

Effect of Al_2O_3 and TiO_2 nanoparticles on aquatic organisms

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Abstract. Environmental toxicity of aqueous disperse systems of nanoparticles of binary compounds of titanium dioxides (with particle size $\Delta_{50}=5$ nm, $\Delta_{50}=50$ nm, $\Delta_{50}=90$ nm), aluminum oxide alpha-forms ($\Delta_{50}=7$ nm and $\Delta_{50}=70$ nm) and macro forms (TiO_2 $\Delta_{50}=350$ nm, Al_2O_3 $\Delta_{50}=4000$ nm) were studied using biological testing methods. The bioassay was performed using a set of test organisms representing the major trophic levels. We found the dependence of the toxic effect concentration degree of nTiO_2 and nAl_2O_3 on the fluorescence of the bacterial biosensor "Ekolyum", the chemotactic response of ciliates *Paramecium caudatum*, the growth of unicellular algae *Chlorella vulgaris* Beijer and mortality of entomostracans *Daphnia magna* Straus. We revealed the selective dependence of nTiO_2 and nAl_2O_3 toxicity on the size, concentration and chemical nature of nanoparticles. The minimal concentration causing an organism's response on nTiO_2 and nAl_2O_3 effect depends on the type of the test-organism and the test reaction under study. We specified L(E)C_{50} and acute toxicity categories for all the studied nanoparticles. We determined that nTiO_2 ($\Delta_{50}=5$ nm) belong to the category «Acute toxicity 1», nTiO_2 ($\Delta_{50}=90$ nm) and nAl_2O_3 ($\Delta_{50}=70$ nm) – to the category «Acute toxicity 2», nAl_2O_3 ($\Delta_{50}=7$ nm) - to the category «Acute toxicity 3». No acute toxicity was registered for nTiO_2 ($\Delta_{50}=50$ nm) and macro form TiO_2 .

1. Introduction

Development and implementation of nanotechnologies in many industries increases the probability of harmful effects of nanoparticles (NPs) on the environment. Wide usage of NPs resulted in a great attention paid lately to NPs of metal oxides; titanium dioxide (nTiO_2) and aluminum oxide (nAl_2O_3) that are very popular among them.

nTiO_2 application is determined by its antibacterial and photocatalytic properties in the first place [1, 2]. nAl_2O_3 has a number of properties – thermal stability, high hardness, resistance and chemical inertness, which makes it possible to use it in various industries. The most wide-spread modification is $\alpha\text{-Al}_2\text{O}_3$ used in optics and laser technologies [3].

It is common knowledge that NP size substances increase in their response ability, which is potentially hazardous for the environment. As there are various ways of polluting water bodies with nanoindustry products, it is necessary to assess NPs toxicity for water ecosystems. The requirement documents in Russia and abroad require this property of NPs to be determined.

The published data concerning the biological effects of nTiO_2 and nAl_2O_3 on aqueous environment are deficient, segmentary and discrepant. This is caused not only by the lack of necessary information about physical and chemical properties of the tested NPs, the method of their synthesis and experiment conditions, but also by the absence of a unified analytical system providing valid ecotoxicological assessment of the hazard the NPs cause to natural objects.



As a result of the programs and research grants implemented earlier (2008-2013) we developed a methodological approach to assessing ecological toxicity that helps to study test-reactions of a wide range of organisms with one algorithm.

2. Materials and methods

We tested nTiO₂ with mean particle size $\Delta_{50}=50$ nm, $\Delta_{50}=90$ nm, nAl₂O₃ (α -form) with particle size $\Delta_{50}=70$ nm and macro forms TiO₂ $\Delta_{50}=350$ nm and Al₂O₃ $\Delta_{50}=4000$ nm obtained with the help of the pneumo circuitual method («Mipor» Ltd, RF). The disperse systems nTiO₂ $\Delta_{50}=5$ nm and nAl₂O₃ $\Delta_{50}=7$ nm were obtained with the help of the method of laser ablation in distilled water, the initial concentration 50 mg/l [4].

Preparation of DS nTiO₂ and nAl₂O₃ on growth medium (GM) with the concentrations 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1 and 1.0 mg/l was done in accordance with the technique [5] with further ultrasonic material dispersion (30 W/l, 5 minutes) just before biological testing.

