CHAPTER «BIOLOGICAL SCIENCES»

DETERMINATION OF MAXIMUM PERMITTED CONCENTRATIONS SUBSTANCES FOR REPRESENTATIVES OF MAIN LINES OF THE TROPHIC CHAIN OF THE WATER ECOSYSTEM

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Abstract. The specificity of the landscape geosystem approach to the establishment of environmental norms determines the need to take into account the properties of landscapes, as a spatio-temporal, heterogeneous in its composition elements of the system. Particular attention in the study is devoted to the question of determining the indicators that serve as criteria for the normalization of loads at different stages of the study of the «impact-effects-changes» chain. For standardization at the «consequences» stage, integrated bioecological indicators with the use of organisms – biotatists, representatives of biocoenoses of aquatic ecosystems were used. Compliance with water quality standards for water bodies of the fishery is intended to provide favorable conditions for the functioning of aquatic ecosystems. The water quality will meet the specified requirements if in any formation of the water object there will be no chronic toxicity of water, ie there will be no negative influence of pollutants on the survival and reproducibility of aquatic organisms. The environmental safety standard for water use is the maximum concentration of pollutants in water of an aquatic object, above which the water is unsuitable for fishery water use. The basic principle of establishing environmental safety standards for water use is the use of a landscape-ecological approach that ensures the integrity of the trophic chain of the

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aquatic ecosystem and their sustainable functioning. Taking into account this approach for setting standards, a test system using a set of test objects that are representatives of producers and consults is used. Maximum permissible concentrations of a substance for individual test objects are determined using the biotesting method. For each test object, mandatory indicators of the toxicity of the substance are determined. Other indicators may be auxiliary, they are used to study the specific features of the substance's impact on the test object. Conclusion on the presence or absence of toxic effects of a substance on a test object in separate experiments according to the relevant indicators of toxicity is made on the basis of the relevant toxicity criterion. The maximum permissible concentration of the substance for the relevant test objects is determined by the results of the synthesis of experimental data on the toxic effects of the substance on all the studied toxicity. In the framework of this study, the testing of biotesting methods using the «basic set» of aquatic organisms was carried out in order to establish the maximum permissible concentration of morpholine. In long-term experiments, chronic morpholin toxicity was studied for *Scenedesmus quadricauda algae, Ceriodaphnia affinis, Cyprinus carpio* and *Brachydanio rerio*. The most sensitive link of the aquatic ecosystem to the action of morpholine, based on the results of experiments, identified crustacean ceriodophytes. In accordance with the criterion for establishing ecological and fishery regulations, the lowest of the maximum allowable concentrations was adopted. The concentration for morpholine is 0.125 mg/dm^3. The limiting indicator of morpholin hazard for the aquatic ecosystem is toxicologically, since the concentration of 0,125 mg/dm^3 is derived from the evaluation of the toxic properties of the substance. Based on the results of experiments in which the cumulative properties of morpholin were determined, the coefficient of material cumulation in organs and tissues of fish was established, which is 0.85-2.4.

1. Introduction

According to European legislation in the EU, the regulation of water quality in surface water bodies is based on the provisions of the WFD 2000/60/EC, the Directives 2008/105/EC and 2013/39/EC [1-3]. In particular, Section 1.2.6 of the WFD 2000/60/EC states that water quality standards for chemicals are established on the basis of the results of ecotoxicological experiments using the «base set» of aquatic organisms, which include algae and / or macrophytes, crustaceans and fish [1].

Article 1 of Directive 2008/105/EC [2] states that «The chemical pollution of surface waters poses a threat to the aquatic environment with consequences such as toxicity to aquatic organisms, the accumulation of harmful substances in the ecosystem and the disappearance of natural habitats and biological diversity, as well as a threat to human health».

Accordingly, in accordance with Article 13 of the above-mentioned Directive, in order to ensure adequate protection of the aquatic environment and human health, water-quality standards are established for environmentally hazardous chemicals, expressed in terms of maximum allowable concentrations (MACs) for substances used in aquatic organisms and their annual average indicators (AAIs). This allows us to assess the short-term and long-term effects of environmentally hazardous substances on the biotic constituent of aquatic ecosystems, to carry out a targeted assessment of the risk of chemical pollution of surface waters for human health through the aquatic environment.

