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Acute Toxicity of Organochlorine Pesticides to Fishes and Shellfishes of a Tropical Estuary

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Abstract In the present study, acute toxicity data were obtained for different organochlorine pesticides (DDT, lindane and endosulfan) to estuarine fishes and shellfishes under continuous flow through system. Median lethal concentration (LC_{50}) was obtained for different time intervals (24, 48, 72, 96 and 120 hr.) and the toxicity curves were drawn using these values to determine the incipient lethal concentration (ILC_{50}) values. Increase in the exposure time decreased the LC_{50} values of the pesticides to the organisms. DDT was found to be highly toxic to the organisms tested followed by lindane and endosulfan. Of the organisms studied, *Mugil cephalus* was found to be most sensitive to the pesticides. Bivalves were highly resistant to the pesticides tested than fishes. Behavioral responses of the test organisms to the toxicant was also described in detail. Safe concentrations and the relative resistance of these organochlorine pesticides to the organisms were discussed.

INTRODUCTION

Bioassay, in its widest sense is the measurement of the potency of any stimulus, physical, chemical, biological, physiological or psychological by means of the reactions that produces in living matter (FINNEY, 1971). Bioassays have been used for centuries and may have began with the first food tasters who protected their masters from food poisoning. Since then, the use of bioassays has been proliferated. With increased interest from public and scientific communities in man's use, abuse and misuse of the aquatic environment, a diverse array of bioassay methods has evolved. This may probably be the most useful method available to toxicologists for predicting the potential hazards of a pollutant.

KATZ (1971) reviewed the role of application of aquatic bioassays. The publications of PERKINS (1972), STEPHEN and MOUNT (1973) and WALDICHUK (1973) were related to marine bioassays. SPRAGUE (1969, '70, '71, '73) presented a critical review of bioassay methods and analyses of the data. NEGILSKI (1975) emphasized that accepted procedures, analyses and data reporting were necessary if bioassay informations were to be meaningful. Different bioassay methods have been reported by KLVERCAMP (1974), CRAIG (1975), PARKER *et al.* (1977) and DAVIS *et al.* (1978) which are of immense use. The status of aquatic toxicology including various end points, fish species used and methodologies was reviewed by TUCKER and Lettzke (1979). Most of the bioassay experiments were designed in accordance with the guidelines given by Sprague (1973), Cmttao (1975), Epa (1978) and Apha (1985).

In recent years, static and continuous flow through bioassays are the two important types of toxicity experiments used largely by toxicologists. The test procedures call for a flow through bioassay where fresh toxicant is continuously presented to test animal and allow the investigation of dynamic environmental processes. The use of this system is essential in bioassays where it is necessary to prevent or reduce changes in the concentration of the test toxicant due to complexation, volatilization, degradation, bioaccumulation or adsorption of the toxicant (Sprague, 1973; Epa, 1978; Apha, 1985). Warlen and Engel (1980) employed both static and continuous flow through bioassays and inferred that the latter generally give more reliable and applicable information than that can be obtained from the former.

Water pollution by pesticides necessitates the establishment of water quality criteria and the estimation of safe concentration for the aquatic organisms. Safe concentration can be defined as the maximum concentration of a pollutant that has no observable harmful effects after long term exposure (Apha, 1985). Sprague (1973) defined the term threshold lethal concentration as the concentration in which there is no evidence of ill effect on test organisms despite prolonged exposures. Incipient lethal level is the concentration at which acute toxicity ceases, that is, the concentration at which 50% of the population can live for an indefinite time. This had been described in different terms by different investigators e. g., incipient lethal level (Fry, 1947; Apha, 1985); ultimate median tolerance limit (Doudorff *et al.,* 1951); lethal threshold concentration (Llyod and Jordan 1963) and asymptotic LC_{50} (Ball, 1967). Bioassays are of primary value in determining the safe concentration of toxicants and it can be calculated by following this formula $AF=MATC/Incipient\ LC_{50}$ followed by $SC=AF \times ILC_{50}$ (Apha, 1985). Application factors (AF) are experimentally derived and are multiplied by acute bioassay results in an attempt to obtain estimates of chronically safe concentration (SC) and MATC is the concentration of toxicant that may be present in the water without causing significant effect to organisms. Among the pesticides, organochlorine pesticides are reported to be highly toxic to nontarget aquatic organisms. Jhingran (1974) in his publication on pollution of aquatic systems in India indicated complete loss of fish life, primarily from increased use of pesticides in agriculture. The toxicity of organochlorine pesticides to zooplankton (Rajendran and Venugopalan, 1988) and to planktonic eggs (Venugopalan and Sasi Bhushana Rao, 1979) of Vellar estuary was reported. Eventhough number of investigations were carried out in India on the toxicity of pesticides to fresh water organisms, very limited studies have been done on estuarine organisms. Since all these experiments were static one, the more realistic continuous flow through system was employed in the present study. Further, residues of organochlorine pesticides in both abiotic and biotic components of Vellar estuary were monitored by Rajendran (1984). In the present study, three organochlorine pesticides such as DDT, lindane and endosulfan (Technical grade supplied by the Union Carbide of India and All India Medical Corporation) were selected to determine the toxicity to the estuarine organisms and an attempt was also made to find out the safe concentration of these pesticides to the organisms.

