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Bioconcentration of Endosulfan in Different Body Tissues of Estuarine Organisms under Sublethal Exposure

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The organochlorine pesticide endosulfan is applied in the agricultural fields as liquid in India, nearly 2,725 metric tons of endosulfan were applied in 1986-87. An average of 600 liters of endosulfan per month was applied in the agricultural fields adjoining the Vellar estuary, India(11° 29'N Lat., 79°51'E Long.). Investigations carried out by Rajendran(1984) revealed the occurrence and distribution of endosulfan in both biotic and abiotic components of Vellar estuary. Considerable 96-hr LC50 data, based on static bioassays were obtained concerning the effects of pesticides on estuarine organisms(Lingaraja and Venugopalan 1978; Lingaraja et al. 1979; SasiBhushanaRao 1980). The results obtained from these static bioassay studies revealed that fishes were susceptible to the organochlorine pesticides than mollusks. However, except the works of Rajendran(1984), Rajendran and Venugopalan(1983) and Rajendran et al (1989), no other information is available on bioassay data for estuarine organisms exposed to endosulfan based on continuous flow through system. Further, though the available information on the uptake of endosulfan by the estuarine organisms are limited to whole body tissues, no attempt has been made to find out the extent of uptake of pesticides by the different body tissues of the estuarine organisms. Hence the present study was planned to determine the bioconcentration of endosulfan in different tissues of fishes Mugil cephalus, Mystus gulio, oyster Crassostrea madrasensis and clam Katelysia opima based on the measured concentration of endosulfan in the experimental medium of the continuous flow through system for a period of 10 d.

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MATERIALS AND METHODS

The test organisms fishes (M. cephalus=100-110 mm, M. gulio=110-120 mm) oysters (15-20 mm) and clams (10-15 mm) were collected from the natural populations of the Vellar estuary, India, and employed for bioconcentration studies. The acclimation and experimental conditions were already described (Rajendran and Venugopalan 1983, Rajendran et al 1989). The collected organisms were held for two weeks in large rectangular aquarium tanks containing estuarine water. During acclimation, the organisms were fed with clam meat and plankton soup. The continuous flow through system was employed for the present study. A series of 35L capacity rectangular fiber glass tanks (considered non-toxic) having smooth inner surface were used. Filtered estuarine water was used as test medium. Pesticide solution was kept in the dosing apparatus and allowed to flow into the mixing tank and then allowed to drain into the experimental tank. The flow rate was 50 ml/min. The physico-chemical parameters of the experimental medium collected from Vellar estuary were: temperature 28 $\pm$ 0.5 C, salinity 27.0 $\pm$ 0.5‰, pH 8.0 $\pm$ 0.2 and dissolved oxygen 4.05 $\pm$ 0.3 mL/L. Based on the acute toxicity data (96hr) obtained by Rajendran et al (1989) for endosulfan to these test organisms, three different sublethal concentrations were selected for the present study. The uptake of endosulfan from the ambient medium by the test organisms in their tissues exposed to three different sublethal concentrations of endosulfan was determined separately by analyzing the tissues at the end of the test period.

Duplicate experiments were conducted with controls. Ten organisms of same size were exposed to each test concentration. The test organisms are not fed during the experiment. At the end of 10-d experimental period, the test organisms were sacrificed and the tissue samples consisting of 4 individuals from each concentration were dissected, pooled and analyzed. The tissues analyzed were gill, mantle, adductor muscle, foot and the rest of the organism for oyster and clam and liver, kidney, brain, muscle, gill and alimentary canal for fishes. Residues of endosulfan were analyzed by following the method described by Rajendran and Venugopalan (1983) and Rajendran (1984). Tissue samples were mixed with anhydrous sodium sulfate and extracted with n-hexane in a soxhlet extractor. The concentrated extracts were eluted in a silica gel column chromatography for clean up and then concentrated for GC analysis. The water samples were analyzed for endosulfan concentration by the method described by Rajendran (1984). Extracts of these samples were analyzed using RLO4 Toshniwal chromatograph, Bombay, India, equipped with a electron capture (^{63}Ni) detector. The pesticide standards supplied by USEPA, Gulf Breeze, U.S.A. were used for quantification of the pesticide. Extracts of water and tissues fortified with endosulfan

provided recoveries greater than 90%. The detection limit of endosulfan in water and tissue samples were 0.01 ug/L and 0.001 ug/g respectively. The concentrations of endosulfan were expressed on a wet weight basis without a correction factor for percent recovery. All samples were fortified with an internal standard (aldrin) prior to analysis in order to evaluate the integrity of the results obtained in the present study. Data were computed for simple correlation and analysis of variance (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

The results in Tables 1 and 2 show that the bioaccumulation of endosulfan by the different tissues of the test organisms was apparent in all the exposed concentrations. The organisms exposed to the highest concentration of endosulfan contained the highest concentration of pesticide while those exposed to the lowest concentration contained the lowest concentration. However, there are differences among the accumulated levels of endosulfan in the test organisms which might be due to the difference in the exposed concentrations of pesticide as they were calculated on the basis of their LC50 value.

