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Bioconcentration Ratio of Diazinon by Freshwater Fish and Snail

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INTRODUCTION

Diazinon (diethyl 2-isopropyl-6-methyl-4-pyrimidinyl phosphorothionate) is one of the organophosphorus insecticide using a great quantities in rice paddy field of Japan. Particularly, the pesticide applied in paddy field is liable to be contaminate aquatic environment. Diazinon is relative highly toxic to fish, for example, the 24-hour LC50 of diazinon to bluegills and rainbow trout was 0.052 ppm at 75°F and 0.380 ppm at 55°F, respectively (Cope, 1965). Moreover, it is well known that diazinon cause the vertebral molformation of fish at relative low concentration, since the report by Nishiuchi (1971). Also diazinon is fairly persistence in aquatic environment, especially in sea water (Kanazawa, unpublished). Therefore, the long term effect of diazinon to aquatic organisms should be investigated.

The present paper described the difference in bioconcentration ratios of diazinon by various species of fishes and snails and the influence by body weight and the concentration of test water.

MATERIALS AND METHODS

Experimental conditions. The experiment was carried out under the continuous flow water system. Water used for this experiment was tap water purified by passing through National PJ-IRFB water cleaner containing active carbon filter. PH and total alkalinity of water were 6.8 and 32 ppm as $CaCO_3$, respectively, Constant flow micropump, Kyowa Seimitsu KHU-94 was used to obtain continuous flow of test chemical solution. Glass aquarium tank (45×24×30 cm) containing 20 liters of water was used for the experiment. Aqueous solution of containing 1 to 5 ppm of diazinon was prepared by using small amounts of acetone, and diluted continuously with one hundred times volume of clean water and poured into the aquarium tank. Flow rate of water was adjusted at about 300 ml/min, and temperature was maintained at 20±2°C. Aquarium water was aerated continuously during the experiment.

Topmouth gudgeon (Pseudorasbora parva), silver crucian carp (Cyprinus auratus), carp (Cyprinus carpio), guppy (Labistes reticulatus), crayfish (Procambarus clarkii), red snail (Indoplanorbis exustus) and pond snail (Cipangopoludina malleata) were used as a test organisms. These were caught at the river or pond in Ibaragi prefecture and used for test after acclimated to laboratory conditions for more than one month. For the experiment of intake and excretion of diazinon by fish, sixteen topmouth gudgeon were placed in each aquarium tank, and exposed

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to 10 or 50 ppb diazinon aqueous solution for 14 or 7 days, and then transfered into clean water and reared for 7 days. Commercial dry feed was given once a day throught the experiment. Concentration of diazinon in water and fish were determined at various intervals of days.

For the experiment on the differences in bioconcentration ratios (partition coefficient between fish body and water) of diazinon by the species of organisms, three or four each organisms were placed in the same aquarium tank, and exposed to about 10 ppb diazinon flow water for 7 days.

Analytical methods. At the fixed sampling time, individual organism or two fishes were taken, and washed with running water and weighed. after adding 10 grams of anhydrous sodium sulfate and 100 ml of acetonitrile, the organisms were blended in Omni-mixer (Sorvall) for 3 minutes, and filtered through 0.5 cm layer of Celite 545 on glass filter 17G3. The filtrate was concentrated below 50°C. The residue was dissolved in 25 ml of n-hexane and transfered into 100 ml separating funnel, and extracted twice with each 25 ml of acetonitrile. The acetonitrile extracts were combined, and concentrated and dissolved in a definite volume of acetone, and applied to gas liquid chromatography (GLC). A Microtek MT-160 gas chromatograph equipped with a flame photometric detector for the phosphorus response with the 526 nm filter was used. Column was 1.0 m×3 mm i.d. pyrex glass, packed with 10% silicone DC-200 on Gas Chrom-Q 60/80 mesh. Operating conditions were as follows : column temperature, 140°C, and carrier gas flow rate, N_2 60 ml/min.

For the determination of diazinon in water, 500 ml of water was sampled, and after adding 100 ml of 4% sodium chloride, extracted twice with 50 ml of dichloromethane. The extracts were combined, and concentrated in vacuo. The residue was dissolved in a definite volume of acetone, and applied to GLC as mentioned above. Recovery on the fortified fishes was 80% at 3 ppm, and that on tap water was 88% at 10 ppb.

RESULTS AND DISCUSSION

The bioconcentration ratios of seven species of freshwater organisms exposed to 10 ppb of diazinon aqueous solution for 7 days were shown in Table 1. Generally, the bioconcentration ratios of fishes were larger than that of crayfish and snails. Among fishes, the bioconcentration ratio of topmouth gudgeon was the highest value, 152 being average. A further investigation will be necessary that the differences in bioconcentration ratio of diazinon by organisms are due to whether the difference of the intake coefficient of chemicals with respiratory or the activity of metabolic enzyme.

