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Lipid Kinetics in Relation to the Toxicity of Three Pesticides in the Climbing Perch, *Anabas testudineus* (Bloch)

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Muscle and liver lipid levels were studied in *Anabas testudineus* (Bloch) exposed to 120 hr LC_0 (Sublethal) and 24 hr LC_{100} (lethal) lindane, disyston and furadan for various periods. Lindane (120 hr LC_0) exposure up to 3 hr caused relatively higher increases in muscle and liver lipid levels than the other two pesticides. Though increases were noted during first 2 periods of exposure (until 3 hr) to lindane, the increases observed at later periods in disyston or furadan topped those induced by lindane. Elevations attained after exposure to any single pesticide in the earlier periods were higher than those observed in later periods. Similar changes were induced by lindane (24 hr LC_{100}). Disyston and furadan at same concentration induced more increase at 6 hr than at 1 and 3 hr.

Key words: *Anabas testudineus*, **Bioassay, Lindane, Disyston, Furadan**

Introduction

Accumulation of pesticides in a fish depends upon the lipid content of its tissues. Anderson and Everhart (1966) reported a direct relationship between fat content and residue levels of DDT in *Salmo salar*. Buhler et al. (1969) and Reinert (1970) examined lipid levels in chinook salmon (*Onchorhynchus tshawytscha*) and coho salmon (*Onchorhynchus kisutch*) and Lake Michigan lake trout (*Salvelinus namaycushi*) respectively after DDT exposure and gave similar conclusion. Fabacher and Chambers (1971) found a direct correlation between endrin tolerance and high lipid content in mosquitofish. Burden (1956) also has observed that a species of fish containing low lipid content was more susceptible to DDT than the one with high fat content.

The affinity of different pesticides for fat is well illustrated by their high residues in fish oil, adipose, brain and liver tissues (Johnson 1968, 1973). Gakstatter (1968) reported dieldrin accumulation more in visceral fat and

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less in muscle of aldrin exposed *Carassius auratus*. Verma et al. (1977) on the other hand, noted more accumulation of chlordane, metasyston and sevin in gills followed by liver, muscle, kidney and intestine of *Labeo rohita* and *Saccobranchus fossilis*. The available evidences for such relationship with regards to organophosphates and organocarbamates are meagre. Hence, the present study was undertaken to elucidate the effect of three different classes of pesticides at different concentrations on lipid profiles of muscle and liver in a teleost.

Material and Methods

Specimens of *Anabas testudineus* (Bloch) ranging 10–12cm in length were collected from the freshwater ponds around Annamalainagar, Chidambaram. They were stocked for at least 15 days in large cement tanks previously washed with potassium permanganate or acriflavine hydrochloride, to clear them from fungal infections, if any. The fishes were fed with boiled eggs and earthworms (free from pesticides) every alternate day. Water in the tank was changed 2 or 3 times a week and after every change 2 mg of acriflavine hydrochloride/litre was added. A week before the commencement of experiments, a suitable number of apparently healthy fishes were transferred from the stock to small cement cisterns and kept under laboratory conditions. These fishes were fed every day and water in the cisterns was renewed daily. Feeding was stopped 2 days before the commencement of the experiment.

The pesticides selected for the present study were lindane (Technical grade, purity 99%; supplied by Bharat Pulverising Mills Private Limited, Bombay), disyston (Technical grade, purity 98%; supplied by Bayer India Limited, Bombay) and furadan (Technical grade, purity 75%; supplied by Rallis India Limited, Bangalore) representing an organochlorine, an organophosphate and a carbamate respectively. After proper bioassay (Static test) as described in methods for Acute Toxicity Tests with fish, macroinvertebrates and amphibians (The committee on methods for toxicity tests with aquatic organisms 1975), ten fishes were exposed to each selected pesticide concentration in 200 litre rectangular fiber glass tanks ($100 \times 50 \times 40$ cm). The water used had the following characteristics: *p*H 7.4–7.6, temperature $28 \pm 1°C$, dissolved oxygen 7–10 ppm, salinity 0.2–0.6 ppm, alkalinity 240–260 mg/l as $CaCo_3$ and hardness 360–380 mg/l as Ca Co_3. All the pesticides were dissolved in acetone whose quantity never exceeded 0.25 ml/l of water at any concentration. Pesticide concentration in the water was expressed as mg/l.

