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Accumulation of Tetradifon in an Algae *(Nannochloris oculata)* and the Cladoceran, *Daphnia magna*

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The toxicity and accumulation behavior of persistent chemicals are important factors in studying the influence of these substances on aquatic organisms. The dynamics of the pesticides in aquatic ecosystems are not fully understood. They are very complex because organisms may accumulate these compounds by direct uptake from water as well as from food.

Most efforts to understand the impact of toxic pollution on phytoplankton have usually centered on measurements of such direct or indirect effects by determining various parameters such as the EC50 for photosynthesis or growth. Such approaches have resulted in a compilation of data consisting of a list of species, concentrations of assorted chemical pollutants, and type of responses under different conditions (Macri and Sbardella 1984; Meyerhoff et al. 1985, Mayasich et al. 1987; Nyholm and Källqvist 1989). The first step for toxic injury to primary producers is bioaccumulation. Many studies have been made to understand toxic responses in relation to uptake (Reinert 1972; Walsh et al. 1977; Yi-xiong and Bozen 1987; Rhee and Thompson 1992). The toxic effect of a compound to phytoplankton community is the direct consequence of accumulation. Since algae are food for animals, an effect on them could affect biological properties of aquatic ecosystems.

Cladocerans are filter-feeding crustaceans which have been extensively used in toxicity testing because they are readily available, adaptable to laboratory conditions, require little space and frequently are one of the more sensitive groups of animals to chemicals (Mokry and Hoagland 1990). *Daphnia magna* has historically been used in toxicity testing establishing a large data base for this species. *D. magna* and other crustaceans are more closely related to insects than vertebrates, including fish. Therefore, highly selective pesticides like tetradifon may pose a greater hazard to arthropods than to vertebrates.

The research reported here was designed to study the rate of accumulation of tetradifon, acaricide commonly used on many fruits, citrus and vegetables of the Valencia Community (Spain), by two organisms from different trophic levels: a green alga *Nannochloris oculata* and the cladoceran *Daphnia magna.*

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MATERIALS AND METHODS

The tetradifon (4-chlorophenyl 2,4,5trichlorophenyl sulfone) used in these experiments was 99% pure as assayed by AFRASA Company (Spain). Stock solutions were prepared by dissolving the toxicant in acetone immediately prior to each experiment.

The freshwater algae cultures of *Nannochloris oculata* were grown in 3-L wide-mouth jars in a nutrient medium consisting of 100 mL of BBM medium (5 g $NaNO_3$, 0.5 g $CaCl_2 2H_20$, 1.5 g $MgSO_4 7H_20$, 1.5 g K_2HPO_4, 3.5 g KH_2PO_4, 0.5 g NaCl); 2 mL of trace metals (5 g NaFe EDTA, 180 mg $MnCl_2 4H_20$, 10 mg $CuSO_4 5H_2O$ mg $ZnSO_4 7H_2O$, 10 mg $CoCl_2 6H_2O$, 6.4 mg $NaMoO_4 2H_20$) and 2 mL of vitamins (200 mg thiamine, 10 mg biotine, 10 mg B_{12}) per liter of water (Bischoff and Bold 1983). Compressed air was continuously bubbled through the solution. The constant light source for the stock bottles consisted on two white fluorscent tubes (5000 lux ilumination); temperature was kept at 22°C.

Before each experiment, a portion of the culture used was concentrated in a centrifuge and rinsed with water. Then, algae concentration was determined using a hemacytometer, and calculations made to have a final algae density of $5x10^5$ cells/ml in the experimental aquaria (5L). The temperature of the cultures during the tests was 22°C.

Algae were exposed to 1.5, 1.8, 2.2 and 4.4 mg/L tetradifon respectively. After 24, 48, 72 and 96 hr the algae (100 mL) were collected and centrifugated (5 min.; 3000 rev/min.) and both the pellet (algae cells) and the media were analyzed for tetradifon by gas chromatography. Each value in Fig. 1 is the average of five replicates.

