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ORIGINAL RESEARCH

Oral Toxicity Study of Silicon Dioxide Nanoparticles on Fertility and Early Embryonic Development in Mice

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Background: Silicon dioxide nanoparticles (SiO_2NPs) are one of the most commonly used non-metal oxide nanomaterials because of their biocompatibility, biodegradability, and surface functionalization. However, the potential toxicological effects of SiO_2NPs have not been comprehensively investigated, and the available toxicological data are inconsistent. In particular, the toxicological effects of SiO_2NPs on reproductive and developmental functions are limited, and little information is available regarding their potential effects on fertility and early embryonic development.

Methods: In this study, we investigated the potential reproductive and developmental toxicity of SiO_2NPs in mice, in accordance with the fertility and early embryonic development study design of International Council for Harmonization (ICH) S5 (R3) guideline. SiO_2NPs were administered once daily via oral gavage at doses of 0, 60, 250, and 1000 mg/kg during the pre-mating, mating, and early gestation periods. Endpoints related to fertility and early embryonic development were evaluated, including female fertility, tubal transport, implantation, early embryonic development, and functional aspects of male fertility. Additionally, the general health of the parental animals was also investigated.

Results: Oral administration of SiO_2NPs did not induce any SiO_2NPs-related adverse effects on female fertility indices, tubal transport, implantation outcomes, early embryonic development, and male fertility function. Additionally, no SiO_2NPs-related adverse effects were observed in clinical signs, body weight, food consumption, clinical pathology, macroscopic observations, and organ weights at any dose level.

Conclusion: The results demonstrated that oral exposure to SiO_2NPs at dose levels up to 1000 mg/kg did not induce any significant adverse effects on fertility, early embryonic development, or general systemic health. These findings contribute to the human health risk assessment of SiO_2NPs, and further toxicological studies should address different physicochemical properties and additional exposure routes to resolve remaining uncertainties.

Keywords: nanotoxicology, E 551, hazard identification, reproductive toxicology, developmental toxicology

Introduction

Silicon dioxide nanoparticles (SiO_2NPs), also known as nanoforms (<100 nm) of silicon dioxide or silica, are being widely used owing to their characteristic physicochemical properties.[1,2] SiO_2NPs have favorable properties, such as biocompatibility, biodegradability, and surface functionalization.[3,4] SiO_2NPs are among the most extensively utilized non-metal oxide nanomaterials, with a global market size of approximately 4.65 billion dollars in 2021, and the market is projected to grow to around 8.65 billion dollars by 2030.[5] SiO_2NPs are extensively used in the food industry, cosmetics, biomedical applications, and drug carriers.[6,7] Owing to their extensive application in daily-use products and various

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industrial areas, concerns regarding the safety and potential toxicological health effects of SiO_2NPs have been raised.[8,9] However, these potential toxicological effects of SiO_2NPs have not been extensively investigated.

A few reviews have summarized the potential toxicological effects of SiO_2NPs on human health.[10,11] Napierska et al reported SiO_2NP exposure-related toxicological effects on health, both in vitro and in vivo; the physicochemical properties of SiO_2NPs, including particle size, surface area, and surface features, were considered critical factors in regulating these toxicological effects.[10] The in vitro experiments revealed that SiO_2NPs exposure were cytotoxic which induces oxidative stress and mediates apoptosis via the intrinsic or mitochondrial pathway; the in vivo experiments revealed that short-term SiO_2NPs exposure exerts toxicological effects on the lungs, liver, and heart.[11] Morris et al reported that a single intratracheal instillation of SiO_2NPs (approximately 50 nm; 0.5 mg/animal) in mice can elicit a significantly higher inflammatory response in the lungs.[12] Zhuravskii et al reported that a single intravenous administration of SiO_2NPs (approximately 13 nm; 7 mg/kg) in rats can lead to mast cell abundance in the liver, lungs, and heart and can lead to liver fibrosis.[13] In contrast to these SiO_2NPs-related toxic effects observed in in vivo experiments, several other in vivo studies have also reported no significant toxicity following chronic oral or dermal exposure to SiO_2NPs.[11,14] These conflicting results imply the need for further toxicological investigations to clarify the potential health risks associated with SiO_2NPs.

Although previous studies have predominantly focused on the systemic toxicological effects of SiO_2NPs, their potential toxicological effects on reproductive and developmental functions remain poorly investigated. Zheng et al conducted a review on the potential genetic and epigenetic toxicities of SiO_2NPs on germ cells.[1] Ye et al reported SiO_2NPs significantly impaired embryo development via increased oxidative stress in zebrafish.[15] Another zebrafish study also reported chronic waterborne exposure to SiO_2NPs detrimentally affected reproductive performance via triggered immune and oxidative stress responses.[16] In vivo mice study reported that juvenile exposure to SiO_2NPs caused significant testicular and epididymal damage, impaired sperm quality, disrupted testosterone synthesis via triggered oxidative stress, inflammation, and apoptosis.[17] However, in contrast to these SiO_2NPs-related toxic effects, several studies have also reported no significant toxicity in developmental and reproductive functions.[18,19] These conflicting results also imply the need for further toxicological investigations to clarify the potential toxicological effects of SiO_2NPs in developmental and reproductive functions. In particular, there is a significant lack of data from in vivo studies regarding the potential effects of SiO_2NPs on fertility and early embryonic development which is critical windows that play an important role in determining reproductive success and offspring health.

Oral exposure of SiO_2NPs is one of the most critical routes of human exposure, considering their extensive use in consumer products directly intended for ingestion.[20] SiO_2NPs are widely used as food additives (E 551, the food additive number for SiO_2), anti-caking agents, and carriers for bioactive compounds in processed foods and dietary supplements.[21,22] Additionally, SiO_2NPs are utilized in pharmaceutical formulations to enhance drug stability and bioavailability to facilitate their use via the oral exposure route.[23] Actually, according to the human exposure assessment, the estimated daily intake of SiO_2NPs from food consumption ranges from approximately 0.3 to 1.2 mg/kg body weight/day although this may vary depending on regional differences and dietary patterns.[24] Moreover, the European Food Safety Authority (EFSA) re-evaluated silicon dioxide as a food additive (E 551) and reported that the estimated dietary intake of silicon dioxide ranges from 20 to 50 mg per day, corresponding to approximately 0.3 to 0.8 mg/kg body weight per day for a 60 kg individual.[22] Accordingly, SiO_2NPs can be absorbed through the gastrointestinal tract and subsequently affected to reproductive organs and developing embryo. Considering that germ cells undergo active differentiation processes and early embryonic development is highly vulnerable, comprehensive assessment of their potential effects on fertility and early embryonic development should be conducted.

Therefore, the purpose of this study was to investigate the potential toxicological effects of SiO_2NPs on fertility and early embryonic development in mice, following a repeated oral exposure during the pre-mating, mating, and early gestation periods. Most previous toxicological studies have focused on systemic toxicological effects of SiO_2NPs. In contrast, data on the reproductive and developmental risks of SiO_2NPs, particularly under realistic exposure scenarios like dietary ingestion, remain limited. Moreover, the conflicting results on SiO_2NPs-related toxicity highlight the importance of conducting well-designed, regulatory guideline-based in vivo studies to support the human risk assessment in developmental and reproductive functions. To address these gaps, this study was investigated the potential effects of

SiO_2NPs on fertility and early embryonic development in mice, in accordance with the ICH S5(R3) guideline.[25] SiO_2NPs were administered daily to mice by oral gavage at doses of 0, 60, 250, and 1000 mg/kg, and female fertility, tubal transport, implantation, early embryonic development, and functional effects on male fertility were investigated.

Materials and Methods

SiO_2NPs and Physicochemical Characterization

SiO_2NPs were purchased from Sigma-Aldrich (USA) in nanopowder form with 99.5% purity. The primary size of SiO_2NPs was 15 nm, based on the information provided by the manufacturer.

The physicochemical characterization of SiO_2NPs was performed as described in our previous study.[26] Briefly, the primary size and shape were confirmed by transmission electron microscopy (TEM, JEM-2100F, JEOL, Japan), and purity was confirmed by energy-dispersive X-ray spectroscopy (TEM equipped with a silicon drift detector, Oxford Instruments, UK). The hydrodynamic diameter of particles in the vehicle was analyzed on an ELS-8000 (Otsuka Electronics, Japan) using the dynamic light scattering (DLS) method.

