Nanomaterials in consumer's goods: the problems of risk assessment

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Abstract. Nanotechnology and engineered nanomaterials are currently used in wide variety of cosmetic products, while their use in food industry, packaging materials, household chemicals etc. still includes a limited number of items and does not show a significant upward trend. However, the problem of priority nanomaterials associated risks is relevant due to their high production volumes and an constantly growing burden on the environment and population. In accordance with the frequency of use in mass-produced consumer goods, leading priority nanomaterials are silver nanoparticles (NPs) and (by a wide margin) NPs of gold, platinum, and titanium dioxide. Frequency of nanosized silica introduction into food products as a food additive, at the moment, seems to be underestimated, since the use of this nanomaterial is not declared by manufacturers of products and objective control of its content is difficult. Analysis of literature data on toxicological properties of nanomaterials shows that currently accumulated amount of information is sufficient to establish the safe doses of nanosized silver, gold and titanium dioxide. Data have been provided in a series of studies concerning the effect of oral intake of nanosized silica on the condition of laboratory animals, including on the performance of the immune system. The article examines the existing approaches to the assessment of population exposure to priority nanomaterials, characteristics of existing problems and risk management.

1. Introduction

The unique properties of nanoparticles (NPs) and nanomaterials (NMs) open up broad prospects for their targeted use in such areas of science and technology as materials science, microelectronics, optics, energy, chemical technology, environmental protection, medicine, consumer goods, including household chemicals, perfumes, cosmetics and food products. In accordance with the forecasts made in the early 2000s, the use of artificial NPs and NMs in food technology promises breakthrough achievements in the field of packaging materials with improved properties, new forms of nutrients with high bioavailability, effective and low-toxic food additives [1, 2].

Physicochemical properties and biological activity of the compounds in the nano-dispersed or nano-structured form may be significantly different from their "traditional forms", presented solutions, bulk phases and macroscopic dispersion [3-5]. Since many of the important new properties of NMs currently are still insufficiently studied, the problem rises their risks assessment to human health and the environment. Suggesting the observed progress of nanotechnology industries it can be assumed
that in the short-term exposure of human to NMs grows rapidly, both in terms of quantity and in terms of the number of affecting nanoscale factors [6,7].

Research on the risks of nanotechnology (NT) and NMs is conducted since the early 2000s in the United States (under the supervision of the FDA and EPA), in the EU (IEC, EFSA, ECETOC et al.), as well as in a number of international organizations (OECD, WHO, FAO, ILSI) [8-10]. In Russia, the problem is studied of NT and NM related risk since the end of 2006. In 2007 the "Concept of toxicological research, risk assessment methodology, identification and quantification of nanomaterials" has been adopted [11], which defined the methodological principles and direction of research in this area in the next future. A total of 50 regulatory and guidance documents were developed and subsequently approved as a part of NTs and NMs safety assessment system in progress of the Federal Target Program "Development of infrastructure nanotechnology industry in the Russian Federation for 2008-2011".

Consensus is achieved in modern scientific literature that the traditional four-tier system of risk assessment is applicable to the risk assessment of NMs and NTs which includes stages of 1) dangerous factor identification (which is a source of risk?), 2) hazard estimation (how and in what quantities of the factor being studied produce the harmful effects for the human body?), 3) exposure estimation (how large is the load of harmful factors on the human body in the real conditions, and how the load is distributed in the population), and 4) the risk estimation (its quantitative expression followed by scaling as negligible small, moderate, high and very high) [12-14]. A block diagram is shown in figure 1 of the system of risk assessment in relation to the NPs and NMs peculiarities.

![Block Diagram of Risk Assessment System](image)

**Figure 1.** Schematic diagram of the nanoparticles (NPs) and nanomaterials (NMs) risk assessment

It is believed that there is no fundamental difference between the applicability of this scheme to the NPs and NMs on the one hand, and to the toxic chemicals of traditional dispersion stage - on the other [15], but the implementation of these steps should be specific in all cases to the properties of NPs and NMs defined in the first place by a high degree of dispersion of their structures.

The objectives of this article include consideration of problems of risk assessment of NPs and NMs used in consumer products.
2. Hazard identification

Variety of NPs and NMs produced with modern nanotechnology industry is extremely high. Thus, according to the resource NanoWerk (http://www.nanowerk.com/phpscripts/n_dbsearch.php), registering only NPs and NMs as individual substances, as of April 2015, production in the world includes 616 varieties of carbon nanotubes, 122 - fullerenes, 107 - derivatives of graphene, 567 – NPs of elementary substances (metals and non-metals), 609 – NPs of binary compounds (excluding quantum dots), 161 – NPs of ternary and more complex compounds, 276 – quantum dots, 42 – nanofibers, 5 – non-carbon nanotubes (2504 positions in total). Since the same NM can be used in practice in several types of products, the potential number of species of nanotechnology products can now exceed $10^4$ items. However, this estimate does not take into account large differences in the degree of commercialization of some kinds of nanotechnology products in the manufacturing of consumer goods. It is interesting from this point of view to consider the actual state of the nanotechnology products on market, identifying the types of NPs and NMs, which are priorities from the perspective of the consumer product exposure.

A computer database (registry) is maintained in ION to solve this problem since 2010, that accumulates information on NT products presented in turnover and use in the Russian Federation. The database is updated from public sources, including registries certificate of state registration of products in the Russian Federation and the Customs Union (http://fp.crc.ru); Websites of "RUSNANO" Fund of Infrastructure and Educational Programs (part of the Group RUSNANO) and the Russian nanotechnology network (http://www.rusnanonet.ru/products/list/; http://www.rusnanonet.ru/goods/; http://www.portalnano.ru/), sites of companies that implement in the Russian Federation different kind of consumer nanotechnology products (food supplements, perfumes, cosmetics and others.). Database on NMs is in free access on the website of institute, www.ion.ru.

Analysis of the data provided by the products applicants declared as nanotechnology products, does not allow to reliably identify the NPs and NMs in the composition of the product in a significant number of cases. This may be due, on the one hand, to the desire of producers to maintain technological secrets and know-how, and on the other hand, to the common practice of using the notation "nanotechnology" or "nanotechnology products" in advertising and marketing purposes without a sufficient factual basis.

The database contains information on 1,035 kinds of products manufactured using NTs and NMs as of April 2015. 958 items included (92 % of the total number of titles of nanotechnology products) presented variety of consumer products, and 77 (12%) – non-consumer (pure materials, industrial raw materials, components, manufacturing equipment). The predominant place in the structure of nanotechnological consumer products take perfumes and cosmetics (792 items, 83%) as can be seen from the data obtained. They are followed by food products (47 items, 5%); it is total 55 titles (6%) combining this class of products with packaging materials for food products. Then follow household chemicals (28 items, 3%), fuel components, additives and converters for motor vehicles (24, 2.5%), car cosmetics (20 titles, 2%) and construction materials presented mostly varnishes and paints for exterior use - (17 items, 2%) in descending order. The share is insignificant of other classes of products which production is represented only by single names.

The most common commodity groups are a means for skin care (creams, masks and balms), toilet water, coloring agents, decorative cosmetics and nail polish among perfumery and cosmetic products manufactured using NTs and NMs. In many cases applicants of these products declare the production to be manufactured "with use of NMs and NTs" for purposes of state registration but the composition of these NMs can’t be disclosed on the basis of available information. The products for which the composition of the NM can be set include 90 kinds of perfumes and cosmetic products with silver NPs, 35 – gold NPs, 17 – platinum NPs. In 33 kinds of perfume and cosmetic products (skin care) the applicant declares the presence of “nanosomes”, which are presumably nanometer-sized capsules jacketed with molecules of hyaluronic acid and/or β-cyclodextrin containing inside various biologically active agents. Biologically active supplements dominate (36 items) in the structure of food nanoindustry production. Half of their number (18) presented products, which are the sources of
colloidal (nanoscale) silver. The remaining commodity groups of products (food additives, complex food additives, processing aids) are represented by single items.