The toxicity of nTiO₂ and nAl₂O₃ was studied by the change in luminescence of bacterial biosensor «Ekolyum» [6], the chemotactic response of ciliates *Paramecium caudatum* [6], the growth of unicellular algae *Chlorella vulgaris* Beijer [7] and mortality of entomostracans *Daphnia magna* Straus [8]. The assessment of toxicity of the analysed DS nTiO₂ and nAl₂O₃ with various particle sizes was done in accordance with the criteria given in certified methods' outlines [7, 8]. The research design is similar to the described in [9, 10].

The properties of nTiO₂ and nAl₂O₃ were verified with the help of transmission electronic microscop «Philips CM-30» (France), using the method of dynamic light scattering (DLS) with the help of the disperse particles sizes analyser «Zetasizer Nano ZS» (USA), the method of BET («TriStar 3000», USA) to measure specific surface and the method of X-ray structure analysis of the phase composition and structural characteristics (X-ray diffractometer Shimadzu XRD-6000, Japan). The research was conducted with the application of metrologically gauged instruments: Autolumat LB953 (Germany), spectrofluorometer «Shimadzu» RF-5301 PC (Japan), photoelectric colorimeter DMS-03 (Russia), concentration meter BIOTESTER-2 (Russia). The statistical processing of the obtained data was done with probit analysis.

3. Results

The results of our investigation showed that the dynamics of the fluorescence of bacterial biosensor «Ekolyum» in GM with nTiO₂ concentration did not depend on the size and concentration of NPs. The toxicity of particles of nTiO₂ ($\Delta_{50}=5$ nm, $\Delta_{50}=50$ nm and $\Delta_{50}=350$ nm) practically for the whole tested concentration range (C) changed insignificantly (figure 1). The exception was the tested DS nTiO₂ $\Delta_{50}=5$ nm with C=0.1 mg/l, the toxicity of which was above “permissible level” and, in accordance with the criteria of a standard method, was defined as a sample with “moderate” degree of toxicity ($I=27.5\pm0.5\%$). In DS TiO₂ ($\Delta_{50}=350$ nm) we noted “suppression” of bio fluorescence within the interval of the tested concentrations. The fluorescence of the biosensor decreased with the increase in DS TiO₂ concentration, but the toxicity index value was above the “permissible level” by 1.0-2.0 % only.

Similar effects were registered during introducing in GM NPs Al₂O₃ $\Delta_{50}=7$ nm and $\Delta_{50}=70$ nm: no sufficient changes in the fluorescence of bacterial biosensor «Ekolyum» (figure 2) were found. In contrast to nTiO₂ $\Delta_{50}=5$ nm the «moderate» degree of toxicity ($I=23.5\pm8.5\%$) was registered for DS with lower concentration of nAl₂O₃ $\Delta_{50}=70$ nm (C=0.001 mg/l).

The macro form Al₂O₃ $\Delta_{50}=4000$ nm unlike the nanoform Al₂O₃ of all the analysed concentrations of DS quenched the fluorescence of the biosensor. Within the interval C from 0.0001 mg/l to 1.0 mg/l the toxicity index exceeded the «permissible» level and changed within the range from $I=20.5\pm0.5\%$ to $I=27.9\pm0.4\%$, which is defined as the sample with the «moderate» toxicity.

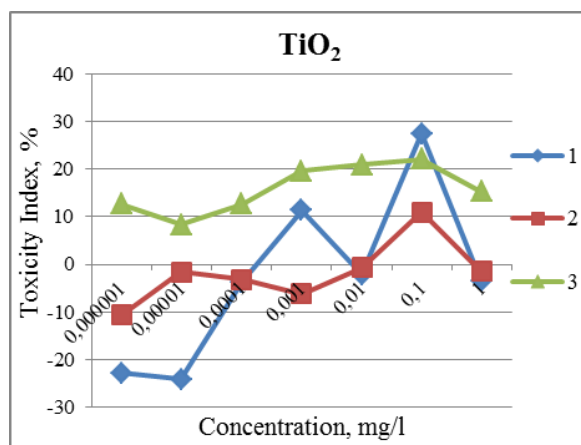


Figure 1. Toxicity index of DS TiO_2 for bacterial biosensor «Ekolyum»: 1 - $\Delta_{50} = 5$ nm, 2 - $\Delta_{50} = 50$ nm, 3 - $\Delta_{50} = 350$ nm