In accordance with the foregoing, the purpose of this study is to test the methods of biotesting using the «basic set» of aquatic organisms in order to establish the maximum permissible concentration of morpholine.

2. Procedure for determining the maximum allowable concentrations of the substance for algae

Maximum permissible concentrations of the substance for representatives of the main parts of the trophic chain of the aquatic ecosystem are determined using appropriate bioassay techniques. Detailed procedures for determining the maximum permissible concentrations of substances for the corresponding test objects are presented in [4].

Maximum permissible concentration of substance for algae Scenedesmus quadricauda (Turp.) Breb is determined by the method of bioassay according to the indicator of the growth rate of algae culture. The method is based on the determination of the difference between the intensity of the growth of algae in the solutions of a substance that is prepared on the nutrient medium (experiment) and in the medium (control) [5; 6].

To establish the maximum allowable concentration of a substance, a series of short-term (determination of acute toxicity) and long-term (determination of chronic toxicity) of experiments are carried out.

The criterion for acute toxicity is the reduction in the number of algae cells by 50% or more in the experiment compared with the control over 72 hours of biotesting.

The criterion for chronic toxicity is a statistically significant deviation of the number of algae cells in the experiment compared with control over 10 days of biotesting.

For conducting experiments, the nutrient medium of the Assumption № 1 is used, which prepares solutions with different concentrations of matter and control. To do this, sterile flasks with a capacity of 250 ml are filled with nutrient medium. In the test flasks add the appropriate aliquots of the working solution of the substance, bringing the total volume to 100 ml. In control flasks, pour 100 ml of nutrient medium. In each experimental and control flask, 2.0 ml of a suspension of algae of 1.0-2.5 ml/ml is added. The number of algae in the experiment and control before the start of the experiment should be 20-50 thousand cc/ml. Repeat for each concentration of substance and control 3-5x.

Control and test flasks are placed in a thermoluminescent unit at a temperature of $(22 \pm 4)^{\circ}$C and illumination intensity of 2500-5000 lux. Twice a day the flasks shake. Replacement of solutions during the experiment is not carried out.

Calculation of the number of algae cells in the experiment and control is carried out using cameras Goryaev or Fuchs-Rosenthal. To calculate the number of cells in the chamber Goryaev content of each flask is thoroughly mixed and taken 4 drops, in each drop count the number of cells in 25 large squares of the chamber. The number of cells in 1 ml is calculated by the formula:

$$X = m \cdot 10^4, \quad (1)$$

where X – number of cells in 1 ml;

m – the number of cells in 25 large squares.

Cell counting can also be performed in a 3.2-ml Fuchs-Rosenthal chamber. When a high number of cells count the number of cells in 16 squares diagonally, at low – counting the number of cells carried across the field of the chamber. The number of cells in 1 ml is calculated by the formula:

$$M = \frac{m \cdot 10^3}{n \cdot V}, \quad (2)$$

where M – number of cells in 1 ml;

m – total number of counted cells;

n – the number of calculated small squares of the camera;

V is the volume of the part of the chamber that has an area of a small square.

The number of cells expressed in thousands or millions per 1 ml.

The experiments are carried out in two stages.

In the first stage, in a wide range of concentrations, the inactive concentration of a substance that does not cause a decrease in the number of algae cells in 72 hours of biotesting is determined, and a concentration that completely suppresses the growth of algae.

At the second stage (determination of acute toxicity), a series of experiments (not less than 6) in the range of concentrations of a substance, selected on the basis of the results of the first stage, are conducted. According to the results of experiments, using one of the statistical methods [4], for each experiment, the value of the average effective concentration of the substance in which the number of algae cells in the experiment is reduced by 50% compared to the control over 72 hours of biotesting are calculated (EC50-72).

