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Comparative Sensitivity to the Insecticides Deltamethrin and Esfenvalerate of Some Aquatic Insect Larvae (Ephemeroptera and Odonata) and *Daphnia magna*

M. A. Beketov

Institute of Animal Systematics and Ecology, Siberian Division, Russian Academy of Sciences, ul. Frunze 11, Novosibirsk, 630091 Russia

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Abstract—Sensitivity to the pyrethroids deltamethrin and esfenvalerate (aqueous solution) and LC_{50} have been determined in acute (96-h) toxicological tests on mayfly larvae (*Cloeon dipterum* and *Caenis miliaria*), damselfly larvae (*Lestes sponsa* and *Cordulia aenea*), and juveniles from a laboratory culture of *Daphnia magna.* Sensitivity to deltamethrin increases in the series *C. aenea* (Odonata) < *D. magna* (Cladocera) < *L. sponsa* (Odonata) < *C. miliaria* (Ephemeroptera) < *C. dipterum* (Ephemeroptera), and that to esfenvalerate, in the series *C. aenea* < *D. magna* < *L. sponsa* ≈ *C. miliaria* ≈ *C. dipterum*. The values of LC_{50} about 0.01 μg/l determined for mayfly larvae are below those known for various hydrobionts from the literature, indicating a very high sensitivity of these insects to pyrethroids.

Key words: pyrethroids, toxicity, *Daphnia magna*, mayflies, Odonata.

The species *Daphnia magna* Straus (Cladocera, Crustacea) is the test object most widely used in various ecotoxicological studies and biotesting of various substances in Russia and abroad (*Metodika…*, 1999; *Methods…*, 1993). In Russia, daphnia of this species are used for determining the acute or chronic toxicity of surface fresh water, groundwater, potable water, and raw and purified wastewater (*Metodika…*, 1999). One species cannot yield complete information on the toxicity and potential hazard of a certain substance to living nature. Species often differ in their tolerance as strongly as in other characters (Begon *et al.*, 1989). Hence, the use of meso- and microcosms with multispecies communities as models of natural ecosystems is apparently the best way of assessing the potential hazard presented by various toxicants (Crossland and La Point, 1992; Williams *et al.*, 2002). However, a comparison of the tolerance of individual species studies separately can also provide ample information.

Insecticides, being designed for pest control, affect all kinds of insects as well as pests. The consequences of the wide application of pesticides, especially insecticides, are difficult to assess and describe adequately (Jepson and Moran, 2001). The problem is greatly complicated due to the fact that trace amounts of insecticides remaining in soil and dissolved in water can have a toxic effect on various insects (Schulz and Liess, 2001). The application of stable organochlorine pesticides is prohibited, but even rapidly decomposing pyrethroids can occur in continental water bodies at potentially hazardous concentrations (Williams *et al.*, 1995). For example, the peak concentrations of fenvalerate in water bodies may reach 0.1–6.0 μg/l (Liess *et al.*, 1999; Baughman *et al.*, 1989) in water and up to 100 μg/l in bottom sediments (Liess *et al.*, 1999). Water bodies situated near farmlands especially suffer from the action of insecticides. Stagnant water bodies, particularly those overgrown with vegetation, are a kind of barrier to the spread of insecticides (Schulz, 2001) but, as a consequence, their biota is much impoverished.

This study deals with sensitivity of the larvae of some amphibionts commonly occurring in stagnant water bodies in Palearctic, namely, two species of mayflies, *Cloeon dipterum* L. and *Caenis miliaria* Tshern., the damselfly, *Lestes sponsa* Hans., and the dragonfly *Cordulia aenea* L. Similarly to many other inhabitants of small stagnant water bodies, these insects are rather tolerant to ammonium, nitrates, phosphates (Beketov, 2002), and other substances of natural origin (Galatowitsch *et al.*, 1999). On the other hand, such organisms may be highly sensitive to various specific substances alien to the natural geochemical background, such as pesticides. Due to such selective sensitivity, it is advisable to use, *inter alia,* the larvae of amphibionts in ecotoxicological investigations in order to detect the presence of insecticides and obtain general information on the toxic properties of a certain substance, solution, or mixture.

The tolerance of aquatic insect larvae to deltamethrin and esfenvalerate was studied and compared with that of *D. magna* to estimate the potential hazard of these insecticides for the aforementioned organisms.

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Another purpose was to assess the possibility of using the insect larvae for biomonitoring, ecotoxicological research, and biotesting. Mayfly larvae e.g., *Ephoron virgo* have already been successfully used in biotesting. For example, Greve *et al.* (1999) conducted tests with *Ephoron virgo* larvae immediately after their hatching from the eggs laid by females captured in nature, which allowed these authors to avoid the laborious cultivation of mayflies in the laboratory.

