed and the photos were taken using optical microscope (Nikon U-III Multi-point Sensor System, USA), and the identity and analysis of the pathology slides were blind to the pathologist.

## 2.6. Titanium content analysis

Tissues were taken out and thawed. About 0.1–0.3 g of each tissue were weighed, digested and analyzed for titanium content. Briefly, prior to elemental analysis, the tissues of interest were digested in nitric acid (ultrapure grade) overnight. After adding 0.5 ml H<sub>2</sub>O<sub>2</sub>, the mixed solutions were heated at about 160 °C using high-pressure reaction container in an oven chamber until the samples were completely digested. Then, the solutions were heated at 120 °C to remove the remaining nitric acid until the solutions were colorless and clear. At last, the remaining solutions were diluted to 3 ml with 2% nitric acid. Inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Electron Co.) was used to analyze the titanium concentration in the samples. Indium of 20 ng/ml was chosen as an internal standard element. The detection limit of titanium was 0.074 ng/ml. Data are expressed as nanograms per gram fresh tissue.

## 2.7. Statistical analysis

Results were expressed as mean  $\pm$  standard deviation (S.D.). Multigroup comparisons of the means were carried out by one-way analysis of variance (ANOVA) test. Dunnett's test was used to compare the differences between the experimental groups and the control group. Student's *t*-test was used to compare the means of each nano-group and the corresponding fine group. The statistical significance for all tests was set at  $p < 0.05$ .

# 3. Results

## 3.1. Purity of nanoparticles

The nominal purity of TiO<sub>2</sub> powder is >92%. The sodium and chlorine contents are both below 0.001%. X-ray fluorescence analysis (XRF) was used to check up the purity of TiO<sub>2</sub>. A molybdenum X-ray tube was

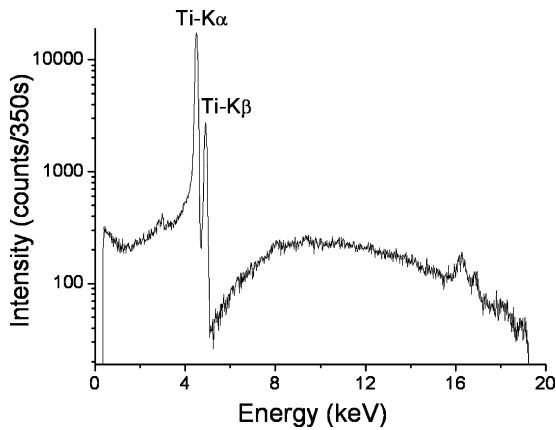


Fig. 1. The XRF spectrum of 25 nm TiO<sub>2</sub> particles.

used to excite samples and the FWHM of the Si(Li) detector for Mn K $\alpha$  peak was 146 eV. The pulse signal of each element's characteristic X-ray emitted from the TiO<sub>2</sub> sample and the Compton scattering are acquired by the Si(Li) detector, and a XRF spectrum is obtained by a multichannel pulse amplitude analyzer which converts the pulse signal to the total counts corresponding to the X-ray energy in each channel. As shown in Fig. 1, only the titanium peaks were observed without any other element peaks in the XRF spectrum of 25 nm TiO<sub>2</sub> particles. The other sized TiO<sub>2</sub> particles give the similar spectra (data not shown). The results show that the purity of different sized particles is better than 99%, which is the same as indicated by businessman.

### 3.2. Coefficients of liver, spleen and kidneys

After 2 weeks, the mice were sacrificed and the weight of body and various tissues/organs were col-

lected. Table 1 shows the coefficients of liver, spleen, and kidneys to body weight which are expressed as milligrams (wet weight of tissues)/g (body weight).

No obvious differences were found in the body weight of four groups. The significant differences were not observed in the coefficients of liver, spleen, and kidneys of the male mice. But, for the female mice, the coefficients of liver in the 80 and 25 nm groups are significantly higher ( $p < 0.05$ ) than the control and fine groups. The increased coefficients indicate that the inflammation might be induced in the female mice after ingestion of TiO<sub>2</sub> particles, which is confirmed by the further morphological examination of liver. There are no significant changes in the coefficients of spleen and kidneys.

### 3.3. Biochemical parameters in serum

In the male mice, there were slightly elevated ALT/AST ratios after exposure to different sized TiO<sub>2</sub> particles (data not shown). The higher serum BUN and CR levels were found in the male mice exposed to the 80 and 25 nm TiO<sub>2</sub> particles.

