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Comparative Assessment of the Effects of Short-Term Inhalation Exposure to Nickel Oxide Nanoparticles and Microdispersed Nickel Oxide

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Abstract—The biological effects of nickel oxide nanoparticles (Nickel (II) oxide, CAS number 1313-99-1, product number 637130) produced by Sigma-Aldrich (United States)) have been analyzed. The nanomaterial that was investigated consists of spherical particles with a hydrodynamic size of 17–40 nm; that is, the particle size is 9- to 38-fold less than the size of the particles of the microdispersed analog (150–1500 nm). CL_{50} of the samples investigated was higher than 5000 mg/m^3 for BALB/C mice; therefore, the substances are classified as low-risk (class 4 hazardous substances). The capacity of nickel oxide nanoparticles used at the absolute concentration of 140194 ± 27768 particles/dm^3 (equivalent to 1.34 ± 0.07 mg/dm^3 nickel oxide) to accumulate in the lungs and blood after 4 h of inhalation exposure is higher than that of the microdispersed analog (39.27 times higher for accumulation in the lungs and 13.71 times higher for accumulation in blood); therefore, the nanoparticles are assumed to be more toxic than the microdispersed analog. This assumption is confirmed by the detection of morphological alterations that include the formation of focal perivascular and peribronchial lymphoid infiltrates with small amounts of macrophages and eosinophils in the lung tissue of BALB/C mice. The microdispersed analog applied at the same actual concentration does not induce the effect described above.

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INTRODUCTION

A considerable amount of research related to the expansion of the range of nanomaterial applications has been performed during the past decade. Nickel oxide nanoparticles are extensively used in instrument engineering, nanooptics, biodiagnostics, chemical industry, and metallurgy [1]. Nickel nanoparticles are produced using spray pyrolysis, which can be accompanied by the formation of an aerosol in the air of the production facility and thus lead to the direct exposure of workers [1]. The "conventional" industrial processes performed at high temperatures, such as galvanic nickel coating or welding, can lead to the formation of aerosols of nickel and/or nickel oxide particles of 0.11–2 μm in size (aerodynamic diameter) [2]. A number of recent studies pointed at the specific physical and chemical properties of nanosized materials, including nickel oxide, as the potential cause for the higher toxicity of such materials as compared to the microdispersed analogs [3–5].

Given the impending development of a range of widely used novel products and the exposure of numerous industrial workers and general population to these products, the elucidation of the mechanism and specific features of the toxic effects of nickel oxide nanoparticles delivered to the organism by means of inhalation appears highly relevant.

MATERIALS AND METHODS

Powdered nanosized nickel oxide (nickel(II) oxide, CAS number 1313-99-1, product number 637130), produced by Sigma-Aldrich (United States)) [6] was used in the study, and microdispersed nickel oxide powder (Nickel (II) oxide, CAS number 1313-99-1, product number 203882) was used for comparison.

Particle size and shape were assessed using electron microscopy on an S-3400N high-resolution scanning microscope (3–10 nm, maximal magnification

300000×, HITACHI, Japan) with an attachment for X-ray dispersion microanalysis (Bruker, Germany).

Aqueous suspensions were prepared from the substances investigated immediately prior to aerosol generation. For this, the powders were added to distilled water that conformed to TU (Technical Requirements) 6-09-2502-77 at 50 mg of the powder per 1 cm^3 water. The suspensions were sonicated in a Sonopuls Hd 3200 ultrasound homogenizer (Bandelin, Germany) at room temperature for 2 min in order to disintegrate the agglomerates of nickel oxide nanoparticles and to ensure the homogenous distribution of the particles in the liquid. The sonication was performed in a continuous pulse mode at 65% power.