Analysis is of great interest of the frequency of various NPs and NMs use in products in turnover, since it allows to identify priority objects for toxicology and hygienic studies aimed by hygienic regulation of NMs in products and environmental samples. Unfortunately, as already noted above, in many cases (for 612 kinds of perfumes and cosmetic products, represented in the database) strict identification of NMs use is not possible. The kind of NMs could be established for 346 types of consumer products, presented in the database. Total 24 types of individual NMs were identified in them. The top of the most "popular" NMs in the consumer goods is as follows (table 1) according to these data.

Nanosized titanium dioxide presented 29 kinds of production. This is, firstly, sunscreen and, secondly - catalytic air purifier. Use of nanosomes and nanoliposomes is sufficiently widespread as components of creams and other skin care products; silicone nanofilms are applied as a water- and dirt-repellent coating on various surfaces - parts of vehicles, building construction, furniture, housewares, textiles and more. Scope of diamond NPs includes decorative cosmetics, metal alloys NPs - remetallizing fuel additive for motor vehicles.

| Nanomaterial                      | Number of products | Ranking position |
|----------------------------------|--------------------|------------------|
| Silver nanoparticles             | 133                | 1                |
| Gold nanoparticles               | 35                 | 2                |
| Nanosomes                        | 33                 | 3                |
| Nanoparticles of titanium dioxide| 29                 | 4                |
| Platinum nanoparticles           | 20                 | 5                |
| Silicone nanofilms               | 19                 | 6                |
| Surfactant micelles              | 10                 | 7                |
| Silica nanoparticles             | 8                  | 8                |
| Diamond nanoparticles            | 7                  | 9-10             |
| Alloy nanoparticles              | 7                  | 9-10             |

Table 1. Ranking of engineered nanomaterials in accordance with the frequency of their use in consumer products.

Nanoscale amorphous silica (SiO$_2$) contains in 8 product items included in the database. However, reason is to assume that the frequency is strongly underestimated of this NM use and hence the degree of the consumers’ exposure to it. As is known, amorphous silica is widely used as a food additive E551, and also as a part of a large number of medicinal tablets together with many types of cosmetic products. There is no information about the size of its particles in the present specification of JECFA on this food additive [16], which allows the use of amorphous superfine silica, obtained by gas-phase hydrolysis of pure tetrachlorosilane. This material known as "Aerosil®" and under a number of other trade names, is characterized by specific surface margined 200-350 m$^2$/g and a primary particle size of 6-30 nm, forming loose aggregates of submicron size, easily destroyable by sonication. Use of this NPs is often not declared by the manufacturers of food production.

Thus, the list of priority NM identified as sources of risk in consumer products includes silver NPs and (by a wide margin in the smaller side in order of importance), NPs of gold, platinum and titanium dioxide. This list should be supplemented with amorphous silica NPs in view of the considerations above. The resulting schedule of NMs coincides in the most number of positions with the list of OECD [17] with the exception that it does not include carbon NMs, such as single- and multi-walled carbon nanotubes and fullerenes. Despite the presence of a plenty potentially useful
properties in these NMs, their use as part of commercialized consumer products is missing or includes
single names. In the list of priority NMs nanosomes aren’t presented which are widely used in the
composition of cosmetic products. This is due to the fact that this type of NPs is biocompatible
according to modern concepts, and within the body they quickly degrade in processes of
bioassimilation.

3. Hazard characterization
Toxicological evaluation of NPs and NMs is based currently on a huge variety of biological test
systems, including models in vitro, in vivo, as well as in silico (computer simulation). The problem is a
difficult task of o these approaches choice for priority NPs and NMs needing in hazard
characterization. Complex hierarchical approaches are developed for this based on the achievements
of genomics, transcriptomics and proteomics, for example, such as program NeoGen in the USA [18].

More traditional methodology is based on the selection of the optimal strategy of toxicological and
hygienic studies based on preliminary data on the potential hazards of the NPs and NM. Criterion of
the potential hazards of nanomaterials should recognize in the first approximation their insolubility
and the ability therefore to the long-term persistence in the biological environment [19]. So, in the
drafting of Annex 18 to the REACH Regulation of the European Union there was proposed [20] a
preliminary assessment criteria of potential danger of NPs and NMs basing only on two indicators: the
volume of annual production and solubility in water and in various biological fluids. The publication
of the European Commission [7] proposed the criteria of NM’s potential danger based on the physico-
chemical characteristics of the particles. Use of the five major external NM properties was
recommended - (1) agglomeration (weakly bound particles) and aggregation (strongly bound
particles), (2) reactivity, (3) the presence of active functional groups on the surface, (4) the particle
size, and (5) the presence of contaminants bound to the surface or in the bulk of the NPs. Factors
include bioavailability, bioaccumulation, potential translocation and potential toxicity that may affect
the risks of artificial NM. A system was developed of pre-classification of NMs according to their
hazard level including very high, high, medium, low and very low risk on this basis.

A more sophisticated system of hazard assessment approach must include information about
structure -activity relationships of artificial NMs [21]. Similar structured approach is applicable in the
Russian Federation to the determination of the required volumes of toxic hygienic and special studies
of NMs, wherein the integrated assessment of the potential hazard is based on forecasting and analysis
procedure that takes into account the entire volume of accessible, reliable and relevant to this NM
scientific and technical information, including data of physico-chemical characteristics of the NPs,
information on effects in cell-free systems and cell cultures, the results of toxicological and
environmental studies, the estimated volume of production [22]. Selected information about the
properties of NM is evaluated, grouped into six functional blocks, ranked in order of importance, as
determined in accordance with peer review. Each estimate is assigned according to its rank with
weighting factor after which the resulting value (index) is calculated of the potential hazard. The
algorithm used includes built-in procedure for evaluating of the uncertainty of the estimation.

As a result, designation of a nanostructured object to the low level of potential hazard require no
any special toxicological-hygienic and medical-biological assessment, and the evaluation should use
the same criteria and approaches applicable to "traditional" analogues of a material manufactured
without use of NT. NPs and NM with an average potential danger are a subject of standard
toxicological and hygienic assessment of their impact on the most important functions of the body.
NPs and NMs characterized by a high degree of potential danger are recommended to be a subject of
more sophisticated studies involving not only the general toxicological research, but also special types
of testing (organotoxicity, immunotoxicity, neurotoxicity, allergenicity, embryotoxicity,
teratogenicity, mutagenicity, carcinogenicity), as well as, possible experiments lifelong laboratory
animals (1.5-2 years), or in a few generations.
One should always take into account in the analysis of dose-effect obtained on biological models that the difficulties and uncertainties are associated with the specific properties of the NPs and NM. So far, there is no consensus about by what units the acting dose of NM should be expressed [15,23].

Value of the results should be considered as a very relative which were obtained in experiments with in vitro models, because there is no information in most cases about whether the dose (concentration) of NPs used in these studies is relevant to situation in vivo related to exposing the organism by the natural ways of intake (inhalation, through the skin, and especially with food and water consumption). A research can play a major role on the penetration (absorption) of NPs through biological barriers, their bioaccumulation, biotransformation and excretion (ADME - research) in the removal of these uncertainties, but the volume is very limited of such information in relation to the practically important NPs and NM currently [22,24]. Information is "scarce" especially about the possibility of absorption of the main types of NPs in the gastrointestinal tract, as well as through uninjured skin [25,26].

