# MEDICAL AND BIOLOGICAL ASPECTS RELATED TO ASSESSMENT OF IMPACTS EXERTED BY RISK FACTORS

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## PECULIARITIES OF BIOACCUMULATION AND TOXIC EFFECTS PRODUCED BY COPPER OXIDE (II) NANOPARTICLES ON THE RESPIRATORY ORGANS UNDER INHALATION EXPOSURE AS OPPOSED TO THEIR MICRO-SIZED CHEMICAL ANALOGUE: ASSESSMENT FOR PREVENTION PURPOSES

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*At present, it is quite relevant to get better insight into parameters and peculiarities of deleterious effects produced by copper oxide nanoparticles (CuO NPs) on the respiratory organs under inhalation exposure. This will help develop more effective prevention measures.*

*The aim of this study was to assess peculiarities of bioaccumulation and toxic effects produced by CuO NPs on the respiratory organs as opposed to their micro-sized analogue in experimental modeling of inhalation exposure for prevention purposes.*

*We established physical properties of the tested materials. Experimental studies were accomplished on Wistar rats. The experimental animals underwent a single 4-hour inhalation exposure to a concentration of ~4 mg/m$^3$; a subchronic inhalation exposure to a concentration of 1.2–1.4 mg/m$^3$; a single intratracheal exposure to a dose of 0.005 grams per one rat. We examined peculiarities of NPs bioaccumulation, their influence on the cellular population of the bronchoalveolar lavage fluid (BALF), development of pathomorphological disorders in tissues, and the lung mass in comparison with the micro-sized analogue.*

*CuO NPs, as opposed to their micro-sized analogue, are smaller in size, have smaller hydrodynamic diameters, greater specific surface area and greater total pore volume; these properties determine their greater permeability. Bioaccumulation in the lungs, which was identified for NPs and MPs, is comparable under a single inhalation exposure. Under chronic exposure, NPs tend to bioaccumulate more intensively. A single intratracheal exposure induces more apparent changes in the BALF cellular population. Exposure to NPs causes emphysema, edema, and erythrocyte exudation in the lungs whereas these effects are not identified under exposure to MPs.*

*Therefore, CuO NPs tend to accumulate more intensively and have more deleterious toxic effects on the respiratory organs (the lungs) than their micro-sized chemical analogue under a single intratracheal exposure (0.005 grams per one rat) and subchronic inhalation exposure (1.2 mg/m$^3$). The study results should be considered when developing activities aimed at preventing negative health outcomes in the respiratory organs under inhalation exposure to the analyzed nanomaterial.*

***Keywords:*** *copper (II) oxide, nanoparticles, microparticles, inhalation exposure, bioaccumulation, toxic effect, cellular phagocytic activity, Wistar rats.*

At present, higher quality of products with a simultaneous decrease in production costs is a challenging task tackled within many various economic activities. Use of nanoparticles (NPs) in products and technological production processes is a possible way to solve it [1]. NPs are objects with different shapes sizes of which fall within 1–100 nanometers range at least in one dimension. Small diameters of NPs result in the quantum-size effect due to which nanomaterials have better adsorption activity and reactivity than their microsized



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chemical analogies as well as electric, optical, magnetic, mechanic and thermodynamic properties not typical for the latter [2–5].

Copper oxide (II) (CuO) nanoparticles are an example of such nanomaterial. According to the Research and Markets analysis, the global nano copper oxide market size reached US$ 122.77 million in 2021. Looking forward, the publisher expects the market to grow four-fold and reach US$ 486.62 million by 2027, exhibiting a compound annual growth rate of 25.8 % [6]. This growth is determined by active CuO NPs implementation in electrical instrument engineering due to their unique conductivity able to ensure high productivity of semiconductor devices, batteries, and microelectronics [6]. In addition to that, CuO NPs are applied in medicine as components of antimicrobial and anticancer drugs [7]; cosmetology, in personal care products [8]; agriculture, as components of fertilizers and pesticides [9, 10]; aerospace industry, as a fuel combustion catalyst [11]; and in structural elements of aerospace applications [12].

According to research results reported in literature, CuO NPs are able to produce some toxic effects, which make them potentially hazardous for human health. Experiments on biological models gave evidence of their ability to induce free radical oxidation [13] that changes protein expression thereby disrupting cellular metabolism [14] and affecting functions and morphology of organ tissues [15]. As a rule, these deleterious effects are more apparent when produced by NPs as opposed to their microsized chemical analogues.

Active use of potentially hazardous CuO NPs in various economic activities facilitates their penetration into the environment, first of all, ambient air. This creates risks of health harm for humans under exposure to airborne NPs. According to the Guide R 2.1.10.1920-04, the respiratory organs are the primary target under acute and chronic inhalation exposure to microsized CuO[1]. Hence, we can assume that the respiratory organs are the target ones under inhalation exposure to CuO NPs as well. Given all the above-stated, it seems quite relevant to get better insight into parameters and peculiarities of deleterious effects produced by copper oxide nanoparticles (CuO NPs) on the respiratory organs under inhalation exposure as opposed to their microsized chemical analogue. This will help develop more effective prevention measures.

