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# The Effect of Malathion, Diazinon, and Various Concentrations of Zinc, Copper, Nickel, Lead, Iron, and Mercury on Fish

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## ABSTRACT

Acute and chronic toxicity tests for malathion, diazinon, copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), nickel (Ni), and iron (Fe) were conducted. Mortalities of *Barilius vagra* and *Cyprinus carpio* (common carp) were variable but LC50-96 hr were similar for pesticides. Adult *B. vagra* seem to be more sensitive to malathion than juvenile carp. Both juvenile carp and adult *B. vagra* were extremely sensitive to diazinon. Long-term exposure to pesticides modified morphology and behavior. The LC50-96 values for Cu, Hg, and Pb were 0.3, 0.16, and 0.44, respectively, for smaller fish and 1.0, 0.77, and 1.33, respectively, for larger fish. Replicate LC50 values for Zn, Ni, and Fe were somewhat variable, and for these metals, the size of the fish seemed to affect response because LC50 values increased as fish size increased. Cooper, Pb, Zn, and Fe residues following exposure to sublethal concentrations of these metals for 15 d were significantly greater in whole juvenile common carp than in controls.

**Index Entries** Common carp; *Barilius vagra*; pesticides; malathion; diazinon; acute toxicity; chronic toxicity; LC50; zinc; copper; nickel; lead; iron; mercury.

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## INTRODUCTION

Pollution of the aquatic environment by toxic substances is a cause of growing concern throughout the world, especially in developing nations. The immediate concern is human health and welfare, but the effect of pollution on aquatic organisms also has ecosystemwide consequences. Several studies addressed the effects of pollution on fish and water quality in the Indo-Pakistan subcontinent (1–6). The two major sources of pollutants that endanger water quality on this subcontinent are agricultural and industrial effluents. In Pakistan, organophosphate, organochlorine, carbamate, and pyrethroid pesticides are commonly used in agriculture and horticulture. The stability and environmental persistence of organochlorine pesticides (e.g., DDT and dieldrin) resulted in their increased agricultural use in Indo-Pakistan (7,9–11). Efforts by the government were intense to stimulate industrial and technological development, which in turn can increase industrial pollution. The benefits and losses of agricultural pesticides and industrial effluents have to be addressed.

Malathion and diazinon are the two most widely used organophosphate pesticides in Pakistan. Malathion {0,0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate} and diazinon {0,0-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate} have moderate to high chemical stability (12). They are contact poisons that are used as foliage sprays to control soft-bodied insects such as cabbage root fly and carrot fly. The primary target of these pesticides is the enzyme acetylcholinesterase (AChE), which normally destroys the neurotransmitter acetylcholine (ACh) at synaptic junctions. Only few studies have been conducted in Pakistan to test the toxic effects of malathion, diazinon, and other organophosphate pesticides on fishes (13–15).

These pesticides regularly enter aquatic environments in amounts considered safe for human health but perhaps hazardous to fish and other aquatic organisms. However, in the absence of experimental work verifying the harm of these chemicals on fish, verification of their effects on the survival of local fish species is impossible. The objective of the experiment reported in this paper was to determine the effects of these two pesticides and selected metals on survival of fishes.

## MATERIALS AND METHODS

Acute toxicity tests were based on mortalities of fish exposed to known concentrations of selected pesticides and several selected metals during 96 h (4 d). Observations continued for surviving fish. The test conditions included acclimation of fish to laboratory tanks (10 gallon glass aquaria and an indoor cement tank), transfer of at least 10 fish to individual aquaria (3 L unless otherwise specified) containing the toxi-

cant, transfer of the same number of control fish to toxicant-free aquaria (3 L unless otherwise specified), continuous aeration of stock and experimental tanks, and daily or bidaily replenishment of the toxicant and water in the test tanks. All fish not undergoing experiments were fed a daily ration of trout chow pellets of appropriate size or a locally prepared mixture of egg yolk, white flour, semolina, and a dash of aquarium fish food. Fish subjected to acute toxicity tests (96 h) and control fish were not fed during the experimental period. Fish mortalities were recorded daily and the median lethal concentration for the test substances determined (LC50-96-h = TLM 50-96 h). The LC50 determinations based on adjusted Probit analysis (16) and Trimmed Spearman-Kärber analysis (17) were computed and compared to one another.

