TOXICOLOGICAL-HYGIENIC ASSESSMENT OF SAFETY OF WATER SUSPENSION OF NANODISPERSED SILICON DIOXIDE PARTICLES SYNTHESIZED BY LIQUID-CRYSTAL TEMPLATING[1]

N. Zaitseva[1], M. Zemlyanova[1], V. Zvezdin[1], Ye. Sayenko[2]

[1] Federal Scientific Center for Medical and Preventive Health Risk Management Technologies,
[2] Institute of Technical Chemistry, the Ural Branch of the Russian Academy of Sciences, Perm, Russia

Abstract. Experimental research has shown that after intragastric administration of a water suspension of nanodispersed silicon dioxide particles synthesized by the method of templating the tested product falls in hazard class 3 by criteria LD50. A number of morphological changes were detected in the least researched dose of 500 mg/kg, in particular, gross changes in the blood circulatory system in the form of vascular distention, kidney, liver, and thymus. Other changes included lymphoid and macrophage proliferation and degenerative changes in the liver, kidney, splenic cords and lungs.
After administration of a microdispersed analogue by the same method, no deaths of experimental animals were recorded (hazard class 4). A number of morphological changes were detected in dose 500 mg/kg including moderate vascular changes in liver, lymphoid proliferation, and lymphoid infiltration in the tissues of the esophagus, liver, kidney, large intestine, and gastric tissues.
Key words: water suspension of nanodispersed silicon dioxide particles, potential hazard, toxicological-hygienic assessment, public health.

Introduction. Following the global IT industry trends, domestic nanotechnology industry in Russia is a strategic priority, as indicated in a Program to develop nanotechnology industry in the Russian Federation by 2015, which determines new approaches to transformation of the national industry. The volume of nanoproduction in Russia must increase ten-fold by 2016 to reach 900 billion roubles in order to make the domestic product competitive [1].

Intensive growth of nanoclusters abroad and in the Russian Federation, development of a global nanoproducts market in terms of nanomaterials, and increase in commercial application in the key industries – medicine, biotechnology, energy, electronics, IT, processing industry, consumer's sector [2] – call for systematic studies of potential hazards to human wellbeing related to large-scale development and proliferation of nanotechnologies and nanobiotechnologies.

Today nano-toxicity and biosafety of nanomaterials are one of the most pressing issues faced by the global community which results in a large number of studies and research works. The studies of interactions between the nanostructures and biological systems focus on the connections between the physical and chemical properties of nanomaterials (including size, shape, surface structure, composition, aggregation, and solubility) and induction of toxic responses in biological structures [3-6]. This area is being researched by the Federal State Research Institution "Federal Research Center for Medical and Preventative Technologies of Public Health Risk Management"; namely, the Institute is conducting a toxicological-hygienic assessment of safety of synthesized nanomaterials [7].

The list of synthesized nanomaterials includes amorphous nanodispersed silicon dioxide

[1] *Translated by Ksenya Zemnlyanova*

particles. This material has a high potential of an active substance that provides targeted drug delivery [8]. Production and utilization capacity of this product is estimated at 1000 tons per year which is considered a mass product [9]. This implies direct exposure of the personnel involved in the production process (inhalation) and the population as consumers of the product (peroral administration of a formulated product). Mesoporous nanodispersed silicon dioxide particles can be also use as biosensors – contrast agents in MRT [10]. In this case, the production and utilization capacity of the product is estimated at up to 1 ton per year - potential personnel exposure.

Preliminary assessment of potential health risks of nanodispersed silicon dioxide particle conducted on the basis of analytic generalization of physic-chemical, molecular and biological, biochemical, cytological, and environmental characteristics and based on the in-house studies and available literature referenced below indicates an average hazard level by 'personal' hazard criteria (D). The D value at 1.75 falls in the range of 1.111 – 1.779 which is considered 'an average level of potential hazard' [9] and requires further toxicological-hygienic studies.

