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EFFECT OF MOSQUITO KILLER INSECTICIDES ON FRESHWATER MUSSELS

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Abstract—1. The effect of four insecticides (Fyfanon, K-Othrin, Unitox 7 and Unitox 20) was investigated on the freshwater mussels *Anodonta cygnea* L., *Anodonta anatina* L. and *Unio pictorum* L. The studies were performed in hard water (total salinity of 310 mg/l). The LC_{50} values for 24, 48, 72, 96 hr and 7 days were determined with a static test at 22°C.

2. All the three mussel species proved to be extremely tolerant against the insecticides tested.

3. The effect of sublethal concentrations of these insecticides on the periodic activity of the mussels was also analyzed, comparing the changes of the active and resting periods.

4. In a concentration of 0.1 μl/l, all the four insecticides affected the periodic activity of *U. pictorum*, whereas in *A. anatina* three of them (Fyfanon, K-Othrin and Unitox 7) and in *A. cygnea* two of them (K-Othrin and Unitox 7) evoked alterations. This concentration is lower by three to five orders than those of the LC_{50} values of 96 hr.

INTRODUCTION

In the course of their application, the insecticides used against mosquitoes inevitably get into the lakes and rivers, where their toxicity possibly causes damages to different species in aquatic ecosystems. An example is the freshwater mussel, especially because of its water filtration activity and its capability to concentrate toxic substances (Lowe *et al.*, 1971; Roberts, 1976; Maki and Johnson, 1976). Only a little is known about the toxicity of pollutants on aquatic invertebrates, concerning both acute and subacute tests (Sprague, 1976; Johnson and Finley, 1980). A special difficulty in the unequivocal evaluation of the toxicity is that the mussels are very sensitive against some pollutant substances, while being extremely tolerant against others (Bedford *et al.*, 1968; Scott and Major, 1972; Epifanio and Srna, 1975; Okazaki, 1976).

The LC_{50} value is generally accepted to express the degree of toxicity; the concentration resulting in the death of 50% of the experimental animals. Different physiological process can be used experimentally for studying the sublethal effects on mussels, like the analysis of the following: (1) changes in growth (Butler *et al.*, 1960; Gilfillan, 1975; Lowe *et al.*, 1971), (2) the gonadal activity (Roberts, 1972), (3) the production of the byssal-thread (Roberts, 1973; Martin *et al.*, 1975) and (4) the degree of the filtration and respiration (Thurnberg *et al.*, 1975; Gilfillan *et al.*, 1976). A further indicator of the damaging effects is the periodic activity, changing the length of the active and resting periods upon the application of toxic agents (Salánki, 1966; Salánki and Varanka, 1976, 1978).

The effect of heavy metals and certain insecticides on the periodic activity of the freshwater mussel *Anodonta cygnea* has already been analyzed in our earlier studies (Salánki and Varanka, 1976, 1978). It could be stated that the length of the active periods decreases even at a relatively low concentration of the toxic substances. Recently, data have been presented concerning the effect of four mosquito killer insecticides (Fyfanon, K-Othrin, Unitox 7 and Unitox 20) on the mortality and tryptamine-induced activity of the larvae of two closely related freshwater mussel species, *A. cygnea* and *A. anatina* (Varanka, 1986).

The aim of our present studies was partly to determine the LC_{50} values of the above mentioned four insecticides in the adults of three freshwater mussel species. Furthermore, the effect of the sublethal concentrations of these insecticides on the periodic activity has also been studied, with particular attention to their tolerance and the species dependence against these insecticides. Our data may provide certain help for practical employers as well, drawing their attention to the amounts applicable without harmful side-effects and to the importance of keeping the technological disciplines.

MATERIAL AND METHODS

Adult mussels (*Anodonta cygnea* L., *Anodonta anatina* L. and *Unio pictorum* L.) collected from Lake Balaton were used for our studies. The animals were kept in flowing and aerated lake water before use (at least for 2 weeks).

