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Effects of Lethal and Sublethal Concentrations of Lindane on the Behaviour and Energy Reserves of the Freshwater Fish, *Oreochromis niloticus*

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Abstract. The fish, *Oreochromis niloticus*, was exposed to lethal and sublethal concentrations of lindane. Lc-50 was found to be 3.16 mg/l for lindane -6 and 0.074 mg/l for technical grade lindane. The fish showed remarkable changes in behaviour such as cough, yawn, fin flickering, S-and partial jerk, nudge and nip. They also showed hyperexcitation alternating with sluggishness and occasional darting movements. Fish exposed to sublethal concentrations of lindane showed difficulty in respiration and aberrant behaviour mentioned above was also increased. It was also noted that the glycogen content of liver and muscle of lindane exposed fish depleted significantly ($P<0.001$). On the otherhand the level of circulating glucose was increased in the fish exposed to lindane ($P<0.01$).

Introduction

Pesticides are deliberately added to the environment for the purpose of killing undesirable organisms. These pesticides should ideally be highly specific in their toxicity to undesirable, target organisms and harmless to nontarget, desirable animals. Unfortunately most of the pesticides are not highly selective and are toxic to many nontarget organisms including fish and other species inhabiting the environment [1, pp. 355-408].

Among the insecticides, organochlorines are very persistent in the environment and tend to accumulate in biological and non-biological materials. Chronically the organochlorine insecticides have more toxic potential than organophosphorus and carbamade.

Alterations in the chemical composition of the aquatic environments affects the behavior and biochemical system of fish [2]. The physiological and biochemical changes in fish and other aquatic animals due to insecticidal poisoning are known to be brought about by influencing the activity of certain enzymes [3].

Lindane (γ-isomer of hexachlorocyclohexane, HCH) is an insecticide widely used for agricultural purposes and a major pollutant of freshwater [4], and has devastating effects on aquatic animals, including fishes inhabiting the feshwater environment. Previous studies indicate that lindane varies in its toxic potential for different fish species. Herbst and Van Esch [5, pp. 1-189] have summarized the 96h Lc-50 values of lindane for 38 species of fish which range from 2 to 1486 µg/l. Geyer ,*et al.* [6] have also reported the 48 hours Lc-50 values for 14 species which range between 22 µg/l and 900 µg/l. The species most sensitive to lindane is *Barbus stigma* [7] with a 96 hours Lc-50 of 1.5 µg/l.

Effects of sublethal and chronic intoxication with lindane on the health of fish and other aquatic animals have also been studied. Survival, growth and reproduction of blue gills and brook trout were studied by Macek, *et al.* [8]. Changes in enzyme activity and alterations in glycogen, glucose and lipid metabolism in fish due to lindane intoxication have also been studied [3; 9]. Lindane intoxication has been shown to result in reduced growth and survival of fish [10]. Suppression of antibody production in fish after lindane exposure was reported by Dunier and Siwicki [4]. Lindane exposure enhances the swimming activity of fish [11].

In the present study an attempt was made to assess the toxicity of lindane (γ–isomer of hexachlorocyclohexane, HCH) to a freshwater fish, *Oreochromis niloticus.* Mortality and changes in energy reserves (glycogen), plasma glucose and behavioral pattern of fish exposed to lindane were investigated.

Materials and Methods

Healthy specimens of *Oreochromis niloticus* of an average length 14.25 ± 1.3 cm and weight 25.87 ± 1.5 g were procured from a fish farm located at Deerab about 80 km south-west of Riyadh. They were transported to Riyadh in plastic bags filled with oxygenated water. In the laboratory, fishes were kept in glass aquaria containing dechlorinated and aged water. Mechanical air pumps were used to aerate the aquarium water. The fish were left for 4 weeks to become acclimatized to laboratory conditions. During the period of acclimation the fish were fed a commercial fish food *ad libitum* twice daily.

When the period of acclimation was complete, ten active and healthy specimens of *Oreochromis niloticus* were transferred into each of a number of aquaria containing 30 liters of water. The working concentrations (2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mg/l) of lindane-6 were prepared by adding known quantities of it directly into aquarium water and mixing thoroughly with a glass rod. Waters with different concentrations (0.05-0.110 mg/l) of technical grade lindane (γ-isomer of hexachlorocyclohexane, HCH, 98% purity) were prepared by adding known volumes of stock solution prepared in absolute acetone. A control set was also run with the same volume of water and same number of fish. A volume of acetone equal to that required for the preparation of the highest concentration (0.11 mg) of pure lindane (technical grade) was also added to the control. The experiment was run in duplicates.