To determine the concentration of a substance that, when it enters a water object, can cause inhibition of producer organisms, calculate the lower bound of the confidence interval of the arithmetic mean values of the average effective concentrations ($EC_{50\text{-}72}$) by the formula:

$$\overline{EC}_{50-72} \pm ts_{\overline{EC}50-72}, \quad (3)$$

where t is the value of Student's criterion for a probability of 0.95 and a degree of freedom n-1; $s_{\overline{EC}50-72}$ – the mean arithmetic error.

Long-term experiments determine the chronic toxicity of the substance. For this purpose, a series of (at least 6) experiments using a series of concentrations is conducted, with the maximum of them being approximately ½ $EK_{50\text{-}72}$, minimal – do not exert a chronic toxic effect on algae.

According to the results of each experiment, using one of the statistical methods [4], determine the minimum active concentrations of the substance that cause a statistically significant deviation of the number of algae cells in the experiment compared with the control for 10 days of biotesting.

Based on a number of values from the minimum active concentrations, determine the maximum allowable concentration of the substance for algae. At the maximum allowable concentration, take that from the minimum effective concentrations, which by frequency of occurrence did not exceed 20%.

3. Procedure for determining the maximum permissible concentrations of substance for crustaceans

Maximum permissible concentration of substance for crustacean *Ceriodaphnia affinis Lilljeborg* is determined by methods of biotesting according to their survival and fertility indices [7-10].

To determine the maximum permissible concentration of substance, a series of short-term (determination of acute toxicity) and long-term (determination of chronic toxicity) of experiments are carried out.

The determination of acute toxicity is based on the determination of the difference between the amount of surviving ceriodophytes in the solutions of the substance (experiment) and in the water in which the ceriodophytes are held (control). The criterion of acute toxicity is the loss of 50% ceriodophytes and more in the experiment compared with control over 48 hours of biotesting.

The determination of chronic toxicity is based on the determination of the difference between survival and (or) fertility of the ceriodophyne in the solutions of the substance (experiment) and in the water in which the ceriodophyne is retained. The criterion for chronic toxicity is a statistically significant reduction in survival and (or) fertility of the seredicin in the experiment as compared to control during biotesting. Experiments on the determination of chronic toxicity in one generation continue (7±1) days until 60% of the initial ceriodophytic three litters. Experiments in a number of generations last up to 30 days. Their duration is determined by the time during which each of the generations (one weekend and three subsequent) will receive 4 pimples.

Additional indicators that characterize the toxicity of a substance for a ceriodophyna may be: the time of the appearance of the first litter, the total number of litters in the experiment compared with control, the appearance of males, the presence of latent eggs (Epiphytes), the nature of the movement.

Experiments are carried out in vessels of 20 ml capacity. Each vessel is poured into 15 ml of test solutions of a substance or control water and contains one ceriodophyne. Dehydrated drinking water is used to prepare substance and control solutions. Repeat experiment and control tenfold. The vessels are placed in a thermoluminescent unit at a temperature (25±2)°C with a light intensity of 600 to 800 lux, a light period of 16 hours, and a dark period of 8 hours.

Conduct short-term and long-term experiments.

Short-term experiments last 48 hours. They are carried out in ten repetitions. During experiments, they are not fed with ceriodophytes. After 24 and 48 years, at the beginning of the experiment, in the experimental and control vessels, the number of live ceriodaphne was counted. Animals are considered to be individuals who move freely in the water column or expose from the bottom of the vessel after light shaking.

Short-term experiments are carried out in two stages.

At the first stage in the experiment, 4-5 test concentrations of the substance are used, differing by 10 times. According to the results of the experiment, determine the concentration of the substance in which all ceriodophyans survive, and the concentration in which the death of 100% ceriodaphina occurs in 48 hours.

At the second stage (determination of acute toxicity), a series of (at least 6) experiments is conducted in the range of concentrations of the substance, which is chosen based on the results of the first stage, namely: at the lowest concentration, all ceriodophyans should survive, and the highest concentration should cause a 100% ceriodaphne loss. The number of concentrations should not be less than 5. At the end of the experiment, live ciryadaphny count in control and experimental vessels and calculate the mean lethal concentration values for 48 hours of biotesting ($\overline{IC}_{50-48}$), using one of the statistical methods.

