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Toxicological Investigation of New Synthesized Reagents - Functionalized Phosphonates with Photocontrolled Biological Activity

L. G. Bakina[1], N. V. Mayachkina[1*], Yu. M. Polyak[1], A. G. Pilip[1], A. O. Gerasimov[1], A. V. Egorova[1,2] and A. A. Manshina[3]

[1]Department of Toxicology, St. Petersburg Federal Research Center of the Russian Academy of Sciences (SPC RAS), Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences, St. Petersburg, 197110, Russia; korshun25@mail.ru

[2]Department of Chemistry, St. Petersburg State Institute of Technology (Technical University), St. Petersburg, 190013, Russia

[3]Department of Chemistry, Institute of Chemistry of St. Petersburg State University, St. Petersburg, Universitetsky Pr., 26, St. Petersburg, 198504, Russia

Abstract

The toxic properties of newly synthesized vinylphosphonates, namely, diene compounds with ethyl and isopropyl substituents in the phosphonate group were examined in this study. Previous studies have revealed laser-induced cis-trans isomerization of these compounds. In addition, the high biological activity of vinylphosphonates and its significant dependence on photoirradiation have been found. The proven possibility of controlling the biological activity of synthesized phosphonates under laser irradiation opens prospects for applying them in photopharmacology and producing drugs with the capability of remote and precise control over the area and duration of their therapeutic effect. Due to the promising application of synthesized phosphonates in photopharmacology, the relevant task for the first stages of research was ascertaining the toxic effects of these synthesized compounds. The toxicity of the substances, including daphnia, ciliates, and unicellular green algae, was tested using a bioassay system. Considering the insolubility of the synthesized phosphonates in water, experiments were carried out after the preliminary dissolution of the phosphonates in dichloromethane at a harmless dichloromethane concentration in water for the selected test organisms.

Major Findings: Based on the results obtained, the toxicological parameters of phosphonates were calculated including lethal concentration LC_{50} (0.2×10^{-5} M/L) and effective concentration EC_{50} (3.3×10^{-5}M/L), as well as no observed effect concentration NOEC (0.9×10^{-6} M/L), which do not cause a statistically significant toxic effect. Daphnia were the most sensitive to synthesized phosphonates.

Keywords: Bioassays, *Daphnia magna*, *Paramecium caudatum*, Photopharmacology, Unicellular Green Algae, Vinylphosphonates

**Author for correspondence*

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1. Introduction

Currently, photopharmacology is known to be one of the most rapidly developing branches of modern science. It is based on controlling the biological activity of compounds through exposure to optical radiation[1]. The main advantage of this approach is the ability to fine-tune biological activity by controlling the parameters of optical radiation such as spectral and energetical characteristics, duration of exposure, and spatial localization. In addition, light can be considered a non-invasive tool for controlling biological processes. All of these things open the way to the creation of "smart drugs" with the capability to remotely and accurately control the duration of their therapeutic effect. In this regard, newly synthesized compounds belonging to the group of functionalized phosphonates are very promising[2-5]. The introduction of "smart drugs" into medical practice may solve the problem of uncontrolled drug activity, which stipulates side effects in healthy organs, as well as the accumulation of biologically active substances in the environment and severe natural pollution. This concept of photopharmacology has proven fruitful and has already demonstrated effectiveness in photoswitchable antimicrobial and anticancer therapy, in ophthalmology, as well as in metabotropic glutamate receptor modulation that is potentially eligible for the treatment of psychiatric disorders such as anxiety, depression, and schizophrenia[6,7].

Due to the promising application of different compounds of this class of reagents in photopharmacology and the need to produce new compounds and materials, that would be safe for humans and animals, the relevant objective for the first stages of research was ascertaining the toxic effects of synthesized compounds. Currently, there are virtually no publications on toxicological studies of the compounds potentially applicable in photopharmacology.

The main object was a series of newly synthesized vinylphosphonates, namely, diene compounds with ethyl and isopropyl substituents in the phosphonate group. These compounds exhibit laser-induced cis-trans isomerization when irradiated with a 266 nm laser. Photoisomerization was unequivocally confirmed by one- and two-dimensional Nuclear Magnetic Resonance (NMR) spectroscopy and Raman spectroscopy[4-8].

