



Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox



Oral two-generation reproduction toxicity study with NM-200 synthetic amorphous silica in Wistar rats

André Wolterbeek^{a,*}, Thies Oosterwijk^a, Steffen Schneider^b, Robert Landsiedel^b,
Didima de Groot^a, Renz van Ee^c, Mariëlle Wouters^c, Han van de Sandt^a

^a TNO, P.O. Box 360, 3700 AJ Zeist, The Netherlands

^b BASF SE, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany

^c TNO, P.O. Box 6235, 5600 HE Eindhoven, The Netherlands

ARTICLE INFO

Article history:

Received 1 February 2015

Received in revised form 4 March 2015

Accepted 18 March 2015

Available online xxx

Keywords:

Nanomaterials

Synthetic amorphous silica

NM-200

Reproductive toxicity

Rat

ABSTRACT

Synthetic amorphous silica (SAS) like NM-200 is used in a wide variety of technological applications and consumer products. Although SAS has been widely investigated the available reproductive toxicity studies are old and do not cover all requirements of current OECD Guidelines. As part of a CEFIC-LRI project, NM-200 was tested in a two-generation reproduction toxicity study according to OECD guideline 416. Male and female rats were treated by oral gavage with NM-200 at dose levels of 0, 100, 300 and 1000 mg/kg bw/day for two generations. Body weight and food consumption were measured throughout the study. Reproductive and developmental parameters were measured and at sacrifice (reproductive) organs and tissues were sampled for histopathological analysis. Oral administration of NM-200 up to 1000 mg/kg bw/day had no adverse effects on the reproductive performance of rats or on the growth and development of the offspring into adulthood for two consecutive generations. The NOAEL was 1000 mg/kg body weight per day.

© 2015 Published by Elsevier Inc.

1. Introduction

Synthetic amorphous silica (SAS), a nanostructured material, is a form of silicon dioxide (SiO₂) which is produced for decades without significant changes in its physical–chemical properties. SAS is applied in a wide variety of technological applications and consumer products [1–4].

“Synthetic Amorphous Silica (SAS)” covers pyrogenic silica, silica gel and colloidal silica (EINECS No. 231-545-4).

The CEFIC sector group Association of Synthetic Amorphous Silica Producers recently summarized the properties of SAS [5]: ‘SAS is produced by thermal (pyrogenic/fumed) or wet (precipitated, gel, colloidal) processes. In the initial particle formation step, primary particles with dimensions below 100 nm are formed by nucleation, coagulation and coalescence. These primary particles covalently bond to form indivisible units, called aggregates, which have no physical boundaries among them. The aggregates have external dimensions typically above 100 nm (pyrogenic, precipitated, gel). The aggregates combine to form agglomerates in the micron size

range by physical attraction forces (van der Waals) and H-bridges. SAS powder is placed on the market as micron-sized agglomerates with an internal structure in the nanoscale. This fact is true for all currently known SAS products in powder form, independent of manufacturer, process and trade name. Colloidal silica is placed on the market as aqueous preparations of mono- and poly-dispersed nanoparticles.’

A recent investigation of Dekkers et al. [3] revealed that the total average daily intake of consumers via food is approximately 9.4 mg/kg bw/day, with 1.8 mg/kg bw/day being in the nano-size range.

Only limited information is available on the general toxicity of nanosilica after oral administration. Recently, the available studies have been reviewed by Dekkers et al. [3,6].

In a study of So et al. [7], Balb/c and C57BL/6 mice were fed diets containing 1% silica particles of 0.5–30 or 30–90 nm in size for 10 weeks. Although the daily oral dose was as high as 2 g/kg bw/day, only slight effects on alanine aminotransferase values were observed in the liver of Balb/C mice and some indications of a fatty liver were observed in both strains of mice.

In a more recent study of Van der Zande et al. [8], rats were given synthetic amorphous silica at dose levels of 100 or 1000 mg/kg body weight per day for 28 days or 2500 mg/kg body weight per day for

* Corresponding author. Tel.: +31 88 866 5053.

E-mail address: andre.wolterbeek@tno.nl (A. Wolterbeek).

84 days. The actual concentration of SAS in the nano-size range (5–200 nm) was 33, 328 and 819 mg/kg body weight/day, respectively. No effects on clinical signs and body weights were observed. Biochemical and immunological markers in blood and isolated cells did not indicate signs of toxicity. In the animals treated with the high dose of synthetic amorphous silica for 84 days, histopathologically, the incidence of periportal fibrosis was slightly, but not statistically significantly, increased.

In another recent oral 28 days toxicity study with nanosized silica, performed according to OECD Guideline 407, no adverse effects were observed after dietary administration of 1000 mg/kg body weight per day [9].

This is in agreement with the results of a 90 days toxicity study with colloidal silica nanoparticles recently reported by Kim et al. [10]. In this study, no treatment-related effects were observed in rats which were dosed by gavage with 500, 1000 and 2000 mg/kg body weight/day of nano silica of 20 and 100 nm.

