Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(3): 2563-2576. **OPEN ACCESS**

Changes in the haemostatic system and the activity of digestive enzymes of rats when exposed to Nano-particles (NPs) CuZn

Elena Anatolevna Rusakova[1], Svyatoslav Valerievich Lebedev[1], Irina Alexandrovna Gavrish[1], Olga Vilorievna Kvan[1,2] and Elena Vladimirovna Sheida[1,2]

[1] Federal Research Center of Biological Systems and Agro-technologies of the Russian Academy of Sciences, 9th Yanvarya St., 29, Orenburg, **Russia**.
[2] Orenburg State University, Pobedy pr. 13, Orenburg, **Russia**.

***Correspondence:** elenka_rs@mail.ru **Accepted**: 20 June 2019 **Published online:** 31 July 2019

Nano-particles CuZn (NPs CuZn) were tested *in vivo*. With different physico-chemical characteristics, they had an ambiguous effect on the haemostatic system and enzyme activity in pancreas and chyme in duodenum. There was a decrease in the content of red blood cells and haemoglobin with the introduction of NPs CuZn with a lower specific surface area. The administration of NPs CuZn led to the development of moderate leucocytosis. The growth of enzyme activity (ALT, AST) pointed to developing destructive processes in liver tissue, the intensity of which directly depended on the size and area of specific surface of NPs. Biochemical blood tests confirmed the potential adverse impact of NPs CuZn. In terms of the severity of these phenomena, taking into account the comparable size and specific surface area, the NPs CuZn IV (65 nm) and the NPs CuZn III (50 nm) should be attributed to nanoparticles with unstable biological action in comparison with the NPs CuZn II (43 nm) and the NPs CuZn I (21 nm). Regardless of the size, the introduction of NPs CuZn led to a decrease in amylase activity, both in the pancreas and duodenum, which may indicate a lesion of acinar cells that produce it. At the same time, the observed increase in protease activity by the end of the experiment may indicate tension in the system of adaptation of digestive enzymes. Thus, the examined NPs CuZn in biota dose, but with different physical and chemical characteristics at the maximum degree of impact on the haemostatic system and the activity of digestive enzymes in the pancreas, can be represented as follows: NPs CuZn IV > NPs CuZn III > NPs CuZn II > NPs CuZn I.

Keywords: rats, nanoparticles (NPs) CuZn, blood, amylase, lipase, protease

INTRODUCTION

Research and development of new nanomaterials is high in the US, EU, China, Sweden, Germany and Denmark (Zhao et al., 2008; de Wit, 2009; Kastenhofer, 2011). According to Zhao et al., 2008) and de Wit, 2009), nanomaterials research is an area with enormous socio - economic potential for the development new drugs, electronics, pesticides and fertilizers, and the mitigation of environmental problems.

Nanoparticles (NPs) are particles smaller than 100 nm (Chapman et al., 2010; Gangadoo et al., 2016), and due to their size, have unique physical and chemical properties (Rai et al., 2009), such as increased biocompatibility and a higher surface area-to-volume ratio, often showing lower toxicity than the corresponding bulk metals. The size of nanoparticles makes them a unique platform for the delivery of various substances to the body, thanks to their ability to pass through endocytic,

lymphatic (Chen and Langer, 1998) and cell membranes (Florence et al., 1995). The most important characteristics of nanomaterials, from the point of view of the European Commission assessment, are: size, size distribution, surface area, stability in respective media, surface adsorption properties and solubility in water (SCENIHR, 2010; SCENIHR, 2013).

The most important problem is the lack of information on the degree of influence of nanomaterials on the organism, depending on their physical and chemical characteristics. Hansen (2009) and Ray (2009) mentioned a number of examples of environmental hazards of nanomaterials such as gold, silver, titanium dioxide, zinc and iron oxide to humans, rats, mice, Danio fish, juvenile cattle and various strains of bacteria. Therefore, NPs need different certification categories before they can be certified for safe use. It is important to note that in a limited number of previous studies, nanoparticles showed low ecotoxicity, but most studies showed some degree of adverse effects on tested animals or cells (Sizova, 2014, 2015).

Currently, a significant number of nanomaterials in various forms and aggregate states, including the form of alloys (FeCo, CuZn), have been developed. The combined use based on the different biological effects of metal antagonists in the organism (Sizova et. al., 2018), as well as the beneficial difference between nanoparticles and their analogues in the form of mineral salts.

It has been proven that the addition of copper increases the conjugation of zinc with large molecules and depletes the ratio of zinc in combination with smaller molecules, thereby offering an antagonism between copper and zinc (Pang and Applegate, 2007). The influence of copper and zinc is associated with participation in enzymatic reactions, in particular in the development of digestive enzymes, the activity of which depends on the absorption of nutrients and the development of organs and tissues (De Lisle, 1996; Underwood, 1977).

The aim of this work was to study the effectiveness of nanoparticles of a CuZn alloy with various physico-chemical systems of haemostasis, including organ weights, organ index and activity of digestive enzymes in the pancreas, and the duodenal chyme in rats.

MATERIALS AND METHODS

The experimental part of the work was carried out in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (national standard of the Russian Federation GOST R 53434-2009) with standard procedures for the operation of biological objects. Animal care was carried out according to the rules of laboratory practice during preclinical studies in Russia (GOST 3 51000.4-96). The experiments were carried out in accordance with the requirements of humane treatment of animals. The content of animals and procedures for the experiments were in accordance with the instructions and recommendations provided for by national regulations (order of the Ministry of health of the USSR 755 of 12.08.1977) and «The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, 555 D.C., 1996)».

2.1 Characteristics of nanoparticles (NPs) CuZn

CuZn alloy of four physical and chemical characteristics was used as a source of trace elements (Table 1).

The first 3 CuZn samples were obtained by gas-phase synthesis. The particle sizes were estimated on the basis of specific surface area measurements, using the Sorbi®-M device (Meta®, Russia). The microstructure of the powders was analysed using a transmission electron microscope, Philips CM-30 (Philips, Japan). To determine the phase composition, Rigaku D/MAX-2200VL/PC diffractometer (Rigaku, Japan), Cu Ka radiation.

Table 1 : Characteristics of NPs CuZn

Sample No.	NPs	Specific surface, m^2/g	Size, nm	Phase composition, %		
				Zn	ZnO	Cu
1	CuZn I	36,0	21,0±0,57	17,2	49,8	29,4
2	CuZn II	22,0	43,0±0,55	13,6	34,1	33,7
3	CuZn III	15,0	50,0±0,54	52,3	8,10	7,90
4	CuZn IV	5,0-6,0	65,0±0,54	40,0	-	60,0

The fourth sample of CuZn produced by Advanced Powder Technologies LLC (Tomsk, Russia) was used. NPs CuZn were obtained by the method of electrical explosion of wire in an argon atmosphere.

Aqueous slurry NPs CuZn were treated with an ultrasound disperser of UZDN-2T (NPP Akadempribor, Russia) at 35 kHz, 300/450 W, 10 mA for 30 min. Aqueous slurry NPs CuZn were treated introduced into the feed mixing step.

