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Evaluation of Influence of Metal Nanoparticles And Their Oxides on Elemental Composition of Organs in Laboratory Animals And Their Bioaccumulative Potential

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ABSTRACT

BACKGROUND: Active development of nanotechnology and the use of research in many industries, including agriculture and medicine, require a comprehensive study of the influence of ultra dispersed substances on humans and animals. Today, we have limited evidence of the influence of nanoparticles on the microelement levels in organs and tissues. However, given the growing production and release of nanoparticles into the environment in processes, it is required to consider both the direct and indirect effects of particles of various chemical origin.

AIM: To evaluate the influence of copper, cobalt, and copper oxide nanoparticles on behavior and microelement levels in the liver, kidneys, and reproductive system in laboratory animals and to study their bioaccumulative potential upon intragastric administration.

METHODS: The experiment was conducted on male ICR mice divided into four variable groups of 6 subjects each, who were administered distilled water (control group) or 0.02 mg/kg suspensions of copper, cobalt, and copper oxide nanoparticles intragastrically for 20 days, once a day. We assessed the changes in body weight and anxiety in animals (the number of upright postures with and without support and the number of short-term grooming). At the end of the experiment, the animals were euthanized to sample the liver, kidneys, and reproductive organs and to determine the microelement levels using energy dispersive X-ray fluorescence.

RESULTS: After administration of all tested nanoparticles, the animals showed signs of anxiety, including an increased number of upright postures with support (the cobalt nanoparticle group) and a decreased number of upright postures without support accompanied by increased number of grooming acts (the copper and copper oxide nanoparticle groups). Animals of the same groups (copper and copper oxide) showed a decrease in body weight compared to the control group. An analysis of the microelement level in the liver, kidneys, and reproductive system revealed ambiguous changes in potassium, calcium, and sulfur levels and increased oxygen content in the testes and appendages. We detected no signs of bioaccumulation of copper, copper oxide, and cobalt nanoparticles in the studied organs. Thus, nanoparticles have indirect toxicity, which is manifested by changes in the microelement levels in organs and is characterized by the rapid elimination of nanoparticles.

CONCLUSION: Copper, cobalt, and copper oxide nanoparticles have a multidirectional indirect effect on the physiology and behavior of animals realized by changes in the microelement levels in their organs. We detected no accumulation of copper, cobalt, or copper oxide nanoparticles in the studied organs.

Keywords: nanoparticles; cobalt; copper; copper oxide; bioaccumulation; liver; kidneys; reproductive system; essential elements.

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Оценка влияния наночастиц металлов и их оксидов на элементный состав органов лабораторных животных и их способность к накоплению

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АННОТАЦИЯ

Обоснование. Интенсивное развитие нанотехнологий, использование результатов исследований во многих отраслях промышленности, в том числе сельском хозяйстве и медицине, требует всестороннего изучения воздействия веществ в ультрадисперсном состоянии на человека и животных. В настоящее время сведения о влиянии наночастиц на микроэлементный состав органов и тканей ограничены. Между тем с учётом растущего производства и выброса наночастиц в окружающую среду в ходе технологических процессов необходимо учитывать как прямое, так и опосредованное воздействие частиц различной химической природы.

Цель. Оценить влияние наночастиц меди (Cu), кобальта (Co) и оксида меди (CuO) на поведенческие реакции и микроэлементный состав печени, почек и репродуктивной системы лабораторных животных, а также исследовать их способность к накоплению при внутрижелудочном введении.

Материалы и методы. Эксперимент проведён на самцах мышей линии ICR, разделённых на четыре вариативных группы по 6 особей в каждой, которым вводили внутрижелудочно дистиллированную воду (контроль) или суспензии наночастиц Cu, Co и CuO в течение 20 дней один раз в день в дозах 0,02 мг/кг. Оценивали динамику массы тела, а также уровень тревожности животных (количество вертикальных стоек с опорой и без опоры и количество актов кратковременного груминга). По завершении эксперимента проводили эвтаназию, забор печени, почек и репродуктивных органов, в которых определяли микроэлементный состав методом энергодисперсионного рентгенофлуоресцентного анализа.

