Proc. Indian natn. Sci. Acad. **B51** No. 5 pp 537–542 (1985)

Organophosphate-Induced Biochemical Changes in the Brain, Liver and Ovary of the Fish, *Channa punctatus* (Bloch)

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(Received 8 August 1985)

In the organophosphate pesticide (OPP) cythion-treated *C. punctatus* the brain protein content significantly decreased on the 15th and 30th days, whereas in the ovary and liver such reduction was seen on the 15th and 60th days, respectively. This decrease had direct correlation with exposure time for brain and ovary but inverse for liver. Total RNA level was lowered significantly in the brain on the 15th and 60th days; in liver after 30th to 60th days and in ovary only on the 60th day after treatment. A significant reduction in DNA content was also recorded in brain on the 60th day; liver from 45th to 60th days and ovary from 30th to 60th days after cythion exposure. RNA and DNA depletion has an inverse correlation with exposure time in all the tissues. These results suggest that cythion is capable of impairing the physiological functions of the organs studied, in this species.

Key Words: Cythion, Protein, RNA, DNA, *Channa punctatus*

Introduction

Pesticides and their residues washed into the water system adversely affect the growth and survival of fishes (Holden 1973, Johnson 1973). As chlorinated hydrocarbons persist in the environment for a long time, there is an increase in the use of organo-phosphate pesticides (OPPs) in agriculture, forestry and public health. Histopathological effects of OPPs on different organs of fishes have been studied by several investigators (Anees 1978, Dubale & Shah 1979, Mandal & Kulshresthra 1980, Lakota et al. 1982). In fish, apart from their well known cholinesterase (ChE) inhibitory effect, OPPs modify the activity of several other metabolic enzymes (Dalela et al. 1978, Mukhopadhyay & Dehadrai, 1980a,b, Sastry & Sharma 1980, Joshi & Desai 1981, Dubale & Awasthi 1984, Natarajan 1984), proteins (Mukhopadhyay & Dehadrai 1980 a, b, Oishi et al. 1980, Choudhari & Chakrabarti 1983, Dubale & Awasthi 1984) and free amino acids (Natarajan 1983). The mutagenic effects of OPPs have been reviewed by Wild (1975). However, long term effects of sublethal dose of OPPs on different aspects of the physiology of fishes are little known. In the present communication the changes induced

by cythion which is 50% malathion (O, O-Dimethyl Phosphorodithioate of Diethylmercapto Succinate), a commonly used organophosphate pesticide on protein, RNA and DNA contents of the brain, liver and ovary of the fish *C. punctatus* are reported.

Materials and Methods

Over forty adult female *C. punctatus* weighing 40–45 g and having a length of 10–12 cm were bought from the local fish market at Varanasi. They were kept in 40 L glass aquaria containing chlorine free water of *p*H 7.2, hardness 154 ppm (as $CaCO_3$), alkalinity 68 ppm (as $CaCO_3$), dissolved oxygen 7.2 ppm, and conductivity 0.56 mMhos, and acclimated to laboratory conditions for 10 days prior to using in the experiment. Specimens were divided into two equal groups. Animals in group-I were exposed to a sublethal dose of 4.0 ppm commercial cythion while those in group-II served as controls. The highest pesticide concentration in which no mortality was observed during 96 hrs of exposure, but fish appeared to be in stress, has been expressed as 'sublethal dose'. The pesticide used in this investigation was manufactured by Northern Minerals Pvt. Ltd., Haryana and marketed by Dhanuka Marketing Company, Hyderabad, India. Aquaria water was changed every alternate day after feeding the fish with goat liver and pesticide concentration was renewed. At intervals of 15, 30, 45 and 60 days, five fish from each group were decapitated and tissues were frozen and processed for the assays. The technique of Lowry et al. (1951) and the method of Munro and Fleck (1966) were used for assessing total protein, RNA and DNA levels, respectively. The results are given in terms of the pesticide's commercial formulation and not active ingredient of pure pesticide, because only former is used in agriculture.

Student's t-test was used for analyzing the data. Correlation coefficient (r) and regression line equation ($Y=a+bX$) were calculated using the formulae of Bruning and Kintz (1977).

