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Bioconcentration and Excretion of Diazinon by Eel

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Freshwater fish are normally exposed to a great variety of pollutants in surface water of industrial and agricultural areas. Uptake and degradation of pesticides like PCP and lindane, or herbicides like atrazine, by organs of fish have been investigated in several ways (Gluth et al. 1985; Marcelle and Thome 1983; Ferrando et al. 1992). Such studies are important for the analysis of the significance of pollutants in the food chain and for the estimation of the degradation capacity of higher animals like fish.

Diazinon is one of the organophosphorus insecticides used in great quantities in the rice paddy fields of Valencia (Spain). Diazinon is relatively highly toxic to fish; the 24-hr LC50 for diazinon to bluegills and rainbow trout was 0.052 mg/L and 0.380 mg/L, respectively (Cope 1965). Morever, it is well known that diazinon causes vertebral malformation of fish at relatively low concentrations (Nishiuchi 1971). Diazinon has been reported as a persistent pesticide in aquatic environment (Kanazawa 1978; Ferrando et al. 1991). Therefore, the long term effect of diazinon to aquatic organisms should be investigated.

The eel, *Anguilla anguilla* was used as experimental animal in this investigation to analyse the distribution of diazinon in different organs of a fish.

MATERIALS AND METHODS

Diazinon (diethyl 2-isopropyl-6-methyl-4-pyrimidinyl phosphorothionate) was purchased from Cequisa (Spain). This chemical, reagent grade (95%), was used without further purification.

Eels of species *A. anguilla* (weight 20-30 g; length 16-20 cm), were collected from Albufera Lake in Valencia, Spain. They were acclimatized to laboratory conditions for 2 wk in 300-L glass tanks. The tanks were supplied with a continuous flow of tap water (temperature, 20°C; total hardness, 250 mg/L as $CaCO_3$; pH, 7.9±0.2; alkalinity, 4.1 mmol/L). A 12-hr photoperiod (8.00 to 20.00 hr) was maintained (Ferrando et al. 1987).

Diazinon was not measured (detection limit:0.01 ng/L; analyzed as described by Zweigh and Sherma 1972) in eels before exposure to this chemical.

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The eels did not respond to feeding attempts in our laboratory situation, but all animals were healthy (Larsson and Fange 1969; Holmberg et al. 1972; Van Waarde et al. 1983).

Eels were exposed to a range of concentrations of diazinon (0.06-0.3 mg/L) for 96-hr. They were not fed. Stock solutions were prepared by dissolving diazinon in acetone; appropriate quantities of these solutions were pipetted into glass aquaria (40-L) containing 35-L of test solution and ten fish. Ten more eels used as controls were kept in 35 L of clean water with the same amount of acetone as in the experimental sets (66 μL/L). Four replicates were carried out at each dosage of the insecticide in static conditions (U.S. Environmental Protection Agency 1975). The percentages of mortality were calculated for each concentration after 24, 48, 72 and 96 hr of exposure to the toxicant. The concentrations causing 50% mortality of the test animals (LC50) and their 95% confidence limits were calculated using probit methods with a computer program.

Fish used in this study were acclimated to laboratory conditions during 2 wk prior to testing (see above). Accumulation studies were performed in glass aquaria (100-L) containing 90-L of the test solution and 16 fish, and a control aquarium. The fish were then randomly divided into test groups to the aquaria with constant aeration, and acclimated for 24 hr with no feeding before initiating exposure studies. The studies were performed as static bioassays in dechlorinated tap water. Fish were exposed to a sublethal concentration of 0.056 mg/L diazinon. Stock solution was prepared by dissolving the pesticide in acetone; final acetone concentration in the aquaria was 66 μL/L. During the test, the temperature of each test water was maintained at 22 ± 1°C. Measurements of diazinon concentration in water were carried out at 0, 24, 48, 72 and 96 hr exposure. Four fish were taken after 24, 48, 72 and 96 hr. Muscle and liver tissue were analyzed for diazinon content for each fish.

Excretion experiments of diazinon (96-hr) from eel were separately carried out in dechlorinated tap water after bioconcentration of the test chemical (0.056 mg/L), in the same manner as the above bioaccumulation experiments. In this experiment, 16 fish were exposed to diazinon for 96 hr; then fish were transferred to each aquarium and clean water was supplied to them. The concentrations of the test chemical excreted from the fish to the water in the test tanks were evaluated every 24 hr. Four fish were taken at 24, 48, 72 and 96 hr, and the muscle and liver analyzed.

