

## CHANGES IN NUCLEIC ACIDS AND PROTEIN CONTENT OF *TILAPIA MOSSAMBICA* EXPOSED TO DICHLORVOS (DDVP)

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### ABSTRACT

Changes in nucleic acids and protein content in liver, muscle and brain of a freshwater fish *Tilapia mossambica* Peters, exposed to a sub-lethal concentration of 0.5 mg. l<sup>-1</sup> of the insecticide, Dichlorvos (DDVP) for a period of 21 days were studied. The exposed fish showed a lower brain and liver somatic indices suggesting loss of cells. Post-exposure studies revealed a significant decline in DNA, RNA and protein contents of liver, muscle and brain. The liver exhibited a greater loss in DNA, RNA and protein contents than those of muscle and brain in the exposed fish. Inhibitory effect of the insecticide on nucleic acids and protein content was markedly elevated by the increase in the exposure period.

### INTRODUCTION

Pesticides act either as a selective toxicant or may display a rather broad spectrum of adverse biological activities. Earlier studies pertaining to the effects of Dichlorvos (DDVP) on *T. mossambica* showed a marked decline in the respiratory metabolism, where the response of fish to toxicity was directly proportional to active metabolism (Rath and Misra 1978). However very little information is available concerning the physiological or biochemical effects of pesticides on nucleic acid levels and protein contents. Dave et al (1975) studied some metabolic changes in protein content in European eel induced by polychlorinated Biphenyls (PCB). Significant changes were marked in the nucleic acids and protein contents of DDT and Dieldrin exposed rats (Bhatia et al 1973 and Kohli et al 1975). Lidman et al (1975) observed the effect of PCB on protein metabolism of rainbow trout. Some pesticides induced DNA damage in human cell culture (Ahmed et al 1977). Only a few reports are available relating to sub-lethal effect of organophosphorus pesticides on nucleic acids and protein metabolism in freshwater fishes. The present study underlines the biochemical effects of Dichlorvos (DDVP) on the nucleic acid levels and protein content of a freshwater fish, *Tilapia mossambica* Peters, especially of brain, muscle and liver tissues.

## MATERIAL AND METHODS

*Tilapia mossambica* Peters of average weight of  $10 \pm 1$  g were used in the present study. The fish, brought from a local nursery were acclimatized in aquaria containing tap water (6.0 to 6.5 pH). All fish were fed with goat liver daily and the medium was maintained at 85-100% oxygen saturation.

A batch ( $n = 40$ ) of healthy fish were exposed to a sub-lethal concentration of  $0.5 \text{ mg. l}^{-1}$  Dichlorvos (DDVP) and another group of 40 were grown in tap water as control. The water medium of both the aquaria were changed daily. At each sampling occasion (Both exposed and control) were stunned and decapitated immediately and tissue samples (liver, muscle and brain) were removed for immediate analysis. The analysis was performed at 7, 14 and 21 days of exposure. The disphenylamine method of Burton (1956) was followed to determine tissue DNA content while the amount of RNA was measured following orcinol method of Volkin and Cohn (1954). Protein content was determined according to Folin--Phenol method of Lowry, et al (1951). The amount of DNA, RNA and protein in liver, brain and muscle were expressed as  $\text{mg. g}^{-1}$  wet tissue.

## RESULTS AND DISCUSSION

As evident from table-1 exposure to sub-lethal concentration of Dichlorvos brought about a significant decrease in the fish liver somatic index (LSI =  $\text{Liver weight} \times 100 / \text{Body weight}$ ) and also the brain somatic index (BSI =  $\text{Brain weight} \times 100 / \text{Body weight}$ ). Liver exhibited a greater diminution in somatic index than the brain. The same trend was observed in all the treated fish even after 21 days of exposure.

