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Acute Toxicity of Endosulfan to the Milkfish, *Chanos chanos,* of the Southeast Coast of India

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Effluents of agricultural processes contain highly toxic chemicals like pesticides, which cause pollution to aquatic environments including estuarine and coastal waters. The accumulation and persistence of pesticides in the aquatic environment constitute a threat to biological life, as witnessed by the chronic and acute poisoning of fish and other aquatic organisms (Hemmer et al. 2001; Wirth et al. 2001). In order to evaluate the potential effects of pesticides on estuarine resources, toxicological data are needed at many levels of biological organization. Acute toxicity tests of short duration represent an important and cost effective component in tier one risk assessment (Hall et al. 1998) and to extrapolate environmentally safe levels of anthropogenic chemicals (Okkerman et al. 1991). Among all forms of chemical pesticides, organochlorines are considered to be the most hazardous with respect to environmental pollution, since they are very persistent, non-biodegradable and add to residue buildup in the food chain (Jaffery et al. 1990). This may pose a constant threat to non-target organisms, especially to fish and other aquatic wildlife species. Concentrations of organochlorines in the seas around India have been reported to be high compared to other regions (Tanabe and Tatsukawa 1980). Large amounts of endosulfan residues have been reported in Indian estuaries (Sujatha et al. 1999; Bhattacharya et al. 2003).

Endosulfan ($C_9H_6Cl_6O_3S$ with IUPAC systematic name 6,7,8,9,10,10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3 -benzodioxathiepin-3-oxide) is a cyclodiene organochlorine pesticide that belongs to a group of polycyclic chlorinated hydrocarbons used to control agricultural insect and mite pests on a variety of field, fruit and vegetable crops (US EPA 2002). Presently, it is used in more than 70 countries for over forty years (Abraham 2004). It has been reported that during 1999-2000, about 81,000 metric tons of endosulfan was manufactured in India (Anonymous 2001). The large-scale use of technical grade endosulfan has raised concern over its potential to cause serious damage and needs further investigation to determine its 'safe level'. A number of investigations have been carried out on the toxicity of pesticides to freshwater organisms. However there are only limited studies on estuarine organisms. Therefore, the present study was carried out to determine the 96h LC_{50} value, as well as safe levels of endosulfan to the milkfish, *Chanos chanos*.

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MATERIALS AND METHODS

Healthy specimens of *C. chanos* (Forsskal, 1775) (Family Chanidae; Order Gonorynchiformes) were collected from the brackish waters of Chinnapalam on the Gulf of Mannar side of the southeast coast of India. They were brought to the laboratory within 30 minutes in plastic bags filled with seawater and sufficient oxygen. The plastic bags were cut open and the fish were then transferred to glass aquaria for acclimatization to laboratory conditions. The average wet weight and length of fish were 0.342 ± 0.06 g and 38.77 ± 2.84 mm respectively. Fish were maintained for at least 15 days under normal day and night durations and were fed daily with rice bran, oil cake powder and a mixed culture of diatoms. Feeding was stopped 24h prior to exposure. Every effort was made to provide optimal conditions for the fish during the acclimatization period. Clean seawater used in the study was that collected from half a kilometer offshore at Pudhumadam Coast of Gulf of Mannar. This seawater was stored in the laboratory, filtered through clean sand, activated carbon and 1μm filter and aereated until needed for testing.

Technical-grade endosulfan, as Thiodan® (35% EC) was purchased (manufactured by Bayer Crop Science Limited, Mumbai, India) and used in the present study. The technical-grade endosulfan used was a mixture of two stereo-isomers, ∝ and β endosulfan, in a ratio of 7:3.

