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Effect of Lindane on the Blood of a Freshwater Fish

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Lindane is a commonly used synthetic organic pesticide. It has received much attention over the past few years as potentially important aquatic pollutant (Muirhead-Thomson 1971; Ware 1983). Fishes are of great nutritional significance and their intoxication by pesticides causes retardation of growth and deterioration in the nutritional value (Livingston 1977). In fish, lindane is known to cause a variety of effects like anemia, inhibiton of ATPase activity (Hanke et al. 1983; Gonzalez et al. 1987) and alterations in nervous function (Joy 1982). Apart from this, very little attention has been paid to biochemical changes which develop more quickly in response to toxicants than any apparent morphological changes. Therefore, the present investigation was undertaken to evaluate the effect of lindane on plasma chemistry of freshwater fish *Anguilla anguilla*. This fish was selected because its wide availability, edibility in Spain and its important ecological role in the Albufera Lake of Valencia, Spain.

MATERIALS AND METHODS

Eels of species *Anguilla anguilla* (weight, 20-30 g; length, 16-20 cm), were collected from a fish farm in Valencia, Spain. They were acclimatized to laboratory conditions for 2 wk in 300 L glass tanks. The tanks were supplied with a continuous flow of tap water (temperature, 20°C; total hardness, 250 mg/L as $CaCO_3$; pH, 7.9±0.2; alkalinity, 4.1 mmol/L). A 12 hr photoperiod (light=08.00 to 20.00 hr) was maintained (Ferrando et al. 1989). The fish were then transferred to test aquaria and were not fed. The acute lethal toxicity (96 hr LC50 value) to the fish for lindane, in these conditions, was 0.67 mg/L (Ferrando et al. 1987).

For the study of the effect of lindane on blood metabolism, groups of 10 fish each were exposed to 0.335 mg/L (0.50 of the LC50-96 hr value) and 0.167 mg/L (0.25 of the LC50-96 hr value) lindane. Fish were sampled for the

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various biochemical parameters at 6, 12, 24, 48, 72 and 96 hr following exposure to both lindane concentrations. Groups of control fish in tap water with acetone (66 µl/L) were sampled for each specified parameter for comparison with the treated fish.

Stock solutions were prepared by dissolving lindane (99%, AGRONEXA company) in acetone; appropriate quantities of this solution were pipetted into glass aquaria (40 L) containing 35 L of test solution and ten fish. Ten more eels, used as controls, were kept in 35 L of clean water with the same concentration of acetone. After treatment the fish were quickly anaesthetized (MST 222); blood was removed directly from the heart with a syringe (1 mL), centrifuged (15 min, 5000 rpm), and the plasma used for the metabolite determination inmediately. Glucose was determined spectrophotometrically (610 nm) using the GOD-Period method (Boehringer-Mannheim). Lactate was estimated using Trichloroacetic acid (7‰) (Lang and Michal 1974) following by spectophotometrical determination (340 nm) using the kit of Boehringer-Mannheim. We determined pyruvic acid level using perchloric acid (1M) (Dange and Masurekar 1982) and Boehringer-Mannheim kits (spectophotometrical determination, 340 nm). Cholesterol levels were analyzed spectophotometrically (500 nm) with a test kit from Boehringer-Mannheim and we used the method of Bligh and Dyer (1959) for the extraction process. Results from pesticide exposed animals were rigorously compared with those from controls.

One-way analysis of variance (ANOVA) was used to determine treatment toxic effects, and Duncan's significant difference test was used for mean separation. The significant level was fixed at $p<0.001$ and $p<0.05$. These analysis were performed using Statistical Analysis System (SPSS+) with an IBM computer.

RESULTS AND DISCUSSION

The results of this study showed that lindane intoxication led to significant variations in biochemical composition of plasma in fish, *Anguilla anguilla* (Table 1 and 2).

