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EFFECT OF MALATHION IN IGNACIO RAMIREZ DAM SEDIMENTS ON RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*)

Laura Martinez-Tabche
Alejandro Chehue Romero
Leobardo Gómez-Oliván
Marcela Galar Martínez
Elena Olvera Hernández
Eugenia López López

Departamento de Toxicología, Sección de Graduados
Escuela Nacional de Ciencias Biológicas
Instituto Politécnico Nacional
Del M. Hidalgo, D.F. Mexico

Above the Ignacio Ramirez Dam (IRD), malathion (MA) is used in agricultural activities to control pests. This compound is one of the most widely used organophosphate pesticides in Mexico. Both acute and sublethal toxicity, as well as insecticide kinetic uptake and depuration in juvenile trout (Oncorhynchus mykiss)*were determined using spiked natural sediments. Lipid peroxidation level and acetylcholinesterase activity (AChE) were used as indicator parameters of sublethal toxicity. The results of acute toxicity of MA in rainbow trout showed that 96h-LC_{50} in water was 0.1164 mg/l while in sediments was 0.7650 mg/kg. All MA concentrations produce an increase in lipid peroxidation level and a decrease in AChE activity. The results of the kinetic model showed that the maximum rate of absorption was at 12 h with a rapid bioconcentration. When MA was desorbed from the sediment, the pesticide concentration was increased in the trout gill. The gills are organs with a great capacity of MA uptake in* Oncorhynchus mykiss *to produce a toxic effect in this organism.*

INTRODUCTION

Studies of the uptake and accumulation of toxic chemicals in fish received considerable stimulus when it was shown that it could affect human health by eating fish which contain these toxicants. A fundamental aspect of these studies is understanding the effects that these chemicals have on a specific organ or system in the fish (Borgmann et al., 1996; Akcha et al., 1999; Ramírez et al., 2000).

About 2000 inhabitants use water from the Ignacio Ramírez Dam (IRD) near Toluca, Mexico, for irrigation and domestic supply. Above the dam, organophosphate pesticides, specifically Malathion (MA), are used in pest control. It is necessary to study the behavior of this chemical in the sediments of the IRD reservoir to evaluate impacts on the biota of the reservoir and human health.

MA is one of the most widely used organophosphate pesticides in Mexican crops. It is a dithiophosphate-succinic acid ester. This pesticide is principally used to control aphids and similar sap-sucking insects, although various weevils, small beetles, scale insects and red spider mites are often also susceptible (CICOPLAFES, 1998). This compound may arrive by lixiviation to the IRD and produce toxic effects on the biota of this water body.

The rainbow trout *(Onchorhynchus mykiss)* was used as test organism since it is very sensitive, both to environmental changes and low concentrations of various toxicants (Bayley et al., 1996). This salmonid contains acetylcholinesterase (AChE) in different organs (Smith, 1993) and this enzyme participates in restoring postsynaptic membrane excitability, through the metabolic degradation of acetylcholine (ACh), which is hydrolyzed into acetate and choline. In this way the membrane is repolarized and its permeability is restored (Heath, 1987). It has been shown that MA inhibits the AChE activity, and in the gill produces massive degeneration of filament (Richmonts and Dutta, 1989).

The lipid peroxidation due to free radicals is considered the main mechanism of cellular destruction (Yalcin et al., 1986). The organophosphate pesticides can also produce peroxidation of lipids (Yarsan et al. ,1999).

The objectives of this work were to determine the uptake and depuration of MA in the rainbow trout, *O. mykiss*, and to study the elimination kinetics in the sediment. An additional objective was to determine the toxic effect of MA on AChE activity and lipid peroxidation levels when fish were exposed to sublethal concentrations.

MATERIAL AND METHODS

Study area

This study was conducted in the IRD reservoir, which is constructed on the Gavia River northwest of the city of Toluca, Mexico. This dam receives inflow from the Salitre, Muerto, Cebollas, San Pedro, Guajolota, and Almoloya rivers. The basin area is 505 km^2. The total storage capacity of the IRD is 20.5 x 10^6 m^3. The geographical coordinates are 19º27'35" N and 99º46’25" W. The principal crops are beans, corn, feed, oats, potatoes and forage. The climate is temperate with summer rains in the months of July, August and September. The hottest months are May and June and the yearly average temperature is 12.4 ºC. The annual precipitation is 822.5 mm.