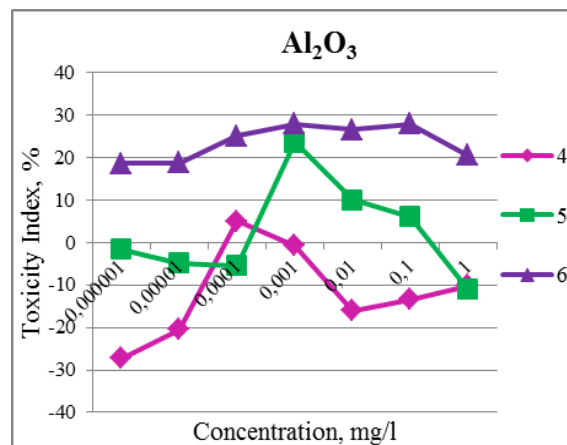


Figure 2. Toxicity index of DS Al_2O_3 for bacterial biosensor «Ekolyum»: 4 - $\Delta_{50} = 7$ nm, 5 - $\Delta_{50} = 70$ nm, 6 - $\Delta_{50} = 4000$ nm

On exposing *Paramecium caudatum* to DS nTiO_2 $\Delta_{50}=5$ nm with $C=0.000001$ mg/l and $C=0.00001$ mg/l the toxicity index value corresponded to control values (figure 3). With the increase in the concentration of nTiO_2 $\Delta_{50}=5$ nm in GM the percentage of ciliates outflow in DS was not monotonic, but decreased definitely, which demonstrated the NPs toxic effect. In DS with the concentrations 0.01, 0.1 and 1.0 mg/l the toxicity index changed within the range from 38.8 ± 6.2 % to 69.9 ± 16.8 % and according to the standard method [6] corresponded to the «moderate» degree of toxicity ($0.40 < I < 0.70$).

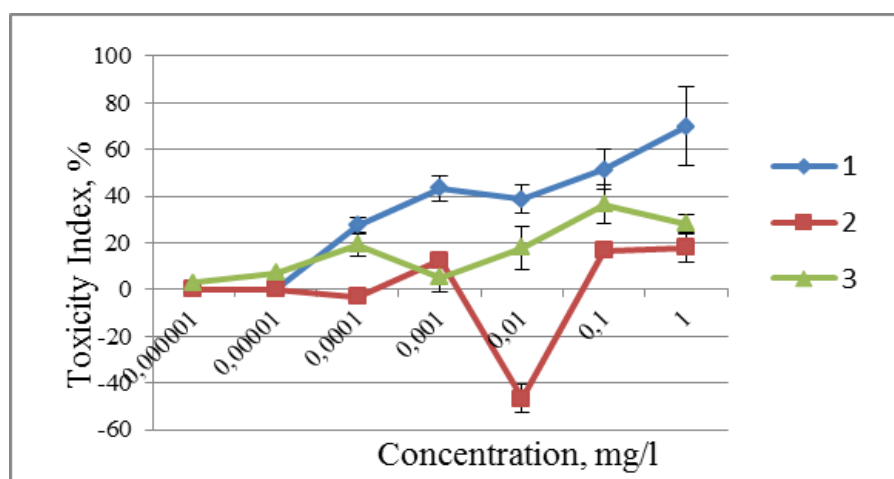


Figure 3. Toxicity index of DS nTiO_2 and nAl_2O_3 for *Paramecium caudatum*: 1 - nTiO_2 $\Delta_{50} = 5$ nm, 2 - nTiO_2 $\Delta_{50} = 50$ nm, 3 - nAl_2O_3 $\Delta_{50} = 70$ nm

We registered the dependence of the chemotactic response of *Paramecium caudatum* on the particle size of TiO_2 in GM. The introduction of particles TiO_2 $\Delta_{50}=50$ nm did not caused toxic impact on *Paramecium caudatum*. We found DS nTiO_2 $\Delta_{50}=50$ nm with $C=0.0001$ mg/l and $C=0.01$ mg/l, in which the ciliates outflow exceeded the control values (figure 3). The highest stimulating effect was registered with $C=0.01$ mg/l ($I = -46.6 \pm 9.4$ %). We stated that the introduction of nAl_2O_3 $\Delta_{50}=70$ nm to GM within the concentration range from 0.000001 to 1.0 mg/l resulted in the change in the

chemotactic response of *Paramecium caudatum* in the range of «permissible» values. The maximal toxicity index was registered with $C=0.1$ mg/l $I=36.4 \pm 1.1$ % (figure 3).

