Research of nickel nanoparticles toxicity with use of Aquatic Organisms

T Morgaleva, Yu Morgalev, I Gosteva, S Morgalev
Centre "Biotest-Nano", Tomsk State University, 36, Lenina ave., Tomsk, 634050, Russia

E-mail: tg.morgaleva@gmail.com

Abstract. The effect of nanoparticles with the particle size $\Delta_{50}=5$ nm on the test function of aquatic organisms was analyzed by means of biotesting methods with the use of a complex of test-organisms representing general trophic levels. The dependence of an infusoria Paramecium caudatum chemoattractant-elicited response, unicellular algae Chlorella vulgaris growth rate, Daphnia magna Straus mortality and trophic activity and Danio rerio fish kill due to nNi disperse system concentration, is estimated. It is determined that the release of chlorella into cultivated environment including nNi as a feed for daphnias raises the death rate of entomostracans. The minimal concentration, whereby an organism response to the effect of nNi is registered, depends on the type of test organism and the analysed test function. $L(E)C_{20}$ is determined for all the organisms used in bioassays. $L(E)C_{50}$ is estimated for Paramecium caudatum ($L(E)C_{50} = 0.0049$ mg/l), for Chlorella vulgaris Beijer ($L(E)C_{50} = 0.529$ mg/l), for Daphnia m. S ($L(E)C_{50} > 100$ mg/l) and for fish Danio rerio ($L(E)C_{50} > 100$ mg/l). According to the Globally Harmonized System hazard substance evaluation criteria and Commission Directive 93/67/EEC, nNi belongs to the “acute toxicity 1” category of toxic substances.

1. Introduction
Currently, metallic engineered nanoparticles (NPs) are prioritized nanomaterials (NM) for investigations. Particularly important is the studying of nickel nanoparticles (nNi) that have widespread application in modern industry and among other things serve as a catalyst, owing to the number of unique physical and chemical properties (high surface energy, low melting point, high specific surface area, magnetism and others). Since nNi is a promising NM, including for the purposes of platina replacing in different catalytic processes, and in the nearest time it may account for a substantial part of NPs produced by manufacture, there emerges the important task of analyzing its environmental safety. The necessity of this task is approved by normative documents both in Russia and the OECD [1, 2]. The information available in the literature concerning this issue is extremely limited, fragmented and controversial. In the last decade, particular attention has been paid to the studying of genotoxic and cancerogenic properties of nNi for cells of homoithermic animals [3, 4]. There are data showing an impact of nNi on protein turnover of mice, including albumin fraction [5]. The authors suppose that albumins take part in nNi transportation.

At the same time, the issue of nNi safety, when it inflows into aqueous environments, practically remains unresolved. An exception is that of research by Griffitt et al. [6]. The authors determined the toxicity of nNi with a particle size of 6 nm with regard to maxilopods Daphnia pulex, Ceriodaphnia...


**dubia** and algae *Pseudokirchneriella subcapitata*. There are data on the toxicological effect of nNiO on growth and morphological changes of *Chlorella vulgaris Beijer*. The authors stated that nNiO triggers cells’ plasmolysis and chlorella’s cellular membrane destruction [7]. In a chronic experiment on *Danio rerio* full-grown fish, Kovrižnych determined the high toxicity and nNiO accumulation in fish [8].

To a greater extent, the topicality in solving the issues concerning the analyzing of the impact of nNi on the test functions of aquatic organisms results from the absence of a unified system of a safety evaluation of NM.

We developed an algorithm of evaluation of ecological toxicity (as a result of the accomplishment of grants of the Federal Target Program in 2008-2013) that is oriented mainly to simultaneously studying the test reactions of an assembly of organisms from different systematic groups depending on the possible distribution sphere, application and utilization of nanoproducts.

### 2. Materials and methods

The nNi disperse system (DS) with an initial concentration of 50 mg/l became available by means of a laser ablation method [9]. According to the data of a BET-analysis and transmission electronic microscopy the average particle size $\Delta_{50} = 5$ nm.

The impact of nNi on the test functions of aquatic organisms was studied on the basis of a chemoattractant-elicited response of single-celled animals *Paramecium caudatum* [10], on the speed of growth of unicellular alga *Chlorella vulgaris Beijer* [11], on the mortality rate of entomostracans *Daphnia magna Straus* [12], on *Daphnia magna Straus* trophic activity rate [10] and on the *Danio rerio* fish kill rate [13]. We were creating DS of nNi according to the technique we developed [14]. The nNi DS toxicity was studied on the concentration line of 0.00001, 0.0001, 0.001, 0.01, 0.1, 1.0, 5.0 and 10.0 mg/l. For the redispersion, DS of nNi was ultrasonicated (30 W/l) for 5 minutes immediately before biotesting. The evaluation of the toxicity of the analysed DS of nNi is implemented in accordance with the criteria according to certified methodologies [11, 12].

