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ASSESSMENT OF ACUTE TOXICITY OF ENDOSULFAN ON A FRESHWATER FISH *NEMACHEILUS AURIUS*

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ABSTRACT
Toxicity of organochlorine insecticide Endosulfan has been tested on a freshwater fish, *Nemacheilus aurius* under laboratory conditions, using static bioassay. LC_{50} values were found to be 0.0060, 0.0050, 0.0047 and 0.0038 ppm for exposure to 24, 48, 72 and 96 hours respectively. The LC_{50} values decreased with the increasing exposure period, thus showing an inverse relationship.

KEYWORDS: Endosulfan, *Nemacheilus aurius* ,Organochloride, Toxicity,

INTRODUCTION

Today many rivers and other water bodies of the world receives millions of liters of sewage, domestic waste, industrial and agricultural effluents containing substances varying from simple nutrients to high toxic substances. These pollutants may enter food web and thus create an environmental hazard to aquatic organisms, especially to fish which is of great importance as far as food value is concerned. The acute toxic effects are generally evolved due to direct action of the toxicants on the target organs of the fish. Toxicological studies with pesticides to fresh water teleost have been studied by many workers (Dalela *et al.*, 1978; Deore and Wagh, 1987; Health, A. G. 1991; Sankar and Konar, 1995).

MATERIAL AND METHODS

The test fish *N. aurius* were collected regularly in live conditions from the river Girna from Dabhadi near Malegaon (near Nashik), Maharashtra (India). Fishes were reared and acclimatized to the laboratory conditions in the glass aquaria containing aerated tap water avoiding overcrowding. The water was renewed every day and the fishes were fed with poultry feed on alternate day. During the experimentation fishes were not fed. The physico-chemical characteristics of the test water such as temperature (22 ± 2^0C), water hardness (420 mg/lit), pH (7±0.2) and Dissolved oxygen (4.27 ml/lit) were analyzed during the experiments according to standard methods suggested by (APHA, 1989). The stock solution of pesticide, endosulfan (35%) was prepared by dissolving a known concentration of this pesticide into the distilled water and required concentrations were made from the stock solution. The acute toxicity as widely accepted for the period of 24, 48, 72 and 96 hrs. have been carried out in the present investigation and the observations were made on the percentage mortality of the fish. The LC_{50} values were calculated by using regression equation method of probit analysis (Finney, 1971) the average values have been put in appropriate tables.

RESULTS AND DISCUSSION

The observations made during the acute toxicity experiments, on the *N. aurius* have been tabulated in Table No. 1-5 and Figure 1. The regression for the relation between the doses of Endosulfan and mortality of fish was calculated for LC_{50} values, and found to be 0.0060, 0.0050 and 0.0047, 0.0038 ppm for 24, 48, 72 and 96 hrs respectively. The LC_{50} values decreased with increasing exposure period. Therefore, the LC_{50} values and exposure period showed an inverse relation (Table 5).

The acute toxicity effects are generally evolved due to direct action of the pesticide on the target organs. Living organisms have ability to adopt themselves with changing environment but serious alterations cause damage to fish life affecting the resistance mechanism of the fish (Manson, 1981). Ellis, 1937 showed that many chemicals induced similar precipitation of mucous which filled the space between gill filaments and gill lamellae ultimately affecting the gaseous exchange leading to stasis of blood and death of the fish. Lloyd and Skidmore (1970) suggested that cytological damage to gills, rather than mucous accumulation caused death by asphyxia. Toxicity, biotransformation and elimination for various concentrations of endosulfan were analyzed in *Anabas testudineus* by Rao and Murthy (1980). It was shown that detoxification of this insecticide was done in the liver and kidney. The metabolites were excreted as, ether, alcohol, sulphate of endosulfan and some residues of endosulfan A and B.

Table 1.
Calculation of log. Dose/probit regression line for some experiments in which mature fish *Nemacheilus aurius* were exposed (24 hrs) to different concentrations of endosulfan. LC_{50} for 24 hrs.

