

DETERMINATION OF THE TOXICITY OF AQUEOUS EXTRACT FROM WASTE PROCESSING OF PHOSPHATE RAW MATERIALS USING BIOTESTING ON HYDROBIONTS

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The biotesting method was used to assess the individual and combined effects of waste from the production of phosphate raw materials at the designed chemical complex of the EuroChem-Karatau company (Republic of Kazakhstan). The goal of the study was to determine the hazard class of waste from the production of phosphate raw materials using biotesting method. Experimental study was carried out on test organisms: *Daphnia magna* Straus freshwater planktonic crustaceans. It has been established that *Daphnia magna* is sensitive to waste from the processing of phosphate raw materials, and the samples under study belong to practically non-hazardous and low-hazard waste with the possibility of processing for the purpose of their further use. On hydrobionts, water extracts without dilution from a mixture of waste module CCP + cake module 1A (1:1), cake module 1A and synthetic gypsum did not have an inhibitory effect. An ecological and toxicological study confirmed that the studied samples belong to the fifth class of environmental hazard (virtually non-hazardous). For the aqueous extract from the waste of the CCP module without its dilution, inhibition of the viability of aquatic organisms was revealed. The death of *Daphnia* may be associated with waste components that cause blockage of the respiratory tract with dispersed particles. It was revealed that with increasing dilution, the mortality rate of *Daphnia* decreases; the 10-times dilution ratio had no effect on hydrobionts.

Keywords bio-testing, toxicity, hazardous industrial waste, hazard class, *Daphnia magna* Straus.

ОПРЕДЕЛЕНИЕ ТОКСИЧНОСТИ ВОДНОЙ ВЫТЯЖКИ ИЗ ОТХОДОВ ПЕРЕРАБОТКИ ФОСФАТНОГО СЫРЬЯ МЕТОДОМ БИОТЕСТИРОВАНИЯ НА ГИДРОБИОНТАХ

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Метод биотестирования впервые использован при оценке отдельного и комбинированного воздействия отходов производства фосфатного сырья проектируемого химического комплекса компании ЕвроХим-Каратау (Республика Казахстан). Цель работы состояла в определении класса опасности отходов производства фосфатного сырья с помощью методов биотестирования. Экспериментальную работу проводили на тест-организмах: пресноводных планктонных рачках *Daphnia magna* Straus. Установлено, что *Daphnia magna* чувствительны к отходам переработки фосфатного сырья, а исследуемые образцы относятся к практически неопасным и малоопасным отходам с возможностью переработки с целью их дальнейшего применения. На гидробионты водные вытяжки без ее

разведения из смеси отхода модуля ССР + кек модуля 1А (1:1), кек модуля 1А и гипса синтетического не оказывают угнетающего эффекта. Эколого-токсикологическое исследование подтвердило, что исследованные образцы относятся к пятому классу опасности для окружающей среды (практически неопасные). Для водной вытяжки из отхода модуля ССР без ее разведения выявлено угнетение жизнеспособности гидробионтов. Гибель дафний, возможно, связана с компонентами отхода, которые вызывают закупорки дыхательных путей дисперсными частицами. Выявлено, что при увеличении разведения смертность дафний понижается, установлена кратность разведения водной вытяжки (в 10 раз), при которой не выявлено воздействие на гидробионты.

Ключевые слова: биотестирование, токсичность, опасные промышленные отходы, класс опасности, *Daphnia magna* Straus.

