Part 2. Biosafety

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TOXICOKINETICS OF ZINC IN RATS AFTER A SINGLE ORAL ADMINISTRATION OF ZINC CARBONATE NANOPARTICLES

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Summary. Creating modern feed additives based on biologically active nanomaterials is an important area of research for improving mineral metabolism in animals and poultry. A significant amount of research focuses on developing zinc nanoparticles with a wide range of pharmacological effects. However, most zinc nanocomposites are toxic, even in low doses, and can accumulate in the body, leading to long-term adverse effects. To address this issue, zinc carbonate nanoparticles stabilized with polyvinylpyrrolidone were synthesized and found to be non-toxic. The study aimed to advance preclinical research on the toxicokinetics of these nanoparticles by conducting an acute toxicity experiment. To this end, 96 male Wistar rats were administered a single oral dose of a colloidal zinc carbonate nanoparticle solution at the following doses: 50 mg/kg b. w. (group I), 500 mg/kg b. w. (group II), and 5,000 mg/kg b. w. (group III), based on the absolute mass of the drug. The toxicokinetic profile of the studied nanoparticles showed typical dynamics of changes in zinc content in the organs and tissues of rats — an increase in the level of this microelement in the blood (only in experimental group III on the 1st day after administration of nanoparticles by 17.5%), liver (on the 1st day in experimental group II by 18.5% and in experimental group III by 52.1%; subsequently, changes were observed only after administration of the maximum dose of the drug — on the 3rd day by 30.7%, on the 7th and 14th days, there was a tendency to increase) and kidneys (by 25.0% in experimental group II and by 36.2% in experimental group III on the 1st day after administration of nanoparticles, on the 3rd day in experimental group II it was higher by 14.9% in experimental group III by 15.9%, on the 7th day of the experiment, the zinc content remained higher than the control values by 13.4% in experimental group II and by 17.7% in experimental group III). Regardless of the dose of administered nanoparticles, zinc did not accumulate in the heart, muscles, or skin with hair. By day 14, the zinc levels in all of the rats' examined organs and tissues were similar to those of the control group. No significant changes in zinc content were observed in experimental group I throughout the experiment. Therefore, zinc carbonate nanoparticles are safe regarding toxicokinetic parameters and do not cause long-term accumulation

Keywords: toxicity, distribution, organs, tissues

Introduction. Bioactive nanoparticles are a leading research topic in veterinary pharmacy, particularly in the field of microelementology. They are being studied as potential agents for improving mineral metabolism with high bioavailability and low toxicity (Jafary, Motamedi and Karimi, 2023; Li Y. et al., 2024b; Yang, Xiong and Long, 2025). However, there are still issues regarding the determination of optimal dosages and particle parameters for different species of animals and birds. Additionally, long-term safety studies are needed to eliminate the risk of nanoparticle accumulation in tissues (Malik, Muhammad and Waheed, 2023; Naumenko et al., 2023; Ashraf et al., 2025).

In recent years, the use of nanostructured zinc, an essential element for metabolic processes, has become widespread. Zinc's bioavailability in its macroform is low, and its toxicity is high (Li Y. et al., 2024a; Yang et al., 2025). One promising approach is the targeted delivery of zinc to specific organs and tissues. This method can increase efficiency, reduce possible side effects, and allow the use of zinc-based nanoparticles as nanocontainers for other pharmacological agents (Do Carmo Neto et al., 2024; Yue et al., 2024).

