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The Influence of Low Concentration of Cypermethrin and Deltamethrin on Phyto- and Zooplankton of Surface Waters*

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Pyrethroids play an important role in modern agriculture but their use is not without risks to non-target organisms and habitats. They have high toxicity to a broad spectrum of aquatic organisms. The aim of the study was to investigate the effect of low concentration (0.02 μg l^{-1}) of two widely used pyrethroids – cypermethrin and deltamethrin on phyto- and zooplankton. The acute bioassays were conducted using *Chlorella vulgaris* and *Thamnocephalus platyurus*. Then, the chronic bioassays were conducted using *Chlorella vulgaris*, *Daphnia magna* and *Brachionus calycilforus*. The 24h LC_{50} values for cypermethrin and deltamethrin to *Thamnocephalus platyurus* were 0.89 μg l^{-1} and 1.51 μg l^{-1}, respectively. The 48h EC_{50} values for cypermethrin and deltamethrin to *Brachionus calycilforus* were 3.828 mg l^{-1} and 8.425 mg l^{-1}, respectively. 13% growth inhibition of *Chlorella vulgaris* (statistically insignificant) after 14 days of exposure to deltamethrin as well as cypermethrin was observed.

Key words: Pyrethroids, zooplankton, phytoplankton, toxicity.

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Pyrethroids are the newest class of broad-spectrum organic insecticides. They are synthetic compounds structurally derived from pyrethrin – an extract from the flower of *Chrysanthemum cinerariaefolium*. Pyrethroids are classified as the fourth generation of insecticides because of their moderate persistence, high potential for control of insect pests and low mammalian and bird toxicity (SMITH & STRATTON 1986).

These insecticides are widely used in agriculture. They can enter aquatic habitats as a result of agriculture runoff, spray drift or erosion (HILL 1989). Such pesticide pollution severely affects non-target aquatic organisms and, consequently, the entire water food chain. A number of studies have evaluated the effects of pyrethroids on freshwater invertebrates. Results of these studies indicate that aquatic arthropods are highly sensitive to pyrethroid poisoning, based on the extremely low concentrations that produce toxic effects (ANDERSON 1989; COATS *et al.* 1989). The LC_{50} values for most pyrethroids are less than 1 μg l^{-1} (SMITH & STRATTON 1986). Toxic effects may be mani-

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fested in different ways. Many studies report an influence on swimming ability, feeding efficiency, reproduction rate and homeostasis (CHRISTENSEN *et al*. 2005; COHEN 2006; KIRKPATRICK *et al*. 2006).

Phytoplankton is the most important group of primary producers in water reservoirs. It is a fundamental part of many food chains. The development of phytoplankton is dependent both on the availability of resources needed for growth (such as light or nutrients), as well as the presence of consumers, mainly zooplankton. The composition of phytoplankton is an important indicator that should be taken into account when assessing the ecological status of waters (KAJAK 1998).

Zooplankton comprises a large portion of the living matter in natural waters and plays an important role in biogeochemical cycles. It is a component of the food chain. On one hand it feeds on phytoplankton and bacterioplankton, on the other hand it is food for many fishes (KAJAK 1998).

Zooplankton is frequently used in ecotoxicological tests due to its high sensitivity to toxic substances and the important position it occupies in the food chain. The responses of zooplankton to toxicity tests are considered to be informative of relative impacts on the ecosystem as a whole (FOCHTMAN *et al.* 2000; HANAZATO 2001). In general, a broad range of aquatic invertebrates are commonly used in toxicity tests, e.g. *Daphnia* spp., *Ceriodaphnia* spp., *Gammarus* spp. and *Brachionus* spp. (FOCHTMAN *et al.* 2000). Daphnids, especially *Daphnia magna*, have been used for many years in standard tests of toxicity because of their high sensitivity, easy handling and high reproduction rate (FOCHTMAN *et al.* 2000; HILL 1989). In the standardized chronic toxicity test using *Daphnia*, proposed by the OECD (1998), reproduction (number of offspring produced) of the tested animals is analyzed. Analysis of reproduction may be useful for assessing the effects of chemicals on population growth (HANAZATO 2001).

Brachionus sp. and *Thamnocephalus platyurus*, like other zooplankton, are a significant food source for many fish species and aquatic invertebrates and they have gained popularity as test organisms because of their easy culture, short generation time, cosmopolitan distribution and commercial availability of their dormant eggs (PERSOONE *et al.* 1989).

