International Journal of Aquatic Biology (2015) 3(4): 199-207
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.NPAJournals.com

Original Article

Acute toxicity and effects of malathion exposure on behavior and hematological indices in Indian carp, *Cirrhinus mrigala* (Hamilton)

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Abstract: Malathion is one of the most commonly used pesticides in agriculture. This study was aimed to investigate the acute toxicity of malathion as an aquatic pollutant on the behavior and hematological indices in Indian carp (*Cirrhinus mrigala*). A static experiment was conducted and 1, 24, 48, 72 and 96 hrs LC_{50} values of malathion for the test fish were estimated as 14.55 mg/L, 12.48 mg/L, 11.56 mg/L, 10.85 mg/L and 9.32 mg/L, respectively. During 96 hrs exposure to 9.32 mg/L of malathion, behavioral abnormalities such as hyperactivity, cough, convulsions, erratic swimming, loss of balance, rapid opercular movements, gill mucous secretion, surfacing and gulping of air were observed in the test fish. The hematological changes in exposed fish after 96 hrs exposure to malathion included a significant decrease in erythrocyte count, hemoglobin content, hematocrit, leukocyte count and a significant increase in neutrophils count as compared to the control fish. In conclusion, acute exposure to 9.32 mg/L of malathion provoked behavioral and hematological abnormalities in Indian carp which offers a valuable tool to monitor malathion induced toxicity in fish.

Article history:
Received 1 April 2015
Accepted 4 June 2015
Available online 25 August 2015

Keywords:
Malathion
Acute toxicity
Behavior
Hematology
Indian carp

Introduction

Among different fresh water pollutants, pesticides are one of the most potentially harmful chemicals introduced into the environment. Organophosphate pesticides are the most commonly used pesticides in the world and require more awareness because of their possible toxic effects on non-target animals (Aktar et al., 2009). Malathion ($C_{10}H_{19}O_6PS_2$) is an organophosphate pesticide widely used in both agriculture and households to control insect pests affecting a number of crops, stored grains and livestock feed via ground and aerial sprays and aerosols. After its application, malathion can contaminate surface water through accidental spillage, spray drift and runoff following rain. The aquatic distribution of malathion can cause harmful effects on aquatic environment and its organisms. Malathion is less persistent in an environment under alkaline conditions and has a higher degree of persistence under acidic conditions. The half-life of malathion is up to 11 days and it degrades into malaxon which is more toxic than the parent (Martinez and Leyhe, 2004). Aquatic distribution of malathion causes significant adverse health effects on fish and other non-target animals. The toxicity potential of malathion varies in different fish and depends upon the differences in absorption, detoxification and inhibition of enzyme acetylcholinesterase (AChE) that breaks down the neurotransmitter acetylcholine so that subsequent impulses can transmit across the synapse. Inhibition of AChE drastically affects growth, feeding and reproductive behaviors and can lead to death of fish usually by asphyxiation (Sparling and Fellers, 2007). Behavioral changes are one of the most important indicators of environmental stress; while hematological parameters are important indicators of disease and are frequently used tools in toxicological

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research, environmental monitoring and in the evaluation of the pathophysiological changes in fish under different stressful conditions (Pimpao et al., 2007). In the last few years, there have been several studies to investigate the malathion toxicity in different fish species including *Glossogobius giuris* (Venkataramana et al., 2006), *Opheocephalus punctatus* (Pugazhvendan et al., 2009), *Oreochromis niloticus* (Al-Ghanim 2012), *Clarias gariepinus* (Ahmed, 2012) and *Heteropneusteus fossillis* (Deka and Mahanta, 2012), but most of these studies were confined to reporting histological changes in fish exposed to sub-lethal concentrations of malathion and very little attention has been paid to the acute effects of malathion exposure on fish hematology. Malathion is a commonly used pesticide and its presence in fresh water reservoirs of Pakistan has previously been reported (Mastoi et al., 2008). However, there is a paucity of scientific documentation regarding the effects of malathion on local fish species. Indian carp (*Cirrhinus mrigala*) is an important edible fresh water fish with substantial economic importance and wide distribution in fresh water reservoirs of Pakistan. Considering the growing use of malathion in Pakistan and lack of knowledge about its potential toxicity in local fresh water fish fauna, present study was carried out to determine acute toxicity of malathion and its effects on behavior and hematological indices of *C. mrigala*.