Результаты. Введение всех протестированных наночастиц вызывало у животных проявление признаков тревожности: наблюдалось увеличение количества стоек с опорой (группа, получавшая наночастицы Co) и снижение числа стоек без опоры, сопровождавшееся увеличением актов кратковременного груминга (группы животных, получавших наночастицы Cu и CuO). В этих же группах (Cu, CuO) наблюдалось снижение массы тела животных по сравнению с контрольной группой. Анализ уровня микроэлементов в печени, почках и репродуктивных органах выявил неоднозначные изменения концентрации калия, кальция и серы, увеличение содержания кислорода в семенниках с придатками. Признаков накопления наночастиц Cu, CuO и Co в исследуемых органах не выявлено. Таким образом, токсичность наночастиц реализуется опосредованно, через изменение микроэлементного состава органов, и характеризуется быстрой элиминацией наночастиц.

Заключение. Наночастицы меди, кобальта и оксида меди оказывают разнонаправленное влияние на физиологические показатели и поведение животных, реализуемое опосредованно, через изменение элементного состава их органов. Накопления наночастиц меди, кобальта, оксида меди в исследуемых органах не обнаружено.

Ключевые слова: наночастицы; кобальт; медь; оксид меди; накопление; печень; почки; репродуктивная система; эссенциальные элементы.

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金属及其氧化物纳米颗粒对实验动物器官元素组成及其蓄积能力的影响评估

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摘要

论证。纳米技术的快速发展，以及其研究成果在包括农业和医学在内的多个工业领域中的广泛应用，促使人们亟需全面研究超微颗粒物对人类和动物的影响。目前，关于纳米颗粒对器官和组织微量元素组成的影响资料仍较有限。与此同时，考虑到在技术过程中纳米颗粒产量和排放量不断增长，其进入环境后所产生的不同化学性质颗粒的直接和间接作用都应予以重视。

目的。评估铜（Cu）、钴（Co）和氧化铜（CuO）纳米颗粒在胃内给予实验动物后，对其行为反应及肝脏、肾脏和生殖系统中微量元素组成的影响，并研究其在相关器官中的蓄积能力。

材料与方法。实验在ICR系雄性小鼠中进行，将其分为四个变异组，每组6只。各组小鼠每日灌胃一次，连续20天，分别给予蒸馏水（对照组）或含有Cu、Co和CuO纳米颗粒的悬浮液，剂量均为0.02 mg/kg。评估小鼠体重变化动态及焦虑水平（包括有支撑和无支撑直立次数及短时梳理行为发生次数）。实验结束后处死动物，采集肝脏、肾脏及生殖器官，采用能量色散X射线荧光分析法测定其微量元素组成。

结果。所有测试的纳米颗粒均引发动物出现焦虑行为表现：观察到有支撑直立次数增加（接受Co纳米颗粒组），无支撑直立次数减少并伴随梳理行为次数增加（接受Cu和CuO纳米颗粒组）。在同一组别（Cu、CuO）中，观察到小鼠体重较对照组有所下降。对肝脏、肾脏和生殖器官中微量元素水平的分析显示：钾、钙和硫的浓度发生多方向变化，附睾睾丸中氧元素含量升高。未在所检测器官中发现Cu、CuO及Co纳米颗粒的蓄积迹象。由此可见，纳米颗粒的毒性是通过改变器官的微量元素组成而间接实现的，其特点是可被迅速清除。

结论。铜、钴和氧化铜纳米颗粒对实验动物的生理指标和行为表现具有多方向影响，其作用机制为通过改变器官的元素组成间接实现。在所研究的器官中未发现铜、钴及氧化铜纳米颗粒的蓄积。

关键词：纳米颗粒；钴；铜；氧化铜；蓄积；肝脏；肾脏；生殖系统；必需元素。

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BACKGROUND

Nanoparticles (NPs) of various chemical origin are both produced in industrial processes and formed naturally [1]. A variety of substances, including those in nanodispersed substances, enter the human body from the environment. In addition to biological activity, promotion of growth and development, protection against diseases, and delivery of medicines to cells, NPs produced in certain processes and manufactured for medical and agricultural applications pose risks to plant and animal organisms as they can cause oxidative stress and cell dysfunction [2, 3].

