A C T A I C H T H Y O L O G I C A E T P I S C A T O R I A

Abbrev.: Acta Icht. Piscat. 30 (1): 117–125, Ann. 2000

Bernard *KŁYSZEJKO*, Grzegorz *HAJEK*

Fish physiology

EFFECT OF DELTAMETRIN AND LAMBDA-CYHALOTRIN ON REACTIVITY OF ISOLATED STRIATED MUSCLES OF COMMON CARP (*CYPRINUS CARPIO* L.)

WPŁYW DELTAMETRYNY I LAMBDA-CYHALOTRYNY NA REAKTYWNOŚC IZOLOWANYCH MIĘŚNI POPRZECZNIE PRĄŻKOWANYCH KARPIA (*CYPRINUS CARPIO* L.)

Department of Fish Physiology, Agricultural University of Szczecin, Poland

Carp of the individual weigh of 92-625 g were subjected to lethal concentrations of deltametrin and lambda-cyhalotrin. Moribund fish were killed and their pectoral fins along with the associated muscles were dissected. Such preparations were stimulated electrically. No effect of the pyrethroids studied was observed on the value of the muscle stimulation threshold. Similarly, no effect was visible on the ergographic records of the work performed.

INTRODUCTION

Toxic effect of synthetic pyrethroids consists in inhibition of conductivity in the nervous system. In the first phase the intoxication symptoms are clonic reactions resulting from overstimulation, while in the next phase—muscle paralysis (Różański 1992). The mechanism of the pyrethroid action on molecular level has not been fully explained. The hitherto conducted studies shown that the toxic effect consisted in blocking in open position ionic transmission canals. It happens probably as a consequence of binding of α-CN group, present in a pyrethroid to nicotine-acetylcholine receptor of GABA receptor. This in turn makes impossible the transfer of the functional potentials in the synapses (Narahashi 1976; Lund 1984; Nishimura 1988; Clark and Brooks 1989; Saldago 1989). Negative temperature coefficient of pyrethroids (higher activity in lower temperatures) results in their moderate toxicity for homoiothermal organisms and increased toxicity for aquatic organisms (Muck et al. 1976; Harris and Kinohita 1977; De Vries and Georghiou 1979; Malinowski 1982).

In fishes sublethal concentrations pyrethroids cause changes becoming visible after certain time-periods (2–4 weeks). Among the changes are: osmoregulation disorders, increase of enzymatic activity in the kidney and liver, decrease in the number of erythrocytes in the blood, and also histopathologic changes of internal organs (Kumaraguru et al. 1982; Łakota et al. 1987–1988; Jungowska-Klin et al. 1992; Kozubek et al. 1992; Przybylska-Wojtyszyn et al. 1992; Wojtaszek et al. 1992).

Synthetic pyrethroids show high toxicity and at the same time they are easily biodegradable and they do not accumulate in an organism (Łakota et al. 1987–1988; Różański 1992). Some tests revealed, however, that some pyrethroids (deltametrin, cypermetrin) can accumulate in Chlorella algae in the amounts several times exceeding their concentrations in the external environment. Deltametrin was also found in low concentrations in the internal organs of carp in this number in the muscle- and adipose tissues (Łakota et al. 1990). The above findings prompted the present authors to find whether fish intoxication with pyrethroids can have a direct effect excitability and work performance of the striated muscles.

MATERIAL AND METHODS

The experiment was conducted on 61 carp of the individual weight of 92–625 g. The fish originated in a cage fish farm situated in a warm-water discharge canal of a power plant. The carp were placed in 150-dm^3 glass tanks filled with tap water. The temperature was 19 ±1°C, pH 7.4–8.2 and oxygen content 8.2–10.4 mg/dm^3. Two pyrethroids were used for intoxication—deltametrin and lambda-cyhalotrin. The concentration was 3.5 μg/dm^3, which in the case of deltametrin is lethal to carp (Łakota et al. 1987–1988).