Animals and Their Maintenance

Male and female ICR mice (CrlOri: CD1, Orient Bio, Republic of Korea) aged 9 weeks were used in this study. The general health of all animals was carefully examined during a 5-day acclimatization period, and healthy animals without any abnormal clinical signs were used in this study. All mice were individually housed in solid-bottom polysulfone cages (W × L × H; 117 × 300 × 134 mm^3) with autoclaved aspen bedding materials. Additionally, mouse retreat and half nylon bones were provided for animal enrichments. The temperature of the animal room was controlled at approximately 20–26°C, with 30–70% humidity, 150–300 Lux, 10–20 times/h ventilation, and a 12-h light/dark cycle, as described in our previous study.[27] A certified rodent laboratory diet (PMI Nutrition International, USA) irradiated with gamma-rays and filtered drinking water irradiated with ultraviolet light were provided ad libitum.

All animal experimental procedures were conducted at the animal experiment facilities of the Korea Institute of Toxicology (KIT), which is fully accredited from the Association for Assessment and Accreditation of Laboratory Animal Care International since 1998. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committees of KIT based on the Animal Protection Act of Korea and the Guide for the Care and Use of Laboratory Animals.[28] This study was conducted in a facility equipped with standard operating procedures following good laboratory practice principles, but was performed as a non-GLP study.

Group Designation and SiO_2NPs Administration

Eighty-eight male and 88 female ICR mice (total 176 mice) were assigned to four groups (one vehicle control group and three SiO_2NPs dose groups) to equalize the mean group body weights before animal experiment initiation. The dose levels in this study were selected as 0, 60, 250, and 1000 mg/kg based on a previous 90-day repeated oral toxicity study of SiO_2NPs in rats, which reported no observed adverse effects even at limit dose (1000 mg/kg) level.[14] The limit dose (1000 mg/kg) was selected as the high dose, in accordance with regulatory non-clinical toxicology guidelines.[25] The middle (250 mg/kg) and low (60 mg/kg) doses were selected using a common ratio of 4, which is general practice in dose selection for regulatory guideline-based non-clinical toxicology studies.[29] Sterile normal saline was administered to the vehicle control (0 mg/kg) animals. SiO_2NPs were weighed and suspended in sterile normal saline to obtain a high dose concentration, following which the high-dose SiO_2NPs were sonicated (amplitude 25%) for 8 minutes using an ultrasonic processor (VC 505, Sonics & Materials Inc., USA). The middle-and low-dose SiO_2NPs were prepared by diluting high-dose SiO_2NPs in vehicle. SiO_2NPs were administered to males by oral gavage once daily, beginning 2 weeks before mating and continuing until the day before sacrifice (approximately 6 weeks in total), and the administration in females began 2 weeks before mating and continued until implantation (gestation day 6). The duration of administration was determined in accordance with the ICH S5(R3) guideline previously referenced.[25] SiO_2NPs were administered based on the most recently measured body weight, with a dose volume of 10 mL/kg. All SiO_2NPs formulations were continuously stirred using a magnetic stirrer during administration.

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In-Life Observations

Mortality and general clinical signs were observed during the in-life period. Mortality was observed twice a day, at the start and end of the animal room procedure. General clinical signs, including general appearance and behavioral changes, were observed at least once daily during the in-life period.

Body weight and food consumption were regularly monitored. Body weight was recorded twice a week during pre-mating, mating, and post-mating periods. Females were additionally weighed on days 0, 3, 7, 10, and 12 during the gestation period. Food consumption was recorded once a week during the pre- and post-mating periods. Female food consumption was additionally measured on gestation days 0, 3, 7, 10, and 12 during the gestation period. Food consumption was calculated by subtracting the amount of leftover food from the amount of food provided.

Estrus cycle examination of females was conducted during the pre-mating and mating periods. A vaginal smear was taken once a day every morning from each female at the beginning of the 14 days prior to mating, and continued until there was evidence of mating. Vaginal smear samples were stained with Wright's stain and examined by light microscopy. Estrus cycles were divided into the following four stages: proestrus, estrus, metestrus, and diestrus; based on the cell types in the vaginal smear, the regularity and length of the estrus cycle were examined.

Mating was conducted with both male and female in the same dose group. Male and female mice were paired in a 1:1 ratio for a period of two weeks in the home cage of male mice each night, and mating confirmation was checked every morning. Females with a vaginal plug observed in situ were considered to be gestation day (GD) 0. Pregnancy was finally confirmed based on the implantation sites in the uterus. According to the mating results, fertility indices and precoital time were calculated.

Terminal Observations

Clinical pathology (hematology and clinical chemistry) examination was conducted with 5 animals/sex/group at the time of sacrifice. Blood samples were collected from designated sacrificed animals (males and pregnant females) from the abdominal vena cava under deep isoflurane anesthesia. The blood samples (approximately 0.5 mL) were placed in tubes containing the potassium salt of EDTA for hematological analysis. Hematological analysis was conducted using a hematology analyzer (ADVIA2020i, Siemens, Germany) for the following parameters: total leukocyte count, total red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean platelet volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, plate count, reticulocyte count, leukocyte differential count, and blood cell morphology. In addition, approximately 1.0 mL of blood samples was placed in tubes without anticoagulant for clinical chemistry analysis. Blood samples for clinical chemistry examination were kept at room temperature for a minimum of 90 min and centrifuged (3000 rpm for 10 min at room temperature) to obtain the serum. Clinical chemistry analysis was conducted using a clinical chemistry analyzer (200FR NEO, Toshiba Co., Japan) for the following parameters: glucose, blood urea nitrogen, creatinine, total protein, albumin, albumin/globulin ratio, globulin, total cholesterol, triglyceride, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, creatinine phosphokinase, calcium, inorganic phosphorus, sodium, potassium, and chloride levels.

Necropsy was conducted for all males and females using isoflurane, for macroscopic observation, organ weight measurement, caesarean section, and sperm analysis. Females were sacrificed on GD 12, and males were sacrificed after the confirmation of dispensability for additional mating (post-mating day 14). Macroscopic observations were carefully conducted for external, abdominal, thoracic, and cranial cavity abnormalities. Organ weights, including those of adrenal glands, brain, epididymides, kidneys, liver, lungs, ovaries, prostate, seminal vesicles with coagulating glands, spleen, testes, thymus, and uterus, were measured; relative organ weight to terminal body weight was also calculated.

Caesarean section was conducted for all necropsied pregnant females on GD 12. The gravid uterus was retrieved from pregnant females; corpora lutea, implantation sites, live/dead embryos, and resorption (early or late) were counted, and pre-implantation loss, post-implantation loss, along with fetal death were calculated.

Sperm analysis, including sperm number, motility, and morphology, was conducted for all necropsied males. The sperm number was counted in left testis and left caudal epididymis. Sperms for motility and morphology analyses were collected from the vas deferens of the left epididymis. Sperm motility was analyzed using a sperm analyzer (HTM-TOX

IVOS, Hamilton-Thorne Research, USA) for the following parameters: motility, path velocity, straight-line velocity, curvilinear velocity, amplitude of lateral head displacement, beat cross-frequency, straightness, and linearity. Sperm morphology was analyzed under a light microscope after staining with 1% eosin for 200 sperms/male.

Statistical Analysis

Statistical analyses were conducted using the SAS program (SAS Institute, USA) or Pristima system (Xybion Medical System, USA), as described in our previous studies.[30,31] Litter data were statistically evaluated based on the statistical unit of a litter. Data from non-pregnant females during gestation were excluded from analysis. The level of significance was set at $p < 0.05$.

Results

Physicochemical Characterization of SiO_2NPs

The physicochemical properties of SiO_2NPs, including primary size, shape, purity, and hydrodynamic size, are summarized in Figure 1 and Table 1. TEM images of the SiO_2NPs showed that the majority of the SiO_2NPs had a generally amorphous shape with a purity of 100%. The mean primary size of SiO_2NPs was 13.6 ± 5.6 nm. The hydrodynamic size of SiO_2NPs in sterile normal saline (6 mg/mL) obtained using DLS was approximately 421.3 nm, and this result indicated that SiO_2NPs formed larger agglomerates in sterile normal saline.

