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TOXIC EFFECTS OF NANOPARTICLES

N. S. LEONENKO, O. B. LEONENKO

State Organization "Institute of Occupational Medicine of the National Academy of Medical Sciences of Ukraine", Kyiv

E-mail: taliya@meta.ua

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The review summarizes the literature data on the assessment of the toxic effects of nanoscale particles at various concentrations and routes of entry into the body. The toxic effect of nanoobjects is more complex and diverse in comparison with the effect of traditional toxicants (heavy metals, organic solvents, poisonous substances, etc.). Despite the growing amount of data, there are no unified approaches to studying the toxic effects of nanoscale particles. This requires the development of specific procedures for their toxicity assessing.

Key words: nanoparticles, methods of toxic effect assessment.

The nanoindustry products are being intensively introduced into all spheres of modern life. It is increasingly used in such areas as pharmacology and medicine, the manufacture of cosmetics and products for personal use, the storage of energy and increasing of its use efficiency, the water purification and air filtration, the environment restoration, the production of chemical and biological sensors, the military and the production of explosives [1–7], as well as the production of consumer goods and materials. The diversity of nanomaterials and nanodevices and the scope of their application are so great that it makes it difficult not only to generalize information on their use, but also limits the ability to predict new applications [2, 3].

Research in the field of health care nanotechnology has steadily increased over the past 15 years and has allowed obtaining a vast amount of knowledge, as evidenced by many scientific publications. However, data on the toxicity of nanomaterials (NMs) and the consequences of their use for human health do not allow for definitive conclusions.

It is generally recognized that changes in the physical properties of a substance in the transition to the form of nanoparticles (NPs) are naturally accompanied by changes in their biological effects. A feature of NPs action on the body is the ability to penetrate easily into all organs and tissues, stimulate metabolic processes in biotic doses and exert an action on living systems that will promote the development of various pathological conditions and even lead to death in toxic doses. The interaction of NPs with living cells may be unpredictable and dangerous [4, 5].

Despite the considerable amount of data on nanotoxicity, the effects of the possible influence of NPs on human health remain unclear. Moreover, taking into account the rapidly growing production of NMs (and, consequently, increasing the risk of their potential impact on the population), there is an urgent need to identify adverse effects on human health, especially in the long-term use of nanotechnology.

For chemical substances, including nanoscale state, three types of biological action are possible: pharmacotoxic - when significant changes are manifested in the activity and state of the body's functional systems, including metabolism and detoxication, the safety zone — when response of the body systems on their impact is not observed, and zone of biotic effects that stimulates metabolism and does not violate the regulatory links inherent in the norm.

Usually toxicological and hygienic assessment of chemical compounds includes

the performance of acute, subacute, chronic experiments with the establishment of lethal, threshold and inoperative levels in different routes of entry, clarifying the damaging effects and the long-term consequences of their action and justifying the permissible levels and doses with the development of recommendations for their safe application or use [6]. When investigating the risk of NMs, considerable attention is paid to screening investigations *in vitro* [7, 8].

Acute toxicity and local irritant effect

According to the literature data, in the acute toxicity study, the LD_{50} value has been determined for oral ingestion of silver gel for *Sprague Dawley* rats — 1 266 mg/kg, and LD_{50} for colloidal silver solution in experiments in mice — 2 820 mg/kg [9] (moderately hazardous substances). According to other studies, nanoscale silver particles (average size is 10 nm, volume concentration (by silver) is 0.27 mg/ml, stabilized with surfactants), according to the value of the average lethal dose with a single intragastric route of entry, have been assessed as low-hazard objects with LD_{50} more than 5 000 mg/kg [10]. Silicon dioxide with single intragastric administration to male mice according to LD_{50} (more than 10 000 mg/kg) is classified as the 4^{th} class of hazard [11].

According to the results of the study of copper NPs (23.5 nm) toxicity with oral administration, LD_{50} for mice was 413 mg/kg. The target organs of copper NPs toxic effect were liver, spleen, kidneys [12]. Nano-sized zinc particles by the value of the average lethal dose, when introduced intragastric one time, are also fallen into low-risk substances with LD_{50} greater than 5 000 mg/kg, and in this dose, they did not have a general toxic effect with obvious signs of intoxication [10]. Alumina-silica nanotubes of 60–150 nm in size (inner diameter is 10–60, length is 400–4 000 nm) when introduced intragastric into rats and mice in doses from 5 000 to 20 000 mg/kg did not cause death and clinical signs of poisoning [13]. The LD_{50} value of TiO_2 NPs for rats in case of intragastric administration was more than 12 000 mg/kg [14], and of carbon nanotubes for rats was 600 mg/kg. Fullerenes C_{60} have total toxicity of 2 500 mg/kg or 1 200–1 500 mg/kg [15].

Thus, the majority of compounds in the nano-sized state by acute oral toxicity belong to the low-hazard objects of the 4^{th} hazard class.

