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Use of Toxicity Tests to Assess the Harmfulness of Selected Herbicides

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ABSTRACT

The aim of the study was to assess the impact of selected herbicides: Roundup Flex Ogrod, Sprinter 350 SL and Chwastox TRIO® 540 SL on the natural environment. The effect of herbicide preparations on the survival and life functions of test organisms was determined using toxicological studies. Various taxa were selected for toxicity tests: Gram-positive bacteria, Gram-negative bacteria, bioluminescent bacteria *Aliivibrio fischeri*, aquatic crustaceans *Daphnia magna* and *Chironomus* sp. larvae. A determination of the minimum inhibitory concentration (MIC) of herbicides on selected micro-organisms was carried out, as well as an effective concentration (EC_{50}) to inhibit the bioluminescence of *Aliivibrio fischeri*, and acute toxicity tests were performed with *Daphnia magna* and *Chironomus* sp., for which a lethal concentration (LC_{50}) was determined. In acute tests, the LC_{50} concentration was calculated by statistical methods. All tested herbicides belong to highly toxic compounds. Sprinter 350 SL showed the highest degree of toxicity, while Roundup Flex Ogrod and Chwastox TRIO® 540 SL showed similar harmfulness. The herbicide formulations tested showed varying degrees of toxicity using *Daphnia* crustaceans and *Chironomus* larvae. Daphnia were more sensitive in acute tests. Based on the conducted research, it was found that regular and detailed control of toxicity and the impact of herbicides on the environment is necessary.

Keywords: herbicides, toxicity, effective concentration, minimum inhibitory concentration, acute tests, environmental impact.

INTRODUCTION

Pesticides are classified as naturally toxic substances that act not only on harmful organisms, but also on beneficial ones. They are usually characterized by high toxicity and the ability to bioaccumulate in the trophic chain. Pesticides are strictly toxicologically controlled, and very harmful ones are regularly withdrawn from use. Regulations concerning their use are contained in directives, acts and national, EU and international resolutions. These substances must meet high requirements for the safety of animals, the environment and, above all, people (Paker, 2013; Jaworska, 2012; Iya and Kwaghe, 2007; Pagliuca et al., 2005).

Pursuant to the Plant Protection Act of 18 December 2003 (Journal of Laws of 2004, No. 11, item 94) in force in Poland, plant protection products include active substances or preparations containing one or more such substances which are intended for:

- protect plants, plant products or objects against harmful organisms or preventing the occurrence of these organisms,
- affecting the life processes of plants in a way other than a nutrient, including a growth regulator,
- securing plant products, if these substances or preparations are not covered by separate regulations,
- destroying unwanted plants,
- destroying plant parts and inhibiting or preventing unwanted plant growth.

The use of pesticides that allow to protect crops, control pests and protect plants from fungi

and bacteria, or stimulate their growth has become a necessity (Jabłońska-Trypuć et al., 2017; Paker, 2013). Considering their use, they can be divided into several groups, these are mainly: herbicides – weed killers, fungicides – fungicidal compounds, and insecticides – insecticidal preparations. Pesticides have found application in many elements of human life, in addition to crop protection they are also used in textile plants, swimming pools and dry cleaners (Rajveer et al., 2019; Yamada, 2017; Biziuk et al., 2001).

Pesticide formulations should show efficacy against organisms considered harmful. However, years of pesticide use have shown their highly non-selective effects and negative impacts also on beneficial species, their habitat, and humans (Zhang et al., 2011; Terry et al,, 2003; Frąk and Wisniewska, 2002). In the environment, pesticides undergo transformations and can be moved between ecosystems in their initial form or as derived metabolites, which often exhibit higher toxicity than the initial compounds. Such forms of pesticides can penetrate soil, water, air, as well as animal feed and food products, posing a direct threat to living organisms (Hu et al., 2013; Walter, 2009; Żelechowska et al., 2001).

With the increase in the use of pesticides in many branches of the economy, there have been serious consequences in the form of toxic effects on living organisms, eutrophication of water reservoirs, and reduction of soil fertility. The large-scale use of pesticides result in environmental pollution in all its elements (Witczak and Pohoryło, 2016; Witczak et al., 2013; Jager et al., 1998; Iman, 1994). The use of pesticides in the world is increasing every year. According to the World Health Organization (WHO), pesticide compounds contribute to the poisoning of approximately 1.5 million people worldwide each year. Consuming food contaminated with pesticides leads to 20,000 deaths annually in the European Union (Makles and Domański, 2008). Currently, 2746 preparations are approved for use in Poland (the register of authorized plant protection products – update 30.06.2023).

