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Prenatal toxicity of synthetic amorphous silica nanomaterial in rats

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ABSTRACT

Synthetic amorphous silica is a nanostructured material, which is produced and used in a wide variety of technological applications and consumer products. No regulatory prenatal toxicity studies with this substance were reported yet. Therefore, synthetic amorphous silica was tested for prenatal toxicity, according to OECD guideline 414 in Wistar rats following oral (gavage) administration at the dose levels 0, 100, 300, or 1000 mg/kg bw/d from gestation day 6–19. At gestation day 20, all pregnant animals were examined by cesarean section. Numbers of corpora lutea, implantations, resorptions, live and dead fetuses were counted. Fetal and placental weights were determined. Fetuses were examined for external, visceral and skeletal abnormalities. No maternal toxicity was observed at any dose level. Likewise, administration of the test compound did not alter cesarean section parameters and did not influence fetal or placental weights. No compound-related increase in the incidence of malformations or variations was observed in the fetuses. The no observed adverse effect level (NOAEL) was 1000 mg/kg bw/d.

1. Introduction

Synthetic amorphous silica (SAS) is a nanostructured material, which is produced and used in a wide variety of technological applications and consumer products like as thickener in pastes, carrier of fragrances, in pharmaceuticals and food [1–4]. Recent investigations revealed that the average daily intake of total silica consumers via food is approximately 9.4 mg/kg bw/d, with 20% being in the nano-size range [3].

SAS covers pyrogenic silica, silica gel and colloidal silica (EINECS No. 231-545-4).

SAS is produced via thermal (e.g. pyrogenic) or wet (e.g. precipitation) processes. Initially primary particles with sizes below 100 nm are built by nucleation, coagulation and coalescence processes. These primary particles form larger particles by covalent bindings, which usually have a dimension above 100 nm. Subsequently these aggregates combine to larger agglomerates via attraction forces (van der Waals, hydrogen bonds). Marketed SAS powder typically has agglomerates in the micrometer size, the internal structure being in the nanoscale range. All currently known products of SAS in powder form have these properties, independent of the producer [5]. Colloidal silica is available on the market as mono- and poly-dispersed nanoparticles in water based formulations [5].

After inhalation exposure of rats to nanonized silica, mainly inflammation in the lung was observed, which was at least partially reversible [6–10].

Regarding the toxicity of nanonized silica via other administration routes, acute inflammation was observed in the liver of mice after single intravenous administration of particles with a particle size of 50, 100, or 200 nm [11]. After repeated intravenous administration of uncoated 70 nm silica particles, in 3 day intervals for four weeks, hepatic fibrosis was reported in mice at a dose of 10 mg/kg body weight [12,13]. Similar changes were described after repeated intravenous administration of non-porous silica particles with a size of 70 nm at 40 mg/kg bw/d, whereas no effects were observed after administration of carboxyl- or amino-modified particles [14].

Two different types of silica particles of 0.5–30 or 30–90 nm in size were administered via food at a concentration of 1% for a period of 10 weeks to Balb/c or C57BL/6J mice [15]. Slightly higher alanine aminotransferase values were observed in Balb/C, but not in C57BL/6J mice. The values were, however, still within the historical control range of Balb/C mice [16]. Animals treated with the silica particles showed signs indicative of a fatty change in the liver.

Recently, a comprehensive oral subchronic toxicity study with SAS in rats was reported [17]. SAS was administered at dose levels of 100 or 1000 mg/kg bw/d for 28 days, or at 2500 mg/kg bw/d for 84 days. There was no evidence for systemic toxicity based on body weights and clinical pathology data. Likewise, no changes

Abbreviation: SAS, synthetic amorphous silica.

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indicative for immunotoxicity were seen based on serum antibody levels or cytokine production of B- and T-cells. The incidence of periportal fibrosis seemed to be slightly higher after treatment for 84 days, when compared to controls, but the difference was not statistically significant. In summary, no adverse effects were attributed to the administration of SAS. In contrast, an increased incidence of liver fibrosis was observed with the reference material NM-202 (hydrophilic pyrogenic silica; Nanomaterials Repository; Joint Research Centre, Italy) and supported by gene expression data. In another recently performed oral 28-day toxicity study with nanonized silica material according to OECD Guideline 407, no adverse effects were observed after administration of 1000 mg/kg body weight/d [18]. Likewise, no compound-related alterations were observed by metabolome analysis in this study.