**In this study, the aim was** to assess peculiarities of bioaccumulation and toxic effects produced by CuO NPs on the respiratory organs as opposed to their micro-sized analogue in experimental modeling of inhalation exposure for prevention purposes.

**Materials and methods.** In this study, we tested commercial samples of powder CuO NPs and microparticles (MPs) manufactured by Sigma Aldrich (USA). The following physical properties of the tested materials were examined: sizes in a powder-like state; hydrodynamic diameters in a water suspension; specific surface areas; total pore volumes. Particle sizes were identified using scanning electronic microscopy (SEM) with JSM-63090LV microscope (JEOL, Japan); hydrodynamic diameters were established by dynamic light scattering with Horiba LB-550 (Horiba, Japan) and Microtrac S3500 (Microtrac, USA) particle size analyzers; specific surface areas and total pore volumes were identified with ASAP 2020 (Micromeritics, USA) analyzer as per Brunauer – Emmett – Teller and Barrett – Joyner – Halenda methods respectively[2].

We conducted a series of experiments to assess bioaccumulation and deleterious effects of the tested materials on the respiratory organs. The experiments were conducted on Wistar rats weighing 185 ± 20.4 grams. The rats were kept in polyethylene cages under

[1] Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals. Moscow, The Federal Center for State Sanitary and Epidemiological Surveillance of the RF Ministry of Health, 2004, 143 p. (in Russian).

[2] Gregg S.J., Sing K.S.W. Adsorption, surface area, and porosity, 2nd ed. Academic Press, 1982, 303 p.; Barrett E.P., Joyner L.G., Halenda P.P. The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms. *Journal of American Chemical Society*, 1951, vol. 73, no. 1, pp. 373–380. DOI: 10.1021/ja01145a126

23 °C approximately and relative air humidity ~ 47 %; light and dark phases interchanged each other each 12 hours. The rats had free access to food and water, apart from exposure hours. The experiments were approved by the Ethics Committee of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. We simulated acute and subchronic inhalation exposure to CuO NPs and MPs. Targeted effects produced by these chemicals on the lungs were investigated using one-time intratracheal introduction. Three groups of animals were created for each experiment: the test group was exposed to CuO NPs; the reference group, CuO MPs; the control group was kept under the same conditions but was not exposed to the tested materials.

Inhalation exposures to CuO NPs and MPs were carried out in a whole-body chamber (TSE Systems GmbH, Germany). An aerosol was generated by inputting CuO NPs and MPs water suspensions in the concentration of 125 mg/cm$^3$ into the inhalation system injector. Air circulation in the inhalation chamber was maintained during all experiments by air inflows and outflows at the speed of 10 dm$^3$ per minute, which allowed total air renewal over 10 minutes. Actual concentrations of the tested materials were identified using DustTrack 8533 (TSI Inc, USA) aerosol analyzer; air samples were taken from the chamber at the speed of 2 dm$^3$ per minute during 5 minutes prior to the beginning of an exposure, and 2 and 4 hours after it started.

Acute inhalation toxicity was simulated in accordance with the State Standard GOST 32646-2014[3]. Each experimental group was made of 6 animals. The prepared suspensions were input into the inhalation system at the speed of 0.4 cm$^3$ per minute, which allowed achieving the maximum possible concentration of the tested materials in the chamber equaling ~ 4 mg/m$^3$. The exposure was a single one and lasted for 4 hours.

Sub-chronic inhalation exposure was carried out in accordance with the Methodical guidelines MU 1.2.2635-10[4]. Each experimental group was made of 10 rats. The prepared suspensions were input into the chamber at the speed of 0.1 cm$^3$ per minute to create NPs and MPs concentrations of one fourth of the maximum possible one (1.2–1.4 mg/m$^3$). The exposure lasted for 28 days 6 hours each day.

Rats that underwent a one-time exposure were kept under observation for 14 days to establish death or some delayed toxic effects. The observation period completed, the animals were euthanized by cervical dislocation followed by immediate decapitation and removal of the lungs. The same procedure was applied to rats that underwent multiple exposures 24 hours after the last one. The mass of the removed lungs was identified using EW-1500i laboratory scales (AND, Japan). NPs and MPs bioaccumulation was comparatively analyzed by measuring copper levels using atomic absorption spectrometry with AAnalyst 400 spectrometer (PerkinElmer, USA).

Pathomorphological changes in lung tissues under multiple inhalation exposure were identified with AxioLab A1 microscope (CarlZeiss, Germany) by examining lung slices 3–4 μm thick painted with hematoxylin and eosin.

An experiment aimed at investigating cellular and phagocytic activity due to targeted effects of CuO Nps and MPs on the respiratory organs was conducted by a one-time intratracheal introduction in accordance with the Methodical guidelines MR No. 01-19/24-17[5]. Each experimental group was made of 10 animals. The experimental suspensions were based

[3] GOST 32646-2014. OECD guidelines for the testing of chemicals. Acute Inhalation toxicity – acute toxic class (ATC) method. Moscow, Standartinform, 2019, 28 p. (in Russian).