Table 2 shows the changes of biochemical parameters in the serum of female mice induced by TiO<sub>2</sub> particles. In the female mice, there are not significant changes for the enzymes of AST and ALP ( $p > 0.05$ ) after oral administration of different sized TiO<sub>2</sub>. However, the ALT level in the three experimental groups increased. And in the 25 nm group, the ratio of ALT/AST increased significantly ( $p < 0.05$ ) comparing with the control group.

It is well known that both the LDH and alpha-HBDH are often used as the markers of cardiovascular damage. Their elevated levels indicate that the heart function might be injured after exposure to TiO<sub>2</sub> particles. Nano-

Table 1  
Coefficients of liver, spleen, and kidneys after oral exposure to TiO<sub>2</sub> particles

Groups	Body weight (g)		Liver (mg/g)	Spleen (mg/g)	Kidneys (mg/g)
	Before	After			
<b>Male</b>					
Control ( $n = 10$ )	20.2 $\pm$ 0.8	26.4 $\pm$ 2.0	45.5 $\pm$ 2.3	2.21 $\pm$ 0.42	19.6 $\pm$ 1.5
25 nm ( $n = 10$ )	20.2 $\pm$ 1.3	26.5 $\pm$ 2.5	44.6 $\pm$ 3.1	2.29 $\pm$ 0.25	18.4 $\pm$ 1.2
80 nm ( $n = 10$ )	21.0 $\pm$ 1.4	26.3 $\pm$ 1.2	44.3 $\pm$ 1.8	2.54 $\pm$ 0.70	18.9 $\pm$ 2.2
Fine ( $n = 9$ )	19.6 $\pm$ 1.6	27.6 $\pm$ 2.0	44.2 $\pm$ 4.0	2.30 $\pm$ 0.29	18.5 $\pm$ 1.0
<b>Female</b>					
Control ( $n = 10$ )	20.1 $\pm$ 1.3	27.4 $\pm$ 2.3	48.1 $\pm$ 2.9	3.54 $\pm$ 0.66	13.5 $\pm$ 0.9
25 nm ( $n = 7$ )	20.6 $\pm$ 1.1	26.7 $\pm$ 2.4	52.4 $\pm$ 1.7 <sup>*,+</sup>	3.10 $\pm$ 0.62	14.0 $\pm$ 0.9
80 nm ( $n = 9$ )	20.1 $\pm$ 0.8	26.8 $\pm$ 1.5	54.5 $\pm$ 3.6 <sup>*,+</sup>	3.95 $\pm$ 1.17	14.5 $\pm$ 1.5
Fine ( $n = 8$ )	19.1 $\pm$ 0.7	25.9 $\pm$ 1.6	49.1 $\pm$ 3.1	3.59 $\pm$ 0.72	13.6 $\pm$ 0.9

\* Represents significant difference from the control group (Dunnett's,  $p < 0.05$ ).

+ Represents significant difference from the fine group (Student's,  $p < 0.05$ ).

Table 2  
Changes of biochemical parameters in the serum of mice induced by TiO<sub>2</sub> particles

Groups	TBIL (μmol/L)	ALT (U/L)	AST (U/L)	ALT/AST	ALP (U/L)	UA (μmol/L)	Cr (μmol/L)	BUN (mmol/L)	CK (U/L)	LDH (U/L)	Alpha-HBDH (U/L)
Female											
Control (n = 10)	0.8 ± 0.3	15.9 ± 4.9	78.2 ± 25.2	0.21 ± 0.05	76.3 ± 18.0	137.0 ± 35.7	36.0 ± 7.3	5.1 ± 0.8	508 ± 219	482.5 ± 159.6	213.2 ± 76.0
25 nm (n = 7)	0.8 ± 0.2	21.3 ± 2.2*	76.0 ± 12.5	0.28 ± 0.03*	83.5 ± 16.3	150.3 ± 21.4	37.8 ± 3.2	6.42 ± 0.65**+	517 ± 140	650.0 ± 116.2*	280.5 ± 43.8*
80 nm (n = 9)	0.7 ± 0.3+	16.6 ± 5.2	85.4 ± 16.5	0.20 ± 0.06	71.1 ± 22.2	152.1 ± 52.5	35.5 ± 3.5	5.48 ± 0.86	605 ± 242	881.5 ± 240.7**+	385.9 ± 130.1**+
Fine (n = 8)	1.0 ± 0.2	18.4 ± 3.7	77.9 ± 12.8	0.25 ± 0.04	73.9 ± 13.2	134.3 ± 35.1	36.3 ± 4.9	5.5 ± 0.9	522 ± 112	512.6 ± 154.2	217.8 ± 52.1

\* Represents significant difference from the control group (Dunnett's,  $p < 0.05$ ).

