Interaction of engineered nanoparticles with toxic and essential elements

AA Shumakova¹, IV Gmoshinski¹, SA Khotimchenko¹ and EN Trushina¹
¹Federal State Budgetary Science Institution «Institute of Nutrition», 2/14, Ust’inskiy proezd, Moscow, 109240 Russia

E-mail: Antonina_sh@list.ru

Abstract. Interaction of engineered nanoparticles with toxic and essential trace elements must be taken into consideration when estimating risks of NPs presented in the natural environment. The purpose of this work was to study the possible influence of silica, titanium dioxide (rutile) and fullerenol NPs on the toxicity of cadmium and to research the status of some trace elements and related indices of immune function in experiments on laboratory animals. Young male Wistar rats received cadmium salt (1 mg/kg b.w. Cd) orally for 28 days separately or in conjunction with the said kinds of NPs in different doses. A number of effects was observed as a result of combined action of Cd together with NPs, increase in bioaccumulation of this toxic trace element in the liver was most evident. The observed effects didn’t show simple dose-dependence in respect to nanomaterials that should be taken into consideration when assessing the possible risks of joint action of nanoparticles and toxic elements existing in the environment in extremely low doses. Violation of microelement homeostasis caused by the combined action of Cd and NPs can have various adverse effects, such as inhibition of T-cell immunity induced by co-administration of Cd with rutile NPs.

1. Introduction
The assessment of potential risk of nanoparticles (NPs) and nanomaterials (NMs) is a difficult challenge, but it is of primary importance [1-3], considering rapid introduction of new products of nanotechnology (food, medicine, cosmetics, household chemicals, fuel components, construction materials and etc.) [4]. At the same time it’s necessary to take into account that NPs are extremely seldom present in the environment and industry in the isolated form, instead they exist in complex with traditional components including contaminants, such as heavy metals, dioxins, etc [5].

NPs have physicochemical properties that are different from the same materials in the form of macroscopic dispersions or solids. One of them is the capability of NPs to penetrate through biological barriers and thereby facilitate trafficking of contaminants adsorbed on NPs surface or bond in their bulk phase. Thus, the question arises about possible risks, consisting in increased toxicity of chemical contaminants of food products and the environment as a whole in their conjunction with NPs. The possibility of such adverse effects was widely discussed [6-13].

Another source of NP’s risk consists in their interaction with trace elements including essential ones (such as Fe, J, Se, Zn, Cu, Mn, Cr, Mo, Co) and other with less obvious biological function (such as Ag, V, B, Si, Br, F). Some of these elements are constituents of commercially manufactured NPs (such as Ag and Si), others may be adsorbed on NPs and penetrate with them through barriers of organism or, on the contrary, linger in the intestinal lumen due to binding to low-absorbable NPs.
This may lead to changes in safety and bioavailability of some trace elements producing several systemic impacts, for example on immune system performance [14].

Therefore, the purpose of this research was to study the possible impact of some widespread NPs such as silica, titanium dioxide (rutile) and fullerene NPs on the toxicity of cadmium together with status of some trace elements and related indices of immune function in experiments on laboratory animals.

2. Materials and methods

2.1. Nanoparticles and nanomaterials

Nanoparticles of titanium dioxide in the form of rutile (NPs TiO$_2$) were purchased from “Sigma-Aldrich” (Germany). The preparation was previously characterized by transmission electron microscopy (TEM) as consisting of partially agglomerated rod-shaped NPs with approximate size of 5×40 nm. Mean hydrodynamic diameter of particles equaled to 44.7 nm (51.5%) and 103.6 nm (48.5%), 90$^{th}$ percentile –129.3 nm according to laser dynamic light scattering (DLS). Fine dispersed amorphous silica “Orisil 300” was purchased in “Silica” LTD (Russia) and had BET specific surface 300 m$^2$/g according to the manufacturer. TEM characterized the sample as loose agglomerates of NPs sized from 5 to 100 nm with a very small number of free particles sized 5-20 nm. DLS-measured mean hydrodynamic diameter in sonicated water slurry was 56.6 ± 32.1 nm; 90$^{th}$ size percentile - 91.7 nm. Fullerol (C$_{60}$(OH)$_{24}$) was purchased from “Fullerene-center” LTD (Russia) and had 99.5% purity in reverse-phase HPLC. DLS revealed marked aggregation of fullerene molecules in water solution with formation of NPs (micelles) sized 2.89 nm (75.1%) and 1.05 nm (24.9 %) in diameter, 90$^{th}$ percentile – 4.68 nm.

