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INTERACTIONS OF INSECTICIDE THIODAN (ENDOSULFAN) AND HEAVY METAL CHROMIUM AND IMPACT ON FISH AND AQUATIC ECOSYSTEM

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Acute 96-hour bioassay tests of a mixture of an organochlorine insecticide thiodan (endosulfan) and heavy metal chromium on fish, *Oreochromis mossambicus*, worm, *Branchiura sowerbyi*, and plankton, *Cyclops viridis*, showed different modes of interaction. The LC_{50} to LC_{95} combined values of thiodan and chromium range from 622.55–756.25 mg l⁻¹ for fish, <12.5–373.8 mg l⁻¹ for worms, and <1.0–36.2 mg l⁻¹ for plankton. The fish sublethal dose (<622.55 mg l⁻¹) of the mixture significantly hampered feeding rate, yield (kg/ha) and reproduction of fish. Free carbon dioxide level of exposed water was significantly increased with sublethal concentrations of mixture and plankton population was significantly decreased.

Keywords: Thiodan; chromium mixture; fish; aquatic ecosystem; antagonism and synergism

INTRODUCTION

Natural waters receive different types of pollutants generating from industrial, agricultural and domestic activities; generally these pollutants mix together and produce a combined impact on fish and other aquatic life. Insecticides used in agricultural fields percolate and drain into natural waters, particularly during the rainy season. Various heavy metals discharged from local industries and sewage are introduced with water and ultimately get mixed with other kinds of pollutants. Studies

reported so far described the effect of each single individual pollutant on local fish and other aquatic life. The objectives of this investigation were to determine the lethal concentrations of two different types of pollutants in a mixture, the modes of interaction between endosulfan and chromium on fish, plankton and worm. This included study of the impact of these pollutants at a sublethal level on fish yield, reproduction, and also on physico-chemical and biological conditions of the exposed water.

MATERIALS AND METHODS:

The fish, *Oreochromis mossambicus*, (size 89.31 ± 1.45 mm; weight 10.19 ± 0.70 g), plankton, *Cyclops viridis*, and worm, *Branchiura sowerbyi*, were used as test organisms. Test chemicals selected were insecticide thiodan (35% emulsifiable concentrate) and heavy metal chromium. Thiodan is an organochlorine insecticide marketed by Hoechst India Limited for agricultural pest control. Chemically the active ingredient of thiodan is α - β -1, 2, 3, 4, 7, 7 hexachlorobicyclo (2, 2, 1)-heptane-(2-bis-(oxymethylene)-5, 6-sulphite). The heavy metal chromium was obtained from the reagent grade of $\text{CrCl}_2 \cdot 3\text{H}_2\text{O}$. Mixture of chromium and thiodan was made at LC_{50} levels of the component pollutants to fish, plankton and worm on the basis of the acute toxicity data of the individual pollutants (Tab. II). All concentrations were given in terms of total active ingredients. Concentrations of mixture were calculated in mg l^{-1} .

Fishes and worms were taken from local farms, and plankton was cultured in the laboratory. They were acclimatized in the laboratory to test conditions for 168, 96 and 24 hours, before test. Aeration was not provided in experimental sets.

Ninety-six hour acute toxicity tests on thiodan + chromium mixture were run in the laboratory using unchlorinated borehole water (pH 7.2 ± 0.2 ; dissolved oxygen 7.6 mg l^{-1} ; total alkalinity 210 mg l^{-1} as CaCO_3 ; hardness 230 mg l^{-1} ; free carbon dioxide 0.8 mg l^{-1} ; temperature $28 \pm 0.1^\circ\text{C}$) following the standard methods (APHA, 1985). Fish were tested in 15 litre glass aquaria each holding 10 litres of water; plankton or worms were tested in 500 ml glass beakers containing 300 ml of water. Respiratory and feeding tests of fish were conducted

in the laboratory for 96 hours with the same water used for acute toxicity test. For each test, five replicates and appropriate controls were maintained. In acute toxicity and respiratory test, five fish (size 72.9 ± 2.8 mm; weight 6.8 ± 0.35 g) were used and for the feeding test, two fish in each aquaria were exposed to the LC_5 dose (622.54 mg l^{-1}) of a mixture which contained $0.00339 \text{ mg l}^{-1}$ thiodan and 622.54 mg l^{-1} chromium. Opercular movements of fish were recorded per minute for all 24 hours. During the feeding test, fishes were given live earthworm, cut into small pieces. Number of pieces consumed by the fish was recorded at all 24 hours. The number of pieces consumed by control fish was taken as the normal feeding rate. Ninety day outdoor ($33.2 \pm 3^\circ\text{C}$) sublethal test on fish was conducted in 60-litre earthen vats containing 50 litre of borehole water and five kg dry pond soil were added to each vat. Fifteen fish fry (size 26.32 ± 2.13 mm; weight 1.02 ± 0.04 g) were kept in each vat and were fed 1:1 mixture of rice bran and mustard oil cake each day at 3% of total body weight. Fish were exposed to a sublethal (LC_5) dose (622.54 mg l^{-1}) of the mixture pollutants and mixed with vat water at fortnightly intervals. Observations on behavioural changes and mortality were made daily.