An important advantage of vinylphosphonates over other photoswitchable molecules is their pronounced bioactive properties. These compounds have been shown to inhibit pronouncedly butyrylcholinesterase, and their effect increased up to 9-fold under photoirradiation. Such a significant difference in inhibition levels for different isomeric states is a decisive factor for the application of new compounds in photopharmacology[4,9].

The performed investigations consisted of a series of experiments aimed at determining the main toxicological parameters of (O)-functionalized phosphonates through biotesting methods.

Biotesting is one of the widely used methods for assessing the toxicity of any substances including wastes and natural environment, for example, surface and ground waters, soils, and sediments. The purposes of bioassays vary across different application areas; in particular, bioassays can be performed to determine general toxicity, mutagenicity, and carcinogenicity. An examined impact in the test system is assessed by simulating the possible routes of entry of a harmful substance into the body; as a consequence, most often the main test objects are aqueous environments. Hydrobionts such as protozoa, algae, crustaceans, mollusks, fish, *etc.*, act as sensitive biological sensors. Currently, more than 50 bioassays are used for environmental research and government environmental monitoring[10-13].

Toxicological investigations are based on assessing the degree of reduction in the values of test parameters in the experimental group relative to the control when diluting aqueous extracts to neutralize the toxic effect. Meanwhile, the toxicometric parameters LC_{50}, EC_{50}, ECx, and NOEC allow performing an independent comparison of the results of toxicity tests on specific substances or mixtures of complex composition, as well as on contaminated soils, waters, *etc*.

The results of bioassays are used in the certification of different biological products, sorbents of petroleum products, and other toxicants[14]. However, not least important is the applicability of bioassays for the rapid assessment of the toxicity of newly synthesized substances in chemistry and pharmacology. At the preliminary stages of the investigation of new substances, bioassay is the most convenient method since it is based on monitoring the vital processes in biological objects in the examined environment and is considered a method of the integral assessment of the toxicity degree of the tested solution. This is related to the fact that, as a rule, biological assays are quite simple, inexpensive, and very sensitive to the effects of different toxicants. In addition, they provide a reliable response to toxic effects in a relatively short period of time[15]. Bioassays are widely

used for toxicological studies of different substances including nanomaterials[16,17].

To obtain the most objective assessment of the toxic properties of the examined substances, the use of several test objects is obligatory, since different living organisms have dissimilar resistance to adverse factors including toxic pollutants[18-20]. The optimal decision for toxicological studies is the formation of a bioassay system including test organisms, which are most sensitive to the examined toxicant, to identify its possible negative effect.

In this regard, the main purpose of this study was the formation of a bioassay system through the selection of test organisms that are most sensitive to the toxicity of the group of examined substances with reference to literature data and the authors' studies; ascertaining the toxicity degree and calculation of the median lethal (median effective) and harmless concentrations of the synthesized phosphonates relying on the experimental results, as well as adjustment of the pilot bioassay system to continue investigation at the following stage.

2. Materials and Methods

Experiments on ascertaining the general toxicity of (O)-functionalized phosphonates and determining their main toxicological parameters were carried out on samples of five synthesized phosphonates, which were assigned conditional numbers Nos. 1, 2, 3, 4, and 5 (Figure 1). We studied the acute toxicity of not photoirradiated vinylphosphonates.

A series of vinylphosphonates, dialkyl[(Z)-2-chloro-2-phenylethenyl]phosphonates, were synthesized in the reaction of phosphorus pentachloride with phenylacetylene, followed by the substitution of chlorine atoms with alcohol groups according to the described principles[8]. The choice of substitution target objects among alcohols for the production of O-functionalized phosphonates was stipulated by the possibility of examining the aliphatic chain effects and the eventuality limitation of the occurring reaction when saturated alcohols are introduced into the reaction. The reaction proceeded with the selective formation of a stable cis-product, for which the nuclear Overhauser effect was identified in the $^{1}H–^{1}H$ NOESY NMR spectra.

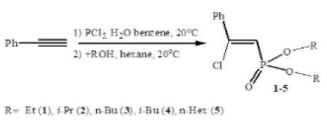


Figure 1. Phosphonate synthesis reaction (samples 1–5).