Experimental animals

Juvenile rainbow trout, *O. mykiss,* 3-5 cm in length (average weight 1-1.5 g), were obtained from the piscicultural center El Zarco located 33 km east of Toluca and were acclimatized to laboratory conditions for 15 d prior to the experiment. In the laboratory, they were maintained in an aquarium with dechlorinated tap water at 9 ± 1 ºC (hardness = 150 mg/l, alkalinity = 31 mg/l, dissolved oxygen = 12 mg/l and pH = 8.2). Water in the aquarium was air-saturated with air pumps.

Sampling

Water

Sampling was carried out between January and March 1999. Water samples were taken from the discharge of the dam. Five samples were collected in sterile polyethylene bottles previously washed with 3 percent nitric acid. Samples were placed on ice and transported back to laboratory for immediate analysis by gas chromatography.

Sediment

Sediment samples of the discharge zone of the dam were taken with a 5.2 x 6 cm diameter conical dredge of stainless steel and stored in nitric acid washed polyethylene bottles in the dark and cooled with ice during transport to the laboratory; they were dried at constant weight at 20 ºC and stored until their use.

Physicochemical characterization

Sediments were characterized by determination of organic matter content (concomitant organic carbon was determined), which was measured using the Walkley-Black method (Secretaría de Agricultura y Recursos Hidraúlicos, 1988). pH was measured using a potentiometer (glass electrode), and a sediment mechanical analysis was conducted to determine percent of sand, clay,

Table 1. Physicochemical Characteristics of Ignacio Ramírez Dam Sediments

PARAMETER	VALUE
pH	6.42±0.35
Organic matter (%)	12.40±0.18
Organic carbon (%)	5.75±0.26
Sand (%)	3.34±0.35
Clay (%)	70.84±1.50
Silt (%)	25.82±0.83
Malathion in water (µg/L)	0.001±0. 0.0002
Malathion in sediment (µg/kg)	0.015±0.0008

and silt by the Bouyoucos method (Bohn, 1993). The MA concentration was determined by gas chromatography. The physicochemical properties are summarized in Table 1.

Equilibrium time

MA was spiked (0.1849 mg/kg 96h-LC_0) in 150 g of IRD sediment, this system was reconstituted with 150 ml of dechlorinated water, then it was shaken during 0, 3, 6, 12, 24, 48, 72, and 96 h. Each

sample was centrifuged at 4500 g x 15 min. Pesticide concentration was determined in water and sediment by gas chromatography.

Acute toxicity

Water system

Ten juvenile organisms were added to an aquarium that contained 15 l of water plus MA. Five MA concentrations were tested (0.108, 0.114, 0.119, 0.125, and 0.130 mg/l) as well as two control groups (dechlorinated water and acetone, which was used to dissolve MA). The duration of exposure was 96 h. The behavior and survival in the different MA concentrations were observed and compared with those of the control.

Sediment system

Ten juvenile organisms were added to an aquarium that contained 150 g of IRD sediment spiked with MA in a collodion bag (14,000 daltons) and reconstituted in 15 l of dechlorinated water. Five MA concentrations were tested (0.1, 0.5, 0.6, 0.8 and 1.0 mg/kg). These systems were equilibrated by mechanic shaken during 3 h (equilibrium time) prior to the addition of the organisms. Two control groups (sediment alone and with acetone) were used. These studies were done in triplicate.

The median lethal concentrations for both systems after 96-h (96h-LC_{50}) were calculated by computerized log-probit analysis.