As a source of deltametrin we used insecticide of the brand name Decis 2.5 EC, manufactured by Hoechst Shering, Germany (toxicity class IV). Another insecticide sold as Karate 0.25 EC manufactured by ZENECA Ltd. Great Britain (toxicity class III) was a source of Lambda-cyhalotrin (Anonymous 1995).

The course of the experiment was as follows: Randomly selected fish was kept in the concentration of pyrethroid until lethal symptoms were observed. Than the fish was killed through decapitation and the material was sampled for studying muscular excitability. As a physiological preparation the pectoral fin with the associated muscles was used (adductor, abductor, arrector) (Grodziński 1961).

Determination of the excitability threshold was conducted in a petri dish. Reactions to supraliminal stimuli were studied using a kimograph setup where the end of the pectoral fin was linked to the lever of the pen (Fig. 1). Stimulator Stymat S-120 was used as a source of stimuli. The stimulation was conducted through needle electrodes 1 mm in diameter, which were inserted in the same sites of the preparations near the attachment sites of the muscles.

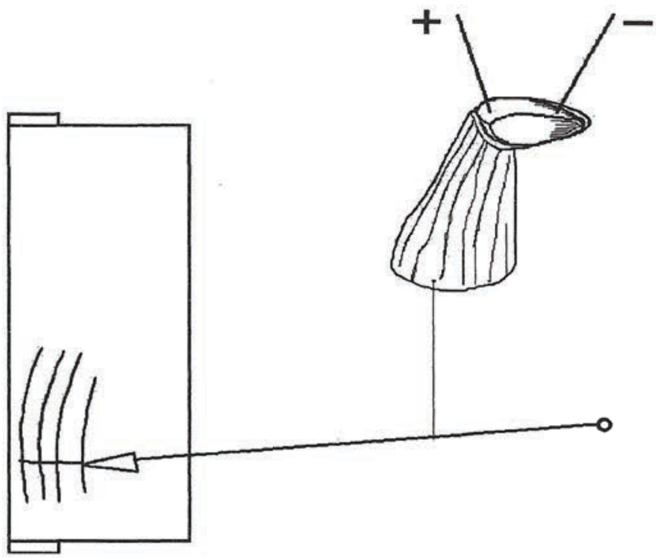

Fig. 1. Diagram of the experimental setup (erogram of the pectoral fin)

Value of the threshold stimulus causing visible "minimal contraction" (Miętkiewski 1973) was determined using single rectangular impulses of the duration of ti = 100 ms and the intensity regulated within 0–1 mA.

Ergograms of isotonic contractions (negligible continuous load of the pen lever) were obtained using a supraliminal stimulus of ti = 100 ms, I = 0.6 mA applied with constant frequency (every 3 s until the complete cessation of fin movements'.

The results were processed statistically with Student t-test using Statgraphics® v. 6.0 package.

RESULTS AND DISCUSSION

Concentrations of 3.5 $\mu g/dm^3$ of deltametrin and lambda-cyhalotrin caused the "stress alarm reaction" syndrome (Seyle 1946). Intense anxiety, attempts of jumping out of the tank, irregular respiratory rhythm, intensified mucous secretion, and some other non-specific reactions typical for fishes responding to a danger were observed (Kłyszejko 1992, 1996; Kłyszejko et al. 1982). Symptoms of the agonal phase (loss of balance, fading respiratory rhythm and fading reactions to mechanical stimuli) occurred as early as after 3 hours of exposure of the fish to lambda-cyhalotrin and after 5 hours of immersion in deltametrin.

Despite the deep intoxication the excitability threshold of the isolated muscular preparations did not change and it was identical for both pyrethroids (Tab. 1).