The research design is similar to the described in [15, 16].

Statistical processing of the acquired data was carried out with the help of probit analysis. The values of L(E)$_{C_{10}}$, as upper limits of NOEC, L(E)$_{C_{20}}$ и L(E)$_{C_{50}}$, were calculated. Work was conducted with the application of metrologically gauged instruments: Autolumat LB953 (Germany), «Shimadzu» RF-5301 PC spectrofluorimeter (Japan), IPS-03 photoelectric colorimeter (Russia), Biotester-2 concentration meter (Russia) and diffractometer «Shimadzu» XRD 6000 (Japan).

### 3. Results

As a result of undertaken studies, we have determined, that cultivated environments with a content of nNi (5 nm) in 0.00001, 0.0001, 0.001, 0.01 and 0.1 mg/l concentrations resulted in changes of test functions in just two organisms: an inhibition of the chemoattractant-elicited response of *Paramecium caudatum* and an inhibition of the growth rate of *Chlorella vulgaris Beijer*. Deviations of the functions from a control did not exceed permissible values. The toxicity index varied from $I=11.6\pm1.8\%$ to $I=34.8\pm1.5\%$ for *Paramecium caudatum* and from $I= -2.2\pm0.06\%$ to $I=7.4 \pm1.44\%$ for *Chlorella vulgaris Beijer*. In the concentrations interval from 0.00001 mg/l to 0.1 mg/l of DS of nNi, the death rate of entomostracans *Daphnia magna Straus* and fish *Danio rerio* was not noted. The trophic activity of *Daphnia magna Straus*, when cultivating entomostracans in DS of nNi with concentrations varying from 0.00001 mg/l to 1.0 mg/l, did not practically differ from the activity of a validation batch (figure 1).

The further increase of a concentration of nNi particles exacerbated the toxic effect of DS. The exposition of *Paramecium caudatum* in DS of nNi with a concentration of 1.0 mg/l during 30 minutes led to the complete inhibition of the chemotaxis of infusoria. The value of the toxicity index comprised 100 % that indicated the high toxicity of the analyzed test system (figure 2).

DS of nNi had a less pronounced toxic effect on the cells growth of *Chlorella vulgaris Beijer* with C=1.0 mg/l. After 22 hours of incubation in DS with the noted concentration, the optical density of
Chlorella decreased in 1.7 times with regard to the control. Moreover, the toxicity index slightly exceeded the permissible level (I=34.5±2.26 %). The exposition of Daphnia magna Straus and Danio rerio in DS of nNi with the concentration of 1.0 mg/l did not result in mortality of entomostracans and fish kill.

![Figure 1. Toxicity Index of DS of nNi according to test functions of organisms of different systematic groups (1- chemotaxis of Paramecium caudatum, 2- weight gain of Chlorella vulgaris Beijer, 3- death rate of Daphnia magna Straus; 4- death rate of Daphnia magna Straus when inserting of chlorella as a feed; 5- trophic activity of Daphnia magna Straus, 6 – death of Danio rerio)](image1)

When increasing the concentration of particles in the cultivated environment, nNi had an acute effect on all the test organisms except for fish. With nNi concentrations of 5.0 mg/l and 10.0 mg/l for Paramecium caudatum and Chlorella vulgaris Beijer, the toxicity of DS of nNi corresponded to the gradation of “High”. The exposition of daphnids in DS of nNi with the noted concentrations resulted in the death rate of entomostracans equal to 40.0±4.1 % and 43.3±6.2 % within 48 hours, and the decreasing of the trophic activity to 77.7±3.5 % and 66.9±6.2 %.

![Figure 2. Toxicity index of DS of nNi according to test functions of organisms of different systematic groups (1- chemotaxis of Paramecium caudatum, 2- weight gain of Chlorella vulgaris Beijer, 3- death rate of Daphnia magna Straus; 4- death rate of Daphnia magna Straus when inserting of chlorella as a feed; 5- trophic activity of Daphnia magna Straus, 6 – death of Danio rerio)](image2)

A growth of the toxic effect of DS of nNi (on the “death rate” indicator) in the series of experiments on analyzing trophic activity of entomostracans in the cultivated environment with nNi is noted. Moreover, the toxicity index increases on 6.7±1.2 % for C=5.0 mg/l and on 10.0±0.7 % for C=10.0 mg/l (figure 1). The growth of toxicity is probably connected with the effect of nNi on entomostracans by means of two ways: immediately from the environment and with contaminated algae. Insertion of chlorella in the cultivated environment as a feed for Daphnia magna Straus is an
During the process of Ni NPs fusion by means of the laser ablation method in water, the authors reported the contamination of the Ni environment. The level of effects of NPs depends on the type of test organisms [24]. It was specified that the tested sample of nNi with the particle size of 5 nm refers to dangerous substances with a high toxicity degree of “acute toxicity 1”.