The synthesized phosphonates are insoluble in water but are highly soluble in an organic substance such as Dichloromethane (DCM). Therefore, the investigation of the possible toxicity of these compounds for aquatic organisms had to be carried out in aqueous solutions of DCM used for the previous dissolution of phosphonates. To account for the solvent matrix effect, it was first necessary to determine the non-toxic concentration of DCM.

At the first stage of the study, with reference to literature data and the experience of the author's own work, a bioassay system was compiled from three test cultures and hydrobionts with increased sensitivity to the toxic effects of different substances. These cultures included the cladocera *Daphnia magna* Str., unicellular green algae *Scenedesmus quadricauda* (Turp.) Breb. and paramecia *Paramecium caudatum* Ehr.

Bioassay with daphnia was performed according to the described method[21]. In the experiment, genetically homogeneous laboratory cultures that regularly underwent sensitivity testing and were kept under special laboratory conditions specified by the standards were used. The assessment technique was based on calculating the mortality of crustaceans under the toxic substance impacts in the examined aqueous environment in comparison to the control (pure aerated cultivation water). The criterion for acute toxicity was the death of 50% or more crustaceans in 48 h relative to the control provided that in the control all crustaceans retained their viability. Synchronized juvenile daphnia 1–2 days old were used in the experiment. Containers with control, examined samples, and test organisms were placed in a rotating cassette of a device UER-03 for exposing crustaceans. The rotation of the cassette provided continuous and uniform aeration of the samples. At the same time, the rotation speed of 6–8 revolutions per minute did not create a stressful situation for daphnia. The experiment was repeated 3 times.

Bioassay with unicellular green algae was carried out according to the method[22]. Cultivation of algae in control and test solutions was performed in a climatostat B-2 at a temperature of 20°C and illumination of 900 lux for 12 h a day. The number of algae in the solution was detected by the solution's optical density, measured at λ=440 nm. The toxicity criterion was a decrease of

20% or more (growth inhibition) or an increase of 30% or more (growth stimulation) in the optical density of algae grown for 45 h (on the 3rd day) in the test sample, compared with growth on a control medium prepared with distilled water. The experiment was repeated 3 times.

Bioassay with ciliates was carried out according to the described method[23]. Here, the test object was the ciliate *P. caudatum* Ehr., which was used in the form of a cell suspension obtained by cultivation under certain conditions with the following washing out of metabolic products and food, and adjusted to a working concentration (1000±500) cells/cm^3. The ascertaining of the toxicity of aqueous environments was based on the chemotactic reaction of ciliates due to their ability to respond to dangerous substances for their vital functions in the aqueous environment by moving directionally along the concentration gradient of these substances to avoid their harmful effects. The toxicity criterion was a reduction of 40% or more in the number of ciliates in the examined sample compared to the control (distilled water). The experiment was repeated 3 times.

For all test objects, the toxicity index T was calculated. In the experiment with daphnia, it represented the proportion of dead individuals; in the experiments with algae and ciliates, it expressed the degree of reduction in the numbers owing to the inhibition of growth or chemotaxis.

The T value (toxicity indicator) was calculated using the formula:

$$T = \left(\frac{\bar{x}_c - \bar{x}_t}{\bar{x}_c} \right) \cdot 100\%, \quad (1)$$

where, $\bar{x}_c$ is the number of surviving daphnia in the control; or the number of ciliates in the control; or the optical density of algae in the control sample (average value of three parallel measurements);

$\bar{x}_t$ is the number of surviving daphnia in the test water solution, or the number of ciliates in the test water solution, or the optical density of the test algae sample (average of three parallel measurements).

The toxicometric parameters LC_{50} and EC_{50}, as well as EC_{20}, were determined by the classical method of probit-analysis[24]. The lethal (effective) and least effective (harmless) dilution of the extracts were previously calculated[21].

The indicators of lethal dilution level LDL_{50} or Effective Dilution Level (EDL_{50}) were calculated using the formula:

$$LDL_{50} = 10^{\frac{(\lg D_h - \lg D_l)\cdot(A_l - 0.5)}{A_l - A_h} + \lg D_l}, \quad (2)$$

where D_h is the highest dilution value, at which the number of dead daphnia or concentration of ciliates and algae in the tested water was less than 50%;

D_l is the lowest dilution value, at which the number of dead daphnia or concentration of ciliates and algae in the tested water solution was more than 50%;

A_l (A_h) is the number of dead daphnia or the proportion of decrease in the concentration of ciliates and algae relative to the control, expressed in fractions of a unit, corresponding to these dilutions.