Table 1

Effect of deltametrin and lambda-cyhalotrin on reactivity of isolated carp muscle

Exposure time in 3.5 μg/dm^3 solution	Number of fish	Fish weight [g]	Threshold intensity of stimulus [mA]
Deltametrin—3 h	27	206–404	0.11 ±0.047
Lambda-cyhalotrin—about 5 h	14	92–617	0.11 ±0.043
Control group	20	208–326	0.11 ±0.038

As a benchmark of the reaction assessment to supraliminal stimulus we assumed the height of the ergogram of the pectoral fin, depicting the power of the muscle contraction. The procedure of obtaining ergograms is diagramatically shown on Fig. 1. The stimulating electrodes were inserted to the preparations in the same sites which was determined visually.

The results of the obtained records (Figs. 2, 3) revealed that in control groups the ergograms had similar shape. The fin movements gradually faded, reaching after about 11 minutes 50% of the initial value. The complete fatigue of the muscles was observed after some 11 minutes. Small displacement of the electrodes did not affect ergographic records in control groups.

Under the same experimental regime, stimulation of the non intoxicated fish preparations gave two types of responses—substantially stronger fin movement (strong reaction) or substantially weaker fin movement (weak reaction) compared to a comparable duration of the control group reaction (Figs. 2, 3). Slight movement or change of needle insertion angle caused changes in reactivity (for example “strong” reaction changing into “weak” one). Concurrently with the fatigue buildup the differences in the reactivity of the preparations faded away. In the case of deltametrin it was after 4 minutes, while 10 minutes for lambda-cyhalotrin.

Contractions of the striated muscles occur in the consequence of stimulation transfer from motor neuron to a muscle fiber through a motor synapse—motor end-plate. Phasing the muscle power is a result of variable recruitment of the motor units in the muscle (Silbernagl and Despopoulus 1994). Toxic action of pyrethroids consists in inhibition of action potential flow in motor neurons (Lund 1984; Clark and Brooks 1989; Różański 1992). Hypersensitivity effect (intensified motion activity, convulsions) is a first sign of pyrethroid intoxication in insects and is linked to disturbances in ionic transmission in the nervous system (Różański 1992).