The data we acquired concerning the toxicity of nNi with a particle size of 5 nm is coherent with the results of single experimental works in the literature. Griffith et al. [6] determined the toxicity of nNi with regard to entomostracans, unicellular algae and fish. However, the figures of L(E)C$^{50}$ (3.89 mg/l, 0.35 mg/l and >10 mg/l correspondingly) given by the authors differ from the data we obtained (table 1). One can suppose that the noted differences for entomostracans and algae are connected with the application by Griffith et al. of other representatives of systematic groups: *Daphnia pulex*, *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*.

According to Gong et al., L(E)C$^{50}$ comprised 32.28 mg/l for the first 72 hours of incubation. Moreover, the authors pointed out that nNiO triggered cells’ plasmolysis and the destruction of cell membranes of *Chlorella vulgaris Beijer.* [7]. In Kovrižnych et al. [8], the toxicity of nNiO was analyzed on full-grown fish *Danio rerio*. The effect of nNiO on *Danio rerio* over 30 days resulted in the accumulation of NPs in fishes’ organisms, and as a consequence, it resulted in the high toxicity of nNiO. The value of L(E)C$^{50}$ for the 30 days of the incubation of *Danio rerio* in a static regime comprised 45.0 mg/l, and L(E)C$^{100}$ (the minimum concentration which resulted in a 100 % death rate of fish) was equal to 6.25 mg/l for the full-grown adult of *Danio rerio*.

The studies we carried out allow us to define “limiting links” of sustainability of an ecosystem to the contamination of the nNi environment. The level of effects of NPs depends on the type of test organism and concentration of NPs. It was found that the vulnerability of separate links of the trophic level decreases in a range of *Paramecium caudatum, Chlorella vulgaris Beijer, Daphnia magna Straus* and *Danio rerio*. The ecotoxicological parameters of DS of nNi are determined for each test organism (table 1): (L(E)C$^{10}$), (L(E)C$^{20}$) and L(E)C$^{50}$.

Biological effects began to appear with nNi concentrations for protozoans *Paramecium caudatum* (L(E)C$^{20}$=0.0047 mg/l), for unicellular algae *Chlorella vulgaris Beijer* (L(E)C$^{20}$=0.067 mg/l), for entomostracans *Daphnia magna Straus* (L(E)C$^{20}$=0.237 mg/l – according to the trophic activity and L(E)C$^{20}$=15.21 mg/l – according to the death rate). As for *Danio rerio*, nNi is not active in terms of biological activity in the analyzed concentrations.

On the basis of the data obtained as a result of ecotoxicological analysis on test systems with an enhanced range of test functions, an integral estimation of the toxicity of nNi with the particle size of 5 nm was conducted.

The ecotoxicological evaluation of the obtained results of the sample toxicity was conducted taking into account the principle of the most expressed reaction. This principle was presented in normative documents [24]. It was specified that the tested sample of nNi with the particle size of 5 nm refers to dangerous substances with a high toxicity degree of “acute toxicity 1”. 

**References:**

[17 - 21]
Table 1. The values of the ecotoxicity of DS of nNi with a size of NPs of 5 nm

| Environment | Test organism                  | Test reaction          | L(Е)C_{50}, (mg/l) | L(Е)C_{20}, (mg/l) | L(Е)C_{10}, (mg/l) | Acute toxicity class |
|-------------|--------------------------------|------------------------|--------------------|--------------------|--------------------|---------------------|
| DS nNi      | *Paramecium caudatum*          | Chemoattractant-elicited | 0.0049             | 0.00047            | 0.00014            | 1                   |
| DS nNi      | *Chlorella vulgaris Beijer*     | Weight gain            | 0.529              | 0.067              | 0.0033             | 1                   |
| DS nNi      | *Daphnia magna Straus*         | Death rate             | >100               | 15.21              | 1.877              | None                |
| DS Ni + Chlorella | *Daphnia magna Straus* | Death rate             | 88.170             | 2.494              | 0.390              | 3                   |
| DS nNi      | *Daphnia magna Straus*         | Trophic activity       | 40.984             | 0.237              | 0.016              | 3                   |
| DS nNi      | *Danio Rerio*                  | Fish kill              | >100               | >100               | >100               | None                |

Conclusion
Acute toxicity class 1

4. Conclusion
It was specified that nNi (5 nm), that was obtained with the method of laser ablation, is a dangerous substance with a high toxicity degree of “acute toxicity 1”.