The aim of the study was to investigate the effects of deltamethrin and cypermethrin in very low concentrations on chosen aquatic invertebrates by using toxicity assays (Toxkits). Population parameters such as growth of green unicellular algae, *Chlorella vulgaris*, and reproduction of the crustacean cladoceran *Daphnia magna* and rotifer *Brachionus calyciflorus* were studied to detect chronic effects. The mortality (24h LC_{50}) of the crustacean anostracan *Thamnocephalus platyurus* was also determined.

Material and Methods

Test organisms

Four test organisms were used in experiments: *Chlorella vulgaris* (Chlorophyceae)*, Daphnia magna* (Crustacea, Cladocera), *Thamnocephalus platyurus* (Crustacea, Anostraca) and *Brachionus calyciflorus* (Rotifera).

A conventional flask test was performed with green unicellular algae *Chlorella vulgaris* Beijerinck 1980, strain A8 obtained from the Silesian Medical Academy, Department of Pharmacy. The algae were cultured in semi-continuous batch cultures according the OECD method: 100 ml Erlenmeyer flasks, continuous uniform illumination of 150 μmol m^{-2} s^{-1} and temperature of 25°C, cultured on OECD test medium (OECD Test Guideline No 201, 1984).

Crustacean cladocerans *Daphnia magna* were hatched from ephippia – a dormant form according to the Daphtoxkit F^{TM} *Magna* procedure. Hatching lasted 72h under continuous illumination of 180 μmol m^{-2} s^{-1}. Then they were conventionally cultured under laboratory conditions (natural photoperiod, temperature of 22 ± 2°C, water hardness of 150 mg $CaCO_3$ l^{-1}, and they were fed with green algae *Chlorella* sp).

Crustacean anostracans *Thamnocephalus platyurus* were hatched from cysts for 22-24h, according to the Thamnotoxkit F^{TM} procedure (temperature of 25°C, under continuous illumination of 180 μmol m^{-2} s^{-1}, water hardness of 150 mg $CaCO_3$ l^{-1}).

Rotifers *Brachionus calyciflorus* were hatched from cysts, incubated under laboratory conditions (temperature of 25°C, under continuous illumination of 180 μmol m^{-2} s^{-1}, water hardness of 150 mg $CaCO_3$ l^{-1}). They were used by Rototoxkit F Chronic procedure and fed with green algae *Raphidocelis subcapitata* during the test.

The test organisms in Toxkits are dormant (cryptobiotic), temporarily immobilized forms that can be hatched on demand within a few minutes up to 3, 4 days depending on the test species. Hence, the test organisms do not have to be cultured in a traditional way and this is one of the most important advantages of this technology (PERSOONE 1991; PERSOONE *et al*. 1994; PERSOONE 1998a; PERSOONE 1998b).

Toxicity tests

Chlorella vulgaris were used in an Alga Growth Inhibition Test according to OECD Guidline No 201

(1984). The low pyrethroid concentration (0.02 μg l^{-1}) was tested. The only modification of the method was prolongation of the test up to 14 days to detect growth inhibition after 3, 7 and 14 days of exposure. This test was conducted only once.

Daphnia magna were used for a chronic toxicity test (21 days) with low pyrethroid concentration (0.02 μg l^{-1}), i.e.: in the Reproduction Test according to the OECD Guideline No 211 (1998) and using Daphtoxkit F^{TM} *Magna*. This experiment was conducted three times.

An acute toxicity test (24h) with *Thamnocephalus platyurus* was performed thrice with the low concentration (0.02 μg l^{-1}) of tested pyrethroids. An acute (24h) test in the full range of concentrations (0.10; 0.32; 1.00; 3.20; 10.00 μg l^{-1}) was done only once. Both tests were performed according to the Thamnotoxkit F^{TM} procedure.

The low concentration (0.02 μg l^{-1}) of cypermethrin and deltamethrin was studied with *Brachionus calyciflorus* as a chronic (48h) reproduction test. It was performed in triplicate. The test was also conducted in the full range of concentrations (0.10; 0.32; 1.00; 3.20; 10.00 μg l^{-1}) – only once. Both tests were performed in standardized conditions according to the Toxkit methodology (Rotoxkit F Chronic).