Our investigations resulted in revealing the dependence of $nTiO_2$ effect on the particle size. The particles $nTiO_2$ $\Delta_{50}=5$ nm and $\Delta_{50}=90$ nm inhibited the growth of *Chlorella vulgaris* Beijer, with $nTiO_2$ $\Delta_{50}=5$ nm in contrast to $nTiO_2$ $\Delta_{50}=90$ nm producing a pronounced toxic effect (figure 4).

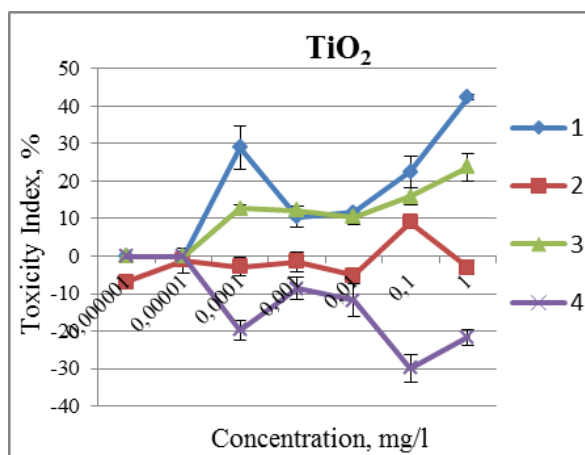


Figure 4. Toxicity index of DS TiO_2 for *Chlorella vulgaris* Beijer: 1 - $\Delta_{50}=5$ nm, 2 - $\Delta_{50}=50$ nm, 3 - $\Delta_{50}=90$ nm, 4 - $\Delta_{50}=350$ nm

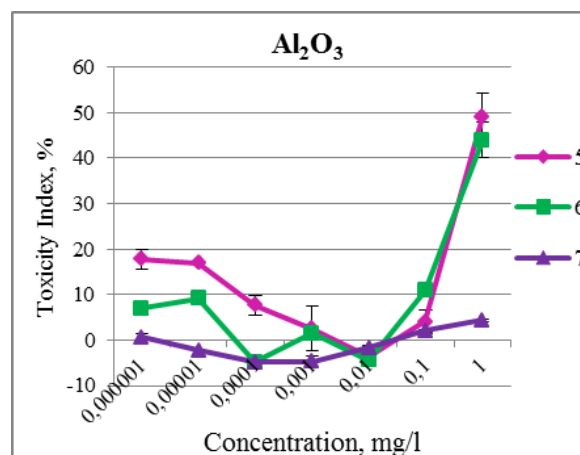


Figure 5. Toxicity index of DS Al_2O_3 for *Chlorella vulgaris* Beijer: 5 - $\Delta_{50}=7$ nm, 6 - $\Delta_{50}=70$ nm, 7 - $\Delta_{50}=4000$ nm

The introduction into GM of the algae DS $nTiO_2$ $\Delta_{50}=50$ nm caused insignificant stimulation of *Chlorella vulgaris* Beijer cells' growth. We registered a non-monotonic decrease in the growth of alga cells while cultivating *Chlorella vulgaris* Beijer in the analysed DS $nTiO_2$ $\Delta_{50}=5$ nm. The toxic effect of $nTiO_2$ $\Delta_{50}=5$ nm showed itself with $C=0.0001$ mg/l ($I=29.0 \pm 5.9$ %) already. With further increase in the concentration the toxicity of DS decreased ($I=10.4 \pm 2.8$ % with $C=0.001$ mg/l and $I=11.7 \pm 0.5$ % with $C=0.01$ mg/l), and then increased again to gain the maximal effect with $C=1.0$ mg/l. The growth of *Chlorella vulgaris* Beijer decreased by 33.8 % compared to the control values. The introduction into GM of the particles TiO_2 $\Delta_{50}=350$ nm in macro form had a stimulating effect on *Chlorella vulgaris* Beijer cells' growth in concentration dependence. The maximal response was registered during introducing particles into $C=0.1$ mg/l. The toxicity of DS TiO_2 $\Delta_{50}=350$ nm was close to significant manifestation.