Transfer of Malathion in the IRD sediment to rainbow trout

Eight juvenile organisms were added to an aquarium that contained 150 g of IRD sediment spiked with 0.1849 mg/kg of MA (corresponds to 96h-LC_0) in a colloidon bag (14,000 dalton) and reconstituted with 15 l of dechlorinated water. These systems were equilibrated by mechanical shaking for 3 h (equilibrium time), prior to the addition of the organisms. A control group was tested also (IRD sediment without insecticide). The duration of the exposures was 0, 12, 24, 48, 72 and 96 h. After exposure periods, 0.05 g of gills were finely cut and mixed with 7 ml of (50:50) methylene chloride-hexane mixture, this system was shaken during 30 minutes. Later it was centrifuged at 4000 g by 5 min, the organic phase was collected and it was introduced through a 15 cm x 15 cm glass column packed with activated fluorisil. The samples were eluted with a methylene chloride-hexane mixture (50:50) and later evaporated with nitrogen. 10 µl of the samples were injected in to a gas chromatograph. On the other hand 0.5 g sediment was tested in the same form. This study was done in triplicate.

Gas chromatographic analysis

For analysis, 10 µl of the extract was injected into a Varian Star 3400 CX gas chromatograph equipped with a thermionic specific detector (TSD) and 30-m J&W DB-1 , 0.53 mm internal diameter infused methyl-silica column (film thickness, 1.5 µm). The carrier gas was hydrogen. The temperature program was as follows: 120 ºC during 1 min; 150 ºC during 30 min; 205 ºC during 10 min; temperature raised 10 ºC/min; 240 ºC during 5 min; temperature raised 2.0 ºC/min (Gluckman et al., 1986).

Sublethal exposure

Eight juvenile organisms were added to an aquarium that contained 150 g of IRD sediment spiked with 0.1849 mg/kg of MA (96h-LC_0) in a colloidon bag (14,000 daltons) and reconstituted with 15 l of dechlorinated water. A control group was tested also. The duration of the exposures

was 0, 12, 24, 48, 72 and 96 h. After exposure periods the fish gills were excised and homogenized with tris buffer at pH 7. The homogenized solution was centrifuged at 12,500 g by 15 min. The supernatant was used for biochemical determinations (lipid peroxidation level and AChE activity). This study was done in triplicate.

Acetylcholinesterase activity

The supernatant was used to determine AChE activity. It was assayed by the Hestrin method (1949). 1 ml of supernatant was added with 2 ml of buffer tris pH 7.0 and 1 ml of acetylcholine standard (80 µmol), this system was incubated at 25 ºC for 35 min. The reaction was stopped by adding 2 ml of alkaline hydroxilamine 2M, 1 ml of HCl 4 N and 1 ml of $FeCl_3$ 0.37 M in 0.1 N HCl. The developed color was assayed at 540 nm in a spectrophotometer (Varian DMS90). Protein concentration was estimated by the Bradford method (1976) using bovine albumin as the protein standard. Enzyme activity was expressed as µmol of hydrolyzed ACh/mg protein/min.

Lipid peroxidation levels

Lipid peroxidation levels were assayed by the modified Beuge and Aust (1978) method. 0.5 ml of supernatant was added with 2.0 ml of 0.37 percent thiobarbituric acid dissolved in trichloroacetic acid. Samples were put in a water bath at 37 ºC for 30 minutes, and then cooled for 15 min in an ice bath. The developed color was assayed at 532 nm in a spectrophotometer. 1,1,3,3-tetramethoxypropane (malondialdehide bis) (MDA) was used as malondialdehide standard. Lipid peroxidation level was expressed as nmol MDA/mg protein.

Statistical analysis

The experimental data were analyzed by unifactorial variance analysis (ANOVA) and the mean differences of each group were compared using the Duncan test for multiple comparisons against a single control. Differences were considered significant at $P < 0.05$.

RESULTS

Physicochemical characterization

The general physicochemical characterization of artificial sediment is given in Table 1. The pH mean value is 6.42, slightly acid. Organic matter and organic carbon are high for aquatic ecosystems, this last parameter represents 46.37 percent of total organic matter. Mechanical sediment analysis showed that this substrate is formed mainly by clay, this parameter gives the sediment a great surface area (particle size < 0.002 mm). The second type of particle prevailing in soil was silt (represent 25.82 percent of total soil), and the third type was sand, which was less than 5 percent of the total.