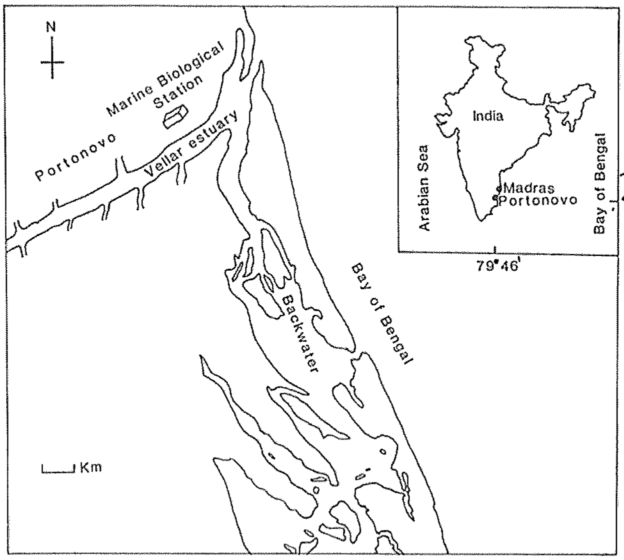

Fig. 1. Map of the study area.

MATERIALS AND METHODS

The continuous flow through system used in the present study was designed as per the descriptions of MOUNT and BRUNGS (1967) and RAJENDRAN (1984). A series of 35 liter capacity rectangular fiber glass tanks (considered nontoxic) having smooth internal surface were used. Estuarine water of required salinity was used after filtering the same through a sintered glass column packed with glass wool. Pesticide solution was prepared and kept in the dosing apparatus and allowed to flow into the first mixing tank. The mixed water was then allowed to drain into the experimental tank. The flow of pesticide solution and water was so adjusted as to get the required concentrations of pesticides. The flow of the ongoing and outgoing test water was adjusted to provide 90% replacement of water in 8 to 12 hours and constant surface level was maintained to keep the dissolved oxygen content at saturation level and also ensure the correct pesticide concentrations.

The test organisms used were of similar size groups with only a narrow range of variation in length. The oyster *Crassostrea madrasensis* (15-20 mm), clams *Katelysia opima* (10-15 mm) and *Meretrix casta* (12-17 mm) and fishes *Mugil cephalus* (100-110 mm) and *Mystus gulio* (110-120 mm) were collected from the natural population of Vellar estuary (11°29' N Lat., 79°46' E Long.), South India (Fig. 1). The test organisms were acclimatized in the laboratory for ten days and fed with shellfish meat and plankton soup. The acclimation water as well as test water were pumped from the Vellar estuary and stored in a sump. The physico-chemical characteristics of the water were: salinity 27.4±0.5‰, pH 8.0±0.2, temperature 28.0±0.5°C and dissolved oxygen 4.12±0.2 ml/l. The test concentrations were initially chosen arithmetically and this was later followed by logarithmic scale. Two sets of controls were maintained with and without solvent. Acetone was used as carrier solvent for all the pesticides. Ten organisms were tested for each test concentration and the experiments were conducted in duplicate. The mortality was observed for a period of five days at regular intervals. The bivalves were considered dead when they kept open their shell valves even after gentle prodding. The fish were considered dead when there was no respiratory movements. The behavioral changes of the test organisms were also monitored. The bioassays were terminated at the end of the test period. The LC_{50} and 95% confidence limits were determined by probit analysis (FINNEY, 1971) using a computer (TDC 316).

RESULTS

Mollusks

DDT was toxic to all the three mollusks tested. The LC_{50} values are shown in Table 1. *C. madrasensis* was more sensitive to DDT than *K. opima* and *M. casta*. 50% survival of oysters was appreciably affected by exposure to a concentration of 9.36 ppb of DDT for 96 hours. The 96 hour LC_{50} values of DDT for *K. opima* and *M. casta* were 13.19 and 14.16 ppb respectively (Table 1).

Lindane was also toxic to all the mollusks, with *K. opima* being the most sensitive of the three and the 96 hour LC_{50} value was 18.26 ppb. *C. madrasensis* was the least sensitive to lindane with a 96 hour value of 26.36 ppb. *M. casta* was slightly more susceptible to lindane than *C. madrasensis* exhibiting a 96 hour LC_{50} value of 22.98 ppb (Table 1). 96 hour LC_{50} value of endosulfan to *C. madrasensis* was 14.13 ppb. *K. opima* and *M. casta* were also more or less equally susceptible to endosulfan, with 96 hour LC_{50} values of 14.22 and 15.20 ppb respectively (Table 1).

In the present study, DDT was found to be more toxic than lindane and endosulfan. The order of toxicity was DDT>endosulfan>lindane. The sensitiveness of the three mollusks studied for the pesticides was in the order of *C. madrasensis*>*K. opima*>*M. casta*. The behavioral responses of the mollusks varied in accordance with the test concentration of the three pesticides. Relatively reduced activity was exhibited during the early hours of exposure at all concentrations. The siphons were extended and food searching movements contributed but eventually the clams appeared to have been paralyzed as they could not retract their siphons even when mechanically stimulated. The pumping activity of the clams was also affected by the pesticides. When compared to controls, the surviving mollusks displayed excessive mucus secretion, sluggishness, gaping of shell valves and per-

Table 1. LC_{50} values of organochlorine pesticides for *Crassostrea madrasensis, Katelysia opima* and *Meretrix casta* (ppb).