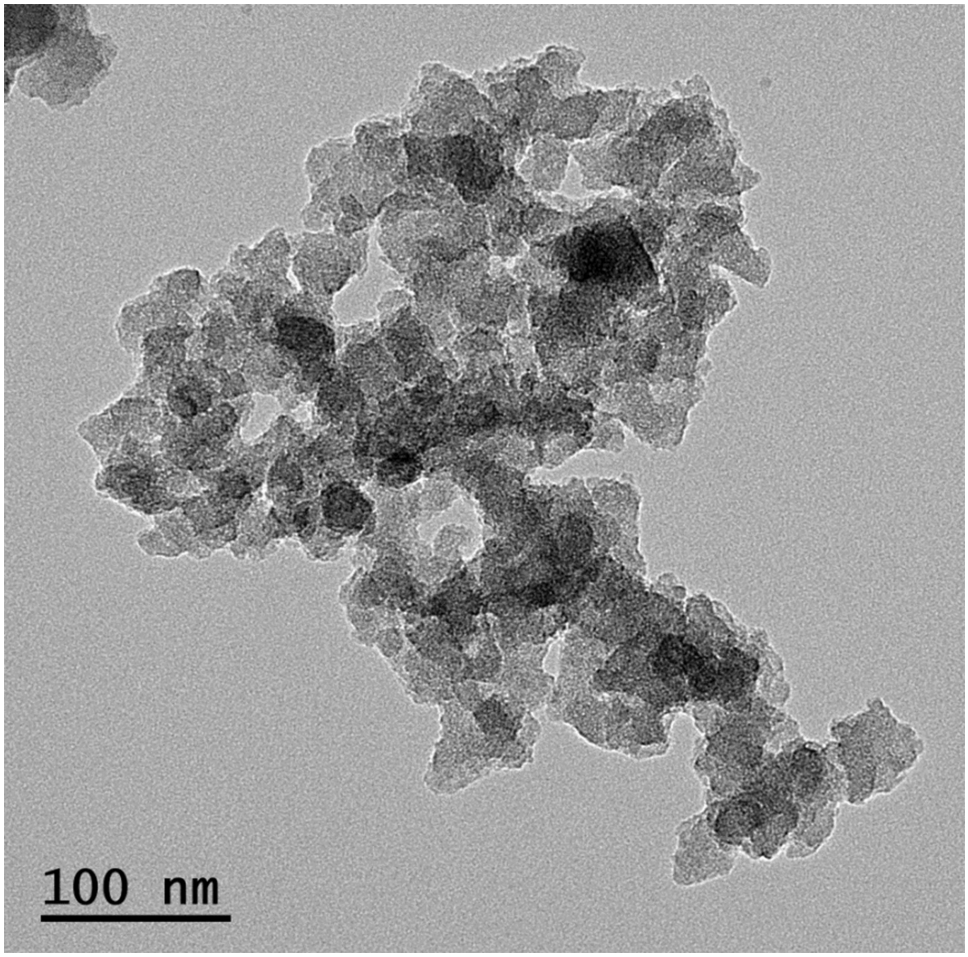

Figure 1 Morphology of silicon dioxide nanoparticles measured by transmission electron microscopy.

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Table 1 Primary Size, Purity, and Hydrodynamic Size of Silicon Dioxide Nanoparticles

Physicochemical Properties	Silicon Dioxide Nanoparticles
Primary size	13.6 ± 5.6 nm
Purity	100%
Hydrodynamic size	421.3 nm

Effects of SiO_2NPs on the Health of Parent Mice

In mortality observations, SiO_2NPs treatment-related found dead or moribund animals were not observed at any dose level evaluated during the study period. In general clinical signs observation, SiO_2NPs treatment-related abnormalities in general appearance and behavioral changes were not observed during the study period.

Body weight and body weight gain measurements between control and SiO_2NPs treatment groups were comparable throughout the in-life study period (Table 2). No statistically significant differences in body weight and body weight gain

Table 2 Body Weight and Body Weight Gain of Silicon Dioxide Nanoparticles-Exposed Male and Female Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	60	250	1000
Males				
Body weight (g)				
Pre-mating period				
Day 1	39.3 ± 2.4	39.0 ± 1.7	39.1 ± 2.4	39.0 ± 2.4
Day 4	39.2 ± 1.6	39.4 ± 1.7	38.7 ± 2.5	39.3 ± 1.8
Day 8	39.2 ± 1.7	39.5 ± 1.8	39.0 ± 1.5	39.1 ± 2.1
Day 11	39.5 ± 1.8	39.8 ± 2.0	39.2 ± 1.6	39.5 ± 1.9
Day 14	39.8 ± 1.8	40.2 ± 1.8	39.3 ± 1.8	39.7 ± 1.8
Mating period				
Day 3	38.6 ± 2.0	39.5 ± 1.6	38.7 ± 2.1	38.7 ± 1.8
Day 7	38.9 ± 2.1	39.7 ± 1.6	38.8 ± 2.0	39.1 ± 2.0
Day 10	39.0 ± 2.2	39.5 ± 1.7	39.0 ± 1.8	39.0 ± 2.0
Day 14	39.2 ± 2.4	39.8 ± 1.8	39.2 ± 1.9	39.3 ± 2.1
Post-mating period				
Day 3	39.6 ± 2.3	40.3 ± 2.0	40.0 ± 1.9	39.8 ± 2.0
Day 7	40.1 ± 2.5	40.6 ± 2.0	40.6 ± 1.9	40.3 ± 2.0
Day 10	40.3 ± 2.4	40.8 ± 2.2	40.9 ± 1.9	40.8 ± 2.2
Day 14	41.1 ± 2.3	41.1 ± 2.2	41.5 ± 2.1	41.2 ± 2.2
Body weight gain during study period (g)				
Pre-mating day 1 to post-mating day 14	1.8 ± 2.2	2.2 ± 2.2	2.4 ± 2.2	2.2 ± 3.5
Females				
Body weight (g)				
Pre-mating period				
Day 1	31.3 ± 2.0	30.4 ± 2.4	31.5 ± 1.4	31.2 ± 2.0
Day 4	31.0 ± 1.8	30.6 ± 1.3	31.1 ± 1.8	30.8 ± 1.6
Day 8	31.2 ± 1.8	30.7 ± 1.5	31.4 ± 1.8	31.1 ± 1.4
Day 11	31.5 ± 1.8	31.1 ± 1.3	31.6 ± 1.6	31.3 ± 1.4
Day 14	31.8 ± 1.9	31.9 ± 1.1	32.0 ± 1.8	31.8 ± 1.4

(*Continued*)

Table 2 (Continued).

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
Gestation period				
Day 0	31.8 ± 1.6	31.4 ± 1.5	31.6 ± 1.6	31.6 ± 1.1
Day 3	33.1 ± 1.7	33.5 ± 1.3	33.1 ± 1.8	33.2 ± 1.4
Day 7	36.0 ± 2.0	36.0 ± 1.4	36.0 ± 1.9	35.7 ± 1.7
Day 10	39.7 ± 1.9	39.8 ± 1.6	39.6 ± 2.3	39.5 ± 1.9
Day 12	45.1 ± 2.2	44.9 ± 2.2	44.8 ± 2.7	44.6 ± 2.2
Body weight gain during study period (g)				
Pre-mating day 1 to gestation day 12	13.9 ± 2.1	14.6 ± 2.6	13.4 ± 2.1	13.4 ± 1.9

Notes: All values are presented as the mean ± standard deviation for 22 mice, except for gestation period values in female mice, where the animal number was 20, 21, 20, and 19 for 0, 60, 250, and 1000 mg/kg, respectively.

between the animals treated with SiO_2NPs and the vehicle control at each data point for both males and females were observed.

Food consumption measurements between control and SiO_2NPs treatment groups were also comparable throughout the in-life study period (Table 3). No statistically significant differences in food consumption between the animals treated with SiO_2NPs and the vehicle control at each data point for both males and females were observed.