[4] MU 1.2.2635-10. Mediko-biologicheskaya otsenka bezopasnosti nanomaterialov [Biomedical assessment of nanomaterials safety]: Methodical guidelines. Moscow, The Federal Center for Hygiene and Epidemiology of the RF Sanitary Service, 2010, 122 p. (in Russian).

[5] MR No. 01-19/24-17. Metodicheskie rekomendatsii po ispol'zovaniyu kletochnykh sistem «invitro» i «in vivo» dlya uskoreniya gigienicheskoi reglamentatsii malorastvorimykh promyshlennykh aerozolei [Methodical guidelines on using cellular systems in vitro and in vivo to accelerate hygienic standardization of poorly soluble industrial aerosols]. Ekaterinburg, 1995, 28 p. (in Russian).

on 0.9 % sterile isotonic sodium chloride solution (0.9 % NaCl) and contained the tested materials in the concentration of 0.013 g/cm$^3$. They were introduced intratracheally into the rats in a volume of 0.4 cm$^3$ per animal. Each animal received 0.005 grams of a tested material. The rats were euthanized 24 hours after the exposure and the bronchoalveolar lavage (BAL) fluid was sampled. Smears to investigate the cellular population of the sampled BAL fluid were prepared as per conventional procedures. Cell count was accomplished using microscopy with an immersion system and magnification 900x.

The research results were statistically analyzed to detect any differences between the experimental groups using Mann – Whitney U-test in STATISTICA 10. The differences were considered statistically significant at a $p \leq 0.05$.

**Results and discussion.** Our examination of physical properties established CuO NPs sizes to be equal to 45.86 nanometers and this was 304.99 times smaller than sizes of the microsized analogue (13,987 nanometers) (Figure 1). An average hydrodynamic diameter of CuO NPs in a water suspension equaled 307.40 nanometers and this was 106.60 times smaller than that of CuO MPs (32,770 nanometers) (Figure 2). The CuO NPs specific surface area was 9.61 times greater than that of CuO MPs and equaled 17.70 m$^2$/g against 1.84 m$^2$/g. The total pore volume of CuO NPs was 0.056 cm$^3$ per g, which was 9.33 bigger than that of CuO MPs (0.006 cm$^3$ per g).

We did not observe any deaths or visible deterioration of the experimental rats' state due to toxic effects of the tested materials after a one-time exposure or during the observation period after multiple exposures. CuO NPs and MPs bioaccumulation was comparable in lung tissues (copper levels being ~ 1.4 times higher against the control group) without any statistically significant differences between the test and reference groups (Table 1).

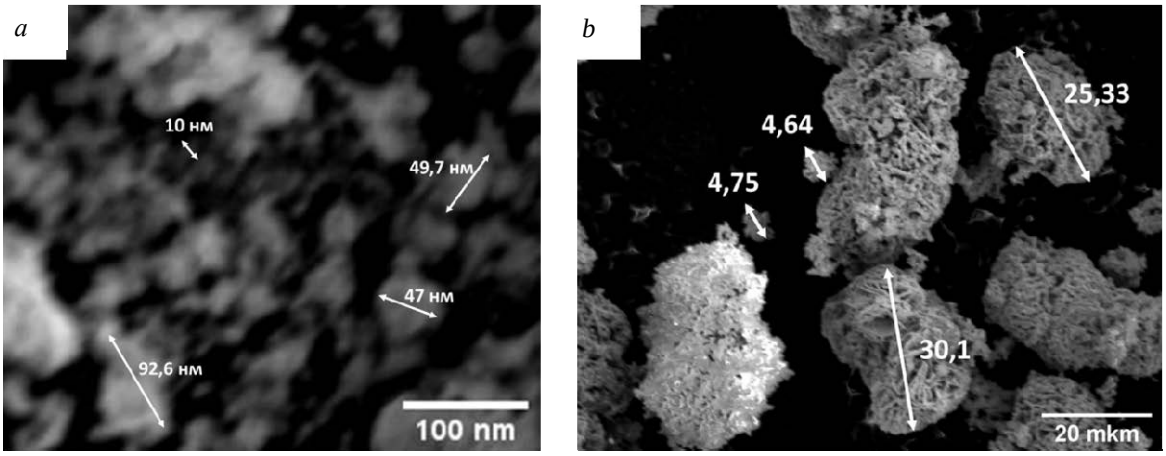
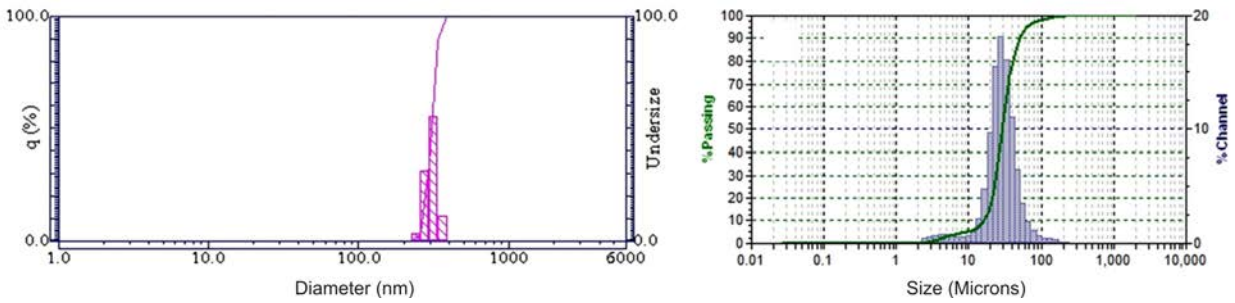