All Toxkit tests have been miniaturized into practical and user friendly microbiotests. They are particularly suited for routine toxicity testing of chemicals as well as aquatic environmental conditions (FOCHTMAN *et al.* 2000). Moreover, due to the standardized conditions of the tests (all materials needed to perform each test are included in these kits) and well known and consistent source of the test organisms, the results obtained are reproducible, repeatable and comparable within and between laboratories (PERSOONE 1991, 1998a,b; PERSOONE *et al.* 1994).

Test chemicals

Two pure pyrethroid chemicals were used in the experiments: deltamethrin (certified analytical standard IPO 127 No 1/99, 99.0% w/w, 0.25 g sample) and cypermethrin (certified analytical standard IPO 109 No 1/00, 98.2 ± 0.2% w/w, 0.25 g sample). Cypermethrin and deltamethrin are the most often used pyrethroids in Polish agriculture. The tested substances were obtained from Promochem Sp. z o. o. in Warsaw. They were used separately.

The pyrethroids were introduced into water as acetone solutions, taking into account that the permitted final concentration of solvent did not exceed 0.1 mg l^{-1}. The added volume of acetone was smaller than 1% of the volume of each sample. Both substances were tested in one concentration of 0.02 μg l^{-1} or in a full range of concentrations specific for the respective test organisms (determined in initial studies). A concentration of 0.02 μg l^{-1} was often detected in surface waters after agriculture applications (SHIRES & BENNETT 1985; TAYLOR & BOGACKA 1979). In all experiments performed according to the Toxkit methodology only the acetone control could be used. Therefore, for the same experimental conditions in the conventional flask test with *Chlorella vulgaris* only acetone control was used as well.

Statistical analysis

Variation was tested for normality (Shapiro-Wilk test) and variance homogeneity (Levene's test). Data were analyzed by non-parametric Kruskal-Wallis ANOVA followed by Mann-Whitney test. STATISTICA 10PL software was used for these analyses. The significance level was set at 0.05. Toxicological endpoints (LC_{50} and EC_{50}) were calculated by log-probit analysis.

Results

The results of acute and chronic toxicity of cypermethrin and deltamethrin to aquatic organisms are presented in Tables I to IV.

Low concentrations (0.02 μg l^{-1}) of cypermethrin or deltamethrin did not cause growth inhibition of *Chlorella* algae after 72h exposure time

Table 1

The influence of low concentration (0.02 μg l^{-1}) of cypermethrin and deltamethrin on green algae *Chlorella vulgaris* growth after 3, 7 and 14 days of exposure

Substance	Parameters	Exposure time (days)		
		3	7	14
Cypermethrin	Number of cells in the control group (10^6)	3.943	4.953	3.470
	Number of cells in concentration of 0.02 μg l^{-1} (10^6)	3.768	4.890	3.005
	Growth inhibition in % as compared with control group	4.44	1.27	13.40
Deltamethrin	Number of cells in the control group (10^6)	3.943	4.953	3.470
	Number of cells in concentration of 0.02 μg l^{-1} (10^6)	3.805	4.758	3.010
	Growth inhibition in % as compared with control group	3.50	3.94	13.26

(Table 1). The same results were observed after 7 days of exposure. Prolonging of the test duration up to 14 days caused about 13% growth inhibition (statistically insignificant) in the test with cypermethrin as well as with deltamethrin.

There were no significant differences in the *Daphnia magna* reproduction test (21 days) for low concentrations of each pyrethroid in comparison to the control group.

Low concentrations (0.02 μg l^{-1}) of the tested pyrethroids did not cause mortality in *Thamnocephalus platyurus* after 24 h of exposure. The acute test of cypermethrin and deltamethrin in the full range of concentrations revealed 24h LC_{50} values of 0.89 μg l^{-1} and 1.51 μg l^{-1} (95%-CL), respectively (Table 2).

The chronic (48 h) test with *Brachionus calyciflorus* showed that a low concentration of cypermethrin caused 2.5% stimulation of reproduction while deltamethrin – 2.2% inhibition (both statistically insignificant). The full range test for *Brachionus calyciflorus* reproduction revealed a 48h EC_{50} value of 3828 μg l^{-1} for cypermethrin (Table 3) and 8425 μg l^{-1} – for deltamethrin (95%-CL) (Table 4).