2.2 Experimental groups and dosage

The studies were carried out on 75 white Wistar rats weighing 70-80 g in the standard conditions of the laboratory where biological tests and examinations were conducted (Federal Scientific Centre of Biological Systems and Agrotechnology of the Russian Academy of Sciences). After a preparatory period of 7 days, the animals were divided into 5 groups (n = 15). Animals in all groups received a basal diet during the experimental period (21 days). Group 1 was fed the addition of NPs sample No. 1, at a dose of 2.3 mg/kg; group 2 was fed NPs sample No. 2, at a dose of 2.3 mg/kg; group 3 – NPs sample No. 3, at a dose of 2.3 mg/kg; group 4 – NPs sample No. 4, at a dose of 2.3 mg/kg. The control group did not receive NPs.

The selected dose was justified by the results of previous experiments, where a pronounced stimulating biological effect was found when using NPs CuZn at a dose of 2.3 mg/kg of feed (Sizova et al., 2016, Rusakova et al., 2016). The feeding of rats was carried out with a semi-synthetic diet (basal diet), consisting of polished rice, 30 g; sucrose 1 g; soy concentrate 1.25 g; refined canola oil 1 g. Watering was carried out with double distilled water. Vitamin and mineral deficiency was balanced by addition of a mixture of fat and water - soluble vitamins.

2.3 Observation and autopsy

The growth of individuals was monitored daily by individual weighing, before feeding (± 2 g). Weight was used as a basis to calculate absolute and average daily growth, and to study the dynamics of growth of experimental animals, as well as the ratio of the mass of the studied organs to body weight. Biomaterial for the study was obtained after decapitation of rats under nembutal anaesthesia. The animals were then dissected and anatomically cut (bones, skin, skeletal muscles, internal organs) followed by weighing, grinding and forming an average sample (10 g) for each individual.

2.4 Haematology research

On the 21st day of the experiment, blood sampling was performed. Biochemical analysis of blood serum was carried out on the automatic biochemical analyser SS-T240 (Dirui Industrial Co., Ltd, China) using commercial biochemical kits for veterinary assessment DiaVetTest (DiaVetTest®, Russia), and commercial biochemical kits Randox (Randox®, USA). The content of erythrocytes (10^{12}/L), leukocytes (10^{9}/L), haemoglobin (g/L) and haematocrit (%) was determined by the automatic haematological analyser URIT-2900 Vet Plus (URIT Medical Electronic Group Co., Ltd, China).

2.5 Determination of enzymatic activity

After the animals were euthanized, the abdominal wall was dissected, and the duodenum and pancreas were removed. By rubbing the pancreatic tissue sample (1 g) in a cold ringer solution (4 mL), a homogeneous suspension was obtained - an organ homogenate that was centrifuged at 3000 rpm for 10 minutes. Aliquots of pancreatic homogenates and duodenum were prepared by rubbing a sample of pancreatic tissue (1 g) in a cold ringer solution (4 mL). A homogeneous suspension was obtained, an organ homogenate, which was centrifuged at 3000 rpm for 10 minutes. Amylase activity was determined by the method of Coles (1986) after incubation of organ homogenate with 1% starch solution, followed by the addition of iodine solution, and then by the determination of the optical density of the samples. The lipase activity of the samples was determined by the Boutwell (1962) method of aerobic incubation of pancreatic homogenate and duodenum with olive oil suspension, followed by titration. Protease in the tissue samples of the pancreas and duodenum 12 was analysed using the method of Batoeva (1971) (Cleavage of 0.1 % solution of casein at colorimetric control). The activity of the studied enzymes was expressed in conventional units (The difference between the readings of the sample spectrophotometer with the substrate and the idle sample per gram of wet sample of the intestinal mucosa per minute).

2.6 Statistical analysis

The statistical analysis compared data obtained from the experimental groups with the control, using software SPSS 19.0 (IBM Corporation, USA) and Statistica 10 (StatSoft Inc., USA.) The value of $p \leq 0.05$ was considered statistically significant. The indicators of body

weight and organs of animals were subjected to an ANOVA and a multiple-comparison test by Sheffa, which was used when the differences were significant.

RESULTS

3.1 Analysis of morphological and biochemical parameters of blood

The results of our studies showed a change in haemostatic system parameters after the introduction of NPs CuZn in relation to the control group (Table 2).

During the analysis of the morphological parameters of the blood, it was revealed that in animals in groups 1, 2, 3 and 4, the content of erythrocytes decreased by 5.63, 4.59, 8.00 and 8.44%, respectively, relative to the control analogue. The content of haemoglobin was positively correlated with the content of red blood cells. Thus, in group 4, the maximum decrease in haemoglobin level was by 6.74%, relative to the control group.

The number of leukocytes in animals of all groups was increased. The maximum significant increase was established in groups 2 and 4 (by 77.8 and 67.9% ($p \leq 0.05$), respectively), compared with the control group. Based on the percentage of leukocytes of different types (neutrophils, eosinophils, basophils, lymphocytes and monocytes), the characteristics of which determine the physiological state of the body, the percentage of lymphocytes and monocytes in group 2 was lower by 5.80 and 2.63%, respectively, against the background of an increase in this indicator in the other groups, relative to control. At the same time, the content of granulocytes in group 2 was higher by 7.58% with the maximum significant decrease in the content of granulocytes in group 1 by 27.8% ($p \leq 0.05$), relative to the control values.

Reduction of platelets results in increased risk of vascular injury, and development of prolonged bleeding. Conversely, high platelets in the blood increased the probability of thrombosis, embolism and the formation of thromboembolism (I.R. Shamsutdinova, 2015). The platelet count of groups 1, 2 and 4 was found to be higher by 6.46, 1.57 and 26.3%, against the background of an decrease by 5.29% in group 3, relative to the control values.

Evaluating biochemical parameters of animal blood at the last stage (after 21 days), several changes were observed. First, in the established hyper-and hypoglycaemic effect, a uniform increase in glucose was observed in groups 1 and 3 at 9.18 and 6.84%, respectively, relative to control. In groups 2 and 4, this figure was reduced by 4.51 and 1.40%, respectively, compared with the control.