When determining the LC_{50} values, the recommendations of Sprague (1969, 1970, 1971) and the Committee on Methods for Toxicity Tests with Aquatic Organisms were considered. The values in question were determined by a static method in glass aquaria at 22 ± 1°C. Following the preliminary experiment, all the determinations were repeated three times. Groups of experimental animals, each consisting of 10 individuals, were selected at random and placed into aerated aquaria. After 24 hr of adaptation, the different concentrations of insecticides were added to the experimental aquaria. During the adaptation and the experiment the animals were not fed. The size and weight ranges of the mussels are shown in Table 1.

The mortality of the mussels was controlled 24, 48, 72, 96 hr and 7 days, respectively, after the start of the tests.

Table 1. Data on size and weight of the experimental mussels

Species	Size (mm)	Weight of soft part (g)
Anodonta cygnea	92 ± 20	22.8 ± 8.5
Anodonta anatina	75 ± 20	18.0 ± 10.2
Unio pictorum	70 ± 10	10.1 ± 4.7

Dead mussels were eliminated daily. As in the preliminary tests, four different concentrations of each insecticide were tested; a further group was used as control. In the latter, we failed to observe spontaneous mortality. The criteria of death were the opening of the shells, the entire lack of the closing reflex as well as that of the mantle edge reflex elicitable with mechanic stimuli (Epifanio and Srna, 1975). The entire cessation of the mantle edge reflex was considered to be of decisive importance. According to our experience, this survived for a longer time in the case of poisoning as compared to the shell-closing reflex, which is realized through the central nervous system.

For checking the physiological effects of the insecticides, the changes ensuing in the adductor muscle activity served as indicator. The studies were applied as static tests. The mussels were fixed with one of their shells in plastic aquaria containing 3 l Balaton-water, whereas the movement of the other shell was continuously recorded by a mussel-actograph (Salánki and Balla, 1964). A 48-hr adaptation was followed by a control period of 7 days. Thereafter, the appropriate concentration of the insecticide to be studied was set in, and the activity of the mussels was recorded for 5 days. All throughout the experiments the aquaria were aerated, partly to ensure that the oxygen concentration required was present and partly to mix the solutions. Three concentration values of the insecticides (0.1, 1.0 and 10 μl/l) were tested. In case of each insecticide and every concentration, measurements were performed on 7–10 individuals. During the course of the determination of the LC_{50} values the temperature of the aquaria was 22 ± 1°C.

The following insecticides were used (also giving active ingredient contents): Fyfanon ULV (95% Malathion)—Cheminova AG, Lemvig, Denmark. K-Othrin ULV (0.12% Dekametrin RU 11679)—made from a Roussel–Uclaf active ingredient, Nitrokémia, Balatonfüzfő, Hungary. Unitox 7 and Unitox 20 (7 and 20% Dichlorvos, DDVP, respectively)—Universal I.Sz., Szeged, Hungary.

The main physical and chemical characteristics of the Balaton-water used were as follows: total hardness (expressed in $CaCO_3$ content): 259.5 mg/l; total salinity 310 mg/ml; specific conductivity: 480 μS; pH: 8.40.

Each solution was prepared in Balaton-water, the insecticides were used in their formula-form. All the concentrations given were also pertaining to the formula. The substances were diluted by intensive shaking for 10 min or in case of higher concentrations, they were emulgeated.

The experimental LC_{50} values of 24, 48, 72, 96 hr and 7 days, respectively, as well as their 95% confidence intervals were determined by probit analysis (Finney, 1971).

To evaluate the effect of the sublethal concentrations, the length of the active and resting periods of the control and experimental periods were recorded. The average of these, obtained during the control time, was taken as unit in each mussel and the changes which ensued due to the effect of the different insecticides were expressed in percent values of the controls. The averages of the parallel data were then calculated and these values are shown in Table 2.