Besides the effects of nanosized silica on general toxicity, an important endpoint under discussion in the context of nanotoxicity is potential reproductive effects of nanomaterials in parents and offspring. Evidence exists that (unborn) children may be more vulnerable than adults to exposure to chemicals as all organs are still in development and several experimental studies have shown that manufactured nanomaterials might adversely affect male and female reproduction and pre- and postnatal development (reviewed by Ema et al. [11]). SAS has been widely investigated in numerous toxicological studies including studies concerning reproductive toxicity [1,12]. There is no indication of reproductive toxic effects of orally dosed SAS based on these data.

Nevertheless, those studies were rather old and not performed according to regulatory requirements (OECD, EU, US-EPA guidelines). To fill this gap, as part of a CEFIC-LRI N3 project, an oral prenatal toxicity study according to OECD guideline 414 (which is reported separately by Hofmann et al. [13]) and a two-generation reproduction toxicity study according to OECD guideline 416 was performed with NM-200, a precipitated synthetic amorphous silica. The aim of the present study was to perform a two-generation reproduction toxicity study according to current OECD guideline 416 [14] and to examine the possible effects of SAS on the reproductive performance of rats and on the growth and development of the offspring into adulthood for two consecutive generations. For these studies the oral route was chosen because it is a major route of human exposure.

2. Materials and methods

2.1. General

This study was performed as part of a European Chemical Industry Council Long-range Research Initiative project (CEFIC-LRI N3 project) entitled 'Testing and Assessment of Reproductive Toxicity of Nanomaterials'.

The study was performed following the principles of the OECD Guideline for Testing of Chemicals 416 [14] and was conducted in accordance with the principles of Good Laboratory Practice (GLP) [15].

Animal care and use was in accordance with Directive 86/609/EEC, which establishes the general principles governing the use of animals in experiments of the European Communities, and with Dutch-specific legislation [16].

2.2. Test compound

NM-200 Synthetic Amorphous Silica (batch PR-A-2) was supplied by the Joint Research Centre (Ispra, Italy) and had the

following characteristics: EINECS No. 231-545-4, CAS numbers 7631-86-9 (old general CAS number for silica including synthetic amorphous silica) and 112945-00-8 (CAS number for precipitated synthetic amorphous silica) and the purity was 96.5% (silicon dioxide content as SiO₂). NM-200 was stored at ambient temperature in the dark under N₂ atmosphere.

The vehicle, methylhydroxypropylcellulose (MHCP) (batch XH171907F1) was supplied by Dow Chemicals and had the following characteristics: CAS number 900-65-3 and the purity was 100% and MHCP was stored at ambient temperature. MHCP was also used as vehicle for NM-200 in general toxicity studies performed by Fraunhofer Institute of Toxicology and Experimental Medicine as part of the CEFIC-LRI N1 project (Tiered Approach to Testing and Assessment of Nanomaterial Safety to Human Health) (personal communication).

2.3. Animals

A total of 116 male and 116 female rats, Wistar outbred (CrI:WI(Han) strain) of about 4–5 weeks of age were obtained from a colony maintained under SPF conditions at Charles River Deutschland (Sulzfeld, Germany).

The animal room was kept under standard laboratory conditions with approximately 10 air changes per hour, temperature and the relative humidity controlled with target ranges of 22 ± 2 °C and 45–65%, respectively and a 12-h light cycle.

A rodent breeding diet (Rat and Mouse 3, Special Diets Services, Witham, England) and water from the public supply were available *ad libitum*. The diet in the feeders was refreshed once per week and topped up when necessary. Animals were housed in macrolon cages with a bedding of wood shavings (Lignocel, Rettenmaier, Rosenberg, Germany) and strips of paper as environmental enrichment (Enviro-dri, Lillico, Betchworth, England).

2.4. Test formulations

Once weekly, until completion of the dosing period of the study, seven bottles per dosing group were prepared, each containing the appropriate amount of NM-200 and stored at ambient temperature in the dark under N₂. On each subsequent day, the required amount of vehicle (0.5% v/v of MHCP in Ultrapure water) was added to achieve concentrations of 0, 10, 30 and 100 mg/ml NM-200 and stirred on a magnetic stirrer (approximately 900 rpm) for at least 60 min. All samples were continuously stirred under the same conditions during the entire daily administration period in order to maintain the homogeneity of the NM-200 in the vehicle.

At various weeks during the study samples were taken from each of the dosing formulations for analytical investigations of the hydrodynamic diameter of the SiO₂ particles using Dynamic Light Scattering (DLS) technique.

2.5. Analysis of NM-200

The particle size and the particle size distribution of the NM-200 particles in the vehicle MHCP were analyzed with a Zetasizer-Nano ZS Instrument (Malvern). Dynamic Light Scattering (DLS) used to determine the size distribution of the particles is based on the quantification of dynamic fluctuations of light scattering intensity caused by Brownian motion of the particles. This technique yields a hydrodynamic diameter that is calculated via the Stokes–Einstein equation from the aforementioned measurements. The result of the measurements is the average hydrodynamic diameter of the particles, the peak value is the hydrodynamic diameter distribution and the polydispersity index (PDI) that describes the width of the particle size distribution. The PDI scale ranges from 0 to 1, with 0 being monodisperse and 1 being polydisperse. Each assigned size

Download English Version:

<https://daneshyari.com/en/article/7252124>

Download Persian Version:

<https://daneshyari.com/article/7252124>

[Daneshyari.com](https://daneshyari.com)