СОЈУЗ НА СТОПАНСКИ КОМОРИ НА МАКЕДОНИЈА
ВОДЕЧКА МАКЕДОНСКА ДЕЛОВНА МРЕЖА

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на

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SILICIUM CARBIDE ENGINEERED NANOPARTICLES: RISKS AND HEALTH EFFECTS

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SILICIUM CARBIDE ENGINEERED NANOPARTICLES: RISKS AND HEALTH EFFECTS

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Abstract

Last decades, the research work on new nano-structured materials and new nanotechnologies has been intensively performed. Nanostructures have shown remarkable properties such as high thermal stability, excellent mechanical and electrical characteristics which allowed them to have a wide application in many areas, starting from industrial application, even in food products, to gene therapy. Thus, tons and tons of nanoparticles enter in the environment and indirectly or directly into the biological systems, including the human body. However, due to their nano-dimensions, nanoparticles are showing environmental and health impacts. There are many controversial papers that describe interactions of the ENPs with the biological systems and rice concern that intentional or unintentional human exposure to certain types of engineered nanoparticles may lead to significant health i.e. toxicological effects.

In this work, the health effects and risk assessment of the SiC nanoparticles were studied. The toxicity of SiC nanoparticles on 4 weeks old female Wistar rats was investigated in comparison with the vehicle-control group treated with 0,5% w/v HPMC. 2, 7 and 14 days later, animals from the control and 2 experimental groups were sacrificed (n=3 per group at each study period) after being anaesthetized by ether. For biochemical and hematological tests, blood samples were collected from the eye vein by removing the eyeball quickly. The tissues and organs such as heart, liver, spleen, kidneys, lung, brain, small and lower intestine, and ovary were excised and prepared in a 10% formalin solution for histopathological diagnosis. All histopathological tests were performed using standard laboratory procedures. The slides were observed and the photos were taken using optical microscopy. The significant changes of serum LDH in both the experimental groups (app. 1580±30) in comparison with the control group (550±70,71) could indicate myocardial damage, which was confirmed by the histological analysis.

Keywords: engineered nanoparticles, nanotoxicology, health effects

1. Introduction

Development of the industrial sector based on application of engineered nanoparticels -ENPs and nanotechnologies is a fast-growing activity. Scientific achievements in many areas of nanotechnology have been successfully transferred into numerous practical applications in the electronics, food, (bio)pharmaceutical, chemical, cosmetic industry, etc.[1,2,3,4] Nanotechnology promises to exceed the impact of the "industrial revolution" and it is projected to become a $1 trillion market by 2015.[5] Due to their unique size and other physicochemical characteristics, ENPs have increased the solicitude for the influence on the

human health and ecological systems.[2,3,4] Researches in this field are with high priority and several reports on the toxicological properties of the ENPs already exist with many documented deleterious effects, particularly in animals.[6-9] The specific physicochemical properties that make nanomaterials useful are the same that may hazard the human body and the environment. Particle size distribution, surface area, porosity, shape, charge density, electrophoretic mobility, strength, flexibility, crystallinity/solubility, stability/ (bio) degradability, bio(muco)adhesivity, bio-compatiility, etc., they all can affect the biodistribution of the NPs and determine their health i.e. toxicological effects.

About the nanoparticles and engineered nanostructures: Nanosized particles (NPs) include all engineered and ambient nanosized spherical particles smaller than 100 nm (Fig. 1). ENPs include only spherical NPs specifically engineered in the laboratory. Other engineered nanosized structures are labelled according to their shape: nanotubes, nanofibres, nanowires, nanorings, etc. Ultrafine particles include ambient and laboratory-generated NPs that are not produced in a controlled, engineered way. Ultrafine nanosized particles are present in the ambient air, while in the working conditions, NSPs can be generated and they can reach high exposure concentrations, up to several hundred micrograms per cubic meter.[10,11] Human exposure can be direct or indirect through contamination of the environment. Even very low concentrations of NPs in the air represent very high particle number concentrations. The development of the nanotechnology has led to development of various strategies for controlled and targeted delivery of drugs, vaccines and diagnostics, which includes administration of nano-sized delivery systems into the human body by different routes.[12] On the other hand, the different physicochemical properties of the engineered nano-sized particles (compared with larger sized particles of the same components) have raised substantial concerns about the safety of the nano-sized materials, intentionally or unintentionally entered into the body, because the nanosized particles have been considered as the most dangerous fraction.[13,14,15,16]

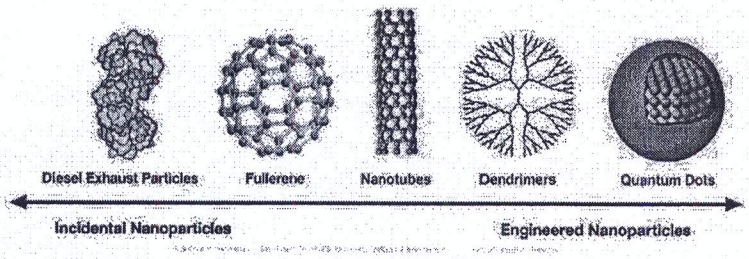


Fig. 1. Various types of nanoparticles

Insoluble or NPs with low solubility are of greatest concern, especially when inhaled, because they usually end up in the bloodstream after crossing all the respiratory or gastrointestinal protective mechanisms. They are then distributed in the various organs and accumulate at specific sites. They can travel along the olfactory nerves and penetrate directly into the brain, as they can pass through the cell barriers. The second NPs feature related to their toxicity seems to be linked with their surface. NPs are so tiny that small quantities (expressed in terms of mass) could have major toxic effects, because of their large surface. In general, the toxicological data specific to ENPs remain insufficient due to the closely specialized-limited number of studies, the short exposure period, the different properties of the NPs tested (size, length and agglomeration) and the often-unusual exposure route in the working environment. Depending on the type of NPs, nephrotoxicity, effects on reproduction, genotoxicity and cytotoxic effects have been reported so far. Some NPs cause granulomas, fibrosis and tumorous reactions in the lungs.[10,11] Therefore, the population

potentially exposed to ENPs should be prudent and apply safe measures of source elimination, exposure control and individual protection, both during the production and use of these products.