Macroscopic analysis revealed no SiO_2NPs treatment-related abnormalities in external, abdominal, thoracic, and cranial cavities. Although minor abnormalities in macroscopic observations, including increased adrenal gland (*N*=1,

Table 3 Food Consumption of Silicon Dioxide Nanoparticles-Exposed Male and Female Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
Males				
Food consumption (g/animal/day)				
Pre-mating period				
Day 1–8	6.3 ± 0.8	6.7 ± 0.9	6.3 ± 0.6	6.2 ± 0.6
Day 8–14	6.8 ± 1.0	7.0 ± 0.6	6.8 ± 0.5	6.9 ± 0.6
Post-mating period				
Day 0–7	6.6 ± 0.7	6.7 ± 0.6	6.7 ± 0.5	6.6 ± 0.7
Day 7–14	6.1 ± 0.5	6.0 ± 0.6	6.3 ± 0.4	6.1 ± 0.3
Food consumption during study period (g/animal/day)				
Pre-mating day 1 to post-mating day 14	6.4 ± 0.6	6.6 ± 0.5	6.5 ± 0.4	6.4 ± 0.4
Females				
Food consumption (g/animal/day)				
Pre-mating period				
Day 1–8	5.7 ± 0.7	5.5 ± 0.8	5.7 ± 0.6	5.7 ± 0.7
Day 8–14	6.3 ± 0.6	6.3 ± 0.4	6.1 ± 0.5	5.9 ± 1.1
Gestation period				
Day 0–3	6.2 ± 0.9	6.5 ± 0.5	6.4 ± 1.1	6.3 ± 0.8
Day 3–7	6.7 ± 0.9	6.6 ± 0.4	6.6 ± 0.6	6.8 ± 0.9
Day 7–10	7.5 ± 0.7	7.5 ± 0.5	7.7 ± 0.8	7.6 ± 1.1
Day 10–12	7.4 ± 0.8	7.4 ± 0.7	7.6 ± 0.8	7.7 ± 0.5
Food consumption during study period (g/animal/day)				
Pre-mating day 1 to gestation day 12	6.4 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	6.3 ± 0.6

Notes: All values are presented as the mean ± standard deviation for 22 mice, except for gestation period values in female mice, where the animal number was 20, 21, 20, and 19 for 0, 60, 250, and 1000 mg/kg, respectively.

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0 mg/kg in males), discoloration of the liver (*N*=1, 250 mg/kg in males), decreased prostate (*N*=1, 0 and 60 mg/kg in males, respectively), decreased seminal vesicles (*N*=1, 1000 mg/kg in males), and cysts in the ovary (*N*=1, 0 and 250 mg/kg in females, respectively), were observed, these findings were considered to be incidental or spontaneous since there were no dose-dependency and/or were observed with low frequency.

Hematology and serum chemistry results between control and SiO_2NPs treatment groups were comparable in clinical pathology analyses (Tables 4 and 5). No statistically significant differences in hematological and serum chemistry results between the animals treated with SiO_2NPs and the vehicle control for each parameter in both males and females.

Organ weight (absolute and relative) measurements between control and SiO_2NPs treatment groups were comparable (Table 6). Although a statistically significant decrease in female absolute lung weights (decreased by 6% from control value) was observed, it was not considered to be SiO_2NPs treatment-related change since there were no correlated changes in the other parameters and the change was relatively small. There were no statistically significant differences in other organ weights between the animals treated with SiO_2NPs and the vehicle control for each organ in both males and females.

Table 4 Hematology Results of Silicon Dioxide Nanoparticles-Exposed Male and Female Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
Males				
RBC (10^6/μL)	9.8 ± 0.5	9.9 ± 0.2	9.8 ± 0.5	9.8 ± 0.3
HGB (g/dL)	14.5 ± 0.5	15.0 ± 0.4	14.4 ± 0.5	14.3 ± 0.6
HCT (%)	48.9 ± 1.6	50.1 ± 1.5	48.5 ± 1.9	48.5 ± 1.6
MCV (fL)	50.1 ± 1.7	50.5 ± 0.5	49.7 ± 1.9	49.8 ± 1.8
MCH (pg)	14.9 ± 0.4	15.1 ± 0.3	14.8 ± 0.6	14.6 ± 0.7
MCHC (g/dL)	29.7 ± 0.4	29.9 ± 0.5	29.7 ± 0.6	29.4 ± 0.4
RDW (%)	12.6 ± 0.3	12.8 ± 0.5	13.2 ± 0.4	12.9 ± 0.3
PLT (10^3/μL)	1188 ± 178	1219 ± 185	1238 ± 187	1208 ± 157
RETA (10^9/L)	317 ± 15	341 ± 58	334 ± 57	363 ± 56
WBC (10^3/μL)	5.3 ± 2.2	5.5 ± 1.6	5.7 ± 3.4	7.0 ± 1.6
NEU (%)	17.2 ± 3.5	21.4 ± 4.9	15.2 ± 2.0	16.7 ± 3.3
NEUA (10^3/μL)	0.9 ± 0.3	1.1 ± 0.2	0.8 ± 0.5	1.1 ± 0.2
LYM (%)	77.4 ± 3.0	73.1 ± 5.4	79.7 ± 2.3	77.6 ± 4.2
LYMA (10^3/μL)	4.1 ± 1.9	4.1 ± 1.4	4.6 ± 2.8	5.4 ± 1.5
EOS (%)	1.6 ± 1.0	2.1 ± 0.7	1.8 ± 0.9	2.3 ± 0.9
EOSA (10^3/μL)	0.07 ± 0.03	0.11 ± 0.04	0.10 ± 0.06	0.16 ± 0.05
MON (%)	3.2 ± 0.7	2.7 ± 0.7	2.4 ± 0.7	2.5 ± 1.0
MONA (10^3/μL)	0.16 ± 0.07	0.14 ± 0.03	0.13 ± 0.06	0.17 ± 0.07
BAS (%)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.0
BASA (10^3/μL)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01
LUC (%)	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.3	0.6 ± 0.1
LUCA (10^3/μL)	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.03	0.04 ± 0.02
HDW (g/dL)	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
MPV (fL)	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.3	4.6 ± 0.1
MAC (%)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
MIC (%)	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
HPE (%)	0.2 ± 0.1	0.3 ± 0.4	0.4 ± 0.3	0.3 ± 0.1
HPO (%)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
RBCFr (10^6/μL)	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
RBCGh (10^6/μL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

(*Continued*)

Table 4 (Continued).

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
Females				
RBC (10^6/μL)	8.3 ± 0.5	8.4 ± 0.4	8.2 ± 0.6	8.6 ± 0.3
HGB (g/dL)	13.2 ± 0.6	13.6 ± 0.7	13.3 ± 0.6	13.4 ± 0.7
HCT (%)	45.4 ± 1.3	46.1 ± 1.9	45.3 ± 2.4	46.1 ± 2.0
MCV (fL)	55.1 ± 2.3	54.8 ± 1.3	55.7 ± 3.3	54.0 ± 1.5
MCH (pg)	16.1 ± 0.5	16.2 ± 0.5	16.4 ± 1.1	15.7 ± 0.7
MCHC (g/dL)	29.2 ± 0.4	29.6 ± 0.5	29.4 ± 0.6	29.1 ± 0.5
RDW (%)	16.4 ± 0.8	16.1 ± 0.4	17.5 ± 0.4	16.7 ± 0.9
PLT (10^3/μL)	1137 ± 24	1137 ± 327	1001 ± 173	1247 ± 232
RETA (10^9/L)	1038 ± 62	1095 ± 89	1177 ± 255	1056 ± 99
WBC (10^3/αL)	4.4 ± 1.2	5.2 ± 1.0	4.5 ± 0.5	5.6 ± 1.2
NEU (%)	27.7 ± 4.3	25.9 ± 4.0	26.2 ± 4.7	24.9 ± 3.9
NEUA (10^3/αL)	1.2 ± 0.3	1.3 ± 0.1	1.2 ± 0.2	1.4 ± 0.4
LYM (%)	66.9 ± 4.1	67.6 ± 5.3	68.6 ± 3.9	69.2 ± 4.2
LYMA (10^3/μL)	2.9 ± 0.9	3.6 ± 0.9	3.1 ± 0.5	3.8 ± 0.8
EOS (%)	1.7 ± 0.4	2.1 ± 0.8	1.7 ± 0.4	2.2 ± 0.9
EOSA (10^3/μL)	0.07 ± 0.03	0.11 ± 0.04	0.07 ± 0.01	0.13 ± 0.07
MON (%)	3.0 ± 1.0	3.5 ± 1.2	2.8 ± 1.0	2.8 ± 0.3
MONA (10^3/μL)	0.14 ± 0.08	0.18 ± 0.04	0.13 ± 0.06	0.16 ± 0.03
BAS (%)	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
BASA (10^3/μL)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
LUC (%)	0.4 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
LUCA (10^3/μL)	0.02 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
HDW (g/dL)	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1
MPV (fL)	5.1 ± 0.3	5.2 ± 0.3	5.2 ± 0.1	5.1 ± 0.3
MAC (%)	2.2 ± 1.7	1.5 ± 0.7	3.5 ± 3.7	1.3 ± 0.3
MIC (%)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
HPE (%)	0.6 ± 0.3	0.6 ± 0.5	0.8 ± 0.5	0.7 ± 0.4
HPO (%)	0.7 ± 0.3	0.6 ± 0.2	0.7 ± 0.3	0.6 ± 0.1
RBCFr (10^6/μL)	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
RBCGh (10^6/μL)	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0