Pesticide	Time (hr)	*C. madrasensis*	*K. opima*	*M. meretrix*
DDT	24	24.82(35.42−17.40)	16.48(22.60−12.02)	24.04(32.68−17.69)
	48	16.19(21.13−12.41)	15.78(22.60−12.02)	20.00(26.26−15.24)
	72	15.75(20.36−12.18)	14.62(21.62− 9.88)	14.42(17.66−11.78)
	96	9.36(11.30− 7.75)	13.19(15.35−11.34)	14.16(16.69−12.00)
	120	9.28(11.04− 7.81)	11.05(13.80− 8.85)	11.28(13.50− 9.42)
Lindane	24	38.31(40.43−36.30)	38.46(53.62−27.59)	25.15(29.45−21.49)
	48	37.17(43.11−32.04)	37.58(48.33−29.22)	24.02(26.87−21.47)
	72	30.57(36.79−25.41)	22.95(32.24−16.34)	23.92(27.42−20.86)
	96	26.36(28.52−24.36)	18.26(22.61−14.74)	22.98(25.22−20.94)
	120	25.09(26.93−23.37)	14.95(18.70−11.95)	20.19(22.21−18.35)
Endosulfan	24	27.24(36.63−20.26)	22.36(27.87−17.94)	23.20(28.40−18.95)
	48	20.54(26.16−16.12)	17.64(21.17−14.70)	16.75(20.87−13.44)
	72	18.09(23.87−13.70)	14.48(16.70−12.33)	16.11(18.16−14.30)
	96	14.13(18.52−10.79)	14.22(17.62−11.47)	15.20(16.80−13.75)
	120	12.58(14.82−10.68)	13.05(14.38−11.84)	12.85(14.28−11.57)

Upper and lower confidence limits were given in parenthesis

Table 2. LC_{50} values of organochlorine pesticides to *Mugil cephalus* and *Mystus gulio* (ppb).

Pesticide	Time (hr)	*M. cephalus*	*M. gulio*
DDT	24	1.19 (1.24− 1.15)	6.38 (6.93− 5.87)
	48	0.72 (0.75− 0.69)	2.61 (3.13− 2.17)
	72	0.40 (0.46− 0.34)	2.18 (2.84− 1.90)
	96	0.26 (0.31− 0.22)	1.13 (1.30− 0.98)
	120	0.20 (0.23− 0.18)	1.08 (1.32− 0.89)
Lindane	24	2.49 (2.81− 2.21)	21.70 (25.26−18.64)
	48	1.86 (2.16− 1.61)	20.16 (24.19−16.80)
	72	1.83 (1.98− 1.69)	16.92 (19.17−14.92)
	96	1.35 (1.64− 1.11)	11.15 (12.65− 9.84)
	120	0.48 (0.62− 0.37)	10.07 (11.74− 8.63)
Endosulfan	24	24.17 (27.39−21.32)	26.05 (31.33−21.66)
	48	23.32 (24.89−21.85)	22.61 (24.36−21.00)
	72	20.14 (21.53−18.84)	20.36 (21.81−19.00)
	96	12.52 (14.25−11.00)	19.52 (22.51−16.92)
	120	12.08 (13.70−10.66)	16.08 (19.50−13.27)

Upper and lower confidence limits were given in parenthesis

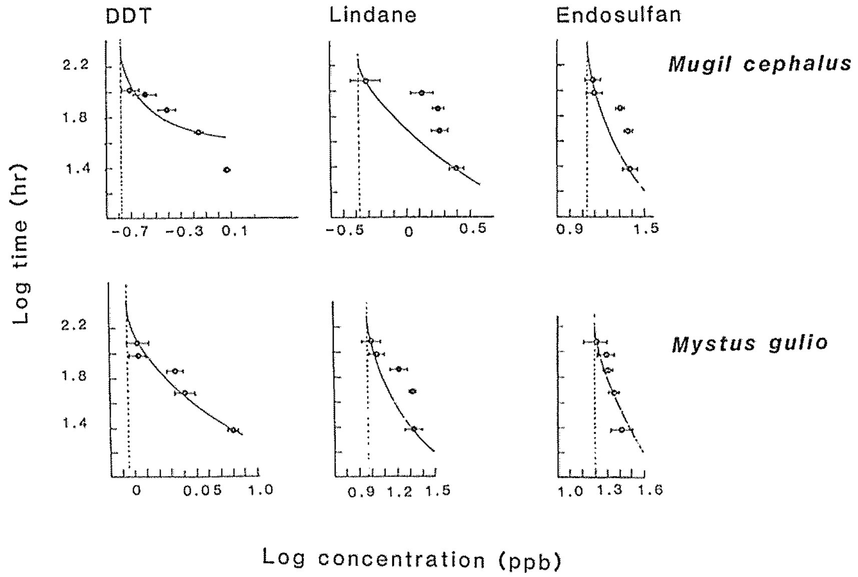

Fig. 2. Toxicity curves for the organochlorine pesticides to estuarine fishes.

manently extended siphons. Oysters usually remained closed when exposed to pesticides and physiological irritation was shown in the form of spasmodic and almost tetanic shell movements and gaping of shell valves was also observed.