In contrast to $nTiO_2$ the introduction of nAl_2O_3 into GM did not cause the expected changes in the growth of *Chlorella vulgaris* Beijer depending on the particle size. The exposure of *Chlorella vulgaris* Beijer to DS nAl_2O_3 $\Delta_{50}=7$ nm and $\Delta_{50}=70$ nm within the range $C=0.000001$ mg/l – 0.1 mg/l caused the inhibition of alga cells' growth вызывала ингибирование роста клеток водоросли within tolerance (figure 5). A pronounced toxic effect was observed only with $C=1.0$ mg/l: *Chlorella vulgaris* Beijer mass growth decreased compared to the control values by 49.1 ± 5.9 % for DS nAl_2O_3 $\Delta_{50}=7$ nm and 44.0 ± 3.9 % for DS nAl_2O_3 $\Delta_{50}=70$ nm. The macro form Al_2O_3 $\Delta_{50}=4000$ nm did not have a toxic effect on *Chlorella vulgaris* Beijer, the toxicity index changing in range of control values.

The researches in the test-organism of *Daphnia magna* Straus in contrast to biosensor «Ekolyum», *Chlorella vulgaris* Beijer and *Paramecium caudatum* did not reveal toxic effects of $nTiO_2$ and nAl_2O_3 . The exposure of *Daphnia magna* Straus in DS with particles TiO_2 ($\Delta_{50}=5$ nm, $\Delta_{50}=50$ nm and $\Delta_{50}=350$ nm) and into DS with particles Al_2O_3 ($\Delta_{50}=7$ nm, $\Delta_{50}=70$ nm and $\Delta_{50}=4000$ nm) did not cause the mortality of more than 10 % of the entomostracans for 48 hours, which indicated the absence of the toxic effect for all the tested concentrations DS TiO_2 and Al_2O_3 (figure 1, 2, 3).

On the basis of the data of the eco toxicological analysis obtained with the help of test-systems with the basic set of test-organisms we did the integral assessment of the toxicity of TiO_2 and Al_2O_3 with various dimension characteristics (table 1).

According to GHS, while making the conclusion on the ecotoxicity of the tested samples of NPs we took into account the principle of assessment by the lowest index available L(E)C_{50} [11].

Table 1. Values of N-dimensional L(E)C_{50} TiO_2 and Al_2O_3

Test-organism \ L(E)C_{50} , (mg/l)	TiO_2				nAl_2O_3		
	NPs		macro form		NPs		macro form
	5 nm	50 nm	90 nm	350 nm	7 nm	70 nm	4000 nm
«Ekolyum»	>100	>100	-	47.6	>100	>100	>100
<i>Paramecium caudatum</i>	0.0256	>100	>100	>100	-	1.22	-
<i>Chlorella vulgaris Beijer</i>	1.153	>100	7.7	>100	30.8	15.15	-
<i>Daphnia magna Straus</i>	>100	>100	>100	>100	>100	>100	>100
Conclusion	Acute toxic class 1	No acute toxicity	Acute toxic class 2	Acute toxic class 3	Acute toxic class 3	Acute toxic class 2	No acute toxicity

Consequently, the research results showed that the toxicity of DS nTiO_2 with dimension $\Delta_{50}=5$ nm were sufficiently higher than the toxicity of DS nTiO_2 with particle size $\Delta_{50}=50$ nm, $\Delta_{50}=90$ nm. A different dependence is observed for nAl_2O_3 . Per totality of the test-parameters under study nAl_2O_3 $\Delta_{50}=70$ nm are most toxic. nAl_2O_3 with dimension $\Delta_{50}=7$ nm are less toxic. The comparison of the toxicity of nTiO_2 and nAl_2O_3 with close dimensional characteristics indicated that the toxicity of nTiO_2 ($\Delta_{50}=5$ nm) is much higher than the toxicity of nAl_2O_3 $\Delta_{50}=7$ nm. At the same time, nAl_2O_3 ($\Delta_{50}=70$ nm) have a more pronounced toxic effect compared to nTiO_2 ($\Delta_{50}=50$ nm) correspond to nTiO_2 ($\Delta_{50}=90$ nm) by toxicity category. The macro form TiO_2 per totality of the test-responses under study belongs to the category «Acute toxicity 3», and the macro form Al_2O_3 is «non-hazardous substance».

4. Discussion

The absence of standardized toxicological methods of assessing the hazard NPs can cause to living objects of nature together with little information in published papers about the size, crystal structure, morphology of NPs, methods of their synthesis, experiment conditions, methods of preparation of DS NPs makes it difficult to compare the obtained results with the literature data.

In the literature available the data on the influence of nTiO_2 and nAl_2O_3 on the test-parameters of aquatic organisms are few. Most researches in the recent years have been devoted to the genotoxic and cytotoxic effect of nTiO_2 and nAl_2O_3 as well as the cancerogenic effect of nTiO_2 [1, 2, 3, 12, 13, 14].

