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ENVIRONMENTAL AND OCCUPATIONAL HAZARDS OF METAL NANOCOMPOUNDS PRODUCTION AND APPLICATION: HYGIENIC, CLINICAL AND TOXICOLOGICAL ASPECTS

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ABSTRACT
Introduction: Nanometals are the most common nanoobjects, used nearly in all industrial branches. Considerable advances in the nanotechnological production have led to progressive use of nanomaterials in industry, though occupational safety of the nanoindustry staff is insufficiently studied.
The aim: Estimation of labor safety during production of metal nanoparticles for the purpose of defining necessary and efficient preventive measures.
Materials and methods: The personnel of the hygienic departments of the O.Bogomolets National medical university have conducted numerous physiological, hygienic, biochemical, morphological and toxicological studies. The scientists have studied and revealed hazardous workplace factors of various metal nanoparticle production technologies, particularly those of metal nanoparticles: nanosilver, titanium nitride, chromium disilicide, lead sulphide, etc.
Results and conclusions: The authors have developed method of the occupational exposure metal nanoparticles exposure air control, assessed and analyzed health of the personnel engaged in production of the nanometals. The paper contains data of the blood cells functional activity assessment, with the detected possible molecular nanoparticles toxicity mechanisms due to the altered gene expression. The authors have studied effect of nanoaerosols onto the laboratory rats respiratory organs, evaluated their blood biochemical characteristics, liver lipid content of fatty acids as well as defined morpho-functional hepatic transformations of the laboratory animals which underwent lead sulfide nanoparticles treatment.

KEY WORDS: metal nanocompounds production, hazardous workplace factors, laboratory animals

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INTRODUCTION
Industrial globalization leads to significantly increased production of new compounds with unique physical, chemical and biological properties. Nanometals take up the priority position among these compounds as they are widely used in medicine, pharmacology, cosmetology, textile production and electronic industry [1]. Considerable economic and scientific development is directly related to increased number of employees affected by new factors [2, 3]. The nanostructure vast specific surface, high surface curvature, polymorphy and insolubility in water and biological media may stipulate for their high bioactivity, higher toxicity and personal human hazards. Unfortunately, high rates of nanoindustry development significantly leave behind scientific trials of nanocompounds production and use negative consequences [3 - 5].

The personnel of Hygienic departments of O.Bogomolets National medical university have recently been studying hygienic effects of nanotechnologies, accepted by leading institutions of the National academy of sciences of Ukraine [6, 7]. The obtained data are offered in the paper.

THE AIM
Aims - estimation of labor safety during production of metal nanoparticles for the purpose of defining necessary and efficient preventive measures.

MATERIALS AND METHODS
The trials on metal nanoparticles production technology were held in the International Center of Electron Beam Technologies, affiliated to E.Paton Institute of Electrical Welding as well as I.Frantsevich Institute for Problems of Materials Science.

The authors, considering basic unfavorable environmental factors, have observed timing, studied psychophysiological factors (particularly, severity of the labor conditions), occupational noise, microclimate, X-ray emission and the operating content of the aerosols containing nanoparticles.

In order to evaluate personnel's health, the authors have analyzed regular medical examination charts and conducted the following clinical and instrumental procedures : objective examination, electrocardiography, electroencephalography and echocardiography. The liver and thyroid gland were examined using the ultrasound.

The range of conducted examination procedures includes: defining the enzymatic activity of aspartate transaminase, alanine-aminotransferase, total bilirubin, cholesterol and glucose blood content. To study nanoparticle effect onto the immune system the enzyme-linked immunosorbent assay ELISA was employed.

Then the authors held trials with the male Wistar rats, weighing 230 – 240g, and white rats, weighing 170-200g. The scientists used nanoparticles of silver in the NaCl matrix, sized 16±5 nm, and suspension of 50mg of titanium nitride ultramicroscopic powder with 1ml of saline solution.

In other trials colloid solutions of lead sulphide nanoparticles, sized 10 and 30nm, were used, which were daily introduced intraabdominally, 5 times a week (working week modeling), concentrated as 1/100 of the ionic lead median effect concentration EC50. To compare the obtained data distilled lead nitrate solution with particles sized 400nm was used.