It was shown that nNi has a selective toxicity and is toxic not for all of the analyzed test organisms. It was specified that the permissible/safe level of tested NPs does not exceed a concentration of 1.0 mg/l. The increase in the concentration of nNi leads to significant toxic damages of test organisms (except for fish). With concentrations of DS of nNi 5.0 mg/l and 10.0 mg/l, significant disorders of organisms’ functions are noted. These disorders were found among representatives of phytoplankton (decrease of mass growth of unicellular algae) and zooplankton (a negative chemotaxis of infusorias, an inhibition of the trophic activity and increasing of the death rate of entomostracans). Phyto- and zooplanctonic community of hydrosphere is at risk. That leads to disorders of trophic and metabolic interrelations, injures to the integrity of natural biocoenosis and ability in terms of rehabilitation.

5. Acknowledgments
This study (research grant No 8.2.57.2015) was supported by Tomsk State University Academic D.I. Mendeleev Fund Program in 2015.
Work was conducted with the application of the Tomsk regional common use centre technical equipment acquired thanks to a grant of the Russian Ministry of the Agreement No.14.594.21.0001 (RFMEFI59414X0001).

References
[1] The concept of toxicological studies, risk assessment methodology, identification and quantification of nanomaterials 2007 Approved by the Resolution of the Chief State Inspector of the Russian Federation dated October 31 79
[2] OECD 2007 Current Developments in Delegations And Other International Organisations on The Safety of Manufactured Nanomaterials Tour de Table 3rd Meeting of the Working Party on Manufactured Nanomaterials

[3] Magaye R, Zhao J, Bowman L and Ding M 2012 Experimental and Therapeutic Medicine 4 551-61

[4] Ahamed M, Alhadlaq H 2014 OncoTargets and Therapy 7 269–280

[5] Atlaskirova A, Baradulina E, Borodulin Ya 2014 Bulletin of Medical Internet conferences 4 (5) 596-600

[6] Griffitt R, Luo J, Gao J, Bonzongo J and Barber D 2008 Environmental Toxicology and Chemistry 27 (9) 1972-78

[7] Gong N, Shao K, Feng W, Lin Z, Liang C, Sun Y 2011 Chemosphere 8 (4) 510-16

[8] Kovrižnych J, Sotnikova R, Zeljenkova D, Rollerova E, Szabova E 2014 Interdiscip Toxicology 7 (1) 23-6

[9] Svetlichnyi V, Lapin I 2013 Russian Physics Journal 56 (5) 581-87

[10] Morgalev Yu, Khoch N, Morgaleva T, Dunaevsky G and Morgalev S 2010 Methodological Guide Tomsk

[11] Morgalev Yu, Morgaleva T and Grigoriev Yu 2010 FR.1.39. 2010.09103 (Moskow:Federal Reestr) p 45

[12] Morgalev Yu, Morgaleva T and Grigoriev Yu 2010 FR.1.39.2010.09102 (Moskow:Federal Register) p 45

[13] The safety assessment of nanomaterials in vitro and in model systems in vivo: Guidelines 2009 Federal Center of Hygiene and Epidemiology

[14] Morgalev S, Morgaleva T, Morgalev Yu, Gosteva I 2015 Journal Advanced Materials Research 1085 424-30

[15] Morgalev Yu, Morgaleva T, Gosteva I, Morgalev S, Kulizhskiy S, Astafurova T 2015 submitted to Journal Advanced Materials Research

[16] Gosteva I, Morgalev Yu, Morgaleva T, Morgalev S 2015 submitted to Journal Advanced Materials Research

[17] Morgalev Yu, Khoch N, Morgaleva T, Gulik E, Borilo G, Bulatova U, Morgalev S and Ponyavina E 2010 Nanotechnologies in Russia 5 11-12

[18] Osborne-Koch M 2009 Environmental Toxicology 44 p

[19] Renault S, Baudrimont M, Mesmer-Dudons N, Gonzalez Pm, Brisson A 2008 Gold Bulletin 41 116-126

[20] Bouldin J, Ingle T, Sengupta A, Alexander R, Hannigan R, Buchanan R 2008 Environmental Toxicology and Chemistry 27 (9) 1958–63

[21] Fabrega J, Luoma S, Tyler Ch, Galloway T, Lead J Environment International 37 517–531

[22] Mahfouz R, Cadete Santos Aires F, Brenier A, Jacquier B, Bertolini J Applied Surface Science 254 5181–90

[23] Sakiyama K, Koga K, Seto T, Hirasawa M, Oriti T 2004 J. Phys. Chem. B. 108 (2) 523-29

[24] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) The third revised edition 2009 UN