ГИДРОБИОНТТАРДА БИОТЕСТІЛЕУ ӘДІСІМЕН ФОСФАТ ШИКІЗАТЫН ҚАЙТА ӨНДЕУ ҚАЛДЫҚТАРЫНАН СУ СЫҒЫНДЫСЫНЫҢ УЫТТЫЛЫҒЫН АНЫҚТАУ

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Биотестілеу әдісі алғаш рет ЕвроХим-Қаратау компаниясының (Қазақстан Республикасы) жобаланатын химиялық кешенінің фосфат шикізатын өндіру қалдықтарының жеке және аралас әсерін бағалау кезінде пайдаланылды. Жұмыстың мақсаты биотестілеу әдістерін қолдана отырып, фосфат шикізатын өндіру қалдықтарының қауіптілік класын анықтау болды. Эксперименттік жұмыс тест-ағзаларда жүргізілді: тұщы су планктонды *Daphnia magna* Straus шаян тәрізділер. *Daphnia magna* фосфат шикізатын қайта өңдеу қалдықтарына сезімтал екендігі анықталды, ал зерттелетін үлгілер оларды әрі қарай қолдану мақсатында қайта өңдеу мүмкіндігімен іс жүзінде қауіпсіз және қауіптілігі төмен қалдықтарға жатады. Гидробионттарға ССР+1А (1:1) кек модулінің, 1А кек модулінің қалдықтарынан және синтетикалық гипстің қоспасынан сұйылтылмаған су сығындылары әсер етпейді. Экологиялық-токсикологиялық зерттеу зерттелген үлгілердің қоршаған ортаға қауіптіліктің бесінші класына жататынын растады (іс жүзінде қауіпті емес). ССР модулінің қалдықтарынан оны өсірусіз су сығындылары үшін гидробионттардың өміршеңдігінің тежелуі анықталды. Дафнияның өлімі тыныс алу жолдарының дисперсті бөлшектермен бітелуіне әкелетін қалдықтардың құрамдас бөліктерімен байланысты болуы мүмкін. Өсіруді көбейткен кезде дафнияның өлімі төмендейтіні, гидробионттарға әсері болмайтын су сығындысының сұйылу жиілігі (10 есе) анықталды.

Түйін сөздер: биотестілеу, уыттылық, қауіпті өндірістік қалдықтар, қауіптілік класы, *Daphnia magna* Straus.

Introduction. In world practice, biotesting methods are widely used to assess the toxicity of water pollution [1]. The use of these methods has a number of advantages over physicochemical analysis, which often does not allow the detection of unstable compounds or the quantification of ultra-low concentrations of toxic substances, as well as taking into account their combined effects.

Biotesting allows to quickly obtain an integral assessment of toxicity at a certain point in time [2].

Traditional, frequently used test objects for monitoring the toxicity of various objects are Cladocera - small planktonic crustaceans, one of the most numerous and diverse orders of the

Branchiopoda class. Well-known representatives of the order, *Daphnia* freshwater planktonic crustaceans (*Daphnia magna* Straus), are among the most sensitive test organisms and are considered the basic object of biotesting [3, 4].

For example, planktonic crustaceans (*Daphnia magna* Straus) were used as a test organism for biotesting of quarry waters in [5]. When studying quarry waters using *D. magna* test organisms, the number of deaths in the tested *Daphnia* samples did not exceed 10%, which indicated the absence of a toxic effect. The tested culture of *Ch. Vulgaris* had a higher sensitivity, however, when the tested samples were diluted 3 times, the toxicity criterion was not exceeded. The calculated values of the dilution coefficient with toxic substances made it possible to rank the water bodies under consideration in increasing order of the possible toxic impact of their waters: Inkerman Quarry Lake, Gasfortskoye Quarry Lake and Kadykovskoye Quarry Lake. However, already with a 3-fold dilution, the tested samples from all reservoirs became completely harmless.

Another work, [6], developed methodological approaches to rapid assessment of the environment, as well as the toxicity of its components in the process of biological monitoring at hazardous industrial facilities. The following biotest objects were used: daphnia (*Daphnia magna* Straus), ceriodaphnia (*Ceriodaphnia affinis* Lilljeborg), ciliates (*Paramecium caudatum*), a preparation of lyophilized luminescent bacteria "Ecolum" based on a recombinant *Escherichia coli* M-17, algae (*Chlorella vulgaris* Beijer, *Scenedesmus quadricauda* (Turp.) Breb.), mammalian germ cells; environmental objects: natural water, bottom sediments, soil samples taken in the sanitary protection zone (SPZ) of the Balakovo region and a nuclear power plant in the Saratov region. The toxicity of samples of bottom sediments, soil, as well as water samples from the cooling pond and the adjacent water area of the Saratov reservoir was revealed.