Nanopowder toxicity was estimated by defining the median lethal dose in intragastric and intraabdominal introduction as well as by studying the nanoparticle effect on the rabbit eye mucosa and skin.

To conduct histological studies the lungs were fixed in 10% neutral formaldehyde solution. Then the material was dewatered in ascending concentration alcohols and treated with chloroform - alcohol, chloroform, chloroform-paraffin (37°C) and paraffin (57°C), the paraffin being poured into the wax. Paraffin sections, 7µm in width, were obtained using sliding microtome. Serial sections were stained with hematoxylin, eosin and Van Gison.

To evaluate nanosilver effect onto expression of SNARK proteinkinase, caseinkinase-1ε and circadian genes, total RNA was obtained from the rat liver, lungs, heart, kidneys and testicles, using the Trizol reagent (Trizol; Invitrogen, USA) according to the manufacturer's instructions. The method was described by O.G.Minchenko et al. [8].

The murine blood serum was studied for such biochemical characteristics as total protein concentration, albumin concentration, total fat concentration, cholesterol concentration, triglyceride concentration and enzymatic activity(ALT and AST), using biochemical analyzer Vitros-250. Sample preparation and biological data estimation were conducted according to the manual.

For histological examination of murine liver, preparations were fixed in 12% formaline solution with the phosphate buffer (pH=7.0-7.2) the sections were stained according to the study needs (Azure II-eosin, Van Gison, PAS reaction) to be further light optically studied. To detect acidic and neutral mucopolysaccharides samples were stained with PAS reagent.

The samples with 30 cells, stained after Einarson technique, were examined for: nucleus intersection area (N area), nucleus specific optical density (N DM), Hertwig index (IH), nucleus integrated optical density (N IntDen), as well as measured nucleic acids in the modified nucleus volume (N Coeff NA) for extrapolation of the results in the section plane: 1: N Coef NA = N IntDen × ¾×N area ×√(N area/π) [9,10].

Cell cytoplasm was studied for: cell intersection area (C area, µm²), cell specific optical density (C DM, units), cell integrated optical density (C IntDen, µm×units), cell nucleic acids in the modified volume (using the same formula as in 1) (C Coef NA). The departure point index for estimation of the nucleic acids content was equal to 1, which is characteristic for the lymphocyte nuclei, situated in the organ stroma. In each trial the paired correlation analysis was conducted.

Morphometric studies were performed using image analyzer microscope Olympus BX51 with digital camera C-4040zoom and PC. Sizing was conducted using micrometer E.LEITZ WETZIAR [11].

The obtained data were statistically analyzed using Microsoft Excel software, mean averages compared regarding the Student's t-criterion. The difference was considered reliable at $p<0.05$.

RESULTS AND DISCUSSION

Introduction of nanotechnologies directly affects labor conditions, so we have decided to study how production affects the operating personnel's health. According to the obtained results, the operating personnel's work refers to the acceptable severity group, hazardous class, "exhausting work" of the 1st degree due to the "continuous attention concentration" parameter, which may lead to increased morbidity with subsequent disability. The next stage was defining health conditions of personnels' work. The staff work under acceptable parameters of microclimate, infrared exposure, production noise and X-ray exposure [12].

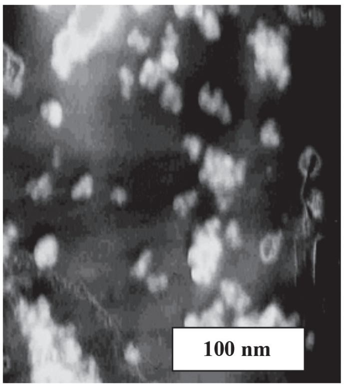
Nanoparticle production is characterized by a real possibility of their invasion into the operating area air and penetration into the personnel's bodies by inhalation. During nanosilver synthesis by electron beam technology method, the highest concentrations of aerosol which contains silver nanoparticles were determined during vacuum chamber purification (0.618±0.004 mg/m³) and during separation of condensate from template (0.0018±0.0001 mg/m³). Estimation of nanosilver concentration is a challenging issue as this is new man-made factor, the health hygienic evaluation of which can't be conducted using maximum concentration limit obtained by gravimetric method for silver metal (1 mg/m³). It is necessary to consider that the silver nanoparticles operating area air concentration during purification of the vacuum chamber purification 61.8 times exceeds permissible standards accepted in the USA, Austria, Germany, Japan and Switzerland (0.01 mg/m³) [12, 13]. The obtained results substantiate the necessity of defining and introducing new health standards for the nanoparticle maximum concentration limit according to their absolute number in the air volume unit, their size, specific surface and other physical-chemical parameters.

