Safety assessment of cerium oxide nanoparticles: Combined repeated-dose toxicity with reproductive/developmental toxicity screening and biodistribution in rats

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Abstract

Background Cerium oxide nanoparticles (CeO₂ NPs) are widely used in various commercial applications because of their characteristic properties. CeO₂ NPs can be easily exposed to humans in real life, but the safety assessment of CeO₂ NPs has not been fully investigated. Therefore, in this study, we conducted a combined repeated-dose and reproductive/developmental toxicity screening study to investigate the potential effects on general health hazards, including reproductive/developmental functions, after repeated CeO₂ NPs oral exposure. In addition, tissues from parental animals and their pups were collected to analyze the internal accumulation of cerium. CeO₂ NPs were orally administered to Sprague-Dawley rats at doses of 0, 100, 300 and 1000 mg/kg during their pre-mating, mating, gestation and early lactation periods.

Results In the general systemic and reproductive/developmental examinations, no marked toxicities were observed in any of in-life and terminal observation parameters in this study. In the biodistribution analysis, cerium was not detected in either parental or pup tissues (blood, liver, lungs and kidneys).

Conclusion Repeated oral exposure of CeO₂ NPs did not induce marked toxicities affecting general systemic and reproductive/developmental functions up to the dose level of 1000 mg/kg and the CeO₂ NPs were not systemically absorbed in parental animals or their pups. This result could be used to risk assessment for human, and additional toxicity studies with CeO₂ NPs will be necessary considering various physicochemical properties and exposure probabilities of this nanoparticles.

1. Introduction

Nanomaterial development and research have precipitously increased recently, and engineered nano-related items are continuously introduced to the market.[1] Cerium oxide nanoparticles (CeO₂ NPs) are one of the 13 priority listed representative manufactured nanomaterials to undergo assessments of the environmental safety and human health implications established by the Organization for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials.[2] CeO₂ NPs have been reported to have redox activity,[3] and their characteristic properties are used in a wide range of industrial areas, including UV-absorbing compounds in sunscreens, solid oxide fuel cells, catalytic converters for removing toxic gases and diesel fuel catalysts.[4, 5, 6, 7] Extensive usage of CeO₂ NPs in various areas has raised human health concerns because of occupational and environmental exposure. However, the potential adverse effects of CeO₂ NPs have not been widely investigated thus far.

Recent publications have suggested that CeO₂ NPs are able to cause environmental toxicity and health problems. CeO₂ NPs exposure in cyanobacterial and green alga was found to induce cytotoxicity through cell wall and membrane disruption.[8] In addition, cytotoxicity was also observed in human lung epithelial cells (BEAS-2B) after CeO₂ NPs exposure through increased reactive oxygen species.[9] In experimental animal studies, acute inhalation exposure of rats to CeO₂ NPs induced cytotoxicity via oxidative stress in the lungs, and these effects were considered able to aggravate chronic inflammation.[10] Another study

showed that exposure of rats to a single intravenous infusion of CeO₂ NPs led to body weight gain impairment, increased spleen weight and hepatic granulomas.[11] Moreover, these study results showed that CeO₂ NPs were able to be retained in internal organs, such as the spleen, liver and bone marrow. Consequently, these results support concerns about the adverse effects of long-term exposure to CeO₂ NPs in humans and the environment.

Nevertheless, the potential adverse effects of repeated CeO₂ NPs exposure have not been thoroughly investigated. In particular, developmental and reproductive toxicity studies with CeO₂ NPs have rarely been conducted. In this regard, the current study was conducted to assess the potential general systemic effects as well as developmental and reproductive effects after repeated CeO₂ NPs oral exposure based on an OECD guideline for testing of chemicals.[12] In addition, we collected maternal samples (blood, liver, lungs and kidneys) and F1 pup samples (blood, liver, lungs and kidneys) to analyze the internal systemic distribution of CeO₂ NPs after repeated oral exposure. Therefore, in this study, CeO₂ NPs were orally administered to Sprague-Dawley (SD) rats at doses of 0, 100, 300 and 1000 mg/kg from pre-mating to lactation periods, and then the CeO₂ NPs-induced potential effects on general systemic, developmental and reproductive functions as well as the internal biodistribution of CeO₂ NPs were observed.

2. Materials And Methods

The current study was conducted in accordance with the OECD Good Laboratory Practice (GLP) regulations.[13] The study design was conducted in general accordance with OECD testing guideline 422, namely, "Combined repeated-dose toxicity study with the reproductive/developmental toxicity screening test".[12] The experimental phase of this study was conducted in 2015, and animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) in accordance with the Animal Protection Act and the Guide for the Care and Use of Laboratory Animals.[14]

CeO₂ NPs and Nanoparticle Characterization

CeO₂ NPs (CAS No. 1306-38-3) were obtained from Sigma-Aldrich (USA). The primary size and shape of CeO₂ NPs were characterized by transmission electron microscopy (TEM, JEM-2100F, JEOL, Japan). The mean particle size of at least 100 CeO₂ NPs was analyzed using a DigitalMicrograph image analyzer program (Gatan, Inc., USA). The purity of the particles was analyzed using energy-dispersive X-ray (EDX) (TEM equipped with a silicon drift detector, Oxford Instruments, UK). The particle hydrodynamic diameter and zeta potentials in vehicle (10 mg/ml concentration) were characterized with ELS-8000 (Otsuka Electronics, Japan) using the dynamic light scattering (DLS) method. CeO₂ NPs in deionized water were identically sonicated by a Vibra-Cell® Model VC 505 sonifier (Sonics & Materials, USA) as dose formulation preparation for oral gavage administration.

Animals and Maintenance

Specific pathogen free naïve male and female SD rats (7 weeks of age) were obtained from Orient Bio, Inc. (Republic of Korea). Animals were housed as 2 (or one) animal(s) per stainless-steel cage (255W × 465L × 200H mm³). Pregnant and lactating dams were housed individually in a poly-sulfone cage (260W × 420L × 180H mm³) with sterilized Aspen animal bedding (Bio Lab, Republic of Korea) during the study period. The animal room was controlled environmental conditions as described previously,[15] with a temperature range of 22 ± 3 °C, a relative humidity range of 50 ± 20%, a 12 hours light-dark cycle and a ventilation range of 10–20 air changes per hour. The water was irradiated by UV light and filtered prior to provide ad libitum. The sterilized commercial rodent feed (PMI Nutrition International, USA) was also provided ad libitum. All animals received an acclimation period of 5 days to become accustomed to the laboratory environment. Then, healthy animals with adequate body weight increase and exhibiting no clinical signs were used in this study.

Dose Selection and Experimental Group

In the previous results of a preliminary study with CeO₂ NPs in SD rats (5 animals/sex/group) were used to select the dose levels of this study (data not shown). Animals were daily dosed CeO₂ NPs with 100, 300 and 1000 mg/kg dose levels for two weeks prior to mating, and dosing was continued through final sacrifice in males (total 28 days) and through gestation day (GD) 15 in females (total of at least 29 days). In a preliminary study, there was no test item-related changes in all examined parameters at any doses tested. Therefore, 1000 mg/kg, which is the limit dose level, was determined to the high dose, and 300 and 100 mg/kg were determined to the intermediate and low doses. Vehicle control animals were administered deionized water. CeO₂ NPs were diluted in deionized water and sonicated by the Vibra-Cell® sonifier with a 13 mm probe at 25% amplitude for 8 min. Dose formulations were mixed by a stirrer during the dosing, and dosing volume was 10 ml/kg.