All the fishes were exposed for 96 hours and monitored for mortality and changes in behavior such as cough (wide opening of mouth with partial extension of fins), yawn (wide opening of mouth along with complete extension of fins), fin flickering (repeated extension and contraction of dorsal fin), threat (movement of a fish towards another fish), nip (bite) and nudge (resting of a fish with its head on the body of other fish). Dead fish were removed immediately after death and their numbers recorded. Fishes were unfed during the period of exposure. The water in aquaria was renewed every 24 hours to keep the concentrations constant. Earlier reports have revealed that lindane concentrations in water do not change within 24 hours [11]. The Lc-50 value was computed by the method of Finney [12, pp. 1-333].

In another set of experiments the fish were exposed to sublethal concentrations (0.02 and 0.03 mg/l) of the γ-isomer of hexachlorocyclohexane, HCH (technical grade lindane - 98.0% purity) for four days. Known quantities of insecticide were dissolved in known volumes of acetone. Required volume of this solution was added to aquaria to obtain the required concentrations (0.02 and 0.03 mg/l). A control set with the same number of fish and same volume of water was run for comparison. The same volume of acetone (as used in preparation of higher concentration of lindane, 0.03 mg/l) was also added to control groups. Fishes were exposed in two groups comprising 15 and 5 fish in group I and II, respectively. Five fish from the first group were sacrificed at 24h, 48h and 72h of exposure and their liver, muscle and blood samples were collected separately for estimation of glycogen and glucose.

Fishes of the second group (in duplicates) were used for studying the behavior and sacrificed after 96 hours of exposure. Similar to the first group, liver and muscle samples were collected and processed for estimation of glycogen. Blood samples were collected in heparinized vials by cutting the caudal peduncle. Clotted blood samples were discarded. Blood samples were centrifuged at 6000 rpm to separate plasma from haematocytes. The glucose in plasma was estimated by the method of Roe [13]. Glycogen in liver and muscle was estimated by the method of Montgomery [14].

The frequency of occurrence of different behavior mentioned earlier was counted for 5 fishes together for 30 minutes on each day. The time of observation was rotated from morning to evening to allow for the diurnal changes in behavior. All the experiments were run in duplicate. Data obtained were subjected to statistical analysis (student 't' test) to determine the significance of the results.

Results and Discussion

Toxicity

The results for the fish exposed to lindane-6 and technical grade lindane (98% purity) showed that this insecticide, in both forms, has toxic effects. The percentages of mortality of fish after 96 hours of exposure to different concentrations of technical grade lindane and lindane-6 are given in Tables 1 and 2, respectively. The 96 hours Lc-50 for technical grade lindane computed from Table 1 was 0.074 mg/l and for lindane-6 computed from Table 2 was found to be 3.16 mg/l. The later value is quite high when compared with the former and with the values reported by previous investigators [5; 6]. This is attributed to the low level (6% w/w) of active ingredient (γ-isomer of hexachlorocyclohexane) in commercial grade lindane-6 and also the method of exposure.

Table 1. Mortality of fish in different concentrations of technical grade lindane (Pure) after 96 hours of exposure.

Concentration of lindane (pure)	No. of fish exposed		No. of dead fish		Percentage mortality		Average mortality
	I	II	I	II	I	II	
Control	10	10	-	-	-	-	-
0.05 mg/l	10	10	2	2	20	20	20%
0.07 mg/l	10	10	4	5	70	50	45%
0.09 mg/l	10	10	7	5	70	50	60%
0.11 mg/l	10	10	8	8	80	80	80%

Data are given for two replicates.

Table 2. Mortality of fish in different concentrations of commercial grade lindane-6 (6% Purity) after 96 hours of exposure

Concentration (mg/l)	No. of fish exposed		No. of fish dead		Percentage mortality %		Average percentage mortality (%)
	I	II	I	II	I	II	
Control	10	10	0	0	0	0	0
2.5	10	10	2	2	20	20	20
3.0	10	10	2	4	20	40	30
3.5	10	10	5	7	50	70	60
4.0	10	10	7	9	70	90	80
4.5	10	10	9	9	90	90	90

Data are given for two replicates.