When applied to the skin of rats, the LD_{50} for silver gel NPs has been determined; it is equal to more than 2 000 mg/kg [9]. Epicutaneous applications of aluminosilicate nanotubes to the skin of rats at doses of 1 250, 2 500 and 3 250 mg/kg did not cause signs of intoxication, animal death, or local irritant effect. The obtained data allowed attributing aluminosilicate nanotubes and silver gel NPs, when applied to the skin, to the substances of the 4^{th} hazard class (low hazard) [13].

Irritant effect on the skin of nano-sized silver particles in a concentration of 5 g/l was not manifested. However, when applied to the mucous membranes of the eyes, an irritating effect has been revealed. Nano-sized zinc particles did not irritate the skin and mucous membranes [10]. At the same time, dermal exposure of NPs can lead to inflammation of the lymphatic system [16], which is the basis for studying and evaluating precisely this way of contact with NPs and NMs as one of the real ones for their entry into the body.

Inhalational administration

The most dangerous way of NPs influence on person may be inhalation. The most important properties that determine the negative impact of NPs on health is their ability to penetrate into alveolar areas of the lungs, causing mechanical, toxic and immunological damage. This can be confirmed by numerous literature data. In particular, there is significant delay of NPs in the lungs, because of the small size; the mechanisms for their removing by respiratory system of organism are ineffective. The ability of NPs to penetrate through the lungs into other body systems has been shown [17]. Cases of rats' death after inhalation of NPs have been noted. It has been found that laboratory animals meet with difficulties in breathing during inhalation of carbon nanotubes. NPs after entering into the alveolar part of the lungs penetrate into the systemic circulatory system and, further, into the brain, which can have a negative impact on the central nervous system [18]. When breathing copper NPs, marked damage of the kidneys, liver and spleen in mice has been detected [19]. Zinc NPs caused more severe kidney damage than microparticles [20].

Nanodispersed manganese oxide in case of inhalation route of entry in aerosol form had an acute toxic effect: LC_{50} at 4-hour exposure for rats was 0.12 mg/dm^3. The greatest number of particles at studied concentrations after 2 hours of exposure corresponded to the dimensions of 20–40 nm, after 4 hours

of exposure corresponded to the dimensions of 30–50 nm, that is, during the experiment, the particle size was changed (increased). The clinical picture of acute intoxication was characterized by irritating, neurotoxic effects, respiratory depression, the combination of which could cause the death of animals. According to the LC_{50} criterion (more than 0.05–0.5 mg/dm^3) for chemical compounds, the test substance can be assigned to the 2nd hazard class (highly hazardous substances) [21], which according to the international hazard classification of chemical compounds (GHS), suggested the presence of negative consequences of such impact.

In case of inhalation exposure of 20–65 nm silver NPs in concentrations of $1.73 \cdot 10^4$–$1.32 \cdot 10^6$ particles/cm^3 for 28 days of the experiment, there were no significant changes in body weight and main biochemical parameters of peripheral blood compared with the control group, or only some biochemical indices were changed [9], and no pathological lesions in lungs and nasal cavity were noted [22].

In another 13-weeks experiment of inhalation exposure, it has been established that the target organs for silver NPs were liver and lungs [23]. It has been also revealed that silver NPs had the ability to penetrate into the olfactory bulb of the brain as a result of axonal transport.

When inhaled intake of iron oxide NPs with dimensions of 22 and 280 nm into the body of rats at doses of 0.8 and 20.0 mg/kg, induction of active oxygen forms in cells, hyperemia, hyperplasia and fibrosis of lung tissue, as well as violation of blood clotting system [24] were observed.

To study the effect of inhalation entry of quantum dots, they were injected into the body by instillation and inhalation. Quantum dots based on CdSe/ZnS at doses of 1.4 to 3 600 pmol/mouse caused thrombosis and pneumonia only in high doses. It has been found that the investigated quantum dots with a diameter of 3.2 nm with the inhalation route of entry penetrated through the olfactory tract into the brain and the central nervous system. In case of intranasal administration of phospholipid-encapsulated CdSe/ZnS quantum dots aqueous solution at a concentration of 7 mg/m^3 for 3 hours, the penetration of NPs along the olfactory nerve through the blood-brain barrier into the cerebral cortex has been revealed [25]. Quantum dots of 2–3 nm in size based on CdSe/ZnSe after 2 hours with inhalation entry can also overcome the blood-brain barrier and penetrate into the cerebral cortex of experimental animals [26].

Thus, the inhalation pathway of NPs entry into the body can be dangerous for a person not only because of getting into the lungs, blood, but also due to the ability of nanoobjects to overcome the blood-brain barrier and penetrate into the cerebral cortex.