Ultra dispersed metals and their oxides have fundamentally different properties compared to the same substances in a macrostate or in the form of ions [4]. NPs are capable of overcoming biological barriers; therefore, they are used in medicine for treatment and diagnostic purposes [5]. However, some studies revealed their ability to cause oxidative stress and disrupt microelemental balance, as well as their cytotoxic effect [6]. Nevertheless, there are reports of rapid elimination of NPs without significant bioaccumulation and pronounced toxic effects [7].

The dialectic of this issue requires a comprehensive study of the toxicological safety of NPs affecting humans, animals, and plants [8, 9]. Industries dealing with nanomaterials should have a realistic understanding of toxicologic risks of a well-known material in a new, ultra dispersed form [10]. Both *in vitro* and *in vivo* studies are needed to verify an acceptable risk profile.

In a series of previous experiments, it was shown that cobalt, copper, iron NPs; and copper, cobalt, and zinc oxides NPs promote plant growth [11]. The studies demonstrated the manifestation of a low-dose mechanism and the relationship between the toxicity or promoting effect and the particle size and concentrations [12]. In most cases, NPs of 35–80 nm in size promoted germination and germinative power and activated inorganic nutrition, which was confirmed by changes in the microelement levels in plants. It was found that NPs of 35–60 nm in size could influence the transmembrane potential of cells and activated enzymes and plant hormones by changing the pH of solutions used for dipping before planting [13]. By activating enzyme synthesis, such particles promoted carbohydrate and nitrogen metabolism, but their bioaccumulation in plants was not recorded. Still, ultra dispersed metal oxides often suppressed plant growth, inhibited their development, and showed a bioaccumulative trend [12].

As such, NPs are used to treat plants, it is highly probable that they enter the body of animals, necessitating an evaluation of their effect on organs and metabolism. Our previous studies on rats and mice investigated the acute and chronic toxicity of copper and cobalt NPs, and copper and zinc oxides NPs. They showed that the optimum dose was 0.02 mg/kg as it did not cause pronounced signs of metabolic and morphologic toxicity in intragastric administration [14, 15].

The liver, kidneys, and reproductive system are generally considered as target organs when studying the toxicity of new compounds due to their key role in detoxification, filtration, and reproduction [16, 17]. In addition, nanodispersed metals both cause a cascade response themselves and may have an indirect effect by changing the elemental composition of organs and tissues; there is insufficient data on such studies in the literature [18].

AIM: To study the influence of copper, cobalt, and copper oxide NPs on the behavior of laboratory animals; changes in body weight; microelement levels in the liver, kidneys, and reproductive system in chronic intragastric administration and to evaluate the bioaccumulative potential of NPs.

METHODS

The study used copper and cobalt NPs of 20–50 nm in size, and copper oxide NPs of 40–60 nm in size. All nanomaterials were synthesized at the National University of Science and Technology MISIS using the electroless metallization of the corresponding hydroxides in a hydrogen stream. The particle surface area was determined by BET adsorption using a Quantachrome NOVA 1200e analyzer (Japan). X-ray phase analysis using a Shimadzu XRD-7000 diffractometer (Japan) was used to verify the ball shape of the particles.

Six weeks old male ICR laboratory mice (n = 24) weighing 18–22 g (Federal State Budgetary Scientific Institution Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency, Moscow Region) were used as subjects. The animals were kept under standard vivarium conditions (temperature: 22 ± 2 °C, relative humidity: 50–60%; light conditions: 12/12 hrs) with access to water and food *ad libitum*.

The experiment was conducted in the vivarium of the Federal State Budgetary Educational Institution of Higher Education Ryazan State Medical University of the Ministry of Health of the Russian Federation in accordance with international regulations (Directive 86/609/EEC) and good laboratory practice (Order No. 199n of the Ministry of Health of the Russian Federation dated April 1, 2016). The study protocol was reviewed and approved by the meeting of the Commission for Control over Laboratory Animal Care and Use (No. 22 dated January 23, 2020). During the experimental period, the mice received complete granulated feed with balanced elemental, energy, vitamin, and mineral content manufactured under GOST R 50258-92.