RESULTS AND DISCUSSION:

Acute Toxicity

The results of the acute toxicity tests of chromium and thiodan mixture on fish, plankton and worm are shown in Table I. The relative

TABLE I Acute toxicity of a mixture of chromium and thiodan to fish, *Oreochromis mossambicus*, plankton, *Cyclops viridis*, and worm, *Branchiura sowerbyi*. The values in parentheses indicate 95% confidence limits

Test Organism	Lethal Concentration (mg l^{-1})			'r' value
	LC_5	LC_{50}	LC_{95}	
Fish	622.55 (618.5-625.0)	688.75 (685.0-692.5)	756.25 (752.5-760.0)	0.9986
Worm	< 12.5 (7.5-12.5)	166.5 (122.5-212.5)	373.8 (328.7-415.9)	0.9460
Plankton	< 1.0 (< 1.0-1.0)	11.0 (2.5-19.3)	36.2 (27.0-48.5)	0.8986

comparison of toxicity of chromium and endosulfan thiodan individually and in combination on fish, plankton and worm is presented in Table II. The plankton, *Cyclops viridis*, was more sensitive to the mixture than worm and fish. The behaviour of the test organisms depended upon the concentration of the mixture and duration of exposure. Initially, the plankton showed irregular lethargic movements but after a few minutes they were found at the bottom of the container with a slow creeping movement. The combined effect of thiodan and chromium was synergistic because the toxicity was increased as compared to their individual effect. The LC_{50} of combined chromium thiodan was 11.0 mg l^{-1} and contained 10.99 mg l^{-1} chromium and $0.000138 \text{ mg l}^{-1}$ thiodan. The above value of chromium and thiodan in the mixture was much lower than the LC_5 of component chromium and thiodan (LC_5 of chromium = 63.0 mg l^{-1} , LC_5 of thiodan = 0.002 mg l^{-1}). In combination, 50% mortality was recorded although below 5% was predicted.

The worms exposed to a mixture of chromium and thiodan at higher concentration were irritated and wrinkled, and took shelter in the precipitated chromium at the bottom of the beaker. Fragmentation of the body of the worm was noticed after 24 hours of exposure. The interaction of chromium and thiodan on the worms were less than

TABLE II Comparative toxicity, mode of interaction of chromium and thiodan together and individual toxic effect on fish, *Oreochromis mossambicus*, plankton, *Cyclops viridis*, and worm, *Branchiura sowerbyi*. Values are expressed in mg l^{-1}

Lethal Level (LC_{50}) of chromium thiodan mixture	Component pollutants in mixture		Lethal level (LC_{50}) of individual pollutant		Modes of Interaction
	chromium	thiodan	chromium	thiodan	
FISH					
688.75	688.74	0.00375	170.0	0.00475	Antagonistic
PLANKTON					
11.00	10.99	0.000138	109.0	0.071	Synergistic
WORM					
166.50	166.40	0.00209	207.5	0.882	Less than Additive

additive. The LC_{50} value of mixture was 166.5 mg l^{-1} which included 166.4 mg l^{-1} chromium and $0.00209 \text{ mg l}^{-1}$ thiodan. The concentration of chromium and thiodan in the mixture was much lower than the LC_{50} of component chromium and thiodan (LC_{50} of chromium are 207.5 mg l^{-1} and thiodan 0.882 mg l^{-1}). In combination, somewhat lower mortality was observed than the expected 70–75% mortality.

Fish exposed to the mixture exhibited severe respiratory distress and tried to swim on the surface water and gulped atmospheric air. They ingested the mixture of treated water and ejected it at regular intervals. The space between the gill filaments of fish was filled with the precipitate of chromium. At acute intoxication, blood was found oozing from gills and the basis of pectoral fins. A thick layer of mucus was also found covering the entire body surface. Acute toxicity of chromium and thiodan mixture on fish indicated a different mode of interaction compared to plankton and worm. The LC_{50} value of the mixture for fish was 688.75 mg l^{-1} which contained 688.74 mg l^{-1} chromium and $0.00375 \text{ mg l}^{-1}$ thiodan. The concentration of chromium and thiodan in mixture was much higher than the LC_{95} of component chromium and LC_{50} of thiodan (LC_{95} of chromium is 201.0 mg l^{-1} ; LC_{50} of thiodan is $0.00475 \text{ mg l}^{-1}$); the resultant interaction produced only 50% mortality, although higher mortalities were predicted. Evidently, the mode of interaction on fish was clearly antagonistic.

