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Original Article

Repeated-dose 28-day dermal toxicity study of TiO_2 catalyst (GST) in Sprague-Dawley rats

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Abstract

TiO_2 have been studied on inhalation and skin exposure due to the properties of the materials' use (cosmetics, paints and other products) and the additional safety information on other intake routes for the potential risk assessment is limited. The aim of this study was to obtain dose-range for subchronic study (repeated 90-day dermal toxicity) new TiO_2 powder, GST produced through sludge recycling of the sewage treatment plant through repeated-dose toxicity in Sprague-Dawley (SD) rats. Three test groups for the GST were administered at 500, 1000, 2000 mg/kg B.W/day in addition to a control group (distilled water for injection). 5 male and 5 female rats were included in each group, and we examined the clinical signs, body weights, food consumption, necropsy (organ weights, macroscopic findings), hematological / biochemical parameters and histopathological findings (eye, skin). As a result of observations, there were no treatment-related effects including clinical signs, mortality, necropsy findings etc. Therefore, the present results suggest that the TiO_2-related effects were not observed for dermal during 28-day and dose selection for repeated 90-day study was considered to be 500, 1000 and 2000 mg/kg B.W/day under the present study conditions.

Keywords: TiO_2, repeated-dose toxicity, NOAEL, GST

Introduction

Currently, TiO_2 (Titanium Dioxide) one of the most frequently used nanomaterials and has been used commercially in cosmetics and skin care products, paints, plastics, paper, toothpicks and other prod [1]. Nanoscale TiO_2 such as photocatalyst represents less than 2% of total consumption and presents physical properties and TiO_2 photocatalyst has been used in field of dye-sensitized solar cells and UV protection agents [2]. One of the main differences between TiO_2 nanoparticles (NPs) and conventional TiO_2 is the much greater surface area of a given mass or volume of nanoparticles compared to an equivalent mass or volume of conventional TiO_2 particles. This greater relative surface area of the TiO_2 NPs affords a greater potential for properties such as catalytic activity and UV absorption at certain wavelengths. Such properties have led to the development or use of TiO_2 NPs for a wide variety of applications, and also due to changes of dimension, TiO_2 NPs may show different biological, chemical, optical, magnetic and structural properties and may induce differential toxicity [3]. Recently, the commercialization of TiO_2 has caused an increase of exposure to human and four main routes of exposure are known for oral / dermal exposures, pulmonary absorption and injection are known for one of the most common forms of route to human and it may translate to systemic organs from the lung and gastrointestinal tract (GIT) [4,5]. The possible biological and safety effects of TiO_2 NPs for dermal exposure and absorption have not been well studied and more investigations on the potential health hazards of the TiO_2 nanoparticles are needed [6]. The exposure can be incidental or intentional. One of the possible effects of chemical substances with human exposures is eye and skin irritation. Especially, the skin is the largest organ of the body and can be an important route for the entry of NPs into mammals [7].

TiO_2 material, which is covered in this paper, the new TiO_2 material, GST (100% anatase) prepared from the precipitated sludge using $TiCl_4$ used as a coagulant to remove total phosphorus in the wastewater was manufactured to have cost-competitive lower than price of commercial material (P-25, Evonik Corp., a flame-made multiphasic TiO_2 nanoparticles containing anatase and rutile) with excellent photocatalytic function [8,9]. We have been studied toxicological test as acute oral / dermal toxicity (TG 402, 423) in female rats by Seol et al. [10], eye or skin irritation/corrosion in rabbit (TG 404, 405) by Kim et al. [11] and 90-day oral repeated toxicity in rats by Kim et al. Through these studies, we confirmed that

GST have no treatment-related effect for oral acute / repeated exposure (90-day, subchronic) or acute dermal and eye or skin. But the study for toxicological information on dermal repeated study (90-day, subchronic) has been not conducted.

Therefore, the present study was performed to provide dose-range information of subchronic study (repeated 90-day dermal toxicity) for establishing safe levels and formulating risk assessments for new TiO_2 catalyst, GST in rats.

Materials and Methods

Test facility

This study was conducted in compliance with the principles of Good Laboratory Practice (GLP) at KTR (Korea Testing & Research Institute), Hwasun based on the Korea Good Laboratory Practice (KGLP) and OECD "Principle of Good Laboratory Practice, ENV/MC/CHEM (98)17 (as revised in 1997)". The study protocol was reviewed and approved (IAC2021-2555) by the Institutional Animal Care and Use Committee (IACUC) of KTR Hwasun based on the Animal Protection Act [Enforcement Date: 2020-02-12] [No.16977 (2020-02-11, partial revision)] [12] and the Laboratory Animal Act [Enforcement Date: 2019-03-12] [No. 15944 (2018-12-11, partial revision)] [13]. The KTR Hwasun has been fully accredited by the association for assessment and accreditation of laboratory animal care (AAALAC).