Oral administration

Assessment of the general toxic effect of nanoscale particles with oral entry into the body was carried out under conditions of acute, subacute, subchronic and chronic effects. According to literature data, when introduced intragastric one time, silver NPs at a dose of 150 mg/animal revealed toxic effects on the central nervous system, despite of the fact that no death was noted [10]. Single oral administration to the mice of iron NPs suspension at doses of 50, 100 and 500 μg/kg did not cause any toxic effects. Only at doses of 1 000, 2 000 and 5 000 μg/kg, the inflammatory process development in the stomach and intestinal mucosa, as well as the violation of hemopoiesis, were identified [27]. With a single exposure of iron oxide NPs, stabilized with dextran and sodium citrate, the toxic effects were detected in rats and dogs in doses exceeding 400 mg/kg. Ferric oxide NPs at a concentration of 100 mg/ml stimulated the respiratory function of the blood, changed the shape of erythrocytes, and induced the conformational rearrangement of hemoglobin [28, 29].

A single oral administration of TiO_2 NPs at a dose of 5 000 mg/kg did not cause the death of animals. Changes in biochemical and other studied blood parameters were more pronounced at a particle size of 25 and 80 nm compared to 250 nm [14].

The evaluation of repeated impacts of lead sulfide NPs (average particle size of 8, 30 and 65 nm) after 30 intraperitoneal injections to rats have shown that the most pronounced changes were initially manifested for NPs of larger size (65 nm). Regardless of the particle size, the administration of PbS caused a disturbance in a number of hematological, biochemical indices in rats, on the basis of which the authors have concluded that precisely nanoparticles cause the toxic damage of the liver, kidney, pancreas and thyroid gland cells. And in the post-exposure period, in animals that received sulphide lead NPs of larger size, the normalization of the fatty acid level in the brain tissue occurred, whereas after the introduction of PbS NPs of

smaller size, the discoordination of the lipid metabolism processes and change in the fatty acid content were revealed [30].

Intragastric administration of 35 nm silver NPs into rats for 28 days at a dose of 0.1 mg/kg of body weight did not lead to significant changes in animal growth, integral, hematological, biochemical indices, detoxification state of xenobiotics and a balance of oxidation-reduction reactions, normal and transient microflora of the large intestine [31, 32]. Under similar conditions, 10–60 nm silver NPs at a concentration of 0.27 mg/ml did not cause significant deviations in the dynamics of the increase in the mass of internal organs, as well as changes in the biochemical composition of the blood compared with the control.

With daily intragastric nanoclay administration to the growing male rats at a dose of up to 100 mg/kg of body weight for 28 days, no effects were found that could be considered potentially toxic [31].

Estimation of the oral effect of long multilayer carbon nanotubes (CNT of 15–40 nm, internal diameter was 3–8 nm, length was 2 and more microns, in concentrations of 0.2 and 0.5 mg/l) was performed in male mice of CBA·C57 BL/6 line within 2 months. It has been established that the prolongation of the experiment from 2 weeks to 2 months caused pathological processes in the body of mice: fatty degeneration in the liver, and villi destruction in the small intestine. At 6 months duration, significant changes were observed in the small intestine of rats: a decrease in the number of villi without compromising the integrity of the suction rim, an increase in the number of destructured villi, and the appearance of villi with apical necrosis [33, 34].

In experiments with oral exposure to rats of 14.3 ± 3 nm silver NPs in concentrations of 0.01; 0.05; 0.5; 5 mg/l for 30 and 90 days, the effects of liver damage, including the morphofunctional state, were observed. The prolongation of silver NPs exposure time from 1 to 3 months led to an increase in destructive changes in the liver of rats, which may be a confirmation of cumulative action of silver compounds [33].

With daily intragastric administration of nanoscale fullerene C_{60} dispersion and fullerenol $C_{60}(OH)_{24}$ solution for 28 and 92 days at doses of 0.1 to 10 mg/kg body weight of rats, the changes in the indices indicating the presence of general toxic effect of these compounds have been found. In the case of C_{60}, they consisted of a dose-dependent decrease in the relative weight of the liver, an increase in the permeability of the small intestine wall for protein macromolecules, an increase in the number of $CD106^{+}$ granular cells in the liver parenchyma. Based on the analysis of the obtained data, the maximum non-active dose of C_{60} with its subacute oral entry was established in the range of 1–10 mg/kg of body weight/day, and of $C_{60}(OH)_{24}$ — 0.1–1.0 mg/kg of body weight/day [31, 35], that is, the fullerenol solution was more toxic.

In long-term experiments carrying out, the general toxic effect was observed when studying the subchronic toxicity of zinc NPs at a dose of 15 mg/animal and in concentrations of 0.45–1.8 mg/ml [10].

Information on the possible toxicity of nano-SiO_2 in vivo is contradictory. According to data obtained during 3-month oral administration of nano-SiO_2 suspension into rats, the main target of the exposure was the immune system, which was confirmed by a decrease in the number of CD^{4+} and an increase in the number of CD^{8+} lymphocytes, an increase in TNF-α production and total leukopenia. The maximum non-active dose of 30–50 nm NPs was more than 10 mg/kg of body weight per day [31].