Materials and Methods

Juveniles of *C. mrigala* weighing 20.4 ± 1.8 g and total length of 5.5 ± 1.8 cm were obtained from Jokhio fish farm (24°16′5″N, 67°35′55″E) Thatta, located northeast of Karachi, Pakistan. Fish were carried to the laboratory in plastic containers and were acclimated to the laboratory conditions for two weeks in a fiberglass tank containing 500 L of continuously aerated fresh water. During this period, temperature, pH, dissolved oxygen and photoperiod were 22.5 ± 1.9°C, 7.5 ± 0.3, 6.8-7.5 mg/L and 12:12 hrs dark-light cycles, respectively. Fish were fed with a commercial fish food twice a day during the period of acclimation but were starved for 24 hrs prior to the experiment and throughout the experiment. Technical grade malathion with 95% active ingredient was purchased from Edgro (Pvt.) Ltd. Karachi and a stock solution at a concentration of 200 mg/L was prepared by dissolving it in the acetone. The stock solution was stored in dark bottles at 4°C and its different dilutions were used to determine acute toxicity, behavioral and hematological effects on experimental fish.

After two weeks of acclimation, a static acute toxicity test was performed. The juvenile specimens were exposed to each of the seven different concentrations (0.5, 1, 2, 4, 8, 16 and 32 mg/L) of malathion to determine 96 hrs LC_{50} values. The experiment was performed in triplicates in 100 L glass aquaria each containing 20 juvenile *C. mrigala*. The physicochemical indices of diluting water used were: temperature 22.5 ± 1.9°C, pH 7.5 ± 0.3, salinity 107 ± 3.2 mg/L, total hardness 112 ± 2.1 mg/L and dissolved oxygen 6.8-7.5 mg/L. These water quality parameters were determined according to the procedures described in standard methods (APHA, 1992). Prior to the introduction of fish, the required volume of malathion was added and water in the aquaria was aerated for one hour to obtain a homogenous mixture of the toxicant. Two control sets were also run containing the same number of fish in the same volume of water but without malathion. Water was renewed daily and fish mortalities were recorded after 1, 24, 48, 72 and 96 hrs of exposure. Fish were considered dead if their gill opercular movement ceased and they could not respond to the stimulus provided by a glass rod. Dead fish were immediately removed from the aquaria and LC_{50} values were calculated using Probit Analysis test (Finney, 1971).

At the end of the acute toxicity test, twenty juvenile *C. mrigala* were exposed to 9.32 mg/L (96 hrs LC_{50}) for 96 hrs in 100 L glass aquaria to determine behavioral and hematological alterations. The experiment was run in triplicate and two control sets were also run concurrently. Fresh test media were provided daily to maintain the concentrations of malathion near to the 80% of nominal concentration.

Table 1. Cumulative mortality of juvenile *Cirrhinus mrigala* exposed to different concentrations of malathion.

Concentrations (mg/L)	Number of dead fish (%)				
	1 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Control	-	-	-	-	-
0.5	-	-	-	-	-
1	-	-	-	-	4
2	8	11	18	24	32
4	20	24	30	32	40
8	28	32	38	41	47
16	78	84	90	96	100
32	100	ND			
χ^2	1.78	2.01	2.29	2.78	3.17
P	<0.05	<0.05	<0.05	<0.05	<0.05

ND= no data because of 100% mortality; (–) no dead fish.