Animal husbandry and maintenance

For the repeated dose toxicity study (Study No. TNK-2021-000584), 44 Sprague-Dawley rats [(5 weeks-old, Crl;CD(SD), SPF] of each sex were obtained from the ORIENT BIO Inc. (8, Hwaaksan-ro 124, Buk-myeon, Gapyeong-gun, Gyeonggi-do, Republic of Korea) and kept carefully following an acclimation period of 8(male)-9(female) days to ensure their suitability for the study. These animals were kept within a well-ventilated and specific pathogen-free (SPF) facility with conditions set to a temperature of 22±3 °C (measured value: 22.1-23.3 °C), a humidity of 50±20% RH (measured value: 44.5-59.7%) with artificial lighting a 12-h light / 12-h dark cycle (08:00-20:00 / 20:00-08:00) and 10-20 air changes per hr. For study, the healthy animals were used after examining conditions included body weight, clinical signs and then 20 rats / sex were randomly divided into four groups listed in Table 1. Animals were kept in stainless steel wire cages and allowed R/O (reverse osmosis) water via a water bottle and irradiation-sterilized pellet diet (Rodent Diet 20 5053, Labdiet, USA), *ad libitum*.

Table 1. Groups for 90-day repeated oral toxicity study.

Group	Dose (mg/kg B.W/day)	Fluid volume (mL/kg)	Number animals (Male)	(Female)
G1	0	5	5 (1101-1105)	5 (2101-2105)
G2	500	5	5 (1201-1205)	5 (2201-2205)
G3	1000	5	5 (1301-1305)	5 (2301-2305)
G4	2000	5	5 (1401-1405)	5 (2401-2405)

Test materials and preparation

The new TiO_2 materials, GST (pale yellow powder, crystalline composition of 100% anatase) was provided by Bentec Frontier Co., Ltd (139, Nanosandan-ro, Nam-myeon, Jangseong-gun, Jeollanam-do, Republic of Korea). The characterization of GST was evaluated as Table 2 [zeta potential, particle size image (SEM), TEM (transmission electron microscopy) image, size distribution).

Table 2. Evaluation for characterization of TiO_2.

Item	Analyzer	Facility
Zeta potential	Particle size & Zeta potential analyzer (Zetasizer Nano ZSP, Malvern Instruments LTD., UK)	Korea TECH[*1]
particle size image	FE-SEM(Field Emission Scanning Electrong Microscope, Tescan Corp., Czech)] equipped with EDS systems (Thermo scientific, USA)	KRICT[*2]
TEM image	FE-EF-TEM (Field Emission Energy Filtered Transmission Electron Microscopy, JEOL, Japan)	Korea Basic Science Institute[*3]
Size distribution	Image J software	-

[*1] Korea TECH: 1600, Chungjeol-ro, Byeongcheon-myeon, Dongnam-gu, Cheonan-si, Chungcheongnam-do, Republic of Korea

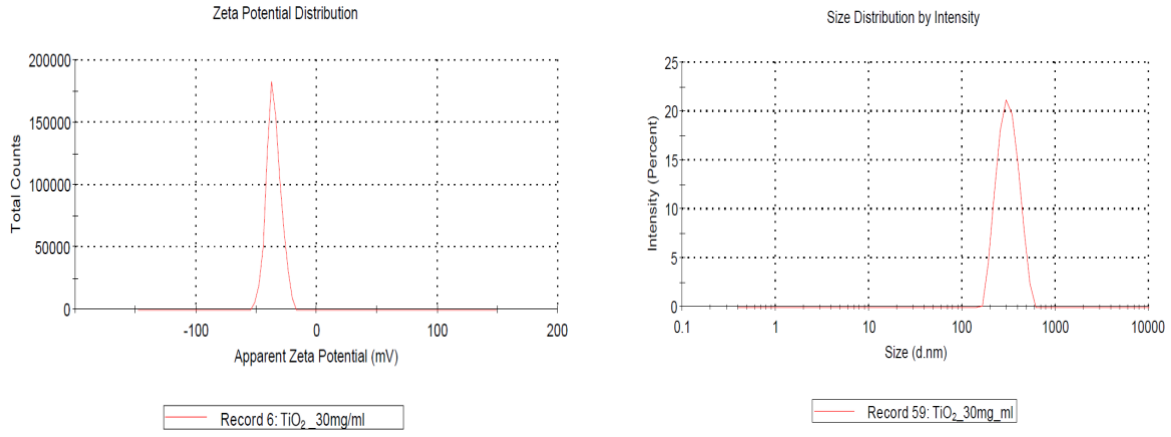
[*2] KRICT: Korea Research Institute of chemical technology Ulsan division, 45 Jonggaro Junggu Ulsan, Republic of Korea