2.2. Toxic heavy metal preparation

Cadmium chloride (CdCl$_2$•2.5 H$_2$O) was used as a source of toxic element with traditional level of dispersion. Preparation was analytical grade, purchased from «Khimmed», Russia.

2.3. Tested solutions preparation

Tested solutions of NPs were freshly prepared daily by dissolving nanomaterials and Cd salt in deionized water with further ultrasound treatment under the following conditions: frequency 44 kHz, power 2 W/ml, time 5 min, temperature 2-4°C.

2.4. Animals

Experiment was performed on 128 adult male Wistar rats, weighing 67-109 g, kept in plastic cages in a temperature controlled (22°C) room, a 12 h light : dark cycle was maintained. Rats received standard full value semisynthetic rat diet prepared on the base of casein (coinciding with the composition to AIN76) and water ad libitum throughout the experiment. The animals were randomized into eight groups of 16 rats. The tested preparations were administered to animals once a day at a fixed time intragastrically by gavage in total volume of deionized water less than 2 ml. The experiment lasted for 28 days. The number of groups and doses of preparation tested are listed in Table 1.

2.5. Methods

Rats’ body weight was registered daily throughout the experiment. Animals were withdrawn from the experiment by exsanguination under deep ether anesthesia, relative organ weights were determined. Hematological parameters of leucocytes were studied in hematology analyzer «Coulter AC TTM 5 diff OV» ( «Beckman Coulter», USA) with a standard set of reagents (manufactured by «Beckman Coulter», France). Expression of CD45RA, CD3, CD4, CD8, CD161a antigens was determined on peripheral blood lymphocytes (Ly) by direct immunofluorescence staining of whole blood with a panel of monoclonal antibodies conjugated with fluorescent dyes manufactured by «Beckman Coulter», USA. Analysis of the stained cells was performed on a flow cytometer “FC-500”, manufactured by «Beckman Coulter» (USA) using program “Cytomics CXP Software”. Ly
populations were isolated by gating on parameters of small angle (FS) and side (SS) light scattering. The gating of CD3 + Ly populations via fluorescence channels FL1 and SS Lin was performed next. The results were recorded on a two-parameter histogram distribution of CD3+ (from gate B) using monoclonal antibodies against CD4 and CD8 detected in fluorescence channel FL5 and FL4, respectively. The expression of CD45RA and CD161a was determined in a separate test similarly. The total content of CD45RA + (B-Ly), CD3 + (T-Ly) and CD161a + (natural killer, NK) was expressed as per cent (%) of the total number of Ly analyzed considering at least 10^4 events per sample. The contents of CD3 + CD4 + (T-helper cells) and CD3 + CD8 + (cytotoxic T-Ly) were determined as % of their share in the total number of CD3 + cells. Dimensionless immunoregulatory index (IRI) was calculated as a ratio of CD4 + / CD8 + cells counts.

| Groups ## | Doses of preparation administered, mg/kg body weight |
|-----------|-------------------------------------------------------|
|           | CdCl₂×2.5H₂O salt as Cd | Silica NPs as SiO₂ | Rutile NPs as TiO₂ | Fullerenol as C₆₀(OH)₂₄ |
| 1         | 0                                      | 0               | 0             | 0                        |
| 2         | 1                                      | 0               | 0             | 0                        |
| 3         | 1                                      | 0               | 0             | 0                        |
| 4         | 1                                      | 100             | 0             | 0                        |
| 5         | 1                                      | 0               | 1             | 0                        |
| 6         | 1                                      | 0               | 100           | 0                        |
| 7         | 1                                      | 0               | 0             | 1                        |
| 8         | 1                                      | 0               | 0             | 10                       |