2. Classes of manufactured nanoparticles

Carbon nanotubes (MWCNT, SWCNT): Multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs), discovered in 1991 by Iijima, are constituted by a unique graphitic sheet or two or more sheets nested together in a tubular multilayer structure.[1] They posses extraordinary properties: high electrical and thermal conductivity, great strength and rigidity, energy storage and field emission.[2] Concerning the toxicity of CNTs, it is related to the presence of metal impurities, which are by-products of the synthesis as well as the use of surfactants to disperse them in an aqueous media. Due to the nanoscale size and carbon backbones, harmful and pathogenic effects from the CNTs can arise due to their ability to enter the respiratory tract, deposit in the lung tissue, redistribute from their site of deposition, and modify the structure of the proteins. In this way, CNTs can potentially activate inflammatory and immunological responses, affecting normal organ functions. Recently, several studies reported for the toxicological effects of the CNTs and other fullerene-based nanostructures.[4-7]

Inorganic nanoparticles of metal and their oxides (Au, Ag, Co, Cr, ZnO, TiO_2, CeO_2, CrO_2): Insoluble inorganic NPs can be composed of pure metals or their oxides, or various inorganic products and alloys. Only their nano-dimensions distinguish them from the same products found on a larger scale. At nano-scale, they display mechanical, electrical and other properties that do not exist when in larger dimensions.[9] Research results shown consistency in the sensitivity of aquatic, fish cell and human cell models to Ag and CeO_2 particles of different size; with the observed sensitivity sequence from highest to lowest as: nano-Ag>microAg>nano CeO2 = micro CeO. Titanium dioxide (TiO_2) ultrafine particles that fall within the nanoparticle size range are commonly used as photocatalysts to clean air and water, as antibacterial agents on glass and steel, and as components of many cosmetics and sunscreens.[17] Last years, intensive study has been performed to find ultrafine TiO_2 aerosols over a range of concentrations, and determine if ultrafine TiO_2 particles are inherently more toxic than larger, fine TiO_2 particles.

Quantum dots and semiconductors (CdSe, CdTe, ZnSe): The quantum dots, so called semiconductor nanocrystals or artificial atoms represent a special form of spherical nanocrystals from 1 to 10 nm in diameter with unique size-dependent optical and electrical properties. They are used as fluorescent probes in diagnostic imaging and in therapeutics, because of their optical properties and their capacity to form covalent bonds with peptides, antibodies, or other low-weight molecules.[9] The authors who have demonstrated in vivo that CdSe/ZnS quantum dots coated with mercaptoacetic acid could bond to blood transferrine were Chan and Nie in 1998, cited by Smith et al.[18] This fluorescent complex was absorbed selectively by cancer cells.

Zero-valent metals (Fe(II)salts): The ferrite (Fe_3O_4) NPs usually used were synthesized from a reaction solution of $FeCl_2$ and $FeCl_3$ and adjusting solution of NH_4OH.[19,20] They have been utilized as supermagnetic NPs for in vivo or in vitro biomedical applications, such as the contrast agents for magnetic resonance imaging, hyperthermia media for tumors treatment, magneto-targeting carrier for chemotherapeutic drugs and immunoassay.[21]

3. Health effect of engineered nanoparticles

Health effects of the ENPs after distribution in the skin: Penetration of the skin barrier is size-dependent with nano-sized particles more likely to enter more deeply into the skin. However, in a broken skin, entry of even larger (0.5-7 μm) particles was observed and there

is a hypothesis that skin when flexed can make the epidermis more permeable to NPs.[22,23] No direct relation between other physicochemical properties and biodistribution of NPs into and via systemic circulation through the skin has been found so far.[24] There are literature data, pointing to the mechanical skin irritation and sensitisation with involvement of the macrophages, Langerhans cells or other cells. For example, exposure to polystyrene NPs under skin barrier dysfunction exacerbated atopic dermatitis-like skin lesions in NC/Nga mice.[25]However, in a study of Park et al., in which human skin equivalent model was used to deduce toxicity of polystyrene and TiO_2 NPs, no phototoxicity, acute cutaneous irritation or skin sensitization was observed.[26]

Health effects of the ENPs after oral ingestion: From the respiratory tract via the mucociliary escalator, ENPs can be ingested into the gastrointestinal tract or they can be directly ingested with water, food or drug delivery system. After the oral ingestion, depending on the size, surface charge and mucoadhesivity, NPs could reach the colon or translocate from the lumen of the intestinal tract via the M cells in the Peyer's patches and isolated follicles of the gut-associated lymphoid tissue and/or via the normal intestinal enterocytes.[27,28] Literature survey suggest that smaller the particle diameter the faster they can permutate the mucus to reach the colonic enterocytes. NPs entering the lymphatic may induce a secretory immune response, while those entering the systemic circulation can distribute to different organs (e.g. kidney, lung, liver, spleen, brain, etc.). For example, in rats dosed orally with radiolabelled functionalized C_{60} fullerenes, 98% were cleared in the faeces within 48 hours, indicating uptake of the particles into the blood.[29]