Most probably the γ-isomer of hexachlorocyclohexane (HCH) present in lindane-6 was not distributed evenly in the aquarium water due to its direct addition into water, resulting in less toxic effects on fish. The Lc-50 value for technical grade lindane (0.074 mg/l) fits in the range (0.002 to 1.486 mg/l) reported earlier [5; 6], for different species of fish. The difference between species in toxic potential of lindane can be attributed to different tolerances related to its metabolization and excretion. It has been reported that fish with a high lipid content can tolerate a higher level of lindane than those containing a low level of lipid and that the lipid content of fish plays an important role in metabolization and excretion of this insecticide [6].

Behavioral and physiological observations

Specimens of *Oreochromis niloticus* exposed to lethal concentration of lindane-6 and technical grade lindane showed remarkable changes in behavior. Uncomfortable movements like burst swimming, S and partial jerking, convulsion and darting movements were increased after the addition of lindane. The fish were hyperexcited and restless. After sometime the fish became sluggish and remained at the bottom calmly. The frequency of other kinds of unusual behavior such as cough, yawn, finflickering, threat, nudge and nip were also increased due to insecticide poisoning. The fish secreted mucus in large quantity and showed loss of balance, which was followed by their succumbing.

The different kinds of behavior of fish exposed to sublethal concentrations of lindane were monitored and their frequencies are presented in Table 3. The data indicate that lindane exposure affected respiratory behavior which is manifested by elevated frequencies of cough and yawn. Discomfort movements and aggressive behavior were also increased due to lindane exposure. The effect of lindane on fish was seen to be dose- and time-dependent as higher frequencies were recorded at higher dose and in the early period of exposure. When the time of exposure was prolonged the frequency of the different behaviors was reduced but it remained higher than the control at all period of exposure (Fig. 1).

Table 3. Frequency of occurrence of different kinds of behavior of *Oreochromis niloticus* exposed to technical grade lindane. Data are mean of two observations made for five fish together for half an hour each day for four days (mean frequency/five fish/2 hours) and ± are standard errors. Values along the row with different letters are significantly different (P<0.01)

Behavior	Concentrations of lindane		
	Control (0.0 mg/l)	0.02 mg/l	0.03 mg/l
Cough	33 ± 2.00^{a}	43.5 ± 2.50^{b}	50 ± 4.00^{b}
Yawn	14 ± 1.00^{a}	17.0 ± 3.00^{ab}	24.0 ± 2.53^{b}
Fin-flickering	200 ± 10.32^{a}	274.5 ± 9.50^{b}	288.0 ± 10.00^{b}
Partial-jerk	41.5 ± 3.5^{a}	51.5 ± 2.50^{ab}	65.5 ± 4.50^{b}
S-jerk	25.5 ± 1.50^{a}	37.5 ± 2.50^{b}	45.5 ± 3.50^{b}
Burst swimming	19.0 ± 3.00^{a}	28.0 ± 4.00^{ab}	32.5 ± 2.50^{b}
Nudge	42.5 ± 2.50^{a}	48.0 ± 4.00^{ab}	44.5 ± 3.50^{b}
Threat	64.0 ± 4.00^{a}	87.0 ± 5.00^{b}	99.0 ± 6.00^{b}
Nip	172.5 ± 7.50^{a}	212.5 ± 7.5^{b}	249.5 ± 7.50^{c}

The fish exposed to different lethal and sublethal concentrations of lindane, undoubtedly experiences stress due to its irritating and neurotoxic effects. The increased erratic movements exhibited by fish may be an attempt to be relieved from such stressful environment. The changes in the animal's behavior after exposure to toxicants may be related to the consequent, alterations in physiological process [15, pp. 1-275]. Mucus