Sr. No.	Conc. In ppm	No. of animals used	Mortality % (p)	Log Conc. +3 X	Emprical probit	Expected probit	nw	y	nwx	nwy	nwx^2	nwxy
1	0.0017	10	10	0.2304	3.7184	3.1	1.5436	4.186	0.3556	6.4615	0.0819	1.4887
2	0.0035	10	20	0.5440	4.1585	4.15	4.7144	4.160	2.5646	19.6119	1.3951	10.6688
3	0.0053	10	40	0.7160	4.7467	4.7	6.1609	4.747	4.4112	29.2457	3.1584	20.9399
4	0.0070	10	50	0.8450	5.0	5.2	6.2742	4.997	5.3017	31.3521	4.4799	26.4925
5	0.0087	10	70	0.9395	5.5244	5.5	5.8090	5.524	5.4575	32.0089	5.1273	30.1472
6	0.0105	10	80	1.0211	5.8416	5.7	5.3159	5.834	5.4280	31.0129	5.5425	31.6673
7	0.0155	10	90	1.0903	6.2816	6.4	3.0199	6.272	3.5945	18.9408	4.2786	22.5452
			∑ =				32.8379		27.1133	168.71	24.0640	143.9497

$$Sxx = \sum nwx^2 - \frac{(\sum nwx)^2}{\sum nw} = 1.6772$$

$$Sxy = \sum nwxy - \frac{(\sum nwx \times \sum nwy(}{\sum nw} = 4.6472$$

$$b = \frac{Sxy}{Sxx} = 2.7707$$

$$\bar{Y} = \frac{\sum nwy}{\sum nw} = 5.13778 \qquad = \frac{\sum nwx}{\sum nw} = 0.8256\ \dot{X}$$

$a = \bar{Y} - b\ \dot{X} = 2.8500$

$$\text{Log } LC_{50} = \frac{5-a}{b} = 0.7759$$

$0.7759 - 3 = -2.2240$ Antilog $(-2.2240) = 0.005969$ $LC_{50} = 0.005969$ ppm

Table 2. Calculation of log. Dose/probit regression line for some experiments in which mature fish *Nemacheilus aurius* were exposed (48 hrs.) to different concentrations of endosulfan.
LC_{50} for 48 hrs.

Sr. No.	Conc. In ppm	No. of animals used	Mortality %	Log Conc. +3 X	Emprical probit	Expected probit	nw	y	nwx	nwy	nwx^2	nwxy
1	0.0017	10	10	0.2304	3.7184	3.25	1.7994	4.012	0.4146	7.2912	0.09552	1.66337
2	0.0035	10	30	0.5440	4.4756	4.4	5.5788	4.477	3.0349	24.9763	1.65098	12.5872
3	0.0053	10	50	0.7160	5.0	5.0	5.3662	5.0	4.5882	31.831	3.26367	22.791
4	0.0070	10	70	0.8450	5.5244	5.5	5.8090	5.524	4.9086	32.0889	4.14778	27.1151
5	0.0087	10	80	0.9395	5.8416	5.85	5.0260	5.841	4.7219	29.3569	4.43622	27.5806
6	0.0105	10	90	1.0211	6.2816	6.2	3.7031	6.278	3.7812	23.2481	3.86098	23.7383
					∑	=	28.2825		21.4194	148.7204	17.45515	116.4757

$$Sxx = \sum nwx^2 - \frac{(\sum nwx)^2}{\sum nw} = 1.2334$$

$$Sxy = \sum nwxy - \frac{(\sum nwx \times \sum nwy)}{\sum nw} = 3.844173$$

$$b = \frac{Sxy}{Sxx} = 3.1166$$

$$\dot{X} = \frac{\sum nwx}{\sum nw} = 0.7573, \quad \bar{Y} = \frac{\sum nwy}{\sum nw} = 5.2583 \qquad a = \bar{Y} - b\,\dot{X} = 2.8980$$

$$\text{Log } LC_{50} = \frac{5-a}{b} = 0.67443$$

0.67443-3 = 2.32557 Antilog (-2.32557) = 0.004725 LC_{50} = 0.004725 ppm

Table 3. Calculation of log. Dose/probit regression line for some experiments in which mature fish *Nemacheilus aurius* were exposed (72 hrs.) to different concentrations of endosulfan.
LC_{50} for 72 hrs.

Sr. No.	Conc. In ppm	No. of animals used	Mortality %	Log Conc. +3 X	Emprical probit	Expected probit	nw	y	nwx	nwy	nwx^2	nwxy
1	0.0017	10	30	0.2304	4.4756	2.8	0.9179	--	0.2114	0.9179	0.0487	0.2114
2	0.0035	10	40	0.5440	4.7467	4.3	5.3159	4.806	2.8918	25.5482	1.5731	13.8979
3	0.0053	10	60	0.7160	5.2533	5.2	6.2742	5.253	4.4923	32.9583	3.2164	23.5980
4	0.0070	10	80	0.8450	5.8416	5.8	5.0260	5.841	4.2499	29.3568	3.5937	24.8236
5	0.0087	10	90	0.9395	6.2816	6.3	3.3589	6.281	3.1556	21.0972	2.9646	19.8203
						∑ =	28.2825		21.4194	148.7204	17.4551	116.4557