The purpose of this research paper is an experimental study and assessment of toxicity and morphological traits of the tissues of internal organs and systems under exposure to nanodispersed silicon dioxide particles synthesized by the method of liquid-crystal templating.

Materials and methods. The experimental studies focused on the water suspension of nanodispersed silicon dioxide particles ($SiO_2 \times 0{,}14H_2O$) synthesized by a standard method of nanostructures synthesis – liquid-crystal templating with a use of cetyl trimethyl ammonium bromide ($C_{16}H_{33}(CH_3)_3NBr$, CTAB) as a structure-directing agent (template) [11, 12]. The synthesis was conducted at a multi-phased dispersed flow lab of the Institute of Technical Chemistry, Ural Branch of the Russian Academy of Sciences. The use of micelles of surface-active agents prevented particle growth and made it possible to synthesize stabilized species of certain sizes correlating with the sizes of micelles [13]. CTAB was removed by repeated ethanol extraction in acidated environment (with hydrochloric acid), the degree of extraction totaled at least 98%. The residual concentration of CTAB in a nanodispersed solution of silicon dioxide was measured on a liquid-crystal chromatographer using the Agilent 6460 Triple Quad Mass Spectrometer (USA). An aqueous solution of microdispersed silicon dioxide ($SiO_2 \times 0{,}08H_2O$) was synthesized by Shtober's method in order to conduct a comparative analysis of toxicity parameters and morphological traits of the tissues of internal organs and tissues in an acute experiment [14].

The assessment of the sizes and shapes of nano- and microdispersed particles of the substance in the water suspension was conducted by the method of dynamic light-scattering using Horiba LB-550 analyzer (Horiba, Japan) and the laser Microtrac S3500 analyzer (Microtrac, USA). Specific surface area of the particles was measured by the Brenauer-Emmett-Teller (BET) method, texture parameters – by the nitrogen adsorption method at the temperature of -196 °C using the ASAP 20z0 analyzer (Micromeritics, США). The pore size distribution was calculated from the adsorption isotherm by the Barrett-Joyner-Halenda method [16]. Silicon concentration in the water suspension was measured by the method of atomic absorption spectrometry with the use of air-acetylene flame on the Perkin-Elmer 3110 spectrometer (Perkin Elmer Inc, USA).

The assessment of toxicity and morphological traits of the tissues of internal organs and systems in an acute experiment with the administration of the tested substances was conducted with the use of C57BL/6J mice, mature males, with a body weight of 27.0±2.0 g. The experimental animals were divided into 10 groups of 10 mice per group. The experimental animals in the 1st, 2nd, and 3rd groups received a one-time dose of silicon dioxide in aqueous suspension intragastrically in a concentration of 41 mg/cm^3 at doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg in the amount of 0.3 ml, 0.6 ml, 0,9 ml respectively. The animals in the 1st, 2nd, and 3rd groups received aqueous solution of the microdispersed substance in a concentration of 15.8 mg/cm^3 administered at the same doses and by the same method in the amount of 0.8 ml, 1.6 ml and 2.4 ml. The animals in the 4th, 5th, and 6th comparison groups received an aqueous suspension containing CTAB administered by the same method in a concentration of 0.96 mg/cm^3 which by 10 times exceeds residual concentration of the substance in the aqueous suspension of nanodispersed silicon dioxide particles, in the amount of 0.3 ml, 0.6 ml, and 0.9 ml (at doses of 1.15 mg/kg, 2.30 mg/kg, 3.46 mg/kg). The control group was administered distilled water in the same amount as the aqueous suspension administered to the animals in the test groups. Toxicity was measured by the following criteria: cidal activity, median lethal time, and clinical manifestations of poisoning. The observation period totaled 14 days.

