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Effect of γ-Hexachlorocyclohexane (Lindane) on Carp (*Cyprinus carpio*)

II. Effects of Chronic Intoxication on Blood, Liver Enzymes, and Muscle Plasmic Membrane

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A 30-day ingestion of γ-hexachlorocyclohexane (lindane) by carp (*Cyprinus carpio*) induced hypoglycemia without activation of two hepatic gluconeogenesis enzymes (fructose diphosphatase, EC 4.1.2.13, and glucose-6-phosphatase, EC 3.1.3.9) and hyponatremia and variations in muscle plasmic membrane-bound enzymes (especially cholinesterases, EC 3.1.1.7). After 109 days carps exhibited a decrease in natremia but no significant hypoglycemia. There was an activation of gluconeogenesis enzymes. Important changes were observed in the activities of muscle plasmic membrane enzymes (especially 5'-nucleotidase, EC 3.1.3.5, and ATPases, EC 3.6.1.3). Lindane, a lipophilic substance, especially disturbed the activity of membrane-bound enzymes enclosed in a phospholipid matrix.

INTRODUCTION

In different fish species, the toxicity of lindane has been frequently measured by the determination of lethal concentrations and the LC_{50}. Several physiological functions are disturbed by this organochlorine insecticide during short-term intoxication: oxygen uptake, blood lactic acid and lipid metabolism (Bakthavathsalam and Reddy, 1981, 1983a,b,c), plasmic ions, tissue glycogen, blood glucose (Srivastava and Mishra, 1982), and regressive changes in the intestine (Lakota *et al.*, 1983).

However, little information is available about changes induced in fish by long-term intoxication, especially regarding the origin of blood glucose. A change in blood glucose can result from food, glycogenolysis, or gluconeogenesis which is easily stimulated during stress in fishes (Demael *et al.*, 1984). Lindane may act on the liver through a stress-like effect.

The first aim of this work was to evaluate the effect of insecticide-contaminated food on the activities of three enzymes involved in gluconeogenesis: phospho*enol*pyruvate carboxykinase (EC 4.1.1.32; PEPCK), fructose diphosphatase (EC 4.1.2.13; FDPase), glucose-6-phosphatase (EC 3.1.3.9; G_6Pase).

The second aim of this work was to control the effects of lindane on molecular interactions in muscle plasmic membrane. Organochlorine insecticides are highly lipophilic and their toxic effects may be partially related to their interactions with the membrane phospholipids.

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In an attempt to answer this question, the activities of 5'-nucleotidase (EC 3.1.3.5), Na^+K^+ ATPases (EC 3.6.1.3), and acetylcholinesterase (EC 3.1.1.7) have been measured on muscle plasmic membrane.

MATERIALS AND METHODS

Animals. Carps (*Cyprinus carpio*) weighing about 60–70 g were the same as those used by Cossarini-Dunier *et al.* (1987, companion paper).

The carps were divided into four lots of 20 animals. In all groups, the food intake was 1% body weight, administered once a day.

The controls (Group I) were fed with commercial pellets. The other groups received pellets contaminated with 10 mg/kg (Group II), 100 mg/kg (Group III), and 1000 mg/kg (Group IV).

Ten animals from each group were sacrificed after 1 month and 10 after 109 days of lindane intoxication.

Measures. Blood samples were taken in order to determine glucose (Saifer and Gerstenfeld, 1958) and plasmic Na^+ with an Eppendorf FCM 6341 flame spectrophotometer.

The liver was crushed and centrifuged for 10 min (100,000*g*, 4°C). The activities of PEPCK (Fleig *et al.*, 1984), FDPase (Latsko and Gibbs, 1974), and G6Pase (Hübscher and West, 1965) and the protein content (Lowry *et al.*, 1951) were determined on the supernatant. The muscle plasmic membrane was isolated (Demael and Lepot, 1983). Proteins (Lowry *et al.*, 1951) and the activities of 5'-nucleotidase (Aronson and Touster, 1974), acetylcholinesterases (Boehringer kit), and Na^+K^+ ATPases (Uesugi *et al.*, 1971) were measured. All enzymatic activities were measured at 20°C. Enzyme activities are given in micromoles or nanomoles per minute per milligram protein.

Statistical analysis. Data were subjected to a one-way analysis of variance. When this analysis showed a significant difference, means were compared two × two by modified *t* statistic (Wallenstein *et al.*, 1980). Significant differences with modified *t* test ($P < 0.05$) between controls and treatments are presented in the tables.