$$Sxx = \sum nwx^2 - \frac{(\sum nwx)^2}{\sum nw} = 0.6259$$

$$Sxy = \sum nwxy - \frac{(\sum nwx \times \sum nwy)}{\sum nw} = 3.4591$$

$$b = \frac{Sxy}{Sxx} = 5.5266$$

$$X = \frac{\sum nwx}{\sum nw} = 0.718, \quad \bar{Y} = \frac{\sum nwy}{\sum nw} = 5.2591 \qquad a = \bar{Y} - b\,x = 1.2910$$

$$\text{Log } LC_{50} = \frac{5-a}{b} = 0.6711$$

0.6711-3 = -2.3289 Antilog (-2.3289) = 0.004689 LC_{50} = 0.004689 ppm

Table 4. Calculation of log. Dose/probit regression line for some experiments in which mature fish *Nemacheilus aurius* were exposed (96 hrs.) to different concentrations of endosulfan.
LC_{50} for 96 hrs.

Sr. No.	Conc. In ppm	No. of animals used	Mortality % (p)	Log Conc. +3 X	Emprical probit	Expected probit	nw	y	nwx	nwy	nwx^2	nwxy
1	0.0017	10	30	0.2304	4.4756	3.3	2.0774	6.016	0.4786	12.4976	0.1103	2.8792
2	0.0035	10	40	0.5440	4.7467	4.7	6.1609	3.969	3.3515	24.4526	1.8232	13.3021
3	0.0053	10	70	0.7160	5.5244	5.5	5.8090	5.496	4.1592	31.9263	2.9780	22.8590
4	0.0070	10	90	0.8450	6.2816	6.2	3.7031	8.387	3.1291	31.0579	2.6441	26.2438
						∑ =	17.7504		11.1186	99.9344	7.5556	65.2841

$$Sxx = \sum nwx^2 - \frac{(\sum nwx)^2}{\sum nw} = 0.5915$$

$$Sxy = \sum nwxy - \frac{(\sum nwx \times \sum nwy)}{\sum nw} = 2.6877$$

$$b = \frac{Sxy}{Sxx} = 4.5439$$

$$\dot{X} = \frac{\sum nwx}{\sum nw} = 0.6264, \quad \bar{Y} = \frac{\sum nwy}{\sum nw} = 5.6300 \qquad a = \bar{Y} - b\,\dot{X} = 2.7837$$

$$\text{Log } LC_{50} = \frac{5-a}{b} = 0.4877$$

0.4877-3 = 2.5122 Antilog (-2.5122) = 0.003074 LC_{50} = 0.003074 ppm

Table 5. Showing regression equation, fudicial limits and comparative toxicity of endosulfan to *N. aurius* at 24, 48, 72 and 96 hrs.

Sr. No.	Hrs. of exposure	Regression equation	Fudicial Limit	Observed LC_{50} Ppm	Calculated LC_{50} Ppm
1	24	Y = 2.85 + 2.77 X	1.26209×10^{-6} - 0.005969	0.0060	0.0059
2	48	Y = 2.89 + 3.12 X	4.4233×10^{-7} - 0.6215	0.0050	0.0047
3	72	Y = 1.29 + 5.52 X	8.869×10^{-5} – 0.2348	0.0047	0.0046
4	96	Y = 2.78 + 4.54 X	3.024×10^{-7} – 0.0415	0.0038	0.0030

Verma *et al.* (1982) conducted a bioassay test with 23 pesticides on a freshwater teleost *Saccobranchus fossilis.* Acute ranges and LC_{50} value for seven organochlorine pesticide (thiotax, endosulfan, heptachlor, chlordane, aldrine, dianidon, rogar, DPVP, molotox, sumithion, ekatin, metasystox, malathion, phoaval, dipteres, formathion and abate) were determined using static test procedures for a period of 24, 48, 72 and 96 hrs. Both by graphical interpolation and probit analysis. The relative potency of all the 23 pesticide was calculated with respect to abate. It was in the decreasing order from organochlorine to organophosphorus and carbonate pesticide respectively. Jeba Kumar et al. (1990) observed the effect of cypermethrine on the organic constituents and its accumulation in the whole fish, *Lepidocephalichthys thermalis* and showed that percentage of mortality is time and dose dependent. Acute toxicity involves the damage to the organisms by the fastest acting mechanism. The result in the present investigation showed percentage of mortality is time and dose dependent. Similar observations were made by Devi *et al.* (1972), Stephenson (1982); Gill *et al* (1991); Kanabav and Banna Durgappa (2001); Joakim Larson et al. (2002); Dussart and Trigwell (2002); Subhendu Datta *et al.* (2003) and Yogesh *et al.* (2004).

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