During the experiment, the animals were maintained in an environment of laboratory vivarium (in polypropylene cages of standard dimensions, 5 animals in each cage) on a half-synthetic ration following the Toxicological-Hygienic Assessment of the Safety of Nanomaterials guidelines (MU 1.2.2520-09). Access to food and water was not limited. During the experiment, the animals were maintained in a room at a temperature of 23.0±2.0 Cº and humidity of 60.0±5.0%.

The experiment was conducted in accordance with the Guidelines for the use of animals in neuroscience research. The remaining rats that survived the experiment were decapitated.

The animals that died during the tests were taken out of the experiment at the end; their liver, spleen, kidney, heart, esophagus, small and large intestine, lungs, testis, thymus, groin glands, cerebral hemispheres, and tentorium were removed consecutively. The organs were fixated in 10% formaldehyde and then embedded in paraffin wax. In order to prepare microsections, serial sections were stained with hematoxylin-eosin, methyl green / pyronin by Brashe and then treated with RNAse, Periodic Acid-Schiff with amylase control for glycogen and neutral glycosaminoglycans (GAG), alcian blue to detect acid containing GAG. Visualization of micro-organisms was conducted using Micros optical microscope (Micros, Austira) with magnification of 100 – 1000^x.

Results. The aqueous suspension of nanodispersed silicon dioxide particles tested ruing the acute experiment contained 67% of the total amount of ellipsoidal particles with the size of a smaller axis of 25-35 nm; 33% of the particles were of a spherical shape with a diameter of 25 nm (Figure 1, 2). The microparticles of silicon dioxide were ‘traditional’ dispersion particles (1 mcm and bigger), with the size of a smaller axis of 3.9 mcm.

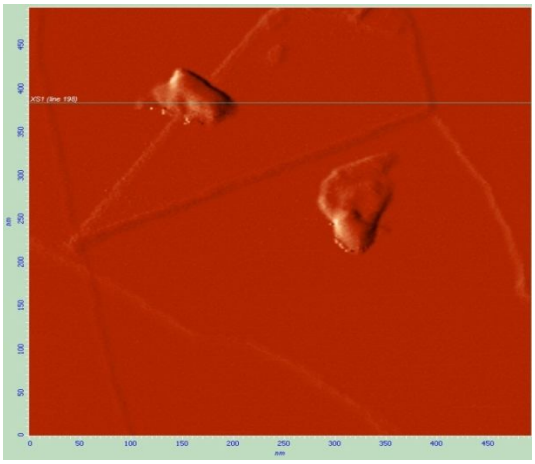

Figure 1. Synthesized nanodispersed silicon dioxide particles shown with a help of atomic force microscopy

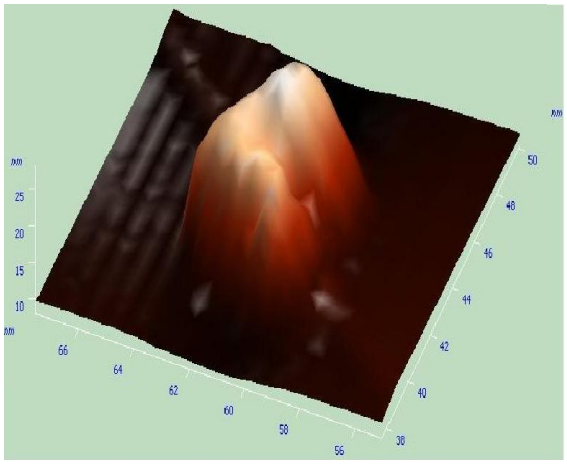

Figure 2. 3D-configuration of the surface of synthesized nanodispersed silicon dioxide particles

Specific surface area of the nanoparticles totaled 96.96 m2/g which correlates with the specific surface area of the particles of nano-sized range (from 50 to 380 м2 per 1 g of substance) and by 7.6 times exceeds the specific surface area of a microsized analogue (12.54 m2/g).

