Organism's responses to a long-term inhalation of silica-containing submicron particles of an industrial aerosol

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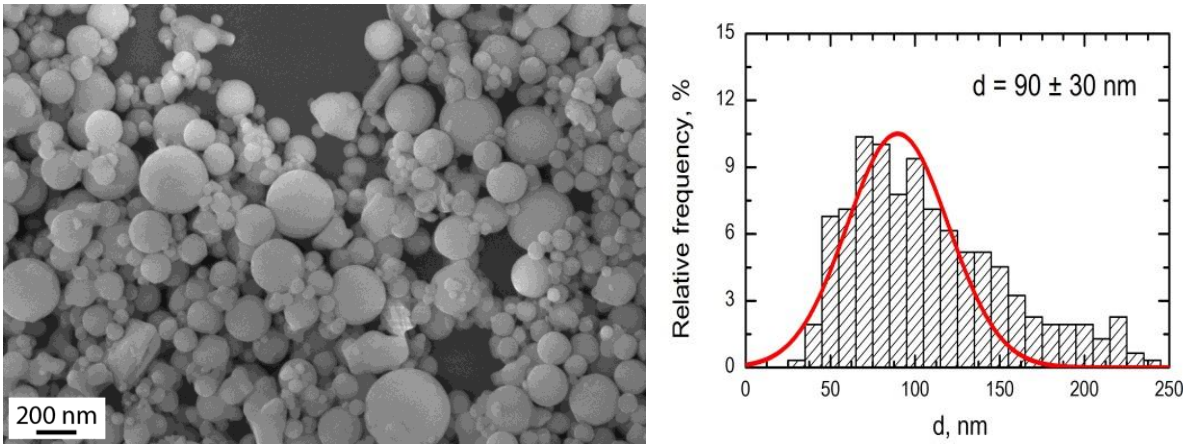
Abstract. Female white rats were exposed during up to 6 months 5 times a week, 4 hr per day in a "nose only" inhalation device to an aerosol containing predominantly submicron (nanoscale included) particles of amorphous silica in the concentration 2.6±0.6 or 10.6±2.1 mg/m^3. In an auxiliary experiment with a single-shot intratracheal instillation of these particles, it was shown that they induced a pulmonary cell response comparable with that to highly cytotoxic and fibrogenic standard quartz powder DQ_{12}. However, in the long-term inhalation test, the studied aerosol proved to be of very low systemic toxicity and fibrogenicity. This paradox may be explained by low SiO_2 retention in lungs and other organs due to a relatively high *in vivo* solubility of these nanoparticles. Nevertheless, their genotoxic action and transnasal penetration into the brain should make one give a cautious overall assessment of this aerosol as an occupational or environmental hazard.

1. Introduction

While engineered SiO_2 nanoparticle toxicity is being widely investigated, mostly on cell lines or in acute animal experiments [1-9], and a lot of others, the practical importance of industrial condensation aerosols with a high SiO_2 particle content and the theoretical interest in it seems to be neglected. Thus, long-term inhalation exposure to nano-SiO_2 has not been, as far as we know, undertaken in experimental nanotoxicology research.

2. Experimental

Outbred female white rats were exposed for 3 or 6 months, 5 times a week, 4 hr a day to an aerosol containing 72% amorphous silica predominantly submicron particles at an exposure concentration of 2.6±0.6 or 10.6±2.1 mg/m^3. This material (Fig. 1a) had been collected from the flue-gas ducts of electric ore smelting furnaces producing elemental silicon, subsequently sieved through a <2 μm screen and re-dispersed in airflow (Fig. 1b) to feed a computerized "nose only" inhalation system (Fig. 2).



