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OCCURRENCE OF LINDANE AND ITS EFFECTS ON SOME HEMATOLOGICAL AND HISTOLOGICAL ASPECTS OF TILAPIA ZILLII (GERV.)

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Key words: Lindane; insecticide; Bioaccumulation; Physiological response; Histological changes; *Tilapia zillii*.

ABSTRACT

*Residues, lethal and sublethal toxicity of organochlorine insecticide lindane to **Tilapia zillii** (Gerv.) were investigated. Lindane was positively detected in all samples collected from Nile River. Its level increased upward from south to north along the River showing its highest mean concentration (10.7 ng/g) in fish from Rosetta while the lowest level (2.7 ng/g) was found in fish from Aswan. Median lethal concentrations of lindane were experimentally estimated at 24 and 96 hr. The LC_{50} values were 32.0 and 21.7 µg/l, respectively. Subjecting fish to sublethal concentrations (10 & 20 µg/l) of lindane induced both hematological and histological changes. In lindane-treated fish, blood glucose level decreased after 3, 6 and 24 hr. whereas hyperglycemia was noticed following 48, 96, and 168 hr. of exposure. Mean cell hemoglobin concentration (MCHC) decreased at all detected time periods following fish exposure to 10 µg/l of lindane. On the other hand, when fish were exposed to 20 µg/l of lindane, MCHC decreased after 3 and 6 hr. then increased by increasing the time of exposure to 24, 48, 96 and 168 hr. Transaminase enzyme (PGOT), plasma protein, sodium and potassium ions increased at all the detected time periods of exposure. Microscopic examination of gills showed hyperplasia in the primary gill lamellae, hypertrophy and shortening in the secondary lamellae with blocking of the inter-lamellar spaces. Livers of lindane-treated fish showed severe fatty deposition with destruction in both hepatocytes and bile ducts.*

with additional solvent 300 ml (3-5 ml/min). The elute was rotary evaporated to 10.0 ml and dried on anhydrous sodium sulphate. The Extractable Organic Matter (EOM) was then determined by weighting a 10.0 µl aliquot on an analytical balance followed by a steam of nitrogen to 1.0 ml and loaded onto a gel.

Residues were analyzed, using a HP-6890 series II GLC equipped with a ^{63}Ni-electron capture detector, SE-45 capillary column (30m X 0.25mm id), with column nitrogen flow of 1.5 ml/min and detector make-up flow of 30 ml/min. The injector and detector temperatures were kept at 210 and 280°C respectively. Initial column oven temperature was 70°C (2 min. hold) and then raised to a final temperature of 260°C (3 °C/min. and 5.0 min hold). Quality assurance measures included analysis of reagent blank with each set of analyses and spike samples. Residues were corrected for percent recovery (80 to 88%). Component identification and determination were matched against authentic standards (BDH grade). Standardization recovery percentage was carried out for each batch. All solvent used were supplied by Merk (pesticide grade). This analysis was performed using an international calibration program IAEA, within MEDPOL-Marine Environment Lab., Monaco, France.

2. Experimental fish study

a. Bioassay test

Live specimens of ***Tilapia zillii*** (Gerv.) weighing 25.8 ± 6.5g were obtained from El-Mex fish farm in Alexandria, kept and acclimatized in the laboratory under appropriate experimental conditions for two weeks. Water temperature, dissolved oxygen and pH were 18.0 ± 1.0°C, 8.5 ± 0.5 mg/l and 7.3 ± 0.2, respectively. Acute toxicity test were carried out according to the Standard Methods for the Examination of Water and Wastewater (1975). Pure technical lindane was used.

To study the effects of sublethal concentrations (10.0 & 20.0 µg/l) of lindane at different time intervals (3, 6, 24, 48, 96 and 168 hr.) on some hematological parameters, blood was obtained directly from the caudal artery into heparinized capillary tubes and plasma was separated by centrifugation. Plasma glutamic oxaloacetic transaminase (PGOT), plasma protein and glucose were measured spectrophotometrically using

Table (1): Lindane and HEOM concentrations in flesh of *Tilapia zillii* from different areas in Nile River.

Sampling site	No. of Fish	HEOM (mg/g)	Lindane (ng/g)	
			Range	Mean
Aswan	12	8-25	0.9-5.9	2.7
Assuite	12	12-33	2.1-11.7	6.1
Beni-Suef	12	18-41	1.4-17.3	7.8
Cairo	12	25-60	4.2-11.8	9.0
El-Mansoura	12	30-70	6.4-15.7.	9.9
Rosetta	12	40-134	9.0-13.4	10.7

Table (2): Haematological changes in *Tilapia zillii* (Gev.) after exposure to sublethal concentrations of lindane for different time periods.