[*3] Korea Basic Science Institute: Jeonju center (regional analytical science), 20, Geonji-ro, Deokjin-gu, Jeonju-si, Jeollabuk-do, Republic of Korea

Test procedure

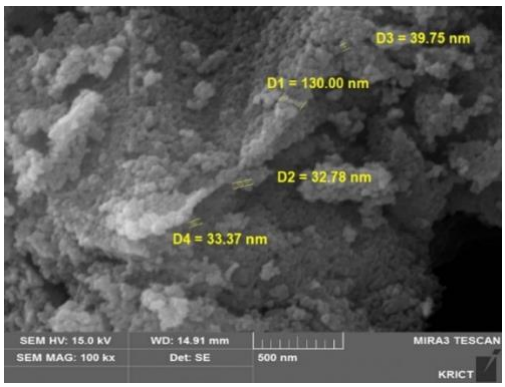
Properties of GST

The characterization of GST showed in Figures 1, 2 and 3, respectively. The zeta potential is the potential between droplet surface and dispersing liquid medium and can be used to estimate surface charge of the droplets in the dispersion medium. Also, it was known for indicator of the droplet stability, where values more positive than +30 mV and more negative than -30 mV indicate good stability against coalescence [14]. The estimated value for GST showed that GST has a negative value (-35.4±5.99 mV) and this value was thought to be good stability and it was thought to be a property to be less agglomeration nature (Figure 1). The particle size value and distribution (95.8±46.3 nm, 46-270 nm) showed that GST was thought to be have materials of various sizes and was difficult to be classified as a nanomaterial considering the definition of nanomaterials (<100 nm) (Figures 2 and 3).



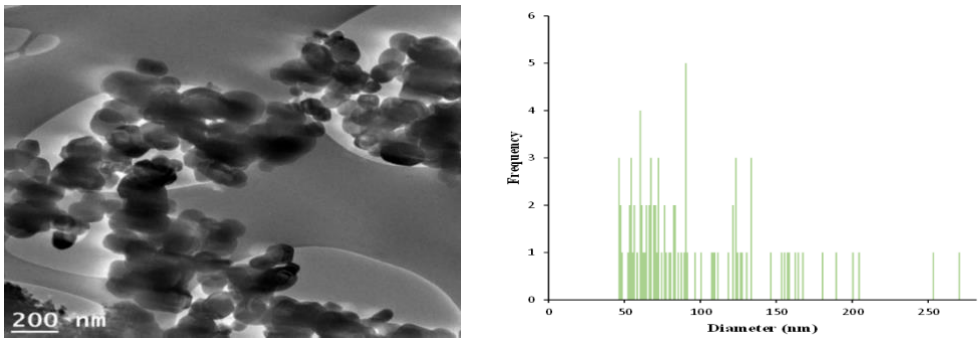
(a) **(b)**

Figure 1. Characterization of TiO_2 particles (GST) analyzed by Korea TECH: (**a**) negative zeta potential (-35.4±5.99 mV, 30 mg/mL); (**b**) size distribution by intensity (mean: 336.8 nm).



(a)

Figure 2. Characterization of TiO_2 particles (GST): (**a**) SEM (scanning electron microscope) image analyzed by KRICT.



(a) **(b)**

Figure 3. Characterization of TiO_2 particles (GST): (**a**) A particles dispersed in 99.9% EtOH was deposited on a copper grid and analyzed using TEM (Transmission electron microscope) image by Korea Basic Science Institute; (**b**) Size distribution (95.8±46.3 nm, 46-270 nm,) of the imaged GST(image J software).

Clinical signs, body weight and food consumption

All animals were observed and recorded daily [treatment period; twice a day (before / after treatment)] throughout the experimental period (28-day treatment) including treatment-related signs (clinical signs, toxicological symptom and mortality). In the food consumptions, the date values were measured per cage (g / cage / day). Body weight was checked at the time of receipt, assignment, at the start of administration, once a week for administration and necropsy (fasting body weight). Food consumption was measured once a week after the initial of administration. After feeding, the feeding amount on the day and the remaining amount on the next day were measured, and the intake amount was calculated from the difference.