The assessment of acute toxicity of the inhaled particles was performed according to GOST (State Standard) 32646-2014 "Methods of Assessing the Effects of Chemical Products on the Human Organism. Acute Inhalation Toxicity: Method for the Assessment of the Class of Acute Toxicity (the ATC Method)" [7]. Male and female white mice of the BALB/C line (body weight 25–30 g; $n = 30$) used as the model organisms were kept in a vivarium at a temperature of 22–24°C and air humidity of 4–50% under natural lighting conditions and fed a standard chow (GOST (State Standard) R 502358-92). All experiments and manipulations were performed according to the relevant manuals and legal acts (GOST (State Standard) Z 51000.3-96 and 51000.4-96; the European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Research Purposes (ETS no. 123)); and the rules of good laboratory practice (GLP) stipulated by Ordinance no. 267 of the Ministry of Healthcare of the Russian Federation from 19.06.2003. The animals were terminated according to the requirements formulated in the "International Recommendations for the Conduction of Biomedical Research on Animals" (1997).

The experimental animals were divided into three groups of ten individuals (experimental group 1, animals that inhaled an aerosol of the aqueous suspension of nickel oxide nanoparticles; reference group 2, animals that inhaled an aerosol of the aqueous suspension of microdispersed nickel oxide; and control group 3, animals kept under similar conditions except for the absence of inhalation exposure to the substances under investigation). The animals were placed into individual pens in order to prevent the ingestion of the particles attached to the fur. The animals were terminated using mild euthanasia by carbon dioxide inhalation: five animals from each group were euthanized immediately after exposure for the assessment of nickel oxide accumulation, and the remaining five animals were terminated on day 14 after exposure for assessing morphological changes in the organs and tissues.

Modeling of the inhalation intake of the substance under investigation into the organism of the experimental animals was performed in an inhalation system with built-in software. The mice were placed into a whole-body chamber (TSE Systems GmbH, Germany). Aqueous suspensions of nickel oxide nanoparticles and microdispersed nickel oxide used to generate the aerosols contained 40 mg/cm^3 of the substance (in nickel oxide equivalents). The rate of air inflow and outflow was maintained at 10 L/min throughout the experiment to provide for the formation and steady circulation of the aerosol in the inhalation chamber. The pressure inside the chamber was maintained at -0.2 ± 0.2 mbar and the temperature was 22–25°C. Oxygen concentration in the chamber was no less than 19%, and the concentration of carbon dioxide did not exceed 1%. The suspensions were fed into the aerosol generator at a rate of 0.05 cm^3/min to ensure the generation of aerosols with nominal concentrations of nanosized and microdispersed nickel oxide at the level of 2 mg/dm^3. Air samples from the inhalation chamber were collected on an AFA-VG-10-1 filter at a flow rate of 2 L/min during 5 min after 2 and 4 h of exposure. Analysis of the aerosol samples captured by the filter involved the incineration of the filters in a muffle furnace and the extraction of soluble nitrates from the ashes by treatment with 70% nitric acid. The liquid was evaporated and the final residue was dissolved in 1% nitric acid. The content (concentration) of nanoparticles in the chamber was assessed using a diffusion aerosol spectrometer (OOO Aeronanotekh, Russia). Air samples were collected for 5 min at a rate of 0.5 L/min after 2 and 4 h of exposure.

The average lethal concentrations of the substances (CL_{50}) were inferred from the survival rate of the animals. An algorithm presented in GOST (State Standard) 32646-2014 "Methods of Assessing the Effects of Chemical Products on the Human Organism. Acute Inhalation Toxicity: Method for the Assessment of the Class of Acute Toxicity (the ATC Method)" was used for the assessment (Fig. 1). The actual concentration of nickel oxide nanoparticles in the chamber was used for the assessment of the CL_{50} value. The class of hazard associated with the substances was assessed according to GOST (State Standard) 12.1.007.76. "Occupational Safety Standards System. Hazardous Substances. Classification and General Safety Requirements."