Table 1. The bioconcentration ratios of diazinon by various species of freshwater organisms

Species	Bioconcentration ratio	
	Individual	Average
Topmouth gudgeon	136, 166, 154	152
Silver crucian carp	32.7, 37.4, 39.4, 36.7	36.6
Carp	80.3, 46.0, 69.1	65.1
Guppy	four bodys gathered	17.5
Crayfish	5.3, 4.5	4.9
Red snail	16.9, 16.4, 17.7	17.0
Pond snail	3.1, 6.2, 8.5	5.9

Exposed to 10 ppb of diazinon aqueous solution for 7 days

Table 2. Effect of the diazinon concentration in test water on the bioconcentration ratios by topmouth gudgeon

Diazinon in water, ppb	Days after exposure	Diazinon in fish, ppb	Bioconcentration ratio
13.5	0		
15.0	1	1450	96.7
11.8	3	2008	170.2
11.5	7	1359	118.2
11.5	14	1725	150.0
Clean water	Days after return to clean water		
	1	719	
	2	410	
	4	80	
	8	5	
53.8	0		
55.0	1	5967	108.0
51.3	2	7458	146.0
53.8	4	11299	210.0
52.5	7	10793	206.0
Clean water	Days after return to clean water		
	1	4787	
	2	1832	
	4	179	
	7	26	

Water temperature : 18±1°C

Intake and excretion of diazinon by topmouth gudgeon exposed to continuous flow water containing 10 ppb or 50 ppb of diazinon were shown in Table 2. The concentration of diazinon in the whole body of this fish was increased gradually after commencement of the experiment, and reached to the equilibrium after 3 days for 10 ppb exposure and after 4 days for 50 ppb exposure, respectively. Those concentration in fishes were 2.01 ppm for 10 ppb exposure and 11.3 ppm for 50 ppb exposure. In that time, a serious differences were not observed in the bioconcentration ratios of these fishes, namely those were 170 and 210, respectively, though there is a difference of five times in the concentration of diazinon in test water. However, all the fishes exposed to 50 ppb diazinon aqueous solution became to cause vertebral deformation. Accordingly this exposure test terminated for 7 days, and test fishes transfered into clean water. The excretion of diazinon was relatively rapid approximate linearly in the fishes of both exposed to 10 ppb and 50 ppb diazinon water. Ultimately, diazinon concentration in fishes were decreased from 1.725 ppm to less than 0.005 ppm on 10 ppb exposure during the period of 8 days in clean water, and from 10.793 ppm to 0.026 ppm on 50 ppb exposure during the period of 7 days, respectively.

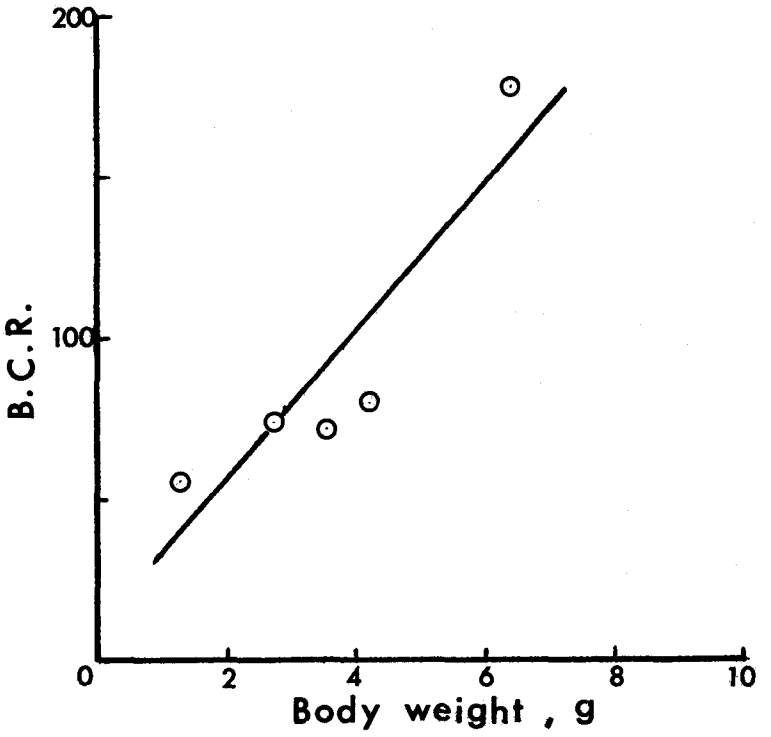

Fig. 1. The relationship between bioconcentration ratio (B.C.R.) of diazinon and body weight of topmouth gudgeon

The relationship between diazinon bioconcentration ratio and body weight of topmouth gudgeon exposed to 10 ppb diazinon aqueous solution for 7 days was shown in Fig. 1. The bioconcentration ratio of whole body of topmouth gudgeon was increased proportional to the body weight. Similarly,Buhler, et al (1969) has been already recognized the DDT residues of whole body of fish do increase with size of fish.

From the results of present study, it is well recognized that the body weight of test organisms should be make uniform for the determination of bioconcentration ratio of chemicals.

SUMMARY

The bioconcentration ratios of diazinon from water by freshwater fishes were generally larger than that of crayfish and snails. Among fishes, the bioconcentration ratio of diazinon by topmouth gudgeon was the highest value, 152 being average. However, elimination of diazinon from fish body was linearly rapid. The influence of test concentration on the bioconcentration ratio was not so much considerable. The bioconcentration ratio of diazinon in whole body of topmouth gudgeon was increased proportional to the body weight.

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