RESULTS

Only results of 109-day controls are presented because no significant difference was observed between 30- and 109-day controls.

Table 1 demonstrates that glycemia in Groups II, III, and IV (30 days) was very low ($P < 0.001$) compared to control and long-term intoxication groups. At 30 days, there was no significant difference among the three groups of intoxicated carps. At 109 days, the blood glucose level in Groups II and IV was equal to that of Group I. Group III alone showed a significant decrease ($P < 0.02$).

Table 1 shows that natremia was always reduced in Groups II, III, and IV after 30 days ($P < 0.001$ for the three lots). After 109 days, natremia was not different between controls and Group II but was very different between controls and Group III ($P < 0.001$) and controls and Group IV ($P < 0.001$).

For a 1-month intoxication period the pesticide significantly decreased the plasma concentration of Na^+. For the longer exposure period, this effect persisted only for the highest doses of pesticide.

TABLE 1

BLOOD GLUCOSE LEVELS AND NA^+ PLASMIC CONCENTRATIONS IN CARPS FED FOR 30 AND 109 DAYS WITH FOOD CONTAMINATED WITH LINDANE

Intoxication period in days	Group	Blood glucose ($mg \cdot ml^{-1}$)	Plasmic Na^+ (mM)
Control	I	0.61 ± 0.06	132.22 ± 1.94
30	II	0.24 ± 0.03****	113.11 ± 3.64****
	III	0.26 ± 0.03****	105.01 ± 3.02****
	IV	0.24 ± 0.02****	117.42 ± 3.67***
109	II	0.64 ± 0.02	127.31 ± 4.15
	III	0.42 ± 0.03***	114.96 ± 2.07****
	IV	0.51 ± 0.03	116.52 ± 1.96****

Note. Means ± SE. Level of contamination of food: Gp I, 0; Gp II, 10 ppm; Gp III, 100 ppm; Gp IV, 1000 ppm. Ten fish in each group.

* $P < 0.05$.
** $P < 0.02$.
*** $P < 0.01$.
**** $P < 0.001$.

The values of neoglucogenesis hepatic enzyme activities are presented in Table 2. A significant increase was found in liver PEPCK activity for nearly all carps exposed to lindane. The FDPase activity showed no statistically significant differences for 30 and 109 days of intoxication, except for Group III (109 days) which showed a significant decrease in this enzyme activity. The liver of 109-day intoxicated carps showed

TABLE 2

HEPATIC NEOGLUCOGENIC ENZYME ACTIVITIES IN CARPS FED 30 AND 109 DAYS WITH LINDANE-CONTAMINATED PELLETS

Intoxication period in days	Group	PEPCK (nmol PEP/mg prot/min)	FDPase (μmol NADP/mg prot/min)	G6Pase (nmol P/mg prot/min)
Control	I	6.23 ± 1.46	3.82 ± 0.37	12.40 ± 0.01
30	II	14.21 ± 2.60*	3.62 ± 0.21	11.53 ± 2.15
	III	14.25 ± 3.40*	3.15 ± 0.37	13.18 ± 2.10
	IV	17.84 ± 3.84*	3.95 ± 0.40	15.33 ± 1.49
109	II	11.91 ± 2.47	4.83 ± 0.69	17.20 ± 0.03**
	III	23.68 ± 6.18***	2.64 ± 0.37*	20.40 ± 3.00**
	IV	15.54 ± 2.81**	3.99 ± 0.73	28.41 ± 2.80****

Note. Means ± SE. Level of contamination in food: Gp I, 0; Gp II, 10 ppm; Gp III, 100 ppm; Gp IV, 1000 ppm. Analyses were made at 20°C. Ten fish in each group.

* $P < 0.05$.
** $P < 0.02$.
*** $P < 0.01$.
**** $P < 0.001$.