Notes: All values are presented as the mean ± standard deviation for 5 mice.

Abbreviations: RBC, total red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelet count; RETA, absolute reticulocyte count; WBC, total leukocyte count; NEU, relative neutrophils count; NEUA, absolute neutrophil count; LYM, relative lymphocyte count; LYMA, absolute lymphocyte count; EOS, relative eosinophil count; EOSA, absolute eosinophil count; MON, relative monocyte count; MONA, absolute monocyte count; BAS, relative basophil count; BASA, absolute basophil count; LUC, relative large unstained cell count; LUCA, absolute large unstained cell count; HDW, hemoglobin distribution width; MPV, Mean platelet volume; MAC, macrocytosis; MIC, microcytosis; HPE, hyperchromacia; HPO, hypochromacia; RBCFr, RBC fragments; RBCGh, RBC ghosts.

Effects of SiO_2NPs on Fertility and Early Embryonic Development

Estrus cycle examination revealed no SiO_2NPs treatment-related abnormalities in the length and regularity of the estrus cycle (Table 7). Similarly, in case of fertility indices, no statistically significant differences were recorded in the mating index, fertility index, fecundity index, pregnancy index, and precoital time between the animals treated with SiO_2NPs and the vehicle control in both males and females (Table 7). One female each at 0 and 1000 mg/kg was not observed to have mated at the initial observation but was later identified as pregnant based on external observation, increased body weight, and the presence of implantation sites in the uterus.

Table 5 Clinical Chemistry Results of Silicon Dioxide Nanoparticles-Exposed Male and Female Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
Males				
GLU (mg/dL)	218.3 ± 39.1	221.8 ± 24.2	217.8 ± 37.3	201.5 ± 32.2
BUN (mg/dL)	24.0 ± 5.6	21.0 ± 1.4	22.5 ± 3.8	24.9 ± 4.1
CREA (mg/dL)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
TP (g/dL)	5.5 ± 0.2	5.7 ± 0.1	5.5 ± 0.2	5.6 ± 0.2
ALB (g/dL)	3.4 ± 0.1	3.5 ± 0.2	3.3 ± 0.1	3.4 ± 0.2
A/G (ratio)	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.0	1.6 ± 0.1
GLO (g/dL)	2.1 ± 0.1	2.2 ± 0.0	2.2 ± 0.1	2.2 ± 0.1
AST (IU/L)	74.0 ± 46.4	52.1 ± 4.6	63.4 ± 30.1	48.9 ± 8.8
ALT (IU/L)	46.3 ± 13.8	36.1 ± 3.8	49.6 ± 27.9	42.8 ± 17.0
TBIL (mg/dL)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
ALP (IU/L)	143.8 ± 33.3	183.7 ± 38.1	165.7 ± 23.2	164.4 ± 25.8
TCHO (mg/dL)	129.8 ± 15.6	121.0 ± 16.9	135.8 ± 18.9	123.2 ± 11.2
TG (mg/dL)	74.1 ± 18.6	87.8 ± 32.8	80.9 ± 28.9	70.9 ± 24.2
Ca (mg/dL))	9.9 ± 0.3	10.0 ± 0.2	10.1 ± 0.4	10.3 ± 0.4
IP (mg/dL)	7.9 ± 0.6	7.4 ± 0.6	7.8 ± 0.7	9.0 ± 0.8
K (mmol/L)	8.3 ± 0.6	8.4 ± 0.3	8.3 ± 0.8	9.0 ± 0.4
CK (IU/L)	133.6 ± 50.7	130.4 ± 37.8	145.6 ± 37.4	118.2 ± 31.6
Na (mmol/L)	156.0 ± 1.4	157.0 ± 1.9	156.8 ± 1.9	157.6 ± 0.9
Cl (mmol/L)	111.6 ± 1.5	112.8 ± 1.3	111.8 ± 1.1	113.2 ± 1.1
Females				
GLU (mg/dL)	125.7 ± 15.5	119.4 ± 9.3	113.3 ± 10.0	131.0 ± 19.6
BUN (mg/dL)	18.2 ± 1.7	19.3 ± 3.8	19.2 ± 3.5	22.2 ± 3.5
CREA (mg/dL)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
TP (g/dL)	4.5 ± 0.1	4.7 ± 0.3	4.8 ± 0.2	4.6 ± 0.4
ALB (g/dL)	2.7 ± 0.1	2.8 ± 0.3	2.8 ± 0.2	2.8 ± 0.3
A/G (ratio)	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
GLO (g/dL)	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
AST (IU/L)	67.5 ± 2.8	78.1 ± 4.8	77.9 ± 9.7	79.7 ± 11.0
ALT (IU/L)	26.7 ± 3.7	27.8 ± 5.4	27.3 ± 2.9	32.1 ± 3.1
TBIL (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
ALP (IU/L)	114.2 ± 20.2	113.4 ± 24.5	138.6 ± 21.8	133.8 ± 18.0
TCHO (mg/dL)	72.8 ± 5.3	85.6 ± 7.4	86.8 ± 13.4	79.4 ± 2.7
TG (mg/dL)	159.9 ± 18.1	194.1 ± 35.6	162.7 ± 31.5	212.5 ± 61.2
Ca (mg/dL))	10.2 ± 0.2	10.5 ± 0.5	10.1 ± 0.2	10.2 ± 0.5
IP (mg/dL)	9.9 ± 1.1	10.0 ± 1.4	9.6 ± 2.2	8.6 ± 1.4
K (mmol/L)	8.4 ± 0.9	8.3 ± 1.0	7.6 ± 0.7	8.2 ± 2.1
CK (IU/L)	151.2 ± 51.7	206.8 ± 68.6	173.8 ± 41.8	168.6 ± 32.5
Na (mmol/L)	153.8 ± 0.8	153.2 ± 1.1	154.2 ± 1.3	152.2 ± 0.8
Cl (mmol/L)	113.8 ± 0.8	112.2 ± 1.3	112.0 ± 1.6	112.2 ± 1.5

Notes: All values are presented as the mean ± standard deviation for 5 mice.
Abbreviations: GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; TP, total protein; ALB, albumin; A/G, albumin/globulin ratio; GLO, globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase; TCHO, total cholesterol; TG, triglyceride; Ca, calcium; IP, inorganic phosphorus; K, potassium; CK, creatine phosphokinase; Na, sodium; Cl, chloride.

Sperm analysis revealed no SiO_2NPs treatment-related abnormalities in sperm number, morphology, and motility in this study (Table 8). Although several endpoints in sperm analysis changed slightly at 1000 mg/kg SiO_2NPs, the changes were not considered SiO_2NPs treatment-related since the alterations were attributed to incidental sperm abnormalities in a single male, which occurred spontaneously.