Progressive pollution of the environment with pesticides imposes the need to carry out thorough toxicological tests of hazardous substances and control over their use. Among the many pesticides used, plant protection products play an important role. Herbicides are chemical substances used to control weeds, among which there are total – destroying all plants and selective – fighting specific plant species.

The research was aimed at evaluating the impact of selected herbicides on the natural environment by determining the impact of the tested preparations on the survival and vital functions of selected organisms using toxicological tests.

MATERIALS AND METHODS

The tests carried out in laboratory conditions consisted in performing toxicity tests on three selected commercial herbicide preparations: Roundup Flex Ogrod, Sprinter 350 SL and Chwastox TRIO® 540 SL (Fig. 1). The selection of preparations was made on the basis of varied composition, availability and popularity of their use.

Three different commercial preparations of herbicides were used for the study, each of which was produced by a different company and differed in composition and content of biologically active substances. The content of active substances in the tested products has been presented is given in Table 1.

Fig. 1. Selected herbicide preparations: Roundup Flex Ogrod, Sprinter 350 SL and Chwastox TRIO® 540 SL

Table 1. The content of biologically active substances in the tested products

Herbicidename	The name of the active substance	The content of the active substance
Roundup FlexOgrod	glyphosate	10.99% - 120 g in 1 l of agent
Sprinter 350 SL	MCPA	7.87% - 90 g in 1 l of agent
	Glyphosate	22.75% - 260 g in 1 l of agent
Chwastox TRIO® 540 SL	dicamba	3.24% - 40 g in 1 l of agent
	MCPA	16.20% - 200 g in1 l of agent
	mecoprop	24.31% - 300 g in 1 l of agent

The first herbicide selected was the most popular and widely used Roundup Flex Ogrod by Substral. It is a herbicide in the form of a concentrate for the preparation of an aqueous solution, which is applied to the leaves by spraying. In particular, it is designed to control couch grass and other monocotyledonous and dicotyledonous weeds (annual and perennial). It can be used by non-professional users in home and allotment gardens. It cannot be used on lawns. It consists of water, auxiliary ingredients and the active substance, which is glyphosate in the form of potassium salt with a content of 10.99% – 120 grams of glyphosate in 1 liter of the agent. Additional ingredients are alkylpolyglycoside and nitroryl, which, as the manufacturer assures, are not classified as dangerous. The dosage of the agent is 30 milliliters of the preparation per 1 liter of water.

The second product selected was Sprinter 350 SL from Target. It is a herbicide, in the form of a liquid for making an aqueous solution. Like Roundup, it is applied foliarly, designed to control annual and perennial monocotyledonous and dicotyledonous weeds in pome and stone tree orchards.

The preparation is in the form of a mixture of various substances. It has two active substances, which are glyphosate in the form of isopropylamine salt of N-(phosphonomethyl)-glycine, the content of which is 22.75% – 260 grams of glyphosate in 1 liter of the agent and MCPA in the form of dimethylamine salt of 4-chloro-o-tolyloxyacetic acid, the content of which is 7.87% – 90 grams of MCPA in 1 liter of the agent. An additional substance that also has a dangerous effect is an ethoxylated tertiary fatty amine. In addition, the manufacturer states that the preparation contains other ingredients, which he does not disclose, but informs that they are not classified as dangerous. The dosage of the preparation is from about 17 to 27 milliliters of the agent per 1 liter of water.

The third selected herbicide preparation was Chwastox TRIO® 540 SL by Ziemovit. It is a plant protection agent with a herbicidal effect in the form of a concentrate for the preparation of an aqueous solution. It is used for foliar control of annual and perennial dicotyledonous weeds in spring and winter cereals as well as on lawns, sports fields and golf courses.

The manufacturer does did not provide the full composition of the preparation, he only informs about the content of active substances. Chwastox in its composition contains three biologically active substances, the most of all three tested preparations. These are: dicamba in the form of potassium 3,6-dichloro-o-anilate, the content of which is 3.24% – 40 grams of dicamba in 1 liter of the agent, MCPA in the form of potassium 4-chloro-o-tolyloxyacetate with a content of 16.20% – 200 grams of MCPA in 1 liter of the agent and mecoprop in the form of 2-(4-chloro-o-tolyloxy) potassium propionate with a content of 24.31% – 300 grams of mecoprop in 1 liter of the agent.