The concentration of diazinon in the water was determined by the following procedure. A measured volume (100 mL) of water was shaken with 30 mL hexane. Each sample was extracted three times. After drying with sodium sulfate, the solvent was evaporated to dryness on a rotatory vacuum evaporator with the water bath at 45°C. The residue was dissolved in 5 mL of hexane, as required for gas chromatography analysis (Zweigh and Sherma 1972). Samples were injected directly into the gas chromatograph (Varian-6000 chromatograph, with a flame fotometric detector. Test conditions: column temperature, 240 °C; injector temperature, 270 °C; detector temperature, 300 °C). Recoveries were in the range of 85-99%. Determination of diazinon in fish samples was carried out by the following method (Zweigh and Sherma 1972). Tissues (1-5 g) were homogenized in 50 mL acetone with a polytron-type homogenizer and keep in maceration during

24 hr. The homogenate was mixed with anhydrous sodium sulphate and filtered (Whatman No 1). The samples were extracted with 30 mL hexane in different funnels. They were shaken and the two phases allowed to separate completely. The lower (aqueous) layer was extracted again with another 30 mL hexane and combined to above hexane. We repeated the process once more. The hexane layer was dehydrated with anhydrous sodium sulphate and subjected to evaporation on a rotatory vacuum evaporator. The samples were dissolved in hexane and cleansed through Florisil cartridges. Then, they were evaporated again. The dried samples were dissolved in 5 mL hexane and injected in a Varian 6000 gas chromatograph. Average recoveries were 80% for diazinon in both tissues.

BCF was calculated by the following equation: BCF=Chemical in each part of the fish/ chemical in the water. The following equation was used for the calculation of excretion rate constants of chemicals from fish (Tsuda et al. 1989): $C= C_o \cdot e^{-kt}$, where: C= chemical concentration in each part of fish ($ng.g^{-1}$) at time t; C_o= chemical concentration in each part of fish ($ng.g^{-1}$) initially; k= excretion rate constant (hr^{-1}) and t= time (hr).

The mean value (M) and the standard deviation (SD) were calculated for each test group based on four values for each tissue from four fishes. These values were compared by analysis of variance (ANOVA), where differences were significant ($p<0.05$), the mean values were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

From the results of mortality readings at 24, 48, 72 and 96 hr exposure (Fig. 1), LC50 values and 95% confidence limits for diazinon were calculated to be 0.16 (0.10-0.23), 0.11 (0.08-0.14), 0.09 (0.07-0.11) and 0.08 (0.06-0.10) mg/L, respectively. Diazinon data from static tests are available for other fish species. Sastry and Malik (1982) conducted tests on *Channa punctatus* and found 96-hr LC50 of 11 μM. Cope (1965) determined 24-hr LC50 value of 0.052 mg/L and 0.380 mg/L respectively for *Lepomis machrochirus* and *Salmo gairdneri*.

When eels were exposed to this pesticide during the acute bioassays, they exhibited signs of restlessness, erratic swimming and convulsions; they lost their balance, secreted mucous and became pale color; they dashed against the walls of the aquaria and sank to the bottom before death. Similar changes in behavior are also observed in various fishes exposed to different pesticides (Haider and Moses 1986; Ferrando et al. 1987; 1989; 1992).

Intake and excretion of diazinon by eel exposed to 0.056 mg/L of this insecticide are shown in Figure 2. The concentration of diazinon in liver and muscle of this fish increased gradually after commencement of the experiment, in both tissues (Fig. 2).

Kanazawa (1983) demostrated that the concentration of diazinon in the whole body of the freshwater fish, topmouth gudgeon (*Pseudorasbora parva*) exposed to 10 μg/L or 50 μg/L of diazinon increased gradually after commencement of the