At present, the pyrethroids deltamethrin and esfenvalerate are widely used both in Russia and abroad (*Ezhegodnik…*, 2001; Thomson, 1992). Esfenvalerate—(S-α-cyano-3-phenoxybenzyl (S)-2-(4-chlorophenyl)-3-methyl butyrate—is a more active (by 70%) SS-isomer of fenvalerate (Antonious, 2002). Preparations of esfenvalerate consist of a mixture of approximately 22% of fenvalerate and some its other isomers, with esfenvalerate being the most toxic (Antonious, 2002). Its half-life in water is about 21 days (Walker and Keith, 1992), depending on insolation. Deltamethrin, S-α-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate, is another highly toxic pyrethroid. Its half-life in surface waters is only several days due to its high absorbability and evaporation (Haug and Hoffman, 1990).

The sensitivity of various organisms, including insect larvae and *D. magna*, to deltamethrin and esfenvalerate has already been assessed, and extensive relevant information is available from databases such as ECOTOX (http://www.epa.gov/ecotox) and EXTOXNET (http://www.ace.orst.edu/info/extoxnet). As far as I know, however, representatives of the Odonata have not been studied in this respect; among the Ephemeroptera, only *Baetis parvus* has been used in experiments with deltamethrin (Mohsen and Mulla, 1981). Representatives of both orders play an important role in freshwater ecosystems and occur in the majority of water bodies. Data on the toxicity of pesticides obtained in the experiments on daphnias were compared with published results (Fairchild *et al.*, 1992; Xiu *et al.*, 1989). These experiments were performed in parallel with those on insect larvae to make the results comparable.

MATERIAL AND METHODS

The experiments on *D. magna* were performed by conventional methods (*Metodika…*, 1999) using the laboratory culture maintained at the Western Siberian Regional Center for Monitoring the Natural Environment (Novosibirsk). Insect larvae were collected from a small pond near the Novosibirsk Zoo immediately before each experiment.

Deltamethrin emulsion (25 g/l) in 2-ml glass ampoules was produced under the trade name Decis by OOO Avgust (Moscow) and packed by ZAO Agrovetservis (Novosibirsk), state registration number 01-2261-0261-1 (F). Esfenvalerate emulsion (50 g/l) in 2-ml ampoules was produced and packed under the trade name Sumi-Alfa by the same companies, state registration number 01-2344-0261-1(F).