Figure 1. SEM image of CuO particles: *a* is nanoparticles, *b* is microparticles




*a* *b*

Figure 2. Measuring hydrodynamic diameters of CuO particles in water suspension: *a* is nanoparticles, *b* is microparticles

Table 1

Copper levels in rats' organs after a one-time inhalation exposure to CuO NPs and MPs ($p \leq 0.05$)

| Experiment | Copper level in the lungs, $M \pm m$, μg/g | | |
|---|---|---|---|
| | Exposure to NPs | Exposure to MPs | Control |
| One-time inhalation exposure | 15.93 ± 3.69* | 16.55 ± 4.31* | 11.50 ± 2.53 |
| Sub-chronic inhalation exposure | 17.10 ± 1.99*^ | 11.64 ± 0.30* | 9.70 ± 0.56 |

Note: * means statistically significant difference from the control group, ^ means statistically significant difference from the reference group.

Cellular and phagocytic activity in rats' airways had significant differences from the control group 24 hours after exposure to CuO NPs. In particular, levels of segmented neutrophils were 2.51 times higher ($p < 0.001$); alveolar macrophages, 4.11 times higher ($p < 0.001$); levels of monocytes were 6.00 times lower ($p < 0.001$); lymphocytes, 10.68 times lower ($p < 0.001$); segmented neutrophils to alveolar macrophages ratio, 1.79 times lower ($p < 0.01$). The structure of cellular population was different from the control after exposure to CuO MPs as well since relative segmented neutorphil count was 2.51 times higher ($p < 0.001$); alveolar macrophages count, 1.59 times higher ($p < 0.01$); monocytes count, 2.59 times lower ($p < 0.001$); lymphocytes count, 4.65 times lower ($p < 0.001$). Comparative analysis revealed that cellular and phagocytic activity under exposure to NPs was different from effects produced by exposure to MPs since relative alveolar macrophages count was 2.58 times higher in the former case ($p < 0.001$), relative monocyte count was 2.31 times lower ($p < 0.001$), lymphocytes count was 2.30 times lower ($p < 0.001$) and segmented neutrophils to alveolar macrophages ratio was 2.52 times lower ($p < 0.001$). The results of investigating cellular and phagocytic activity are given in Table 2.

Sub-chronic exposure did not result in any animal deaths or deterioration of their health. Exposure to CuO NPs created elevated copper levels in the lungs, which were 1.76 times higher than in the control group ($p < 0.05$); exposure to MPs, 1.2 times higher ($p < 0.05$) against the control. Copper levels were 1.45 times higher after exposure to NPs ($p < 0.05$) than in the reference group after exposure to MPs (Table). Histological examination established lymphoid hyperplasia in bronchi walls, acute inflammation in the bronchi, inflammation foci in interstitial tissues, disseminated vasculitis, brown pigmentation of macrophages, emphysema foci, and erythrocyte exudation into alveolar lumen and edema in rats' lungs after exposure to CuO NPs. Similar exposure to CuO MPs did not result in emphysema, edema, or erythrocyte aggregates in the alveoli (Figure 3).

Table 2

The cellular population structure in 100 cells in smears of rats' bronchoalveolar swabs 24 hours after intratracheal instillation of water suspensions with CuO NPs and MPs ($p \leq 0.05$)

| Parameter | The cellular population structure, $M \pm m$ | | |
|---|---|---|---|
| | Exposure to NPs | Exposure to MPs | Control |
| Segmented neutrophils, % | 75.6 ± 0.45* | 75.5 ± 0.37* | 30.1 ± 2.82 |
| Monocytes, % | 3.2 ± 0.25*^ | 7.4 ± 0.58* | 19.2 ± 1.67 |
| Alveolar macrophages, % | 15.2 ± 0.93*^ | 5.9 ± 0.32* | 3.7 ± 0.40 |
| Lymphocytes, % | 4.4 ± 0.54*^ | 10.1 ± 0.35* | 47.0 ± 0.86 |
| Segmented neutrophils / alveolar macrophages ratio, a.u. | 5.21 ± 0.44*^ | 13.13 ± 0.69 | 9.34 ± 1.49 |

Note: * means statistically significant difference from the control group, ^ means statistically significant difference from the reference group.

