PATHOLOGICAL AND TOXICOLOGICAL STUDIES ON MALATHION TO DETERMINE THE OPTIMUM DOSE LEVEL USED TO ERADICATE *LETHOCERUS NILOTICUM* INSECT PREADATING FISH

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ABSTRACT: Malathion is an organophosphate insecticide and acaricide that has been widely used in agriculture practice. Male *Oreochromis niloticus* fish were exposed to different dose levels of malathion for estimation of LC_{50}. The water bugs *(Lethocerus niloticum)* were exposed to the same dose levels for recording the mortality percentage. From the obtained mortality data in both fish and bugs ,the average dose level of malathion that causing death in water bugs without any lethality in fish. Was estimated Another group of *Oreochromis niloticus* fish and water bugs (laboratory introduced to the glass aquaria) were exposed to the average dose level of malathion for 24 & 96-hours. The toxic and pathological effects of such dose level on fish were investigated. Gonadosomatic index, some serum hormones (testosterone, T_3 and T_4) and enzymatic activities in brain tissue (AChE, LDH) were also investigated . The obtained LC_{50} of malathion was 0.70 mg/L while the average dose level causing mortality in water bugs without any lethal effect on fish was 0.26 mg/L. The results revealed significant decrease in gonadosomatic index in the group of fish exposed to the average dose level of malathion for 96-hours. There were marked significant reduction in the serum hormones after the same period of the exposure and inhibition of the enzymatic activities of brain tissue of intoxicated fish, which was obvious at 96-hours for both enzymes (AchE - LDH). histopathological findings, which revealed the presence of vacuolar degenerative changes of the hepatocytes and severe congestion of the hepatoportal vessels. The histopathological changes in spleen include lymphocytic depletion, melanomacrophage center devoid of melanin and activation of haemopoietic elements. Gills showed congestion of the lamellar capillary, oedema and eosinophillic granular cell infiltration. The hyperplasia and fusion of secondary lamellae were also noticed. The histopathological alterations in testis revealed degeneration of germinal epithelium and absence of spermatozoa in the

seminiferous tubule lumenae. Brain of intoxicated fish showed focal gliosis and oedema while thyroid gland showed absence of colloid in the lumenae of some follicles. Statistical analysis of the obtained data denotes that the average dose level of malathion is effective for eradication of water bugs without any adverse effect on fish when applied only for 24-hours.

INTRODUCTION

It is now well known that large amounts of insecticides are used for control operations. Drift from these treatments and accidental over-spraying may contaminate small surface water such as temporary ponds **(Lahr et al., 2008)**. Deliberate or accidental contamination of ponds by widely utilized organophosphorous insecticides, such as malathion, is a potential problem for agriculture in tropical countries **(Pathiratne and George, 2007)**. Malathion is an organophosphate insecticide and acaricide that has been widely used on raw agricultural products including edible grains, fruits, nuts, forage crops, cotton and to control harmful insects **(NIOSH, 1976)**. Malathion seems to be more toxic to insects and fish than to mammals due to the lack of hydrolytic enzymes in insects and fish **(Krueger et al., 1960)**. Several investigations have been made to explore the effects of pesticides on some aspects of the thyroid physiology of fish **(Leatherland and Sonstegard, 1978 and Singh and Singh, 1980)**. On the other hand malathion apparently reduce the thyroid activity **(Yadav and Singh, 1986)**. Malathion acts as a neurotoxin due to its ability to block neurotransmission by inhibiting the enzyme acetylcholinesterase **(O'Brien, 1960)**. Other changes as hematological changes **(Mishra and Srivastava, 1983)** were also reported.

The changes in the enzyme activities of LDH, GIDH and GOT have been used to demonstrate the extent of tissue damage in fish **(Asztalos and Nemcsok, 1985)**. The magnitude of the increase in LDH in the blood sera shows the degree of tissue damage and the LDH isoenzyme pattern reflects which tissue was damaged **(Herbert et al., 1970 and Asztalos and Nemcsok, 1985)**.

Several histopathological studies were performed on the effect of pesticides upon the tissues of aquatic organisms. **Walsh and Ribelin (1975)** revealed that malathion causes necrosis of livers; damage to gill architecture and permeability **(Reddy, 1988)** and slight changes in testis

and adrenal glands without histopathological changes in ovaries and thyroid glands **(Ozmen and Akay, 1993).**

Lahr et al., (2008) used malathion insecticide to control the two abundant organisms that occur in temporary ponds in the African Sahel region, the fairy shrimp *Streptocephalus sudanicus* and the backswimmer, Anisops sardeus. They reported that malathion had slight toxic effect on the two organisms. **Easa and Abu-El-Wafa (1993)** applied malathion a concentration of 0.02 ppm for two times with 15 days interval to eradicate the Caligus curtus parasite, which infests marine fishes (Mugilidae and Sparidae) reared in fish farms in Egypt.

Lethocerus species are large or giant water bugs commonly found in ponds and slowly moving freshwater. *Lethocerus americanus* known as the "toe biter" for its encounters with swimmers. It has the reputation of inflicting painful bites when carelessly handled. They normally feed on other pond life: insects, tadpoles, salamanders, small fish and snails. These large tan or brown bugs may leave their habitat at night in search of mates or another pond **(Huntley, 1998).** A new species of the Kinetoplastid flagellates was isolated from hindgut of the giant water bugs, Lethocerus indicus. So the giant water bugs act as a host for parasites of fish **(De et al., 1995).** Other species of water bugs *(Lethocerus medius)* has been used to isolate the apolipophorin-III, that isolated from hemolymph of *L. medius* had a $M(r) = 19.000$ and an amino composition high in methionine, in comparison with other apolipophorin-IIIs **(Kanost et al., 1995).** In Egypt, the available literature indicated that, some *Lethocerus* species are available. **Sorour (2008)** studied the ultrastructural variations in *Lethocerus niloticum (Insecta: Hemiptera)* caused by pollution in lake Mariut(Alexandria, Egypt). *Lethocerus niloticum* in this study believed to be used as a biomonitor for some pollutants. On the other hand, the pathogenicity of these insects to native fish breeds in Egypt was not carefully studied.

This study was undertaken to evaluate the toxic effects of malathion (one of the widely used pesticides among Egyptian agriculture practices) at the dose level that induced mortality in water bugs without any expected lethality on *Oreochromis niloticus* fish. In the same times studying the effect of this level on gonadosomatic index, level of testosterone and the thyroid hormones, as well as the changes in the activities of brain tissue acetylcholinesterase (AChE) and lactate dehydrogenase (LDH). Histopathological alterations in the affected

organs (liver, spleen, gills, testis, brain and thyroid gland) were also considered and recorded at this dose level.

