Acute Toxicity and Behavioral Changes of the Gold Fish (*Carassius Auratus*) Exposed to Malathion and Hinosan

Saeid Shahbazi Naserabad [*1], *Alireza Mirvaghefi* [1], *Mohammad Hasan Gerami* [2], *Hamed Ghafari Farsani* [1]

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ABSTRACT

Background: Pesticides are widely used in agriculture. Excessive use of pesticides has health risk for human and threatens non-target organisms. This research aimed to determine lethal concentrations of malathion and Hinosan for *Carassius auratus* (5±1 gr) [mean ± SD].

Methods: Experiments were performed according to O.E.C.D for 4 days (96 h) and concentrations of 0, 1, 2, 4, 8 mg L^{-1} Hinosan and 0, 1, 2, 4, 16 mg L^{-1} malathion with three replicates. LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} for 24, 48, 72 and 96 h were determined using a probit analysis.

Results: The results indicated that the 96 h LC50 value of Hinosan and malathion for Gold fish was 4.02 and 4.71 mg/L, respectively. Fishes exhibited irregular, erratic and darting swimming movements, hyper excitability, bruise in the caudal section, loss of equilibrium and sinking to the bottom.

Conclusion: Malathion and Hinosan have medium toxicity for *C. auratus* and could cause irreversible harm and behavioral changes.

Keywords: *Carassius auratus*, Hinosan, LC_{50}, Malathion.

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INTRODUCTION

Aquatic ecosystems are the largest natural environments which are constantly faced with the threats such as deterioration in genetic and biological diversity. Although, these environments are not targets for pesticides, previous studies sighted the presence of pesticides and their metabolites in surface waters [1- 3]. The most commonly used organophosphates (OPs) are parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos and phosmet. Malathion is an organophosphate pesticide that is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication [4]. Hinosan, another organophosphate pesticide (Edifenphos; 0-ethyl S, S-diphenylphos-phorodithioate) was marketed by Bayer AG in 1968 to control *Pyricularia oryzae* on rice [5]. OPs are alkylating agents that chemically reaction with main cell macromolecules such as proteins, nucleic acids and lipids and disturb their performance [6,7]. The main mechanism of OPs is enzyme inhibition of the acetylcholinesterase which can cause disruption in central nervous system (CNS) and peripheral nervous system.

Goldfish (*Carassius auratus*) is a freshwater, benthopelagic fish of the family Cyprinidae of order Cypriniformes which is one of the most commonly kept aquarium fish with a wide distribution in central Asia, China and Japan.In Iran, it has been reported in Atrak, Gorgan, Gharasu, Tajan, Babol, Haraz, Sardab, Aras (including the middle aras and lower reaches of its tributary in Qareh Chai), Tonekabon, Pol-e Rud, and Safid rivers, the Anzali Mordab where it is now the largest population, the Gorgan Bay, the Boojagh Wetland and Alma- and Ala-Gol. The trade of ornamental fish is a significant worldwide economic activity for the aquaculture industry with wild caught freshwater fish being the most commercialized species [8]. *C.auratus* is considered as the most popular and favorable aquarium fish in Iran and approximately 5 million goldfish are reproduced during the new-year's holidays (Nowruz) [9].

This study aimed to investigate the lethal concentration (LC_{50} 96-h) of malathion and

1. Department of Fisheries, University of Tehran, Karaj, Iran.
2. Department of Fisheries, University of Gonbad Kavous, Golestan, Iran.
*Corresponding Author: E-mail: saeid.shahbazi@ut.ac.ir

Hinosan in gold fish *Carassius auratus*. Due to the proximity of breeding places of gold fish to rivers, rice paddies and orchards, this study can to be useful for breeding gold fish.

