# 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).

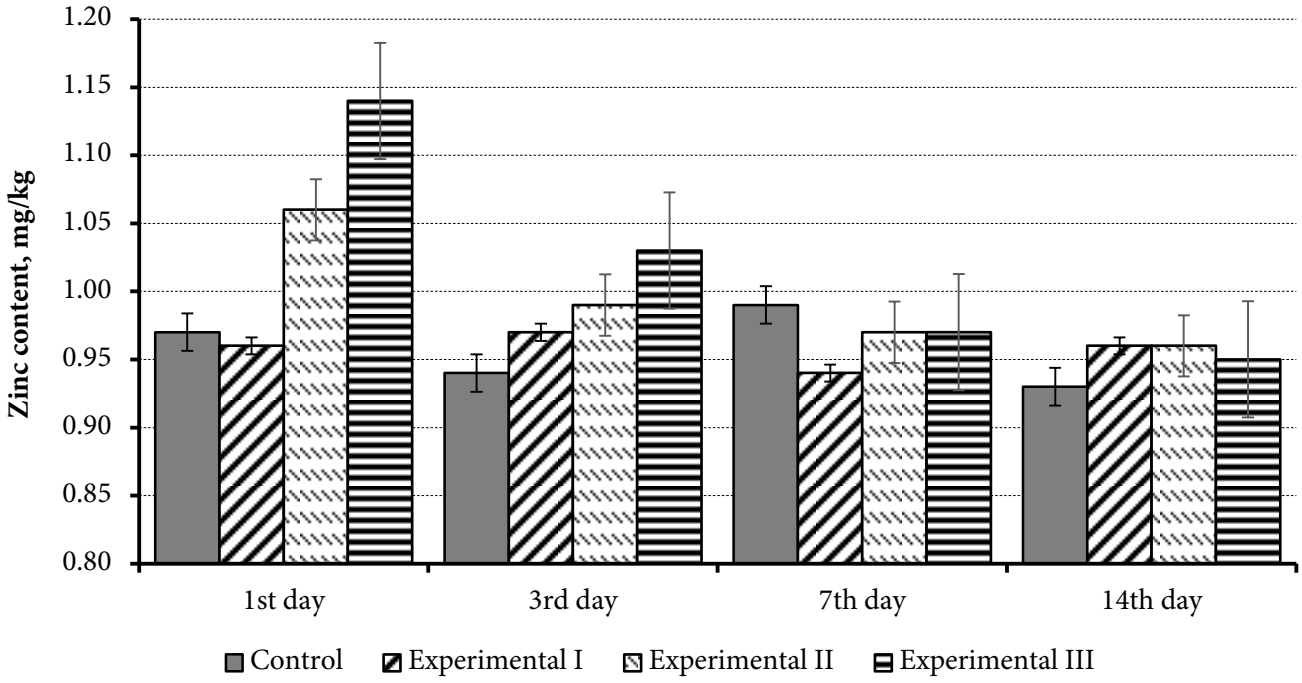


**Figure 1.** Zinc content in the blood of rats after a single oral administration of zinc carbonate nanoparticles.