### **Pesticides**

For acute toxicity tests, two pesticides were selected: 57% E.C malathion (organophosphate pesticide) and 35% E.C diazinon (organophosphate pesticide). The two organophosphates were provided by the Agrochemical company. Appropriate concentrations of the pesticides (Tables 1-4) were established by dilution with water. The pesticides were thoroughly mixed in the test chamber before fish were added. All of the fish in the studies were of the same approximate size. The malathion tests on juvenile common carp were conducted on groups of two different sizes ( $X = 2.5$  and  $5.0$  cm). Diazinon tests were conducted on juvenile common carp ( $X = 7.1$  cm) and adult *B. vagra* ( $X = 6.8$  cm). Daily mortalities, behavior, and gross morphological changes were recorded.

### **Selected Metals**

Acute toxicity tests for several selected metals were conducted with  $\text{CuSO}_4$ ,  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{ZnCl}_2$ ,  $\text{NiCl}_2$ , and  $\text{FeSO}_4$ . All stock solutions (100 ppm) were prepared in deionized water. Predetermined dilutions of the stocks were made to achieve the final test concentrations (Tables 5,6).

### **Chronic Toxicity Tests**

Experiments were conducted to determine the chronic effects of malathion on the survival of juvenile common carp. The final concentrations of malathion in the experiments were between 0.1 and 2.0 ppm. All fish, 10-20/test ( $X = 3.5$  cm), were maintained in 10 gallon glass tanks containing 12 L of tap water. A control tank with comparable numbers of fish was established for each test regime. Mortalities and gross morphological and behavioral alterations were recorded daily. Dead fish were measured (standard length), weighed to the nearest mg on an electric balance (Mettler), and preserved ( $-20^\circ\text{C}$ ) for subsequent bioaccumulation determinations. During the experimental period, fish were fed daily and the tanks were continuously aerated. Pesticide concentrations were replenished weekly following the procedures of Johnson (8).

Table 1  
Mortalities and LC50-96 h of Juvenile *Cyprinus carpio* Exposed to Malathion

Experiment	No. of dead fish at each concentration, ppm					Probit LC50-96 h ppm	Adjusted Probit slope	Trimmed LC50-96 h ppm
	8.55	11.4	14.25	14.8	17.1			
1, X = 2.5 cm	0	2	4	7	9	13.80	12.30	10.21
2, X = 5.0 cm	1	3	3	9	10	12.81	9.46	10.38

X = mean size; N = 10 fish per test; 25°C, O<sub>2</sub> = 6.43 mg/L, pH = 7. LC50-96 hr determined by adjusted Probit analysis and compared with Trimmed Spearman-Kärber method.

Table 2  
Mortalities and LC50-96 Values of *Barilius vagra* Exposed to Malathion

Experiment	No. of dead fish at each concentration, ppm				Probit LC50-96 h ppm	Adjusted Probit slope
	4.00	6.50	8.55	11.40		
1	2	1	7	9	7.39	5.02
2	1	3	4	10	7.66	6.02

X = 6.0 cm; N = 10 fish/test; 20°C, O<sub>2</sub> = 6.5 mg/L, pH = 7.2.

Table 3  
Mortalities and LC50-96 Values of Juvenile *Cyprinus carpio* Exposed to Diazinon

Experiment	No. of dead fish at each concentration, ppb				Probit LC50-96 h ppb	Adjusted Probit slope
	4800	7200	9600	12,000		
1	0	4	7	10	4974.5	1.34
2	1	3	4	9	3426.8	1.67

X = 7.1 cm; N = 10 fish/test; 25°C, O<sub>2</sub> = 6.5 mg/L, pH = 7.1.