Table 1. List of groups of animals and studied preparation doses

The concentration of cadmium (Cd), lead (Pb), arsenic (As), silver (Ag), zinc (Zn), copper (Cu), chromium (Cr), manganese (Mg), aluminum (Al), nickel (Ni) and cobalt (Co) in organs (liver, kidney, brain) was determined by mass-spectrometry with inductively coupled plasma (ICP-MS) with octopole reaction system (ORS) by means of device “Agilent ICP-MS 7700x” (“Agilent Technologies”, Japan). Sample preparation for ICP-MS measurements was carried out in microwave digestion system “TOP WAVE” (“Analytic Jena”, Germany).

3. Results and discussion
Four rats died during the experiment in groups 1, 4 and 5; three – in groups 2 and 3, two – in group 7. A bilateral pneumonia was found on section in all the dead animals which was apparently a result of occasional aspiration of silica suspensions. The remaining animals had visibly normal behavior and performance, condition of hair and mucous surfaces and stool. At day 28 no pronounced and dose-dependent influences were observed on relative organ mass with exception of lung weight 16% (p<0.05) increase in group 8 (fullerenol, 10 mg/kg) compared to group 2 (Cd only).

As it’s seen from Table 2, Cd intoxication didn’t influence WBC indices in rats in the used doses. However, silica NPs (group 3) and rutile NPs (group 5) uniformly decreased Ly count and increased neutrophiles but this effect was not dose-dependent in respect to NPs dose.

| Group # | Number of rats | Total WBC count, 10³/µl | Contents of total WBC, % | Platelets 10⁹ dm⁻³ |
|---------|----------------|----------------------------|---------------------------|-------------------|
|         |                |                            |                           |                   |
| 1       | 8              | 13.3±2.0                   | 14.7±1.2                  | 76.9±1.6          |
|         |                |                            |                           | 7.6±0.6           |
|         |                |                            |                           | 634.0±28.0        |

Table 2. Mean (M ± m) indicators of leukocytes and platelets in rats from the treated groups
Study of cellular immune performance of animals (Table 3) revealed no influence of Cd on the studied parameters. NPs of all types had no effect on B-cells and NK counts. The most pronounced changes were the decrease in T-helper proportion and increase in T-cytotoxic cells with corresponding lowering of IRI in animals subjected to high dose of rutile NPs against the background of Cd intoxication. It’s noticeable that such effect was absent in rats receiving this nanomaterial without Cd as it was shown in compatible conditions of experiment in [15].

ICP-MS studies of trace elements in the organs have clearly shown marked Cd accumulation in all tissues of the animals receiving this toxic element (Table 4). The most pronounced Cd rise was noticed in kidney (33.95%) and liver (45.14%) whereas in brain Cd concentration increased only by 55%. Cd accumulation in liver was strongly affected by silica NPs and (to lesser extent) fullerol in small dose, whereas such effect from rutile NPs wasn’t statistically significant. This result confirms the suggestion that Cd uptake in live organisms may be enhanced due to effect of its concomitant penetration through biological barriers together with some kinds of NPs [16]. Corresponding growth of kidney Cd accumulation in presence of NPs intake was far less pronounced and considered insignificant. Apparently no influence of Cd was noticed on NPs level in brain.

