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Research article

ASSESSING POTENTIAL HAZARD OF ZINC OXIDE NANOPARTICLES TO HUMAN HEALTH

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Wide implementation of nanomaterials in various economic activities and associated contamination of environmental objects create health risks for workers and general population. Nanoparticles have physiochemical properties that are different from their micro-sized analogues, which may cause more pronounced negative effects upon intake. In this regard, assessment of safety of nanoindustry products is an urgent hygienic problem and the basis for developing recommendations aimed at minimizing health risks.

The aim of the study was to assess potential hazards of a nanomaterial for human health using zinc oxide nanoparticles (ZnO NPs) as an example.

Potential hazard of ZnO NPs was assessed using a set of indicators based on implementation of the predictive-analytical procedure in accordance with MR 1.2.2522-09.

The analysis of ZnO NPs properties indicates that they belong to nano-sized particles (from 6 to 100 nanometers). During cell membrane penetration, ZnO NPs were shown to stimulate greater production of free radicals that cause damage to supramolecular structures. They transform proteomic profile and metabolic reactions changing the expression of proteins that regulate the integrity of cytoskeleton, nuclear matrix and apoptotic process, which leads to cell death. Cellular and molecular changes are manifested through morphofunctional tissue impairments in tissues organs where ZnO NPs bioaccumulate (the liver, kidneys and lungs). Negative effects are manifested as redox imbalance, cytolysis, impaired filtration processes, weaker cellular immunity and, as a consequence, developing inflammatory, dystrophic and necrotic processes. Implementation of the predictive-analytical procedure showed that ZnO NPs are potentially highly hazardous for human health (according to the hazard coefficient D = 2.102).

High potential hazard for human health indicates that it is necessary to investigate remote and specific effects of ZnO NPs in order to perform complete hygienic assessment of their safe levels. This will allow achieving more effective development of preventive measures aimed at minimizing health risks caused by ZnO NPs for workers and general population.

Keywords: *nanoparticles, zinc oxide, potential hazard, human health, criterion analysis, predictive-analytical procedure, character assessment, toxicity.*

At present, new materials are being implemented quite actively into various economic activities all over the world. In the Russian Federation, transition to advanced science-intense design technologies and high-tech production based on using them are fixed as a top priority trend in the country development for the next decade in accordance with "…priority trends in scientific and technological development…"[1].

Nano-sized substances are a good example of a new material that can support this

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[1] Ob utverzhdenii prioritetnykh napravlenii nauchno-tekhnologicheskogo razvitiya i perechnya vazhneishikh naukoemkikh tekhnologii: Ukaz Prezidenta Rossiiskoi Federatsii ot 18.06.2024 № 529 [On Approval of Priority Trends in Scientific and Technological Development and the List of the Priority Science-Intense Technologies: The RF President Order issued on June 18, 2024 No. 529]. *President Rossii*. Available at: http://www.kremlin.ru/acts/bank/50755 (August 17, 2024) (in Russian).

transition. Replacement of micro-sized materials with nano-sized ones in products allows improving their mechanical, electrical, optical, thermodynamic and other properties [1]. This is achieved due to nanoparticles (NPs) having smaller sizes, greater specific surface area and porosity.

Advanced physiochemical properties of nanoindustry products ensure high demand for them, which makes for rapid growth in production volumes. This involves higher NPs levels in environmental objects due to their emissions during manufacturing, use and utilization of nano-products. Given that, there is a growth in health risks for workers and general population associated with exposure to nanoparticles.

Nanoparticles are able to penetrate through body protection barriers much more effectively as compared with their micro-sized chemical analogues due to their smaller sizes. They also spread in the body and accumulate in various organs more effectively [2]. Nanoparticles more actively enter chemical reactions in their further interaction with biological structures due to their large specific surface area. Among such reactions, it is noteworthy to mention lipid peroxidation, which leads to greater generation of free radicals, disrupted metabolic processes and damage to cellular ultrastructures [2].

Given all the above-mentioned, assessment of NPs safety for human health is an urgent hygienic task and basis for developing recommendations aimed at minimizing health risks for general population and workers who consume nanoindustry products or deal with manufacturing them.

A criterion analysis can be used as an approach to predicting potential health hazards posed by NPs. It focuses on various indicators obtained on the basis of available literature data on properties of nanoparticles and their peculiar effects on biological systems. Prediction results allow establishing a range of further studies necessary for developing scientifically grounded hygienic recommendations aimed at health risk minimization.