Fish

DDT, lindane and endosulfan were acutely toxic to both the fishes studied (Table 2). *M. cephalus* was more sensitive than *M. gulio* exposed to DDT; the 96 hour LC_{50} value being 0.26 ppb. *M. gulio* had a 96 hour LC_{50} value of 1.13 ppb. Thus *M. cephalus* seemed to be four times more sensitive to DDT than *M. gulio* (Table 2). Lindane was also highly toxic to *M. cephalus*. It was eight times more susceptible to lindane than *M. gulio*. The 96

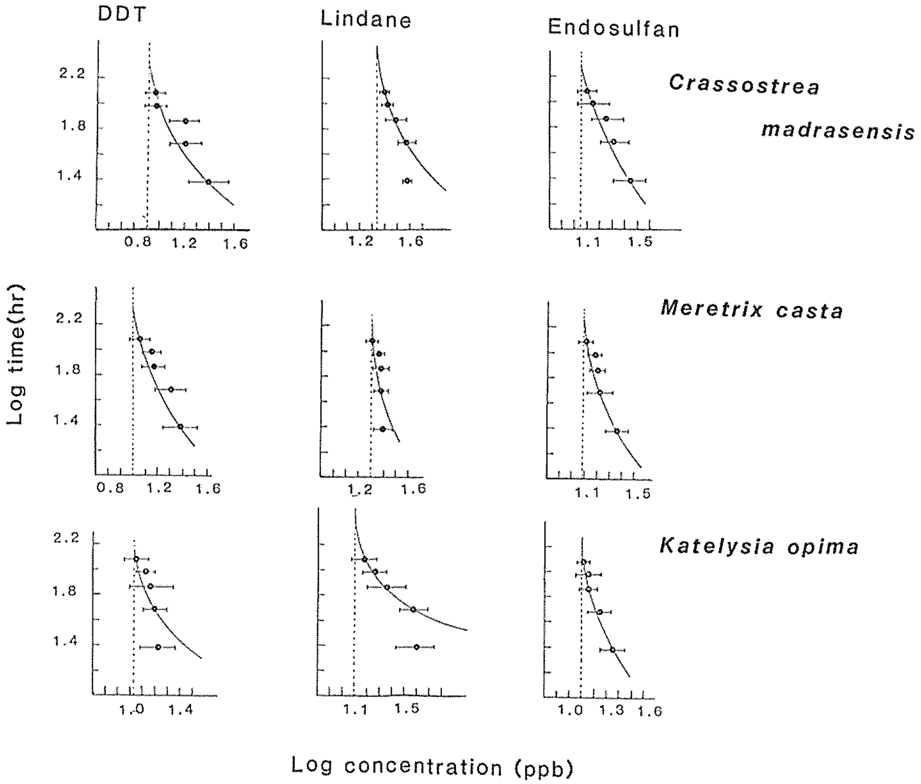

Fig. 3. Toxicity curves for the organochlorine pesticides to oyster and clams.

Table 3. The relative resistance of organochlorine pesticides to the estuarine organisms.

Species	DDT	Lindane	Endosulfan
Mugil cephalus	1.00	1.00	1.00
Mystus gulio	4.35	6.80	1.56
Crassostrea madrasensis	36.00	16.07	1.13
Katelysia opima	50.73	11.13	1.14
Meretrix casta	54.46	14.01	1.21

Table 4. Safe concentration (ppb) of organochlorine pesticides to the estuarine organisms.

Species	DDT	Lindane	Endosulfan
Mugil cephalus	0.03−0.13	0.14− 0.68	1.25−6.26
	(0.17)	(0.43)	(0.97)
Mystus gulio	0.11−0.57	1.12− 5.58	1.95−9.76
	(0.89)	(1.33)	(15.85)
Crassostrea madrasensis	0.94−4.68	2.64−13.18	1.41−7.07
	(8.13)	(21.88)	(11.22)
Katelysia opima	1.32−6.60	1.83− 9.13	1.42−7.11
	(10.72)	(12.59)	(12.59)
Meretrix casta	1.42−7.08	2.30−11.49	1.52−7.60
	(10.00)	(19.95)	(12.30)

ILC_{50} values are given in parenthesis

hour LC_{50} for *M. cephalus* was 1.35 ppb whereas it was 11.15 ppb for *M. gulio*. Both *M. cephalus* and *M. gulio* were relatively less susceptible to endosulfan (Table 2). The 96 hour LC_{50} values being 12.52 and 19.52 ppb respectively for *M. cephalus* and *M. gulio*. The order of toxicity of the pesticides to the fishes was DDT > lindane > endosulfan. *M. cephalus* was more sensitive to all the three pesticides tested.

Toxicity curves drawn using the LC_{50} values determined in the experiments are shown in Figs. 2 and 3. The incipient LC_{50} values obtained from these curves for the test organisms are shown in Table

4. From these results, it could be noticed that an increase in the exposure decreased the LC_{50} values of the pesticides. Behavioral effects observed in the fishes exposed to pesticides were decreased swimming activity and accompanied by abnormal hyperkinetic activity when disturbed. The latter was pronounced at higher concentrations of the pesticides. Hyperkinetic activity was often followed by temporary paralysis which persisted from a few seconds to over a minute before taken to a seminormal state. Most of the fish which died during the experiments exhibited symptoms of poisoning such as change in color as well as behavior. Initially, their color darkened and they swam erratically with their body inclined downwards.