SAS was tested for carcinogenicity in rats and mice at dietary concentrations up to 5%. There was no evidence for a carcinogenic effect [19].

A review of reproductive toxicity data of manufactured nanomaterials is given by Ema et al. [20]. No studies with oral administration were listed in this review.

With regard to the widespread presence of SAS in food, an assessment of the developmental toxicity of SAS after oral administration is important. A literature search revealed the absence of publications on prenatal toxicity studies with SAS performed according to regulatory requirements i.e. OECD, EU or US-EPA guidelines. Therefore, the aim of the present study was to fill this gap and to examine possible maternal and embryo–fetal effects of SAS when administered orally to rats during pregnancy. The oral route was chosen since it is a major route of human exposure (see above).

The present study was performed as part of a CEFIC-LRI N3 project, together with a two-generation reproduction toxicity study in rats [21], which is reported separately.

2. Materials and methods

2.1. General

The study was conducted according to the OECD Principles of Good Laboratory Practice [22], which principally meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)].

The prenatal developmental toxicity study was performed according to OECD Guideline 414 and EPA OPPTS 870.3700 [23,24].

2.2. Test compound

NM-200 synthetic amorphous silica, batch PR-A-2, a white powder, was obtained from EU Joint Research Centre (Ispra). This reference material was produced by precipitation and had a silicon dioxide content of 96.5% (impurities based on energy-dispersive X-ray spectroscopy: Na, Al, S). Primary particles were in the 10–25 nm range, the specific surface area being 230 m^2/g. The test compound was stored under an argon atmosphere at room temperature.

Dosing formulations of synthetic amorphous silica were prepared with highly deionized water containing 10% fetal bovine serum in order to avoid agglomeration. The formulations were analyzed by scanning electron microscopy after shock freezing and in situ analytical ultracentrifugation.

2.3. Animals

Permission for animal studies was obtained from the local regulatory agencies, and all protocols were in compliance with the federal guidelines. The laboratories of BASF's Experimental Toxicology and Ecology, where all the studies were performed, are AAALAC-certified. All procedures for animal care and exposure were conducted under the rule of the German Animal Welfare Act (1998). Sexually mature, virgin Wistar rats (Crl:WI[Han]) were supplied by Charles River Laboratories, Research Models and Services, Germany GmbH. Only animals free from clinical signs of disease were used for the investigations.

During the study period, the rats were housed individually in Makrolon type M III cages supplied by Becker & Co., Castrop Rauxel, Germany (floor area about 800 cm^2). Dust-free wooden bedding was used in this study (Lignocel, supplied by Rettenmaier & Söhne GmbH + Co. KG, Rosenberg, Germany). For enrichment, wooden gnawing blocks were offered (Typ NGM E-022, supplied by Abedd® Lab. and Vet. Service GmbH, Vienna, Austria).

The animals were accommodated in fully air-conditioned rooms in which central air conditioning maintained a range of temperature of 20–24 °C and a range of relative humidity of 30–70%. The air exchange rate was 15 times per hour. There were no deviations from these limits during the entire study. The day/night cycle was generally 12 h (12 h light from 6.00 h to 18.00 h and 12 h darkness from 18.00 h to 6.00 h). Before the study started, the animal room was completely disinfected using a disinfector ("AUTEX" fully automatic, formalin–ammonia-based terminal disinfection). The walls and the floor were cleaned once a week with water containing an appropriate disinfectant. The food used was ground Kliba maintenance diet mouse/rat "GLP", meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland. Food and tap water (from water bottles) were available ad libitum.

After an acclimatization period of at least 5 days, 1–2 untreated female rats were mated with one untreated male animal of the same breed. The male mating partners were kept under the same conditions (air conditioning, feed, water) as the female rats of this study. Mating took place from about 15:30 h to about 7:30 h on the following day. If sperm were detected microscopically in a vaginal smear in the morning, the female was considered impregnated and transferred into the study. This day was referred to as gestation day 0 (GD 0, beginning of the experimental phase) and the following day as GD 1. The random distribution of the animals to the individual groups was carried out on GD 0 by randomly (computer-generated) removing the animals from the cages. On GD 0 the rats were 12–15 weeks old. The body weight of the pregnant animals on GD 0 varied between 195.3 and 271.0 g.