The physicochemical characteristics of water were maintained the same as mentioned above. The behavioral changes of fish were recorded at every 12 hrs during the study and dead fish were removed from the aquaria. At the end of 96 hrs exposure period, a total of twelve surviving fish from experimental aquaria and same number of fish from control group were randomly collected using a small dip net with minimum disturbance in the water. Blood sample of each fish was collected by cardiac puncture using a 18 G needle attached to a plastic syringe. Blood was transferred into heparanized glass vials (50 IU Sodium heparin/ ml of blood) and was immediately used for hematological examinations. The hematological indices examined included erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), leukocyte count (WBC), leukocyte differential counts and were determined according to the unified methods for hematological examination of fish (Svobodova et al., 1991).

All statistical analyses were carried out using SPSS 17.0 computer software for windows (SPSS Inc., Chicago, IL, USA). The difference in fish mortalities was analyzed by $\chi 2$ test. The values of hematological indices were presented as mean ± SD. The data for these parameters was tested for normality (Kolmogorov-Smirnov test) and analyzed by one way analysis of variance (ANOVA) to test significant differences between the hematological parameters of controlled and exposed groups and $P<0.05$ were considered statistically significant.

Results

Cumulative mortality of *C. mrigala* after exposure to different concentrations of malathion at different durations of exposure is summarized in Table 1. The data clearly indicates a time and concentration-dependent significant increase in fish mortality rate ($P<0.05$ in each case). The highest and fastest fish mortalities were recorded after 1 hrs exposure to the highest concentration of malathion (32 mg/L), while lowest fish mortalities were recorded after 96 hrs exposure to 1 mg of malathion. The LC_{50} values with 95% confidence limits of malathion for *C. mrigala* were estimated as 14.55 mg/L (13.98-15.32) for 1 hrs, 12.48 mg/L (11.91-13.39) for 24 hrs, 11.56 mg/L (10.89-12.89) for 48 hrs, 10.85 mg/L (no data as $P>0.05$) for 72 hrs and 9.32 mg/L (no data as $P>0.05$) for 96 hrs; and significant differences were observed in the LC_{10}, LC_{50} and LC_{90} values recorded for different times of exposure ($P<0.05$; Table 2).

The behavioral response observed every 12 hrs of malathion exposure in test fish included hyperactivity, cough, convulsions, erratic swimming, loss of balance, rapid opercular movements, gill mucous secretion, surfacing and gulping of air. Subsequently, exhausted fish sank to the bottom of aquaria and died.

Compared to the control group, fish exposed to 9.32

Table 2. Exposure time dependent lethal concentrations of malathion (mg/L) for juvenile *Cirrhinus mrigala*.

Time	Lethal concentration values with 95% confidence limits (mg/L)*		
	LC_{10}	LC_{50}	LC_{90}
1 hrs	2.41^a (2.98-2.32)	14.55^a (13.98-15.32)	17.52^a (17.11-18.92)
24 hrs	2.18^b (2.34-1.95)	12.48^b (11.91-13.39)	15.27^b (14.85-15.92)
48 hrs	1.75^c (1.92-1.59)	11.56^c (10.89-12.89)	13.39^c (12.64-14.02)
72 hrs	1.56^d (−)	10.85^d (−)	11.21^d (−)
96 hrs	1.32^e (−)	9.32^e (−)	10.52^e (−)

(−) no data because of $P< 0.05$, *Lethal concentration values in columns with different letters significantly differ ($P< 0.05$).

Table 3. Hematological indices of *Cirrhinus mrigala* after 96 hrs exposure to 9.32 mg/L of malathion.

Variables	Control group	Exposed group
RBC (10^6/mm^3)	1.71 ± 0.11^a	1.10 ± 0.20^b
Hb (g/dL)	4.94 ± 0.22^a	2.48 ± 0.37^b
Hct (%)	13.42 ± 2.07^a	8.94 ± 1.32^b
MCV (fL)	214.75 ± 8.39^a	211.34 ± 4.74^a
MCH (pg)	29.42 ± 2.18^a	30.76 ± 1.87^a
MCHC (%)	24.33 ± 2.48^a	26.56 ± 1.96^a
WBC (10^3/mm^3)	36.41 ± 2.68^a	24.44 ± 1.65^b

RBC = erythrocyte count; Hb = hemoglobin concentration; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = leukocyte count. Different superscript letters indicate significant ($P<0.05$) difference between the groups.