A strong dependence exists of NM properties including toxic one on the state of the interface (so-called "surface chemistry") [27]. It should be borne in mind also that partial solubility of toxic chemical compounds (which, by itself, is not a constant but is a function of particle size) plays a large role in the toxic properties of NPs and NM, and the ability is important of NPs to release toxic species adsorbed or chemically bonded to them, such as bacterial endotoxins, heavy metal ions, etc (all of which is defined as the so-called effect of "Trojan horse") [15].

The values of safe doses (concentrations) are calculated after the establishment of non-observed effect maximum exposing dose (NOAEL) for critical organs and systems, as well as whole body by the introduction of appropriate safety coefficients. As a rule, two consecutive 10-fold safety coefficients are introduced when extrapolating to human data obtained in small animals (rats or mice).

The world's scientific literature has accumulated to date a large amount of experimental data on the toxicology of the most important NPs and NM. The amount of information experienced exponential growth over the last decade on the relevant biological and physico-chemical properties of the NPs and NM meeting the criteria of scientific accuracy and completeness as evidenced by the results of scientometric tests. The total number of publications registered in specialized databases (Web of Science, PubMed and others) exceeded 55,000 positions already in 2012 [15]. However, the distribution of publications on the NM species studied does not correlate with their practical importance as constituents of consumer products. In addition there is usually a significant disparity between the use of biological models and test systems and their relation to the most likely scenario of exposure within each sufficiently studied NM. In particular among 63 articles concerning toxicological characterization of the anatase form of titanium dioxide, 27 of papers (43%) were performed on in vitro models of cell cultures and 36 (57%) - in vivo, including 18 papers (29%) on inhalation model, 13 (21%) - when administered parenterally, 4 (6%) - if administered to the gastrointestinal tract, and only one (1%) - when applied to skin according to a meta-analysis [25].

However, sufficient amount of information is now accumulated that enable one to establish safe doses of silver, gold, titanium dioxide NPs, which was reflected in the publications [28-33]. Properties of platinum NPs were studied in much smaller extent; at that almost no data were obtained on biological effects at transdermal way of exposure. The same is true to the NPs of amorphous silica, for which the volume of exposure through food is apparently significantly underestimated.

The majority of nanosized amorphous silica studies were carried out in systems in vitro (cell cultures of various types). Thus, the possibility was demonstrated of catalytic generation of reactive oxygen species in a cell-free system [34] and in keratinocytes [35] and in human alveolar epithelial cells in culture [36]. The ability was set of this NPs type to accumulate in cells of different lines in culture showing a variety of cytotoxic effect. Authors of paper [37] described the actions of amorphous nano-silica on human bronchial epithelial cell line Beas-2B. Reduction in cell viability was close to 20% accompanied with development of peroxidation, changes in the proteomic profile of cells (increase in the expression of several enzymes of intracellular kinase cascade). The summary is presented in table 2 of some other results obtained in cell cultures.
Table 2. Some results of in vitro toxicological evaluation of nanosized amorphous silica

| #  | Experimental model                                                                 | Threshold concentration in media, µg/ml | References |
|----|------------------------------------------------------------------------------------|-----------------------------------------|------------|
| 1  | Normal human bronchial epithelial cells, Beas 2 B                                   | 1.0                                     | [38]       |
| 2  | Normal human lung epithelial and endothelial cells                                  | 300                                     | [39]       |
| 3  | Normal human endothelial cells                                                      | 10.0                                    | [40]       |
| 4  | Platelets                                                                          | 10.0                                    | [41]       |
| 5  | Stem cells                                                                         | 1.0                                     | [42]       |
| 6  | Alveolar epithelial cells of human lung                                             | ≥150 (evaluation)                       | [43]       |
| 7  | Hepatocytes, Kupfer cells                                                           | 100                                     | [44]       |
| 8  | Hepatocytes, Kupfer cells                                                           | 10                                      | [45]       |
| 9  | Human hepatocytes                                                                  | 50                                      | [46]       |
| 10 | Endothelial cells                                                                  | 25                                      | [47]       |
| 11 | Normal and malignant cells of the alveolar epithelium of human lung, fibrosarcoma cells | 10                                      | [48]       |

The mechanism of the cytotoxic effects of silica NPs, as might be expected, is associated with a non-specific deleterious effects on the cell structure of free radicals and reactive oxygen species, catalytically generated on SiO$_2$/water interface [49]. These effects are much more pronounced in NPs when compared to silica of "traditional" degree of dispersion (such as fumed silica, silica gel and so on) in view of silica NPs highly developed interface and a small radius of curvature (which entails an increase in the chemical potential). However, it should be borne in mind that the majority of effects were detected at large concentrations of NPs in cell culture (exceeding 1.0 µg / ml, see table 2).

Quantitative data is now extremely scarce on the extent of absorption, biodistribution and bioaccumulation of nanosized silica due to the high technical difficulties related to the identification and quantification of these NPs in the biological matrix. Accumulation of label was detected after intravenous administration of $[^{125}\text{I}]}$-labeled NPs to mice mainly in cells of the reticulo-endothelial system of liver and spleen [50]. An attempt was undertaken in single study [51] to quantify the absorption and bioavailability of the silica NPs in the organs and tissues of rats after prolonged oral administration using ICP-MS. However, the results obtained were ambiguous due to a high background level of silicon in the bodies of animals of the control group. Significant increase of the silicon content was shown on a qualitative level in the liver and spleen of animals treated with said NPs at doses of 1000 mg / kg body weight or more. Thus, at present data is insufficient which would allow extrapolating the results of silica NPs toxicity studies in cell cultures to the situation in vivo, using the ADME properties of this NM.

Several studies have characterized toxic properties of amorphous silica in vivo. NPs were injected to mice intraperitoneally at very high doses (up to 2 g/kg body weight) in the study [52]. Marked changes were noticed in the function of peritoneal macrophages, increasing production IL-1β, TNF-α, NO, gene expression of IL-1,6, TNF-α, nitric oxide synthase, cyclooxygenase-2. Signs of cell death were observed with the introduction of NM at only the highest doses. 70 nm nanosized silica was hepatotoxic to rats at a dose 30 mg / kg when administered intravenously [53]. Nanoscale SiO$_2$ with particle diameter of 50-100 nm was captured by the liver and kidneys, excreted in the urine and bile when injected intravenously according to [54]. Aim of the study [55] was characterization of silica NPs aerosol toxicity in neonatal rats. Single or 3-day exposure to the aerosol showed no significant pulmonary inflammatory, genotoxic or histopathological effects in rats exposed to particles.
A number of works was devoted to the identification of possible allergenic properties of nanosized silica. For example, these NPs were able to enhance nasal allergic sensitization of mice by ovalbumin allergen according to [56]. Similar data was obtained in a study [57] using a model of asthma in rats sensitized by ovalbumin. A recent study [58] evaluated immunotoxic properties of amorphous SiO$_2$ NPs intraperitoneally administered to mice at a dose of 2-50 mg/kg body weight. Changing proportions were shown of CD3+, CD45+, CD4+ and CD8+ cells in the spleen, shifts in the levels of total IgG and IgM among the effects studied.

Silica NPs were not carcinogenic in rats unlike quartz and carbon NPs according to [59].

Information is scarce in the literature about the possible effects of the silica NPs administered by oral route. Van der Zande M et al. [51] studied subacute (in 84-day experiment) toxicity of two kinds of nanostructured silica administered orally to rats at high doses (100-2500 mg/kg body weight daily). Biochemical and immunological parameters haven’t been studied in blood of animals but fibrosis increase was noticed in a dose-dependent manner and expression of genes responsible for this process also took place in the liver. Authors estimated the threshold subacute oral dose (LOAEL) of silica NPs close to of 2500 mg/kg body weight and NOAEL exceeding 100 mg/kg body weight according to these indicators.

