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THE ANALYSIS OF DIELDRIN RESIDUES IN THE BRAIN, LIVER AND MUSCLES OF THE AFRICAN CATFISH Clarias gariepinus (BURCHELL, 1822) CHRONICALLY EXPOSED TO DIELDRIN.

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ABSTRACT

Mature adult *Clarias gariepinus* were obtained at the **ABRU** hatchery in Sonning (**UK**), were they had been bred and reared for several years. These were exposed to two concentrations of dieldrin in water (2.4μg^{-1} and 4.0μg^{-1}).

The residue analysis of dieldrin in three tissues exposed for one month at two concentrations was carried out. The fish exposed and those under control were killed and liver, brain and muscles samples were removed. These were subjected to GLC analytical process. The results indicated significantly ($p< 0.05$) higher residues in liver than in muscle and brain.

The results also showed that residue levels were dependant on exposure concentration.

INTRODUCTION

The accumulation by fish of appreciable amounts of a pesticide from relatively low aqueous levels is a common phenomenon. Evidence of organochlorine insecticide residues in salmonid fish (underyearling, *Onchorhynchus mykiss* Walbaum) was provided by Holden (1973), who detected low levels of dieldrin in gills, muscle and liver. He showed that prolonged exposure to low concentrations of dieldrin may result in higher concentrations of pesticides in the gills and muscles.

On a comparative basis, his survey of the salmonids from various habitats in Scotland, which received pesticide contaminants from various sources, revealed widely differing pesticide residue levels including dieldrin. Similarly, Koeman *et al.*(1971), made the same observations on the variation in dieldrin residue levels in different Nigerian indigenous freshwater species, in his wild test sprays of some parts of Bauchi State. He observed that levels of dieldrin varied both in total body and between tissues. For example, he found that the total body level of dieldrin in *Synodontis gambiensis* (Gunther) was six times higher than in *Epiplatys bifasciatus* (Steindachner), and in liver, the level in *Synodontis ocellifer* was 80 times higher than in *Hyperopisus bebe occidentalis* (Gunther).

The uptake of dieldrin and dieldrin-induced changes in the activities of microsomal liver enzymes of the marine flatfish *Pleuronectes platessa L.* was investigated by Vink (1975). He observed that dieldrin suppresses the activity of aminopyrine demethylase, at a steady-state level of dieldrin in the liver (5 $mg.kg^{-1}$, wet weight), which is reached after two weeks' exposure.

Whole body accumulation and tissue distribution of dieldrin in rainbow trout through oral-dose tests and subchronic exposures via water, was the subject of study by Curtis et al., (1986). They found that whole body dieldrin residues measured in fish exposed for 96 h or less were proportional to both exposure concentration and duration. Residues in fish exposed for 96 h to 0.15 $\mu g\ l^{-1}$ average 0.548 μg dieldrin/ g fish and residues following exposure to 0.99 μgl^{-1} averaged 5.65 $\mu g\ g^{-1}$. There was some evidence that feeding the fish a growth ration helped to produce greater total percent lipid and therefore more dieldrin residues than did feeding a maintenance ration only.

This part of the work aims at determining how a single dose of dieldrin administered on a regular basis for 30 days was distributed to some tissues of the body of fish that had not been previously exposed to dieldrin.

MATERIALS AND METHODS

Fish for this work were obtained from the ABRC (Aquatic biology Research Centre) in Sonning. They were housed individually as a precaution against aggression which might lead to death. This housing was maintained for throughout the experimental period. The fish were then exposed to two concentrations of dieldrin $2.4\mu h^{-1}$, $4.0\mu g^{-1}$ and controls.

Preparation of tissues for extraction.

After 30-days of exposure, the fish were removed, anaesthetized, weighed and measured (Table 1).

They were then killed and whole liver, whole brain and muscle tissues were removed, lightly dried between tissue papers and weighed (Table 1). For the muscle, a specified weight of 4 grams was taken from the mild latero-dorsal part of the body. The tissues were then placed in 20 ml. glass vials and kept in a freezer until required.

Extraction

The extraction procedure finally adopted was found to recover 75-80% of the dieldrin from control tissue samples to which known amounts of dieldrin had been added. Samples of 250 mg of the tissues were taken. Each tissue was then sliced into small pieces in a beaker. A little clean acid-washed sand, and 4 g of anhydrous sodium sulphate were added to the tissue. This was ground in the beaker with a glass rod to a free flowing mix, which was then extracted five times with small volumes (4 ml) of 7:3 n-hexane: acetone at 60 °C. The five extracts were combined in a glass stoppered graduated 25 ml. test tube. This was filtered through a glass wool plug in a filter funnel and a solution of 2% Na_2SO_4 was added (4 vols to 1 vol of extract). This was shaken for one minute in a separating funnel, then allowed to separate, the lower water part was then drained out and the upper layer was transferred to a 15 ml test tube via an anhydrous sodium sulphate filter to remove any water. The residual aqueous acetone mixture was then washed with a further 3 ml of hexane, which was further dehydrated by passing it through the anhydrous sodium sulphate filter. This final extract was added to the main extract.