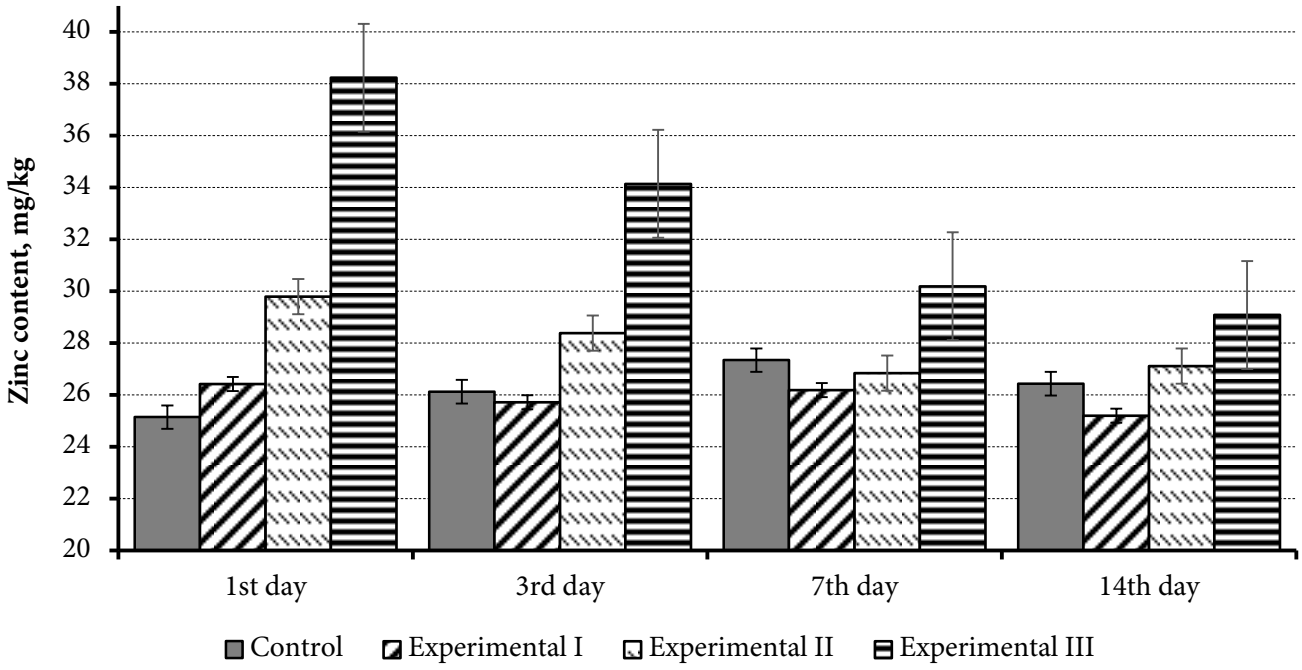


**Figure 2.** Zinc content in the liver of rats after a single oral administration of zinc carbonate nanoparticles.

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).