Tananova ON et al [60] examined proteome of microsomal fraction of liver cells from rats, orally receiving nanostructured silica in three doses (from 1 to 100 mg / kg body weight) during 28 days. NPs caused both the disappearance and appearance of new components of the proteome (protein spots on the 2D- electrophoregram) compared to the control group of animals in all doses studied. Mass spectrometric identification revealed the dominant protein peak which disappeared under the influence of NPs at dose 10-100 mg / kg body weight, which was identified as the glucose-regulated protein precursor (GRP 78).

Zaitseva NV and co-workers [61,62] studied the acute oral toxicity in mice of silica NP obtained by method of liquid crystal templating in the presence of cetyltrimethylammonium bromide. $LD_{50}$ was 4638 mg/kg body weight by oral route of administration. Nano disperse silica has expressed high cumulatively (the cumulative index $I_k = 0.45$). Said NM demonstrated also marked toxic effect on animals, judging by the complex biochemical, hematological and morphological parameters at a dose of 0,1-0,3 $LD_{50}$.

Thus, the data available are inconsistent on the toxicity of silica NP in vivo. It should be noted that most of the samples of nanosized silica studied in the literature do not correspond to nanosized silica such as "AEROSIL ®", which is most widely used as a food additive. In view of this, joint research has been conducted by FSBI "Institute of Nutrition", FSBI "FNTS health-care technology risk management to public health" of Rospotrebnadzor and A.N.Bach Institute of biochemistry in 2013-2014 on establishment of maximum no observed effect and threshold doses of this particular form of NM [63-66].

The object of investigation was the amorphous silica “Orisil 300”. The specific surface area of the sample was equal to 300 m$^2$/g when determined by method of BET according to the manufacturer data, which corresponds to calculated diameter of the primary nonporous spherical particles of 7.5 nm. This material presented a white, light, x-ray amorphous powder giving after ultrasonic development opalescent colorless solution in water, which was stable for at least 2 days. Examination of this silica slurry by TEM showed at concentration of 1 mg/ml (figure 2 a-c) that particles of the disperse phase presented mainly large aggregates consisting of distinguishable primary particles sized from 5 to 100 nm, and a very small amount of free particles sized 5-20 nm. AFM showed on the scanned image of the sample slurry dried on a substrate the presence of NPs predominantly in the aggregated state. NPs aggregates varied in size on all the scans from area 20x20 mm$^2$, reaching a maximum of up to 2 μm in one dimension. The structural element was a particle sized in diameter less than 100 nm for all the aggregates. NP’s population had uniform morphology consisting of spherical particles with sizes from 20 to 60 nm (figure 2 d,e). Spektroacoustic study of sonicated aqueous silica slurry showed a bimodal particle size distribution with a predominance of the NP’s fraction with an average diameter of 20-40 nm at concentration of the 5% by weight. Particle size analysis by means of laser dynamic light
scattering showed that the same sample presented predominant fraction of particles in preparation with number average hydrodynamic diameter of 56.6 ± 32.1 nm with 90th percentile - 91.7 nm (figure 3 a,b). The content of particles with a diameter greater than 100 nm did not exceed 10% of the total number of particles after sonication.

Figure 2. Characteristics of nanosized silica particles by transmission electron microscopy, device «JEM-100CX» («JEOL», Japan); (a-c), and atomic force microscopy, device «SmartSPM» («AIST-NT», Russia), (d, e) (data taken from [63,65])
Biological experiments were conducted in accordance with Russian and international rules for work in laboratory animals. Acute toxicity of nanosized silica was studied by a single point method in 20 adult male BALB/C mice weighing 25.0 ± 2.0 g aged 2 months (data taken from [65]). Analysis of the results obtained led to conclusion that an aqueous suspension of nanosized silica did not cause death and of intoxication at a dose of 10,000 mg / kg. Changes in the integrated performance of experimental animals weren’t observed. No any morphological changes were identified in histological preparations of colon and jejunum. Thus, the value of \( LD_{50} \) of the studied nanosized silica exceeded 10,000 mg / kg body weight in oral route.

Subacute oral toxicity of nanosized silica was studied in the growing male Wistar rats in 3-month (92 days) experiment. Animals were divided into five groups (№№ 1-5), treated with this NM at doses, respectively, 0 (control); 0.1; 1.0, 10 and 100 mg / kg of body weight daily as aqueous sonicated slurry. Within 1 month NM was administered intragastrically by gavage, and then added to the experimental semisynthetic balanced diet. Death occurred of one rat in group 4 and three – in group 5 during the 1st month of intragastric administration. The autopsy of animals showed that the death was caused by bilateral pneumonia, which developed as might be expected due to accidental inhalation of traces of suspension containing a high concentration of NPs when administered by gavage. Furthermore, one rat died in group 3 on the 3rd month of experiment. Other animals of all experimental groups had well appearance of skin, hair and mucous membranes, normal motor activity, behavior and stool and did not differ from the control group. Determination of average monthly gains of body weight showed that animals of all groups grew by almost the same rate (\( p > 0.1, \) ANOVA) during the 1st and 2nd month, however a slight (less than 15%) but significant lag in weight gain was noticed in all four experimental groups as compared with control group at the end of the third month. This effect was not dose-dependent and was apparently associated with a reduction of fat mass gain in these groups of aged animals.

Testing showed no negative impact on cognitive function studied by the method of the conditioned reflex of passive avoidance (CRPA) in terms of CRPA resistance at all doses of nanosized silica studied, but at the largest dose of 100 mg/kg the elevation could be suggested of animals discomfort.

**Figure 3.** The size distribution of silica NPs in the sonicated aqueous dispersion according to spektroacoustic studies on device «DT-1202» («Dispersion technology Inc.», USA) (a); and dynamic light scattering on device Nanotrack Wave (Microtrack Inc., USA) (b) (taken from [63,65]).
(anxiety), which resulted in significant reductions in the number of initial visits to dark compartment of the experimental setup.

Integral indices (permeability of the intestinal barrier, the relative weight of internal organs), biochemical, hematological, immunological parameters, the composition of colon microbiota, oxidative DNA damage by excretion of 8-oxo-2-deoxy guanosine (8-oxoG) in urine, liver hepatocyte apoptosis by flow cytometry were evaluated after removal of the animals from the experiment. Effects were studied in separate series of experiments of silica NPs on allergic sensitivity in rats immunized with chicken ovalbumin using model of systemic anaphylaxis (duration of treatment in this case made 28 days).

The results obtained were used to determine NOAEL, taking into consideration for each effect its statistical significance, absolute value, the direction (i.e., the interpretation as an adverse effect like), as well as the presence of a monotonic dependence on the dose of NM. Table 3 presents summary of basic experimental data. The results showed that the most sensitive target for silica NPs is the immune system of animals (figure 4). The total number of leukocytes was significantly lowered in animals of group 5 treated with silica NPs at 100 mg/kg body weight. At the same time, the qualitative composition of Ly was significantly modified despite only a marginal and insignificant decrease of the relative number of lymphocytes (Ly) (7%, \( p > 0.05 \)) in animals of group 5. Proportion significantly decreased of T-helper cells (13%, \( p < 0.05 \)), increased of cytotoxic Ly (19%, \( p < 0.05 \)), all of this leading to the decrease (27%, \( p<0.05 \)) in the immunoregulatory index (dimensionless ratio CD4/CD8) as shown by flow cytometry. TNF-\( \alpha \) level in serum of animals of group 5 increased by an average of 590%, \( p < 0.05 \), and the concentration of IL-10 showed pronounced (36%) trend to decrease.