Plasma glucose level increase from the controls (80.28 mg/100mL) in fish exposed to 0.335 mg/L (Table 1) and 0.167 mg/L (Table 2). The increase in glucose level was found to be significant ($p<0.001$; $p<0.05$) in the fish exposed to 0.335 and 0.167 mg/L at 6, 12, 24, 48, 72 and 96 hr. Thus, lindane exposure caused hyperglycemia in fish. The percentage of increase in glucose level after 0.335 mg/L exposure was 197.8 and 219.8% at 72 and 96 hr respectively; and for 0.167 mg/L exposure was 191.8 and 214.9% at the same time period. Singh and Srivastava (1981) in a series of experiments observed significant and persistent hyperglycemia in endosulfan exposed fish (*Heteropneustes fossilis*). Sastry and Sharma (1981) also noted hyperglycemia in fish (*Ophiocephalus punctatus*) exposed to diazinon.

Table 1. Effect of 0.335 mg/L of Lindane on biochemical profile of fish, *Anguilla anguilla*.

Parameter	Control	Exposure period (hr)					
		6	12	24	48	72	96
Glucose	80.28	180.93**	126.4**	182.36**	219.51**	239.09**	256.75**
(mg/100 mL)	±7.62	±5.86	±7.46	±13.6	±8.22	±6.16	±25.56
Lactate	12.98	14.05	19.51**	22.23**	25.6**	26.21**	29.89**
(mg/100 mL)	±1.24	±1.97	±3.85	±4.71	±2.31	±2.41	±2.39
Pyruvate	0.471	0.511	0.696*	0.898**	1.032**	0.875**	0.582
(mg/100 mL)	±0.075	±0.050	±0.273	±0.300	±0.189	±0.280	±0.080
Cholesterol	559.24	428.60*	305.66**	309.78**	369.49**	296.81**	348.39**
(mg/100 mL)	±18.35	±66.82	±31.38	±34.99	±31.56	±34.25	±40.47

Table 2. Effect of 0.167 mg/L of Lindane on biochemical profile of fish, *Anguilla anguilla*.

Parameter	Control	Exposure period (hr)					
		6	12	24	48	72	96
Gucose	80.28	176.77**	119.99*	168.56**	211.33**	234.29**	252.81**
(mg/100 mL)	±7.62	±7.57	±11.50	±19.23	±11.87	±8.77	±19.87
Lactate	12.98	15.34	16.90	18.32	24.97**	34.87**	34.70**
(mg/100 mL)	±1.24	±0.47	±6.90	±2.95	±1.51	±7.74	±8.60
Pyruvate	0.471	0.467	0.752*	1.074**	0.933**	0.769**	0.529
(mg/100 mL)	±0.075	±0.111	±0.226	±0.179	±0.089	±0.128	±0.077
Cholesterol	559.24	349.83**	390.54**	409.28**	368.45**	335.68**	420.09**
(mg/100 mL)	±18.35	±16.35	±4.15	±24.75	±10.37	±32.29	±46.69

The values are means ± SD of 10 observations. * $p<0.05$. ** $p<0.001$

Similar results were found in *Ciprinus carpio* exposed to lindane (Gluth and Hanke 1985). The hyperglycemia response exhibited by lindane intoxicated fish might be due to the fact that cholinergic inhibitors also affect secondary adrenergic reactions. According with Gupta (1974) the hyperglycemia induced by pesticide might be explained in part by inhibition of cholinesterase at neuroeffector sites in the adrenal medulla leading to hypersecretion of adrenaline which stimulates the breakdown of glycogen to glucose.

Blood sugar levels are elevated in fish during acute exposure to a variety of compounds, including pesticides. Stressful stimuli elicit rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue of fish; both hormones produce a rapid hyperglycemia (Singh and Srivastava 1981). The hyperglycemia observed at these times after exposure to lindane might be a result of glycogenolysis in muscle and liver causing a significant increase in blood glucose levels.

Significant elevation was observed in blood pyruvate levels at 12, 24, 48 and 72 hr in treated fish (Table 1 and 2) as compared with controls (0.471 mg/mL). Hyperglycemia was observed (Table 1 and 2) at 12, 24, 48, 72 and 96 hr following exposure to 0.335 mg/L ; and at 48, 72 and 96 hr in fish subjected to 0.167 mg/L of lindane (12.98 mg/100 mL in controls). The elevated pyruvate level in fish was possibly due to rapid glycolysis under hypoxia. The increase activity of lactic acid content in this study suggests this assumption. Endosulfan also induced elevation of both blood pyruvate and lactate in *Heteropneustes fossilis* (Singh and Srivastava 1981). It has been reported that the rate of oxygen consumption increases by as much as 40-70% in several teleosts treated with sublethal concentrations of both organochlorine and organophosphate pesticides (Srivastava et al. 1977; Pandey et al. 1979).