MATERIALS AND METHODS

Experimental Designe:

One hundred and thirty *Tilapia nilotica* fish *(Oreochromis niloticus)* weighing 85 ± 2 gm and measured 15 ± 3 cm total length, purchased from a local commercial producer [Fouky Farm, an intensive fish farm in Kalyobiya governorate] were used for this study. Seventy water bugs *(Lethocerus niloticum) (Insecta : Hemiptera)* were also used.

Experimental fish were distributed in glass aquaria [30 X 40 X 80 cm]. Each of which contains 50 liter of dechlorinated water at constant temperature of 27 ± 2 °C with oxygen source achieved by air pumps for continuous aeration. In addition, a 12-hours photoperiod is maintained. Fish were fed during the experimental period once daily on a commercial dry pellet ration. Fish were acclimatized to the laboratory conditions for 2 weeks before the beginning of each experiment. Water bugs were purchased from a local commercial specific experimental animal house producer, and were distributed in similar glass aquaria . These aquaria have the same conditions as previously mentioned in fish. Water bugs were acclimatized to the laboratory conditions for 2 weeks before the beginning of experiment and were fed on fish offales during this period.

Tested Compound:

Malathion, insecticide [diethyldimethoxy thiophosphoryl thiosuccinate], was used in this experiment. It was used in its commercial grade consisting of 57 % malathion and 43 % organic and inert solvents.

Experiments:

Pilot Experiment:

A pilot study was made on five groups of fish. Each group contained ten fish, in order to select the dose ranges needed for the determination of the LC_{50} / 96 h **(Ghosh, 1971).**

Determination of Acute Lethal Concentration Dose (LC_{50}):

The following experiment was carried out to determine the 96-hours median lethal concentration (LC_{50}) of malathion for *Oreochromis*

niloticus fish. For this purpose, 60 fish were used, and divided into six equal groups. One group was served as a control while the other five groups were used for malathion exposure. The LC_{50} of the tested pesticides was determined according to **Doudoroff et al., (1951).**

Determination of Malathion Dose Levels Causing Mortality in Water Bugs:

This experiment was performed to determine the dose level of malathion that induced mortality in water bugs without any lethal effects on fish. For achieving this goal, the same dose levels of malathion were used in this experiment. Sixty water bugs were used and divided into six equal groups. One group was served as control, while the other five groups were exposed to the different dose levels of malathion [0.08, 0.17, 0.35, 0.70 and 1.40 mg/L] for 96 hours.

Assay of the Adverse Effects of the Suggested Average Dose Level of Malathion on Fish:

This assay was used to screen the toxicity of the suggested average dose level of malathion [24-hours and 96-hours exposure periods] on the health and the pathological conditions of *Oreochromis niloticus* fish. Twenty fish and ten water bugs were used in this experiment. They were classified into two groups; the first one contained ten fish only and served as a control, while the second group contained 10 fish and 10 insects. Close observation was performed during the whole period of experiment in order to record the mortality in both fish and insects. After 24 and 96 hours, 5 fish were taken from each group (control and treated groups) for the determination of the following parameters:

The Gonadosomatic Index:

The gonadosomatic index was determined according to the method given by **Munkittrick and Dixon (1988).**

The Hormonal Changes [Testosterone, Triiodothyronine (T_3), Thyroxin (T_4)]::

Testosterone concentration in serum was determined by radioimmunoassay (RIA) according to the method of **Tremblay et al., (1972).** On the other hand, T_3 and T_4 were determined according to the method of **Brown and Eales, (1977).**

The Enzymatic Activities in Brain [Cholinesterase and Lactate Dehydrogenase]:Cholinesterase activity in brain was determined

according to **Szasa (1968);** while the LDH activity was determined according to the method of **Annon (1971).**

The Pathological Changes:

Live fish were collected from the experimental groups treated with the suggested dose level of malathion after 24 hours and 96 hours exposure periods, to detect the safety of this dose level on the fish tissues. Thus, fish were subjected to post-mortem examination and any lesion seen was recorded. For the histopathological study, samples from liver, spleen, gills, testis, brain and thyroid were fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin. About 4u-thick sections were prepared and stained with Hematoxlene and Eosine **(Carleton et al., 1967).**

Statistics:

Student's t-test was used to compare the means of control and treated groups according to **Snedecor (1971).**

RESULTS AND DISCUSSION

Pilot Experiment:

The mortality data of fish used in the pilot experiment were recorded 96-hours after exposure to successive dosage levels of malathion. These results are recorded and tabulated in Table (1), from which it was indicated that the approximate LC_{50} for malathion lies at the dose level 0.70 mg/ l

Table (1): Mortality data in *Oreochromis niloticus* fish exposed to malathion in pilot experiment:

Group No.	Fish/group	Dose **mg/L**	Dead fish	Survived fish	Mortality %
1 st	10	0.35	1	9	10
2 nd	10	0.70	5	5	50
3 rd	10	1.40	7	3	70
4 th	10	2.80	10	0	100
5 th	10	5.60	10	0	100

Acute Lethal Concentration Dose (LC$_{50}$ /96 hr) For Fish Exposed to Malathion:

Confirmatory experiment was carried out to estimate the LC$_{50}$ /96 h of malathion for *Oreochromis niloticus* fish. Results obtained and recorded in Table (2) indicated that the LC$_{50}$ was found to be equal to 0.70 mg/L.

Table (2) : Mortality data of *Oreochromis niloticus* fish exposed to different dose levels of malathion:

Fish Group	Fish/group	Dose mg/L	Dead fish	Mortality %
1 st	10	0.08	0	0
2 nd	10	0.17	0	0
3 rd	10	0.35	1	10
4 th	10	0.70	5	50
5 th	10	1.40	6	60

Determination of Malathion Dose Levels Causing Mortality Percentage in Water Bugs:

The mortality percentage of water bugs exposed to different dose levels of malathion was determined and the obtained data were recorded in Table (3).The mortalities among water bugs exposed to different dose levels of malathion were occurred within 24-h from the beginning of exposure. The dose level of malathion which caused mortalities in water bugs and may be had no lethal effect on *Oreochromis niloticus* fish lies between the dose levels of 0.17 and 0.35 mg/L as seen in the same Table.