Health effects of the ENPs after distribution in the respiratory tract: Physicochemical properties of the inhaled particles, especially size distribution, surface charges and surface chemistry, are important for the pathogenic effects into the lung as they influence both the deposition and the clearance rates.[30]The main mechanism for deposition of inhaled ENPs in the respiratory tract is diffusion due to the displacement when the particles collide with the air molecules. The clearance of deposited particles in the respiratory tract is manifested by physical translocation of the particles by different mechanisms and chemical dissolution. Particles size was confirmed as the key parameter in their deposition into the lung. The smaller the particles the deeper they can travel into the lung. So, in the alveoli, particles smaller than 2.5 μm can be found, while inhaled ultrafine particles (an aerodynamic diameter < 100 nm), deposited manly in the alveolar region, are subject to easy and rapid translocation through the systemic circulation to other vital organs fractional deposition of the NPs in the lung is up to 70%, larger of them are being recognized and phagocytised by alveolar macrophages and translocated into the intersticium (Fig. 2 and Fig. 3). On the other hand, 5-nm particles showed equal deposition of app. 30% in all three regions (nasopharyngeal, tracheobronchial and alveolar region), 20-nm particles had the highest deposition efficiency in the alveolar region (~50%), whereas in the tracheobronchial and nasopharyngeal regions, the deposition efficiency was app. 15% .[32]

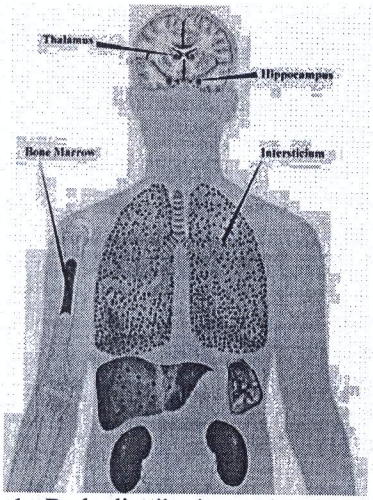


Fig. 1. Body distribution and main target organs after administration of ENPs via respiratory tract (coronal plain)

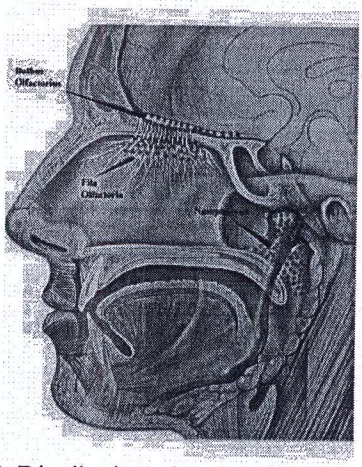


Fig. 2. Distribution of ENPs after nose breathing (sagittal plain)

Considering the brain toxicity, extrapulmonary translocation of the NPs through the olfactory nerve was reported to be the most probable pathway for NPs to the brain. [31,32] Research data point that intranasally instiled TiO_2 ENPs could be also translocated into the central nervous system of mice via the olfactory nerve tract and accumulated in the olfactory nerve layer, olfactory ventricle, cerebral cortex, thalamus and CA1 and CA3 regions of hippocampus. As previously said, air borne NPs (ultrafine particles) have been also found to penetrate into the systemic circulation following inhalation. In this way they can reach other extrapulmonary organs, such as the liver, spleen, kidney, etc.[30] For example, pulmonary exposure to diesel particles with ultrafine range resulted in severe atrophy of renal glomerulus and infiltration of inflammatory cells in the lumen of the Bowman's capsules.

Health effects of ENPs after intravenous injection: Literature data suggest that ENPs easily reach liver after intravenous administration and accumulate and retain there for a long period of time. Biodistribution of two types of radiolabelled (^{111}In) functionalized single-walled carbon nanotubes (f-SWNT) after intravenous administration was followed. Rapid first-order clearance from the blood compartment through the renal excretion route (3-3.5 hours) was observed without any toxic side effects or mortality. Radioactivity in all organs examined after 30 min of administration were detected, with higher levels of radioactivity found in the muscle, skin, kidney, lung and blood.

4. Exposure methods for the assessment of biodistribution and health effects of enps

The available studies have shown that the selection of the exposure methods for the assessment of the health effects of the NPs depends from several factors, such as the type of the NPs and nanostructures, their composition, physicochemical properties and the exposure route and period, including the often-unusual exposure route in the working environment. The exposure methods are directly related to the translocation, disposition and deposition of the NPs in the human body and they are all studied in toxicokinetics. In the group of the most frequently studied, the following exposure methods are included: the inhalation, the ingestion, intravenous injection, intra-tracheal instillation, cutaneous exposure, dermal and in vitro exposure.

