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Effects of the Polychlorinated Biphenyl Aroclor 1242 on Locomotor Activity and on the Neurotransmitters Dopamine and Norepinephrine in the Brain of the Gulf Killifish, *Fundulus grandis*

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Several environmental pollutants have been found to alter brain neurotransmitters. SHARMA (1973) found that chronic dietary exposure of mallard ducks to dieldrin caused depletion of brain 5-hydroxytryptamine (5-HT), norepinephrine (NE), and dopamine (DA). However, SHARMA (1976) found that chronic administration of dieldrin to mice failed to alter significantly the brain 5-HT, NE, or DA, but the 5-hydroxyindoleacetic acid (5-HIAA) level increased. TAYLOR & DISTEFANO (1976) found that sublethal doses of methylmercury produced a decrease in brain 5-HT, 5-HIAA, NE and DA in rat pups. HRDINA et al. (1973) found that acute doses of p,p'-DDT caused a decrease in brain NE in rats, but no changes in DA or 5-HT. The polychlorinated biphenyl (PCB), Aroclor 1254, has been shown to suppress avoidance behavior in coturnix quail chicks suggesting an effect on the nervous system (KREITZER & HEINZ 1974). Injections of low level doses of DA into rats resulted in stimulation of locomotor activity, while NE depressed activity (PIJNENBURG et al. 1976). MCDONALD (1979) found that intraperitoneal injections of DDT and parathion into goldfish (Carassius auratus) altered the concentrations of brain neurotransmitters 5-HT, DA, and NE. Dopamine and NE levels were decreased in the brains of ring doves (Streptopelia risoria) fed DDE, dieldrin or Aroclor 1254 (HEINZ et al. 1980).

Only a few studies have been conducted to determine the effects of sublethal levels of environmental pollutants on the locomotor activity of fishes. This is surprising because locomotor activity of animals has been suggested as a sensitive indicator of toxicity since locomotor activity occurs naturally and thus not only reflects the functional status of the nervous system but also represents behavior relevant to the animal's survival. More surprising is the lack of studies attempting to correlate the effects of environmental pollutants on brain neurotransmitters and locomotor behavior. DAVY et al. (1972) found that a sublethal concentration of DDT reduced the locomotor activity of goldfish. KLEEREKOPER et al. (1972) and TIMM et al. (1972) reported that sublethal concentrations of copper changed the locomotor behavior of goldfish and catfish (Ictalurus punctatus); the average size of the turns made increased when the fish were exposed to copper, although the velocity of the locomotor activity was unaltered. RAND (1977) found that a sublethal concentration of parathion caused a decline in the general locomotor behavior of goldfish. Sublethal concentrations of DDT, cadmium, chromium or zinc caused hyperactivity in the bluegill (Lepomis macro-

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chirus) (ELLGAARD et al. 1977, 1978).

In the ongoing experiments with pollutants in this laboratory, hyperactivity has been observed in crabs, Uca pugilator, exposed to sublethal concentrations of two organochlorine compounds, DDT (FINGERMAN et al. 1979) and the Aroclor 1242 (unpublished results). Aroclor 1242 was also found to produce an increase in the level of melanin-dispersing hormone in the eyestalk neuroendocrine cells of fiddler crabs, suggesting an effect on eyestalk neurotransmitters that control release of this hormone (FINGERMAN & FINGERMAN 1978, NAGABHUSHANAM et al. 1979). In view of the observation described above and because of the persistence in the environment of PCBs the following experiments were designed to determine the effects, if any, of the Aroclor 1242 on both the brain neurotransmitter levels and locomotor activity of the Gulf killifish, Fundulus grandis.

MATERIALS AND METHODS

Specimens of adult male F. grandis were obtained from bait dealers in Louisiana. The stock supply of animals was maintained in aerated, filtered 50% artificial sea water (Instant Ocean, Aquarium Systems, Inc., Eastlake, OH) and on a 12 h light (1050 lx) and 12 h dark schedule. Water temperature was 25°C. The stock fish were fed fish flakes daily. However, the fish used in the experiments were not fed from the time they were first selected from the stock tank.

The PCB used was Aroclor 1242 (Monsanto Lot No. G266K). It was first dissolved in acetone (8 mg/mL) and diluted 1:2000 with 50% artificial sea water to provide the desired final concentration of 0.0004%. Control fish were exposed to 50% artificial sea water containing 0.05% acetone, the same concentration as in the PCB-containing solution.