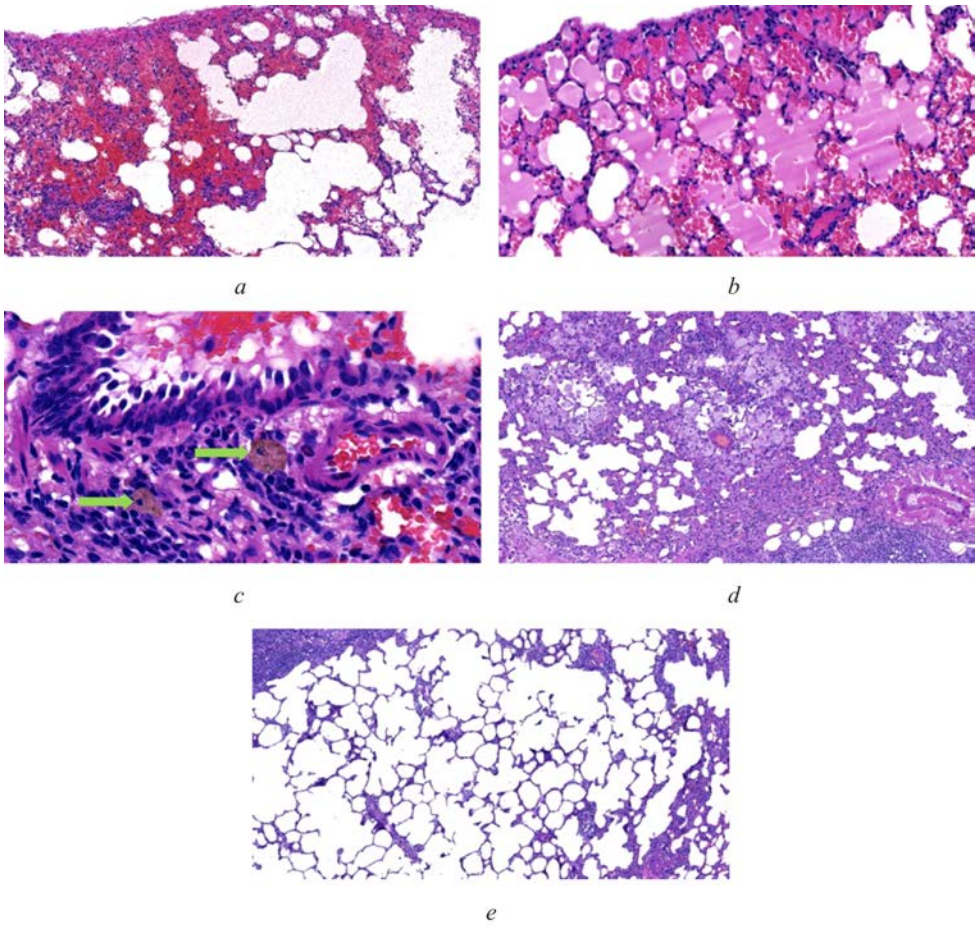


Figure 3. Microphotos of histological specimens of rats' lungs after multiple inhalation exposure to CuO NPs and MPs, painted with hematoxylin-eosin: (*a*) the test group, areas with emphysema and hemorrhages, magnification 100x; (*b*) the test group, alveolar edema, magnification 200x; (*c*) the test group, the green arrow shows macrophages with brown pigment in a bronchial wall, magnification 400x; (*d*) the reference group, magnification 100x; (*e*) the control group, magnification 100x

Pathomorphological changes in lung tissues caused by multiple inhalation exposure to the tested materials are also accompanied with a lower mass of the organ. Under exposure to NPs, mass of the lungs was 1.6 times lower ($p < 0.05$) (2.1 ± 0.1 g); under exposure to MPs, 1.9 times lower ($p < 0.05$) (1.8 ± 0.1 g) against the control group without any statistically significant differences between the test and reference groups.

By analyzing and generalizing the results of the accomplished investigations, we established that the tested CuO sample was a nanomaterial judging by particle sizes, specific surface area, and the total pore volume and had physical properties obviously different from those of its microsized chemical analogue. Stability of the CuO NPs nano-sized phase is known to directly depend on electrostatic repulsive force between the particles, which tends to decline as pH moves towards 6 [16]. Presumably, electrostatic repulsive force is weaker in a water medium with pH 7 and this makes NPs be drawn to each other and agglomerate. This is evidenced by measurements of hydrodynamic diameters by dynamic light scattering (DLS), according to which CuO NPs have 6.7 times greater sizes in a water medium against the same indicator in a native powder identified by SEM. Physical properties typical for CuO NPs imply their greater permeability and, consequently, greater ability to bioaccumulate and produce more deleterious toxic effects as opposed to MPs.