Inhalation exposure: Inhalation exposure was one of the first studied methods. Researchers have used this method to assess the influence of the particle size of TiO_2 NPs on their deposition/translocation in the lung. Fine (250 nm) and ultrafine (20 nm) TiO_2 NPs were compared after inhalation in rats and the results pointed to greater pulmonary retention of ultrafine particles when the same concentrations of fine and ultrafine NPs were administered (22.3±4.2 and 23.5±2.9 mg/m^3, respectively). Until these studies, TiO_2 was considered to be nontoxic dust and it was used as an inert control in several toxicological studies. However, significant damage to the pulmonary epithelium, development of interstitial fibrosis and alteration of macrophage functions was observed pointing that, when nano-scaled, inert particles can easily become biologically active i.e. toxic. The distribution of iridium-192 NPs by inhalation in anesthetized rats when exposed by ventilation to 15 and 80 nm aerosols (2.5 μg/cm^3) was studied. Radioactivity in the liver, heart and brain was detected and this observation was twice as great for 15 nm NPs. Considering the insolubility of iridium NPs and therefore absence of absorption in the intestine, they concluded that NPs were translocated to the mentioned organs by the pulmonary blood vessels. In a long-term inhalation study in rats, Oberdörster et al have studied the cerebral distribution of carbon-13 (insoluble) particles. In the exposure chambers, the rats were exposed for 6 h to concentrations of 0, 150 and 170 μg/cm^3, and then sacrificed on days 1, 3, 5 and 7. Significant capture of the NPs in the brain, the cerebellum and the olfactory bulbs of the rats was observed on day 1 and on the 7th day, the NPs were detected in the olfactory bulbs only. To explain cerebral capture of C-13 NPs, the authors postulated translocation from the lung to the bloodstream, and then crossing the blood-brain barrier. Inhalation of 35 nm radiolabelled carbon particles led to a significant accumulation in the olfactory bulb of rats seven days after exposure.

Ingestion exposure: Using the ingestion exposure, Chen et al. have studied the acute and subacute toxicity of C60 polyalkylsulfonate in rats. No mortality was observed in an acute oral toxicity test with doses up to 2500 mg/kg. Wang et al. have also used ingestion exposure of carbon nanotubes to assess the toxicokinetics effects. Hydroxylated single-walled carbon nanotubes (SWCNT) administered by gavage in mice (100 μL of a 15 μg/mL solution) were distributed to most of the organs and tissues, except the brain. Hillyer and Albrecht have reported blood and tissue distribution of ingested colloidal gold NPs in mice. Significant amount of NPs in the animals' brain, lung, heart, kidneys, intestine, stomach, liver and spleen were observed, with higher content of NPs smaller than 10 nm in comparison with particles bigger than 28 nm.

Cutaneous exposure: Lademann et al. have used cutaneous exposure to study the effects of TiO_2 NPs. They have not found significant absorption of coated TiO_2 nanocrystals (17 nm) beyond the stratum corneum of the skin of human volunteers, except for a small quantity (< 1%), which had penetrated via the hair follicles. This was confirmed by the isolation of the follicles from the living tissue. Using the same cutaneous exposure, the application of three formulations with different particulate characteristics has been tested (T805: 20 cubic nm; Eusolex T200: 10-15 cubic nm, agglomerated into needle-shaped 100 nm NPs; Tioveil AQ-10P: 100 nm, in the form of coated needles of Al_2O_3 and SiO_2 and particulate forms of TiO_2; variable affinities for water and oil; coated or not). These results suggested a low probability for absorption of nanoparticulate TiO_2 beyond the dermis and distribution to the bloodstream.

Intravenous injection: Rajagopalan et al. have used intravenous injection to study the pharmacokinetics of a water-soluble fullerene, p,p'-bis (2-amimoethyl)-diphenyl-C60, administered in rats (15 and 25 mg/kg). Injection of 25 mg/kg caused death of two tested rats in 5 minutes, while five other rats survived when a dose of 15 mg/kg was administered. Using the intravenous injection, Douglas et al. studied the biodistribution of polymer-coated or uncoated poly(butyl 2-cyanoacrylate) NPs in rabbits. About 60% of the NPs were located

in the liver and the spleen and app. 30% remained in the bloodstream. The coating had no significant influence on NPs biodistribution.

Intratracheal instillation: Using the single intratracheal instillation (0,1 and 5 mg/kg) Warheit et al. have studied the pulmonary toxicity of acute exposure to a SWCNT preparation in male rats. There was no effect at 1 mg/kg, while at 5 mg/kg, high mortality rate (~15 %) was observed. The mortality was caused by the mechanical blockade of the upper airways, increased pulmonary cell proliferation and occurrence of multifocal pulmonary granulomas. In addition, significant increases in lung weight was also observed.
In the study performed by intratracheal instillation of 20-30 nm TiO_2 NPs in rats, an increase in pulmonary neutrophiles as early parameter of inflammation was observed. Coating by methylation to render the particles hydrophobic, slightly reduced the neutrophile production (for the 2 particulate dimensions of TiO_2) when a dose of 1 mg was administered. The authors concluded that the surface characteristics of the NPs are the determining factor in the pulmonary inflammation, while the coating by methylation played a marginal role.

Dermal exposure: The dermis is rich in blood supply and macrophages, lymph vessels, dendritic cells and five different types of sensory nerve endings. It was reported that interadermally injected ENPs are distributed into the local lymph nodes. For the skin, there is a possibility for translocation of the ENPs through the skin sensory nerves; this pathway has been previously demonstrated for the nasal and tracheobronchial regions of the respiratory tract.

