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Sublethal Effects of Malathion on Channel Catfish, *Ictalurus punctatus*

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Malathion [O, O-dimethyl-S-1, 2-di-(ethoxycarbamyl) ethyl phosphorodithioate] is an organophosphate which is a widely used pesticide in the United States. This pesticide 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). Oxygen analogs of malathion (malaoxon) appear to be the active part that will bind vigorously to acetylcholinesterase (O'Brien 1976). In mammals, this malaoxon is hydrolyzed rapidly thus becoming inactive, but this hydrolysis does not occur in insects and it proceeds very slowly in fish.

Malathion clearly affects the hematology of fish. Mukhopadyhay and Dehadrai (1980) reported that malathion caused erythropenia as well as other changes in the blood. Histopathological studies of rainbow trout Oncorhyncus mykiss (Walsh and Ribelin 1975), the snake head Channa punctatus (Dubale and Shah 1979), walking catfish Clarius batrachus (Mandal and Kalshrestha 1983) and tilapia Saratherodon mossambicus (Shukla et al. 1984) indicated malathion causes necrosis, hyperplasia, and edema of gills with vacuolation and necrosis of livers. Also, malathion has been associated with spinal injury (Kubota et al. 1982). The study reported here was designed to determine the effects of a commercially available malathion solution on channel catfish Ictalurus punctatus. Objectives were to measure hematological changes and histopathological effects of a sublethal concentration of malation on the fish.

MATERIALS AND METHODS

Channel catfish fingerlings from the Alabama Agricultural Experiment Station, Auburn, Alabama used in these experiments averaged 16.5 cm in length and 26.8 gm in weight. The fish were

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acclimated at 25°C in troughs with flowing dechlorinated (activated charcoal filtered) municipal water for 2 wk before the experiment started. The experiment was conducted in static glass aquaria filled with 48 L of dechlorinated water at a temperature of 25±1C. Other water quality parameters were: total hardness of 50 mg/L, alkalinity of 26 mg/L as $CaCO_3$ and pH of 7.8. The fish were not fed during the experiment.

Malathion was a commercial grade consisting of 56.1% malathion, 35.2% organic solvent and 8.7% inert solvent. The commercial grade was used to more closely duplicate the compound most likely to contaminate natural waters.

Channel catfish fingerlings were exposed to 4.5 mg/L of malathion (active ingredient) for 96 h. The 96-h LC_{50} of malathion for channel catfish determined in a separate study was 9.65 mg/L (Areechon 1987). Twenty aquaria were stocked with 10 fish each; there was a total of 100 malathion-exposed fish and another 100 unexposed control fish. Treated water was changed every 24 h to maintain the same calculated malathion concentration throughout the experimental period.

Blood was collected from 20 fish at 12, 24, 48, 72 and 96 h after exposure to malathion and from 20 fish of the control group at each sampling period. Total numbers of erythrocytes and leukocytes were counted at each sampling period using a Spencer-bright line hemocytometer. Differential leukocyte counts were made from smears of blood fixed in methanol and stained with Camco Quik stain (Cambridge Chemical Products, Inc., Ft. Lauderdale, Florida) and Hemal Stain I and II (Hemal Stain Co., Inc., Danbury, Connecticutt). Identification of leukocytes was based on the classification of Grizzle and Rogers (1976).

Hemoglobin content was determined by the cyanmethemoglobin method using Drabkin's reagent (Sigma Chemical Co., St. Louis, Missouri) and the correction method of Larsen (1964). Hematocrit was obtained by microcentrifugation of the sealed heparinized capillary tubes at about 8000 rpm for 10 min and the hemotocrit was measured by using a Spiracrit (Aloe Scientific, St. Louis, Missouri).

Morphologic indices for erythrocyte, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Weinberg et al. (1972). Plasma protein was determined by using the Biuret reaction as described by Wedemeyer and Yasutake (1977). Plasma glucose was measured as described by Hyvarinen and Nikkila (1962). Statistical analyses were made between hematological parameters by analysis of variance of the means.