Malathion concentration in water and sediment

The study of pesticide recovery showed a recovery level between 75 and 105 percent for the three substrates (water, sediment, and organisms) with a coefficient of variation of 7 percent at zero time. The mean MA value from IRD water expressed in µg/l was 0.001 ± 0.0002 (representing the average of three replicates). The MA content in sediment was 0.015 µg/kg (Table 1). The MA content in sediment is 15 times higher than the pesticide concentration in water. The MA in water exceed the threshold effective limit established by the EPA (1976), which is 0.0001 mg/l.

Acute toxicity

In the control group (dechlorinated tap water) rainbow trout displayed typical behavior. At other concentrations of MA after 24 h of exposure, the fish presented apnea signs (gasping) and white stains in the skin. The 96h-LC_{50} value for MA in water test against *O. mykiss* was 0.1164 mg/l (95 percent confidence limits: 0.112 and 0.120). The value of 96h-LC_{50} for MA in sediment on *O. mykiss* was 0.765 mg/kg (95 percent confidence limits: 0.698-0.890). The 96h-LC_{50} in sediment was 6.57 times higher than LC_{50} in water.

Elimination of MA in IRD sediment and rainbow trout gills

The behavior of MA in spiked IRD sediments can be observed in Figure 1. These results showed a decrease in the pesticide concentration with respect to time. However in the fish, a rapid absorption of the insecticide is observed. MA in fish exhibited a rapid bioconcentration, since the time between the maximal absorbed concentration and the beginning of depuration was long (Figure 1). The one-compartment model gave a good description of MA elimination from gill organisms and sediments. The elimination constants from sediment (Kes) and gill (Keg) were obtained by linear regression analysis (Kes =-0.0065; r^2=0.998; Keg=-0.0038, r^2=0.991) and the half-life from sediment (T1/2s) and gill (T1/2g) were T1/2s =106.63 ±5.5 and T1/2g =182.4±9.3 h.

Acetylcholinesterase activity

O. mykiss controls produced an average of 0.0079 ± 0.00065 µmol of ACh hydrolyzed/mg of protein/min. When fishes were intoxicated with 0.1849 µg/Kg of MA (LC_0) at 96 h of exposure, AChE activity was decreased 93.80 percent with respect to control group at all exposure times monitored (12-96 H) (Figure 2) ($P < 0.05$).

Lipid peroxidation level

When the trout *O. mykiss* was exposed to 0.184 mg/kg of MA during 12, 24, 48, 72 and 96 h, the lipid peroxidation levels were increased in 78.91, 85.97, 91.95, 92.28 and 94.3 percent respectively comparing to control group (Figure 3) ($P < 0.05$).