In vitro: In vitro exposure to the C60 fullerene (12.5 μg C60-cyclodextin) induced oxidative damage in rat hepatic microsomes. It was shown that this damage can be modulated by antioxidants and free radical scavengers. Researchers have studied the cytotoxicity (CL50) of four water-soluble fullerenes on human cells in vitro (skin fibroblasts and hepatic caricinoma cells). They showed that the toxicity varies with the nature of the functional group. Pantarotto et al. studied the intracellular transport of functionalized SWCNT, conjugated with lysine, in human and mouse fibroblasts in vitro (1, 5 and 10 mM).They showed that these carbon nanotubes could pass through the cellular membrane, accumulate in the cell and end up in the cell nucleus. The same capability for penetration through the cell membrane was found for MWCNTs.

5. The health effects of sic nanoparticles

5.1. Materials and methods

Nano-sized Silicium Carbide particles were used in this experiment. A 0,5% hydroxypropylmethylcellulose K4M (HPMC, K4M) was used as a suspending agent. A 1,5 g of the particles powder was dispersed onto the surface of 0,5%, w/v HPMC solution (10 ml), and then the suspending solutions containing SiC particles were treated by ultrasonic for 15-20 min and mechanically vibrated for 2-3 min. The size of the particles was determined using laser diffractometry (Mastersizer Hydro-2000S, Malvern Instruments Ltd., UK). The size of the nanoparticles was 10-20 nm.

Animals and treatment: Four weeks old female Wistar rats (average body mass 276±28,38 g) were acclimated during the 2 week period before starting the experiment. During the experimental period, the rats were housed in polycarbonate cages (maximum of 3 rats per cage) in a room with controlled temperature (23±2°C) and humidity (55±7%), and a 12-h light/dark cycle. The rats were fed rodent diet and filtered water ad libitum. At 5th week, the rats were divided into four groups: 2 control groups, one control, not-treated group (n=3) and the second one, vehicle-control group (n=9), treated with adequate volume of the solution of HPMC in water (0,5% m/v), and 2 experimental groups treated with different dose of nanoparticles, one (n=9) with a dose of 1 g/kg body weight and the second one (n=9) treated with a dose of 5 g/kg body weight. After vigorous stirring, suspension of SiC nanoparticles containg the correspondent dose of nanoparticles was given to rats as a single dose via

intragastric tube in a minute (according to OECD procedure). Food and water were provided 2 h later. The symptoms and mortality were observed and recorded carefully during the whole period of study. No rat died during the study, and they all were active and non-anorectic. 2, 7 and 14 days later, animals from the vehicle-control and 2 experimental groups were sacrificed (n=3 per group at each study period) after being anaesthetized by ether. Blood samples were collected from the eye vein by removing the eyeball quickly. The tissues and organs such as heart, liver, spleen, kidneys, lung, brain, small and lower intestine, and ovary were excised, part of the tissues and organs were stripped and immediately fixed in a 10% formalin solution for histopathological diagnosis.

Histopathological examination: For pathological studies, all histopathological tests were performed using standard laboratory procedures. The tissued were embedded in paraffin blocks, then sliced into 5 μm in thickness and placed onto glass slides. After hematoxylin-eosin staining, the slides were observed and the photos were taken using optical microscope Lieca. The identity and analysis of the pathology slides were blind to the pathologist.

Biochemistry and hematology: In the present study, liver function was evaluated with serum levels of total bilirubin (TBIL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST). Nephrotoxicity was determined by uric acid, urea and creatinine. The enzymes creatine kinase and lactate dehydrogenase were assayed for evaluating cardiac damage. In addition, the following parameters were determined: for hematological status, white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HTC), platelet count (PLT), number of lymphocytes (LY), number of monocytes (MO), number of neutrophils (NE); glucose (GLU); for protein status, total protein (TP), albumin (ALB), globulins; for lipid status, cholesterol (CHO), triglyceride (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL); for electrolyte status, sodium (Na), potassium (K-S), calcium (Ca-S),iron (Fe-S).

Statistical analysis: Results were expressed as mean $\pm$ standard deviation ($\pm$SD). Multigroup comparison of the means was carried out by one-way analysis of variance (ANOVA) test. Dunnett's test was used to compare the differences between the experimental groups and the control group. Student's t-test was used to compare the means of each group. The statistical significance for all tests was set at $p<0.05$.

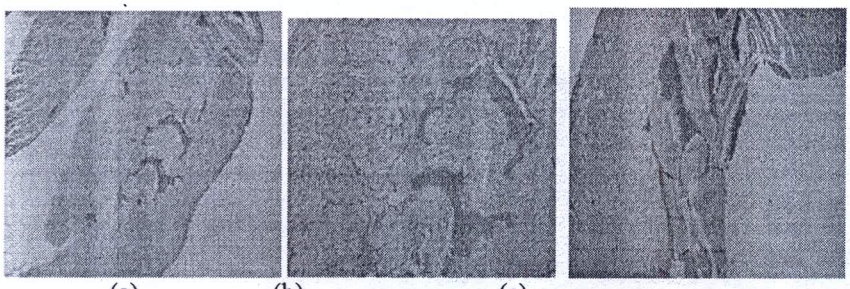
5.2. Results

Loss of weight during the study ranged from 6,67$\pm$5,29 (the second day of nanoparticles administration) to 25,14$\pm$3,3 (2 weeks of nanoparticles administration), while for the control group, weight loss was between 3 and 5 g. Tables 1-3 show the biochemical and hematological data for the serum of the Wistar rats.

