Vet.Med.J.,Giza. Vol.49, No.1. (2001) 131-146.

BIOCHEMICAL DISORDERS IN SERUM OF TILAPIA NILOTICA EXPOSED TO DIAZINON FOR MEDIUM TERM

M.A. GHONEIM *; E.N. EL-KHATIB **; and M.Z. ATTIA***

Depts. of Biochemistry * and Physiology***, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt and Dept. Mammalian Toxicology**, Central Agricultural Pesticides Laboratory, Egypt.

Received: 2.12.2000.
Accepted: 16.12.2000.

SUMMARY

Diazinon is an organophosphorous pesticide that commonly used in eradication of insects. Fish is one of the non-target organisms that may be exposed to diazinon toxicity. In this investigation we studied the effect of exposure of Tilapia nilotica to diazinon at concentrations of 40, 80 and 160 PPM for one, two and four weeks. Lipids profile, proteins content, proteins fingerprint, bilirubin, thyroid hormones and certain enzymes activity (GST, CK and AchE) of treated fish serum were determined. The same parameters were followed also after a period of ten days of recovery. Dramatic changes were found in most of the measured parameters at all tested concentrations and times of exposure. Serum protein fractionation by SDS-PAGE revealed disappearance of certain protein fractions at the different concentrations of diazinon. Some of these fractions were reappeared again after the recovery period indicating the depressing effect of diazinon on the expression of certain genes.

Key words: Diazinon - *Tilapia nilotica* - SDS-PAGE - Medium term - Biochemistry.

INTRODUCTION

Pesticides are introduced into water system by direct application, spray drift, agricultural run-off and industrial effluents. Aquatic organisms uptake of pesticides from water takes place through gills and skin during respiration, and orally when feeding. Pesticides are then distributed through various organs, metabolized and finally excreted partially back to the water system.

$ To whom correspondence should be addressed. Fax: 202-5725240 E-mail: mghoneima® hotmail. com.

Organophosphorous insecticides are added to the global environment at an annual rate of tens of thousands of tons (Melnikov et al., 1975). These compounds, apart from controlling agricultural pests, are highly toxic to fish causing mass mortalities (Folmar et al., 1979). Odenkirchen and Eisler (1988) suggested that, accidental or careless applications of chlorpyrifos (an organophosphorous insecticide) have resulted in the death of many species of non-target organisms such as fish, aquatic invertebrates, birds and human. It is widely accepted that organophosphates kill both vertebrate and invertebrate animals by inhibiting the cholinesterase activity with consequent disruption of nervous activity under the effect of the accumulating acetylcholine at the nerve ending, and are hence regarded as nerve poisons (El-Benhawy et al., 1990 and De-Bruijn and Hermens, 1993).

Fish is one of the most important non-target organisms in the ecosystem and showed high response to many pollutants. Many authors found that most pesticides are toxic to aquatic invertebrates and fish (Schimmel et al., 1983 and Franson, 1984). The increased production and use of pesticides might cause adverse effects to fish production. Many pesticides increase the level of enzymes: acid phosphatase (Saxena and Sarin, 1980 and Abou-Donia et al., 1986), glutathione-s-transferase (Oeschet et al., 1982) and transaminases (El-Gendy, 1990) in living organisms.

Diazinon is one of the commonly used organophosphates that has many applications in the field. It might reach to the Nile stream through drainage systems.

The purpose of the present investigation is to determine the effect of continuous exposure of diazinon at different concentrations over a period of one month on thyroid hormones, serum proteins concentration and fingerprint, lipid profile, bilirubin content and certain serum enzymes activities (creatine phosphokinase CPK, acetylcholinesterase AChE and glutathione-s-transferase GST).

MATERIALS AND METHODS

Pesticide:

Formulated diazinon 60% EC was used in this study. It was obtained from the Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Experimental fish:

Living fish, Bolti (Tilapia nilotica) were obtained from ìFoki Farmî, Kaliob City, Egypt. The fish weighting 80 ± 15 gm each and measuring 20 ± 4 cm in length. The fish were kept in 200-L aquariums with aerated chlorine-free tap water and acclimatized to the laboratory condition for about two weeks prior to the experiment. During rearing, the fish were fed on commercial diet.

Experimental procedures:

I- Determination of LC_{50} of diazinon:

Healthy fish were used in an experiment for determination of the median lethal concentration (LC_{50}) of the tested pesticide in its formulated form. Five fish were used in each replicate and three replicates of concentration for each treatment. Mortality was recorded after 96 hours. The LC_{50} was calculated according to the method of Weil (1952).

II- Subchronic Toxicity (one month exposure):

Fish were divided into four groups (one control group and three treated ones). Fish in the treated groups were exposed to one of three concentrations (40, 80 and 160 PPM). Sets of ten fish each from control and treated groups were taken at 7, 14, and 28 days of exposure, and ten days after the end of exposure (i.e after recovery). Blood samples were obtained from the caudal vein and collected in clean glass tubes then centrifuged at 3000 rpm for 15 minutes at 4°C. Sera were separated and kept frozen at - 80°C until analysis.