Table 2

Acute toxicity of cypermethrin and deltamethrin to crustacean anostracan *Thamnocephalus platyurus* (24h LC_{50}, 95%-CL, n=40)

Concentration (μg l^{-1})	Cypermethrin		Deltamethrin	
	Number of dead organisms	Mortality (%)	Number of dead organisms	Mortality (%)
Control	0/40	0	0/40	0
0.10	1/40	2.5	0/40	0
0.32	9/40	22.5	3/40	7.5
1.00	19/40	47.5	9/40	22.5
3.20	37/40	92.5	33/40	82.5
10.00	40/40	100	40/40	100
24h LC_{50}	0.829 μg l^{-1}		1.51 μg l^{-1}	

Table 3

Effects of cypermethrin exposure on *Brachionus calyciflorus* reproduction (48h EC50, 95%-CL, n=10)

Concentration (μg l^{-1})	Number of live organisms in replication								Mean (±SD)
	1	2	3	4	5	6	7	8	
Control	7	6	3	3	3	3	4	4	4.125 (1.55)
0.10	7	3	4	5	6	3	2	2	4.000 (1.85)
0.32	3	1	3	1	1	1	0	1	1.375 (1.06)
1.00	0	0	0	1	1	1	1	1	0.625 (0.52)
3.20	1	0	0	0	0	0	0	0	0.125 (0.35)
10.00	0	0	0	0	0	0	0	0	0 (0.00)
48h EC_{50}	3.828 mg l^{-1}								

Table 4

Effects of deltamethrin exposure on *Brachionus calyciflorus* reproduction (48h EC_{50}, 95% CL, n=10)

Concentration (μg l^{-1})	Number of live organisms in replication								Mean
	1	2	3	4	5	6	7	8	
Control	7	4	5	2	7	2	3	4	4.250 (1.98)
0.10	6	7	4	6	7	4	3	2	4.875 (1.89)
0.32	5	6	3	3	3	2	2	4	3.500 (1.41)
1.00	2	0	2	1	1	1	2	1	1.250 (0.71)
3.20	0	0	0	0	0	0	0	0	0.000 (0.00)
10.00	0	0	0	0	0	0	0	0	0.000 (0.00)
48h EC_{50}	8.425 mg l^{-1}								

Discussion

Concern about the presence and detection of toxic chemicals in water ecosystems has increased in recent years. Different test organisms such as algae and planktonic invertebrates are used for measuring and controlling water quality.

Phytoplankton are crucial in surface water because of their ecological position at the base of most aquatic food webs and their essential roles in nutrient and phosphorus cycling. Typical phytoplankton include *Chlorella* spp. A few papers have been published about the toxicological aspects of pesticides on green algae. According to MA (2005) *Chlorella vulgaris* and other green algae were more sensitive to beta-cyfluthrin then cyanobacteria. Growth inhibition of *Chlorella vulgaris* was observed at 4409.5 μg l^{-1} beta-cyfluthrin per litre after 96h of exposure which is over two orders of magnitude in comparison to SÁENZ *et al.* (2012). Significant inhibition of algal growth was observed from 30 μg l^{-1} of cyfluthrin per litre (96h EC_{50}). This result is similar to toxicity data on pyrethroids published by WHO. For cypermethrin and deltamethrin, the 72h EC_{50} values were 15.26 and 22.77 μg l^{-1}, respectively. The present study shows a lack of growth inhibition of *Chlorella vulgaris* after 72h of exposure to very low concentrations of cypermethrin and deltamethrin (0.02 μg l^{-1}). However, in the same experiment 13% of algae growth inhibition (statistically insignificant) was observed after a longer time of exposure (14 days). A similar situation may occur in the natural environment after application of high doses of these pesticides in agriculture. LUTNICKA *et al.* (1999) showed that the application of high doses of different pyrethroids in an aquatic ecosystem model caused long term persistence of the chemicals in very low concentrations in this environment (the median time was about 50 days). Low concentrations of these pesticides can be toxic for phytoplankton after a longer duration. Reduction in the population of green algae can lead to a decrease of the amount of zooplankton (KAJAK 1998) and plankton community structure (RÓŻAŃSKI 1992).