Parameter	Time of exposure in hours					
	3	6	24	48	96	168
	Glucose (mg/100 ml)					
Control	30.4 ± 1.1	31.5 ± 1.9	30.7 ± 1.4	29.9 ± 1.6	28.8 ± 1.7	32.9 ± 1.3
10.0 µg/l	28.6 ± 1.7	29.1 ± 1.9	26.1 ± 1.6*	39.5 ± 2.3**	31.1 ± 1.7	33.3 ± 1.8
20 µg/l	27.8 ± 1.6	26.2 ± 1.7*	23.9 ± 1.8**	42.3 ± 2.0**	36.6 ± 4.0*	38.2 ± 2.3*
	Mean cell hemoglobin concentration (%)					
Control	23.7 ± 0.3	23.4 ± 0.6	23.1 ± 0.6	20.6 ± 0.6	22.5 ± 0.5	21.8 ± 0.7
10.0 µg/l	20.3 ± 0.9**	21.2 ± 0.4**	22.9 ± 0.7	20.5 ± 0.5	21.8 ± 0.5	21.6 ± 0.9
20 µg/l	19.4 ± 0.6**	18.7 ± 1.2**	28.5 ± 0.7**	21.4 ± 0.5	24.0 ± 1.1	23.4 ± 1.2
	PGOT (u/l)					
Control	10.0 ± 0.6	12.2 ± 0.8	15.0 ± 1.1	10.7 ± 0.7	10.0 ± 0.5	12.1 ± 0.7
10.0 µg/l	12.4 ± 1.9	13.5 ± 1.2	16.8 ± 1.9	15.3 ± 2.1*	13.8 ± 2.4	13.0 ± 1.7
20 µg/l	16.8 ± 1.9**	18.0 ± 1.3**	20.3 ± 1.3**	16.0 ± 1.8**	14.5 ± 1.9*	13.1 ± 1.6
	Plasma protein (g/100 ml)					
Control	5.57 ± 0.3	5.56 ± 0.2	5.48 ± 0.4	5.50 ± 0.4	4.90 ± 0.2	5.00 ± 0.3
10.0 µg/l	5.80 ± 0.4	6.15 ± 0.3*	5.70 ± 0.3	5.63 ± 0.2	5.40 ± 0.2*	5.70 ± 0.2*
20 µg/l	6.88 ± 0.3**	7.10 ± 0.2**	5.88 ± 0.3	6.10 ± 0.2	5.50 ± 0.2*	5.85 ± 0.2*
	Plasma sodium (mmol/l)					
Control	208.0 ± 3.2	211.0 ± 2.8	218.3 ± 6.1	213.5 ± 6.7	236.8 ± 8.2	218.3 ± 8.9
10.0 µg/l	226.7 ± 10.1*	259.2 ± 7.6**	250.1 ± 5.1**	241.8 ± 8.4*	248.9 ± 5.2	252.8 ± 10.6*
20 µg/l	238.4 ± 4.3*	268.3 ± 8.4**	247.2 ± 3.0**	254.9 ± 3.5**	254.4 ± 6.6*	277.3 ± 15.4**
	Plasma potassium (mmol/l)					
Control	18.4 ± 0.9	18.9 ± 0.5	19.3 ± 0.9	17.2 ± 0.9	17.0 ± 0.6	19.3 ± 1.4
10.0 µg/l	18.9 ± 0.8	19.7 ± 1.3	19.5 ± 1.7	18.2 ± 1.0	17.7 ± 0.5	19.5 ± 1.3
20 µg/l	20.6 ± 0.7*	21.2 ± 0.8*	20.3 ± 1.1	18.0 ± 1.5	17.5 ± 1.2	20.2 ± 1.1

-Values are expressed in the Table as mean ± standard error (n = 5).
* $P < 0.05$, ** $P < 0.01$

lindane. Mean cell hemoglobin concentration (MCHC) decreased at all time intervals when fish were exposed to 10.0 µg/l lindane. This same trend was observed when the fish were exposed to 20.0 µg/l lindane for 3 and 6 hr. However, these values increased between 24 and 168 hr. Plasma transaminase (PGOT), protein, sodium and potassium ions increased at all time intervals as compared to controls.

Histological investigations indicated that, gills of the control fish have a regular architecture (Fig. 1). Each gill unit is composed of primary gill filament and laterally originating secondary lamellae with clear wide interlamellar spaces in between.