The entry of iron NPs at doses of 20 and 40 μg/kg for 90 days into the body of laboratory animals did not lead to significant deviations in biochemical and hematological indices compared to the control group. When studying the chronic toxicity of iron oxide, an increase in the activity of aminotransferases in the blood associated with cytomorphological changes in the liver was detected. [29].

The chronic six-month oral entry of non-functionalized spherical particles of nanosilver with a diameter of 20–40 nm (10–90 percentile) at doses of 0.056 mg/kg/day, 0.011 mg/kg/day and 0.0022 mg/kg/day has been assessed using a wide range of physiological, histomorphological, hematological, biochemical and immunological methods of investigation [36]. It has been established that the long-term action of the hydrosol of the nanosilver exerted a dose-dependent general toxic effect developing against the background of NPs accumulation in the organs of animals. The threshold dose for the systemic effect (Lim_{ch} integr) was 0.0022 mg/kg. In case of prolonged oral nanosilver entry into the body, the cumulative properties were manifested [36].

In another 6-month experiment, the dynamics of the effect of 4 doses of 14.3 ± 0.05 nm silver nanoparticles [37] has been studied [37]. It allowed evaluating the effect on the liver at a

dose of 0.3 mg/kg as pronounced harmful, at a dose of 0.023 mg/kg — as LOAEL, and at a dose of 0.0028 and 0.0006 mg/kg as NOEL.

A confirmation of the increased danger with prolonged administration of NPs can also be the results according to which the signs of silver nanoparticles general toxic effect in experiments in vivo were observed in doses of 30–150 mg/animal in the study of acute toxicity, in doses of 0.9–2.7 mg/animal in the study of subchronic toxicity, and in concentration of 0.005 mg/ml in chronic experiments carrying out [10].

Thus, with oral NPs entry into the organism of laboratory animals, the features of their influence depend on the concentration, the duration of the impact and the dimension of NPs, and also the chemical nature. The changes can be multifactorial and ambiguous. The difficulty in comparing the presented data was that there was no systematic research in general, the choice of doses and experimental conditions, in most cases the characteristics of the substances studied and the corresponding controls were not presented.

In general, it can be said that a single acute impact of NPs in high doses depends on their size, is manifested by inflammation and is characterized by damage of the central nervous system. With repeated subacute and subchronic NPs entry into the body of laboratory animals (from 28 days to 3 months), the changes are not unambiguous — from pronounced liver, kidney, pancreas and thyroid gland cells damage (in case of intraperitoneal action of lead sulfide NPs) to the absence of significant changes, when aluminum NPs are introduced orally, and their ambiguity under the influence of silver, silicon dioxide, iron NPs and the manifestation of general toxicity and fatty degeneration for NPs CNT and fullerenes. In case of chronic NPs action, an increasing dose-dependent danger of general toxicity with the prolongation of administration duration has been established, which develops against the background of NPs accumulation in the organs of animals.

Effects of nanoparticles in biotic doses

The effects of NPs at these doses can be either negative, for example, gold NPs of size 20 and 30 nm significantly changed the ATPase activity of the membrane fraction and the β-lactamase activity of the cells; and positive — they also stabilized the cell membrane [38, 39]. The latter is used to develop and create a new generation of drugs that will allow for the correction of a number of pathologies [40]. An example of this can be copper-based NPs with low toxicity, prolonged and multifunctional action. When studying the effect of copper NPs introduced into the animals' organism in different concentrations, it has been found that their biotic action area was within the dose range from 0.01 mg/kg to 15 mg/kg, "safety zone" — in the dose range of 15–25 mg/kg, and the zone of pharmacotoxic action began with a dose of 25 mg/kg. Copper and copper oxide NPs in biotic doses with high biological activity had a spherical shape, the average particle diameter was 124 nm and 119 nm, respectively.

Copper NPs, in case of subcutaneous single administration into animals in biotic doses, contributed to an increase in rats' survivability after an experimental myocardial infarction. It was also established that copper and copper oxide NPs had a pronounced antibacterial effect against gram-positive and gram-negative bacteria.

The influence of copper NPs on the body of rats at increasing concentrations has been studied. To do this, NPs were used with the following physicochemical characteristics: the average size of copper NPs, having a spherical shape, was 103.0 ± 2.0 nm; the core of the particles contained 96.0 ± 4.5% of crystalline copper, 4.0 ± 0.4% of copper oxide; the thickness of oxide film on the surface of NPs was 6 nm [41].

In case of copper NPs introduction 1 day after the first injection (at a dose of 2 mg/kg of body weight), a sharp increase 2.93 times of copper content itself in the liver of the animals occurred, which resulted in a change in the concentrations of 25 elements studied on the 1st and the 7th days (iron, calcium, zinc, cadmium, cobalt, tin, iodine, selenium, manganese, lead, arsenic, mercury, magnesium, potassium, sodium, lithium, aluminum, silicon, chromium and vanadium, strontium, boron, nickel, phosphorus, arsenic). The changes showed consistency (with the exception of several elements, for example, zinc) and were built on antagonistic relationships [42], which allowed the authors to conclude that the system of the regulation of trace elements level after single introduction of copper NPs is stimulated.