Table 6 Absolute and Relative Organ Weights of Silicon Dioxide Nanoparticles-Exposed Male and Female Mice

		Silicon Dioxide Nanoparticles (mg/kg)			
		0	**60**	**250**	**1000**
Males (N)		22	22	22	22
Brain	Absolute (mg)	481.9 ± 17.2	484.5 ± 22.4	482.4 ± 25.5	474.4 ± 18.7
	Relative[a] (%)	1.21 ± 0.08	1.21 ± 0.07	1.19 ± 0.07	1.19 ± 0.09
Spleen	Absolute (mg)	99.8 ± 15.2	105.3 ± 17.7	108.1 ± 16.4	102.0 ± 12.3
	Relative (%)	0.25 ± 0.04	0.26 ± 0.04	0.27 ± 0.04	0.25 ± 0.03
Adrenal glands	Absolute (mg)	5.3 ± 0.9	5.2 ± 1.2	5.3 ± 1.1	5.4 ± 0.9
	Relative (%)	0.013 ± 0.002	0.013 ± 0.003	0.013 ± 0.003	0.013 ± 0.002
Liver	Absolute (mg)	2181.8 ± 181.1	2192.4 ± 152.4	2246.3 ± 168.2	2201.9 ± 163.9
	Relative (%)	5.46 ± 0.39	5.47 ± 0.35	5.56 ± 0.39	5.48 ± 0.27
Lungs	Absolute (mg)	217.7 ± 17.1	216.4 ± 11.0	219.0 ± 20.0	215.7 ± 20.6
	Relative (%)	0.55 ± 0.05	0.54 ± 0.04	0.54 ± 0.03	0.54 ± 0.05
Thymus	Absolute (mg)	43.0 ± 10.7	38.1 ± 7.4	41.9 ± 10.0	40.3 ± 8.3
	Relative (%)	0.11 ± 0.03	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02
Right testis	Absolute (mg)	125.3 ± 18.8	129.5 ± 19.8	129.9 ± 21.0	124.0 ± 11.8
	Relative (%)	0.31 ± 0.05	0.32 ± 0.05	0.32 ± 0.05	0.31 ± 0.03
Left testis	Absolute (mg)	118.3 ± 17.8	124.3 ± 21.5	124.0 ± 19.4	119.4 ± 14.0
	Relative (%)	0.30 ± 0.05	0.31 ± 0.05	0.31 ± 0.05	0.30 ± 0.03
Right epididymis	Absolute (mg)	52.9 ± 7.4	52.9 ± 4.8	51.3 ± 6.9	49.8 ± 4.4
	Relative (%)	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.12 ± 0.01
Left epididymis	Absolute (mg)	51.8 ± 6.0	53.3 ± 6.0	52.6 ± 7.0	50.3 ± 4.8
	Relative (%)	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01
Seminal vesicles with coagulating glands	Absolute (mg)	394.1 ± 57.0	381.0 ± 58.7	376.1 ± 83.7	390.5 ± 88.0
	Relative (%)	0.99 ± 0.15	0.95 ± 0.13	0.93 ± 0.21	0.97 ± 0.20
Prostate	Absolute (mg)	20.1 ± 6.0	18.8 ± 7.0	18.9 ± 6.1	19.9 ± 6.2
	Relative (%)	0.050 ± 0.015	0.047 ± 0.017	0.047 ± 0.014	0.050 ± 0.016
Right kidney	Absolute (mg)	371.8 ± 43.5	393.7 ± 55.1	387.4 ± 41.6	386.5 ± 46.8
	Relative (%)	0.93 ± 0.11	0.98 ± 0.12	0.96 ± 0.10	0.96 ± 0.10
Left kidney	Absolute (mg)	356.2 ± 36.1	381.6 ± 46.8	369.2 ± 38.2	380.9 ± 56.5
	Relative (%)	0.89 ± 0.10	0.95 ± 0.11	0.91 ± 0.09	0.95 ± 0.14
Females (N)		20	21	20	19
Brain	Absolute (mg)	506.8 ± 18.2	507.5 ± 17.4	505.3 ± 21.7	501.6 ± 24.6
	Relative (%)	1.15 ± 0.07	1.16 ± 0.07	1.16 ± 0.08	1.15 ± 0.07
Spleen	Absolute (mg)	328.3 ± 44.6	314.7 ± 69.7	325.0 ± 56.0	326.5 ± 50.8
	Relative (%)	0.74 ± 0.11	0.71 ± 0.13	0.74 ± 0.11	0.75 ± 0.10
Adrenal glands	Absolute (mg)	12.1 ± 2.2	12.5 ± 2.8	13.2 ± 2.7	11.9 ± 2.6
	Relative (%)	0.027 ± 0.005	0.028 ± 0.006	0.030 ± 0.006	0.027 ± 0.006
Liver	Absolute (mg)	2855.2 ± 178.1	2851.1 ± 211.4	2849.9 ± 223.5	2756.8 ± 203.0
	Relative (%)	6.47 ± 0.37	6.49 ± 0.31	6.51 ± 0.31	6.31 ± 0.24
Lungs	Absolute (mg)	236.8 ± 13.9	229.8 ± 14.0	228.1 ± 15.1	222.6 ± 14.2**
	Relative (%)	0.54 ± 0.04	0.52 ± 0.03	0.52 ± 0.03	0.51 ± 0.04
Thymus	Absolute (mg)	52.4 ± 10.6	50.1 ± 13.9	51.1 ± 8.6	51.2 ± 9.4
	Relative (%)	0.12 ± 0.02	0.11 ± 0.03	0.12 ± 0.02	0.12 ± 0.02
Uterus/cervix	Absolute (mg)	5959.8 ± 757.2	5789.7 ± 1011.0	5313.3 ± 918.3	5556.3 ± 1048.2
	Relative (%)	13.5 ± 1.4	13.1 ± 2.1	12.1 ± 1.8	12.7 ± 2.1
Right ovary	Absolute (mg)	10.9 ± 2.2	11.3 ± 2.4	11.1 ± 3.2	10.0 ± 1.7
	Relative (%)	0.025 ± 0.005	0.026 ± 0.005	0.025 ± 0.007	0.023 ± 0.004
Left ovary	Absolute (mg)	10.3 ± 3.3	10.9 ± 3.3	9.9 ± 2.6	8.8 ± 2.4
	Relative (%)	0.023 ± 0.007	0.025 ± 0.007	0.023 ± 0.006	0.020 ± 0.005
Right kidney	Absolute (mg)	265.4 ± 20.1	266.7 ± 20.9	266.6 ± 20.2	260.1 ± 20.9
	Relative (%)	0.60 ± 0.04	0.61 ± 0.05	0.61 ± 0.04	0.60 ± 0.04
Left kidney	Absolute (mg)	261.2 ± 16.6	258.7 ± 21.6	256.9 ± 17.3	248.4 ± 20.3
	Relative (%)	0.59 ± 0.04	0.59 ± 0.05	0.59 ± 0.04	0.57 ± 0.04

Notes: [a]Organ weight/terminal body weight ratio. All values are presented as the mean ± standard deviation, $^{**}p < 0.01$, compared with the control group.

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Table 7 Estrus Cycle Examination and Fertility-Related Results of Silicon Dioxide Nanoparticles-Exposed Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
No. of males	22	22	22	22
No. of females	22	22	22	22
Estrus cycle examinations				
Length (day)				
Mean	4.4	4.1	4.3	4.5
Sdev	0.5	0.3	0.5	0.4
Regularity				
Regular (%)	21 (95.5)	21 (95.5)	22 (100.0)	21 (95.5)
Irregular (%)	1 (4.5)	1 (4.5)	0 (0.0)	1 (4.5)
Fertility indices				
Males				
Mating index[a] (%)	22/22 (100.0)	21/22 (95.5)	20/22 (90.9)	20/22 (90.9)
Fertility index[b] (%)	21/22 (95.5)	21/22 (95.5)	20/22 (90.9)	20/22 (90.9)
Fecundity index[c] (%)	21/22 (95.5)	21/21 (100.0)	20/22 (90.9)	20/22 (90.9)
Females				
Mating index[d] (%)	22/22 (100.0)	21/22 (95.5)	20/22 (90.9)	20/22 (90.9)
Fertility index[e] (%)	21/22 (95.5)	21/22 (95.5)	20/22 (90.9)	20/22 (90.9)
Pregnancy index[f] (%)	21/22 (95.5)	21/21 (100.0)	20/22 (90.9)	20/22 (90.9)
Precoital time (day)				
Mean	2.6	1.9	2.1	2.4
Sdev	1.9	1.0	1.1	1.4

Notes: [a] (No. of males with evidence of mating/No. of males paired) × 100. [b] (No. of males impregnating a female/No. of males paired) × 100. [c] (No. of males impregnating a female/No. of males with evidence of mating) × 100. [d] (No. of females with evidence of mating/No. of females paired) × 100. [e] (No. of pregnant females/No. of females paired) × 100. [f] (No. of pregnant females/No. of females with evidence of mating) × 100.