Fifty channel catfish fingerlings were exposed to 4.5 mg/L of malathion for 96 h in static aquaria (10 fish in each aquarium) and 50 channel catfish were used as non-exposed controls. Gills, brain, head and posterior kidney, liver, spleen, intestine, and

stomach were collected from 10 treated and 10 control fish at five different periods (12, 24, 48, 72, and 96 h). All organs were fixed in Bouin's solution and embedded in Paraplast for sectioning. Sections were stained with hematoxylin and eosin for microscopic examination. Also, part of these organs from each fish were preserved in 10% formalin, sectioned while frozen, and then stained with oil red O.

RESULTS AND DISCUSSION

Fish exposed to malathion were lethargic, however, they were very sensitive to disturbances. A slight tap on the aquarium resulted in severe contraction of the trunk muscle, especially in the middle part of the body. Exposed fish appeared to lose equilibrium and direction of movement. About 80% of exposed fish developed vertebral deformities (Fig. 1) that were clearly visible in radiographs.

The deformity of vertebrae of fish exposed to malathion were observed in other studies by Kumar and Ansari (1984), who suggested that malnutrition could be related to the skeletal deformity. They reported that malathion-exposed zebrafish Brachydanio rerio stopped feeding, consequently resulting in vitamin C deficiency which compounded the effect of malathion.

Another explanation of spinal deformity is the effect of malathion on the central nervous system and/or the peripheral nervous system. The persistent activity of acetylcholine due to inhibition of acetylcholinesterase during exposure to malathion could cause continuous contraction of the trunk muscles. Malathion-exposed fish in this study exhibited these severe muscular contractions which could have caused continuous pressure on the vertebrae and damage. Kubota et al. (1982) reported spinal fractures of medaka Oryzias latipes exposed to an organophosphate that also caused damage to the lateral musculature which was followed by production of collagen. This indicated that collagen synthesis was not affected by organophosphate poisoning. In the present study, malathion apparently caused the spinal deformities rather than a nutritional deficiency, because the fish were not starved for several months required to precipitate a vitamin C deficiency and spinal deformity (Lim and Lovell 1978).

Exposed fish had significantly ($P < 0.05$) increased numbers of erythrocytes and decreased numbers of leukocytes at 48, 72 and 96 h after exposure (Table 1). Hematocrit and hemoglobin of the exposed fish were significantly increased at the 72- and 96-h sampling periods (Table 1). The differential leukocyte counts did not show any significant change for the control or treated fish at any of the sampling periods. Lymphocytes in control fish ranged from 43.5% ± 2.34 [standard deviation (SD)] to 57.45% ± 3.55 while lymphocytes of treated fish ranged from 46.45 ± 2.56 to 52.34% ± 3.45. Thermbocytes of control fish ranged from 40.25% ± 2.11 to 54.24% ± 3.40 while those of treated fish were

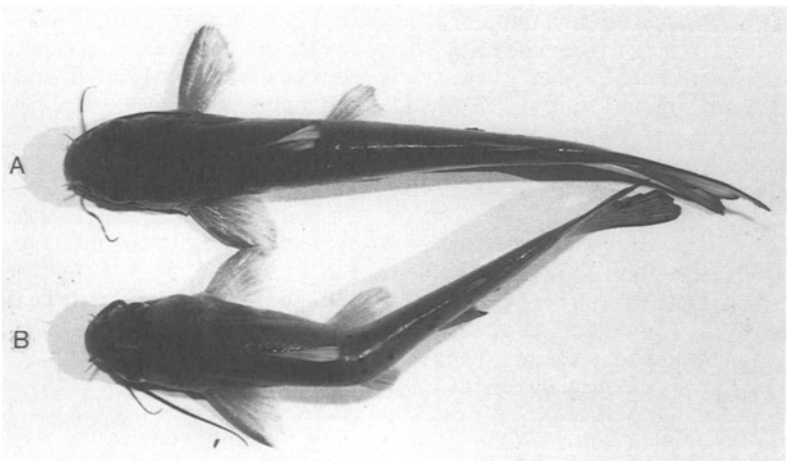

Fig. 1. Channel catfish fingerling with deformed vertebrae after malathion exposure (B) and unexposed control fish (A).

43.45% ± 3.45 to 51.45% ± 2.45. Neutrophiles of control fish composed 1.95% ± 0.55 to 2.30% ± 0.45 of the leukocyte population while neutrophiles of treated fish ranged from 1.82% ± 0.66 to 2.07% ± 0.55. Hemoblasts of control fish were 0.15% ± 0.14 to 0.33% ± 0.19 while hemoblasts of treated fish were 0.18% ± 0.15 to 0.28% ± 0.20. Generally the percentage of leukocytes increased with time, thrombocytdes decreased, while neutrophils and hemoblasts remained unchanged in control and treated fish.