Nowadays, the scientists possess sufficient range of nanoparticle visualization methods as well as methods of estimating nanoparticles air concentration, though most of them have drawbacks. The authors have offered a new approach to the hygienic control of nanoparticle operating area air content and battle-tested the polyvinyl pyrolidone water-soluble nanofilters, which may arrest the aerosol particles, sized 1nm-10 µm, with air aspiration speed below 30 l/min. Chemical properties of polyvinyl pyrolidone water-soluble filters provide for obtaining suspension with already stable nanoparticles. This permits for using the obtained suspension to analyze the nanoparticles shape and dispersion content by electron microscopy and atom absorption spectral measurement (fig. 1). Besides defining shape and size of nanoobjects, water-soluble filters permit to calculate their total surface area, which is required for complete toxicological assessment [14, 15].

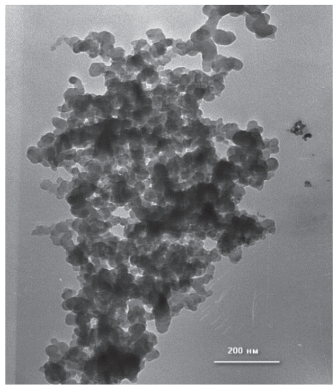
The size and shape of metal nanocompounds have been assessed by the authors in collaboration with the O.Bogomolets National medical university department of microbiology and virusology personnel (head of the department – academician Shyrobokov V.P.). Nanopowders are presumably of round shape, with capacity for aggregation (fig.1) [12, 16].

To establish the upper toxicity parameters, we introduced to the laboratory animals (mice and rats) nanopowders of refractory metal compounds (titanium nitride and chromium disilicide) dosed as 5000, 10000 and 15000 mg/kg. None of nanopowders caused animals death upon its introduction into the stomach, only slight general depression and lethargy were noted in 24 hours after introduction. Introduction of nano $CrSi_2$, nano TiN and nano ZrO_2 in the conjunctival eye sac and on the skin of the rabbit didn't lead to visible changes on the conjunctiva or skin [16].

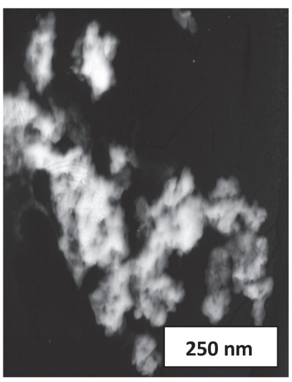
Clinical assessment of hepatobiliary system showed increased activity of alanine aminotransferase and aspartate



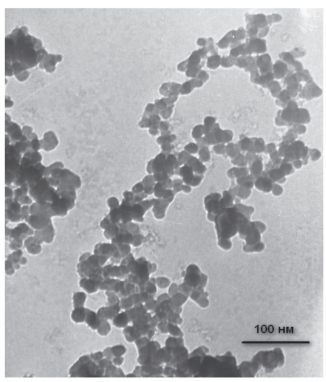
Silver nanoparticles.
Magnification: 80 000



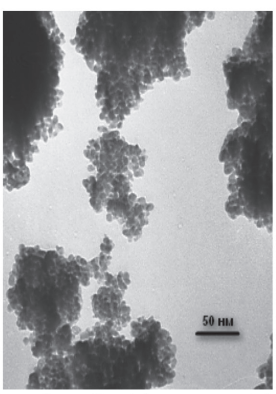
Chromium disilicide particles
Magnification: 144000



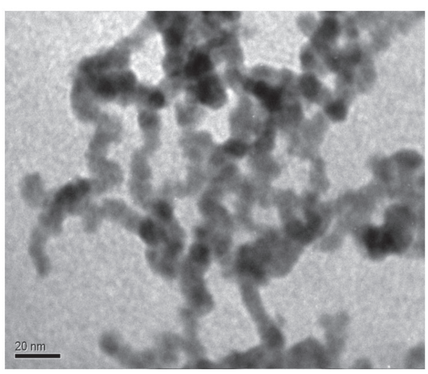
Barium titanate nanoparticles
Magnification: 40000



Titanium nitride nanoparticles
Magnification:108000



Zirconium dioxide nanoparticles
Magnification: 130000



Electron microscopic photo of nanoparticles after distillation of the lead sulfide colloid solution

Fig.1. Electron-diffraction photographs of studied nanoparticles of metals and their compounds.