Toxicity tests

Three tests were selected to assess the harmful effects of herbicides on the environment and to determine their toxicity: a Minimal Inhibition Concentration (MIC) test using microorganisms, a test using the Microtox system with *Aliivibrio-fischeri* bacteria and an acute toxicity test with *Daphnia magna* and chironomid larvae.

Performing a test determining the MIC of the tested microorganisms

In order to perform the test, a Mueller-Hinton agar medium and broth cultures of bacterial strains of the following types were prepared: *Escherichia, Staphylococcus, Pseudomonas, Enterobacter, Citrobacter, Klebsiella, Proteus, Bacillus, Sarcina* and *Candida* yeast. Then, initial solutions of the three tested herbicide preparations

with a concentration of 300 mg/dm^3 each were made. Subsequent, two-fold dilutions were made from the stock solutions using distilled water. In this way, stock solutions of each of the tested preparations with concentrations from 150 mg/dm^3 to 0.2929 mg/dm^3 were prepared. Then, the prepared solutions in the amount of 1 cm^3 were poured into sterile Petri dishes and diluted with 19 cm^3 of sterile Mueller-Hinton medium, obtaining 20 times lower concentrations for testing. In the next stage, cultures of the tested microorganisms were made and the plates were incubated at 37°C for 24 hours. After this time, the MIC was determined for each type of microorganism and each tested preparation. The lowest concentration of the tested agent for which no growth of the microorganism occurred was taken as the MIC value.

Performing a test using the Microtox system with Aliivibrio fischeri bacteria

The aim of the study was to perform a basic acute test with dilutions recommended by the Microtox system. The system includes the Microtox M500 analyzer from SDI with the Omni 4.1 software. According to the procedure, the luminescence of the samples was read twice: Io (initial luminescence of the bacterial suspension itself) and It (luminescence after incubation of the bacteria at time *t* with the tested sample of herbicides). The system generated reports and the result of the test was the EC_{50} value – the concentration that inhibits 50% of the light emission of the *Aliivibrio fischeri* bacteria.

The tests made it possible to determine the EC_{50} value of the tested herbicides. Then, the obtained EC_{50} values were converted into toxicity units according to the formula:

$$TU = (1/EC_{50}) \cdot 100$$

where: TU – toxic unit,
EC_{50} – toxic effect (%).

On the basis of the obtained TU values, the toxicity classes of the tested preparations were determined using the system proposed by Persoone (Mantins et al. 2015). Depending on the obtained TU value, Persoone distinguished five classes of toxicity:

- class 0 TU = 0 – non toxic-sample,
- class 1 0 < TU < 1 – no significant toxicity,
- class 2 1 < TU < 10 – significant toxicity,
- class 3 10 < TU < 100 – high acute toxicity,
- class 4 TU > 100 – very high toxicity.

Acute toxicity test with Daphnia magna and Chironomus sp. larvae

In order to perform the test, stock solutions of the tested preparations with a concentration of 100 mg/dm^3 each were prepared, and then diluted solutions in the concentration range from 50 mg/dm^3 to 0.0031 mg/dm^3 were made from each of them. Dilutions were made using chemically pure water. Then, 50 cm^3 of diluted solutions were poured into the crystallizers. The control sample was dilution water. Next 10 specimens of the tested organisms were carefully transferred to each crystallizer using a micropipette – parallel *Daphnia magna* daphnia and *Chironomus* sp. larvae. The tested samples were left for 24 hours at room temperature. After the test time, the number of dead organisms was counted and recorded for each dilution of the test preparations. Then, the lethal concentration LC_{50} for each organism was calculated using two statistical methods: the probit method and the Trimmed Spearman-Karber method using the Speraman software.

RESULTS AND DISCUSSION

For a comprehensive assessment of the threats resulting from the presence of herbicides in the environment, predicting their fate and analyzing the risk associated with their occurrence, it is necessary to determine both the compounds that are components of herbicides, their metabolites and products formed during degradation processes taking place at the place of their application, e.g. in the soil. The toxicity of the metabolites of many herbicides can be much higher than the starting compound (Golombieski et al., 2016; Carles et al., 2017). For this reason, supplementing chemical tests with acute toxicity tests is a rational solution.

