Risk assessment of silver nanoparticles

V A Shipelin¹, I V Gmoshinski¹, S A Khotimchenko¹
¹Federal State Budgetary Research Institution «Institute of Nutrition», 2/14, Ust’inskiy proezd, Moscow, 109240 Russia
E-mail: v.shipelin@ya.ru

Abstract. Nanoparticles of metallic silver (Ag) are among the most widely used products of nanotechnology. Nanosized colloidal silver (NCS) is presented in many kinds of production as solutions of particles with diameter less than 100 nm. NCS is used in a variety of fields, including food supplements, medicines, cosmetics, packaging materials, disinfectants, water filters, and many others. Problems of toxicity and related safety of NCS for humans and environmental systems are recently overestimated basing on data of numerous toxicological studies in vitro and in vivo. The article discusses the results of current studies in recent years and the data of author’s own experiments on studying the safety of NCS, that allows to move on to risk assessment of this nanomaterial presented in consumer products and environmental samples.

1. Introduction
Among the most widely used nanotechnology objects there are metallic silver nanoparticles (Ag NPs) with dimension less than 100 nm that are manufactured in the form of colloidal solutions of nano-sized colloidal silver (NCS). Despite the fact that the history of colloidal Ag production dates back to antiquity, it was in the past 2 decades, that the use of this nanomaterial as part of consumer products became massive, which was in most cases justified by the presence of potent biocidal properties in Ag NPs against pathogenic bacteria, fungi and even some viruses [1].

Biocidal effect of Ag NPs by themselves is likely to be negligible [2] according to carefully conducted recently control experiments, however, the ability of this NP to release high concentrations of Ag + ions in oxidative condition gives them in practice pronounced antimicrobial properties including that in respect of microorganisms having resistance to the vast majority of modern antibiotics. [3] The notion has been overestimated that the NCS is a hazard to humans and higher animals based on the recent works on numerous toxicological data obtained in studies in vitro and in vivo [4]. In addition, the growing concern is caused by the possible consequences of entering Ag NPs into the environment as a part of various waste products. In some foreign countries NCS is currently considered as a pesticide that is a subject of the regulation applicable to the highly dangerous chemicals [5]. In Russia, despite a significant increase in production volumes and the use of the NCS, its regulation is missing, which makes actual the task of the risk assessment of this priority nanomaterial.

2. Hazard identification
NCS is commercially available in several forms, differing in size and shape of NPs, and the composition of their surface coatings (functionalization). The most common are, firstly, the so-called "Classical" NCS stabilized by adsorbed citrate ions, second, preparation obtained by biochemical
synthesis in the reverse micelle system comprising sodium dioctyl sulfosuccinate (food additive E480), thirdly, so-called "Cluster" NCS produced by photocatalytic method in the presence of polyvinylpyrrolidone (PVP, food additive E1201) and, fourthly, containing no stabilizers "ion-free" colloidal Ag, formed under the action of high-energy dispergation in distilled water (under the influence of the laser pulse on metal silver target or electrical explosion of silver wire) [6]. When assessing the risks of all these forms of NCS, it is important to keep in mind that their actions in relation to toxic doses of biological objects can vary significantly. Thus it is the "cluster" NCS stabilized with PVP that is apparently one of the most popular forms of this product, because of the relative ease of preparation technology, stability and biocompatibility of the stabilizer used.

Applications of NCS include food supplements, medicines, bandage, packaging materials, cosmetic products, disinfectants, paints and varnishes, textiles, water filters [7-9]. There are registered in the Russian Federation more than 120 types of consumer products containing the Ag NPs as of 2015/Cosmetic products predominate among them (70%). In addition to the direct impact in the composition of products, the Ag NPs may expose the population through various environmental objects, such as water, air, soil, agricultural raw materials.

3. Hazard characterization

NCS has toxic effects on the eukaryotic cells in culture [10-13], water and soil organisms [4,13], in laboratory animals at inhalation [14,15] epicutaneous [16] and oral [17-20] administration. Mechanism of Ag nano-toxicity, according to modern concepts, lies in Ag NPs ability to penetrate through biological barriers [21], accumulate in cells and release silver ions (Ag+) in very high local concentrations under the influence of oxidizing agents [22] including endogenous. This mode of action has been called in the literature as a "Trojan horse effect" [23]. Ag+ is a potent cellular poison due to its ability to inhibit enzymes and membrane transporters containing thiol groups in irreversible manner [13].

Our analysis of numerous literature data on the cytotoxic effect of the Ag NPs on models of cell cultures in vitro showed that the threshold concentration of NPs, corresponding to the minimum observable effect on cells is in any case not less than 3 μg/g of the incubation medium. The question remains open whether such a concentration of NPs can arise in the tissue during the natural ways of exposure (inhalation, oral ingestion or skin resorption). In several studies it has been shown by means of mass spectrometry [21] radioisotope methods [24], transmission electron microscopy [25,26] that the Ag NPs were bioavailable when entering through the gastrointestinal tract, capable to penetrate through the intestinal wall, reach blood circulation and accumulate in internals, primarily in the liver and spleen. Application of methods of radioactive tracers and neutron activation analysis allowed to quantify the transfer of orally administered Ag NPs from pregnant and nursing rats to their offspring through the fetoplacental barrier and via breast milk [27], as well through the blood-brain barrier into the brain [28].