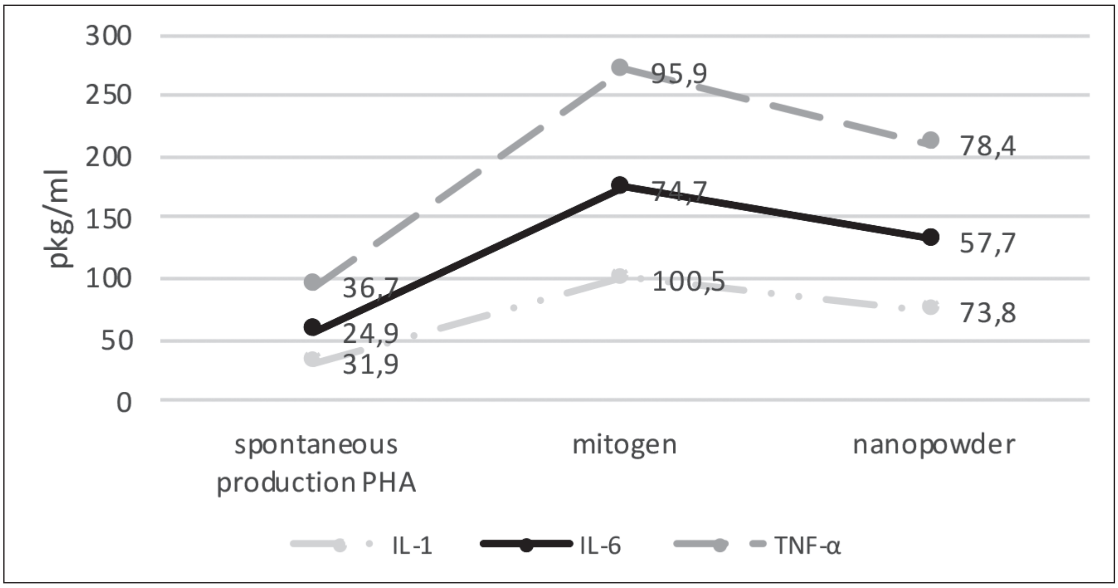


Fig. 2. Production of IL-1, IL-6 and TNF-α by mononuclear cells in vitro, by operating personnel dealing with nanocomposites.

aminotransferase, combined with hypercholesterolemia and hyperglycemia. Continuous action of nonoxide refractory metal compound nanopowders causes systemic autoimmune response with cytokines. Such processes make up a pathogenetic factor of onset and development of structural vascular changes as well as toxic digestive conditions [16] (fig. 2, 4).

The parallel trial detected that after 30 introductions of lead sulphide nanoparticles, sized 10 and 30 nm, to the animals, murine blood alanine tranferase activity reliably increased ($p \leq 0.05$) 2.5 times, after 60 introductions – almost threefold, compared to the control animals.

Murine blood aspartate transferase activity, after 60 introductions of toxins, reliably increased, compared to the control ($p \leq 0.05$, fourfold).

The authors have also established that with 30 and 60 toxins introductions, total lipids and cholesterol concentration tended to decrease, while triglycerides and glucose increased, regardless of the studied toxicants action duration [17].

Morphological examination showed reinforcement of dystrophy in the animals' hepatocytes, according to the lead sulphide nanoparticles action duration. After 30 introductions of the substances the authors revealed granular cytoplasm and off-centered nuclei, congestion of the central vein and inconsiderable amount of erythrocytes in sinusoids (fig. 3). After 60 introductions the dystrophy was even more expressed [18].

Morphometric and densitometric studies of the hepatocyte nuclei and cytoplasm after 30 and 60 introductions of the substances revealed decompensation processes.