TABLE 1. *Brain and Liver somatic Index of T. mossambica treated with Dichlorvos at different days of exposure.*

Organs	Treatment	Exposure period		
		7 days	14 days	21 days
Brain (BSI)	Control	$0.559 \pm 0.0123$	$0.663 \pm 0.0245$	$0.718 \pm 0.01$
	Exposed	$0.503 \pm 0.002$	$0.627 \pm 0.0174$	$0.689 \pm 0.01$
Liver (LSI)	Control	$1.150 \pm 0.01$	$1.459 \pm 0.05$	$1.622 \pm 0.0332$
	Exposed	$0.818 \pm 0.0245$	$1.180 \pm 0.3498$	$1.258 \pm 0.2654$

NS\* Not Significant.

DNA, RNA and protein contents showed a sharp decline in the exposed fish. The concentration of DNA was markedly higher in brain tissue as compared to muscle and liver, while RNA and protein contents were observed to be more in liver than in muscle and brain. Liver exhibited a maximum loss in DNA, RNA and protein and the loss was more pronounced in longer exposure period. (Fig. 1, 2 and 3). The RNA/DNA ratio also decreased in exposed fish (Fig. 4) and it showed a positive correlation ( $P < 0.01$ ) with protein (Fig. 5). The percentage of protein inhibition gradually increased with the increase in exposure period and liver exhibited a maximum inhibition as compared to muscle and brain (Table 2).

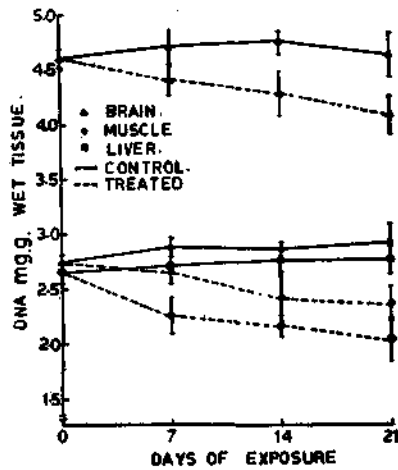


FIG. 1. DNA mg. g<sup>-1</sup> wet tissue of *T. mossambica* exposed to Dichlorvos for 21 days.

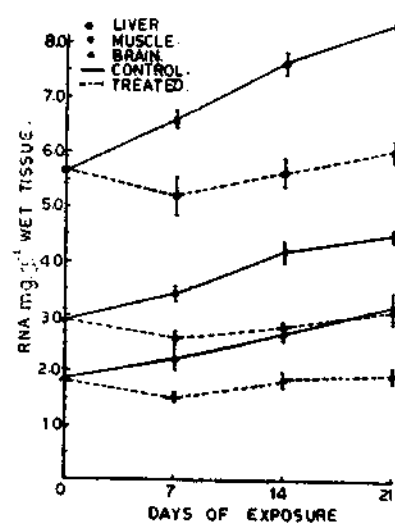


FIG. 2. RNA mg. g<sup>-1</sup> wet tissue of *T. mossambica* exposed to Dichlorvos for 21 days.

TABLE 2: Percentage of protein inhibition in brain, muscle and liver of *T. mossambica* exposed to Dichlorvos at different days.

Organs	Exposure period		
	7 days	14 days	21 days
Brain	3.85 ± 1.36	7.73 ± 0.98	9.38 ± 1.78
Muscle	10.64 ± 2.63	15.57 ± 1.56	17.52 ± 3.53
Liver	16.13 ± 1.89	24.91 ± 2.73	29.5 ± 1.81

The decrease in somatic index of liver and brain of exposed fish suggested some loss of somatic cells. The rate of decrease in somatic index in liver was found to be higher than in brain. The liver is the main site for active metabolism and also for storage. However LSI was always higher exhibiting liver enlargement in PCB treated European eel (Dave et al 1975) and rainbow trout (Lidman et al 1975).