The Lowest Ineffective Dilution (LID) is a dilution, accompanied by no inhibition or only minor effects, which do not exceed the variability inherent in the experiment. Guided by the principle of maintaining maximum environmental safety and based on many years of experimental experience in biotesting, 20% of the control value (LID_{20}) was chosen as this variability magnitude.

LID_{20} indicators were calculated using the formula:

$$LID_{20} = 10^{\frac{(\lg D_h - \lg D_l)\cdot(A_l - 0.2)}{A_l - A_h} + \lg D_l}, \quad (3)$$

where D_h is the highest dilution value, at which the number of dead daphnia or concentration of ciliates and algae in the examined water solution was less than 20%;

D_l is the lowest dilution value, at which the number of dead daphnia or concentration of ciliates and algae in the examined water solution was more than 20%;

A_l (A_h) is the number of dead daphnia or the proportion of a decrease in the concentration of ciliates and algae relative to the control, expressed in fractions of a unit, corresponding to these dilutions.

The values of LC_{50}, EC_{50}, and NOEC were calculated as the quotient of dividing the initial concentration of an aqueous solution of phosphonate in DCM by the lethal (effective) or harmless dilution level LDL_{50}, EDL_{50}, LID_{20}.

Mathematical processing of the obtained data was carried out using the method of variation statistics in the Excel software, calculating the arithmetic mean (M) and the relative error of the mean S_x. The significance of the differences was assessed using Student's t-test with

Table 1. Toxicity of DCM solutions for living organisms

DCM, %	Daphnia *D. magna* Str.		Ciliates *P. caudatum* Ehr.		Green algae *S. quadricauda* (Turp.) Breb.	
	T, %	Toxicity	*T*, %	Toxicity	*T*, %	Toxicity
0 (control)	0	non-toxic	0	non-toxic	0	non-toxic
1.0	100	toxic	87±4	toxic	100	toxic
0.1	3±0.3	non-toxic	29±3	non-toxic	14±4	non-toxic
0.01	0	non-toxic	10±2	non-toxic	-5±3*	non-toxic

* Note. The minus sign in front of the T value means that there was an increase in algae concentration in the test sample compared to the control.

Table 2. Toxicity of solutions of phosphonates in DCM for living organisms

Phosphonates, Sample No.	Daphnia *D. magna* Str.		Ciliates *P. caudatum* Ehr.		Green algae *S. quadricauda* (Turp.) Breb.	
	Dilution	*T*, %	Dilution	*T*, %	Dilution	*T*, %
Control	w/d*	0	w/d	0	w/d	0
1	w/d	100	w/d	86±1	w/d	93±3
	10	100	10	11±4	3	61±2
	100	7±3			9	41±2
					27	37±6
					81	18±7
2	w/d	100	w/d	47±5	w/d	100
	10	90±6	10	39±4	3	100
	100	10±6	100	7±3	9	66±4
					27	55±4
					81	9±5
3	w/d	100	w/d	58±1	w/d	100
	10	100	10	52±1	3	100
	100	0	100	3±1	9	86±5
					27	48±4
					81	38±4
					243	7±2
4	w/d	100	w/d	51±2	w/d	100
	10	57±3	10	26±6	3	71±9
	100	0	100	10±4	9	43±5
					27	29±6
					81	19±5
5	w/d	100	w/d	37±1	w/d	100
	10	100	10	4±1	3	100
	100	100			9	96±6
	1000	23±3			27	52±2
	10000	0			81	24±6
					243	5±3

* Note: Hereinafter w/d has a meaning without dilution.

the significance level of P <0.05, calculated for the two compared values.

3. Results

The first stage of the experiment involved determining the safe concentration of DCM as a solvent for the synthesized phosphonates. The results of DCM testing with the selected bioassay system, including daphnia, ciliates, and algae, showed that an aqueous solution of DCM at a concentration of 1.0% brought about the complete death of daphnia, while the number of ciliates lowered by 87% and the algae concentration decreased by 52% (Table 1). This DCM concentration can be characterized as acutely toxic for the selected test objects. Meantime, aqueous solutions of DCM at concentrations of 0.1 and 0.01% did not have a significant inhibitory effect on these living organisms and, consequently, were non-toxic. Thus, a DCM concentration of 0.1% (or lower) is safe (non-toxic) for daphnia, ciliates, and algae and can be recommended for dissolving synthesized phosphonates. Herein, a solution of phosphonates in DCM at this concentration does not have the matrix effect and the possible toxicity is determined only by the phosphonates.

