Research of nickel nanoparticles toxicity with use of Aquatic Organisms

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Abstract. The effect of nanoparticles with the particle size Δ_{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 Beijer* 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 maxillopods *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 Δ_{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 Wt/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±1.8 % to I=34.8±1.5 % for *Paramecium caudatum* and from I= -2.2±0.06 % to I=7.4 ±1.44 % 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.

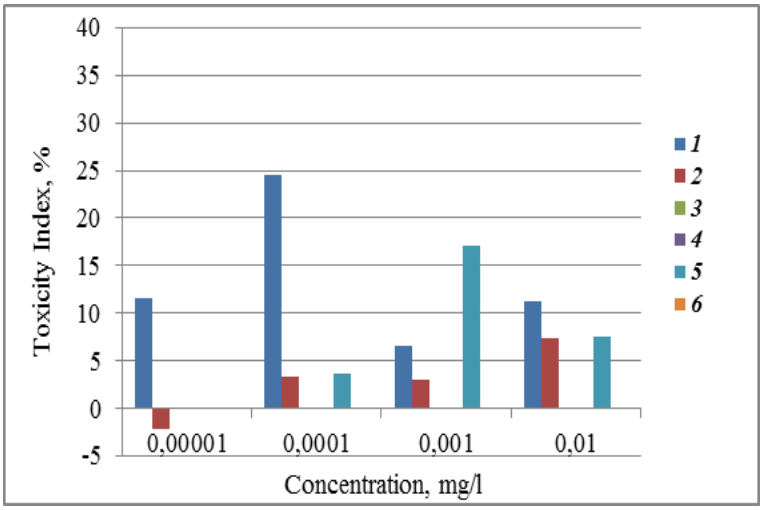

Figure 1. Toxicity Index of DS of nNi according to test functions of organisms of different systematic groups (1- chemotaxis of *Paramecium caudatum*, 2- weight gain of *Chlorella vulgaris Beijer*, 3- death rate of *Daphnia magna Straus*; 4- death rate of *Daphnia magna Straus* when inserting of chlorella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

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 %.

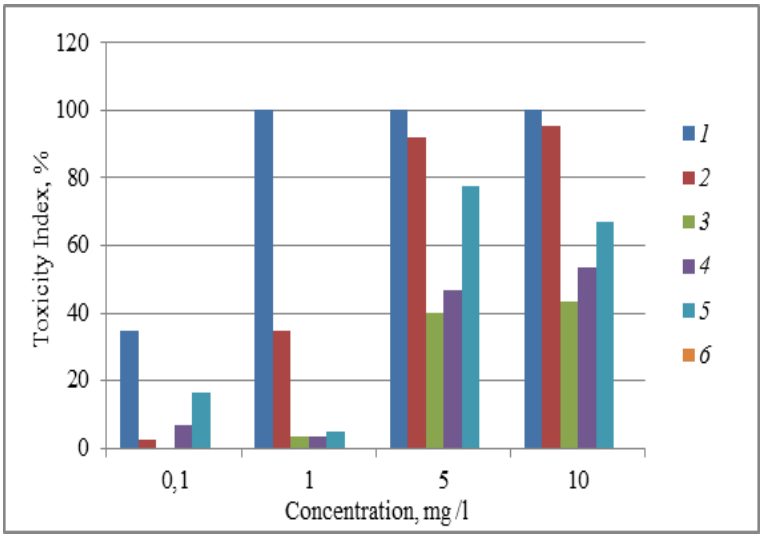

Figure 2. Toxicity index of DS of nNi according to test functions of organisms of different systematic groups (1- chemotaxis *of Paramecium caudatum*, 2- weight gain of *Chlorella vulgaris Beijer*, 3- death rate of *Daphnia magna Straus*; 4- death rate of *Daphnia magna Straus* when inserting of chlorella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

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

essential condition for the methodology of conducting experiments on analyzing of an effect of nNi on the trophic activity of *Daphnia magna Straus.* According to the data obtained by scientists of our laboratory during parallel trials (unpublished data), the concentration of nNi connected with chlorella's cells exceeded control values by more than 10000 times even after 24 hours of exposition. The ability of fresh-water algae to accumulate a significant amount of NPs and to serve as a source of their transition to consumers through the food chain was noted by the number of authors on the example of Au, TiO_2 and quantum dots [17 - 21].

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. Griffitt 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 Griffitt et al. of other representatives of systematic groups: *Daphnia pulex, Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata.*

The data that we obtained can be compared with the results of Gong et al. and Kovrižnych et al. [7, 8], who analyzed the effect of nNiO on the test functions of aquatic organisms. The structural analysis of nNi that we tested showed that during ablation in water, nNi is acidized. Moreover, the particles with a diameter of <8 nm fully consisted of nickel oxide, and NPs with a diameter of >8 nm, a central metal core with an oxide shell is observed [22]. The same results were obtained by Sakiyama et al. [23]. During the process of Ni NPs fusion by means of the laser ablation method in water, the authors obtained shell particles with a diameter of 5÷20 nm with a core consisting of metallic Ni and an oxide layer 2nm thick.

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 etbal. [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 an 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 100.0 mg/l, and $L(E)C_0$ (the maximum concentration which did not resulted in deaths 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 ecotoxiological 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 partical 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".

Table 1. The values of the ecotoxicity of DS of nNi with a size of NPs of 5 nm

Environ-ment	Test organism	Test reaction	$L(E)C_{50,}$ (mg/l)	$L(E)C_{20,}$ (mg/l)	$L(E)C_{10,}$ (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 phytoplancton (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).

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