Mathematical modeling of Ag NPs administered to the gastrointestinal tract bioaccumulation and biodistribution was performed in [29] using kinetic equations of 1-st order for describing of interorgan exchange of this NPs. In this way it was shown that potentially dangerous levels of Ag NPs can be achieved in critical organs (liver, spleen) after acute or subacute oral administration of these NPs in a daily dose of at least 5-10 mg / kg body weight.

Direct estimations of toxic doses of Ag NP in experiments on mammals are inconsistent, which may be due to differences in particle size and functionalization, insufficient duration of the experiment and a limited set of biomarkers of toxicity. In a series of papers by single or relatively short repeating (28 days) oral administration of various kinds of surface functionalized Ag NP adverse effects were detected only in extremely high doses ranging from 125 mg/kg body weight; critical organ, apparently, was the liver of animals. In particular, the analysis of the morphological changes of the liver tissue and the biliary system of mice after exposure to Ag NP showed vacuolization, focal necrosis of liver, bile duct hyperplasia, increased cell infiltration, inflammation, central veins extension [19].
Ag NP caused marked increase in expression of IL-1, IL-6, IL-4, IL-10, IL-12 and TGF-β, together with signs of histopathological effects on kidney when administered to mice intragastrically in a dose of 1 mg/kg body weight for 14 days[20]. In research [17] it was shown that the 28-day administration of Ag NP to growing male rats, aged about one month at the beginning of the experiment, resulted in decreased fasting glucose, increased gut macromolecular permeability and suppression of gut symbiotic micro flora. In NCS doses of 250 mg / kg body weight and more no significant signs have been detected of reproductive toxicity [18]. Unlike this in other studies, unfunctionalized Ag NPs, orally administered to rats for 6 months, expressed marked cumulation and had a threshold dose for systemic effect much smaller than 0.1 mg / kg body weight per day [30].

So named “cluster” NCS presenting the most common and practically important form of such nanomaterial in Russia has been studied in joint research of FSBI "Institute of Nutrition", FSBI "FNTS health-care technology risk management to public health" of Rospotrebnadzor and A.N.Bach Institute of biochemistry. NCS preparation stabilized with PVP was introduced into the gastrointestinal tract of laboratory animals (rats and mice) in 92-day lasting experiment using a wide variety of integral, functional, morphological, biochemical, cytological (ex vivo) and proteomic indicators. The aim of this current research is to evaluate the safe doses of Ag NPs, which will further create a framework for their hygienic rationing in production.

4. Exposure assessment, risk characterization
Accurate assessment of human exposure to Ag NPs is currently hindered by the lack of reliable statistical information on the volume of production and use of this nanomaterial, as well as reliable exposure scenarios.

According to [31] the annual quantity of NCS production in the world totaled more than 500 tones in terms of Ag in 2011 whereas in 2015 it could exceed 1,000 tones, which corresponded to about 140 mg of silver for each inhabitant of the Earth for one year. It is important to bear in mind that the distribution of Ag NPs in the consumer’s production as well as in biosphere can be extremely uneven. It is assumed that the general population is exposed to Ag NPs mainly by the oral and epicutaneous route when consuming food supplements, cosmetics and also by NPs migrating into food from packaging materials [32], while for the employees of enterprises producing products with NCS apparently predominates inhaled route of exposure [33].

The assumption did not receive experimental confirmation that large amounts of Ag NPs could enter atmosphere in the form of aerosols during disposal of wastes containing NCS textiles, medical supplies and other products at waste incineration plants. In fact, a major amount of these NPs accumulated in the ash and in some extent in sludge formed in wet cleaning systems of flue gases [34]. Subsequently this NCS can be transferred to the field in the composition of surrogate fertilizers, rinsed in water and accumulate in different soil and water organisms [31], and, ultimately, come to human through the food chain as part of agricultural production. However, data is currently insufficient to quantify the exposure proceeds in such scenarios.

Ag NPs migrating into the food from packaging materials was studied in [35] using data of mass spectrometry confirmed by electron and atomic force microscopy and it was shown that the exposure value of consumer via packaged products does not exceed current upper allowable level of Ag consumption as a chemical element if using the packaging material for intended purposes (i.e. for packing products in accordance with the manufacturer's recommendations).

Apart this question is raised of risk assessment of NCS for the environment, related to the impact of said nanomaterial on aquatic and soil micro- and macro-organisms - normal components of ecosystems. To solve all these problems, further accumulation and systematization of data is necessary on the toxic effects of Ag NPs at the level of organism and population that enables refinement of their safe doses.
5. Conclusion
Thus, the analysis of nanotechnology products assortment presented in the turnover in the Russian Federation indicates that various forms of nano-sized colloidal silver are the most widely used in consumer products far superior in this regard to all other types of mineral engineered nanomaterials. This makes of paramount importance the assessment of risks, produced by Ag NPs for health of consumers and workers employed in nanotechnology facilities, as well as for the environment.

Analysis of the literature indicates that currently nanoscale silver is one of the most widely studied nanomaterials from the point of view of its toxic effect on biological objects including laboratory animals in vivo. However, the available results of toxicological studies are largely inconsistent, which may be due either to differences in the samples of used NPs (with different particle size and shape, functionalization of the surface), and lack of a unified methodology in the planning the biological experiment. Upon completion of the ongoing 3-month study on the effect of Ag NP stabilized with polyvinylpyrrolidone on laboratory animals using a combination of laboratory tests included in official guidelines approved for the safety assessment of nanomaterials in Russia, it will be possible to establish safe levels of exposure with this widely manufactured and used form of nano-sized silver in Russia. In the future, it will go to its hygienic rationing in products and environmental objects.

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