Bioanalysis has also been successfully used to determine the hazard of pollution of environmental objects with oil and waste from the oil refining

industry containing petroleum hydrocarbons, represented by persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) [7]. The ecotoxicological study used the test culture *Daphnia magna* Straus - a sensitive test organism that reacts to the presence of petroleum products of various fractions and mass concentrations in water. When conducting biotesting, the work took into account specific methodological nuances: the death of daphnia in the aqueous extract from waste, established within 48 hours, did not exceed 10%, but the morphological state of the tested object indicated a significant negative impact: daphnia individuals are smaller than in the control test, their trophic activity is minimal, and only longer exposure (96 hours) led to the death of the test organism. It has been established that biotesting, in this case, remains a mandatory method for determining total toxicity.

The authors of [8] carried out an environmental and toxicological assessment of the waste generated as a result of protein production by growing *Hermetia illucens* fly larvae and determined the hazard class of zoocompost using biotesting methods. The hazard class was determined by the dilution coefficient of the aqueous extract, at which no harmful effect on the test objects was detected. To study the toxicity of zoocompost, the species of lower crustacean *Daphnia magna* Straus were used. As a result of the experiments, the dilution ratio of zoocompost extracts was established (equal to 10), at which the death of daphnia was not observed, therefore, the waste under study was classified as hazard class 4.

An ecotoxicological assessment of six drill cuttings (waste from the oil industry) using certified methods of contact and eluate biotesting using test organisms of different trophic levels was carried out in [9]. Biotesting carried out on *Daphnia magna* Straus showed that aqueous extracts from all samples of drill cuttings did not have an inhibitory effect on these organisms.

The authors of [10] studied the soil of a quarry in the Neva Lowland, reclaimed using loamy soil and municipal solid waste. When conducting biotesting of aqueous extracts of soil samples, the presence of

acute toxicity was established at one of the studied sites. The presence of chronic toxicity in water extracts was identified at all sites.

In [11], the soils of agricultural lands and nearby reservoirs of the Prokhorovsky district of the Belgorod region were studied. Watercress *Lepidium sativum* and crustacean *Daphnia magna* Straus were used as a bioindicator. According to the results of biotesting, the water in reservoirs adjacent to agricultural land corresponds to hazard class II, the “slightly polluted” category. It was revealed that the soils of agricultural lands of the holding have initial manifestations of toxic effects.

The toxicity of ash and slag waste obtained after burning coal at power plants was determined experimentally using test organisms [4]. When analyzing the toxicity of the ash and slag mixture after burning the culm of the Donetsk deposit (Russia), an acute toxic effect of aqueous extract from waste on the test organisms *Daphnia magna* Straus and the weed *Scenedesmus quadricauda* was revealed. A non-hazardous dilution factor for the water extract from waste was revealed, causing the death of no more than 10% of daphnia in 96 hours and a deviation in the number of weeds by no more than 20% in 72 hours compared to the control value. Ash and slag waste was classified as waste of III-IV environmental hazard class.

The influence of fluoropolymer production wastewater on test objects of different systematic groups was studied in [12]. Toxicity assessment was carried out by tetrazole-topographic, anaphase-metaphase analysis methods and micronuclear test of micropreparations. It has been established that the germination of white mustard seeds (*Sinapis alba* L.), the survival of *Daphnia Magna* Straus and cyanobacteria (*Nostoc paludosum* Kutz) are reduced under the influence of fluoropolymer production wastewater with a decrease in the dilution ratio. In Scots pine (*Pinus sylvestris* L.), mitotic activity decreases, and the proportion of cytogenetic disorders increases with a decrease in the degree of dilution of the test solution. The negative impact of fluoropolymer production wastewater on biota and the need for disposal, excluding discharge into the environment, have

been determined.