Bioaccumulation, which was identified for NPs and MPs, is comparable under a single inhalation exposure that created elevated copper levels in rats' lungs; this is likely due to

shirt exposure duration. This is further evidenced by investigating bioaccumulation under sub-chronic exposure when copper levels in the lungs were higher after exposure to NPs as opposed to their microsized chemical analogue. Similar changes were reported by other authors in their study accomplished on mice. The experiment established growing levels of the tested chemical element in the lungs during the entire exposure to CuO NPs [17]. NPs penetrate the lungs and then are able to move into the airways epithelium by transcytosis penetrating the interstitial tissues and thus getting access to blood and lymph circulation. This allows them to reach various organs and systems in the body [18]. Given that, we can assume that CuO NPs are more toxic than CuO MPs. This is evidenced by targeted effects on the lungs under intratracheal NPs and MPs introduction; as a result, it was established that CuO NPs had greater influence on the structure of cellular population as opposed to MPs, which manifested itself through higher relative alveolar macrophage count and lower monocyte and lymphocyte count. Overall, greater levels of alveolar macrophages and segmented neutrophils detected under exposure to NPs are rather typical for a developing neutrophilic variant of acute inflammation[6] [19, 20]. This may induce inflammation in lung and bronchi tissues under multiple inhalation exposure such as pneumonia, bronchitis, and vasculitis. The pathway of this inflammation is presumably associated with oxidative stress induced by effects of intracellular free radicals, generation of which is facilitated by exposure to CuO NPs [13, 21, 22]. This process stimulates greater activity of pro-inflammatory cytokines that induce an inflammatory reaction [23]. Inflammation in the lungs is accompanied with lymphoid hyperplasia [24, 25]. A more apparent decrease in segmented neutrophils to alveolar macrophages ratio is observed in the cellular population of the BAL fluid under exposure to CuO NPs as opposed to their microsized chemical analogue. This difference is due to a growing relative alveolar macrophage level and a neutrophil level remaining steady. The alveolar macrophage level is known to depend on a number of particles that have penetrated the lungs and, given a dose is the same, the smaller a particle diameter, the higher is this level[7]. Given that, when much smaller NPs penetrate the lungs, the body needs to make clearance more effective and this is achieved by reinforced mobilization of alveolar macrophages and neutrophils. In addition to that, growing numbers of these cells allow distributing a cytotoxic burden due to a smaller number of particles absorbed by one cell[7].

Greater toxicity of CuO NPS as opposed to CuO MPs is evidenced under multiple inhalation exposure. Lungs of rats exposed to NPs have obvious signs of developing emphysema, erythrocyte aggregates in alveolar lumen, and edemas; all this was not found after exposure to microsized particles. Emphysema detection is in line with the results reported in a study accomplished by a team of authors and involving experiments on rats. In these experiments, animals underwent an inhalation nose-only exposure to the tested nanomaterial [26]. This pathology typically develops under elevated activity of alveolar macrophages and neutrophils in lung tissues [27, 28]. Serine proteinases, including neutrophil elastase (NE), cathepsin G and proteinase 3 (PR3), are released from azurophil granules of a neutrophil into the extracellular space; they are able to induce tissue lesions [28]. Alveolar edema accompanied with blood exudation into alveolar lumen can result from disrupted blood circulation [29] caused by poorer blood vessel permeability due to metabolic syndrome [30]. The identified pathomorphological changes are accompanied with a declining mass of the lungs. We have not found any data in published works on effects produced by CuO NPs on

[6] Hawkins E.C., DeNicola D.B., Kuehn N.F. Bronchoalveolar lavage in the evaluation of pulmonary disease in the dog and cat. State of the art. *J. Vet. Intern. Med.*, 1990, vol. 4, no. 5, pp. 267–274. DOI: 10.1111/j.1939-1676.1990.tb03120.x

[7] Katsnelson B., Alexeyeva O., Privalova L.I., Polzik E. Pneumoconiosis: pathogenesis and biological prophylaxis. Ekaterinburg, Urals Branch of RAS Publ., 1995, 328 p. (in Russian).

masses of organs under inhalation exposure; however, this aspect was investigated in experiments on rats that involved multiple oral exposures. As reported in a study [31], mass of the lungs with inflammatory changes in them went down against the same indicator in the control group.

**Conclusion.** The study results indicate that CuO NPs accumulate in the lungs under acute and sub-chronic inhalation exposure in concentrations of ~ 4 mg/m$^3$ and 1.2–1.4 mg/m$^3$ respectively. Bioaccumulation, which was identified for NPs and MPs, is comparable under a single inhalation exposure; this is likely due to short exposure duration. This is evidenced by sub-chronic inhalation exposure when NPs tend to accumulate in the lungs more intensively as opposed to MPs since copper levels are 1.45 times higher in the former case. CuP NPs are more toxic for the respiratory organs than their microsized chemical analogue. This fact is evidenced by more intensive development of inflammation established as per changes in the cellular population structure in the BAL fluid. Greater toxic effects of CuO NPs on lung tissues are evidenced by occurring emphysema, edema, and erythrocyte aggregates in alveoli under sub-chronic inhalation exposure; all these adverse outcomes were not identified under exposure to MPs. The study results should be considered when developing activities aimed at preventing negative health outcomes in the respiratory organs (the lungs) under inhalation exposure to the analyzed nanomaterial.