From these data, it was suggested that the average dose level could be calculated as follows:

The average dose level =(0.17+ 0.35) ÷ 2 = 0.26 mg/L.

Table (3): Mortality data of water bugs exposed to different dose levels of malathion:

Water bug Groups	Water bugs /group	Dose mg/L	No. of dead water bugs	Mortality Percentage
1 st	10	0.08	0	0
2 nd	10	0.17	0	0
3 rd	10	0.35	8	80
4 th	10	0.70	10	100
5 th	10	1.40	10	100

The Adverse Effects of The Average Dose Level (0.26mg/L) On *Oreochromis niloticus* Fish:

Mortalities:

The average dose level had no lethal effect on fish but caused death in 7 water bugs from the total number of 10 insects i.e. (70 %) mortality.

Gonadosomatic index:

The effect of the average dose level on gonadosomatic index was determined and recorded in Table (4). A non-significant decrease in the gonadosomatic index after 24-h (0.013 ± 0.0004) was scared in relation to the control group (0.014 ± 0.0002). On contrary, the same index showed significant decrease (0.012 ± 0.001) at $p < 0.05$ after 96-h compared with control group.

Hormonal Changes in Serum:

Concerning the effect of the average dose level on the testosterone and thyroid hormones, Table (4) showed significant decrease in testosterone level (79.2 ± 1.68) at $p < 0.05$ after 96-h exposure period compared with the control group (81.4 ± 1.97). A similar trend was noticed with respect to the thyroid hormones levels (T_4 & T_3); i.e. significant decrease (62.76 ± 1.08 and 1.82 ± 0.025, respectively) at $p < 0.05$ and $p < 0.01$ after the same period of exposure, in comparison with the control group (64.62 ± 0.49 and 2.30 ± 0.02, respectively). Moreover, the levels of hormones showed non-significant decrease during 24-h exposure to malathion.

Table (4): Effect of average dose level of malathion on gonadosomatic index, hormonal changes and enzymatic activities of *Oreochromis niloticus* fish:

Group	Time	Gonad somatic Index			Hormonal Changes			Enzymatic Changes	
		Body weight (g)	Testis weight (g)	Index	Testoster one (ng/dl)	T_3 (ng/dl)	T_4 (ng/dl)	AChE (u/l)	LDH (u/l)
Control	24-h	85 ± 0.70	1.15 ± 0.010	0.014 ± 0.0002	81.4 ± 1.97	65.52 ± 0.15	2.3 ± 0.02	68 ± 0.31	61.2 ± 1.24
Control	96-h	84.6 ± 0.68	1.14 ± 0.005	0.014 ± 0.0002	81.4 ± 1.97	64.62 ± 0.49	2.3 ± 0.02	68 ± 0.31	61.2 ± 1.24
Treated (0.26mg/L)	24-h	84 ± 0.31	1.12 ± 0.02	0.013 ± 0.0004	81 ± 0.97	63 ± 1.12	2.28 ± 0.03	65.8 ± 1.59	59.8 ± 0.97
Treated (0.26mg/L)	96-h	81** ± 1.05	0.92* ± 0.08	0.012* ± 0.001	79.2* ± 1.68	62.76* ± 1.08	1.82** ± 0.025	64* ± 1.41	59.6* ± 0.98

No. of fish / group = 5 Probability level : * significant at p < 0.05 ** significant at p < 0.01
Values indicate mean ± S.E

Enzymatic Changes in Brain:

The present study revealed that the average dose level of malathion (0.26 mg/L) to *Oreochromis niloticus* fish induced significant inhibition in the activities of acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) in the brain tissue at 96-h where the values being $64.0 = 1.41$ and 59.6 ± 0.89 u/L in comparison to the control values, 68.0 ± 0.31 and 61.2 ± 1.24 u/l

AChE and LDH, respectively (Table, 4). In the same time, the results denoted non-significant changes in both AChE and LDH activities of brain tissue at 24-h exposure period.

3-4-5- The Histopathological Changes:

3-4-5-1-Histopathological changes of malathion-exposed fish (0.26 mg/L for 24-h):

Histopathological examination of fish in this group revealed non-significant alterations than normal in the most of the examined organs especially testis and thyroid appeared normal (Fig. 1,2).

3-4-5-2-Histopathological changes of malathion-exposed fish (0.26 mg/L for 96-h):

The gross examination of fish exposed to the average dose level (0.26 mg malathion/ L) for 96-h; revealed abnormal black discoloration of the body, severely congested gills and rough scales.

The histopathological examination of such group showed the following changes:

In liver, vacuolar degeneration of the hepatocytes and severe congestion of hepatoportal blood vessels were observed (Fig. 3). Mononuclear cell infiltration was noticed in portal area and around the hepatoportal blood vessels. In such cases, mild degenerative changes were seen in hepatopancreatic region. In other cases, histopathological examination showed mild pathological alterations including congestion of the hepatic blood vessels and granular degeneration of some hepatocytes.

The examination of spleen revealed depletion of lymphocytes with decreased melanin intensity of melanomacrophage centers (Fig. 4). In other cases, splenic ellepsoids showed marked thickening of their capillary wall, swelling of the endothelial lining with activation of the haematopoietic cells around the blood vessels of the ellepsoids (Fig. 5).

The examined gills revealed severe congestion of the lamellar capillaries (Fig. 6), while in other fish, gill oedema and aggregation of eosinophillic granular cells (EGC) was the common picture (Fig. 7). In other cases, hyperplasia of the cells constituting gill lamellae together with fusion of secondary lamellae was a common lesion observed (Fig. 8).

The examined testis of this group showed degeneration and atrophy of the germinal epithelium of seminiferous tubules and a few spermatozoa in their lumen (Fig. 9).

The brain of the fish in this group showed focal gliosis with oedema (Fig. 10). Thyroid gland examination revealed devoidence of some follicles from colloid (Fig. 11), but without evidence of cellular hyperplasia.

Pesticides represent the most environmental pollutants all over the world. Serious problems were created due to the application of pesticides either for mammals or aquaculture. The presence of pesticides in the environment is of potential toxicological concern not only for fish (**Jackson, 1976**) but also for human health.