The second change was in activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Their increased activity is a key indicator of liver disease and destructive (damaging) changes in the liver parenchyma. Despite the absence of pronounced organ specificity, the determination of the activity of AST and ALT has a great diagnostic value. The high activity of AST was noted, which was greater than the control value by 47.3 and 63.3% ($p \leq 0.05$) in groups 2 and 3, against the background of a decrease in this indicator in groups 1 and 4 by 6.96 and 17.1%, compared with the control. In addition, the level of ALT was increased (17.3 ($p \leq 0.05$) and 1.94% in groups 3 and 4), with a decrease in the activity in groups 1 and 2 (13.3 and 5.32% ($p \leq 0.05$)), relative to the control (Fig 1)

Table 2 : The effect NPs CuZn in various forms on the morphological parameters of blood of rats

	Control	2,3 мг/кг CuZn I (1group)	2,3 мг/кг CuZn II (2 group)	2,3 мг/кг CuZn III (3 group)	2,3 мг/кг CuZn IV (4group)
Leukocytes, 10^9/l	8,10±1,56	9,25±0,78	14,4±0,57*	9,75±0,86	13,6±0,29*
Lymphocytes, %	41,4±1,66	50,7±1,96*	39,0±1,55	41,5±0,20	42,6±1,47
Monocytes, %	19,0±1,39	20,7±0,29	18,5±0,94	20,4±0,24	19,9±1,39
Granulocytes, %	39,6±2,79	28,6±1,67*	42,6±0,61	38,4±0,04	37,6±2,86
Lymphocytes, 10^9/l	6,23±0,58	4,65±0,20	5,60±0,00	4,00±0,33	5,80±0,35
Monocytes, 10^9/l	2,83±0,12	1,90±0,16	2,70±0,24	2,00±0,16	2,70±0,23
Granulocytes, 10^9/l	2,50±0,98	2,70±0,41	6,10±0,33**	3,75±0,37	5,10±0,29
Erythrocytes, 10^{12}/l	6,75±0,42	6,37±0,16	6,44±0,13	6,21±0,19	6,18±0,02
Haemoglobin, g/l	141,0±7,09	140,5±2,86	143,0±3,27	137,5±4,49	131,5±2,59
Haematocrit, %	34,3±2,10	34,1±0,78	33,5±0,37	34,0±1,71	31,9±0,64
Platelets, 10^9/l	204,3±71,4	217,5±27,4	207,5±6,12	193,5±8,57	258,0±13,3

* – the results are statistically reliable ($p \leq 0,05$);

** – the results are statistically reliable($p \leq 0,01$);

*** – the results are statistically reliable (p≤0,001)

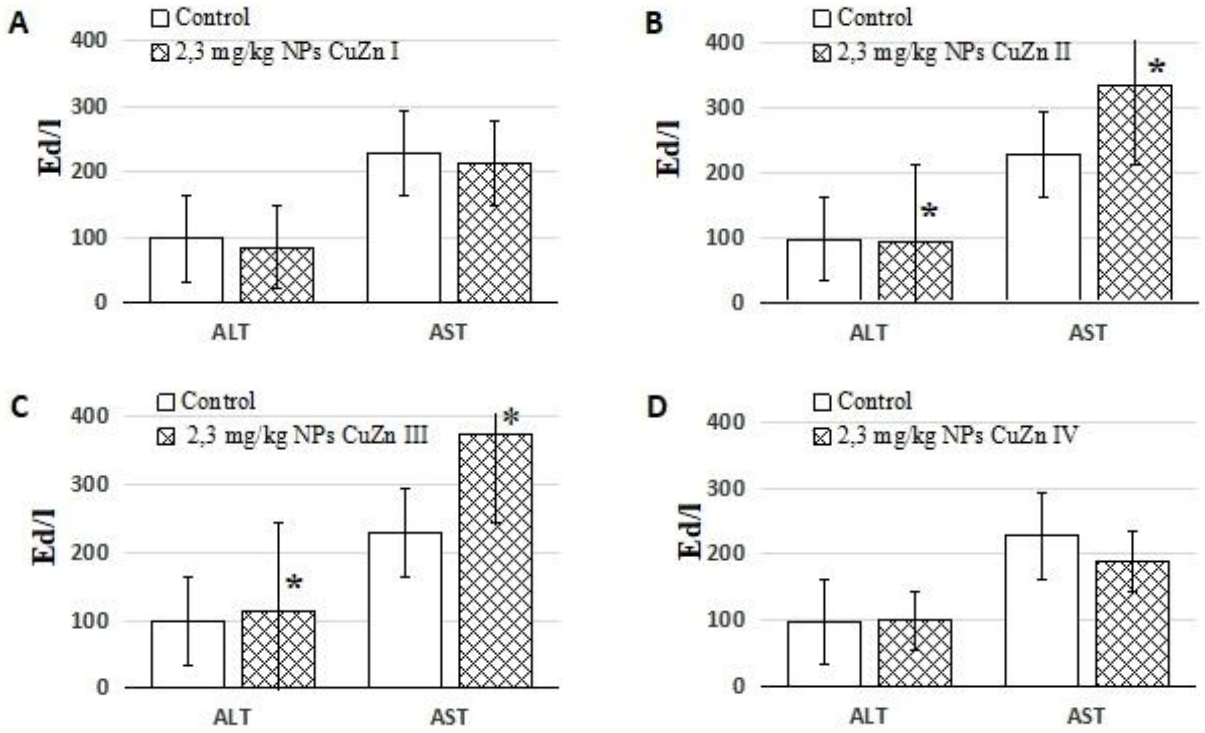

Figure 1; The content of transaminase enzymes (ALT and AST) in the blood of rats after the introduction of NPs CuZn, Ed/l. A) 2,3 mg/kg NPs CuZn I. B) 2,3 mg/kg NPs CuZn II. C) 2,3 mg/kg NPs CuZn III. D) 2,3 mg/kg NPs CuZn IV. * – the results are statistically reliable (p≤0,05); ** – the results are statistically reliable(p≤0,01); * – the results are statistically reliable (p≤0,001).**

Other changes were as follows: The decrease of g-GT activity in groups 3 and 4 was found to be 3.62 and 3.43 (p≤0.001) times, against the background of 10 times (p≤0.001) decrease in 1 group, relative to control. The different reactivity of nanoparticles depending on the specific surface area was manifested by an increase in creatinine in groups 2, 3 and 4 against the background of its decrease by 5.10% in group 1, when compared with the control. The effect on the mineral composition of blood NPs CuZn was accompanied by an increase in the Mg level in all groups, with a significant increase in the Mg level by 3.28 and 3.20 (p≤0.01) times in groups 2 and 4, relative to control.

Metabolism of Ca and P, when included in the diet of NPs CuZn, was affected with a decrease in Ca content by 7.25% in group 4, and P by 22.8 and 31.2% in groups 1 and 3 and an increase in P by 3.96 (p≤0.01) times in group 4, compared with the control indicators. The Fe content tended to decrease in all groups, with a maximum decrease in group 1 by 47.6% and in group 3 by 6.05% (Fig 2).

The conducted research has shown that all studied parameters in laboratory animals were within the limits of admissible physiological norms. However, the changes in morphological and biochemical parameters of the blood were not limited to physiological standards, and it is important to assess the emerging trends and minor changes occurring within this norm against the background of the introduction of NPs CuZn in various forms.

3.2 Influence of NPs CuZn on the mass of internal organs

The biotic dose of 2.3 mg/kg of NPs alloy CuZn I (group 1) and CuZn II (group 2) contributed to the maximum weight reduction of the kidneys, heart, liver and spleen. The lungs and brain were less exposed to nanoparticles, which was expressed as a smaller change in their mass.