The next link of the surface water food chain is zooplankton. Cypermethrin caused changes in this community structure at a concentration from 0.13 μg L^{-1} after an 11-day period (WENDT-RASCH *et al.* 2003). One of the most important groups of zooplankton are *Daphnia* spp. (*D. carinata*, *D. cucullata*, *D. galeata*, *D. hyalina*, *D. laevis*, *D. longispina*, *D. magna*, *D. obtusa*, *D. pulex*, *D. pulicarina*, *D. similis*, *D. spinulata*). *Daphnia magna* is a commonly used standard test organism in ecotoxicological studies (FOCHTMAN *et al*. 2000; MARTINS *et al.* 2007). This species has been tried on 92% of all organic pollutants (SÁNCHEZ-BAYO 2006). In studies conducted by RATUSHNYAK *et al*. (2005) the 96h EC_{50} value for cypermethrin for *Daphnia magna* was 0.00061 μg l^{-1}. CHRISTENSEN *et al*. (2005) reported that cypermethrin at a concentration higher than 0.1 μg l^{-1} disturb the swimming ability of *Daphnia magna* after 6, 24, and 48 h of exposure. This is explained as a result of nervous system dysfunction (WARE 1983). According to FRIBEG-JENSEN *et al*. (2003), cypermethrin increased the total abundance of *Daphnia* spp. at a concentration of 0.47-6.1 μg l^{-1} after 4 and 12h of application. The 24h LC_{50} for cypermethrin was 2 μg l^{-1} (MARTINS *et al.* 2007). ŁAKOTA *et al*. (1988) revealed the 48h LC_{50} value as 0.36 and 0.05 μg l^{-1} for cypermethrin and deltamethrin, respectively. Deltamethrin caused immobilization of *Daphnia magna* at concentrations ranging from 0.05 to 1.01 μg l^{-1} (24h EC_{50}) and from 0.27 to 4.65 μg l^{-1} (48h EC_{50}) (MARTINS *et al.* 2007). In our study low concentrations (0.02 μg l^{-1}) of the tested pyrethroids did not cause any toxic effects on *Daphnia magna* reproduction. In studies performed by RATUSHNYAK *et al*. (2005) cypermethrin at a concentration of 0.002-0.2 μg l^{-1} did not affect population growth rate 21 days after application. LOZANO *et al.* (1992) obtained similar results. Esfenvalerate did not modify the total abundance of cladocerans below a concentration of 1 μg l^{-1} after 18 days of exposure.

In analysis of *Thamnocephalus platyurus*, the 24h LC_{50} (μg l^{-1}) were 0.89 and 1.51 for cypermethrin and deltamethrin, respectively. Very low concentrations of pyrethroids (0.02 μg l^{-1}) did not cause mortality in this species. In contrast to the above results, SÁNCHEZ-FORTÚN and BARAHONA (2005) revealed 24h LC_{50} (μg l^{-1}) value as 670 for cypermethrin. There is no other available data on the toxicity of cypermethrin and deltamethrin to *Thamnocephalus platyurus*. The toxic effects of Tempo SC Ultra (11.8% a.i. cyfluthrin) was investigated by BRAUSCH & SMITH (2009). The 48h LC_{50} value was 10.06 μg l^{-1}.

The next representative of zooplankton is *Brachionus* sp. It is reported that 24h LC_{50} (μg l^{-1}) values for *Brachionus calyciflorus* were 80, 220, and 40 for cypermethrin, permethrin, and resmethrin, respectively (SÁNCHEZ-FORTÚN & BARAHONA 2005). Available data on the toxicity of cypermethrin and deltamethrin on *Brachionus calyciflorus* is insufficient. According to our studies, low concentrations of cypermethrin and deltamethrin did not cause statistically significant growth inhibition after 48h of exposure; 48h EC_{50} (μg l^{-1}) was 3828 for cypermethrin and 8425 for deltamethrin. In studies conducted by MEDINA *et al*. (2004) rotifer populations exposed to cypermethrin (at a concentration of about 5 μg l^{-1}) followed a different pattern, decreasing moderately 2 days after application, increasing markedly shortly thereafter (on

day 6), and decreasing again to control levels on day 14. According to TIDOU *et al.* (1992) deltamethrin at a concentration of 13 μg l^{-1} caused the immediate disappearance of rotifers in pond conditions. They were also rare 30 days after application. After 2 months of duration, the rotifer community started to recover.

Summarizing the results obtained from our study and previous contributions, it is difficult to make clear conclusions on the toxic effects of low and very low concentrations of different pyrethroids on aquatic organisms. In our study, a very low concentration (0.02 μg l^{-1}) of cypermethrin and deltamethrin did not cause detectable and statistically significant toxic effects on reproduction and growth rate (in constant conditions). Differences in data obtained by investigators are probably due to various experimental conditions. The sensitivity to pyrethroids is dependent on the species being tested, the size and age of the organism, type of tested pyrethroid, the time of exposure etc.

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