The terminal phases of both high and low intoxications were characterized by highly agitated movements, intense opercular activity, tremors, convulsions, upside down swimming and loss of sense of direction. The increased opercular activity and frequent excursions to the surface of the water were the responses of the fishes exposed to high concentrations of pesticides. Frequently extreme restlessness was shown by the fishes and they tried to jump out of the tank. This was followed usually by violent movements of the pelvic fins and erratic and jerky swimming. These activities intensified with increase in the exposure period at higher concentrations. Difficulty in swimming progressed until the fish swam with their tails and dorsal fin breaking the water surface. Finally the fish lost its equilibrium, swam upside down and died. The respiratory process might have been adversely affected as a result of gill damage.

DISCUSSION

In situations where no toxicity data are available, acute bioassays become extremely important. NEGILSKI (1975) suggested that the development of acute toxicity bioassay data must be viewed as a necessary first step for providing comparative toxicity information on different toxicants and species of organisms. The published data on the toxicity of pesticides to mollusks were chiefly concerned with the rate of shell growth in relation to sublethal exposures to pesticides (BUTLER, 1963, '66, '71; EDWARDS, 1973; ENGEL *et al.*, 1971; SCHIMMEL *et al.*, 1977a, b). But in the present study, the mortality data were determined since it would be a convenient and objective criterion for evaluating the organism's response to lethal concentrations. BUTLER (1971) found that DDT, although toxic to oysters and clams at a concentration of 10 ppb seemed to cause adverse effects even at a concentration of 1.0 ppb regardless of the length of exposure. The 48 hour LC_{50} values reported by PORTMANN (1972) to *Cardium edule* for DDT (10 ppb), lindane (10 ppb) and endosulfan (10 ppb) were lower than the 48 hour LC_{50} values obtained for oyster and clams in the present investigation (Table 1). The complacent explanation for the high values in the present study might be that those experiments were static bioassays.

Since most of the reported data on toxicity to mollusks are EC_{50} values (effective concentration that brings about reduction or inhibition of growth in 50% of the test organisms). Hence there is no meaning in interpreting those data with the mortality data obtained in the present study and this restricts further discussion in this line. The pumping activity of the clams exposed to pesticides was affected. This was evident from the non retraction of the siphons even when they mechanically stimulated. RAJENDRAN (1984) also observed similar behavioral responses in clams exposed to organochlorine pesticides. EISLER and WEINSTEIN (1967) also made similar observations in *Mercenaria mercenaria*. The LC_{50} values reported by EISLER (1970) for different pesticides and various fishes and crustaceans ranged from 8 to 3250 ppb respectively. He concluded that clams were highly resistant to the pesticides since they survived even at higher concentrations.

The bioassay results showed that the toxicity of the pesticides to *M. cephalus* and *M. gulio* was a function of concentration and duration of exposure. In the present study, the LC_{50} values observed for DDT, lindane and endosulfan were not close to each other and the range of concentrations was wide. However comparison of the toxicity data collected in the present study with those of other results may not be meaningful because of the major factors influencing bioassays like temperature, species susceptibility and variability in bioassay techniques adopted i. e. static or continuous flow. Results of acute toxicity tests of the present study generally corroborate those of several other investigators who conducted bioassays with organochlorine pesticides using estuarine animals. Further data pertaining to LC_{50} values for estuarine fish and shellfish in India are also rather sparse. LINGARAJA and VENUGOPALAN (1978) reported fairly high values of DDT (3.6 ppb) for the estuarine perch *Therapon jarbua*. SASI BHUSHANA RAO (1980) reported LC_{50} values of lindane for the juveniles of the *Therapon jarbua* (57 ppb), *Mugil cephalus* (74 ppb) and *Ambasis commersoni* (37 ppb) of Vellar estuary. 48 hour endosulfan LC_{50} values reported for *Mugil curema* was 0.6 ppb (SCHOETTGER, 1970) and 96 hour value for *M. cephalus* was 0.38 ppb by continuous flow system (SCHIMMEL *et al.*, 1977b). The reported toxicity data for temperate organisms are higher than the values obtained in the present study and it indicates that *M. cephalus* and *M. gulio* are more sensitive than their temperate counterparts.

Behavioral responses observed in the present study in *M. cephalus* and *M. gulio* were similar to those observed in *Ictalurus puntatus* by CARTER (1971); in *Salmo gairdneri* by WILDISH *et al.* (1971); in *Carassius auratus* by DAVY *et al.* (1972); in *Colisa fasciatus* by VERMA *et al.* (1974, '75); in *Therapon jarbua* by LINGARAJA and VENUGOPALAN (1978) and LINGARAJA *et al.* (1979); in *M. cephalus, T. jarbua, A. commersoni* by SASI BHUSHANA RAO (1980) and in *Cyprinus carpio* by TOOR and KAMALDEEP KAUR (1974) and SINGH *et al.* (1981) and in some estuarine fish and shellfish by RAJENDRAN (1984). It is known that generally fishes respond to toxic chemicals by increased opercular movements (LINGARAJA and VENUGOPALAN, 1978). Therefore the intense opercular activity exhibited by *M. cephalus* and *M. gulio* after exposure to pesticides may be attributed to the hypoxic stress accompanied by a sequential inhibitory influence of these toxicants on the respiratory system. The route of entry of pesticides in toxicity tests is generally agreed to be via the gill (HOLDEN, 1962; FERGUSON *et al.*, 1966) and hence the respiratory process may be adversely affected. This might arise as a result of gill damage which might at first appear as mucus secretion. LINGARAJA and VENUGOPALAN (1978) observed copious mucus secretion in the test organisms in order to reduce the irritating effect of the pesticides. Changes in the color of the test fish in the experimental tank might be due physiological discomfort exercised by the fish (LINGARAJA *et al.*, 1979). Loss of equilibrium usually proceeds death of fish exposed to lethal concentrations of toxicants.