The *in vitro* studies showed that the nanocomposites may increase functional activity of the monocyte-macrophage line cells regarding production of IL-1, IL-6 and TNF-α, which standardly coordinate interaction of the immune cells and activate inflammation.

IL-4, inducing the B-lymphocytes proliferation, is supposed to increase receptor expression for the IgE Fc-fragment, being an antagonist of IFN-α, suppressing production of IL-1, TNF-α, IL-6, IL-8 and cytotoxic effect of T-lymphocytes. It is of extreme importance in the development of immediate allergic reactions.

In order to reveal the silver nanoparticles possible genotoxic and cancerogenic action the authors, in cooperation with O.Palladin Biochemistry institute personnel, studied gene expression. The article covers only some issues of the study of nanosilver effect onto the caseinkinase-1ε gene expression, more detailed information on the trials is represented in further publications [8, 19, 20]. Silver nanoparticles may affect caseinkinase-1ε gene expression in the murine lungs, liver, myocardium and testicles in 24 hours after introduction of silver nanoparticles. Silver nanoparticles stimulating effect onto the caseinkinase-1ε mPNA expression in these organs was also detected in 3 and 14 days after the nanoparticles introduction.

The authors have revealed that the caseinkinase-1ε gene expression intensifies in kidneys under the silver nanoparticles effect, but only in 3 and 14 days after their introduction. The obtained results evidence about possible effect of silver nanoparticles on important cell metabolic processes regulation within the level of caseinkinase-1ε gene expression, which may lead to disordered signaling cascade in cells and development of pathological conditions, as well as serve as a sensitive marker of metal nanoparticles biosafety [20].

According to the conducted hygienic trials of the production process, the authors have established that the thermosynthesis operating personnel underwent titanium nitride influence. The conducted electron miscoscopic analysis showed present titanium nitride powder conglomerates in intraalveolar septae macrophages, viewed as large or small aggregations which decreased in number throughout the experiment (fig. 6, 7) [16, 21].

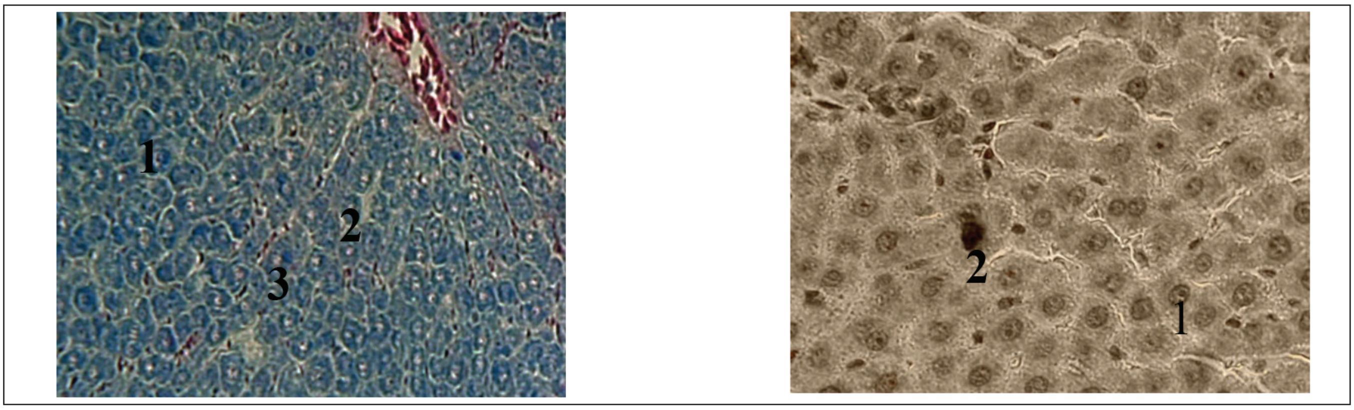


Fig. 3. Murine hepatic structure after introduction of lead sulphide nanoparticles, sized 30nm, after 30 introductions (A – staining with Azure II-eosin, magn. 200; B – staining with picro-fuchsin according to Van Gieson, magn. 400). 1 – hepatocyte, 2 – hepatocyte nucleus, 3 – blood corpuscles.