The present results in this study revealed that the 96-h LC_{50} of malathion was 0.70 mg/L . Several investigations were carried on fish to determine the LC_{50} of malathion in aquatic organisms. **Shim and Self (2004)** reported 96-hours LC_{50} values for the herbivorous fish to be from 0.14 to 9.7 mg/L for different organophosphorous compounds including malathion. **Shukla et al., (2002)** determined the LC_{50} of malathion for the fingerlings of *Channa punctatus and found* it was in the range of 10.95to3.22 mg/L for 24to96 hours exposure periods. **Jagan et al., (1989)** recorded the LC_{50} of malathion for carp fish *(Cyprinus carpio)* to be 0.138 ppm and the corresponding LC_{90} figure was 0.53 ppm. **Tsuda et al. (1997)** determined the 48-h LC_{50} for malathion in Killfish to be 1.8 mg/L. Variation between the obtained results concerning the estimation of LC_{50} of malathion and those of other investigators may be attributed to several factors such as species, age, body weight, time of exposure, the method used and the sensitivity of fish as well as stress facttors. These suggestions were in agreement with those mentioned by Hoekstra et al., **(1994)** who recorded that the LC_{50} originate from toxicity tests was differed not only with the test species, but also with the experimental conditions which may affect the bioavailability of the toxicants. **Pathiratne and George (1998)** reported that tilapia were very sensitive to malathion (96-h LC_{50} = 2 ppm) .

The obtained results concerning the reduction of gonadosomatic index of fish exposed to malathion were found to be resembled with the findings obtained by many investigations. **Haider and Upadhyaya (2005)** reported that the commercial formulation of four organophosphorous insecticides birlane, jardona, phosdrin and malathion

caused loss of stage II and III oocytes accompanied by significant decline in gonadosomatic index of fresh water teleost, *Mystus vittatus* (bloch) exposed to malathion. **Shukla et al., (2002)** revealed that the sublethal concentration of malathion had adverse effects on growth and metabolism, and exposure of fingerling *Channa punctatus* fish to 2.5 mg/L malathion induced significant reduction in growth. **Khillare and Wagh (1988)** showed that the fish *Puntius stigma* collected from the river Kham Aurangabad, India showed changes in survival, feeding, growth rate and oxygen consumption during chronic exposure to endosulfan, malathion and sevin (carbaryl). **Ramakrishnan et al., (1997)** exposed the *Oreochromis mossambicus* to the sublethal concentration of malathion (0.3-1.2 ppm). They found significant reduction in feeding and growth rate by 61 % and 73 %, respectively in the greatest concentration of malathion.

Significant reduction in the testosterone level of 96-h malathion exposed fish was also denoted. The significant decrease in the androgenic hormone could be supported by the obtained histopathological findings of the affected testes, which revealed the presence of marked degeneration and atrophy of the germinal epithelium of the seminiferous tubules with a few spermatogonia in their luminae. On the other hand, it was proved that some types of pesticides as organophosphates, pyrethroids, fungicides and herbicides may possess antiandrogenic effect in different livestock. Pesticides can interact competitively with androgen receptors and sex hormones binding globulin, a mechanism by which exposure to pesticides may result in disturbances in endocrine effect related to androgen action **(Brody et al., 1983; Eil and Nisula, 1990 and Wolf et al., 2003).**

Singh and Singh (1987) studied the effect of cythion (organophosphorous) and BHC (organochlorine) on the level of sex hormones (testosterone, estradiol-17 beta and estrone) in catfish *Clarias batrachus*. Sublethal concentrations of either pesticide apparently affected sex hormones production after 4 weeks of exposure, as was evident by the decrease of their levels in blood plasma. They suggested that cythion and BHC toxicity affected sex hormones production due to the impairment in the synthesis and/or release of these steroids. The previous investigations proved that some of the organophosphorous compounds have antiandrogenic effect.

Altering thyroid gland function by organophosphorous insecticides has been recognized in fish by some authors: **Leatherland and**

Sonstegard (1978) and Singh and Singh (1980). In the present investigation, the reduced serum T_3 & T_4 levels in 96-hours exposed fish clearly indicated altered thyroid function. Results obtained within the scope of the study regarding the significant reduction in T_3 & T_4 were in agreement with **Sinha et al., (1991) and Aktar et al., (1996).**

Sinha et al., (1991) concluded that malathion decreased the level of T_3 & T_4 and T_3/T_4 ratio in *Clarias batrachus* fish without altering the level of T_4 in circulation. **Aktar et al., (1996)** exposed rats to malathion and observed that malathion induced significant decrease ($p < 0.01$) in serum concentration of T_3 & T_4. The reduced plasma T_4 level by malathion might be due to the sequential retardation of iodine accumulation and its conversion into hormonal form (i.e. hormone biosynthesis) **(Singh and Singh, 1980). Leatherland and Sonstegard (1978)** observed significant reduced levels of both T_3 & T_4 in *Oncorhynchus kaisutch* fed with an organophosphorous, mirex. They suggested that the extrathyroidal conversion of T_4 to T_3 was inhibited .Such previous trend was parallel with the suggestion that revealed inhibitory effect of malathion on biosynthesis of T_4 and the extrathyroidal conversion of T_4 to T_3 a pattern which also matched with the results obtained within the scope of the study

Inhibition of AChE activity in fish brain after toxic exposure to organophosphorous pesticides is very specific effect, which can be utilized for diagnostic purposes. This inhibition effect was taken as an index of organophosphorous insecticide toxicity **(Chakraborty et al., 1978).** Moreover, inhibition of AChE in fish exposed to organophosphates may serve as an indicator of hazard due to application of these chemicals in the natural environment. The inhibition of AChE was maximal in the brain, muscles and gill **(Nagat Ali, 1995).** The inhibition of AChE activity that recorded within the obtained results was in agreement with **Ansari and Kumar (1984); Johnson and Wallace (1987); Sulaiman et al., (1989); Nemcsok et al., (1990).**

Ansari and Kumar (1984) reported that a 7-day exposure to 0.5-1.1 mg/L malathion significantly inhibited the brain AChE activity in the zebra fish *(Brachydanio rerio)*. **Johnson and Wallace (1987)** recorded that malathion caused inhibition of brain acetylcholinesterase activity in fat head minnows and rainbow trout.**Sulaiman et al.,(1989)** mentioned that acetylcholinesterase activity of the brain was depressed significantly in *Tilapia nilotica* and *Tilapia mossambica* exposed to malathion.