Organochlorine pesticides affect the brain and the nerves which result in neurophysiological alterations thus affecting the behavior of the fish and the functions controlled by the central nervous system. DDT is supposed to exert its toxic effects on the nervous system by inhibiting adenosine triphosphatase (Matsumura and Patil, 1969). Janicki and Kinter (1971) suggested that the effects of organochlorine pesticides to fish may be related to disruption of osmoregulatory transport mechanisms. The threshold or incipient LC_{50} may be termed as the asymptotic part of the toxicity curve drawn using LC_{50} for different exposures (Apha, 1985). It has greater theoretical significance than an LC_{50} for some arbitrary time. Eaton (1973) defined the term application factor as the ratio between the concentration of a toxicant causing 50% mortality in two days to two weeks. Chronically safe concentration can be calculated for a particular toxicant and for a given species by using these application factors where only acutely lethal concentrations have been estimated (Eaton, 1973). The tremendous differences in the ratios obtained from the results of acute to chronic exposure indicates that the utility of these experimentally determined application factors are more useful than the use of arbitrarily determined factors. Hansen *et al.* (1977a, b) supported the concept of scientifically derived application factor. They also opined that scientifically calculated application factor for one species and body of water will be similar to the factor determined for another species from another body of water. The relative resistance of organochlorine pesticides to these organisms are calculated using 96 hour LC_{50} values of the pesticides and are shown in Table 3. From these results, it could be inferred that *M. cephalus* was highly sensitive to all the pesticides. The oyster and clams are found to be resistant to these pesticides (Table 3). The differences in the toxicity values may be due to biological variables and the chemical nature and hence it could be concluded that the toxic nature may be species specific and/or chemical specific.

Estimated ILC_{50} values and the safe concentration (SC) derived therefrom are tabulated for the three pesticides used and for the respective bioassay organism in Table 4. The reported ILC_{50} values of lindane for juveniles of *A. commersoni, T. jarbua* and *M. cephalus* (Sasi Bhushana Rao, 1980) were lower than the values observed in the present study. The ILC_{50} values of endosulfan determined in the present study for *M. cephalus* (10.97 ppb) and *M. gulio* (15.85 ppb) were also higher than the ILC_{50} value reported previously for *Pimephales promelas* (0.86 ppb) by Macek *et al.* (1976). This might be expected to be due to the constant flow, longer duration of the experiment, size of the organisms and species used in the present investigation. In this study, the safe concentration ranged from 0.03 to 1.42 ppb for DDT, 0.14 to 2.64 ppb for lindane and 1.25 to 1.95 ppb for endosulfan were found to have no harmful effect (mortality) to the test organisms. Further the reported concentration of these pesticides in Vellar estuary (Rajendran, 1984) were lower than the safe concentration obtained in the present study. Hence it could be inferred that the residual concentration may not pose any immediate threat to aquatic life. However the build up of these pesticides in their tissues as reported by Rajendran and Venugopalan (1983) for *C. madrasensis,* may pose any danger to their physiological activities.

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REFERENCES

American Public Health Association, 1985, In "Standard Methods for the examination of water and wastewater" (ed. by A. E. GREENBERG, R. R. TRUSSELL, and L. S. CLESCERI) pp, 689-819 16th ed., APHA, Washington.

BALL, I. R., 1967, The relative susceptibility of some species of fresh water fish to poisons. *Water Res.*, 1:767-775.

BUTLER, P. A., 1963, A review of fish and wildlife service investigation during 1961 and 1962. *U. S. Fish. Wildl. Serv. Circ.*, 167:11-25.

BUTLER, P. A., 1966, Pesticides in the marine environment. *The Appl. Ecol.*, 3:253-259.

BUTLER, P. A., 1971, Influence of pesticides on marine ecosystems. *Proc. R. Soc. Lond. Ser. B*, 177: 321-329.

CARTER, F. L., 1971, In vivo studies of brain acetylcholine esterase inhibition by organophosphate and carbamate insecticides in fish. *Diss. Abstr. Int.*, 32:2772-2773.

The Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975, Methods for acute toxicity tests with fish, macro-invertebrates and amphibians. *US EPA Publ. No.* 600/3-75-009, 1-61.

CRAIG, G. R., 1975, Proc. 2nd Annual Aquatic Toxicity Workshop. 1-340.

DAVIS, I. C., GREER, G. L. and BIRTWELL, I. K., 1978, Proc. of the 4th Annual Workshop. *Fish. Mar. Serv. Tech. Rep.*, 818:1-211.