+ Represents significant difference from the fine group (Student's,  $p < 0.05$ ).

sized TiO<sub>2</sub> particles induce more severe myocardial damage than fine particles in this experiment. The serum LDH and alpha-HBDH enzymes of the 80-nm group not only are statistically higher ( $p < 0.05$ ) than the control group, but also higher ( $p < 0.05$ ) than the fine group. Similarly, the 25 nm TiO<sub>2</sub> particles induce the slightly higher LDH and alpha-HBDH levels compared with the control and fine groups.

Although there is no statistically differences in the experimental groups, the serum BUN and CK showed increased levels compared with the control group. Nevertheless, the 25 nm TiO<sub>2</sub> particles induced the significantly higher ( $p < 0.05$ ) BUN levels than the control.

### 3.4. Histopathological evaluation

The histological photomicrographs of the brain, kidneys, liver, and stomach sections are shown in Figs. 2–5. Only the photographs of female mice are shown because both female and male mice show the same pathological changes. The mice had a slight brain lesion associated with exposure to TiO<sub>2</sub> particles. The vacuoles were observed in the neurons of hippocampus and their number was increased in the 80 nm and fine groups compared with the control mice, which indicated the fatty degeneration induced in the hippocampus of brain tissue (Fig. 2). However, the vacuoles are not typical in the 80 nm group. This symptom did not appear in the mice exposed to 25 nm TiO<sub>2</sub> nanoparticles.

The histopathological changes of kidneys in the female mice are shown in Fig. 3. In the 80 nm group, the renal tubule was filled with the proteinic liquids. In addition, the serious swelling in the renal glomerulus was observed in the group treated with fine particles.

In liver tissue, the hydropic degeneration around the central vein was prominent and the spotty necrosis of hepatocyte was also found in the female mice post-exposure 2 weeks to the 80 nm and fine TiO<sub>2</sub> particles (Fig. 4). There were some inflammation cells in the chorion layer of stomach in the mice of the 80 nm group (Fig. 5), which could be ascribed to the overload of particles in the stomach after a single oral administration of mass TiO<sub>2</sub> particles. In one of the control mice, the inflammation cells in the mucosa layer of stomach were also observed (Fig. 5A), but it was not of representative. Maybe this effect was induced by the immune self-deficient of this mouse.

In addition, there are no abnormal pathology changes in the heart, lung, testicle (ovary), and spleen tissues. To our surprise, no significant histopathological change was observed in any tissues of mice exposed to the 25 nm TiO<sub>2</sub> particles compared with the control mice. The rea-

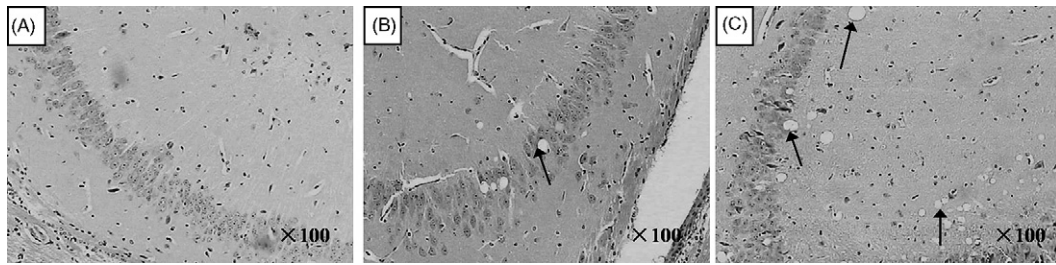


Fig. 2. Histopathology of the brain tissue (100 $\times$ ) in female mice 2 weeks post-exposure to different sized TiO<sub>2</sub> particles by a single oral administration of control group (only exposure to 0.5% HPMC) (A), 80 nm group (B), and fine group (C). Arrows indicate the fatty degeneration of hippocampus in the brain tissue.

