World Applied Sciences Journal 24 (12): 1616-1620, 2013
ISSN 1818-4952
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DOI: 10.5829/idosi.wasj.2013.24.12.13298

Development of Methodology for Detection of Ecological Maximum Permissible Concentration of Xenobiotics (On the Example of Pyrethroid Insecticides)

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Submitted: Aug 22, 2013; **Accepted:** Sep 18, 2013; **Published:** Sep 22, 2013

Abstract: The current aquatic legislation in Russia is based on two basic strategies of defense, conservation and improvement of quality of nature reservoirs. Leading direction in the area of experimental and practical ecology is development of methodology for detection of ecological MPC of xenobiotics considering physiological properties of aquatic population since this methodology is not created all over the world yet. Considering the revealed effect of the action of low and ultralow doses of pyrethroid insecticides to aquatic arthropodas, we corrected previous approach for assessment of ecologic MAC for hazardous substances. On the present stage of investigations, the following basic universal principles for detection of aquatic ecologic MAC for xenobiotics were stated.

Key words: Ecological maximum permissible concentration · Xenobiotics · Pyrethroid · Daphnia · Pesticide

INTRODUCTION

The current aquatic legislation in Russia is based on two basic strategies of defense, conservation and improvement of quality of nature reservoirs. Firstly, they are standards of quality of aquatic environment. The regulation of natural surface waters quality is performing from viewpoint of their suitability only for some types of water use and does not oriented to provide optimal structural-functional organization of aquatic ecosystems as a main factor for formation of natural waters quality. Secondly, nature reservoirs are standards for waste discharge considering abidance of maximum permissible concentrations (MPC) for polluting compounds with the aim of providing a quality for water environment in the areas of waste discharge disposal. But, at present, the conception of maximum permissible discharge (MPD) is unsatisfactory since it is based on ingredient control [1]. Integral evaluation for summarized action of unfavorable factors of the environment (including pollutants) may be achieved only using biological methods.

Leading direction in the area of experimental and practical ecology is development of methodology for detection of ecological MPC of xenobiotics considering physiological properties of aquatic population since this methodology is not created all over the world yet.

The perspective of such a development is in the ability for solving of a range of problems of ecologic and economic characters:

- To reveal before-threshold concentrations of pollutants (especially of newly synthesized) without toxic effects to trophic chains of aquatic ecosystems;
- To reduce chemical pressure to environment and to have a significant economic effect owing to reduction pf pesticide utilization without loss of their toxic effects.

The present situation needs the further toxicological research with the attraction of representatives of aquatic biocenosis that are most sensitive to the action of pollutants considering the combined effect of chemical compounds and various factors (temperature, pH, etc).

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Beginning from the measurement of acute toxicity and residual amounts of xenobiotics, it is necessary to shift to more complex integral investigation of ecological aspects of chronic toxicity. As a result of these investigations, it will be possible to evaluate a hazard from pollutants toward the environment before measurement of acute toxicity and to predict harmful effects.

With the aim to escalate or generalize assessment of toxicity, some attempts were made to study the problem “from the side of chemicals” to predict biological efficiency using physico-chemical features. At that, it is necessary to consider that the most relevant value of biological activity should reflect properties of interaction between organisms and chemicals correlating with toxicity of these substances. Survival of organisms and their fertility are such biological values.

Widely used criterion for detection of hazard for pollutants is a character and degree of water use restriction or toxicity to fish. Frequently, toxicity of xenobiotics (including insecticides) to other representatives of aquatic ecosystem such as bacteria, phytoplankton, arthropodas, etc.

It is known that synthesis of pesticides, referring to a class very dangerous toxicants, is based on the obtaining of compounds with selective action toward insects (insecticides), plants (herbicides), fungi (fungicides), cotton-plants (defoliants), etc. Use of defoliants in Russian Federation is limited, fungicides are used during storage of agricultural crops; therefore, namely insecticides and herbicides are entered to waters with surface flow.