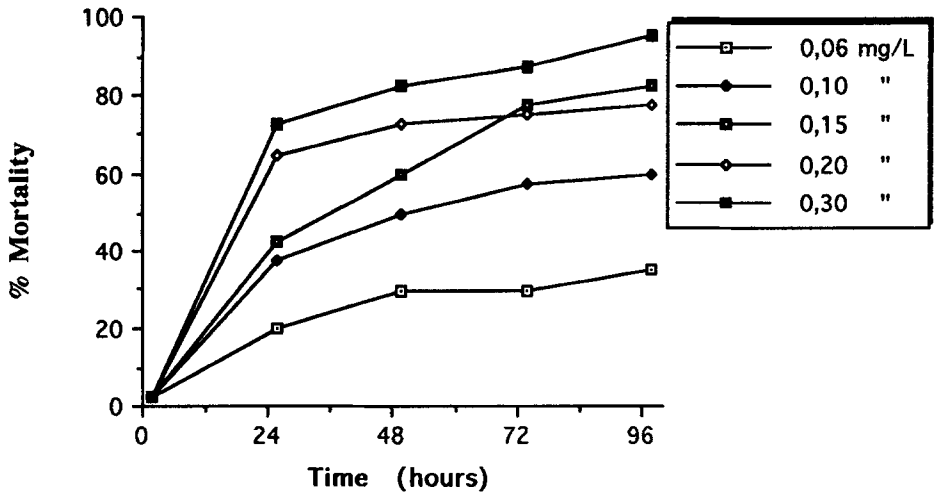

Figure 1. Dose-response curves in *A. anguilla* after 24, 48, 72 and 96 hr exposure to diazinon.

experiment, and reached the equilibrium after 3 days for 10 μg/L exposure and after 4 days for 50 μg/L exposure, respectively. The concentration of diazinon in the fish was 11.3 mg/L for 50 μg/L exposure, and the BCF value was 210.

Serious differences ($p<0.05$) were observed in the bioconcentration ratios of liver and muscle, namely those were 800 and 1600, respectively (Table 1 and 2).

The eels in this investigation were not fed, so the uptake of diazinon from the water took place through the gills and/or the skin. Such an uptake of pesticides is common in fish (Holmberg et al. 1972). Diazinon is probably distributed by the blood to different organs, where the main part is stored in fat tissues (Johnson 1968). Adsorption process of diazinon to skin (outer epidermis) could lead to an uptake via dermis to muscle, and the high accumulation rate of skeletal muscle, in this study, gives a good indication for this mechanism.

On the other hand, the bioaccumulation of diazinon in the muscle tissue may be due to the solubility of this insecticide in the muscle fat, quite high in *A. anguilla* (Ferrando and Andreu 1990).

As the muscle is possibly the largest store of fat in eel, it effectively protects the fish from the initial influx of diazinon, which is advantageous to the survival of the fish.Our results are in agreement with those found by Gunkel and Streit (1979) who studied the bioaccumulation process of the herbicide atrazine in the fish *Coregonus fera.*

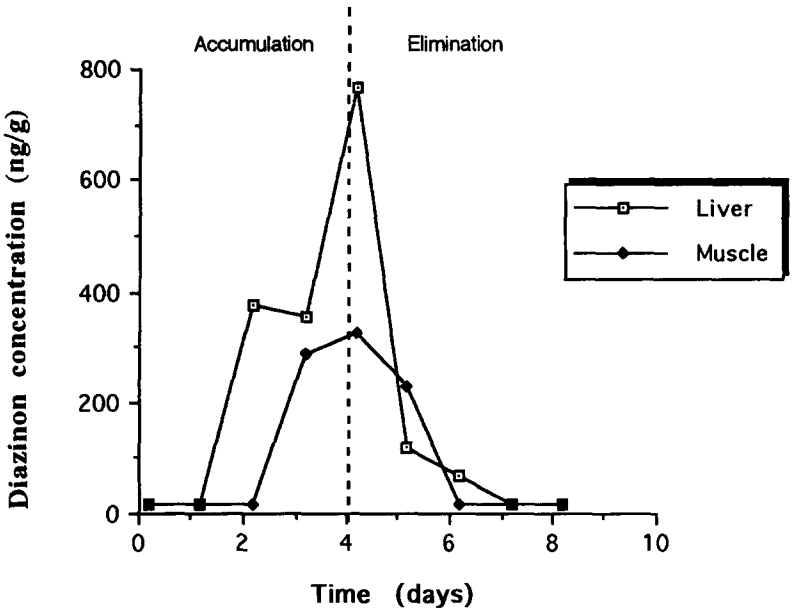

Figure 2. Bioaccumulation and elimination of diazinon in eel tissues during exposure to 0.056 mg/L.