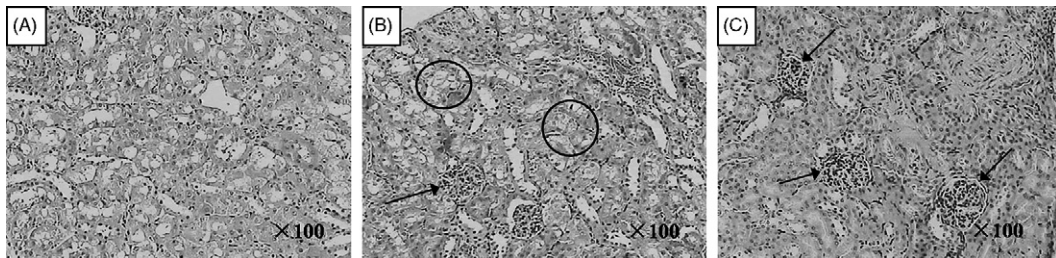


Fig. 3. Histopathology of the kidneys tissue (100 $\times$ ) in female mice 2 weeks post-exposure to different sized TiO<sub>2</sub> particles by a single oral administration of control group (only exposure to 0.5% HPMC) (A), 80 nm group (B), and fine group (C). Circles indicate the proteinic liquid in the renal tubule; arrows indicate the swelling in the renal glomerulus.

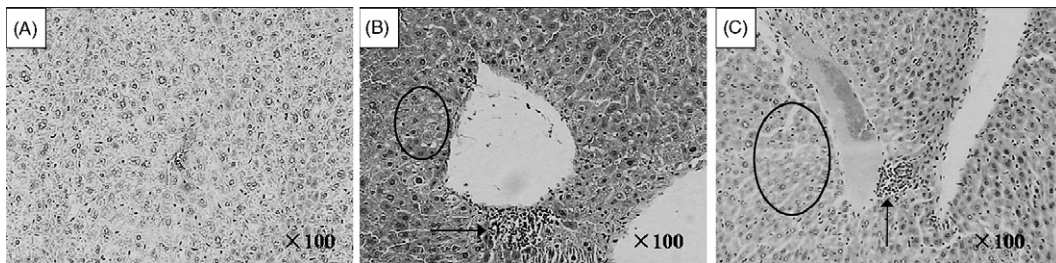


Fig. 4. Histopathology of the liver tissue (100 $\times$ ) in female mice 2 weeks post-exposure to different sized TiO<sub>2</sub> particles by a single oral administration of control group (only exposure to 0.5% HPMC) (A), 80 nm group (B), and fine group (C). Circles indicate the hydropic degeneration around the central vein; Arrows indicate the spotty necrosis of hepatocytes.

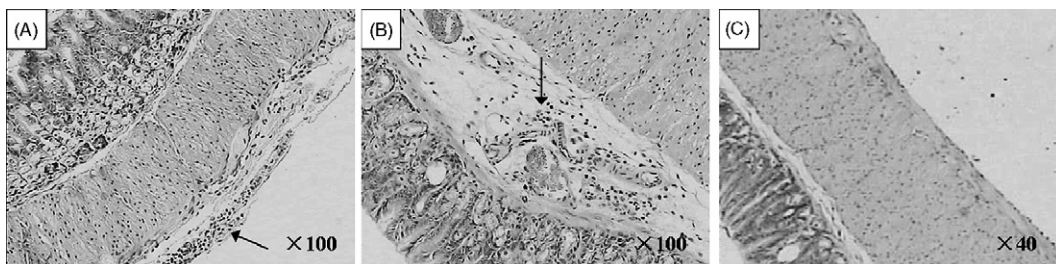


Fig. 5. Histopathology of the stomach tissue (100 $\times$  for A and B; 40 $\times$  for C) in female mice 2 weeks post-exposure to different sized TiO<sub>2</sub> particles by a single oral administration of control group (only exposure to 0.5% HPMC) (A), 80 nm group (B), and fine group (C). Arrows indicate the inflammation cells.