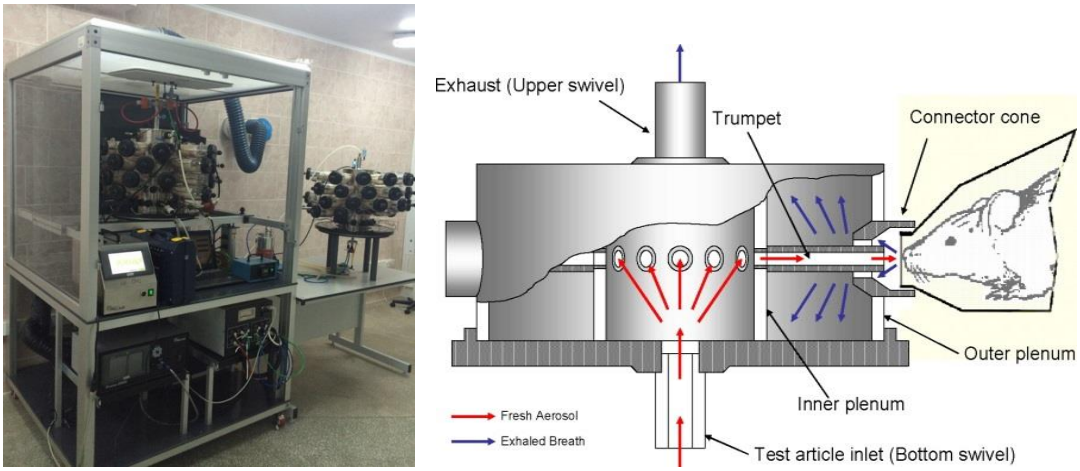
(a) (b)

Figure 1. Particles of the powder collected in the flue gas duct from the hood over a silicon smelting ore-thermal furnace and sieved through a < 2 μm screen. (a) SEM image, (b) particle size distribution.

Preliminarily, we carried out an experiment to study the response of the lower airways free cells obtained from rats exposed, 24 h before bronchoalveolar lavage, to a single-shot intratracheal instillation of the same particles suspended in normal saline in comparison with the response to the highly cytotoxic and fibrogenic standard quartz dust DQ_{12}. A batch of each powder was incubated during 24 h at 37°C together with either the normal saline, or the Ringer-Locke's solution, or the cell-free bronchoalveolar lavage fluid (BALF) supernatant. Samples of each system were taken at several time points and analysed for the Si content with the help of atomic absorption spectrometry (AAS).

3. Results and discussion

Judging by a 3-4-fold increase in the total cell count of the BALF after intratracheal instillation of particles due to enhanced recruitment of alveolar macrophages (AM) and, especially, of neutrophil leukocytes (NL) to the lower airways free surface (Table 1), the cytotoxicity of Nano-Silica Containing Aerosol (NSCA) investigated and that of quartz DQ_{12} are quite similar.



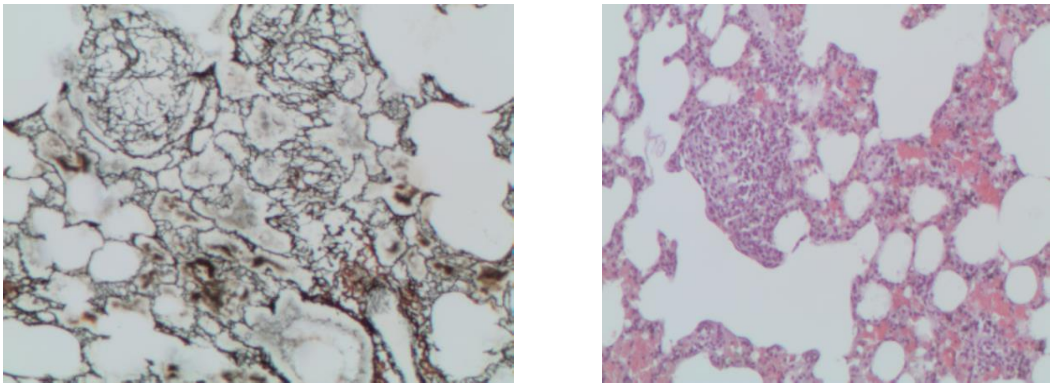
(a) (b)

Figure 2. (a) General view of the experimental setup (photographed with the front chamber doors removed). Next to the exposure unit, a similar system setup for sham exposure of control rats is seen. (b) Diagram of aerosol flows in the "nose only" inhalation exposure system (courtesy CH Technology, USA).

Table 1. Number of cells in the bronchoalveolar lavage fluid (BALF) 24 hours after intratracheal instillation of NSCA pr DQ_{12} particle suspensions administered to rats at a dose of 7 mg in 1mL normal saline (x ± s.e.).

NB: *statistically significant difference from the control group (P <0.05 by Student's t-test).