The toxicokinetics of zinc nanoparticles (ZnNPs) in rats differ from those of traditional zinc salts, demonstrating distinct biological patterns based on physicochemical parameters (Zhang et al., 2018; Bautista-Pérez et al., 2024). Depending on the route of administration, ZnNPs are rapidly absorbed and pass through epithelial barriers in the intestine or respiratory tract to enter the systemic bloodstream (Fujihara and Nishimoto, 2024). They are mainly distributed in the liver, spleen, lungs, and kidneys, where ultrastructural changes in cells are observed, including mitochondrial damage and DNA fragmentation (Chen et al., 2016; Abo-El-Sooud et al., 2023). The metabolism of ZnNPs is associated with interaction with metallothioneins and activation of antioxidant systems — they can induce oxidative stress, enhance the formation of reactive oxygen species, and disrupt the balance of enzymatic processes, affecting various types of metabolism in the body (Cho et al., 2013; Kausar et al., 2023). Elimination occurs through bile and urine; however, ZnNPs tend to remain in tissues longer than soluble salts due to their lower solubility and intracellular accumulation. This increases the risk of subchronic intoxication with prolonged use (Baek et al., 2012; Pei et al., 2022). In contrast, some ZnNPs forms, particularly zinc oxide nanoparticles (ZnONPs), release Zn^{2+} ions too quickly, causing toxic effects. ZnNPs can accumulate in their original form without dissociation when interacting with animals or birds, contributing to increased zinc content in organs and tissues as a trace element (Liu et al., 2016). In contrast, low toxicity is exhibited by zinc carbonate nanoparticles (ZnCNPs) synthesized by the authors of the article — a modern feed additive that is an effective method for correcting mineral metabolism with antioxidant action (Koshevoy et al., 2025a), which requires the determination of toxicokinetic parameters and strict control of dosages to complete a series of preclinical studies.

When assessing toxicokinetics, it is necessary to establish differences in the routes by which ZnNPs enter the body and to determine their *in vivo* distribution. ZnNPs initially enter the systemic circulation rapidly and circulate in both bound and free forms. They are then distributed and accumulated in organs and tissues, primarily in the liver, the main organ of deposition and detoxification. There, ZnNPs interact with metallothioneins, forming intracellular complexes that alter mitochondrial structure and energy metabolism (Paek et al., 2013; Ali et al., 2023). The second most important target organ for ZnNPs is the spleen, where they are retained by macrophages and other reticuloendothelial system cells and are accompanied by immune activation and, in some cases, damage to cellular organelles (Wang et al., 2016). The lungs also accumulate significant amounts of ZnNPs, particularly when inhaled or administered intratracheally, where they elicit local inflammatory responses and can persist for extended periods in alveolar macrophages (Rahman et al., 2022). In the kidneys, ZnNPs are primarily distributed to the cortical tissue, where they affect tubular transport and may cause nephrotoxicity with prolonged exposure (Hashim et al., 2025). To a lesser extent, they are found in the heart and brain, and in endocrine and sex glands, but with chronic administration, their translocation across histohematological barriers is possible (Yun et al., 2015; Deore et al., 2021; Rehman et al., 2024).

Numerous studies in recent years have characterized in detail the toxicological profile of ZnNPs in laboratory animals, including four sequential ADME parameters: A — absorption, D — distribution, M — metabolism, E — excretion, mainly focusing on the route of administration — intratracheal, intraperitoneal, intragastric, etc. (Fujihara et al., 2015; Li et al., 2017; Liang et al., 2022).

The primary objective of our study was to characterize the ADME profile of ZnNPs following oral administration. First, ZnNPs are absorbed via rapid penetration through intestinal epithelial barriers. Due to their small size and their ability to interact with cell membranes, ZnNPs exhibit more rapid and greater systemic uptake than macrostructural salts (Park et al., 2017). Second, ZnNPs are distributed in hepatocytes and Kupffer cells in the liver, in macrophages and other reticuloendothelial system cells in the spleen, in alveolar macrophages in the lungs, and in the cortical substance of the kidneys (Bayat et al., 2023).

Thirdly, in most cases, the typical mechanism of zinc metabolism in nanoform is binding to blood proteins, metallothioneins, and other substrates; ultimately, ZnNPs are eliminated primarily through bile and urine (Lee et al., 2016; Hadrup, Vogel and Jacobsen, 2025). Thus, the ADME profile of ZnNPs in rats demonstrates high absorption, accumulation in the liver and kidneys, metabolic activation of antioxidant systems, and slow excretion. These characteristics make ZnNPs an effective source of microelements, but also pose toxic risks associated with their use, necessitating long-term safety studies (Keerthana and Kumar, 2020; Czyżowska and Barbasz, 2022).