The acute bioassay test procedure used was based on standard methods (Sprague 1973; OECD 1993; APHA/AWWA/WEF 1998). The experiments were conducted in replicate bioassay chambers and repeated three times. The stock solution of endosulfan was prepared by dissolving it in 100% acetone and the doses were administered to obtain a final acetone concentration of 0.1% in each treatment. Initially a preliminary range-finding bioassay was performed. Based on the results obtained, a concentration gradient series of 0.1, 0.2, 0.4, 0.8 and 1.6 μg/L was prepared from the stock solution. The vehicle control (for acetone) and negative control (unexposed) were also set in all the experiments simultaneously. In each test concentration twenty organisms were introduced in a 25L glass aquarium containing 20L of test medium. Mortality was recorded at 24, 48, 72 and 96h of exposure and no specimen was used more than once. Fish which showed no respiratory movements and no response to a tactile stimulus were recorded as dead and were removed as soon as possible after death. Measurements of pH, dissolved oxygen, salinity and temperature of the test water were done at the beginning and every 24h just before renewal of the test medium. No feed was provided during experiments to avoid contamination by excrements in the test solutions. The test solutions were replaced every 24h by fresh solution of respective concentrations of endosulfan to keep the concentrations of endosulfan near the nominal level.

As an estimate of relative lethal toxicity, LC_{50} values and their respective 95% confidence limits were calculated for 48 and 96h time points for each test series using the probit analysis software (US EPA 1992). Linear regression analysis for

concentration versus (%) mortality of *C. chanos* was carried using Excel in MS Office 2000. The safe level estimation, at 96h exposure for endosulfan, was based on methods by Sprague (1971), CWQC (1972), NAS/NAE (1973), IJC (1977) and CCREM (1991).

RESULTS AND DISCUSSION

The physicochemical characteristics of the test water were, temperature 32.15 ± 1.0° C, dissolved oxygen 5.5 ± 0.2 mg/L, salinity 31.14 ± 0.5 ‰ and pH 8.41 ± 0.4. The calculated 48 and 96h acute LC_{50} values and the 95% confidence limits (Table 1) of endosulfan, dissolved in acetone using a static bioassay system to milkfish, *C. chanos* was 0.687 µg/L (0.504-0.943) and 0.566µg/L (0.425-0.754) respectively. No mortality was observed in acetone and seawater controls. The relationship between concentration of test chemicals and percentage of fish mortality and the respective linear regression equation for concentration versus (%) mortality as curves are given in Fig.1. A dose-dependent increase and a time-dependent decrease were observed in mortality rate such that, as the exposure time increases from 24 to 96h, the median lethal concentration was reduced (Fig.1), which indicated a direct proportional relationship between mortality and concentration of test chemical. It is obvious from the results that endosulfan is highly toxic even at very low concentrations (µg/L levels).

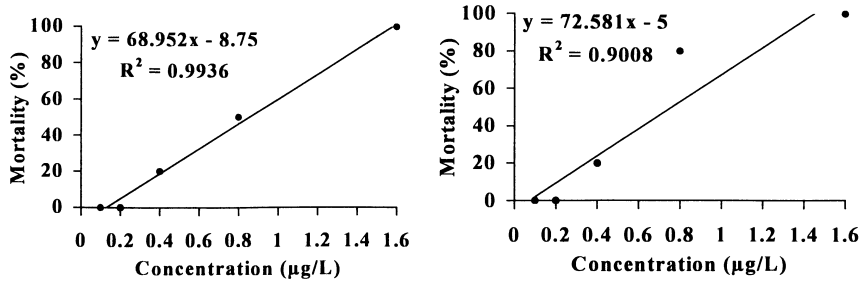
The estimated safe levels of endosulfan, as calculated by multiplying the 96h LC_{50} with different application factors (AF) are given in Table 2. There was a large variation in the safe levels estimated by different methods for endosulfan varying from 0.0566 to 5.66 x 10^{-5} µg/L.