Hematology, biochemistry and hormone analysis

All animals were fasted overnight before blood sampling. The blood samples were collected from the abdominal aorta under anesthesia and transferred to the tubes with anti-coagulant; CBC bottle (EDTA 2K, BD, USA) for hematological test using blood analyzer (ADVIA 2101i, Siemens, Germany), multi-channel microplate reader (Synergy HT, BioTek) and vacutainer (9NC Sodium citrate, BD, USA) for coagulation test using blood coagulation analyzer (ACL ELITE PRO, Instrumentation Laboratory, U.S.A.), respectively. And then, the remaining samples were placed in tubes without an anticoagulant for biochemistry (TBA-120FR, TOSHIBA, Japan) and for hormone analysis (Immunite 2000xpi, Siemens, Germany). To get plasma for coagulation examination, blood samples were centrifuged for 10 minutes (3000 rpm, 4 °C) and the sera for biochemistry / hormone analysis were centrifuged in the same way after the tube were kept at room temperature.

- Hematology; Red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Reticulocyte (Retic), platelet count (PLT), total leucocyte counts (WBC), differential count [neutrophils (Neut), lymphocytes (Lymph, monocytes (Mono), eosinophils (Eos) and basophils (Baso)]
- Coagulation test: prothrombiN (PT), activated partial thromboplastin time (APTT)
- Biochemistry: total protein (TP), albumin (ALB), A/G ratio (Albumin/Globulin), total bilirubin (T-BIL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl-transpepti dase (GGT), creatinine (CRE), blood urea nitrogen (BUN), Urea, total cholesterol (T-CHO), triglycerides (TG), glucose (GLU), calcium (Ca), inorganic phosphorus (IP), creatine kinase (CK), cholinesterase (CHE), total bile Acid (TBA), sodium (Na), potassium (K) and chloride (Cl)

Necropsy and histopathology

On the day scheduled for necropsy, all animals were anaesthetized with anesthetic drug (Ifran Liquid for Inhalation, Hana Pharm. Co., Ltd., Republic of Korea) and device (MatrxTM VIP 3000, MIDMARK, USA) followed by blood sampling. And then, gross examinations were performed on external surface, all internal organs of the cranial, thoracic, and abdominal cavities. Absolute and relative weights (organ weight to fasted body weight ratio, %) of organs were measured for including organs; liver, thymus, brain, pituitary gland, heart, spleen, uterus with cervix, kidneys (*), adrenal glands (*), thyroid gland (*), ovaries (*), prostate + seminal vesicles with coagulating glands, testes (*) and epididymides (*). The bilateral organs (*) were measured, respectively and then the measured weights were summed.

The organs were fixed in 10% neutral buffered formalin, bouin's fixation (for testes / epididymides), davidson's solution (for eyes with the harderian gland) as following organs; liver, kidney, adrenal glands, heart, lung (with bronchus), trachea, brain (with pituitary gland), spleen, ovary, testis, epididymis, prostate / seminal vesicles, ovary, uterus (vagina), urinary bladder, tongue, (para)thyroid gland, (small / large) intestine, pancreas, stomach, sternum / femur, skeletal muscle (sciatic nerve), (submandibular / mesenteric) lymph node, spinal cord skin (with mammary gland). The processing for fixed tissues were conducted in eye and skin through trimming, embedding in paraffin, section, and stained with hematoxylin & eosin (H&E). The slide specimens for eye and skin were microscopically examined using the image analyzing system (Leica, Germany) in high dose and control dose group.

Statistical analysis

Data were presented as means ± standard deviation (S.D.) for each group. The body weight, food consumption, organ weights and hematological/biochemical data were analyzed using SPSS (Ver 19.0, Chicago, IL, USA) program. In treatment group, the Leven's test was performed to derive the homogeneity of variances and one way ANOVA test was performed to determine the significant differences between study groups. In the case of confirming significant difference, post-hoc test was proceeded according to the result of homogeneity (homogeneity; Scheffe tset, heterogeneity; Dunnett's T3 test). In recovery group, data were performed using independent *t*-test. *P* value <0.05 were considered statistically significant.

Results and Discussion

Clinical signs

During the experimental period, there were no abnormal clinical signs and mortality such as treatment-related moribund and dead animals in any of the treatment groups.

Body weight and food consumptions

For the experimental period (28-day treatment period), the body weight showed a normal increase and there were no treatment-related effects in food consumptions between the treatment and recovery group Figure 5.