Further increase in the load (the total dose of 4 mg/kg of animal weight) with NPs on the body also did not disturb the system of homeostatic regulation of microelements level in the tissue, and the antagonistic relationships of trace elements were preserved. After the third injection (the total dose of

6 mg of the NPs/kg of the animal weight, close to the maximal threshold dose — 10 mg/kg), the content of copper in the liver increased by 29% compared to the control, and the antagonistic relationship between the elements was already violated. As a result, there was an increase in the concentration of the following elements in the liver as compared to the control: Fe, Zn, Mg, Na, Co, Al, Li, K, V, I, Se, B, P, As, Sn, Cd; and a decrease of: Ca, Si, Sr, Pb, Cr. The concentrations of elements Ni, Hg did not differ from control. Finally, after 12-fold administration of copper NPs into the body, when the total dose of copper NPs was approaching LD_{100}, an increase in the concentration of all elements in the liver, except iodine and selenium (an element of antioxidant protection), was noted. Thus, the introduction of copper NPs led to a change in microelement status of the liver. In addition, a dose increase close to LD_{100} caused an imbalance of microelements [43].

In other experiments, as a result of the preincubation of Ag and Fe NPs at a concentration of 1 μg/ml with CHO-K1 test culture, it has been established that there is no inhibition of membrane ATPase activity and an increase of cytosol LDHase activity relative to their values for intact cells [44]. In addition, it has been shown that iron nanopowder in concentrations of 2–6 μg/kg of body weight stimulated the growth of animals, bactericidal activity of blood serum and total protein increase in blood [27].

Analyzing the results of studying the safety of metal NPs using genotoxic, mutagenic and biochemical markers under *in vitro* conditions, a composite mixture of Ag, Cu, Fe, and Mn NPs (MeNc) has been created. In the experiment on male rats Wistar ($n = 144$), the biocompatibility and bioavailability of this mixture after a single per os administration at a dose of 3 ml of metals mixture/kg, as well as toxic — 10, 20 and 40 ml/kg of body weight were studied. When pathoanatomical autopsy on the 1st, the 3rd, the 7th and the14th day of the experiment, bloating of the stomach and thick intestine due to influence in the entire dose range was recorded. Exceeding the coefficients of mass of internal organs indicated that the main "targets" of biological influence were the spleen and lungs; with an increase of the composite dose the kidneys, liver and heart were involved under the complex influence of NPs [44].

Resulting data correlated with the dynamics of hematopoietic system parameters in animals injected with nanoparticles at doses of 10, 20 and 40 ml/kg. In the blood of rats, leukocytosis was recorded at all periods of the study (an average up to 23.7%), erythropoiesis suppression (an average up to 38.2%) with a decrease in the total hemoglobin content (an average up to 17.6%); an increase in the level of seromucoids (on average by 32.3%) with respect to control ($P \leq 0.05$). The level of circulating immune complexes (CIC) of the average molecular weight increased an average by 58% in all animals that received metals in both ionic and nanoscale form. Moreover, more pronounced changes in indices were recorded in rats after administration of nanoforms at doses of 10 and 40 ml/kg.

There were also significant changes in the functional state of the liver of rats starting from the1st day of experiment: against the background of urea formation activation and glycolysis inhibition, the detoxification enzyme activity intension was recorded, which was accompanied by inhibition of ALAT activity and hyperfermentation of ASAT, GGT, and ALKP ($P \leq 0.05$). Changes in the indices were more pronounced in case of the introduction of MeNc at a dose of 10 ml/kg for 14 days, which was regarded as selective toxicity of the latter and imbalance in the processes of detoxification in the animals.

The excessive formation of derivatives of proteins' oxidative modification throughout the experiment, and, starting from the 3rd day, the level of lipid peroxidation (LPO) products' decrease with respect to control ($P \leq 0.05$) indicated the violation of cell membranes functional-structural state due to the administration in rats of biotic dose of metals mixture and increased doses of MeNc. At the same time, normalization of these processes was not observed, which was manifested in the activation of catalase and the consumption of endogenous antioxidants pool ($P \leq 0.05$) in the blood of rats.

In other experiments, based on the results of comparative assessment of the biogenic metals toxicity, it has been found that they were less toxic in the form of NPs than their ionic analogs, in particular copper — 7 times, zinc — 30 times and iron — 40 times, which may be due to stabilization of NPs or solubility of the latter [30].

At the same time, a narrow security zone between the biotic and pharmacotoxic effects of copper NPs is worth noting: 0.01–15 mg/kg and more than 25 mg/kg, respectively. Therefore, when correcting the conditions, it is necessary to consider carefully both the choice of doses and the number of injections, as well as the assemblage of different NP combinations.