Total reduction in body weight was observed when NPs CuZn III was added to the diet (group 3): heart (33.5%), kidneys (27.4%) and liver

(22.1%) of lungs (7.88%) and spleen (4.48%) when compared with intact animals. There was less influence of NPs on the mass of the brain. When NPs CuZn IV was provided to group 4 (heart - 25.7%, and kidney-19.8%, liver - 13.5%). Early development of liver and lung tissue pathology was described with the inhalation administration of ZnO to rats at a dose of 2.5 mg/kg and Cu (Huang Y., Yan G, et. al., 2008). Perhaps these elements caused a disruption in energy metabolism, mitochondria and cell membranes of the parenchymal organs.

3.3 The effect of NPs CuZn on body weight (BW), pancreatic mass (PM), and ratio of pancreatic mass to body weight (PM/BW) of rats

Due to the special role of copper and zinc in the function of the pancreas and its enzymatic activity, the relative body weight of rats to the mass of the pancreas (PM/BW) was calculated (Fig 3).

The use of NPs CuZn IV (group 4) was accompanied by a maximum decrease of BW by 16.2% compared with the control. A similar trend of a uniform decrease in BW continued when using NPs CuZn I (group 1), NPs CuZn II (group 2) and NPs CuZn III (group 3) at 12.9, 12.7 and 11.9%, respectively. Compared with the control values, the mass of the pancreas (PM) in the group receiving the NPs CuZn IV (group 4) alloy was 13.9% lower, against the background of the stimulating effect of the NPs CuZn III (group 3) by 23.7%, and correlated with the glucose content in these groups.

Evaluation of relative values largely determines compliance with the physiological level of development. The highest indicators of PM/BW were established using the NPs CuZn III (group 3), they differed by 40.4% relative to the control values. The indicator of the PM/BW using the NPs CuZn IV (group 4), NPs CuZn I (group 1) and NPs CuZn II (group 2) was 2.70 %, 14.0% and 12.5%, relative to control.

3.4 Activity of digestive enzymes in the pancreas and the chymus of the duodenum

The dynamics of enzymatic activity of the pancreas, as a primary agent in the digestive system, was characterized by an increase in the activity of amylase in rats of group 1 for 7 days (93.7%) and a decrease in group 2 (48.8%). On day 14, there was a significant suppression of amylase activity in groups 3 and 4 (by 47.9, 50.0%, respectively) compared to the control (Fig 4).

Adaptation of amylase activity occurred by day 21, except for group 3, which when compared with the control values was 44.9% lower. In the duodenum there were more pronounced changes in the activity of amylase, manifested by a decrease of 7 and 14 days in groups 2, 3 and 4 by 49.0-50.9%. By day 21, the activity of amylase also decreased, 75.8 and 86.2% in groups 3 and 4, respectively.

In the pancreas, another proteolytic enzyme pattern was observed. Regardless of the dimension of the nanoparticles on day 7, the decrease in protease activity relative to control values ranged from 35.7 to 70.5%, while on day 14, they ranged from 68.6 to 74.6%, compared with the control. On 21 day, protease activity was higher than the control values in groups 2 and 3 by 57.8 and 61.9%, respectively.

A similar mixed effect of nanoparticles on the activity of protease was observed in the intestine, which on day 7 and 14 decreased in all groups, with group 1 experiencing the biggest effect (97.38%). A subsequent stimulatory effect was observed for day 21 in group 2 (46.3%) (Fig 5).

Lipolytic activity in the pancreas in all periods decreased, with a minimum of 7 days (33.5-58.9%), with some stabilization after 14 days, and subsequent decrease at 21 days, with a significant difference between the control and groups 3 and 4, at 41.4 and 3%, respectively.

In the duodenum, lipase activity on day 7 was significantly lower than control in all experimental groups (12.1-41.5%). On day 14, lipase activity varied in different directions, with group 1 growing higher than control by 3.2%, and groups 3 and 4 – lower by 52.2-55.1%. On day 21 in groups 2 and 3, lipase activity increased, compared with the control by 2-14%, while in groups 1 and 4, activity decreased.

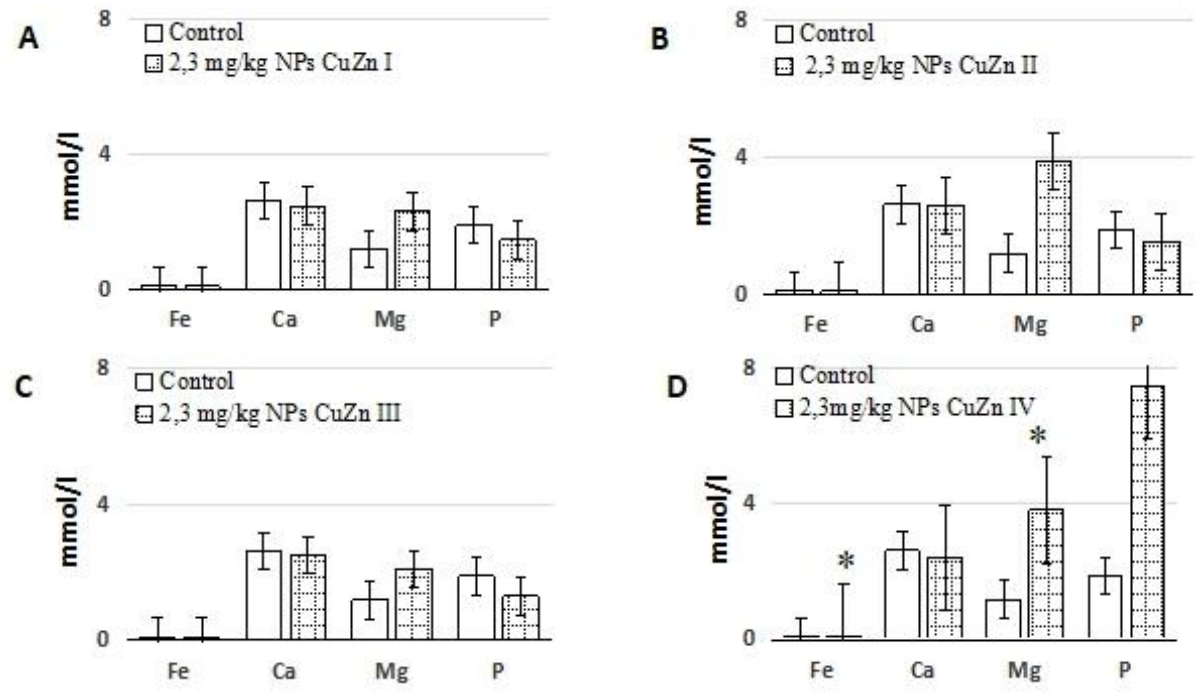

Figure 2; Concentration of chemical elements in the blood of rats after the introduction of NPs CuZn, mmol/l. A) 2, 3 mg/kg NPs CuZn I. B) 2,3 mg/kg NPs CuZn II. C) 2, 3 mg/kg NPs CuZn III. D) 2,3 mg/kg NPs CuZn IV. * – the results are statistically reliable (p≤0,05); ** – the results are statistically reliable(p≤0,01); * – the results are statistically reliable (p≤0,001).**