No significant differences in the parameters in the vehicle-control group during the study was observed as well as between the control group treated with adequate volume of 0,5%m/v HPMC and the control group of rats without any treatment. The parameters for the vehicle-control group are presented in Tables 1-3, expressed as means $\pm$ SD from all three study periods (n=9). Loss of weight during the study ranged from 6,67$\pm$5,29 g (the 2nd day of nanoparticles administration) to 25,14$\pm$3,3 g (after 2 weeks of nanoparticles administration) in the experimental groups, while for the control group, weight loss was 5,89$\pm$3,20 g during the whole period of study. Significant increase in the platelet count (thrombocytosis) was observed at the 2nd day of the study (679,5$\pm$70,0 in the control and 1032,67$\pm$106,31 in the experimental group treated with a dose of 5 g/kg). However, the analysis performed at the end of the 2nd week pointed to normalization of this parameter, where the platelet count dropped to 626,33$\pm$297,73 in the experimental group treated with a dose of 5 g/kg body weight. The levels for the enzymes CK, LDH, AST were increased at the end of the study (the 2nd week) in both the experimental groups, without significant difference between the experimental groups. The level of ALT was slightly increased by the end of the 2nd week and no elevation in the ALT/AST ratios (in a range from 0,5-0,6) was observed after exposure to

different dose of nanoparticles in comparison with the control group (average ratio 0,7). No parameter indicating nephrotoxicity was increased. The significant change of serum LDH in both the experimental groups (app. 1580±30) in comparison with the control group (550±70,71) could indicate myocardial damage, which can be confirmed by the histological analysis.

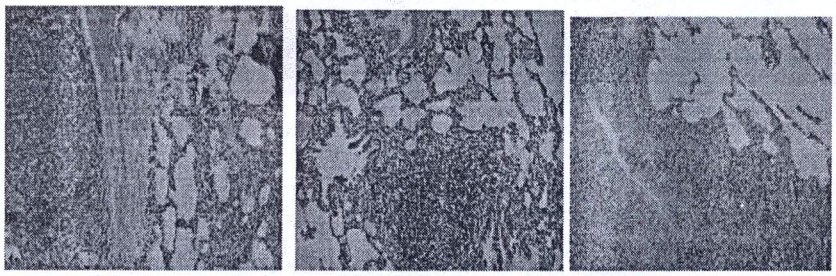
Characteristic photos of each tested organ (heart, liver, spleen, kidneys, lungs, intestines and ovaries) are shown in next series of photos. In Figure 13, characteristic changes registered at the heart of tested animals are shown. To this organ, micro bleedings are registered as consequence of nanoparticles presence, there is no appearance of significant effects.



(a) (b) (c)

Fig. 13. Characteristic changes registered at the heart of tested animals

In figure 14, characteristic microscopic pictures of nanoparticles effects towards lungs are shown. In the lungs, the biggest influence and effects are registered, from the presence of nanoparticles. Micro bleedings are registered in the lungs (interstitial, intra alveolar). Also, widen interstitial was noted infiltrated with inflammatory cells, lymphocytes, eosin files, neutrophiles, macrophages, with focuses of consolidation and fibrosis, as macrophages with ingested nuclear debris "starry sky", and in part broken inter alveolar seeps and poor stroma.

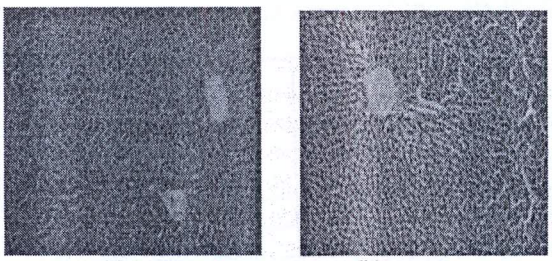


(a) (b) (c)

Fig. 14. Characteristic changes registered at the lungs of tested animals.

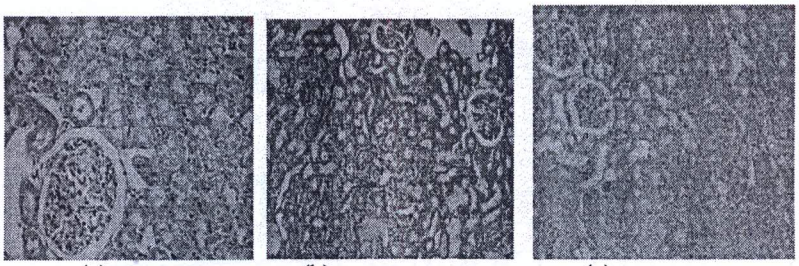
In figure 15, characteristic changes are registered at the liver of tested animals. In this organ, like consequence of the presence of nanoparticles, the stasis of micro bleedings are registered, then hepatocytes degenerative changed, sinus spaces in part narrowed, in portal spaces there is a presence of lymphocytes and eosin files polymorphs.

On figure 16, characteristic changes are registered at the kidneys of tested animals. At the kidneys, because of nanoparticles presence, stasis, micro bleedings, degenerative changed tubular cells intra cells with yellow-brown pigment, are noticed. On figure 17, characteristic changes registered at the spleen of tested animals. At the spleen, because of nanoparticles presence, stasis, and intra cells with yellow-brown pigment, is noticed.