To determine the concentration of a substance that, when it enters a water object, can cause the death of crustaceans, calculate the lower boundary of the confidence interval for the mean arithmetic mean values of the mean lethal concentrations ($\overline{LC}_{50-48}$) by the formula:

$$\overline{LC}_{50-48} \pm ts_{\overline{LC}50-48}, \quad (4)$$

where t is the value of Student's criterion with a probability of 0.95 and degree of freedom n-1; $s_{\overline{LC}50-48}$ – the mean arithmetic error.

Long-term experiments determine the chronic toxicity of the substance. Experiments on the determination of chronic toxicity are carried out on one generation of ceriodophytes and in a number of generations.

Experiments on one generation. Experiments are carried out in ten repetitions. Everyday in each vessel with ceriodafniami carry out the replacement of control water and appropriate solutions of the substance to freshly prepared solutions and make feed. During the replacement of the control water and substance solutions, the number of live outflow and newborns is cedar. After counting newborns, the ceriodophyllum is

removed. The duration of the experiments is (7±1) days until 60% of the initial ceriodophytic three litters appeared.

To determine chronic toxicity in one generation, a series of experiments (at least 6) using at least 5 concentrations of a substance are carried out. In this case, the highest concentration should be approximately ½ $\overline{LC}_{50-48}$ (of course, this concentration exhibits chronic toxicity). The smallest of the concentrations should not have a chronic toxic effect on the ceriodophyte.

At the end of the experiments, the number of surviving surviving ceriodophytes and the number of newborns in each replication of control and experiment are counted. According to the calculation results, for each experiment, the minimum active concentrations of a substance are determined using one of the statistical methods [4]. On the basis of the data obtained, determine the one with the minimum effective concentrations, which in frequency of occurrence did not exceed 20%.

Experiments on a number of generations. Experiments in a number of generations are carried out in the same way as experiments on one generation. From the latter they differ in that they are carried out in twenty repetitions; the experiment on each individual generation continues until the appearance of the four ceremony of four litters, usually up to 10 days.

In the course of experiments, generations follow the following scheme: newborns of the first litter of ceriodophytes emerging from the initial ceriodophyllus are planted in one copy to the corresponding aqueous solutions of the substance and control water (the first generation). Similarly, they receive the second and third generations. In general, experiments for three generations last for up to 30 days.

For the determination of chronic toxicity in a number of generations, a series of (at least 6) experiments using at least 3 concentrations of a substance is carried out. The largest of them should be equal to the minimum effective concentration, which, in the frequency of occurrence in experiments in one generation did not exceed 20%, and the smallest – should not have a chronic toxic effect on any of the studied generations of ceriodaphnia.

During experiments, the effect of the substance on the survival and fecundity of the ceriodophy and on the quality of the offspring are studied: survival and fertility of the following generations of ceriodophytic, the time of the first litter, the total number of litters, the appearance of males, the presence or absence of latent eggs (Epiphytes). The character of the ceriodophytic motion is also fixed.

At the end of the experiment, the number of surviving females was counted and the number of young people born in experimental and control vessels. Based on the results of the calculation determine the effective concentrations for each generation of ceriodophyllum. Among the obtained active concentrations, it is determined which of them is the minimum. The values of this concentration are taken for the minimum active concentration in the experiment for several generations.

On the basis of a number of values from the minimum active concentrations obtained in experiments on generations of ceriodaphne, determine the maximum permissible concentration of substance for crustaceans. At the maximum allowable concentration, take that from the minimum effective concentrations, which by frequency of occurrence did not exceed 20%.

4. Procedure for determining the maximum allowable concentrations of substances for fish

Fish, along with water mammals, are the closest link in the trophic chain of the aquatic ecosystem. The determination of the possible adverse effects of a regulated substance on fish is extremely important from the point of view of the assessment of the hazard of a substance for the existence of fish populations in water bodies and the threat to human health when using fish as a food product.