III- Determination of Biochemical Markers:

The total proteins was determined in the serum of treated and untreated fish using colorimetric method based on the principle of the Biuret reaction (copper salts in an alkaline medium) according to Henry (1964). Serum albumin was determined by colorimetric method using bromocresol green at pH 4.2 according to Doumas (1972). Serum globulins were calculated as the difference between total proteins and albumin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure described by Laemmli (1970), using vertical slab gel. The gels were stained using Coomassi blue R250. The gels were scanned and analyzed by Gel-Pro analyzer 3.1 program (Multimedia, USA). The colorimetric determination of serum acetylcholinesterase activity was made according to the method of Ellman et al. (1961) and modified by Bisso et al. (1991). Activity of creatine kinase was measured according to Gruber (1978) while GST activity was determined as mentioned by Vessey and Boyer (1984). Total lipids was determined according to Chabrol and Charonnat (1937), triacylglycerol by the method of Fossati and Prencipe (1982), cholesterol by Richmond (1973), LDL-cholesterol by Steinberg (1981) and HDL-cholesterol by Burstein (1970). Total circulating tri-iodothyronine (T3) and tetra-iodothyronine (T4) were determined in serum by the Microparticle Immunoassay (MIEA) technology based on the method of Glinoer et al. (1978). Total and direct bilirubins were determined in serum according to the method of Walters and Gerarde (1970).

IV- Statistical Analysis:

Results are expressed as mean ± standard error (S.E.). Data were subjected to Studentís t- test according to Snedecor and Chochran (1980).

RESULTS

1- Serum Lipids Profile:

Table (1) demonstrates the lipids profile as determined in the serum of control and treated fish. Total lipids were significantly decreased in the group received 40-PPM diazinon for four weeks, in all groups received 80 PPM except the recovery group and in all groups received 160 PPM. Triacylglycerol was significantly decreased in the group of fish received 40 PPM for one, two and four weeks while significantly increased in the recovery group. It was also significantly decreased in the groups received 80 PPM for one and two weeks while it was significantly increased in the group received 160 PPM for one week. In relation to the cholesterol level, it was significantly increased in the groups received 40 PPM diazinon for one and two weeks and in the group received 80 PPM for two and four weeks while it was significantly decreased in all groups received 160 PPM diazinon. LDL-cholesterol was significantly increased in groups received 40 PPM diazinon for one, two weeks and after recovery, group received 80 PPM for two weeks and groups received 160 PPM for one week and after recovery. On the other hand, HDL-cholesterol was found to be significantly decreased in all treated groups by different concentrations except 80 PPM after recovery and 160 PPM for one week.

2- Activities of Serum Creatine Kinase (CK), Acetylcholinesterase (AChE) and Glutathione-s-transferase (GST):

Table (2) is showing the measured activities of CK, AChE and GST enzymes in the serum of control and treated fish. Creatine kinase was significantly inhibited by diazinon added at concentration of 40 PPM for one week, four weeks and after recovery. When added at 80 PPM, CK was significantly inhibited after recovery. At the same time, 160 PPM significantly decreased CK at two, four weeks and after recovery. On the contrary, CK activity was significantly elevated at 40 PPM and 80 PPM after two weeks of treatment.

Concerning acetylcholinesterase, it was significantly inhibited at all tested concentrations and at all times of exposure.

Diazinon was found to have dose and time dependent effect on GST activity. Significant increases in GST activity are produced in groups exposed to diazinon at 40 PPM for one and two weeks and 80 PPM for one week. On the other hand, significant decreases in GST activity was found in groups received diazinon at 40 PPM and 80 PMM for four weeks and 160 PMM for one, two and four weeks.

3- Serum Proteins Concentration:

Significant decreases in total proteins content were found in all treated groups of fish except the group received 40 PPM diazinon after

recovery period and the group received 160 PPM diazinon for one week as demonstrated in table (3). Albumin contents were significantly decreased in most of the treated groups with notable exception of the groups received 80 and 160 PPM after recovery .

Table (1): Lipids profile in the serum of control and treated *T. nilotica* fish.

	Total lipids (g/l)	Triacylglycerol (mg/dl)	Cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
Control group	7.01±0.26	74.72±5.52	119.80±4.68	7.92±1.38	381.50±6.50
40 PPM/ one week	6.39±0.24	37.70±2.37***	147.20±4.85***	15.10±1.82***	329.02±21.00*
40 PPM/ two weeks	6.15±0.32	59.50±1.81*	148.00±5.93**	13.77±0.93**	296.92±19.00**
40 PPM/ four weeks	4.82±0.26***	32.06±2.84***	116.80±2.84	10.07±1.62	253.08±3.30***
40 PPM/ Recovery	7.15±0.35	106.91±3.62***	130.20±5.11	15.69±0.34***	328.80±5.00***
80 PPM/ one week	4.46±0.25***	58.20±1.08*	122.30±1.34	7.51±0.48	302.12±23.00**
80 PPM/ two weeks	4.33±0.29***	52.95±2.49**	135.70±3.20*	12.54±1.20*	320.83±25.20*
80 PPM/ four weeks	5.06±0.49***	75.01±4.98	135.50±3.31*	5.00±0.14	312.00±21.28**
80 PPM/ Recovery	6.47±0.26	77.60±2.00	115.30±2.00	9.56±0.77	345.00±26.77
160 PPM/ one week	5.18±0.32***	121.20±3.07***	102.60±3.06**	17.63±0.39***	378.13±31.00
160 PPM/ two weeks	3.73±0.27***	79.30±2.33	81.10±4.95***	8.96±1.45	253.86±22.00***
160 PPM/ four weeks	4.16±0.28***	87.59±4.38	96.35±5.57**	9.98±0.99	191.59±7.00***
160 PPM/ Recovery	3.85±0.39***	85.06±4.64	74.50±6.46***	12.63±1.02*	207.53±14.00***

* Significant than control group at $P < 0.05$.
** " " " " " $P < 0.01$.
*** " " " " " $P < 0.001$.