Table1. 48 and 96h acute toxicity of endosulfan to milkfish, *C. chanos*

Point	Concentration (µg/L)	95% Confidence limit Lower - Upper	Slope ± SE	Intercept ± SE
48h exposure				
LC 50.00	0.687	0.504 - 0.943	4.73 ± 1.24	5.77 ± 0.35
96h exposure				
LC 50.00	0.566	0.425 - 0.754	6.00 ± 1.72	6.48 ± 0.53

Note: Control group (theroretical spontaneous response rate) 0.0000

Static acute 48 and 96h LC_{50} values from the literature for endosulfan to various fish species are listed in Table 3. The test result of 0.566µg/L as the LC_{50} in the present study at 96h indicated that endosulfan is highly toxic at very low concentration. In other fish species, the reported 96h LC_{50} values of endosulfan are 2.7µg/L in *C. variegatus*, 1.53µg/L in *M. beryllina*, 1.3µg/L in *A. affinis* and 1.15µg/L in *F. heteroclitus*. The 96h LC_{50} value of 0.38µg/L in *M. menidia* and 0.09 µg/L in *M. cephalus* were the only lower values. Thus, the 96h LC_{50} value of endosulfan found in the present study is within the range of values (0.09 –



(a) (b)

Figure 1. Percentage mortality of *C. chanos* after 48 (a) and 96h (b) exposure to nominal concentrations of endosulfan

Table 2. Estimates of safe level (µg/L) of endosulfan at 96h exposure time

Chemical	96h LC_{50}	Method	AF	Safe level
Endosulfan	0.566	Sprague (1971)	0.1	0.0566
		CWQC (1972)	0.01	5.66×10^{-3}
		NAS/NAE (1973)	0.1 to 0.0001	0.0566 to 5.66×10^{-5}
		IJC (1977) and CCREM (1991)	0.05	0.0283

Table 3. Comparison of acute toxicity estimates (LC_{50}) for fish species at 48 and 96h exposure to endosulfan, listed in order of decreasing concentration

Test Species	Common name	LC_{50} (µg/L)	Reference
48h exposure			
Chanos chanos	Milkfish	0.68	This study
Mugil curema	White Mullet	0.60	Schoettger (1970)
96h exposure			
Cyprinodon variegatus	Sheepshead Minnow	2.70	Schimmel (1981)
Menidia beryllina	Inland silverside	1.50	Hemmer et al. (1992)
Atherinops affinis	Topsmelt Silverside	1.30	Hemmer et al. (1992)
Fundulus heteroclitus	Mummichog	1.15	Trim (1987)
Chanos chanos	Milkfish	0.56	This study
Menidia menidia	Atlantic silverside	0.38	Schimmel et al. (1983)
Mugil cephalus	Flathead Mullet	0.09	Schimmel et al. (1977)

2.7µg/L) for various5 fish species reported. This limited data set available (Table 3) indicates that *M. cephalus* appears to be the most sensitive species followed by *M. menidia, C. chanos, F. heteroclitus, A. affinis, M. beryllia* and *C. variegates.* Toxicity data for *M. curema* at 48h exposure showed similar sensitivity to endosulfan with that of *C. chanos* obtained in the present study.

A. Affinis, M. berylli and *F. heteroclitus* produced a 96h lethal response two

orders of magnitude higher and *C. variegates* produced a 96h lethal response four orders of magnitude higher than that observed for *C. chanos.* Values for *M. curema* at 48h and *M. menidia* at 96h to endosulfan were an order of magnitude lower than that observed for *C. chanos*. In *M. cephalus* the 96h exposure was six orders of magnitude lower than that observed for *C. chanos*. Thus, it is evident that the difference in the toxicity values may be due to biological variables and the chemical formulation. Hence it could be concluded that the toxic nature may be species specific and/or chemical specific.

Sunderam et al. (1994) suggested that acute toxicity data could be used with an application factor in deriving water quality guidelines. In this study, the safe concentration ranged from 0.0566 to 5.66 x 10^{-5} µg/L of endosulfan that they have no harmful effect (mortality) to the test organisms. With regard to the current US EPA water quality criteria (WQC) for protection of saltwater organisms, the safe levels are 0.034 µg/L (acute) and 0.0087µg/L (chronic). The reported concentrations of endosulfan in Indian estuaries (Sujatha et al. 1999; Bhattacharya et al. 2003) were higher than the marine WQC and the safe concentration values obtained in the present study. Hence it could be inferred that the endosulfan concentration may pose any intermediate threat to aquatic life in the Indian context.

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