UDC 669.85/.86, 574.21, 504.5 https://doi.org/10.23947/2541-9129-2020-4-56-67

Biotechnical toxicity assessment system of rare earth metals compounds

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Introduction. Expanding the scope of application of rare-earth metal compounds that are unique in their properties increases the interest of many researchers in studying the impact of rare-earth metals and their compounds on human health and the environment. One of the most relevant and modern methods for assessing the safety of the studied media for a biological test object is bioassay.

Problem Statement. The objective necessity of determining the combined effect of rare earth metals and their compounds on human health and the environment involves the use of biological systems. Modern methods of bioassay are extremely sensitive, which is sufficient to determine sub-threshold concentrations of hazardous substances in accordance with international standards. Thus, the use of these methods can make it possible to determine the index and the degree of toxicity of rare earth metal compounds with high accuracy in order to prepare a package of necessary documentation on industrial safety of products.

Theoretical Part. Based on the studied toxicological effects of rare earth metals, the authors proposed to conduct a toxicity assessment based on the concept of biotechnical systems. The object of research was oxides and carbonates of rare earth metals. The results of the study to determine the index and the degree of toxicity of rare earth metal compounds, as well as to assess the lethal concentration of LC50 (24 h) by biotesting using test organisms Paramecium Caudatum were used to write a safety data sheet for cerium oxide and carbonate.

Conclusion. The studies have shown that a certain modification of the technical solutions embedded in the devices of the Biotester series makes it possible to correctly solve the problem of assessing the toxicity of rare earth metals and their compounds. Based on the research results, the safety data sheets were developed.

Keywords: biotest analysis, infusorias, paramecium caudatum, rare-earth metals, oxides and carbonates, toxicity, toxicity index.

For citation: Semenova M. I., Smirnov A. V., Sokolov A., Kovalevskaya A. S., Smolova O. V. Biotechnical toxicity assessment system of rare earth metals compounds: Safety of Technogenic and Natural Systems. 2020;4:56–67. https://doi.org/10.23947/2541-9129-2020-4-56-67

Introduction. In the modern world, the scope of application of rare earth metals is increasing every year. Due to their unique physical and chemical properties, these materials have already found their application in such industries as metallurgy, oil production, textiles and agriculture. However, with the development of technologies, the range of applications of rare earth materials is constantly growing.

The discovery of unique catalytic, electrical, magnetic, and optical properties paved the way for the introduction of metals into more technologically complex processes for the production of mobile devices, hybrid cars, and wind turbines. In addition, the use of rare earth metals in clean energy technologies and safety systems has attracted global attention to these elements.

According to many studies on the effects of rare earth metals and their compounds on human health and the environment, it can be concluded that they are not safe.

One of the most relevant and modern methods for assessing the safety of the studied media for a biological test object is bioassay. Toxicity assessment is based on the concept of biotechnical systems (BTS). They are a combination of biological and technical elements combined into a single functional system of goal-oriented behavior.

Thus, the research to determine the index and degree of toxicity of rare earth metal compounds by bioassay to prepare a package of necessary documentation on industrial safety of products is very relevant.

Problem Statement. The subject of control and monitoring in the field of environmental protection, industry and pharmacology are often objects of not just variable, but fundamentally indeterminate composition, characterized by a large number of multicomponent ingredients that can also change their particular properties under the influence of external factors. The objective need to determine the cumulative effect of the entire complex of factors requires the use of new operational controls using biological systems that can simulate the effect of adverse factors on living organisms and, ultimately, on humans. Thus, the purpose of the research activity was to determine the index and degree of toxicity of rare earth metal compounds by bioassay to prepare a package of necessary documentation on industrial safety of products.

To achieve this goal, the following tasks are set:

— analysis of the possibility of using bioassay on protozoa to assess the toxicity of substances;

— determination of the toxicity index of the studied compounds;

— determination of the average lethal concentration of LC50 (24 h) using paramecium caudatum test organisms to determine the degree of toxicity of the studied substances;

— development of safety data sheets for cerium oxide and carbonate.

Theoretical Part. Properties of rare earth metals. Rare earth metals (REM) consist of seventeen elements, 15 of which are called lanthanides. Two additional elements, scandium (Sc) and yttrium (Y), are included in the list of rare earth metals because of their similar chemical and toxicological properties and because they are often found in the same ore deposits as other rare earth metals.