Table (2) showing the activities of the enzymes measured in control and different treated groups.

	Creatine Kinase (U/L)	Cholinesterase (U/L)	Glutathione-s-transferase (OD/mg protein/min)
Control group	44.6 ± 4.5	516±47	0.0022±0.0003
40 PPM/ one week	27.5 ± 3.2***	141±16***	0.0045±0.0005***
40 PPM/ two weeks	63.1 ± 4.5**	188±23***	0.0039±0.0004**
40 PPM/ four weeks	29.7 ± 3.2*	117±7***	0.0014±0.0001**
40 PPM/Recovery	14.9 ± 3.7***	352±21*	0.0019±0.0001
80 PPM/ one week	41.6 ± 2.9	164±12***	0.0089±0.0008***
80 PPM/ two weeks	111.4 ± 7.4***	188±21***	0.0022±0.0004
80 PPM/ four weeks	49.7 ± 7.4	281±19***	0.0015±0.0001*
80 PPM/ Recovery	25.3 ± 1.5***	375±23*	0.0025±0.0003
160 PPM/ one week	47.5 ± 5.9	258±7***	0.0011±0.0002**
160 PPM/ two weeks	31.2 ± 3.2*	188±21***	0.0014±0.0001*
160 PPM/ four weeks	14.9 ± 2.2***	235±14***	0.0011±0.0001**
160 PPM/ Recovery	26.0 ± 3.9***	282±14***	0.0025±0.0004

* Significant than control group at $P < 0.05$.
** " " " " " $P < 0.01$.
*** " " " " " $P < 0.001$.

Table (3): Mean±S.E. of serum total proteins, albumin and globulins (gm/dl) in different groups of *T. nilotica* exposed to diazinon.

	Total proteins	Albumin	Globulins
Control group	3.56±0.17	1.22±0.05	2.34±0.14
40 PPM/ one week	2.29±0.09***	0.92±0.02***	1.37±0.09***
40 PPM/ two weeks	2.81±0.05***	1.04±0.09	1.77±0.10**
40 PPM/ four weeks	2.35±0.05***	0.65±0.04***	1.70±0.05***
40 PPM/Recovery	3.26±0.05	0.94±0.02***	2.32±0.03
80 PPM/ one week	2.56±0.10***	1.00±0.05**	1.56±0.11***
80 PPM/ two weeks	2.74±0.06***	0.99±0.07*	1.75±0.03**
80 PPM/ four weeks	2.56±0.13***	0.86±0.02***	1.70±0.12**
80 PPM/ Recovery	2.62±0.06***	1.16±0.05	1.46±0.11***
160 PPM/ one week	3.02±0.16	1.21±0.09	1.81±0.14*
160 PPM/ two weeks	2.93±0.09**	0.94±0.07**	1.99±0.14
160 PPM/ four weeks	2.63±0.16**	0.77±0.05***	1.86±0.11*
160 PPM/ Recovery	2.46±0.21**	1.13±0.09	1.33±0.10***

* Significant than control group at $P < 0.05$.
** " " " " " $P < 0.01$.
*** " " " " " $P < 0.001$.

and 160 PPM for one week. Concerning globulins level, significant reductions were found in all groups except recovery group received 40 PPM and group received 160 PPM for two weeks.

4- Serum Proteins Fingerprinting:

Results of serum protein fractionation using SDS-PAGE revealed dramatic changes in all treated groups as compared with the control one. As shown in table (4), some fractions were disappeared as a result of diazinon treatment with certain concentration and reappeared after recovery period. For example, the protein band at molecular weight of 323.1 Kda in the control untreated group was disappeared in the group treated with 40 PPM of diazinon for four weeks and reappeared after recovery. The same band was disappeared in the group received 80 PPM for two and four weeks and reappeared again after recovery. On the other hand, that band was permanently disappeared in all groups treated with 160 PPM and was not reappeared after recovery. Another pattern of effect involved the disappearance of certain fraction as a result of treatment with certain concentration. For example, the band at 152.6 Kda was disappeared only in the group received 80 PPM for all periods including that after recovery. Surprisingly, only one protein fraction revealed resistance to the effect of diazinon at all its different concentrations and time of exposure. This band was at molecular weight of 66 Kda so, it is supposed to be the albumin fraction.

5- Bilirubin and Thyroid Hormones:

As shown in table (5), total and direct bilirubin were significantly increased in groups of fish received 40 PPM diazinon for four weeks and after recovery. Significant increases were also found in total bilirubin of groups that received 80 PPM at all periods while direct bilirubin was only elevated in the recovery group. Similarly, addition of 160-PPM diazinon significantly increased total bilirubin after one, two weeks and recovery while significantly increased direct bilirubin after recovery only.