Lungs and blood were used for the analysis of nickel accumulation in the organism of the experimental animals. Samples of lung tissue and whole blood were obtained from five animals from each group. The samples were incinerated in a muffle furnace for 9 h at 450–500°C to ensure the extraction of nickel into the solution used for further analysis. Organ mass and blood volume were taken into account

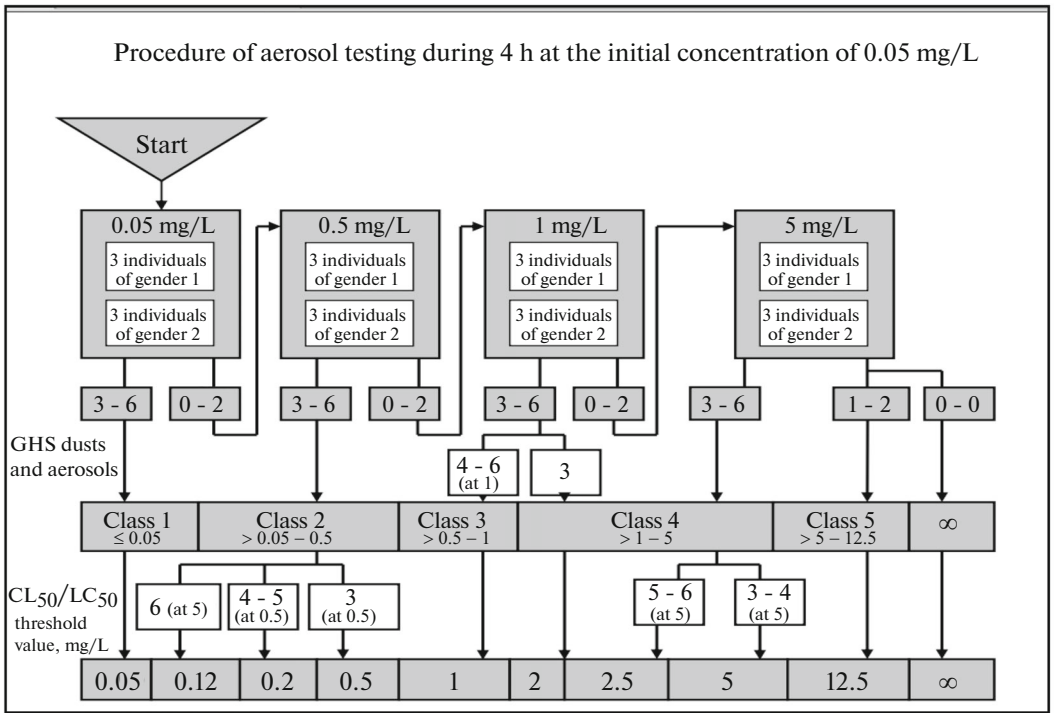

Fig. 1. Scheme of the assessment of acute toxicity for the inhalation of aerosols during 4 h at the initial concentration of 0.05 mg/L.

as the concentration of nickel was calculated. The concentrations measured were compared to the concentration of the substance under investigation in the lungs and blood of the animals from the control group.

The actual concentration of the substances of interest in the inhalation chamber and the biological samples was assessed using mass spectrometry with inductively coupled plasma. An Agilent 7500cx device (United States) with an octuple reaction/collision chamber was used in the experiment and helium was used as the reactant gas.

The viscera (heart, lungs, spleen, liver, kidneys, pancreas, stomach, and small and large intestines) were retrieved from five animals from each group using Shor's procedure for complete evisceration. A special tool was used to extract the brain for subsequent analysis. Tissue and organ samples were fixed in 10% neutral buffered formalin immediately after dissection. The fixed tissue fragments were dehydrated in an Excelsior ES automatic histological processor (Thermo Scientific, Germany). The histological sections were prepared from 3–4 μm thick paraffin sections stained by hematoxylin-eosin according to the conventional procedure; a Varistain Gemini ES stainer robot (Thermo Scientific, Germany) was used in this part of the study. The histological preparations obtained were analyzed using an Axio Lab A1 light microscope (Carl Zeiss, Germany) and photographed at 400× magnification using a Mikroskopkamera AxioCam ERc 5s camera (Carl Zeiss, Germany). The morphological changes in the organs of the experimental animals from groups 1 and 2 were identified using a comparison to the morphological structure of tissues and organs of the animals from the control group 3.