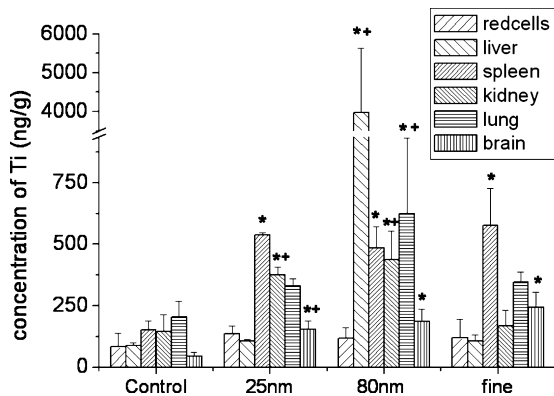


Fig. 6. The contents of titanium in each tissue of female mice 2 weeks post-exposure to different sized  $\text{TiO}_2$  particles by a single oral administration. \*Represents significant difference from the control group (Dunnett's,  $p < 0.05$ ), and +represents significant difference from the fine group (Student's,  $p < 0.05$ ).

son was not clear and the detailed mechanism would be investigated further.

### 3.5. Titanium content analysis

The contents of titanium in each tissue of female mice 2 weeks post-exposure to different sized  $\text{TiO}_2$  particles by oral are shown in Fig. 6. In the experimental

groups, the titanium was mainly accumulated in the liver, kidneys, spleen, and lung. In the red cells, there are slight increases of titanium content in the experimental groups, but no significant difference was found between the experimental groups and the control group (Fig. 7A). Titanium is mainly accumulated in the liver tissue ( $3970.4 \pm 1670.1$  ng/g) of the 80 nm group, whereas the titanium content is  $106.3 \pm 7.8$  ng/g in the 25 nm group and  $106.7 \pm 25.1$  ng/g in the fine group, respectively (Fig. 7B). In the kidneys, the Ti concentrations in the 80 and 25 nm  $\text{TiO}_2$  group are significantly higher than those in the control and fine groups ( $p < 0.05$ ) (Fig. 7C). This phenomenon showed that  $\text{TiO}_2$  particles were entrapped in the reticuloendothelial system and excreted by kidneys *in vivo*.

## 4. Discussion

Titanium dioxide is an inert and poorly soluble matter. In 1969, WHO (1969) reported that the  $\text{LD}_{50}$  of  $\text{TiO}_2$  for rats is larger than 12,000 mg/kg body weight after oral administration. Ferin et al. (1992) reported that the ultrafine  $\text{TiO}_2$  particles (20 nm) penetrated more easily into the pulmonary interstitial space than the fine particles (250 nm) at equivalent masses. Therefore, in the present study, the different sized  $\text{TiO}_2$  particles (25, 80