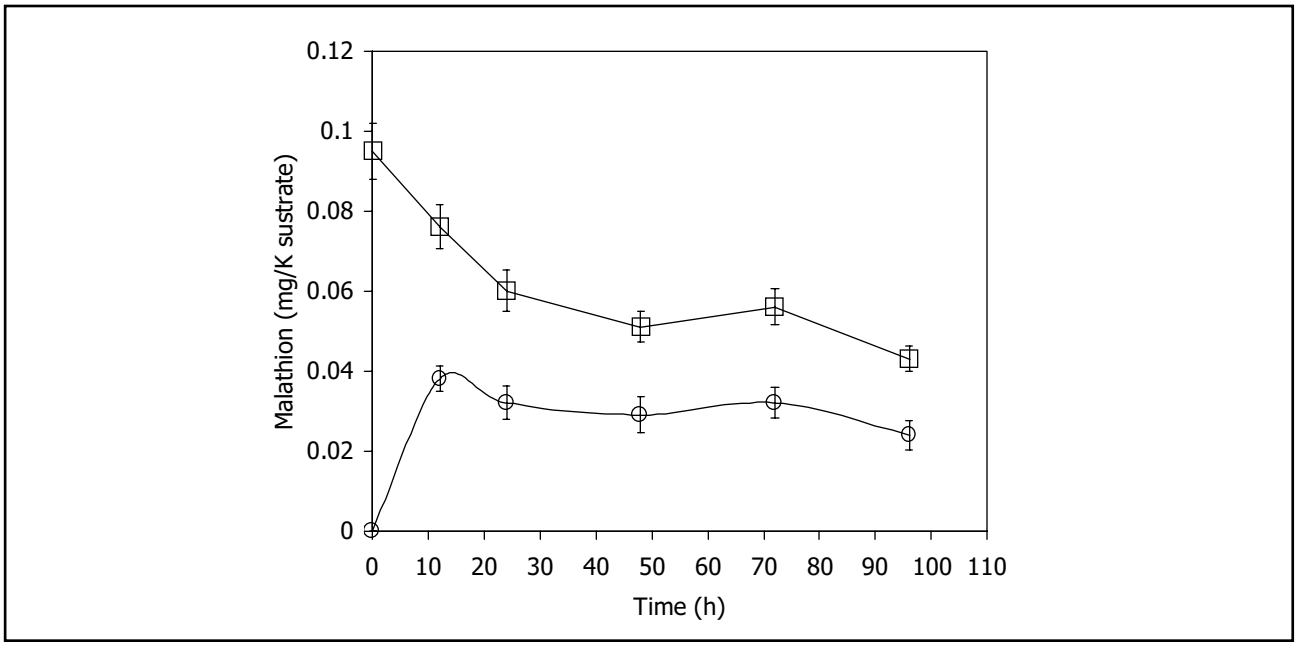

Figure 1. Temporary course of spiked malathion in sediments of Ignacio Ramírez Dam (•) and rainbow trout *O. mykiss* gill (O). Each value represents an average of three replicates± standard deviation.