RESULTS

Beside the most frequently used LC_{50} value of 96 hr, the values of 24, 48, 72 hr and 7 days exposures are shown in Table 2. The time dependence of these values may give information on the accumulating character and the temporal development of the effect of the insecticides applied. The most significant differences between the LC_{50} values of shorter and longer times were found in the case of Fyfanon. In *Anodonta cygnea*, the LC_{50} value of the longer time was found to be 110-fold lower than that of the shorter one; whereas in *A. anatina* it was 70-fold

Table 2. LC_{50} values and 95% confidence intervals (μl/l) obtained on mussels at 22 ± 1°C

Insecticide	24 hr	48 hr	72 hr	96 hr	7 days
			Anodonta cygnea		
Fyfanon	~25,000	14,500	6350	975	225
		9570–20100	5300–7480	820–1150	190–267
K-Othrin	nd	nd	~20,500	10,000	6300
				7800–12,500	5040–7680
Unitox 7	2153	658	408	331	288
	1720–2680	538–788	348–472	284–381	246–336
Unitox 20	2533	1222	523	385	217
	2200–2930	1000–1450	433–617	330–443	185–252
			Anodonta anatina		
Fyfanon	nd	~35,000	14,250	5000	500
			9740–19,300	4250–5880	428–580
K-Othrin	nd	nd	nd	~19,500	8600
					6850–10,500
Unitox 7	4500	660	426	336	286
	3830–5200	528–797	362–498	290–388	247–338
Unitox 20	3110	2203	567	439	269
	2500–3850	1800–2670	470–673	360–525	230–313
			Unio pictorum		
Fyfanon	nd	nd	nd	nd	~31,250
K-Othrin	nd	~26,500	8100	5800	5000
			7470–8810	4850–6950	4230–5900
Unitox 7	~10,000	7116	1008	685	590
		5720–8600	850–1180	583–795	500–695
Unitox 20	5650	2773	1010	497	358
	4910–6460	2450–3180	845–1190	420–580	305–420

Data represent the average of three experiments.
~ = Data from one or two experiments.
nd = No data; there was no 50% death in any of the highest concentrations of the three parallel experiments.

lower. *U. pictorum* proved to be less sensitive against Fyfanon by orders, as compared to the other two species.

All the three species showed a very good tolerance against K-Othrin, which exerted the least toxic effect on the two *Anodonta* species. K-Othrin only caused mortality after a relatively longer (48–72 hr) exposure, and the accumulation of the effect was also of a slighter degree. An 8–17-fold accumulation of the effect, that is the decrease of the LC_{50} values determined from 24 hr to 7 days, could be detected when applying Unitox 7 or Unitox 20. The almost 3-fold difference in the active ingredient content between the two insecticides was not reflected in the LC_{50} values. Moreover, the LC_{50} values of Unitox 20 were lower in some cases. Based on 7 days LC_{50} values, *U. pictorum* was more tolerant against Unitox 7 and Unitox 20, whereas both *Anodonta* species exhibited a higher and near to identical sensitivity. In the case of shorter (24–48 hr) exposures, *A. cygnea* was, however, even more sensitive than *A. anatina*.

Death of the mussels was sometimes preceded by characteristic physiological alterations. Upon the effect of K-Othrin, the adductor muscles first became relaxed; their closing reflex decreased or ceased entirely. The foot and the mantle were markedly swollen. When applying mechanical stimuli, the withdrawal-reflex of the foot remained generally active for a longer time than the adductor muscle reflex. Following the application of Unitox 7 and Unitox 20, the adductor muscles relaxed, and the foot and the mantle also exhibited a swollen appearance. In the mantle cavity, a large amount of viscous mucus could be observed. Finally, the foot and the mantle became shrunken and first the foot-reflex and then the mantle-edge reflex ceased.

The effects of three concentrations of the insecticides on the length of the active and resting periods are summarized in Table 3.