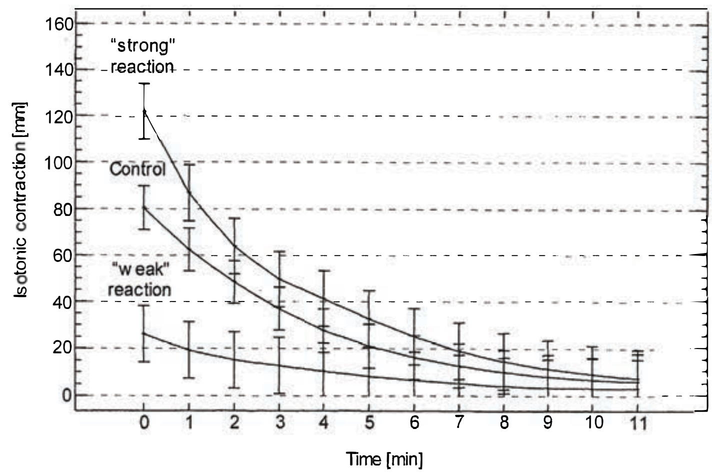

Fig. 2. Effect of deltametrin on reactions of pectoral fin of carp

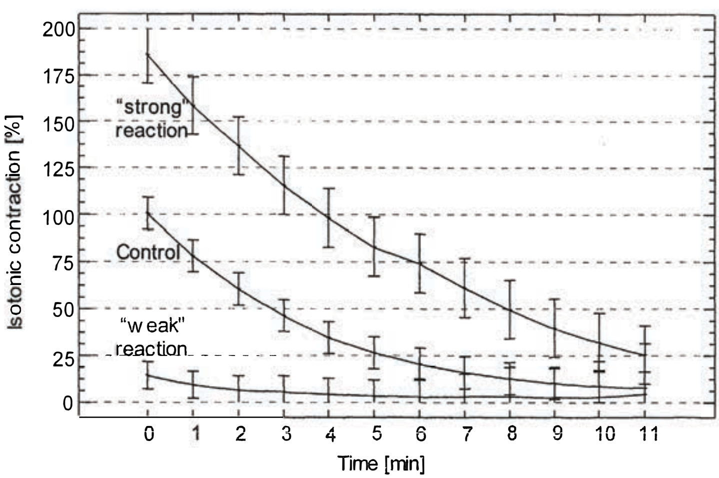

Fig. 3. Effect of lambda-cyhalotrin on reactions of pectoral fin of carp

Discussing the observed changes in the reactivity one might conclude that they were caused by insufficient accuracy of the electrode insertion. The experiment was based on a preparation constituting a functional entity of three muscles, thus containing also nerve structures (lower parts of motor neurons, collaterals, motor end-plates) which were indistinguishable from the muscle tissue.

It can be assumed that decreased reactivity of a preparation occurred when the stimulating electrodes came into contact with the nerve structures where the conductivity was blocked by pyrethroids. Elevated reactivity of the preparation occurred when the electrodes, due to slight shifting conducted stimuli to the muscle tissue. Taking into account, however, the diameter of the electrode (1 mm) and the depth of the penetration of the muscle tissue (about 3 mm), it can be concluded that in each case the stimulation reached a similar number of the muscle fibers. The differences in the reactivity of the preparations made of intoxicated fish could have been caused simultaneously by:

- different level of paralysis of the nervous structures;
- different topography of the electrodes in relation to these structures and in relation to the muscle tissue.

The obtained results would suggest that the action of pyrethroids is not only limited to the nervous system and in the case of striated muscles it does not change significantly the threshold excitability. It has effect, however on biochemical processes responsible for the strength of a contraction. Possible confirmation of that would require the use of more precise stimulation methods involving intrusion of microelectrodes into single muscle fibers.

Changes in reactivity of the preparations had wider range and they persisted longer after the intoxication of the fish with lambda-cychalotrin (Fig. 3). Shorter was also the time of immersion required for inflicting agonal phase (Tab. 1). This would indicate a higher sensibility of carp to this pyrethroid compared to deltametrin.

CONCLUSIONS

In the isolated complex of the striated muscles of carp subjected to the action of lethal concentrations of deltametrin and lambda-cyhalotrin there have been:

- no changes in the reactivity threshold and
- no effect of the electrode topography on the ergographic record of the work performed.

REFERENCES

Anonymous, 1995: Zalecenia ochrony roślin na lata 1995/96 dotyczące zwalczania chorób, szkodników oraz chwastów roślin uprawnych. Cz. I. [Plant protection recommendations for the years of 1995/96 concerning combating diseases, pests, and weeds of crops. Part. I] Instytut Ochrony Roślin, Zakład Upowszechniania Postępu i Doskonalenia Zawodowego, Poznań. (In Polish).

Clark M., M. Brooks, 1989: Role of ion channels and introterminal calcium homeostasis in the action of deltametrin at presinaptic nerve terminals. Biochem. Pharm., **39**, 14: 2233–2245.

De Vries D., G. Georghiou, 1979: Influence of temperature on the toxicity of insecticides to susceptible and resistant house flies. J. Econom. Entomol., **72**: 148–150.

Grodziński Z., 1961: Anatomia i embriologia ryb [Anatomy and embriology of fishes]. PWRiL, Warszawa. (In Polish).

Harris C.R., G.B. Kinohita, 1977: Influence postreatment temperature on the toxicity of pyrethroid insecticides. J. Econom. Entomol., **70**, 2: 215–218.

Jungowska-Klin B., S. Łakota, A. Raszka, J. Wojtaszek, 1992: Wpływ deltametryny na narybek karpia. Cz. II. Wpływ subletalnych dawek deltametryny na aktywność aminotransferazy tyrozynowej (TAT) w wątrobie karpia (*Cyprinus carpio* L.) [Effect of deltamethrin on carp fry. Part II. Effect of sublethal doses of deltamethrin on the activity of tyrosine aminotransferase (TAT) in the carp (*Cyprinus carpio* L.) liver]. Archiwum Ochrony Środowiska, **1**: 121–128. (In Polish).