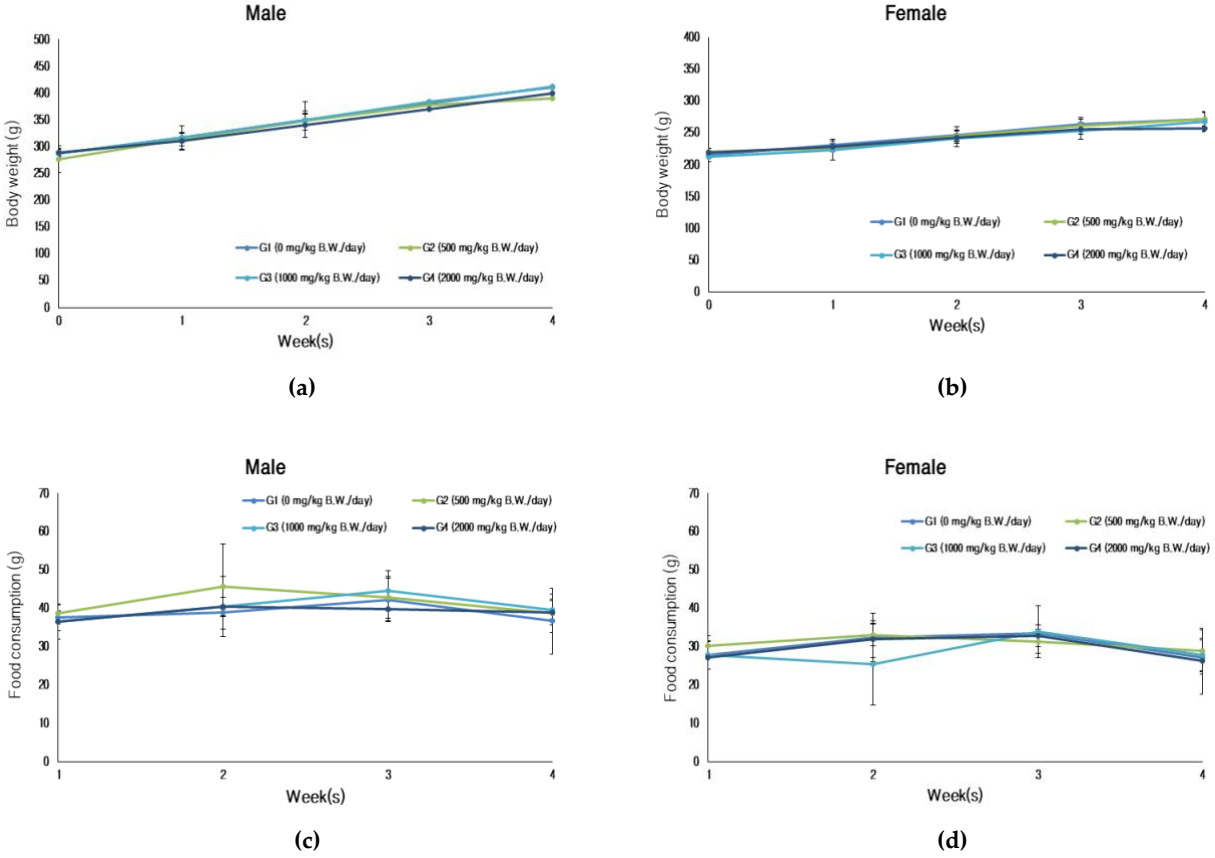


Figure 4. Changes for body weight and food consumptions: (**a**) body weight (male); (**b**) Body weight (female); (**c**) Food consumptions (male); (**d**) food consumptions (female).

Hematology and biochemistry

There were no treatment-related significant effects in both sexes in any of treatment group as Tables 3 and 4.

Necropsy and organ weight

At the end of treatment, in gross necropsy, there were no abnormal opinions in both external and internal observation in all administration group of both sexes. Also, as a result of measuring absolute or relative organ weight, there were no significant differences in male and female administration group compared to vehicle control.

Histopathological examination

The histopathological examination was performed to identify the treatment-related effects for treatment group.

Considering retinopathy finding (presence of nanomaterials) observed in previous research of ZnO (particle size: 20 nm, charge: negative) for 90-day in rats [15], we examined the eyes carefully including harderian gland and there were no TiO_2-related effects as Figure 5. Also, skin exposed with TiO_2, there were no treatment-related changes considered to be toxicological lesion occurred by test substance administration as Figure 6.

Table 3. Hematological parameters.

