Egyptian J. Anim. Prod., 33, Suppl. Issue, Nov. (1996): 499-506

ACUTE TOXICITY AND HEMATOLOGICAL EFFECTS OF MALATHION IN NILE TILAPIA (*OREOHROMIS NILOTICUS*)

M.A. Danasoury and Laila Reda

Faculty of Agriculture, Suez Canal University

SUMMARY

The acute toxicity and hematological effects of malathion on organophosphorus pesticide, were examined in Nile tilapia *(Oreochromis niloticus)*. The 96 hr. LC_{50} 's malathion was 3.8 ppm. Exposure of an adult fresh water fish to sublethal and chronic levels (ranging from 1/6 LC_{50} to 2/3 LC_{50}) of malathion at intervals 48h, 96h., 15 and 30 days, each resulted in many alterations in the blood picture. Malathion also decreased some blood parameters like total RBC count, Haemoglobin, Haematocrite and Mean cell haemoglobin concentration (McHc). On the other hand increase was observed in total WBC count, mean cell volume (Mcv) and mean cell Haemoglobin (McH) which was statistically ($P<0.05$) significant. Possible reasons for these changes are discussed.

Keywords: Nile tilapia, malathion, haemoglobin, haematocrit, red blood cells, white blood cells.

INTRODUCTION

The hazards of pesticides to fish are of great concern. In recent years, incidences of fish mortality due to pesticides, industrial effluents, and sewage pollution have been reported a number of times (Johnson, 1968; Mawdesley , 1971; Katz *et al.*, 1972 and Coppage and Braidech, 1976). Pesticides have been found to be highly toxic not only to fishes, but also to fish food organisms.

The susceptibility of the various fish species to a number of organophosphorus insecticides is well documented (Sprague, 1971; Pimentel 1971; Katz *et al.*, 1972). However, little attention has been paid to elucidate the blood-pesticide relationship in fishes, particularly with reference to organophosphorus formulations. It is, therefore desirable to extend work on sublethal and chronic effects of these toxic substances on blood of the most important edible fish in Egypt (*Oreochrouis niloticus*).

In the present investigation an attempt is made to study the sublethal and chronic effects of malathion (0.0 dimethyl phosphorothioate s- (1.2 dicarbonthoxy ethyl) on hematological parameters of the Nile tilapia (*Oreochromis niloticus*) fish.

This study examined the quantitative variation in PCV (packed cell volume or haematocrit), haemoglobin concentration blood (Hb), mean corpuscular haemoglobin concentration (McHc), red blood cell count (RBC), white blood cell count (WBC), mean cell haemoglobin content (McH) and the mean cellular volume (MCV).

The indiscriminate use of pesticides in agricultural operations adversely affects the aquatic environment to a very great extent. This posses a great danger to freshwater organisms including fishes.

MATERIALS AND METHODS

Nile tilapia (*Oreochromis niloticus*) that averaged 50±2 g in weight and 16 cm in length were obtained from Fish Research Center in Suez Canal University. Fish were stocked and maintained during adaptation period (7 days) to laboratory condition in dechlorinated tap water. The water of the aquarium was aerated continuously through stone diffusers; connected to a mechanical air-compressor. Water temperature was (27.7±/°C). The pH was 7.51 ± 0.08, and the salinity was (0.19%). Fish were maintained under natural photoperiod conditions. Fish were fed with market artificial feed formulated in the dry pelleted form, (25% protein, 58% CHO, 6% ether extract, 6% C. fiber, 7% mineral) miner daily feed rate was 1.4% of body weight (Bresch, 1971). Fish density was 10 fish per aquarium containing 60 liter water.

Acute Toxicity Of Malathion To Nile Tilapia (*Oreochromis niloticus*)

The experiment was performed with apparent healthy, Nile tilapia fish after being acclimatized to the laboratory conditions and were not fed two days before starting and durated then for 96 hours test period.

A 96 hours bioassay was performed to determine LC_{50} values, using a series of malathion (57% active ingredient) concentrations, two replicates for each concentration of pesticide were performed. Control check, which did not receive any chemicals, was included. Dead fish were removed as soon as observed from the aquaria to avoid deterioration and were recorded, after 96 hours from treatment.

The results of fish toxicity were analyzed by the probit analysis method of Finney (1971) to determine the fish LC_{50}.

Eight fish (*Oreochromis niloticus*) were exposed to sublethal concentrations 1/6, 1/3, 2/3 LC_{50} of the malathion as determined from toxicity experiment. The blood parameters were measured after 48 hrs., 96 hrs., 15 days and 30 days.