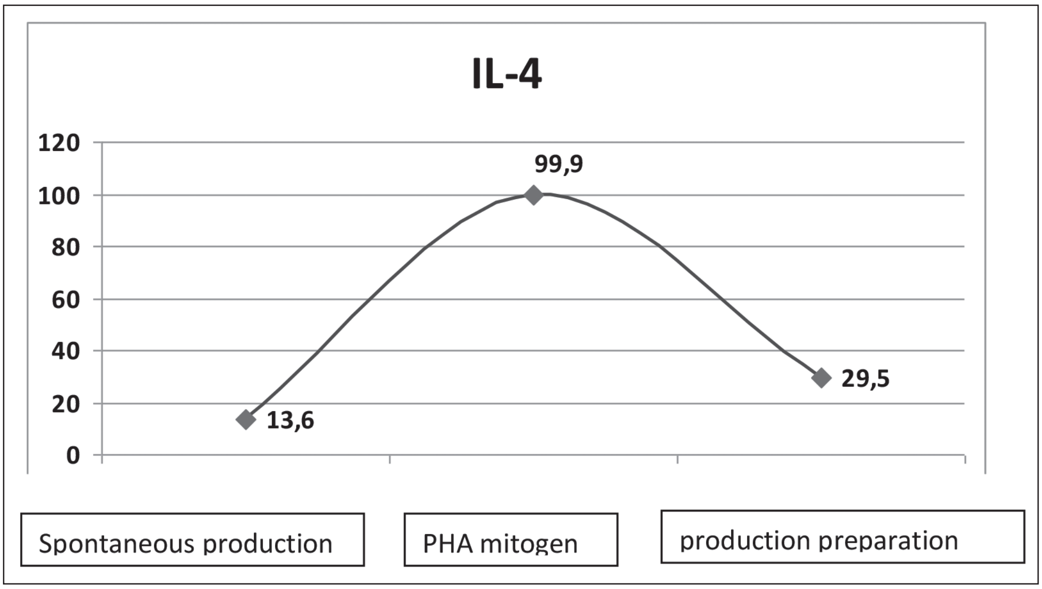


Fig. 4. Production of IL-4 by mononuclear cells in vitro by donors who contact with nanocomposites.

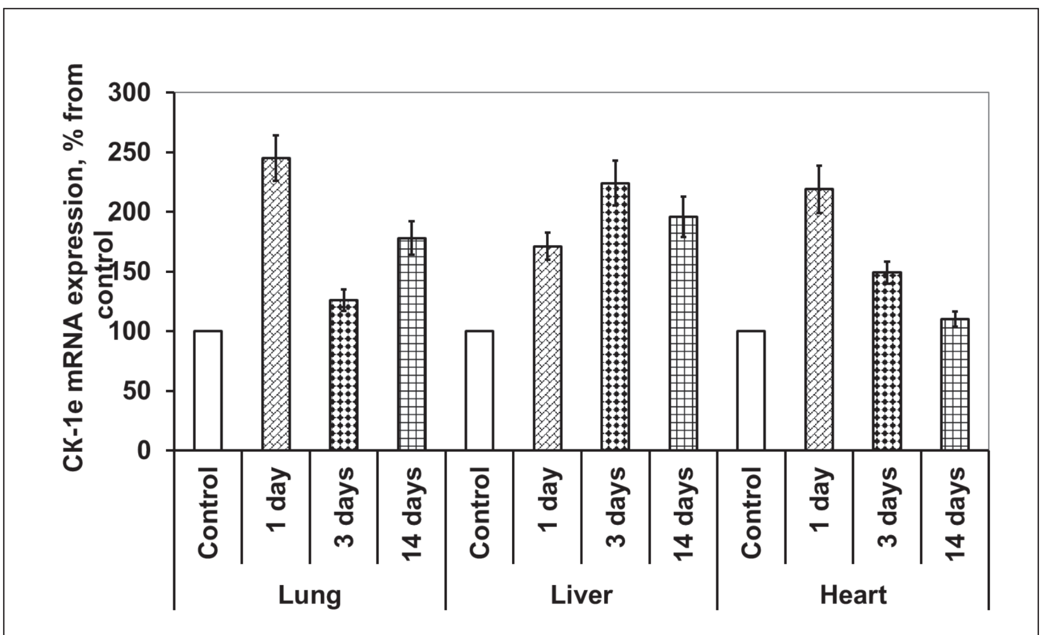


Fig. 5. Analysis of silver nanoparticles effect onto the caseinkinase-1ε mRNA expression in the lungs, liver and myocardium of rats.