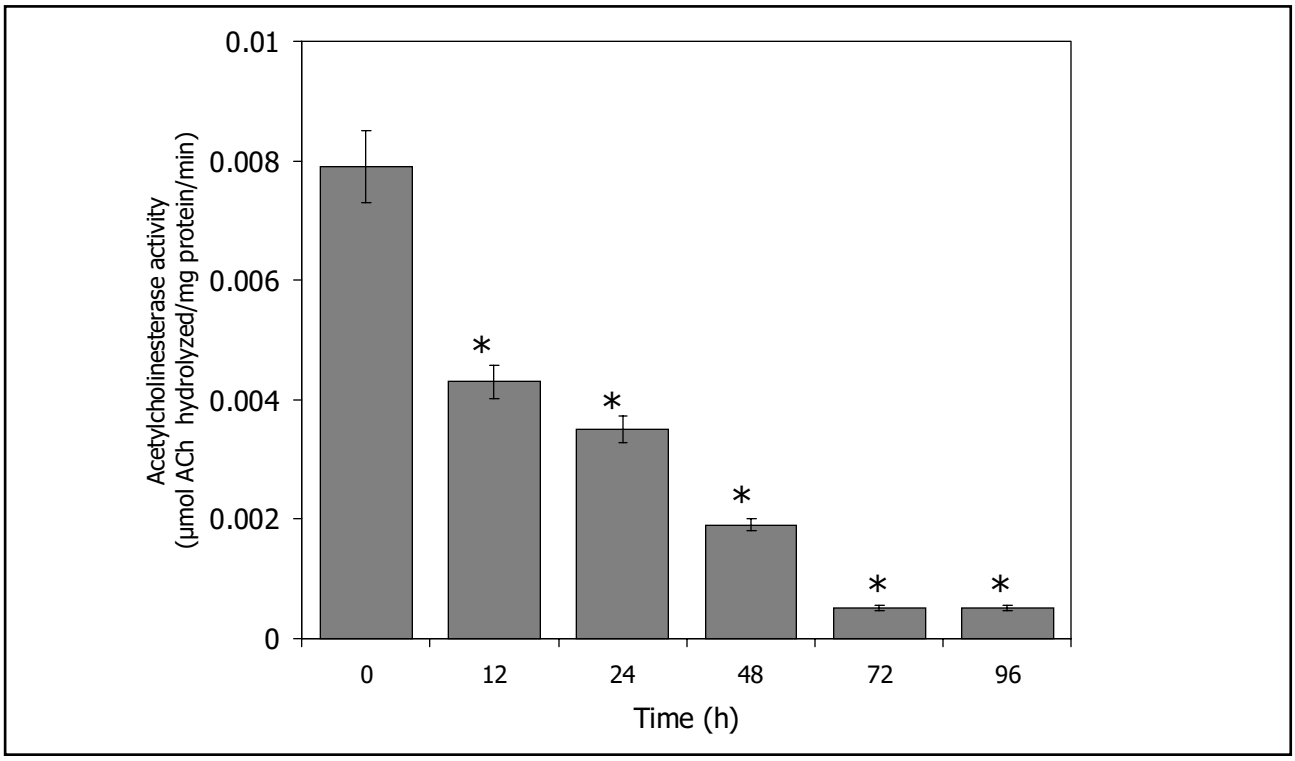

Figure 2. Toxic effect of malathion spiked in Ignacio Ramirez sediment on acetylcholinesterase activity of rainbow trout*O. mykiss*gill. Each value represents an average of three replicates ± standard deviation; *$P < 0.05$.