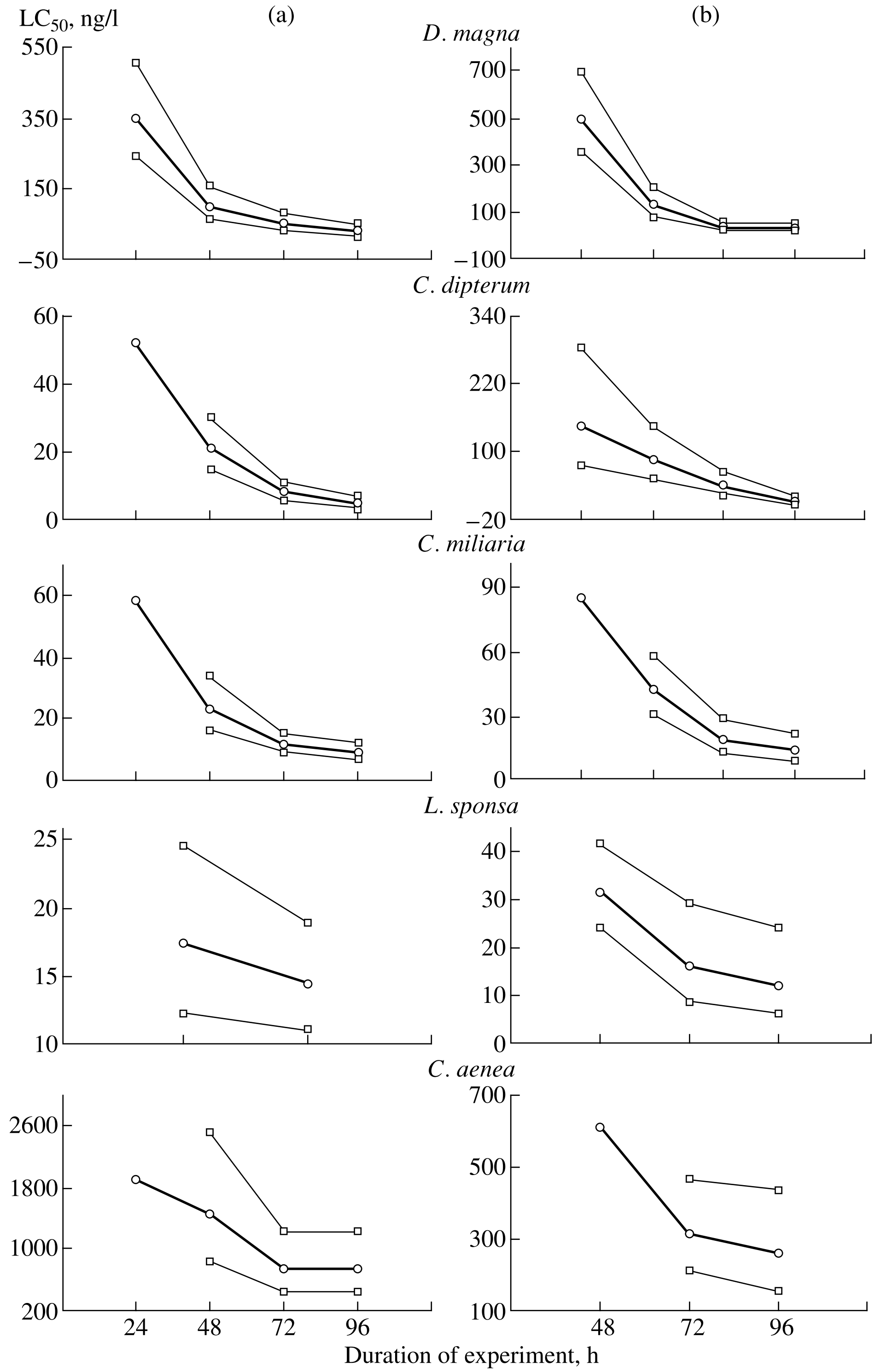
Serial dilutions of these pyrethroids were prepared so that mortality among test objects did not differ significantly from the control at the lowest concentration and reached 100% at the highest concentration (as determined in preliminary experiments). Each concentration was tested in three parallel series. In the control (three series), cultivation in water was used. The age of daphnia did not exceed 24 h. The duration of experiments on daphnia and insects was 96 hours.

The scheme of experiments with mayfly and damselfly larvae was the same. Pond water used for preparing test solutions was filtered through paper filters (pore size 1–2.5 nm) to prevent the adsorption of insecticides on suspended particles. The hydrochemical parameters of pond water analyzed immediately before use are shown in Table 1. Water temperature was 20 ± 3°C. The content of oxygen in the water used in the experiments with insect larvae was much lower than in the experiments with daphnia cultures. However, considering the high tolerance of larvae and their high survival rate (no less than 90%) in the control, experimental conditions could be regarded as acceptable. In the control, three series of experiments with pond water were performed. To prevent adsorption of pesticides, experimental vessels contained no substrata, which was an additional stress factor for test organisms. Death was recorded if an animal failed to move in response to mechanical stimulation. After 96 h, the animals that remained motionless, in unnatural position (upside down), or made slight convulsive movements when taken with pincers were also considered perished. Such somewhat ambiguous criteria were used because the moribund period after exposure to toxic substances could continue for several days, being manifested in weak convulsions.

All experiments and chemical analyses were made at the Western Siberian Regional Center for Monitoring the Natural Environment (Novosibirsk).

Table 1. Main hydrochemical parameters of pond water

Parameter	Mean values ± error
Dissolved oxygen, mg/l	3.68 ± 0.92
BOD5, mg/l	4.66 ± 1.00
Total ammonium ($N - NH_4^+ + NH_3$), mg/l	1.68 ± 0.17
Nitrites, ($N - NO_2^-$), mg/l	0.007 ± 0.001
Nitrates ($N - NO_3^-$), mg/l	0.23 ± 0.19
pH	7.5 ± 0.70
Hardness, mg-equiv	2.34 ± 0.23



Values of LC$_{50}$ (○) and 95% confidence intervals (□) of (a) deltamethrin and (b) esfenvalerate for in *D. magna, C. dipterum, C. miliaria, L. sponsa*, and *C. aenea.*

Toxicity was estimated quantitatively by the standard parameter LC$_{50}$ (96 h), i.e., the concentration of a toxic agent causing death of 50% of test organisms within 96 h. To determine this parameter, mortality at each tested concentration is taken into account. The values of LC$_{50}$ and the corresponding 95% confidence intervals were calculated by the improved Trimmed Spearman–Karber method (Hamilton *et al.*, 1977)

Table 2. Values of LC_{50} (96 h) of deltamethrin and esfenvalerate and the corresponding 95% confidence intervals for *D. magna*, *C. dipterum*, *C. miliaria*, *L. sponsa*, and *C.aenea*