### Table 3. Mean indices of cellular immunity performance in rats from the treated groups

| Groups ## | Number of rats | Percentage of total lymphocytes, M±m | Percentage of total CD3+, M±m | CD4/CD8 (IRI) |
|-----------|----------------|--------------------------------------|-------------------------------|---------------|
|           |                | CD45RA + (B-Ly) | CD3+ (T-Ly) + (NK-cells) | CD161a | CD3+ (Th-cells) | CD4+ | CD3+ (T-cytotoxic) | CD4+ | CD8+ (IRI) |
| 1         | 8              | 27.1±2.0          | 50.6±4.4                      | 7.7±1.0 | 53.7±4.8          | 44.4±4.8 | 1.4±0.3 |
| 2         | 7              | 25.7±2.4          | 54.2±4.6                      | 8.5±1.8 | 55.8±5.6          | 42.5±5.5 | 1.6±0.4 |
| 3         | 6              | 29.0±2.8          | 50.8±4.9                      | 8.4±1.7 | 55.6±7.8          | 41.9±7.3 | 1.7±0.4 |
| 4         | 6              | 32.9±5.5          | 50.4±6.0                      | 9.1±1.7 | 46.1±5.8          | 51.7±5.2 | 1.0±0.2 |
| 5         | 6              | 29.1±3.3          | 51.4±2.8                      | 9.1±1.2 | 50.1±8.0          | 47.5±8.1 | 1.5±0.5 |
| 6         | 10             | 25.5±1.3          | 56.9±2.6                      | 6.9±1.1 | 38.4±4.8          | 60.1±4.8 | 0.8±0.2 |
| 7         | 6              | 29.1±3.2          | 50.0±3.6                      | 9.5±1.4 | 49.6±7.0          | 48.6±7.2 | 1.3±0.3 |
| 8         | 10             | 27.2±1.9          | 53.1±3.3                      | 6.9±0.6 | 50.4±5.1          | 47.9±5.0 | 1.3±0.3 |

1,2Superscript - group numbers, the difference with which was significant, p<0.05

### Table 4. Cadmium accumulation in rat organs in presence of various NP types

| Groups ## | Number of rats | Concentration μg/g tissue |
|-----------|----------------|--------------------------|
|           | Liver          | Kidney                   | Brain                    |
| 1         | 0.083±0.011    | 0.101±0.006             | 0.009±0.002             |
Exposition of the animals to Cd demonstrated different impacts on levels of toxic, essential and conditionally essential trace elements (Tables 5, 6) that coincided with the data known from literature [17]. The effect of decrease in Pb accumulation was detected in liver, especially in rats additionally treated with low doses of silica (group 3), fullerenol (group 7) and high doses of rutile NPs (group 6). Unlike this Zn level significantly grew in following groups: 4 (high dose of silica), 5 (low dose of rutile) 7 and 8 (both doses of fullerenol). Consequently, this NPs markedly enhance effect of Cd on Zn accumulation that was yet insignificant in group 2 (Cd only). Cu levels significantly grew in presence of fullerenol treatment (group 7) whereas Ag accumulation diminished and As increased in group 4 (high dose of silica NPs). Any differences in liver levels of Al, Ni and Cr weren’t found in animals developed by both Cd and all kinds of NPs (data not shown).

Accumulation of Mn in liver was apparently not influenced by Cd itself but significantly enhanced by low dose of silica (group 5) and both doses of fullerenol (groups 7,8).