The volume of the extract was then blown down through a slow stream of oxygen free nitrogen to obtain a volume of 2 ml. This process was done to all tissue samples.

Column Clean-up

The 2 ml extract obtained from the preceding process was subjected to a clean-up using florisil (60-200 mesh). To clean-up, florisil was heated in an oven at 60°C for 15 hours. It was then removed, partially cooled and mixed in 3% w/w of distilled water to deactivate the materials. In the meantime, glass columns were prepared by cutting glass tubes (3 cm id) into 10cm lengths, which were then heated at one end to produce narrow openings of about 2 mm (id). These were then plugged at that end with cotton wool. The columns were placed on a double deck wooden rack supported on either side by a stand and the whole frame was placed in a fume cupboard. The florisil was then topped with 1 g of anhydrous sodium sulphate, and washed through with 10 ml of n hexane.

After washing through with the n hexane, the 2 ml in hexane extract was then added and was followed by another 10 ml of 5% diethylether:n hexane, the elute (10 ml) was rejected. This was then followed by 30ml of 10% diethylether, the elute was collected and examined by electron-capture GLC.

GLC analysis

The cleaned-up extracts, were taken from the fume cupboard to the GLC. bench for injection. The injection was carried out using a 10 µl Hamilton syringe, a volume of 3 µl being injected at the port.

Table 1: Final weights and lengths of *C. gariepinus* exposed to dieldrin at 2.μgl^{-1} and 4.0μgl^{-1} over a 30-day period, and also the weights of tissues removed. Liver and brain tissues' weight are total and that of fat is that found at the main depot covering the viscera.

Concentration 2.4μgl^{-1}

Fish No	Sex (g)	Wt. (mm)	Len (g)	Muscle sam (g)	Liver (g)	brain	Fat (g)
1	m	140.30	293	4	1.04	0.25	0.03
2	f	138.40	290	4	1.10	0.35	0.08
3	f	169.50	300	4	1.12	0.24	0.09
4	f	155.70	295	4	0.79	0.41	0.05
5	m	248.70	344	4	1.30	0.30	0.06
6	m	207.07	320	4	1.92	0.28	1.78

Concentration 4.0μgl^{-1}

Fish No	Sex (g)	Wt. (mm)	Length (g)	Muscle sam (g)	Liver (g)	brain	Fat (g)
1	m	200.60	330	4	1.15	0.62	0.81
2	f	126.60	272	4	0.82	0.59	-
3	f	142.70	282	4	0.93	0.28	-
4	f	146.30	286	4	1.07	0.33	-
5	m	122.20	297	4	0.65	0.41	-
6	m	134.60	272	4	0.83	0.30	-
Cont. 1	m	129.80	277	4	1.10	0.27	1.81
Cont. 2	f	126.56	256.6	4	1.21	0.24	1.73

Table 2: The effects of dieldrin on weights of adult C. gariepinus exposed to dieldrin at 2.4 and 4.0 μ l^{-1} for 30 days.

Concentration 2.4 $\mu g l^{-1}$

Fish No	Sex	Int. Wt. (g)	Final wt. (g)	Wt. Diff. (g)	+ /- (%)	Remark
1	m	151.60	140.3	11.26	-7.43	loss
2	m	124.79	138.4	13.61	+10.91	gain
3	f	154.46	169.5	15.04	+9.737	gain
4	f	153.30	155.7	2.40	+1.566	gain
5	f	211.08	248.7	37.62	+17.823	gain
6	m	189.80	207.07	17.27	+9.099	gain

Concentration 4.0 $\mu g l^{-1}$

Fish No	Sex (g)	Int. Wt.	Final wt.	Wt. Diff.	+ /- (%)	Remark
1	m	151.40	200.6	49.20	+32.497	gain
2	f	146.50	126.60	19.90	-13.584	loss
3	f	134.10	142.70	8.60	+6.418	gain
4	f	133.70	146.30	12.60	+8.975	gain
5	m	166.82	122.2	44.62	-26.747	loss
6	f	133.90	134.60	0.72	+0.538	gain
Cont. 1	m	112.20	129.80	17.60	+15.686	gain
Cont. 2	f	114.30	126.56	12.26	+10.726	gain

Table 3: Dieldrin ($\mu g\ mg^{-1}$) found in samples of tissue from fish exposed to 2.4 and 4.0 μgl^{-1} dieldrin per litre for 30 days.

