Original Article

Acute Toxicity and Behavioral Changes Associated with Diazinon in *Rutilus rutilus caspicus* and *Hypophthalmicthys molitrix*

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ABSTRACT

Background: Diazinon is an organophosphorous pesticide which widely found in municipal, agricultural, and urban storm water discharges. The present study was conducted to achieve lethal concentration (LC_{50}) and behavioral changes of *Rutilus rutilus caspius* and *Hypophthalmicthys molitrix* after exposure to lethal concentration of diazinon.

Methods: The experiment was carried out in static conditions, based on instructions of OECD in 4 days under controlled water physicochemical conditions with pH of 7.2±0.2, oxygen of 7±0.3 mg/l, total hardness of 180 mg CaCo3 and temperature of 24±1 C°. All fishes were acclimatized in 400 L aquaria for 10 days. Treated aquaria had concentrations of 0.5, 1, 2, 4, 8, 10, 20, 40, 60, and 80 ppm of diazinon for *H. molitrix*, and 1, 2, 4, 8, 10, and 20 for *R. rutilus caspicus*, while there was no toxic concentration for the control group. LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90}, and LC_{99} were calculated for 24, 48, 72, and 96 hours.

Results: LC_{50} 96h diazinon values were 3.93 and 1.71 ppm for *H. molitrix* and *R. rutilus caspicus*, respectively. Clinical observation revealed that the poisoned fishes suffered from nerve paralysis syndrome. The fishes exhibited irregular, erratic, and darting swimming movements, severe aching, and collapse to the bottom of the aquarium.

Conclusion: These findings suggest that diazinon has medium toxicity at low concentrations for thede two species and causes morbidities.

Keywords: Diazinon, Lethal Toxicity, Organ-Phosphorus Pesticide, Roach, Silver Carp.

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INTRODUCTION

Aquatic ecosystems constantly face threats, such as genetic restrictions and biological diversity. Although these environments are not the target for pesticides, some studies have indicated the presence of pesticides and their metabolites in surface water [1-3]. Organophosphorous pesticides compounds are widely used in agriculture and, thus, permeate into fish farms and aquatic ecosystems which cause contamination [4].

Diazinon (O, O-Diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate, INN - Dimpylate) is a non-systemic organophosphate insecticide formerly used to control cockroaches, silverfish, ants, and fleas in residential, non-food buildings [5, 6]. It is of medium stability in environment and can affect the immune system through influencing antigens and antibodies, production of T toxic lymphocytes, lymphocyte proliferation, cytokine and hydrogen peroxide production by macrophages, and disrupting activity of the nervous system [7]. Diazinon can be stable in environment for six months at low temperatures with humidity, high alkalinity, and lack of appropriate microbiological degradation [5]. Existence of diazinon in aquatic environments can adversely affect non-target organisms, such as invertebrates, mammals, birds, and particularly aquatic living organisms including fishes [8].

The "Silver carp" *Hypophthalmichthys molitrix* is a species of fishes in the family Cyprinidae which is the main species used in warm water culture in almost all provinces of Iran [9]. Rutilus is a genus of fishes in the family Cyprin-

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idae which is found in Europe and western Asia where there are about 15 species [9]. *Rutilus rutilus caspicus* Jakowlew, 1870, (Caspian roach) is native to the Caspian Sea and rivers leading to it. *R. rutilus caspicus* is widely distributed in the Caspian Sea but because of overfishing and deterioration of its spawning grounds, it is considered for being listed as an endangered species for the region [10].

Several studies have been conducted on acute toxicity and the destructive effect of toxicant in fishes [11-14], but sensitivity of various fish species is different for various toxic substances. Therefore, toxicology tests are needed for different fishes [15]. In addition, behavior serves as the link between physiological and ecological processes and it may act as an ideal for studying environmental pollutants effects for which fishes are considered an excellent model [16]. Many researchers have proposed using behavioral indicators in fish for ecologically relevant monitoring of environmental contamination [17].

Accordingly, the present study was conducted to determine the effect of acute toxicity with diazinon on *R. rutilus caspicus* and *H. molitrix*. Data on acute toxicity due to diazinon is scarce for these species and this study was carried out to collect such data.