MATERIAL AND METHODS

Two hundred and fifty *Carassius auratus* with an average weight 5±1 g were acclimatized to the laboratory condition in 80×40×30 cm aquarium for 15 days. In order to measure biological capability and determine survival, the specimens were kept in natural and toxin-free environment to determine natural mortality. Physicochemical properties of water used for these experiments were as follows: temperature 24±1 ºC, dissolved oxygen 7 to 7.5 ppm, pH 7.5 to 8 and $CaCO_3$ 215 mg/l. During the main experiment, water was not changed. The specimens were fed twice a day with Biomar ® at 2% of the body weight, before the test. All Experiments were performed on 16-h light and 8 hours of darkness. Fish behavior and clinical signs were recorded. Experiments were performed according to the OECD standard method [10], to determine 96 h LC_{50} of malathion and Hinosan to *Carassius auratus*. To determine the lethal concentration of Hinosan fishes were divided to 5 treatments (4 toxin and 1 control treatment) and were exposed to concentrations of 0, 1, 2, 4, 8 mg L^{-1}. For malathion the specimens were exposed to 6 treatments (5 toxin and 1 control treatment) i.e. 0, 1, 2, 4, 16 mg L^{-1}. Mortality rates were recorded after 24, 48, 72 and 92 hours and dead specimens were removed from the aquarium. The nominal concentration of toxin causing mortality (LC_1،LC_{10} ، LC_{30} ،LC_{50} ،LC_{70} ،LC_{90} and LC_{99}) within 24, 48, 72 and 92 hours for each toxin were computed on the basis of probit analysis version 16/0 with SPSS 18 [11]. Eventually, maximum allowable concentration (MAC), the lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC) were also determined.

RESULTS

No mortality was observed in the control group in the main experiment and during the acclimatization. Hours after poison exposure absolute mortality (hundred percent) with Hinosan occurred in 8 ppm concentration; while, this value was 16 ppm for malathion (Table 1). The values of LC_{50} 96h for malathion and Hinosan in *Carassius auratus* were 4.71 and 4.02 mg L^{-1}, respectively (Table 2-4). Behavioral changes and clinical symptoms in fishes that were exposed to malathion started at 8 mg L^{-1} after 5 hours, but at 16 mg L^{-1} symptoms were observed after 2 hours. These changes occurred for Hinosan toxicity in 4 and 8 mg L^{-1} after 2 hours. The control group and treatments with concentrations of 1 and 2 mg L^{-1} had normal behavior during the experiment. Fishes with acute toxicity had asthenia, imbalance, spiral swimming and collapse to the bottom of the aquarium. Abnormal swimming, increasing respiratory motions, bruise in the caudal fin was the symptoms of Hinosan acute toxicity in fishes. No Observed Effect Concentration (NOEC) and Lower Observed Effect Concentration (LOEC) are shown in Figure 1 and 2.

Based on computed LC_{50} 96h for every species, contaminants MAC in natural environments is 0.1 LC_{50}. In fact, MAC is the amount concentration in the range concentrations of a toxic substance in an environment that is between NOEC and the LOEC level. Therefore, the calculated values were 0.402 and 0.471 mg/l for Hinosan and malathion respectively, in 24±1 C°. In addition, LOEC which is the lowest concentration or amount of a toxic substance found by experiment or observation that causes an adverse effect on function, capacity, growth, development, or the lifespan of a target species under defined conditions of exposure was calculated to be 4 mg/l for both toxins. NOEC, which represent the toxicant consecration that has no effect on the organisms exposed to and yields no statistically significant deviation from a control group; was determined to be 2 mg/l for both toxins.

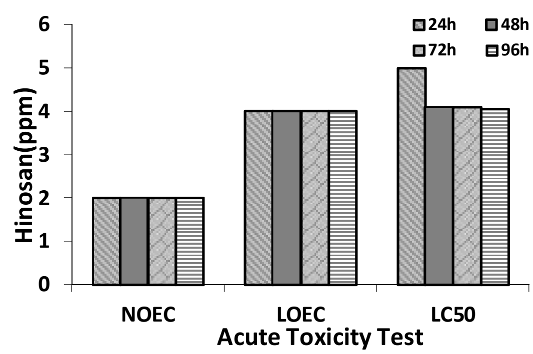

Figure 1. Acute toxicity test of Hinosan on *Carassius auratus*.

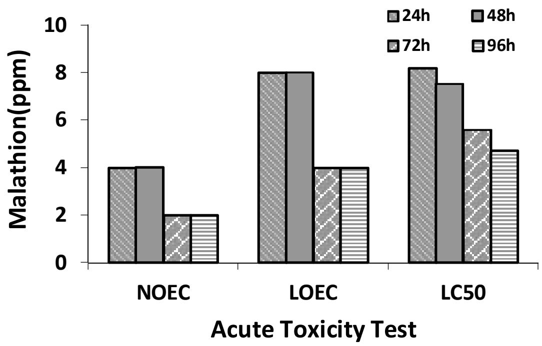

Figure 2. Acute toxicity test of malathion on *Carassius auratus*.