Plasma glucose of the exposed fish was significantly higher than the controls throughout the sampling period (Table 2). MCV of exposed fish was significantly lower at 48 h but then returned to normal (Table 1). MCH decreased significantly at 48, 72 and 96 h after exposure (Table 1) and MCHC values of exposed fish decreased significantly at 72 h (Table 1).

An increase in erythrocytes found in this study differed from other studies in which malathion caused erythropenia (Mukhopadyhay and Dehadrai 1980), however elevated erythrocyte number appeared to result from malathion exposure rather than handling, diseases, temperature or anesthetics. Leukopenia was also observed in this study, which is inconsistent with other studies involving malathion toxicity to fish.

Table 1. Hematological parameters (Mean ± standard deviation) of channel catfish fingerlings exposed to 4.5 mg/L malathion. Mean values that differ significantly ($P < 0.05$) from control within each sampling period are indicated by *. Twenty fish were sampled at each sampling period.

Time After Exposure (hr)	Erythrocyte (10^6/mm^3)	Leukocyte (10^3/mm^3)	Hematocrit (%)	Hemoglobin (g/100ml)	MCV[1] (micron^3)	MCH[1] (picograms)	MCHC[1] %
12							
Control	2.17±0.17	121.89±28.42	29.87±2.41	6.47±0.70	135.45±10.91	29.12±3.48	22.10±2.98
Treated	2.25±0.26	117.56±24.43	29.26±1.72	6.61±0.45	130.72±12.80	27.30±3.38	22.65±2.22
24							
Control	2.27±0.14	131.89±23.28	28.81±2.15	6.94±0.87	127.43±11.86	30.51±3.84	23.42±3.18
Treated	2.33±0.23	149.33±26.94	29.76±2.86	6.82±0.95	126.30±13.83	28.81±3.58	22.76±2.08
48							
Control	2.22±0.41	139.67±30.35*	26.92±3.35	7.36±0.66	123.14±13.02*	35.69±7.07*	28.10±4.66
Treated	2.54±0.24*	94.67±19.03*	27.96±2.19	6.66±0.76	111.08±10.85*	27.34±2.79*	23.95±4.10
72							
Control	2.20±0.31	137.89±31.42*	23.27±3.39*	6.89±0.78*	106.02±7.45	33.00±5.55*	31.46±5.38*
Treated	2.61±0.32*	107.22±39.13*	26.17±2.46*	7.85±0.44*	108.66±6.90	27.57±2.75*	25.64±2.69*
96							
Control	2.33±0.30	135.18±28.64*	25.79±3.91*	7.04±0.67*	111.51±18.11	30.79±3.75	28.08±5.39
Treated	2.80±0.25*	95.67±21.63*	28.93±2.00*	7.76±0.39*	105.45±17.82	23.37±2.60*	23.93±2.35

[1] MCV - mean corpuscular volumne; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.