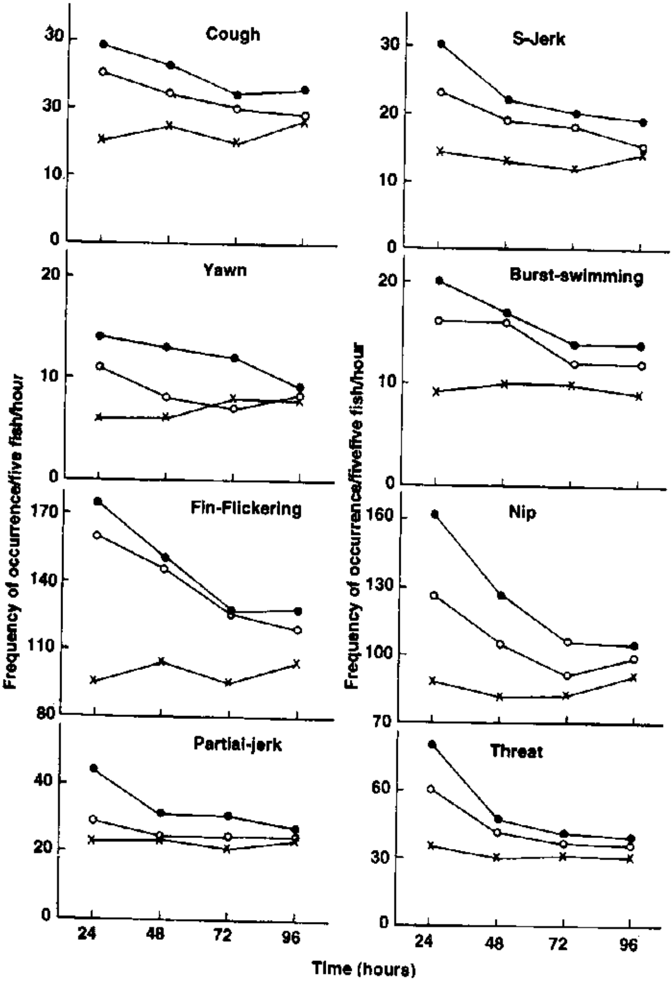

Fig. 1. Frequency of occurrence of different behavior of lindane exposed (0.02 mg/l, O———O; and 0.03 mg/l, ●———●) and control (X———X) fish, *Oreochromis niloticus*, at different intervals of time.

secretion is a common response of fish to stressful conditions. It is secreted in high quantity, probably, for coating the body and other organs like gills to reduce their contact with the toxic environment and get relief from stress and the irritating effects of toxicants. Reduction in the frequency of different behaviors with time may be attributed to acclimation and adaptation of fish to a stressful environment.

The increased physical activities due to lindane exposure certainly require more energy which can be obtained by oxidation of fuel molecules (glucose) in the presence of oxygen. Mucus deposition on the gills will reduce the diffusion of gases accross them. The increased demand for oxygen and reduced supply of it can lead to an increased frequency of cough and yawn. Both of these behaviors have a clearing effect on gills and thus increase the diffusion of oxygen across them [16].

Carbohydrate metabolism

Data presented in Table 4 indicate that the levels of glycogen in the liver of lindane exposed fish were depleted ($P<0.001$) at all the concentrations and period of exposure. More pronounced effects were noticed at the beginning of exposure and at higher concentration. Gradually the extent of depletion lowered after prolongation of the treatment period. Muscle glycogen of the lindane exposed fish was also depleted ($P<0.001$) in the same pattern as noted for liver glycogen. The depletion was greater in muscle compared with liver. Previous studies have shown that due to the physical disturbance of fish [17] and stress of acute hypoxia [18; 19], glycogen level of liver and muscle of fish become depleted rapidly.

It is also reported that exposure to various toxicants considered stressful such as heavy metals [20-22], pulpmill effluents [23] and pesticides [24- 28], causes reduction in the muscle and liver glycogen of the fish. Glycolysis in the fish exposed to different pollutants may be expected tq meet the energy requirements of the animal for the increased level of physical activities (discussed in section on behavioral observations) and enhanced physiological processes for metabolizing and eliminating the toxicants. It has been shown that during actual or potential situations of stress both catecholamines [17] and adrenocorticosteriods [29-31] are secreted in increased amount and induce marked changes in carbohydrate energy reserves of the fish [32; 33]. Thus, reduction in the level of glycogen in muscle and liver of fish exposed to lindane in present study may be related to stress-induced increase in circulating catecholamines and adrenocarticosteroids.

The significant ($P<0.001$) elevation of blood glucose level in the fish exposed to lindane may be due to mobilization of muscle and hepatic glycogen [27; 34, pp. 1-943]. Stressful stimuli elicit rapid secretion of glucocorticoids [31; 35] and catecholamines [17] from adrenal tissue of the fish. Both hormones are known to produce hyperglycemia in animals. Therefore, the hyperglycemia condition registered in the present study may be ascribed to lindane induced hyper-secretion of these hormones which cause glycolysis in the liver and muscle of lindane exposed fish.

Table 4. Changes in glycogen and glucose level in fish, *Oreochromis niloticus*, after lindane (technical grade) exposure. Values are mean ± standard error.