Zinc oxide (ZnO) NPs are a nanomaterial, which is being actively used in economic activities. It is employed as a component in various industries including chemical and pharmaceutical industry (drug delivery, antibiotics, and anti-diabetic drugs) [3], electronic industry (light-emitting UV-range devices, solar batteries, gas sensors, electrical energy storage systems, and transistors), agriculture (as a component in fertilizers, extranutrition for agricultural crops and forage for farm animals) [4, 5], food industry (as a preservative or a component in food packaging) [6, 7] and textile industry (as a component in textiles with UV-protection, antibacterial and anti-fungi properties, with protection against microwaves and electromagnetic radiation) [8]. The annual global production volume of ZnO NPs reaches 33,400 tons, which means the material is produced in massive quantities (production volume > 1000 tons per year) [9]. A wide range of economic activities where products containing ZnO NPs are used and high volumes of their production as well as these NPs penetrating environmental objects (ambient air, drinking water, and foods) from emissions and discharge make it possible to assume large-scale exposure to them for workers and general population.

In this regard, assessment of potential hazards posed by ZnO NPs for human health is an urgent hygienic problem and the basis for developing recommendations aimed at minimizing health risks for workers and general population.

This study continues the cycle of research works accomplished at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies and aimed at assessing safety of new materials, nano-sized particles included, for human health [10, 11].

The aim of this study was to assess potential hazards of a nanomaterial for human health relying on available literature data and using zinc oxide nanoparticles (ZnO NPs) as an example

Materials and methods. The study was accomplished in conformity with methodical approaches described in the Methodical guide-

lines MR 1.2.2522-09[2]. Information sources were selected according to the criteria stipulated in the Methodical guidelines in requirements to employed information resources (item 4.4). Information about ZnO NPs was grouped in several functional blocks that described physical, physiochemical, molecular-biological, cytological, physiological, toxicological, and ecological properties.

A partial hazard quotient (D_k) was calculated for each functional block as a ratio of the summated intensity of nanomaterial characters included into a functional block to their summated maximum possible intensity taking the weighing function into account per the following formula:

$$D_k = \frac{\sum_{i=1}^{N} R_i \varphi_i}{\sum_{i=1}^{N} R_i^{\max} \varphi_i}, \quad (1)$$

where k is the serial number of a functional block;

i is the serial number of a character;

N is the total number of characters in a block;

R_i is estimation of a character intensity in scores;

$R_i^{\max}$ is the maximum possible score estimate for a given character;

φ_i is the value of the 'weighing function' for the i-th character depending on its rank.

A potential hazard quotient (D) of ZnO NPs for human health was calculated as a square root of the summated values of partial hazards identified for each block and raised to the second power per the following formula:

$$D = \sqrt{\sum_{k=1}^{6} D_k^2}, \quad (2)$$

where D_k is the value of a partial hazard of a functional block, k is the serial number of a block.

A level of potential hazard (low, medium, or high) was established depending on a calculated value of the quotient D.

Statistical significance of accomplished calculations was established using the assessment incompleteness coefficient (U) per ratios of the sums of the weighing functions for uncertain and known characters of ZnO NPs:

$$U = \frac{\sum_{i=1}^{25} u_i \varphi_i}{\sum_{i=1}^{25} \varphi_i}, \quad (3)$$

where U is the assessment incompleteness coefficient;

u_i is the coefficient that is equal to '1', if the i-th character is assumed to be uncertain (information is unavailable) and '0' in any other assessment;

φ_i is the value of the 'weighing function' for this character.

The accomplished estimations were considered significant at the U coefficient value < 0.250.

Results and discussion. We estimated physiochemical properties of ZnO NPs and peculiarities of their interactions with biological systems. As a result, we established that analyzed data covered particles from practically the whole nano-sized range (6~100 nanometers) with predominant shapes being close to spherical[3, 4]. When introduced into a water medium, ZnO NPs seem poorly soluble (0.0029 g/dm^3 at 20 °C) and form small aggregates, which is evidenced by a growing hydrodynamic size by more than twofold against particle sizes in a powder-like nanomaterial[4] [12]. Poor solubility in water may indicate the chemical is more likely hydrophobic than hydrophilic.

[2] MR 1.2.2522-09. Metodicheskie rekomendatsii po vyyavleniyu nanomaterialov, predstavlyayushchikh potentsial'nuyu opasnost' dlya zdorov'ya cheloveka; utv. Glavnym gosudarstvennym sanitarnym vrachom RF 1 iyulya 2009 g. [Methodical guidelines on identifying nano-materials that are potentially hazardous for human health; approved by the RF Chief Sanitary Inspector on July 1, 2009]. *GARANT.RU: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/4088803/?ysclid=m35rww92ox710213070 (September 02, 2024) (in Russian).

[3] Tsink nanooksid 40 nm [Zinc nano-oxide 40 nanometers]. *Osobo chistye veshchestva*. Available at: https://ochv.ru/magazin/product/cink-nanooksid (August 12, 2024) (in Russian).

[4] Zinc oxide. *Merck*. Available at: https://www.sigmaaldrich.com/TR/en/product/aldrich/544906 (August 12, 2024).