Table 1. Cumulative mortality of goldfish (n=21, each concentration) exposed acutely to Hinosan.

Mortality (No.)				Concentration (ppm)
96h	72h	48h	24h	
0	0	0	0	control
0	0	0	0	1
0	0	0	0	2
10	9	9	3	4
21	21	21	21	8

Table 2. Lethal Concentrations (LC_{1-99}) of Hinosan depending on time (24-96h) for goldfish (mean ± SE).

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_1	2.95 ± 0.15	2.68 ± 0.81	2.68 ± 0.81	2.64 ± 0.74
LC_{10}	3.82 ± 0.15	3.31 ± 0.81	3.31 ± 0.81	3.26 ± 0.74
LC_{30}	4.45 ± 0.15	3.77 ± 0.81	3.77 ± 0.81	3.71 ± 0.74
LC_{50}	**4.88 ± 0.15**	**4.09 ± 0.81**	**4.09 ± 0.81**	**4.02 ± 0.74**
LC_{70}	5.32 ± 0.15	4.41 ± 0.81	4.41 ± 0.81	4.33 ± 0.74
LC_{90}	5.95 ± 0.15	4.87 ± 0.81	4.87 ± 0.81	4.78 ± 0.74
LC_{99}	6.82 ± 0.15	5.51 ± 0.81	5.51 ± 0.81	5.39 ± 0.74

Table 3. Cumulative mortality of goldfish (n=21, each concentration) exposed acutely to malathion.

Mortality (No.)				Concentration (ppm)
96h	72h	48h	24h	
0	0	0	0	control
0	0	0	0	1
0	0	0	0	2
4	1	0	0	4
21	21	14	9	8
21	21	21	21	16

Table 4. Lethal Concentrations (LC1-99) of malathion depending on time (24-96h) for Goldfish (mean ± SE).

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_1	5.29 ± 0.80	5.01 ± 0.70	3.53 ± 0.36	2.83 ± 0.36
LC_{10}	6.59 ± 0.80	6.13 ± 0.70	4.45 ± 0.36	3.68 ± 0.36
LC_{30}	7.54 ± 0.80	6.94 ± 0.70	5.11 ± 0.36	4.29 ± 0.36
LC_{50}	**8.19 ± 0.80**	**7.50 ± 0.70**	**5.58 ± 0.36**	**4.71 ± 0.36**
LC_{70}	8.84 ± 0.80	8.06 ± 0.70	6.04 ± 0.36	5.13 ± 0.36
LC_{90}	9.78 ± 0.80	8.88 ± 0.70	6.70 ± 0.36	5.74 ± 0.36
LC_{99}	11.08 ± 0.80	9.99 ± 0.70	7.62 ± 0.36	6.59 ± 0.36

DISCUSSION

Result of LC_{50} 96h for both toxins showed that it decreases with increasing toxin concentration and duration of exposure. It means that by increasing the duration, lower concentrations of the toxins were required to kill 50% of fish and LC_{50} values in the first 24 hours of the experiment were more than the end of the 96-hour LC_{50}. Exposure time is one of the effective factors in OPs toxicity [12]. When fish are exposed to a constant concentration of toxins, their tolerance diminishes over time and the toxin has more time to affect. When the toxin accumulates in fish tissues, adverse effects increase which thereby causes a decrease in LC_{50} 96h. Overall, LC_{50} for both toxins used in *C. auratus*, showed a decreasing trend over 96 hours and in listed physicochemical conditions. Previous studies on acute toxicity of Diazinon and Deltamethrin in *Cyprinus carpio* [13,14] also reported similar decreasing trends in LC_{50} 96h.