Blood Samples

Individual fish was anaesthetized in 93 mg/l Ms222 (Sandoz) solution for blood sampling. Heparin at a concentration of 3 mg/ml blood was used as anticoagulant (Barham *et al.*, 1979) and blood was obtained anaerobically by cardiac puncture. The sampling procedure did not exceed 3 min.

Analytical Procedures

Blood for white blood cell (WBC) count, red blood cell (RBC) count and haemoglobin (Hb) content was obtained by cardial puncture with a 2.5 ml syringe and 21gauge needle previously rinsed with 10% sodium heparin.

Blood cell counts were estimated using an improved Neubauer haemocytometer (Hesser, 1960). Haemoglobin was measured using the cyan-methaemoglobin method (Snieszko, 1961).

Haematocrit values were read with a microhaematocrit reader to avoid the swelling of cells, which can occur in fish blood stored anaerobically, haematocrit was determined completely within 15 mm after 15 minutes from sampling (Soivivo, *et al.*, 1974).

Calculated Blood Variables

From the blood variables determined (Hb, PCV and RBC), mean corpuscular haemoglobin concentration (McHc), mean corpuscular haemoglobin content (McH) and mean cell volume (McV) were calculated according the methods of Dacie and Lewis (1975).

The mean cell haemoglobin concentration (McHc) was determined by dividing the haemoglobin concentration by the haematocrit. This measure was used to asses the amount of red cell swelling present (Milligan and Wood, 1982).

Averages of eight individual estimations were taken and the mean values of control and experimental fishes, were subjected to statistical analysis using student's test described by Bailey (1965).

RESULTS AND DISCUSSION

Percentage of mortality was recorded after 96 hours, normal equivalent deviate (mortality/log conc.) regression was obtained by the method of Finney (1971). In the present study one formulated pesticides, malathion (57 E.C) was used to determine the toxicity of this compound. Table (1) and Fig (1) represent the 96 hours LC_{50} value of Nile Tilapia (*Oreochromis niloticus*), where the 96 h. LC_{50}'s malathion was 3.8 ppm.

Table 1. Toxicity of malathion (cythion 57% EC) on Nile Tilapia *Oreochromis niloticus* after 96 hr. according to Finney's (1971) method of analysis.

Toxicity	Concentration (ppm)
LC_{10}	1.7
LC_{25}	2.5
LC_{50}	3.8
LC_{90}	8.8
Slop	3.6
MLC 50 ×103	115.1

MLC_{50}: Molar LC_{50} = LC_{50}/molecular weight of compound.

Most organophosphorus compounds showed their toxic effects against the aquatic system. Kumar and Ansari (1984) found that the LC_{50} values of malathion against zebra fish at the different exposure times of 24, 48 and 96 hours, show a time dependent decrease with increasing the exposure period. They reported that the LC_{50} of 96 hrs. was 1.05 mg/l which was lower than results obtained with malathion.

In fact the toxicity and action of organophosphorus insecticides had been investigated by O'Brien (1960). He demonstrated that the toxicity of phosphorothioate insecticides depends on metabolic conversion into their corresponding oxygen analogues.

Oxon malathion was more toxic inside fish because this compound was converted to maloxon. Thus mortality mainly occurred due to accumulation of neurotransmitter

substance (acetylcholine). Since the active site of hydrolyzing enzyme (acetyl-cholinesterase) of the nerve cells is phosphorylated by dimethyl or methyl phosphate. This means that acetylcholinestrase (AchE) inhibitor probably causes death by blocking neurotransmission in respiratory center of brain or neuro- muscular function of respiratory.

From Table (1) and Fig. (1) the results indicated that there was no difference between the observed and calculated mortality values.

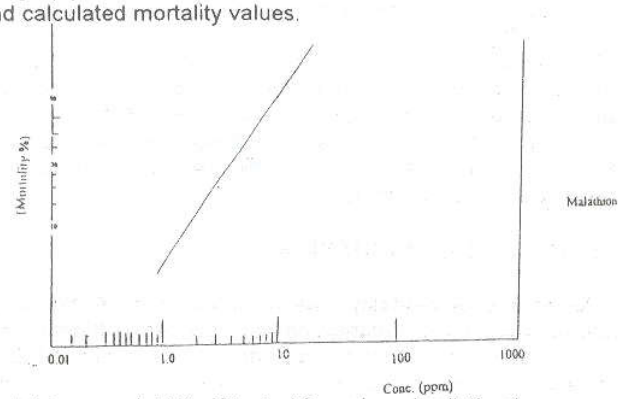

Figure 1. Toxicity of malathion aganist Nile Tilapia (*Oreochromis niloticus*)

Blood Parameters

Table (2) summarizes the examination of organophosphorus insecticide "malathion" both at sublethal for acute and chronic levels as a response on hematological parameters of Nile Tilapia fish (*Oreochromis niloticus*) that in the blood picture.