A study [13] reported that ZnO NPs solubility reached the peak within 6 hours in artificial fluids that simulated gastric (pH 1.5), intestinal (pH 6.8) and plasma (pH 7.4) media and amounted to ~ 24, 0.2 and 2.8 % respectively. Solubility was ~ 9, 12 and 2 % respectively in these media obtained from rats as established in an *ex vivo* experiment. Presumably, a lower proportion of dissolved NPs in gastric fluid in an *ex vivo* experiment is due to its higher pH value (3.2). Higher solubility in intestinal fluid is assumed to be associated with gastric fluid present in it, which results in lower pH in comparison with an artificial medium.

A medium's pH value is a factor that influences adsorption capability of ZnO particles. When a pH value changes from acidic to neutral, ZnO NPs adsorption capability grows for such dyes as acid fuchsin and Congo red. Transition to an alkaline medium decreases the adsorption capability. When pH grows from 3 to 6, adsorption of malachite green goes down. Values of the indicator reached 3307 mg/g, which proves high adsorption capability of ZnO NPs [14].

A study [15] reported some effects produced by a medium pH on a surface charge of ZnO NPs. The zeta-potential is positive in a neutral medium. When pH reaches 10.1, a charge becomes zero and then turns into negative as a pH value grows further. ZnO NPs are charged negatively in fluids that simulate the body internal environment (pH 1.5–7.4) [13].

ZnO NPs charge has an influence on their biological introduction. In an *in vivo* experiment, negatively charged ZnO NPs are absorbed into the blood stream in larger quantities than positively charged ones after oral administration [16]. Having penetrated the blood stream, NPs are carried over the whole body and are accumulated in the liver, kidneys, and lungs. Elevated zinc levels were also established in the liver and kidneys in an experiment on mice orally exposed to ZnO NPs daily for 13 weeks and in an experiment on rats after inhalation exposure [12, 17]. These findings show that ZnO NPs are able to penetrate through the gut-vascular and blood-air barriers in the body.

At the cellular-molecular level, membranes are a biological system barrier, which ZnO NPs encounter first. Upon contact with a cell membrane, ZnO NPs induce reorganization of lipid components, which results in breaking its integrity as evidenced in an *in vitro* study on cellular lines of human and mice melanoma [18https://pubmed.ncbi.nlm.nih.gov/34235764/]. Lipid destruction can also be due to peroxidation by free radicals produced by ZnO NPs [19].

A study [20] reported ZnO NPs to be able to penetrate through cell membranes by an endocytosis-like pathway. Having penetrated a cell, they spread through cytoplasm and are localized in lysosomes, vesicles and nucleus due to intracellular transportation [20–22]. There is a decline in mitochondrial potential of exposed cells, which results in excessive production of free radicals, reactive oxygen species included [21].

Excessive generation of intracellular free radicals initiates damage to supramolecular structures, such as DNA and proteins. Oxidative damage to DNA was confirmed in an *in vivo* study in rat brain cells after daily oral exposure to ZnO NPs for 7 days [23]. The study results showed increased levels of malonic dialdehyde and decreased levels of superoxide dismutase, glutathione, and catalase, which is typical for developing redox imbalance. Simultaneously, a study that used the comet assay as a research method established a longer and more intensive 'tail', which was a sign of DNA fragmentation. Use of the method yielded comparable results in an *in vitro* study where DNA damage was confirmed in *Scaphechinus mirabilis* sperm [24].

ZnO NPs are able to interact with amino-acid residues in proteins (glycin, phenyl alanine, arginine, aspartic acid, glutamine, and asparagine) forming a hydrogen, electrostatic or metal-acceptor bond with them [25]. Amino-acids associated with NPs undergo

oxidative changes under exposure to free radicals, which is evidenced by enhanced protein carbonylation in *Deinococcus radiodurans* bacteria exposed to nano-sized ZnO [26].

Oxidative damage to proteins stimulates changes in the proteome profile and metabolic processes. A study performed on *Saccharomyces cerevisiae* yeast [27] reported disrupted expression of proteins that regulated carbon metabolism, biosynthesis of co-factors, amino-acids, fatty acids, purines, pyrimidines, nucleosides, and nucleotides. This may impair antioxidant activity, energy metabolism, and cell membrane stability and result in DNA and protein damage.

Changes in protein expression and disrupted metabolism can lead to changes in morphology and cell death. In a study [28], carcinoma cells of human alveoli basal epithelium transformed after exposure to ZnO NPS changing their shapes from prolate to spherical. Cell death caused by exposure to ZnO NPs can follow the apoptosis pathway. Exposure of HepG2 human liver cancer cells resulted in a decline in mitochondria membrane potential, which, in its turn, induced greater Bax protein expression and lower Bcl2 expression [19]. A growing Bax / Bcl2 ratio is evidence of activated apoptosis.