Table 4  
Mortalities and LC50-96 Values of *Barilius vagra* Exposed to Different Concentrations of Diazinon

Experiment	No. of dead fish at each concentration, ppb				Probit LC50-96 h ppb	Adjusted Probit slope
	1200	2400	4800	7200		
1	3	8	4	10	1914.3	1.68
2	0	4	8	10	2939.1	5.26

X = 6.0 cm.

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Table 5  
Mortalities and LC50-96 h Values of *Cyprinus carpio* Exposed to Different Concentrations of Cu, Hg, and Pb

Metal	Size, cm	No. of dead fish at each concentration, ppm					Probit LC50-96 h ppm	Adjusted Probit slope	Trimmed LC50-96 h ppm
		0.1	0.5	1.0	1.5	2.0			
Cu	3.5	2	6	8	10	—	0.30	2.036	—
	6.5	—	2	4	6	10	1.00	3.640	—
Hg	3.5	4	7	10	—	—	0.16	2.014	0.57
	3.5	0	3	6	10	—	0.71	4.450	0.62
	6.5	—	2	6	10	10	0.77	5.580	0.94
Pb	3.5	2	4	6	10	—	0.44	1.723	—
	6.5	—	3	5	9	10	0.80	4.020	—
	6.5	—	1	4	3	9	1.33	3.330	—

Smaller juvenile fish ( $X = 3.5$ ) were exposed to concentrations of 0.1, 0.5, 1.0, and 1.5 ppm. Water temp., 20°C. Larger juvenile fish ( $X = 6.5$ ) were exposed to concentrations of 0.5, 1.0, 1.5, and 2.0 ppm at 15°C.  $N = 10$  fish per test,  $O_2 = 6.4$  mg/L,  $pH = 7.1$ .

Table 6  
Mortalities and LC50-96 h Values of *Cyprinus carpio* Exposed to Different Concentrations of Zn, Ni, and Fe at 15°C Water Temperature

Metal	Size, cm	No. of dead fish at each concentration, ppm							Probit LC50-96 h ppm	Adjusted Probit slope	Trimmed LC50-96 h ppm
		0.1	0.5	1.0	2.0	2.5	3.0	3.5			
Zn	3.2	2	4	6	9	10	10	—	0.45	1.93	0.83
	3.2	0	3	3	2	9	10	—	1.34	2.30	1.24
	6.0	—	0	3	4	—	9	10	1.64	4.30	1.64
	6.0	—	0	4	3	—	4	9	2.25	2.40	1.66
Ni	3.2	0	1	4	4	6	10	—	1.54	2.70	—
	3.2	1	1	3	4	8	10	—	1.30	1.89	—
	6.0	—	0	3	4	—	9	10	1.64	4.30	—
	6.0	—	0	2	4	—	4	9	2.30	3.00	—
Fe	3.2	2	3	5	9	9	10	—	0.56	1.80	—
	3.2	0	6	4	4	4	9	—	1.36	1.20	—
	6.0	—	1	4	6	—	10	10	1.22	3.70	—
	6.0	—	0	4	3	—	4	9	2.25	2.37	—

Smaller fish (3.2 cm) were exposed to concentrations of 0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 ppm. Larger fish (6.0 cm) were exposed to 0.5, 1.0, 2.0, 3.0, and 3.5 ppm concentrations.