A careful analysis of “structure of pesticides-physiological organization of aquatic organisms” based on literature data allowed us to state the following. The modern herbicides and insecticides - two classes of agricultural chemicals are principally different in mechanisms of their action:

- Herbicides inhibit photosynthesis (photosystem II) that may mediate their algicide activity
- In insecticides selectivity of action is linked with principal differences in the structure of the nervous system in vertebrates and invertebrates. There are two types of neuro-muscular transition in various animals (Walker, Holden-Due, 1989): type I (vertebrates) - animals use acetylcholine as mediator, type II (invertebrates) - animals use glutamate as activating mediator while γ-aminobutyric acid as braking mediator and type III (annelids) - animals use acetylcholine as activating mediator while γ-aminobutyric acid as braking mediator.

Phosphor- organic insecticides inhibit acetylcholinesterase both in vertebrates (guppi fish) and invertebrates (daphnia).

Chlor-organic insecticides destroy neuro-muscular transition due to blockade of receptors for gamma-aminobutyric acid. The presence to blood-brain barrier restricts the action of these compounds in the CNS of vertebrates. This includes a selectivity of action.

Insecticides of a new generation (pyrethroids) act on sodium canals of membranes from nervous cells. Toxic action of these compouns is also related with their influence on the activity of neurosecretory cells via modulating trnsport of calcium.

A direct synthesis of insecticides and herbicides by companies-producers aimed at creation of compounds with increased the basic effect and decreased side effects (toxic action toward humans and other vertebrates). Therefore specifity of action is increasing in every next generation of pesticides. Moreover, all modern pesticides are hydrophobic chemicals entering through biological membranes. Increasing of hydrophobicity owing to chemical adapters is one of ways to reduce their application in agriculture.

The different mechanisms of pesticide action toward living organisms mediated their complex sensitivity of organisms to the compounds. For examples, insecticides in low concentrations do not affect micrflora and protozoa since these organisms have not a nervous system - a target for insecticides. Analogously, low concentrations of herbicides will not toxic to fish, crustaceans since it have not a phytosyntethic apparatus (a target for herbicides). This clear fact - link between structure and function - is not taken into account in many cases. Thus, to detect ecological maximum allowable concentrations (MAC), it is important to use a proper biological object.

Aquatic arthropodas (but not fish and other vertebrates: it have 3-5-order low sensitivity) are targets for insecticides [2]; herbicides target organisms that have a photosynthetic systems, phosphor-organic compounds (inhibiting acetylcholinesterase) act on aquatic vertebrates (guppi fish) and arthropodas (*Daphnia magna*) in the same manner. So, the main requirement to a set of test organisms for bioindication is a principal difference onphysiological organization mediating a sensitivity to pollutants with various chemical structure.

The aim of this work was to develop a methodology for detection of ecological maximum permissible concentration of xenobiotics on the example of pyrethroid insecticides.

MATERIALS AND METHODS

Organisms of *Daphnia magna* straus were used as experimental organisms. The choice was mediated by a short life cycle that allow to simulate the action of toxicants at the population level and on all stagers of development. Also, daphnia are targets for insecticides and the most sensitive organisms within a trophic chain of aquatic ecosystem. *D. magna* animals were provided by Department of Invertebrate Zoology and Functional Histology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia. Homogenous culture of daphnia was obtained from one female concerning methodological manual [3]. In experiments, third generation of daphnia was used.

Type II pyrethroids were used in this study: deltamethrin ((*S*)-α-cyano-3 - phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopro panecarboxylate); fenvalerate ((*RS*)-α-cyano -3-phenoxybenzyl (*RS*)-2 - (4-chlorophenyl) -3-methylbutyrate); and cypermethrin ((*RS*)-α-cyano-3-phenoxybenzyl (1*RS*, 3*RS*; 1*RS*,3*SR*)-3-(2,2-dichlorovinyl) - 2,2-dimethylcyclopropanecarboxylate).

Third generation of pyrethroids are analog of natural pyrethrins with ester chemical nature. These compounds photochemically are more stable [4].

The above-mentioned chemicals were dissolved in 1 mL of 96% ethanol and then diluted to the necessary doses. Special experiments showed that spirit did not affect physiological conditions of daphnia. 80-100 animals were analyzed. On the basis of [5-8] we developed methodology for working with low an ultralow concentrations of the insecticides.