Mice were randomized into four groups of six subjects. Animals in group 1 (control group) received distilled water intragastrically; group 2 received suspended copper NPs; group 3 received cobalt NPs, and group 4 received copper oxide NPs. 0.002 mg/mL NPs were suspended in distilled water by dispersing nanopowders in an ultrasonic bath (Grad 13–35, STC Soltek, Moscow) with a power of 150 W and a frequency of 35 kHz for 15 min. 10 mL/kg of suspended NPs were administered daily in the morning, before feeding, for 20 days, according to Guidelines (MU) 1.2.2869-11 (Moscow, 2011).

The animals' body weight was measured using OHAUS laboratory scales (resolution 0.01 g) before the experiment and at day 6, 11, 16, and 21. Visual examination was performed daily. Motor activity was assessed every three days by counting upright postures (with and without support) for 2 min in a plexiglass arena (diameter: 135 mm, height: 350 mm). In addition, we recorded the number of grooming acts lasting up to 5 s [19].

At day 21, animals were euthanized by decapitation after inhalation anesthesia with isoflurane. The liver, kidneys, and reproductive system (testicles and appendages) were sampled in accordance with MU 1.2.2745-10. The organs were dried in a cabinet dryer for 72 h at 75 °C and ground in porcelain mortars with porcelain pestles.

The elemental composition of the tissues was determined by energy dispersive X-ray fluorescence using an Arl QuantX spectrometer (Switzerland) at the Regional Center for Scanning Probe Microscopy of the Federal State Budgetary Educational Institution of Higher Education Ryazan State Radio Engineering University named after V.F. Utkin. The test was performed using UniQuant software with automatic spectral fitting. In addition, we performed spot analysis of samples using a JEOL JSM-6610LV scanning electron microscope with an INCA X-MAX system (identification of all elements except hydrogen, helium, and lithium).

Statistical data processing was performed using the Statistica 12.0 software. Normality of distribution was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The data were processed after variance analysis (normal distribution) or the Kruskal–Wallis test (anomalous distribution). The corresponding parameters recorded in each experimental group on a specific day were compared with the control group on the same day (Dunnett's test for body weight and behavior of animals in normal distribution; Mann–Whitney test with Bonferroni correction for the essential microelement level in abnormal distribution). Data are presented as the arithmetic mean and standard error of the mean (normal distribution) or the median and lower/upper quartiles (anomalous distribution). Differences were considered significant at $p < 0.05$ for normal distributions and $p < 0.017$ for anomalous distributions.

RESULTS

Body Weight

The average baseline body weight of mice in all groups was 19.07±0.10 g and did not differ between groups (p=0.987). As early as at day 4 of the experiment, the group of animals given copper oxide NPs showed low appetite, which was recorded by an increased amount of food left in the feeders. At day 7, the mice that were given nanodispersed copper showed low appetite as well. Weighing of animals at day 6, 11, 16, and 21 recorded weight loss in the groups receiving copper and copper oxide suspensions. The weight in the copper NPs group of animals decreased by 38.6% (the average weight difference was 12.27±1.21 g; p <0.001) and by 43.9% (the difference of 13.97±1.46 g with the control group; p <0.001) in the copper oxide NPs group compared to the control group at the last day of the experiment. The experimental group subjects that were administered nanodispersed cobalt consumed all food and showed weight gain that did not differ significantly from the control group (p=0.492; see Fig. 1).