Acute toxicity (LD50) of nano-sized silicon dioxide in a dispersed solution after one-time intragastric administration totaled 4638 mg/kg which puts it in hazard class 3. The clinical picture of acute intoxication effects in the first 20 minutes of the experiment included decrease in motor activity and weak response to sound stimulus. The effects on the 2-3 days included significant swelling of the abdominal region, shallow breathing, and restriction of movement. Most deaths of the experimental animals took place on the 2-4 days (Table 1). No deaths were registered during the observation period in the studied ranges of doses for the aqueous suspension of microdispersed silicon dioxide and CTAB solution (hazard class 4).

Table 1

Dynamics of deaths in experimental animals after one-time intragastric administration of nano- and microdispersed aqueous suspension of silicon dioxide and CTAB solution

№	Experimental Group	Dose, mg/kg	Animals in a group	Observation period in days					The number of animals that died	Mortality %
				1	2	3	4	14		
1	Test	500.0	10	0/10	0/10	0/10	1/10	0/10	1	10
2	Test	1000.0	10	0/10	0/10	0/10	4/10	0/10	4	40
3	Test	1500.0	10	0/10	0/10	2/10	2/10	2/10	2	20
4	Comparison	500.0	10	0/10	0/10	0/10	0/10	0/10	0	0
5	Comparison	1000.0	10	0/10	0/10	0/10	0/10	0/10	0	0
6	Comparison	1500.0	10	0/10	0/10	0/10	0/10	0/10	0	0
7	Comparison	1.15	10	0/10	0/10	0/10	0/10	0/10	0	0
8	Comparison	2.30	10	0/10	0/10	0/10	0/10	0/10	0	0

9	Comparison	3.46	10	0/10	0/10	0/10	0/10	0/10	0	0
10	Control	-	10	0/10	0/10	0/10	0/10	0/10	0	0

It was determined that nanosized silicon dioxide particles in aqueous suspension at the dose of 1000 mg/kg and 1500 mg/kg have a toxic effect on blood corpuscles of the experimental animals including the presence of polychromatophil cells (up to 25% of the total erythrocyte count), pathological Jolly bodies in the red blood cells (up to 10% of the total erythrocyte count), and significant thrombocyte aggregation. Microdispersed silicon dioxide administered at the same dose did not have any negative effects on the blood corpuscles.

The analysis of the morphological changes of the tissues of internal organs after one-time intragastric administration of nanodispersed silicon dioxide in aqueous suspension at the least studied dose (500 mg/kg) showed significant changes in the blood circulatory system including excessive venous plethora in liver, kidney, thymus, meninx vasculosa, cardiac muscle; sudden expansion of the blood vessels in the mucous membrane and submucous tissue of the esophagus and stomach (Figures 1, 3).

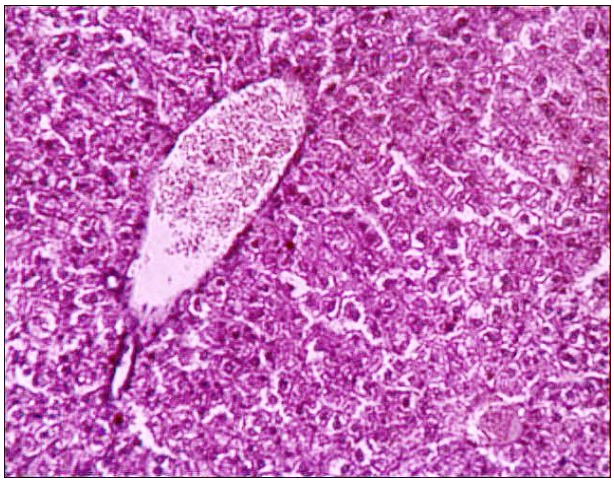

Figure 1. Liver of a survived mouse (x200) after one-time intragastric administration of nanodispersed silicon dioxide in aqueous suspension at the dose of 500 mg/kg