Table 8 Sperm Analysis Results of Silicon Dioxide Nanoparticles-Exposed Male Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
No. of males	22	22	22	22
Sperm number				
Testis (10^6/testis)	61.4 ± 5.3	59.0 ± 3.5	59.7 ± 5.8	57.7 ± 6.1
Epididymis (10^6/epididymis)	50.2 ± 6.2	48.3 ± 6.3	47.4 ± 3.1	46.5 ± 10.7
Sperm morphology				
Normal shape (N)	194.4 ± 2.6	194.3 ± 3.0	194.5 ± 2.8	185.1 ± 40.6
Abnormal shape (N)	5.6 ± 2.6	5.7 ± 3.0	5.5 ± 2.8	14.9 ± 40.6
Morphological abnormality of sperms (%)	2.8 ± 1.3	2.8 ± 1.5	2.7 ± 1.4	7.5 ± 20.3
Sperm motility				
MOT (%)	95.5 ± 2.8	96.5 ± 2.7	96.8 ± 2.0	92.2 ± 20.7
VAP (μm/s)	188.1 ± 21.0	173.0 ± 24.7	172.9 ± 29.5	174.7 ± 48.3
VSL (μm/s)	169.2 ± 22.5	152.4 ± 25.0	150.5 ± 31.3	155.0 ± 46.0
VCL (μm/s)	297.6 ± 30.5	287.1 ± 34.7	288.8 ± 37.1	282.9 ± 70.6
ALH (μm)	12.1 ± 1.5	12.6 ± 1.3	12.7 ± 1.3	12.1 ± 3.1
BCF (Hz)	32.6 ± 3.1	32.3 ± 3.0	30.6 ± 4.2	30.3 ± 7.2
STR (%)	88.5 ± 3.8	86.3 ± 3.8	85.2 ± 5.5	83.0 ± 19.1
LIN (%)	57.3 ± 6.0	53.6 ± 5.4	52.3 ± 7.6	52.4 ± 14.3

Notes: All values are presented as the mean ± standard deviation.

Abbreviations: MOT, percentage of motile sperm; VAP, average velocity of the smoothed cell path; VSL, average velocity measured in a straight line from the beginning to the end of the track; VCL, the average velocity measured over the actual point-to-point track followed by the cell; ALH, the mean width of the head oscillation as the sperm cells swim; BCF, frequency of sperm head crossing the average path in either direction; STR, average value of the ratio VSL/VAP; LIN, average value of the ratio VSL/VCL.

Table 9 Caesarean Section Results of Silicon Dioxide Nanoparticles-Exposed Female Mice

	Silicon Dioxide Nanoparticles (mg/kg)			
	0	**60**	**250**	**1000**
No. of pregnant females	20	21	20	19
Corpora lutea (N)	16.0 ± 1.8	16.0 ± 2.1	15.1 ± 2.0	15.0 ± 1.6
Implantation (N)	15.6 ± 1.9	15.3 ± 2.9	14.6 ± 2.7	14.4 ± 1.7
Early resorption (N)	0.7 ± 0.7	0.8 ± 1.0	1.0 ± 0.9	0.5 ± 0.9
Late resorption (N)	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Dead embryo (N)	0.1 ± 0.2	0.0 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
Fetal death[a] (N)	0.8 ± 0.7	0.9 ± 1.0	1.0 ± 1.0	0.6 ± 0.9
Live embryo (N)	14.9 ± 1.8	14.5 ± 3.0	13.6 ± 2.8	13.8 ± 2.1
Pre-implantation loss[b] (%)	2.2 ± 4.5	4.6 ± 12.0	3.7 ± 10.4	3.9 ± 4.6
Post-implantation loss[c] (%)	4.7 ± 4.4	6.1 ± 7.4	7.2 ± 8.0	4.2 ± 6.8

Notes: [a]No. of resorptions/litter + No. of dead embryo/litter. [b][(No. of corpora lutea/litter – No. of implantation/litter) / No. of corpora lutea/litter] × 100. [c][(No. of implantation/litter – No. of live embryo/litter) / No. of implantation/litter] × 100. All values are mean ± standard deviation.

Caesarean section results between control and SiO_2NPs treatment groups were comparable in this study (Table 9). No statistically significant differences in all endpoints of caesarean section between the animals treated with SiO_2NPs and the vehicle control were observed.

Discussion

The objective of this study was to investigate the potential toxicological effects of SiO_2NPs on fertility and early embryonic development in mice. SiO_2NPs are the most extensively utilized non-metal oxide nanoparticles and widely applied in food industry, cosmetics, biomedical applications, and drug carriers.[5,32,33] Oral exposure to SiO_2NPs is a major human exposure route due to their widespread use in food additives (E 551), dietary supplements, and pharmaceuticals to enhance stability, bioavailability, and ingestion safety.[21–23] While previous studies have mainly focused on the systemic toxicity of SiO_2NPs, their potential effects on developmental and reproductive functions remain poorly understood. Additionally, conflicting findings on SiO_2NPs toxicity highlight the need for well-designed in vivo studies to clarify their effects on reproductive and developmental functions. Therefore, in this study, SiO_2NPs were administered to ICR mice by oral gavage at doses of 0, 60, 250, and 1000 mg/kg during the pre-mating, mating, and early gestation periods in accordance with the ICH S5(R3) guideline.[25] The results of this study showed no substantial SiO_2NPs-related toxicological effects on female fertility, tubal transport, implantation, early embryonic development, and functional effects on male fertility.

In vitro studies on SiO_2NPs reported significant cytotoxic effects in various cell lines. For example, Peivandi et al reported a dose-dependent decreased cell viability at SiO_2NPs concentrations ranging from 1 to 100 μg/mL in lung epithelial cells (A549).[7] Lee et al also reported cytotoxic effects of SiO_2NPs at a concentration of 30 μg/mL in human hepatocellular carcinoma cells (HepG2).[34] Additionally, Kobayashi et al reported SiO_2NPs induced lysosomal dysfunction in human choriocarcinoma cells (JEG-3) at 25 μg/mL.[35] From a mechanistic perspective, oxidative stress has been identified as one of the main causes of SiO_2NPs-induced toxicity.[36,37] Similar to SiO_2NPs, oxidative stress induced by other types of nanoparticles also damages cellular components, leading to cytotoxicity.[38,39] Yang et al recently suggested that the main mechanism of nanotoxicity is the generation of reactive oxygen species, which can cause oxidative stress and damage cellular organelles, DNA, cell membranes, ion channels, and cell surface receptors, leading to toxicological effects.[40]

In vivo studies on SiO_2NPs suggest that they can induce toxicological effects, but the results across studies remain inconsistent.[11] In addition to the previously described SiO_2NPs-related toxicological results in in vivo studies, a single oral gavage administration of SiO_2NPs (83 nm, 40 mg/kg) in mice resulted in renal damage characterized by renal tubular necrosis, hemorrhage, and vascular congestion.[41] Another study reported that 50-day repeated intraperitoneal

injection of SiO_2NPs (10 nm, 2 mg/kg) in rats decreased body weight gain, altered clinical pathology parameters, induced liver histopathological damage, and significantly downregulated the expression of drug-metabolizing enzyme genes.[42] Sasai et al also reported that 13-week repeated (twice weekly) oropharyngeal aspiration of SiO_2NPs (200 and 300 nm, 4 mg/dose) in rats induced histopathological abnormalities in lung and kidney.[43] In contrast to these study results, a 90-day repeated oral gavage toxicity study in rats administered SiO_2NPs (20 and 100 nm) at high doses (500, 1000, and 2000 mg/kg/day) reported no adverse toxicological effects at any evaluated endpoint.[14] Similarly, another 90-day repeated oral gavage study using differently sized SiO_2NPs (25.9 nm) at doses of 166.7, 500, and 1500 mg/kg in rats also found no significant toxic effects.[44] These contradictory results indicate that SiO_2NPs toxicity may be highly influenced by study designs, including the species of laboratory animals, exposure durations, dose levels, routes of administration, as well as the physicochemical properties of nanoparticles.