Kłyszejko B., 1992: Ocena wpływu chlorfenwinfosu na aktywny wysiłek fizyczny karpia (*Cyprinus carpio* L.) metodą testu wysiłkowego [Assessment of the effect of chlorfenvinphos on active physical performance of carp (*Cyprinus carpio* L.) using a physical effort test]. Zesz. Nauk. AR w Szczecinie, Rybactwo Morskie i Technologia Żywności, 19 (150): 3–10. (In Polish).

Kłyszejko B., 1996: Impact of chlorine on performance ability of carp (*Cyprinus carpio* L.). Acta Ichth. Piscat., **26**, 2: 137–142.

Kłyszejko B., R. Węgrzynowicz, G. Głębocka, R. Zbanyszek, 1982: Wpływ wysokich stężeń nieoczyszczonej ropy naftowej na objawy behawioralne oraz zmiany wskaźników hematologicznych (erytrocyty, hemoglobina, hematokryt, Na^+, K^+, Ca^{++}, Mg^{++}) węgorza *Anguilla anguilla* (L.) [Effects of high concentration of untreated oil on the behavioural symptoms and changes of hematological parameters (erythrocyte count, hemoglobin, hematocrit, Na^+, K^+, Ca^{++}, Mg^{++}) of European eel, *Anguilla anguilla* (L)]. Zesz. Nauk. AR w Szczecinie, Rybactwo Morskie i Technologia Żywności, 12 (93): 195–206. (In Polish).

Kozubek Z., S. Łakota, A. Poloczek-Adamowicz, A. Raszka, 1992: Wpływ deltametryny na narybek karpia. Cz. III. Wpływ subletalnych dawek deltametryny na aktywność fosfatazy kwaśnej i zasadowej w narządach wewnętrznych karpia (*Cyprinus carpio* L.) [Effect of deltamethrin on carp fry. Part III. Effect of sublethal doses of deltamethrin on the activity of acid- and alkaline phosphatase in the internal organs of carp (*Cyprinus carpio* L.)]. Archiwum Ochrony Środowiska, **2**: 83–90. (In Polish).

Kumaraguru A.K., W.H. Beamish, H.W. Ferguson, 1982: Direct and circulatory paths of Permetrin (NRDC-143) causing histopathological changes in the gills of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol., **20**: 87–91.

Lund A.E., 1984: Pyrethroid modification of sodium channel: current concepts. Pestic. Biochem. Physiol., **22**: 161–168.

Łakota S., A. Raszka, T. Utracki, Z. Chmiel, 1987–1988: Wpływ toksyczny deltametryny i cypermetryny na wybrane organizmy wodne [Toxic effect of deltamethrin and cypermethrin on selected aquatic organisms]. Organika. Prace Nauk. Inst. Przem. Org.: 71–77. (In Polish).

Łakota S., E. Kołodziejczyk, A. Raszka, 1990: Badania nad ubocznym działaniem pyretroidów na środowisko wodne [Study on side effect of pyrethroids on the aquatic environment]. Pestycydy, **4**: 19–25. (In Polish).

Malinowski J., 1982: Wpływ temperatury na aktywność owadobójczą fotostabilnych pyretroidów [Effect of temperature on insecticidal activity on photo-stable pyrethroids]. Roczn. Nauk Roln., Ser. E, **12**: 245–255. (In Polish).

Miętkiewski E., 1973: Kurs fizjologii doświadczalnej [A course of experimental physiology]. PZWL, Warszawa. (In Polish).

Muck W.L., E. Olson, L. Marking, 1976: Toxicity of natural pyrethrin and five pyrethroids to fish. Arch. Environ. Contam. Toxicol., **4**: 18–29.