2.4. Experimental procedure

Groups of 25 mated rats received SAS orally via gavage once daily from implantation to end of gestation (GD 6–GD 19) at dose levels of 0 (vehicle, highly deionized water containing 10% fetal bovine serum), 100, 300, or 1000 mg/kg body weight at a dose volume of 10 mL/kg body weight. The high dose was chosen as limit dose according to the guidelines. Clinical signs, body weights and food consumption were recorded regularly throughout the study. On GD 20, females were sacrificed and examined macroscopically. Uteri and ovaries were removed. Gravid uterus weights and corrected body weight gain of the dams (terminal body weight – gravid uterus weight – body weight on GD6) were determined. The number of corpora lutea, as well as the number, status and distribution of implantation sites (live fetuses, early resorptions, late resorptions, dead fetuses) were also determined. Conception rate, pre-implantation loss (%), and post-implantation loss (%) were calculated using the litter as unit in the latter two parameters. Placental diameters were measured during gross pathological assessment.

At necropsy each fetus was weighed, sexed, and external tissues and all orifices were examined macroscopically. The sex was determined by observing the distance between the anus and the base of the genital tubercle. Furthermore, the viability of the fetuses and

the condition of placentae, umbilical cords, fetal membranes, and fluids were examined. Individual placental weights were recorded. Thereafter, the fetuses were sacrificed by subcutaneous injection of a pentobarbital (Narcoren®; dose: 0.1 mL/fetus). After these examinations, approximately one half of the fetuses per dam were eviscerated, skinned and placed in ethanol, the other half was placed in Harrison's fluid for fixation.

The fetuses fixed in Harrison's fluid were examined for any visceral findings according to the method of Barrow and Taylor [25]. After this examination these fetuses were discarded. The skeletons of the fetuses fixed in ethanol were stained according to a modified method of Kimmel and Tramell [26]. Thereafter, the skeletons of these fetuses were examined under a stereomicroscope morphologically referring to the glossary of Wise et al. [27] and its updated version of Makris et al. [28]. Classifications of findings were based on terms and definitions proposed by the 'Berlin Workshops' [29–31] and divided into the categories malformations (permanent structural changes, likely adversely affecting survival or health) and variations (changes spontaneously occurring and considered unlikely to adversely affect survival or health). Fetal findings not classifiable as malformation or variation were described as 'unclassified observations'. After this examination the stained fetal skeletons were individually retained.

2.5. Statistical analysis

The data for food consumption, maternal, litter weight and body weight change, carcass and gravid uterus weight, number of corpora lutea and implantations, number of resorptions, number of live fetuses, percent pre- and post-implantation loss, percent live fetuses per litter, number of pups per litter, post-implantation loss, and mean placental weights were analyzed by the two-sided Dunnett's test for the hypothesis of equal means [32,33]. The female mortality, number of pregnant females, and number of litters with fetal findings, were analyzed by pairwise comparison of each dose group with the control group using the one-sided Fisher's exact test for the hypothesis of equal proportions [34]. Proportions of fetuses per litter with findings were analyzed by pairwise comparison of each dose group with the control group by a one-sided Wilcoxon test for the hypothesis of equal medians [35–37].

3. Results

3.1. Size stability analyses

The correct size stability of the test material in the vehicle was shown. In situ analytical ultracentrifugation showed an onset of the size distribution at 40 nm, the weight-average around 300 nm, and a long tail of agglomerates up to some μm's. The range of the size distribution was confirmed by scanning electron microscopy after shock freezing, with agglomerates measuring from sub-100-nm to 3 μm.

3.2. Mortality and clinical signs

No compound-related mortality or clinical signs were observed. Three rats of the control group, four rats of the low dose group and two animals of the high dose group were not pregnant.

3.3. Body weight and food consumption

Body weight development (Fig. 1) and food consumption (Fig. 2) were not influenced by the treatment during the study. Likewise, average carcass weight and corrected body weight gain were not influenced by the treatment during the study.