All metals in this group have very similar physical and chemical properties. Here are some of these properties:

— silvery-white soft metals that fade when they come into contact with air;

— solubility increases with increasing atomic number;

— high melting and boiling point;

— strong paramagnets;

— high electrical conductivity;

— active reducing agents;

— capable of exploding in the air;

— capable of fluorescence under ultraviolet light;

— dust of these compounds can be fire hazardous and explosive [1, 2].

Use of rare earth metals and their compounds. Rare earth metals play an increasingly important role in modern production. Their main application is in the production processes of "high technologies". Examples of such technologies include mobile phones, optical lenses, digital cameras, high-performance magnets, batteries, automotive catalytic converters, metal alloys, lasers, medical images, green energy devices, and aerospace weapons systems [1].

Some of the most commonly used forms of rare earth metals are carbonates and oxides. Oxides are mainly used to create laser installations, ceramic products and glass due to their unique optical properties. For example, lanthanum oxide is used to make special optical glasses, infrared adsorbing glass, and phosphors. Yttrium oxides are used in the production of ceramics and glass. They have high melting points and give the glass impact resistance and low expansion [2, 3].

Carbonates are mainly used for surface coatings or in the production of thermoelectric materials. For example, cerium carbonate covers the exhaust pipes of cars, since this compound absorbs exhaust gases [4].

Toxicological effect of rare earth metals and their compounds. Based on the atomic mass, REM is divided into two groups: "light" (Ce, Eu, Gd, La, Nd, Pr, and Sm) and "heavy" (Dy, Er, Ho, Lu, Tb, Tm, Y, and Yb). The role of REM in living systems is not fully understood. Light REM accumulates mainly in the liver, while heavy REM tends to replace calcium and accumulates in the bones. The toxicity of REM generally decreases with increasing atomic mass [1-3].

REM can affect the lungs, blood, destroy platelets, change the structure of DNA, and lead to disorders of the kidneys and brain. For example, gadolinium can accumulate in the soft tissues of the brain in the subarachnoid space of the brain. In addition, experiments were conducted that showed the effect of REM on fetal development in pregnant mice. However, none of the REM is currently classified as a carcinogen [1, 5].

Materials and methods for assessing the toxicity of REM compounds. The extensive use of REM in various production processes, as well as their ability to have a negative impact on the environment and the human body, lead to the need to assess the degree of harmful effects of metal and draw up the necessary documentation on product safety.

A measuring bioassay system is a set of methodological and hardware tools aimed at assessing the toxicity of substances based on the biological activity of test objects.

Bioassay is a procedure for detecting environmental toxicity using test objects that signal danger, regardless of what substances and in what combinations changes in the vital functions of the test objects were caused [6].

The construction of the bioassay system is based on the general principles of creating biotechnical systems (Fig. 1).

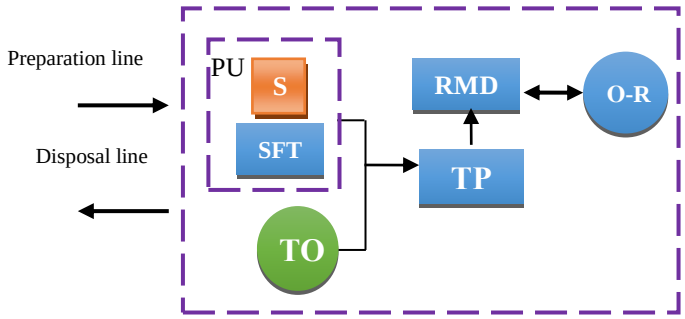

Fig. 1. Bioassay biotechnical system:
S — sample; TO — bioobject used as a test object;
PU — sample preparation unit; SFT — sample forming tools;
RMD — reaction monitoring devices; O–R — operator-researcher;
* special disposal is not required due to non-pathogenicity of the culture

The main function of the bioassay channel is to obtain a numerical indicator with certain accuracy at the output. From the point of view of biotechnical systems, bioassay is a measurement task that has some measured parameters and its own metrological support. The principle of operation of the bioassay system can be represented as a mathematical model in which the function of distribution of the concentration of a toxic substance in space and time will be implemented by the function of distribution of toxicity. At the same time, an important part in the development of bioassay systems is the creation of a measuring converter. Optical measurement converters are most often used, since they have a minimum error. The optical-acoustic method can also be used to directly measure the optical characteristics of scattering media. Among other things, the bioassay system must also include the environment of the laboratory where the testing is performed, since it is the environment that must meet certain conditions for temperature and humidity, and must not contain toxic substances.