RESULTS

Analysis of electron microscopic images of the powdered substances under investigation showed that most of the nickel oxide nanoparticles had a spherical shape at an average form-factor (the ratio of the maximal linear dimension of the particle to the minimal of three dimensions) of 2.5. Cubic particles predominated in the powder of the microdispersed analog, and the form-factor for the material was 4 (Figs. 2, 3). The diameter of nickel oxide nanoparticles was shown to range from 17 to 40 nm, whereas the size of the particles of the microdispersed analog ranged from 150 to 1500 nm and thus was 9- to 38-fold higher than the nanoparticle size (Figs. 2, 3).

Assessment of the parameters of inhalation exposure showed that the actual concentration of nickel oxide in the inhalation chamber was 1.34 ± 0.07 mg/dm^3 at the nominal concentration of 2 mg/dm^3.

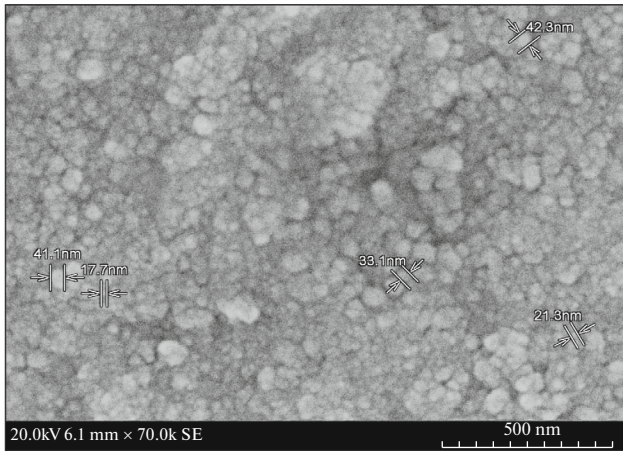

Fig. 2. Scanning electron microscopy image of nickel oxide nanoparticles (nonprocessed powder).

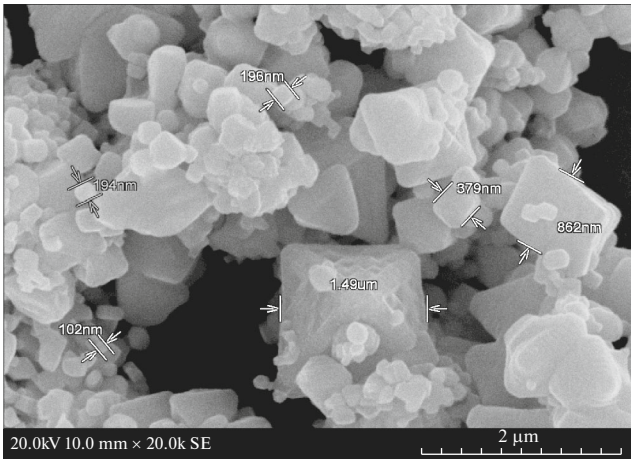

Fig. 3. Scanning electron microscopy image of microdispersed nickel oxide (nonprocessed powder).

The assessment of the content of nanoparticles in the air of the inhalation chamber revealed the absence of agglomeration of the nanoparticles into micrometer-sized particles upon the injection of the aerosol into the chamber (Fig. 4). The size of the majority (98%) of the particles did not exceed 100 nm after 2 and 4 h of exposure at the actual concentrations tested, and the average concentration of the particles during the experiment was 140 194 ± 27 768 particles/dm^3.

The size of 98% of microdispersed nickel oxide particles that were fed into the chamber as an aerosol was in the range of 1–10 μm, and the actual concentration of nickel oxide in the air of the chamber was 1.30 ± 0.02 mg/dm^3.