Twelve male and twelve female SD rats were divided to each of the groups to have a similar mean body weight using the Pristima system (Xybion Medical System Co., USA). CeO₂ NPs were daily administered by oral gavage in a volume of 10 mL on each animals. Males were administered during a 2-week premating period and during mating and up to the final sacrifice in males (total of 38 days). Females were administered during a 2-week premating period and during mating, gestation and up to lactation day (LD) 4 (total of at least 41 days).

In-life Observations

Clinical examinations including mortality and general clinical signs were examined twice daily. In addition, detailed clinical signs were examined once weekly during the study period. Animal body weights were measured twice weekly during the pre-mating and mating periods, on days 0, 7, 14 and 20 of gestation, and on days 0 and 4 of lactation. Food consumption was also measured in the same days except for the mating and was calculated as g/animal/day. During mating, males and females were mated on a one-to-one basis for a period of up to 2 weeks. Mating was confirmed by the presence of sperm in the vaginal smear and/or the vaginal plug, and this was considered GD 0. Based on these

mating results, the number of days the animals were confirmed to mate (precoital time) and fertility-related data, including mating, fertility, fecundity and pregnancy index, were calculated. Functional observations of animals, including sensory function tests (tail pinch, approach and touch response, pupillary reflex and acoustic startle response), grip strength and motor activity, were conducted with 6 animals/sex/group before necropsy. The progress and completion of parturition was monitored twice daily, including signs of parturition, premature delivery, abortion, and prolonged or difficult parturition. Pregnant females were allowed to access their litters, and then the gestation duration, number of dead and live pups, runts, sexing of live pups, live pup body weight and pup external abnormalities were recorded. After parturition, pup mortality and general clinical signs were examined once daily. Based on the parturition and pup mortality results, the delivery index (% of dams with live pups among pregnant dams) and viability index (% of survival pups on post-natal day 4 after birth) were calculated. Pup individual body weight and sex were recorded on post-natal day (PND) 0 and 4, and these data were reported for each litter.

Terminal Observations

All surviving males on the day after final dosing and females on LD 5 were humanely sacrificed with isoflurane. Blood for clinical pathology were collected from the caudal vena cava from 5 randomly selected animals/sex/group. Animals for blood collection were fasted approximately 16 hours (overnight) prior to sacrifice. Blood for hematology was placed into tubes containing potassium salt of ethylenediaminetetraacetic acid (EDTA) and then analyzed with an ADVIA2120i hematology analyzer (Siemens, Germany) for the following parameters: total red blood cell count (RBC), mean corpuscular volume (MCV), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet count (PLT), reticulocyte count, total white blood cell count (WBC) and WBC differential count (absolute and relative counts of neutrophils [NEU], lymphocytes [LYM], monocytes [MON], basophils [BAS] and eosinophils [EOS]). Blood for coagulation was put into tubes containing 3.2% sodium citrate and centrifuged (approximately 3,000 rpm, 10 min, at room temperature) to obtain plasma. A coagulation test was conducted with an ACL 9000 coagulation analyzer (Instrumentation Laboratory, Italy) for the following parameters: activated partial thromboplastin time (APTT) and prothrombin time (PT). Blood samples for clinical chemistry were placed into tubes without anticoagulant and kept at room temperature for a minimum of 90 min and then centrifuged (approximately 3000 rpm, 10 min, at room temperature) to obtain serum. Clinical chemistry analysis was conducted with a Toshiba 200 FR NEO chemistry analyzer (Toshiba Co., Japan) for the following parameters: glucose (GLU), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), total cholesterol (TCHO), triglyceride (TG), albumin/globulin ratio (A/G), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (CREA), phospholipid (PL), creatine phosphokinase (CK), sodium (Na), inorganic phosphorus (IP), calcium (Ca), potassium (K) and chloride (Cl). After blood collection for clinical pathology, all animals were subjected to macroscopic observations, and the following organs were examined and preserved in 10% neutral buffered formalin or an appropriate fixative for histopathology: ovaries, testes, uterus with cervix, brain, stomach, ileum, duodenum, jejunum, colon,

cecum, rectum, liver, kidneys, adrenal glands, spinal cord (cervical, thoracic, lumbar), prostate, epididymides, seminal vesicles with coagulation glands, thyroid with parathyroid glands, trachea, lungs with bronchi, mesenteric lymph nodes, mandibular lymph nodes, urinary bladder, femur with marrow, sciatic nerve, spleen, heart, thymus and abnormal lesions. All reproductive organs and the other organs from 5 animals per sex in each group were further processed to slides and stained with hematoxylin and eosin for histopathological examinations. Kidneys were also examined in the low- and intermediate-dose groups to further investigate the treatment-related changes. All male reproductive organs (testes, epididymides, seminal vesicles with coagulation glands and prostate) were weighed, and the following organs were weighed from 5 animals per sex in each group: liver, kidneys brain, pituitary gland, heart, thymus, spleen, ovaries, adrenal glands, lungs and uterus with cervix. Paired reproductive organs were weighed separately.

Tissue Collection and Cerium Analysis

Parental animal tissues (blood, liver, lungs and kidneys) and pup tissues (blood, liver, lungs and kidneys) were collected and weighed (approximately 200 mg) for cerium content analysis. Pup tissues were collected from at least 5 individual pups and pooled by litter. All collected tissues were stored in a deep freezer (approximately -80 °C) until analysis. Cerium content analysis was conducted according to our previous study.[16] Briefly, thawed tissues were digested in a solution of 1 ml 30% H₂O₂ and 7 ml 70% HNO₃ with a microwave digestion system (Milestone, Italy). After wet digestion, the tissue concentrations of cerium were analyzed with an inductively coupled plasma atomic emission spectroscopy (ICP-AES) equipped with an Ultima 2 apparatus (Horiba Jobin, France).

Statistical Analysis

Statistical analyses were conducted based on the general statistical method used in this types of toxicology study and our previous study.[17] Statistical analysis was performed using the Prisma System or Statistical Analysis Systems (SAS Institute, USA), and the level of significance was taken when $p < 0.05$ or $p < 0.01$. Litter data were statistically evaluated using the statistical unit as a litter. Pup body weight was analyzed using one-way analysis of covariance (ANCOVA), and the litter size was used as the covariate.

3. Results

Physicochemical Properties of CeO₂ NPs

The physicochemical properties of CeO₂ NPs, including analyses of primary size, shape, purity and hydrodynamic size with zeta potential, are summarized in Table 1. The average primary size of the CeO₂ NPs was 14.2 ± 5.03 nm, and the majority of the CeO₂ NPs had a polyhedral shape with a purity of 100%. The hydrodynamic size of the CeO₂ NPs was 76.3 nm, and the zeta potential of the CeO₂ NPs in deionized water was 47.37 mV.

General Systemic Observations

All animals survived to the scheduled sacrifice. Furthermore, observations of animals during the study period did not reveal any differences in clinical examinations among the treatment and control groups (data not shown).