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Nemcsok et al., (1990) studied the *in vivo* effect of 2 ppm of malathion on the carp *(Cyprinus carpio)* brain and liver AChE. They found that the enzyme activity decreased significantly in the investigated tissues.. The kinetic studies of AChE in the brain showed that malathion acted as a mixed inhibitor in mesencephalon and medulla oblongata while it acted as an uncompetitive inhibitor in cerebrum and cerebellum (Panneerselvam **et al., 1996). Das and Sengupta (2003)** reported that the inhibition of AChE of brain was a dose-dependent. **Beauvais et al., (2000)** reported that cholinesterase activity was significantly decreased with increasing the concentration of malathion and significantly differed among exposure durations.

Concerning the result of LDH, it was found that the brain LDH activity was significantly reduced in the treated fish than control group. The result was in agreement with those of **Rani et al., (1990)**. They exposed *Clarias batrachus* fish to malathion at 5 ppm for 96-h. The results indicated reduction in the activity of LDH in liver, gills and brain . They reported that the significant alterations in metabolite content and enzyme activity under malathion toxicity may suggest a transient shift from aerobic to anaerobic metabolism. LDH has a role for interconversion of lactate and pyruvate in the presence of NADH and NADH$_2$. Reduction of LDH activity in brain tissues or other tissues indicated tissue damage to the affected organs with elevation in serum LDH activity **(Murray et al., 1988)**. The brain tissue damage was confirmed by the obtaind histopathological findings since the brain showed focal gliosis and oedema.

Water pollution induces pathological changes in fish. As an indicator of exposure to contaminants, histology represents a useful tool to assess the degree of pollution particularly for sublethal and chronic effects **(Bernet et al.,2009)**. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs **(Bucke et al., 1996)**. Our findings revealed that *Oreochromis niloticus* exposed to malathion resulted in marked and significant histopathological findings in the liver, spleen, gills, tests, brain and thyroid gland of the tested fish .

The histopathological alterations in the liver of treated fish were in agreement with the findings reported by **Anees (1978); Ramalingam (1988) and Al-Hamdanne (1998).**

The study of **Anees (1978)** revealed that malathion caused

vacuolation and necrosis of hepatocytes in channel catfish. **Ramalingam, (1988)** reported that malathion caused comparatively more liver damage of fish *Sarotherodon mossambicus* than mercury and several changes such as necrosis, fatty degeneration and red cell occlusion in portal vessels were also observed. Several histopathological changes in carp fish exposed to malathion were recorded by **Al-Hamdanne (1998)**. These changes include vacuolation of hepatic cells with sinusoidal congestion, kidney congestion, haemorrhage of intestinal submucosal layer and coagulative necrosis of muscles.

Regarding the histopathological changes in the spleen of malathion-treated fish; **Abu-Hadeed (1978)** exposed Nile catfish *(Clarias lazera)* to the organophosphorous insecticide (Curacron). His histopathological results revealed severe congestion and destruction of some lymphoid follicles of spleen. Similar findings were given by **Abou El-Magd et al., (1998)** who observed congestion, moderate depletion of the lymphocytes of the splenic white pulp, thickening of splenic blood vessels, activation of the melanomacrophage centers and infiltration of the red pulp with lymphocytes in Nile catfish treated with glyphosate. The results of the problem at hand were nearly similar to those recorded by **Hussain (1993)** who showed depletion of the lymphocytes in the white pulp of spleen in rats treated with some pesticides.

Marked histopathological lesions in the gills of *Oreochromis niloticus* fish that were exposed to malathion were observed through the study. Several investigators reported similar alterations in the gills of the affected fish. **Walsh and Ribelin (1975)** for instance mentioned that malathion caused lesions in the gills of exposed fish including necrosis, hyperplasia, hypertrophy and oedema. **Reddy (1988)** showed also that sublethal concentration of malathion (2 ppm) damage to the gill architecture in carp fish after 15 days from exposure. He also reported that this damage decreased rates of O_2 consumption rates and opercular movement of fish.

In malathion-treated fish, the histopathological examination of brain revealed focal gliosis and oedema. Closely similar observations were mentioned by **El-Swak et al., (1992); Hussain (1993) and Abou El-Magd et al., (1998). El-Swak et al., (1992)** observed focal gliosis in brain of ametryn (Gespax)-treated common carp. **Hussain (1993) and Abou El-Magd et al., (1998)** reported that the focal gliosis of brain was observed in glyphosate-treated rats and fish, respectively.