Assays for brain NE and DA were conducted following the methods of MAICKEL et al. (1968) and ANSELL & BEESON (1968). Brain tissues were placed on dry ice as they were dissected out. Whole brains were weighed and homogenized in acidified n-butanol. In addition to tissue sample tubes, blank and recovery tubes were also prepared. Blank tubes contained 0.3 mL of distilled water. Recovery tubes contained 0.1 mL of a standard solution of either DA or NE. Fluorescence of the NE was measured at 385 nm activation and 485 nm emission and DA at 320 nm activation and 385 nm emission. The following formula was used to calculate neurotransmitter concentrations:

$$\frac{X - Y}{Z - Y} \times \frac{1000}{M} = \text{neurotransmitter concentration } (\mu g/g)$$

where, X = fluorescence reading of sample, Y = fluorescence reading of blank, Z = fluorescence reading of recovery, and M = weight of tissue sample in mg.

The swimming activity of F. grandis was observed in activity chambers kept in constant illunination (1050 lx) that were patterned after those devised by HOAR et al. (1955) for use with fishes. Each chamber was 53 x 26 x 6 cm and the bottom of each was marked with 4

equally spaced transverse black lines. Activity was determined by counting the number of lines a fish crossed during 10 min of observation. In both sets of experiments statistical analyses involved both calculations of standard deviation and Student's t test.

EXPERIMENTS AND RESULTS

The first set of experiments was designed to determine the effects of a sublethal level of Aroclor 1242 on brain neurotransmitters of F. grandis. Experiments were performed twice. Male fish were exposed at noon to the PCB-solution or control solution. At noon on the following day, after a 24-h exposure, the brains were removed and assayed for NE and DA, a total of 10 control and 10 experimental fish were used in the assays. Assays for 5-HT and 5-HIAA were also run, however, there were no statistically significant differences between the levels in the control and PCB-exposed fish.

After a 24-h exposure to Aroclor 1242, the whole brain concentration levels of both NE and DA were significantly decreased as compared with the controls (TABLE 1). The average NE concentration in the control fish was 2.5 µg/g and in the PCB-exposed fish, 0.61 µg/g ($p < 0.01$). The DA level was 0.91 µg/g in the control fish and 0.44 µg/g in the PCB-exposed fish ($p < 0.001$).

TABLE 1. Averages (mean ± standard error) of the brain neurotransmitters, DA and NE, in F. grandis.

	Controls			Aroclor 1242-exposed			
	$\bar{x}$ ± S.E.	(No.)		$\bar{x}$ ± S.E.	(No.)	p
NE	2.5 ± 0.6	(10)		0.6 ± 0.1	(10)	0.01
DA	0.9 ± 0.1	(10)		0.4 ± 0.1	(10)	0.001

The second set of experiments was designed to determine the effects of a sublethal level of Aroclor 1242 on the locomotor activity of F. grandis. Experiments were performed three times. Four control and 4 experimental males were used in each experiment with 2 fish in each activity tank. The fish were placed in the activity chambers containing 50% artificial sea water for 24 h before being observed. Their locomotor activity was recorded for 10 min at noon for two days (-day 2 and -day 3) before they were exposed to the PCB-solution or control solution. After the second observation on -day 1, the medium in the activity chambers was changed; half the fish were exposed to the PCB solution and half to the control solution of acetone in 50% sea water. Locomotor activity was again observed at noon 24 h later (+day 1) and the next two days (+day 2 and +day 3), thereafter. The results from each experiment were averaged for each day and are presented in TABLE 2.

The averaged activity for the controls was 6.8 and 4.6 lines crossed in 10 min, respectively, for -day 2 and -day 1; for the groups that would be exposed to the PCB-solution, the averages were 15.6 and 9.7 for -day 2 and -day 1, respectively (TABLE 2). The groups were not statistically significantly different even though the group destined to be exposed to Aroclor 1242 was slightly more active than the controls. On +day 1 the average locomotor activity of the control groups was 3.6, while the PCB-exposed fish averaged 94.0 ($p < 0.001$). On +day 2 the control group averaged 9.0 and the PCB-group 78.9 ($p < 0.001$); on +day 3 the control group averaged 13.7 and the PCB-group 44.8 ($p < 0.01$). Although the PCB-groups averaged less on +day 2 and +day 3 than on +day 1 there was still a statistically significant difference between the PCB-groups and controls. A comparison of the -day 2 control data with the +day 3 control data was not statistically significant, however, a comparison of the -day 2 PCB-to-be group with the +day 3 PCB-exposed group was significantly different ($p < 0.01$).