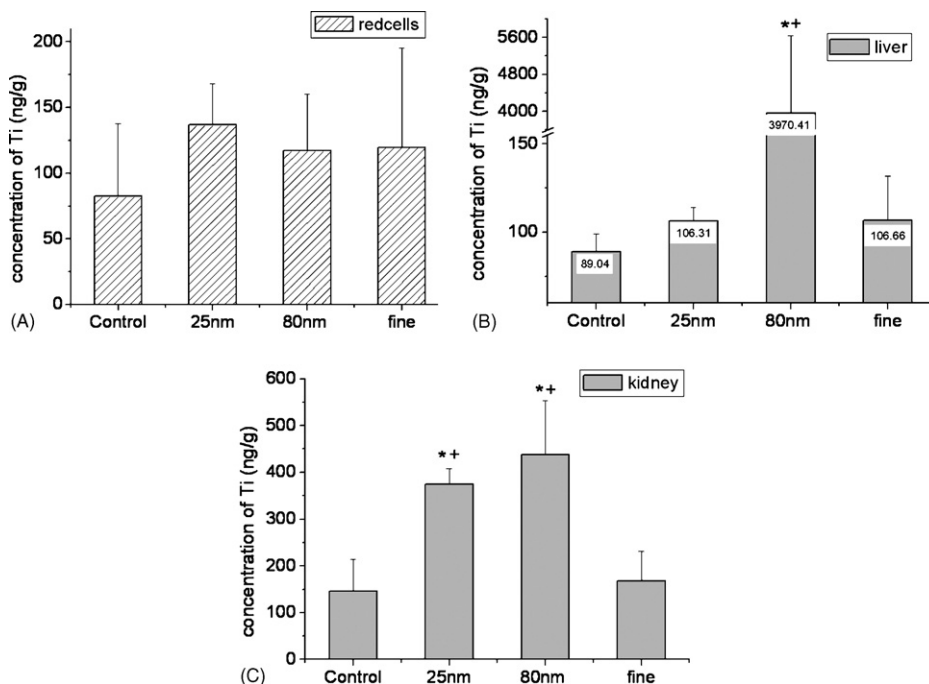


Fig. 7. The contents of titanium in red cells (A), liver (B) and kidney (C) of female mice 2 weeks post-exposure to different sized  $\text{TiO}_2$  particles by a single oral administration. \*Represents significant difference from the control group (Dunnett's,  $p < 0.05$ ), and +represents significant difference from the fine group (Student's,  $p < 0.05$ ).

and 155 nm) were used to evaluate the acute toxic effect on adult mice by oral administration.

Epidemiology researches have reported that TiO<sub>2</sub> is low toxicity and shows no carcinogenic effect and/or nonmalignant respiratory disease for human (Boffetta et al., 2004; Chen and Fayerweather, 1988). But, recently, Baan's working group of the International Agency for Research on Cancer (IARC) classified pigment-grade titanium dioxide as possibly carcinogenic to human beings (group 2B, Baan et al., 2006). In our acute toxicity research of 25, 80 nm and fine TiO<sub>2</sub> particles, abnormal activities were not observed in these mice, and there were no cancer and carcinogenic symptoms after autopsy because of the short exposure time. Although the levels of AST, ALT, and TBIL enzymes for liver function were not changed much, the ratio of ALT/AST, a more sensitive indicator for hepatic injury, was increased after oral ingestion of TiO<sub>2</sub> particles. The increased liver weight and the hepatocyte necrosis in the pathological examination also warranted liver injury because of the intervention of TiO<sub>2</sub> particles. The result of titanium content indicated that it was mainly accumulated in liver of mice treated with the 80 nm TiO<sub>2</sub> particles. Previously, some papers reported that the retention half-time of TiO<sub>2</sub> particles *in vivo* was long because of its difficult excretion. Oberdörster et al. (1994) reported that the retention half-times of TiO<sub>2</sub> in rat lung were 117 days for fine particles (250 nm) and 541 days for ultrafine particles (20 nm). After intravenous injection of rats with 250 mg/kg of TiO<sub>2</sub> (size, 0.2–0.4 μm), about 69% of the injected TiO<sub>2</sub> at 5 min and 80% at 15 min were accumulated in the liver (Huggins and Froehlich, 1966). In this experiment, after oral ingestion of massive TiO<sub>2</sub> particles once, the difficult clearance of 80 nm TiO<sub>2</sub> *in vivo* may directly result in the particle deposition in the liver and lead to the hepatic lesion. Surprisingly, the 25 nm TiO<sub>2</sub>, the same as the fine particles, do not retain in the liver. It is mainly accumulated in the spleen, kidneys, and lung tissues.

There is no significant difference in the kidneys' coefficients between the control and experimental groups. But the kidneys dysfunction was found in the treated mice because of the high serum BUN and CR levels and the serious pathological change of kidneys. Generally speaking, serum BUN was excreted out through the renal glomerulus by the blood transportation. In this study, the renal glomerulus swelled and the renal tubule was filled with the proteinic liquid because of the retained TiO<sub>2</sub> particles, which led to the high BUN concentration in the serum and the serious pathological change of kidneys. International Programme on Chemical Safety

(IPCS, 1982) had showed that most ingested titanium was excreted with urine and unabsorbed by the organism. Because of the small size and difficult clearance of TiO<sub>2</sub>, we found that the retention of different sized TiO<sub>2</sub> particles *in vivo* induced the damage of liver and kidneys after exposure to 5 g/kg TiO<sub>2</sub> particles by a single oral gavage. Similarly, after respiratory exposure to ultrafine TiO<sub>2</sub> aerosols (0.8 μm, 10 mg/m<sup>3</sup>) for 1 year, the rats exhibited significantly increased lung weights compared with clean-air control animals (Heinrich et al., 1995). The body weights decreased and life-time were shortened because of the particles retention *in vivo*. In the same way, the overload of TiO<sub>2</sub> could induce the inflammation, pulmonary epithelial proliferation and fibroproliferative lung lesion after inhalation or instillation of TiO<sub>2</sub> particles (Bermudez et al., 2002; Warheit et al., 1996).