Concerning the effect of diazinon on the thyroid hormones level of Tilapia nilotica, significant decreases in T3 level were obtained in all groups except that received 40 PPM for one week and the recovery group received 160 PPM. However, T4 level was significantly increased in all groups treated with 40 PPM except the recovery one while those received 160 PPM were found to have significant decreased levels in T4 after one and two weeks then significantly elevated in the recovery group.

DISCUSSION

1- Serum enzymes activity:

Inhibition of acetylcholinesterase activity in all diazinon-treated groups with different concentrations and periods of exposure reveals high sensitivity of the enzyme to diazinon. Organophos-

phorous insecticides are known to cause inhibition of acetylcholinesterase so that in some cases is accompanied by the inhibition of neurotarget esterase (Repetto et al., 1988). El-Gendy et al., (1990b) pointed out that inhibition of AchE in fish exposed to organophosphorous compounds may serve as an indicator for the hazard due to application of these chemicals in the environment.

Dembele et al., (2000) found that high concentration of diazinon killed all the tested carp after only 4h.while the lowest concentration significantly inhibited brain AChE after 96 h. The results suggested that in biomonitoring programs

Table (4): demonstrates the pattern of serum proteins fingerprint by SDS-PAGE.

Cont.	40/w	40/2w	40/4w	40/R	80/w	80/2w	80/4w	80/R	160/w	160/2w	160/4w	160/R
323.1	+	+	-	+	+	-	-	+	-	-	-	-
269.9	-	-	-	-	+	-	+	+	-	-	+	+
244.7	-	-	+	+	-	-	+	+	+	+	+	+
213	-	-	+	-	-	-	-	+	-	-	-	+
172	-	-	-	+	-	-	-	-	+	+	-	+
152.6	+	+	+	+	-	-	-	-	+	+	+	+
133.5	-	-	+	+	+	-	-	-	+	+	-	-
120	-	-	+	+	-	+	+	+	+	-	-	-
110.4	+	+	+	+	+	+	-	-	+	-	+	-
99.4	-	-	+	+	-	-	+	+	+	-	-	+
88.1	+	+	+	+	-	-	+	+	+	-	+	-
83.9	-	-	-	+	-	-	-	+	+	+	-	+
76.3	-	-	+	+	-	-	+	+	+	-	-	+
66	+	+	+	+	+	+	+	+	+	+	+	+
63.4	+	+	+	-	+	+	+	+	+	+	-	+
56.3	+	+	+	+	-	+	+	+	+	-	+	+
53	-	+	+	-	+	-	-	-	-	+	+	+
49.4	-	+	+	+	-	-	+	+	+	+	+	+
44.9	+	+	+	+	+	-	+	-	+	-	-	+
42.1	+	+	+	+	+	+	+	+	+	-	+	-
35.6	+	+	+	+	+	+	+	+	+	+	-	+
29.7	+	+	+	+	+	+	+	+	+	-	+	+
24	+	+	+	+	+	+	+	-	+	+	+	+
21.6	+	+	+	+	+	+	+	+	+	-	+	+
16.6	-	+	+	+	+	+	-	-	+	-	+	+
12.9	+	+	+	-	+	+	+	+	+	+	+	+
10.3	+	+	+	-	+	+	-	+	-	+	+	+
8.5	-	-	-	+	+	+	+	-	+	+	-	+
5.4	+	-	+	-	+	+	-	+	+	+	+	+
3.4	+	-	+	+	-	-	+	+	+	+	+	+
0.96	-	-	-	+	-	-	-	+	+	-	-	-

Cont.= Molecular weights in Kda of serum fractions in control group.
40/w, 40/2w, 40/4w and 40/R= groups received 40 PPM diazinon for one week, two weeks, four weeks and after recovery, respectively.
80/w, 80/2w, 80/4w and 80/R= groups received 80 PPM diazinon for one week, two weeks, four weeks and after recovery, respectively.
160/w, 160/2w, 160/4w and 160/R= groups received 160 PPM diazinon for one week, two weeks, four weeks and after recovery, respectively.
(+) = protein fraction is present. (-) = protein fraction is absent.

Table (5): Serum levels of bilirubins and thyroid hormones in different groups of *T. nilotica* exposed to diazinon.

	Total bilirubin umol/	LDirect bilirubin umol/L	T3 ug/dl	T4 Ng/d
Control	130±2.94	98±5.47	83.00±1.60	0.39±0.02
40 PPM/ one week	125±8.43	113±6.54	85.67±1.30	0.53±0.02***
40 PPM/ two weeks	133±2.01	108±1.62	74.0±1.40***	0.49±0.01***
40 PPM/ four weeks	187±3.90***	124±1.46***	77.67±1.23*	0.53±0.01***
40 PPM/Recovery	240±3.20***	139±2.08***	40.0±0.20***	0.33±0.02
80 PPM/ one week	99±2.94***	92.5±3.17	48.33±1.13***	040±0.01
80 PPM/ two weeks	158±8.85*	112±8.88	64.33±1.43***	0.36±0.07
80 PPM/ four weeks	146±2.00***	93±4.20	67.67±0.91***	0.41±0.01
80 PPM/ Recovery	208±3.70***	142±7.93***	73.0±0.61***	0.37±0.01
160 PPM/ one week	163±10.93**	119±8.55	67.0±0.40***	0.30±0.01***
160 PPM/ two weeks	160±3.63***	123±4.16	38.67±2.30***	0.24±0.02***
160 PPM/ four weeks	137±3.84	105±2.32	78±1.12*	0.45±0.02
160 PPM/ Recovery	194±4.24***	128±2.50***	81.67±1.22	0.54±0.02***