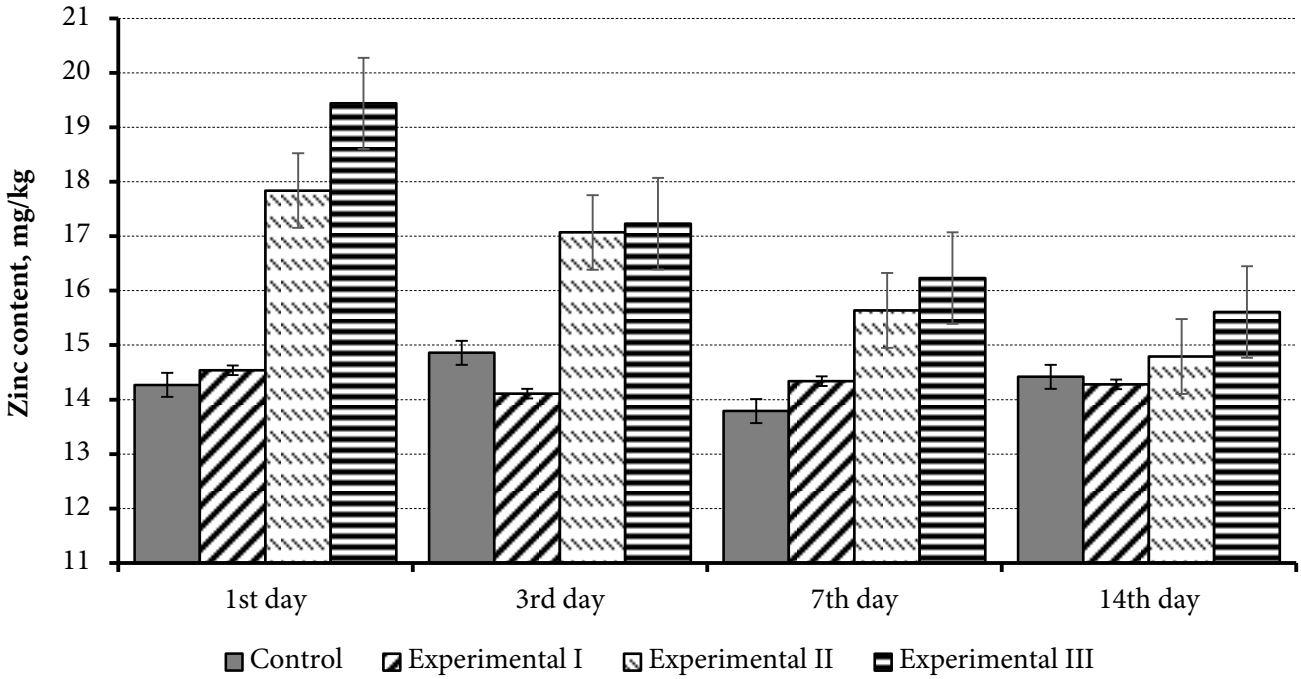


**Figure 3.** Zinc content in rat kidneys after a single oral administration of zinc carbonate nanoparticles.

**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.

**References**


Abdelhakeem, E., Mohamed, S. A., Beherei, H. H., Moaness, M. and Hegazy, D. (2025) 'Novel biocompatible hyaluronic acid (HA)/polyvinylpyrrolidone (PVP) composites containing silver decorated-zinc MOF nanoparticles: antimicrobial activity, drug delivery and wound healing', *AAPS PharmSciTech*, 27(1), p. 5. doi: 10.1208/s12249-025-03243-z.

Abo-El-Sooud, K., Abd-El Hakim, Y. M., Hashem, M. M. M., El-Metwally, A. E., Hassan, B. A. and El-Nour, H. H. M. (2023) 'Restorative effects of gallic acid against sub-chronic hepatic toxicity of co-exposure to zinc oxide nanoparticles and arsenic trioxide in male rats', *Heliyon*, 9(6), p. e17326. doi: 10.1016/j.heliyon.2023.e17326.

Ali, A., Saeed, S., Hussain, R., Afzal, G., Siddique, A. B., Parveen, G., Hasan, M. and Caprioli, G. (2023) 'Synthesis and characterization of silica, silver-silica, and zinc oxide-silica nanoparticles for evaluation of blood biochemistry, oxidative stress, and hepatotoxicity in albino rats', *ACS Omega*, 8(23), pp. 20900–20911. doi: 10.1021/acsomega.3c01674.

Ashraf, M., Zulfiqar, F., Ijaz, U., Rauf, U., Riaz, M., Arshad, S., Jamil Khan, M. and Sahin, T. (2025) 'Nanotechnology, nano-systems and applications of nanoparticles in novel drug delivery — a comprehensive review', *Pakistan Journal of Pharmaceutical Sciences,* 38(2), pp. 669–677. Available at: https://www.pjps.pk/uploads/2025/06/1749142227.pdf.

Baek, M., Chung, H. E., Yu, J., Lee, J. A., Kim, T. H., Oh, J. M., Lee, W. J., Paek, S. M., Lee, J. K., Jeong, J., Choy, J. H. and Choi, S. J. (2012) 'Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles', *International Journal of Nanomedicine,* 7, pp. 3081–3097. doi: 10.2147/IJN.S32593.

Bautista-Pérez, R., Cano-Martínez, A., Herrera-Rodríguez, M. A., Ramos-Godinez, M. D. P., Pérez Reyes, O. L., Chirino, Y. I., Rodríguez Serrano, Z. J. and López-Marure, R. (2024) 'Oral exposure to titanium dioxide E171 and zinc oxide nanoparticles induces multi-organ damage in rats: role of ceramide', *International Journal of Molecular Sciences*, 25(11), p. 5881. doi: 10.3390/ijms25115881.