Parameters	Group(Dose)*	G1(0)	G2(500)	G3(1000)	G4(2000)
	Sex/Week	4 weeks	4 weeks	4 weeks	4 weeks
Total leucocyte count (10^3 cells/μL)	Male	7.28±4.29	7.53±2.01	8.38±2.79	7.19±1.62
	Female	5.28±1.36	6.09±1.84	4.97±0.58	5.09±1.59
Total erythrocyte count (10^6 cells/μL)	Male	8.18±0.34	8.34±0.38	8.25±0.47	8.22±0.24
	Female	7.83±0.22	8.35±0.33	7.98±0.31	7.76±0.32
Hemoglobin concentration (g/dL)	Male	15.3±0.3	15.5±0.5	15.4±0.3	15.0±0.2
	Female	14.5±0.6	15.3±0.5	14.8±0.5	14.4±0.5
Hematocrit (%)	Male	48.0±0.9	49.2±1.8	48.8±1.3	47.8±0.7
	Female	45.3±1.3	47.8±1.5	46.0±1.9	45.1±1.6
Mean corpuscular volume (fL)	Male	58.8±1.8	59.0±1.0	59.3±2.6	55.1±1.3
	Female	57.9±1.0	57.3±1.2	57.6±0.4	58.0±1.4
Mean corpuscular hemoglobin (pg)	Male	18.7±0.7	18.6±0.5	18.7±0.7	18.2±0.4
	Female	18.5±0.5	18.3±0.3	18.6±0.2	18.6±0.5
Mean corpuscular hemoglobin concentration (g/dL)	Male	31.7±0.2	31.6±0.4	31.6±0.4	31.4±0.2
	Female	32.0±0.7	32.0±0.2	32.3±0.5	32.0±0.2
Reticulocyte (10^9cells/μL)	Male	173.9±33.5	188.5±39.2	195.6±32.3	178.3±49.5
	Female	204.4±79.4	190.6±45.0	165.0±35.2	171.3±46.0
Reticulocyte (%)	Male	2.12±0.38	2.26±0.44	2.38±0.45	2.18±0.64
	Female	2.62±1.07	2.29±0.60	2.08±0.49	2.23±0.69
Platelet (10^3cells/μL)	Male	1107±95	1158±108	1130±87	1174±144
	Female	1297±133	1143±247	1106±197	1110±102
Prothrombin time (sec)	Male	19.1±4.0	16.2±0.9	19.7±3.3	20.3±3.3
	Female	12.0±0.4	12.1±0.9	11.7±0.5	12.6±0.7
Activated partial thromboplastin time (sec)	Male	18.6±1.1	18.1±1.5	18.0±2.3	18.2±2.1
	Female	16.1±1.2	15.1±2.7	16.4±1.3	15.5±1.0
Neutrophils (%)	Male	13.9±4.2	12.5±4.0	12.7±4.0	11.8±3.6
	Female	12.1±6.8	11.7±6.3	10.2±3.3	13.3±5.7
Lymphocytes (%)	Male	81.6±3.3	82.7±4.8	83.4±4.4	84.1±4.7
	Female	84.2±7.1	83.9±6.2	85.7±3.5	83.1±5.5
Monocytes (%)	Male	1.2±0.2	1.8±0.4	1.4±0.5	1.3±0.7
	Female	1.4±1.0	1.9±1.2	1.2±0.8	1.0±0.4
Eosinophils (%)	Male	1.4±0.4	1.4±0.6	1.2±0.7	1.4±0.2
	Female	1.1±0.2	1.2±0.2	1.4±0.6	1.4±0.4
Basophils (%)	Male	0.2±0.0	0.1±0.0	0.1±0.1	0.2±0.1
	Female	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1

Values are in mean±standard deviation
*: mg/kg

Table 4. Blood chemical parameters.