The effects of lindane were represented by histopathological symptoms (Figures 2 & 3), that include hypertrophy of secondary lamellar cells, hyperplasia of primary lamellar cells and blocking of interlamellar spaces and severe gill epithelial damage including hypertrophy and fusion of secondary lamellae.

Livers of the control fish group (Fig. 4) showed normal hepatocytes with a usual polygonal shape, homogenous cytoplasm and rounded nuclei. On the contrast, , severe fatty accumulation, hepatocellular necrosis, nuclei destruction (Fig. 5) and bile duct destruction (Fig. 6) were detected in livers of lindane-exposed fish.

Fig. (1): Frontal section through gill filament of control fish showing the normal gill structure with wide interlamellar spaces. x=400

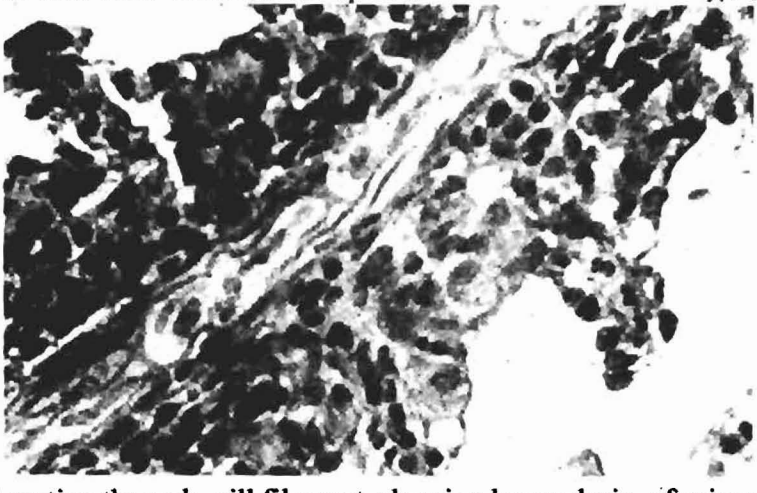

Fig. (2): Frontal section through gill filament showing hyperplasia of primary lamellar cells and hypertrophy of secondary lamellar cells. x=1000

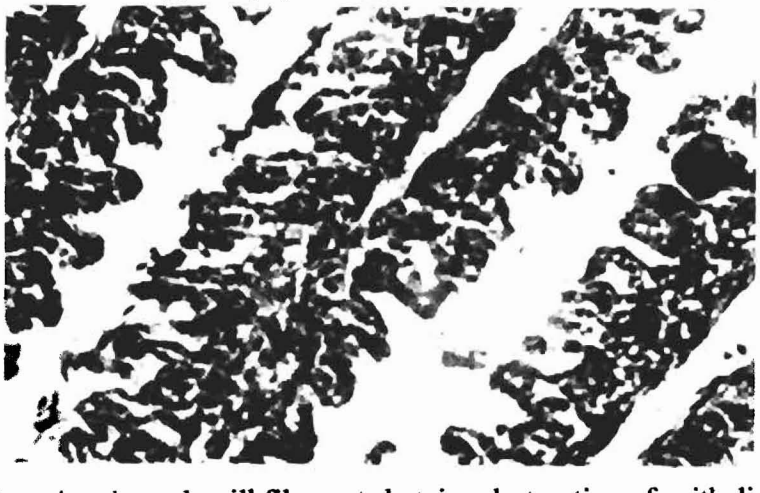

Fig. (3): Frontal section through gill filament showing destruction of epithelial cells and blocking of interlamellar spaces. x=100

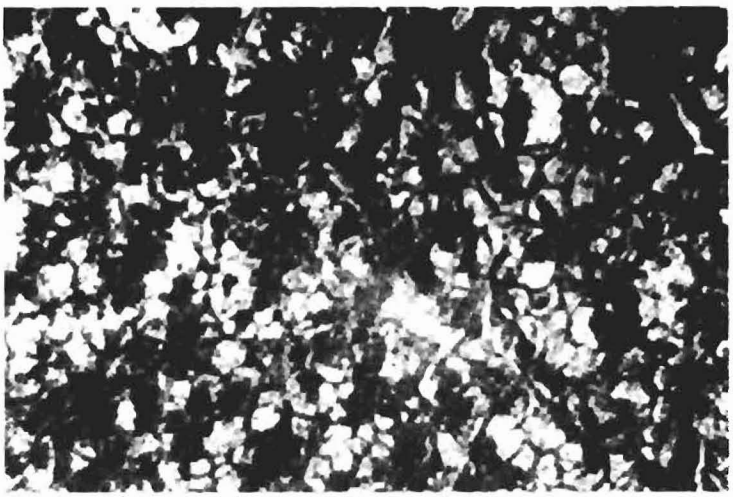

Fig. (4): Cross section through liver of control fish showing the characteristic uniform hepatocytes. x=300