Another cell death pathway can be associated with affected cytoskeleton and nuclear matrix morphology. Vital capacity of GC-01 spermagonia cell line was established to decline after exposure to ZnO NPs [29]. The study results confirmed multidirectional changes in expression of α-tubulin, β-tubulin, F-actin and β-actin. This indicates that cytoskeleton integrity was broken due to changes in expression of microtubule and microfilament proteins. A change in nucleus morphology was established, which was combined with increased expression of nuclear matrix proteins SUN1, LAP1 and lamin A/C supporting an organelle structure.

Damage done by ZnO NPs at the cellular-molecular levels may cause organotoxic effects. An *in vivo* study established that exposure to ZnO NPs caused morphofucntional impairments in liver, kidney, and lung tissues. ZnO NPs hepatotoxicity was also confirmed in experiments on rats after intraperitoneal exposure for 21 days at the dose of 2 mg/kg/day. In comparison with the control rats, the following histological and histochemical alterations were demonstrated in the hepatic tissues of rats exposed to ZnO NPs: sinusoidal dilatation, Kupffer cells hyperplasia, lobular and portal triads inflammatory cells infiltration, necrosis, hydropic degeneration, hepatocytes apoptosis, anisokaryosis, karyolysis, nuclear membrane irregularity, glycogen content depletion and hemosiderosis [30]. In another study [31], elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was established in serum of rats after intraperitoneal injections of ZnO NPs for 7 days at the dose of 25 mg/kg/day. This may result from liver dysfunction.

Nephrotoxic effects of ZnO NPs were confirmed after oral exposure for 14 days at the dose of 1000 mg/kg/day. Biochemical blood tests established higher creatine and urea nitrogen levels indicating kidney dysfunction. Pathomorphological changes were manifested as tubular epithelial cell necrosis [32].

One-time intratracheal exposure to 20 μg of ZnO NPs resulted in higher levels of malonic dialdehyde (MDA), higher lactate dehydrogenase (LDH) activity and weaker antioxidant activity (AOA) in mice bronchoalveolar lavage fluid [33]. Developing inflammation was established in lung tissues.

Results of *in vivo* studies established some specific effects of ZnO NPs, such as immunotoxicity. A decline in NK-cell (natural killers) activity was found in mice after oral administration of 750 mg/kg/day dose of ZnO NPs for 2 weeks, which indicates immunosuppression [34]. Weaker cellular immunity can be confirmed by lower expression of pro- and anti-inflammatory cytokines established in exposed mice.

An average lethal dose of ZnO NPs amounts to > 2000 mg/kg of body weight upon oral exposure (hazard class 3 in accordance

with the State Standard GOST 12.1.007-76[5]); > 1,79 mg/dm^3 upon inhalation exposure (hazard class 2); > 2000 mg/kg upon percutaneous exposure (hazard class 3)[6].

We have created a descriptive master table that provides data on estimated intensities of identified ZnO NPs characters (Table).

We obtained the overall potential hazard value (D) for ZnO NPs, which equaled 2.102, by calculating 'partial' hazard (D_k) for each block in the Table.

This resulting potential hazard value D is within the range of 1.780–2.449, which is estimated as 'high potential hazard'.

Estimated intensities of ZnO NPs characters

Character	Rank	Weighing function value (φ)	Character description	Estimation of character intensity(R)	Short description of ZnO NPs provided in a source	Source
1	2	3	4	5	6	7
Block 1. Physical properties						
Size	1	2	6–100 nanometers	2	Size is 6–100 nanometers	[footnotes 3 and 4, 12–24, 26–34]
Particle shape	1	2	Close to spherical	2	Size is 6–90 nanometers	[footnote 3, 16, 21–23, 28, 33, 34]
Block 2. Physiochemical properties						
Solubility in water	1	2	Poorly soluble	3	Size is < 100 nanometers	[footnote 4]
Solubility in body fluids	2	1	Poorly soluble	2	Size is 28.2 nanometers, hydrodynamic size is 1976 nanometers	[23]
Charge	1	2	Negative	3	Size is 28.2 nanometers, hydrodynamic size is 1976 nanometers	[13]
Adsorption capability	3	0.75	High	4	Size is10 nm, specific surface area is 26.9 m^2/g	[14]
Resistance to aggregate formation	3	0.75	Low	0	Size is 40 nm, hydrodynamic size is 201.8 nm, specific surface area is 60 m^2/g	[12]
Water repellence	4	0.5	Rather hydrophobic	3	Size is < 100 nanometers	[footnote 4]
Adhesion to surfaces	5	0.3125	Unknown	3	-	-
Free radical generation	2	1	Established	4	Size is 90 nm, spherical shape	[21]
Block 3. Molecular and biological properties						
Interaction with DNA	1	2	Established	4	Size is 30 nm, hydrodynamic size is 272 nm, spherical shape	[23]

[5] State Standard GOST 12.1.007-76. Sistema standartov bezopasnosti truda. Vrednye veshchestva. Klassifikatsiya i obshchie trebovaniya bezopasnosti: Mezhgosudarstvennyi standart, utv. postanovleniem Gos-standarta SSSR ot 10 marta 1976 g. № 579 [The system for work safety standards. Adverse chemicals. Classification and general safety requirements: Interstate standard, approved by the order of the USSR State Standard on March 10, 1976 No. 579]. *GARANT: information and legal support*. Available at: https://base.garant.ru/3922227/ (August 28, 2024) (in Russian).