The periodic activity of *A. cygnea* was practically not influenced by Fyfanon in 0.1 μl/l concentration. Applying 1 μl/l concentration, the duration of the active periods slightly increased, whereas those of the resting ones were shortened (Fig. 1). Both active and resting periods were considerably shorter (by 36% and 28%, respectively) upon the effect of 10 μl/l concentration of Fyfanon. In *A. anatina*, all the three concentrations applied increased the duration of the resting periods. In contrast, the active periods could only be influenced by 1 μl/l concentration, decreasing their duration. The effect of Fyfanon on the resting periods of *U. pictorum* was similar, though of slighter degree than that exerted on *A. anatina*. Active periods of *U. pictorum* became shortened when adapting any of the three Fyfanon concentrations.

All the three concentrations of K-Othrin decreased the length of the active periods of *A. cygnea* (in the case of 10 μl/l by 65%). Duration of the resting periods was also reduced by the two higher concentrations. The effect of K-Othrin was found to be weaker in *A. anatina*. Here, the length of the active periods decreased significantly (by 18%) in the case of the application of the highest (10 μl/l) K-Othrin concentration. On the contrary, the resting periods were already influenced by the lower concentrations, their duration increased. In *U. pictorum*, duration of the active periods increased, whereas those of the resting periods were reduced at 0.1 μl/l concentrations. One μl/l concentration caused a slight increase of the active periods and a considerable increase of the resting periods. However, the highest (10 μl/l) concentration of K-Othrin reduced the length of the active periods and slightly increased that of the resting periods.

Low (0.1 μl/l) concentration of Unitox 7 had no effect on the duration of the active periods in *A. cygnea*, but it increased those of the resting ones. Beside increasing the duration of the resting periods, the medium (1 μl/l) concentration of this insecticide decreased the length of the active periods. When applying the highest (10 μl/l) concentration, the length of the active periods increased significantly (59%), whereas that of the resting periods remained unchanged. In *A. anatina*, all the three concentrations

Table 3. Changes in length of resting and active periods of the mussels upon the effect of different concentrations of insecticides

Insecticide	Length of active and resting periods expressed in the per cent of the control (A_1/A%: P_1/P%) 0.1 μl/l	1.0 μl/l	10 μl/l
		Anodonta cygnea	
Fyfanon	96:96	108:84*	64*:772*
K-Othrin	70*:106	71*:73*	35*:63*
Unitox 7	99:124*	86*:142*	159*:105
Unitox 20	99:102	110:125*	134*:85*
		Anodonta anatina	
Fyfanon	103:147*	80*:148*	105:168*
K-Othrin	105:125*	96:110	82*:96
Unitox 7	96:74*	119*:80*	97:75
Unitox 20	95:99	111:79*	150*:84
		Unio pictorum	
Fyfanon	76*:135*	81*:134*	61*:137*
K-Othrin	143*:84	107:154*	90:114
Unitox 7	86*:104	77*:92	206*:79*
Unitox 20	121*:156*	124*:103	213*:80*

A = Average length of the active periods in controls.
A_1 = Average length of the active periods after treatment.
P = Average length of the resting periods in controls.
P_1 = Average length of the resting periods after treatment.
*$P \leq 0.05$.

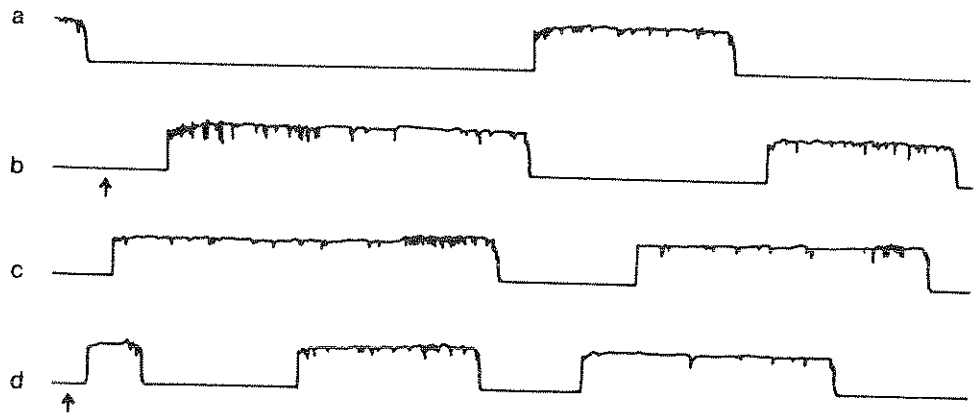

Fig. 1. Effect of Fyfanon on the periodic activity of *Anodonta cygnea*. (a) and (c)—Control, (b) and (d)—after the addition of 1.0 and 10 μl/l concentrations of Fyfanon, respectively.