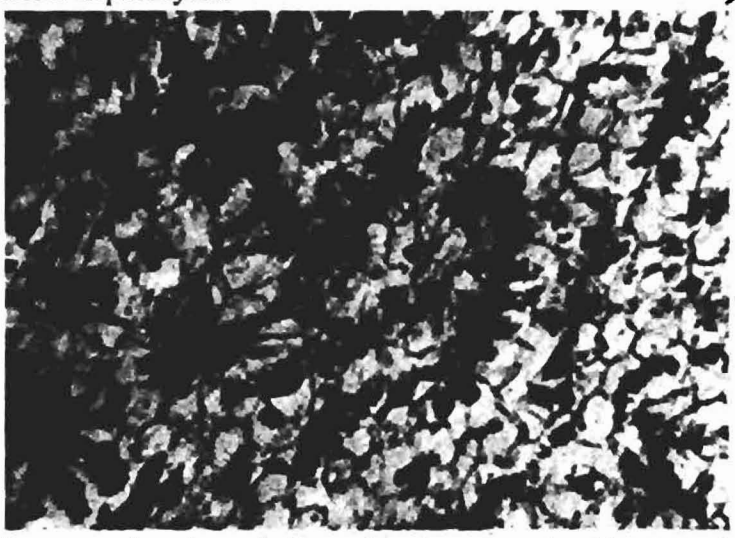

Fig. (5): Cross section through liver showing necrosis of hepatocytes and sever fatty deposition. x=300

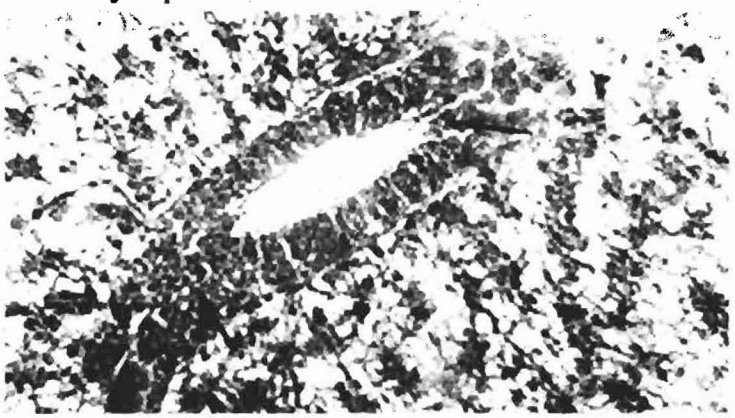

Fig. (6): Cross section through liver showing bile duct destruction (arrows). x=300

DISCUSSION

The analytical results of lindane residue in muscle of ***Tilapia zillii*** revealed a south to north increasing gradient of lindane level along the Nile. This may be due to the agricultural waste dumping to the water body of the River. In addition, there was a good positive relation between lindane concentrations and fish fat content, as judged by the Extractable Organic Matter (EOM) data. The obtained results agree with those obtained by Abd-Allah ***et al.***, (1998). Zhou ***et al.***, (1999) found that the level of HCH in Tilapia muscle was 2.0-3.7 ng/g. In the present study, the presence of lindane in all detected samples indicates that the isomer is still used in our environment.

In the present study, the effects of lindane on the behaviour of fish were similar to those observed in several fish species exposed to various organochlorine pesticides (Gupta ***et al.***, 1984; Alkahen, 1996). The hyperactivity, convulsion and intermittent paralysis shown by lindane-exposed fish support the findings of Muller ***et al.***, (1981) who reported a neurotoxic effect of lindane on fish similar to that observed in insects and mammals. It is postulated that both the imbalance of the central monoaminergic system and the lindane-induced GABAergic blockade may be the basis of the behaviour alteration (Rivera ***et al.***, 1998).

Several researchers had estimated median lethal concentrations of lindane for fish. Schimmel ***et al.***, (1977) estimated 96-hr. LC_{50} of lindane as 0.30 and 0.10 mg/l for sheephead minnow and pinfish, respectively. Srivastava and Mishra (1982) reported that the 96-hr. LC_{50} of lindane for Indian catfish was 0.26 mg/l. Mourad and Abd-Allah (1995) estimated 24 and 96-hr. LC_{50} of lindane for carp as 42.0 and 25.8 µg/l, respectively. The data on acute toxicity of lindane in ***Tilapia zillii*** show that the fish were more sensitive. In general, fish show a wide range of toxic response to insecticides since the acute toxicity is not only species specific but also influenced by various factors e.g. temperature, pH, hardness, dissolved oxygen, etc. (Schoettger, 1970) . The toxicity of individual insecticide is, therefore, difficult to compare (Schimmel ***et al.***, 1976). The present data are generally in agreement with those obtained by El-Sebae (1981).