* value is significantly different than control group at P<0.05.
** " " " " " " " " P<0.01.
*** " " " " " " " " P<0.001.

carp brain AChE could be used as a good diagnostic tool for chronic organophosphate pollution. Our results providing serum AchE as an easier diagnostic test for diazinon toxicity in *T. nilotica.*

Hamm et al., (1998) reported that recent monitoring of the Sacramento-San Joaquin River system (CA) indicates that levels of the organophosphate pesticide, diazinon, exceed National Academy of Science guidelines. Diazinon exposure significantly inhibited AChE activity within whole embryos and in homogenates of retinas from treated animals. Enzyme histochemistry localized AChE activity to regions equivalent to sites of necrosis.

Pan and Dutta (1998) found that juvenile brain acetylcholinesterase activities were significantly inhibited by sublethal doses of diazinon. They suggested that inhibition of brain AChE would definitely cause physiological and behavioral modifications that reduce survival ability of the animals at an early stage of growth.

Diazinon toxicity was found to differ among fish species and can largely be explained in relation to metabolic balances in the liver and with the features of the target enzyme (Keizer et al., 1995).

Glutathione-s-transferase is thought to play a

physiological role in the detoxification and elimination of toxic and undesirable foreign compounds including pesticides and drugs, (Chasseaud, 1973). El-Gendy et al., (1990a) reported that brain, gill and liver contained the highest level of GST. They found also that, liver GST activity was increased in fishes intoxicated with pyrazophos or glyphosate (organophosphorus compounds) to protect the fish from pesticide injury. However, in this study effect of diazinon on serum GST activity of T.nilotica was found to be dose and time-dependent. Small and medium doses have increasing effect on GST activity after one and two weeks of exposure. By increasing the period of exposure or increasing the dose of diazinon, GST activity is found to be inhibited. Interestingly, GST activity is fully recovered after ten days of the end of diazinon exposure. GST is therefore considered one of the phase two enzymes that involved in detoxification of environmental chemicals (Goldstein and Faletto, 1993).

El-Gendy, et al., (1990b) found that GST activity was inhibited in the brain and gill, while it was stimulated in the liver of fish treated with other organophosphates.

Similar findings to that obtained in the present investigation was reported by Kim et al., (1993). They found that the detoxifying enzyme (GST) activity of carp (Cyprinus carpio L) was increased at the sublethal doses of diazinon in different organs (liver, head and gut), but were declined by increasing doses. In addition, the activity of the enzyme was declined by increasing protein content.

Creatine kinase activity in serum of diazinon treated T. nilotica showed different pattern according to the dose and time of exposure. However, it seems that diazinon has cumulative effect where it is significantly inhibited after the recovery period. Similarly, Wilkinson et al., (1986) studied the effect of low levels of diazinon treatment on four marker enzymes in rat heart and skeletal muscle. Typical differences in Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), Phosphofructo kinase (PFK) and Hexokinase (HK) activities were observed between heart and skeletal muscles. Although these results demonstrate that chronic low levels of diazinon have little effect on the glycolytic and oxidative activity in heart and skeletal muscle, but it may have pronounced effect on energy store in muscles as a result of myocardial infarction to which CK activity can be considered as a mirror reflecting its occurrence.

2- Serum Proteins:

Our finding that treatment with diazinon has depressing effect on the serum proteins is consistent with several previous reports. Patil et al., (1990) studied the biochemical changes in the liver and muscles of fish B. dussumieri exposed to 2 and 4 ppm monocrotophose for up to 7 days. They found that the protein levels

were lower than those of control. Ansari and Kumar (1988) reported that, diazinon have deleterious effects on nucleic acids and protein content in fish. They stated that, zebra fish, Brachydanio rerio treated with four concentrations of diazinon insecticide (o.64, 1.06, 1.4, and 1.9 mg/L) for up to 168 hours resulted in a significant reduction of DNA, RNA and protein content, whereas the amino acid content was significantly enhanced. They also showed that all these changes showed dose- as well as time- dependent responses. El-Gendy et al., (1998) reported that, 1/1000 field recommended concentration of the organophosphorous compounds; edifenphos and glyphosate decreased the serum total protein content in Tilapia nilotica treated fishes.

Saxena et al., (1989) reported that some pesticides are considered more toxic to fish where it inhibited the de novo synthesis of certain lipids and proteins by the liver. The liver of Tilapia mossambica exposed to monocrotophos, organophosphate insecticide, showed a decrease in protein content by 45% after 5 days, and returned to control level at 10-30 days then decreased again by 45 days (Sherekar and Kulkarni, 1989).

Murthy et al., (1986) found that lethal and sublethal dosages of the pesticide, lindane, reduced the protein content in tissues of Tilapia mossambica, with a corresponding increase in neutral protease activity leading to increase in the tissue amino acid level.