Parameters	Group(Dose)* Sex/Week	G1(0) 4 weeks	G2(500) 4 weeks	G3(1000) 4 weeks	G4(2000) 4 weeks
Total protein (g/dL)	Male	5.5±0.3	5.5±0.1	5.5±0.2	5.4±0.2
	Female	5.9±0.3	6.0±0.3	5.9±0.3	5.7±0.1
Albumin (g/dL)	Male	4.0±0.1	4.0±0.1	3.9±0.2	4.0±0.1
	Female	4.3±0.4	4.4±0.3	4.3±0.3	4.1±0.1
A/G ratio	Male	2.7±0.3	2.6±0.2	2.6±0.6	2.8±0.3
	Female	2.8±0.5	2.8±0.2	2.7±0.4	2.7±0.4
Total bilirubin (mg/dL)	Male	0.01±0.01	0.02±0.02	0.01±0.01	0.05±0.05
	Female	0.04±0.03	0.04±0.02	0.03±0.03	0.03±0.03
Alkaline phosphatase (U/L)	Male	522±105	553±103	535±97	511±120
	Female	307±52	299±74	287±79	291±67
Aspartate aminotransferase (U/L)	Male	126±8	124±14	119±7	121±10
	Female	120±54	100±14	91±15	109±19
Alanine aminotransferase (U/L)	Male	31±4	30±4	32±4	37±6
	Female	39±14	34±8	30±4	26±4
Creatinine (mg/dL)	Male	0.28±0.02	0.30±0.02	0.31±0.03	0.31±0.03
	Female	0.38±0.01	0.34±0.03	0.35±0.03	0.35±0.04
Blood urea nitrogen (mg/dL)	Male	13.7±1.1	13.7±1.6	14.3±1.1	14.6±1.5
	Female	16.1±3.3	12.7±0.6	15.9±2.6	15.7±3.1
Total cholesterol (mg/dL)	Male	57±9	48±13	50±11	48±9
	Female	58±14	55±2	62±9	52±12
Triglycerides (mg/dL)	Male	18±4	27±6	23±12	16±6
	Female	10±	10±2	11±1	11±7
Glucose (mg/dL)	Male	157±9	132±4	167±37	147±13
	Female	152±16	155±6	153±16	149±30
Calcium (mg/dL)	Male	9.0±0.2	9.2±0.3	9.4±0.2	9.3±0.3
	Female	9.5±0.5	9.6±.4	9.6±0.5	9.4±0.3
Inorganic phosphorus (mg/dL)	Male	8.0±0.4	7.9±0.3	8.0±0.4	7.9±0.5
	Female	7.6±0.4	7.2±0.3	7.4±0.4	7.3±0.7
γ-Glutamyl transpeptidase (IU/L)	Male	1.47±1.01	2.36±1.42	1.87±1.49	2.10±2.39
	Female	1.79±0.82	1.49±0.88	0.83±0.36	1.33±0.43
Creatine kinase (U/L)	Male	694±185	698±144	577±138	526±94
	Female	570±781	310±100	336±219	376±300
Bile acid (μmol/L)	Male	15.9±6.9	25.7±8.9	21.3±13.7	15.0±6.9
	Female	20.5±5.9	25.3±7.0	19.3±12.7	12.3±5.1
Sodium (mmol/L)	Male	146.6±0.7	147.1±0.9	146.0±1.1	146.1±0.6
	Female	145.2±1.1	144.9±0.4	144.6±0.7	145.5±1.3
Potassium (mmol/L)	Male	4.82±0.10	4.98±0.15	4.95±0.20	4.93±0.17
	Female	4.64±0.21	4.59±0.26	4.65±0.27	4.44±0.40
Chloride (mmol/L)	Male	101.0±1.0	102.1±1.3	100.8±1.4	102.1±0.7
	Female	102.8±1.6	101.8±1.1	101.6±0.6	102.9 ±1.5
Cholinesterase (U/L)	Male	4.2±.0.8	4.4±0.8	3.7±0.8	3.9±0.5
	Female	3.8±2.5	3.6 ±1.4	3.6±1.3	4.3±1.7