## RESULTS

### *Acute Toxicity Tests: Pesticides*

#### *Malathion*

Mortalities were variable, but LC50-96-h values in response to malathion were similar in juvenile and adult carp. According to Probit analysis, the LC50-96-h values were 13.8 and 12.81 ppm for the two sizes of fishes, but the LC50 values by Trimmed Spearman-Kärber analysis were 10.21 and 10.38 ppm for these two sizes (Table 1). The LC50-96-h values from Probit analysis were greater for the smaller sized fish than for the larger fish. Adult *B. vagra* seem to be more sensitive to malathion than juvenile common carp. The LC50-96-h values in replicate tests were 7.39 and 7.66 ppm (Table 2). Control fish of both species appeared unaffected and suffered no mortalities in all tests. Fish that survived longer than 96 h showed behavioral modifications. Impaired locomotion was noted at concentrations as low as 0.57 mg/L, and some fish developed deformed vertebral columns (scoliosis of the posterior body) and dark skin at a concentration of 8.5 mg/L. The fish ultimately died even if transferred to toxicant-free water. *Basilus vagra* showed darkening of the skin at a concentration as low as 4.0 mg/L (ppm) for longer than 7 d but did not develop scoliosis.

#### *Diazinon*

Both juvenile common carp and adult *B. vagra* were extremely sensitive to this pesticide. The LC50-96-h values for common carp in replicate tests were 4974.5 and 3426.8  $\mu\text{g/L}$  (ppb) (Table 3). Fish surviving (at 4800 ppb) for 7 d or more developed impaired swimming, darkening of skin, and scoliosis. The LC50-96-h values in replicate tests on *B. vagra* were 1941.3 and 2939.1  $\mu\text{g/L}$  (Table 4). Adults of this species seem to be more sensitive to the pesticide than juveniles of the common carp. Although body darkening was evident in some individuals that survived exposure to lower concentrations (1200  $\mu\text{g/L}$ ) beyond 4 d, no scoliosis was evident.

### *Chronic Tests*

Exposure to malathion for 40 d yielded a LC50-30-d value of 1.4 mg/L for juvenile common carp ( $X = 3.5$  cm). Chronic tests when repeated exposing the juveniles ( $X = 4.0$  cm) for 70 d to concentrations of 0.1 mg/L, 0.57 mg/L, 1.0 mg/L, and 2.0 mg/L yielded a LC50-60-d value of 0.8 mg/L. The exposed fish again showed morphological and behavioral modifications. Concentrations as low as 0.57 mg/L caused darkening of the skin after 30 d and in rare cases scoliosis. The incidence of scoliosis increased with concentration. The first fish to die at any test concentration suffered scoliosis and weight loss.

### Acute Toxicity Tests: Selected Metals

The LC50-96-h values for each metal are based on adjusted Probit analysis (Tables 5,6). The LC50-96-h values for smaller fish (3.5 cm) were 0.3, 0.16, 0.71 (replicate for Hg), and 0.44 ppm for Cu, Hg, and Pb, respectively. The LC50-96-h values for larger fish (6.5 cm) were 1.0, 0.77, 0.8, and 1.33 (replicate for Pb) for Cu, Hg, and Pb, respectively (Table 5). Larger fish seem to be substantially less sensitive, based on LC50 values, than smaller fish. Figures 1 and 2 show the mortality of smaller and larger juvenile carp when exposed to different concentrations of Cu, Hg, and Pb. Replicate LC50 values for Zn, Ni, and Fe were variable. The LC50 values increased with fish size (Table 6). Relationship between the concentration of metals (Zn, Ni, and Hg) and mortality for different sized fish is shown in Fig. 3. Probit analysis revealed an appreciable difference in response by different sized fish. Such a difference was not evident when the same data were analyzed by the Spearman-Kärber method (Tables 5,6). Analysis with the Spearman-Kärber technique rendered less pronounced differences in LC50 values between replicate tests than analysis with the Probit method. Significant differences in levels of residues were detected between the control and treated groups (Table 7). The residues of Fe, Cu, and Pb were respectively eightfold, fivefold, and nearly twofold higher in the treated group than in the control group. The concentrations of Zn and Pb were comparable.