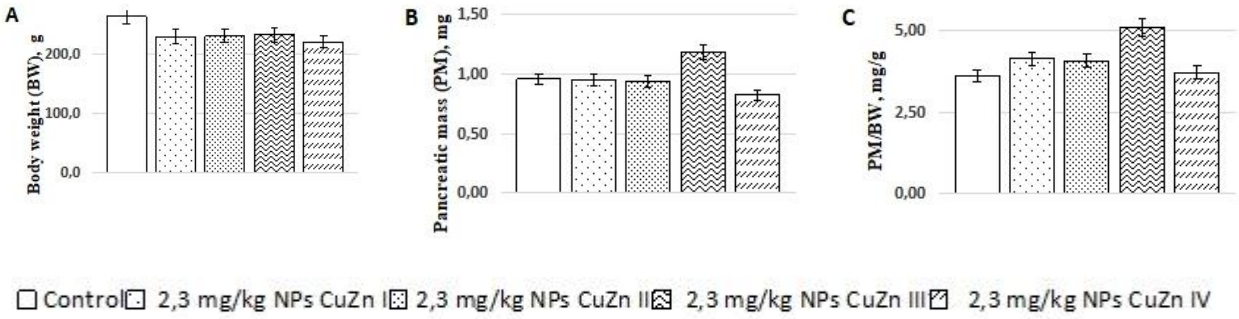

Figure 3; Quantitative analysis of the last body weight (BW) (A),the pancreas mass (PM) (B) and pancreas-to-body mass (PM/BW) (C) of the rats. * – the results are statistically reliable (p≤0,05); ** – the results are statistically reliable(p≤0,01); * – the results are statistically reliable (p≤0,001).**

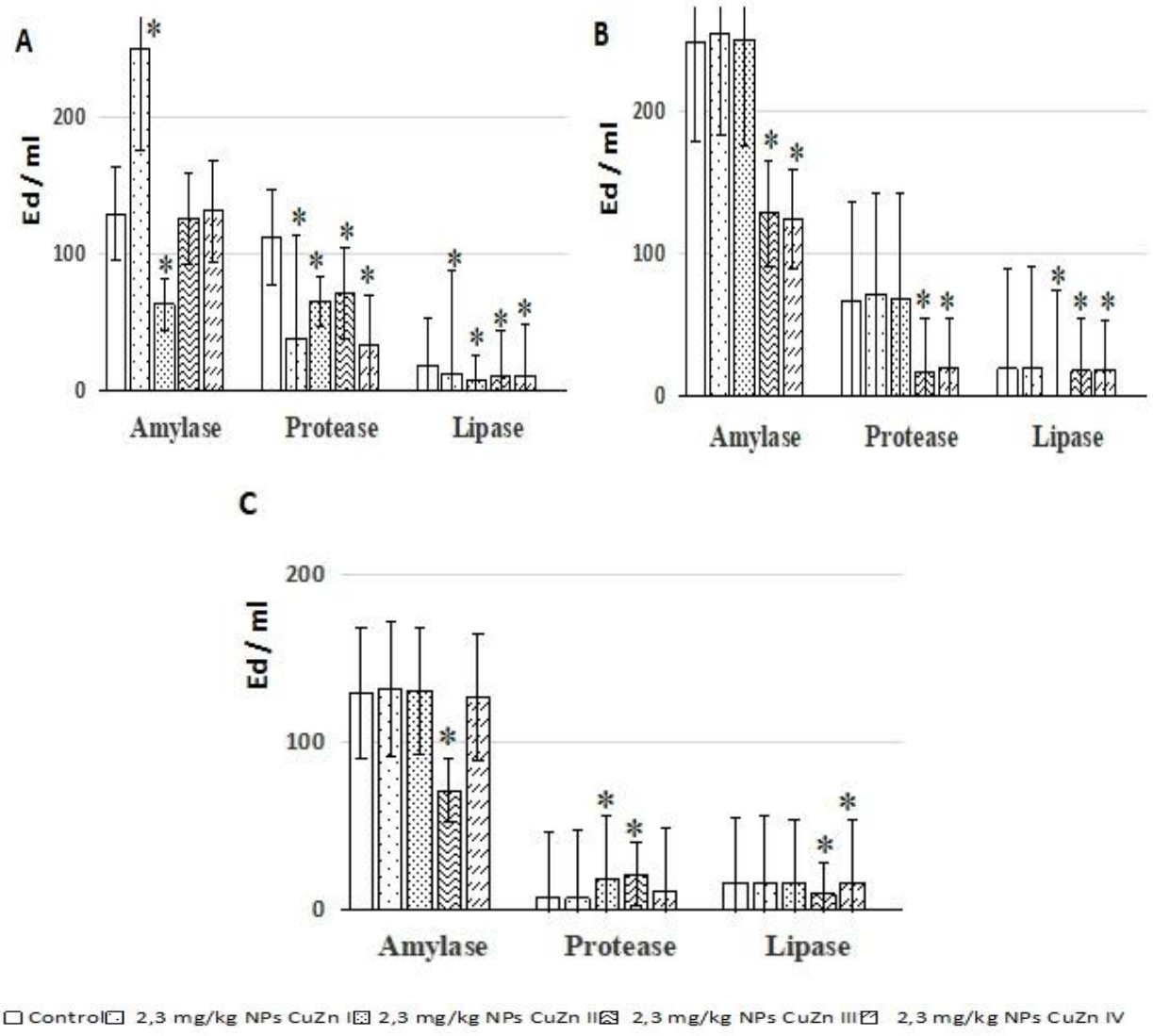

Figure 4; Activity of digestive enzymes in the pancreas. A) 7 days of the exhibition. B) 14 days of the exhibition. C) 21 days of the exhibition. * – the results are statistically reliable (p≤0,05); ** – the results are statistically reliable(p≤0,01); * – the results are statistically reliable (p≤0,001).**