What was instilled:	**Number of cells * 10^6**			**NL/AM**
	Total	**Alveolar macro-phages (AM)**	**Neutrophil leukocytes (NL)**	
NSCA	5.85±0.56*	3.03±0.66	2.86±0.79*	2.24±1.16*
DQ_{12}	7.44±1.30*	3.79±0.94*	3.10±0.80*	1.69±0.64*
Normal saline (control group)	1.96±0.29	1.49±0.28	0.52±0.24	0.37±0.16



(a) (b)

Figure 3. Rat lungs after inhalation exposure to the NSCA at the concentration of 10.6 mg/m^3 during 6 months. (a) Focal coarsening of the argyrophylic framework of the lung tissue and appearance of a net of thin argyrophylic fibres within a macrophagal-lymphocytic nodule. (b) Alveolar septa are thickened, with lymphocytic infiltration, but round macrophage-lymphocytic clusters without detectable collagen fibres within or around them seen in this preparation occur as solitary observations only. Hematoxylin and eosin staining, magnification X50.

Table 2. Some indices for rat lungs status after inhalation exposure to particles of NSCA (X ± s.e.).
NB: *statistically significantly difference from corresponding control value (p<0.05 by Student's t-test); •statistically significantly difference from the low-exposure group of the same exposure period (p<0.05 by Student's t-test).

Indices	3 months			6 months		
	Controls	**NSCA 2.6 mg/m^3**	**NSCA 10.6 mg/m^3**	**Controls**	**NSCA 2.6 mg/m^3**	**NSCA 10.6 mg/m^3**
Dry lung mass, g/100g body mass	0.08 ±0.01	0.08 ±0.01	0.09 ±0.01•	0.17 ±0.02	0.17 ±0.02	0.16 ±0.01
Total lipid content of lungs, mg	33.82 ±2.74	31.84 ±2.98	37.66 ±4.57	68.02 ±5.63	36.98 ±4.81*	60.64 ±4.56
Hydroxyproline content of lungs, µg	2060 ±85	4978 ±1187*	4641 ±1022*	4112 ±578	3048 ±533	6142 ±946•
Hydroxyproline content of lungs, µg/100 g body mass	776 ±42	1898 ±460*	1711.9 ±381*	1653 ±209	1146 ±209	2227 ±342•

Table 3. SiO_2 content of the rat organs, blood and excreta (x±s.e.).
NB: [1]Determined in each group of rats' pooled tissue, divided by the number of rats in the group;
[*]Statistically significantly different from the control value for the same exposure period;
[+]Statistically significantly different from the index obtained for the same exposure period at the lower concentration;
[@]Statistically significantly different from the value obtained for the previous exposure period at the same concentration ($p< 0.05$ by Student's t-test).

Exposure period, months	Group	Organs, mg						Blood, mg/L	Urine, mg/L	Feces, mg/g
		lungs	lungs-associated lymph nodes	Liver	kidneys	spleen	brain			
Exposure concentration of NSCA 2.6 mg/m^3										
3	Exposed	0.11± 0.02*	0.01	0.02± 0.00*	0.03± 0.00*	0.02± 0.00	0.02± 0.00	0.39± 0.16*	1.47± 0.21*	2.18± 0.12*
	Control	0.02± 0.01	0.00	0.01± 0.01	0.01± 0.01	0.02± 0.01	0.01± 0.00	0.24± 0.06	0.44± 0.07	1.36± 0.10
6	Exposed	0.20± 0.01*+	0.06	0.04± 0.01*+	0.04± 0.01*+	0.05± 0.01*+	0.02± 0.00	0.46± 0.04*+	1.68± 0.07*+	2.32± 0.28*
	Control	0.04± 0.006	0.01	0.01± 0.002	0.01± 0.001	0.02± 0.003	0.01± 0.00	0.27± 0.04	0.44± 0.07	1.38± 0.08
Exposure concentration of NSCA 10,6 mg/m^3										
3	Exposed	0.63± 0.07*@	0.02	0.03± 0.00*@	0.04± 0.01*@	0.06± 0.01*@	0.02± 0.00	0.60± 0.14*@	4.57± 0.23*@	7.24± 0.30*@
	Control	0.02± 0.01	0.00	0.01± 0.01	0.01± 0.01	0.02± 0.01	0.01± 0.00	0.24± 0.06	0.44± 0.07	1.36± 0.10
6	Exposed	0.74± 0.05*+@	0.06	0.06± 0.01*+@	0.07± 0.01*+@	0.07± 0.00*+@	0.02± 0.00	0.71± 0.05*+@	3.06± 0.11*+@	4.39± 0.53*+@
	Control	0.04± 0.01	0.01	0.01± 0.00	0.01± 0.00	0.02± 0.00	0.01± 0.00	0.27± 0.04	0.44± 0.07	1.38± 0.08

However, under long-term inhalation exposures, the NSCA proved to be of a very low systemic toxicity as assessed with a lot of functional and biochemical indices, and of a negligible pulmonary fibrogenicity as assessed both morphologically (Fig. 3) and biochemically (Table 2).