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**Competing interests.** The author declares no competing interests.

## References

1. Mobasser S., Firoozi A.A. Review of nanotechnology applications in science and engineering. *J. Civil Eng. Urban.*, 2017, vol. 6, no. 4, pp. 84–93.

2. Khan I., Saeed K., Khan I. Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, 2019, vol. 12, no. 7, pp. 908–931. DOI: 10.1016/j.arabjc.2017.05.011

3. Zaitseva N.V., Zemlyanova M.A. Identification of health effects caused by environmental chemical exposure. In: G.G. Onishchenko ed. Perm, Knizhnyi format, 2011, 532 p. (in Russian).

4. Zolina L.I., Gracheva K.O. Physicochemical and biochemical properties of metallic nanoparticles and their applications. *Industrial Processes and Technologies*, 2022, vol. 2, no. 1, pp. 29–38. DOI: 10.37816/2713-0789-2022-2-1-29-38 (in Russian).

5. Kovaleva N.Yu., Raevskaya E.G., Roshchin A.V. Aspects of nanomaterial safety: nanosafety, nanotoxicology, nanoinformatics. *Khimicheskaya bezopasnost'*, 2017, vol. 1, no. 2, pp. 44–87. DOI: 10.25514/CHS.2017.2.10982 (in Russian).

6. Global nano copper oxide market report 2022 to 2027: industry trends, share, size, growth, opportunities and forecasts. *Research and Markets*. Available at: https://www.globenewswire.com/news-release/2022/12/23/2579082/0/en/Global-Nano-Copper-Oxide-Market-Report-2022-to-2027-Industry-Trends-Share-Size-Growth-Opportunities-and-Forecasts.html (August 30, 2023).

7. Naz S., Gul A., Zia M., Javed R. Synthesis, biomedical applications, and toxicity of CuO nanoparticles. *Applied Microbiology and Biotechnology*, 2023, vol. 107, pp. 1039–1061. DOI: 10.1007/s00253-023-12364-z

8. Vats M., Bhardwaj S., Chhabra A. Green synthesis of copper oxide nanoparticles using *Cucumis sativus* (Cucumber) extracts and their bio-physical and biochemical characterization for cosmetic and dermatologic applications. *Endocrine, Metabolic & Immune Disorders Drug Targets*, 2021, vol. 21, no. 4, pp. 726–733. DOI: 10.2174/1871530320666200705212107

9. Margenot A.J., Rippner D.A., Dumlao M.R., Nezami S., Green P.G., Parikh S.J., McElrone A.J. Copper oxide nanoparticle effects on root growth and hydraulic conductivity of two vegetable crops. *Plant Soil*, 2018, vol. 431, pp. 333–345. DOI: 10.1007/s11104-018-3741-3

10. Rahman A., Pittarate S., Perumal V., Rajula J., Thungrabeab M., Mekchay S., Krutmuang P. Larvicidal and antifeedant effects of copper nano-pesticides against *Spodoptera frugiperda*

(J.E. Smith) and its immunological response. *Insects*, 2022, vol. 13, no. 11, pp. 1030. DOI: 10.3390/insects13111030

11. Chen W., Zhu B., Sun Y., Guo P., Liu J. Nano-sized copper oxide enhancing the combustion of aluminum/kerosene-based nanofluid fuel droplets. *Combustion and Flame*, 2022, vol. 240, pp. 112028. DOI: 10.1016/j.combustflame.2022.112028

12. Rita A., Sivakumar A., Martin Britto Dhas S.A. Influence of shock waves on structural and morphological properties of copper oxide NPs for aerospace applications. *J. Nanostruct. Chem.*, 2019, vol. 9, pp. 225–230. DOI: 10.1007/s40097-019-00313-0

13. Sarkar A., Das J., Manna P., Sil P.C. Nano-copper induces oxidative stress and apoptosis in kidney via both extrinsic and intrinsic pathways. *Toxicology*, 2011, vol. 290, no. 2–3, pp. 208–217. DOI: 10.1016/j.tox.2011.09.086

14. Edelmann M.J., Shack L.A., Naske C.D., Walters K.B., Nanduri B. SILAC-based quantitative proteomic analysis of human lung cell response to copper oxide nanoparticles. *PLoS One*, 2014, vol. 9, no. 12, pp. e114390. DOI: 10.1371/journal.pone.0114390

15. Sajjad H., Sajjad A., Haya R.T., Khan M.M., Zia M. Copper oxide nanoparticles: In vitro and in vivo toxicity, mechanisms of action and factors influencing their toxicology. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 2023, vol. 271, pp. 109682. DOI: 10.1016/j.cbpc.2023.109682

16. Peng C., Shen C., Zheng S., Yang W., Hu H., Liu J., Shi J. Transformation of CuO nanoparticles in the aquatic environment: influence of pH, electrolytes and natural organic matter. *Nanomaterials (Basel)*, 2017, vol. 7, no. 10, pp. 326. DOI: 10.3390/nano7100326