Values are in mean±standard deviation
*: mg/kg

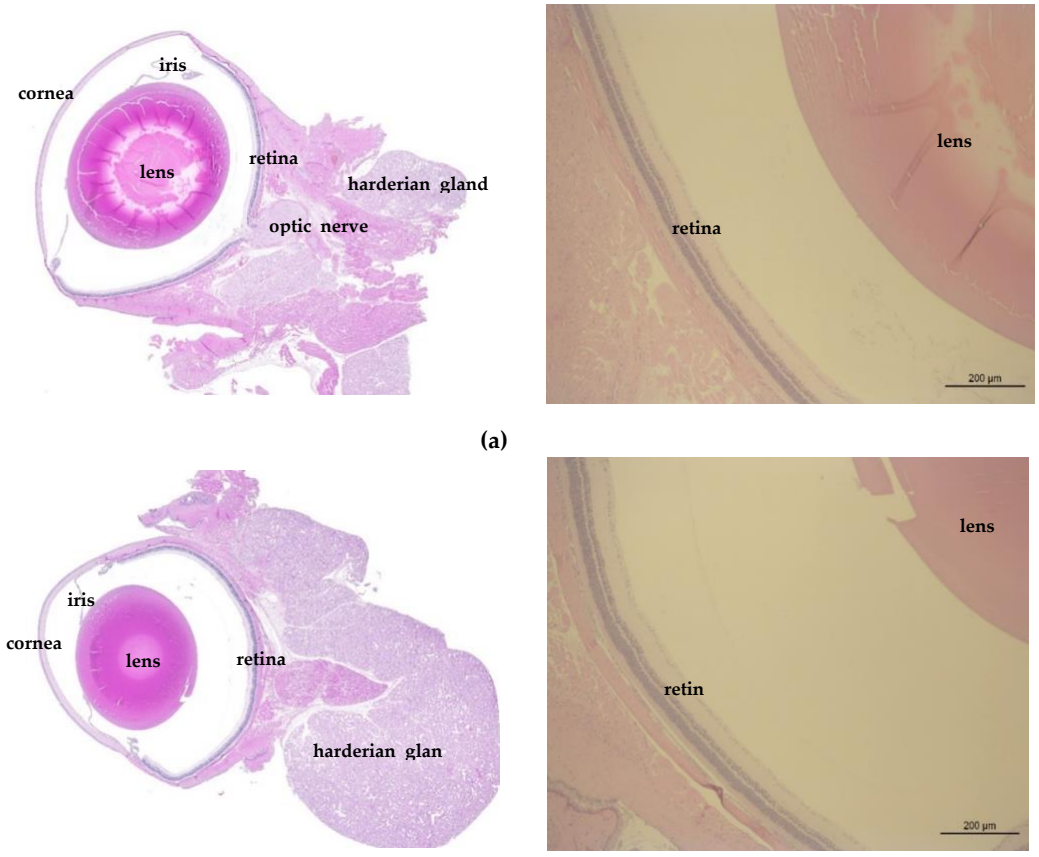


Figure 5. Eye photographs (Left: Slide scanner image, ZEISS Axio Scan. Z1, right: Optical microscope, X50) including the cornea, iris, lens, retina and accessory organs (optic nerve, harderian gland): (**a**) Control group (animal number 2101); (**b**) High dose group (animal number 2405).

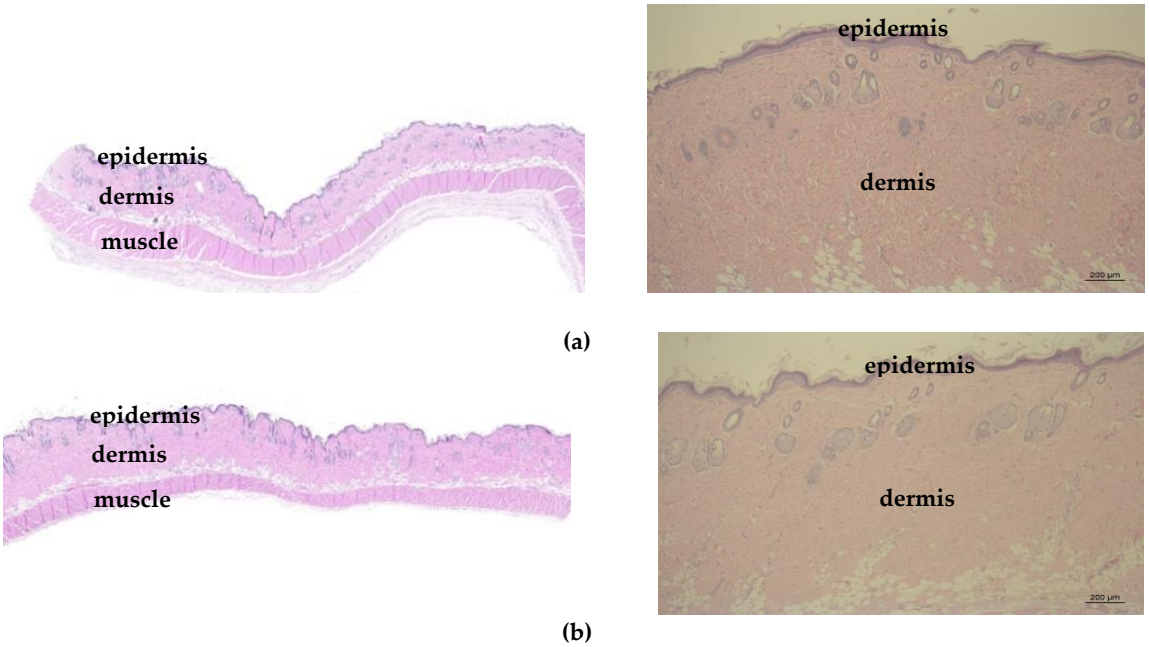


Figure 6. Skin photographs (Left: Slide scanner image, ZEISS Axio Scan. Z1, right: Optical microscope, X50) including the epidermis and dermis: (**a**) Control group (animal number 2101); (**b**) High dose group (animal number 2405).

Conclusions

This study was performed to investigate dose-range for subchronic study (90-day repeated dermal study) and evaluate the systemic toxicity for the test substance, new TiO_2 (GST), manufactured from sludge recycling of the sewage treatment plant, on male and female Sprague-Dawley rats. 28 days repeated oral dosing via gavage was conducted at dose levels of 0 (vehicle control), 500 (low dose group), 1000 (middle dose group), and 2000 (high dose group) mg/kg B.W/day.

There were no treatment-related effects in body weight, food consumption, hematological examination, biochemical examination, organ weight, gross necropsy and histopathological examination (eye, skin).

In conclusion, the effect related with GST was not observed on male and female Sprague-Dawley rats followed by 28 days of administration period at dose levels of 0, 500, 1000, and 2000 mg/kg body weight. Therefore, dose selection for repeated 90-day study was considered to be 500, 1000 and 2000 mg/kg B.W/day under the present study conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

CRediT author statement

JHK: Conceptualization, Methodology, Writing- Original draft preparation, MKP: Supervision, Writing- Reviewing and Editing, JMI: Visualization, HSS: Visualization, HJP: Resources, SSN: Project administration, Writing- Reviewing and Editing

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