Motor Activity

The upright motor activity of experimental animals was determined by counting the number of times they stood on their hind legs without support and with support on the side in a special setup at day 3, 6, 9, 12, and 18. Starting from day 7 of the experiment, we observed a reliable decrease in the number of unsupported upright postures by 21.4% (day 9, p=0.017), 38.7% (day 12, p=0.037), and 43.8% (day 18, p=0.002) in the copper NPs group; and by 28.1% (day 9, p=0.055), 32.25% (day 12, p=0.011), and 40.6% (day 18, p=0.002) in the copper oxide NPs group. The number of short-term (less than 5 s) grooming acts increased, indicating a lower cognitive activity and anxious behavior. The supported upright postures on the side in these groups did not differ significantly from the control group (p=0.567). In the cobalt NPs group, starting from day 9, the animals tended to increase the average number of supported upright postures by 1.53 times (41.93–53.57%; p=0.013) compared to the control group. This trend persisted until the end of the experiment. However, the number of grooming acts and unsupported upright postures did not differ from the control group (p=0.352).

Elemental Composition of Biological Material

Measurements of copper (p=0.288), copper oxide (p=0.323), and cobalt (p=0.596) levels in the liver, kidneys, and reproductive system did not reveal significant differences between the experimental and control groups, indicating the absence of bioaccumulation of the studied NPs. The analysis of other elements showed reliable differences of potassium and calcium levels in all studied organs, sulfur levels in the liver and reproductive system, and oxygen in the reproductive system.

Potassium level in the liver of animals was reliably higher in all experimental groups compared to the control group: by 111.8% (p=0.004) in the copper NPs group, by 159.8% (p=0.001) in the cobalt NPs group, and by 208.1% (p=0.002) in the copper oxide NPs group. Calcium levels in the cobalt NPs and copper oxide NPs groups were higher by 86.2% (p=0.004) and 89.4% (p=0.004), respectively, compared to the control group (see Table 1).

In the kidneys, there was a significant increase in elemental sulfur (copper oxide NPs) by 34.4% (p <0.001) and

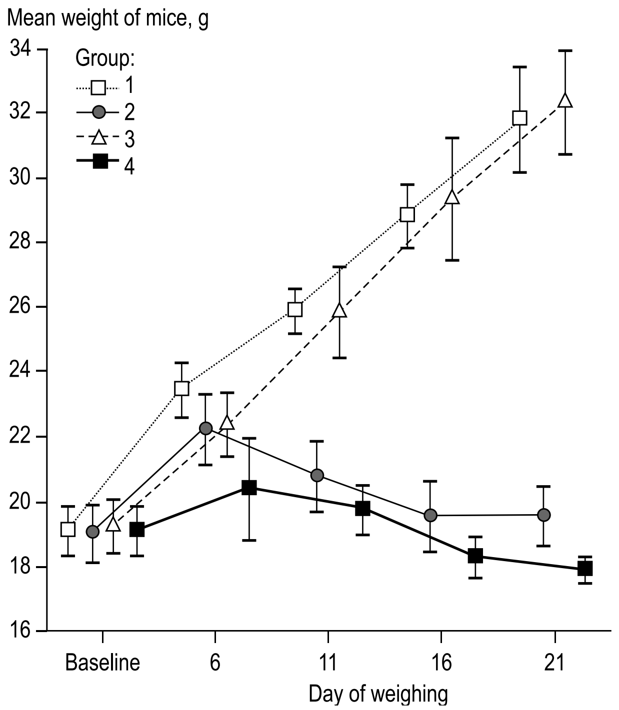

Fig. 1. Changes in body weight of mice (g) with intragastric administration of nanoparticles (NPs) for 21 days (mean value ± standard error of the mean). * Significant differences in relation to the control group (each experimental group was compared with the control group on a specific day of observation using the Dunnett's test); $p < 0.01$.

calcium (cobalt NPs) by 30.8% (p=0.001) compared to the control group; in the copper oxide NPs group, the calcium level decreased by 37.83% (p=0.001). In the nanodispersed copper and cobalt groups, the potassium level was lower (by 66.1% and 42.6% [p=0.001], respectively) (see Table 2).

We observed a significant decrease in the sulfur level in the testes of animals in the cobalt NPs group (by 83.7% [$p < 0.001$]) and the copper oxide NPs group (by 54.3% [$p < 0.001$]) compared to the control group. However, the calcium level increased in all experimental groups: by 52.4% (copper NPs; p=0.005), by 44.4% (cobalt NPs; p=0.004), and by 107.9% (copper oxide NPs; $p < 0.001$; see Table 3).