17. Areecheewakul S., Adamcakova-Dodd A., Haque E., Jing X., Meyerholz D.K., O'Shaughnessy P.T., Thorne P.S., Salem A.K. Time course of pulmonary inflammation and trace element biodistribution during and after sub-acute inhalation exposure to copper oxide nanoparticles in a murine model. *Part. Fibre Toxicol.*, 2022, vol. 19, no. 1, pp. 40. DOI: 10.1186/s12989-022-00480-z

18. Oberdoster G., Oberdoster E., Oberdoster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.*, 2005, vol. 113, no. 7, pp. 823–839. DOI: 10.1289/ehp.7339

19. Nazarenko G.I., Kishkun A.A. Klinicheskaya otsenka rezul'tatov laboratornykh issledovanii [Clinical estimation of laboratory results]. Moscow, Meditsina, 2006, 544 p. (in Russian).

20. Stanzel F. Bronchoalveolar Lavage. In book: Principles and Practice of Interventional Pulmonology. In: A. Ernst, F.J.F. Herth eds. New York, Springer, 2013, pp. 165–176. DOI: 10.1007/978-1-4614-4292-9_16

21. Anreddy R.N.R. Copper oxide nanoparticles induces oxidative stress and liver toxicity in rats following oral exposure. *Toxicol. Rep.*, 2018, no. 5, pp. 903–904. DOI: 10.1016/j.toxrep.2018.08.022

22. Keshavarzi M., Khodaei F., Siavashpour A., Saeedi A., Mohammadi-Bardbori A. Hormesis Effects of Nano- and Micro-sized Copper Oxide. *Iran. J. Pharm. Res.*, 2019, vol. 18, no. 4, pp. 2042–2054. DOI: 10.22037/ijpr.2019.13971.12030

23. Bulua A.C., Simon A., Maddipati R., Pelletier M., Park H., Kim K.-Y., Sack M.N., Kastner D.L., Siegel R.M. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). *J. Exp. Med.*, 2011, vol. 208, no. 3, pp. 519–533. DOI: 10.1084/jem.20102049

24. Khatami M. Developmental phases of inflammation-induced massive lymphoid hyperplasia and extensive changes in epithelium is an experimental model of allergy: implications for a direct link between inflammation and carcinogenesis. *Am. J. Ther.*, 2005, vol. 12, no. 2, pp. 117–126. DOI: 10.1097/01.mjt.0000143699.91156.21

25. Samsonova M.V., Chernyaev A.L. Primary lymphoid lung lesions. *Prakticheskaya pul'monologiya*, 2016, no. 3, pp. 71–75 (in Russian).

26. Gosens I., Cassee F.R., Zanella M., Manodori L., Brunelli A., Costa A.L., Bokkers B.G.H., de Jong W.H. [et al.]. Organ burden and pulmonary toxicity of nano-sized copper (II) oxide particles after short-term inhalation exposure. *Nanotoxicology*, 2016, vol. 10, no. 8, pp. 1084–1095. DOI: 10.3109/17435390.2016.1172678

27. Goldklang M., Stockley R. Pathophysiology of Emphysema and Implications. *Chronic Obstr. Pulm. Dis.*, 2016, vol. 3, no. 1, pp. 454–458. DOI: 10.15326/jcopdf.3.1.2015.0175

28. Jasper A.E., Mclver W.J., Sapey E., Walton G.M. Understanding the role of neutrophils in chronic inflammatory airway disease. *F1000 Res.*, 2019, vol. 8, pp. 557. DOI: 10.12688/f1000research.18411.1

29. Kaptein F.H.J., Kroft L.J.M., Hammerschlag G., Ninamber M.K., Bauer M.P., Huisman M.V., Klok F.A. Pulmonary infarction in acute pulmonary embolism. *Thromb. Res.*, 2021, vol. 202, pp. 162–169. DOI: 10.1016/j.thromres.2021.03.022

30. Willson C., Watanabe M., Tsuji-Hosokawa A., Makino A. Pulmonary vascular dysfunction in metabolic syndrome. *J. Physiol.*, 2019, vol. 597, no. 4, pp. 1121–1141. DOI: 10.1113/JP275856

31. Stepankov M.S., Zemlyanova M.A., Zaitseva N.V., Ignatova A.M., Nikolaeva A.E. Features of Bioaccumulation and Toxic Effects of Copper (II) Oxide Nanoparticles Under Repeated Oral Exposure in Rats. *Pharm. Nanotechnol.*, 2021, vol. 9, no. 4, pp. 288–297. DOI: 10.2174/2211738509666210728163901

*Stepankov M.S. Peculiarities of bioaccumulation and toxic effects produced by copper oxide (II) nanoparticles on the respiratory organs under inhalation exposure as opposed to their micro-sized chemical analogue: assessment for prevention purposes. Health Risk Analysis, 2023, no. 4, pp. 124–133. DOI: 10.21668/health.risk/2023.4.12.eng*

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