For residue analysis (Zweigh and Sherma 1972 method), algae samples were homogenized with 50 mL acetone with a Model PT 10-ST Polytron and kept during 24 hr in 100 mL of 2% aqueus sodium sulphate. The extract was transferred to a 250-mL funnel and 30 mL hexane was added; then the mixture was shaken for 1 min and this process was repeated three times. After the solvent phases separated, the lower aqueous phase was drained into a beaker and the upper layer (hexane) collected in a 100-mL erlenmeyer flask. The extracts were concentrated to dryness by placing the flasks in a rotatory vacuum evaporator with the water bath at 45°C. The samples were dissolved in hexane and cleaned through Florisil packed; then they were evaporated again. The dried samples were dissolved in 5-mL hexane and injected directly into a gas chromatograph (Perkin Elmer F-17). The average recovery rate of tetradifon was 85% (the detection limit was 0.1 µg/L).

Daphnia magna were obtained from continuous cultures maintained in our laboratory in 5-L aquaria at 22±l°C, in dechlorinated tap water (total hardness, 250 mg/L as $CaCO_3$; pH 7.9±0.2; alkalinity, 4.1 mmol/L), 12hr:12hr 1ight:dark photoperiod and a density of below 50 animals/L. The medium was renewed three times each week and the daphnids were fed daily with the algae *Nannochloris oculata.*

Preliminary acute toxicity tests were conducted according to the EEC standard procedure (EEC 1984) for determining the 24 hr-LC50 for tetradifon in *D. magna.* Five test concentrations plus a control and a solvent control (acetone: 0.1 mL/L) was used. A single brood was used for each concentration series.

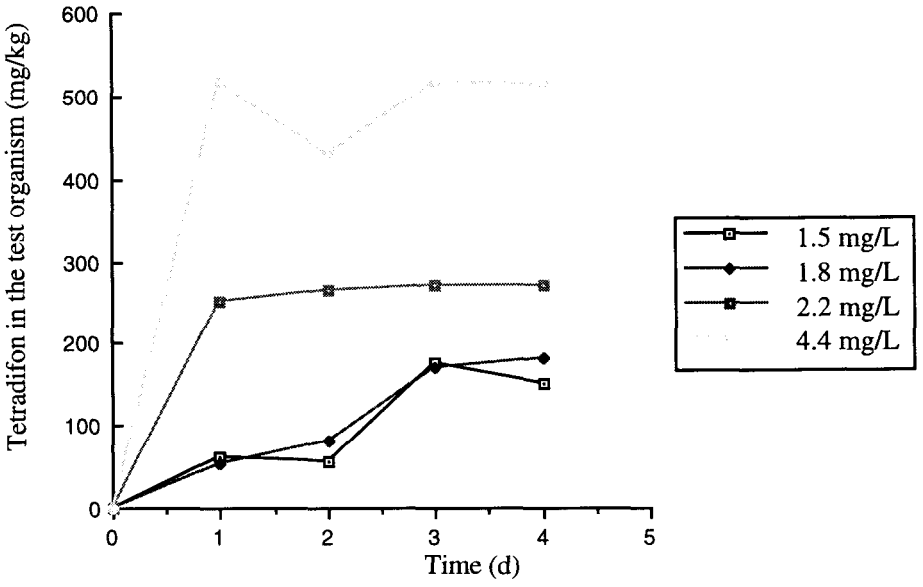

Figure 1. *Nannochloris oculata:* accumulation of different tetradifon concentrations as a function of time.

For each replicate, ten neonates from a designated brood were placed in a 30 mL glass beaker containning 25 mL for each test concentration and control. Test organisms were not fed during the testing period. Animals were introduced into the test solutions with a glass pipette, beginning with the controls and continuing up through successively increasing treatment concentrations. Observations were made at 24 and 48 hr, and mortality results recorded.

D. magna accumulation of tetradifon was evaluated under two different experimental conditions. In the first experiment, pesticide accumulation from water was tested by placing about 300 organisms (< 24 hr old) in each of four 5-L test aquaria containing concentrations of 1.5, 1.8, 2.2 and 4.4 mg/L pesticide in water. *D. magna* were not fed during the test. *In* the second test, *D.magna* were fed on N. *oculata* ($5x10^5$ cells/ml) for 21 d as well as exposed to the same tetradifon concentrations as in the first experiment. Since *D. magna* reproduced during this experiment; only the parent adults were used for the residue analysis. We repeated both experiments five times.