Body weights during the study period are presented in Figure 1. There were no treatment-related changes in body weight or weight gain during the study. Food consumption during the study period is presented in Table 2. In male rats of the 300 mg/kg dose group, food consumption during the pre-mating day 1-4 was significantly lower (0.93% of control) than in vehicle control animals.

In functional observations including sensory function tests (tail pinch, approach and touch response, pupillary reflex and acoustic startle response), grip strength and motor activity, there were no treatment-related changes during the study (data not shown).

In macroscopic observations, there were no treatment-related changes among the treatment and control animals (data not shown). Hematology results are presented in Table 3. In female rats of the 100 mg/kg dose group, the PT value was significantly higher (1.14-fold over control) than the respective level in the vehicle control animals. Clinical chemistry results are presented in Table 4. In male rats of the 1000 mg/kg dose group, the GGT value was significantly higher (2.24-fold over control) than the respective level in the vehicle control animals. Other hematology and clinical chemistry values for the CeO₂ NPs-treated animals were comparable to those of the vehicle control animals. Organ weights are presented in Tables 5 and 6. There were no treatment-related changes in organ weights among the treatment and control animals.

Histopathological examination results are presented in Table 7. In male rats of the 1000 mg/kg dose group, an increased incidence of tubular basophilia in kidneys was observed.

Developmental and Reproductive Observations

Fertility results with precoital time are presented in Table 8. There were no treatment-related changes in fertility results with precoital time. Reproductive and littering findings are presented in Table 9. There were no treatment-related changes in reproductive and littering finding parameters during the gestation and lactation periods. In general clinical signs and external examination of F1 pups at necropsy, there were no treatment-related changes among the treatment and control animals (data not shown). F1 pup body weights are presented in Table 10. An increase in F1 male and female pup covariate-adjusted body weights (up to 1.11-fold over control) during the post-natal period (PND 0 and 4) was observed at 1000 mg/kg.

Tissue Distribution of Cerium

Tissue distribution analysis of cerium in parental and pup tissues revealed that CeO₂ NPs were not detected in almost all of the samples (data not shown).

4. Discussion And Conclusion

CeO₂ NPs have been used in a wide range of industrial areas as a consequence of their characteristic properties. Indeed, they are used as a diesel fuel additive in Europe and are increasingly being employed as a catalyst in the automotive industry.[18, 19] The increasing numbers of CeO₂ NPs used are expected to cause direct or indirect exposure in humans. However, their potential toxicological effects in human health are not well investigated. An in vitro study with CeO₂ NPs showed that they did not induce genotoxicities, including DNA and chromosomal damage, in human lens epithelial cells.[20] However, other in vitro studies imply the opposite results with respect to CeO₂ NPs toxicity. CeO₂ NPs exposure in human lung cancer cells yielded elevated oxidative stress and cell membrane damage.[21] In addition, CeO₂ NPs exposure in human hepatocellular carcinoma SMMC-7721 cells induced damage and apoptosis via oxidative stress and the activation of MAPK signaling pathways.[22]

In vivo studies, such as acute oral gavage toxicity studies with CeO₂ NPs (30 nm; 100 and 5000 mg/kg) in rats revealed that CeO₂ NPs did not have overt toxicities.[23] Another acute oral gavage exposure study of CeO₂ NPs (< 25 nm; 30 and 300 mg/kg) in rats reported that no significant toxicities were observed with respect to general clinical signs, clinical pathology parameters and histopathological examinations.[16] Repeated-dose oral gavage exposure of mice to CeO₂ NPs (3–5 nm; weekly 0.5 mg/kg for 2 or 5 weeks) did not induce overt toxicities.[24]. Conversely, another repeated-dose oral gavage exposure study with CeO₂ NPs (< 25 nm; 30, 300 and 600 mg/kg for 28 days) in rats revealed that prolonged exposure to high concentrations has the potential to cause biochemical alterations, genetic damage and histological changes in the liver, spleen and brain.[25] In addition, single oral gavage exposure to CeO₂ NPs (4 ± 1 nm; 0.14 mg to 2.57 mg/kg based on the representative animal weight) induced the disruption of microvascular smooth muscle signaling.[26] These inconsistent and insufficient toxicity results obtained with CeO₂ NPs indicate the necessity of standard toxicity studies that are acceptable by the regulatory authorities of various countries.

Combined repeated-dose toxicity with reproductive/developmental toxicity screening tests are required by OECD test guidelines for testing chemicals that are reviewed in light of scientific progress by the various regulatory authorities.[12] This toxicity study is needed for industrial chemicals with a production or import level of more than 10 tonnage per year according to the European Registration, Evaluation, Authorisation and Restriction of Chemicals (EU REACH) legislation.[27] This study design is able to assess various developmental and reproductive toxicity endpoints as well as general toxicity endpoints in subacute toxicity studies. Thus far, since controversial toxicity results have been obtained with CeO₂ NPs in previous in vitro and in vivo studies, the combined repeated-dose toxicity with reproductive/developmental toxicity screening test could be considered one of the most appropriate toxicity studies to evaluate the potential effects of CeO₂ NPs on general functions as well as developmental and reproductive functions. In addition, we also analyzed the internal systemic distribution of CeO₂ NPs in major organs (blood, liver, lungs and kidneys) of parental animals and their pups after repeated oral exposure.

In general systemic evaluations after repeated CeO₂ NPs oral exposure, no CeO₂ NPs-related dead or moribund animals were observed during the study period. Although food consumption was decreased during the pre-mating day 1–4 with statistical significance in 300 mg/kg dose group males, this finding was considered to be incidental since it was transient and did not have a dose-response. As for hematology, the significantly increased PT in 100 mg/kg dose-group females was considered to be incidental since it did not have a dose-response. Regarding clinical chemistry, a statistically significant increase in GGT in 1000 mg/kg dose-group males was also considered to be incidental since there were no correlated changes in organ weights and histopathological examinations. In the histopathological examinations, the increased incidence of tubular basophilia in the kidneys in 1000 mg/kg dose-group males was considered to be incidental since it also occurred sporadically in normal animals, did not have an obvious dose-response and yield no correlated clinical chemistry changes. In addition, there were no toxicologically significant CeO₂ NPs-related changes in other examinations for general systemic effects.

In developmental and reproductive observations after repeated CeO₂ NPs oral exposure, there were no CeO₂ NPs-related changes in any of the parameters except the body weights of the F1 pups. The covariate-adjusted body weights of the pups increased in both males and females during PND 0 and 4 at the 1000 mg/kg. A change in pup growth compared with that of the concurrent control is one of the endpoints that can be indicates toxicity in a reproductive toxicity study.[28] It may be secondarily influenced by gestation length, maternal nutritional status and litter size.[29, 30] However, there were no concurrent changes in maternal body weight, litter size or gestation length in this study. It has also been reported that test substances with endocrine modes of action cause increased pup body weights. However, these endocrine effects were not clear in this study since there were no concurrent mode of action-related changes, including estrus cycle abnormalities and histopathological changes in reproductive organs.[31] In addition, biodistribution results indicated that CeO₂ NPs were not systemically observed in parental animals or their pups. Therefore, an increased pup body weight was not considered treatment-related, although the mechanism of CeO₂ NPs induced changes in the body weight of pups is not clear. Additional endpoint evaluations with an extended postnatal period was considered necessary to clearly elucidate the potential toxicological effects of CeO₂ NPs on the development of pups.