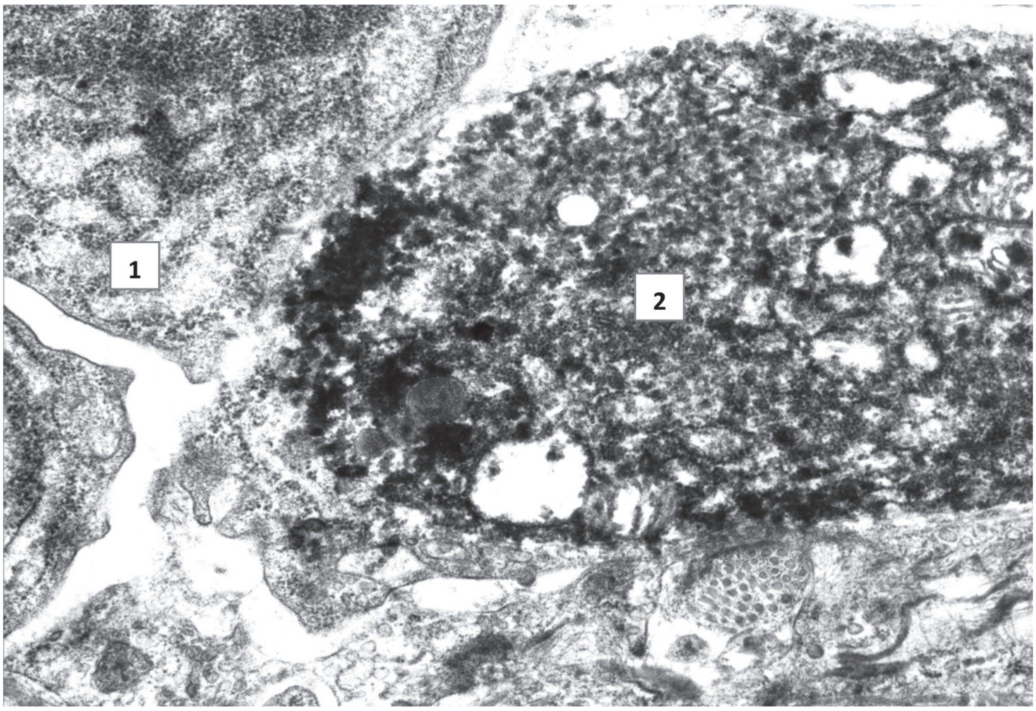


Fig. 6. Lungs in 7 days after introducing titanium nitride nanoparticles.
Cell debris (1). Macrophage necrosis (2).
Electron microscopic photo. Magnif.:A-18000,B- 18000.