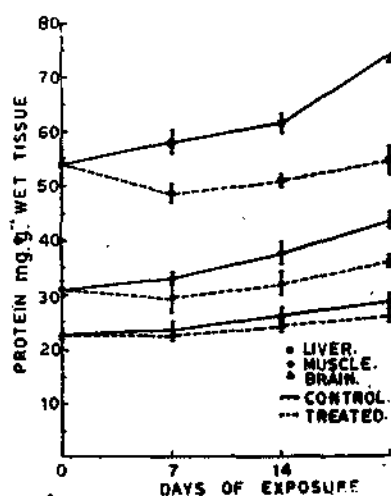


FIG. 3. Changes in Protein mg g<sup>-1</sup> wet tissue of *T. mossambica* exposed to Dichlorvos for 21 days.

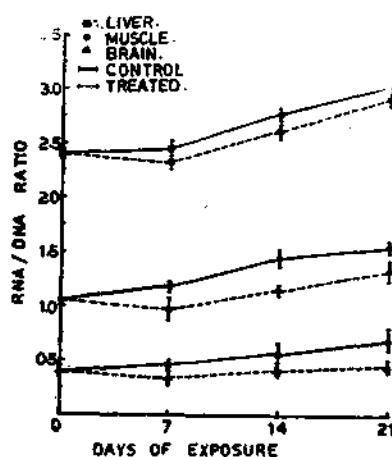


FIG. 4. RNA:DNA ratio of control and exposed fish, *T. mossambica*

Exposed fish showed a significant low value in the amount of nucleic acids (DNA and RNA) and proteins and liver exhibited a maximum inhibition as compared to muscle and brain. The higher decreasing trend was evident with the increase in the exposure period. Since DNA is the chemical estimate of cell number its decline also suggested some loss of cells. And the loss of DNA content of fish shows the possible interference of Dichlorvos with nucleic acid synthesis. DDT was also shown to induce chromosomal damage in mice (Johnson and Jahl 1963). But Bhatia et al (1973) reported that DNA of rat liver was unaffected by Dieldrin treatment. Usually the RNA content and the RNA/DNA ratio of a tissue are considered as to indicate the intensity of protein synthesis (Misra and Patnaik 1974). And these 2 parameters show a positive correlation with the protein content. RNA content indicates the intensity of protein synthesis in a tissue (Brachet 1955). High level of RNA reflects its involvement in cellular growth in control fish. In view of the significant correlation of RNA and protein, a deficient synthesis of any type of RNA should have its reflection in a corresponding failure of protein synthesis.

Not only the reduction in the amount of m RNA but also a lesion of its functional capacity brings about such a failure (Brain 1976). But in rat exposed to DDT and Dieldrin hepatic RNA content increased significantly (Sanchez 1967, Bhatia et al 1973 and Kohli et al 1975).

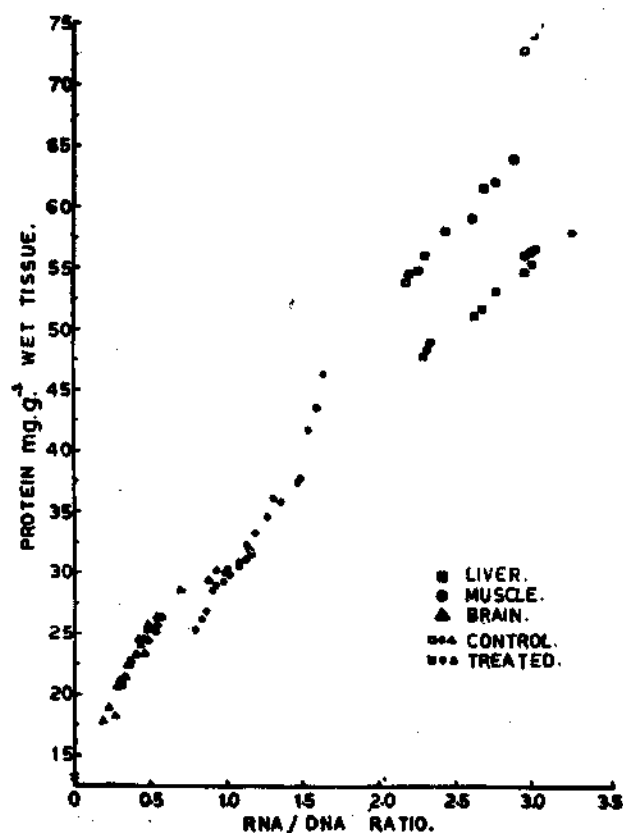


FIG. 5. Correlation ( $P < 0.01$ ) of RNA/DNA ratio with protein content of control and exposed fish, *T. mossambica*.