Bayat, M., Daei, S., Ziamajidi, N., Abbasalipourkabir, R. and Nourian, A. (2023) 'The protective effects of vitamins A, C, and E on zinc oxide nanoparticles (ZnO NPs)-induced liver oxidative stress in male Wistar rats', *Drug and Chemical Toxicology*, 46(2), pp. 209–218. doi: 10.1080/01480545.2021.2016809.

Cao, F., Liang, K., Tang, W. W., Ni, Q. Y., Ji, Z. Y., Zha, C. K., Wang, Y. K., Jiang, Z. X., Hou, S., Tao, L. M. and Wang, X. (2024) 'Polyvinylpyrrolidone-curcumin nanoparticles with immune regulatory and metabolism regulatory effects for the treatment of experimental autoimmune uveitis', *Journal of Controlled Release*, 372, pp. 551–570. doi: 10.1016/j.jconrel.2024.06.047.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Chen, A., Feng, X., Sun, T., Zhang, Y., An, S. and Shao, L. (2016) 'Evaluation of the effect of time on the distribution of zinc oxide nanoparticles in tissues of rats and mice: a systematic review', *IET Nanobiotechnology*, 10(3), pp. 97–106. doi: 10.1049/iet-nbt.2015.0006.

Cho, W. S., Kang, B. C., Lee, J. K., Jeong, J., Che, J. H. and Seok, S. H. (2013) 'Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration', *Particle and Fibre Toxicology*, 10(1), p. 9. doi: 10.1186/1743-8977-10-9.

Choi, S.-J. and Choy, J.-H. (2014) 'Biokinetics of zinc oxide nanoparticles: toxicokinetics, biological fates, and protein interaction', *International Journal of Nanomedicine*, 9(2), pp. 261–269. doi: 10.2147/ijn.s57920.

Ćurlin, M., Barbir, R., Dabelić, S., Ljubojević, M., Goessler, W., Micek, V., Žuntar, I., Pavić, M., Božičević, L., Pavičić, I. and Vinković Vrček, I. (2021) 'Sex affects the response of Wistar rats to polyvinyl pyrrolidone (PVP)-coated silver nanoparticles in an oral 28 days repeated dose toxicity study', *Particle and Fibre Toxicology*, 18(1), p. 38. doi: 10.1186/s12989-021-00425-y.

Czyżowska, A. and Barbasz, A. (2022) 'A review: Zinc oxide nanoparticles — friends or enemies?', *International Journal of Environmental Health Research*, 32(4), pp. 1–17. doi: 10.1080/09603123.2020.1805415.

Deore, M. S., Keerthana, S., Naqvi, S., Kumar, A. and Flora, S. J. S. (2021) 'Alpha-lipoic acid protects co-exposure to lead and zinc oxide nanoparticles induced neuro, immuno and male reproductive toxicity in rats', *Frontiers in Pharmacology*, 12, p. 626238. doi: 10.3389/fphar.2021.626238.

Ding, X. Y., Hong, C. J., Liu, Y., Gu, Z. L., Xing, K. L., Zhu, A. J., Chen, W. L., Shi, L. S., Zhang, X. N. and Zhang, Q. (2012) 'Pharmacokinetics, tissue distribution, and metabolites of a polyvinylpyrrolidone-coated norcantharidin chitosan nanoparticle formulation in rats and mice, using LC-MS/MS', *International Journal of Nanomedicine,* 7, pp. 1723–1735. doi: 10.2147/IJN.S29696.

Do Carmo Neto, J. R., Franco, P. I. R., Braga, Y. L. L., de Oliveira, J. F., Perini, H. F., Albuquerque, L. F. D., Martins, D. B., Helmo, F. R., Andrade, A. A., Miguel, M. P., Celes, M. R. N., Rocha, T. L., Almeida Silva, A. C., Machado, J. R. and da Silva, M. V. (2024) 'Toxicity assessment of new Ag-ZnO/AgO nanocomposites: An *in vitro* and *in vivo* approach', *Journal of Functional Biomaterials*, 15(3), p. 51. doi: 10.3390/jfb15030051.

