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Relative Toxicity of the Technical Grade Material, Isomers, and Formulations of Endosulfan to the Fish *Channa punctata*

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Endosulfan is a broad-spectrum, non-systemic, organochlorine compound of the cyclodiene group. It is being widely used in India because of its lower mammalian toxicity and shorter persistence in the environment than many other organochlorine pesticides. However, it is known to be highly toxic to fish.

Much of the earlier work on the toxicity of endosulfan to fish was conducted with either technical grade material or some formulations (MAIER-BODE 1968, SCHOETTGER 1970). However, the two isomers of endosulfan are known to differ in their toxicity to insects (LINDQUIST & DAHM 1957, BARNES & WARE 1965); isomer-A is more toxic than isomer-B. Moreover, isomer-A has been reported to be less firmly bound to the soil and hence to move into the aquatic ecosystem earlier than isomer-B, (BYERS et al. 1965, RAO & MURTY 1980). Further, it is well known that the toxicity of a pesticide is altered when it is formulated. Yet, there is only one report on the relative toxicity of technical endosulfan, the two isomers and formulations to fish (RAO et al.). Hence, a comparative study of technical endosulfan, its isomers and two formulations viz., 35% EC (emulsifiable concentrate) and 4% dust, to the freshwater fish Channa punctata (Bloch) was attempted. Further, the degradation of technical endosulfan by this fish is also being reported.

MATERIALS AND METHODS

Technical grade endosulfan (96% pure) was obtained from the National Chemical Laboratory, Pune, India. Samples of the two formulations were obtained from the Government Pesticide Testing Laboratory, Guntur (S. India) and the active ingredient was calculated on the basis of colorimetric analysis (MAITLEN et al. 1963) of serial dilutions.

The isomers were separated on a Florisil column (22 mm i.d., containing about 20 g of Florisil activated at 110°C for 24 h and deactivated just before use with 1 mL of water). About 500 mg of technical grade endosulfan was dissolved in 5 mL of hexane and transferred to the column which was eluted with 100 mL of n-hexane followed by 150 mL of 0.5% acetone in hexane and 150 mL of 2% acetone in hexane. Isomer-A was eluted in hexane and 0.5% acetone in hexane fractions. Separation of the two isomers was confirmed by thin-layer chromatography.

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TLC plates (250 µm thick) were prepared according to the method of MOATS (1966) and were developed in 4% acetone in heptane. Spots were visualised by exposure to UV light. The R_f values on TLC plates were reported earlier (RAO et al. 1980).

Toxicity tests.

Experiments were conducted employing continuous flow of pesticide containing water, as recommended by the Committee on the "Methods for toxicity tests with fish, macroinvertebrates and amphibians" (ANON. 1975). Channa punctata (6 to 9 cm length) were obtained from a tank near the Nagarjuna University Campus (Guntur Dt., S. India). Fish were acclimatized in the laboratory at 30°± 2°C. Fish were not fed during the period of acclimatization and test. Different tissues of randomly selected fish were extracted with acetonitrile, transferred into hexane, cleaned-up and concentrated. The extract was spotted on TLC plates to test for the presence of endosulfan residues and the fish were found to be free from the residues of endosulfan (sensitivity of the method is 0.1 µg).

The chemical constituents of the water used for test were: turbidity - 8 silica units; pH - 8.4; total hardness - 152 mg/L; carbonate hardness as $CaCO_3$ - 152 mg/L; chloride as Cl - 54 mg/L; Calcium - 25.6 mg/L; Magnesium - 21.1 mg/L; phenolphthalein alkalinity - 10 mg/L; methyl orange alkalinity - 320 mg/L; sulfate as $BaSO_4$ - 148 mg/L; dissolved oxygen 8-10 ppm and chemical oxygen demand - 0.6 mg/L. This water was free from residues of endosulfan.

Aliquots of the stock solution of the pesticide in acetone (10 µg/mL) were added to 16 L glass reservoirs, to obtain desired concentrations of the pesticide in water (calculated on the basis of the active ingredient of the pesticide). Pesticide containing water was let into the test containers (12 L capacity), using thin-walled polyethylene tubes, with flow-regulators. On an average, the flow rate was about 3 L/h and about 70 L of water flowed through the test containers in a 24-h period. The loading of the test tanks was maintained within the range recommended by the above cited committee.

To the control fish, acetone in an amount equal to that present in the highest concentration of the toxicant tested was added. If there was more than 5% mortality in any batch of fish during acclimatization, that batch was discarded.

Pilot experiments were conducted with a wide range of test concentrations from which five concentrations that resulted in a mortality in 10-90% range (necessary for the method of calculating the LC 50 values followed) viz., 3.5-6.5 ppb for technical endosulfan, 1-5 ppb AI for 35% EC, 0.05-0.45 ppb for endosulfan-A, 3-11 ppb for endosulfan-B and 12-20 ppb AI for 4% dust, were chosen. Each test was repeated twice with 10 fish in each concentration. Since the mortality recorded for a particular concen-

tration was the same in all the three tests, the results were pooled and the 96-h LC 50 values were calculated by using the unweighted regression method of probit analysis (FINNEY 1971).