During 10-d exposure period, endosulfan was accumulated by fishes M. cephalus and M. gulio 10 to 100 times of the concentration of endosulfan measured in the exposure medium(Table 1). In both the fishes, liver was found to concentrate high levels of endosulfan and the gill accumulated low concentration. The differences among the accumulated levels of endosulfan in different tissues of fishes were statistically significant ($P > 0.01$). Increased uptake of endosulfan was obvious in all the tissues of the fishes with an increase in the exposure concentration. The relationship obtained in the present study between the exposure concentration and the endosulfan concentration in the tissues was linear($P > 0.01$). Unlike the levels of endosulfan in tissues, the bioconcentration factors showed an inverse relationship with the exposure concentration. No residues of endosulfan were determined in control organisms in the present study and the levels of the pesticide in control organisms might be lower than the detection limits. The levels of endosulfan in M. cephalus were in descending order: liver, brain, kidney, alimentary canal, muscle, gill. The similar trend was also observed in M. gulio.

As observed in fishes, the oyster and clam were also able to accumulate endosulfan in all the body tissues analyzed (Table 2). Linear relationships($P > 0.01$) were also observed between the exposure and accumulated concentrations of endosulfan in oyster and clam as noticed in fishes. In oyster, the adductor muscle concentrated to higher levels of endosulfan whereas in clam, the foot and adductor muscle were able to accumulate the maximum concentrations of the

Table 1. Accumulation of endosulfan in different tissues of fishes exposed to various sublethal concentrations for a period of 10 d (n=5 for tissue samples).

Tissue/organ	*Mugil cephalus*			*Mystus gulio*		
	Water concentration (ug/L)	Tissue concentration (ng/g) $\bar{X}\pm SD$	Concentration factor	Water concentration (ug/L)	Tissue concentration (ng/g) $\bar{X}\pm SD$	Concentration factor
Gill	0.13	1.37±0.15	10.5	0.20	2.01±0.07	10.1
	0.63	6.34±0.94	10.1	0.98	9.49±0.36	9.7
	1.25	13.03±0.83	10.4	1.95	15.41±0.84	7.9
Liver	0.13	12.55±1.22	96.5	0.20	42.47±0.97	21.2
	0.63	61.10±6.90	97.0	0.98	186.65±4.29	19.0
	1.25	110.17±7.15	88.1	1.95	347.63±7.09	17.8
Kidney	0.13	2.73±0.24	21.0	0.20	7.39±0.36	37.0
	0.63	13.24±0.72	21.0	0.98	24.82±1.56	25.3
	1.25	24.94±0.85	19.9	1.95	42.19±0.73	21.6
Brain	0.13	4.22±0.46	32.5	0.20	10.16±0.76	50.8
	0.63	19.89±1.46	31.6	0.98	36.84±1.12	37.6
	1.25	38.10±0.80	30.5	1.95	53.51±0.96	27.4
Muscle	0.13	2.42±0.20	18.6	0.20	3.50±0.35	17.5
	0.63	11.42±0.53	18.1	0.98	16.71±0.55	17.1
	1.25	23.06±1.80	18.4	1.95	35.42±0.75	16.6
Alimentary canal	0.13	2.67±0.36	20.5	0.20	4.25±0.29	21.3
	0.63	12.61±0.58	20.0	0.98	19.64±0.69	20.0
	1.25	23.69±1.03	19.0	1.95	38.47±0.91	19.7

Table 2. Accumulation of endosulfan in different tissues of mollusks exposed to various sublethal concentrations for a period of 10 d (n=5 for tissue samples).