A biodistribution analysis after CeO₂ NPs exposure has been conducted in a few previous studies. Single- and 28-day repeated inhalation exposure to CeO₂ NPs (< 5000, 40 and 5–10 nm; 55.00, 19.95 and 10.79 mg/m³) resulted in their distributed to the lungs, liver, kidneys, spleen, brain, testes and epididymides [32]. Hirst et al. [24] also reported that CeO₂ NPs (3–5 nm; weekly 0.5 mg/kg for 2 or 5 weeks) were deposited in the spleen, liver, lungs and kidneys after intravenous and intraperitoneal administration. However, CeO₂ NPs were not considered to be deposited in internal organs after oral exposure. Most of the orally exposed CeO₂ NPs were excreted through feces within 24 hours. Other oral exposure studies with CeO₂ NPs (< 25 nm; 30 and 300 mg/kg for 1 day and 6.6 nm; 1 mg/animal for 1 day) suggested that they could hardly be absorbed in the gastrointestinal tract, and most of the orally exposed CeO₂ NPs were excreted in feces within a few days.[16, 33] Our results also confirmed these

previous studies showing that CeO₂ NPs were not deposited in parental internal organs after repeated oral exposure. In addition, we confirmed that CeO₂ NPs did not accumulate in the internal organs of the pups.

A lack of marked toxicity of CeO₂ NPs was observed in this study, but this finding does not mean that CeO₂ NPs are safe for humans and the environment. The toxicity of nanoparticles can be changed depending on their physicochemical properties, such as particle size, chemical composition and surface structure.[34] In fact, size-dependent toxicity and internal distribution differences of nanoparticles have been reported in previous studies. [35, 36, 37] Furthermore, nanoparticles exposure in humans and the environment can occur through various exposure routes and scenarios considering the wide range of CeO₂ NPs application. [18, 38] Therefore, additional toxicity studies of CeO₂ NPs would be necessary considering their various physicochemical properties and exposure scenarios.

In conclusion, under the experimental conditions of this study design, there were no CeO₂ NPs-related adverse effects in terms of general systemic signs as well as development and reproduction, at doses up to 1000 mg/kg. In addition, CeO₂ NPs were not deposited in the parental or pup internal organs after repeated oral exposure. These results in this study could be used for the risk assessment of repeated CeO₂ NPs exposure in humans, and further toxicity studies will be necessary to understand the potential risk to humans considering the various physicochemical properties and exposure scenarios of CeO₂ NPs.

Abbreviations

AAALAC:Association for Assessment and Accreditation of Laboratory Animal Care International, ANCOVA:Analysis of covariance, ANOVA:Analysis of variance, A/G:Albumin/globulin ratio, ALB:Albumin, APTT:Activated partial thromboplastin time, AST:Aspartate aminotransferase, BAS:Basophils, BUN:Blood urea nitrogen, Ca:Calcium, CeO₂ NPs:Cerium oxide nanoparticles, Cl:Chloride, CREA:Creatinine, CK:Creatine phosphokinase, DLS:Dynamic light scattering, EDTA:Ethylenediaminetetraacetic acid, EDX:Energy-dispersive X-ray, EOS:Eosinophils, EU REACH:European Registration, Evaluation, Authorisation and Restriction of Chemicals, GD:Gestation day, GGT:Gamma glutamyl transpeptidase, GLP:Good laboratory practice, GLU:Glucose, HCT:Hematocrit, HGB:Hemoglobin, IACUC:Institutional Animal Care and Use Committee, ICP-AES:Inductively coupled plasma atomic emission spectroscopy, IP:Inorganic phosphorus, K:Potassium, KIT:Korea Institute of Toxicology, LD:Lactation day, LYM:Lymphocytes, MCH:Mean corpuscular hemoglobin, MCHC:Mean corpuscular hemoglobin concentration, MCV:Mean corpuscular volume, MON:Monocytes, Na:Sodium, NEU:Neutrophils, OECD:Organization for Economic Co-operation and Development, PL:Phospholipid, PLT:Platelet count, PND:Post-natal day, PT:Prothrombin time, RBC:Red blood cell count, SD:Sprague-Dawley, TCHO:Total cholesterol, TEM:Transmission electron microscopy, TG:Triglyceride, TP:Total protein, WBC:White blood cell count

Declarations

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Availability of data and materials

The relevant datasets supporting the conclusions of this article are included within the article, and all datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JL, EJJ, SYN and WJY conceived and designed the present study. JL, JSJ, SJL and SYK managed the in-life observation experiments, and WJI managed the terminal observation experiments. KP conducted the cerium distribution analysis. JL, JSJ and SHK analyzed the physicochemical properties of particles. JL, YJS and WJY interpreted the data and wrote the manuscript. All authors reviewed and approved the final manuscript.

Ethics approval

All animal protocols were prepared in accordance with the Animal Protection Act of Korea and Guide for the Care and Use of Laboratory Animals published by the Institute for Laboratory Animal Research (ILAR). This animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of KIT.

Competing interests

The authors declare that they have no competing interests.

CONSENT FOR PUBLICATION

Not applicable

CONSENT TO PARTICIPATE

Not applicable

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Tables

[Due to technical limitations, table 1 could not be displayed here. Please see the supplementary files to access table 1.]

Table 2

Food consumption of CeO₂ NPs-treated males and females during the study period

	CeO ₂ NPs (mg/kg)			
	0	100	300	1000
Males				
Pre-mating day 1-4 (g)	30.6 ± 1.94	29.3 ± 0.76	28.6 ± 1.53*	30.5 ± 1.55
Pre-mating day 4-8 (g)	31.3 ± 2.17	30.4 ± 1.17	30.2 ± 1.61	31.2 ± 1.36
Pre-mating day 8-11 (g)	31.4 ± 2.27	31.5 ± 1.48	30.5 ± 2.34	32.2 ± 1.16
Pre-mating day 11-14 (g)	32.3 ± 2.04	32.6 ± 1.81	31.4 ± 2.38	32.7 ± 1.13
Total period (g, pre-mating day 1 to 14)	31.4 ± 2.01	30.9 ± 1.13	30.2 ± 1.85	31.7 ± 1.04
Females				
Pre-mating day 1-4 (g)	21.1 ± 1.62	20.2 ± 1.09	21.3 ± 1.07	21.2 ± 1.62
Pre-mating day 4-8 (g)	21.8 ± 1.19	21.4 ± 0.77	22.7 ± 1.22	22.2 ± 1.63
Pre-mating day 8-11 (g)	21.9 ± 1.65	22.3 ± 1.35	23.0 ± 2.22	22.1 ± 1.85
Pre-mating day 11-14 (g)	23.0 ± 1.50	23.0 ± 1.47	23.7 ± 0.76	23.3 ± 1.93
Gestation day 0-7 (g)	25.6 ± 1.70	26.0 ± 2.56	25.7 ± 2.15	26.8 ± 1.40
Gestation day 7-14 (g)	25.5 ± 1.52	25.9 ± 2.31	25.9 ± 2.26	25.8 ± 1.97
Gestation day 14-20 (g)	29.8 ± 2.13	29.2 ± 1.89	29.3 ± 1.87	31.5 ± 2.02
Post-natal day 0-4 (g)	35.8 ± 5.01	36.8 ± 3.19	35.9 ± 6.13	40.1 ± 4.06
Total period (g, pre-mating day 1 to lactation day 4)	25.0 ± 1.13	25.0 ± 1.36	25.4 ± 1.23	25.9 ± 1.28

* Represent a significant difference at the $p < 0.05$ level compared to the vehicle control (n = 12, mean ± SD).