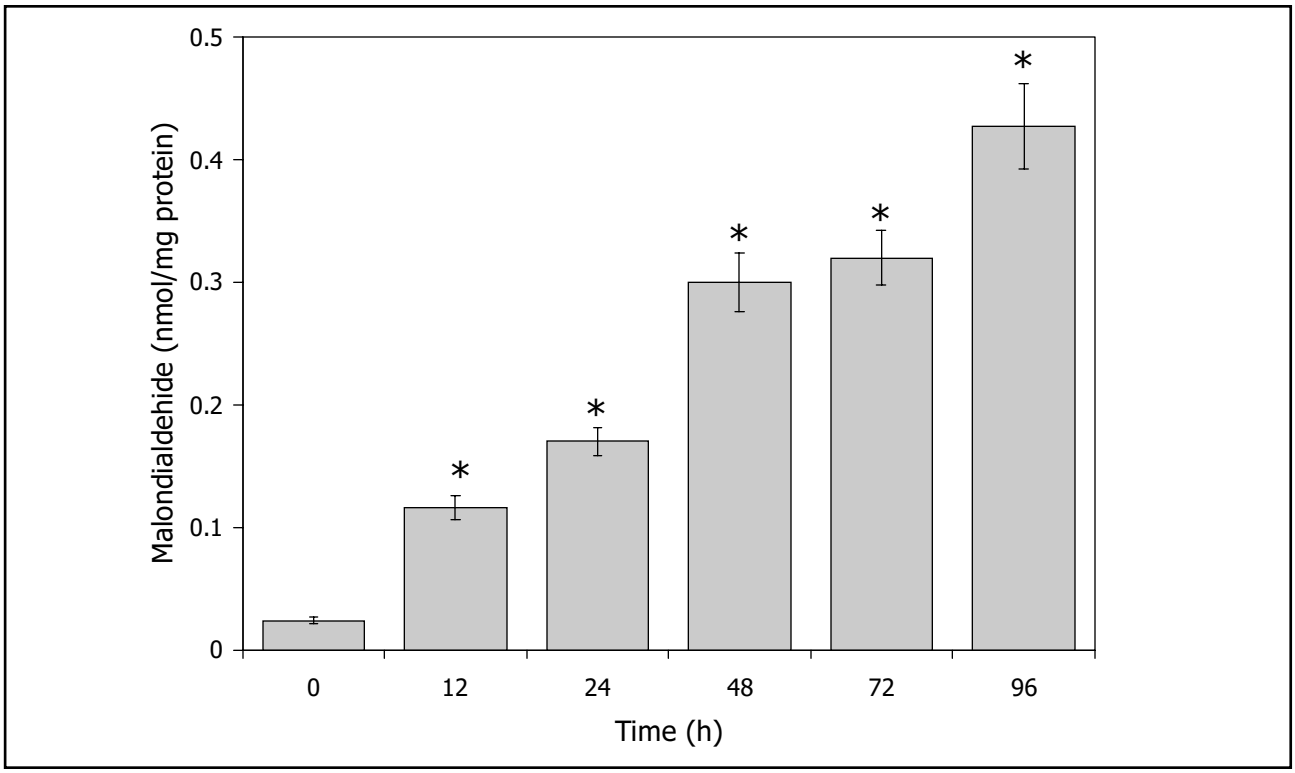

Figure 3. Toxic effect of malathion spiked in Ignacio Ramírez sediment on lipid peroxidation level of rainbow trout*O. mykiss*gill. Each value represents an average of three replicates ± standard deviation; *$P < 0.05$.

DISCUSSION

There exists a specific scale of pH that can produce the death of test organisms and also affect the contaminants bioavailability in sediments (SETAC, 1993). The value of pH in the IRD sediments was 6.42, which favors MA persisting longer in sediments. Freed et al. (1979) found that the MA T½ as pH 8 was in the range of 2.0-5.3 d, but at pH 7.4 was 11 d.

The organic matter content was high and this property can contribute to the adsorption of MA in the sediment. Together with this, the silt and clay percentages may favor adsorption with absorption being able to form complexes that permit MA persistence in sediments. The sand content was lower than silt and clay, and this characteristic may contribute to the vertical and horizontal availability in sediment (Freed et al., 1979).

MA concentration in water and sediment was 0.001 µg/l and 0.015 µg/kg respectively (Table 1). Organism studies have indicated an association between the presence of this pesticide residue and its toxicity. Mishra and Srivastava (1983) found MA caused a severe drop in erythrocyte count in catfish *Heteropneustes fossilis.* In gill fish, massive degeneration of filament from malathion exposure has been shown (Richmonts and Dutta, 1989).

The results of acute toxicity of malathion in rainbow trout showed that 96h-LC_{50} in water was 0.1164 mg/l while in sediments was 0.765 mg/kg. The 96h-LC_{50} in water value is smaller than that reported by Macek and Mcallister (1970) whose value was 0.170 mg/l, similar to that reported by Post and Shroeder (1971), whose value was 0.122 mg/l. Both studies used trout. For sediments there are no values of acute toxicity reported.

The processes by which a foreign chemical is taken up into a terrestrial animal cannot be applied to fish. This is because the aquatic environment imposes several constraints. One of the most critical is the matter of obtaining sufficient oxygen. A fish must breathe roughly 20 times more than a terrestrial animal in order to obtain an equivalent amount of oxygen. The site of oxygen uptake in fish is primarily the gills (Heath, 1987). Therefore the gill tissue of *O. mykiss* (which is the main point of entry for dissolved substances) was used to determine the MA uptake.

Kanasawa (1975) reported 50 percent degradation of MA in fish after 3-4 d of exposure, which is similar to that reported in the present case (T½ of MA in *O. mykiss* was 182.4 H, corresponding to 7.6 d). These results indicated that MA was eliminated slowly from the organism (Figure 1). Sujatha et al (1995) reported that MA could be stored in lipids and therefore stayed in the organism, increasing the . In Figure 1 it can be observed that the first phase represents a period of transfer of MA from sediment to the gill. Lastly, the plateau represents a steady-state period where the uptake and elimination of the pesticide are in equilibrium. These results suggest that MA in fish exhibited a rapid bioconcentration, since the time between the maximal absorbed concentration and the beginning of depuration was long (70 h). It can be suggested that the elimination of the xenobiotic was produced slowly. The T½ of MA in *O. mykiss* was of 182.4 h, which corroborates the last suggestion.

Concomitantly when MA was absorbed by trout, a decrease of this pesticide was observed in the sediment (Figure 1). MA concentration spiked in sediment diminished importantly in the first 24 h (near of 50 percent). The T½ in IRD sediment was 106.63 h, which reflects the low persistence of this pesticide. Howard (1971) classifies this chemical as a low persistence pesticide because 50 percent is degraded before 30 d.