## DISCUSSION

Responses to experimental exposure to organophosphate pesticides (7,8,10,18–22) varied considerably by species of fish. The observed range of acute malathion toxicity (LC50-96-h) is 12.5 ppm for fathead minnows *Primephales promelas*, 0.1 ppm for rainbow trout *Oncorhynchus mykiss*, and 0.02 ppm for bluegills *Lepomis macrochirus* (18). Bluegills are extremely sensitive to malathion and died at a concentration of 0.1 ppm in 96 h (23), in contrast to fathead minnows, which died at concentrations of 9.0–10.45 ppm. Javaid and Munawar (13) reported wide differences in response to *Channa punctatus* to diazinon (TLm 50 = 0.456 ppm) and malathion (TLm 50 = 0.92 ppm). The acute LC50-96-h for malathion in juvenile common carp was 13.8 and 12.81 ppm for fish with mean size of 2.5 and 5.0 cm. These values are comparable to those for the fathead minnows (10,23). Adult *B. vagra* were considerably more sensitive to malathion than juvenile common carp. The 96-h LC50 values for *B. vagra* were slightly greater than 7.0 ppm.

Diazinon seemed to be more toxic than malathion; 96-h LC50s ranged between 3.42 and 4.97 ppm for juvenile common carp and between 1.9 and 2.9 ppm for adult *B. vagra*. Considerably lower levels have been reported for the 96-h LC50 for rainbow trout juveniles (0.38 ppm)

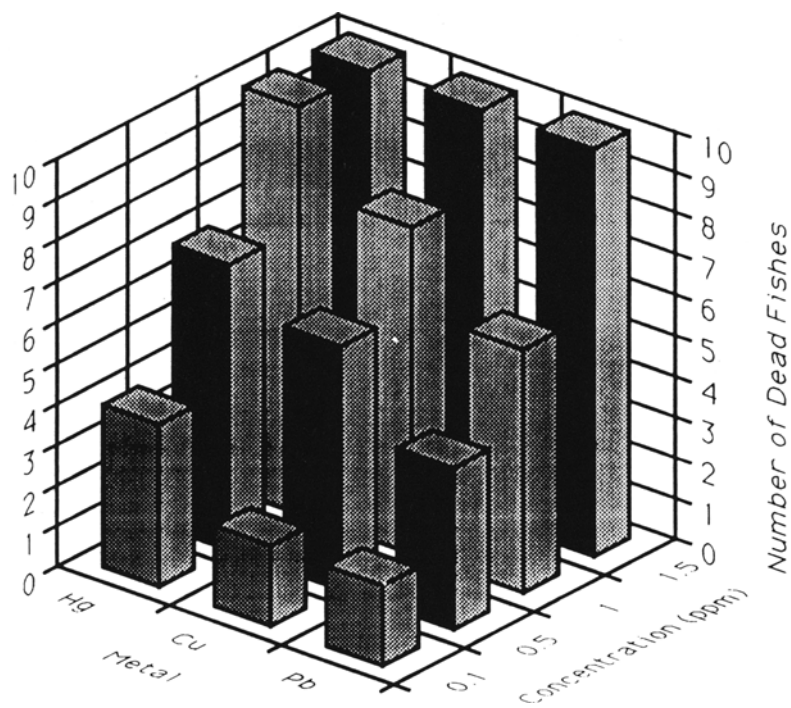


Fig. 1. Mortality of smaller juvenile carp at different concentrations of Hg, Cu, and Pb.