had a decreasing effect on the duration of the resting periods. At the same time, changes in the active periods could only be evoked by 1 μl/l concentration. In *U. pictorum*, lower concentrations (0.1 and 1.0 μl/l) of Unitox 7 slightly decreased the length of the active periods, without causing any remarkable changes in the length of the resting periods. High (10 μl/l) concentrations of the insecticide evoked about a 2-fold increase in the duration of the active periods, while decreasing that of the resting ones by 20%.

Low (0.1 μl/l) concentrations of Unitox 20 proved to be ineffective in *A. cygnea*. At 1 and 10 μl/l concentrations, the duration of the active periods increased by 10 and 34%, respectively, and the length of the resting periods increased by 25 and 15%, respectively. In *A. anatina* and *U. pictorum*, the effect of Unitox 20 was of a similar character. There was only one exception; its medium (1 μl/l) concentration caused about a 20% reduction in the length of the resting periods. In *U. pictorum*, the duration of the active periods was enhanced by all three concentrations of Unitox 20; 10 μl/l concentration increased it by 213%. The length of the resting periods increased at the lowest concentrations, whereas it decreased at the highest concentrations (Fig. 2).

DISCUSSION

It is widely accepted that in mussels, the LC_{50} values of short (24 and 48 hr) durations can be interpreted only with certain restrictions. By closing the shells, the animals under disagreeable circumstances are capable of temporarily reducing the toxic effect of the surroundings (Epifanio and Srna, 1975). This capability, however, greatly depends on the characteristics and the mechanisms of effects of the toxic substances. The decrease of contractility of the adductor muscles, due to the effect exerted either on the central nervous system or directly on the muscles, inhibits the animals

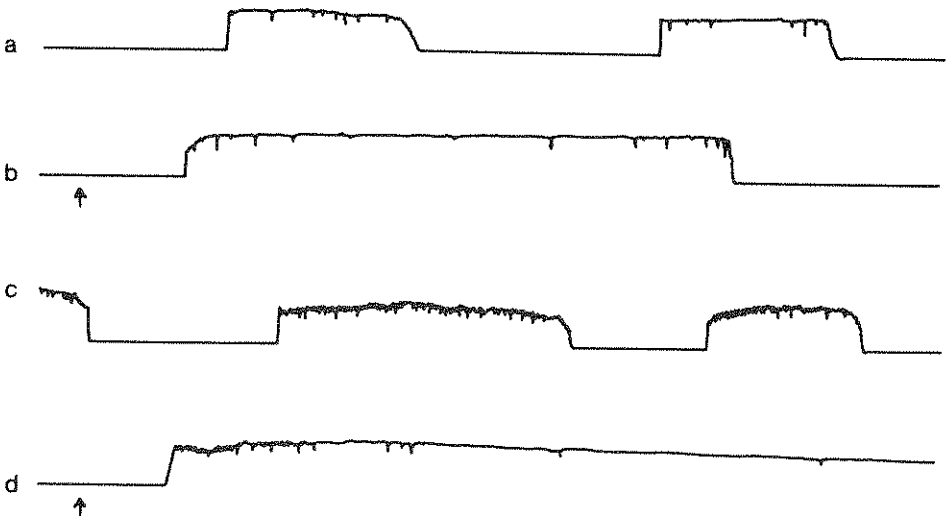

Fig. 2. Effect of Unitox 20 on the periodic activity of *Unio pictorum*. (a) and (c)—Control, (b) and (d)—after the addition of 1.0 and 10 μl/l concentrations of Unitox 20, respectively.

in using this preventing mechanism. The considerable individual differences of the LC_{50} values, as compared to other animal species, can also be a consequence of this preventing mechanism as well as of the changes and individually different durations of the opened (active) and closed (resting) periods.