Table 3. Summary table of the effects of subacute oral administration of nanosized silica to rats in the 92- and 28-day trials (taken from [63,64,66])

| №№ | Indices studied                                                                 | Presence (+) or absence (-) of effect | Effect can (+) or can’t (-) be considered as harmful | NOAEL evaluation | Note         |
|-----|---------------------------------------------------------------------------------|---------------------------------------|-----------------------------------------------------|------------------|-------------|
| 1   | Body weight gain                                                                | +                                     | -                                                   | >100 mg/kg       |             |
| 2   | Relative organ’s mass                                                           | +                                     | +                                                   | ?                | No dose-dependent |
| 3   | Intestinal wall macromolecular permeability                                      | -                                     | -                                                   | >100 mg/kg       |             |
| 4   | Liver microsome system of xenobiotic detoxication                                |                                       |                                                     |                  |             |
|     | Total CYP450                                                                     |                                       |                                                     | >100 mg/kg       |             |
|     | Total Cytochrome b5                                                              |                                       |                                                     | >100 mg/kg       |             |
|     | CYP 1A1 activity                                                                 | +                                     | -                                                   | >100 mg/kg       |             |
|     | CYP 1A2 activity                                                                 | -                                     | -                                                   | >100 mg/kg       |             |
|     | CYP 2B1 activity                                                                 | +                                     | +                                                   | ?                | No dose-dependent |
|     | Glutathione-S-transferase activity                                               | +                                     | -                                                   | >100 mg/kg       |             |
|     | UDP-glucuronosyl transferase activity                                             | -                                     | -                                                   | >100 mg/kg       |             |
| 5   | Activity of lysosomal hydrolases total                                           |                                       |                                                     | >100 mg/kg       |             |
|     | non-sedimentable                                                                |                                       |                                                     | >100 mg/kg       |             |
| 6   | Serum dyene conjugates of PUFA                                                   | +                                     | -                                                   | >100 mg/kg       |             |
|     | Antioxidant enzymes activity                                                     | -                                     | -                                                   | >100 mg/kg       |             |
|   | Liver pool of non-protein thioles | - | - | >100 mg/kg |
|---|-----------------------------------|---|---|-------------|
| 8 | Blood biochemistry                |   |   |             |
|   | ALAT activity                     | + | - | >100 mg/kg  |
|   | ASAT activity                     | - | - | >100 mg/kg  |
| 8 | Serum albumin                     | + | + | ?            |
|   | Total serum protein               | + | + | ?            |
|   | Serum glucose                     | + | + | ?            |
|   | Serum creatinine                  | - | - | >100 mg/kg  |
|   | Serum uric acid                   | - | - | >100 mg/kg  |
|   | Alkaline phosphatase activity     | - | - | >100 mg/kg  |
| 9 | Total hemoglobin                  | - | - | >100 mg/kg  |
| 10| Hematological indices (red blood cells) |
|   | Hemoglobin concentration in cell  | - | - | >100 mg/kg  |
|   | Red blood cells count             | + | - | >100 mg/kg  |
|   | Hematocrit                        | - | - | >100 mg/kg  |
|   | Mean red blood cell volume        | + | - | >100 mg/kg  |
|   | Hemoglobin amount in cell         | + | - | >100 mg/kg  |
| 11| Hematological indices (white blood cells) |
|   | Total white cells count           | + | + | 10-100 mg/kg|
|   | Lymphocytes ratio                 | - | - | >100 mg/kg  |
|   | Same                              | + | + | >100 mg/kg  |
|   | Other (monocytes, neutrophyles, basophiles etc) | - | - | >100 mg/kg |
| 12| Platelets                         | - | - | >100 mg/kg  |
| 13| DNA oxidative damage (urinary 8-oxoG) | + | - | >100 mg/kg |
| 14| Hepatic cells apoptosis (flow cytometry) | - | - | >100 mg/kg |
| 15| Allergic sensitivity (active anaphylactic shock) | - | - | >100 mg/kg |
| 16| IgG to ovalbumine level, ELISA, in sensitized animals | - | - | >100 mg/kg |
| 17| Cytokines                         |   |   |             |
|   | TNF-α                             | + | + | 10-100 mg/kg|
| 18| Neutrophilic leucocytes           | - | - | >100 mg/kg  |
|   | Phagocytosis activity             |   |   |             |
| 19| Cellular immunity                 |   |   |             |
|   | CD45RA+ (B-Ly)                    | - | - | >100 mg/kg  |
|   | CD161a+ (Natural killers)         | + | - | >100 mg/kg  |
|   | CD3+ (T-Ly)                       | - | - | >100 mg/kg  |
|   | CD3+CD4+ (T-helpers)              | + | + | 10-100 mg/kg|
|   | CD3+CD8+ (T-cytotoxic)            | + | + | 10-100 mg/kg|
|   | CD4+/CD8+ ratio                   | + | + | 10-100 mg/kg|
| 20| Cognitive function (CRPA)         | - | - | >100 mg/kg  |
|   | Anxiety level (CRPA)              | + | + | 10-100 mg/kg|

No dose-dependent same same at sensitization
Figure 4. Effect of 92-day receiving various doses of nanoscale silica on the performance of the immune system of rats (data taken from [66])

Data obtained in animals sensitized with ovalbumin demonstrated similar yet less pronounced shifts in immunological parameters induced by silica and no significant increase was noticed in the severity of the allergic reaction after administration of antigen challenge dose (p>0.05). The same could be noted for the level of antibodies to ovalbumin.

Thus, these data allow estimating LOAEL of nanosized amorphous silica corresponding to used as a food additive E551, by the value of 100 mg / kg body weight / day, and NOAEL value of 10 mg / kg body weight / day. Size of safe dose of amorphous nanosized silica must be reduced for human ingestion in accordance with the relevant safety coefficients to 100 times and thus is equal to 0.1 mg / kg of body weight per day, or 7-10 mg per day for an adult.

4. Exposure assessment

Assessing exposure to NPs and NMs needs measuring their number (quantity) entering the body by different ways (inhalation, oral, dermal) as a result of contact with various objects of the environment (air, water, soil) and products (food, perfume and cosmetic products, goods household chemicals, etc). The most important steps in the evaluation of exposure are: identification of scenarios and routes of exposure; identification of the medium that carries the NPs/NMs; determination of their concentrations in the control points; timing, frequency, and duration of exposure; identification of affected population. Exposure scenario can often be simplified in the real conditions of risk assessment and reduced to the flow of NPs/NMs by only one way (eg, ambient air, drinking water, food, cosmetic products, etc.). In some cases an exposure scenario may be limited to NPs/NMs income from certain source of emissions (eg waste of nanoindustry enterprises stationed in the area, the packaging material containing NPs and so on).

With regard to NMs used in the composition of consumer’s goods direct impact of production on population should be considered both in the case of food products - by oral exposure, perfume and cosmetics - primarily transdermal. Indirect impact should also be considered through objects of the environment. The significance increases of the latter group of scenarios with the volume of priority NM production. Thus annual production of silver NP totaled in the world more than 500 tons in terms
of Ag in 2011, and could exceed 1,000 tons in 2015 according to [34], which corresponds to about 140 mg per year for every person on Earth. Silica NPs annual production is estimated at 25,000 tons in the world [67], which is about 40% of global issue volume of nanopowders. Daily load on human with silica NPs from food can reach 1.8 mg / kg body weight according to recent evaluation [51]. A more accurate estimate of the load is not possible at the moment, because the specification of silica used as a food additive E551 isn’t disclosed in many cases by the manufacturers of products, and its nanoscale nature isn’t declared. Selective control of a number of food and food supplements samples containing E551 and its products held in ION by means of dynamic light scattering showed that this dietary additive is known to present NPs having particle size in the range below 100 nm in many cases.