MATERIALS AND METHODS

Three hundred live specimens of *H.molitrix* (mean weight 15 gr and total length 8 cm) and *R. rutilus caspicus* (mean weight 3.5 gr and total length 6 cm) were obtained. The samples were acclimatized in 400 L aquaria for 10 days. In order to measure biological capability and determine survival rates. The fishes were also kept in natural and toxin-free environments to determine their natural mortality rate. Dissolved oxygen was fixed at 7-7.3 ppm, pH: 7.2 to 7.5, temperature (24±1) and hardness of 180 ppm. The fishes were fed twice daily with Biomar feed at 2% body weight before the test. Feeding was discontinued 24 h prior to the test and throughout the test. All fishes were kept in 12 hr light and 12 h darkness conditions. Fish behavior and clinical signs were recorded. Static acute toxicity test was performed following the guideline of OECD standard method [18]. Pretest analysis was carried out to determine the concentration of the toxin in treated aquaria at concentrations of 0.5, 1, 2, 4, 8, 10, 20, 40, 60, and 80 ppm of diazinon for *H. molitrix* and 1, 2, 4, 8, 10, and 20 for *R. rutilus caspicus*, and the control group with no toxic concentration. Mortality rates were recorded after 24, 48, 72, and 92 hours and the dead fishes were quickly removed from the aquarium. The nominal concentrations 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 were calculated. LC_{50} values for 24, 48, 72, and 96 h exposures were computed on the basis of probit analysis using TOXISAT software. LC_{50} was calculated based on Hotos and Vlahos [19] and 95% confidence interval was calculated by the following equation:

$$LC_{50}\ (95\%\ CL) = LC_{50} \pm 1.96\ [SE\ (LC_{50})]$$

$$SE\ (LC_{50}=1/b \sqrt{pnw}$$

Where b was Slope of the toxin / probit regression line, p was the amount of toxicant, n was the number of fishes, and w was mean weight of samples.

During the experiment, clinical observations were recorded three times a day and at the end of the experiment, maximum allowable concentration (MAC; $LC_{50}/10$), the lowest observable effect concentration (LOEC), and no observable effect concentration (NOEC) were also determined.

Ethical Considerations

The experiments were performed on fishes complied with the protocols of the Society of Toxicology (code of ethics January 31, 1985; Revised June 1, 2005; Reviewed and Reaffirmed September 14, 2011; Revised November 5, 2012) and Canadian Council on Animal Care (CCAC, 1998). All analyses and experiments were performed to minimize suffering. The study was conducted with the minimal number of fishes.

RESULTS

No mortality was observed during the study. The results showed that within 96 h, mortality increased with increasing toxin concentrations and duration of exposure (Tables 1 and 2). According to the pretest analysis, lethal concentration ranges were 0.5-80 and 1-40 ppm for *H. molitrix* and *R. rutilus caspicus*, respectively. In addition, LC_{50} 96h for diazinon in *H. molitrix* and *R. rutilus caspicus* were 3.93 and 1.71 ppm, respectively (Tables 3 and 4).

The results showed that LC_{50} value for both fishes declined with increases in time and the amount of toxicant. According to LC_{50} value, maximum allowable concentrations were calculated 0.39 and 0.17 ppm for *H. molitrix* and *R. rutilus caspicus*, respectively (Figure 1).

Table 1. Mortality rate in acute toxicity (LC_{50}24h) rates for *Hypophthalmicthys molitrix* (n=21 each treatment).

Concentration (ppm)	n	24 h	48 h	72 h	96 h
Control	21	0	0	0	0
0.5	21	0	0	0	0
1	21	0	3	4	6
2	21	0	6	7	10
4	21	3	6	9	13
8	21	5	7	12	15
10	21	7	10	16	21
20	21	18	21	21	21
40	21	17	21	21	21
60	21	21	21	21	21
80	21	21	21	21	21

Table 2. Mortality rate in acute toxicity (LC_{50}24h) rates for *Rutilus rutilus caspicus* (n=21 each treatment).

Concentration (ppm)	n	24 h	48 h	72 h	96 h
Control	21	0	0	0	0
1	21	5	7	10	10
2	21	8	12	12	16
4	21	13	14	18	18
8	21	14	18	19	20
10	21	15	18	19	21
20	21	19	21	21	21

Table 3. Lethal Concentrations (LC1-99) of diazinon depending on time (24-96h) for *Hypophthalmicthys molitrix* (mean ± SE).

Point	Concentration (ppm)+ SE			
	24h	**48h**	**72h**	**96h**
LC_1	0.87 ± 0.6	0.01 ± 0.33	0.01 ± 0.34	0.01 ± 0.34
LC_{10}	5.39 ± 0.6	2.25 ± 0.33	0.01 ± 0.34	0.01 ± 0.34
LC_{30}	8.67 ± 0.6	5.74 ± 0.33	3.73 ± 0.34	1.94 ± 0.34
LC_{50}	10.90 ± 0.6	9.31 ± 0.33	6.44 ± 0.34	3.93 ± 0.34
LC_{70}	13.20 ± 0.6	12.80± 0.33	9.14 ± 0.34	5.92 ± 0.34
LC_{90}	16.40 ± 0.6	18.00± 0.33	13.00 ± 0.34	8.80 ± 0.34
LC_{99}	21 ± 0.6	25.10± 0.33	18.40 ± 0.34	12.70± 0.34

Table 4. Lethal concentrations (LC1-99) of diazinon depending on time (24-96h) for *Rutilus rutilus caspicus* (mean ± SE).