An increased level of elemental oxygen was found in the reproductive system of animals in all experimental groups compared to the control group. Significant differences were observed in two groups; an increase of 50.5% (cobalt NPs, p=0.001) and 30.9% (copper oxide NPs, p=0.014), which may indicate a specific effect of such NPs on these tissues (see Table 3). Levels of other elements did not differ significantly between the groups (p=0.392).

DISCUSSION

Copper and cobalt are important for cell function. For example, cytochrome c oxidase, consisting of several subunits with a copper atom, is an element of the electron transport chain, a universal catabolic pathway.

Cobalt is primarily contained in cyanocobalamin molecules; it is present in the active centers of important enzymes, such as methyltransferase and ribonucleoside triphosphate reductase; it is a coenzyme of some proteolytic enzymes, and influences heme metabolism [20].

According to the literature, when administered intragastrically, the tested NPs may be absorbed enterally, primarily by clathrin-dependent endocytosis [10, 21]. Subsequently, they enter the bloodstream and are delivered to organs and tissues, where they can accumulate [22, 23]. Mononuclear phagocytes, especially macrophages, are largely responsible for the elimination of NPs from the body [24, 25].

Macro- and microelements, including calcium, potassium, and sulfur, are markers of toxicity [26]. According to the literature, copper and cobalt NPs can increase the level of essential elements in tissues, such as calcium, magnesium, iron, manganese, zinc, potassium, and phosphorus [27, 28]. As laboratory animals received identical feed and water

Table 1. Measurements of the levels of elements in weight samples of liver tissue, %

Group	Potassium	Calcium
Group 1 (control group)	0.259 (0.247; 0.262)	0.094 (0.077; 0.111)
Group 2 (copper NPs)	0.546 (0.532; 0.574)*	0.132 (0.114; 0.152)
Group 3 (cobalt NPs)	0.673 (0.648; 0.709)*	0.175 (0.142; 0.183)*
Group 4 (copper oxide NPs)	0.798 (0.751; 0.836)*	0.178 (0.157; 0.185)*

Note. * Significant differences in comparison with the control ($p < 0.01$).

Table 2. Measurements of the levels of elements in weight samples of kidney tissue. %

Group	Sulfur	Potassium	Calcium
Group 1 (control group)	0.442 (0.411; 0.458)	0.183 (0.177; 0.191)	0.185 (0.169; 0.193)
Group 2 (copper NPs)	0.407 (0.387; 0.442)	0.062 (0.059; 0.071)*	0.122 (0.119; 0.137)
Group 3 (cobalt NPs)	0.518 (0.470; 0.528)	0.105 (0.103; 0.112)*	0.242 (0.238; 0.263)*
Group 4 (copper oxide NPs)	0.594 (0.575; 0.613)*	0.228 (0.221; 0.236)	0.115 (0.091; 0.120)*

Note. * Significant differences in comparison with the control ($p < 0.01$).

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Table 3. Measurements of the levels of elements in weight samples of reproductive tissue (testes and appendages). %

Group	Sulfur	Potassium	Calcium	Кислород
Group 1 (control group)	0.527 (0.518; 0.536)	0.229 (0.221; 0.236)	0.126 (0.112; 0.141)	15.18 (14.70; 15.71)
Group 2 (copper NPs)	0.648 (0.629; 0.707)	0.503 (0.483; 0.531)*	0.192 (0.172; 0.214)*	22.48 (14.01; 25.64)
Group 3 (cobalt NPs)	0.086 (0.079; 0.103)*	0.287 (0.265; 0.302)	0.182 (0.171; 0.194)*	22.85 (20.77; 23.87)*
Group 4 (copper oxide NPs)	0.241 (0.233; 0.271)*	0.271 (0.261; 0.277)	0.262 (0.251; 0.277)*	19.87 (19.12; 20.14)*

Note. * Significant differences in comparison with the control (p <0.01).

throughout the experiment, changes in the levels of elements in organs may be associated with changes in their excretion by the kidneys and redistribution between different organs under the influence of the administered NPs.

