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Changes in Selected Biochemical Parameters in the Brain of the Fish, *Anguilla anguilla* (L.), Exposed to Lindane

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Pesticides used in pest control programs have been shown to produce many physiological and biochemical changes in freshwater organisms by influencing the activities of several enzymes. Among these physiological changes, energy metabolism has a key role as the animal is forced to expend more energy to mitigate toxic stress. Based on this, an attempt was made to study the sublethal effect of lindane (an organochlorine insecticide) on the carbohydrate metabolism of the eel. This fish was selected because of its wide availability, edibility in Spain and its important ecological role in the Albufera Lake of Valencia, Spain.

Since brain plays an important role in fish physiology and it is a rather interesting tissue in fish toxicology, especially when pesticides are involved (because of their mode of action in the nervous system (Ware 1983)), the study of brain was carried out to elucidate the intricate metabolic compensatory mechanisms in this tissue during lindane exposure.

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 (8.00 to 20.00 hr) was maintained (Ferrando et al. 1987). 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 brain 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 anaesthetized (MST 222); brain tissue was removed and we made the metabolite determination. For glycogen determination, tissue homogenization was made using KOH (60% and 30‰), and glycogen content was estimated by anthrone reagent method (Seifter et al. 1950). Glucose was determined from the sobrenadants of glycogen determination samples, spectrophotometrically (610 nm), using the GOD-Period method (Boehringer-Mannheim). Lactate was estimated spectrophotometrically (340 nm) using kits from Boehringer-Mannheim. Previously, tissues were homogenized with trichloroacetic acid (7‰) (Lang and Michal 1974). To determine pyruvic acid levels we homogenized tissues in perchloric acid (1M) (Dange and Masurekar 1982) and using kits from Boehringer-Mannheim. The lipids were analyzed spectophotometrically (530 nm) with a test kit from Boehringer-Mannheim and we used the method of Bligh and Dyer (1959) for the extraction process (chloroform, methanol, water).

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 metabolic changes in eel brain under lindane stress are summarized in Table 1 and 2. No eels died during exposure to both insecticide concentrations.

There was a gradual depletion in glycogen levels in brain after 6, 12, 24 and 72 hr of treatment with 0.167 mg/L of lindane (Table 2). Decrease in glycogen content is indicative of increased rate of glycogenolysis. On the other hand, we found a decrease in glucose levels after 72 and 96 hr that can be indicative of its exit into the blood. Normally the decrease of tissue glycogen content runs parallel to a elevation of glucose in the serum (Hanke et al. 1983). There are no data about the effect of pesticides on brain carbohydrate metabolism. Sreenivasula et al. (1986) reported hyperglycemia in Malathion exposed *Oziotelphusa senex senex* accompanied by decrease of glycogen levels in liver and muscle. Thus, a decreased carbohydrate

Table 1. Alterations in the metaolic levels of brain in control *Anguilla anguilla* and those exposed to 0.335 mg Lindane L^{-1}.

Parameter	Control	Exposure period (hr)					
		6	12	24	48	72	96
Glycogen	18.22	21.54*	21.02*	18.14	21.14*	21.93*	16.05
(mg/g wet wt)	±2.50	±2.64	±2.29	±1.80	±1.75	±3.22	±2.92
Glucose	0.85	1.55**	1.57**	1.48**	1.55**	1.03*	0.95
(mg/g wet wt)	±0.10	±0.21	±0.14	±0.10	±0.19	±0.15	±0.14
Lactate	599.1	639.8	712.86*	781.35**	785.7**	641.4	550.11
(mg/g wet wt)	±38.18	±22.47	±32.96	±73.4	±69.3	±42.8	±50.91
Pyruvate	3.33	3.97	3.92	3.99	4.61*	5.28**	4.15
(mg/g wet wt)	±0.46	±1.20	±0.96	±0.95	±0.91	±0.91	±0.61
Lipids	530.04	211.26**	190.13**	361.90*	444.7*	559.71	529.44
(mg/g wet wt)	±143.7	±49.79	±45.09	±99.73	±64.87	±63.28	±72.10

The values are means ± SD of 10 observations

* $p<0.05$

** $p<0.001$

Table 2. Alterations in the metaolic levels of brain in control *Anguilla anguilla* and those exposed to 0.167 mg Lindane L^{-1}.