DAVY, F. B., KLEEREKOPER, H. and GENSTER, J., 1972, Effects of exposure to sublethal DDT on the locomotor behavior of the gold fish (*Carassius auratus*). *J. Fish. Res. Bd. Canada*, 29:1333-1341.

DOUDOROFF, P., ANDERSON, B. G., BURDICK, G. E., GALTSOFF, P. S., HART, W. B., PATRICK, R., STRONG, E. R., SARBER, W. W. and VAN HOM, H. W., 1951, Bioassay methods for the evaluation of acute toxicity of industrial waste to fish. *Sewage Ind. Wastes*, 23:1380-1397.

EATON, J. G., 1973, Recent developments in the use of laboratory bioassays to determine safe levels of toxicants for fish. *Bioassay Techniques and Environmental Chemistry*, 1:101-115.

EDWARDS, C. R., 1973, Environmental Pollution by Pesticides, 542pp. Plenum Press, London.

EISLER, R., 1970, Acute toxicities of organochlorine and organophosphate insecticides to estuarine fishes. *U. S. Fish. Wildl. Ser. Tech. Rep.*, 46:1-12.

EISLER, R. and WEINSTEIN, M. P., 1967, Changes in metal composition of the Quahaug clam, *Mercenaria mercenaria* after exposure to insecticides. *Chespeake Sci.*, 8:253-258.

ENGEL, R. H., NEAT, M. J. and HILLMAN, R. E., 1971, Sublethal and chronic effects of DDT and lindane on glycolytic and glyconeogenic enzymes to the quahaug clam *Mercenaria mercenaria. FAO Fish. Rep.*, 99:1-7.

Environmental Protection Agency, 1978, Bioassay procedures for the ocean disposal permit program. US EPA 600/9-78-010, 1-121.

FERUGUSON, D. E., LUNDKE, J. L. and MURPHY, G. G., 1966, Dynamics of endrin uptake and release by resistant and susceptible strains to mosquito fish. *Trans. Amer. Fish. Soc.*, 95:335-344.

FINNEY, D. J., 1971, Probit Analysis, 333pp. University Press, Great Britain.

FRY, F. E. J., 1947, Effect of the environment on animal activity. *Ont. Fish. Res. Lab.*, 68:1-5.

HANSEN, D. J., GOODMAN, L. R., and WILSON, A. J., 1977a, Kepone: Chronic effects on embroyo, fry, juvenile and adult sheephead minnows (*Cyprinodon variegatus*). *Chesapeake Sci.*, 18:227-232.

HANSEN, D. J., SCHIMMEL S. C., and FORESTER, J., 1977b, Endrin: Effects on the entire life cycle of a salt water fish, *Cyprinodon variegatus. J. Toxicol. Environ. Helth.*, 3:721-733.

HOLDEN, A. V., 1962, A study of the absorption of labelled DDT from water by fish. *Ann. Appl. Biol.*, 50:467-472.

JANIKI, R. H. and KINTER, W. B., 1971, DDT- distrupted osmoregulatory events in the intestine of the eel *Anguilla rostrata*. *Science,* 173:1146-1148.

JHINGRAN, V. G., 1974, Fisheries in India in the context of aquatic pollution. *J. Ecol. Environ. Sci.,* 1: 1-15.

KATZ, L., 1971, In "Water and Water pollution hand book" (LEONARD L. CIACCIO ed.), pp. 763-800, Marcel Dekker Inc., New York.

KLAVERCAMP, J., 1974, Proc. 1st Annual Aquatic Toxicity Workshop, 1-301.

LINGARAJA, T. and VENUGOPALAN, V. K., 1978, Pesticide induced physiological and behavioural changes in an estuarine teleost *Therapon jarbua* (Forskal). *Fish. Technol.,* 15:115-119.

LINGARAJA, T., SASI BHUSHANA RAO, P. and VENUGOPALAN, V. K., 1979, DDT induced ethological changes in estuarine fish. *Environ. Biol. Fish.* 4:83-88.

LLOYD, R. and JORDAN, D. H. M., 1963, Predicted and observed toxicities of several sewage effulents to rainbow trout. *J. Proc. Inst. Sewage Purif.,* 2:167-173.

MACEK, M. J., LINDBERG, M. A., SANTER, S., BUXTON, K. S. and CASTA, P. A., 1976, Toxicity of four pesticides to water fleas and fathead minnows. *Ecol. Res. Ser.,* EPA 600/3-76-099, 1-58.

MATSUMURA, F. and PATIL, K. C., 1969, Adenosine tri-phosphate sensitive to DDT in synapses of rat brain. *Science,* 166:121-122.

MOUNT, D. I. and BRUNGS, W. A., 1967, A simplified dosing apparatus for fish toxicology studies. *Water Res.,* 1:21-29.

NEGILISKI, D. S., 1975, Foundamentals of aquatic bioassays. *Proc. R. Austr. Chem. Inst.,* 42:50-54.

PARKER, W. R., PESSIAH, E., WELLS, P. G. and WESTLAKE, G. F., 1977, Proc. 3rd Aquatic Toxicity Workshop, 1-176.

PERKINS, E. J., 1972, Some problems of marine toxicity studies. *Mar. Pollut. Bull.,* 3:13-15.