Results

Protein: In *C. punctatus* exposed to cythion, the brain protein content decreased significantly initially at 15th ($P<0.01$) and 30th days ($P<0.05$), and returned to normal on the 45th day. Decrease in liver protein ($P<0.05$) level occurred only on the 60th day of exposure. Ovarian protein was reduced ($P<0.05$) on the 15th day of treatment and it returned to pretreatment level on 30th day. Reduction in protein content correlated directly with exposure time in brain ($r=+0.859$, $P<0.01$), ovary ($r=+0.095$) and inversely in liver ($r=-0.666$) (table 1).

RNA: Brain RNA content was significantly depleted ($P<0.05$) on the 15th day of exposure, returned to normal level between 30th to 45th days and again reduced ($P<0.01$) on the 60th day. Liver RNA level exhibited a significant decrease on the 30th ($P<0.05$), 45th ($P<0.001$) and 60th ($P<0.01$) days. However, decrease in the levels of ovarian RNA was noticed only on the 60th day of the experiment termination ($P<0.001$). RNA depletion in the brain ($r=-0.131$), liver ($r=-0.818$, $P<0.025$) and ovary ($r=-0.715$) was inversely correlated with exposure time (table 2).

DNA: Its content in the brain decreased significantly on the 60th day ($P<0.001$) of exposure whereas in liver it was lowered on the 45th ($P<0.01$) and 60th ($P<0.001$) days. Ovarian DNA content was decreased on 30th ($P<0.01$), 45th ($P<0.001$) and 60th ($P<0.05$) days. The DNA reduction was inversely correlated with exposure time and the correlation coefficient (r) values for brain, liver and ovary were -0.447, -0.823 ($P<0.025$) and -0.701, respectively (table 3).

Table 1 *Changes in the brain, liver and ovarian protein content (mg/g wet tissue) of fish exposed to 4.0 ppm of cythion for various exposure intervals. Values are the mean±SD of five fish in treated and control groups*

Tissue/Treatment		Exposure time intervals (Days)				Correlation coefficient (r) value and regression line equation*
		15	30	45	60	
Brain	Control	82.50±7.65	80.40±7.89	84.22±7.84	86.61±2.42	$r=+0.859(P<0.01)$
	Treated	64.41±3.11 ($P<0.01$)	67.19±3.86 ($P<0.05$)	89.21±3.68 (NS)	84.47±3.62 (NS)	$Y=55.77+0.54X$
Liver	Control	133.71±5.35	136.13±3.97	137.53±4.13	139.88±10.20	$r=-0.666$ (NS)
	Treated	132.64±3.42 (NS)	133.50±8.46 (NS)	134.96±4.11 (NS)	121.72±4.79 ($P<0.05$)	$Y=138.52-0.20X$
Ovary	Control	108.34±4.01	106.86±9.02	108.56±8.34	106.58±9.94	$r=+0.095$ (NS)
	Treated	94.54±8.17 ($P<0.05$)	107.40±3.81 (NS)	103.95±6.45 (NS)	97.16±3.20 (NS)	$Y=99.66+0.02X$

*Regression line equation between changes in protein content (Y) and exposure time (X).

Table 2 *Changes in the brain, liver and ovarian RNA content (mg/g wet tissue) of fish exposed to 4.0 ppm of cythion for various exposure intervals. Values are the mean±SD of five fish in treated and control groups*

Tissue/Treatment		Exposure time intervals (days)				Correlation coefficient (r) value and regression line equation*
		15	30	45	60	
Brain	Control	3.58±0.66	3.40±0.13	3.61±0.40	3.53±0.48	$r=-0.131$(NS)
	Treated	2.58±0.28 ($P<0.05$)	3.28±0.30 (NS)	3.25±0.15 (NS)	2.45±0.14 ($P<0.01$)	$Y=3.00-0.002X$
Liver	Control	12.25±0.44	12.11±0.29	12.00±0.47	11.99±0.51	$r=-0.818(P<0.025)$
	Treated	11.78±0.73 (NS)	11.10±0.79 ($P<0.05$)	9.82±0.64 ($P<0.001$)	10.41±0.73 ($P<0.01$)	$Y=12.12-0.03X$
Ovary	Control	30.82±0.45	30.28±0.63	29.53±1.82	30.45±2.03	$r=-0.715$(NS)
	Treated	28.08±2.48 (NS)	30.75±1.02 (NS)	27.81±1.14 (NS)	23.63±1.17 ($P<0.001$)	$Y=31.64-0.10X$

*Regression line equation between changes in RNA content (Y) and exposure time (X)