No apparent deterioration of the animal state during the experiment was observed in either group 1 or group 2. The state of the fur coat and locomotor activity did not change during the 14-day follow-up period and neither polyuria nor polyphagia was observed. None of the animals died during the experiment or during the follow-up period. The average lethal concentration CL_{50} for BALB/C mice exceeded 5000 mg/m^3 both in case of the aqueous suspension of nickel oxide nanoparticles and in the case of an aque-

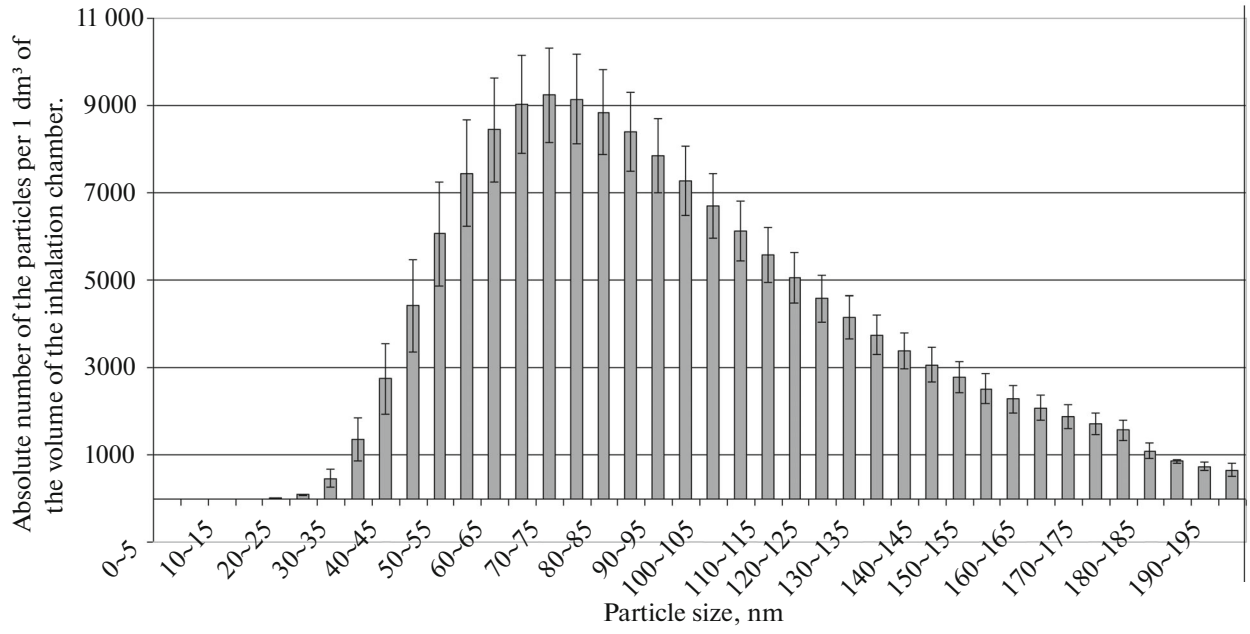

Fig. 4. Nanoparticle concentration in the air of the inhalation chamber at a nickel oxide concentration of 1.34 ± 0.07 mg/dm^3.

Results of a comparative assessment of nickel oxide concentrations in the lungs and blood of experimental animals after 4 h of exposure to nanosized and microdispersed nickel oxide particles

Group of animals	Parameter	Concentration in the lungs, μg/g	Concentration in the blood, μg/dm^3
Control group	Mean value ($M \pm m$)	30.20 ± 4.58	6.25 ± 0.64
Group 1 exposed to nickel oxide nanoparticles	Mean value ($M \pm m$)	8089.88 ± 3586.9	141.10 ± 33.7
	p-Value for difference from the control group	0.0012	0.0001
	Increase relative to the value for the control group	276.81	22.57
	p-Value for difference from the group 2	0.0001	0.0001
	Increase relatively to the value for group no. 2	39.27	13.71
Group 2 exposed to microdispersed nickel oxide	Mean value ($M \pm m$)	206.03 ± 50.95	10.23 ± 1.16
	p-Value for difference from the control group	0.0001	0.0001
	Increase relatively to the value for the control group	6.82	1.63

ous suspension of the microdispersed analog; therefore, the substances were assigned to class 4 of hazardous substances (weakly hazardous).