Concentrations occurring in the environment, e.g. in water or soil, are usually very low. An example is the research conducted by Grygiel et al. (2012) in 2005–2009. The authors examined 172 soil samples taken from experimental plots where winter rapeseed was grown. Herbicides commonly used to protect this crop were applied to the plots, following the manufacturer's recommendations regarding, among others: date and dose of the preparation. Samples for testing were taken at the time of harvesting the crop. It was found that the maximum herbicide residues in the soil

ranged from 0.008 to 0.032 mg/kg. Residues at this level were detected in 11% of samples. Residues at a level of 0.002 mg/kg were present in 75% and 14% of the samples had no detectable herbicide residues.

It should be emphasized that the results obtained in the conducted experiments did not concern the determination of the toxicity of herbicides contained in environmental samples. The aim of the research was to determine the toxicity of the most popular herbicides and the possibility of using three different toxicological tests to evaluate them. For some herbicides, there are no acute test results in their safety data sheets. The concentrations adopted in the tests resulted from the regulations included on the labels of the three tested herbicides. Table 2 summarizes the MIC results of the tested herbicides Roundup Flex Ogrod, Sprinter 350 SL and Chwastox TRIO® 540 SL. Based on the obtained results, MIC values were determined for all tested microorganisms. If the growth of the microorganism was noted at the highest tested concentration of the preparation, the following was assumed: MIC > the highest concentration.

Table 3 shows the results of the acute toxicity test of the examined tested herbicides using the Microtox system. It presents the EC_{50} concentration values of the tested preparation, which inhibit 50% of the bioluminescence of the *Aliivibrio fischeri* bacteria, which were measured after 5 and 15 minutes in the Microtox analyzer. EC_{50} values listed in Table 3 were read from reports generated by the Microtox system. EC_{50} results were converted to toxicity units.

Table 4 presents the calculated LC_{50} values of the tested herbicides, which were determined based on the performance of acute tests with daphnia and chironomes. LC_{50} calculations were carried out using two calculation methods: probit and the Spearman-Karber Trimmed program.

Herbicides should be effective against specific plants. Nevertheless, the preparations are also toxic to many other organisms. In the test determining

Table 2. MIC values determined for all tested preparations and tested microorganisms

Microorganism	Herbicide name		
	Roundup	Sprinter	Chwastox
	MIC value [mg/dm^3]		
Pseudomonas(G-)	3.75	7.5	3.75
Enterobacter (G-)	3.75	0.9375	7.5
Candida (G+)	1.875	0.1171	0.9375
Bacillus (G+)	> 7.5	> 7.5	3.75
Staphylococcus (G+)	3.75	1.875	7.5
Citrobacter (G-)	1.875	< 0.0146	< 0.0146
Escherichia (G-)	3.75	3.75	> 7.5
Sarcina (G+)	> 7.5	3.75	7.5
Klebsiella (G-)	< 0.0146	< 0.0146	< 0.0146
Proteus (G-)	7.5	3.75	7.5

Note: „G-" – gram negative bacteria, „G+" – gram positive bacteria or *Candida* yeast.

Table 3. Microtox toxicity test results

Luminescence drop measurementtime	EC_{50} value [%]	TU
Herbicide – Roundup Flex Ogród		
5 minutes	0.4005	249.69
15 minutes	0.4040	247.52
Herbicide – Sprinter 350 SL		
5 minutes	0.3064	326.37
15 minutes	0.3272	305.62
Herbicide – Chwastox TRIO® 540 SL		
5 minutes	0.4644	215.33
15 minutes	0.4072	245.57

Table 4. LC_{50} results of the tested herbicide determined in acute tests with *Chironomus sp.* and *Daphnia magna*

Herbicidename	LC_{50} value [mg/l]			
	Probit method		Spearman-Karber method	
	Daphnia magna	*Chironomus* sp.	*Daphnia magna*	*Chironomus* sp.
Roundup	0.068	0.733	0.07	0.78
Sprinter	0.006	0.024	0.01	0.02
Chwastox	0.202	0.745	0.37	0.58

the minimum concentration inhibiting the growth of the tested microorganisms, concentrations of herbicides recommended by manufacturers were used. Based on the tests carried out, it was found that the Sprinter 350 SL agent turned out to be the most toxic, because the MIC values obtained were by far the lowest. Only *Bacillus* and *Pseudomonas* bacteria showed growth in the Sprinter herbicide solution 7.5 mg/dm^3.