Table 4. Leukocytes differential count of *Cirrhinus mrigala* after 96 hrs exposure to 9.32 mg/L of malathion.

Variables	Control group (n = 12)	Exposed group (n = 12)
Neutrophils (%)	13.74 ± 3.85^a	18.62 ± 2.15^b
Lymphocytes (%)	73.12 ± 4.42^a	72.42 ± 3.25^a
Monocytes (%)	11.56 ± 1.23^a	11.74 ± 1.65^a
Eosinophils (%)	7.12 ± 0.95^a	7.68 ± 1.05^a
Basophils (%)	3.41 ± 0.55^a	3.52 ± 0.84^a

Different superscript letters indicate significant ($P<0.05$) difference between the groups

mg/L of malathion for 96 hrs showed significantly lower values for RBC count, Hb, Hct and WBC count ($P<0.05$); but the values recorded for MCV, MCH and MCHC were not significantly different between the two groups (Table 3). Neutrophils of exposed fish group increased significantly ($P<0.05$) while no significant difference was observed in the lymphocytes, monocytes, eosinophils and basophils counts of both groups (Table 4).

Discussion

In the present study, the 1 and 96 hrs LC_{50} values of malathion for juvenile *C. mrigala* were found to be 14.55 mg/L and 9.32 mg/L, respectively. In view of this, malathion can be included in a group of moderately toxic substances for this fish. An increase in the toxicity of malathion for the test fish was observed with increase in duration of exposure and concentration of pesticide. For instance 4% fish died when they were exposed to 1 mg/L of malathion for 96 hrs, whereas 100% fish died when exposed to 32 mg/L of malathion for 1 hrs. The acute toxicity of malathion varies in different fish species and ranges from few ppb to several mg/L. Office of the pesticide programme (USEPA, 2006) has reported 96 hrs LC_{50} values of malathion for Western mosquito fish (*Gambusia affinis*) as 0.7 ppb, for rainbow trout (*Onchorhynchus mykiss*) as 4.1 ppb, for bluegill sunfish (*Lepomis macrochirus*) as 30 ppb, for cutthroat trout (*O. clarki*) as 174 ppb, for yellow perch (*Perca flavescens*) as 263 ppb, for tilapia (*Tilapia mosambica*) as 2000 ppb, for common carp (*Cyprinus carpio*) as 6590 ppb and for medaka (*Oryzia latipes*) as 40,000 ppb. On the other hand, very high 96 hrs LC*50* values of malathion for black bullhead (*Ameiurus melas*) as 11.8 mg/L and for Indian catfish (*Hetereopneustes fossilis*) as 15.3 mg/L have also been documented (Martinez and Leyhe, 2004). The toxicity potential of pesticides changes with respect to age, size and exposure time in different fish species. The difference in the toxicity of malathion among different fish species can be attributed to the difference in susceptibility and tolerance regarding absorption, biotransformation and excretion of pesticide (Dutta et al., 1992).