El-Saadony, M. T., Fang, G., Yan, S., Alkafaas, S. S., El Nasharty, M. A., Khedr, S. A., Hussien, A. M., Ghosh, S., Dladla, M., Elkafas, S. S., Ibrahim, E. H., Salem, H. M., Mosa, W. F., Ahmed, A. E., Mohammed, D. M., Korma, S. A., El-Tarabily, M. K., Saad, A. M., El-Tarabily, K. A. and AbuQamar, S. F. (2024) 'Green synthesis of zinc oxide nanoparticles: Preparation, characterization, and biomedical applications — A review', *International Journal of Nanomedicine*, 19, pp. 12889–12937. doi: 10.2147/IJN.S487188.

Fatima, A., Zaheer, T., Pal, K., Abbas, R. Z., Akhtar, T., Ali, S. and Mahmood, M. S. (2024) 'Zinc oxide nanoparticles significant role in poultry and novel toxicological mechanisms', *Biological Trace Element Research*, 202(1), pp. 268–290. doi: 10.1007/s12011-023-03651-x.

Fennell, T. R., Mortensen, N. P., Black, S. R., Snyder, R. W., Levine, K. E., Poitras, E., Harrington, J. M., Wingard, C. J., Holland, N. A., Pathmasiri, W. and Sumner, S. C. (2017) 'Disposition of intravenously or orally administered silver nanoparticles in pregnant rats and the effect on the biochemical profile in urine', *Journal of Applied Toxicology*, 37(5), pp. 530–544. doi: 10.1002/jat.3387.

Ferdous, Z., Beegam, S., Tariq, S., Ali, B. H. and Nemmar, A. (2018) 'The *in vitro* effect of polyvinylpyrrolidone and citrate coated silver nanoparticles on erythrocytic oxidative damage and eryptosis', *Cellular Physiology and Biochemistry*, 49(4), pp. 1577–1588. doi: 10.1159/000493460.

Fujihara, J. and Nishimoto, N. (2024) 'Review of zinc oxide nanoparticles: toxicokinetics, tissue distribution for various exposure routes, toxicological effects, toxicity mechanism in mammals, and an approach for toxicity reduction', *Biological Trace Element Research*, 202(1), pp. 9–23. doi: 10.1007/s12011-023-03644-w.

Fujihara, J., Tongu, M., Hashimoto, H., Yamada, T., Kimura-Kataoka, K., Yasuda, T., Fujita, Y. and Takeshita, H. (2015) 'Distribution and toxicity evaluation of ZnO dispersion nanoparticles in single intravenously exposed mice', *The Journal of Medical Investigation*, 62(1–2), pp. 45–50. doi: 10.2152/jmi.62.45.

Hadrup, N., Vogel, U. and Jacobsen, N. R. (2025) 'Biokinetics of inhaled silver, gold, copper oxide, and zinc oxide nanoparticles: A review', *Nanotoxicology*, 19(3), pp. 259–289. doi: 10.1080/17435390.2025.2476994.

Hashim, M., Anjum, S., Mujahid, H., Alotaibi, K. S., Albattal, S. B., Ghamry, H. I. and Soliman, M. M. (2025) 'Thymoquinone loaded zinc oxide nanoformulations synthesis, characterization and evaluation of their efficacy against carbon tetrachloride induced hepatorenal toxicity in rats', *Toxicology Research,* 14(2), p. tfaf037. doi: 10.1093/toxres/tfaf037.

Iqbal, R., Qureshi, O. S., Yousaf, A. M., Raza, S. A., Sarwar, H. S., Shahnaz, G., Saleem, U. and Sohail, M. F. (2021) 'Enhanced solubility and biopharmaceutical performance of atorvastatin and metformin via electrospun polyvinylpyrrolidone-hyaluronic acid composite nanoparticles', *European Journal of Pharmaceutical Sciences*, 161, p. 105817. doi: 10.1016/j.ejps.2021.105817.

Jafary, F., Motamedi, S. and Karimi, I. (2023) 'Veterinary nanomedicine: Pros and cons', *Veterinary Medicine and Science*, 9(1), pp. 494–506. doi: 10.1002/vms3.1050.