Increased potassium and calcium levels in the liver and reproductive system may indicate nephrotoxicity of NPs [29, 30]. Calcium is a universal regulator of humoral response [31]. There is evidence that exposure to NPs causes oxidative stress, depleting antioxidant ability and disrupting calcium homeostasis [32]. Possible mechanisms include extracellular calcium influx due to lipid peroxidation-induced membrane damage and calcium channel blockade [33]. Increased calcium levels may also be associated with its mobilization from bone tissue due to the direct effect of NPs on osteoclasts and osteoblasts or indirect effect involving parathyroid hormones.

Lower sulfur levels, especially in the reproductive system, may be associated with a disrupted antioxidant protection and protein metabolism. Increased kidney sulfur (copper oxide NPs) can be interpreted as an adaption—an attempt to compensate for oxidative stress by increasing the production of antioxidant sulfur compounds, especially glutathione [34]. However, its lower levels in the reproductive system (cobalt NPs and copper oxide NPs) indicates a possible coenzyme deficiency and depletion of protective systems [35].

Increased oxygen levels in the reproductive system of animals in all experimental groups may be associated with increased lipid peroxidation, which is a typical effect of metal NPs [36]. This is confirmed by lower sulfur levels in the system and indicates the development of uncompensated oxidative stress. This effect may indicate both higher sensitivity of the reproductive system to NP exposure and a possible increase in blood flow, promoting local oxygen saturation of tissues.

Increased anxiety in animals observed at administration of NPs may be associated with the nephrotoxicity described above and, similar to the NPs described above, the penetration of blood-brain barrier by the particles and their central nervous system toxicity [37].

The normal weight gain in animals receiving cobalt NPs and its decrease in subjects receiving copper NPs and copper oxide NPs may be associated with the latter's gastrotoxicity [38, 39]. The absence of bioaccumulated copper and cobalt in organs can be explained by their rapid elimination, mainly by the intestines [40]. Excretion of NPs by the kidneys is unlikely due to the damaging effect on these organs.

Thus, at a dose of 0.02 mg/kg, all studied NPs show signs of toxicity, especially reproductive, which is consistent with the literature [41, 42]. Therefore, when using NPs in agriculture, e.g. for seed treatment, it is required to consider the possible risks of their subsequent entry into the body of animals and humans. It is advisable to evaluate the doses, frequency, and duration of exposure to NPs and the vegetative season, when they should be used. It is worth noting that the prooxidant effect of NPs can be reduced by improving their physicochemical properties, including size, shape, content, and pH of solutions.

CONCLUSION

Copper, cobalt and copper oxide NPs are toxic in case of continuous intragastric intake. Its toxicity is manifested by nephrotoxicity and activation of lipid peroxidation.

The absence of significant bioaccumulation of copper, copper oxide, and cobalt in tissues is consistent with the hypothesis of the predominantly transient effect of the studied NPs and their possible rapid elimination from the body. Our study emphasizes the need for further research into the mechanisms of NP exposure on organs and systems, especially in long-term exposure.

ADDITIONAL INFORMATION

Author contributions: Inna V. Obidina: investigation, data curation, formal analysis, writing—original draft; Gennady I. Churilov: conceptualization; Yulia N. Ivanycheva: methodology; Elizaveta M. Pronina and Tamriko I. Matua: investigation; Ivan V. Chernykh: formal analysis, writing—review & editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors made substantial contributions to the conceptualization, investigation, and manuscript preparation, and reviewed and approved the final version prior to publication).

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ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

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Этическая экспертиза. Протокол исследования рассмотрен и утверждён на заседании комиссии по контролю за содержанием и использованием лабораторных животных ФГБОУ ВО РязГМУ Минздрава России № 22 от 23.01.2020.

Источники финансирования. Отсутствуют.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).

Доступ к данным. Редакционная политика в отношении совместного использования данных к настоящей работе не применима, новые данные не собирали и не создавали.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внешних рецензента, член редакционной коллегии и научный редактор издания.

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