Delayed effects

When assessing the safety of chemical compounds, including in the nanoscale state, it is necessary to take into account possible long-term effects of the action. According to literature data, spherical silver NPs with an average diameter of 23.2 nm and a content of $1.1 \cdot 10^{11}$ in 1 ml, exerted an embryotoxic effect, as they caused a number of pathological changes in the body of pregnant females. Nevertheless, deviations in fetal development in utero were small and limited to an increase in postimplantation and total intrauterine death. The consequences of prenatal exposure were manifested in offspring after birth in the form of persistent inhibition of weight gain, hematological shifts, and behavioral and metabolic phenotype changes [45]. When administered orally at a dose of 100 mg/kg, titanium nanooxide and its macro analog showed embryotoxic properties. The consequences of prenatal contact in both cases were revealed in offspring in the postnatal stage of ontogenesis. The nature of deviations in live-born individuals had "dimensionally-determined" specificity [46]. Experimental evidences of silver NPs transfer from mother to offspring through the placenta and breast milk have been also obtained [47]. In the experiments to assess the penetration through these barriers, low molecular weight silver NPs with a size of 34.9 ± 14.8 nm stabilized with low molecular weight polyvinylpyrrolidone were used. The average level of NPs accumulation in the fetus was 0.09–0.15% of the administered dose, which was comparable to the accumulation of the label in liver, blood and muscle frame of adult animals, and at least 10-100 times higher than the penetration of NPs through the blood-brain barrier in the brain of females. In lactating females, the total intake of [110mAg]-NPs in milk was not less than 1.9 ± 0.3% of the administered dose for 48 hours of lactation; at least 25% of this amount was absorbed in the digestive tract of young rats.

When assessing the embryotoxic effect of gold NPs with an average diameter of 20.3 nm and a content of $7.7 \cdot 10^{11}$/ml, it has been found in rats that, when administered intragastrically calculated as 1.0 ml per 100 g of body weight, in pregnant females a number of pathological shifts was registered. However, deviations in the development of fetuses in utero were few and limited only to an increase in postimplantation and general intrauterine death without fertility decreasing. The effects of prenatal exposure were similar to the effects of silver [45].

In experiments on zebra fish embryos, it has been established that their exposure to 1.5 nm gold NPs for 5 days caused embryo death in a concentration of 10 ppm, and to 0.8 nm gold NPs — in a concentration of 400 ppb [48]. That is, embryotoxic properties of gold NPs were more pronounced in smaller particles, whereas the pronounced teratogenic effect of gold NPs in a concentration of 50 ppm was independent of their size [49]. When exposed to iron oxide NPs, there was a teratogenic effect and embryotoxicity [29].

When studying the influence of NPs on reproductive function, the object of the study was a sample of a titanium dioxide nanopowder with a particles size of 40 ± 3 nm. Work solutions of titanium nanooxide and macroforms of titanium dioxide were administered intragastrically at a dose of 100 mg/kg/day in an amount of 1.0 ml per 100 g of body weight. Control rats, in the same manner, obtained a solvent (deionized water). At the end of the experiment, the duration of which was 2 months, intra-group mating was conducted and offspring was received. Despite the fact that prolonged contact of parental individuals with nano- or macroform of titanium dioxide did not affect the ability of animals to mate and conceive, it subsequently had its delayed negative effect on the postnatal development of young rats in both experimental groups. Titanium dioxide in the nanoform caused additional impairments in the offspring, which are not characteristic for the macroanalogue, in the form of the rate of puberty in females and males inhibition, a decrease in the strength of the muscle grip and hematological shifts [50].

The effect of 5 nm gold NPs and 20 nm silver NPs on spermatogenesis has been studied [51, 52]. Solutions of gold NPs at a dose of 0.57 mg/kg/day and silver NPs at a dose of 0.28 mg/kg/day were administered into rats per os for 8 weeks. As a result of the conducted studies, it has been established that gold and silver NPs penetrated through the hemato-testicular barrier and accumulated in the interstitial tissue between the seminiferous tubules. At the same time, there was no negative effect of gold and silver NPs on the proliferative function of the generative epithelium. It has been concluded that the introduction of gold and silver NPs for 8 weeks at these concentrations and in this experiment did not cause changes in the properties of the generative epithelium of the seminiferous tubules.

There are also other data on the absence of nanosilver direct influence on the reproductive function [47].

There are well-founded evidences of the carcinogenicity of SiO_2 crystalline form, but amorphous NPs are much less studied and their results are often ambiguous and contradictory. It is believed that a serious prognostic sign of a tumor transformation can be a cytotoxic effect that causes an increased frequency of polynuclear cells, which can be confirmed by reports of the potential carcinogenicity of SiO_2 NPs [53, 54].