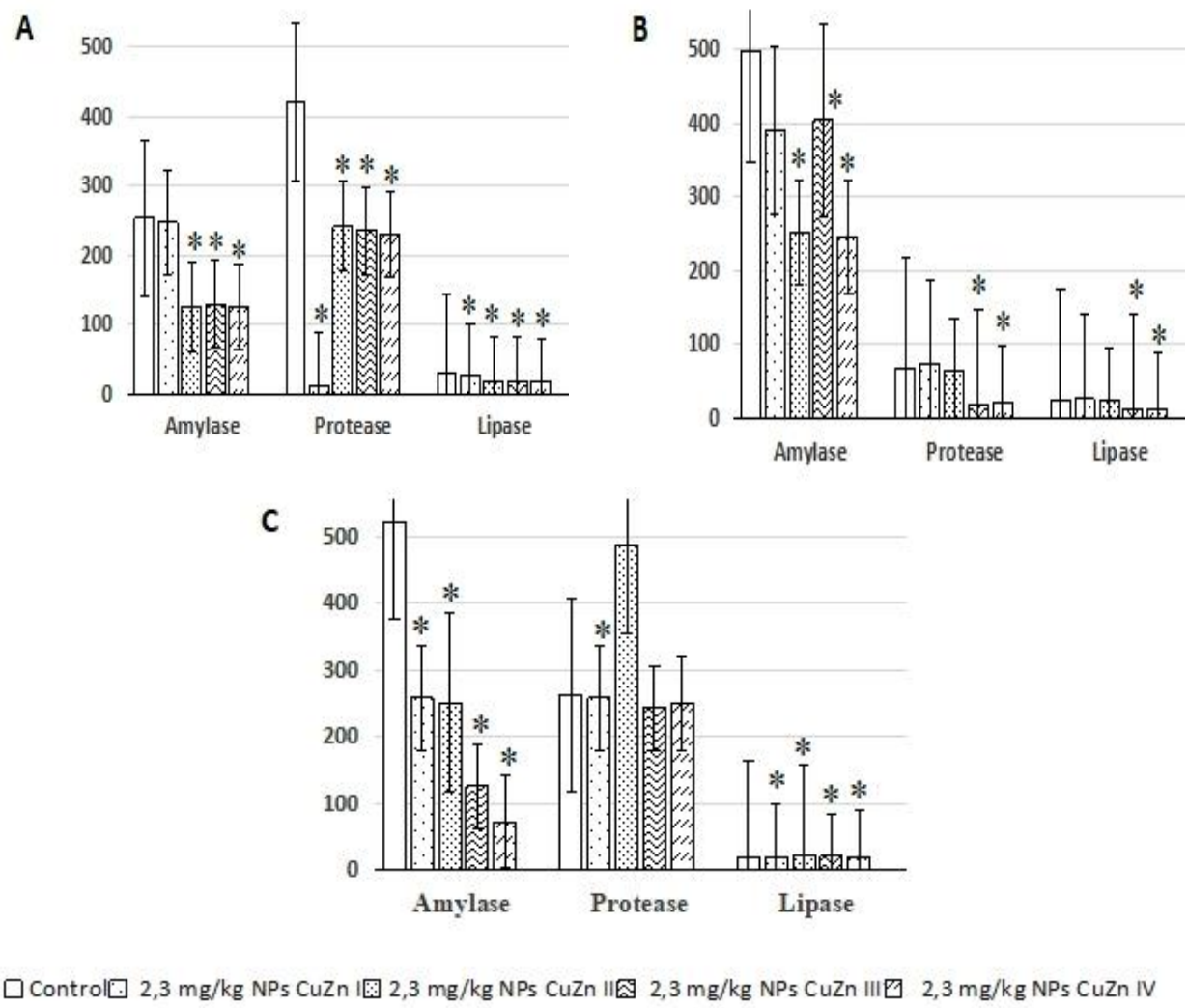

Figure 5; Activity of digestive enzymes in the chymus of the duodenum. A) 7 days of the exhibition. B) 14 days of the exhibition. C) 21 days of the exhibition. * – the results are statistically reliable (p≤0,05); ** – the results are statistically reliable(p≤0,01); * – the results are statistically reliable (p≤0,001).**

In general, the activity of enzymes was lower in the pancreas than in the duodenum, which is due to the mechanisms of enzyme activation in the duodenum.

DISCUSSION

In this study, we have shown for the first time that NPs CuZn, with different physical and chemical characteristics, has different effects on the haemostatic system, as well as on the activity of digestive enzymes in the pancreas and the duodenal chyme of laboratory animals (Wistar rats).

Based on the results obtained, a stable dependence was formed, with a decrease in the specific surface area and an increase in the size of nanoparticles. Additionally, the number of erythrocytes and haemoglobin decreased, which can be explained by the kinetics of particles in the gastrointestinal tract, the rate of penetration into the bloodstream (Hot et. al., 2004) and possible dysfunction in the haematopoiesis system. The absorption of nanoparticles by erythrocytes depends on the size of nanoparticles (Peters et. al., 2006), while the charged material type plays a small role (Rothen-Rutishauser et. al., 2006).

Based on toxicological studies, small nanoparticles (up to 30 nm) cause more adverse effects on living organisms than larger particles

made from the same material (Donaldson and Stone, 2004; Ferin et. al., 1992; Gurr et. al., 2005; Oberdörster et. al., 1994). Since smaller nanoparticles are easier to penetrate through cell membranes, they are grouped to form aggregates of more than 100 nm in size and thus disrupt cellular nutrition (Oberdörster, 1988; Takenaka et. al., 2001). Metal nanoparticles less than 30 nm rapidly move in the blood circulation system, spread in organs and tissues and then subsequently disrupt metabolic processes, both in the cell and in the body (Chen et. al., 2006; Wiebert et. al., 2006).

Biochemical blood analysis is a recognized informative test that reflects the general condition of animals, and allows us to judge the immunological reactivity of the body (Shamsutdinova, 2015). Evaluating the biochemical parameters of animal blood, the increase of enzyme transaminases activity (ALT and AST) was established. In our opinion, the degree of increase in the activity of aminotransferases can be due to the intensive activity of the cardiovascular system and damage to liver cells, which is associated with the process of particle exit from damaged organs and tissues into the bloodstream, and indicates the possible presence of developing destructive processes in hepatocytes and cardiomyocytes (Komarov, 2002). At the same time, an increase in the activity of aminotransferases in the blood of animals can be an adaptive reaction of the body, one of the protective measures in the adaptation process to additional stress effects of external factors.

Evaluating biochemical parameters of animal blood at the last stage (after 21 days), the following changes were recorded: first, in the established hyper-and hypoglycaemic effect. It is known that 65-70% of circulating blood glucose is utilized by the central nervous system. This is due to the risk of hypoglycaemic conditions, resulting in changes in brain metabolism and, ultimately, leading to the death of neurons and as a consequence of brain dysfunction (Malone and Ullrich, 2007). Neurotoxic action of nanoparticles is inversely proportional to their size. The smaller the particle, the greater the surface-to-volume ratio, while the larger surface area affects the increase in reactivity (Roduner, 2006), oxidation (Donaldson and Stone, 2002), and DNA damage (Risom, 2005).

The suppression of weight characteristics may be associated with different degrees of accumulation of nanoparticles. In studies of De Jong, W. H., et.al., 2008, it was found that nanoparticles of 10 nm, accumulated in almost all organs, while larger nanoparticles (50-250 nm) were localized primarily in the liver, spleen and blood. Particle aggregation is also an essential factor in cytotoxicity. High concentrations of nanoparticles were aggregated (Churg et. al, 1998; Gurr et. al., 2005), and hence the toxic effects were reduced, compared to lower concentrations (Takenaka et. al., 2001) since the area of the reactive surface was reduced and the translocation of particles was limited.