While comparing the degree of toxicity of individual pollutant and their combination based on LC_{50} value (Tab. II), it may be suggested that the mixture of chromium and thiodan is more toxic to plankton than to the worm and the fish which might be due to variation in the mode of interaction with the respective test organisms. The present investigation corroborates with the findings of Spehar and James (1986) who reported that a mixture of heavy metal (containing heavy metal chromium) showed more toxic effect to Daphnids (*Ceriodaphnia dubia*) than to fathead minnows during acute exposure although they were exposed four to eight times greater concentration and caused 15–60% mortality. In the present study, the decreased toxicity of the mixture to fish was during acute exposure, possibly due to visible precipitation of the chromium when combined with thiodan, in test water.

SUB-LETHAL IMPACT:

Feeding and Respiratory Rate

Feeding rates of fish exposed to sublethal concentration of mixture were reduced significantly ($p < 0.05$); this was 42.63% compared to 100% in control set. The food pieces were found covered with the precipitated chromium, causing difficulty by fish in identify and locate the pieces.

No significant changes was observed in opercular movement of fish exposed to sublethal level of mixture.

Behaviour and Survival

No remarkable change in behaviour other than slower movement observed in fish exposed to sublethal level of mixture. A peculiar aggressive behaviour in some fish was noticed after successive treatments of the mixture of pollutants.

Fish Production and Reproduction

The yield (kg^{-1}ha) of fish was reduced significantly (Tab. III). Non-availability of the required fish food organism or disability to detect and consume the natural and supplementary feed in pollutant mixed water might be a possible cause for reduced growth of fish. The condition factor (K) and GSI of fish were significantly reduced in comparison to that in control fish (Tab. III). The maturity index (MI) of both male and female fishes were also reduced significantly ($p < 0.05$). Obviously, the combined chromium and thiodan at a sublethal con-

TABLE III Influence of combined chromium and thiodan at sublethal concentration on yield, gastrosomatic index (GSI), maturity indices (MI) and fecundity of fish, *O. mossambicus*. Statistical significance is shown at $P < 0.05^*$

Concentration mg l^{-1}	Yield	GSI	K	MI of O +	♂	Fecundity
0.00 (Control)	2022.89	5.810	1.52	4.77	0.32	118
622.55	1178.25*	10.423*	1.20*	0.72*	0.09*	26*

centration of 622.54 mg l^{-1} produced stress on fish gonads which reduced the normal reproductive ability. Individually, chromium is one of the most toxic metals for gametes (Billard, 1979).

AQUATIC ECOSYSTEM:

The physicochemical factors such as dissolved oxygen, total alkalinity, hardness and pH of exposed water did not vary significantly except free carbon dioxide (FCO_2) as compared to control value (Tab. IV). The significant increase in the FCO_2 level was possibly due to release of carbon dioxide in the process of biodegradation of the combined pollutants. The delayed decomposition process of unused food matter might have produced noxious gases in exposed water.

Phytoplankton and zooplankton population (no l^{-1}) was decreased significantly during chronic exposure possibly due to mass mortality of phytoplankton. The values of phytoplankton and zoo-plankton in exposed water was 1330 and 229 (no l^{-1}) while in control set it was 2355 and 517. Similarly significant reduction in zooplankton population might be attributed to the influence of combined pollutant on growth, survival and reproduction.

Chironomid larvae population (no/m^2 dry and wet weight g/m^2) in exposed water was not significantly influenced. This result confirms the findings of Kaviraj (1983) and Pal and Konar (1989) who reported no significant variation when exposed to a sublethal level of heavy metal mercury and the pesticide (phosphamidon) individually.

From the present investigation, it may be concluded that chromium and thiodan if allowed to interact in natural waters at estimated sublethal concentration, the consequent interaction among them are the

TABLE IV Influence of combined chromium and thiodan on physicochemical parameters of exposed water. Statistical significance is shown at $P < 0.05^*$. Values are expressed in mg l^{-1} except pH

Concentration mg l^{-1}	Dissolved oxygen	Free Carbon dioxide	Total alkalinity	Hardness	pH
0.00 (Control)	10.80	1.15	150.84	162.62	7.51
622.55	9.00	1.98*	135.60	136.20	7.75

potential to hamper growth and reproduction of fish and may destroy primary fish food organisms of the receiving water. It may also be suggested that only short-term toxicity test on fish and aquatic organisms are not sufficient while formulating standards of water quality. Evidently, both short term and long term tests on the pollutant mixture should be considered for establishing the safe level. Obviously, there is urgent need for additional research on the mechanisms of multiple toxicity in various environmental conditions for safeguarding the vital aquatic resources.

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