Noticeable differences were observed in the hematological parameters of exposed *O. niloticus*. In control fish the total RBC count was ($3.60 \pm 0.11 \times 10^6/mm^3$).

The total RBC count showed a significant ($P< 0.05$) decreasing trend with increasing exposure time and concentration of malathion reported for indian cat fish (Heteropneu fossillis) exposed to near 1/10 LC_{50} of malathion for 24, 48 and 72 hrs. (Dutta *et al.*, 1992). The reduction in RBC number may be due to microcytic or normocytic anemia (Tuschiya 1979). In 30 days exposure the WBC count increased ($7.16 \pm 0.05 \times 10^3/mm^3$) with low concentration as compared with control ($5.80 \pm 0.19 \times 10^3/mm^3$), whereas, in the high concentration of malathion there was a slight increase ($7.51 \pm 0.05 \times 10^3/mm^3$) in WBC count. The increase of WBC count may be attributed to the response of fish to malathion exposure where malathion may work as an antigen. The haemoglobin concentration in control was 7.51 ± 0.42 g/100 ml which decreased to 7.31 ±0.05, 7.14 ± 0.05 and 6.89 ± 0.09 g/100 ml during 48 hr. exposure for 0.64, 1.26 and 2.53 mg/ml concentration of malathion, respectively. This decreasing trend continued after 96 hr. 15, and 30 days. A similar decrease in the haemoglobin content of blood was noted in Nile tilapia exposed to fenitrothion by Koundinya and Rama-murthi (1979). In the present study the haemoglobin concentration showed a decreasing trend with increasing concentration of malathion and exposure time.

Table2. Effect of exposure time to different concentrations of malathion on hematological changes in Nile tilapia (Oreochromis niloticus).

Exposure time (hrs or day)	Pesticicde (mg/L)	Total RBC count ($x10^6/mm^3$)	Total WBC count ($x\ 10^3/mm^3$)	Haemoglobin mg/100ml	Haematocrit % (Ht)	Mean cell volumes (MCV)	Mean cell Haemoglobin (McH)	Mean cell Haemoglobin Concentration (McHc)
0 time (control)	0	3.60±0.11	5.80±0.19	7.51±0.42	26.80±0.18	74.55±2.27	20.90±1.71	28.01±1.65
A	0.64	3.25±0.09	6.18±0.05	7.31^{ab} ±0.05	26.11±0.06	80.31±2.003	22.47±0.49	27.97^{a} ±0.18
48 B	1.26	3.05±0.07	6.73±0.08	7.14^{b} ±0.05	25.996±0.08	85.37±1.88	23.45^{b} ±0.60	27.47^{b} ±0.18
C	2.53	2.95±0.06	7.02±0.05	6.89±0.09	25.58±0.16	86.90±2.01	23.39^{a} ±0.29	26.92^{b} ±0.39
A	0.64	3.02±0.06	6.61±0.13	7.17^{b} ±0.06	25.79±0.02	85.48±2.22	23.76^{a} ±0.60	27.80^{a} ±0.24
96 B	1.26	2.84±0.03	6.93±0.09	6.97^{b} ±0.09	25.54±0.10	89.98±0.52	24.56^{a} ±0.26	27.29^{a}±0.31
C	2.53	2.74±0.05	7.23^{b}±0.08	6.61±0.07	25.38±0.08	92.82±1.81	24.18^{a} ±0.28	26.05^{b}±0.29
A	0.64	2.85±0.07	6.79±0.09	6.22±0.11	25.38±0.08	89.10±2.02	21.83^{Cb} ±0.61	24.50±0.35
15 DAYS B	1.26	2.61±0.09	7.14±0.08	5.86^{c} ±0.09	25.12±0.14	96.54±3.62	22.52^{b} ±0.72	23.34±0.29
C	2.53	2.49±0.07	7.34^{b} ±0.06	5.36±0.08	24.30±0.09	97.50±2.46	21.51^{b} ±0.39	22.07±0.28
A	0.64	2.48±0.05	7.16±0.05	5.97±0.13	24.21±0.08	97.54±2.11	24.06^{a} ±0.82	24.66±0.34
30 DAYS B	1.26	2.31±0.08	7.28±0.06	5.66^{c} ±0.16	23.78±0.09	103.68±2.41	24.66^{a} ±0.56	23.80±0.41
C	2.53	2.15±0.05	7.51±0.05	4.45±0.06	22.03±0.10	102.83±2.04	20.68^{b} ±0.50	20.20±0.35