[6] Zinc oxide. *Merck*. Available at: https://www.sigmaaldrich.com/TR/en/product/aldrich/544906 (August 12, 2024).

End of the Table

1	2	3	4	5	6	7
					Size is 40–50 nm, hydrodynamic size is 200 nm, specific surface area is 58 m^2/g	[24]
Interaction with proteins	3	0.75	Established	4	Size is 1 nm (simulated with a computer program)	[25]
					Size is < 100 nm	[26]
Interaction with membranes	2	1	Established	4	Size is 15 nm, specific surface area is 65 m^2/g	[18]
					Size is 30 nm, hydrodynamic size is 267 nm	[19]
					Size is 95 nm, hydrodynamic size is 262 nm, variable shapes	[20]
Block 4. Cytological properties						
Intracellular localization	2	1	Localized in organelles and cytoplasm	4	Size is 95 nm, hydrodynamic size is 262 nm, variable shapes	[20]
					Size is 90 nm, spherical shape	[21]
					Size is 30–50 nm, hydrodynamic size is 284.76 nm, spherical shape	[22]
Transforming impact on cells	1	2	Established	4	Nano-spheres: size is 6–38 nm, hydrodynamic size is 183–505 nm, specific surface area is 7–90 m^2/g, spherical shape; Nanorods: size (diameter / length) is 6–7 nm / 8–19 nm, hydrodynamic size is 481–593 nm, specific surface area is 37–88 m^2/g, cylindrical shape	[28]
Transformation of the proteome profile and metabolic processes	3	0.75	Established	4	Size is < 70 nm, hydrodynamic size is 60–150 nm	[27]
Cytotoxicity	1	2	Death of unchanged cells	4	Size is 30 nm, hydrodynamic size is 267 nm	[19]
					Size is 88 nm, specific surface area is 12 m^2/g	[29]
Block 5. Physiological properties						
Penetration through body protection barriers	4	0.5	Established	4	Size is 40 nm, hydrodynamic size is 201.8 nm, specific surface area is 60 m^2/g	[12]
					Size is 20–70 nm, spherical shape	[16]
					Size is 20 nm	[17]

End of the Table

1	2	3	4	5	6	7
Bioaccumulation	2	1	Accumulates in some organs	3	Size is 40 nm, hydrodynamic size is 201.8 nm, specific surface area is 60 m^2/g	[12]
					Size is 20–70 nm, spherical shape	[16]
					Size is 20 nm	[17]
Makes protective barriers more penetrable for other toxicants	3	0.75	Unknown	3	-	-
Acute toxicity	1	2	Hazard class 2	4	Size is < 100 nm	[footnote 4]
Chronic toxicity	1	2	Toxic for warm-blooded animals	4	Size is 35 nm	[30]
					Size is 20–50 nm, hydrodynamic size is 169.2 nm	[31]
					Size is 50 nm	[32]
					Size is 12.9 nm, hydrodynamic size is 304 nm, spherical shape	[33]
Specific toxic effects	1	2	Established	4	Size is 29–79 nm, spherical shape	[34]
Block 6. Ecological properties						
Global production volume	1	2	Produced in large-scale volumes	4	-	[9]
Possible exposure scales	1	2	General population at the national level	4	-	[9]
Bioaccumulation in organisms	2	1	Unknown	2	-	-
Bioaccumulation in environmental objects	3	0.75	Unknown	3	-	-

We have not been able to find any information about the following ZnO NPs characters: adhesion to surfaces; ability to make protection barriers more penetrable for other toxicants; accumulation in organisms and environmental objects. The assessment incompleteness coefficient (U) was equal to 0.079, which is within the range of 0–0.250 and allow considering the accomplished assessment to be significant.

Analytical assessment of aggregated data confirms that the analyzed ZnO particles fall within the nano-sized range, that is, below 100 nanometers. Smaller particle sizes than those of micro-sized chemical analogues (more than 100 nanometers) can ensure more pronounced introduction, spread over the body and bioaccumulation of ZnO NPs. Solid nanoparticles are known to be able to spread evenly in the lungs upon inhalation exposure and their highest levels are observed in the bronchi whereas the greatest proportion of PM_{10} particles is deposited in upper airways mucosa [35].