It’s important to bear in mind when evaluating the exposure that the distribution of artificial mineral NP in the production and in the biosphere can be extremely uneven. It is assumed that the general population is exposed to NPs mainly by oral and transdermal route when consuming foodstuffs, food supplements and cosmetics containing these NPs, as well NPs migrating into food from packaging materials [68, 69], whereas inhaled route of exposure apparently predominates in employees of enterprises producing products with NMs [70]. The assumption does not receive experimental confirmation that a large number of mineral NPs can enter the atmosphere in the form of aerosols during the disposal of waste of textiles, medical supplies and other products of NT on waste-incineration plants. In fact, a major amount of NPs is accumulated in ash together with the sludge from wet treatment system of exhaust gas clarification [71], which can be supplied subsequently to fields as a surrogate fertilizer, washed away into water, accumulate in different soil and water organisms [72], and eventually come to man through the food chain as a part of agricultural production. However, data is currently insufficient to quantify the exposure that proceeds in such a scenario.

5. Risk characterization
Several models are available to characterize quantitatively the risks of exposure to NPs and NMs depending on various scenarios of exposure and the rendered character of harmful effects [73-77]. Special attention should be given when choosing a model that is adequate to the current situation to information about the presence of cumulative effects produced by NPs/NMs (accumulation in organs and tissues, accumulation of toxic action) and certain types of remote effects on the body (mutagenic, carcinogenic effects, etc.), synergistic effects of various NPs and NMs in conjunction with toxic substances of traditional dispersity.

The risk profiling of NPs/NMs is qualitative in nature when based on a model that takes into account deterministic effects, since all the risks are considered insignificant that are associated with the current dose (concentration) of the NPs/NMs at a level below the reference values (norms). If one exceed the reference value there is a significant risk, but it’s not currently possible to establish clearly the nature of the quantitative relationship between excess dose over reference level and value of risk (probability of harmful effect). In view of this, the risk assessment is based on qualitative model using so-called “Hazard quotient” (HQ) and “hazard index” (HI) [78]. This model is basically the same that is applicable to non-carcinogenic risk of chemicals for which one can set the threshold for safe exposure. In this case, the hazard ratio is calculated by the formula

$$HQ = \frac{Ed}{RfD}$$

when $Ed$ – exposure, a $RfD$ – safe referent level of exposure (dose or concentration). It is clear that both values used in the calculation must match in dimension, so that the resulting hazard ratio remains dimensionless.

The aggregative risk is characterized of a number of nanoscale factors in the case of their combined action by the hazard index (HI) which is calculated by the formula
\[ HI = \sum_{i=1}^{N} HQ_i \]

when \( i \)- is a serial number of nanoscale factors taken into account.

The total hazard index (THI) is calculated when the complex flow take place of nanoscale chemicals into the human body from the environment by a number of ways, as well as in the multimedia and multipath impact as follows:

\[ THI = \sum_{j=1}^{M} HI_j \]

when \( HI_j \) - hazard index for way or route of action number \( j \) from \( M \) possible.

If risk calculated according to the specified parameters (coefficient index or the total hazard index) is 0.1 or less, the significance is negligible of harmful effects in humans, hence this impact is characterized as acceptable and does not require any measures for its decline. If the calculated hazard index of NPs/NMs is between 0.1 and 1.0 it’s possible that the risk of harmful effects in man throughout life must be regarded as significant taking into consideration the uncertainty in evaluating the exposure setting and the reference levels, and decision-making must provide a set of measures to reduce the impact of NPs/NMs on population/person. If the calculated ratio exceeds 1.0, the risk of harmful effects in humans is high (unacceptable), but it's apparently not possible to pinpoint the precise value of this due to the qualitative nature of this model.

Authors of paper [79] have shown with respect to the silver NPs migrating into the food from packaging materials, using the data of mass spectrometry confirmed by TEM, AFM and DLS, that exposure amount of consumer through packaged products didn’t not exceed the upper allowable levels of Ag consumption as a chemical element in the conditions when the packaging material was used by proper destination (e.g. for package of products in accordance with the manufacturer's recommendations). It is essential to note in this regard, that the silver nanoparticles could be successfully detected in the food product by DLS in modern method modification [80].

The results of the risk assessment of the NPs and NM should be taken into account in the design of decision-making to reduce risk levels for the population by controlling the production, trafficking and use of nanotechnology products, which is the generally termed as "risk management".

6. Risk management

Management is possible of risks posed by the NMs and NPs presented in consumer products on the basis of the assessment of the risks and in the presence of the regulatory framework to control NT and NM on the state, municipal (local) and the corporate level.

Regulation of NT, which is now developing in most industrialized countries in the world, can be divided into the so-called "Horizontal" and "Vertical" regulation [81]. The subject of "Horizontal" regulation is formulated extremely broad and covers all kinds of products of certain functional purpose, for example, all food products, all cosmetics, medicines, etc., in the total amount of which NM and NT products occupy usually only a small section. In the case of "Horizontal" legislation specific properties of NPs and NMs are commonly not specified, and the rules are not stated applicable specifically to them. NM and nanotechnology products are covered by this legislation mainly because of their functional purpose to the extent that they perform the same tasks and have in some sense similar consumer properties as products not containing NM and produced by "traditional" technologies [82-84]. "Vertical" legislation is specifically designed for the purpose of NT and its products regulation and is applicable only in those industries. In view of this specificity is provided of the "Vertical" regulations, as opposed to the "horizontal", as a rule, by well-developed terminology and definitions, which allows one’s to apply nanotechnology products with much greater uniqueness and fewer discrepancies than "Horizontal" legislation.
The analysis of legal acts and special literature has shown that currently "vertical" legislation is developed abroad in a very small extent in relation to NPs and NT [81,85,86]. Perhaps the only example, to fully meet these criteria, is Regulation EC No 1223/2009 on cosmetic products, in which extensive sections are, providing specific procedures governing placing on the market and labeling of this type of NT products [87].

In the EAEC explicitly formulated articles are defined in a number of Technical regulations of the Customs Union adopted during the 2011-2012 that apply to NM and NT products specified to their procedures-to-market (associated usually with the state registration of products). Thus, the elements of "Vertical" "nano" - legislation are already presented in these documents. As for lower level regulations, according to which the state registration and necessary expertise of products is held a whole system of recommendations and guidance documents was approved by Rospotrebnadzor that consider all aspects of control procedures implemented to nanotechnology products, including classification, examination procedures, state registration and supervision, quantitative analysis and valuation, toxicological and special studies, risk assessment. The system allows risk management of NMs and nanotechnology products at level of the most possible certainty at actual scientific knowledge in accordance with uniform, unified approach.

7. Conclusion
Genuine “flowering” of NT and NM application has been expected in the manufacturing of a great many consumer products, including food according to the forecasts for the period 2012-2014 which had been made at the beginning of the 2000s, mainly on the basis of trend analysis of scientific and patent information. However, studies have shown of commercialized range of consumer products, that there is the massive introduction of NT and NM predominantly in the manufacture of cosmetics, while their use in food products, packaging materials, household chemicals, etc. still includes a limited number of items and shows no significant upward trend. However, the problem is relevant of exposing population to priority NM and the associated risks due to high production volumes of some NMs and their entrance to the environment. Silver NPs and (by a wide margin) NPs of gold, platinum, and titanium dioxide have leading priority in this respect in accordance with the frequency of use in mass-produced consumer goods. Nanosized silica introduction is now apparently underestimated into food products as a food additive since the use of this NM is not declared by manufacturers of products and objective control of its content is difficult.

Analysis of literature data on toxicological properties of NMs shows that sufficient amount of information is currently accumulated enabling establishment of safe exposing doses of silver, titanium dioxide and gold NPs. In series of recent studies evidence have been provided on the effect of silica NPs oral intake on the state of the organism of laboratory animals, including the performance of the immune system. These results allow establishing a NOAEL for this practically important NM and move on to their hygienic regulation.