Point	Concentration (ppm)+ SE			
	24h	**48h**	**72h**	**96h**
LC_1	0.01 ± 0.31	0.01 ± 0.32	0.01 ± 0.33	0.01 ± 0.45
LC_{10}	0.01 ± 0.31	0.01 ± 0.32	0.01 ± 0.33	0.01 ± 0.45
LC_{30}	1.91 ± 0.31	1.35 ± 0.32	0.66 ± 0.33	0.91 ± 0.45
LC_{50}	4.92 ± 0.31	3.68 ± 0.32	2.35 ± 0.33	1.71 ± 0.45
LC_{70}	7.92± 0.31	6.00± 0.32	4.05 ± 0.33	2.51 ± 0.45
LC_{90}	12.20± 0.31	9.36± 0.32	6.49± 0.33	3.67 ± 0.45
LC_{99}	18.20± 0.31	13.90± 0.32	9.86 ± 0.33	5.26± 0.45

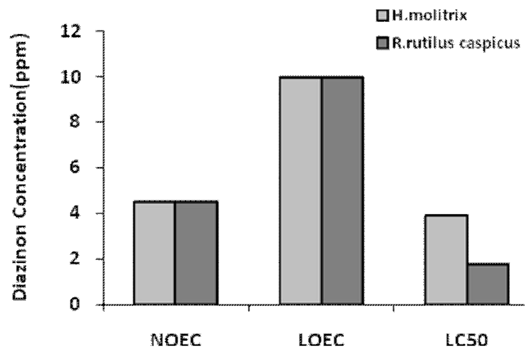

Figure 1. Acute toxicity testing statistical endpoints in *Rutilus rutilus caspicus* and *Hypophthalmicthys molitrix* exposed to crude diazinon.

Nerve paralysis syndrome was observed as the most common clinical sign in the fishes. At the beginning of the experiment, the fishes exhibited fast movements with severe impatience. However, gradually and at the end of the experiment, the fishes showed less movement. Semicircle swimming, abnormal rotating, and increasing of opercular movements were also observed in the fishes.

DISCUSSION

Exposure time is an effective factor in organophosphorous toxic ratios [6]. When fishes are exposed to a constant concentration of the toxin, their tolerance diminishes over time and the toxin is more respite to exert an influence. However, when the toxin accumulates in fish tissue, the adverse effects on the body also increase and thereby cause reductions in LC_{50} 96h. Dissolved oxygen, pH, size and age, type of species, water quality, and concentration and formulation of test chemicals are other major factors in toxicity of chemicals to aquatic organisms [11]. The results indicated that in similar conditions, Caspian roach was more sensitive that silver carp to diazinon.

The toxicity of diazinon in fishes was evident and various LC_{50} values were obtained for different fish species. LC_{50} 69 h diazinon values were 0.16-0.09 ppm for *Anguilla Anguilla* [20], 7.83 ppm for *Orechromis niloticus* [21], 6.6 ppm for *Clarias gariepinus* [22], 1.53 ppm for *Cyprinus carpio* [23], 1.17 ppm for *Oncorhynchus mykiss* [7], and 4.14 for *Silurus glanis* [5]. Fishes sensitivity to diazinon depends on their adsorption, inhibition of acetylcholinesterase, and disposal of toxic substances ability [24]. Therefore, different fishes have various sensitivities to diazinon.

Behavior is considered a promising tool in ecotoxicology [25, 26]. Brain neurotransmitter levels and enzyme functions correlate with behavioral states [27, 28]. Therefore, neurological dysfunction induced by toxicant exposure can cause abnormal behavior. Pesticides are lipophilic and are rapidly absorbed in fish gills which cause respiratory limitations [29]. They inhibit brain cholinesterase activity and disrupt physiological functions at cholinergic synapses by altering cholinergic receptor number [16]. In addition, they decrease muscarinic cholinergic receptor numbers in the brains of several different fish species, though the mechanisms of action remain unclear [30]. Furthermore, organophosphorous toxicants can decreases catalase activity in the brain and electrophysiological properties of the brain and alter several antioxidant enzyme activities in fishes [31, 32]. In the present study, fishes were exposed to diazinon had abnormal swimming movements with respiratory disorders, fast opercular movements, and abnormal rotation. These findings are reported by other authors who studied acute toxicities other than organophosphorous pesticides [11, 33-35].

Maximum allowable concentration (MAC) in natural environments is 0.1 LC_{50} according to LC_{50} 96h. The lowest observable effect concentration (LOEC) and MAC was determined 5 and 10 ppm for both species respectively (Figure 1). The 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 represents the concentration of the toxicant that will not cause any effect. Sensitivity of bioassays, toxicity evaluation, and comparative evaluation of the effects of pesticides was done using LOEC values [36].

CONCLUSION

According to Table 5 (determination of toxicity in different pesticides), diazinon presents medium toxicity for *H. molitrix* and *R. rutilus caspicus*. Diazinon is abundant in the environment and it is of high stability. Therefore, management programs should be conducted to reduce this pesticide in the environment. As it was mentioned, this pesticide has high behavioral toxicity to these two species.

Table 5. Determination of toxicity in different pesticides [37].

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

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