Organophosphorus compounds were reported to cause marked suppression in both humoral and cell mediated immune responses fish (El-Gendy et al., 1998). This may be attributed to depressed number of lymphocytes, direct effect on the function of T-and B cells, inhibition of biosynthesis of critical proteins involved in immune functions or to impairment of immune regulatory mechanisms. Khalaf-Allah (1999) found also that total protein, globulin as well as macrophage phagocytic index and antibody titer were lower in vaccinated as compared to the non-vaccinated groups of T. nilotica that were exposed to 1/10 LC_{50} of diazinon for 30 days.

3- SDS-polyacrylamide gel electrophoresis :

The present SDS-PAGE of serum proteins revealed disappearance of some protein fractions and appearance of another new ones. Serum proteins from fish treated with (1/1000 field recommended concentration) of edifenphos and glyphosate, exhibit also changes in the electrophoretic patterns compared with control serum (El-Gendy et al., 1998). Similar electrophoretic findings were reported by Shimaila (1989). The causes for the observed changes in fish serum protein due to pesticides may take place as a result of physiological, genetical and environmental factors (Harries, 1974) or resulted from their changes in the DNA and RNA of the brain, liver, muscle and gill proteins, (Duraray and Selvarajan, 1992). Thus this pattern of band disappearance and reappearance found

in our study may be due to temporary inhibition of certain gene expression that code for such protein fragment. This effect may be on the transcription and/or translation level. In some cases, this inhibitory effect is persisted even after the recovery period that may be attributed to overall gene dysfunction.

4- Lipids profile:

McGill et al., (1981) performed an experiment for 26 months, fed baboons a high saturated fat, high cholesterol diet that contained very low concentrations of diazinon. They detected no effect of pesticide on body weight, serum lipid, or lipoprotein cholesterol concentrations, or experimental atherosclerosis. However, very low density lipoprotein plus low density lipoprotein cholesterol concentration showed a positive association with fatty streaks in the aorta and its major branches, including the coronary arteries, while high density lipoprotein cholesterol concentration showed a consistently negative association. These results are consistent with epidemiological evidence suggesting that high density lipoprotein cholesterol concentration is inversely related to probability of developing clinically manifest atherosclerotic disease. However, in our study diazinon was found to decrease the level of total lipids in general. This is sometimes associated either with increasing or decreasing level of triacylglycerol. LDL-cholesterol is found to be increased antiparallel to HDL-cholesterol level. This might lead to atherosclerosis and confirm the finding of McGill et al., (1981).

Ghioni et al., (1997) studied the effects of sublethal doses of dichlorvos on lipid composition and metabolism of rainbow trout skin cells in primary culture. They reported that, dichlorvos had a range of effects on rainbow trout skin cell cultures that may affect cell proliferation and fatty acid metabolism, and significantly inhibited desaturation of fatty acids.

5- Thyroid hormones and bilirubin content:

Cranmer et al., (1978) found that prenatal exposure to pesticides of diazinon initiated persistent postnatal endocrine dysfunction. Adrenal function and hepatic metabolism of corticosterone were studied in adult hybrid mice exposed during development to an organophosphate (Diazinon). Animals were exposed to relatively low levels of the toxins in utero and neonatally via the mothers' milk. Exposure to lower doses of the anticholinesterase compounds, e.g diazinon, resulted in impairment of hepatic metabolism of corticosterone in vitro due to a loss in reductive capacity per unit liver weight. Plasma levels of corticosterone were also elevated in these animals, but without a concomitant increase in adrenal steroidogenesis in vitro.

In the present study, thyroid gland function was found also to be affected with diazinon

treatment. This effect is represented in decreased levels of T3 accompanied by elevated levels of T4 hormones in some doses and times. High dose of exposure revealed irreversible declined levels of both hormones even after recovery period. This affected level of thyroid hormones indicates either thyroid gland dysfunction or abnormal hepatic metabolism of the hormones.

The finding that diazinon treatment increased total bilirubin and in some conditions (dose and time exposure) direct bilirubin, direct us to the fact that diazinon not only affect liver biosynthetic activity but also affect the metabolism of steroid compounds.

From the present study, we can conclude that direct and indirect contamination of water by the tested pesticide may cause fish killing, reduced reproduction and elevated concentration of undesirable chemicals in edible fish tissues. Therefore, there is an increasing need to minimize the adverse effects of these compounds on environmental quality. These chemicals should also be restricted to field of agriculture far from water sources.

REFERENCES

Abou-Donia, M. B.; K. M. Abdo; P. R. Timmons and J. E. Roctor, (1986): Brain acetylcholinesterase, acid phosphatase and 2, 3, cyclic nucleotide-3-phosphohydrolase andplasma butyryl-cholinesterase activities in hens treated with a single dermal neurotoxic doseof s, s, s-tri-n-butyl phosphorotrithioate, Toxicol. Appl. Pharmacol., 83: 461- 473.

Ansari, B.A. and Kumar, K. (1988): Diazinon toxicity; effect on protein and nucleic acid metabolism in the liver of zebrafish, Brachydanio rerio (Cyprinidae). Sci. Total Environ.; 76(1):63-8.