When setting maximum permissible concentrations, it is recommended to use freshwater species of Cyprinus carpio Linaeus and Brachydanio rerio Hamilton-Buchanan.

Cyprinus carpio (hereinafter carp) is a widespread industrial species of fish grown in fish farms in Ukraine. They are easy enough to adapt to laboratory conditions of detention. It is convenient for them to conduct research on morphological, hematological, pathoanatomical parameters and to study cumulative properties.

The maximum permissible concentration of a substance for a carp is determined by the following indices [11]:

survival (recording live and dead fish);

the state of the gyre apparatus (color, blood filling, the form of gyrium petals);

clinical picture (position of the rays of swimmers, coordination of movements, abdomen and scales, rhythm of breathing);

pathoanatomical characteristic (the consistency of the liver and kidneys, the presence of the exsudate, the condition of the mucous membrane);

hematological characteristic (hemoglobin content, determination of leukocyte formula and blood elements);

organoleptic properties of broth and meat of fish (color, smell, taste);

material cumulation (accumulation of matter in organs and tissues, calculation of concentrations of matter in organs and tissues in relation to concentration in water).

The method for determining the toxicity of a carp material is based on the determination of the difference between the values of the abovementioned indicators of the state of fish in the solutions of the substance compared with the control in pure water.

The criterion for the toxicity of the substance for a carp is to evaluate the change of the listed indices in the fish of the experimental group compared with the control group, which is kept in clean water. In addition, the values of hematological parameters in the experiment are compared with the normative ones that are set for the carp.

To establish the maximum allowable concentration of the substance, determine the concentrations that do not lead to changes in the condition of the carp on the studied parameters.

The maximum permissible concentration of matter for carp is set in a series of long-term experiments for up to 20 days.

For experiments, the concentration of a substance is used, which is defined as the maximum permissible for other representatives of the trophic chain of the aquatic ecosystem.

Experiments are carried out consistently in the same years, single-parent plants and two-year-old carp.

In order to assess the suitability of water for conducting hydrochemical studies. To do this, at the beginning of experiments, determine the gas regime, the hydrogen index (pH) of water, content of biogenic elements (NH_4^+, NO'_2, NO'_3, PO^{-3}_4), water-soluble organic matter (permanganate and bichromate oxidation), basic ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, HCO_{3^-}, Cl^-, SO_4^{2-}), general hardness and mineralization of water. The listed hydrochemical indicators of water quality are also determined at the end of experiments to assess the influence of the substance on the hydrochemical regime.

To control the conditions of fish containment in pools, experimental and control aquariums of each day, determine the concentration of oxygen dissolved in water, which must be maintained at 6-8 mgO2 / l.

The obtained values of hydrochemical indicators of water quality are estimated in comparison with control and normative indicators.

Hydrochemical studies are carried out according to the generally accepted method [12].

The survival of different age groups of fish is determined during experiments by systematically recording live and dead fish. The obtained data is summarized and expressed in absolute and percentile values for each variant of experiment and control. Determine the highest concentrations of substances that do not lead to the death of fish of different age groups.

The clinical picture (symptoms of poisoning) is estimated by changing the behavior of fish in the aquatic environment and the response to external stimuli during periodic review during experiments.

The state of fish in the course of its intoxication is estimated by general methods (autopsy, inspection, odor determination, measurement, photographing).

Also use special methods of research (microscopy, electrocardiography, radiography, and others).

According to the results of experiments, determine the maximum permissible concentration of matter, in which no signs of intoxication of fish are revealed.

The state of the gyro apparatus is investigated during experiments. Normally, the gills are full-blooded, bright red, clean, gill lobes are level, lie in parallel, their free edges form evenly half-circle around the gill bracket. At poisoning of fish gills acquire various shades – dark cherry, red, pale red, pale yellow-pink, dark brown, grayish. An overview of the gills is carried out at the dead fish and at the end of the experiments.