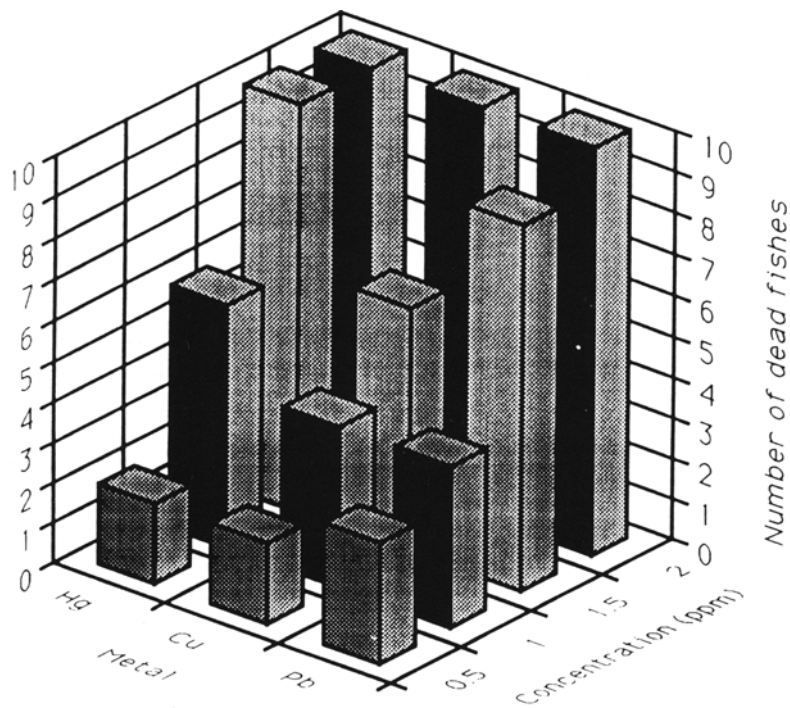


Fig. 2. Mortality of larger juvenile carp at different concentrations of Hg, Cu, and Pb.



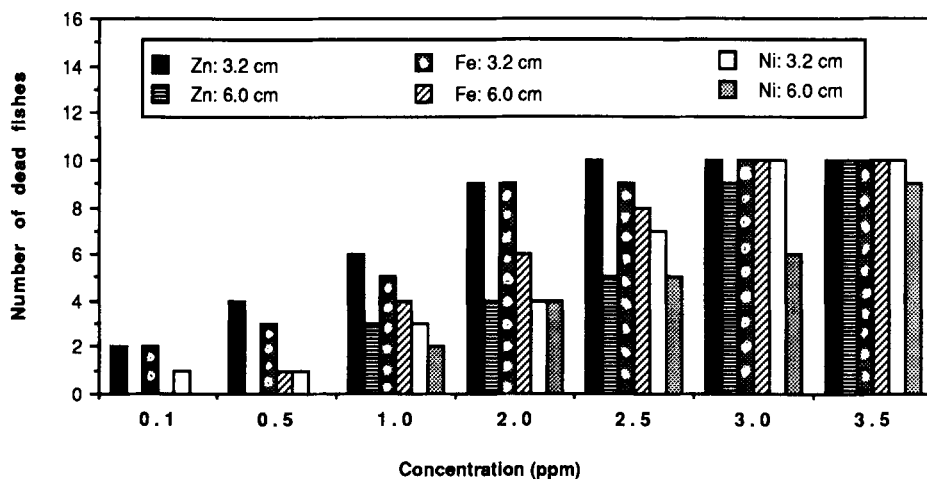


Fig. 3. Relationship between different concentrations of Zn, Ni, and Fe and number of dead fishes. Size of fish is mentioned next to the metal.

and adult *C. punctatus* (0.445 ppm) (13). An earlier study of juvenile common carp (20) showed the 24- and 48-h LC50 values for diazinon to be 7.0 and 3.2 ppm. Based on these data, one expects the 96-h LC50 to be lower than the levels obtained in this study. Possible explanations might involve differences between the two studies in oxygen levels, pH, temperature, and water hardness. Differences existed also in the mean size and perhaps the adaptive history of the carp used in the two studies. Local or regional differences in responses of carp to diazinon were reported previously by investigations of Sastry and Malik and Javaid and Munawar (13,24). Sastry and Malik (24) estimated the 96-h LC50 to be 3.1 ppm but (13) showed it to be 0.445 ppm. These data collectively support the hypothesis that local conditions and differences in fish can influence the responses of a given species to particular pesticides; data from one geographical region or locality may not be applicable in other geographical regions or localities.