Bisso, M., Braincesco, R. and Michalek, H. (1991): Size and Charge Isomers of Acetylcholinesterase in the Cerebral Cortex of Young and Aged Rats. Neurochemical Research, 16(5): 571-575.

Burstein, M. (1970): Separation of high density lipoproteins and determination of cholesterol and phospholipids bound to these fractions. Lipid Res., 11; 583.

Chabrol. E. and Charonnat, R. (1937): Colorimetric determination of total lipids with sulfophosphovanillic mixture. Presse. Med., 96: 1713.

Chasseaud, L.F. (1973): The nature and distribution of enzymes catalyzing the conjugation of glutathione with foreign compounds. Drug Metab. Rev., 2: 185-220.

Cranmer, J.S., Avery, D.L., Grady, R.R. and Kitay, J.I. (1978): Postnatal endocrine dysfunction resulting from prenatal exposure to carbofuran, diazinon or chlordane. J. Environ. Pathol. Toxicol., 2(2):357-69.

De-Bruijn, J. and Hermens, J. (1993): Inhibition of acetylcholinesterase and acute toxicity of organophosphorous compounds to fish: a preliminary structure-activity analysis. Aqua. Toxicol., 24 (3-4): 257-274.

Dembele, K., Haubruge, E., Gaspar, C. (2000): Concentration effects of selected insecticides on brain acetylcholinesterase in the common carp (Cyprinus carpio L). Ecotoxicol. Environ. Saf. 45(1): 49-54.

Doumas, B. (1972): In Standard Methods of Clinical Chemistry, Acad. Press, N.Y., 7:175.

Durairay, S. and Selvarajan, V.R. (1992): Influence of quinalphos, an organophosphorous pesticide, on the biochemical constituents of the tissues of fish, Oreochromis mossambicus. Environ. Ecol., 13(3): 181 - 185.

El-Benhawy, M.A.;El-Tounsy, M.; Farid, N. and El-Salkh, B. (1990): Histochemical studies on the effect of curacron on the adrenal gland of the rat. Egypt. J. Histol.; 13 (2): 207-217.

El-Gendy, K. S. (1990): The effects of thiodicarb and cypermethrin on some liver enzymes of rats. Bull. Alex. Fac. Med., 35(4): 607-612.

El-Gendy, K. S.; N. M. Aly; N. S. Ahmed and A. H. El-Sebae (1990a): Comparative toxicity of some pesticides to common carp and their effects on biochemical targets in living tissues. J. Pest. Control Environ. Sci., 2: 29-41.

El-Gendy, K. S.; N. S. Ahmed; N. M. Aly; N. Saber and A. H. El-Sebae (1990b): Effect of some pesticides on the antioxidant enzymes and lipid peroxidation in carp tissues. J. Pest. Cont. Environ. Sci., 2: 21-27.

El-Gendy, K.S., Aly, N.M., El-Sebae, A.H. (1998): Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (Tilapia nilotica). J. Environ. Sci.Health B, 33(2): 135-49.

Ellman, G. L.; K. D. Courtney; V. J. Andres and R. M. Featherstone (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Folmar, L.C., Sanders, H.O. and Julin, A.M. (1979): Toxicity of the herbicide glyphosphate and several of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol.; 8(3): 269-78.

Fossati, P. and Prencipe, L. (1982): Fully enzymatic determination of triglycerides in serum. Clin. Chem., 28: 2077.

Franson, J.C. (1984): Postmortem changes in liver weight of Japanese quail. Bull. Environ. Contam. Toxicol., 33 (3):313-6.

Ghioni, C., Bell, J.G., Bell, M.V. and Sargent, J.R. (1997): Fatty acid composition, eicosanoid production and permeability in skin tissues of rainbow trout (Oncorhynchus mykiss) fed a control or an essential fatty acid deficient diet. Prostaglandins Leukot. Essent. Fatty Acids, 55(6): 479-89.

Glinoer, D., Fernandez, M. and Ermans, A.M. (1978): Use of a direct thyroxine binding globulin measurement in the evaluation of thyroid function. J. Endocrinol. Invest., 1; 329.

Goldstein, J.A. and Faletto, M.B. (1993): Advances in mechanisms of activation and deactivation of environmental chemicals. Environ. Health Perspect.; 100: 169 - 176.

Gruber, W. (1978): Inhibition of creatine kinase activity by Ca2+ and reversing effect by EDTA letter to editor], Clin. Chem., 24:177.

Hamm, J.T., Wilson, B.W., Hinton, D.E. (1998): Organophosphate-induced acetylcholinesterase inhibition and embryonic retinal cell necrosis in vivo in the teleost (Oryzias latipes). Neurotoxicology; 19(6): 853-69.

Harries, J.E. (1974): Biochemical studies on carp plasma protein. I: Isolation and nature of albumin. Bull. Japan. Soc. Of Sci.Fishers.; 42(6): 677 - 685.

Henry, R. (1964): Clim. Chem. Principles and techniques. Harper-Row, N. York, p. 182.

Keizer, J., D'Agostino, G., Nagel, R., Volpe, T., Gnemi, P. and Vittozzi, L. (1995): Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species. Sci Total Environ.,171(1-3): 213-20.

Khalaf-Allah, S.S. (1999): Effect of pesticide water pollution on some haematological, biochemical and immunological parameters in Tilapia nilotica fish. Dtsch. Tierarztl. Wochenschr.; 106(2): 67-71.