Samples (50 animals each) of *D. magna* were collected from each test aquarium after 24, 48, 72 and 96 hr exposure to the pesticide (in the first experiment), and after 1, 2, 3, 4, 7, 14, 21 d (in the second experiment); rinsed in water, dried to constant weight, and stored in glass vials in a freezer (T= -25°C). Water samples were also analyzed at the same exposure times.

Tetradifon residues in dried animals were determined following the method previously described for algae samples. The average recovery rate of tetradifon was 90%.

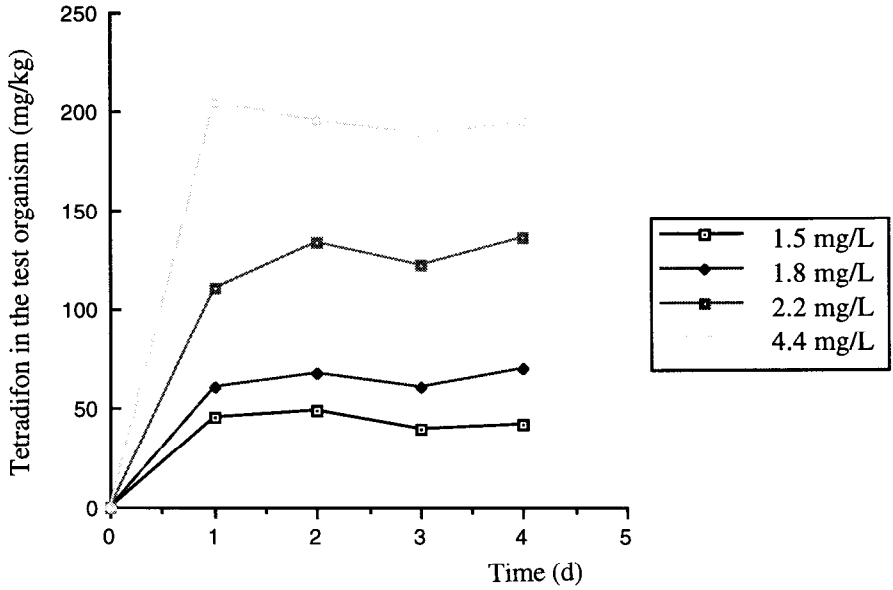

Figure 2. Tetradifon accumulation in *Daphnia magna* after exposure to different concentrations of this pesticide in the water.

Consistent water quality characteristics were maintained by transferring the cladocerans to fresh test solutions every day. A 12:12 hr 1ight:dark photoperiod was maintained. Test temperature was 22°C. Enough compressed air was bubbled through the aquaria to maintain oxygen concentration between 90 and 100%.

Data derived from the experiments were analyzed using analysis of variance followed by Duncan test ($p<0.05$) with the SPSS computer program (Nie and Hull 1981).

RESULTS AND DISCUSSION

The experiments carried out in our laboratory showed that tetradifon-LC50 (24-hr) on *Daphnia magna* was 8.9 mg/L (7.2-9.1). Based on that result we selected the sublethal pesticide concentrations for bioaccumulation experiments.

All organisms tested accumulated tetradifon from water during an initial period of exposure, until a nearly stable equilibrium was attained. The amounts accumulated were directly proportional to the amounts in water.

Nannochloris oculuta exposed to initial nominal concentrations of 1.5, 1.8, 2.2 and 4.4 mg/L of tetradifon in water accumulated high pesticide levels after 24 hr of exposure (Fig. 1); concentrations appeared to reach equilibrium at 72 hr with 1.5 and 1.8 mg/L tetradifon, and at 24 hr with the highest concentrations. It was not possible to determine how much of the pesticide accumulated by the algae was absorbed and how much was simply adsorbed to the outer surface of the organisms. Although samples of algae were rinsed with distilled water before