Parameter	Control	Exposure period (hr)					
		6	12	24	48	72	96
Glycogen	18.22	10.55*	10.01**	9.02**	17.65	14.84*	17.02
(mg/g wet wt)	±2.50	±1.90	±1.53	±1.80	±2.95	±3.65	±3.42
Glucose	0.85	0.787	0.797	0.820	0.755	0.726**	0.565**
(mg/g wet wt)	±0.10	±0.054	±0.053	±0.066	±0.17	±0.15	±0.13
Lactate	599.1	625.98	711.12*	751.16**	589.14	459.92*	484.16*
(mg/g wet wt)	±38.18	±22.31	±50.03	±71.51	±15.44	±57.92	±52.37
Pyruvate	3.33	4.98**	5.35**	6.86**	4.15*	3.97	3.96
(mg/g wet wt)	±0.46	±1.04	±0.51	±0.87	±1.24	±0.25	±0.32
Lipids	530.04	1330.5**	1266.6**	1434.3**	759.61*	483.22	559.9
(mg/g wet wt)	±143.7	±64.1	±48.5	±238.5	±134.3	±20.42	±63.47

The values are means ± SD of 10 observations

* $p<0.05$

** $p<0.001$

content in lindane intoxicated eel (Table 2) may be due to the rapid utilization of carbohydrates by the tissue, possibly to overcome the pesticide induced stress. Dezwaan and Zandee (1972) reported that anoxic or hypoxic conditions were known to elevate carbohydrate consumption. Lactate levels increased to maximum extent at 12-24 hr with 0.167 mg/L of lindane (Table 2). High lactic acid content in brain is suggestive of the emphasis laid on glycolysis during pesticide stress. The rate of lactate production is considered as an index of physiological stress in the biological system (Krishna et al. 1987). Prevalence of stress conditions in the brain tissue might have result in the elevation in lactate content.

The elevated pyruvate levels in fish after 6 hr exposure were possible due to rapid glycolysis under hypoxia (Table 2). Endosulfan also induced elevation of both pyruvate and lactate in *Heteropneustes fossilis* (Narendra et al. 1981). Perhaps, the oxygen debt by this tissue was paid off so that lactate would be reconverted to pyruvate and metabolized through the Kreb's cycle. Gut et al. (1984) showed that acrylonitrile induced significant increases in brain lactate and pyruvate in rats.

Increased levels of total lipids content (Table 2) at 6, 12, 24 and 48 hr of exposure to 0.167 mg/L of lindane suggests lipogenesis under pesticidal intoxication.

The results were different after treatment with 0.335 mg/L of lindane. We found an increase in brain glycogen content at 6, 12, 24, 48 and 72 hr (Table 1) suggesting an inhibition of glycogenolysis or an increase in glycogenesis or gluconeogenesis. The high brain glucose levels support this argument (Table 1). In the catfish, *Heteropneustes fossilis* acute methyl parathion poisoning induced hyperglycemia followed by increase in hepatic glycogen content (Srivastava and Singh 1981). Hanke et al. (1983) found a decrease in their liver glycogen content of *Cyprinus carpio* with low doses of DDT and an increase in this parameter with high levels of this pesticide suggesting a clear correlation between glycolysis and gluconeogenesis.

Lactate and pyruvate content were markedly increased during the exposure time (Table 1). The results are similar to those found with 0.167 mg/L. Both elevated lactic and pyruvic acid content suggest a severe respiratory stress in the fish tissue. According to Dange and Masurekar (1982), an upward trend in lactic acid in the tissues may be taken to indicate that oxygen supply to the tissues is not adequate for the normal metabolic functions.

The brain total lipids level in *Anguilla anguilla* was significantly lowered after 6, 12, 24 and 48 hr of exposure to 0.335 mg/L of lindane (Table 1). The observed decrease could be due to its used as an energy source instead of glycogen reserves. The utilization of lipid reserves may constitute a carbohydrate source. As we can see in Table 1, the decrease in lipid levels was correlated with an increase in glycogen and glucose levels.

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