Behavioral changes and hematological profile are sensitive indicators that may provide information about potential toxic effects of pesticides and other pollutants in aquatic organisms. The investigation of behavioral and hematological changes has become an important means of understanding toxicological impacts and pathological processes in fish (Singh et al., 2009). The results of present study showed that malathion exposure at a concentration of 9.32 mg/L for 96 hrs exerted a certain influence on fish behavior that include hyperactivity, seizures, erratic swimming, loss of buoyancy, increased cough rate and gill mucous secretion. These behavioral changes correspond to the observations by other authors dealing with the toxicity of organophosphate pesticides. It regards abnormal fish behavior in *Danio rerio* exposed to chlorpyrifos (Levin et al., 2004) and *C. mrigala* exposed to diazinon (Rauf and Arain, 2013). Behavioral changes in the exposed fish appear to be the manifestation of malathion toxicity. Increased surfacing and gulping of surface water appear to be an attempt by fish to overcome hypoxia and to avoid breathing in the toxic water. Erratic swimming, convulsions, loss of equilibrium and abnormal swimming in exposed fish are caused by deficiency in nervous and muscular coordination which can be attributed to the neurotoxic effects of malathion. Similar observations have been reported in *C. carpio* and fingerling *Silurus glanis* after acute exposure to dimethoate and diazinon, respectively (Singh et al., 2009; Koprucu et al., 2006). Increased mucous secretion in malathion exposed fish during present study is probably an adaptive response of fish to reduce the irritating effect of the toxicant to eliminate it through epidermal mucous secretion. Similar fish response has also been reported in *C. carpio* exposed to dimethoate (Singh et al., 2009), *C. mrigala* exposed to diazinon (Rauf and Arain, 2013) and *Labeo rohita* exposed to malathion (Patil and David, 2008). In fact, malathion as other organophosphate pesticides exerts its effects by inhibiting the activity of enzyme acetylcholinesterase that leads to the accumulation of acetylcholine in cholinergic synapses and ends up with hyperstimulation; this decreased activity of acetylcholinersterase affects optomotor responses of fish that can disrupt the overall survivability of the animal in their natural environment (Dutta et al., 1994).

In the present study, the main hematological response of *C. mrigala* after acute exposure to malathion include significantly lower RBC count, Hb concentration, Hematocrit and WBC count as compared to the control. A similar response in these hematological indices to give evidence for suppressed hemotopoiesis, followed by anemia induction has previously been reported in fish exposed to other organaophosphate pesticides. It regards, changes in erythrocyte indices after exposure to chloropyrifos in *Clarias gariepinus* (Nwani et al., 2013), monocrotophos in *Catla catla* (Jeyapriya et al., 2013) and diazinon in fingerlings of *S. glanis* (Koprucu et al., 2006). Similarly, a significant decrease in erythrocyte count, hemoglobin content, hematocrit and WBC count after 96 hrs exposure to 8.15 mg/L of diazinon and 30 days exposure to 0.815 mg/L of diazinon has also been reported in immature *C. mrigala* (Rauf and Arain, 2013; Haider and Rauf, 2014). Ahirwar et al. (2012) reported decreased RBC count, hemoglobin content and WBC count in *Notopterus notopterus* following 3 hrs exposure to lethal concentrations of malathion, while decreased RBC count, hematocrit and hemoglobin content have also been reported in *C. gariepinus* following sub-lethal exposure to malathion (Ahmed, 2012). In consistence with the present study, a decreased RBC count, hemoglobin content and hematocrit levels in *Channa punctatus* following three days exposure to malathion and a similar response in *H. fossilis* after four days exposure to malathion has previously been reported (Parveen and Shadab, 2011; Singh et al., 2009). Malathion induced decreased RBC count, Hb content and hematocrit values in fish are indicator of anemia that can be a result of disruptive iron-synthesizing mechanism, destruction of mature erythrocytes and malfunctioning of the hemopoeitic system (Adhikari et al., 2004). Since hemopoeitic system of fish is mainly located in the interstitium of the kidney, the hematological response of test fish can be a result of malathion induced morphological alterations in renal interstitium of fish (Dutta, 1992). Changes in leukocyte differential counts are recognized as sensitive indicators of environmental stress as monocytes and neutrophils generally increase while lymphocytes decrease in response to a stressor (Shah and Altindg, 2005). In the present study, the decrease in total leukocytes count and alterations in the leukocyte differential count of fish after acute exposure to malathion are in consistence with the previous reports. It regards, decreased WBC count after acute exposure to diazinon in *C. mrigala* (Rauf and Arain, 2013) and fingerlings of *S. glanis* (Koprucu et al., 2006), *Oreochromis mosambicus* exposed to phosalaone (Ali and Rani, 2009) and *O .mykiss* treated with deltamethrin (Velisek et al., 2007). Similar to the present study, a decrease in total leukocyte count and increase in neutrophil count after five days exposure to diazinon has been reported in *C. gariepinus* (Nwani et al., 2012). Svoboda et al. (2001) reported a similar decreased non-specific immunity in *C. carpio*, following acute exposure to diazinon. In contrast to the present study, three and four days exposure to malathion induced increase in total leukocyte count in *N. notopterus* and *C. punctatus*, respectively (Ahirwar et al., 2012; Parveen and Shadab, 2011) and authors attributed this increase to the acute response of immune system due to tissue injury caused by malathion exposure in fish. However, the decrease in total WBC count of test fish after acute exposure to malathion during present study may be attributed to the rapid destruction or failure in the delivery to the circulation due to reduced production of these cells; while, increase in neutrophil count may be due to the defensive response of fish to overcome malathion induced stress in fish.