Long-term tests of contaminant toxicity should extend over at least one generation and are best conducted in a flow-through system (23,25). Long-term tests in this study had to be restricted to relatively shorter periods (at most 3 mo duration). Thirty-day LC50 and 60-d LC50 values dropped to 1.4 and 0.8 ppm, respectively, when juveniles of common carp were exposed to 1.0 ppm concentration of malathion. A 70-d exposure to 0.1 ppm malathion caused no mortalities or behavioral changes. Similar results were obtained in long-term tests on several other species of fish by Javaid and Munawar, Eaton, and Pickering and Henderson (13, 23, 26); concentrations close to 1.0 ppm were innocuous in long-term situations.

Table 7  
Bioconcentration of Metals in Whole Juvenile Carp Exposed  
to Cu, Pb, Zn, and Fe for 15 d

Group	Cu, ppm	Pb, ppm	Zn, ppm	Fe, ppm
	0.1	0.5	0.5	0.5
Control (toxicant free water)	0.00435 ±0.0007 (4)	0.0660 ±0.004 (4)	0.0950 ±0.007 (4)	0.0370 ±0.005 (4)
Treated	0.0244 ±0.005 (4)	0.1032 ±0.008 (5)	0.1330 ±0.015 (4)	0.3300 ±0.06 (6)

Mean concentrations expressed as ppm ±SEM. Figures in parenthesis indicate the numbers of fishes per test.

Acute toxicity tests (96 h) provide a measure of the toxicity of compounds to a given species under specific environmental situations (water chemistry, pH, temperature, and so on) (13,24). They also reflect the severe and rapid damage from sudden exposure to lethal concentrations of contaminants. Accidental spillage of pesticides or the release of large amounts of pesticides in agricultural runoff creates conditions that are comparable to the results of the acute tests. On the other hand, chronic tests provide a measure of the effects of usually sublethal exposures over extended time (7,25). Although, sudden mortalities of fish from toxic amounts of pesticides in agricultural runoff are more profound (7), long-term exposure to extremely low levels (far below acute LC50 values) may reduce longevity, reproductive vigor, and size. These data on malathion suggest that concentrations below 0.1 ppm have no discernable effect on juvenile common carp.

One of the major questions in contaminant research is the concentration of a pesticide that has no effect (i.e., assessment of environmentally safe concentrations) on the sustained fisheries potential (7,10,23,25). A calculation of the environmentally safe concentration of maximum acceptable toxicant concentration (MATC) is usually determined by dividing the lowest concentration of a toxicant by the 96-h LC50 concentration. These procedures give a no-effect concentration called *the application factor of the toxicant*. This value can also be applied to other species of fish for which 96-h LC50 values are known (10,23). Accordingly, an application factor may be derived from the acute toxicity and chronic test data (70-d test) from this investigation on the common carp. Because the chronic tests should extend over a generation, these values should be used with caution. The application factor of malathion is 1/13.8 of the 96-h LC50 value. Because 0.57 ppm of malathion had some effects on the behavior of fish, the application factor must be between 1/13.8 and 0.57/13.8 of the 96-h LC50 value. According to Mount and Stephan (10), the application factor obtained from chronic tests on one species can be

used to derive application factors for other species as well. Whether the concentrations based on the malathion application factor for the common carp are indeed safe for the entire generation and for other species is unknown.

Undoubtedly, the acute lethality of pesticides (malathion and diazinon) is manifested by mortalities noted in the acute tests. The observed sublethal effects preceding death were escape behavior, increased locomotor activity, distress, and loss of equilibrium. Fish also exhibited a visible but often transient change in body coloration (darkening). These concentrations of malathion and diazinon resulted in persistent darkening of the body and deformities of the posterior back bone (crooked backbone or scoliosis). At malathion concentrations as low as 1.0 mg/L of water, scoliosis and body darkening were minimal but increased with the concentration. Behavioral and morphological changes also have been recorded in other species of fish exposed to organophosphates, especially malathion and diazinon. These neuromuscular and skeletomuscular effects involve altered function of the AChE enzyme (7,10,23,27,28).

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