The effect of malathion on thyroid gland revealed devoidence of some follicles from colloid without evidence of cellular hyperplasia. **Ram et al., (1989)** described the effect of cythion (malathion) on the histophysiology of the thyroid and thyrotrophs in *Channa punctatus* fish. The pharyngeal thyroid of the treated fish exhibited hypertrophy and hyperplasia of the follicular epithelium and reduction of colloid content. Some of the follicles were exhausted and transformed into degenerated cystic masses. These histopathological changes could be correlated with a significant and dose-dependent reduction of thyroid radioiodine uptake and conversion ratio values.
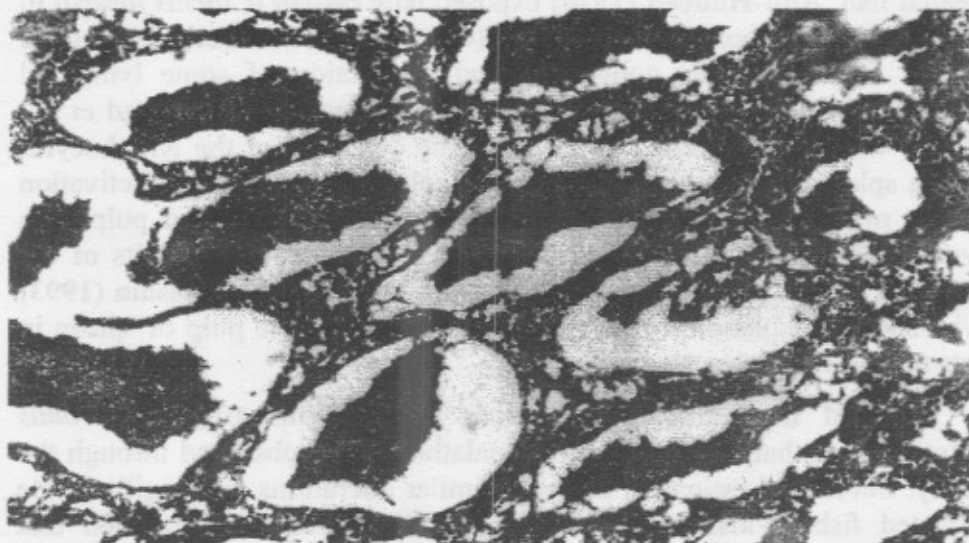
Fig. (1): Testis of Oreochromis niloticus fish exposed to 0.26 mg/L malathion for 24-h, showing normal seminiferous tubules impacted with spermatozoa. [H&E stain, X40]
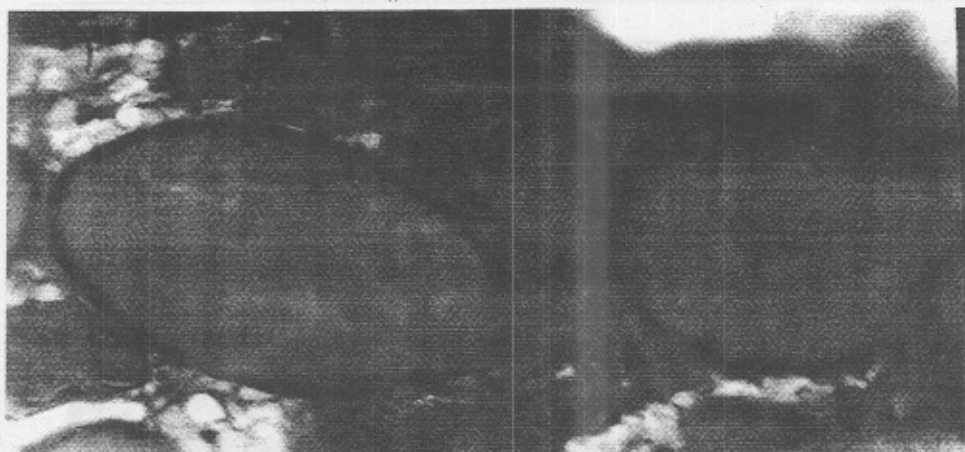
Fig. (2): Thyroid gland of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 24-h, showing normal follicles. [H&E stain, X40]
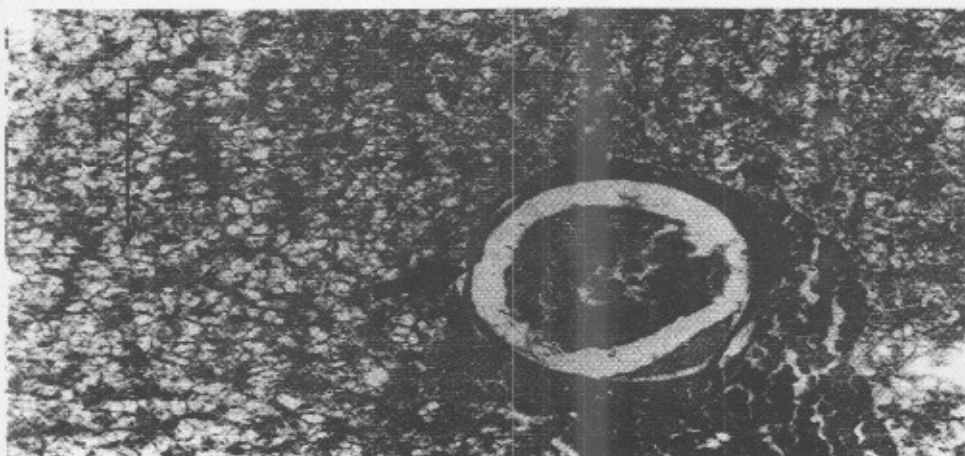
Fig. (3): Liver of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing vacuolar degeneration of the hepatocytes and severe congestion of hepatoportal blood vessels. [H&E stain, X40]
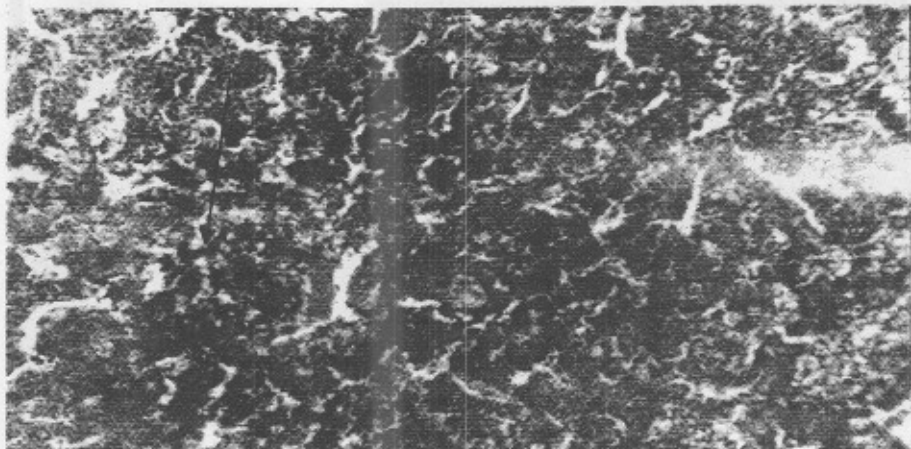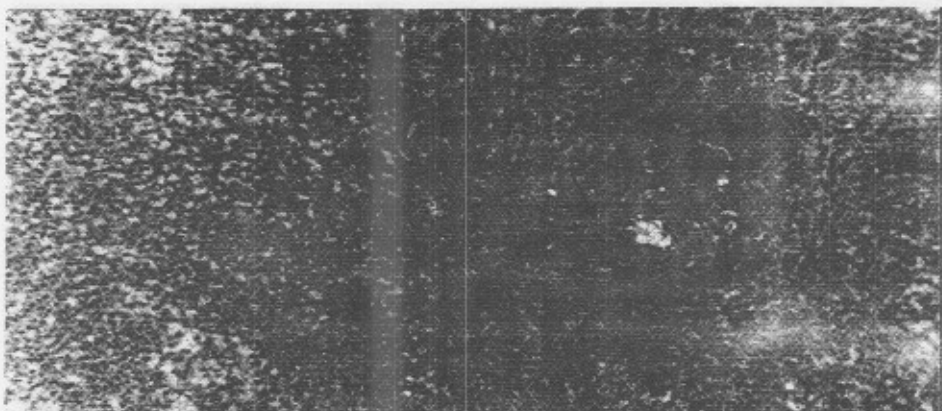
Fig. (4): Spleen of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing lymphocytic depletion and melanomacrophage center devoid of melanin. [H&E stain, X40]