Cytotoxic and DNA-damaging effect

Considerable attention has been paid to evaluation of the cyto- and DNA-damaging effects of NPs [55]. However, the data on these effects of NPs are ambiguous. For example, in the reactions of chorioallantoic membrane vessels (CMV), the DNA damaging effect of aluminum oxide, silicon dioxide, titanium dioxide NPs, quantum dots, dendrimers, fullerenes, nanofibres [44] has been detected. At the same time, under the influence of iron oxide NPs, no DNA-damaging effect has been detected [29]. It has been found that SiO_2 NPs at concentrations of 100 and 200 μg/ml did not induce cyto- and DNA-damaging effects in human peripheral blood lymphocytes culture, since the proliferative activity of cells, the frequency of pycnoses, apoptosis, and the number of micronuclei did not differ from the control ones [54].

In another study, it has been shown that the addition of SiO_2 NPs to the culture of lymphocytes at concentrations of 200 and 100 μg/ml did not affect the mitotic activity of the cells, the number of pycnotic and apoptotic nuclei. At the same time, in case of the action of NPs at a concentration of 200 μg/ml, a statistically significant ($P < 0.05$; $P < 0.01$; $P < 0.001$) increase in the number of two-, three- and four-nucleated cells relative to the control value was observed. In general, it has been concluded that the NPs studied at concentrations of 100 μg/ml and 200 μg/ml had cyto- and DNA-damaging/mutagenic effects [56]. According to other works, silicon dioxide NPs (70 nm in size) caused more pronounced cytotoxic effect in comparison with micro-sized analogues, penetrated into the cytoplasm and nucleus of cells, interacted with DNA, suppressed replication, transcription and proliferation [57].

When studying the cytotoxicity of silicon dioxide in the form of nanowire and NPs in vitro on two lines of human epithelial cells, a concentration of 190 μg/ml was threshold, below which no toxic effects were observed. Higher concentrations caused destruction of the membrane (cytosol LDH is marker) and cell necrosis. Using cell culture of human bronchoalveolar carcinoma, a dose-dependent cytotoxic effect and an oxidative stress of 15 and 46 nm silicon dioxide NPs in doses of 10 and 100 μg/ml were detected for 48 hours [58]. It has been found that SiO_2 NPs (100 nm) are not cytotoxic at a concentration of up to 30 μg/ml in water, however, with increasing content, cytotoxicity increases significantly.

According to the literature, 20, 30 and 45 nm sized gold NPs with single intravenous administration into rats did not cause DNA-damaging effect in liver, kidneys, intestines and bone marrow cells, but the smallest (of 20 nm) particles damaged DNA in spleen cells [59]. Particles of Au metal with a diameter of about 15 nm were not toxic to the animals even at high concentrations. At the same time, NPs with a diameter of 1.2 nm caused cell death within 12 hours. Genetoxic effects of nano-metals are due to the ability of particles, in particular copper, gold, to penetrate into the nuclear apparatus of the cell, to bind to the guanine residues of DNA molecules and to disrupt its structure [60, 61]. The genotoxic effects of nano-gold can be explained by the ability to specifically integrate into the DNA structure, but this effect was observed only when 1.4 nm particles were used [62]. Thus, the smaller gold NPs, the more dangerous their impact on the body. In assessing the gold NPs damaging effects, it has been established [63] that the type and modifying method of their surface affects the development of toxicity in laboratory conditions, in particular the functional activity of macrophages.

It has been also found that quantum dots caused pronounced inflammatory changes and DNA damage in bronchoalveolar lavage, possibly due to direct cadmium exposure [26].

When studying the genotoxicity of metal NPs of hexacyanoferrate Co and Zn in concentrations of 0.16–830.0 and 0.25–802.0 μg/ml, respectively, on the culture of CHO-K1 cells, it has been shown that they disrupted the DNA structure. The values of I_{DNA} were within the limits of those for the cells treated with the test mutagen, and indicated the manifestation of the DNA-damaging effect of these NPs [44]. Samples of other NPs (Ag, Fe, Cu, and Mn) in similar concentrations were safe because of genotoxic effects absence (the I_{DNA} values were significantly close to those for intact cells). Although the concentrations used differed thousands-fold, the dosage or other concentration dependence was not indicated, so, in all likelihood, it has not been detected.

The obtained data of the genotoxic effect of hexacyanoferrate Co and Zn metal NPs correlated with the results of the determination of NPs mutagenic properties. In the model of Allium cepa apical meristem, it has been found that Co and Zn NPs significantly inhibited the activity of mitotic processes: the level of mitotic index decreased 2.9 and 4.6 times, and the number of aberrant cells exceeded the control level 5 and 10 times, which was the basis for asserting the presence of pronounced mutagenic properties of these metals NPs.