To conduct toxicological examinations, solutions of phosphonates were prepared in DCM at 0.1% concentration. The initial concentration of phosphonates in solutions was 10^{-3} M/L. If the initial solution showed toxicity, then the dilution was carried out according to the methodology as follows: 10-fold for daphnia and ciliates or 3-fold for algae.

The results of determining the toxicity of aqueous solutions of phosphonates in DCM for daphnia, ciliates, and algae were calculated using Formula (1) and summarized in Table 2.

The initial solutions of all examined phosphonate samples were revealed to be acutely toxic to daphnia. This toxicity was neutralized for all samples, except for No. 5, with a 100-fold dilution of the solutions, while for sample No. 5, the neutralization was reached with a 1000-fold dilution.

Bioassay with ciliates showed moderate toxicity of the initial solutions of synthesized phosphonates, except for sample No. 1, which demonstrated strong toxicity, and sample No. 5, which revealed weak toxic properties. In the meantime, the differences between the latter and the control (pure cultivation water) were significant ($p < 0.05$); however, according to the scale developed for this method, the degree of its toxicity can be assessed as acceptable.

To neutralize the identified toxicity, a 10-fold dilution was required for samples No. 1, 4 and 100-fold for samples No. 2 and 3.

Assessing the toxicity of phosphonate solutions to algae revealed that all initial solutions of phosphonates in DCM were toxic to them. However, the effects of the examined reagents on algae differed. In particular, sample No. 1 inhibited algae growth; toxicity was gradually neutralized to safe levels at 81-fold dilution. Meanwhile, solutions of phosphonates Nos. 2, 3, 4 and 5 not only inhibited algae growth but also caused cell lysis and destruction of chlorophyll, which led to complete suspension discolouration.

To neutralize the toxicity of phosphonate solutions to algae, the following dilutions were required: for samples No. 1, 2, and 4 by 81 times, and for samples No. 3 and 5 by 243 times.

Based on the results of integrated bio-testing, it can be affirmed that phosphonate sample No. 5 was the most toxic to daphnia and algae. The same sample No. 5 showed minimal toxicity among the examined compounds for paramecia. Thus, different sensitivity to sample No. 5 was revealed in the test organisms used in the experiment.

Relying on the performed bioassays, the toxicity indicators of the examined phosphonate solutions were calculated. This involved finding a lethal concentration of LC_{50} for daphnia, which corresponded to the death of 50% of individuals and, similar to it, an average effective concentration of EC_{50} for ciliates and algae, which corresponded to a decrease in the cell number by 50%.

The results of calculating toxicometric parameters using Formula (2) are given in Table 3.

Sample No. 5 turned out to be the most toxic as its LC_{50} was 0.2×10^{-5} M/L. Samples No. 1, 2, and 3 were comparable and characterized by lethal concentration an order of magnitude lower; their LC_{50} values fluctuated within the range of (2.9-–3.2) $\times10^{-5}$ M/L. Sample No. 4 turned out to be the least toxic; its LC_{50} was equal to 6.8×10^{-5} M/L. The resulting LC_{50} values were determined from the experiments with daphnia as the most sensitive test organisms.

Finally, No Observed Effect Concentrations (NOEC) of the examined phosphonate solutions were determined. As indicated earlier, these concentrations were determined by the dilution factor of the solutions required to neutralize toxicity within less than 20% value

Table 3. Toxicity indicators of phosphonates in DCM solutions for living organisms