Kausar, S., Jabeen, F., Latif, M. A. and Asad, M. (2023) 'Characterization, dose dependent assessment of hepatorenal oxidative stress, hematological parameters and histopathological divulging of the hepatic damages induced by Zinc Oxide Nanoparticles (ZnO-NPs) in adult male Sprague dawley rats', *Saudi Journal of Biological Sciences*, 30(9), p. 103745. doi: 10.1016/j.sjbs.2023.103745.

Keerthana, S. and Kumar, A. (2020) 'Potential risks and benefits of zinc oxide nanoparticles: A systematic review', *Critical Reviews in Toxicology*, 50(1), pp. 47–71. doi: 10.1080/10408444.2020.1726282.

Kermanizadeh, A., Powell, L. G., Stone, V. and Møller, P. (2018) 'Nanodelivery systems and stabilized solid-drug nanoparticles for orally administered medicine: current landscape', *International Journal of Nanomedicine*, 13, pp. 7575–7605. doi: 10.2147/ijn.s177418.

Koshevoy, V., Naumenko, S., Orobchenko, O. and Bespalova, I. (2023) 'Acute toxicity of zinc carbonate nanocrystals on white mice model' [Hostra toksychnist nanokrystaliv tsynku karbonatu na modeli bilykh myshei], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj. Series: Veterinary Sciences [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho. Seriia: Veterynarni Nauky]*, 25(112), pp. 123–130. doi: 10.32718/nvlvet11220. [in Ukrainian].

Koshevoy, V. I., Naumenko, S. V., Bespalova, I. I., Radzikhovskyi, M. L. and Balym, Yu. P. (2024a) 'Dynamics of the activity of hepato-specific enzymes and the state of protein synthesizer function of the liver in rats during chronic intake of zinc carbonate hydroxide nanoparticles' [Dynamika aktyvnosti hepatospetsyfichnykh enzymiv ta stan proteinsyntezuvalnoi funktsii pechinky u shchuriv za khronichnoho nadkhodzhennia nanochastynok tsynku hidrokarbonatu], *Veterinary Medicine [Veterynarna medytsyna],* 110, pp. 188–196. doi: 10.36016/VM-2024-110-29. [in Ukrainian].

Koshevoy, V. I., Naumenko, S. V., Bespalova, I. I. and Yefimova, S. L. (2024b) 'Biochemical parameters of nephrotoxicity of zinc hydrocarbonate nanocrystals' [Biokhimichni parametry nefrotoksychnosti nanokrystaliv tsynku hidrokarbonatu], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii. Seriia: Veterynarni nauky],* 26(116), pp. 270–277. doi: 10.32718/nvlvet11639. [in Ukrainian].

Koshevoy, V., Naumenko, S., Bespalova, I. and Yefimova, S. (2025a). 'Effect of zinc carbonate nanoparticles subchronic intake on antioxidant status of male rabbits', *Journal for Veterinary Medicine Biotechnology and Biosafety*, 11(4), pp. 3–11. doi: 10.36016/jvmbbs-2025-11-4-1.

Koshevoy, V. I., Naumenko, S. V., Bespalova, I. I. and Yefimova, S. L. (2025b) 'Hematotoxicity of zinc carbonate nanoparticles in the Wistar rat model', *Ukrainian Journal of Veterinary and Agricultural Sciences*, 8(1), pp. 21–26. doi: 10.32718/ujvas8-1.04.

Lee, J., Yu, W. J., Song, J., Sung, C., Jeong, E. J., Han, J. S., Kim, P., Jo, E., Eom, I., Kim, H. M., Kwon, J. T., Choi, K., Choi, J., Kim, H., Lee, H., Park, J., Jin, S. M. and Park, K. (2016) 'Developmental toxicity of intravenously injected zinc oxide nanoparticles in rats', *Archives of Pharmacal Research*, 39(12), pp. 1682–1692. doi: 10.1007/s12272-016-0767-z.