Table 3

Hematology results of CeO₂ NPs-treated males and females during the study period

CeO₂ NPs (mg/kg)

	0	100	300	1000
Males				
RBC (10 ⁶ /mL)	9.1 ± 0.38	9.1 ± 0.28	8.9 ± 0.53	8.9 ± 0.10
HGB (g/dL)	16.6 ± 0.61	16.9 ± 0.43	16.8 ± 0.55	16.8 ± 0.63
HCT (%)	51.0 ± 2.50	51.4 ± 2.04	50.7 ± 2.10	50.4 ± 2.13
MCV (fL)	56.1 ± 1.69	56.2 ± 0.93	57.2 ± 1.77	56.9 ± 1.86
MCH (pg)	18.3 ± 0.43	18.5 ± 0.30	18.9 ± 0.68	18.9 ± 0.52
MCHC (g/dL)	32.5 ± 0.42	33.0 ± 0.73	33.1 ± 0.59	33.3 ± 0.35
PLT (10 ³ /mL)	1130.8 ± 149.70	1024.2 ± 133.65	940.4 ± 41.25	1101.6 ± 84.75
RET (%)	2.5 ± 0.46	2.5 ± 0.31	2.5 ± 0.28	2.5 ± 0.55
RETA (10 ⁹ /mL)	223.8 ± 41.42	226.0 ± 22.21	223.8 ± 31.62	218.4 ± 48.74
WBC (10 ³ /mL)	10.6 ± 3.32	11.6 ± 4.11	12.4 ± 1.03	11.0 ± 2.17
NEU (%)	14.8 ± 6.90	11.4 ± 1.72	12.1 ± 2.95	13.4 ± 2.47
NEUA (10 ³ /mL)	1.5 ± 0.74	1.4 ± 0.68	1.5 ± 0.47	1.5 ± 0.48
LYM (%)	81.3 ± 6.68	84.7 ± 2.09	83.2 ± 3.63	81.7 ± 2.02
LYMA (10 ³ /mL)	8.7 ± 2.82	9.8 ± 3.26	10.3 ± 0.72	9.0 ± 1.74
EOS (%)	0.8 ± 0.24	0.9 ± 0.18	0.9 ± 0.33	1.0 ± 0.36
EOSA (10 ³ /mL)	0.09 ± 0.052	0.10 ± 0.030	0.12 ± 0.052	0.11 ± 0.038
MON (%)	1.9 ± 0.55	1.9 ± 0.62	2.3 ± 0.73	2.5 ± 0.56
MONA (10 ³ /mL)	0.2 ± 0.13	0.3 ± 0.15	0.3 ± 0.09	0.3 ± 0.03
BAS (%)	0.6 ± 0.11	0.5 ± 0.09	0.7 ± 0.10	0.6 ± 0.20
BASA (10 ³ /mL)	0.06 ± 0.021	0.06 ± 0.028	0.09 ± 0.005	0.06 ± 0.009
LUC (%)	0.7 ± 0.27	0.6 ± 0.12	0.7 ± 0.10	0.8 ± 0.13
LUCA (10 ³ /mL)	0.07 ± 0.050	0.07 ± 0.035	0.09 ± 0.023	0.09 ± 0.012
PT (sec)	14.0 ± 1.68	13.7 ± 0.84	16.0 ± 2.74	13.6 ± 0.73
APTT (sec)	17.5 ± 0.72	18.0 ± 0.78	18.3 ± 0.87	17.8 ± 0.99
Females				
RBC (10 ⁶ /mL)	7.8 ± 0.50	7.7±0.33	7.9±0.51	8.1±0.17
HGB (g/dL)	14.9 ± 0.89	14.8±0.25	14.8±0.86	15.3±0.52
HCT (%)	46.0 ± 2.56	45.6±1.35	45.5±2.72	47.0±1.48
MCV (fL)	59.4 ± 0.91	59.4 ± 1.22	57.9 ± 1.96	58.2 ± 1.17
MCH (pg)	19.3 ± 0.23	19.3 ± 0.51	18.8 ± 0.67	18.9 ± 0.29
MCHC (g/dL)	32.5 ± 0.51	32.5 ± 0.45	32.5 ± 0.42	32.4 ± 0.39
PLT (10 ³ /mL)	1351.0 ± 167.25	1213.2 ± 174.16	1258.4 ± 222.70	1305.0 ± 253.02
RET (%)	5.8 ± 1.26	6.4 ± 1.72	6.3 ± 1.44	6.2 ± 1.65
RETA (10 ⁹ /mL)	448.3 ± 78.31	492.2 ± 142.86	489.7 ± 77.76	502.9 ± 136.39
WBC (10 ³ /mL)	9.5 ± 2.47	9.8 ± 2.40	11.2 ± 1.14	12.8 ± 3.22
NEU (%)	12.2 ± 4.95	13.3 ± 2.15	12.8 ± 3.21	13.2 ± 2.78
NEUA (10 ³ /mL)	1.2 ± 0.68	1.3 ± 0.30	1.4 ± 0.21	1.7 ± 0.75
LYM (%)	81.5 ± 5.15	80.5 ± 1.99	80.7 ± 4.04	80.3 ± 3.15

CeO₂ NPs (mg/kg)

	0	100	300	1000
LYMA (10 ³ /mL)	7.7 ± 2.04	7.9 ± 2.06	9.1 ± 1.36	10.2 ± 2.30
EOS (%)	0.8 ± 0.35	0.6 ± 0.24	0.7 ± 0.26	0.7 ± 0.32
EOSA (10 ³ /mL)	0.07 ± 0.021	0.06 ± 0.038	0.07 ± 0.023	0.09 ± 0.038
MON (%)	4.3 ± 0.68	4.0 ± 1.17	4.6 ± 0.94	4.6 ± 0.77
MONA (10 ³ /mL)	0.4 ± 0.09	0.4 ± 0.11	0.5 ± 0.08	0.6 ± 0.21
BAS (%)	0.5 ± 0.11	0.5 ± 0.21	0.4 ± 0.08	0.4 ± 0.08
BASA (10 ³ /mL)	0.04 ± 0.013	0.05 ± 0.031	0.05 ± 0.008	0.05 ± 0.020
LUC (%)	0.8 ± 0.26	1.1 ± 0.13	0.8 ± 0.29	0.8 ± 0.23
LUCA (10 ³ /mL)	0.07 ± 0.013	0.10 ± 0.025	0.09 ± 0.030	0.10 ± 0.026
PT (sec)	13.6 ± 1.17	15.5 ± 0.29*	15.3 ± 0.94	14.4 ± 1.54
APTT (sec)	16.1 ± 0.72	15.6 ± 1.15	14.8 ± 1.01	15.0 ± 0.93

* Represent a significant difference at the **p** < 0.05 level compared to the vehicle control (n = 5, mean ± SD).