When we transferred the animals to diazinon-free water, we observed a rapid elimination of this insecticide from the tissues (Fig.2). For example, 24 hr after transferred, 57 and 63% of the initially bioconcentrated insecticide were eliminated by the liver and muscle, respectively. The excretion of diazinon was relatively rapid approximate linearly in the fish *Pseudorasbora parva* exposed to 10 and 50 ng/g diazinon water (Kanazawa 1978).

Table 1. Accumulation and elimination of diazinon in eel liver after exposure to 0.056 mg/L diazinon (n=4; ND= no detected levels)

Diazinon in water (μg/L)	Days of exposure	Diazinon in liver ng/g	Bioconcentration ratio (BCF)
56.0	0		
0.53	1	ND	
0.10	2	80.0	800
N.D	3	90.0	
N.D.	4	70.0	
	Days after return to clean water		
	1	40.0	
	2	ND	
	3	ND	
	4	ND	

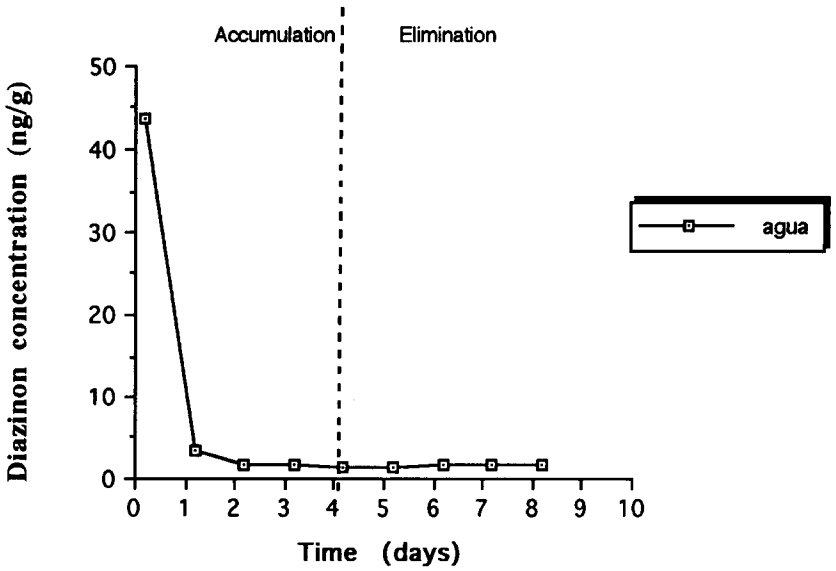

Figure 3. Diazinon levels in water in the accumulation and elimination periods.

Table 2. Accumulation and elimination of diazinon in eel muscle after exposure to 0.056 mg/L diazinon (n=4; ND= no detected levels).

Diazinon in water (μg/L)	Days of exposure	Diazinon in muscle (ng/g)	Bioconcentration ratio (BCF)
56.0	0		
0.53	1	ND	
0.10	2	160	1600
N.D	3	290	
N.D.	4	100	
		Days after return to clean water	
	1	63	
	2	ND	
	3	ND	
	4	ND	

The diazinon concentration measured in water is shown in Figure 3. During the first four days a decrease of the diazinon concentration in water can be observed. This decrease is correlated with the increase of insecticide concentration in the fish. Similar results were found by Thybaud and Le Bras (1988) who studied the absorption and elimination of the insecticide lindane by *Asellus aquaticus*.

The rate constant (k) and biological half-lives of diazinon in the selected tissues are

shown in Table 3. The excretion rate of diazinon for liver (k=0.023) was slightly faster compared with the rate for muscle (k=0.019).

A biological half-life of 25 and 26 hr was estimated for diazinon in eel liver and muscle, respectively. The half-life derived for diazinon in our species is shorter than that reported by Kanazawa (1978) whose estimates was 33 hr in the fish *Pseudorasbora parva* for the same insecticide.

Table 3. Excretion rate constants and biological half-lives of diazinon by *A. anguilla.*

	Diazinon	
Tissue	k (hr^{-1})	Half-life (hr)
Liver	0.023	25
Muscle	0.019	26

Interspecific differences could influence the elimination rates of diazinon. Some species appear to eliminate pesticides through biliary excretion (Glickman et al. 1977), while others eliminate these compunds primarily through branchial excretion although renal and biliary routes are also used (Kobayashi and Nakamura 1979).

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