Total lipid content of liver and muscle tissues of fish was estimated following the semi-micro determination method of Pande et al. (1963). The tissues were isolated on ice and were quickly weighed in a cold room (10°C). The homogenate (1ml containing 25 mg liver or 50 mg muscle) was prepared in cold chloroform–methanol mixture (2 : 1). The homogenate was filtered through a filter paper soaked in chloroform–methanol mixture. One of the filtrate was transferred into a glass stoppered graduated tube ($6'' \times 3/4''$) and the solvent was removed by rapid evaporation under reduced pressure in a vacuum desiccator (care was taken while creating vacuum in the desiccator to prevent spurting by careful and controlled manipulation of the stopcock of the desiccator). After complete removal of the solvent, the vacuum was released carefully by putting a piece of white paper at the mouth of the stopcock to make the ingoing air free from dust. The tubes were taken out and 3.0 ml of 2% potassium dichromate in 98% sulphuric acid was added. The tubes were placed in a boiling water bath for 15 min and then cooled in ice-water bath. When the contents were sufficiently cooled, 4.5 ml of

distilled water was added and the tubes were cooled again in running tap water. The colour intensity was measured at 590 nm in a Linear Readout Grating Spectrophotometer (Cecil, model CE 373) against a reagent blank and the values are expressed as mg total lipid/g wet weight of tissue.

To determine the extent of utilization/storage, lipid contents of the tissues of the fish were estimated at various periods, viz. 1, 3, 6, 12, 24, 48, 72, 96 and 120 hr for 120 hr LCo (0.075, 4.0 & 0.56 mg/l respectively for lindane, disyston & furadan) and at 1, 3 and 6 hr for 24 hr LC_{100} (0.59, 10.5 & 1.56 mg/l respectively for lindane, disyston & furadan) exposure. The concentrations selected for the study are of two extremes, viz. sublethal and lethal. Controls were also maintained simultaneously with 0.25 ml of acetone/litre (maximum aliquot used in test concentrations) and their lipid levels examined at the time intervals as in 120 hr LC_0 exposure.

The lipid contents of exposed fish were compared statistically with unexposed population using student's '*t*' test and significance was taken at $p < 0.05$ and $p < 0.01$. Data were also analysed statistically by analysis of variance (*F* test) using one way classification (Steel & Torrie 1960) and significance was taken at $p < 0.05$ and $p < 0.01$.

Results and Discussion

The mean lipid levels of muscle (tables 1 & 3) and liver (tables 2 & 3) tissues of fish were significantly ($p < 0.05$) increased on exposure to pesticides (*F* test). Lipid content was relatively higher in liver than in muscle of