Given the large amount of research on the toxicity of ZnONPs both *in vitro* and *in vivo* in laboratory and productive animals and poultry, the authors of this article decided to synthesize zinc-based NPs with reduced toxicity parameters, both through the use of a safe synthesis method that complies with the provisions of 'green chemistry' and the use of a biocompatible stabilizer (El-Saadony et al., 2024; Fatima et al., 2024).

Thus, zinc carbonate nanoparticles (ZnCNPs), stabilized with polyvinylpyrrolidone (PVP) and previously shown not to exhibit acute toxicity in white mice at a maximum dose of 40,000 mg/kg b. w., were synthesized (Koshevoy et al., 2023). The use of PVP as a stabilizer can significantly alter the toxicokinetic profile of ZnNPs. PVP is widely used in pharmaceuticals and biomedicine as a polymer capable of forming a protective shell around nanoparticles and preventing their aggregation (Sarcinelli et al., 2021; Shahrousvand et al., 2023; Abdelhakeem et al., 2025).

In theory, using PVP as a stabilizer for NPs may affect their ADME profile, especially during the absorption phase. PVP stabilization increases the dispersibility and stability of NPs in biological environments. This allows for a more uniform passage through epithelial barriers and reduces the risk of local aggregation in the intestine. The result is more controlled and less traumatic for tissue absorption (Iqbal et al., 2021; Cao et al., 2024).

During the distribution phase, PVP-modified NPs are less likely to precipitate rapidly in the liver and spleen because the protective polymer shell reduces their interaction with plasma proteins and cell membranes. This reduces the likelihood of large intracellular aggregates forming. This may lead to a more even distribution across organs and reduce the severity of local toxic effects (Choi and Choy, 2014; Li W. et al., 2024b).

During metabolism, PVP acts as a barrier, slowing direct contact between HPs and cell organelles. This reduces the intensity of induced oxidative stress and the risk of mitochondrial and DNA damage (Ding et al., 2012; Ferdous et al., 2018).

During elimination, PVP may promote the slower removal of HPs because stabilized complexes are more stable and less soluble. This prolongs their circulation time in the blood, delays their uptake in tissues, and reduces the likelihood of acute organ damage (Kermanizadeh et al., 2018; Li W. et al., 2024a).

Thus, the toxicokinetics become prolonged, and using PVP as a stabilizer contributes to shifting the toxicokinetic profile of NPs from rapid and aggressive to milder and more prolonged (Fennell et al., 2017; Ćurlin et al., 2021).

The study **aimed** to determine the toxicokinetics of zinc in the organs and tissues of rats exposed to zinc carbonate nanoparticles stabilized with polyvinylpyrrolidone in an acute toxicological experiment.

Materials and methods. This study employed zinc carbonate nanoparticles (ZnCNPs), stabilized with polyvinylpyrrolidone and synthesized at the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine. The colloidal ZnCNPs solution used had a concentration of 2.5 g/dm^3, a pH value of 7.5, and contained spherical nanoparticles. The experiment was conducted on 96 sexually mature male Wistar rats, which were divided into four groups: a control group (C) and three experimental groups (E I, E II, and E III), with 24 rats in each group. Experimental group I received a single dose of 50 mg/kg b. w. of colloidal ZnCNPs solution, experimental group II received a single dose of 500 mg/kg b. w., and experimental group III received a single dose of 5,000 mg/kg b. w., all administered orally. The control group received a similar volume of distilled water. Throughout the study, the clinical condition and ethological parameters of all animals were monitored.

To determine the toxicokinetics of zinc on days 1, 3, 7, and 14 after the start of the experiment, six animals from each group were anesthetized and decapitated. A pathomorphological study was performed and samples of organs and tissues were collected, including blood, liver, kidney, heart, muscle, and hair with skin.

The parenchymal organs were separated from the connective tissue and the muscle from the tendon. The samples were then treated with concentrated nitric acid. The zinc content was subsequently determined using inductively coupled plasma mass spectrometry.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University.

The results were analyzed statistically. A Student's *t*-test was used. A difference was considered significant at $P < 0.05$. (Van Emden, 2019).