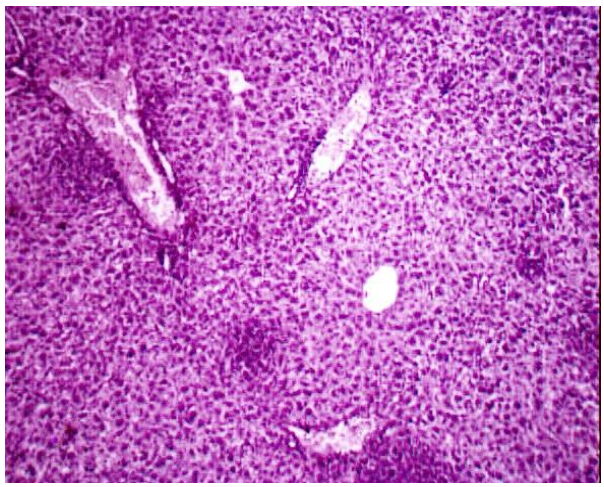

Figure 2. Liver of a survived mouse (x400) after one-time intragastric administration of microdispersed silicon dioxide in aqueous suspension at the dose of 500 mg/kg

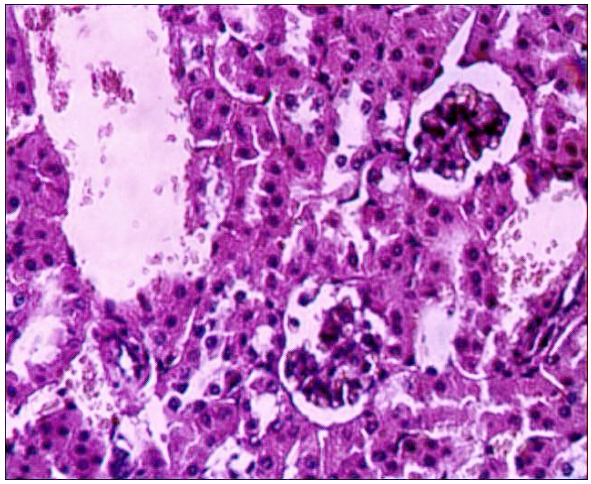

Figure 3. Liver of a survived mouse (x400) after one-time intragastric administration of nanodispersed silicon dioxide in aqueous suspension at the dose of 500 mg/kg

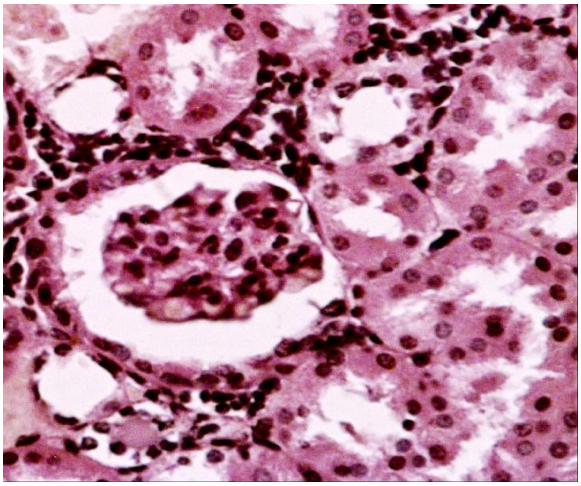

Figure 4. Liver of a survived mouse (x400) after one-time intragastric administration of microdispersed silicon dioxide in aqueous suspension at the dose of 500 mg/kg

Active proliferative processes registered in the lymphoid and macrophage systems included multiple microfocal and, more rarely, small-focal lympho-histiocytic infiltration in the liver with periportal small-focal infiltrations; few small diffusive intratubular infiltrations in the kidney, vibrant response of mesangial cells and moderate expansion of cavities of Bowman-Shumlyansky's capsules of the kidney corpuscles; lymphatization of the red pulp in the spleen; confluence of the splenic lymphoid nodules; near-zero presence of brain matter in the lobules of the thymus which made the lobules look homogenous.