Table 3 *Changes in the brain, liver and ovarian DNA content (mg/g wet tissue) of fish exposed to 4.0 ppm of cythion for various exposure intervals. Values are the mean ± SD of five fish in treated and control groups*

Tissue/Treatment		Exposure time intervals (days)				Correlation coefficient (r) value and regression line equation*
		15	30	45	60	
Brain	Control	3.47±0.45	3.56±0.35	3.43±0.14	3.45±0.37	$r=-0.447$ (NS)
	Treated	2.92±0.49 (NS)	3.63±0.30 (NS)	3.51±0.29 (NS)	2.20±0.21 ($P<0.001$)	$Y=3.63-0.01X$
Liver	Control	5.21±0.29	5.23±0.44	5.20±0.27	5.09±0.18	$r=-0.823$ ($P<0.025$)
	Treated	5.02±0.22 (NS)	5.22±0.47 (NS)	3.92±0.66 ($P<0.01$)	4.04±0.31 ($P<0.001$)	$Y=5.60-0.02X$
Ovary	Control	3.24±0.28	3.36±0.43	3.46±0.40	3.36±0.24	$r=-0.701$ (NS)
	Treated	3.14±0.22 (NS)	2.56±0.09 ($P<0.01$)	2.35±0.18 ($P<0.001$)	2.60±0.44 ($P<0.05$)	$Y=3.11-0.01X$

*Regression line equation between changes in DNA content (Y) and exposure time (X)

Discussion

In general, results of the present investigation revealed that cythion induced reduction in the protein, RNA and DNA contents in different tissues of *C. punctatus*. OPPs are known to methylate and phosphorylate cellular protein (Wild 1975). Choudhari and Chakrabarti (1983) reported a decrease in serum and liver proteins in rats exposed to OPPs. Reduction of cytochrome oxidase activity is observed in different tissues of *Channa striatus* treated with OPP metasystox (Natarajan 1984). Dubale and Awasthi (1984) recorded time dependent depletion of protein content in liver and kidney of *Heteropneustes fossilis* treated with OPP dimethoate. The above authors observed decreased values during the first week which became normal by the end of third week. Dose dependent increase in serum protein levels in rat administered with tri-n-butyl phosphate is reported by Oishi et al. (1980). The protein content in the brain of cythion exposed *C. punctatus* decreased up to the 30th day and returned to normal level on the 45th and 60th days. In the ovary and liver significant reduction of protein was seen only on the 15th and 60th days, respectively. Mukhopadhyay and Dehadrai (1980 a,b) have reported decreased serum protein level and protein biosynthesis in liver in *Clarias batrachus* exposed to sublethal dose of malathion.

OPPs inhibit acid and alkaline phosphatase activity in different tissues of fishes (Dalela et al. 1978, Sastry & Malik 1979, Mukhopadhyay & Dehadrai 1980a, Sastry & Sharma 1980), which may adversely affect nucleic acid synthesis (Sastry & Sharma 1980). In *C. punctatus* exposed to cythion, RNA and DNA content of brain, liver and ovary were depleted significantly showing inverse correlation with exposure time. Patankar and Vaidya (1980) reported considerable genetic damages induced by phosphamidon in cultured human leucocytes and *in vivo* bone marrow cells of mice.

OPPs are known to inhibit cholinesterase activity in mallard ducklings (Fleming & Bradburg 1981). Gupta and Paul (1977) reported more accumulation of ^{32}P-labelled

malathion in liver than other organs of *Gallus domesticus*. In cythion treated *C. punctatus* significant positive correlation in brain protein reduction ($r = +0.859$, $P<0.01$), and inverse correlation in liver RNA ($r = -0.818$, $P<0.025$) and DNA ($r = -0.823$, $P<0.025$) contents depletion were noticed at different exposure intervals. These observations are suggestive of the primary action of cythion on brain during initial period of exposure and cumulative toxic effect in liver during prolonged exposure resulting subsequent more pronounced decrease of liver RNA and DNA. Thus cythion is capable of inducing alterations in the normal physiological functions in different organs possibly through impairing the synthesis and metabolism of protein, RNA and DNA in this species. Further, the toxicity seems to differ in relation to organs as well as biomolecules and the duration of exposure.

Acknowledgements

First author is grateful to the Council of Scientific and Industrial Research of India for the award of a Senior Research Fellowship, which made this work possible.

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