Phosphonate solutions, sample no.	Daphnia *D. magna* Str.		Ciliates *P. caudatum* Ehr.		Green algae *S. quadricauda* (Turp.) Breb.	
	LDL_{50} Lethal dilution level of the initial solution	LC_{50}, M/L Lethal concentration (death of 50% of daphnia in 48 h)	EDL_{50} Effective dilution level of the initial solution	EC_{50}, M/L Effective concentration (50% reduction in the number of ciliates in 30 min)	EDL_{50} Effective dilution level of the initial solution	EC_{50}, M/L Effective concentration (50% reduction in the number of algae cells in 72 h)
1	34.7	2.9×10^{-5}	2.8	36×10^{-5}	5.5	18×10^{-5}
2	31.6	3.2×10^{-5}	w/d	100×10^{-5}	30.2	3.3×10^{-5}
3	31.6	3.2×10^{-5}	11	9.1×10^{-5}	26.3	3.8×10^{-5}
4	14.8	6.8×10^{-5}	1.2	83×10^{-5}	6.8	14.7×10^{-5}
5	426.6	0.2×10^{-5}	w/d	100×10^{-5}	29.5	3.4×10^{-5}

Table 4. No observed effect concentrations of phosphonate solutions for different test organisms

Phosphonate solutions, sample no.	Daphnia *D. magna* Str.		Ciliates *P. caudatum* Ehr.		Algae *S. quadricauda* (TURP.) BREB.	
	LID_{20} Lowest ineffective dilution	NOEC, M/L No observed effect concentration (death of less than 20% of daphnia in 48 h)	LID_{20} Lowest ineffective dilution	NOEC, M/L No observed effect concentration (less than 20% reduction in the number of ciliates in 30 min)	LID_{20} Lowest ineffective dilution	NOEC, M/L No observed effect concentration (less than 20% reduction in algae cell numbers in 72 h)
1	72.4	1.4×10^{-5}	7.2	13.9×10^{-5}	72.4	1.4×10^{-5}
2	75.9	1.3×10^{-5}	38.9	2.6×10^{-5}	60.3	1.7×10^{-5}
3	63.1	1.6×10^{-5}	44.7	2.2×10^{-5}	154.9	0.6×10^{-5}
4	45.7	2.2×10^{-5}	24	4.2×10^{-5}	72.4	1.4×10^{-5}
5	1122	0.09×10^{-5}	3	33.3×10^{-5}	102.3	1.0×10^{-5}

of the control for all test organisms, regardless of the scales adopted in the methods for determining the toxicity threshold. This is related to the fact that all statistically insignificant differences in all cases of biotesting were less than 20% of the control. The results obtained with Formula (3) are shown in Table 4.

For daphnia and green algae, no observed effect concentrations were very close and in most cases amounted to 10^{-5} M/L. For ciliates, NOEC were higher by 2 times for samples No. 2, 3, and 4; 10 times more for sample No. 1; and 374 times more for sample No. 5, thereby confirming the lower sensitivity of ciliates to synthesized phosphonates. Summarizing the results obtained allows deriving the safe concentration of the examined substances common to the entire experiment. It turned out to be the smallest, 0.9×10^{-6} M/L, for sample No. 5, while for the remaining samples of phosphonates, it ranged from 1.3×10^{-5} to 2.2×10^{-5} M/L.

4. Discussion

Generalization of the results of bioassays on five examined samples of synthesized phosphonates revealed that all three test organisms used in experiments (daphnia, ciliates, and algae) showed high and comparable sensitivity to these substances. The most sensitive were daphnia, and the least sensitive were ciliates. However, the available sample size is not enough for a conclusion; therefore, the authors consider it advantageous to use all three test organisms in biotesting in further investigations.

It is known that the comparative sensitivity of aqueous organisms to the effects of various toxicants can vary quite

significantly. Nevertheless, all researchers recognize the importance of selecting the most sensitive test organisms. Testing of aqueous solutions by using sensitive aquatic organisms helps to explicitly assess their toxicity[25,26]. Bioassays with organisms from different systematic groups, including crustaceans (*D. magna*), ciliates (*P. caudatum*), and green algae (*Chlorella or Scenedesmus*), are aimed at increasing the accuracy of assessing the toxicity of solutions.

There are many examples in the literature indicating different sensitivities of test organisms to the impact of the same toxicant. Thus, an investigation of the toxicity of certain types of surfactants, in particular sodium dodecyl sulfate, revealed that daphnia was the most sensitive test culture[19]. Japanese scientists have identified the high sensitivity of *D. magna* to the effects of heavy metals and proposed to consider a bioassay with daphnia as a key test in determining the target values of water quality[27]. Daphnia is sensitive to unpurified cyanobacterial extracts at concentrations ranging from 1 to 50 μg/L[28].