Analysis of the concentration of the substances of interest in the lungs and blood of the experimental animals showed that nickel concentration (converted to

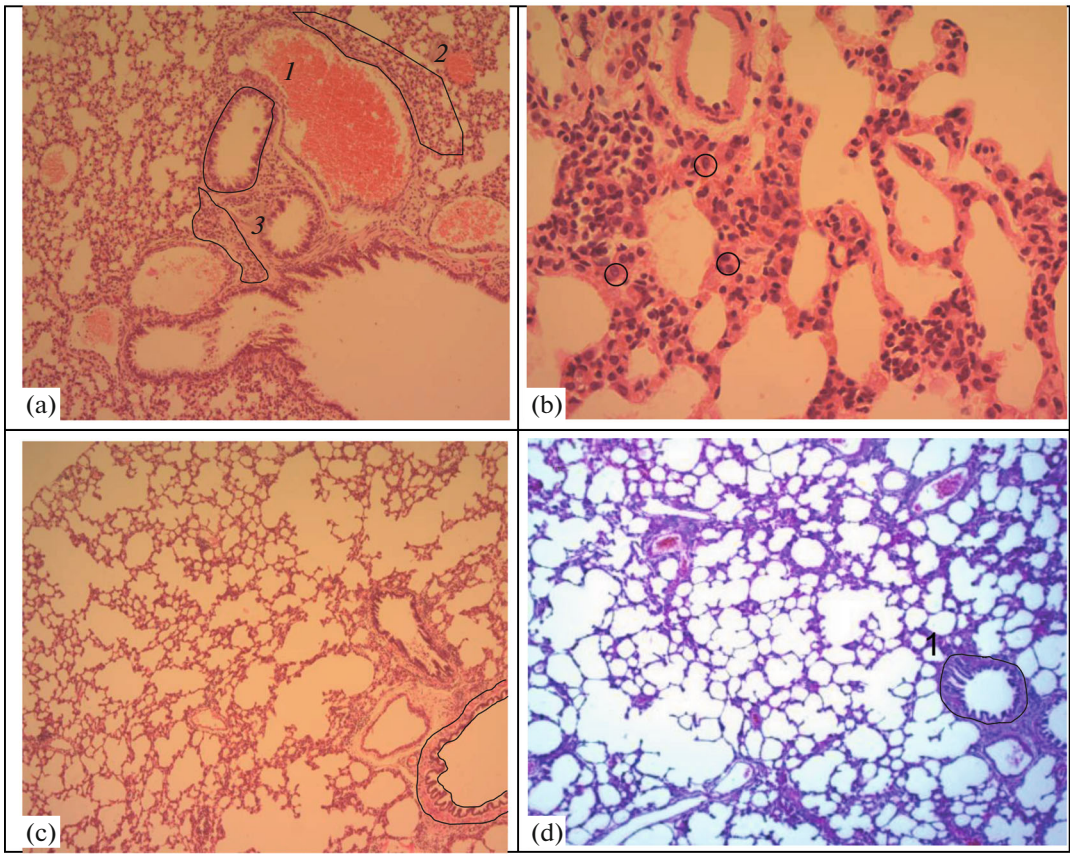

Fig. 5. (Color online) Bronchi of BALB/C mice (hematoxylin-eosin staining, magnification 200–400×).The morphological changes were most pronounced in the bronchi of animals from experimental group 1 (a, b), as is evident from the comparison to reference group 2 (c) and the control group (d). Weak and moderate peribronchial infiltration by lymphocytes and eosinophils was detected in the walls of the bronchi (A1). The inflammatory infiltrate spread to the alveoli adjacent to the bronchi (A2, B). The bronchial wall in mice of the reference group (C1) and in animals of the control group (D1) was smooth, devoid of bulges and signs of endocytosis, bronchial luminae were clear, and the outlines were not altered. Eosinophils with a bright pink cytoplasm and multilobular basophilic nuclei were present in the tissues of mice from the experimental group (B1). Sites of endocytosis were apparent (A3).