Among the four insecticides used against mosquitoes, Fyfanon, Unitox 7 and Unitox 20 are the least toxic in *U. pictorum* (see Table 2). At the same time, this species shows the highest sensitivity against K-Othrin. In the two *Anodonta* species, there is no significant difference between the toxicity of Unitox 7 and Unitox 20 after exposures of 24 and 48 hr. Contrary to this, Fyfanon and K-Othrin are more toxic in *A. cygnea* than in *A. anatina*.

Since static tests were used in our studies, it was necessary to know the speed of decomposition of the insecticides to be able to interpret our results. According to Pfeifer's (1980) data, the half-time of the insecticide Malathion (Cheminova AG) is 8 ± 2 hr in Balaton-water at 100 μl/l concentration, while in the case of the Dekametrin (Roussel-Uclaf) this is 4 days at a concentration of 200 μl/l. The entire decomposition of both compounds takes place in 8 days. The half-time of Unitox 7 with 5% active ingredient content is 18 hr at a concentration level of 100 μl/l. Accordingly, it can be established that during the course of the determination of the LC_{50} values and the studies on the effect of sublethal ranges, the concentration of the substances applied decreases, though their decomposition is far from complete. Hence, the static test imitates a one-time, impulse-like pollution of the standing waters.

With the exception of Fyfanon, the insecticides cause the relaxation of the adductor muscles at LC_{50} concentrations. Therefore, the defensive reflex of shell-closing is not functional. A similar effect producing the relaxation of the adductor muscle has been described by Epifanio and Srna (1975) in *Mercenaria mercenaria* and *Crassostrea virginica* following the application of nitrite ions. An exception is the effect of Unitox 7 in *A. anatina*.

According to the LC_{50} values, it can be established that among the invertebrates, mussels exhibit an extreme tolerance againt Fyfanon and both products of Unitox. It is also clear that the mussel species tested tolerate K-Othrin well, being considered one of the most effective insecticides against mosquitoes (Roussel-Uclaf, 1979).

The active ingredient substances of Fyfanon and the Unitox products, as organic phosphoresters, firstly have a cholinesterase inhibiting effect, while Dekametrin possesses a direct neurotoxic effect. In the case of mussels, however, one has to count with non-specific effects on the basis of the long-lasting effects of the insecticides.

As one of their most characteristic physiological processes, the periodic activities of the mussels are much better indicators of the effects of insecticides, as compared to the mortality. At 0.1 μl/l concentrations, the periodic activity of *A. anatina* is already changed by K-Othrin and Unitox 7, that of *A. cygnea* is additionally affected by Fyfanon, whereas that of *U. pictorum* is significantly altered by all the four insecticides. The alterations do not represent similar tendencies even in the case of different concentrations of a given insecticide (e.g. the effect of Unitox 7 on *A. cygnea*, see Table 3). The durations of the active and resting periods are functionally connected to each other between certain limits; the alteration of one of them is not completely independent from the other. The exact regulatory mechanism is still not known. It is remarkable that, whereas in respect of mortality based on LC_{50} values *U. pictorum* has been found to be the most tolerant, its periodic activity exhibits the highest sensitivity against the insecticides tested.

By detecting changes ensuing in the periodic activity at insecticide concentrations three–five orders lower than the LC_{50} values of 96 hr, one may be cautious to draw conclusions as to the seemingly extreme tolerance of mussels. These insecticides are generally used against mosquitoes in a quantity of 0.5 l/ha, which, supposing a uniform dispersion, results in a concentration of 0.5 μl/l in 10 cm water depth. This final concentration may already influence the periodic activity of the mussels. Therefore, the application of insecticides requires considerable attention as well as the acceptance of the technological disciplines.

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