According to the results of experiments, determine the maximum permissible concentration of matter, in which there is no change in the state of fish gills.

The patho-anatomical section is performed using live fish, as well as fish that are in an agony or just died. Pay attention to the consistency of parenchymal organs, the presence of an exudate. When examining the digestive tract, the nature of the contents and condition of the mucous membrane are marked: the presence of edema, hyperemia, color.

According to the results of experiments, determine the maximum permissible concentration of matter, in which no signs of changes in the state of the internal organs of fish are shown in comparison with the control group of fish.

In the study of organoleptic properties of the broth, attention is drawn to the color of the broth and the presence or absence of a foreign smell in it.

According to the results of experiments, determine the maximum permissible concentration of the substance, in which there are no changes in the organoleptic properties of broth and meat of fish compared with the control group of fish.

Hematologic studies are carried out on the following indicators:

determination of hemoglobin content; counting the number of red blood cells; Counting the number of leukocytes; determination of leukocyte formula; conducting qualitative analysis of the formed elements of blood of fish.

The obtained values of the indices in the experimental groups are compared with the parameters of the control group and determine the maximum permissible concentration of the substance, in which the hematological parameters of the fish do not change. In addition, the obtained values are compared with the normative ones, which are established for multi-age groups of carp.

At the maximum permissible concentration of the substance for *Cyprinus carpio* fish, the lowest value is taken from the determined maximum allowable concentrations for the individual indices studied.

Brachydanio rerio (further danio) is an aquarium species of fish. They are easily cultivated in the laboratory throughout the year, are sensitive to the action of many chemicals, especially in the early stages of development (embryos and larvae).

When determining the maximum permissible concentration of a substance for *Brachydanio rerio*, use of mature data and early stages of their development (embryos and larvae) [13; 14].

The maximum permissible concentration of substance for sexually mature data is determined in the short-term (determination of acute lethal toxicity) and long-term (determination of chronic toxicity) experiments.

In short-term experiments, acute lethal toxicity is determined, which is based on establishing the difference between the number of surviving fish in the solutions of the substance (experiment) and in the water in which the fish are kept (control).

The criterion of acute lethal toxicity is the death of 50% or more fish in the experiment compared with control for 96 hours of biotesting.

In long-term experiments, the duration of which is 30 days, chronic toxicity is determined, which is based on establishing the difference

between the values of fish state indicators in the solutions of the substance (experiment) and in the water in which the fish are kept (control). These indicators are:

survival; behavior (coordination of movements, location, reaction to irritation, activity of eating food); morphological features (appearance, position of the rays of swimmers, abdomen, gill apparatus and scales); physiological parameters (character and frequency of breathing, growth of ichthyomasis, fatness).

The criterion of chronic toxicity for mature data is a statistically significant deviation of the above indicators in the experiment compared with the control.

The maximum permissible concentration of the substance for data at early stages of development (embryos and larvae) is determined by the following indicators:

survival of embryos and larvae; curling of larvae; teratological indices of larvae.

Determination of the toxicity of the substance is based on establishing the difference between the values of the above listed indicators of the state of embryos and larvae given in the solutions of the substance (experiment) and in the water in which they are contained (control).

The criterion of toxicity of a substance for data in the early stages of development is a statistically significant deviation of the listed parameters in the experiment compared with the control.

Conduct short-term and long-term experiments. The duration of short-term experiments is 96 hours.

Short-term experiments are carried out in two stages. At the first stage (preliminary experiment) use 4-5 test concentrations of the substance, differing 10 times. According to the results of experiments, determine the range of concentrations of matter in which there is a death of fish from 0 to 100%.

At the second stage (determination of acute lethal toxicity), a series of experiments is carried out (at least 6). Use at least 5 concentrations of test solutions that decrease in geometric progression and include the highest non-lethal and lowest lethal concentrations obtained during 96 hours in the previous experiment. According to the results of experiments, calculate the value of the average lethal concentration of substance for 96 hours of biotesting ($\overline{LC}_{50-96}$) for each experiment.