RESULTS

While studying concentration dependences of hazardous action of pyrethroids to daphnia, we detected a significant toxic effect in the area of low doses. This was a reason for detailed analysis of Russian and international literature devoted to action of low and ultralow doses [9-23] and subsequent development of methodic approaches for investigation of actions of low and ultralow doses of pyrethroid insecticides to aquatic organisms.

In conditions of acute experiments, the following acting doses were detected:

- At optimal temperatures (23°±0,5°C) - 6.0×10^{-21}M on fenvalerate); 6.0×10^{-22}M on cypermethrin); 5.0×10^{-31}M on deltamethrin but in more late period (by 5-7 days) (Figs. 1, 2, 3).

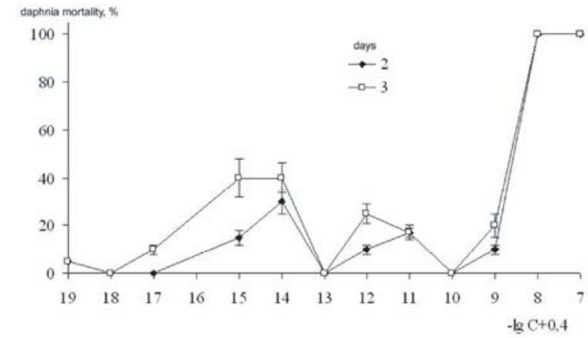

Fig. 1: Concentration dependence of acute toxicity of fenvalerate in compare with a control at 23°C.

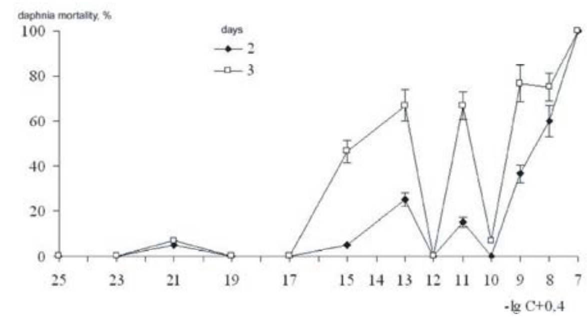

Fig. 2: Concentration dependence of acute toxicity of cypermethrin in compare with a control at 23°C.

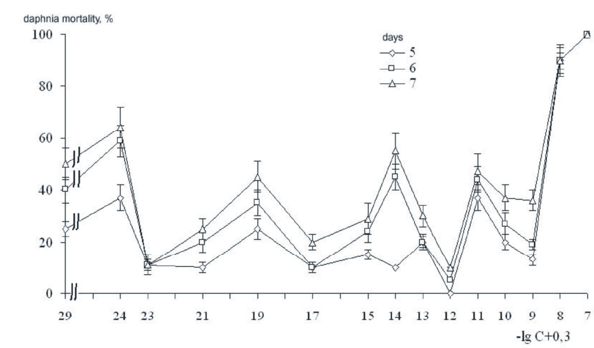

Fig. 3: Concentration dependence of acute toxicity of deltamethrin in compare with a control at 23°C (data is shown while toxic effect was maximal).

- At increased temperature (28°±0,5°C) characteristic to reservoirs- refrigerants $6,0\times10^{-23}$M on fenvalerate), $5,0\times10^{-31}$M on deltamethrin) (Figs. 4, 5).

At combined action of increased temperature (28°C), deltamethrin 2.0×10^{-13}M, oil 10 mg/L and copper 5 microg/L or deltamethrin (2.0×10^{-12}M), fenvalerate (2.5×10^{-17}M) and cypermethrin ($2,4\times10^{-17}$M) minimal acting concentrations in acute experiment were 10-fold lower than individual ones [6].