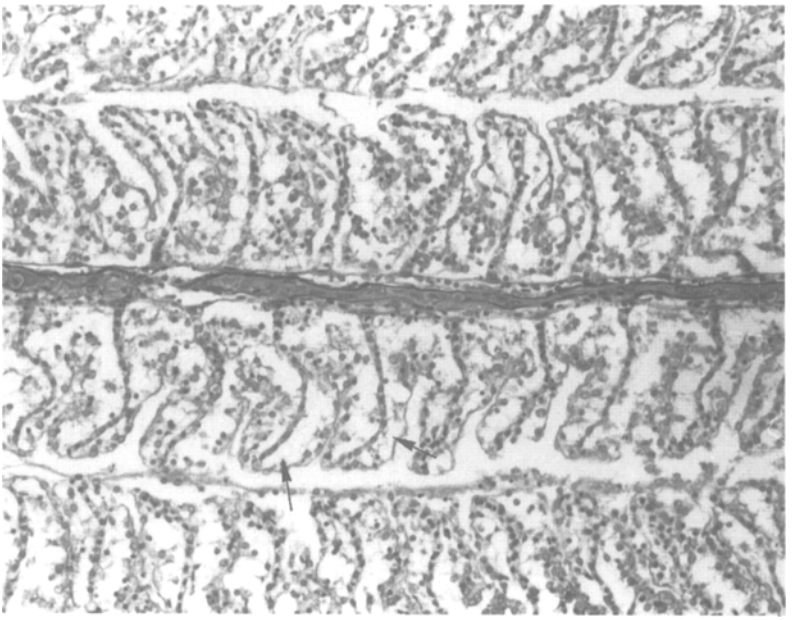

Figure 2. Necrosis (arrow) of gill lamellae from channel catfish fingerling exposed to 4.5 mg/L malathion for 96. H&E X 200.

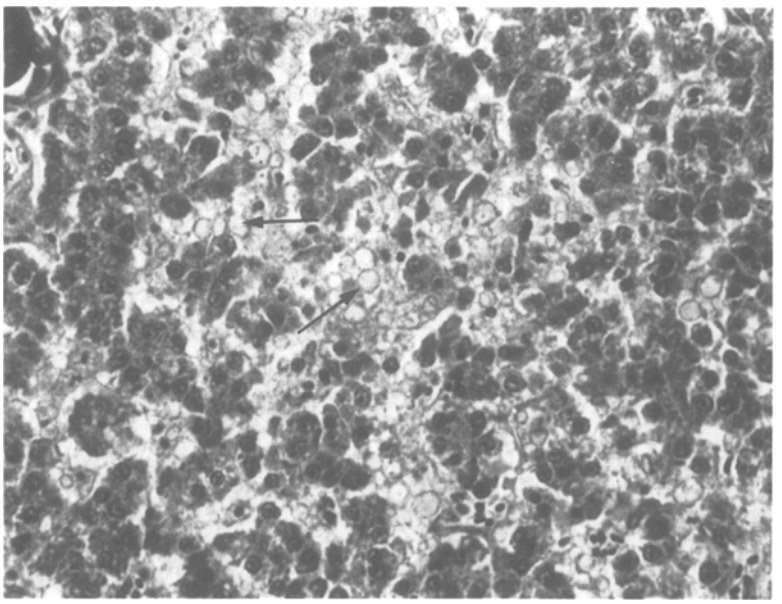

Figure 3. Necrosis and remnants of dead cells (arrows) in the liver of channel catfish fingerling exposed to 4.5 mg/L malathion for 96. H&E X 400.

Table 2. Hematological variables (Mean ± standard deviation) of channel catfish fingerlings exposed to 4.5 mg/L of malathion. Mean value that differs significantly ($P < 0.05$) from control within each sampling period is indicated by *. Twenty fish were sampled at each sampling period.

Hours After Exposure	Plasma Glucose (mg/100ml)		Plasma protein (g/100ml)	
	Control	Treated	Control	Treated
12	34.18±11.58	60.17±9.64*	2.79±0.24	2.82±0.03
24	39.50±8.38	51.50±9.52*	2.51±0.11	2.97±0.09*
48	38.67±9.07	53.67±7.12*	2.60±0.23	2.70±0.15
72	33.33±4.9	47.00±7.59*	2.76±0.16	2.78±0.09
96	35.90±6.49	45.67±6.13*	2.86±0.18	3.00±0.19

Epithelium of the gill lamellae of treated fish became necrotic and began to slough from the supporting structures (Fig. 2). The liver of treated fish appeared necrotic with various levels of accumulation of remnants of dead cells giving the liver a foamy appearance as early as 12 h after exposure (Fig. 3). Oil-red-O stain indicated that this vacuolation was fatty change. No sigfnificant histopathology was observed in kidneys or other tissues of treated fish other than the previously discussed spinal injury.

Malathion and other pesticides cause lesions in the gills of exposed fish, including necrosis, hyperplasia, hypertrophy and edema (Walsh and Ribelin 1975). Liver of channel catfish exposed to malathion in this study developed vacuolation and focal necrosis, which is in agreement with other studies concerned with the effects of malathion and other organophosphates (Anees 1978).

The results from this study indicate that malathion does affect blood components especially by increasing hemotological components of channel catfish erythrocytes and decreasing leukocytes. Correspondingly the hemoglobin and mean corpuscular hemoglobin increases. Malathion also increases the plasma glucose of catfish.

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