In long-term experiments, chronic toxicity of a substance is determined for sexually mature data. For this purpose, a series of (at least 6) experiments in a range of concentrations, the maximum of which is equal to $\overline{LC}_{50-96}$, the following decreases in geometric progression, for example 1/2, 1/4, 1/8, 1/16 $\overline{LC}_{50-96}$.

The duration of long-term experiments is 30 days. Two times a day, fish feed dry or live food, the dead fish are removed. Every 3 days, control water and test solutions are replaced by freshly prepared.

During experiments, observations are made on survival, behavior, morphological characteristics and physiological parameters of fish.

Daily in each aquarium count the number of surviving fish, and remove those that perished.

Observations of behavior are carried out every 10 days of experiments.

In normal condition, fish do not show abnormal deviations in behavior: they behave calmly, swim across the entire thickness of water. When knocking on the walls of the aquarium, the fish quickly sink in the opposite direction, actively moving along the entire thickness of the water, after a few minutes they calm down and begin to swim slowly. Fish actively eating food.

At intoxication, fish behave irritably, their movements become swift, gushing, they hold on to the surface of the water, shortly before the death, they fall to the bottom and do not move. The general reaction of fish is somewhat slowed down: they slip away from the stimulus, some fish begin to rush across the entire water column, sometimes bumping into the walls of the aquarium or other fish; loss of orientation is observed. The activity of eating food is reduced, or fish at all refuse to feed.

Morphological signs. Changes in external signs of fish are recorded every 10 days of experiments.

Normally, the rafts of the swimmers are pressed to the body, the mouth is completely closed. The fish have a bright color, clear lines of coloring. Gill apparatus of bright red color. The body of fish is smooth, with uniform lining, the scales are not lagging behind the body.

At intoxication of fish their external signs change, the color is dull, lines of color are eroded. The gill apparatus becomes lighter or gets a different color. In some cases, the scales are lagging behind the body, the swim is widely diluted in the sides of the body, the mouth is not completely closed.

Physiological parameters. The nature and frequency of breathing. At intoxication, the respiration rate in the experimental fish differs from the control, respiratory movements become accelerated, in some cases – convulsive.

Observations on the nature and frequency of breathing of fish are carried out every 10 days of the experiment.

Growth of ichthyomasis, coefficient of fattening. To determine the ichthyomass at the beginning of the experiments and every 10 days fish are weighed and measured. Weigh and measure also the fish that died during the experiments. Before weighing and measuring, each fish is dried on gauze.

Growth or reduction of ichthyomas are determined in percentages relative to the initial ichthyomass.

5. Determination of the maximum permissible concentration for morpholine

Characteristic of the substance. Morpholine, synonyms: tetrahydro-1,4-oxazine; diethylene dihydrogen oxide; empirical formula – C_4H_9NO; the class of compounds is an organic compound, refers to a class of cyclic bases having a secondary amino group; molecular weight 87.12; aggregate state – hygroscopic oily colorless or yellowish-transparent liquid; dissolved in water, ethanol, diethyl ether; relative density – ρ_4 = 1,0000 – 1,0030 g/cm^3 (20°C); refractive index – $n_д$ = 1,4535 – 4,1555 (20°C); melting point – (-3,1)°C; boiling point – 127-130°C for 760 mm Hg. Morpholine is used in nuclear power plants to reduce corrosion and erosion processes and the level of pollution of steam generators.

In long-term experiments, chronic morpholin toxicity was studied for *Scenedesmus quadricauda* algae, *Ceriodaphnia affinis, Cyprinus carpio* and *Brachydanio rerio*.

According to the results of long-term experiments, the maximum permissible concentrations of morpholine for all test objects used in the experiment, which are presented in Figure 1, were determined.

The figure shows that the lowest of the maximum permissible concentrations of morpholine was obtained on crustaceans *Ceriodaphnia affinis*.

The most sensitive link of the aquatic ecosystem to the action of morpholine is crustacean ceriodophyne. In accordance with the criterion on which the established ecological and fishery standards adopt the lowest of the specified maximum allowable concentrations. There is such a concentration for morpholine 0,125 mg/dm^3.