As can be seen from Table 3, the AAS- determined silica content of the lungs and lungs-associated lymph nodes is substantially higher than in the controls, this increase being dependent on both the level and the duration of the inhalation exposure, which dependences confirm its causal relationship with the latter.

However, according to Ref. [10], approximately the same extent of retention of SiO_2 in rat lungs (0.550 mg) was observed over 6 months under a similar inhalation exposure to standard quartz dust DQ_{12} at a concentration of 1 mg/m^3, which is 10 times lower than in our experiment with the NSCA. In other words, if calculated per unit concentration of aerosol in the air, the accumulated mass of particles in the lungs under chronic inhalation exposure to NSCA is approximately 10 times less than that under similar inhalation exposure to quartz dust DQ_{12}.

The most consistent explanation of this phenomenon (which, in its turn, is a sufficient explanation of weak silica-induced pathology) is the dissolution of submicron (nanoscale, in particular) silica particles *in vivo*, especially in the lining fluid of the pulmonary area, on which they deposit when inhaled. This mechanism was modelled by us *in vitro* by a noticeable solubilisation of particles in the BALF of intact rats.

Even though the scanning transmission electron microscopy (STEM) confirmed that NSCA particles are retained in the lungs (Fig. 4), this retention was evidently not sufficient to cause any essential pathological changes in the pulmonary tissue.

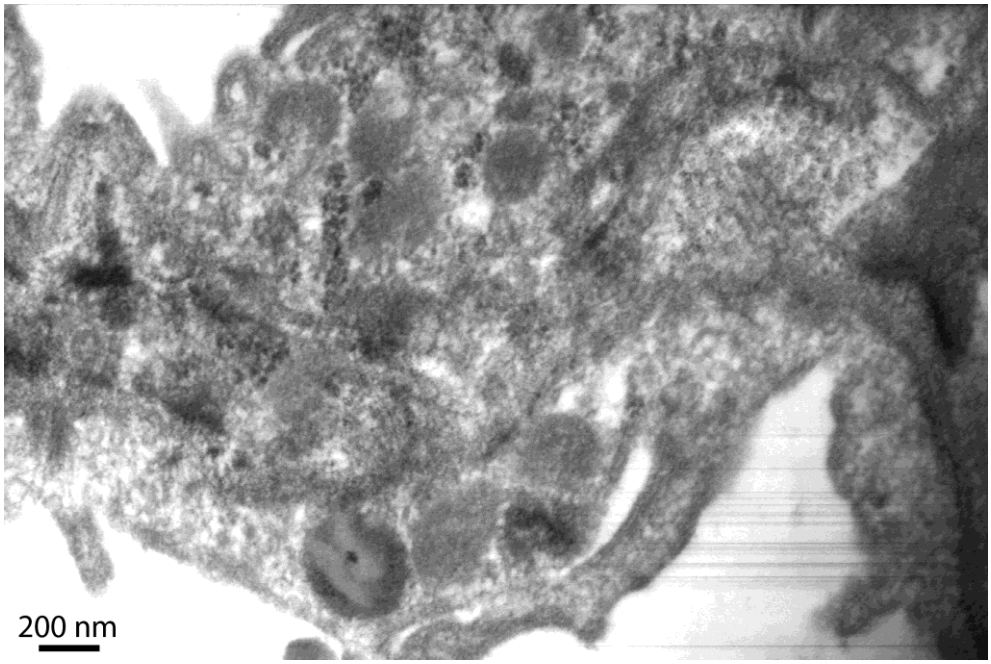


Figure 4. Nanoparticles within a type I alveolocytes of a rat exposed to the NSCA at the concentration of 10.6 mg/m^3 during 3 months. STEM image.