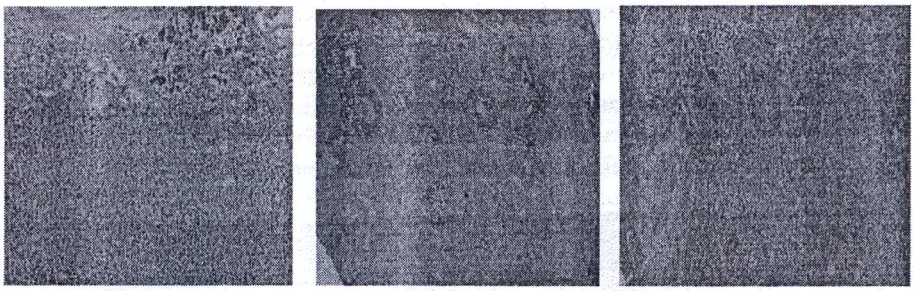
(a) (b)

Fig. 15. Characteristic changes registered at the liver of tested animals



(a) (b) (c)

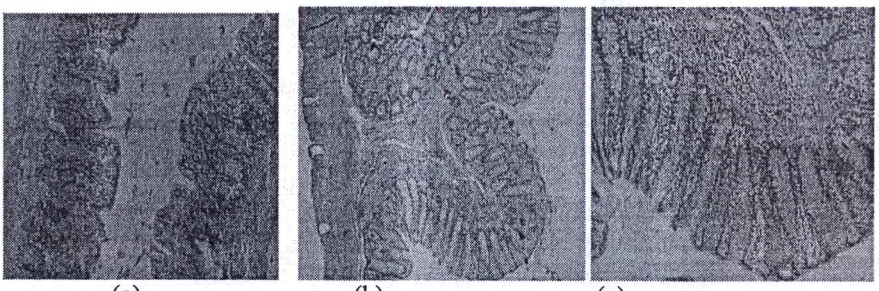
Fig. 16. Characteristic changes registered at the kidneys of tested animals



(a) (b) (c)

Fig. 17. Characteristic changes registered at the spleen of tested animals

In figure 18, changes are registered at the gastro-intestinal tract - GIT of tested animals. At the GIT because of nanoparticles influence, effects in intestinal mucosa and sub mucosa are registered. Also, there are present lymphocytes, eosinophils and plasma cells.



(a) (b) (c)

Fig. 18. Characteristic changes registered at the at the gastro-intestinal tract – GIT of tested animals.

Table 1. Changes of biochemical parameters in the serum of the rats induced by SiC nanoparticles after 2 days of administration

Laboratory Parameter	Control group (treated with adequate volume of 0,5% m/v HPMC) n=9	**2 days** (treated with dose of nanoparticles 1 g/kg) n=3	**2 days** (treated with dose of nanoparticles 5 g/kg) n= 3
Loss of mass (g)	**5,89±3,20**	**6,67±5,2**	**16,67±10,3**
Hematological status			
White blood cells (WBC) x10^9/L	4,11±0,1	5,33±0,99	5.67±0,85
Red blood cells (RBC) x 10^{12}/L	7,54±0,91	7,62±0,54	7,49±0,42
Hemoglobin (Hb) (g/L)	139±26,87	135,33±7,57	145±14,73
Platelet count (PLT) x 10^9/L	**679,50±70,00**	**1032,67±86,31**	**1031,67±106,53**
Glycide status			
Glucose (GLU) (mmol/L)	4,00±0,28	4,87±0,85	6,03±0,21
Degradation products (Nephrotoxicity)			
Urea (U) (mmol/L)	9,10±0,7	9,97±1,39	9,73±1,33
Creatinine (CRE) (µmol/L)	47,45±4,88	47,95±5,59	49,33±4,04
Uric acid (UA) (µmol/L)	118±16,57	103,33+15,31	105,5+9,99
Enzymatic status			
Creatine kinase (CK-S) (U/L)	236,50±94,04	296,17±151,45	**373,6+217,02**
Gamma-glutamyl transpeptidase (GGT-S) (U/L)	3,65±0,49	3±1	2,53+1,44
Lactate dehydrogenase (LDH-S) (U/L)	550±70,71	481,67±224,84	583,67±211,80
Aspartate aminotransferase (AST-S) (U/L)	121±12,73	133,67±18,47	123,67±13,01
Alanine aminotransferase (ALT-S) (U/L)	86,50±21,92	67,33±21,94	73,5+19,16
Alkaline phosphatase (ALP-S) (U/L)	420±25,05	279,33±76,79	289,67±83,17
Lipid status			
Cholesterol (CHO) (mmol/L)	2±0,15	2,33±0,23	2,49+0,36
Triglyceride (TG) (mmol/L)	0,62±0,03	0,60±0,09	0,74+0,06
Protein status			
Total protein (TP) (g/L)	78,65±4,74	90,30±3,30	77,73+9,11
Albumin (ALB) (g/L)	45±1,41	43,0±1,0	41,67+0,58

Table 2. Changes of biochemical parameters in the serum of the rats induced by SiC nanoparticles after 7 days of administration

Laboratory parameter	Control group (treated with adequate volume of 0,5% m/vHPMC) n=9	7 days (treated with dose of nanoparticles 1 g/kg) n=3	7 days (treated with dose of nanoparticles 5 g/kg) n=3
Loss of mass	**5,89±3,20**	**8,67±5,2**	**16,44±4,3**
Hematologic status			
White blood cells (WBC)x10^9/L	4,11±0,1	3,83±0,49	4,34±2,35
Red blood cells (RBC) x 10^{12}/L	7,54±0,91	8,62±0,18	7,72±0,99
Hemoglobin (Hb) (g/L)	139±26,87	146,67±3,53	141,67±19,86
Platelet count (PLT) x 10^9/L	**679,50±70,00**	839,5±104,56	**1046,67±369,00**
Glycide status			
Glucose (GLU) (mmol/L)	4,00±0,28	6,23±0,40	6,4±0,85
Degradation products			