FIG(5): Spleen of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing swelling of endothelial cells of ellipsoidal capillaries thickening of capillary wall and activation of hemopoietic element. [H&E stain, X40]
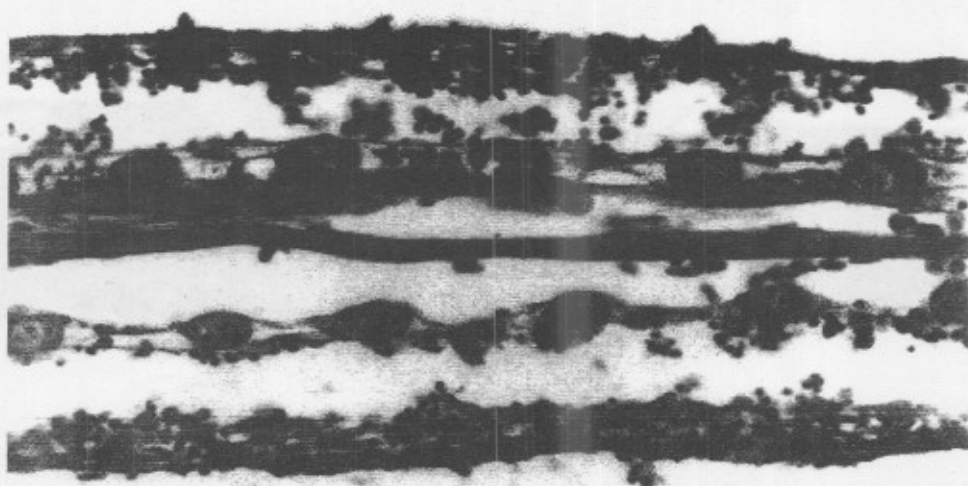
Fig. (6): Gills of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing severe lamellar capillary congestion. [H&E stain, X40]

Fig. (7): Gills of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing oedema and eosinophillic granular cells infiltration. [H&E stain, X40]
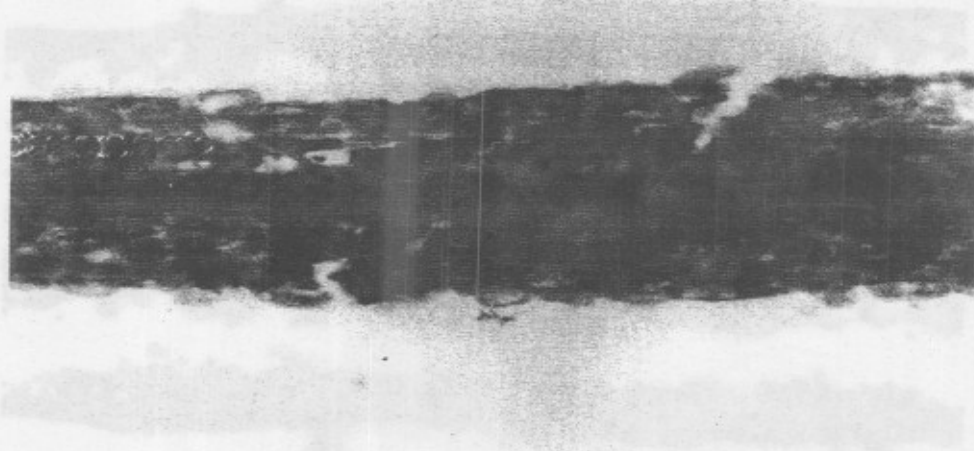
Fig. (8): Gills of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing hyperplasia and fusion of secondary lamellae. Notice: mucous secreting cells activation and eosinophillic granular cell aggregation. [H&E stain, X40]
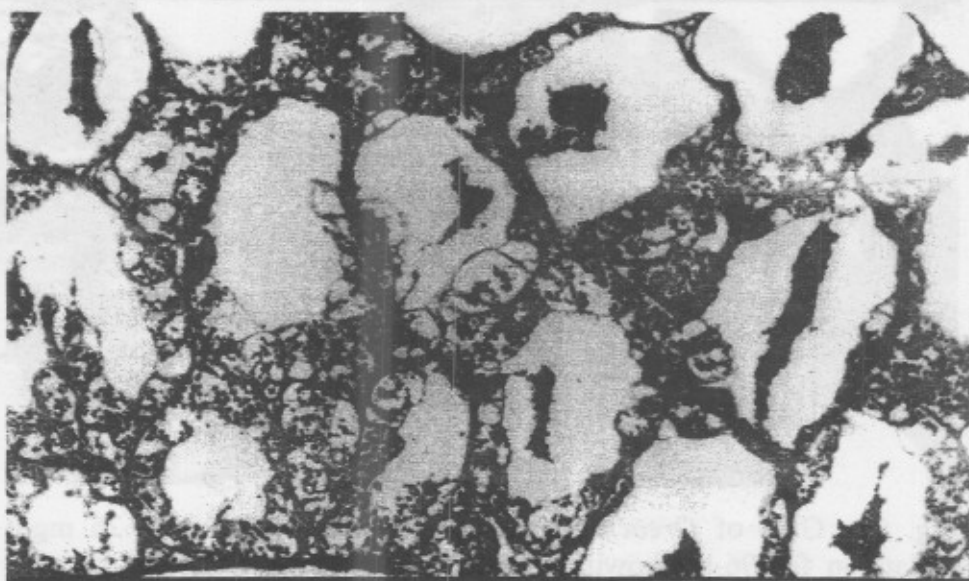