We should mention established pH effects on ZnO NPs introduction. The results obtained by investigating how NPs behave in liquid media simulating gastric fluid, intestinal fluid and plasma make it possible to conclude that solubility grows as a pH value declines. However, solubility was not above 24 and 12 % of the total amount of particles in artificial and natural media respectively. Given that, we can

assume that both solid NPs and Zn^{2+} ions make their contributions to bioaccumulation and toxic processes initiated by ZnO NPs. NPs surface charge depends on pH since the zeta-potential goes down as pH values grow. ZnO NPs absorption into the blood stream increases as a charge declines.

Upon penetration into the body, ZnO NPs interact with macromolecules and supramolecular structures. ZnO NPs are able to produce oxidative effects on cell structures by stimulating greater generation of free radicals. Damage to these structures and disruption of their normal functioning determines cytotoxic effects of the analyzed nanomaterial including its impact by changing the proteome profile and metabolic processes. This can ultimately lead to cell death. Exposure to ZnO NPs induces changes in expression of microtubule and microfilament proteins, nuclear matrix and apoptosis regulators, which results in cell death.

When we consider peculiar interactions between ZnO NPs and biological systems at the tissue-organ level, we should mention growing zinc levels in the liver, kidneys and lungs of exposed animals both upon oral and inhalation exposure. Bioaccumulation in these organs and cytotoxic properties determine development of negative effects initiated by ZnO NPs. Hepatotoxicity is manifested through cytolytic effects (increased ALT and AST activity) and developing pathomorphological changes. Hepatocytes with changed nucleus sizes and prone to apoptosis are detected in parenchymal tissue. Inflammatory, dystrophic and necrotic processes develop in the liver. Morphofunctional changes found in the kidneys involve disrupted filtration (growing creatine and urea nitrogen) and developing epithelial necrosis. Toxic effects produced by ZnO NPs on the lungs are manifested through developing redox imbalance (growing MDA levels and weaker AOA), cytolysis (growing LDH activity in bronchoalveolar lavage fluid), and tissue inflammation. Given the aforementioned peculiarities of bioaccumulation and morphofucntional impairments, we can assume the liver, kidneys and lungs to be primary targets under exposure to ZnO NPs.

Negative impact on cells determines immunotoxic expression of ZnO NPs. Simultaneous decrease in NK-cell activity and lower production of pro- and anti-inflammatory cytokines were established in mice exposed to ZnO NPS. These results are evidence of lymphocyte death and imbalance between pro- and anti-inflammatory cytokine systems, which may stimulate immunosuppression.

Conclusion. We have established high potential hazard of ZnO NPs for human health based on implementation of the predictive-analytical procedure (D = 2.102). The obtained assessment is supported by the whole set of known properties of zinc oxide particles from the whole nano-sized range (6~100 nanometers) and has statistical significance (U = 0.079). The results of the present study indicate that it is necessary to investigate remote and specific toxic effects of ZnO NPs (genotoxicity, teratogenic effects, embryotoxicity, and gonadotoxicity) in order to perform complete hygienic assessment of their safe levels for particles of different sizes within the nano-sized range. This will allow achieving more effective development of scientifically grounded preventive measures aimed at minimizing health risks caused by ZnO NPs for workers and general population who manufacture and consume products containing this nanomaterial.

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References

1. Adekoya J.A., Ogunniran K.O., Siyanbola T.O., Dare E.O., Revaprasadu N. Band structure, morphology, functionality, and size- dependent properties of metal nanoparticles. In book: *Noble and precious metals – properties, nanoscale effects and applications*; M.S. Seehra, A.D. Bristow eds., 2018, pp. 15–42. DOI: 10.5772/intechopen.72761

2. Sukhanova A., Bozrova S., Sokolov P., Berestovoy M., Karaulov A., Nabiev I. Dependence of nanoparticle toxicity on their physical and chemical properties. *Nanoscale Res. Lett*., 2018, vol. 13, no. 1, pp. 44. DOI: 10.1186%2Fs11671-018-2457-x

3. Bala N., Saha S., Chakraborty M., Maiti M., Das S., Basub R., Nandy P. Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. *RCS Advances*, 2015, vol. 5, no. 7, pp. 4993–5003. DOI: 10.1039/C4RA12784F

4. Adil M., Bashir S., Bashir S., Aslam Z., Ahmad N., Younas T., Asghar R.M.A., Alkahtani J. [et al.]. Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress. *Front. Plant. Sci*., 2022, vol. 13, pp. 932861. DOI: 10.3389/fpls.2022.932861

5. Gauba A., Hari S.K., Ramamoorthy V., Vellasamy S., Govindan G., Arasu M.V. The versatility of green synthesized zinc oxide nanoparticles in sustainable agriculture: A review on metal-microbe interaction that rewards agriculture. *Physiological and Molecular Plant Pathology*, 2023, vol. 125, pp. 102023. DOI: 10.1016/j.pmpp.2023.102023