Thus, biotesting methods make it possible to assess the biological usefulness of the studied water bodies, their suitability for the life of aquatic organisms that ensure self-purification processes in the reservoir and biological oxidation during wastewater treatment [13]. The results of biotesting for toxicity make it possible to quickly determine the dangerous effects of chemical pollution on the life of aquatic organisms, not by individual components, but by their mixtures, often of unknown nature and not detected by other methods of analyzing toxic substances.

Toxic effects recorded by biotesting methods include complex, synergistic, antagonistic and additional effects of all chemical, physiological and biological components present in the test water, adversely affecting the physiological, biochemical and genetic functions of the test organisms. As a result of the laboratory biotesting procedure using classical methods, the acute or chronic toxicity of the test water is established in experiments of various durations. Acute toxicity is expressed when the intensity of the acting agent is so great that the body's compensatory and adaptive reactions do not have time to manifest themselves, and it dies. Chronic toxicity is determined by intense but longer exposure to toxicants; in this case, an imbalance occurs between the decay and synthesis of substances in the body of aquatic organisms, the destruction of the genome and the cessation of reproduction. Based on the data from the experiments, an acutely lethal concentration of the substance (or the dilution factor of the test water) is established, at which the death of organisms does not exceed that in the control.

Materials and methods. Determination of the toxicity of aqueous extract by biotesting on hydrobionts was carried out for samples of waste from the processing of phosphate raw materials in the production of simple superphosphate (SSP), obtained by processing by hydrochloric acid decomposition of phosphorites at a semi-industrial Technophos installation (Technophos, Prayon Technophos EAD, Devnya, Bulgaria) for the projected production plant of mineral fertilizers

at EuroChem-Karatau (Kazakhstan).

EuroChem-Karatau chemical complex for processing phosphates is designed for processing phosphate rock to produce dicalcium phosphate (calcium hydrogen phosphate dihydride). The production of dicalcium phosphate consists of the following main technological stages:

1) Processing of phosphorite raw materials by a solution of hydrochloric acid with the transfer of the valuable component into a soluble state in the form of a product solution and the production of a solid residue, which is a technology waste (Module 1A);

2) Treatment of the product solution with ground limestone with precipitation of dicalcium phosphate and drying of the resulting product DCP (dicalcium phosphate) and obtaining a solution of calcium chloride (Module 1B);

3) Treatment of the calcium chloride solution with slaked lime with the precipitation of

magnesium, aluminum and iron impurities into the hydrate cake and obtaining a purified calcium chloride solution (CCP module), which is removed from the process for further processing;

4) Treatment of part of the calcium chloride solution with sulfuric acid to obtain a regenerated hydrochloric acid solution and gypsum precipitate (Module 4).

In this study, the toxicity assessment was carried out based on the potential danger to aquatic organisms of the following waste from the processing of phosphate raw materials:

- Sample No.1 – cake of Module 1A;
- Sample No.2 - synthetic gypsum;
- Sample No.3 - waste of the CCP module - magnesium hydroxide;
- Sample No.4 - cake of the Module 1A and waste of module CCP (mixture 1:1) (Figure 1).



Fig.1 – Initial samples of the phosphate raw material processing waste

When conducting these studies, an aquatic laboratory culture of *Daphnia magna* of 1-day age was used

as a test object (Figure 2).



Fig.2 - Daphnia magna

The studies were carried out in accordance with ST RK 17.1.4.01-95 [14] standard and the Methodological Guide for Biotesting of Water [15]. We used a method for determining the toxicity of water and aqueous extracts from soils, sewage sludge, waste by mortality and changes in the fertility of daphnia.

The survival rate of test objects is the average number of daphnia that survived in the tested extracts and in the control over a certain time. The criterion for acute toxicity of the test sample (DL_{50}) is the death of 50 percent or more of the daphnia in the analyzed water sample compared to the control during the biotesting period (48 hours). If the result is more than 50% death of individuals, the test sample is considered toxic. The calculation of dead daphnia in the test water sample in comparison with the control is calculated using the formula [13]:

$$A = \frac{(X_c - X_t)}{X_c} \cdot 100, \% \quad (1)$$

where X_c is the arithmetic mean number of daphnia that survived in the control sample;

X_t – arithmetic mean number of daphnia surviving in the tested water.