Another part of the bioassay system is the test object (TO).

When conducting a bioassay analysis, various organisms can serve as a test object: infusoria, daphnia, algae, fish, etc. The choice of the test object depends on the test reaction that is planned to be recorded and the environment that is being biotested.

One of the most common and convenient test objects for bioassay is the Paramecium Caudatum infusoria. Infusoria has a number of properties, due to which its use is most appropriate:

— biological significance: infusoria P. Caudatum is one of the most common types of laboratory organisms;

— harmlessness to humans and animals;

— good knowledge;

— high sensitivity to toxic substances;

— availability of cultivation for any practical laboratory;

— low cost of culture;

— pronounced taxis [6].

This organism belongs to the protozoa subkingdom, the Ciliphora type. In the natural environment, Paramecium caudatum is common in fresh water bodies, such as lakes, swamps, ponds, etc. The shape of the cell is ellipsoid, the dimensions are 200 × 40 microns. The main food of the infusoria is bacteria, yeast, etc. Reproduction of the infusoria occurs by transverse cell division. The surface of the body of infusoria is covered with cilia, which serve both to move in the aquatic environment and serve as receptors that perceive chemical stimuli.

A distinctive feature of the infusoria is its continuous movement. Moreover, the nature of this movement may change depending on the surrounding factors. A special feature of infusoria is the presence of negative geotaxis — the property of infusoria to float into the upper layers of the liquid, which is characterized as movement against the Earth's gravitational field. But what is most important is the reaction of protozoa to toxic substances. The constant desire of infusoria to find their comfort zone leads to motor responses to chemical stimuli. If toxic substances are harmful to protozoa, infusoria will try to swim away from them. This phenomenon is called chemotaxis.

It is worth noting that the chemotactic reaction of infusoria is stronger than the geotactic one [7]. The chemotactic reaction is realized if there is a stable gradient of chemical concentrations over time.

When bioassay is conducted on infusoria, the toxicity of the test sample is judged by the survival rate, the intensity of reproduction, changes in motor activity, behavioral (taxic) reactions, etc. [8].

In this study, the assessment was based on the index and degree of toxicity of rare earth metal compounds based on the chemotactic reaction.

The lethal concentration of LC50 was also determined. The duration of the experiment in this case was 24 hours.

As a safety assessment of the product (sample), the following rare earth metals were studied: neodymium, yttrium, cerium, lanthanum, praseodymium and gadolinium.

To obtain objective quantitative information about the test reaction, a reaction monitoring device (RMD) was used, which is a Boitester-2M device.

Application of the "Biotester" series device for assessing the toxicity of rare earth metals. The study was conducted in accordance with PND F T 16.3.1-10 "Methodology for determining the toxicity of production and consumption waste by express method using a device of the "BIOTESTER" series, since the compounds under study are inorganic powdery chemicals [9].

Taking into account the fact that the chemotactic reaction is realized under the condition that there is a stable gradient of chemical concentrations in time, in this method, such a gradient is created by superimposing two media on

each other: a suspension containing infusoria and the test sample. The two media are arranged vertically relative to each other: the upper one will be a sample, and the lower one will be a suspension of infusoria. To ensure that the media do not mix with each other, it is necessary to achieve a difference in the densities of the two media, while leaving the possibility of free movement of infusoria between them.

The criterion of toxic effect is a significant difference in the number of infusoria cells between the two zones. If the test sample does not contain toxic substances, the concentration of infusoria cells in the upper zone will be observed in the cuvette. And the higher the toxicity of the sample, the less infusoria will be distributed in it.

Biotester-2M is a device that determines the concentration of particles in a homogeneous medium by the amount of the transmitted radiation. The main principle of the device is to read changes in the flow of the transmitted radiation. To realize this phenomenon, a light source or emitter and a photodetector must be present.

A cuvette containing a gradient medium, i.e., the overlay of the toxic sample under study on the solution with infusoria, is placed between the light source (LS) and the receiver (R). The emitter emits a light flux of a certain area S. Each object interacts with the radiation at the intersection of the flow, like a transparent sphere obeying the laws of geometric optics (Fig. 2) [10].