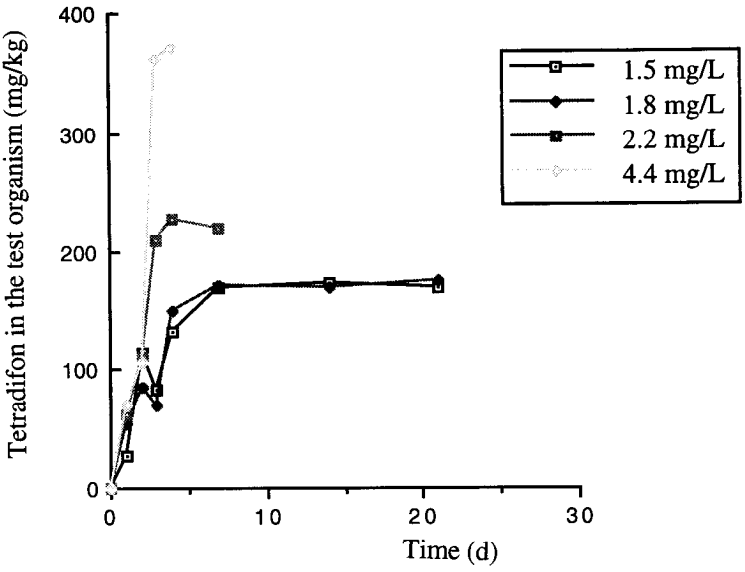

Figure 3. Tetradifon accumulation in *Daphnia magna* fed on *Nannochloris oculata* after exposure to different concentrations of this pesticide.

pesticide extraction, it is possible that a portion of the tetradifon was adsorbed. The average tetradifon concentration factor (concentration in organism divided by concentration in water when equilibrium has been reached), for all dosage levels, was about 100 (95-120) for *N. oculuta.*

Daphnia magna accumulated tetradifon during an initial period of exposure (1 d), until the equilibrium was reached (Fig. 2 and 3). We found a clear difference between daphnids exposed to the pesticide in water (without algae) and daphnids exposed to tetradifon when there were algae in the medium as food.

In the first experiment (Fig.2), *D. magna* exposed to initial concentrations of 1.5 and 1.8 mg/L tetradifon accumulated 45 and 60 mg/L after 24 hr, and the equilibrium was reached at this time. Organisms exposed to 2.2 and 4.4 mg/L pesticide accumulated 110 and 205 mg/L at 24 hr too. In the second experiment (Fig. 3), the daphnids accumulated higher pesticide amounts, and equilibrium levels were reached later (after 5 or 6 d). Organisms exposed to 1.5 and 1.8 mg/L tetradifon accumulated 168 and 171 mg/L, respectively, after 6 d treatment, while those exposed to 2.2 and 4.4 mg/L pesticide accumulated 210 and 361 mg/L after 5 d, respectively. Tetradifon concentration factors, when equilibrium was reached, were about 50 (35-62) in the first experiment and 100 (84-l 15) in the second.

Thus, the tetradifon concentrations in *D. magna* attributed to direct uptake from water were about 2-fold less than in presence of food (algae), probably because in the second experiment daphnids incorporated the pesticide both from water and from algae.

The observation that *Daphnia* accumulated more tetradifon from water plus food than directly from water confirms observations made in other experiments. Macek and Korn (1970) reported that *Salvelinus fontinalis* concentrated more DDT from food than from water. Bahner et al. (1977) observed that the accumulation of the pesticide kepone from water by algae was the dominant source of pesticide to *Mysidopsis bahia* and *Palaemonetes pugio.* Reinert (1972), on the other hand, reported that *D. magna* concentrated more dieldrin from water than from food. In his experiment, however, algae had incorporated dieldrin in a separate test and afterwords were used as daphnid food.

In the present study, the concentration factors for accumulation of tetradifon were similar in both species, so we did not observe higher accumulation in daphnids with respect to algae as is reported in other studies. Hamelink et al. (1971) observed that concentrations of DDT residues increased from one trophic level to the next. Similar results were found by Reinert (1972), he reported that dieldrin concentration factors increased from algae *(Scenedesmus obliquus)* to *Daphnia magna.*

Although the present study indicates that pesticide accumulation in organisms from food is a very important factor to be taken into account, direct accumulation of these compounds from water can contribute significantly to the total concentrations accumulated by aquatic organisms. So, it is very difficult to predict the amounts of pesticides that aquatic organisms will accumulate in the environment because many chemical and biological factors will interact.

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