Liver being the active site of metabolism the percentage of protein inhibition was more in comparison to muscle and brain being least affected. Bhatia et al (1973) observed that the protein content increased in dieldrin induced rat liver whereas no change in protein of liver and muscle was observed in PCB treated European eel (Dave et al 1975) and rainbow trout (Lidman et al 1975). As protein synthesis is required for DNA synthesis (Balis, 1968) Dichlorvos might block the synthesis of DNA as well as all other functions dependent on protein synthesis. Thus Dichlorvos might enhance the synthesis of proteins and DNA-directed RNA formation.

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## REFERENCES

- AHMED, F. E., R. W. HART AND N. J. LEWIS. 1977. Pesticide induced DNA damage and its repair in cultured human cells. *Mut Res.* 42, 161.
- BALIS, M. 1968. Antagonists and nucleic acids, In: *Frontiers of Biology* (Eds. Neuberger, A. and Tatum, E. L.), p. 206, North-Holland Publishing Company-Amsterdam. The Netherlands.
- BHATIA, S. C. S. C. SHARMA AND T. A. VENKITASUBRAMANIAN. 1973. Effects of Dieldrin on hepatic carbohydrate metabolism and protein biosynthesis in vivo. *Toxicol. Appl. Pharmacol.* 24, 216.
- BRACHET, J. 1955. In: *The nucleic acids*, Vol. II, Academic Press, New York.
- BRUIN, A. DE. 1976. In: *Biochemical toxicology of environmental agents*, pp. 612. Elsevier/North-Holland Biomedical Press. The Netherlands.
- BURTON, K. 1956. A study of the conditions and mechanisms of diphenylamine reaction for estimation of DNA. *Biochem. J.* 62, 315.
- DAVE, G., A. BOMAN, M. L. JOHANSON-SJOBECK AND U. LINDMAN. 1975. Metabolic effects of PCB (Poly Chlorinated Biphenyls) in the european eel *Anguilla anguilla*, L. In: *Sub-lethal effects of toxic chemicals on aquatic animals*. (Eds. Koeman, J. H. and Strik, J. J. T. W. A.) Elsevier Scientific Publishing Company - Amsterdam/Netherlands.
- JOHANSON, G. A. AND S. M. JAHIL. 1963. DDT induced chromosomal damage in mice. *J. Hered.*, 63, 147.
- KOHLI, K. K., S. C. SHARMA, S. C. BHATIA AND T. A. VENKITASUBRAMANIAM. 1975. Biochemical effects of chlorinated insecticides DDT & Dieldrin. *J. Sci. Ind. Res.*, 34(8), 462.
- LIDMAN, U., G. DAVE, AND M. L. JOHANSON-SJOBECK. 1975. Metabolic effects of PCB Poly chlorinated Biphenyls) in rainbow trout, *Salmo gairdnerii*, Rich. In: *Sub-lethal effects of toxic chemicals on aquatic animals* (Eds. Koeman, J. H. and Strik, J. J. T. W. A.), Elsevier Scientific Publishing Company|Amsterdam-Netherlands.
- LOWRY, C. H., N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL. 1951. Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.* 193, 265.
- MISRA B. N. AND B. K. PATNAIK. 1974. Age changes in nucleic acids, protein, free amino acids and water content of the skin in male garden lizard in natural surroundings. *Gerontologia*, 20, 1.
- SANCHEZ, E. 1967. DDT-induced metabolic changes in rat liver. *Canadian J. Biochem.* 45, 1809.
- VOLKIN, E. AND W. E. COHN. 1954. *Methods of biochemical analysis* (Ed. Glick, D) Vol. 1, 287, Wiley (Interscience) New York.