6. Youn S.-M., Choi S.-J. Food additive zinc oxide nanoparticles: dissolution, interaction, fate, cytotoxicity, and oral toxicity. *Int. J. Mol. Sci*., 2022, vol. 23, no. 11, pp. 6074. DOI: 10.3390/ijms23116074

7. Espitia P.J.P., Otoni C.G., Soares N.F.F. Zinc oxide nanoparticles for food packaging applications. In book: *Antimicrobial Food Packaging*; J. Barros-Velázquez ed. Elsevier, 2016, pp. 425–431. DOI: 10.1016/B978-0-12-800723-5.00034-6

8. Nisansala H.M.D., Rajapaksha G.K.M., Dikella D.G.N.V., Dheerasinghe M.J., Sirimuthu N.M.S., Patabendige C.N.K. Zinc oxide nanostructures in the textile industry. *Indian Journal of Science and Technology*, 2021, vol. 14, no. 46, pp. 3370–3395. DOI: 10.17485/IJST/v14i46.1052

9. Rahman H.S., Othman H.H., Abdullah R., Edin H.Y.A.S., Al-Haj N.A. Beneficial and toxicological aspects of zinc oxide nanoparticles in animals. *Vet. Med. Sci*., 2022, vol. 8, no. 4, pp. 1769–1779. DOI: 10.1002/vms3.814

10. Zemlyanova M.A., Zaitseva N.V., Stepankov M.S. Research and assessment of the molybdenum oxide (VI) nanoparticles toxicity under inhalation in Wistar line rats in comparison with the micro-sized chemical analog. *Gigiena i sanitariya*, 2023, vol. 102, no. 10, pp. 1119–1124. DOI: 10.47470/0016-9900-2023-102-10-1119-1124 (in Russian).

11. 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

12. Cho W.-S., Kang B.-C., Lee J.K., Jeong J., Che J.-H., Seok S.H. Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. *Part. Fibre Toxicol*., 2013, vol. 10, pp. 9. DOI: 10.1186/1743-8977-10-9

13. Yu J., Kim H.-J., Go M.-R., Bae S.-H., Choi S.-J. ZnO Interactions with biomatrices: effect of particle size on ZnO-protein corona. *Nanomaterials (Basel)*, 2017, vol. 7, no. 11, pp. 377. DOI: 10.3390/nano7110377

14. Zhang F., Chen X., Wu F., Ji Y. High adsorption capability and selectivity of ZnO nanoparticles for dye removal. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2016, vol. 509, pp. 474–483. DOI: 10.1016/j.colsurfa.2016.09.059

15. Zhang F., Lan J., Yang Y., Wei T., Tan R., Song W. Adsorption behavior and mechanism of methyl blue on zinc oxide nanoparticles. *J. Nanopart. Res.*, 2013, vol. 15, no. 11, pp. 2034. DOI: 10.1007/s11051-013-2034-2

16. Baek M., Chung H.-E., Yu J., Lee J.-A., Kim T.-H., Oh J.-M., Lee W.-J., Paek S.-M. [et al.]. Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. *Int. J. Nanomedicine*, 2012, vol. 7, pp. 3081–3097. DOI: 10.2147/IJN.S32593

17. Wang L., Wang L., Ding W., Zhang F. Acute toxicity of ferric oxide and zinc oxide nanoparticles in rats. *J. Nanosci. Nanotechnol*., 2010, vol. 10, no. 12, pp. 8617–8624. DOI: 10.1166/jnn.2010.2483

18. Czyzowska A., Barbasz A., Rudilphi-Szydlo E., Dyba B. The cell membrane as the barrier in the defense against nanoxenobiotics: Zinc oxide nanoparticles interactions with native and model membrane of melanoma cells. *J. Appl. Toxicol*., 2022, vol. 42, no. 2, pp. 334–341. DOI: 10.1002/jat.4216

19. Sharma V., Anderson D., Dhawan A. Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2). *Apoptosis*, 2012, vol. 17, no. 8, pp. 852–870. DOI: 10.1007/s10495-012-0705-6

20. Liu Z., Lv X., Xu L., Liu X., Zhu X., Song E., Song Y. Zinc oxide nanoparticles effectively regulate autophagic cell death by activating autophagosome formation and interfering with their maturation. *Part. Fibre Toxicol*., 2020, vol. 17, no. 1, pp. 46. DOI: 10.1186/s12989-020-00379-7

21. Choudhury S.R., Ordaz J., Lo C.-L., Damayanti N.P., Zhou F., Irudayaraj J. From the cover: zinc oxide nanoparticles-induced reactive oxygen species promotes multimodal cyto- and epigenetic toxicity. *Toxicol. Sci*., 2017, vol. 156, no. 1, pp. 261–274. DOI: 10.1093/toxsci/kfw252