Degenerative changes were exhibited in the liver – in the form of pronounced polymorphism of hepatocyte nuclei; in the kidney – in the form of a sharp color contrast between the proximal and distal canaliculi, in the spleen – in the form of increased count of apoptotic cells and cell debris, in the lungs – in the form of thickening of interalveolar septum due to the growth of interstitial tissue.

Administration of microdispersed suspension at the dose of 500 mg/kg caused moderate vascular changes predominantly in the kidney, heart, and brain. Liver exhibited expansion of large portal vessels (Figures 2, 4). Insignificant proliferative changes were registered in the liver in the form of singular microfocal perivascular infiltrates. Proliferative responses of the lymphoid tissue included signs of hypertrophy and proliferation in the spleen including enlarged area of lymphoid nodules, lymphatization of the red pulp in the spleen, presence of a large amount of blastic variants; lymphoid infiltrations in the tissues of the internal organs including diffusive infiltrations in the esophagus, and stomach, and focal infiltrations in the liver, kidney, in the passage between the esophagus and stomach, and in the large intestine.

Conclusions and Recommendations. After intragastric administration, water suspension of nanodispersed silicon dioxide particles synthesized by the method of templating the tested product falls in hazard class 3 by criteria LD50 (LD50 – 4638 mg/kg). A microdispersed

analogue falls in hazard class 4. Histological changes in the tissues of internal organs and systems of the experimental animals after one-time intragastric administration of nanodispersed silicon dioxide in aqueous suspension at the least studied dose (500 mg/kg) included significant changes in the blood circulatory system including excessive venous plethora in liver, kidney, and thymus which were not exhibited after administration of a microdispersed analogue. Active proliferative processes were registered in the lymphoid and macrophage systems, liver, kidney, the red pulp of the spleen; at the same time, the administration of a microdispersed analogue caused only insignificant proliferative changes in the liver. Degenerative changes were exhibited in the liver – in the form of pronounced polymorphism of hepatocyte nuclei; in the kidney – in the form of a sharp color contrast between the proximal and distal canaliculi, in the spleen – in the form of increased count of apoptotic cells and cell debris, which were not exhibited after administration of a microdispersed analogue.

Peroral administration of nanodispersed silicon dioxide that was synthesized by the templating method and has a mesoporous structure in the practical application for the purpose of direct drug delivery requires revision of the chronic toxicity characteristics. In order to provide a safe work environment for the personnel involved in the production process it is necessary to revise the acute and chronic toxicity characteristics exhibited after inhalation.

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About the authors:

Zaitseva Nina Vladimirovna – Academician, the Russian Academy of Medical Sciences, Doctor of Medical Sciences, Professor; Director; e-mail: znv@fcrisk.ru, root@fcrisk.ru; phone: +7 (342) 237-25-34; Federal Research Center for Medical and Preventative Technologies of Public Health Risk Management; 614045, Perm, Monastyrskaya, 82.

Zemlyanova Marina Aleksandrovna – Doctor of Medical Sciences, Professor; Head, Biochemical and Cytogenetic Diagnostics Laboratory; e-mail: zem@fcrisk.ru; phone: +7 (342) 236-39-30; Federal Research Center for Medical and Preventative Technologies of Public Health Risk Management; 614045, Perm, Monastyrskaya, 82.

Zvezdin Vasiliy Nikolayevich – Research Associate at the Biochemical and Cytogenetic Diagnostics Laboratory; email: zvezdin@fcrisk.ru; phone: + 7 (342) 237-18-15; Federal Research Center for Medical and Preventative Technologies of Public Health Risk Management; 614045, Perm, Monastyrskaya, 82.

Yekaterina Vladimirovna Sayenko – Candidate of Chemical Sciences; research associate at the multi-phased dispersed flow lab; email: saenko_ekaterina@mail.ru; phone: + 7 (342) 237-82-80; Institute of Technical Chemistry, Ural Branch of the Russian Academy of Sciences; 614013, Perm, Akademika Korolyova, 3.