High sensitivity to the toxic effects of herbicides and acaricides at sublethal concentrations was revealed in the ciliates *P. tetraurelia*[18,20]. The work on testing the membrane-stabilizing activity of herbal medicines dihydroquercetin and silymarin, in which the ciliates *P. caudatum* were used as a test culture, also deserves great attention[29].

In addition, freshwater microalgae of the genus *Chlorella* (*C. vulgaris, C. pyrenoidosa, C. fusca*) and the genus *Scenedesmus* is very informative when assessing the toxicological parameters of various substances according to numerous literature data. They exhibited sensitivity in the toxicological assessment of heavy metals, surfactants, drugs, rare earth elements, and heterocyclic compounds[30-33].

Regarding the different sensitivities of test organisms to toxicants, the lowest concentration of a substance that causes a toxic effect on the entire bioassay system is considered an indicator of toxicity. In this way, the principle of the greatest environmental safety is implemented in practice.

The calculation of toxicometric parameters (lethal and effective concentrations) was carried out according to the probit analysis model[24] with a history of more than half a century. Concurrently, there is evidence in the literature that a comparative analysis for calculations of LD_{50} toxicometric indicators using the Belenky method and modern versions of probit regression did not reveal significant differences in the final results[34]. Thus, the modern development of this methodology has not increased the accuracy of assessing toxicometric parameters, although it has created prerequisites for the adaptability of powerful and effective methods for controlling the effects of random factors. Therefore, the authors of this study used M.L. Belenky's calculation method as a classic one, proven by decades of toxicological studies and making it possible to determine the necessary parameters with satisfactory accuracy. Sample No. 5 turned out to be the most toxic (LC_{50} 0.2×10^{-5} M/L) while sample No. 4 turned out to be the least toxic (LC_{50} 6.8×10^{-5} M/L).

No observed effect concentrations in most cases amounted to 10^{-5} M/L. NOEC were very close for daphnia and green algae while for ciliates NOEC were significantly higher, thereby confirming the lower sensitivity of ciliates to synthesized phosphonates. The safe concentrations of the examined phosphonates were 0.9×10^{-6} M/L for sample No. 5, and $(1.3\text{-}2.2)\times10^{-5}$ M/L for other samples.

Thus, this study found the high biological activity of newly synthesized vinylphosphonates against different groups of organisms (daphnia, ciliates and unicellular green algae). The non-toxic concentration of the solvent DCM for the test cultures was 0.1%, which allowed for the neutralization of the matrix effect of the solvent. This concentration was used to prepare solutions of synthesized phosphates. Daphnia was the most sensitive to the impact of synthesized phosphonates through biotesting and toxicity ascertaining. Unicellular green algae were less sensitive to a certain extent, and the least sensitive ones turned out to be ciliates.

The performed toxicological studies made it possible to calculate the average lethal concentrations LC_{50} of synthesized phosphonates. The most toxic was sample No. 5 with LC_{50} equal to 0.2×10^{-5} M/L. Samples No. 1, 2, and 3 were characterized by almost an order of magnitude lower lethal concentration, in which the LC_{50} values fluctuated within the range of $(2.9\text{–}3.2)\times10^{-5}$ M/L. Sample No. 4 turned out to be the least toxic with LC_{50} of 6.8×10^{-5} M/L. All LC_{50} values were determined from the results of experiments with daphnia as the most sensitive test organism. Calculation of the No Observed Effect Concentrations (NOEC) revealed that the lowest value corresponded to sample No. 5 and was 0.9×10^{-6} M/L; for the remaining phosphonate samples, the values ranged from 1.3×10^{-5} to 2.2×10^{-5} M/L. The previously proven possibility of controlling the biological activity of synthesized phosphonates under laser irradiation opens

prospects for applying them in photopharmacology and producing drugs with the capability of remote and precise control over the area and duration of their therapeutic effect.

5. Data Availability

The data analyzed during the current study are available from the corresponding author on request.

6. Funding

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7. Author Contributions

L.B., N.M., A.M., A.E., and A.P. were involved in conception and design. L.B., N.M., and Y.P. collected and analyzed the data. L.B. was involved in writing—original draft preparation. L.B., A.M., Y.P., and A.G. contributed to writing—review and editing. All authors read and approved the final manuscript.

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