In the present study, repeated oral gavage administration of SiO_2NPs did not exert toxicological effects in female fertility, tubal transport, implantation, early embryonic development, and functional effects on male fertility. Although studies on the developmental and reproductive toxicity of SiO_2NPs remain limited, results of this study are consistent with previous oral gavage administration-based developmental and reproductive toxicity studies of SiO_2NPs. Hofmann et al reported that oral gavage administration of SiO_2NPs (10–25 nm, 100, 300, and 1000 mg/kg) from GD 6 to 19 in rats did not cause any toxicological effects on general health of pregnant females, caesarean section parameters, and fetal morphological examinations.[18] Similarly, Wolterbeek et al reported that oral gavage administration of SiO_2NPs (409–-1664 nm, 100, 300, and 1000 mg/kg) across two generations of rats did not exert toxicological effects on reproductive performance and offspring development.[19] However, in contrast to these oral gavage administration studies on rats, Yamashita et al reported that intravenous administration of SiO_2NPs (70 nm, 0.8 mg/animal) from GD 16 to 17 in mice resulted in developmental toxicity, including an increased resorption rate and decreased fetal body weight.[45] Additionally, Liu et al reported that 15-day repeated (every 3 days, a total of five times) tracheal perfusion of SiO_2NPs (43 nm, 7, 21, and 35 mg/kg) in pregnant mice resulted in ovarian damage, imbalance of sex hormones, and increase the number of atretic follicles.[46]

The discrepancies in the toxicological effects of nanoparticles can be attributed to multiple factors, as supported by previous studies. Previous studies suggested that discrepancies in reproductive and developmental toxicity results among studies on manufactured nanoparticles may result from differences in the physicochemical properties of nanoparticles, experimental models, exposure timing across developmental stages, and the selection of evaluation endpoints.[6,47–49] Additionally, Albanese et al suggested that key physicochemical properties of nanoparticles, including size, shape, chemical functionality, surface charge, and composition, significantly influence the toxicities of nanoparticles.[50] Pan et al further demonstrated that nanoparticle-induced cytotoxicity exhibits a size-dependent effect across multiple cell lines.[51] In this study, gold nanoparticles with a diameter of 1.4 nm induced the highest cytotoxicity, whereas those with a diameter of 15 nm showed no cytotoxicity. Beyond physicochemical properties, the route of exposure also plays a critical role in nanoparticle toxicity. Park et al reported that exposure routes directly impact the internal tissue distribution of nanoparticles.[52] In this study, orally administered cerium oxide nanoparticles were rarely detected in the bloodstream or internal tissues, whereas intravenously administered nanoparticles led to a relatively high systemic distribution of cerium. Additionally, Wang et al reported that susceptibility to nanoparticle toxicity varies with age, demonstrating that titanium dioxide nanoparticles caused severe liver edema, heart injury, and mast cell activation in the stomach tissues of young rats, while only mild injuries were observed in the liver and kidney tissues of adult rats.[53] These findings underscore the complexity of extrapolating nanoparticle toxicity data from animal models to human risk assessment.

Risk assessment is a multi-step process used to support decision-making regarding the safety of chemicals in commercial, industrial, and environmental contexts.[54] Risk assessment of nanoparticles for both human health and the environment is essential, considering their increasing incorporation into consumer products and industrial applications. However, accurate nanoparticle risk assessment remains a significant challenge considering the complex interplay of multiple influencing factors, such as the physicochemical properties of nanoparticles and study designs, all of which determine their biological and toxicological effects.[55] Additionally, nanoparticles have a long and complex value chain, including synthesis, manufacturing, product use, disposal, and environmental release, which results in multiple potential

exposure pathways for both humans and the environment.[56] To address these complexities, the scientific community is actively working to improve the standardization and transparency of nanotoxicological research. Recent initiatives emphasize the importance of high standards of characterization in reporting bio-nano experimental studies.[49,57] In addition to these efforts, further toxicological studies using a wide variety of nanoparticles with different physicochemical properties and study designs are needed to ensure a comprehensive risk assessment for human health and the environment.

In this study, we investigated the potential toxicological effects of SiO_2NPs on fertility and early embryonic development in mice. However, the study did not assess other critical reproductive and developmental endpoints, such as prenatal development during pregnancy, parturition, and post-natal development of offspring. Additionally, it is necessary to evaluate the potential toxicological effects of SiO_2NPs via inhalation, as this represents one of the most common exposure routes to nanoparticles in humans. These limitations suggest that further toxicological studies are needed to investigate the effects of SiO_2NPs with diverse physicochemical properties and study designs to better understand their potential risks to human health and the environment.

Conclusion

In summary, this study is the first to investigate the potential toxicological effects of SiO_2NPs on fertility and early embryonic development in accordance with the ICH S5(R3) guideline. Oral administration of SiO_2NPs during the pre-mating, mating, and early gestation periods did not induce any adverse effects on female fertility indices, tubal transport, implantation outcomes, early embryonic development, and male fertility function at doses up to 1000 mg/kg. Additionally, no SiO_2NPs-related adverse effects on the general systemic health of parental animals were observed at doses up to 1000 mg/kg, including clinical signs, body weight, food consumption, clinical pathology, macroscopic observations, and organ weights. The results of this study provide important data to support the human health risk assessment of SiO_2NPs. Further toxicological studies are needed to address their diverse physicochemical characteristics, widespread applications, and various potential exposure routes.

Abbreviations

ALB, Albumin; ALH, The mean width of the head oscillation as the sperm cells swim; ALP, alkaline phosphatase; ALT, Alanine aminotransferase; A/G, Albumin/globulin ratio; AST, Aspartate aminotransferase; BAS, Relative basophil count; BASA, Absolute basophil count; BCF, Frequency of sperm head crossing the average path in either direction; BUN, Blood urea nitrogen; Ca, Calcium; CK, Creatine phosphokinase; Cl, Chloride; CREA, Creatinine; DLS, Dynamic light scattering; EOS, Relative eosinophil count; EOSA, Absolute eosinophil count; GD, Gestation day; GLO, globulin; GLP, Good laboratory practice; GLU, Glucose; HCT, Hematocrit; HDW, Hemoglobin distribution width; HGB, Hemoglobin; HPE, Hyperchromacia; HPO, Hypochromacia; IP, Inorganic phosphorus; K, Potassium; KIT, Korea Institute of Toxicology; LIN, Average value of the ratio VSL/VCL; LUC, Relative large unstained cell count; LUCA, Absolute large unstained cell count; LYM, Relative lymphocyte count; LYMA, Absolute lymphocyte count; MAC, Macrocytosis; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; MCV, Mean corpuscular volume; MIC, Microcytosis; MON, Relative monocyte count; MONA, Absolute monocyte count; MOT, Percentage of motile sperm; MPV, Mean platelet volume; Na, Sodium; NEU, Relative neutrophils count; NEUA, Absolute neutrophil count; PLT, Platelet count; RBC, Total red blood cell count; RBCGh, RBC ghosts; RBCFr, RBC fragments; RDW, Red cell distribution width; RETA, Absolute reticulocyte count; SiO_2NPs, Silicon dioxide nanoparticles; STR, Average value of the ratio VSL/VAP; TBIL, Total bilirubin; TCHO, Total cholesterol; TEM, Transmission electron microscopy; TG, Triglyceride; TP, Total protein; VAP, Average velocity of the smoothed cell path; VCL, The average velocity measured over the actual point-to-point track followed by the cell; VSL, Average velocity measured in a straight line from the beginning to the end of the track; WBC, Total leukocyte count.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no potential conflicts of interest in this article.

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