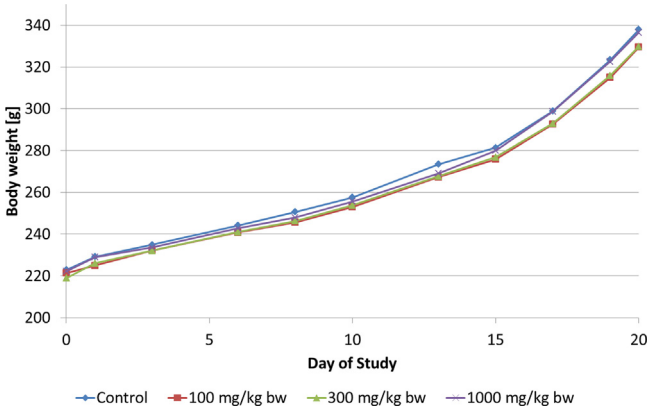

Fig. 1. Body weights of pregnant rats.

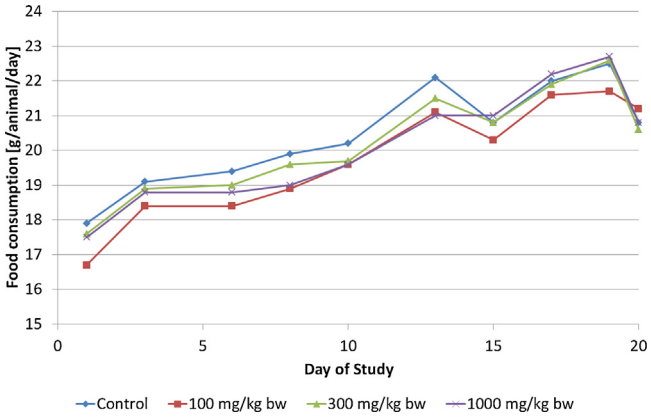

Fig. 2. Food consumption of pregnant rats.

3.4. Cesarean section and necropsy

Gravid uterus weights were not influenced by the treatment (Table 1).

No compound-related macroscopic findings were observed at necropsy. The conception rate, mean number of corpora lutea, implantation sites, and post-implantation loss were comparable in all groups. The only minor difference to control values was pre-implantation loss (slightly higher in the groups treated with SAS); however, a compound-related effect can be excluded as treatment started after implantation. No dead fetuses were noted. The sex distribution of the fetuses was comparable in all groups. Mean fetal weights did not show any biologically relevant differences between the test substance-treated groups and the control (Table 2).

3.5. Fetal morphology examinations

3.5.1. Fetal morphology examinations

Fetal malformations are summarized in Table 3 (for a list of all findings, see Tables S1, S3 and S5).

External malformations were recorded for one fetus each in the low- and high-dose group. Both fetuses concerned had multiple malformations. Mandibular micrognathia mirrored the severely malformed skull bones found during skeletal examination in one fetus; these findings were considered related to each other. The cleft palate observed in the other fetus is present in the historical control data at a comparable incidence. Both findings were considered to be spontaneous in nature and without a relation to dosing. The total incidence of external malformations in treated animals did not differ significantly from that of the control group.

Table 1
Gravid uterus weights and maternal body weight change.

Dose [mg/kg bw/d]		0 (Vehicle)	100	300	1000
Gravid uterus [g][D]	Mean ± SD	65.9 ± 12.74	62.5 ± 13.32	61.7 ± 12.16	67.9 ± 11.53
	N	22	21	25	23
Carcass [g][D]	Mean ± SD	272.1 ± 20.05	267.0 ± 19.71	268.1 ± 16.29	268.8 ± 12.04
	N	22	21	25	23
Net weight change from day 6 [g][D]	Mean ± SD	27.9 ± 9.07	26.1 ± 6.08	27.1 ± 7.66	26.1 ± 4.81
	N	22	21	25	23

$^{*}p \leq 0.05$; $^{**}p \leq 0.01$ (D, two-sided Dunnett test [32,33]).

Table 2
Summary of reproduction data.