Table 4

Clinical chemistry results of CeO₂ NPs-treated males and females during the study period

CeO₂ NPs (mg/kg)

	0	100	300	1000
Males				
GLU (mg/dL)	158.9 ± 20.30	149.2 ± 28.84	157.6 ± 45.06	148.5 ± 29.05
BUN (mg/dL)	15.1 ± 1.63	13.7 ± 1.52	14.3 ± 1.73	13.6 ± 1.81
CREA (mg/dL)	0.49 ± 0.042	0.48 ± 0.030	0.47 ± 0.042	0.47 ± 0.058
TP (g/dL)	6.6 ± 0.30	6.7 ± 0.22	6.6 ± 0.22	6.6 ± 0.38
ALB (g/dL)	4.3 ± 0.16	4.2 ± 0.11	4.2 ± 0.10	4.2 ± 0.20
A/G (ratio)	1.8 ± 0.16	1.7 ± 0.06	1.8 ± 0.12	1.8 ± 0.08
AST (IU/L)	149.4 ± 21.87	137.5 ± 34.53	129.9 ± 4.30	153.9 ± 18.45
ALT (IU/L)	33.4 ± 5.71	28.6 ± 5.63	32.5 ± 2.95	31.5 ± 3.89
TBIL (mg/dL)	0.14 ± 0.016	0.12 ± 0.012	0.15 ± 0.019	0.13 ± 0.023
GGT (IU/L)	0.41 ± 0.226	0.65 ± 0.132	0.60 ± 0.119	0.92 ± 0.179**
ALP (IU/L)	581.6 ± 179.23	510.3 ± 110.03	467.4 ± 57.25	514.4 ± 66.37
TCHO (mg/dL)	70.8 ± 26.86	64.2 ± 19.52	60.0 ± 8.86	68.4 ± 10.21
TG (mg/dL)	31.8 ± 9.27	34.2 ± 11.80	23.9 ± 9.97	21.4 ± 10.88
Ca (mg/dL))	11.1 ± 0.27	11.3 ± 0.43	11.2 ± 0.23	11.0 ± 0.66
IP (mg/dL)	10.2 ± 0.52	10.3 ± 0.60	10.5 ± 0.40	10.3 ± 0.70
K (mmol/L)	8.3 ± 0.92	7.5 ± 0.85	8.4 ± 0.87	7.8 ± 1.58
CK (IU/L)	715.6 ± 197.55	584.2 ± 203.16	483.6 ± 79.36	652.0 ± 217.99
PL (mg/dL)	102.4 ± 25.02	93.6 ± 22.69	92.0 ± 7.28	99.2 ± 10.43
Na (mmol/L)	148.0 ± 0.71	147.8 ± 1.92	147.4 ± 1.14	147.8 ± 1.64
Cl (mmol/L))	102.4 ± 1.67	101.6 ± 0.55	102.6 ± 1.34	102.4 ± 0.89
Females				
GLU (mg/dL)	131.2 ± 22.43	116.0 ± 15.10	108.8 ± 10.68	139.1 ± 17.09
BUN (mg/dL)	24.1 ± 4.71	19.4 ± 3.38	21.2 ± 4.13	20.3 ± 3.60
CREA (mg/dL)	0.57 ± 0.077	0.51 ± 0.032	0.54 ± 0.041	0.56 ± 0.034
TP (g/dL)	7.0 ± 0.42	7.0 ± 0.41	7.00 ± 0.21	7.1 ± 0.25
ALB (g/dL)	4.5 ± 0.22	4.6 ± 0.27	4.5 ± 0.13	4.6 ± 0.12
A/G (ratio)	1.8 ± 0.10	2.0 ± 0.16	1.9 ± 0.06	1.9 ± 0.08
AST (IU/L)	142.7 ± 44.27	129.1 ± 23.12	135.6 ± 17.69	121.3 ± 8.02
ALT (IU/L)	40.2 ± 8.01	41.5 ± 8.14	45.1 ± 5.18	39.8 ± 2.57
TBIL (mg/dL)	0.13 ± 0.018	0.12 ± 0.015	0.13 ± 0.016	0.12 ± 0.018
GGT (IU/L)	0.59 ± 0.224	0.78 ± 0.104	0.67 ± 0.268	0.71 ± 0.384
ALP (IU/L)	332.3 ± 111.04	293.1 ± 58.04	232.7 ± 31.73	267.1 ± 83.92
TCHO (mg/dL)	49.8 ± 16.04	58.0 ± 16.46	53.8 ± 10.78	55.2 ± 12.79
TG (mg/dL)	38.9 ± 22.46	36.9 ± 13.14	41.7 ± 14.91	54.7 ± 25.54
Ca (mg/dL))	11.7 ± 0.38	11.8 ± 0.46	11.7 ± 0.26	11.9 ± 0.20
IP (mg/dL)	9.9 ± 0.81	10.2 ± 0.05	10.3 ± 1.19	9.8 ± 0.71
K (mmol/L)	8.6 ± 0.52	8.3 ± 0.45	8.2 ± 1.12	8.2 ± 1.67
CK (IU/L)	611.8 ± 331.76	518.0 ± 112.86	570.6 ± 129.05	448.8 ± 130.31
PL (mg/dL)	106.8 ± 26.92	116.8 ± 24.76	108.6 ± 14.77	115.8 ± 19.10
Na (mmol/L)	144.8 ± 1.30	145.0 ± 1.87	145.0 ± 1.41	146.0 ± 1.58
Cl (mmol/L))	101.4 ± 0.55	101.2 ± 1.48	101.6 ± 1.14	102.2 ± 2.39

** Represent a significant difference at the $p < 0.01$ level compared to the vehicle control (n = 5, mean $\pm$ SD).

Table 5
Absolute and relative organ weights of CeO₂ NPs-treated males during the study period

		CeO ₂ NPs (mg/kg)			
		0	100	300	1000
Terminal weight (g)	body (g)	427.0 ± 38.68	439.2 ± 23.73	436.4 ± 36.00	448.9 ± 28.64
	N	12	12	12	12
Adrenal glands	Absolute (g)	0.06 ± 0.010	0.07 ± 0.005	0.06 ± 0.013	0.07 ± 0.004
	Relative (%)	0.016 ± 0.0027	0.016 ± 0.0016	0.016 ± 0.0021	0.015 ± 0.0009
	N	5	5	5	5
Brain	Absolute (g)	1.97 ± 0.119	1.99 ± 0.065	2.03 ± 0.092	2.00 ± 0.116
	Relative (%)	0.484 ± 0.0680	0.460 ± 0.0233	0.497 ± 0.0302	0.461 ± 0.0126
	N	5	5	5	5
Heart	Absolute (g)	1.25 ± 0.124	1.29 ± 0.144	1.33 ± 0.130	1.32 ± 0.106
	Relative (%)	0.304 ± 0.0233	0.300 ± 0.0302	0.325 ± 0.0244	0.303 ± 0.0278
	N	5	5	5	5
Kidneys	Absolute (g)	3.25 ± 0.192	3.40 ± 0.150	3.50 ± 0.334	3.47 ± 0.415
	Relative (%)	0.794 ± 0.0711	0.788 ± 0.0501	0.854 ± 0.0468	0.797 ± 0.0703
	N	5	5	5	5
Liver	Absolute (g)	11.71 ± 2.072	12.74 ± 0.706	12.12 ± 2.086	12.51 ± 1.408
	Relative (%)	2.825 ± 0.2167	2.950 ± 0.1063	2.940 ± 0.2455	2.870 ± 0.1972
	N	5	5	5	5
Pituitary gland	Absolute (g)	0.01 ± 0.002	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.001
	Relative (%)	0.003 ± 0.0001	0.003 ± 0.0002	0.003 ± 0.0002	0.003 ± 0.0003
	N	5	5	5	5
Prostate	Absolute (g)	0.62 ± 0.122	0.59 ± 0.170	0.66 ± 0.128	0.65 ± 0.093
	Relative (%)	0.144 ± 0.0258	0.135 ± 0.0407	0.151 ± 0.0314	0.146 ± 0.0177
	N	12	12	12	12
Spleen	Absolute (g)	0.66 ± 0.131	0.67 ± 0.152	0.65 ± 0.133	0.69 ± 0.082
	Relative (%)	0.158 ± 0.0134	0.155 ± 0.0331	0.158 ± 0.0199	0.158 ± 0.0127
	N	5	5	5	5
Thymus	Absolute (g)	0.34 ± 0.024	0.39 ± 0.076	0.35 ± 0.106	0.32 ± 0.057
	Relative (%)	0.083 ± 0.0099	0.090 ± 0.0179	0.084 ± 0.0193	0.072 ± 0.0112