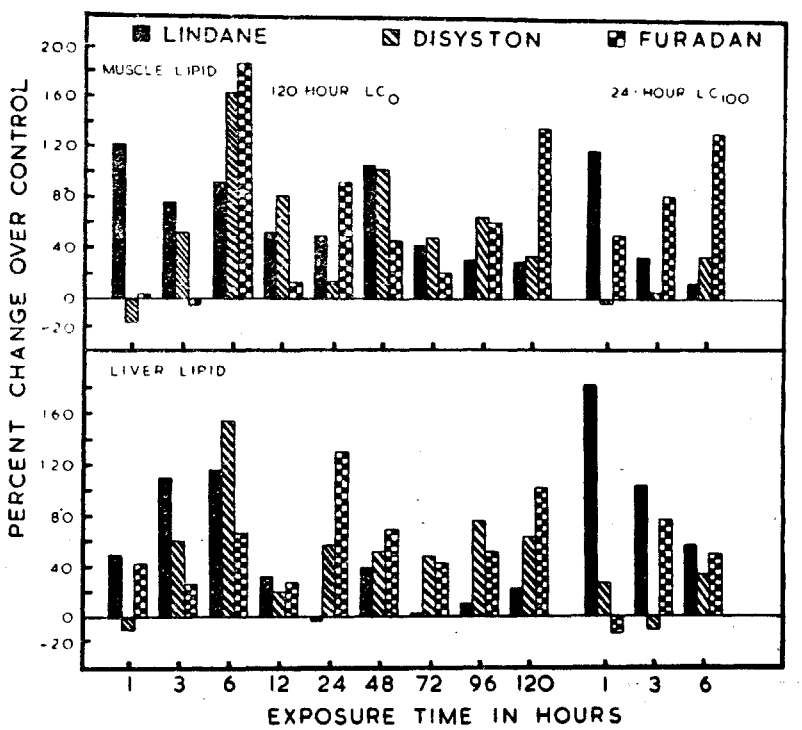

Figure 1

Figure 2

Per cent changes in lipid contents in the muscle (Figure 1) and liver (Figure 2) of *Anabas testudineus* exposed to 120 hr LC_0 and 24 hr LC_{100} of lindane, disyston and furadan for various time periods

Anabas testudineus which was attributed to the different rate of lipid metabolism in these tissues. Lindane at 120 hr LC_0 and 24 hr LC_{100} caused relatively higher increases in muscle or liver lipid levels than the other two pesticides until 3 hr of exposure (figures 1 & 2). Though increases were noted until 3 hr of exposure to lindane, the increases observed at later periods in disyston or furadan topped those induced by lindane. Probably the tissue lipid levels reflect the rates of metabolism of the pesticides since a direct relation between the residue concentrations and lipid levels was conceived by many authors (Anderson & Everhart 1966, Gakstatter 1968, Johnson 1968, Reinert 1970, Fabacher & Chambers 1971). The rates of metabolism of pesticides are different in the body of the host animal (Hollingworth 1971). O'Brien (1967) compiled evidence to show that insects avoid poisoning by storing an insecticide in a region where it can do no harm. Organophosphate insecticide, 'schradan' is less toxic to the female American cockroach because she has a larger fat body than the male, and stores a large fraction of the dose there.

When dynamics of lipid metabolism was considered, some generalizations could be made. In 120 hr LC_0 furadan, the elevation reached either in the muscle or in the liver

Table 1 *Levels of lipid in the muscle of* Anabas testudineus *treated with acetone (0.25ml/l) and 120 hr LC_0 of lindane (0.075 mg/l), disyston (4.0 mg/l) and furadan (0.56 mg/l) for various hours (Values expressed as mg total lipid/g wet weight of tissue)*

Hours of exposure	Control	Lindane	Disyston	Furadan
1	5.03 ± 0.71	11.23 ± 2.20 + 123.2	4.15 ± 1.23a — 17.5	5.23 ± 0.68a +4.0
3	4.94 ± 0.76	8.75 ± 1.65 + 77.1	7.55 ± 0.52 + 52.8	4.73 ± 0.45a — 4.3
6	4.84 ± 0.40	9.35 ± 0.83 + 93.2	12.73 ± 2.04 + 163.0	13.83 ± 2.46 + 185.7
12	5.06 ± 0.75	7.72 ± 0.59 + 52.9	9.23 ± 0.90 + 82.4	5.70 ± 0.45a + 12.6
24	4.78 ± 0.82	7.20 ± 0.62 + 50.6	5.45 ± 0.52a + 14.0	9.20 ± 1.59 + 92.5
48	4.75 ± 0.23	9.80 ± 0.62 + 106.3	9.63 ± 0.55 + 102.7	6.93 ± 1.62b + 45.9
72	4.54 ± 0.50	6.50 ± 0.57 + 43.2	6.77 ± 0.49 + 49.1	5.47 ± 0.85a + 20.5
96	4.43 ± 0.53	5.78 ± 0.49 + 30.5	7.30 ± 0.45 + 64.8	7.08 ± 0.78 + 59.8
120	4.39 ± 0.33	5.70 ± 0.45 + 29.8	5.88 ± 0.78 + 33.9	10.32 ± 0.59 + 135.1
F-value	1.306	19.502	43.000	35.179

Mean ± SD of 6 individual observations; + or — indicates per cent increase or decrease over control; a = $P > 0.05$; b = $P < 0.05$; Others = $P < 0.01$; F values at 2.16 corresponds with P 0.05; F values at 2.62 corresponds with P 0.01