Dose [mg/kg bw/d]		0 (Vehicle)	100	300	1000
Females mated	*N*	25	25	25	25
Pregnant	*N*	22	21	25	23
Conception rate	%	88	84	100	92
Aborted	*N*	0	0	0	0
Premature births	*N*	0	0	0	0
Dams with viable fetuses	*N*	22	21	25	23
Dams with total litter loss	*N*	0	0	0	0
Female mortality[F]	*N*	0	0	0	0
Pregnant at terminal sacrifice	*N*	22	21	25	23
	%	88	84	100	92
Corpora lutea[D]	Mean ± SD	14.2 ± 2.40	13.9 ± 2.08	14.0 ± 1.99	14.7 ± 2.03
Implantation sites[D]	Mean ± SD	13.5 ± 2.56	12.5 ± 2.80	12.8 ± 1.62	13.4 ± 2.27
Pre-implantation loss [%][D]	Mean ± SD	4.9 ± 7.31	9.4 ± 17.69	7.7 ± 11.81	8.4 ± 12.31
Post-implantation loss [%][D]	Mean ± SD	8.3 ± 9.96	5.2 ± 5.22	9.9 ± 16.20	6.0 ± 3.56
Early resorptions [%][D]		7.6 ± 9.27	5.2 ± 5.22	9.9 ± 16.20	5.6 ± 3.75
Late resorptions [%][D]		0.7 ± 2.26	0	0	0.3 ± 1.49
Dead fetuses		0	0	0	0
Dams with viable fetuses	*N*	22	21	25	23
Live fetuses[D]	Mean ± SD	12.4 ± 2.59	11.8 ± 2.71	11.5 ± 2.47	12.7 ± 2.23
Live fetuses [% implantations][D]	Mean ± SD	91.7 ± 9.96	94.8 ± 5.22	90.1 ± 16.20	94.0 ± 3.56
Males[D]	Mean ± SD	5.9 ± 1.70	5.5 ± 1.91	6.5 ± 1.85	5.7 ± 1.79
Males [%][D]	Mean ± SD	43.7 ± 10.64	46.5 ± 18.79	50.9 ± 14.23	43.2 ± 13.09
Females [D]	Mean ± SD	6.5 ± 1.95	6.3 ± 2.76	5.0 ± 2.34	6.9 ± 2.21
Females [%][D]	Mean ± SD	48.0 ± 11.17	48.3 ± 18.55	39.2 ± 15.98	50.9 ± 11.85
% Males		47.4	46.8	56.3	45.4
Placental weights[D]	Mean ± SD	0.47 ± 0.040	0.47 ± 0.042	0.46 ± 0.038	0.47 ± 0.038
	N	22	21	25	23
Of male fetuses[D]	Mean ± SD	0.49 ± 0.038	0.48 ± 0.036	0.47 ± 0.043	0.48 ± 0.037
	N	22	21	25	23
Of female fetuses[D]	Mean ± SD	0.46 ± 0.042	0.46 ± 0.052	0.46 ± 0.044	0.46 ± 0.041
	N	22	20	25	23
Fetal weights[D]	Mean ± SD	3.4 ± 0.26	3.4 ± 0.15	3.4 ± 0.24	3.4 ± 0.14
	N	22	21	25	23
Males[D]	Mean ± SD	3.5 ± 0.27	3.4 ± 0.18	3.5 ± 0.24	3.5 ± 0.14
	N	22	21	25	23
Females[D]	Mean ± SD	3.3 ± 0.25	3.3 ± 0.17	3.4 ± 0.28	3.3 ± 0.15
	N	22	20	25	23

$^{*}p < 0.05$; $^{**}p < 0.01$ (D, two-sided Dunnett test [32,33]).

Table 3
Fetal morphology data: summary of fetal malformations.

Dose [mg/kg bw/d]		0 (Vehicle)	100	300	1000
Litters evaluated	*N*	22	21	25	23
Fetuses evaluated	*N*	272	248	288	291
Total malformations					
Fetal incidence	*N*	3	1	0	1
	%	1.1	0.4	0.0	0.3
Litter incidence[F]	*N*	2	1	0	1
	%	9.1	4.8	0.0	4.3
Affected fetuses per litter[W]	Mean% ± SD	1.3 ± 4.07	0.4 ± 1.98	0.0 ± 0.00	0.3 ± 1.60

$^{*}p < 0.05$; $^{**}p < 0.01$ (F, one-sided Fisher's exact test [34]; W, one-sided Wilcoxon [35–37]).

One soft tissue malformation (i.e. supernumerary liver lobes – unilateral) was observed in the control group.

