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Toxicity of Endosulfan to the Freshwater Fish *Cirrhinus mrigala*

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Widespread application of endosulfan (Thiodan(R), 6,7,8,9,10,10-hexachloro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) to control bollworms of cotton, resulted in the contamination of the aquatic environment in Guntur district, S. India with the residues of endosulfan (RAO et al. 1979). Of the many pesticides whose toxicity to fish has been reported, endosulfan is known to be one of the most toxic to fish (MAIER-BODE 1968, SCHOETTGER 1970, RAO & MURTY 1980, RAO et al. 1980, DEVI et al. in press). However, the toxicity of this compound to many Indian fish has not been studied and very little is known about the relative toxicity of the technical grade material and the two isomers of endosulfan viz., endosulfan-A and endosulfan-B, to fish. The metabolism and elimination of this compound by fish and also the biochemical changes induced by this pesticide in the various tissues of fish are little known. We are reporting the toxicity of the technical grade material and the two isomers of endosulfan (96-h LC 50 value) to the major carp, Cirrhinus mrigala (Ham.) and the metabolism and elimination of endosulfan when the fish is exposed to sublethal concentrations. The changes induced by technical endosulfan in the total protein content and the Na^+ and K^+ concentrations in the different tissues of the fish are also being described.

MATERIALS AND METHODS

Toxicity Studies:

The fish, C. mrigala (6 to 8 cm in length) were caught in Guntur channel, near Nagarjuna University campus and acclimatized to the laboratory conditions ($28^0 \pm 2^0$ C) in well aerated water. Continuous flow systems were employed to determine the 96-h LC 50 values. Technical endosulfan was obtained from National Chemical Laboratory, Pune, India. The two isomers present in the technical material were separated on a Florisil column using hexane and acetone as eluting mixtures (RAO et al. 1980). The recommendations of

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the Committee on "Methods for toxicity tests with fish, macroinvertebrates and amphibians" (ANON. 1975), were followed in conducting the toxicity tests. Examination of the well water used for conducting the tests and tissues of randomly chosen control fish revealed that they were free from the residues of endosulfan (limits of detection, 0.1 ppm). The fish were not fed during the period of acclimatization. Any batch of fish in which the mortality exceeded 5% during the period of acclimatization, was discarded. The toxicant, dissolved in acetone, was added to water in large glass reservoirs (24 to 30 L capacity) and the water containing the toxicant was let into the test chambers (10 L capacity), through thin walled polyethylene tubes at a rate of 4 L/h. Acetone was added to the controls in an amount equal to that present in the highest concentration of the toxicant employed; the maximum concentration of acetone added to the control or test tanks was less than 0.1 mL/L. The chemical parameters of water used were: total solids, 560 ppm; total hardness as $CaCO_3$, 123 ppm; total alkalinity as $CaCO_3$, 304 ppm; chlorides as Cl^-, 52 ppm; free chlorine, not detectable; dissolved oxygen, 7 to 8 ppm; chemical oxygen demand for 4 h, 0.2 ppm; pH, 8.4; turbidity, 12 silica units.

Pilot experiments were conducted to choose 5 test concentrations that resulted in mortality in the range of 10-90%; 10 fish were exposed to each concentration and the experiment was repeated thrice. The fish that did not respond to tactile stimulus were considered as dead and were removed immediately. The data were pooled to calculate the LC 50 value, which was calculated by the unweighted regression method of probit analysis (FINNEY 1971). The difference between the observed and the calculated values was tested for significance using the Chi-square test.

Metabolism of endosulfan:

The fish that survived 96-h exposure to 1.1 ppb concentration of technical endosulfan in the acute toxicity tests were employed to study the metabolism of endosulfan in the different tissues of C. mrigala. The fish were killed by decapitation and like tissues from 5 fish were pooled for the analysis of endosulfan residues and its metabolites.

The tissues were mixed with ca 15 g of anhydrous Sodium sulfate and macerated. They were extracted with acetonitrile (2 mL solvent/g of tissue), on a wrist action shaker for one hour. The acetonitrile extract was shaken with 700 mL of 2% Sodium sulfate solution and the residues were serially extracted into hexane.

The hexane extracts were pooled and dried over a column of anhydrous Sodium sulfate. The extract was further cleaned-up with activated carbon and was concentrated in a Kuderna-Danish evaporator. Thin layer chromatography with two different solvent systems (RAO et al. 1980), was employed to identify the residues and metabolites of endosulfan in the different tissues.

Total protein and Na^+ and K^+ concentration:

The fish that survived 96-h exposure to 1.1 ppb concentration of technical endosulfan in the toxicity experiments were employed to study the pesticide induced biochemical changes. The protein content of brain, kidney, liver and muscle was estimated by the method of LOWRY et al. (1951). Purified bovine albumin serum (supplied by Sigma chemical co., U.S.A), was used to prepare the protein standards. The Sodium and Potassium content of the tissues were estimated respectively by the method of TRINDER (1951) and JACOBS & HOFFMAN (1931). The difference in the tissue protein, Na^+ and K^+ values of the test and control fish was tested for significance, employing Student's t-test.