Li, M., Zou, P., Tyner, K. and Lee, S. (2017) 'Physiologically based pharmacokinetic (PBPK) modeling of pharmaceutical nanoparticles', *The AAPS Journal*, 19(1), pp. 26–42. doi: 10.1208/s12248-016-0010-3.

Li, W., Lu, X., Jiang, L. and Wang, X. (2024a) 'Biosafety and pharmacokinetic characteristics of polyethylene pyrrolidone modified nano selenium in rats', *BMC Biotechnology*, 24(1), p. 98. doi: 10.1186/s12896-024-00915-9.

Li, W., Lu, X., Jiang, L. and Wang, X. (2024b) 'Radioprotective effect of polyvinylpyrrolidone modified selenium nanoparticles and its antioxidation mechanism *in vitro* and *in vivo*', *Frontiers in Bioengineering and Biotechnology*, 12, p. 1392339. doi: 10.3389/fbioe.2024.1392339.

Li, Y., Li, J., Li, M., Sun, J., Shang, X. and Ma, Y. (2024a) 'Biological mechanism of ZnO nanomaterials', *Journal of Applied Toxicology,* 44(1), pp. 107–117. doi: 10.1002/jat.4522.

Li, Y., Lu, Y., Li, J., Li, M., Gou, H., Sun, X., Xu, X., Song, B., Li, Z. and Ma, Y. (2024b) 'Screening of low-toxic zinc oxide nanomaterials and study the apoptosis mechanism of NSC-34 cells', *Biotechnology Journal,* 19(2), p. e2300443. doi: 10.1002/biot.202300443.

Liang, C., Fang, J., Hu, J., Geng, X., Liu, H., Feng, Y., Wang, W., Cui, W., Yu, Z. and Jia, X. (2022) 'Toxicokinetics of

zinc oxide nanoparticles and food grade bulk-sized zinc oxide in rats after oral dosages', *NanoImpact,* 25, p. 100368. doi: 10.1016/j.impact.2021.100368.

Liu, J., Feng, X., Wei, L., Chen, L., Song, B. and Shao, L. (2016) 'The toxicology of ion-shedding zinc oxide nanoparticles', *Critical Reviews in Toxicology,* 46(4), pp. 348–384. doi: 10.3109/10408444.2015.1137864.

Malik, S., Muhammad, K. and Waheed, Y. (2023) 'Emerging applications of nanotechnology in healthcare and medicine', *Molecules,* 28(18), p. 6624. doi: 10.3390/molecules28186624.

Naumenko, S., Koshevoy, V., Matsenko, O., Miroshnikova, O., Zhukova, I. and Bespalova, I. (2023) 'Antioxidant properties and toxic risks of using metal nanoparticles on health and productivity in poultry', *Journal of World`s Poultry Research*, 13(3), pp. 292–306. doi: 10.36380/jwpr.2023.32.

Paek, H. J., Lee, Y. J., Chung, H. E., Yoo, N. H., Lee, J. A., Kim, M. K., Lee, J. K., Jeong, J. and Choi, S. J. (2013) 'Modulation of the pharmacokinetics of zinc oxide nanoparticles and their fates *in vivo*', *Nanoscale,* 5(23), pp. 11416–11427. doi: 10.1039/c3nr02140h.

Park, E. J., Jeong, U., Yoon, C. and Kim, Y. (2017) 'Comparison of distribution and toxicity of different types of zinc-based nanoparticles', *Environmental Toxicology*, 32(4), pp. 1363–1374. doi: 10.1002/tox.22330.

Pei, X., Jiang, H., Xu, G., Li, C., Li, D. and Tang, S. (2022) 'Lethality of zinc oxide nanoparticles surpasses conventional zinc oxide via oxidative stress, mitochondrial damage and calcium overload: A comparative hepatotoxicity study', *International Journal of Molecular Sciences,* 23(12), p. 6724. doi: 10.3390/ijms23126724.