nickel oxide concentration) in the lungs of animals that inhaled an aerosol of nickel oxide nanoparticles for 4 h was 276 times higher than the value for the control group and 39 times higher than the value for the reference group (table). Similar changes of the concentration of the substance under investigation were observed for the blood: the concentration of nickel (converted to nickel oxide concentration) in the blood of experimental animals was 22.5 times higher than in the blood of animals of the control group and 13.7 times higher than the value for the reference group.

Comparative assessment of the morphological changes in the tissues and organs under investigation in the animals of experimental group 1 and reference group 2 allowed for the identification of significant changes in lung tissue. Focal perivascular and peribronchial lymphoid infiltration with admixed macrophages and eosinophils was detected in the lung tissues from the experimental animals (group 1) (Fig. 5). The general features of the morphology of lung tissue in experimental animals from the reference group 2 and the control group 3 were similar and corresponded to a morphological variant of the physiological norm. No morphological changes in the tissues of the brain, spleen, kidneys, heart, liver, pancreas, stomach, and small and large intestines were detected in samples from the animals from groups 1 and 2 when compared to the controls.

DISCUSSION

The results of the recent study point to the capacity of nickel oxide nanoparticles under investigation for accumulation in the lung tissue and entry into the bloodstream during a 4-h inhalation exposure to the nanoparticles at a concentration equivalent to 1.34 ± 0.07 mg/dm^3 nickel oxide. The data on the capacity of nickel oxide nanoparticles for accumulation in lung tissue are in agreement with the existing theories on the toxic mechanisms that underlie the effects of nanoparticles [8–10].

The character of the morphological changes is consistent with earlier observations of an effect related to the activation of cellular and phagocytic defense mechanisms [8, 11]. The active accumulation of nickel oxide nanoparticles in the lung tissue is apparently related both to the high capacity of the particles for penetration and to the activation of phagocytes, since the active migration of phagocytes can result in the formation of peribronchial infiltrates. The penetration of nanoparticles into the blood during the actual exposure may be mediated by diffusion from the alveoli to the capillaries. Further migration of the particles may be due to the migration of phagocytes from the peribronchial spaces.

CONCLUSIONS

The sample of nickel oxide nanoparticles investigated (Nickel (II) oxide, CAS number 1313-99-1, product number 637130), produced by Sigma-Aldrich (United States)) consisted of spherical nanoparticles with a hydrodynamic size ranging from 17 to 40 nm. The CL_{50} value for nickel oxide nanoparticles was 5000 mg/m^3 in case of 4-h administration of the nanoparticle aerosol to BALB/C mice by inhalation. This corresponded to a class 4 hazard (low hazard). The capacity of the nanosized material for accumulation in the lungs and blood was higher than that of the microdispersed analog (39 times higher for accumulation in the lungs and 13 times higher for accumulation in the blood), and therefore the toxicity of the former material is expected to be higher as well. Inhalation of an aerosol of nickel oxide nanoparticles used at the absolute concentration of 140194 ± 27768 particles/dm^3 (equivalent to 1.34 ± 0.07 mg/dm^3 nickel oxide) for 4 h caused the formation of focal perivascular and peribronchial lymphoid infiltrates with admixed macrophages and eosinophils in the lungs of BALB/C mice. The microdispersed analog did not induce a similar effect when administered at a concentration equivalent to 1.3 ± 0.02 mg/dm^3 of nickel oxide.

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