In conclusion, malathion can be classified as a moderately toxic substance for *C. mrigala*. Exposure to malathion in a concentration of 9.32 mg/L for 96 hrs was caused severe stress and resulted in significant behavioral and hematological alterations in the test fish. Malathion exposure, even in small concentrations has the potential to impair physiological activities leading to observed behavioral and hematological pattern and can ultimately lead to the death of fish. This fact should be taken into consideration when this pesticide is used in agricultural fields surrounding fresh water reservoirs to avoid malathion related toxicity in aquatic organisms.

Acknowledgement

Author is thankful to Miss. Qurat-Ul-Ain Aslam of Bahria University Islamabad, for her valuable comments to improve manuscript and Prof. M. Ayub Qureshi, Head Department of Zoology and Principal Govt. Superior Science College, Shah Faisal Colony, Karachi, Pakistan, for providing all the facilities for the successful completion of this study.

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International Journal of Aquatic Biology (2015) 3(4): 199-207
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.NPAJournals.com

چکیده فارسی

سمیت حاد و اثرات در معرض قرارگیری مالاتونین بر رفتار و شاخص‌های خون‌شناسی کپور هندی مریگال (***Cirrhinus mrigala***)

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چکیده:
مالاتیون یکی از مهمترین آفت‌کش‌های مورد استفاده در کشاورزی می‌باشد. این مطالعه با هدف بررسی سمیت حاد مالاتیون به‌عنوان یکی از آلاینده‌های اکوسیستم‌های آبی بر روی رفتار و شاخص‌های خون‌شناسی کپور هندی مریگال (*Cirrhinus mrigala*) اجرا شد. یک آزمایش استاتیک به اجرا درآمد و مقادیر LC_{50} ۱، ۲۴، ۴۸ و ۶۹ ساعت مالاتونین برروی ماهی مورد آزمایش به‌ترتیب در غلظت‌های ۱۴/۵۵، ۱۲/۴۸، ۱۱/۵۶، ۱۰/۸۵ و ۹/۳۲ میلی‌گرم در لیتر تخمین زده شد. در طول ۹۶ ساعت در معرض قرارگیری در غلظت ۹/۳۲ میلی‌گرم در لیتر مالاتونین، ناهنجاری‌های رفتاری شامل تحرک بالا، سرفه، تشنج، شنای نامنظم، از دست دادن تعادل، حرکات سریع سرپوش‌آبششی، ترشح موکوس آبششی، به‌سطح آب آمدن و بلعیدن هوا در ماهیان مورد آزمایش مشاهده شد. تغییرات خونی در ماهیان در معرض سم مالاتیون بعد از ۹۶ ساعت شامل یک کاهش معنی‌دار در تعداد اریتروسیت، هموگلوبین، هماتوکریت و لوکوسیت و افزایش معنی‌دار در تعداد نوتروفیل در مقایسه با ماهیان تیمار بود. به عنوان نتیجه‌گیری می‌توان بیان داشت که قرارگرفتن ماهیان در معرض ۹۶ میلی‌گرم در لیتر مالاتیون ناهنجاری‌های رفتاری و خون‌شناسی را در کپور هندی مریگال تحریک می‌کند که به‌عنوان یک ابزار با ارزش برای پایش سمیت ناشی از مالاتیون در ماهیان می‌تواند مدنظر قرارگیرد.

کلمات کلیدی: مالاتونین، سمیت حاد، رفتار، خون‌شناسی، کپورهندی.