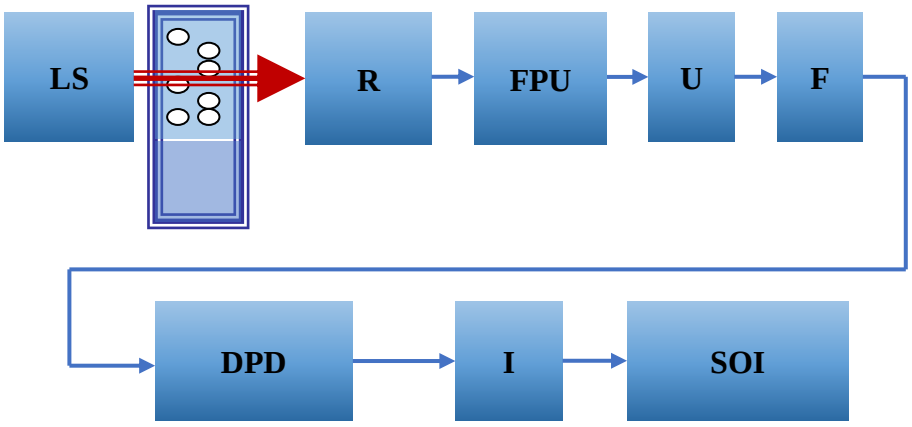

Fig. 2. Block diagram of the Biotester-2M device

In other words, each infusoria that falls under the light beam will change its intensity. The change in intensity will be detected by the photodetector that receives the light beam. In this case, the quantitative change in light intensity is proportional to the number of infusoria.

Each of the studied samples was analyzed in 3 cuvettes, 5 readings of the BIOTESTER-2M device were taken from each cuvette.

According to PND F T 16.3.16-10, to prevent gross errors during the analysis, the acceptability of the control sample was promptly evaluated according to the following inequality:

$$|Ikmax - Ikmin| \leq 0{,}2Icp.k, \quad (1)$$

where $Ikmax$ — the maximum readings of the device for control samples, $Ikmin$ — the minimum readings of the device for control samples, $Icp.k$ — the average readings of the device for control samples [9].

The toxicity of the sample was assessed by the relative difference in the number of infusoria in the upper zone of the cuvette in the control and the analyzed samples. In accordance with PND F T 16.3.16-10, the toxicity index is calculated by the formula:

$$T = \frac{|I\text{ср.к} - I\text{ср.пр}|}{I\text{ср.к}} \times K, \quad (2)$$

where $I_{cp.k}$ — the average instrument reading for control samples, $I_{cp.пр}$ — the average instrument reading for analyzed samples, K — the sample dilution coefficient. The toxicity index T is a dimensionless value and can take values from 0 to 1 in accordance with the degree of toxicity of the analyzed sample.

According to PND F T 16.3.16-10, depending on the index value, the samples are classified according to their degree of toxicity into 3 groups:

— I. Acceptable degree of toxicity ($0.00 < T \leq 0.40$).

— II. Moderate degree of toxicity ($0.40 < T \leq 0.70$).

— III. High degree of toxicity ($T > 0.70$).

Determination of the average lethal concentration of LC50 (24 h) using test organisms Paramecium caudatum. The method consists in recording the survival rate of freshwater test organisms Paramecium caudatum in the analyzed sample of the object under study relative to the control sample, determining its toxicity and toxicological parameters during 6, 24 or 96 hours of testing [11].

Determination of toxicity and average lethal concentration of LC50 (24 h) was carried out in accordance with GOST R 57166-2016 "Water. Determination of toxicity by survival of freshwater infusoria Paramecium caudatum".

To prepare the analytical solution, it is necessary to use 50 grams of a dry rare earth metal compound and 450 ml of distilled water. Then, for 6-7 hours, mixing takes place using a magnetic stirrer at a minimum speed. After the end of mixing, the mixture settles for 14 hours. The supernatant is passed through the filter paper. After 24 hours, the containers with the analyzed solution are visually examined and the number of surviving infusoria is calculated [11].

Research Result. The toxicity indices (excluding the dilution coefficient) obtained by ten-fold dilution of carbonates of the studied substances are shown in fig. 3 (the red line on the diagram indicates the level of non-toxicity of the sample).