Narahashi T., 1976: Nerve membrane as a target of pyrethroids. Pestic. Sci., **7**: 267–272.

Nishimura K., T. Kitahara, Y. Ikemoto, T. Fujita, 1988: Quantitative structure-activity studies of pyrethroids. 16. Physicochemical and strustural effects of tetramethrin and related compounds on the decay of sodium tail currents in crayfish gland axons. Pestic. biochem. Physiol., **31**: 155–165.

Przybylska-Wojtyszyn M., M. Łozińska-Grabska, S. Łakota, A. Raszka, J. Wojtaszek, 1992: Wpływ deltametryny na narybek karpia. Cz. I. Wpływ subletalnych dawek deltametryny na aktywność aminotransferazy alaninowej i asparaginianowej w narzadach wewnętrznych i surowicy karpia (*Cyprinus carpio* L.) [The effect of deltamethrin on carp fry. Part I. Effect of sublethal doses of deltamethrin on the activity of alanine- and aspartate aminotransferase in the internal organs and blood serum of carp (*Cyprinus carpio* L.)]. Archiwum Ochrony Środowiska, **1**: 107–120. (In Polish).

Różański L., 1992: Przemiany pestycydów w organizmach żywych i środowisku [Pesticide transformation in living organisms and in the environment]. PWRiL, Warszawa. (In Polish).

Saldago V., M. Herman, T. Narahashi, 1989: Interaction of pyrethroid fenvalerate with nerve membrane sodium channels: temperature dependence and mechanism of depolarization. Neurotoxicol., **10**: 1–14.

Seyle H., 1946: The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocr., **6**: 117–230.

Silbernagl S., A. Despopoulus, 1994: Kieszonkowy atlas fizjologii [Pocket physiology atlas]. PZWL, Warszawa. (In Polish).

Wojtaszek J., S. Łakota, M. Łozińska-Grabska, M. Przybylska-Wojtyszyn, A. Raszka, 1992: Wpływ deltametryny na narybek karpia (*Cyprinus carpio* L.). Cz. IV. Zmiany wybranych parametrów hematologicznych pod wpływem subletalnych stężeń deltametryny [Effect of deltamethrin on carp fry (*Cyprinus carpio* L.). Part IV. Changes of selected hematological parameters in response to sublethal concentrations of deltamethrin]. Archiwum Ochrony Środowiska, **2**: 91–101. (In Polish).

Bernard *KŁYSZEJKO*, Grzegorz *HAJEK*

WPŁYW DELTAMETRYNY I LAMBDA-CYHALOTRYNY NA REAKTYWNOŚĆ IZOLOWANYCH MIĘŚNI POPRZECZNIE PRĄŻKOWANYCH KARPIA (*CYPRINUS CARPIO* L.)

STRESZCZENIE

Badano wpływ deltametryny i lambda-cyhalotryny na reaktywność izolowanych mięśni poprzecznie prążkowanych karpia. Doświadczenia wykonano w warunkach akwaryjnych na 61 szt. karpi o masie jednostkowej 92–625 g w temperaturze 19 ±10°C. Karpie przetrzymywano w letalnej koncentracji obu pyretroidów – 3,5 $\mu g/dm^3$. Po pojawieniu się fazy agonalnej ryby uśmiercano i pobierano preparaty składające się z płetwy piersiowej i zespołu mięśni odpowiedzialnych za jej ruchy. Izolowane preparaty poddano stymulacji elektrycznej. Stwierdzono brak wpływu deltametryny i lambda-cyhalotryny na wartość progu pobudliwości mięśni oraz wpływ topografii elektrod na ergograficzny zapis wydajności pracy w grupach doświadczalnych.

Received: 10 May 1999

Author's address:

Bernard Kłyszejko PhD DSc
Grzegorz Hajek MSc
Department of Fish Physiology
Agricultural University of Szczecin
Kazimierza Królewicza 4, 71-550 Szczecin, Poland