The liver, as a main detoxification tissue, is activated to eliminate the side effects induced by the mass ingested TiO<sub>2</sub> particles. And part of these particles should be excreted out by the kidneys. However, the small size and difficult clearance of 25 and 80 nm TiO<sub>2</sub> particles resulted in the long-time retention of nanoparticles *in vivo* and induced the damage of liver and kidneys after oral exposure to 5 g/kg TiO<sub>2</sub> particles.

LDH test in serum is often used to detect tissue alterations and diagnose heart attack, anemia, and liver diseases. Generally, high LDH level shows the myocardial lesion when combined with CK and alpha-HBDH, and the hepatocellular damage are expressed when combined with AST and ALT enzymes. In the 25 and 80-nm groups, the high LDH and alpha-HBDH enzymes implied that 25 and 80 nm particles resulted in more myocardial lesion than fine particles though the pathological change of heart was not observed. The particulate matter exposures (PM10 and PM2.5) had an impact on the LDH level in BAL fluid (Gerlofs-Nijland et al., 2005). In the study of pulmonary responses to pigment-TiO<sub>2</sub> particles, researchers also found that the increased LDH level in BAL fluid of rats after subchronic and long-term inhalation of TiO<sub>2</sub> particles (Bermudez et al., 2002; Warheit et al., 1996). It is to say that the inhaled particulate matter increased the hypoxia of cells in the lung tissues, which resulted in the high LDH level in BAL fluid. In this study, the overload of nano-sized TiO<sub>2</sub> particles *in vivo* induced the hypoxia in the liver and heart and the over-produced LDH leaked into the serum of blood in treated animals. Additionally, some papers reported that particles could induce cardiovascular disease and impact autonomic nervous system after inhaling much aerosol particles (Donaldson et al., 2001; Neas, 2000; Peters et al., 2006).

The toxicity induced by particles is not only correlated with the sizes but the shapes (Oberdörster et al., 1994; Yamamoto et al., 2004). Yamamoto et al. showed that dendritic and spindle TiO<sub>2</sub> particles had a higher cytotoxicity than spheric particles. In previous study, we found that the 25 and 80 nm TiO<sub>2</sub> particles were column/spindle shape, whereas the phase of fine TiO<sub>2</sub> was octahedral by transmission electron microscope (Wang et al., in press). The 80 nm TiO<sub>2</sub> particles showed the more serious damage than the fine ones, which was consistent with the previous published results (Yamamoto et al., 2004), whereas, the 25 nm TiO<sub>2</sub> particles was not.

However, because of the large surface area of nanoparticles, researchers (RS & RAE, 2004; Tran et al., 2000) stated that the surface area of the particles appears to be a better dose determinant. The previous work with two poorly soluble dusts with different specific surface areas has shown that the particles toxicity was highly relevant to ultrafine particles because at a relatively low mass ultrafine particles have a high surface area (Tran et al., 2000). Whereas, Warheit et al. (2006) showed that exposures to nanoscale TiO<sub>2</sub> rods and dots produced transient inflammatory and cell injury effects in the rats at 24 h and were not different from the pulmonary effects of larger-sized TiO<sub>2</sub> particles exposures. Similarly, in this study, we did not observe the significant difference or trend on the size-dependent toxic effects for TiO<sub>2</sub> particles. However, the gender-dependent effects were obvious after exposure to TiO<sub>2</sub> particles.

## 5. Conclusion

In our experiment, the acute toxicity of 25, 80 nm and fine TiO<sub>2</sub> particles was investigated according to the standard procedure (OECD Guidelines, No. 420) for testing the chemicals. No obvious acute toxicity was observed after a single oral exposure to 5 g/kg TiO<sub>2</sub> particles. However, the female mice showed higher coefficients of liver in the nano-sized (25 and 80 nm) groups than the fine group. From the changes of biochemical parameters (ALT/AST, BUN, and LDH), we demonstrated that TiO<sub>2</sub> particles induced the significant lesions of liver and kidneys in female mice. TiO<sub>2</sub> particles mainly retained in liver, kidneys, spleen, and lung by determining the titanium content using ICP-MS, The obvious hepatic injury (hydropic degeneration around the central vein and the spotty necrosis of hepatocyte) and renal lesion (proteinic liquids in the renal tubule and swelling in the renal glomerulus) were observed in the histopathological examination.

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