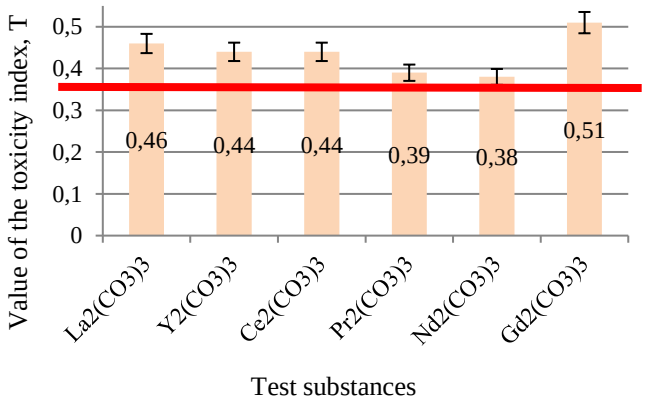

Fig. 3. Value of toxicity indices of carbonates of the studied metals at 10-fold dilution of the sample

As it can be seen from the diagram, when diluted tenfold, praseodymium and neodymium carbonate are considered non-toxic in accordance with PND F T 16.3.16–10 ($T \leq 0.40$). And the remaining samples of carbonates of the studied rare earth metals must be diluted 100 times. The values of the toxicity indices of a hundredfold dilution (excluding the dilution coefficient) are shown in fig. 4.

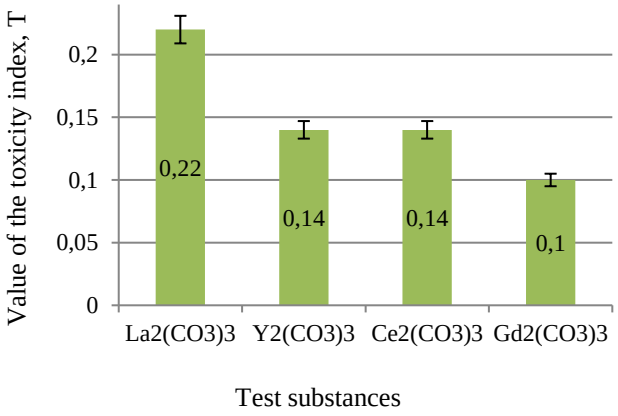

Fig. 4. Value of the toxicity indices of carbonates of the studied metals at 100-fold dilution of the sample

The resulting diagram shows that at 100-fold dilution, the carbonates of the studied rare earth metals are non-toxic in accordance with PND F T 16.3.16–10 ($T \leq 0.40$).

The final values of the toxicity indices of carbonates of the studied rare earth metals are presented in table 1.

Table 1

Toxicity indices of the studied rare earth metal carbonates

Determined indicator	Analysis result		Toxicity index (excluding degree of dilution)	Degree of toxicity of the sample, T
	Test substance	Dilution degree		
Toxicity of the sample on Paramecium caudatum infusoria	$La_2(CO_3)_3$	100	0.22	High, T = 22
	$Y_2(CO_3)_3$	100	0.14	High, T = 14
	$Ce_2(CO_3)_3$	100	0.14	High, T = 14
	$Pr_2(CO_3)_3$	10	0.39	High, T = 3.9
	$Nd_2(CO_3)_3$	10	0.38	High, T = 3.8
	$Gd_2(CO_3)_3$	100	0.1	High, T = 10

In addition to carbonates, the toxicity indices were calculated for oxides of the studied compounds. The toxicity indices (excluding coefficient 0.22; 0.14; 0.14; 0.1; 0; 0.05; 0.1; 0.15; 0.2; La2(CO3) 3 Y2(CO3) 3 Ce2(CO3)3 Gd2(CO3) 3. The values of the toxicity indeces obtained by tenfold dilution of the oxides of the studied metals are shown in fig. 5 (the red line on the diagram indicates the level of non-toxicity of the sample).

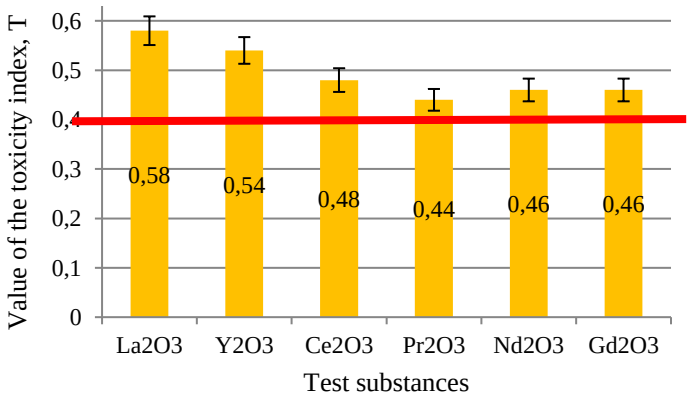

Fig. 5. Value of toxicity indices of oxides of the studied metals at 10-fold dilution of the sample

As it can be seen from the diagram, with a tenfold dilution, all the oxides under consideration are toxic compounds in accordance with PND F T 16.3.16-10 (T ≤ 0.40); therefore, it is necessary to dilute the samples of the studied substances 100 times. The values of the toxicity indices of a hundredfold dilution (excluding the dilution coefficient) are shown in fig. 6.