TABLE 3

ENZYMATIC ACTIVITIES OF MUSCLE PLASMA MEMBRANE IN CARPS FED 30 AND 109 DAYS WITH LINDANE-CONTAMINATED PELLETS

Intoxication period in days	Group	Cholinesterase (mmol/mg prot/min)	5-Nucleotidase (nmol P/mg prot/min)	$Na^+ K^+$ ATPases (nmol P/mg prot/min)
Control	I	124.18 ± 3.49	21.01 ± 4.03	95.59 ± 11.95
30	II	163.94 ± 13.59*	22.05 ± 6.79	73.80 ± 6.36
	III	105.14 ± 5.49	23.00 ± 5.55	62.79 ± 7.85*
	IV	175.38 ± 22.81*	20.15 ± 4.30	71.13 ± 7.02
109	II	143.83 ± 13.12	28.17 ± 3.53	62.74 ± 8.32*
	III	175.31 ± 11.86***	42.36 ± 5.42***	71.16 ± 14.35
	IV	137.76 ± 12.57	49.01 ± 8.95***	55.56 ± 4.68**

Note. Means ± SE. Level of contamination in food: Gp I, 0; Gp II, 10 ppm; Gp III, 100 ppm; Gp IV, 1000 ppm. Analyses were made at 20°C. Ten carps in each group.

* $P < 0.05$.

** $P < 0.02$.

*** $P < 0.01$.

a statistically significant G6Pase increase, while no difference was observed after 30 days.

The activities of the membrane-bound enzymes analyzed were dependent on the presence of lindane in food. Cholinesterase activity slightly increased with two exceptions: Group III on Day 30 and Group IV on Day 109.

5′-Nucleotidase activity increased significantly in Groups III and IV after 109 days only. Muscle plasmic membrane Na^+K^+ ATPase activity decreased in all groups after 30 and 109 days.

The addition of lindane to pellets ingested by carps led to changes in the activities of muscle membrane-bound enzymes.

DISCUSSION

The differences in results observed between the two periods of exposure resulted not from an excessive pesticide accumulation in long-term intoxication but only from the length of exposure, because the tissue concentrations of lindane were the same, especially in the liver and whole body (Cossarini-Dunier *et al.*, 1987, companion paper).

Hypoglycemia in carps was very severe after 30 days and less pronounced after a longer time. Gluth and Hanke (1985) have also shown that lindane had no effect on glucose elevation. According to Gupta and Singh (1982) and Lakota *et al.* (1983), the first decrease could be the result of regressive changes in the intestines. Glucose absorption in the small intestine was decreased by different agricultural insecticides (Sastry and Siddiqui, 1982).

At 30 days, gluconeogenesis was not stimulated since liver PEPCK activity alone was significantly stimulated. At this time hypoglycemia in intoxicated carp resulted in the reduction of intestinal glucose absorption and in the nonactivation of gluconeogenesis. This last observation has also been made in scorpion fish fed for 21 days

with food contaminated with lindane (Escoubet and Vicente, 1975). During acidic or classic thermal stress, this metabolic pathway is quickly stimulated (Demael *et al.*, 1984). Thus lindane given for 30 days does not act as a stress factor on carp but as a pollutant which modifies liver metabolism after tissue accumulation.

The effect of lindane on glycemia was reduced or abolished after 109 days. The possibilities of gluconeogenesis were higher than after 1 month intoxication. However, this metabolic pathway was not stimulated to the same extent in all groups. We can observe that G6Pase, a microsome enzyme, was activated much more after a 109-day exposure to lindane than after 30 days.

According to Gupta and Singh (1982) histological lesions in the intestines and gills resulting from lindane exposure were responsible for the hyponatremia observed in all lots of intoxicated carps. Sugar entry in the enterocyte is bound to Na^+ and this is a membrane process. When glucose absorption is disrupted, Na^+ entrance is disrupted as well. A similar situation may exist with ATPase activity in the gills (Gupta and Singh, 1982).

In fact, the effect of pesticide on histological perturbations was linked to its highly lipophilic nature. Their toxic effects may be partially related to their accumulation in membranes and to changes in fluidity which may modify membrane protein exposure (Momchilova *et al.*, 1985; Antunes-Madeira and Madeira, 1985).

In carp contaminated with lindane, ATPase activities showed variations which could result from changes in membrane fluidity (Cornelius and Skou, 1984). Lindane exposure seems to modify the level of muscular membrane cholinesterases. This result is in agreement with other observations (Bakthavathsalam and Reddy, 1983a,b,c). Momchilova *et al.* (1985) reported that 5'-nucleotidase activity was also strongly dependent on environmental lipids. With the concentrations of lindane used here, no severe perturbation was observed in the muscle membrane-bound enzymes after 1 month of exposure. After 3 months of lindane exposure, these enzyme activities are more affected.

In conclusion, the results of the present study support the idea that lindane affects the function of cellular structures by modifying the properties of the membrane lipidic matrix leading to metabolic dysfunctions.

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