Serum cholesterol concentration was lowered with both treatments mainly after 72 hr of exposure (Table 1 and 2). A reduction of 47-40% was determined. The concentration of cholesterol in the serum of eel (controls) was 559.24 mg/100 mL. The fish exposed to various concentrations of lindane thus manifested hypocholesterolemia.. This finding is in accordance with the findings of Gluth and Hanke (1985) who also observed decreased cholesterol level in fish (*Cyprinus carpio*) during lindane exposure. It is presumed that reduction in circulating cholesterol level is either because of more utilization of cholesterol during corticosteroidogenesis, as it is precursor for steroid hormones, or depressed *de novo* synthesis. Methylmercury has also been reported to decrease the serum cholesterol level in bluegill fish, *Lepomis machrochirus* (Dutta and Haghighi 1986). Simultaneously an increase in protein level was also observed in the same study. This might have resulted in high density lipoprotein in serum and was suggested to be the cause of hypocholesterolemia in mercury exposed fish.

This study suggests that exposure of fish to lindane leads to disturbance in carbohydrate metabolism which might be responsible for its toxic action.

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REFERENCES

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Dange AD, Masurekar VB (1982) Naphthalene-induced changes in carbohydrate metabolism in *Sarotherodon mossambicus* Hydrobiologia 94:163-172

Dutta HM, Haghighi AZ (1986) Methylmercuric chloride and serum cholesterol level in bluegill (*Lepomis machrochirus*). Bull Environ Contam Toxicol 36:181-185

Ferrando MD, Andreu E, Almar M, Cebrian C, Nuñez A (1987) Acute toxicity of organochlorine pesticides to the european eel, *Anguilla anguilla* : The dependency on exposure time and temperature. Bull Environ Contam Toxicol 39:365-369

Ferrando MD, Andreu E (1989) Effects of temperature, exposure time and other water parameters on the acute toxicity of endosulfan to european eel, *Anguilla anguilla*. J Environ Sci Health B(24):219-224

Gluth G, Hanke W (1985) A comparison of physiological changes in carp, *Cyprinus carpio* induced by several pollutants at sublethal concentrations. Ecotox Environ Saf 9:179-188

Gonzalez JM, Cabanas JM, Martinez M, Muñoz J (1987) Alterations in acetylcholinesterase activity in plasma and synaptosomal fractions from C.N.S. of rats acutely intoxicated with Lindane. Effect on Succinylcholine. Bull Environ Contam Toxicol 39:647-655

Hanke W, Gluth G, Blubet H, Muller R (1983) Physiological changes in carps induced by pollution. Ecotox Environ Saf 7:229-241

Joy RM (1982) Mode of action of lindane, dieldrin and related insecticides in the central nervous system. Neurobehav Toxicol Teratol 4:813-823

Lang G, Michal G (1974) Methods of enzymatic analysis. 2nd edn Academic Press London

Livingston RJ (1977) Review of current literature concerning the acute and chronic effects of pesticides on aquatic organisms. CRC critical reviews in environmental control: 325-351

Muirhead-Thomson RC (1971) Pesticide and freshwater fauna. Academic Press London and New York. 248 p

Narendra NS, Srivastava AK (1981) Effects of endosulfan on fish carbohydrate metabolism. Ecotox Environ Saf 5:412-417

Pandey BN, Chanchal AK, Singh SB, Prasad S, Singh MP (1979) Effect of some biocides on the blood and oxygen consumption of *Channa punctatus* Proc Symp Environ Biol:343-348
Sastry KV, Sharma K (1981) Diazinon-induced changes in *Ophiocephalus punctatus.* Ecotox Environ Saf 5:191-200
Seifter S, Dayton S, Novic B, Muntwyler I (1950) Estimation of glycogen with the anthrone reagent. Arch Biochem 25:191-200
Srivastava GN, Gupta RA, Mohamed MP, Nath D (1977) Effects of sublethal ethyl parathion on the metabolism and activity of *Colisa fasciata.* Indian J Environ Health 19:63-66
Ware GW 1983) Pesticides. Theory and application. WH Freeman and Company. 308 p
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