The resulting diagram shows that at 100-fold dilution, the oxides of the studied rare earth metals are non-toxic in accordance with PND F T 16.3.16-10 (T ≤ 0.40).

The final values of the toxicity indices of the oxides of the rare earth metals under consideration are presented in table 2.

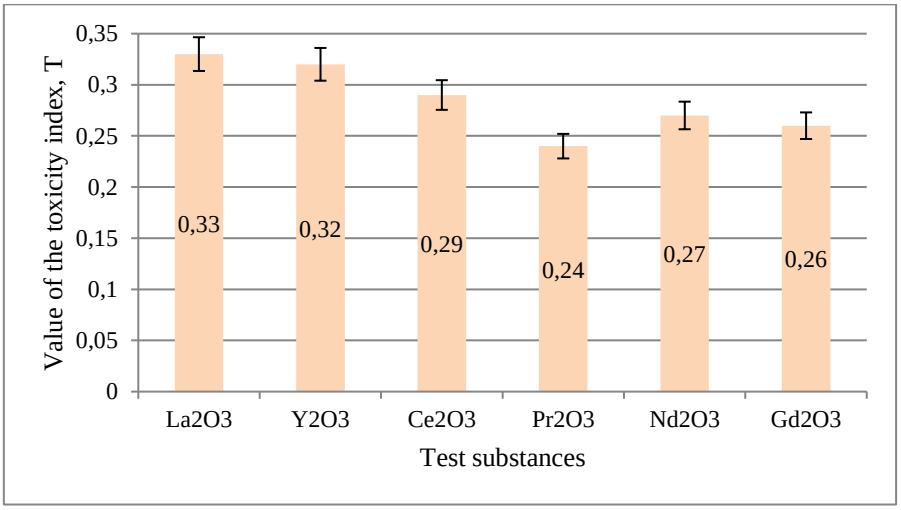

Fig. 6. Value of toxicity indices of oxides of the studied metals at 100-fold dilution of the sample

Table 2

Toxicity indices of the studied rare earth metal oxides

Determined indicator	Analysis result		Toxicity index (excluding degree of dilution)	Degree of toxicity of the sample, T
	Test substance	Dilution degree		
Toxicity of the sample on Paramecium caudatum infusoria	La_2O_3	100	0.33	High, T = 33
	Y_2O_3	100	0.32	High, T = 32
	Ce_2O_3	100	0.29	High, T = 29
	Pr_2O_3	100	0.24	High, T = 24
	Nd_2O_3	100	0.27	High, T = 27
	Gd_2O_3	100	0.26	High, T = 26

The measurement results of the toxicity indeces of carbonates and oxides of rare earth metals were evaluated for acceptability in accordance with the recommendations presented in PND F T 16.3.16–10. The convergence of the results of parallel definitions was checked by the formula:

$$|T - Tmax, \min| \le r, \quad (3)$$

where r — the ratio of operational control of convergence (r = 0.43T); T — the arithmetic average of the results of 3 parallel measurements of toxicity index in arbitrary units; T_{max} — the maximum value of the toxicity index of the sample of three parallel measurements; T_{min} — the minimum value of the toxicity index of the sample of three parallel measurements.

According to the evaluation, the condition (3) is met for each of the results, which means that the results obtained can be considered reliable.

Determination of toxicity and average lethal concentration of LC50 (24 h) was carried out in accordance with GOST R 57166-2016 "Water. Determination of toxicity by survival of freshwater infusoria Paramecium caudatum".

The results of the analysis are presented in table 3.