Nephrotoxicity			
Urea (U) (mmol/L)	9,10±0,7	11,47±0,57	9,1±1,55
Creatinine (CRE) (μmol/L)	47,45±4,88	64,5±7,07	60,93±3,43
Uric acid (UA) (μmol/L)	118±16,57	97,33+13,32	105,5+9,98
Enzymatic status			
Creatine kinase (CK-S) (U/L)	236,50±94,04	213,5±48,79	**157,5±35,86**
Gamma-glutamyl transpeptidase (GGT-S) (U/L)	3,65±0,49	4,23±2,80	4,96+1,98
Lactate dehydrogenase (LDH-S) (U/L)	550±70,71	621,00+11,31	542,5±214,24
Aspartate minotransferase (AST-S) (U/L)	121±12,73	86,33±25,97	90,67+8,08
Alanine aminotransferase (ALT-S) (U/L)	86,50±21,92	59,00±11,13	63,67+12,01
Alkaline phosphatase (ALP-S) (U/L)	420±25,05	234,33±120,81	177+72,51
Lipide status			
Cholesterol (CHO) (mmol/L)	2±0,15	2,15±0,73	2,05±0,62
Triglyceride (TG) (mmol/L)	0,62±0,03	0,60±0,02	0,49+0,19
Protein status			
Total protein (TP) (g/L)	78,65±4,74	84,97±7,54	85,23+5,46
Albumin (ALB) (g/L)	45±1,41	40,0±2,65	41,00+3,46

Table 3. Changes of biochemical parameters in the serum of the rats induced by SiC nanoparticles after 2 weeks of administration

Laboratory parameter	Control group (treated with adequate volume of 0,5% m/vHPMC)n=9	2 weeks (treated with dose of nanoparticles 1 g/kg) n=3	2 weeks (treated with dose of nanoparticles 5 g/kg) n=3
Loss of mass (g)	**5,89±3,20**	10,89±8,2	25,14±3,3
Hematological status			
White blood cells (WBC)x10^9/L	4,11±0,1	5,60±1,70	5,45±0,07
Red blood cells (RBC) x 10^{12}/L	7,54±0,91	7,41±0,18	7,67±0,03
Hemoglobin (Hb) (g/L)	139±26,87	140,5±2,12	143,00±2.83
Platelet count (PLT) x 10^9/L	**679,50±70,00**	**620,0±283,25**	**626,33±297,73**
Glycide status			
Glucose (GLU) (mmol/L)	4,00±0,28	4,45±0,64	3,76±1,4
Degradation products Nephrotoxicity			
Urea (U) (mmol/L)	9,10±0,7	9,2±1, 7	9,4±3,25
Creatinine (CRE) (μmol/L)	47,45±4,88	58,91±1,55	51,25±6,72
Uric acid (UA) (μmol/L)	118±16,57	190,0+28,28	147,9+11,17
Enzymatic status			
Creatine kinase (CK-S) (U/L)	236,50±94,04	**368,0±109,30**	**365,5±63,64**
Gamma-glutamyl transpeptidase (GGT-S) (U/L)	3,65±0,49	4,63±1,80	4,66+1,68
Lactate dehydrogenase (LDH-S) (U/L)	550±70,71	1580,00+28,28	1520±28,28
Aspartate aminotransferase (AST-S) (U/L)	121±12,73	168,0±50,03	182,5+31,82
Alanine aminotransferase (ALT-S) (U/L)	86,50±21,92	90,00±3,25	109,5+13,43
Alkaline phosphatase (ALP-S) (U/L)	420±25,05	320,5±112,43	388,5+10,51
Lipid status			

Cholesterol (CHO) (mmol/L)	2±0,15	2,27±0,53	2,13±0,31
Triglyceride (TG) (mmol/L)	0,62±0,03	0,58±0,11	0,53+0,08
Protein status			
Total protein (TP) (g/L)	78,65±4,74	95,35±3,75	75,75+13,79
Albumin (ALB) (g/L)	45±1,41	45,0±1,41	48,00+8,48

6. Summary

In this paper, in the first part an attempt was made, biodistribution and health effects i.e. toxicity profile after different routes of exposure and entry into the human body of several engineered nanostructures and NPs to be presented and compared. This report should contribute for easy identification and use of the main risk factors for predicting toxicity of newly generated NPs. An improved understanding of the risks for toxicity and health effects of the ENPs will aid in future development and exploitation of a variety of nanomaterials. Well summarized results in the field of study are especially important for the industry and sectors where NPs are produced or used in terms of providing guidance on the assessment of exposure to these materials.

In the second part, the health effects and risk assessment of the SiC nanoparticles were studied. The toxicity of nano-sized SiC particles on 4 weeks old female Wistar rats was investigated in comparison with the vehicle-control group treated with 0,5% w/v HPMC. 2, 7 and 14 days later, animals from the control and 2 experimental groups were sacrificed (n=3 per group at each study period) after being anaesthetized by ether. Biochemical and hematological tests, as well as histopathological tests were performed. The significant changes of serum LDH in both the experimental groups (app. 1580±30) in comparison with the control group (550±70,71) could indicate myocardial damage, which was confirmed by the histological analysis. Due to the presence of nanoparticles, the stasis of micro bleedings were registered, then hepatocytes degenerative changed, sinus spaces in part narrowed, in portal spaces there is a presence of lymphocytes and eosin files polymorphs.

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