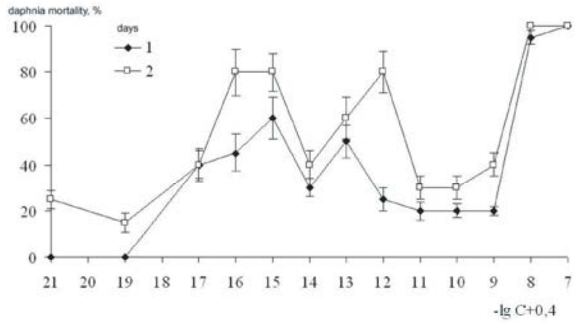

Fig. 4: Concentration dependence of acute toxicity of fenvalerate in compare with a control at 28°C.

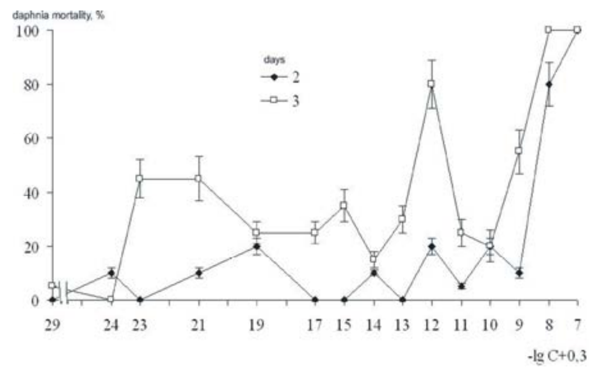

Fig. 5: Concentration dependence of acute toxicity of deltamethrin in compare with a control at 28°C.

In chronic experiment (1st and 2 nd generation of daphnia) we revealed toxicity of the insecticides 2-order lower that in acute experiment [6, 7, 22].

On the basis of the results, it is possible to conclude the following. Ecologic MAC of the insecticides under study (minimal acting doses considering temperature factor, combined and chronic action) are as follow: 5.0×10^{-34}M on deltamethrin); 6.0×10^{-26}M on fenvalerate) and $6.0\times10^{-}$ M ^{25} on cypermethrin) [6- 8].

At the condition of short-term contact between hydrobionts and xenobiotocs, it is necessary to consider their adaptation to the action of toxicant. For example, while culturing daphnia in decis solution (1×10^{-9}M) for 4 days (23±0.5°C) and then in pure water for 21 days, corrective for the value of daphnia mortality of the 1st generation 9in comparison with initial value) was 10%, on the parameter of fertility (number of offsprings per 1 female) of the 2nd generation (in comparison with the 1st generation) was 40% [6].

In the literature, data on the action of insecticides in ultralow doses lower than minimal constants of dissociation of ligand-receptor complexes toward aquatic arthropodas are absent. This is possible connected with the fact the effects of action of these doses toward aquatic arthropodas were not investigated due to the probable lacking of their biological significance as well as due to the possible effect mask characteristic to the action of ultralow doses [11].

Well-accepted methods of chemical analysis for nature waters cannot reveal these doses, so their hazardous effects are not considered. Trace impurities, consistently persisting in water, result in more expressed alterations in vitality of daphnia in the next generations.

Considering the revealed effect of the action of low and ultralow doses of pyrethroid insecticides to aquatic arthropodas, we corrected previous approach for assessment of ecologic MAC for hazardous substances. On the present stage of investigations, the following basic universal principles for detection of aquatic ecologic MAC for xenobiotics were stated (it is oriented o the maintenance of optimal structural-functional organization of aquatic ecosystems, conservation of the most sensitive junctions of trophic chain):

- Identification of the components of aquatic ecosystems most sensitive to the action of xenobiotics with the use of analysis "structure of chemical reagent - physiological organization of aquatic organisms, their function".
- Development of test-system allowing to detect aquatic ecologic MAC for xenobiotics with various mechanisms of toxic action to hydrobionts with different toxicoresistance.
- Investigation of hazardous action of xenobiotics including in the area of low and ultralow doses considering their interaction with other factors regulating physiological condition of hydrobionts, primarily with temperature, combined action with another the most toxic compounds both in acute and chronic experiments.
- Quantitative assessment of the degree of adaptation of aquatic organisms to unfavorable conditions of the environment considering a time of contact with the corresponding correction of values for ecologic MAC.

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