Table 3

Results of bioassay for acute toxicity

Substance	Testing duration, h	Mass concentration of the substance, mg/dm^3	Number of surviving organisms in containers, PCs			Arithmetic mean of the number of surviving organisms, PCs.	Percentage of death of text-organisms, %
			1	*2*	*3*		
La_2O_3	24	0(control)	30	30	30	30	0
		0.00424	17	16	16	16	46
		0.02924	16	17	14	16	48
		0.05424	15	13	17	15	50
		0.07924	12	13	12	12	59
		0.10424	11	12	13	12	60
Y_2O_3	24	0(control)	30	30	30	30	0
		0.0064	16	18	18	17	42
		0.0314	15	16	18	16	46
		0.0564	14	14	17	15	50
		0.0814	12	11	14	12	59
		0.1064	12	11	13	12	60
Ce_2O_3	24	0(control)	30	30	30	30	0
		0.062	18	17	16	17	43
		0.087	14	17	14	15	50
		0.112	14	11	13	13	58
		0.137	13	11	12	12	60
		0.162	10	9	11	10	67
Pr2O3	24	0(control)	30	30	30	30	0
		0.047	26	27	26	26	12
		0.072	23	23	24	23	22
		0.097	16	12	17	15	50
		0.122	13	11	14	13	58
		0.147	11	10	12	11	63
Nd2O3	24	0(control)	30	30	30	30	0
		0.06	19	20	21	20	33
		0.085	18	18	17	18	41
		0.11	17	16	18	17	43
		0.135	15	13	17	15	50
		0.16	11	13	16	13	56

Gd2O3	24	0(control)	30	30	30	30	0
		0.4	14	16	17	16	48
		0.425	13	17	15	15	50
		0.45	11	12	10	11	63
		0.475	10	11	8	10	68
		0.5	9	8	9	9	71
La2(CO3)3	24	0(control)	30	30	30	30	0
		0.009	19	19	20	19	36
		0.034	18	17	19	18	40
		0.059	16	18	15	16	46
		0.084	15	17	13	15	50
		0.109	13	15	11	13	57
Y2(CO3)3	24	0(control)	30	30	30	30	0
		0.0155	18	19	20	19	37
		0.0405	17	16	17	17	44
		0.0655	15	15	18	16	47
		0.0905	13	13	18	15	50
		0.1155	12	11	14	12	59
Ce2(CO3)3	24	0(control)	30	30	30	30	0
		0.101	17	20	18	18	39
		0.126	17	18	15	17	44
		0.151	15	18	12	15	50
		0.176	14	13	12	13	57
		0.201	12	13	11	12	60
$Pr_2(CO_3)_3$	24	0(control)	30	30	30	30	0
		0.078	17	20	19	19	38
		0.103	16	18	17	17	43
		0.128	14	17	16	16	48
		0.153	14	16	15	15	50
		0.178	11	12	14	12	59
$Nd_2(CO_3)_3$	24	0(control)	30	30	30	30	0
		0.16	18	20	18	19	38
		0.185	16	18	16	17	44
		0.21	14	15	15	15	50
		0.235	13	14	14	14	54
		0.26	12	13	13	13	58
$Gd_2(CO_3)_3$	24	0(control)	30	30	30	30	0
		0.59	18	16	20	18	40
		0.615	16	15	18	16	46
		0.64	16	13	17	15	50

		0.665	14	12	15	14	54
		0.69	11	10	15	12	60

According to the results of bioassay performed in accordance with GOST R 57166-2016, it is clear that the samples are highly toxic and pose a danger to the environment and humans.

Conclusion. Based on the results of the research, the toxicity indices of compounds of a number of rare earth metals were determined. According to PND F T 16.3.16-10 "Methodology for determining the toxicity of production and consumption waste by express method using a device of the "BIOTESTER" series", these substances are highly toxic compounds. Based on the results of the analysis of the average lethal concentration of LC50 (24 h) using Paramecium caudatum test organisms, it was proved that these compounds are highly toxic in accordance with GOST R 57166-2016.

Based on the research results, safety data sheets were developed in accordance with GOST 30 333-2007 "Safety data sheet for chemical products. General requirements" and R 50.1.102-2014 "Preparation and registration of a safety data sheet for chemical products". The results obtained are included in section 12 "Information on environmental impact".

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Submitted 17.09.2020
Scheduled in the issue 19.10.2020

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M. I. Semenova — research to determine the toxicity index of rare earth metal compounds; A. V. Smirnov — research to determine the average lethal concentration of LC50, preparation of the text; A. Sokolov —calculations performance, analysis of the results, formulation of the conclusions; A. S. Kovalevskaya — scientific supervision, correction of the conclusions; O. V. Smolova — formulation of the main concept, goals and objectives of the study, finalizing the text.