Contrasting results are limited on acute toxicity of malathion and Hinosan for *Carassius auratus*. Sailatha et al. observed LC_{50} 48h for technical and commercial grades of malathion on *Tilapia mossambica* as 5.52 and 0.337 ppm, respectively [15]. They declared that commercial grade malathion was more toxic than the technical one. Forget et al. reported mortality and LC_{50} values for several stages of the marine copepod *Tigriopus brevicornis* (Müller) exposed to malathion as 24.3 μg $liter^{-1}$ [16]. They indicated that dichlorvos was the most toxic substance for *T. brevicornis*, followed by malathion. Pandey et al. determined LC_{50} value for malathion in *Channa punctatus* to be 6.61 ppm [17]. They declared that LC50 value for malathion was higher than the value reported (1.8 mg/L) by Tsuda et al. at 48h in killifish (*Oryzias latipes*) [18] and Khangarot and Ray reported a lower LC50 value (1.738, 1.39–2.353 ppm) at 96h in *C. punctatus* [19]. Also acute toxicity of malathion for some fishes like, *Labeo rohita, Ptychocheilus Lucius*, *Colisa fasciatus* and *Ctenopharyngodon idella* were found to be 0.009, 3.71, 2.2 and 2.138 mgl/L, respectively [20- 23].

Data on Hinosan toxicity in fish are limited too. Shamoushaki determined LC_{50} values of Hinosan for *Acipenser nudiventris* as 28 ppb and reported that A. nudiventris are severely irritated by Hinosan [24]. Also Alinezhad determined LC_{50} values of Hinosan for A. persicus and *A. stellatus* as 307 ppb and 0.206 ppb, respectively [25].

Behavior is considered a promising tool in ecotoxicology [26, 27]. In toxic environments, fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium which is due to inhibition of acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine in cholinergic synapses causing hyperstimulation [28]. Pesticides are lipophilic and are rapidly absorbed in fish gills which cause respiratory limitations [29]. They also attack neural organs resulting in imbalance, spiral swimming and collapse to the bottom of the aquarium. Our observed behavioral changes were in acordance with previous researches on organophosphorus pesticides [30- 33] and malathion on *CtenoPharyngodon idella*[26]. Change in respiration rate is one of the common physiological responses to toxicants [22]. Gulping air at the surface and swimming at the water surface were also observed in acute toxicity by malathion in *Labeo rohita* [34]. Abnormal swimming pattern, intense opercular movements and loss of coordinated movement with fish lying on their side in acute toxicity of diazinon and malathion was reported by Banaee et al. in *Oncorhynchus mykiss* [31], Uner et al. in *Oreochromis niloticus* [30], Pandey et al. in *Channa punctatus* [17] and Salwa and El Ella in *Ctenopharyngodon idella*[26].

Maximum Allowable Concentration (MAC) in natural environments according to LC_{50} 96h is 0.1 LC_{50} level. The value of MAC for malathion and Hinosan in mean temperature 24±1 C° were calculated as 0.471 and 0.402 mg/l, respectively. The lowest observable effect concentration (LOEC) is analogous to the "limit of detection" of the conventional methods of analysis. LOEC represents the initial toxicity threshold of a chemical while NOEC represent the concentration of toxicant that will not cause any effect. Sensitivity of bioassays, toxicity evaluation and comparative evaluation of the effects of pesticides was evaluated using the LOEC values [35]. The acute toxicity response, LOEC and NOEC are shown in Figure 1 and 2.

Also, according to Table 5 (determination of toxicity in different pesticides) both malathion and Hinosan have medium toxicity for *Carassius* *auratus*. Malathion and Hinosan are abundant in many ecosystems and are used extensively in farmlands. Employing biological methods to reduce pests instead of chemical pesticides could help to improve environment [36].

Table5. Determination of toxicity in different pesticides.

LC_{50} (mg/L)	Degree of toxicity
Up to 100	Nearly no poison
10-100	Toxicity Low
1-10	Toxicity Medium
0.1-1	Toxicity High
Less to 0.1	Toxicity Very high

CONCLUSION

Acute toxicity studies are the first step in toxicological reaserch. Our results demonstrated that malathion and Hinosan have harmful effects on the behavior of fish. This study showed that these toxicants at relatively low concentrations cause mortality in fishes even at short periods of exposure. Thus, it can be concluded that Gold fishes are sensitive to Hinosan and malathion, and their mortality rate is dose dependent, as well.

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