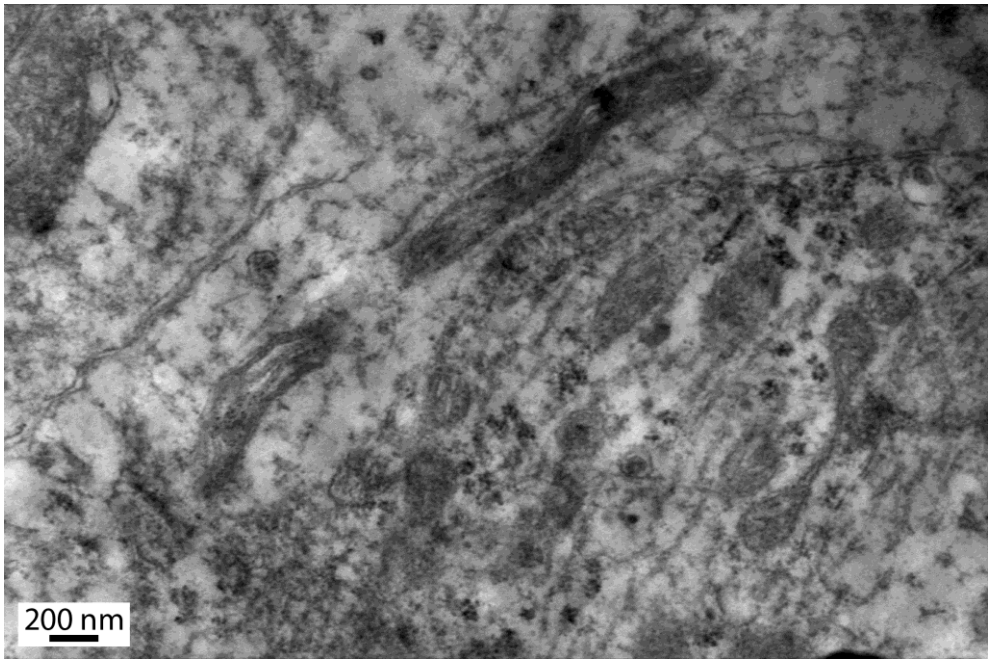


Figure 5. Nanoparticles in the neuron body under inhalation exposure to NSCA at the concentration of 2.6 mg/m^3 during 3 months. STEM image.

The ability of inhaled nanoparticles initially deposited in the nasal passages to penetrate along the olfactory nerve fibres into the olfactory area of the brain known from the literature [11-13] has been confirmed in our previous experiment with nano-Fe_2O_3 as well [14]. As follows from Figure 5, even in the rats that were exposed to the lowest concentration of the NSCA we revealed a considerable number of nanoscale electron-dense round formations in some neurons of this area. These formations are most likely to be nanoparticles of the aerosol under study.

Table 4. Genomic DNA fragmentation coefficient for blood and bone marrow cells of rats exposed to the NSCA per inhalation during 6 months (RAPD-test) (x ± s.e.)
NB: *Statistically significant difference from corresponding control value;
•Statistically significant difference from the low-exposure group ($p < 0.05$ by Student's t-test).

Cells	Controls	Exposure level	
		2.6 mg/m^3	**10.6 mg/m^3**
Nucleated blood cells	0.4240±0.0005	0.4622±0.0004*****	0.4704±0.0005***•**
Bone marrow	0.3995±0.0005	0.4043±0.0003*****	0.4316±0.0003***•**

As can been seen from Table 4, the enhanced fragmentation of the genomic DNA was found in both nucleated blood cells and bone marrow cells, the latter clearly displaying the dependence of this effect on the exposure level.

4. Conclusion

The silica (mostly amorphous) containing submicron particles with a prevailing proportion of those in the nanoscale range induce, when instilled intratracheally, a pulmonary cell response comparable with that to highly cytotoxic and fibrogenic standard quartz powder DQ_{12}. Nevertheless, in long-term inhalation experiment at realistic concentrations, they proved to be of very low systemic toxicity and negligible pulmonary fibrogenicity. This paradox may be explained by low SiO_2 retention in lungs and other organs due to a relatively high solubility of these nanoparticles in relevant biological and model milieus.

However, their genotoxic action and transnasal penetration into the brain found in the same inhalation experiment should make one give a cautious overall assessment of this aerosol as an occupational or environmental hazard.

Acknowledgements

The research was partially supported by the Ministry of Science and Higher Education of the Russian Federation (project 3.9534.2017/8.9) and by Government of the Russian Federation (Act 211, Agreement 02.A03.21.0006). The equipment of the Ural Centre for Shared Use "Modern Nanotechnology" UrFU was used.

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