Results and discussion. Evaluation of rat internal organs and tissues revealed differences in zinc content after a single dose of zinc carbonate nanoparticles (ZnCNPs). While the obtained data indicate typical changes in the toxicokinetics of zinc after the oral administration of its compounds — namely, an increase in zinc content in the blood, liver, and kidneys, which are the main organs involved in its metabolism and excretion — the effect of ZnCNPs exhibited certain peculiarities. First, we studied the zinc content in the rats' blood because it is the primary route for the absorption and distribution of trace elements in the body.

On the first day after the NPs solution was administered, the zinc content in the blood of the rats in experimental group III increased by 17.5% (1.14 ± 0.04 mg/kg, $p < 0.01$). A tendency toward an increase in this indicator was observed in experimental group II. In experimental group I, the zinc content was at the level of the control values (Fig. 1). From the third to the fourteenth day of the main period of the experiment, there were no significant changes in zinc content in the experimental animals. These results are consistent with a previous study on the hematotoxicity of ZnCNPs conducted by the authors of this article. The study's results indicate no toxic effects on the blood system (Koshevoy et al., 2025b).

Secondly, it was important to determine changes in zinc content in the liver and kidneys because they are responsible for excreting this metal in bile and urine. Additionally, the liver is the site of zinc deposition, metabolism, and related enzymatic systems. Fig. 2 shows that introducing ZnCNPs into the livers of rats caused changes in zinc content.

Administration of the minimum dose (50 mg/kg b. w.) to rats in experimental group I did not cause changes in zinc content during the experiment. In animals in experimental group II, zinc content in the liver increased by 18.5% (29.79 ± 1.12 mg/kg, $p < 0.01$) only on the first day of the study. On the third day, this indicator tended to increase. On the seventh and fourteenth days, zinc content in the liver was at the level of the control group. Significant changes were observed in experimental group III. After the administration of ZnCNPs at a dose of 5,000 mg/kg b. w. on the first day of the study, the zinc content in the rats' livers exceeded that of the control group by 52.1% (38.23 ± 1.16 mg/kg, $p < 0.001$). On the third day, the zinc content remained 30.7% higher (34.14 ± 1.03 mg/kg, $p < 0.001$). Subsequently, on days 7 and 14 of the experiment, the zinc content in the animals tended to increase within the same group. This increase demonstrates the bioavailability and ability to accumulate ZnCNPs during acute intake. Previous studies by the authors of this article also indicate the absence of ZnCNP toxicity, particularly concerning liver protein synthesis and hepatospecific enzyme activity (Koshevoy et al., 2024a).