Fish that showed no respiratory movements and no response to tactile stimulus were recorded as dead and were removed immediately after death.

Uptake and metabolism.

Brain, gills, kidney and liver of the fish that survived 96-h exposure to 6.5 ppb concentration of technical endosulfan were used for studying the uptake and metabolism of endosulfan. Whole organs from a number of fish were pooled and the tissues were extracted with acetonitrile in a mechanical flask shaker, after grinding the tissues with sodium sulfate (solvent to tissue ratio was 2:1). The extract was filtered through a cotton plug and partitioned into n-hexane in a separatory funnel. The extract was cleaned-up, following the method of KATHPAL & DEWAN (1975). The cleaned-up extract was concentrated in a Kuderna-Danish evaporator and the concentrate was spotted on TLC plates.

The metabolites of endosulfan were prepared in the laboratory according to the method of LINDQUIST & DAHM (1957) and were identified on TLC plates.

RESULTS AND DISCUSSION

There were no deaths in the controls. The test fish swam erratically, often surfacing, followed by loss of equilibrium. Subsequently they sank to the bottom of the test container and erratic opercular movements preceded death. The color of the skin became progressively pale during the period of test. These symptoms were common for all the toxicants tested. In the case of technical endosulfan, isomer-A and 35% EC, the skin became very dark after the death of the fish.

The toxicity of the different toxicants is shown in Table 1.

The regression equations for calculating the LC 50 values are: $Y = 2.1X + 2.48$ for endosulfan-A; $Y = 2.58X - 1.16$ for 35% EC; $Y = 7.75X - 15.8$ for technical endosulfan; $Y = 2.86X - 3.07$ for endosulfan-B and $Y = 8.78X - 23.1$ for 4% dust.

The decreasing toxicity of different toxicants to C. punctata was in the following order: isomer-A, 35%EC, technical endosulfan, isomer-B and 4% dust. Chi-square test showed that the difference between the observed and calculated values was not significant.

LINDQUIST & DAHM (1975) and BARNES & WARE (1965) with houseflies and RAO et al. (1980) with the fish Labeo rohita, reported that technical endosulfan was less toxic than isomer-A and more toxic than isomer-B. However, WOLFENBARGER & GUERRA (1972) reported that technical material was more toxic than either of the

TABLE 1

Relative toxicity of technical endosulfan, isomers and formulation products of endosulfan.

Compound	96-h LC 50 value (ppb)	95% confidence limits	Calculated X^2 value*	Relative to technical material
Isomer-A	0.16	0.11-0.21	4.2	30 times more toxic
35% EC	2.5	1.9-3.2	1.7	2 times more toxic
Technical material	4.8	4.4-5.2	1.5	-----
Isomer-B	6.6	6.6-6.7	1.2	0.7 times as toxic
4% dust	16	15-17	0.8	0.3 times as toxic

* not significant at p = 0.5

isomers to the bollworm. The results of the present work agree with the former reports. Further, in the present study 35% EC was observed to be more toxic than the technical material. This may be attributed to the greater toxicity of the emulsifiers in the former and also the uniform distribution of the toxicant molecules through the test medium.

The relatively higher toxicity of isomer-A than isomer-B is ecologically very significant. It was reported that isomer-A is less strongly bound to the soil and hence is carried by run-off into the aquatic environment earlier than isomer-B (BYERS et al. 1965, RAO & MURTY 1980).

Uptake and metabolism of the pesticide.

Thin-layer chromatograms confirmed the uptake of endosulfan by all the tissues studied i.e., brain, gills, kidney and liver. The metabolites of endosulfan observed in the different tissues were endosulfan lactone and endosulfan alcohol in all the tissues studied, endosulfan hydroxyether in brain, gills and kidney and endosulfan ether in kidney and liver. Both isomers were recorded in all the tissues; the largest quantities were present in liver and kidney where the principal metabolite was endosulfan ether. Endosulfan sulfate, earlier reported as one of the metabolites from various experimental animals (GORBASCH et al. 1968, DEEMA et al. 1966, SCHOETTGER 1970, WAYMAN et al. 1978 and RAO et al. 1980) was not recorded in the present study.

The observation that non-toxic endosulfan ether was the chief metabolite in liver and kidney indicates the active involvement of these two tissues in the process of detoxification.

DEEMA et al. (1966) and WAYMAN et al. (1978) also suggested that kidney and liver were active in the metabolism and elimination of this pesticide by rodents.

In conclusion, we found that technical endosulfan, its isomers and two formulations were highly toxic to the fish Channa punctata, the toxicity being in the parts per billion range, whereas for many organochlorine pesticides, the toxicity is in parts per million range. Hence, although endosulfan is considered as a safe pesticide, it is likely to pose serious problems in the aquatic environment.

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