It is well known that carbohydrate energy reserves in fish are influenced by a variety of situations such as hypoxia (Burton *et al.*, 1972), exercise (Nakano

and Tomlinson, 1967) or exposure to organochlorine insecticides (Singh and Srivastava, 1981). It has also been shown that catecholamine are secreted during periods of stress which deplete glycogen energy reserves in fish (Larsson, 1973). Thus the marked hyperglycemia in fish after exposure to lindane was probably caused by a stress. The hypoglycemic phase is probably due to a rapid utilization of blood glucose during hyperexcitability and convulsion, characteristics of organochlorine pesticide toxicity. The obtained results are in agreement with that recorded by Soengas ***et al.***, (1997).

In this study, the decrease in the MCHC after exposure to lindane may be due to the haemolysing action of lindane leading to profound anoxaemia. A similar result was reported by Gromysz-Kalkowska ***et al.***, (1985) who found a reduction in the MCHC after exposure of Japanese quail to different insecticides. On the other hand, the increase of MCHC in fish by exposure to 20.0 µg/l of lindane between 24 and 168 hr. may be a result of the increased hemoglobin percentage, that hypoxic conditions caused by prevailing in the fish (Madhu ***et al.***, 1984).

The influence of lindane on the concentration of total plasma protein was very pronounced. Fluctuation of plasma protein concentrations with changes of physical and chemical properties of the environment are well known (Assem ***et al.*** 1992). In this study, the increase of plasma protein concentration could be attributed to the increase in liver protein synthesis, disturbances in liver function or the immune response to lindane that lead to an increase in the formation of protein-insecticide complex. This agrees with the findings of Smith and Schuring (1999) who reported that lindane inhibits immune cell of ***Oreochromis niloticus*** and induces immuno toxicity.

The close relationship between enzymes and organ toxicity has made enzymes estimations are important in the diagnosis of several diseases. It was found that PGOT estimation is one of the most sensitive criteria of the liver function test to evaluate the hepatocellular function and follow up the hepatic diseases. It is likely that the initial changes in PGOT is due to cellular degradation by lindane perhaps in the liver or heart muscle. Sulik ***et al.*** (1997) demonstrated that acute lindane intoxication produces morphological changes in the liver that evidence as disturbances in energetic processes of hepatocytes. Mourad (1990) studied the electrocardiograms of eels and recorded a considerable damage in the cardiac muscle following exposure to lindane. The

reversal PGOT activity with time shown in this experiment may be due to a relatively slow but constant inhibition of the enzymes by lindane.

The increase of sodium and potassium ions during exposure to lindane indicated the impairment of osmoregulatory and electrolyte regulatory system that are well known in fish following exposure to organochlorine pesticides (Leadem ***et al.***, 1974). In agreement with the previous findings, changes in cations concentrations observed in this study may be attributed to interaction of lindane with plasma resulting in a loss of the Na/K selectivity (Schefczif and Simonis, 1980) or disruption of liver metabolism (Helmy ***et al.***, 1983).

It is apparent that the studied fish were under considerable stress during exposure to lindane. However, an accommodation or adaptation seems to occur subsequently for some blood parameters. This means that the blood changes observed during the experiments seem to be transient although the exact length of the transient period is unknown. Therefore, there is no dogmatism that short-term alteration will affect the fish survival, reproduction or growth potential.

Microscopic examination after exposure of ***Tilapia zillii*** to sublethal concentration of lindane revealed that extensive damage had occurred in gills structure ranging from destruction of epithelial cells to blocking of the interlamellar spaces. These observed changes led to partial or severe blocking of the water passage, diminishing the free surface of secondary lamellae and decreasing respiratory exchange surface. The obtained results are in agreement with Studnicka ***et al.*** (1981). Our results agree also with Matei and Malgin (1972) who reported that lindane (at medium lethal concentration) increased the chloride cells in number and size.

The most important changes showed in the liver after exposure to lindane were severe fatty accumulation (as indicated by vacuolization) and destruction of both bile ducts and nuclei of hepatocytes. These obtained results indicate that lindane intoxication produces harmful changes in liver that cause disturbances in energetic processes of hepatocytes. Similar histopathological changes of liver after exposure to lindane were recorded by Drewett and Abel (1983) and Sulik ***et al.*** (1997).

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