Table 5. Trace elements accumulation in rat liver in presence of cadmium and NPs

| Groups | Number of rats | Pb     | As      | Ag      | Zn      | Cu      | Mn      | Co      |
|--------|---------------|--------|---------|---------|---------|---------|---------|---------|
| 1      | 8             | 0.064± | 0.020±  | 4.14±   | 34.74±  | 5.487±  | 2.164±  | 0.073±  |
| 2      | 8             | 0.008± | 0.002±  | 1.402±  | ±1.883± | 0.318±  | 0.106±  | 0.004±  |
| 3      | 8             | 0.046± | 0.018±  | 2.306±  | 37.272± | 5.094±  | 2.212±  | 0.064±  |
| 4      | 7             | 0.009± | 0.001±  | 0.746±  | ±2.223± | 0.434±  | 0.126±  | 0.005±  |
| 5      | 8             | 0.038± | 0.018±  | 1.941±  | 39.277± | 5.201±  | 2.347±  | 0.071±  |
| 6      | 8             | 0.007± | 0.002±  | 0.747±  | ±1.432± | 0.274±  | 0.115±  | 0.002±  |
| 7      | 7             | 0.051± | 0.024±  | 2.272±  | 48.111± | 6.597±  | 2.516±  | 0.073±  |
| 8      | 8             | 0.009± | 0.002±  | 1.440±  | ±2.369± | 0.630±  | 0.073±  | 0.005±  |
| 9      | 8             | 0.042± | 0.020±  | 1.465±  | 43.246± | 6.522±  | 2.319±  | 0.083±  |
| 10     | 8             | 0.007± | 0.001±  | 0.724±  | ±2.315± | 0.798±  | 0.122±  | 0.005±  |
| 11     | 8             | 0.031± | 0.020±  | 5.280±  | 38.748± | 5.007±  | 2.132±  | 0.067±  |
| 12     | 8             | 0.008± | 0.002±  | 1.585±  | ±1.566± | 0.281±  | 0.080±  | 0.002±  |
| 13     | 8             | 0.030± | 0.023±  | 1.001±  | 44.196± | 6.320±  | 2.612±  | 0.073±  |
| 14     | 8             | 0.006± | 0.002±  | 0.803±  | ±2.297± | 0.163±  | 0.104±  | 0.006±  |
| 15     | 8             | 0.045± | 0.020±  | 3.189±  | 42.694± | 5.623±  | 2.466±  | 0.077±  |
| 16     | 8             | 0.009± | 0.002±  | 0.825±  | ±1.791± | 0.221±  | 0.088±  | 0.010±  |

Superscript –see comment to table 3

Table 6. Trace elements accumulation in rat kidneys in presence of cadmium and NPs

| Groups | Number of rats | Pb     | As      | Zn      | Cu      | Mn      | Co      |
|--------|---------------|--------|---------|---------|---------|---------|---------|
| 1      | 8             | 0.066± | 0.014±  | 21.722± | 4.611±  | 0.915±  | 0.131±  |
|        |               | 0.004± | 0.001   | 0.481±  | 0.064±  | 0.023±  | 0.003±  |

Superscript –see comment to table 3
The landscape of trace elements distribution in kidneys was similar to some extent in regard to Pb, Zn and Mn, but the changes caused by NPs were less pronounced and could be explained in most cases by effect of Cd itself. In any way Cd strongly enhanced Co accumulation in kidney irrespective to NPs development. Effects on Al, Ni, Cr and Ag accumulation in all groups were insignificant (data not shown).

Most changes in trace elements levels in brain were small in dimension and insignificant that reflects high degree of brain-blood barrier impermeability to chemical factors and stability of brain media homeostasis (data not shown). The only exception was decrease in Co accumulation from 12.0±1.5 ng/g tissue (group 1) to 8.5±0.7 ng/g tissue (group 2) caused by Cd. Said effect was obvious also in combined treatment with all 3 types of NPs.

The results obtained confirm the presence of interactions of different types of NPs with toxic trace element Cd during its absorption and biodistribution in the bodies, which coincides with the data obtained earlier on alternative biological models [16,18,19]. Said changes of Cd accumulation may result in different manifestations of Cd toxicity, in particular its immunosuppressive action [20]. In this work it was demonstrated when joint treatment with Cd and rutile (TiO$_2$) NPs displayed pronounced decrease in Th mediated immunity in rats. The fact is noticeable that in many cases the effects of NPs appear to be dose independent i.e. pronounced at low rather than high doses. Today the data is insufficient to explain comprehensively this observation but one should keep in mind the instability in time and the variability of t properties of NPs depending on the biological environment [21]. It can be suggested in this case that NPs aggregation in gastrointestinal tract (that is more pronounced at high concentrations/doses in accordance with the law of mass action) is a factor that counteracts with NPs uptake and concomitant Cd trafficking. This may mean that effects potentiating toxic elements action may be more pronounced at extremely low doses of NPs that apparently take place in the real environmental conditions.