The data we obtained are in accord with the data of Liu et. al. [15], who studied the effect of nTiO_2 ($\Delta_{50}<20$ nm and $\Delta_{50}=30$ nm) on the survival rate of *Daphnia magna Straus* and stated that nTiO_2 with particle size $\Delta_{50}<20$ nm induced a higher toxicity compared to nTiO_2 $\Delta_{50}=30$ nm, which contradicted the data of L. Adams et. al. [16] and J. Verran [17], who showed in their studies that the particle size of nTiO_2 does not influence its toxicity. The inconsistency of the data on the dependence of nTiO_2 toxicity on the particle size may be caused by the authors' employing different methods of assessing the toxicity of NPs. For example, Liu et. al. are known to have studied the survival rate of *Daphnia*

magna Straus in a long-term experiment. The research in a long-term toxicity of nTiO₂ for 21 days displayed the disorder of fertility and the increase in the mortality of entomostracans up to 70 % [18, 19]. The analysis of toxicity of nAl₂O₃ with dimensional characteristics Δ_{50} =40-100 nm on immobilisation and further death of *Ceriodaphnia dubia* displayed a clear temporal dose-dependent trend to reveal a toxic effect taking into account aggregation and bioavailability reduction of NPs [20]. One of the basic mechanisms of the toxic effect of nTiO₂ and nAl₂O₃, in the authors' view, is an oxidative stress that arises in the condition of NPs impact on aquatic organisms.

According to the literature, the absence of toxic effect of nTiO₂ with particle size Δ_{50} <50 nm was found for luminescent bacteria *Vibrio fischeri* [21], which is in accord with the data we obtained.

V. Aruoja et.al. [22] studied the influence of nTiO₂ on the freshwater alga *Pseudokirchneriella subcapitata*. It was stated that the macro form TiO₂ (L(E)C₅₀=35.9 mg/l) was less toxic than nTiO₂ (L(E)C₅₀=5.83 mg/l). I.M. Sadiq et.al. [23] revealed the inhibitory effect of nAl₂O₃ with particle size Δ_{50} <50 nm on the growth of microalgae *Scenedesmus sp.* and *Chlorella sp.* The value of L(E)C₅₀ for 72 hours of incubation was 45.4 mg/l for *Chlorella sp.* and 39.4 mg/l for *Scenedesmus sp.* The macro form Al₂O₃ (Δ_{50} <5 mkm) also displayed toxicity, though to less extent (L(E)C₅₀= 110.2 mg/l for *Chlorella sp.* and L(E)C₅₀=100.4 mg/l for *Scenedesmus sp.*). The researchers revealed selective sensitivity of various algae species to nAl₂O₃. The authors suppose that nTiO₂ and nAl₂O₃ form specific aggregates settling on the surface of algae cells, which causes toxic effect.

We were the first to show the influence of nTiO₂ and nAl₂O₃ with various size characteristics on the chemotactic response of ciliates *Paramecium caudatum*.

5. Conclusion

Eco toxicological analysis showed that nTiO₂ Δ_{50} =5 nm belong to the category « Acute toxicity 1», nTiO₂ Δ_{50} =90 nm and nAl₂O₃ Δ_{50} =70 nm - to the category «Acute toxicity 2», macro form TiO₂ Δ_{50} =350 nm and nAl₂O₃ Δ_{50} =7 nm - to the category «Acute toxicity 3». The absence of acute toxicity was found for nTiO₂ Δ_{50} =50 nm and the macro form Al₂O₃ Δ_{50} =4000 nm.

In most test-systems with both nTiO₂, and nAl₂O₃ having various size characteristics there is no monotonic dependence between the concentration of DS and the level of the test-response change, the general trend being the increase in the test-response with the concentration increased. The degree of hazard of DS containing nTiO₂ and nAl₂O₃ is determined by the size of NPs, their chemical nature and concentration in DS.

The toxicity of nTiO₂ and nAl₂O₃ is different for various test-organisms. Most sensible to the presence of nTiO₂ Δ_{50} =5 nm and nAl₂O₃ Δ_{50} =70 nm in the environment are the ciliates *Paramecium caudatum*, of nTiO₂ Δ_{50} =90 nm and nAl₂O₃ Δ_{50} =7 nm – the unicellular algae *Chlorella vulgaris* Beijer, of macro form TiO₂ Δ_{50} =350 nm – bacterial sensor «Ekolyum».

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