A, B, and C: 1/6, 1/3, and 2/3 LC_{50} Numbers have same letter not significant different $P < 0.05$ (Mean ± SD) (N = 8)

These findings on (*Oreochromis niloticus*) are in partial agreement with the results of other researchers (Dalela *et al.*, 1981; Mishra and Srivastava 1983; El-Domiaty 1987, Gill *et al.*, 1991, and Dutta *et al.*, 1992). This study showed the decrease in haematocrit values in malathion-treated fish for all exposure both acute and chronic. These decreased values may be attributed to disequilibrium of osmotic pressure inside and outside the cells (Hanke 1980). McV significantly ($P < 0.05$) increased with increasing exposure time, which may be caused by a reduction of RBC count, plasma volume and red cell swelling.

A similar decrease in haematocrit values in sumithion, treated fish (*Cyprinus carpio*) has been reported by Tamura *et al.* (1964). However, Yasuda (1965) and Ishihara *et al.* (1967) found that sumithion caused an increase in haematocrit values in carp. Marked red cell swelling is present (Milligan and Wood, 1982).

Table (2) shows the mean of a decreasing trend of cell haemoglobin concentration (McHc) with increasing concentration of malathion and exposure time. The (McHc) was 20.20 ± 0.35 after 30 days of exposure in the case of high concentration of malathion as compared with control 28.01 ± 1.65.

The lowered erythrocyte counts (RBC), haemoglobin concentration and elevated mean cell haemoglobin concentration (McHc), indicate that the insecticides exerted an effect similar to that of anaemia. Similar results have been reported by Eisler (1967) for fishes exposed to methyl parathion.

REFERENCES

Aness, A.A., 1975. Acute toxicity of from organophosphorous insecticides to a freshwater teleost chana punctatus (Bloch). Pak J. Zool 7: 135-141.

Bailey, N.T.J., 1965. "Statistical Methods in Biology" English Language Book Society, Great Britain.

Barham, W.T., G.L. Smith, and H. Schoonbee, 1979. The effect of heparin concentration on certain blood parameters of the rainbow trout samlo gairdneri Richardson. Comp. Biochem. Physiol. 63: 369-371.

Bresch, H.,1971. Investigation of long- term action of xenobiotics on fish with special regard to reproduction. Ecotoxicol. Environ. Safety, 6: 102-112.

Coppage, D.L. and T.E. Braidech, 1976. River pollution by anticholinesterase agents, Water Res. 10:19.

Dacie, J.V. and S.M. Lewis, 1975. Practical Haematology 5th edn. London. J & A Churchill.

Dalela, R.C., S. Rani, V. Kumar and S.R. Verma, 1981. Effect of *in vivo* haematological alterations in freshwater teleost, *Mystus vittatus* following subacute exposure to pesticide and their combinations. J. Environ Biol 2: 76-86.

Dutta, H.M., J.V.V. Dogra, N.K. Singh, P.K. Roy, SS.T. Nasar, S. Adhikari, J.S.D. Munshi and C. Richmonds, 1992. Malathion induced changes in the serum proteins and Hematological parameters of an Indian Catifish Heteropneustes fossilis (Bloch). Bull. Environ. Conta. Toxicol. 49: 91-97.

Eisler, R., 1967. Tissue changes in puffers exposed to methoxychlor and methyl parathion. U.S. Sport. Fish. Wildl. Tech. Pap. 17. P. 1-15.

El-Domiaty, N.A.,1987. Stress response of Juvenile elarias larva elecited by copper. Comp. Biochem. Physiol 88C: P 259-262.

Finney, D.J. , 1971. Probit analysis PP 333. Combridge Univ. Press.

Gill T.S., H. Tewari and J.C. Pant, 1991. Effect of water-copper and Lead on the peripheral blood in rosy bard, Barbus (Puntius) conchonius Hamilton. Bull. Environ. Contan Toxicol 46: P. 606-612.

Hesser, E.F., 1960. Methods for routine fish haematology. Progve. Fish. Cult. 22: 164-171.

Ishihara, T., M. Yasuda and O. Tamura, 1967. Effects of pesticides on blood characters and oxygen consumption of carp (Japanese). Bull. Fac. Fish. Nagasaki, Univ. 24: P. 65-78.