Numerous data on changes in the bioavailability of trace elements may be placed among the effects of "the interaction of trace elements" in animals treated with Cd [17]. Their explanation implies that trace elements compete for cellular sites responsible for uptake, transport, retention (fixation) and clearance both to influence of toxic metal (i.e. Cd) on activity of enzyme systems responsible for essential elements metabolism.

As shown by the data obtained in this work, various types of NPs, like silica, rutile and fullerol are not neutral in respect to these processes, and may modify them to some extent, which is an additional source of risk of breaking the element homeostasis in the combined action of Cd and these NMNs.

4. Conclusion
Thus, a number of effects were observed in combined action of Cd and NPs of practical importance including silica, titanium dioxide (rutile) and fullerenol on laboratory animals, the most significant effect was the strengthening of bioaccumulation of this toxic trace element in the liver. The effects observed do not show a simple dose-dependence towards NMs that should be considered when assessing the possible risks of joint action of NPs and toxic elements present in the environment in extremely low doses. Violation of microelement homeostasis caused by the combined action of Cd and NPs can have various adverse effects, such as inhibition of T-cell immunity induced by co-administration of cadmium with rutile NPs.

References
[1] Oberdörster G, Oberdorster E and Oberdorster J 2005 Environ. Health Perspect. 113 823
[2] 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 Particle Fibre Toxicol. 3 (11) 1–35
[3] Onischenko GG and Tutelyan VA 2007 Voprosy pitaniya(Problems of nutrition) 76 (6) 4 (in Russian)
[4] Wise K and Brasuel M 2011 Nanotechnol Sci Appl 4 73
[5] Holsapple MP, Farland WH, Landry TD, Monteiro-Riviere NA, Carter JM, Walker NJ and Thomas KV2005 Toxicol Sci. 88 (1) 12-17
[6] Cui F, He C, Yin L, Qian F, He M, Tang C and Yin C2007 Biomacromolecules 8(9) 2845-2850
[7] El-Shabouri M.H, 2002 Int. J. Pharm. 249 (1-2) 101-108
[8] Thomas KV, Farkas J, Farmen E, Christian P, Langford K, Wu QandTollefsen KE 2011 J Toxicol. Environ. Health A. 74 (7-9) 466-477
[9] Cronholm P, Karlsson HL and Hedberg J2013 Small.9(7)970-982
[10] Limbach LK, Wick P, Manser P, Grass RN, Bruinink A and Stark WJ2007 Environ Sci Technol.41(11)4158-4163
[11] Ying Zhu, Yu Zhang, Jing Li, Xiaoyong Zhang, Chunhai Fan and Qing Huang 2014 Material of «The 7th International Nanotoxicology Congress «NanoTox2014»256
[12] Melnik EA, Buzulukov YP, Demin VF, Demin VA, Gmoshinski IV, Tyshko NV and Tutelyan VA2013 ActaNaturae (Russian version) 5 (3) 111
[13] Quadros ME and Marr LC2010 J Air Waste Manag. Assoc. 60 (7) 770-81
[14] Benetti F, Bregoli L, Olivato I and Sabbioni E 2014 Metallomics 6 729
[15] Arianova EA, Shumakova AA, Tanaanova ON, Trushina EN, Mustafina OK, Sharanova NE, Gmoshinski IV and Khotimchenko SA 2012 Voprosy pitaniya (Problems of nutrition) 84 (6) 47
[16] Zhang X, Sun H, Zhang Z, Niu Q, Chen Y, and Crittenden JC 2007 Chemosphere 67 160
[17] Avtsin AP, Zhavoronkov AA, Rish MA and Strochkova LS 1991 Human microelementoses Moscow, “Meditsina” edition 496 P.
[18] Sun H, Zhang X, Zhang Z, Chen Y and Crittenden JC 2009 Environ Pollut157 1165
[19] Yang WW,  Miao AJ and Yang LY2012 PLoS One7e32300
[20] Demenesku J, Mirkov I, Ninkov M, Popov-Aleksandrov A, Zolotarevski L, Kataranovski D and Kataranovski M 2014 Toxicology 326 96
[21] Maynard RL 2012 Emerg. Health Threats J 5 10