Experimental. To obtain ready-made samples during the study, before starting the tests, the initial

samples were air-dried, then crushed and sifted through a 1-mm sieve.

At the next stage, the moisture content of all studied samples was determined. The moisture content of the samples was: CCP module – 12%, cake of module 1A – 2%, synthetic gypsum – 3%, CCP module waste + cake of module 1A mixture (1:1) – 7%. A certain moisture characteristic was used to calculate the mass of an air-dry sample intended for preparing an aqueous extract.

Water-soluble compounds were extracted from the samples using dechlorinated tap water taken in a solid-to-liquid ratio of 1:10 (by weight). Aqueous extracts from phosphate raw material processing waste were prepared in the following ratio:

- 88 g of air-dried sample (waste of the CCP module) and 880 cm³ of cultivation water;
- 98 g of air-dried sample (module 1A cake) and 980 cm³ of cultivation water;
- 97 g of air-dried sample (synthetic gypsum) and 970 cm³ of cultivation water;
- 93 g of air-dried sample (mixture (1:1) of CCP module waste + cake of module 1A) and 930 cm³ of cultivation water.

Stirring of the prepared suspensions was carried out for 7 hours. Then the suspensions settled for 15-20 minutes.

The resulting aqueous extracts from samples of phosphate raw material processing waste were filtered through a “white ribbon” filter on a Buchner funnel. A weak vacuum was used for filtration using an electric pump. The resulting leach extract was tested for toxicity.

The biotesting procedure (determination of acute toxicity) was carried out for the test samples under the conditions specified in Table 1.

Using a 2-cm³ glass pipette with a cut off and melted end, 10 specimens of daphnia at the age of 24 hours were transplanted into a glass and transferred into beakers with analyzed and cultivation water using a plankton net or a Pasteur pipette, the liquid was sucked out of the glass and the measured

volume of test water was added carefully not to damage the daphnia.

For testing, 100 cm³ of analyzed and cultivation water were taken into chemical cups with a capacity of 250 cm³. Each sample of the test

water was analyzed in triplicate. Repetition for control samples is also threefold. 10 one-day-old daphnia were placed in each glass and exposed at a temperature of 22±2 °C for 48 hours. Before the start of biotesting, daphnia were fed.

Table 1 - Conditions for the biotesting procedure

Sample No.	Date: 22.02.2023	Temperature, °C	Dissolved oxygen, mg/dm ³	pH
1	Control	24	7.5	8.21
2	Waste of the CCP module	24	6.3	8.28
3	Waste of the CCP module + module 1A cake	24	6.1	8.19
4	Cake of module 1A	24	6.5	7.16
5	Synthetic gypsum	24	6.9	7.55

Table 2 - Results of calculating the survival rate of daphnia

Duration of biotesting	Number of surviving daphnia, specimens														
	Control			Waste of the CCP module			CCP module waste + cake of module 1A			Cake of module 1A			Synthetic gypsum		
	No. of experiment			No. of experiment			No. of experiment			No. of experiment			No. of experiment		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
22.02.2023	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
23.02.2023 (24 hours)	10	10	10	2	1	2	10	7	7	10	10	10	6	6	8
24.02.2023 (48 hours)	10	10	10	2	1	2	10	6	5	10	10	10	6	6	8
Average number of surviving daphnia, specimens	10			1.7			7			10			6.7		
A, %	0			83			30			0			33		

During the entire biotesting period, the crustaceans were not fed. Surviving daphnia were counted after 1, 6, 12, 24, and 48 hours. Crustaceans

were considered survivors if they moved freely in the water or surfaced from the bottom no later than 15 s after a slight rocking of the glass.

Results of testing are presented in Table 2. The biotesting results were considered correct if the death of daphnia in the control did not exceed 10% for the entire observation period and the oxygen concentration in the test water at the end of the experiment was at least 2 mg/dm³ (Table 3).