Tissue/organ	*Crassostrea madrasensis*			*Katelysia opima*		
	Water concentration (ug/L)	Tissue concentration (ng/g) $\bar{X}\pm SD$	Concentration factor	Water concentration (ug/L)	Tissue concentration (ng/g) $\bar{X}\pm SD$	Concentration factor
Gill	0.14	4.58±0.32	32.7	0.14	4.51±0.37	31.4
	0.71	22.23±0.68	31.3	0.71	15.74±1.11	22.2
	1.41	42.30±1.25	30.0	1.42	24.34±2.08	17.1
Mantle	0.14	1.97±0.13	13.8	0.14	2.89±0.52	20.6
	0.71	8.92±0.56	12.6	0.71	11.25±1.17	15.8
	1.41	16.13±0.64	11.4	1.42	15.23±1.90	10.7
Adductor muscle	0.14	11.15±0.40	79.6	0.14	9.41±0.79	67.2
	0.71	47.73±0.99	67.2	0.71	31.41±1.67	29.3
	1.41	87.15±0.94	61.8	1.42	41.56±1.63	29.3
Foot	0.14	9.80±0.41	70.0	0.14	8.77±1.00	61.1
	0.71	47.87±1.29	67.4	0.71	33.55±1.78	47.1
	1.41	58.85±0.75	41.7	1.42	42.55±1.61	30.0
Rest of the organism	0.14	7.22±0.17	51.6	0.14	5.76±1.06	41.1
	0.71	34.77±0.60	49.0	0.71	22.37±1.96	31.5
	1.41	64.79±3.01	46.0	1.42	35.01±2.23	24.6

pesticide in all the exposure concentrations. As observed for fishes, statistically significant differences ($P > 0.01$) were observed among the accumulated pesticide concentrations in different tissues of clams and oysters. At the lowest sublethal concentration used in the present study, the different tissues could concentrate low concentration of the pesticide (Table 2). The descending trend observed in C. madrasensis was as follows: adductor muscle, foot, rest of the organisms, gill, mantle and in K. opima, the same was in the following order: foot, adductor muscle, rest of the organism, gill, mantle.

The test organisms exposed to all concentrations of pesticide contained significantly ($P > 0.01$) higher concentrations of pesticide. Organisms exposed to the lowest concentration of endosulfan were significantly different from animals exposed to the other two higher concentrations ($P > 0.01$). No mortality was observed in controls as well as in the different sublethal concentrations of endosulfan as the selected pesticide concentrations were approximately from 0.01 to 0.1 toxic unit of the median lethal concentration of endosulfan for these test organisms reported by Rajendran et al. (1989).

Information on the uptake of pesticides, in general, by the tropical organisms and endosulfan in particular are rather scanty. Though results were available on the extent of accumulation of endosulfan in their whole body tissues of M. cephalus (Macek and Korn, 1970; Rajendran, 1984), Carassius auratus (Schoettger, 1970), Mytilus edulis (Roberts,1972), and fish and oyster (Schimmel et al 1977), studies on the extent of accumulation in different tissues of the organisms are very limited (Rajendran and Venugopalan, 1983). Macek and Korn (1970) observed maximum bioconcentration of endosulfan in M. cephalus, the factors being 2429 in edible tissues and 2755 in whole body tissues for an exposure of 28 days. Ernst (1977) reported factors of 600 for M. edulis in 50 hr. Studies of Roberts (1972) showed that M. edulis concentrated endosulfan to a maximum of 22 times that much of the water in their tissues. Rajendran and Venugopalan (1983) reported that the adductor muscle and foot of the oyster could accumulate higher amount of organochlorine pesticides under sublethal exposure. In the present study also, the adductor muscle and foot of the clam and oyster could accumulate higher amounts of endosulfan. In the present study, mantle and gill of oyster and clam concentrated low concentration of endosulfan as observed by Rajendran and Venugopalan (1983) for the oyster.

Schoettger (1970) reported the higher endosulfan levels in brain, liver and gill of gold fish Carassius auratus. The liver, brain and kidney of M. cephalus and M. gulio accumulated higher amounts of endosulfan. Higher concentrations of DDT and lindane accumulated in liver and

brain of estuarine fishes under laboratory conditions as already reported by Rajendran (1984). In fishes, studies with labeled DDT revealed that 85% of DDT was stored in the liver. Liver of fishes acts as a large sink of the lipid soluble pesticide, a function seemed to be taken by the adipose tissues in mammals. The gill and skin of the test organisms are first exposed to the pollutants, which may damage severely the cell structure of the organs as pointed by Lingaraja et al(1979) and it might have reduced the uptake and storage capacity of the gill. The results of the present study revealed that there were considerable differences in the levels of the pesticide and it could be inferred that the uptake of pesticide mainly depends upon the storage capacity of the different organs. The high concentration of endosulfan in different tissues of fishes and shellfishes may be detrimental to the biological activities of the organisms. In future, in addition to the uptake studies, the depuration of the pesticides by the tissues should also be studied.

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