22. Jain A.K., Singh D., Dubey K., Maurya R., Pandey A.K. Zinc oxide nanoparticles induced gene mutation at the HGPRT locus and cell cycle arrest associated with apoptosis in V-79 cells. *J. Appl. Toxicol*., 2019, vol. 39, no. 5, pp. 735–750. DOI: 10.1002/jat.3763

23. Attia H., Nounou H., Shalaby M. Zinc oxide nanoparticles induced oxidative DNA damage, inflammation and apoptosis in rat's brain after oral exposure. *Toxics*, 2018, vol. 6, no. 2, pp. 29. DOI: 10.3390/toxics6020029

24. Kukla S.P., Chelomin V.P., Mazur A.A., Slobodskova V.V. Zinc oxide nanoparticles induce DNA damage in sand dollar *Scaphechinus mirabilis* sperm. *Toxics*, 2022, vol. 10, no. 7, pp. 348. DOI: 10.3390/toxics10070348

25. Singh K.P., Dhasmana A., Rahman Q. Elucidation the toxicity mechanism of zinc oxide nanoparticle using molecular docking approach with proteins. *Asian Journal of Pharmaceutical and Clinical Research*, 2018, vol. 11, no. 3, pp. 441–446. DOI: 10.22159/ajpcr.2018.v11i3.23384

26. Singh R., Cheng S., Singh S. Oxidative stress-mediated genotoxic effect of zinc oxide nanoparticles on Deinococcus radiodurans. *3 Biotech*, 2020, vol. 10, no. 2, pp. 66. DOI: 10.1007/s13205-020-2054-4

27. Babele P.K. Zinc oxide nanoparticles impose metabolic toxicity by de-regulating proteome and metabolome in *Saccharomyces cerevisiae*. *Toxicol. Rep*., 2019, vol. 6, pp. 64–73. DOI: 10.1016/j.toxrep.2018.12.001

28. Hsiao I.-L., Huang Y.-J. Effects of various physicochemical characteristics on the toxicities of ZnO and TiO nanoparticles toward human lung epithelial cells. *Sci. Total Environ*., 2011, vol. 409, no. 7, pp. 1219–1228. DOI: 10.1016/j.scitotenv.2010.12.033

29. Pinho A.R., Martins F., Costa M.E.V., Senos A.M.R., da Cruz E Silva O.A.B., de Lourdes Pereira M., Rebelo S. In vitro cytotoxicity effects of zinc oxide nanoparticles on spermatogonia cells. *Cells*, 2020, vol. 9, no. 5, pp. 1081. DOI: 10.3390/cells9051081

30. Almansour M.I., Alferah M.A., Shraideh Z.A., Jarrar B.M. Zinc oxide nanoparticles hepatotoxicity: histological and histochemical study. *Environ. Toxicol. Pharmacol*., 2017, vol. 51, pp. 124–130. DOI: 10.1016/j.etap.2017.02.015

31. Pei X., Jiang H., Xu G., Li C., Li D., Tang S. Lethality of zinc oxide nanoparticles surpasses conventional zinc oxide via oxidative stress, mitochondrial damage and calcium overload: a comparative hepatotoxicity study. *Int. J. Mol. Sci*., 2022, vol. 23, no. 12, pp. 6724. DOI: 10.3390/ijms23126724

32. Yan G., Huang Y., Bu Q., Lv L., Deng P., Zhou J., Wang Y., Yang Y. [et al.]. Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng*., 2012, vol. 47, no. 4, pp. 577–588. DOI: 10.1080/10934529.2012.650576

33. Guo T., Fang X., Liu Y., Ruan Y., Hu Y., Wang X., Hu Y., Wang G., Xu Y. Acute lung inflammation induced by zinc oxide nanoparticles: evolution and intervention via NRF2 activator. *Food Chem. Toxicol.*, 2022, vol. 162, pp. 112898. DOI: 10.1016/j.fct.2022.112898

34. Kim C.-S., Nguyen H.-D., Ignacio R.M., Kim J.-H., Cho H.-C., Maeng E.H., Kim Y.-R., Kim M.-K. [et al.]. Immunotoxicity of zinc oxide nanoparticles with different size and electrostatic charge. *Int. J. Nanomedicine*, 2014, vol. 9, suppl. 2, pp. 195–205. DOI: 10.2147/ijn.s57935

35. Li D., Li Y., Li G., Zhang Y., Li J., Chen H. Fluorescent reconstitution on deposition of $PM_{2.5}$ in lung and extrapulmonary organs. *Proc. Natl Acad. Sci. USA*, 2019, vol. 116, no. 7, pp. 2488–2493. DOI: 10.1073/pnas.1818134116

Stepankov M.S., Zemlyanova M.A. Assessing potential hazard of zinc oxide nanoparticles to human health. Health Risk Analysis, 2024, no. 4, pp. 123–134. DOI: 10.21668/health.risk/2024.4.11.eng

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