Kim, I.S.; Lee, K.B.; Shim, J.H.; and Suh, Y.T. (1993): Synergistic effects of pesticides of detoxifying enzyme activity of carp Cyprenius carpio L.; J. Kor. Agric. Chem. Soc., 36(1): 64 - 69.

Laemmli, U.K. (1970): Cleavage of Structural proteins during the Assembly of the Head ofBacteriophage T4.Nature, 227: 680-685.

Melnikov, N.N., Stonov, L.D., Grapov, A. F. and Sergeyeva, T.A. (1975): New herbicides of the isophos group: chemistry, biological activity, mode of action. Environ. Qual. Saf. Suppl., 3: 707-12.

McGill, H.C. Jr., McMahan, C.A., Kruski, A.W. and Mott, G.E.(1981): Relationship of lipoprotein cholesterol concentrations to experimental atherosclerosis in baboons. Arteriosclerosis; 1(1): 3-12.

Murthy, B. N., Reddy, M.S., Venkateswarly, Y. and Rao, K.V. (1986): Lindane induced altertion in the protein breakdown and utilization in the selected tissue of fresh water fish, Tilapia mossambica (Peters): Nation. Acad. Sci. Letters - India; 9 (1): 27 - 30.

Odenkirchen, E.W. and Eisler, R. (1988): Chlorpyrifos hazards to fish, wildlife and invertebrates: a synoptic review. Contaminant hazard reviews, Report No. 13, Fish and Wildlife service, U.S. Department of the Interior.

Oeschet, F.; Friedberg, T.; Herbst, M.; Paul, W.; Wilhelm, N.; and Bentley, P. (1982): Effect of lindane treatment on drug metabolizing enzyme and liver weight of CFI mice in which it evoked hepaticas and in non-susceptible rodents; Chem. Biol. Interact., 40: 1

Pan, G. and Dutta, H.M. (1998): The inhibition of brain acetylcholinesterase activity of juvenile largemouth bass Micropterus salmoides by sublethal concentrations of diazinon. Environ. Res. 79 (2): 133-7.

Patil, V.T., Shinde, V.S. and Kulkarni, A.B. (1990): Biochemical Changes induced by monocrotophos on the estuarine edible mudskipper Boleophthalmum dussumieri; Environ. Ecol., 8 (4): 1167 - 1173.

Repetto, G., Sanz, P. and Repetto, M. (1988): In vivo and in vitro effect of triclorfon on esterases of the red Cary fish Procambarus Clarkii. Bull. Environ. Contam. Toxicol., 41(4): 597-603.

Richmond, W. (1973): Enzymatic determination of cholesterol. Clin. Chem., 19; 1350-1356.

Saxena, A. K. and K. Sarin, (1980): Histopathological and biochemical changes in the liver and testes of desert gerbil after repeated exposures of thimet (phorate), Toxicol., 18: 133.

Saxena, P.K.; Singh, V.P.; Kondal, J.K. and Soni, G.L. (1989): Effects of some pesticides on in vitro lipid and protein synthesis by the liver of the freshwater teleost, Channa punctatus.; Environmetal - Pollution; 58(4): 273 - 280.

Schimmel, S. C.; R. L. Garnas; J. M. Patrick and J. C. Moore, (1983): Acute toxicity,bioconcentration and persistence of AC222, 705, benthiocarb, chlorpyriphos, fenvalerate, methyl parathion and permethrin in the estuarine environment, J. Agric. Food Chem., 31(1):104-113.

Snedecor, G.W and Cochran, W.G. (1980): Statistical Methods. 6th edition, Oxford and J.B.H. Publishing Co. USA.

Sherekar, P.Y. and Kulkarni, K.M. (1989): Biochemical changes in the liver of fish, Tilapia mossambica (Peters), during continuous exposure to monocrotophos. Ecotoxicol. Environ. Saf.; 15 (3): 272 - 276.

Shimaila, A.M.A. (1989): Effect of some additives on the toxicity of certain rodenticides against white rat fed on different diets. Ph. D. Thesis. High Institute of Public Health, Alexandria University, Egypt.

Steinberg, D. (1981): Metabolism of lipoproteins at the cellular level in relation to atherogenesis. In lipoproteins, Atherosclerosais and Coronary Heart disease, 1; 2:31-48, Elsevier, North Holland.

Vessey, D. A. and Boyer, T.D. (1984): Differential activation and inhibition of different forms of rat liver glutathione-S- transferase by the herbicides 2,4 dichlorphenoxyacetate (2, 4-D) and 2, 4, 5-trichlorophenoxyacetate (2, 4, 5-T). Toxicol. Appl. Pharmacol.,73: 492-499.

Walters, M. and Gerarde, H. (1970): Determination of total and direct bilirubin in serum. Microchem. J., 15: 231.

Weil, C. S. (1952): Tables for convenient calculation of median effective dose (ED 50 or LD 50)and instructions in their use. Biometrics, 8: 249.

Wilkinson, J.G., Rajendra, W., Oloffs, P.C., Banister, E.W. (1986): Diazinon treatment effects on heart and skeletal muscle enzyme activities. J. Environ. Sci. Health B; 21(2): 103-13.