In general, enzyme activity was lower in the pancreas than in the duodenum, which is due to the mechanisms of activation of proenzymes in the duodenum and being in the pancreas in an inactive state, a form of self-defence of the gland from digestion (Saunders and Wormsley, 1975).

As known, one of the mechanisms of toxic effects of nanoparticles is the release of ions, for example, zinc ions have a pronounced effect on the activity of pancreatic lipase. Repression of enzyme activity on day 7 and 14 may be associated with the development of pathological processes in the gland and a decrease in the acinar cells producing it (Calvano et al., 2014).

In general, the literature discusses the effect of zinc as having a distinct inhibitory effect on the activity of enzymes. Depression of enzyme activity under the action of zinc may be due to the ability of proteins for nonspecific binding. The mechanism of inhibition of activity, apparently, is the formation of metal ion compounds with functional groups of protein amino acids and changes in their physical and chemical properties (Gureeva, 2009).

On the contrary, the addition of copper ions contributed to the increase in the level of lipase in the intestine (Dove, 1995). It has been shown that high concentrations of dietary copper increase the growth of pigs by stimulating the activity of enzymes involved in the use of nutrients (Luo and Dove, 1996; Xia et al., 2004). It is interesting that the inhibition of proteases in the experiment of Chen (1998), occurred only after the dissolved concentration of copper in the intestinal liquid reached the threshold. Before reaching the same threshold, amino acids in the intestine formed complexes with copper, preventing the inhibition of protease by interacting with enzymatically active sites.

A decrease in the activity of amylase, both in the pancreas and the duodenum, may indicate a lesion of acinar cells that produce it. At the same time, the observed increase in protease activity by

the end of the experiment may indicate an adaptive reaction. Along with the stimulating pancreatic secretion effect, there is also an inhibitory influence. It is transmitted by sympathetic excitation and vasoconstriction, as well as by the release of hormonal factors — inhibitors — from the lower part of the small intestine and colon (Cheng, 2016).

The high enzyme activity also can indicate lesions of pancreatic tissue or obstruction to the outflow of pancreatic fluid, and low enzyme activity associated with a decrease in the number of acinar cells that produce the enzymes.

Activation of enzymes can be associated with the activation of the sympathetic-adrenal system of the body and the release of catecholamines in the blood under the introduction of various substances, since the enzymes are sensitive to the action of a number of hormones, including adrenaline, which causes cAMP-dependent activation of this enzyme, which indicates the development of stress reaction (Brokerhof and Jensen, 1978). At the same time, the stimulation of the digestive system may be important in feeding, since the activation of the enzymatic system leads to a more complete use of the diet, and as a consequence, a decrease in feed consumption.

Excessive or toxic amounts of zinc reduce the secretion of enzymes in the pancreas, so in our study we can also talk about the adverse effects of this alloy. At the same time, it is very interesting that zinc is an antagonist of copper, contributing to the levelling of pro-oxidant effects arising under the influence of zinc (Reid et al., 1987; Powell et al., 1999).

CONCLUSION

Thus, the examined NPs CuZn in biota doses, with different physical and chemical characteristics at the maximum degree of impact on the haemostatic system and the activity of digestive enzymes in the pancreas, can be represented as follows: NPs CuZn IV > NPs CuZn III > NPs CuZn II > NPs CuZn I.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The study was supported by the Program of Basic Scientific Research of the Russian Academy of Sciences (No. 0761-2018-0031).

AUTHOR CONTRIBUTIONS

IAG and OVK designed and performleed the experiments. EAR wrote the manuscript. SVL performed enzymes activity. EVSh performed data analysis. All authors read and approved the final version.

REFERENCES

Batoyev CJ, 1971. Photometric determination of the activity of proteolytic enzymes in the pancreas, the juice to reduce the concentration of casein. Agricultural Institute. 25:122-126.

Boutwell Jr JA. 1962. Clinical chemistry – Laboratory manual methods. Philedelphia: 212-214.

Brockerhoff H, Jensen R, 1978. Lipolytic enzymes. M:Mir. pp. 396.

Calvano J, Edwards G, Hixson C, Burr H, Mangipudy R, Tirmenstein M, 2016. Serum microRNAs-217 and− 375 as biomarkers of acute pancreatic injury in rats. Toxicology. 368: 1-9.

Chapman J, Weir E, Regan F, 2010. Period four metal nanoparticles on the inhibition of biofouling. Colloids Surf B Biointerfaces. 78(2): 208–216.

Chen H, Langer R, 1998. Oral particulate delivery: status and future trends. Adv Drug Deliv Rev. 34(2): 339–350.

Chen Z, Mayer LM, 1998. Digestive proteases of the lugworm, Arenicola marina, inhibited by Cu from contaminated sediments. Environ Toxicol Chem. 17: 433–438.

Cheng HM, 2016. Neuro-Gastroenterology. In Physiology Question-Based Learning (pp. 199-213). Springer, Singapore.

Churg A, Stevens B, Wright J. 1998. Comparison of the uptake of fine and ultrafine TiO2 in a tracheal explant system. Am. J. Physiol. 274: 81–86.

Coles H, 1986. Pancreatic function test In: Veterinary clinical pathology. Philadelphia. pp: 420-426.

De Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJ, Geertsma RE, 2008. Particle size-dependent organ distribution of gold nanoparticles after intravenous ad-ministration. Biomaterials 29: 1912–1919.

De Lisle RC, Sarras Jr MP, Hidalgo J and Andrews GK, 1996. Metallothionein is a component of exocrine pancreas secretion: implications for zinc homeostasis. Am. J. Physiol. Cell Physiol. 271:C1103-C1110.

De Wit C, 2009. New nano-materials environmental risks and possibilities," Summary of a Symposium at the Royal Swedish Academy of Sciences. May 2008, arranged by the Environmental Committee and sponsored by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), the Swedish Environmental Protection Agency and the Swedish Chemicals Agency. 592.

Donaldson K, Stone V, 2002. Current hypotheses on the mechanisms of toxicity of ultrafine particles. Annali dell'Istituto superiore di sanitÃ 39: 405–410.

Donaldson K, Stone V, Tran C, Kreyling W, Borm PJ, 2004. Nanotoxicology. Occup. Environ. Med. 61: 727–728.

Duncan TV, 2011. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. J. Colloid Interface Sci. 363: 1-24.

Ferin J, Oberdörster G, 1992. Polymer degradation and ultrafine particles: potential inhalation hazards for astronauts. Acta Astronautica 27: 257–259.

Florence AT, Hillery AM, Hussain N, Jani PU, 1995. Nanoparticles as carriers for oral peptide absorption: studies on particle uptake and fate. J Control Release 36(1): 39–46.

Gangadoo S, Stanley D, Hughes RJ, Moore RJ, Chapman J, 2016. Nanoparticles in feed: progress and prospects in poultry research. Trends Food Sci Technol 58:115–126.

Gangadoo S, Stanley D, Hughes RJ, Moore RJ, Chapman J, 2017. The synthesis and characterisation of highly stable and reproducible selenium nanoparticles. Inorg and Nano-Met Chem 47(11):1568–1776.