Skeletal malformations were noted in single fetuses at 0, 100 and 1000 mg/kg body weight/d. Each of them affected individual fetuses and neither statistically significant differences between the test groups nor a dose–response relationship was observed. The overall incidences of skeletal malformations were comparable to those found in the historical control data.

3.5.2. *Fetal morphology examinations*

Fetal variations are summarized in Table 4 (for a list of all findings, see Tables S4 and S6).

Three soft tissue variations, i.e. short innominate, enlarged atrial chamber of the heart and uni- or bilateral dilation of renal pelvis, were detected. These findings observed in 1–5 fetuses of 1–4 litters at 0, 100, 300 and 1000 mg/kg bw/d showed no dose–response

Table 4
Fetal morphology data: summary of fetal variations.

Dose [mg/kg bw/d]		0 (Vehicle)	100	300	1000
Litters evaluated	*N*	22	21	25	23
Fetuses evaluated	*N*	272	248	288	291
Total variations					
Fetal incidence	*N*	143	136	151	154
	%	53	55	52	53
Litter incidence[F]	*N*	22	21	25	23
	%	100	100	100	100
Affected fetuses per litter[W]	Mean% ± SD	52.7 ± 3.66	55.2 ± 5.66	52.3 ± 2.80	53.3 ± 6.18

$^{*}p < 0.05$; $^{**}p < 0.01$ (F, one-sided Fisher's exact test [34]; W, one-sided Wilcoxon [35–37]).

relationship. The observable differences between the groups reflect the usual fluctuation for this parameter and were clearly within the range of the historical control data.

For all groups, skeletal variations of different bone structures were observed, with or without effects on corresponding cartilage. The observed skeletal variations were related to several parts of fetal skeletons and appeared without a relation to dosing. The overall incidences of skeletal variations were comparable to the historical control data.

The incidence of the variations observed showed no dosing-related statistical significance and were within historical control ranges. Therefore these observations are not considered toxicologically relevant.

3.5.3. *Unclassified findings*

Unclassified findings are listed in Tables S2 and S7.

Two unclassified external observations, i.e. blood coagulum around placenta or fused placenta, were recorded for 1–2 littermates of the control, the mid and the high dose group and was assessed as spontaneous in nature.

Additionally, some isolated cartilage findings without impact on the respective bony structures, which were designated as unclassified cartilage observations, occurred in all groups including the controls. The observed unclassified cartilage findings were related to the skull and the sternum. Although the incidence of notched manubrium was statistically significantly higher in the high-dose group, it was well within the historical control range. Thus, an association to the test substance and a toxicological relevance for these findings is not assumed.

4. Discussion

In the present prenatal developmental toxicity study the test compound synthetic amorphous silica was administered to pregnant Wistar rats daily by gavage from implantation to one day prior to the expected day of parturition (GD 6–19) to evaluate its potential maternal and prenatal developmental toxicity. Analyses confirmed the particle size and the correctness of the prepared concentrations in vehicle. No toxicologically-relevant differences were observed between dams receiving 100, 300, or 1000 mg/kg bw/d synthetic amorphous silica and those receiving vehicle only. Furthermore, there were no alterations to gestational parameters, including number of implantations, resorptions, and live or dead fetuses at any dose. Finally, no toxicologically relevant adverse developmental effects were detected during this study. Prenatal development of Wistar rats occurred normally following dosing with up to 1000 mg/kg bw/d of SAS.

Likewise, no adverse effects were observed in parental animals and their offspring after oral administration of SAS at doses up to 1000 mg/kg bw/d in a two-generation study with Wistar rats [21].

The results of the present study are in line with those of another recently conducted study, in which there was no evidence for any systemic effect after daily oral administration of 1000 mg/kg body weight nanonized silica and other nanomaterials (ZrO_2, $BaSO_4$) for 28 days in rats; there was also no alteration by analysis of metabolome data [18]. Likewise, no systemic toxicity was observed in another recently conducted study, in which rats received two negatively charged colloidal SiO_2 particles (20 nm or 100 nm in size) by daily oral administration for a period of 90 days at doses up to 2000 mg/kg body weight [38]. One possible explanation for the lack of toxicity could be a lack of absorption of nanonized silica after oral exposure. However, kinetic studies were carried out with the two colloidal SiO_2 particle types used in the above-mentioned 90-day study, and gastrointestinal absorption ranged from 6.6% to 9.7% after single oral administration of 500 or 1000 mg/kg body weight in rats [39]. Increased Si levels were present in liver, kidneys and spleen within three days post dosing. Transmission electron microscopy revealed silica nanoparticles in liver and kidneys [40].