Exposure time (h)	Liver glycogen (Mg/g)			Muscle glycogen (mg/g)			Serum glucose mg/100 ml		
	Control	0.02 mg/l	0.03 mg/l	Control	0.02 mg/l	0.03 mg/l	Control	0.02 mg/l	0.03 mg/l
24	9.33	8.74*	8.68*	4.91*	4.43*	4.39*	75.80	91.82*	97.28*
	±0.141	±0.055	±0.086	±0.088	±0.117	±0.123	±3.14	±3.60	±3.71
48	9.23	8.54*	8.50*	4.85	4.28*	4.24*	74.82	89.32*	92.50*
	±0.183	±0.069	±0.075	±0.063	±0.073	±0.062	±2.44	±3.24	±2.16
72	9.20	8.45*	8.44*	4.79*	4.15	4.15*	74.00	88.48*	89.66*
	±0.137	±0.125	±0.132	±0.072	±0.083	±0.040	±2.56	±4.29	±3.63
96	9.10	8.40	8.37*	4.78	4.13	4.12*	72.60	85.26*	86.04*
	0.135	±0.072	±0.070	±0.039	±0.079	±0.147	±2.49	±4.17	±3.86

*Treated mean values differ significantly from control (<0.001).

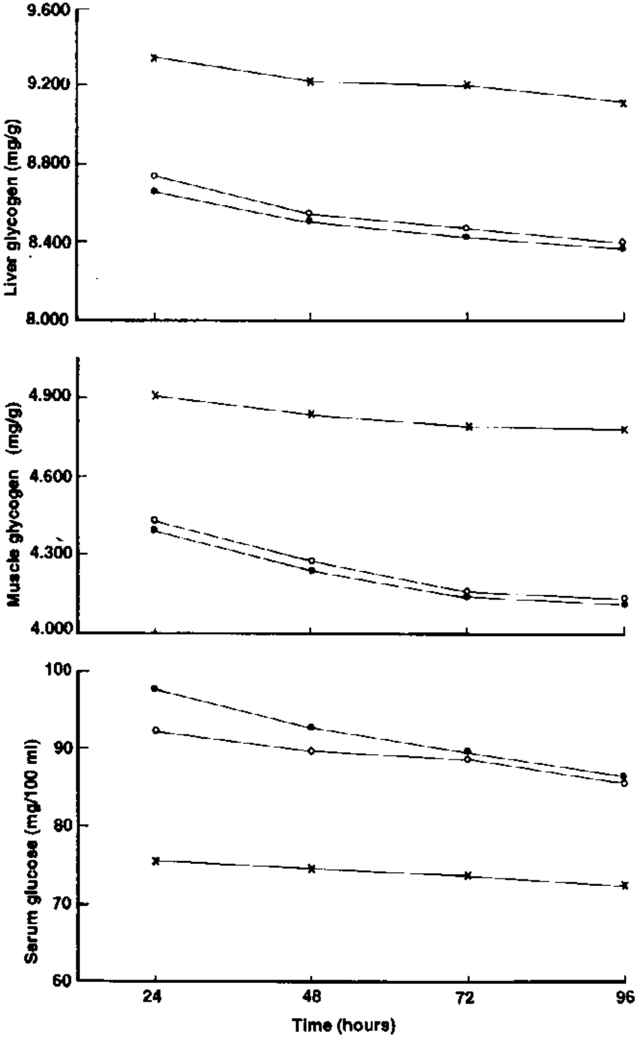

Fig. 2. Changes in glycogen and serum glucose levels in fish, *Oreochromis niloticus*, exposed to 0.02 mg/l (○——○) and 0.03 mg/l (●——●) lindane and control (×——×).

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تأثير اللندين على المخزون الطاقي والسلوك لأسماك البلطي النيلي

Oreochromis niloticus

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(سُلِّم في ٢١ شعبان ١٤١٥هـ؛ وقُبِل للنشر في ٢٦ ذي القعدة ١٤١٦هـ)

ملخص البحث. غُمرت عدّة عيّنات من أسماك البلطي النيلي في تراكيز حرجة من اللندين التجاري والخالص. وتبيّن من التجربة النتائج التالية:

(١) التركيز الحرج (LC 50) للندين التجاري ٣,١٦ ملجرام/ لتر.

(٢) التركيز الحرج (LC 50) للندين الخالص ٠,٠٧٤ ملجرام/ لتر.

(٣) تغيّرات سلوكية واضحة.

(٤) نوبات سلوكية حادة يتبعها هدوء للأسماك.

(٥) صعوبة في التنفس.

(٦) نقص في الجليكوجين في الكبد ($P < 0.001$).

(٧) نقص في الجليكوجين في العضلات ($P < 0.001$).

(٨) زيادة في تركيز الجلكوز في الدم ($P < 0.001$).