References
[1] Bouwmeester H, Brandhoff P, Marvin HJP, Weigel S, Peters RJB. 2014 Trends in Food Science & Technology 40(2) 200,
[2] Popov KI, Filippov AN and Khurshudyan SA 2009 Russian Chem. J. 53 86
[3] Borm PJ, Robbins D, Haubold S , Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D and Oberdorster E 2006 Part. Fibre Toxicol. 3, 11
[4] Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D and Yang H 2005 Part. Fibre Toxicol. 2 8
[5] Oberdörster G, Oberdorster E, and Oberdorster J 2005 Environ. Health Perspect. 113 823
[6] Gmoshinski IV, Smirnova VV and Khotimchenko SA 2010 Nanotechnologies in Russia 5 6
[7] Henkler F, Tralau T, Tentschert J, Kneuer C, Haase A, Platzek T, Luch A and Gotz ME 2012 *Arch. Toxicol.* **86** 1641

[8] Risk Assessment of Products of Nanotechnologies Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) 2009 *European Commission Health and Consumers DG.* Brusseles 71 p.

[9] Brief P 2009 Appropriate Risk Governance Strategies for Nanotechnology Applications in Food and Cosmetics. *Geneva: International Risk Governance Council* 44 P.

[10] Grobe A, Renn O and Jaeger A 2008 Risk Governance Strategies for Nanotechnology Applications in Food and Cosmetics. *Geneva: International Risk Governance Council* 52 P

[11] Onishchenko GG and Tutrelyan VA 2007 *Voprosy pitaniya (Problems of nutrition)* **76**(6) 4

[12] Zaitseva NV, Trusov PV, Shur PZ, Kyryanov DA, Chigvintsev VM and Cinker MJu 2013 *Analizy riska zdorov'yu (Health risk analysis)* 1 15.

[13] Yokell1 RA and MacPhail RC 2011 *J.Occupational Med. Toxicol.* **6** 74

[14] Stander L and Theodore L 2011 *Int. J. Environ. Res. Public Health.* **8** 470

[15] Maynard RL 2012 *Emerg. Health Threats J* **5** 10

[16] Silicon dioxide, amorphous 1973-1992 *Rome: JECFA.* 2 P. http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

[17] OECD 2010 List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship programme for the testing of manufactured nanomaterials: revision.-*Paris: OECD Environment Directorate* 13

[18] Cote I, Anastas PT, Birnbaum LS, Clark RM, Dix DJ, Edwards SW and Preuss PW 2012 *Environ. Health Perspect* **120** 1499

[19] Utembe W, Potgieter K, Byron SA and Mary G 2015 *Part. Fibre Toxicol.* **12** 11

[20] Oksel C, Ma CY and Wang XZ 2015 *Procedia Engineering* **102** 1500

[21] Nanomaterials and REACH 2012 *Background Paper on the Position of German Competent Authorities* **52** P. http://www.bfr.bund.de/cm/349/nanomaterials-and-reach.pdf

[22] Onishchenko GG, Tutrelyan VA, Gmoshinski IV and Khotimchenko SA 2013 *Gigiena & sanitariya (Hygiene and Sanitation)* **1** 4

[23] Draft scientific opinion. Guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed 2011 *EFSA Scientific Committee.*- Parma, Italy.: European Food Safety Authority (EFSA) 32 P. http://www.efsa.europa.eu/en/consultationsclosed/call/scaf110114.pdf

[24] Oostingh GJ, Casals E, Italiani P, Colognato R, Stritzinger R, Ponti J, Pfuller T, Kohl Y, Ooms D, Favilli F, Leppens H, Lucchesi D, Rossi F, Nelissen I, Thielecke H, Puntes VF, Duschl A and Boraschi D 2011 *Part. Fibre Toxicol.* **8** 18

[25] Chang X, Zhang Y, Tang M and Wang B 2013 *Nanoscale Res Lett.* **8**(1) 51

[26] Fröhlich E and Roblegg E 2012 *Toxicology* **291** 10

[27] Levard C, Hotze EM, Lowry GV, Gordon E and Brown GE 2012 *Environ Sci Technol.* **46** 6900

[28] Stensberg MC, Wei Q, McLamore ES, Porterfield DM, Wei A and Sepúlveda MS 2011 *Nanomedicine (London)* **6** 879

[29] Schluesener JK and Schluesener HJ 2013 *Arch Toxicol.* **87** 569

[30] Gerber A, Bundschuh M, Klingelhofer D and Gronberg DA 2013 *J.Occupational Med. Toxicol.* **8** 32

[31] Khlbotov N and Dykman L 2011 *Chem. Soc. Rev.* **40** 1647

[32] Shi H, Magaye R, Castranova V and Zhao J. 2013 *Part. Fibre Toxicol.* **10** 15

[33] Gaillet S, Rouanet J-M. 2015 *Food and Chemical Toxicology* **77** 58

[34] Thomassen LC, Aerts A, Rabolli V, Lison D, Gonzalez L, Kirsch-Volders M, Napierska D, Hoet PH, Kirschhock CE and Martens JA 2010 *Langmuir* **26** 328

[35] Nabeshi N., Yoshikawa T., Matsuyama K. Nakazato Y, Tochigi S, Kondoh S, Hirai T, Akase T, Nagano K, Abe Y, Yoshioka Y, Kamada H, Itoh N, Tsunoda S and Tsutsumi Y 2011 *Part. Fibre Toxicol.* **8** 1
[36] Eom HJ and Choi J 2009 *Toxicol. In Vitro* **23** 1326
[37] Eom HJ and Choi J 2011 *Environ. Health Toxicol* **26** e2011013
[38] Kasper J, Hermanns MI, Bantz C, Maskos M, Stauber R, Pohl C, Unger RE and Kirkpatrick JC 2011 *Part. Fibre Toxicol.* **8** 6
[39] Corbalan JJ, Medina C, Jacoby A, Malinski T and Radomski MW 2011 *Int. J. Nanomedicine* **6** 2821
[40] Shi J, Karlsson HL, Johansson K Gogvadze V, Xiao L, Li J, Burks T, Garcia-Bennett A, Uheida A, Muhammed M, Mathur S, Morgenstern R, Kagan VE and Fadeel B. 2012 *ACS Nano* **6** 631
[41] Napierska D, Thomassen LC, Rabolli V, Lison D, Gonzalez L, Kirsch-Volders M, Martens JA and Hoet PH 2009 *Small* **5** 846
[42] Ye Y, Liu J, Xu J, Sun L, Chen M and Lan M 2010 *Toxicol. In Vitro* **24** 751
[43] Sohaebuddin SK, Thevenot PT, Baker D, Eaton JW and Tang L 2010 *Part. Fibre Toxicol.* **7** 22
[44] Yang X, Liu J and He H 2010 *Part. Fibre Toxicol.* **7** 1
[45] Xue Y, Chen Q, Ding T and Sun J 2014 *Int. J. Nanomedicine* **9** 2891
[46] Wang W, Li Y, Liu X, Jin M, Du H, Liu Y, Huang P, Zhou X, Yuan L and Sun Z 2013 *Int. J. Nanomedicine* **8** 3533
[47] Duan J, Yu Yongbo, Yu Yang, Li Y, Huang P, Zhou S and Sun Z 2014 *Part. Fibre Toxicol.* **11** 50
[48] Fede C, Selvestrel F, Compagnin C, Mognato M, Mancin F, Reddi E and Celotti L 2012 *Anal. Bioanal. Chem.* **404** 1789
[49] Napierska D, Thomassen LCJ, Lison D, Martens JA and Hoet PH 2010 *Part Fibre Toxicol.* **7** 39
[50] Yu T, Hubbard D, Ray A and Ghandehari H 2012 *J. Control Release* **163** 46
[51] Van der Zande M, Vandebriel RJ, Groot MJ, Kramer E, Rivera ZEH, Rasmussen K, Ossenkoppele JS, Tromp P, Gremmer ER, Peters RJB, Hendriksen PJ, Marvin HJP, Hoogenboom RLAP, Peijnenburg AAM and Bouwmeester H 2014 *Part. Fibre Toxicol.* **11** 8
[52] Park EJ and Park K 2009 *Toxicol. Lett.* **184** 18
[53] Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y and Yagi K 2009 *Eur. J. Pharm. Biopharm* **72** 496
[54] Cho M, Cho WS, Choi M, Kim SJ, Han BS, Kim SH, Kim HO, Sheen YY and Jeong J 2009 *Toxicol. Lett.* **189** 177
[55] Sayes CM, Reed KL, Glover KP, Swain KA, Ostraat ML, Donner EM and Warheit DB 2010 *Inhal. Toxicol.* **22** 348
[56] Yoshida T, Yoshioka Y, Fujimura M Yamashita K, Higashisaka K, Morishita Y, Kayamuro H, Nabeshi H, Nagano K, Abe Y, Kamada H, Tsunoda S, Itoh N, Yoshikawa T and Tsutsumi Y. 2011 *Nanoscale Res. Lett.* **6** 192
[57] Han B, Guo J, Abrahaley T, Qin L, Wang L, Zheng Y, Li B, Liu D, Yao H, Yang J, Li C, Xi Z and Yang X 2011 *PLoS One* **6** e17236
[58] Lee S, Kim M-S, Lee D, Kwon TK, Khang D, Yun H-S and Kim S-H 2013 *Int. J. Nanomedicine* **8** 147
[59] Kolling A, Ernst H, Rittinghausen S, Heinrich U 2011 *Inhal. Toxicol.* **23** 544
[60] Tananova ON, Arianova EA, Gmoshinski IV, Toropygin IVu, Kryapova EV, Trusov NV, Khotimchenko SA and Tutelyan VA 2014 *Biochemistry (Moscow) Suppl.Ser.B: Biomedical Chem.* **8** 125
[61] Zaitseva NV, Zemlyanova MA, Zvezdin VN and Saenko EV 2013 *Analyz riska zdorov'yu (Health risk analysis)* **1** 65
[62] Zaitseva NV, Zemlyanova MA, Lebedinskaya OV, Zvezdin VN, Melekhin SV and Akaf'yeva TI 2013 *Morfologiya (Morphology)* **144**(5) 78
[63] Shumakova AA, Arianova EA, Shipelin VA, Sidorenko JuS, Selifanov AV, Trushina EN, Mustafina OK, Safenkova IV, Gmoshinski IV and Tutelyan VA 2014 *Voprosy pitaniya (Problems of nutrition)* **83**(3) 52
[64] Shumakova AA, Avren’eva LI, Guseva GV, Trusov NV, Maltsev GYu, Soto SH, Vorozhko IV, Solomatina VL, Gmoshinski IV, Khotimchenko SA and Tuteluan VA 2014 Voprosy pitaniya (Problems of nutrition) 83(4) 58