		CeO ₂ NPs (mg/kg)			
		0	100	300	1000
Lungs	N	5	5	5	5
	Absolute (g)	1.50 ± 0.126	1.58 ± 0.120	1.57 ± 0.173	1.62 ± 0.067
	Relative (%)	0.367 ± 0.0411	0.367 ± 0.0314	0.383 ± 0.0205	0.374 ± 0.0205
Right testis	N	5	5	5	5
	Absolute (g)	1.70 ± 0.169	1.74 ± 0.134	1.69 ± 0.109	1.65 ± 0.120
	Relative (%)	0.400 ± 0.0492	0.396 ± 0.0313	0.391 ± 0.0514	0.369 ± 0.0280
Left testis	N	12	12	12	12
	Absolute (g)	1.73 ± 0.157	1.74 ± 0.124	1.71 ± 0.110	1.67 ± 0.124
	Relative (%)	0.407 ± 0.0510	0.397 ± 0.0260	0.394 ± 0.0443	0.372 ± 0.0295
Right epididymis	N	12	12	12	12
	Absolute (g)	0.66 ± 0.063	0.69 ± 0.064	0.68 ± 0.064	0.66 ± 0.070
	Relative (%)	0.155 ± 0.0168	0.158 ± 0.0164	0.156 ± 0.0208	0.148 ± 0.0207
Left epididymis	N	12	12	12	12
	Absolute (g)	0.67 ± 0.065	0.68 ± 0.061	0.66 ± 0.061	0.65 ± 0.058
	Relative (%)	0.157 ± 0.0186	0.156 ± 0.0141	0.152 ± 0.0194	0.145 ± 0.0178
Seminal vesicles with coagulating glands	N	12	12	12	12
	Absolute (g)	1.68 ± 0.205	1.65 ± 0.286	1.68 ± 0.216	1.69 ± 0.212
	Relative (%)	0.396 ± 0.0468	0.378 ± 0.0720	0.388 ± 0.0569	0.377 ± 0.0478
	N	12	12	12	12

(n = 5 or 12, mean ± SD)

Table 6

Absolute and relative organ weights of CeO₂ NPs-treated females during the study period

		CeO ₂ NPs (mg/kg)			
		0	100	300	1000
Terminal weight (g)	body (g)	314.5 ± 19.91	316.7 ± 24.09	311.0 ± 23.68	316.8 ± 19.84
Adrenal glands	Absolute (g)	0.08 ± 0.007	0.07 ± 0.011	0.08 ± 0.010	0.08 ± 0.007
	Relative (%)	0.028 ± 0.0033	0.024 ± 0.0029	0.027 ± 0.0034	0.027 ± 0.0024
Brain	Absolute (g)	1.94 ± 0.090	1.93 ± 0.079	2.02 ± 0.083	1.96 ± 0.048
	Relative (%)	0.652 ± 0.0356	0.650 ± 0.0220	0.692 ± 0.0283	0.654 ± 0.0298
Heart	Absolute (g)	1.00 ± 0.064	0.95 ± 0.037	0.97 ± 0.071	1.04 ± 0.063
	Relative (%)	0.334 ± 0.0130	0.321 ± 0.0170	0.334 ± 0.0262	0.346 ± 0.0194
Kidneys	Absolute (g)	2.28 ± 0.191	2.23 ± 0.210	2.14 ± 0.141	2.23 ± 0.066
	Relative (%)	0.763 ± 0.0282	0.750 ± 0.0512	0.734 ± 0.0357	0.744 ± 0.0273
Liver	Absolute (g)	10.52 ± 1.205	10.40 ± 0.865	10.19 ± 0.297	10.91 ± 0.730
	Relative (%)	3.522 ± 0.2660	3.503 ± 0.2766	3.495 ± 0.1690	3.638 ± 0.2589
Pituitary gland	Absolute (g)	0.02 ± 0.002	0.02 ± 0.001	0.02 ± 0.004	0.02 ± 0.002
	Relative (%)	0.006 ± 0.0006	0.006 ± 0.0005	0.006 ± 0.0014	0.006 ± 0.0004
Spleen	Absolute (g)	0.62 ± 0.072	0.69 ± 0.090	0.65 ± 0.081	0.70 ± 0.123
	Relative (%)	0.209 ± 0.0205	0.231 ± 0.0285	0.223 ± 0.0323	0.232 ± 0.0429
Thymus	Absolute (g)	0.31 ± 0.029	0.31 ± 0.054	0.31 ± 0.079	0.38 ± 0.124
	Relative (%)	0.104 ± 0.0134	0.104 ± 0.0209	0.105 ± 0.0270	0.125 ± 0.0407
Lungs	Absolute (g)	1.34 ± 0.040	1.29 ± 0.058	1.42 ± 0.049	1.36 ± 0.108
	Relative (%)	0.451 ± 0.0129	0.435 ± 0.0179	0.486 ± 0.0222	0.453 ± 0.0339
Uterus	Absolute (g)	0.78 ± 0.123	0.78 ± 0.090	0.83 ± 0.116	0.73 ± 0.062
	Relative (%)	0.260 ± 0.0387	0.262 ± 0.0295	0.284 ± 0.0354	0.243 ± 0.0262
Right ovary	Absolute (g)	0.07 ± 0.009	0.06 ± 0.009	0.06 ± 0.008	0.06 ± 0.011
	Relative (%)	0.021 ± 0.0024	0.021 ± 0.0037	0.019 ± 0.0027	0.019 ± 0.0036

CeO ₂ NPs (mg/kg)					
		0	100	300	1000
Left ovary	Absolute (g)	0.07 ± 0.019	0.06 ± 0.005	0.06 ± 0.012	0.05 ± 0.007
	Relative (%)	0.023 ± 0.0058	0.020 ± 0.0021	0.020 ± 0.0046	0.018 ± 0.0024

(n = 5, mean ± SD)