TABLE 2. Average number of lines (mean ± standard error) crossed by male F. grandis.

	Controls			Experimentals			
	$\bar{x}$ ± S.E.	(No.)		$\bar{x}$ ± S.E.	(No.)	p	
-Day 2	6.8 ± 3.4	(12)		15.6 ± 4.9	(12)	N.S.	
-Day 1	4.6 ± 1.9	(11)		9.9 ± 2.2	(12)	N.S.	
+Day 1	3.6 ± 1.8	(11)		94.0 ± 13.4	(11)	0.001	
+Day 2	8.0 ± 2.9	(7)		78.9 ± 7.2	(8)	0.001	
+Day 3	13.7 ± 6.8	(3)		44.8 ± 2.7	(4)	0.01	

DISCUSSION

Where investigations of other pollutants have revealed changes in 5-HT, NE and DA, usually all three are either increased or decreased. Chronic exposure to dieldrin caused depletion of brain 5-HT, NE and DA in mallard ducks (SHARMA 1973). SHARMA suggested that changes in the brain biogenic amines could be related to behavioral disorders following exposure to environmental contaminants. Interestingly, SHARMA (1976) found that chronic administration of dieldrin to mice did not produce any alteration in brain 5-HT, NE or DA, but did cause an increase in the 5-HIAA levels. Nonlethal doses of methylmercury produced an initial decrease in brain 5-HT, 5-HIAA, NE and DA in rat pups on day 8; the concentrations increased and exceeded the control values by day 15 of exposure (TAYLOR & DISTEFANO 1976). In the present investigation both NE

and DA in the fish brains decreased after a 24-h exposure of the fish to Aroclor 1242, but as stated above, there was no effect on brain 5-HT or 5-HIAA. One investigation in which only one of the brain neurotransmitters was affected by a pollutant was that of HRDINA et al. (1973) who used an acute dose of p,p'-DDT. They found that p,p'-DDT caused a decrease in brain NE in rats, but no change in the DA or 5-HT concentrations. MCDONALD (1979) found that intraperitoneal injections of DDT into goldfish reduced the brain NE and DA, but elevated the level of 5-HT. In the same study, parathion decreased brain NE and 5-HT, but had no effect on DA. He concluded that these pollutants produced their effects on the transmitter levels by affecting the activities of the enzymes involved in the synthesis and degradation of these biogenic amines. The levels of DA and NE were measured in one-half of the brain of ring doves after graded dietary exposures to DDE, dieldrin, or Aroclor 1254; the levels of the pollutants were determined in the other half of the brain (HEINZ et al. 1980). They found that all three pollutants had a dose-related effect on the DA and NE levels in the brain. As the levels of DDE, dieldrin or Aroclor 1254 increased in the brain, the DA and NE decreased.

In the present study, the Aroclor 1242 caused highly significant increases in the locomotor activity of fish that had been exposed for 24 h. It is not surprising that the average number of lines crossed on +day 2 and +day 3 were less than on +day 1 since the PCB solution was not changed once the fish were exposed to it. DAVY et al. (1972) found that returning goldfish to clean water after 4 days of exposure to DDT did not result in restoration of their normal locomotor behavior. Similarly, when bluegills were returned to clean water free of DDT for 2 weeks, the effects of DDT on their locomotor behavior was not reversed (ELLGAARD et al. 1977). KREITZER & HEINZ (1974) found that when they again fed coturnix quail chicks food free of Aroclor 1254, there was no recovery toward their normal avoidance behavior.

In a study by PIJNENBURG et al. (1976), injections of low level doses of DA caused stimulation of locomotor activity in rats, while low level doses of NE depressed activity. The DA metabolites 3-methyoxytyramine and homovanillic acid (HVA) tested did not produce an increase in activity, while another DA metabolite, dihydroxyphenylacetic acid (DOPAC) produced a slight initial increase, but far less than did the effect of DA. If, indeed, DA stimulates locomotor activity in F. grandis as in the rat, then Aroclor 1242 may be causing the release of excessive DA, thereby not only increasing locomotion but also decreasing the brain concentration of DA, the effects seen herein. And if NE depresses locomotor activity as in the rat, Aroclor 1242 may be destroying noradrenergic neurons or preventing the synthesis of NE, resulting in a lowered brain concentration of NE and allowing increased locomotor activity to occur, just the effects observed herein with this Aroclor. The results obtained in this study show that not only may locomotor activity be a useful tool in monitoring pollution but may also be a significant indicator of alterations in the brain levels of important biogenic amines.

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