Table 2 *Levels of lipid in the liver of* Anabas testudineus *treated with acetone (0.25 ml/l) and 120 hr LC_0 of lindane (0.075 mg/l) disyston (4.0 mg/l) and furadan (0.56 mg/l) for various hours (Values expressed as mg total lipid/g wet weight of tissue)*

Hours of exposure	Control	Lindane	Disyston	Furadan
1	16.12 ± 1.08	24.00 ± 1.54 + 48.9	14.57 ± 1.46a — 9.6	22.90 ± 2.19 + 42.1
3	16.13 ± 1.42	33.88 ± 3.18 + 110.0	25.83 ± 1.78 + 60.1	20.28 ± 3.15b +25.7
6	15.47 ± 2.19	33.45 ± 2.73 + 116.2	39.35 ± 6.69 + 154.4	25.67 ± 6.22 + 65.9
12	15.42 ± 1.63	20.28 ± 1.08 + 31.5	18.37 ± 1.09 + 19.1	19.60 ± 1.22 + 27.1
24	16.10 ± 0.90	15.60 ± 2.45a — 3.1	25.13 ± 2.01 + 56.1	36.92 ± 1.55 +129.3
48	16.12 ± 1.38	22.18 ± 1.26 + 37.6	24.27 ± 3.93 + 50.6	27.63 ± 0.94 + 67.7
72	15.02 ± 1.48	15.25 ± 1.54a + 1.5	22.02 ± 1.25 + 46.7	21.32 ± 1.08 + 41.9
96	15.25 ± 1.59	16.82 ± 1.79a + 10.3	26.70 ± 2.68 + 75.1	23.08 ± 0.83 + 51.3
120	14.67 ± 1.51	17.87 ± 1.69 + 21.8	23.78 ± 1.25 + 62.1	29.48 ± 1.26 +101.0
F-value	1.86	74.41	30.90	26.28

Mean ± SD of 6 individual observations; + or — indicates per cent increase or decrease over control; a = $P > 0.05$; b = $P < 0.05$; Others = $P < 0.01$; F values at 2.16 corresponds with P 0.05; F values at 2.62 corresponds with P 0.01

lipid was greater when compared with that of 120 hr LC_0 disyston (tables 1 & 2). The difference is more pronounced in the case of 24 hr LC_{100}. Whether the difference is due to the toxicity of the two pesticides or the response of the fish to the pesticides is not known.

In 24 hr LC_{100} lindane exposure, high per cent elevations were observed in both muscle and liver lipid levels (table 3) at the first hour of exposure which gradually decreased in the later periods of exposure. In disyston and furadan treated fish, a reverse trend was noted; the per cent elevation in first hour being low when compared to later periods. Differences in the rate of uptake and metabolism of pesticides and the response of the fish might be the reasons for these differences in the lipid levels.

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Table 3 *Levels of lipid in the muscle and liver tissues of* Anabas testudineus *treated with 24 hr LC_{100} of lindane (0.59 mg/l), disyston (10.5 mg/l) and furadan (1.56 mg/l) for various hours (Values expressed as mg total lipid/g wet weight of tissue)*

Tissues	Hours of exposure			F-value
	1	3	6	
		LINDANE		
Muscle	10.93 ± 1.14 + 117.3	6.58 ± 1.89a + 33.2	5.42 ± 1.29a + 12.0	23.29
Liver	45.40 ± 8.67 + 181.6	32.58 ± 3.81 + 101.0	23.97 ± 2.87 + 54.9	21.38
		DISYSTON		
Muscle	4.85 ± 0.85a — 3.6	5.20 ± 0.45a + 5.3	6.42 ± 0.73 + 32.6	8.43
Liver	20.28 ± 1.08 + 25.8	14.37 ± 1.21 — 10.9	20.47 ± 1.43 + 32.3	46.75
		FURADAN		
Muscle	7.53 ± 0.96 + 49.7	8.93 ± 1.65 + 80.8	11.17 ± 4.04 + 130.8	3.03
Liver	13.87 ± 2.76 — 14.0	28.23 ± 7.77 + 75.0	22.88 ± 4.38 + 47.9	10.88

Mean ± SD of 6 individual observations; + or — indicates per cent increase or decrease over control; a = $P > 0.05$; Others = $P < 0.01$; F values at 2.16 corresponds with P 0.05; F values at 2.62 corresponds with P 0.01

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