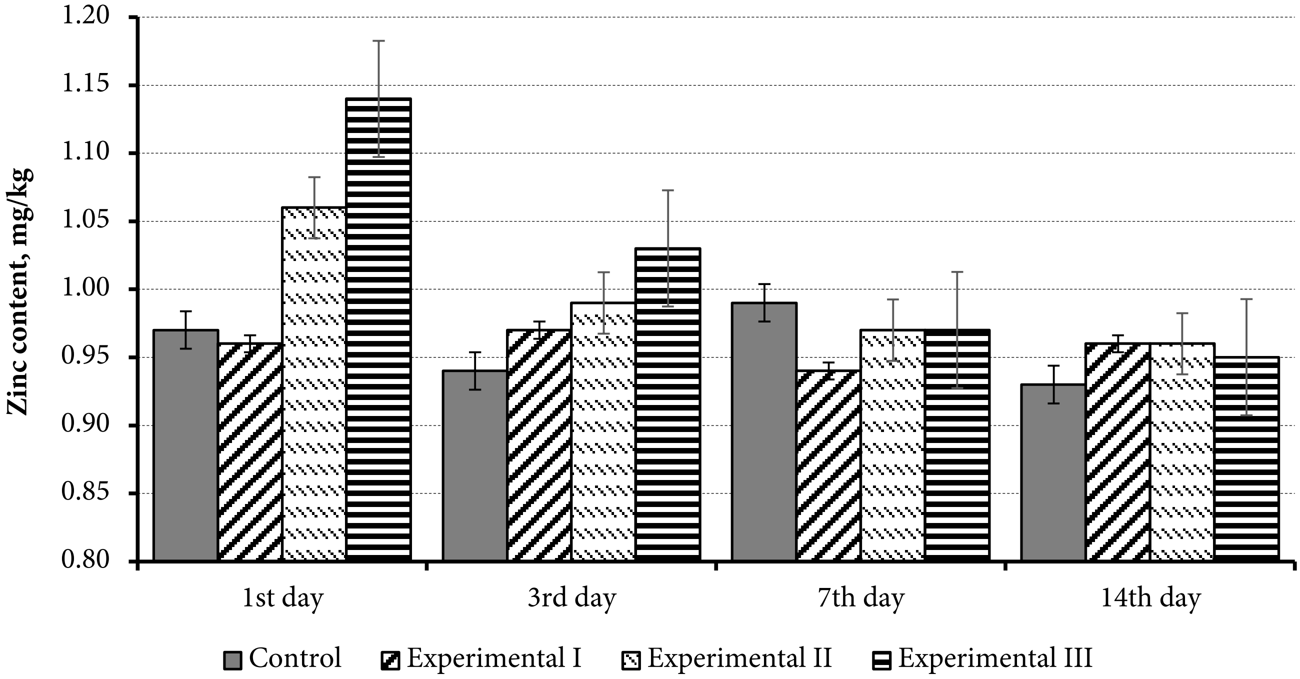

Figure 1. Zinc content in the blood of rats after a single oral administration of zinc carbonate nanoparticles.

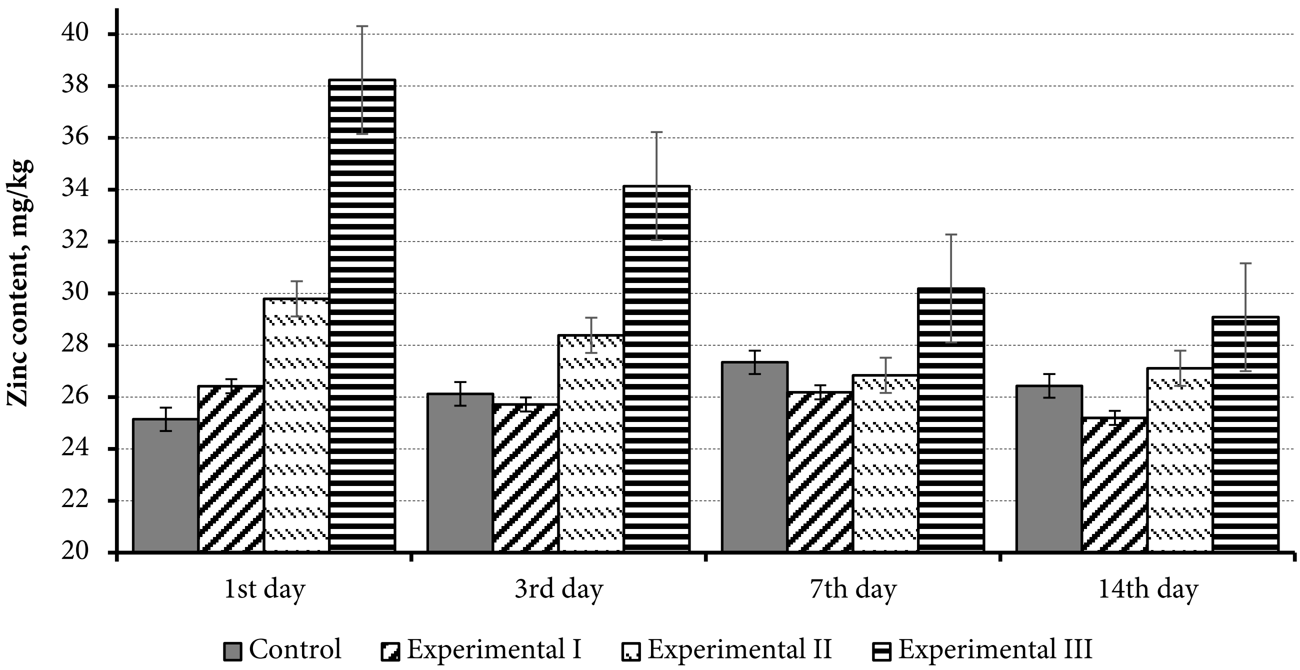

Figure 2. Zinc content in the liver of rats after a single oral administration of zinc carbonate nanoparticles.