In our studies, the assessment of NPs cytotoxicity has been performed in experiments in vitro on bovine spermatozoids using the AT-05 analyzer [64, 65]. The toxicity criterion was the toxicity index (I_t) value, equal to the ratio of indicator cells mobility in the test sample to their mobility in the control. The aim of the work was to evaluate the effect of various stabilizers (mercaptopropionic acid — MPA, sodium polyphosphate — NaPP, polyethylenimine — PEI, gelatin) on the cytotoxicity of lead and cadmium sulfides NPs. The size-effect dependence of cytotoxicity of cadmium sulfide NPs (1.8–2.0 nm, 4.0–6.0 nm, 15.0–20.0 nm) in various stabilizers has not been revealed. The native solutions of cadmium sulfonamide NPs of 4.0–6.0 nm in size, stabilized with MPA, and of 15.0–20.0 nm in size, stabilized with NaPP, had a cytotoxic effect. The most toxic were the cadmium sulfide NPs (15–20 nm) in PEI, since the cytotoxic effect was observed when they were diluted.

It has been found also that 10.0–15.0 nm lead sulfide NPs in 1% gelatin showed no cytotoxic effect, while at the same time, 40.0–50.0 nm lead sulfide NPs in 0.1% gelatin had a cytotoxic effect. Thus, the data obtained indicated a complex dependence of biological effects of NMs, due to both the chemical nature of the latter and to their surroundings [66].

Specific features that allow suggesting the cytotoxic and DNA-damaging effects of NMs can be high penetrating ability at the level of the body, organs, tissues and cells; generation of free radicals, including active forms of oxygen and nitrogen; cytoskeleton damage; the ability of some NMs to overcome the cariolema and be located in the nucleus of the cell; conjugation with DNA [44,67].

Thus, despite the growing number of experiments in the field of nanotoxicology [5, 68–70], there is still no single approach to assessing the toxicity and dangers of NPs and NMs. Based on that, the effect of the latter on living organisms and biological systems depends on their physicochemical properties, size, shape, surface characteristics, objects of research and experimental conditions, the effects of their damaging effects are not always linear in nature [70, 71], and even contradictory, unreliable and unregulated. Their determinism [72] is not expressed, and the consequences of their release into the environment can be completely unexpected [73, 74]. It has been concluded that these factors make it difficult to analyze and compare the results obtained. It is believed that the use of ultra-high doses of NPs in vitro on cell cultures to determine toxicity is difficult to extrapolate to human data. An open problem of nanotoxicology can also be a large number of studies using NPs different in size, shape and composition. In many works, it is noted that at the current stage of the development of knowledge about NMs and NPs, it is impossible to systematize the links between the obtained properties of NPs and the effects they cause.

It is problematic to obtain NPs the same exactly in size and maintain this size and properties when ingested. Upon the application of condensation methods for NPs obtaining (in the reactions of reduction, substitution, oxidation, hydrolysis), the concentrations of the latter are insignificant: from μg/ml to mg/ml. Naturally, under these conditions, the values of the average lethal doses are not determined, most likely due to the limited possible volume of administration to the animals; and with repeated inflows into the body, the damaging effects manifest themselves in the subsequent periods of the experiments, rather than after several first injections. This may be the reason for a longer stay of NPs in the body and the possibility of getting them into cellular organelles, including the nucleus.

Thus, the effects of nanoobjects toxic action on living organisms are more complex and diverse, and perhaps even more dangerous than those of macroobjects. In general, they cannot be single-factorial and can be conditionally divided into organo-cyto-systemic, dose-size-structurally-time, delayed. Therefore, an assessment of the manifestation of nanoparticles and nanomaterials toxicity and hazards requires the development of special procedures and approaches as compared with those for macro forms of chemicals.

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ТОКСИЧНА ДІЯ НАНОЧАСТИНОК

Н. С. Леоненко, О. Б. Леоненко

ДУ «Інститут медицини праці НАМН України», Київ

E-mail: taliya@meta.ua

В огляді узагальнено дані літератури, що стосуються оцінки токсичних ефектів нанорозмірних частинок за різних концентрацій і шляхів надходження до організму. Токсична дія нанооб'єктів є більш складною і різноманітною порівняно з такою традиційних токсикантів (важкі метали, органічні розчинники, отруйні речовини тощо). Незважаючи на зростаючу кількість даних, єдиних підходів до вивчення токсичних ефектів нанорозмірних частинок не існує, що вимагає розроблення специфічних процедур оцінювання їхньої токсичності.

Ключові слова: наночастинки, методи оцінки токсичної дії.

ТОКСИЧЕСКОЕ ДЕЙСТВИЕ НАНОЧАСТИЦ

Н. С. Леоненко, О. Б. Леоненко

ДУ «Институт медицины труда НАМН Украины», Киев

E-mail: taliya@meta.ua

В обзоре обобщены данные литературы, касающиеся оценки токсических эффектов наноразмерных частиц при различных концентрациях и путях поступления в организм. Токсическое действие нанообъектов более сложное и разнообразное по сравнению с таковым традиционных токсикантов (тяжелые металлы, органические растворители, отравляющие вещества и т. д.). Несмотря на растущее количество данных, единых подходов к изучению токсических эффектов наноразмерных частиц не существует, что требует разработки специфических процедур оценки их токсичности.

Ключевые слова: наночастицы, методы оценки токсического действия.