Gureeva NB, 2009. Pancreatic lipase: activation, inhibition and Association with lipid peroxidation. Chemical and pharmaceutical journal. 11: 30-35.

Gurr J-R, Wang AS, Chen C-H, Jan K-Y, 2005. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213: 66–73.

Hansen SF, 2009. Regulation and Risk Assessment of Nanomaterials- Too Little, Too Late? PhD Thesis. Technical University of Denmark, Lyngby, Denmark.

Hansen SF, Britt HL, Olsen SI, Baun A, 2007. Categorization framework to aid hazard Identification of nanomaterials. Nanotoxicology. 1: 243-250.

Hoet PH, Brüske-Hohlfeld I, Salata OV, 2004. Nanoparticles – known and unknown health risks. J. Nanobiotechnol. 2: 12.

Jigach AN, Leypunsky IO, Kuskov ML, Stanko N, Storozhev VB, 2000. Setup for production and study of physicochemical properties of metal nanoparticles. Devices and techniques of experiment. 6: 122-127.

Kastenhofer K, 2011. Risk assessment of emerging technologies and post-normal science. Sci., Technol. & human values. 3: 307-333.

Komarov FI, Korovkin BF, 2002. Biochemical indices in the clinic of internal diseases. Moscow "Medpress - information": 134 – 136.

Lebedev SV, Kudasheva AV, Ryabov N, Miroshnikov SA, 2016. On the prospects of nanopreparations based on alloys of microelements-antagonists (on the example of Fe and Co). Agricultural biology. 51. 14: 553-562.

Luo XG, Dove CR, 1996. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. Journal of animal science. 74: 1888-1896.

Malone J, Ullrich R, 2007. Novel radiation response genes identified in gene-trapped MCF10 Amammary epithelial cells. Radiat. Res. 2: 176-184

National Nanotechnology Initiative. 2011. Environmental Health and Safety Research Strategy. http://www.nano.gov/sites/default/files/pub_resource/nni_2011 _ehs_research_strategy.pdf /.

Nielsen FH, 1986. Other Elements. X. Tin (Sn). Trace Elements in Human and Animal Nutrition. 2.: 441-446.

Noonan CW, Kathman SJ, Sarasua SM, White MC, 2003. Influence of environmental zinc on the association between environmental and biological measures of lead in children. J Expo Sci Environ Epidemiol. 13:318–23.

Oberdörster G, 1988. Lung clearance of inhaled insoluble and soluble particles. J. Aero. Med. 1: 289–330.

Oberdörster G, Ferin J, Lehnert BE, 1994. Correlation between particle size, in vivo particle persistence, and lung injury. Environ. Health Perspect. 102:173.

Oberdörster G, Oberdörster E, Oberdörster J, 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 823–839.

Pang Y, Applegate TJ, 2007. Effects of dietary copper supplementation and copper source on digestive pH, calcium, zinc, and copper complex size in the gastrointestinal tract of the broiler chicken. Poult Sci. 86: 531–7.

Peters A, Veronesi B, Calderón-Garcidueñas L, Gehr P, Chen LC, Geiser M, Reed W, Rothen-Rutishauser B, Schurch S, Schulz H, 2006. Translocation and potential neurological effects of fine and ultrafine particles a critical update. Part. Fibre Toxicol. 3:1–13.

Powell SR, Gurzenda EM, Wingertzohan MA, Wapnir RA, 1999. Promotion of copper excretion from the isolated perfused rat heart attenuates post ischemic cardiac oxidative injury. Am J Physiol. 277:956–62

Rai M, Yadav A, Gade A, 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 27(1):76–83.

Ray PC, Yu H, Fu PP, 2009. Toxicity and environmental risks of nanomaterials: challenges and future needs. J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev. 27(1): 1-35.

Reid LS, Gray HB, Dalvit C, Wright PE, Saltman P, 1987. Electron transfer from cytochrome b5 to iron and copper complexes. Biochemistry. 26:7102–7.

Risom L, Møller P, Loft S, 2005. Oxidative stress-induced DNA damage by particulate air pollution. Mutation Res. 592: 119–137.

Roduner E, 2006. Size matters: why nanomaterials are different. Chem. Soc. Rev. 35:583–587.

Rothen-Rutishauser BM, Schürch S, Haenni B, Kapp N, Gehr P, 2006. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ. Sci. Technol. 40:4353–4359.

Rusakova EA, Sizova EA, Miroshnikov SA, Sipaylova OYu, Makaev SA, 2016. Hepatotoxic, hematological changes and Elemental status in pregnant Wistar rats under the action of Zn and ZnO Agricultural biology. 51(14): 524-532.

Saunders JH, Wormsley KG, 1975. Pancreatic extracts in the treatment of pancreatic exocrine insufficiency. Gut. 16(2):157.

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). 2010. Scientific basis for the definition of “nanomaterial” http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_032.pdf/:46.

Scientific Committees on Consumer Safety (2013). Health and environmental risks, emerging and newly identified health risks, rules of procedure. http://ec.europa.eu/health/scientific_committees/docs/rules_procedure_2013_en.pdf /:51.

Shamsutdinova IR, 2015. Changes in blood parameters of laboratory animals with the introduction of silver nanoparticles. news of the Orenburg state agrarian University. 6 (56): 122.

Shamsutdinova IR, 2015. Evaluation of the effects of biodoses of silver nanoparticles on the metabolism of proteins in animals. Innovative science . 10(3):17-20.

Sizova EA, Korolev VL, Mack WA, Miroshnikova EP, Shakhov VA, 2016. Morphological and biochemical blood parameters in broilers during correction of the diet with salts and Cu nanoparticles. Agricultural biology. 51: 903-911.

Sizova EA, Miroshnikov SA, Lebedev SV, Levakhin YuI, Babicheva IA, Kosilov VI, 2018. Comparative tests of various sources of microelements in feeding chicken-broilers. Sel’skokhozyaistvennaya Biologiya [Agricultural Biology]. 53(2): 393-403.

Snedecor GW, Cochran WG, 1976. Statistical Methods, The Iowa State Univ. Press. Ames. USA. : 298–300.

Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J, 2001. Pulmo nary and systemic distribution of inhaled ultrafine silver particles in rats. Environ. Health Perspect. 109: 547.

Underwood EJ, 1977. Iron. In: Trace Elements in Human and Animal Nutrition:13-35.

Zhang H, Yang D, Yang H, Liu H, 2008. Effect on

conception and offspring development in female parenatal rats following intratracheal instillation of nano-C/ZnO and C-ZnO composite nanoparticles. Wei Sheng Yan Jiu. (Chinese). 37(6): 654-656.

Zhao F, Zhao Y, Wang C, 2008. Activitiesrelated to health, environmental and societal aspects of nanotechnology in China. J. Cleaner Prod**.** 16: 1000-1002.

Zhao L, Seth A, Wibowo N, Zhao CX, Mitter N, Yu C, Middelberg APJ, 2014. Nanoparticle vaccines. Vaccine. 32: 327-337.