Tissue Conc.(μgl^{-1})->	Muscle 2.4	4.0	Liver 2.4	4.0	Final wt. 2.4	4.0
Fish no 1	0.0996	0.550	0.903	2.0396	0.059	0.721
2	0.128	0.162	0.9185	4.046	0.0604	0.166
3	0.121	0.211	1.138	3.780	0.0226	0.297
4	0.195	0.378	0.890	3.411	0.0896	0.067
5	0.123	0.508	0.754	4.217	0.0502	0.545
6	0.136	0.268	0.358	3.482	0.004	0.151
Mean	0.134	0.346	0.827	3.496	0.048	0.325
SD	±0.03	±0.16	+0.26	±0.78	±0.03	±0.26
Control	0. 00	0.00	0. 00	0.00	0. 00	0.00
Control	0. 00	0.00	0. 00	0.00	0. 00	0.00

Table 4: Percentage distribution of dieldrin in samples of three tissues (muscle, liver and brain) of C. gariepinus, exposed to two chronic levels (2.4 & 4.0 μg^{-1}) of dieldrin over a period of 30 days.

Concentration 4.0 μgl^{-1}

Fish No	Tot.diel.rec. ($\mu g\ mg^{-1}$)	Muscle %	Liver %	Brain %
1	1.0613	9.384	85.047	5.568
2	1.107	11.572	82.972	5.456
3	1.2813	9.412	88.823	1.764
4	1.1736	16.581	75.784	7.635
5	0.9273	13.232	81.354	5.414
6	0.4969	27.349	71.946	0.704
Tot.	6.0474	27.349	485.926	26.541
Mean	1.008	87.47	80.988	4.424

4.0 μgl^{-1} exposure level

Fish No	Tot.diel.rec. ($\mu g\ mg^{-1}$)	Muscle %	Liver %	Brain %
1	3.3111	16.614	61.599	21.787
2	4.374	3.704	92.501	3.795
3	4.2875	4.916	88.152	6.932
4	3.8556	9.809	88.461	1.729
5	5.270	9.638	80.013	10.35
6	3.9004	6.858	89.272	3.87
Tot.	24.999	51.534	499.938	48.042
Mean	4.166	8.589	83.323	8.077

RESULTS

Mortality & weight loss

No mortality was observed, however, a few fish (e.g. 2 and 5 at 4.0 μgl^{-1}) exhibited extreme weight loss (Table 2). This was apparently due to depressed appetite probably as a result of dieldrin toxicity. The rest of the fish appeared healthy and fed fairly well, however, all treated fish accepted food less frequently than the control fish. Fish 5 (4.0μgl^{-1} did not start accepting food until the 14th day and fish 1 (2.4μgl^{-1} did not accept food for nine days. This was reflected in their small fat depot (Table 1). Rates of growth appeared to be generally lower at the higher concentration (apart from fish 1 which grew spectacularly well) than at the lower concentration or in the control fish.

Regression analysis was carried out but did not show any relationship between final weight and levels in the other tissues at the two levels of treatment. Similarly too, no relationship was found between the weights of liver and brain taken and the level of dieldrin.

Fat depot weights are lower in the higher concentration than in the lower concentration, although they are generally low in all treated fish than control (Table 1). There was a relationship between % weight of liver and fat in the lower concentration. The controls had bigger liver and more fat.

Recovery levels.

The total computed recoveries made from the three tissues of each fish are given in Table 3. The values for each fish ranged from 0.497μl^{-1} - 1.281μl^{-1} at 2.4μl^{-1} exposure level, while at 4.0μl^{-1}, the range was 3.311μgmg^{-1}. - 5.27 $Ngmg^{-1}$

Tissue distribution of dieldrin

Analysis of tissue residues indicated varying levels of dieldrin, with the lowest values in brain tissue and highest in liver. This is rather conspicuous in the mean values given (Table 3). The liver concentrations were particularly high when compared to the brain, especially at 2.4 μgl^{-1}, where the mean value was 18 times higher than in brain, while at the 4.0 μgl^{-1} exposure level, the concentration was 10 times higher. The analysis of variance showed a significantly high concentration levels.

A computation of comparative percent distribution in tissues, shows that individual fish had higher dieldrin levels in liver than other tissues at the two aqueous concentration levels. Also mean percentage values reflected the same trend. However, comparing individual tissues, it was observed that the mean percentage value in muscle at the lower aqueous concentration was higher compared to that at the higher aqueous concentration. Conversely, it was observed that the mean percentage level in both brain and liver was higher at the higher aqueous concentration than at the lower concentration.