RESULTS AND DISCUSSION

Toxicity studies:

The test fish showed a gradually increasing irritability and aggressiveness as the test progressed. The 96-h LC 50 value and its 95% confidence limits (indicated in parentheses) of technical endosulfan, isomers A and B were respectively 1.3 (1.22 - 1.32), 0.6 (0.5 - 0.9) and 8.8 (7.8 - 10) ppb. The regression equation for calculating the LC 50 value was $Y = 19.1X - 34.99$ for technical endosulfan, $Y = 4.99X - 3.98$ for endosulfan-A and $Y = 5.2X - 10.36$ for endosulfan-B. Isomer-A was twice as toxic as the technical material, whereas isomer-B was about seven times less toxic than the technical grade material. RAO et al. (1980) reported that for the freshwater fish Labeo rohita, endosulfan-A is about 20 times more toxic than the technical grade material, whereas isomer-B was about 7 times less toxic. The more toxic nature of endosulfan-A than B may be a potential threat to fisheries as it is known that isomer-A is less strongly bound to soil and hence is carried by runoff into the aquatic environment, where it accumulates to a greater extent than isomer-B (BYERS et al. 1965).

Metabolism and elimination of endosulfan:

Except liver and kidney, no other tissue contained the isomers of endosulfan. The only metabolite recorded was endosulfan ether which was found in the extracts of brain, kidney and liver. Muscle extracts contained neither the known metabolites nor the isomers of endosulfan.

Earlier experiments with fish (western whitesuckers and goldfish)by Schoettger (1970) revealed that endosulfan sulfate is an intermediary product in the detoxification process, the end product being endosulfan alcohol. Endosulfan ether was reported as the principal metabolite in three tropical fish viz., Anabas testudineus (RAO & MURTY 1980), Labeo rohita (RAO et al. 1980) and Channa punctata (DEVI et al. in press). In Cirrhinus mrigala, endosulfan was degraded into nontoxic endosulfan ether as in the case of the other warm water fish studied hitherto.

It has been reported that in various animals, the metabolites of endosulfan were eliminated either through feces or urine or both. The work of SCHOETTGER (op cit.) showed that in western whitesuckers and rainbow trout, endosulfan was eliminated with bile through gut. The experiments of WAYMAN et al.(1978) with rats showed that liver and kidney were the main storage organs for the residues and metabolites of endosulfan, which were eliminated through feces and urine. The presence of isomers of endosulfan and its degradation product-endosulfan ether- in the liver and kidney, observed in the present study, suggests that these organs play a vital role in the elimination of endosulfan in C. mrigala.

Total protein and Sodium and Potassium concentration:

The biochemical changes induced in some tissues of C. mrigala, exposed to 1.1 ppb concentration of technical endosulfan are shown in Table 1.

Although there were slight changes in the protein content of the tissues of test fish when compared with those of controls, only the change observed in the kidney of test fish was statistically significant. As has already been pointed, liver and kidney appear to be the principal organs of detoxification of endosulfan in C. mrigala, and a significant decrease in the protein levels in these organs seems to indicate severe stress experienced in the process of elimination of endosulfan.

TABLE 1

Changes in the protein, Na^+ and K^+ concentrations (ug/mg wet weight of the tissue) of some selected tissues of C. mrigala[1] exposed to 1.1 ppb concentration of technical endosulfan.

Tissue	Protein		Na^+		K^+	
	Control fish	Test fish	Control fish	Test fish	Control fish	Test fish
Brain	106 ± 6	92 ± 4	10.7 ± 0.8	10.5 ± 1.2	5.4 ± 0.2	8.1* ± 0.2
Gill	114 ± 6	96 ± 12	4.7 ± 0.1	8.8* ± 1.5	5.2 ± 0.2	6.8* ± 0.1
Kidney	190 ± 18	102* ± 10	not determined		-	-
Liver	158 ± 38	148 ± 20	6.9 ± 0.3	10.6* ± 0.2	4.3 ± 0.1	14* ± 0.4
Muscle	138 ± 4	146 ± 18	9 ± 0.6	4.8* ± 1.6	4.9 ± 0.2	6.6* ± 0.1

1. Each result is the mean of three replications, with the standard deviation indicated.

* change is significant at p = 0.05 level.

There was a marked change in the Sodium and Potassium concentration of the various tissues. The Sodium concentration increased significantly in gill and liver and decreased sgnificantly in muscle of the exposed fish. The Potassium concentration was elevated in all the tissues of the test fish.

Several workers (WALKER 1963, EISLER & EDMONDS 1966, MACEK 1968) reported changes in the ionic balance in the tissues of fish exposed to pesticides. Exposure of goldfish to endrin (1 and 3 ppm) increased the Sodium ion concentration in the serum whereas a concentration of 0.1 ppb of DDT decreased Sodium and Potassium ions in the hepatopancreas of Penaeus aztecus and P. duoram by 25% (NIMMO & BLACKMAN 1972).

Sodium and Potassium have an effect on muscular irritability. The increased irritability of test fish,

which is reflected in the aggressiveness of the fish with the progress of the tests, seems to be the result of the drastic changes in the Na^+ and K^+ levels in the various tissues.

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