In conclusion, neither maternal nor embryo–fetal toxicity could be observed after oral administration of synthetic amorphous silica to pregnant rats from implantation throughout gestation at a daily dose up to 1000 mg/kg bw/d. In addition, no compound-related effects were observed in the fetuses by external, visceral and skeletal examinations. Based on the results of the present study the no observed adverse effect level (NOAEL) was established at 1000 mg/kg bw/d.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.reprotox.2015.04.006

References

[1] OECD. SIDS dossier on synthetic amorphous silica and silicates. UNEP 2004:1–254. http://www.chem.unep.ch/irptc/sids/oecdsids/Silicates.pdf
[2] EU. The European Parliament and the Council of the European Union: Regulation (EC) No 1333/2008 of the European parliament and of the council of 16 December 2008 on food additives. Off J Eur Union 2008;L354:16–33. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0016:0033:en:PDF
[3] Dekkers S, Krystek P, Peters RJ, Lankveld DX, Bokkers BG, van Hoeven-Arentzen PH, et al. Presence and risks of nanosilica in food products. Nanotoxicology 2011;5:393–405.

[4] Fruijtier-Polloth C. The toxicological mode of action and the safety of synthetic amorphous silica – a nanostructured material. Toxicology 2012;294:61–79.
[5] Association of Synthetic Amorphous Silica Producers (ASASP). Synthetic amorphous silica – ASASP's current interpretation as a nanomaterial.; 2013. http://www.asasp.eu/docs/ASASP%20Nano%20Statement%20201304.pdf
[6] Reuzel PG, Bruijntjes JP, Feron VJ, Woutersen RA. Subchronic inhalation toxicity of amorphous silicas and quartz dust in rats. Food Chem Toxicol 1991;29:341–54.
[7] Rosenbruch M. Inhalation of amorphous silica: morphological and morphometric evaluation of lung associated lymph nodes in rats. Exp Toxicol Pathol 1992;44:10–4.
[8] Lee KP, Kelly DP. The pulmonary response and clearance of Ludox colloidal silica after a 4-week inhalation exposure in rats. Fundam Appl Toxicol 1992;19:399–410.
[9] Arts JH, Muijser H, Duistermaat E, Junker K, Kuper CF. Five-day inhalation toxicity study of three types of synthetic amorphous silicas in Wistar rats and post-exposure evaluations for up to 3 months. Food Chem Toxicol 2007;45:1856–67.
[10] Landsiedel R, Ma-Hock L, Hofmann T, Wiemann M, Strauss V, Treumann S, et al. Application of short-term inhalation studies to assess the inhalation toxicity of nanomaterials. Part Fibre Toxicol 2014;11:16.
[11] Cho M, Cho WS, Choi M, Kim SJ, Han BS, Kim SH, et al. The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. Toxicol Lett 2009;189:177–83.
[12] Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K. Silica nanoparticles as hepatotoxicants. Eur J Pharm Biopharm 2009;72:496–501.
[13] Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K. Histological analysis of 70-nm silica particles-induced chronic toxicity in mice. Eur J Pharm Biopharm 2009;72:626–9.
[14] Isoda K, Hasezaki T, Kondoh M, Tsutsumi Y, Yagi K. Effect of surface charge on nano-sized silica particles-induced liver injury. Pharmazie 2011;66:278–81.
[15] So SJ, Jang IS, Han CS. Effect of micro/nano silica particle feeding for mice. J Nanosci Nanotechnol 2008;8:5367–71.
[16] Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. Br J Radiol 2006;79:248–53.
[17] Van der Sande M, Vandebriel RJ, Groot MJ, Kramer E, Herrera Rivera ZE, Rasmussen K, et al. Sub-chronic toxicity study in rats orally exposed to nanostructured silica. Part Fibre Toxicol 2014;11:8.
[18] Buesen R, Landsiedel R, Sauer UG, Wohlleben W, Gröters S, Strauss V, et al. Effects of SiO_2, CeO_2, and $BaSO_4$ nanomaterials with or without surface functionalization upon 28-day oral exposure to rats. Arch Toxicol 2014;88:1881–906.