DISCUSSION

Growth and fat depot

There was a general mean weight increase from 164.17±30.9 g to 176.61±43.34 g in the lower concentration compared to a mean weight decrease of 125.5±61.1g 144.4±13.32g in the higher concentration over the 30 days exposure. The two concentrations taken together, the effect of dieldrin on weight could be seen in fish 1 at 2.4 μgl^{-1}, and 2 & 5 at 4.0μgl^{-1}, where a mean % loss of between 7- 26.3 g was recorded, while those fish that showed weight gain was between 0.5-32.5g. Fish 1 at 4.0μgl^{-1} showed a spectacular weight gain because unlike most fish, it fed well throughout the experimental period. These observations show a high rate of variation between fish, and agree with the observations of many fish toxicologists.

It is possible that due to increased activity (Holden, 1973; Srivastaya and Mishra, 1987) and depressed appetite as a result of dieldrin toxicity, most treated fish must have used up their fat reserve. This is reflected in the fat depot as only the fastest growing fish (fish 1) at the higher concentration had fat (Table 1).

The effect of dieldrin accumulation on the weight gain or loss was clearly demonstrated in Table 2 Fish no. 2 and 5 exposed at 4.0 μgl^{-1}, were particularly emaciated after the experiment. They had lost weight considerably, this was reflected in the residue analysis results as can be seen in Table 3 especially liver values. There may be a relationship between the amount of dieldrin accumulated in the liver and weight loss. In an earlier experiment on the effects of dieldrin on growth, it was observed that dieldrin had a depressed growth effect.

Tissue distribution of dieldrin

GLC analysis of tissues for dieldrin revealed a pattern of variation in distribution of the chemical. Variation in the distribution of dieldrin in body tissues has been a common observation by toxicologists, whether in field or laboratory exposures. The dieldrin tissue concentration variation observed in this work is similar to that observed by Holden (1973). He found that when rainbow trout were exposed to an aqueous concentration of 0.0056 ppm dieldrin for 5-7 days, the gills accumulated 13.8 ppm, muscle, 3.5 ppm and 20.8 ppm in liver. His results indicated that the liver had about 6 times the level in the muscle. This is similar to my results. Holden (1973), also recorded similar trends in the same fish which he had exposed at a concentration of 0.0023 ppm for 17-23 days, that was 18.0 ppm in gills, 7.7 ppm in muscles and 16.0 ppm in the liver. One striking thing, however, in these results was that the levels had varied with exposure time. For example in the liver, the level was lower than in the shorter exposure period. In reporting on this observation, he stated that, under acutely toxic conditions, the liver, spleen and fatty tissues usually contain much higher concentrations of pesticide residues than the gill or muscle tissues, but that long exposures to low concentrations result in concentrations in the liver being similar to, or even less than, those in the gills or muscle. The fall in liver concentration might be attributed to the fish's metabolic activity in which the dieldrin is broken down to other metabolites. Edwards and Millburn (1965), believed that this might be the case since it was estimated that the half-life of (^{14}C) dieldrin for individual tissues is around 20 days. Alternatively dieldrin loss might be due to excretion which is rather slow, between 3-4 weeks in some fish, to establish an equilibrium with the surrounding water (Walker, 1985).

Tissue distribution variation of dieldrin was also observed in the perch from Loch Leven by Holden (1973). He reported the following residue ranges: gills, 0.87-3.7 ppm; muscle, 0.08-1.61; liver, 0.46 - 1.98 ppm; spleen, 2.8-20.3 ppm; fat, 11.4-38.5 ppm and testes, 0.11- 1.2 ppm. He suggeted that the variation could be due to the presence of fat depots in the tissues. This suggestion is consistent with the lipophilic nature of OCPs.

The liver is a fatty tissue as well as a centre for anabolic and catabolic activities. Epoxidation of aldrin to dieldrin for example, occurs in liver microsomes of several freshwater species of fishes (Edwards and Millburn, 1985), its microsomal activity can be induced by dieldrin (Vink, 1975). Microsomal liver stimulation by dieldrin in mammals has been a usual observation among pharmacologists and toxicologists alike (Edward and Millburn, 1985; Walker, 1985).

Recovery levels

The figures obtained from this experiments could be increased by 20-25% (correction factor for extraction method). In the course of extraction (about three different preliminary samples were each added known concentrations of dieldrin, these were subjected to the extraction process and recoveries made were computed and found to average 75-80%).

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