Table 7

Selected histopathological examination results of CeO₂ NPs-treated males and females during the study period

		CeO ₂ NPs (mg/kg)			
		0	100	300	1000
Males					
Adrenal glands					
Vacuolation, corticomedullary	Grade 1	0	-	-	1
Epididymis					
Infiltration, mononuclear cell	Grade 1	0	-	-	1
Heart					
Cardiomyopathy	Grade 1	3	-	-	1
Jejunum					
Mineralization, Peyer's patch	Grade 1	0	-	-	1
Kidneys					
Basophilia, tubules	Grade 1	1	3	2	4
	Grade 2	0	1	0	0
Casts	Grade 1	0	1	0	0
Cyst(s)	Grade 1	1	0	0	0
Dilation, tubules	Grade 1	0	0	0	1
Infiltration, mononuclear cell	Grade 1	2	0	2	0
Mineralization	Grade 1	0	0	1	0
Liver					
Infiltration, mononuclear cell	Grade 1	4	-	-	0
Necrosis, focal	Grade 1	1	-	-	0
Vacuolated area	Present	0	-	-	1
Lungs with bronchi					
Aggregation, foamy macrophage	Grade 1	1	-	-	2
Infiltration, inflammatory cell	Grade 1	1	-	-	0
Osseous metaplasia	Present	1	-	-	0
Lymph node, mesenteric					
Mineralization	Grade 1	1	-	-	0
Prostate					
Infiltration, inflammatory cell	Grade 1	3	-	-	3
	Grade 2	1	-	-	0
Spleen					
Hemopoiesis	Grade 1	1	-	-	0
Stomach					
Dilation, glands	Grade 1	0	-	-	1
Testes					
Atrophy, tubules	Grade 1	1	-	-	0
Thyroid and parathyroid glands					
Ultimobranchial cyst	Present	0	-	-	2
Females					
Heart					
Cardiomyopathy	Grade 1	1	-	-	0
Kidneys					
Basophilia, tubules	Grade 1	0	1	1	0
Dilation, tubules	Grade 1	1	0	1	1

		CeO ₂ NPs (mg/kg)			
		0	100	300	1000
Hyperplasia, oncocytic, tubule	Grade 1	0	1	0	0
Hypertrophy, tubule	Grade 1	0	1	0	0
Infiltration, mononuclear cell	Grade 1	0	2	0	0
Infiltration, mononuclear cell, pelvis	Grade 1	0	0	0	1
Mineralization	Grade 1	1	0	1	1
Liver					
Infiltration, mononuclear cell	Grade 1	0	-	-	1
Necrosis, focal	Grade 1	0	-	-	2
Vacuolation, focal	Grade 1	1	-	-	0
Lungs with bronchi					
Granuloma	Present	1	-	-	0
Granulomatous inflammation	Grade 3	0	-	-	1
Lymph node, mesenteric					
Mineralization	Grade 1	0	-	-	1
Ovaries					
Cyst(s)	Present	1	-	-	1
Spleen					
Hemopoiesis	Grade 1	0	-	-	1
Thyroid and parathyroid glands					
Ultimobranchial cyst	Present	0	-	-	2

Grades: 1 (minimal), 2 (slight), 3 (moderate), 4 (marked), 5 (severe), -: Not examined, (n = 5)

Table 8

Fertility with precoital time results of CeO₂ NPs-treated males during the study period

	CeO ₂ NPs (mg/kg)			
	0	100	300	1000
Males				
Mating index ^{a)}	100	100	100	100
Fertility index ^{b)}	100	100	100	100
Fecundity index ^{c)}	100	100	100	100
Females				
Mating index ^{d)}	100	100	100	100
Fertility index ^{e)}	100	100	100	100
Pregnancy index ^{f)}	100	100	100	100
Precoital Time (day)	3.3 ± 3.58	1.8 ± 0.62	2.2 ± 1.11	1.9 ± 1.08

^{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

(n = 12, mean ± SD)

Table 9

Reproductive and littering findings results of CeO₂ NPs-treated females during the study period

CeO ₂ NPs (mg/kg)				
	0	100	300	1000
Gestation period (day)	21.5 ± 0.43	21.5 ± 0.30	21.6 ± 0.31	21.8 ± 0.40
Corpora lutea (day)	17.8 ± 2.76	16.5 ± 2.58	16.3 ± 1.66	17.4 ± 2.02
Implantations (N)	15.3 ± 3.05	15.3 ± 2.42	14.8 ± 2.08	15.4 ± 2.02
Pups born (N)	14.8 ± 3.10	14.4 ± 2.39	13.9 ± 2.02	14.8 ± 2.12
Perinatal death (N)	0.08 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.45
Unaccounted-for sites ^{a)} (%)	2.8 ± 4.45	5.5 ± 5.82	6.2 ± 4.47	3.9 ± 4.52
Sex ratio ^{b)} (%)	106.9 ± 62.62	93.9 ± 28.20	105.3 ± 55.49	130.7 ± 66.77
Live litter size (N)				
PND 0	14.8 ± 3.05	14.4 ± 2.39	13.9 ± 2.02	14.6 ± 2.11
PND 4	14.8 ± 3.05	14.3 ± 2.39	13.8 ± 1.96	14.4 ± 1.98
Viability index ^{c)} (%)	100.0	99.4 ± 1.92	98.9 ± 2.61	99.0 ± 2.39
Delivery index ^{d)} (%)	100.0	100.0	100.0	100.0
Pups with external abnormalities	0	0	0	0

^{a)} (No. of implantation sites/litter) – (No. of live pups at birth/litter) / No. of implantation sites/litter × 100

^{b)} (No. of male pups on PND 0/litter) / (No. of female pups on PND 0/litter) × 100

^{c)} (No. of live pups on PND 4/litter) / (No. of live pups at birth/litter) × 100

^{d)} (No. of dams with live pups) / (No. of pregnant dams) × 100

(n = 12, mean ± SD)

Table 10

F1 pups body weights of CeO₂ NPs-treated parental animals during the study period

		CeO ₂ NPs (mg/kg)			
		0	100	300	1000
F1 male pups					
PND 0					
Body weight (g)		6.5 ± 0.38	6.7 ± 0.33	6.8 ± 0.59	7.0 ± 0.47
Covariate-adjusted mean (g)		6.50	6.67	6.80	7.04*
PND 4					
Body weight (g)		10.1 ± 0.82	10.7 ± 1.07	11.0 ± 1.14	11.0 ± 1.21
Covariate-adjusted mean (g)		10.07	10.64	10.87	11.20*
F1 female pups					
PND 0					
Body weight (g)		6.2 ± 0.41	6.3 ± 0.29	6.4 ± 0.55	6.7 ± 0.56
Covariate-adjusted mean (g)		6.20	6.29	6.39	6.69*
PND 4					
Body weight (g)		9.5 ± 0.89	10.0 ± 0.97	10.4 ± 1.20	10.6 ± 1.39
Covariate-adjusted mean (g)		9.56	10.05	10.38	10.52

* Represent a significant difference at the **p** < 0.05 level compared to the vehicle control.
(n = 12, mean ± SD)

Figures

Figure 1

Body weights of CeO₂ NPs-treated males (A) and females (B) during the study period (n = 12, mean ± SD)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)