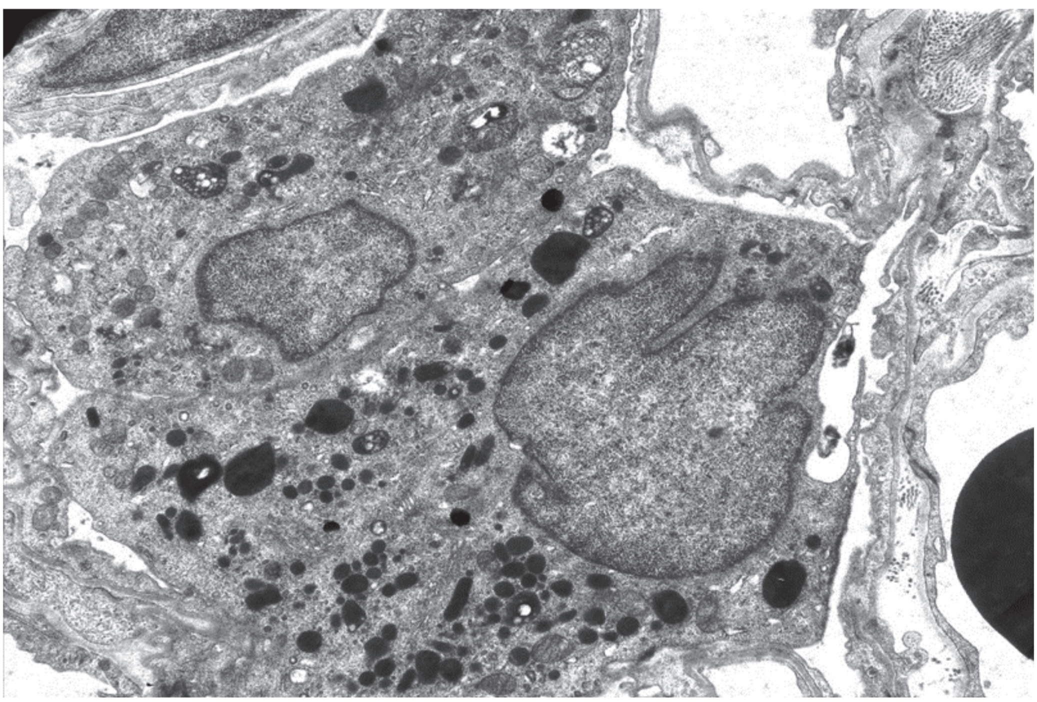


Fig. 7. Lungs in 1 year after introduction of the titanium nitride nanoparticles. Macrophage. Electron-microscopic photo. Magnif.:A-12000, B-9000.

The intraalveolar septae within the material accumulation were thickened, infiltrated with lymphocytes and plasmocytes, tissue basophils, macrophages and rarely neutrophils. Also, morphological signs of chronic bronchitis were noted: thickening, swelling and infiltration of all wall layers, dystrophic changes and exfoliation of ciliated epithelium.

The data on health and occupational morbidity of the personnel engaged in nanoparticle production are poorly represented in medical sources or absent at all. As for the personnel affected by the barium titanate nanocrystal powder, they are most commonly affected with respiratory and haemopoetic diseases. The clinical-instrumental assessment detected changes in the bronchopulmonary system: chronic obstructive pulmonary disease and the right ventricular hypertrophy. The other common diseases are asthenic syndrome with cerebral angiospasm and cerebral functional disorder signs [22, 23].

The obtained data make up a basis for preparing the substantiated professional safety principles aimed at the nanoproduction personnel healthcare.

CONCLUSIONS

According to the conducted hygienic trials, the authors have established that the operating conditions of the nanoproduction personnel are characterized with many hazardous production factors: physical, dynamic, static and nervous overload, production noise, X-ray exposure, effect of aerosols with silver or any other metal nanoparticles and their oxygen-free compounds.

The described methods of air nanoparticles content assessment are quite informative and easy to use, they may be applied during production technological operations, which are characterized by high dust exposure.

To establish the nanoaerosols effect on the development of personnel health disorders it is recommended to supervise health dynamic patterns of the personnel.The authors have offered new sequence of periodical personnel's medical check-ups in order to improve their quality.

A single introduction of titanium nitride ultramicroscopic particles leads to structural metamorphoses in the bronchial tree and respiratory organs, the degree of expression of which depends on aerosol action.

The authors have established basic law principles of lead nanoparticles effect, which is a global pollutant, onto the body: in 30-fold and 60-fold introduction of studied substances we observed impaired liver protein synthesis, leading to dysproteinosis, disordered lipid and carbohydrate metabolism, which is related to cytotoxic effect of the studied nanoparticles. While evaluating liver morphology, pronounced dystrophic changes, dependent on exposure, were noticed.

After 30 introductions of lead sulphide the effect was observed in ALT, while after 60 introductions both ALT and AST increased their activity equally.

Continuous effect of non-oxide refractory metal compound nanopowders leads to systemic immune-inflammatory response with cytokines, which make up a pathogenetic factor of onset and progress of the vascular structural changes and hepatobiliary toxic disorders.

It has been established that molecular mechanisms of metabolic abnormalities which affect the circadian rhythms, necessary for human activity cycle, may, in their turn, provoke various pathological conditions and trigger oncogenesis. Altered expression of these genes is a genetic marker of nanoparticles hazardous effect.

The reliable nanoindustry development should be supported with a long-term studies of potential toxicological risks and their management, basing on the scientifically substantiated standardization of the nanoparticles production environment composition.

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Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

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