Hank, W., 1980. Fishes as test parameters for environmental chemicals. Karlsruher Beitraege zur Zoophysiologie. Heft 5.

Johnson, D.W., 1968. Pesticides and fishes. Areview of selected literature. Trans. Amer. Fish. Sco. 97, P. 398.

Katz, M., R.S. Legore, K. Weitkamp, J.M. Cumin, D. Andersonand D.R. May, 1972. Water pollution: Effects on freshwater fish. J. Water. Pollut. Cont. Fed. 44:1226-1250.

Koundinya, P.R. and R. Ramamurlhi, 1979. Haematological studies in sarotherodon (Tilapia) mossambica (peters) exposed to lethal (LC_{50} 48hr. concentrations of sumithion and sevin). Curr. Sci. India 48: 877.

Kumar, K. and B.A. Ansari, 1984. Malathion toxicity skeletal deformities in zebra fish (Brachydanio rerrio, cyprinidae). Pesti. Sci. 15: 107-111.

Mawdesley, L. and E. Thomas, 1971. Toxic chemicals: The risk to fish, New Scient. 49:74.

Milligan, C.L. and C. M. Wook, 1982. Disturbances in haematology, fluid valume distribution and circulatory function associated with low environmental pH in the rainbow trout, salmo gairdneri. J. exp. Biol. 99, P. 397-415.

Mishra, J. and A.K. Srivastava, 1983. Malathion induced haematological and biochemical changes in the Indian catfish *Heteropensutes fossilis*. Enviorn Res. 30: 399-411.

O'Brien, R.D., 1960. Toxic phosphorus esters chemistry. Metabolism and biological effects. Academic press. New York an London 1st Edition, 166.

Pimentel, D., 1971. Ecological effects of pesticides on Non-target species. V.S. Government printing office, Wahington, 220 P.

Snieszko, S.F., 1961. Microhematocrit values in rainbow troct, brown trout, and brook trout. Prog. Fish. Cult. 23: 114.

Soivivo, A., K. Westman and K. Nyholm, 1974. Changes in haematocrit values in blood samples treated with and without oxygen: a comparative study with four salmonid species. J. Fish Biol. 6, 763-769.

Sprague, J.B., 1971. Measurement of pollutant toxicity to fish. Water Res. :145-166.

Tamura O., M. Kitadi and S. Maeda, 1964. On the influnce of some agricultural chemical etc. on the haematological chracters of fish. Bull. Fac. Fish. Nagasaki, Univ. 17. P. 87-96.

Tuschiya, K., 1979. Lead in Friberg L, Nordberg GF, Vouk VB (eds) Handbook on the toxicity of metals. Elsevier/ North Holland Biomedical Press, Amsterdam.

Yasuda, T., 1965. Physiological and pathological effects of agricultural chemicals on a quatic organisms (Japanese). Bull. Fab. Soc. Sci. Fish. 31: 715.

السمية الحادة وبعض التأثيرات فى الدم التى يحدثها تعرض أسماك البلطى النيلى *(Oreochromis niloticus)* للملاثيون

محمد الدناصورى و ليلى رضا

جامعة قناة السويس ـ كلية الزراعة ـ الإسماعيلية

تمت دراسة أثر السميه الحادة بالملاثيون على أسماك البلطى النيلى وكذا درس تأثير التعرض للمبيد على بعض قياسات الدم لهذه الأسماك.

و أوضحت الدراسة أن تركيز الملاثيون المميت لـ ٥٠٪ من الأسماك هو ٣,٨ جزء فى المليون.

وتعريض الأسماك الناضجة لمستويات حادة ومزمنة تتراوح ما بين ٦/١ إلى ٣/٢ الجرعة المميتة لـ٥٠٪ من الأسماك المعاملة لمدة ٢٤، ٤٨، ٩٦ ساعة، ١٥ و ٣٠ يوم قد أدت إلى تغير صورة الدم للأسماك المعاملة بالملاثيون.

وظهرت التأثيرات المعنوية فى بعض قياسات الدم مثل نقص عدد كرات الدم الحمراء- الهيموجلوبين- مكداس الدم- متوسط هيموجلوبين خلية الدم نتيجة للمعاملة بالملاثيون.

من جهة أخرى لوحظ زيادة معنوية فى العدد الكلى لكرات الدم البيضاء- متوسط حجم الخلية متوسط تركيز هيموجلوبين الخلية ونوقشت الأسباب المحتملة لهذه التغيرات.