Sprinter 350 SL is a non-selective systemic herbicide. It is absorbed by the leaves and then moved to the roots and runners, inhibiting the development and growth of plants. The first symptoms of the agent's effect on plants are visible 7–14 days after the treatment, and the plants die completely after about 30 days. It was also found that Roundup Flex Ogrod and Chwastox TRIO® 540 SL had a similar effect on the tested microorganisms. Most of the tested microorganisms show growth in very similar concentrations under the influence of both tested preparations. Roundup Flex Ogrod is a systemic herbicide. It is taken up by the green parts of plants (leaves, green shoots and non-woody bark), and then it moves throughout the plant and reaches its underground parts (roots, runners, etc.), causing it to die. The first symptoms of the agent (yellowing and wilting) are visible 7–10 days after the treatment. The plants die completely after about 3 weeks. Chwastox TRIO® 540 SL, on the other hand, is taken up by the leaves of plants, causing their deformation, and then inhibition of growth and death of the plants.

The highest resistance to the tested herbicides was shown by bacteria of the following genera: *Pseudomonas, Enterobacter, Bacillus, Staphylococcus, Escherichia, Sarcina* and *Proteus*, for which the MIC values were the highest. However, the most sensitive microorganisms were yeasts of the genus *Candida* and bacteria of the genus *Citrobacter* and *Klebsiella*, for which no growth was observed at the lowest tested concentration of each of the tested agents. Gram-negative bacteria cells of the genera *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Escherichia*, *Klebsiella* and *Proteus* are surrounded by an additional outer sheath that increases resistance to herbicides. For this reason, some of them show low sensitivity to the tested herbicides. In addition, *Pseudomonas* bacteria produce biologically active compounds (e.g. antibiotics or lytic enzymes), contributing to the detoxification of the environment in which they live, which allows them to survive at higher doses of the herbicide (Berry et al., 2014; McSpadden Gardener, 2007; Banaszkiewicz, 2003).

In turn, the growth of Gram-positive bacteria of the genera *Staphylococcus*, *Bacillus* and *Sarcina* was possible due to the presence of a thicker cell wall consisting of many layers of murein in their structure. In addition, bacilli can produce spores that enable them to survive in unfavorable conditions.

The herbicidal preparations used showed toxicity in various concentrations towards the tested types of microorganisms, which is also confirmed by the studies of other researchers. As reported by Wyszkowska and Kucharski (2004), when examining the effect of the herbicide Chwastox TRIO® 540 SL on various groups of microorganisms, the use of the optimal dose of herbicide recommended by the manufacturer may cause changes in the activity of microorganisms. The lowest doses contribute to an increase in the number of copiotrophic bacteria and actinomycetes and a decrease in the number of fungi. On the other hand, doses higher than optimal have an adverse effect on bacteria of the genus *Azotobacter*, oligotrophic, copiotrophic, cellulolytic, spore-forming bacteria and fungi (Baćmaga et al., 2007; Wyszkowska, 2002, 2004).

The tested herbicides are also toxic to marine bioluminescent bacteria *Aliivibrio fischeri*. They cause the destruction of bacterial cells, which is manifested by the inhibition of the emission of light produced by bacteria. The obtained EC_{50} values showed that the Sprinter 350

SL preparation turned out to be the most toxic, for which the EC_{50} after 5 minutes was 0.3064%, and after 15 minutes it slightly increased to 0.3272%. In the case of the herbicides Roundup Flex Ogrod and Chwastox TRIO® 540 SL, the concentration that inhibited 50% of the bioluminescence of the *Aliivibrio fischeri* ranged from 0.4040% to 0.4072% after 15 minutes. After converting these values into toxicity units (TU) and comparing them with the Persoone's system, it was found that all tested herbicide preparations belong to the highest – fourth toxicity class. This means that the tested agents are very toxic and harmful to the natural environment.

The third type of tests used to test herbicides were acute tests using the aquatic organisms *Daphnia magna* and *Chironomus sp.* The tests also highlighted differences in the sensitivity of the test organisms to herbicides. Two methods were used to assess the correctness of calculating LC_{50} values, with very similar results. Regardless of the herbicide tested, the LC_{50} values obtained for the test organisms were below the value of 1 mg/L. The guidelines in effect in the European Union (Table 5) and those used by the EPA (Table 6) were used to assess the degree of toxicity.

The herbicide LC_{50} results obtained in all acute tests indicate their high toxicity. *Daphnia magna* was a more sensitive bioindicator, regardless of the herbicide used in the study. *Chironomus sp.* larvae occur in an environment with a higher degree of pollution, which is why they are more resistant to toxic substances, which was confirmed by the conducted research. The most toxic herbicide was Sprinter 350 SL, for which the calculated LC_{50} value was the lowest and amounted to 0.006 mg/l.