Test organism	LC_{50} (95% CI), μg/l	
	Deltamethrin	Esfenvalerate
D. magna	0.0293 (0.0179–0.0478)	0.029(0.017–0.050)
C. dipterum	0.0050 (0.0036–0.0069)	0.0096 (0.0051–0.0181)
C. miliaria	0.0091 (0.0067–0.0124)	0.0147 (0.0092–0.0222)
L. sponsa	0.0145 (0.0110–0.0190)	0.0122 (0.0062–0.0239)
C. aenea	0.7600 (0.4600–1.2400)	0.262 (0.156–0.439)

using SPEARMAN software (Montana State University). Data on the mortality in different replications of the same experiment were pooled before calculation. The significance of differences between LC_{50} values was determined by analyzing the overlap of 95% confidence intervals with the aid of STATISTICA software.

RESULTS AND DISCUSSION

In the control, mortality among all test animals did not exceed 10%. The values of LC_{50} (96 h) are shown in Table 2. *Cloeon dipterum* was most sensitive to deltamethrin and esfenvalerate. The LC_{50} of deltamethrin for this species significantly differed from that for other species ($p < 0.05$), but the LC_{50} of esfenvalerate for *C. dipterum, C. miliaria*, and *L. sponsa* did not differ statistically ($p > 0.05$). Unexpectedly, this parameter for *C. aenea* larvae had the highest value. The sensitivity of *D. magna* to the insecticides was higher than that of *C. aenea* larvae but lower than that of other insects (Table 2).

The sensitivity of daphnias to deltamethrin was slightly lower that the values known from publications (Xiu *et al.*, 1989) but their sensitivity to esfenvalerate proved to be higher (Fairchild *et al.*, 1992). An analysis of modern detailed databases (ECOTOX and EXTOXNET) showed that LC_{50} (96 h) obtained for *C. aenea* larvae were the highest among invertebrates. However, a considerable part of the LC_{50} values in these databases were determined in experiments of different durations (24 or 48 h). Therefore, I also calculated the LC_{50} for 24, 48, and 72 h. The LC_{50} (24 h) of deltamethrin for *C. aenea* proved to be lower only than that for *Dicrotendipes californicus* larvae (Diptera) (Ali and Mulla, 1980). According to my data, the tolerance of *C. aenea* to esfenvalerate was the highest among invertebrates and almost the same as in aquatic vertebrates.

The values of LC_{50} calculated for 24-, 48-, 72-, and 96-h exposure (when it was possible) reflect the dynamics of toxicity manifestations in the course of experiments (figure). The curves of mortality as a function of time for all test objects generally have the same (inverse logarithmic) form demonstrating a somewhat delayed manifestation of the acute toxic effect, which is characteristic of pyrethroids.

The experiments demonstrated that *C. dipterum* larvae were most sensitive to deltamethrin. Pyrethroids dissolved in water are more toxic than those adsorbed on solid particles (Schulz and Liess, 2001). Therefore, the scheme of experiments (filtered water, the absence of substrata and food, and low content of dissolved oxygen) could have a significant effect on their results. However, it was aimed at making the solution of insecticide, rather than its absorbed fraction, the principal toxic factor. In particular, the absence of food could be an important factor of decreased tolerance (Liess *et al.*, 2001). Sensitivity of *L. sponsa* and *C. miliaria* to deltamethrin was also rather high: the values of LC_{50} for these species were lower than those for dipteran larvae indicated in the ECOTOX and EXTOXNET databases.

As noted above, the tolerance of *C. dipterum* to esfenvalerate was relatively low and did not differ statistically from that of *C. militaria* and *L. sponsa*. Unfortunately, the available data were insufficient for reliably comparing the corresponding LC_{50} values with those for other insects. However, the aforementioned species proved to be more sensitive to the pyrethroids than fish, amphibians, and crustaceans indicated in ECOTOX and EXTOXNET.

The results of this study allow the conclusion that the aqueous solutions of deltamethrin and esfenvalerate are extremely toxic for the larvae of mayflies and damselflies under experimental conditions, exerting their toxic effect at concentrations as low as 0.01 μg/l. Although the larvae were exposed to additional stress factors in the experiments, their high sensitivity to these insecticides indicates that they are hazardous to the ecosystems of continental stagnant water bodies. It should be mentioned that it is difficult to detect insecticides in solutions at concentrations below 0.1 μg/l using analytical chemistry methods. The high selective sensitivity of insect larvae to insecticides makes them a promising test objects for biomonitoring and various ecotoxicological investigations.

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