Table 3 - Oxygen concentration in the tested samples at the end of the experiment

Sample No.	After 96 hours	Dissolved oxygen, mg/dm ³
1	Control	2.6
2	Waste of the CCP module	2.4
3	CCP module waste + cake of module 1A	2.0
4	Cake of module 1A	2.4
5	Synthetic gypsum	2.5

For the waste of the CCP module, a repeated biotesting procedure was carried out (dilution factor 10 times). The biotesting procedure (determination of acute toxicity) was carried out for the test sample under the conditions specified in Table 4. The results of the calculation after 48 hours are presented in Table 5.

Table 4 - Conditions for the biotesting procedure

Date: 28.02.2023	Temperature, °C	Dissolved oxygen, mg/dm ³	pH
Control	24	7.5	8.15
Waste of the CCP module	24	6.5	8.20

Table 5 - Results of calculating the survival rate of daphnia

Duration of biotesting	Number of surviving daphnia, specimens					
	Control			CCP module waste		
	repetition			repetition		
	1	2	3	1	2	3
28.02.2023	10	10	10	10	10	10
01.03.2023 (24 hours)	10	10	10	10	10	10
02.03.2023 (48 hours)	10	10	10	10	9	9
Average number of surviving daphnia, specimens	10			9.3		
A, %	0			7		

Results and discussion. As a result of the experiment, it was established that the survival rate of aquatic organisms in experimental samples (compared to the control sample) is:

- CCP module waste - 17%;
- CCP module waste + cake of module 1A - 70%;

- Cake of module 1A - 100%;
- Synthetic gypsum - 67%.

Thus, determining the impact of only the aqueous extract of waste without its dilution allows to classify the waste: waste of CCP module + cake of module 1A, cake of module 1A and synthetic gypsum to the fifth class of environmental hazard.

For the waste of the CCP module, a repeated

biotesting procedure was carried out (dilution factor 10 times). According to the results of the study for the waste of the CCP module (survival rate of aquatic organisms was 93%) based on the dilution factor of the aqueous extract (10 times), at which no impact on aquatic organisms was detected in accordance with the dilution factor ranges (Table 6), hazard class 4 was established.

Table 6 - Assignment of hazard class according to the dilution factor of the aqueous extract

Waste hazard class	Dilution factor of the aqueous extract from hazardous waste, at which there is no harmful effect on aquatic organisms
I	10,000
II	From 10,000 to 1,001
III	From 1,000 to 101
IV	100
V	1

Conclusion. Even though there is no universal test system for determining all existing toxicants, biotesting as an integral assessment method is successfully used as an environmental monitoring tool. Biotesting is one of the research techniques in the field of toxicology, widely used nowadays to assess the degree of toxicity of various ecosystems. Biotesting does not cancel the analytical control system, but effectively complements it with qualitatively new indicators, since from an environmental point of view, the results of determining the concentration of pollutants themselves have only relative value. It is important to know not the levels of pollution, but the biological effects they cause. As a result of biotesting, it was established that *Daphnia magna* is sensitive to waste from the processing of phosphate raw materials. Aqueous extracts (without dilution) from the mixture of CCP module waste + cake of module 1A (1:1), cake of module 1A and

synthetic gypsum do not have an inhibitory effect on hydrobionts. An ecological and toxicological study confirmed that the studied samples belong to the fifth class of environmental hazard (virtually non-hazardous). For the aqueous extract from the waste of the CCP module without its dilution, inhibition of the viability of aquatic organisms was revealed. The death of daphnia may be associated with waste components that cause blockage of the respiratory tract with dispersed particles. It was revealed that with increasing dilution, the mortality rate of daphnia decreases; the dilution ratio of the aqueous extract was established (10 times), having no effect on hydrobionts. Thus, the waste from the CCP module, in accordance with the dilution factor ranges, is assigned to the fourth hazard class (low hazard) for the environment. The results obtained allowed to conclude that the studied waste is acceptable for safe storage (under controlled conditions) and further processing.

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