Summarizing the test results, it was found that all tested herbicide preparations showed very strong toxicity to aquatic organisms and bacteria. Each of the products was characterized by a different, although extremely high, degree of toxicity, which was influenced by: the chemical composition of the agent, the amount of the active substance contained, the presence of additional and supporting substances and the concentration of the dosed solutions. The test organisms also showed different sensitivity to the tested preparations. Their resistance to herbicides was conditioned by the condition of the individual, its size, natural habitat and the concentration of the preparation acting on them.

The most toxic agent in all tests turned out to be Sprinter 350 SL, which was caused by the presence of the largest amount of glyphosate – 22.75% and additionally MCPA – 7.87%. Chwastox TRIO® 540 SL, despite the content of the largest amount of biologically active substances in its composition: dicamba – 3.24%, MCPA – 16.20% and mecoprop – 24.31%, was characterized by a similar degree of toxicity to the Roundup Flex Ogrod preparation, containing only one active substance – glyphosate (10.99%). Glyphosate and its metabolites, i.e. aminomethylphosphonic acid (AMPA) are detected in both water and soil. Data on occurrence and levels of glyphosate residues in EU soils is very limited. 21% of the tested EU topsoils contained glyphosate, and 42% contained AMPA. Both glyphosate and AMPA had a maximum concentration in soil of 2 mg kg^{-1} (Silva et al.,2018). The glyphosate concentration in ground waters were generally low – <2,5 µg/l in several European and Asia countries, but higher in France (Geng et al.,2021, Poiger et al., 2017, Bruggen et al., 2018). It should also be mentioned that glyphosate and AMPA are commonly detected in drinking-water (Mas et al., 2020; Parvez et al., 2018).

Herbicides, as producers assure, are safe for the environment, but not for all elements of the environment. Although they are assigned to the control of specific, harmful organisms, they can also show toxicity to other beneficial plants or animals, as well as to humans. Therefore, regular and detailed control of their toxicity and impact on the environment is necessary, and only those preparations that meet a number of requirements and standards should be allowed for use.

Table 5. Classification of chemical compounds in terms of toxicity to aquatic biocenosis according to European Union guidelines (Łebkowska, 1999)

LC_{50} [mg/l]	Compound toxicity assessment
< 1	Highly toxic
> 1 – 10	Toxic
> 10 – 100	Harmful

Table 6. Classification of chemical compounds in terms of toxicity for aquatic biocenoses according to the guidelines of the American Environmental Protection Agency (Łebkowska, 1999)

$LC(EC)_{50}$ [mg/l]	Compound toxicity assessment
< 1	Highly toxic
> 1 – 100	Moderately toxic
> 100	Slightly toxic

Reducing the effects of herbicide use will only be possible if the rules of their use are strictly followed. This especially applies to the appropriate concentration made in accordance with the label on the packaging. Spraying should be carried out at the appropriate time and under appropriate weather conditions in such a way as to limit the penetration of herbicides into other environments, e.g. water. In addition, the environment in and around the spraying area should be monitored. Educating the public, especially farmers, about the harm caused by the use of specific herbicides may also play an important role. An alternative solution to limit the effects of herbicides should be the development of safe and ecological plant protection products.

CONCLUSIONS

On the basis of toxicological tests carried out and the results obtained, the following conclusions were drawn:

1. Tested herbicide preparations have a negative impact on the natural environment. High concentrations of these agents inhibit the growth of microorganisms, reduce the bioluminescence of *Aliivibrio fischeri* and kill lower order aquatic organisms.
2. The chemical composition of herbicides significantly affects their toxicity. Herbicide formulations containing glyphosate have been found to be more harmful.
3. The most toxic agent turned out to be the Sprinter 350 SL containing in its composition MCPA and the largest amount of glyphosate.
4. Chwastox TRIO® 540 SL and Roundup Flex Ogrod showed a similar, very high degree of toxicity in relations to the tested organisms.
5. The concentrations of the working mixture recommended by the manufacturers of the preparations for all the tested agents significantly exceed the lethal concentrations (LC_{50}) of the tested aquatic organisms and the concentrations that inhibit the growth of microorganisms and the bioluminescence of the *Aliivibrio fischeri* bacteria.

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