Fig. 3 shows the effect of ZnCNPs administration on zinc content in rat kidneys. After the maximum dose of the test compound was administered orally, the zinc content increased by 36.2% (19.44 ± 0.84 mg/kg, $p < 0.001$) in rats in experimental group III. The changes in experimental group II, which received a lower dose, were less pronounced; the zinc content exceeded the control data by 25.0% (17.84 ± 0.56 mg/kg, $p < 0.01$). Meanwhile, no significant changes in zinc content were observed in rats of experimental group I on the first day of the experiment.

Subsequently, on the third day, the zinc content demonstrated similar changes. In experimental group III, the zinc content was 15.9% higher (17.23 ± 0.71 mg/kg, $p < 0.05$). In experimental group II, the zinc content was 14.9% higher (17.07 ± 0.49 mg/kg, $p < 0.05$). In experimental group I, the zinc content only tended to increase compared to the control rats. This study noted prolonged excretion of zinc from the rats' bodies after ZnCNPs administration, as the zinc content in the kidneys was higher than the control values on the seventh day of the experiment: by 17.7% in group III (16.23 ± 0.68 mg/kg, $p < 0.05$), by 13.4% in group II (15.64 ± 0.61 mg/kg, $p < 0.05$), and at the control level in group I. By the end of the experiment on day 14, there were no significant changes in microelement content among any of the experimental groups. The accumulation of zinc in kidney tissue indicated that the kidneys were excreting it over a longer period of time. Additionally, biochemical studies revealed that ZnCNPs exhibited no signs of nephrotoxicity (Koshevoy et al., 2024b). The effect of ZnCNPs on zinc content in the hearts, muscles, skin, and hair of rats was also determined (Table 1).