Rahman, H. S., Othman, H. H., Abdullah, R., Edin, H. A. S. and Al-Haj, N. A. (2022) 'Beneficial and toxicological aspects of zinc oxide nanoparticles in animals', *Veterinary Medicine and Science*, 8(4), pp. 1769–1779. doi: 10.1002/vms3.814.

Rehman, N., Jabeen, F., Asad, M., Nijabat, A., Ali, A., Khan, S. U., Luna-Arias, J. P., Mashwani, Z. U., Siddiqa, A., Karthikeyan, A. and Ahmad, A. (2024) 'Exposure to zinc oxide nanoparticles induced reproductive toxicities in male Sprague Dawley rats', *Journal of Trace Elements in Medicine and Biology*, 83, p. 127411. doi: 10.1016/j.jtemb.2024.127411.

Sarcinelli, M. A., Gullo, M. P., Gentile, G., Cocca, M. C., Errico, M. E., Avella, M. and Tavares, M. I. B. (2021) 'Polyvinylpyrrolidone/ Montmorillonite/ Zinc oxide bionanosystems prepared by spray drying', *Journal of Nanoscience and Nanotechnology*, 21(9), pp. 4830–4839. doi: 10.1166/jnn.2021.19284.

Shahrousvand, M., Mirmasoudi, S. S., Pourmohammadi-Bejarpasi, Z., Feizkhah, A., Mobayen, M., Hedayati, M., Sadeghi, M., Esmaelzadeh, M., Mirkatoul, F. B. and Jamshidi, S. (2023) 'Polyacrylic acid/ polyvinylpyrrolidone hydrogel wound dressing containing zinc oxide nanoparticles promote wound healing in a rat model of excision injury', *Heliyon,* 9(8), p. e19230. doi: 10.1016/j.heliyon.2023.e19230.

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Wang, C., Lu, J., Zhou, L., Li, J., Xu, J., Li, W., Zhang, L., Zhong, X. and Wang, T. (2016) 'Effects of long-term exposure to zinc oxide nanoparticles on development, zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice', *PloS One,* 11(10), p. e0164434. doi: 10.1371/journal.pone.0164434.

Yang, J., Xiong, D. and Long, M. (2025) 'Zinc oxide nanoparticles as next-generation feed additives: bridging antimicrobial efficacy, growth promotion, and sustainable strategies in animal nutrition', *Nanomaterials,* 15(13), p. 1030. doi: 10.3390/nano15131030.

Yang, K., Zhu, R., Bao, H., Xu, S., Gao, Y., Xue, Y., Wang, J., Wang, X., Pan, Y., Hong, L. and Zhao, K. (2025) 'Chitosan-based nanomaterials: Pioneering a review in veterinary medicine applications', *International Journal of Biological Macromolecules,* 320(4), p. 146011. doi: 10.1016/j.ijbiomac.2025.146011.

Yue, W., Wang, X., Zhang, J., Bao, J. and Yao, M. (2024) 'Construction of immobilized laccase system based on ZnO and degradation of mesotrione', *Toxics,* 12(6), p. 434. doi: 10.3390/toxics12060434.

Yun, J. W., Yoon, J. H., Kang, B. C., Cho, N. H., Seok, S. H., Min, S. K., Min, J. H., Che, J. H. and Kim, Y. K. (2015) 'The toxicity and distribution of iron oxide-zinc oxide core-shell nanoparticles in C57BL/6 mice after repeated subcutaneous administration', *Journal of Applied Toxicology,* 35(6), pp. 593–602. doi: 10.1002/jat.3102.

Zhang, S. Q., Yu, X. F., Zhang, H. B., Peng, N., Chen, Z. X., Cheng, Q., Zhang, X. L., Cheng, S. H. and Zhang, Y. (2018) 'Comparison of the oral absorption, distribution, excretion, and bioavailability of zinc sulfate, zinc gluconate, and zinc-enriched yeast in rats', *Molecular Nutrition & Food Research*, 62(7), p. e1700981. doi: 10.1002/mnfr.201700981.

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