Fig. (9): Testis of Oreochromis niloticus fish exposed to 0.26 mg/L malathion for 96-h, showing degeneration of germinal epithelium. Notice: the seminiferous tubules are devoid of spermatozoa. [H&E stain, X40]
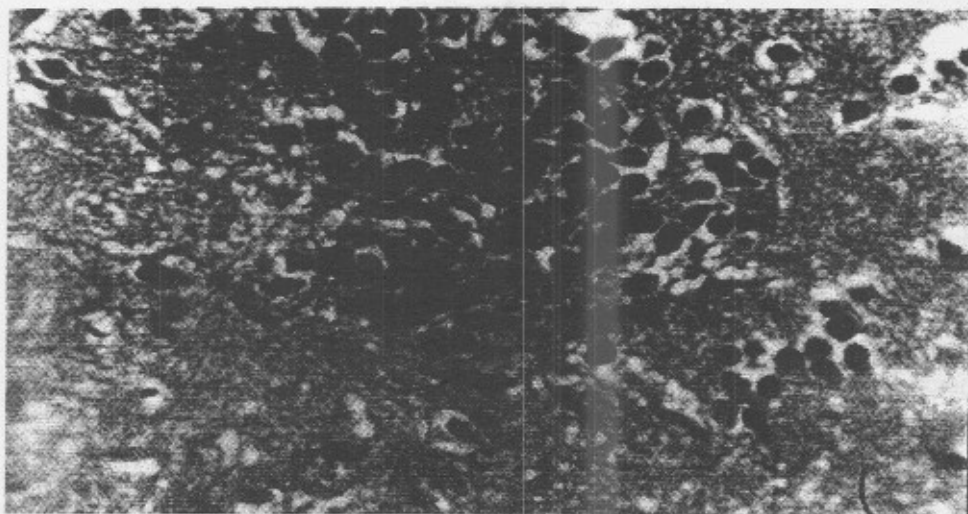
Fig. (10): Brain of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing focal gliosis. [H&E stain, X40]
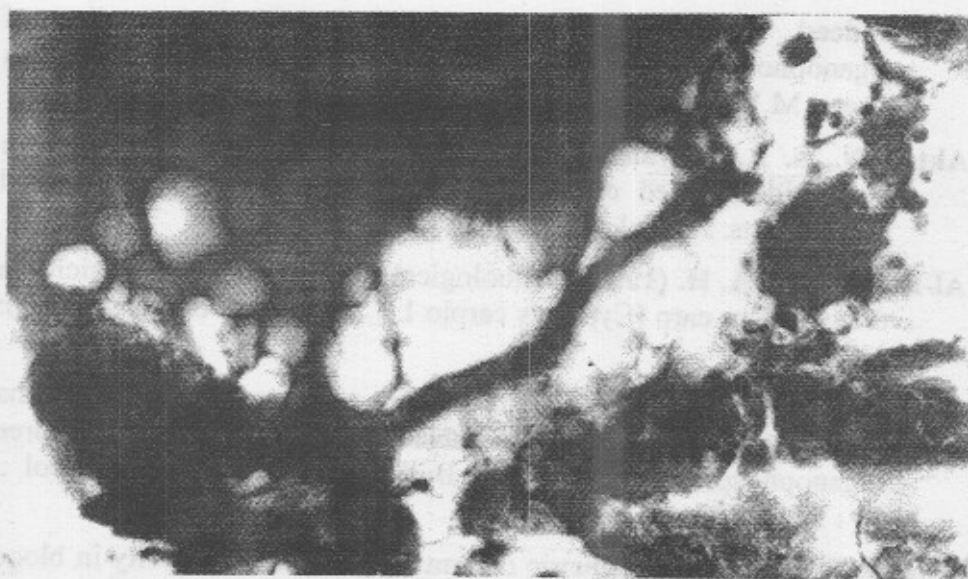

Fig. (11): Thyroid gland of *Oreochromis niloticus* fish exposed to 0.26 mg/L malathion for 96-h, showing colloidal devoid in the lumen of thyroid follicle. [H&E stain, X40]

CONCLUSION

In this investigation, some trials were adopted to achieve the optimum dose level of malathion that causes lethality in water bugs without causing any toxic effect in fish. After these trials, the average dose level of malathion was found to be 0.26 mg/L. In order to eradicate the water bugs intentionally introduced to the laboratory glass aquaria, the average dose level of malathion (0.26 mg/L) was applied for 24 h. These observations can be applied in the field of fish farm to avoid the hazardous effect induced by water bugs, but it may be needed further investigations to realize this object

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الملخص العربى

دراسات سمية وباثولوجية على الملاثيون لتحديد مستوى الجرعة الأمثل لاستخدامه في إبادة حشرة اليثوسيرس النيلى المفترسة للأسماك

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المركز الاقليمى للأغذية والأعلاف بمركز الأبحاث الزراعية .

يعتبر وجود بق الماء (ليثوسيرس النيلي) في أحواض الأسماك من الخطورة حيث أنه يتغذى على الأسماك وخاصة في منطقة الرأس وعليه يهدف هذا البحث إلى تحديد جرعة المبيد الحشري الفوسفورى ملاثيون التي تسبب نفوق لبق الماء دون أى تأثير عكسي على الأسماك . لتحقيق ذلك الهدف تم تعريض ذكور أسماك البلطي النيلي إلى جرعات مختلفة من الملاثيون لتحديد التركيز نصف المميت للأسماك . ومن ناحية أخرى تم تعريض بق الماء (ليثوسيرس نيلي) إلى نفس الجرعات لمعرفة نسبة النفوق الحادثة في بق الماء عند التعرض لهذه الجرعات . ومن نتائج التجربتين السابقتين يمكن تحديد مستوى الجرعة من الملاثيون الذي يسبب نفوق في بق الماء دون حدوث نفوق للأسماك . هذا وقد تم تعريض مجموعة أخرى من الأسماك وبق الماء إلى هذه الجرعة لمدة ٢٤ساعة و ٩٦ ساعة لدراسة التأثير السام وكذلك التأثير الهستوباثولوجى على أنسجة الأسماك المعرضة لهذه الجرعة . هذا وقد تم تحديد معامل وزن الخصية إلى الجسم وكذلك بعض هرمونات السيرم مثل التستوستيرون والتراى أيودو ثيرونين (تى ٣) والثيروكسين (تى ٤) وأيضاً النشاط الأنزيمى لبعض خمائر إنسجة المخ مثل الكولين استيريز واللاكتات ديهيدروجيناز . وقد أوضحت الدراسة أن التركيز نصف المميت للملاثيون هو ٠,٧٠ مجم / لتر من الماء بينما مستوى الجرعة الأمن من الملاثيون الذي يسبب نفوق لبق الماء ولا يسبب نفوق للأسماك هو ٠,٢٦ مجم / لتر . أيضاً هناك نقص معنوي في معامل وزن الخصية بالنسبة للجسم بعد ٩٦ ساعة من التعرض مع نقص في مستوى الهرمون بالسيرم وكذلك تثبيط لنشاط خمائر أنسجة المخ . وقد دعمت هذه النتائج بالدراسة الهستوباثولوجيا والتي أوضحت وجود تغيرات باثولوجية بجميع الأنسجة التي تم دراستها بعد هذه الفترة من التعرض لهذه الجرعة . ومن التحليل الاحصائى لهذه النتائج وجد أن مستوى الجرعة الأمن من الملاثيون للأسماك والفعال في إبادة بق الماء هو ٢٦ مجم / لتر ويطبق لمدة ٢٤ ساعة فقط حيث أنه لا يوجد أي تأثير عكسي لهذه الجرعة على الأسماك خلال هذه الفترة من التعرض .