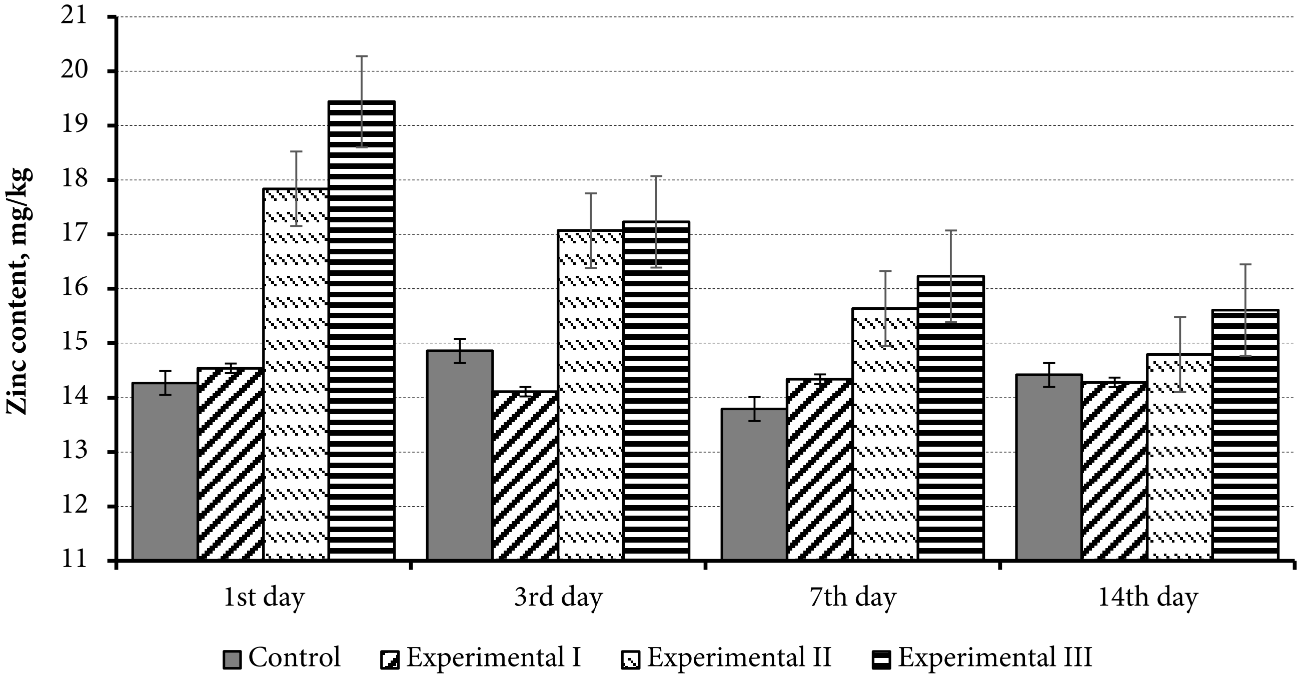

Figure 3. Zinc content in rat kidneys after a single oral administration of zinc carbonate nanoparticles.

Table 1 — Dynamics of zinc content in the heart, muscles, skin, and hair in rats following a single oral administration of zinc carbonate

Research day / group		Zinc content, mg/kg (M ± m, n = 6)		
		Heart	Muscles	Skin and hair
1	C	16.23 ± 0.51	11.78 ± 0.41	110.47 ± 3.36
	E I	16.31 ± 0.57	11.71 ± 0.39	107.62 ± 3.24
	E II	16.91 ± 0.53	11.84 ± 0.37	114.59 ± 3.51
	E III	17.04 ± 0.54	12.49 ± 0.47	120.34 ± 4.14
3	C	16.41 ± 0.49	11.44 ± 0.37	109.18 ± 3.71
	E I	16.37 ± 0.52	11.74 ± 0.43	109.88 ± 3.34
	E II	16.59 ± 0.51	12.08 ± 0.46	113.17 ± 3.41
	E III	16.87 ± 0.57	13.23 ± 0.54*	114.23 ± 4.34
7	C	16.31 ± 0.48	11.51 ± 0.44	111.36 ± 4.08
	E I	16.34 ± 0.56	11.68 ± 0.52	112.58 ± 3.47
	E II	16.47 ± 0.53	11.92 ± 0.57	111.89 ± 3.38
	E III	16.69 ± 0.62	12.06 ± 0.51	112.74 ± 3.91
14	C	16.21 ± 0.54	11.32 ± 0.39	110.12 ± 3.47
	E I	16.29 ± 0.52	11.64 ± 0.51	111.74 ± 3.42
	E II	16.36 ± 0.61	11.81 ± 0.49	109.94 ± 3.32
	E III	16.33 ± 0.78	11.89 ± 0.43	111.41 ± 3.86

Note. * — $p < 0.05$ statistically significant changes compared to the control group data.

As shown in Table 1, the administration of ZnCNPs solution did not increase zinc content in the heart. Only in experimental groups II and III on the first and third days of observation was there a tendency for this indicator to increase. However, there were no significant changes compared to the control group during the experiment in any of the experimental groups. Similar dynamics were observed in the study of wool samples with skin; throughout the 14-day experiment, zinc content did not differ significantly. Administering ZnCNPs did not lead to zinc accumulation in the muscles of rats. Only the maximum dose of 5,000 mg/kg b. w. increased the content of this trace element by 15.6% three days after administration. Thus, a single oral administration of ZnCNPs has no significant effect on zinc content in the hearts, muscles, skin, or hair of male rats.

Conclusions. The toxicokinetic profile of newly synthesized zinc carbonate nanoparticles stabilized with polyvinylpyrrolidone exhibited characteristic changes in zinc content in the organs and tissues of male rats. Administering dosages to three groups of animals at tenfold increases of 50–500–5,000 mg/kg b. w., compared to the control group, caused an increase in zinc levels in the blood, liver, and kidneys of rats in experimental groups II and III. There was no accumulation of zinc in the heart, muscles, or skin with fur in any of the experimental groups of rats. On day 14, zinc levels in all examined organs and tissues did not differ from those of the control group. No significant changes in zinc content were observed in group I during the experiment.

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