[19] Takizawa Y, Hirasawa F, Noritomi E, Aida M, Tsunoda H, Uesugi S. Oral ingestion of syloid to mice and rats and its chronic toxicity and carcinogenicity. Acta Med Biol 1988;36:27–56.
[20] Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod Toxicol 2010;30(3):343–52.
[21] Wolterbeek A, Oosterwijk T, Schneider S, Landsiedel R, de Groot D, van Ee R, et al. Oral two-generation reproduction toxicity study with NM-200 synthetic amorphous silicia in Wistar rats. Reprod Toxicol 2015;56:147–54.
[22] OECD. Decision of the council concerning the mutual acceptance of data in the assessment of chemicals [C(81)30(Final)] (Adopted by the council at its 535th meeting on May 12, 1981). In OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 1. In: The OECD principles of good laboratory practice, environment monograph No. 45. Paris: Environment Directorate, Organisation for Economic Co-operation and Development; 1992.
[23] OECD. OECD guidelines for testing of chemicals, section 4: health effects. No. 414 prenatal developmental toxicity study. Paris: Organisation for Economic Cooperation and Development; 2001.
[24] US Environmental Protection Agency. Health effects test guidelines OPPTS 870,3700 prenatal developmental toxicity study; 1998.
[25] Barrow MV, Taylor WJ. A rapid method for detecting malformations in rat foetuses. J Morphol 1969;127:291–306.
[26] Kimmel CA, Tramell C. A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. Stain Tech 1981;56:271–3.
[27] Wise D, Beck S, Beltrame D, Beyer B, Chahoud I, Clark RL, et al. Terminology of developmental abnormalities in common laboratory mammals (version 1). Teratology 1997;55:249–92.
[28] Makris SL, Solomon HM, Clark R, Shiota K, Barbellion S, Buschmann J, et al. Terminology of developmental abnormalities in common laboratory mammals (version 2). Birth Defects Res B 2009;86:227–327.
[29] Chahoud I, Buschmann J, Clark R, Druga A, Falke H, Faqi A, et al. Classification terms in developmental toxicology: need for harmonisation. Reprod Toxicol 1999;13:77–82.
[30] Solecki R, Bürgin H, Buschmann J, Clark R, Duverger M, Fialkowski O, et al. Harmonization of rat fetal skeletal terminology and classification. Report of the third workshop on the terminology in developmental toxicology—Berlin, 14–16 September 2000. Reprod Toxicol 2001;15:713–21.
[31] Solecki R, Bergmann B, Bürgin H, Buschmann J, Clark R, Druga A, et al. Harmonization of rat fetal external and visceral terminology and classification. Report of the fourth workshop on the terminology in developmental toxicology—Berlin, 18–20 April 2002. Reprod Toxicol 2003;17:625–37.
[32] Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. JASA 1955;50:1096–121.
[33] Dunnett CW. New tables for multiple comparisons with a control. Biometrics 1964;20:482–91.
[34] Siegel S. Non-parametric statistics for the behavioural sciences. New York: McGraw-Hill; 1956.
[35] Nijenhuis A, Wilf SW. Combinatorial algorithms. New York: Academic; 1978.
[36] Miller RG. Simultaneous statistical inference. New York: Springer; 1981.
[37] Hettmansperger TP. Statistical inference based on ranks. New York: Wiley; 1984.
[38] Kim YR, Lee SY, Lee EJ, Park SH, Seong NW, Seo HS, et al. Toxicity of colloidal silica nanoparticles administered orally for 90 days in rats. Int J Nanomed 2014;9(Suppl. 2):67–78.
[39] Paek HJ, Chung HE, Lee JA, Kim MK, Lee YJ, Kim MS, et al. Quantitative determination of silica nanoparticles in biological matrices and their pharmacokinetics and toxicokinetics in rats. Adv Sci Mater 2014;6(7):1605–10.
[40] Lee JA, Kim MK, Paek HJ, Kim YR, Kim MK, Lee JK, et al. Tissue distribution and excretion kinetics of orally administered silica nanoparticles in rats. Int J Nanomed 2014;9(Suppl. 2):251–60.