The limiting indicator of the morpholin hazard for the aquatic ecosystem is toxicological, since the concentration 0,125 mg/dm^3 obtained on the

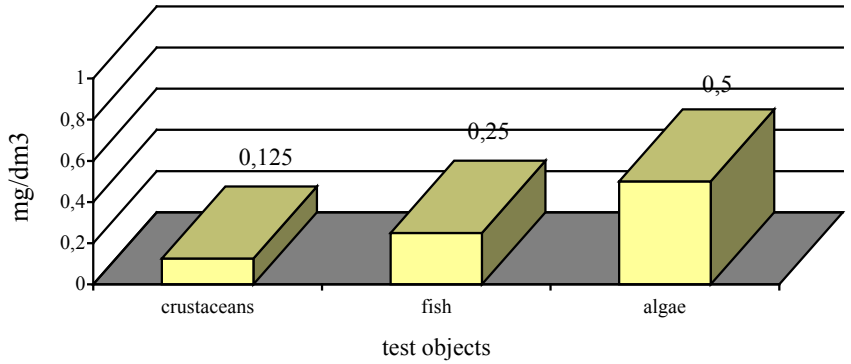

Figure 1. Maximum permissible concentrations of morpholine for various test objects

basis of evaluation of toxic properties of a substance. Based on the results of experiments in which the cumulative properties of morpholin were determined, the coefficient of material cumulation in organs and tissues of fish was established, which is 0.85-2.4.

In experiments to determine the stability of morpholin in aqueous medium, it was found that a decrease in its concentration by 95% occurs in 32 days. According to the classification, morpholine is a moderately stable substance, since its stability is in the range of 11 to 60 days.

6. Conclusions

Experiments on determining the maximum permissible concentrations of a substance for test objects are carried out in three stages. The results obtained in experiments during the test substance on the test objects (experiment) are compared with the results of experiments in the absence of this substance in the medium (control).

At the first stage, the concentration of the substance from the inactive, which does not cause death of the test objects or change of the investigated index of the life of the test-object, to the lethal concentration for all test objects, or the suppression of their vital functions by the corresponding indicators, is determined. It uses a wide range of concentrations, which may vary in order of magnitude, for example: 0,01; 0,1; 1,0; 10,0; 100,0 mg/dm^3.

At the second stage (determination of the acute toxic effect of the substance on test objects), a series of (at least 6) short-term experiments is conducted, the results of which determine the concentration of matter in which 50% of test objects are killed ($\overline{LC}_{50}$) or at 50% ($\overline{EC}_{50}$) the studied indicators of life-test test objects are suppressed. To do this, use a narrow range of concentrations of matter. Each concentration is tested in 2-10 times repetitions.

In the third stage (determination of the chronic toxic effect of the substance on the test objects), a series of (at least 6) long-term experiments is conducted, in which a number of concentrations are used, among which the maximum should be approximately ½ $\overline{LC}_{50}$ ($\overline{EC}_{50}$), minimal – do not cause chronic toxic effects on the test object with appropriate indicators of livelihoods. According to the results of the experiments, the minimum active concentration of the substance in each experiment is found, which causes a statistically significant deviation (inhibition or stimulation) of each of the studied indicators of the life of the test object (toxicity indicators) in the experiment compared with the control. Using a series of values obtained from the minimum active concentrations of a substance in a series of experiments, determine the maximum permissible concentration of substance for each indicator of the life of the test object.

For the maximum permissible concentration of matter for individual test objects, take the lowest value from the maximum allowable concentrations obtained in the experiments for each of the studied indicators of the life of the test object.

As a result of the testing of biotesting techniques using the «basic set» of aquatic organisms in order to establish the maximum permissible concentration of morpholin, the following results were obtained: the most sensitive link of the aquatic ecosystem to the action of morpholin are crustacean ceriodophytes, the maximum permissible concentration for morpholine was 0,125 mg/dm^3.

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