A variety of other tissues besides brain also have AChE. The extent of methyl parathion inhibition of this enzyme in these tissues was compared, in which *Tilapia* were exposed for 48 h to a concentration 1/3 the LC_{50}. Following this treatment, the relative amount of AChE inhibition was found to be greatest in the brain, followed by muscle, gill, and liver in that order (Rao and Rao, 1984). In this study, it can be observed that with time the AChE activity was diminished up to 93.80 percent with respect to the control group ($p < 0.05$). Ahammad-Said and Ramana (1980) reported that when *Tilapia mossambica* was exposed to MA and parathion a decrease in AChE activity was observed. Post and Schroeder (1971) noted a 50 percent reduction in *Coho salmon* following exposure to MA, when rainbow trout was exposed to this pesticide the reduction in AChE activity was lower. This investigation reaffirms the action mechanism of MA, since is well known that organophosphate pesticides affect the central nervous system by inhibition of AChE, the enzyme that prevents the buildup of acetylcholine (Murty and Remni, 1992).

Lipid peroxidation is a degenerative process, in organisms it is an important factor that contributes to aging and degenerative processes, including cancer and a multitude of pathological problems. It affects the polyunsaturated fatty acids of membrane phospholipids. The general mechanism of this process involves the formation of toxic aldehydes, which react with protein and nonprotein substances resulting in widespread changes in cellular membranes. In this study, the results of lipid peroxidation are showed in Figure 3, where it can be seen that as time passes the lipid peroxidation levels increased. When the rainbow trout was exposed to MA over 96 h, an increase of 94.32 percent was observed with respect to control group ($P < 0.05$). These findings are compatible with studies of Yarsan et al. (1999). These investigators had proposed that the damage produced by MA stimulate lipid peroxidation, via the consumption of the superoxide dismutase, glutathione peroxidase, and catalase enzymes for prevention of peroxidation cases. Another possibility would be that mentioned by Parkinson (1996) that during biotransformation processes of MA to malaoxon by microsomal enzymes, free radicals are produced.

Another explanation could be that MA, as well as other organophosphate pesticides, require metabolic activation for the expression of its toxic effects (Ecobichon, 1996). The microsomal enzymes on many occasions could generate free radicals (0_2 and H_20_2) and it is probable that these reactive groups provoke a stimulation in the lipid peroxidation processes; therefore, it may be suggested that MA could act in the same way.

Finally, the fish exhibited a lack of recovery in AChE activity and in lipid peroxidation level, although the MA elimination was more than 75 percent of these organisms. Wallace and Herzberg (1988) observed a lack of significant spontaneous reactivation of dimethyl phosphorylated serum esterases from birds dosed with demeton-S-methyl. They noted that it may be due to either (1) the aging of the dimethyl phosphorylated enzyme or (2) the presence in excess of the parent compound, demeton-S-methyl, or its sulfoxide or sulfone derivatives, in a free state or nonspecifically bound to nonesterasic protein , e.g., albumin (Martin et al, 1981). In the latter case, reactivation would be masked by further inhibition.

CONCLUSIONS

The results show that the MA is bioconcentrated in fish. MA quantification in water, sediments and organisms from the IRD is not enough to determine the impact of this pesticide on this water body, due to the fact that the toxic effect remained even though the MA concentration had diminished in the three components. It is necessary to develop toxokinetics, toxodynamic and

chemical analysis in order to establish safety limits for aquatic biota. Additionally, it is recommended to use the gills and the determination of AChE activity and lipid peroxidation levels for toxic response evaluation, because this organ and damage biomarkers are very sensitive to effects produced by MA at sublethal concentrations in fish.

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ADDRESS FOR CORRESPONDENCE
Laura Martinez-Tabche
Departamento de Toxicología, Sección de Graduados
Escuela Nacional de Ciencias Biológicas
Instituto Politécnico Nacional
Apartado Postal 105-314
Del M. Hidalgo,
C.P. 11340 D.F. Mexico

E-Mail: ltabche@bios.encb.ipn.mx