PORTMANN, J. E., 1972, In "Marine pollution and sea life" (RUIVO, M. ed) pp. 212-217, Fishing News Ltd., London.

RAJENDRAN, N., 1984, Organochlorine pesticide residues in Vellar estuary, Ph. D. Thesis, Annamalai University, India, 317pp.

RAJENDRAN, N., and VENUGOPALAN, V. K., 1983, In "Indian Ocean—Biology of Benthic Marine Organisms" (THOMPSON, M. F., SAROJINI, R., and NAGABHUSHANAM, R., eds.), pp. 495-500, Oxford & IBH Publ. Co., New Delhi.

RAJENDRAN, N., and VENUGOPALAN, V. K., 1988, Toxicity of organochlorine pesticides to zooplankton of Vellar estuary. *Indian J. Mar. Sci.,* 17:168-169.

SASI BHUSHANA RAO, P., 1980, Pesticide Toxicity to Fish Eggs and Juveniles, Ph. D. Thesis, Annamalai University, India, 210pp.

SCHIMMEL, S. C., PATRICK Jr, J. M. and FORESTER, J., 1977a, Toxicity and bioconcentration of BHC and lindane in selected estuarine animals. *Arch. Environ. Contam. Toxicol.,* 6:355-363.

SCHIMMEL, S. C., PATRICK Jr. J. M., and WILSON, A. J., 1977b, In "Aquatic Toxicology and Hazard Evaluation" (MAYER, F. L. and HAMELINK, J. L. eds.) pp. 241-252. American Society for Testing Materials.

SCHOETTGER, R. A., 1970, Toxicology of thiodon in several fish and aquatic invertebrates. *Fish. Wildl. Sci.,* USDI no. 35:1-31.

SINGH, D. N., TYAGI, R. K., and PANWAR, R. S., 1981, Toxicity of some organic biocides to a fresh water fish *Cyprinus carpio communis* (Linn.). *J. Environ. Biol.,* 2:41-46.

SPRAGUE, J. B., 1969, Measurement of pollutant toxicity to fish. I Bioassay methods of acute toxicity. *Water Res.,* 3:793-821.

SPRAGUE, J. B., 1970, Measurement of pollutant toxicity to fish. II Utilizing and applying bioassay results. *Water Res.,* 4:3-32.

SPRAGUE, J. B., 1971, Measurement of pollutant toxicity to fish. III Sublethal effects and safe concentrations. *Water Res.,* 5:245-266.

SPRAGUE, J. B., 1973, The ABC's of pollutant bioassay using fish. Biological methods for the assessment of water quality. ASTM STP., 528:6-30.

STEPHEN, C. E., and MOUNT, D. I., 1973, Use of toxicity tests with fish in water pollution control. ASTM STP., 528:164-177.

TOOR, H. S., and KAMALDEEP KAUR, 1974, Toxicity of pesticides to the fish *Cyprinus corpio communis* (Linn.). *Indian J. Exp. Biol.,* 12:334-336.

VENUGOPALAN, V. K. and SASI BHUSHANA RAO, P., 1979, Pesticide induced impairment in incubation and post embryonic development of planktonic eggs of estuarine fish of Porto Novo (south India) waters. *Proc. Symp. Environ. Biol.,* 1:397-408.

VERMA, S. R., GUPTA, S. and TYAGI, M. P., 1975, Studies on the toxicity of lindane on *Colisa fasiatus. Jahrp. Leipzig.,* 121:38-54.

VERMA, S. R., BANSAL, S. K. and DALELA, R. C., 1977, Bioassay trials with a few organic biocides on fresh water fish *Labeo rohita. Indian J. Environ. Hlth.,* 19:107-115.

WALDICHUK, M., 1973, Trends in methodology for evaluation of effects of pollutants on marine organisms and ecosystems.*CRC Crit. Rev. Env. Control,* 167-211.

WAREN, S. M., and ENGEL, D. M., 1980, Flow through versus static bioassay systems: an evaluation. *J. Elisha Mitchell Sci. Soc.,* 94:108-114.

WILDISH, D. J., CARSON, W. G., CUNNINGHAM, T. and LISTER, N. J., 1971, Toxicological effects of some organophosphorous insecticides to Atlantic salmon fish. *Fish. Res. Bd. Canada Manuscr. Rep. Ser.* 1157:1-22.

熱帯河口域の魚貝類におよぼす有機塩素系殺虫剤の急性毒性

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3種の有機塩素系殺虫剤（DDT，リンデン，エンドサルファン）が熱帯河口域の魚貝類5種（二枚貝3種，魚類2種）におよぼす急性毒性を連続フローシステムの実験系を用いて明らかにした。まず各殺虫剤ごとに半数致死濃度 LC_{50} の24，48，72，96，120時間値を測定し，これらの値から初期半数致死濃度 ILC_{50} (Incipient Median Lethal Concentration) を推定した。毒性試験の結果，供試殺虫剤の中では DDT の毒性が最も強かった。5種の試験生物の中で，殺虫剤に対する感受性は魚類の *M. cephalus* で最も高く，一方抵抗性は二枚貝で高かった。試験生物にとって無害な各殺虫剤の濃度 (Safe Concentration) を求め，またこれらの殺虫剤が試験生物の行動におよぼす影響を詳述した。