[65] Zaitseva NV, Zemlyanova MA, Zvezdin VN, Dovbish AA, Gmoshinski IV, Khotimchenko SA, Safenkova IV and Akafyeva TI 2014 Voprosy pitaniya (Problems of nutrition) 83(2) 42

[66] Gmoshinsky IV, Khotimchenko SA, Trushina EN and Khanferyan RA 2014 Toxicological assessment of silica nanoparticles in subacute experiment in rats 7 International Nanotoxicology Congress (Nanotox 2014) Antalya-Turkey, April 23-26, 2014. Program & abstracts 160

[67] http://www.abercade.ru/research/analysis/67.html

[68] Hansen SF, Michelson ES, Kamper A, Borling P, Stuer-Lauridsen F and Baun A 2008 Ecotoxicology 17 438

[69] Keller AA, Vosti W, Wang H, Lazareva A. 2014 J. Nanoparticle Res. 16, 1

[70] Christensen FM, Johnston HL, Stone V, Aitken RJ, Hankin S, Peters S and Aschberger K 2010 Nanotoxicology 4 284

[71] Vejerano EP, Leon EC, Holder AL and Marr LC 2014 Environ. Sci.: Nano 1 133

[72] Fabrega J, Luoma SN, Tyler CR, Galloway TS and Lead JR 2011 Environ. Int. 37 517

[73] Shatkin JA 2008 Nanotechnology: health and environmental risks. Boca Raton, London, NY: CRC Press 167 P.

[74] Linkov I, Steevens J, Adlakha-Hutcheon G., Bennett E, Chappell M, Colvin VJ, Davis M, Davis T, Elder A, Hansen SF, Hakkinen PB, Hussain SM, Karkan D, Korenstein R, Lynch I, Metcalfe C, Ramadan AB and Satterstrom K 2009 J. Nanoparticle Res. 11 513

[75] Tolaymat T, El Badawy A, Sequeira R, and Genaidy A. 2015 Journal of Hazardous Materials 298, 270

[76] Kazak AA, Stepanov EG, Gmoshinski IV and Khotimchenko SA 2012 Voprosy pitaniya (Problems of nutrition) 81(4) 11

[77] Sotiriou GA, Singh D, Zhang F, Wohlleben W, Chalbot M-CG, Kavouras IG, Demokritou P. 2015 Environmental Science: Nano 2(3) 262

[78] Risk assessment of the impact of nanomaterials and nanoparticles on the human body 2011 Guidelines MR 1.2.0038-11 Moscow: Rospotrebnadzor 98 P.

[79] Smirnova VV, Kricnavoarova OV, Pridvorova SM, Zherdev AV, Gmoshinski IV, Kazydub GV, Popov KI and Khotimchenko SA 2012 Voprosy pitaniya (Problems of nutrition) 81(2) 40

[80] Rykhik OV., Panferov VG, Kotova NN and Popov KI 2013 Khranenie i pererabotka selkhozsyrya 6 43

[81] Cushen M, Kerry J, Morris M, Cruz-Romero M and Cummins E 2012 Trends Food Sci. Technol. 24 30

[82] Directive 2011/65/EU of the European Parliament and of the Council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment (recast). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:174:0088:0110:en:PDF.

[83] Falkner R and Jaspers N 2012 Global Environmental Politics 12 30

[84] Malloy TF Soft Law and Nanotechnology. A Functional Perspective. Essay. http://www.nanolawreport.com/tags/soft-law-and-nanotechnology-a/#axzz 24N3I7Zj0.

[85] Classification, labeling and packaging of nanomaterials in REACH and CLP. Annex II: Final version. Doc. CA/90/2009 Rev2. 2009. European Commission, Environment Directorate-General Water, Chemical and Biotechnology, Brussels, 3 December 6 P http://ec.europa.eu/environment/chemicals/reach/pdf/classif_nano.pdf.

[86] Preparing for our future: Developing a common strategy for key enabling technologies in the EU. 2009 Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the regions.- Brussels:
Commission of the European Communities 12 P. http://ec.europa.eu/enterprise/sectors/ict/files/communication_key_enabling_technologies_en.pdf.

[87] Cosmetics companies to face new rules in Europe. 2009 International Herald Tribune, 24 March, http://iht.com/bin/printfriendly.php?id=21022812

Abbreviations list:

ADME - absorption, distribution, metabolism and excretion (research); AFM - atomic force microscopy; BET - Brunauer–Emmett–Teller (method); CRPA- conditioned reflex of passive avoidance; DLS - dynamic light scattering; HI - hazard index; HQ - hazard quotient; ICP-MS - mass-spectrometry with inductively coupled plasma; ION- Institute of nutrition (Moscow); LOAEL - the lowest observable adverse effect dose (threshold dose); NM(s) - nanomaterial(s); NOAEL - not observable adverse effect dose (safe dose); NP(s) -nanoparticle(s); NT - nanotechnology; TEM - transmission electron microscopy; THI - total hazard index;